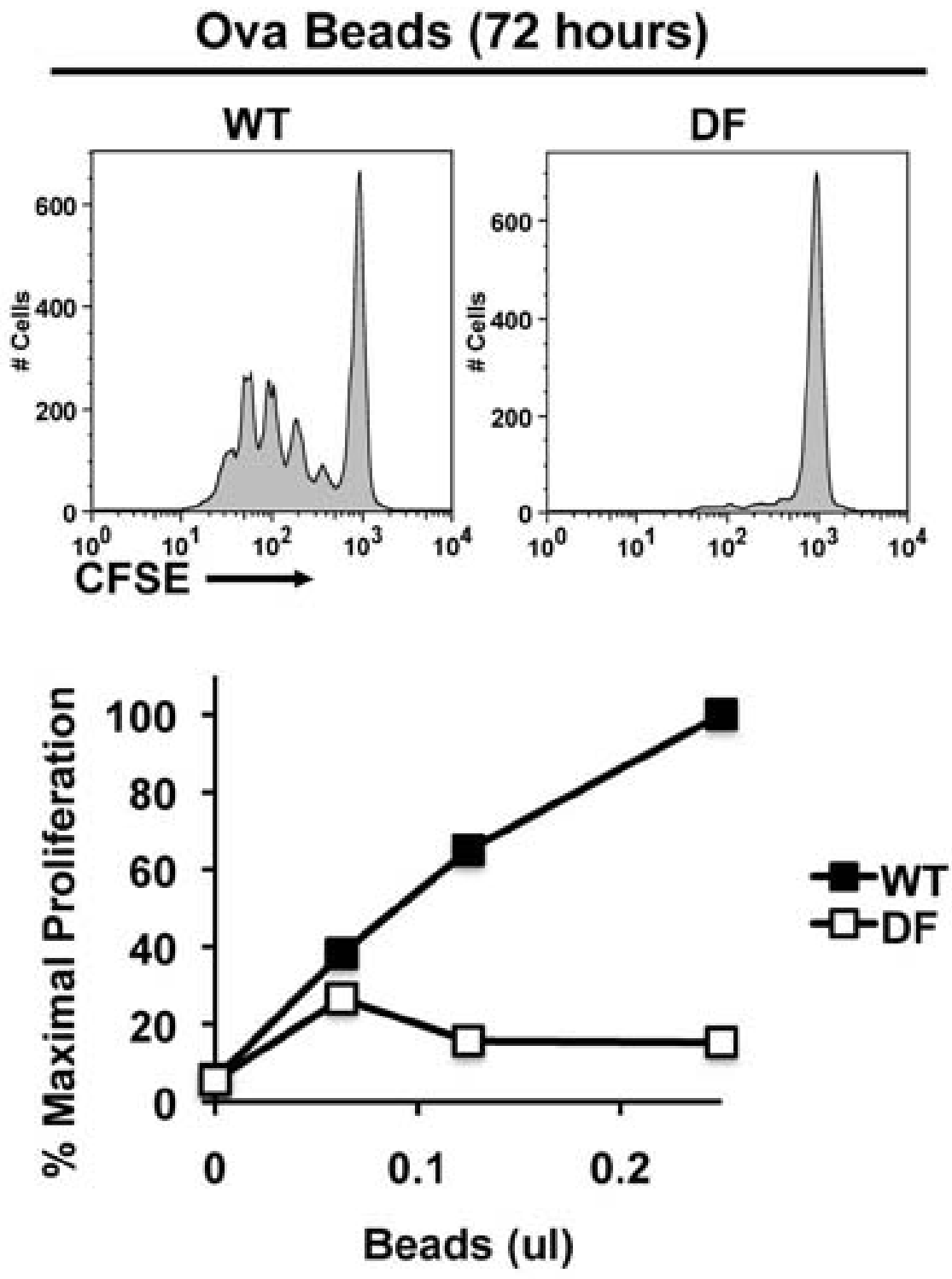
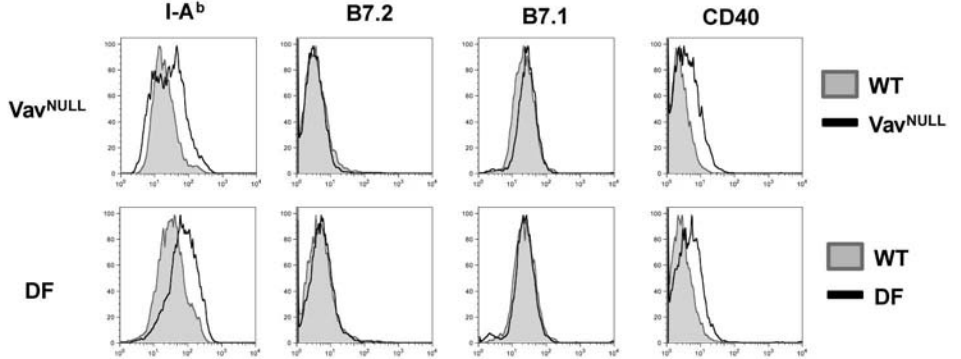
Supplemental Figure 1.



Supplemental Figure 2.



