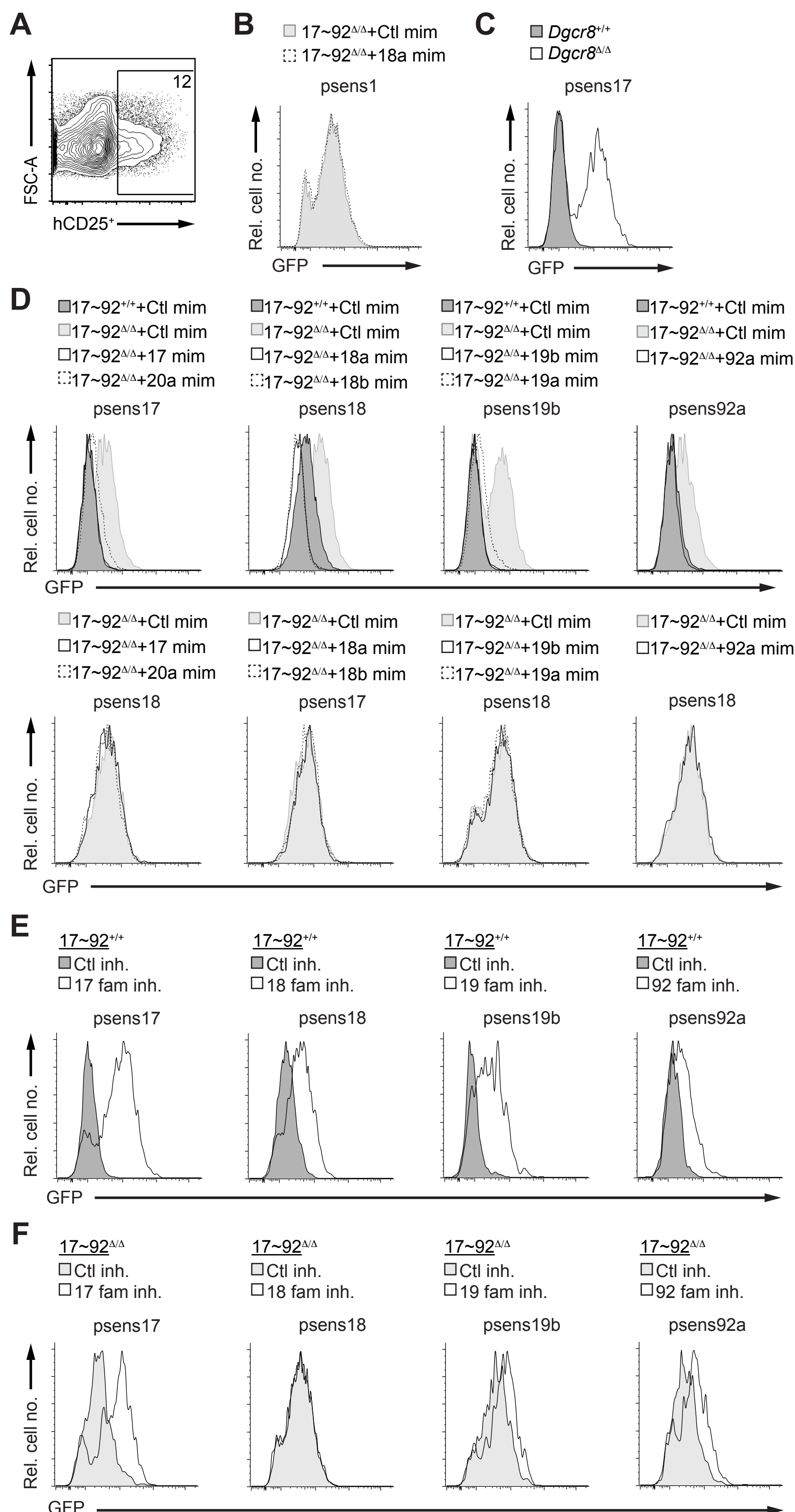
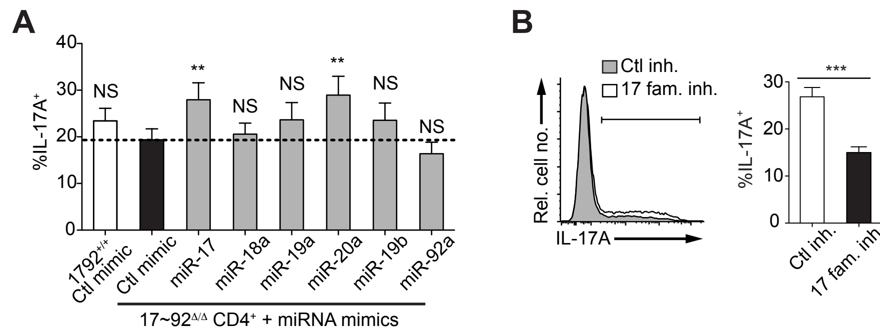
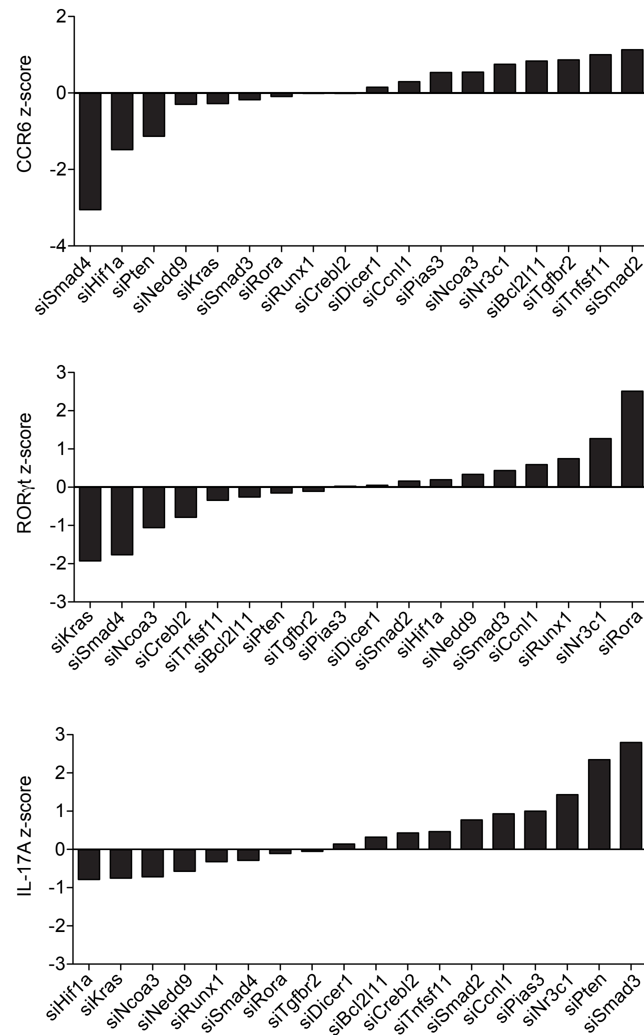
Montoya_SupFig1



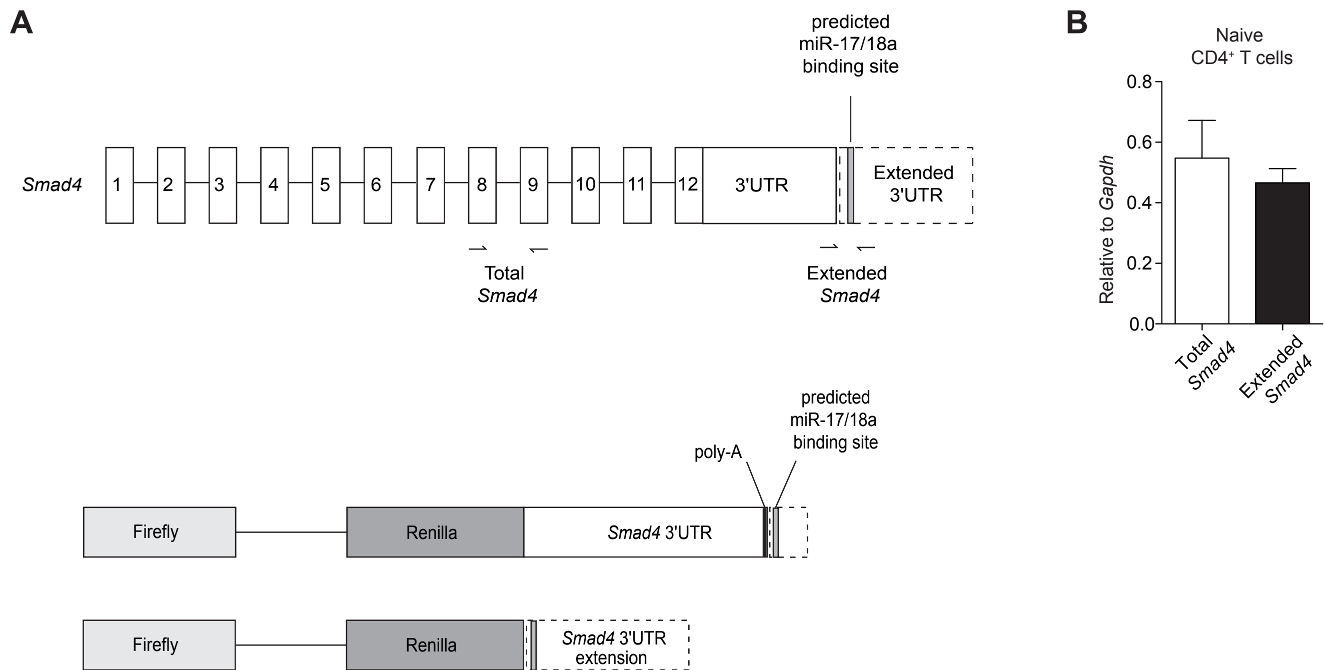
Supplementary Figure 1. Specific miRNA sensors reveal residual miR-17 and miR-92 activity in miR-17~92-deficient $CD4^+$ T cells. (**A**) Pre-gating strategy for murine $CD4^+$ T cells retrovirally transduced with MSCV-PGK-hCD25 miRNA sensors. Successfully transfected cells stain positive for human CD25. (**B**) Murine $17\sim92^{\Delta/\Delta}$ $CD4^+$ T cells transduced with a retroviral sensor expressing EGFP with 4 perfectly complementary binding sites for miR-1 (psens1) and transfected with control mimic (Ctl mim) or miR-18a mimic (18a mim). Gated on live $CD25^+$ cells. miR-1 is not expressed in $CD4^+$ T cells, thus representing maximum GFP expression of retroviral sensors. (**C**) $Dgcr8^{+/+}$ and $Dgcr8^{\Delta/\Delta}$ $CD4^+$ T cells transduced with retroviral sensors expressing EGFP with 4 perfectly complementary binding sites for miR-17 (psens17) in the 3'UTR and analyzed by flow cytometry. (**D**) Histograms show EGFP fluorescence in $17\sim92^{+/+}$ and $17\sim92^{\Delta/\Delta}$ $CD4^+$ T cells transduced with the indicated retroviral miRNA sensors (psens) and transfected with the indicated control (Ctl) and miR-17~92 cluster miRNA mimics. Results demonstrate the sensitivity of each sensor for its corresponding miRNA family (top row) and the specificity of each miRNA mimic (bottom row). (**E, F**) $17\sim92^{+/+}$ $CD4^+$ T cells (**E**) or $17\sim92^{\Delta/\Delta}$ $CD4^+$ T cells (**F**) were