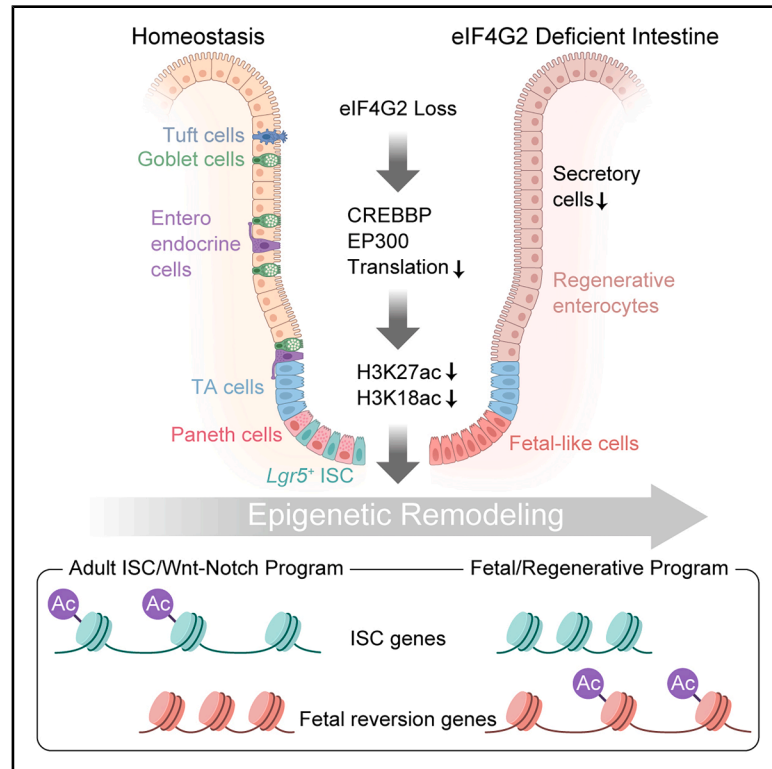


eIF4G2-mediated selective translation of chromatin regulators safeguards adult intestinal stem cell identity and differentiation

Graphical abstract



Authors

Haruko Kunitomi, Aye Myat Khaine, Radia Jamee, ..., Yuichi Shichino, Shintaro Iwasaki, Shinya Yamanaka

Correspondence

haruko.kunitomi@gladstone.ucsf.edu (H.K.), syamanaka@gladstone.ucsf.edu (S.Y.)

In brief

Yamanaka and colleagues show that the non-canonical translation factor eIF4G2 preserves adult intestinal identity by supporting selective translation of chromatin regulators. Loss of eIF4G2 shifts the epithelium into a durable fetal-like regenerative state with enhancer remodeling and impaired stem cell and secretory maturation.

Highlights

- eIF4G2 stabilizes adult intestinal epithelial identity
- *Eif4g2* loss triggers durable fetal-like and YAP-associated reprogramming
- Ribosome profiling links eIF4G2 to selective translation of chromatin regulators
- Translational buffering helps preserve adult enhancer and lineage programs



Article

eIF4G2-mediated selective translation of chromatin regulators safeguards adult intestinal stem cell identity and differentiation

Haruko Kunitomi,^{1,*} Aye Myat Khaine,¹ Radia Jamee,¹ Vanessa Arreola,¹ Mariselle Lancero,¹ Amba Raychaudhuri,¹ Samuel Perli,¹ Yoshiko Sato,² Mio Iwasaki,² Pedro Ruivo,³ Kiichiro Tomoda,^{1,2} Mari Mito,⁴ Yuichi Shichino,^{4,5} Shintaro Iwasaki,^{4,6} and Shinya Yamanaka^{1,2,7,*}

¹Gladstone Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA 94158, USA

²Center for iPS Cell Research and Application, Kyoto University, Kyoto 606-8507, Japan

³Comparative Pathology Laboratory, University of California, Davis, Davis, CA 95616, USA

⁴RNA Systems Biochemistry Laboratory, Pioneering Research Institute, RIKEN, Saitama 351-0198, Japan

⁵Department of RNA Biochemistry, Institute of Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan

⁶Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Chiba 277-8563, Japan

⁷Lead contact

*Correspondence: haruko.kunitomi@gladstone.ucsf.edu (H.K.), syamanaka@gladstone.ucsf.edu (S.Y.)

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SUMMARY

eIF4G2 (DAP5/NAT1) is a non-canonical translation initiation factor, but its role in homeostasis is unclear. Using inducible *Eif4g2* knockout mice and intestinal organoids, we show that eIF4G2 loss collapses *Lgr5*⁺ intestinal stem cell (ISC) and secretory maturation programs while preserving villus architecture. Transcriptomic and single-nucleus multiome analyses reveal a durable fetal-like/regenerative state with YAP-TEAD activation and regenerative absorptive cells. Ribosome profiling identifies selective translation-efficiency loss among chromatin regulators, especially the KAT3 coactivators CREBBP and EP300, resulting in reduced KAT3 abundance and global histone acetylation; chemical KAT3 inhibition phenocopies this state. CUT&Tag and assay for transposase-accessible chromatin sequencing (ATAC-seq) demonstrate that reduced eIF4G2-KAT3 output drives locus-selective enhancer remodeling, with loss of adult ISC/Wnt-Notch elements and activation of TEAD-enriched fetal loci, without inflammatory or integrated stress response programs driving the transition. Fetal intestinal spheroids remain viable despite similar biochemical defects, highlighting a stage-specific requirement for translational buffering in maintaining adult identity.

INTRODUCTION

Protein translation initiation is rewired during development, stress responses, and regeneration^{1–3} and can rapidly reshape cell state by selectively altering protein output.^{4,5} eIF4G2 (DAP5/NAT1), a non-canonical eIF4G-family member, has been implicated in the selective translation of mRNAs harboring features such as structured 5' UTRs and upstream open reading frames (uORFs),^{6–8} but its physiological function in adult tissues remains unclear due to embryonic lethality in *Eif4g2*-null mice.⁹ We therefore generated an inducible loss-of-function model to interrogate the consequences of *Eif4g2* deletion at the tissue level.

We focused on the intestinal epithelium, a rapidly renewing tissue maintained by *Lgr5*⁺ intestinal stem cells (ISCs),¹⁰ in which precise translational control is likely critical for sustaining self-renewal and differentiation. When ISC identity is disrupted, intestinal epithelial cells can transiently adopt a regenerative fetal-like program^{11,12} associated with YAP (Yes-associated protein)/TAZ (transcriptional coactivator with PDZ-binding motif)-TEAD (TEA domain) signaling.¹³

Here we show that eIF4G2 contributes to adult intestinal homeostasis by sustaining selective translation of dosage-sensitive epigenetic regulators, including the KAT3 (lysine acetyltransferase 3) coactivators CREBBP (CREB-binding protein) and EP300. Loss of this buffering decommissions adult ISC-associated enhancers, activates fetal-like regulatory programs, and drives a stable alternative epithelial state. These findings provide a framework for understanding how selective translation initiation interfaces with epigenetic regulation to control stem cell identity in adult tissues.

RESULTS

Generation of a tamoxifen-inducible *Eif4g2*-KO mouse line

We generated a conditional *Eif4g2* allele by flanking exon 5 (103 bp) with loxP sites; Cre-mediated excision is predicted to introduce a frameshift and premature stop codon (Figure 1A). *Eif4g2*^{fllox} mice were recovered at Mendelian ratios (Figure S1A), indicating no overt viability defect associated with the floxed allele.



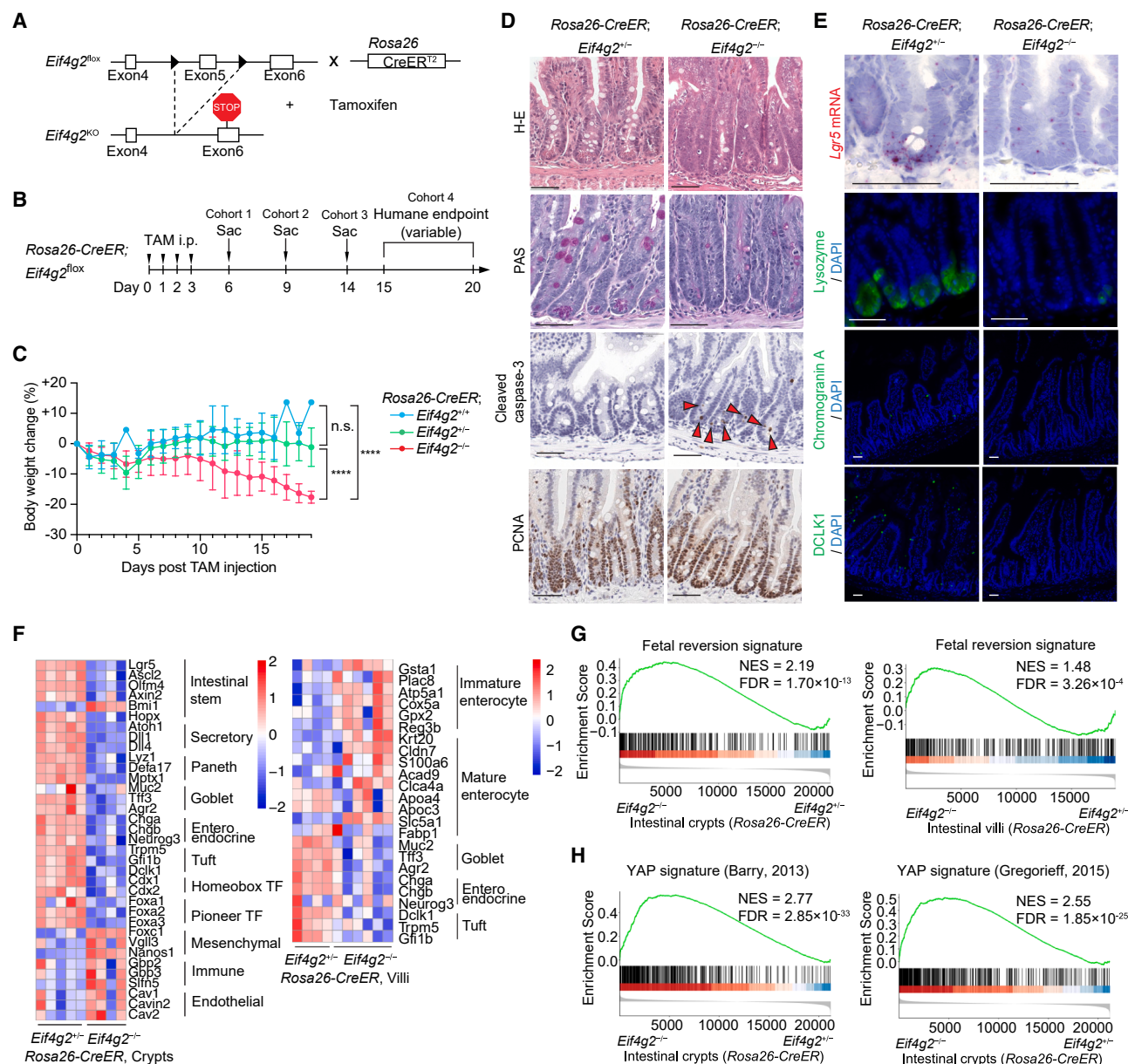


Figure 1. *Eif4g2*-KO intestines lose *Lgr5*⁺ ISCs and adopt a fetal-like, YAP-associated regenerative state

(A) Conditional *Eif4g2* floxed allele and CreER KO strategy.

(B) Systemic deletion timeline and analysis cohorts (*Rosa26-CreER*). Humane endpoint is defined as >15% weight loss relative to day 0.

(C) Body weight after tamoxifen in *Rosa26-CreER* mice (WT *n* = 11, heterozygote *n* = 24, homozygote *n* = 29). Mixed-effects model (REML) with Tukey multiple comparisons where indicated.

(D) Jejunum H&E, PAS, cleaved caspase-3, and PCNA staining at humane endpoint. Red arrowheads indicate cleaved caspase-3-positive cells.

(E) *Lgr5* RNAscope and lysozyme, chromogranin A, and DCLK1 immunofluorescence. *Lgr5*⁺ cells and secretory lineages are reduced in *Rosa26-CreER*; *Eif4g2*^{-/-} epithelium. *n* = 4 per genotype.

(F) Bulk RNA-seq heatmaps of jejunal villus and crypts at day 9 post-TAM (*Rosa26-CreER*). Values are gene-wise Z scores. Crypts: *Eif4g2*^{+/-} *n* = 5, *Eif4g2*^{-/-} *n* = 4; villi: *n* = 5 per genotype.

(G and H) GSEA of fetal spheroid-enriched (fetal reversion) signature and YAP-target signatures in crypts and villi ranked by differential expression (*Rosa26-CreER*; *Eif4g2*^{-/-} versus *Eif4g2*^{+/-}).

Data are mean ± SD **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. Scale bars, 50 μm.

See also [Figure S1](#) and [Tables S1](#) and [S2](#).

Crossing to B6.129-Gt (*ROSA*)26Sor^{tm1(cre/ERT2)Tyj/J} (*Rosa26-CreER*) mice¹⁴ yielded a tamoxifen-inducible systemic *Eif4g2*-knockout (KO) mouse model with efficient recombination across tissues (except the brain) and near-complete intestinal eIF4G2 protein loss by day 6 (Figures 1B, S1B, and S1C).

eIF4G2 is indispensable for ISCs in mice

After tamoxifen administration, wild-type (WT) (*Eif4g2*^{+/+}) and heterozygous (*Eif4g2*^{+/-}) mice remained stable, whereas homozygous (*Eif4g2*^{-/-}) mice developed a progressive clinical decline and reached humane endpoints (>15% weight loss) within 3 weeks (Figure 1C). Consistent with prior work,⁹ histopathological analysis of major organs in *Eif4g2*^{+/-} mice showed no overt abnormalities. In contrast, *Eif4g2*^{-/-} mice showed hypocellularity in bone marrow (Figure S1D) and a prominent intestinal phenotype marked by reduction of goblet and Paneth cells (Figure 1D). We therefore focused subsequent studies on the small intestine.

PAS (periodic acid-Schiff) staining confirmed the reduction of goblet cells, and RNAscope/immunostaining showed marked depletion of *Lgr5*⁺ ISCs, lysozyme⁺ Paneth cells, chromogranin A⁺ enteroendocrine cells, and DCLK1⁺ tuft cells (Figures 1D and 1E). Immunoblotting of isolated crypts confirmed efficient eIF4G2 depletion and reduced OLFM4 (olfactomedin 4) and lysozyme protein levels (Figure S1E). Cleaved caspase-3⁺ apoptotic cells increased in *Eif4g2*^{-/-} crypts, whereas PCNA⁺ (proliferating cell nuclear antigen-positive) proliferating cells were largely preserved, suggesting relative sparing of transient amplifying (TA) cell proliferation and villus structure despite loss of ISC and secretory lineages (Figure 1D).

Blinded pathology scoring using a modified system from Erben et al.¹⁵ confirmed increased lesion severity in *Eif4g2*^{-/-} mice, driven mainly by goblet- and Paneth-cell loss, crypt necrosis, and increased crypt proliferation (Figure S1F; Table S1). Goblet-cell loss was also evident in the colon (Figure S1G). Collectively, systemic eIF4G2 deletion depletes ISCs, impairs secretory differentiation, and increases crypt cell death, while proliferative subpopulations and enterocytes are relatively maintained.

The *Eif4g2*-deficient epithelium adopts a fetal-like reversion program

To define transcriptional programs sustaining villus architecture despite ISC loss, we performed RNA sequencing (RNA-seq) on crypt and villus fractions 9 days after tamoxifen (Figure 1B, cohort 2). *Eif4g2*^{-/-} crypts showed reduced ISC and secretory-lineage markers, together with downregulation of intestinal lineage regulators such as homeobox factors (*Cdx1*, *Cdx2*)¹⁶ and pioneer factors (*Foxa2*, *Foxa3*)¹⁷ (Figure 1F, left). In parallel, we observed increased expression of mesenchymal, immune, and endothelial programs, primarily consistent with erosion of epithelial lineage programs, although altered cellular composition cannot be excluded (Figure 1F, left). In contrast, *Eif4g2*^{-/-} villus fractions showed increased immature enterocyte markers and reduced secretory-lineage markers, indicating aberrant enterocyte gene expression despite preserved morphology (Figure 1F, right).

Loss of ISCs following irradiation, toxin-mediated ablation, and chemotherapy can trigger a transient regenerative fetal-like reversion, in which progenitor and differentiated cells adopt fetal-like transcriptional states that support epithelial repair, a process linked to YAP/TAZ-TEAD signaling.^{11–13} Gene set enrichment analysis (GSEA) showed enrichment of a published fetal-like crypt signature¹⁸ in *Eif4g2*^{-/-} crypts (NES [normalized enrichment score] = 2.19, false discovery rate [FDR] <0.01; Figure 1G, left; Table S2) and of two independent YAP-target gene sets^{19,20} (Figure 1H; Table S2). These data indicate that *Eif4g2* loss triggers an early fetal-like program with YAP-target induction.

Bmi1—an adult “+4” reserve stem cell marker²¹ also linked to fetal ISC populations²²—was among the upregulated stem-associated transcripts in *Eif4g2*^{-/-} crypts (Figure 1F, left). RNA-scope confirmed broad crypt-wide *Bmi1* expression in *Eif4g2*^{-/-} crypts, whereas in *Eif4g2*^{+/-} controls, *Bmi1* signal was detected only in rare, scattered cells (Figure S1H). Together, the fetal-like stem/progenitor state induced by eIF4G2 depletion is characterized by increased *Bmi1* expression.

Eif4g2^{-/-} villus fractions also showed enrichment of the fetal-like crypt gene signature (NES = 1.48, FDR < 0.01; Figure 1G, right). This suggests that the fetal-like reversion extends across the crypt-villus axis.

Single-nucleus multiome profiling reveals the emergence of a regenerative cycling population following *Eif4g2* loss

To define the impact of eIF4G2 deletion in the intestine at higher resolution, we performed single-nucleus multiome (snMultiome; paired RNA + assay for transposase-accessible chromatin [ATAC]) profiling at days 6 and 9 after tamoxifen (Figure 1B, cohorts 1 and 2). Villus epithelium was removed before crypt isolation, enriching the dataset for crypt cells. Because day 6 and day 9 samples showed similar structure without major timepoint-specific effects, we combined them for the subsequent analyses.

Weighted nearest neighbor (WNN) analysis identified distinct subpopulations segregating by genotype (Figure 2A) and cell type (Figure 2B). Within the three major populations shown in Figure 2A (stem/proliferative, absorptive, and goblet/Paneth), *Eif4g2* loss generated distinct subpopulations (Figures 2A, 2B, S2C, and S2E). In contrast, other subpopulations—including absorptive progenitors, secretory progenitors, tuft, and enteroendocrine—co-clustered between *Eif4g2*^{+/-} and *Eif4g2*^{-/-} samples (Figure 2A), arguing against major batch effects. *Eif4g2* expression was comparable across subpopulations (Figure S2A), indicating that the observed differences do not reflect basal expression.

