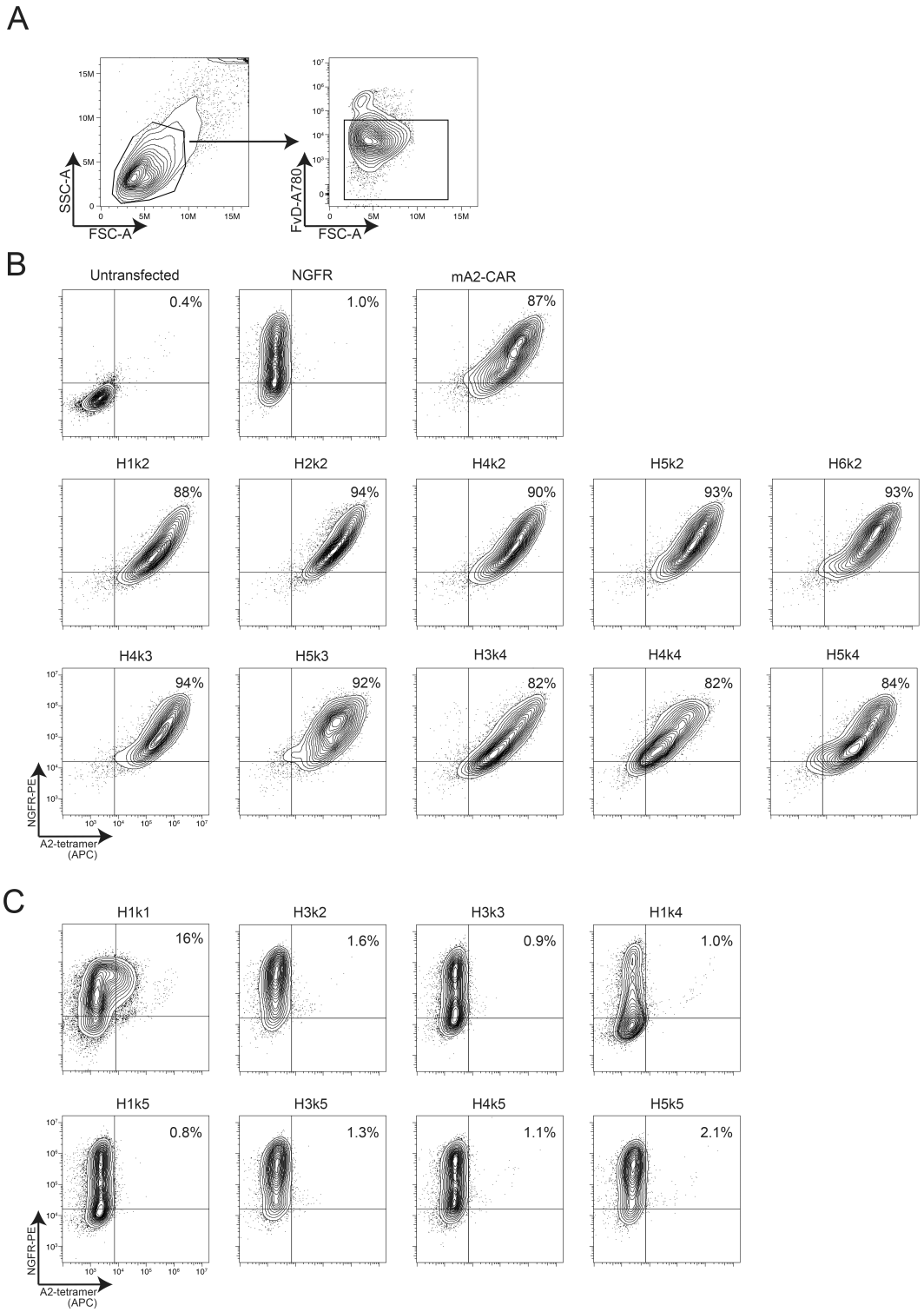
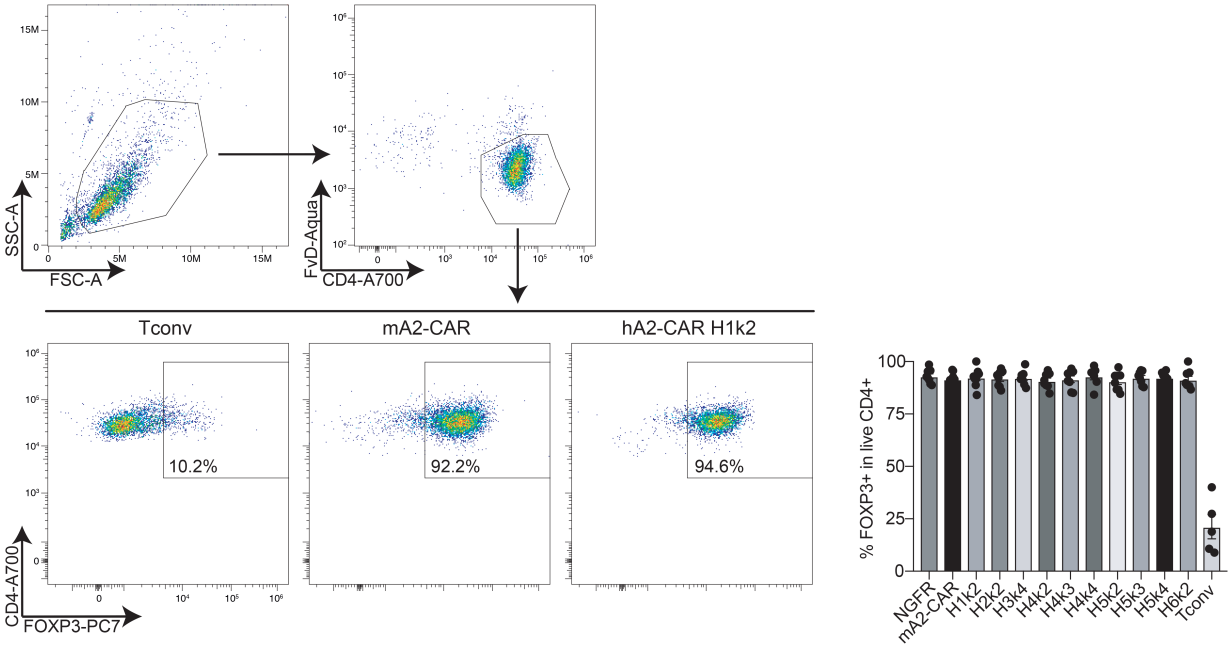
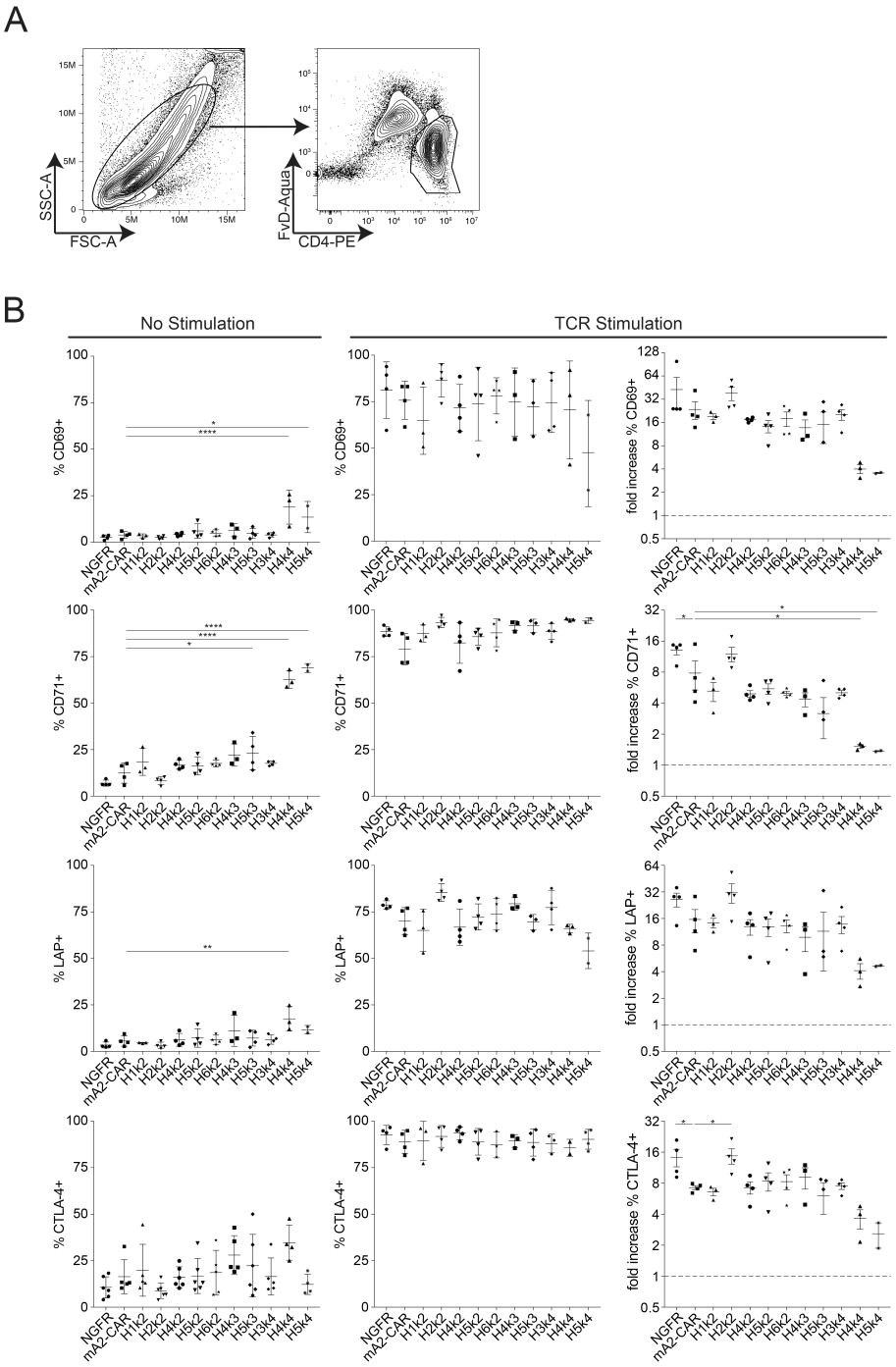


