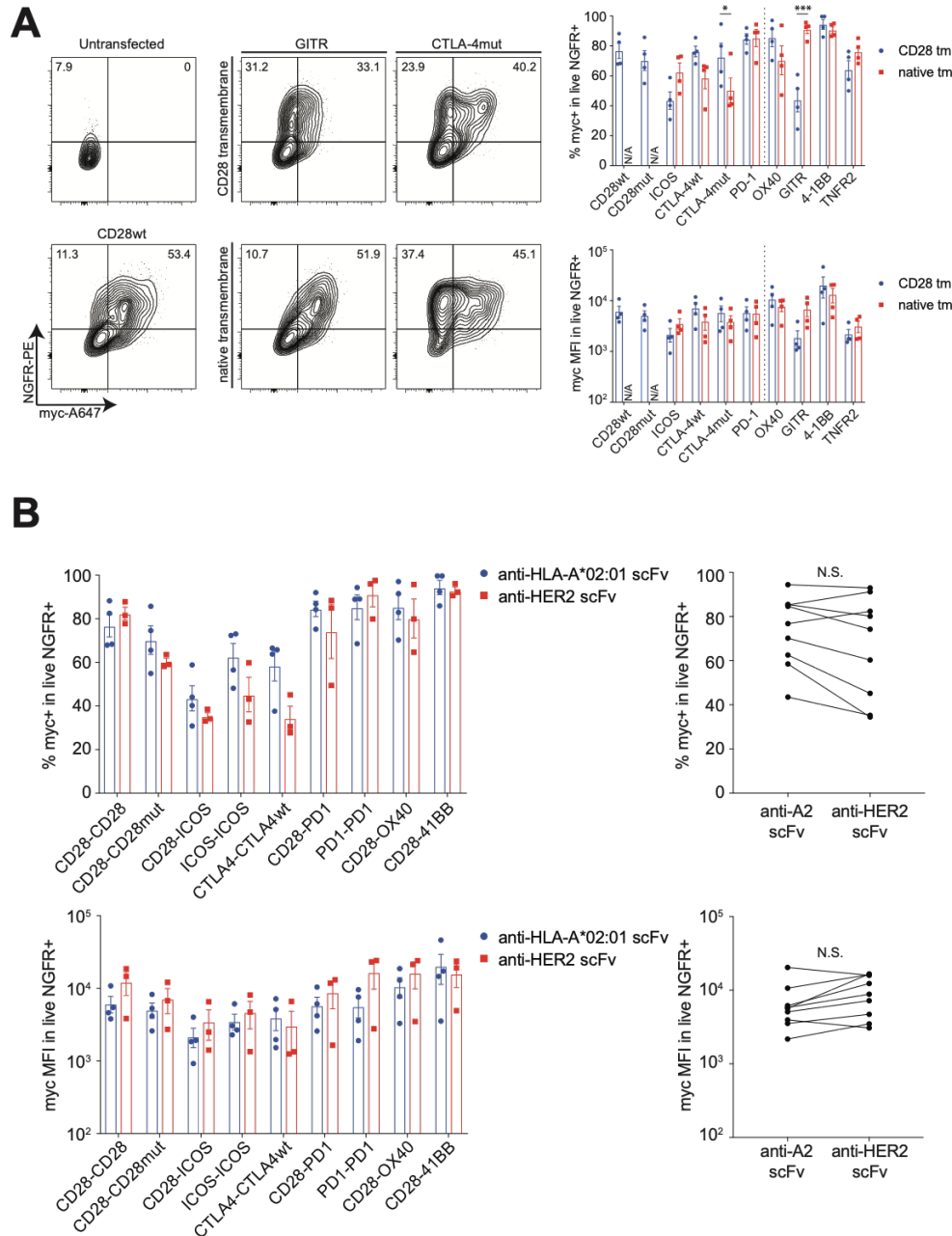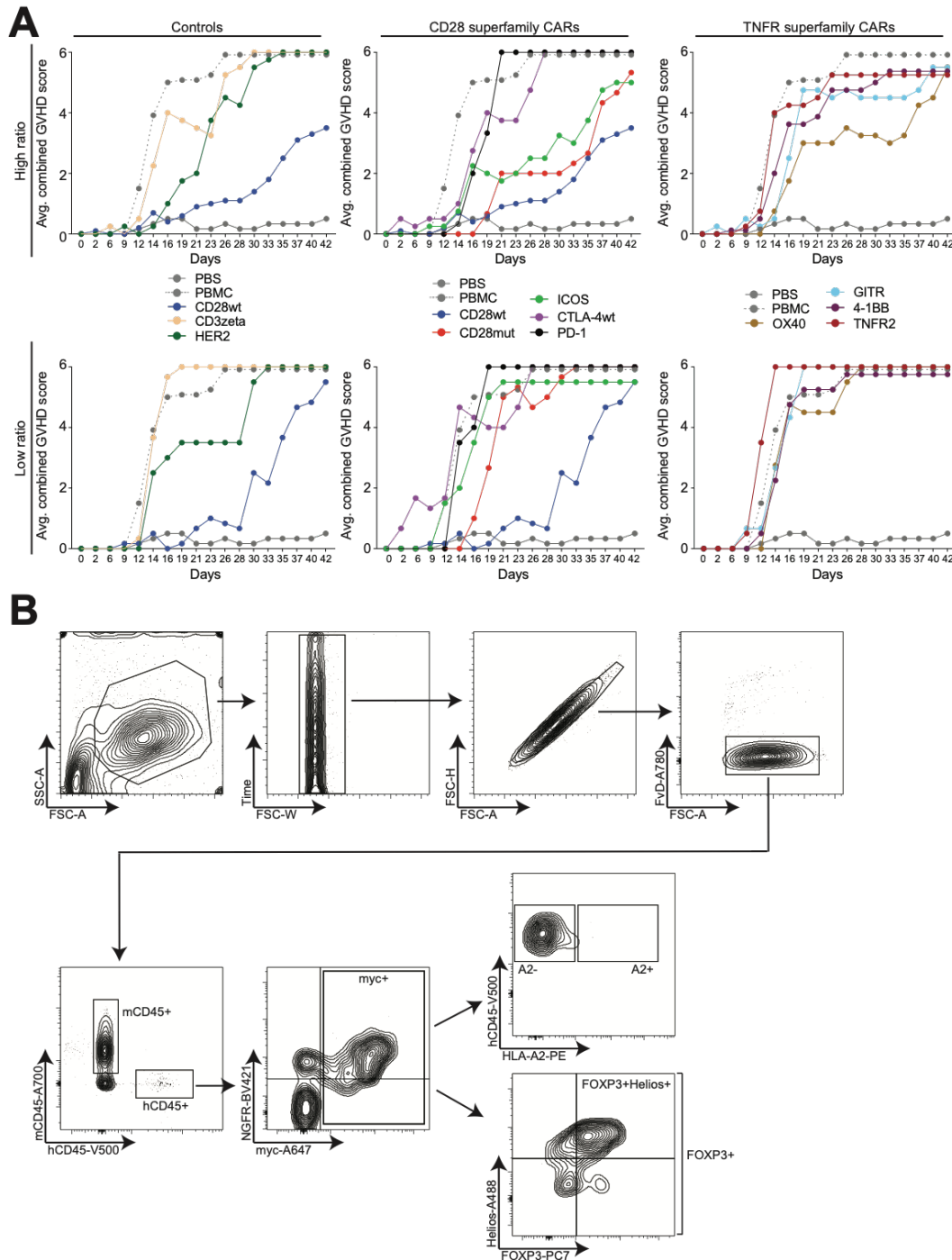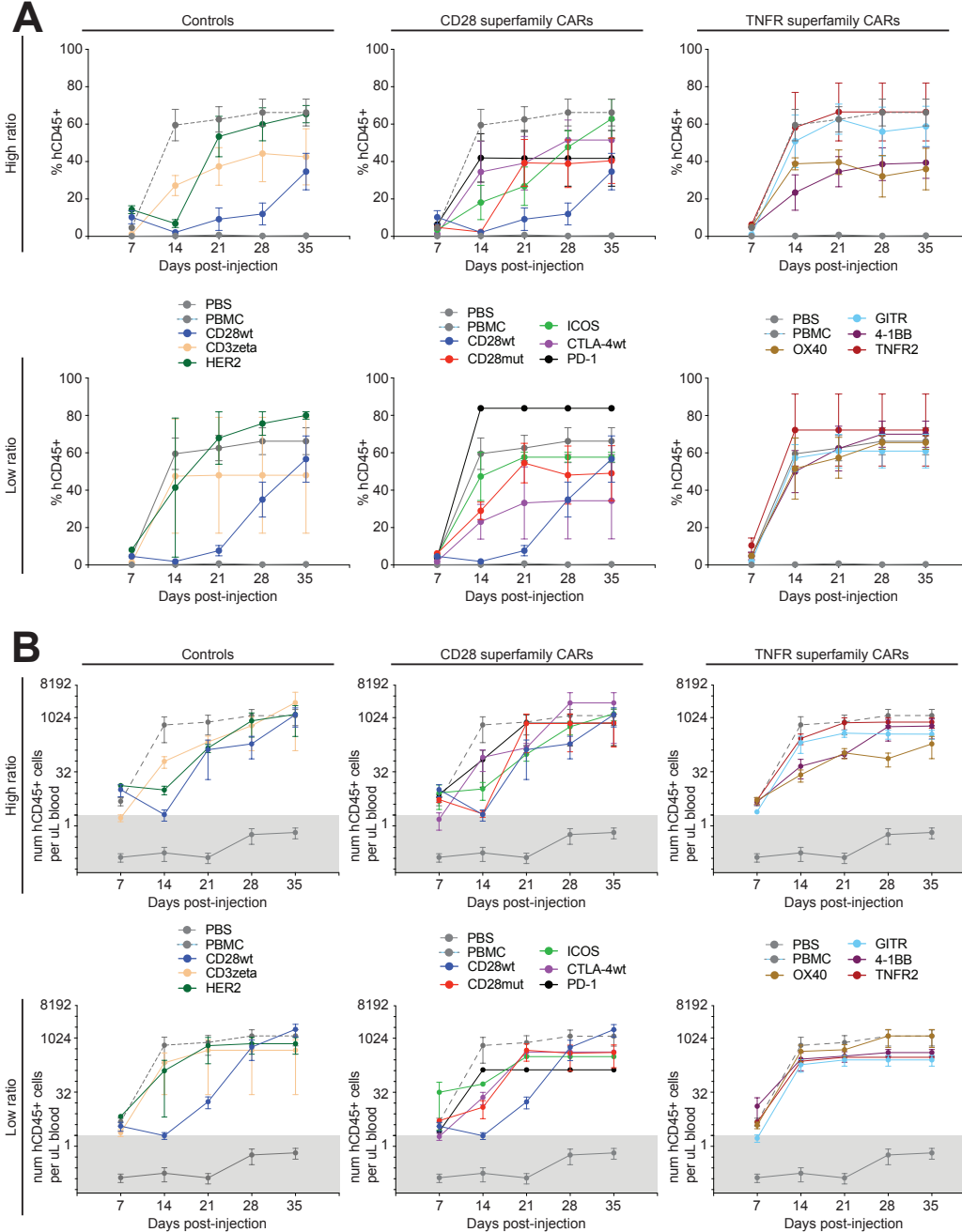# SUPPLEMENTAL FIGURES & TABLES

