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Supplementary Information for

DNA origami demonstrate the unique stimulatory power of single pMHCs as T-cell antigens

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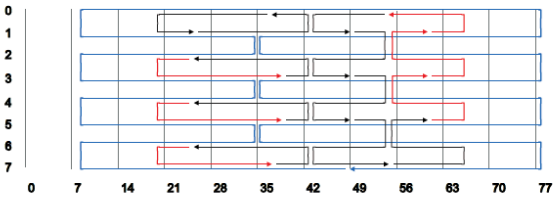
This PDF file includes:

Figures S1 to S21
Tables S1 to S10
SI References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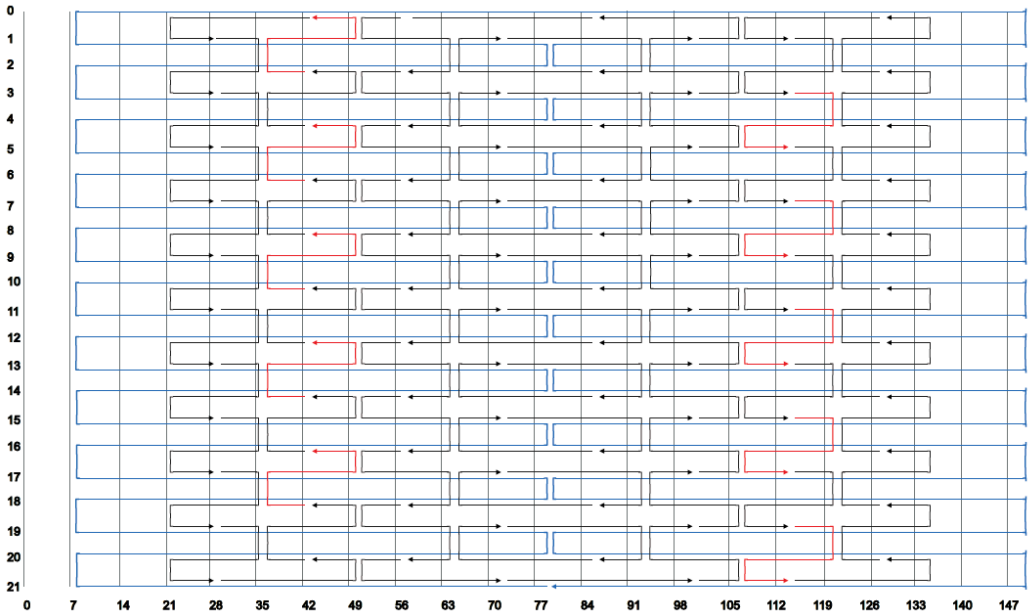
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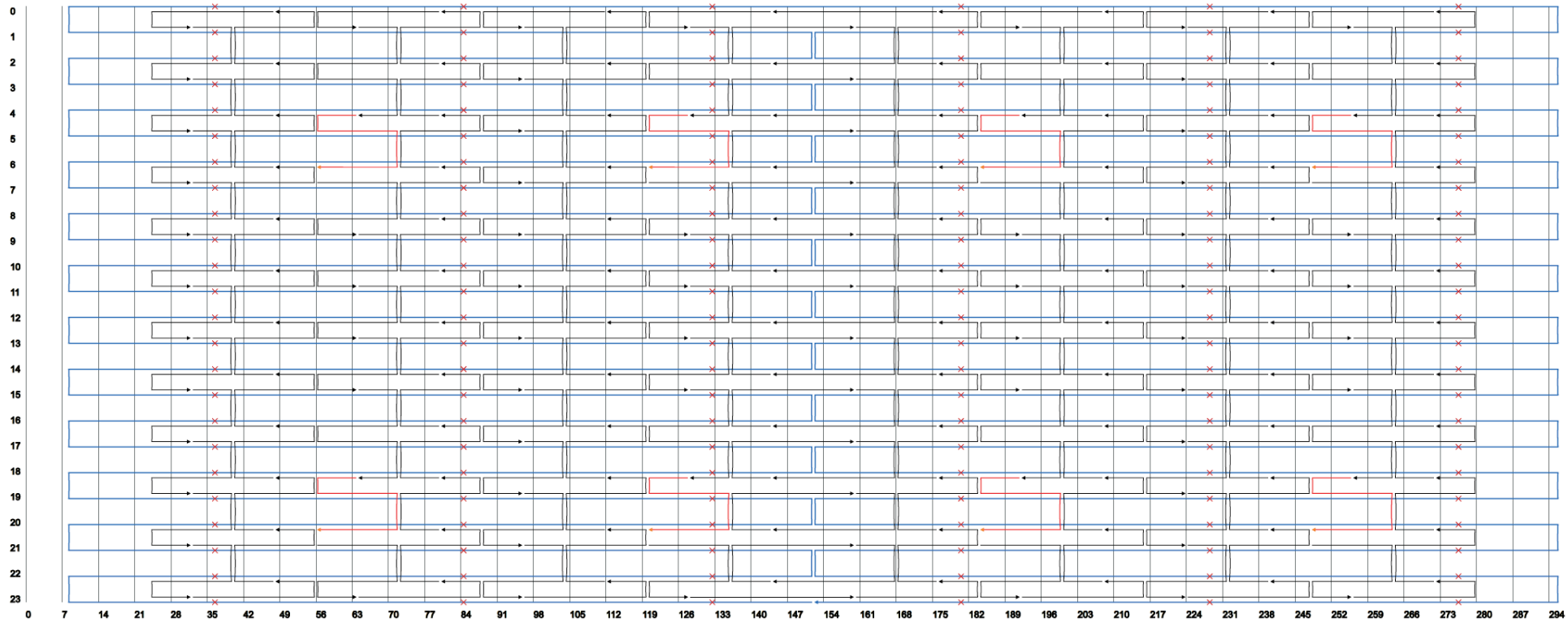

Fig. S1. DNA origami scaffold routing. DNA origami (30x20 nm, 65x54 nm, 100x70 nm (1)) were designed based on the M13mp18 scaffold (2) using caDNAno (3). At sites chosen for ligand attachment, staple strands were elongated at their 3'-end with 21 bases. For attachment to the SLB via complementary cholesterol-oligonucleotides staple strands were elongated at predefined distinct positions at their 5'-end with 25 bases (red arrows).

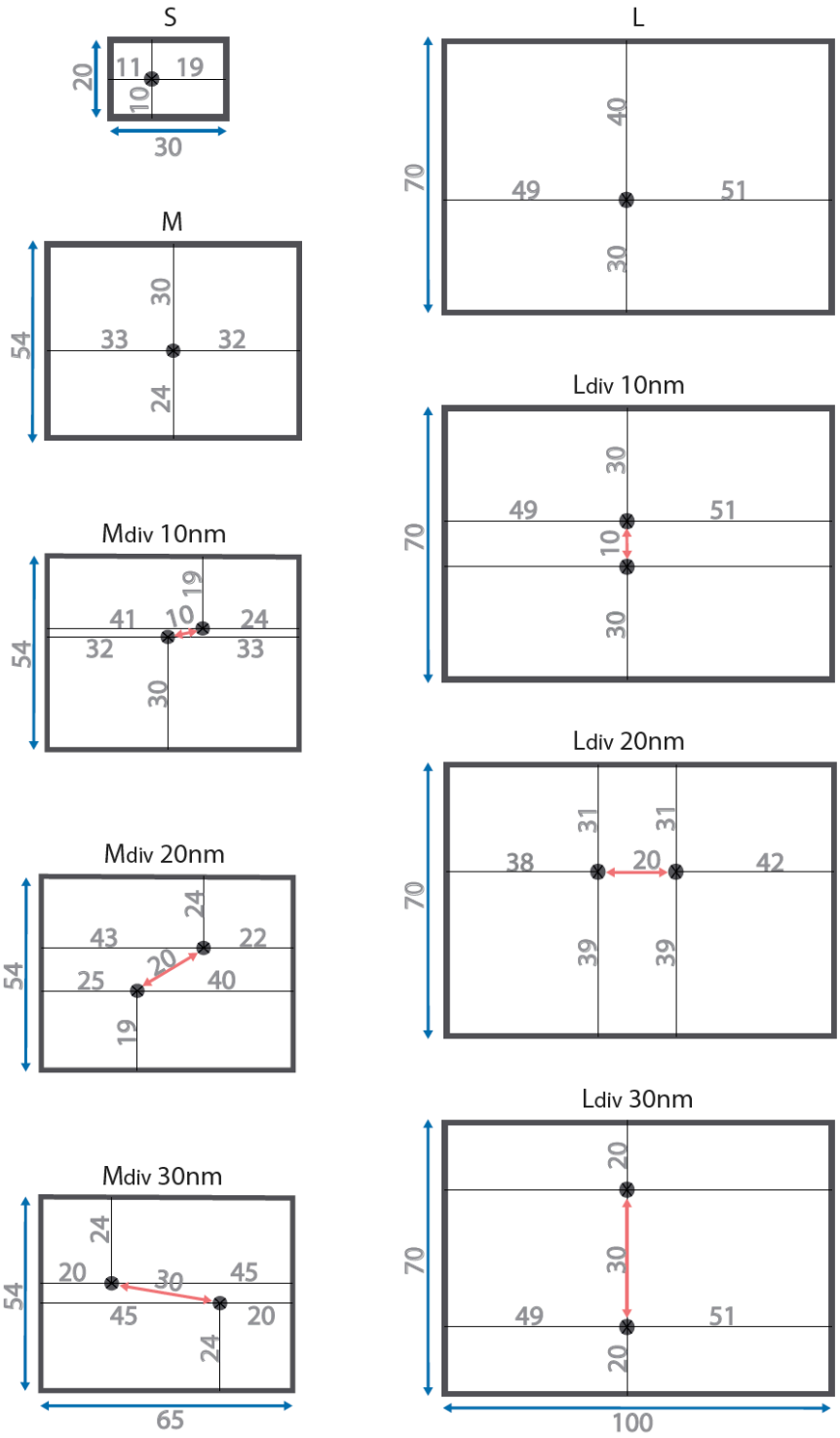

Fig. S2. Schematics of DNA origami used in this study. Black dots indicate modification sites on the top side of DNA origami platforms. The distances are approximated with 0.34 nm per base pair along the helical axis and with 2 nm per helix perpendicular to the helical axis (2). Interhelical gaps were assigned 1 nm, depending on the spacing of crossovers between helices. Distances are given in nm. The distances between the ligand position and the platform edges define the minimum possible ligand spacing permitted by a given DNA origami platform.

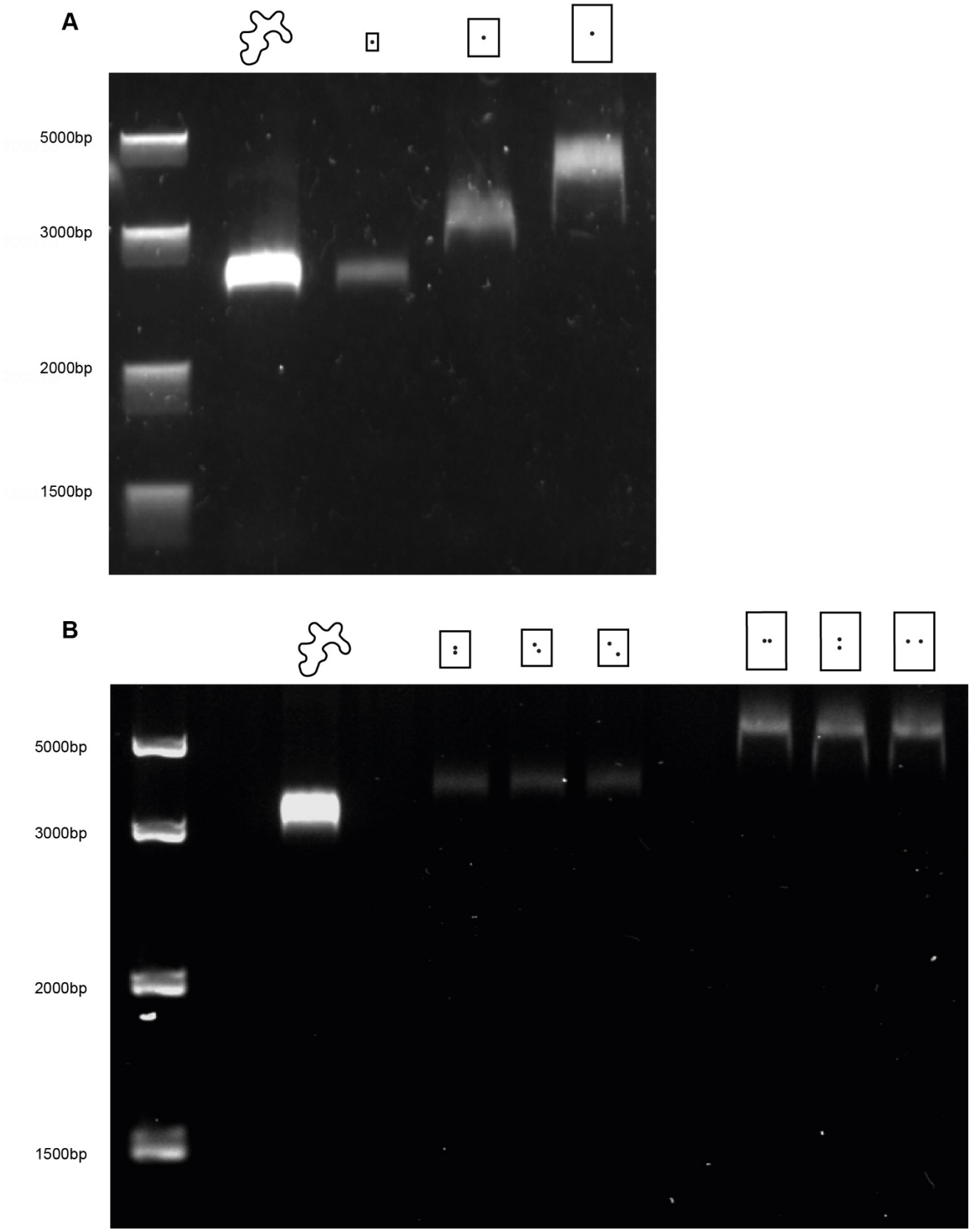

Fig. S3. Agarose gel electrophoresis of DNA origami platforms. *(A)* 1% agarose gel (1xTAE, 10 mM $MgCl_2$) pre-stained with Sybr™ gold nucleic acid gel stain was run at 100V for 75 min. A FastRuler middle range DNA ladder was used as a reference. Schematic sketches above the individual bands indicate the different DNA origami layouts functionalized with dSA. *(A),* from left to right: M13mp18 scaffold, S, M, L. *(B)* M13mp18 scaffold, M_{div} 10nm, M_{div} 20nm, M_{div} 30nm, L_{div} 10nm, L_{div} 20nm, L_{div} 30nm.

