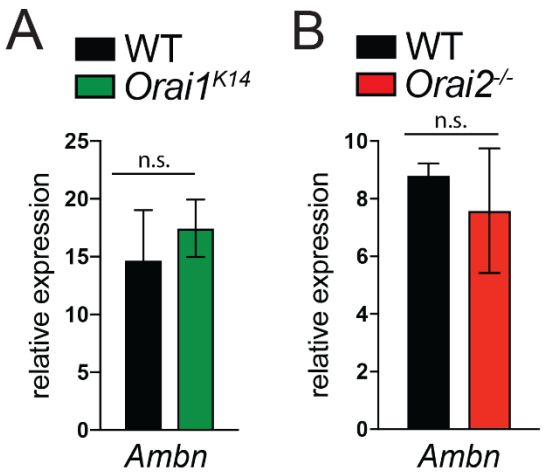
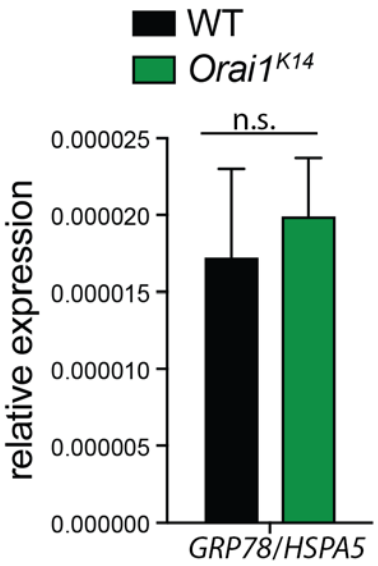
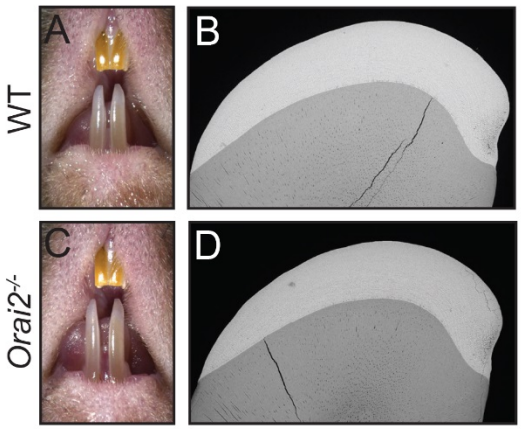
Supplementary Figures



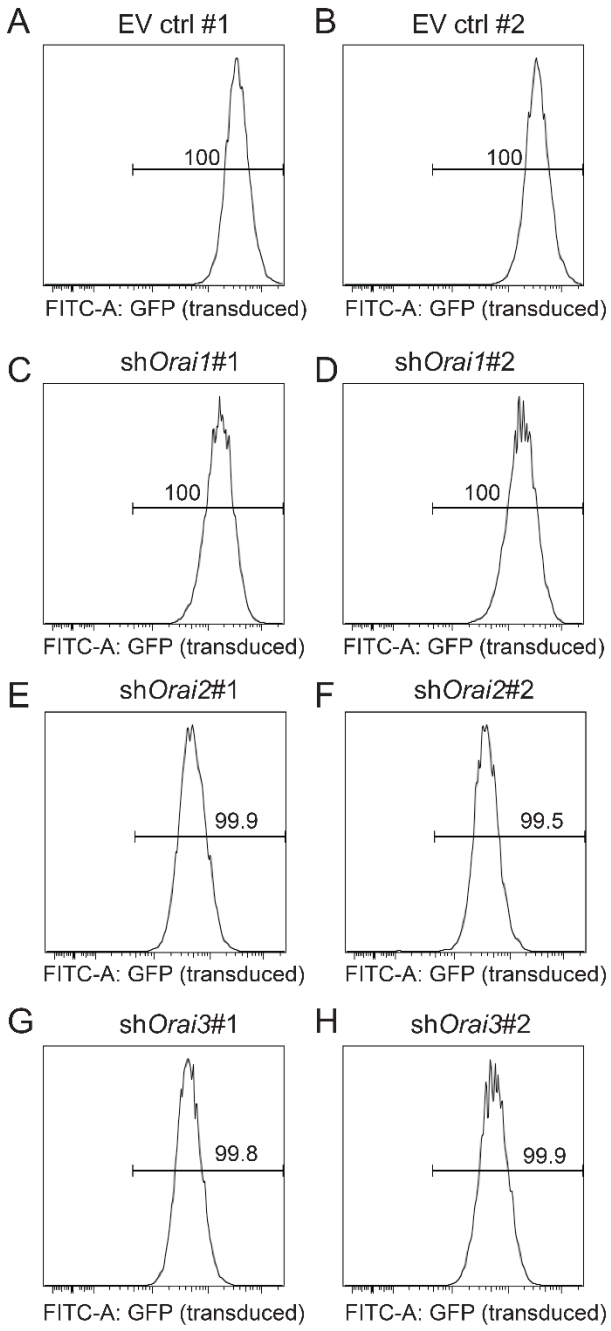
Supplementary Figure 1. Unchanged expression of ameloblastin in ORAI-deficient cells. The expression of the ameloblastin gene (*Ambn*) was analyzed in enamel organ cells of *Orai1*^{K14} (A) and *Orai2*$^{-/-}$ mice (B) by RT-PCR showing no significant differences. Data represent averages (+/- SEM) of N=5 mice analyzed using Mann-Whitney (A) or Welch's t-test (B).



