

Supplementary Material

Metabolic profile in endothelial cells of chronic thromboembolic pulmonary hypertension and pulmonary arterial hypertension.

V.F.E.D. Smolders*^{1,2}, C. Rodríguez*^{1,3}, I. Blanco^{1,4}, R. Szulcek^{5,6}, Wim Timens⁷, L. Piccari¹, Y. Roger¹, X. Hu¹, Constanza Morén^{8,9}, C. Bonjoch¹, L. Sebastián¹, M. Castellà¹⁰, J. Osorio^{1,4}, V. I. Peinado^{1,4}, Harm Jan Bogaard¹¹, P. H. A. Quax², M. Cascante^{12,13+}, J.A. Barberà^{1,4+}, O. Tura-Ceide^{1,3,4+}.

¹ Department of Pulmonary Medicine, Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); University of Barcelona; Barcelona, Spain. ² Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands. ³ Biomedical Research Institute-IDIBGI, Girona, Spain. ⁴ Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Respiratorias, Madrid, Spain. ⁵ Laboratory of in vitro modeling systems of pulmonary diseases, Institute of Physiology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charitéplatz, Berlin, Germany. ⁶ German Heart Center Berlin, Berlin, Germany. ⁷ University of Groningen, University Medical Center Groningen, Dept. of Pathology and Medical Biology, Groningen, the Netherlands ⁸ Laboratory of Muscle Research and Mitochondrial Function, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona (UB), Department of Internal Medicine, Hospital Clínic of Barcelona (HCB), Barcelona, Spain. ⁹ Biomedical Research Networking Centre on Rare Diseases (CIBERER), Madrid, Spain. ¹⁰ Department of Cardiovascular Surgery, Institut Clínic Cardiovascular, Hospital Clínic, Barcelona, Spain. ¹¹ Amsterdam UMC, VU University Medical Center (VUmc), Dept. of Pulmonary Diseases, Amsterdam Cardiovascular Sciences (ACS), Amsterdam, the Netherlands. ¹² Department of Biochemistry and Molecular Biomedicine and Institute of Biomedicine (IBUB), Faculty of Biology, Universitat de Barcelona, Diagonal 643, 08028 Barcelona, Spain. ¹³ CIBER of Hepatic and Digestive Diseases (CIBEREHD), Institute of Health Carlos III (ISCIII), 28029 Madrid, Spain.

*Both authors have contributed equally

+Correspondence: olgaturac@gmail.com, martacascante@ub.edu and jbarbera@clinic.cat

Servei de Pneumologia, Hospital Clínic. Villarroel, 170. Barcelona 08036. Spain. Phone 34-93-227-5747; Fax. 34-93-227-5455.

Running title: Metabolic profile in pulmonary hypertension.

Table of contents: Supplementary Tables and figures.

Supplementary table 1. Primers used in this manuscript

Gene name	Forward (5'-3')	Reverse (5'-3')
G6PD	CCAAGCCCATCCCCTATATTT	CCACTTGTAGGTGCCCTCAT
GLS1	GCTGTGCTCCATTGAAGTGACT	TTGGGCAGAAACCACCATTAG
GLUD1	GGGATTCTAACTACCACTTGCTCA	AACTCTGCCGTGGGTACAAT
GLUT1	GGTTGTGCCATACTCATGACC	CAGATAGGACATCCAGGGTAGC
HK2	TCCCCTGCCACCAGACTA	TGGACTTGAATCCCTTGGTC
LDHA	GCAGATTTGGCAGAGAGTATAATG	GACATCATCCTTTATCCGTAAAGA
PDHA1	CCTGACTTTATATGGCGATGG	CTGCCATGTTGTAAGCTTCG
PDK1	GGTTACGGGACAGATGCAGT	CGTGGTTGGTGTGTAATGC
PFKFB3	CCTACCTGAAATGCCCTCTTC	GTCCCTTCTTTGCATCCTCTG
HOXD3	CGTAAGGATTGCATCGGACT	TCCTAAGCTCGGCTGGATAA
HOXD8	TAAACCAGCTTGCTGTGTGC	GTGAGGCTATCGCTTTCCTG
HOXD9	CCTGCTCCATTGGTTCCTTA	TCAGAAACATGGGGGACATT

