Supporting Information

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SI Materials and Methods

Antibodies. Antibodies specific for mouse CD4 (RM4-5), CD11c (HL3), and CD69 (H1.2F3) were purchased from BD Pharmingen. Antibodies recognizing mouse CD8α (53-6.7), DEC205 (205yekta), and CD45.1 (A20) were purchased from eBiosciences. Antibody recognizing IFN-γ (XMG1.2) was purchased from Invitrogen. Horseradish peroxidase (HRP)-conjugated anti-His_6 tag was purchased from Qiagen (cat. 1014992). HRP-conjugated streptavidin was purchased from GE Healthcare (cat. RPN1231V). HRP-conjugated anti-HA tag was purchased from Roche Diagnostics (clone 3F10, cat. 12013819001). Polyclonal anti-PDI serum (rabbit) was generated with bacterially expressed human PDI. Antibody against GAPDH was purchased from Abcam. Anti-cathepsin L serum (rabbit) is a gift from Dr. Sanja Sever, Massachusetts General Hospital, Charlestown, MA.

Mice. C57/BL6, BALB/c, OTII, OTI Rag2$^{-/-}$, and LCMV-specific mice were purchased from Jackson Laboratory. Rop7-I transnuclear mice have been described elsewhere (1). All mice were bred in the animal facility of the Whitehead Institute for Biomedical Research (Cambridge, MA).

Protein Production and Purification. The cDNA encoding the heavy chain of the antibody recognizing the mouse DEC205 (2, 3) was modified by PCR mutagenesis to introduce an LPETG motif and a His_6 tag at the C terminus. Modified cDNA was cloned into the original AbVec-hIgG1 plasmid. αDEC205 light chain and modified heavy chain were expressed in CHO-S cells (cat. R80007; Invitrogen) according to the manufacturer's instructions. The culture media was centrifuged, and the resultant supernatant was filtered through a 0.22-μM membrane filter and applied to a Protein G-Sepharose column (cat. P3296; Sigma), washed with PBS solution, eluted with 0.1 M glycine-HCl, pH 2.8, and immediately neutralized with 0.4 M Tris·Cl, pH 11. The antibody was dialyzed against 50 mM Tris·Cl, 150 mM NaCl, pH 7.0, and concentrated (cat. UFC901024; Millipore). Sortase A from *Staphylococcus aureus* (4) and G_5GFP (5) were expressed and purified as described previously. All peptides were synthesized by the Biopolymers Laboratory at the Koch Institute for Cancer Research at the Massachusetts Institute of Technology.

Purification of αDEC205 Adducts. Following the sortase reaction, αDEC205 was purified by incubation of the reaction mixture with fivefold excess binding capacity of protein G-coupled Sepharose beads for 2 h at 4 °C. Sepharose beads were washed five times with 50 mM Tris·Cl, 150 mM NaCl, pH 7.0. Antibody was eluted with 0.1 M glycine-HCl, pH 2.8, and immediately neutralized with 0.4 M Tris·Cl, pH 11. In some experiments, reactions were desalted on spin columns (cat. 89862; Thermo Scientific) before binding to protein G-coupled Sepharose. Alternatively, αDEC205 was purified by gel filtration chromatography (Superdex 200 10/300GL; cat. 17-5175-01; GE Healthcare) in 50 mM Tris·Cl, 150 mM NaCl, pH 7.0. After purification, the antibody concentration was measured by sandwich ELISA against IgG1: coating antibody goat anti-mouse Ig (heavy and light chain; cat. 1010-01; SouthernBiotech), revealing antibody HRP-conjugated goat anti-mouse IgG1 (cat. 1070-01; SouthernBiotech), standard mouse IgG1 (cat. 0102-01; SouthernBiotech).

Cleavage Prediction Analysis. H-2K^b, H-2K^d, H-2K^k, H-2D^b, H-2D^d, H-2D^k, and H-2L^d-restricted T-cell epitopes from the SYFPEITHI database (www.syfpeithi.de) were inserted at the C-terminus fragment of αDEC205 heavy chain (SDMAKKETVWRLEEFGRF-GGGGSLPET) separated by a GGG or GGGFR linker. Cleavage predictions were made by using an open access algorithm (www.mpiib-berlin.mpg.de/MAPPP/cleavage.html) (6). The following settings were used: minimal fragment size, 3; maximal fragment size, 20; minimum possibility for cleavage after a single residue, 0.5; and minimum possibility for cleavage of a fragment, 0.9. A null score corresponds to the generation of the final epitope. Negative scores correspond to the digestion in the epitope. Positive scores correspond to digestion upstream of the first residue of the epitope.