transduced with miR-17 (psens17), miR-18a (psens18a), miR-19b (psens19b) or miR-92a (psens92a) sensors and transfected with control inhibitor (Ctl inh.) and miR-17 family inhibitor (17 fam inh.), miR-18 family inhibitor (18 fam inh.), miR-19 family inhibitor (19 fam. inh.) or miR-92 family inhibitor (92 fam. inh.). Data are representative of four (A-D, F) or seven (E) independent experiments each with one mouse per genotype.



Supplementary Figure 2. miRNAs of the miR-17 family promote IL-17 production. (**A**) Naive 17~92$^{+/+}$ and 17~92$^{\Delta/\Delta}$ CD4^{+} T cells were cultured under Th17-polarizing conditions and transfected on day 2 with control miRNA mimics (Ctl mimic) or miRNA mimics of the individual miR-17~92 cluster members. Cells were analyzed by flow cytometry on day 3.5 for IL-17A production after restimulation with PMA/ionomycin. Gated on live CD4^{+} T cells. (**B**) Flow cytometric analysis of IL-17A expression of 17~92$^{+/+}$ CD4^{+} T cells polarized under Th17-conditions and transfected with control inhibitor (Ctl inh.) or miR-17 family inhibitor (17 fam. inh.) on day 2, and analyzed on day 3.5. Representative histograms show IL-17 production after restimulation with PMA/ionomycin and the frequency of IL-17A^{+} cells is quantified in the bar graph. **P<0.01 and ***P<0.001 (one-way ANOVA with Dunnett's multiple comparison post-test comparing all columns to control miRNA mimic-transfected 17~92$^{\Delta/\Delta}$ CD4^{+} T cells (A) or two-tailed paired t-test with pre-assigned littermate pairs (B)). Data are pooled from five (A) or three (B) independent experiments, with one or two to three mice per experiment, respectively (mean and s.e.m.).