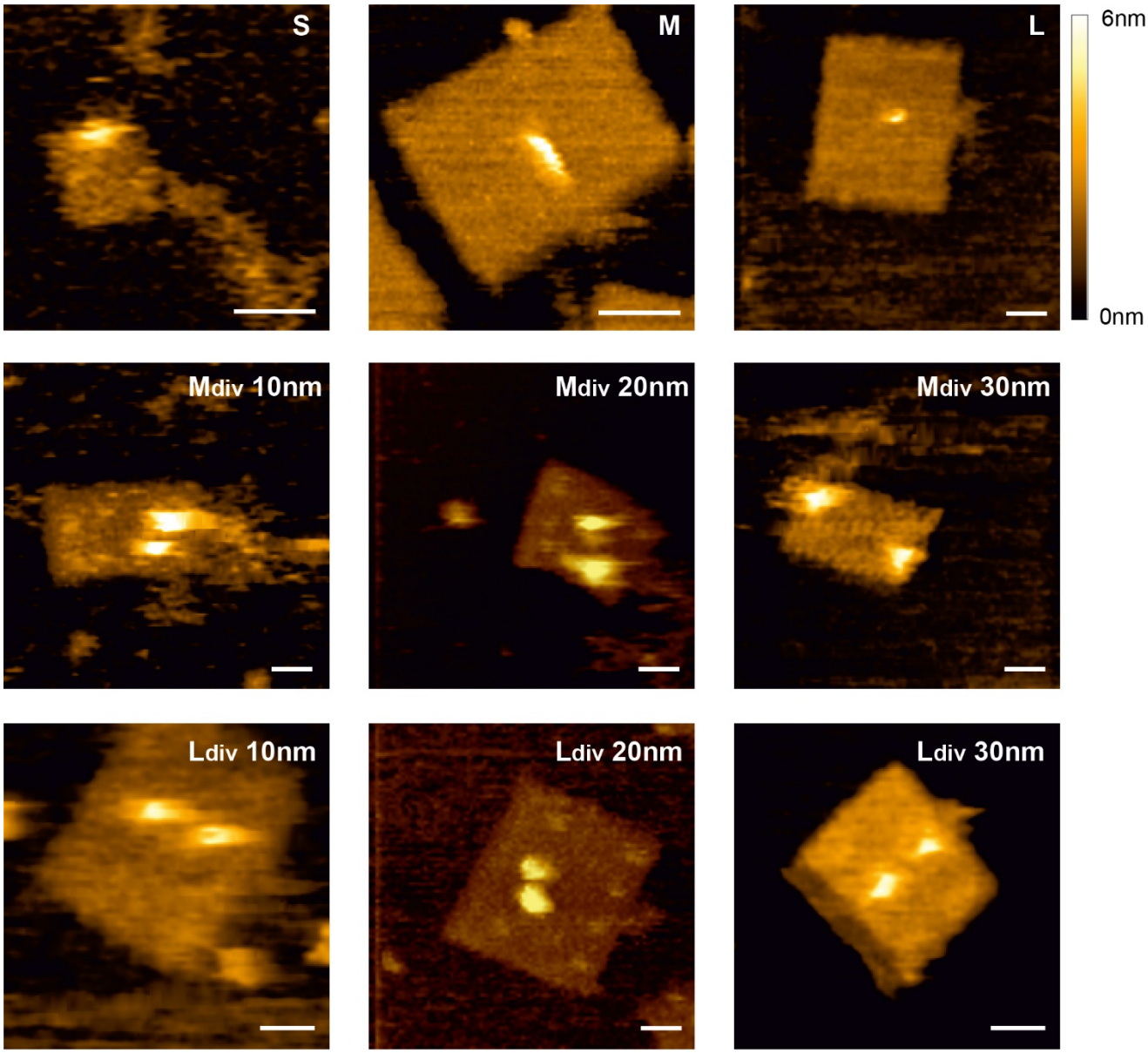

Fig. S4. AFM images of functionalized DNA origami platforms. The different DNA origami platforms used in this study were functionalized with dSA as indicated in Fig. S2 and imaged using highspeed-AFM (HS-AFM). Scale bar, 25 nm.

Note

Unfolded scaffold, which is unstructured and highly flexible, is imaged as a blur with HS-AFM. While almost all of the M13mp18 Phage DNA is used to fold the large “L” DNA origami structure, half (“M” DNA origami) or 90% (“S” DNA origami) remain unfolded. Note that the divalent streptavidin used for attachment of biotinylated TCR-ligands was placed on top of a ~5nm linker. During AFM imaging, the AFM tip pushes the streptavidin molecule towards the DNA origami platform surface, sometimes even ripping it off from the structure, thereby distorting the distances.

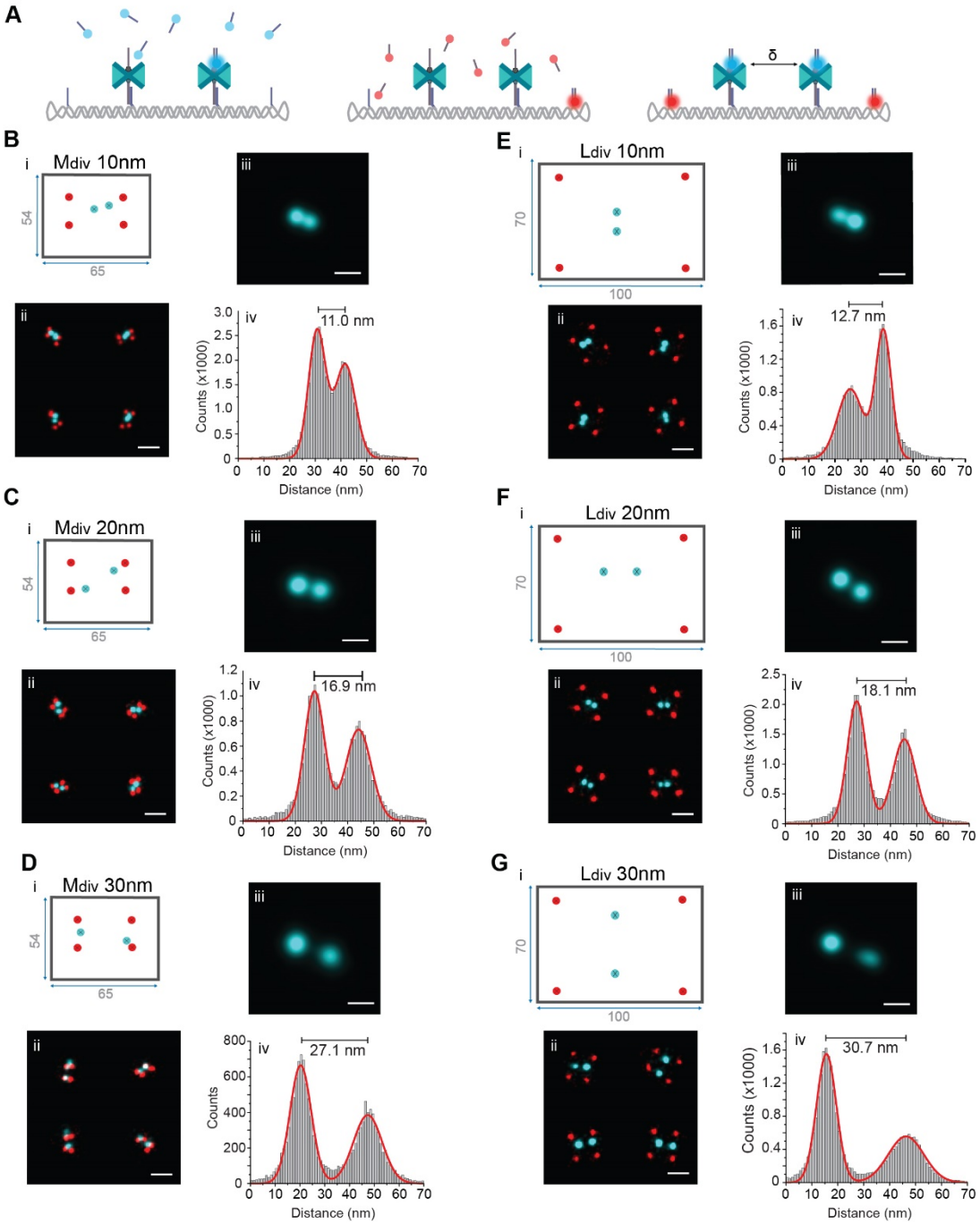

Fig. S5. Mapping ligand positions on divalent DNA origami platforms via DNA-PAINT super-resolution microscopy. *(A)* Principle of DNA-PAINT. Freely diffusing dye-labeled oligonucleotides ("imager" strands) transiently bind to their target-bound complements ("docking" strands) to achieve the necessary target "blinking" for stochastic super-resolution microscopy. For this, biotinylated TCR ligands were replaced with biotinylated DNA-PAINT docking strands (cyan) on divalent DNA origami platforms. Additionally, four staple strands at the corners were extended with DNA-PAINT docking sites as platform markers (red). Ligand (cyan) and platform (red) positions were imaged consecutively by Exchange-PAINT (4) using Cy3B imager strands. **i,** DNA origami layouts showing ligand and platform positions for the following constructs: *(B),* M_{div} 10nm, *(C),* M_{div} 20nm, *(D),* M_{div} 30nm, *(E),* L_{div} 10nm, *(F),* L_{div} 20nm, *(G),* L_{div} 30nm. **ii,** 4 exemplary pseudo-color DNA-PAINT super-resolution images of DNA origami platforms. Scale bar, 50 nm. **iii,** DNA-PAINT sum images of ligand positions n ≥ 49. Scale bar, 20 nm. **iv,** Intensity profiles across ligand positions indicated in (iii).

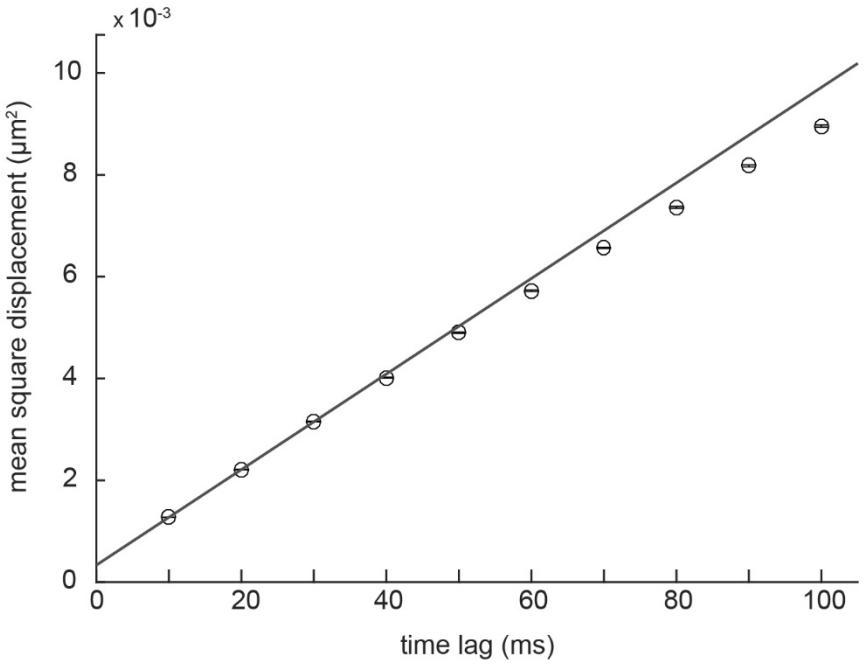

Fig. S6. Determination of diffusion behavior of DNA origami on SLBs. Diffusion constants were evaluated by performing single-molecule tracking experiments. Mean square displacements were determined and plotted as a function of time lags. Assuming pure Brownian motion, the diffusion coefficient D was derived by fitting the first two data points with a linear fit. An exemplary plot for M-H57 is shown.

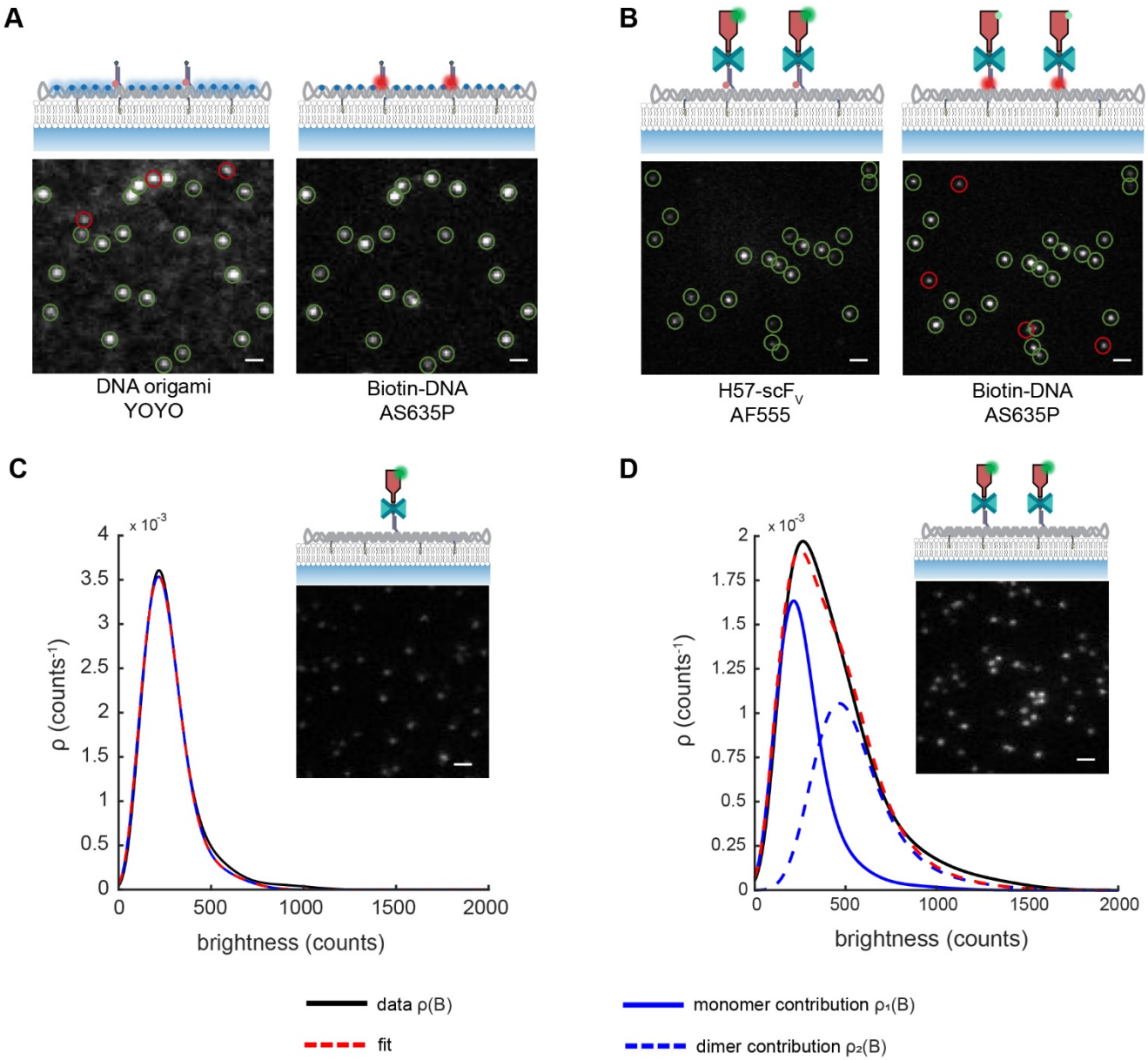

Fig. S7. Determination of DNA origami ligand occupancy. *(A)* To determine the fraction of attachment sites on DNA origami available for modification, elongated staple strands were hybridized with fluorescently labeled biotinylated oligos (Biotin-DNA-AS635P) and two-color colocalization with DNA origami, pre-stained with YOYO, was assessed. Exemplary TIRF images of M_{div} 30nm DNA origami on a SLB are shown. Green open circles indicate signals detected in both color channels; red open circles indicate signals detected only in one channel. *(B)* We quantified the colocalization of hybridized biotin-DNA-AS635P and AF555-labeled H57-scF$_V$s to determine the functionalization efficiency of existing biotin modifications with ligand (here shown for M_{div}-H57$_{30nm}$). *(C,D)* Exemplary TIRF images and corresponding brightness distributions ρ_n of DNA origami on SLBs decorated with AF555-labeled H57-scF$_V$. Data for medium-sized DNA origami bearing 1 (M-H57, 800 signals, *(C))* or 2 (M_{div}-H57$_{30nm}$, 708 signals, *(D))* possible ligand attachment sites are shown. The detected signals were fitted and deconvolved into monomer and dimer contributions (see methods section) (5). Only ~ 0.5% of signals originate from non-specifically bound ligand. Scale bar, 2 µm.