Supplementary table 2. Antibodies used in this manuscript

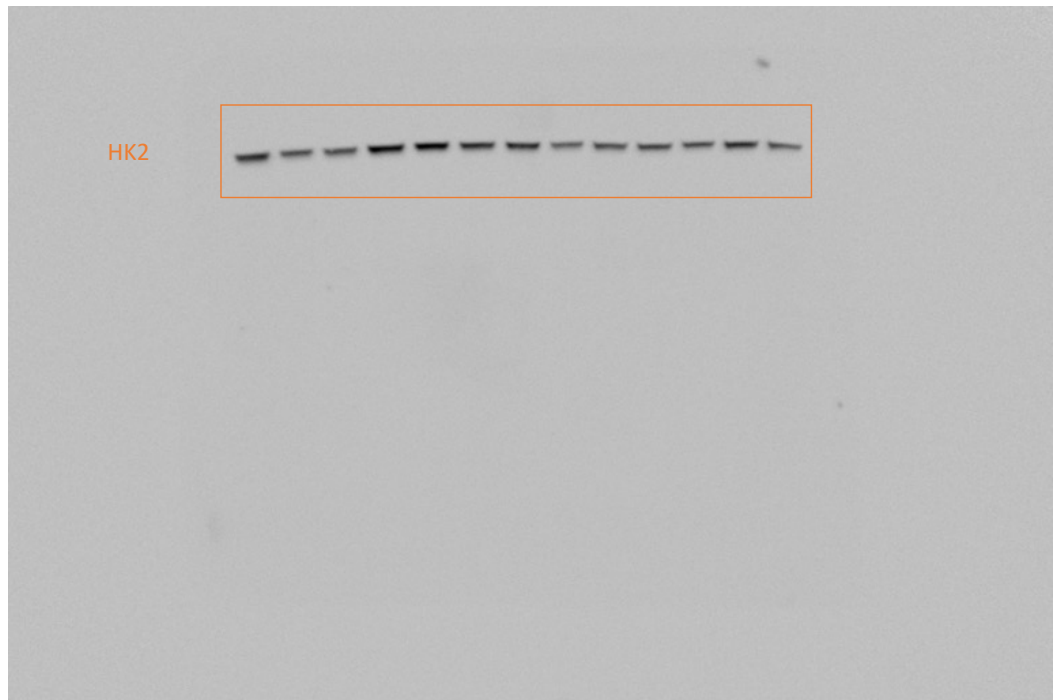
Name	Dilution	Company	Code	Characteristics	Host	Reactivity	kDa
HK2	1/800	santa cruz	sc-374091	monoclonal	mouse	human	100
LDHA	1/800	santa cruz	sc-137243	monoclonal	mouse	human	35
G6PD	1/10000	abcam	ab993	polyclonal	rabbit	human	59
PDH-E1α	1/1000	santa cruz	sc-377092	monoclonal	mouse	human	43
p-PDH-E1α	1 μ g/mL	abcam	ab92696	polyclonal	rabbit	human	43
Total OXPHOS Cocktail	1/250	abcam	ab110413	monoclonal	mouse	human	/ ^a
PDK1	1/2000	abcam	ab207450	monoclonal	rabbit	human	49
β-actin	1/1000	novus biologicals	NB600-503	polyclonal	rabbit	human	37
Vinculin	1/10000	abcam	ab129002	monoclonal	rabbit	human	124

^a This kit contains 5 antibodies. The kDa for each antibody is indicated in figure 4.

Full-length gels represented in Figure 3 in order of appearance.