Within the stem/proliferative population, the *Eif4g2*^{-/-} subpopulation showed high proliferative activity, as indicated by *Mki67* expression (Figure S2A) and proliferating score (Figure S2B), and was therefore designated “regenerative cycling.” This subpopulation also showed reduced *Lgr5* and increased *Bmi1* and *Ly6a* (Sca-1), consistent with a regenerative state distinct from canonical *Lgr5*⁺ ISCs (Figure 2C).

To quantify genotype-dependent changes in the stem/proliferative population, we compared per-mouse pseudobulk UCell scores (Figure 2D). *Eif4g2* loss reduced ISC signature scores

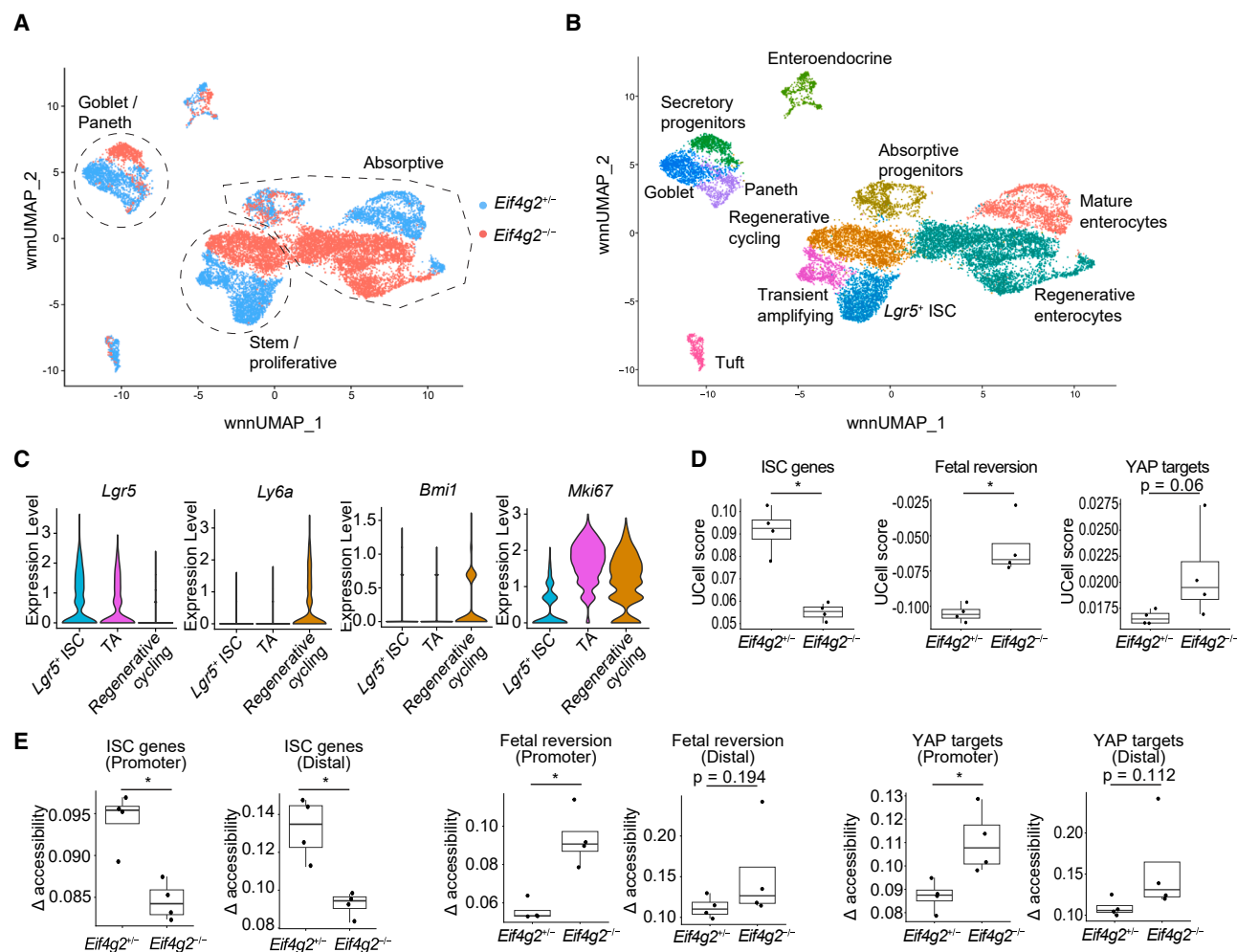


Figure 2. Single-nucleus multiome profiling reveals regenerative cell-state and chromatin remodeling following *Eif4g2* loss

(A and B) WNN/UMAP of intestinal epithelial snMultiome data colored by genotype or annotated cell type. Dashed outlines indicate major stem/proliferative, absorptive, and goblet/Paneth subpopulations. Biological replicates: day 6, $n = 2$ per genotype; day 9, $n = 2$ per genotype. ~3,000 nuclei per sample.

(C) Violin plots of *Lgr5*, *Ly6a*, *Bmi1*, and *Mki67* across stem/proliferative subpopulations.

(D) Per-mouse pseudobulk UCell scores for ISC, fetal-reversion, and YAP-target signatures within the stem/proliferative subpopulation ($n = 4$ per genotype; two-sided Wilcoxon rank-sum test).

(E) Per-mouse Δ chromatin accessibility for promoter-proximal and distal regulatory elements associated with the indicated signatures within the stem/proliferative subpopulation (median ATAC TF-IDF of query peaks minus GC/length-matched background; $n = 4$ per genotype; two-sided Wilcoxon rank-sum test). Promoters are defined as peaks within ± 2 kb of TSS; distal peaks are LinkPeaks-linked peaks excluding promoter overlaps.

Data are mean $\pm$ SD. * $p < 0.05$.

See also Figure S2 and Table S2.

and increased fetal-reversion scores, with YAP-target scores also trending upward, consistent with adult ISC collapse and fetal-like reversion.

To test whether this remodeling is accompanied by epigenetic reprogramming, we analyzed the ATAC modality in the stem/proliferative population. For each mouse, we computed accessibility changes for ISC, fetal reversion, and YAP-target regulatory elements as the difference between median ATAC TF-IDF (term frequency-inverse document frequency) scores of signature-associated peaks and GC- and length-matched background peaks, separately for promoter-proximal peaks (± 2 kb from the transcription start site) and distal elements linked by LinkPeaks. *Eif4g2* reduced accessibility at ISC-associated regu-

latory elements across promoter-proximal and distal regions, consistent with collapse of the ISC regulatory program. In contrast, fetal-reversion and YAP-target signatures showed increased accessibility, most prominently at promoter-proximal elements, suggesting promoter-centered chromatin opening during activation of these programs (Figure 2E).

Together, *Eif4g2* loss drives coordinated transcriptional and epigenetic remodeling within the stem/proliferative population.

***Eif4g2* loss induces lineage maturation defects in absorptive and secretory subpopulations**

Within the absorptive population, the absorptive progenitor subpopulation contained both *Eif4g2*^{+/+} and *Eif4g2*^{-/-} cells

(Figure S2C), indicating that early absorptive lineage cells emerge even without eIF4G2. In contrast, two differentiated enterocyte subpopulations showed strong genotype bias: “mature enterocytes” were largely derived from *Eif4g2*^{+/−} cells, whereas “regenerative enterocytes” were predominantly *Eif4g2*^{−/−} (Figure S2C). To quantify these differences, we compared per-mouse pseudobulk UCell scores across genotypes (Figure S2D). *Eif4g2*^{−/−} enterocytes showed increased fetal-reversion scores and a trend toward higher YAP-target activity, together with a significant decrease in the enterocyte maturation program (Figure S2D), consistent with the regenerative/fetal-like state and impaired maturation seen in bulk villus RNA-seq (Figure 1G).

Within the goblet/Paneth population, *Eif4g2*^{−/−} epithelium showed striking depletion of mature goblet and Paneth subpopulations, consistent with the *in vivo* histology (Figure S2E). Secretory progenitors (*Atoh1*⁺; Figure S2A), however, were maintained and even relatively expanded in *Eif4g2*^{−/−} samples (Figure S2E), suggesting that *Eif4g2* loss permits entry into the secretory lineage but impairs terminal maturation. Consistent with this model, per-mouse pseudobulk UCell scoring within the secretory progenitor subpopulation showed significantly increased fetal-reversion and YAP-target programs in *Eif4g2*^{−/−} samples (Figure S2F), linking the regenerative/YAP state to impaired terminal maturation.

We next asked whether cellular stress responses contribute to the secretory maturation defect. Because secretory lineages have high secretory output, they carry a substantial translational and proteostasis load.²³ We quantified the integrated stress response (ISR) program by UCell score within stem/proliferative, mature, and regenerative enterocytes, and secretory progenitors (Figure S2G). Secretory progenitors exhibited higher ISR scores than other subpopulations, even in controls, and ISR was further increased in *Eif4g2*^{−/−} secretory progenitors (Figure S2G). This suggests that *Eif4g2* loss exacerbates pre-existing stress in secretory progenitors, potentially contributing to defective terminal differentiation into mature goblet and Paneth cells. Increased ISR, however, does not explain the regenerative/fetal-like state in *Eif4g2*^{−/−} stem/progenitor and absorptive cells.

Intestinal epithelial *Eif4g2*-KO induces sustained fetal-like reversion

Rapid weight loss in systemic *Eif4g2*^{−/−} mice prevented analysis of long-term intestinal consequences. We therefore generated a tamoxifen-inducible, intestinal epithelium-specific *Eif4g2*-KO model by crossing *Eif4g2*-floxed mice with B6.Cg-Tg(*Vil1-Cre/ERT2*)23Syr/J mice.²⁴ Unlike systemic deletion, epithelial-restricted eIF4G2 deletion did not cause weight loss on normal chow for at least three months (Figure 3A). BaseScope *in situ* hybridization confirmed efficient loss of *Eif4g2* transcripts three months after tamoxifen (Figure 3B), with signal retained only in rare “escaper” crypts (Figure S3A). Epithelial-restricted deletion reproduced the key intestinal features of systemic *Eif4g2*^{−/−} mice, including marked depletion of OLFM4⁺ ISCs and MMP7⁺ Paneth cells (Figure 3B). These findings indicate that the intestinal phenotype is not secondary to systemic abnormalities.

We performed bulk RNA-seq on crypt and villus fractions from *Vil1-CreER; Eif4g2*^{fllox} mice three months after tamoxifen. Line-

age-marker changes closely resembled those at the early *Rosa26-CreER* time point (Figure 3C), including reduced ISC markers *Lgr5* and *Olfm4*, suppression of secretory-lineage markers, increased *Bmi1* expression, and persistence of fetal-like and YAP-associated signatures (Figures S3B and S3C). Thus, whereas fetal-like reversion is often transient after acute injury, *Eif4g2* loss establishes a durable epithelial state maintained for months.

snMultiome analysis suggested abnormal enterocyte maturation in *Eif4g2*^{−/−} mice (Figure S2D). In *Vil1-CreER Eif4g2*^{−/−} mice, genes involved in lipid uptake, microvillus structure, and nutrient transport were reduced, whereas chylomicron assembly-related programs were increased, potentially reflecting compensation for reduced lipid acquisition (Figure 3D). Although these mice maintained body weight for months on a chow diet (Figure 3A), whether they can tolerate other dietary conditions remains unknown.

Collectively, eIF4G2 deficiency induces a sustained fetal-like reversion program across the crypt-villus axis. Crypts suppress canonical *Lgr5*⁺ ISC programs and adopt a *Bmi1*-enriched fetal-like progenitor state, whereas villi retain enterocyte identity but show transcriptional attenuation of absorptive programs.

Small intestinal organoids recapitulate the *in vivo* findings

To assess epithelial-intrinsic effects of *Eif4g2* loss *in vitro*, we established small intestinal organoids (SIOs) from *Rosa26-CreER; Eif4g2*^{+/+}, *Eif4g2*^{fllox/+}, and *Eif4g2*^{fllox/flox} mice and induced recombination with 4-hydroxytamoxifen (4-OHT) (Figure 4A). eIF4G2 protein was efficiently depleted within 2 days in *Eif4g2*^{fllox/flox} SIOs (Figure S4A). RNA-seq at day 6 mirrored the *in vivo* transcriptome, with downregulation of ISC and secretory-lineage markers, increased *Bmi1*, reduced intestinal lineage regulators, and induction of transcripts associated with non-epithelial programs (Figure 4B). GSEA showed enrichment of a fetal-like crypt signature and YAP-target programs (Figures 4C and 4D), accompanied by increased active/nuclear YAP (Figures S4B and S4C).

Upon serial passaging after 4-OHT treatment (Figure 4E), most *Eif4g2*-deficient SIOs progressively lost budding morphology and failed to expand; however, a subset survived and adopted a cystic, fetal-like spheroid morphology (Figure 4F). These cystic spheroids remained expandable for at least 30 days. RT-qPCR (reverse-transcription quantitative PCR) time-course analyses showed progressive loss of *Lgr5* and induction of the fetal-like spheroid marker *Tacstd2* (Figure 4G). Collectively, these data indicate that *Eif4g2* loss drives fetal-like reprogramming in adult SIOs, with organoids adopting this state selectively retained during long-term culture.

Eif4g2-loss-induced fetal-like reversion occurs independently of canonical inflammatory and stress pathways

Because injury-associated fetal-like reversion has been linked to inflammatory cytokine signaling and cellular stress responses,^{11,12} we asked whether such pathways are activated upon eIF4G2 loss in SIOs. Single-sample GSEA of MSigDB Hallmark gene sets showed no significant increases in IFN- α ,

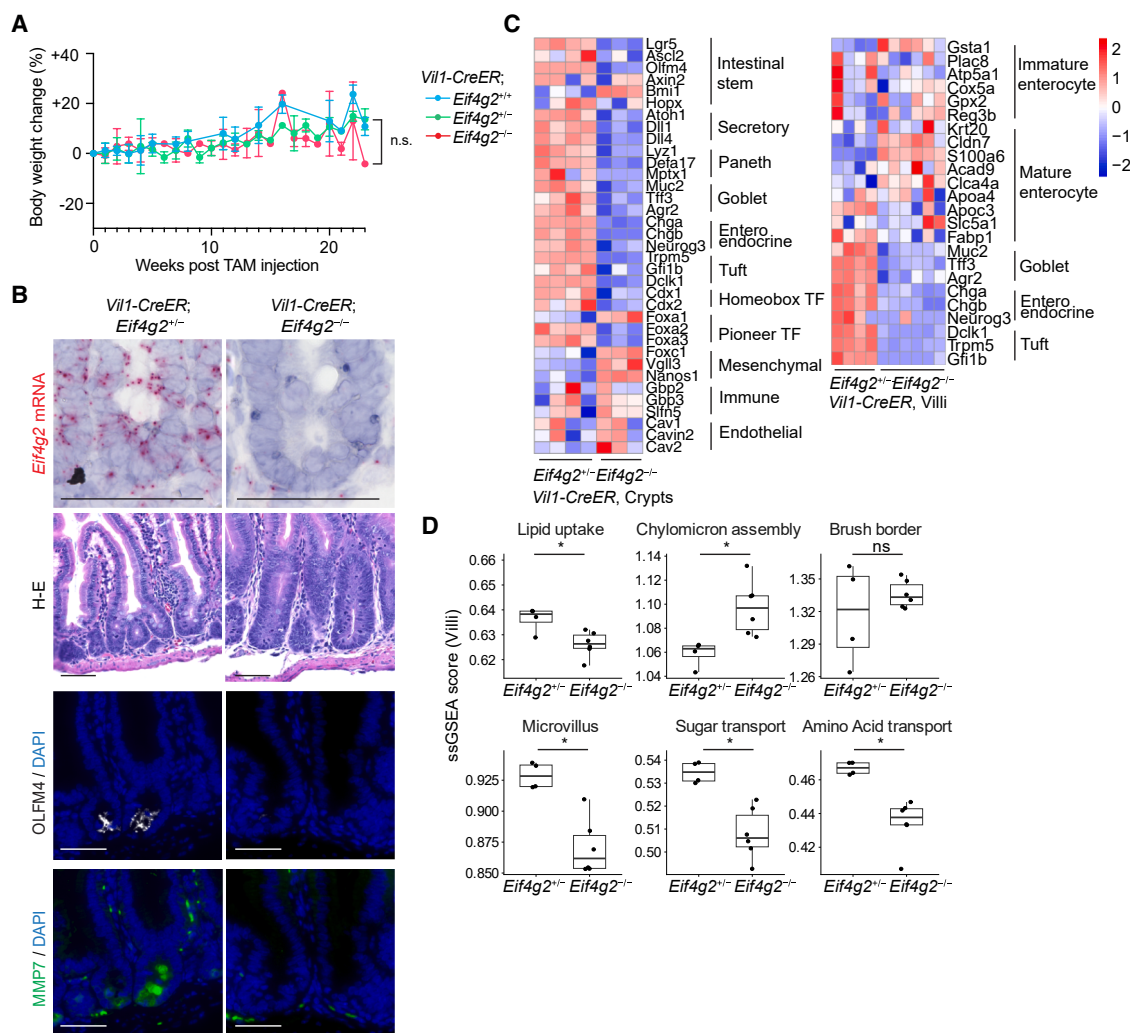


Figure 3. Fetal reversion caused by *Eif4g2*-KO is a durable state

(A) Body weight change over weeks following tamoxifen in *Vil1-CreER; Eif4g2* mice (WT $n = 7$, heterozygote $n = 7$, homozygote $n = 5$). Mixed-effects model (REML) with Tukey multiple comparisons where indicated.

(B) Jejunal *Eif4g2* BaseScope, H&E, OLFM4 and MMP7 immunofluorescence 12 weeks post-TAM (*Vil1-CreER*). Heterozygote $n = 4$, homozygote $n = 6$.

(C) Bulk RNA-seq heatmaps of jejunal crypts and villi at 12 weeks post-TAM (*Vil1-CreER*). Values are gene-wise Z scores. Crypts, *Eif4g2^{+/+}* $n = 4$, *Eif4g2^{-/-}* $n = 3$; villi: *Eif4g2^{+/+}* $n = 4$, *Eif4g2^{-/-}* $n = 6$.

(D) ssGSEA of indicated pathways at 12 weeks post-TAM (*Vil1-CreER*). Two-sided Wilcoxon rank-sum test with Benjamini-Hochberg adjustment. *Eif4g2^{+/+}* $n = 4$, *Eif4g2^{-/-}* $n = 6$.

Data are mean $\pm$ SD. Scale bars, 50 μ m. * $p < 0.05$.

See also Figure S3 and Table S2.

IFN- γ , TNF- α /NF- κ B, or IL-6-STAT3, although TNF- α /NF- κ B and IL-6-STAT3 trended upward (Figure S4D). We also evaluated translational stress-linked antiviral and stress pathways, including double-stranded RNA (dsRNA)/pattern recognition receptor (PRR) signaling and the ISR²⁵ (Figure S4D). We detected neither increased dsRNA levels (Figure S4E) nor enrichment of dsRNA/PRR-response signatures, and unfolded protein response (UPR) and ISR gene-set scores were unchanged (Figure S4D). Phosphorylation of eIF2 α was decreased in *Eif4g2*-deficient SIOs (Figure S4F). Together, these data do not support canonical cytokine-, dsRNA/PRR-, or ISR-driven pathways as dominant triggers of the fetal-like program,

instead suggesting that *Eif4g2* loss more directly activates the regenerative pathway.