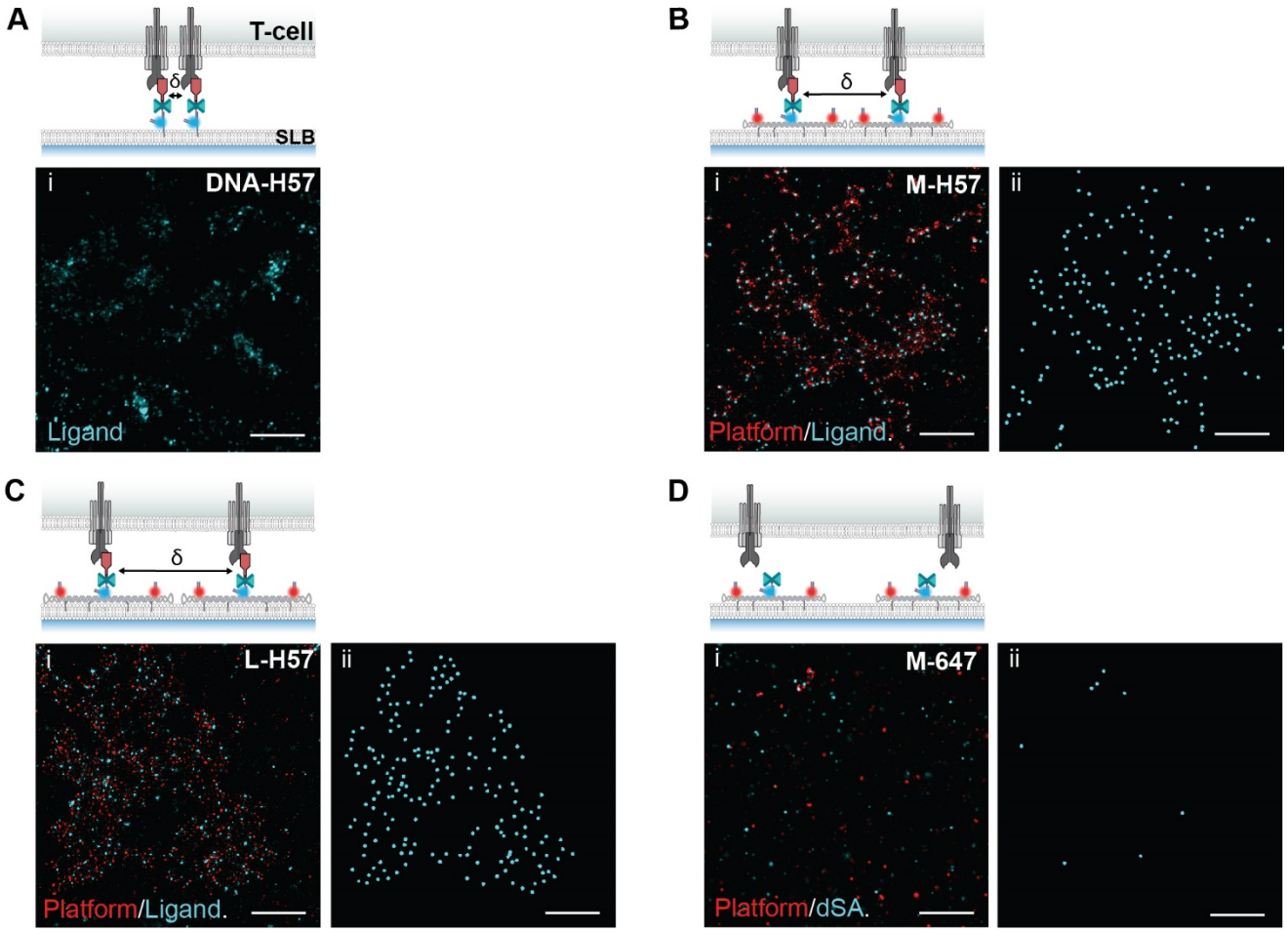

Fig. S8. DNA-PAINT super-resolution imaging of H57-scF$_V$ within microclusters. DNA origami platforms impose minimum distances δ on ligands in microclusters. For DNA-PAINT, biotinylated oligos were extended with DNA-PAINT docking sites at their 3' end for imaging ligand positions (cyan). Additionally, four staple strands at the platform corners were extended with DNA-PAINT docking sites to serve as platform markers (red). *(A)* DNA-anchored H57-scF$_V$s free to move without restrictions (DNA-H57, δ ~ 5 nm), *(B)* H57-scF$_V$s on medium-sized DNA origami platforms (M-H57, δ = 48 nm), *(C)* H57-scF$_V$s on large DNA origami platforms (L-H57, δ = 60 nm), *(D)* medium-sized DNA origami platforms without ligands labeled with AF647 (M-647).
i, Exemplary pseudo-color DNA-PAINT super-resolution images of H57-decorated constructs underneath fixed T-cells. Ligand (cyan) and marker (red) positions were imaged consecutively by Exchange-PAINT using Cy3b imager strands. Characteristic microcluster formation could be observed for all constructs except for DNA origami platforms without ligands. **ii**, Detected ligand positions after filtering. Ligand positions from (i) were classified as «true» or «false» based on their blinking statistics (see methods section and Fig. S9). Note that individual positions of the freely arrangeable DNA-H57 could not be resolved via DNA-PAINT at the experimental conditions applied. Scale bars, 500 nm.

Note on DNA super-resolution imaging of S-H57 platforms

The smallest DNA origami platform (S-H57, 30x20 nm) could not be successfully imaged via DNA-PAINT due to undesired binding events to the structures. This is likely caused by transient hybridization of the DNA imager strand to the unfolded scaffold (note that for the S DNA origami platform only 10% of the scaffold was used for the platform design).

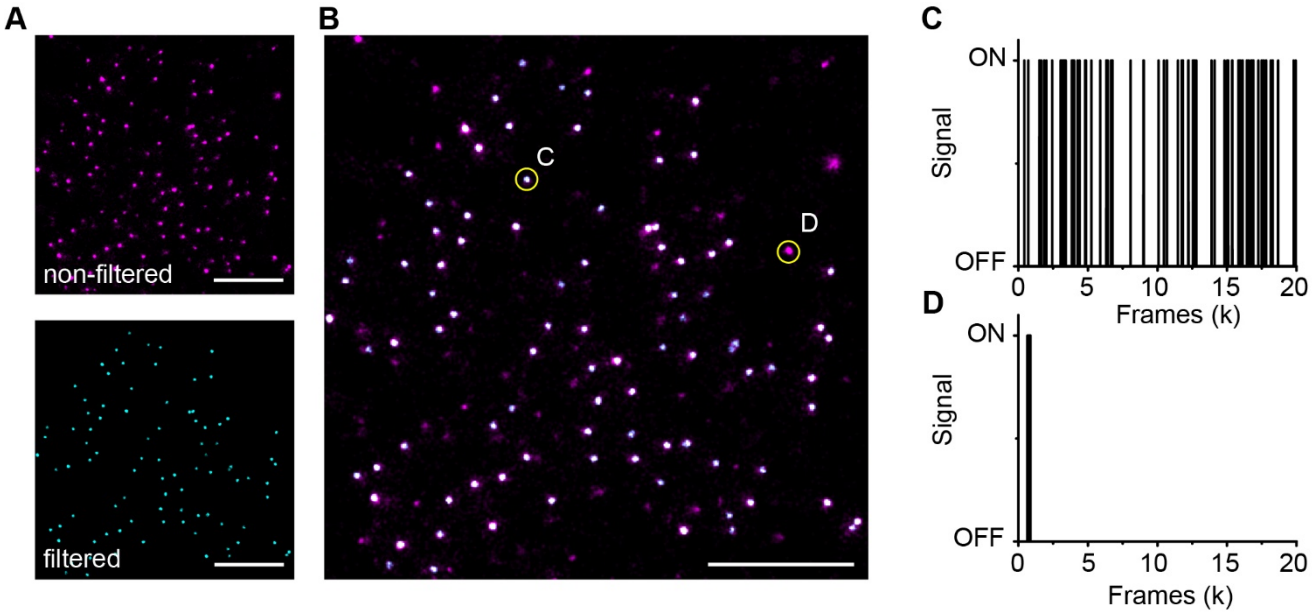

Fig. S9. Filtering of DNA-PAINT data. *(A)* An exemplary DNA-PAINT super-resolution image is shown with non-filtered (magenta) and filtered data (cyan). *(B)* Overlay of images from *(A)*. Signals arising from specific binding (after filtering) classified as «true» localizations are shown in white. *(C)* Specific binding (indicated in *(A)*) is characterized by repetitive (n ≥ 12) single-molecule detection events within a 10 nm radius due to transient binding of the imager strand to a complementary docking site. *(D)* Non-specific binding (indicated in *(B)* is characterized by a single binding event of longer duration. These non-specific signals were filtered out and discarded for further analysis. Scale bar, 500 nm

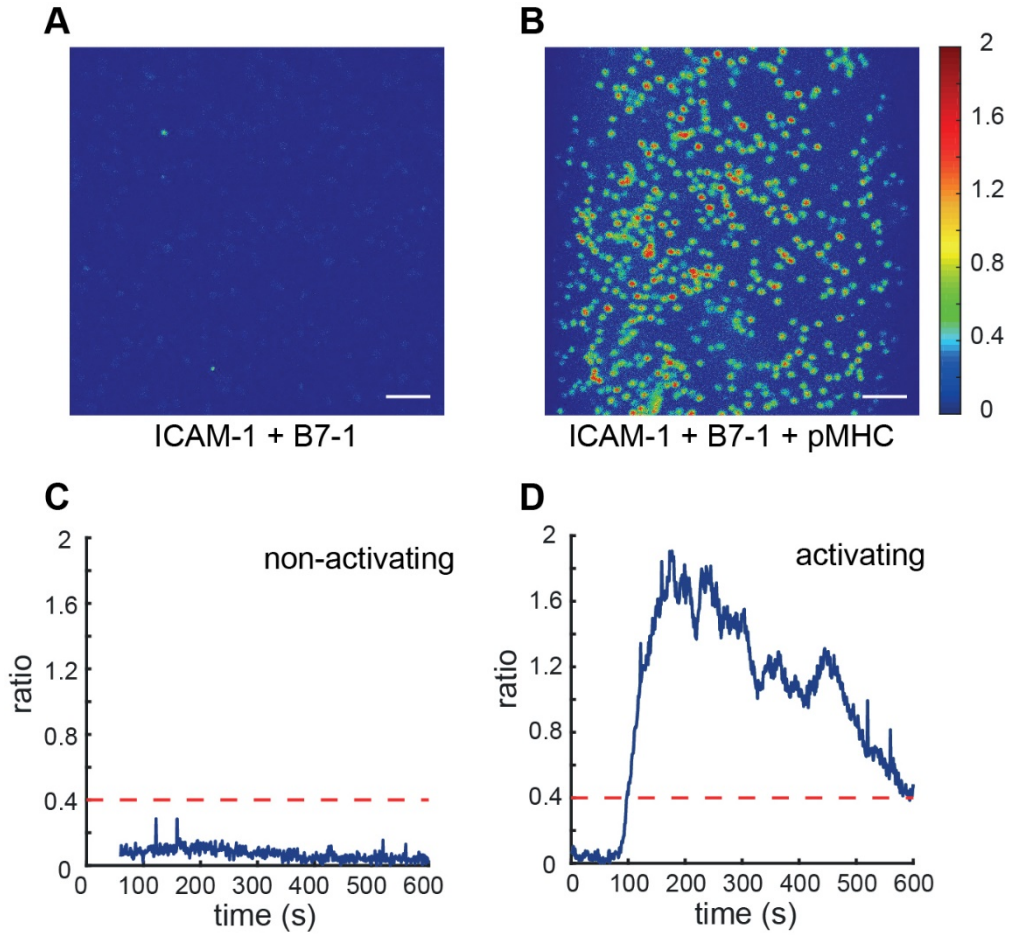

Fig. S10. Calcium imaging experiments to assess the T-cell activation state. T-cells were loaded with the ratiometric Ca^{2+}-sensitive dye Fura-2 AM, seeded onto SLBs and fluorescence emission was recorded at excitation wavelengths 340 nm and 380 nm over 10 min. Activation was tracked via a change of the intensity ratio (340/380nm). Exemplary ratio images recorded at activating (ICAM-1 100 μm^{-2}, B7-1 100 μm^{-2}, pMHC 150 μm^{-2}, *(A))* and non-activating (ICAM-1 100 μm^{-2}, B7-1 100 μm^{-2}, *(B))* conditions are shown 5 min after cell seeding. *(C,D)* For each cell, the intensity ratio 340/380nm was determined and plotted over time. Exemplary calcium traces for a T-cell under non-activating *(C)* and activating *(D)* conditions are shown. The threshold ratio for counting a cell as "activated" was set to 0.4 for all experiments, indicated by a red dashed line. Scale bar, 4 µm.

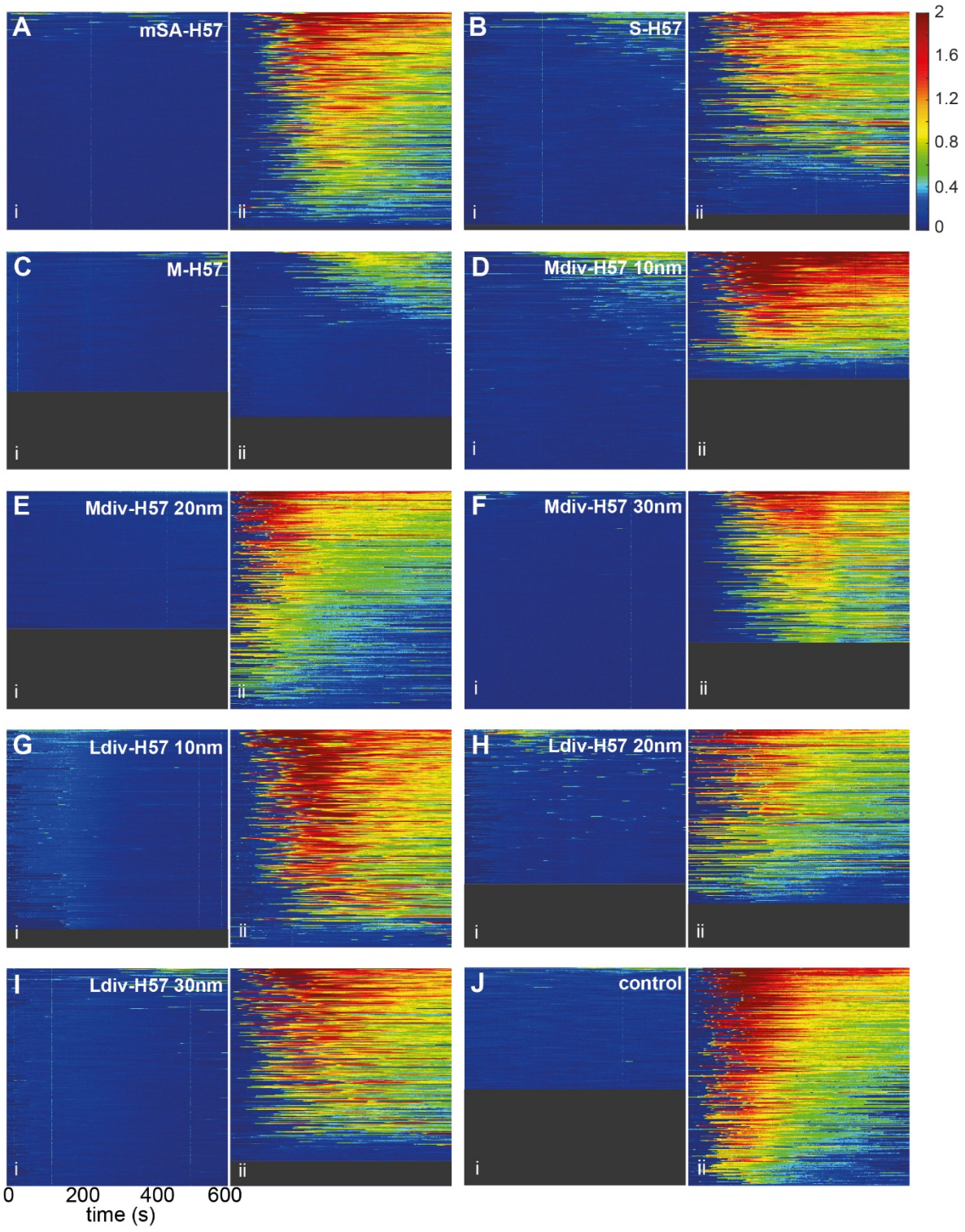

Fig. S11. Population calcium flux on H57-scF$_V$ biointerfaces. Pseudo-colored calcium flux (Fura-2 AM ratio) traces of >100 cells per region for: mSA-H57 *(A)*, S-H57 *(B)*, M-H57 *(C)*, M_{div}-$H57_{10nm}$ *(D)*, M_{div}-$H57_{20nm}$ *(E)*, M_{div}-$H57_{30nm}$ *(F)*, L_{div}-$H57_{10nm}$ *(G)*, L_{div}-$H57_{20nm}$ *(H)*, L_{div}-$H57_{30nm}$ *(I)* at non-activating (**i**) and activating conditions (**ii**). For M-H57, traces at the highest measured ligand density of 248 μm^{-2} are shown. *(J)* A negative control containing ICAM-1 and B7-1 at 100 μm^{-2} (i) and a positive control containing ICAM-1 and B7-1 at 100 μm^{-2}, and His-pMHC at 150 μm^{-2} (ii) are shown as a reference. Each horizontal line represents one cell. Cell traces are ranked by their integrated calcium flux in descending order. Calcium was imaged at 1 Hz for 10 min at 24°C.

