Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary data for

Negatively Curved Cellular Membranes Promote BAIAP2 Signaling Hub Assembly

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Contents

SUPPLEMENTARY TEXT

Enrichment of PS biosensor at negatively curved membranes.

We find enrichment of the biosensor directed against PS at negatively curved membrane sections. However, it remains elusive whether the observed enrichment of the lipid biosensor is the cause or the consequence of curvature-dependent protein enrichment. In vitro and numerical studies probing lipid dynamics in the absence of protein-lipid interactions clearly show that curvature-driven lipid sorting only occurs in proximity to a demixing point, which is not the case in biological membranes[1,2]. Hence, while binding of curvature-sensitive cytosolic proteins critically relies on the electrostatic interactions between negatively charged lipids and positive residues of the BAR domain[3,4], under physiological conditions individual lipids in the plasma membrane are very unlikely to enrich in a curvature-dependent manner unless aided by curvature-sensitive proteins."

SUPPLEMENTARY REFERENCES

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3 M. P. Ebrahimkutty and M. Galic, Receptor-Free Signaling at Curved Cellular Membranes, *BioEssays : news and reviews in molecular, cellular and developmental biology*, 2019, **41**, e1900068.

4 E. Berganza, M. P. Ebrahimkutty, S. K. Vasantham, C. Zhong, A. Wunsch, A. Navarrete, M. Galic, and M. Hirtz, Multiplexed phospholipid membrane platform for curvature sensitive protein screening, *Nanoscale, 2022,* **13**, 12642-12650.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Spatial confinement does not increase cell stress. (a) Cells plated on nanopattern stained with Annexin V (green) and 7-AAD (magenta). From left to right, merged image, gray scale image of Annexin staining, gray scale image of 7-AAD staining, and bright field image are shown. **(b)** Cells subjected to high stress stained with Annexin V (green) and 7-AAD (magenta). **(c)** Negative control stained with Annexin V and 7-AAD. As above, representative images are shown. Note that all images were acquired using the same exposure times. Scale bars (a-c), 10 μm.

Supplementary Figure 2: Lipid sensors at the negatively curved membranes. (a) Cells co-expressing the phosphatidylinositol (3,4,5) triphosphate sensor PH Akt Venus and a cytosolic reference does not show enrichment of the biosensor in grooves of the nanoridge array. Representative image (left) and quantification (right) are shown. **(b)** Cells co-expressing the phosphatidylinositol (4,5) bisphosphate sensor PH PLCD and a cytosolic reference does not show enrichment of the biosensor in grooves of the nanoridge array. As above, representative image (left) and quantification (right) are shown. **(c)** Cells co-expressing the phosphatidyserine sensor Lact C2 and a cytosolic reference show slight enrichment of the biosensor (Lact C2/Cytosol +5%) in grooves of the array. Again, representative image (left) and quantification (right) are shown. Scale bars (a-c), 5 μm.

Supplementary Figure 3: FRAP control and VASP staining on nanoridge arrays. (a) FRAP experiments show no apparent differences in recovery time and immobile fraction for cytosolic protein in negatively curved grooves compared to adjacent regions. **(b)** VASP enriches in puncta at nanoridge arrays. From left to right, merged

image and single channel images are shown. **(c)** Full-length BAIAP2 and its adaptor protein VASP co-localize in puncta at nanoridge arrays. From left to right, merged image and single channel images are shown. Scale bars (a-c), 5 μm.

Supplementary Figure 4: BAIAP2 mutant selectively enrich at the nanogrooves. **(a)** Triple-transfection of full length BAIAP2 (green) with the actin marker f-tractin (magenta) and a cytosolic reference. Ratiometric analysis shows enrichment of BAIAP2 and actin over a cytosolic reference along negatively curved membrane sections of the nanoridge arrays (BAIAP2/Cytosol +26%; f-tractin/Cytosol +26%). **(b)** Triple-transfection of mutant BAIAP2-I403P (green) with f-tractin (magenta) and cytosolic reference. Ratiometric analysis shows enrichment of mutant BAIAP2-I403P and actin over cytosolic reference (BAIAP2-I403P/Cytosol +23%; f-tractin/Cytosol +25%). **(c)** Triple-transfection of mutant BAIAP2-I268N (green) with f-tractin (magenta) and cytosolic reference. Ratiometric analysis shows enrichment of mutant BAIAP2-I268N and actin over cytosolic reference (BAIAP2-I268N/Cytosol +11%; f-tractin/Cytosol +22%). Scale bars (a-c), 5 μm.