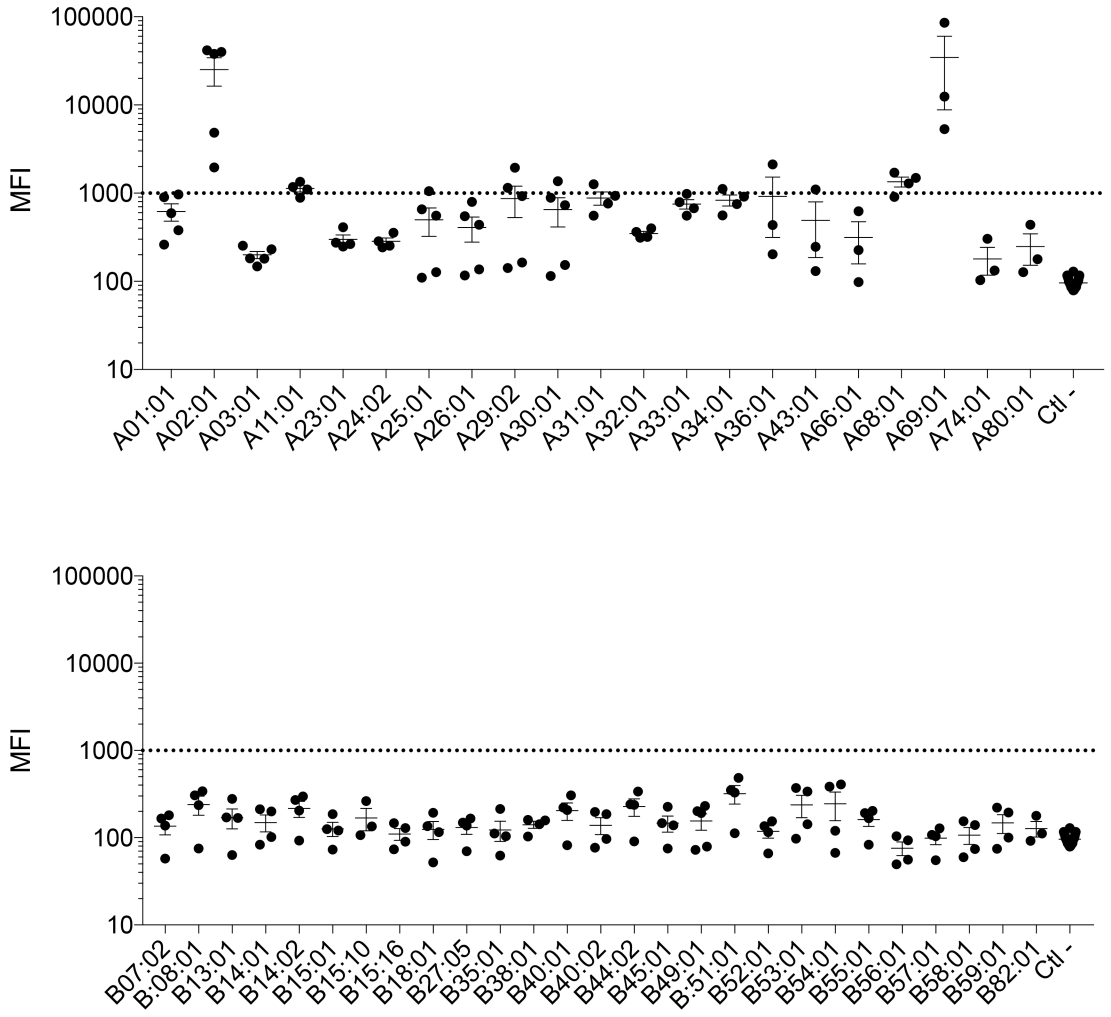
Supplemental Figure 1. ***Cell surface expression and tetramer binding of hA2 CARs in 293T cells.*** 293T cells were transiently transfected with the indicated constructs. 48 hours later, expression and tetramer binding were analysed by flow cytometry. The gating strategy is shown in (**A**). Representative flow plots are shown for constructs which do (**B**) or do not (**C**) retain their ability to bind to HLA-A*02:01. Data are representative of two independent experiments.