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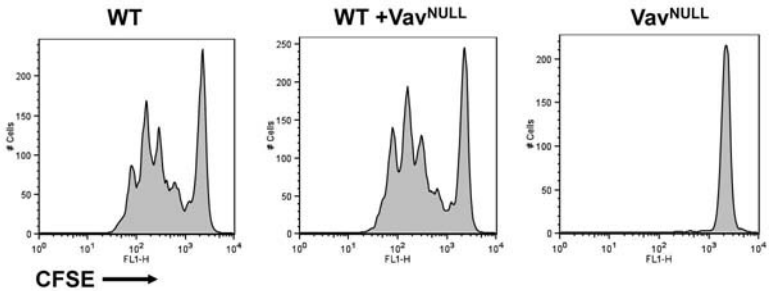

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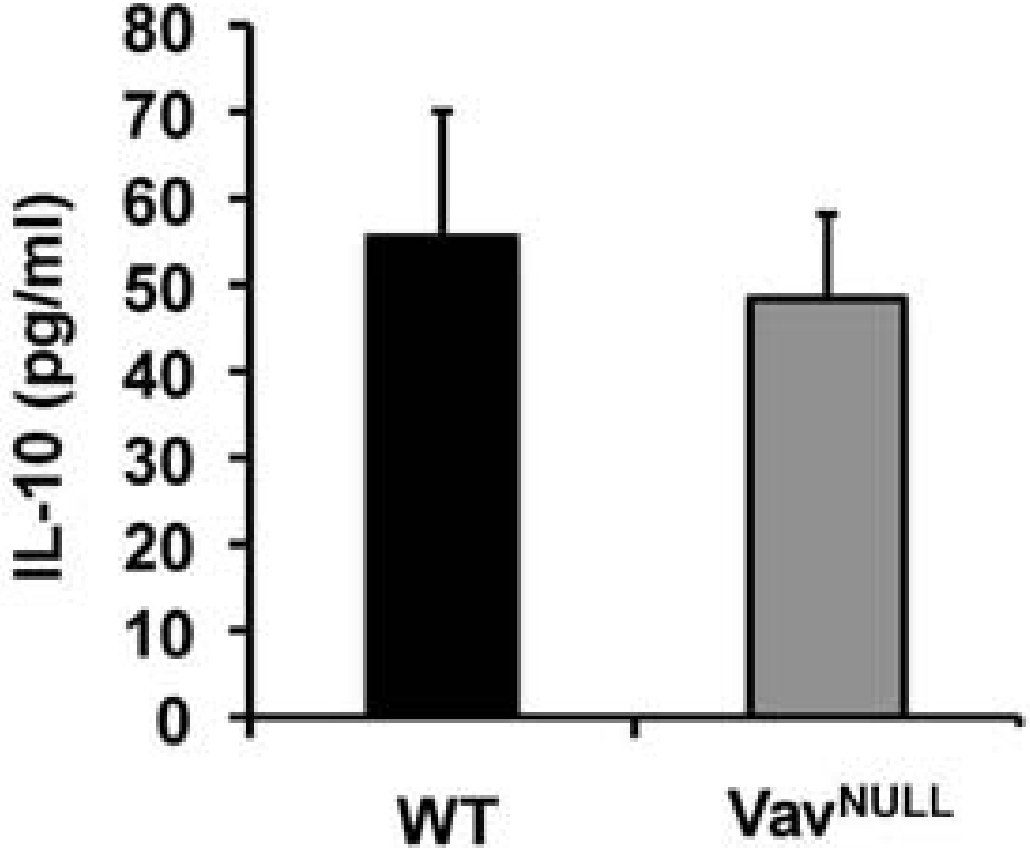

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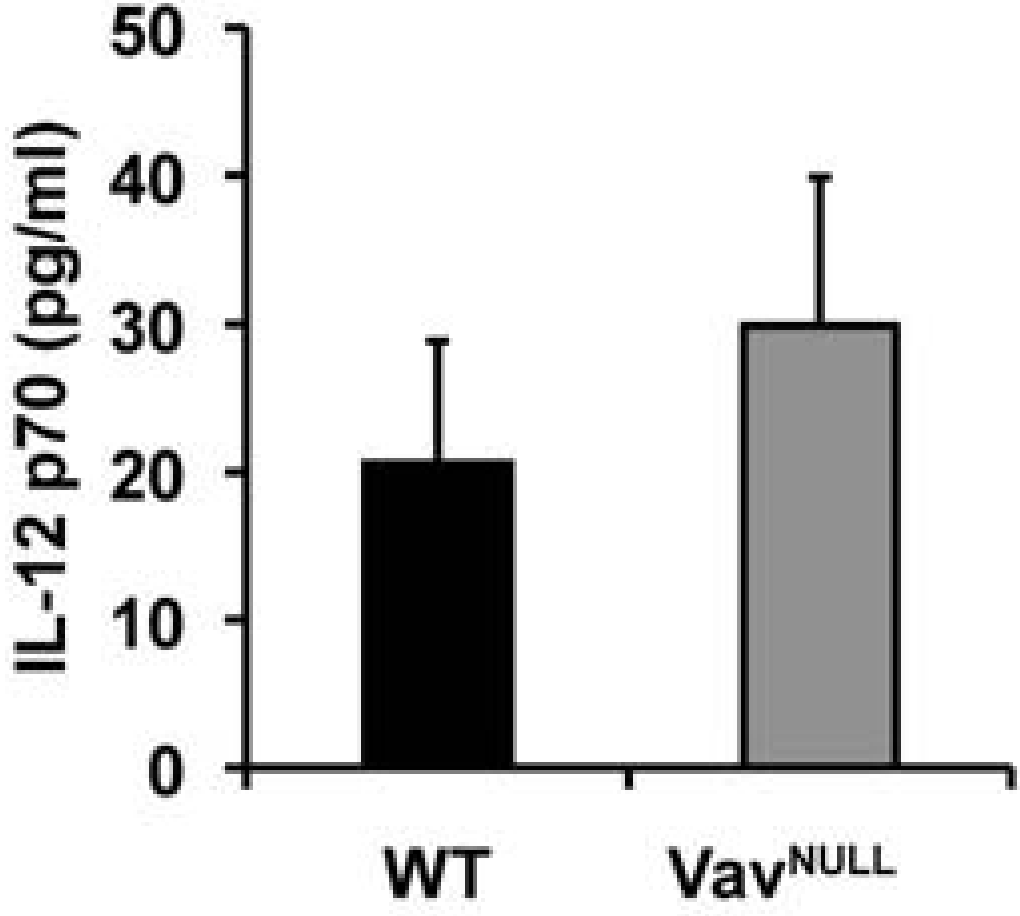

