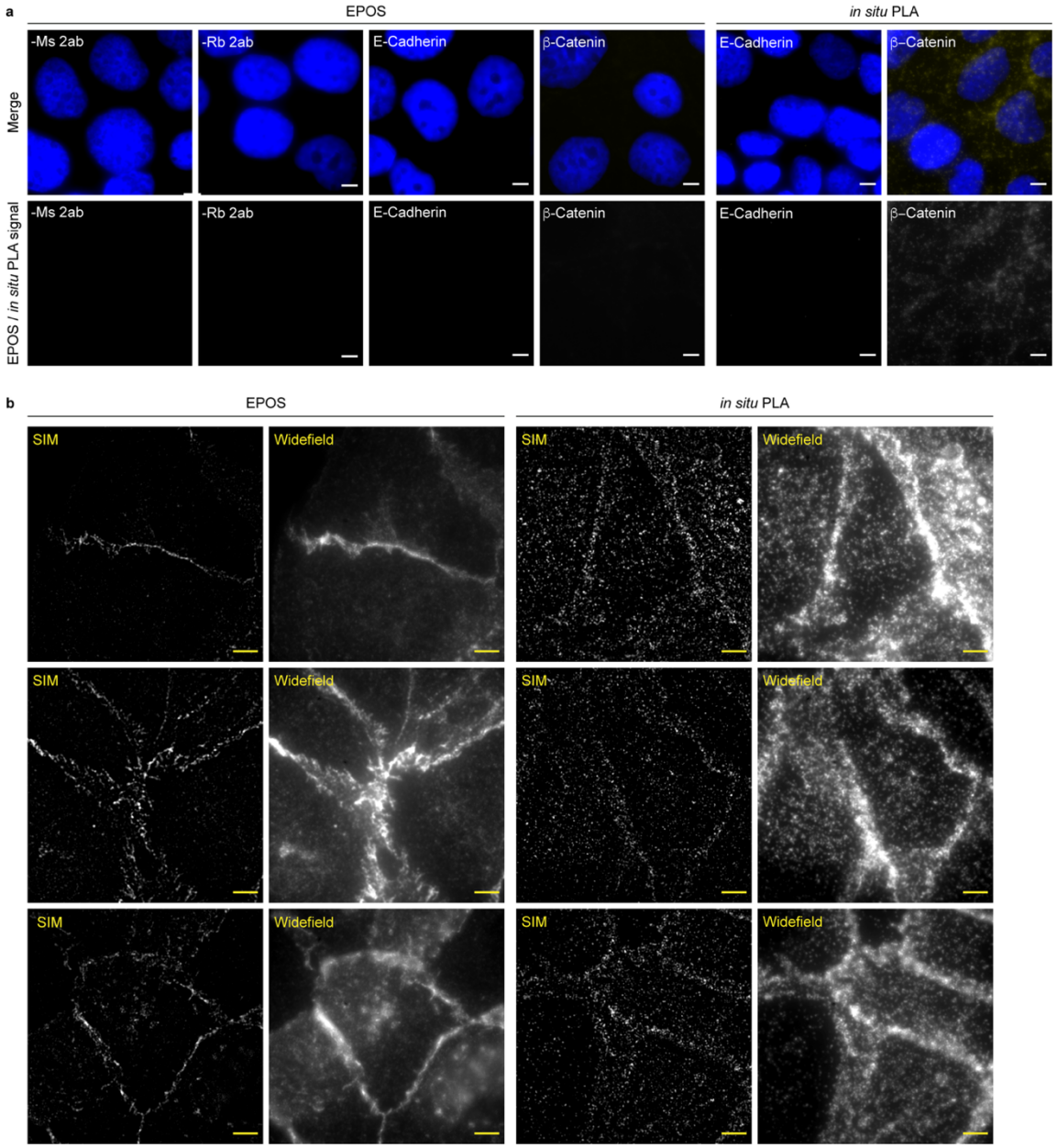
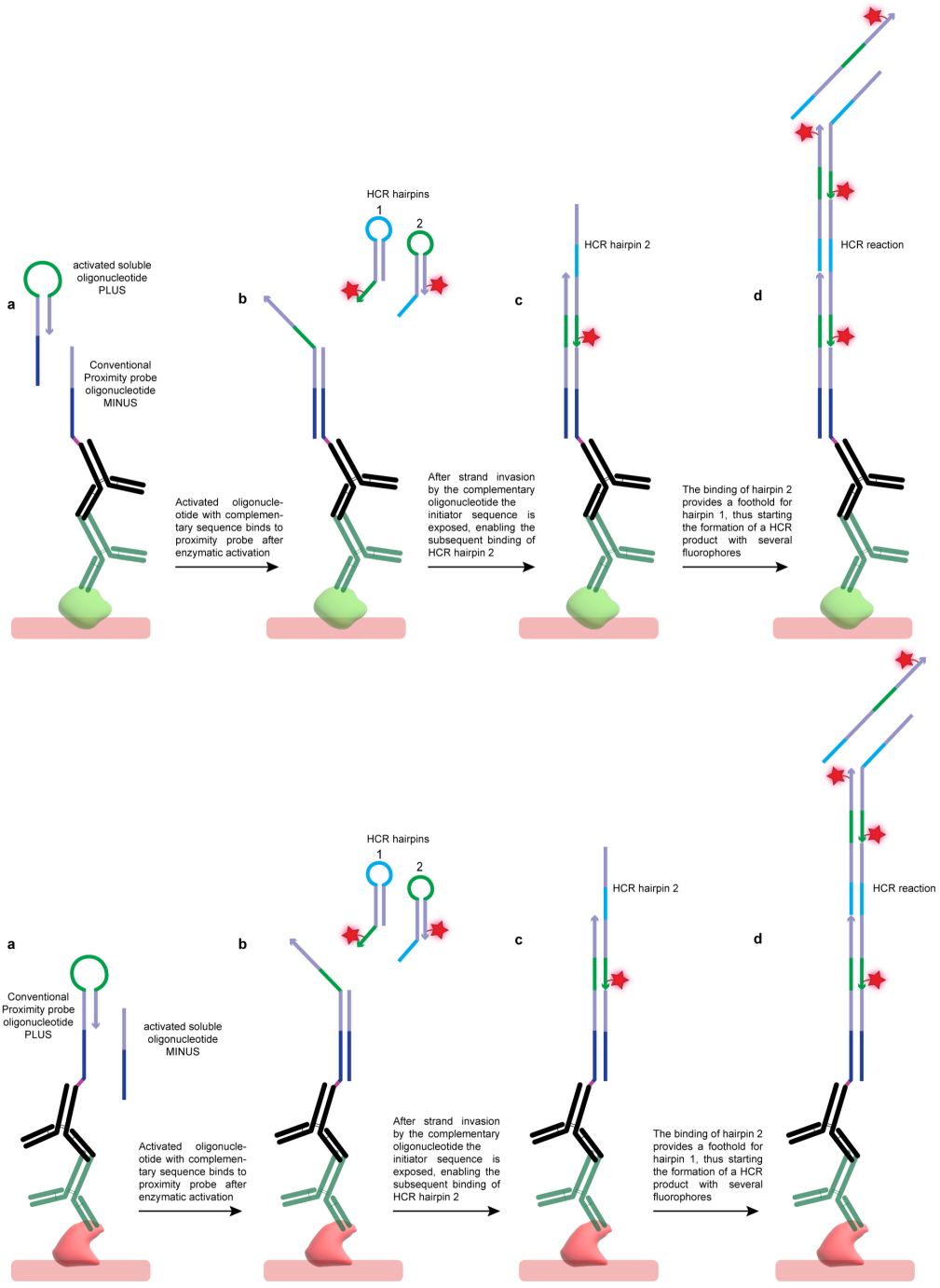
