Expanded View Figures

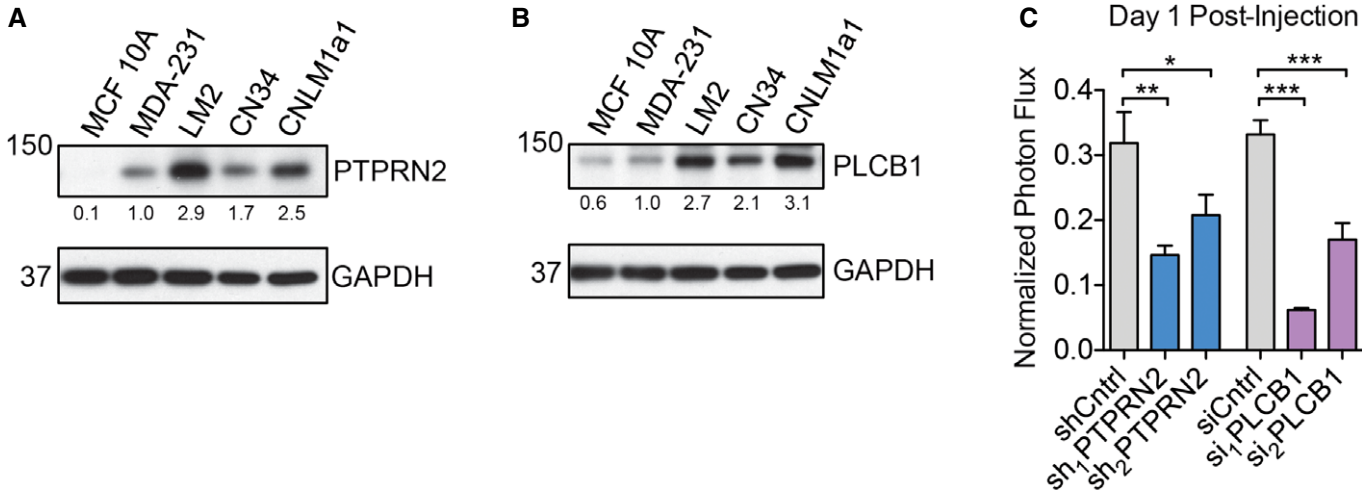

Figure EV1. PTPRN2 and PLCβ1 promote breast cancer metastasis.

A, B Western blot analysis of MCF 10A, MDA-MB-231, LM2, CN34, and CNLM1a1 cell lysate using anti-PTPRN2 (A) or anti-PLCβ1 (B). GAPDH was used as a loading control. Densitometry analysis below the blots is adjusted for GAPDH levels and normalized to MDA-MB-231 values.

C Bioluminescence imaging quantification of lung colonization 1 day after injection of 40,000 LM2 cells with knockdown of PTPRN2, PLCβ1, or control cells. For shCntrl, sh_1PTPRN2: $N = 5$ mice/group. For sh_2PTPRN2: $N = 6$ mice. For siCntrl: $N = 5$ mice. For si_1PLCb1, si_2PLCb1: $N = 6$ mice/group. Error bars represent SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