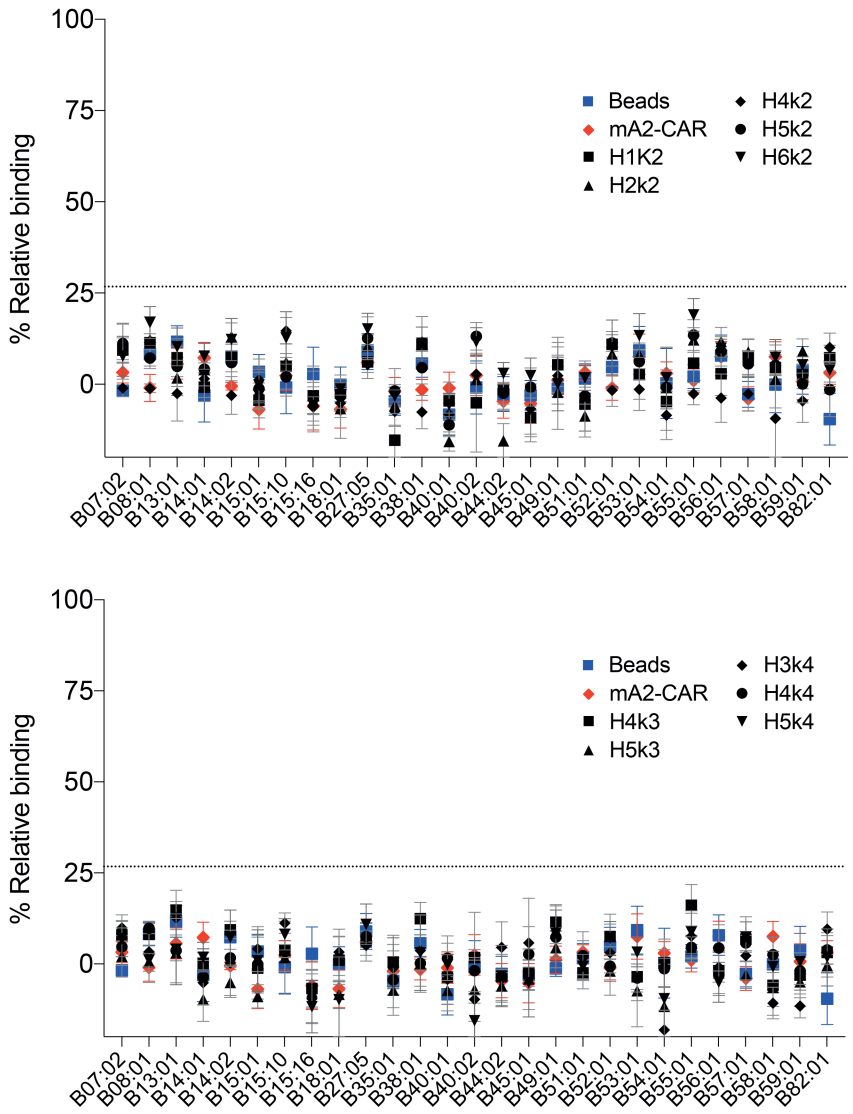
Supplemental Figure 2. ***FOXP3 expression on m/hA2-CAR Tregs after in vitro expansion.*** CAR Tregs and untransduced Tconv cells were expanded as described in the Methods. Prior to use in experiments the cells were analyzed for FOXP3 expression. The gating strategy and representative data are on the left, with averaged data on the right. Mean ± SEM. n=5-9 pooled from at least 5 independent experiments.



