iScience, Volume *25*

Supplemental information

Forced expression of the non-coding RNA miR-17∼92 restores activation and function in CD28-deficient $CD4^+$ T cells

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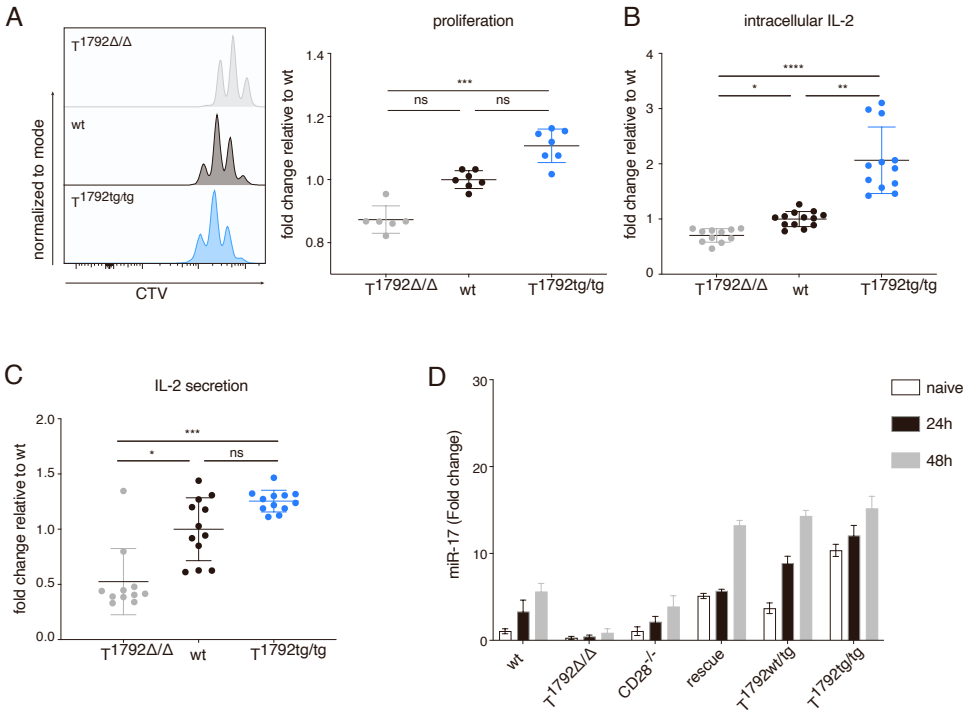

Figure S1. MiR-17~92 regulates T cell proliferation and Interleukin-2 production, Related to Figure 1
A) Proliferation of CTV labelled $T^{1792\Delta/\Delta}$ (grey), wt (black) and $T^{1792tg/tg}$ (light blue) $CD4^+$ T cells activated for 48h with plate-bound anti-CD3/anti-CD28 mAbs. B) Quantification of flow cytometric intracellular IL-2 staining in $CD4^+$ cells stimulated for 3h with PMA/Iono/BFA. C) IL-2 secretion measured by ELISA in culture supernatants at 48h. Data represent 2-3 independent experiments with 3-4 biological replicates per group. D) miR-17 expression of different genotypes and timepoints as indicated, assessed by quantitative PCR. Data from an experiment with 3-9 biological replicates per condition. Error bars show mean ±SD, Dunn's multiple comparison test, p values: ns=not significant, *<0.05 **<0.002 ***<0.0002 ****<0.0001.

