Figure S1

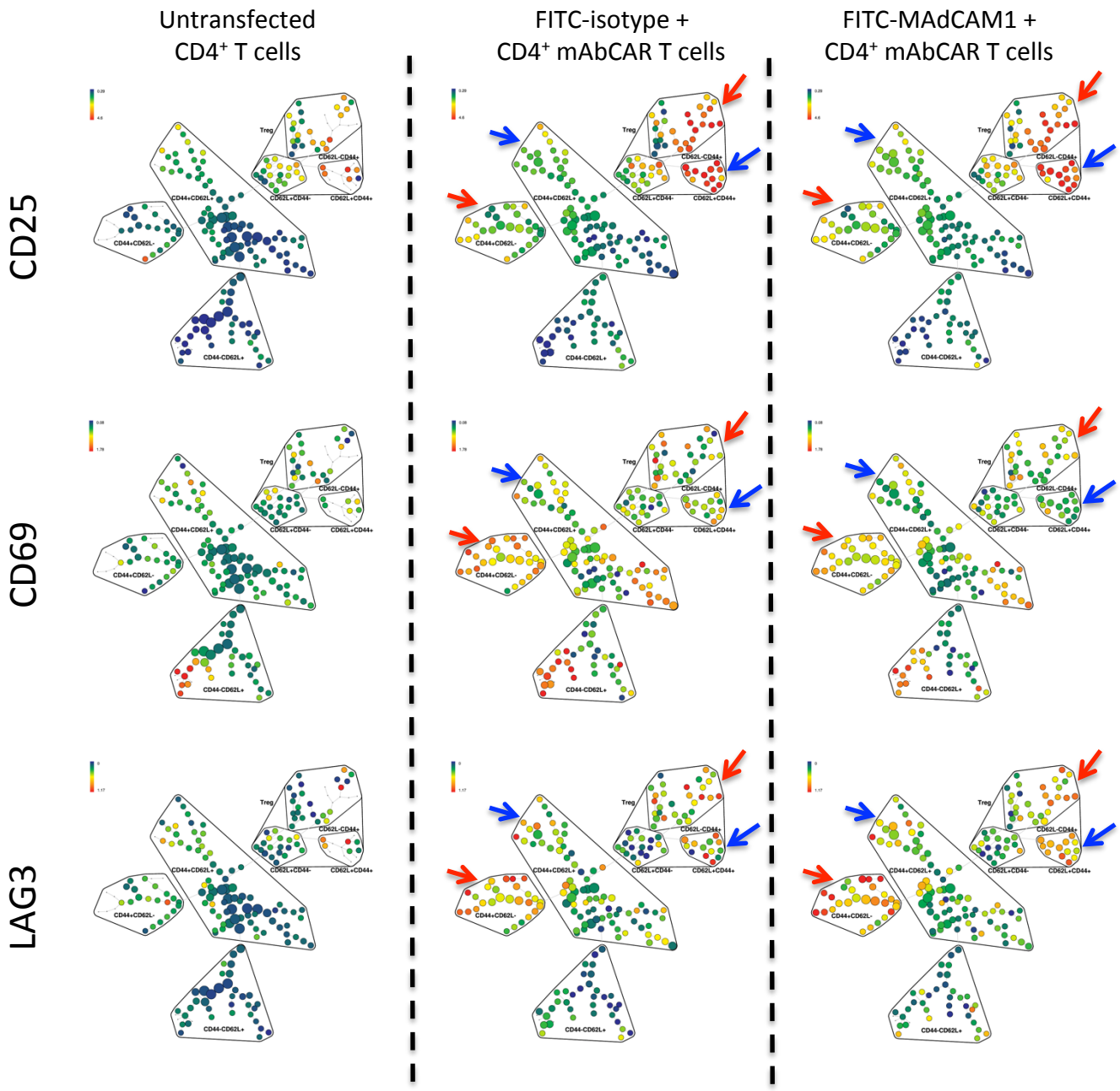

Untransfected
CD4+ T cells
FITC-isotype +
CD4+ mAbCAR T cells
FITC-MAdCAM1 +
CD4+ mAbCAR T cells
CD25
CD69
LAG3
Treg
CD62L-CD44+
CD62L+CD44-
CD62L+CD44+
CD44+CD62L+
CD44+CD62L-
CD44-CD62L+

Figure S1. Mass cytometry reveals FITC induced activation of mAbCAR T cell subsets

Spade analysis of mAbCAR T cells that have been analyzed through mass cytometry after incubation with FITC conjugated isotype or anti-MAdCAM1 antibodies revealed increased CD25, CD69 and LAG3 expression in central memory ($CD44^{+}CD62L^{+}$, blue arrows) and effector memory ($CD44^{+}CD62L^{neg}$, red arrows) subsets of $CD4^{+}FoxP3^{neg}$ T cells and $CD4^{+}FoxP3^{+}$ Treg cells. Reported analysis has been performed on the live $CD4^{+}$ T cell population and cells have been gated for $CD4^{+}FoxP3^{-}$ cells, $CD4^{+}FoxP3^{+}$ cells as indicated. Shown data is one representative sample of three samples of $CD4^{+}$ T cells cultured in the presence or not of FITC conjugated isotype or anti-MAdCAM1 antibodies. Dot size is representative of the size of the single homogenous cell population. Plot color intensity refers to the grade of the expression of the reported markers as shown. Data are representative of 1 of 2 consecutive experiments.

