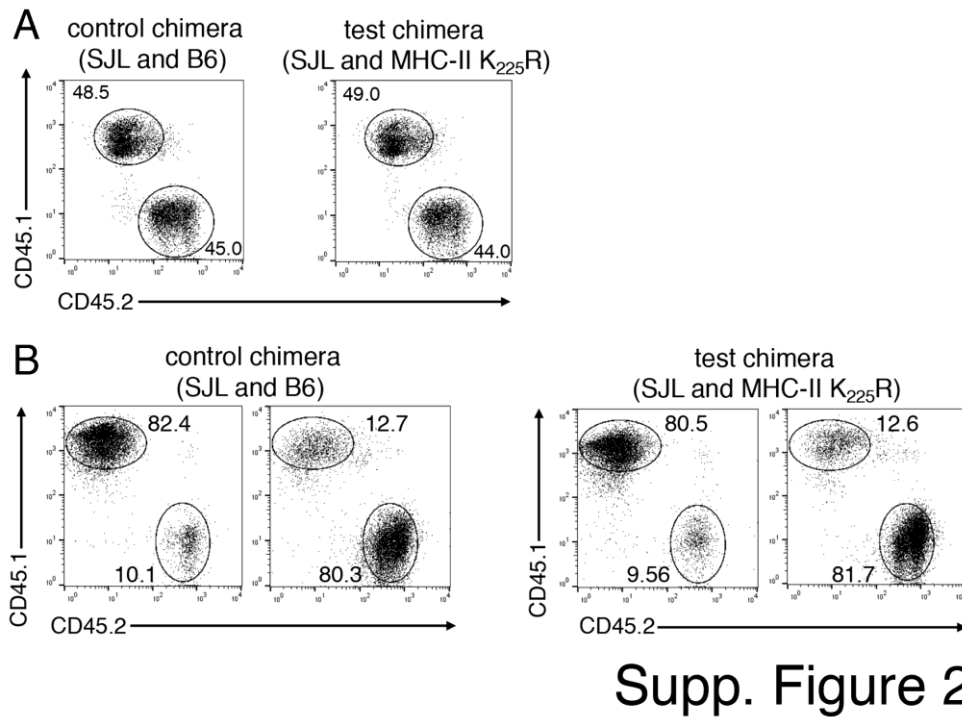


Supp. Figure 1

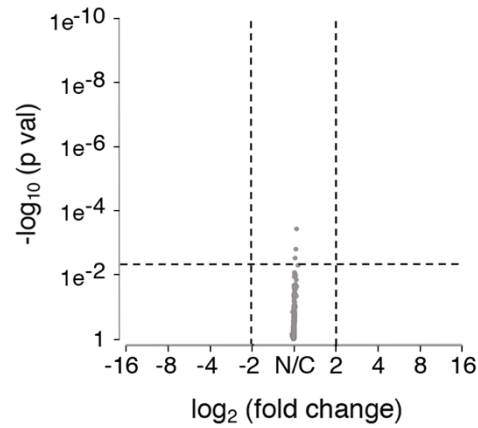
Supplemental Fig. 1. MHC-II ubiquitination mutant DCs make inefficient conjugates with CD4 T cells.

Purified spleen DCs isolated from WT and MHC-II K₂₂₅R mutant mice DCs were pulsed with 20 µg/ml OVA protein for 3 hr, washed, and cultured with OT-II CD4⁺ T cells for 30 min. Conjugates were fixed and stained with APC-CD3 mAb and e450-MHC-II mAb for analysis by confocal immunofluorescence microscopy. Conjugates was scored by a blinded observer, and the percentage of cells with significant amounts of conjugates was calculated.



Supplemental Fig. 2. Analysis of mixed bone marrow chimeric mice.

(A) Mixed BM chimeric mice were generated by reconstitution of irradiated CD45.1⁺ mice with a 1:1 mixture of BM cells from CD45.1⁺ B6.SJL mice and BM cells from either CD45.2⁺ WT or MHC-II K₂₂₅R ubiquitination mutant donors. The chimerism from chimera 1 (SJL:B6) or chimera 2 (SJL:MHC-II K₂₂₅R) was determined 8-12 weeks post reconstitution by FACS analysis. (B) Purified spleen DCs from chimeric mice were subjected to additional separation into CD45.2⁺ DCs (using anti-CD45.2 microbeads; Miltenyi Biotech) and CD45.1⁺ DCs (the flow-through from the anti-CD45.2 microbead column). Representative FACS experiment of total spleen cells, gated on CD11c^{Hi} cells, examining DC chimerism and purity (based on CD45.1 and CD45.2 expression).