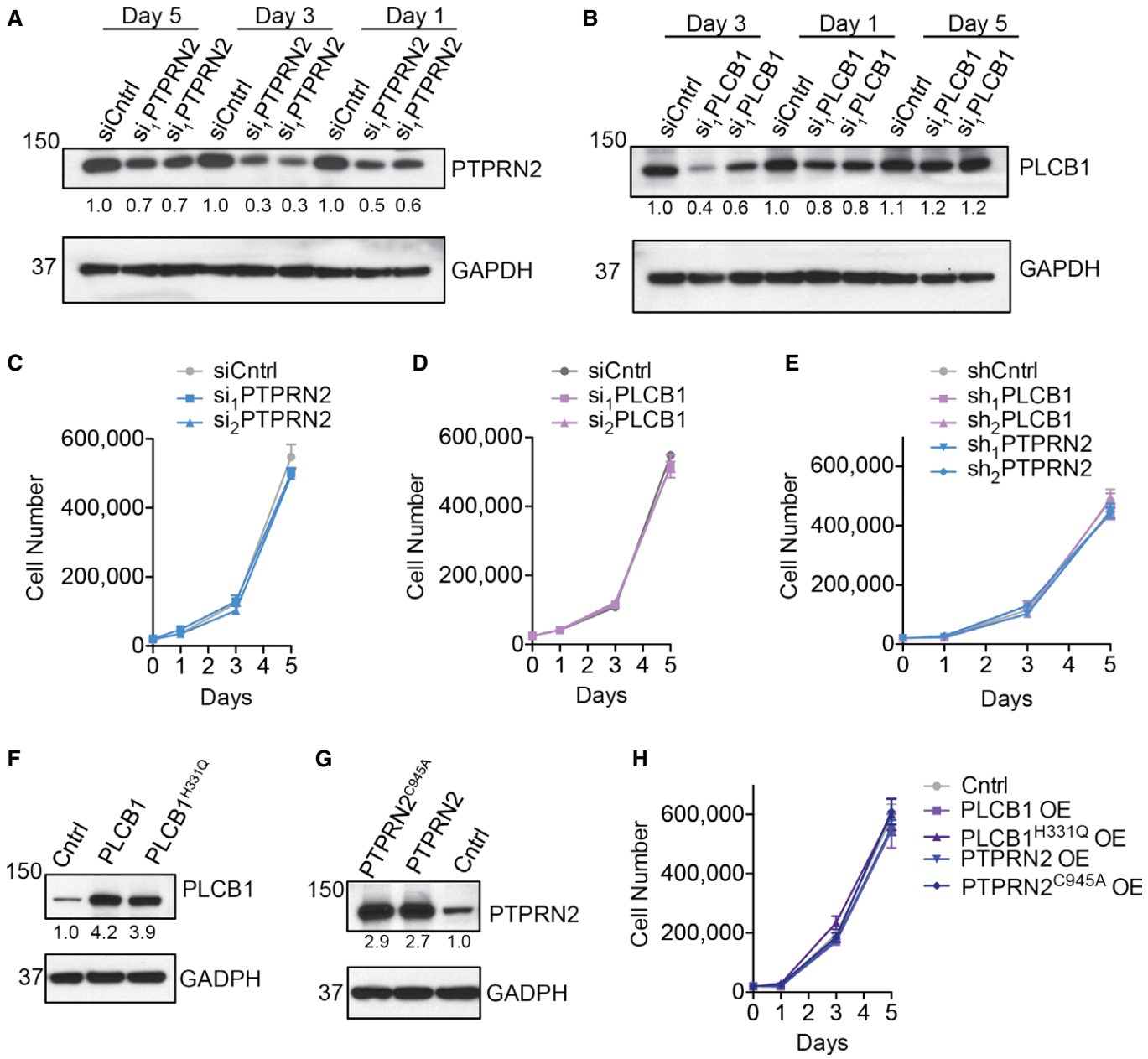

Figure EV2. PLCβ1 and PTPRN2 drive metastatic migration and invasion.

A, B Western blot analysis of LM2 cells transfected with siRNA targeting PTPRN2, PLCβ1, or a control siRNA using anti-PTPRN2 (A) and anti-PLCβ1 (B) at various time points post-transfection. GAPDH was used as a loading control. Densitometry analysis below the blots is adjusted for GAPDH levels and normalized to siCntrl values on day 1.

C, D Proliferation of 20,000 LM2 cells transfected with siRNA targeting PTPRN2 (C), PLCβ1 (D), or a control siRNA. *N* = 3 wells/group.

E Proliferation of 20,000 LM2 cells transduced with hairpins targeting PLCβ1, PTPRN2, or a control hairpin. *N* = 3 wells/group.

F, G Western blot analysis of MDA-MB-231 cells overexpressing PLCβ1, PLCβ1^{H331Q} (F), PTPRN2, PTPRN2^{C945A} (G), or a control vector using anti-PTPRN2 (F) or anti-PLCβ1 (G). GAPDH was used as a loading control. Densitometry analysis below the blots is adjusted for GAPDH levels and normalized to Cntrl values.

H Proliferation of 20,000 MDA-MB-231 cells overexpressing PLCβ1, PLCβ1^{H331Q}, PTPRN2, PTPRN2^{C945A}, or a control vector. *N* = 3 wells/group.

Data information: Error bars represent SEM.

