FigS1

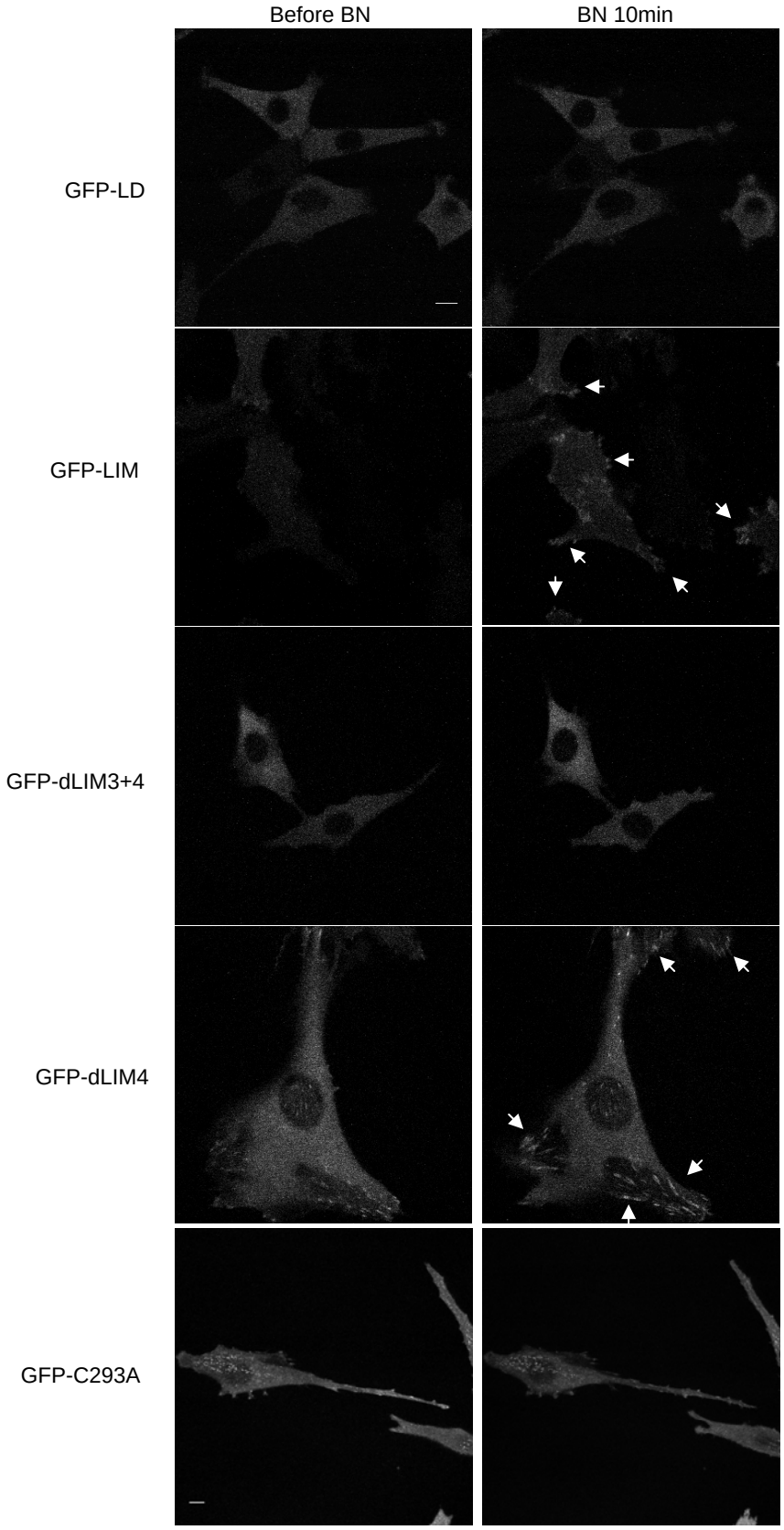

Fig. S1. LIM3 is essential for LPXN focal adhesion targeting.
BALB/c fibroblasts stably expressing GRPr and GFP-tagged LPXN constructs were imaged in real time by confocal microscopy before and after addition of BN. Results shown are the representative images. Arrows indicate translocated LPXN. Bars, 10 μm.

