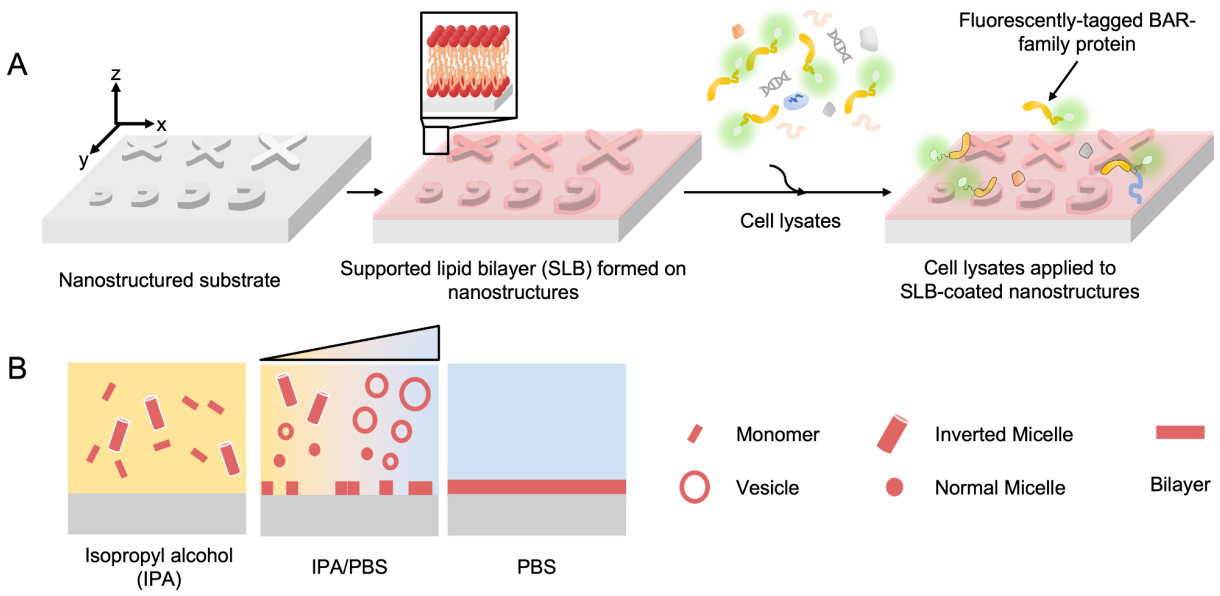
Electronic Supplementary Material (ESI) for Biomaterials Science.