Fetal spheroids propagate in the absence of *Eif4g2*

Eif4g2 KO in adult SIO induces cystic growth and fetal-like reversion. In contrast, fetal intestinal spheroids are inherently cystic and display a fetal-like transcriptional state.^{18,26}

We therefore asked whether eIF4G2 is required to maintain the fetal-like state. We established fetal spheroids from the E14.5 small intestine of *Rosa26-CreER; Eif4g2^{fllox/+}* and *Eif4g2^{fllox/fllox}* embryos and induced recombination with 4-OHT (Figure 5A). The eIF4G2 protein was

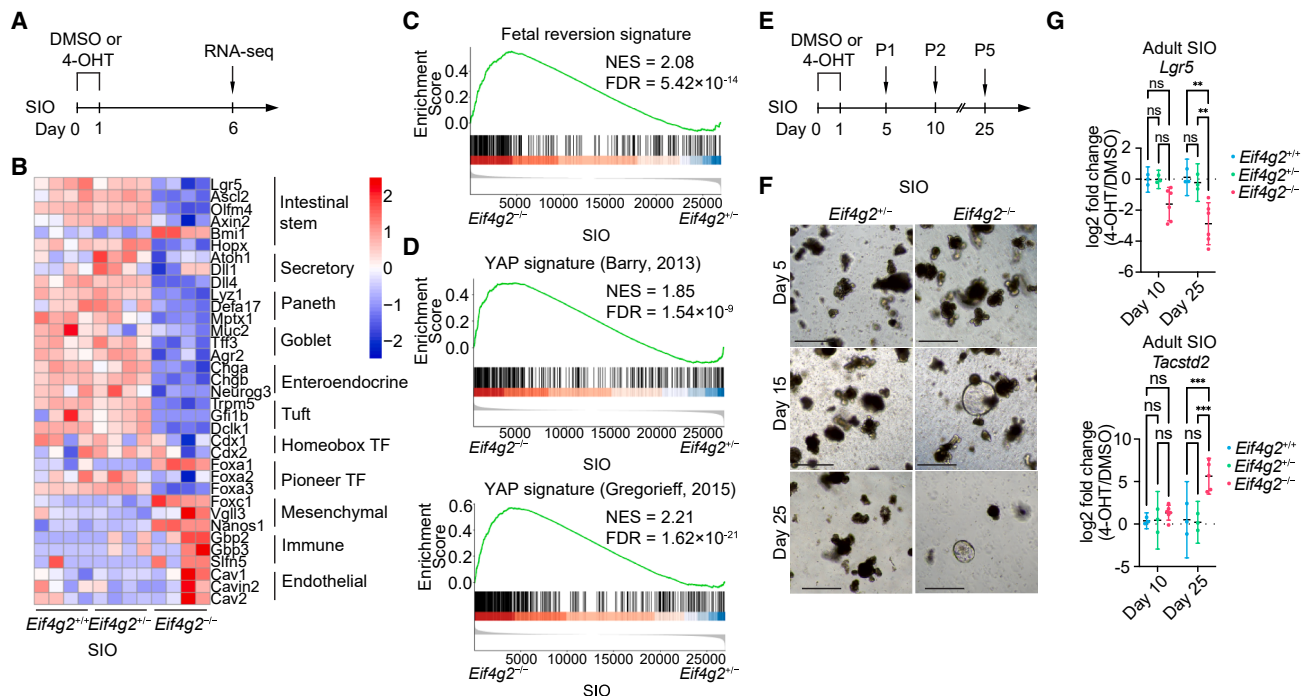


Figure 4. Adult SIOs recapitulate the *in vivo* phenotype

(A) Adult SIO experimental scheme: 4-OHT or DMSO on day 0 for 24 h; RNA-seq at day 6.

(B) Bulk RNA-seq heatmap in adult SIOs at day 6. Values are gene-wise Z scores. $n = 4$ per genotype.

(C and D) GSEA of fetal-reversion and YAP-target signatures ranked by differential expression (SIOs; Eif4g2^{-/-} versus Eif4g2^{+/+}).

(E) Passaging scheme after induction.

(F) Representative brightfield images at days 5, 15, and 25.

(G) *Lgr5* and *Tacstd2* RT-qPCR across time points, shown as log₂ fold change (4-OHT/DMSO) normalized to *Gapdh*; Brown-Forsythe/Welch ANOVA (lognormal distribution) with Dunnett correction. $n = 4$ each for Eif4g2^{+/+} and Eif4g2^{+/-}, $n = 6$ for Eif4g2^{-/-}.

Data are mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Scale bars, 500 μ m.

See also Figure S4 and Table S2.

readily detectable at baseline and comparable to adult SIOs (Figure 5B) and was efficiently depleted by 4-OHT (Figure 5C). Despite efficient KO, Eif4g2^{-/-} fetal spheroids propagated for >30 days while maintaining cystic morphology and growth, and *Tacstd2* expression remained stable (Figures 5D and 5E).

Bulk RNA-seq performed 6 days after 4-OHT revealed principal component separation by both genotype and developmental context (Figure 5F). In the presence of eIF4G2, fetal spheroids showed lower expression of adult ISC, Paneth, enteroendocrine, and tuft cell markers and homeobox transcription factors, but higher expression of pioneer transcription factors and non-intestinal lineage-associated markers, than adult SIOs (Figure 5G). Upon Eif4g2 deletion, ISC, secretory, and homeobox programs were further reduced, pioneer transcription factors decreased, and non-intestinal lineage-associated markers increased further. GSEA confirmed enrichment of the fetal-reversion signature in control fetal spheroids relative to adult SIOs (Figure 5H), which was further enhanced after Eif4g2 deletion (Figure 5I).

Across systems, fetal spheroids exhibited fewer differentially expressed genes (DEGs) than adult SIOs (2,167 versus 6,814), yet the DEG sets overlapped extensively (1,490 shared; Figure 5J). Shared DEGs were highly directionally concordant (98.7%) and showed strongly correlated effect sizes (Pearson's

$r = 0.89$; Figure S5A). Collectively, eIF4G2 loss elicits a conserved core transcriptional response in adult and fetal intestinal epithelial cultures, while adult SIOs mount a broader secondary response consistent with more extensive downstream remodeling.

eIF4G2 loss does not reduce bulk translation output in SIOs

To investigate the molecular basis of ISC depletion and secretory-lineage defects after Eif4g2 deletion, we assessed the impact of eIF4G2 depletion on global and selective protein translation (Figure 6A). Using the SUnSET (surface sensing of translation) assay²⁷ at day 6 after 4-OHT, puromycin incorporation normalized to total protein was not significantly altered in Eif4g2^{-/-} SIOs (Figure 6B), indicating preserved nascent protein synthesis at this time point. eIF4G1 and eIF4G3 protein levels were unchanged (Figure S6A), and nascent RNA synthesis assessed by EU incorporation also showed no significant genotype-dependent differences (Figure S6B).

Ribo-seq identifies eIF4G2-dependent transcripts with reduced TE

We performed paired Ribo-seq²⁸ and RNA-seq in Eif4g2^{+/+}, Eif4g2^{+/-}, and Eif4g2^{-/-} SIOs at day 4 after 4-OHT to quantify

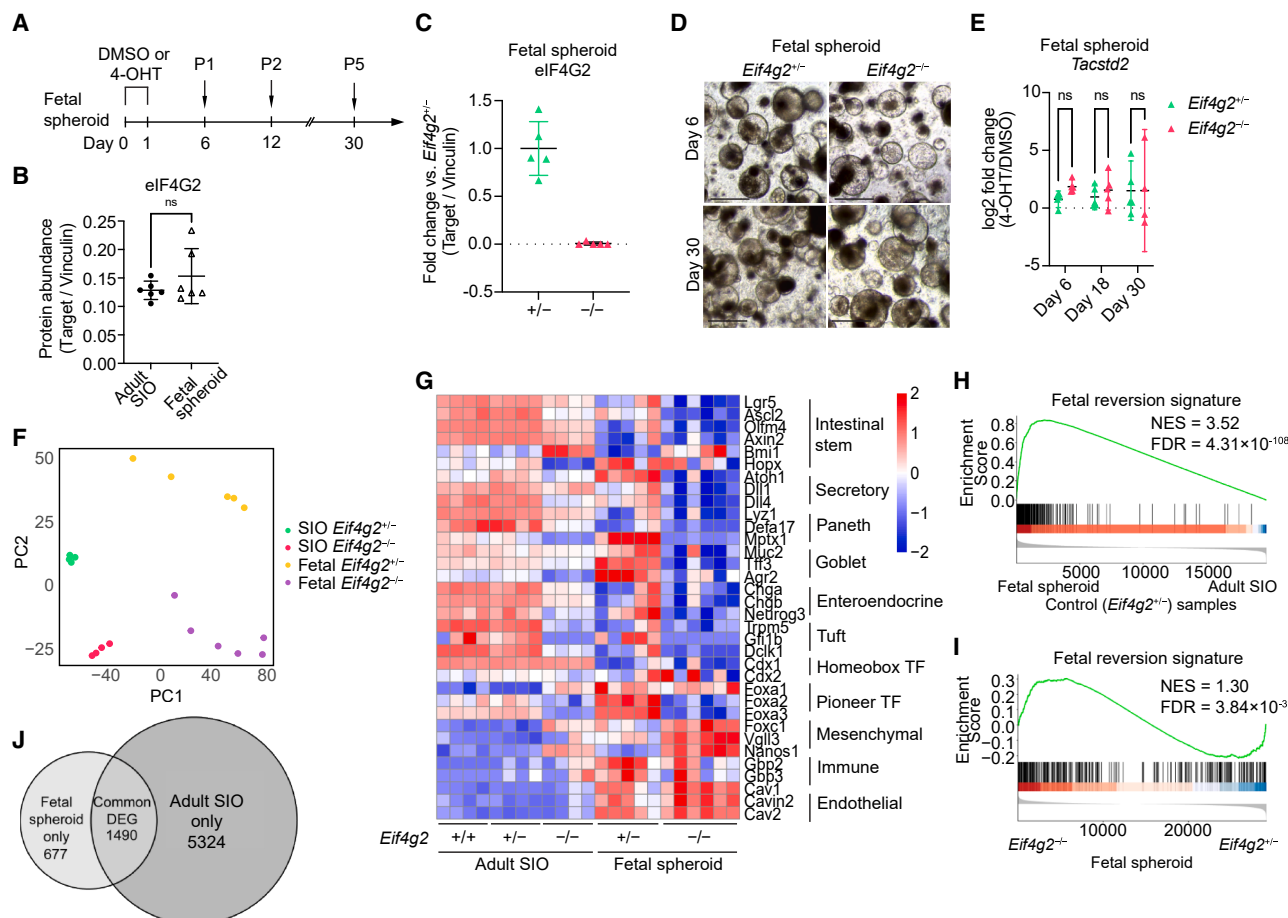


Figure 5. Fetal spheroids propagate in the absence of eIF4G2

(A) Fetal spheroid 4-OHT induction and passaging scheme.
(B) Baseline eIF4G2 protein abundance in adult SIOs and fetal spheroids, normalized to vinculin. $n = 6$ each.
(C) eIF4G2 protein abundance in fetal spheroids at day 6, normalized to vinculin. $n = 5$ each.
(D) Representative brightfield images at day 6 and 30.
(E) *Tacstd2* RT-qPCR across time points in fetal spheroids, shown as log₂ fold change (4-OHT/DMSO) normalized to *Gapdh*. $n = 5$ each.
(F) PCA of bulk RNA-seq in adult SIOs and fetal spheroids. $n = 4$ each for adult SIOs, $n = 5$ for *Eif4g2*^{+/-} fetal spheroids, $n = 6$ for *Eif4g2*^{-/-} fetal spheroids.
(G) Bulk RNA-seq heatmap of adult SIOs and fetal spheroids at day 6 post-induction. Values are gene-wise Z scores. $n = 4$ per genotype for SIOs; *Eif4g2*^{+/-} fetal spheroids $n = 5$; *Eif4g2*^{-/-} fetal spheroids $n = 6$.
(H) GSEA of fetal reversion signature in control fetal spheroids versus adult SIOs ranked by differential expression.
(I) GSEA of fetal reversion signature in fetal spheroids ranked by differential expression (*Eif4g2*^{-/-} versus *Eif4g2*^{+/-}).
(J) Venn diagram of shared differentially expressed genes (DEGs; FDR < 0.05, |log₂FC| > 1) between adult SIOs and fetal spheroids after *Eif4g2* loss.
Data are mean ± SD. * $p < 0.05$. Scale bars, 500 μm.
See also Figure S5 and Table S2.

translation efficiency (TE) of individual transcripts. TE profiles were largely unchanged in *Eif4g2*^{+/-} SIOs relative to WT controls (Figure 6C, left). In contrast, *Eif4g2*^{-/-} SIOs exhibited widespread TE remodeling, with 283 genes showing decreased TE and 155 genes showing increased TE (Figure 6C, right; Table S3).

To relate TE changes to protein output, we performed matched quantitative proteomics and RNA-seq 6 days after 4-OHT. Protein-to-mRNA ratios showed modest concordance with Ribo-seq-based TE (Jaccard = 0.072; Fisher's exact test OR = 2.41, $p = 2.0 \times 10^{-13}$), but agreement was stronger for transcripts with decreased TE than for those with increased TE (down: OR = 4.14, $p = 2.2 \times 10^{-16}$; up: OR = 1.90, $p = 0.013$; Table S4).

Based on this observation, we focused on transcripts with significantly decreased TE and termed them “eIF4G2-dependent transcripts” (Table S3), many of which were reported previously across diverse cell types.^{29,30} These include *Crebbp*, *Ep300*, *Kmt2d*, and *Wnk1*, supporting a conserved role for eIF4G2 in selective translation. Consistent with reported features of eIF4G2-regulated mRNAs,^{7,29} these transcripts have longer 5' untranslated regions (UTRs) and higher prevalence of uORFs (Figures S6C and S6D). They also exhibited increased ribosome footprint density in the 5' UTR relative to the coding sequence (CDS) in *Eif4g2*^{-/-} SIOs (Figure S6E). Together, eIF4G2 is required for efficient translation initiation of mRNAs with long 5' UTRs, and its depletion impairs translation of the main CDS.

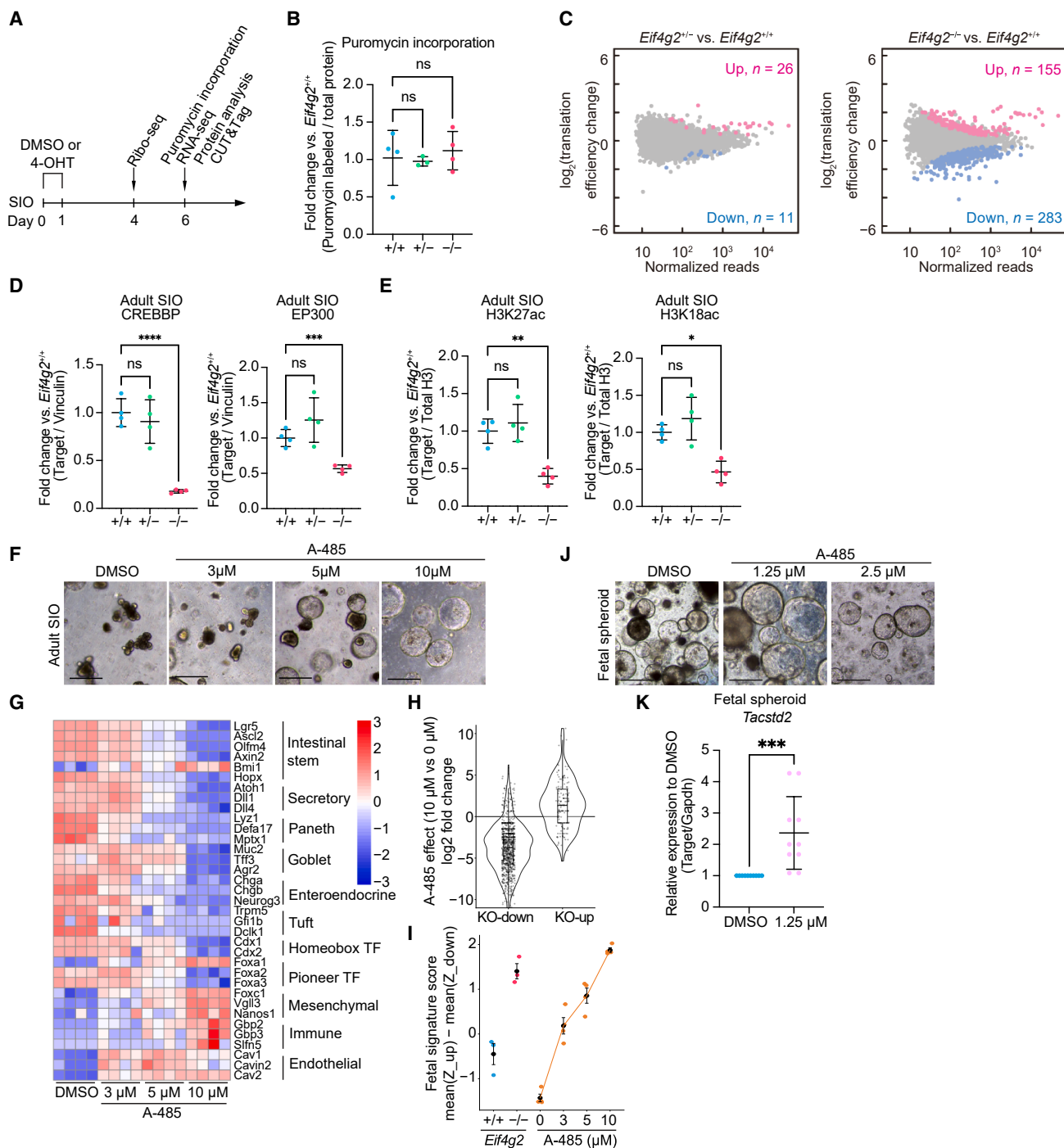


Figure 6. Decreased translation of eIF4G2-dependent transcripts *crebbp* and *Ep300* contributes to the loss of ISC programs

(A) Experimental scheme for Ribo-seq/RNA-seq, puromycin incorporation, protein analysis, and CUT&Tag in adult SIOs after 4-OHT induction. (B) SUNSET assay of global translation at day 6 after 4-OHT in adult SIOs. $n = 4$ each for *Eif4g2*^{+/+} and *Eif4g2*^{-/-}, $n = 3$ for *Eif4g2*^{+/-}. (C) MA plots of translation efficiency (TE) in *Eif4g2*^{-/-} versus *Eif4g2*^{+/+} and *Eif4g2*^{+/-} versus *Eif4g2*^{+/+} SIOs. Genes with significantly increased (red) and decreased (blue) TE (FDR < 0.05) are indicated. Lists of up/down TE genes are in Table S3. (D) CREBBP and EP300 protein abundance in adult SIOs 6 days after induction, normalized to vinculin. $n = 4$ each. (E) H3K27ac and H3K18ac abundance in adult SIOs 6 days after induction, normalized to total H3. $n = 4$ each. (F) Brightfield images of adult SIOs treated with A-485 for 4 days. (G) Bulk RNA-seq heatmap of adult SIOs treated with A-485 for 4 days. Values are gene-wise Z scores. $n = 4$ each. (H) Distribution of A-485 effects on genes down- or upregulated by *Eif4g2* loss (KO-down and KO-up). (I) Fetal-signature score across genotypes and A-485 doses. $n = 4$ each.

