

A functional interaction between liprin- α 1 and B56 γ regulatory subunit of protein phosphatase 2A supports tumor cell motility

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The authors have no competing interests.

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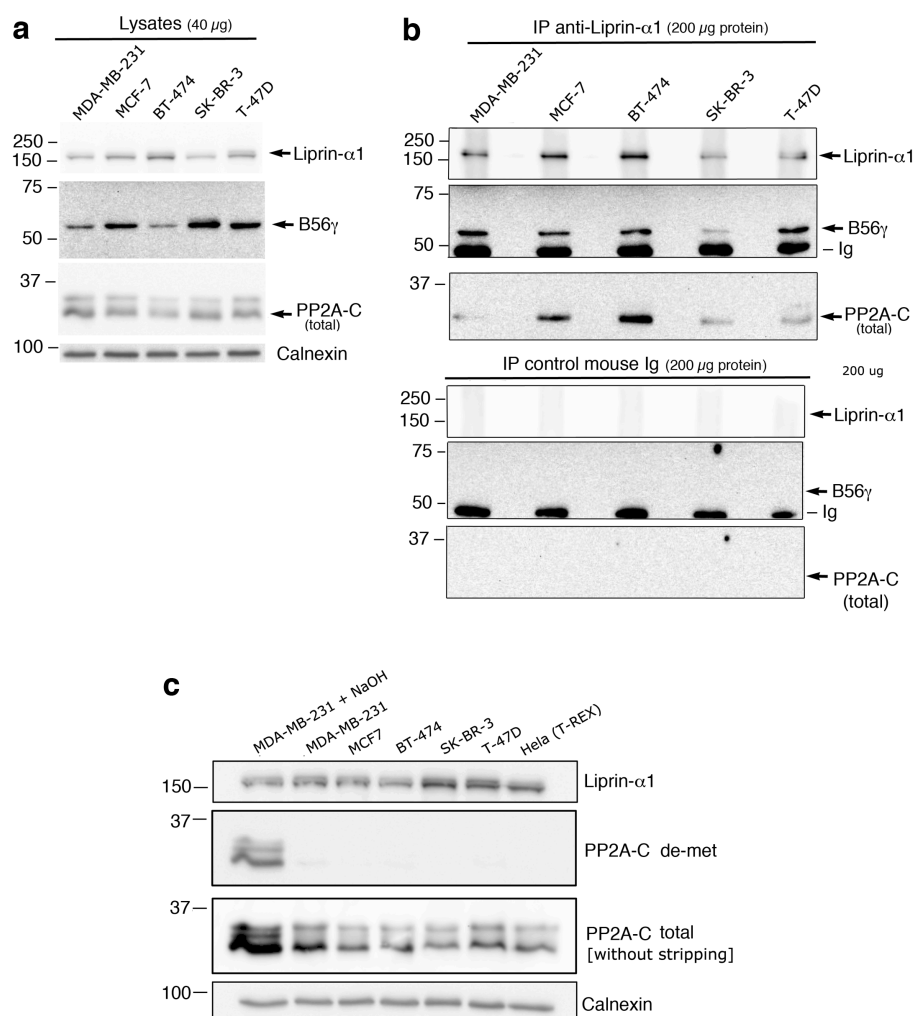
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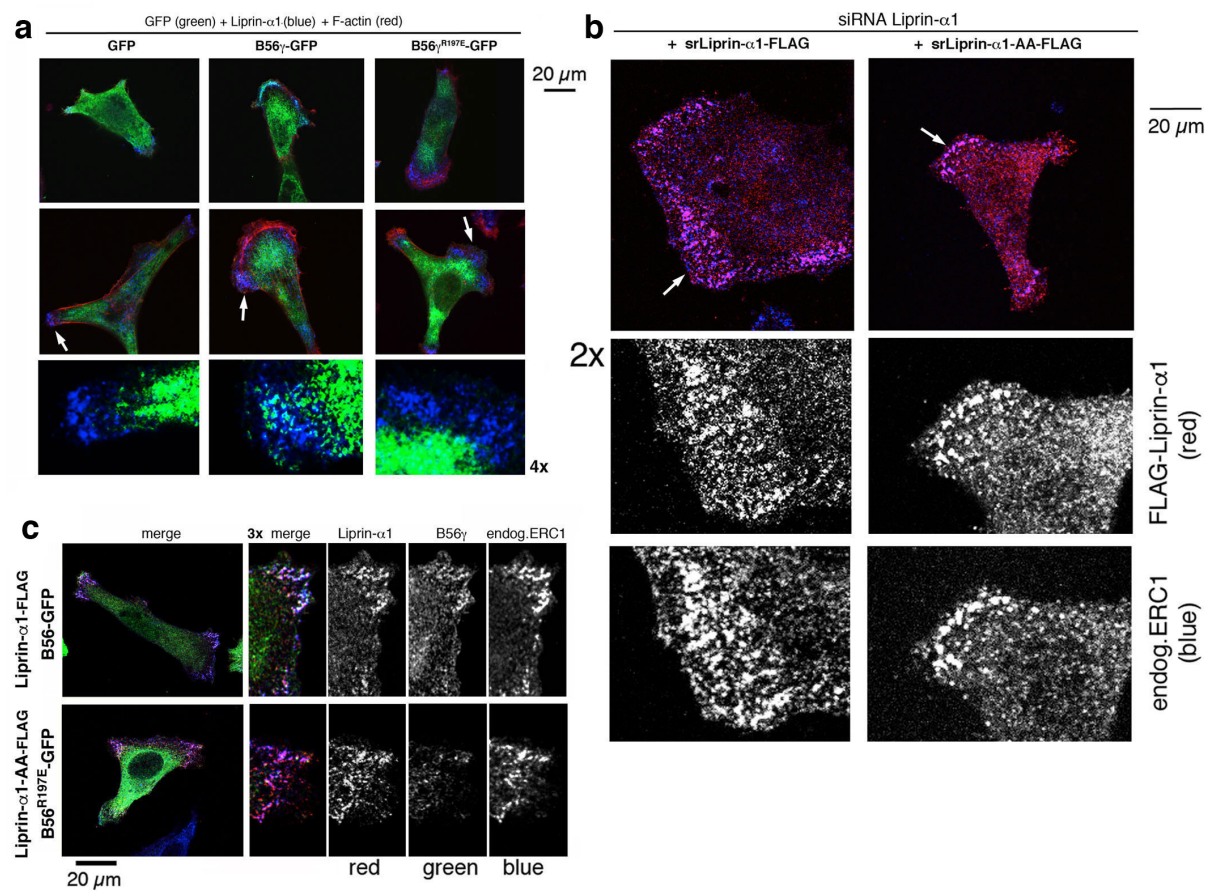
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Supplementary Figure 1. Expression of the liprin- α 1/B56 γ /PP2A-C complex in different breast cancer cell lines.



