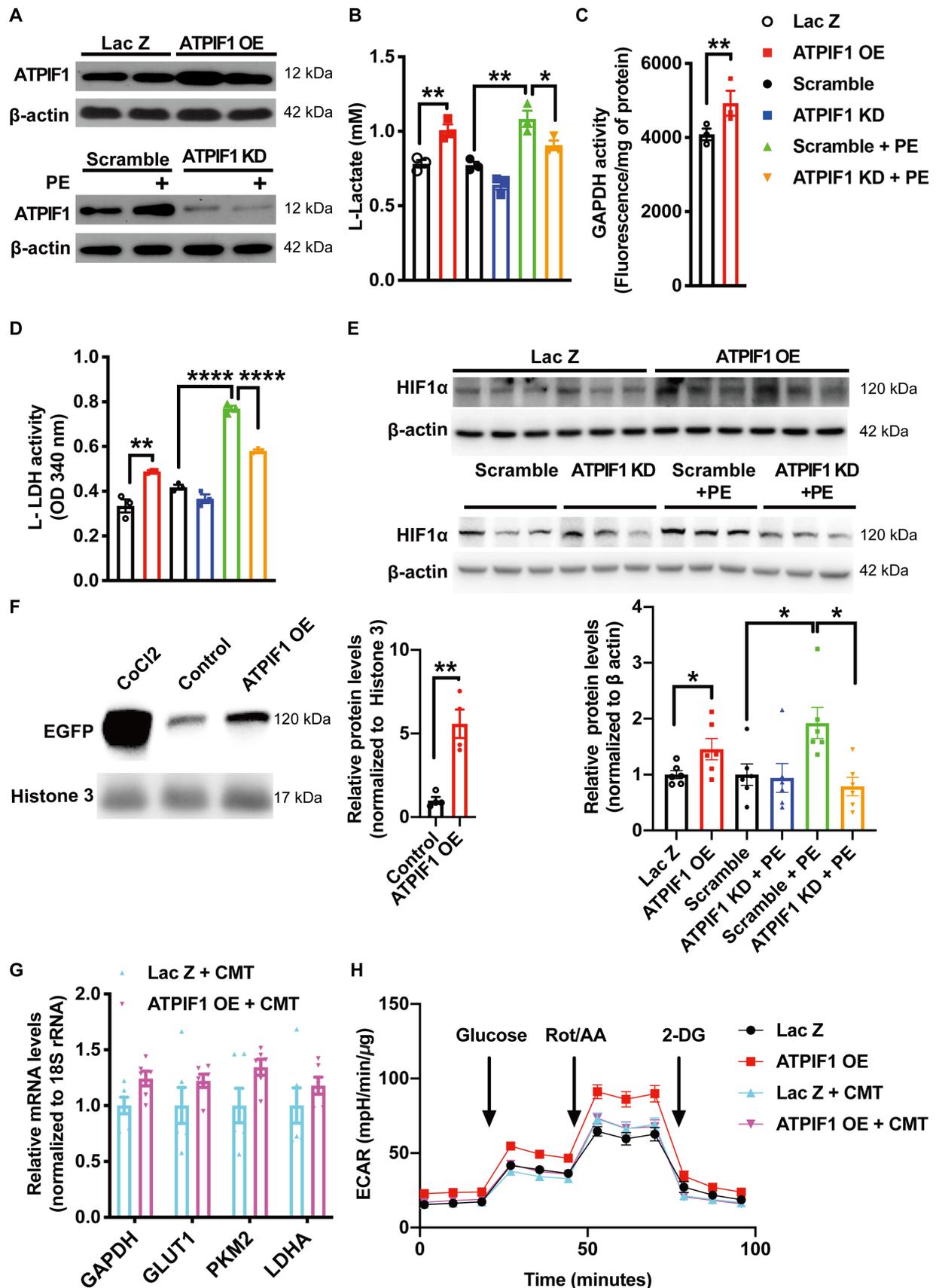


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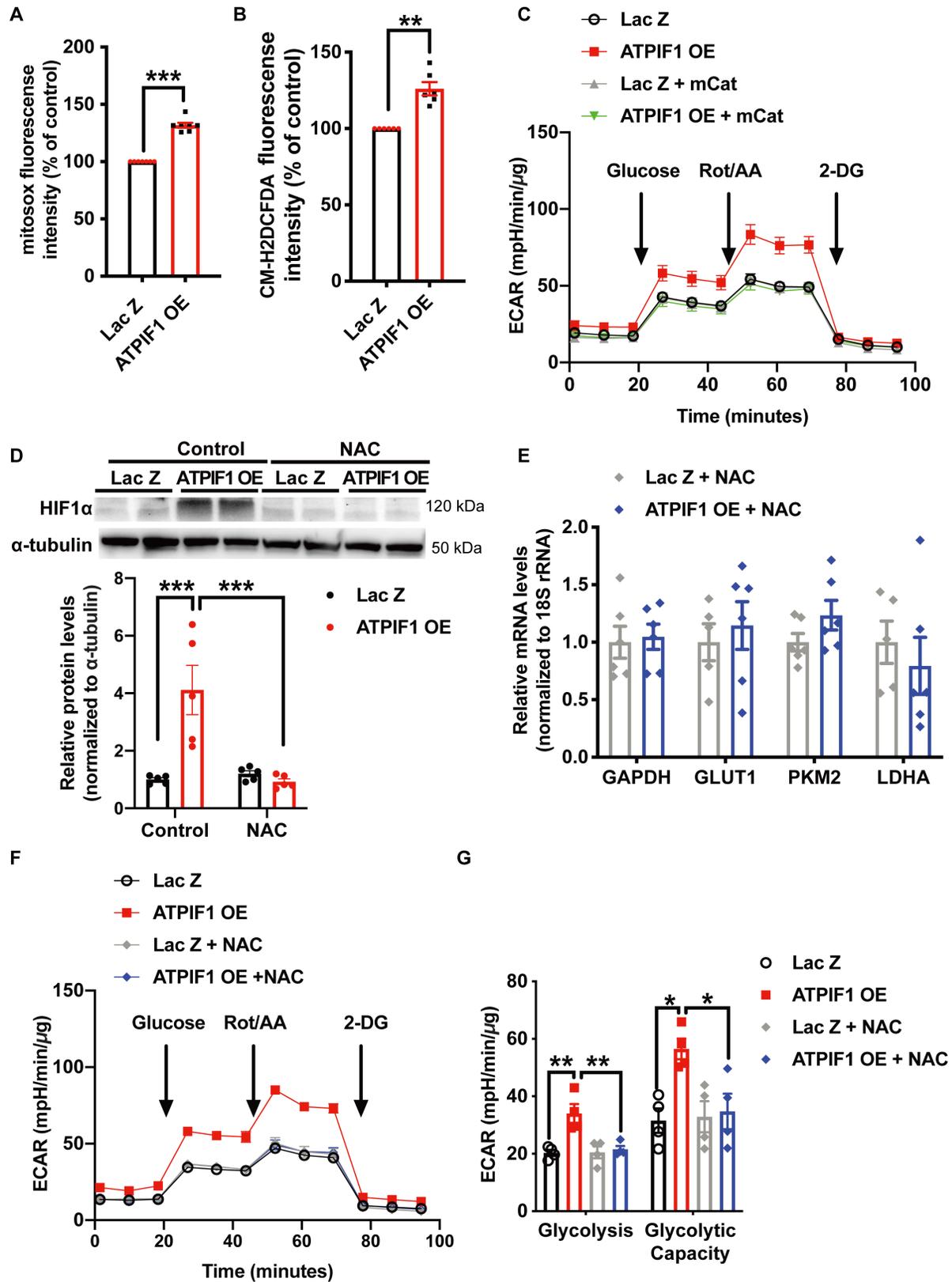
Supplementary 1. Assessment of cardiac hypertrophy and ATPIF1 gene expression.

(A) Brain natriuretic peptide (BNP) mRNA levels (n=4-6); (B) Heart weight (HW) to tibia length ratio (n=7); (C) Cross sectional area of primary adult rat cardiomyocytes treated with 10 μ M phenylephrine (PE) for 48 hrs (n=4); and (D) ATPIF1 mRNA expression were assessed (n=4-6) in each group. (E) c-fos mRNA expression was assayed in primary adult rat cardiomyocytes (n=4). (F) The upstream nucleotide sequence (-928/-1bp) of mouse ATPIF1 gene. The candidate AP-1 complex binding sites are denoted in red and underlined. 18S rRNA was served as mRNA internal control. Data are means \pm SEM of the values. P values were determined using unpaired Student's t-test comparing the treatment group with its respective controls; *p < 0.05; **p<0.01; ***p<0.001; ****p<0.0001.



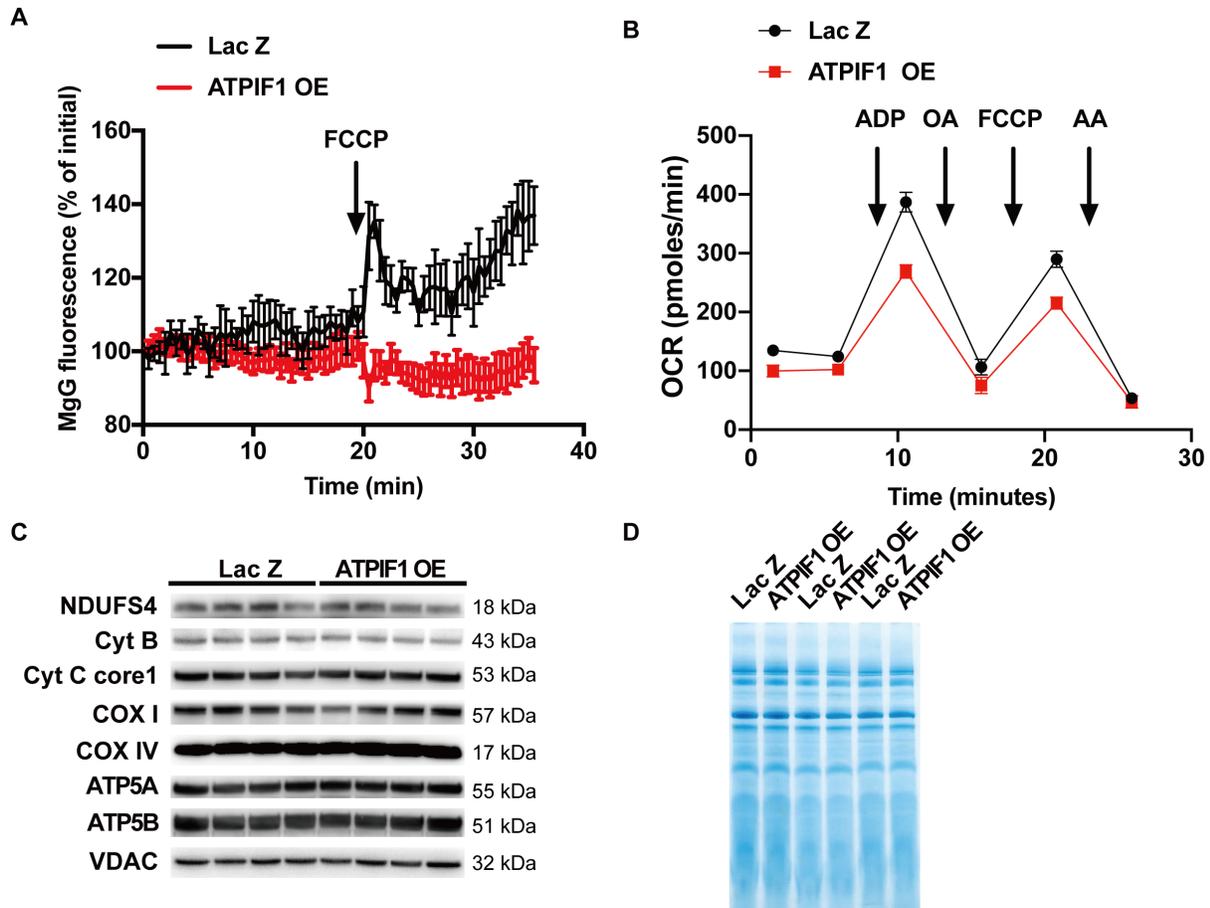
Supplementary 2. Upregulation of ATPIF1 enhanced glycolytic activity in cardiomyocytes

(A) ATPIF1 protein level assay in cardiomyocytes transduced by ATPIF1 adenovirus or ATPIF1 adenoviral shRNA with or without phenylephrine (PE, 10 μ M) after 48 or 72 hrs culture. (B) Measurement of lactate level from the culture medium of cardiomyocytes (n=3). (C) GAPDH activity assay in cardiomyocytes after 48 hrs of culture (n=3). (D and E) LDHA activity assay (D) and HIF1 α immunoblot analysis (E) of cardiomyocytes in the overexpression or knockdown of ATPIF1 with or without PE after 48 or 72 hrs culture (Lac Z, control for overexpressing cells; Scramble, control for knockdown cells; n=3-6). (F) Representative immunoblot and group average of EGFP level from isolated nuclei in HEK293 expressing HIF1 α -EGFP and ATPIF1 or empty vector controls (n=4). (G) Glycolytic gene expression in cardiomyocytes overexpressing ATPIF1 after the inhibition of HIF1 α activity by CMT (n=6). (H) ECAR tracing of cardiomyocytes overexpressing ATPIF1 or LacZ with or without the addition of HIF1 α inhibitor (chetomin, CTM). Data represent the average from 4 independent experiments, each experiment has 4-5 replicate wells. All values were normalized to 18S rRNA expression and shown as the change over LacZ + CMT. Data are means \pm SEM of the values. P values were determined using unpaired Student's t-test for Lac Z vs. ATPIF1 OE comparison or two-way ANOVA followed by Tukey's multiple comparison test for comparison of Scramble vs. ATPIF1 KD vs. Scramble+PE vs. ATPIF1 KD+PE; *p < 0.05; **p < 0.01; ****p < 0.0001).



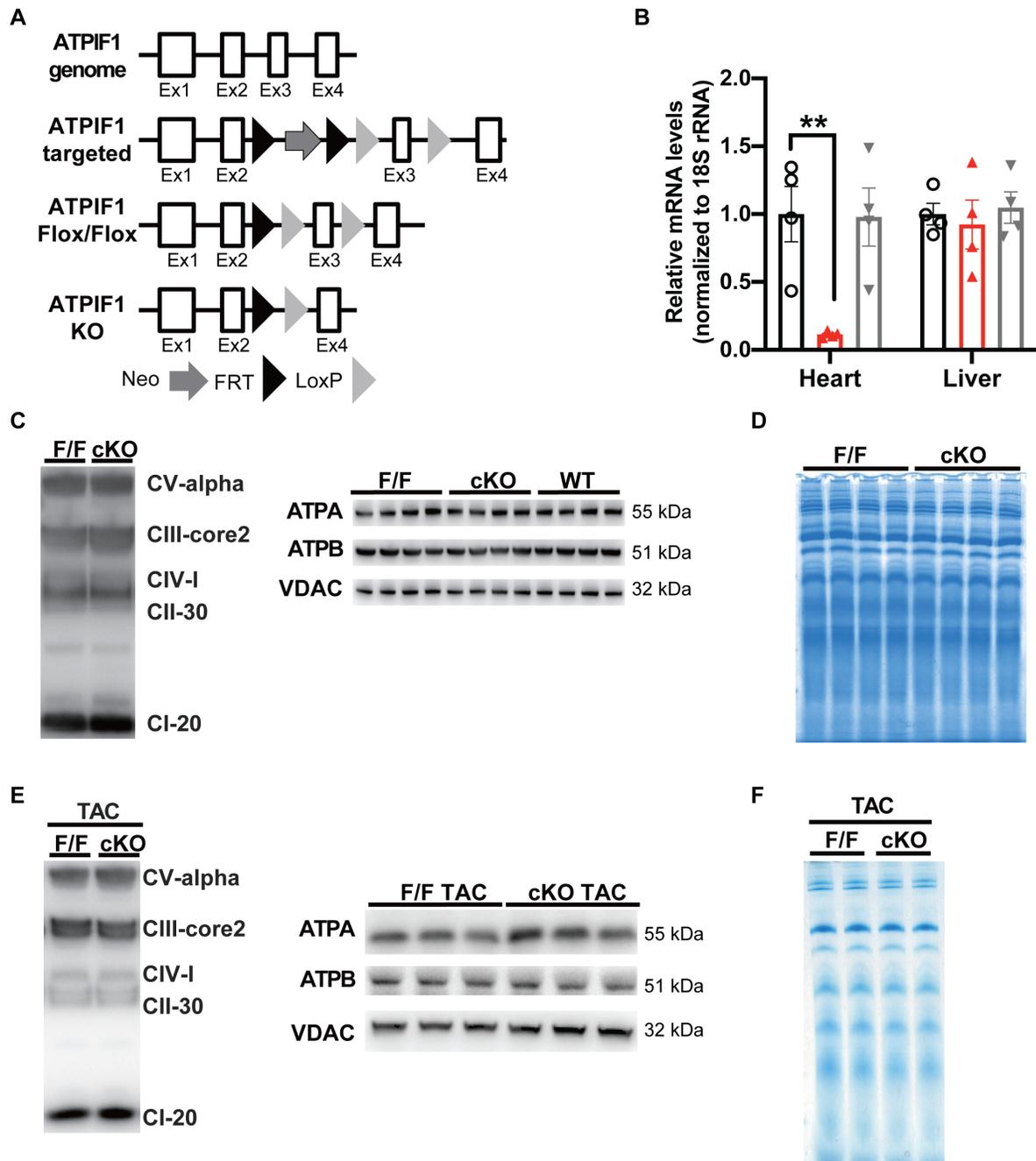
Supplementary 3. ROS accumulation contributed to increased glycolysis by ATPIF1

(A and B) Mitochondrial superoxide production assessed with mitoSOX (A, n=5) and reactive oxygen species (ROS) assessed with CM-H2DCFDA (B, n=3) in H9C2 cells. (C) ECAR tracing of cardiomyocytes with co-expression of ATPIF1 and mitochondrial catalase (mCat). Data represent the average from 5 independent experiments, each experiment has 4-5 replicate wells. (D, E, F and G) Representative immunoblot of HIF1 α protein (D, n=5), real-time PCR analysis of glycolytic gene expression (E, n=6) and ECAR tracing and tabulated data (F and G, n=4) in cardiomyocytes with or without 0.2 mM NAC (N-acetylcysteine) treatment for 24 hrs. Data represent the average from 4 independent experiments, each experiment has 4-5 replicate wells (F and G). Gene expression values were normalized to 18S rRNA expression and shown as relative change over the average of the control sets (E). Data are means \pm SEM of the values. P values were determined using unpaired Student's t-test (A, B, E) or two-way ANOVA followed by Tukey's multiple comparison test (D); *p < 0.05; **p<0.01; ****p<0.0001.



