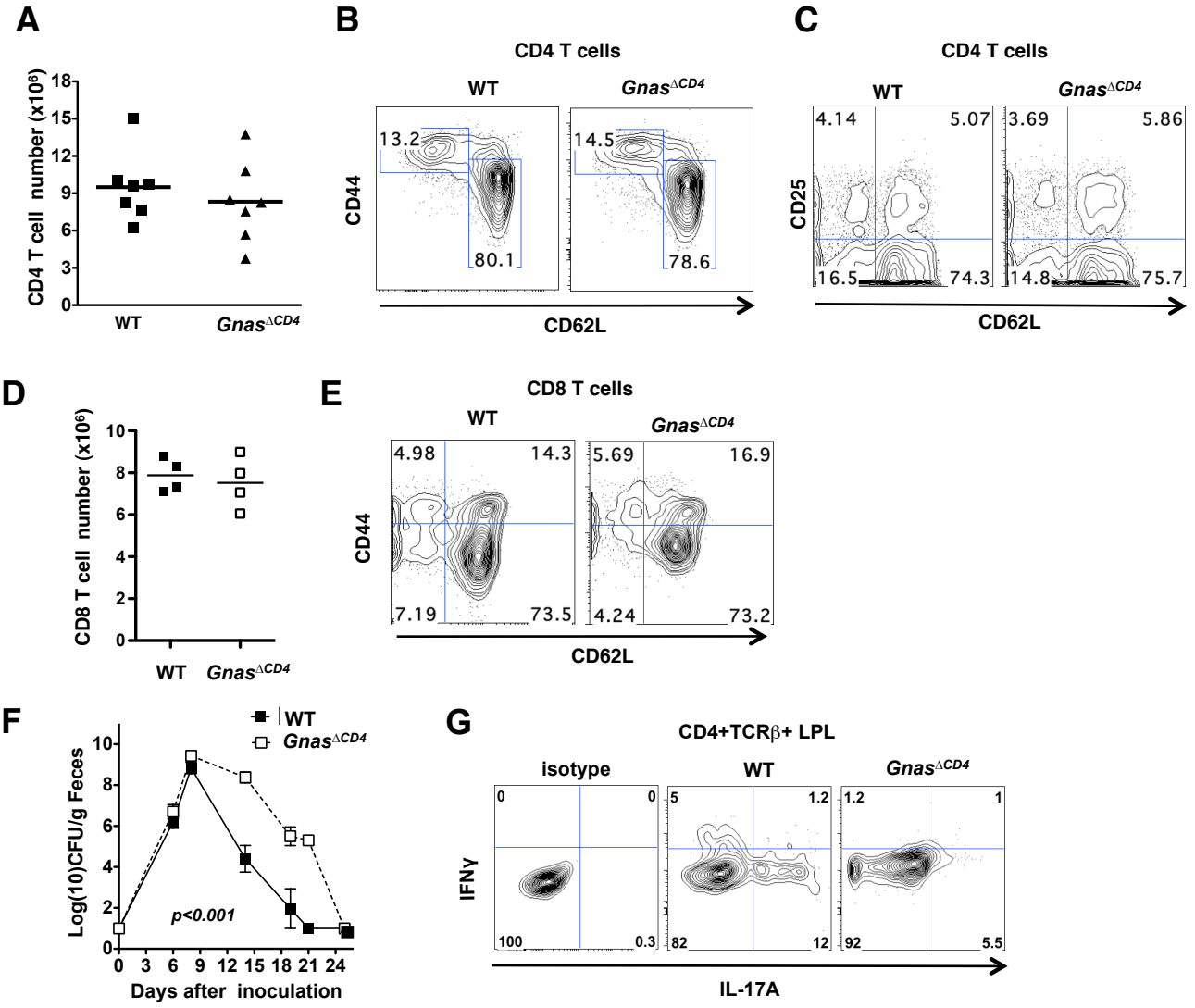
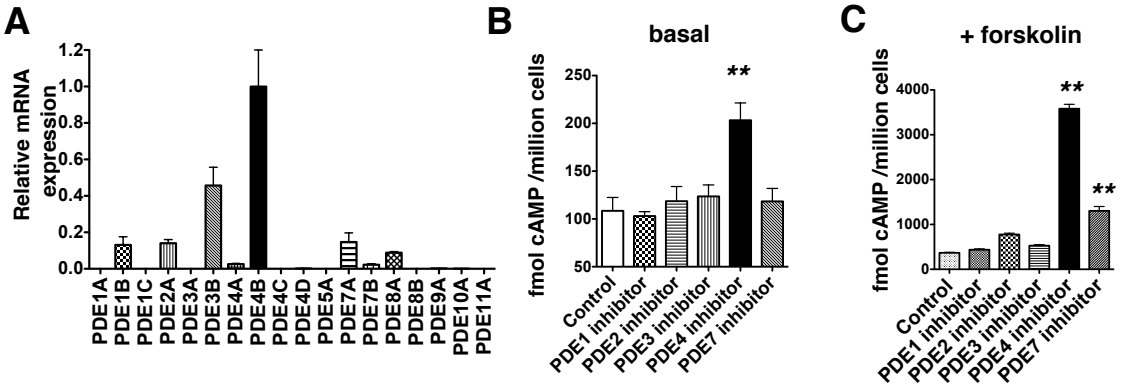
Supplemental Figure 1.



