Supplemental Methods

Human monocyte-derived DC (MoDC) generation

Peripheral blood mononuclear cells (PBMC) were isolated from normal leukopacks (Central Blood Bank, Pittsburgh, PA) by Ficoll-Hypaque density gradient centrifugation followed by CD14 immunomagnetic bead selection (Miltenyi Biotec, Auburn, CA). PBMC ($1.1x10^6$ cells/ml) were cultured in RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with pooled human AB serum (5% v/v, Gemini Bio-Products, West Sacramento, CA), L-glutamine (2 mM, Cellgro Manassas, VA), penicillin/streptomycin (100 U/ml, Lonza, Walkersville, MD), rhGM-CSF (1000 U/ml, R&D Systems, Minneapolis, MN) and rhIL-4 (1000 U/ml, R&D Systems) for 6 d. One half of the medium was refreshed every 2 d. Cultures were stimulated with TLR4-specific LPS (100 ng/ml; *S. minnesota* R595, Alexis Biochemicals, San Diego, CA) on d4. RAPA (10 ng/ml; LC Laboratories, Woburn, WA) or Torin1 (Tocris Bioscience, Minneapolis, MN) were added on d2 and d4.

Human $CD34^+$ cell-derived DC

Human $CD34^+$ cell-derived DC were generated from immunomagnetic bead-selected (Miltenyi Biotec, Germany), cryopreserved $CD34^+$ cells from blood donors after informed consent, as described[1] with minor modifications. Briefly, $4x10^4$ $CD34^+$ cells/ml were expanded in 6-well plates (Greiner, Germany) for 6 d with GM-CSF (50 ng/ml, Miltenyi Biotec), stem cell factor (25 ng/ml, Miltenyi Biotec) and TNFα (2.5 ng/ml, Miltenyi Biotec) in RPMI-1640 supplemented with L-glutamine, penicillin/streptomycin and 10% heat-inactivated FCS (PAA, Germany). On d6, cells were washed then cultured in fresh medium for an additional 7 d with GM-CSF (50 ng/ml) and IL-4 (50 ng/ml, eBioscience, Germany). Vehicle control or mTOR inhibitors were

added on d2 at the indicated concentration and refreshed every 2 d. DC were stimulated with LPS (100 ng/ml; 0111:B4 strain, Sigma-Aldrich, Germany) for 24 h.

Flow cytometric analysis of absolute T_{reg} numbers

The absolute number of cells in MLR was determined on d5 by trypan blue exclusion or flow cytometry using CountBright Absolute Counting Beads (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Total cell numbers were multiplied by T_{reg} frequency to determine the absolute number of T_{reg} in MLR.

