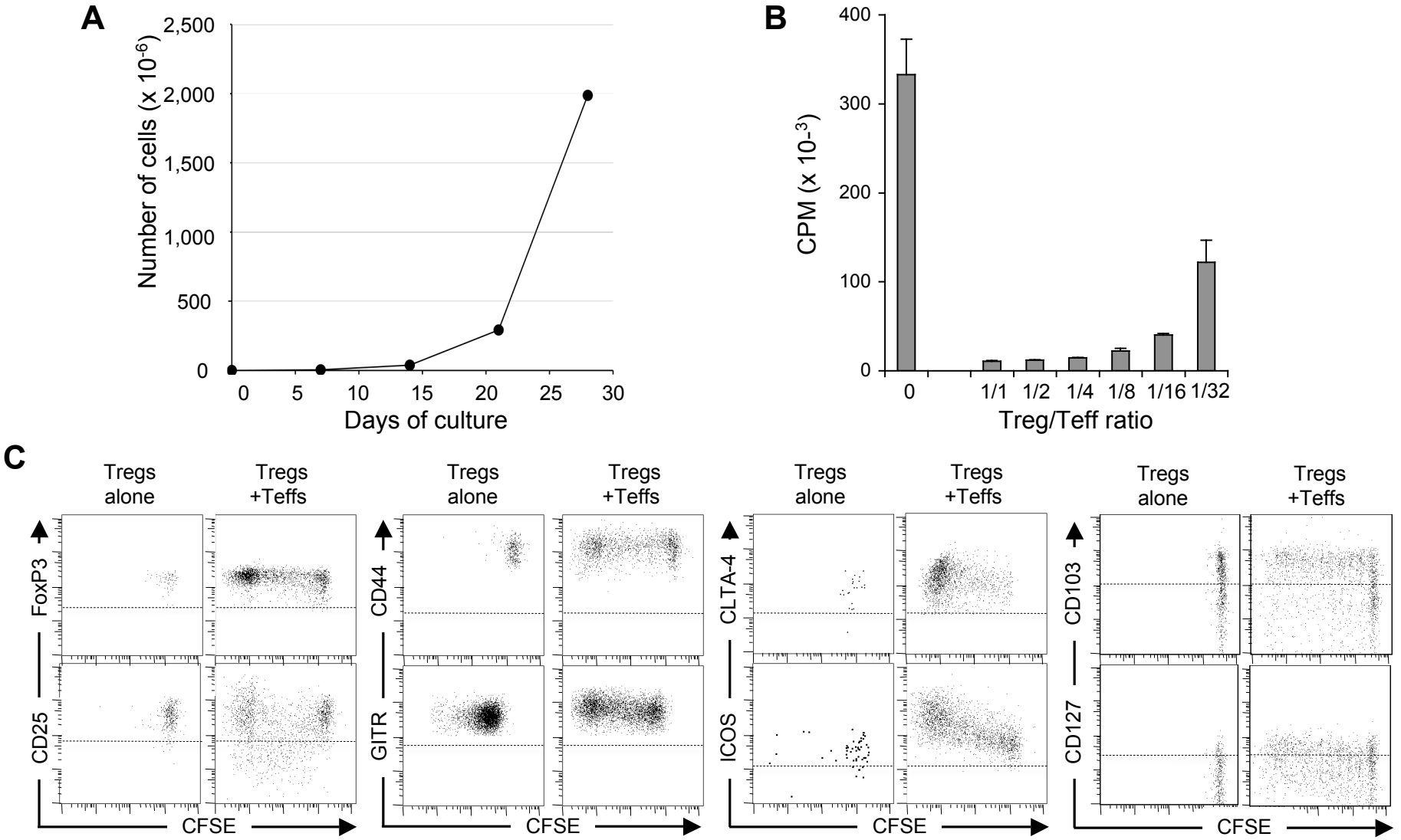
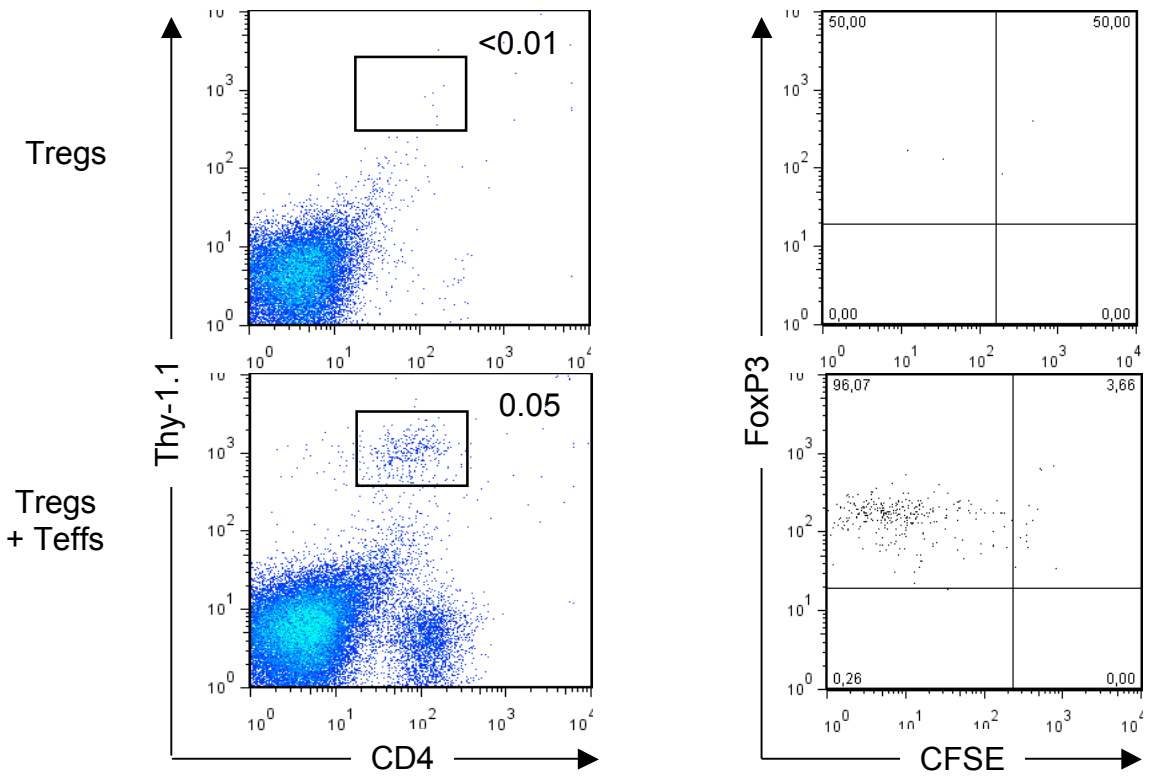
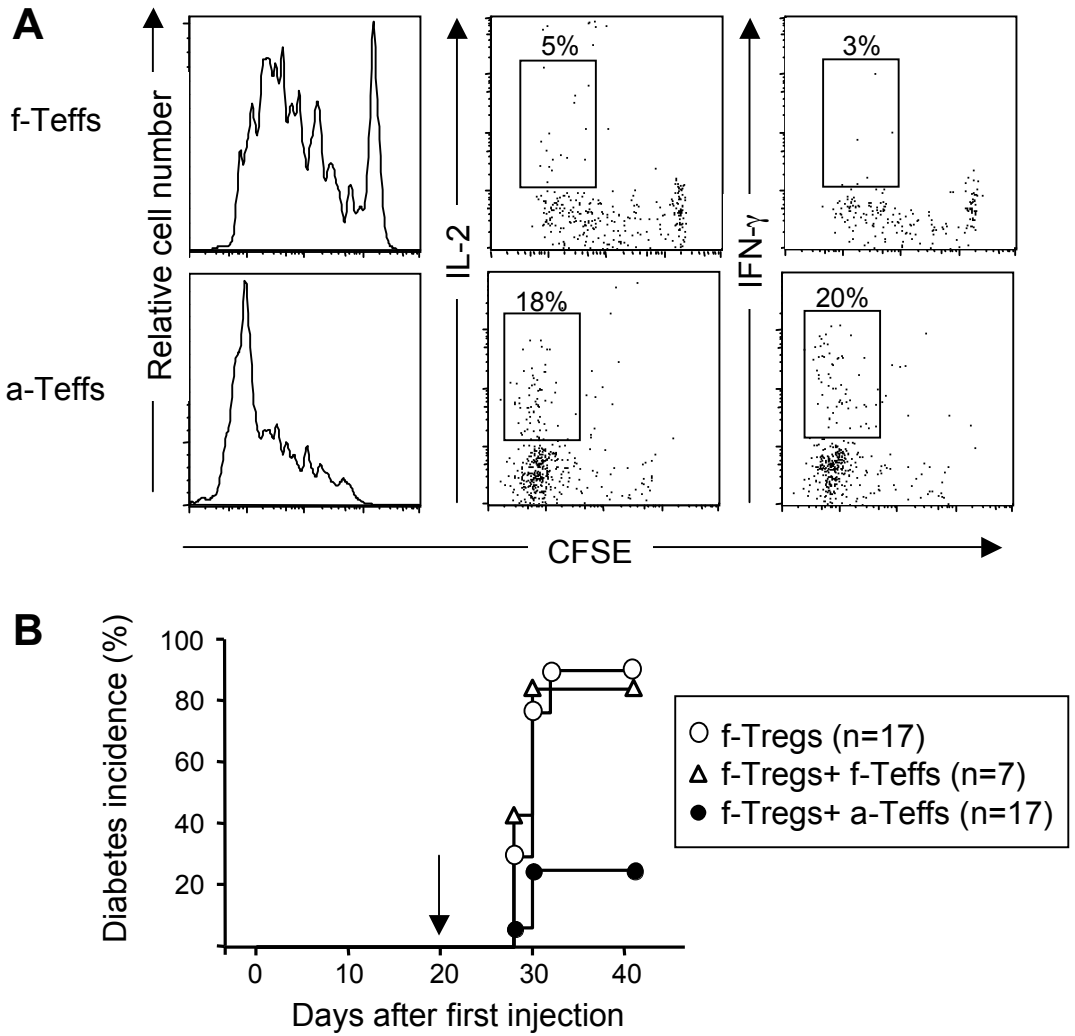


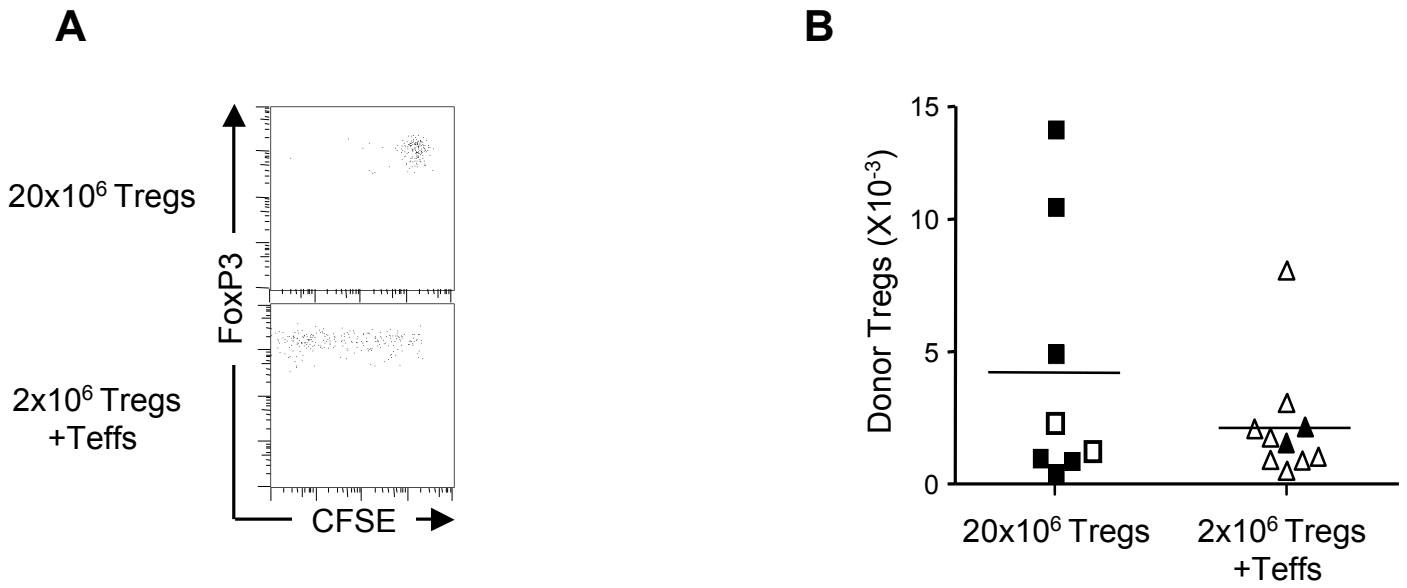
Supplemental Figure 1. In vitro Treg expansion and phenotype of expanded boosted Tregs. (**A**) In vitro expansion of purified HA-Tregs stimulated by HA-pulsed DCs. Viable cell numbers were determined after trypan blue staining. (**B**) The in vitro suppressive activity of HA-Tregs was determined after 3 weeks of culture. HA-Teffs (10^5 cells) stimulated by DCs (2 x 10^4 cells) and anti-CD3 mAb (1 µg/ml of 2C11) were co-cultured with various numbers of *in vitro* expanded HA-Tregs for 68 hours in flat bottom 96 well plates. Proliferation was measured by thymidine DNA incorporation during the last 12 hours. (**C**) Ins-HA mice were injected with CFSE-labeled Thy-1.1$^+$ in vitro expanded HA-Tregs alone or co-transferred with pre-activated HA-Teffs. Representative expression out of 3 independent experiments of the indicated markers on donor Tregs (CD4$^+$Thy-1.1$^+$) in PLNs 7 days after cell injection. Horizontal dashed lines delineate positive staining.



