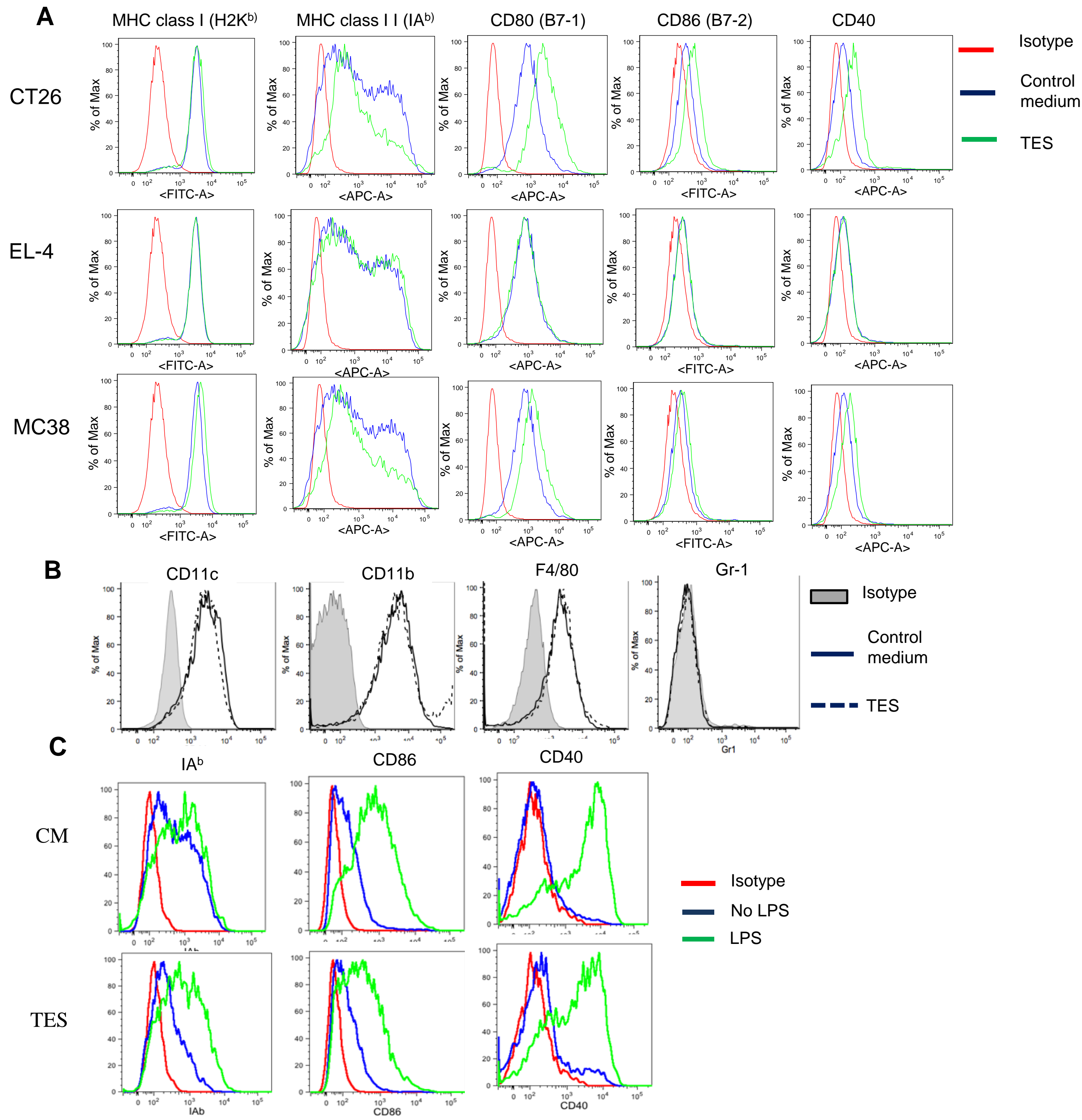
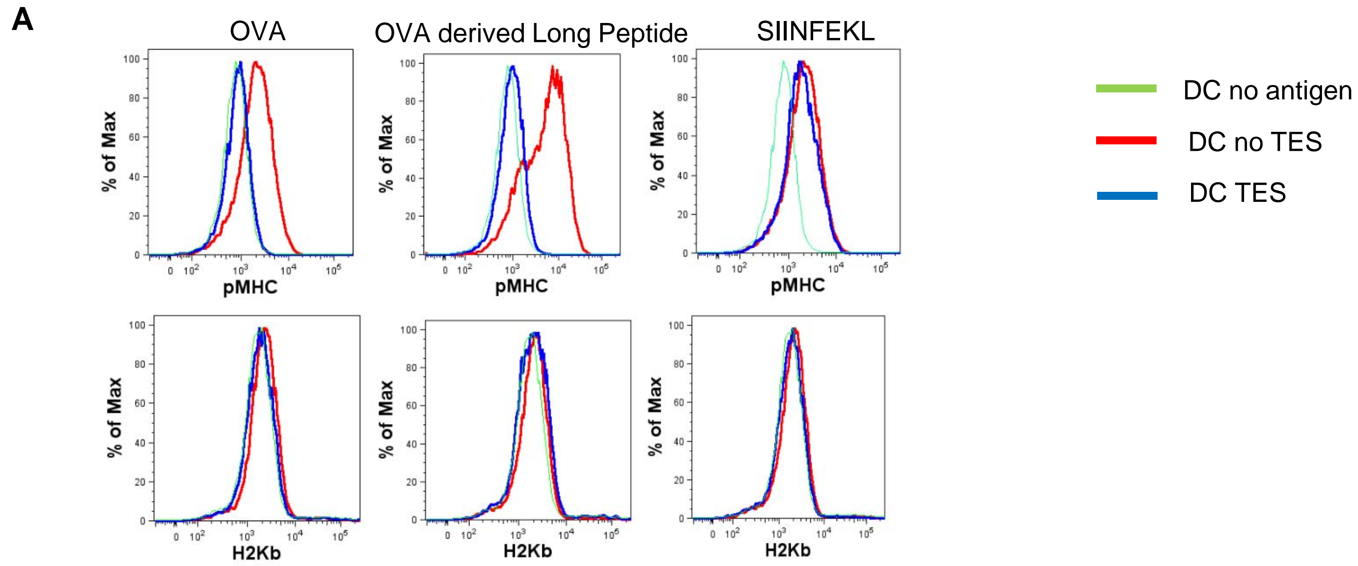