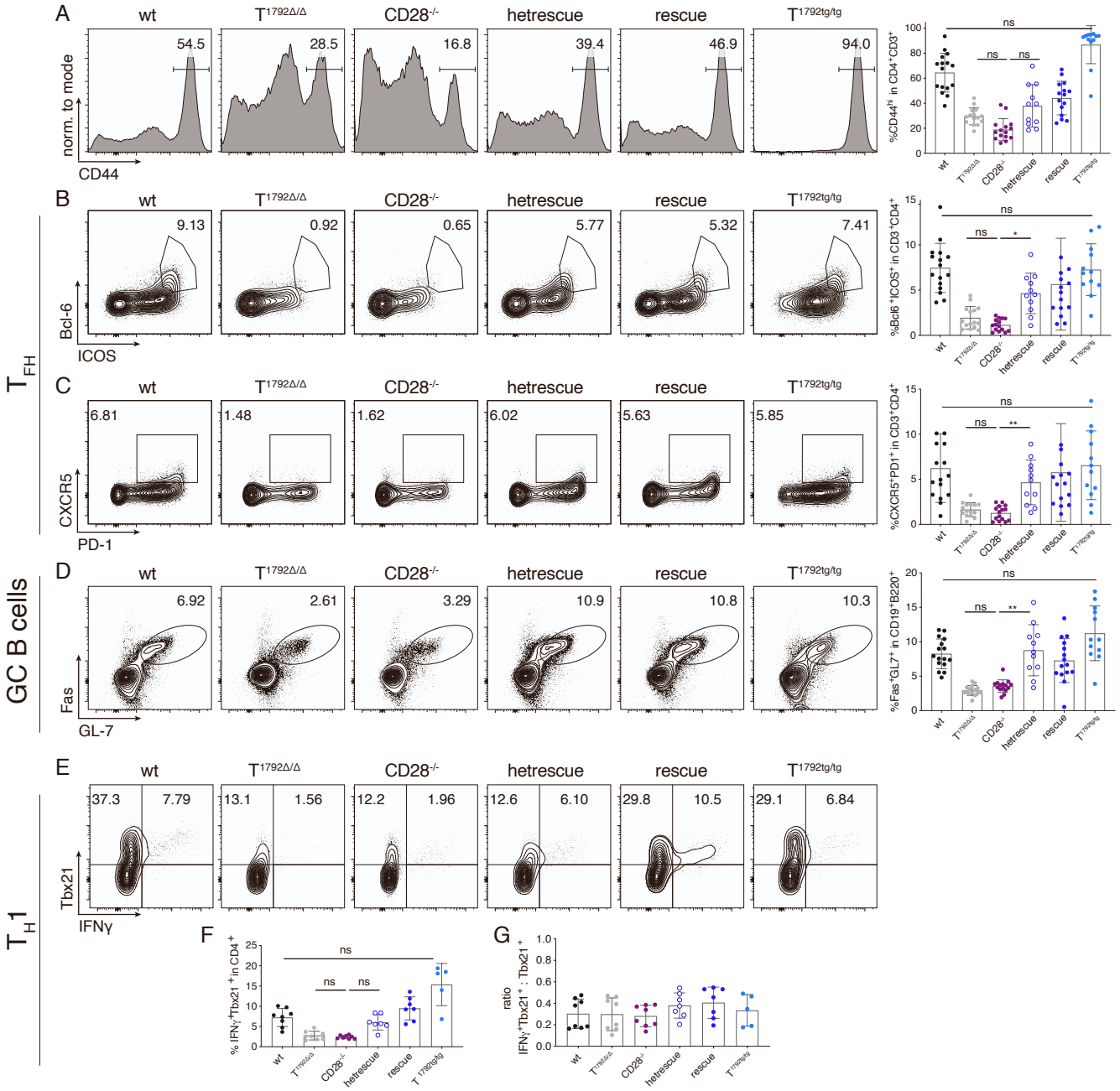

Figure S2. MiR-17~92-mediated gene regulation is very sensitive to precise miR-17~92 expression in vivo, Related to Figure 2

6-8 week old mice were infected with $2*10^5$ PFU LCMV Armstrong and spleens were analyzed at d8 post infection. wt (black), $T^{1792\Delta/\Delta}$ (grey), $CD28^{-/-}$ (purple), $CD28^{-/-}$ with heterozygous transgenic miR-17~92 expression (hetrescue; dark blue empty circles), $CD28^{-/-}$ with homozygous transgenic miR-17~92 expression (rescue; dark blue), $T^{1792tg/tg}$ (light blue). A-D) represent data from 4 independent experiments with 3-4 mice per group, gated on viable $CD4^+CD3^+$ or viable $CD19^+B220^+$ cells. Representative FACS plots and quantification of relative numbers of A) CD44 expression, B) quantification of % $Bcl6^+ICOS^+$ (T_{FH}), C) % $CXCR5^+PD\text{-}1^+$ (T_{FH}) and D) % Fas^+GL7^+ (GC B cells). E-G) Splenocytes were restimulated with GP-64 and BFA for 4h and investigated for T_H1 phenotype, pre-gated on viable $CD3^+CD4^+$ cells. Shown are 2-3 independent experiments with 3-4 biological replicates per group. E) representative FACS plots of Tbx21 and IFNγ expression F) quantification of relative numbers of $Tbx21^+IFN\gamma^+$ $CD3^+CD4^+$ cells. G) ratio of $Tbx21^+IFN\gamma^+$ to total $Tbx21^+$ cells. Error bars represent mean with SD, Dunn's multiple comparison test, p values: ns=not significant, *<0.0332, **<0.0021, ***<0.0002, ****<0.0001.