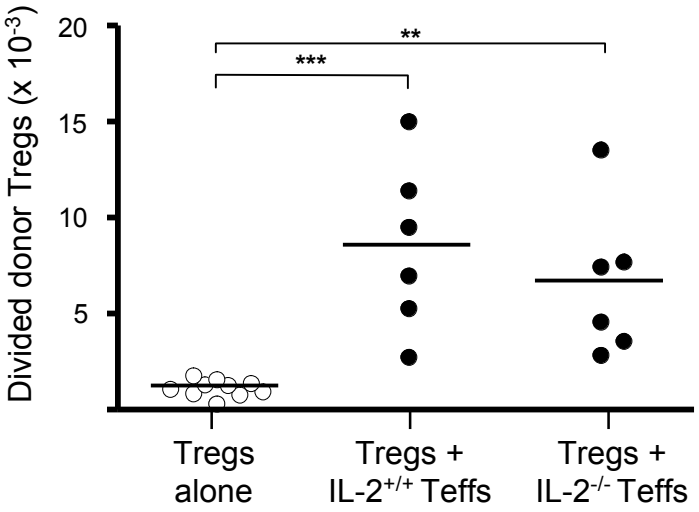
Supplemental figure 2. High numbers of divided islet-specific Tregs in the pancreas of mice co-transferred with islet-specific Teffs and Tregs. Ins-HA mice were injected with CFSE-labeled Thy-1.1+ expanded HA-Tregs alone (Tregs) or with pre-activated HA-Teffs (Tregs +Teffs). Representative dot plot of CD4 vs Thy-1.1 among total lymphocytes (left) and CFSE vs FOXP3 within CD4+Thy-1.1+ cells (right) in the pancreatic islets 7 days after transfer. Data were representative of 3 independent experiments with 2 to 3 mice per group.



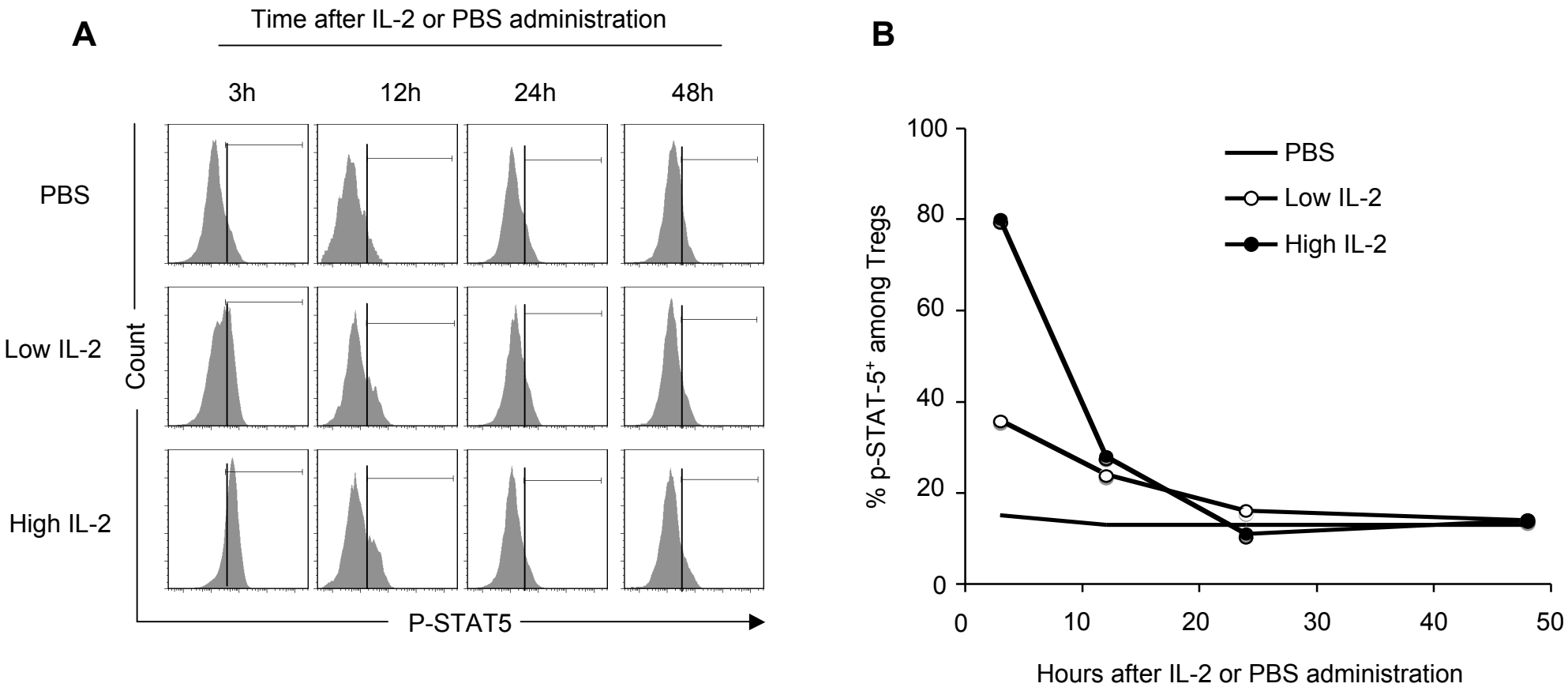
Supplemental Figure 3. Pre-activated, but not freshly-purified HA-Teffs, were strongly re-activated in PLNs of ins-HA mice and induced long-term protection from diabetes challenge when combined with freshly purified Tregs. (**A**) Four days after injection of CFSE-labeled Thy-1.1$^+$ freshly purified HA-Teffs (f-Teffs) or pre-activated HA-Teffs (a-Teffs), ins-HA mice were sacrificed for analyses of CFSE profile, IL-2 and IFN-γ production among CD4$^+$Thy-1.1$^+$ cells in PLNs. Representative data out of 4 