# Supplementary Materials for

## TFAM knockdown-triggered mtDNA-nucleoid aggregation and a decrease in mtDNA copy number induce the reorganization of nucleoid populations and mitochondria-associated ER-membrane contacts

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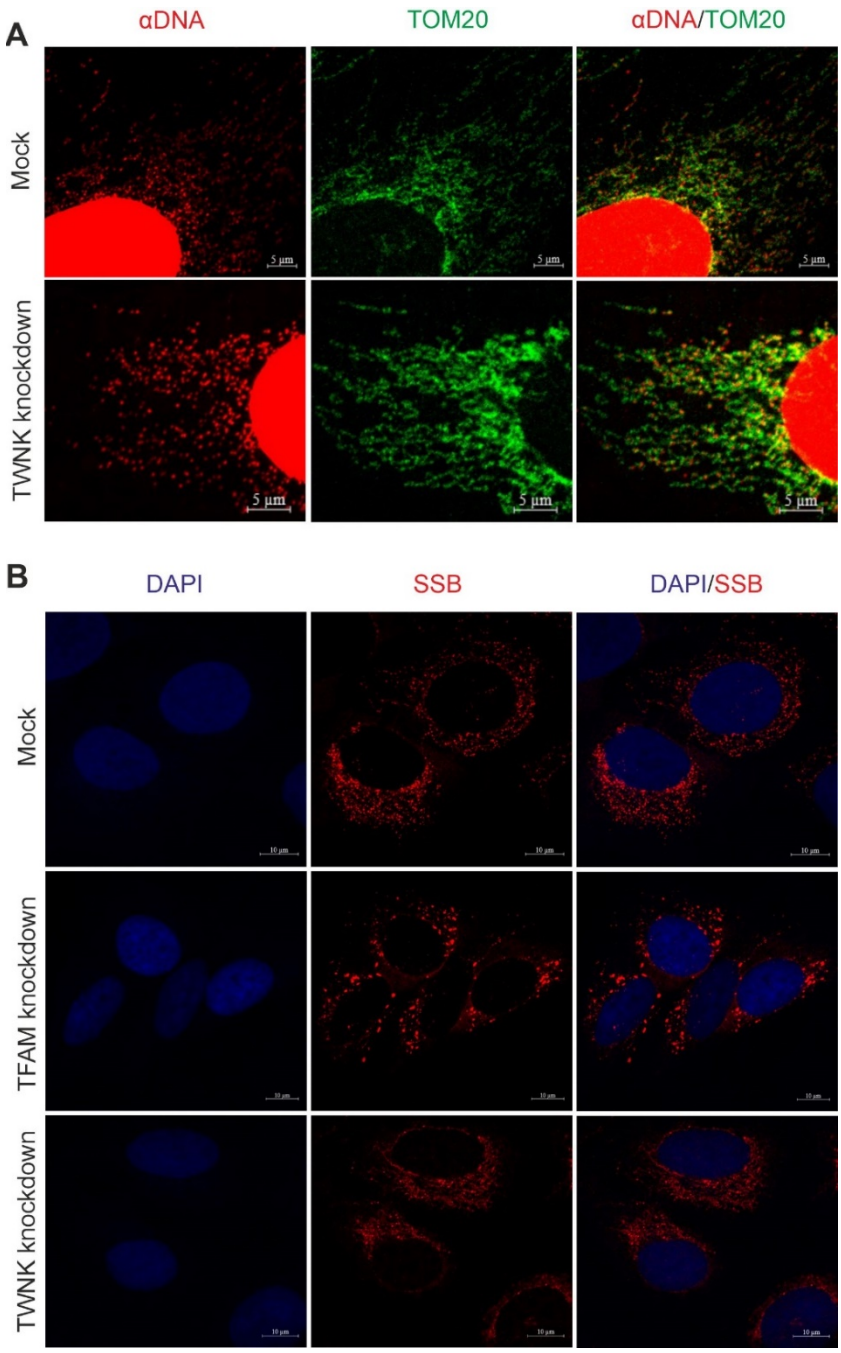
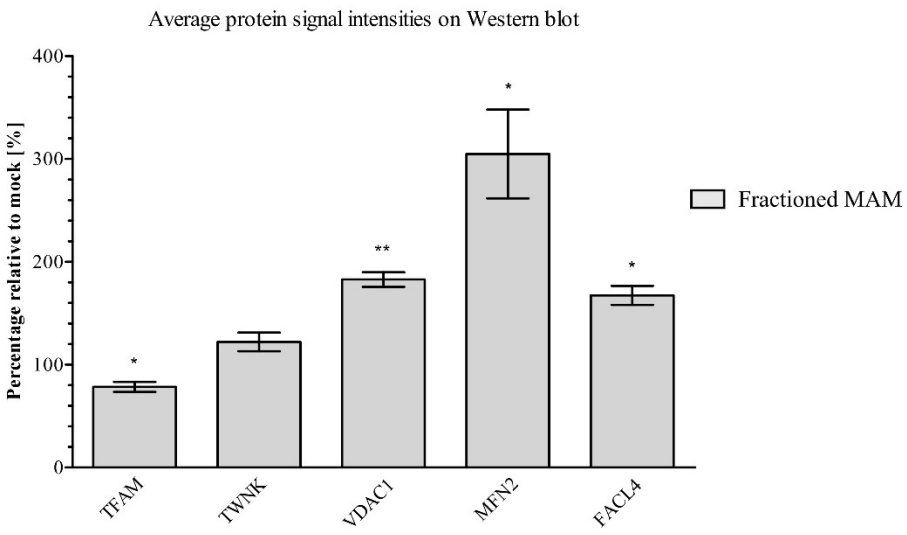
**This PDF file includes:**

Figs. S1 to S5

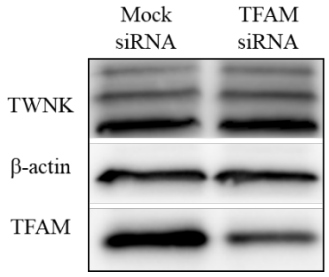
Figs. of electrophoretic blots



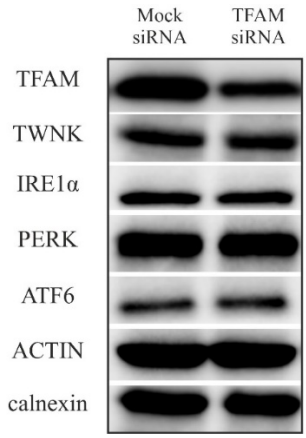

**Supplementary figure 1.** Confocal images of TFAM and TWNK knockdown in U2OS cells. Knockdown of the mtDNA replicative helicase TWNK does not cause enlarged mtDNA-nucleoids (A). TFAM knockdown-caused enlarged nucleoids are also positive for SSB (B), whereas in TWNK knockdown cells SSB signals do not accumulate and are weaker compared to the control transfected cells. TOM20 was used as mitochondrial network marker. Image acquisition using confocal microscopy used a Zeiss LSM710 confocal microscope equipped with 4 solid-state lasers (405 nm, 458/488/514 nm, 561 nm and 633 nm). Slides were imaged with a 63X oil immersion objective.



