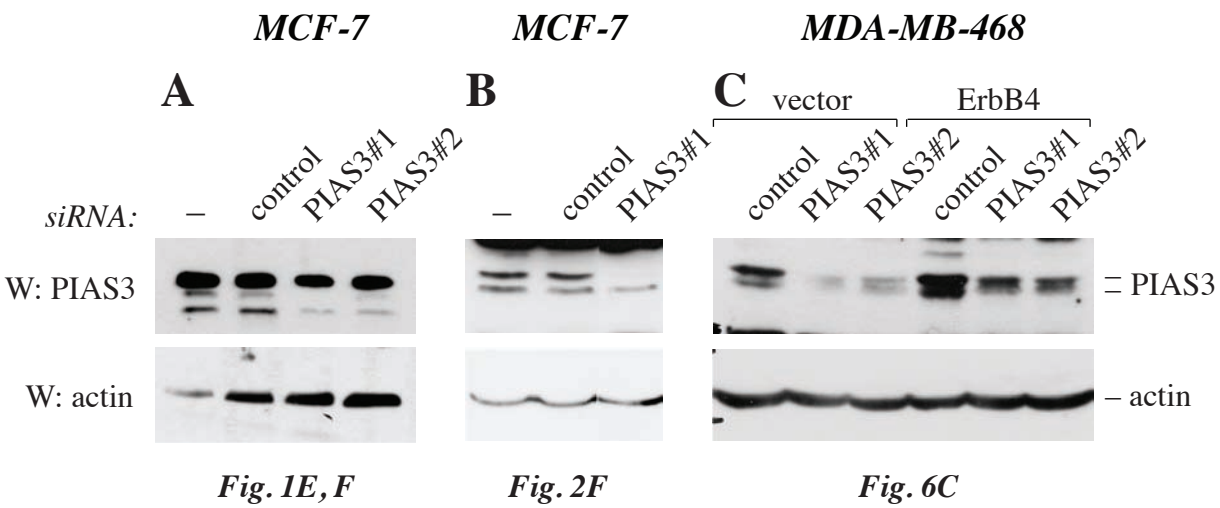

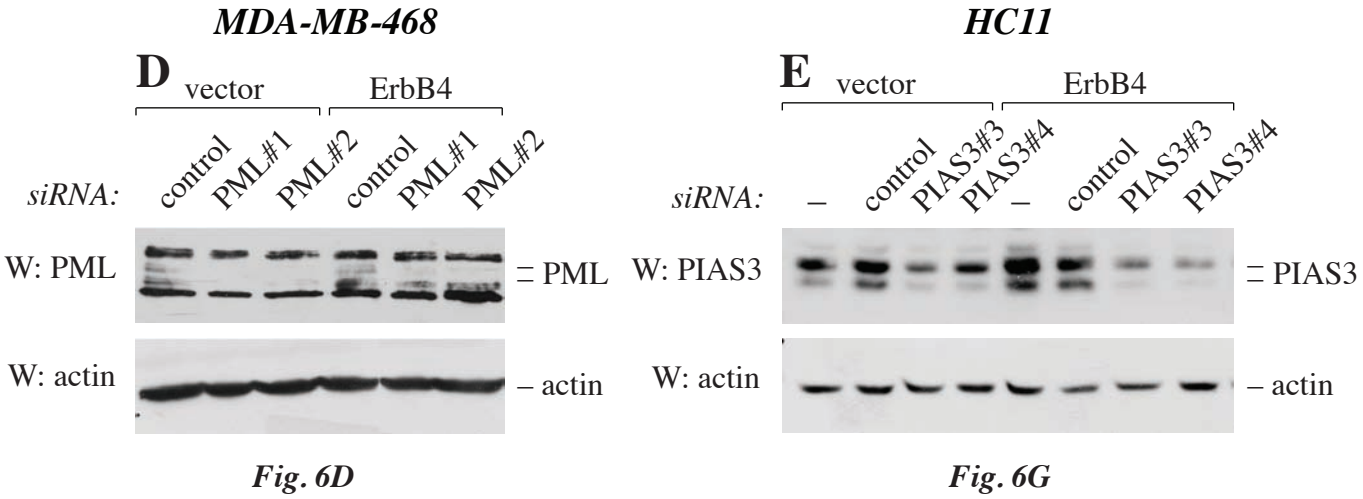

Figure S1. Effect of RNA interference on target protein expression. MCF-7, MDA-MB-468, and HC11 cells were transfected with siRNAs targeting PIAS3 or PML, or with non-specific control siRNAs, and analyzed for expression of PIAS3 or PML, and actin, as described in Experimental Procedures. The Western analyses were carried out simultaneously with the experimentation described in the depicted figures; *i.e.* A) corresponds to Fig. 1E and F, B) to Fig. 2F, C) to Fig. 6C, D) to Fig. 6D, and E) to Fig. 6G.