experiments. (**B**) Ins-HA mice were transferred with freshly purified HA-Tregs alone or co-injected with freshly purified HA-Teffs or pre-activated HA-Teffs. Three weeks later (arrow), mice were challenged with pre-activated HA-Teffs, to test their sensitivity to diabetes. Data were pooled from 5 independent experiments.



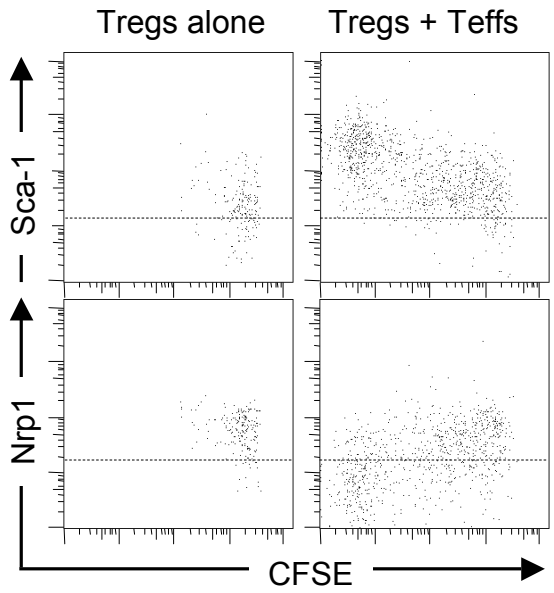
Supplemental Figure 4. Numbers of boosted and non-boosted Tregs 3 weeks after transfer. Ins-HA mice were transferred with 20 x 10^6 CFSE-labeled expanded Thy-1.1$^+$ HA-Tregs alone, or with 2 x 10^6 CFSE-labeled expanded Thy-1.1$^+$ HA-Tregs and 2 x 10^6 pre-activated HA-Teffs. Mice were sacrificed either at day 19 or were challenged with pre-activated HA-Teffs at day 19 and sacrificed 4 days after for flow cytometry analyses in PLNs. (**A**) Representative profile of FoxP3 staining vs CFSE dilution among donor-Tregs (CD4$^+$Thy-1.1$^+$FoxP3$^+$) at day 19. (**B**) Absolute number of donor Tregs (CD4$^+$Thy-1.1$^+$FoxP3$^+$ cells) before (▫,Δ) and after (■,▲) the challenge with HA-Teffs. Each symbol represents an individual mouse from 3 independent experiments.



