

Differential remodeling of the electron transport chain is required to support TLR3 and TLR4 signaling and cytokine production in macrophages

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Supplementary Information:

Supplementary Table S1: List of reagents, chemical or kit used in this study, referenced from Methods section.

Supplementary Figure S1: PIC activation is associated with increased OXPHOS function under low glucose conditions. BMDMs were plated onto Seahorse XFp miniplates and treated with 10 μ g/mL PIC for 18 hours. OXPHOS function was assessed *via* successive Oligomycin (Oligo), Carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), and Rot/AA injections **(a)**. Quantification of the spare respiratory capacity percentage (SRC%) **(b)** and ATP production **(c)**. Data shown represents a test run using one representative animal.

Supplementary Figure S2: TRIF and TRAF6 expression during PIC activation is not affected by glucose levels. Macrophages stimulated either with LPS or PIC for 18 hours under high glucose **(a)** or low glucose **(b)** media conditions were examined for differences in TLR signaling. Protein levels of TRIF and TRAF6 were measured via immunoblotting. Data shown represents blots from a representative animal while data shown represents mean \pm SEM of two individual mice.

Supplementary Figure S3: Targeting ETC function leads to complete loss of IFN responses. LPS- **(a)** or PIC- **(b)** stimulated BMDMs were co-treated with a panel of ETC (Rotenone, Antimycin, Cyanide) inhibitors to assess the importance of mitochondrial function to antiviral responses. CXCL10 and TNF- α cytokine secretion was measured after 18 hours in high glucose or low glucose media conditions. Data shown represents a mean of two individual animals.

Supplementary Figure S4: Targeting mitochondrial ROS leads to loss of type I IFN production. LPS- **(a)** or PIC- **(b)** stimulated BMDMs were co-treated with a panel of mtROS (MT, S3QEL, NAC) modulators to assess the importance of mitochondrial function to antiviral responses. CXCL10 and TNF- α cytokine secretion was measured after 18 hours in high glucose or low glucose media conditions. Data shown represents a mean of two individual animals.

Supplementary Figure S6: Uncropped Images of Figure 4

Supplementary Figure S7: Uncropped Images of Figure 5

Supplementary Figure S8: Uncropped Images of Figure 7

Supplementary Figure S9: Uncropped Images of Supplementary Figure S2

Supplementary Table S1: List of reagents, chemical or kit used in this study.