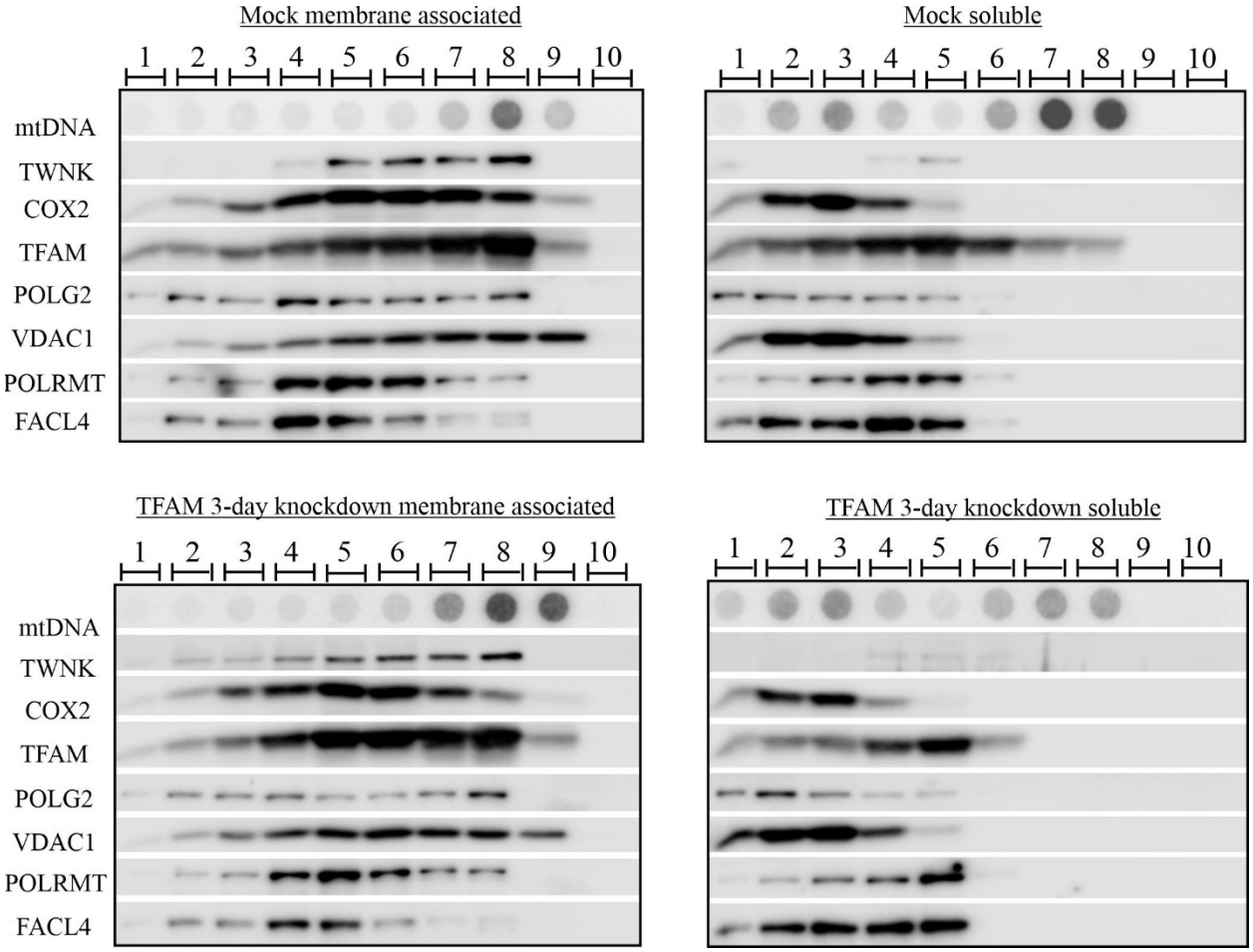

**Supplementary figure 2**. Sub-fractioned MAM from TFAM knockdown cells analyzed by Western blot were quantified, normalized to the loading control β-actin and expressed as a percentage relative to control siRNA transfections (n=3). For statistics a two-tailed paired Student's t-test was used. Error bars indicate +/- SEM (n=3); * indicates a p-value ≤ 0.05, ** indicates a p-value ≤ 0.01.




**Supplementary figure 3.** A Western blot of HEK293 control-transfected and TFAM knockdown total cell extracts showing that TWNK is slightly more abundant in TFAM knockdown cells. β-actin is used as a loading control.




**Supplementary figure 4.** Detection of early ER-stress related proteins by Western analysis. 3 days after TFAM knockdown no changes were observed in the ER-stress related proteins IRE1α, PERK, ATF6 in total cell extracts of HEK293 cells. IRE1α - inositol-requiring enzyme 1 alpha; PERK - PKR-like ER kinase; ATF6 - activating transcription factor 6.



**Supplementary figure 5. Replicating nucleoid components are mainly found in fraction 8.** Crude mitochondrial fractions from both TFAM knockdown and control siRNA-treated cells were floated in iodixanol gradients by isopycnic centrifugation. Replicating nucleoid components are mainly detected in fraction 8 of membrane-associated protein gradients, representing the pool of replicating nucleoids (see references Gerhold et al., 2015; Rajala et al., 2014). Fraction 5 in the soluble protein gradients contains only little TWNK and mtDNA, but instead transcription components, such as TFAM and POLRMT, hence it represents the pool of transcriptionally active nucleoids. MtDNA signals in TFAM knockdown membrane-associated fractions (especially in fractions 8 and 9) are stronger compared to the control, while they are clearly weaker in the soluble fractions. This illustrates that more mtDNA is localized in membrane-associated replicating nucleoids to compensate the loss of mtDNA. COX2 shows a decrease in the nucleoid fraction of TFAM knockdown mitochondria compared to controls, as does mitochondrial RNA polymerase, possibly indicating a decrease in transcriptional activity due to the loss of TFAM.