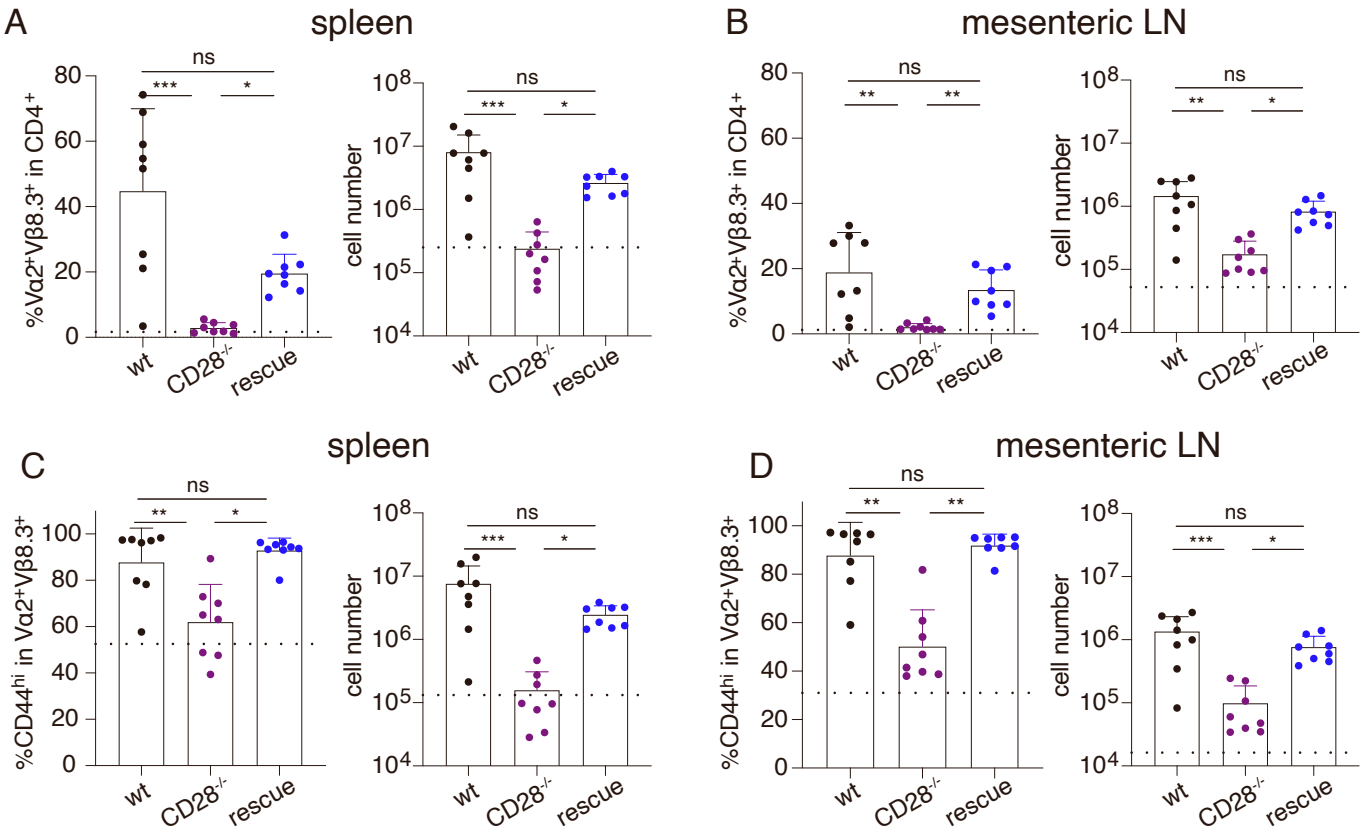

Figure S3. Restoration of T cell activation of CD28-deficient T cells by miR-17~92 is cell intrinsic, Related to Figure 3

Adoptive transfer of naïve SMARTA^{+} CD4^{+} T cells into CD28$^{-/-}$ hosts, subsequent LCMV Armstrong infection and analysis of organs at d8 post infection. Donor genotypes wt (black), CD28$^{-/-}$ (purple), rescue (dark blue). Dotted line indicates recipient's intrinsic Vα2^{+}Vβ8.3^{+} population measured in a non-transferred host. Vα2^{+}Vβ8.3^{+} cells in viable CD4^{+} population from spleen (A) and mesenteric LN (B). CD44 expression in Vα2^{+}Vβ8.3^{+} population from spleen (C) and mesenteric LN (D). 2 independent experiments, 4 recipients per group. Error bars represent mean ±SD, Dunn's multiple comparison test, p values: ns=not significant, *<0.05, **<0.002, ***<0.0002.