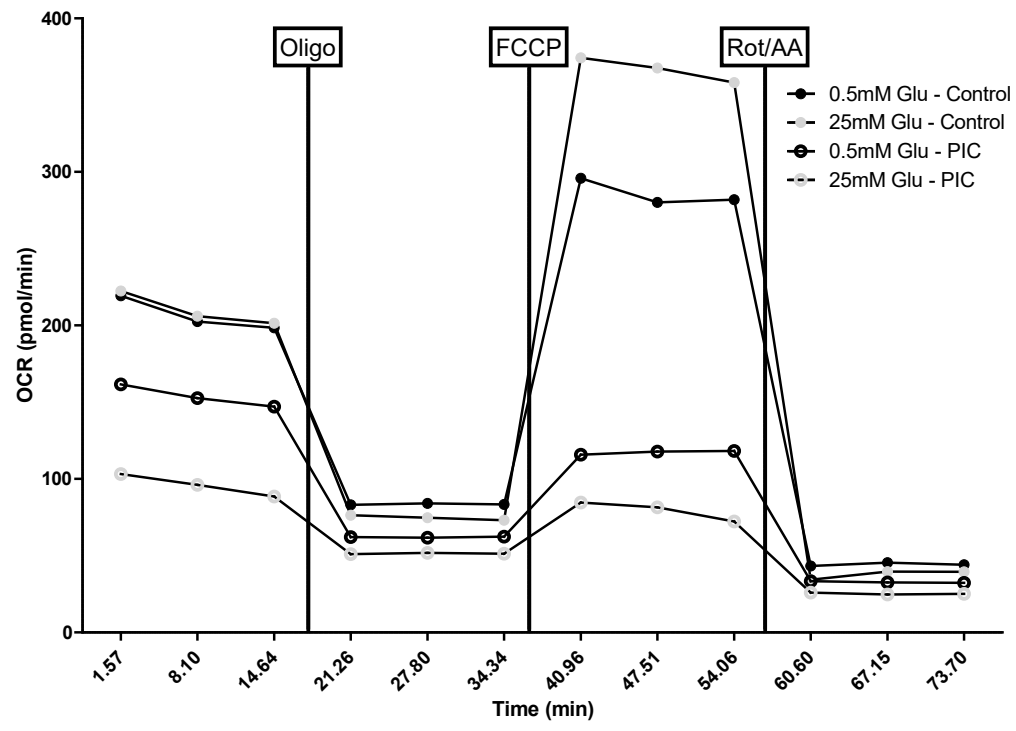
Reagent or Resource				
Antibodies	Supplier	Catalog Number	Additional Information	
			Isotype	Host
SDHB Monoclonal Antibody	Abcam	ab14714	IgG2a	Mouse
SOD2 (D3X8F) XP® monoclonal antibody	Cell Signalling Technology	13141	IgG	Rabbit
IRF3 Polyclonal Antibody	ThermoFisher	PA5-20086	IgG	Rabbit
Phospho-IRF3 (Ser385) Polyclonal Antibody	ThermoFisher	PA5-38285	IgG	Rabbit
IRF7 Polyclonal Antibody	ThermoFisher	PA5-79519	IgG	Rabbit
Phospho-IRF7 (Ser477) Polyclonal Antibody	ThermoFisher	PA5-64834	IgG	Rabbit
NDUFB8 Monoclonal Antibody (Complex I)	ThermoFisher	459210	IgG1, kappa	Mouse
UQCRC2 Polyclonal Antibody (Complex III)	ThermoFisher	PA5-30204	IgG	Rabbit
COX4/Complex IV Monoclonal Antibody	ThermoFisher	A21348	IgG2a, kappa	Mouse
GPX4 Polyclonal Antibody	ThermoFisher	PA5-79321	IgG	Rabbit
Ikbα Polyclonal Antibody	ThermoFisher	PA5-22120	IgG	Rabbit
Anti-Mouse IgG HRP-linked Secondary Antibody	Abcam	ab6789	IgG	Goat
Anti-Rabbit IgG, HRP-linked Secondary Antibody	Cell Signalling Technology	7074	IgG	Goat
Cell Culture	Supplier	Catalog Number		
DMEM, high glucose, pyruvate	Life Technologies	11995-073		
DMEM, no glucose, no glutamine, no pyruvate	Life Technologies	A1443001		
FBS	Life Technologies	12484-028		
Penicillin-Streptomycin (10,000 U/mL)	Life Technologies	15140-122		
Glucose Solution (200g/L)	Life Technologies	A2494001		
Sodium Pyruvate (100 mM)	Life Technologies	11360070		
L-Glutamine (200 mM)	Life Technologies	25030081		
Chemicals	Supplier	Catalog Number		