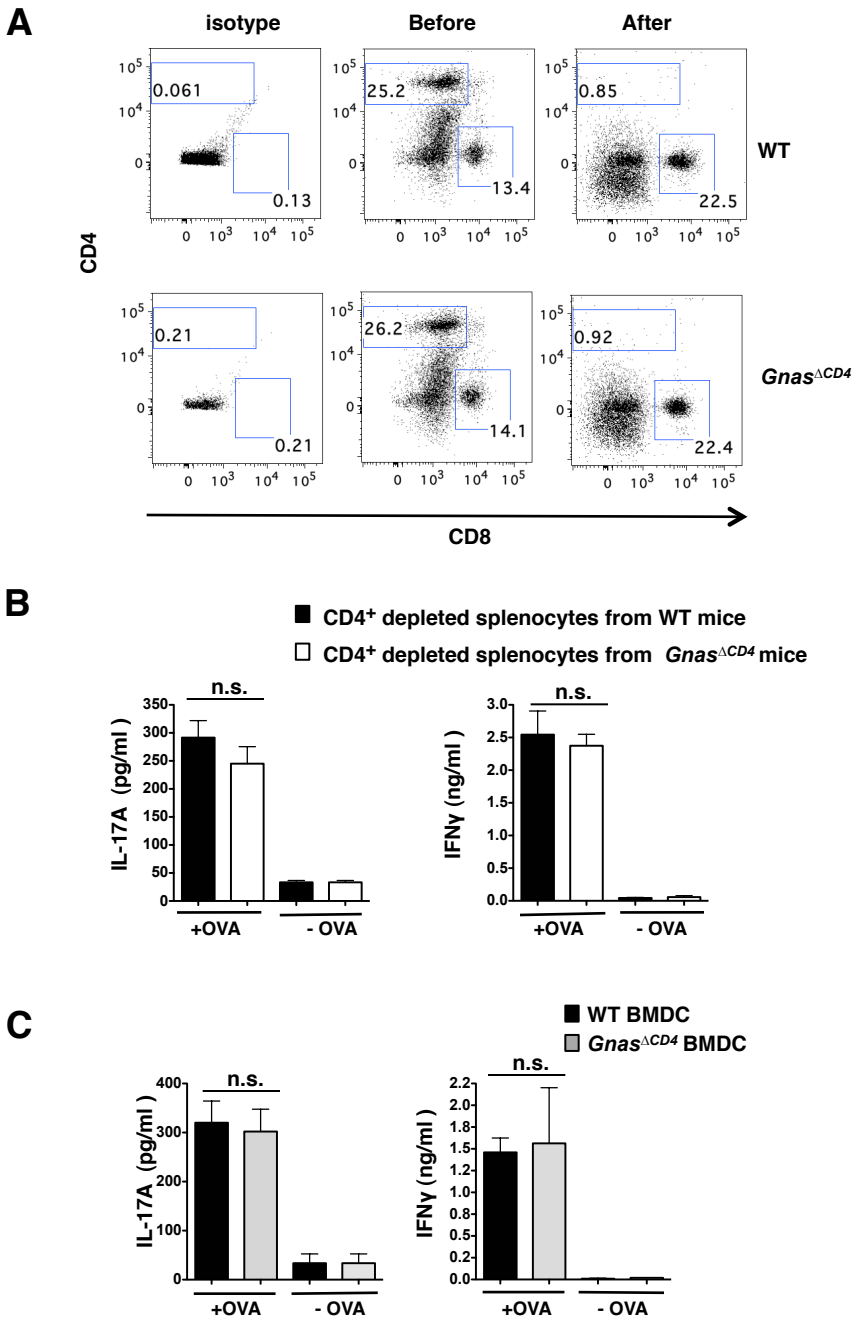
Supplemental Figure 1: Characterization of *Gnas*$^{\Delta CD4}$ CD4 and CD8 T cells
Splenocytes from 8-week old WT and *Gnas*$^{\Delta CD4}$ mice were labeled with CD4 Pacific blue, TCRβ percp-cy5.5, CD44 PE-cy7, CD62L APC and CD25 Alexa 488 Abs. **(A)** The number of CD4 T cell and **(B)** the percentage of effector and memory CD4 T cells ($CD44^{high}CD62L^{low}$) in *Gnas*$^{\Delta CD4}$ spleens are comparable to those in WT spleens. The cells were gated on $CD4^+TCR\beta^+$ T cells. **(C)** Comparable frequencies of $CD25^+$ CD4 T cells in the two strains. Data is representative of two independent experiments. Splenocytes from 8-week old WT and *Gnas*$^{\Delta CD4}$ mice were labeled with CD8a Pacific blue, TCRβ percp-cy5.5, CD44 PE-cy7, CD62L APC. **(D)** The CD8 T cell numbers and **(E)** the proportions of effector memory CD8 T cells ($CD44^{high}CD62L^{low}$) in *Gnas*$^{\Delta CD4}$ spleens are comparable to those in WT spleens. The cells were gated on $CD8^+TCR\beta^+$ T cells. **(F)** Bacterial counts in fecal homogenates from WT or *Gnas*$^{\Delta CD4}$ mice infected orally with *C. rodentium* ($2x10^8$ CFU) were determined at various time points as described previously (Dann, S.M., et al. 2008. *J Immunol* 180:6816-6826). Data are mean ± s.e.m, n=3, from a representative experiment out of three that were performed. * *p* value was calculated by two way ANOVA. **(G)** The reduced frequency of IL-17A$^+$ CD4$^+$ LPL or IFNγ$^+$ CD4$^+$ LPL in *Gnas*$^{\Delta CD4}$mice in *C. rodentium* infection. LPLs were isolated from the colons of WT or *Gnas*$^{\Delta CD4}$mice at day 8 post-infection as described elsewhere (Mucida, D, et al. 2007. *Science* 317:256-260). Briefly, the colonic tissues from the host mice that received either WT or *Gnas*$^{\Delta CD4}$T cells (n=3) were pooled, cut and then digested by collagenase (type VIII; Sigma). To collect the LPL cells, the cell suspensions were carefully layered onto a 40% and 80% discontinuous Percoll solution and centrifuged for 20 min at 1,000g. LPL cells were recovered from the interface and were stimulated with PMA/ionomycin for 4 h. The intracellular cytokines in CD4$^+$ TCRβ$^+$-gated LPL were determined (FACS). Data of a representative mouse/group are displayed.

Supplemental Figure 2.