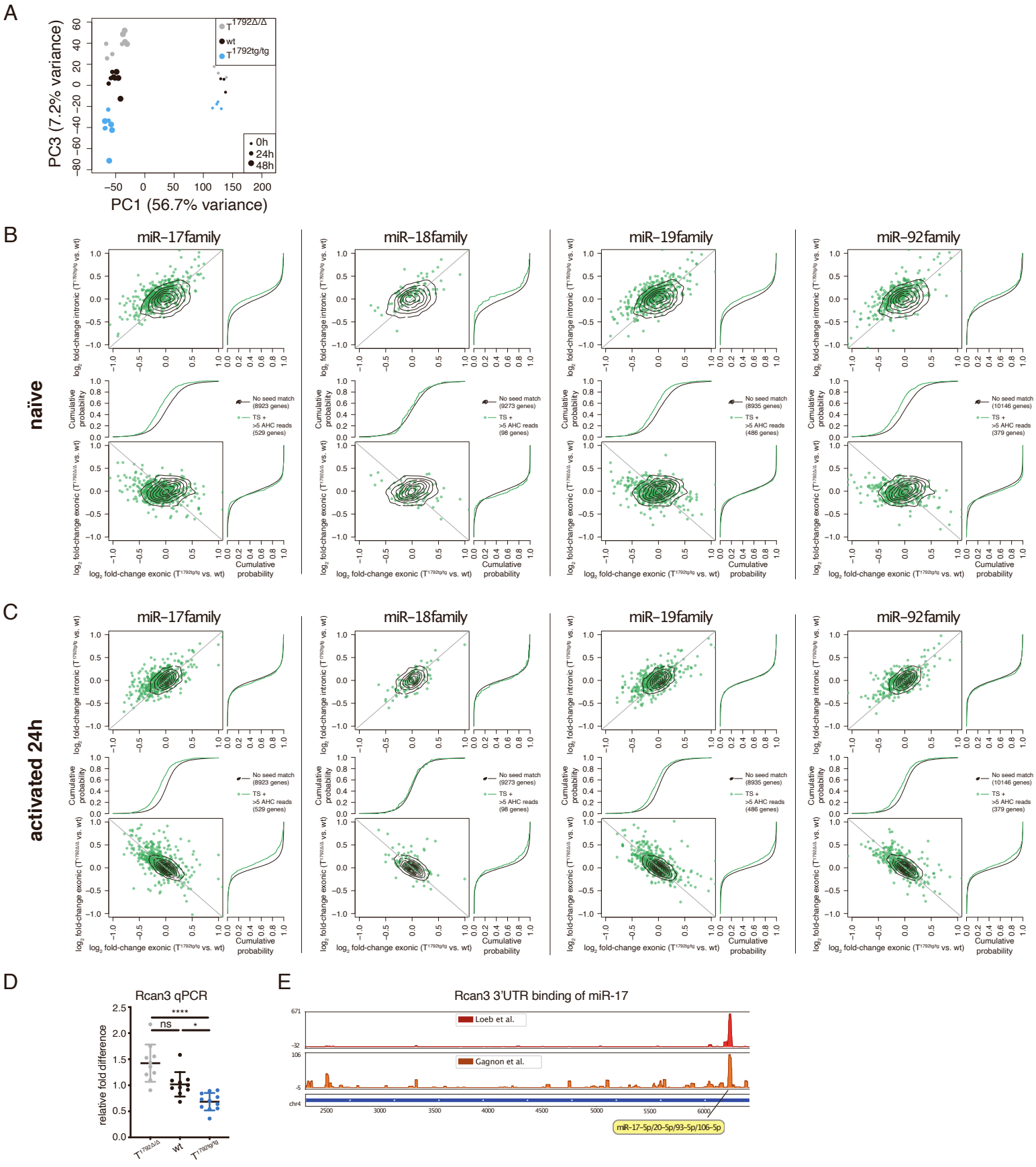

Figure S4. MiR-17~92 shapes the transcriptome after CD4+ T cell activation, Related to Figure 4

