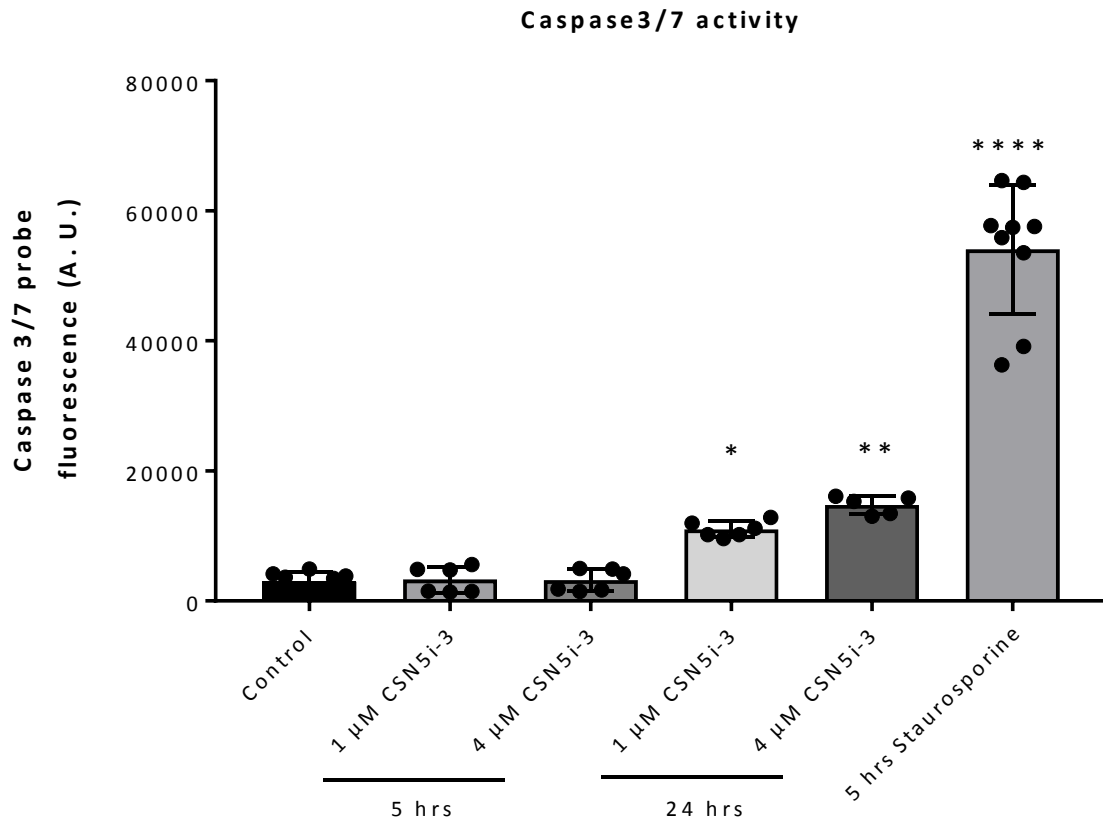


**CSN5 inhibition triggers inflammatory signaling and Rho/ROCK-dependent loss of endothelial integrity**

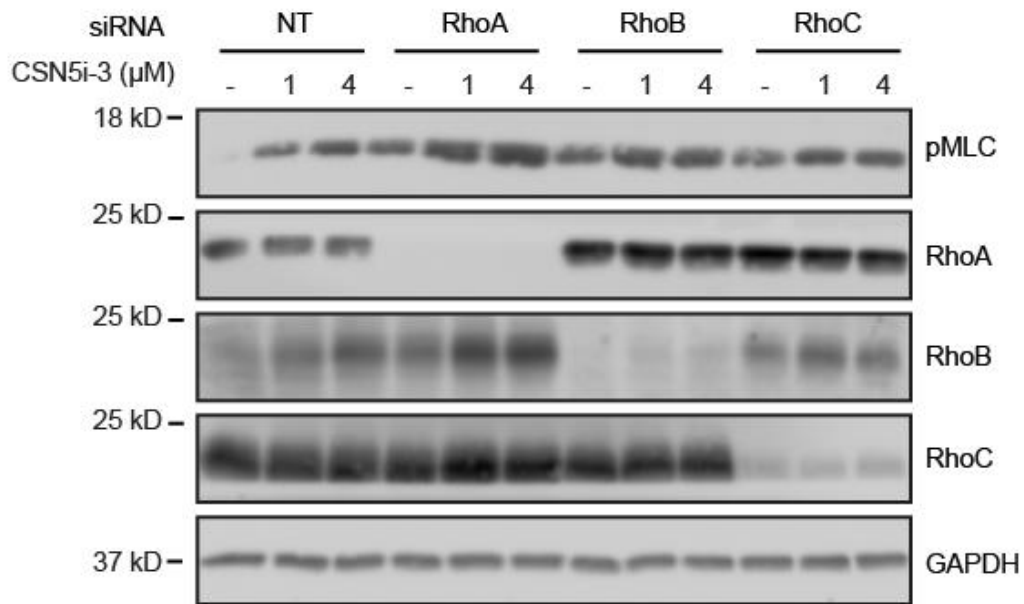
Jisca Majolée<sup>1¶</sup>, Manon C.A. Pronk<sup>1¶</sup>, Kin K. Jim<sup>2</sup>, Jan S.M. van Bezu<sup>1</sup>, Astrid M. van der Sar<sup>2</sup>, Peter L. Hordijk<sup>1</sup>, Igor Kovačević<sup>1\*</sup>