(legend continued on next page)

TE of epigenetic regulators is suppressed by *elf4g2* depletion

Gene Ontology (GO) enrichment analysis on *elf4g2*-dependent transcripts identified chromatin and epigenetic regulatory pathways alongside core RNA and protein homeostasis terms (Table S5), consistent with our snMultiome data showing genotype-associated chromatin accessibility remodeling in the stem/proliferative population (Figure 2E). Among these “*elf4g2*-dependent transcripts,” we focused on *Crebbp* and *Ep300*, which encode the sole lysine acetyltransferase family 3 (KAT3) proteins responsible for H3K18 and H3K27 acetylation³¹ and are dose-sensitive.^{32,33}

Intestinal organoids show decreased acetylation of H3K18 and H3K27 upon *elf4g2* depletion

CREBBP and EP300 protein levels were markedly reduced in *Eif4g2*^{-/-} SIOs at day 6 after 4-OHT (Figure 6D). Global H3K18ac and H3K27ac levels (normalized to total histone H3) were also significantly decreased (Figure 6E), indicating reduced KAT3-dependent acetylation following *elf4g2* depletion.

Because adult SIOs undergo progressive failure after *Eif4g2* deletion, whereas fetal spheroids remain viable, we asked whether baseline KAT3 levels or acetylation status differ by developmental stages. Control fetal spheroids displayed comparable or modestly higher CREBBP/EP300 and H3K18ac/H3K27ac levels relative to adult SIOs (Figures S6F and S6G). After *Eif4g2* loss, fetal spheroids showed reductions in CREBBP/EP300 and H3K18ac/H3K27ac at day 6 (Figures S6H and S6I). Thus, *elf4g2* loss elicits a common molecular signature—reduced KAT3 abundance and decreased H3K18/H3K27 acetylation—in both adult and fetal intestinal epithelial cultures. However, fetal spheroids tolerate these chromatin changes and remain viable.

CREBBP/EP300 inhibitor A-485 recapitulates key aspects of the *Eif4g2*^{-/-} loss phenotype

To test whether reduced CREBBP/EP300 activity contributes to the intestinal phenotypes caused by *Eif4g2* loss, we treated WT adult SIOs with A-485, a selective CREBBP/EP300 inhibitor.³⁴ A-485 dose-dependently reduced H3K18ac and H3K27ac (Figure S6J) and induced a cystic morphology similar to *Eif4g2*^{-/-} SIOs (Figure 6F). Transcriptome profiling showed that A-485 reproduced key expression changes observed upon *Eif4g2* loss, including downregulation of intestinal epithelial lineage markers and lineage-specifying transcription factors, with reciprocal upregulation of non-epithelial lineage-associated transcripts (Figure 6G).

At the transcriptome level, A-485 induced a response concordant with the *Eif4g2*^{-/-} program: across the top 1,000 genes ranked by the KO effect size, genes decreased in *Eif4g2*^{-/-} samples (KO-down) tended to be downregulated by A-485, whereas genes upregulated in *Eif4g2*^{-/-} samples (KO-up) tended to be upregulated by A-485 (Figure 6H, Pearson's

$r = 0.57$, $p < 2.2 \times 10^{-16}$; cosine similarity = 0.77). Consistently, GSEA comparing A-485-treated versus DMSO-treated adult SIOs showed enrichment of KO-up genes (Figure S6K; Table S2), fetal-like crypt gene signature (Figure S6L), and YAP-target programs (Figure S6M) in the A-485 condition. The fetal-signature score increased dose-dependently after A-485 treatment and reached levels comparable to *Eif4g2*^{-/-} KO at 10 μ M (Figure 6I).

We next asked whether fetal spheroids show similar sensitivity to KAT3 inhibition. Because fetal spheroids exhibited reduced H3K27ac at lower A-485 doses than adult SIO (Figure S6N), we tested a lower dose range. A-485-treated fetal spheroids largely maintained cystic morphology (Figure 6J), although viability was reduced at 2.5 μ M. *Tacstd2* expression was maintained (Figure 6K), suggesting that fetal-state identity is more resistant to KAT3 inhibition than adult SIOs. Together, these results indicate that reduced KAT3 activity contributes, at least in part, to the intestinal phenotypes caused by *elf4g2* depletion.

elf4g2 loss redistributes H3K27ac and KAT3 occupancy from ISC to YAP/fetal regulatory programs

To define how reduced KAT3 activity reshapes the intestinal chromatin landscape after *Eif4g2* loss, we performed CUT&Tag for H3K27ac, CREBBP, and EP300 in adult SIOs at day 6 after 4-OHT. Because global H3K27ac and KAT3 levels are reduced upon *Eif4g2* loss (Figure 6E), depth-based normalization can confound locus-specific redistribution with genome-wide signal loss. We therefore applied greenlist-based size-factor normalization (de Mello et al.³⁵; see STAR Methods), as spike-in normalization was unsuccessful. Differential peak analysis (*Eif4g2*^{-/-} versus *Eif4g2*^{+/+}) identified both gains and losses of H3K27ac (FDR < 0.05), consistent with active chromatin redistribution. MA plots (Figures 7A and 7E) show only significantly differential H3K27ac peaks (FDR < 0.05). Regions losing H3K27ac were enriched for ISC and Wnt/Notch motifs (ASCL2, LEF/TCF, RBPJ), whereas regions gaining H3K27ac were enriched for TEAD motifs, consistent with activation of a YAP/TEAD-associated regulatory program (Table S6).

Using a gene-set-centric analysis, we assigned each H3K27ac peak to its nearest gene and classified peaks as promoter-proximal (± 2 kb from the nearest transcription start site) or distal. For each gene set, we summarized per-peak H3K27ac log2 fold changes (*Eif4g2*^{-/-} versus *Eif4g2*^{+/+}) and compared them with housekeeping peaks using a two-sided Wilcoxon rank-sum test and Cliff's Δ . H3K27ac was reduced at loci associated with ISC genes³⁶ (Table S2) and increased at fetal-like loci relative to housekeeping controls (Figure 7B; Table S2), indicating coordinated remodeling across promoters and enhancers.

Because H3K27ac output is directly linked to KAT3 coactivator occupancy, we asked whether CREBBP/EP300 binding tracked with H3K27ac remodeling. Across H3K27ac-lost and

(J) Brightfield image of fetal spheroids treated with A-485 for 4 days.

(K) *Tacstd2* RT-qPCR normalized to *Gapdh* in fetal spheroids under A-485 treatment. $n = 5$ each.

Data are mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Scale bars, 500 μ m.

See also Figure S6 and Table S2, S3, S4, and S5.

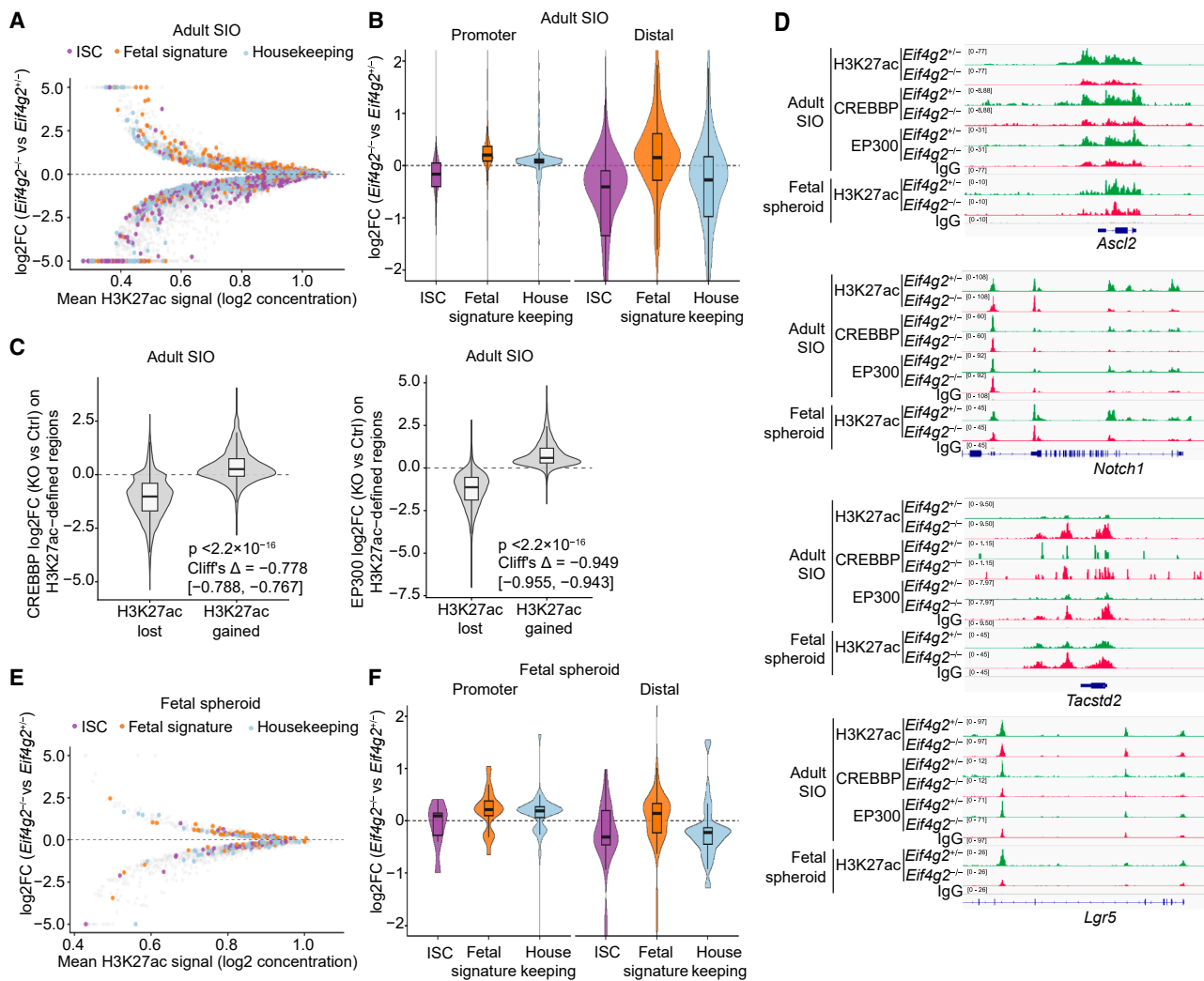


Figure 7. eIF4G2 loss remodels active chromatin and redistributes KAT3 coactivator occupancy

(A and B) Gene set-centric differential H3K27ac analysis at promoter-proximal and distal regions in adult SIOs after *Eif4g2* deletion ($n = 4$ per genotype). Only differentially enriched peaks (FDR < 0.05) are shown in the MA plot. Peaks were assigned to the nearest gene and classified as promoter-proximal (± 2 kb of TSS) or distal. Per-peak log₂ fold changes (*Eif4g2*^{-/-} versus *Eif4g2*^{+/+}) of normalized H3K27ac signal were compared with two-sided Wilcoxon rank-sum tests using housekeeping as reference; effect sizes are reported as Cliff's Δ . Relative to housekeeping, ISC-associated regions showed reduced H3K27ac at promoters ($p = 1.342 \times 10^{-13}$, $\Delta = -0.509$) and distal elements ($p = 2.232 \times 10^{-4}$, $\Delta = -0.147$), whereas fetal-like regions showed increased H3K27ac at promoters ($p = 3.63 \times 10^{-11}$, $\Delta = 0.415$) and distal elements ($p = 3.343 \times 10^{-11}$, $\Delta = 0.311$). Within the ISC gene set, promoter and distal distributions also differed ($p = 4.139 \times 10^{-5}$, $\Delta = 0.278$), with promoter log₂FC values less negative than distal values.

(C) CREBBP and EP300 redistribution relative to H3K27ac remodeling in adult SIOs. CREBBP/EP300 log₂ fold changes (*Eif4g2*^{-/-} versus *Eif4g2*^{+/+}) are summarized over H3K27ac-lost and H3K27ac-gained regions, defined by differential H3K27ac intervals. Two-sided Wilcoxon rank-sum tests; Cliff's Δ reported.

(D) Genome browser tracks showing H3K27ac, CREBBP, and EP300 occupancy at representative adult ISC/Wnt-Notch loci (*Ascl2*, *Notch1*) and fetal/regenerative loci (*Tacstd2*), with *Lgr5* shown as indicated. Matched IgG controls are shown for adult SIOs and fetal spheroids.

(E and F) Gene set-centric H3K27ac analysis in fetal spheroids after *Eif4g2* deletion ($n = 3$ per genotype). Only differentially enriched peaks (FDR < 0.05) are shown in the MA plot. Promoters were defined as ± 2 kb from TSS. Two-sided Wilcoxon rank-sum tests using housekeeping as reference; effect sizes are reported as Cliff's Δ . Relative to housekeeping, fetal-signature distal regions showed increased H3K27ac ($p = 1.046 \times 10^{-3}$, $\Delta = 0.363$), whereas ISC-associated regions showed nonsignificant decreases (promoter $p = 0.231$; distal $p = 0.717$). Within the ISC gene set, promoter-distal differences were not significant ($p = 0.288$). See also Table S6.

H3K27ac-gained intervals, both CREBBP and EP300 showed concordant redistribution of CUT&Tag signals. Occupancy of both coactivators decreased at H3K27ac-lost intervals and increased at H3K27ac-gained intervals (Figure 7C), supporting coordinated genome-wide relocation of KAT3 coactivators.

Locus-level examples illustrate coordinated loss of ISC enhancers and gain of fetal/regenerative elements

To illustrate this remodeling at single-locus resolution, we examined representative ISC and fetal loci. In ISC/Wnt-Notch-associated genes such as *Ascl2* and *Notch1*, H3K27ac was markedly reduced in *Eif4g2*^{-/-} SIOs, accompanied by

concordant losses of CREBBP and EP300 occupancy. In contrast, at the fetal marker *Tacstd2*, *Eif4g2* loss induced de novo accumulation of H3K27ac, together with increased CREBBP/EP300 signal, whereas *Lgr5* showed a comparatively modest change (Figure 7D). These examples reinforce the genome-wide analyses and illustrate a shift from adult ISC/Wnt-Notch regulatory elements toward fetal/regenerative programs.

Directionally conserved H3K27ac reprogramming in fetal spheroids with distinct baseline dependencies

We next asked whether the directionality of H3K27ac remodeling observed in adult SIOs is preserved in the fetal epithelium. We profiled H3K27ac by CUT&Tag in fetal spheroids following *Eif4g2* deletion (*Eif4g2*^{-/-} versus *Eif4g2*^{+/-}, within the fetal context). Because CREBBP and EP300 CUT&Tag profiles in fetal spheroids did not meet quality thresholds for differential analysis, we used H3K27ac as the primary readout of active regulatory remodeling. In fetal spheroids, H3K27ac changes were directionally consistent with those observed in the adult dataset, showing a trend toward reduced acetylation at ISC-associated loci, while fetal-signature regions, particularly distal elements, exhibited significant gains in H3K27ac (Figures 7E and 7F).

Representative loci further highlighted distinct baseline chromatin states between fetal and adult epithelium, which may modulate the impact of *Eif4g2* loss. *Ascl2* and *Notch1* exhibited comparatively low baseline H3K27ac in fetal spheroids and showed limited genotype-dependent change, whereas *Tacstd2* displayed high basal H3K27ac and remained acetylated upon *Eif4g2* loss. Conversely, *Lgr5* showed reduced H3K27ac in fetal spheroids despite limited change in adult SIOs (Figure 7D), suggesting that fetal spheroids may be less dependent on canonical adult ISC/Wnt-Notch elements. Collectively, these results indicate broadly conserved directional H3K27ac reprogramming across adult and fetal intestinal epithelial cultures, while context-specific baseline states and dependencies likely shape the functional consequences of losing select adult ISC-associated enhancers following *elF4G2* deletion.

DISCUSSION

Here, we identify *elF4G2* as a critical regulator of adult intestinal homeostasis. Inducible *elF4G2* loss collapses canonical *Lgr5*⁺ ISC programs and secretory maturation, while villus architecture remains relatively preserved. Epithelial-restricted deletion reproduces this dissociation for months, indicating that epithelial maintenance can persist despite erosion of adult stem cell identity and lineage maturation, and that *elF4G2* loss establishes a durable alternative epithelial state rather than a transient injury response.

Across *in vivo* and *in vitro* transcriptomic analyses, *elF4G2* deficiency is associated with persistent fetal-like and YAP/TEAD-associated programs. snMultiome further showed a shift away from *Lgr5*-driven homeostasis toward regenerative states, including a highly proliferative progenitor population, together with coordinated chromatin accessibility remodeling.

elF4G2 loss also upregulates transcripts associated with mesenchymal, immune, and endothelial-like programs in both tissue and epithelial organoids. We favor a model in which

fetal-like reprogramming entails partial dedifferentiation and blurred lineage boundaries, consistent with regenerative responses reusing developmental programs and plasticity modules.^{12,37}

Mechanistically, our results support a model in which *elF4G2* stabilizes epithelial cell state by selectively supporting translation of a subset of transcripts. Ribosome profiling reveals transcript-specific changes in TE, whereas puromycin incorporation assays show preserved global protein synthesis, indicating selective rather than global translational impairment. *elF4G2*-dependent transcripts are enriched for chromatin and epigenetic regulators, including *Crebbp* and *Ep300*, the two KAT3 acetyltransferase coactivators critical for enhancer acetylation and identity-associated transcription.^{38–40} Consistent with this, *elF4G2*-deficient adult organoids exhibited reduced KAT3 abundance together with decreased global H3K18ac and H3K27ac.

Pharmacological perturbation supports this axis. CREBBP/EP300 inhibition with A-485 induced morphologic and transcriptomic changes that recapitulated key features of *elF4G2* loss, including activation of fetal-like and YAP-associated programs. Although additional *elF4G2*-dependent targets likely contribute, these results highlight KAT3 as a prominent and tractable node in this selective translation network.

At the level of regulatory architecture, CUT&Tag reveals extensive yet non-uniform active chromatin remodeling after *elF4G2* loss. Despite reduced CREBBP/EP300 and H3K18ac/H3K27ac levels, the response is highly locus-selective, consistent with prior work indicating that KAT3 depletion disproportionately impacts identity-linked regulatory elements.³⁹ Loci that lost H3K27ac were enriched for adult ISC/Wnt-Notch motifs and regulatory elements, whereas loci that gained H3K27ac were enriched for TEAD motifs, consistent with activation of fetal/regenerative modules. CREBBP/EP300 occupancy tracked with these changes, and representative loci illustrated a shift from adult ISC-associated elements (e.g., *Ascl2*, *Notch1*) toward fetal/regenerative loci (e.g., *Tacstd2*). *In vivo* snATAC-seq profiles further supported reduced accessibility at ISC-linked elements.