Naïve CD4+ T cells from $T^{1792\Delta/\Delta}$ (grey), wt (black) and $T^{1792tg/tg}$ (light blue) mice were activated with plate-bound αCD28 and αCD3 mAbs for 0, 24h and 48h. Total RNA was extracted for bulk sequencing. A) PCA (PC1 vs. PC3) based on the 25% most variable genes. B, C) For each seed family, dot plots compare the log2 value of the exonic expression ratio for each gene in $T^{1792tg/tg}$ vs. wt (x-axis) versus either exonic expression ratio for each gene in $T^{1792\Delta/\Delta}$ vs. wt comparison on the y-axis (bottom row) or intronic expression ratio for each gene in $T^{1792tg/tg}$ vs. wt (top row) in naïve (B) and 24h post activation (C). Each ratio is compared to the cumulative fraction of all log2 ratios in the corresponding comparison. Black curve: all genes of our data set without a seed match and ≤5 AHC reads; green: subset of genes with a seed sequence for the indicated seed family and >5 reads in the AHC. D) Rcan3 mRNA expression detected by qPCR 24h after activation; shown are pooled data from three independent experiments, normalized to wt. $T^{1792\Delta/\Delta}$ (grey), wt (black), $T^{1792tg/tg}$ (light blue). mRNA expression normalized to 18S rRNA. Values are means ±SD, Dunn's multiple comparison test, p values: ns=not significant, *<0.05 ****<0.0001. E) Binding sites of Argonaute 2 in 3'UTR of Rcan3 detected by HITS-CLIP; predicted miR17 binding site indicated by yellow flag. See also Suppl. Table S1.

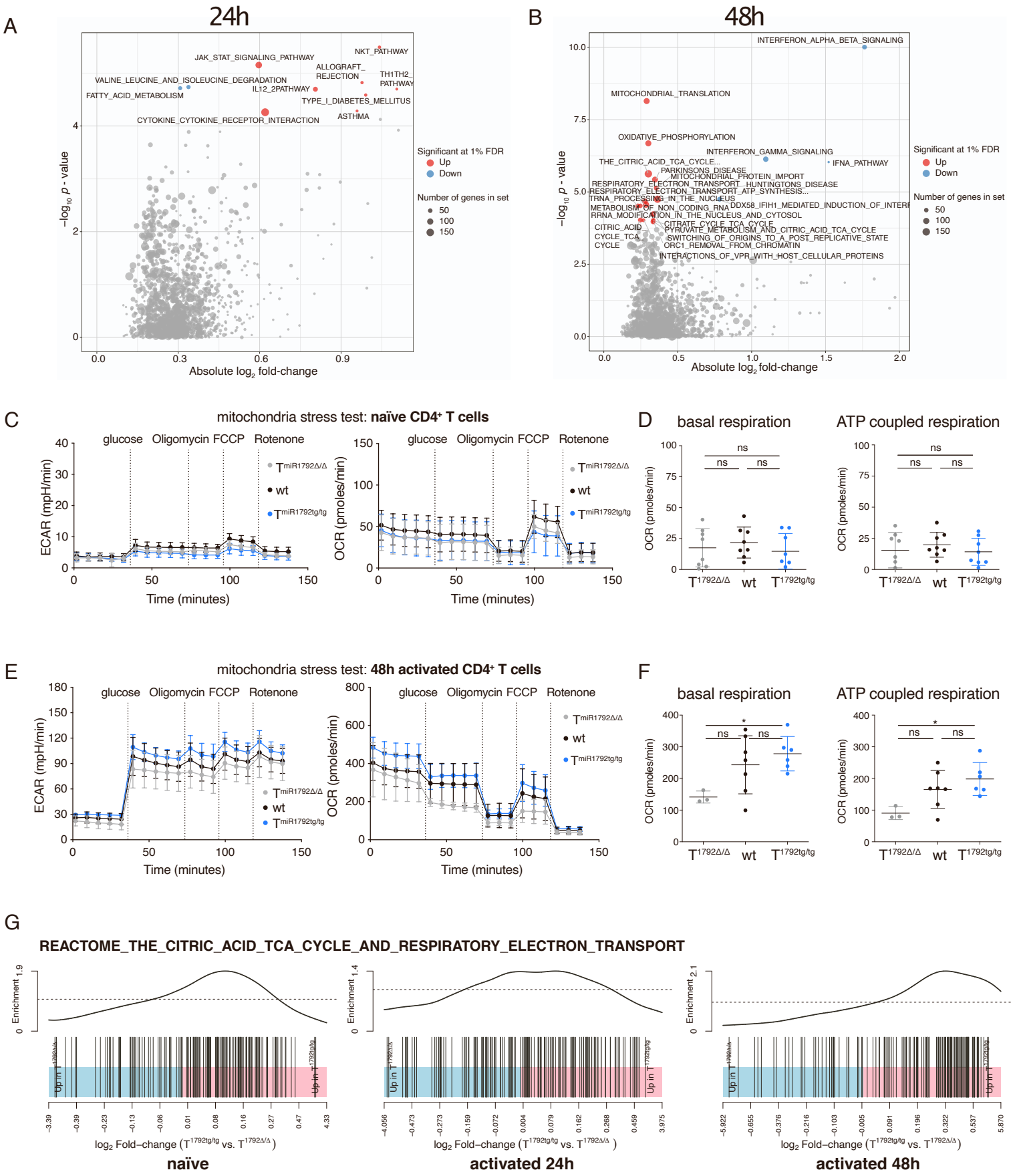

Figure S5. MiR-17~92 expression modifies metabolism in activated CD4+ T cells after 48h, Related to Figure 5

