Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information for

Dynamic blue light-switchable protein patterns on giant unilamellar vesicles

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Protein expression and purification

pQE-80L iLID (C530M) and pQE-80L MBP-SspB Nano were gifts from Brian Kuhlman (Addgene plasmids # 60408 and # 60409 respectively). pQE-80L iLID (C530M) expresses iLID with an N-terminal His6-tag and pQE-80L MBP-SspB Nano expresses Nano with N-terminal His6-MBP-TEV tag (His6-MBP-TEV-Nano). The mOrange-GGS was inserted into the pQE-80L MBP-SspB Nano plasmid after the BamH1 cutting site to yield His6-MBP-TEV-mOrange-Nano. The TEV cutting site was used cleave the His6-tag after Ni^{2+}-NTA affinity purification to yield Nano and mOrange-Nano. His6-tagged TEV protease originated from the Wombacher Lab and was kindly provided as a glycerol stock of *E. coli* BL21 (DE3) co-transformed with pLysS (chloramphenicol) and pET N_TEV234 (kanamycin) plasmids.

All proteins were recombinantly expressed in *E. coli* following a standard protocol. In short, 10 ml overnight cultures were inoculated into 1 l LB medium with the appropriate antibiotic and grown at 37 ^{o}C shaking at 200 rpm till the OD_{600} = 0.6-0.8. The protein expression was induced with 500 µM IPTG and the cultures were grown overnight at 16 ^{o}C (with an exception for the TEV protease, which was expressed at room temperature overnight). 1 l of harvested bacteria was resuspended in 20 ml of Buffer A (50 mM Tris, 300 mM NaCl, pH 7.4) with 100 mM PMSF (in methanol) and lysed by sonication. The lysate was separated by centrifugation and the proteins in the supernatant were purified using Ni^{2+}-NTA affinity columns with consequent gel filtration (HiLoad 16/600 Superdex 200 pg, GE Healthcare). For the purification of all proteins Buffer A with 250 mM Imidazole was used as elution buffer for the Ni^{2+}-NTA affinity columns and all proteins were stored in Buffer A. Only for the His6-TEV protease a different storage buffer (50 mM Tris, 400 mM NaCl, 2 mM EDTA, pH 7.4) was used. The purity of proteins was checked on custom-made 12% SDS-PAGE gels. The protein concentration was determined by UV-Vis spectral analysis.

To ensure that the protein recruitment to the GUV membrane is occurring due to the iLID/Nano binding and not because of the His6-tag-Ni^{2+}-NTA interaction,[1] the His6-tag was cut off from His6-MBP-TEV-Nano and His6-MBP-TEV-mOrange-Nano using His6-TEV protease. For this the purified proteins were incubated with His6-TEV protease at 1:50 concentration ratio (protein : TEV protease) overnight at 4 ^{o}C (reaction buffer: 50 mM Tris, 0.5 mM EDTA, 1 mM DTT, pH 8.0). Then, the His6-TEV protease and the cut off fragment His6-MBP were removed using a Ni^{2+}-NTA column. The mOrange-Nano and Nano proteins without the His6-tag were collected in the flow through.

GUV formation and functionalization with iLID

GUVs were prepared using the assisted gel formation method.[2] First 50 µL of polyvinyl alcohol (PVA, 145000 g/mol, 5% w/w in water + 100 mM sucrose) were dried as a thin layer on top of a 60 x 24 mm glass slide at 50 ^{o}C for 30 min. Then, 5 µL of a lipid solution (10 mg/ml 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) + 10 mol% 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG) + 0.1 mol%

1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid) succinyl] Ni^{2+} Salt (DGS-NTA-Ni^{2+}) + 1 mol% 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine (DiD) dye in chloroform, all lipids are purchased from Avanti Polar Lipids) were dried on the PVA layer at 30 ^{o}C or under vacuum for 1 hour. After this a chamber was built on top of the slide using silica grease as spacer and a second glass slide. The lipids were hydrated using ca. 1 ml of rehydration buffer (50 mM Tris, 100 mM NaCl, pH 7.4) for 1 hour at room temperature allowing GUV formation. The chamber was then inverted and GUVs were harvested. iLID was immobilized on the GUVs through the His-tag-Ni^{2+}-NTA interaction by adding iLID to a final concentration of 10 nM to 100 µL harvested GUVs in suspension and incubating for 30 min in the dark.