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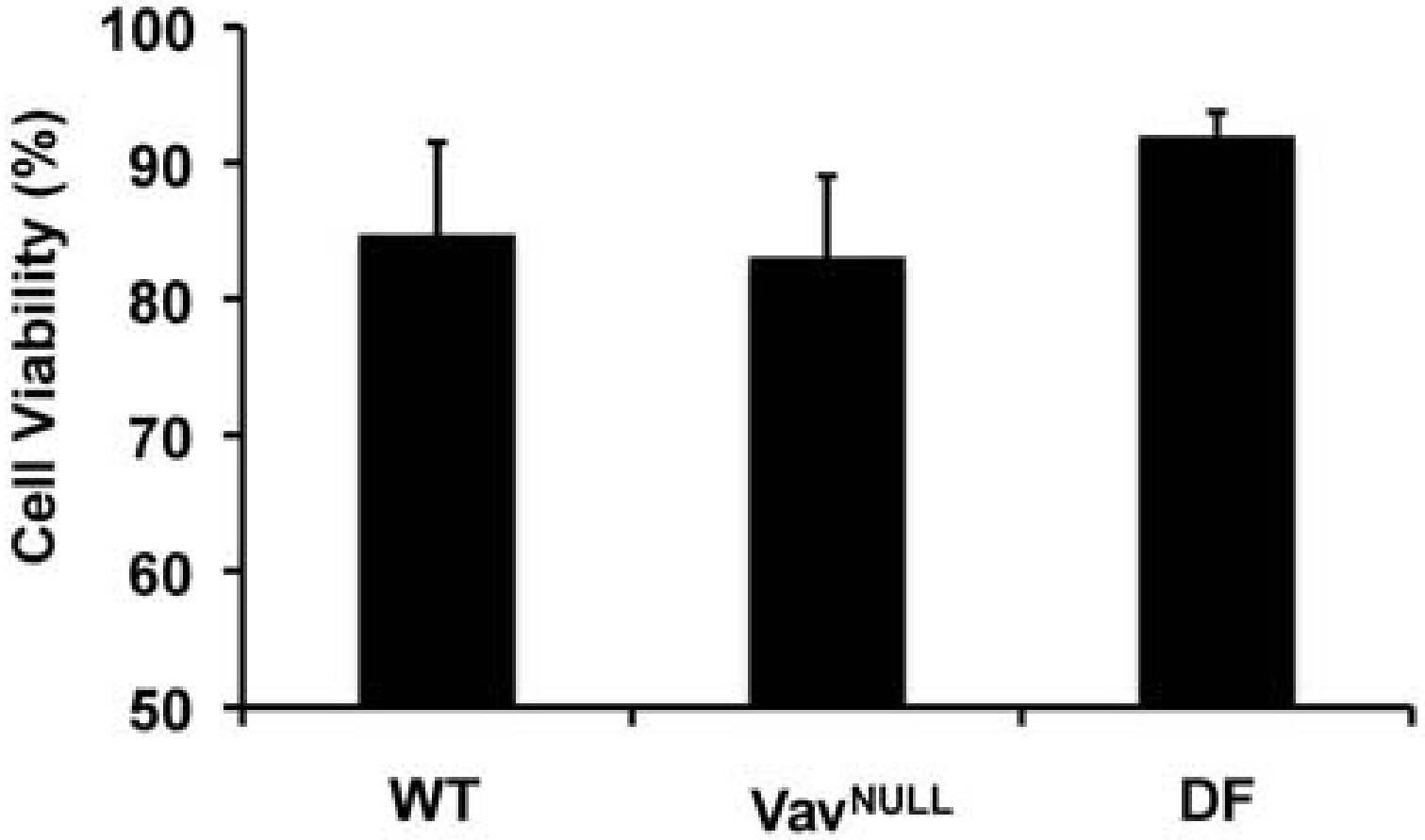