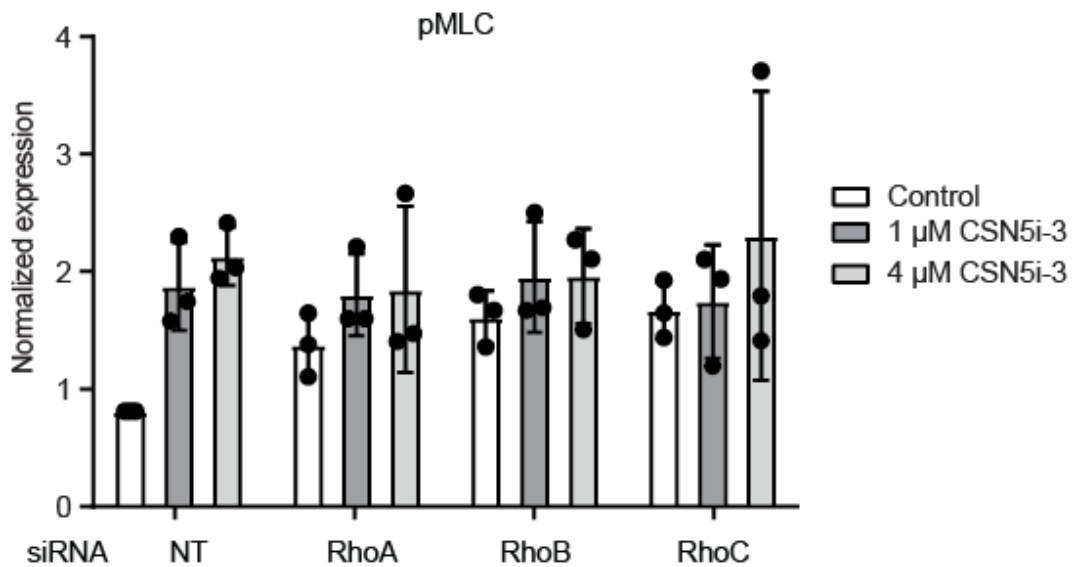
**Supplemental Figure 1 – CSN5i-3 induces apoptosis after 24 hours, but not after 5 hours of treatment.**

Caspase-3/7 assay of HUVEC monolayers treated with CSN5i-3 (1 or 4 μM) for 5 or 24 hours or 200 nM Staurosporin for 5 hours as a positive control. \* p<0.05, \*\* p<0.01, \*\*\*\* p<0.0001 compared to control after Dunnet post-hoc analysis of one-way ANOVA.