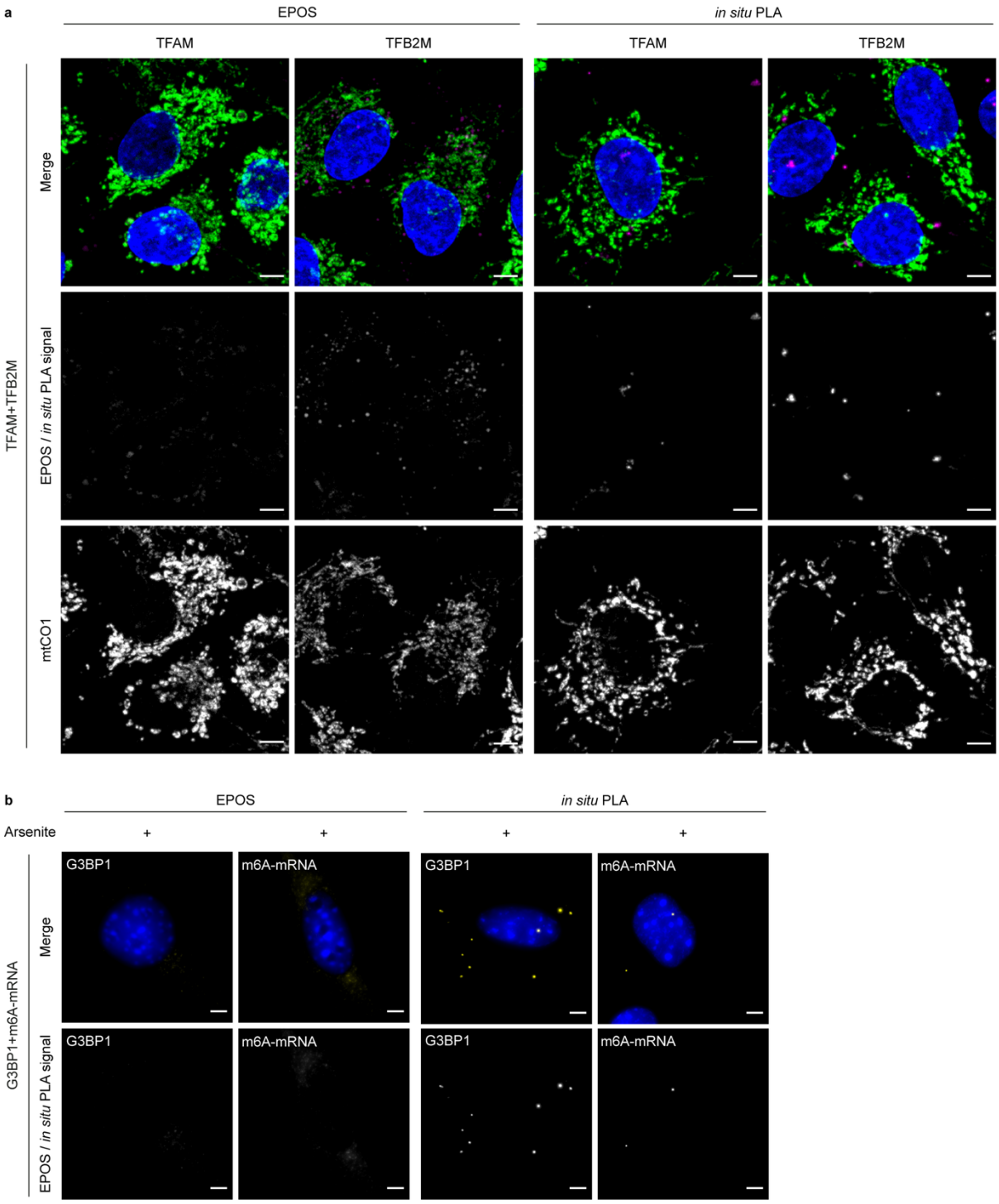
Supplementary figure legends