Lipopolysaccharide	Invivogen	tIrl-eklps
Poly(I:C), High molecular weight	Invivogen	tIrl-pic-5
MitoTempo	Sigma	SML0737
N-Acetyl-L-cysteine	Sigma	A9165
Antimycin A	Sigma	A8674
Rotenone	Sigma	R8875
Oligomycin	Sigma	O4876
FCCP	Sigma	C2920
Potassium Cyanide	Sigma	60178
2-deoxyglucose	Sigma	D6134
S3QEL	Cedarlane	5735/10
Commerical Kits	Supplier	Catalog Number
Mouse IL-1 beta/IL-1F2 DuoSet ELISA Kit	R&D Systems	DY401
Mouse IL-6 DuoSet ELISA Kit	R&D Systems	DY406
Mouse IL-10 DuoSet ELISA Kit	R&D Systems	DY417
Mouse TNF-alpha DuoSet ELISA Kit	R&D Systems	DY410
Mouse CXCL10/IP-10/CRG-2 DuoSet ELISA Kit	R&D Systems	DY466
IFN alpha/IFN beta 2-Plex Mouse ProcartaPlex Panel	Life Technologies	EPX020-22187-901
Hydrogen Peroxide Assay Kit (Cell-based)	Abcam	ab138874
Seahorse XFp Glycolytic Rate Assay Kit	Aglient	103346-100
Seahorse XFp Cell Mito Stress Test Kit	Aglient	103010-100
Reagents	Supplier	Catalog Number
Image-iT™ TMRM Reagent	Invitrogen	I34361
MitoSOX™ Red Mitochondrial Superoxide Indicator	Invitrogen	M36008
CellROX™ Orange Reagent	Invitrogen	C10443
Western Blot Accessories	Supplier	Catalog Number
Halt™ Protease and Phosphatase Inhibitor Cocktail	ThermoFisher	78440

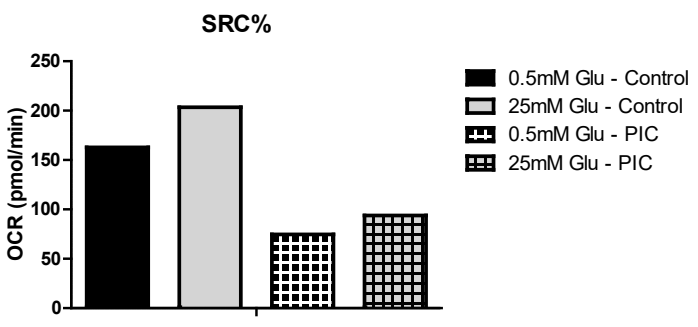
RIPA Lysis and Extraction Buffer	ThermoFisher	89900
DC™ Protein Assay Kit II	Bio-Rad	5000112
TGX Stain-Free™ FastCast™ Acrylamide Kit, 12%	Bio-Rad	1610185
Trans-Blot® Turbo™ RTA Mini PVDF Transfer Kit	Bio-Rad	1704272
Clarity™ Western ECL Substrate	Bio-Rad	1705060