H3K27ac marks active enhancers and is largely mediated by KAT3 acetyltransferases,⁴¹ whereas YAP/TEAD complexes engage distal enhancers with increased H3K27ac at YAP-bound loci.⁴² A redistribution framework may explain these locus-selective patterns: adult identity and maturation programs may rely on KAT3-sensitive elements that lose H3K27ac as coactivator dosage declines, whereas fetal/regenerative programs may retain or gain H3K27ac and accessibility. This is consistent with prior work showing that distal enhancers can be particularly sensitive to KAT3 depletion.^{43–45} Given the rapid turnover of H3K27ac,^{46,47} even modest local shifts between KAT3-mediated deposition and HDAC-mediated removal could generate the gains we observe, providing a mechanistic basis for how disrupted *elF4G2*-dependent translational buffering reshapes enhancer landscapes.

This framework also helps explain the strong state dependence of *elF4G2* loss. Adult organoids progressively lose budding morphology and fail to expand following *Eif4g2* deletion, whereas fetal spheroids propagate robustly despite efficient *elF4G2* depletion and comparable reductions in KAT3 protein levels and H3K18 and H3K27 acetylation. These observations suggest that adult intestinal identity depends more

heavily on KAT3-dependent adult ISC/Wnt-Notch enhancer architecture, whereas fetal epithelial states are less dependent and therefore tolerate similar biochemical defects. More broadly, this state dependence is consistent with models in which regeneration-associated fetal-like programs represent a conserved repair mode that reuses developmental logic,^{12,37} whereas adult homeostasis may be uniquely vulnerable to disruptions of identity-stabilizing epigenetic control.³⁹

Finally, our data suggest that sustained fetal-like reversion can preserve epithelial architecture while failing to fully restore mature physiological programs. In epithelium-specific KO mice, villus architecture is maintained for months without overt weight loss; however, enterocytes exhibit aberrant gene expression, and secretory-lineage maturation is impaired. Secretory progenitors—cells with inherently high proteostatic demand⁴⁸—display elevation of ISR-like signatures, and both fetal-reversion and YAP-target programs are increased within this subpopulation. Prior work supports the concept that YAP/TAZ-driven regeneration can remodel differentiation trajectories,^{13,49} and that ER/UPR stress pathways are tightly coupled to goblet-cell secretory capacity and differentiation.²³ These observations are consistent with a model in which eIF4G2 loss engages a durable regenerative/fetal-like state that maintains tissue architecture but imposes a chronic trade-off: regenerative competence and survival at the expense of full terminal secretory maturation.

Together, our results identify eIF4G2 as a critical post-transcriptional regulator of adult intestinal stem-cell homeostasis and highlight selective translation of epigenetic regulators as a mechanism for stabilizing adult cell identity. The *Eif4g2* loss-of-function mouse and organoid systems establish a long-lived regeneration-associated epithelial state, providing a tractable model for how translational control and epigenetic coactivator dosage govern adult identity and chronic regenerative reprogramming *in vivo*.

More broadly, our findings add to the growing evidence that core translation factors do not function solely as housekeeping components of protein synthesis but can selectively shape specific cellular programs through translation of defined mRNA subsets. In this context, eIF4G2 emerges as a selective translational regulator that links initiation control to epigenetic state stability in adult tissues.

Limitations of the study

Reduced KAT3 output is strongly associated with the eIF4G2-loss phenotype, but direct genetic epistasis will be needed to establish causality; large KAT3 CDSs currently limit these approaches. eIF4G2-dependent transcripts extend beyond KAT3, and additional targets may reveal parallel pathways affecting stemness and secretory maturation. *In vivo* snMultiome showed strong genotype-state coupling, limiting within-state comparisons and cell-type-specific chromatin inference. In fetal spheroids, CREBBP/EP300 CUT&Tag did not meet quality thresholds, limiting direct assessment of coactivator occupancy. Canonical IFN, dsRNA, or ISR pathways were not dominant at ISC program collapse, but subtler stress contributions remain possible, and how selective translation interfaces with YAP/TEAD activation remains unresolved. Finally, the basis for the severe systemic phenotype after whole-body eIF4G2 deletion remains unresolved and will require additional tissue-specific models.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Shinya Yamanaka (shinya.yamanaka@gladstone.ucsf.edu)

Materials availability

Unique/stable reagents generated in this study are available from the [lead contact](#) under a completed materials transfer agreement.

Data and code availability

Thor-Ribo-Seq (GSE294619), RNA-seq (GSE294512), CUT&Tag (GSE318066), and single-nucleus multiome data (GSE318068) have been deposited in the NCBI database. Mass spectrometry data were deposited in the ProteomeXchange Consortium via jPOSTrepo⁵⁰ under accession JPST004351 (PXD073625). Original data for other experiments have been deposited to Mendeley Data: <https://doi.org/10.17632/zymh4y3w9h>. Custom scripts for ribosome profiling mapping and quantification are available at <https://github.com/ingolia-lab/RiboSeq>.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.K., S.P., K.T. and S.Y.; methodology, H.K., M.I., P.R., Y. Shichino., and S.I.; investigation, H.K., A.M.K., R.J., V.A., M.L., A.R., Y. Sato., M.I., P.R., K.T., M.M., Y. Shichino., and S.I.; data curation, H.K., Y. Shichino., and M.I.; writing – original draft, H.K.; writing – review & editing, H.K., M.I., P.R., Y. Shichino., S.I., and S.Y.; visualization, H.K. and Y. Shichino.; funding acquisition, Y. Shichino., S.I., and S.Y.; supervision, H.K. and S.Y.

DECLARATION OF INTERESTS

S.Y. is a scientific advisor of iPS Academia Japan, Orizuru Therapeutics, and Altos Labs without salary. S.I. is a member of the Scientific Reports editorial board.

STAR★METHODS

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- Single-nucleus Multiome data analysis
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

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REFERENCES

1. Sonenberg, N., and Hinnebusch, A.G. (2009). Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 136, 731–745. <https://doi.org/10.1016/j.cell.2009.01.042>.
2. Amiri, M., Sonenberg, N., and Tahmasebi, S. (2025). mRNA translational control of regeneration. *Curr. Opin. Genet. Dev.* 93, 102367. <https://doi.org/10.1016/j.cde.2025.102367>.
3. Genuth, N.R., and Dillin, A. (2025). Translational regulation in stress biology. *Nat. Cell Biol.* 27, 1609–1621. <https://doi.org/10.1038/s41556-025-01765-z>.
4. Saba, J.A., Liakath-Ali, K., Green, R., and Watt, F.M. (2021). Translational control of stem cell function. *Nat. Rev. Mol. Cell Biol.* 22, 671–690. <https://doi.org/10.1038/s41580-021-00386-2>.
5. Teixeira, F.K., and Lehmann, R. (2019). Translational Control during Developmental Transitions. *Cold Spring Harb. Perspect. Biol.* 11, a032987. <https://doi.org/10.1101/cshperspect.a032987>.
6. Liberman, N., Gandin, V., Svitkin, Y.V., David, M., Virgili, G., Jaramillo, M., Holcik, M., Nagar, B., Kimchi, A., and Sonenberg, N. (2015). DAP5 associates with eIF2beta and eIF4AI to promote Internal Ribosome Entry Site driven translation. *Nucleic Acids Res.* 43, 3764–3775. <https://doi.org/10.1093/nar/gkv205>.
7. Weber, R., Kleemann, L., Hirschberg, I., Chung, M.Y., Valkov, E., and Igreja, C. (2022). DAP5 enables main ORF translation on mRNAs with structured and uORF-containing 5' leaders. *Nat. Commun.* 13, 7510. <https://doi.org/10.1038/s41467-022-35019-5>.
8. de la Parra, C., Ernlund, A., Alard, A., Ruggles, K., Ueberheide, B., and Schneider, R.J. (2018). A widespread alternate form of cap-dependent mRNA translation initiation. *Nat. Commun.* 9, 3068. <https://doi.org/10.1038/s41467-018-05539-0>.
9. Yamanaka, S., Zhang, X.Y., Maeda, M., Miura, K., Wang, S., Farese, R.V., Jr., Iwao, H., and Innerarity, T.L. (2000). Essential role of NAT1/p97/DAP5 in embryonic differentiation and the retinoic acid pathway. *EMBO J.* 19, 5533–5541. <https://doi.org/10.1093/emboj/19.20.5533>.
10. Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., and Clevers, H. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 449, 1003–1007. <https://doi.org/10.1038/nature06196>.
11. Nusse, Y.M., Savage, A.K., Marangoni, P., Rosendahl-Huber, A.K.M., Landman, T.A., de Sauvage, F.J., Locksley, R.M., and Klein, O.D. (2018). Parasitic helminths induce fetal-like reversion in the intestinal stem cell niche. *Nature* 559, 109–113. <https://doi.org/10.1038/s41586-018-0257-1>.
12. Viragova, S., Li, D., and Klein, O.D. (2024). Activation of fetal-like molecular programs during regeneration in the intestine and beyond. *Cell Stem Cell* 31, 949–960. <https://doi.org/10.1016/j.stem.2024.05.009>.
13. Yui, S., Azzolin, L., Maimets, M., Pedersen, M.T., Fordham, R.P., Hansen, S.L., Larsen, H.L., Guiu, J., Alves, M.R.P., Rundsten, C.F., et al. (2018). YAP/TAZ-Dependent Reprogramming of Colonic Epithelium Links ECM Remodeling to Tissue Regeneration. *Cell Stem Cell* 22, 35–49.e7. <https://doi.org/10.1016/j.stem.2017.11.001>.
14. Ventura, A., Kirsch, D.G., McLaughlin, M.E., Tuveson, D.A., Grimm, J., Lintault, L., Newman, J., Reczek, E.E., Weissleder, R., and Jacks, T. (2007). Restoration of p53 function leads to tumour regression in vivo. *Nature* 445, 661–665. <https://doi.org/10.1038/nature05541>.
15. Erben, U., Loddenkemper, C., Doerfel, K., Spieckermann, S., Haller, D., Heimesaat, M.M., Zeitz, M., Siegmund, B., and Kühl, A.A. (2014). A guide to histomorphological evaluation of intestinal inflammation in mouse models. *Int. J. Clin. Exp. Pathol.* 7, 4557–4576.
16. Beck, F. (2004). The role of Cdx genes in the mammalian gut. *Gut* 53, 1394–1396. <https://doi.org/10.1136/gut.2003.038240>.
17. Friedman, J.R., and Kaestner, K.H. (2006). The Foxa family of transcription factors in development and metabolism. *Cell. Mol. Life Sci.* 63, 2317–2328. <https://doi.org/10.1007/s00018-006-6095-6>.
18. Mustata, R.C., Vasile, G., Fernandez-Vallone, V., Strollo, S., Lefort, A., Libert, F., Monteyne, D., Pérez-Morga, D., Vassart, G., and Garcia, M.I. (2013). Identification of Lgr5-independent spheroid-generating progenitors of the mouse fetal intestinal epithelium. *Cell Rep.* 5, 421–432. <https://doi.org/10.1016/j.celrep.2013.09.005>.
19. Barry, E.R., Morikawa, T., Butler, B.L., Shrestha, K., de la Rosa, R., Yan, K.S., Fuchs, C.S., Magness, S.T., Smits, R., Ogino, S., et al. (2013). Restriction of intestinal stem cell expansion and the regenerative response by YAP. *Nature* 493, 106–110. <https://doi.org/10.1038/nature11693>.
20. Gregorieff, A., Liu, Y., Inanlou, M.R., Khomchuk, Y., and Wrana, J.L. (2015). Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. *Nature* 526, 715–718. <https://doi.org/10.1038/nature15382>.
21. Sangiorgi, E., and Capecchi, M.R. (2008). Bmi1 is expressed in vivo in intestinal stem cells. *Nat. Genet.* 40, 915–920. <https://doi.org/10.1038/ng.165>.
22. Smith, N.R., Giske, N.R., SenGupta, S.K., Conley, P., Swain, J.R., Nair, A., Fowler, K.L., Klocke, C., Yoo, Y.J., Anderson, A.N., et al. (2025). Dual states of murine Bmi1-expressing intestinal stem cells drive epithelial development utilizing non-canonical Wnt signaling. *Dev. Cell* 60, 2177–2191.e5. <https://doi.org/10.1016/j.devcel.2025.03.014>.
23. Ma, A. (2008). Unresolved ER Stress Inflames the Intestine. *Cell* 134, 724–725. <https://doi.org/10.1016/j.cell.2008.08.023>.
24. el Marjou, F., Janssen, K.P., Chang, B.H.J., Li, M., Hindie, V., Chan, L., Louvard, D., Chambon, P., Metzger, D., and Robine, S. (2004).

- Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis* 39, 186–193. <https://doi.org/10.1002/gene.20042>.
25. Pakos-Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A., and Gorman, A.M. (2016). The integrated stress response. *EMBO Rep.* 17, 1374–1395. <https://doi.org/10.15252/embr.201642195>.
 26. Garcia, M.I., Ghiani, M., Lefort, A., Libert, F., Strollo, S., and Vassart, G. (2009). LGR5 deficiency deregulates Wnt signaling and leads to precocious Paneth cell differentiation in the fetal intestine. *Dev. Biol.* 331, 58–67. <https://doi.org/10.1016/j.ydbio.2009.04.020>.
 27. Schmidt, E.K., Clavarino, G., Ceppi, M., and Pierre, P. (2009). SUNSET, a nonradioactive method to monitor protein synthesis. *Nat. Methods* 6, 275–277. <https://doi.org/10.1038/nmeth.1314>.
 28. Ingolia, N.T., Ghaemmaghami, S., Newman, J.R.S., and Weissman, J.S. (2009). Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* 324, 218–223. <https://doi.org/10.1126/science.1168978>.
 29. David, M., Olender, T., Mizrahi, O., Weingarten-Gabbay, S., Friedlander, G., Meril, S., Goldberg, N., Savidor, A., Levin, Y., Salomon, V., et al. (2022). DAP5 drives translation of specific mRNA targets with upstream ORFs in human embryonic stem cells. *RNA* 28, 1325–1336. <https://doi.org/10.1261/rna.079194.122>.
 30. Sugiyama, H., Takahashi, K., Yamamoto, T., Iwasaki, M., Narita, M., Nakamura, M., Rand, T.A., Nakagawa, M., Watanabe, A., and Yamanaka, S. (2017). Nat1 promotes translation of specific proteins that induce differentiation of mouse embryonic stem cells. *Proc. Natl. Acad. Sci. USA* 114, 340–345. <https://doi.org/10.1073/pnas.1617234114>.
 31. Jin, Q., Yu, L.R., Wang, L., Zhang, Z., Kasper, L.H., Lee, J.E., Wang, C., Brindle, P.K., Dent, S.Y.R., and Ge, K. (2011). Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. *EMBO J.* 30, 249–262. <https://doi.org/10.1038/emboj.2010.318>.
 32. (ClinGen), C.G.R. CREBBP: Dosage Sensitivity Curation (Haploinsufficiency score 3) <https://search.clinicalgenome.org/kb/gene-dosage/CREBBP>.
 33. (ClinGen), C.G.R. EP300: Dosage Sensitivity Curation (Haploinsufficiency score 3) <https://search.clinicalgenome.org/kb/gene-dosage/HGNC%3A3373>.
 34. Lasko, L.M., Jakob, C.G., Edalji, R.P., Qiu, W., Montgomery, D., Digiammarino, E.L., Hansen, T.M., Risi, R.M., Frey, R., Manaves, V., et al. (2017). Discovery of a selective catalytic p300/CBP inhibitor that targets lineage-specific tumours. *Nature* 550, 128–132. <https://doi.org/10.1038/nature24028>.
 35. de Mello, F.N., Tahira, A.C., Berzoti-Coelho, M.G., and Verjovski-Almeida, S. (2024). The CUT&RUN greenlist: genomic regions of consistent noise are effective normalizing factors for quantitative epigenome mapping. *Brief. Bioinform.* 25, bbad538. <https://doi.org/10.1093/bib/bbad538>.
 36. Muñoz, J., Stange, D.E., Schepers, A.G., van de Wetering, M., Koo, B.K., Itzkovitz, S., Volckmann, R., Kung, K.S., Koster, J., Radulescu, S., et al. (2012). The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent '4' cell markers. *EMBO J.* 31, 3079–3091. <https://doi.org/10.1038/emboj.2012.166>.
 37. Fazilat, H., and Basler, K. (2023). Reactivation of embryonic genetic programs in tissue regeneration and disease. *Nat. Genet.* 55, 1792–1806. <https://doi.org/10.1038/s41588-023-01526-4>.
 38. Yao, T.P., Oh, S.P., Fuchs, M., Zhou, N.D., Ch'ng, L.E., Newsome, D., Bronson, R.T., Li, E., Livingston, D.M., and Eckner, R. (1998). Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* 93, 361–372. [https://doi.org/10.1016/S0092-8674\(00\)81165-4](https://doi.org/10.1016/S0092-8674(00)81165-4).
 39. Lipinski, M., Muñoz-Viana, R., Del Blanco, B., Marquez-Galera, A., Medrano-Relinque, J., Caramés, J.M., Szczepankiewicz, A.A., Fernandez-Albert, J., Navarrón, C.M., Olivares, R., et al. (2020). KAT3-dependent acetylation of cell type-specific genes maintains neuronal identity in the adult mouse brain. *Nat. Commun.* 11, 2588. <https://doi.org/10.1038/s41467-020-16246-0>.
 40. Vannam, R., Sayilgan, J., Ojeda, S., Karakyriakou, B., Hu, E., Kreuzer, J., Morris, R., Herrera Lopez, X.I., Rai, S., Haas, W., et al. (2021). Targeted degradation of the enhancer lysine acetyltransferases CBP and p300. *Cell Chem. Biol.* 28, 503–514.e12. <https://doi.org/10.1016/j.chembiol.2020.12.004>.
 41. Creighton, M.P., Cheng, A.W., Welstead, G.G., Kooistra, T., Carey, B.W., Steine, E.J., Hanna, J., Lodato, M.A., Frampton, G.M., Sharp, P.A., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl. Acad. Sci. USA* 107, 21931–21936. <https://doi.org/10.1073/pnas.1016071107>.
 42. Stein, C., Bardet, A.F., Roma, G., Bergling, S., Clay, I., Ruchti, A., Agarinis, C., Schmelzle, T., Bouwmeester, T., Schübeler, D., and Bauer, A. (2015). YAP1 Exerts Its Transcriptional Control via TEAD-Mediated Activation of Enhancers. *PLoS Genet.* 11, e1005465. <https://doi.org/10.1371/journal.pgen.1005465>.
 43. Hsu, E., Zemke, N.R., and Berk, A.J. (2021). Promoter-specific changes in initiation, elongation, and homeostasis of histone H3 acetylation during CBP/p300 inhibition. *eLife* 10, e63512. <https://doi.org/10.7554/eLife.63512>.
 44. Raisner, R., Kharbada, S., Jin, L., Jeng, E., Chan, E., Merchant, M., Haverly, P.M., Bainer, R., Cheung, T., Arnott, D., et al. (2018). Enhancer Activity Requires CBP/P300 Bromodomain-Dependent Histone H3K27 Acetylation. *Cell Rep.* 24, 1722–1729. <https://doi.org/10.1016/j.celrep.2018.07.041>.
 45. Martire, S., Nguyen, J., Sundaresan, A., and Banaszynski, L.A. (2020). Differential contribution of p300 and CBP to regulatory element acetylation in ESCs. *BMC Mol. Cell Biol.* 21, 55. <https://doi.org/10.1186/s12860-020-00296-9>.
 46. Weinert, B.T., Narita, T., Satpathy, S., Srinivasan, B., Hansen, B.K., Schölz, C., Hamilton, W.B., Zucconi, B.E., Wang, W.W., Liu, W.R., et al. (2018). Time-Resolved Analysis Reveals Rapid Dynamics and Broad Scope of the CBP/p300 Acetylome. *Cell* 174, 231–244.e12. <https://doi.org/10.1016/j.cell.2018.04.033>.
 47. Pelham-Webb, B., Polyzos, A., Wojenski, L., Kloetgen, A., Li, J., Di Giammartino, D.C., Sakellaropoulos, T., Tsigos, A., Core, L., and Apostolou, E. (2021). H3K27ac bookmarking promotes rapid post-mitotic activation of the pluripotent stem cell program without impacting 3D chromatin reorganization. *Mol. Cell* 81, 1732–1748.e8. <https://doi.org/10.1016/j.molcel.2021.02.032>.
 48. Hetz, C., Silva-Agüero, J.F., and Ellerby, L.M. (2024). Essential roles of the unfolded protein response in intestinal physiology. *eGastroenterology* 2, e100129. <https://doi.org/10.1136/egastro-2024-100129>.
 49. Imajo, M., Ebisuya, M., and Nishida, E. (2015). Dual role of YAP and TAZ in renewal of the intestinal epithelium. *Nat. Cell Biol.* 17, 7–19. <https://doi.org/10.1038/ncb3084>.
 50. Okuda, S., Watanabe, Y., Moriya, Y., Kawano, S., Yamamoto, T., Matsumoto, M., Takami, T., Kobayashi, D., Araki, N., Yoshizawa, A.C., et al. (2017). jPOSTrepo: an international standard data repository for proteomes. *Nucleic Acids Res.* 45, D1107–D1111. <https://doi.org/10.1093/nar/gkw1080>.
 51. Chen, E.Y., Tan, C.M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G.V., Clark, N.R., and Ma'ayan, A. (2013). Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinform.* 14, 128. <https://doi.org/10.1186/1471-2105-14-128>.
 52. Xu, S., Hu, E., Cai, Y., Xie, Z., Luo, X., Zhan, L., Tang, W., Wang, Q., Liu, B., Wang, R., et al. (2024). Using clusterProfiler to characterize multiomics data. *Nat. Protoc.* 19, 3292–3320. <https://doi.org/10.1038/s41596-024-01020-z>.
 53. Hänzelmann, S., Castelo, R., and Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinform.* 14, 7. <https://doi.org/10.1186/1471-2105-14-7>.
 54. Kim, D., Langmead, B., and Salzberg, S.L. (2015). HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods* 12, 357–360. <https://doi.org/10.1038/nmeth.3317>.

55. Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930. <https://doi.org/10.1093/bioinformatics/btt656>.
56. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
57. Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
58. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. j.* 17, 10. <https://doi.org/10.14806/ej.17.1.200>.
59. Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. <https://doi.org/10.1093/bioinformatics/bts635>.
60. Smith, T., Heger, A., and Sudbery, I. (2017). UMI-tools: modeling sequencing errors in Unique Molecular Identifiers to improve quantification accuracy. *Genome Res.* 27, 491–499. <https://doi.org/10.1101/gr.209601.116>.
61. Meier, F., Brunner, A.D., Frank, M., Ha, A., Bludau, I., Voytik, E., Kaspar-Schoenefeld, S., Lubeck, M., Raether, O., Bache, N., et al. (2020). diaPASEF: parallel accumulation-serial fragmentation combined with data-independent acquisition. *Nat. Methods* 17, 1229–1236. <https://doi.org/10.1038/s41592-020-00998-0>.
62. Demichev, V., Messner, C.B., Vernardis, S.I., Lilley, K.S., and Ralser, M. (2020). DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat. Methods* 17, 41–44. <https://doi.org/10.1038/s41592-019-0638-x>.
63. Di Tommaso, P., Chatzou, M., Floden, E.W., Barja, P.P., Palumbo, E., and Notredame, C. (2017). Nextflow enables reproducible computational workflows. *Nat. Biotechnol.* 35, 316–319. <https://doi.org/10.1038/nbt.3820>.
64. Ewels, P.A., Peltzer, A., Fillinger, S., Patel, H., Alneberg, J., Wilm, A., Garcia, M.U., Di Tommaso, P., and Nahnsen, S. (2020). The nf-core framework for community-curated bioinformatics pipelines. *Nat. Biotechnol.* 38, 276–278. <https://doi.org/10.1038/s41587-020-0439-x>.
65. Ramírez, F., Ryan, D.P., Grüning, B., Bhardwaj, V., Kilpert, F., Richter, A.S., Heyne, S., Dündar, F., and Manke, T. (2016). deepTools2: a next generation web server for deep-sequencing data analysis. *Nucleic Acids Res.* 44, W160–W165. <https://doi.org/10.1093/nar/gkw257>.
66. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R.; 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
67. Quinlan, A.R., and Hall, I.M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842. <https://doi.org/10.1093/bioinformatics/btq033>.
68. Yu, G., Wang, L.G., and He, Q.Y. (2015). ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. *Bioinformatics* 31, 2382–2383. <https://doi.org/10.1093/bioinformatics/btv145>.
69. Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell* 38, 576–589. <https://doi.org/10.1016/j.molcel.2010.05.004>.
70. Satija, R., Farrell, J.A., Gennert, D., Schier, A.F., and Regev, A. (2015). Spatial reconstruction of single-cell gene expression data. *Nat. Biotechnol.* 33, 495–502. <https://doi.org/10.1038/nbt.3192>.
71. Hao, Y., Stuart, T., Kowalski, M.H., Choudhary, S., Hoffman, P., Hartman, A., Srivastava, A., Molla, G., Madad, S., Fernandez-Granda, C., and Satija, R. (2024). Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat. Biotechnol.* 42, 293–304. <https://doi.org/10.1038/s41587-023-01767-y>.
72. Stuart, T., Srivastava, A., Madad, S., Lareau, C.A., and Satija, R. (2021). Single-cell chromatin state analysis with Signac. *Nat. Methods* 18, 1333–1341. <https://doi.org/10.1038/s41592-021-01282-5>.
73. Hafemeister, C., and Satija, R. (2019). Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. *Genome Biol.* 20, 296. <https://doi.org/10.1186/s13059-019-1874-1>.
74. Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., and Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 459, 262–265. <https://doi.org/10.1038/nature07935>.
75. Mizutani, T., and Clevers, H. (2020). Primary Intestinal Epithelial Organoid Culture. *Methods Mol. Biol.* 2171, 185–200. https://doi.org/10.1007/978-1-0716-0747-3_11.
76. Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., and Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods* 5, 621–628. <https://doi.org/10.1038/nmeth.1226>.
77. Mito, M., Shichino, Y., and Iwasaki, S. (2023). Thor-Ribo-Seq: ribosome profiling tailored for low input with RNA-dependent RNA amplification. Preprint at bioRxiv. <https://doi.org/10.1101/2023.01.15.524129>.
78. Mito, M., Mishima, Y., and Iwasaki, S. (2020). Protocol for Disome Profiling to Survey Ribosome Collision in Humans and Zebrafish. *Star Protoc.* 1, 100168. <https://doi.org/10.1016/j.xpro.2020.100168>.
79. Xie, Z., Bailey, A., Kuleshov, M.V., Clarke, D.J.B., Evangelista, J.E., Jenkins, S.L., Lachmann, A., Wojciechowicz, M.L., Kropiwnicki, E., Jagodnik, K.M., et al. (2021). Gene Set Knowledge Discovery with Enrichr. *Curr. Protoc.* 1, e90. <https://doi.org/10.1002/cpz1.90>.
80. Kuleshov, M.V., Jones, M.R., Rouillard, A.D., Fernandez, N.F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S.L., Jagodnik, K.M., Lachmann, A., et al. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* 44, W90–W97. <https://doi.org/10.1093/nar/gkw377>.
81. Rumbaugh, G., and Miller, C.A. (2011). Epigenetic changes in the brain: measuring global histone modifications. *Methods Mol. Biol.* 670, 263–274. https://doi.org/10.1007/978-1-60761-744-0_18.
82. Batth, T.S., Tollenaere, M.X., Rütger, P., Gonzalez-Franquesa, A., Prabhakar, B.S., Bekker-Jensen, S., Deshmukh, A.S., and Olsen, J.V. (2019). Protein Aggregation Capture on Microparticles Enables Multipurpose Proteomics Sample Preparation. *Mol. Cell. Proteomics* 18, 1027–1035. <https://doi.org/10.1074/mcp.TIR118.001270>.
83. Iwasaki, M., Nishimura, R., Yamakawa, T., Miyamoto, Y., Tabata, T., and Narita, M. (2024). Differences in Uniquely Identified Peptides Between ddaPASEF and diaPASEF. *Cells* 13, 1848. <https://doi.org/10.3390/cells13221848>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-NAT1	BD Biosciences	Cat#610742; RRID: AB_398065
Rabbit monoclonal anti-OLFM4	Cell Signaling Technology	Cat#14369S; RRID: AB_2798465
Rabbit monoclonal anti-Lysozyme	Abcam	Cat#Ab108508; RRID: AB_10861277
Goat polyclonal anti-MMP7	R&D	Cat#AF2967SP; RRID: AB_664120
Rabbit polyclonal anti-Chromogranin A	Abcam	Cat#Ab283265; RRID: N/A
Rabbit monoclonal anti-DCMKL1	Abcam	Cat#Ab109029; RRID: AB_10864128
Rabbit polyclonal anti-cleaved caspase-3	Cell Signaling Technology	Cat#9661; RRID: AB_2341188
Rabbit monoclonal anti-PCNA	Cell Signaling Technology	Cat#13110; RRID: AB_2636979
Recombinant Rabbit anti-active YAP	abcam	Cat#Ab205270; RRID: AB_2813833
Mouse monoclonal anti-Puromycin	Sigma-Aldrich	Cat#MABE343; RRID: AB_2566826
Rabbit monoclonal anti-phospho-eIF2 α	Cell Signaling Technology	Cat#3398T; RRID: AB_2096481
Rabbit monoclonal anti-eIF2 α	Cell Signaling Technology	Cat#5324; RRID: AB_10692650
Rabbit polyclonal anti-GAPDH	Abcam	Cat#Ab9485; RRID: AB_307275
Rabbit monoclonal anti-Histone H3	Cell Signaling Technology	Cat#4499T; RRID: AB_10544537
Rabbit monoclonal anti-acetyl-histone H3 (Lys18)	Cell Signaling Technology	Cat#13998S; RRID: AB_2783723
Rabbit monoclonal anti-acetyl-histone H3 (Lys27)	Cell Signaling Technology	Cat#8173S; RRID: AB_10949503
Rabbit polyclonal anti-Histone H3K27ac antibody	Active Motif	Cat#39034; RRID: AB_2722569
Rabbit monoclonal anti-CBP antibody	Cell Signaling Technology	Cat#7389; RRID: AB_2616020
Rabbit monoclonal anti-CREBBP antibody	Abcam	Cat#Ab283283; RRID: N/A
Rabbit monoclonal anti-p300 antibody	Cell Signaling Technology	Cat#57625; RRID: AB_3068009
Rabbit monoclonal anti-p300 antibody	Cell Signaling Technology	Cat#54062; RRID: AB_2799450
IgG Rabbit control	Cell Signaling Technology	Cat#2729; RRID: AB_1031062
Rat monoclonal anti-mouse CD31; Pacific Blue	eBioscience	Cat#RM5228; RRID: AB_1500466
Rat monoclonal anti-mouse CD45; Pacific Blue	BioLegend	Cat#103125; RRID: AB_493536
Rat monoclonal anti-mouse CD326; Brilliant Violet 605	BioLegend	Cat#118227; RRID: AB_2563984
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody; Alexa Fluor 488	Invitrogen	Cat#A-21206; RRID: AB_2535792
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody; Alexa Fluor 647	Invitrogen	Cat#A-31573; RRID: AB_2536183
Chemicals, peptides, and recombinant proteins		
Tamoxifen	Sigma-Aldrich	T5648
(Z)-4-Hydroxytamoxifen	Sigma-Aldrich	H7904
Fluoromount G mounting medium	Invitrogen	00-4958-02
DirectPCR Lysis Reagent (Tail)	Viagen Biotech	102-T
Proteinase K solution	Viagen Biotech	501-PK
KOD Xtreme Hot Start DNA Polymerase	Sigma-Aldrich	71975
Advanced DMEM/F12	Gibco	12634028
B-27 Supplement	Gibco	17504044
HEPES	Gibco	15630106

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
GlutaMAX	Gibco	35050-061
N-acetyl-L-cysteine	Sigma-Aldrich	A9165-5G
Penicillin/Streptomycin solution	Corning	30-002-CI
Zeocin	Gibco	R25001
Matrigel Growth Factor Reduced Basement Membrane Matrix, Phenol Red-free, LDEV-free	Corning	356231
Mouse recombinant EGF	Invitrogen	PMG8043
Recombinant human Noggin	Peprtech	120-10C-250UG
A-485	Tocris	6387
Cell Recovery Solution	Corning	CB-40253
TrypLE Express	Gibco	12-604-013
Y-27632	Cayman Chemical	10005583
1M Tris-HCl pH 7.5	Fujifilm Wako Pure Chemical	318-90225
1M Magnesium Chloride Solution	Nacalai tesque	20942-34
USB Dithiothreitol (DTT), 0.1M solution	Thermo Fisher Scientific	707265ML
Cycloheximide	Merck	C4859-1ML
Triton X-100	Nacalai tesque	12967-32
Turbo DNase	Thermo Fisher Scientific	AM2238
RNase I	LGC Biosearch Technologies	N6901K
TRIzol LS reagent	Thermo Fisher Scientific	15596018
MPER	Thermo Scientific	78501
Pierce RIPA buffer	Thermo Scientific	89901
1M Tris-HCl pH9.0	TEKNOVA	T1090
Sodium deoxycholate	Sigma-Aldrich	30970
N-Lauroylsarcosine sodium salt	Sigma-Aldrich	61743
2X Laemmli sample buffer	Bio-Rad	1610737
Hydrochloric acid, 1N	Sigma-Aldrich	H9892-100ML
Protease inhibitor	Sigma-Aldrich	P8340-1ML
Phosphatase inhibitor cocktail 2	Sigma-Aldrich	5726-1ML
Phosphatase inhibitor cocktail 3	Sigma-Aldrich	P0044-1ML
Target Retrieval Solution, Citrate pH 6.1	Dako	S169984-2
Alt-R S.p. Cas9 Nuclease V3	Integrated DNA technologies	1081058
Puromycin Dihydrochloride	Gibco	A1113803
5-ethynyl uridine	Invitrogen	E10345
Sodium butyrate	Sigma-Aldrich	B5887-250MG
Ghost dye red 710	BioLegend	420403
Critical commercial assays		
Anti-Rabbit Detection Module for Jess, Wes, Peggy Sue or Sally Sue	proteinsimple	DM-001
Anti-Mouse Detection Module for Jess, Wes, Peggy Sue or Sally Sue	proteinsimple	DM-002
20X Anti-Rabbit HRP Conjugate	proteinsimple	043-426
2-40kDa Jess or Wes Separation Module, 8 x 25 capillary cartridges	proteinsimple	SM-W012
12-230kDa Jess or Wes Separation Module, 8 x 25 capillary cartridges	proteinsimple	SM-W004
66-440kDa Jess or Wes Separation Module, 8 x 25 capillary cartridges	proteinsimple	SM-W008
RePlex Module	proteinsimple	RP-001

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Total Protein Detection Module for Chemiluminescence based total protein assays	proteinsimple	DM-TP01
RNeasy Mini Kit	QIAGEN	74104
RNeasy Plus Micro Kit	QIAGEN	74034
RNase-free DNase	QIAGEN	79256
PrimeScript RT Master Mix	Takara Bio	RR036A
Taqman Fast Advanced Master Mix	Applied Biosystems	4444557
riboPOOL rRNA Depletion Kit (Mouse-Rat Ribo-Seq)	siTOOLS Biotech	dp-P012-052
T7-Scribe Standard RNA IVT Kit	CELLSCRIPT	C-AS2607
SEQuoia Express Stranded RNA Library Prep Kit	Bio-Rad	12017297
Direct-zol RNA Microprep Kit	Zymo Research	R2062
Aurora column (250mm length, 75µm i.d.)	IonOptics	AUR3-25075C18-CSI
nanoElute	Bruker	NA
BaseScope Reagent Kit v2-Red Assay	Advanced Cell Diagnostics	323900
BaseScope Probe - BA-Mm- <i>Eif4g2</i> -2zz-st-C1	Advanced Cell Diagnostics	1090831-C1
RNAscope 2.5 High Definition (HD)-RED Assay	Advanced Cell Diagnostics	322350
RNAscope Probe Mm-Bmi1-O1	Advanced Cell Diagnostics	466021
RNAscope Probe Mm-Lgr5	Advanced Cell Diagnostics	312171
Click-iT™ Plus EdU Alexa Fluor™ 647 Flow Cytometry Assay Kit	Molecular Probes	C10635
SCISONS anti-dsRNA ELISA kit (K1 based)	Vector Laboratories	10623002
CUT&Tag-IT Assay Kit	Active Motif	53170
CUT&Tag-IT spike-in control, Anti-Rabbit	Active Motif	53168

Deposited data

Raw and analyzed RNA-seq data	This paper	GSE294512
Raw and analyzed CUT&Tag data	This paper	GSE318066
Raw and analyzed single-nucleus multiome (Gene expression + ATAC) data	This paper	GSE318068
Raw and analyzed Ribo-seq and paired RNA-seq data	This paper	GSE294619
Raw and analyzed data (mass spectrometry)	This paper	jPOSTrepo (https://repository.jpostdb.org/), JPST004351 (PXD073625)
Raw images/data	Mendeley	https://doi.org/10.17632/zymh4y3w9h

Experimental models: Cell lines

HEK293 R spondin-1	Sigma-Aldrich	SCC111
<i>Rosa26</i> -CreER; <i>Eif4g2</i> ^{+/+} , <i>Eif4g</i> ^{flox/+} , and <i>Eif4g2</i> ^{flox/flox} mouse intestinal organoids and fetal spheroids	This paper	N/A

Experimental models: Organisms/strains

<i>Eif4g2</i> ^{flox} mouse	This paper	N/A
B6.129-Gt(<i>ROSA</i>)26Sor ^{tm1(cre/ERT2)Tyj/J} (<i>ROSA26</i> -CreERT2)	Jackson Laboratories	#008463
B6.Cg-Tg(<i>Vil1</i> -cre/ERT2)23Syr/J (<i>Villin</i> -CreERT2)	Jackson Laboratories	#020282

Oligonucleotides

Mouse <i>Eif4g2</i> crRNA #1	Integrated DNA technologies	TCAACTGATACTCTCCGGTG
Mouse <i>Eif4g2</i> gRNA #2	Integrated DNA technologies	AACCAAATGGTTACACCCCT

