

## SUPPLEMENTARY MATERIAL

### **PLCy2 regulates TREM2 signalling and integrin-mediated adhesion and migration of human iPSC-derived macrophages**

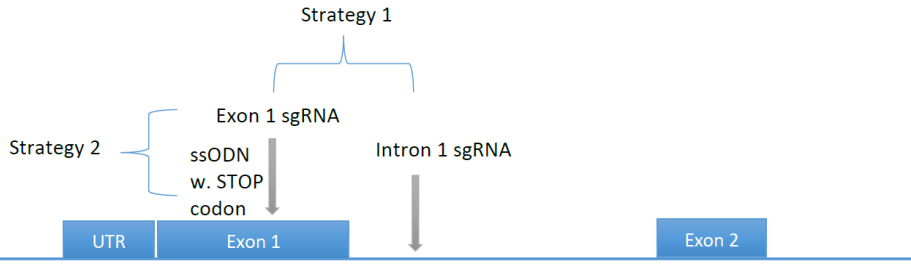
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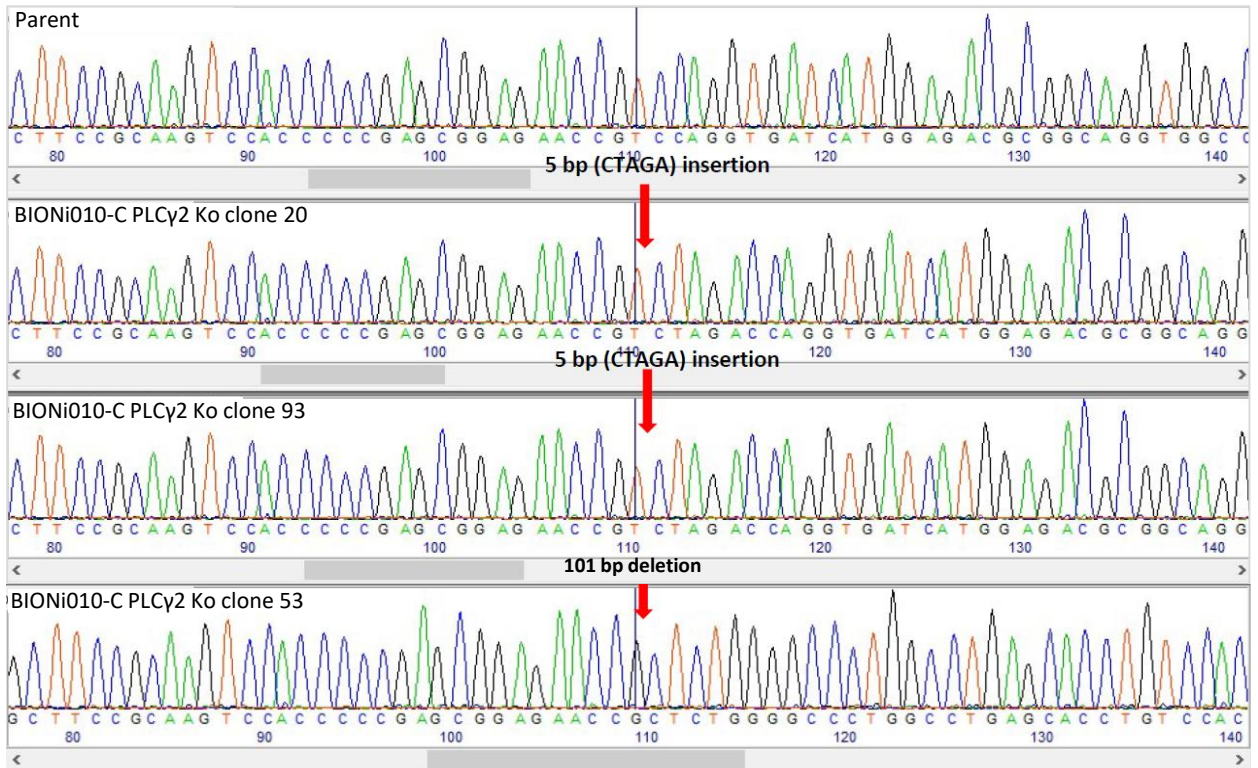
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# Supplementary figure 1:

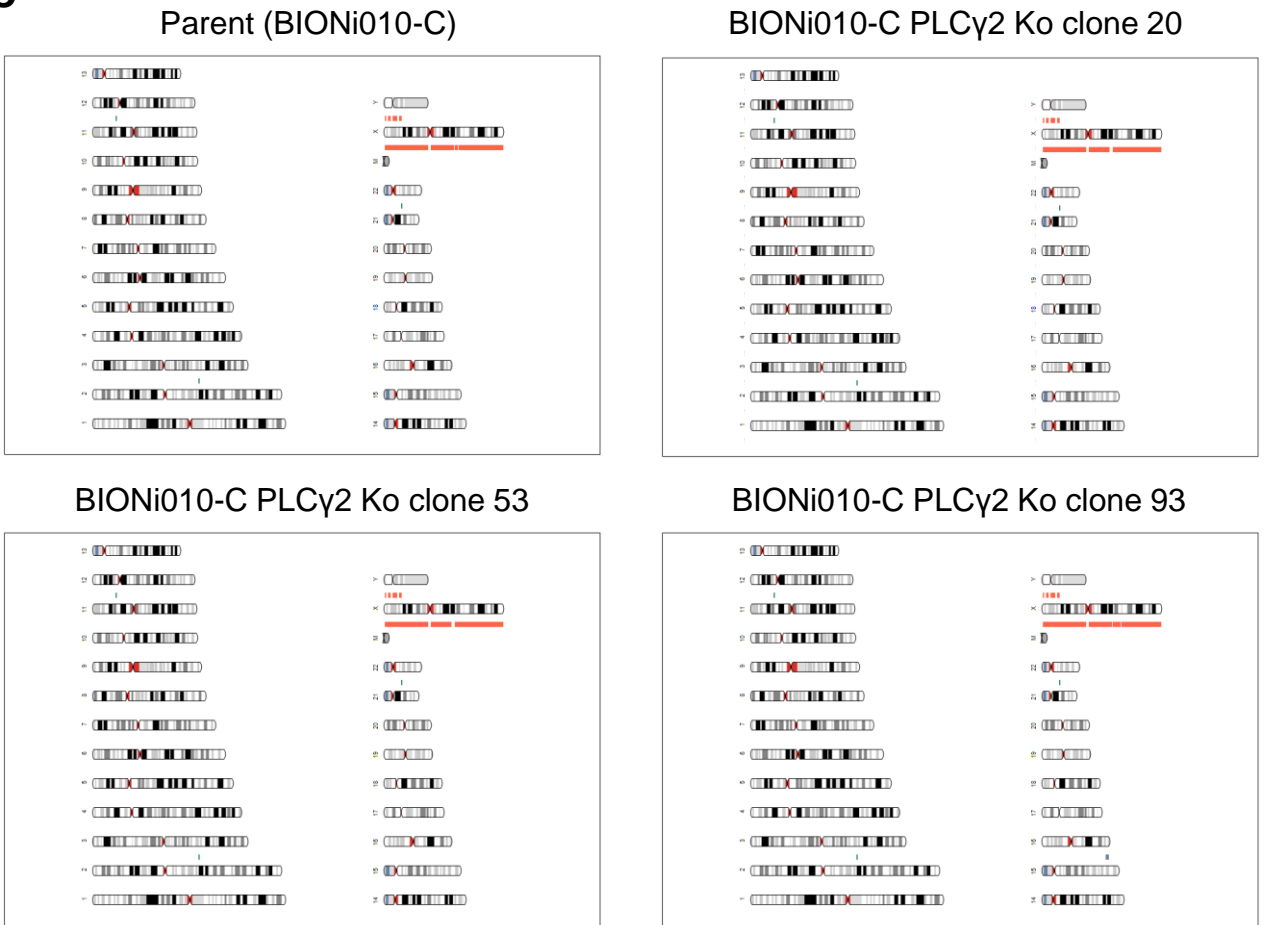
**A**



**B**



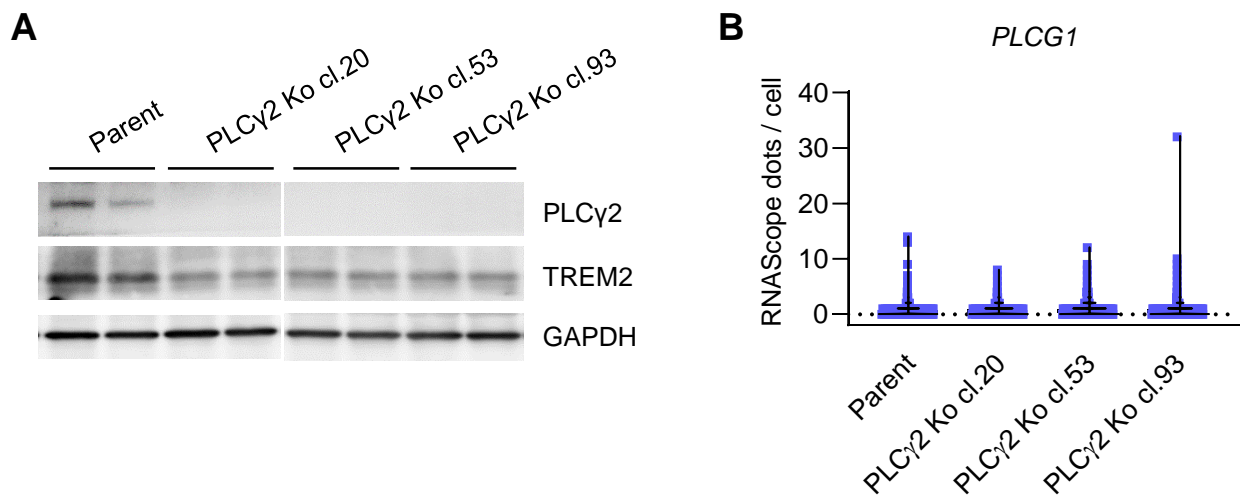
**C**



## Supplementary figure 1: Generation of genetically modified iPSC lines.

[A] Two strategies were applied that lead to a premature stop of translation: Exon 1 and Intron 1 sgRNA's were used to cut out the last part of Exon 1 in *PLCG2* or Exon 1 sgRNA and