Supplementary 4. ATPIF1 upregulation has no effect on mitochondrial electron transport chain protein

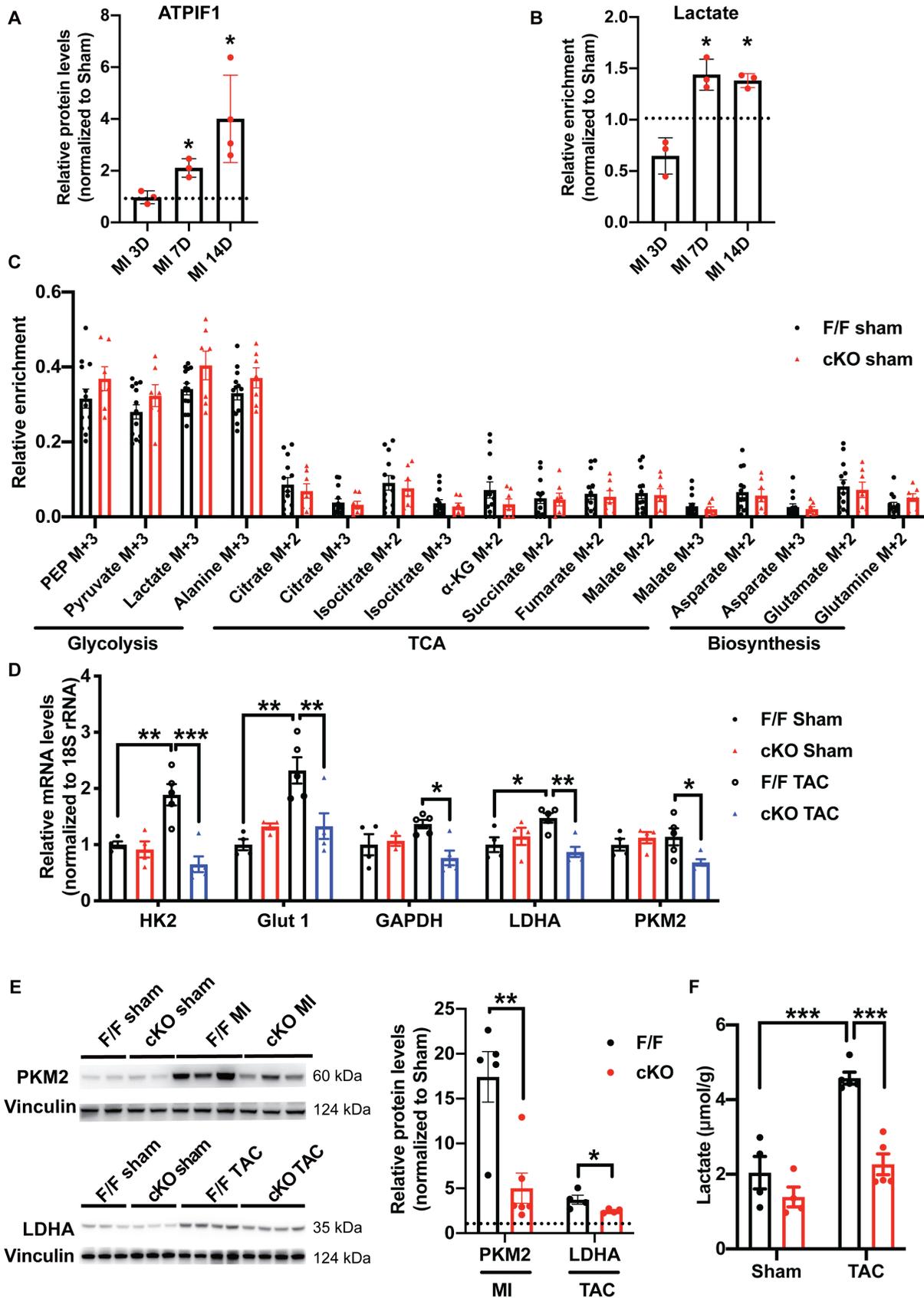
(A) Rate of ATP hydrolysis assessed by real-time assay of Mg^{2+} efflux-based fluorescence signal using Magnesium green (MgG) in cardiomyocytes during $5\mu M$ FCCP treatment (n=4). (B) OCR tracing of cardiomyocytes overexpressing ATPIF1. Data represent the average from 5 independent experiments, each experiment has 4-5 replicate wells. (C) Western blot assay of mitochondrial OXPHOS protein (n=4). (D) Measurement of supercomplex abundance in $50\mu g$ isolated mitochondria from cardiomyocytes, using Clear-Native PAGE followed by Coomassie staining and destaining.

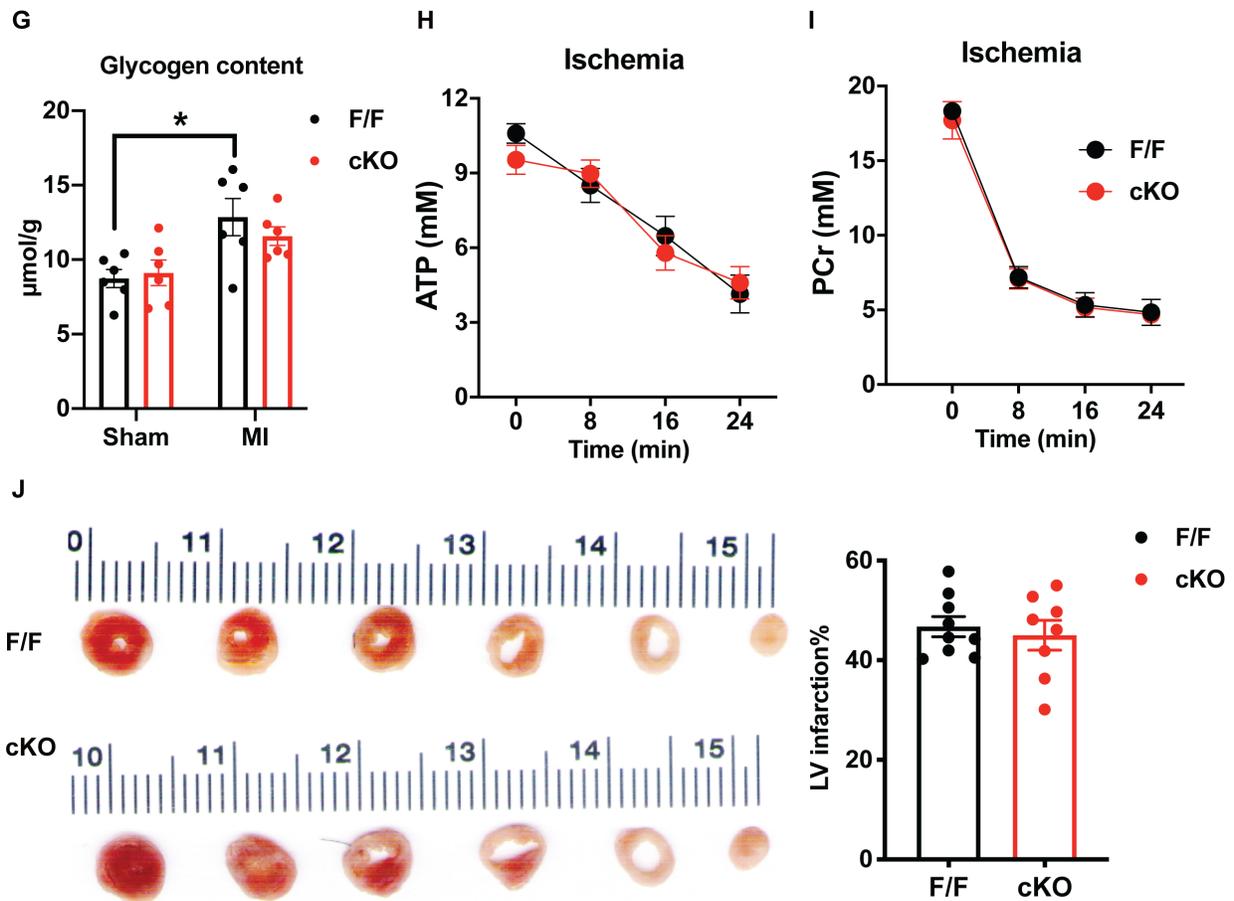


Supplementary 5. Cardiac-specific deletion of ATPIF1 in mice does not change protein levels of the mitochondrial electron transport chain

(A) Strategy to generate cardiac-specific ATPIF1 KO mice is shown. Exon 3 of ATPIF1 was flanked by LoxP sequences and deleted after α MHC-Cre-mediated homologous recombination. (B) Real-time PCR analysis of ATPIF1 expression from liver or ventricular lysates (n=4-5). (C

and D) Representative immunoblot analysis of OXPHOS protein (C, n=4), α and β subunit of F₀F₁-ATP synthase (C, n=3-4) and measurement of supercomplex abundance (D, n=4) using clear-Native PAGE followed by Coomassie staining and destaining in isolated mitochondria. (E and F) Representative immunoblot analysis of OXPHOS protein (E, n=6) and measurement of supercomplex abundance (F, n=3-4) using clear-Native PAGE followed by Coomassie staining and destaining in isolated mitochondria at 28 days after TAC surgery. 18S rRNA was served as mRNA internal control. VDAC was used as protein loading control. Data are means \pm SEM of the values. P values were determined using one-way ANOVA followed by Dunnett's multiple comparison (F); **p<0.01.





Supplementary 6 Cardiac-specific deletion of ATPIF1 prevented the switch to increased glycolysis induced by pathological cardiac hypertrophy

(A) Immunoblot analysis for ATPIF1 protein expression at day 3, 7 and 14 after MI. (B) Quantification of lactate level in the ventricular tissue at day 3, 7 and 14 after MI by GC/MS (n=3). (C) Relative ¹³C enrichment of glycolysis, TCA and anaplerosis metabolites from F/F and cKO ventricular tissue at day 7 after sham operation (n=7-13). (D, E and F) Real-time PCR assay of glycolytic genes (D, n=4-5), immunoblot analysis for PKM2 and LDHA protein expression (E, n=3-6), quantification of lactate level (F, n=4-5) in ventricular lysates 14 days after TAC or MI and sham operation. (G) Glycogen content in the ventricular tissue 7 days after MI or sham surgery (n=6). (H and I) ATP (H) and Phosphocreatine concentration (I) measured by ³¹P NMR

spectroscopy in isolated perfused hearts before and during global no-flow ischemia (n=11). (J) Representative image of heart sections and percentage of infarcted area to total left ventricular area 3 days after MI surgery. 18S rRNA was served as mRNA internal control. Vinculin was used as protein loading control. Data are means \pm SEM of the values. P values were determined using Student unpaired t-test (A, B and E) or two-way ANOVA followed by Tukey's multiple comparison test (D, F and G); *p < 0.05; **p<0.01; ***p<0.001.

B) Supplemental Table:

Table 1. Quantitative analysis of the crosslinks by pair-wise comparison of TAC and sham hearts.

Protein XLs	Peptide XLs	T1/S1	T2/S2	T3/S3	T4/S4	T5/S5
ATPA ₄₂₇ _ATPA ₄₉₈	AMKQVAGTMK_	-0.04		0	-0.02	-0.06
	GYLDKLEPSK	(0.259)		(0.363)	(0.3)	(0.132)
ATPA ₄₂₇ _ATPA ₅₃₁	AMKQVAGTMK_	0.24	0.36	0.07	0.13	0.25
	SDGKISEQSDAK	(0.039)	(0.091)	(0.074)	(0.106)	(0.08)
ATPA ₄₂₇ _ATPA ₅₃₁	AMKQVAGTMK_					0.06
	SDGKISEQSDAKLK	-0.03	0.45	0.77	0.19	(0.17)
ATPA ₄₂₇ _ATPA ₅₃₁	AMKQVAGTM(Ox)K_	-0.12	0.34	0.38	0.03	0.34
	SDGKISEQSDAK	(0.346)	(0.182)	(0.166)	(0.108)	(0.076)
ATPA ₄₂₇ _ATPA ₅₃₁	AM(Ox)KQVAGTMK_	0.17	0.16	0.04	0.24	0.22
	SDGKISEQSDAK	(0.069)	(0.12)	(0.121)	(0.071)	(0.082)
ATPA ₄₂₇ _ATPA ₅₃₁	AM(Ox)KQVAGTM(Ox)K_	0.17	0.47	0.13	0.14	0.29
	SDGKISEQSDAK	(0.387)	(0.308)	(0.117)	(0.099)	(0.161)

ATPA ₅₃₁ _ATPA ₄₂₇	SDGKISEQSDAK_ AMKQVAGTMKLELAQYR	-0.26 (0.039)	0.66 (0.255)	0.06 (0.18)	0.59 (0.638)	0.36 (0.11)
ATPA ₅₃₁ _ATPA ₄₂₇	SDGKISEQSDAK_ AMKQVAGTMKLELAQYREVA AFAQFGSDLDAATQQLLSR				0.52	
ATPA ₅₃₁ _ATPA ₄₂₇	SDGKISEQSDAK_ AMKQVAGTM(O _x)KLELAQYR		0.4 (0.625)	0.23 (0.149)	0.18 (0.175)	0.65
ATPA ₅₃₁ _ATPA ₄₂₇	SDGKISEQSDAK_ AM(O _x)KQVAGTMKLELAQYR				0.52 (0.638)	
ATPA ₅₃₁ _ATPA ₄₃₄	SDGKISEQSDAK_ AMKQVAGTMKLELAQYREVA AFAQFGSDLDAATQQLLSR				0.47	
ATPA ₅₃₁ _ATPA ₄₃₄	SDGKISEQSDAK_ QVAGTMKLELAQYR	0.39 (0.141)	0.2 (0.096)	0.07 (0.08)	0.34 (0.069)	0.42 (0.065)
ATPA ₅₃₁ _ATPA ₄₃₄	SDGKISEQSDAK_ QVAGTM(O _x)KLELAQYR	0.22 (0.376)	-0.1 (0.185)	0.41 (0.055)	0.38 (0.199)	0.39 (0.106)

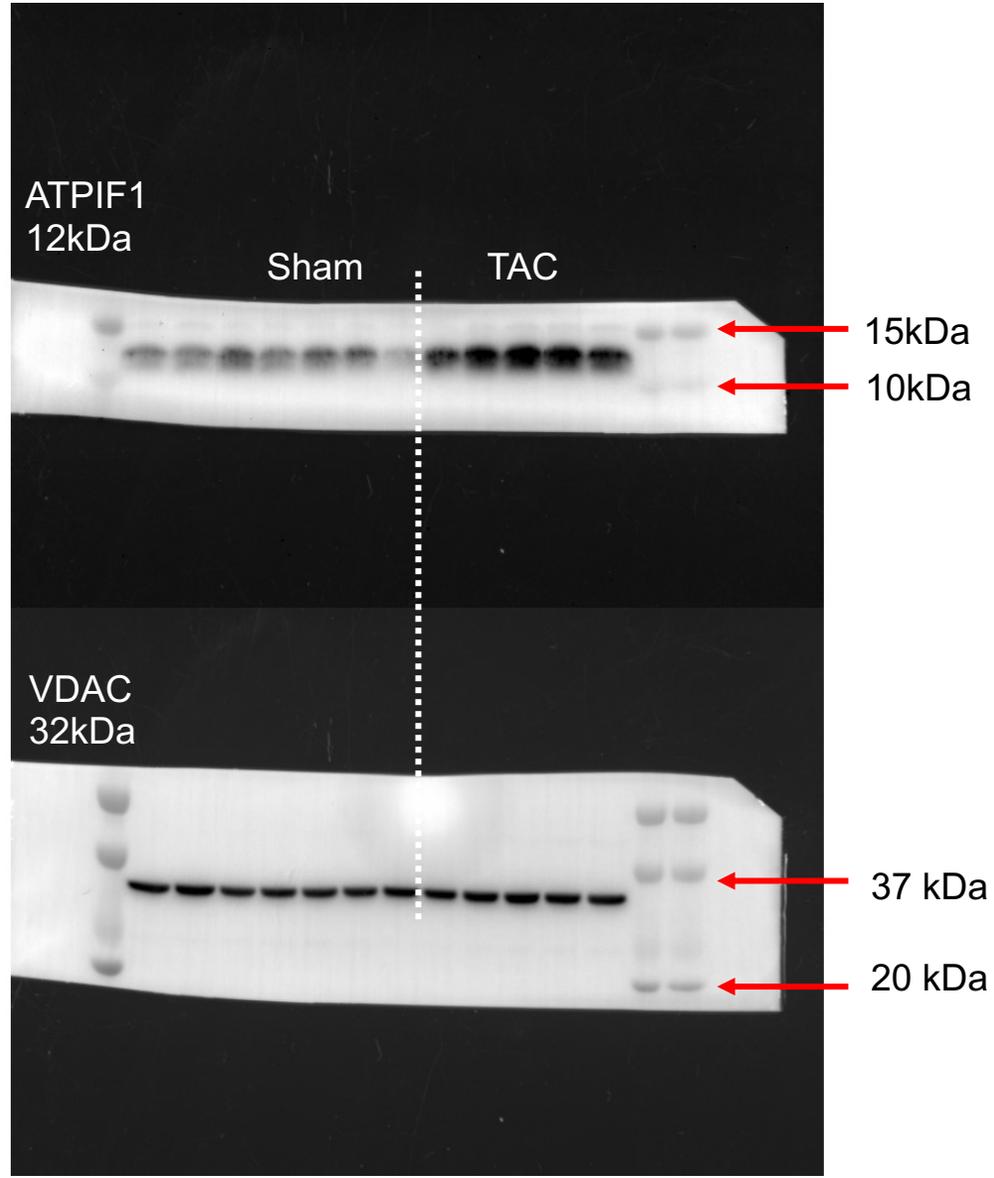
ATPA ₄₉₈ _ATPA ₅₃₁	GYLDKLEPSK_ SDGKISEQSDAK		0.39 (0.119)	0.13 (0.29)	-0.02 (0.181)	-0.03 (0.079)
ATPB ₅₂₂ _ATPA ₄₃₄	ADKLAEEHGS_ QVAGTMKLELAQYR	0.03 (0.218)		-0.19 (0.471)	-0.16 (0.164)	-0.26 (0.149)
ATPB ₅₂₂ _ATPA ₄₃₄	ADKLAEEHGS_ QVAGTM(Ox)KLELAQYR	0.12 (0.62)			-0.33 (0.588)	
ATPB ₄₈₅ _ATPB ₅₂₂	LVPLKETIK_ ADKLAEEHGS	-0.27 (0.117)	0.41 (0.134)	-0.21 (0.226)	-0.62 (0.274)	-0.56 (0.07)
ATPB ₅₂₂ _ATPA ₄₉₈	ADKLAEEHGS_ GYLDKLEPSK	-0.06 (0.103)			-0.51 (0.128)	-0.56 (0.333)
ATPB ₅₂₂ _ATPA ₅₃₁	ADKLAEEHGS_ SDGKISEQSDAK	0.13 (0.265)	0.4 (0.301)	0.02 (0.096)	-0.03 (0.061)	0.09 (0.098)
ATPB ₄₈₅ _ATPA ₄₂₇	LVPLKETIK_ AMKQVAGTMK	0.15 (0.255)	0.48 (0.069)	0.09 (0.074)	-0.09 (0.086)	-0.19 (0.16)
ATPB ₄₈₅ _ATPA ₄₂₇	LVPLKETIK_ AMKQVAGTM(Ox)K	0.04 (0.18)				

ATPB ₄₈₅ _ATPA ₅₃₁	LVPLKETIK_ SDGKISEQSDAK	0.04 (0.028)	0.32 (0.223)	0.03 (0.167)	-0.02 (0.063)	-0.13 (0.041)
ATPB ₅₂₂ _ATPA ₄₂₇	ADKLAEEHGS_ AMKQVAGTMK	-0.07 (0.091)	0.52 (0.098)	0.01 (0.078)	-0.07 (0.054)	0.04 (0.074)
ATPB ₅₂₂ _ATPA ₄₂₇	ADKLAEEHGS_ AMKQVAGTM(Ox)K		0.32 (0.085)	-0.24 (0.203)	-0.03 (0.248)	-0.12 (0.201)
ATPB ₅₂₂ _ATPA ₄₂₇	ADKLAEEHGS_ AM(Ox)KQVAGTMK	0 (0.159)	0.39 (0.118)	0.15 (0.193)	-0.12 (0.045)	-0.09 (0.086)
ATPB ₅₂₂ _ATPA ₄₂₇	ADKLAEEHGS_ AM(Ox)KQVAGTM(Ox)K	0.2 (0.298)	0.52 (0.411)	-0.04 (0.128)	-0.03 (0.136)	-0.11 (0.082)

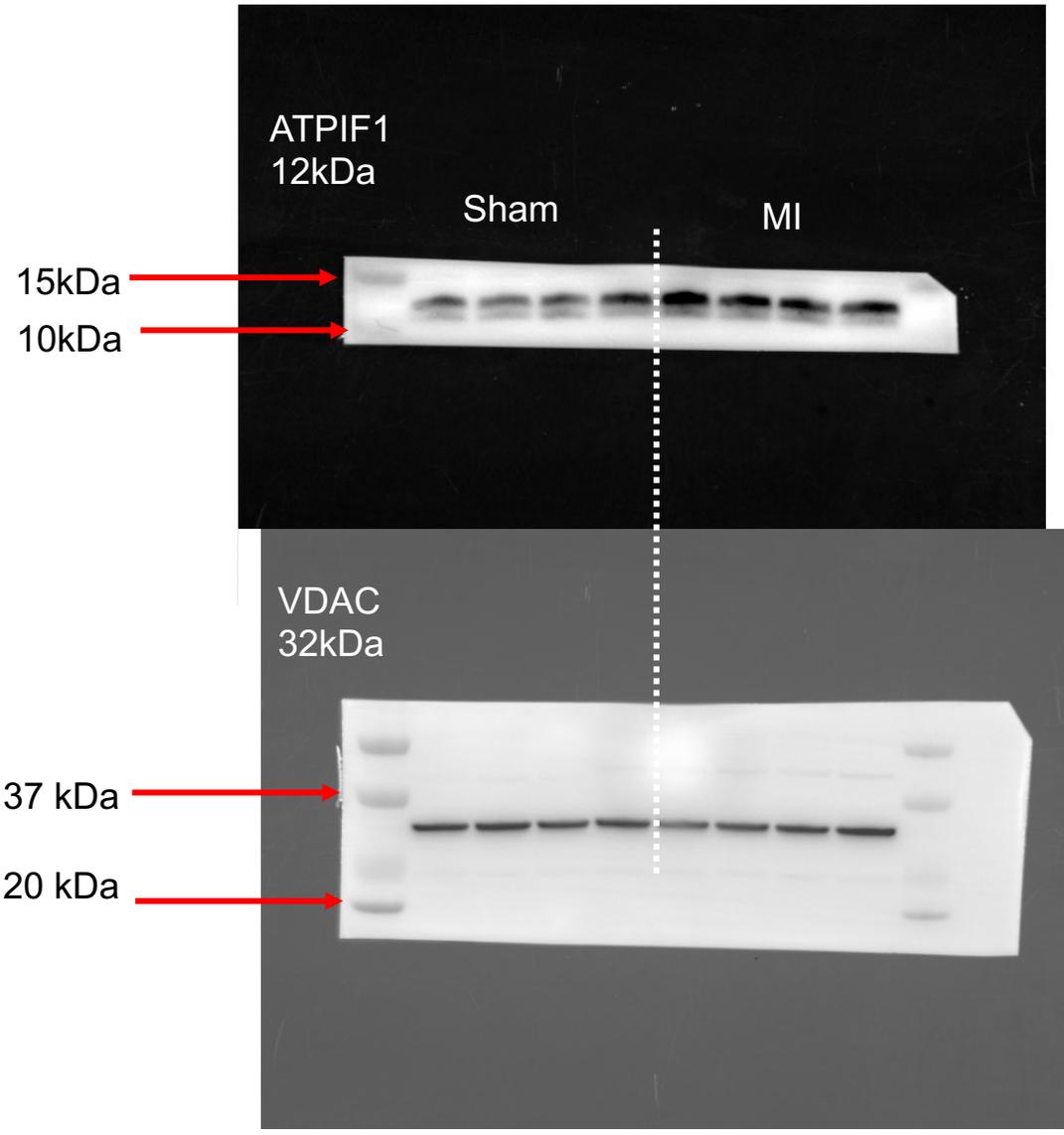
Log₂ ratios of TAC vs sham from 5 biological replicates for crosslinks identified between ATPA dimer, ATPB dimer, ATPA-ATPB.

