C-type natriuretic peptide facilitates autonomous Ca<sup>2+</sup> entry in growth plate chondrocytes for stimulating bone growth C型ナトリウム利尿ペプチドは 自発的な Ca<sup>2+</sup>流入を介して骨伸長を促進する

Graduate School of Pharmaceutical Sciences, Kyoto University

宮崎 侑

# **Contents**

Abstract	1
Introduction	2-3
Results	4-26
Discussion	27-30
Materials and methods	31-37
References	38-41
Publication list	42
Acknowledgments	43

### <u>Abstract</u>

The growth plates are cartilage tissues found at both ends of developing bones, and vital proliferation and differentiation of growth plate chondrocytes are primarily responsible for bone growth. C-type natriuretic peptide (CNP) stimulates bone growth by activating natriuretic peptide receptor 2 (NPR2) which is equipped with guanylate cyclase on the cytoplasmic side, but its signaling pathway is unclear in growth plate chondrocytes. I previously reported that transient receptor potential melastatin-like 7 (TRPM7) channels mediate intermissive Ca<sup>2+</sup> influx in growth plate chondrocytes, leading to activation of Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) for promoting bone growth. In this report, I provide experimental evidence indicating a functional link between CNP and TRPM7 channels. My pharmacological data suggest that CNP-evoked NPR2 activation elevates cellular cGMP content and stimulates big-conductance Ca<sup>2+</sup>-dependent K<sup>+</sup> (BK) channels as a substrate for cGMP-dependent protein kinase (PKG). BK channel-induced hyperpolarization likely enhances the driving force of TRPM7-mediated Ca<sup>2+</sup> entry and seems to accordingly activate CaMKII. Indeed, ex vivo organ culture analysis indicates that CNP-facilitated bone growth is abolished by chondrocyte-specific *Trpm7* gene ablation. The defined CNP signaling pathway, the NPR2-PKG-BK channel-TRPM7 channel-CaMKII axis, likely pinpoints promising target proteins for developing new therapeutic treatments for divergent growth disorders.

## **Introduction**

The development of skeletal long bones occurs through endochondral ossification processes, during which chondrocyte layers form the growth plates at both ends of bone rudiments, and then the expanded cartilage portions are gradually replaced by trabecular bones through the action of osteoclasts and osteoblasts [1]. Therefore, bone size largely depends on the proliferation of growth plate chondrocytes during endochondral development. On the other hand, atrial (ANP), brain (BNP) and C-type (CNP) natriuretic peptides regulate diverse cellular functions by activating the receptor guanylate cyclases, NPR1 and NPR2 [2]. Of the natriuretic peptides, CNP exclusively stimulates bone development by acting on growth plate chondrocytes expressing the CNP-specific receptor NPR2 [2-4]. Indeed, loss- and gain-of-function mutations in the human NPR2 gene cause acromesomelic dysplasia and skeletal overgrowth disorder, respectively [5, 6]. Furthermore, translational studies have been probing the benefits of CNP treatments in various animal models with impaired skeletal growth, and a phase III clinical trial of CNP therapy has recently been completed and approved for treatment of patients with achondroplasia primarily resulting from mutations in the FGFR3 gene [7]. It is thus likely that NPR2 guanylate cyclase controls chondrocytic cGMP content during growth plate development. Downstream of NPR2 activation, cGMP-dependent protein kinase (PKG) seems to phosphorylate target proteins to facilitate growth plate chondrogenesis [4]. Activated

PKG is postulated to stimulate the biosynthesis of growth plate extracellular matrix by playing an inhibitory role in the mitogen-activated protein kinase Raf-MEK-ERK cascade [8]. In parallel, glycogen synthase kinase  $3\beta$  (GSK3 $\beta$ ) is likely activated by PKG-mediated phosphorylation, leading to the hypertrophic maturation of growth plate chondrocytes [9]. However, it is still unclear how CNP promotes bone growth at the molecular level, and it is important to further address CNP signaling cascade in growth plate chondrocytes.

In the transient receptor potential channel superfamily, the melastatin subfamily member 7 (TRPM7) forms a mono- and divalent cation-permeable channel in various cell types and participates in important cellular processes including cell growth and adhesion [10]. My research group recently reported that growth plate chondrocytes generate autonomic intracellular Ca<sup>2+</sup> fluctuations, which are generated by the intermittent gating of TRPM7 channels, and also that TRPM7-mediated Ca<sup>2+</sup> entry activates Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII), facilitating chondrogenesis for endochondral bone development [11]. Based on these observations, I explored the link between CNP signaling and TRPM7-mediated Ca<sup>2+</sup> entry through the experiments described in this report. My data obtained clearly indicate that big-conductance Ca<sup>2+</sup>-dependent K<sup>+</sup> (BK) channels play a key role in the functional coupling between NPR2 and TRPM7 channels in growth plate chondrocytes.

## **Results**

## CNP facilitates spontaneous Ca<sup>2+</sup> fluctuations in growth plate chondrocytes

In the growth plates of developing bones, proliferating cartilage cells, designated as round and columnar chondrocytes, frequently exhibit weak increases and decreases in intracellular  $Ca^{2+}$  concentration under resting conditions [11]. On the other hand, previous *in vivo* studies demonstrated that CNP application (>1 µmol/kg) stimulates endochondral bone growth [2]. In my Fura-2 imaging of round chondrocytes within femoral bone slices prepared from wild-type mice, CNP pretreatments (30~300 nM for 1 hr) dose-dependently facilitated spontaneous  $Ca^{2+}$  fluctuations (Figure 1A). In particular, fluctuation-positive cell ratio and fluctuation amplitude were remarkably elevated in response to the CNP treatments. In contrast, ANP treatments exerted no effects on  $Ca^{2+}$  fluctuations in growth plate chondrocytes.

In chondrocyte-specific *Npr2*-knockout mice (*Npr2*<sup>fl/fl</sup>, *Col2a1-Cre*<sup>+/-</sup>), Cre recombinase is expressed under the control of the collagen type  $2\alpha 1$  gene promoter and thus inactivates the floxed *Npr2* alleles in a chondrocyte-specific manner [12]. My RT-PCR analysis indicated that the floxed *Npr2* gene was largely inactivated in the growth plates prepared from the E17.5 mutant embryos, but such recombination events were not detected in other tissues examined (Figure 2A and B). Accordingly, *Npr2* mRNA contents in the mutant growth plates were reduced to less than 40% of controls (Figure 2C), despite the growth plate preparations contain not only chondrocytes but also perichondrium-resident cells including undifferentiated mesenchymal cells and immature chondroblasts. In contrast to the imaging observations in wild-type and control bone slices, CNP treatments failed to enhance  $Ca^{2+}$  fluctuations in the mutant round chondrocytes prepared from the chondrocyte-specific *Npr2*-knockout mice (Figure 1B). Therefore, CNP seems to facilitate spontaneous  $Ca^{2+}$  fluctuations downstream of NPR2 activation in growth plate chondrocytes.

#### Figure 1



### CNP-induced facilitation of Ca<sup>2+</sup> fluctuations in growth plate chondrocytes.

(A) Fura-2 imaging of round chondrocytes pretreated with or without natriuretic peptides. Femoral bone slices prepared from wild-type C57BL embryos were pretreated with or without CNP and ANP, and subjected to Ca<sup>2+</sup> imaging. Representative recording traces from three cells are shown in each pretreatment group (upper panels). The effects of CNP and ANP pretreatments on spontaneous Ca<sup>2+</sup> fluctuations are summarized (lower graphs). The fluctuation-positive cell ratio, fluctuation amplitude and frequency were statistically analyzed, and significant differences from the control vehicle pretreatment are marked with asterisks (\*p<0.05 and \*\*p<0.01 in one-way ANOVA and Dunnett's test). The data are presented as the means ± SEM. with *n* values indicating the number of examined mice. (B) Fura-2 imaging of round chondrocytes prepared from chondrocyte-specific *Npr2*-knockout (*Npr2*fl/fl, *Col2a1-Cre*<sup>+/-</sup>) and control (*Npr2*fl/fl, *Col2a1-Cre*<sup>-/-</sup>) mice. The bone slices were pretreated with CNP, and then subjected to Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panels) and the CNP-pretreated effects are summarized (right graphs); significant differences from the wild-type group are marked with asterisks (\*p<0.05 in one-way ANOVA and Tukey's test). The data are presented as the means ± SEM. with *n* values





#### Chondrocyte-specific Npr2 ablation.

(A) Organization of floxed and deleted *Npr2* alleles. The chondrocyte-specific *Npr2*-knockout (*Npr2*<sup>fl/fl</sup>, *Col2a1-Cre<sup>+/-</sup>*) mice were previously generated [12]. In this study, genotyping primers were newly designed, and *Npr2* ablation was evaluated in growth plates. The genomic map shows PCR primers for detecting the mutated *Npr2* alleles and *Npr2* mRNA. (B) *Npr2* gene ablation in various tissues from the chondrocyte-specific *Npr2*-knockout mice. Genomic DNAs were prepared from tissues (Gp, humeral growth plate; Br, brain; Lu, lung; Hr, heart; Lv, liver; Ki, kidney) from the E17.5 chondrocyte-specific *Npr2*-knockout and control embryos, and subjected to PCR analysis to detect the floxed and deleted *Npr2* alleles; the *Col2a1-Cre* transgene was also examined. (C) Reduction of *Npr2* mRNA in mutant growth plates prepared from the chondrocyte-specific *Npr2*-knockout mice. Total RNAs were prepared from humeral growth plates from the E17.5 embryos, and subjected to RT-PCR analysis for estimating *Npr2* mRNA content. 18S ribosomal RNA was examined as an internal control. The relative mRNA contents were estimated from cycle thresholds in RT-PCR reactions and are summarized in the bar-graph. The data represent means  $\pm$  SEM, and the numbers of mice examined are shown in parentheses. A significant difference between the genotype is marked with an asterisk (\*\**p*<0.01 in *t*-test).

## Activated PKG facilitates spontaneous Ca<sup>2+</sup> fluctuations

CNP binds to NPR2 to activate its intrinsic guanylate cyclase and thus stimulates PKG by elevating cellular cGMP contents [2]. CNP also binds to NPR3 which acts as a decoy receptor for ligand clearance, but the *Npr3* gene seemed to be inactive in growth plate chondrocytes (Figure 3). Next, I pharmacologically verified the contribution of PKG to CNP-facilitated  $Ca^{2+}$  fluctuations. The cGMP analog 8-(4-chlorophenylthio)-cyclic GMP (8-pCPT-cGMP) is widely used as a PKG-selective activator, while KT5823 is a typical PKG inhibitor. In wild-type growth plate chondrocytes pretreated with 8-pCPT-cGMP (100  $\mu$ M for 1 hr), spontaneous  $Ca^{2+}$  fluctuations were remarkably facilitated (Figure 4A); both fluctuation-positive cell rate and fluctuation amplitude were highly increased. In contrast, the bath application of KT5823 (2  $\mu$ M) clearly attenuated CNP-facilitated  $Ca^{2+}$  fluctuations within a short time frame (Figure 4B). Therefore, PKG activation seems to be essential for CNP-facilitated  $Ca^{2+}$  fluctuations in growth plate chondrocytes.





#### Gene expression analysis in wild-type growth plate chondrocytes.

Total RNAs were prepared from growth plate sections packed with round chondrocytes or enriched with columnar and hypertrophic chondrocytes, and subjected to RT-PCR analysis. The cycle threshold (Ct) was determined for each RT-PCR reaction for estimating relative mRNA content. The data represent the mean  $\pm$  SEM, and the numbers of mice examined are shown in parentheses. Significant differences between the growth plate sections are marked with asterisks (\**p*<0.05 and \*\**p*<0.01 in *t*-test). n.d.: not detectable.

#### Figure 4



Contribution of PKG to CNP-facilitated Ca<sup>2+</sup> fluctuations.

(A) Facilitated Ca<sup>2+</sup> fluctuations in round chondrocytes pretreated with the PKG activator 8-pCPT-cGMP. Wild-type bone slices were pretreated with or without the cGMP analog, and then subjected to Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panels), and the pharmacological effects are summarized (right graphs). Significant differences between control and 8-pCPT-cGMP pretreatments are marked with asterisks (\*\*p<0.01 in *t*-test). The data are presented as the means ± SEM. with *n* values indicating the number of examined mice. (B) Attenuation of CNP-facilitated Ca<sup>2+</sup> fluctuations by the PKG inhibitor KT5823. Wild-type bone slices were pretreated with CNP, and then subjected to Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panel), and KT5823-induced effects are summarized (right graphs). Significant KT5823-induced shifts are marked with asterisks (\*\*p<0.01 in *t*-test). The data are presented as the means ± SEM. The data are presentative recording traces are shown (left panel), and KT5823-induced effects are summarized (right graphs). Significant KT5823-induced shifts are marked with asterisks (\*\*p<0.01 in *t*-test). The data are presented as the means ± SEM. With *n* values indicating the number of examined mice are shown (left panel), and KT5823-induced effects are summarized (right graphs). Significant KT5823-induced shifts are marked with asterisks (\*\*p<0.01 in *t*-test). The data are presented as the means ± SEM. with *n* values indicating the number of examined mice.

## Activated BK channels contribute to CNP-facilitated Ca<sup>2+</sup> fluctuations

Spontaneous Ca<sup>2+</sup> fluctuations are facilitated by activated BK channels in growth plate chondrocytes [11]. Previous studies have established a functional link between PKG and BK channels in several cell types including smooth muscle and endothelial cells; activated PKG enhances BK channel gating by directly phosphorylating the  $\alpha$  subunit KCNMA1 protein [13-15]. I thus examined whether altered BK channel activity is associated with CNP-facilitated Ca<sup>2+</sup> fluctuations. The BK channel inhibitor paxilline (10  $\mu$ M) exerted no obvious effects on basal Ca<sup>2+</sup> fluctuations in non-treated chondrocytes. However, the same paxilline treatments remarkably inhibited CNP-facilitated  $Ca^{2+}$  fluctuations (Figure 5A); both fluctuation-positive cell ratio and fluctuation amplitude were clearly decreased after paxilline application. On the other hand, the BK channel activator NS1619 (30 µM) stimulated basal Ca<sup>2+</sup> fluctuations in the growth plate chondrocytes prepared from control mice. The NS1619-induced effects were preserved in the mutant chondrocytes prepared from chondrocyte-specific Npr2-knockout mice (Figure 5B). Therefore, BK channel activation is likely involved in CNP-facilitated  $Ca^{2+}$  fluctuations in growth plate chondrocytes.

Figure 5



Contribution of BK channels to CNP-facilitated Ca<sup>2+</sup> fluctuations.

(A) Attenuation of CNP-facilitated Ca<sup>2+</sup> fluctuations by the BK channel inhibitor paxilline in round chondrocytes. Wild-type bone slices were pretreated with or without CNP, and then subjected to Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panels), and paxilline-induced effects are summarized (right graphs). Significant paxilline-induced shifts are marked with asterisks (\*p<0.05 and \*\*p<0.01 in one-way ANOVA and Tukey's test). The data are presented as the means ± SEM. with *n* values indicating the number of examined mice. (B) Ca<sup>2+</sup> fluctuations facilitated by the BK channel activator NS1619 in *Npr2*-deficient chondrocytes. Bone slices were prepared from the chondrocyte-specific *Npr2*-knockout and control embryos, and NS1619-induced effects were examined in Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panels), and the effects of NS1619 are summarized (right graphs). Significant NS1619-induced shifts are marked with asterisks (\*\*p <0.01 in one-way ANOVA and Tukey's test). The data are presented as the means ± SEM. With *n* values indicating the number of examined in Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panels), and the effects of NS1619 are summarized (right graphs). Significant NS1619-induced shifts are marked with asterisks (\*\*p <0.01 in one-way ANOVA and Tukey's test). The data are presented as the means ± SEM. with *n* values indicating the number of examined mice.

## PLC seems unrelated to CNP-facilitated Ca<sup>2+</sup> fluctuations

 $Ca^{2+}$  fluctuations are maintained by phosphatidylinositol (PI) turnover in growth plate chondrocytes [11]. Although it has been reported that activated PKG inhibits phospholipase C (PLC) in smooth muscle [16-19], it might be possible that NPR2 activation enhances basal PLC activity to facilitate  $Ca^{2+}$  fluctuations. The PLC inhibitor U73122 (10  $\mu$ M) remarkably inhibited basal  $Ca^{2+}$ fluctuations in non-treated chondrocytes: the fluctuation-positive cell ratio and fluctuation amplitude reduced less than half in response to U73122 application (Figure 6). U73122 was also effective for CNP-facilitated  $Ca^{2+}$  fluctuations, but the inhibitory efficiency seemed relatively weak compared to those on basal fluctuations. Given the different inhibitory effects, it is rather unlikely that PLC activation accompanies CNP-facilitated  $Ca^{2+}$  fluctuations.

PKG stimulates sarco/endoplasmic reticulum  $Ca^{2+}$ -ATPase (SERCA) by phosphorylating the  $Ca^{2+}$  pump regulatory peptide phospholamban (PLN) in smooth and cardiac muscle cells [20-22], and activated  $Ca^{2+}$  pumps generally elevate stored  $Ca^{2+}$  content and thus stimulate store  $Ca^{2+}$  release. RT-PCR data suggested that the *Pln* gene and the *Atp2a2* gene encoding SERCA2 are weakly active in growth plate chondrocytes (Figure 3). To examine the effects of CNP treatments on  $Ca^{2+}$  stores, I examined  $Ca^{2+}$  responses to the activation of Gq-coupled lysophosphatidic acid (LPA) receptors (Figure 7A) and the  $Ca^{2+}$  pump inhibitor thapsigargin (Figure 7B). CNP- and vehicle-pretreated chondrocytes exhibited similar LPA-induced  $Ca^{2+}$  release and thapsigargin-induced  $Ca^{2+}$  leak responses. Therefore, CNP treatments seem ineffective for store  $Ca^{2+}$  pumps in growth plate chondrocytes. Moreover, the dose-dependency of  $Ca^{2+}$  release by LPA (1~10  $\mu$ M) was not altered between CNP- and vehicle-pretreated chondrocytes, implying that CNP does not affect basal PLC activity.