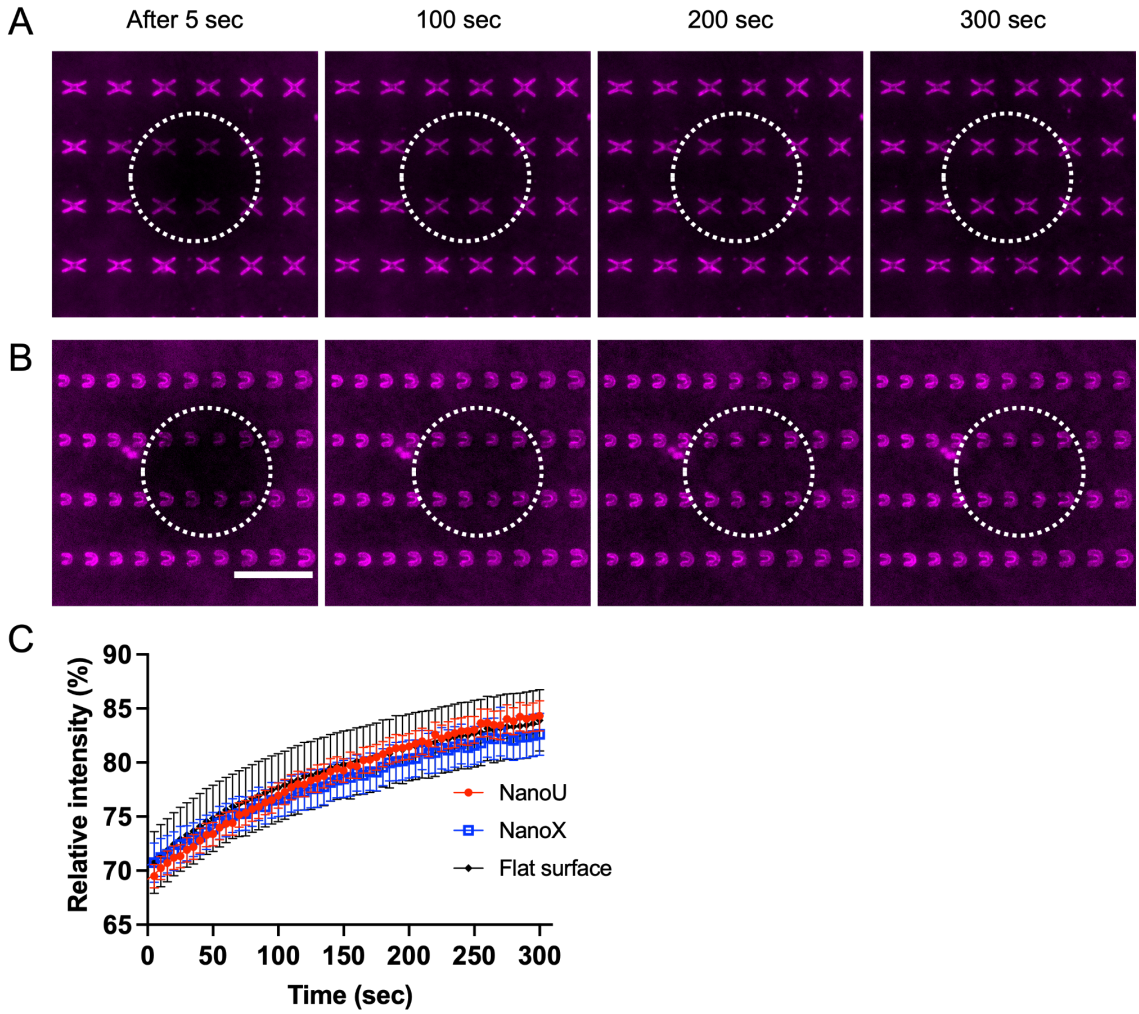
SUPPLEMENTARY INFORMATION for

A NanoCurvS platform for quantitative and multiplex analysis of curvature-sensing proteins

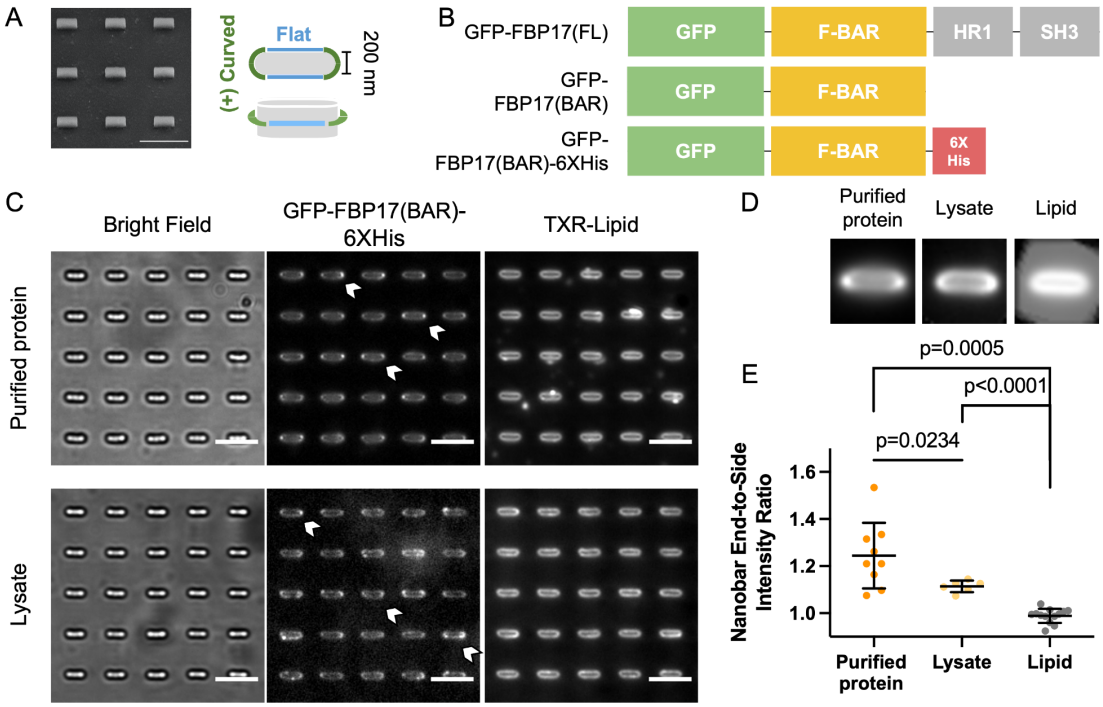


Supplementary Figure 1. Schematic illustrations of SLBs on nanostructures.

(A) Schematic illustrations of the NanoCurvS assay. **(B)** Illustrations of solvent-assisted lipid bilayer (SALB) formation method.

