

Mitochondrial Quality Control impairment is a hallmark of TDP-43^{G376D} ALS patient-derived fibroblasts

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Supplementary Text

S1. Collection of Skin Biopsies and Isolation of Patient-Derived Dermal Fibroblasts

Skin biopsies were obtained using a sterile disposable punch biopsy instrument (4–5 mm in diameter) and immediately transferred into a sterile solution. For fibroblast isolation, tissue specimens were mechanically minced into small fragments and seeded onto 35 mm culture dishes preconditioned with Fetal Bovine Serum (FBS), followed by incubation overnight at 37°C in a humidified atmosphere containing 5% CO₂.

On the following day, tissue explants were maintained in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 15% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 1% non-essential amino acids. Explants were cultured under standard conditions for primary dermal fibroblasts until cell growth from the tissue fragments was observed. Once established, fibroblasts were expanded in 100 mm culture dishes and passaged upon reaching approximately 80% confluence.

S.2 Plasmid Constructs and Transfection

Full-length TDP-43 cDNA was used as template for PCR amplification of the complete TDP-43 coding sequence, which was subsequently subcloned into the pEGFP-C1 vector to generate the N-terminal GFP-tagged full-length TDP-43 construct (GFP-TDP-43). The GFP-TDP-43^{G376D} construct was generated by site-directed mutagenesis starting from the full-length GFP-TDP-43 plasmid using PfuTurbo DNA Polymerase (600252-52, Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's instructions.

Mutagenesis was performed using the following primers:

5'-GGAAATAACTCTTATATAGTGACTCTAATTCTGGTGCAGC-3'

5'-GCTGCACCAGAATTAGAGTCACTATAAGAGTTATTTCC-3'.

The empty pEGFP-C1 vector was used as control in transfection experiments.

S.3 Genomic DNA isolation and qPCR

Genomic DNA was isolated from 5×10^6 HEK293T and Neuro2a cells using the Genomic-tip 20/G kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's instructions. DNA concentration and purity were assessed by spectrophotometric measurement at 260/280 nm using a NanoDrop 2000C (Waltham, MA, USA). mtDNA integrity was evaluated by amplification of a long (13.4 kb) fragment and a short (235 bp) fragment, the latter used as an internal control.

Long fragment (13.4 kb): 5'-AAAATCCCCGCAAACAATGACCACCC-3' (forward) and 5'-GGCAATTAAGAGTGGGATGGAGCCAA-3' (reverse) (accession number NC_005089.1)

Short fragment (235 bp): 5'-CCTCCCATTTCATTATCGCCGCCCTGC-3' (forward) and 5'-GTCTGGGTCTCCTAGTAGGTCTGGGAA-3' (reverse) (accession number NC_005089.1)

qPCR products were quantified using PicoGreen dsDNA fluorescence assay (PicoGreen dsDNA Assay Kit), and fluorescence intensity was measured using a fluorescence microplate reader BioTek Synergy H1 (Agilent Technologies, Santa Clara, CA, USA). Mitochondrial DNA damage was quantified by comparing the relative amplification efficiency of the long mtDNA fragment normalized to the short fragment within the same sample. Specifically, relative lesion frequencies per 10 Kbp DNA was calculated by applying the Poisson distribution according to the formula:

$$\text{Lesion frequency} = -\ln Ad / A_o$$

where Ad means amplification of treated samples, and Ao represents amplification of controls.

91 **Supplementary Table**92 **Table S1. Antibodies used for Western blot**

Antibody	Reference number
APE1	cod.NB100-116 (Novus Biologicals, LLC, Centennial, CO, USA)
ATF4	cod.#A18687 (ABclonal Technology, Woburn, MA, USA)
ATF5	cod.#A3563 (ABclonal Technology, Woburn, MA, USA)
B-ACTIN	cod.bs-0061R (Bioss Antibodies, Woburn, MA, USA)
Hsp90	sc-13119 (Santa Cruz Biotechnology, Dallas, TX, USA)
CHOP	cod.#A0221 (ABclonal Technology, Woburn, MA, USA)
CLPP	cod.#A32214 (ABclonal Technology, Woburn, MA, USA)
DNA pol γ	cod.sc-5931 (Santa Cruz Biotechnology, Dallas, TX, USA)
eiF2 α (Ser51)	cod.#A0764 (ABclonal Technology, Woburn, MA, USA)
FOXO3A	cod.#A0102 (ABclonal Technology, Woburn, MA, USA)
GFP	sc-9996 (Santa Cruz Biotechnology, Dallas, TX, USA)
LONP1	cod.#A4293 (ABclonal Technology, Woburn, MA, USA)
MICOS Complex Subunit MIC10 (C1ORF151)	cod.PA5-116347 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA)
MICOS Complex Subunit MIC60 (IMMT)	cod.abx235199 (Abbexa, Cambridge, UK)
OGG1	cod.#NB100-106 (Novus Biologicals, LLC, Centennial, CO, USA)
P-eiF2 α (Ser51)	cod.#3398 (Cell Signaling Technology, Danvers, MA, USA)
PERK	cod.#3192 (Cell Signaling Technology, Danvers, MA, USA)
PINK1	cod.#6946 (Cell Signaling Technology, Danvers, MA, USA)
P-PERK (Thr980)	cod.#3179 (Cell Signaling Technology, Danvers, MA, USA)