Among diverse Ca<sup>2+</sup> handling-related proteins, PLC, PLN and BK channels have been reported as PKG substrates, however, my observations suggested that both PLC and PLN receive no obvious functional regulation in CNP-treated chondrocytes. On the other hand, the paxilline treatments diminished CNP-facilitated Ca<sup>2+</sup> fluctuations down to non-treated control levels (Figure 5A), suggesting that activated BK channels predominantly contribute to CNP-facilitated Ca<sup>2+</sup> fluctuations in growth plate chondrocytes.

### Figure 6



Effects of PLC inhibitor U73122 on CNP-facilitated Ca<sup>2+</sup> fluctuations.

In Ca<sup>2+</sup> imaging, U73122 was bath-applied to wild-type round chondrocytes pretreated with or without CNP. Representative recording traces are shown (left panels), and the effects of U73122 are summarized (right bar-graphs). Data represent means  $\pm$  SEM, and the numbers of cells and mice examined are shown in parentheses in the keys and graph bars, respectively. Significant differences between before and after the U73122 treatment are marked with asterisks (\*p<0.05 and \*\*p<0.01 in one-way ANOVA and Tukey's test).





(A) Store  $Ca^{2+}$  release triggered by 1-oleoyl lysophosphatidic acid (LPA) in wild-type round chondrocytes pretreated with or without CNP. Representative recording traces are shown (left panels), and LPA-evoked  $Ca^{2+}$ responses are summarized (right graphs). Data represent means  $\pm$  SEM, and the numbers of cells and mice examined are shown in parentheses in the keys and graph bars, respectively. No significant differences were observed between CNP- and vehicle-pretreated groups (one-way ANOVA and Tukey's test). (B)  $Ca^{2+}$  leak responses evoked by the SERCA pump inhibitor thapsigargin (TG) in wild-type round chondrocytes pretreated with or without CNP. Representative recording traces are shown (left panels), and TG-evoked  $Ca^{2+}$ responses are summarized (right bar-graphs). Data represent means  $\pm$  SEM, and the numbers of cells and mice examined are shown in parentheses in the keys and graph bars, respectively. No significant differences were observed between CNP- and vehicle-pretreated groups (t-test).

### **CNP induces BK channel-mediated hyperpolarization**

To confirm the contribution of activated BK channels to CNP-facilitated Ca<sup>2+</sup> fluctuations, I conducted confocal imaging using the voltage-dependent dye oxonol VI. In this imaging analysis, depolarization results in the accumulation of the dye into cells, in which the fractional fluorescence intensity, normalized to the maximum intensity monitored in the bath solution containing 100 mM KCl, is thus increased (Figure 8A left panel). The fractional intensity of CNP-pretreated cells was significantly lower than that of non-treated cells in a normal bath solution (Figure 8A middle graph), although both cells exhibited similar intensity shifts in high K<sup>+</sup> bath solutions. Based on the recording data, I prepared a calibration plot for the relationship between the fractional intensity and theoretical membrane potential (Figure 8A right panel). In the tentative linear correlation, resting potentials of  $-46.4 \pm 0.2$  and  $-43.6 \pm 0.3$  mV were estimated in CNP-treated and non-treated cells, respectively. The estimated potentials closely approximate the reported value from monitoring articular chondrocytes using sharp microelectrodes [23].

In pharmacological assessments, paxilline elevated fractional intensities to the same levels in CNP-and non-treated chondrocytes (Figure 8B). Moreover, NS1619 decreased fractional intensities to the same levels in both cells under 20 mM KCl bathing conditions, which enabled us to reliably

evaluate the reducing intensity shifts (Figure 8C). The oxonol VI imaging data suggested that CNP treatments induce BK channel-mediated hyperpolarization and thus facilitate spontaneous  $Ca^{2+}$  fluctuations by enhancing  $Ca^{2+}$ -driving forces in growth plate chondrocytes.

#### Figure 8



BK channel-mediated hyperpolarization induced by CNP.

(A) Oxonol VI imaging of round chondrocytes pretreated with or without CNP. Wild-type bone slices were pretreated with or without CNP, and then subjected to membrane potential imaging. During contiguous treatments with high-K<sup>+</sup> solutions, cellular fluorescence intensities were monitored and normalized to the maximum value in the 100 mM KCl-containing solution to yield the fractional intensity (left panel). The resting fractional intensities were quantified and statistically analyzed in CNP- and vehicle-pretreated cells (middle graph). For preparing the calibration plot (right panel), the data from ten cells in bathing solutions containing 4 (normal solution), 20, 40, 60 and 100 mM KCl are summarized; red and black lines indicate the estimated resting membrane potentials of CNP- and vehicle-pretreated cells, respectively. (B) Effects of the BK channel inhibitor paxilline on resting membrane potential in round chondrocytes. Recording data from ten cells pretreated with or without CNP were averaged (left panel), and the fractional intensities elevated by paxilline are summarized (right graph). (C) Effects of the BK channel activator NS1619 on membrane potential in round chondrocytes. Recording data from ten cells pretreated with or without CNP were averaged (left panel), and the fractional intensities in normal, 20 mM KCl and NS1619-containing 20 mM KCl solutions are summarized (right graph). Significant differences between CNP- and vehicle-pretreated cells are indicated by asterisks in A (\*\*p<0.01 in t-test) and in C (\*\*p<0.01 in one-way ANOVA and Dunn's test). The data are presented as the means  $\pm$  SEM. with *n* values indicating the number of examined mice.

## CNP enhances TRPM7-mediated Ca<sup>2+</sup> entry and CaMKII activity

Spontaneous Ca<sup>2+</sup> fluctuations are predominantly attributed to the intermissive gating of cell-surface TRPM7 channels in growth plate chondrocytes [11]. For pharmacological characterization of TRPM7 channels, FTY720 is used as a typical inhibitor, while NNC550396 is an activator. As reasonably expected, bath application of FTY720 (10  $\mu$ M) clearly diminished CNP-facilitated Ca<sup>2+</sup> fluctuations in round chondrocytes (Figure 9A). On the other hand, NNC550396 (30  $\mu$ M) remarkably facilitated Ca<sup>2+</sup> fluctuation in non-treated chondrocytes, and this facilitation was preserved in the mutant chondrocytes prepared from chondrocyte-specific *Npr2*-knockout mice (Figure 9B). Therefore, CNP treatments likely facilitate TRPM7-mediated Ca<sup>2+</sup> influx in growth plate chondrocytes.

TRPM7-mediated Ca<sup>2+</sup> entry activates CaMKII in growth plate chondrocytes toward bone outgrowth [11], and cellular CaMKII activity can be estimated by immunochemically quantifying its autophosphorylated form. In immunocytochemical analysis, CNP-pretreated growth plate chondrocytes were more decorated with the antibody against phospho-CaMKII than non-treated control cells (Figure 10A). This CNP-facilitated decoration was abolished by the cotreatment of the CaMKII inhibitor KN93 (30 μM). This observation was further confirmed by Western blot analysis; CNP treatments increased the phospho-CaMKII population without affecting total CaMKII content in the cell lysates prepared from growth plates (Figure 10B). Therefore, CaMKII is likely activated downstream of enhanced TRPM7-mediated Ca<sup>2+</sup> entry in CNP-treated growth plate chondrocytes.



#### Figure 9

## Enhanced TRPM7-mediated Ca<sup>2+</sup> entry by CNP treatments.

(A) Inhibition of CNP-facilitated Ca<sup>2+</sup> fluctuations by the TRPM7 inhibitor FTY720 in round chondrocytes. Wild-type bone slices were pretreated with CNP, and then subjected to Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panel), and the effects of FTY720 are summarized (right graphs). Significant FTY720-induced shifts are marked with asterisks (\*\*p<0.01 in *t*-test). The data are presented as the means ± SEM. with *n* values indicating the number of examined mice. (B) Ca<sup>2+</sup> fluctuations facilitated by the TRPM7 channel activator NNC550396 in *Npr2*-deficient round chondrocytes. Bone slices were prepared from the chondrocyte-specific *Npr2*-knockout and control embryos, and NNC550396-induced effects were examined in Ca<sup>2+</sup> imaging. Representative recording traces are shown (left panels) and the effects of NNC550396 on Ca<sup>2+</sup> fluctuations are summarized (right graphs). Significant NNC550396-induced shifts in each genotype are marked with asterisks (\*\*p <0.01 in one-way ANOVA and Tukey's test). The data are presented as the means ± SEM. with *n* values indicating the number of examined mice.

#### Figure 10



#### CaMKII activation in CNP-treated round chondrocytes.

(A) Immunohistochemical staining against phospho-CaMKII (p-CaMKII) in round chondrocytes. Wild-type bone slices were pretreated with or without CNP and the CaMKII inhibitor KN93, and then subjected to immunostaining with antibody to p-CaMKII. DAPI (4', 6-diamidino-2- phenylindole) was used for nuclear staining. Lower panels show high-magnification views of white-dotted regions in upper panels (scale bars, 10  $\mu$ m). (B) Immunoblot analysis of total CaMKII and p-CaMKII in growth plate cartilage. Growth plate lysates were prepared from wild-type bone slices pretreated with or without CNP, and subjected to immunoblot analysis with antibodies against total CaMKII and p-CaMKII (upper panel). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also analyzed as a loading control. The immunoreactivities observed were densitometrically quantified and are summarized (lower graph). A significant difference between CNP- and vehicle-pretreatments is marked with an asterisk (\*p<0.05 in one-way ANOVA and Tukey's test). The data are presented as the means  $\pm$  SEM. with *n* values indicating the number of examined mice.

## Pharmacologically activated BK channels facilitate bone outgrowth

Based on the present data from in vitro experiments, the novel CNP-signaling route, represented as the NPR2-PKG-BK channel-TRPM7 channel-CaMKII axis, can be proposed in growth plate chondrocytes. I attempted to examine the proposed signaling axis in metatarsal bone culture, a widely used ex vivo model system for analyzing bone growth and endochondral ossification [24]. In chondrocyte-specific Trpm7-knockout mice (Trpm7<sup>fl/fl</sup>, 11Enh-Cre<sup>+/-</sup>), Cre recombinase is expressed under the control of the collagen type XI gene enhancer and promoter, and thus inactivates the floxed *Trpm7* alleles in cartilage cells [11]. The bone rudiments prepared from control embryos (*Trpm7*<sup>fl/fl</sup>, 11Enh-Cre<sup>-/-</sup>) regularly elongated during ex vivo culture, and their outgrowth was significantly stimulated by the supplementation with CNP (30 nM) into the culture medium (Figure 11A). In contrast, the mutant rudiments prepared from the chondrocyte-specific Trpm7-knockout embryos were reduced in initial size and did not respond to the CNP supplementation. Therefore, CNP-facilitated bone outgrowth seems to require TRPM7 channels expressed in growth plate chondrocytes.

In my proposed signaling axis, activated BK channels exert an essential role by converting the chemical signal into the electrical signal. I finally examined the effect of the BK channel activator NS1619 on bone outgrowth (Figure 11B). NS1619 supplementation ( $30 \mu$ M) significantly stimulated

the outgrowth of wild-type bone rudiments. In contrast, under the same culture conditions, no stimulation was detected in the mutant rudiments from the chondrocyte-specific *Trpm7*-deficient embryos. The observations in the bone culture support my conclusion that CNP activates BK channels and thus facilitates TRPM7-mediated  $Ca^{2+}$  influx in growth plate chondrocytes, stimulating bone growth.

## Figure 11



#### Contribution of TRPM7 and BK channels to CNP-facilitated bone outgrowth.

(A) Loss of CNP-facilitated outgrowth in *Trpm7*-deficient bones. Metatarsal rudiments isolated from the chondrocyte-specific *Trpm7*-knockout (*Trpm7*<sup>fl/fl</sup>, *11Enh-Cre*<sup>+/-</sup>) and control (*Trpm7*<sup>fl/fl</sup>, *11Enh-Cre*<sup>-/-</sup>) embryos were precultured in normal medium for 6 days, and then cultured in medium supplemented with or without CNP for 3 days. Representative images of cultured metatarsals are shown (left panels; scale bar, 0.3 mm), and longitudinal bone outgrowth during the CNP-supplemented period was statistically analyzed in each genotype group (right graphs). Significant CNP-supplemented effects are marked with asterisks (\**p*<0.05 in *t*-test). The data are presented as the means  $\pm$  SEM. with *n* values indicating the number of examined mice. (**B**) Stimulated bone outgrowth by the BK channel activator NS1619. Metatarsal rudiments isolated from wild-type and the chondrocyte-specific *Trpm7*-knockout embryos were precultured in normal medium for 5 days, and then cultured in medium supplemented with or without NS1619 for 4 days. Representative images of cultured metatarsals are shown (left panels; scale bar, 0.3 mm), and Instituted metatarsals are shown (left panels; scale bar, 0.3 mm), and statistically analyzed in each genotype group (right graphs). A significant S1619-supplemented period was statistically analyzed in each genotype group (right graphs). A significant NS1619-supplemented effect is marked with asterisks (\**p*<0.05 in *t*-test). The data are presented as the means  $\pm$  SEM. with *n* values indicating the number of examined metaarsals are shown (left panels; scale bar, 0.3 mm), and longitudinal bone outgrowth during the NS1619-supplemented period was statistically analyzed in each genotype group (right graphs). A significant NS1619-supplemented effect is marked with asterisks (\**p*<0.05 in *t*-test). The data are presented as the means  $\pm$  SEM. with *n* values indicating the number of examined mice.

## **Discussion**

I reported that in growth plate chondrocytes, PLC and BK channels maintain autonomic TRPM7-mediated Ca<sup>2+</sup> fluctuations, which potentiate chondrogenesis and bone growth by activating CaMKII [11]. Based on the present data, together with the previous reports, I proposed a new CNP signaling axis in growth plate chondrocytes (Figure 12A). CNP-induced NPR2 activation elevates cellular cGMP content and thus activates PKG, leading to the phosphorylation of BK channels. The resulting BK channel activation likely induces cellular hyperpolarization to facilitate TRPM7-mediated  $Ca^{2+}$  entry by enhancing the  $Ca^{2+}$  driving force, leading to CaMKII activation. Therefore, it is likely that CaMKII activity is physiologically regulated by BK channels as a key player of the CNP signaling cascade. In a recent genetic study, several patients carrying loss-of-function mutations in the KCNMA1 gene encoding BK channel a subunit were characterized by a novel syndromic growth deficiency associated with severe developmental delay, cardiac malformation, bone dysplasia and dysmorphic features [25]. In the KCNMA1-mutated disorder, CNP signaling likely fails to facilitate TRPM7-mediated Ca<sup>2+</sup> fluctuations in growth plate chondrocytes and resulting insufficient Ca<sup>2+</sup> entry may lead to systemic bone dysplasia associated with stunted growth plate cartilage. On the other hand, the origin of CNP may still be ambiguous in the signaling scheme. Transgenic mice overexpressing CNP in a chondrocyte-specific manner develop a prominent skeletal overgrowth phenotype, suggesting autocrine CNP signaling [26]. However, several genechip data in public databases indicate that prepro-CNP mRNA is abundantly expressed in the placenta among embryonic tissues (for example, see the records under accession number GSE28277 in NCBI database). Therefore, it may be important to further examine which cell type primarily produces CNP to facilitate bone growth during embryonic development.

From a physiological point of view, it is interesting to note that the proposed CNP signaling axis has clear overlap with the nitric oxide (NO) and ANP/BNP signaling cascades for vascular relaxation [27-29]. In blood vessels, NO is produced by endothelial cells in response to various stimuli including shear stress and acetylcholine, and activates soluble guanylate cyclase in neighboring vascular smooth muscle cells. ANP and BNP are released from the heart in response to pathological stresses, such as atrial distension and pressure overload, and are delivered to activate the receptor guanylate cyclase NPR1 in vascular muscle. In either case, the resulting cGMP elevation followed by PKG activation induces BK channel-mediated hyperpolarization and thus inhibits L-type Ca<sup>2+</sup> channel gating, leading to vascular dilation due to decreased Ca<sup>2+</sup> entry into vascular muscle. Therefore, activated BK channels inhibit the voltage-dependent Ca<sup>2+</sup> influx in vascular muscle cells regarded as excitable cells (Figure 12B). In contrast, activated BK channels reversely stimulate TRPM7-mediated  $Ca^{2+}$  entry in growth plate chondrocytes classified as nonexcitable cells, because the channel activity is voltage-independently maintained by the intrinsic PI turnover rate.

CNP is an effective therapeutic reagent for achondroplasia and divergent short statures [26, 30, 31], and vosoritide, a stable analog of CNP has recently been approved for the treatment of achondroplasia [32]. The proteins contributing to the CNP signaling axis may be new pharmaceutical targets for developing medications; in addition to NPR2, BK and TRPM7 channels are reasonably considered promising targets. Moreover, phosphodiesterase subtypes might be useful targets, although the subtypes responsible for cGMP hydrolysis remain to be identified in growth plate chondrocytes. Chemical compounds specifically targeting the signaling axis defined in this study would be useful drugs for not only clinical treatment of developmental disorders but also artificially modifying body sizes in farm and pet animals.