Supplemental Figure 1

Supplemental Figure 1. Surface expression of signaling domain CAR variants with different transmembrane domains or scFvs. (A) Anti-HLA-A2 CAR variants were generated differing in their co-stimulatory domains, with either their original (or "native") transmembrane domains or a CD28 transmembrane domain. These constructs were transiently transfected into 293T cells and assessed by flow cytometry 48 hours after transfection, for surface expression of the CAR, evidenced by myc-tag, and the transduction marker, truncated NGFR (CD271). Left: representative flow cytometry plots. Right: summarized data of percent of mean fluorescence intensity of myc-tag detection in the live NGFR+ fraction. Data are n=3-4, pooled from at least three independent experiments. **(B)** scFvs from selected anti-HLA-A2 CAR constructs from (A) were replaced with an anti-HER2 scFv, and resultant constructs were transiently transfected into 293T cells and assessed by flow cytometry 48 hours after transfection for surface expression of the CAR, evidenced by myc-tag, and the transduction marker, truncated NGFR (CD271). Top: percent myc positive cells in live NGFR+ cells. Bottom: mean fluorescence intensity of myc in live NGFR^{+} cells. Individual data (left) and summarized data (right) are shown. Statistics show one-way ANOVA with Holm-Sidak post-test comparing each transmembrane variant or scFv pair. Mean ± SEM. * $p < 0.05$, *** $p < 0.001$. "n.s." denotes not significant.