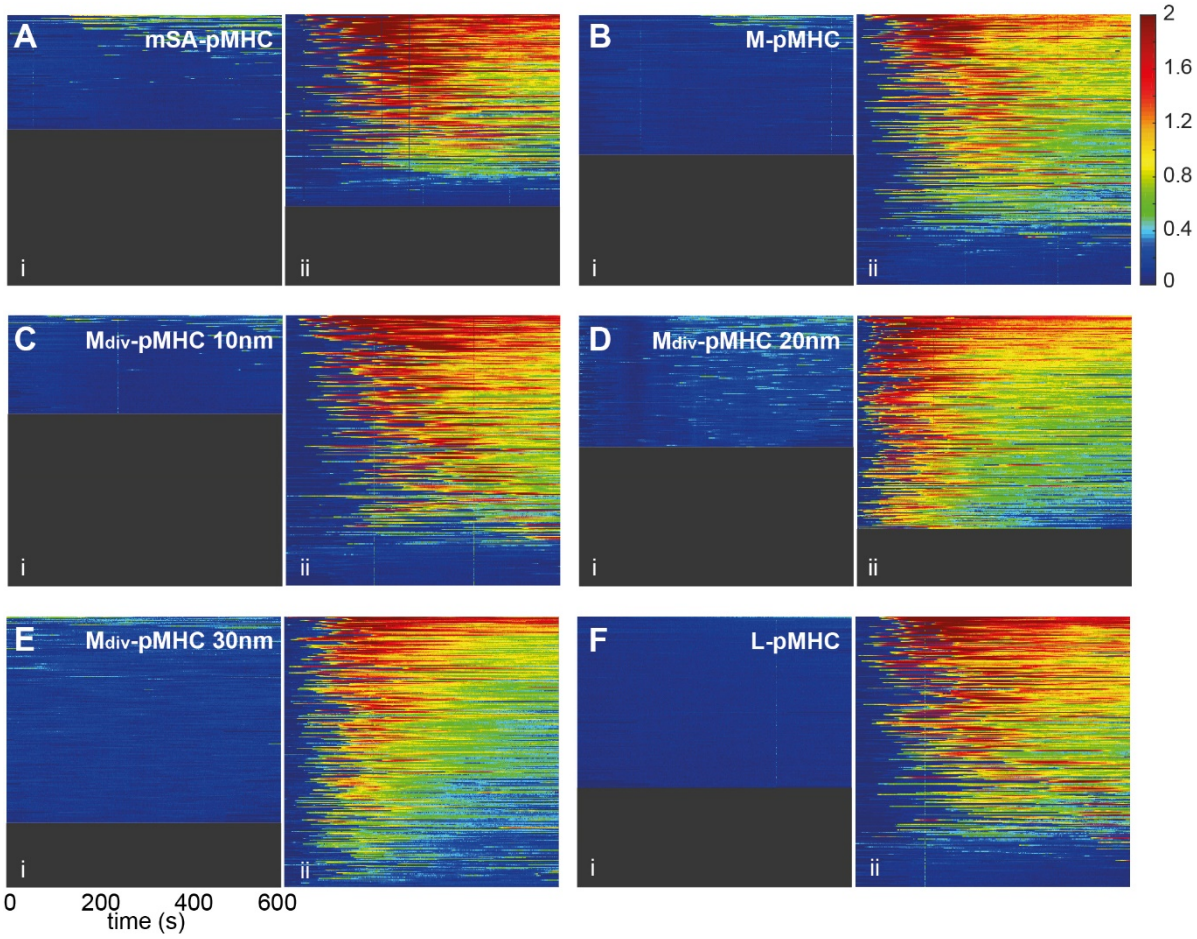

Fig. S12. Population calcium flux on pMHC biointerfaces. Pseudo-colored calcium flux (Fura-2 AM ratio) traces of >100 cells per region for: mSA-pMHC *(A)*, M-pMHC *(B)*, L-pMHC *(C),* M_{div}-pMHC$_{10nm}$ *(D)* M_{div}-pMHC$_{20nm}$ *(E)* and M_{div}-pMHC$_{30nm}$ *(F)* at non-activating (**i**) and activating conditions (**ii**) are shown. Each horizontal line represents one cell. Cell traces are ranked by their integrated calcium flux in descending order. Calcium was imaged at 1 Hz for 10 min at 24°C.

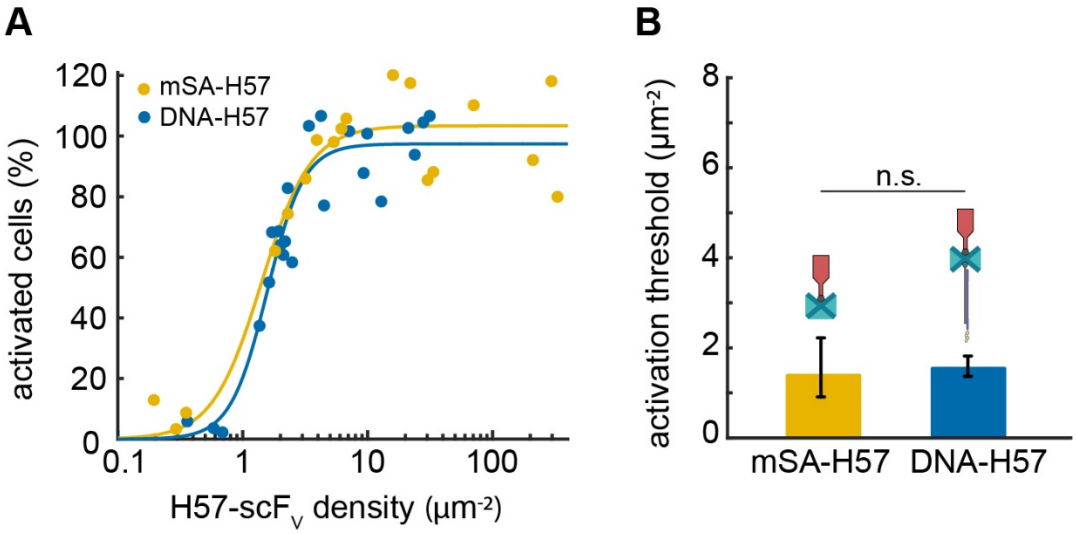

Fig. S13. Comparison of signaling potency of mSA-H57 and DNA-H57. *(A)* Percentage of activated T-cells at different ligand surface densities are shown. Data were normalized (=100%) to a positive control containing ICAM-1 and B7-1 at 100 µm^{-2}, and His-pMHC at 150 µm^{-2}. *(B)* Dose-response curves were fitted with Eq. 6 to extract activation thresholds. For each construct, data are from at least three independent experiments and three different mice. Error bars represent the 95% CI.

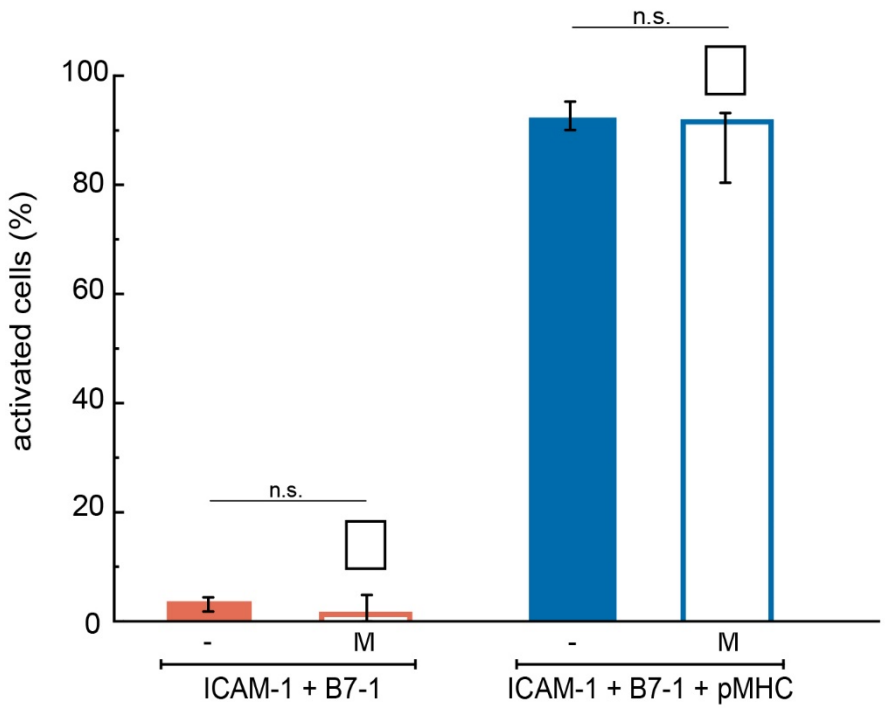

Fig. S14. Ligand-free DNA origami platforms do not influence T-cell activation behavior. The presence of ligand-free medium-sized DNA origami platforms (M) at a density of ~50 μm^{-2} did not alter the intracellular calcium response at either non-activating (ICAM-1 100 μm^{-2}, B7-1 100 μm^{-2}) or activating (ICAM-1 100 μm^{-2}, B7-1 100 μm^{-2}, His-pMHC 150 μm^{-2}) conditions. Data are the median of at least three independent experiments and three different mice (± 95% C.I.).

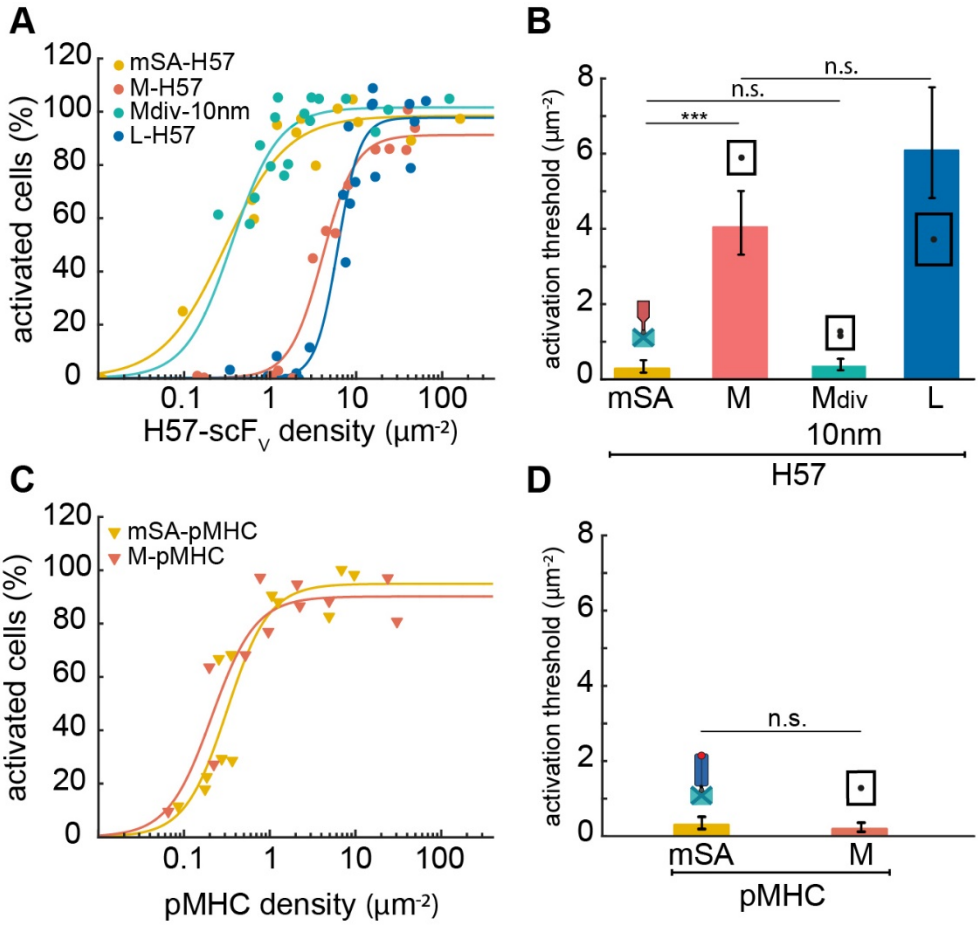

Fig. S15. T-cell activation on biointerfaces at 37°C. The percentage of activated cells at different surface densities of H57-scFv *(A)* or pMHC *(C)* was measured at 37°C. Data were normalized to a positive control containing His-tagged ICAM-1 and B7-1 at 100 µm^{-2}, and His-pMHC at 150 µm^{-2} (=100%). For each construct, data are from at least two independent experiments and two different mice. *(B,D)* Activation thresholds determined from fits of data from *(A)* and *(C)*. $t_{1/2}$ for the H57:TCR and pMHC:TCR interaction at 37°C are 6 min and 100 ms, respectively (6). Error bars represent the 95% CI. *** $p < 0.001$. A matrix containing the results of significance tests for all combinations of ligands is shown in Dataset S1.