**Supplementary images for electrophoretic gels and blots**

Fig. 1E

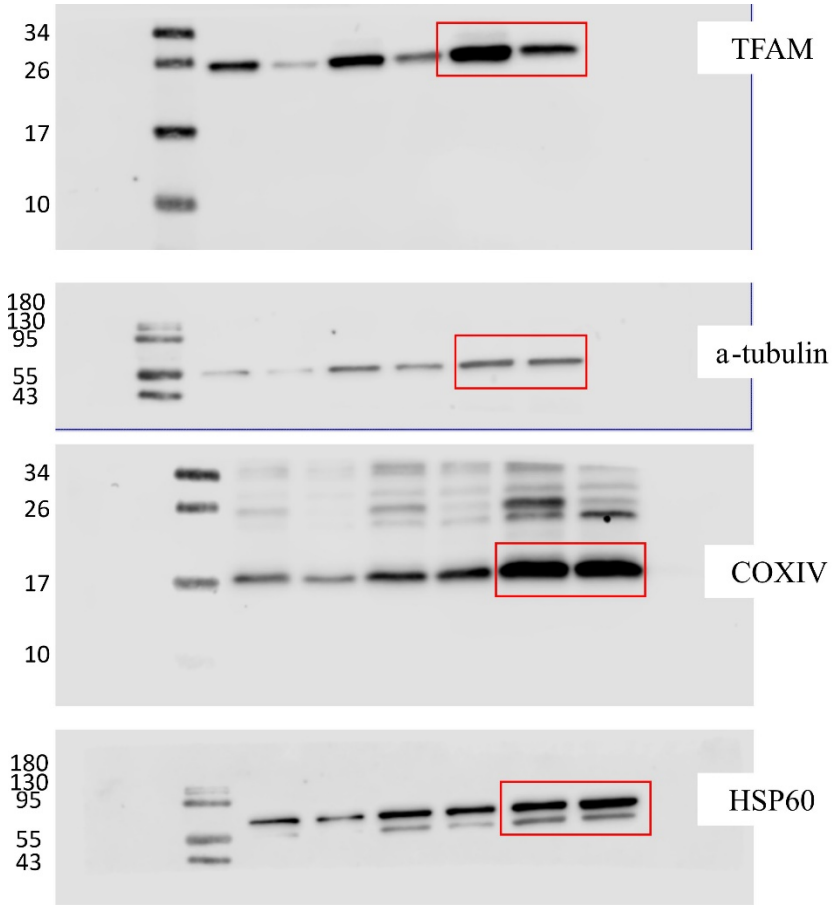


Fig. 2A

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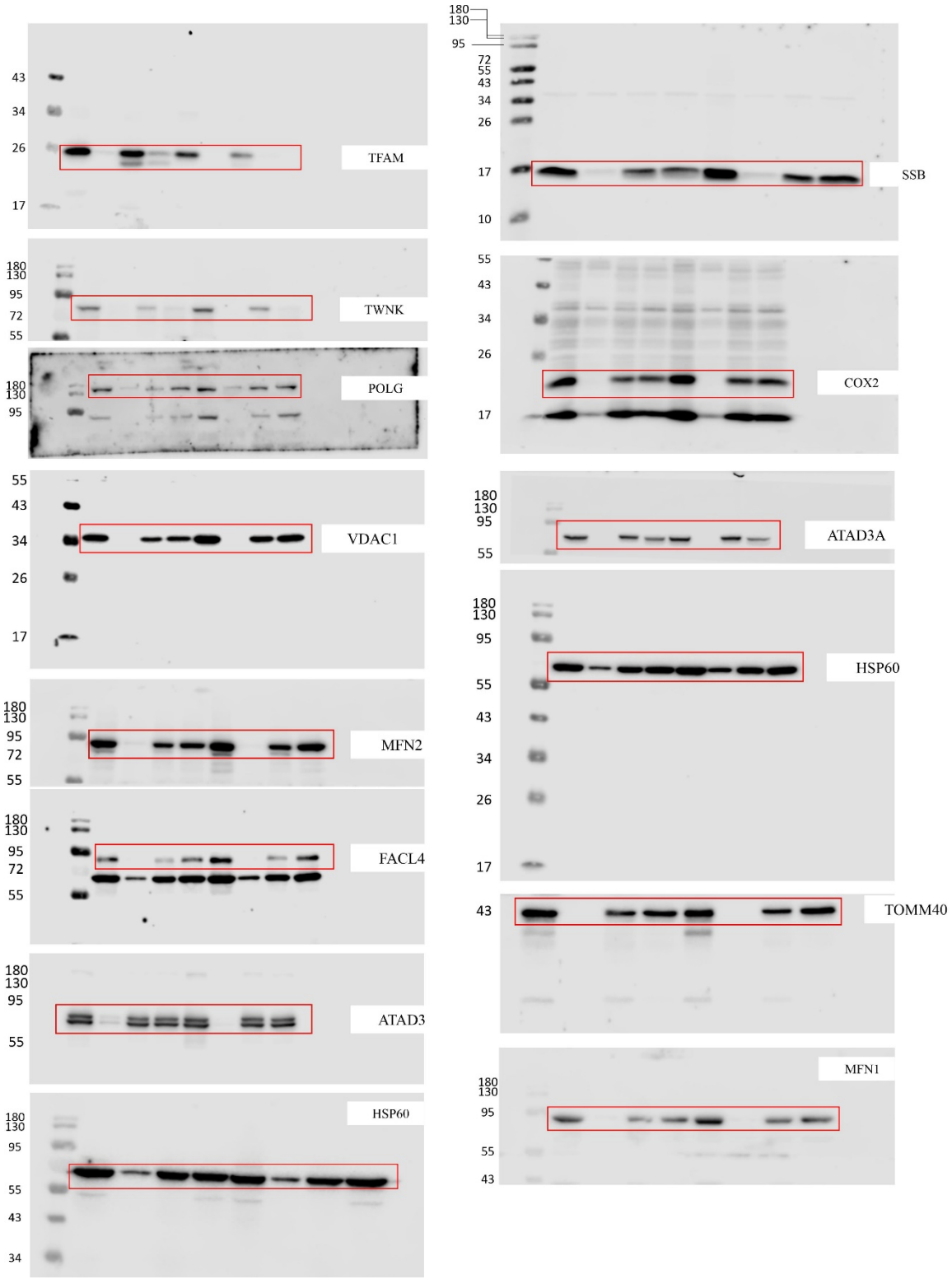

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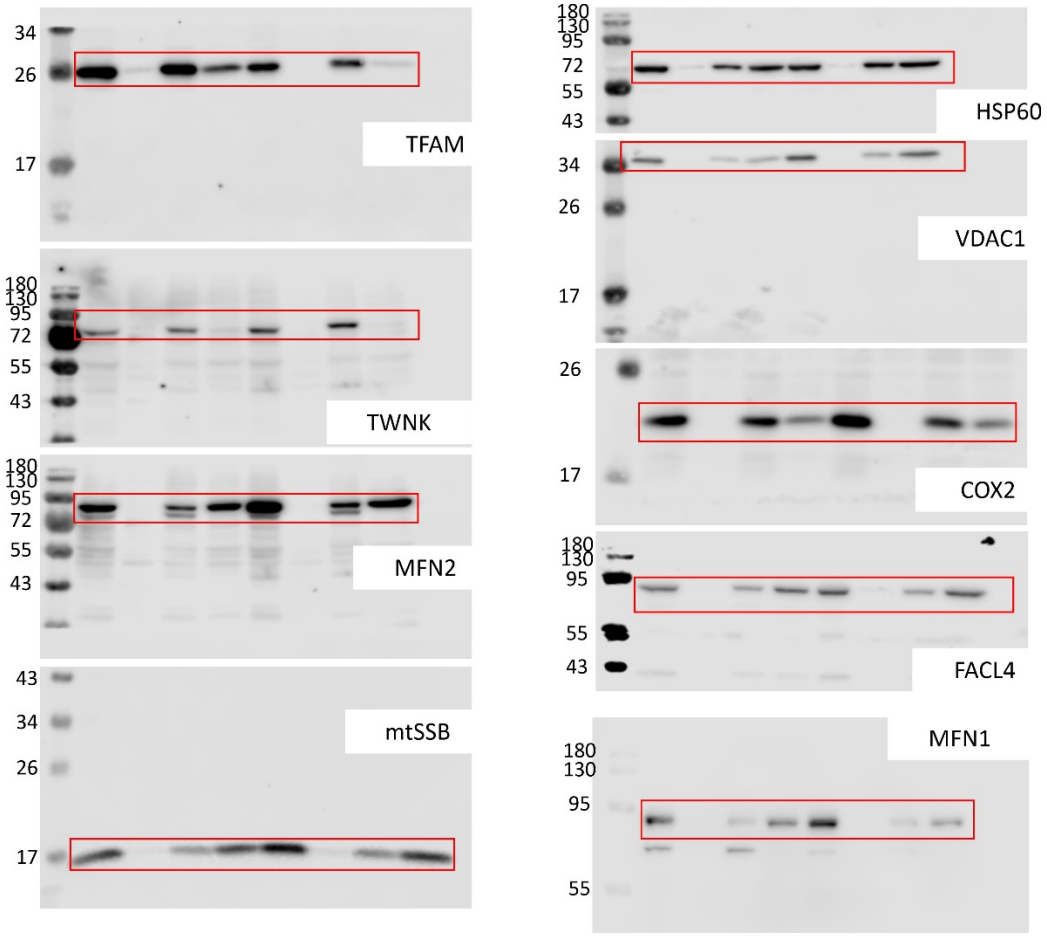
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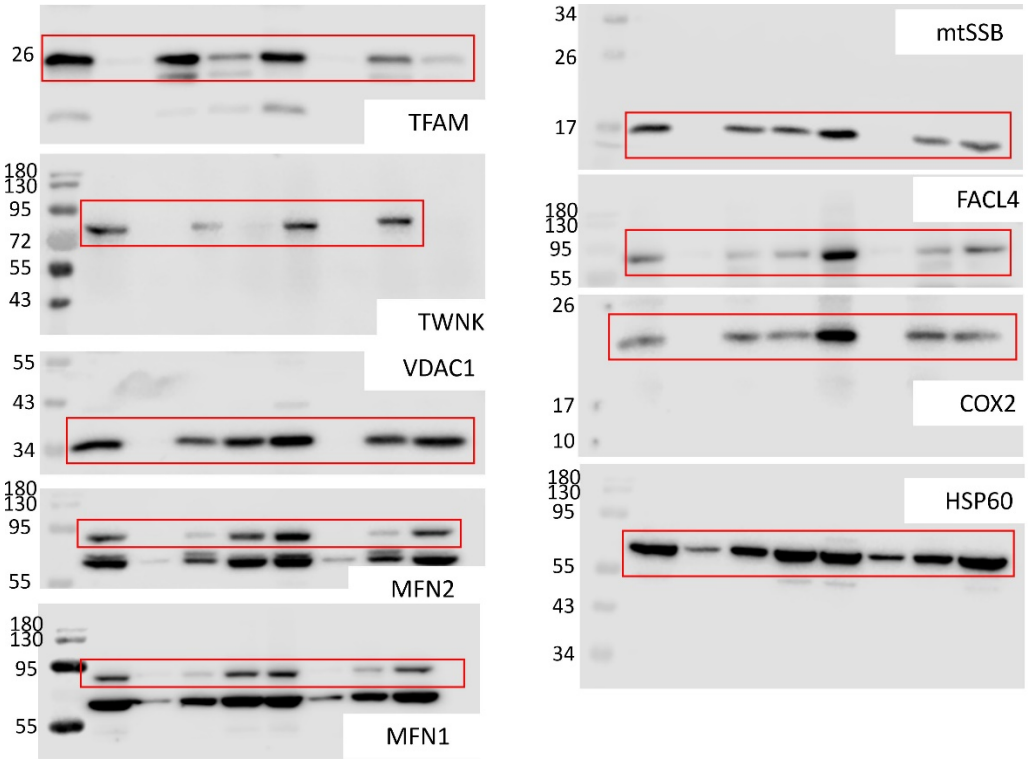
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Fig. 2B

