Supplementary Information

Supplementary Methods

Flow Cytometry and antibodies

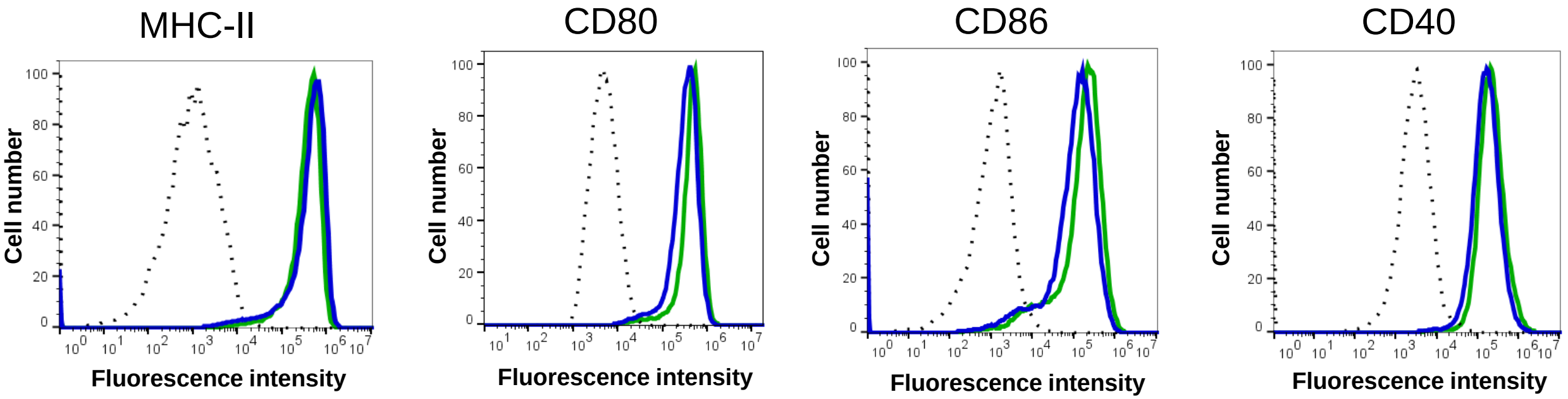
The following fluorescently-conjugated anti-mouse primary antibodies were purchased from Biolegend: CD45 (Clone 30-F11, Cat. 103134), I-Ab (clone AF6-120.1, Cat. 116416), CD80 (clone 16-10A1, Cat. 104714), CD86 (clone GL-1, Cat. 105028), CD40 (clone 3/23, Cat. 124622), CD11c (clone N418, Cat. 117310), CD54 (ICAM-1, clone YN1/1.7.4, Cat. 116108), CD102 (ICAM-2, clone 3C4, Cat. 105609), CD19 (clone 6D5, Cat. 115520), CD11b (clone M1/70, Cat. 101208), CD40 (clone3/23, Cat. 124622), CD4 (clone RM4-5, Cat. 100526), CD8a (clone 53-6.7, Cat. 100723), CD103 (clone 2E7, Cat. 121406), and isotype controls: IgG2b,κ (clone MRG2b-85, Cat. 408214) and IgG2a,κ (clone RTK2758, Cat. 400506, 407112). Unconjugated CD40 mAb (Bio X cell, clone FGK4.5/FGK45, Cat. BE0016-2) was intrafootpad injected to activate DCs in vivo as previously described [13]. For analysis of surface expression of various proteins and ex vivo proliferation assay, cells were labeled with fluorescent primary antibodies (10μg/ml), washed, resuspended in fluorescence-activated cell sorting (FACS) buffer (PBS-/-, 1% BSA, 5mM EDTA, and 0.01% sodium azide), and analyzed on the CytoFLEX S Flow Cytometer (Beckman Coulter). Data was acquired with CytExpert software (Beckman Coulter) and post-acquisition analysis was performed using FlowJo software (Tree Star,Inc.).

Supplementary Video Legends

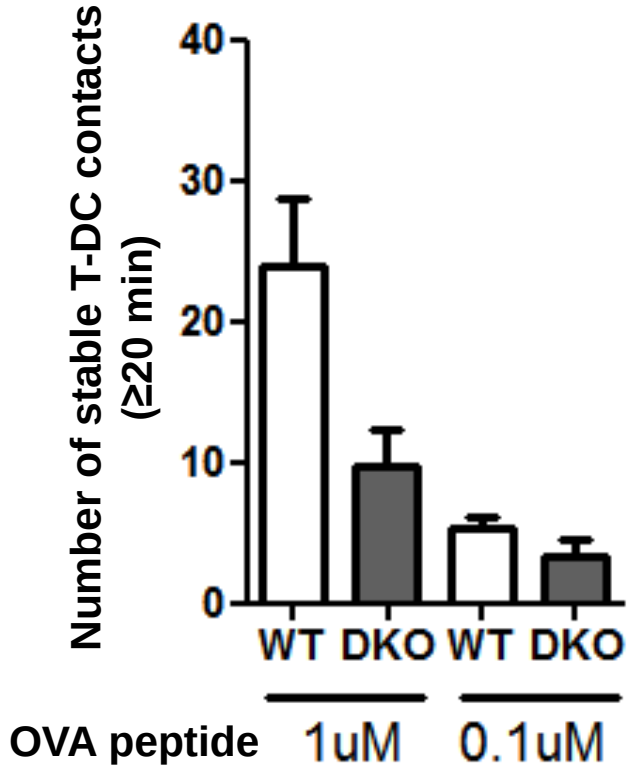
Video 1. Representative movie of dsRed OT-II (red) stably conjugated to either LPS stimulated CFP WT BMDCs (marked by white circles) or to CFSE ICAM DKO BMDCs (marked by orange circles). Both types of DCs were loaded with saturating doses of OVA peptide and co-injected into the footpad 24 hrs before intravital imaging. The movie corresponds to Fig. 2B. Bar, 50 μm.

Video 2. Representative movie of dsRed OT-II (red) and GFP-polyclonal (green) $CD4^+$ T cells migrating inside the T zone of the popliteal lymph node into which LPS stimulated CFP BMDCs saturated with OVA peptide were injected into the footpad 24 hrs before intravital imaging as described in the Materials and Methods section and with results depicted in Fig. 3B. White arrows denote stable OT-II arrests on representative DCs. Bar, 30 μm.

Video 3. Representative movie of dsRed OT-II (red) T cells migrating inside the T zone of the popliteal lymph node into which LPS stimulated CFP WT BMDCs and CFSE ICAM DKO BMDCs each saturated with OVA peptide were co-injected into the footpad 24 hrs before intravital imaging as described in the Materials and Methods section and with results depicted in Fig. 3C. White and orange arrows denote stable OT-II arrests on WT and ICAM DKO DCs, respectively. Bar, 20 μm.



Supplementary Figure 1. Effect of ICAM deficiency on MHC-II and co-stimulatory receptor expression by LPS stimulated BMDCs. DCs were stimulated with LPS for 24 hrs as described in the Materials and Methods section and stained for the indicated antibodies.



Supplementary Figure 2. Long lasting OT-II conjugates with co-transferred LPS stimulated OVA peptide loaded WT and ICAM1/2 DKO DCs. Effect of OVA peptide loadings. Images were taken 3-6 hrs post i.v. injection of OT-T cells. Results are mean values from 4 different fields of view analyzed in two independent experiments.