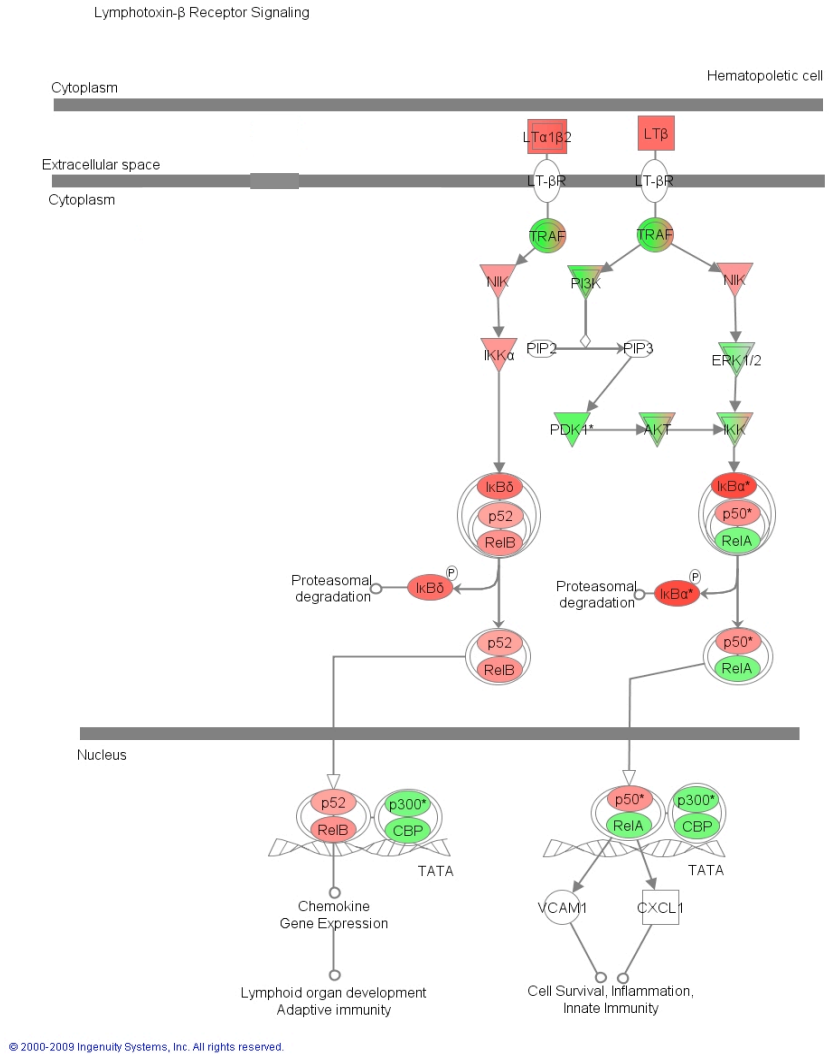
Supplemental Figure 5. IL-2 deficient Teffs strongly boost freshly purified Tregs. Ins-HA mice were transferred with CFSE-labeled Thy-1.1^{+} freshly purified HA-Tregs alone (Tregs alone) or co-injected with IL-2$^{+/+}$ or IL-2$^{-/-}$ pre-activated HA-Teffs. Divided HA-Treg numbers (CFSE^{dim}CD4^{+}Thy-1.1^{+} cells) were quantified in PLNs 10 days later. Each dot represents individual mice from at least 2 independent experiments. ***p<0.0001; **p<0.001.



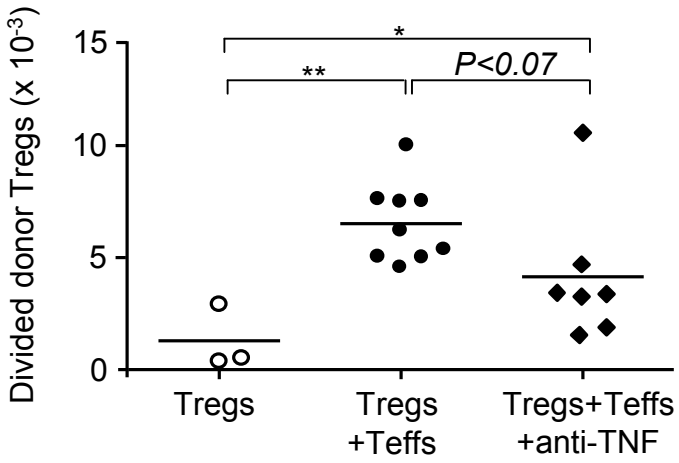
Supplemental Figure 6. Persistence of p-STAT-5 signal after IL-2 administration. Percentage of p-STAT-5+ cells among FoxP3+ cells were determined by flow cytometry **v**arious time after one i.p. injection of low (25,000 IU) or high (250,000 IU) dose of human IL-2 in BALB/c mice. (A) Representative STAT-5 staining among Tregs ($CD4^+FoxP3^+$). Bars delimitate positive staining. (B) Percentage of p-STAT-5+ cells among Tregs ($CD4^+FoxP3^+$).