Protein recruitment to GUVs

For imaging the chamber was incubated with 3% bovine serum albumin (BSA w/v in water) for 20 min prior to the experiment to prevent protein adsorption and the bursting of the GUVs. The chamber was washed 3 times with buffer (50 mM Tris, 100 mM NaCl, pH 7.4). Afterwards, a solution of iLID functionalized GUVs mixed in a 1:1 ratio with a solution of 50 µM mOrange-Nano was transferred into the chamber.

The imaging was performed either on a Leica SP5 laser scanning confocal microscope (for all the experiments except for the patterning) or a Leica SP8 laser scanning confocal microscope with a FRAP (Fluorescence Recovery after Photobleaching) module (for the patterning experiments). An argon laser (488nm) was used to illuminate the samples with the blue light continuously and the TRITC channel was used to detect mOrange (excitation max: 557 nm; emission max: 576 nm). A HeNe laser was used to excite the DiD dye (excitation max: 644 nm; emission max: 665 nm) in the GUVs. There is DiD bleed through to the TRITC channel, however despite the bleaching of the DiD dye over time, the intensity of the GUV fluorescence increases under blue light illumination. Illumination with far-red light was done with a standard far-red LED lamp, 15 W, 700-800 nm.

The light power was measured using a LabMax-TOP meter with an OP-2 VIS power sensor (Coherent Inc.). The sensor was positioned over the microscope objective and

the laser power (Argon laser, 488 nm) was measured for different power percentages (1%, 2%, 5%, 10% and 20%) through the 63x water objective (Table S1).

Table S1. Light power at different laser power percentage.

Laser power percentage	**Mean power ± Standard deviation [nW]**
1%	70.8 ± 2.7
2%	151.3 ± 5.6
5%	575.5 ± 21
10%	1790 ± 71
20%	6330 ± 238

For the quantification of the protein recruitment to GUVs the mean fluorescence intensity of a whole GUV was analysed. The fluorescence intensity of the data point t=0 was subtracted from all other data points. All the data was then normalized by setting the maximum value to 100 and calculating the percentage of intensity change. The experiments were repeated multiple times, minimum 3 GUVs analysed in each case.

Effect of lipid fluidity on the protein recruitment

GUVs with a different fluidity were prepared to compare the dependence of protein recruitment on the lipid membrane fluidity (Fig. S4). To modulate the fluidity we added 20 mol% or 40 mol% to the used lipid preparation (10 mg/ml POPC + 10 mol% POPG + 0.1 mol% DGS-NTA-Ni^{2+} + 1 mol% DiD). Additionally, a 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) (10 mg/ml DOPC + 10 mol% POPG + 0.1 mol% DGS-NTA-Ni^{2+} + 1 mol% DiD) lipid preparation was used for the comparison.

The GUVs were prepared, hydrated with buffer (50 mM Tris, 100 mM NaCl, pH 7.4), harvested and functionalized with iLID protein (10 nM) as described above. After 30 min incubation in the dark 25 nM final concentration of mOrange-Nano was added to the chamber. The GUVs were illuminated with a 488 nm laser (Argon laser, 5%) through 63x water objective for 15 min, while continuously acquiring fluorescent

images for mOrange-Nano in TRITC channel (excitation max: 557 nm; emission max: 576 nm) and DID in Cy5 channel (excitation max: 644 nm; emission max: 665 nm) .