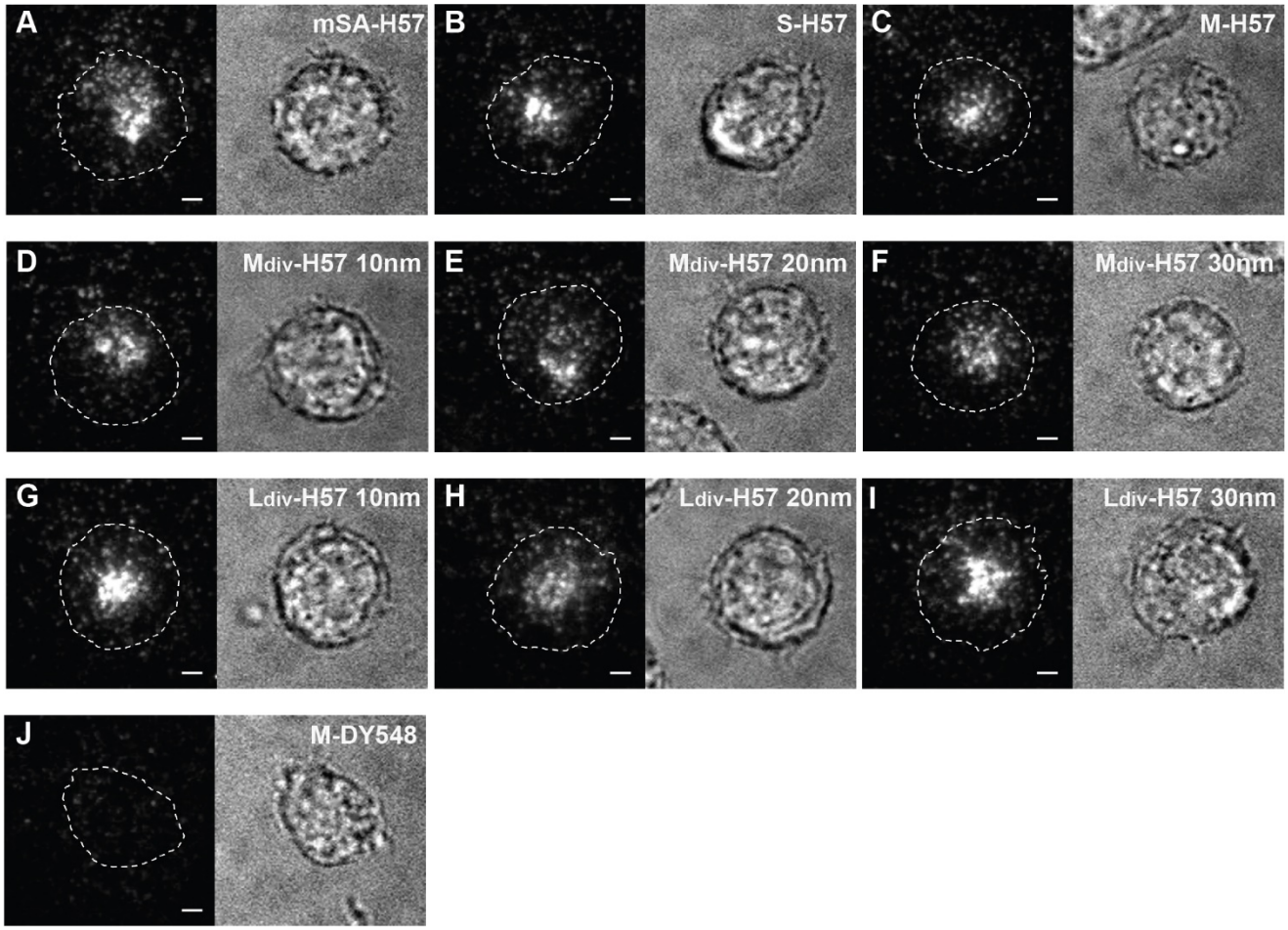

Fig. S16. H57-scF$_V$ organization at the T-cell-SLB interface. Representative TIRF images showing the organization of AF555-labeled H57-scF$_V$ at densities of ~4 μm^{-2} for the different constructs. The cell outline was derived via brightfield images and is indicated by a dashed white contour line. Images were recorded 10 min after cell seeding. All constructs bearing H57-scF$_V$ (mSA-H57*(A),* S-H57*(B),* M-H57*(C)*, M$_{div}$-H57$_{10nm}$ *(D),* M$_{div}$-H57$_{20nm}$ *(E)* M$_{div}$-H57$_{30nm}$ *(F),* L$_{div}$-H57$_{10nm}$ *(G)*, L$_{div}$-H57$_{20nm}$ *(H)*, L$_{div}$-H57$_{30nm}$ *(I)*) showed characteristic microcluster formation. For ligand-free DNA origami platforms visualized by labeling with a DY548-oligo (M-DY548 *(J)*) no microcluster formation could be observed. Scale bar, 2μm.

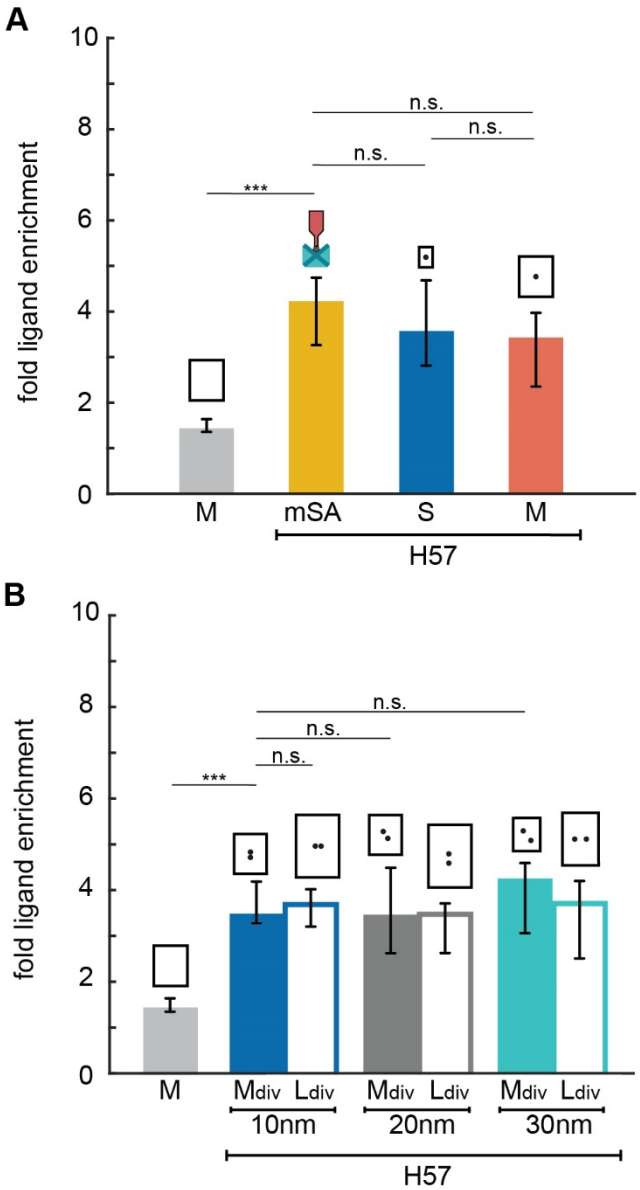

Fig. S17. H57-scF$_V$ enrichment at the T-cell – SLB interface. Images shown in Fig. S16 were analyzed and the relative enrichment of ligands at cell contacts is shown for monovalent *(A)* and divalent constructs *(B)*. For each construct, data are the median of n ≥15 cells from at least two independent experiments and two different mice. Error bars indicate the 95% CI. *** $p < 0.001$. A matrix containing the results of significance tests for all combinations of ligands is shown in Dataset S3.

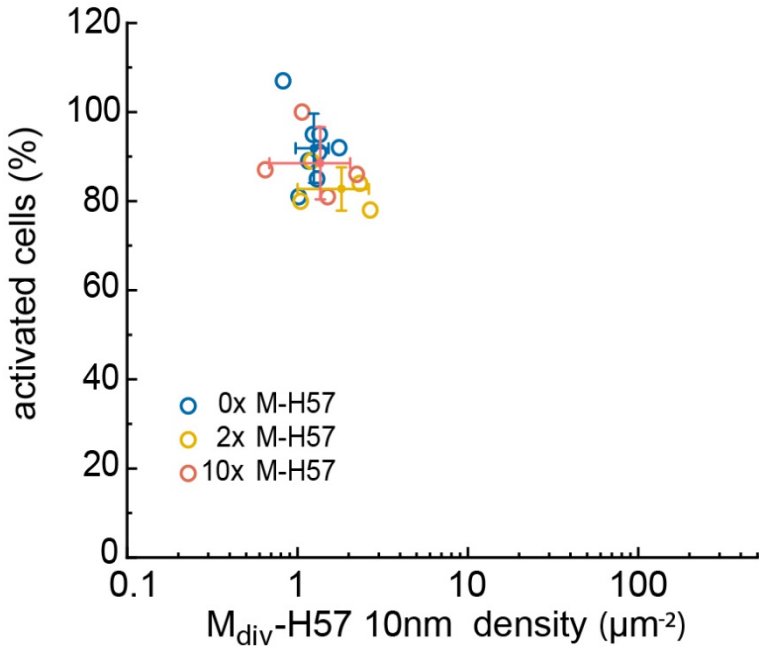

Fig. S18. On biointerfaces featuring M_{div}-$H57_{10nm}$, only DNA origami platforms functionalized with two scF$_V$s contribute to signaling. A 2- or 10-fold molar excess of M-H57 was added at surface densities of M_{div}-$H57_{10nm}$ between ~0.5 and 2 μm^{-2} and the percentage of activated cells was determined. For each condition, the median (± SD) is indicated. Note that addition of a 10-fold molar excess of M-57 also leads to an increased displacement of ICAM and B7 from the T-cell – SLB interface, which did, however, not affect activation thresholds of M_{div}-$H57_{10nm}$. Data are from at least two independent experiments and two different mice.

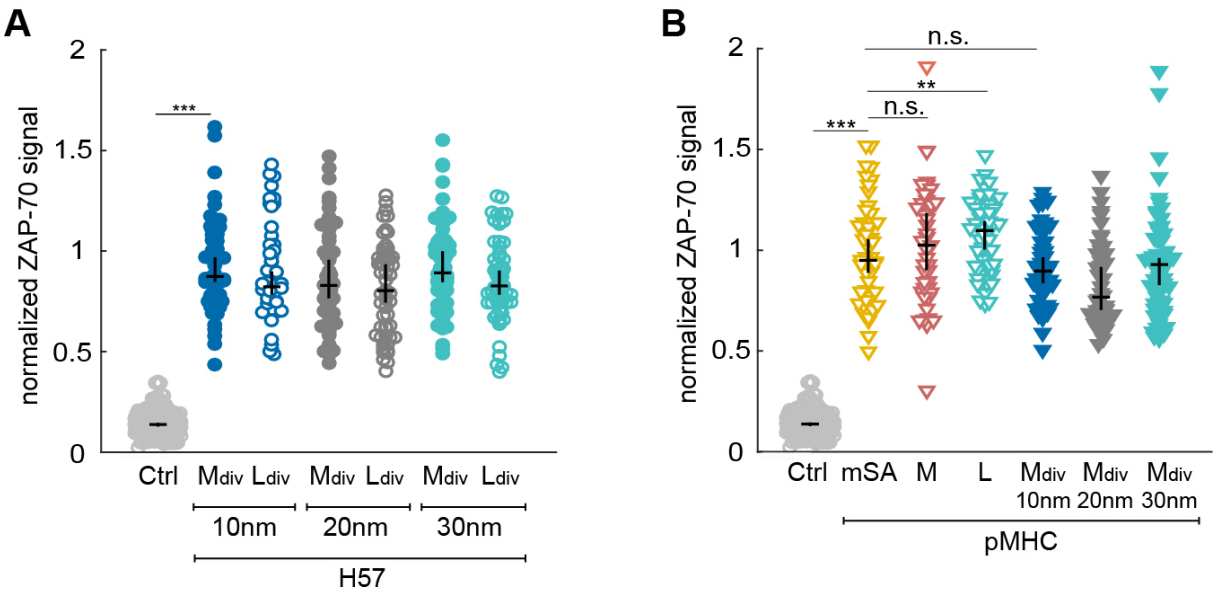

Fig. S19. ZAP-70 recruitment to the TCR. Immunostaining of ZAP-70 recruited to the T-cell plasma membrane on biointerfaces featuring divalent H57-scF$_V$ DNA origami platforms *(A)* or pMHC constructs *(B)*. Ligand densities were ~50 μm^{-2} for all constructs. T-cells were fixed 10 min after cell seeding and immunostained for ZAP-70. For each construct, the TIRF signal of the cells was analyzed and normalized using a positive control containing His-tagged ICAM-1 and B7-1 at 100 μm^{-2}, and His-pMHC at 150 μm^{-2}. Cells on bilayers containing only ICAM-1 and B7-1 at 100 μm^{-2} are shown as a negative control (Ctrl). Medians and their 95% CIs are shown. Each data point represents a single cell, $n > 45$ cells from at least two independent experiments and two different mice. *** for $p < 0.001$, ** for $p < 0.005$. A matrix containing the results of significance tests for all combinations of ligands is shown in Dataset S2.

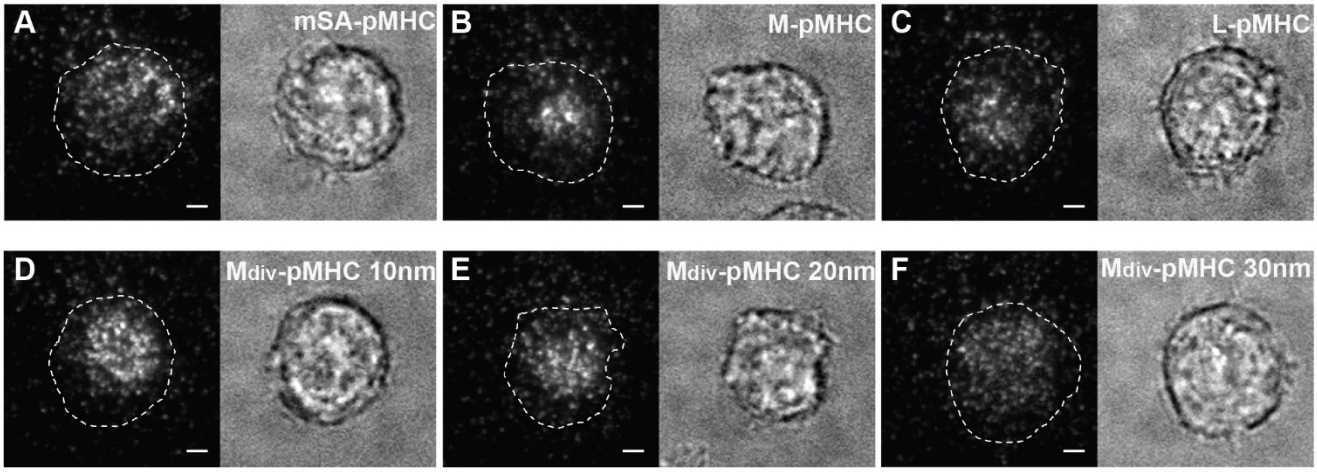

Fig. S20. pMHC organization at the T-cell-SLB interface. Representative TIRF images showing the organization of pMHC (labeled with AF555) at densities of ~4 μm^{-2} for the different constructs. The cell outline was derived via brightfield images and is indicated by a dashed white contour line. Images were recorded 10 min after cell seeding. All constructs bearing pMHC (mSA-pMHC *(A)*, M-pMHC *(B)*, L-pMHC *(C)*, M_{div}-$pMHC_{10nm}$ *(D)*, M_{div}-$pMHC_{20nm}$ *(E)*, M_{div}-$pMHC_{30nm}$ *(F)*) showed characteristic microcluster formation. Scale bar, 2µm.

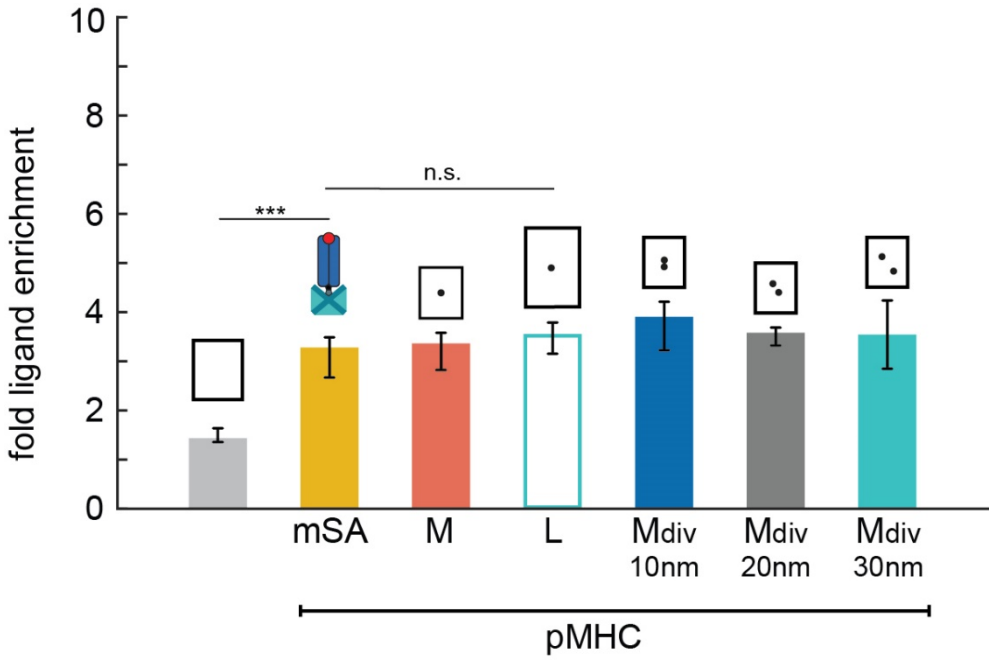

Fig. S21. pMHC enrichment at the T-cell – SLB interface. Images shown in Fig. S20 were analyzed and the relative enrichment of ligands at cell contacts is shown. For each construct, data are the median of n ≥15 cells from at least two independent experiments and two different mice. Error bars indicate the 95% CI. *** for $p < 0.001$. A matrix containing the results of significance tests for all combinations of ligands is shown in Dataset S3.