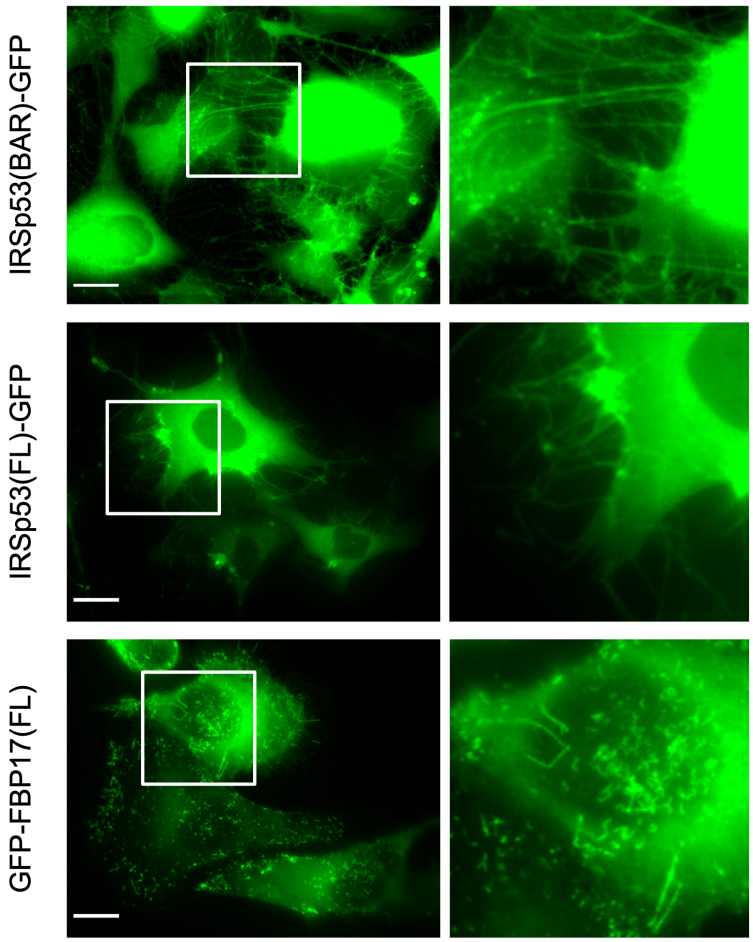


Supplementary Figure 2. SLBs on nanostructures show similar fluidities as surrounding flat areas. **(A)** Representative fluorescence images of lipid bilayers on a nanoX array at different time points after photobleaching. **(B)** Representative fluorescence images of lipid bilayers on a gradient nanoU array at different time points after photobleaching. For both (A) and (B), the bilayer consists of 70% DOPC, 15% DOPS, 15% PI(4,5)P_2 and ~1% Texas Red-DHPE. White-dashed circles indicate the bleached region. **(C)** A plot of the time-lapsed fluorescence recovery signals. Scale bar: 20 μm.

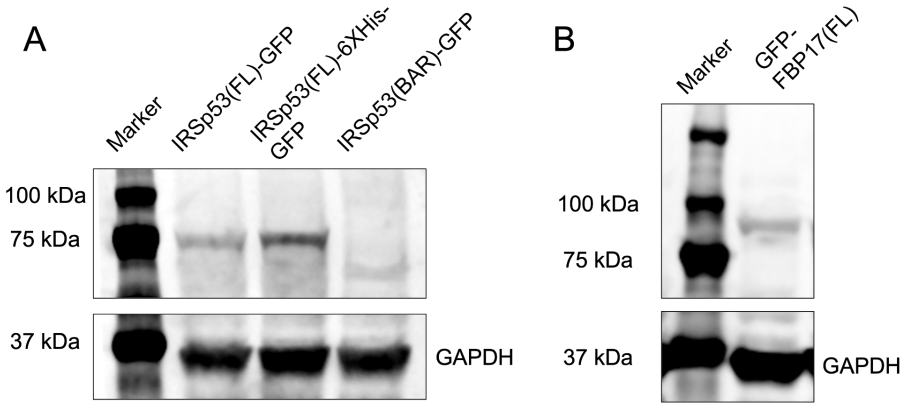


Supplementary Figure 3. Purified protein and lysates of FBP17 BAR show similar curvature sensitivity on nanobar arrays.

(A) Illustration and a SEM image of a 200-nm nanobar array. All nanobars are 200 nm in width, 1 μm in height and 5 μm in spacing. Scale bar: 5 μm. **(B)** Constructs of the GFP-FBP17 and GFP-FBP17(BAR)-6XHis variants used in this study. **(C)** Representative fluorescence images of GFP-FBP17(BAR)-6XHis purified protein and lysates on SLB-coated nanobar arrays. The lipid bilayer is made of 90% DOPC, 10% DGS-Ni-NTA and doped with ~1% Texas Red-DHPE for bilayer visualization. Scale bar: 5 μm. **(D)** Averaged images of GFP-FBP17(BAR)-6XHis purified protein and lysates on SLB-coated nanobar arrays. **(E)** Quantification of fluorescence intensity of GFP-FBP17(BAR)-6xHis purified protein and lysates on SLB-coated nanobar arrays. Error bars represent standard deviation. Welch's t tests (unpaired, two-tailed, not assuming equal variance) are applied for all statistical analyses.

