Supplementary Figure and Table Legends

Extended Data Fig. S1: Dynamic transcriptional regulation during Th1 differentiation

(**a**) Expression dynamics of Th1 or Th2 signature genes. Cells were collected at the indicated time points during 3 days of *in vitro* culture for RNA extraction and qPCR. Error bar denotes SD.

(**b**) Three major dynamic phases at global transcriptome level during T helper cell *in vitro* differentiation. Spearman correlation matrix across collected time points is shown as a heatmap.

(**c**) Relative expression of dynamically expressed genes during *in vitro* Th2 differentiation.

(**d**) Heterogeneity among $CD4^+$ T cells at day 8 of Armstrong infection. Cells are colored by the relative expression of signature genes.

(**e**) Global lineage structure inferred by Slingshot using minimum spanning tree on the clusters of interest.

Extended Data Fig. S2: Determination of Th1 unique regulators under Th1/Th2 dichotomous culture condition

(**a**) Modification of Th1/Th2 dichotomous culture condition by titrating cytokines. Error bar denotes SD.

(**b**) Th1/Th2 balance is not related to TCR clonality. Similar percentages of $IFN\gamma^+$ and $IL\text{-}13^+$ cells were detected in wild type and OT-II TCR transgenic cells under dichotomous condition. ns, not significant, by two-tailed Student's t test. Error bar denotes SD.

(**c**) UMAP embedding of the cells collected at 64 hours from two technical replicates. Cells (dots) are colored by clusters, technical replicates, cell cycle and relative expression of naïve, Th1 or Th2 signature genes.

(**d**) Among the cells collected from 64 hours, there is exclusive expression between *Ifng* and *Il13* (top), between Th1 and Th2 program (bottom).

(**e**) Differentially expressed genes between defined Th1 and Th2 cells at 64 hours are shown with their expression profiles in each single cell as a heatmap.

Extended Data Fig. S3: Validation of the function of Th1 regulator candidates using pooled genetic screens

(**a**) The performance of *in vitro* gene perturbation strategy was evaluated by targeting positive control genes *Tbx21* and *Gata3* in Th1 and Th2 cells respectively. Naïve $CD4^+$ T cells from Rosa26-Cas9 mice were activated for 24 hours (anti-CD3 and anti-CD28), transduced with lentiviral-sgRNA and rested for 2 days with IL-2 for gene perturbation. The effect of each sgRNA was subsequently evaluated after 2 days *in vitro* Th1 (or Th2) polarization. **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**b**) Technical replicates show high correlation of sgRNA distribution. From *in vivo* screening, total activated ($CD44^+$) cell population was sorted as control sample and randomly split into two fractions for following steps to assess technical variations.

(**c**) All detected sgRNAs were ranked with their unique functions in regulating IFNγ expression. Delta LFC value was calculated for each sgRNA in each screening setting by comparing LFC value in distinct sorted cell populations. sgRNAs ranking in the top with lower delta LFC values in Th1 screenings are IFNγ positive regulators, while sgRNAs ranking in the bottom with higher delta LFC values in Th1 screenings are IFNγ negative regulators.

(**d**) All screen candidates were ranked based on their unique function in regulating IFNγ expression. Top-ranking IFNγ positive regulators (top left 30 genes) and negative regulators (top right 20 genes) are shown in the heatmap with their sgRNA enrichment score in distinct screening settings.

Extended Data Fig. S4: Neuropeptide CGRP and ADM modulate T cell differentiation through Ramp3

(**a**) Expression levels of RAMP family in ILC2 cells using data set from GSE136154. Error bar denotes SD.

(**b**) The expression of RAMP family genes in $CD4^+$ T cells after 4 hours *in vitro* activation with anti-CD3 and anti-CD28. Cells were collected for RNA extraction to assess the expression of receptors (*Ramp1, Ramp2, Ramp3*) with RT-qPCR. Error bar denotes SD.

(**c**) The expression levels of RAMP family in $CD8^+$ T cells after 4 hours *in vitro* activation with anti-CD3 and anti-CD28. Cells were collected for RNA extraction to assess the expression of receptors (*Ramp1, Ramp2, Ramp3*) with RT-qPCR. Error bar denotes SD.

(**d**) Expression dynamics of *Ramp3* during T cell *in vitro* differentiation under Th0, Th1 and Th2 conditions.

(**e**) Expression pattern of GPCRs during T helper cell differentiation.

(**f**) *Calcrl* gene expression shows similar expression dynamics as *Ramp3* during Th1 *in vitro* differentiation.

(**g**) *Ramp3* sgRNAs are further depleted in IFNγ^{high} cells and IL-13$^{low/neg}$ cells from both *in vitro* and *ex vivo* screens.