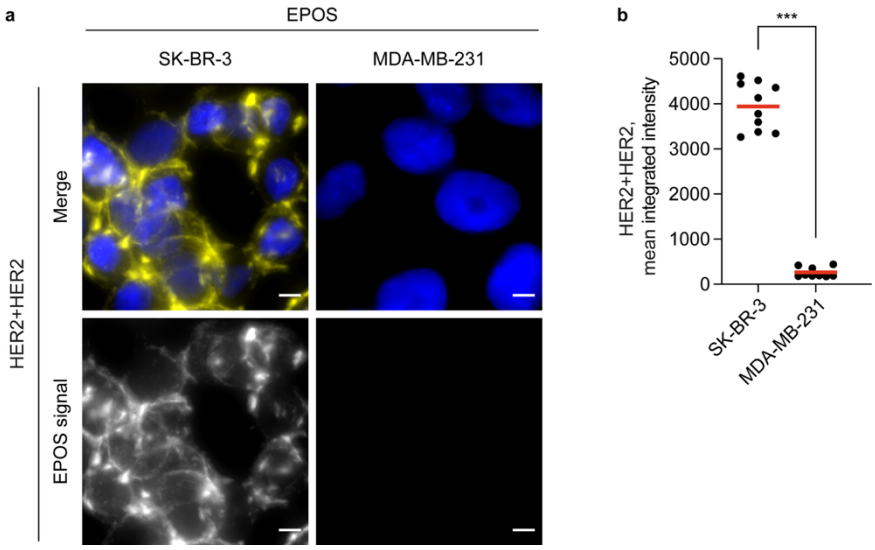
Supplementary figure 1: a) Technical controls, omitting proximity probes (prox probes) or primary antibodies. Omitting the anti-mouse prox probe (-Ms 2ab) or the anti-rabbit prox probe (-Rb 2ab) totally abolished all signals. When only one primary antibody was used, with both prox probes present, no background was detected in both EPOS and *in situ* PLA, except when only the rabbit anti-β-Catenin primary antibody was used (i.e. the mouse anti-E-cadherin primary antibody was omitted). b) E-cadherin and β-catenin interactions in HaCaT cells visualised with EPOS or *in situ* PLA imaged with structured illumination microscopy (SIM) and super-resolution widefield microscopy. The EPOS and *in situ* PLA signals are shown in yellow, and Hoechst 33342 nuclear staining is shown in blue in the merged images. The scale bar is 5 μm.