A



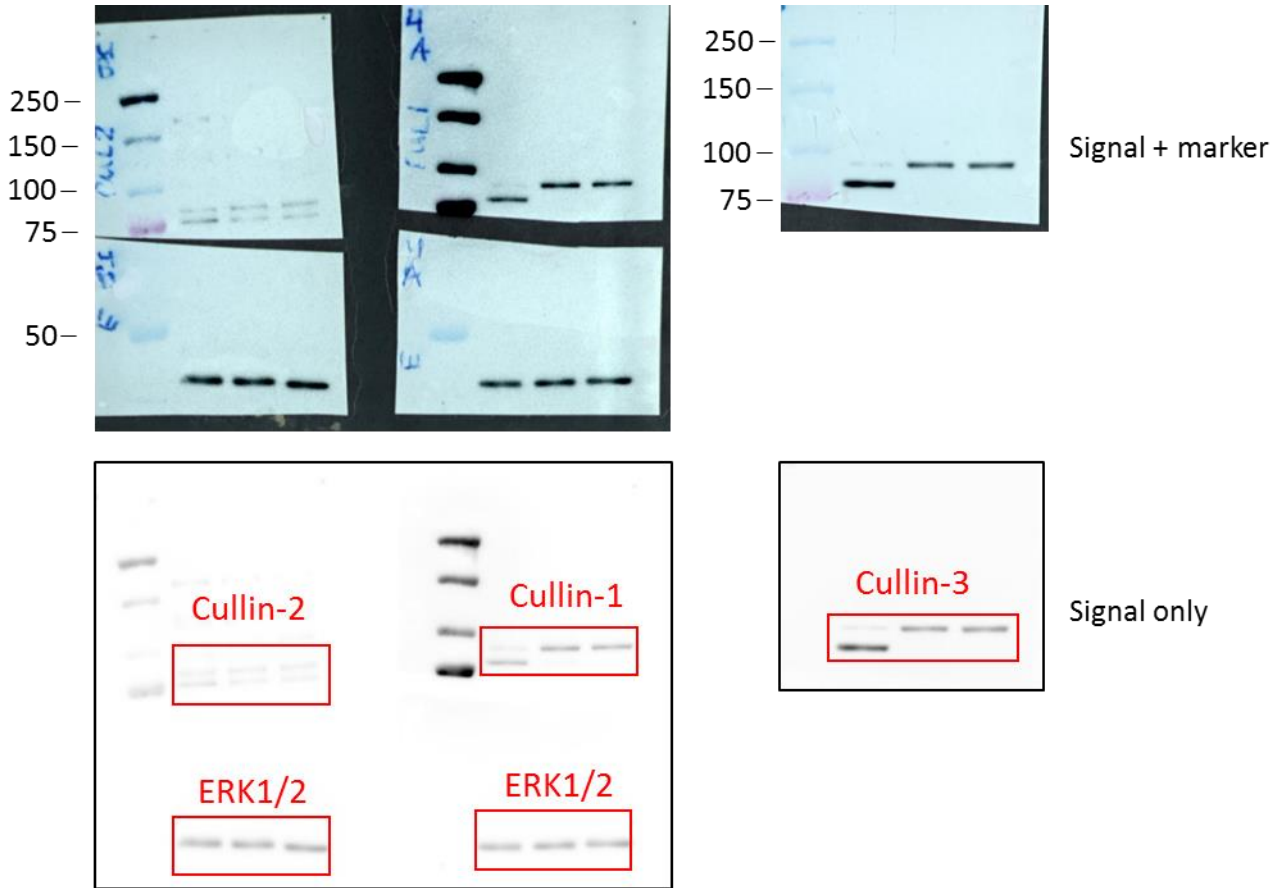
B



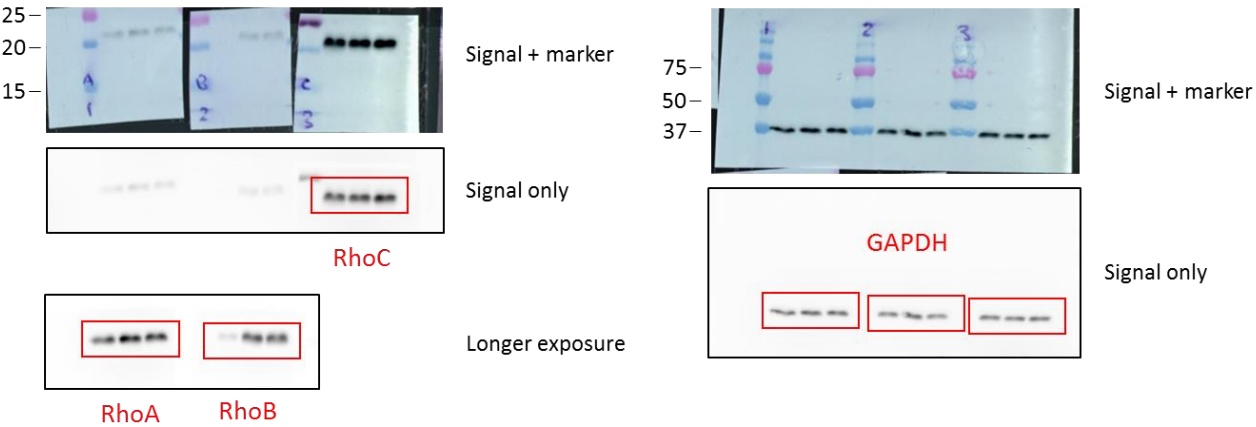
**Supplemental Figure 2 – single knockdown of RhoA, RhoB or RhoC does not effectively prevent MLC phosphorylation by CSN5i-3.**

A) Western blot analysis of pMLC, RhoA, RhoB, and RhoC expression in RhoA, RhoB and RhoC knockdown cells after 5 hr of CSN5i-3 (1 μM or 4 μM) treatment. GAPDH was used as loading control. Blot images were cropped for clarity of presentation. B) Quantification of pMLC levels compared to control.