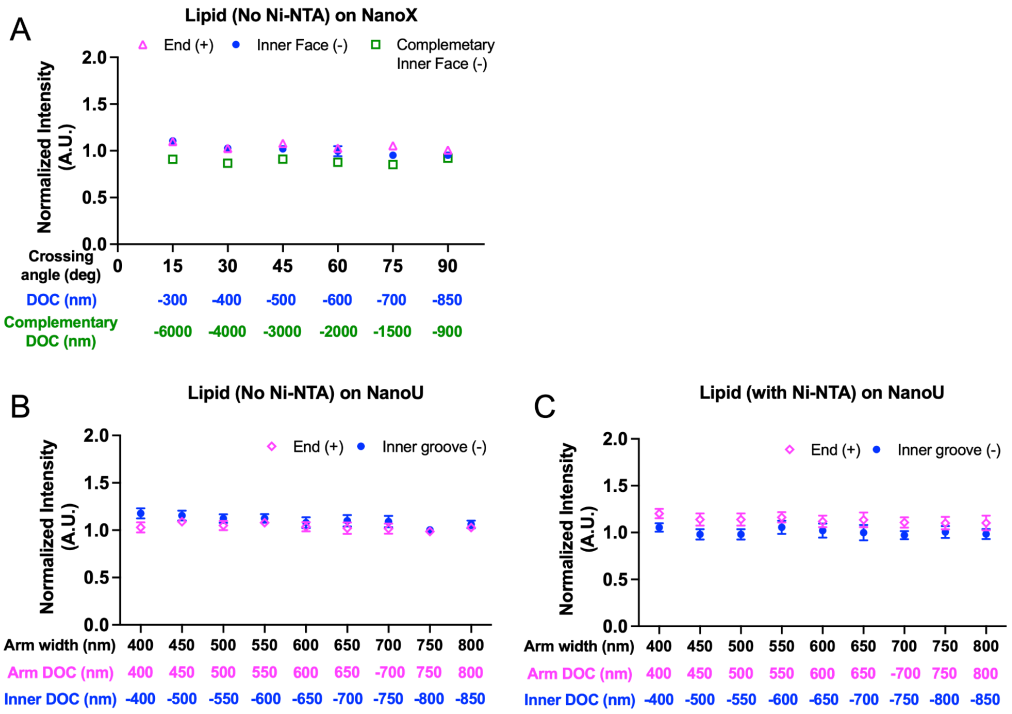


Supplementary Figure 4. Representative fluorescence images of U2OS cells expressing IRSp53(BAR)-GFP, IRSp53(FL)-GFP, and GFP-FBP17(FL). Scale bar: 20 μm.



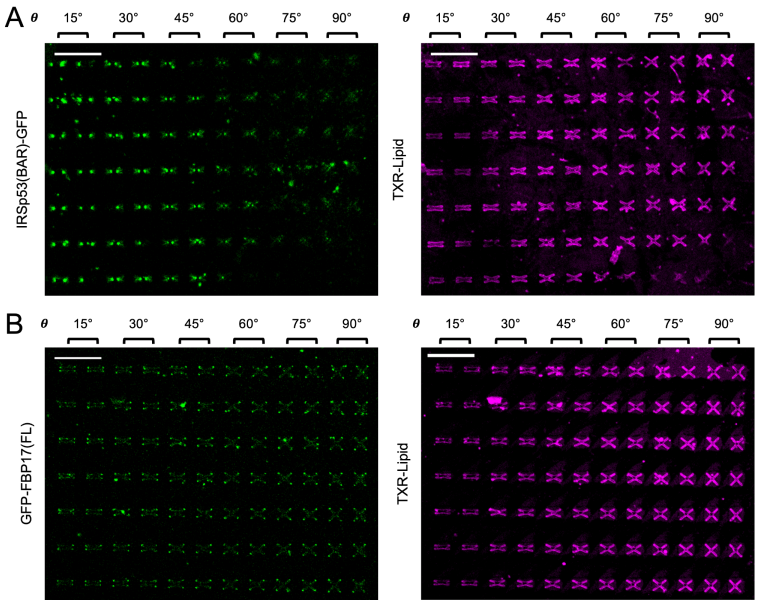
Supplementary Figure 5. Western blots of protein lysates confirming the presence of transfected proteins.

(A) IRSp53-GFP variants. **(B)** Full-length GFP-FBP17. Both GFP-FBP17 and IRSp53-GFP variants were probed by rabbit anti-GFP antibodies. GAPDH was stained with mouse anti-GAPDH antibodies as a control.