Supp. Figure 3

Supplemental Fig. 3. Maturation reverses the gene expression differences between MHC-II K₂₂₅R and wild-type DCs.

Volcano plot showing the differentially expressed genes from activated cDCs (both CD8⁻ and CD8⁺ cDCs) isolated from spleens of WT and MHC-II K₂₂₅R mutant mice. The DCs were activated by culture with LPS for 16 hr prior to scRNA-Seq analysis. Gray dots indicate non-significant genes. X and Y axes show log₂ fold change and $-\log_{10}$ p-value, respectively. Thresholds are shown as dashed lines.

Supplemental Table 1. PANTHER analysis of gene expression differences between MHC-II K₂₂₅R and wild-type DCs

Analysis Type:	PANTHER Overrepresentation Test (Released 20200728)						
Annotation Version and Release Date:	GO Ontology database DOI: 10.5281/zenodo.4033054 Released 2020-09-10						
Analyzed List:	Client Text Box Input (Mus musculus)						
Reference List:	Mus musculus (all genes in database)						
Test Type:	FISHER						
Correction:	FDR						
GO biological process complete	Mus musculus - REFLIST (22265)	Input (159)	(expected)	(over/under)	(fold Enrichment)	(raw P-value)	(FDR)
immune system process (GO:0002376)	2430	43	17.35	+	2.48	2.40E-08	3.79E-04
immune response (GO:0006955)	1533	30	10.95	+	2.74	5.07E-07	4.01E-03
regulation of immune system process (GO:0002682)	1515	29	10.82	+	2.68	1.26E-06	4.98E-03
leukocyte differentiation (GO:0002521)	384	14	2.74	+	5.11	1.00E-06	5.28E-03
cell activation (GO:0001775)	624	17	4.46	+	3.81	3.27E-06	7.39E-03
leukocyte activation (GO:0045321)	541	16	3.86	+	4.14	2.35E-06	7.43E-03
regulation of response to external stimulus (GO:0032101)	912	21	6.51	+	3.22	2.91E-06	7.66E-03
lymphocyte activation (GO:0046649)	436	14	3.11	+	4.5	4.18E-06	8.27E-03
hemopoiesis (GO:0030097)	674	17	4.81	+	3.53	8.79E-06	1.39E-02
lymphocyte differentiation (GO:0030098)	284	11	2.03	+	5.42	8.74E-06	1.54E-02
antigen receptor-mediated signaling pathway (GO:0050851)	307	11	2.19	+	5.02	1.76E-05	2.53E-02
hematopoietic or lymphoid organ development (GO:0048534)	732	17	5.23	+	3.25	2.47E-05	3.25E-02
response to external stimulus (GO:0009605)	2360	35	16.85	+	2.08	2.74E-05	3.33E-02
immune response-activating signal transduction (GO:0002757)	332	11	2.37	+	4.64	3.53E-05	3.72E-02
immune response-activating cell surface receptor signaling pathway (GO:0002429)	331	11	2.36	+	4.65	3.43E-05	3.88E-02
immune response-regulating cell surface receptor signaling pathway (GO:0002768)	343	11	2.45	+	4.49	4.69E-05	4.12E-02
immune response-regulating signaling pathway (GO:0002764)	346	11	2.47	+	4.45	5.06E-05	4.21E-02
negative regulation of cell population proliferation (GO:0008285)	692	16	4.94	+	3.24	4.56E-05	4.24E-02
immune system development (GO:0002520)	768	17	5.48	+	3.1	4.44E-05	4.39E-02

Supplemental Table 1. PANTHER analysis of gene expression differences between MHC-II K₂₂₅R and wild-type DCs.

Overrepresentation Analysis with PANTHER. The results from PANTHER show the number of pathway related genes in the mouse genome, the number of pathway genes found in the differentially regulated subset, the expected number of pathway genes in the subset, fold change of discovered genes over expected genes, and the raw and adjusted p-values (i.e. FDR).