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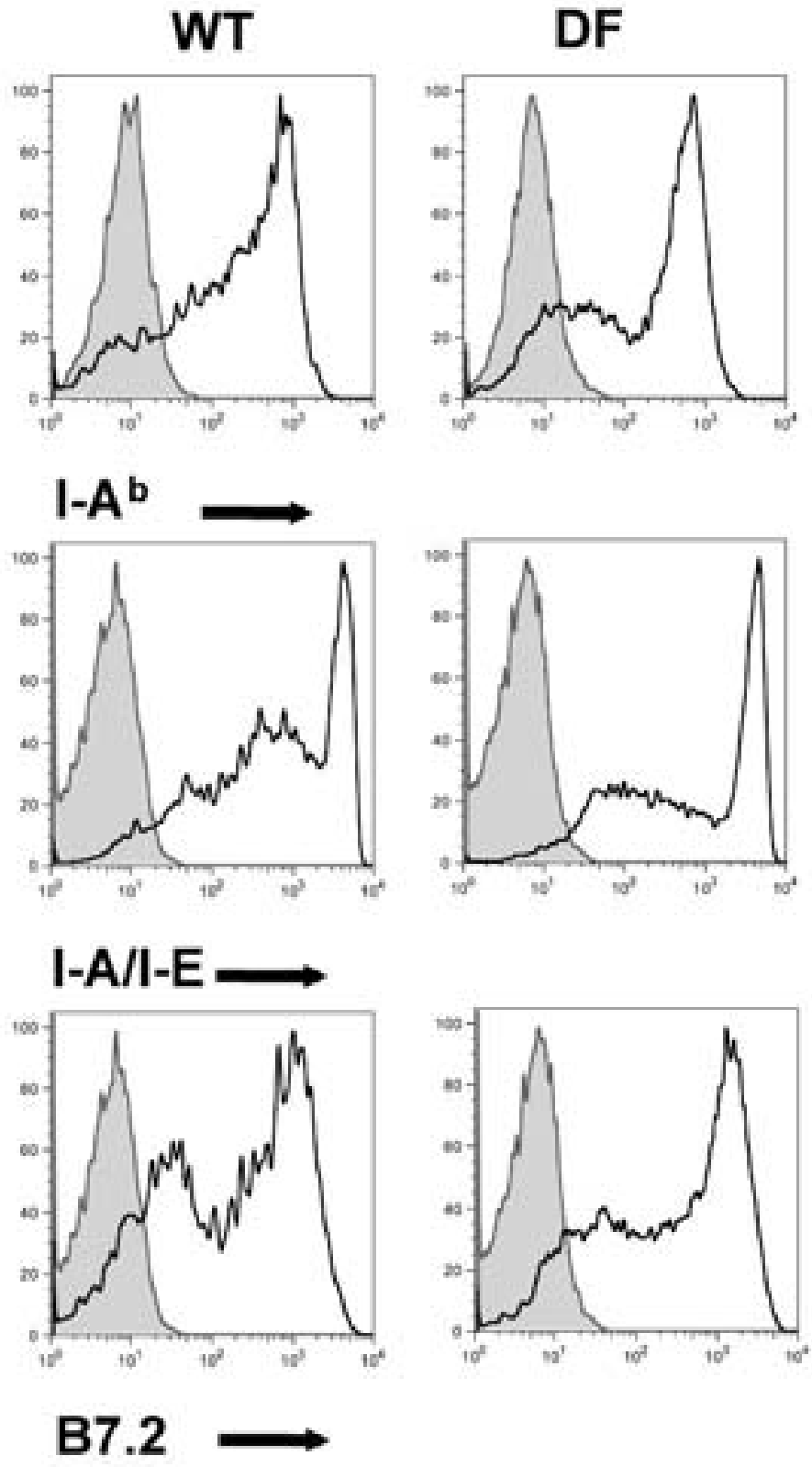

