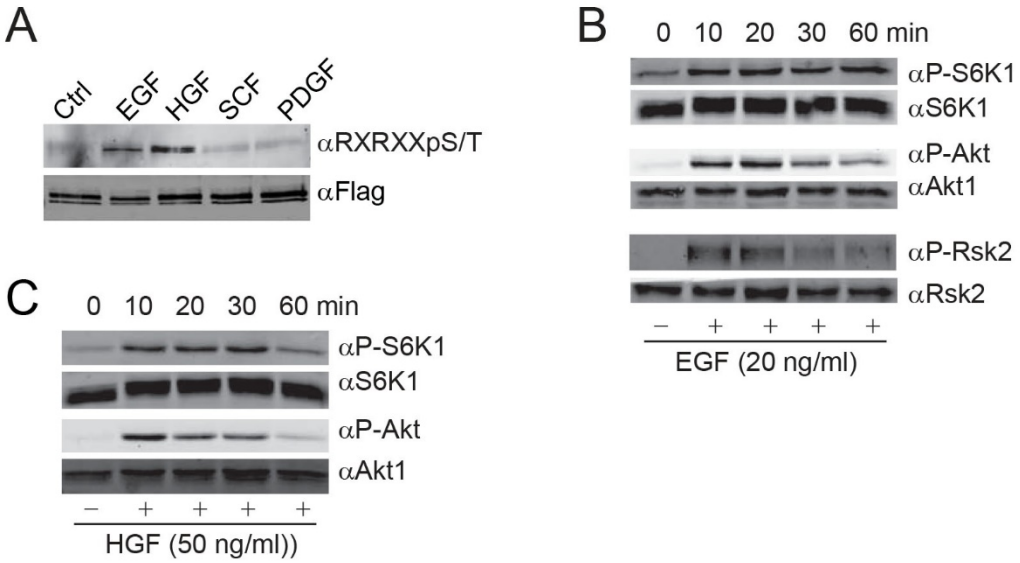
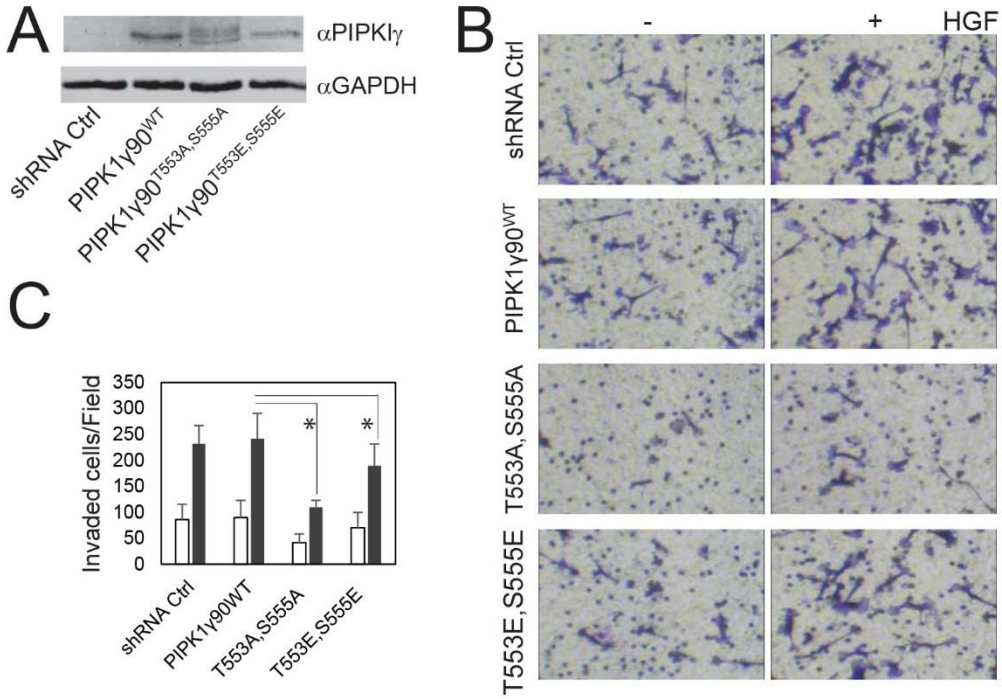
Jafari et al. Supplementary Figures S1-S3

