Figure S1

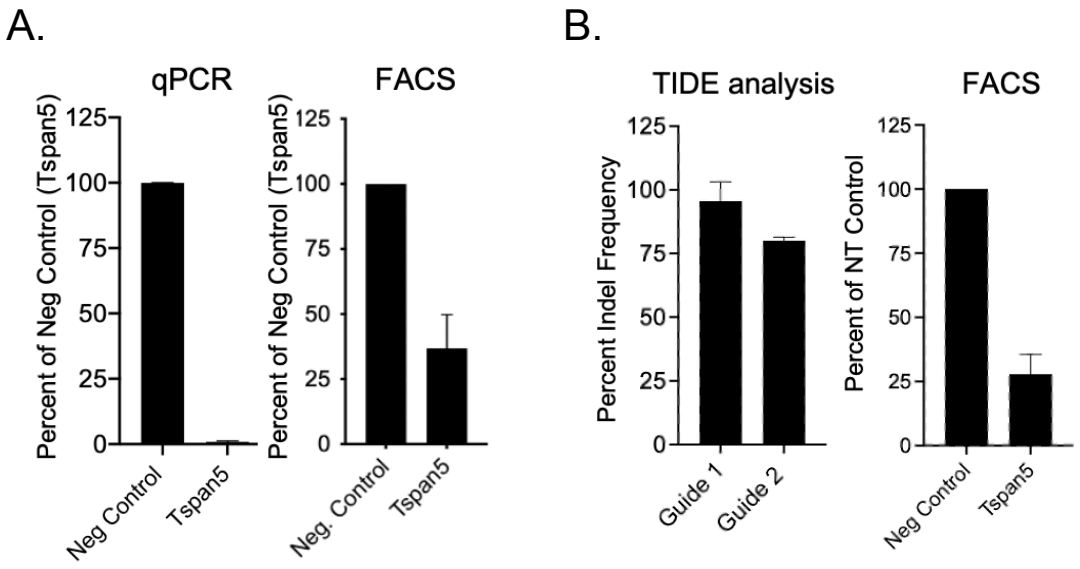

Figure S1
(**A**) Tspan5 knockdown efficiency in DC3.2 cells as determined by qPCR (left) and staining for Tspan5 expression by flow cytometry (right). SiRNA targeting H-2 I-A beta chain (Neg. Control) and Tspan5 are shown. (**B**) Efficiency of CRISPR-mediated KO in DC3.2 cells was determined by TIDE analysis. Bars graph indicates the percent indel frequency present following transduction of a dual CRISPR construct with guides targeting 2 unique sites (left panel) and surface expression of Tspan5 shown on right. Data are representative of ≥3 independent experiments.