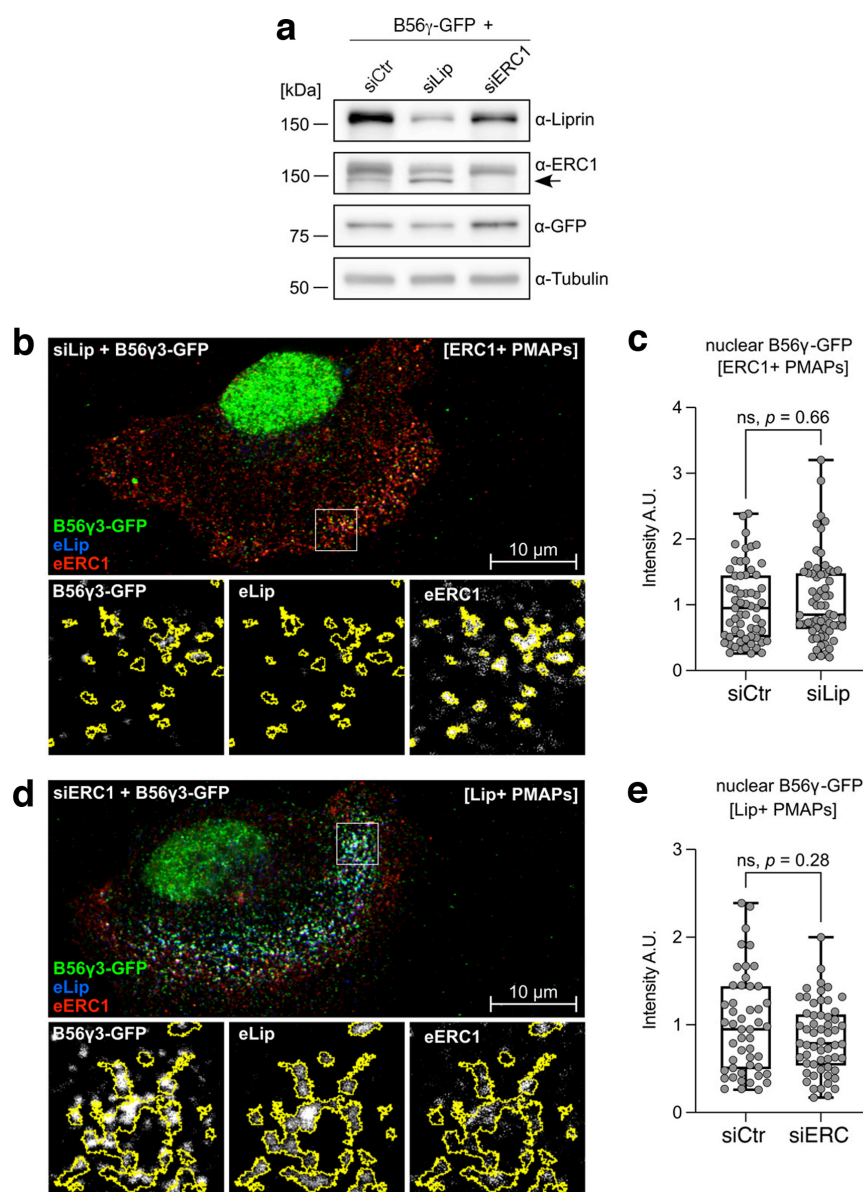
a Immunoblotting on lysates from indicated breast cancer cell lines. **b** Immunoprecipitation of endogenous liprin- α 1 to detect the endogenous liprin- α 1/B56 γ /PP2A-C complexes. **c** The endogenous catalytic PP2A-C subunit in different breast cancer cell lines is methylated. Filters with cell lysates were incubated with Abs for the indicated antigens. The second filter from the top was incubated with an Ab specific for the demethylated form of PP2A-C; the first lane on the left has been loaded with an aliquot of lysate treated with NaOH to demethylate PP2A-C (positive control). The same filter was then re-blotted with an Ab recognizing both methylated and demethylated PP2A-C (PP2A-C total, third blot from the top).