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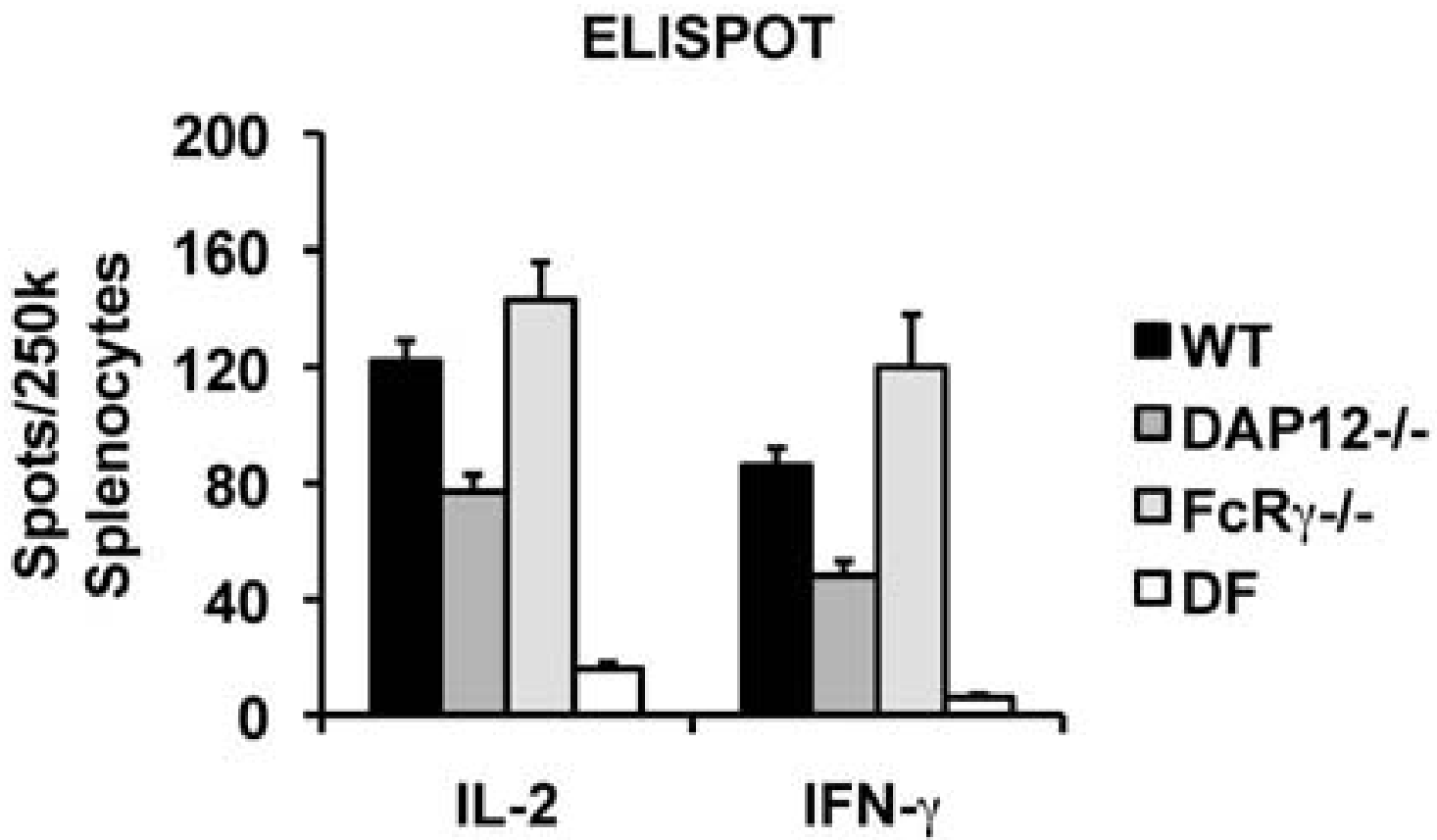

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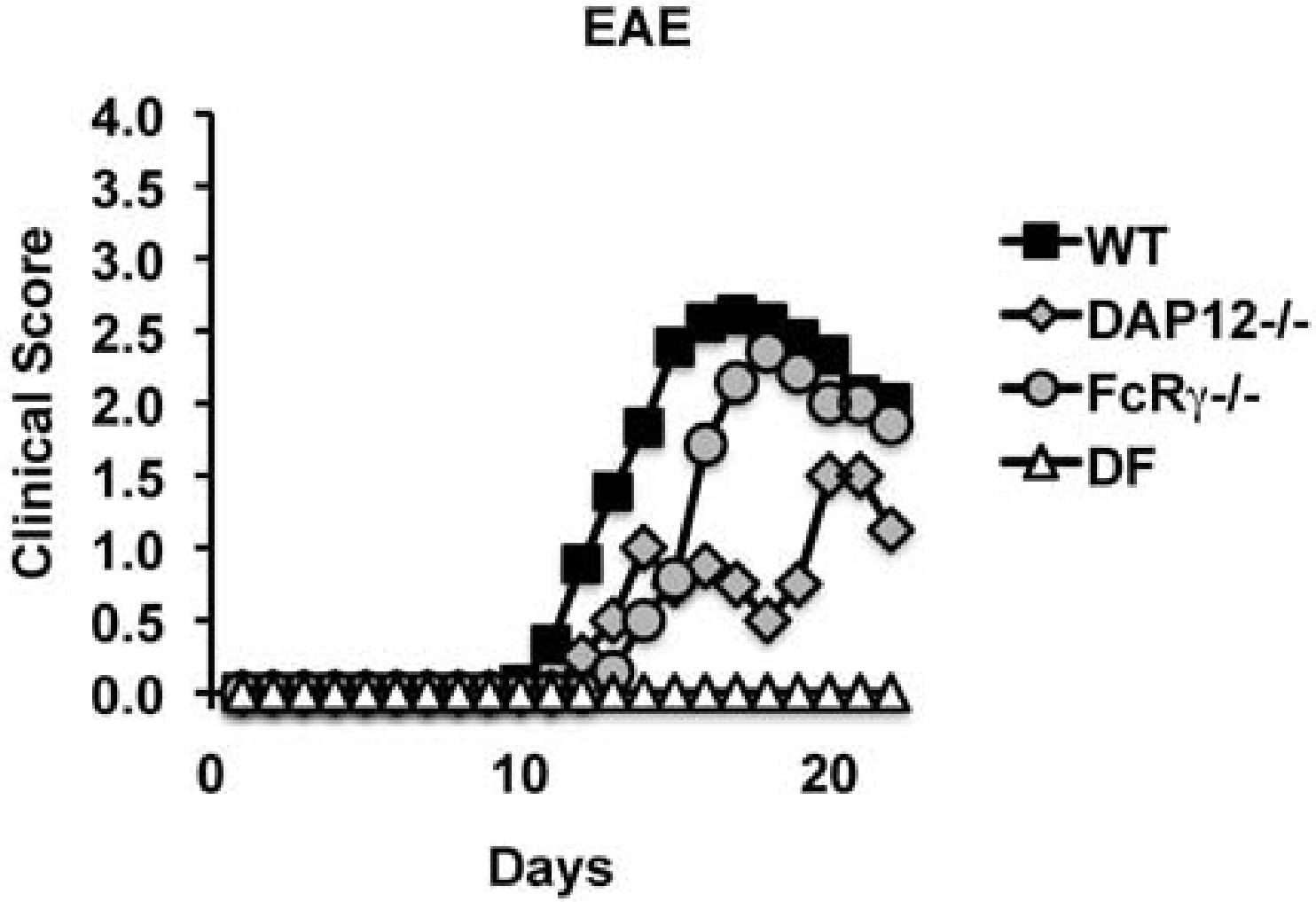