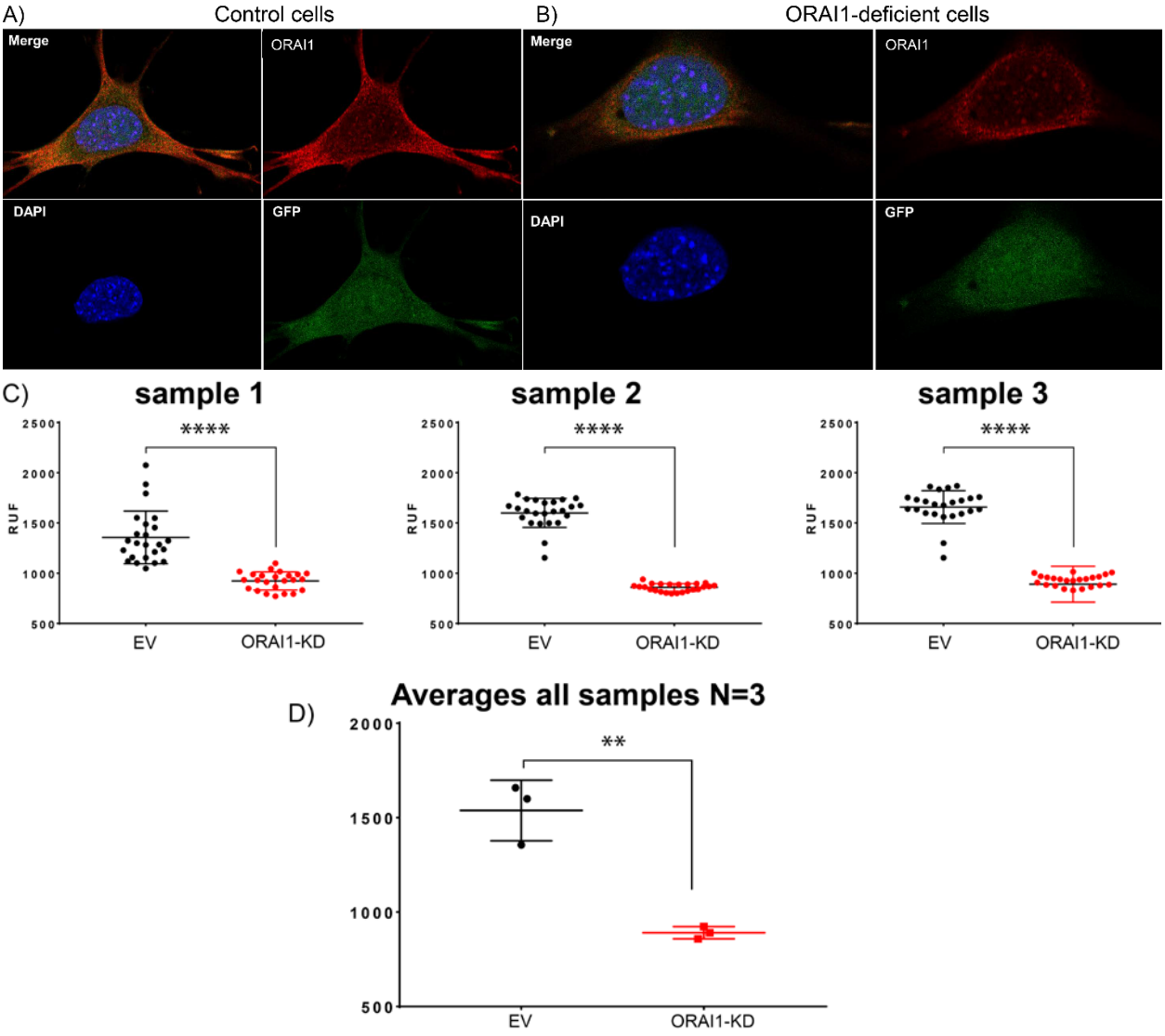
Supplementary Figure 2. Enamel cells of *Orai1*^{K14} mice do not show increased expression of unfolded protein response (UPR) markers. The expression of GRP78 (gene name *HSPA5*) associated with UPR was analyzed in enamel organ cells of *Orai1*^{K14} mice by RT-PCR showing no significant differences. Data represent averages (+/- SEM) of N=5 mice analyzed using unpaired Student's t-test.