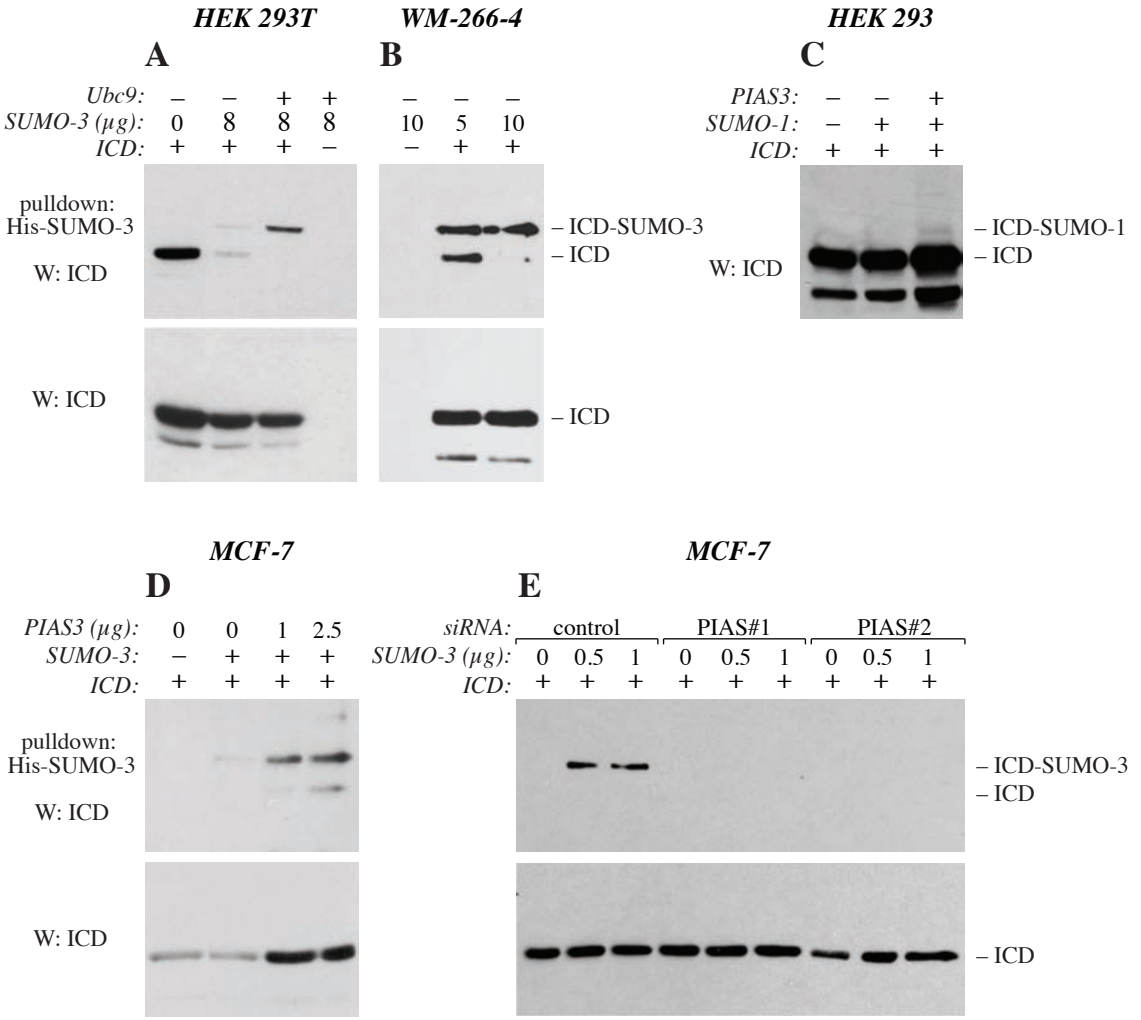

Figure S2. Sumoylation of ErbB4 ICD. A) HEK 293T cells were transfected with constructs encoding ICD2-Myc (1), His-SUMO-3, and/or Ubc9 (kindly provided by Dr. Erik Meulmeester, (2)) as indicated. Cells were lysed in a denaturing buffer (8 M urea, 0.1 M Na_2HPO_4/NaH_2PO_4, 10 mM Tris-HCl pH 7.0, 10 mM imidazole, 10 mM β-mercaptoethanol), and lysates were incubated with Ni-NTA agarose (Qiagen) to pull down His-SUMO conjugates. After extensive washing, conjugates were eluted with elution buffer (200 mM imidazole, 5 % SDS, 150 mM Tris-HCl pH 6.8, 30 % glycerol, 720 mM β-mercaptoethanol) and analyzed for sumoylation of ErbB4 ICD by Western blotting with anti-ErbB4 (sc-283). B) WM-266-4 cells expressing ICD2-HA with or without His-SUMO-3 were analyzed as described in (A) with anti-ErbB4 (E200; Abcam). C) HEK 293 cells expressing ICD2-HA, His-SUMO-1, and/or Flag-PIAS3 were analyzed for ErbB4 sumoylation as described in Experimental Procedures. D) MCF-7 cells expressing ICD2-HA, His-SUMO-3, and/or Flag-PIAS3 were analyzed as described in (A) with anti-HA (ab18181; Abcam). E) MCF-7 cells were cotransfected with indicated siRNAs together with plasmids encoding ICD2-HA and His-SUMO-3, using Lipofectamine 2000 according to the manufacturer's instructions. Sumoylated ErbB4 ICD was detected with anti-HA (ab18181; Abcam).

References:

1. Sundvall, M., Peri, L., Määttä, J. A., Tvorogov, D., Paatero, I., Savisalo, M., Silvennoinen, O., Yarden, Y., and Elenius, K. (2007) *Oncogene* ***26,*** 6905–6914

