

Supplementary Information

TMCO1 is upregulated in breast cancer and regulates the response to pro-apoptotic agents in breast cancer cells

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Supplementary Tables

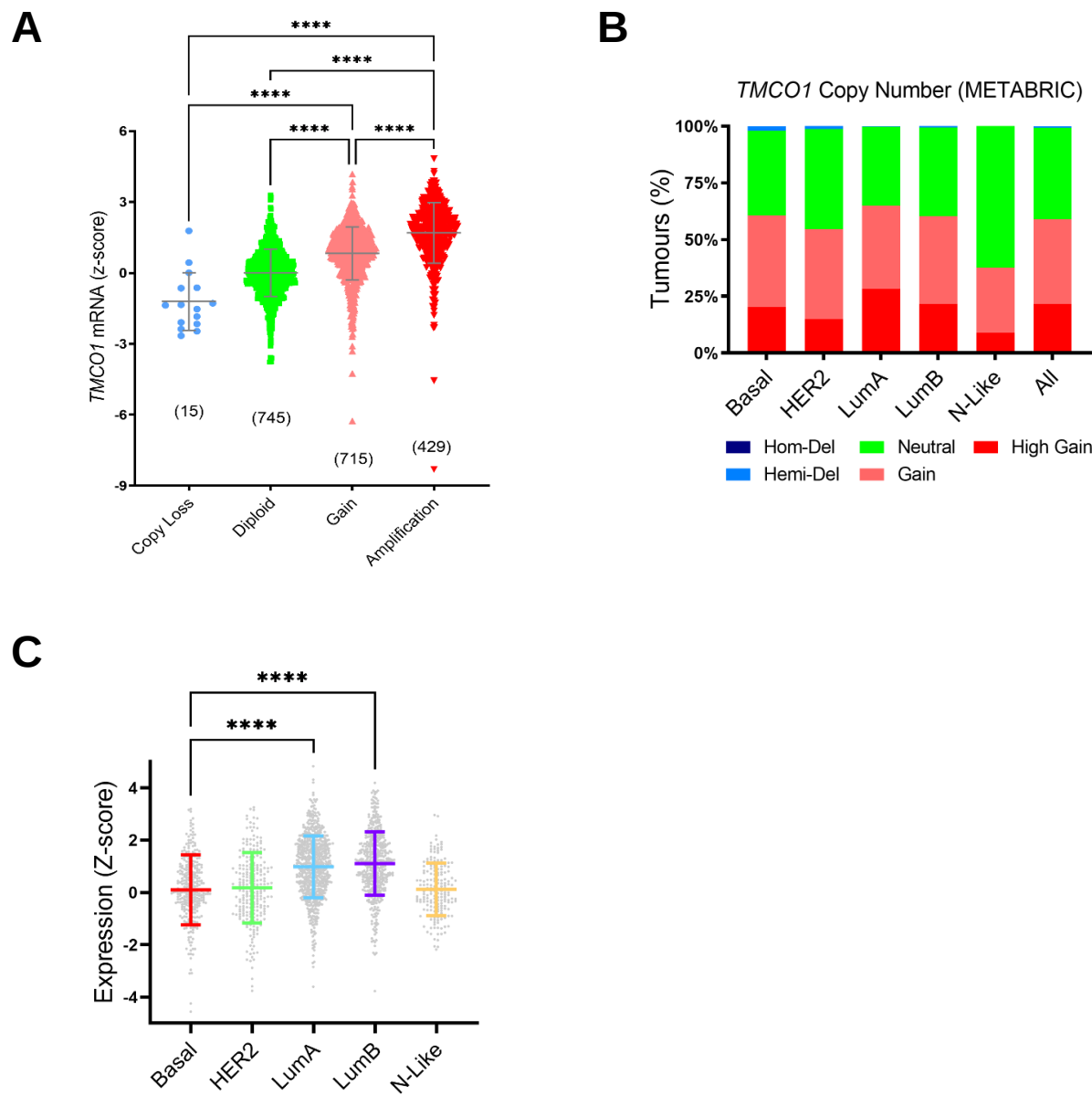
Table S1: Table shows the survival analysis of breast cancer patients with high or low *TMCO1* expression based on their PAM50 molecular subtypes and lymph node involvement using Kaplan-Meier Plotter.