FigS2

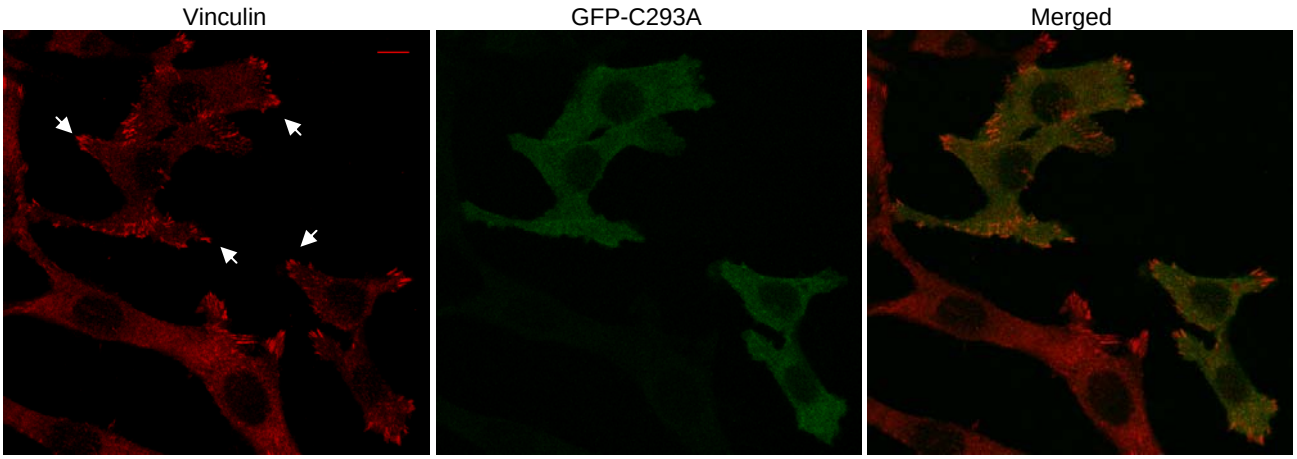

Fig. S2. BN stimulates focal adhesion formation in GFP-C293A-expressing cells. BALB/c fibroblasts stably expressing GRPr and GFP-C293A (green) were treated with 100 nM BN for 10 min, fixed and stained with anti-vinculin antibody (red). Arrows indicate some focal adhesions. Bars, 10 μm.