2. Meulmeester, E., Kunze, M., Hsiao, H. H., Urlaub, H., and Melchior, F. (2008) *Mol. Cell* ***30,*** 610–619

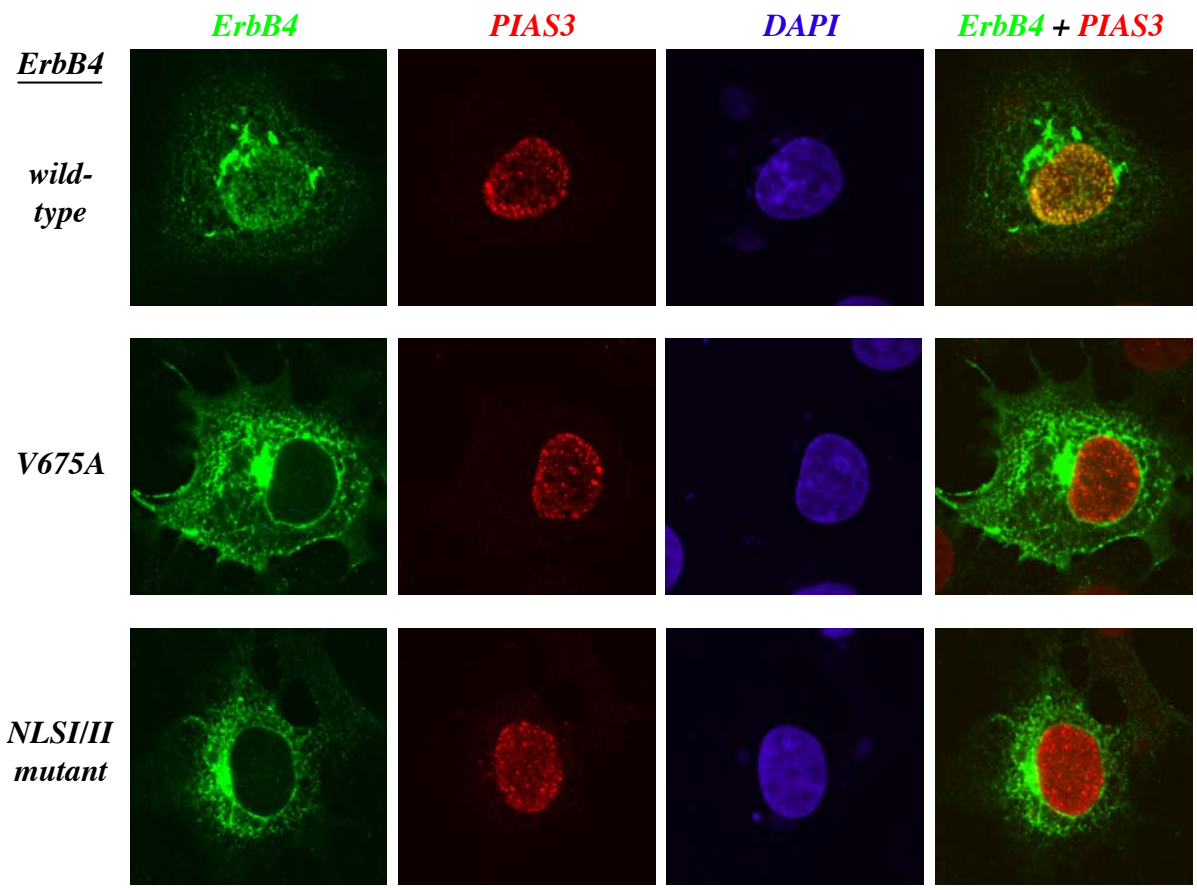

Figure S3. Effect of ErbB4 cleavage and NLS on PIAS3-promoted nuclear sequestration. COS-7 cells were transfected with HA-tagged constructs of wild-type ErbB4 JM-a CYT-2, a mutant ErbB4 not susceptible to RIP (V675A), or ErbB4 with mutated nuclear localization signal (NLSI/II mutant), together with Flag-tagged PIAS3, and stained with anti-ErbB4 (HFR-1; green) and anti-PIAS3 (AV32762, Sigma-Aldrich; red). The nuclei were stained with DAPI (blue). The cells were visualized by confocal microscopy with a 100x objective.

