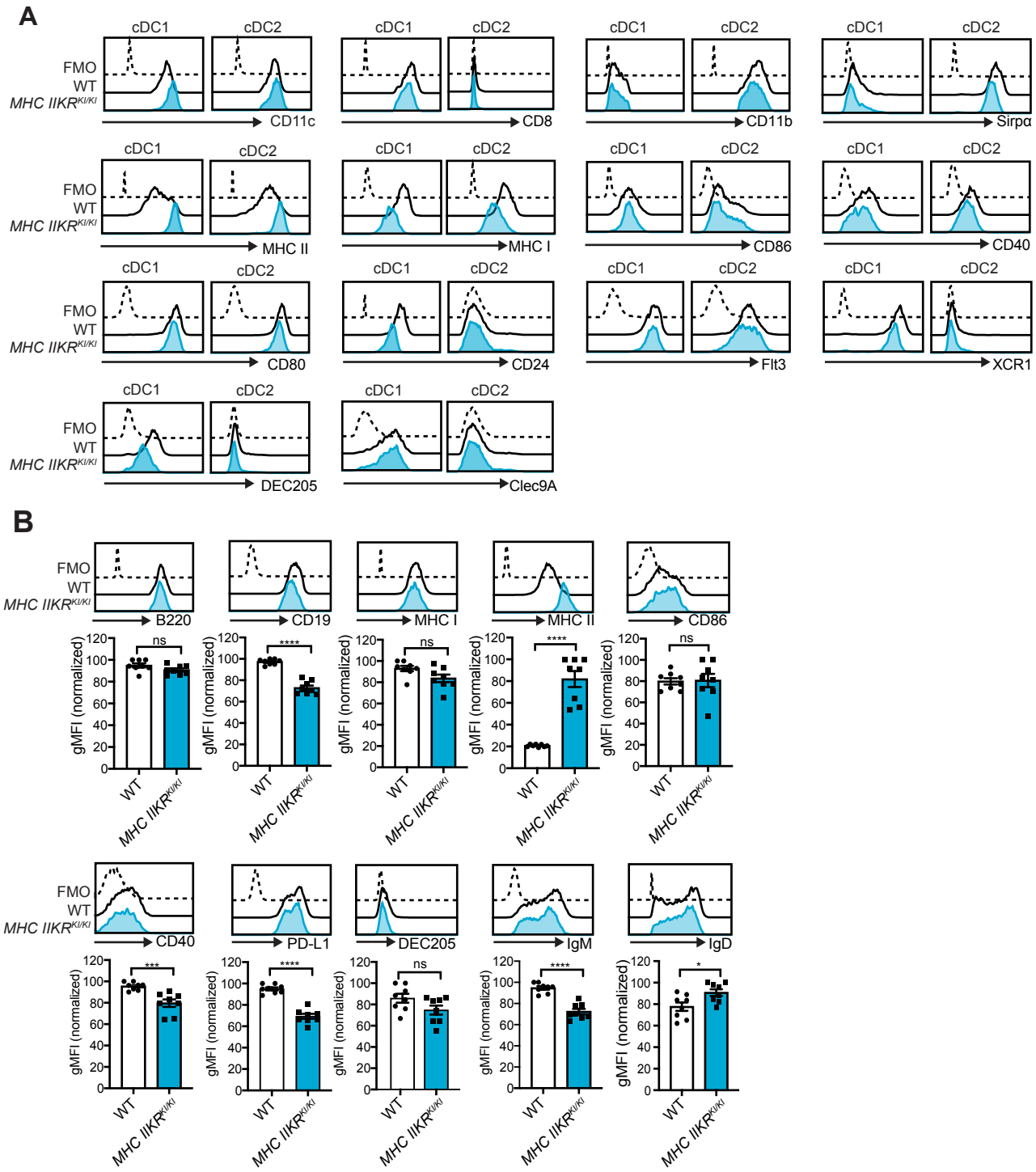
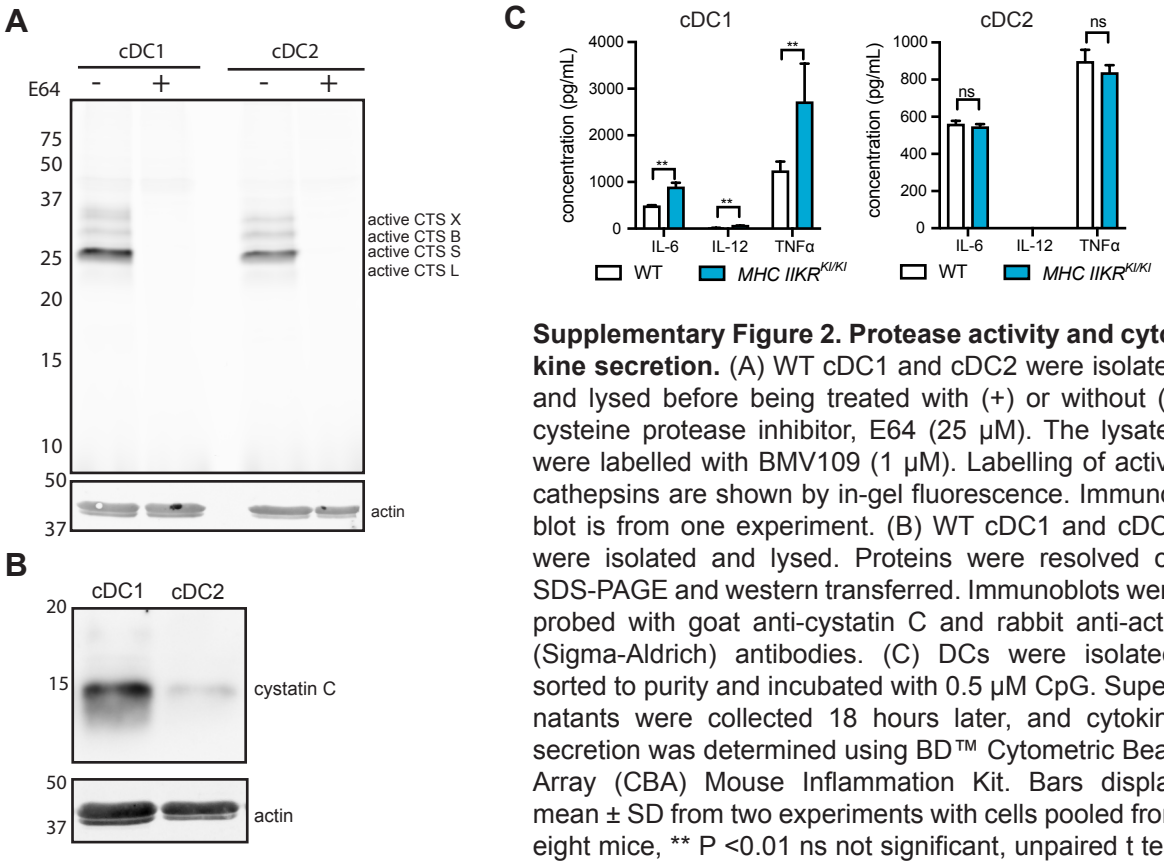
Supplementary Figure 1