Supplemental Figure 2

Supplemental Figure 2. Supporting information for GVHD model. (A) GVHD score for mice in GVHD model. Mice were scored on a scale from 0-3 for the following factors: weight loss, skin inflammation, fur maintenance, pain, deteriorating posture. If a score of 3 in any category or a combined score of 6 occurred, the mouse was sacrificed. Plotted are the average rolling combined GVHD scores for each experimental group, split by CAR signaling-domain superfamily and Treg:PBMC ratio. If a mouse was sacrificed for a score of 3 in one category, a value of 6 was assigned. Numbers of replicates are as in Figure 2, pooled from three independent experiments. Means without error are shown. **(B)** Gating strategy for flow cytometry analysis of GVHD experiments. Gates are as marked. Gating of CD28wt CAR Tregs from a high ratio mouse at day 7 is shown as an example.

Supplemental Figure 3

**Supplemental Figure 3. hCD45 engraftment in GVHD model.** Mice were bled weekly and at experimental endpoint. Flow cytometry analysis of blood was performed to determine the blood composition. **(A)** Percent of hCD45$^+$ cell engraftment is shown over time, split by Treg:PBMC ratio and CAR signaling domain superfamily. **(B)** Absolute number of hCD45$^+$ cells per uL of blood is shown over time. Mean ± SEM.

Supplemental Figure 4
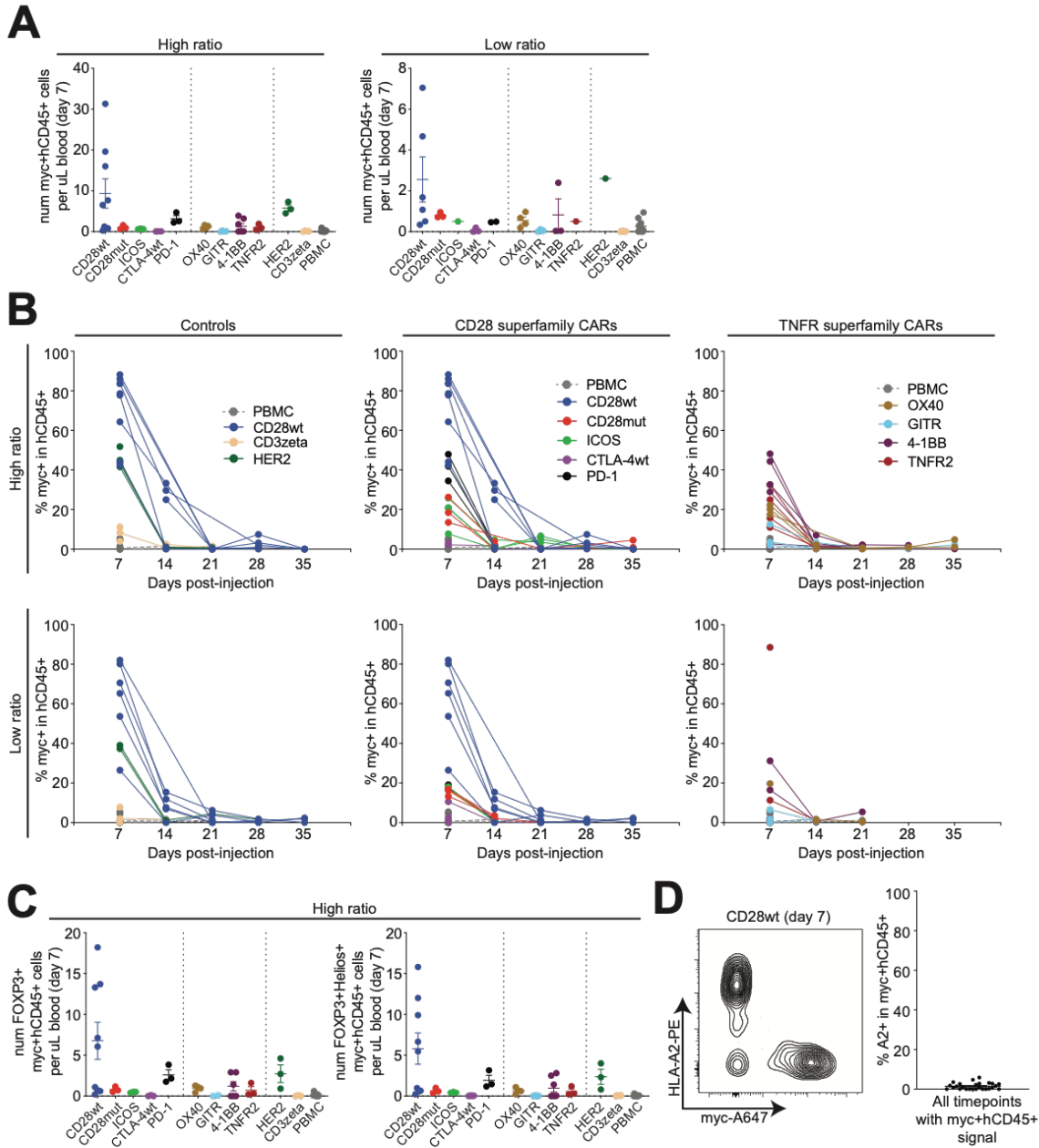
Supplemental Figure 4. Absolute number of Myc+hCD45+ cells in GVHD model. Mice were bled on day 7 as per Figure 2A. **(A)** Proportion of myc+ cells in hCD45+ subset is shown over time, split by Treg:PBMC ratio and CAR signaling domain superfamily. **(B)** Absolute number of myc+hCD45+ cells per uL of blood was determined on day 7 post-cell injection. Left: high Treg:PBMC ratio mice are shown. Right: low Treg:PBMC ratio mice are shown. **(C)** Absolute number of FOXP3+ myc+hCD45+ (left) FOXP3+Helios+myc+hCD45+ (right) cells per uL of blood was determined on day 7 post-cell injection. High Treg:PBMC ratio is shown. **(D)** Comparison of HLA-A2- and myc-expressing cells. Left: example flow plot of mutually-exclusive HLA-A2 and myc staining. Right: Summary data are shown. Each dot is the average A2+ expression of the myc+hCD45+ population at a single timepoint. Mean ± SEM.