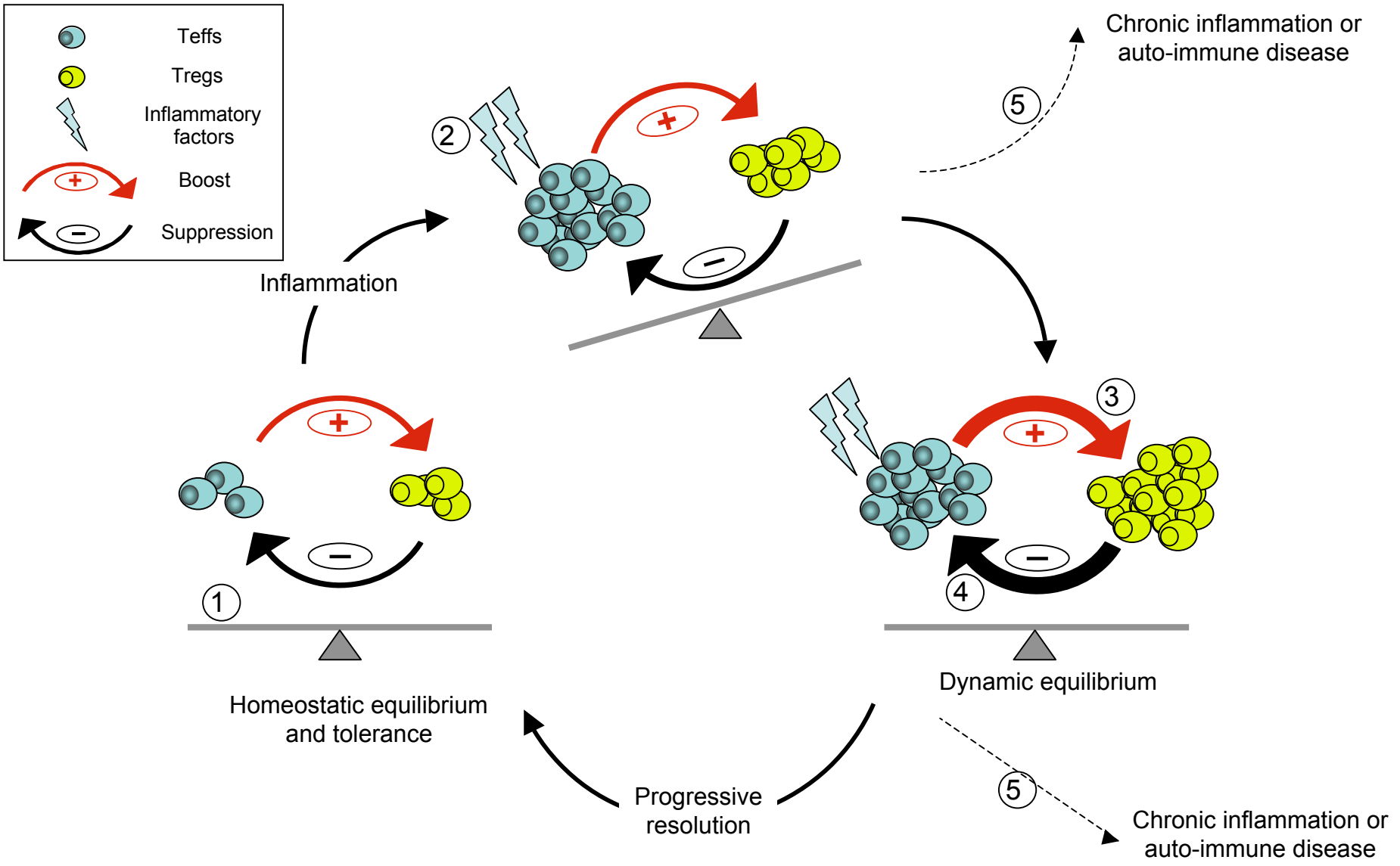
Supplemental Figure 7. HA-Tregs boosted *in vivo* acquired phenotypic modifications as *in vitro*. Ins-HA mice were transferred with CFSE-labeled Thy-1.1^{+} expanded HA-Tregs alone or were co-transferred with pre-activated HA-Teffs. Expression of Sca-1 and Nrp1 on donor Tregs (CD4^{+}Thy-1.1^{+}FoxP3^{+}) was assessed 10 days later. Representative data of 2 independent experiments.



Supplemental Figure 8. Boosted Tregs upregulated the expression of molecules of the alternative NF-kB pathway. Graphic representation of the NF-kB signaling pathway downstream of the lymphotoxin-ß receptor. Genes are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). The node color indicates the degree of up- (red) or down- (green) regulation of gene expression in boosted Tregs versus non-boosted Tregs generated from the transcriptome data and analyzed using the Ingenuity software.