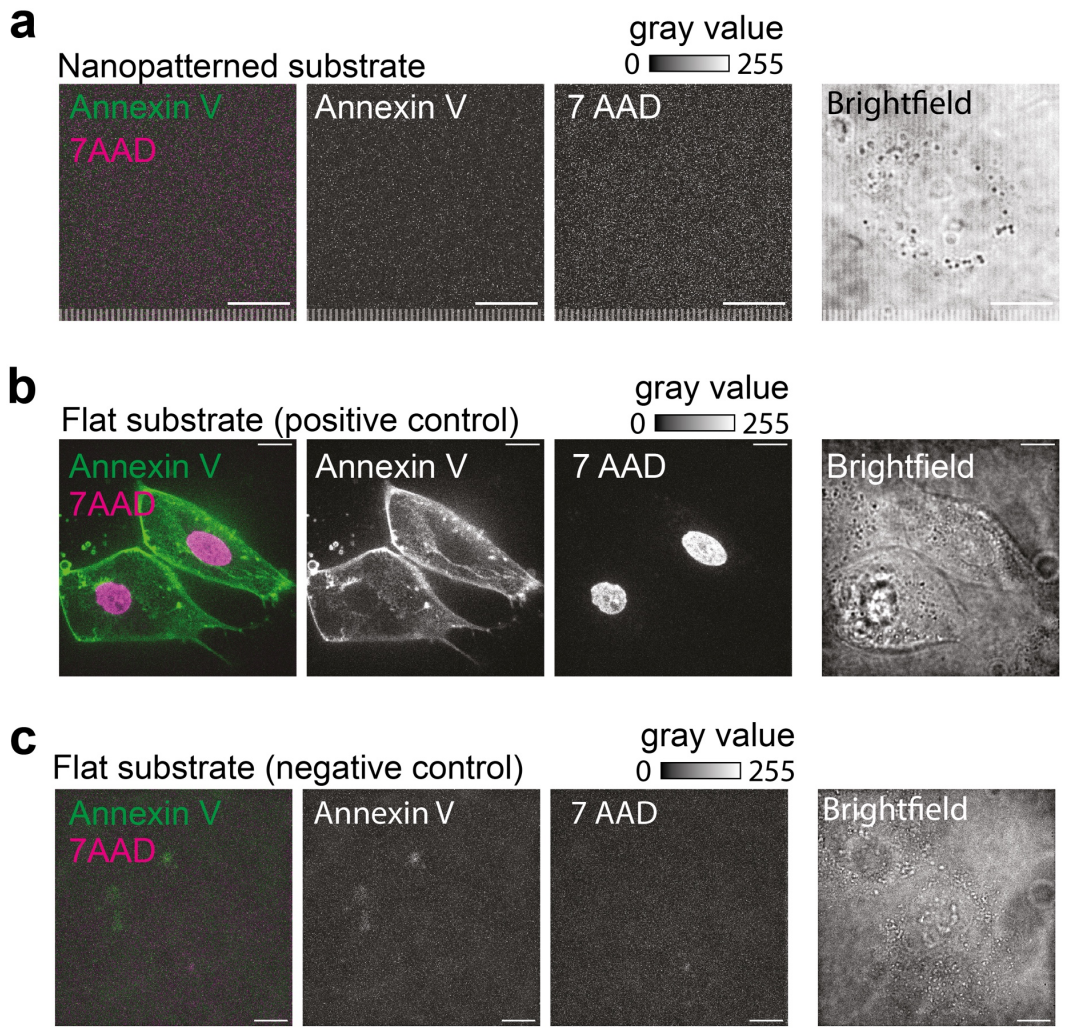

Figure S1