(**h, i**) CGRP and ADM promote Th1 / CD8$^+$ T cell differentiation but antagonize Th2 differentiation. (**h**) Naïve CD8$^+$ T cells were *in vitro* differentiated under Tc0 condition (anti-CD3 and anti-CD28) with / without CGRP Representative flow cytometry left panel, quantitation, right panel. (**i**) ADM treatment show similar effects as CGRP in both CD4$^+$ and CD8$^+$ T cells. *, $P < 0.05$; **, $P < 0.01$, ns, not significant, by two-tailed Student's t test. Error bar denotes SD.

(**j**) CGRP and ADM also inhibit IL-5 expression under Th2 condition after 4 days *in vitro* culture. Representative flow cytometry left panel, quantitation, right. *, $P < 0.05$; **, $P < 0.01$, by two-tailed Student's t test.

(**k**) Naïve CD4$^+$ T cells were cultured with Th1 / Th2 dichotomous condition for 3 days before analyzing cytokine expression by flow cytometry, (representative flow cytometry left panel, quantitation, right panel).

(**l**) CGRP induces the positive feedback loop of CGRP-RAMP3 in CD8$^+$ T cells. Isolated naïve CD8$^+$ T cells were treated with the neuropeptide for 4 hours and collected for qPCR analysis. **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**m**) CGRP inhibits Th2 differentiation in an intrinsic manner. Naïve CD4$^+$ T cells from three WT or *Calca*$^{-/-}$ mice were sorted and cultured *in vitro* with Th2 condition. Intracellular cytokine staining was performed after 3 days culture. Representative flow cytometry left panel, quantitation, right panel. Error bar denotes SD.

(**n**) CGRP modulates T helper cell differentiation *in vitro* in both an autocrine and paracrine manner. Naïve CD4$^+$ T cells were sorted from WT or *Calca*$^{-/-}$ mice for 3 days *in vitro* culture under Th1 or Th2 condition with CGRP treatment. Intracellular cytokine staining was performed to detect cytokine expression. Points represent individual mice (representative flow cytometry left panel, quantitation, right panel). *, $P < 0.05$; ns, not significant, assessed by two-tailed Student's t test. Error bar denotes SD.

Extended Data Fig. S5: Neuropeptide CGRP and ADM enhance Th1 differentiation through cAMP signaling

(**a-b**) CGRP and ADM induce cAMP signaling in T cells. (**a)** Differentially expressed genes between CGRP and Vehicle group (after 4 hours treatment) are presented in the heatmap. (**b)** Gene ontology enrichment analysis was performed with the upregulated genes in CGRP treatment group. (**c**) CGRP and ADM induce intracellular cAMP accumulation in $CD4^+$ T cells. Naïve T cells were treated with CGRP or ADM for 30 minutes and intracellular cAMP was measured using ELISA. *, $P < 0.05$; **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**d-e**) cAMP is involved in the CGRP-Ramp3 positive feedback loop. The expression of *Ramp3* (**d**) and *Calca* (**e**) are significantly induced upon dbcAMP treatment for 4 hours. NS, not significant; *, $P < 0.05$; **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**f**) Well differentiated T cells are less responsive to the CGRP / ADM treatment. *, $P < 0.05$, by two-tailed Student's t test. Fold change of *Calca* (upper), *Ramp3* (mid) and *Crem* (lower) expression relative to control. Error bar denotes SD.

(**g**) dbcAMP treatment recapitulates CGRP and ADM effects in promoting IFNγ expression in $CD8^+$ T cells. Cells were collected for intracellular cytokine staining after 3 days *in vitro* culture. Representative flow cytometry left panel, quantitation, right panel. **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**h**) Enhancement of Th1 differentiation by CGRP is abrogated with CREB inhibitor 666-15 (50 μM), (representative flow cytometry left panel, quantitation, right panel). *, $P < 0.05$; **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**i**) Enhancement of Th1 differentiation by ADM is abrogated with CREB inhibitor 666-15 (50 μM). Cells were collected for intracellular cytokine staining after 3 days *in vitro* culture. Representative flow cytometry left panel, quantitation, right panel. **, $P < 0.01$, by two-tailed Student's t test.

(**j-k**) Enhancement of Th1 differentiation by CGRP (**j**) and ADM (**k**) is blocked with adenylyl cyclase inhibitor SQ22536 (20 μM). Cells were collected for intracellular cytokine staining after 3 days *in vitro* culture. **, $P < 0.01$, by two-tailed Student's t test.