A, B) Naïve CD4+ T cells from $T^{1792\Delta/\Delta}$, wt and $T^{1792tg/tg}$ mice were activated with plate-bound αCD28 and αCD3 mAbs for 24h and 48h. Total RNA was extracted for sequencing. Volcano plot of GSEA pathway (KEGG,REACTOME,PID,BIOCARTA) enrichment for genes differentially expressed after 24h (A) and 48h (B) activation between $T^{1792\Delta/\Delta}$ and $T^{1792tg/tg}$. Red dots indicate pathway enriched for genes up regulated whereas blue dots are pathways enriched for down regulated genes. Only significantly enriched pathways (1% FDR) are colored. Dot width indicates the gene set size for each pathway. C-G) CD4+ T cells from $T^{1792\Delta/\Delta}$ (grey), wt (black), $T^{1792tg/tg}$ (light blue) were assessed for their metabolic activity by mitochondria stress test with the seahorse machine. C) Mitochondria stress test measured with a 96-well seahorse in naïve CD4+ T cells. 2 experiments with 3-4 biological replicates per group and experiment are shown. D) Basal respiration and ATP coupled respiration in naïve CD4+ T cells E) Mitochondria stress test measured in CD4+ T cells activated for 48h. F) Basal respiration and ATP coupled respiration in activated CD4+ T cells. Pooled 3-8 biological replicates from 4 experiments are shown. Tukey's multiple comparison test, p values: *<0.05. G) RNA sequencing data shows an enrichment of genes associated with TCA. Shown is gene set REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT enrichment with genes differentially expressed between $T^{1792\Delta/\Delta}$ and $T^{1792tg/tg}$ in naïve, 24h and 48h post activation. Colors indicate fold change direction with blue being upregulated in $T^{1792\Delta/\Delta}$ and red downregulated.