Supplementary Figure 2. Subcellular localization of wildtype and mutant B56 γ and liprin- α 1 in migrating MDA-MB-231 cells.



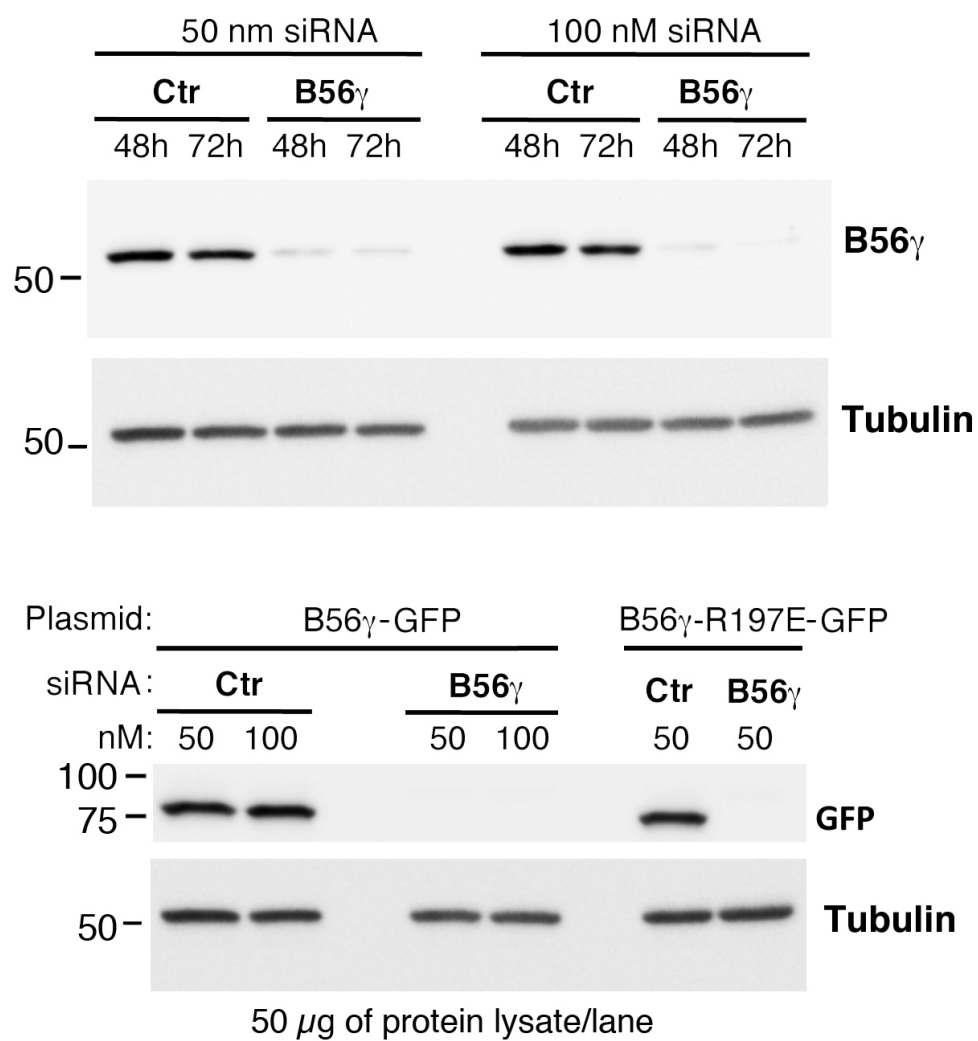
a Colocalization of B56 γ -GFP with endogenous liprin- α 1 at PMAPs in migrating MDA-MB-231 cells plated on 2.5 μ g/ml fibronectin. **b** Both wildtype (left) and SLiM mutant liprin- α 1 (right) localize at PMAPs. **c** B56 γ -GFP colocalizes with liprin- α 1-FLAG and endogenous ERC1 at PMAPs in migrating MDA-MB-231 cells.