Supplemental Figure 2: PDE4 inhibition increases cAMP levels in CD4 T cells
(A) WT CD4 T cells were enriched from splenocytes using CD4-negative selection immunomagnetic beads. Total RNA was extracted for qPCR analysis. Expression of mRNA levels of PDE isoforms (mean ± s.e.m.) was normalized to 18S rRNA housekeeping gene and expressed relative to PDE4B (the highest expressed PDE in CD4 T cells). (**B)** WT CD4 T splenocytes were treated for 30 min with PDE type-specific inhibitors as follows: PDE1 inhibitor 8-MM-IBMX, 30 μM; PDE2 inhibitor, EHNA, 10 μM; PDE3 inhibitor, milrinone, 10 μM; PDE4 inhibitor, rolipram, 10 μM and PDE7 inhibitor, BRL-50481, 30 μM, and the cAMP levels determined. Data shown are from 4 independent experiments (Mean ± s.e.m.), $p<0.006$ vs. control treatment. (**C)** The enriched WT CD4 T cells were incubated with PDE inhibitors for 30 min and then with forskolin (1 μM) for 10 min. The cAMP levels were measured as described in Experimental procedures.

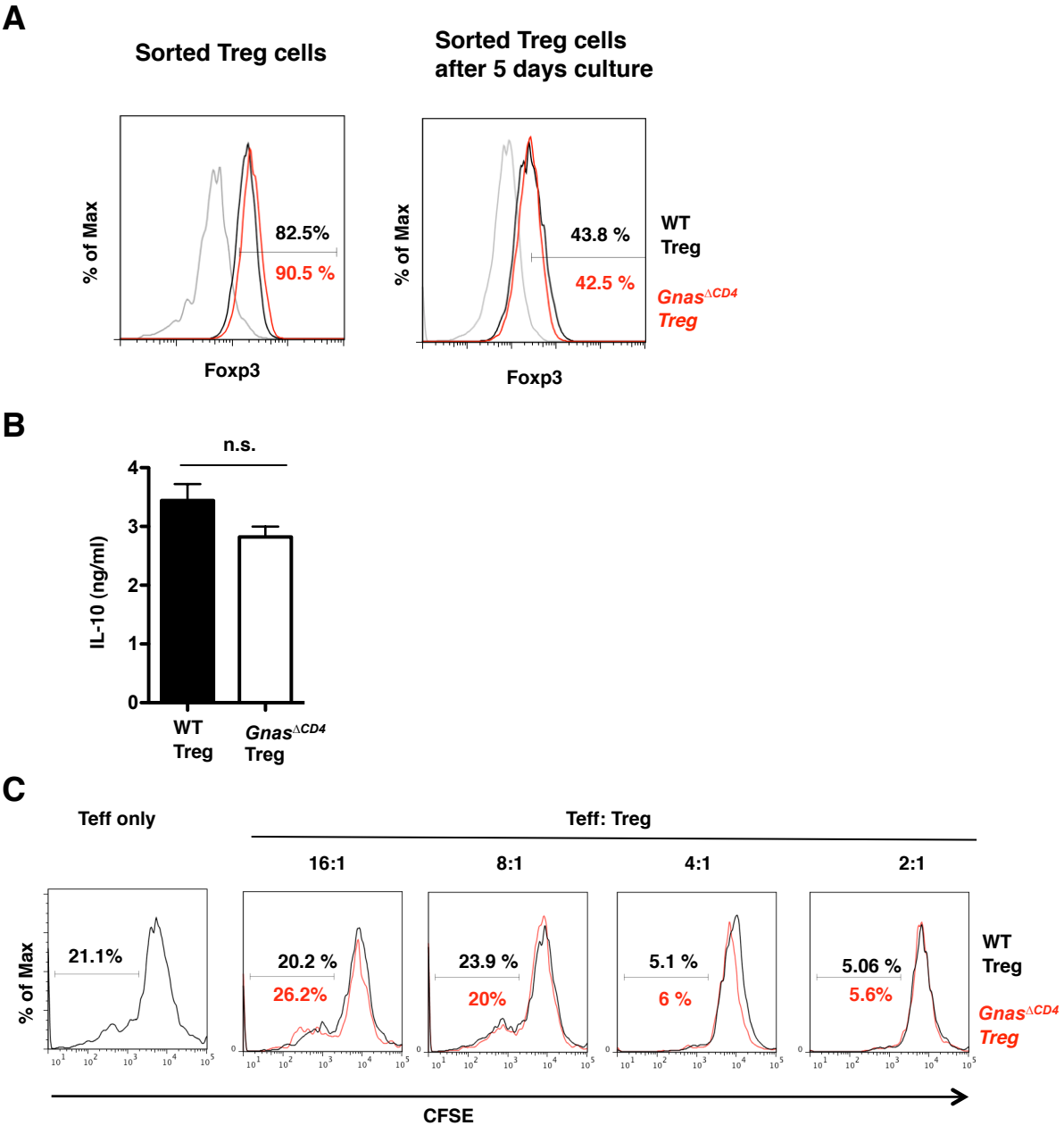
Supplemental Figure 3.



Supplementary Figure 3: **Deletion of Gαs in CD8 and BMDCs do not affect OVA-specific CD4 T cells responses.**