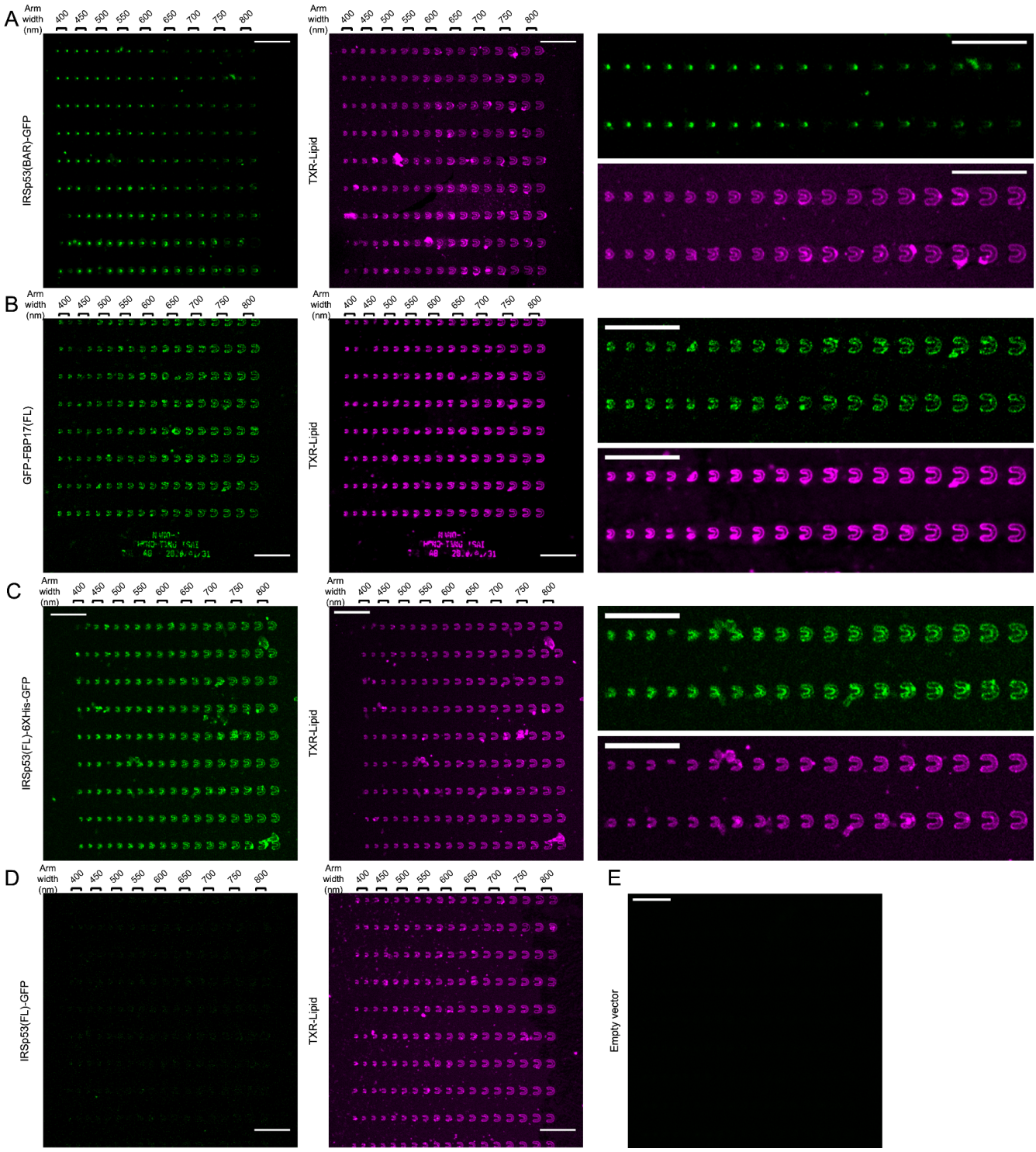


Supplementary Figure 6. Quantification of TXR-lipid bilayer signals on gradient nanoU and nanoX arrays.

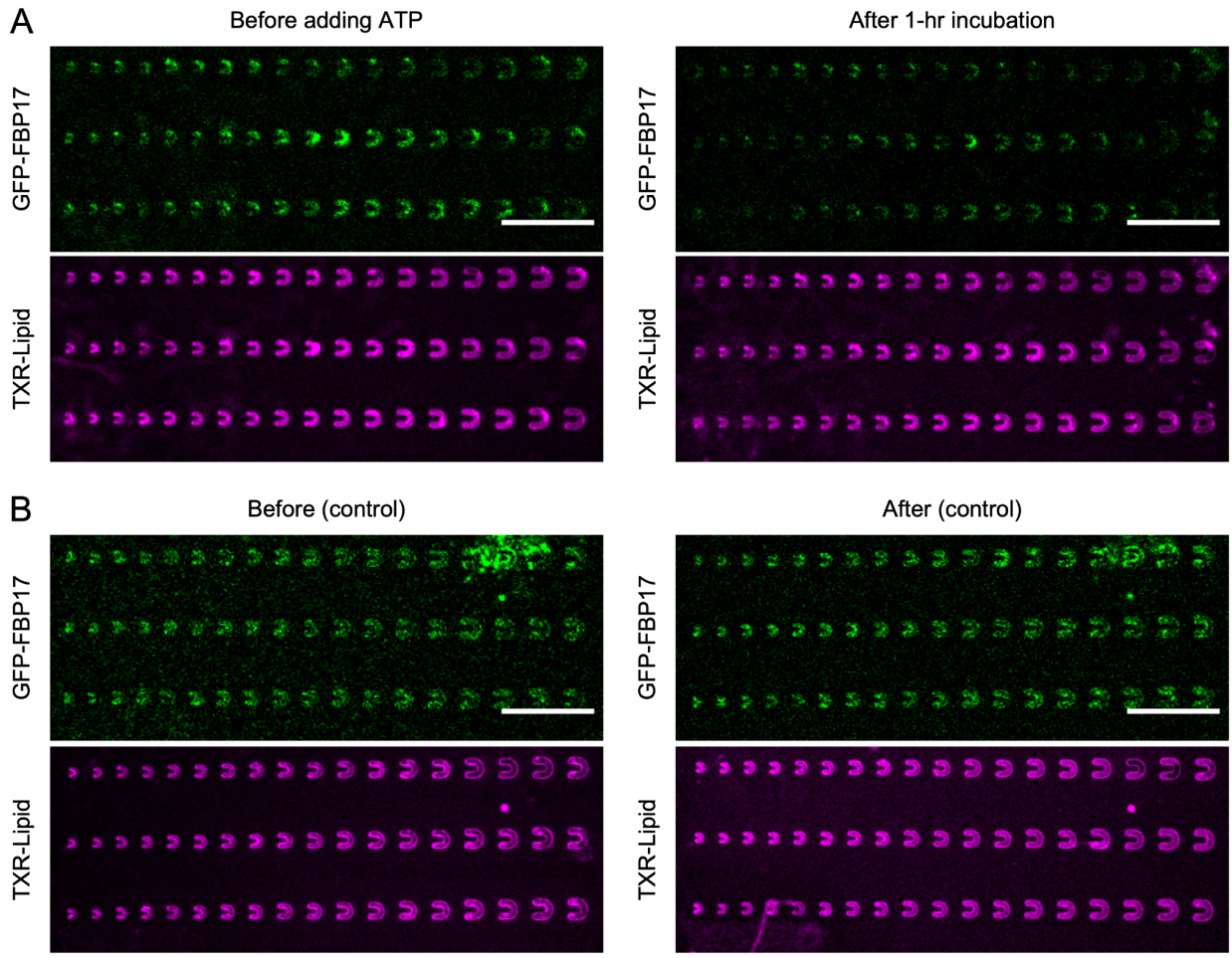
(A-B) 70% DOPC, 15% DOPS, 15% PIP_2 and ~1% Texas Red-DHPE on (A) gradient nanoX arrays or (B) gradient nanoU arrays. **(C)** 55% DOPC, 15% DOPS, 15% PIP_2, 15% DGS-Ni-NTA, and ~1% Texas Red-DHPE on gradient nanoU arrays. Error bars represent standard error of mean.



