

## Supplementary Data

**Supplementary Figure 1. Comparative analysis of additional signature genes of auto Ag-specific iPSC-Tregs and nTregs.** Experimental procedures are described in Fig. 1. Data shown are representative of three identical experiments. The values represent mean  $\pm$  S.E.M. ( $n = 3$ ). ns,  $p > 0.05$ , multiple Student's 1-tailed t test.

**Supplementary Figure 2. Comparison of cell survival between iPSC-Tregs and nTregs.** iPSC-Tregs (DsRed<sup>+</sup>) or nTregs (RFP<sup>+</sup>) from the FoxP3-IRES-mRFP (FIR) reporter mice were stimulated *in vitro* with  $\alpha$ -CD3 plus  $\alpha$ -CD28 antibodies, or adoptively transferred into naive C57BL/6 recipient mice ( $1 \times 10^5$ / mouse) that were subsequently challenged *i.p.* with  $\alpha$ -CD3 antibody. (A) Data shown is the percentage T cell recovery, calculated based on assigning the input number of cells in each culture as 100%. (B) On days 7, 14, and 21, DsRed<sup>+</sup> / RFP<sup>+</sup> Tregs were enumerated from pooled lymph nodes and spleen. Data are the mean number of DsRed<sup>+</sup>/RFP<sup>+</sup> Tregs  $\pm$  S.D. from three independent experiments. ns,  $p > 0.05$ , multiple Student's 1-tailed t test.

**Supplementary Figure 3. Significant induction of autoimmune diabetes post VACV infection.** Experimental procedures are described in Fig. 2. Mice that were at age of 9 weeks, including a week in which mice were challenged with VACV-OVA or control PBS. Blood sugar was measured. The values represent mean  $\pm$  S.E.M. \*\*\*\*,  $p < 0.0001$ , multiple Student's 1-tailed t test.

**Supplementary Figure 4. Reduction of effector CD4<sup>+</sup> T cells in the pancreas by tissue-associated iPSC-Tregs.** Experimental procedures are described in Fig. 3. At week 10, Ag-specific

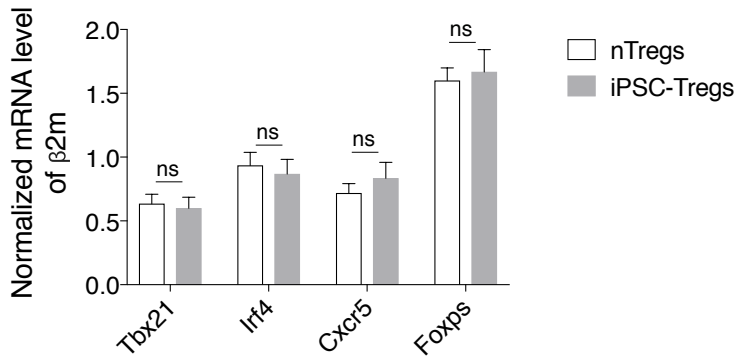
pre-iPSC-Tregs were transferred into diabetic mice. At week 16, mice were sacrificed and their pancreases were used for analysis of CD4, FoxP3 and TCR. TCR<sup>+</sup> (V $\alpha$ 2<sup>+</sup>V $\beta$ 5<sup>+</sup> for OVA-specific, V $\alpha$ 2<sup>+</sup>V $\beta$ 8<sup>+</sup> for SM1-specific) cells in CD4<sup>+</sup> FoxP3<sup>-</sup> or CD4<sup>+</sup> FoxP3<sup>+</sup> populations were shown. Data shown are representative of three individual experiments (n=5).

**Supplementary Figure 5. Appearance of Ag-specific iPSC-Tregs in the lymph nodes (LNs)**

**and spleen.** Experimental procedures are described in Fig. 3. At week 10, OVA-specific pre-iPSC-Tregs were transferred into diabetic mice. Before or after the cell transfer, mice were sacrificed and their pooled LNs or spleen were isolated for analysis of CD4, FoxP3 and TCRV $\alpha$ 2/TCRV $\beta$ 5. Percentages of TCRV $\alpha$ 2<sup>+</sup>TCRV $\beta$ 5<sup>+</sup> cells in CD4<sup>+</sup> population were shown at week 10, 13 and 16. Data shown are representative of three individual experiments (n=5).

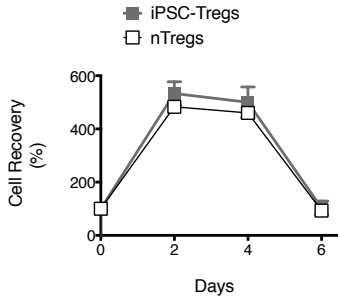
The values represent mean  $\pm$  S.D. \*,  $p < 0.05$ , ns,  $p > 0.05$ , Student's 1-tailed t test.