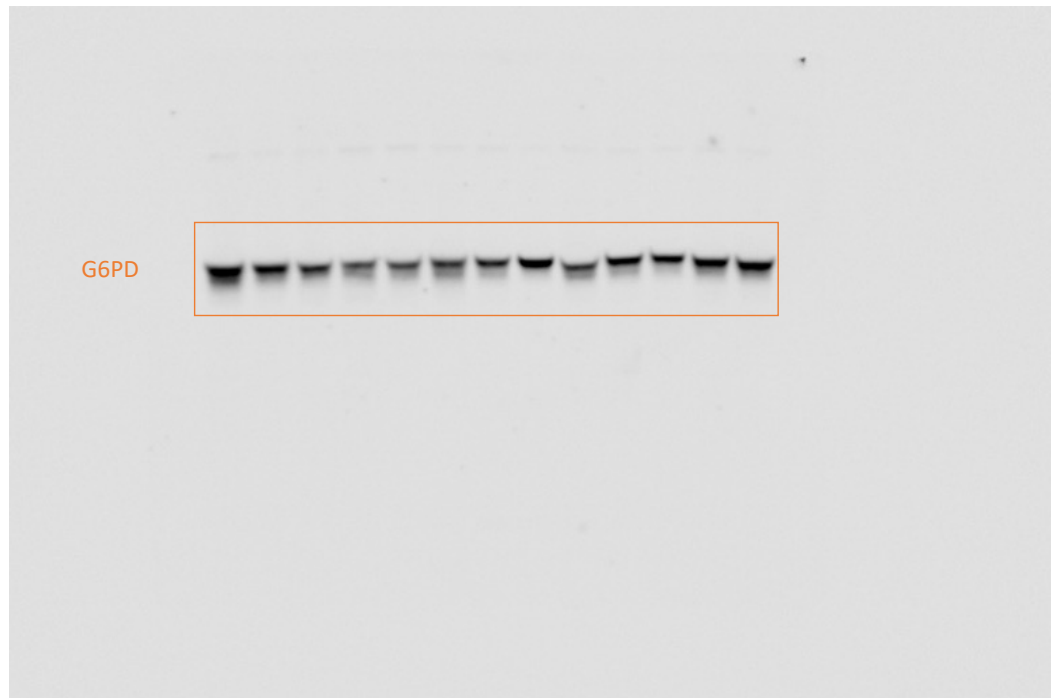
a)

Figure 3A-B



b)

Figure 3A-B



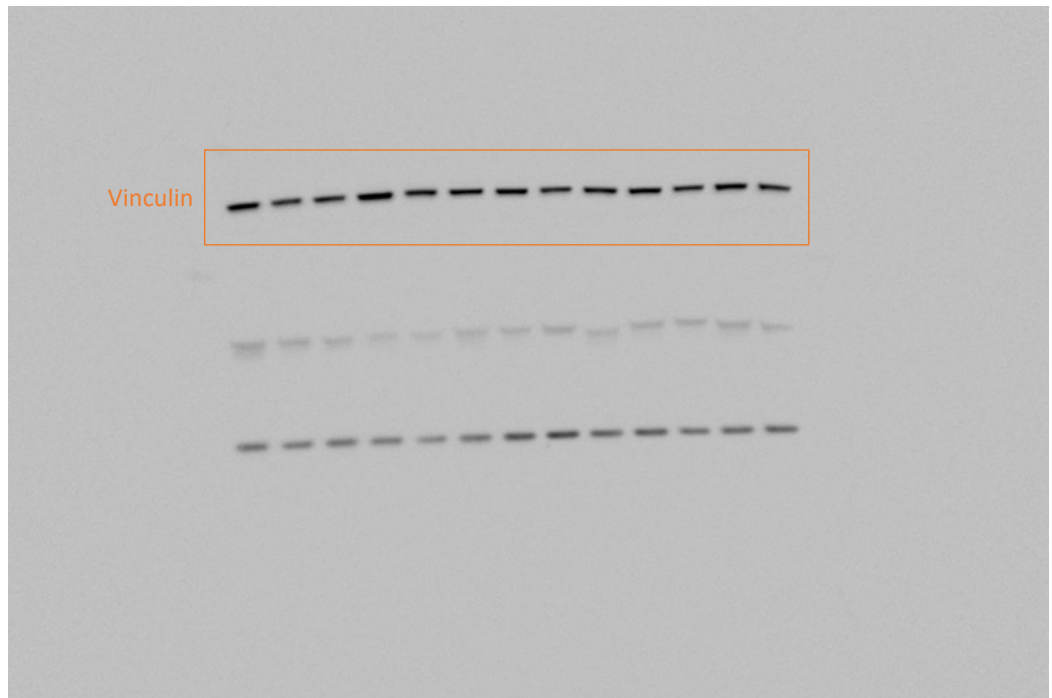
c)