(TAC/sham: T/S; 95% confidence interval for each cross-linked peptide pairs are shown in parenthesis.)

Full unedited gel for Figure 1A

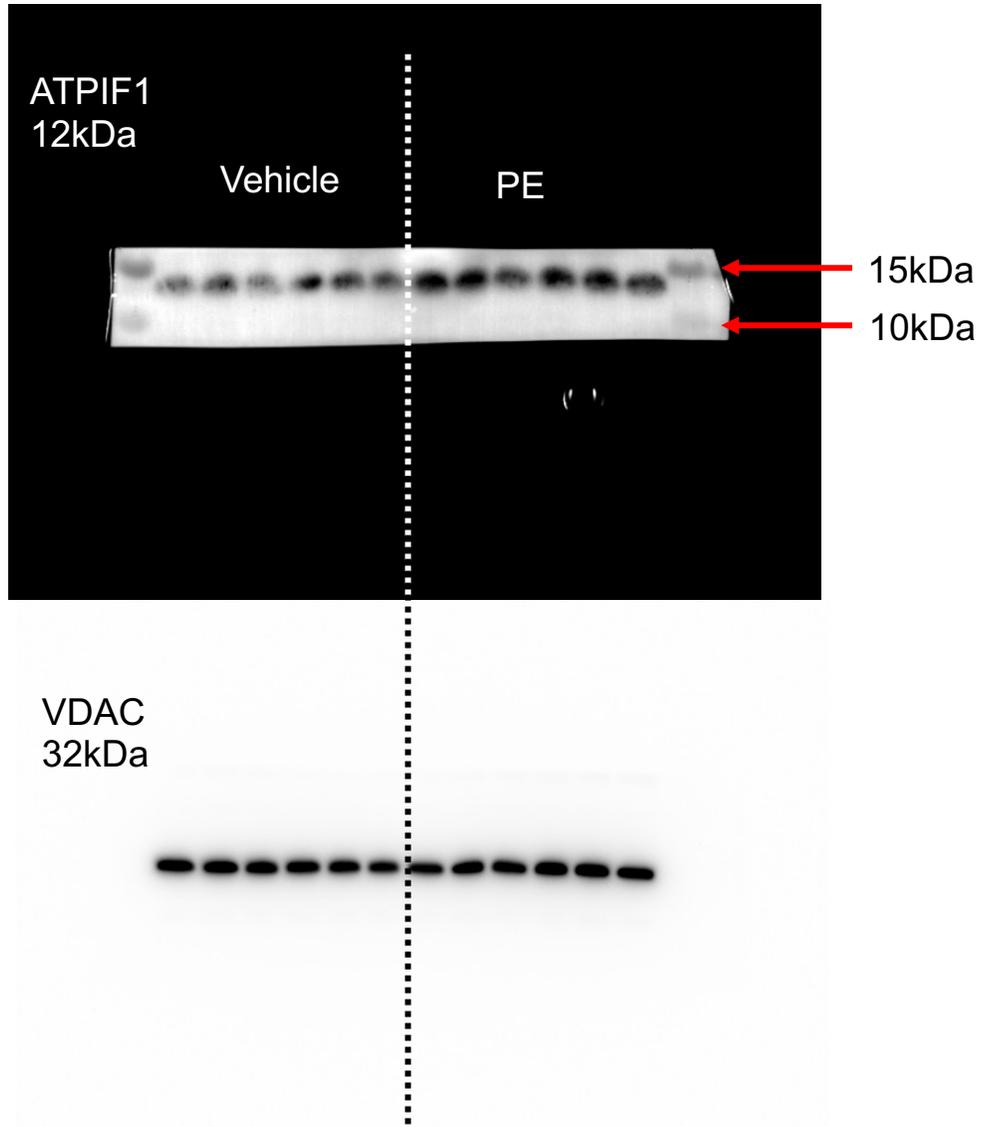


Full unedited gel for Figure 1B

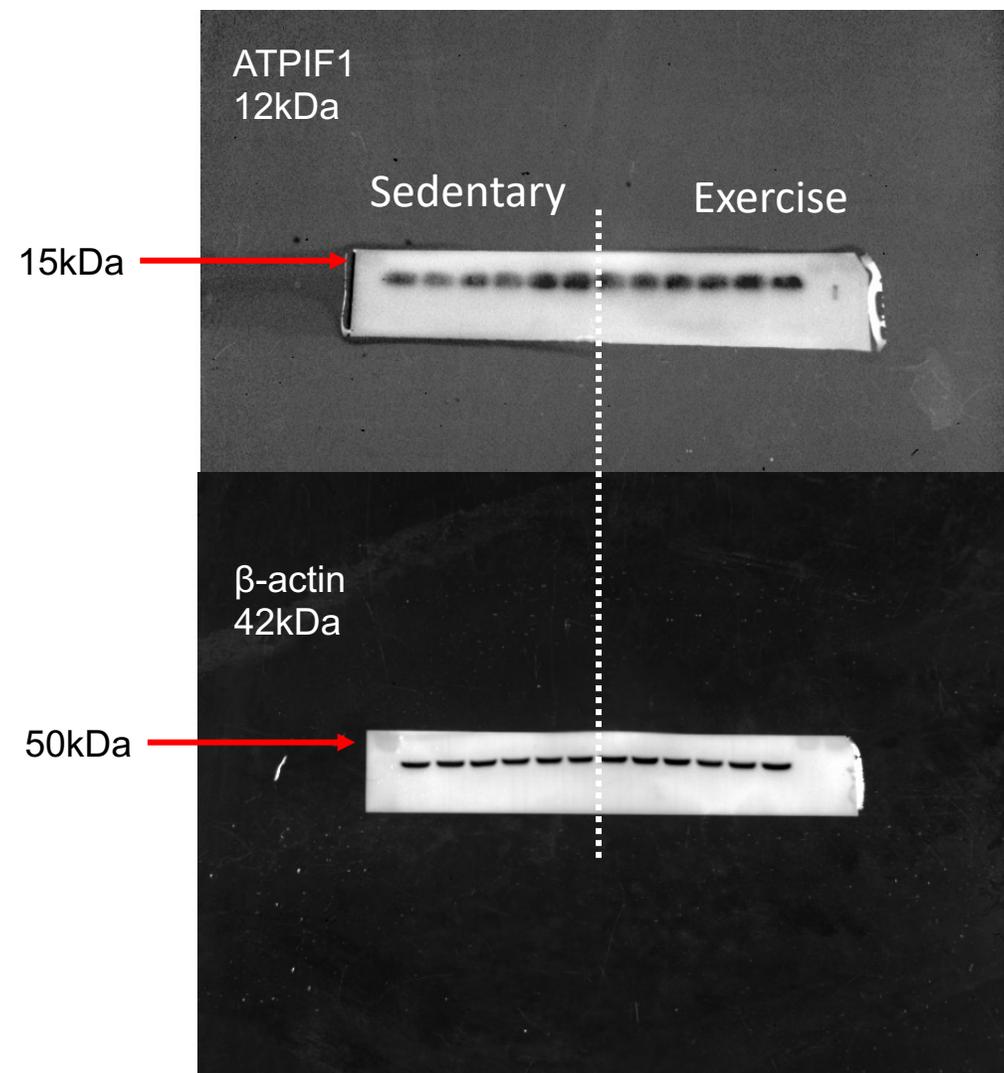


ATPIF1: Cell Signaling Technology 8528
VDAC: Cell Signaling Technology 4661

Full unedited gel for Figure 1C

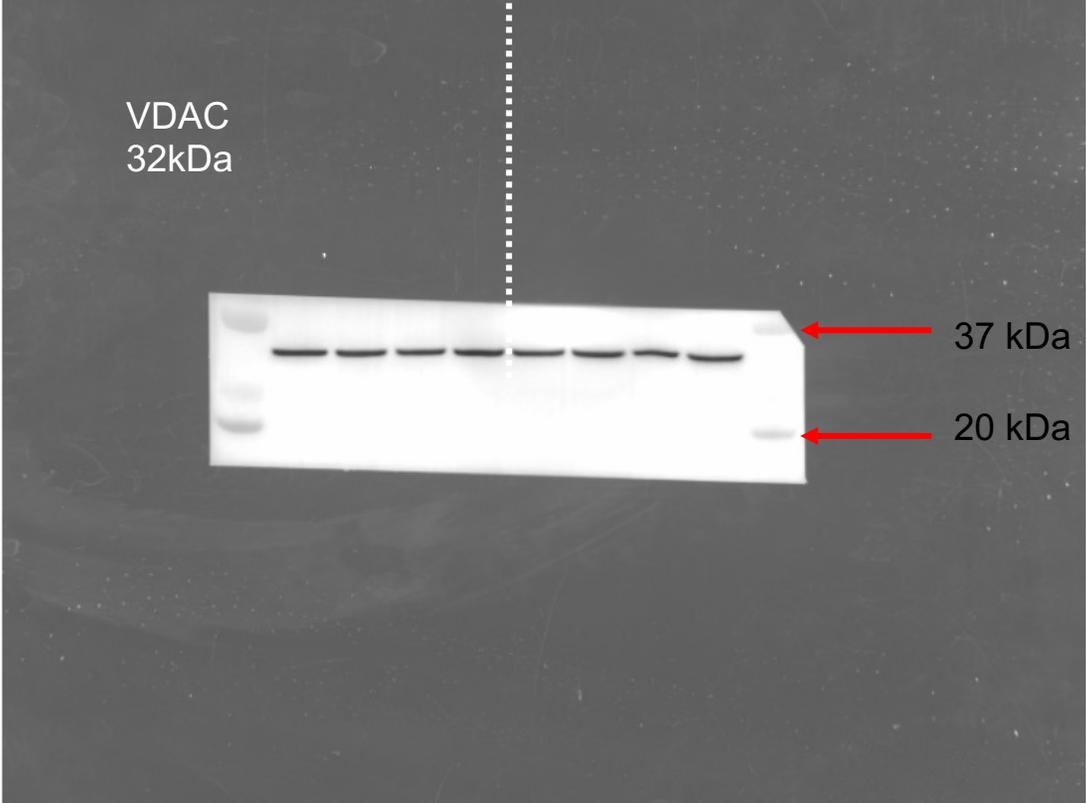
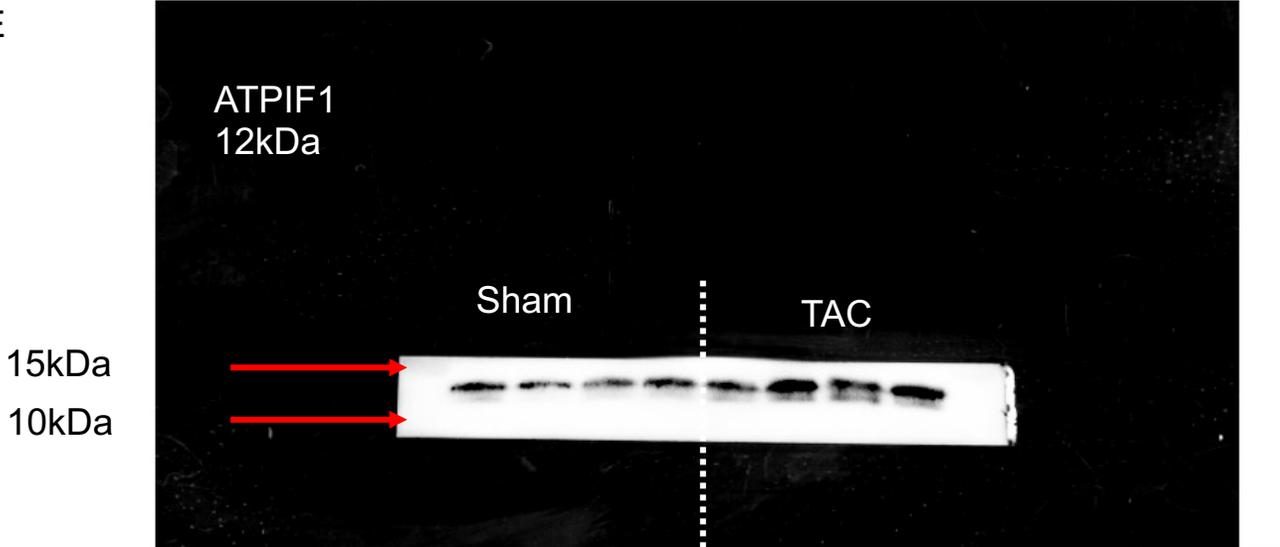


Full unedited gel for Figure 1D



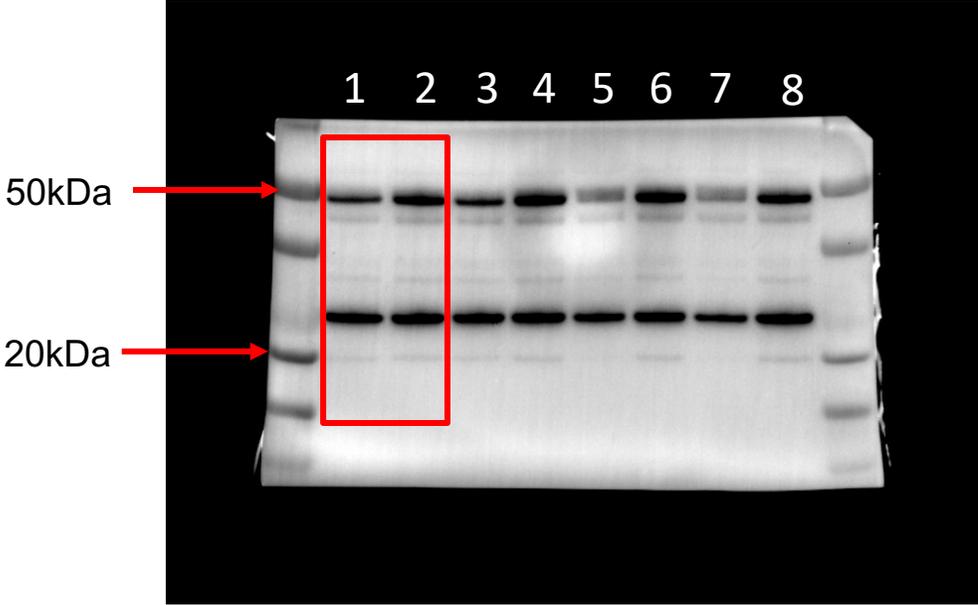
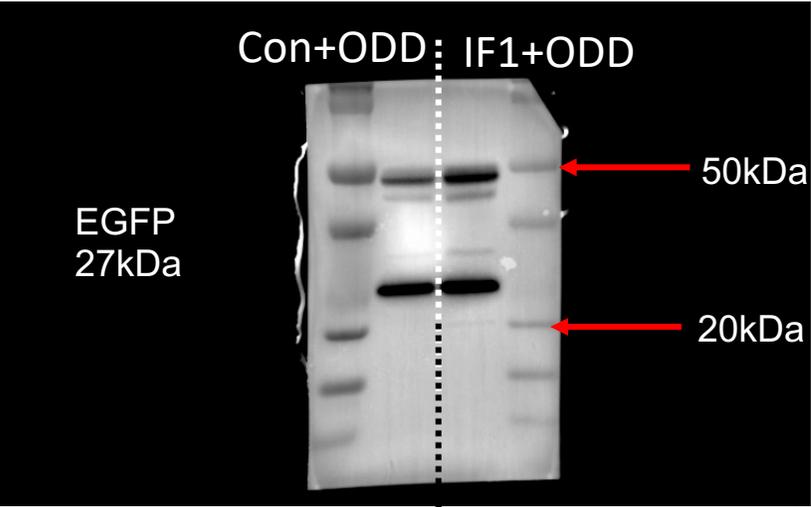
ATPIF1: Cell Signaling Technology 8528
VDAC: Cell Signaling Technology 4661
 β -actin: Sigma A2103

Full unedited gel for Figure 1E



ATPIF1: Cell Signaling Technology 8528
VDAC: Cell Signaling Technology 4661

Full unedited gel for Figure 2C



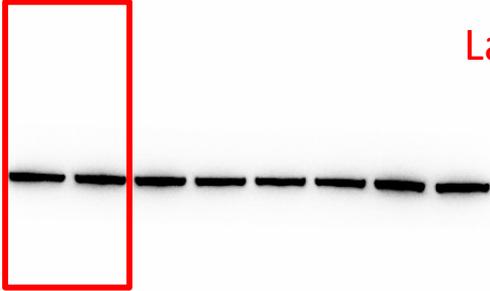
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Lane 2,4,6,8:IF1+ODD