Table S1. Diffusion coefficients of H57-scF$_V$ constructs on SLBs. Single-molecule trajectories of TCR ligand constructs were recorded on SLBs, pooled and diffusion coefficients were determined by mean square displacement analysis. DNA origami without modification were stained with YOYO. Diffusion coefficients are given as mean ± s.e.m.. Data are from at least two independent experiments.

layout	ligand	D (μm^2/s)	trajectories (n)
S	-	0.257 ± 0.046	19,615
M	-	0.273 ± 0.063	32,582
L	-	0.236 ± 0.040	46,441
mSA	H57-scF$_V$	0.548 ± 0.021	10,070
DNA	H57-scF$_V$	0.454 ± 0.009	2,218
mSA	pMHC	0.562 ± 0.018	5,582
S	H57-scF$_V$	0.284 ± 0.057	20,474
M	H57-scF$_V$	0.239 ± 0.010	10,047
L	H57-scF$_V$	0.322 ± 0.028	6,561
M	pMHC	0.288 ± 0.011	8,482
L	pMHC	0.264 ± 0.010	2,420
M$_{div}$ 10nm	H57-scF$_V$	0.238 ± 0.009	17,187
M$_{div}$ 20nm	H57-scF$_V$	0.305 ± 0.008	6,090
M$_{div}$ 30nm	H57-scF$_V$	0.233 ± 0.002	11,418
M$_{div}$ 10nm	pMHC	0.261 ± 0.023	9,959
M$_{div}$ 20nm	pMHC	0.256 ± 0.011	10,770
M$_{div}$ 30nm	pMHC	0.241 ± 0.059	4,653
L$_{div}$ 10nm	H57-scF$_V$	0.287 ± 0.017	22,094
L$_{div}$ 20nm	H57-scF$_V$	0.261 ± 0.033	5,000
L$_{div}$ 30nm	H57-scF$_V$	0.249 ± 0.045	9,746

Table S2. Ligand occupancies of DNA origami platforms. Efficiency of functionalization with TCR ligands was determined via sequential two-color colocalization experiments as described in the Methods section. Data are the mean of at least two independent experiments (± SEM).

layout	ligand	no ligand (%)	single occupancy (%)	double occupancy (%)
S	H57-scF$_V$	58.7 ± 1.1	41.3 ± 1.1	n.a.
M	H57-scF$_V$	40.0 ± 0.7	60.0 ± 0.7	n.a.
L	H57-scF$_V$	39.0 ± 1.0	61.0 ± 1.0	n.a.
M	pMHC	39.8 ± 1.2	60.2 ± 1.2	n.a.
L	pMHC	39.5 ± 1.4	60.5 ± 1.4	n.a.
M$_{div}$ 10nm	H57-scF$_V$	23.7 ± 1.3	42.7 ± 2.4	33.6 ± 2.4
M$_{div}$ 20nm	H57-scF$_V$	19.4 ± 1.1	46.5 ± 0.9	34.1 ± 0.9
M$_{div}$ 30nm	H57-scF$_V$	16.3 ± 1.8	46.5 ± 3.5	37.2 ± 3.5
M$_{div}$ 10nm	pMHC	28.6 ± 1.5	38.4 ± 4.7	33.0 ± 4.7
M$_{div}$ 20nm	pMHC	33.7 ± 1.0	28.8 ± 0.6	37.5 ± 0.6
M$_{div}$ 30nm	pMHC	26.0 ± 1.3	38.7 ± 3.3	35.3 ± 3.3
L$_{div}$ 10nm	H57-scF$_V$	13.9 ± 1.4	48.3 ± 2.2	37.8 ± 2.2
L$_{div}$ 20nm	H57-scF$_V$	28.7 ± 2.9	37.9 ± 2.3	33.4 ± 2.3
L$_{div}$ 30nm	H57-scF$_V$	12.7 ± 2.6	44.4 ± 2.6	42.9 ± 2.6

Table S3. Fitting parameters of fits to dose-response curves. Dose-response curves recorded for the different constructs featuring one (mSA, DNA, S, M, L) or two (M_{div}, L_{div}) TCR ligands (H57-scF$_V$s, pMHCs) were fitted with Eq. 6 to extract the activation threshold T_A, the maximum response A_{max} and the Hill coefficient n. The 95% confidence intervals are shown. The mean number of cells per region (± SEM) and the number of animals used to generate dose-response curves are shown. *For fitting of data recorded for M-H57 at 24°C, A_{max} was fixed at 100%.

layout	ligand	modification	T (°C)	n	n_{low}	n_{high}	A_{max} (%)	$A_{max, low}$	$A_{max, high}$	T_A (μm^{-2})	$T_{A, low}$	$T_{A, high}$	# cells	# animals
mSA	H57-scF$_V$	-	24	2.20	0.46	3.94	103.39	95.38	111.40	1.42	0.91	2.20	229 ± 40	4
DNA	H57-scF$_V$	-	24	2.95	1.51	4.40	97.42	89.91	104.93	1.57	1.36	1.82	350 ± 54	3
S	H57-scF$_V$	-	24	1.32	0.71	1.92	98.19	87.53	108.84	0.79	0.55	1.14	288 ± 66	4
M	H57-scF$_V$	-	24	2.41	-0.38	5.20	100.00*	100.00	100.00	271.76	232.61	317.50	188 ± 55	5
M_{div} 10nm	H57-scF$_V$	-	24	2.32	1.57	3.07	100.08	93.90	106.26	0.98	0.86	1.11	212 ± 46	4
M_{div} 20nm	H57-scF$_V$	-	24	2.66	1.63	3.69	102.99	97.02	108.96	2.17	1.67	2.82	206 ± 35	3
M_{div} 30nm	H57-scF$_V$	-	24	4.26	-0.10	8.62	108.41	80.20	136.61	18.75	14.11	24.91	190 ± 56	4
L_{div} 10nm	H57-scF$_V$	-	24	1.90	1.31	2.50	101.24	94.17	108.31	2.16	1.79	2.60	362 ± 63	4
L_{div} 20nm	H57-scF$_V$	-	24	5.76	2.15	9.38	96.84	89.82	103.87	2.63	2.36	2.94	195 ± 36	3
L_{div} 30nm	H57-scF$_V$	-	24	8.59	2.19	14.99	98.28	91.51	105.06	16.07	14.20	18.19	367 ± 52	4
mSA	pMHC	-	24	2.48	1.17	3.79	95.07	88.52	101.61	0.73	0.57	0.94	208 ± 39	3
M	pMHC	-	24	2.90	1.78	4.03	91.55	84.98	98.13	0.78	0.63	0.95	204 ± 53	3
L	pMHC	-	24	2.57	1.48	3.67	105.61	95.25	115.98	0.91	0.78	1.07	208 ± 32	3
M_{div} 10nm	pMHC	-	24	1.65	1.31	1.99	100.46	94.94	105.98	2.11	1.83	2.44	187 ± 44	3
M_{div} 20nm	pMHC	-	24	6.18	3.03	9.34	95.88	90.88	100.89	1.14	1.05	1.23	191 ± 55	3
M_{div} 30nm	pMHC	-	24	4.21	2.71	5.72	99.53	93.48	105.57	1.00	0.90	1.13	301 ± 73	3
mSA	H57-scF$_V$	-	37	1.20	0.58	1.82	98.46	89.29	107.63	0.30	0.18	0.51	147 ± 40	3
M	H57-scF$_V$	-	37	2.26	1.27	3.25	91.27	82.96	99.57	4.07	3.31	5.01	174 ± 49	2
L	H57-scF$_V$	-	37	3.39	0.76	6.01	97.75	87.15	108.36	6.12	4.82	7.77	243 ± 66	2
M_{div} 10nm	H57-scF$_V$	-	37	1.61	0.70	2.52	101.63	90.30	112.95	0.36	0.24	0.54	198 ± 58	2
mSA	pMHC	-	37	1.76	0.07	3.46	94.93	74.85	115.02	0.32	0.19	0.52	157 ± 40	2
M	pMHC	-	37	1.69	0.05	3.34	90.16	75.47	104.85	0.21	0.12	0.36	184 ± 72	2
mSA	H57-scF$_V$	CD4-blocked	24	6.25	2.97	9.52	102.94	97.55	108.32	2.14	1.92	2.39	177 ± 36	4
mSA	pMHC	CD4-blocked	24	7.34	4.63	10.04	105.16	99.19	111.13	33.22	31.59	34.93	260 ± 47	4
M	pMHC	CD4-blocked	24	2.73	1.49	3.97	111.29	88.70	133.88	37.46	29.47	47.63	241 ± 53	4

Table S4. Minimum distances of ligands as permitted by DNA origami platforms. DNA origami platforms act as spacers that limit the approach of ligands to a minimum distance δ, representing the smallest possible distance between two individual ligands. Note that for divalent DNA origami featuring two ligands, δ is a fixed value. Here, **d** is the smallest possible distance between ligands on adjacent DNA origami platforms. Quasi-crystalline packing of DNA origami was assumed for all cases.

layout	δ (nm)	d (nm)
S	20	n.a.
M	48	n.a.
L	60	n.a.
M_{div} 10nm	10	38
M_{div} 20nm	20	38
M_{div} 30nm	30	40
L_{div} 10nm	10	60
L_{div} 20nm	20	62
L_{div} 30nm	30	40

Table S5. List of staple strands

DNA origami layout [nm]	Name	Sequence
30x20	7[56]-BLK	GAAAAACCAAGGGCGATCGCGAGATAGGGTTG
30x20	7[80]-BLK	CAGCCAGCTTTCCGGCCCATTCGCCATTCAGGCTGGCGAA
30x20	3[58]-BLK	GCATGCTCCACACAACAGAGACGGGCAACA
30x20	1[40]-BLK	TCTTTTCACCAGTTACGAGCCGGATGCCAGCT
30x20	3[72]-BLK	CGACTCTAGACGTTGTAAAACGACTTCGCTAT
30x20	0[55]-BLK	GCATTAATGAATCGGCCAACGCGCGTGGTTTT
30x20	0[79]-BLK	GTCGGGAAACCTGTCGAGCATAAA
30x20	5[58]-BLK	GGCCTCGGCCAGTGCCACCCCAGCAGGCGA
30x20	5[72]-BLK	TACGCCAGCTGCGCAACTGTTGGGGTCTATCATCGCACTC
65x54	10[63]-BLK	CAGCTTTCGGGCGCATCGTAACCGATCGGCCT
65x54	11[112]-BLK	AGCGCGAACAATCATAAGGGAACCCGGTGTAC
65x54	12[95]-BLK	ACACTCATAGGGGACGACGACAGTTGCATCTG
65x54	13[80]-BLK	CGCCAGCTAAAGACAGCATCGGAAGTCACCCT
65x54	14[63]-BLK	GCGATTAAGTACCGAGCTCGAATTAATTGTTA
65x54	15[112]-BLK	TTTCTTAACCGCTTTTGCGGGATCCGAGGGTA
65x54	16[95]-BLK	GTATCGGTGCTGTTTCCTGTGTGACGTAATCA
65x54	19[112]-BLK	TAGCAAGCCGATCTAAAGTTTTGTGTATGGGA
65x54	2[63]-BLK	TATTTTCATTAAGCAATAAAGCCTACATTATG
65x54	5[80]-BLK	CGGAGACATTCATCAGTTGAGATTATTACAGG
65x54	6[63]-BLK	TCAACCGTATCGATGAACGGTAATGGTTGATA
65x54	7[112]-BLK	TAATTTCACTAACGGAACAACATTTAGGAATA
65x54	8[95]-BLK	AGAACGAGCAATCATATGTACCCCCGTAAAAC
65x54	9[80]-BLK	CAAAAATAAAAGAGGACAGATGAAGAACTGAC
65x54	1[32]-BLK	TGATTCCCAAAAGGTGGCATCAATAATCATAC
65x54	17[32]-BLK	CATTAATTGGGCGCCAGGGTGGTTATTGCCCT
65x54	20[143]-BLK	AGGAGGTTTACCGTAACACTGAGTCATTCCAC
65x54	4[143]-BLK	TACCAGACCGGAATCGTCATAAATGTTCAGAA
65x54	0[143]-BLK	ACAGGTCAGGATTAGAGAGTACCTGAAGCCCG
65x54	0[47]-BLK	GCTCAACATGTTTTAAATATGCAAATAACAGT
65x54	0[63]-BLK	TGAATATAAGATTTAGTTTGACCATTTAGCTA
65x54	0[95]-BLK	TCATTTTTGCGGATGGCTTAGAGCTTAATTGC
65x54	1[112]-BLK	ATTAAGAGTTAATTGCTCCTTTTGATAAGAGG
65x54	1[128]-BLK	AAAGACTTTGACCATAAATCAAAAATAGCGTC
65x54	1[32]-BLK	TGATTCCCAAAAGGTGGCATCAATAATCATAC
65x54	1[80]-BLK	ATTTCGCAAGCAAAGCGGATTGCAGACTATTA
65x54	10[143]-BLK	TAGCCGGACCTTCATCAAGAGTAATCAACGTA
65x54	10[95]-BLK	CAACTTTGATTCGCGTCTGGCCTTAACGCCAT
65x54	11[32]-BLK	GGGATAGGTTTCCGGCACCGCTTCCATTCAGG
65x54	11[80]-BLK	CCAGTTTGCTTTGACCCCCAGCGAACACTAAA
65x54	12[143]-BLK	CTACGAAGATTTGTATCATCGCCTATGTTACT
65x54	12[47]-BLK	CAGCCAGCTCACGTTGGTGTAGATATCAACAT
65x54	12[63]-BLK	CAGGAAGATCGGTGCGGGCCTCTTGCTGCAAG
65x54	13[112]-BLK	GCAACGGCAAAGAGGCAAAAGAATTTATACCA
65x54	13[128]-BLK	CTTTGAGGTGCAGGGAGTTAAAGGACAGCTTG
65x54	13[32]-BLK	CTGCGCAAGTTTTCCCAGTCACGAATGCCTGC
65x54	14[143]-BLK	CTGAGGCTACTAAAGACTTTTTCATAATGCCA
65x54	14[95]-BLK	CAGCAGCGGGCGAAAGGGGGATGTCGCTATTA
65x54	15[32]-BLK	AGGTCGACACGAGCCGGAAGCATACTAACTCA
65x54	15[80]-BLK	TGGTCATATTATCAGCTTGCTTTCCTTTAATT