Supplemental Figure 5
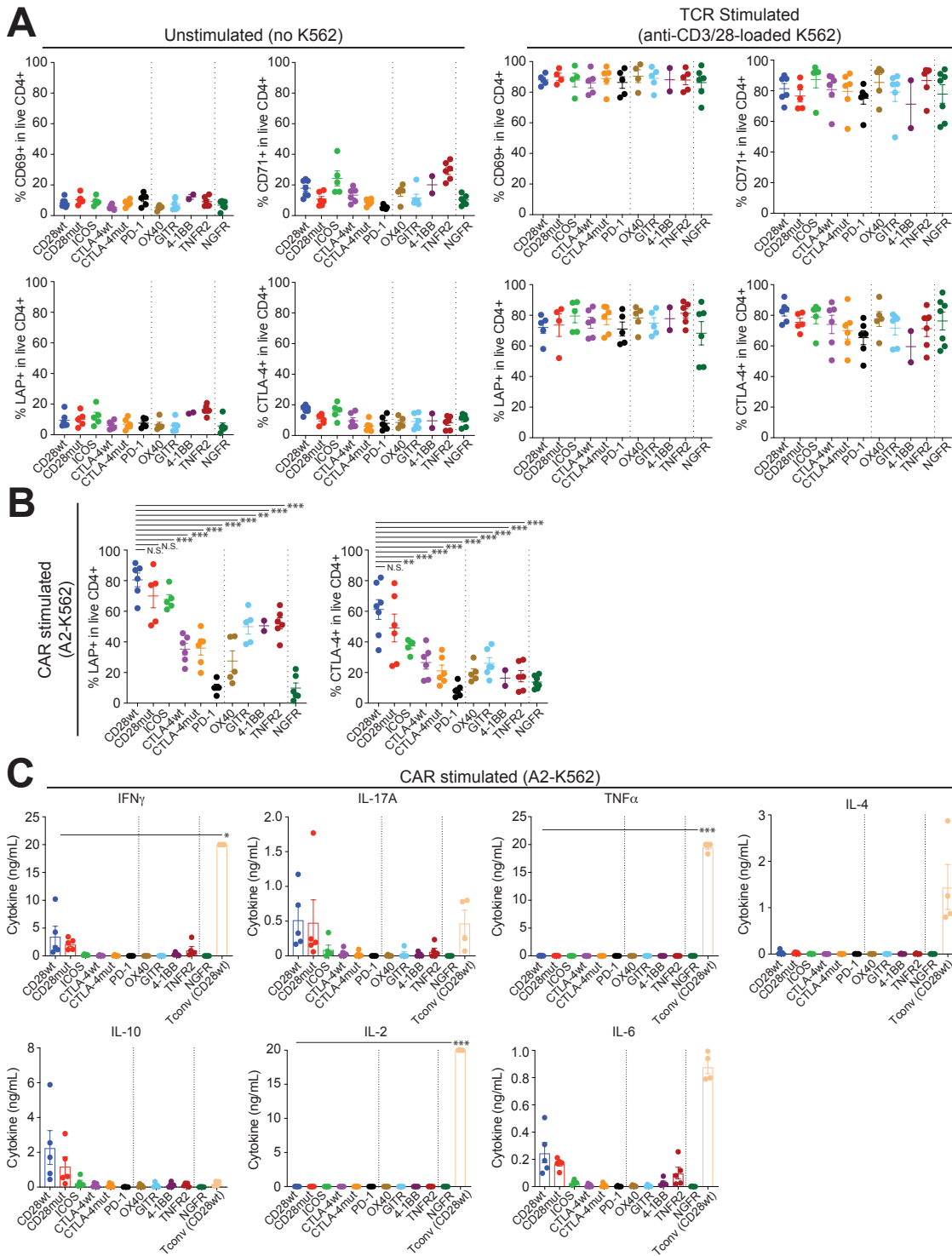
Supplemental Figure 5. Activation and cytokine production by signaling domain CAR variants on human Tregs. (A-C) Human Tregs were transduced and expanded as in Figure 1B, rested overnight in low IL-2 conditions, then co-cultured with either no K562 cells (unstimulated), irradiated K562 cells expressing HLA-A2 (CAR-stimulated) or irradiated anti-CD3/CD28-loaded K562 cells expressing CD64 (TCR-stimulated) at a ratio of 1 K562 to 2 CAR Tregs. **(A-B)** After 24 hours, percent CD69, CD71, LAP and CTLA-4 positive in live CD4 cells was determined. Summary data for **(A)** unstimulated and TCR-stimulated cells or **(B)** CAR-stimulated cells are shown. Data are n=2-7 donors, pooled from at least two independent experiments. **(C)** After 72 hours, amounts of the indicated cytokines were determined by cytometric bead array in CAR-stimulated cells. Results for all examined cytokines are expressed as a concentration in the supernatant. n=4-5 from at least two independent experiments. Statistics show one-way ANOVA with Holm-Sidak post-test comparing all constructs to CD28wt Tregs. Mean ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001. "n.s." denotes not significant.

Supplemental Figure 6
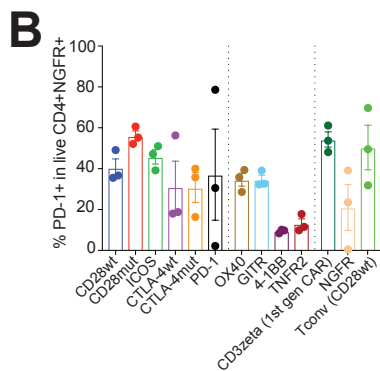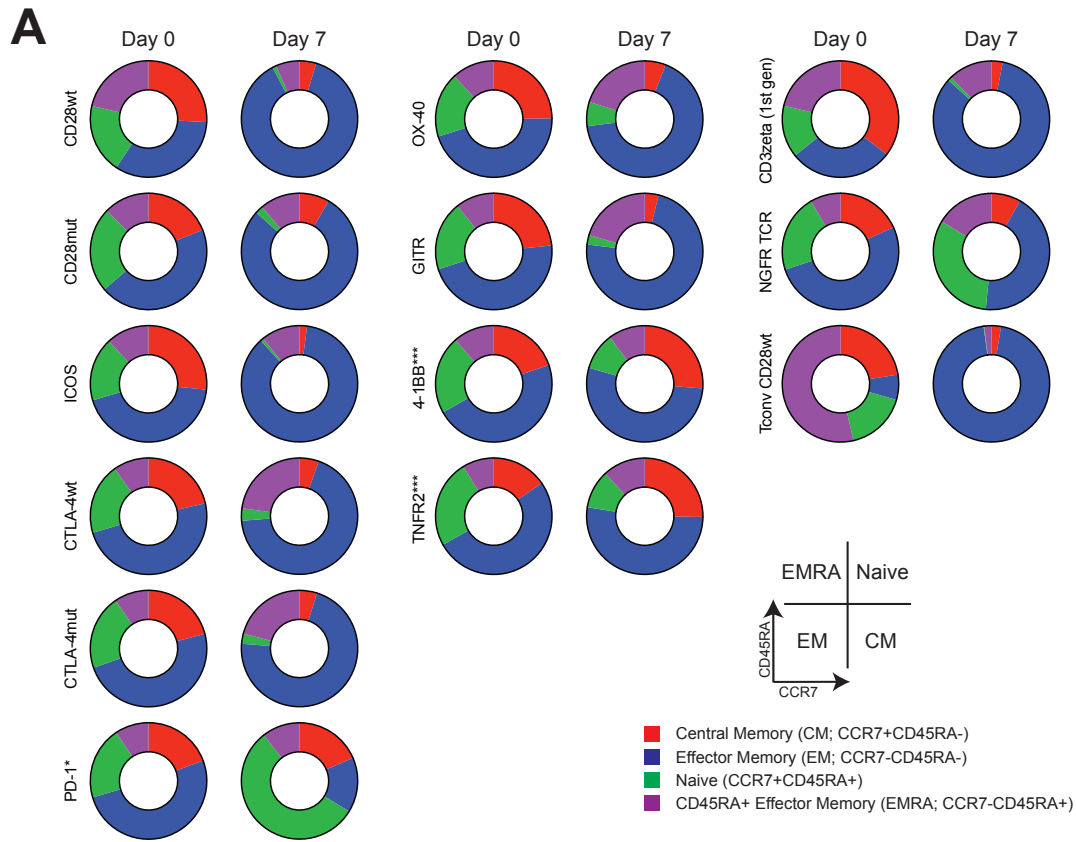
Supplemental Figure 6. Memory subset and exhaustion of signaling variant CAR Tregs after 7-day CAR-mediated expansion. Signaling-variant CAR Tregs derived from naïve Tregs were produced as in Figure 5, then co-cultured for 7 days with irradiated K562 cells expressing HLA-A2 at a ratio of 1 K562 cell to 2 CAR Tregs. **(A)** Using flow cytometry analysis of CD45RA and CCR7 expression in live CD4$^+$NGFR$^+$ cells, memory subset polarization after CAR stimulation was determined. Average subset values are shown for baseline (day 0) and day 7. Statistics show a one-way ANOVA with Holm-Sidak post-test comparing proportion of central memory cells (CD45RA$^-$CCR7$^+$) from CD28wt to all other groups. **(B)** PD-1 expression of CD4$^+$NGFR$^+$ cells. Data are from n=3-4 donors, pooled from at least 2 independent experiments. *** p < 0.001.