Figure S2

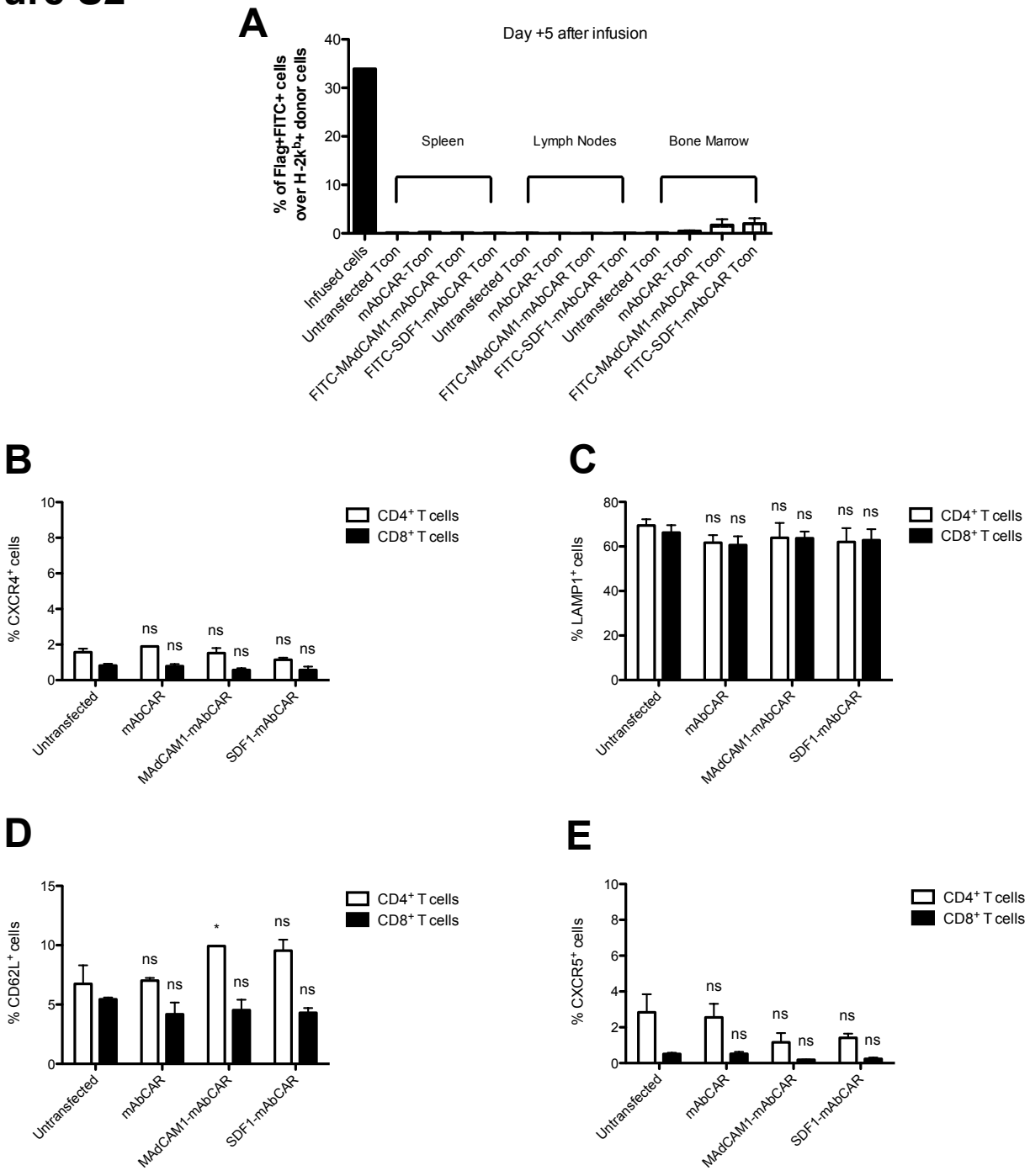
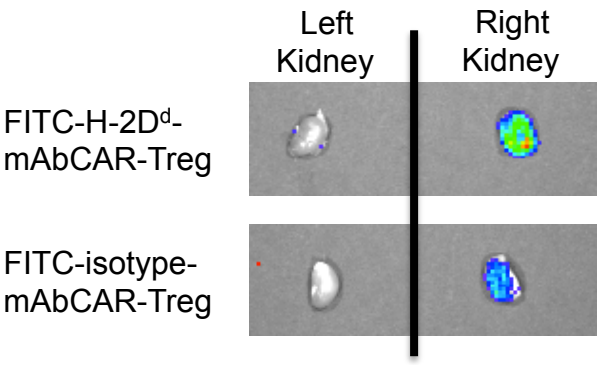