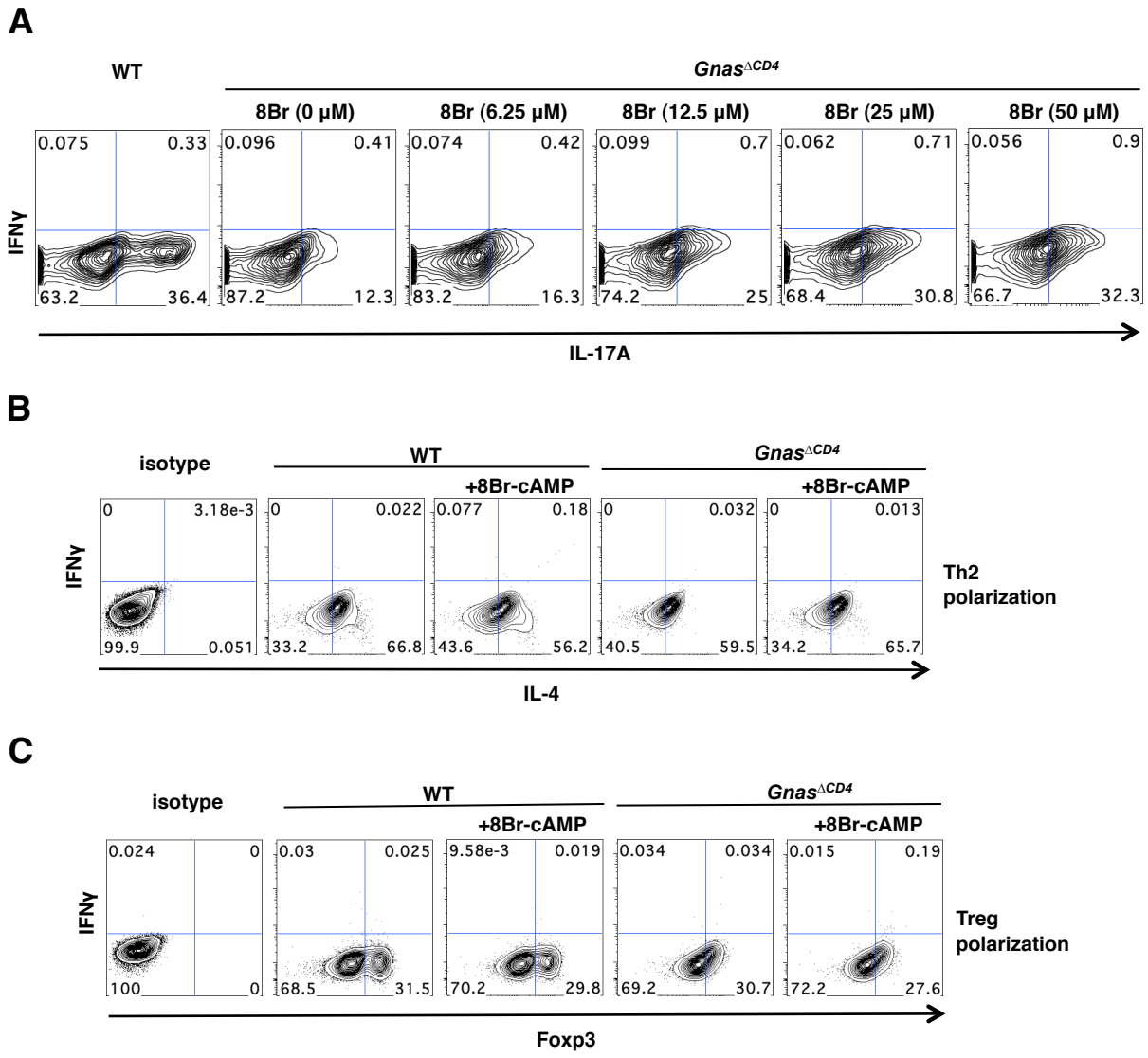
(A) Depletion of CD4+ cells in splenocytes from WT and *Gnas*$^{\Delta CD4}$ mice. The CD4$^+$ cells were labeled by anti-CD4 biotin Abs and depleted from splenocytes by biotin+ selection kit. The cells before and after CD4 depletion were stained with anti-CD4 APC and anti-CD8a PE antibodies. **(B)** Effect of CD4$^+$ cell depleted splenocyte on differentiation of OT-2 CD4 T ells. The naïve OT-2 CD4 T cells (2.5×10^5 cells) were enriched by magnetic beads (CD8$^-$B220$^-$ CD11c$^-$ CD11b$^-$ CD25$^-$) and co-cultured for 5 days with WT or *Gnas*$^{\Delta CD4}$ splenocytes (1.25×10^5 cells) that were depleted of CD4$^+$ cells in the presence or absence of class II-derived OVA peptide (10 μg/ml). Cytokine concentration (IL-17A and IFNγ) in the supernatant was detected by ELISA (mean ± s.e.m, n=3). **(C)** Effect of *Gnas*$^{\Delta CD4}$ BMDCs cells on differentiation of OT-2 cells. The naïve OT-2 cells (2.5×10^5 cell) were enriched and co-cultured for 5 days with WT or *Gnas*$^{\Delta CD4}$ BMDCs (1.25×10^5 cell) in presence or absence of OVA peptide (10 μg/ml). Cytokine concentrations (IL-17A and IFNγ) in the supernatant were detected (ELISA) (mean ± s.e.m, n=3).

Supplementary Figure 4:



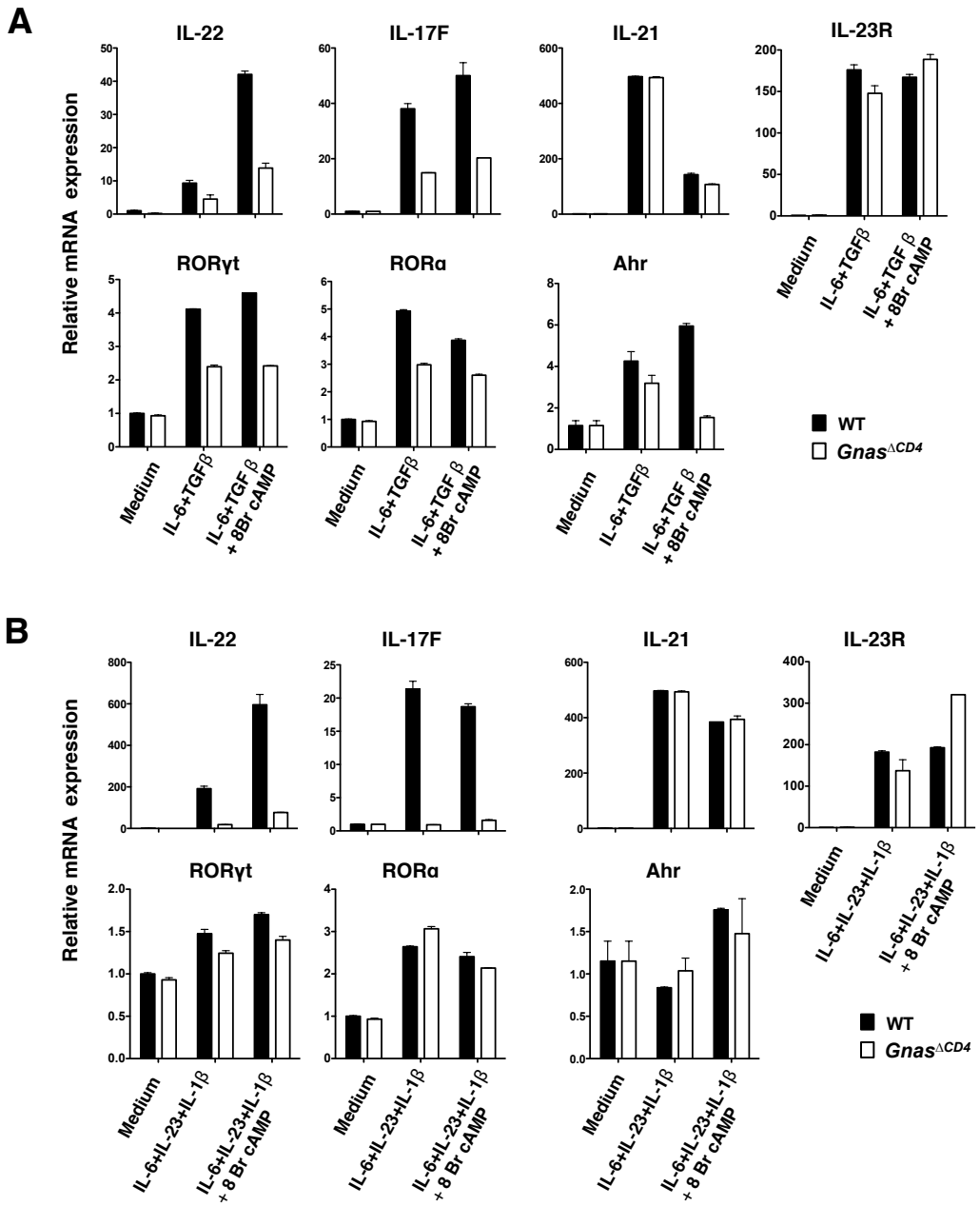
Supplementary Figure 4: *Gnas*$^{\Delta CD4}$ CD4 T cells do not regulate Treg cell differentiation.
(A) Stability of Foxp3 in WT and *Gnas*$^{\Delta CD4}$ T regulatory cells *in vitro*. Splenic Treg cells (CD4^{+}CD45RB^{low} CD25^{+}) were sorted from WT or *Gnas*$^{\Delta CD4}$ mice and co-cultured for 5 days with effector CD4 T cells (CD4^{+}CD45RB^{high} CD25^{-}) from congenic CD45.1 mice for 3 days at different ratios in the presence of anti-CD3 (1µg/ml) antibody and irradiated CD45.1+ splenocytes (3000 rad). The Treg cells (CD45.2^{+}) before and after cultured *in vitro* were gated and intracellular Foxp3 expression were analyzed by FACS. **(B)** IL-10 production in WT and *Gnas*$^{\Delta CD4}$ T reg cells. The sorted naïve WT and *Gnas*$^{\Delta CD4}$ CD4T cells were differentiated into Treg cells *in vitro* (TGFβ 10 ng/ml+hIL-2 100 U/ml) for 4 days. The cells were stimulated with anti-CD3/28 antibodies for 24h and IL-10 was measured in the supernatants (ELISA). Results are showed in mean ± s.e.m in triplicates. **(C)** Suppressive function of WT and *Gnas*$^{\Delta CD4}$ Treg cells *in vitro*. Splenic Treg cells (CD4^{+}CD45RB^{low} CD25^{+}) were sorted from WT or *Gnas*$^{\Delta CD4}$ mice and co-cultured for 3 days with CFSE (5 µM) labeled effect T cells (CD4^{+}CD45RB^{high} CD25^{-}) from congenic CD45.1 mice for 3 days at different ratios in the presence of anti-CD3 antibody (5 µg/ml) and irradiated WT splenocytes (3000 rad). The effector CD4 T cells (CD45.1^{+}) were gated and the CFSE dilution was analyzed (FACS).

Supplemental Figure 5.



Supplementary Figure 5: cAMP has divergent effects on Th subset differentiation
(A) 8Br-cAMP increase Th17 differentiation in *Gnas*$^{\Delta CD4}$ CD4 T cells in a dose-dependent manner. The sorted naïve CD4 T cells were cultured under Th17 differentiation condition (IL-6 and TGFβ, anti-CD3 and anti-CD28 Abs and neutralized Abs for IL-4 and IFNγ) with the indicated concentration of 8Br-cAMP for 4 days. The cells were further stimulated by PMA and inomycin for 4h in presence of GolgiStop. **(B-C)** Cyclic AMP does not affect Th2 and Treg differentiation *in vitro***.** FACS-sorted naive CD4 T cells from WT or *Gnas*$^{\Delta CD4}$ spleens were cultured for 4 days under Th2 **(B)** or Treg **(C)** differentiation conditions as described in Experimental procedures with and without 8Br-cAMP (25 μM). CD4 T cells were then stimulated with PMA/ionomycin for 4h and the intracellular cytokines IL-4, IFNγ and Foxp3 were determined (FACS). Data is representative of 2 independent experiments with similar results.

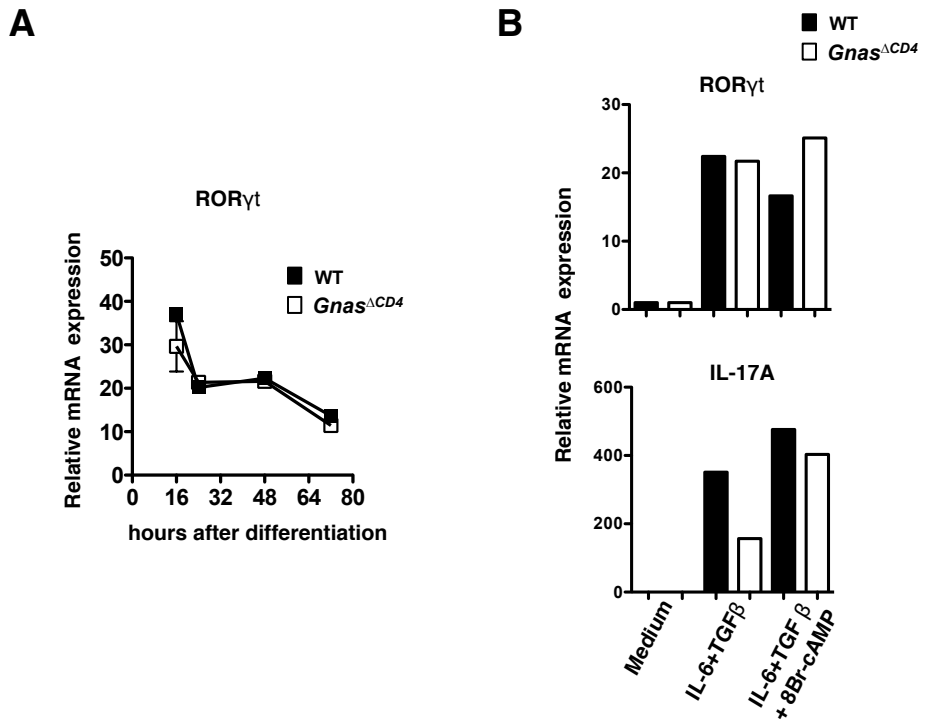
Supplemental Figure 6.