Figure 3A-B



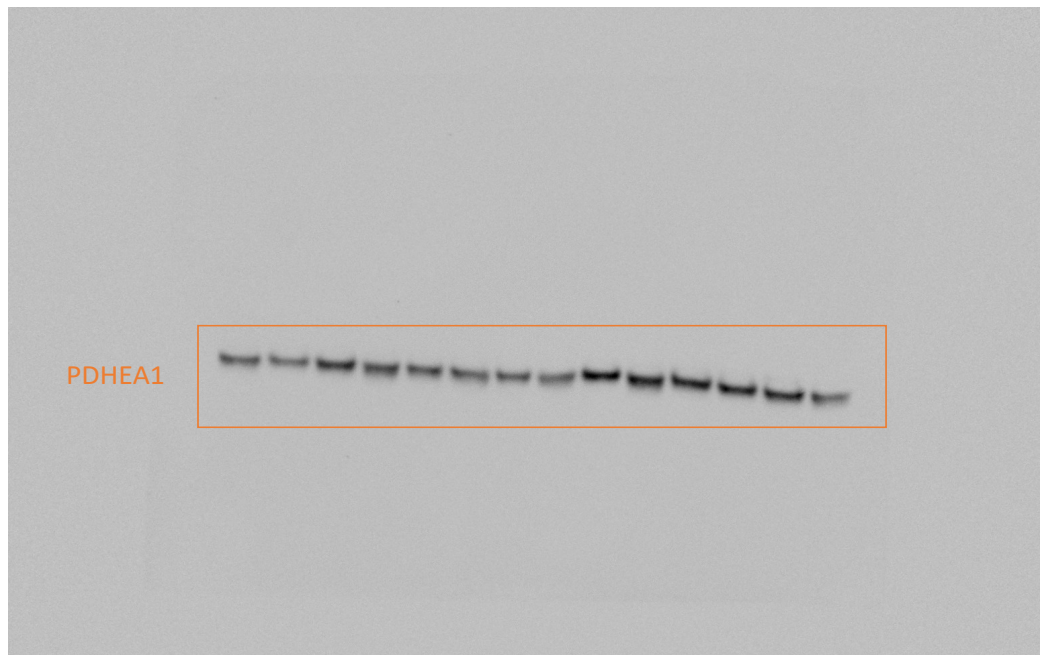
d)

Figure 3A-B



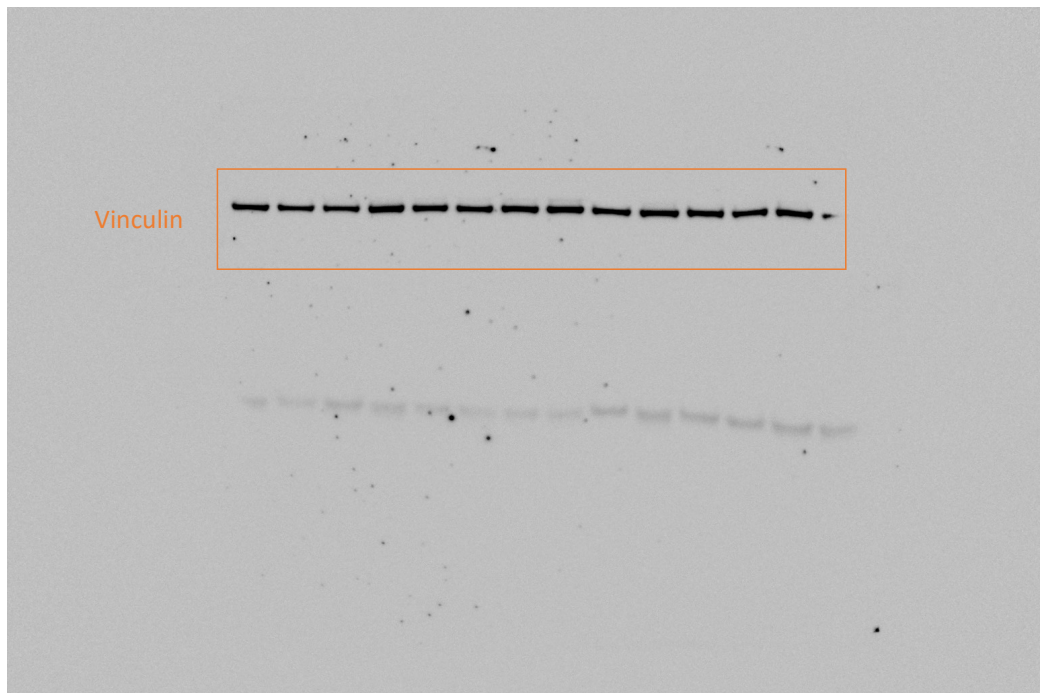
e)

