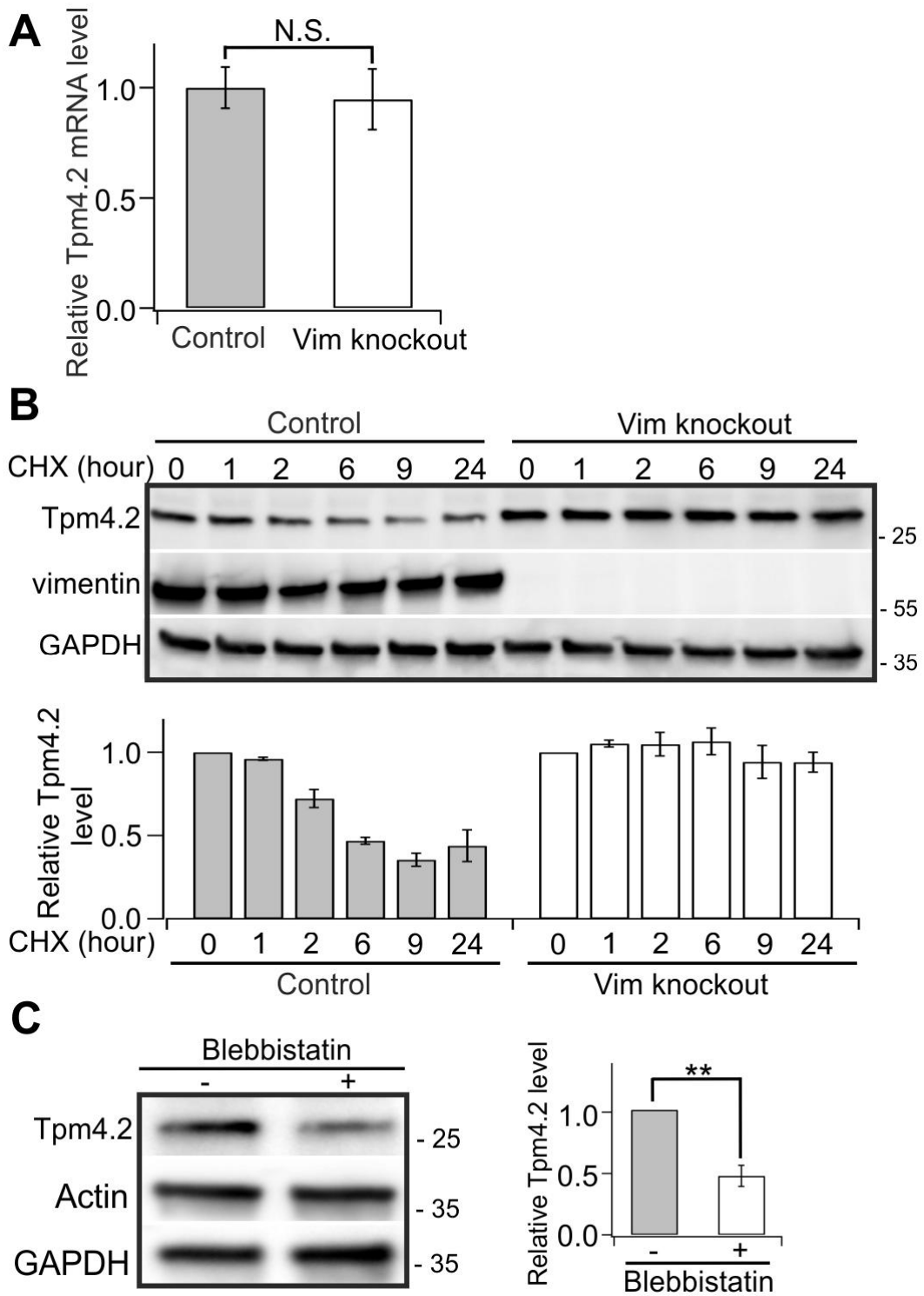
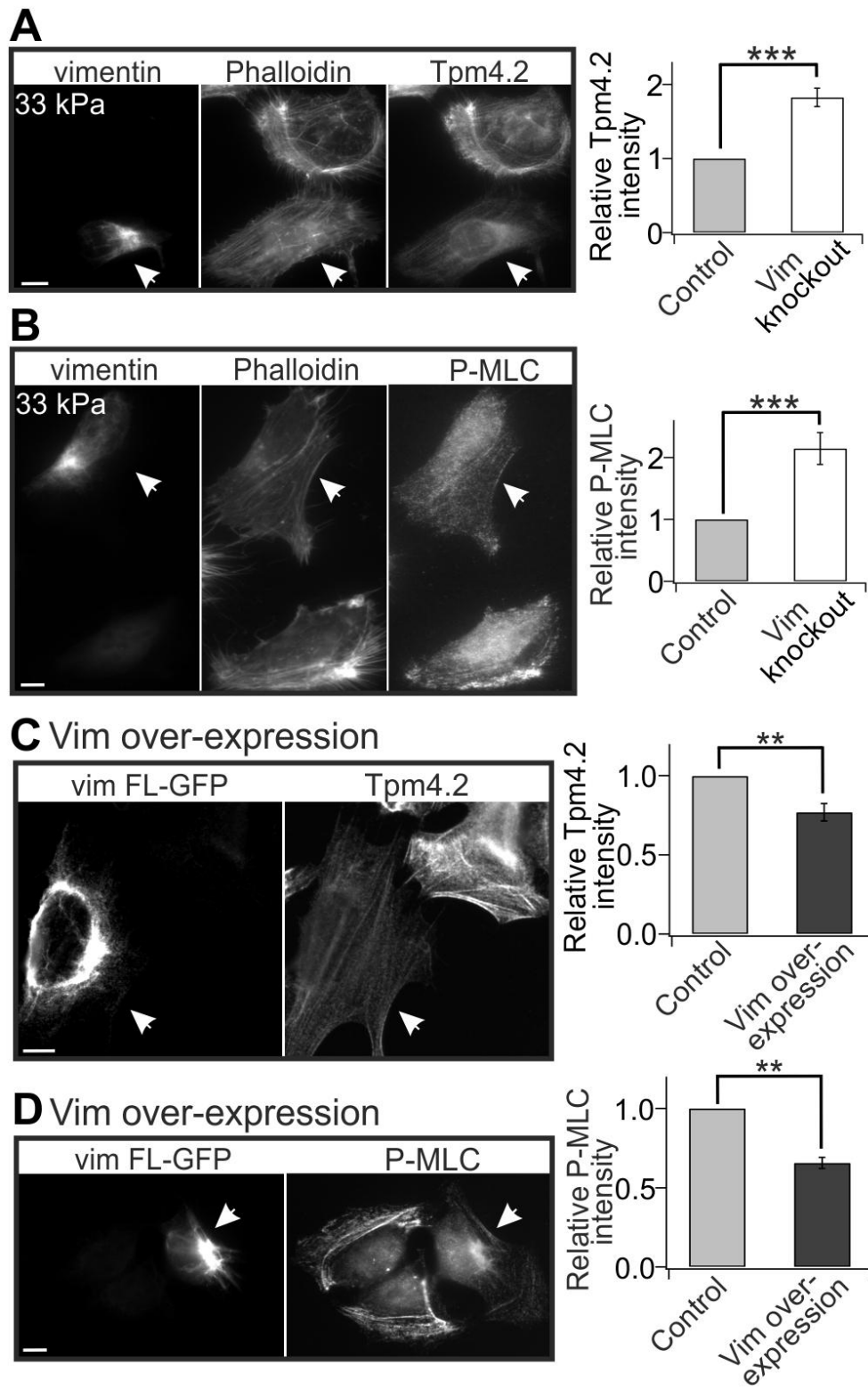


Figure S1



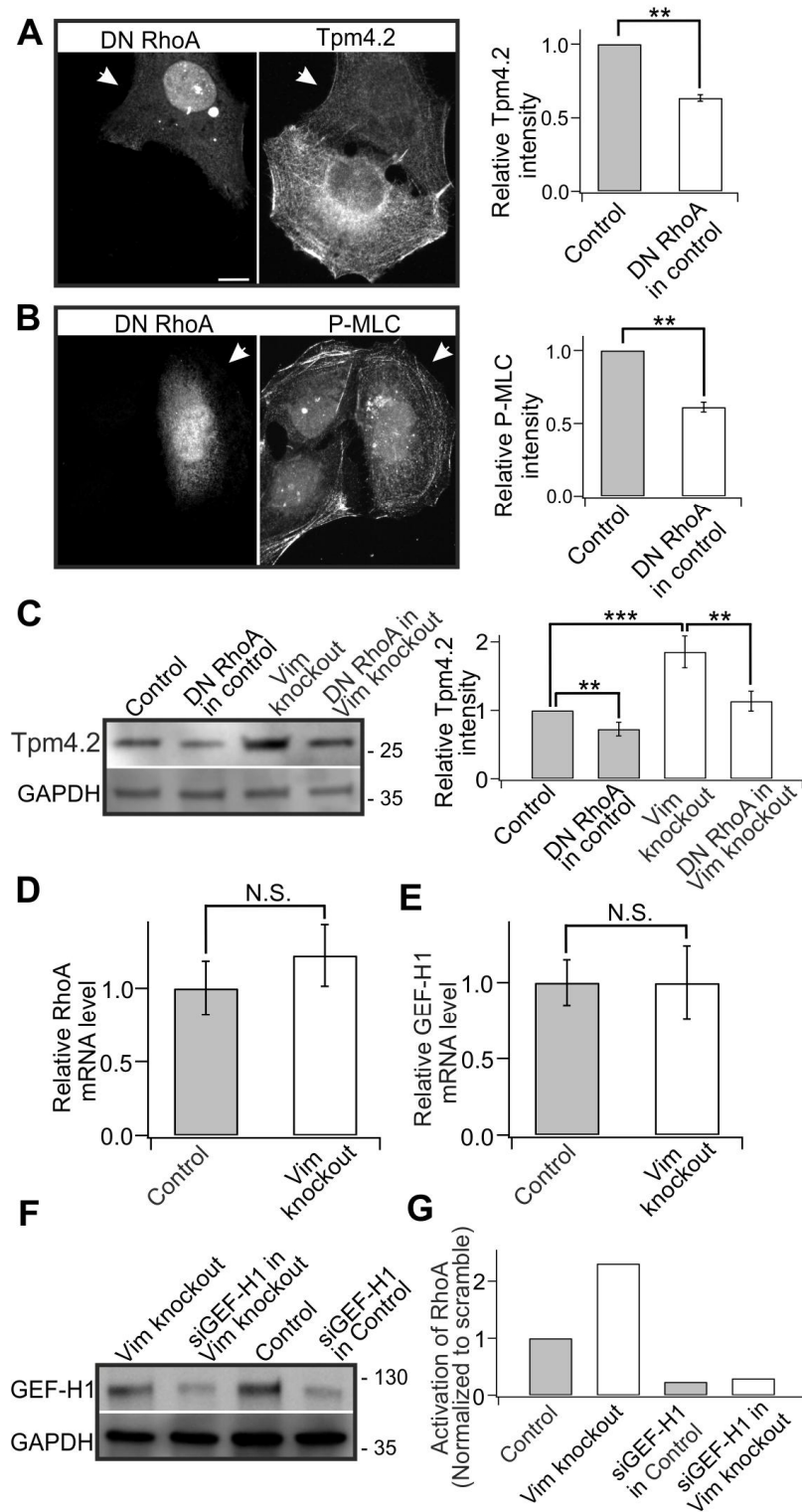
**Figure S1. Vimentin depletion results in diminished turnover of Tpm4.2 protein.** (A). Quantitative RT-PCR (qRT-PCR) analyses of Tpm4.2 mRNA levels in control and vimentin knockout cells. The data are from three independent experiments. (B). Control and vimentin knockout cells were treated with cycloheximide (CHX) and the total Tpm4.2 levels were analyzed by Western blot at the indicated time points. The blot was also probed with vimentin antibody to confirm that the vimentin knockout U2OS cell culture is not contaminated by wild-type U2OS cells, and with GAPDH antibody to control equal sample loading. Molecular weights in kilodaltons (kDa) are indicated. Relative levels of Tpm4.2 normalized to internal control GAPDH from three Western blots are shown in the graph below the blots. (C). Western blot analysis of Tpm4.2 levels in control U2OS cells and in U2OS cells incubated for 30 minutes with 10  $\mu$ M blebbistatin. The blot was also probed with actin, and GAPDH antibody to verify equal sample loading. Molecular weights in kilodaltons (kDa) are indicated. Panel on the right shows the quantification of relative levels of Tpm4.2 normalized to internal control GAPDH from three Western blots. \*\* $P < 0.01$  (paired *t*-test). The data are presented as mean  $\pm$  SEM. N.S. = not significant.