VDAC 32kDa



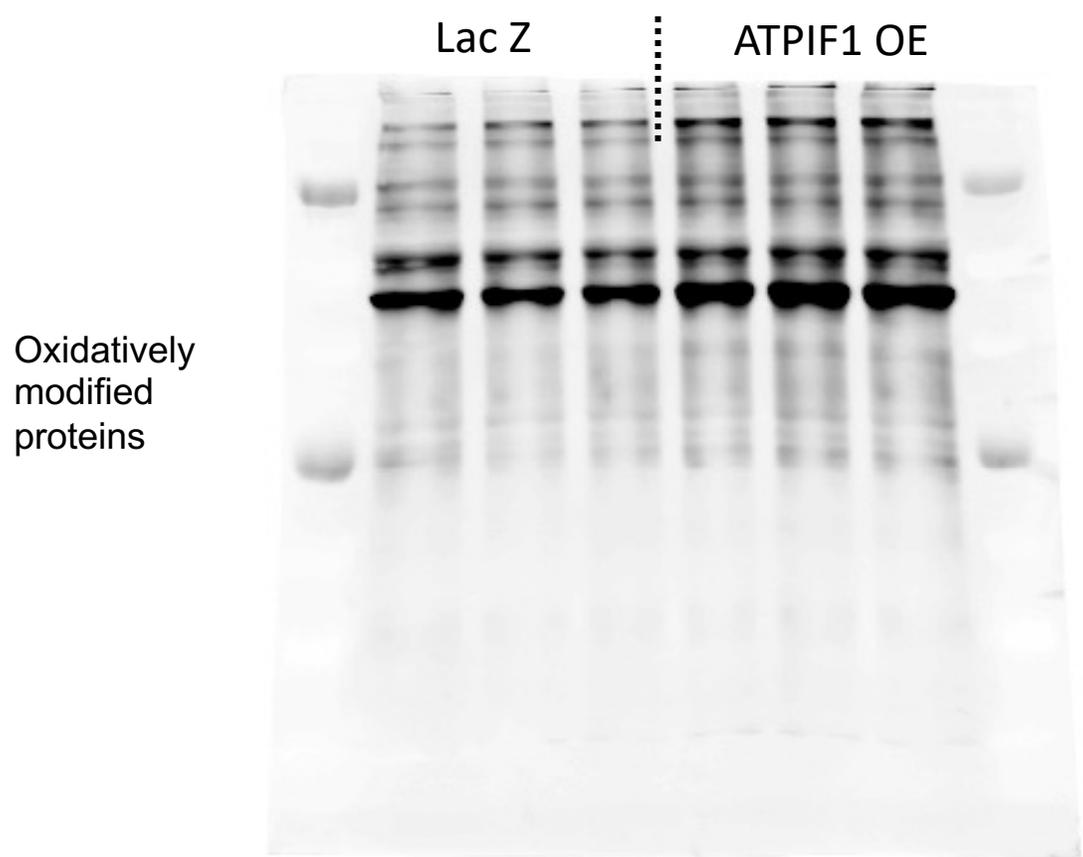
VDAC 32kDa



Lane 1,2: shown in the figure 2C

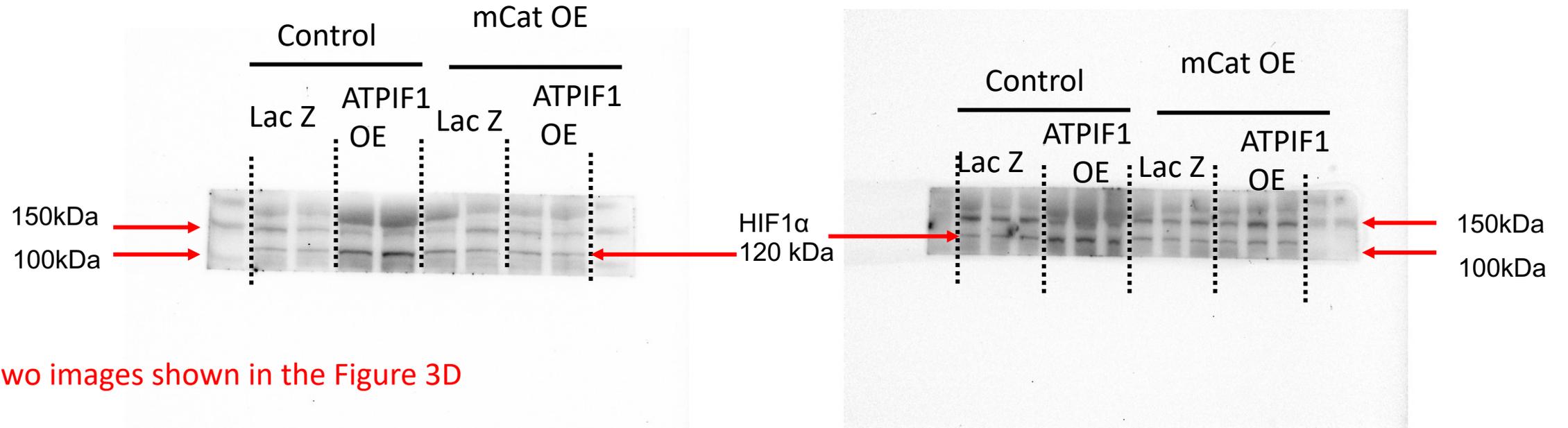
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VDAC: Cell Signaling Technology 4661

Full unedited gel for Fig 3B

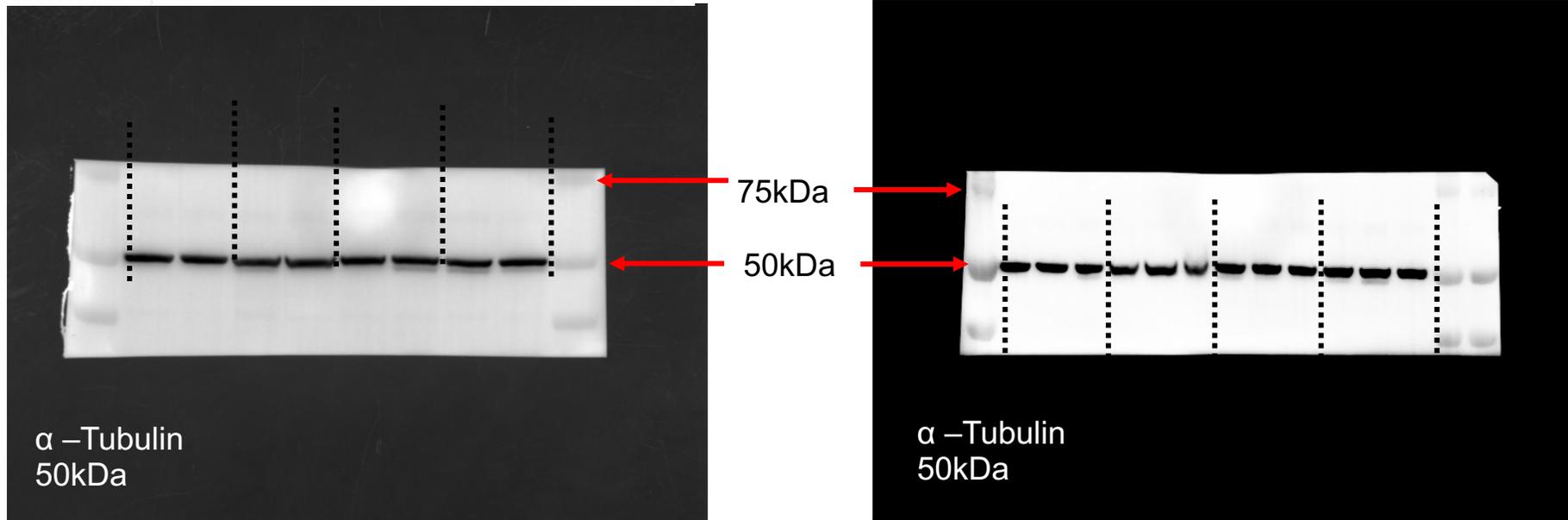


OxyBlot Protein Oxidation Detection Kit : Sigma S7150
VDAC: Cell Signaling Technology 4661

Full unedited gel for Fig 3D

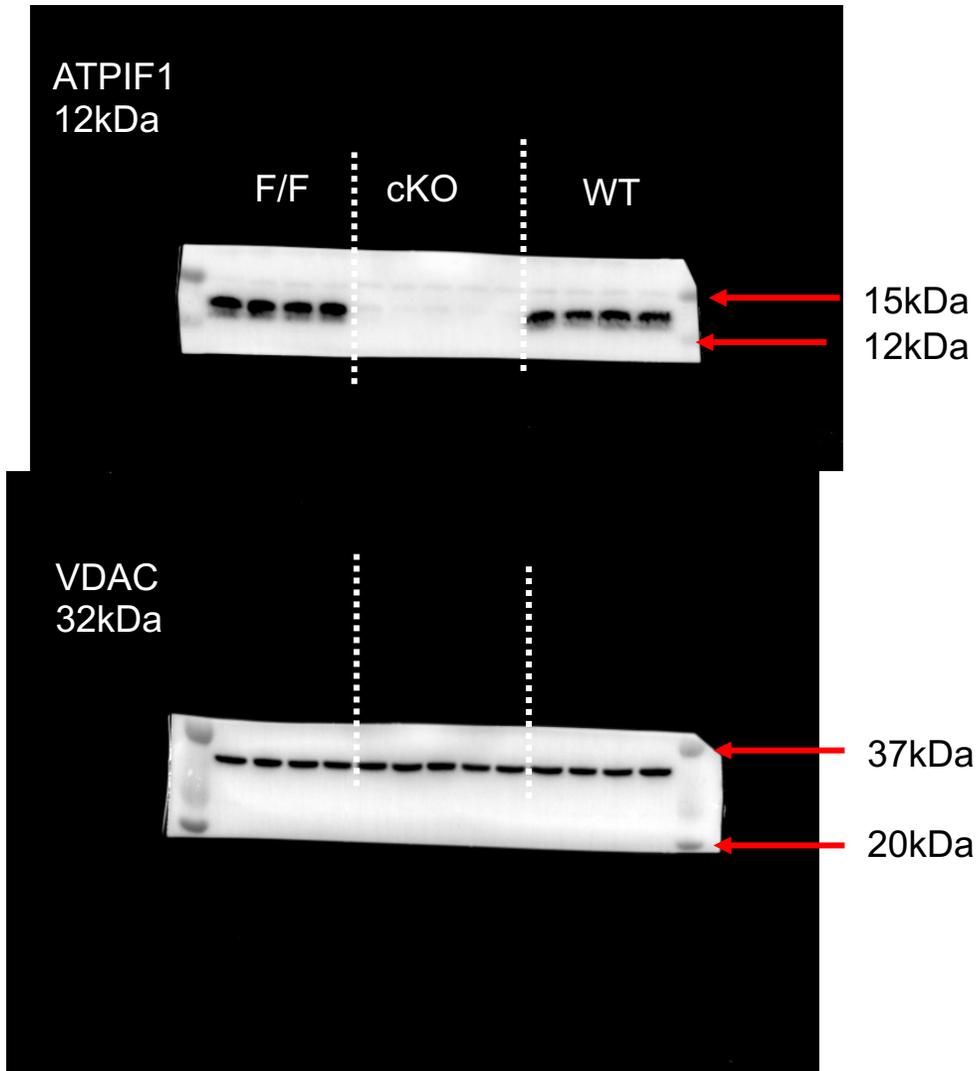


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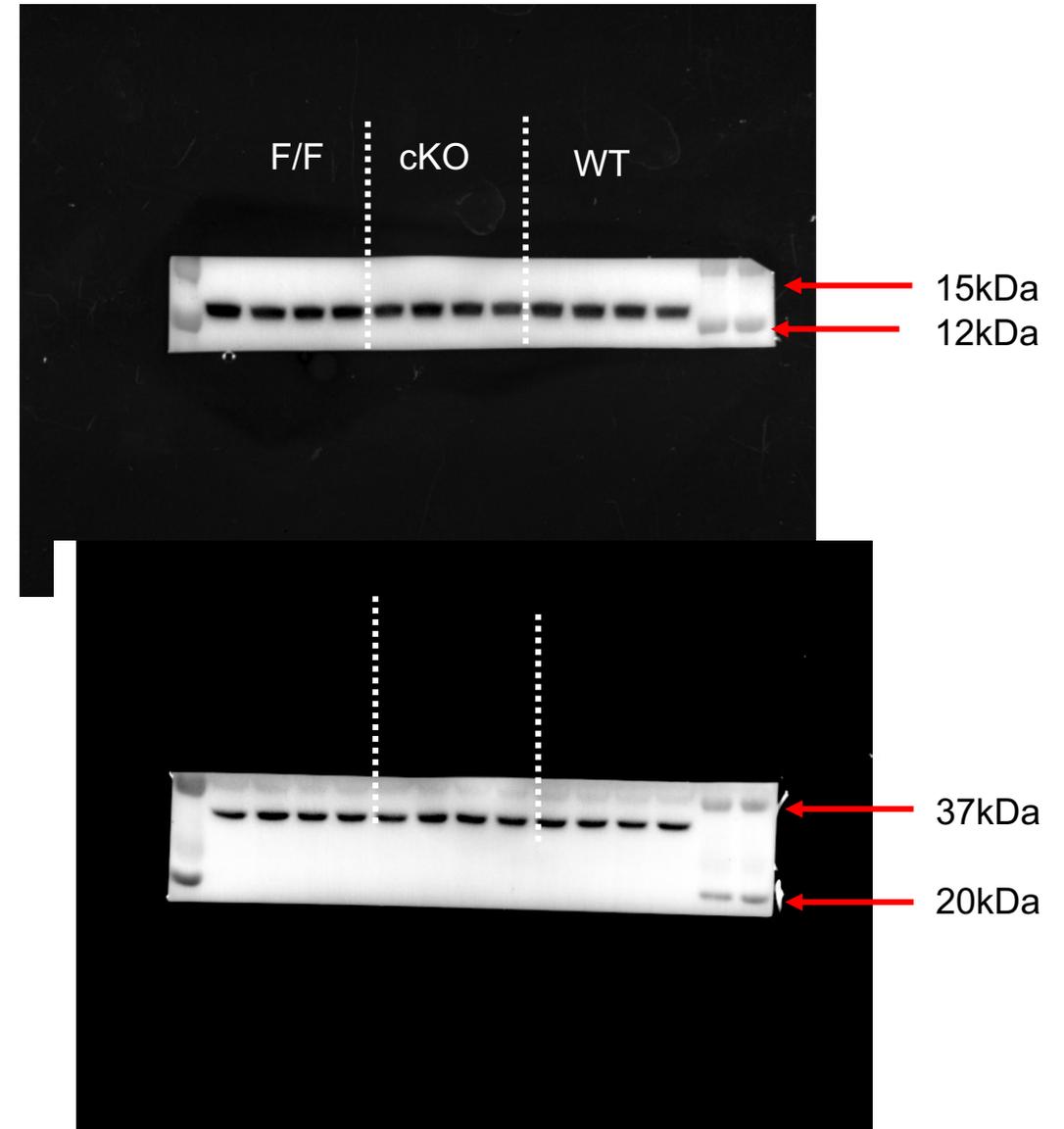


HIF1α: Abcam ab1
α-Tubulin: Sigma T6199

Heart tissue

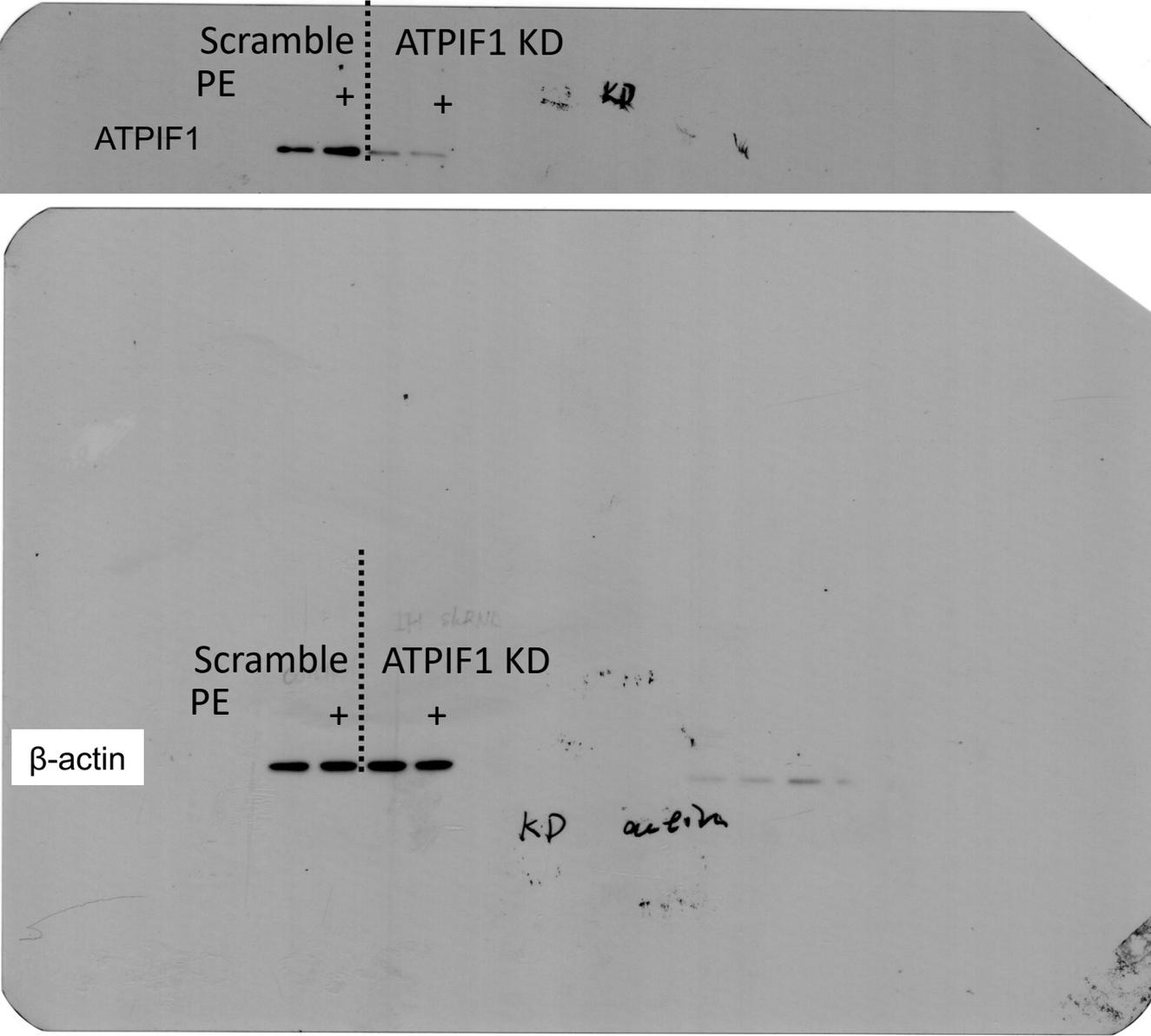
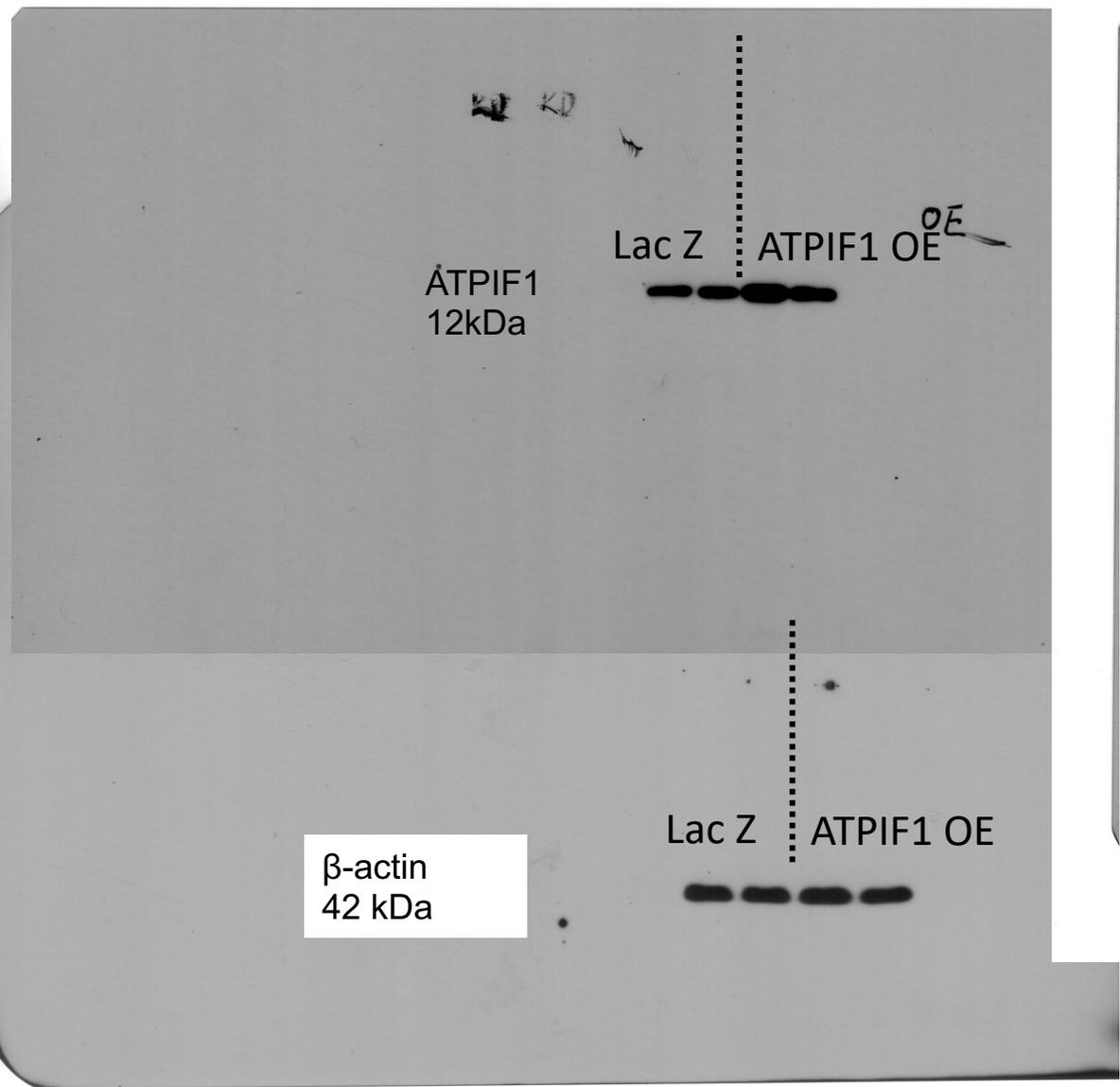