Supplementary Figure 3. Liprin- α 1 silencing affects the localization of B56 γ at PMAPs.



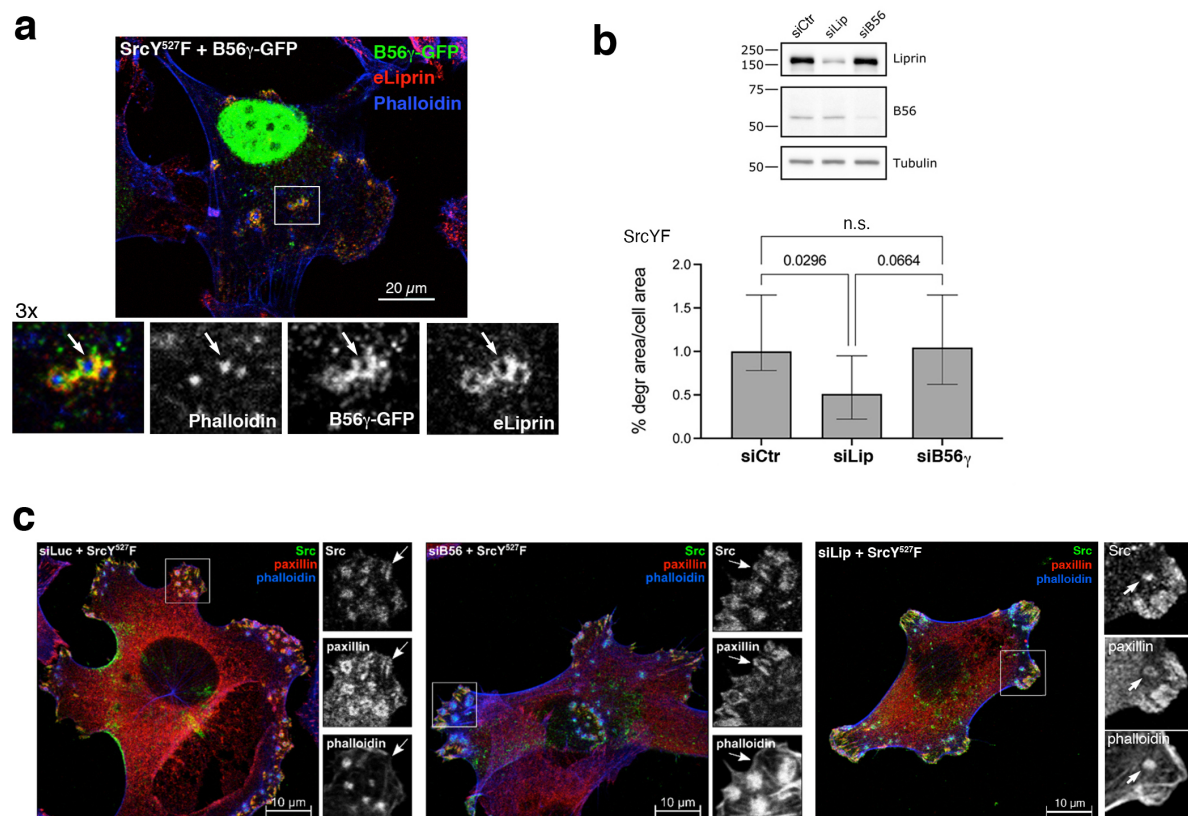
a Western blot of MDA-MB-231 cells cotransfected with B56 γ -GFP and control (siCtr), liprin- α 1 (siLip), or ERC1 (siERC1) siRNA. Immunoblotting for liprin- α 1 and ERC1 (top filter reblotted for ERC1). **b** Quantification of signal for B56 γ -GFP in ERC1-positive PMAPs: ROIs (yellow) were defined according to the signal of ERC1, and subsequently applied to B56 γ -GFP and Liprin- α 1 channels. **c** Quantification of nuclear signal of B56 γ -GFP. **d** Quantification of B56 γ -GFP in liprin- α 1-positive PMAPs: ROIs (yellow) were defined for the signal of ERC1, and subsequently applied to B56 γ -GFP and liprin- α 1 channels. **e** Quantification of B56 γ -GFP nuclear signal.

