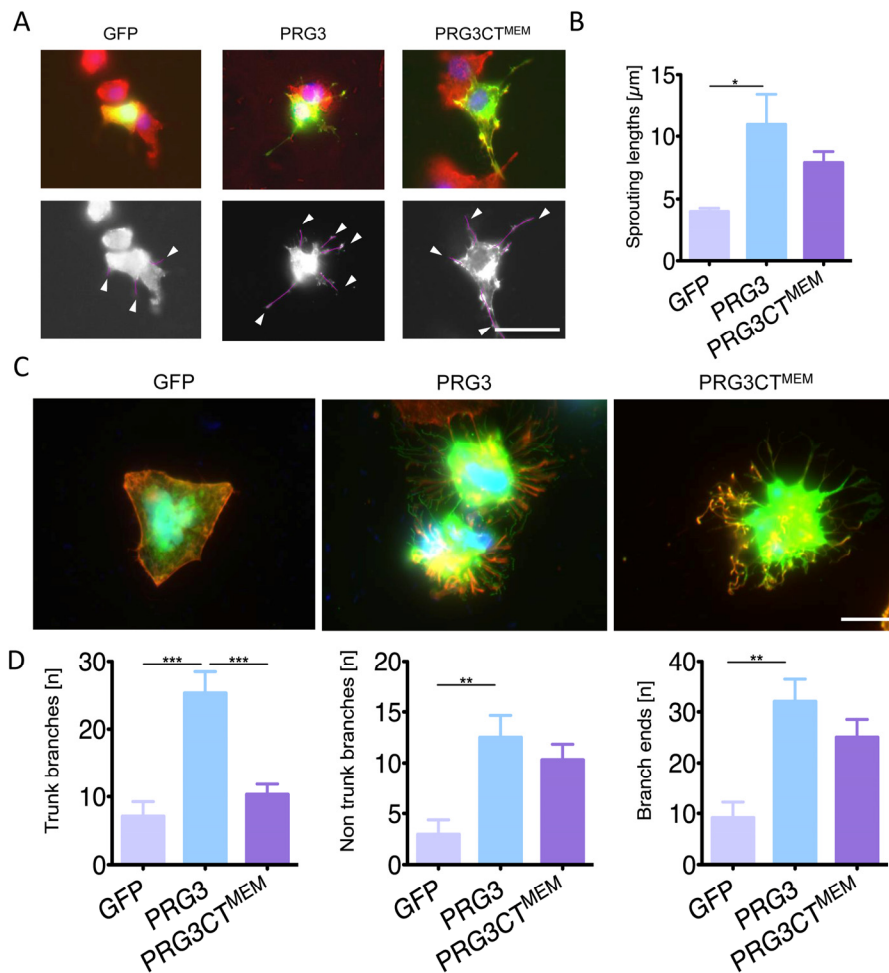


**Supplementary Figure S2. PRG3 and actin staining in primary cortical neurons.** Primary neurons were stained positive for PRG3 in primary mixed neuron-astrocyte cultures. In comparison, astrocytes were negative for PRG3 immunostaining (Scale bar Neuron = 20 $\mu$ m, scale bar astrocyte = 50  $\mu$ m).