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Alt-R CRISPR-Cas9 tracrRNA	Integrated DNA technologies	1072532
Mouse <i>Eif4g2</i> F primer	Integrated DNA technologies	GGTCTACAGAGTGAGTTCCAGGACAGC
Mouse <i>Eif4g2</i> R primer	Integrated DNA technologies	GCTCAAGCCAGACAAAAGCCCTACTCC
Lgr5 TaqMan Assay	Thermo Fisher Scientific	Mm00438890_m1
Tacstd2 TaqMan Assay	Thermo Fisher Scientific	Mm01183793_s1
Gapdh TaqMan Assay	Thermo Fisher Scientific	Mm99999915_g1
Software and algorithms		
Prism 10	GraphPad	https://www.graphpad.com/scientific-software/prism/
Compass for Simple Western software	Protein Simple	https://www.bio-technie.com/resources/instrument-software-download-center
FlowJo (v10.10.0)	BD Life Sciences	https://www.flowjo.com/
R software for statistical computing v4.5.1	R Foundation for Statistical Computing	https://www.r-project.org/
Python 3.10.19	Python Software Foundation	https://www.python.org/
EnrichR	Chen et al. ⁵¹	https://maayanlab.cloud/Enrichr/
clusterProfiler (v4.12.6)	Xu et al. ⁵²	https://bioconductor.org/packages/clusterProfiler/
GSVA (ssGSEA)	Hänzelmann et al. ⁵³	https://bioconductor.org/packages/GSVA/
Msigdbr (v25.1.1)	Dolgalev, 2025	https://CRAN.R-project.org/package=msigdbr
Hisat2 (v2.0.5)	Kim et al. ⁵⁴	https://github.com/DaehwanKimLab/hisat2
featureCounts (v1.5.0-p3)	Liao et al. ⁵⁵	https://subread.sourceforge.net/
DeSeq2 (v1.20.0)	Love et al. ⁵⁶	https://doi.org/10.1186/s13059-014-0550-8
Fastp	Chen et al. ⁵⁷	https://github.com/OpenGene/fastp
Cutadapt 3.7	Martin et al. ⁵⁸	https://cutadapt.readthedocs.io/
STAR v2.7.0a	Dobin et al. ⁵⁹	https://github.com/alexdobin/STAR
UMItools v1.1.2	Smith et al. ⁶⁰	https://github.com/CGATOxford/UMI-tools
diaPASEF	Meier et al. ⁶¹	TimsTOF Pro (Bruker)
DIA-NN (v1.8.2 beta27)	Demichev et al. ⁶²	https://github.com/vdemichev/DiaNN
Nextflow v25.04.8	Di Tommaso et al. ⁶³	https://www.nextflow.io/
nf-core cutandrun v3.2.2	Ewels et al. ⁶⁴ , nf-core community	https://nf-co.re/cutandrun/
deepTools v3.5.6	Ramírez et al. ⁶⁵	https://deeptools.readthedocs.io/
Samtools v1.22.1	Li et al. ⁶⁶	https://www.htslib.org/
DiffBind 3.16.0	Stark and Brown, 2011	https://bioconductor.org/packages/DiffBind/
BEDTools v2.31.1	Quinlan and Hall ⁶⁷	https://bedtools.readthedocs.io/
ChIPseeker v1.42.0	Yu et al. ⁶⁸	https://bioconductor.org/packages/ChIPseeker/
TxDb.Mmusculus. UCSC.mm10.knownGene / org.Mm.eg.db	Carlson M (Bioconductor annotation package) Carlson M et al. (Bioconductor annotation package)	https://bioconductor.org/packages/TxDb.Mmusculus.UCSC.mm10.knownGene/ https://bioconductor.org/packages/org.Mm.eg.db/
HOMER v4.11	Heinz et al. ⁶⁹	https://homer.ucsd.edu/homer/
Cell Ranger ARC (v2.0.2)	10x Genomics	https://www.10xgenomics.com/support/software/cell-ranger-arc
refdata-cellranger-arc-mm10-2020-A-2.0.0	10x Genomics	https://www.10xgenomics.com/support/software/cell-ranger-arc
Seurat 5.4.0	Satija et al., ⁷⁰ Hao et al. ⁷¹	https://satijalab.org/seurat/
Signac 1.16.0	Stuart et al. ⁷²	https://stuartlab.org/signac/
SCTransform (glmGamPoi) 0.4.3	Hafemeister and Satija ⁷³	https://cran.r-project.org/package=sctransform
SeuratObject 5.3.0	Hoffman et al., 2025	https://cran.r-project.org/package=SeuratObject

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committees at the University of California, San Francisco and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were maintained on a predominantly C57BL/6 background and housed in a specific pathogen-free, temperature- and humidity-controlled facility under a 12-hour light/dark cycle with food and water *ad libitum*. Mice were 20 to 40 weeks old for knockout experiments and at least 8 weeks old for cell isolations. Both sexes were used, and littermates served as controls.

Generation of *Eif4g2^{fllox}* mice

Eif4g2^{fllox} mice were generated by blastocyst injection of Cas9 ribonucleoproteins (purified Cas9 protein, crRNA targeting the *Eif4g2* locus, universal tracrRNA; Integrated DNA Technologies (IDT)) together with a single-stranded oligonucleotide donor for homology-directed repair (IDT) to insert two loxP sites flanking exon 5. Injected blastocysts were transferred to pseudopregnant females. Founders were screened by PCR and sequencing, and positive founders were outcrossed to C57BL/6J mice to establish germline transmission. Tamoxifen-inducible knockout models were generated by crossing *Eif4g2^{fllox}* mice with *Rosa26-CreER* and *Vil1-CreER* mice (see [key resources table](#)).

METHOD DETAILS

Tamoxifen induction

To delete floxed *Eif4g2* alleles (*Rosa26-CreER*; *Eif4g2^{fllox}* and *Vil1-CreER*; *Eif4g2^{fllox}*), tamoxifen (100mg/kg body weight, Sigma-Aldrich) dissolved in corn oil was administered by intraperitoneal injection once daily for 4 consecutive days.

DNA extraction and genotyping with PCR

Mouse tissue was lysed in DirectPCR Lysis Reagent and Proteinase K solution (Viagen Biotech) and digested overnight at 56°C. Genotyping PCR was performed using KOD Xtreme Hot Start DNA Polymerase (Sigma-Aldrich) using the primers listed in the [key resources table](#). PCR products were resolved on a 1.8% agarose gel to distinguish wild type (730 bp), floxed (798 bp), and excised (191 bp) alleles.

Histology and immunohistochemistry

Mouse organs were fixed in 4% paraformaldehyde (PFA) in PBS at 4°C overnight, washed in PBS, and transferred through 25%, 50% and 70% ethanol (30 min each) before storage in 70% ethanol at 4°C. Tissue dehydration, embedding, sectioning (4 μm), H&E and PAS staining, cleaved caspase 3 and PCNA immunohistochemistry, and slide imaging was performed at Histowiz Inc (NY, USA).

Immunofluorescence

Paraffin sections were deparaffinized in xylene and rehydrated through graded ethanol to water. Heat-induced antigen retrieval was performed in citrate buffer (Target Retrieval Solution, pH 6.1; DAKO). Sections were blocked in PBS containing 5% donkey serum, 1% BSA, and 0.1% Tween20 for 1 hour at room temperature, incubated with primary antibodies overnight at 4°C, and then with Alexa Fluor-conjugated secondary antibodies for 1.5 hours at room temperature. Nuclei were counterstained with DAPI, and sections were mounted with Fluoromount-G (Invitrogen). Images were acquired on a Keyence BZ-X810 microscope. Antibodies are listed in the [key resources table](#).

Pathological evaluation of the small intestine

Small intestinal lesion severity was scored using a semiquantitative system modified from a published method.¹⁵ Slides were examined by a board-certified veterinary pathologist blinded to experimental groups. Each mouse was scored on a 0–4 severity scale (0, absent; 1, minimal, <10%; 2, mild, 10–25%; 3, moderate, 25–50%; 4, marked, >50%) for the following parameters: goblet cell depletion, Paneth cell depletion, crypt proliferation, crypt single cell necrosis, epithelial erosion, epithelial ulceration, crypt loss, villus blunting, inflammatory cell infiltration and inflammatory cell extension.

RNAscope and BaseScope

RNA in situ hybridization was performed on paraffin sections using RNAscope 2.5 HD-RED Assay or BaseScope Reagent Kit v2 RED (Advanced Cell Diagnostics (ACD)) according to the manufacturer's protocol. After deparaffinization and rehydration, sections were treated with hydrogen peroxide (RT, 10 min), target retrieval (100°C, 15 min), and protease (Protease plus for RNAscope or Protease III for BaseScope; 40°C, 30 min). Probes ([key resources table](#)) were hybridized at 40°C for 2 hours and slides were counterstained with hematoxylin. Images were acquired on a Keyence BZ-X810 microscope.

Quantification of *Bmi1* along the crypt-villus axis

Ten well-oriented crypts were analyzed per mouse (n=3 mice/genotype; 30 crypts/genotype). Cell positions were assigned using an absolute coordinate method in which the nucleus contacting the basement membrane was position 0, and positions increased by

one towards the villus tip. *Bmi1* signal intensity was scored in each epithelial cell using the ACD semi-quantitative guideline (0, 0 dots/cell; 1, 1–3 dots/cell; 2, 4–9 dots/cell; 3, 10–15 dots/cell; 4, ≥ 15 dots or $>10\%$ dots in clusters). Statistical analyses were performed in GraphPad Prism 10 using a mixed-effects two-way ANOVA (cell position $\times$ genotype) with mouse as a random effect, followed by Sidák correction for pairwise comparisons at each position.

Production of R spondin-conditioned media

R-spondin 1 expressing HEK293T cells (Sigma-Aldrich) were maintained in DMEM containing 20% FBS, 1% penicillin/streptomycin, and 125 $\mu\text{g}/\text{mL}$ Zeocin at 37°C, 5% CO_2 . For conditioned medium production, cells were cultured in organoid basal medium (Advanced DMEM/F12 supplemented with 10 mM HEPES, 1x Glutamax, 1x B27, and 1 mM N-acetylcysteine). After 5 days, the supernatant was collected, cleared by centrifugation and filtration (0.22 μm), and stored at -80°C until use.

Organoid and fetal spheroid establishment and culture

Adult small intestinal organoids were established from crypts isolated from the proximal small intestine as described previously.⁷⁴ Briefly, the proximal small intestine was harvested, villi were removed, and tissue fragments (~ 5 mm) were incubated in 2.5 mM EDTA in PBS for 20 min at 4°C. Crypts were released by vigorous shaking in PBS containing 10% FBS, filtered through a 40- μm strainer, and pelleted. Crypts were embedded in growth factor-reduced Matrigel (Corning) and plated in 12-well plates (200 crypts per 20 μL dome, four domes per well). After Matrigel polymerization, 1 mL of ENR medium (organoid basal medium supplemented with EGF (50 ng/mL), Noggin (100 ng/mL) and 10% (v/v) R-spondin conditioned medium) was added. Organoids were passaged every 4–5 days by mechanical disruption at a 1:4 – 1:6 ratio.⁷⁵

Fetal intestinal spheroids were established as described previously.^{18,26} Briefly, embryos were collected at E14.5, fetal small intestines were dissected and minced, and tissue was digested with 1 mg/mL collagenase/dispase (Roche) for 10 min at 37°C. Cells were filtered (40 μm), embedded in Matrigel, and cultured in ENR medium. Spheroids were passaged every 4–6 days by mechanical disruption at 1:4 – 1:8 split ratio.

To activate CreER, organoids and spheroids were treated with 0.5 μM 4-OHT (Sigma) for 24 hours. For CREBBP/EP300 inhibition, organoids and spheroids were treated with A-485 (3–10 μM ; Tocris) or DMSO in ENR medium for 4 days.

Organoid whole-mount staining

Organoids were fixed and permeabilized as described previously,⁷⁵ then incubated with primary antibodies overnight at 4°C, followed by Alexa Fluor-conjugated secondary antibodies and DAPI counterstaining. Images were acquired on a Zeiss LSM880 confocal microscope. Antibodies are listed in the [key resources table](#).

RNA isolation and RT-qPCR

Intestinal crypt and villi samples were collected as described in ‘Organoid and fetal spheroid establishment and culture’. Then, approximately 10 mg (wet weight) of tissue was homogenized in 350 μL of RLT Plus using TissueLyser II. Organoids were recovered from Matrigel using Cell Recovery Solution at 4°C and lysed in RLT Plus. Total RNA was purified using RNeasy with on-column DNase digestion. cDNA was synthesized from 0.1–1 μg RNA. RT-qPCR was performed using TaqMan chemistry on StepOne or Lightcycler 96 instrument. Target transcripts were normalized to *Gapdh*, and relative expression was calculated using the $\Delta\Delta\text{Ct}$ method (fold-change relative to control). Assay details are provided in the [key resources table](#).

RNA sequencing

Total RNA was sent to Novogene (Sacramento, CA) for library preparation, sequencing (Illumina, PE150, 30M paired reads) and bioinformatics analyses. Raw FASTQ reads were processed using fastp to remove adapter-containing reads, then aligned to the mouse reference genome (GRCm38/mm10, Genome Reference Consortium, https://www.ncbi.nlm.nih.gov/assembly/GCF_000001635.20/) using Hisat2.⁷⁶ Gene expression quantification was performed using featureCounts.⁵⁵ Differential expression analysis was performed using DESeq2.⁵⁶ P-values were adjusted using the Benjamini–Hochberg method for controlling the false discovery rate. Genes with an adjusted P-value ≤ 0.05 were considered differentially expressed. Gene set enrichment analyses were performed using clusterProfiler.⁵² The fetal spheroid signature gene set comprised genes upregulated (>2 -fold) in fetal tissue-derived spheroids versus adult tissue-derived organoids,¹⁸ and YAP-activated signatures were curated from published intestinal YAP target gene sets in intestinal cells.^{19,20} Genes upregulated in *Eif4g2*^{−/−} SIOs were defined from the SIO RNA-seq differential expression results using a >2 -fold threshold. Single-sample GSEA (ssGSEA) was computed with GSVA (method = “ssgsea”) using normalized expression values. Gene sets used for ssGSEA were derived from Gene Ontology (GO) and Reactome pathway collections, and Hallmark gene sets (MSigDB) were used for inflammatory signaling programs (e.g., IFN- α /IFN- γ , TNF- α /NF- κB , IL-6-STAT3, and UPR). Additional compact custom gene sets were defined for stress-response programs (ISR/ATF4–CHOP) and dsRNA/PRR signaling. The final gene lists used for ssGSEA are provided in [Table S2](#). ssGSEA scores were compared between genotypes using a two-sided Wilcoxon rank-sum test, with Benjamini–Hochberg correction across gene sets.

Library preparation for Thor-Ribo-seq and paired RNA-seq

For Ribo-seq and paired RNA-seq experiments, Matrigel domes containing organoids were scraped off using spatulas and snap frozen in liquid nitrogen. Thor-Ribo-Seq libraries were prepared as previously described with modifications.^{77,78} Twelve frozen

domes containing organoids were combined with frozen droplets of 300 μ L lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 100 μ g/mL cycloheximide, 100 μ g/mL chloramphenicol, and 1% Triton X-100) and pulverized using Multi-beads Shocker (Yasui Kikai) at 2,800 rpm for 15 sec. Lysates were incubated with 15 U of Turbo DNase (Thermo Fisher Scientific) for 10 min on ice and clarified by centrifugation at 20,000 g for 10 min at 4°C. Lysates containing $\sim$ 2 μ g of RNA were treated with 20 U of RNase I (LGC Biosearch Technologies) for 45 min at 25°C. Ribosomes were precipitated by 1 M sucrose cushion followed by ultracentrifugation for 1 h at 100,000 rpm (408,800 $\times$ g) at 4°C using Optima MAX-TL and a TLA-110 rotor (Beckman Coulter). RNAs ranging from 17 to 34 nt were excised from 15% TBE-urea gels, purified, and ligated with DNA linkers containing the T7 promoter sequence. After the rRNA depletion using the riboPOOL rRNA Depletion Kit (Mouse-Rat Ribo-Seq) (siTOOLs Biotech), an oligonucleotide to the T7 promoter region was hybridized and in vitro transcription was performed using a T7-Scribe Standard RNA IVT Kit (CELLSCRIPT). Amplified RNAs were subsequently ligated with the second linker, reverse-transcribed, and PCR-amplified.

For paired RNA-seq, total RNA was purified using TRIzol LS reagent (Thermo Fisher Scientific) and Direct-zol RNA Microprep Kit (Zymo research). Following the removal of rRNA by riboPOOL Mouse-Rat rRNA depletion kit (siTOOLs Biotech), the sequencing library was prepared with SEQuoia Express Stranded RNA Library Prep Kit (Bio-Rad). The libraries were sequenced on a NovaSeq X Plus platform (Illumina) with paired-end 150 bp reads.

Data processing was performed as previously described⁷⁷ with modifications. For Thor-Ribo-Seq, after the base correction with overlap analysis, the quality filtering and the adapter removal were performed on read 1 using fastp.⁵⁷ For RNA-seq, both paired reads were used. Reads were then demultiplexed by Cutadapt 3.7⁵⁸ and mapped to noncoding RNAs using STAR v2.7.0a⁵⁹ to exclude them from the analysis. The remaining reads were aligned to the mouse genome mm39 and assigned to the GENCODE Mouse release VM36 reference obtained from the UCSC Genome Browser (<https://genome.ucsc.edu/index.html>) using STAR. UMI suppression was performed using UMItools v1.1.2.⁶⁰ Read counting was performed using the custom scripts (<https://github.com/ingolia-lab/RiboSeq>). The offsets of the A site from the 5' end of the ribosome footprint were determined to be 15 for fragments of 19–34 nt. For RNA-Seq, an offset of 15 was used for all mRNA fragments. The canonical transcripts were defined based on Ensembl Canonical.

Fold-change calculations were performed with the DESeq2 package.⁵⁶ Translation efficiency (TE), which is Ribo-Seq counts normalized by RNA-seq counts, was also measured by DESeq2. The significance was calculated by a likelihood ratio test in a generalized linear model. The reads corresponding to the first and last five codons in the coding sequences were excluded from the calculation. mRNAs were defined as uORF-containing if they included at least one uORF annotated in Ribo-uORFs with a strong Kozak sequence. Gene ontology analyses were performed using EnrichR.^{51,79,80} All custom scripts used in this study are available upon request.