Supplementary Figure 7. Representative whole-field fluorescence images of BAR protein lysates on SLB-coated nanoX arrays. (A) IRSp53(BAR)-GFP; **(B)** GFP-FBP17(FL). Scale bar: 20 μm.

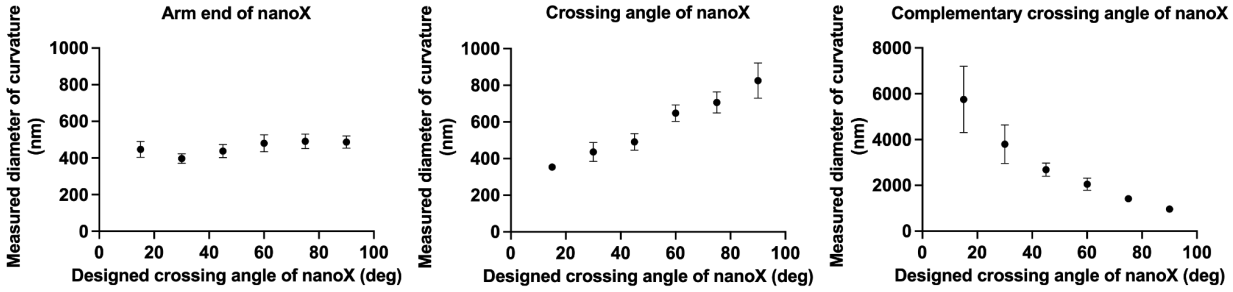


Supplementary Figure 8. Representative whole-field and zoom-in fluorescence images of BAR protein lysates on SLB-coated nanoU arrays. (A) IRSp53(BAR)-GFP; **(B)** GFP-FBP17(FL); **(C)** IRSp53(FL)-6XHis-GFP; **(D)** IRSp53(FL)-GFP; **(E)** Empty vector. Scale bar: 20 μm.



Supplementary Figure 9. Representative fluorescence images from the c-Abl kinase experiments on SLB-coated nanoU arrays. (A) c-Abl-catalyzed phosphorylation upon ATP addition significantly reduces the intensity of GFP-FBP17 on the lipid bilayer. **(B)** GFP-FBP17 fluorescence intensity does not change upon the addition of a control buffer. Scale bar: 20 μm.

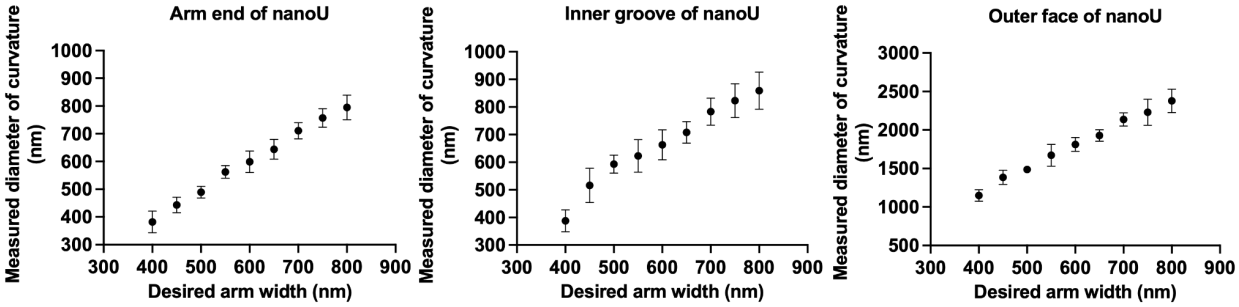
Designed values			Measured values (Diameter of curvature)			Number of nanostructures measured
Arm width (nm)	Crossing angle (θ)	Complementary crossing angle (θ)	Arm end (nm)	Crossing angle (nm)	Complementary crossing angle (nm)	
400	15	165	447±43.2	354±15.8	5749±1449	10
	30	150	397±26.3	436±51.8	3797±840	10
	45	135	438±36.2	491±45.4	2680±293	10
	60	120	480±46.1	648±45.2	2050±270	10
	75	105	491±39.7	706±57.9	1416±125	10
	90	90	487±33.1	825±96.2	966±83.2	10