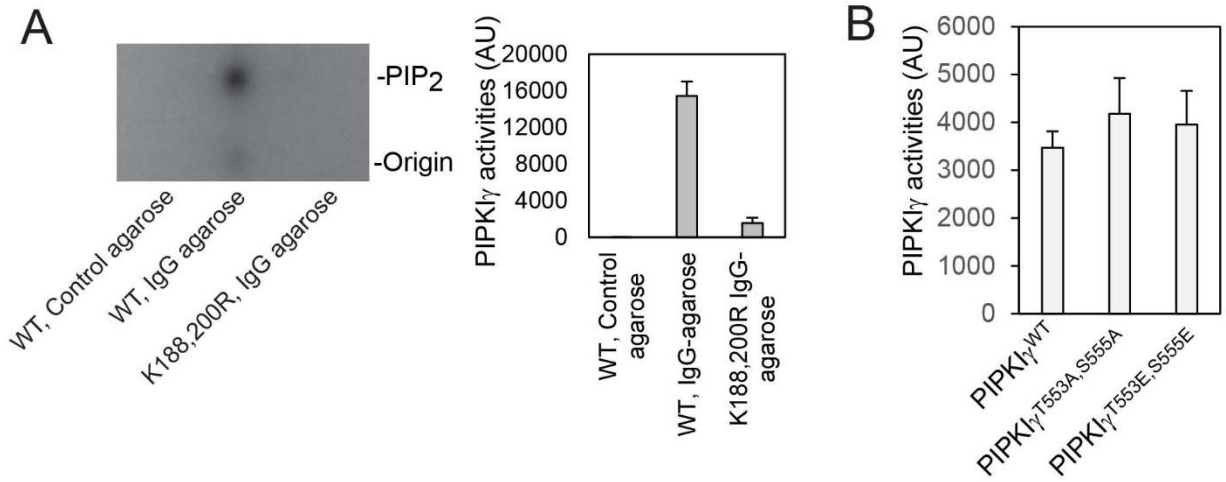


Supplementary Fig. S1. HGF and EGF stimulate PIPKI90 phosphorylation. A. MDA-MB-468 cells that stably express Flag-PIPKIγ90 were serum-starved and stimulated with EGF (20 ng/ml), HGF (50ng/ml), SCF (20 ng/ml) and PDGF (20 ng/ml) for 20 min, respectively. Flag-PIPKIγ90 was immunoprecipitated and the phosphorylation was detected with an anti-RXRXXpS/T motif antibody. **B.** Time-course of Akt, S6K1 and Rsk2 activation in EGF-stimulated MDA-MB-231 cells. **C.** Time-course of Akt and S6K1 activation in HGF-stimulated MDA-MB-231 cells.



Supplementary Fig. S2. Overexpression of PIPKIγ90^{T553A,S555A} in MDA-MB-231 cells suppressed cell invasion. A. Expression of ZZ-PIPKIγ, -PIPKIγ^{T553A,S555A}, or -PIPKIγ^{T553E,S555E} in parental MDA-

MB-231 cells. MDA-MB-231 cells were infected with retroviruses that express ZZ-PIPKIγ90, -PIPKIγ90^{T553A,S555A}, or PIPKIγ90^{T553E,S555E}, and then selected with neomycin. **B.** MDA-MB-231 cells that express ZZ-PIPKIγ90, ZZ-PIPKIγ90^{T553A,S555A}, and ZZ-PIPKIγ90^{T553E,S555E}, respectively, were examined for their Matrigel invasive activities in the absence and presence of HGF. **C.** Quantification of Experiment "B". White bar, without HGF, grey bar, 20 ng/ml HGF. Data are presented as mean±SEM , n=3. *P<0.05.



Supplementary Fig. S3. Mutation at T553 and S555 did not affect PIPKIγ activity in vitro. **A.** ZZ-PIPKIγ90^{WT} and ZZ-PIPKIγ90^{K188,200R} were transfected into CHO-K1 cells, respectively, and immunoprecipitated with IgG-conjugated-agarose beads or protein A agarose. The activities of PIPKIγ and the mutants were determined using PI(4)P and [γ-^{32}P]ATP as substrates. **B.** ZZ-PIPKIγ, -PIPKIγ^{T553A,S555A}, and -PIPKIγ^{T553E,S555E} were immunoprecipitated using IgG-Agarose beads from CHO-K1 cells. The activities of PIPKIγ90 and mutants were determined as described above.