Protein recruitment to ROI

The protein recruitment to region of interest (ROI) was performed following the same protocol as in the protein recruitment to the whole GUV. In this case only a small ROI on the side of a GUV was constantly illuminated with blue light (488 nm, 5% power), while acquiring images at 1 image/s in the TRITC channel (excitation max: 557 nm; emission max: 576 nm) for up to 30 min.

Protein recruitment to a single GUV

For the protein recruitment to a single GUV experiments the GUVs (10 mg/ml 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) + 10 mol% 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG) + 0.1 mol% 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid) succinyl] (DGS-NTA) Ni^{2+} Salt + 1 mol% 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbo-cyanine (DiD) dye in chloroform, from Avanti Polar Lipids) were prepared, hydrated with buffer (50 mM Tris, 100 mM NaCl, pH 7.4) and harvested as described above. The GUVs were then incubated with 10 nM iLID for 30 min in the dark and afterwards 25 nM final concentration of mOrange-Nano was added to the chamber. The GUVs were allowed to settle and a region with several GUVs close but not touching each other was chosen. One of the GUVs in the field of view was illuminated with a 488 nm laser (Argon laser, 5%) through 63x water objective for 15 min, while the other GUVs were not illuminated. Fluorescent images for mOrange-Nano in TRITC channel (excitation max: 557 nm; emission max: 576 nm) and DID in Cy5 channel (excitation max: 644 nm; emission max: 665 nm) were acquired simultaneously for the whole field of view.

Patterning on GUV carpet

For the patterning experiments a layer of GUVs (4 mM total lipid concentration, POPC + 10% POGP + 1 % N-succinyldioctadecylamine with three NTA groups (trisNTA-Suc-DODA)[4]) was prepared as described above, leaving out the harvesting

step and working directly with the hydrated layer of GUVs in the hydration chamber. For the hydration step a modified buffer with lower salt concentrations was used to prevent the GUVs from bursting (10 mM Tris, 150 mM NaCl, pH 7.4). The GUVs were then incubated with 10 nM iLID for 30 min in the dark and afterwards 50 µM mOrange-Nano was flushed into the chamber. To create a pattern the FRAP-Module of the Leica SP8 laser scanning confocal microscope was used to define the ROI. The regions of interest were illuminated with a 496 nm laser for up to 1 min, and fluorescent images for mOrange-Nano were acquired simultaneously.

Protein recruitment to functionalized glass surfaces

The 20x20 mm glass surfaces were functionalized with Ni^{2+}-NTA terminated PEG as previously described.[3] Subsequently, each surface was incubated with 100 µl of 10 µM iLID, which can bind to the surface through His-tag-Ni^{2+}-NTA interaction, and briefly washed with Buffer A. The surfaces were incubated either in the dark or under blue light (488nm, light intensity of 0.63 mW/m^2) for 30 min with 1 µM mOrange-Nano solution, briefly washed with excess of Buffer A, and mounted on glass slides with 50 µL Mowiol 4-88. Fluorescence images were acquired in TRITC channel using an inverted fluorescence microscope (DMi8, Leica) through the 40x air objective. The mean fluorescence of each surface was then quantified with ImageJ and the results were statistically analysed for significance with the Mann-Whitney test (Fig. S1).

QCM-D

All experiments were performed on a Qsense Analyzer with flow modules for dark measurements and window modules for blue light experiments. Blue light was shone through the window module with a standard 15 W blue light LED lamp. The flow rate was kept at 250 µl/min throughout the experiments.