A
Day +5 after infusion
% of Flag+FITC+ cells over H-2k^b+ donor cells
Spleen
Lymph Nodes
Bone Marrow
Infused cells
Untransfected Tcon
mAbCAR-Tcon
FITC-MAdCAM1-mAbCAR Tcon
FITC-SDF1-mAbCAR Tcon
B
% CXCR4+ cells
C
% LAMP1+ cells
D
% CD62L+ cells
E
% CXCR5+ cells
CD4+ T cells
CD8+ T cells
ns
*
Untransfected
mAbCAR
MAdCAM1-mAbCAR
SDF1-mAbCAR

Figure S2. mAbCAR T cells do not show different patterns of homing marker expression after in vivo transfer

Percentage of $FLAG^+FITC^+$ T cells over total donor cells (A) and surface expression of CXCR4 (B), LAMP1 (C), CD62L (D), CXCR5 (E) in $CD4^+$ (white bars) and $CD8^+$ T cells (black bars) reisolated 5 days after infusion from transplanted mice that received adoptive transfer of untransfected T cells, mAbCAR T cells, FITC-MAdCAM1 mAbCAR T cells, or FITC-SDF1 mAbCAR T cells are reported. Data are representative of one of two consecutive experiments. Data are representative of 1 of 2 consecutive experiments. 2-tailed Student *t* test was used for statistical analysis; ns = not significant; * $p<0.05$.

Figure S3


Left Kidney
Right Kidney
FITC-H-2D^{d}-mAbCAR-Treg
FITC-isotype-mAbCAR-Treg

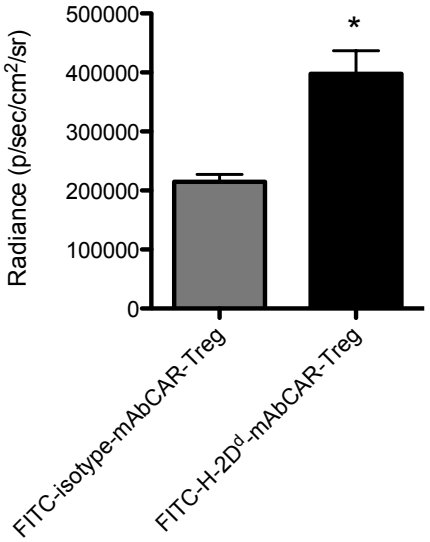

Radiance (p/sec/cm^2/sr)
500000
400000
300000
200000
100000
0
*
FITC-isotype-mAbCAR-Treg
FITC-H-2D^{d}-mAbCAR-Treg

Figure S3. Ex vivo BLI demonstrates higher frequencies of H-2D^{d}-mAbCAR Treg cells in kidneys transplanted with allogeneic pancreatic islet grafts

BLI signal is reported from left kidney of mice that received *luciferase*$^{+}$ H-2D^{d}-mAbCAR Treg cells (black bar) or isotype-mAbCAR Treg cells (white bar) and allogeneic pancreatic islet graft in the right kidney capsule. A representative image is also reported were the transplanted kidneys are shown in the right side of the pictures and control kidneys in the left side. Data are representative of 1 of 2 consecutive experiments. 2-tailed Student *t* test was used for statistical analysis; * $p<0.05$.