PAM50 subtype	Lymph node status	Logrank P	Hazards ratio (HR)	95% CI	Patient numbers (n)	
					Low <i>TMCO1</i>	High <i>TMCO1</i>
Luminal A	Negative	0.1825	1.24	0.9 – 1.7	359	630
	Positive	0.3792	0.84	0.57 – 1.24	198	333
Luminal B	Negative	0.4008	1.13	0.85 – 1.48	264	346
	Positive	0.0732	1.34	0.97 – 1.83	190	291
HER2	Negative	0.231	0.74	0.45 – 1.22	163	101
	Positive	0.3366	0.84	0.58 – 1.21	94	183
Basal	Negative	0.1118	0.73	0.49 – 1.08	324	132
	Positive	0.0063	1.68	1.15 – 2.44	233	90

Table S2: Table showing number of total and unique peptides as well as peptide coverage for proteins identified to interact with FLAG-TMCO1 in LC-MS/MS.

(separate Excel file)

Supplementary Figures

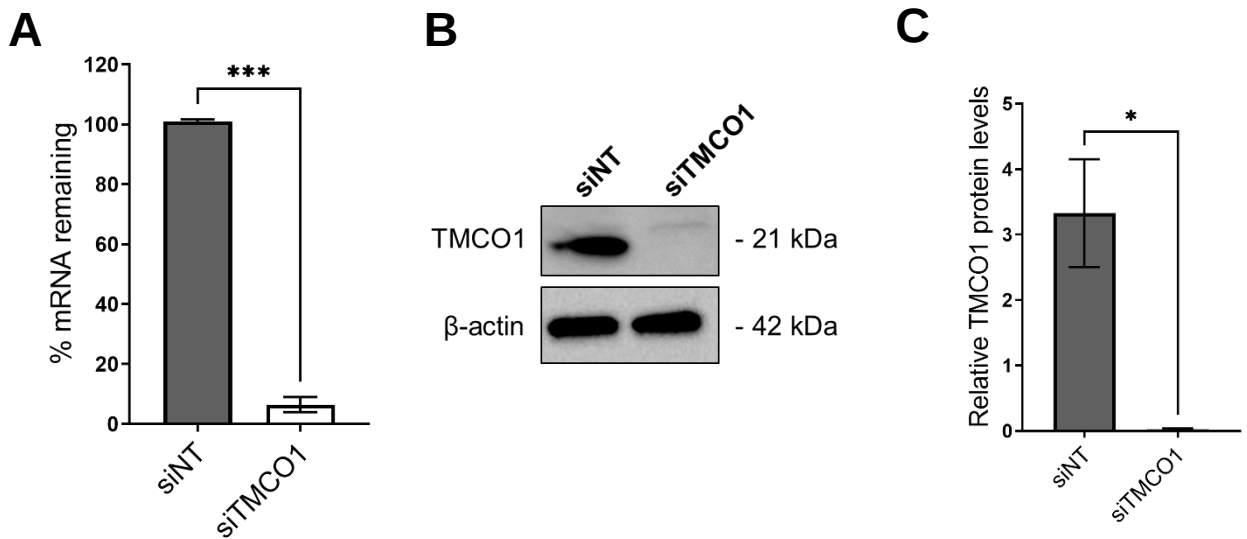


Supplementary Fig. 1: *TMCO1* gene copy number and gene expression in the METABRIC breast cancer patient dataset

A *TMCO1* mRNA levels in METABRIC breast cancer patient dataset stratified to gene copy number status. Sample sizes for individual gene copy number groups are shown in the brackets.

