Supporting Information

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SI Materials and Methods

Activation of T-Cell Hybridomas. Bone marrow–derived DCs were pulsed for 16 h with 10 μM of either peptide VSV (RGYVYQGL), VSV-E6 (RGYVYEGL), VSV-R6 (RGYVYRGL), or VSV I1E6 (IGYVYEGL) and then cocultured with N30.7 T-cell hybridomas in 96-well U-bottom plates (10^5 N30.7 T cells and 10^5 DCs per well). After 24 h, IL-2 release was measured by cytokine ELISA. Alternatively, DCs were pulsed for 16 h with 10 μM with 10 μM of the peptides and then cocultured with N30.7 T-cell hybridomas in 96-well U-bottom plates (10^5 N30.7 T cells and 10^5 DCs per well) for 5 h. N30.7 T-cell hybridomas were then harvested, stained with anti-CD8α and anti-Vβ13 antibodies (BD PharMingen), and analyzed by flow cytometry.

N30.7 T-cell Hybridoma Antagonism Assays. OT-I N30.7 T-cell hybridomas (10^5) were stimulated simultaneously with 10^5 DCs pulsed with 10 μM peptide VSV and 10^5 DCs unpulsed or pulsed with 10 μM VSV-E6, VSV-R6, or VSV I1E6. After 24 h of coculture, IL-2 release was measured by ELISA in the culture supernatants. Alternatively, after 5 h of coculture, TCR expression was measured by staining with anti-CD8α and anti-Vβ13 antibodies (BD PharMingen) and analyzing by flow cytometry.

Dynamics of Golgi Apparatus in N30.7 T-Cell Hybridomas. DCs were pulsed with 10 μM of peptide VSV, VSV-E6, VSV-R6, or VSV I1E6 for 16 h. After peptide pulse, DCs pulsed with VSV were stained with 0.5 μM CMTMR-Orange (Molecular Probes). Unpulsed DCs or DCs pulsed with either peptide VSV-E6, VSV-R6 or VSV I1E6 were stained with 0.5 μM BODIPY 630 (Molecular Probes). Golgi apparatus of N30.7 T-cell hybridomas was labeled with 5 μM BODIPY FL C5-Ceramide (Molecular Probes) at 37° C for 30 min. After staining, DCs were seeded on microchambers (Nalge Nunc International) previously coated with poly-D-lysine (Sigma-Aldrich). Measurements were started immediately after the addition of N30.7 T cells to the microchamber containing DCs. Fluorescence measurements were done on an Olympus Fluoview 1000 confocal microscope. Image sequences of the time-lapse recording were processed using FV10-ASW 1.6 Viewer and LSM Image Browser software.

Determination of Golgi Apparatus Polarization in T-Cell Hybridomas. R8 APCs were pulsed with 10 μM peptide VSV for 16 h. After peptide pulse, DCs were stained with 0.5 μM BODIPY 630 (Molecular Probes). Golgi apparatus of N30.7, G99A, or V98L T-cell hybridomas was labeled with 5 μM BODIPY FL C5-Ceramide (Molecular Probes) at 37°C for 30 min. After staining, DCs and T-cell hybridomas were seeded on microchambers (Nalge Nunc International) previously coated with poly-D-lysine (Sigma-Aldrich) and incubated for 1 h at 37°C. After this time, fluorescence measurements were made in an Olympus Fluoview 1000 confocal microscope. Images were processed using FV10-ASW 1.6 Viewer and LSM Image Browser software.