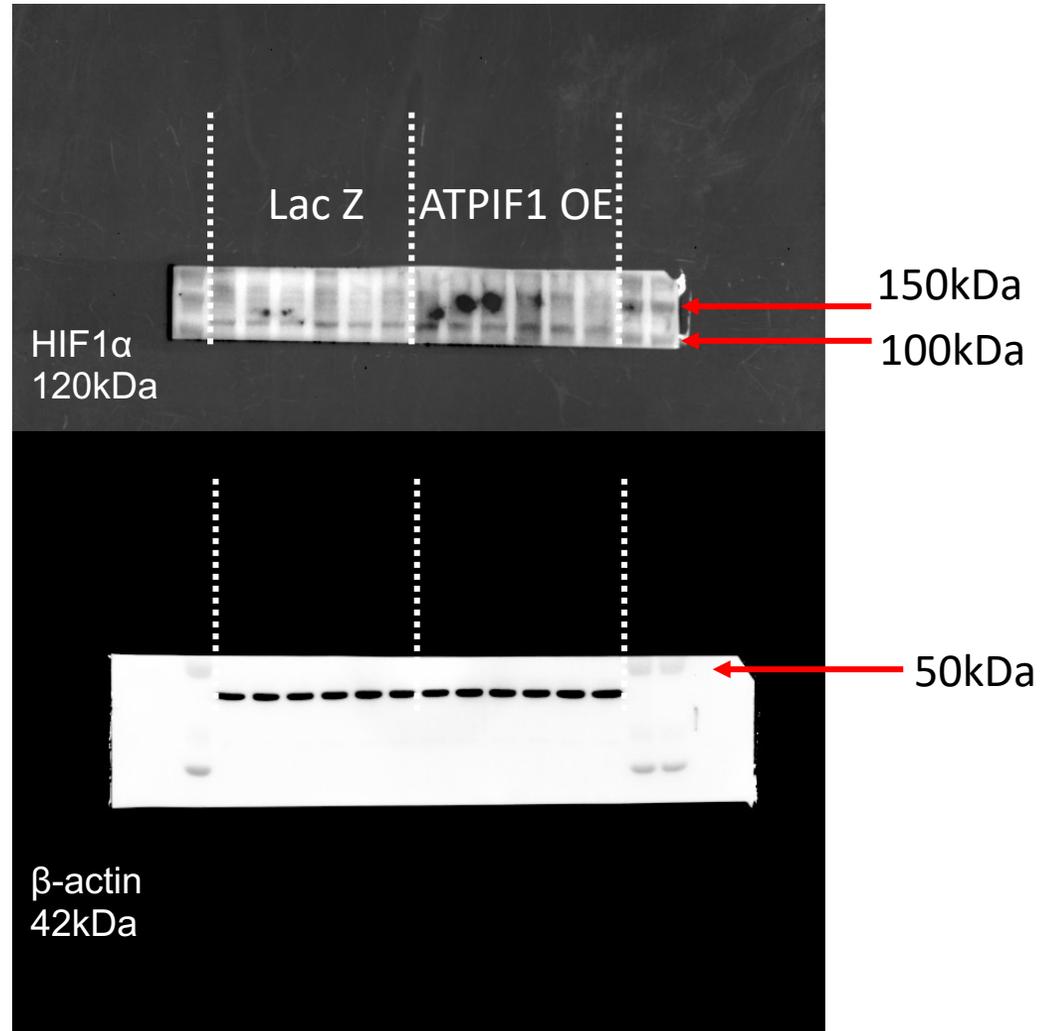
Liver tissue



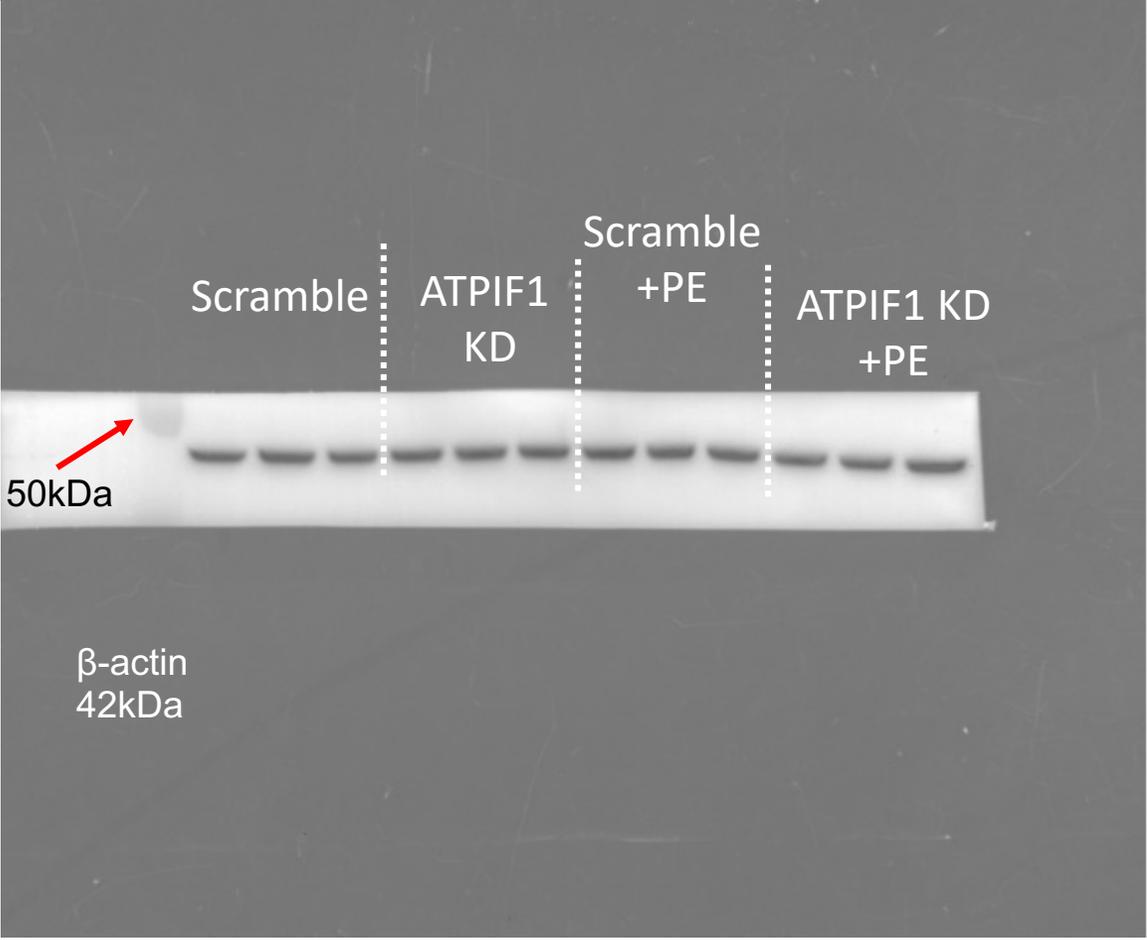
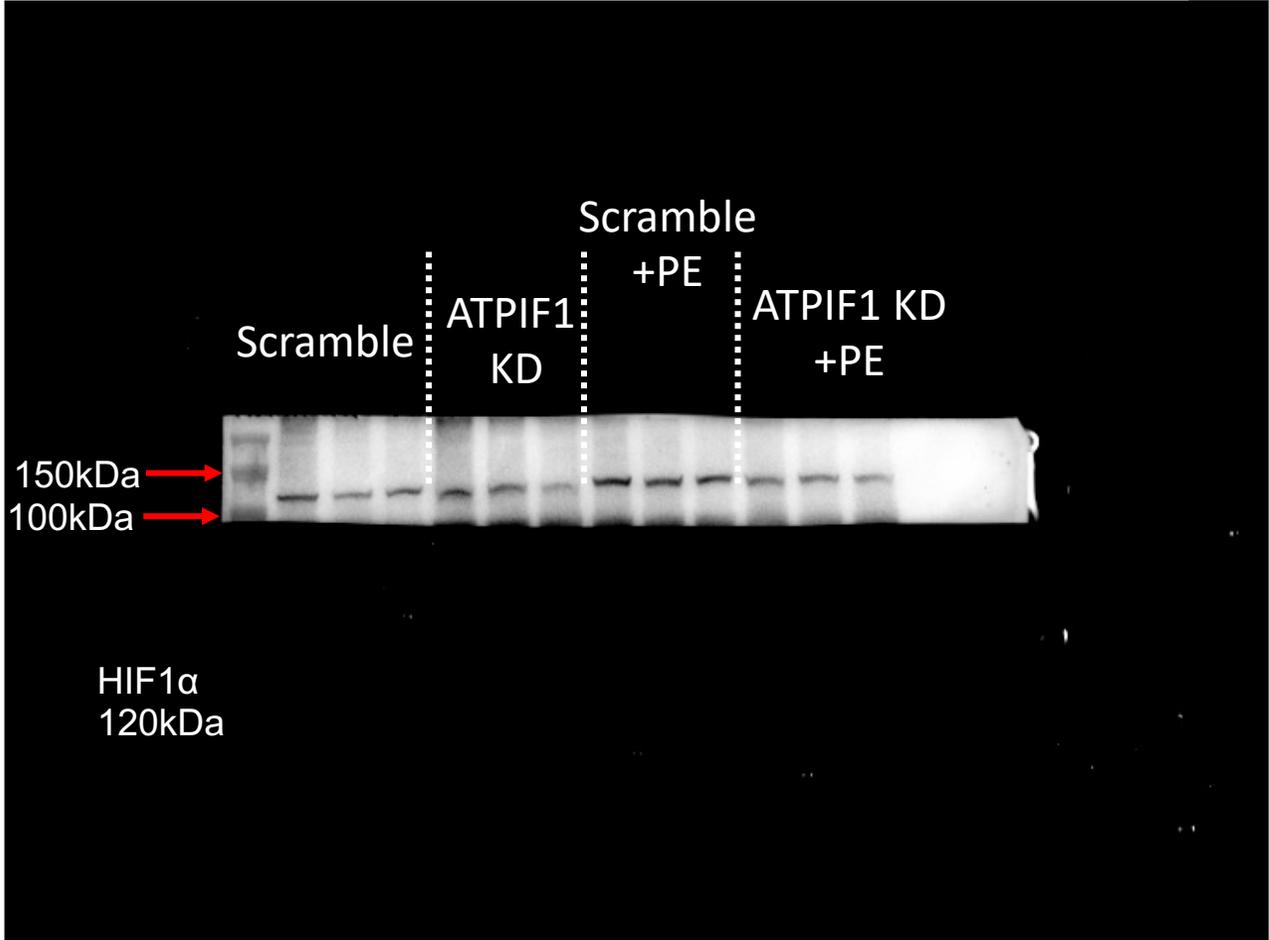
Full unedited gel for Supplementary 2A

ATPIF1: Cell Signaling Technology 8528
β-actin: Sigma A2103



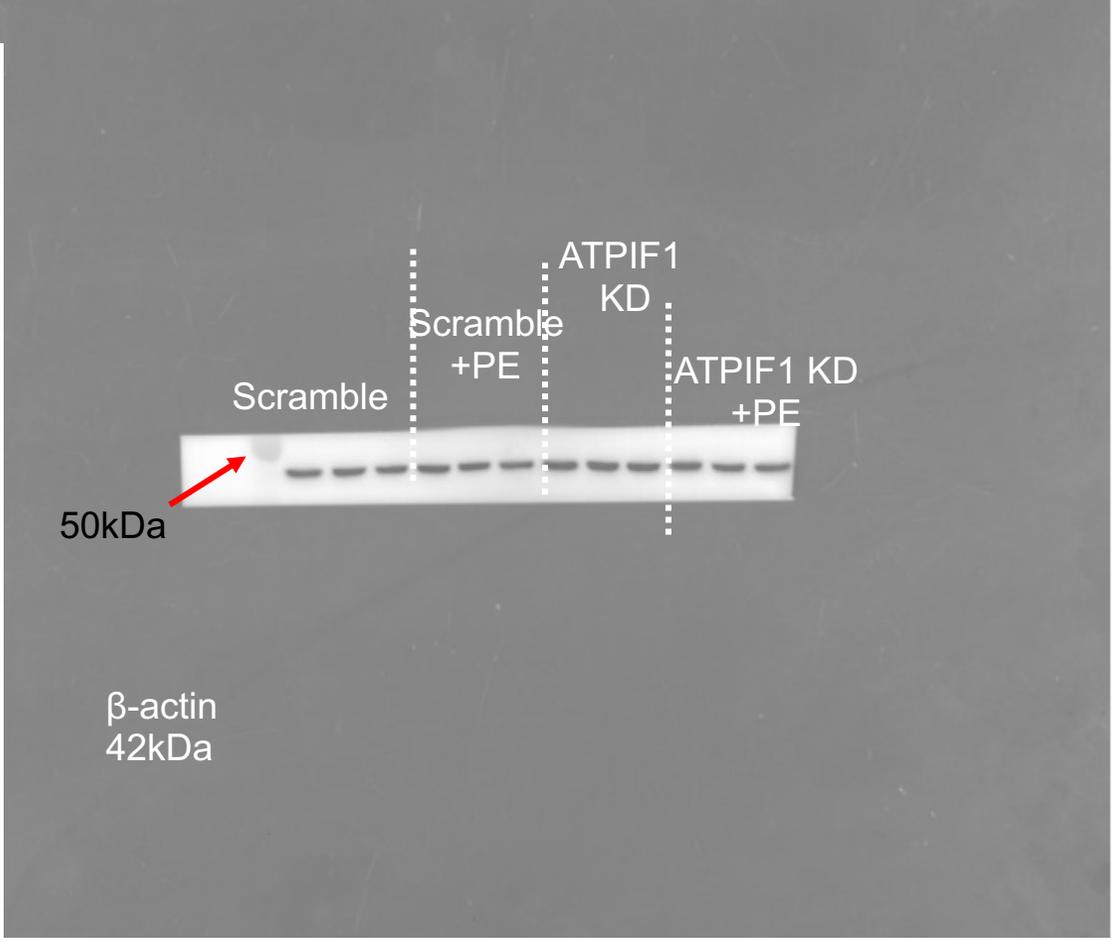
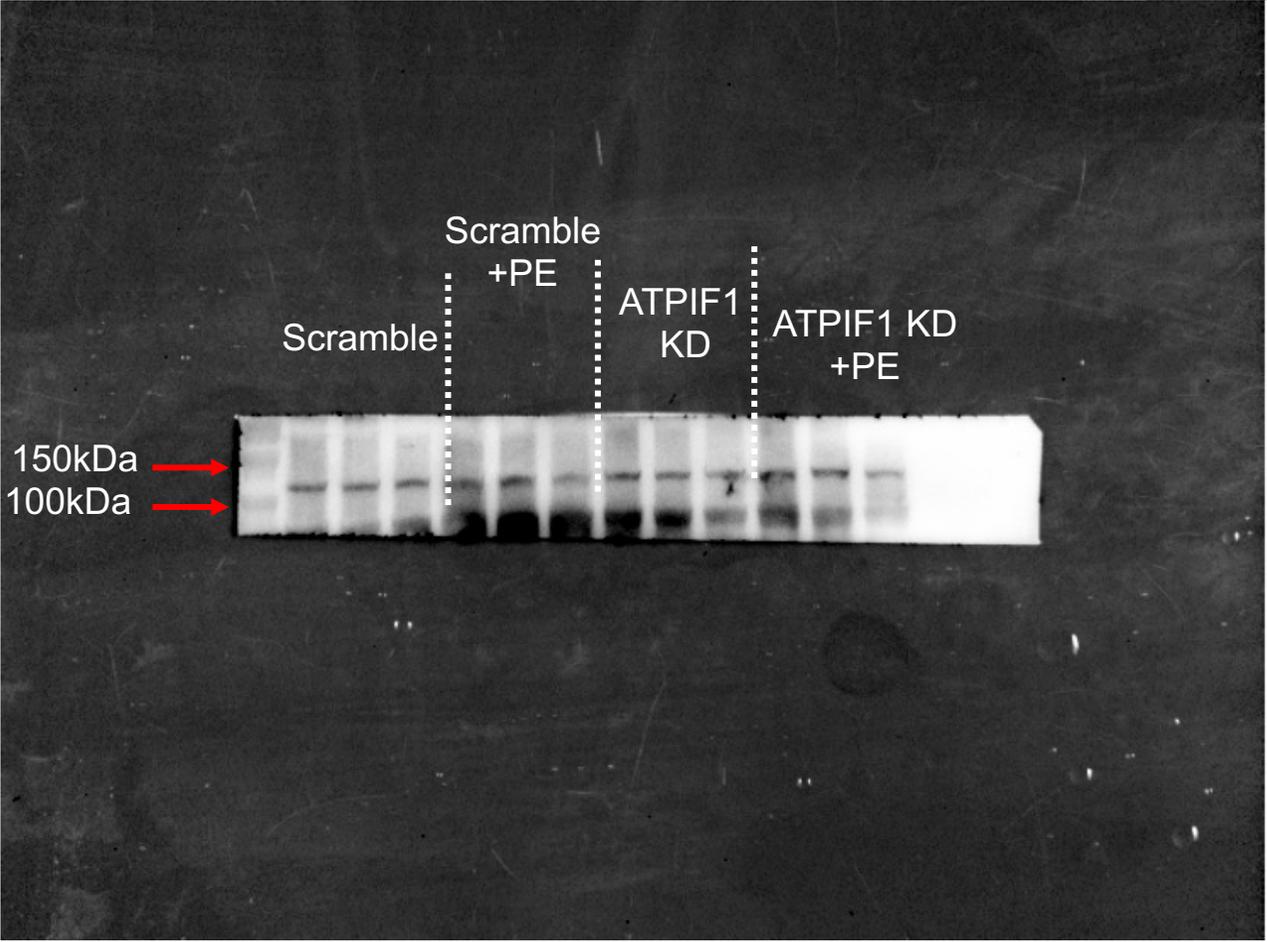