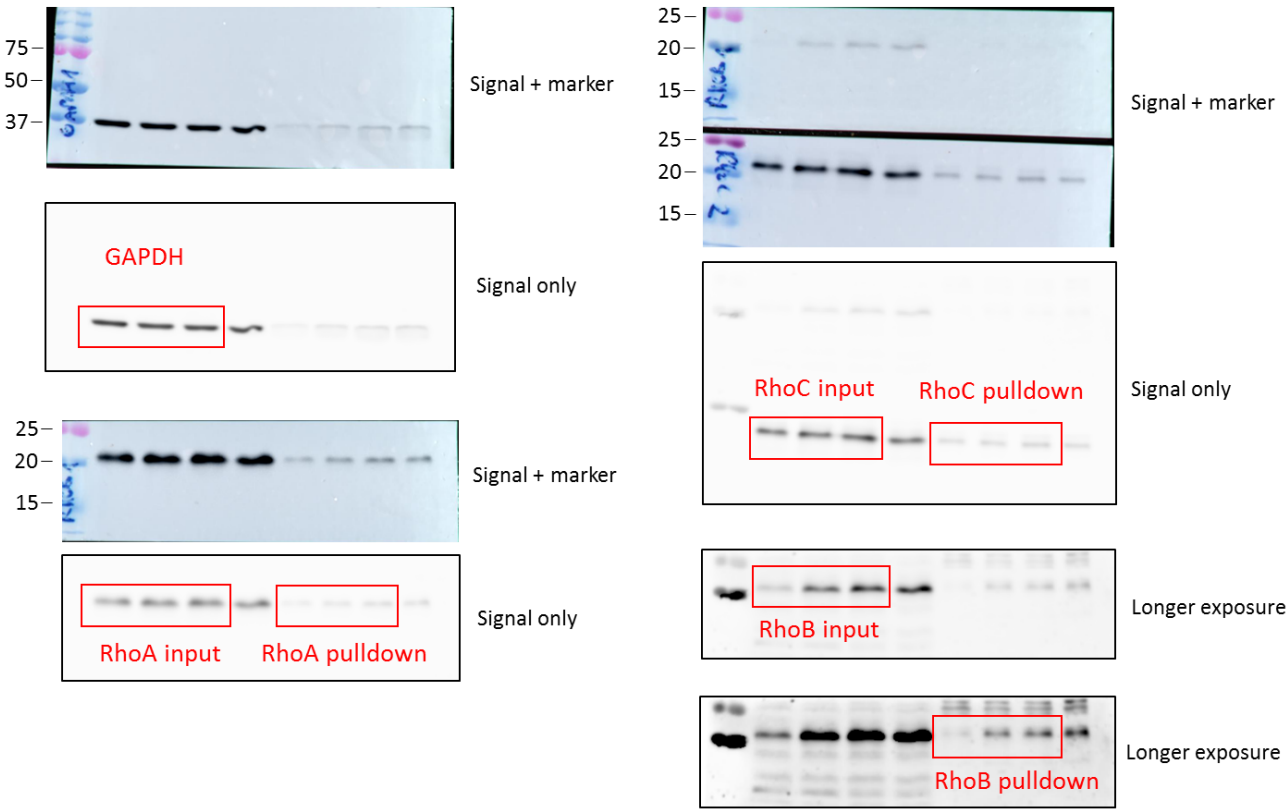
**Supplemental Figure 3 – full blots of Figure 1D**