Figure S2

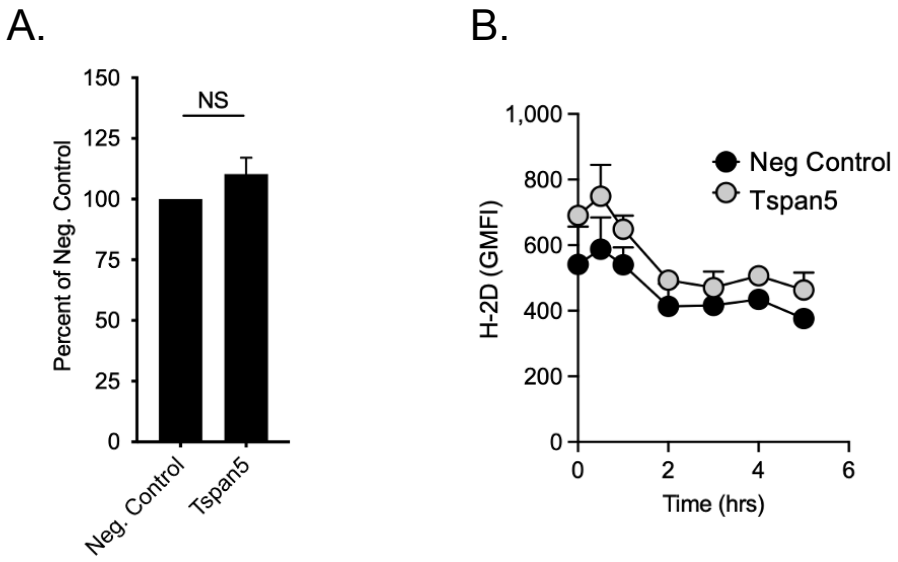

Figure S2
(**A**) DC2.4 cells were treated with SiRNA targeting the MHC class II gene H-2 I-A beta chain (Neg. Control) or Tspan5. H-2D expression at the plasma membrane was assessed by flow cytometry 48 hrs. after silencing. Data are expressed as the percent MHC I expression compared to Neg. Control treated samples. Means + SD of 3 independent experiments are shown. (**B**) DC2.4 cells were treated with SiRNA targeting H-2 I-A beta chain (Neg. Control, black) or Tspan5 (gray). Cells were treated with BFA to block surface expression of new MHC I molecules trafficking from the ER. Remaining surface MHC I molecules were analyzed over time by flow cytometry. Data are shown as means of duplicates and are representative of 3 independent experiments.