Figure S1

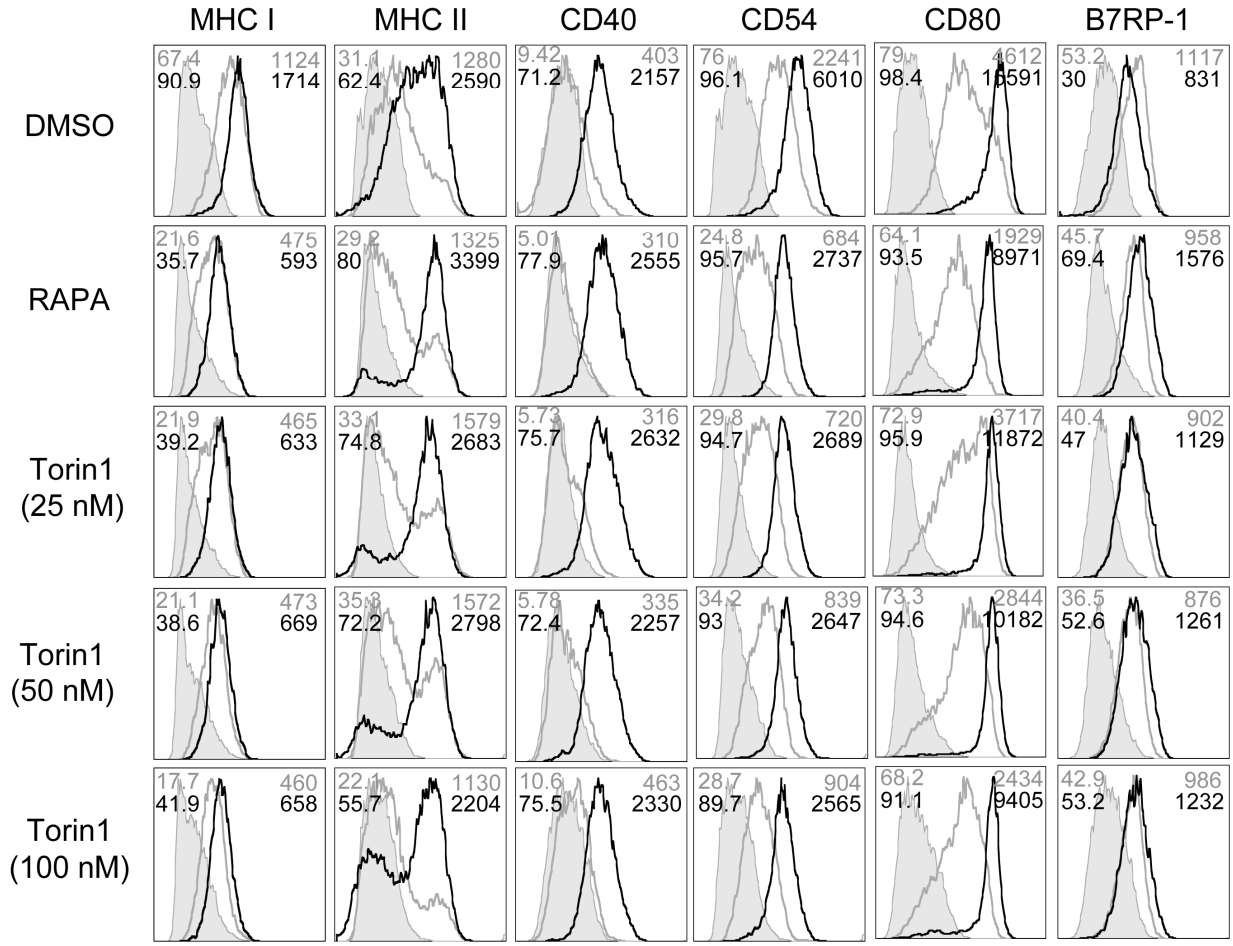

Figure S1. RAPA- and Torin1-exposed DC phenotype. (A) RAPA or the indicated concentration of Torin1 was added to BM cell cultures from d2-d8, and the phenotype of CD11c-gated cells analyzed on d8 following overnight LPS stimulation. Isotype controls are indicated by the shaded histograms, and unstimulated (gray) and LPS-stimulated (black) conditions are indicated by solid lines. The percent of positive cells and MFI are indicated in the upper left and right corner, respectively. Data are representative of n≥3 experiments.