a ssODN was used to insert a STOP codon in Exon 1. [B] Sequencing analysis of PLCy2 Ko clones 20, 53 and 93. The red arrow indicates the site of the CRISPR cutting in the first allele [C] Chromosome karyograms of Parent and PLCy2 Ko clones 20, 53 and 93 from Illumina microarray SNP analysis.

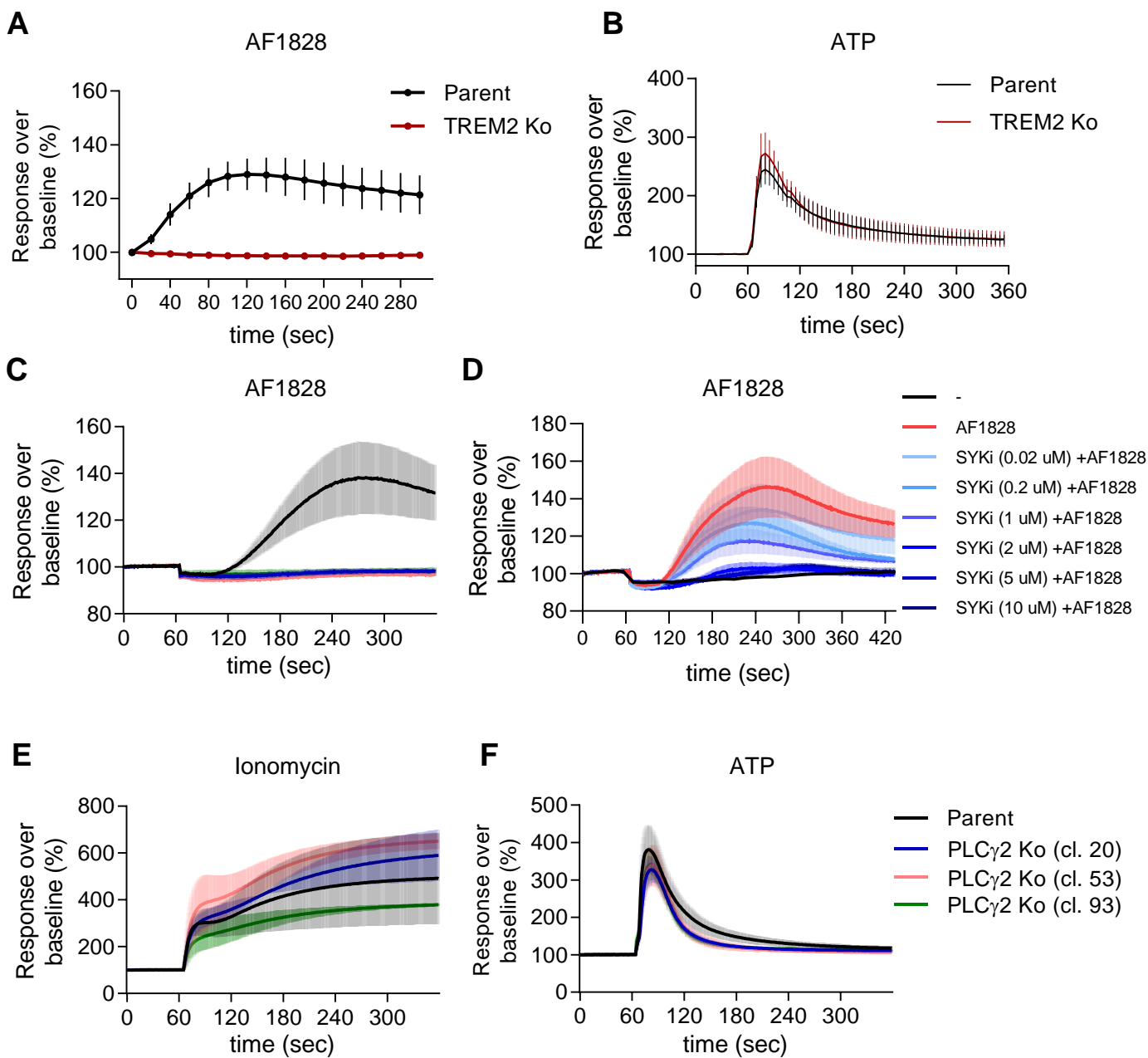
## Supplementary figure 2:



### Supplementary figure 2: Validation of PLCγ2 Ko iPSC macrophages clones 20, 53 and 93.

[A] Levels of total PLCγ2 in Parent line and Ko clones. [B] RNAScope analysis of PLCG1 transcripts in Parent vs. Ko lines.

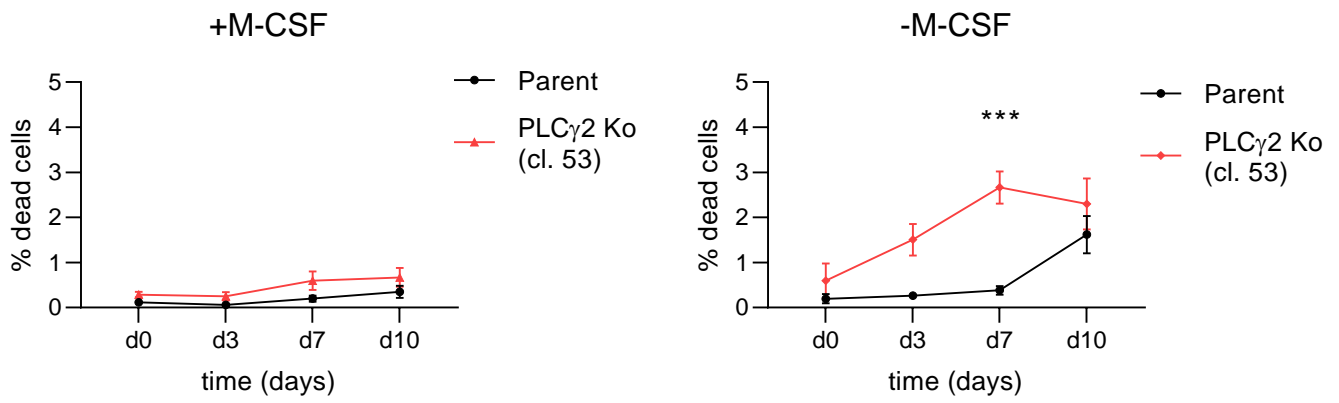
### Supplementary figure 3:



### Supplementary figure 3: Characterisation of the Ca<sup>2+</sup> assay.

[A] Kinetics of calcium response to TREM2 antibody (AF1828, 5 ug/ml) in Parent and TREM2 Ko line. N=4 [B] Kinetics of calcium response to ATP (0.5 mM) in Parent and TREM2 Ko line. N=4 [C] Calcium kinetics upon stimulation with 5 ug/ml TREM2 antibody in Parent and PLCγ2 Ko (clones 20, 53 and 93). N=3 [D] Concentration dependent inhibition of TREM2- induced calcium response using a SYK inhibitor (BIB-057). N=3 [E] Kinetics of calcium response to ionomycin (5 uM) in Parent and PLCγ2 Ko clones. N=2 [F] ATP- induced calcium flux in Parent and PLCγ2 Ko clones. n=4, all data shown represent mean ± SEM.