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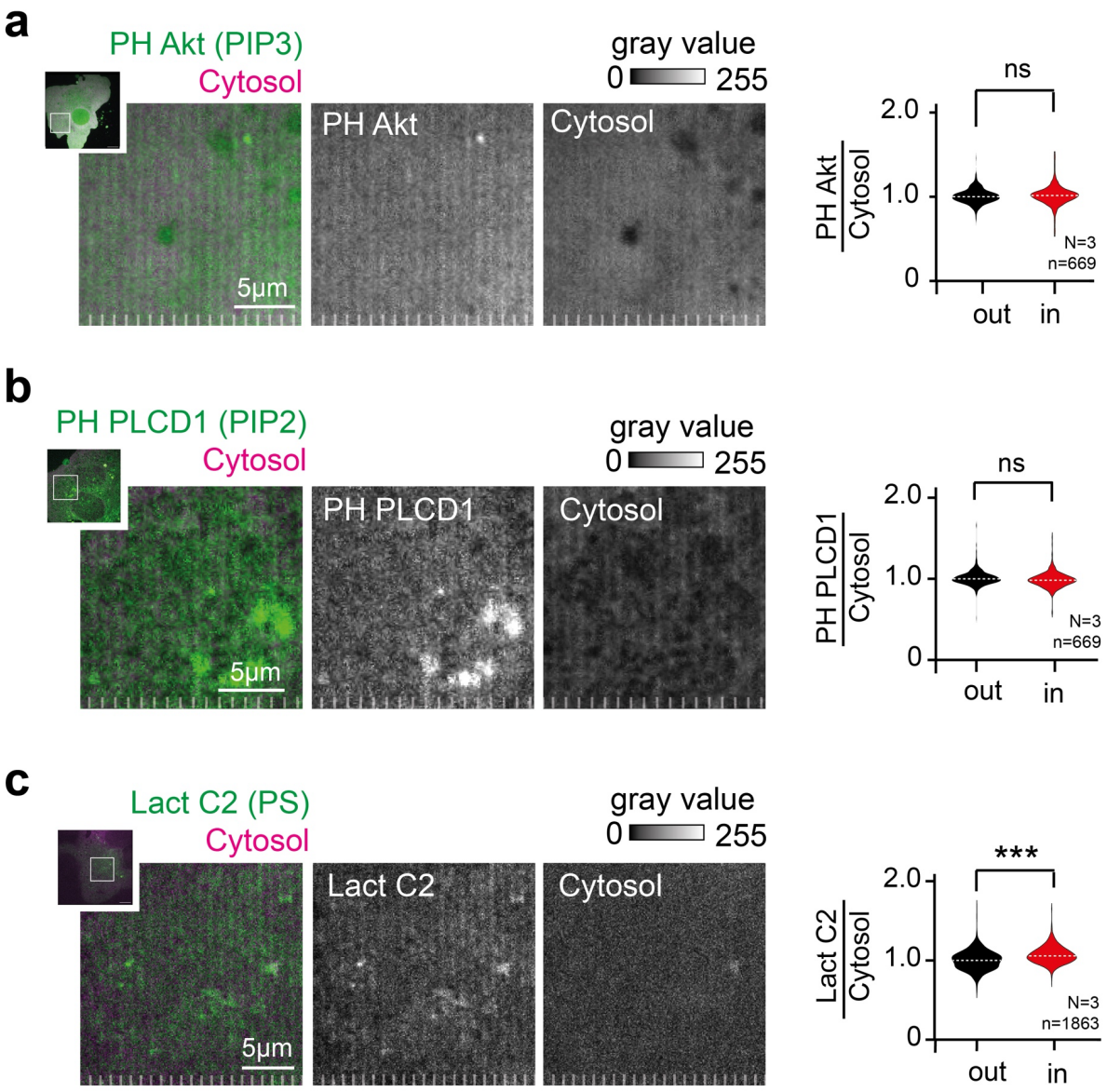