### Figure 12

Α



#### Growth plate chondrocyte

Vascular dilation



(A) The schematic diagram representing the NPR2-PKG-BK channel-TRPM7 channel-CaMKII axis proposed as an essential CNP signaling cascade in growth plate chondrocytes. Previous studies proposed that the RAF-MEK-ERK axis is also involved in growth plate CNP signaling [8]. (B) The schematic diagram representing the NO- and ANP/BNP-induced relaxation signaling in vascular smooth muscle.

## **Materials and Methods**

#### **Reagents**, primers and mice

Reagents and antibodies used in this study are listed in Table 1. Synthetic primers used for RT-PCR analysis and mouse genotyping are listed in Table 2. C57BL mice were used as wild-type mice in this study. Chondrocyte-specific *Trpm7*-knockout mice with C57BL genetic background were generated and genotyped as previously described [11]. Chondrocyte-specific *Npr2*-knockout mice with C57BL background were generated as previously described [12], and I newly designed primers for detecting the *Col2a1-Cre* transgene and the floxed *Npr2* gene in this study (Figure 2). All experiments in this study were conducted with the approval of the Animal Research Committee according to the regulations on animal experimentation at Kyoto University.

## **Bone slice preparations**

Femoral bones were isolated from E17.5 mice and immersed in a physiological salt solution (PSS): (in mM) 150 NaCl, 4 KCl, 1 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 5.6 glucose, and 5 HEPES (pH 7.4). Longitudinal bone slices (~40 μm thickness) were prepared using a vibrating microslicer (DTK-1000N, Dosaka EM Co., Japan) as previously described [11].

## Ca<sup>2+</sup> imaging

Fura-2 Ca<sup>2+</sup> imaging of bone slices was performed as previously described [11]. Briefly, bone slices placed on glass-bottom dishes (Matsunami, Japan) were incubated in PSS containing 15 µM Fura-2 AM for 1 hr at 37°C. For ratiometric imaging, excitation light of 340 and 380 nm was alternately delivered, and emission light of >510 nm was detected by a cooled EM-CCD camera (Model C9100-13; Hamamatsu Photonics, Japan) mounted on an upright fluorescence microscope (DM6 FS, Leica, Germany) using a 40x water-immersion objective (HCX APO L, Leica). In typical measurements, ~30 round chondrocytes were randomly examined in each slice preparation to select the  $Ca^{2+}$  fluctuation-positive cells generating spontaneous events (>0.025 in Fura-2 ratio) using commercial software (Leica Application Suite X), and recording traces from the positive cells were then analyzed using Fiji/ImageJ software (US. NIH) for examining Ca<sup>2+</sup> fluctuation amplitude and frequency. Imaging experiments were performed at room temperature (23-25 °C) and PSS was used as the normal bathing solution. For the pretreatments of CNP, ANP and 8-pCPT-cGMP, bone slices were immersed in PSS with the indicated compound for 1 hr at room temperature after Fura-2 loading.

### Membrane potential monitoring

Bone slices were perfused with the PSS containing 200 nM oxonol VI at room temperature and analyzed as previously described [33]. To prepare the calibration plot showing the relationship between the fluorescence intensity and membrane potential, saline solutions containing 20 mM, 40 mM, 60 mM or 100 mM KCl were used as bathing solutions. Fluorescence images with excitation at 559 nm and emission at >606 nm were captured at a sampling rate of ~7.0 s using a confocal laser scanning microscope (FV1000; Olympus).

### Immunochemical analysis of CaMKII

Bone slices were pretreated with or without CNP were subjected to immunochemical assessments as previously described [34]. Briefly, for immunohistochemical analysis, bone slices were fixed in 4% paraformaldehyde and treated with 1% hyaluronidase to enhance immunodetection [35, 36]. After blocking with fetal bovine serum-containing solution, bone slices were reacted with primary and Alexa 488-conjugated secondary antibodies and observed with a confocal microscope (FV1000; Olympus). For immunoblot analysis, bone slices were lysed in the buffer containing 4% sodium deoxycholate, 20 mM Tris-HCl (pH 8.8) and a phosphatase inhibitor cocktail (100 mM NaF, 10 mM Na<sub>3</sub>PO<sub>4</sub>, 1 mM Na<sub>2</sub>VO<sub>3</sub> and 20 mM  $\beta$ -glycerophosphate). The resulting lysate proteins were electrophoresed on SDS-polyacrylamide gels and electroblotted onto nylon membranes for

immunodetection using primary and HRP-conjugated secondary antibodies. Antigen proteins were visualized using a chemiluminescence reagent and image analyzer (Amersham Imager 600, Cytiva). The immunoreactivities yielded were quantitatively analyzed by means of Fiji/ImageJ software.

## Metatarsal organ culture

Metatarsal bone rudiments were cultured as previously described [24]. Briefly, the three central metatarsal rudiments were dissected from E15.5 mice and cultured in  $\alpha$ MEM containing 5 µg/ml ascorbic acid, 1 mM β-glycerophosphate pentahydrate, 100 units/ml penicillin, 100 µg/ml streptomycin and 0.2% bovine serum albumin (fatty acid free). The explants were analyzed under a photomicroscope (BZ-X710, Keyence, Japan) for size measurements using Fiji/ImageJ software.

## Gene expression analysis

Quantitative RT-PCR analysis was performed as previously described [37]. Total RNA was prepared from mouse tissues using a commercial reagent (Isogen) and reverse-transcribed using a commercial kit (ReverTra ACE qPCR-RT kit). The resulting cDNAs were examined by real-time PCR (LightCycler 480 II, Roche), and the cycle threshold was determined from the amplification curve as an index for relative mRNA content in each reaction.

## Quantification and statistical analysis

All data obtained are presented as the means  $\pm$  SEM. with *n* values indicating the number of examined mice. Student *t*-test and ANOVA were used for two-group and multiple group comparisons, respectively (Prism 7, GraphPad Software Inc.): *p*<0.05 was considered to be statistically significant.

Reagent/Resource	Source	Identifier	
Antibodies			
Anti-phospho-CaMKII (Thr 286)	Cell Signaling Technology	Cat#12716; RRID: AB_2713889	
Anti-CaMKII	Abcam	Cat#EP1829Y; RRID: AB_868641	
Anti-GAPDH	Sigma-Aldrich	Cat#G9545; RRID: AB_796208	
Anti-rabbit IgG-HRP	Santa Cruz	Cat#sc-2357; RRID; AB_628497	
Anti-rabbit Alexa Flour 488	Invitrogen	Cat#A-11008; RRID: AB_143165	
Chemicals			
Amersham ECL Prime Western Blotting Detection	Cytiva	Cat#RPN2232	
ANP (Human, 1-28)	Peptide Institute	Cat#4135	
CNP-22 (Human)	Peptide Institute	Cat#4229	
FTY720	Sigma-Aldrich	SML0700; CAS: 162359-56-0	
Fura-2AM	Dojindo	F025; CAS: 108964-32-5	
Hyaluronidase from sheep testes	Sigma-Aldrich	H2126; CAS: 37326-33-3	
ISOGEN	NipponGene	Cat#319-90211	
KN93	Wako	115-00641; CAS: 139298-40-1	
КТ5823	Cayman Chemical	10010965; CAS: 126643-37-6	
NNC 550396 dihydrochloride	Tocris Bioscience	2268; CAS: 357400-13-6	
NS1619	Sigma-Aldrich	N170; CAS: 153587-01-0	
1-oleoyl lysophosphatidic acid	Cayman Chemical	62215; CAS: 325465-93-8	
Oxonol VI	Sigma-Aldrich	75926; CAS: 64724-75-0	
Paxilline	Tocris Bioscience	2006; CAS: 57186-25-1	
8-pCPT-cGMP	Biolog	C009; CAS: 51239-26-0	
ReverTra Ace® qPCR RT Master Mix with gDNA	ТОУОВО	Cat#FSQ-301	
Thapsigargin	Nacalai Tesque	33637-31; CAS: 67526-95-8	
U73122	Sigma-Aldrich	U6756; CAS: 112648-68-7	