HIF1α: Abcam ab1
β-actin: Sigma A2103



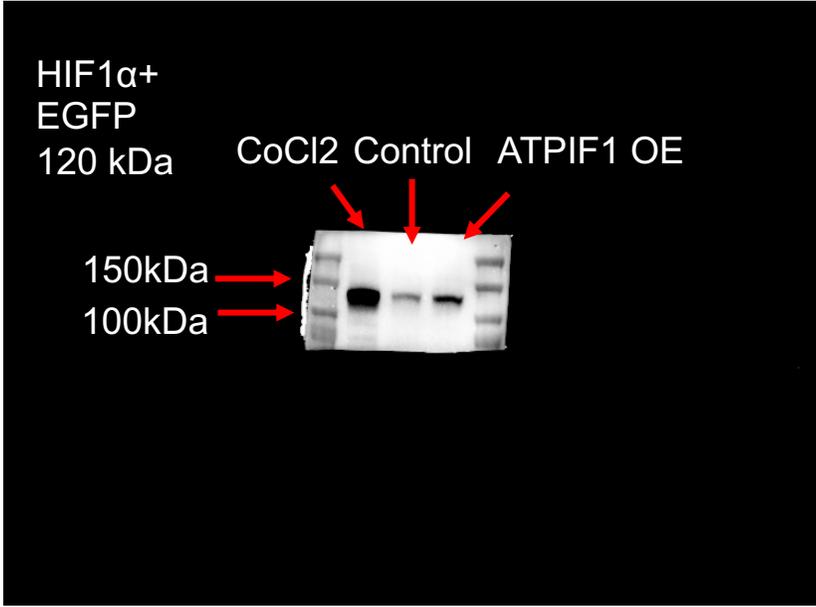
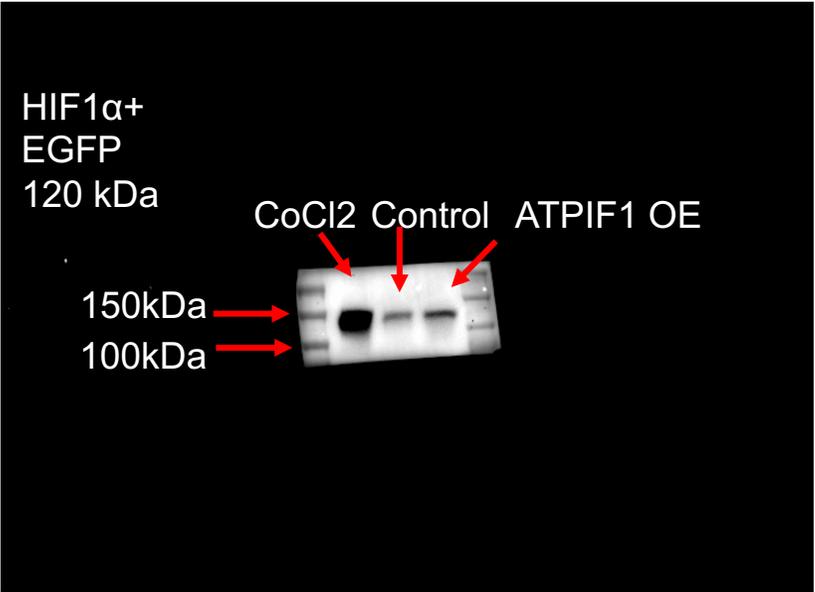
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HIF1α: Abcam ab1
β-actin: Sigma A2103

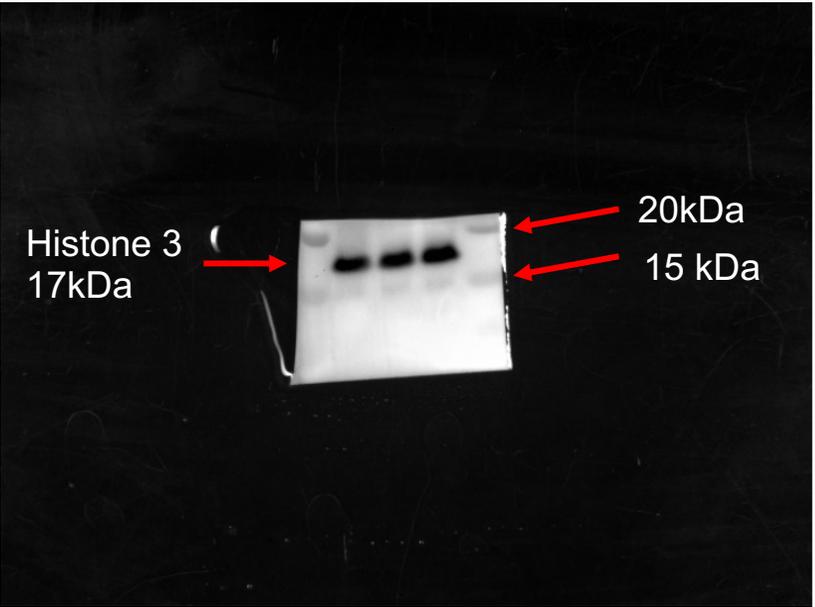
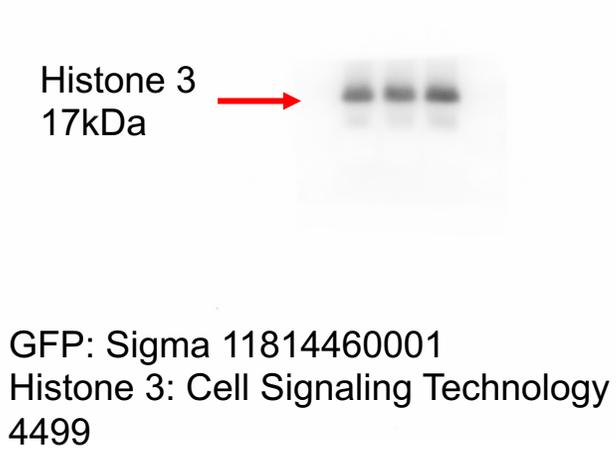


HIF1 α : Abcam ab1
 β -actin: Sigma A2103

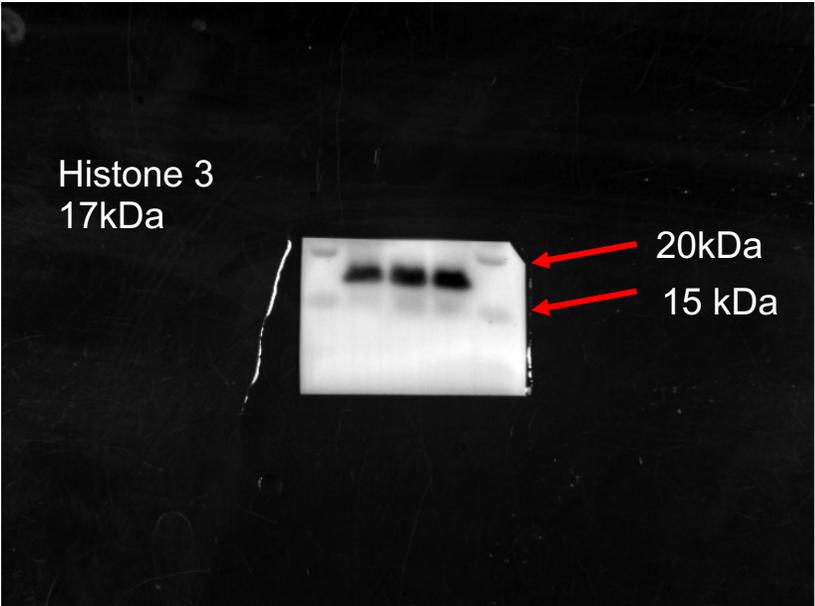
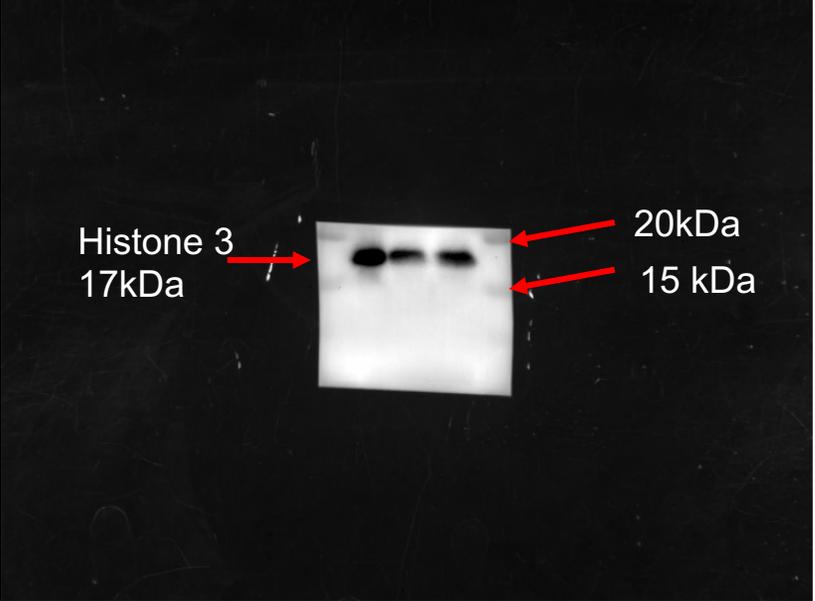
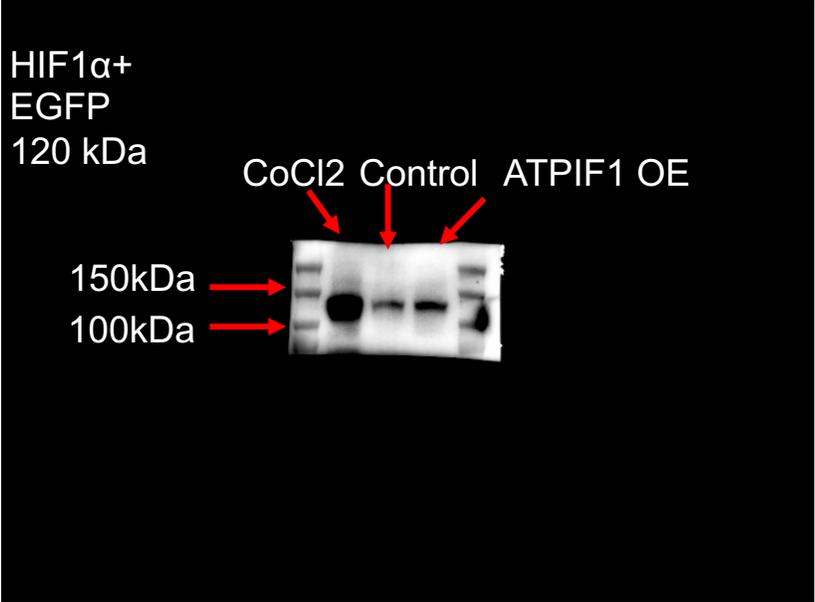
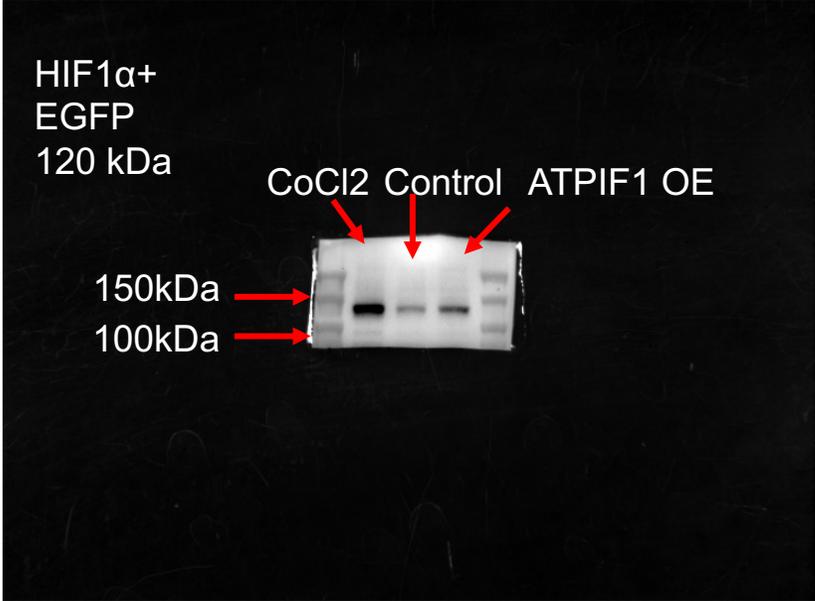
Full unedited gel for Supplementary 2F



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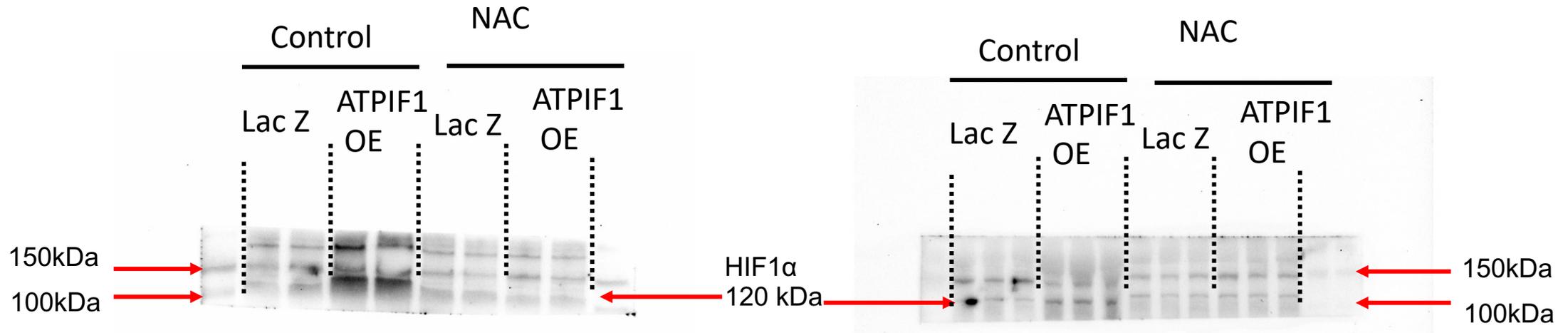


Full unedited gel for Supplementary 2F

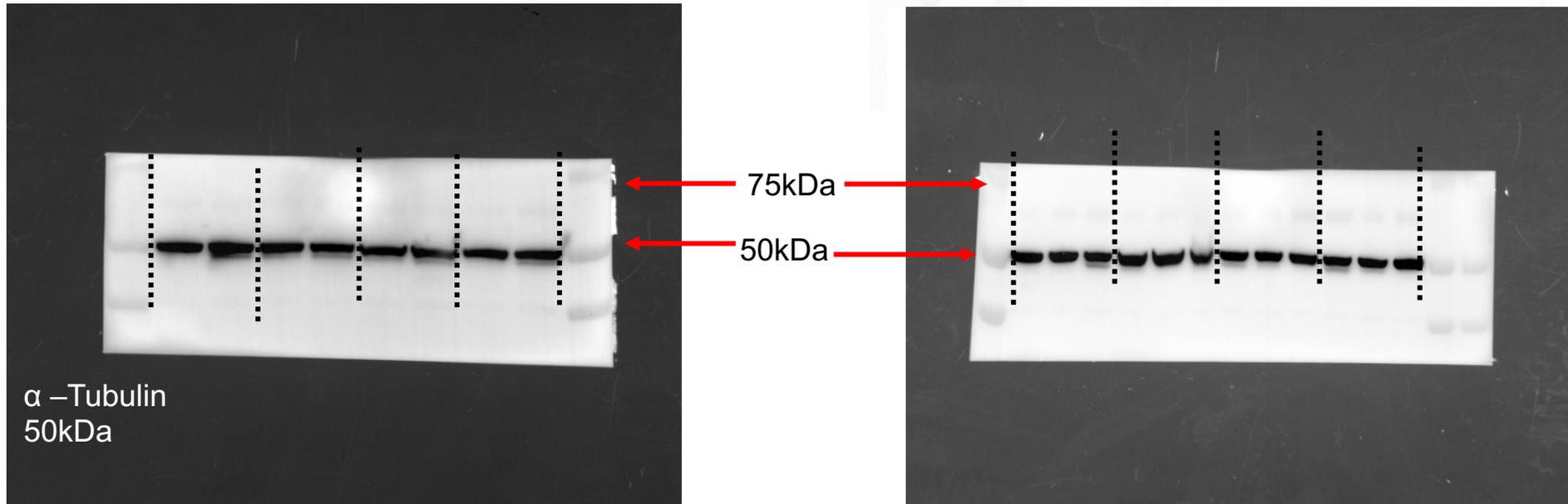


GFP: Sigma 11814460001
Histone 3: Cell Signaling Technology
4499

Full unedited gel for Supplementary 3D

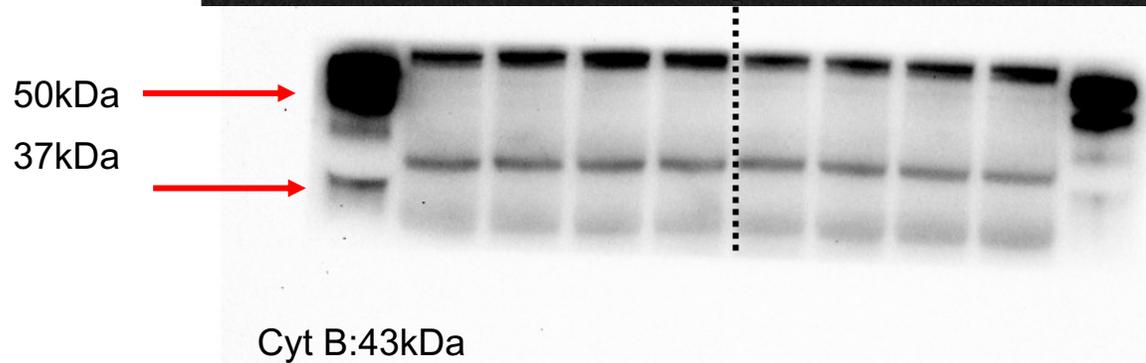
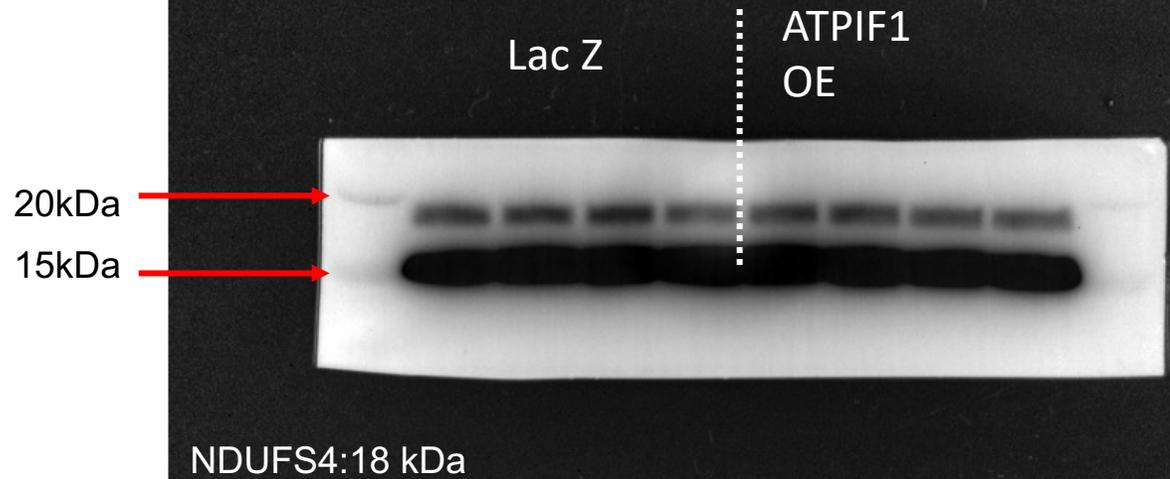