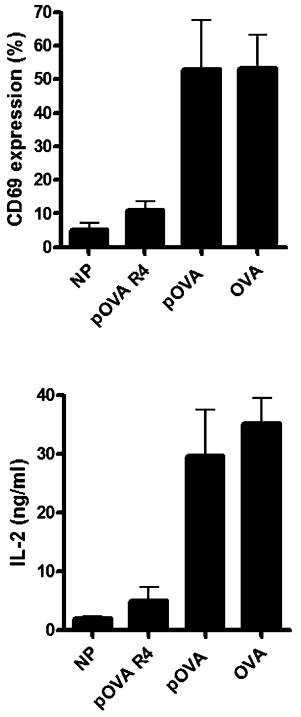


Fig. S1. APL pOVA-R4 fails to induce T-cell activation. pOVA- or pOVA-R4–pulsed DCs were cocultured with OT-I T cells, and then IL-2 secretion and CD69 expression were determined. Unpulsed DCs (NP) and DCs pulsed with the OVA protein (OVA) were included as positive and negative controls, respectively. Graphs represent average of three independent experiments; error bars represent SE.

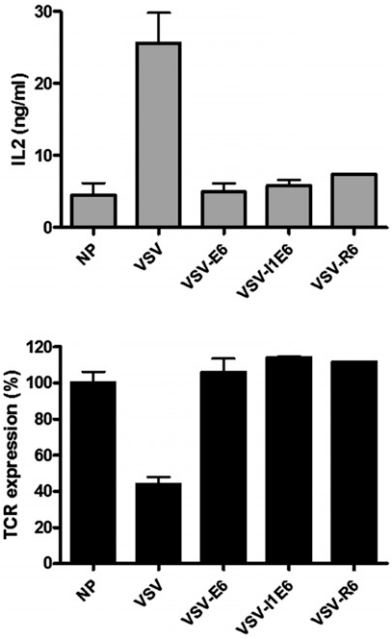


Fig. S2. pVSV induces N30.7 T-cell activation. pVSV-, pVSV-E6–, pVSV-R6–, or pOVA-I1E6–pulsed DCs were cocultured with N30.7 T-cell hybridomas and then IL-2 secretion and TCR expression were determined. Graphs represent average of three independent experiments; error bars represent SE.

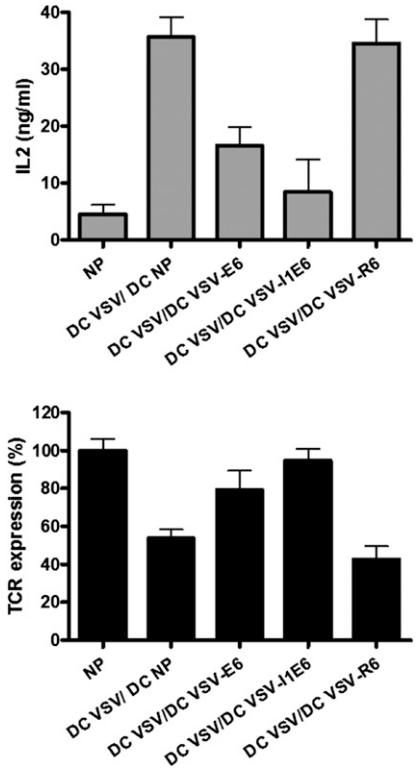


Fig. S3. pVSV APLs E6 and I1E6 are capable of cross-antagonizing N30.7 T cells. N30.7 T-cell hybridomas were simultaneously stimulated with DCs pulsed with pVSV and DCs pulsed with pVSV-APLs, and then IL-2 release and TCR expression were determined. Graphs represent average of three independent experiments; error bars represent SE.