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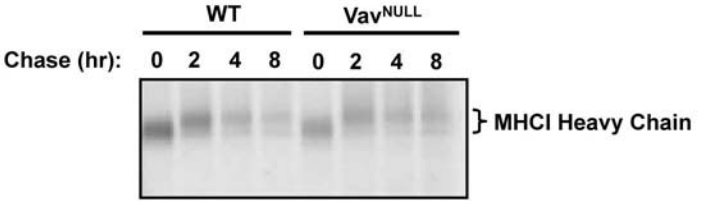

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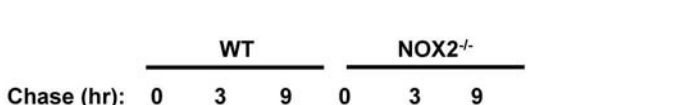

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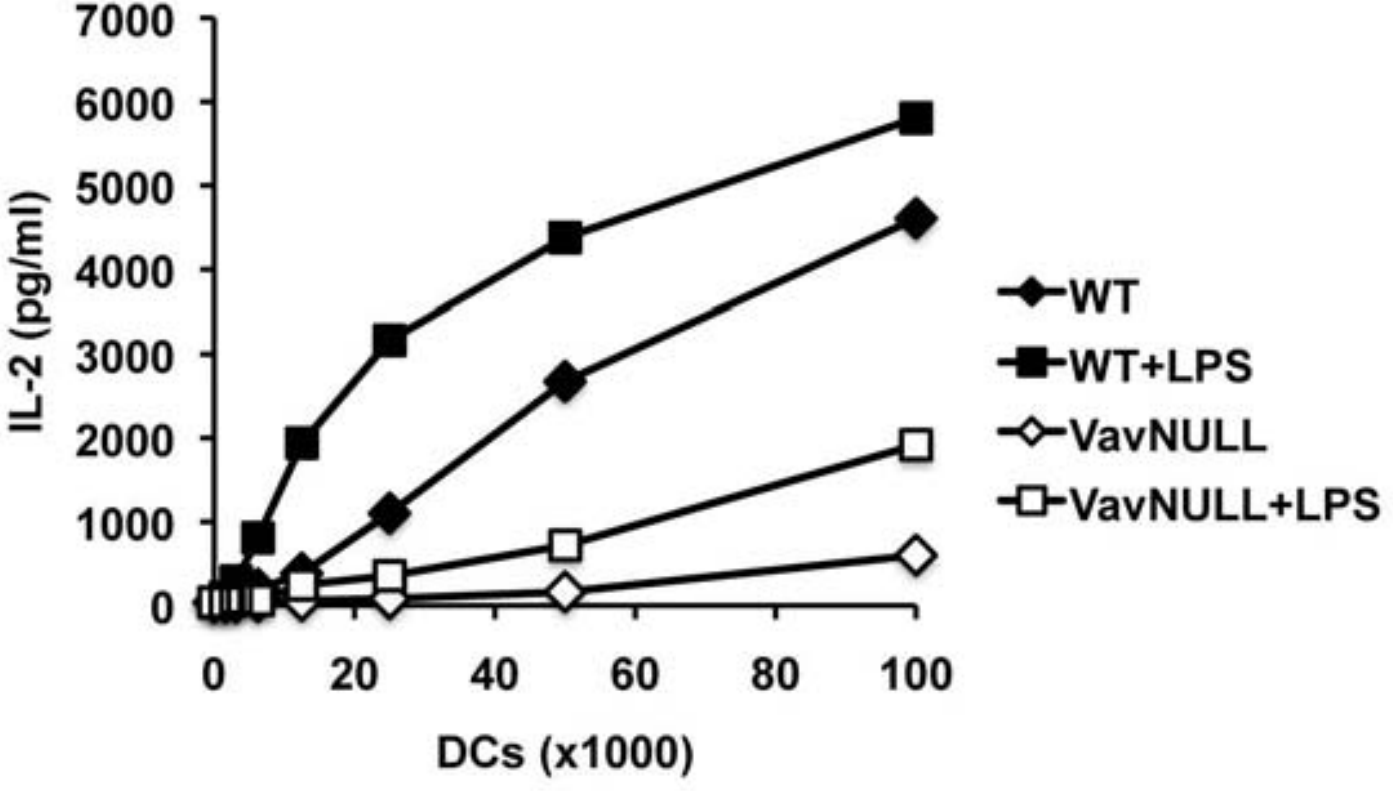