65x54	16[143]-BLK	TCACGTTGAGTTGCGCCGACAATGTTCGGTCG
65x54	16[47]-BLK	CACAACATTCTAGAGGATCCCCGGGTTGGGTA
65x54	16[63]-BLK	TCCGCTCACCGCTTTCCAGTCGGGGCCAACGC
65x54	17[112]-BLK	TTTTGCTAAGGCTCCAAAAGGAGCGAGGTGAA
65x54	17[128]-BLK	TCAACAGTCTCATAGTTAGCGTAACCAATAGG
65x54	17[32]-BLK	CATTAATTGGGCGCCAGGGTGGTTATTGCCCT
65x54	17[80]-BLK	CGTGCCAGAGTAAATGAATTTTCTCGTCTTTC
65x54	18[143]-BLK	AGACAGCCTTCAGCGGAGTGAGAATAATTTTT
65x54	18[63]-BLK	GCGGGGAGGCAGCAAGCGGTCCACTGATGGTG
65x54	18[95]-BLK	CAGACGTTCTGCATTAATGAATCGAAACCTGT
65x54	19[32]-BLK	TCACCGCCATAAATCAAAAGAATAGGAACAAG
65x54	19[80]-BLK	GCCCCAGCAGCCACCACCCTCATTGAACCGCC
65x54	2[143]-BLK	AACGAGAACAAATATCGCGTTTTAAAACTCCA
65x54	2[95]-BLK	TAGTCAGAAATGGTCAATAACCTGTTAGATAC
65x54	20[143]-BLK	AGGAGGTTTACCGTAACACTGAGTCATTCCAC
65x54	20[47]-BLK	AATCCCTTTGGCCCTGAGAGAGTTAGGCGGTT
65x54	20[63]-BLK	GTTCCGAATCCAACGTCAAAGGGCGAAAAACC
65x54	20[95]-BLK	ACCCTCAGAGGCGAAAATCCTGTTGCTGGTTT
65x54	21[112]-BLK	TTTTGCTCAGAACCGCCACCCTCATTCAGGGA
65x54	21[128]-BLK	GCGGATAAGTGCCGTCGAGAGGGTCCGTACTC
65x54	21[32]-BLK	AGTCCACTATTAAAGAACGTGGACATCGGCAA
65x54	21[80]-BLK	GTCTATCAAGAGAAGGATTAGGATTAGCGGGG
65x54	3[112]-BLK	TAGACTGGATCAGGTCTTTACCCTTCAAAAAG
65x54	3[32]-BLK	AGGCAAGGAGCCTTTATTTCAACGTTTAAATG
65x54	3[80]-BLK	AAAGCTAACAGAGGGGGTAATAGTGCAAAAGA
65x54	4[143]-BLK	TACCAGACCGGAATCGTCATAAATGTTCAGAA
65x54	4[47]-BLK	GCGGGAGACAAAGAATTAGCAAAATTTGGGGC
65x54	4[63]-BLK	ACCCTGTAAAGATTCAAAAGGGTGTATGATAT
65x54	4[95]-BLK	AGTTTTGCATCGGTTGTACCAAAACAGAGCAT
65x54	5[112]-BLK	CCACATTCATAGCGAGAGGCTTTTAAAATGTT
65x54	5[128]-BLK	CAGATACAACGTTAATAAAACGAAACTTTAAT
65x54	5[32]-BLK	CAATGCCTATGCCGGAGAGGGTAGTCATTGCC
65x54	6[143-BLK]	AAAAATCTTAACGCCAAAAGGAATCCCTCGTT
65x54	6[95]-BLK	TAGAAAGAGTCAAATCACCATCAAAGAAAGGC
65x54	7[32]-BLK	TGAGAGTCAGATTGTATAAGCAAAAATTCGCA
65x54	7[80]-BLK	TAGCATGTTAGTAAATTGGGCTTGGAAACACC
65x54	8[143-BLK]	ACAAAGCTATTACCTTATGCGATTTTGGGAAG
65x54	8[47]-BLK	AAACAGGATGGAGCAAACAAGAGATCTAGCTG
65x54	8[63]-BLK	ATCAGAAATTTTTTAACCAATAGGCCTGTAGC
65x54	9[112-BLK]	AGACCAGGAGGCTTGCCCTGACGAAGATGGTT
65x54	9[128-BLK]	CTGGCTGAACGAGGCGCAGACGGTACAAAGTA
65x54	9[32]-BLK	TTAAATTTAGCGAGTAACAACCCGACCGTAAT
100x70	0[111]-BLK	TAAATGAATTTTCTGTATGGGATTAATTTCTT
100x70	0[143]-BLK	TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA
100x70	0[175]-BLK	TCCACAGACAGCCCTCATAGTTAGCGTAACGA
100x70	0[207]-BLK	TCACCAGTACAAACTACAACGCCTAGTACCAG
100x70	0[239]-BLK	AGGAACCCATGTACCGTAACACTTGATATAA
100x70	0[271]-BLK	CCACCCTCATTTTCAGGGATAGCAACCGTACT
100x70	0[47]-BLK	AGAAAGGAACAACTAAAGGAATTCAAAAAAA
100x70	0[79]-BLK	ACAACTTTCAACAGTTTCAGCGGATGTATCGG

100x70	1[128]-BLK	TGACAACTCGCTGAGGCTTGCATTATACCAAGCGCGATGATAAA
100x70	1[160]-BLK	TTAGGATTGGCTGAGACTCCTCAATAACCGAT
100x70	1[192]-BLK	GCGGATAACCTATTATTCTGAAACAGACGATTGGCCTTGAAGAGCCAC
100x70	1[224]-BLK	GTATAGCAAACAGTTAATGCCCAATCCTCA
100x70	1[256]-BLK	CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCGGGAACCAG
100x70	1[32]-BLK	AGGCTCCAGAGGCTTTGAGGACACGGGTAA
100x70	1[64]-BLK	TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGAGGTCAATC
100x70	1[96]-BLK	AAACAGCTTTTTGCGGGATCGTCAACACTAAA
100x70	10[111]-BLK	TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT
100x70	10[143]-BLK	CCAACAGGAGCGAACCAGACCGGAGCCTTTAC
100x70	10[175]-BLK	TTAACGTCTAACATAAAAACAGGTAACGGA
100x70	10[207]-BLK	ATCCCAATGAGAATTAACTGAACAGTTACCAG
100x70	10[239]-BLK	GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA
100x70	10[271]-BLK	ACGCTAACACCCACAAGAATTGAAAATAGC
100x70	10[47]-BLK	CTGTAGCTTGACTATTATAGTCAGTTCATTGA
100x70	10[79]-BLK	GATGGCTTATCAAAAAGATTAAGAGCGTCC
100x70	11[128]-BLK	TTTGGGGATAGTAGTAGCATTAAAAGGCCG
100x70	11[160]-BLK	CCAATAGCTCATCGTAGGAATCATGGCATCAA
100x70	11[192]-BLK	TATCCGGTCTCATCGAGAACAAGCGACAAAAG
100x70	11[224]-BLK	GCGAACCTCCAAGAACGGGTATGACAATAA
100x70	11[256]-BLK	GCCTTAAACCAATCAATAATCGGCACGCGCCT
100x70	11[32]-BLK	AACAGTTTTGTACCAAAAACATTTTATTTC
100x70	11[64]-BLK	GATTTAGTCAATAAAGCCTCAGAGAACCCTCA
100x70	11[96]-BLK	AATGGTCAACAGGCAAGGCAAAGAGTAATGTG
100x70	12[111]-BLK	TAAATCATATAACCTGTTTAGCTAACCTTTAA
100x70	12[143]-BLK	TTCTACTACGCGAGCTGAAAAGGTTACCGCGC
100x70	12[175]-BLK	TTTTATTTAAGCAAATCAGATATTTTTTGT
100x70	12[207]-BLK	GTACCGCAATTCTAAGAACGCGAGTATTATTT
100x70	12[239]-BLK	CTTATCATTCCCGACTTGCGGGAGCCTAATTT
100x70	12[271]-BLK	TGTAGAAATCAAGATTAGTTGCTCTTACCA
100x70	12[47]-BLK	TAAATCGGGATTCCCAATTCTGCGATATAATG
100x70	12[79]-BLK	AAATTAAGTTGACCATTAGATACTTTTGCG
100x70	13[128]-BLK	GAGACAGCTAGCTGATAAATTAATTTTTGT
100x70	13[192]-BLK	GTAAAGTAATCGCCATATTTAACAAAACTTTT
100x70	13[224]-BLK	ACAACATGCCAACGCTCAACAGTCTTCTGA
100x70	13[256]-BLK	GTTTATCAATATGCGTTATACAAACCGACCGT
100x70	13[32]-BLK	AACGCAAAATCGATGAACGGTACCGGTTGA
100x70	13[64]-BLK	TATATTTTGTCATTGCCTGAGAGTGGAAGATT
100x70	13[96]-BLK	TAGGTAAACTATTTTTGAGAGATCAAACGTTA
100x70	14[111]-BLK	GAGGGTAGGATTCAAAAGGGTGAGACATCCAA
100x70	14[143]-BLK	CAACCGTTTCAAATCACCATCAATTCGAGCCA
100x70	14[175]-BLK	CATGTAATAGAATATAAAGTACCAAGCCGT
100x70	14[207]-BLK	AATTGAGAATTCTGTCCAGACGACTAAACCAA
100x70	14[239]-BLK	AGTATAAAGTTCAGCTAATGCAGATGTCTTTC
100x70	14[271]-BLK	TTAGTATCACAATAGATAAGTCCACGAGCA
100x70	14[47]-BLK	AACAAGAGGGATAAAAATTTTTAGCATAAAGC
100x70	14[79]-BLK	GCTATCAGAAATGCAATGCCTGAATTAGCA
100x70	15[128]-BLK	TAAATCAAAATAATTCGCGTCTCGGAAACCAGGCAAAGGGAAGG
100x70	15[160]-BLK	ATCGCAAGTATGTAAATGCTGATGATAGGAAC
100x70	15[192]-BLK	TCAAATATAACCTCCGGCTTAGGTAACAATTTCATTTGAAGGCGAATT

100x70	15[224]-BLK	CCTAAATCAAAATCATAGGTCTAAACAGTA
100x70	15[256]-BLK	GTGATAAAAAGACGCTGAGAAGAGATAACCTTGCTTCTGTTCGGGAGA
100x70	15[32]-BLK	TAATCAGCGGATTGACCGTAATCGTAACCG
100x70	15[64]-BLK	GTATAAGCCAACCCGTCGGATTCTGACGACAGTATCGGCCGCAAGGCG
100x70	15[96]-BLK	ATATTTTGGCTTTCATCAACATTATCCAGCCA
100x70	16[111]-BLK	TGTAGCCATTAAAATTCGCATTAAATGCCGGA
100x70	16[143]-BLK	GCCATCAAGCTCATTTTTTAACCACAAATCCA
100x70	16[175]-BLK	TATAACTAACAAAGAACGCGAGAACGCCAA
100x70	16[207]-BLK	ACCTTTTTATTTTAGTTAATTTCATAGGGCTT
100x70	16[239]-BLK	GAATTTATTTAATGGTTTGAAATATTCTTACC
100x70	16[271]-BLK	CTTAGATTTAAGGCGTTAAATAAAGCCTGT
100x70	16[47]-BLK	ACAAACGGAAAAGCCCCAAAAACACTGGAGCA
100x70	16[79]-BLK	GCGAGTAAAAATATTTAAATTGTTACAAAG
100x70	17[224]-BLK	CATAAATCTTTGAATACCAAGTGTTAGAAC
100x70	17[32]-BLK	TGCATCTTTCCCAGTCACGACGGCCTGCAG
100x70	17[96]-BLK	GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC
100x70	18[111]-BLK	TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC
100x70	18[143]-BLK	CAACTGTTGCGCCATTCGCCATTCAAACATCA
100x70	18[175]-BLK	CTGAGCAAAAATTAATTACATTTTGGGTTA
100x70	18[207]-BLK	CGCGCAGATTACCTTTTTTAATGGGAGAGACT
100x70	18[239]-BLK	CCTGATTGCAATATATGTGAGTGATCAATAGT
100x70	18[271]-BLK	CTTTTACAAAATCGTCGCTATTAGCGATAG
100x70	18[47]-BLK	CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA
100x70	18[79]-BLK	GATGTGCTTCAGGAAGATCGCACAATGTGA
100x70	19[160]-BLK	GCAATTCACATATTCCTGATTATCAAAGTGTA
100x70	19[224]-BLK	CTACCATAGTTTGAGTAACATTTAAAATAT
100x70	19[32]-BLK	GTCGACTTCGGCCAACGCGCGGGGTTTTTC
100x70	19[96]-BLK	CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC
100x70	2[111]-BLK	AAGGCCGCTGATACCGATAGTTGCGACGTTAG
100x70	2[143]-BLK	ATATTCGGAACCATCGCCCACGCAGAGAAGGA
100x70	2[175]-BLK	TATTAAGAAGCGGGGTTTTGCTCGTAGCAT
100x70	2[207]-BLK	TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG
100x70	2[239]-BLK	GCCCGTATCCGGAATAGGTGTATCAGCCCAAT
100x70	2[271]-BLK	GTTTTAACTTAGTACCGCCACCCAGAGCCA
100x70	2[47]-BLK	ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT
100x70	2[79]-BLK	CAGCGAAACTTGCTTTCGAGGTGTTGCTAA
100x70	20[111]-BLK	CACATTAAAATTGTTATCCGCTCATGCGGGCC
100x70	20[143]-BLK	AAGCCTGGTACGAGCCGGAAGCATAGATGATG
100x70	20[175]-BLK	ATTATCATTCAATATAATCCTGACAATTAC
100x70	20[207]-BLK	GCGGAACATCTGAATAATGGAAGGTACAAAAT
100x70	20[239]-BLK	ATTTTAAAATCAAAATTATTTGCACGGATTCG
100x70	20[271]-BLK	CTCGTATTAGAAATTGCGTAGATACAGTAC
100x70	20[47]-BLK	TTAATGAACTAGAGGATCCCCGGGGGGTAACG
100x70	20[79]-BLK	TTCCAGTCGTAATCATGGTCATAAAAGGGG
100x70	21[120]-BLK	CCCAGCAGGCGAAAAATCCCTTATAAATCAAGCCGGCG
100x70	21[160]-BLK	TCAATATCGAACCTCAAATATCAATTCCGAAA
100x70	21[184]-BLK	TCAACAGTTGAAAGGAGCAAATGAAAAATCTAGAGATAGA
100x70	21[224]-BLK	CTTTAGGGCCTGCAACAGTGCCAATACGTG
100x70	21[248]-BLK	AGATTAGAGCCGTCAAAAAACAGAGGTGAGGCCTATTAGT
100x70	21[32]-BLK	TTTTCACTCAAAGGGCGAAAAACCATCACC