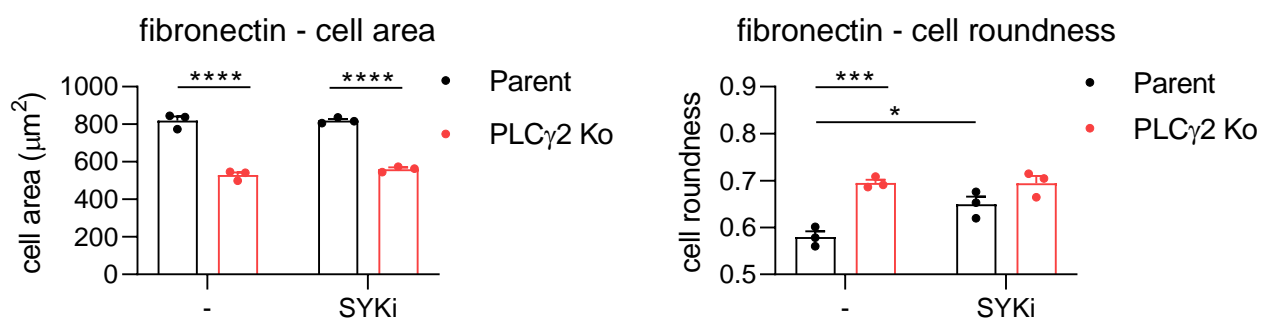
## Supplementary figure 4:



### Supplementary figure 4: Survival analysis of PLCγ2 Ko clone 53 in the presence or absence of M-CSF.

Cell death under normal culture conditions (+M-CSF) after  $\geq 7$  days in the PLCγ2 Ko clone 53 compared to the Parent. In the absence of M-CSF (-M-CSF), PLCγ2 Ko clone 53 showed enhanced sensitivity to cell death than the Parent line.  $n = 3$ , all data shown represent mean  $\pm$  SEM. two-way ANOVA followed by Bonferroni's multiple comparison test. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

## Supplementary figure 5:

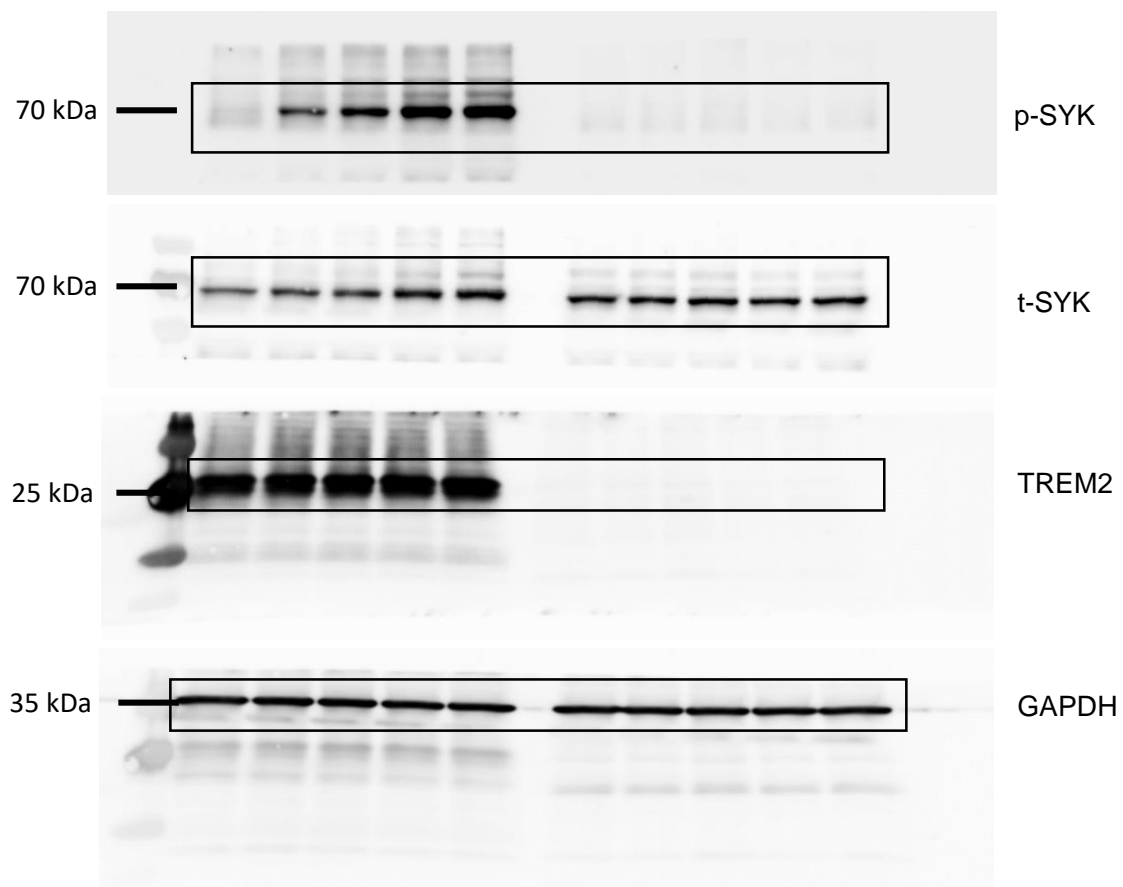


### Supplementary figure 5: Effect of PLCγ2 Ko on cell spreading on fibronectin.

Fibronectin coating does not rescue cell area or cell roundness in PLCγ2 Ko cells. N=3, all data shown represent mean ± SEM. two-way ANOVA followed by Bonferroni's multiple comparison test. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