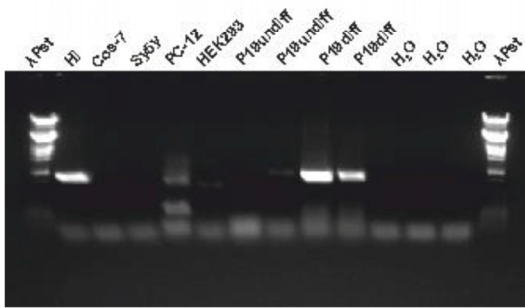


**Supplementary Figure S3. PRG3 promotes neurite-like sprouting and hedgehog-like morphology in non-neuronal cells.** (A) P19 embryonic carcinoma cells expressing PRG3 and PRG3<sup>MEM</sup> show long membrane protrusions (upper row). Traces of outgrowing protrusions for quantification are shown in bottom row (arrows). Scale bar represents 10 μm. (B) Quantification of neurite-like membrane protrusions (sprouting length) in GFP, PRG3 and PRG3CT<sup>MEM</sup> expressing P19 cells. Three independent experiments were carried out and differences were considered statistically significant with values mean ± SEM (one way anova, Bonferroni correction, multiple groups comparison; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. (C) PRG3 and PRG3CT<sup>MEM</sup> induce hedgehog-like morphological phenotype in HeLa cells. Representative images of GFP transfected cells (GFP), PRG3 overexpressing HeLa cells (PRG3) and membrane targeted C-terminus of PRG3 (PRG3CT<sup>MEM</sup>). Scale bar equals 10 μm. (D) Quantitative analysis of hedgehog-like extensions in GFP, PRG3, and PRG3CT<sup>MEM</sup> expressing HeLa cells. Measurements of trunk branches (close to cell bodies), non-trunk branches (peripheral branches) and tip branches are given. Statistical analysis was performed with one way anova (Bonferroni correction for multiple comparisons from three independent experiments); \* p<0.05, \*\* p<0.01, \*\*\* = p<0.001. Error bars are given as ± SEM of each group.

A



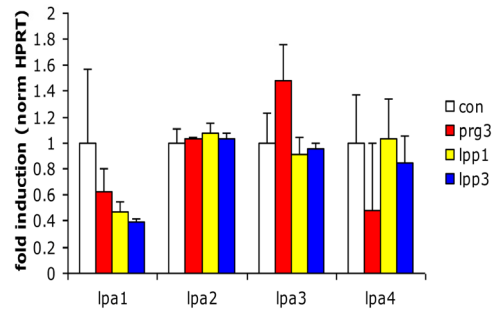
B



**Supplementary Figure S4. RasGRF1 siRNA knockdown control and cell line PRG3 expression profiling.** (A) Control RasGRF1 immuno-pulldown shows effective RasGRF1 knockdown after transfection (B) mRNA expression of PRG3 in different cell lines, hippocampal tissue (Hi) served as positive control.

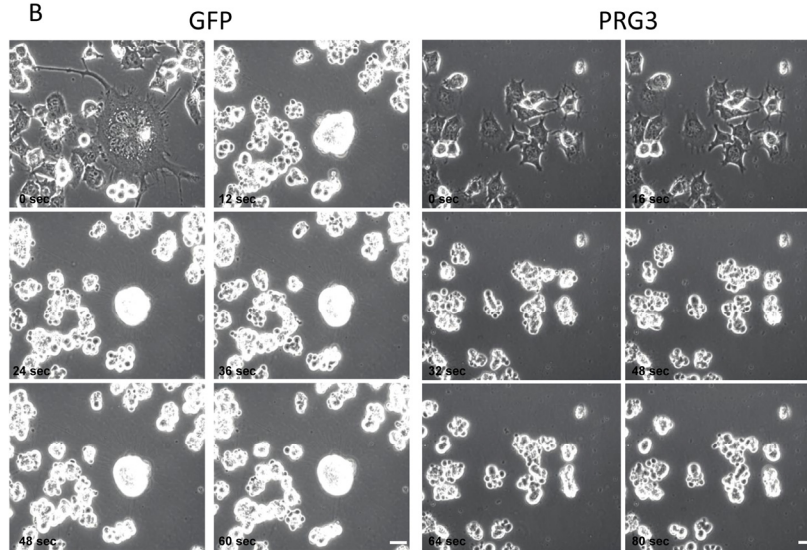
A

Expression of LPA receptors in N1E-115 cells



Calyculin A 100nM

B



**Supplementary Figure S5. N1E-115 LPA receptor expression and Cell contraction control using calyculin A.** (A) Expression analysis of PRG3, LPP1, LPP3 and control N1E-115 cells shows expression of all 4 receptors. (B) Representative time laps microscopy image series out of three independent experiments are shown of 100nM Calyculin A induced cell collapse in GFP and PRG3 cells after 80 seconds. Scale bar represents 50µm.