Supplementary Figure 3. siRNA screen identifies functionally relevant miR-18a target genes. Primary screen of miR-18a target genes, presented as analysis of $17{\sim}92^{\Delta/\Delta}$ $CD4^+$ T cells cultured under Th17-polarizing conditions and transfected with an siRNA non-targeting control or 18 different targeting pools each comprising 4 individual siRNAs. Cells were analyzed by flow cytometry on day 3.5 for surface CCR6 expression (top), intracellular RORγt expression (middle), and IL-17A production after restimulation with PMA/ionomycin (bottom). Results were normalized to negative control siRNA. z-score = x - mean/s.d., where x represents the RORγt gMFI or percentage of $CCR6^+$ or $IL\text{-}17A^+$ cells for each siRNA treatment and the mean is across all treatments.



Supplementary Figure 4. Smad4 extended transcript is expressed in CD4⁺ T cells. (**A**) Positions of qPCR primers used for total *Smad4* and extended *Smad4* transcript quantification (top). Dual luciferase constructs for the annotated *Smad4* 3'UTR and the *Smad4* 3'UTR extension (bottom). Bolded line indicates polyA signal sequence at the end of the annotated 3'UTR. Dashed lines represent the *Smad4* extended transcript supported by human and mouse EST (expressed sequence tag) data. (**B**) mRNA expression of *Smad4* and the *Smad4* extended transcript in naive $CD4^+$ T cells by qPCR; expression was normalized to Gapdh in each sample. Data in (B) are pooled from eight independent experiments with one to three mice per experiment (mean and s.e.m).