1. Kirak O, et al. (2010) Transnuclear mice with predefined T cell receptor specificities against Toxoplasma gondii obtained via SCNT. *Science* 328(5975):243–248.
2. Hawiger D, et al. (2001) Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J Exp Med* 194(6):769–779.
3. Kraal G, Breel M, Janse M, Bruin G (1986) Langerhans' cells, veiled cells, and interdigitating cells in the mouse recognized by a monoclonal antibody. *J Exp Med* 163 (4):981–997.
4. Popp MW, Antos JM, Grotenbreg GM, Spooner E, Ploegh HL (2007) Sortagging: A versatile method for protein labeling. *Nat Chem Biol* 3(11):707–708.
5. Antos JM, et al. (2009) A straight path to circular proteins. *J Biol Chem* 284(23): 16028–16036.
6. Holzhütter HG, Kloetzel PM (2000) A kinetic model of vertebrate 20S proteasome accounting for the generation of major proteolytic fragments from oligomeric peptide substrates. *Biophys J* 79(3):1196–1205.

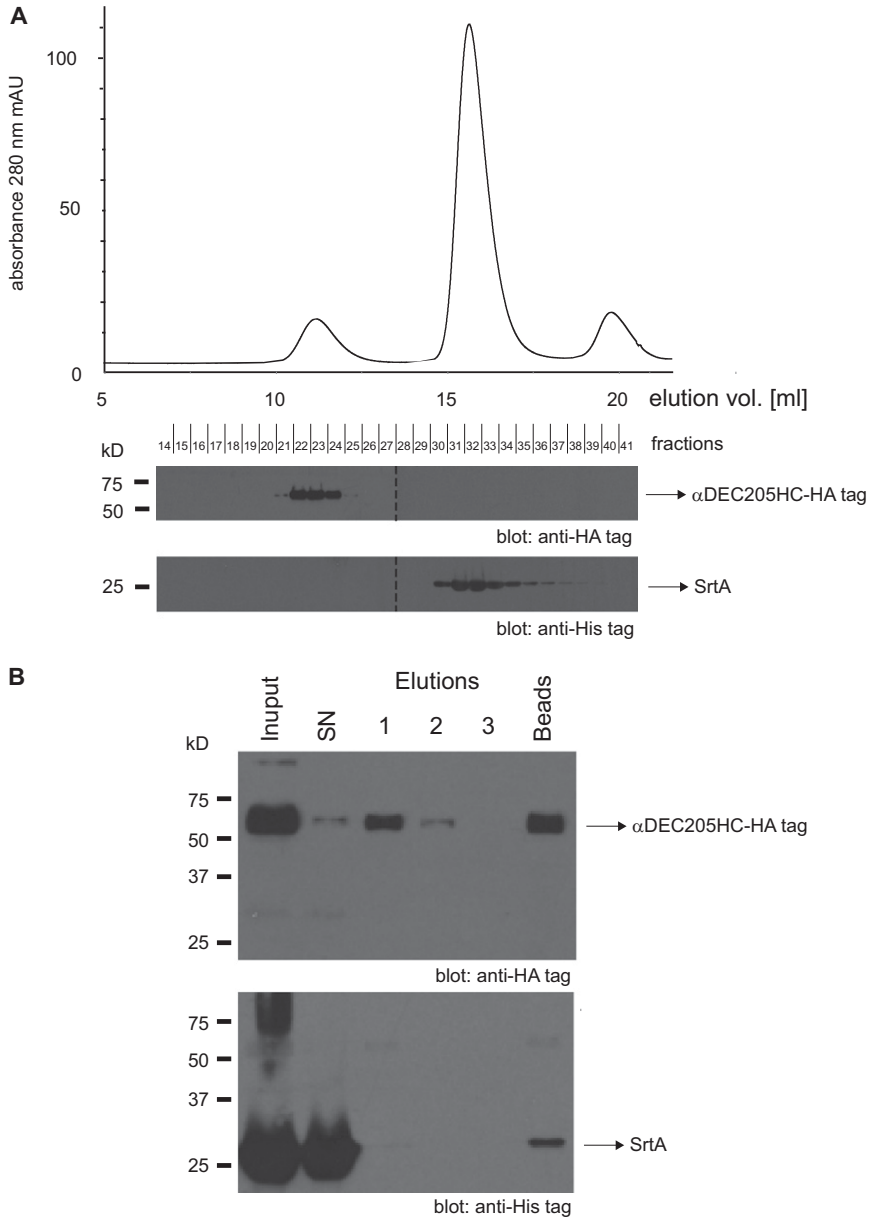

Fig. S1. Purification of αDEC205 adducts. (*A*) Elution profile of αDEC205 sortagged with a probe containing an HA tag as resolved by gel filtration chromatography. The presence of αDEC205-HA