## Table 1. Chemical reagents for pharmacological analysis

NpT       Rev TATCAAATGCCTCACGCTIGGA       Np2       Rev CCTGGTACCCCCCTCTITA         Np3       For GGTATGCCGACTTCTCTIGG       FIFor       GTAACCTGGGTAGCATGTTIGG         DelFor       TGTTATTTGTGAGATGACG       Rev       ATGGTGGAGGAGGCTTTTGTGG         Cot2a1-Cr       Rev CATGGTGGAGAAATGATGCG       Prkg1       For ATGGCATCAGGAGAAATGATGCG         Prkg2       For TGCGGAGAGAAATGATGCG       Kcmm1       For AGGCCGGAGAAATGAGAAATGATGCG         Kcmmb1       Rev CACTGTGCTGCCCCCTCTA       Kcmm2       For TCACGAGAGACACCAAACGCCAAAGAC         Kcmmb3       Rev GCACTTGGGGGGTGGTCGCCG       Kcmm4       For CTCAGAGACACCCCAACGCCCAAAG         Kcmn1       Rev CGCACTGGGGGTGGTCGTCGA       Kcmn2       For CACACGCCGAAATGCCCAAAGCCCCAAGAC         Kcmn3       For CCCAAGTGCGGAAGGAGAA       Kcm12       For GCACAGGACCCAAGAGACACTG         Picb1       For CCCAAGTGCGGAAGGAACACTA       Picb2       For AACCCCAACACACTGAAGC         Picb3       For CAGGCACCACACACAGACACTA       Picb1       For CAGGCCACACACACACACAA         Rev GGTGGGAGGCGCACACACACACACA       Picb2       For AACCCCTACACACACACGGGAGGA         Rev GGAAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	New	For AACAAGGAGAACAGCAGCAAC		For GGCCCCATCCCTGATGAAC
Npr3       For Galance Galance Control of C	Npr1	Dr1 Rev TATCAAATGCCTCAGCCTGGA Npr2	Npr2	Rev CCTGGTACCCCCTTCCTGTA
Null       Rev TCIGGICTCATCTAGTGICA       PH/00         DelFer       TGTATTITIGTGACATGACG       Rev       ATGGTGGAGAGAGGACTTTAATTCC         Col2at-Cre       For CGTTGGGAAGTTGGICG       Prkg1       For ATGGACTCTGGGCGTCTA         Rev CATTGGGGAAGAMAATGATGCG       Kenmat       For ATGGACTCGAGGAGGCTA         Rev GATGGGGCAGGCTGAGGAGGA       Kenmat       Rev TCTGGGGACACCAAACTTC         Rev GATGGGGCGGGGGGGGGGGGCGACTT       Kenmat       For TCAGGAGACAACCACAACTCTC         Rev GGCCTGGGGGTGGGCCTGGA       Kenmat       For CTCGGAGACCAAAGCCCAAGGC         Kennth3       For GTGGATGAGGGGCGGGAGACT       Kennat       For CTCGGAGACCAAAGCCCAAGGC         Kennth3       For GTGGAGAGGGGCGGGGAGGA       Kennat       For CTCGGCAGCCCAAGGC         Kennth3       For ACCCCAAGAGGGGATGGAGAA       Kennat       For GCCCCCAGGAGA         Kennth3       For GTGAGGCAGGCGGGAGAACTTC       Picb2       For ACCCCAAGAGGGGATGGAGAACTTC         Picb1       For CAGGCCCAAGCAGAAGACTC       Picb2       For AACCCCAAGGAGGGGGGGGGAAGACTT         Picb2       For AACCCCAAGCAGAAGACTC       Picb2       Rev GGGAGGGGGGGGAAGACTA         Rev GGAGGGGGGGGGAAGCATA       Picb2       Rev AGGAGGGGGGGGGAAGCCAGAGGCGCGGGGGAGAGCCCAGGGGGG	Ninr2	For GGTATGGGGACTTCTCTGTG	ElEor	GTAACCTGGGTAGACTAGTTGTTGG
Defer       TGTTATTTGTGAGATGAGG       Rev       ATGGTGGAGGAGGAGTGTTTATCC         Collat-Cre       For GGTGTGAGTGGATGGATGGT       Prkg1       For ATGGCGTTTTTTGTGGGAGCT         Fig2       For GTGTGGAGGAGGAGAA       For ATGGCATTTTTTTGTGGGAGCTA         Rev GATGGGGAGGAGAAATGATGTGG       Kenmal       For ATGGACTTTTTTTGTGGGAGCTA         Rev GAATGGGAGGAGGAGGAGAA       Kenmal       For ATGGACATCTAGGGGGCTG         Kenmb1       For GTGGAGGAGGGCTGGACTT       Kenmb2       For TCAGAGAGACCCAAACCAA         Kenn1       For TGGGAGTGAGGGGCTGGACTT       Kenmb1       For CCCAAGTGGGTGTGCAAC       Kenmb1         Rev CACTGTGGCTGCGAAC       Kenn2       For GAGCCCCAAGGAGACGTGTGTGC       Kenn3       For ACTCCAAAGCCCCGATCGTC         Rev TGCTGACACCCCGATGGTGGAGAA       Ken14       For ACACTCCAAGGTGAAACCTC       Picb1       For CCCAAGTTGGAGAGAA         Picb1       For CAGCCCAAGCCAAGAGAATA       Picb2       For AAGCCATTGAGGAGGAG       Picb1         Pic CAGCCCAAGCCAAGAGAAGA       Picg1       For AAGCCATTGCGGGAGA       Picb1         For CACCCAAGCCCAACCAGAGACATA       Picg2       For AAGCCATTGCAGGAGGAGAG       Picb2       For AAGCCATTGCAGGAGGAGGAGGAGGTGT         Picg2       For CACCCCAAGCCACACGAGAGCACTATCC       Picg2       For AAGCCCAAGCCAAGCGAGGAGGA	Npr3	Rev TCTGGTCTCATCTAGTCTCA	FIFO	
Colzat-Cer       For CSTIGTIGAGTIGGATAGTTG       Prkg1       For ATGGACTTTTTGGAGACTC         Prkg2       For TIGCGGAAGAAAATGATGTGG       Kenmat       For ATGCACTTCGAGGAGGCTA         Rev CATTGCTGCTGCCCCTCTA       Kenmbt       For TAGCACTGTGGCGCCCCTA       Rev CATGTGGAGTTGCAGGAGAA         Kenmb1       For TGCAAGCGGGGTGGACTT       Kenmbt       For TGCAAGCGGAGTGGACTGAC       Kenmbt         Rev CACTGTGGGTTGCGCCTGA       Kenmbt       For GGATGAGAAGACCCAACAGCACCAAGACCCCCAAGA         Rev TGCTTCAACAGCGGATTGGTC       Kennt       For GGATGGACGCAAGACCCCAAGAAGACCTGA         Rev GGAAGGACGTGATGGGAAACTC       Picb1       For CACGCCAAGCCGAAGGACTG       For ACCCCCTACAGAACACCCCT         Picb1       For CACGCAAGCCCCAAGGAGACTC       Picb2       For AACCCCTAAGCCGAATGCTC         Picb3       For CACGACCCCAAGCGAAGGACTC       Picb1       For CACGACCCCCAACGAGAGCT         Rev AGATGCTGGGCAACTAATC       Picg1       For AACCCCTAAGGAGGAGGAGTGATGGAGGA         Rev AGATGCTGGGGCAACCCAAGGAGACT       TpmT       For AACCCCTAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGT         Rev ATAGCTGACCCAACAGGAGAGCT       TpmT       For AACCGCAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	DelFor	TGTTATTTTGTGAGATGACG	Rev	ATGGTGGAGGAGGTCTTTAATTCC
Construction       Rev CATTGCTGCACTTGGTCGT       Pring?       Rev GGTTTCCGGAGAGAAATGCTGG         Pring2       For TGCGGAGAGAAATGCTGG       Kcmma1       For ACGCGTGCGGACGTGAGCAGAA         Kcmmb1       For ACGCGTGGGTTGGTCGCCCCTGTA       Kcmmb2       For CAGGGCGGACACTTCC         Kcmmb1       For GCGACTGGGGTTGGTCCCCG       Kcmmb2       For CCCGGGTGGACCACCCAAGTG         Kcmmb3       For GCGACTGGGGTTGGTCCCGC       Kcmmb4       For GCACCTGGCAAGC         Kcmn1       For TCCAAAATGCTGCTGCAAAC       Kcmn2       For GCACCTGGCGAACC         Kcmn3       For CCAAGGTGAAACCCCGTTGC       Kcmn4       For GCACCTGCGGAACCTG         Rev GGAAGGGAACGCGAAGAGGAATA       Picb1       For CCAAGGTGAAAACCTC       Picb2         For ACTCCAACACCCGATCGGCAACAATC       Picg1       For ACATCCAAGGGAAGGGAGA         Picb2       For ACACCCAAGCGAAGGACATA       Picg1       For ACACCCAAGCGAAGGGAGT         Picg2       For ACACCCAAGCAACAATC       Picg1       For ACACCCAAGCTGGAAGGGGTGGAGT         Picg2       For ACACCCCAAGCGAAGGACATC       Picg1       For ACACCCCAAGCGAAGGGAGGAGT         Picg2       For ACACCCCAAGCGAAGGACAGT       Picg3       For ACACCCCAAGCCGAAGGAGGAGT         Picg2       For ACACACACACAGAGGAGGAGT       Picg4       For AC	Collant Cro	For CGTTGTGAGTTGGATAGTTG	Drka1	For ATGGACTTTTTGTGGGACTC
Prkg2       For TIESCGGAMGAAAATGATGTGG       Kommat       For ANTECACTTCGAGGAGGTA         Rev GAATGGGAGGTTGAGGAGAA       For ACAACTGTGGTGCCCCCTCIA       Kommbt       For TCAGGAGACAAACCAACACTTC         Rev CCACGGGTGAATTCCAAA       For ACAACTGTGGCTTGACCCCCTCIA       Kommbt       For TCAGGAGACACAACCAACCACACCTC         Kanmb3       For GTGGTGGTGGCGCTGAAC       Kommbt       For CTCAGAAAGCGCAACGAC       Kommbt         Kann1       For TCAAAATGCTGCTGGCAACC       Konmbt       For GACTGGACACACCAGAA       For GACTGGCAACACAGAG         Kann3       For ACCAACCCGGATGGTGC       Konmbt       For GACTGCAACGACACGTG       For GACCCTACAGAACACACGTG         Picb1       For CAGGCAAGCAGAACGTGATGGAGA       Kcnn1       For GACCCTACAGAACACACGTG       For AACCCCACACTTGGTGGAACTTC         Picb2       For CAGGCCAGCCAACAGAGACATA       Picg1       For AACCCCACACGAGAGAGACTA       Picg1       For AACCCCACACGCAGAGAGAGA         Picg2       For AACCCCACCCACCAGAGAGAGCATA       Picg1       For AACCCACACTTGGCGCCTG       Tpm7       For AACCACACATGGAGGAGAGAGA         Rev GTAGCCACCACCACGAGAGAGGACG       Camk20       For AACGAAGAGCAACCCACCACGAGAGGAGGAGAGA       Fer AACTATCCTTGGGAGAGTGACGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	Colza1-Cre	Rev CATTGCTGTCACTTGGTCGT	Pikgi	Rev GGTTTTCATTGGATCTGGGC
Pring2       Rev GARTGGGGAGGTTGAGGAGAA       Primat       Rev CTCAGCCGGTAATTCCAAA         Kenmb1       For CACACTGTGGCTGCCCTCTA Rev CACTGTTGGTTTGATCCCG       Kenmb2       For TCAGAGCAGCAACAACAACAACAACAACAACAACAACAACA	Dukan	For TTGCGGAAGAAAATGATGTCG	Kenmal	For AATGCACTTCGAGGAGGCTA
Kenmb1       For ACAACTG TEGTTIGGTCCCG CTGTA       Kenmb2       For TCAGGAGACACAACACTTC         Rev CACTG TEGTTIGGTCCTGA       Kenmb4       For CTCCCTGACACACACCCAAGTG       Rev TAAATAGCAAGCCAACCCCAAGTG         Kenn1       For CTGATAGACGGGCTGGACTT       Kenmb4       For CTCCCTGACACCCAAGTGGTG       Rev TAAATAGCAACTGCCAGATGGGC         Kenn3       For CTCAAACACCCGAATCGTC       Kenn4       For GGCACCCCAAGACCGGAA       Rev GGGAAGCCCAAGACGGAA         Kenn3       For ACTCAAACACCGGTAAACTTC       Plcb2       For ACTCCAGGAGCGGAAAACCTC       Plcb2         Picb3       For CACCCAAGCGGAAAAACCTC       Plcg1       For ACTCCAGGAGCGAGAAGAGAGATA       Plcg1         Picb3       For CACCCACCCAACCCAAGAGAGATCA       Plcg1       For AACGCCTTGGGAGTGTCTTC       Plcg2         For AACGCCAACCCAACACGAGAGATCA       Plcg1       For AACGCCTTGGGAGTGTCTTC       Plcg1       For AACGCCAAGCAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	FINYZ	Rev GAATGGGGAGGTTGAGGAGAA	Kennar	Rev CTCAGCCGGTAAATTCCAAA
Rev ACTIGETTIGATECCG       Network       Rev ACTIGETTIGATECCATAGCAA         Kamb3       For GTGGGARACGGGGGGGACT       Kamb4       For GTGGGARACCGAACGGACTGAAC         Kann1       For GTGGACAACCCTAACCTGCAAAC       Kann1       For GTGGACAACCTGACACGGA         Rev GACTTCAACACCCGATTCGTC       Kann4       For GGCACTGGGAAACCTG       Rev GAGAAGGAACGTGAGAAACCTC         Picb1       For ACTCAACCCCAGATTCGTC       Kann4       For AGGCACCTTAACAACCGAGAAACCTC       Picb2       For ACTCCAGGAAACCTCAGAAAACCTC         Picb1       For CAAGCCAACCAACAAGAACATA       Picb2       For AAGCCCAACCCAACCAACAGAAACCTC       Picb2       For AAGCCCAACCCAACCAACAAGCT       Rev GGACCTTTAGGGATGAAGC         Picb3       For AACCCCAACCCAACCACACGAGACT       Picp1       For AAGCCCAACCCAACCACACGAGAC       Tym7       For ATTGCTTGCGGAAACCCCA         Rev AGGTTCAACCCCATGAGGACCAACG       Camk2b       For AAGCAACAGCAACACAAGCCCAACG       Rev CTCTCTAACTGCGGAAGCCAACGC       Rev CTCTCAACCACACGAGCCAACGC         Camk2d       For ATTTTGCACACTTCCTCG       Pde3a       For AACCACACCACACAGAGCCAACG       Rev GTGTGGAGACTGCAACGCAACGCAACGC         Pde2a       For ATTTTGCACACTTCCTCG       Pde3a       For AACCACACACGACACCACGAGCACCACGA       Rev GTTGTGCGGAATGCCACTC         Pde6a       For AACCAACCACACGCACCACG       Pde5a       Fo	Konmh1	For ACAACTGTGCTGCCCCTCTA	Kenmh2	For TCAGGAGACACCAACACTTC
Kennb3       For GTGGATGACCGGCTGGACTT       Kennb4       For CTCGTACCACCCCAATG         Rev GACATTGGGGTTGGTCGGAAAC       Kenn1       For TCAAAAATGCTGCTGCTGAAAC       Kenn2       For GATCTGGACAGCCGATGGC         Kenn3       For CTCAACACCCGATTCGTC       Kenn4       For GATCTGACACCCGATTGGTC       For GACCTCACAGCGGATGGAGCAT         Picb1       For CCCAAGGTGAAACCTC       Picb2       For ACATCACAGGGAAAACCTC       Picb2         For ACGCCAAGCGGAAAACCTC       Picb2       For ACGCCAGCGACACAGGAGCATCA       Picg1       For ACGCCCAGCCCAGCAGAGAGAGCATC         Picb3       For CACGCAAGCGGAACAAGAGCATC       Picg1       For AACGCCTTGGGAGTGTGGAGCT         Picb3       For CACGCAACCGAGCACAAGAGGCAGC       Trpm7       Rev GATGTGCGGAAGTGGAGGAG         Picg2       For AAGCCACCACTGATGAGGC       Camk2a       For ATGCTAGAGGCTGTCATCC       Rev GATGTCGGGAACTCC         Rev GATGTGAAGCCTACAGCCCCAT       Camk2a       For ATGCAAGACACAAGCCAAGG       Camk2a       For ATGCAAGCACAGTTCCCCCAT         Pde2a       For ATGCAAGACACATGCCCCAT       Pde3a       For AACTATACCAGCGGACCACGG       Rev GTAGGAGAGCGAGCACCCCCCCCCCCCCCCCCCCCCCCC	Kennor	Rev CACTGTTGGTTTTGATCCCG	Kenningz	Rev AGTTAGTTTCACCATAGCAA
Number       Rev GCACTTGGGGTTGGTCGTGA       Number       Rev TAAATAGCAAATGGATGGC         Kenn1       For TCAAAATAGCGAATGCATGGC       Kenn2       For GGCACCTCGCAGACCAGATG         Rev GGAAGGACGTGGTGGGACTCT       Picb1       For CACGCCAGACGACGATGGAC       Kenn4       For GGCACCTCGCAGACACAGAGGA         Picb1       For CACGCCAGCACGAGAGCAT       Picb2       For AGCCCAGAGCGAGAGGAG       For AGCCCAGAGGAGAG         Picb3       For CACGCCACCACGAGGCA       Picb1       For AGCCCAGCCACCAGAGGAC       Picb1       For AGCGCAGCCACGAGGAG       Picb1         Picb3       For CAGGCCCACCACGAGGCA       Picb1       For AGCCCAGCCACCAGAGGAC       Picb1       For AGCGCAGCCACCACGAGGCA         Picg2       For AGCCCACCCACCAGAGGCAT       Picg1       For AGCGCTTAGGGCAGAG       Rev GTTCGTGGGAGAGGAG       Rev GTTCGTGGGAGAGGAGGAG       Rev GTTCGTGGGAGAGGAGC       Rev GTTCGTGCGGAGAGGCAGC         Camk2a       For AGTCGTGGAGAGGCAGTC       Pide3a       For ATTCCTAGCCCCAGCCCCAT       Rev GTTCGTGGGAGAGGAGTC       Pide3a         Por ATTCTGCAGAGCGTGACCACTG       Pide3a       For AGGGGAGCAGCCAGCCAGC       Pide3a       For CCCAGGCCAGCCCAGCT         Pide6a       For ATTCCAAGCAGGGTGTTC       Pide3a       For CCCAGGCCAGCCCAGCT       Pide3a       For CCCAGGCCAGCCCAGCT	Kenmb3	For GTGGATGACGGGCTGGACTT	Kcnmb4	For CTCCTGACCAACCCCAAGTG