FigS3

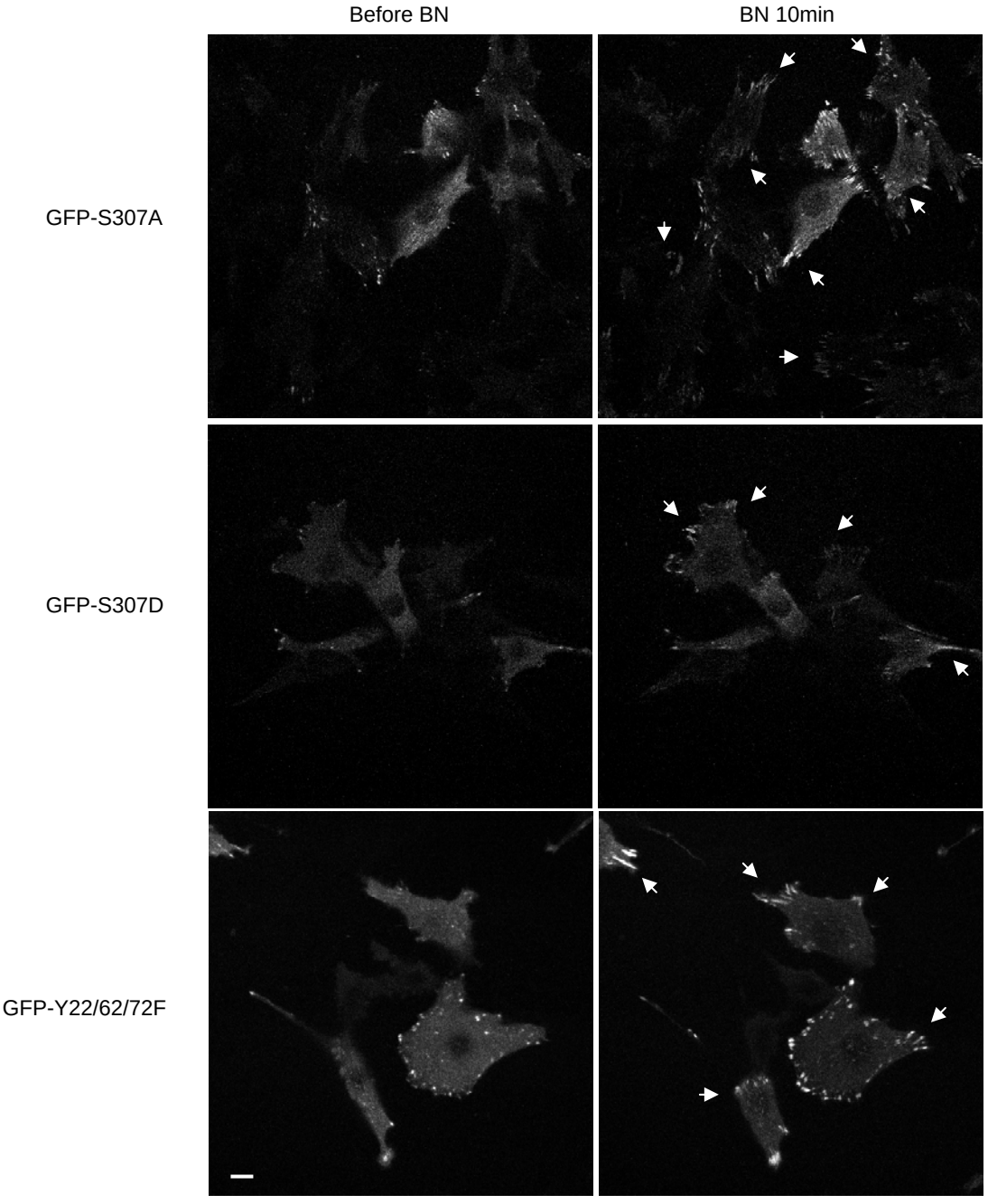

Fig. S3. Phosphorylation of S307, Y22, Y62 and Y72 is not required for LPXN FA targeting.

BALB/c fibroblasts stably expressing GRPr and GFP-tagged LPXN point mutants were imaged in real time by confocal microscopy before and after addition of BN. Results shown are representative images. Only one of the non-phosphorylatable mutants (GFP-S307A) is shown. Bars, 10 μm.