Supplemental Figure 7

A

Sequencer
- Sequencing
- Basecalling (.bcl)

BaseSpace
- Readseq
- Reads (.fastq)
- STAR
- Alignment to reference sequence (.bam)

- Transgene Sequences (.fasta)
- Burrows-Wheeler Aligner
- Alignment to transgene (custom .bam with transgene alignment info)

Remove reads which map to the transgene

R
- Filtered Alignment (.bam)
- htseq-count
- Raw Counts
- limma-voom
- DE analysis

B

-log(p-value) vs log(fold change)

Treg CAR vs TCR stimulation (CD28+CD3 signals)

← TCR Treg   CAR Treg →

Transcription factors with enriched targets

| None | HIF1A |
| | CREB1 |

Selected enriched hallmark pathways

MYC_TARGETS_V2	MTORC1_SIGNALING
DNA_REPAIR	G2M_CHECKPOINT
	IL2_STAT5_SIGNALING
	FATTY_ACID_ METABOLISM
	INTERFERON_ALPHA_RESPONSE
	INTERFERON_GAMMA_RESPONSE
	TNFA_SIGNALING_VIA_NFKB

Genes labeled: SOX4, MYOF, CTTN, HMOX1, VIM-AS1, MGC12916, SLC7A11, QPRT, KIAA0125, KIF26B, PHEX, LMF1, MAML3, DUSP8, LOC606724, DNM1P46, NGFR, MET, CXCL8, IL3, TBC1D30, TUB, CXCR1, MIR613

C

-log(p-value) vs log(fold change)

Tconv CAR vs TCR stimulation (CD28+CD3 signals)

← TCR Tconv   CAR Tconv →

Transcription factors with enriched targets

MYB	NFKB1
E2F4	REL
	RELA
	FOS

Selected enriched hallmark pathways

G2M_CHECKPOINT	IL6_JAK_STAT3_SIGNALING
MYC_TARGETS_V2	P53_PATHWAY
E2F_TARGETS	TNFA_SIGNALING_VIA_NFKB
MTOR1_SIGNALING	IL2_STAT5_SIGNALING
MITOTIC_SPINDLE	INTERFERON_ALPHA_RESPONSE
	INTERFERON_GAMMA_RESPONSE
	KRAS_SIGNALING_UP

Genes labeled: LTA, CAPG, NFKBID, PTP4A3, TNFSF10, IL4I1, TNFSF4, CXCL9, NRP1, MYO3B, TRIM66, IL13, IL5, ITGA1, CCL20, EBI3, SOX4, IL2, PDGFRA, GZMH, MET, IL18RAP, NPTX1, IFI44L, HDAC9, DNM1P46, MX1, PTGS2, GPR34, PTPRG, IRF7, KRT7, TNFAIP8L3, CCL1, LOC440028, CYP1B1, HPGDS, ABCG1, NDUFA4L2, TEX29, NXN, CCL22
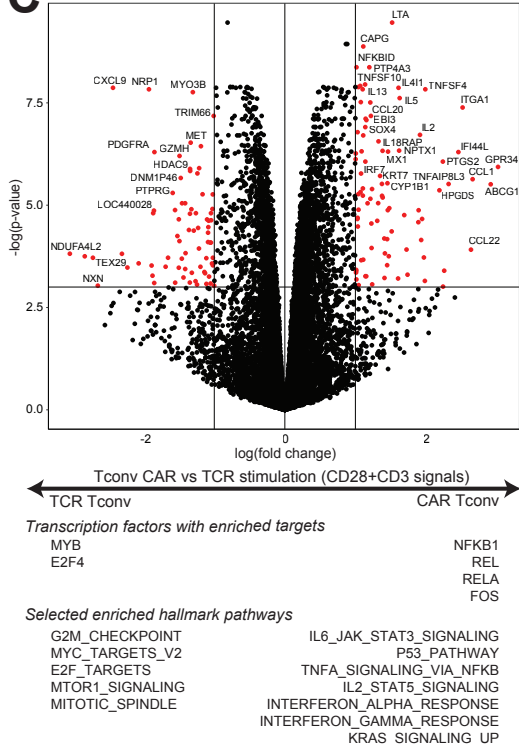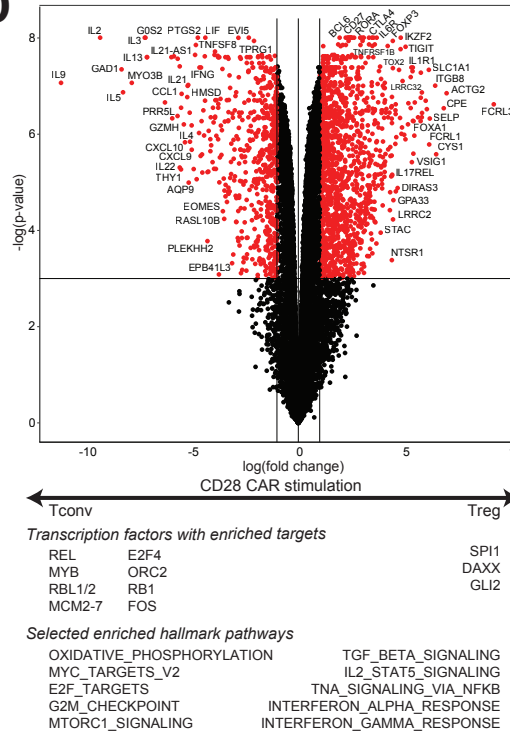
D