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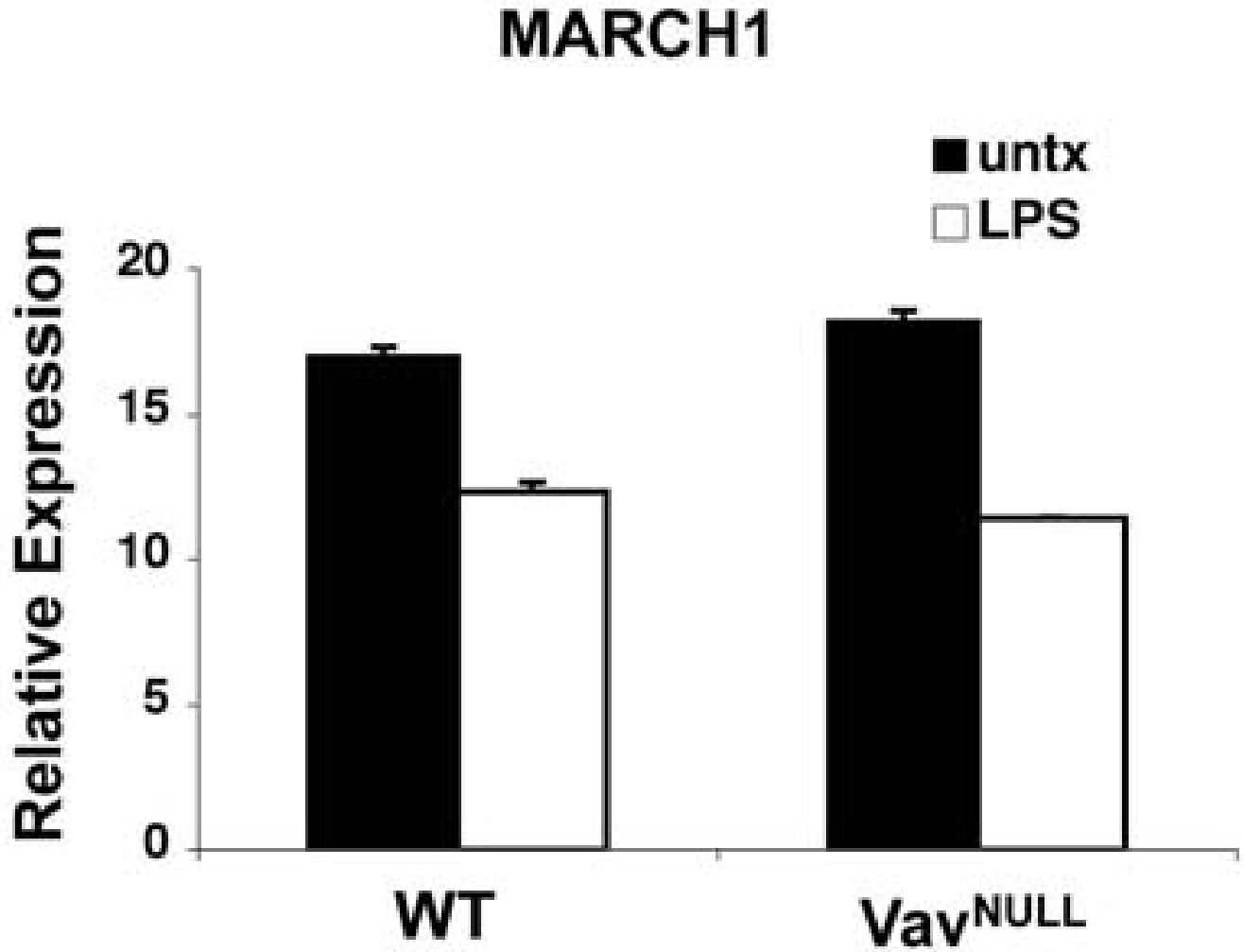

Figure S1. ITAM Signaling is Required for Presentation of Particulate Antigen by Dendritic Cells. WT and DF DCs (50,000) were cultured with the indicated doses of ovalbumin-coated latex beads and purified OT2 T cells. T cells were analyzed for proliferation by CFSE dye dilution after 72 hours of stimulation.

Figure S2. MHCII and Costimulatory Molecule Expression in WT and ITAM Signaling deficient Dendritic Cells. WT, Vav^{NULL}, and DF DCs were cultured for 5-7 days and analyzed for $I\text{-}A^b$ (Clone AF6-120.1), B7.2 (Clone GL1), B7.1 (Clone 16-10A1), and CD40 (Clone 3/23) expression by flow cytometry. Knock-out expression profiles (black) are overlayed with wildtype DC expression profiles (gray).