The following components in buffer(150 mM NaCl, 10 mM Tris, pH 7.4) were washed over a SiO_2 QCM-D crystal at the respective time points and excess proteins or chemicals was washed off with buffer after every step (indicated with b): 1) 10 mg/ml DOPC + 5 mol% DGS-NTA with 5 mM $CaCl_2$ to from a supported lipid bilayer,

2) 10 mM $NiCl_2$ for 5 min to load the NTA-groups with Ni^{2+}, 3) 1 ml of 1 µM iLID was flown over the sensor and the pump was stopped until the frequency stabilized. 1 ml of Nano (4) 250 nM, 5) 500 nM, 6) 1 µM and 7) 2 µM) was washed onto the sensor at increasing concentrations in a stepwise manner, where the pump was stopped and the next concentration was only added after the frequency was stable. Nano was washed away by flushing an excess of Buffer A. 8) 250 mM imidazole for 3 min to confirm that iLID specifically binds to the lipid bilayer through the His-tag-Ni^{2+}-NTA interaction.

To calculate thermodynamic and kinetic constants the thickness of each layer was calculated using changes in frequency and dissipation in QTools (Bioline Scientific Inc.) according to standard modelling protocol. The K_d was obtained by fitting the layer thickness at the respective Nano concentrations to a Hill-fit with one binding site. The k_{off} was computed from the wash off behaviour of Nano in QTools. The k_{on} was calculated from the relationship of K_d to k_{off}. The mean values were calculated from three technical replicates and the errors are given as the standard deviation.

References

1. E.P. Beers and J. Callis, *J. Biol. Chem*., 1993, **268**, 21645.
2. A. Weinberger, F.C. Tsai, G.H. Koenderink, T.F. Schmidt, R. Itri, W. Meier, T. Schmatko, A. Schröder and C. Marques. *Biophys J.,* 2013, **105**, 154.
3. F. C. Schenk, H. Boehm, J. P. Spatz and S. V. Wegner, *Langmuir*, 2014, **30**, 6897.
4. C. Grunwald, K. Schulze, A. Reichel, V. U. Weiss, D. Blaas, J. Piehler, K.-H. Wiesmülle and R. Tampé, *Proc. Natl. Acad. Sci.*, 2010, **107**, 14, 6146.

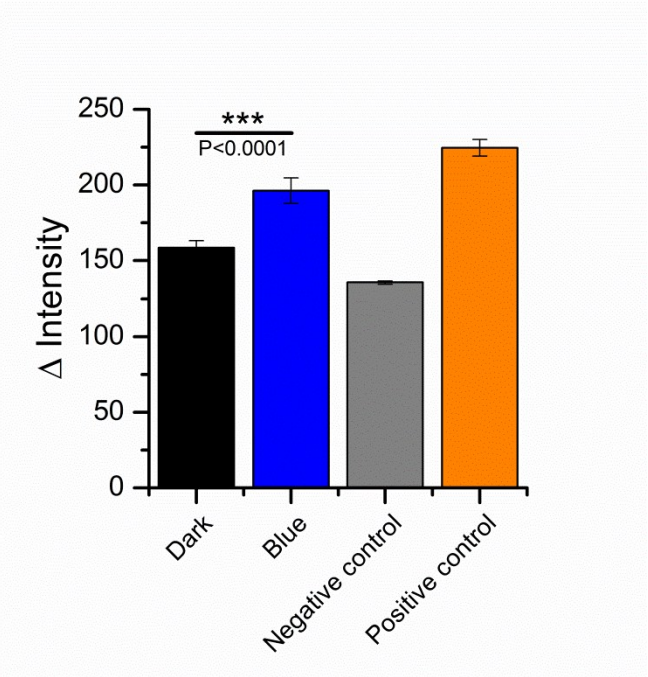

Fig. S1 Protein recruitment to iLID immobilized Ni^{2+}-NTA PEG functionalized glass surfaces under blue light and in the dark. Surfaces without immobilized iLID incubated with mOrange-Nano and mOrange-Nano with a His6-tag were used as negative and positive controls, respectively. A non-parametric Mann-Whitney test was performed to analyze the statistical difference. The error bars are the standard error from 3 technical replicates.