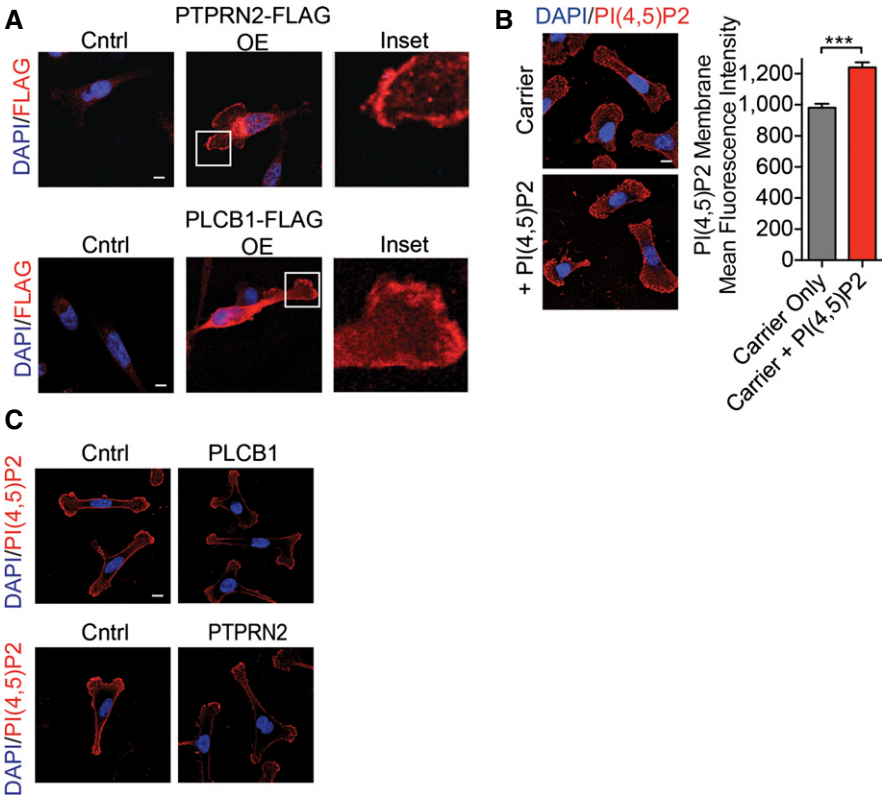

Figure EV3. PLCβ1 and PTPRN2 regulate membrane PI(4,5)P_2 levels.

A Representative images of MDA-MB-231 cells retrovirally transduced with PTPRN2-FLAG, PLCβ1-FLAG, or control vector and immunostained with anti-FLAG (red) and DAPI (blue). Scale bar, 10 μm.

B Mean fluorescence intensity of membrane PI(4,5)P_2 was analyzed in LM2 cells treated with carrier incubated with PI(4,5)P_2 or carrier alone. Cells were immunostained with anti-PI(4,5)P_2 antibody (red) and DAPI (blue) and analyzed using fluorescence microscopy. N = 50 cells/group. Scale bar, 10 μm. Error bars represent SEM. ***$P < 0.001$.

C Representative fluorescence images of MDA-MB-231 overexpressing PTPRN2, PLCβ1, or control vector immunostained with anti-PI(4,5)P_2 antibody (red) and DAPI (blue). Scale bar, 10 μm.

Figure EV4. PLCβ1 and PTPRN2 facilitate cofilin localization and activity.

A Western blot analysis of whole-cell lysate of LM2 cells transfected with a control siRNA or siRNAs targeting PTPRN2 or PLCβ1 using anti-CFL1, anti-β-actin, and anti-GAPDH.

B Western blot analysis of whole-cell lysate of MDA-MB-231 cells overexpressing PTPRN2, PLCβ1, or a control vector using anti-CFL1, anti-β-actin, and anti-GAPDH.

C LM2 cells transfected with siRNA targeting PTPRN2, PLCβ1, or a control siRNA were immunostained for CFL1 (red) and DAPI (blue). Left, quantification of membrane mean fluorescence intensity of CFL1. Right, representative images. N = 50 cells/group. Scale bar, 10 μm. Error bars represent SEM. ***$P < 0.001$.

D MDA-MB-231 cells overexpressing PTPRN2, PLCβ1, or a control vector were immunostained for CFL1 (red) and DAPI (blue). Left, quantification of membrane mean fluorescence intensity of CFL1. Right, representative images. N = 50 cells/group. Scale bar, 10 μm. Error bars represent SEM. ***$P < 0.001$.

E Representative images for Fig 5F. LM2 cells were transfected with siRNA targeting PLβ1 or a control siRNA and subjected to the barbed end assay. Cells were stained for biotin–actin (red), DAPI (blue). Scale bar, 20 μm.