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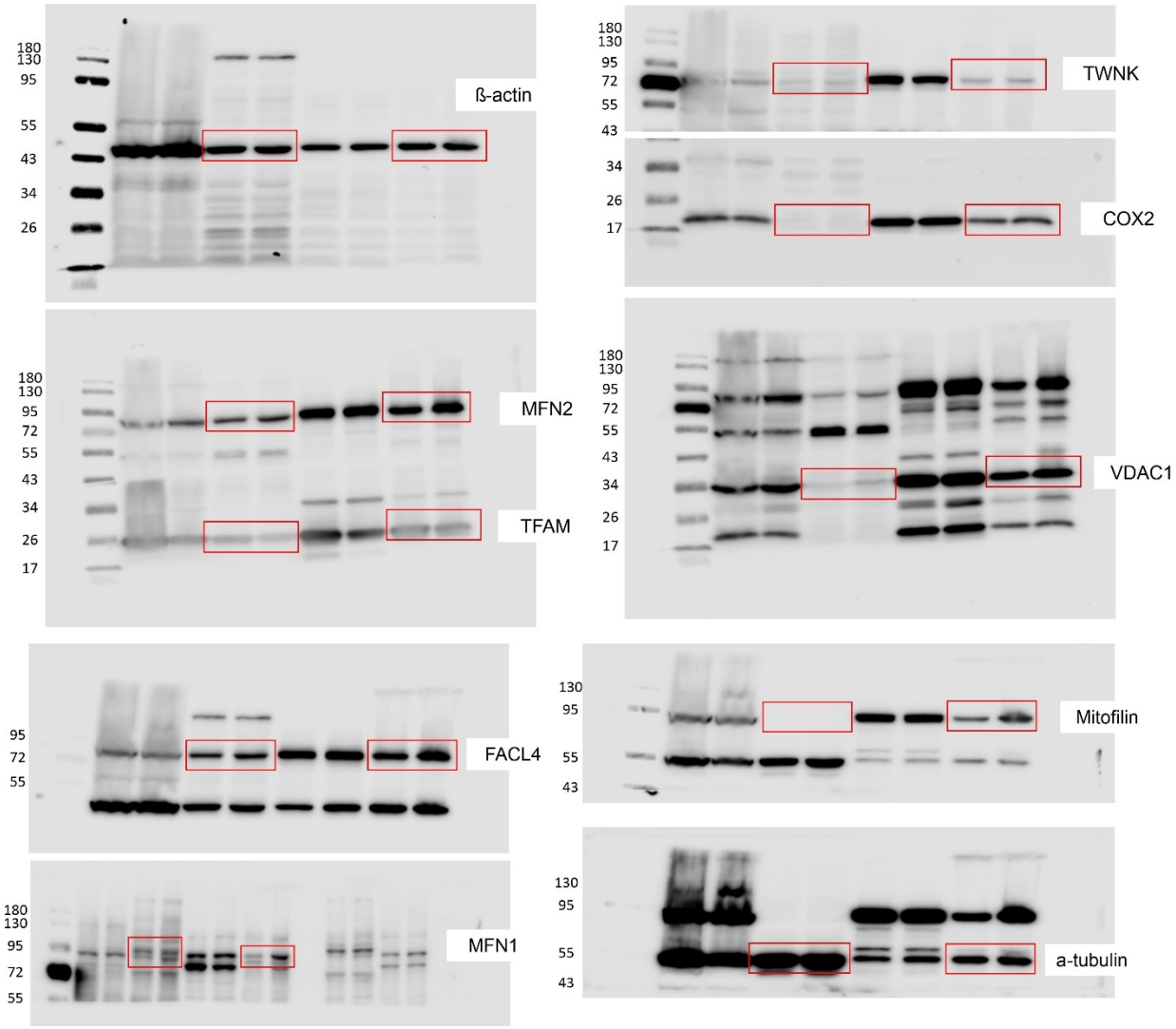

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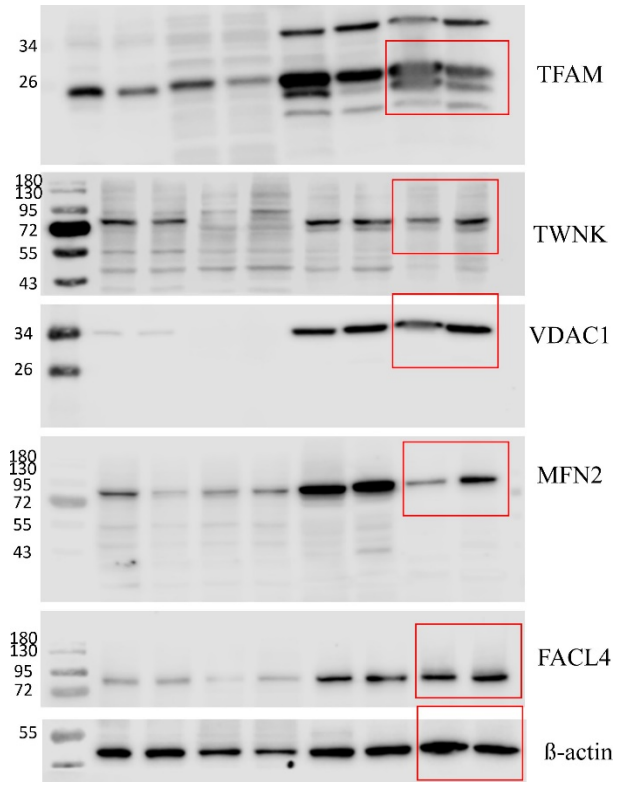


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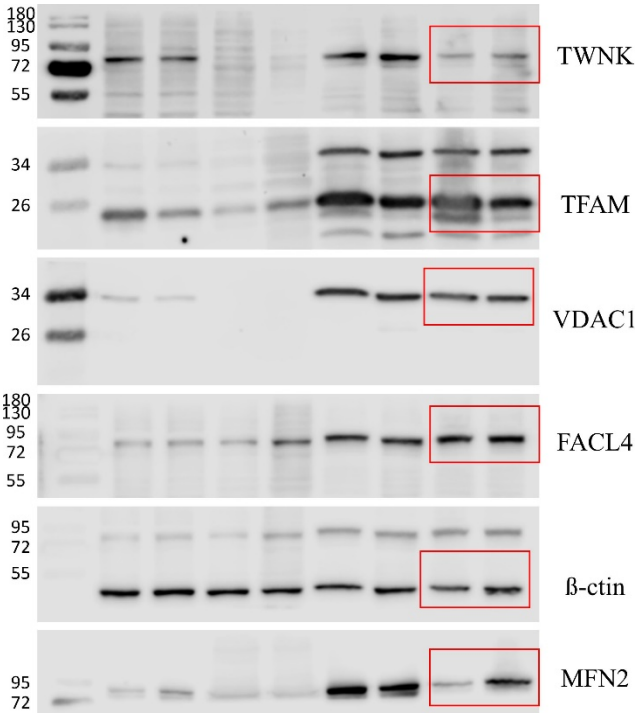