tag and sortase A was monitored by immunoblotting against the HA and His_6 tags. (*B*) Protein G-Sepharose purification of αDEC205 sortagged with a probe containing HA tag. The purification of sortagged αDEC205 using protein G-Sepharose pull-down was monitored by immunoblotting for HA (*Upper*) and His_6 tag (*Lower*) in equivalent sample containing equivalent amount of each purification steps. Beads, remaining protein, retrieved from protein G-Sepharose beads after elution; SN, supernatant from protein G-Sepharose incubation.

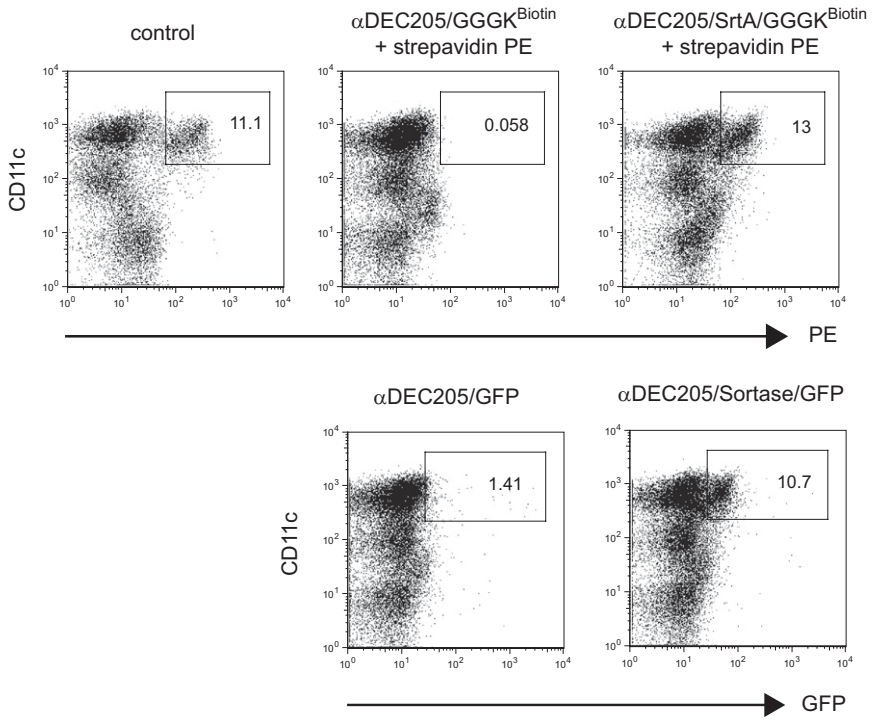

Fig. S2. Binding of sortagged αDEC205 to DEC205$^+$ dendritic cells (DCs). The CD11c-positive enriched fraction of the spleen of a C57/BL6 mouse was stained for CD11c together with αDEC205 of a commercial source ("control"), αDEC205 incubated with biotin-containing probes without (*Upper Middle*) or with sortase A (*Upper Right*), or αDEC205 incubated with GFP-containing probes without (*Lower Middle*) or with sortase A (*Lower Right*). αDEC205HC, heavy chain of the αDEC205 antibody; αDEC205LC, light chain of the αDEC205 antibody; SrtA, sortase A from *S. aureus*.