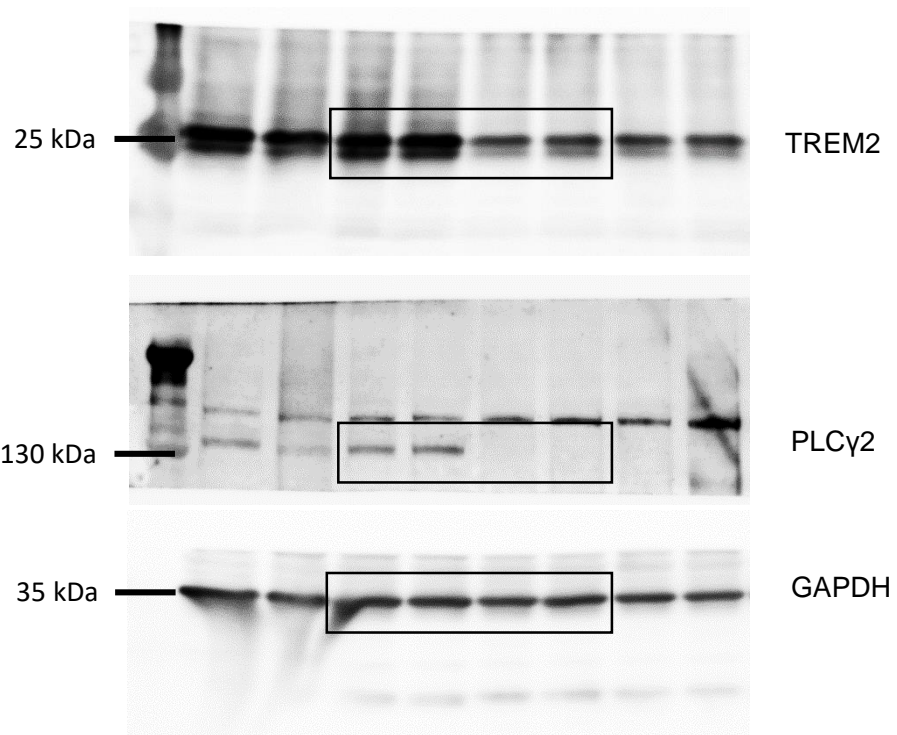
## Supplementary figure 6:

This figure shows the full original uncropped Western blot images of Figure 1A (results)



## Supplementary figure 7:

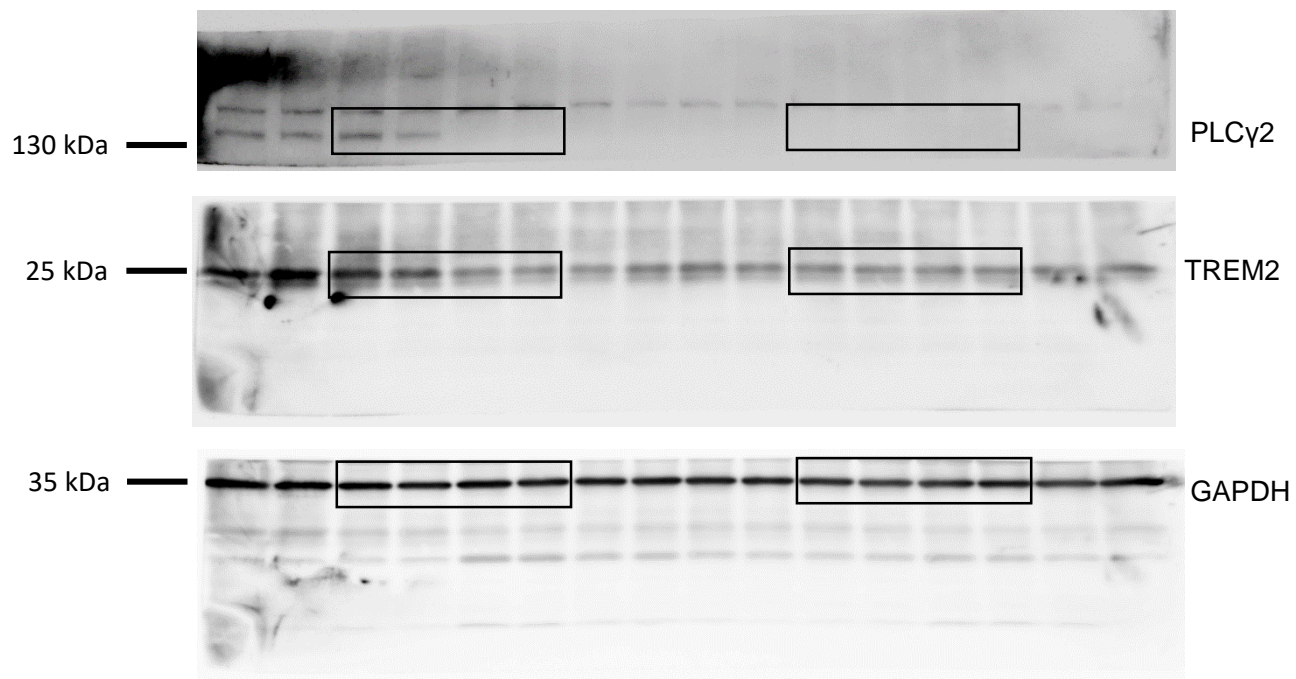
This figure shows the full original uncropped Western blot images of Figure 2A (results)