-log(p-value) vs log(fold change)

CD28 CAR stimulation

← Tconv   Treg →

Transcription factors with enriched targets

REL	E2F4	SPI1
MYB	ORC2	DAXX
RBL1/2	RB1	GLI2
MCM2-7	FOS	

Selected enriched hallmark pathways

OXIDATIVE_PHOSPHORYLATION	TGF_BETA_SIGNALING
MYC_TARGETS_V2	IL2_STAT5_SIGNALING
E2F_TARGETS	TNA_SIGNALING_VIA_NFKB
G2M_CHECKPOINT	INTERFERON_ALPHA_RESPONSE
MTORC1_SIGNALING	INTERFERON_GAMMA_RESPONSE

Genes labeled: IL2, G0S2, PTGS2, LIF, EVI5, BCL2, CCR8, CTLA4, FOXP3, IL9, IL3, IL21-AS1, TNFSF8, TPRG1, TNFRSF1B, IKZF2, TIGIT, IL13, GAD1, IFNG, IL21, TOX2, IL1R1, SLC1A1, IL5, MYO3B, CCL1, HMSD, LRRC32, ITGB8, PRR5L, IL4, CPE, ACTG2, GZMH, SELP, FCRL3, CXCL10, FOXA1, FCRL1, CYS1, CXCL9, VSIG1, IL22, L17REL, THY1, DIRAS3, AQP9, GPA33, EOMES, LRRC2, RASL10B, STAC, PLEKHH2, NTSR1, EPB41L3

Supplemental Figure 7. Transcriptome analysis of CAR- and TCR-stimulated Tregs and Tconv. Purified ΔNGFR/CAR Tregs or Tconv were stimulated with anti-CD3/CD28-dynabeads or HLA-A2-coated beads for 16 hours, then processed for RNA sequencing. **(A)** Schematic of RNA-sequencing pre-processing strategy to remove transgene reads. Complete description of actions performed is written in Methods. To observe direct functional consequences of CAR-stimulation, any sequencing reads that mapped to the CAR transgene or ΔNGFR transduction marker were removed from further differential gene expression analysis. **(B)** Differentially expressed genes between CD28wt-CAR- and TCR-stimulated Tregs. **(C)** Differentially expressed genes between CD28wt-CAR- and TCR-stimulated Tconv. **(D)** Differentially expressed genes between CAR-stimulated Tregs and Tconv expressing a CD28wt-CAR. Full lists, normalized enrichment scores and adjusted p values are found in **Supplemental Table 5-7**.

Supplemental Table 1. Gene set enrichment analysis of transcription factor targets and hallmark pathways supporting Figure 7A

Group: PD-1, TNFR2, CTLA-4mut, CTLA-4wt, CD3zeta | | | **Group: CD28wt** | | |

Enriched transcription factor targets	NES	Adj. p val.	Enriched transcription factor targets	NES	Adj. p val.
CREBBP	-1.92	0.0333	MYB	2.00	0.0192
EP300	-1.92	0.0333	ORC2	1.99	0.0213
			RB1	1.94	0.0329
			E2F4	1.90	0.0192
			MCM2	1.87	0.0402
			MCM3	1.87	0.0402
			MCM4	1.87	0.0402
			MCM5	1.87	0.0402
			MCM6	1.87	0.0402
			MCM7	1.87	0.0402
			RELA	1.62	0.0402
			NFKB1	1.45	0.0449

Enriched hallmark pathways	NES	Adj. p val.	Enriched hallmark pathways	NES	Adj. p val.
HALLMARK_INTERFERON_ALPHA_RESPONSE	-2.01	0.001	HALLMARK_MYC_TARGETS_V1	3.43	0.001
HALLMARK_INTERFERON_GAMMA_RESPONSE	-1.73	0.001	HALLMARK_MYC_TARGETS_V2	3.08	0.001
HALLMARK_HEME_METABOLISM	-1.60	0.004	HALLMARK_E2F_TARGETS	2.97	0.001
			HALLMARK_MTORC1_SIGNALING	2.62	0.001
			HALLMARK_UNFOLDED_PROTEIN_RESPONSE	2.58	0.001
			HALLMARK_G2M_CHECKPOINT	2.50	0.001
			HALLMARK_OXIDATIVE_PHOSPHORYLATION	2.07	0.001
			HALLMARK_GLYCOLYSIS	2.05	0.001
			HALLMARK_DNA_REPAIR	2.04	0.001
			HALLMARK_UV_RESPONSE_UP	1.86	0.001
			HALLMARK_ADIPOGENESIS	1.83	0.001
			HALLMARK_FATTY_ACID_METABOLISM	1.48	0.022

Supplemental Table 2. Gene set enrichment analysis of transcription factor targets and hallmark pathways supporting Figure 7B

Group: 4-1BB, TNFR2

Group: CD28wt

Enriched transcription factor targets	NES	Adj. p val.	Enriched transcription factor targets	NES	Adj. p val.
IKBKG	-1.93	0.0194	ORC2	1.98	0.0190
CREBBP	-1.90	0.0190	MCM2	1.83	0.0473
EP300	-1.90	0.0190	MCM3	1.83	0.0473
			MCM4	1.83	0.0473
			MCM5	1.83	0.0473
			MCM6	1.83	0.0473
			MCM7	1.83	0.0473
			E2F4	1.71	0.0190
			MYB	1.63	0.0194