Supplementary Figure 6: Transcriptional regulation of Th17 differentiation in *Gnas*$^{\Delta CD4}$ CD4 T cells

FACS-sorted naive CD4 T cells were cultured for 4 days under two Th17 differentiation conditions; IL-6/TGFβ **(A)** or IL-6/IL-23/IL-1β (**B**). The CD4 T cells were stimulated by anti-CD3/28 Abs for 4h. The mRNA levels of cytokines and transcriptional factors were determined by qPCR. The expression levels were normalized to the expression of *Rplp0* housekeeping gene. Data are presented as mean ± s.e.m of duplicates from one of two similar experiments**.**

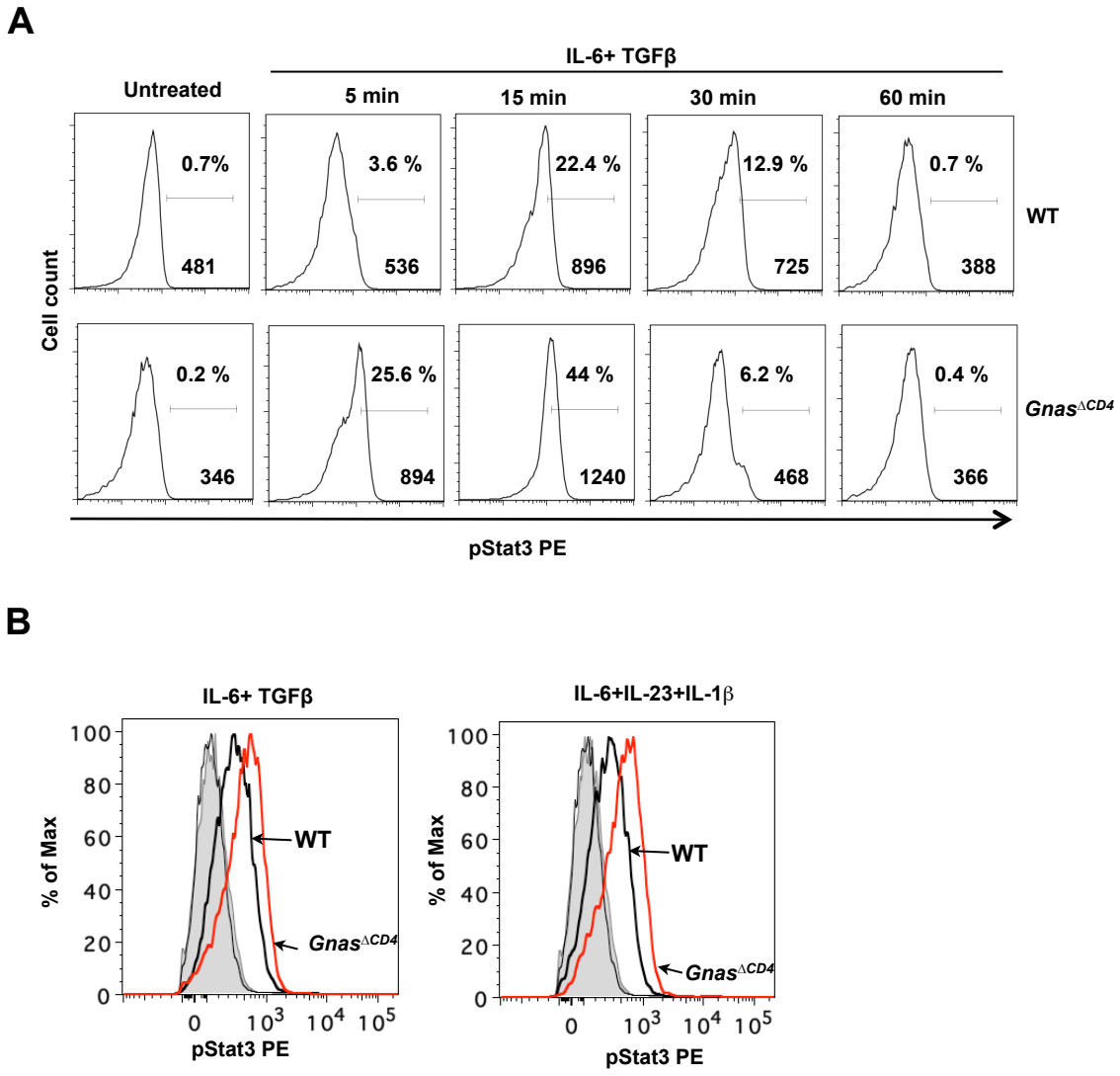
Supplementary Figure 7.