Supplementary Figure 4. Silencing of B56 γ .



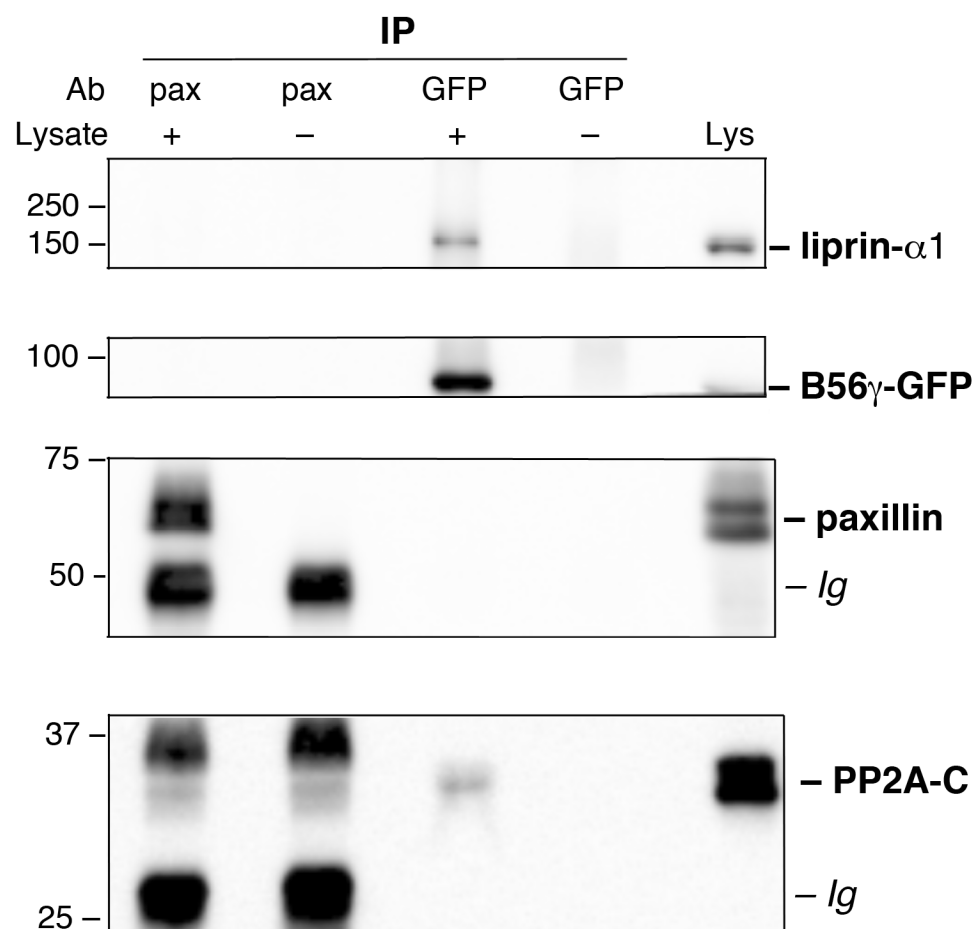
Silencing of endogenous (top) and overexpressed (bottom) B56 γ in MDA-MB-231 cells transfected with the indicated siRNAs and plasmids.

Supplementary Figure 5. Localization of B56 γ at PMAPs near invadosomes.