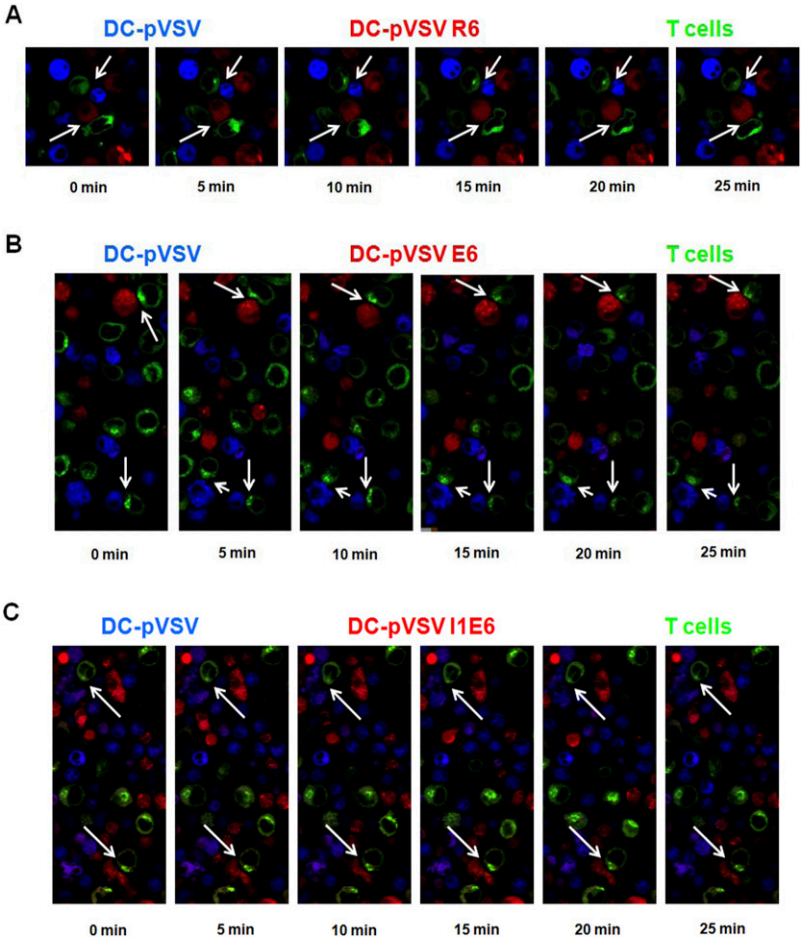


Fig. S4. Antagonist ligands for N30.7 T cells are capable of promoting efficient T-cell Golgi polarization. pVSV- or pVSV APLs–pulsed DCs were stained with BODIPY 630 (blue) or CMTMR orange (red) and cocultured with N30.7 T-cell hybridomas stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy. Data shown are representative sequences of snapshot images for pVSV-pulsed DCs vs. pVSV-R6- (*A*), pVSV-E6- (*B*), or pVSV-I1E6- (*C*) pulsed DCs, derived from one of three experiments.

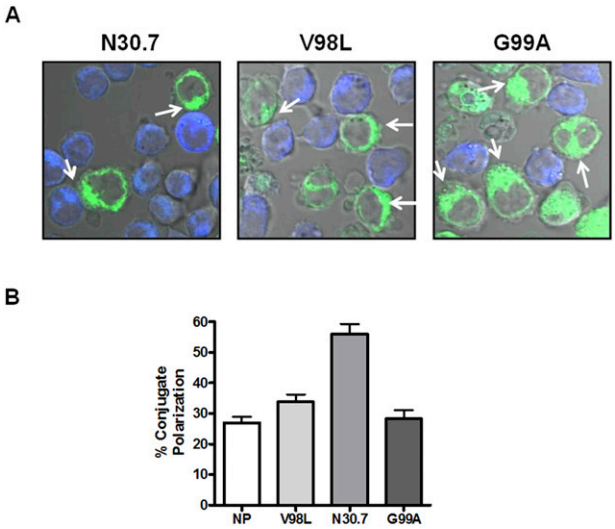


Fig. S5. T-cell Golgi polarization is impaired by long half-lives of TCR/pMHC interaction. (*A*) pVSV-pulsed R8 APCs were stained with BODIPY 630 (blue) and cocultured with N30.7 T-cell hybridomas, the long half-life mutant G99A hybridomas, or the short half-life mutant V98L hybridomas stained for Golgi apparatus (green). Golgi apparatus polarization was determined by confocal microscopy. Representative images are shown. (*B*) Quantification of confocal data showing percentages of T cells polarizing Golgi apparatus toward APCs in T cell–R8 conjugates.

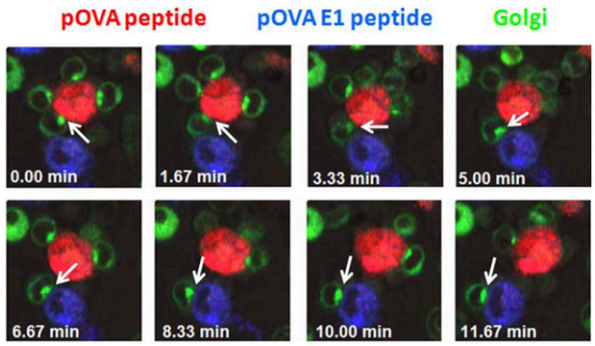


Fig. S6. T cells repolarize from DCs presenting agonist pMHC ligands to DCs presenting antagonist pMHC ligands. Peptide-pulsed DCs were stained with BODIPY 630 (blue) or CMTMR orange (red) and cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy. Data shown are representative sequences of snapshot images for pOVA-pulsed DCs (red) vs. pOVA-E1–pulsed DCs (blue), derived from one of three independent experiments.

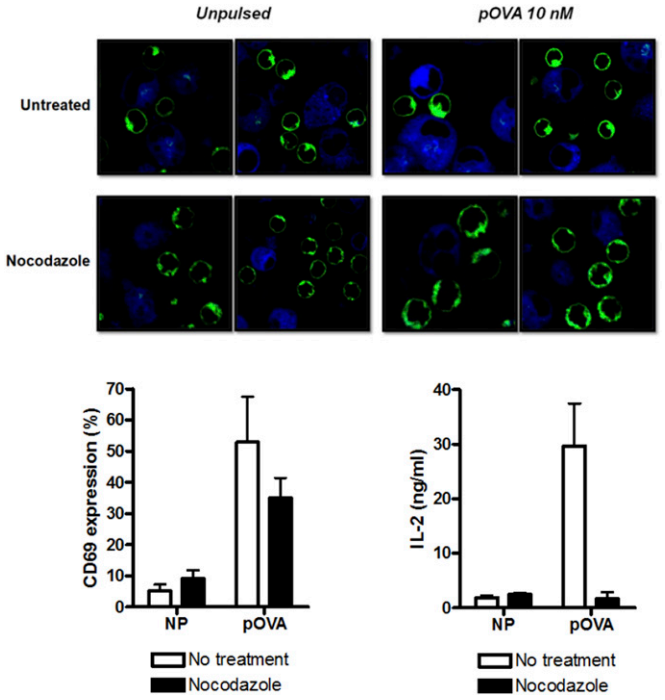


Fig. S7. MTOC disruption impairs IL-2 secretion but does not abolish CD69 upregulation. Unpulsed or pOVA-pulsed DCs were stained with BODIPY 630 (blue) and cocultured with untreated or nocodazole-treated OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by confocal microscopy. The same untreated or nocodazole-treated OT-I T cells were stimulated with unpulsed or pOVA-pulsed DCs, and then IL-2 release and CD69 upregulation were determined. Graphs represent average of two independent experiments; error bars represent SE. Photomicrographs represent one of two representative experiments.

Table S1. Measurement of Golgi apparatus polarization toward contact site between T-cell and pOVA- and pOVA APL–pulsed DCs

Single conjugates

Polarization toward	Polarized (%; N° conjugates)	Nonpolarized (%; N° conjugates)
DC-NP	28.4; 28	71.6; 71
DC-pOVA-K4	36.72; 87	63.28; 148
DC-pOVA-E1	77.12; 276	22.88; 84
DC-pOVA-R4	67.7; 44	32.3; 21
DC-pOVA	83.84; 2018	16.16; 384
DC-pOVA-A2	77.17; 216	22.83; 62
DC-pOVA-G4	34.21; 156	65.79; 293