Figure S3. ITAM Signaling is Intrinsically Required for Dendritic Cell Antigen Presentation. WT and Vav^{NULL} DCs were pulsed with ovalbumin peptide or in plain media prior to coculture with CFSE-labeled OT2 T cells for determination of T cell proliferation after three days. Left panel: $25x10^3$ peptide-pulsed WT DCs and $25x10^3$ unpulsed WT DCs. Middle panel: $25x10^3$ peptide-pulsed WT DCs and $25x10^3$ peptide-pulsed Vav^{NULL} DCs. Right panel: $25x10^3$ peptide-pulsed Vav^{NULL} DCs and $25x10^3$ unpulsed Vav^{NULL} DCs.

Figure S4. IL-10 Production is Similar Between WT and Vav^{NULL} Dendritic cells. WT and Vav^{NULL} DCs were cultured with ovalbumin antigen in the presence of OT-II T cells for 24 hours. Supernatants were collected and assayed for production of IL-10 by Cytometric Bead Array analysis (BD Biosciences). Data from 9 different samples were analyzed and are shown as mean ± s.d. (p value= 0.211).

Figure S5. IL-12 Production is Similar Between WT and Vav^{NULL} Dendritic Cells. WT and Vav^{NULL} DCs were cultured with ovalbumin antigen in the presence of OT-II T cells for 24 hours. Supernatants were collected and assayed for production of IL-12p70 by Cytometric Bead Array analysis (BD Biosciences). Data from 6 different samples were pooled and are shown as mean ± s.d. (p value= 0.115).

Figure S6. Cell Viability of WT and ITAM Signaling Deficient Dendritic Cells After Peptide Pulse, Wash, and Overnight Culture. WT, Vav^{NULL}, and DF DCs were pulsed with a saturating dose of OT2 peptide for 2 hours at 37°C, washed in media, and cultured overnight in the absence of peptide. DCs were then analyzed for cell viability by light scatter (FSC vs SSC). Data are shown from at least 3 independent experiments and expressed as mean ± s.d.

Figure S7. MHCII and B7.2 Molecule Expression on Dendritic Cells After CFA Immmunization of WT and DF Mice. Draining lymph nodes of WT and DF mice were analyzed by flow cytometry on day 5 post footpad immunization with CFA. MHCII and B7.2 expression was evaluated specifically on CD11c+ cells. I-A^b (Clone AF6-120.1), I-A/I-E (Clone M5/114.15.2), and B7.2 (Clone GL1) expression levels are shown in black, and isotype controls are shown in gray.

Figure S8. Deletion of Both DAP12 and FcRγ Results in CD4 T Cell Priming Defects That are More Severe Than Single Deletion of DAP12 or FcRγ. Mice were immunized s.c. with MOG_{35-55} peptide in adjuvant. After 3 weeks, spleens were recovered and restimulated *in vitro* with MOG peptide to determine antigen-specific T cell frequencies by ELISPOT. Data represent the mean$\pm$s.e.m. for n$\geq$4 mice per group.

Figure S9. Deletion of Both DAP12 and FcRγ Results in More Severe EAE Than Single Deletion of DAP12 or FcRγ. Mice were immunized with MOG_{35-55} peptide and pertussis toxin to induce disease. During the course of disease, mice were assigned a clinical score with grade 1 = tail weakness, grade 2 = hind limb weakness sufficient to impair righting, grade 3 = one limb plegic, grade 4 = hind limb paralysis, grade 5 = moribund. Data are expressed as mean clinical score for $n \geq 4$ mice per group.

Figure S10. ITAM Signaling Does Not Regulate the Half-Life of MHCI. WT and Vav^{NULL} DCs were pulse-labeled with [^{35}S] for 30 minutes and chased for the indicated time points. Subsequently, cells were lysed and MHCI was immunoprecipitated (clone 28-8-6). Samples were then resolved by PAGE and analyzed by autoradiography.

Figure S11. NOX2 Does Not Regulate the Half-Life of MHCII. WT and $NOX2^{-/-}$ DCs were pulse-labeled with [^{35}S] for 30 minutes and chased for the indicated time points. Subsequently, cells were lysed and MHCII was immunoprecipitated (clone Y3P). Samples were then resolved by PAGE and analyzed by autoradiography.

Figure S12. LPS Treatment Enhances Antigen Presentation in the Absence of ITAM Signaling But Does Not Restore Responses to WT Levels. WT and Vav^{NULL} DCs were pulsed with beta-gal peptide (10 uM), washed, and chased for 24 hours in plain media with or without LPS (100 nM). After chase, DCs were fixed and cultured with the B11 T cell hybridoma overnight. Culture supernatants were analyzed for the presence of IL-2 by ELISA.

Figure S13. Expression of March1 is Similar Between WT and Vav^{NULL} Dendritic Cells. WT and Vav^{NULL} DCs were cultured for 7 days and analyzed for March1 expression by quantitative PCR. Where indicated, DCs were treated for 20 hours with LPS (100ng/ml). Data from triplicate samples were normalized to GAPDH expression.