Supplementary Table 1. Characterization of gradient nanoX arrays.

Error bars represent standard deviation.

Desired Arm width (nm)	Measured values (Diameter of curvature)			Number of nanostructures measured
	Arm end (nm)	Inner groove (nm)	Outer face (nm)	
400	382±39.4	388±39.9	1148±75.5	10
450	443±28.0	516±62.2	1384±92.0	10
500	489±21.1	593±32.8	1487±31.9	10
550	562±23.0	623±59.2	1672±141	10
600	599±38.6	663±54.2	1811±89.4	10
650	644±36.0	708±39.1	1928±74.2	10
700	711±29.5	783±49.3	2136±85.2	10
750	757±33.5	823±61.2	2230±168	10
800	795±44.1	859±67.1	2378±152	10



Supplementary Table 2. Characterization of gradient nanoU arrays.

Error bars represent standard deviation.

A

Target	Desired Crossing angle (θ)	Normalized intensity (A.U.)				Number of fields of view considered (N)	Number of nanostructures measured (n)	Corresponding figure
		NanoX region	Mean	SD	SEM (SD/$\sqrt{N}$)			
IRSp53 (BAR)-GFP	15	End	1.56	0.80	0.23	12	168	Fig. 1E
		Inner face	5.50	3.49	1.01			
		Complementary inner face	0.83	0.56	0.16			
	30	End	0.88	0.15	0.04	12	182	
		Inner face	4.29	2.71	0.78			
		Complementary inner face	0.71	0.30	0.09			
	45	End	0.83	0.12	0.03	12	196	
		Inner face	3.91	2.16	0.62			
		Complementary inner face	0.92	0.25	0.07			
	60	End	0.86	0.10	0.03	12	186	
		Inner face	3.36	1.62	0.47			
		Complementary inner face	1.31	0.19	0.05			
	75	End	0.73	0.13	0.04	11	171	
		Inner face	2.53	0.95	0.29			
		Complementary inner face	1.55	0.19	0.06			
	90	End	0.73	0.14	0.04	11	167	
		Inner face	1.98	0.55	0.17			
		Complementary inner face	1.96	0.54	0.16			

B

Target	Desired Crossing angle (θ)	Normalized intensity (A.U.)				Number of fields of view considered (N)	Number of nanostructures measured (n)	Corresponding figure
		NanoX region	Mean	SD	SEM (SD/$\sqrt{N}$)			
GFP-FBP17 (FL)	15	End	2.22	0.32	0.16	4	72	Fig. 1H
		Inner face	1.18	0.15	0.07			
		Complementary inner face	1.08	0.04	0.02			
	30	End	2.20	0.56	0.25	5	90	
		Inner face	0.99	0.24	0.11			
		Complementary inner face	0.97	0.12	0.05			
	45	End	2.21	0.47	0.21	5	90	
		Inner face	0.94	0.09	0.04			
		Complementary inner face	0.85	0.13	0.06			
	60	End	2.64	0.38	0.17	5	90	
		Inner face	0.93	0.10	0.04			
		Complementary inner face	0.92	0.09	0.04			
	75	End	2.44	0.22	0.10	5	90	
		Inner face	0.90	0.17	0.07			
		Complementary inner face	1.16	0.18	0.08			
	90	End	2.36	0.46	0.21	5	90	
		Inner face	0.99	0.13	0.06			
		Complementary inner face	0.98	0.14	0.06			

Supplementary Table 3. Statistical analysis for Figure 1.

A

Target	Desired Arm width (nm)	Normalized intensity (A.U.)				Number of fields of view considered (N)	Number of nanostructures measured (n)	Corresponding figure
		NanoU region	Mean	SD	SEM (SD/$\sqrt{N}$)			
IRSp53 (BAR)-GFP	400	End	3.03	1.12	0.35	10	165	Fig. 2D
		Inner groove	16.3	9.18	2.90			
	450	End	2.27	0.98	0.28	12	198	
		Inner groove	14.6	12.2	3.53			
	500	End	2.11	0.90	0.26	12	196	
		Inner groove	13.5	10.6	3.07			
	550	End	1.67	0.47	0.14	12	187	
		Inner groove	12.4	7.98	2.30			
	600	End	2.10	0.93	0.27	12	196	
		Inner groove	13.5	8.43	2.43			
	650	End	1.78	0.67	0.19	12	188	
		Inner groove	11.1	6.39	1.85			
	700	End	1.60	0.65	0.20	11	172	
		Inner groove	10.0	7.07	2.13			
	750	End	2.00	1.34	0.39	12	173	
		Inner groove	9.37	4.96	1.43			
	800	End	1.51	0.66	0.19	12	180	
		Inner groove	7.53	4.90	1.42			