Fig. 3A

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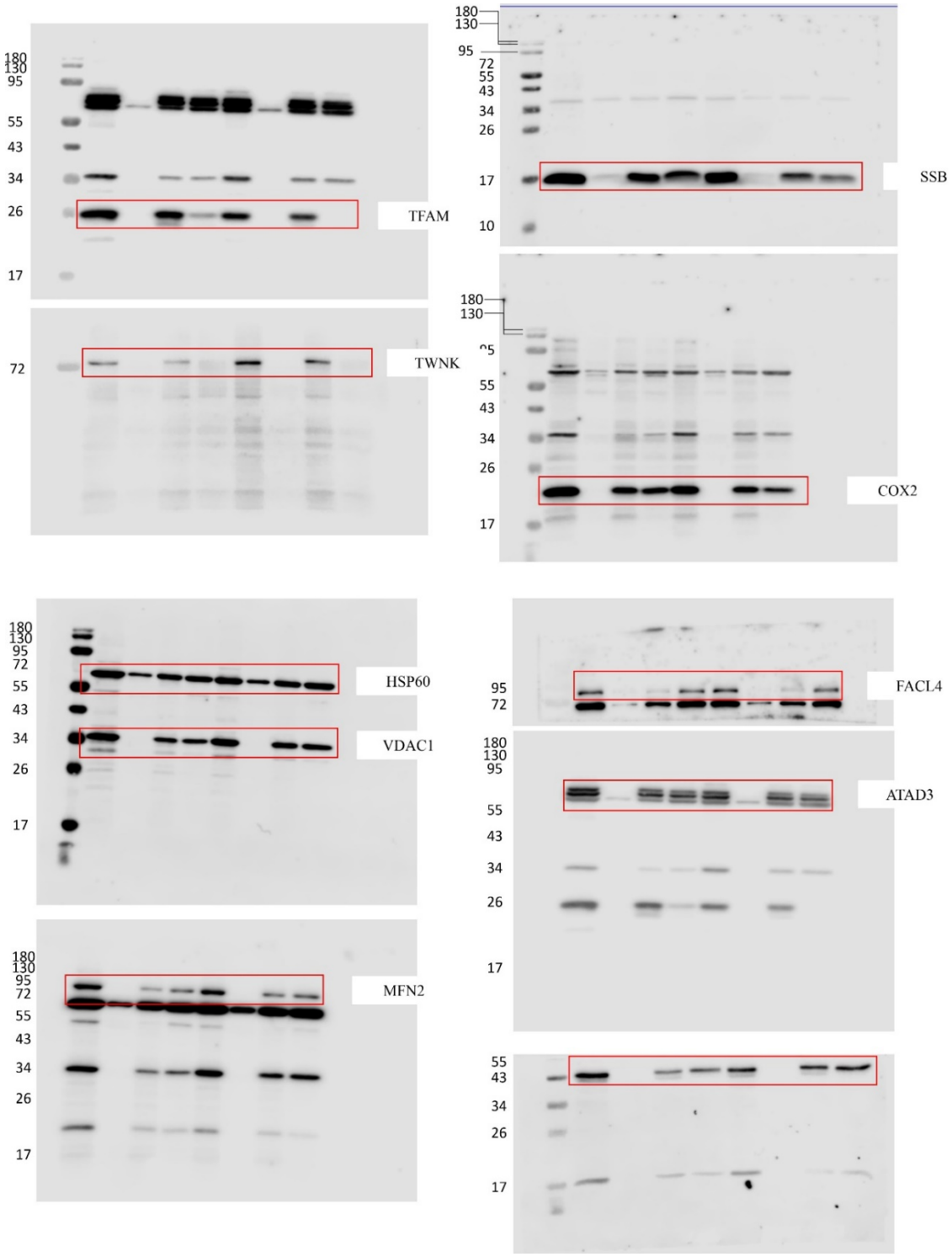

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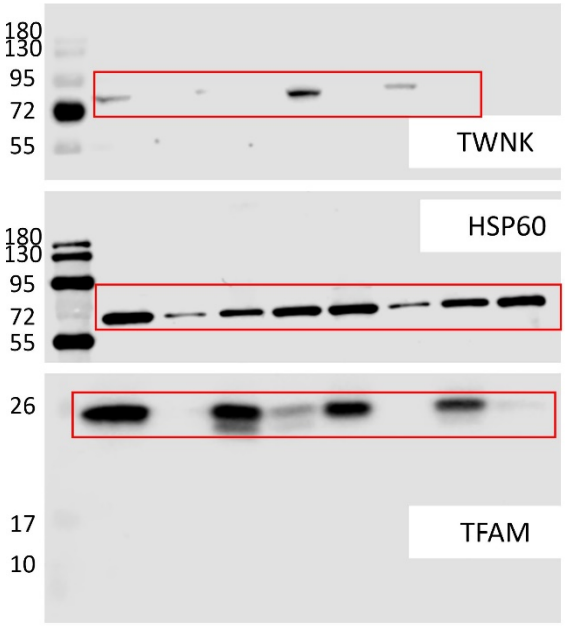


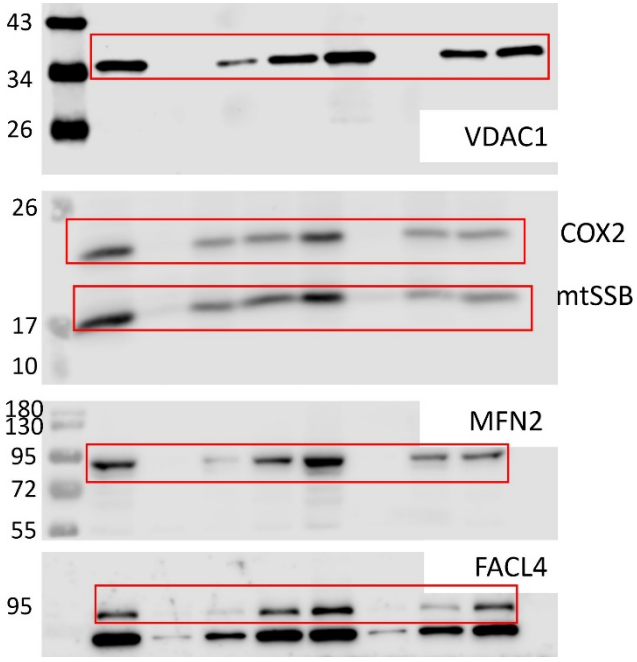


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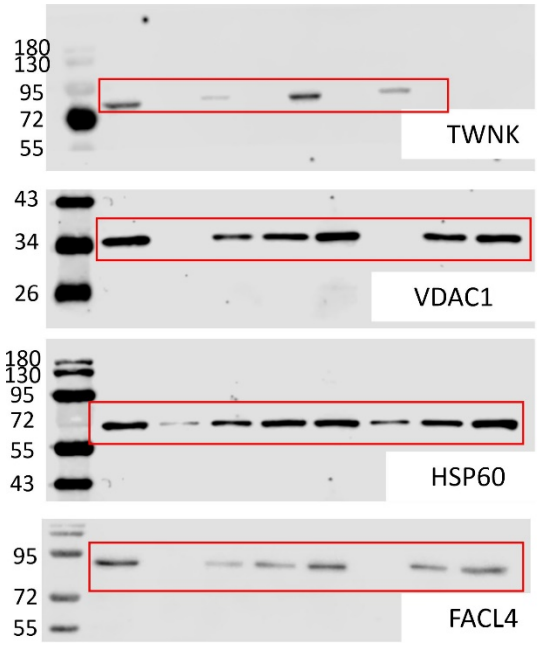