Figure S4

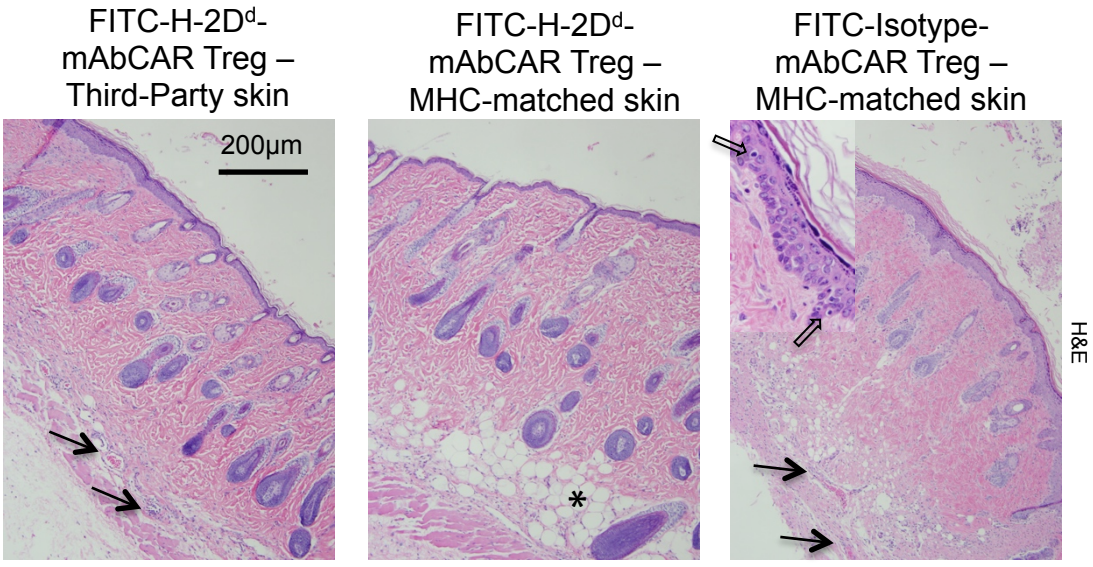

FITC-H-2Dd-
mAbCAR Treg –
Third-Party skin
FITC-H-2Dd-
mAbCAR Treg –
MHC-matched skin
FITC-Isotype-
mAbCAR Treg –
MHC-matched skin
200µm
*
H&E

Figure S4. H-2D^{d}-mAbCAR Treg cells reduce occurrence of rejection in H-2D^{d+} skin grafts

Hystologic sections of different skin grafts as reported at day +14 after skin transplant. Hematoxilin-Eosin staining is shown. Skin MHC-matched with pancreatic islet graft from mice that were treated with FITC-H-2D^{d}-mAbCAR-Treg show less signs of graft rejection (lymphoid infiltration, full arrows; picnotic bodies, bold open arrows) and better persistence of normal subcutaneous adipose tissue (*). Data are representative of 1 of 2 consecutive experiments.

Figure S5

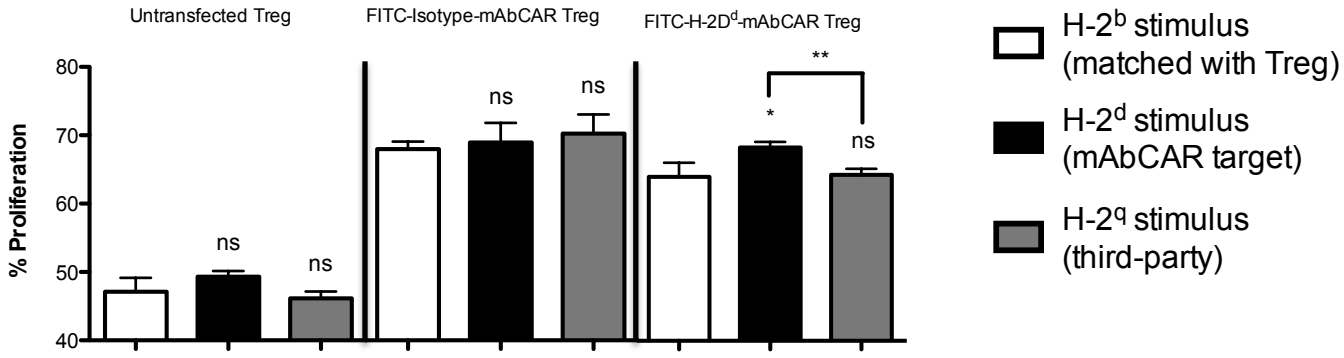

Untransfected Treg
FITC-Isotype-mAbCAR Treg
FITC-H-2D^d-mAbCAR Treg
% Proliferation
80
70
60
50
40
ns
ns
ns
ns
*
**
ns
H-2^b stimulus (matched with Treg)
H-2^d stimulus (mAbCAR target)
H-2^q stimulus (third-party)

Figure S5. FITC-H-2D^{d} mAb allows for H-2D^{d}-mAbCAR Treg antigen specificity

Proliferation of untransfected, isotype-mAbCAR, and H-2D^{d}-mAbCAR Treg in response to irradiated splenocytes derived from C57BL/6 mice (H-2^{b}) MHC-matched with the Treg (white bars), BALB/c mice (H-2^{d}) that represent a target for FITC-H-2D^{d} mAb (black bars), and FVB/N mice (H-2^{q}) that were third-party (grey bars) was measured via cell trace violet. Stimulation with irradiated splenocytes derived from BALB/c mice (H-2^{d}) increased proliferative response of H-2D^{d}-mAbCAR Treg. Data are representative of 1 of 2 consecutive experiments. 2-tailed Student *t* test was used for statistical analysis; * $p<0.05$; ** $p<0.01$.