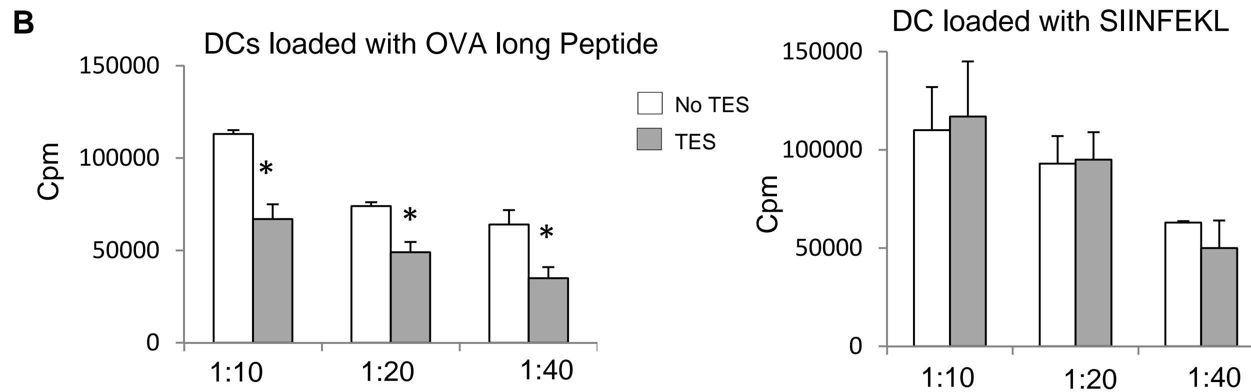
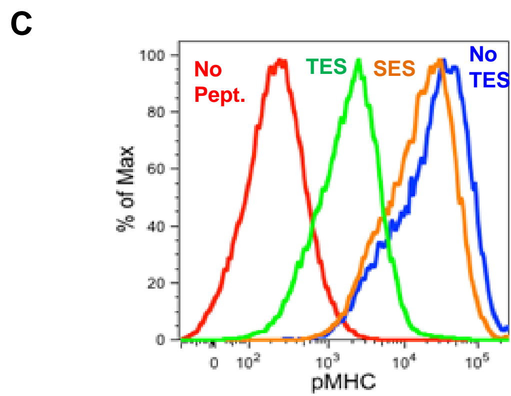