Figure S2

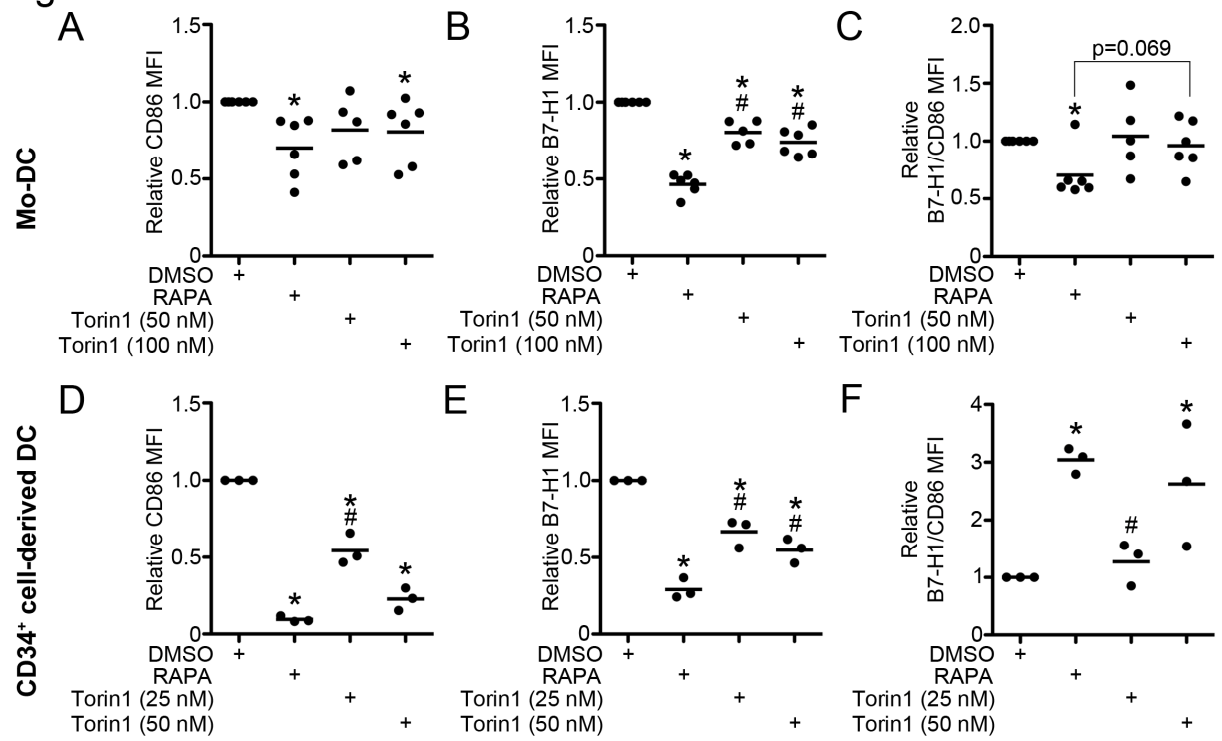

Figure S2. Torin1 spares B7-H1 while similarly reducing CD86 expression compared to RAPA on human DC. (A,B) Human Mo-DC were cultured in RAPA or Torin1 and matured overnight with LPS. (A) CD86 and (B) B7-H1 expression was normalized to DMSO. (C) The B7-H1 to CD86 expression ratio was calculated and normalized to DMSO. (D,E) $CD34^+$ cell-derived DC were stimulated with LPS overnight and (D) CD86 and (E) B7-H1 expression determined. (F) Normalized B7-H1 to CD86 expression was calculated. * and # indicate $p<0.05$ when compared to control and RAPA, respectively. Each data point represents one individual donor.