Figure S3

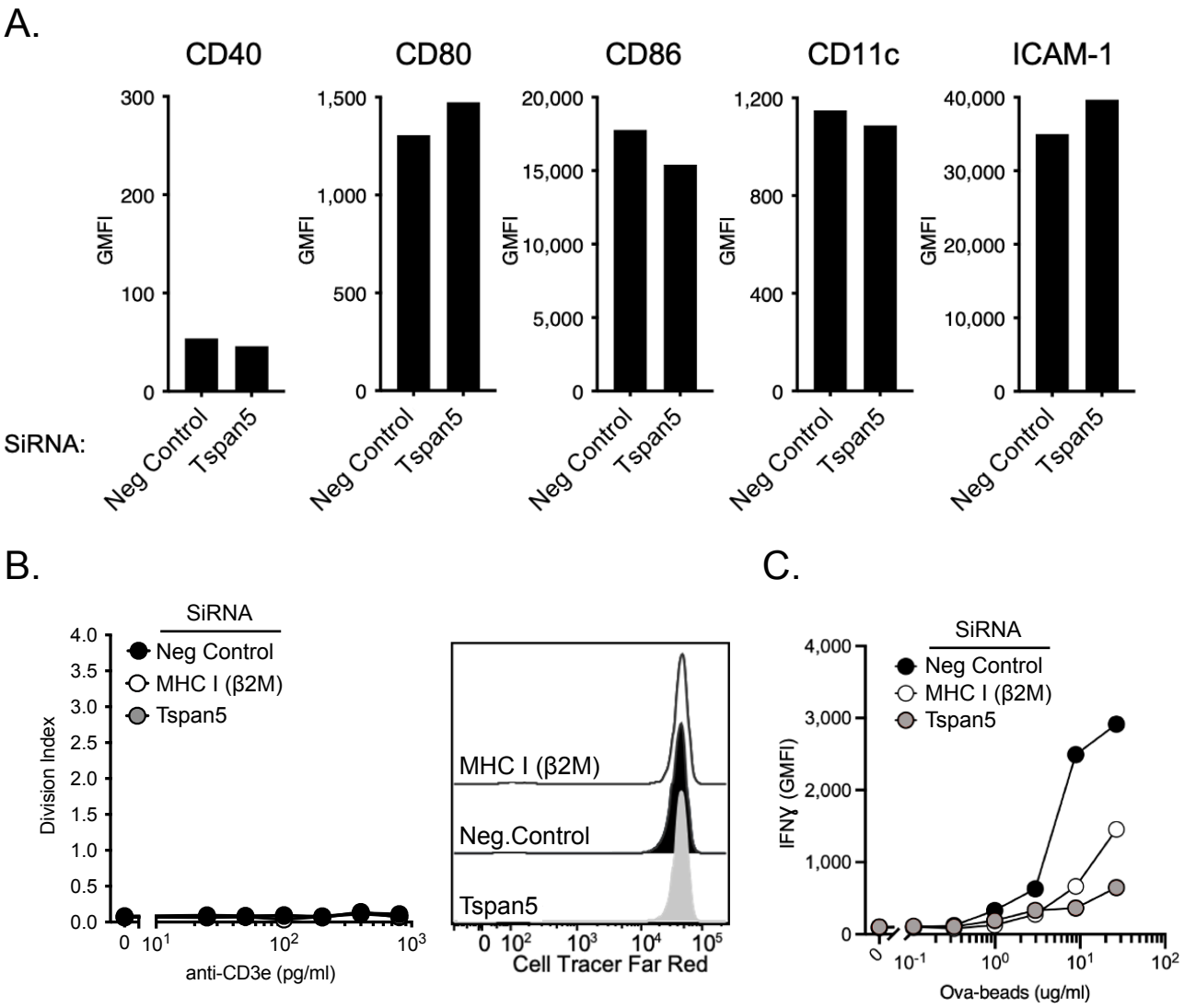

Figure S3

(**A**) DC3.2 cells were silenced for the MHC II I-A beta chain (Neg Control) or Tspan5, as indicated. Flow cytometry was used to determine the influence of SiRNA knockdown on surface expression of co-stimulatory molecules and adhesion molecules. Representative data are shown. (**B**) OT-1 T cell proliferation was assessed in DCs treated for the indicated SiRNAs. Cells were treated with a titration of anti-CD3e in the presence of blocking antibody for LFA-1. Left graph shows data from a representative experiment and the right graph shows a representative flow histogram at one dose of anti-CD3e. Data are from the same experiment as shown in Fig. 3A and B. (**C**) Naïve OT-1 TCR Tg CD8 T cells were expanded in culture following the addition of 10nM S8L peptide, as indicated in the Materials and Methods. DCs were silenced for the indicated targets and cells were incubated with effector OT-1 T cells and a titration of Ova-beads as a source of antigen. CD8 T cell activation was assessed 24hrs later by intracellular staining for IFNɣ production. Data are representative of 3 independent experiments.