Enriched hallmark pathways	NES	Adj. p val.	Enriched hallmark pathways	NES	Adj. p val.
HALLMARK_MYC_TARGETS_V2	-2.76	0.003	HALLMARK_INTERFERON_ALPHA_RESPONSE	2.20	0.003
HALLMARK_MYC_TARGETS_V1	-2.74	0.003	HALLMARK_INTERFERON_GAMMA_RESPONSE	2.08	0.003
HALLMARK_E2F_TARGETS	-2.60	0.003	HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.52	0.007
HALLMARK_G2M_CHECKPOINT	-2.21	0.003	HALLMARK_HEME_METABOLISM	1.41	0.037
HALLMARK_MTORC1_SIGNALING	-2.08	0.003			
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	-2.08	0.003			
HALLMARK_UV_RESPONSE_UP	-1.59	0.006			
HALLMARK_GLYCOLYSIS	-1.48	0.013			
HALLMARK_IL2_STAT5_SIGNALING	-1.44	0.013			

Supplemental Table 3. Gene set enrichment analysis of transcription factor targets and hallmark pathways supporting Figure 7C

Group: PD-1			Group: CD3zeta		
Enriched transcription factor targets	**NES**	**Adj. p val.**	**Enriched transcription factor targets**	**NES**	**Adj. p val.**
STAT6	-1.96	0.0325	MYB	2.06	0.0276
STAT2	-1.88	0.0420	E2F4	1.46	0.0430
STAT3	-1.69	0.0420			
Enriched hallmark pathways	**NES**	**Adj. p val.**	**Enriched hallmark pathways**	**NES**	**Adj. p val.**
HALLMARK_P53_PATHWAY	-1.42	0.0140	HALLMARK_MYC_TARGETS_V1	2.70	0.0007
			HALLMARK_MYC_TARGETS_V2	2.50	0.0007
			HALLMARK_MTORC1_SIGNALING	2.32	0.0007
			HALLMARK_E2F_TARGETS	2.11	0.0007
			HALLMARK_UNFOLDED_PROTEIN_RESPONSE	2.09	0.0007
			HALLMARK_CHOLESTEROL_HOMEOSTASIS	2.03	0.0007
			HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.90	0.0007
			HALLMARK_ESTROGEN_RESPONSE_LATE	1.83	0.0007
			HALLMARK_G2M_CHECKPOINT	1.81	0.0007
			HALLMARK_OXIDATIVE_PHOSPHORYLATION	1.79	0.0007
			HALLMARK_GLYCOLYSIS	1.76	0.0007
			HALLMARK_FATTY_ACID_METABOLISM	1.71	0.0007
			HALLMARK_IL2_STAT5_SIGNALING	1.60	0.0019
			HALLMARK_ANDROGEN_RESPONSE	1.56	0.0125
			HALLMARK_UV_RESPONSE_UP	1.43	0.0181
			HALLMARK_HYPOXIA	1.43	0.0181
			HALLMARK_ADIPOGENESIS	1.41	0.0170
			HALLMARK_XENOBIOTIC_METABOLISM	1.41	0.0249
			HALLMARK_INFLAMMATORY_RESPONSE	1.38	0.0340
			HALLMARK_DNA_REPAIR	1.38	0.0340

Supplemental Table 4. Gene set enrichment analysis of transcription factor targets and hallmark pathways supporting Figure 7D

Group: CD28mut			Group: CD28wt		
Enriched transcription factor targets	**NES**	**Adj. p val.**	**Enriched transcription factor targets**	**NES**	**Adj. p val.**
STAT1	-1.91	0.0336	E2F4	1.70	0.0269

Enriched hallmark pathways	**NES**	**Adj. p val.**	**Enriched hallmark pathways**	**NES**	**Adj. p val.**
HALLMARK_WNT_BETA_CATENIN_SIGNALING	-1.75	0.0124	HALLMARK_E2F_TARGETS	2.52	0.0013
HALLMARK_INTERFERON_GAMMA_RESPONSE	-1.67	0.0014	HALLMARK_MYC_TARGETS_V1	2.45	0.0013
HALLMARK_ALLOGRAFT_REJECTION	-1.56	0.0064	HALLMARK_G2M_CHECKPOINT	2.35	0.0013
HALLMARK_INTERFERON_ALPHA_RESPONSE	-1.51	0.0208	HALLMARK_MTORC1_SIGNALING	2.28	0.0013
			HALLMARK_MYC_TARGETS_V2	2.02	0.0013
			HALLMARK_PROTEIN_SECRETION	1.96	0.0013
			HALLMARK_MITOTIC_SPINDLE	1.78	0.0013
			HALLMARK_UNFOLDED_PROTEIN_RESPONSE	1.72	0.0030
			HALLMARK_FATTY_ACID_METABOLISM	1.68	0.0032
			HALLMARK_XENOBIOTIC_METABOLISM	1.67	0.0032
			HALLMARK_SPERMATOGENESIS	1.56	0.0209
			HALLMARK_ANDROGEN_RESPONSE	1.54	0.0209
			HALLMARK_PEROXISOME	1.54	0.0209
			HALLMARK_OXIDATIVE_PHOSPHORYLATION	1.50	0.0093
			HALLMARK_COMPLEMENT	1.50	0.0148
			HALLMARK_ADIPOGENESIS	1.50	0.0129
			HALLMARK_GLYCOLYSIS	1.43	0.0239

Supplemental Table 5. Gene set enrichment analysis of transcription factor targets and hallmark pathways supporting Supplemental Figure 7B

Group: Treg TCR (CD3+CD28)			Group: Treg CAR CD28wt		
Enriched transcription factor targets	**NES**	**Adj. p val.**	**Enriched transcription factor targets**	**NES**	**Adj. p val.**
			HIF1A	1.88	0.0216
			CREB1	1.81	0.0216
Enriched hallmark pathways	**NES**	**Adj. p val.**	**Enriched hallmark pathways**	**NES**	**Adj. p val.**
HALLMARK_MYC_TARGETS_V2	-2.41	0.0019	HALLMARK_CHOLESTEROL_HOMEOSTASIS	1.84	0.0024
HALLMARK_MYC_TARGETS_V1	-2.32	0.0019	HALLMARK_MITOTIC_SPINDLE	1.79	0.0019
HALLMARK_DNA_REPAIR	-1.44	0.0167	HALLMARK_HYPOXIA	1.77	0.0019
			HALLMARK_INTERFERON_GAMMA_RESPONSE	1.74	0.0019
			HALLMARK_IL2_STAT5_SIGNALING	1.74	0.0019
			HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.72	0.0019
			HALLMARK_SPERMATOGENESIS	1.67	0.0043
			HALLMARK_HEME_METABOLISM	1.65	0.0019
			HALLMARK_INFLAMMATORY_RESPONSE	1.64	0.0024
			HALLMARK_INTERFERON_ALPHA_RESPONSE	1.62	0.0055
			HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	1.60	0.0197
			HALLMARK_MTORC1_SIGNALING	1.60	0.0024
			HALLMARK_KRAS_SIGNALING_UP	1.60	0.0024
			HALLMARK_P53_PATHWAY	1.56	0.0024
			HALLMARK_APOPTOSIS	1.54	0.0044
			HALLMARK_PROTEIN_SECRETION	1.50	0.0182
			HALLMARK_ANDROGEN_RESPONSE	1.50	0.0182
			HALLMARK_G2M_CHECKPOINT	1.44	0.0043
			HALLMARK_FATTY_ACID_METABOLISM	1.42	0.0192
			HALLMARK_ALLOGRAFT_REJECTION	1.39	0.0182
			HALLMARK_COMPLEMENT	1.38	0.0245
			HALLMARK_XENOBIOTIC_METABOLISM	1.38	0.0254