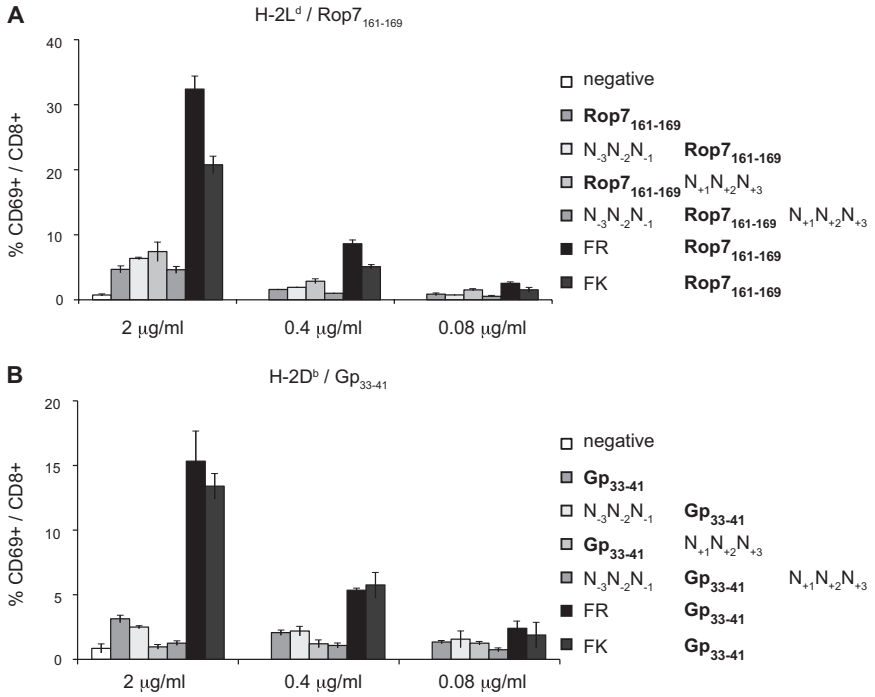

Fig. S3. In vitro stimulation of antigen-specific CD8 T cells by DCs incubated with sortagged αDEC205. Bone marrow-derived DCs were incubated in vitro with medium alone (negative) or αDEC205 sortagged with Rop7$_{161-169}$ (*A*) or Gp$_{33-41}$ (*B*)-containing probes (Table S1) at various concentrations, washed, and incubated together with splenocytes from Rop7 (*A*) or LCMV (*B*) transnuclear or transgenic mice. Histogram shows percentage of CD69-positive CD8 T cells for each concentration of αDEC205 adduct used after 16 h. Errors bars show SDs of three independent measurements; representative of three independent experiments.

Table S1. Peptide probes: Amino acid sequences of peptide probes (N to C terminus)

Probe	Sequence
1	GGGYPYDVPDYA
2	GGGISQAVHAAHAEINEAGR
3	GGGSLKISQAVHAAHAEINEAGR
4	GGGISQAVHAAHAEINEAGREVV
5	GGGSLKISQAVHAAHAEINEAGREVV
6	GGGFRISQAVHAAHAEINEAGR
7	GGGFKISQAVHAAHAEINEAGR
8	GGGV(Cit)ISQAVHAAHAEINEAGR
9	GGGSIINFEKL
10	GGGQLESIINFEKL
11	GGGSIINFEKLTEW
12	GGGQLESIINFEKLTEW
13	GGGFRSIINFEKL
14	GGGFKSIINFEKL
15	GGGV(Cit)SIINFEKL
16	GGGSIINFEK*L
17	GGGIPAAAGRFF
18	GGGRGHIPAAAGRFF
19	GGGIPAAAGRFFRRV
20	GGGRGHIPAAAGRFFRRV
21	GGGFRIPAAAGRFF
22	GGGFKIPAAAGRFF
23	GGGKAVYNFATC
24	GGGTSIKAVYNFATC
25	GGGKAVYNFATCGIL
26	GGGTSIKAVYNFATCGIL
27	GGGFRKAVYNFATC
28	GGGFKKAVYNFATC
29	GGGFRKSLTYYKL
30	GGGFRSCLDYSHL
31	GGGFRRSYIYYAL
32	GGGFRLGSVYYKL
33	GGGFRAILKFKSL
34	GGGFRKNYIFEEKL
35	GGGFRCNRIYARL
36	GGGFRLSPPMAHL
37	GGGFRAGYIYYQL
38	GGGFRTGFRYSYM
39	GGGFRTNYKFSLV
40	GGGFRVNLVFPSV
41	GGGFRSVYGFTGV
42	GGGFRAIYSFRNA
43	GGGFRTSINFVKI
44	GGGFRAGPHNDMEI
45	GGGFRSAPMKTVTI
46	GGGFRSAITNHAAF
47	GGGFRSAIENYETF
48	GGGK*

Cit, citrulline.
*Biotin.