Kenn1       For TCAMAAATGCTGCTGCAAAC       Kenn2       For GATCTGGCAAAGACCCAGAA         Rev TCGTTCAACATCCCTTGTC       Kenn3       For ACTTCAACAACCCGATTGGTC       Rev TGGTCACGAGACACACTG         Picb1       For CACCAAGCTGATGGAGA       Kenn4       For GGCACCCTCACAGACACACTG         Picb1       For CCCAAGCTGATGGAGAA       Picb2       For ACTCCAGGAGATGGTCAGAGACATA         Picb3       For CAGGCCAGCACAGAGACATA       Picg1       For ACCCCTTCGCCACTGCAGAGAGACATA         Picg2       For AACCCCAACCCACAGAGACATA       Picg1       For AACCCCTTGCGCACTCCACAGG         Picg2       For AACCCCACCCACCAGAGAGC       Camk2b       For AAGCCGAGAGAGAGGAGAG         Rev GTCAAAGCCTTCCCCCCG       Trpm7       For AAGCCGAGAGAGAGTCAAGCC         Rev GTTAAACAAAGCAAAGC       Camk2b       For AAGCCGAGAGAGAGTCACCC         Rev GTAAACAAAGCCACAGGG       Camk2g       For AATCTGCGGAACTCCCCCAT         Rev GTAAGCCACACTTCTCAGGCATC       Pde3a       For AACCTATGCTGGAGCACC         Rev GTATACCAAGCAGAGAGGTCATC       Pde5a       For AACCTACCAGCGAGAGCC         Rev GTATACCAAGCCAGAGAGGCATC       Pde5a       For CACCTACCAGTGCCAGCAGACACC         Pde6a       For ATTCCCAAGCCAGAGGCACC       Pde5a       For CATCGGGACACCCAGCAGACACC         Rev GTAAGCAAACCATCAGCGAGAGGCACCCG		Rev GCACTTGGGGTTGGTCCTGA	11011104	Rev TAAAATAGCAAGTGAATGGC
Rev TCGTTCACCTTCCCTTGTTC       Nume       Rev GAAGTCCCTTGCTCCTGCT         Kcnn3       For ACTTCAACACCCGATTCGTC       Kcnn4       For GGCAACCCAACGTG         Picb1       For ACTTCAAGACCCAATTCGTC       Picb2       For ACTCCAGGAAGTGGTCAAGT         Picb3       For CAGGCCAACGAGACAGATA       Picg1       For AACTCCATGCAGAGGGAG       Rev CTTTCCCGCAGGGGAG         Picb3       For ACGCCAACCAACCACCACGAGGTC       Picg1       For AACCCCTTTGAGGACTGAGG       Rev CATGTTTCCCGCAGGGG         Picg2       For AACGCCAACCAACCACACGAGGGC       Trpm7       For AAGCCATTCCCGGGAGGTC       Rev GGTGAAACGCAACG         Camk2a       For CACCACCACTTAGGACCAAG       Camk2b       For AAGCCATTCCCGGGAGGTTCC       Rev GTGTGAAGACCAAGCCAACG         Pde2a       For ATCTTCAAGGACGAGGCCAAC       Camk2g       For CACGACCCTTGCTGGGCAA         Pde3b       For ATCTCAAGGAAGGCCAACC       Pde3a       For AACTATACCTGCTGGAGCC         Pde3a       For AACCACCACCCGCGGACACC       Pde3a       For CCCTGACGTGAACTCC       Pde3a         Pde6a       For TACTCAAGGAAGGCAACC       Pde5a       For CCCCCAGGGACCAC       Pde5a       For CCCCCCAGGCTGAACACCG         Pde6a       For AAGCGTTGAAGCCAGCGAGGTATCC       Pde5a       For TCCGGGCCATTCAACTGC       Pde6b       For CCCCAGGGACATTCCCCAG	Kenn1	For TCAAAAATGCTGCTGCAAAC	Kcnn2	For GATCTGGCAAAGACCCAGAA
Kenn3       For ACTTCAACACCCGATTCGTC       Kenn4       For GGCACCTCACGACGACACGTG         Picb1       For CCCAAGTTGCGTGAACTTCT       Picb2       For ACATCCAGGACAGTGGTCAGACTT         Picb3       For CCGCACGCACGACAGACTA       Picb2       For ACATCCAGGAGAGTGGGAGA         Picb3       For ACGCCAGCACGACAGACATA       Picg1       For ACGCCTTGAGGACTGGGAA         Picg2       For AACCCCAACCCACACGAGGTC       Trpm7       For ATGCTGGGAGAGGGAGGA         Rev AGGTTCAACGCCCCCTG       Trpm7       For AAGCAGAGAGGAGAG       Camk2b         For GATAACACACAAGCCACCATC       Camk2b       For AAGCAGAGAGTCCAAGG       Rev GTGTGAAAGCCCACACGAGG         Camk2a       For ATTCTTGACCACTTCCCCAT       Camk2b       For AAGCAGAAGCCCACGGG       Rev GTAAGCCCCACTCAAGGCCCATC         Pde2a       For ATTCTTGACCACTTCTCCG       Pde3a       For ACTCTAAGCCACGTCGAGGAG       Rev CTATGCTGGTGCGCATC         Pde3b       For ATTCCAAAGCAGAGGCCATC       Pde5a       For GATACTTTGCTGGTGGCATC       Pde5a         Pde6a       For ATTCCAAGGAGAGTGTATG       Pde5a       For CCCCAGGAGAGACCCATCGAGGAGAGC       Rev TGTGTGCGGCATTTAACTGC         Pde6a       For ATTCCAAGCAGAGAGTGTATG       Pde5a       For CATCCACCGCGTGAGCACTG       Rev GAAGACAATTCCCGAGGGGAGAG         Pde6a <td< th=""><th></th><th>Rev TCGTTCACCTTCCCTTGTTC</th><th></th><th>Rev GAAGTCCCTTTGCTGCTGTC</th></td<>		Rev TCGTTCACCTTCCCTTGTTC		Rev GAAGTCCCTTTGCTGCTGTC
Rev GGAAAGGAACGTGATGGAGA       Name       Rev TTTCTCGCCTTGTTGAACT         Picb1       For CCCAAGTTGCGTGAAACCTTCT       Picb2       For ACATCCAGGAAGTGGTCAGG         Picb3       For CAGGCCAGCAAGAGGAGATA       Picg1       For AAGCCTTTGGGAGAGG         Picg2       For CAGGCCAGCAACGAGGAGTC       Pirm7       For AAGCGTTTGGGGAGG         Picg2       For CACCCCCATTGAGGACGAGG       Trpm7       For AAGCGTTTGGGGAGGGAGG         Camk2a       For CACCACCATTGAGGACGAAGG       Camk2b       For CACGACGAAGATCCCCGG         Rev GTTCTGAGGACGCAACGAAGGCCCCCAT       Camk2b       For CACGACGAGAGGCCATCC       Pde3a         Rev GTAGCCCTCAAAGCCCACCCAT       Camk2b       For AACCTTGCGGGAGCCC       Rev GTAGGCGCTTATGCGGGGAG         Pde2a       For ATCCTAAGGAGGCATC       Pde3a       For GACCCACCCGCCGCACACCA       Pde5a         Rev CTTGCTGGGAAGGCCACCC       Pde5a       For TCCGGGCCTTTATGCTGG       Rev GTAGGCCTTCAAGCCACCCCCCAT         Pde6a       For AACCCACCCCCCCCACGCGGCACCA       Pde5a       For CCCAGGAATTCCAAGCCACCCCCAT         Pde6a       For AACCCACCCCCCCTGCCCAGG       Pde5a       For GCCAGGAATTCCAAGCCACCCCGCCATCACCG         Pde6a       For AACCCACCCCCCCCACCCGCGCACCACCGGAGC       Pde6b       For CCCCAGAACCCAACCGAGCCACCCCCACCCCCACCCCGCATCACCG	Kcnn3	For ACTTCAACACCCGATTCGTC	Kcnn4	For GGCACCTCACAGACACACTG
Picb1       For CCCAAGTTGCGTGAAACTTCT       Picb2       For ACATCCAGGAAGATGGTGCAG         Picb3       For CAGGCCAGCAAGGAGATA       Picg1       For AACGCTTTGAGGACTGAAACCTC       Picg1       For AACGCTTGAGGACTGAAGG         Picg2       For AACCCCAACCCAACGAGGC       Trpm7       Rev GATTGTCGGGAAGTGGAGG       Rev ATGTTTCCGGGAAGTGAAGGC         Camk2a       For CACCACCATGAGGCAAGG       Camk2b       For AACCCGAAGCCAAGGCAAGG       Rev GATGTGGGAAGAGCAAGG         Camk2a       For GATAACACAAAGAAGCCAACG       Camk2b       For AACTAACACAAAGAGCCAACG       Rev GTGGGGAAGCGCAAGG         Pde2a       For ATTCTTGACCACTTCCTCC       Pde3a       For AACTAACGCCATTGGGGAGACT       Rev CTCTGACGGAAGCCACG         Pde3b       For ATCTTTGACCACTTCCTCCG       Pde3a       For AACCTAACCGCACTGACGCACG       Pde5a         Rev CTAAGCCACCACTGACGCAGCACAC       Pde5a       For AACCAAACGCATTCGAGAAAGGCATACGCACGCAGGACACC       Pde5a         Pde6ba       For AACCCAGGCAGGCACACC       Pde6b       For CCCAAGAAAGCAATAGCACAGG       Rev CTGCGGGGCATTCTGACTGG         Pde6ba       For AACCGAACCGCAGCAGCACC       Pde6ba       For CCCAGAAAGCATAACGCA       Rev CTGCTGCGGGCATTCGCACGG         Pde6ba       For AACCGAACCGCAGGCACC       Pde6bb       For CCCAGAAGAAGCAAGAGAA       Rev TCAAAGCCAAAGCGCATCGCACG	1.01110	Rev GGAAAGGAACGTGATGGAGA	i territ	Rev TTTCTCCGCCTTGTTGAACT
NoticRev GTTGCCAAGCTGAAAACCTCPiculRev CGCACCGACTCCTTTACTTCPicb3For CAGGCCAGCACAGAGACATA Rev AGGACTGCGCACACCAAATCPicg1For AACCCTTGACGCAGGAGA Rev CTCTCTCAATCTCTCGCAAGGPicg2For AACCCCAACCACAGGAGTC Rev ATGTTTCCACTGTGCCACCCACGAGGAGTrpm7For ATTGCTTAGGTGTGCCamk2aFor CACCACCATTGAGGACGAAG 	Picb1	For CCCAAGTTGCGTGAACTTCT	Plcb2	For ACATCCAGGAAGTGGTCCAG
Picb3   For CAGGCCAGCAGAGAGCATA Rev AGATGCTGGCAATCGAATC   Picg1   For AACGCTTGAGGATGGAGA Rev CTCTCATCTCGCAAGG     Picg2   For AACCCCAACCGACGAGAGC   Trpm7   For ATTGCTTAGTTTGGCGCTG Rev AATGTTTCACCTTGCCCTG     Camk2a   For CACCACCCATTGAGGACGAAG Rev GTTCAAAGGCTGCATTCC   Camk2b   For AAGGAGTGCAGCAGG Rev GTAAGCCTCAAAGCCCACG     Camk2d   For GATAAACAAAAACAAAAGCCAACG Rev GTAAGCCTCAAAGCCCACT   Camk2b   For AACTGTGCGGAGAGTCCAGG     Pde2a   For AATTCCAAAGCCTCTCTCG Rev CATAACCCACTTCAGCCATC   Pde3a   For AACTATACCTGCTGGGACTC Rev TGATGGAGAGCACGACAC     Pde3b   For ATTCCAAAGCCACAGCACAC   Pde5a   For ACCTTGCTGCGGACTCC Rev TGATGGAGGACAGGACAC     Pde6a   For AACCCACCCGCTGACCACTG Rev CTTCCTTCTTCTTGTGACGGA   Pde6b   For TCCGGGCTATCTAAGCACAGGACAC     Pde6b   For TGCTGAGGAGATGGTATG Rev CTTCCTCTCTTCTTGTGTGACGGA   Pde6b   For GCCGGGCTATCTAAGCACTGC Rev AGAAGCAATTCCTCAAGC     Pde6c   For TGCTCAGGGAATGGTTATG Rev CTCACCCCAACCCTGCACCG   Pde6b   For GCTGGGGGCTAACGCA Rev AGAACCCAACCTCAAGG     Pde6b   For ACCCCCACCCCCTGACCACTG   Pde6b   For GCTGGGGCAACTCAAGGA     Pde6b   For ACCCCAAGCCCACTGC   Pde6b   For GCTGGGGCAACTCAACGC     Pde40a   For AAGGGTGAGATTCGGTCAGC   Pde6b   For GCTGGGGCAACTCCAAGGA     Pde40a   For ACCTCCCCAAACCCTTCACCAGC   Pde6b   For GCTGGGGCGCAATTCCCCAAGCCACCG     Rev TGCTGGGGGTATTG		Rev GTTGCCAAGCTGAAAACCTC		Rev CGCACCGACTCCTTTACTTC
RevRevAGGATGCTGGGAATCCAAACCProfRevCarCCCAACCCTACTCTCCGCAAGGPlcg2For AACCCCAACCCACACGAGTCTrpm7For ATTGCTGGTAGTTTGGTGTCRevRevAGTTTCACCTTGCCCCTGTrpm7For ATTGCTGGGAGAGTGAGGGGCamk2aFor CACCACCATTGAGGACGAAGCamk2gFor CAAGACAGCAAGCCRevGTTACAAAGCCAACGCCamk2gFor CAAGACAGCAAGCCTATCCRevFor ATTCTTGACCACTTCTCTGPde3aFor AATAACCACAAAGCAGCAAGCPde2aFor ATTCTTGCAAAGCCAAGGCACCPde5aFor AACCCCACCGTGGCGACACCCRevFor AATCCCACCCCCCCGTGACCACTGPde5aFor CCCAAGGATACAGCAPde6aFor ATCCTTGCAGGAGACACCPde5aFor CCCAAGGAAAATCCCCACTGCPde6aFor TCTCTGCGGCGCTATCTGACCACTGPde6bFor CCCAAGGAATTCCCGGCCATPde6aFor TCTCTGCGGACCACTGPde6bFor CCCAAGGACTCGACAGCCPde6aFor TCTCCGGAGAATTGGTTATGPde6bFor CCCAAGGACTCGACAGTGPde6aFor CATCCCCCAACCCCTGCACCPde6hFor GCCAAGACTCGAAGGCPde6gFor CATCCGCCAAAGCCATCGCPde6hFor GCTTGGTGCTGACAGTGPde10aFor CATCCGCAAAGCCATCGGCAGLpar1For CCCAAGGATGATGGTGAGGGLpar2For AAGCGAGGTGGTATTCAGLpar3For CAACCGAGACCAGAGCRev TCCTCACGGAAGCAGGGAGTTCACGGRev GGTTGCTGGAAGACCAGAGCRev GGTGGTGATGCCAGAGGCLpar4For CACCAGAGAACAGAAACATLpar5For CAAACCGAGACCAGAGCRev TGGTGGCAGTTCACCGGCGTTAAtp2a1For CAACCCAGAGACCACAGGCRev TGAGGAGGCACTCACCGGCTTGAtp2a3For CCCCCGGAAGACCGGCAGAGCCGCAGAGCCG <th>Plcb3</th> <th>For CAGGCCAGCACAGAGACATA</th> <th>Plca1</th> <th>For AACGCTTTGAGGACTGGAGA</th>	Plcb3	For CAGGCCAGCACAGAGACATA	Plca1	For AACGCTTTGAGGACTGGAGA
Picg2       For AACCCCAACCCACCGAGTC Rev AATGTTTCACCTTGCCCCTG       Trpm7       For ATGCTTGCTTGGTTGTTC Rev GATTGCGGAGAGTGGAGTGCAGGTG Rev GGTTCAAAGGCGACGAAG         Camk2a       For CACCACCATTGAGGACGAAG Rev GGTTCAAAGGCTGCATTCC       Camk2b       For AAGCAGATGGAGTCAAGCC Rev TGCTGTCGAGAGCAAGGC Rev CTGATAACAAAGCCAACG         Camk2d       For AACCCACACGATTGCAGCCATTCC Rev GTAAACCAACAAGCCCACT       Pde3a       For AACTATACCTGCTCGGCGA Rev CTAACCCACTTCAGCCATT         Pde2a       For ATCTTTGACCACTTCTCG Rev CATAACCCACCTTCAGCCATC Rev CATAACCCACCTCAGCGAGACAC       Pde3a       For AACTATACCTGCTCGGACACC         Pde3b       For ATTCCAAAGCAGAGGTCATC Rev CTGTGGGAGTGACACC       Pde5a       For TGCGGGCTTATCAAAGCA Rev TGATGGAGGTGACACC         Pde6a       For AACCCACCCCGCTGACCACTG Rev CTCTTCCTTCTTGTTGTGACGA       Pde6b       For TCCGGGACTTCAAACTGC Rev ACAAAGCAAATTCCTCAAGC Rev CTCAAGGAGATTCGTACAGGT         Pde6b       For AACGCAGACACCACTGG Rev CTCCCCAAACCCATTGGACAC       Pde6b       For CCCCAAGACACTCAACGG Rev ACAAAGCCAAACTCGAAAGCAACT         Pde6g       For AACGGTGAGATTCGGTCAGC Rev TCATCCCCCAAACCCTTGCAC       Pde6b       For CATCCGCAAACCCACAGGCA Rev CTCCAGATGGTATTGCTGAC         Pde10a       For CCTCAGTGGTGTTTGTGAC       Lpar1       For AACGAGATGATGGGGA       For CCTCAGAGAGCAGCAGCAGC         Lpar4       For CCTCAGTGGTGTTTCTGAC       Lpar5       For AACACGAGACCTCACCA       For CACCCAGAAGACAAGAAACAT       Lpar5<	11000	Rev AGGATGCTGGCAATCAAATC		Rev CTCCTCAATCTCTCGCAAGG
Rev AATGTTTCACCTTGCCCCTGMmRev GATTGTCGGGAAGAGTGGAGTCamk2aFor CACCACCATTAGGGACGAAGCamk2bFor AAGCAGATGGAGTCAAGCCRev GGTTCAAAGGCTGTCATTCCCamk2bFor CACGACGAAGGCATCCAAGPde2aFor GATAAACAACAAGCCCCCTPde3aFor CACGACGCGCGCGAPde3bFor ATTTGACCACTTCTCGGCATCPde3aFor GACCCTTGCGGCAGTGCGAPde3bFor ATTCCAAGGCCAGCAGCACCACPde5aFor GACCCTGCGTGCGACAGTCCPde6aFor AACCAACCCGCGGCAGCAGACACPde5aFor GACCCTTGCGTGCGCATTGPde6aFor AACCAACCCGCGGACAGACACPde6bFor TGCGGCCTATCAAAGTGCPde6aFor TGCTCCGGGAATGGTATGPde6bFor CCCCAAGAAATCGCAGGACAGGACAGTPde6bFor TGCCCGGGAAATGGTTATGPde6bFor CCCCAAGAAATCCGAAGGAAGGACACPde6gFor ATCCCCCAAACCCTGCACCPde6hFor CCCCAAGACCCGAGAAAPde6gFor CATCCCCCAAAGCCCATGGCPde6hFor CATCCGCAAAGCCATCGACAGGAPde10aFor CATCCCCCAAAGCCATGGCACPde6hFor CCTTGGCGCTTATTGTCTPde10aFor CCTCAGTGGTGGTATTCGGCAGLpar3For ACTTTCCCAGCAGAGAACAGPar4For CCTCAGTGGTGGTATTCGGCAGLpar3For CCTCAGGAGAGCAGAACAGRev GCAGTTCCTCCCATCACTGTAtp2a3For CCTCGGTCGTTAGCACACAGPlnFor AACCCAGCTGCCGCATTPth/hFor CCTCCGCAAACCCGCAACCGRev GGAGGCGCCCCAACTGCCCACACARev GGTGGTAGTGGGGGGGAACCCGRev GGTGGTGGTGTGTGCAACAGGGAGCCCCGAATGGTGGGGGGAACCGGPlnFor CAACCCAGGGCAAAGGTCGCCACACAFor CCCCGGTAAAGGGGAACCGCRev GGAGGCGCCCAATTCCCCTTCCol2a1For CACCCGGAAACCGC <td< th=""><th>Plca2</th><th>For AACCCCAACCCACACGAGTC</th><th>Trpm7</th><th>For ATTGCTTAGTTTTGGTGTTC</th></td<>	Plca2	For AACCCCAACCCACACGAGTC	Trpm7	For ATTGCTTAGTTTTGGTGTTC