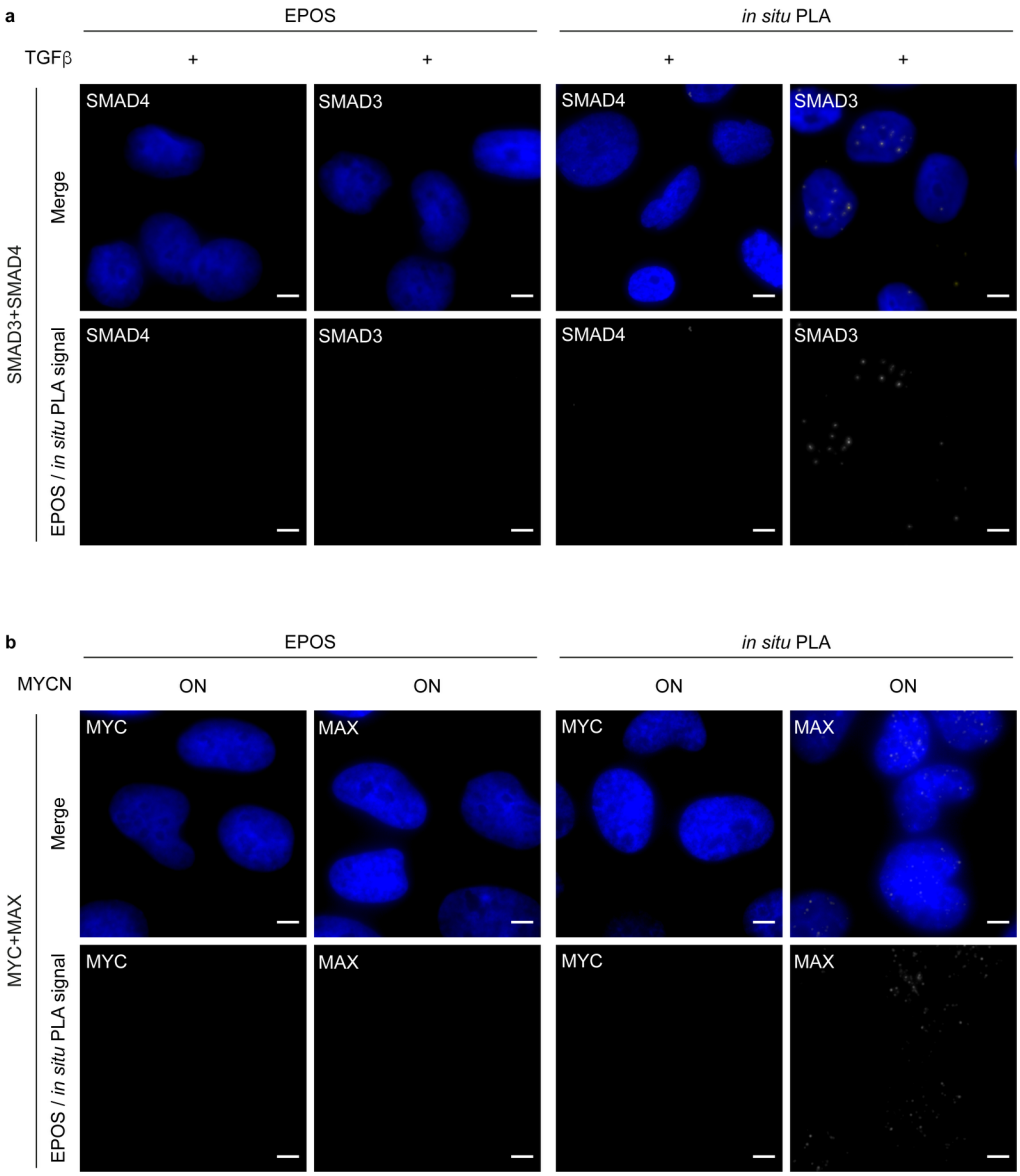
Supplementary Figure 2: **Schematic representation of the EPOS method utilizing an activated soluble oligonucleotide PLUS or an activated soluble oligonucleotide MINUS to initiate an HCR. a**) Activated proximity probes (prox probes) after digestion with uracil-DNA glycosylase (UDG) and endonuclease IV (EndoIV) are incubated with the corresponding activated soluble oligonucleotide MINUS or activated soluble oligonucleotide PLUS. **b**) The oligonucleotide hybridizes to the prox probe and exposes the initiator sequence. **c**) The exposed initiator sequence hybridizes to and invades HCR hairpin 2, **d**) which templates an HCR reaction.