Sup. Fig. 1

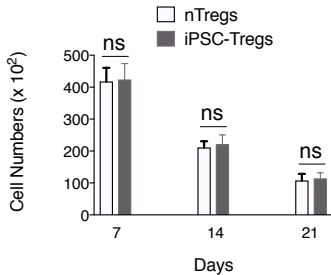


# Sup. Fig. 2

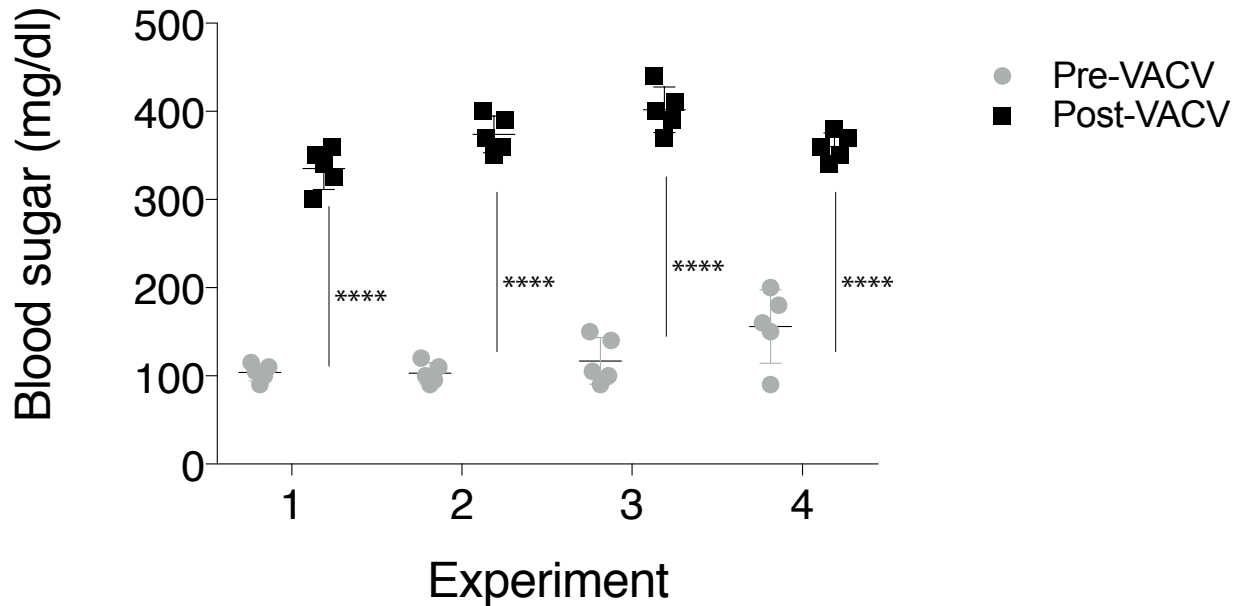
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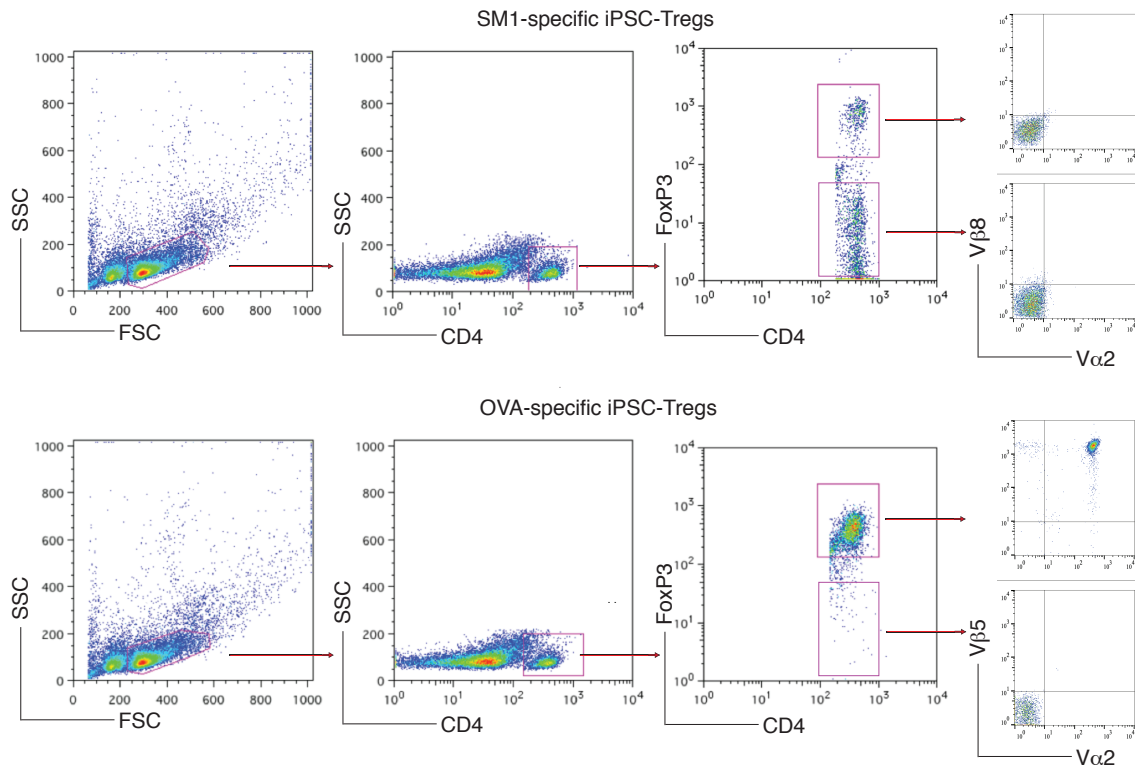
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Sup. Fig. 3



Sup. Fig. 4



Sup. Fig. 5