Camk2aFor CACCACCATTGAGGAGGAGGCamk2bFor AAGCAGATGGAGTCAAGCC Rev GCTGCGGAAGATTCCAGGCamk2dFor GATAAACAACAAAGCCAAAGCCAATCCRev GCTGCGGAACGCTATCCRev GTAAGCCATTCTAGCCATTRev CTCTGACTGGCGGAGTCAAGCCPde2aFor ATCTTTGACCACTTCAGCCATCPde3aFor GACTAACCTGCTGGCGGARev TCGTGCGGGCTTAGCTGGGGGPde3bFor ATCTTGCAAGGCAGGCCATCPde5aFor GACCTTGCTGCTCATTGRev TGGTGGGGCTTATGGTGGGPde6aFor AACCCACCCGCTGACCACTGPde5aFor GACCCTTGCTCATTGRev TGGTGGGGCTATCAAGCCPde6aFor AACCCACCCCGCTGACCACTGPde6bFor CCCGAGAGAACGCCACAGGARev TGCGGGCCTATCTAAACTGCPde6aFor TTGCTCAGGAAATGGTTATGPde6dFor CCCCAAGAAAATCCCCAGGGRev AGAAGCCAAATCCCCAGGGPde6gFor AAGCGTGAGACTGGTCAGCPde6hFor GGCAGACTCGACAGAGRev CTCCAGATGGCTGAACGCTPde6gFor CATCCCCAAAGCCATCATCGLpar1For GCCTGGGCGTATCAAGARev CTCCAGATGGTGAACGCTPde10aFor CATCCCCAAAGCCATCATCGLpar3For ACTTTCCACACCAGGGGGFor ACTTTCCACACCAGAGGCCAGAGCLpar4For CCTCAGTGGTGGTATTCAGLpar5For AACCAGCATCATCGTRev GTGGGAGAGACGAGAGCAGAGACAGAGAACATLpar6For CAACCAGAGAACAGAAAACATLpar5For CAACCAGGAGACCCGAGAGCRev GCAGTGCCCGCACACAGGGCAACAGAGAACATAtp2a1For CCTCCGGCAGAGCCRev GGGGGCATTCCCCCACACAGGGAAGACGCTTARev GGGGGGAGGCCCCACACAGAGAGCACAGAGAGACAG		Rev AATGTTTCACCTTGCCCCTG		Rev GATTGTCGGGAGAGTGGAGT
Rev GGTTCAAAGGCTGTCATTCC     Rev RGCTGTCGGAAGATTCCAGG       Camk2d     For GATAACAAAGGCCTGTCATTCC     Rev GTAAGCCTCAAGTCCCAT     Rev GTAAGCCTCAAGTCCCAT       Pde2a     For ATCTTTGACCACTTCTCTCG     Pde3a     For AACTATACCTGCTGGACCAC       Pde3b     For ATTCCAAAGGCAGGGTCATC     Pde3a     For GACCTTGCGTGGGGCTTTG       Pde6a     For AATCCCACCGCTGACCACTG     Pde3a     For GACCTTGCGTGCGGACTACC       Pde6a     For AACCCACCCGCTGACCACTG     Pde6b     For TCCGGGCCTATCTAAACTGC       Pde6a     For AACCCACCCGCTGACCACTG     Pde6b     For TCCGGGCCTATCTAAACTGC       Pde6a     For AACCCACCCGCTGACCACTG     Pde6b     For TCCGGGCCTATCTAAACTGC       Pde6b     For TGTCTCCAGGAAGCTGTACACG     Pde6b     For CCCAGAGACACTCTAAGCA       Pde6c     For TGTGCTCAGGAATCGTACGC     Pde6h     For GCCCAGAGCACTCAAGCA       Pde6g     For AAGGTGTGCTGGTATTGCTGAC     Pde6h     For GCTGGTGCCCTTTATGCT       Pde10a     For AGTGTGCTGGTATTGCTGAC     Pde6h     For ACTTTCCCAGAAGCACT     Rev GTGTGCGGCCTTTATGCT       Pde10a     For AGTGTGCTGGTATTCAGC     Lpar1     For AGTGGGCCCTTACACCGA     Rev TTGATGGAGACCTGGACAGC     Rev GTGTGCTCGCACAAGCCT       Lpar2     For AGTGTGCTGGTGTTTCAGC     Lpar3     For ACCCGAGCAGAA	Camk2a	For CACCACCATTGAGGACGAAG	Camk2b	For AAGCAGATGGAGTCAAGCC
Camk2dFor GATAAACAACAACAAGCCAACG Rev GTAAGCCTCAAAGTCCCCATCamk2gFor CAAGAACAAGCAAGCAAGCCATATCC Rev CCTCTGACTGACTGGTGGCGAPde2aFor ATCTTTGACCACTTCTCTCG Rev CATAACCCACTTCAGCCACTCPde3aFor AACTATACCTGCTGGGAGCTC Rev TTCGTGCGGGCTTTATGCTGGPde3bFor ATTCCAAAGCAGAGGCACCAGCAGCACPde5aFor GACCCTTGGGTGCTCAATG Rev TGATGGAGTGACAGTACAGCPde6aFor AACCCACCGGTGACCACTG Rev CTCTTCTTGTTGACGAPde6bFor CCCGGGCCTATCAAACTGC Rev AGAAGACAATTCCCCGGCCATPde6aFor TTGCTCAGGAATGGTTATG Rev GTAAGCGAGACTCGTACAGGTPde6dFor CCCAAGACACACTGGAAGAAPde6gFor TTGCTCAGGAATCGGTCAGC Rev CAACAGCCAACCCTGACAGCPde6dFor GCCAGACTCGAACGCTPde6gFor AACGCGCAAAGCCATCATCG Rev TCATCCCCCAAAGCCATCATCG Rev TCTCATCACCCCTAGCCCAGPde6hFor GCCTGGGCAGCTCAAGAGPde10aFor CATCCGCAAAGCCATCATCG Rev TTGCATGGTGGTGTATTCGGCAGLpar1For GCTTGGTGCCTTTATTGTCT Rev GGTAGGAGTGAGTGATGGGGGLpar2For ACTGTGGCGGTGGTGTTTTCAG Rev CACAGAAGAACAAGAAAAACATLpar5For ACACCGAGAGGCCAGAGCCLpar6For TACTTGCCCATCACGGTTT Rev GATGGCAGTGCAGCCTAtp2a1For CAAACAAGGGACCCTCACCA Rev GCAGGTAGGAGGCCAGAGCPlnFor TACCTCACTCGCTGGGCAT Rev TGATGGCCAGGCAATGGTGGAAGTCAtp2a3For CACCGGGTAAGCGGAAGACCG Rev GGTGGTCTGCTTGGAC Rev GGTGGTCCCGAAAACCGCCG Rev GGTGGTCCCGAAAACCGCCG Rev GGTGGTCCCCACAAAAACCAC Rev GGTGGGCCAACACCAAAACCACCA Rev GGTGGGCCAACACCAAAACCACCA Rev GGTGGGCCAACACCAAAACCACCA Rev GGTGGTCCCGCAAAATCGCTCACCAACACACACACACACA		Rev GGTTCAAAGGCTGTCATTCC		Rev TGCTGTCGGAAGATTCCAGG
Rev GTAAGCCTCAAAGTCCCCAT   Painted   Rev CCTCTGACTGACTGACTGACGGA     Pde2a   For ATTCTTTGACCACTTCTCG   Pde3a   For AACTAACCCACTGCGGACTC     Pde3b   For ATTCCAAAGCAGAGGTCATC   Pde5a   For AACCTAACCCGCTGCGGCACACAGCAC     Pde6a   For ATTCCAAAGCAGAGGTCATC   Pde5a   For GACCCTTGCGGCTTATTGCTGACGACGC     Pde6a   For AACCCACCCGCTGACCACTG   Pde5a   For TCCGGGGCTATCAAACTGC     Pde6a   For AACCCACCCGCTGACCACTG   Pde6b   For TCCCGGACACATACTGC     Pde6a   For AACCGACCGCTGACACAGGA   Pde6b   For CCCCAGAAATCCTGACAGGT     Pde6g   For AAGGGTGAGATTCGGTCACC   Pde6b   For GCAGACTCGACACGTGACACCT     Pde6g   For AACGGTGAGATTCGGTCACC   Pde6b   For GCCAGAAACGCACACTGACACTG     Pde10a   For CATCCCCCAAACCCATCG   Pde6b   For GCTGGTGGCAGCTTCACCCTTACTACAGGG     Pde10a   For AGTGTGCTGGTATTGCTGAC   Lpar1   For ACTTTCCCTTCATCACAGGGG     Lpar2   For AGTGTGGTGGTGTGTGATTTCGGAG   Lpar3   For ACTTTCCCACAGAGGCCAGGAGC     Lpar4   For TACTTGCCCCAGAAGAAACAT   Lpar3   For CACTGCCCAAAACGGGACCCCACACACACAAACCAC     Lpar6   For TACTTGCCCCCCATCACTGT   Atp2a1   For CACAGAGGCCAGGAACCCCACACACACACACACACACAC	Camk2d	For GATAAACAACAAAGCCAACG	Camk2a	For CAAGAACAGCAAGCCTATCC
Pde2a   For ATCTTTGACCACTTCTCTCG Rev CATAACCCACTTCAGCCATC   Pde3a   For AACTATACCTGCTCCGGACTC Rev TTGAGAGCCAGCACC     Pde3b   For ATTCCAAAGCAGAGGTCATC   Pde5a   For GACCTTGCGTGCTGCTCATTG     Pde6a   For AACCCACCGCGTGACCACTG Rev CTTTGCTTGTGTGACGACAC   Pde6b   For TCCGGGCTATCTAAACTGC Rev TGATGGAAACCGCACACGG Rev CTCTTCCTTCTTGTTGAGGA     Pde6a   For TTGCTCAGGAAATGGTTATG Rev GAAACAGGAACTCGTACAGGT   Pde6b   For CCCAGGGCAATTCCCGACGCAT     Pde6b   For TTGCTCGGGAAGCTCGTCAGG   Pde6b   For CCCAGAGACTCCGAGAGA     Pde6g   For AAGGGTGAGATTCGGTCAGC Rev TCATCCCCAAACCCTTGCAC   Pde6h   For GGCAGACTCGACAGATTCAGGAG Rev TCATCCCCCAAACCCTTGCAC     Pde10a   For ACTTCGGCAAAGCCATCATCG Rev TTGATGGAGAGCCTGGCAG   Lpar1   For GCTTGGTGCTTTATTGTT Rev GTTGGTGGTGTGTGTGCAC     Lpar2   For AGTGTGCTGGTATTGCTGAC   Lpar3   For AACCAGAAGCAATACC     Lpar4   For TACTTTGCCATTGCGGCATT   Lpar5   For CAACGAGAGCCAGAGCC     Lpar6   For TACTTGCCATTCGGGTATT CCCCAAGGCAGTCCGCGGCTAT   Atp2a3   For CCTCGGTGATGGGAACCCG Rev GCAGTGGAGACCCGGAACCCG Rev GACTGGCACTTCACTGGCTTT   For AACCCAACACGAGGCTGGGGAAGACCG Rev GGATGGTGGCATATGCACC   For CAACCGGGAGACCTCACCA Rev GGTGGTGCCAAAACCCAC Rev GGATGGTGGGCATATGCCTT   For CAACCGGAAGGCCGGGGAAGACCG Rev GGATGGTGGCCAACACCACA Rev GGTGGTGCCACATATCCCTT   For CACCCGGGAAGACCCCA Rev GGTGGTGGTGGGATGGGGGGGGGGGGGGGGGGGGGGGG		Rev GTAAGCCTCAAAGTCCCCAT		Rev CCTCTGACTGACTGGTGCGA
Rev CATAACCCACTTCAGCCATC   Poice   Rev TTCGTGCGCGCTTTATGCTGG     Pde3b   For ATTCCAAAGCAGAGGTCATC   Pde5a   For GACCCTTGCGTTGCTATG     Pde6a   For AACCCACCCGCTGACCACACG   Pde6b   For GACCCTTGCGTTGCTATG     Pde6a   For TTGCTCAGGAAATGGTTATG   Pde6b   For TCCCGGGCCTATCTAACTGC     Pde6c   For TTGCTCAGGAAATGGTTATG   Pde6d   For CCCAAGAAAATCCTCAAGTG     Pde6g   For AAGCGAGATCGTCAGCAGC   Pde6d   For CCCAAGAAAATCCTCAAGGA     Pde6g   For AAGGGTGAGATTCGGTCAGC   Pde6h   For GCTGGTGGCTGACAGAGTCAAGA     Pde10a   For AGTGTGCTGGAGATTCGGTCAGC   Pde6h   For GCTTGGTGCTGTTTATGTT     Rev TCTCATCACCCTCAGCCCAG   Pde6h   For ACTTTCCAGAGAGAGCCTGAGA     Pde10a   For ACTTGCGGAAAGCCATCATCG   Pde7h   For ACTTTCCATGCGAAGACCATCATCG     Lpar2   For AGTGTGGTGGTATTGCTGAC   Lpar1   For ACTTTCCAAGCAAGAACAT   For ACTTTCCAAGGAGACCAGAGACAT     Lpar4   For CCTCAGGAGAACAGAAACAT   Lpar5   For ACACCAGAGGACCAGAGA     Lpar6   For TACTTGCCCATCACTGGTATT   Atp2a1   For CCTCCGGAAACCGCAAGAC     Rev TGATGGGAGTGTCCGTGTGCA   Atp2a3   For CCTCGGCAAATCGCTCACCA     Rev GACTTCCTCCCCATCACTGGCTTT   Atp2a3   For CCTCCGCAAAACCGCACAC     Rev TGATGGGGGGGCAATATCCGTCGTGGCATT   Ptilh   For CACACTGGTAGGAGCCGCAGACG <t< th=""><th rowspan="3">Pde2a Pde3b</th><th>For ATCTTTGACCACTTCTCTCG</th><th>Pde3a</th><th>For AACTATACCTGCTCGGACTC</th></t<>	Pde2a Pde3b	For ATCTTTGACCACTTCTCTCG	Pde3a	For AACTATACCTGCTCGGACTC
Pde3bFor ATTCCAAAGCAGAGGTCATC Rev GTTAGAGAGCCAGCAGCACCPde5aFor GACCCTGCGTGCTCATTG Rev TGATGGAGTGACAGTACAGCPde6aFor AACCCACCGCTGACCACTG Rev CTCTTCTTTGTTGACGAPde6bFor TCCGGGCCTATCTAAACTGC Rev AGAAGACAATTCCCGGCCATPde6cFor TTGCTCAGGAAATGGTTATG Rev GAAACAGAACTCGTACAGGTPde6dFor CCCAAGAAAATCCTCAAGTG Rev ACAAAGCCAAACTCGAAGAAPde6gFor AGGGTGAGATTCGGTCAGC Rev TCATCCCCAAACCCTTGCACPde6hFor GGCAGACTCGACAGTTCAAGAPde6gFor CATCCGCAAACCCTTGCAC Rev TCTCATCACCCTCAGCCCAGPde6hFor GGCAGACTCGACAGTTCAAGAPde10aFor CATCCGCAAAGCCTTGCGC Rev TCTCATCACCCTCAGCCCAGLpar1For ACTTTCCCTTATTGTCT Rev GTAGGAGTAGATGAGGGLpar2For AGTGTGCTGGTATTGCTGAC Rev TTGATGGGAGAGACAGGAACAGGALpar3For ACTTTCCCACAGCACAGALpar4For CACCAGAGAGACAGAAAACATLpar5For AACCAGAGATCACAGALpar6For TACTTTGCCATTCCGCACTGT Rev GAACCAGAGAACCAGGAACCTGTAtp2a1For CCTCGGGAAACCGAAtp2a2For AAACCAGATGTCCGTGTGCA Rev TGATGGCAGTTCCTCCCCATCACTGGCTTA Rev TGATGGCAGTCCGGCTAT Rev TGATGGCAGTCCGGCTAT Rev TGACGGAGTGCTCGGCTATA Rev TGACGGAGTGCTCGGCTATA Rev TGACGGAGTGCTCGGCTATA Rev GACCAGGGAGCCCCGAACACCAC Rev GACTGCCTTCTTCTTCTTCFor CACACCAGAAACCAC Rev GCTGGCTATGGGGCAAGACCG Rev GGATGGTGTGGGCAAGACCG Rev GGATGGTGTGGTGAAGTGGGGCAAGACCG Rev GGATGGTGTGGTGGATTGGGGCAAGACCTGAC Rev GGAGCCACCGATCGCACACAC Rev GGAGGCCACCGATCCACACAC Rev GGAGGCCACCGATCCACACAC Rev TGGGGCCAAAATCGCTCCACCACAC Rev TGGAGCCACCGATCCACACAC Rev TGGCGTGGTGGAACCCGAGAGGCFor CACCCGGAACCCGAACCCCACACAC Rev ATGGAGCCACCGATCCACACACACACACACACACACACAC		Rev CATAACCCACTTCAGCCATC		Rev TTCGTGCGGCTTTATGCTGG
Rev GTTAGAGAGCCACCACTG Rev CTCTACCTCCTTCTTGTTGACCACTG Rev CTCTTCCTTCTTGTTGACCACTG Rev CTCTTCCTTCTTGTTGACCACACGPde6bRev TGATGGACACTGACACGCPde6aFor ACCCACCCGCTGACCACTG Rev CAGAAGACACGGAAATGGTTATG Rev GAAACAGAACACGGAAATGGTTATG Rev GAAACAGAACCCGAACGGTPde6bFor TCCGGGCCATCTCACGCGCATPde6gFor AAGGGTGAGATTCGGTCAGC Rev TCATCCCCAAACCCTTGCACPde6hFor GGCAGACTCGACAGGTTCAAGAPde10aFor CATCCGCAAACCCTTGCAC Rev TCATCCCCCAAACCCTCACGCCAGPde6hFor GGCTGGCGCTTTATTGTCT Rev GGTAGGAGTAGAGGGGGGGPde10aFor AGTGTGCTGGTATTGCTGAC Rev TTGATGGAGAGCCTGGCAGLpar1For GCTTGGTGCTTTATTGTCT Rev GTAGGAGTAGAGGGGGGLpar2For AGTGTGCTGGTATTTCAG Rev TTGATGGAGAGCCTGGCAGLpar3For AACCCAGAGAGCACAGAAACATLpar4For CACCAGAAGAACAAGAAACAT Rev CACAGAAGAACAAGAAACAATLpar5For AAACCAGGACCCTGACAG Rev AAGACCCAGAGACCCGAGAGCLpar6For TACTTTGCCATTTCGGATTT Rev TGATGGCACTTCACCGGCTAT Rev TGACGGACTTCACCGGCTAT Rev TGACGGACTTCACCGGCTAT Rev CACAGAAGAACACCGAtp2a3For CAAAACAGGAACCACA Rev CGTGGTACCCGAAATAGTGGGAPlinFor TACCTCACTGGCTGGCA Rev AGCTGGGCCAATACTCCTTPthlh Rev GCTTGCTTCTTCTTCTTCTFor CAACCCGGAATGGTGGGG18SFor AGACAAGGGACTCGCACCACAC Rev TGGCTGGCGCAATACTCCCACACAC Rev TTGCTGTGAAACCACGGAACCTCACACACACCACACACAC		For ATTCCAAAGCAGAGGTCATC	Pde5a	For GACCCTTGCGTTGCTCATTG
Pde6a   For AACCCACCCGCTGACCACTG Rev CTCTTCTTGTTGACGA   Pde6b   For TCCGGGCCATTCTAAACTGC Rev AGAAGACATTCCGGCCAT     Pde6c   For TTGCTCAGGAAATGGTTATG Rev GAAACAGAACTCGTACAGGT   Pde6d   For CCCAAGAAATCCTAAGGA Rev ACAAAGCCAAATCCGAAGAA     Pde6g   For AAGGGTGAGATTCGGTCAGC Rev TCATCCCCAAACCCTTGCAC   Pde6h   For GGCAGACTCGACAGTTCAAGA Rev CTCCAGATGGCTGACAGCT     Pde10a   For CATCCGCAAAGCCATCATCG Rev TCTCATCACCCTAGCCAG   Lpar1   For GCTTGGTGCCTTTATTGTCT Rev GGTAGGAGTGAGATGATGGGG     Lpar2   For AGTGTGCTGGTATTGCTGAC Rev TTGATGGAGAGACCAGCAGCAT   Lpar3   For ACTCTTCCCACAGCAATAACC     Lpar4   For CCTCAGTGGTGGTATTTCAG Rev CACAGAAGAACAAGAAACAT   Lpar5   For AACCCAGGAGACCCAGAGC     Lpar6   For TACTTTGCCATTTCGGATTT Rev GCACTTCTCCCCATCACTGT   Atp2a1   For CACAAACAGGGACCCCCAACAG Rev CACAGAAGACAAGGAAACAT   Rev GCACTGGTAGGAGACTCGT     Lpar6   For AAACCAGGTGTCCGGTGTGCA Rev GAACCAGGAGTGCCCGGTGTGCA Rev GAAGCAGAGTCTCACCGGCTTT   Atp2a1   For CCTCAGTGGAGACCCG Rev CGTGGTACCCGAAAGGACCCG Rev GGGTGACCCGAAAACCAC Rev CGTGGTACCCGAAAAACCAC   For CACACAGGGAGACCCG Rev GGGTACCCGAAAAAACCAC Rev GGGTGGCTACCGCAAAAACCAC Rev GGGTGGCTACCGCAAAAACCAC Rev GGATTGTGTTCTTCTTC   For CACACTGGTAAGTGGGGCAAGACCG Rev GGATTGTGTTGTTCAGGGGC     Bas   For AAACCAAGGGCAAGACCTCAC Rev TGAGGCAAATCGCTCCACCAAC Rev TGGAGCCAACCGGGAAACCTCAC   Actb   For CACCGGAGGCACCACAC Rev ATGGAGCCACCGATGCAACCTCAC     Bap   For TGTGTCCGTCGTGGAGAACCTCAC   Actb   For CACCCGTGGTAAGAGCCCTCACCAA Rev TGGAGCCACCGATGG		Rev GTTAGAGAGCCAGCAGACAC		Rev TGATGGAGTGACAGTACAGC