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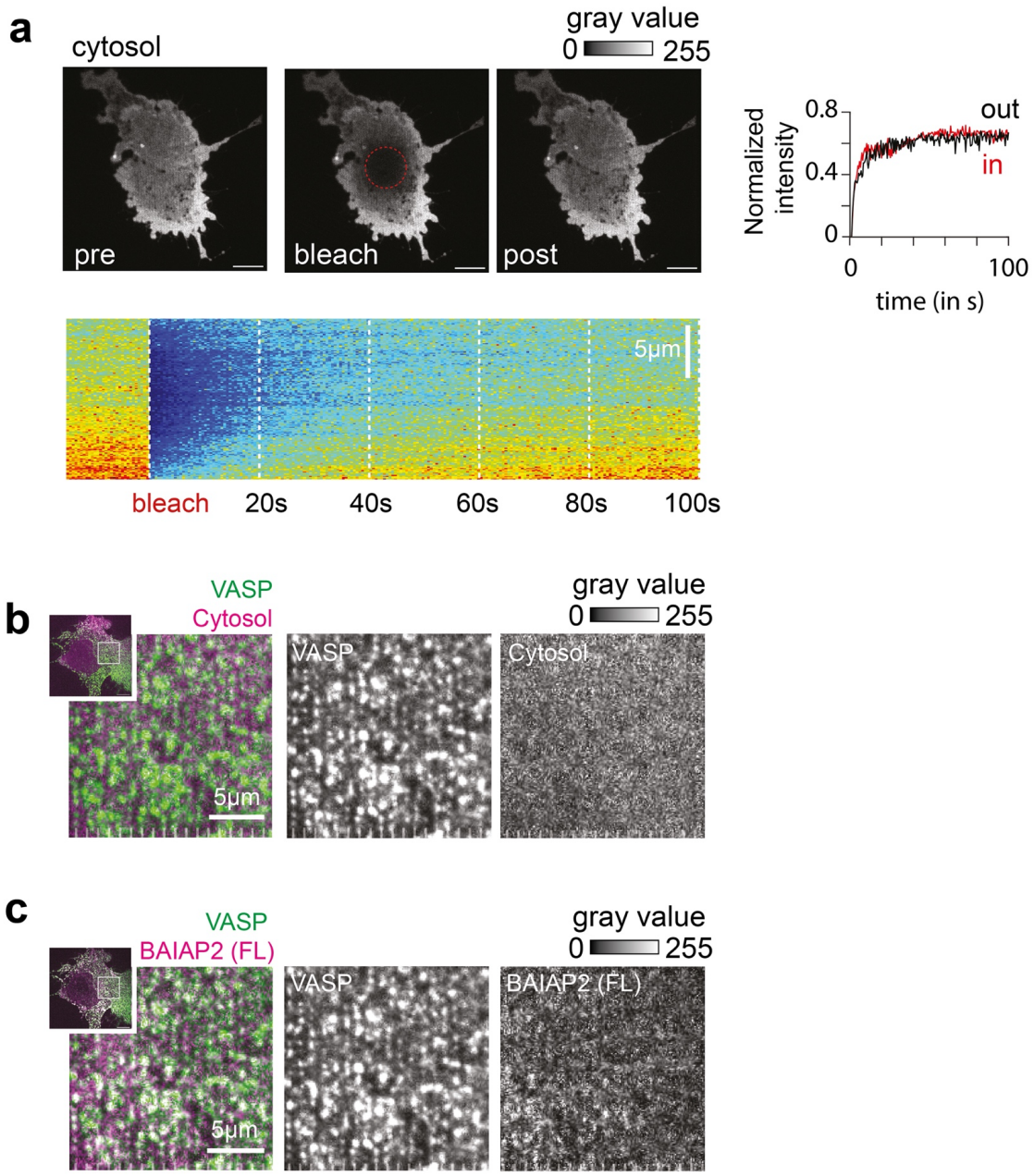