Supplemental Figure 9. The Teff→Treg boost was inhibited by an anti-TNF-a mAb. Four to 6 week-old NOD mice were transferred with freshly isolated CFSE-labelled CD45.2+ BDC2.5-Tregs alone or with pre-activated CD45.1+ BDC2.5-Teffs with or without an anti-TNF-α mAb treatment. Absolute numbers of divided donor Tregs ($CFSE^{dim}CD4^{+}CD45.2^{+}FoxP3^{+}$ cells) were quantified in PLNs 5-6 days after transfer. Each dot represents individual mice pooled from 3 independent experiments. *p<0.05, **p<0.001.



Supplemental Figure 10. A new model of regulation in inflammation and auto-immunity. At the steady state (1), rare auto-reactive Teffs and Tregs co-exist in lymphoid tissues, generating a homeostatic Teff/Treg equilibrium and tolerance. During sustained inflammation (2), Teffs are strongly activated. These cells thus produce or induce factors favoring a strong Teff→Treg boost (3), leading to subsequent Treg expansion. This expansion strengthens Treg-mediated suppression exerted on Teffs (4). This inhibition of Teff activation probably reduces Treg boost factors, eventually leading to a progressive resolution and a return to the basal homeostatic Teff/Treg equilibrium. In some conditions, the response may progress toward chronic inflammation or autoimmune disease (5). This may be due to either ineffective Treg-mediated suppression or a defect of the Teff→Treg boost.

Ingenuity Canonical Pathways	-Log(P-value)	Ratio	Molecules
Glycolysis/Gluconeogenesis	3,99E+00	4,96E-02	TPI1, ACSS1, PFKL, ENO3, PFKP, HK2, GALK1
Fructose and Mannose Metabolism	3,92E+00	3,60E-02	TPI1, PFKL, PFKP, HK2, GALK1
Galactose Metabolism	2,80E+00	3,57E-02	PFKL, PFKP, HK2, GALK1
CD40 Signaling	2,67E+00	7,35E-02	NFKBIA, FOS, ICAM1, TRAF5, LTA
CCR5 Signaling in Macrophages	2,64E+00	5,81E-02	CCL4, FOS, CCL5, CCL3, GNG12
CD27 Signaling in Lymphocytes	2,47E+00	8,16E-02	NFKBIA, FOS, TRAF5, CD70
Lymphotoxin β Receptor Signaling	2,12E+00	7,02E-02	LTB, NFKBIA, TRAF5, LTA
Antigen Presentation Pathway	2,08E+00	7,69E-02	CALR, HLA-DQB2, HLA-DRA
Activation of IRF by Cytosolic Pattern Recognition Receptors	1,86E+00	5,48E-02	ZBP1, NFKBIA, ISG15 (includes EG:9636), LTA
Chemokine Signaling	1,69E+00	5,19E-02	CCL4, CXCR4, FOS, CCL5
p53 Signaling	1,43E+00	4,60E-02	SERPINE2, GADD45G, GADD45B, E2F1
Glycosphingolipid Biosynthesis - Neolactoseries	1,40E+00	2,99E-02	ST8SIA4, ST3GAL6
Eicosanoid Signaling	1,32E+00	3,57E-02	PTGER2, ALOX5AP, AKR1C3
Glucocorticoid Receptor Signaling	1,21E+00	2,54E-02	ADRB2, NFKBIA, FOS, ICAM1, CCL5, CCL3, ANXA1
4-1BB Signaling in T Lymphocytes	1,11E+00	5,41E-02	NFKBIA, TNFRSF9
Aryl Hydrocarbon Receptor Signaling	8,53E-01	2,58E-02	FOS, MCM7, TGM2, E2F1
IL-6 Signaling	8,48E-01	3,12E-02	NFKBIA, FOS, IL6R
CXCR4 Signaling	8,05E-01	2,44E-02	CXCR4, FOS, EGR1, GNG12
IL-2 Signaling	7,47E-01	3,57E-02	IL2RB, FOS
G-Protein Coupled Receptor Signaling	5,00E-01	1,86E-02	ADRB2, P2RY1, NFKBIA, DUSP4
NF-κB Signaling	4,90E-01	2,08E-02	NFKBIA, TRAF5, LTA
IL-8 Signaling	3,79E-01	2,19E-02	ICAM1, MMP9, PLD4, GNG12
Role of NFAT in Regulation of the Immune Response	3,79E-01	1,61E-02	NFKBIA, FOS, GNG12
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	3,62E-01	2,33E-02	CDC25B

Supplemental Table 1. Top canonical pathways differently used by boosted Tregs. Gene array data were processed using Ingenuity pathway analysis software. The table shows the canonical pathways, identified from the Ingenuity canonical pathways library, that were most significant to the data set according to the following 2 criteria: 1) Fischer's exact test was used to calculate a p-value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone ("Log(P-value)"). 2) A ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway is displayed ("Ratio"). "Molecules" show the genes from the data set included in the corresponding canonical pathway