Figure S4

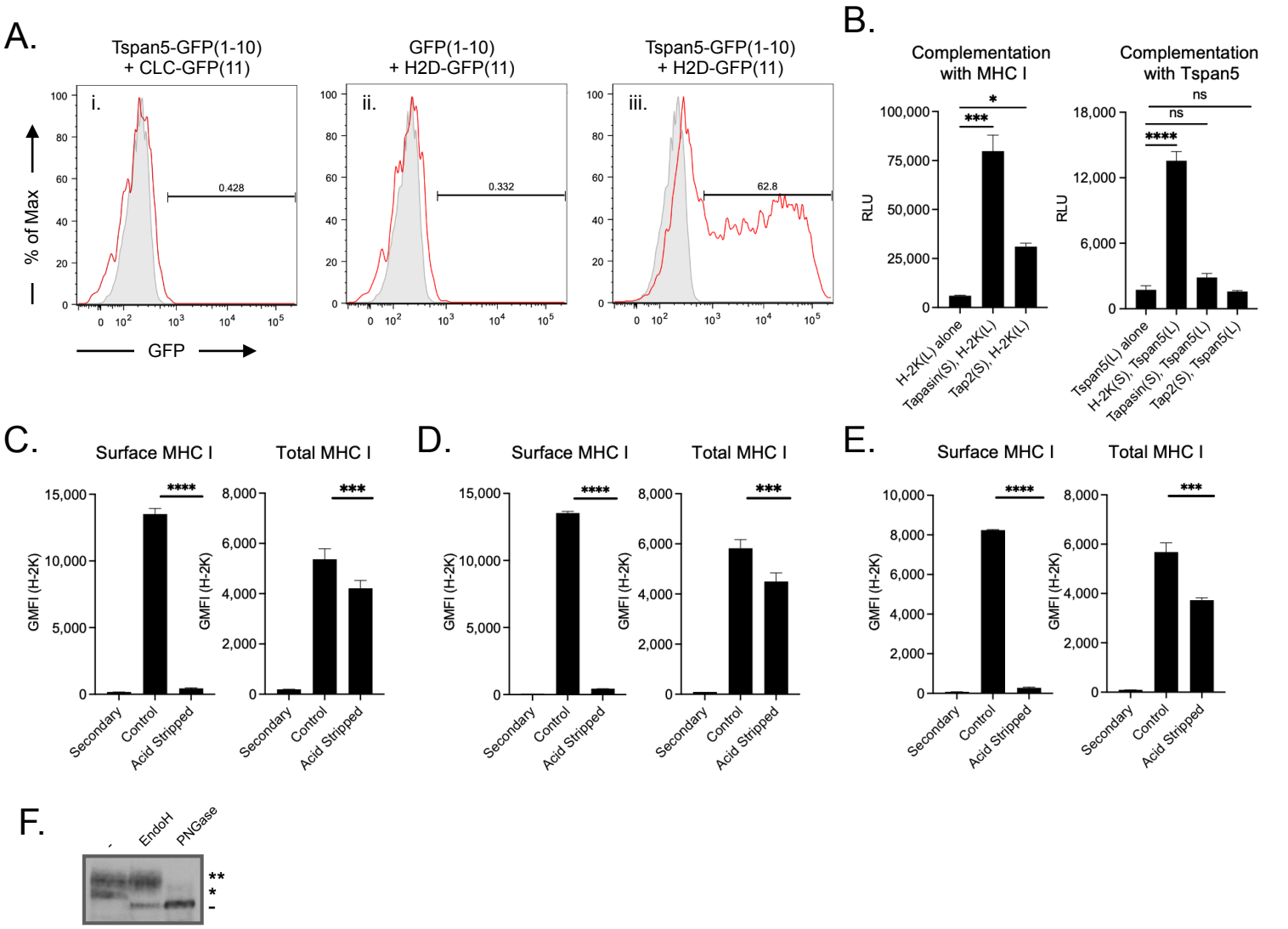

Figure S4

(A) L929 cells were transfected to co-express the indicated fusion proteins with complementing superfold GFP (hereafter, GFP) fragments to determine the subcellular localization of complemented proteins. (i) As a negative control, Tspan5-GFP (exon 1-10) was co-expressed in L929 cells with the clathrin light chain (CLC) fused to exon 11 of GFP (left panel, CLC-GFP (11)); (ii) GFP exon 1-10 was co-expressed with H-2D fused to exon 11 of GFP (center panel, H-2D-GFP (11)); and (iii) Tspan5-GFP was co-expressed with H-2D-GFP (11) (iii). (**B**) Constructs tagged with the short (S) or long (L) NanoBit luciferase fragments were transfected alone or were co-transfected in 3T3 cells, as indicated. Protein-protein interaction was assessed by measuring luciferase activity (RLU). (**C**) Surface and total MHC class I molecules from L929 cells expressing H-2K tagged with GFP (exon 11) and Tspan5 tagged with GFP (exons 1-10) were measured via flow cytometry. Control or acid-stripped cells were fixed in 4% PFA, then stained for surface MHC I (left panel). Alternatively, control or acid-stripped cells were fixed and permeabilized in BD Cytofix/ Cytoperm, and then stained for total MHC I in the presence of 0.25% Saponin (right panel). **(D)** Same as (C), except for expression of HA-tagged Tspan5 and myc-tagged H-2K. (**E**) Same as (D) except expressing endogenous Tspan5 and myc-tagged H-2K. (**F**) Three species of Tspan5 are shown via western blot on a non-reducing SDS-PAGE gel. Lane 1 shows Tspan5 migration from lysates containing 1% NP40 (-). Lysates from lane 2 were treated with EndoH which remove species that have not trafficked beyond the ER/cis-Golgi compartment (EndoH). Lane 3 represents lysates that were treated with PNGase F which removes all glycans except for the terminal GlycNac (PNGase). ** EndoH resistant, * EndoH sensitive, - unglycosylated.