SIRT3	cod.#2627 (Cell Signaling Technology, Danvers, MA, USA)
TRAP1	cod.#A2748 (ABclonal Technology, Woburn, MA, USA)
Anti-rabbit IgG	cod.#ab97051 (Abcam, Cambridge, UK)
Anti-mouse IgG	cod.#ab97023 (Abcam, Cambridge, UK)

Supplementary Figures

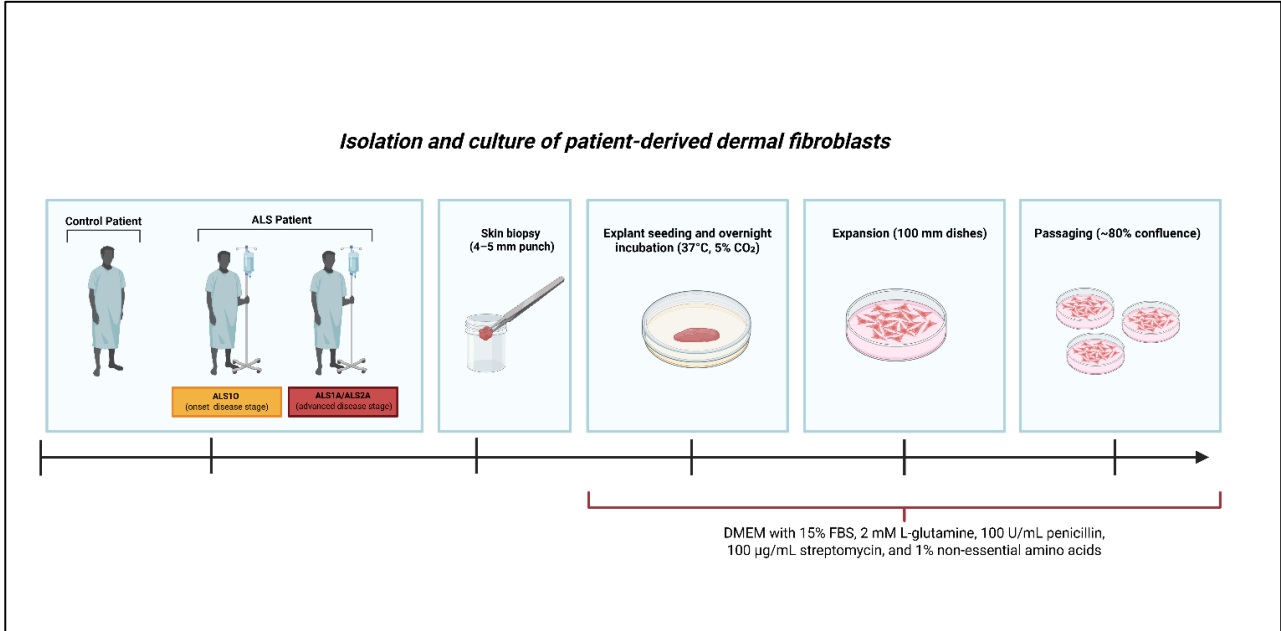


Figure S1. Schematic workflow of skin biopsy collection and dermal fibroblast isolation. ALS-derived fibroblasts were designated as ALS1A and ALS2A, corresponding to advanced symptomatic stages (A = advanced), and ALS1O, corresponding to the disease onset stage (O = onset).