Figure S2



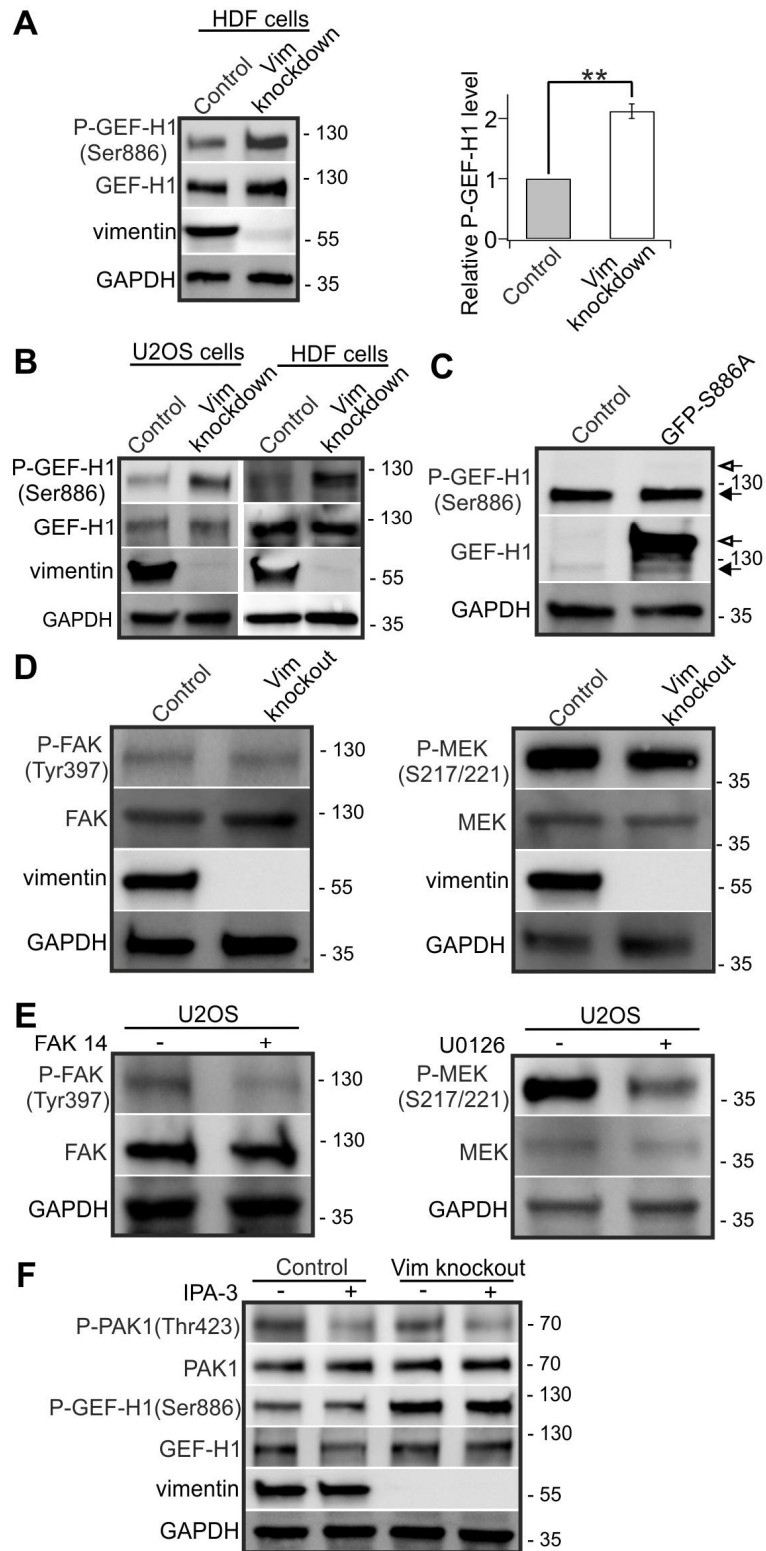
**Figure S2. Effects of vimentin on Tpm4.2 and P-MLC levels. (A, B).** Vimentin depletion results in increased Tpm4.2 and P-MLC levels also in a more compliant matrix. Panels on the left show representative images of control (indicated by arrows) and vimentin-depleted cells that were co-cultured on same plates and stained with Tpm4.2 (A) and P-MLC (B) antibodies, respectively. Panels on the right show the quantification of normalized relative Tpm4.2 (26 control cells from 9 images and 28 vimentin knockout cells from 9 images) and P-MLC (25 control cells from 9 images and 28 vimentin knockout cells from 9 images) fluorescent intensities. Mean intensity values of control and vimentin knockout cells from each image were used for statistical analysis. \*\*\* $P < 0.001$  (paired  $t$ -test). **(C, D).** Over-expression of FL-vimentin-GFP in control U2OS cells results in a decrease in both Tpm4.2 (C) and P-MLC (D) intensities. Panels on the left show representative images of non-transfected and vimentin-GFP expressing (marked with arrows) cells. Panels on the right show the quantification of normalized relative Tpm4.2 (36 control cells from 9 images and 38 vimentin knockout cells from 9 images) and P-MLC (32 control cells from 9 images and 31 vimentin knockout cells from 9 images) fluorescent intensities. Mean intensity values of control and vimentin over-expression cells from each image were used for statistical analysis. \*\* $P < 0.01$  (paired  $t$ -test). The data are presented as mean  $\pm$  SEM. Scale bars, 10  $\mu$ m.

