Supplemental information

Supplemental figure legend

Figure S1. Related to Figure 1

(a) The efficiency of p38a deletion in splenic DC subsets. Splenic DC subsets were sorted from 5 pairs of mice. (b) The Ag cross-presentation ability of $CD8^+$ cDCs from $p38\alpha^{\Delta HPC}$ mice was reduced. (c) The cross-presentation of OVA protein (100 ug/ml) by BM-derived $CD24^+$ cDCs of $p38\alpha^{\Delta HPC}$ mice was reduced. (d-f) Splenic $CD8^+$ cDCs from WT or $p38\alpha^{\Delta DC}$ mice were coated with $OVA_{323-339}$ (1 ug/ml) or OVA protein (100 ug/ml) for 2 hrs at 37 ℃, then $CD8^+$ cDCs ($0.25\text{-}1\times10^4$/well) were incubated with CFSE-labeled OT-II $CD4^+$ T cells (1×10^5/well) in a 96-well plate for 60-80 h. T cell proliferation was measured by flow cytometry analysis. The medium used was RPMI1640-10%FBS-1% P/S (penicillin and streotomycin) with GM-CSF (20 ng/ml). (b-f) The results were representative of two independent experiments, each included duplicated samples. The results were presented as mean +/- SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure S2. Related to Figure 2

(a)The Ag direct-presentation ability of $CD8^-$ cDCs from $p38\alpha^{\Delta HPC}$ mice was reduced. (b)The direct-presentation of OVA protein by BM-derived $CD24^-$ cDCs of $p38\alpha^{\Delta HPC}$ mice was reduced. All of the results were representative of two independent experiments, each included duplicated samples. The results were presented as mean +/- SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure S3. Related to Figure 3

(a) The expressions of co-stimulatory molecules on splenic $CD8^+$ cDCs and $CD8^-$ cDCs from WT or $p38\alpha^{\Delta DC}$ mice after stimulation by Pam2 (50 nM) and CpG2216 (100 nM) for 16 h. (b and c) p38α deletion did not influence the apoptosis in DCs. $CD8^+$ cDCs (b) and $CD8^-$ cDCs (c), were incubated with OVA protein (100 ug/mL) or Pam2 (50nM) for various times. The cell death was detected by 7-Amino-actinomycin

D (7-AAD) staining. (a-c) The DCs were sorted from more than 5 pairs of mice. All of the results were representative of two independent experiments, each included duplicated samples.

Figure S4. Related to Figure 4

(a) The mRNA levels of Nox2 and TAP1 were reduced in BM-derived $CD24^+$ cDCs of $p38\alpha^{\Delta HPC}$ mice after incubation with OVA protein. The results were representative of two independent experiments, each included duplicated samples. (b) Negative control of Ag degradation. BM-derived $CD24^+$ cDCs were incubated with protease inhibitors at 37 ℃ for 10 min, and then pulsed with OVA-coated beads at 37 ℃ for 30 min. After that, cells were removed off free beads and lysed. The recovered beads were stained with FITC conjugated anti-OVA antibody. The results were presented as mean +/- SEM. *, $p < 0.05$; ***, $p < 0.001$.