Figure 1A

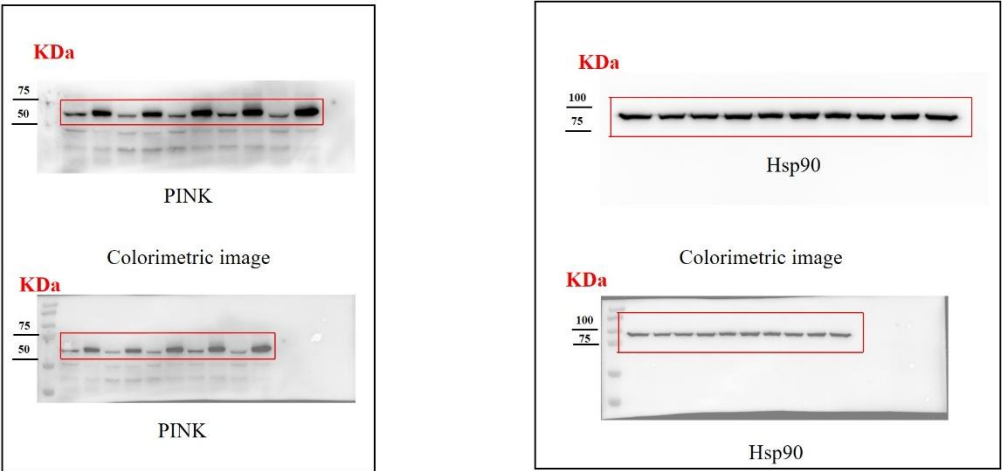


Figure 2A

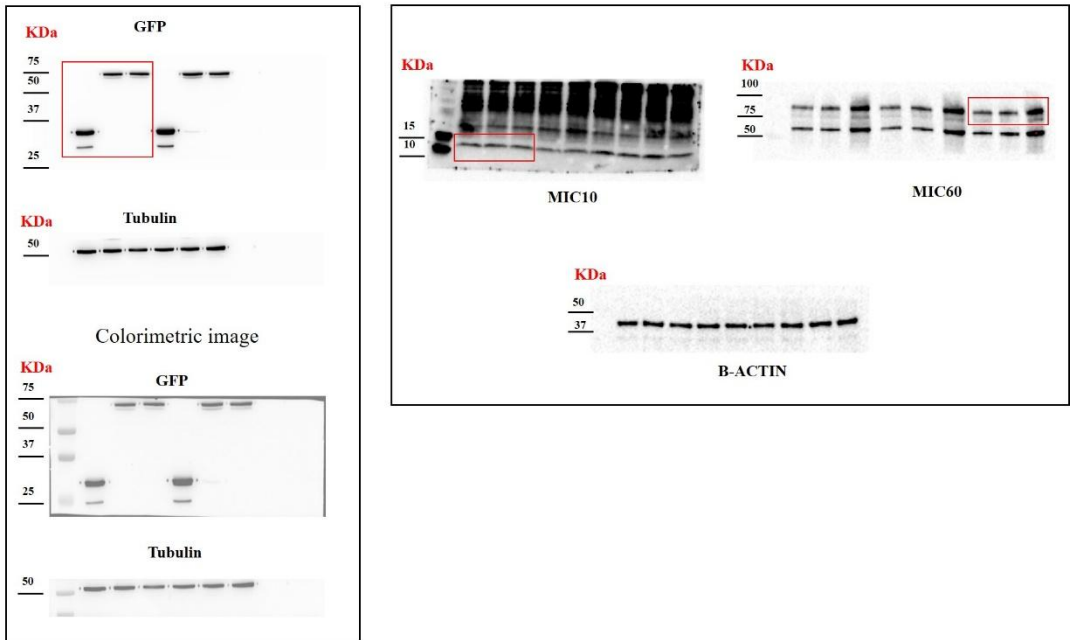


Figure S2. Uncropped Western blot images

Figure 2B

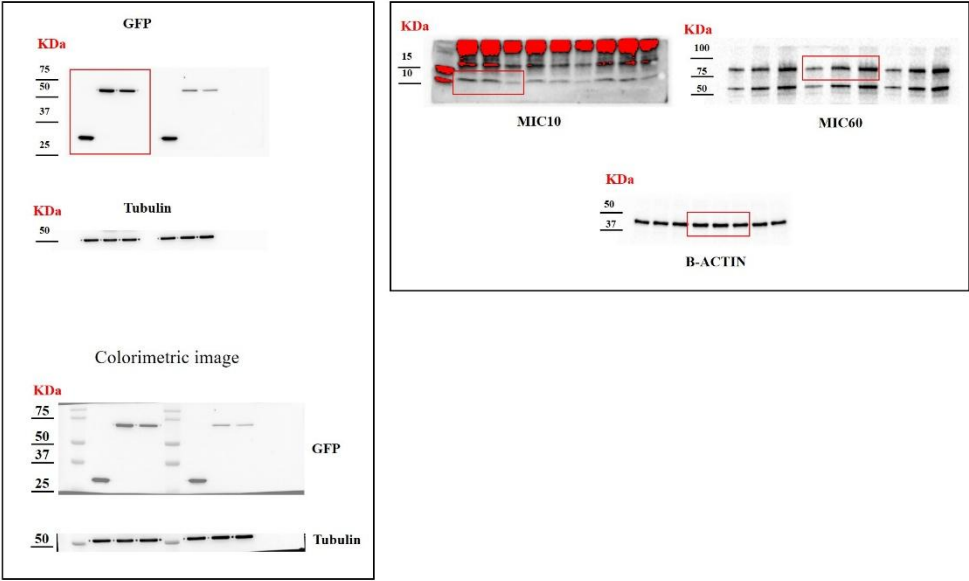


Figure 2C

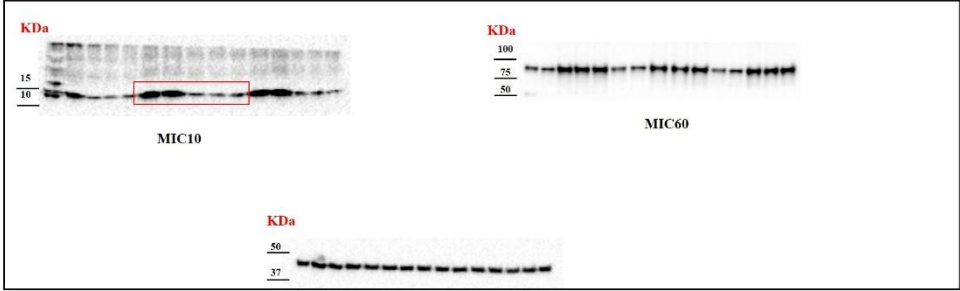


Figure S3. Uncropped Western blot images

Figure 5C