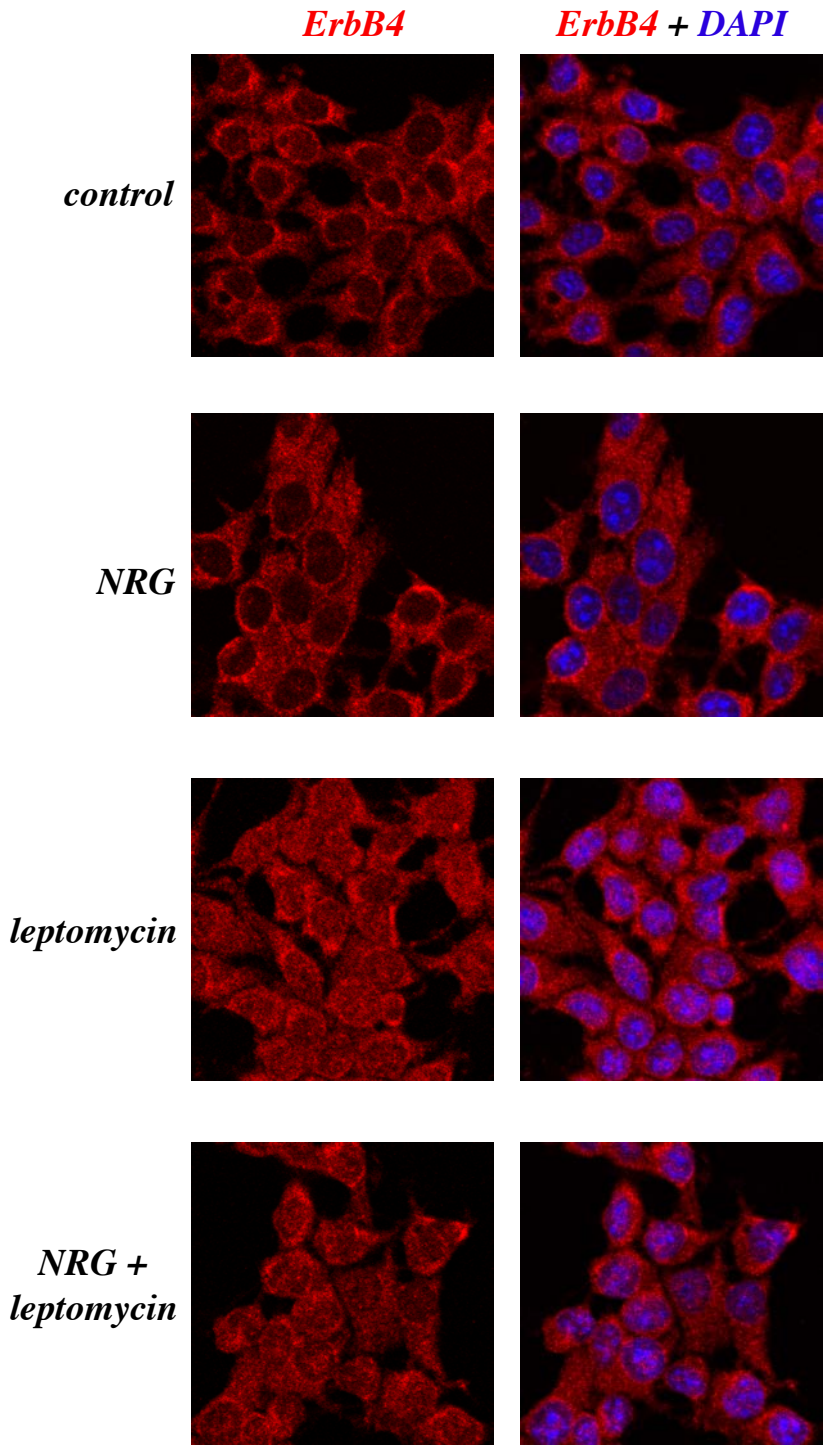

Figure S4. Subcellular localization of ErbB4 in MCF-7 cells. MCF-7 cells were treated for 3 hours with 0 or 25 ng/ml leptomycin B and for 45 min with 0 or 50 ng/ml NRG-1, and stained with anti-ErbB4 (HFR-1; red). The nuclei were stained with DAPI (blue). The cells were visualized by confocal microscopy with a 40x objective.