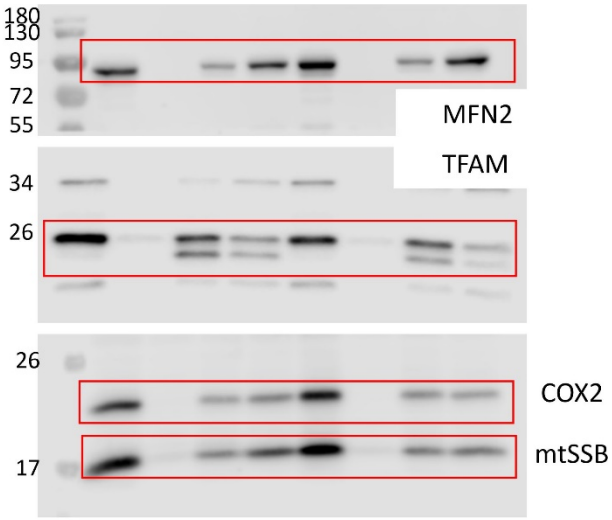

Fig. 3B

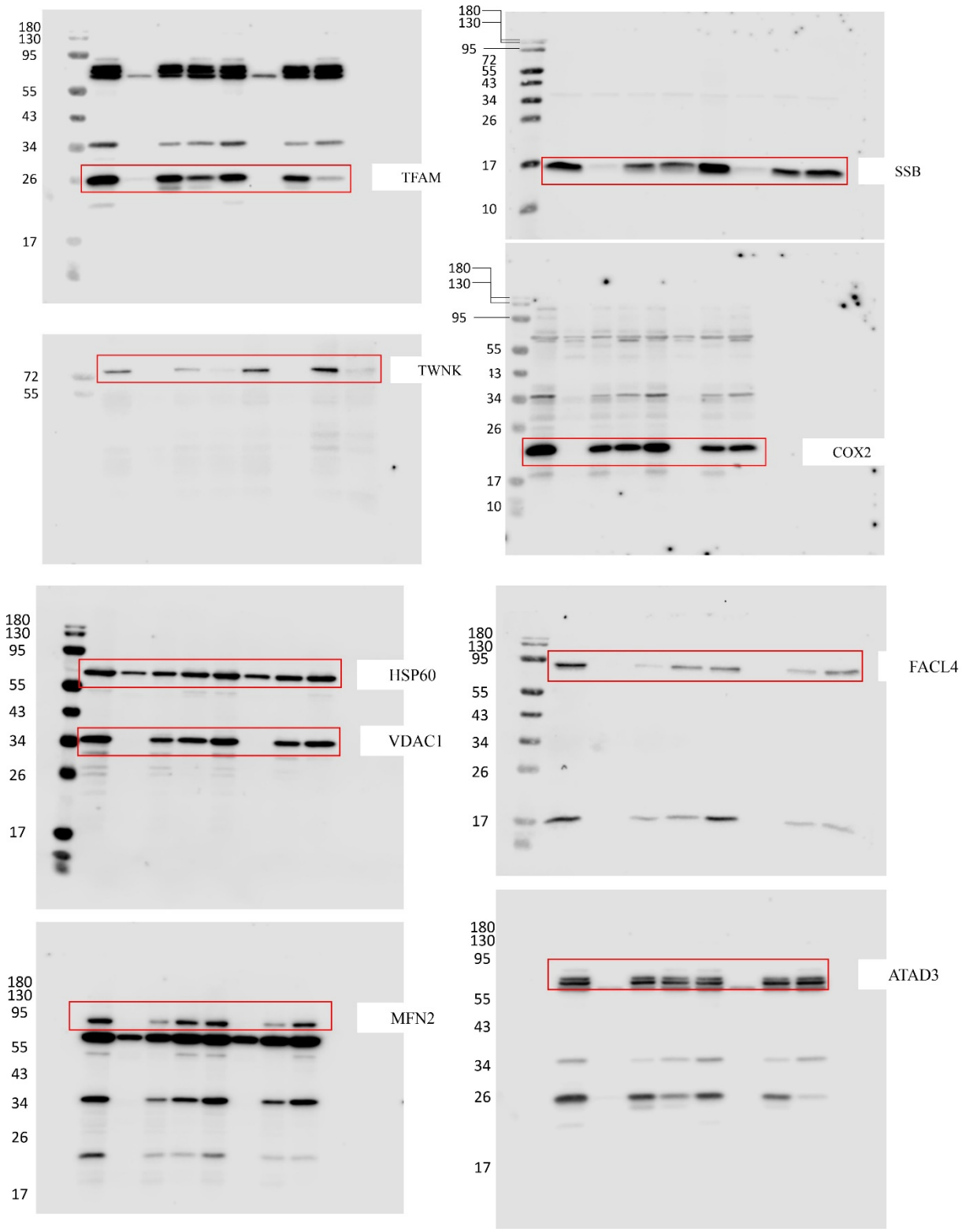
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Fig. 4A

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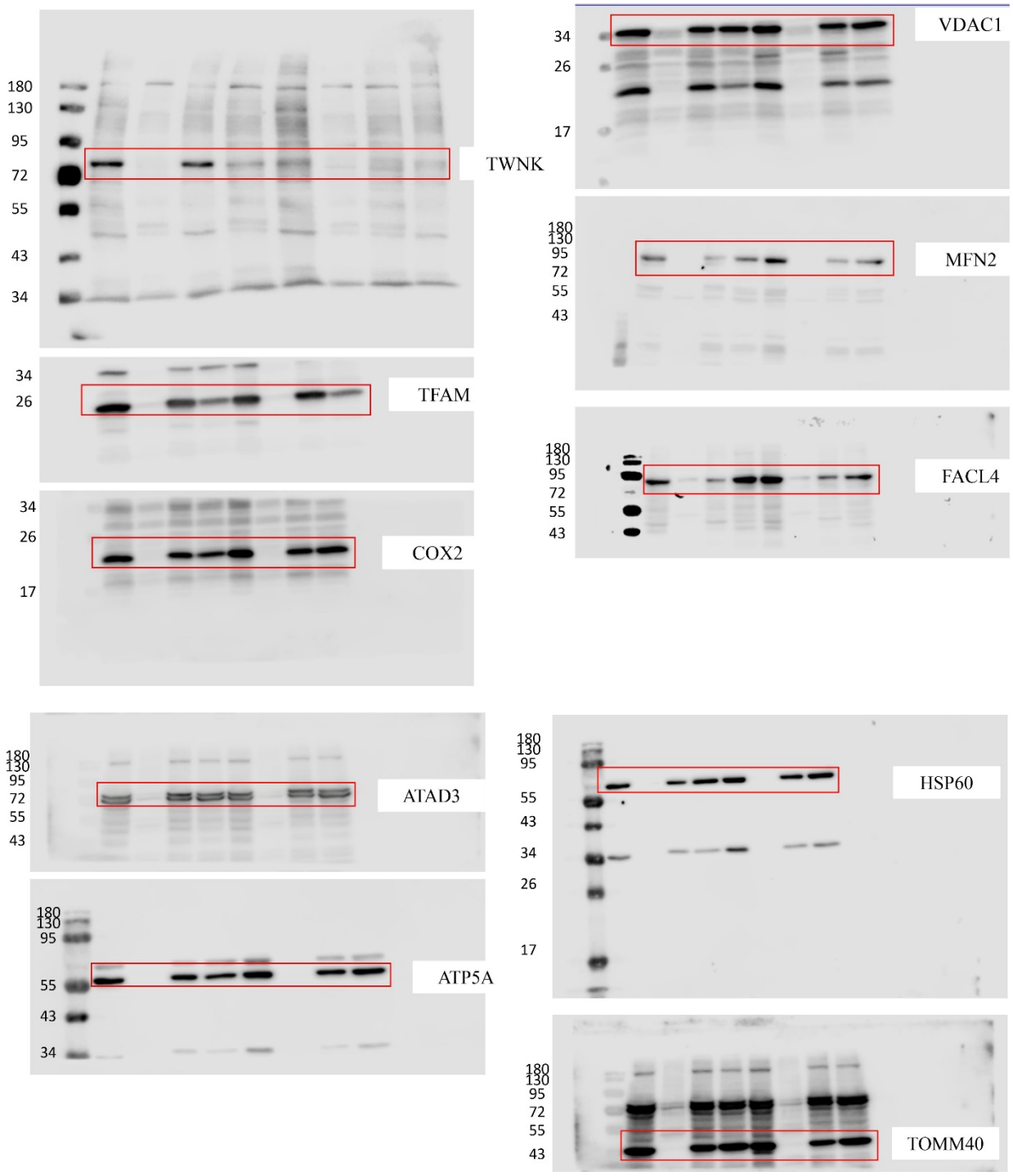