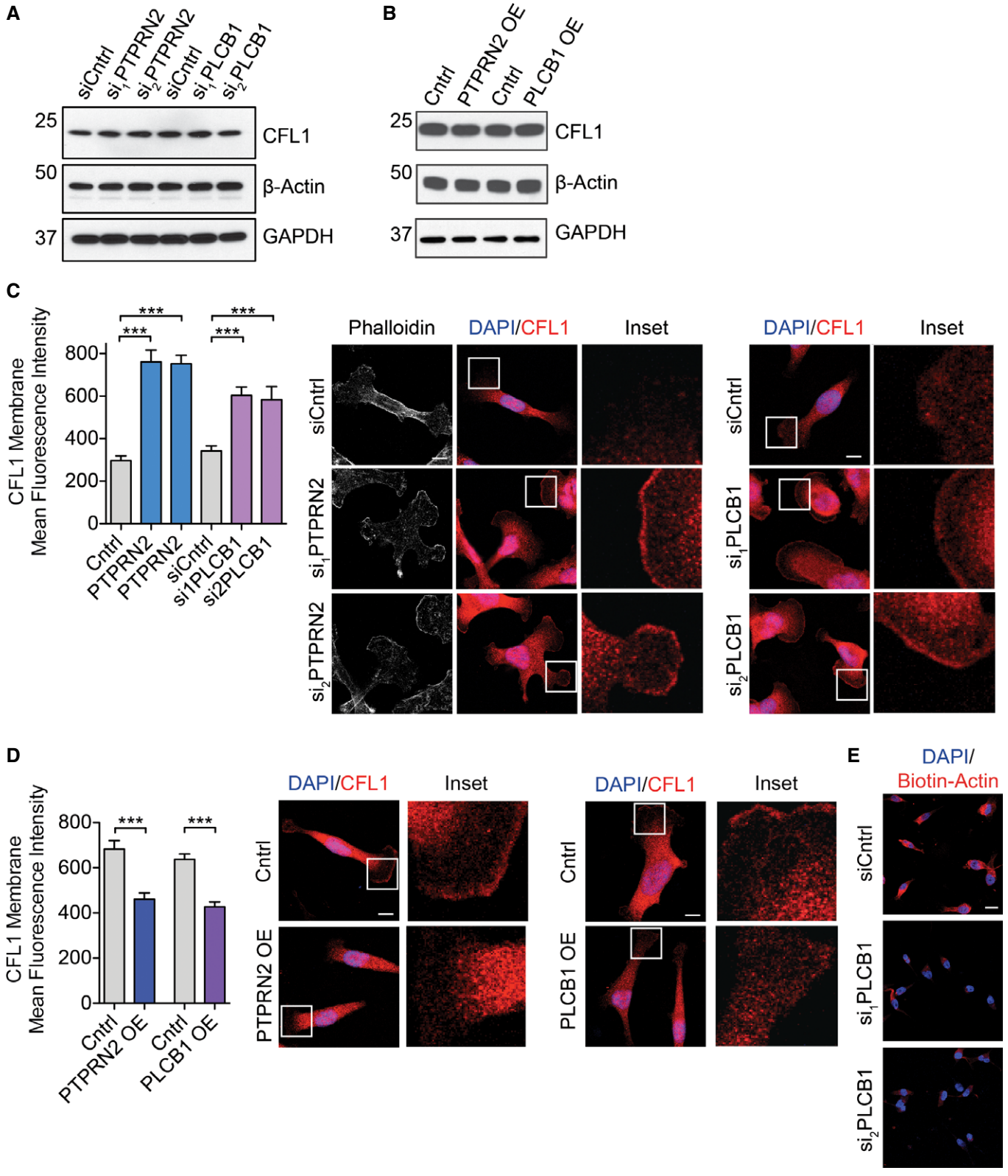

Figure EV4.