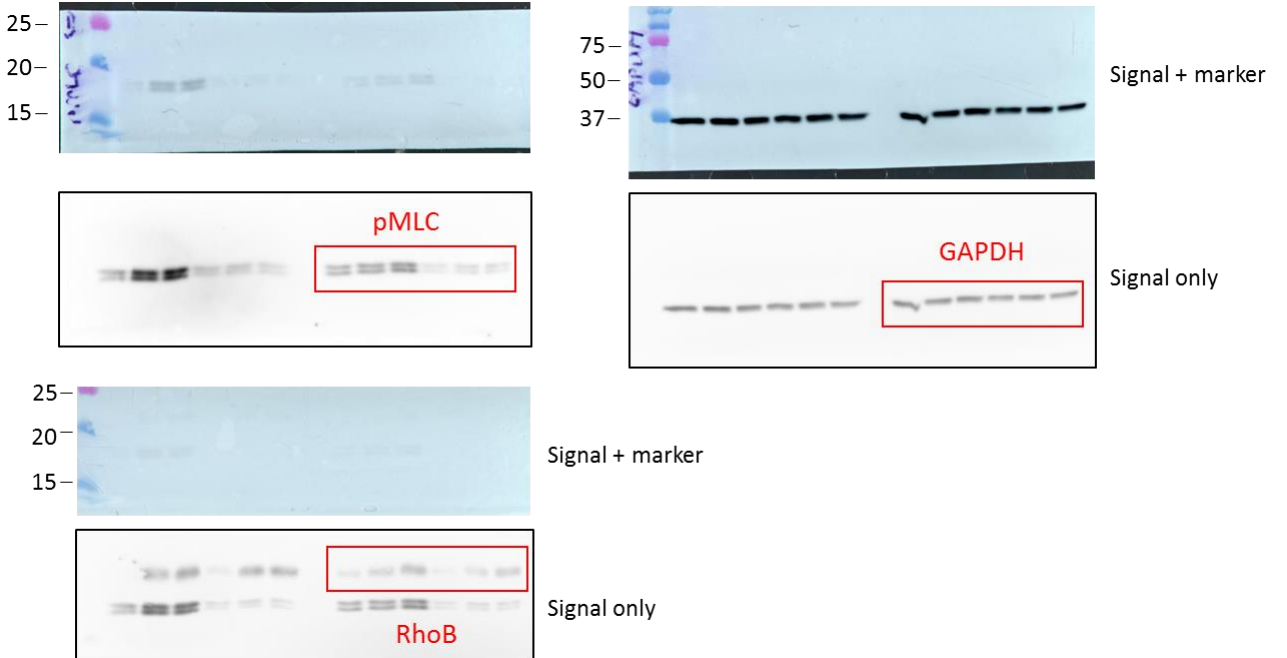
**Supplemental Figure 4 – full blots of Figure 2ABC**



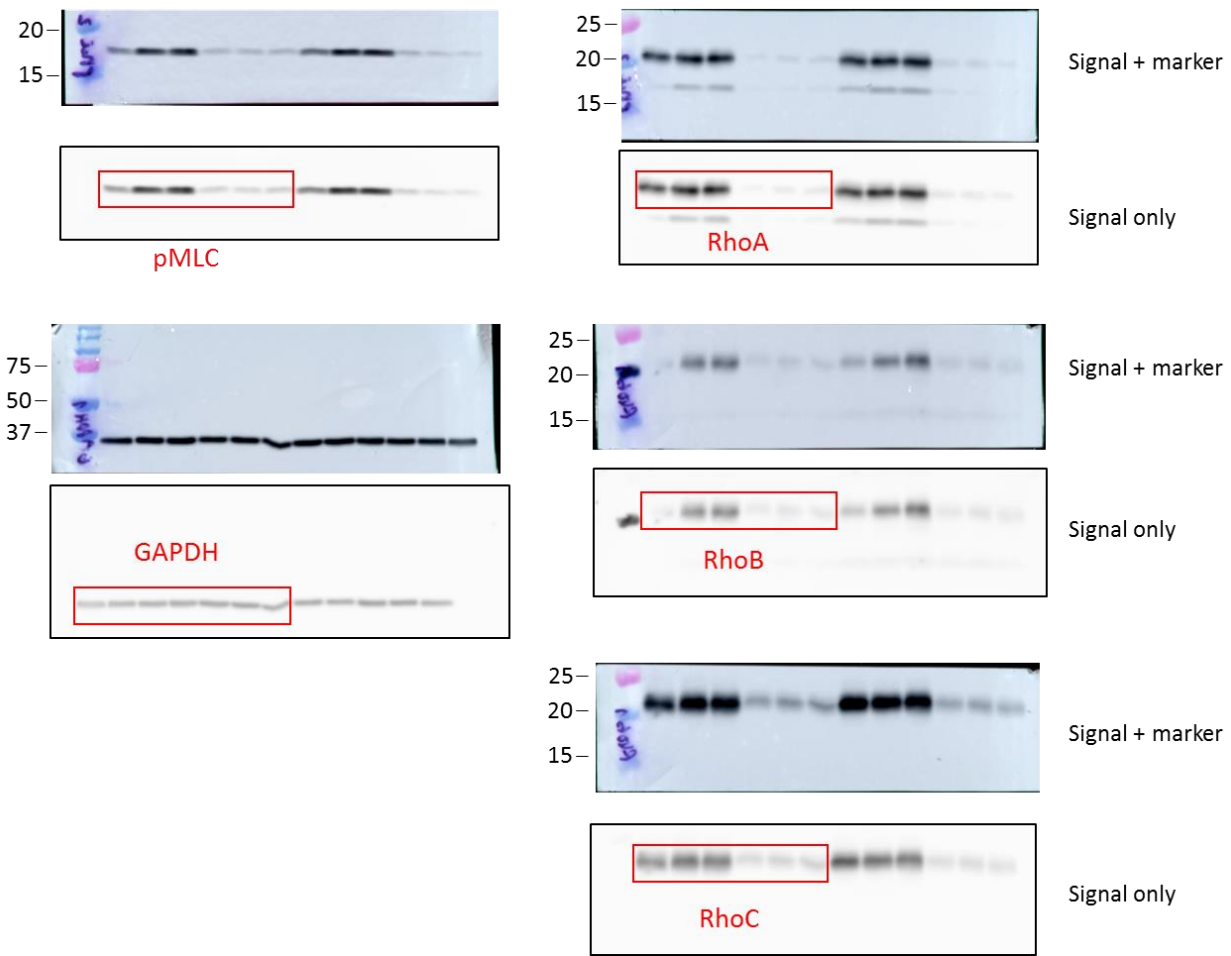
**Supplemental Figure 5 – full blots of Figure 2E**