Figure S1

Figure S1. Effect of tumor explant supernatants on the expression of MHC class I, II and co-stimulatory molecules on DCs and cross-presentation of OVA. A. DCs were generated from bone marrow progenitor with GM-CSF. On day 3 medium was replaced with one containing 20% v/v tumor explant supernatants (TES) from three different tumors: CT 26, EL4, or MC38. Two days later, cells were collected and analyzed. Expression of MHC class I, II, and co-stimulatory molecules was analyzed in gated $CD11c^+$ cells. Typical example of 4 experiments is shown. **B.** The phenotype of cells cultured in the presence of TES. DCs were generated as described above in complete medium during 3–day culture with GM-CSF. On day 3 $CD11c^+$ cells were isolated using magnetic beads and cultured for additional 48 hr in the prescence of TES from EL-4 tumors. The phenotype of the cells was evaluated by flow cytometry. **C.** DCs generated as described above were cultured with 500 ng/ml LPS overnight. Expression of indicated molecules within gated $CD11c^+$ cells was evaluated.







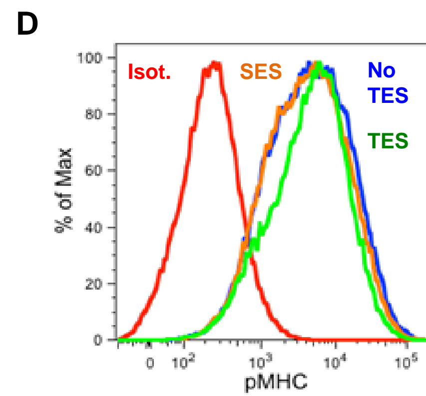


Figure S2

Figure S2. Effect of tumor-derived factors and control spleen explant supernatant (SES) on pMHC and cross-presentation in DCs. A. DCs were differentiated from BM progenitors with GM-CSF and IL-4. At day 5, $CD11c^{+}$ cells were isolated and cultured for 2 days with TES from EL4 tumors. DCs were loaded with OVA or long peptide during the last 24 hours of culutre. **A.** Expression of pMHC and MHC class I (H2Kb) on DCs. **B**. Stimulation of OT-1 $CD8^{+}$ T-cell proliferation. T-cell proliferation was measured in triplicates. * - $p<0.05$. **C.** DCs from WT mice cultured for 48 hr with either TES or explants from control splenocytes (SES). During last 24 hr in culture cells were loaded with long OVA-derived peptide and pMHC complexes were evaluated using 25-D1 antibody. **D.** DCs from OVA-Tg mice. Experiments were performed as above. Three experiments with the same results were performed.