**** $P < 0.0001$ (one way ANOVA, Kruskal-Wallis test) **B** Proportion of *TMCO1* copy number alterations within the different breast cancer molecular subtypes in the METABRIC dataset **C**

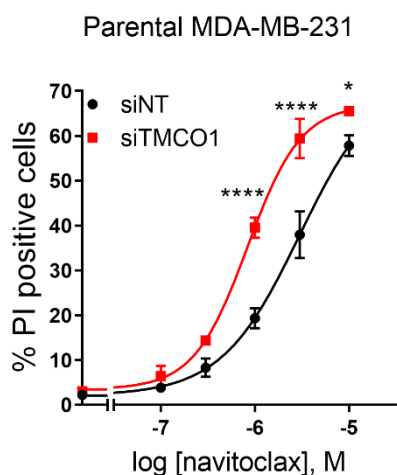
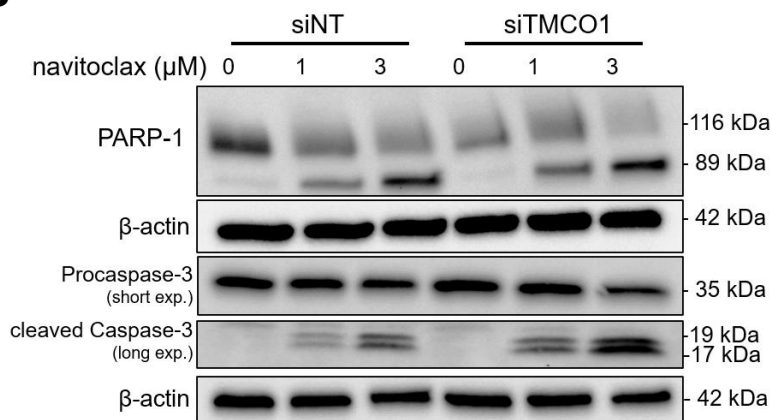
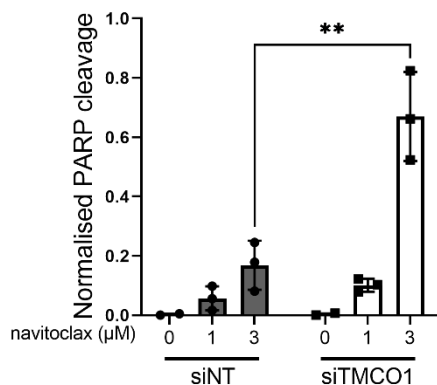
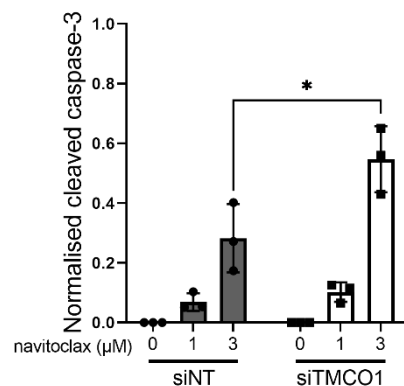
TMCO1 gene expression classified to breast cancer molecular subtypes. (one way ANOVA, Tukey's test) Error bars represent mean $\pm$ S.D.



Supplementary Fig. 2: Validation of *TMCO1* silencing using Dharmacon ON-TARGETplus

TMCO1 siRNA

A Bar graph shows percentage of *TMCO1* mRNA remaining in GCaMP6m-MDA-MB-231 cells at 48 – 72 h following transfection with Dharmacon ON-TARGETplus *TMCO1* siRNA. **B, C** Representative immunoblot and densitometric analysis showing *TMCO1* protein silencing at 96 h post-siRNA transfection. * $P < 0.05$, **** $P < 0.0001$ (paired t-test). Bar graphs show mean $\pm$ S.D.