Figure 3C



f)

Figure 3C



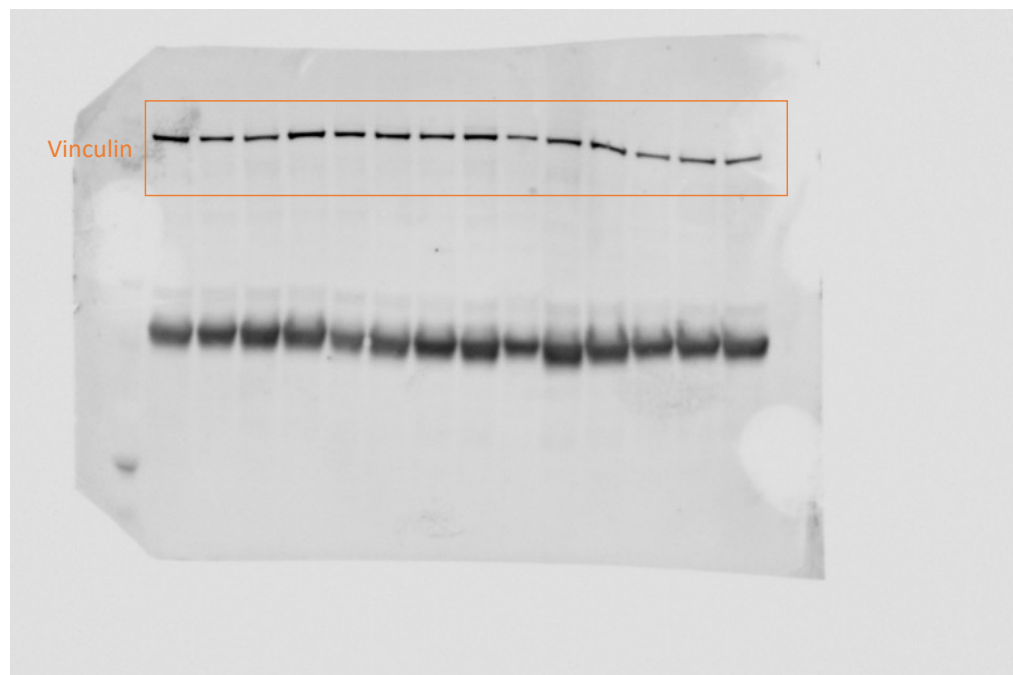
g)

Figure 3C



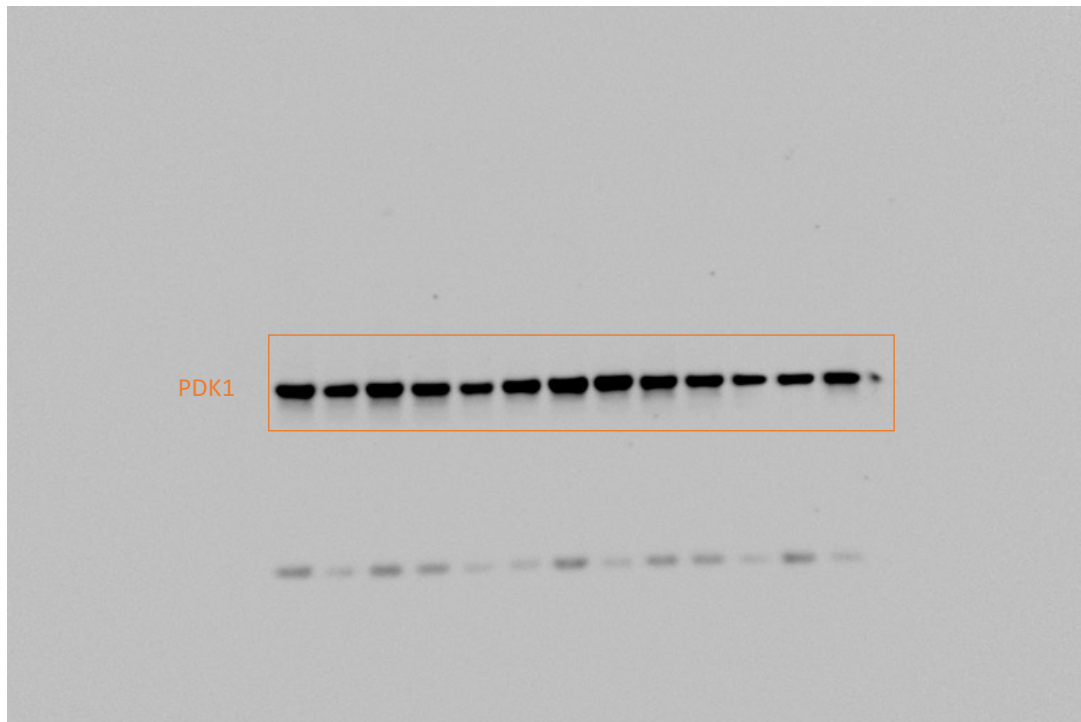
h)