Supplementary Figure 7: The RORγt mRNA expression during Th17 differentiation in *Gnas*$^{\Delta CD4}$ CD4 T cells

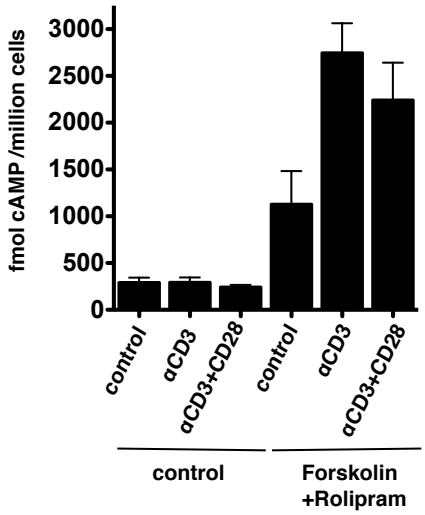
(A) The RORγt mRNA expression in WT and *Gnas*$^{\Delta CD4}$ CD4 cells during Th17 differentiation. The sorted naïve CD4 T cells were treated by Th17 condition (IL-6 and TGFβ, anti CD3/28 Abs and neutralized Abs for IL-4 and IFNγ) for indicated the times (16, 24, 48, 72h after differentiation). **(B)** The mRNA expression of RORγt and IL-17A at 48h after Th17 differentiation. The sorted naïve CD4 T cells were differentiated by Th17 condition (IL-6 and TGFβ) with or without 8Br-cAMP (25 μM) for 48h. The mRNA expression was normalized to the housekeeping gene *Rplp0*. The mRNA expressions are shown as mean ± s.e.m of duplicated samples.

Supplemental Figure 8.



Supplemental Figure 8: Stat3 phosphorylation in *Gnas*$^{\Delta CD4}$ CD4 T cells
(A) Activation of Stat3 in WT and *Gnas*$^{\Delta CD4}$ CD4 T cells. Enriched naive CD4 T cells were cultured in IMDM medium for indicated periods after treatment of IL-6 (20 ng/nl)/ TGFβ (4 ng/ml) and the levels of p-Stat3 (pY705) were determined (FACS). The numbers in the bottom of the histogram are the mean fluorescence intensity. The numbers displayed above the gate represents the percentage of positively stained cells. **(B)** CD4 T cells from WT and *Gnas*$^{\Delta CD4}$ mice were stimulated by anti-CD3 (10 μg/ml) and CD28 (1 μg/ml) Abs with the indicated cytokines (IL-6, 20ng/ml; IL-23,10 ng/ml; IL-1β, 10 ng/ml and TGFβ, 4 ng/ml) for 15 min, and p-Stat3 (pY705) levels were determined (FACS). The data shown are representative of one out of two independent experiments.

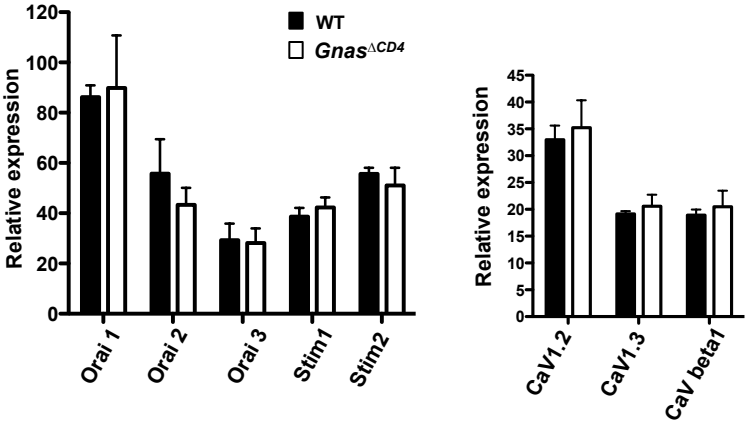
Supplemental Figure 9.



Supplemental Figure 9: TCR cross-linking enhances intracellular cAMP production

Splenic CD4 T cells were stained with anti-CD3 Ab (5 μg/ml), placed on ice for 1h, and then incubated with rolipram (10 μM) for 45 min at 37 °C. Cells were further treated with forskolin (1 μM) and a combination of goat anti-hamster Ab (10 μg/ml) with or without anti-CD28 Ab (1 μg/ml) as indicated, for 10 min. Cyclic AMP levels were determined (RIA). The data are presented as mean ± s.e.m of duplicate samples.

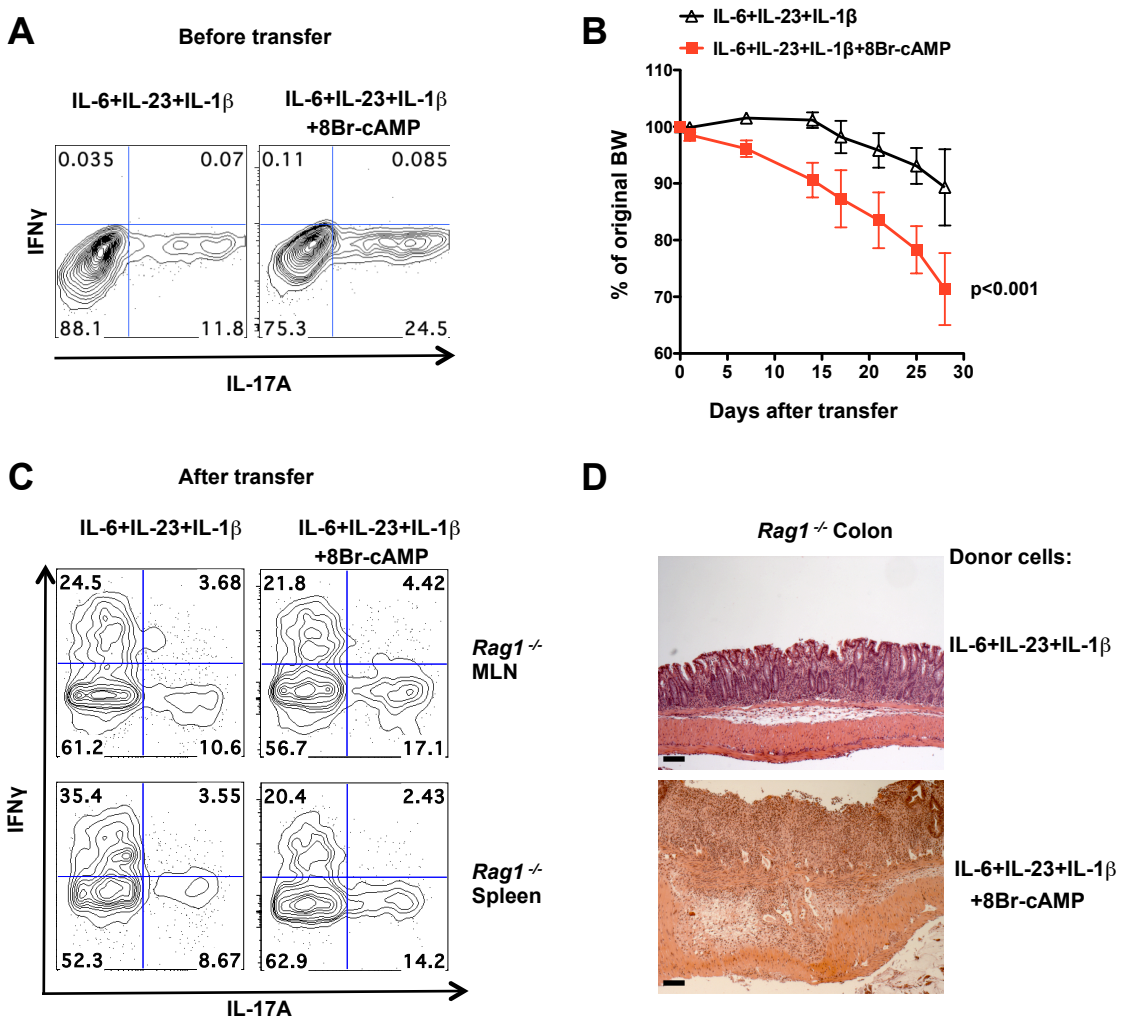
Supplemental Figure 10.