100x70	21[56]-BLK	AGCTGATTGCCCTTCAGAGTCCACTATTAAAGGGTGCCGT
100x70	21[96]-BLK	AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC
100x70	22[111]-BLK	GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT
100x70	22[143]-BLK	TCGGCAAATCCTGTTTGATGGTGGACCCTCAA
100x70	22[175]-BLK	ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA
100x70	22[207]-BLK	AGCCAGCAATTGAGGAAGGTTATCATCATTTT
100x70	22[239]-BLK	TTAACACCAGCACTAACAACTAATCGTTATTA
100x70	22[271]-BLK	CAGAAGATTAGATAATACATTTGTCGACAA
100x70	22[47]-BLK	CTCCAACGCAGTGAGACGGGCAACCAGCTGCA
100x70	22[79]-BLK	TGGAACAACCGCCTGGCCCTGAGGCCCGCT
100x70	23[128]-BLK	AACGTGGCGAGAAAGGAAGGGAAACCAGTAA
100x70	23[160]-BLK	TAAAAGGGACATTCTGGCCAACAAAGCATC
100x70	23[192]-BLK	ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG
100x70	23[224]-BLK	GCACAGACAATATTTTTGAATGGGGTCAGTA
100x70	23[256]-BLK	CTTTAATGCGCGAACTGATAGCCCCACCAG
100x70	23[32]-BLK	CAAATCAAGTTTTTTGGGGTCGAAACGTGGA
100x70	23[64]-BLK	AAAGCACTAAATCGGAACCCTAATCCAGTT
100x70	23[96]-BLK	CCCGATTTAGAGCTTGACGGGGAAAAAGAATA
100x70	3[160]-BLK	TTGACAGGCCACCACCAGAGCCGCGATTTGTA
100x70	3[224]-BLK	TTAAAGCCAGAGCCGCCACCCTCGACAGAA
100x70	3[32]-BLK	AATACGTTTGAAAGAGGACAGACTGACCTT
100x70	3[96]-BLK	ACACTCATCCATGTTACTTAGCCGAAAGCTGC
100x70	4[111]-BLK	GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA
100x70	4[143]-BLK	TCATCGCCAACAAAGTACAACGGACGCCAGCA
100x70	4[175]-BLK	CACCAGAAAGGTTGAGGCAGGTCATGAAAG
100x70	4[207]-BLK	CCACCCTCTATTCACAAACAAATACCTGCCTA
100x70	4[239]-BLK	GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT
100x70	4[271]-BLK	AAATCACCTTCCAGTAAGCGTCAGTAATAA
100x70	4[47]-BLK	GACCAACTAATGCCACTACGAAGGGGGTAGCA
100x70	4[79]-BLK	GCGCAGACAAGAGGCAAAAGAATCCCTCAG
100x70	5[224]-BLK	TCAAGTTTCATTAAAGGTGAATATAAAAGA
100x70	5[32]-BLK	CATCAAGTAAAACGAACTAACGAGTTGAGA
100x70	5[96]-BLK	TCATTCAGATGCGATTTTAAGAACAGGCATAG
100x70	6[111]-BLK	ATTACCTTTGAATAAGGCTTGCCCAAATCCGC
100x70	6[143]-BLK	GATGGTTTGAACGAGTAGTAAATTTACCATTA
100x70	6[175]-BLK	CAGCAAAAGGAAACGTCACCAATGAGCCGC
100x70	6[207]-BLK	TCACCGACGCACCGTAATCAGTAGCAGAACCG
100x70	6[239]-BLK	GAAATTATTGCCTTTAGCGTCAGACCGGAACC
100x70	6[271]-BLK	ACCGATTGTCGGCATTTTCGGTCATAATCA
100x70	6[47]-BLK	TACGTTAAAGTAATCTTGACAAGAACCGAACT
100x70	6[79]-BLK	TTATACCACCAAATCAACGTAACGAACGAG
100x70	7[120]-BLK	CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGA
100x70	7[160]-BLK	TTATTACGAAGAACTGGCATGATTGCGAGAGG
100x70	7[184]-BLK	CGTAGAAAATACATACCGAGGAAACGCAATAAGAAGCGCA
100x70	7[224]-BLK	AACGCAAAGATAGCCGAACAAACCCTGAAC
100x70	7[248]-BLK	GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCA
100x70	7[32]-BLK	TTTAGGACAAATGCTTTAAACAATCAGGTC
100x70	7[56]-BLK	ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG
100x70	7[96]-BLK	TAAGAGCAAATGTTTAGACTGGATAGGAAGCC
100x70	8[111]-BLK	AATAGTAAACACTATCATAACCCTCATTGTGA

100x70	8[143]-BLK	CTTTTGCAGATAAAAACCAAAATAAAGACTCC
100x70	8[175]-BLK	ATACCCAACAGTATGTTAGCAAATTAGAGC
100x70	8[207]-BLK	AAGGAAACATAAAGGTGGCAACATTATCACCG
100x70	8[239]-BLK	AAGTAAGCAGACACCACGGAATAATATTGACG
100x70	8[271]-BLK	AATAGCTATCAATAGAAAATTCAACATTCA
100x70	8[47]-BLK	ATCCCCCTATACCACATTCAACTAGAAAAATC
100x70	8[79]-BLK	AATACTGCCCAAAAGGAATTACGTGGCTCA
100x70	9[128]-BLK	GCTTCAATCAGGATTAGAGAGTTATTTTCA
100x70	9[192]-BLK	TTAGACGGCCAAATAAGAAACGATAGAAGGCT
100x70	9[224]-BLK	AAAGTCACAAAATAAACAGCCAGCGTTTTA
100x70	9[256]-BLK	GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA
100x70	9[32]-BLK	TTTACCCCAACATGTTTTAAATTTCCATAT
100x70	9[64]-BLK	CGGATTGCAGAGCTTAATTGCTGAAACGAGTA
100x70	9[96]-BLK	CGAAAGACTTTGATAAGAGGTCATATTTCGCA

Table S6. List of elongated staple strands

DNA origami layout [nm]	Name	Sequence	Docking Sequence
30x20	Z'-4T-6[39]	AGAGTCCTAGCATATTTAGCCTTTTAGTGTTGTACGTGGACTCCAACGTCAAAGGGC	AGAGTCCTAGCATATTTAGCC
30x20	Z'-4T-3[88]	AGAGTCCTAGCATATTTAGCCTTTTCCGGGTACTTTCCTGTGTGAAATTCCTGGGGT	AGAGTCCTAGCATATTTAGCC
30x20	Z'-4T-1[88]	AGAGTCCTAGCATATTTAGCCTTTTGCCTAATGCACTGCCCGCTTTCCA	AGAGTCCTAGCATATTTAGCC
30x20	Z'-4T-2[39]	AGAGTCCTAGCATATTTAGCCTTTTGCTGATTGCAAGCGGTCCACGCTGGTTTGAGCTT	AGAGTCCTAGCATATTTAGCC
30x20	Z'-4T-5[88]	AGAGTCCTAGCATATTTAGCCTTTTAGGGGGATGGGTTTTCCCAGTCACGAGGATCC	AGAGTCCTAGCATATTTAGCC
30x20	Z'-4T-4[39]	AGAGTCCTAGCATATTTAGCCTTTTAAATCCTGTTATAAATCAAAAGAATAGCCGTGCG	AGAGTCCTAGCATATTTAGCC
30x20	1[72]-4T-V'	GTGTAAAGGTTATCCGCTCACAATCTGCAGGTTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
65x54	9[80]-4T-V'	CAAAAATAAAAGAGGACAGATGAAGAACTGACTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
65x54	16[95]-4T-V'	GTATCGGTGCTGTTTCCTGTGTGACGTAATCATTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
65x54	5[80]-4T-V'	CGGAGACATTCATCAGTTGAGATTATTACAGGTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
65x54	15[112]-4T-V'	TTTCTTAACCGCTTTTGCGGGATCCGAGGGTATTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
65x54	15[112]-4T-X'	TTTCTTAACCGCTTTTGCGGGATCCGAGGGTATTTTGCCAAGATAGACAGAAG	GCCAAGATAGACAGAAG
65x54	8[95]-4T-V'	AGAACGAGCAATCATATGTACCCCCGTAAAACTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
65x54	Z'-10[47]	AGAGTCCTAGCATATTTAGCCTTTTTAAATGTGTTGTTAAATCAGCTCAAGCCCCAA	AGAGTCCTAGCATATTTAGCC
65x54	Z'-11[128]	AGAGTCCTAGCATATTTAGCCTTTTCAACGGAGGCACCAACCTAAAACGTACAGAGG	AGAGTCCTAGCATATTTAGCC
65x54	Z'-14[47]	AGAGTCCTAGCATATTTAGCCTTTTACGCCAGGCTGTTGGGAAGGGCGATCGCACTC	AGAGTCCTAGCATATTTAGCC
65x54	Z'-15[128]	AGAGTCCTAGCATATTTAGCCTTTTATACCGATAAAATCTCCAAAAAAAAACAACTT	AGAGTCCTAGCATATTTAGCC
65x54	Z'-18[47]	AGAGTCCTAGCATATTTAGCCTTTTTGCGTATTGCGTTGCGCTCACTGCCAATTCCA	AGAGTCCTAGCATATTTAGCC
65x54	Z'-19[128]	AGAGTCCTAGCATATTTAGCCTTTTAACCCATGTAGTACCGCCACCCTCAGTACCAG	AGAGTCCTAGCATATTTAGCC
65x54	Z'-2[47]	AGAGTCCTAGCATATTTAGCCTTTTGCGAGCTGAATTCTGCGAACGAGTATGCTGTA	AGAGTCCTAGCATATTTAGCC
65x54	Z'-3[128]	AGAGTCCTAGCATATTTAGCCTTTTCAATACTGGACGATAAAAACCAAAAACTAATG	AGAGTCCTAGCATATTTAGCC
65x54	Z'-6[47]	AGAGTCCTAGCATATTTAGCCTTTTATAAATTAGAGTAATGTGTAGGTAATACTTTT	AGAGTCCTAGCATATTTAGCC
65x54	Z'-7[128]	AGAGTCCTAGCATATTTAGCCTTTTCATTGTGAGCTCATTCAGTGAATACGCATAGG	AGAGTCCTAGCATATTTAGCC
100x70	5[160]-4T-V'	GCAAGGCCTCACCAGTAGCACCATGGGCTTGATTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
100x70	9[160]-4T-V'	AGAGAGAAAAAAATGAAAATAGCAAGCAAACTTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
100x70	13[160]-4T-V'	GTAATAAGTTAGGCAGAGGCATTTATGATATTTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
100x70	17[160]-4T-V'	AGAAAACAAAGAAGATGATGAAACAGGCTGCGTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
100x70	12[111]-4T-V'	TAAATCATATAACCTGTTTAGCTAACCTTTAATTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
100x70	12[175]-4T-V'	TTTTATTTAAGCAAATCAGATATTTTTTGTTTTTGTGGAGTAGTGTCATGT	GTGGAGTAGTGTCATGT
100x70	18[63]-4T-Z'	ATTAAGTTTACCGAGCTCGAATTCGGGAAACCTGTCGTGCTTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC
100x70	4[63]-4T-Z'	ATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAATTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC
100x70	18[127]-4T-Z'	GCGATCGGCAATTCCACACAACAGGTGCCTAATGAGTGTTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC
100x70	4[127]-4T-Z'	TTGTGTCGTGACGAGAAACACCAAATTTCAACTTTAATTTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC
100x70	18[191]-4T-Z'	ATTCATTTTTGTTTGGATTATACTAAGAAACCACCAGAAGTTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC
100x70	4[191]-4T-Z'	CACCCTCAGAAACCATCGATAGCATTGAGCCATTTGGGAATTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC
100x70	18[255]-4T-Z'	AACAATAACGTAAAACAGAAATAAAAATCCTTTGCCCGAATTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC
100x70	4[255]-4T-Z'	AGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAATTTTAGAGTCCTAGCATATTTAGCC	AGAGTCCTAGCATATTTAGCC

Table S7. List of DNA-PAINT staple strands

DNA origami layout [nm]	Name	Sequence	DNA-PAINT Sequence
65x54	14[63]-6T-P1	GCGATTAAGTACCGAGCTCGAATTAATTGTTATTTTTT ATACATCTA	ATACATCTA
65x54	19[112]-6T-P1	TAGCAAGCCGATCTAAAGTTTTGTGTATGGGATTTTTT ATACATCTA	ATACATCTA
65x54	2[63]-6T-P1	TATTTTCATTAAGCAATAAAGCCTACATTATGTTTTTT ATACATCTA	ATACATCTA
65x54	7[112]-6T-P1	TAATTTCACTAACGGAACAACATTTAGGAATATTTTTT ATACATCTA	ATACATCTA
100x70	23[32]-6T-P1	CAAATCAAGTTTTTTGGGGTCGAAACGTGGATTTTTTATACATCTA	ATACATCTA
100x70	4[47]-6T-P1	GACCAACTAATGCCACTACGAAGGGGGTAGCATTTTTTATACATCTA	ATACATCTA
100x70	23[224]-6T-P1	GCACAGACAATATTTTTGAATGGGGTCAGTATTTTTTATACATCTA	ATACATCTA
100x70	4[239]-6T-P1	GCCTCCCTCAGAATGGAAAGCGCAGTAACAGTTTTTTTATACATCTA	ATACATCTA

Table S8. List of modified oligonucleotides

Designation	Sequence	Modification
Biotin-TEG-V	ACATGACACTACTCCAC	5'-Biotin-TEG
Biotin-TEG-V-4T-AS635P	ACATGACACTACTCCACTTTT	5'-Biotin-TEG; 3'- AS635P
Biotin-TEG-2T-P3*'	TTTCTTCATTA	5'-Biotin-TEG
Biotin-TEG-V-2T-P3*'	ACATGACACTACTCCAC TTTCTTCATTA	5'-Biotin-TEG
AF647-4T-X	TTTTCTTCTGTCTATCTTGGC	5'-AF647
DY549-4T-V	TTTTACATGACACTACTCCAC	5'-DY548
Z-TEG-Cholesterol	GGCTAAATATGCTAGGACTCT	3'-Cholesterol
Cholesterol-TEG-Z	GGCTAAATATGCTAGGACTCT	5'-Cholesterol
Z-TEG-Biotin	GGCTAAATATGCTAGGACTCT	3'-TEG-Biotin
Biotin-TEG-Z	GGCTAAATATGCTAGGACTCT	5'-TEG-Biotin
P1-Cy3b	CTAGATGTAT	3'-Cy3b
P3-Cy3b	GTAATGAAGA	3'-Cy3b
P3*'-2T-Z'-4T-TEG-Biotin	TCTTCATTATTAGAGTCCTAGCATATTTAGCCTTTT	3'TEG-Biotin

Table S9. DNA-PAINT imaging parameters for divalent DNA origami platforms. In divalent DNA origami platforms (M_{div}, L_{div}), biotinylated TCR ligands were replaced by biotinylated DNA-PAINT sequences (Biotin-TEG-2T-P3') and imaged with P3-Cy3B. Additionally, four staple strands at the corners were extended with P1' DNA-PAINT docking strands for barcoding and imaged with P1-Cy3B by Exchange-PAINT. DNA origami were immobilized on neutravidin-coated glass slides and imaged under following conditions:

Layout	Imager strand & Concentration (nM)	Frames (n)	Integration Time (ms)	Laser power density (W/cm^2)	Setup	Analyzed DNA origami (n)
M_{div} 10nm	8nm P1-Cy3B; 10nM P3-Cy3B	20,000; 20,000	150, 200	1,038	1	49
M_{div} 20nm	8nm P1-Cy3B; 10nM P3-Cy3B	15,000; 15,000	150, 200	1,038	2	49
M_{div} 30nm	8nm P1-Cy3B; 10nM P3-Cy3B	15,000; 15,000	150, 150	1,038	2	49
L_{div} 10nm	8nm P1-Cy3B; 10nM P3-Cy3B	20,000; 20,000	150, 200	1,038	2	100
L_{div} 20nm	8nm P1-Cy3B; 10nM P3-Cy3B	15,000; 15,000	150, 200	1,038	2	100
L_{div} 30nm	8nm P1-Cy3B; 10nM P3-Cy3B	15,000; 15,000	150, 150	1,038	2	100

Table S10. DNA-PAINT imaging parameters for T-cell experiments. For DNA-PAINT, biotinylated oligos were extended with P3' DNA-PAINT docking sites at their 3' end and imaged using Cy3B-labeled imager strands (P3-Cy3B). Additionally, four staple strands at the corners were extended with P1' DNA-PAINT docking strands for barcoding and imaged with P1-Cy3B by Exchange-PAINT to verify the integrity of DNA origami platforms upon T-cell interaction as well as to ensure that derived ligand positions could be allocated to the nanoplatforms. All experiments were performed using super-resolution microscope 1 under following conditions:

Construct	Imager strand concentration (nM)	Frames (n)	Integration Time (ms)	Laser power density (W/cm^2)	Analyzed cells (n)	Number of ligands (n)
DNA-H57	10 nM P3-Cy3B	15,000	200	1,038	n.a.	n.a.
M-H57	8 nM P1-Cy3B 10 nM P3-Cy3B	15,000 15,000	100 150	1,038	21	2,390
L-H57	8 nM P1-Cy3B 10 nM P3-Cy3B	15,000 15,000	100 100	1,038	17	1,270
M-647	8 nM P1-Cy3B 10 nM P3-Cy3B	15,000 15,000	100 150	1,038	22	627

Dataset S1. Results of significance tests of activation thresholds for all combinations of ligands.

Dataset S2. Results of significance tests of ligand enrichment for all combinations of ligands.

Dataset S3. Results of significance tests of ZAP-70 recruitment for all combinations of ligands.

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