Rev CICITICCTICITICITIGITIGACGARev AGAAGACAATTICCCGGGCCATPde6cFor TTGCTCAGGAATGGTTATG Rev GAAACAGAACTCGTACAGGTPde6dFor CCCAAGAAAATCCTCAAGTG Rev ACAAAGCCAAACTCGAAGAAPde6gFor AAGGGTGAGATTCGGTCAGC Rev TCATCCCCCAAACCCTTGCACAPde6hFor GGCAGACTCGACAGTTCAAGAPde10aFor CATCCGCAAAGCCATCATCG Rev TCTCATCACCCTCAGCCAGLpar1For GCTTGGTGCCTTTATTGTCT Rev GTAGGAGATGATGGGGGLpar2For AGTGTGCTGGTATTGCTGAC Rev TTTGATGGAGAGCCTGGCAGLpar3For ACTTTCCCTTCACACGAGAGACAAGALpar4For CCTCAGTGGTGGTATTTCAG Rev CACAGAAGACAAGAACATLpar5For AACTTCACCAGAGGACCAGAGCLpar6For ACTTTGCCATTTCCGATTT Rev GCACTTCTCCCACAGAGAGACAAGAACATAtp2a1 Rev GCAGTGAGAGCCTGGCAGFor CACAAACAGGAACCCAAPlnFor AACCAGATGTCCGGTGTGCA Rev TGACGGAGTGCTCGGCATTA Rev TGATGGCACTTCACCTGGCTTTAAtp2a1 Rev GCAGTGTACTCGGTGTGAFor CACACCGAAACCAAAAACACPlnFor CACCCACAGAGGCCATGGCAGATAtp2a3 Rev GCTGCCTTCTCTTCTTCTTCTTCTFor CACACCGAAAACCAC Rev GCTGGTACCCGAAAACCACFor CACACCGGAAACCAC Rev GCTGGTACCCGAAAAACCACPlnFor CAAGCCAGGCTATGGAAGTC Rev TGACGGGCCAATATCTCCTTPthlhFor CACACTGGTAGGGGCAAGACCG Rev GGATTGTGTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT	Pde6a	For AACCCACCCGCTGACCACTG	Pde6b	For TCCGGGCCTATCTAAACTGC
Pde6cFor TIGCTCAGGAAATGGTTATG Rev GAAACAGAACTCGTACAGGTPde6dFor CCCAAGAAAATCCTCAAGTG Rev ACAAAGCCAAACTCGAAGAAPde6gFor AAGGGTGAGATTCGGTCAGC Rev TCATCCCCAAACCCTTGCACPde6hFor GGCAGACTCGACAGTCAAGGAPde10aFor CATCCGCAAAGCCATCATCG Rev TCCATCACCCTCAGCCAGGLpar1For GCTTGGTGCTCTTTTTGTT Rev GGTAGGAGTAGATGATGGGGLpar2For AGTGTGCTGGTATTGCTGAC Rev TTTGATGGAGAGACCAGAGCALpar3For ACTTTCCCTTCACACCGGLpar4For CCTCAGTGGTGGTATTTCAG Rev TTTGATGGAGAGACAGAACAACATLpar3For ACTTTCCCTTCACACCGGLpar4For CCTCAGTGGTGGTATTTCAG Rev CACAGAGAACACAAGAAACATLpar5For AACACGACTTCACAAGGLpar6For ACTTTGCCATTCCGACTTT Rev GCACTTCCTCCCATCACTGGTAtp2a1For CAAAACAGGGACCCTCACCA Rev GCAGTGATGGGGAACTCGTAtp2a2For TACTTTGCATTTCGGCATT Rev TGATGGCACTCACTGGTCGGCATTA Rev TGACGGAGTGCTCGGCTATT Rev TGACGGAGTGCTCGGCTAT Rev TGACGGGAGTGCTCGGCTATTAAtp2a3For CTCCCCAACACCAAAAACAC Rev GCTGGTACCCGAAATGGTGAPlnFor CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTTAtp2a3For CACCCGGAAAACCAC Rev GCTGCTTTCTTTTCTTCTTCTTCTTCTTCTTCTTCTTCTT		Rev CTCTTCCTTCTTGTTGACGA		Rev AGAAGACAATTTCCCCGGCCAT
Rev GAAGCAGAACTCGIACAGGTRev ACAAAGCCCAAAGCCCGAGAAPde6gFor AAGGGTGAGATTCGGTCAGC Rev TCATCCCCAAAGCCAACCTTGCACPde6hFor GGCAGACTCGACAGTTCAAGA Rev CTCCAGGTGGTGGTTATTGTCT Rev GTAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	Pde6c	For TTGCTCAGGAAATGGTTATG	Pde6d	For CCCAAGAAAATCCTCAAGTG
Pde6gFor AAGGGTGAGATTCGGTCAGCPde6hFor GGCAGACTCGACAGTTCAGACAGTPde10aFor CATCCCCAAAGCCATCATCGLpar1For GCTTGGTGGTGCTGAACGCTPde10aFor CATCCGCAAAGCCATCATCGLpar1For GCTTGGTGGCCTTATTGTCTRev TCTCATCACCCCCAGCCCAGLpar3For ACTTGCGCAGAGTACGGGGLpar2For AGTGTGCTGGTATTGCTGACLpar3For ACTTTCCACAGCAATAACCLpar4For CCTCAGTGGTGGTATTCGGTAGTTTCAGLpar5For AACACGACTTCTACCAACAGALpar6For TACTTTGCATTCGGATTTAtp2a1For CAAAACAGGGACCCTGACAGAAtp2a2For AACCCAGATGTCCGTGTGCA Rev TGATGGCACTTCACCACTGGCTTAAtp2a3For CCTCCGGTCATCGGTGAPlnFor TACCTCACTCGCTCGGCTAT Rev TGACGGAGTGCTGGCAAGAGTCPthlhFor CCTCCCAACACCAAAAACCAC Rev GCTGGTTCTTCTTCTTCTTCTTCCol10a1For CAAGCAAGCAGGCAGAGTC Rev AGACCAGGGAAACCTCACCA Rev CTCAACACGGGAAACCTCACCA Rev CTCAACACGGGAAACCTCACCAACA Rev CTCAACACGGGAAACCTCACCAACAACCAC Rev AGGTGGTCCGTGGCAAACCTCACCAACAAACCACCA Rev CTCAACACGGGAAACCTCACCAACAAACCACCACACACAAAACCACCACACACACAC				Rev ACAAAGCCAAACTCGAAGAA
Pde10aRev TCATCCCCAAACCCCTTGCACLpar1Rev CTCCAGATGCCTGAACGCTPde10aFor CATCCGCAAAGCCATCATCG Rev TCTCATCACCCTCAGCCCAGLpar1For GCTTGGTGCCTTTATTGTCT Rev GGTAGGAGTAGATGATGAGGGGLpar2For AGTGTGCTGGTATTGCTGAC Rev TTTGATGGAGAGCCTGGCAGLpar3For ACTTTCCCTTCACAGCAATAACCLpar4For CCTCAGTGGTGGTATTTCCG Rev CACAGAAGAACAAGAAACATLpar5For AACACGACTTCTACCAGCAAGAACAALpar6For TACTTTGCCATTTCGGATTT Rev GCACTTCCTCCCATCACTGTAtp2a1For CAAACAGGAACCAAGAACCAAAtp2a2For AAACCAGATGTCCGTGTGCA Rev TGATGGCACTTCACCGCTTACACTGGCTTAtp2a3For CCTCGGTCATCTGGACA Rev CGTGGTACCCGAAAAACCACPlnFor TACCTCACTCGCTCGGCTAT Rev TGACGGAGTGCTGGGCAATATCCCTTPth/hFor CCCCAACACCAAAAACCAC Rev GCTGCCTTCTTCTTCTCol10a1For AAACCAGGGCAATATCTCCTTCol2a1For CACACTGGTAAGTGGGGCAAGACCG Rev AGCTGGGCCAATATCCCCAACA Rev TGCAGGAGCCTCACCAAC Rev TGCAGGACCTCATCGGGGAAACCTCAC Rev TGCTGTGTGTTCAGGGGAAACCTCAC Rev AGCTGGGCCACCCACCAAAACCACC Rev AGCTGGGCCACCCACCAACA Rev TTGCTGTTCAGGGAACCCG Rev AGCTGGGCCACCCACCAACA Rev TTGCTGTTGTGTGCGTGGGAACCCCACAAAACCACC Rev AGCGGAGCCACCCACCAACACCAAC Rev AGCGGAGCCACCCACCAACACCAACACCACACACACACA	Pde6g	For AAGGGTGAGATTCGGTCAGC	Pde6h	For GGCAGACTCGACAGTTCAAGA
Pde10a   For CATCCGCGAAGCCATCATCG   Lpar1   For GCTTGGTGCCTTTATTGTCT     Rev TCTCATCACCCCTCAGCCCAG   Lpar3   For ACTTTCCCTTCATCACCTG     Lpar2   For AGTGTGCTGGTATTGCTGAC   Lpar3   For ACTTTCCCTTCACACGCAAGAACCC     Lpar4   For CCTCAGTGGTGGTATTTCAG   Lpar3   For ACCTTTCCCACAGGCAATAACC     Lpar4   For CCTCAGTGGTGGTATTTCAG   Lpar5   For AACACGACTTCACCAACAG     Lpar6   For TACTTTGCCATTTCGGATTT   Atp2a1   For CAAAACAGGGACCCTCACCA     Atp2a2   For AAACCAGATGTCCGTGTGCA   Atp2a3   For CCTCCGGTCATCGGCTAT     Pln   For TACCTCACTGGCTGGCATT   Pthlh   For CACACGACAGAGAACCAC     Pln   For CAAGCCAGGCTATGGAAGTC   Col2a1   For CACACTGGTAGGGCAAAGCCG     Rev AGCTGGGCCAATATCTCCTT   Col2a1   For CACACTGGTAAGTGGGGCAACCGA     Rev AGCTGGGCCAATACCCAAC   Actb   For CATCCGTAAGACCACAACAAGACCACACACACACACACAC	_			Rev CTCCAGATGGCTGAACGCT
Lpar2For AGTGTGCTGGTATTGCTGAC Rev TTGATGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	Pde10a		Lpar1	
Lpar2For AGTGGTGGTGGTGGTGGTGGTGGAGLpar3For ACTTTCCCTCCTACTACTACTG Rev GTCTTTCCACAGCAATAACCLpar4For CCTCAGTGGTGGTATTTCAG Rev CACAGAAGAACAAGAAACAATLpar5For AACACGACTTCTACCAACAG Rev AGACCCAGAGGGCCAGAGCLpar6For TACTTTGCCATTTCGGATTT Rev GCACTTCCTCCCATCACTGTAtp2a1For CAAAACAGGGACCCTCACCA Rev GCCAGTGATGGAGAACCGGTAtp2a2For AAACCAGATGTCCGTGTGCA Rev TGATGGCACTTCACTGGCTTAtp2a3For CCTCGGTCATCTGCTCTGAC Rev CGTGGTACCCGAAAACAGCGAA Rev CGTGGTACCCGAAAACCAC Rev GCTTGCCTTCTTCTTCTTCPlnFor TACCTCACTCGCTCGGCTAT Rev AGCTGGGCCAATGTCGGCCAATATCTCCTTPthlhFor CACACTGGTAAGTGGGGCAAGACCG Rev GGATTGTGTTGTTTCAGGGTCCGGGCol10a1For CAAGCCAGGCTATGGAAGTC Rev CTCAACACGGGAAACCGCCACAAATCGCTCACCA Rev CTCAACACGGGAAACCTCACCCol2a1For CATCCGTAAGTGGGGCAAGACCG Rev ATGGAGCCACCGATCCACAGapdhFor TGTGTCCGTCGTGGGATCTGA Rev TIGCTGTTGAAGTCGCAAGGCGAGGAGActbFor CATCCGTAAGACCACCAAA				
Lpar4For CCTCAGTGGTGGTATTTCAG Rev CACAGAAGAACAAGAACAATLpar5For AACACGACTTCTACCAACAG Rev AAGACCCAGAGGCCAGAGCLpar6For TACTTTGCCATTTCGGATTT Rev GCACTTCCTCCCATCACTGTAtp2a1For CAAAACAGGGACCCTCACCA Rev GCCAGTGATGGAGAACTCGTAtp2a2For AAACCAGATGTCCGTGTGCA Rev TGATGGCACTTCACTGGCTTAtp2a3For CCTCCGGTCATCTGCTCGAC Rev CGTGGTACCCGAAAAAACCAC Rev CGTGGTACCCGAAAAAACCAC Rev TGACGGAGTGCTCGGCTATA Rev TGACGGAGTGCTCGGCTATAGAAGTC Rev AGCTGGGCCAATATCTCCTTPthlhFor CCCCAACACAAAAACCAC Rev GCTGGTACCCGAAAAAACCAC Rev GGATTGTGTTGTTTCAGGGTCGGG18SFor AGACAAATCGCTCCACCAAC Rev TGGTGGTCCGTCGTGGAAACCTCACActbFor CATCCGTAAGACCCTACAC Rev ATGGAGCCACCGATCCACAGapdhFor TGTGTCCGTCGTGGATCTGA Rev TGCTGTTGAAGTCGCGCAAGACCGAGAGCFor CATCCGTAAGACCCACAA	Lpar2		Lpar3	
Lpar4For ConcentrationLpar5For AACACGACTIC TACCAACAG Rev AAGACCCAGAGAGCCAGAGCLpar6For TACTTTGCCATTTCGGATTT Rev GCACTTCCTCCCCATCACTGTAtp2a1For CAAAACAGGGACCCTCACCA Rev GCCAGTGATGGAGAACTCGTAtp2a2For AAACCAGATGTCCGTGTGCA Rev TGATGGCACTTCACTGGCTAT Rev TGACGGAGTGCTCGGCTAT Rev TGACGGAGTGCTCGGCTATA Rev AGCTGGGCCAATATCTCCTTAtp2a3For CCTCGGTCATCTGGTGAC Rev CGTGGTACCCGAAAACACAC Rev CGTGGTACCCGAAAAACCAC Rev GCTTGCCTTTCTTCTTCCol10a1For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTTCol2a1For CACACTGGTAAGTGGGGCAAGACCG Rev GGATTGTGTTGTTTCAGGGTTCGGG18SFor AGACAAATCGCTCCACCAAC Rev TGGTGGTCCGTCGTGGAAACCTCACActbFor CATCCGTAAGACCCCAAC Rev ATGGAGCCACCGATCCACAGapdhFor TGTGTCCGTCGTGGATCTGA Rev TGCTGTTGAAGTCGCGCAGGAGFor CATCCGTAAGACCCGATCCACA	-			
Lpar6     For TACTTTGCCATTTCGGATTT Rev GCACTGCCCATCACTGT     Atp2a1     For CAAAACAGGGACCCTCACCA Rev GCCAGTGATGGAGAACTCGT       Atp2a2     For AAACCAGATGTCCGTGTGCA Rev TGATGGCACTTCACTGGCTT     Atp2a3     For CCTCGGTCATCTGCTCGAC Rev CGTGGTACCCGAAATGGTGA       Pln     For TACCTCACTCGCTCGGCTAT Rev TGACGGAGTGCTCGGCTTTA     Pth/h     For CTCCCAACACCCAAAAACCAC Rev GCTTGCCTTTCTTCTTC       Col10a1     For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTT     Col2a1     For CACACTGGTAAGTGGGGCAAGACCG Rev GGATTGTGTTGTTTCAGGGTTCGGG       18S     For AGACAAATCGCTCCACCAAC Rev TGGTGTCCGTCGTGGATCTGA Rev TGGTGTCCGTCGTGGATCTGA Rev TGCTGTTGAAGTCGCAGGAG     Actb     For CATCCGTAAGACCCGATCCACA	Lpar4		Lpar5	
Lpar6   Foi TACTITISCEATTICEGRATIT   Atp2a1   Foi CAAAACAGGGACCCTCACGAC     Rev GCACTTCCTCCCATCACTGT   Atp2a1   Foi CAAAACAGGGACCCTCACCGT     Atp2a2   For AAACCAGATGTCCGTGTGCA Rev TGATGGCACTTCACTGGCTT   Atp2a3   For CCTCGGTCATCTGCTCGAC Rev CGTGGTACCCGAAATGGTGA     Pln   For TACCTCACTCGCTCGGCTAT Rev TGACGGAGTGCTCGGCTTTA   Pthlh   For CTCCCAACACCAAAAACCAC Rev GCTTGCCTTTCTTCTTC     Col10a1   For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTT   Col2a1   For CACACTGGTAAGTGGGGCAAGACCG Rev GGATTGTGTTGTTCAGGGTTCGGG     18S   For AGACAAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC   Actb   For CATCCGTAAGACCCGATCGAC Rev ATGGAGCCACCGATCCACA     Gapdh   For TGTGTCCGTCGTGGATCTGA Rev TTGCTGTTGAAGTCGCAGGAG   For CACCGTAAGACCCCCACAA				
Atp2a2     For AAACCAGATGTCCGTGTGCA Rev TGATGGCACTTCACTGGCTT     Atp2a3     For CCTCGGTCATCTGCTCGAC Rev CGTGGTACCCGAAATGGTGA       Pln     For TACCTCACTCGCTCGGCTAT Rev TGACGGAGTGCTCGGCTTTA     Pthlh     For CTCCCAACACCAAAAACCAC Rev GCTTGCCTTTCTTCTTC       Col10a1     For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTT     Col2a1     For CACACTGGTAGTGGGGCAAGACCCG Rev GGATTGTGTTGTTCAGGGTTCGGG       18S     For AGACAAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC     Actb     For CATCCGTAAAGACCTCATGCAAC Rev ATGGAGCCACCGATCCACA       Gapdh     For TGTGTCCGTCGTGGATCTGA Rev TTGCTGTTGAAGTCGCAGGAG     For CACACTGGAGCAACCTCAC     Actb	Lpar6		Atp2a1	
Atp2a2   Poi AAACCAGATGTCCGTGTGCA   Atp2a3   Poi CCTCGGTCATCTGCTCGCTCGAC     Rev TGATGGCACTTCACTGGCTT   Atp2a3   For CCTCGGTACCCGAAATGGTGA     Pin   For TACCTCACTCGCTCGGCTAT Rev TGACGGAGTGCTCGGCTTTA   Pth/h   For CTCCCAACACCAAAAACCAC Rev GCTTGCCTTTCTTCTTCTTC     Col10a1   For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTT   Col2a1   For CACACTGGTAAGTGGGGGCAAGACCG Rev GGATTGTGTTGTTCAGGGTTCGGG     18S   For AGACAAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC   Actb   For CATCCGTAAAGACCTCATGCCAAC Rev ATGGAGCCACCGATCCACA     Gapdh   For TGTGTCCGTCGTGGATCTGA Rev TTGCTGTTGAAGTCGCAGGAG   For CATCGGAGCCACCGATCCACA				
Pin   For TACCTCACTCGCTCGGCTAT Rev TGACGGAGTGCTCGGCTTTA   Pth/h   For CTCCCAACACCAAAAACCAC Rev GCTTGCCTTTCTTCTTCTTC     Col10a1   For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTT   Col2a1   For CACACTGGTAAGTGGGGGCAAGACCG Rev GGATTGTGTTGTTCAGGGTTCGGG     18S   For AGACAAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC   Actb   For CATCCGTAAGACCCTATGCCAAC Rev ATGGAGCCACCGATCCACA     Gapdh   For TGTGTCCGTCGTGGAGTCTGA Rev TTGCTGTTGAAGTCGCAGGAG   For CATCCGTAAGACCCCACAA	Atp2a2		Atp2a3	
Pin   For TGACGGAGTGCTCGGCTTTA   Pth/h   For CACACCGAAAAACCAC     Col10a1   For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTT   Col2a1   For CACACTGGTAAGTGGGGCAAGACCG Rev GGATTGTGTTGTTTCAGGGTTCGGG     18S   For AGACAAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC   Actb   For CATCCGTAAGACCCTATGCCAAC Rev ATGGAGCCACCGATCCACA     Gapdh   For TGTGTCCGTCGTGGAGTCTGA Rev TTGCTGTTGAAGTCGCAGGAG   For CATCCGTAAGGCCACCGATCCACA	Pin			
Col10a1   For CAAGCCAGGCTATGGAAGTC Rev AGCTGGGCCAATATCTCCTT   Col2a1   For CACACTGGTAAGTGGGGCAAGACCG Rev GGATTGTGTTGTTTCAGGGTTCGGG     18S   For AGACAAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC   Actb   For CATCCGTAAGACCCTCATGCCAAC Rev ATGGAGCCACCGATCCACA     Gapdh   For TGTGTCCGTCGTGGATCTGA Rev TTGCTGTTGAAGTCGCAGGAG   For CATCCGTAAGACCCCACAA			Pthlh	Rev GCTTGCCTTCTTCTTCTTC
Col10a1   For CACCAC TGG TAAG TGG GGC AAGACCG     Rev AGCTGGGCCAATATCTCCTT   Col2a1     18S   For AGACAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC     Gapdh   For TGTGTCCGTCGTGGATCTGA Rev TTGCTGTTGAAGTCGCAGGAG				
18S   For AGACAAATCGCTCCACCAAC Rev CTCAACACGGGAAACCTCAC   Actb   For CATCCGTAAAGACCTCTATGCCAAC Rev ATGGAGCCACCGATCCACA     Gapdh   For TGTGTCCGTCGTGGAGTCGA Rev TTGCTGTTGAAGTCGCAGGAG   For CATCCGTAAAGACCTCACA	Col10a1		Col2a1	
18S   For CATCCGTAAGAGCCTCAC   Actb     Rev CTCAACACGGGAAACCTCAC   Actb     Gapdh   For TGTGTCCGTCGTGGATCTGA Rev TTGCTGTTGAAGTCGCAGGAG				
Gapdh       For TGTGTCCGTCGTGGATCTGA       Rev TTGCTGTTGAAGTCGCAGGAG	18S		Actb	
Gapdh Rev TTGCTGTTGAAGTCGCAGGAG				
	Gapdh	Rev TTGCTGTTGAAGTCGCAGGAG		