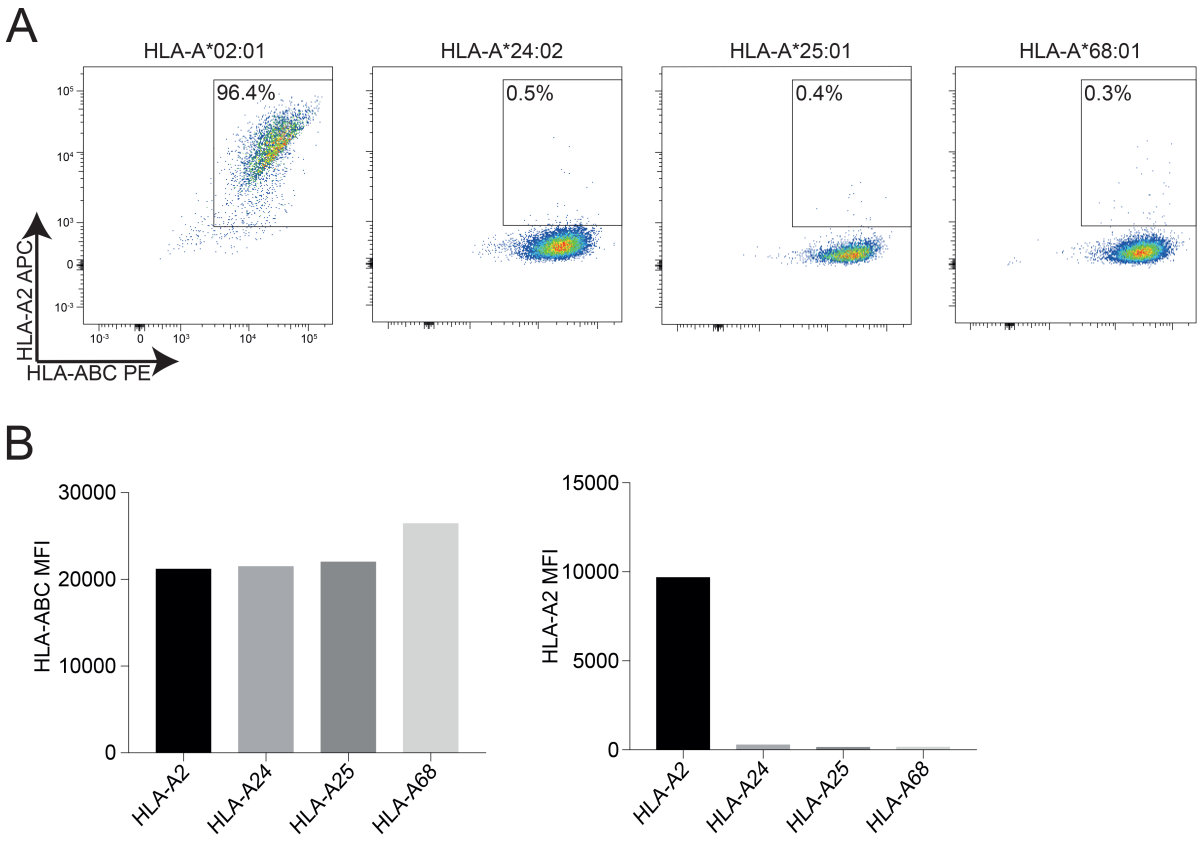
Supplemental Figure 3. ***Activation of hA2-CAR Tregs using artificial antigen presenting cells.*** In tandem with the experiment in Figure 2B&C, ΔNGFR control/CAR Tregs were co-cultured for 16 hours with either no stimulation or a 2:1 (Tregs: K562) ratio of CD64-expressing K562 cells loaded with anti-CD3 and anti-CD28 monoclonal antibodies (TCR stimulation). **(A)** Example gating strategy. **(B)** Expression of CD69, CD71, CTLA-4 and LAP were measured by flow cytometry. Fold increase of each activation marker over baseline (no stimulation, left) was calculated (right). Data are n=2-4 for each construct from at least two independent experiments. Mean ± SEM. One-way ANOVA and Holm-Sidak's multiple comparisons test comparing all constructs to mA2-CAR Tregs. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.