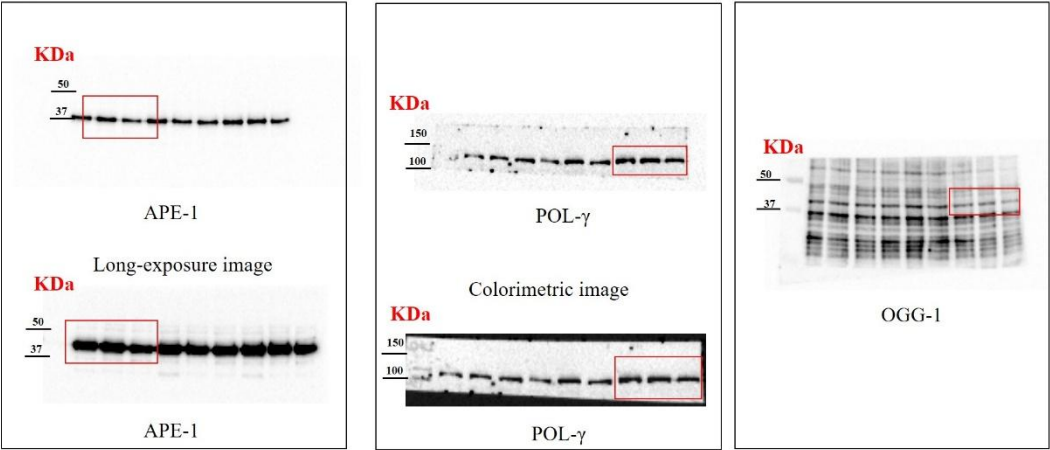


Figure 5D

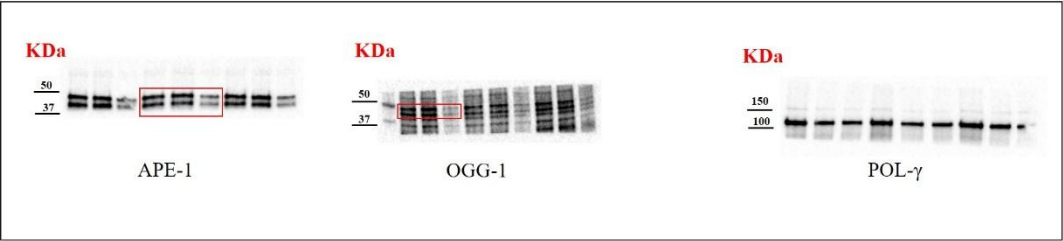


Figure 5E

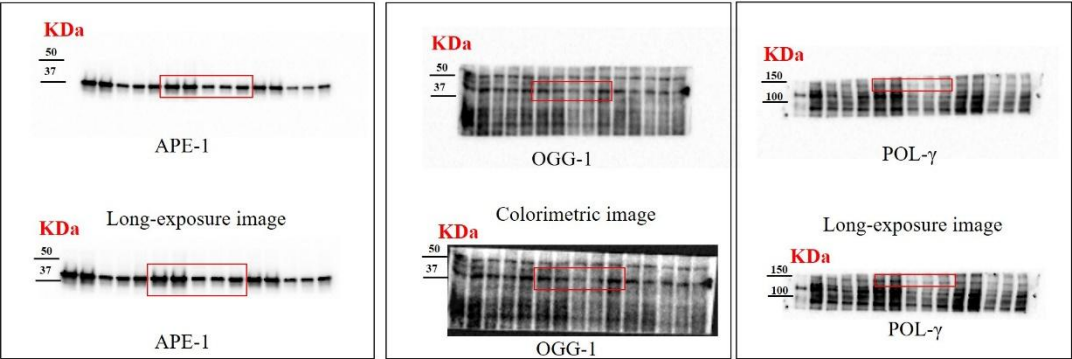


Figure S4. Uncropped Western blot images

Figure 6A

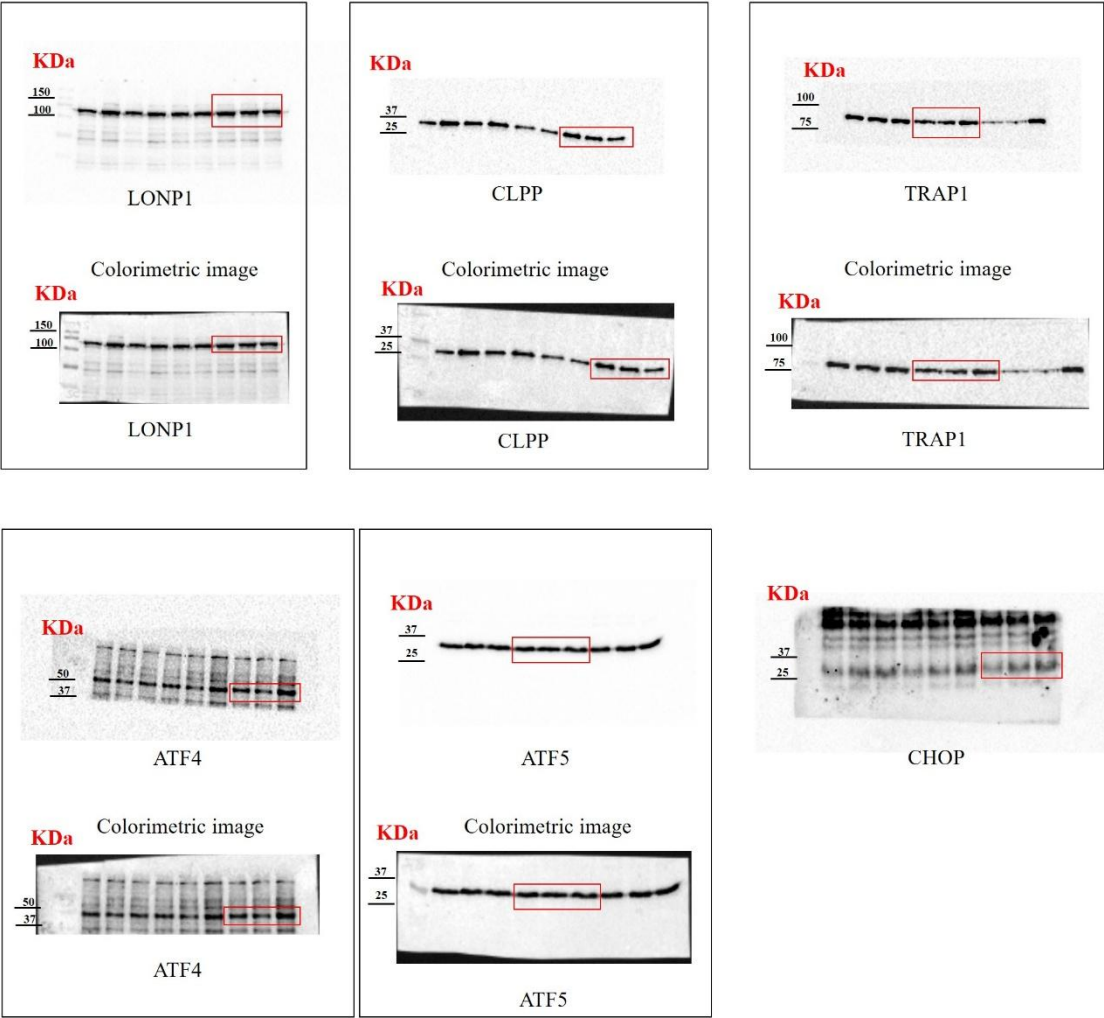


Figure S5. Uncropped Western blot images

Figure 6B

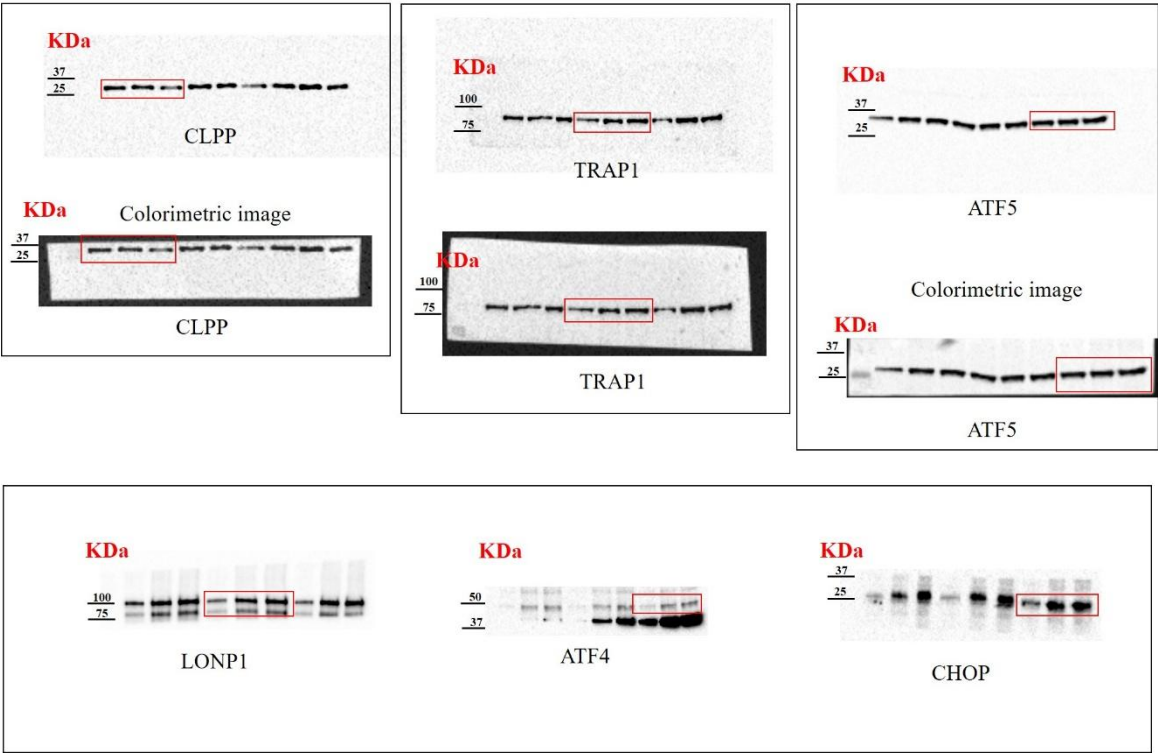