Supplementary Figure S1

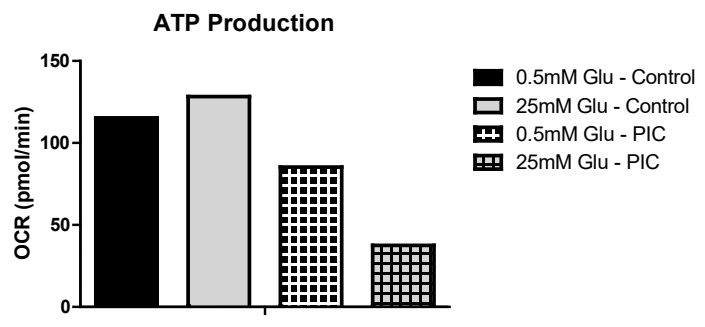
a



b

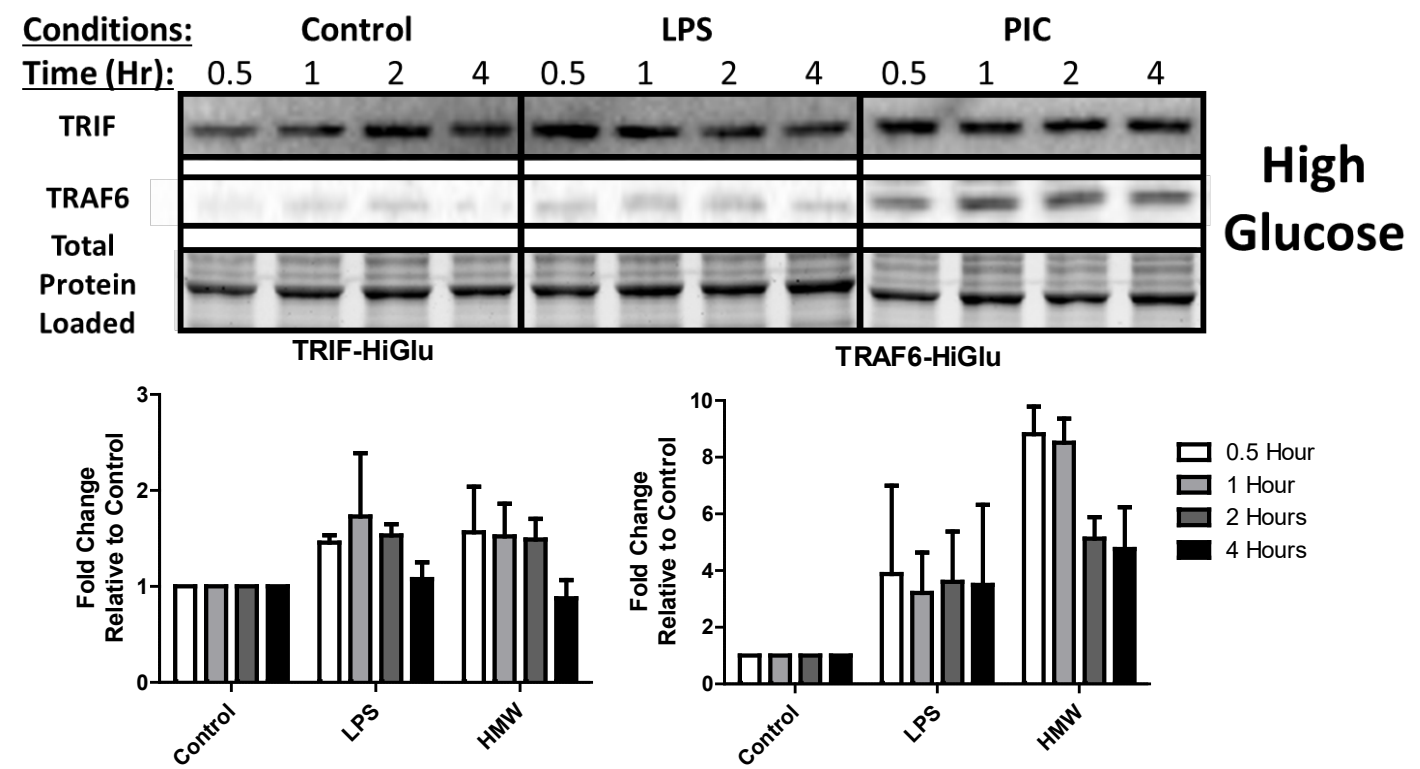