Figure S3

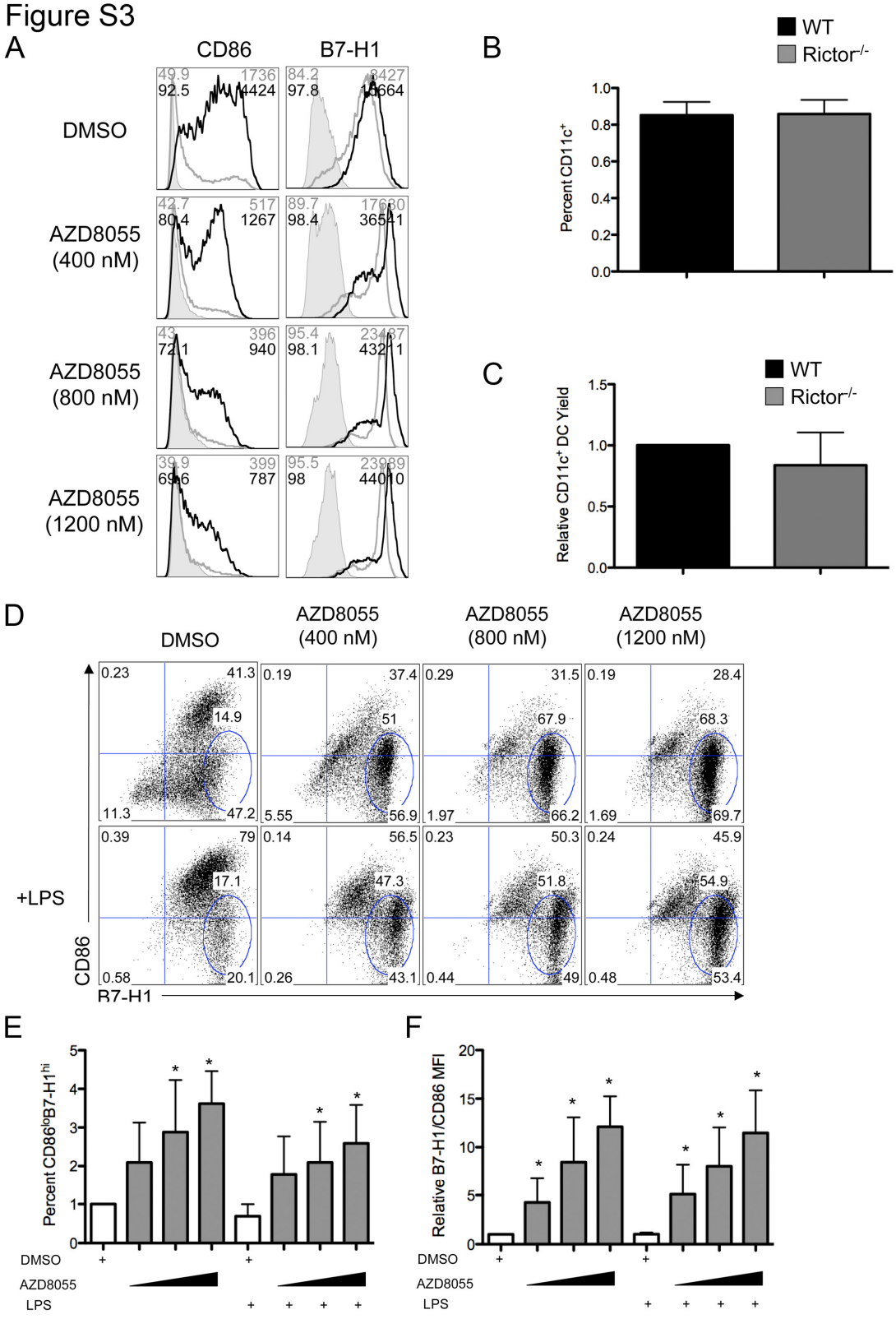
A
CD86
B7-H1
DMSO
AZD8055 (400 nM)
AZD8055 (800 nM)
AZD8055 (1200 nM)
B
WT
Rictor-/-
Percent CD11c+
C
Relative CD11c+ DC Yield
D
DMSO
AZD8055 (400 nM)
AZD8055 (800 nM)
AZD8055 (1200 nM)
+LPS
CD86
B7-H1
E
Percent CD86hiB7-H1hi
DMSO
AZD8055
LPS
F
Relative B7-H1/CD86 MFI

Figure S3. AZD8055 enhances the ratio of B7-H1 to CD86 expression and promotes CD86^{lo}B7-H1^{hi} DC. (A) CD86 and B7-H1 expression was analyzed on CD11c-gated cells following culture with AZD8055 at the indicated dose. Unstimulated DC (gray lines) or those stimulated with LPS overnight (black lines). The percent of positive cells and MFI are indicated in the upper left and right corners, respectively. Isotype controls are indicated by shaded histograms. (B) Percentage of CD11c^{+} DC in WT and rictor$^{-/-}$ BM cultures. (C) Absolute number of CD11c^{+} DC isolated from WT and rictor$^{-/-}$ BM cultures. (D) Co-expression of CD86 and B7-H1 was analyzed on CD11c-gated cells cultured in AZD8055. (E) CD86^{lo}B7-H1^{hi} DC gated in (D) were quantified relative to DMSO controls. (F) B7-H1 and CD86 expression was normalized to DMSO control DC expression and the ratio determined for each treatment group. AZD8055 concentrations ranged from 400 nM to 1200 nM. Data are from n≥3 independent experiments. *, $p<0.05$ compared to DMSO or DMSO+LPS controls.