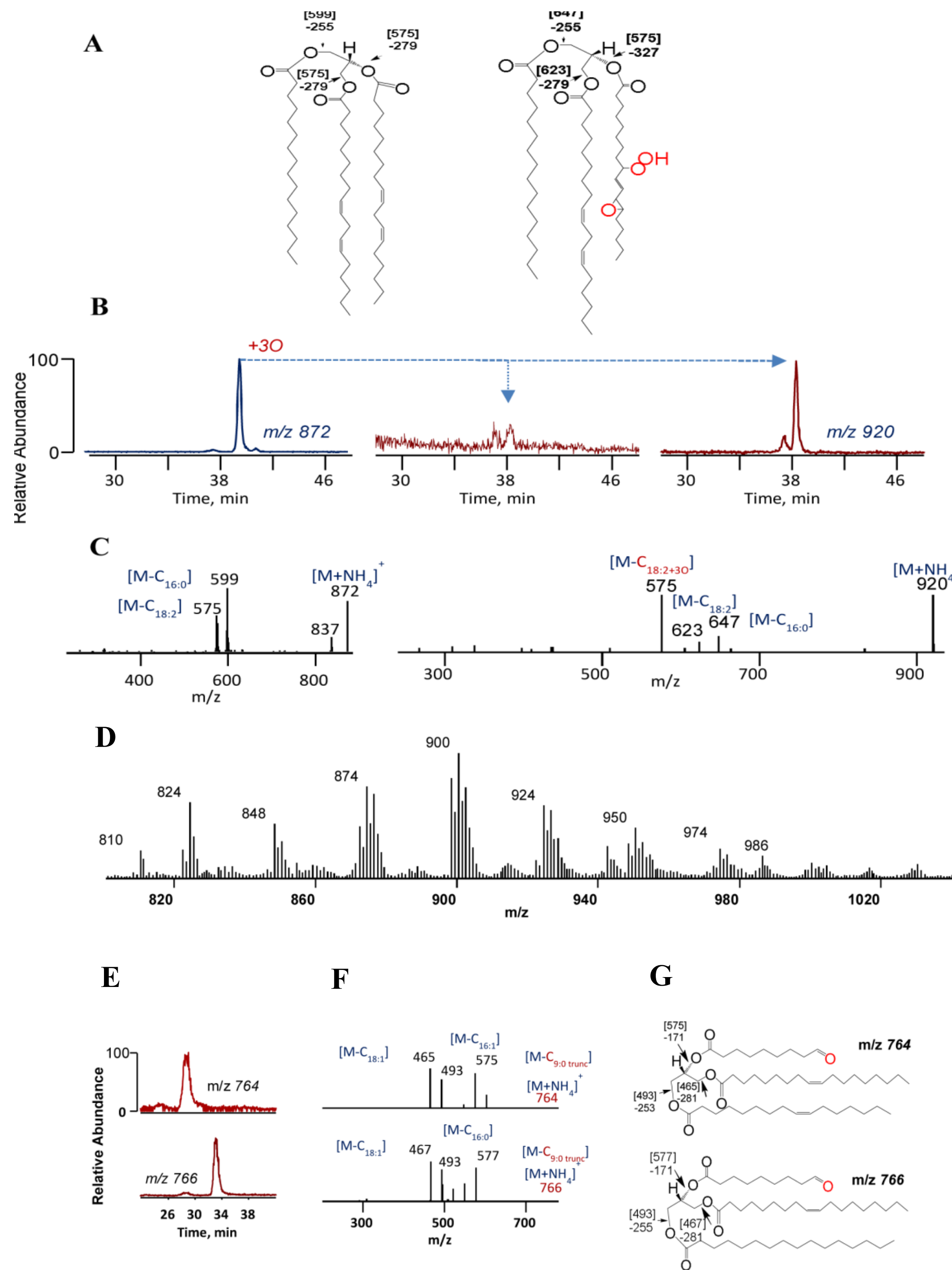


Figure S3

Figure S3. Profile of oxidized lipids in DCs. A-C. DCs isolated from TB mice. **A**. Suggested structure of TAGs at m/z 872 and its oxygenated metabolite at m/z 920. **B.** Typical LC-MS spectra of TAG ($C_{18:2}/C_{18:2}/C_{16:0}$) at m/z 872 (left panel) and its oxygenated species ($C_{18:2}/C_{18:2+3O}/C_{16:0}$) at m/z 920 in control (middle panel) and tumor-derived (left panel) DCs. **C**. MS^2 spectra of TAGs at m/z 872 (left panel) and m/z 920 (right panel). **D-G.** Fragmentation LC-MS analysis of oxidized lipids in human DCs. **D**. Typical MS^1 spectrum of TAGs from human DCs cultured with TCM. **E**. Typical LC-MS profiles of oxidized TAGs at m/z 764 and 766. **F**. MS^2 spectra of oxidized TAGs with m/z 764 and 766, respectively, containing molecular species $C_{16:1}/C_{18:1}/C_{9:0\ +O}$ and $C_{16:0}/C_{18:1}/C_{9:0+O}$ with truncated 9-oxo-nonanoic acid from DCs. **G.** Possible structures of oxTAGs m/z 764 and 766