B

Target	Desired Arm width (nm)	Normalized intensity (A.U.)				Number of fields of view considered (N)	Number of nanostructures measured (n)	Corresponding figure
		NanoU region	Mean	SD	SEM (SD/$\sqrt{N}$)			
GFP-FBP17 (FL)	400	End	2.01	1.14	0.33	12	152	Fig. 2F
		Inner groove	1.05	0.56	0.16			
	450	End	1.70	0.59	0.17	12	155	
		Inner groove	1.10	0.69	0.20			
	500	End	1.49	0.30	0.09	10	162	
		Inner groove	0.92	0.17	0.05			
	550	End	1.36	0.32	0.09	12	177	
		Inner groove	0.93	0.26	0.08			
	600	End	1.35	0.24	0.07	12	183	
		Inner groove	0.98	0.22	0.06			
	650	End	1.37	0.23	0.07	11	182	
		Inner groove	0.93	0.21	0.06			
	700	End	1.25	0.15	0.05	10	158	
		Inner groove	0.89	0.18	0.06			
	750	End	1.31	0.22	0.06	12	193	
		Inner groove	0.98	0.20	0.06			
	800	End	1.13	0.18	0.05	12	188	
		Inner groove	0.90	0.28	0.08			

Supplementary Table 4. Statistical analysis for Figure 2.

A

Target	Normalized intensity (A.U.)		Number of fields of view considered (N)	Corresponding figure
	Mean	SD		
Empty vector	2.47	0.29	4	Fig. 3C
IRSp53(FL)-GFP	2.26	0.08	4	
IRSp53(FL)-6XHis-GFP	10.3	2.03	13	
IRSp53(BAR)-GFP	14.1	4.57	15	

B

Target	Desired Arm width (nm)	Normalized intensity (A.U.)				Number of fields of view considered (N)	Number of nanostructures measured (n)	Corresponding figure
		NanoU region	Mean	SD	SEM (SD/$\sqrt{N}$)			
IRSp53 (FL)-6XHis-GFP	400	End	0.99	0.31	0.08	13	159	Fig. 3E
		Inner groove	2.43	0.86	0.24			
	450	End	1.06	0.25	0.07	13	175	
		Inner groove	2.33	1.05	0.29			
	500	End	0.94	0.14	0.04	13	196	
		Inner groove	1.96	0.96	0.27			
	550	End	0.93	0.18	0.05	13	207	
		Inner groove	1.78	0.71	0.20			
	600	End	0.91	0.19	0.05	13	203	
		Inner groove	1.82	0.65	0.18			
	650	End	0.97	0.16	0.04	13	209	
		Inner groove	1.71	0.68	0.19			
	700	End	0.96	0.18	0.05	13	213	
		Inner groove	1.76	0.75	0.21			
	750	End	0.98	0.18	0.05	13	200	
		Inner groove	1.50	0.44	0.12			
	800	End	0.98	0.17	0.05	13	214	
		Inner groove	1.58	0.57	0.16			

Supplementary Table 5. Statistical analysis for Figure 3.

A

Target	Desired Arm width (nm)	Normalized intensity (A.U.)				Number of fields of view considered (N)	Number of nanostructures measured (n)	Corresponding figure
		NanoU region	Mean	SD	SEM (SD/$\sqrt{N}$)			
GFP-FBP17 (FL) Before adding ATP	400	End	1.38	0.21	0.07	9	162	Fig. 4E
	450		1.30	0.22	0.07	9	162	
	500		1.21	0.18	0.06	9	162	
	550		1.16	0.16	0.05	9	162	
	600		1.23	0.13	0.04	9	162	
	650		1.17	0.20	0.07	9	162	
	700		1.12	0.13	0.04	9	162	
	750		1.13	0.14	0.05	9	162	
	800		1.11	0.21	0.07	9	162	

B

Target	Desired Arm width (nm)	Normalized intensity (A.U.)				Number of fields of view considered (N)	Number of nanostructures measured (n)	Corresponding figure
		NanoU region	Mean	SD	SEM (SD/$\sqrt{N}$)			
GFP-FBP17 (FL) 1-hr After adding ATP	400	End	1.34	0.24	0.09	7	124	Fig. 4F
	450		1.27	0.23	0.09	7	124	
	500		1.23	0.27	0.10	7	124	
	550		1.16	0.18	0.07	7	124	
	600		1.01	0.18	0.07	7	124	
	650		1.09	0.18	0.07	7	124	
	700		1.06	0.16	0.06	7	124	
	750		1.02	0.17	0.06	7	124	
	800		1.04	0.19	0.07	7	124	

Supplementary Table 6. Statistical analysis for Figure 4.