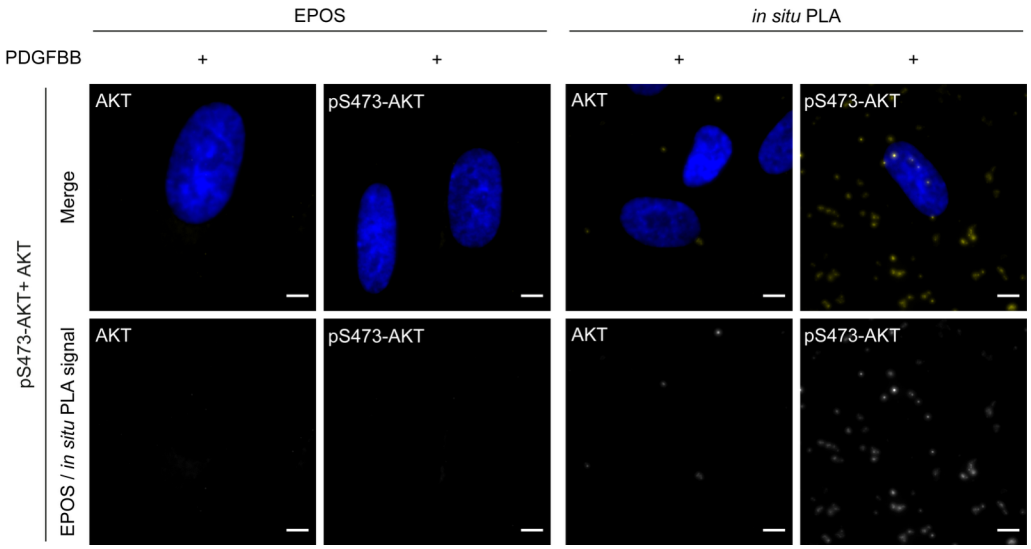
Supplementary figure 3: Technical controls omitting primary antibodies against a) TFAM or TFB2M in A549 lung adenocarcinoma cells and b) G3BP1 or m6A-mRNA interactions in HT22 mouse hippocampal neuronal cells after arsenite treatment. The signal is shown in magenta (a) or yellow (b), Alexa Fluor® 488 conjugated anti-MTCO1 antibody (Mitochondria, green) and Hoechst33342 staining of nuclei is shown in blue in the merged images. The scale bar is 5 µm.