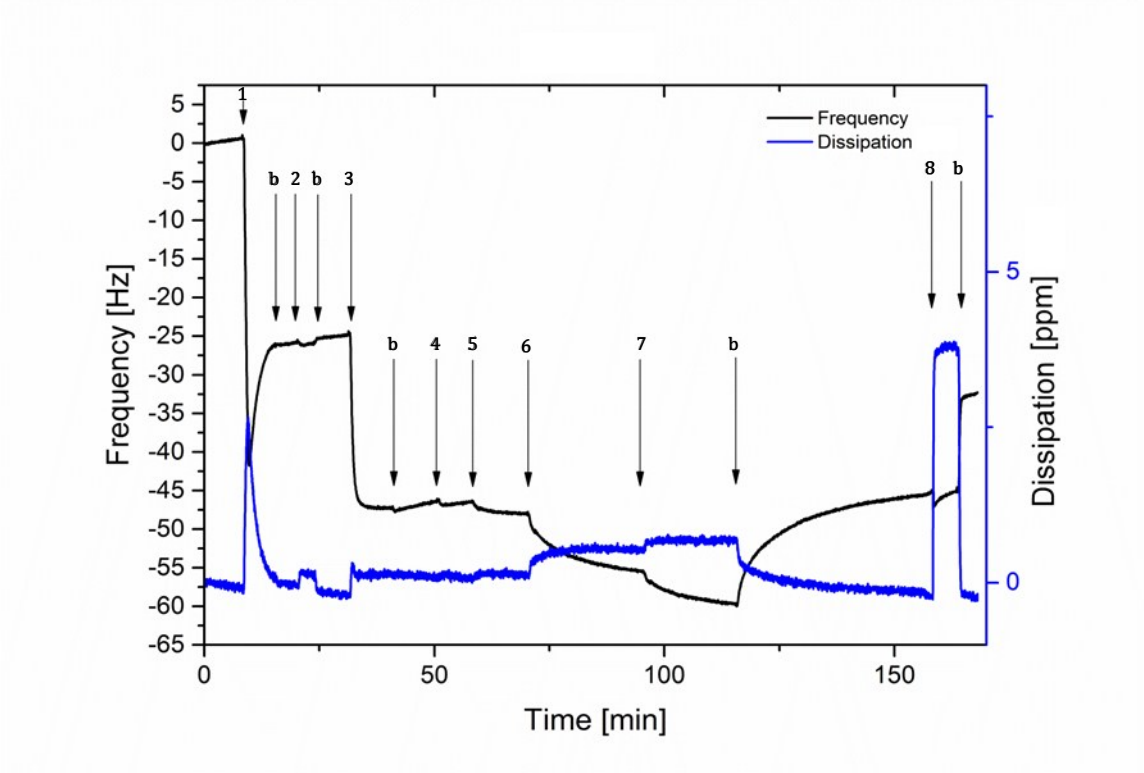

Fig. S2 Exemplary changes of frequency and dissipation (7 th overtone) in a QCM-D measurement. Arrows indicate the start of addition of the following components in buffer (150 mM NaCl, 10 mM Tris, pH 7.4). 1) 10 mg/ml Lipids (DOPC + 5 mol% DGS-NTA) with 5 mM $CaCl_2$, 2) 10 mM $NiCl_2$, 3) 1 µM iLID, 4) 250 nM Nano, 5) 500 nM Nano, 6) 1 µM Nano, 7) 2µM of Nano, 8) 250 mM imidazole. b indicates washing steps with buffer.