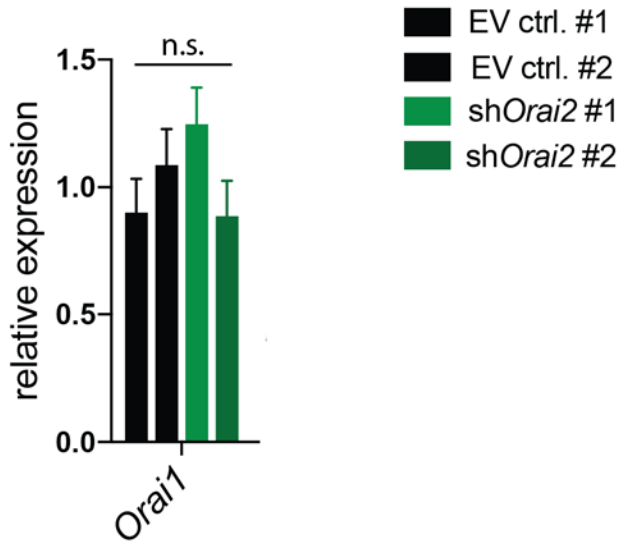
Supplementary Figure 3. Normal dental phenotype in ORAI2-deficient mice. (A, C) Macroscopic images of incisors of WT (**A**) and *Orai2*$^{-/-}$ (**C**) mice. **(B, D)** Scanning electron microscopy (SEM) images of cross-sections from incisors of WT (**B**) and *Orai2*$^{-/-}$ (**D**) mice imaged one millimeter from the tip. Data in **(A-D)** are representative of three mice per group.



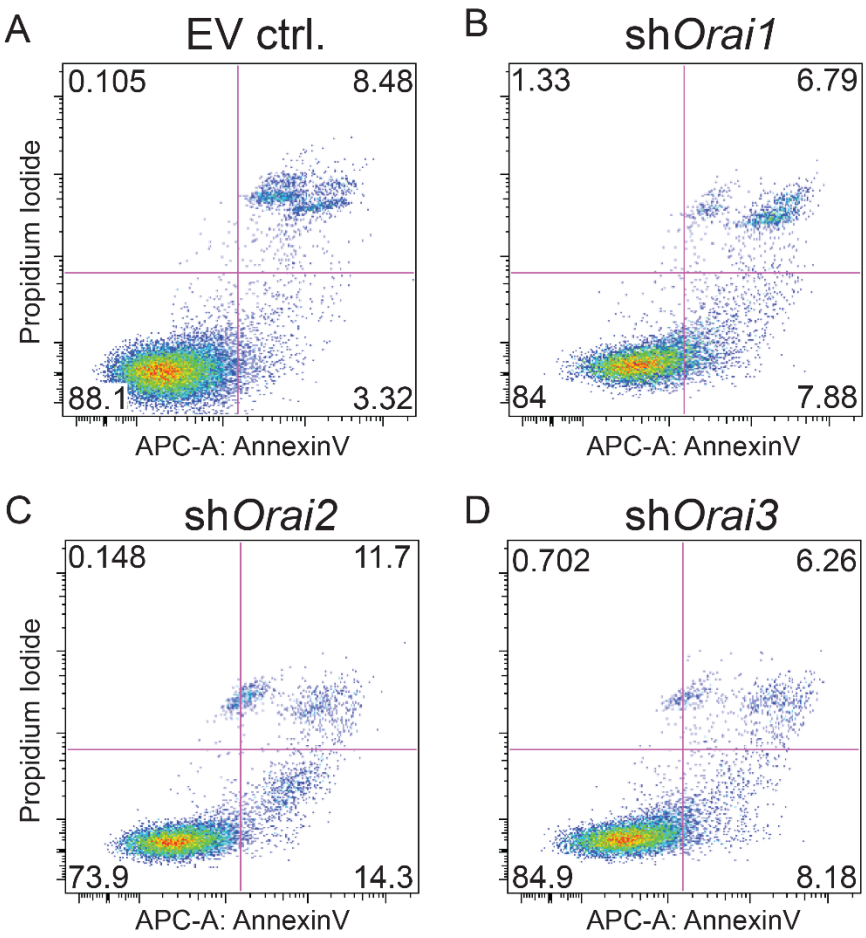
Supplementary Figure 4. Transduction efficiency in LS8 cells. Cells were transduced with either empty vector (EV ctrl., **A** and **B**) or shRNAs against *Orai1* (sh*Orai1*, **C** and **D**), *Orai2* (sh*Orai2*, **E** and **F**), and *Orai3* (sh*Orai3*, **G** and **H**) and analyzed for GFP expression by flow cytometry. Data are representative of N=3 experiments.