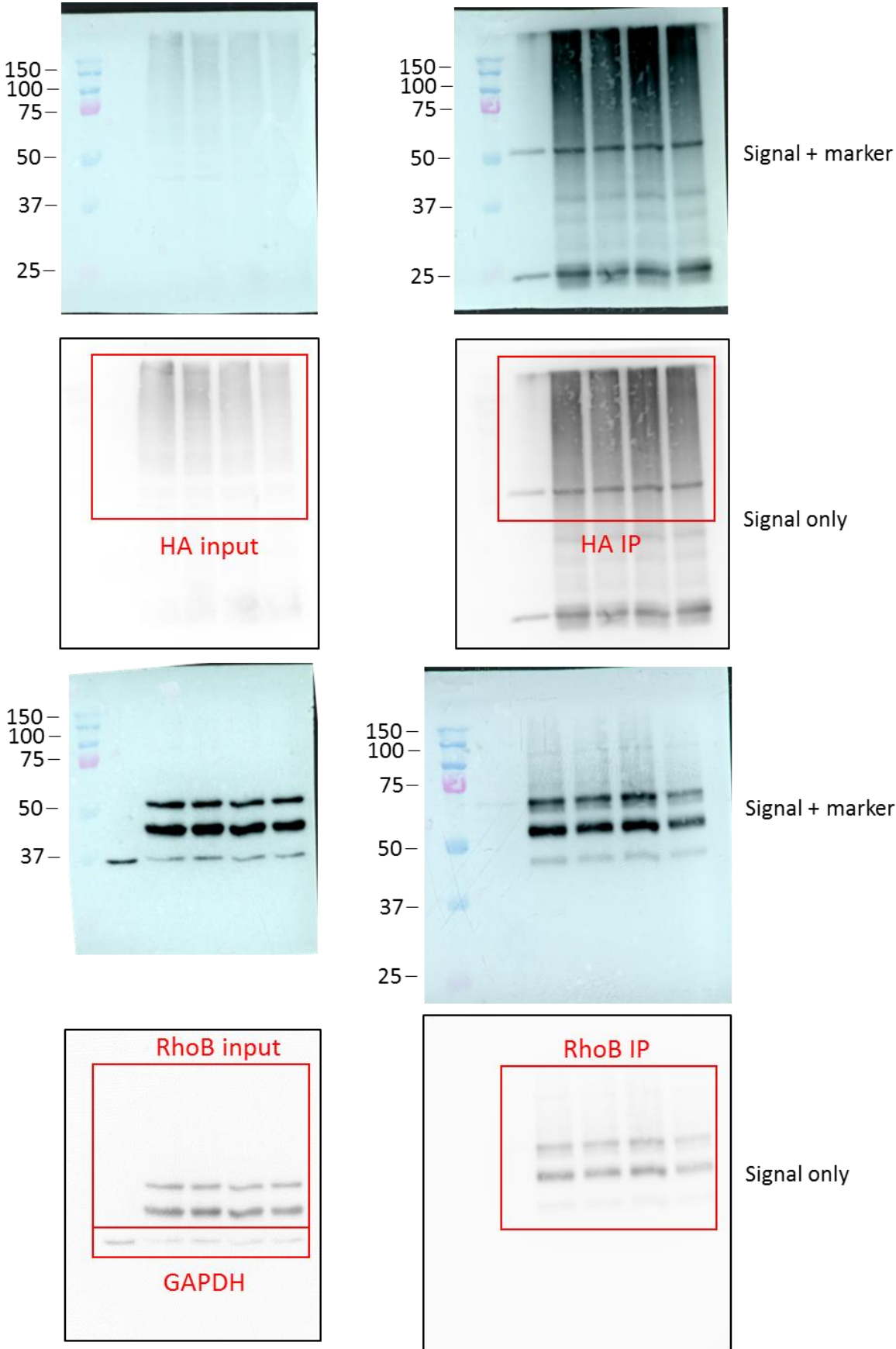
**Supplemental Figure 6 – full blots of Figure 3D**