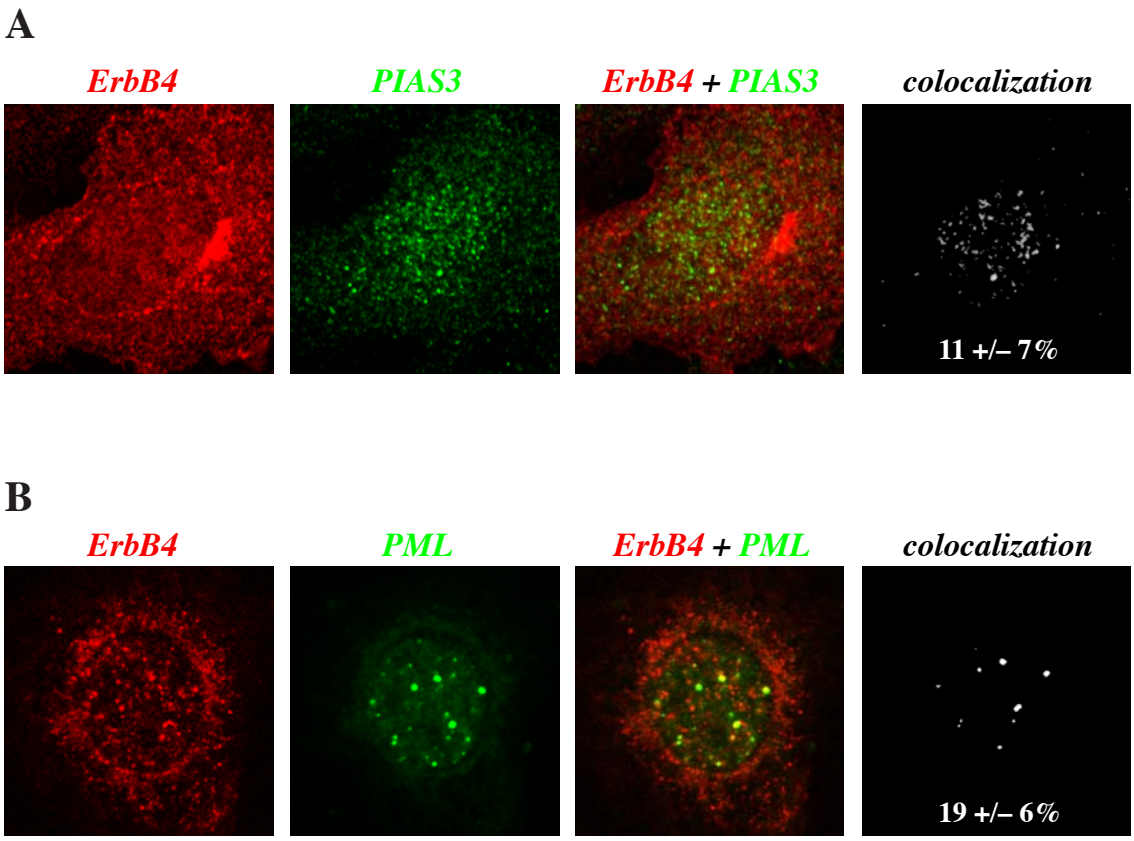

Figure S5. Colocalization of ErbB4 with endogenous PIAS3 and PML. A) HC11 cells retrovirally expressing HA-tagged ErbB4 JM-a CYT-2 were treated for 15 min with 50 ng/ml NRG-1, and stained with anti-HA (ErbB4; red) and anti PIAS3 (ab22856; green). Immunofluorescence signals were visualized by confocal microscopy with a 100x objective. In quantitative colocalization analysis, 17 +/- 7 % of nuclear ErbB4 colocalized with nuclear PIAS3, and 11 +/- 7% of nuclear PIAS3 colocalized with nuclear ErbB4 (n = 15). Colocalization analysis was carried out with BioimageXD (1). Data are described as colocalization coefficients after Manders et al (2). B) HC11 cells expressing ErbB4 JM-a CYT-2 were stained with anti-ErbB4 (HFR-1; red) and anti-PML (Millipore; green). Quantitative colocalization analysis demonstrated that 19 +/- 6 % of PML signal was also positive for ErbB4 (n = 10) .

References:

1. Kankaanpää P., Pahajoki K., Marjomäki V., Heino J., White DJ. (2006) BioImageXD – free open source software for analysis and visualization of multidimensional biomedical images. (http://www.bioimagexd.net)
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