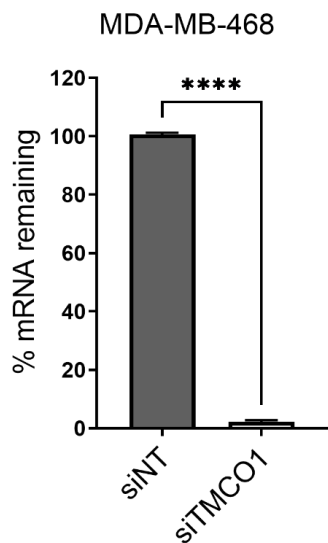
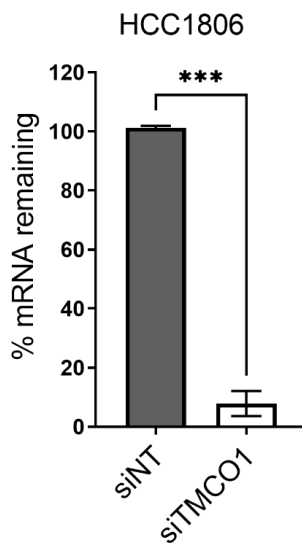
A**B****C****D**

Supplementary Fig 3: Effect of *TMCO1* silencing on cell death in parental MDA-MB-231 cells

A Concentration-effect curve showing %PI positivity at different concentrations of navitoclax.

Data points represent mean $\pm$ S.E.M ($n = 3$) **B** Representative immunoblots showing increased PARP-1 and caspase-3 cleavage as a result of *TMCO1* silencing in parental MDA-MB-231 cells treated with navitoclax. **C, D** Densitometric analyses showing the effect of *TMCO1* silencing on PARP-1 and caspase-3 cleavage with navitoclax treatment. Bar graphs show mean $\pm$ S.D. ($n = 3$)

* $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$ (two-way ANOVA with Sidak's post-hoc test).

A**B**

Supplementary Fig 4: Validation of *TMCO1* silencing in MDA-MB-468 and HCC1806 cell lines

A, B Bar graphs show percentage of *TMCO1* mRNA remaining in MDA-MB-468 and HCC1806 cells at 48 h following transfection with Dharmacon ON-TARGETplus *TMCO1* siRNA. **** $P < 0.0001$ (paired t-test). Bar graphs show mean $\pm$ S.D.