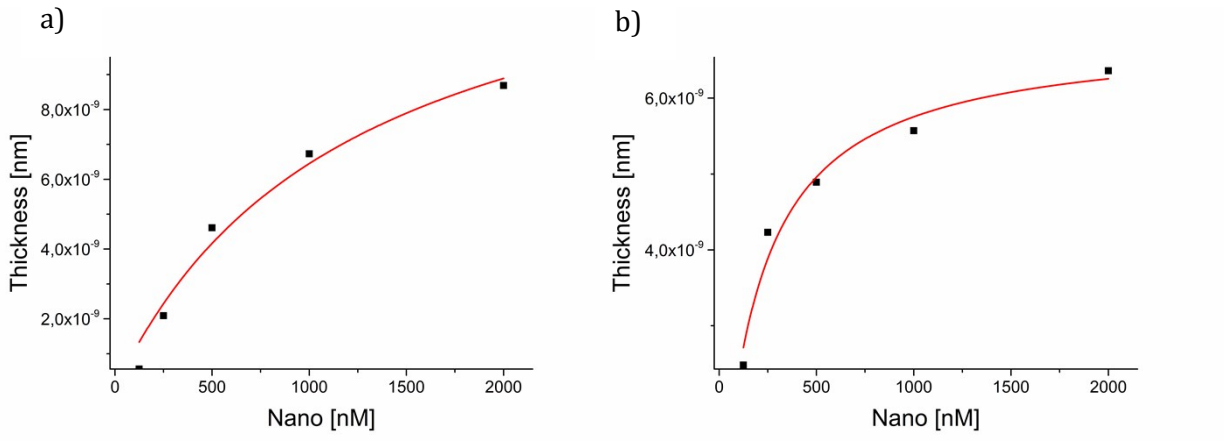

Fig. S3 Film thickness vs. Nano concentrations with Hill-Fit a) in dark and b) under blue light illumination.

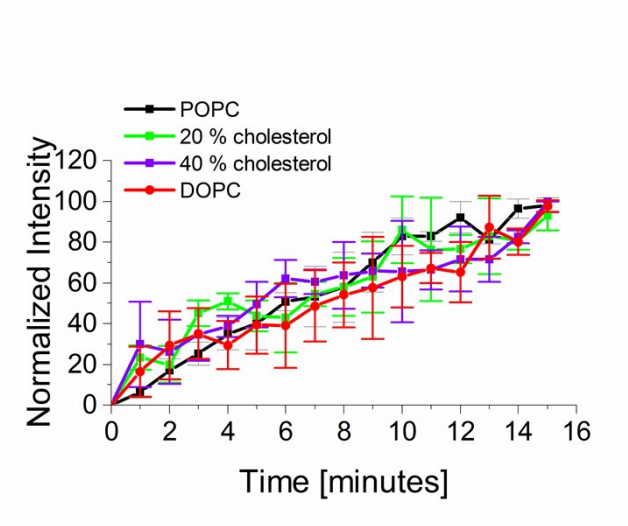

Fig. S4 Protein recruitment to GUVs with different lipid composition and membrane fluidity. POPC-GUVs (used in all other the protein recruitment experiments to GUVs) aew shown in black, POPC-GUVs with additional 20 mol% cholesterol are shown in green, POPC-GUVs with additional 40 mol% cholesterol are shown in purple and DOPC-GUVs are shown in red. All GUVs contain 0.1 mol% DGS-NTA to immobilize iLID on the GUVs. The error bars show standard deviation from 3 different experiments.

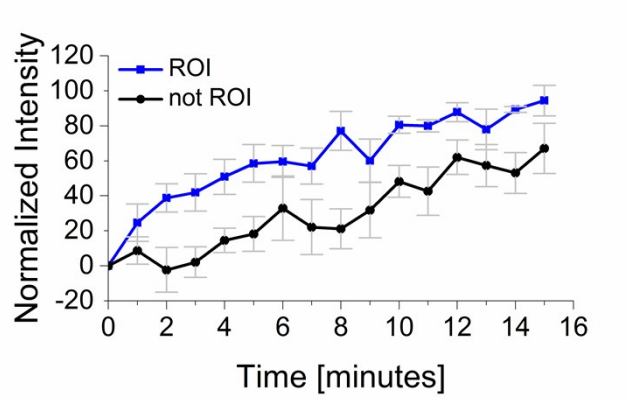

Fig. S5 Quantitative analysis of local recruitment of mOrange-Nano to the ROI (indicated in blue) and the opposite side of the GUV (indicated in black). The error bars show the standard error from 5 independent experiments.