These two images shown in the Supplementary 3D

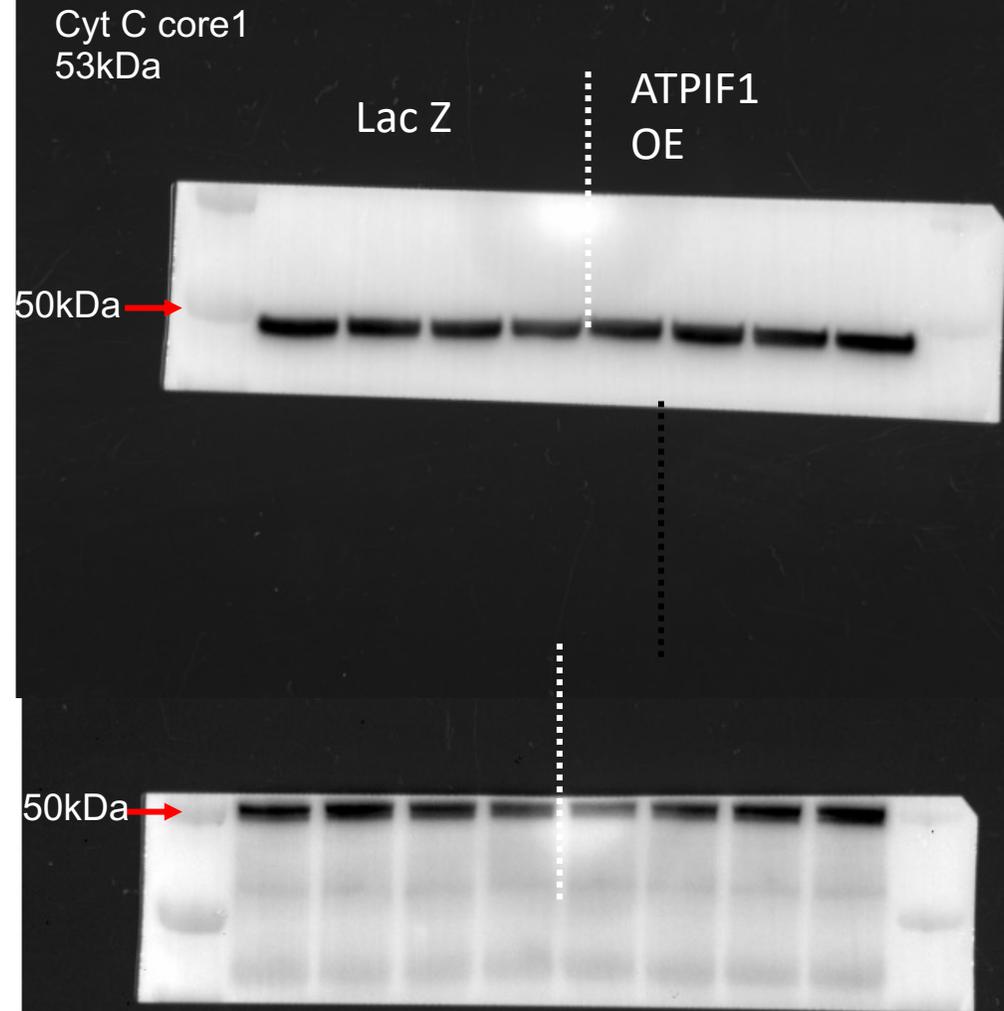


HIF1α: Abcam ab1
α-Tubulin: Sigma T6199

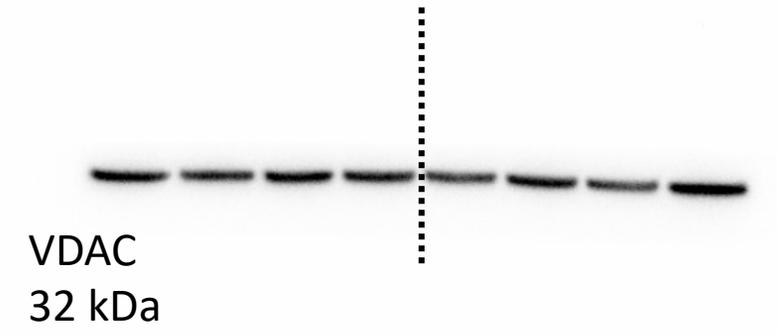
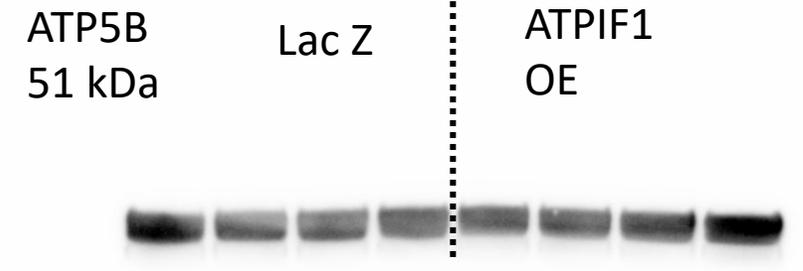
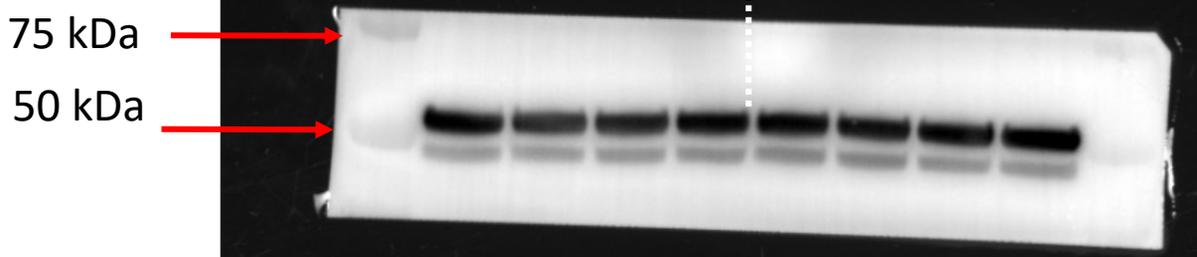
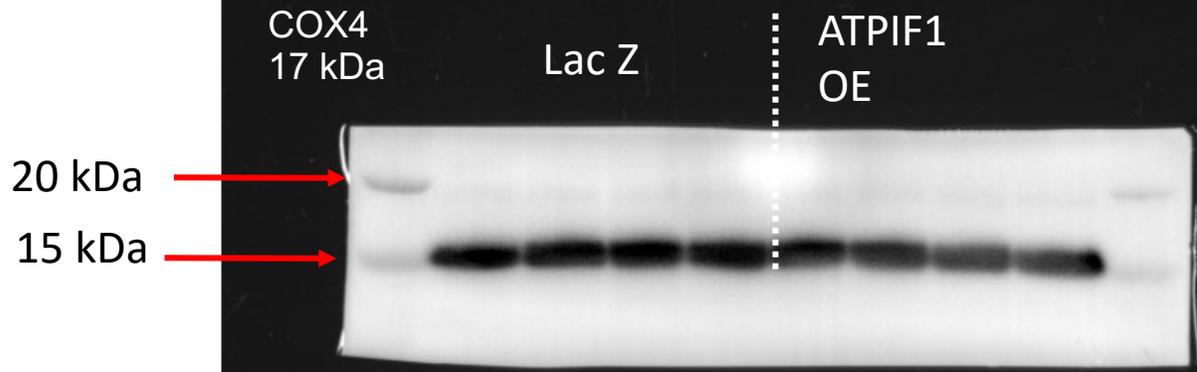
Full unedited gel for Supplementary 4C



NDUFS4: Thermo fisher MA5-19432;
Cyt B: Santa Cruz Biotechnology sc-11436;
Cyt C core 1: Abcam ab110252;
COX-1: Thermo fisher PA5-26688;



Full unedited gel for Supplementary 4C

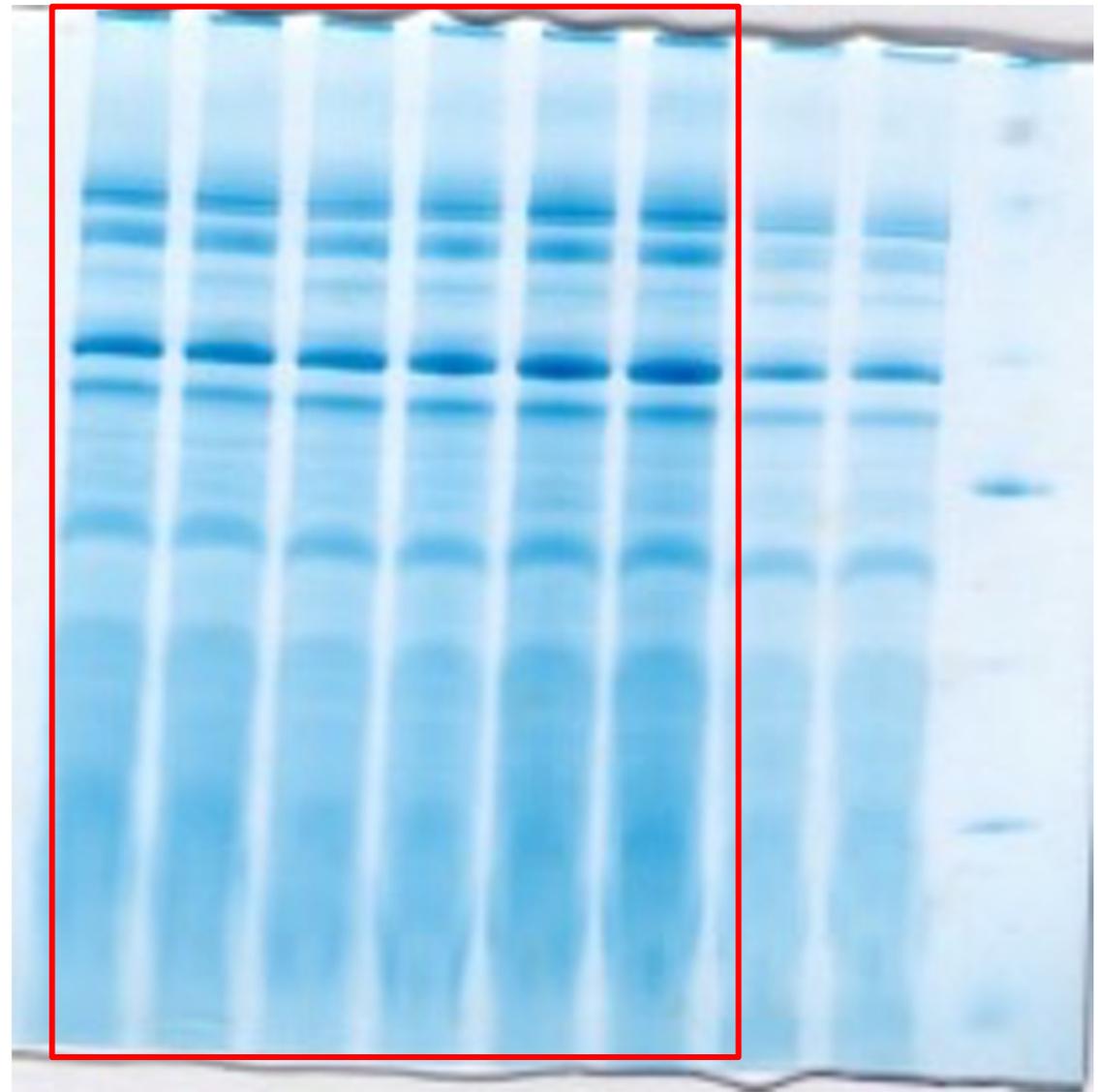


COX 4: Abcam ab14744;
ATP5A: Santa Cruz Biotechnology sc-136178
ATP5B: Santa Cruz Biotechnology sc-33618
VDAC: Cell Signaling Technology 4661

Full unedited gel for Supplementary 4D

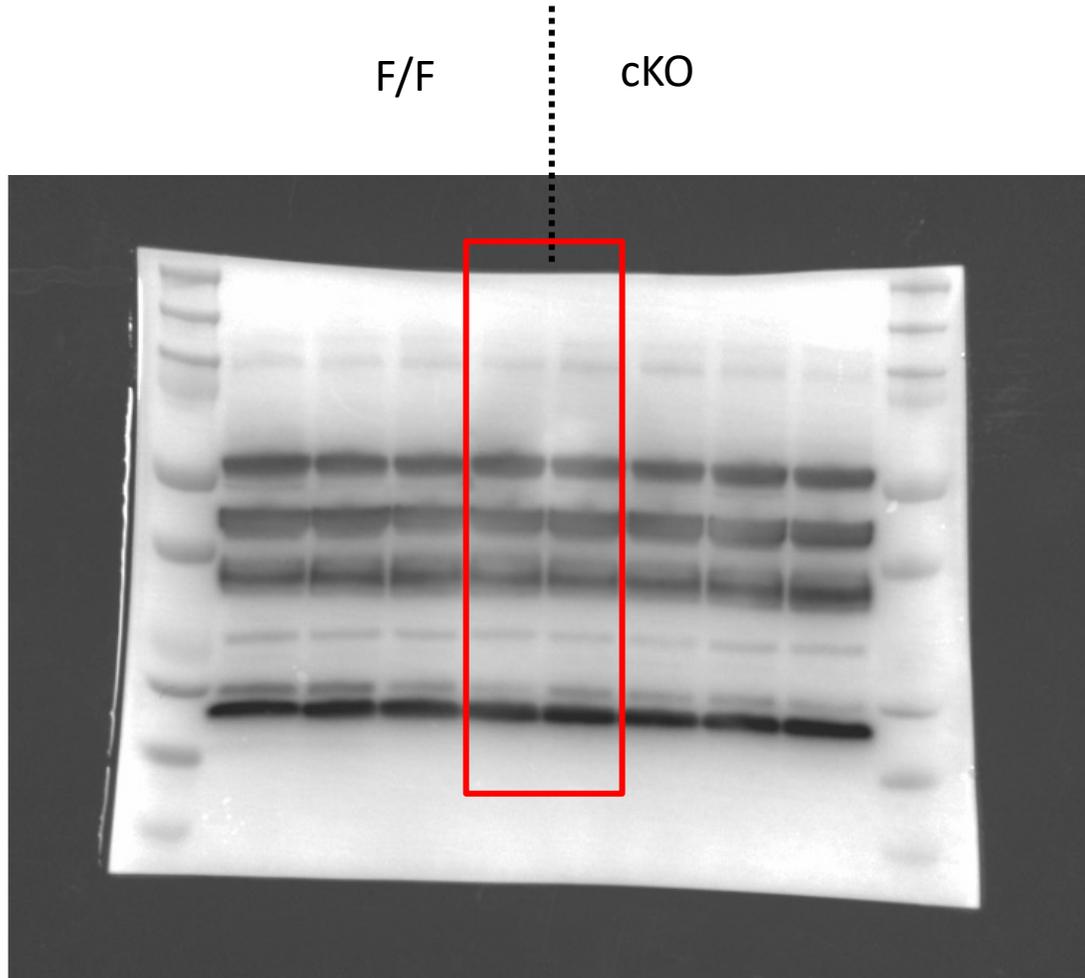
IF1 OE Lac Z IF1 OE Lac Z IF1 OE Lac Z IF1 OE Lac Z Marker

Blue Native gel



These lanes shown in the Supplementary 4D

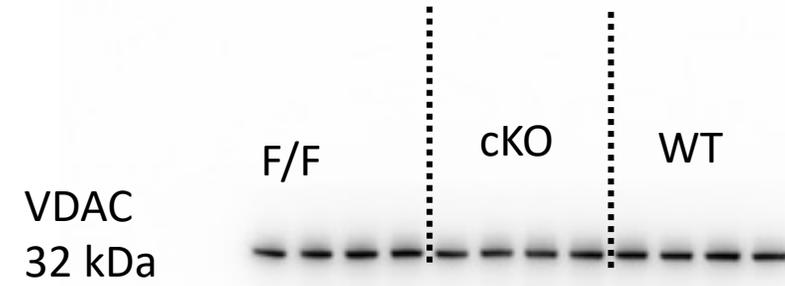
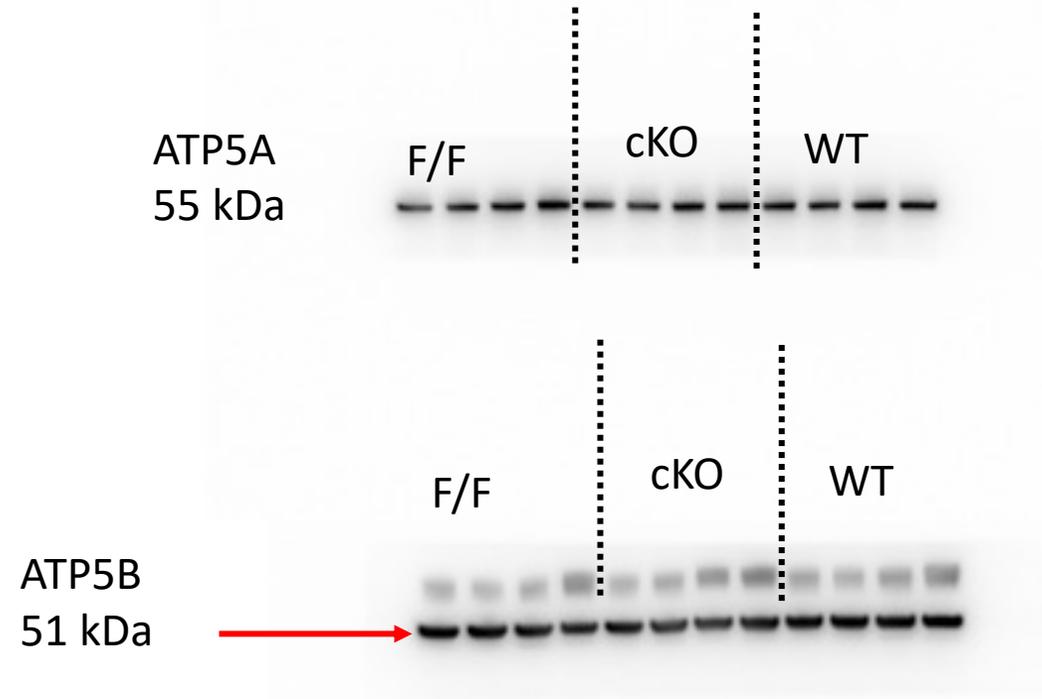
Full unedited gel for Supplementary 5C



OXPHOS cocktail antibody: Abcam ab110413

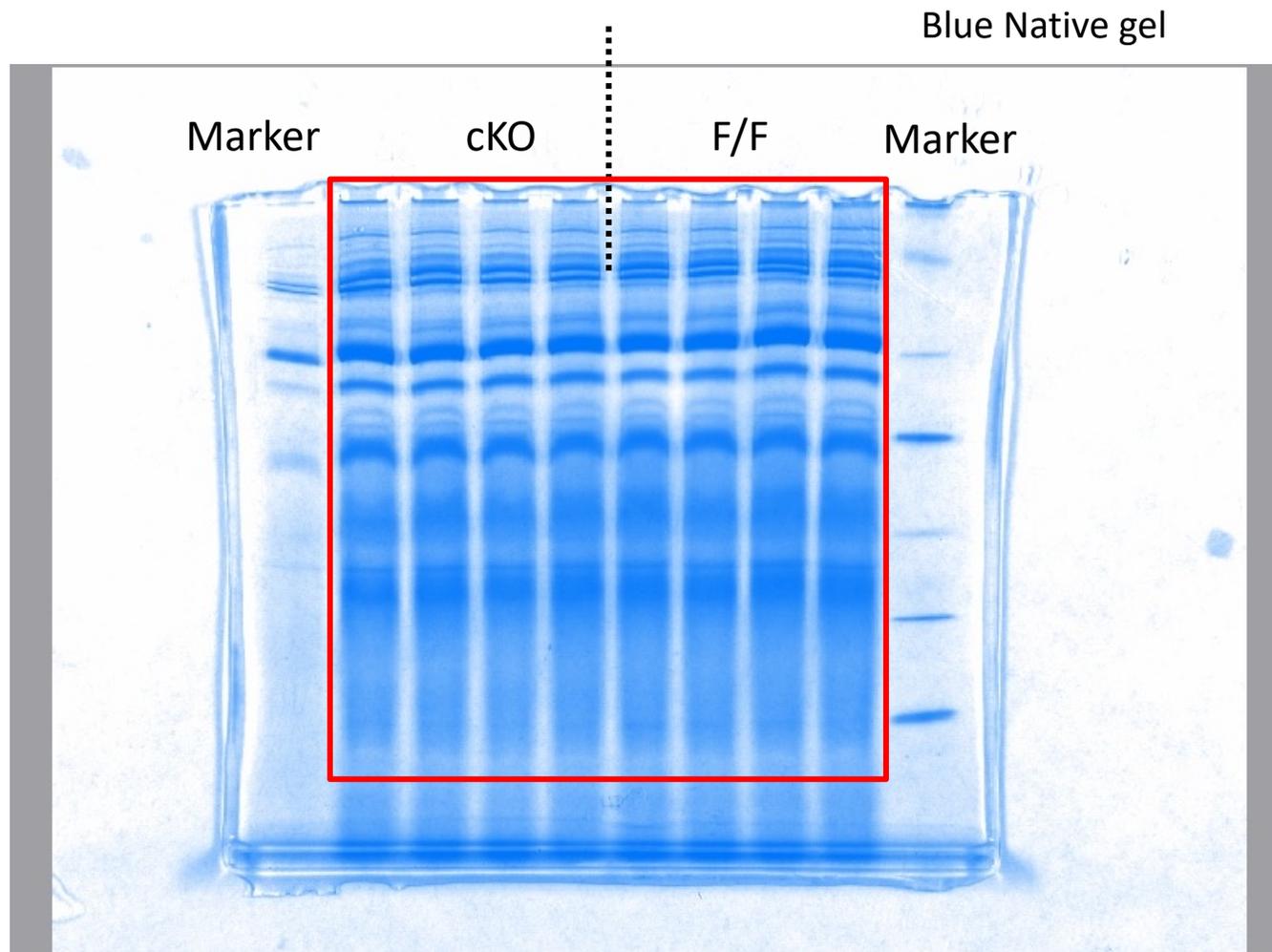
These 2 lanes shown in the Supplementary 5C

Full unedited gel for Supplementary 5C



ATP5A: Santa Cruz Biotechnology sc-136178
ATP5B: Santa Cruz Biotechnology (sc-33618)
VDAC: Cell Signaling Technology 4661