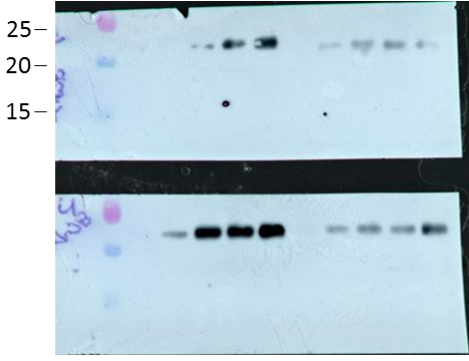
**Supplemental Figure 7 – full blots of Figure 3G**



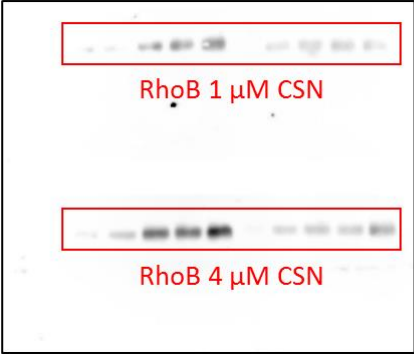
Supplemental Figure 8 – full blots of Figure 4A



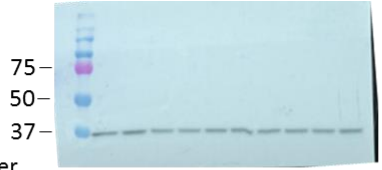
**Supplemental Figure 9 – full blots of Figure 4D**