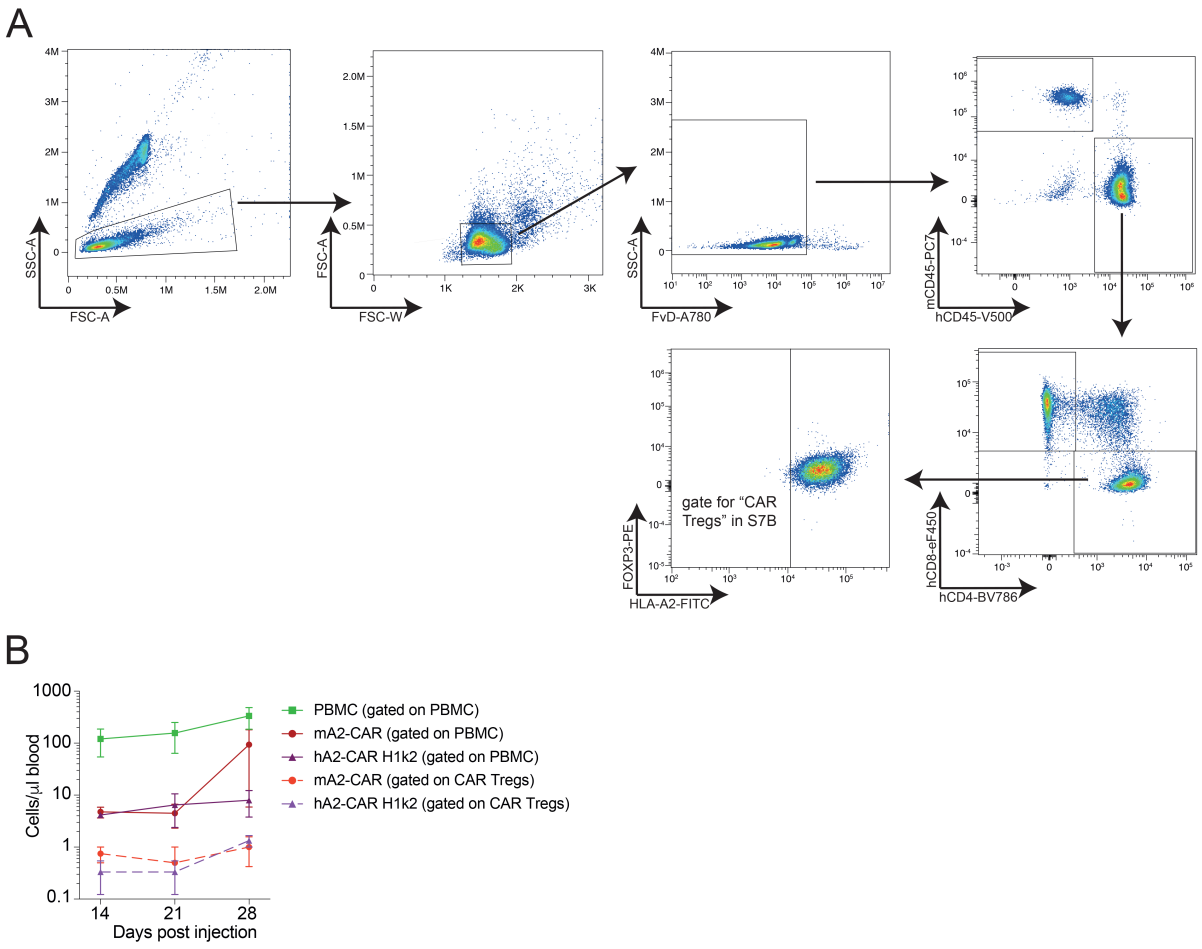
Supplemental Figure 4. ***Cross-reactivity of BB7.2 mAb with other HLA-A allelic variants.*** FlowPRA beads were incubated with APC-conjugated BB7.2 monoclonal antibody (Thermo Fisher Scientific, 17-9876-41) as recommended by manufacturer, and then analyzed by flow cytometry. Mean fluorescence intensity (MFI) of the reporter anti-FITC human IgG antibody is shown. Higher MFI indicates binding to the indicated HLA alleles. n=3-5 from 2-3 independent experiments.