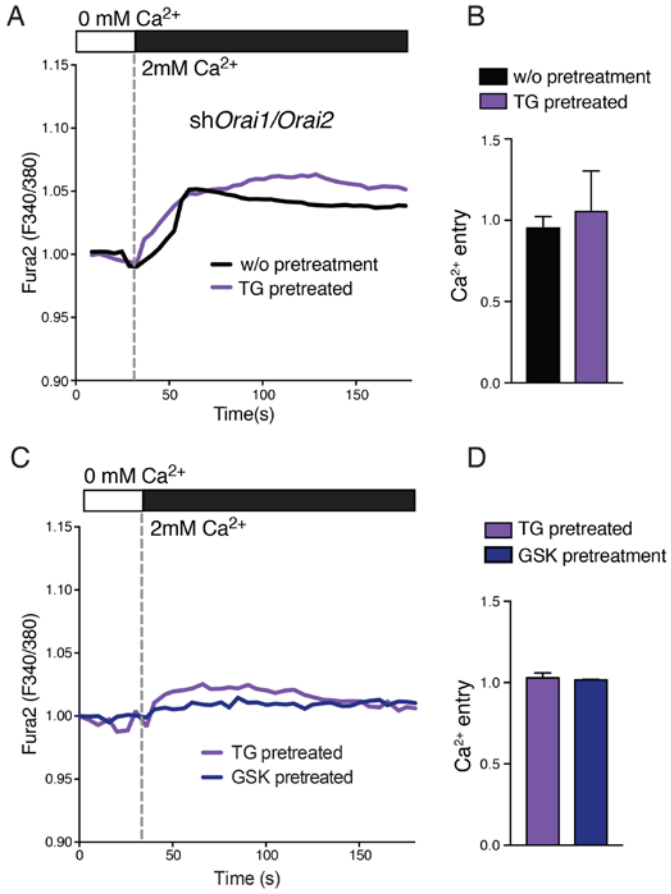
Supplementary Figure 5. Immunofluorescence analysis showing downregulation of ORAI1 in GFP positive sh*Orai1* cells. A-B) Downregulation of ORAI1 protein was confirmed by blinded immunofluorescence in GFP positive control cells (A) and sh*Orai1* cells (B) using anti-ORAI1 (Sigma SAB3500126) following protocols reported in (*1*). **C-D)** Quantification of differences in fluorescence between control and sh*Orai1* cells following protocols reported in (*2*). Three independent experiments were performed with $p<0.01$ (**) and $p<0.0001$ (****) using Mann-Whitney (C) or unpaired Student's t-test (D)