c

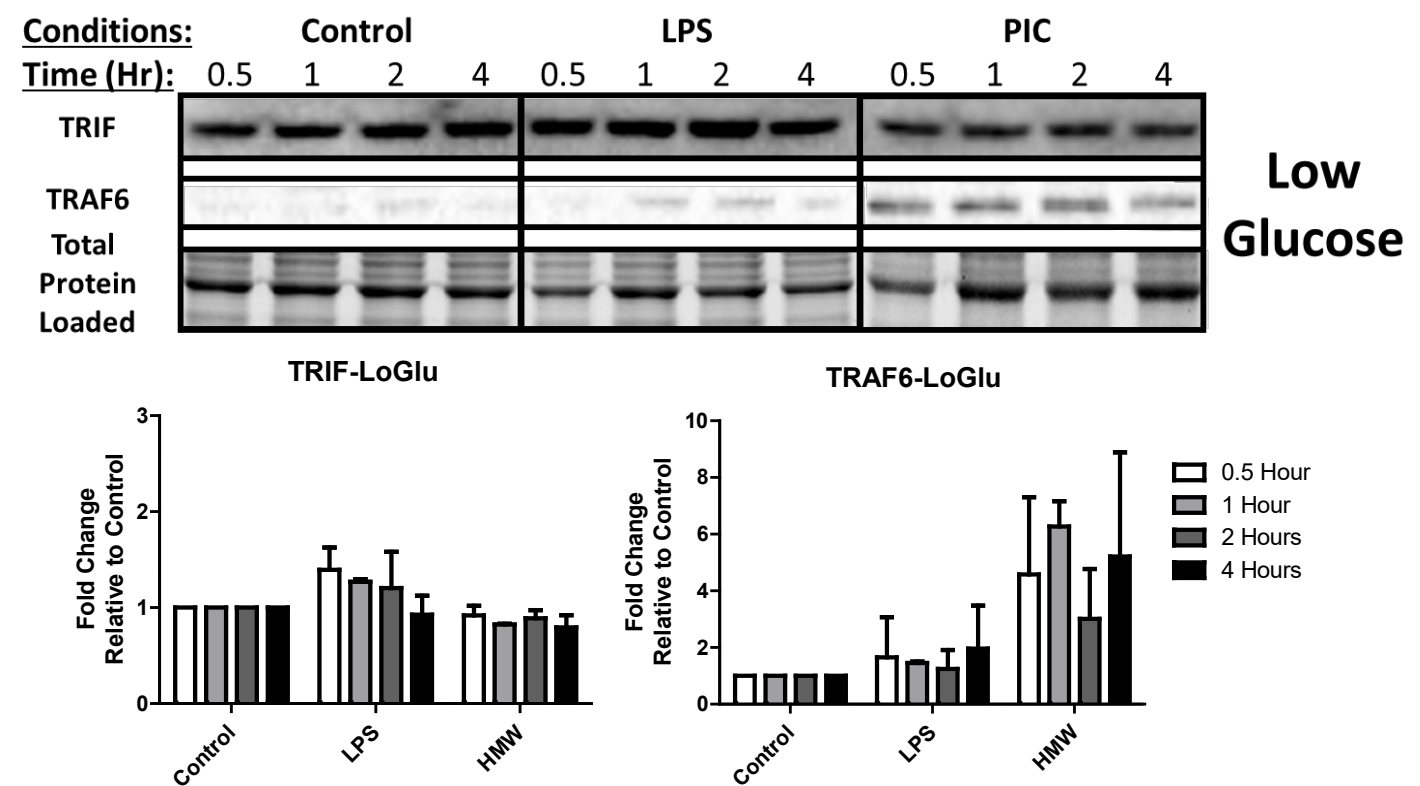


Supplementary Figure S2

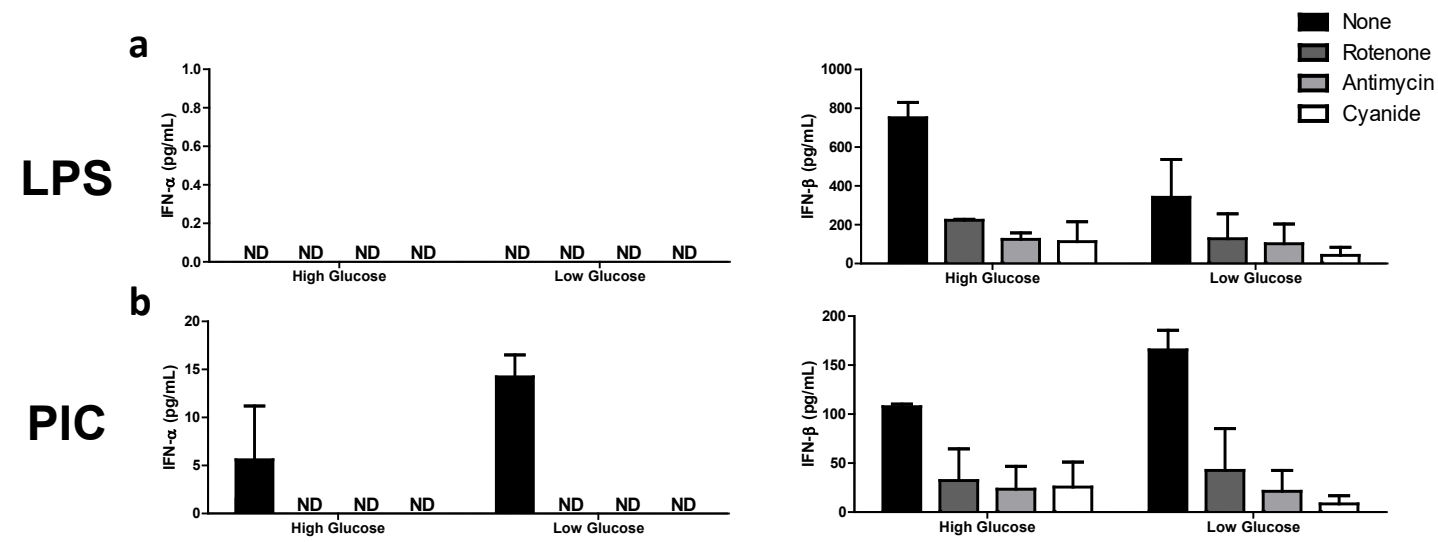