Supplemental Table 6. Gene set enrichment analysis of transcription factor targets and hallmark pathways supporting Supplemental Figure 7C

Group: Tconv TCR (CD3+CD28)			Group: Tconv CAR CD28wt		
Enriched transcription factor targets	**NES**	**Adj. p val.**	**Enriched transcription factor targets**	**NES**	**Adj. p val.**
MYB	-1.69	0.0124	REL	1.97	0.0175
E2F4	-1.59	0.0175	NFKB1	1.86	0.0124
			RELA	1.84	0.0124
			FOS	1.75	0.0234

Enriched hallmark pathways	**NES**	**Adj. p val.**	**Enriched hallmark pathways**	**NES**	**Adj. p val.**
HALLMARK_G2M_CHECKPOINT	-2.57	0.0010	HALLMARK_TNFA_SIGNALING_VIA_NFKB	2.04	0.0010
HALLMARK_MYC_TARGETS_V1	-2.42	0.0010	HALLMARK_INTERFERON_ALPHA_RESPONSE	2.03	0.0010
HALLMARK_E2F_TARGETS	-2.17	0.0010	HALLMARK_INFLAMMATORY_RESPONSE	1.90	0.0010
HALLMARK_MYC_TARGETS_V2	-1.96	0.0010	HALLMARK_P53_PATHWAY	1.84	0.0010
HALLMARK_MITOTIC_SPINDLE	-1.83	0.0010	HALLMARK_IL2_STAT5_SIGNALING	1.80	0.0010
HALLMARK_UV_RESPONSE_DN	-1.72	0.0010	HALLMARK_INTERFERON_GAMMA_RESPONSE	1.77	0.0010
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	-1.36	0.0487	HALLMARK_XENOBIOTIC_METABOLISM	1.62	0.0026
HALLMARK_MTORC1_SIGNALING	-1.32	0.0429	HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	1.50	0.0451
			HALLMARK_APOPTOSIS	1.50	0.0104
			HALLMARK_IL6_JAK_STAT3_SIGNALING	1.46	0.0475
			HALLMARK_UV_RESPONSE_UP	1.46	0.0194
			HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	1.44	0.0248
			HALLMARK_COMPLEMENT	1.41	0.0248
			HALLMARK_KRAS_SIGNALING_UP	1.40	0.0328
			HALLMARK_APICAL_JUNCTION	1.37	0.0429

Supplemental Table 7. Gene set enrichment analysis of transcription factor targets and hallmark pathways supporting Supplemental Figure 7D

Group: Tconv CD28wt			Group: Treg CD28wt		
Enriched transcription factor targets	**NES**	**Adj. p val.**	**Enriched transcription factor targets**	**NES**	**Adj. p val.**
REL	-2.34	0.0082	SPI1	2.03	0.0082
MYB	-2.07	0.0082	DAXX	1.93	0.0230
RBL1	-2.04	0.0230	GLI2	1.85	0.0331
RBL2	-2.04	0.0230			
MCM2	-1.93	0.0230			
MCM3	-1.93	0.0230			
MCM4	-1.93	0.0230			
MCM5	-1.93	0.0230			
MCM6	-1.93	0.0230			
MCM7	-1.93	0.0230			
E2F4	-1.90	0.0082			
RB1	-1.83	0.0294			
ORC2	-1.82	0.0331			
FOS	-1.71	0.0309			

Enriched hallmark pathways	**NES**	**Adj. p val.**	**Enriched hallmark pathways**	**NES**	**Adj. p val.**
HALLMARK_MYC_TARGETS_V1	-3.71	0.0011	HALLMARK_INTERFERON_ALPHA_RESPONSE	2.06	0.0011
HALLMARK_MYC_TARGETS_V2	-3.38	0.0011	HALLMARK_CHOLESTEROL_HOMEOSTASIS	1.88	0.0013
HALLMARK_E2F_TARGETS	-2.88	0.0011	HALLMARK_HYPOXIA	1.87	0.0013
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	-2.41	0.0011	HALLMARK_HEME_METABOLISM	1.80	0.0013
HALLMARK_DNA_REPAIR	-2.18	0.0011	HALLMARK_APOPTOSIS	1.80	0.0011
HALLMARK_G2M_CHECKPOINT	-2.16	0.0011	HALLMARK_INTERFERON_GAMMA_RESPONSE	1.80	0.0013
HALLMARK_OXIDATIVE_PHOSPHORYLATION	-1.95	0.0011	HALLMARK_UV_RESPONSE_DN	1.75	0.0013
HALLMARK_MTORC1_SIGNALING	-1.78	0.0011	HALLMARK_COMPLEMENT	1.75	0.0016
HALLMARK_UV_RESPONSE_UP	-1.50	0.0079	HALLMARK_IL6_JAK_STAT3_SIGNALING	1.70	0.0063
			HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.69	0.0013
			HALLMARK_P53_PATHWAY	1.65	0.0013
			HALLMARK_MITOTIC_SPINDLE	1.57	0.0016
			HALLMARK_IL2_STAT5_SIGNALING	1.57	0.0016
			HALLMARK_MYOGENESIS	1.55	0.0074

Enriched hallmark pathways	NES	Adj. p val.	Enriched hallmark pathways	NES	Adj. p val.
			HALLMARK_APICAL_JUNCTION	1.53	0.0063
			HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	1.49	0.0114
			HALLMARK_BILE_ACID_METABOLISM	1.49	0.0244
			HALLMARK_TGF_BETA_SIGNALING	1.49	0.0385
			HALLMARK_ALLOGRAFT_REJECTION	1.48	0.0087
			HALLMARK_PROTEIN_SECRETION	1.47	0.0241
			HALLMARK_KRAS_SIGNALING_DN	1.43	0.0337
			HALLMARK_KRAS_SIGNALING_UP	1.40	0.0275