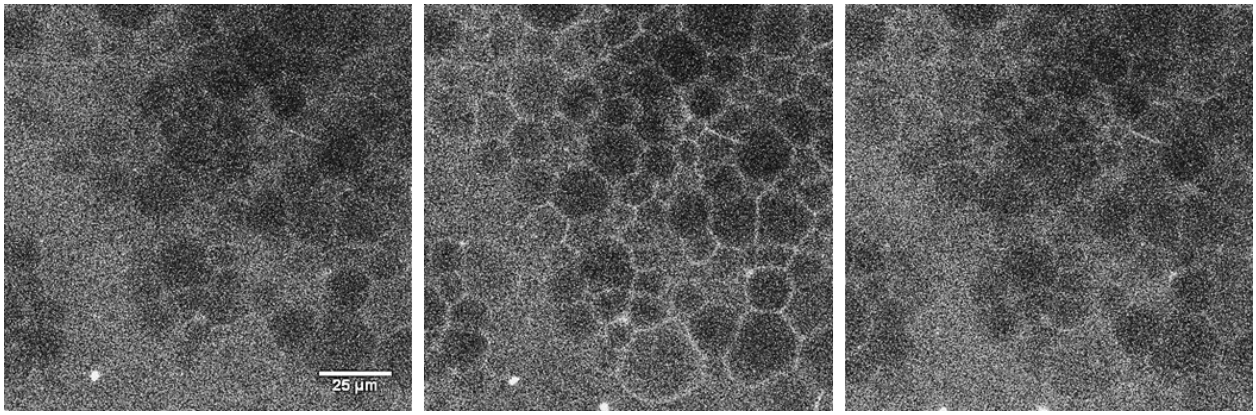

Fig. S6 Reversibility of patterns. GUV carpet a) before illumination, b) after blue light illumination for ca. 1 sec. and c) after approximately 3 minutes in the dark.

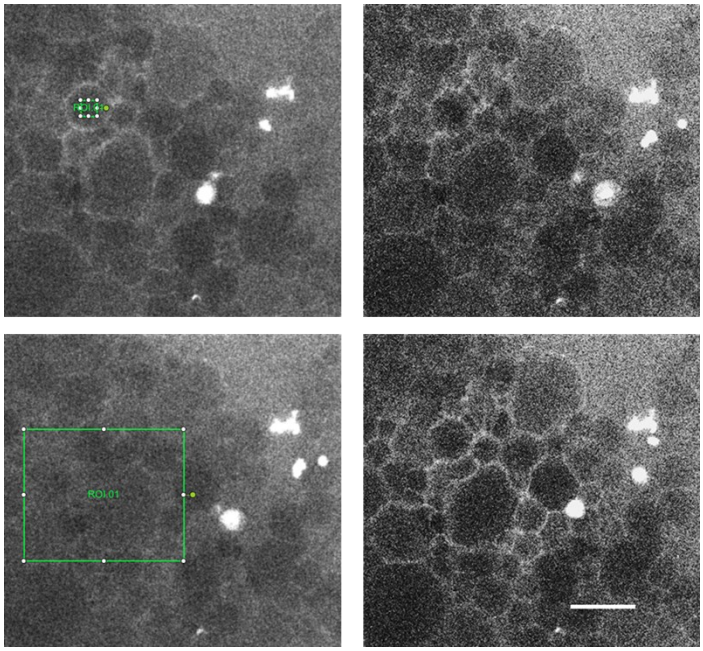

Fig. S7 Illumination of regions of interest in different sizes on a GUV carpet. Left panel indicates the area and size of illuminated region. Right panel shows the respective area with mOrange-Nano recruited to the GUV membrane. The scale bar is 25 µm.