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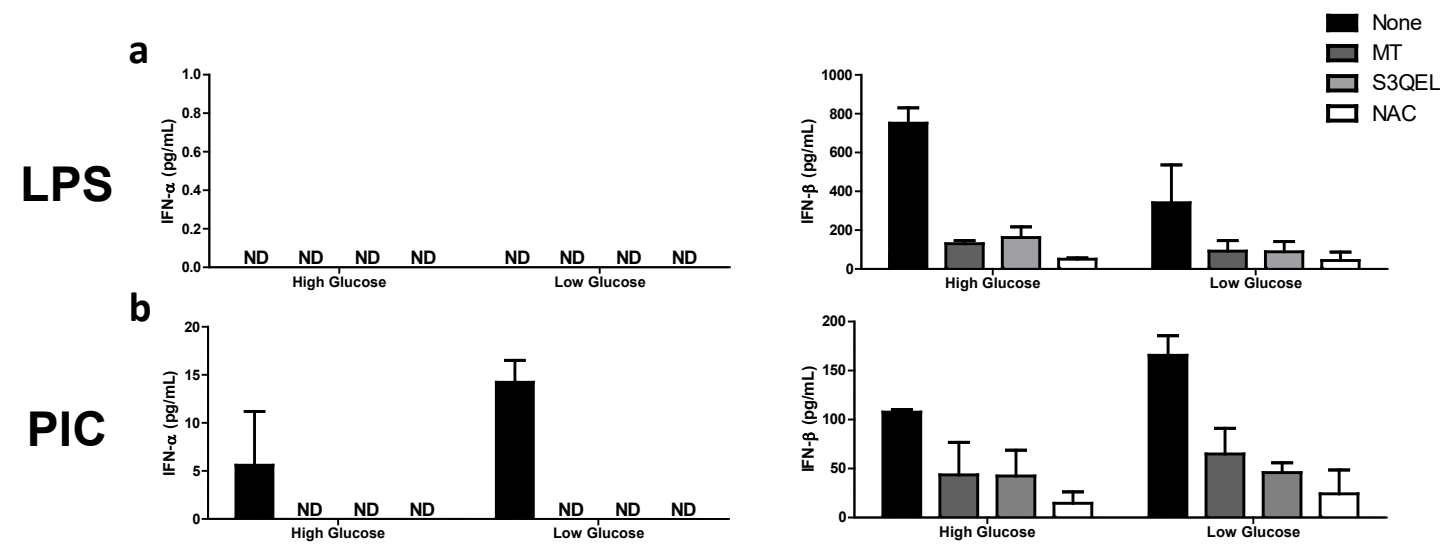