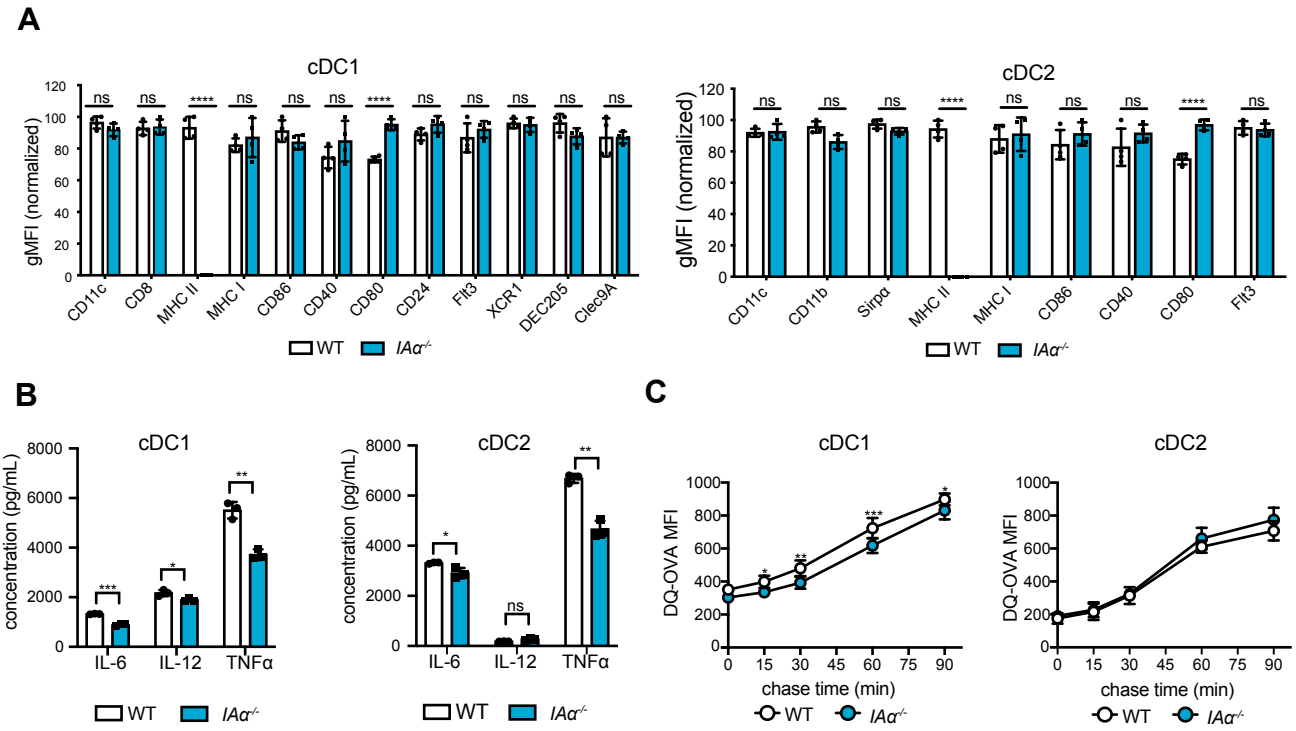
Supplementary Figure 1. Lack of MHC II ubiquitination alters splenic DC and B cell cell-surface phenotype. WT and *MHC IIKR*$^{KI/KI}$ splenic (A) DC or (B) B cell surface marker expression determined by flow cytometry. Representative histograms are shown for fluorescence minus one (FMO, dashed line), WT (black line) and *MHC IIKR*$^{KI/KI}$ (blue filled). gMFI for B cell markers has been quantified by normalizing the gMFI signal to the maximum gMFI signal. Symbols represent individual mice, with data pooled from a minimum of 2 independent experiments. **** P <0.0001*** P <0.001* P <0.05, ns not significant, unpaired t test.