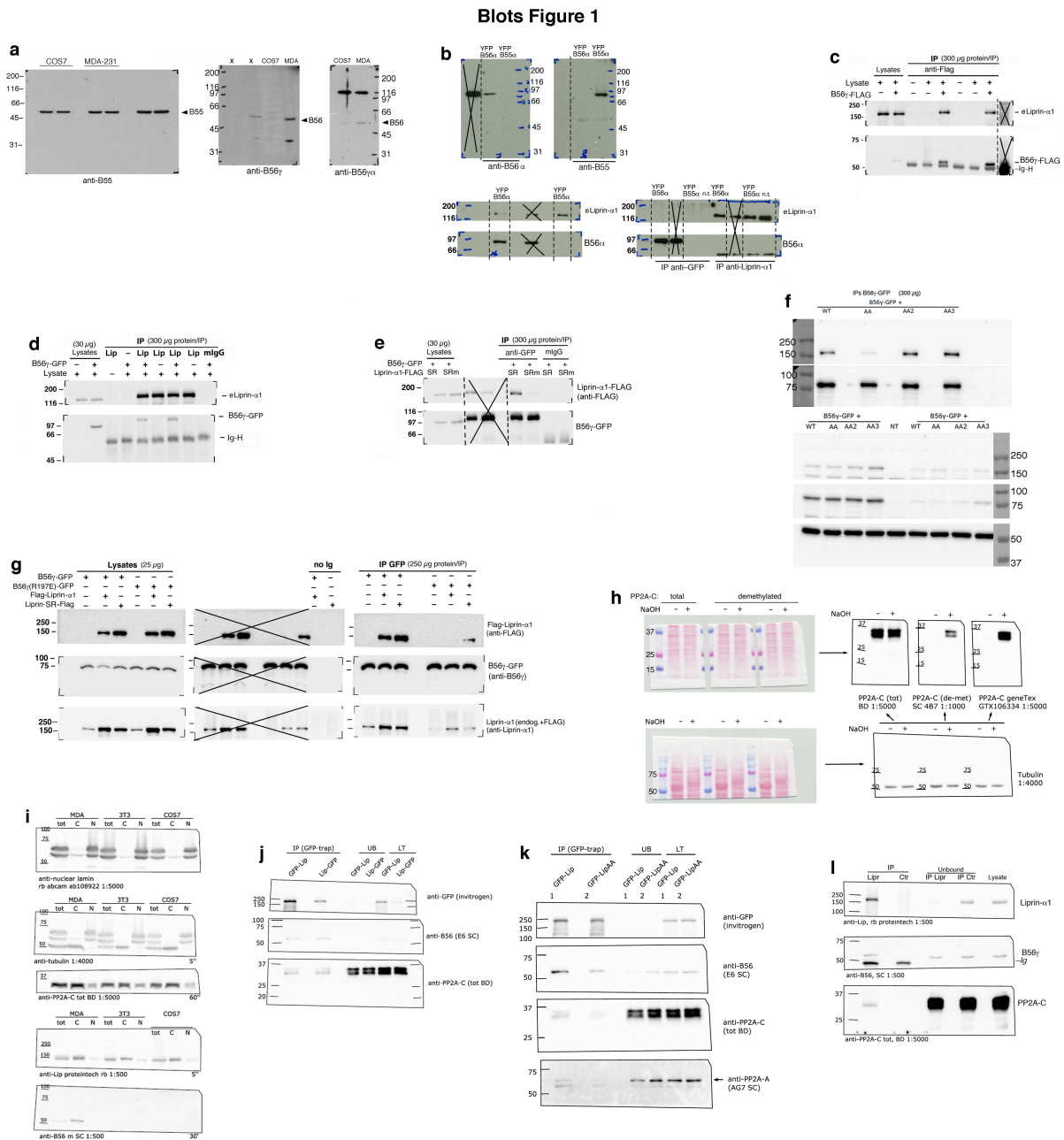
a Cotransfection of B56 γ -GFP and constitutive active Src (SrcY⁵²⁷F) showed the colocalization of B56 γ -PP2A with liprin- α 1 PMAPs near invadosomes. **b** Representative western blot of MDA-MB-231 cells cotransfected with constitutive active Src (SrcY⁵²⁷F) and control (siCtrl), liprin- α 1 (siLip), or B56 γ (siB56) siRNA. Graphs: quantification of the area of ECM degradation per cell (left) and of ECM degradation/cell area (right) (left graph, medians + 95% CI; n = 201-217 cells from 3 experiments; Kruskal-Wallis test and Dunn's correction for multiple comparisons; right graph, means + SEM). **c** Confocal images of SrcY⁵²⁷F-transfected MDA-MB-231 cells with control, B56 γ , or liprin- α 1 siRNA. Paxillin accumulates at adhesions and around Src- and phalloidin-positive invadosomes. Merge images: Src (green), paxillin (red), phalloidin (blue).