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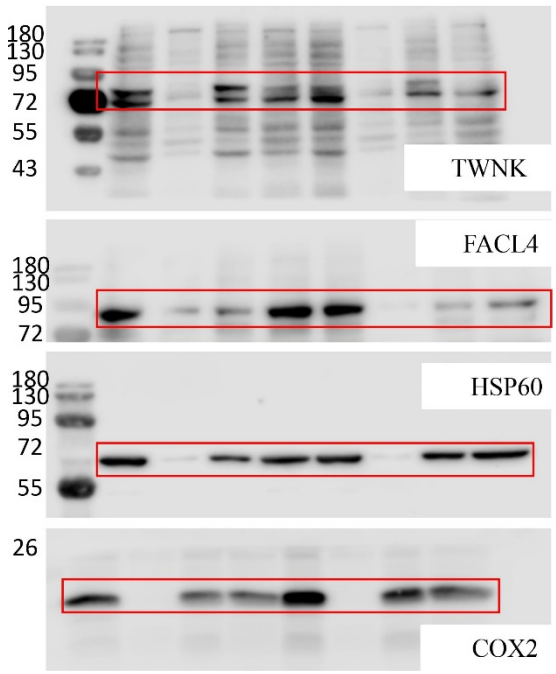


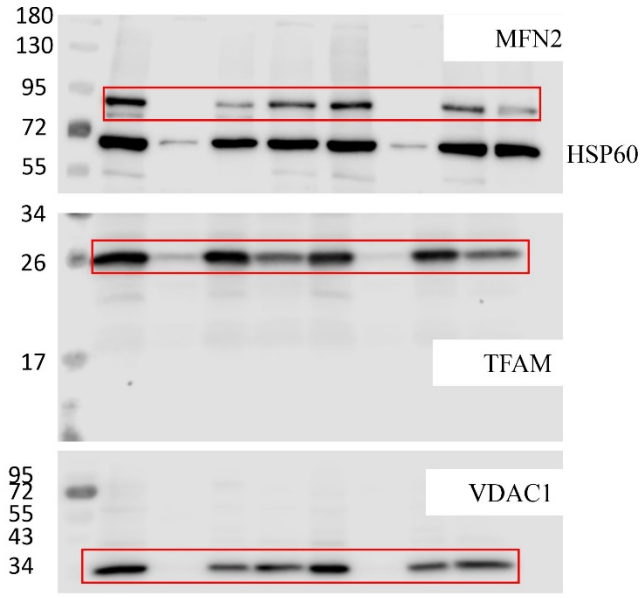


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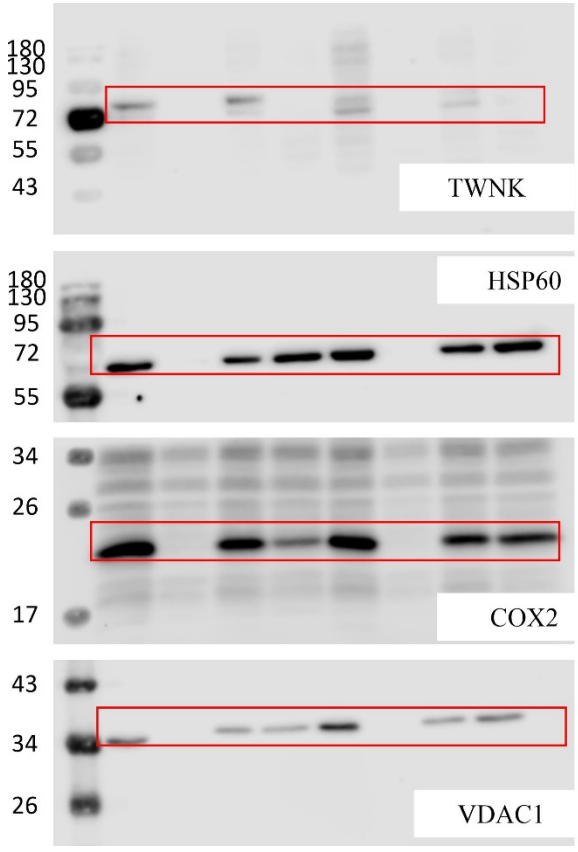