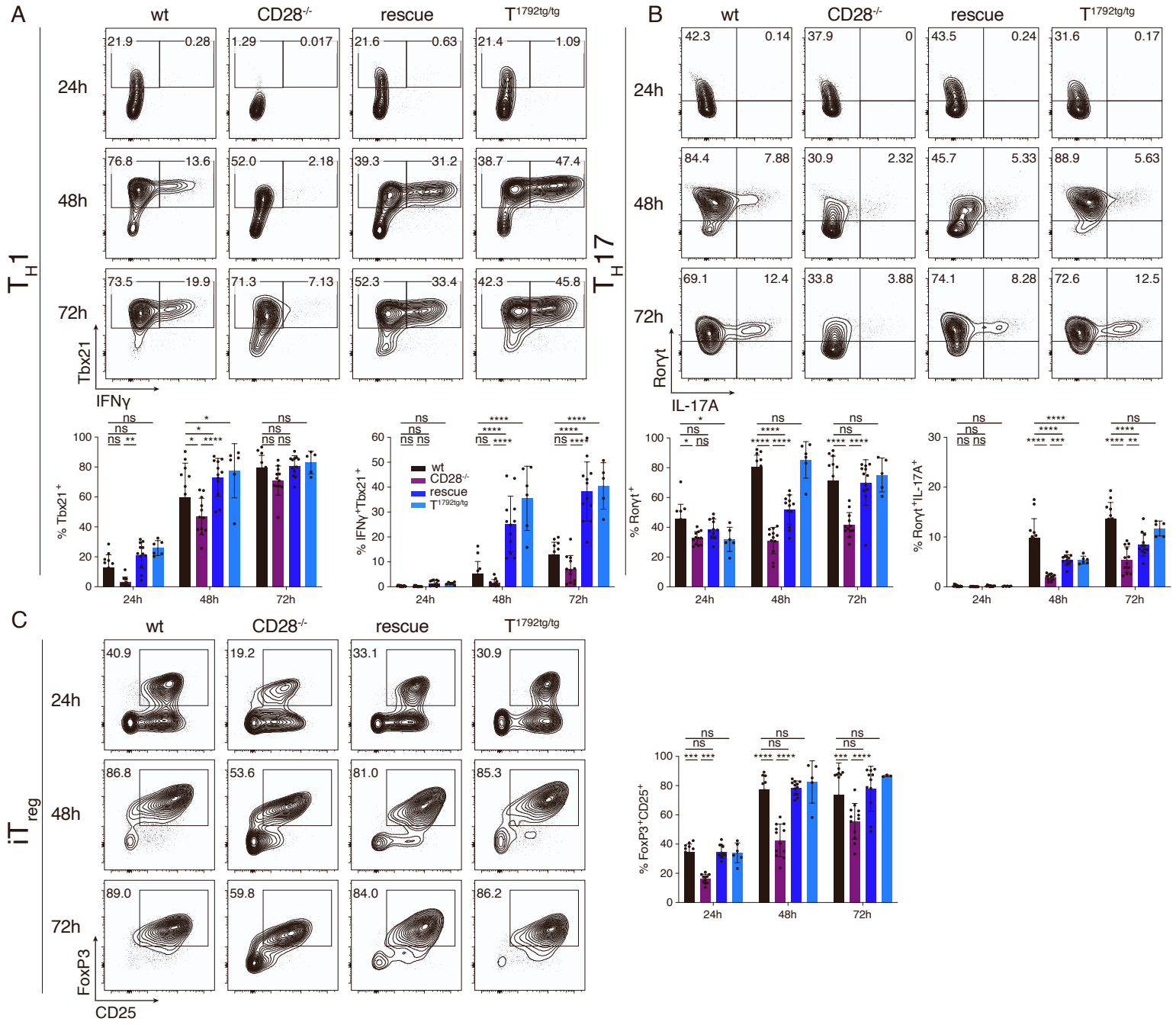

Figure S6. Transgenic miR-17~92 functionally corrected the defects of CD28-/- T cells to differentiate into TH1, TH17 and iTreg in vitro, Related to Figure 5

Naïve CD4+ T cells were activated for 24h, 48h, and 72h with plate-bound anti-CD28 and anti-CD3 under in vitro skewing conditions for T_H1, T_H17, and iT_{reg} differentiation. wt (black), $CD28^{-/-}$ (purple), rescue (dark blue) and $T^{1792tg/tg}$ (light blue). A) T_H1 differentiation assessed by IFNγ/Tbx21 staining in viable CD4+ T cells B) T_H17 differentiation assessed by IL-17A/Rorγt staining in viable CD4+ T cells C) iT_{reg}s characterized by CD25/ Foxp3 expression. Data from 2 independent experiments.