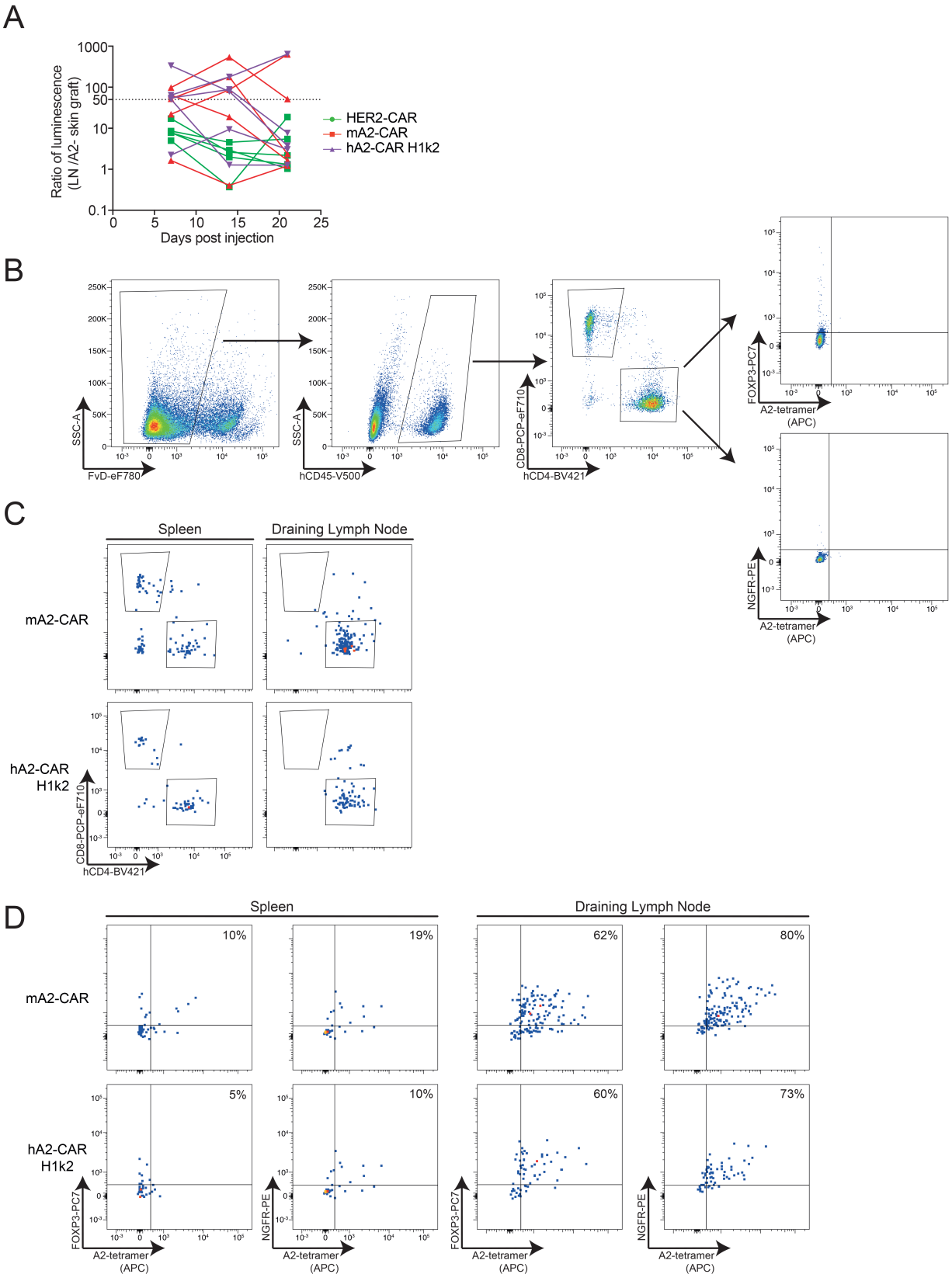
Supplemental Figure 5. ***Cross-reactivity of m/hA2 CARs with common HLA-B allelic variants.*** NGFR control, m/hA2-CAR Tregs were incubated with HLA-B single antigen FlowPRA beads, analyzed and normalized as in Figure 3. Results shown are n=2-6 from at least 2 independent experiments.