Supplementary figure 4: a) Detection of HER2 homodimerization with detection probes conjugated to sdAbs in SK-BR-3 and MDA-MB-231 cells. b) quantification of the EPOS staining in (a). The EPOS signals are shown in yellow, and Hoechst 33342 nuclear staining is shown in blue in the merged images. The scale bar is 5 µm. Statistical analysis was performed using the Mann–Whitney test. n= 3 experiments. Each dot represent an image frame, mean values per cell, ***=$p<0.005$.



Supplementary figure 5: Technical controls omitting primary antibodies against a) SMAD3 or SMAD4 after the cells were stimulated with 5 ng/ml TGF-β in MDA-MB-231 breast cancer cells and b) MYCN or MAX in Tet21N neuroblastoma cells in the absence of doxycyclin (MYCN on). Omission of any primary antibody results in no signal with EPOS, while *in situ* PLA produces a signal even in the absence of the SMAD4 or the MYC antibody. The EPOS and *in situ* PLA signals are shown in yellow, and Hoechst 33342 nuclear staining is shown in blue in the merged images. The scale bar is 5 µm.



Supplementary figure 6: Technical controls omitting primary antibodies against phosphorylation on serine 473 (pS473-AKT) or total AKT after the cells were stimulated with 20 ng/ml PDGF-BB for 15 minutes. Omission of any primary antibody results in no signal with EPOS, while *in situ* PLA produces a signal even in the absence of the total AKT antibody. The EPOS and *in situ* PLA signals are shown in yellow, and Hoechst 33342 nuclear staining is shown in blue in the merged images. The scale bar is 5 μm.

Supplemental Tables

Supplemental Table 1: Oligonucleotides used

Oligo name	5´-> 3´	Vendor
Proximity probe oligonucleotide MINUS	GCACUUGACUCGUAGCCCUUACUCCCUUUUUGGGAGTAAGG GCTACGAGTCAAGTGCAAAAA-Azide	QDS
Proximity probe oligonucleotide MINUS part 1	GCACUUGACUCGUAGCCCUUACUCCCUUUUUGGGAGTAAGG	IDT
Proximity probe oligonucleotide MINUS part 2	Phosphate-GCTACGAGTCAAGTGCAAAAA-Azide	IDT
Proximity probe oligonucleotide PLUS	Azide-AAAAAGCACTTGACTCGTAGCCCT TACTCCCTGTGATGGGAGTAAGGGCUAUGAGUCAAGUGCUUU	QDS
Proximity probe oligonucleotide PLUS part 1	Azide-AAAAAGCACTTGACTCGTAGCCCT	IDT
Proximity probe oligonucleotide PLUS part 2	Phosphate- TACTCCCTGTGATGGGAGTAAGGGCUAUGAGUCAAGUGCUUU	IDT
HCR-hairpin 1	GGAATTGCCCTTACTCCCTGTGATGGGAGTAAGGGC-TexasRed	IDT
HCR-hairpin 2	GGGAGTAAGGGCAATTCCGCCCTTACTCCCATCACA-TexasRed	IDT
Activated soluble oligonucleotide MINUS	GGGAGTAAGGGCTACGAGTCAAGTGC	IDT
Activated soluble oligonucleotide PLUS	GCACTTGACTCGTAGCCCTTACTCCCTGTGATGGGAGTAAGGGC	IDT

Supplemental Table 2: Primary antibodies used

Target	Host	Dilution	Product number	Company
AKT	Mouse	1:100	2920	Cell Signaling Technology
AKT (pS473)	Rabbit	1:50	4060	Cell Signaling Technology
β-catenin	Rabbit	1:100	8480	Cell Signaling Technology
Clathrin	Mouse	1:200	ab2731	Abcam
Clathrin	Rabbit	1:10 -> 1:7290	4796	Cell Signaling Technology
E-cadherin	Mouse	1:100	610182	BD Transduction Laboratories
G3BP1	Rabbit	1:500	45656	Cell Signaling Technology
Her2	Llama	2 μg/ml	Q17	QVQ
m6A-mRNA	Mouse	1:500	ab208577	Abcam
MAX	Rabbit	1:400	ab101271	Abcam
MYCN	Mouse	1:400	B8.4.B	Santa Cruz Biotechnology
SMAD3	Rabbit	1:100	9523	Cell Signaling Technology
SMAD4	Mouse	1:100	sc-7966	Santa Cruz Biotechnology
TFAM	Mouse	1:200	sc-376672	Santa Cruz Biotechnology
TFB2M	Goat	1:200	46 481	Biozol