Supplementary Figure 6. B56 γ does not interact with paxillin.



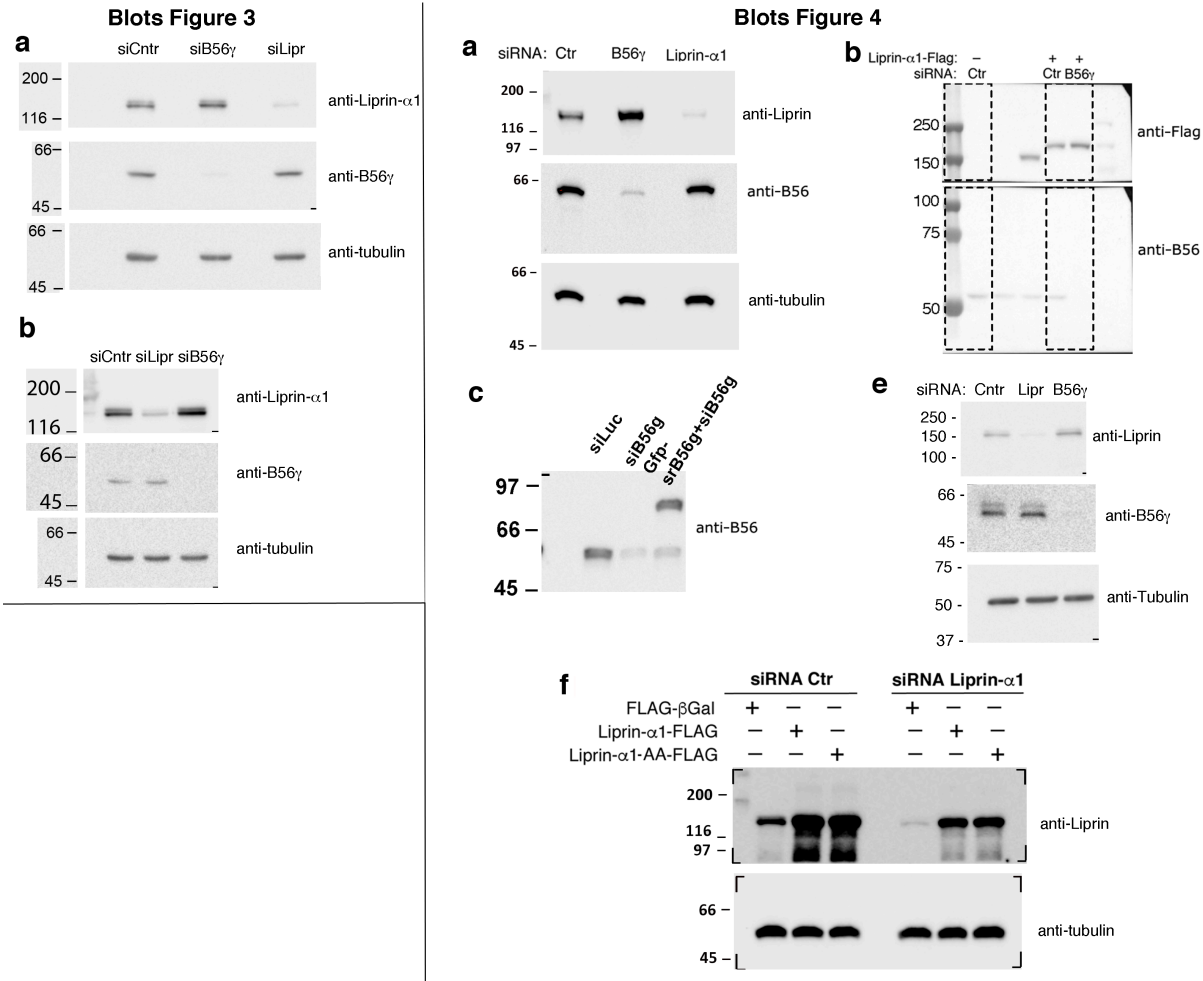
Immunoprecipitation with anti-paxillin (pax) or anti-GFP (GFP-trap) Abs from lysates of MDA-MB-231 cells transfected with B56 γ -GFP. Filters were cut and incubated with the indicated Abs.

Supplementary Figure 7. Full, uncropped blot images of panels presented in Figure 1.