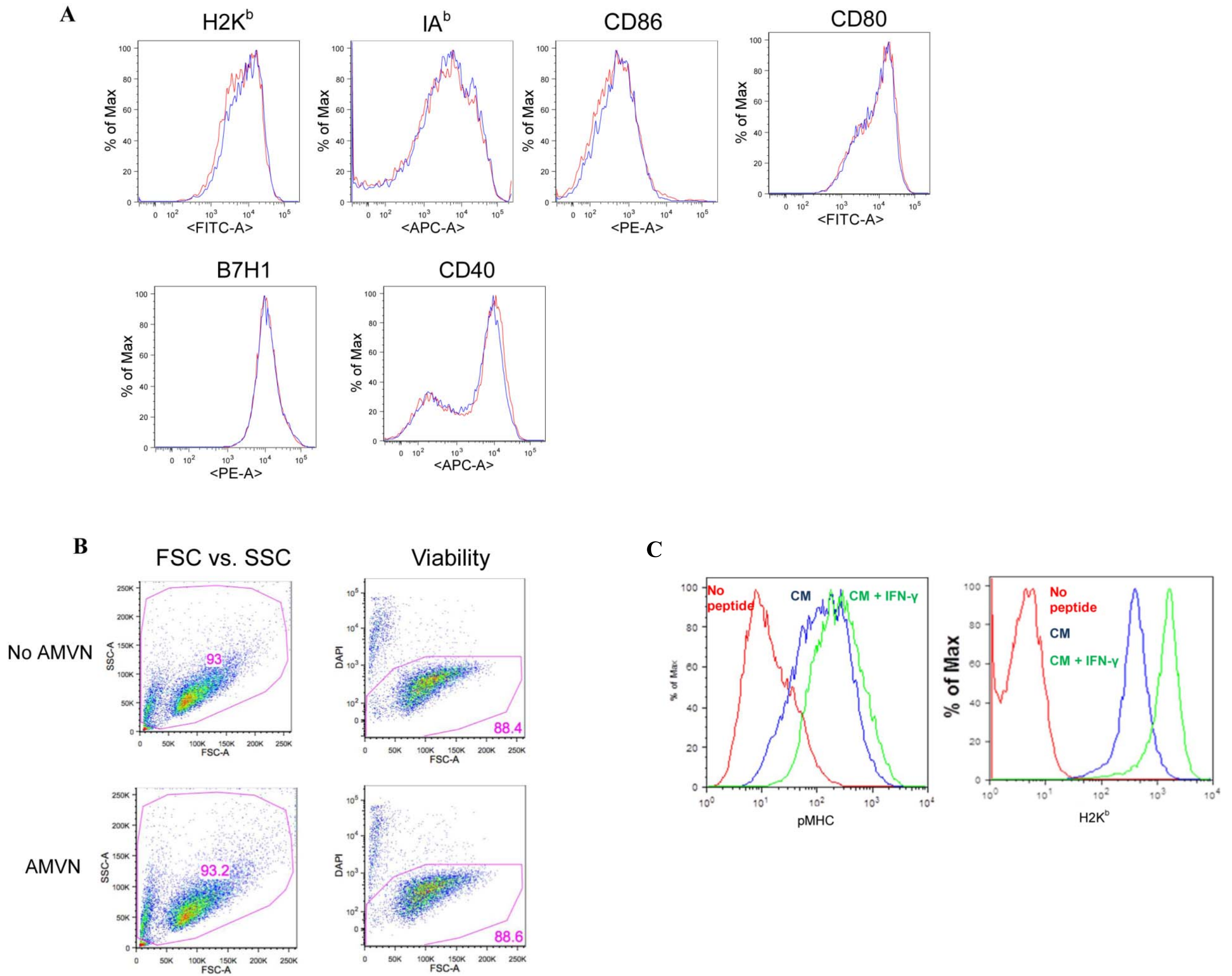


Figure S4

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Figure S4. Effect of AMVN on the expression of surface molecules on DCs and IFN-γ on cross-presentation of long OVA-derived peptide. A. DCs were generated from BM progenitors and treated for 2 hr with 0.5 mM AMVN. Cells were then washed and cultured in complete medium supplemented with GM-CSF for additional 24 hr. Cell phenotype was evaluated within $CD11c^{+}$ cell population. Red line – control, blue line cells treated with AMVN. Three experiments with the same results were performed. Similar results were obtained with AMVN concentration 0.2 mM. **B.** Viability of DCs treated with 0.5 mM AMVN. **C.** DCs were cultured with IFN-γ (250 ng/ml) for 1 day, followed by loading with long OVA-derived peptide. Left panel – pMHC expression; right panel –MHC class I ($H2K^{b}$) expression.