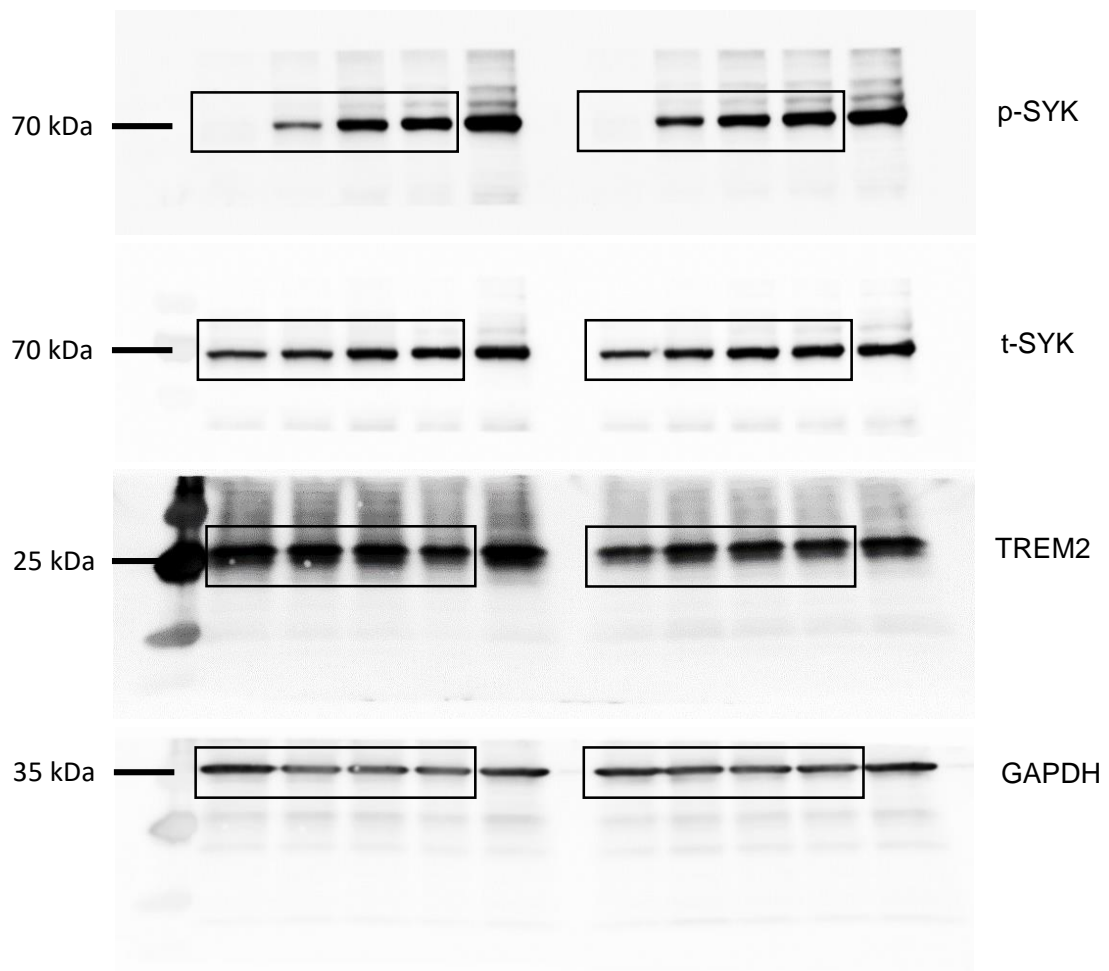
## Supplementary figure 8:

This figure shows the full original uncropped Western blot images of Suppl. Figure 2



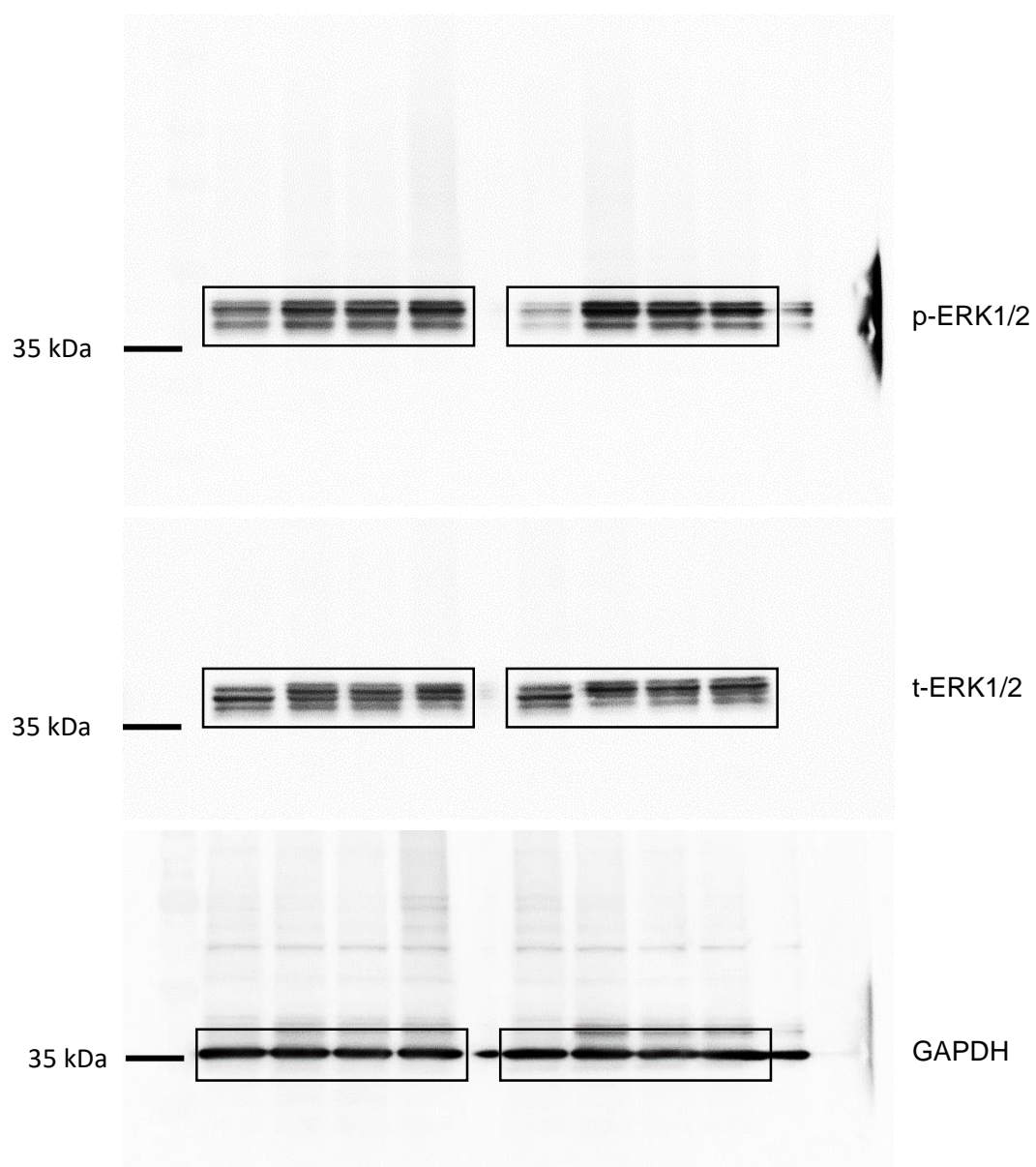
## Supplementary figure 9:

This figure shows the full original uncropped Western blot images of Figure 3A (results)



## Supplementary figure 10:

This figure shows the full original uncropped Western blot images of Figure 3F (results)



## Supplementary figure 11:

This figure shows the full original uncropped Western blot images of Figure 5E (results)