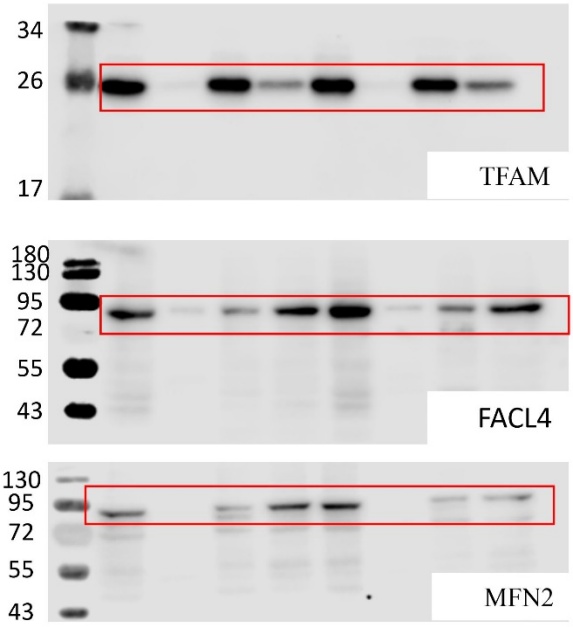

Fig. S6

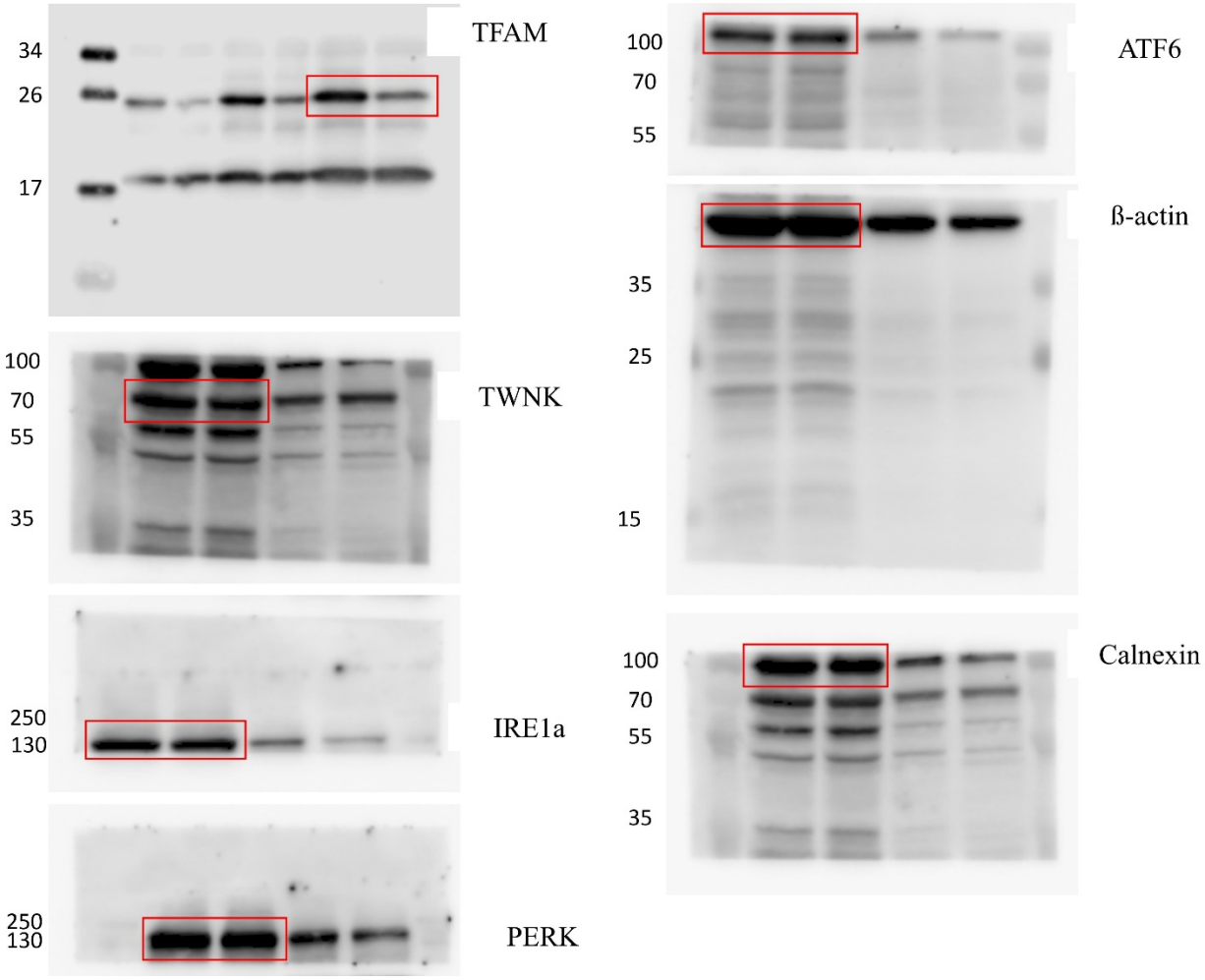
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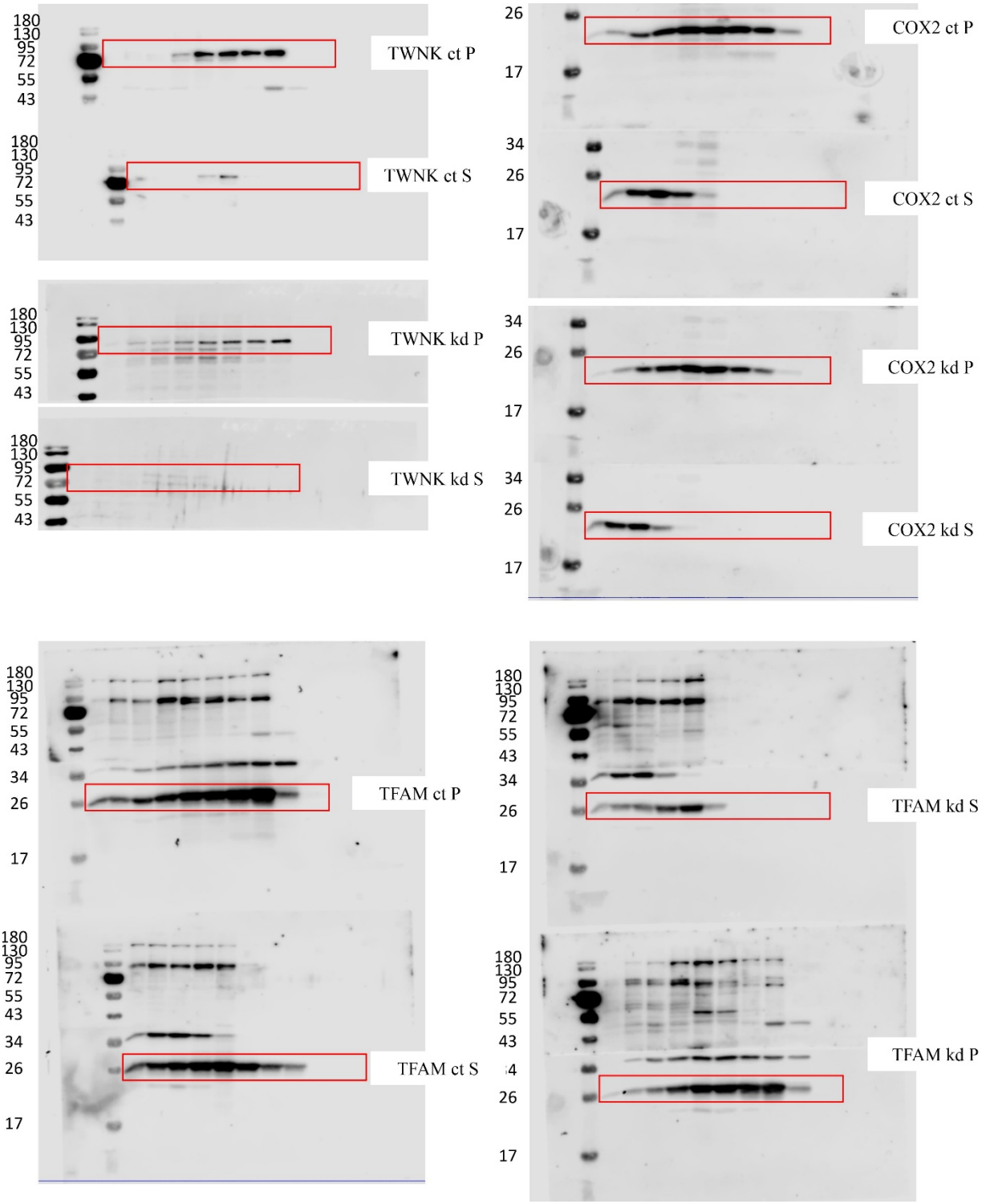
Fig. S8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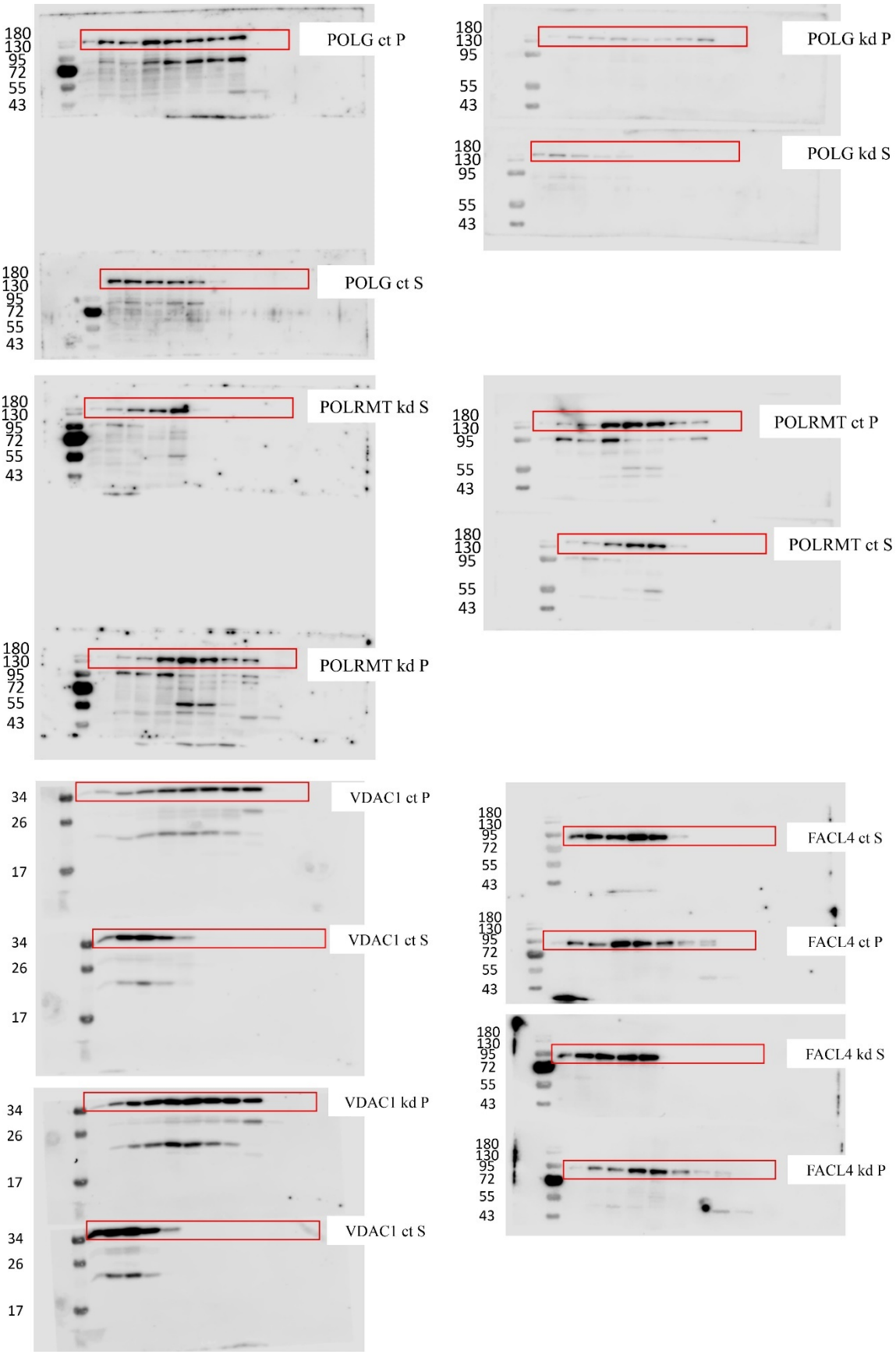
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