Figure S4

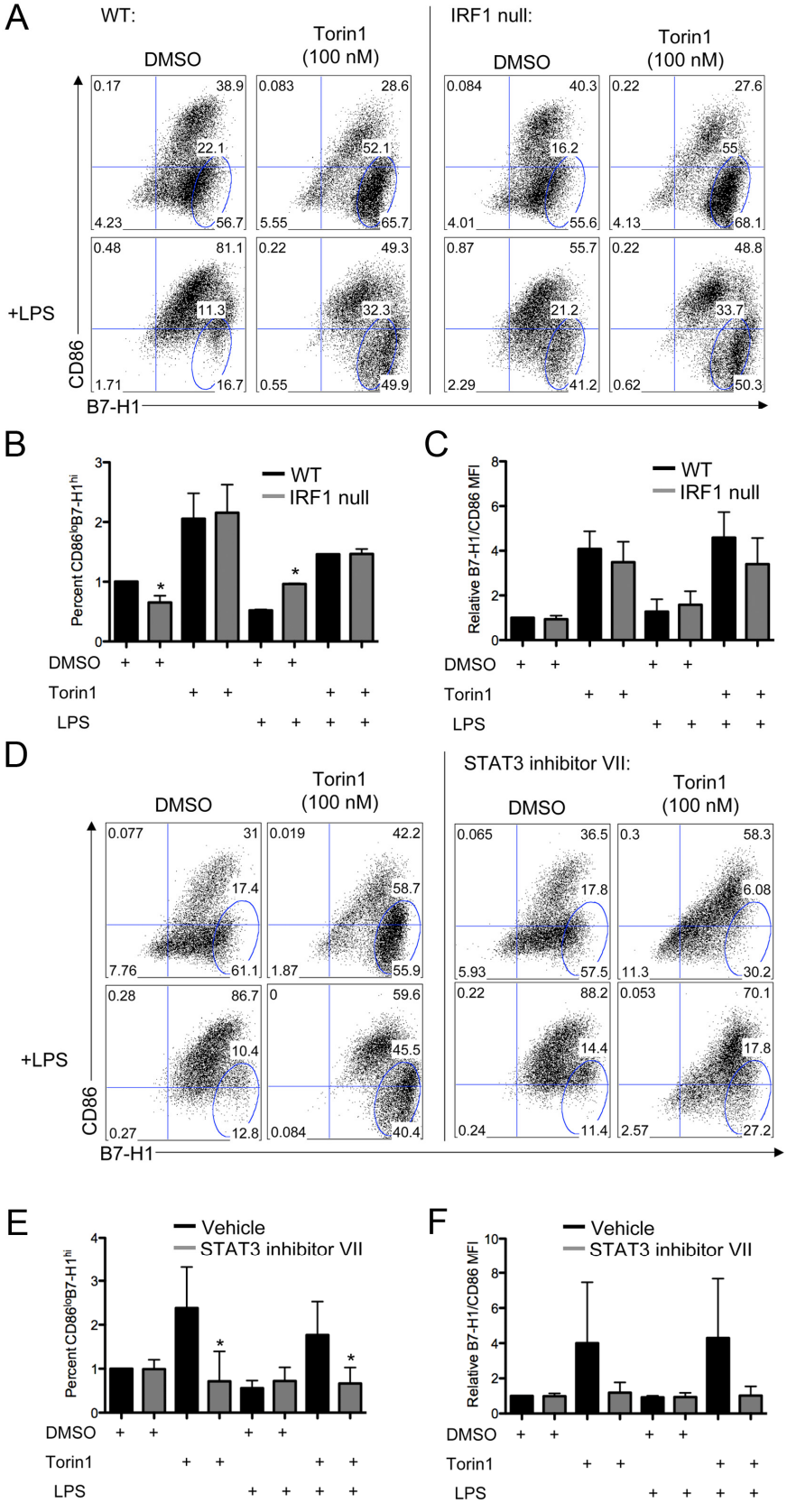
A
WT:
IRF1 null:
DMSO
Torin1 (100 nM)
+LPS
CD86
B7-H1
B
C
WT
IRF1 null
Percent CD86loB7-H1hi
Relative B7-H1/CD86 MFI
DMSO
Torin1
LPS
D
STAT3 inhibitor VII:
E
F
Vehicle
STAT3 inhibitor VII