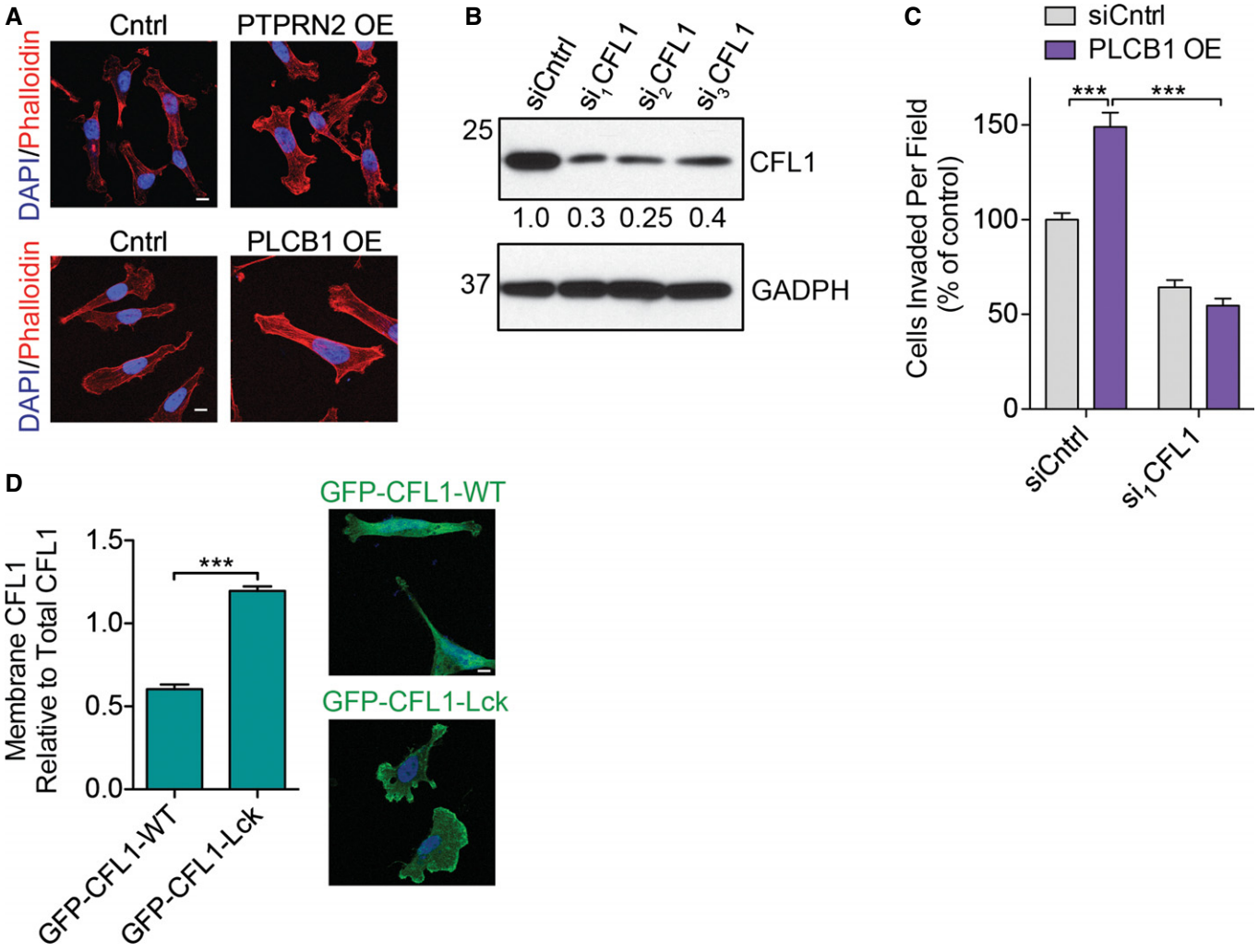

Figure EV5. PLCβ1 and PTPRN2 act upstream of cofilin-mediated actin dynamics.

A Representative images for Fig 6B. MDA-MB-231 overexpressing PTPRN2, PLCβ1, or control vector were stained with phalloidin (red) and DAPI (blue) and analyzed using fluorescence microscopy. Scale bar, 10 μm.

B Western blot analysis of MDA-MB-231 cells transfected with siRNA targeting CFL1 or a control siRNA using anti-CFL1. GAPDH was used as a loading control. Densitometry values below the blot are adjusted for GAPDH levels and normalized to siCntrl value.

C MDA-MB-231 cells were transfected with siRNAs targeting CFL1 or a control siRNA in the setting of control or PLCβ1 overexpression and subjected to the invasion assay. $N = 5$ inserts/group. Error bars represent SEM. ***$P < 0.001$.

D MDA-MB-231 were transfected with siRNA targeting the 3′ UTR of *CFL1* to deplete endogenous CFL1 and further transfected with plasmids encoding either GFP-CFL1-WT or GFP-CFL1-Lck (green) and immunostained with DAPI (blue). Left, quantification of membrane mean fluorescence intensity of GFP-CFL1 as analyzed by fluorescence microscopy. Right, representative images. $N = 50$ cells/group. Scale bar, 10 μm. Error bars represent SEM. ***$P < 0.001$.