FigS4

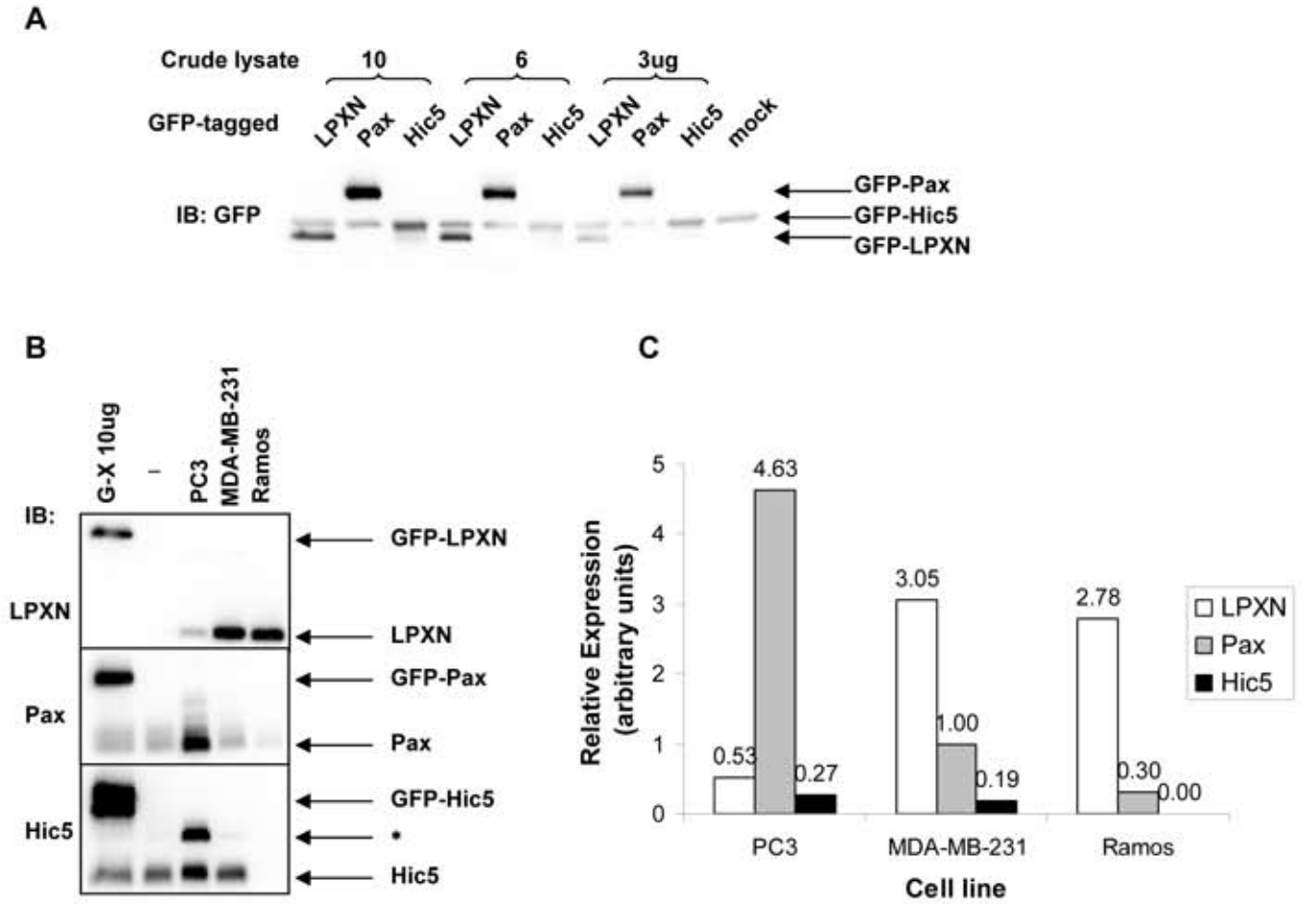

Fig. S4. Relative expression level of 3 paxillin family members in PC3, MDA-MB-231 and Ramos cells.

(A) CHOP cells were transiently transfected with plasmids expressing GFP-tagged LPXN, paxillin (Pax), or Hic5. Mock-transfected CHOP cells (mock) were included as a background control. 3, 6 or 10 μg of cell lysates were analyzed by anti-GFP immunoblotting to determine an expression ratio (GFP-LPXN: GFP-paxillin: GFP-Hic5; ratio1). (B) 10 μg of transfected CHOP cell lysates (G-X refers to GFP-LPXN (top), GFP-paxillin (middle), and GFP-Hic5 (bottom)) were resolved on SDS-PAGE concurrently with 20 μg of cell lysates from non-transfected CHOP cells (–), PC3 cells, MDA-MB-231 cells and Ramos cells. Western blotting was performed using anti-LPXN, anti-paxillin, or anti-Hic5 antibody, relative protein levels quantitated to obtain the ratio between GFP-tagged and endogenous protein (G-X: X; ratio2) as well as the relative expression of one family member among three cell lines (XPC3: XMDA-MB-231: XRamos; ratio3). * Paxillin detected by anti-Hic5 antibody. (C) Using ratios 1-3 generated from (A, B), relative expression levels of endogenous LPXN, paxillin and Hic5 were calculated. Briefly, a comparison of endogenous expression of a family member with its GFP-tagged construct was made using a family member specific antibody (ratio2), then a comparison of GFP-tagged construct with each other using a GFP antibody (ratio1). Finally, a comparison of one family member level among three cancer cell lines was made (ratio3). Numbers above the bars refer to relative endogenous protein levels of paxillin family members.

FigS5

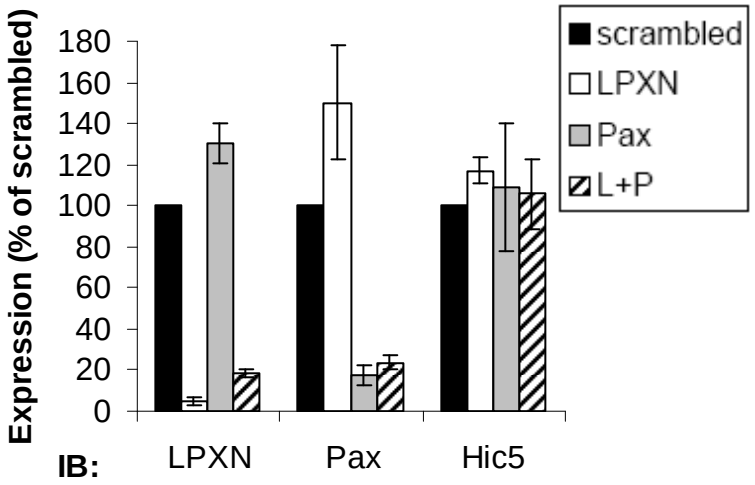

Fig. S5. siRNA-mediated knockdown of LPXN or/and paxillin in MDA-MB-231 cells. MDA-MB-231 cells were transiently transfected with scrambled, LPXN, paxillin (Pax) or both LPXN and paxillin (L+P) siRNA duplexes as described under *Materials and Methods*. 20 μg of cell lysates were resolved on SDS-PAGE, subjected to anti-LPXN, anti-paxillin or anti-Hic5 Western blotting, and expression of each protein quantified relative to scrambled siRNA-treated cells. The mean ± s.e.m. from six experiments is shown.