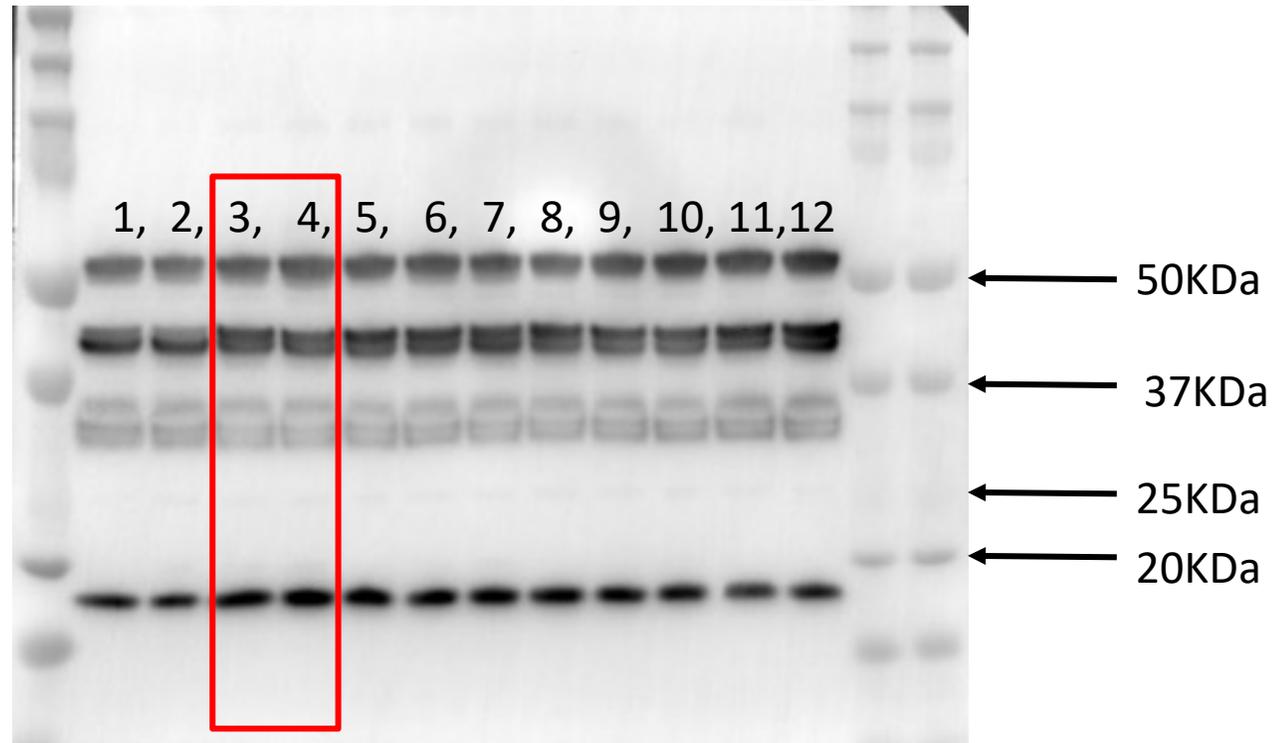
Full unedited gel for Supplementary 5D



These lanes shown in the Supplementary 5D

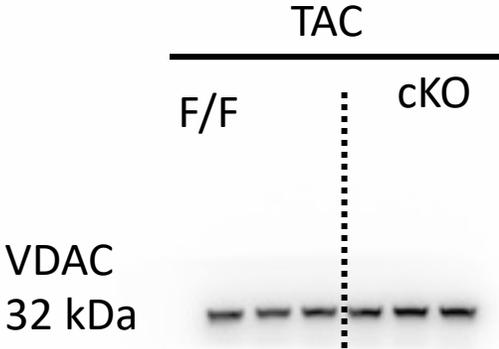
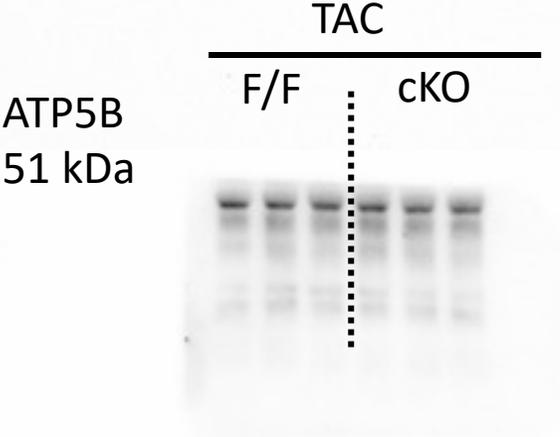
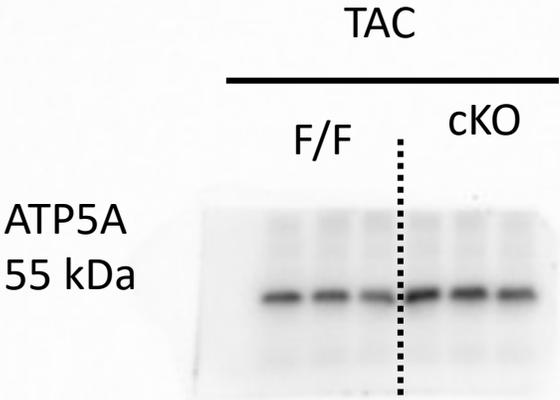
F/F: Lane 1,3,5,7,9,11;
cKO: 2,4,6,8,10,12

TAC

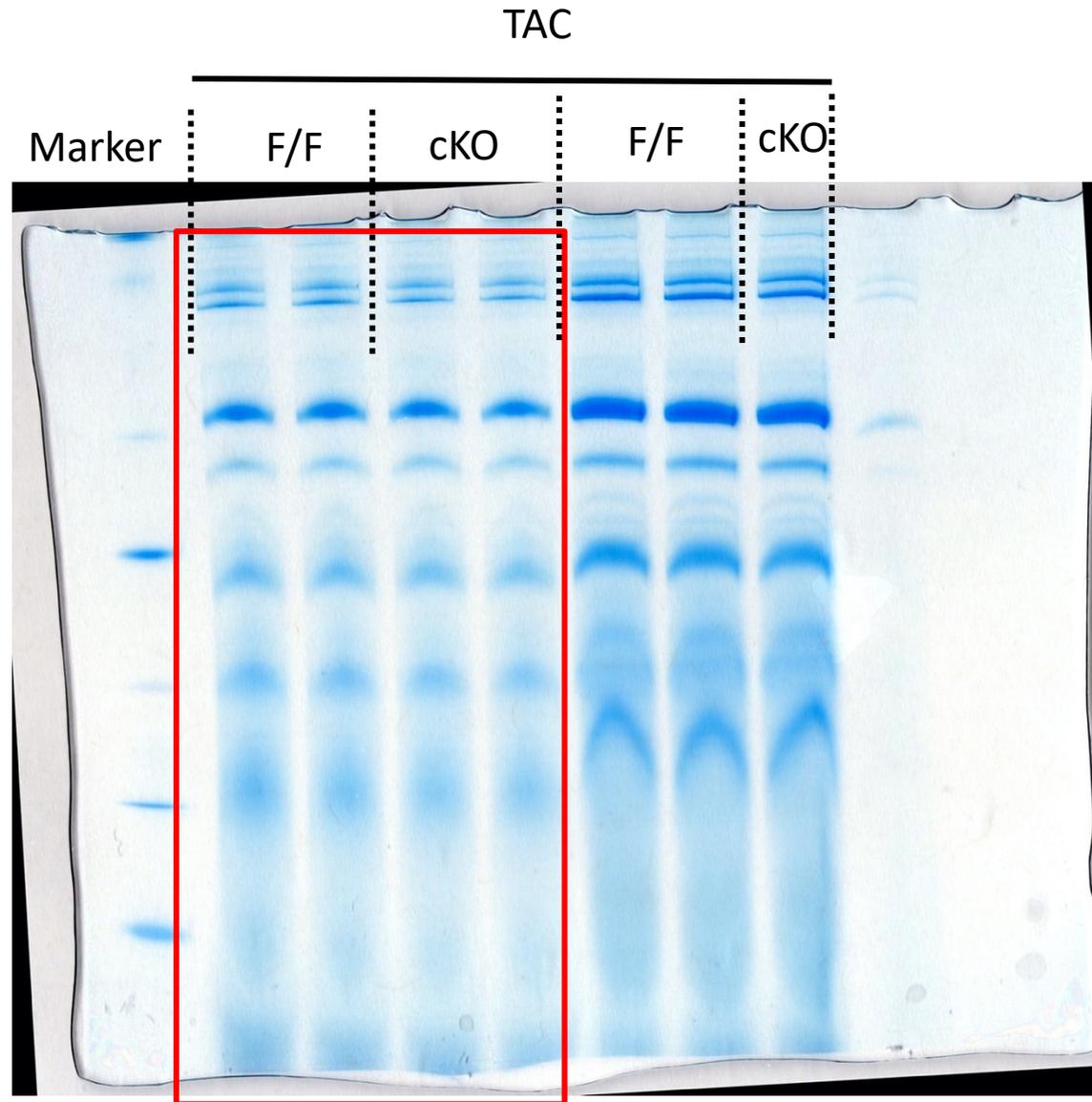


- Complex I subunit NDUFB8 - "CI-20" ~ [20kD](#)
- Complex II subunit 30kDa - "CII-30" ~ [30kD](#)
- Complex III subunit Core 2 - "CIII-core2" ~ [47kD](#)
- Complex IV subunit I - "CIV-I" ~ [39kD](#)
- ATP synthase subunit alpha - "CV-alpha" ~ [53kD](#)

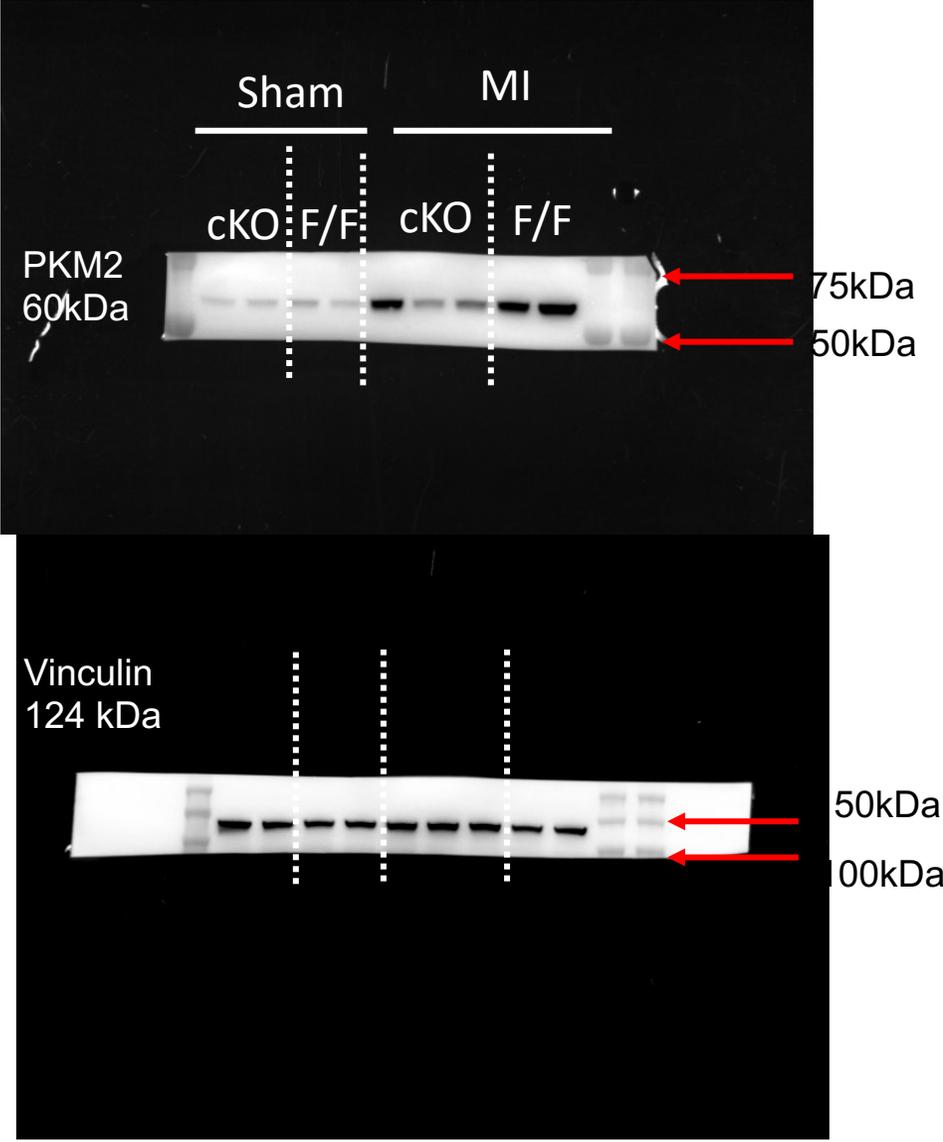
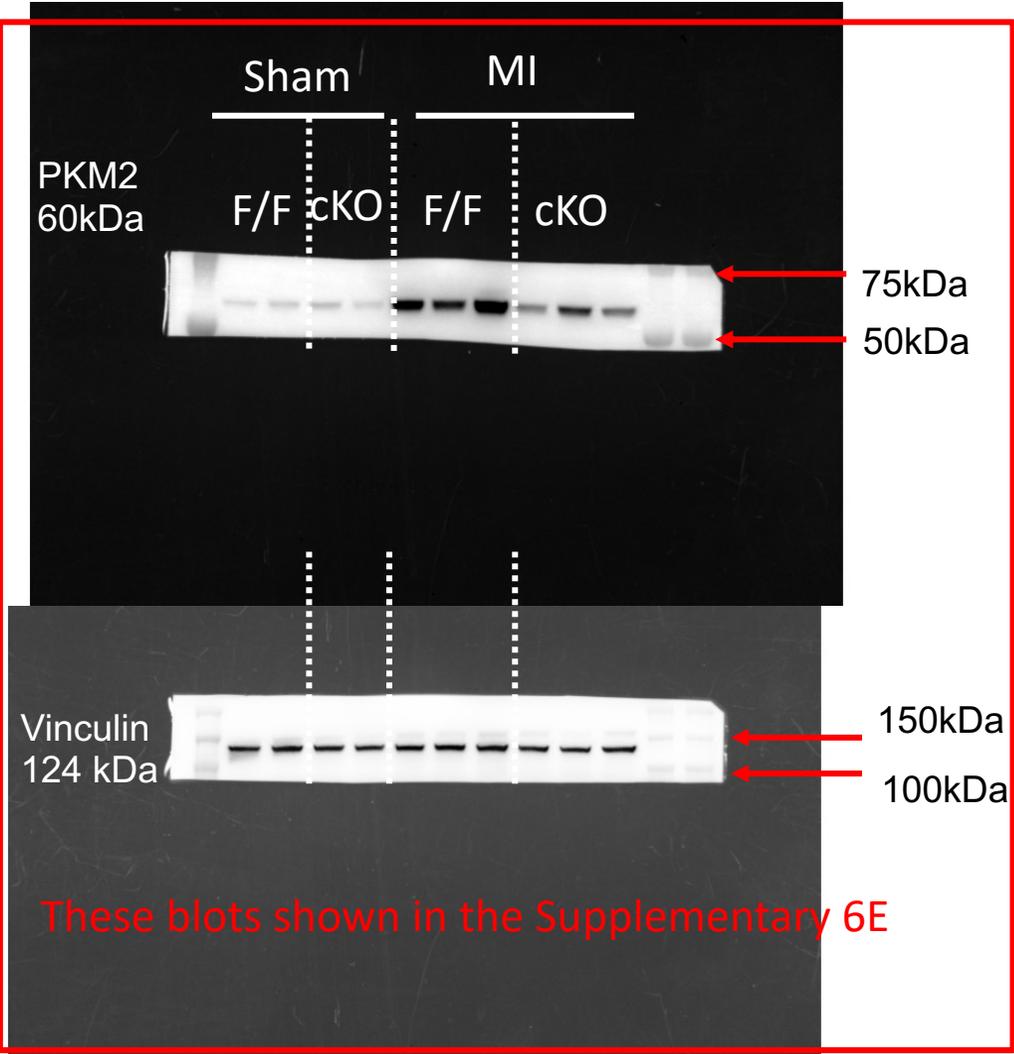
These 2 lanes shown in the Supplementary 5E



ATP5A: Santa Cruz Biotechnology sc-136178
ATP5B: Santa Cruz Biotechnology (sc-33618)
VDAC: Cell Signaling Technology 4661

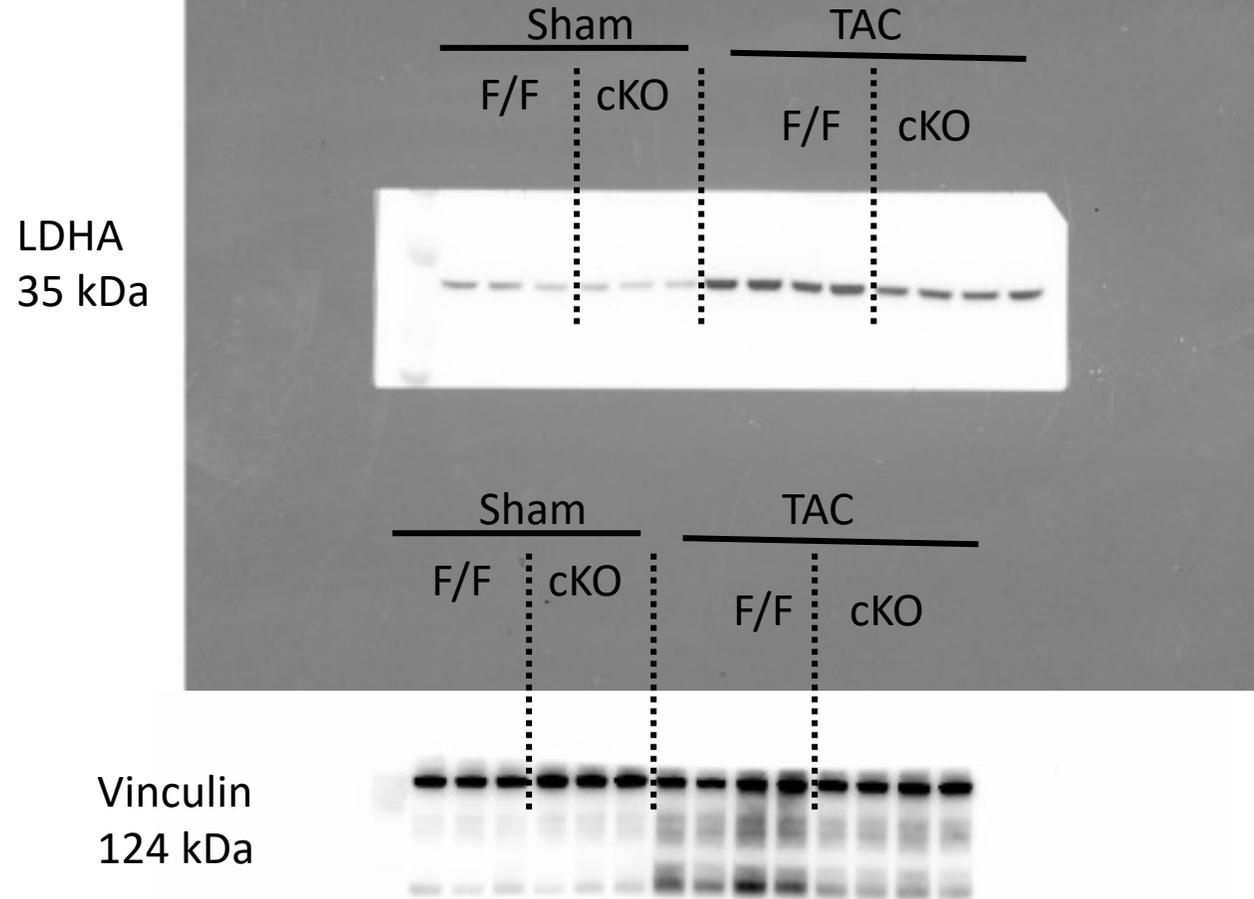


These lanes shown in the Supplementary 5F



PKM2: Cell Signaling Technology 4053
Vinculin: Cell Signaling Technology 13901

Full unedited gel for Supplementary 6E



LDHA: Cell Signaling Technology 2012;
Vinculin: Cell Signaling Technology 13901