Figure S5

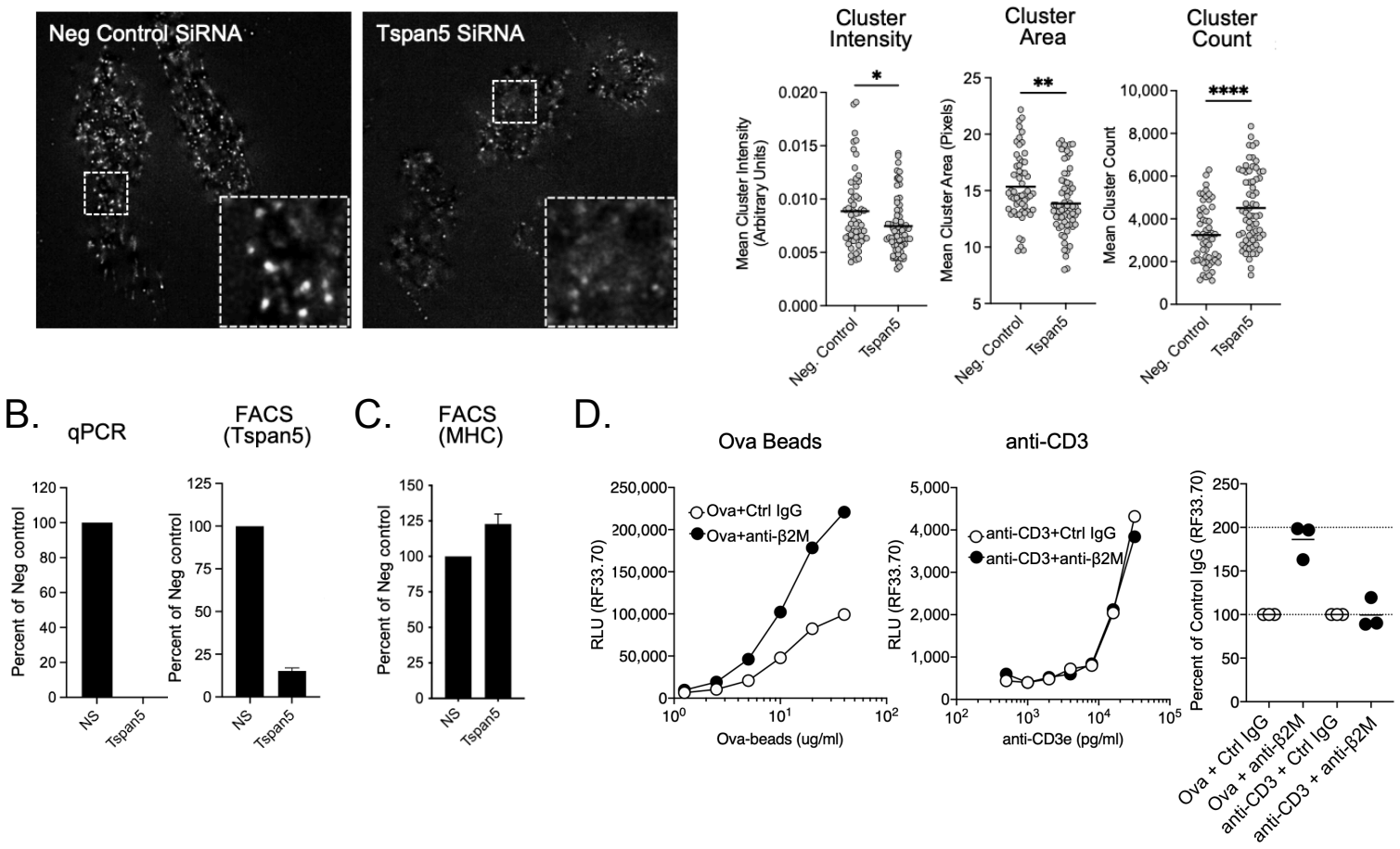

Figure S5

(**A**) DC2.4 cells were transfected with siRNA targeting Tspan5 or I-A beta chain (Neg. control). The distribution of H-2K molecules on the cell surface of live cells was assessed using a fluorophore conjugated Fab-fragment (Y3-Fab), as described. Fluorescent images were acquired from multiple 0.125μm z-sections followed by deconvolution using the DV OMX V4 microscope, as described. Images shown are a single z-section at the plasma membrane and are representative of >50 cells per group. Graphs shown are mean values from > 50 individual cells, expressed as mean intensity of MHC I clusters (arbitrary units, left graph), mean area of MHC I clusters (pixels, middle graph), or cluster count (each point represents the cluster count/cell, (right graph)). p-values determined by two-tailed, unpaired t-test. * < 0.015, ** < 0.005, ****< 0.001. (**B**) Human U2OS cells were transfected with the indicated SiRNAs and Tspan5 expression was evaluated by qPCR (left panel) and flow cytometry for surface expression of Tspan5 (right panel). Data are representative of ≥3 independent experiments. (**C**) HLA expression in U2OS cells following transfection with non-silencing control SiRNA (NS) or SiRNA targeting Tspan5 (Tspan5) was assayed by flow cytometry. Bar graph shows the mean+SD of 3 independent experiments as a percentage of NS control treated cells. (**D**) DCs were treated with control mouse antibodies (Ctrl IgG, white) or mouse anti-β2M antibodies (anti-β2M, black) in the presence of titrated Ova coupled beads (left) or anti-CD3e (center) and the CD8 T cell hybridoma RF33.70. Average T cell activation from 3 independent experiments are shown as a percentage of cells treated with Control IgG (right panel).