Full-length Western blots

Figure 4A

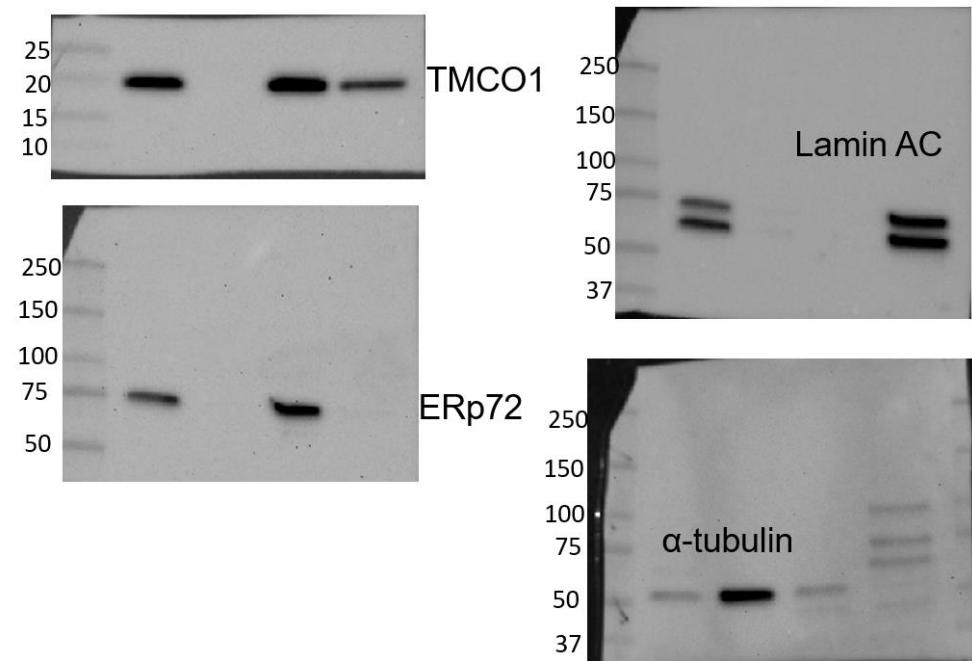


Figure 4F

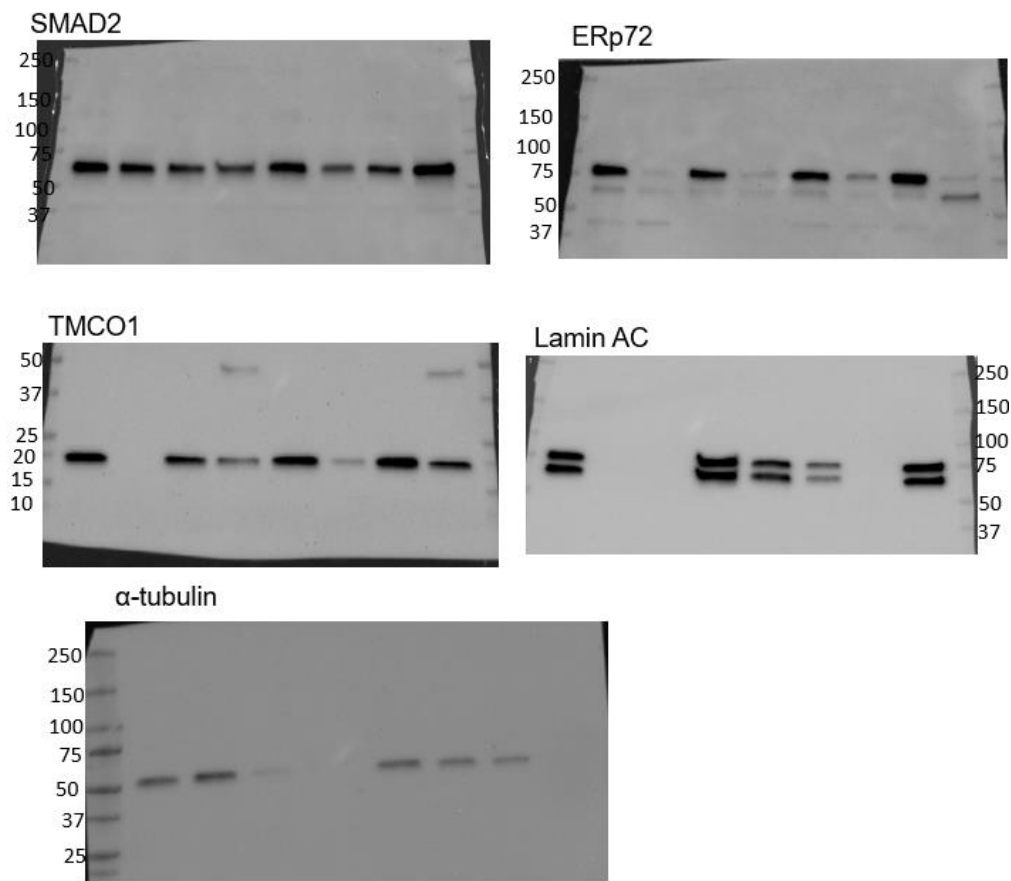
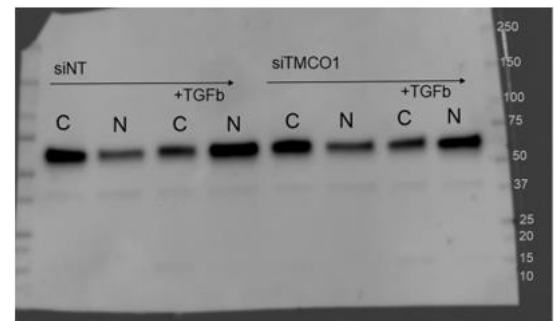
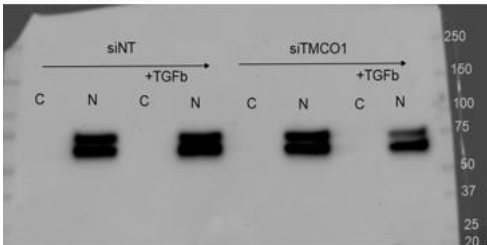