Extended Data Fig. S6: CGRP-Ramp3-cAMP loop enhances Th1 differentiation through epigenomic remodeling and activating STAT1

(**a**) Th1 pioneer program was induced by CGRP and ADM. Isolated naïve $CD4^+$ T cells were treated with CGRP or ADM for 4 hours and collected for qPCR analysis. **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**b**) Th1 pioneer program was induced by cAMP in a dose dependent manner. Isolated naïve $CD4^+$ T cells were treated with dbcAMP for 4 hours and collected for qPCR analysis. **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**c**) Categories of all cis-element open chromatin regions detected in ATAC-seq data of Th1 and Th2 cells.

(**d**) Shared and unique Th1/Th2 differentially accessible chromatin regions were identified in the CGRP treated and control group.

Extended Data Fig. S7: CGRP-RAMP3 axis enhances antigen-specific Th1 and Tc1 response during LCMV Armstrong infection.

(**a**) Co-localization of beta-III tubulin and CGRP in the spleen of WT and $Calca^{-/-}$ mice. Scale bar, 10 μm.

(**b, c**) IFNγ expression was restrained in $CD8^+$ T cells of *Calca*$^{-/-}$ mice. Splenocytes were collected from spleen at Day8 post Armstrong infection, followed by restimulation with PMA / Ionomycin for another 4 hours for intracellular cytokine staining within (**b**) total $CD8^+$ T cells and (**c**) antigen specific $CD8^+$ T cells (LCMV GP_{33-41} $tetramer^+$). Representative flow cytometry left panel, quantitation, right panel. **, $P < 0.01$, ns, not significant, by two-tailed Student's t test. Error bar denotes SD.

(**d**) Gating strategy of distinct cell types in spleen upon LCMV Armstrong infection. *, $P < 0.05$, by two-tailed Student's t test. Error bar denotes SD.

(**e**) Flow cytometric quantification of immune cells in spleen from *Calca*$^{-/-}$ and WT mice at day 8 post LCMV Armstrong infection. *, $P < 0.05$, by two-tailed Student's t test. Error bar denotes SD.

(**f**) At steady state, there is no difference on Th1 or Tc1 differentiation between *Ramp3*$^{-/-}$ and WT mice. ns, not significant, by two-tailed Student's t test.

(**g**) At steady state, *Ramp3*$^{-/-}$ mice have similar spleen size as WT mice. ns, not significant, by two-tailed Student's t test. Error bar denotes SD.

(**h, i**) Tc1 response was restrained in $CD8^+$ T cells of *Ramp3*$^{-/-}$ mice upon LCMV Armstrong infection without affecting cell activation. At Day8 post Armstrong infection, splenocytes were

restimulated with PMA / Ionomycin for intracellular cytokine expression analyses within (**h**) total $CD8^+$ T cells and (**i**) antigen-specific $CD8^+$ T cells (LCMV GP_{33-41} $tetramer^+$). Representative flow cytometry left, quantitation, right *, $P < 0.05$; **, $P < 0.01$, ns, not significant, by two-tailed Student's t test. Error bar denotes SD.

(**j**) Antigen-specific Th1 response was reduced in *Calca*$^{-/-}$ mice during LCMV Armstrong infection. Splenocytes were collected from spleen at Day8 post Armstrong infection, followed by restimulation with LCMV GP_{61-80} for another 4 hours for intracellular cytokine staining. Representative flow cytometry left panel, quantitation, right *, $P < 0.05$; **, $P < 0.01$, by two-tailed Student's t test. Error bar denotes SD.

(**k**) RAMP1 did not show function in regulating T cell response during LCMV Armstrong infection. At Day8 post Armstrong infection, splenocytes were restimulated with PMA / Ionomycin (upper) or LCMV GP_{61-80} / LCMV GP_{33-41} (lower) for intracellular cytokine expression analyses. ns, not significant, by two-tailed Student's t test. Error bar denotes SD.

Supplementary Table S1. Dynamic kmeans clusters during T helper cell differentiation.

Supplementary Table S2. DEGs of cell clusters during LCMV Armstrong infection, related to Figure 1G.

Supplementary Table S3. sgRNA-sequences in CRISPR screens.

Supplementary Table S4. sgRNA sequencing depth from CRISPR screens.

Supplementary Table S5. Oligo sequences for qPCR and individual CRISPR-KO.

Supplementary Table S6. Differential expression results for CGRP versus Vehicle in Th1 and Th2.

Supplementary Table S7. Differential chromatin accessibility results for CGRP versus Vehicle in Th1 and Th2.

Supplementary Table S8. Differential expression results for Th1 versus Th2 in CGRP and Vehicle.

Supplementary Table S9. Differential chromatin accessibility results for Th1 versus Th2 in CGRP and Vehicle.