Figure 3C



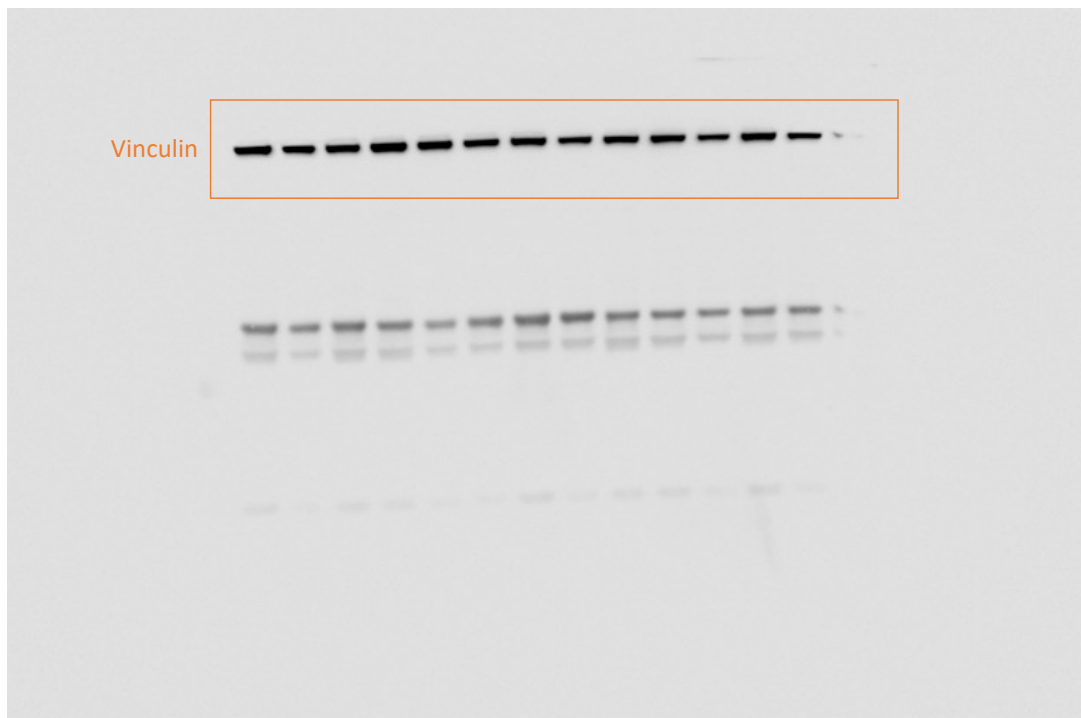
i)

Figure 3C



j)