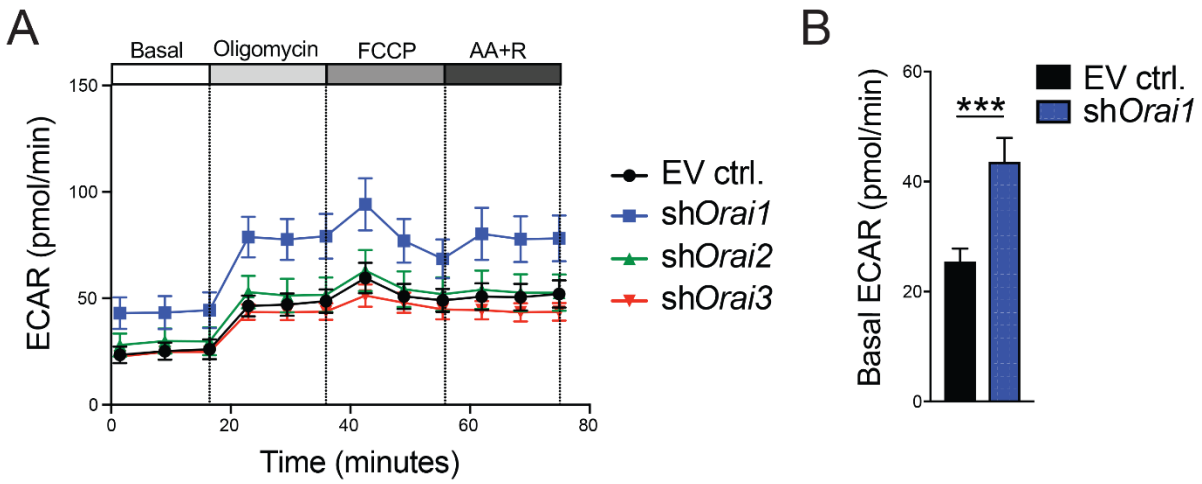
Supplementary Figure 6. *Orai1* expression is not significantly altered in sh*Orai2* cells. The expression of *Orai1* was analyzed in sh*Orai2* cells showing no significant differences. Data represent averages (+/- SEM) of N= 3 independent experiments analyzed using unpaired Student's t-test.



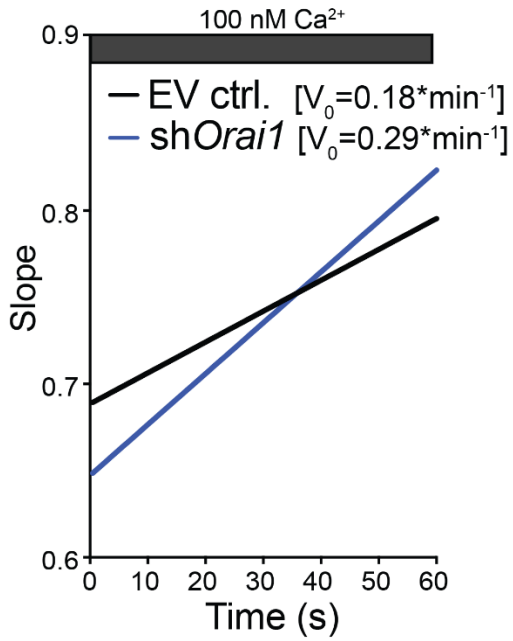
Supplementary Figure 7. Apoptosis and cell death. Apoptosis and cell death were analyzed in LS8 cells transduced with either empty vector (EV ctrl., **A**) or shRNAs against *Orai1* (sh*Orai1*, **B**), *Orai2* (sh*Orai2*, **C**), and *Orai3* (sh*Orai3*, **D**). Cells were stained with Annexin V and propidium iodide (PI) and analyzed by flow cytometry. Representative dot blots shown in this Figure are the basis of data shown in main Fig.4I.



Supplementary Figure 8. Small Ca^{2+} leak is present in sh*Orai1/Orai2* cells. (A) Cytosolic Ca^{2+} uptake was measured in Fura2-loaded LS8 cells transduced with shRNA against *Orai1* and *Orai2* (sh*Orai1/Orai2*) using a Flexstation III. Purple tracings are sh*Orai1/Orai2* cells that had been pretreated with thapsigargin as described in our SOCE measurements shown in Fig 5. Black tracings represent Ca^{2+}uptake in sh*Orai1/Orai2* cells but without prior thapsigargin treatment. This minor uptake results from a change in the external Ringer solution of 0 mM Ca^{2+} with a solution containing 2mM Ca^{2+}, thus potentially reflecting a plasma membrane leak. (**B**) Statistical analyses of Ca^{2+} leak in sh*Orai1/Orai2* cells. (N=3) independent experiments; data are mean +/- SEM. **(C)** In a separate experiment, re-addition of Ca^{2+} to thapsigargin stimulated sh*Orai1/Orai2* showed a similar elevation to that observed in cells preincubated with the CRAC channel inhibitor GSK-7975A**. (D)** Statistical analyses of Ca^{2+} peak in sh*Orai1/Orai2* cells. (N=3) independent experiments; data are mean +/- SEM.