Figure S4. STAT3 but not IRF-1 is required for generation of CD86^{lo}B7-H1^{hi} DC by ATP-competitive mTOR inhibition. (A) WT or IRF-1 null BM cell cultures were exposed to Torin1 (100 nM) from d2-d8 as described in the methods. Cultures were stimulated with LPS (100 ng/ml) overnight on d7 and CD11c^{+} DC interrogated for CD86 and B7-H1 expression by flow cytometry. (B) Quantification of CD86^{lo}B7-H1^{hi} DC. (C) The ratio of B7-H1 to CD86 expression was determined on CD11c^{+} DC on d8. (D) DC were differentiated in the presence of Torin1 and exposed to STAT3 inhibitor VII on d7 for 2h before stimulation with LPS overnight. Representative dot plots of CD86 vs. B7-H1 expression are shown. (E) Quantification of CD86^{lo}B7-H1^{hi} gate in (D) normalized to DMSO controls. (F) Ratio of B7-H1 to CD86 MFI normalized to DMSO controls. Bar graphs demonstrate the mean + SD from n=2-4 independent experiments with * indicating $p<0.05$ when compared to WT DC (A-C) or samples not treated with STAT3 inhibitor (D-F).

Figure S5

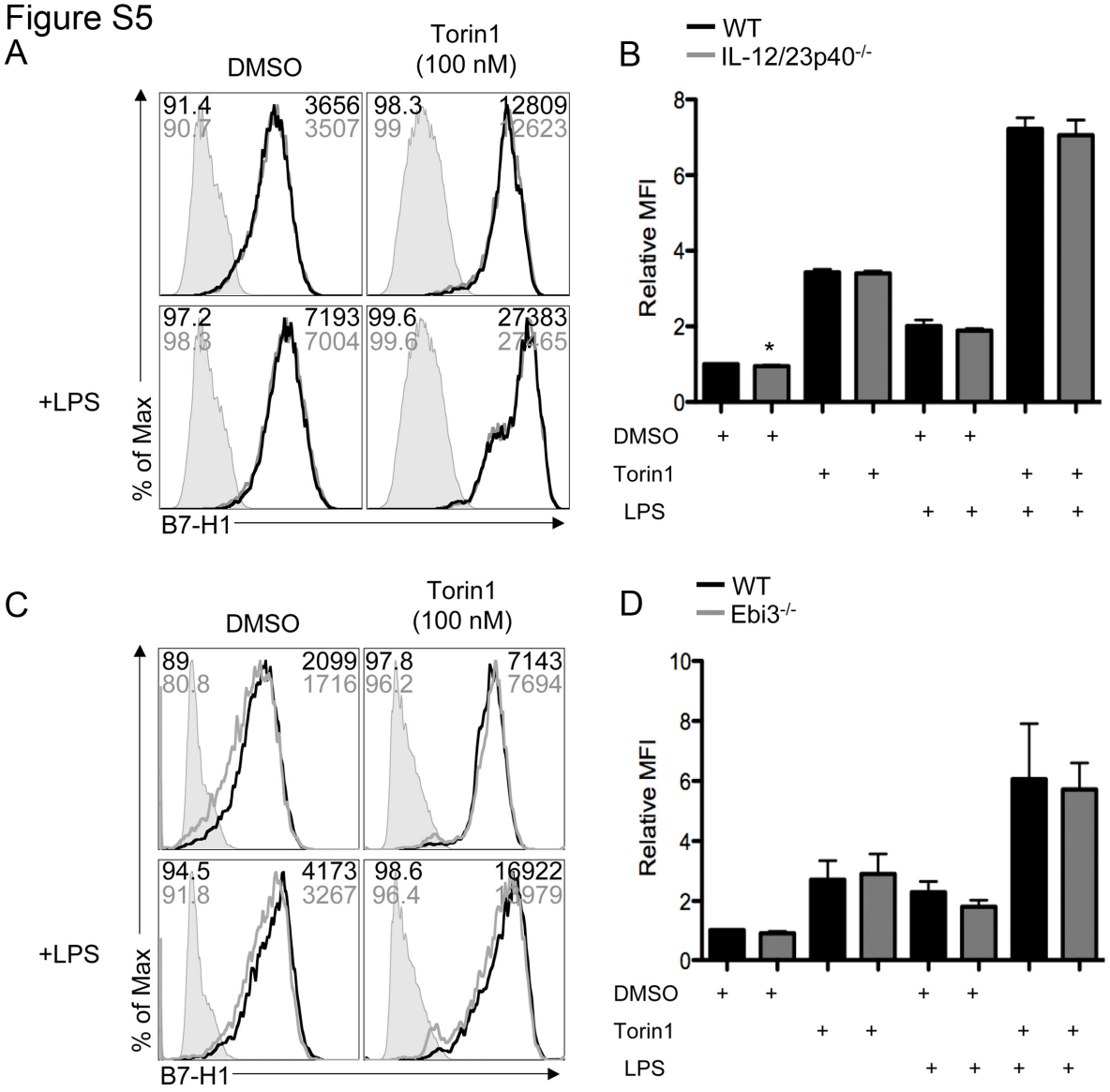

Figure S5. B7-H1 upregulation by Torin1 does not require autocrine IL-12/23 or IL-27. CD11c^{+} DC differentiated from (A,B) IL-12/23$^{-/-}$ (IL-12/23p40$^{-/-}$) or (C,D) IL-27$^{-/-}$ (Ebi3$^{-/-}$) BM cells differentiated in Torin1 from d2-d8 were analyzed for expression of B7-H1. *, $p<0.05$ when compared to WT DC. Data are from n=3 independent experiments.