Figure S6. Uncropped Western blot images

Figure 6C

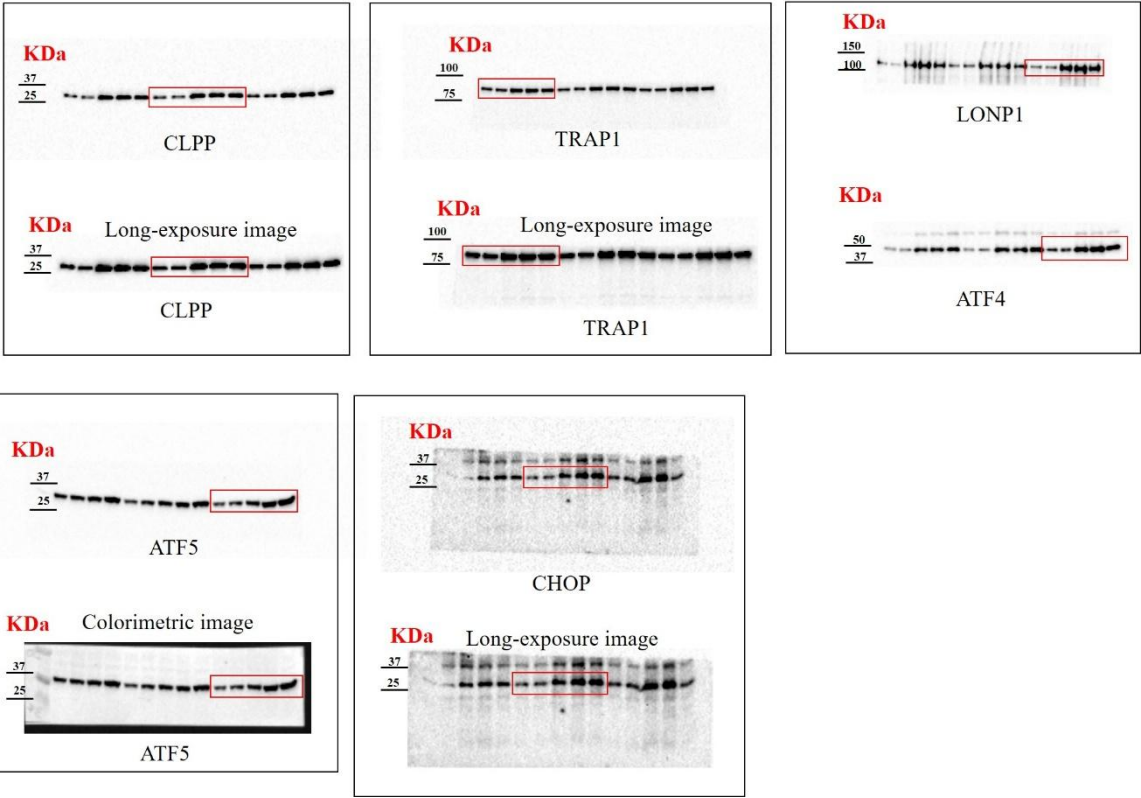


Figure S7. Uncropped Western blot images

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Figure 6D

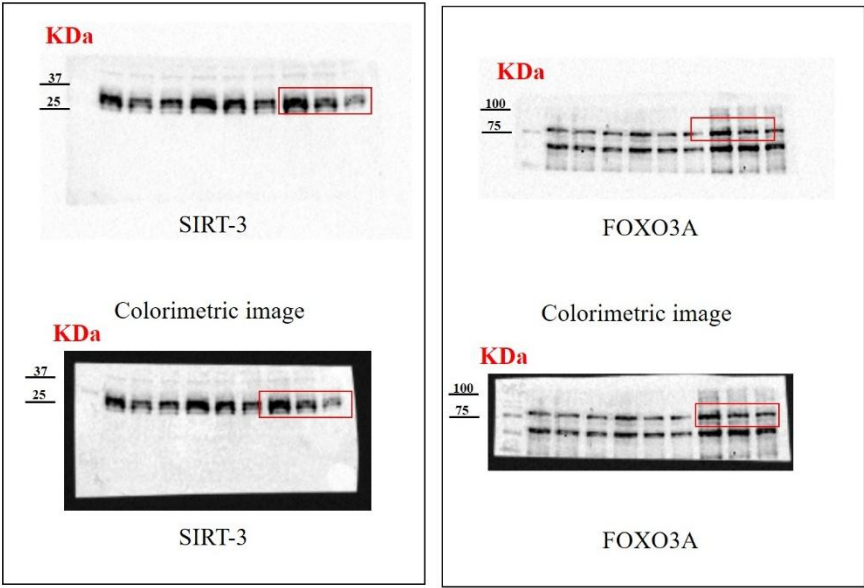


Figure 6E

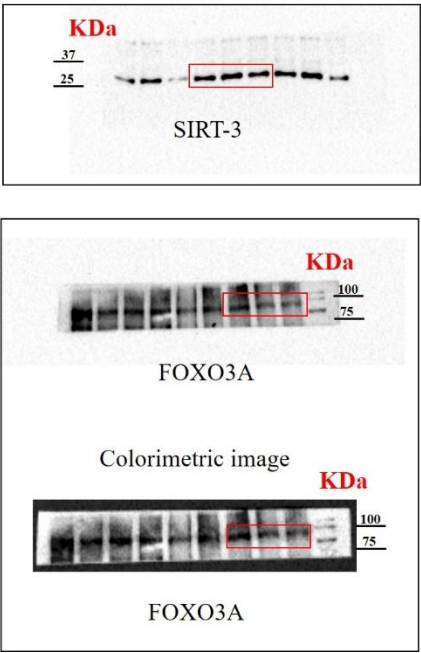


Figure 6F