Signal + marker



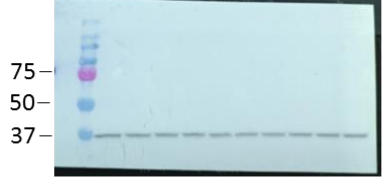
Signal only



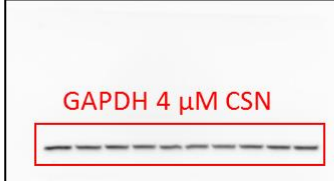
Signal + marker



Signal only



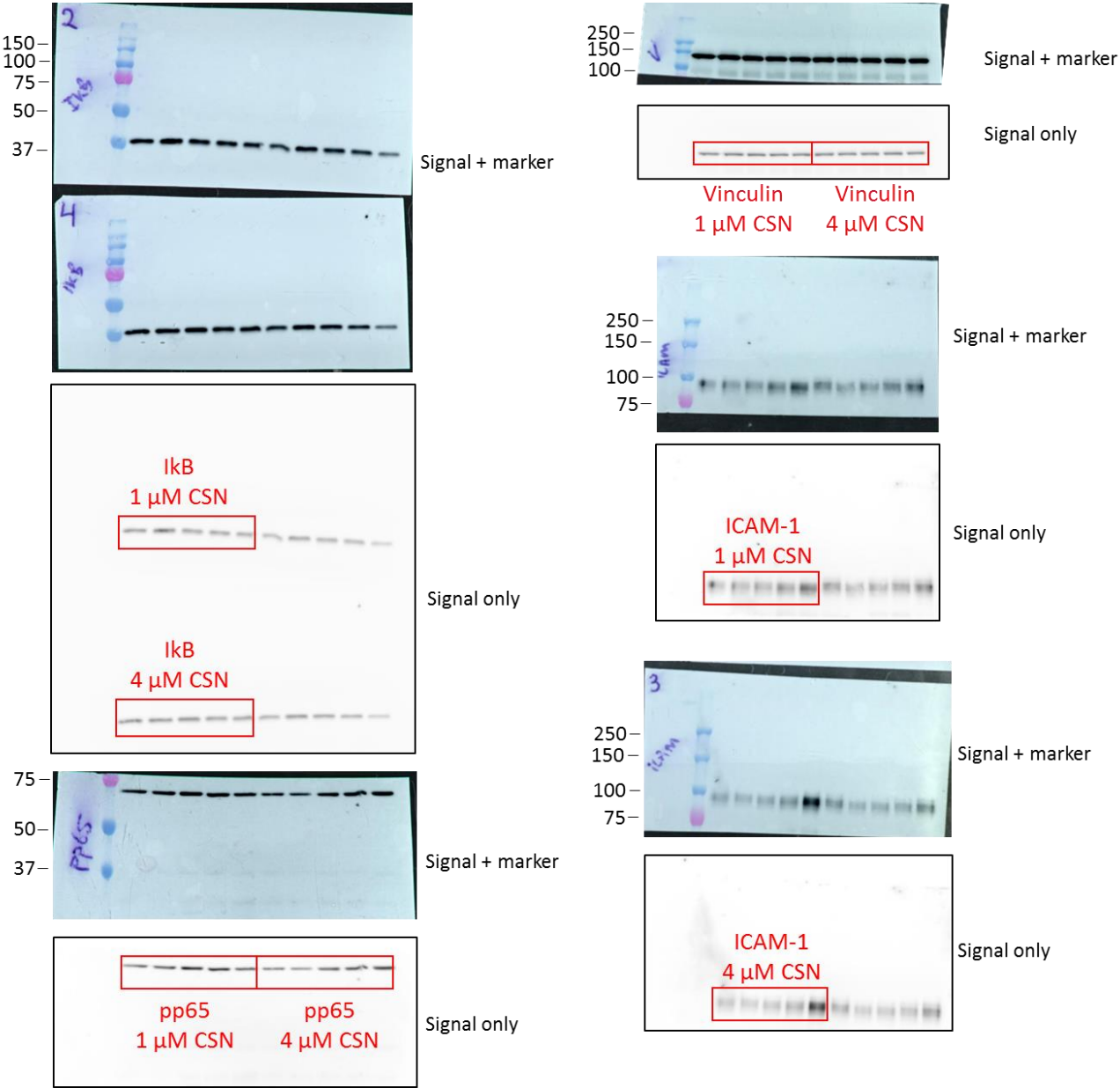
Signal + marker



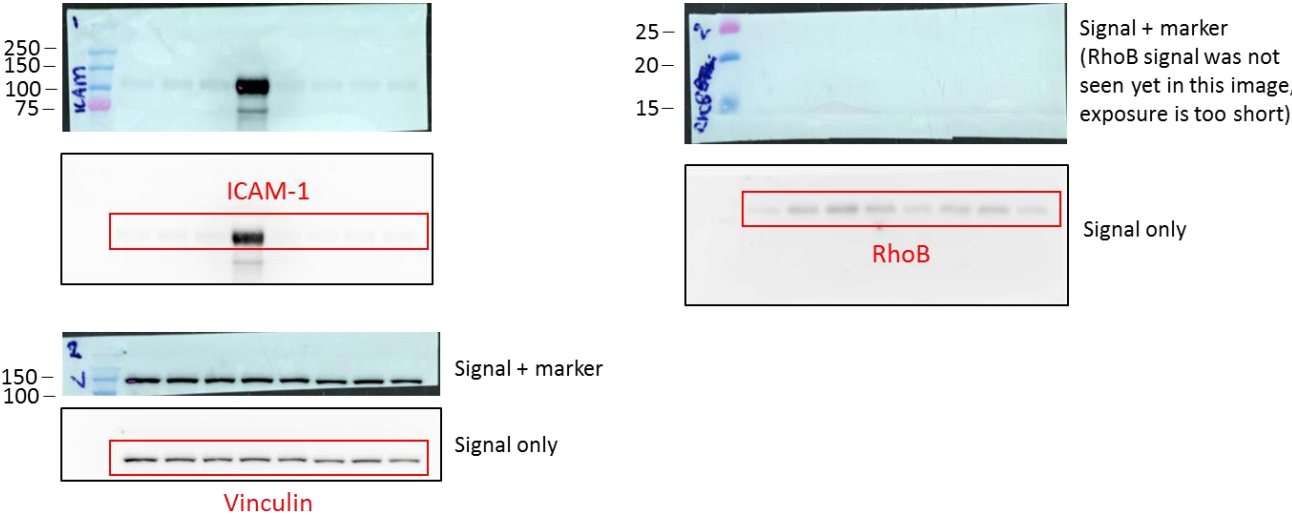
Signal only



**Supplemental Figure 10 – full blots of Figure 5A**



**Supplemental Figure 11 – full blots of Figure 5E**



**Supplemental Figure 12 – full blots of Figure 6A**