Figure 3C

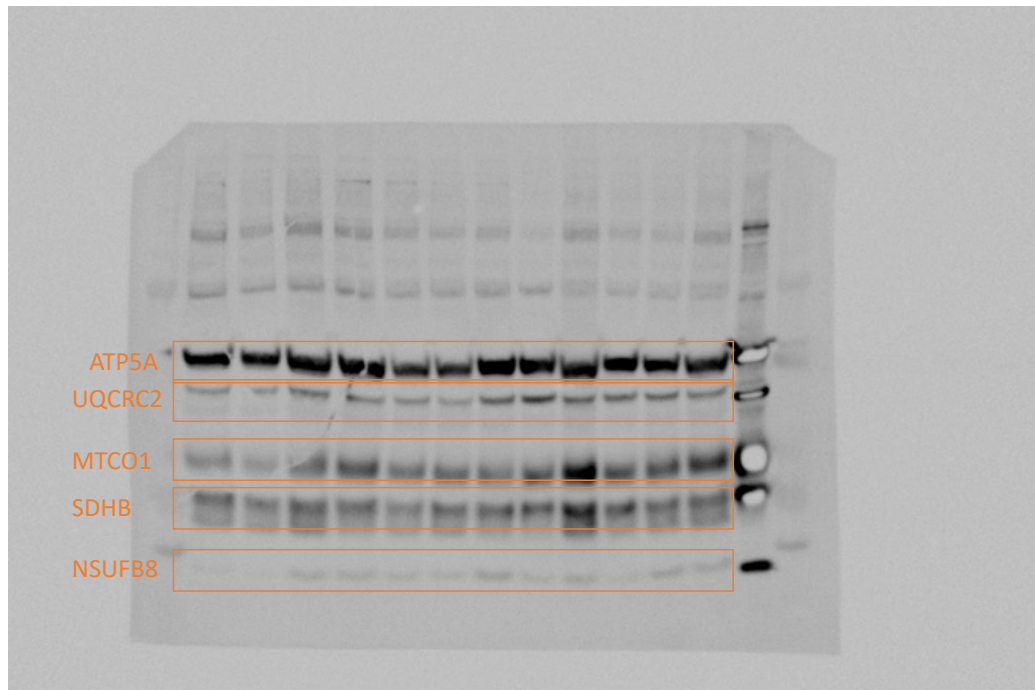


Supplementary Fig. 1. Full-length gels represented in main Figure 3A-C, a) HK2, b) G6PD, c) LDHA, d) Vinculin, e) PDHEA1, f) Vinculin, g) p-PDHEA1, h) Vinculin, i) PDK1 and j) Vinculin.

Full-length gels represented in Figure 4 in order of appearance.

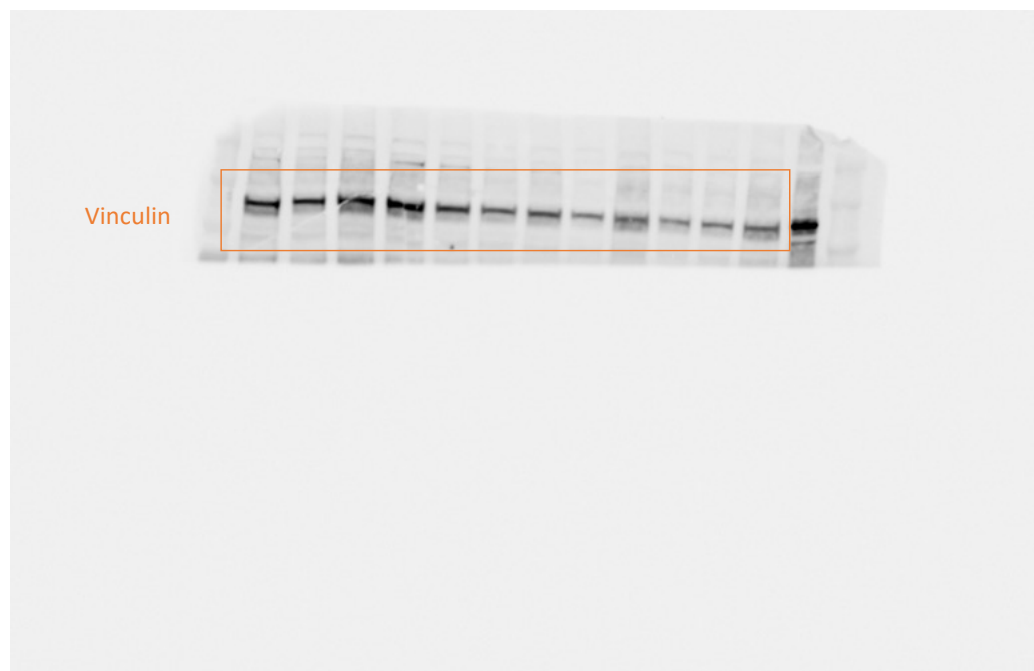
a)

Figure 4



b)

Figure 4



Supplementary Fig. 2. Full-length gels represented in main Figure 4, a) ATP5A, UQCRC2, MTCO1, SDHB and NSUFB8 oxidative phosphorylation associated proteins and b) Vinculin.