Supplemental Figure 10: Expression levels of ORAI, STIM and L-type Ca^{2+} channels subunits in WT and *Gnas*$^{\Delta CD4}$ CD4 T cells

Total RNA was extracted from splenic CD4 T cells sorted from WT and *Gnas*$^{\Delta CD4}$ mice. The mRNA expression levels for ORAI, STIM and L-type Ca2+ channels were normalized to the expression of the *Rplp0* housekeeping gene. Data are presented as mean ± s.e.m from 4 mice.

Supplemental Figure 11.



Supplemental Figure 11: Cyclic AMP enhances the colitogenicity of *in vitro* differentiated Th17 cells (IL-6/IL-23/IL-1β) in an adoptive transfer model of colitis.
FACS-sorted naive CD4 T cells from WT or *Gnas*$^{\Delta CD4}$ spleens were cultured for 4 days under Th17 differentiation condition (IL-6/IL-23/IL-1β with/without 8Br-cAMP, 25 μM). The differentiated Th17 cells (1×10^5 cells per mouse) were adoptively transferred into *Rag1*$^{-/-}$ mice. **(A)** The intracellular expression of IFNγ and IL-17A in Th17 cells before transfer. **(B)** Percentage of initial body weight of *Rag1*$^{-/-}$ recipients transferred with Th17 cells. Data shown are mean ± s.e.m, n=5 in each group. The *p* value indicates two away ANOVA of the difference between recipients receiving either Th17 or 8Br-cAMP-treated Th17 cells. **(C)** Intracellular expression of IFNγ and IL-17A in Th17 cells 28 days post-transfer. CD4+ cells harvested from the spleens or MLN of recipient mice were stimulated with PMA/ionomycin for 4h. Intracellular cytokine levels were determined by flow cytometry. **(D)** Histological analysis in the colons of recipients receiving *in vitro* differentiated Th17 cells with or without 8Br-cAMP (original magnification, ×50, scale bar: 100μM).

Supplementary Table 1: Primers sequence:

Gene	Forward primer (5'-3')	Reverse primers (5'-3')
Rplp0	AGATGCAGCAGATCCGCAT	GTTCTTGCCCATCAGCACC
Gnas	GCAGAAGGACAAGCAGGTCT	CCCTCTCCGTTAAACCCATT
Ahr	CCGTCCATCCTGGAAATTCGAACC	CCTTCTTCATCCGTTAGCGGTCTC
Rorc	CAGCCAACATGTGGAAAAGCT	GGGAAGGCGGCTTGGA
Rora	TGTGATCGCAGCGATGAAAG	AACAGTTCTTCTGACGAGGACAGG
Foxp3	TCAAGTACCACAATATGCGACC	AACATGCGAGTAAACCAATGG
Tbx21	AGCCAGCCAAACAGAGAAGAC	AATGTGCACCCTTCAAACCCT
Gata3	ACATGTCATCCCTGAGCCACATCT	AGGAACTCTTCGCACACTTGGAGA
Il17a	AGTGTTTCCTCTACC	GAAAACCGCCACCGCTTAC
IL22	CATGCAGGAGGTGGTACCTT	CAGACGCAAGCATTTCTCAG
IL21	TCGCCTCCTGATTAGACTTCGTCA	AGGGTTTGATGGCTTGAGTTTGGC
Il23r	GCCAAGAAGACCATTCCCGA	TCAGTGCTACAATCTTCTTCAGAGGACA
Il17f	TGCCATGCACACCTTACTGAGAGT	AGCAAGAAATCCTGGTCCTTCGGA
Stim1	GCTCTCAATGCCATGCCTTCCAAT	TCTAGGCCATGGTTCAACGCCATA
Stim2	AGCAGTAGTTTATGCCGCTCTCGT	AGGGCAACTTGACACAGACAG GAT
Cacna1c	GAGCCACGGTGAATCAGGA	GCAGTACTCGGCTTCTTCACTCA
Cacna1d	CCCATCGGAGGTCCTCCT	GACATCGTCTTGGCTGCTTTG
Cacnb1	ACGCCATGTAGCCGATGTC	GCCGGCTTCGTTGTTTGA
Orai1	CCAAGCTCAAAGCTTCCAGC	GCACTAAAGACGATGAGCAACC
Orai2	TCCTCAGACACACCAAGG	CAAAAGACACCAATCATGTTCTGC
Orai3	GGATCCTGGGTTAAATGAGAG	TTGAGGACAGTTGTGCAGAC
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG