Improving health and viability in mitochondrial disease through inhibition of cytosolic translation and autophagy

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Supplemental Files

Fig S1. Effects of RC inhibition, probucol, and nutrients on AMPK activation.

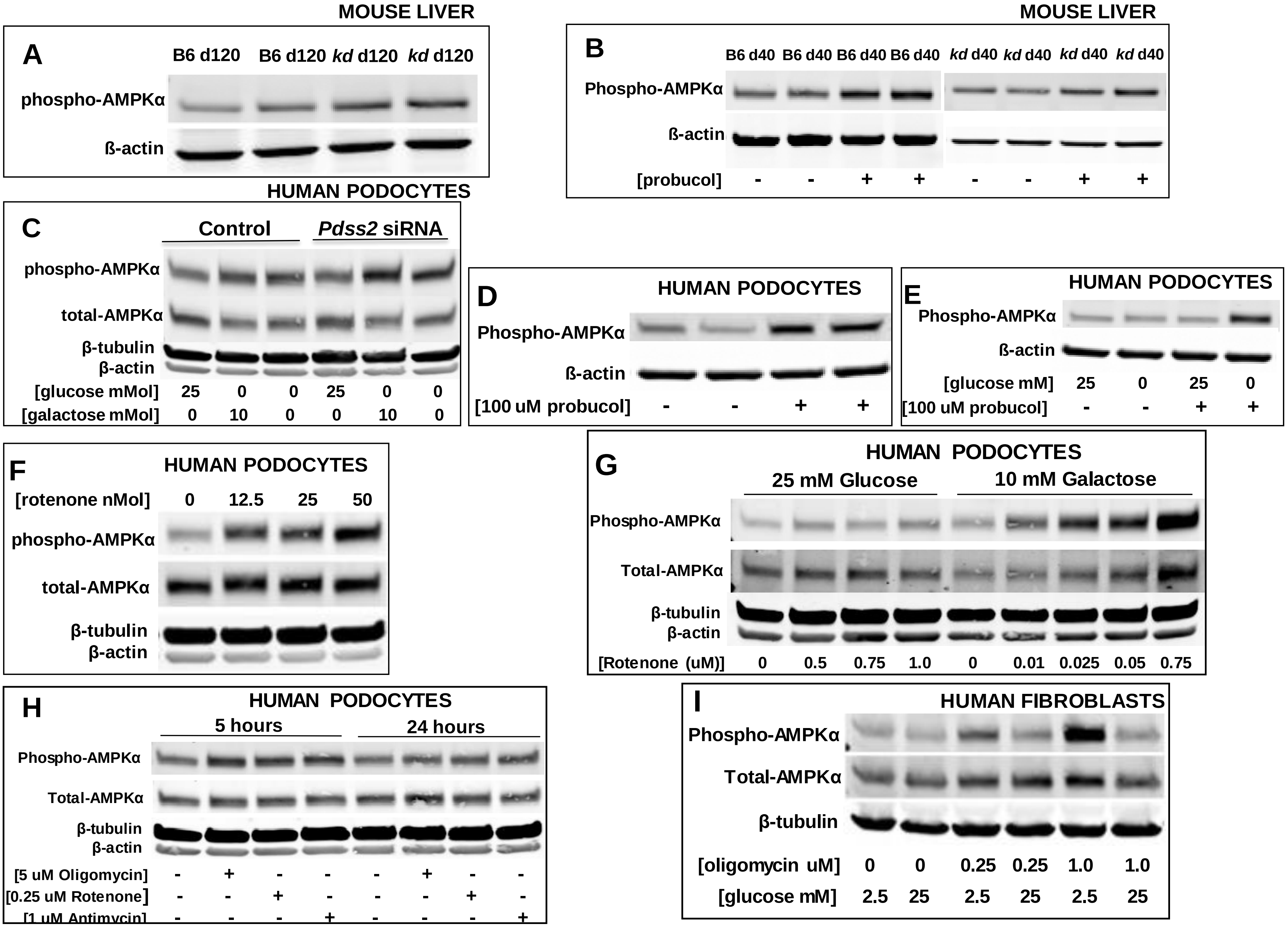


Table S1. Table of all lifespan results in *C. elegans gas-1(fc21)* worms treated with probucol, rapamycin, and cycloheximide

STUDY DRUG	Concentration	Buffer	Worm Stage at Treatment Initiation	Worm # Studied	Experimental Replicate #	Study Date	Lifespan Percent Change Relative to Untreated *gas-1* Median	p value vs *gas-1*	p value vs N2	NOTES
Probucol	5 mM	2.7% DMSO	Dev	60	1	Oct-12	NA	NA	NA	TOXIC DMSO
	5 mM	2.7% DMSO	YA	60	1	Oct-12	NA	NA	NA	TOXIC DMSO
	500 uM	Ethanol	Dev	60	1	Dec-12	85	< 0.0001	< 0.0001	
	500 uM	Ethanol	YA	60	1	Dec-12	85	0.0869	< 0.0001	
	50 uM	Ethanol	Dev	60	1	Mar-13	117	0.0034	0.3994	
	50 uM	Ethanol	YA	60	1	Mar-13	108	0.0354	0.0961	
	5 uM	Ethanol	Dev	60	1	Mar-13	133	< 0.0001	0.4285	
	5 uM	Ethanol	YA	60	1	Mar-13	108	0.0003	0.894	
Rapamycin	2.5 uM	Ethanol	Dev	56	1	Dec-12	85	< 0.0001	< 0.0001	
	2.5 uM	Ethanol	YA	60	1	Dec-12	85	0.0025	< 0.0001	
	250 nM	Ethanol	Dev	56	1	Dec-12	85	0.0157	< 0.0001	
	250 nM	Ethanol	YA	56	1	Dec-12	85	0.0313	< 0.0001	
	25 nM	Ethanol	Dev	56	1	Dec-12	100	0.5081	< 0.0001	
	25 nM	Ethanol	YA	60	1	Dec-12	100	0.648	< 0.0001	
	25 nM	Ethanol	Dev	59	2	Mar-13	100	0.114	0.0419	
	25 nM	Ethanol	YA	58	2	Mar-13	92	0.9537	0.0005	
	2.5 nM	Ethanol	Dev	54	1	Mar-13	117	0.0151	0.133	
	2.5 nM	Ethanol	YA	57	1	Mar-13	107	0.0066	0.1154	
	2.5 nM	Ethanol	Dev	60	2	Aug-14	100	0.512	< 0.0001	
	2.5 nM	Ethanol	YA	60	2	Aug-14	100	0.0689	< 0.0001	
	2.5 nM	Ethanol	YA	60	3	Sep-14	100	0.7236	< 0.0001	
	250 pM	Ethanol	Dev	60	1	Sep-14	82	0.0096	< 0.0001	
Cycloheximide	2.5 uM	Water	Dev	60	1	Jul-14	82	0.0006	< 0.0001	
	2.5 uM	Water	YA	60	1	Aug-14	100	0.1294	< 0.0001	
	250 nM	Water	YA	60	1	Aug-14	100	0.8881	< 0.0001	
	250 nM	Water	YA	60	2	Sep-14	100	0.4995	< 0.0001	
	25 nM	Water	YA	60	1	Aug-14	118	0.3689	< 0.0001	
	25 nM	Water	Dev	60	1	Sep-14	100	0.7099	< 0.0001	

*Dev, Development; YA, young adult day 1

Fig S2. Probucol effects on lifespan of *C. elegans gas-1(fc21)* CI deficient worms

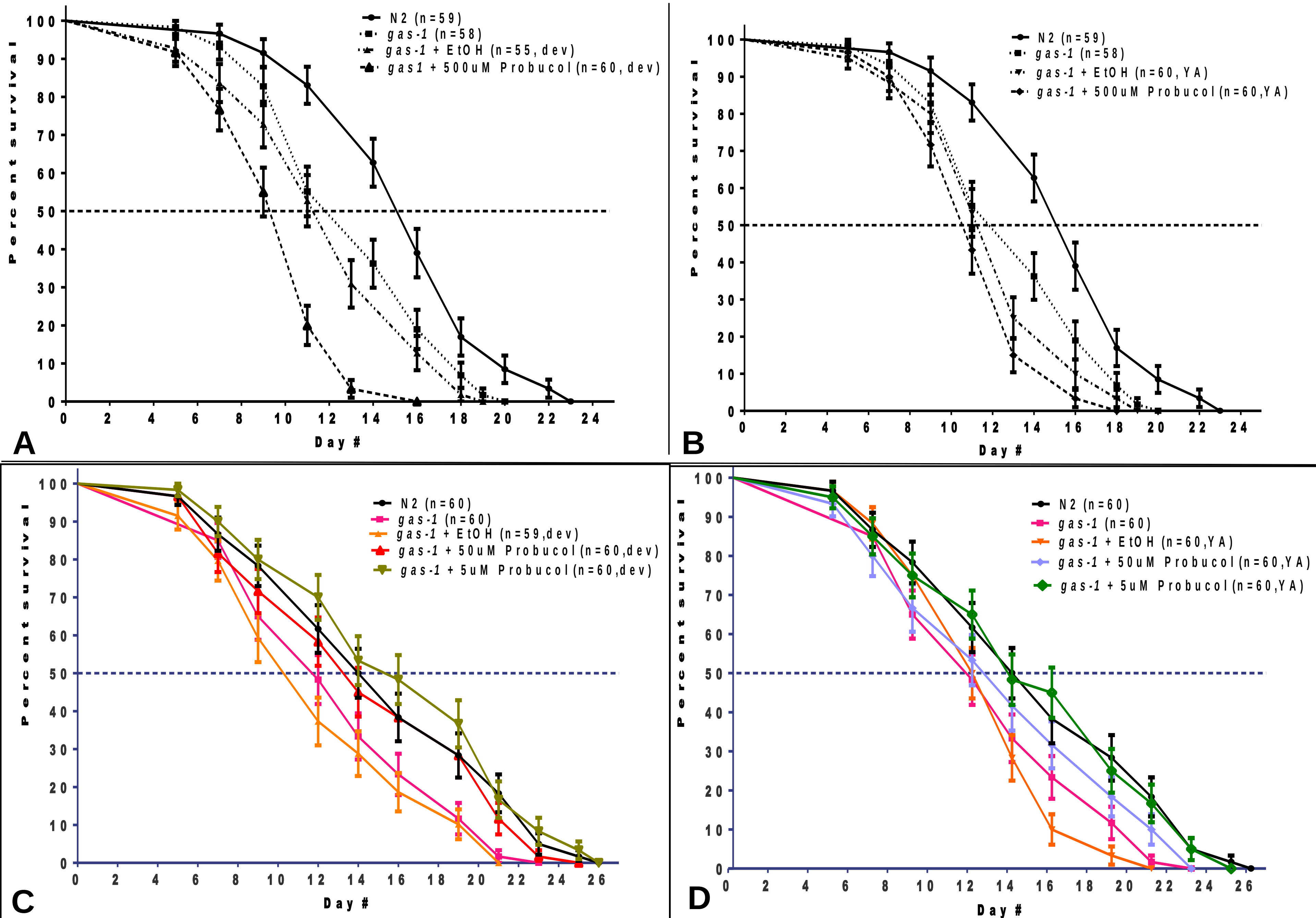


Fig S3. Rapamycin effects on lifespan of *C. elegans gas-1(fc21)* CI deficient worms

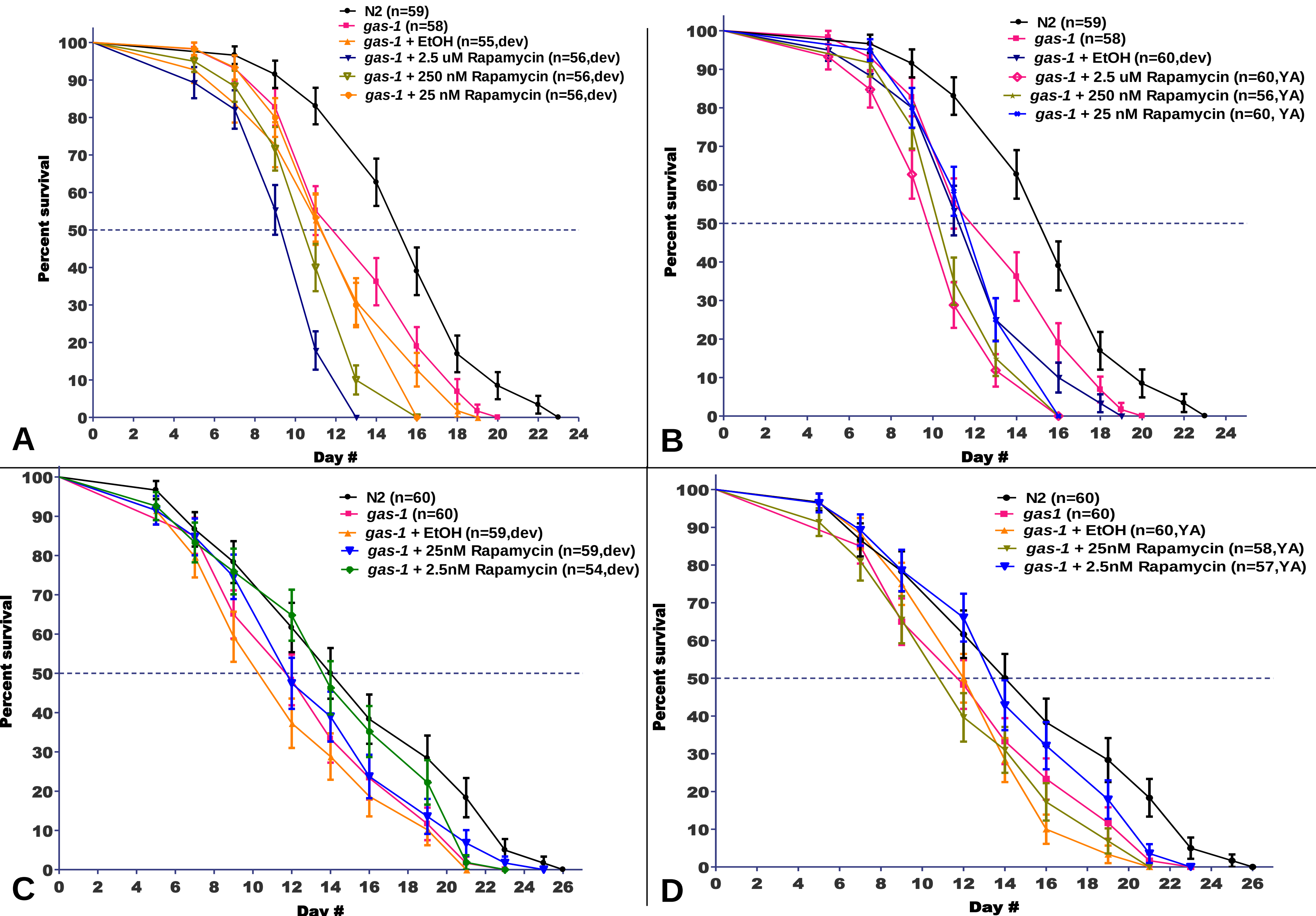


Fig S4. Cycloheximide effects on *C. elegans* lifespan of *gas-1(fc21)* CI deficient worms

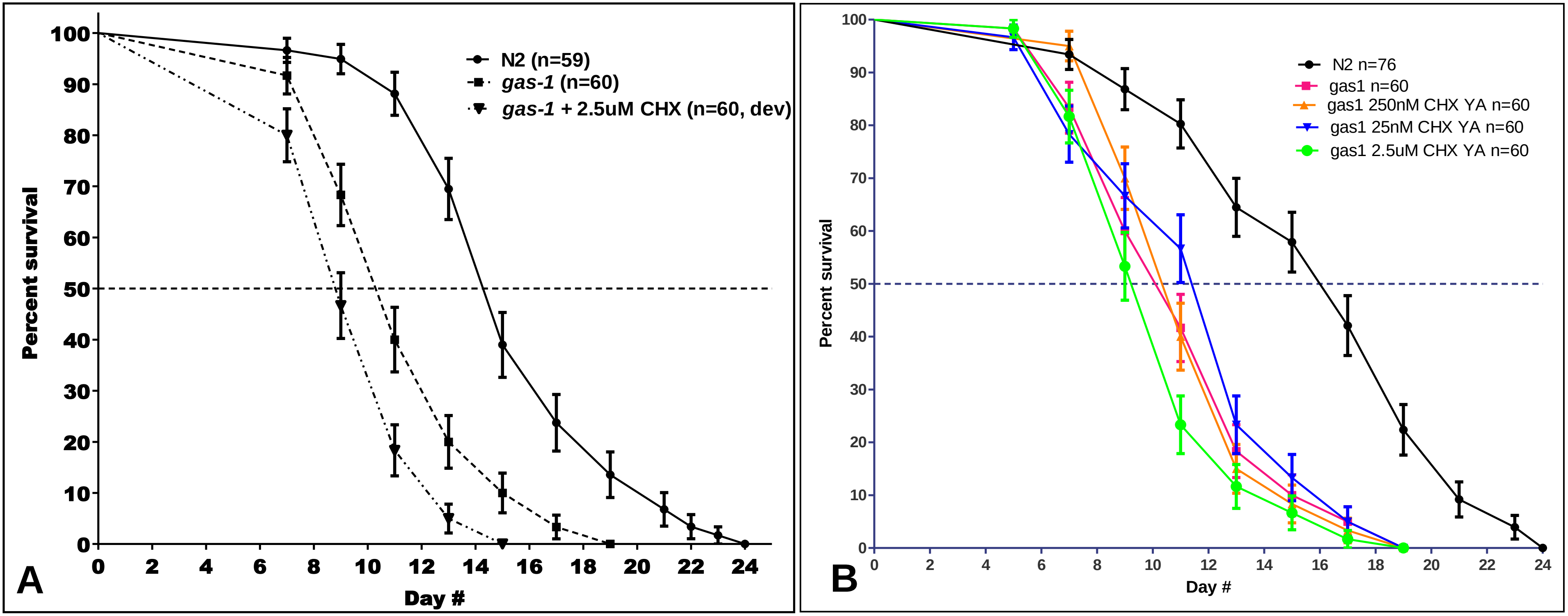


Fig S5. Cytosolic translation activity effects in human podocytes.

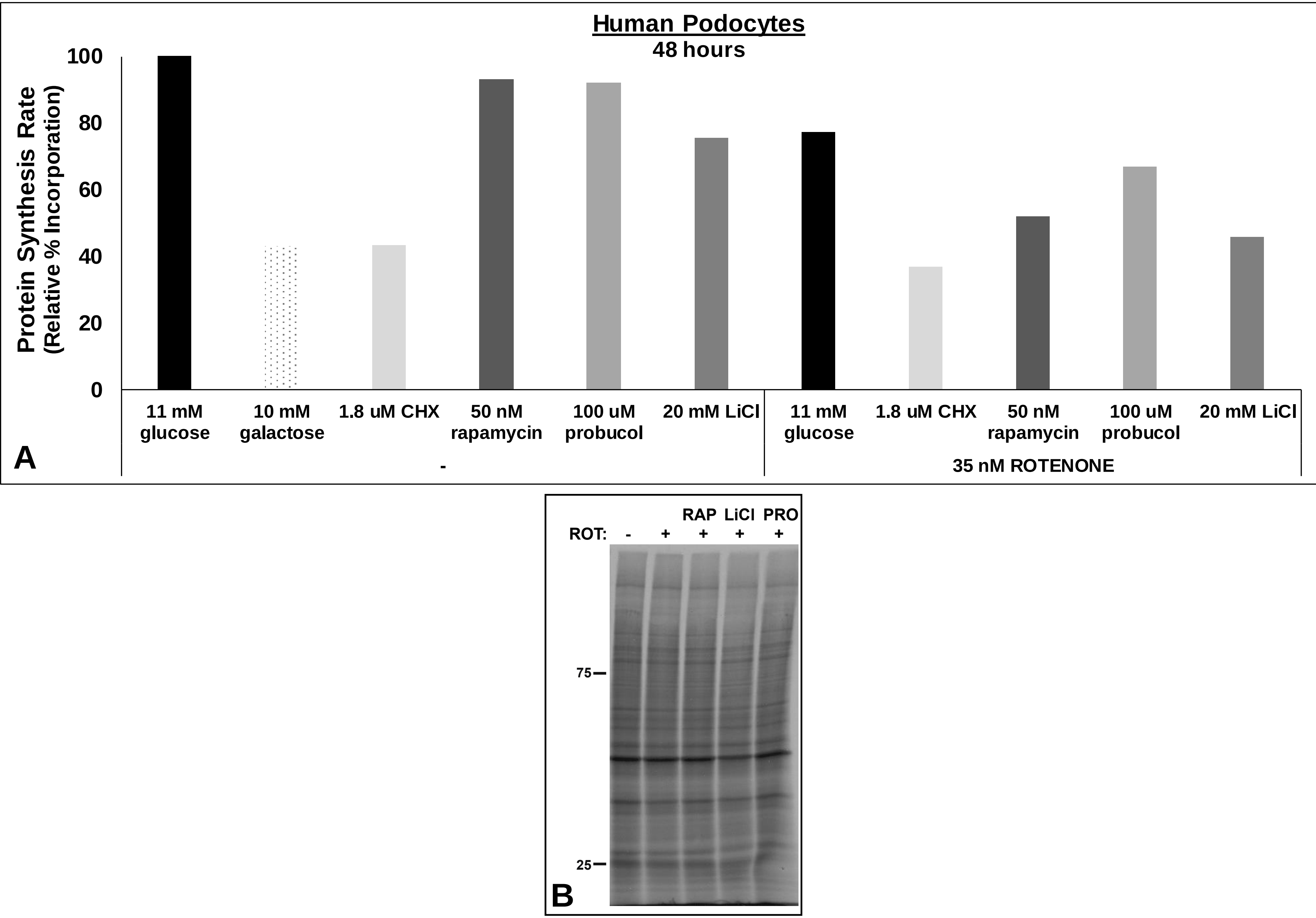


Fig S6. Cycloheximide effects in CIII (Antimycin A) or CV (oligomycin) inhibition & in CI-III (*FBXL4*) disease

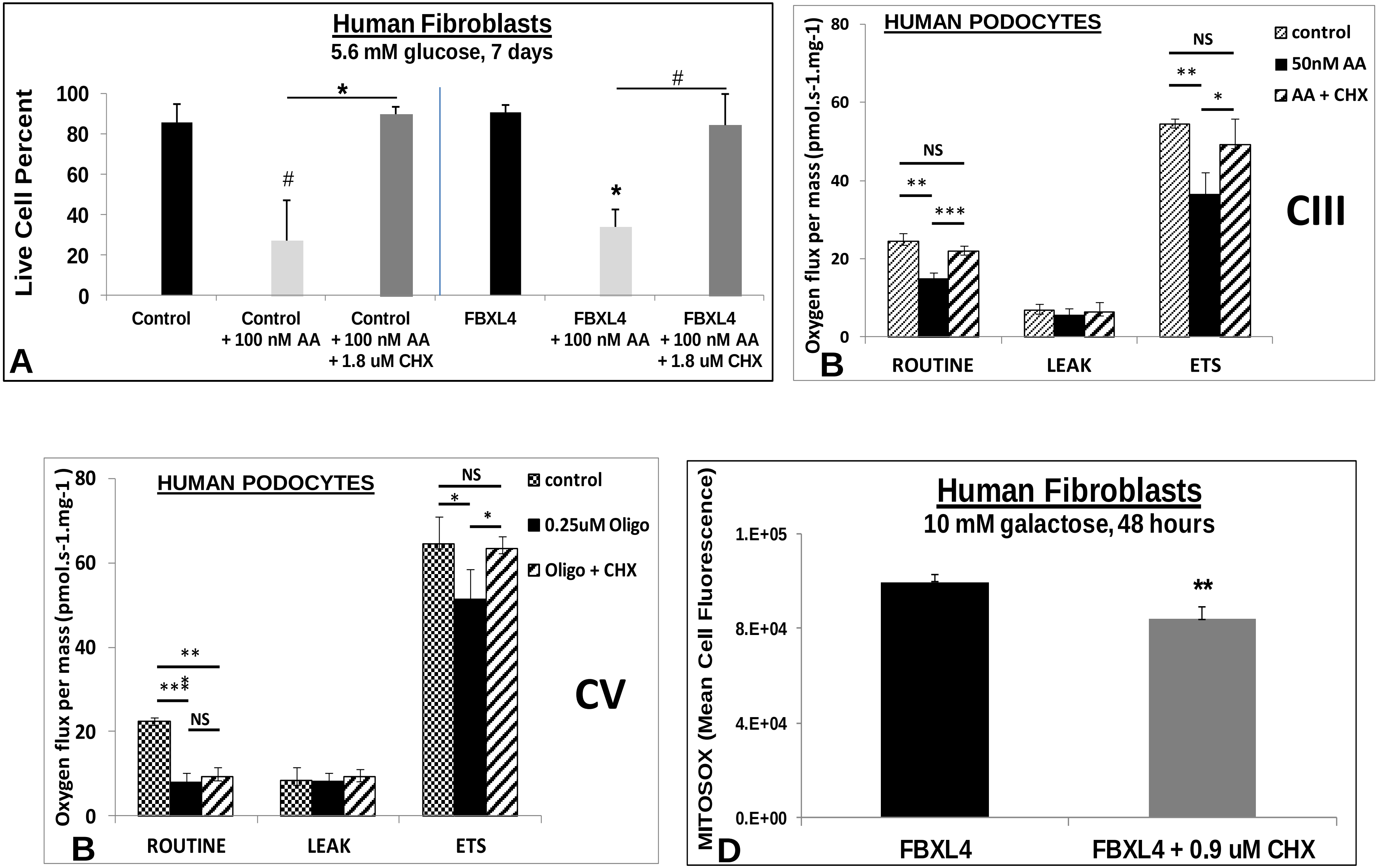


Fig S7. Cycloheximide effects on mitochondrial physiology in human cells with pharmacologic or genetic based RC deficiencies.

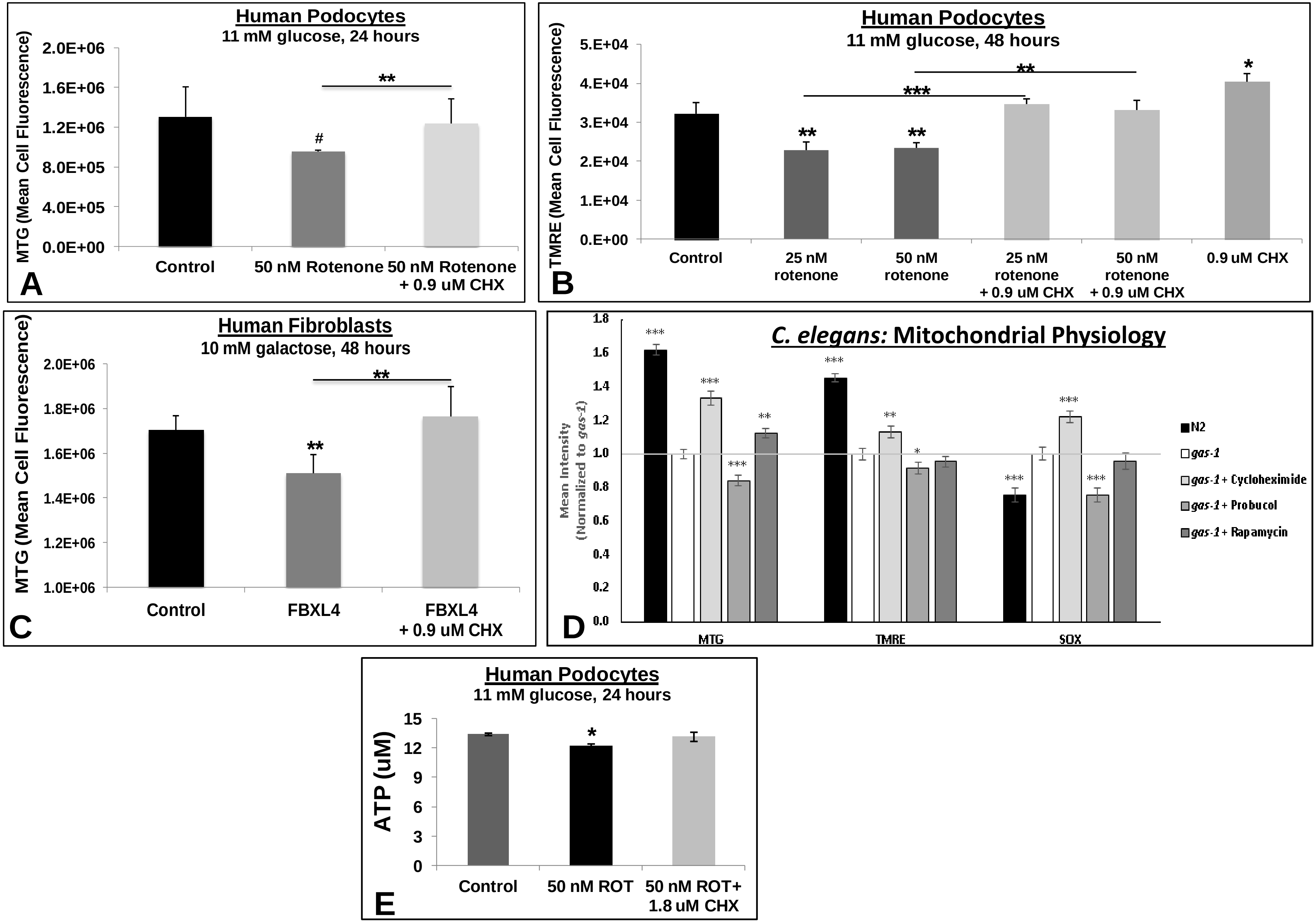


Fig S8. Apoptosis is induced only by high-dose, short-duration rotenone

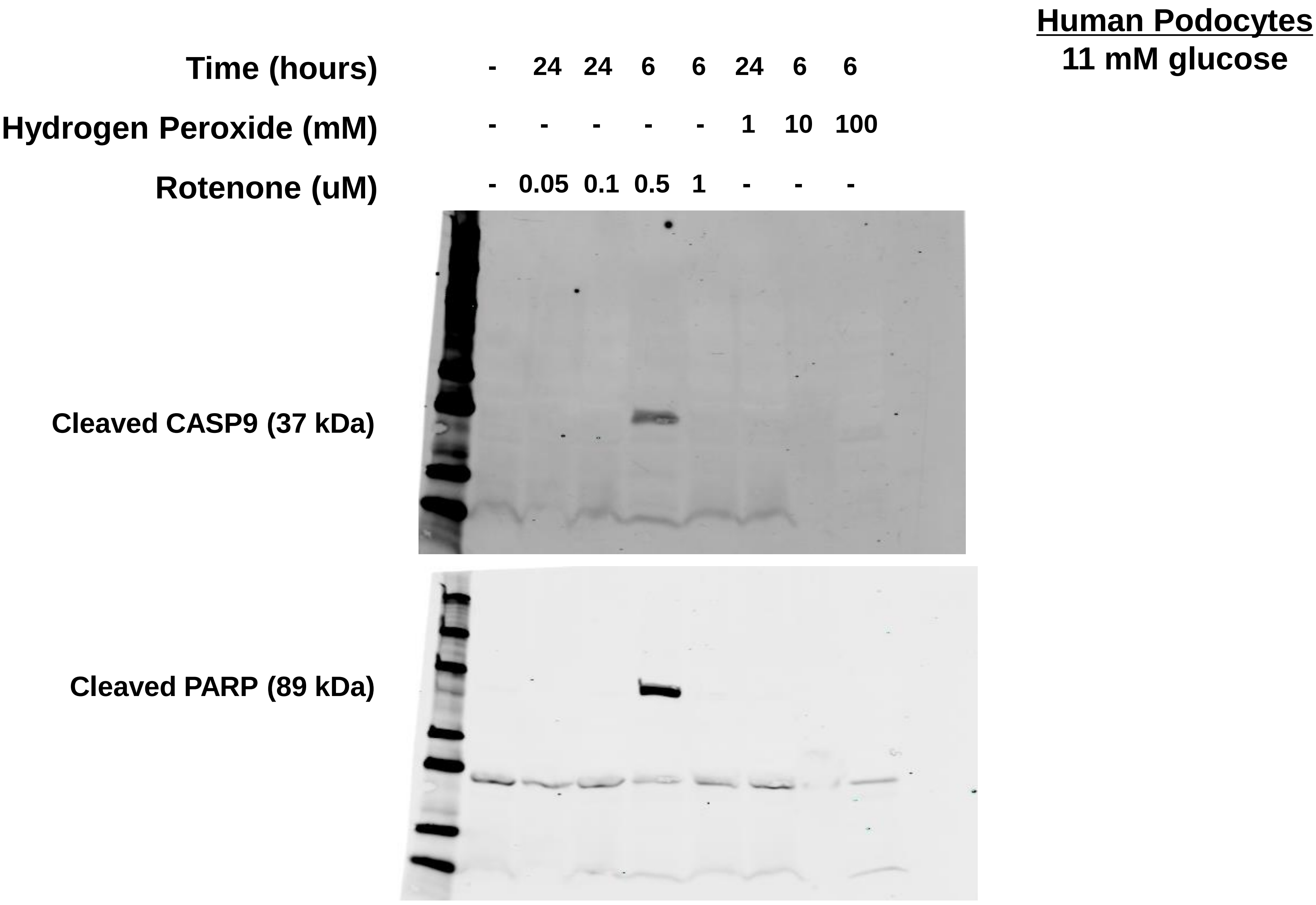


Fig S9. Mitophagy is activated in *FBXL4* deficient patient fibroblasts with RC complex I-III dysfunction.

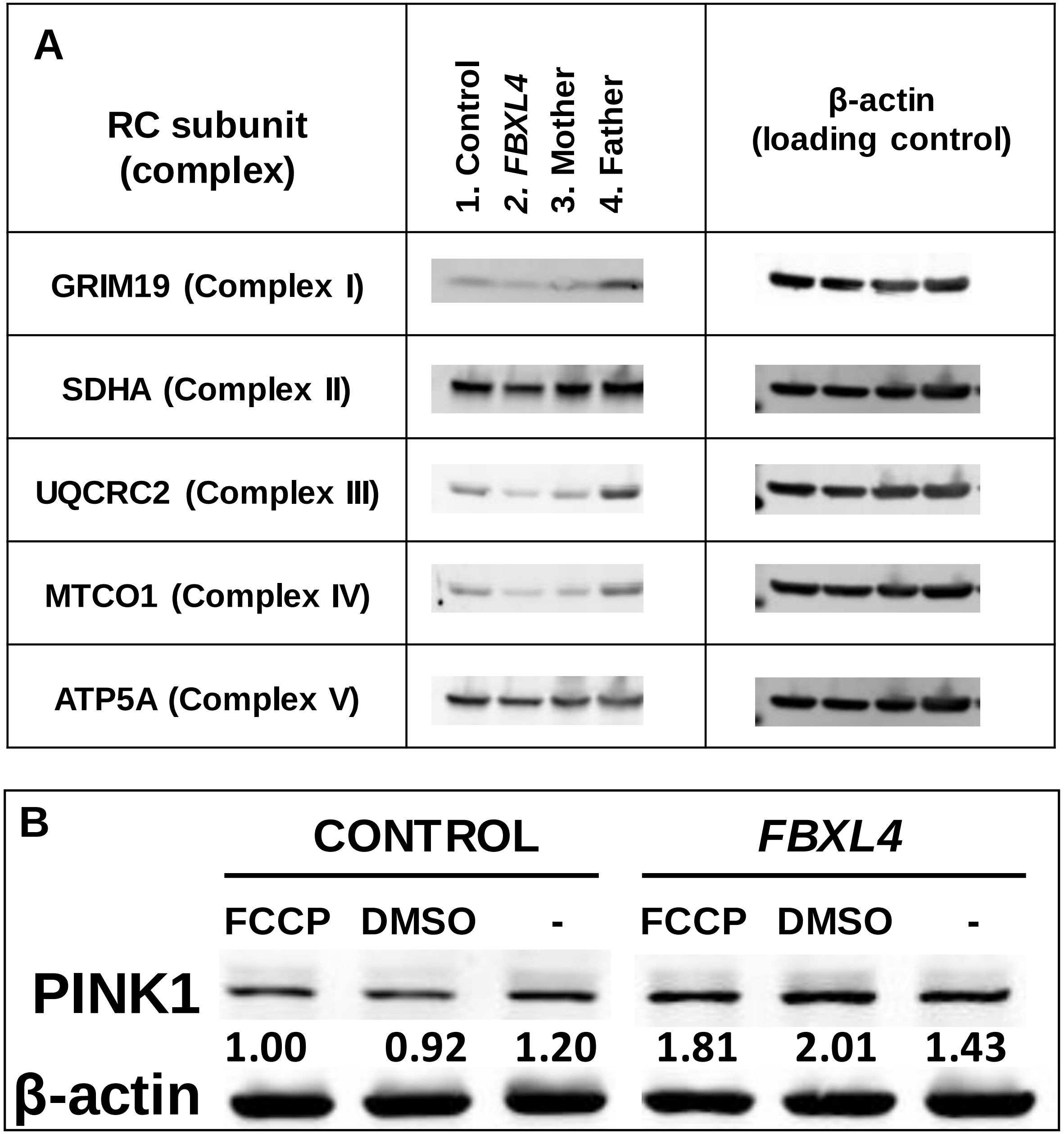


Fig S10. Chloramphenicol effects on cell viability with RC inhibition and therapies

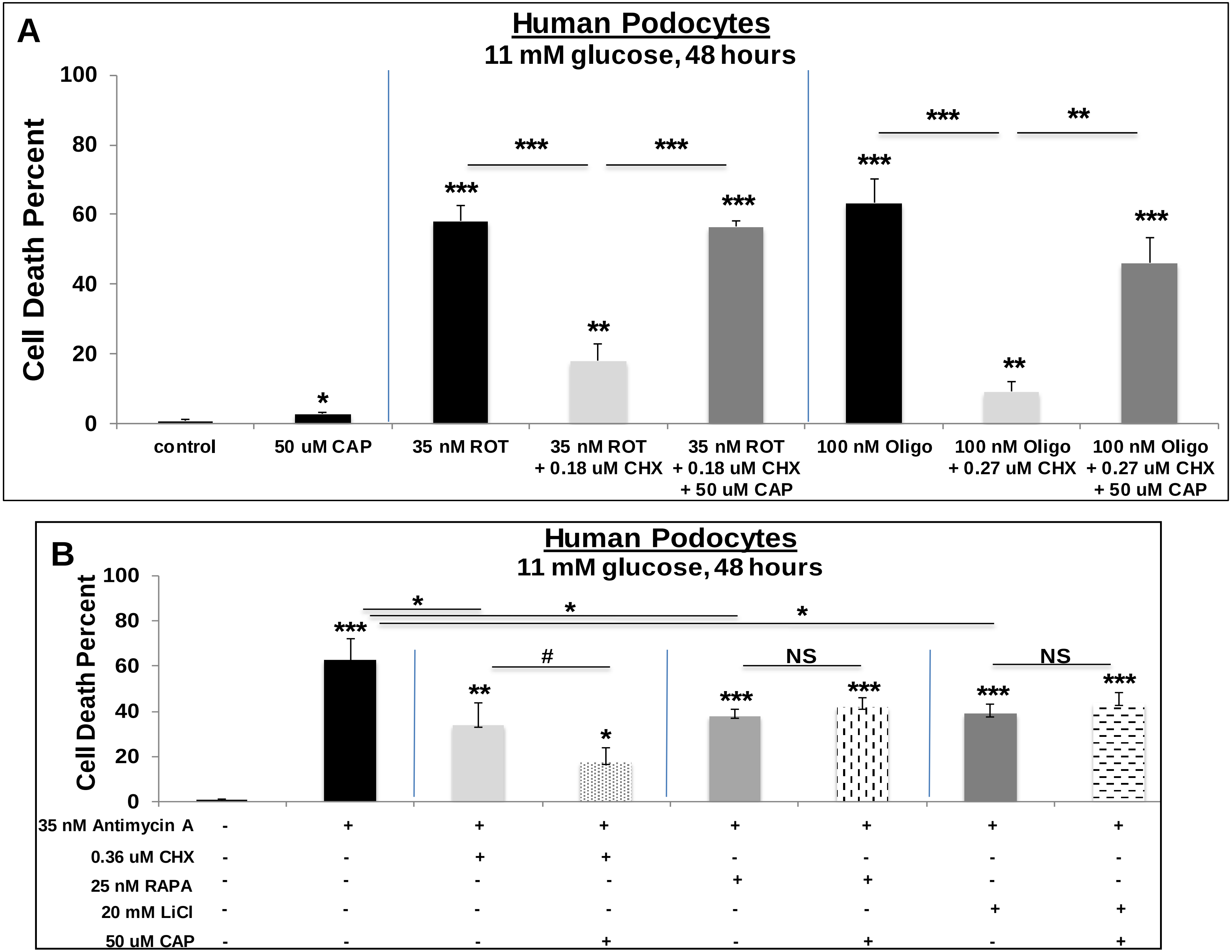


Fig S11. Long-term (24-48 hours) mitochondrial RC inhibition activates the ER stress response (GRP78 and PERK) in human podocytes and is prevented by cycloheximide.

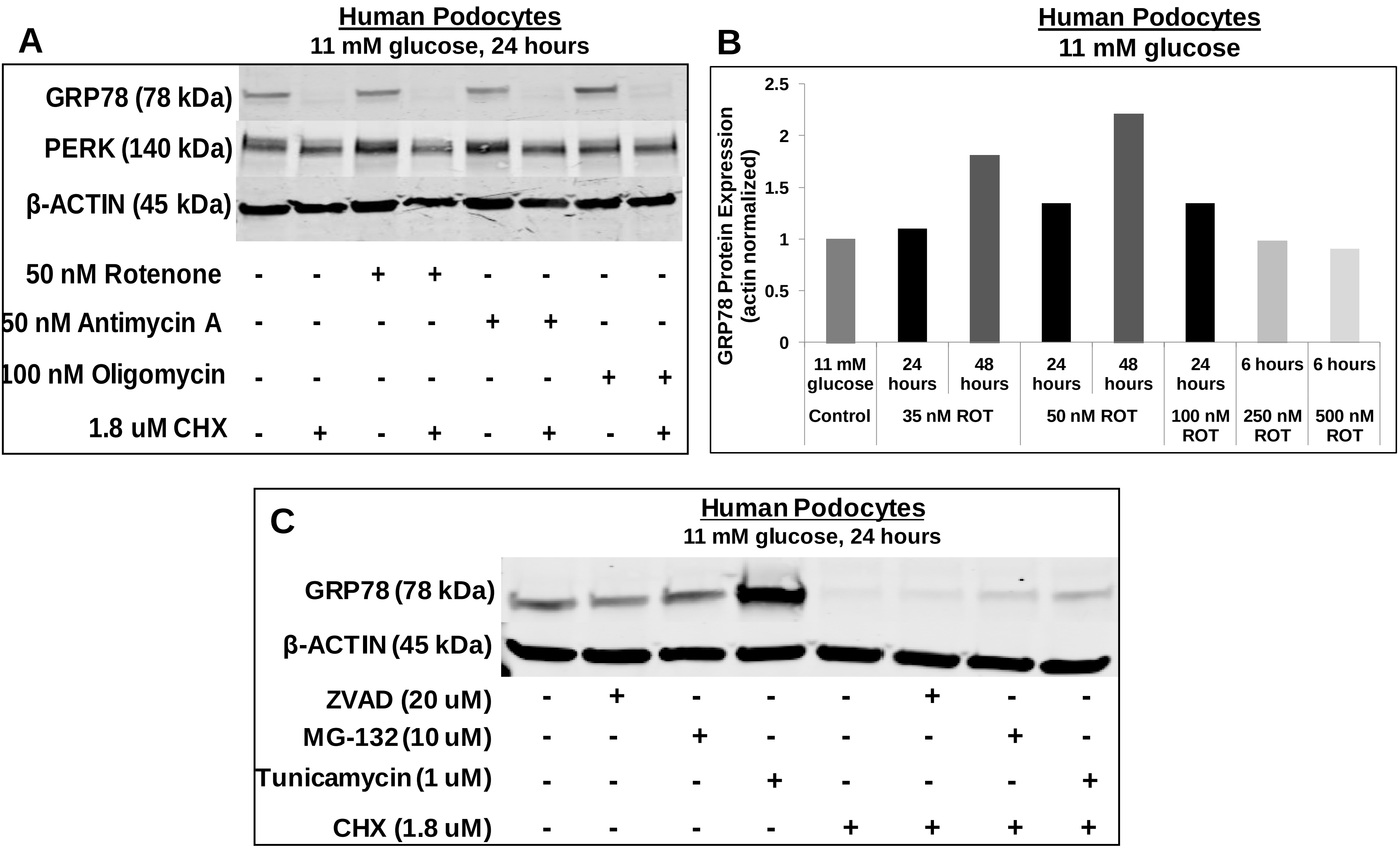


Fig S12. Short-term mitochondrial RC inhibition induces the ER stress response (PERK and phosphor-EIF2α) in human podocytes.

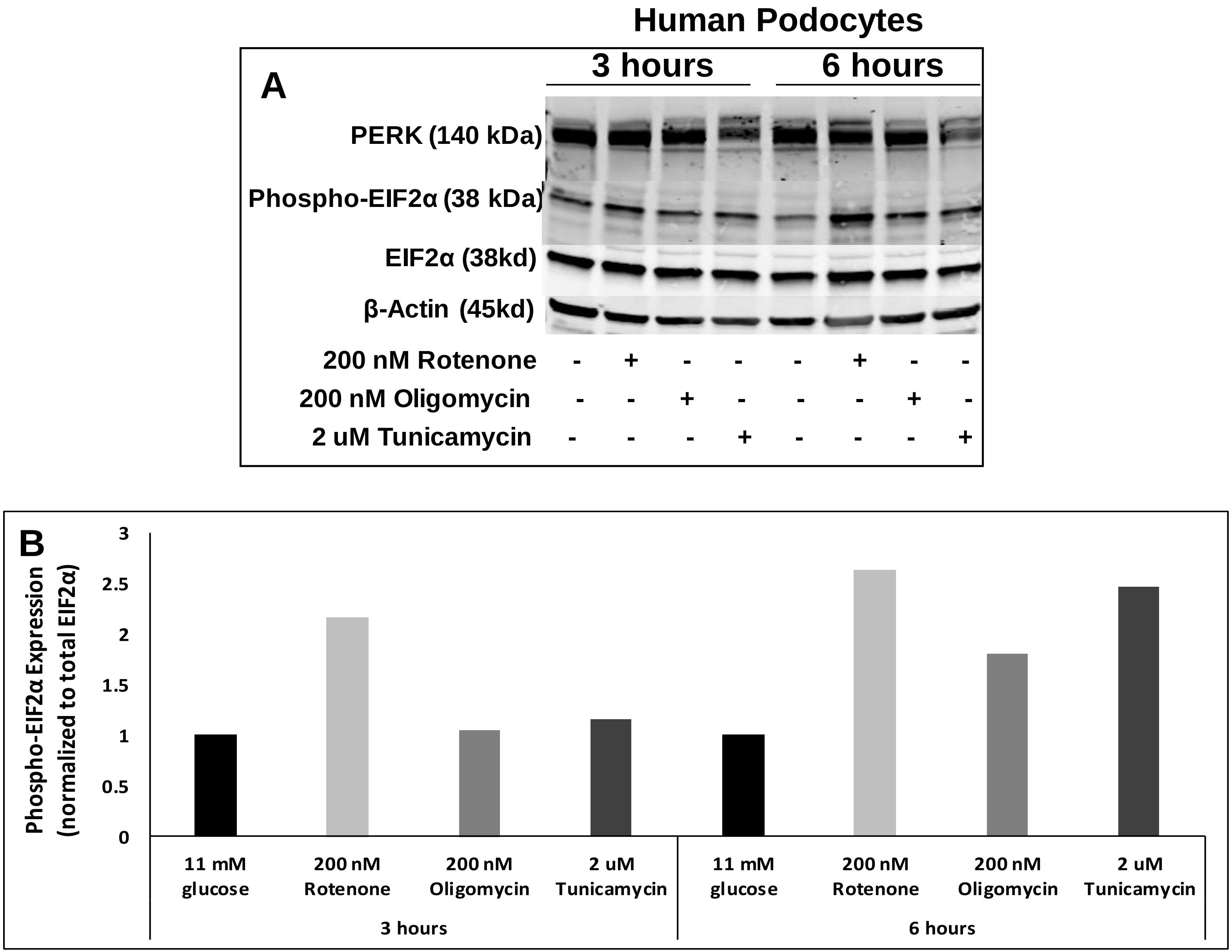


Fig S13. Gene expression profiling in *C. elegans gas-1(fc21)* adult worms following 24 treatment with (A) 5 uM probucol and (B-C) 2.5 uM cycloheximide

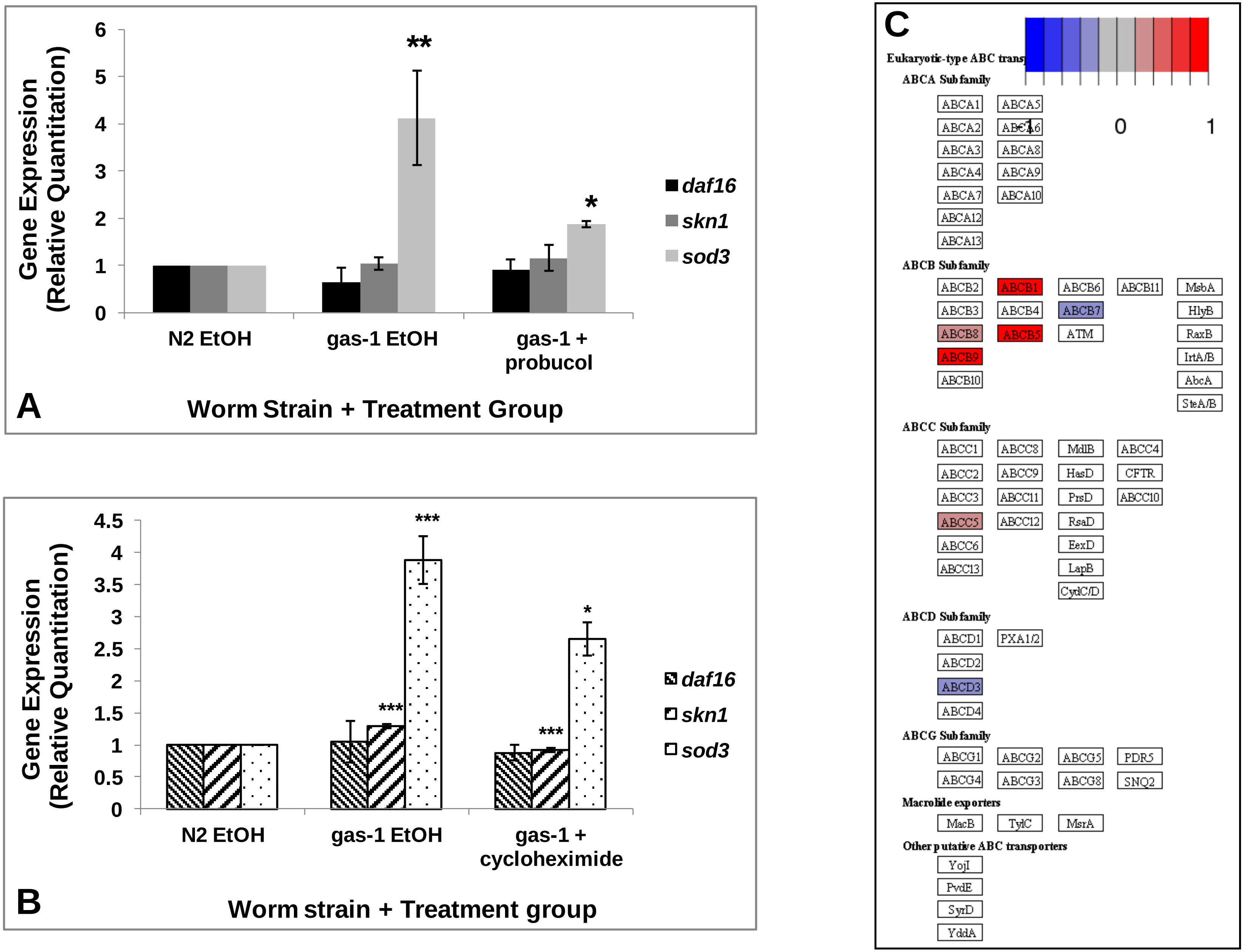


Fig S14. Pathway-level RNAseq transcriptome effects of treating *C. elegans gas-1(fc21)* mutant adult worms with cycloheximide, probucol or rapamycin.

A Cycloheximide effects in *gas-1(fc21)*

UPREGULATED by CHX in *gas-1*	KEGG Pathway ID	# Genes Total	#Genes Measured	Enrichment Score (ES)	P Value	FDR	KEGG Pathway Name
1	cel02010	16	16	2.03	7.85E-05	0.71%	ABC transporters
2	cel03010	142	115	1.61	9.49E-04	4.27%	Ribosome
3	cel04350	34	32	1.5	1.86E-03	5.57%	TGF-beta signaling pathway
4	cel04310	58	56	1.26	6.57E-03	14.78%	Wnt signaling pathway
5	cel04141	124	119	0.81	5.56E-02	92.23%	Protein processing in endoplasmic reticulum
6	cel03030	32	30	0.76	6.75E-02	92.23%	DNA replication
7	cel00350	22	21	0.73	7.67E-02	92.23%	Tyrosine metabolism
8	cel03040	106	102	0.65	1.05E-01	92.23%	Spliceosome
9	cel03060	21	20	0.63	1.12E-01	92.23%	Protein export
10	cel03008	79	58	0.61	1.17E-01	92.23%	Ribosome biogenesis in eukaryotes
11	cel03018	44	43	0.54	1.48E-01	92.23%	RNA degradation
12	cel00970	113	32	0.48	1.76E-01	92.23%	Aminoacyl-tRNA biosynthesis
13	cel00590	13	13	0.45	1.90E-01	92.23%	Arachidonic acid metabolism
14	cel00600	25	24	0.44	1.96E-01	92.23%	Sphingolipid metabolism
15	cel04145	56	55	0.38	2.28E-01	92.23%	Phagosome

DOWNREGULATED by CHX in *gas-1*	KEGG Pathway ID	# Genes Total	#Genes Measured	Enrichment Score (ES)	P Value	FDR	KEGG Pathway Name
1	cel01040	15	14	-1.78	4.03E-04	3.62%	Biosynthesis of unsaturated fatty acids
2	cel00190	111	98	-1.14	1.31E-02	59.02%	Oxidative phosphorylation
3	cel00520	32	30	-1.06	1.99E-02	59.66%	Amino sugar and nucleotide sugar metabolism
4	cel04146	60	59	-0.95	3.09E-02	59.66%	Peroxisome
5	cel00450	11	10	-0.93	3.96E-02	59.66%	Selenocompound metabolism
6	cel00051	23	22	-0.87	4.28E-02	59.66%	Fructose and mannose metabolism
7	cel00500	26	25	-0.87	4.64E-02	59.66%	Starch and sucrose metabolism
8	cel00380	29	27	-0.79	6.19E-02	62.71%	Tryptophan metabolism
9	cel04140	15	15	-0.8	6.27E-02	62.71%	Regulation of autophagy
10	cel00062	15	14	-0.72	8.20E-02	70.21%	Fatty acid elongation
11	cel04020	37	37	-0.68	9.09E-02	70.21%	Calcium signaling pathway
12	cel00260	26	26	-0.68	9.36E-02	70.21%	Glycine, serine and threonine metabolism

B Probucol effects in *gas-1(fc21)*

UPREGULATED by Probucol in *gas-1*	KEGG Pathway ID	# Genes Total	#Genes Measured	Enrichment Score (ES)	P Value	FDR	KEGG Pathway Name
1	cel04145	56	55	1.55	1.36E-03	12.23%	Phagosome
2	cel03022	33	32	0.84	5.13E-02	97.95%	Basal transcription factors
3	cel03030	32	30	0.79	6.10E-02	97.95%	DNA replication
4	cel04130	23	23	0.74	7.61E-02	97.95%	SNARE interactions in vesicular transport
5	cel00590	13	13	0.74	8.16E-02	97.95%	Arachidonic acid metabolism
6	cel03018	44	43	0.71	8.39E-02	97.95%	RNA degradation
7	cel03420	33	33	0.69	8.92E-02	97.95%	Nucleotide excision repair
8	cel00770	11	11	0.71	9.18E-02	97.95%	Pantothenate and CoA biosynthesis

DOWNREGULATED by Probucol in *gas-1*	KEGG Pathway ID	# Genes Total	#Genes Measured	Enrichment Score (ES)	P Value	FDR	KEGG Pathway Name
1	cel00982	36	35	-2.15	1.88E-05	0.17%	Drug metabolism - cytochrome P450
2	cel00071	44	41	-1.58	9.86E-04	4.44%	Fatty acid degradation
3	cel00980	32	31	-1.39	3.94E-03	8.11%	Metabolism of xenobiotics by cytochrome P450
4	cel04020	37	37	-1.34	3.98E-03	8.11%	Calcium signaling pathway
5	cel00062	15	14	-1.42	4.50E-03	8.11%	Fatty acid elongation
6	cel01040	15	14	-1.34	6.41E-03	8.53%	Biosynthesis of unsaturated fatty acids
7	cel00061	13	12	-1.29	6.63E-03	8.53%	Fatty acid biosynthesis
8	cel04146	60	59	-1.23	8.69E-03	9.78%	Peroxisome
9	cel00900	15	13	-1.23	1.12E-02	11.20%	Terpenoid backbone biosynthesis
10	cel00830	18	18	-1.16	1.38E-02	12.38%	Retinol metabolism
11	cel00350	22	21	-1.04	2.29E-02	18.70%	Tyrosine metabolism
12	cel00650	20	19	-1.01	2.71E-02	20.29%	Butanoate metabolism
13	cel00040	20	19	-1	3.03E-02	20.96%	Pentose and glucuronate interconversions
14	cel00051	23	22	-0.91	3.80E-02	23.12%	Fructose and mannose metabolism
15	cel04122	12	11	-0.93	3.85E-02	23.12%	Sulfur relay system
16	cel00010	39	33	-0.9	4.46E-02	23.63%	Glycolysis / Gluconeogenesis
17	cel04140	15	15	-0.9	4.46E-02	23.63%	Regulation of autophagy
18	cel00500	26	25	-0.83	5.90E-02	29.50%	Starch and sucrose metabolism
19	cel00020	33	31	-0.79	6.59E-02	31.21%	Citrate cycle (TCA cycle)
20	cel04010	64	62	-0.71	8.06E-02	36.29%	MAPK signaling pathway
21	cel00380	29	27	-0.68	9.36E-02	36.82%	Tryptophan metabolism
22	cel00280	40	38	-0.69	9.38E-02	36.82%	Valine, leucine and isoleucine degradation
23	cel00920	14	14	-0.68	9.41E-02	36.82%	Sulfur metabolism

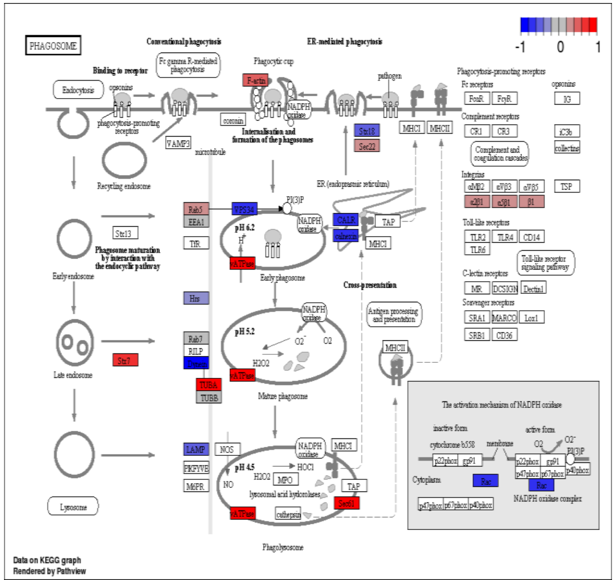
C Rapamycin effects in *gas-1(fc21)*

UPREGULATED by RAPA in *gas-1*	KEGG Pathway ID	# Genes Total	#Genes Measured	Enrichment Score (ES)	P Value	FDR	KEGG Pathway Name
1	cel04145	56	55	0.93	3.33E-02	97.55%	Phagosome
2	cel03050	38	37	0.72	7.87E-02	97.55%	Proteasome
3	cel00590	13	13	0.71	8.40E-02	97.55%	Arachidonic acid metabolism

DOWNREGULATED by RAPA in *gas-1*	KEGG Pathway ID	# Genes Total	#Genes Measured	Enrichment Score (ES)	P Value	FDR	KEGG Pathway Name
1	cel00982	36	35	-1.29	6.27E-03	56.39%	Drug metabolism - cytochrome P450
2	cel03020	24	23	-0.93	3.54E-02	61.93%	RNA polymerase
3	cel03010	142	115	-0.87	4.31E-02	61.93%	Ribosome
4	cel04120	77	73	-0.87	4.45E-02	61.93%	Ubiquitin mediated proteolysis
5	cel04080	24	24	-0.83	5.13E-02	61.93%	Neuroactive ligand-receptor interaction
6	cel04140	15	15	-0.81	6.13E-02	61.93%	Regulation of autophagy
7	cel00040	20	19	-0.78	6.48E-02	61.93%	Pentose and glucuronate interconversions
8	cel00450	11	10	-0.78	6.83E-02	61.93%	Selenocompound metabolism
9	cel04310	58	56	-0.75	7.38E-02	61.93%	Wnt signaling pathway
10	cel04020	37	37	-0.73	7.56E-02	61.93%	Calcium signaling pathway
11	cel00980	32	31	-0.7	8.71E-02	61.93%	Metabolism of xenobiotics by cytochrome P450
12	cel03040	106	102	-0.67	9.35E-02	61.93%	Spliceosome

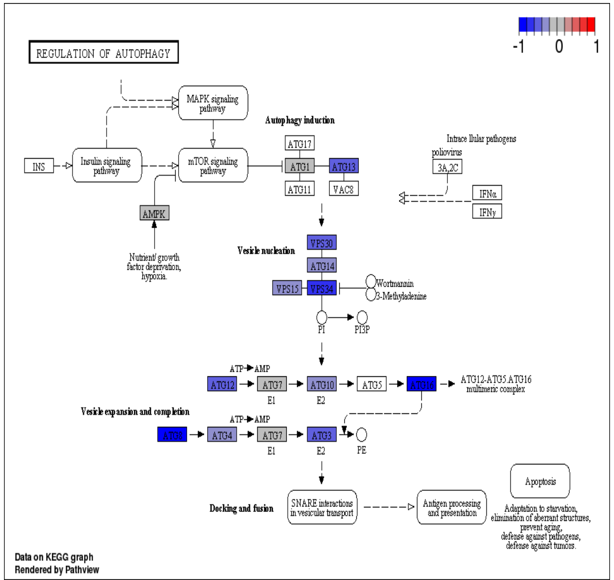
D Consistent pathway-level effects in *gas-1(fc21)* by all 3 drugs (depicted for Probucol)

'PHAGOSOME' UPREGULATION



Data on KEGG graph
Rendered by Pathview

'REGULATION OF AUTOPHAGY' DOWNREGULATION



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