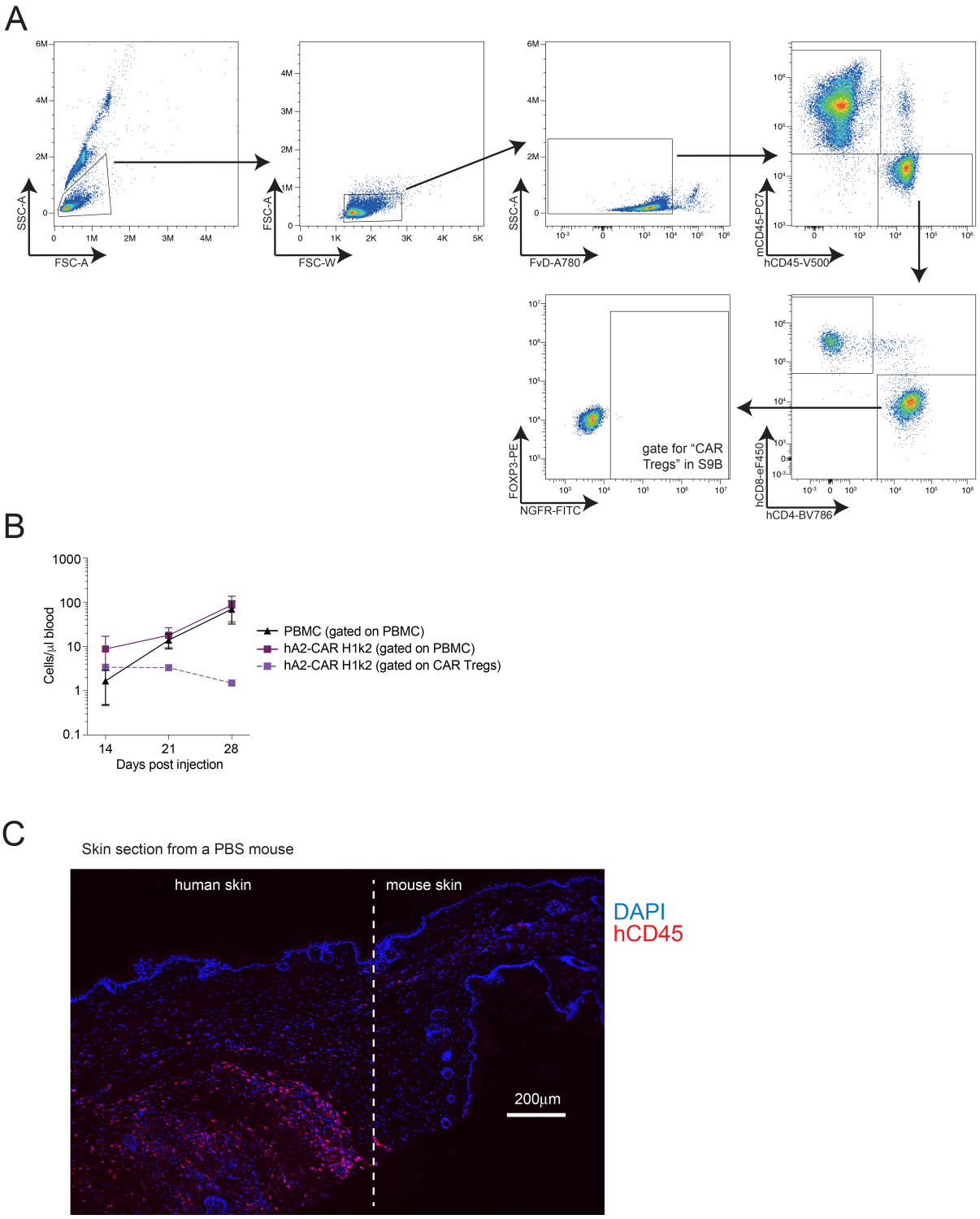
Supplemental Figure 6. ***Characterization of K562 cells expressing HLA-A allelic variants.*** **(A)** Representative flow cytometry plots from staining HLA-A2, A24, A25 and A68-expressing K562s with anti-HLA-ABC or anti-HLA-A2 (BB7.2 clone) mAbs. Gated on live cells. **(B)** Mean fluorescence intensity of HLA-ABC (left) and HLA-A2 (right) for each K562 cell line.



Supplemental Figure 7. ***In-vivo cell tracking after adoptive transfer in a xenogeneic GVHD mouse model.*** Irradiated NSG mice were injected with cells as described in Figure 5B-D. **(A)** Gating strategy to measure human $CD45^+$ and CAR Treg ($hCD45^+hCD4^+HLA\text{-}A2^-$) cell engraftment. Shown are representative data from a PBMC + H1k2-CAR Treg mouse. **(B)** Absolute number of PBMC and CAR Treg engraftment per µL of blood over time. Number of PBMCs were calculated as $hCD45^+$ minus CAR Treg counts, as gated in (A).