Double conjugates

DC_1/T cell/DC_2	Polarized toward DC_1 (%; N° conjugates)	Polarized toward DC_2 (%; N° conjugates)
DC-pOVA/T cell/DC-NP	80.4; 61	19.6; 15
DC-pOVA/T cell/DC-pOVA-K4	83.47; 48	16.53; 11
DC-pOVA/T cell/DC-pOVA-E1	55.42; 21	44.58; 14
DC-pOVA/T cell/DC-pOVA-R4	54.5; 6	45.5; 5
DC-pOVA/T cell/DC-pOVA-A2	53.89; 63	46.11; 50
DC-pOVA/T cell/DC-pOVA-G4	88.91; 63	11.09; 7

T-cell/DC single conjugates (formed by one T cell conjugated with one DC pulsed with a pOVA APL peptide) and double conjugates (formed by one T cell simultaneously conjugated with two DCs pulsed with different pOVA APL peptides) were randomly selected from at lest four independent experiments. Polarization of Golgi apparatus was scored blindly by visual quantification.

Table S2. Phenotype of N30.7 TCR/H-2K^b for VSV-derived peptides*

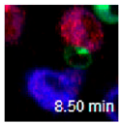
Peptide	Peptide sequence	Phenotype
pVSV	RGYVYQGL	Agonist
pVSV-E6	RGYVYEGL	Antagonist
pVSV-R6	RGYVYRGL	Null
pVSV-I1E6	IGYVYEGL	Antagonist

*Data from Lee SJ, Hori Y, Groves JT, Dustin ML, Chakraborty AK (2002) Correlation of a dynamic model for immunological synapse formation with effector functions: Two pathways to synapse formation. *Trends Immunol* 23:492–499.

Table S3. Binding kinetics of N30.7 TCR mutants for the H-2K^b/VSV complex*

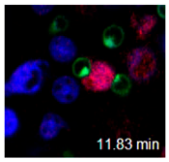
TCR	Ligand	Half-life, min†
N30.7	H-2K^b/ RGYVYQGL	5.1 ± 0.7
G99A	H-2K^b/ RGYVYQGL	10.8 ± 3.7
V98L	H-2K^b/ RGYVYQGL	0.5 ± 0.1

*Data from refs. 8 and 10.
†Half-lives of TCR–ligand interaction were measured with H-2K^b/VSV tetramers.



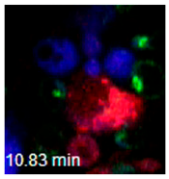
Movie S1. Unpulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S1.



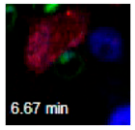
Movie S2. Unpulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S2.



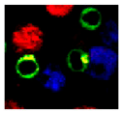
Movie S3. pOVA-K4–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S3.



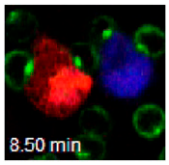
Movie S4. pOVA-K4–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S4.



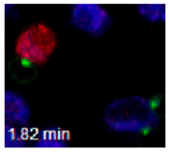
Movie S5. pOVA-R4–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S5.



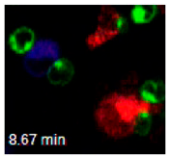
Movie S6. pOVA-E1–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S6.



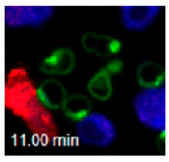
Movie S7. pOVA-E1–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S7.



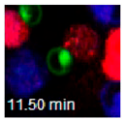
Movie S8. pOVA-A2–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S8.



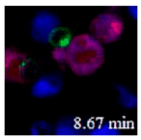
Movie S9. pOVA-A2–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S9.



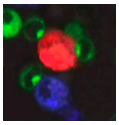
Movie S10. pOVA-G4–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S10.



Movie S11. pOVA-G4–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S11.



Movie S12. pOVA-E1–pulsed DCs and pOVA-pulsed DCs were stained with BODIPY 630 (blue) and CMTMR orange (red), respectively. Red- and blue-stained DCs were subsequently cocultured with OT-I T cells stained for Golgi apparatus (green). Golgi apparatus polarization was determined by time-lapse video microscopy.

Movie S12.