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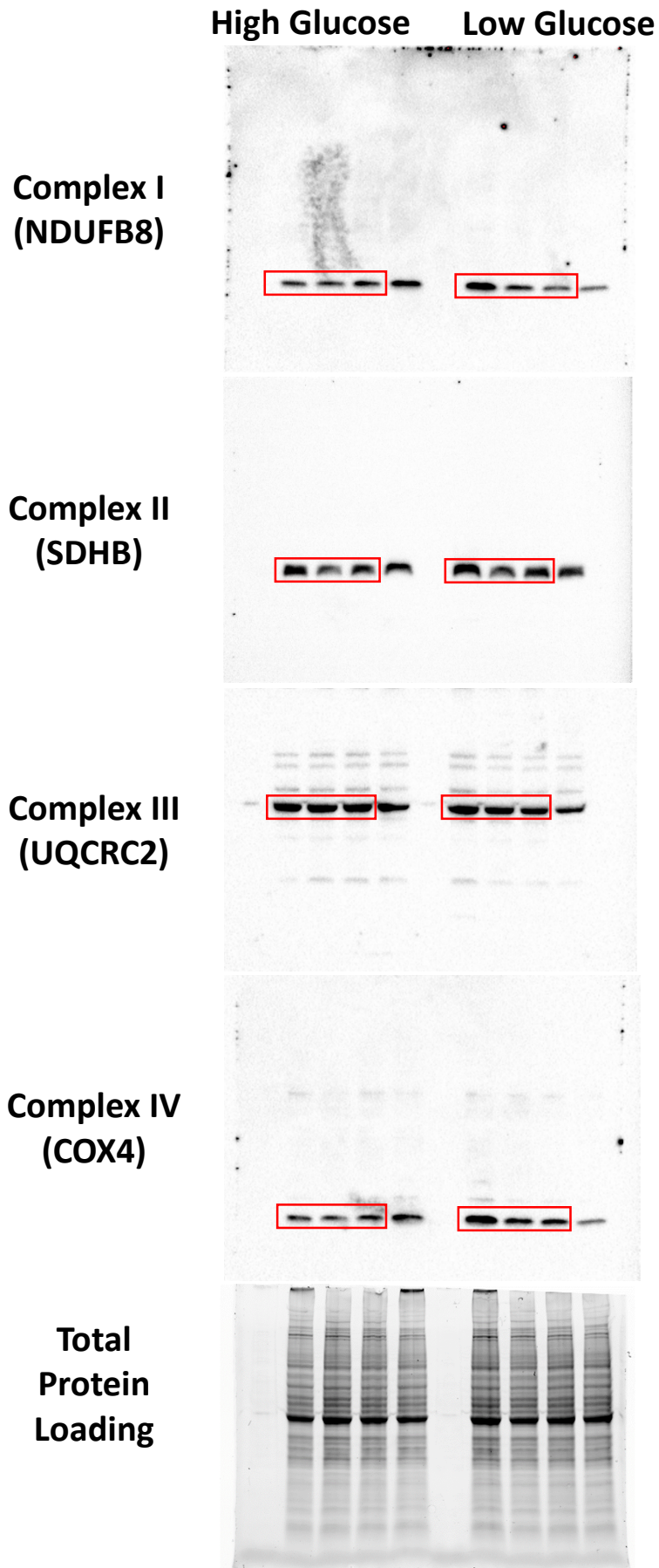
Supplementary Figure S3