## Table 2. Primers used for PCR analysis

## **References**

- 1. Berendsen, A.D., and Olsen, B.R. (2015). Bone development. *Bone*. 80, 14-18.
- 2. Nakao, K., Itoh, H., Saito, Y., Mukoyama, M., and Ogawa, Y. (1996). The natriuretic peptide family. *Curr Opin Nephrol Hypertens.* 5, 4-11.
- 3. Wit, J.M., and Camacho Hübner, C. (2011). Endocrine regulation of longitudinal bone growth. *Endocr Dev.* 21, 30-41.
- Peake, N.J., Hobbs, A.J., Pingguan-Murphy, B., Salter, D.M., Berenbaum, F., and Chowdhury, T.T. (2014). Role of C-type natriuretic peptide signalling in maintaining cartilage and bone function. *Osteoarthritis Cartilage*. 22, 1800-1807.
- 5. Vasques, G.A., Arnhold, I.J.P., and Jorge, A.A.L. (2014). Role of the natriuretic peptide system in normal growth and growth disorders. *Horm Res Paediatr.* 82, 222-229.
- Wit, J.M., Oostdijk, W., Losekoot, M., van Duyvenvoorde, H.A., Ruivenkamp, C.A.L., and Kant, S.G. (2016). MECHANISMS IN ENDOCRINOLOGY: Novel genetic causes of short stature. *Eur J Endocrinol.* 174, R145-R173.
- Savarirayan, R., Tofts, L., Irving, M., Wilcox, W., Bacino, C.A., Hoover-Fong, J., Ullot Font, R., Harmatz, P., Rutsch, F., Bober, M.B., et al. (2020). Once-daily, subcutaneous vosoritide therapy in children with achondroplasia: a randomised, double-blind, phase 3, placebo-controlled, multicentre trial. *Lancet.* 396, 684-692.
- Krejci, P., Masri, B., Fontaine, V., Mekikian, P.B., Weis, M., Prats, H., and Wilcox, W.R. (2005). Interaction of fibroblast growth factor and C-natriuretic peptide signaling in regulation of chondrocyte proliferation and extracellular matrix homeostasis. *J Cell Sci.* 118, 5089-5100.
- Kawasaki, Y., Kugimiya, F., Chikuda, H., Kamekura, S., Ikeda, T., Kawamura, N., Saito, T., Shinoda, Y., Higashikawa, A., Yano, F., et al. (2008). Phosphorylation of GSK-3β by cGMP-dependent protein kinase II promotes hypertrophic differentiation of murine chondrocytes. *J Clin Invest*. 118, 2506-2515.
- 10. Fleig, A., and Chubanov, V. (2014). Trpm7. Handb Exp Pharmacol. 222, 521-546.
- Qian, N., Ichimura, A., Takei, D., Sakaguchi, R., Kitani, A., Nagaoka, R., Tomizawa, M., Miyazaki, Y., Miyachi, H., Numata, T., et al. (2019). TRPM7 channels mediate spontaneous Ca<sup>2+</sup> fluctuations in growth plate chondrocytes that promote bone development *Sci Signal*. 12, eaaw4847.
- 12. Nakao, K., Osawa, K., Yasoda, A., Yamanaka, S., Fujii, T., Kondo, E., Koyama, N., Kanamoto, N., Miura, M., Kuwahara, K., et al. (2015). The local CNP/GC-B system in

growth plate is responsible for physiological endochondral bone growth. Sci Rep. 5, 10554.

- De-Li, D., Peng, Y., Bao-Feng, Y., and Wen-Hui, W. (2008). Hydrogen peroxide stimulates the Ca<sup>2+</sup>-activated big-conductance K channels (BK) through cGMP signaling pathway in cultured human endothelial cells. *Cell Physiol Biochem.* 22, 119-126.
- Fukao, M., Mason, H.S., Britton, F.C., Kenyon, J.L., Horowitz, B., and Keef, K.D. (1999).
   Cyclic GMP-dependent protein kinase activates cloned BK Ca channels expressed in mammalian cells by direct phosphorylation at serine 1072. *J Biol Chem.* 274, 10927-10935.
- 15. White, R.E., Kryman, J.P., El-Mowafy, A.M., Han, G., and Carrier, G.O. (2000). cAMP-dependent vasodilators cross-activate the cGMP-dependent protein kinase to stimulate BK(Ca) channel activity in coronary artery smooth muscle cells. *Circ Res.* 86, 897-905.
- Guo, J.Y., Zhang, M.H., Jiang, J.Z., Piao, L.H., Fang, X.S., Jin, Z., and Cai, Y.L. (2018). The role of CNP-mediated PKG/PKA-PLCβ pathway in diabetes-induced gastric motility disorder. *Peptides*. 110, 47-55.
- Huang, J., Zhou, H., Mahavadi, S., Sriwai, W., and Murthy, K.S. (2007). Inhibition of Gαq-dependent PLC-β1 activity by PKG and PKA is mediated by phosphorylation of RGS4 and GRK2. *Am J Physiol Cell Physiol*. 292, C200-C208.
- Nalli, A.D., Kumar, D.P., Al-Shboul, O., Mahavadi, S., Kuemmerle, J.F., Grider, J.R., and Murthy, K.S. (2014). Regulation of Gβγi-dependent PLC-β3 activity in smooth muscle: Inhibitory phosphorylation of PLC-β3 by PKA and PKG and stimulatory phosphorylation of Gαi-GTPase-activating protein RGS2 by PKG. *Cell Biochem Biophys.* 70, 867-880.
- Xia, C., Bao, Z., Yue, C., Sanborn, B.M., and Liu, M. (2001). Phosphorylation and regulation of G-protein-activated phospholipase C-β3 by cGMP-dependent protein kinases. *J Biol Chem*. 276, 19770-19777.
- Bibli, S.I., Andreadou, I., Chatzianastasiou, A., Tzimas, C., Sanoudou, D., Kranias, E., Brouckaert, P., Coletta, C., Szabo, C., Kremastinos, D.T., et al. (2015). Cardioprotection by H2S engages a cGMP-dependent protein kinase G/phospholamban pathway. *Cardiovasc Res.* 106, 432-442.
- Luc, R., Franz, H., and Rik, C. (1988). Cyclic GMP-dependent protein kinase phosphorylates phospholamban in isolated sarcoplasmic reticulum from cardiac and smooth muscle. *Biochem J.* 252, 269-273.
- Lalli, M.J., Shimizu, S., Sutliff, R.L., Kranias, E.G., and Paul, R.J. (1999). [Ca<sup>2+</sup>]<sub>i</sub> homeostasis and cyclic nucleotide relaxation in aorta of phospholamban-deficient mice. *Am J Physiol.* 277, H963-970.
- 23. Clark, R.B., Hatano, N., Kondo, C., Belke, D.D., Brown, B.S., Kumar, S., Votta, B.J., and Giles, W.R. (2014). Voltage-gated K<sup>+</sup> currents in mouse articular chondrocytes regulate

membrane potential. Channels. 4, 179-191.

- Houston, D.A., Staines, K.A., MacRae, V.E., and Farquharson, C. (2016). Culture of murine embryonic metatarsals: A physiological model of endochondral ossification. *J Vis Exp.*, e54978.
- 25. Liang, L., Li, X., Moutton, S., Schrier Vergano, S.A., Cogné, B., Saint-Martin, A., Hurst, A.C.E., Hu, Y., Bodamer, O., Thevenon, J., et al. (2019). De novo loss-of-function KCNMA1 variants are associated with a new multiple malformation syndrome and a broad spectrum of developmental and neurological phenotypes. *Hum Mol Genet.* 28, 2937-2951.
- Yasoda, A., Komatsu, Y., Chusho, H., Miyazawa, T., Ozasa, A., Miura, M., Kurihara, T., Rogi, T., Tanaka, S., Suda, M., et al. (2004). Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. *Nat Med.* 10, 80-86.
- 27. Martel, G., Hamet, P., and Tremblay, J. (2009). Central role of guanylyl cyclase in natriuretic peptide signaling in hypertension and metabolic syndrome. *Mol Cell Biochem.* 334, 53-65.
- 28. Zois, N.E., Bartels, E.D., Hunter, I., Kousholt, B.S., Olsen, L.H., and Goetze, J.P. (2014). Natriuretic peptides in cardiometabolic regulation and disease. *Nat Rev Cardiol.* 11, 403-412.
- Kubacka, M., Kotańska, M., Kazek, G., Waszkielewicz, A.M., Marona, H., Filipek, B., and Mogilski, S. (2018). Involvement of the NO/sGC/cGMP/K<sup>+</sup> channels pathway in vascular relaxation evoked by two non-quinazoline α1-adrenoceptor antagonists. *Biomed Pharmacother*. 103, 157-166.
- Ueda, Y., Yasoda, A., Yamashita, Y., Kanai, Y., Hirota, K., Yamauchi, I., Kondo, E., Sakane,
   Y., Yamanaka, S., Nakao, K., et al. (2016). C-type natriuretic peptide restores impaired skeletal growth in a murine model of glucocorticoid-induced growth retardation. *Bone.* 92, 157-167.
- Yamashita, T., Fujii, T., Yamauchi, I., Ueda, Y., Hirota, K., Kanai, Y., Yasoda, A., and Inagaki, N. (2020). C-Type natriuretic peptide restores growth impairment under enzyme replacement in mice with mucopolysaccharidosis VII. *Endocrinology*. 161, bqaa008.
- 32. Fafilek, B., Bosakova, M., and Krejci, P. (2021). Expanding horizons of achondroplasia treatment: current options and future developments. *Osteoarthritis Cartilage*. S1063-4584, 00980-00988.
- Yamazaki, D., Tabara, Y., Kita, S., Hanada, H., Komazaki, S., Naitou, D., Mishima, A., Nishi, M., Yamamura, H., Yamamoto, S., et al. (2011). TRIC-A channels in vascular smooth muscle contribute to blood pressure maintenance. *Cell Metab.* 14, 231-241.
- Li, Y., Ahrens, M.J., Wu, A., Liu, J., and Dudley, A.T. (2011). Calcium/calmodulin-dependent protein kinase II activity regulates the proliferative potential of growth plate chondrocytes. *Development*. 138, 359-370.

- 35. Ahrens, M.J., and Dudley, A.T. (2011). Chemical pretreatment of growth plate cartilage increases immunofluorescence sensitivity. *J Histochem Cytochem*. 59, 408-418.
- Mouser, V.H.M., Melchels, F.P.W., Visser, J., Dhert, W.J.A., Gawlitta, D., and Malda, J. (2016). Yield stress determines bioprintability of hydrogels based on gelatin-methacryloyl and gellan gum for cartilage bioprinting. *Biofabrication*. 8, 035003.
- 37. Chengzhu, Z., Ichimura, A., Qian, N., Iida, T., Yamazaki, D., Noma, N., Asagiri, M., Yamamoto, K., Komazaki, S., Sato, C., et al. (2016). Mice lacking the intracellular cation channel TRIC-B have compromised collagen production and impaired bone mineralization. *Sci Signal.* 9, ra49.

## **Publication list**

<u>Miyazaki, Y.</u>, Ichimura, A., Kitayama, R., Okamoto, N., Yasue, T., Liu, F., Ueda, Y., Yamauchi, I., Hakata, T., Nakao, K. Kakizawa, S., Nishi, M.,Mori, Y., Akiyama, H., Nakao, K. and Takeshima, H. (2021). C-type natriuretic peptide facilitates autonomic Ca<sup>2+</sup> entry in growth plate chondrocytes for stimulating bone growth. *bioRxiv*.

## **Reference Thesis**

Miyazaki, Y., Ichimura, A., Sato, S., Fujii, T., Oishi, S., Sakai, H. and Takeshima, H. (2018). The natural flavonoid myricetin inhibits gastric H<sup>+</sup>, K<sup>+</sup>-ATPase. *Eur J Pharmacol.* 820, 217-221.

Qian, N., Ichimura, A., Takei, D., Sakaguchi, R., Kitani, A., Nagaoka, R., Tomizawa, M., <u>Miyazaki,</u> <u>Y.</u>, Miyachi, H., Numata, T., Kakizawa, S., Nishi, M., Yasuo Mori, Y. and Takeshima, H. TRPM7 channels mediate spontaneous Ca<sup>2+</sup> fluctuations in growth plate chondrocytes that promote bone development. (2019). *Sci Signal.* 12, eaaw4847.

## **Acknowledgments**

I appreciate gratefully to Professor Hiroshi Takeshima, Graduate School of Pharmaceutical Science, Kyoto University for his immediate guidance through this paper. I also appreciate to Assistant Professor Atsuhiko Ichimura, Graduate School of Pharmaceutical Science, Kyoto University for his immediate guidance and encouragement through the course of this work. My appreciation is also given to Associate Professor Sho Kakizawa and, Dr. Miyuki Nishi, Graduate School of Pharmaceutical Science, Kyoto University, Dr. Hiromu Itoh, Kurashiki Central Hospital, Dr. Kazuwa Nakao, Dr. Kazumasa Nakao, Dr. Ichiro Yamauchi and Dr. Yohei Ueda, Graduate School of Medicine, Dr. Yasuo Mori, Graduate School of Engineering, Kyoto University, Dr. Haruhiko Akiyama, Graduate School of Medicine, Gifu University. I thank Mr. Hitoshi Miyachi, Ms. Satsuki Kitano, and Mr. Jun Matsushita for mouse in vitro fertilization. I appreciate to the late Tetsuro Fujita and his family or persons concerned for giving Fujita Jinsei Scholarship. And I express my acknowledgment to co-workers of my research group: Mr. Ryo Kitayama, Mr. Naoki Okamoto, Mr. Tomoki Yasue, Mr. Feng Liu, Ms. Yitong Wang, Mr. Takaaki Kawabe and Mr. Hiroki Nagatomo. I also appreciate to the members of the department of Biological Chemistry, Graduate School of Pharmaceutical Science, Kyoto University for their continuous encouragement helpful discussions and technical supports. I also appreciate to my friends. Lastly, I would like to express my gratitude to my parents and brother for mental encouragement and persistent understanding.