Figure 4I

SMAD2



Lamin A/C



α -tubulin

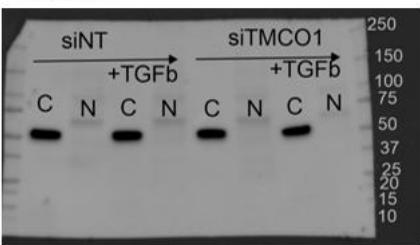
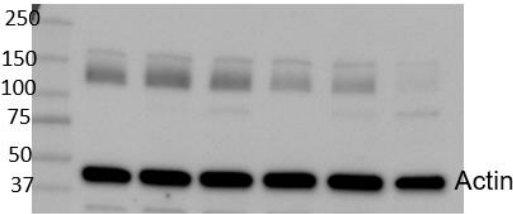
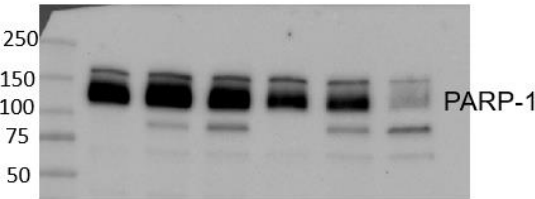
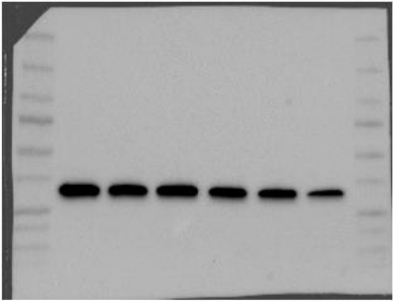


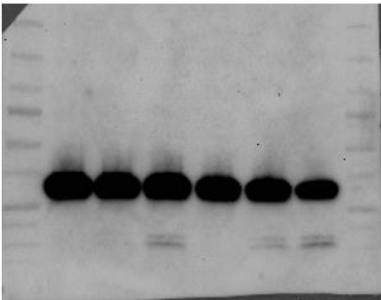
Figure 5C



Caspase 3 (short exposure)



Caspase 3 (long exposure)