Supplementary Figure 2



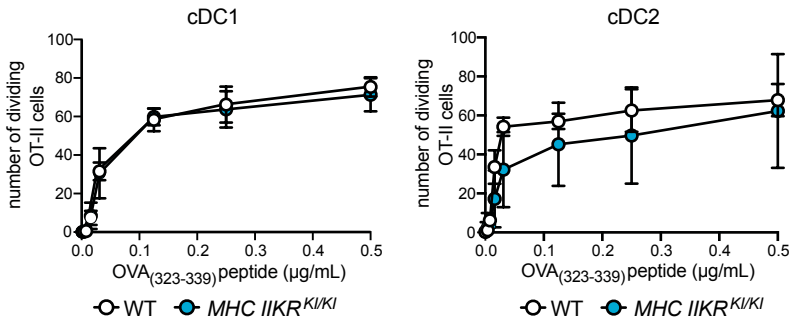
Supplementary Figure 2. Protease activity and cytokine secretion. (A) WT cDC1 and cDC2 were isolated and lysed before being treated with (+) or without (-) cysteine protease inhibitor, E64 (25 μM). The lysates were labelled with BMV109 (1 μM). Labelling of active cathepsins are shown by in-gel fluorescence. Immunoblot is from one experiment. (B) WT cDC1 and cDC2 were isolated and lysed. Proteins were resolved on SDS-PAGE and western transferred. Immunoblots were probed with goat anti-cystatin C and rabbit anti-actin (Sigma-Aldrich) antibodies. (C) DCs were isolated, sorted to purity and incubated with 0.5 μM CpG. Supernatants were collected 18 hours later, and cytokine secretion was determined using BD™ Cytometric Bead Array (CBA) Mouse Inflammation Kit. Bars display mean ± SD from two experiments with cells pooled from eight mice, ** P <0.01 ns not significant, unpaired t test with Holm-Sidak correction for multiple comparisons.

Supplementary Figure 3



Supplementary Figure 3. DC lacking MHC II have altered function. (A) WT and *IAα*$^{-/-}$ splenic cDC1 and cDC2 cell-surface marker expression determined by flow cytometry. gMFI has been normalized to the maximum gMFI signal. Symbols represent individual mice. Data are from one experiment. **** P <0.0001, ns not significant, unpaired t test. (B) WT and *IAα*$^{-/-}$ splenic cDC1 and cDC2 cytokine secretion. DC were sorted to purity and incubated with 0.5 μM CpG, 50 ng/mL IFNγ and 20 ng/mL GM-CSF. Supernatants were collected 18 hours later, and cytokine secretion was determined using BD™ Cytometric Bead Array (CBA) Mouse Inflammation Kit. Bars display mean ± SD from two experiments with cells pooled from eight mice, *** P <0.001 ** P <0.01 * P <0.05, ns not significant, unpaired t test. (C) *IAα*$^{-/-}$ and WT cDC1and cDC2 were isolated, sorted to purity and pulsed with 20 μg/mL DQ-OVA for 15 min. Cells were washed twice and DQ-OVA signal was measured by flow cytometry at different chase time points. **** P <0.0001*** P <0.001* P <0.05, two-way ANOVA with Bonferroni's test for multiple comparisons.

Supplementary figure 4



Supplementary figure 4. MHC II ubiquitination does not impact in vitro presentation of $OVA_{(323-339)}$ peptide. 2.5×10^4 sorted WT and *MHC IIKR*$^{KI/KI}$ cDC1 and cDC2 were incubated with increasing concentration of $OVA_{(323-339)}$ peptide and 2.5×10^4 OT-II cells. 68 hours later, the number of divided OT cells was determined as described in Materials and Methods. Data is pooled from two independent experiments.