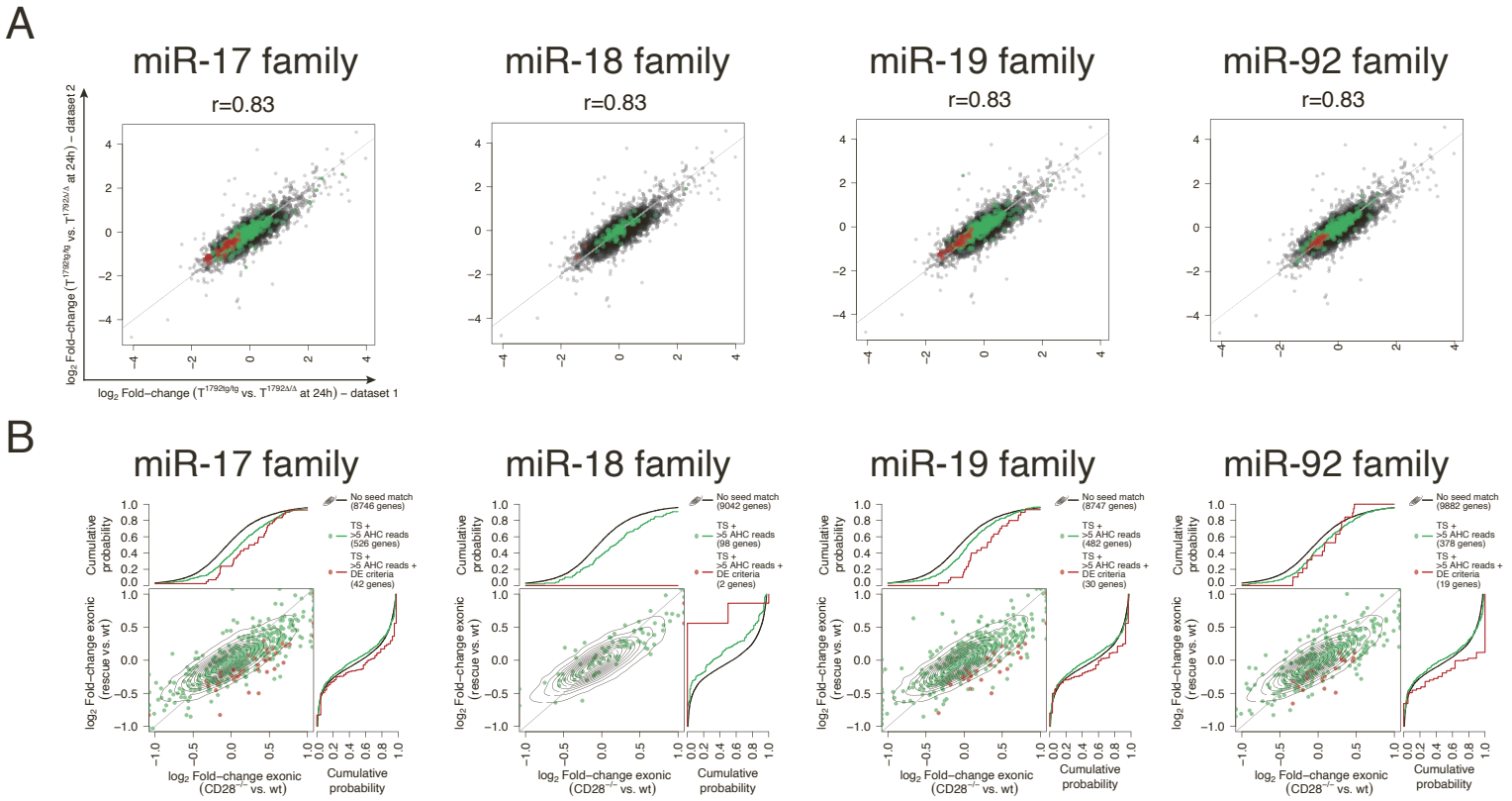

Figure S7. All miR-17~92 seed families are necessary for CD28-induced gene repression, Related to Figure 6

Total RNA was extracted for sequencing from CD4$^+$ T cells. A) Correlation of gene expression levels between RNA-seq experiment 1 (Fig. 4) and 2 (Fig. 6). Black: genes without seed match, <5 AHC reads and no differential expression in the second RNA sequencing. Green: genes with a conserved binding site for each indicated seed family and >5 reads in the AHC; red: genes with a conserved binding site for each indicated seed family, >5 reads in the AHC and differential expression in the second RNA sequencing data set. B) For each seed family, dot plots compare the log2 value of the exonic expression ratio for each gene in the CD28$^{-/-}$ vs. wt comparison (x-axis) versus the exonic expression ratio for each gene in rescue vs. wt (y-axis) in CD4$^+$ T cells 24h post activation. Each ratio is compared to the cumulative fraction of all log2 ratios in the corresponding comparison. Black curve: all genes of our data set without a seed match and five or less AHC reads; green: subset of genes with a seed sequence for the indicated seed family (TS) and >5 reads in the AHC; red: genes with a conserved binding site for the corresponding seed family (TS), >5 reads in the AHC and differential expression in the second RNA sequencing data set.

Table S2. List of genes rescued by miR-17~92 expression, Related to**Figure 5**

List of genes in which "rescue" T cells were more similar to wt and $T^{1792tg/tg}$ cells than $CD28^{-/-}$ and $T^{1792\Delta/\Delta}$ cells. Corresponds to genes in box in figure 7D.

Gene name
Tgtp2
Gbp6
Mir6974
Oaf
Smim3
AW112010
Coro2a
Slco3a1
Tspan9
Ly6a
H2-Q6
Slc13a3
Atf3
Cacnb3
Casp1
Casp4
Runx2
Cd44
Socs1
Cxcr3
Ccr5
Eomes
H2-T23
Hbegf
Icam1
Irgm1
Cxcl10
Ifng
Igtp
Il12a
Il12rb2
Il4
Ajuba
Cxcl9
Gbp4
Penk
Ppic
Ptger3
Ell2
Rab20
Rpl29

Sox5
Serpinb6b
Serpina3g
Serpinb9
Stra6
Tcf4
Fam26f
Tgtp1
Soat2
Xlr3c
Gm4841
C1ql3
Ppp1r16b
Gbp7
Asap3
Picalm
Ccdc102a
Serpina3f
Gadd45g
Mgll
Gm4951
Ubd
Ehd2
Insl6
Batf3
Gm996
Rnf225
Brsk1
Mir18
Mir20a
AA467197
Dlc1
Irgm2
Gbp3
Espn
Ube2l6
Ly6i
Iigp1
Il21
Gm12185
Gbp10
Gm12250
4930428O21Rik
Fkbp11
Tma7
Arrdc4
Tmem35a
Fam212a

Tln2
Zbtb46
Mir17
Nuak2
Rnf19b
Abhd14b
Sytl3
Trps1
Tnfrsf25
Gm1966
Gm8989
Il10ra
Zbp1
Gbp8
Tgfbr3
Nckap5l
Gprin3
Gm18853
Gvin1
Ptprn
Parp3
Cysltr2
H2-DMa