Actin for caspase 3

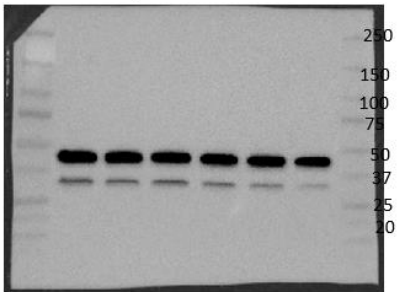


Figure 5F

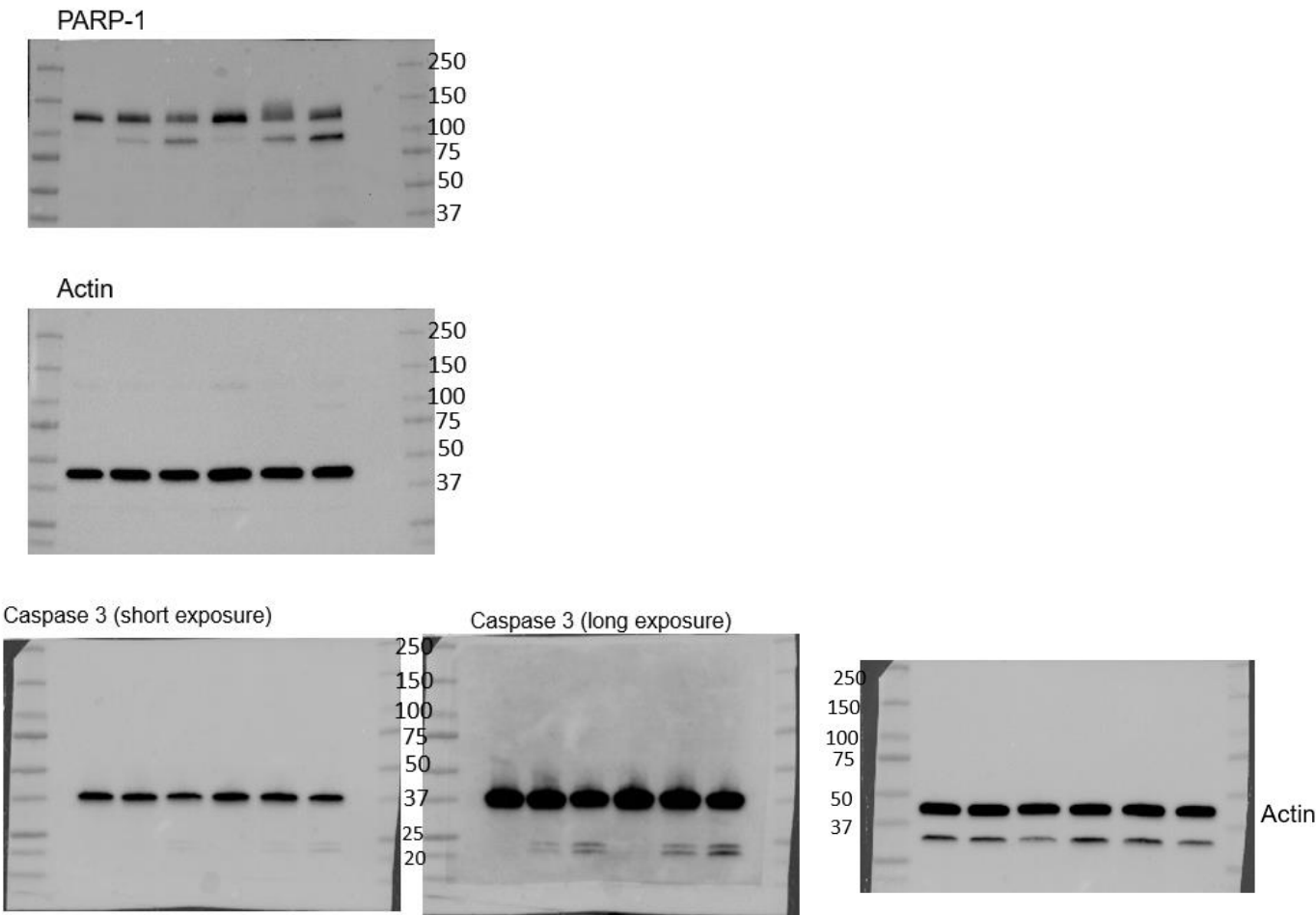


Figure 6C