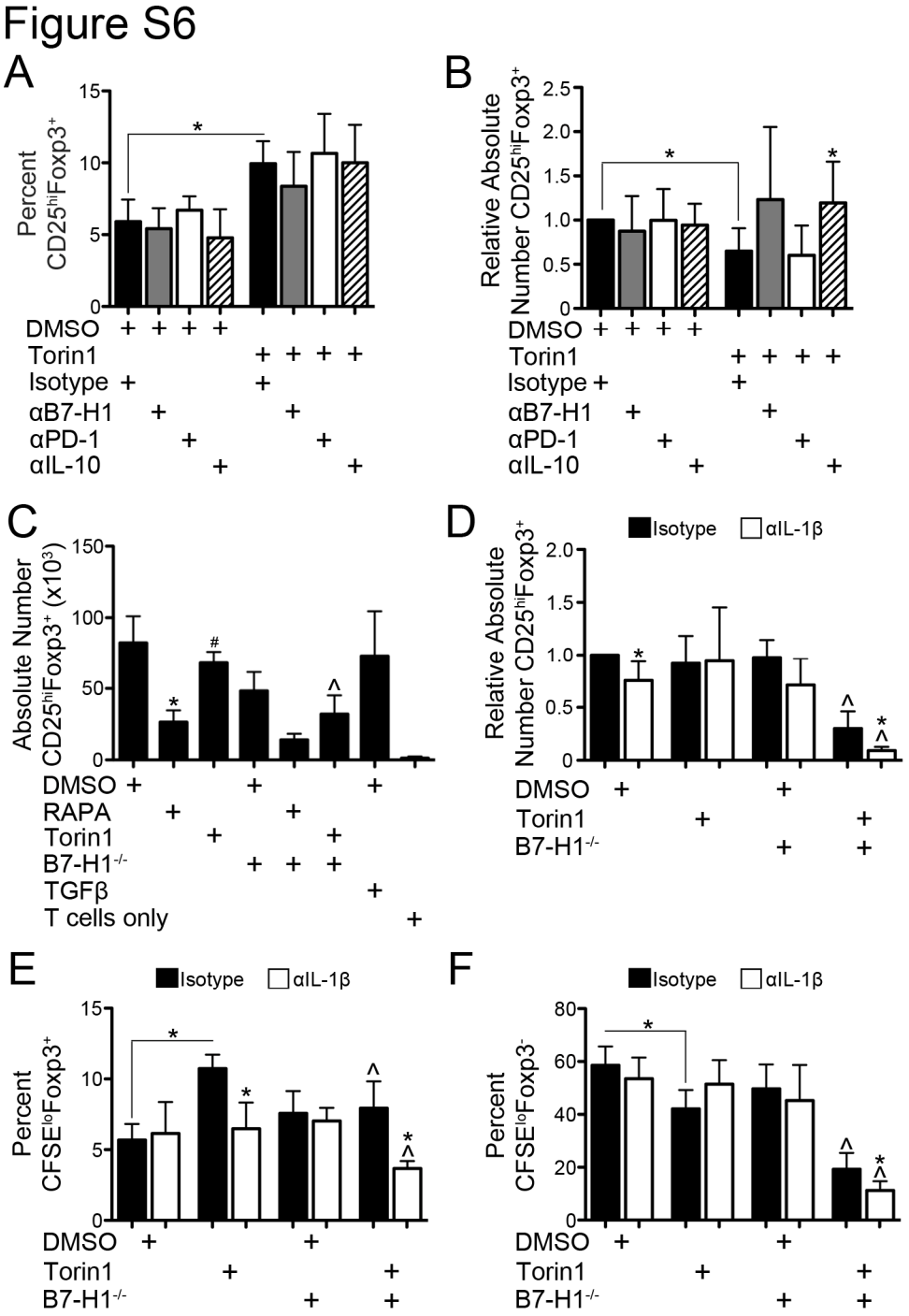

Figure S6. Enhanced T_{reg} induction by Torin1-DC is PD-1- and IL-10-independent. (A-D) WT or B7-H1$^{-/-}$ B6 DC generated in DMSO, RAPA or Torin1 were used as stimulators of CFSE-labeled CD4^{+}CD25^{-} BALB/c T cells in 5d MLR. (A) B7-H1, PD-1 or IL-10 were neutralized by addition of Ab at the start of MLR and the percent of T_{reg} determined. (B) Absolute numbers of

T_{reg} in MLR from (A) were determined by flow cytometry and normalized to control. (C) Total viable cell numbers were determined on d5 by trypan blue exclusion, and the absolute number of induced T_{reg} was calculated. Exogenous TGFβ (10 ng/ml) was added to cultures as a positive control, where indicated. (D) IL-1β was neutralized in MLR. The absolute number of T_{reg} was determined by flow cytometry and normalized to control. (E,F) Quantification of (E) $CFSE^{lo}Foxp3^{+}$ and (F) $CFSE^{lo}Foxp3^{-}$ cells in MLR with IL-1β neutralization. (A-B and D-F) *, ^ $p<0.05$ compared to corresponding isotype control and WT condition, respectively. (C) *, #, and ^ $p<0.05$ compared to WT DMSO, WT RAPA, and the corresponding WT condition, respectively. Data are representative of $n\geq3$ independent experiments.

References

1. Ratta M, Rondelli D, Fortuna A, et al. Generation and functional characterization of human dendritic cells derived from CD34 cells mobilized into peripheral blood: comparison with bone marrow CD34+ cells. *Br J Haematol*. 1998;101(4):756-765.