**Figure S3**



**Figure S3. Interplay between vimentin, GEF-H1, and RhoA.** (A, B). Expression of dominant negative (DN) RhoA causes a decrease in Tpm4.2 (A) and P-MLC (B) levels in control U2OS cells. Panels on the left show representative examples of DN RhoA expressing cells (indicated by arrows). Panels on the right show the quantification of normalized relative Tpm4.2 (A, 25 control cells from 8 images and 29 DN RhoA expressing cells from 8 images) and P-MLC (B, 31 control cells from 9 images and 27 DN RhoA expressing cells from 9 images) fluorescence intensities. Mean intensity values of control and DN RhoA over-expression cells from each image were used for statistical analysis.  $**P < 0.01$  (paired *t*-test). (C). Western blot analysis verifying that DN RhoA results in a decrease in Tpm4.2 levels in both control U2OS and vimentin knockout cells. The blot was also probed with GAPDH antibody to verify equal sample loading. Molecular weights in kilodaltons (kDa) are indicated. Panel on the right shows the quantification of relative levels of Tpm4.2 normalized to internal control GAPDH from three Western blots.  $**P < 0.01$ ,  $***P < 0.001$  (paired *t*-test). (D, E). qRT-PCR analyses of RhoA (C) and GEF-H1 (D) mRNA levels in control and vimentin knockout cells. The data are from three independent experiments. (F). Western blot analysis verifying that GEF-H1 was efficiently depleted by GEF-H1 siRNA oligonucleotide (that is a different from the one used in Fig. 4) in both control and vimentin knockout cells. The blot was also probed with GAPDH antibody to verify equal sample loading. (G). G-LISA analysis of the levels of active RhoA in GEF-H1 silenced control and vimentin knockout cells generated by GEF-H1 siRNA oligonucleotides used in (E). The data are presented as mean  $\pm$  SEM. N.S. = not significant. Scale bars, 10  $\mu$ m.

**Figure S4**



**Fig S4. GEF-H1 and kinases control experiments.** (A). Western blot analysis of GEF-H1 phosphorylated on Ser886 and total GEF-H1 levels in control and vimentin knockdown HDF cells generated using an siRNA pool as in Fig. 1B and C. Panel on the right shows the quantification of normalized relative levels of P-GEF-H1 (Ser886) compared to total GEF-H1 levels from five Western blots and presented as mean  $\pm$  SEM. \*\* $P < 0.01$  (paired *t*-test). (B). A different vimentin siRNA oligonucleotide (compared to the siRNA pool used in experiments presented in panel A) was applied to verify the vimentin knockout/knockdown induced increase in the levels of Ser886 phosphorylated GEF-H1 in both U2OS and HDF cells. (C). Western blot analysis of control cells over-expressing phospho-deficient GFP-GEF-H1 (S886A) mutant demonstrates the specificity of the P-GEF-H1 (Ser886) antibody. Black arrows show the position of endogenous GEF-H1 (~120 kDa) and hollow arrows show the position of GFP-GEF-H1 (S886A) mutant (~147 kDa). (D). Vimentin depletion drastically affected neither the total protein levels nor the levels of active FAK (left panel) and MEK (right panel). (E). Western blots verified the efficiencies of inhibitors for FAK (FAK-14, left panel) and its downstream kinases MEK1 and MEK2 (U0126, right panel), respectively. (F). Western blot analysis of P-GEF-H1 (Ser886) levels in control and vimentin knockout U2OS cells incubated in the presence or absence of PAK1 inhibitor IPA-3. The blot was probed with P-PAK1 (Thr423) and PAK1 antibodies to verify the inhibitor efficiency. The blots in (D, E, F) were probed with vimentin antibody to confirm that the vimentin knockout cell cultures were not contaminated by wild-type cells, and with GADPH antibody to verify equal sample loading. Molecular weights in kilodaltons (kDa) are indicated.