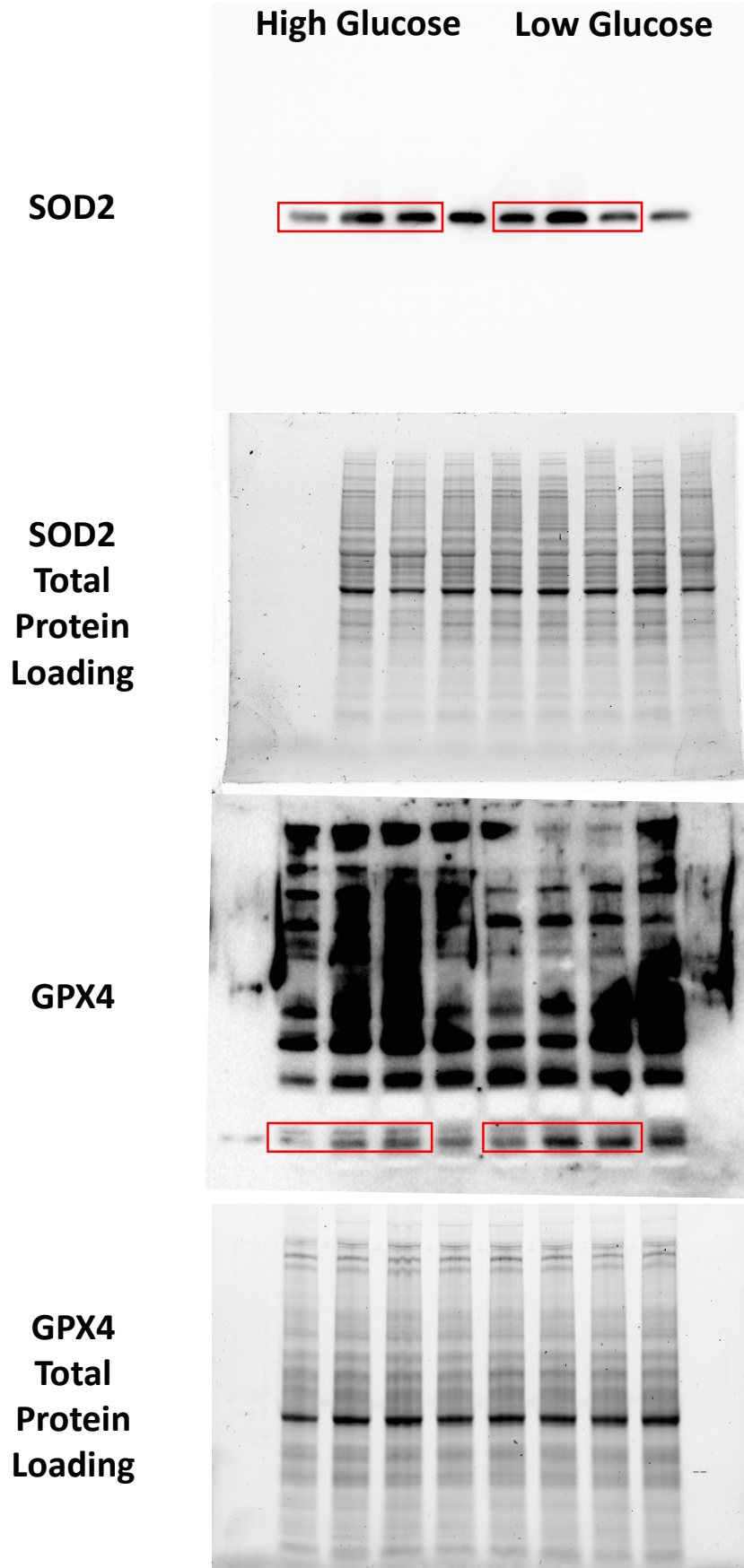
Supplementary Figure S4



Supplementary Figure S6 – Uncropped Images of Figure 5



Supplementary Figure S7 – Uncropped Images of Figure 7



Supplementary Figure S8 – Uncropped Images of Supplementary Figure S2