Protein extraction

Mouse intestinal crypts and organoids were isolated as above and washed in ice-cold PBS. Approximately 10 mg (wet weight) of crypts were lysed in 500 μ L RIPA buffer (Thermo Scientific) supplemented with protease and phosphatase inhibitor cocktails (1 $\times$; Sigma-Aldrich) and homogenized using TissueLyser II (QIAGEN). Organoids were recovered with Cell Recovery Solution and then lysed with ice-cold lysis buffer (MPER (Thermo Scientific) or Laemmli buffer (Bio-Rad), depending on the downstream targets) with protease and phosphatase inhibitor cocktails (1 $\times$). Lysates were clarified by centrifugation, and protein concentration was determined. Samples were snap frozen and stored at -80°C until analysis. For histone extraction, an acid extraction method was used as previously described.⁸¹

Proteomics

Organoids were lysed in PTS buffer (12 mM SDC, 12 mM SLS, and 100 mM Tris-HCl (pH 9.0)) containing a protease and phosphatase inhibitor mixture (Sigma-Aldrich). The lysates were used as protein samples and processed using a modified protein aggregation capture method⁸² as previously described.⁸³ A total of 200 ng of desalted peptides were loaded and separated on an Aurora column (250 mm length, 75 μ m i.d., IonOpticks) using a nanoElute (Bruker) for subsequent analysis on a timsTOF Pro2 system (Bruker). The mobile phases were composed of 0.1% formic acid (solution A) and 0.1% formic acid in acetonitrile (solution B). A flow rate of 400 nL/min of 2–17% solution B for 60 min, 17–25% solution B for 30 min, 25–37% solution B for 10 min, 37–80% solution B for 10 min, and 80% solution B for 10 min was used (120 min in total). The applied spray voltage was 1400 V, and the interface heater temperature was 180°C. To obtain MS and MS/MS spectra, the Parallel Accumulation Serial Fragmentation (PASEF) acquisition method with data-independent acquisition (DIA) mode was used (diaPASEF).⁶¹ For diaPASEF settings, 1.7 s per one cycle with precursor ion scan and 16 times diaPASEF scans were conducted with the MS/MS isolation width of 25 m/z, precursor ion ranges of 400–1200 m/z, ion mobility ranges of 0.57–1.47 V-s-cm⁻². The obtained DIA data were searched by DIA-NN (v1.8.2 beta27)⁶² against selected mouse entries of UniProt/Swiss-Prot release 2022_03 with the carbamidomethylation of cysteine as the fixed modification and protein N-terminal acetylation and methionine oxidation as the variable modification. For the other DIA-NN parameters, Trypsin/P protease, one missed cleavage, peptide length range of 7–30, precursor m/z range of 300–1800, precursor charge range of 1–4, fragment ion m/z range of 200–1800, and 1% precursor FDR were used. The values “PG.MaxLFQ” from the results were used as representative protein area values for the comparison of protein expression levels.

Protein expression analysis by antibody-based protein quantification

Antibody-based protein quantification was performed using a WES/Jess automated capillary electrophoresis system (ProteinSimple) with 2–40 kDa, 12–230 kDa or 66–440 kDa separation modules, according to the manufacturer's instructions. Lysates were diluted to

0.4 mg/mL. Signals were normalized either to GAPDH or histone H3, or to total protein using the Replex module and Total Protein Assay. Antibodies are listed in the [key resources table](#). Data were analyzed using Compass for Simple Western software.

SUnSET assay

SIOs were incubated in ENR medium supplemented with 10 μ g/mL Puromycin for 15 minutes at 37°C. Then, they were washed with ice-cold PBS and recovered in cell recovery solution and incubated for 20 min at 4°C with gentle rotation. SIOs were lysed in MPER (as described above) and puromycin incorporation was analyzed using the WES/Jess automated capillary electrophoresis system (as described above).

EU incorporation assay

Nascent RNA synthesis was assessed by 5-ethynyl uridine (EU) incorporation followed by click-chemistry detection and flow cytometry. EU was added by a half medium change to a final concentration of 1 mM, and organoids were incubated for 1 hour at 37°C. Organoids were dissociated with TrypLE Express supplemented with Y-27632 for 10 min at 37°C, washed with ice-cold PBS, and processed for fixation/permeabilization and click labeling according to the manufacturer's instructions using the Click-iT™ Plus Alexa Fluor™ 647 flow cytometry reagents (Molecular Probes). Protector RNase inhibitor (Roche; 40 U/mL) was included in all solutions used before fixation. Samples were analyzed on a BD LSRFortessa X. Data were analyzed in FlowJo (v10.10.0).

dsRNA ELISA

dsRNA levels were assessed using the SCICONS anti-dsRNA ELISA kit (K1-based; Vector Laboratories) according to the manufacturer's instructions. Total RNA (1 μ g/well) was applied to the plate. *Eif4g2*^{+/+}, *Eif4g2*^{+/-}, and *Eif4g2*^{-/-} samples were analyzed with four biological replicates per genotype, and each sample was measured in technical triplicates. Kit-provided poly(I:C) control and an RNase III-digested poly(I:C) were included as controls for dsRNA-specific detection. Because ELISA signal depends on the dsRNA source, results were normalized to the wild-type group and reported as relative dsRNA signal. Absorbance was measured using a microplate reader (Molecular Devices).

CUT&Tag analysis

CUT&Tag was performed using the CUT&Tag-IT Tissue Kit (Active Motif) according to the manufacturer's protocol with minor modifications. Matrigel domes were mechanically disrupted by repeated pipetting, and organoids were collected, washed with ice-cold PBS containing 10 mM sodium butyrate, and processed immediately. Organoids were lysed and homogenized using a Dounce homogenizer in kit lysis buffer supplemented with 0.5 μ M spermidine and 10 mM sodium butyrate; these additives were included in all subsequent buffers unless noted otherwise. Nuclei were stained with acridine orange/propidium iodide (AOPI) and counted using an EVOS microscope. For each reaction, 450,000 mouse nuclei were combined with 22,500 CUT&Tag-IT Spike-In Drosophila nuclei (Active Motif), bound to Concanavalin A beads for 10 min at room temperature with rotation, and subjected to antibody incubations. Replicate numbers were as follows: for SIOs, n = 4 biological replicates per genotype for H3K27ac, CREBBP, and EP300, and n = 2 per genotype for rabbit IgG controls; for fetal spheroids, n = 3 per genotype for H3K27ac, and rabbit IgG controls were performed in heterozygotes only (n = 3). Primary antibodies are listed in the [key resources table](#). Anti-H3K27ac and rabbit IgG control were incubated overnight at 4°C, whereas anti-CREBBP and anti-EP300 were incubated for 2 hours at 4°C. The CUT&Tag-IT Spike-In antibody was added to all samples as instructed. Secondary antibody incubation, pA-Tn5 binding, tagmentation, DNA extraction, and library preparation were performed according to the kit protocol. Libraries were sequenced on an Illumina NextSeq 2000 (P2 flow cell; paired-end 2 $\times$ 50 bp) at a depth of ~14 million read pairs per sample.

CUT&Tag data preprocessing

CUT&Tag libraries were processed using the nf-core/cutandrun pipeline (v3.2.2) executed with Nextflow v25.04.8 using the Docker profile. Unless otherwise specified, default pipeline settings were used. Briefly, paired-end FASTQ files were quality-controlled and aligned to the mouse genome (mm10/GRCm38) using Bowtie2, and duplicate-marked BAM files and standard QC metrics were generated. Spike-in reads were additionally aligned to Drosophila dm6, and spike-in-based scale factors were computed. Peaks were called using SEACR in relaxed mode with an mm10 blacklist applied.

For signal normalization across samples, we initially evaluated spike-in-based scaling derived from the CUT&Tag-IT Spike-In Drosophila nuclei. However, spike-in-scaling showed an apparent systematic bias between genotypes that could confound differential binding analyses. Therefore, downstream differential analyses used a greenlist-based library-size normalization strategy (see below) and the resulting size factors were applied during DiffBind/DESeq2 normalization.

Construction of a mouse mm10 CUT&Tag “greenlist” for normalization

To define a robust set of background regions for normalization, we constructed an mm10 CUT&Tag greenlist based on the published framework³⁵ and its reference implementation (https://github.com/fndemello/CUT-RUN_greenlist), with minor modifications. Public mouse CUT&Tag datasets were identified by searching GEO for studies containing ≥ 4 IgG control samples per series. SRA metadata tables were obtained via the SRA Run Selector and IgG control runs were retained. Runs with very low sequencing depth ($< 5 \times 10^5$ reads) were excluded. Selected IgG FASTQs were downloaded from SRA and aligned to mm10 using Bowtie2 to generate BAM files.

Genome-wide background signal was quantified with deepTools multiBamSummary (bins mode; 1 kb bins) while excluding mm10 blacklist regions (Boyle-Lab mm10 blacklist v2). Raw bin-level count matrices were quantile-normalized, and Shannon entropy (maximum-likelihood estimator) was computed per bin to identify regions with uniform background coverage across IgG datasets. Candidate bins were initially defined using the top 0.1% entropy threshold; however, this yielded too few regions after gene-proximity filtering, and we therefore used the top 1% entropy threshold to obtain a sufficiently large and stable greenlist. Bins within ± 5 kb of annotated genes (GENCODE vM34 basic) were removed, and the remaining bins were sorted and merged to generate the final greenlist BED file (mm10_cutntag_1percent_final_greenlist.bed). GEO/SRA accessions used for its construction (Table S7) and the final BED file are provided as Data S1.

Greenlist-based size factor estimation for experimental CUT&Tag libraries

For experimental adult SIO and fetal spheroid CUT&Tag libraries, read counts over the final mm10 greenlist regions were quantified using deepTools multiBamSummary (BED-file mode) from individual replicate BAM files. Greenlist counts were then used to estimate library-size normalization factors using DESeq2 (estimateSizeFactors; design ~ 1). Size factors were estimated separately for each target (H3K27ac, CREBBP, and EP300) and separately for adult SIO and fetal spheroid datasets.

Differential binding analysis with DiffBind using greenlist-derived size factors

Differential CUT&Tag peak analyses were performed in R using DiffBind with DESeq2 statistics, with adult SIO and fetal spheroid datasets analyzed independently. For each target, DiffBind objects were created from sample sheets and read counts were generated using dba.count with SummarizeOverlaps. Greenlist-derived DESeq2 size factors were loaded and matched to samples by the basename of the BAM filename, rescaled to a geometric mean of 1, and supplied to DiffBind via dba.normalize (method = DESeq2; normalize = ordered_sf). Differential binding was tested by specifying a contrast between homozygous vs heterozygous samples (dba.contrast, category = Condition), followed by DESeq2 analysis (dba.analyze, method = DESeq2). Differential peak tables were obtained using dba.report. Significant differential peaks (FDR < 0.05) were exported as BED files and separated into gained (Fold > 0) and lost (Fold < 0) sets. For downstream analyses, H3K27ac peaks were annotated to the nearest gene and genomic feature using ChIPseeker (annotatePeak; tssRegion = c(-2000, 2000)) with TxDb.Mmusculus.UCSC.mm10.knownGene and org.Mm.eg.db.

Gene set-stratified analysis of differential CUT&Tag peaks

Annotated DiffBind/DESeq2 peak tables (H3K27ac; adult SIO and fetal spheroid datasets analyzed separately) were imported into R. Peaks were classified as promoter-proximal (≤ 2 kb from TSS) or distal (>2 kb) based on distanceToTSS, and each peak was assigned to its nearest gene. Peaks were stratified by gene-set membership (ISC-core, fetal signature, or housekeeping control). The ISC-core and fetal-signature gene lists are provided in Table S2. The housekeeping genes set (HOUNKPE_HOUSEKEEPING_GENES, MSigDB Mouse, version 2025.1) was downloaded from MSigDB and restricted to genes with at least one assigned peak in the corresponding dataset.

For gene set comparisons, all peaks were included, and distributions of peak log₂ fold changes (HOMO vs HET; DiffBind output) were compared between gene sets using a two-sided Wilcoxon rank-sum test, with effect sizes quantified by Cliff's delta. When directionality was summarized, peaks were labeled as gained or lost only among significant differential peaks (FDR < 0.05), with gained defined as Fold > 0 and lost as Fold < 0.

HOMER motif enrichment analysis

Motif enrichment was performed using HOMER (findMotifsGenome.pl) on H3K27ac peaks that were significantly decreased ("lost") or increased ("gained") in *Eif4g2* homozygous knockout compared with heterozygous controls (FDR < 0.05). To enable comparable motif scanning across peak sets, SEACR peak intervals were converted to fixed-length windows (± 100 bp; 200 bp total) centered on each peak summit. Known motif enrichment was assessed against a background composed of all tested consensus H3K27ac peaks processed identically to control for sequence composition and interval length (H3K27ac_TESTED_UNIVERSE_from_DiffBind_center200.bed). HOMER was run on mm10 using the provided fixed-length intervals (-size given) with repeat masking (-mask) and default settings for motif enrichment.

CBP/EP300 signal at H3K27ac-defined peak sets

To assess whether CBP and EP300 occupancy changed at sites where H3K27ac was altered in *Eif4g2*-KO, H3K27ac "lost" and "gained" peak sets were defined from the adult SIO DiffBind/DESeq2 output (lost: FDR < 0.05 and Fold < 0; gained: FDR < 0.05 and Fold > 0). CBP and EP300 CUT&Tag signals were then quantified over these fixed H3K27ac peak regions using DiffBind (dba.count with SummarizeOverlaps) with greenlist-derived DESeq2 size factors applied via dba.normalize (method = DESeq2). Differential binding across these regions was tested for each factor (CBP or EP300) using a homozygous vs heterozygous contrast (dba.contrast) followed by DESeq2-based testing (dba.analyze).

Single-nucleus Multiome (ATAC + Gene Expression) library preparation and sequencing

Crypts were isolated from the mid-jejunum as described in 'Organoid and fetal spheroid establishment and culture'. Crypt epithelial cells were dissociated by incubating crypt fractions in PBS containing Dispase (0.4 U/mL) for 12 min at 37°C with intermittent shaking.

Cells were stained with anti-CD31, anti-CD45, anti-CD326 (EpCAM) antibodies and Ghost Dye Red 710, and epithelial cells were purified by FACS (BD Aria Fusion; 100 μ m nozzle) as Ghost Dye[−] CD31[−] CD45[−] CD326⁺ events. Sorted cells were collected into ENR supplemented with Y-27632 and maintained at 4°C during sorting and collection.

Single-nucleus multiome libraries were prepared according to the manufacturer's protocol using the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Bundle, with Single Index Kit N Set A for ATAC libraries, and Dual Index TT Set A for GEX libraries. The target nuclei number was 3,000. Libraries were quantified using Qubit dsDNA High Sensitivity assays and assessed for fragment size distribution using an Agilent Bioanalyzer High Sensitivity chip.

Libraries from all 8 samples were pooled by modality (ATAC pool and GEX pool) and sequenced on an Illumina NovaSeq X 10B flow cell, loading all 8 samples in a single lane per modality. ATAC libraries were sequenced using 51 $\times$ 12 $\times$ 24 $\times$ 51 and gene expression libraries using 28 $\times$ 10 $\times$ 10 $\times$ 90 read configurations. Base call demultiplexing and FASTQ generation were performed by the UCSF CAT core facility using standard Illumina/10x Genomics workflows. FASTQ files were processed using Cell Ranger ARC (cell-ranger-arc count, v2.0.2) with the mouse reference package refdata-cellranger-arc-mm10-2020-A-2.0.0.

Single-nucleus Multiome data analysis

Downstream analyses were performed in R using Seurat and Signac. For each sample, nuclei were filtered by RNA- and ATAC-based quality metrics (RNA; nFeature_RNA > 500, nFeature_RNA < 8,000, nCount_RNA > 1,000, and percent.mt < 25, and ATAC; TSS.enrichment > 2 and nucleosome_signal < 4). As timepoint-driven differences were minor and key conclusions were concordant across the two time points, we integrated day 6 and day 9 datasets after per-sample QC and merged into a combined object.

For gene expression, the merged object was processed using SCTransform with regression of percent.mt (method = glmGamPoi), followed by PCA (50 PCs computed). For chromatin accessibility, the ATAC assay was processed using TF-IDF normalization and latent semantic indexing (LSI). Highly variable ATAC features were selected using FindTopFeatures (min.cutoff = "q0"), and LSI was computed by RunSVD. To jointly embed multiome nuclei, a weighted-nearest neighbor (WNN) graph was constructed using RNA PCs (dims 1–30) and ATAC LSI dimensions (dims 2–30, excluding LSI1 to reduce sequencing depth-associated effects). UMAP visualization was performed on the WNN graph, and clustering was performed on the WNN shared nearest neighbor graph (wsnn) using the SLM algorithm (algorithm = 3). Cell types were annotated in the integrated dataset using canonical marker genes and cluster structure.

To define ISC-associated regulatory elements from the multiome data, ATAC–RNA peak–gene links were inferred within the Lgr5+ ISC subset. A curated ISC-related gene list was first filtered to include genes detected in the dataset and further restricted to genes expressed in Lgr5+ ISCs (fraction of expressing cells > 0.10 and mean expression > 0.05 in the RNA data layer). To reduce computational burden, the ATAC assay was downsampled to the top 50,000 variable peaks (FindTopFeatures; min.cutoff = "q50"), and region-level covariates were computed with RegionStats using the mm10 genome. Peak–gene links were then computed using LinkPeaks with the filtered ISC gene set. The resulting linked peaks were aggregated to define an ISC-associated peak set (ISC elements). For each nucleus in the combined dataset, an ISC element accessibility score was computed as the mean ATAC accessibility (data layer) across peaks in the ISC element set.

To test concordance between chromatin accessibility and orthogonal chromatin profiling, genomic regions showing decreased signal in homozygous samples from separate CUT&Tag experiments (e.g., H3K27ac-lost, CBP-lost, and EP300-lost regions in BED format) were imported as genomic ranges and intersected with the ATAC peak set. For each nucleus, mean ATAC accessibility (data layer) was computed across peaks overlapping each CUT&Tag-defined region set, yielding region-set accessibility scores (e.g., K27acLost_mean, CBPLost_mean, EP300Lost_mean). Genotype effects were evaluated using sample-level pseudo-bulk medians and two-sided Wilcoxon rank-sum test as described above.

To test whether ISC-associated regulatory elements showed preferential accessibility loss, we compared accessibility changes at ISC element peaks against GC- and/or length-matched background peaks. Within the stem/progenitor subpopulation (*Lgr5*⁺ ISC, transient amplifying, and KO-enriched proliferative cells), GC (and where applicable length) distributions were computed from peak meta-features, and background peak sets were selected using MatchRegionStats. For each peak, pseudo-bulk ATAC accessibility was calculated per sample as the mean accessibility across nuclei, and a peak-level genotype shift was computed as Δ = mean(Homo) – mean(Hetero) across samples. The distribution of Δ values for ISC element peaks (or for ISC element peaks intersecting CUT&Tag lost regions) was compared to the distribution for matched background peaks using a one-sided Wilcoxon rank-sum test (alternative = "less"), testing whether ISC-associated peak sets exhibited a more negative accessibility shift than background.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data in the figure panels are presented as mean $\pm$ standard deviation. Sample number (n) indicates the number of biological replicates in each experiment. For RT-qPCR and Wes data, comparisons between two groups were evaluated using two-sided, lognormal Welch's t tests. Comparisons between three groups were evaluated using one-way ANOVA assuming a lognormal distribution, with Welch's correction to account for unequal variances (Brown–Forsythe/Welch ANOVA). Post hoc comparisons were performed using Dunnett's multiple-comparisons test with WT as the control, and multiplicity-adjusted P values were reported.

To compare body weight change over time, a mixed-effects model (REML) was used with genotype as the between-subject factor and time (days post-TAM injection) as the within-subject factor. Geisser–Greenhouse correction was applied for repeated measures. Where indicated, Tukey's multiple-comparisons test was performed to compare genotypes at each time point.

To compare intestinal pathological scores within different groups, Kruskal-Wallis test was performed. For analyses in which multiple crypts were quantified per mouse, crypt-level measurements were treated as repeated measures nested within mouse (biological replicate), and statistical testing was performed using repeated-measures two-way ANOVA or mixed-effects models in GraphPad Prism with Šidák's correction for multiple comparisons. Above analyses were performed using GraphPad Prism 10 software (San Diego, CA, USA). Significant differences are noted at $p < 0.05$, and the range of statistical significance is shown by an asterisk within the figure panels: ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.