Figure S3
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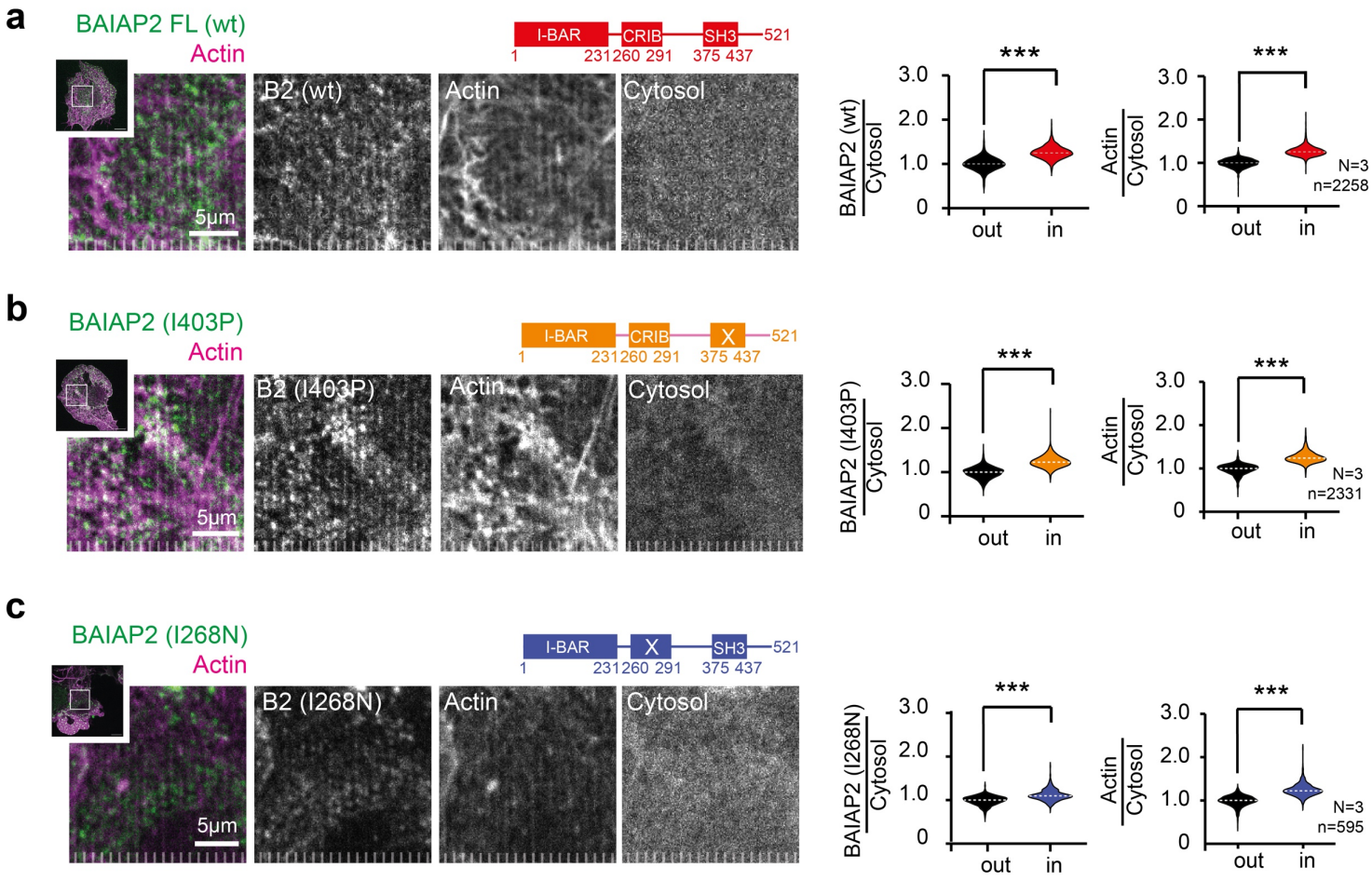

Figure S4
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