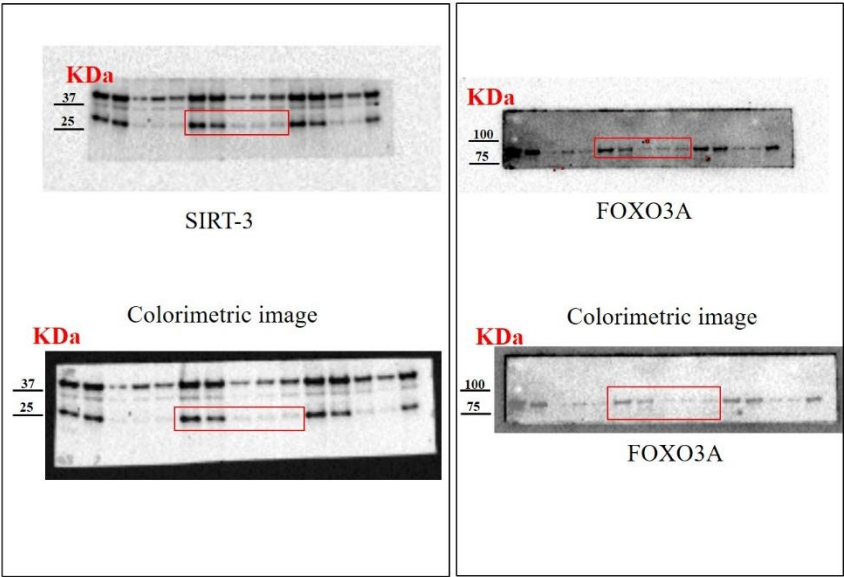


Figure S8. Uncropped Western blot images

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Figure 7A

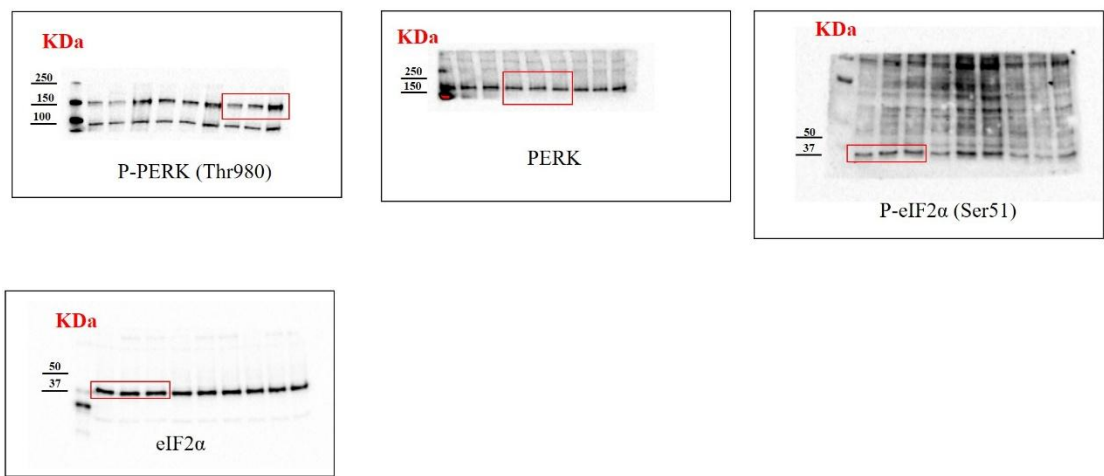


Figure 7B

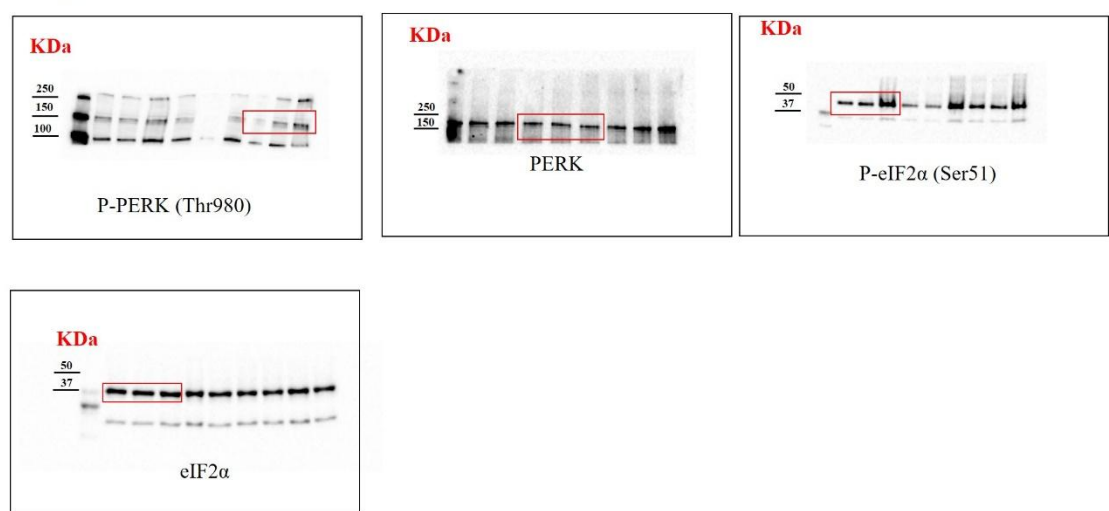


Figure S9. Uncropped Western blot images

Figure 7C

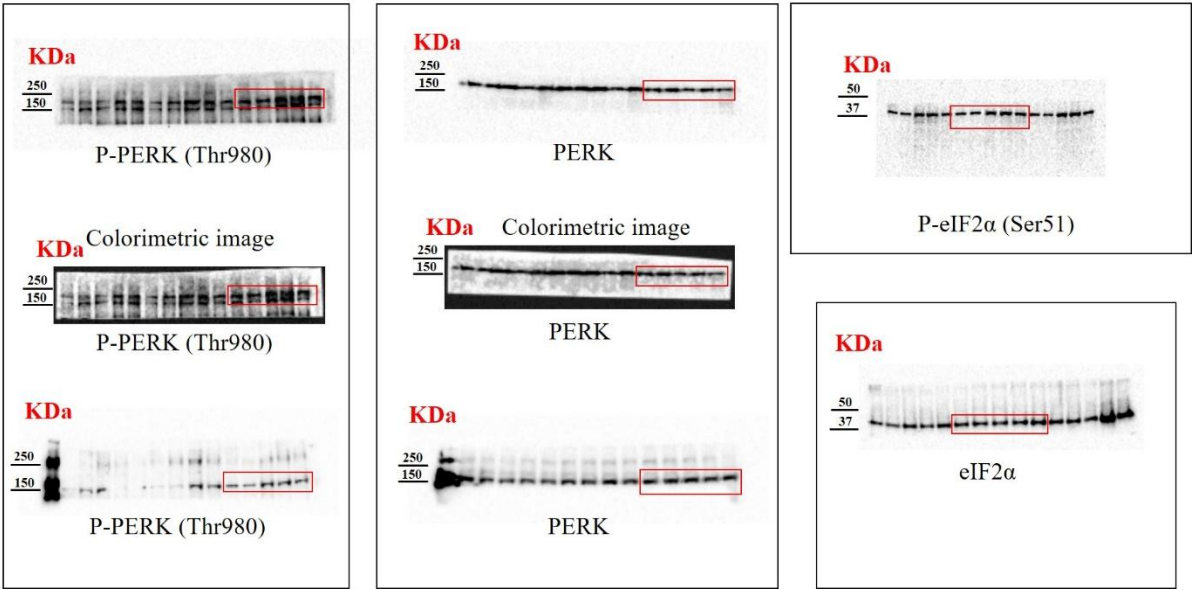