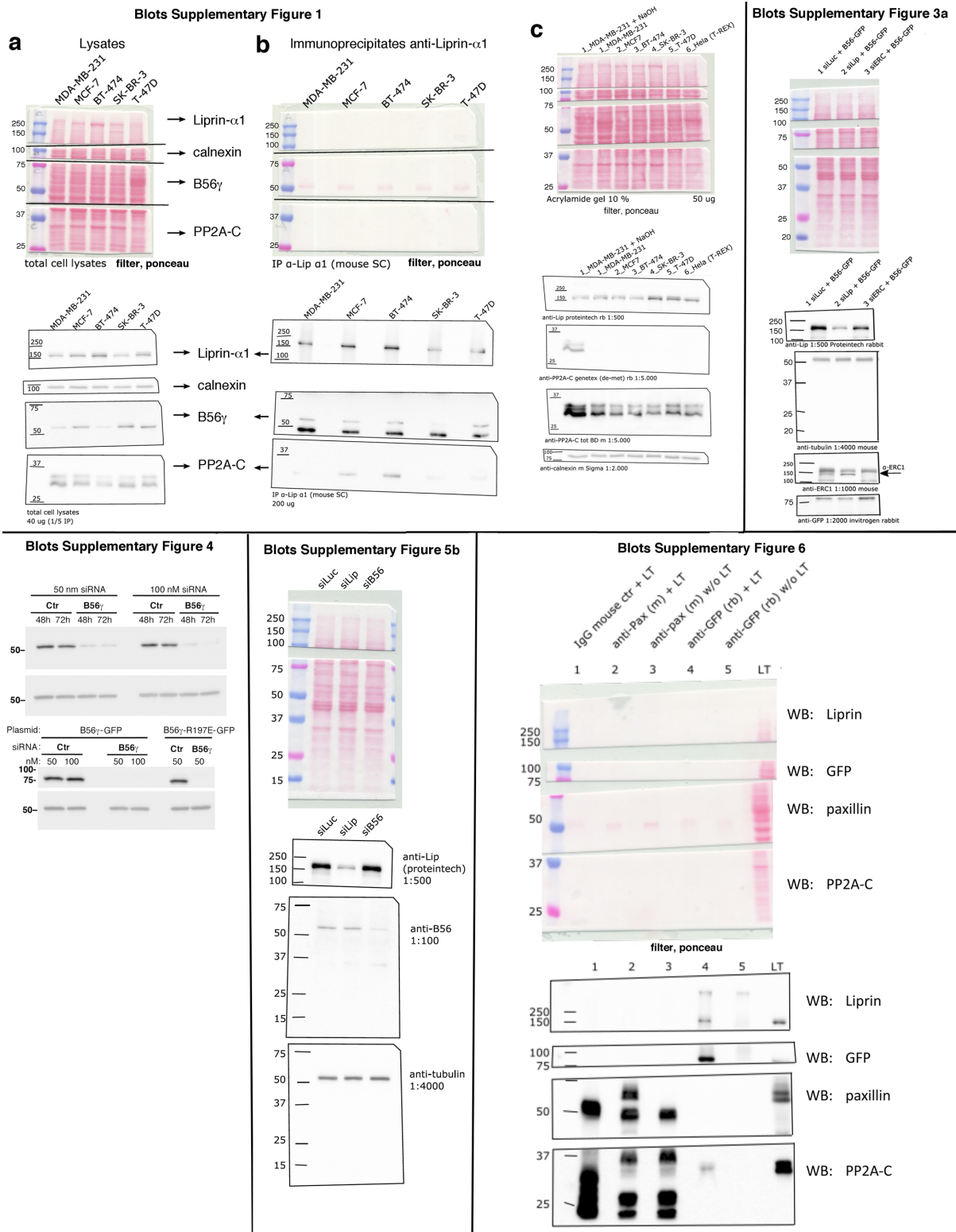
Unedited images of the blots shown in the indicated panels of **Figures 1**. The description of the experimental conditions are described in the legend of **Figure 1**.

Supplementary Figure 8. Full, uncropped blot images of panels presented in Figures 3 and 4.



Unedited images of the blots shown in the indicated panels of **Figures 3** and **4**. The description of the experimental conditions are described in the legends of the respective figures.

Supplementary Figure 9. Full, uncropped blot images of panels presented in Supplementary Figures.



Unedited images of the blots shown in the indicated panels of **Supplementary Figures 1, 3, 4, 5, 6**. The description of the experimental conditions are described in the legends of the respective Supplementary Figures.