Supplementary Figure 9. Increased extracellular acidification rate (ECAR) in sh*Orai1* cells. **(A-B)** Extracellular acidification rates (ECAR) in LS8 cells transduced with either empty vector (EV ctrl.) or shRNAs against *Orai1*, *Orai2* or *Orai3*. ECAR was analyzed using a Seahorse XFe analyzer and sequential treatment with Oligomycin, FCCP and Antimycin A / Rotenone. Representative ECAR traces (**A**) and quantification of basal ECAR values **(B)**. Data represent 4 independent experiments (mean +/- SEM). $p<0.001$ (***) using using Mann-Whitney test (B).



Supplementary Figure 10. **Quantification of velocity (slope) of ER Ca^{2+} refilling.** The slope of Ca^{2+} refilling in EV ctrl. and sh*Orai1* cells was analyzed during the first 60s after tBHQ application in 100 nM Ca^{2+} (V_0)**.** Data represent the mean +/- SEM of 3 independent experiments. sh*Orai1* cells showed a significant faster refilling (V_0 = 0.29×min^{-1} ± 0.00137) compared to ctrl cells (V_0 = 0.18×min^{-1} ± 0.00059) with $p<0.05$ (*) using an unpaired Student's t-test).

Table S1. Primer and shRNA sequences used in this study.

Specific mouse Primers designed for Genotyping

Primer	**Forward sequence**	**Reverse sequence**
Cre	ACGACCAAGTGACAGCAATG	CTCGACCAGTTTAGTTACCC
Orai1	CAGCGTGCATAATATACCTAACT CTACCCG	GTATTGATGAGGAGAGCAAGCGT GAATC
Orai2	CTCGGCAGTTGCCTGTTTG	ACAGCCACCACGCTCATC

shRNA sequences for stable knowdown of Orai1-3

Target gene	shRNA sequences
Orai1 #1	TGCTGTTGACAGTGAGCGCGGAGATTTTCTTCTGTGAGAATAGTGAAGCCACAGATGT ATTCTCACAGAAGAAAATCTCCATGCCTACTGCCTCGGA
Orai1 #2	TGCTGTTGACAGTGAGCGCCCTGGCGCAAGCTCTACTTAATAGTGAAGCCACAGATGT ATTAAGTAGAGCTTGCGCCAGGATGCCTACTGCCTCGGA
Orai2 #1	TGCTGTTGACAGTGAGCGACGCCACAACCGTGAGATCGAATAGTGAAGCCACAGATGT ATTCGATCTCACGGTTGTGGCGCTGCCTACTGCCTCGGA
Orai2 #2	TGCTGTTGACAGTGAGCGCGAGCAACATCCACAACCTCAATAGTGAAGCCACAGATGTA TTGAGGTTGTGGATGTTGCTCATGCCTACTGCCTCGGA
Orai3 #1	TGCTGTTGACAGTGAGCGCCAGCTGGAGAACGATCATGAATAGTGAAGCCACAGATGTA TTCATGATCGTTCTCCAGCTGTTGCCTACTGCCTCGGA
Orai3 #2	TGCTGTTGACAGTGAGCGCCTCCCTTAGTTTAGCTTCTAATAGTGAAGCCACAGATGTAT TAGAAGCTAAACTAAGGGAGATGCCTACTGCCTCGGA

Specific mouse Primers designed for qPCR

Primer	**Forward sequence**	**Reverse sequence**
Hprt1	GCTGACCTGCTGGATTACAT	TTGGGGCTGTACTGCTTAAC
Orai1	AGACTGCCTGATCGGATGGC	TTGTCCCCGAGCCATTTCCT
Orai2	GCAGCTACCTGGAACTCGTC	GTTGTGGATGTTGCTCACCG
Orai3	CAGTCAGCACTCTCTGCGG	TGGCCACCATGGCGAAG
Enam	TATGGTCTTCCACCAAGGAA	TAGGCACACCATCTCCAAAT
AmelX	CCCTGAGCTTCAGACAGAAA	CTGCCTTATCATGCTCTGGT
Atp2a2	CAGCCTGTCTGAGAACCAGT	CTCCAGATAGTTCCGAGCAA
Hspa5	ACTTGGGGACCACCTATTCCT	ATCGCCAATCAGACGCTCC