A
Ratio of luminescence (LN /A2- skin graft)
1000
100
50
10
1
0.1
0
5
10
15
20
25
Days post injection
HER2-CAR
mA2-CAR
hA2-CAR H1k2
B
SSC-A
FvD-eF780
hCD45-V500
CD8-PCP-eF710
hCD4-BV421
FOXP3-PC7
A2-tetramer (APC)
NGFR-PE
C
Spleen
Draining Lymph Node
mA2-CAR
hA2-CAR H1k2
D
10%
19%
62%
80%
5%
10%
60%
73%

Supplemental Figure 8. ***Trafficking of luciferase+ m/hA2 CAR Tregs to lymph nodes.*** Tregs were co-transduced with lentivirus encoding luciferase and either HER2-CAR, mA2-CAR or hA2-CAR constructs, expanded and injected into transplanted NSG mice as in Figure 6. **(A)** Amount of luciferase radiance in the draining lymph node area was quantified using the average amount of photons/sec/cm^2/steradian and plotted as a ratio between the lymph node area and the adjacent background signal from NSG (A2-negative) skin grafts over time. Each line represents one mouse from the indicated group. Points above the dotted line at 50 indicate a visually detectable signal. (**B**) Lymph nodes and spleen were harvested at Day 23 and analyzed using the indicated gating strategy with gates set on the basis of cells isolated from the spleen of mice injected with HER2-CAR Tregs. (**C&D**) Flow cytometry profiles of cells isolated from spleen or draining lymph nodes of the indicated mice. (**C**) Plots were pre-gated on FvD^-hCD45^+ as in (A) and analyzed for proportion of hCD4/CD8 cells. **(D)** Flow cytometry plots were pre-gated as $FvD^-hCD45^+CD4^+$ cells and analyzed for proportions of $FOXP3^+CAR^+$ cells using the gating strategy in Supplemental Figure 7. For B-D, n=1 per group from one independent experiment.



Supplemental Figure 9. ***Tracking of hA2 CAR Tregs in the human skin transplant model.*** NSG mice were transplanted with human HLA-A*02^{+} skin and injected with cells as described in Figure 7. **(A)** Gating strategy to measure human CD45^{+} (PBMC) and CAR Treg

($hCD45^+hCD4^+NGFR^+$) cell engraftment, shown are representative data from a PBMC + hA2-CAR Treg mouse. **(B)** Absolute number of PBMCs and CAR Treg engraftment per µL of blood over time. Number of PBMCs were calculated as hCD45+ minus total CAR Treg counts, as gated in (A). **(C)** Immunofluorescence staining for hCD45 on a section from a mouse that received a saline (PBS) injection. The section contains the human graft and the adjacent mouse skin.

Supplemental Table 1. Summary of p values showing CAR Tregs that had significantly higher relative binding to denoted HLA alleles compared with beads only (supporting data in Figure 3E). Two-way ANOVA and Dunnett's multiple comparisons test.

HLA-A*02:01		**HLA-A*69:01**		**HLA-A*03:01**		**HLA-A*33:01**	
mA2-CAR	0.0001	mA2-CAR	0.0001	mA2-CAR	0.0315	mA2-CAR	0.0019
H4k4	0.0006	H4k2	0.0001	**HLA-A*25:01**		**HLA-A*36:01**	
H4k2	0.0001	H5k2	0.0001	mA2-CAR	0.0001	mA2-CAR	0.0035
H5k2	0.0001	H1k2	0.0001	H1k2	0.0130	H1k2	0.0082
H1k2	0.0001	H3k4	0.0013	**HLA-A*29:02**		**HLA-A*68:01**	
H3k4	0.0001	H2k2	0.0084	mA2-CAR	0.0001	mA2-CAR	0.0001
H2k2	0.0001	H5k3	0.0001	**HLA-A*30:01**		H1k2	0.0001
H5k3	0.0001	H4k3	0.0001	mA2-CAR	0.0001	H5k2	0.0337
H4k3	0.0001	H6k2	0.0060	**HLA-A*31:01**		H6k2	0.0009
H6k2	0.0001			mA2-CAR	0.0001		
H5k4	0.0001			H1k2	0.0077		

Supplemental Table 2. Primers used in qPCR analysis of Figure 7E.

Oligo name	**Direction**	**Oligo sequence (5' to 3')**
human *IL17*	FWD	TCA ACC CGA TTG TCC ACC AT
human *IL17*	RVS	GAG TTT AGT CCG AAA TGA GGC TG
human *IL6*	FWD	TCC AAA GAT GTA GCC GCC CCA
human *IL6*	RVS	CCA GTG CCT CTT TGC TGC TTT CA
human *IL1B*	FWD	CTG AGC TCG CCA GTG AAA TGA TG
human *IL1B*	RVS	TGC TGT AGT GGT GGT CGG AGA
human *DEFB4*	FWD	ACC TGC CTT AAG AGT GGA GCC A
human *DEFB4*	RVS	ACA TGT CGC ACG TCT CTG ATG A
human *IFNG*	FWD	TGC CCA GAG CAT CCA AAA GA
human *IFNG*	RVS	TGT ATT GCT TTG CGT TGG AC
human *TNFA*	FWD	AGG CGC TCC CCA AGA AGA CA
human *TNFA*	RVS	GGG CTG ATT AGA GAG AGG TCC CT
18S ribosomal RNA	FWD	CAA GAC GGA CCA GAG CGA AA
18S ribosomal RNA	RVS	GGC GGG TCA TGG GAA TAA C