Figure S10. Uncropped Western blot images

Figure 5C

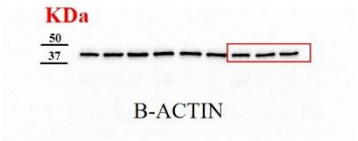


Figure 5D

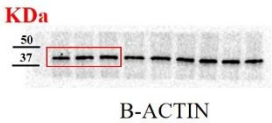


Figure 5E



Figure 6A

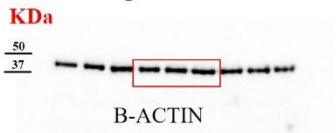


Figure 6B

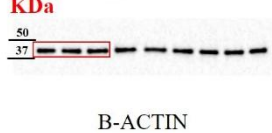


Figure 6C

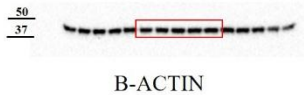


Figure 6D

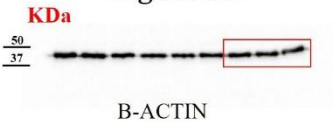


Figure 6E

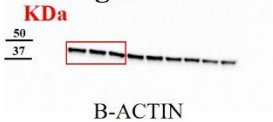


Figure 6F

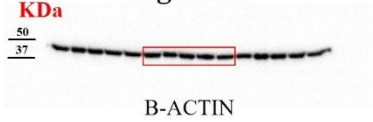


Figure 7A

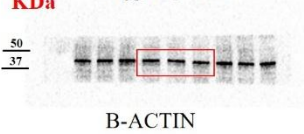


Figure 7B



Figure 7C

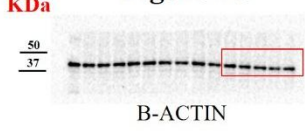


Figure S11. Uncropped Western blot images