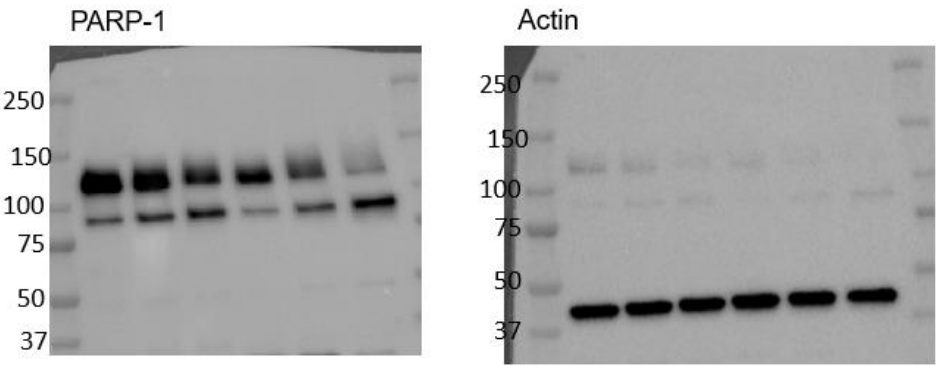
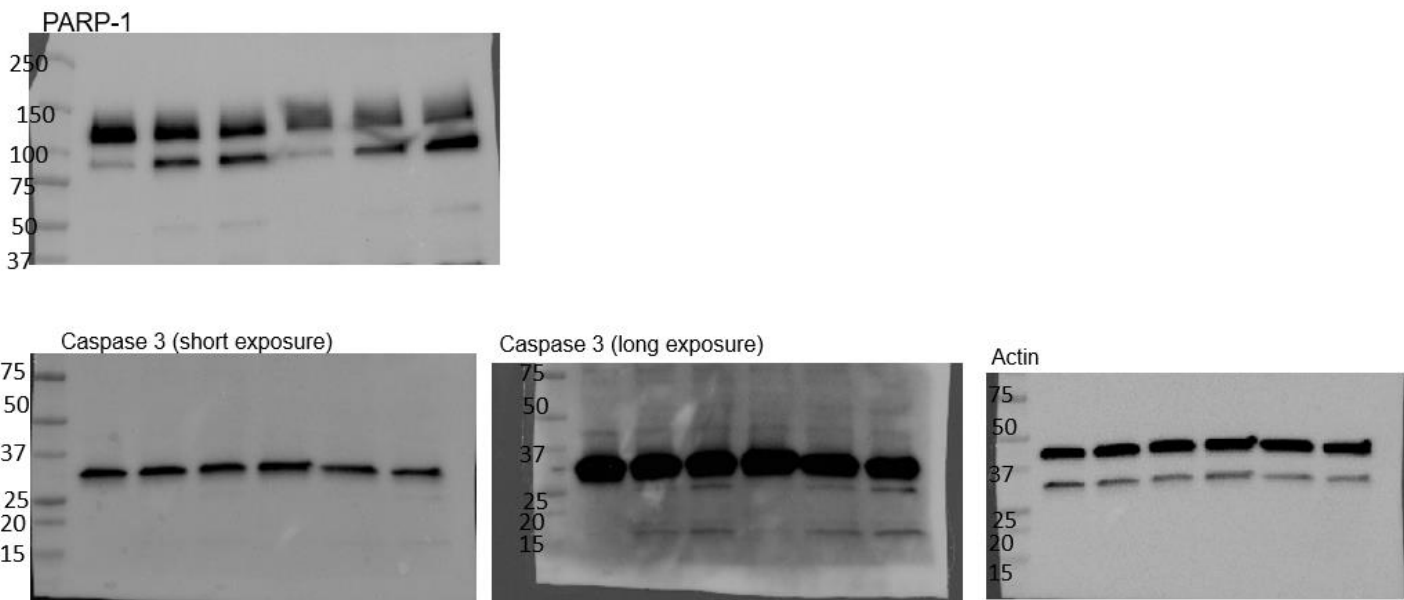
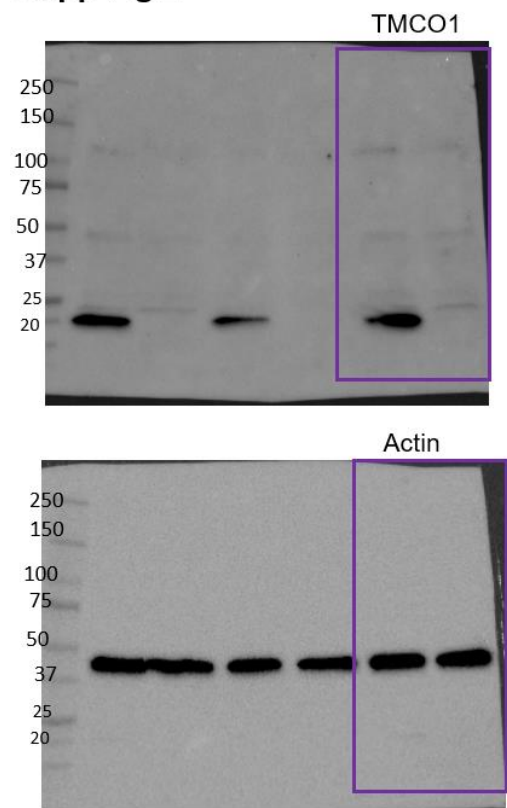


Figure 6E



Supp Fig 2



Supp Fig 3

