A

CELL LINE CHARACTERIZATION (DNA)

Target Gene	NEK10	Mutation	35bp deletion in exon 4
Guide RNA Sequence	TGCTTGATTGGACGTTCAAA	Genomic location	chr3:27352473
Target Transcript	NM_199347		
Synonyms			

Forward Primer	Reverse Primer	Sequencing Primer
CAGAGAAACAGCCAGATGAAGAAAG	GTGCTTACTGAAACATCTGGTCATT	GTGCTTACTGAAACATCTGGTCATT

Sequencing result

AGACATATCATTCATAGATATGTTAAATGGAGCCCTTTGGAAACATATGATTTTTCTTTTATTACCAAGTGAACATTCTTTGAAAACG
CACCGAAGTCTTTTAAGATCTGAATAGTCCCTAGGAGAGAGAATAACACAATGTGAACCCACAGAAGGCTCCAGGTGAACAAGAT
CGTGTTACCCACTGATGGCATTGCCACTTGGAGGCCTGCTTTTATCAACTGGAATGAATACACACACTAAACATTTTCTTTAGAAC
ACTAGAAATGACCAGATGTT

Sequence alignment

AACATCTGGTCATTTCTAGTGTTCTAAAGAAAATGTTTAGTGTGTGTATTCATTCCAGTTGATAAAAGCAGGCCTCCAAGTGGCA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AACATCTGGTCATTTCTAGTGTTCTAAAGAAAATGTTTAGTGTGTGTATTCATTCCAGTTGATAAAAGCAGGCCTCCAAGTGGCA

ATGCCATCAGTGGGTAACACGATCTTGTTCACCTGGAGCCTTCTGTGGGTTCACATTGTGTTATTCTCTCTCCTAGGGACTATTC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATGCCATCAGTGGGTAACACGATCTTGTTCACCTGGAGCCTTCTGTGGGTTCACATTGTGTTATTCTCTCTCCTAGGGACTATTC

AGATCTTAAAAGACTTCGGTGC--------------------------------GTTTTCAAAGAATGTTCACTTGGTAATA
|||||||||||||||||||||| ||||||||||||||||||||||||||||
AGATCTTAAAAGACTTCGGTGCCTTTTGAACGTCCAATCAAGCAAACAACAGGTAGCGTTTTCAAAGAATGTTCACTTGGTAATA

AAAGAAAAATCATATGTTTCCAAAGGGCTCCATTTAACATATCTATGAATGATATGTCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAAGAAAAATCATATGTTTCCAAAGGGCTCCATTTAACATATCTATGAATGATATGTCT

B

35 bp deletion

F1 F2 B2 B1

NEK10 - EXON 4

C

Parental   NEK10 KO

MW F1+B1 F1+B2 F2+B1 F2+B2 F1+B1 F1+B2 F2+B1 F2+B2

200 bp

100 bp

D

MW HAP1 NEK10 KO HAP1 Control

kDa
100
75
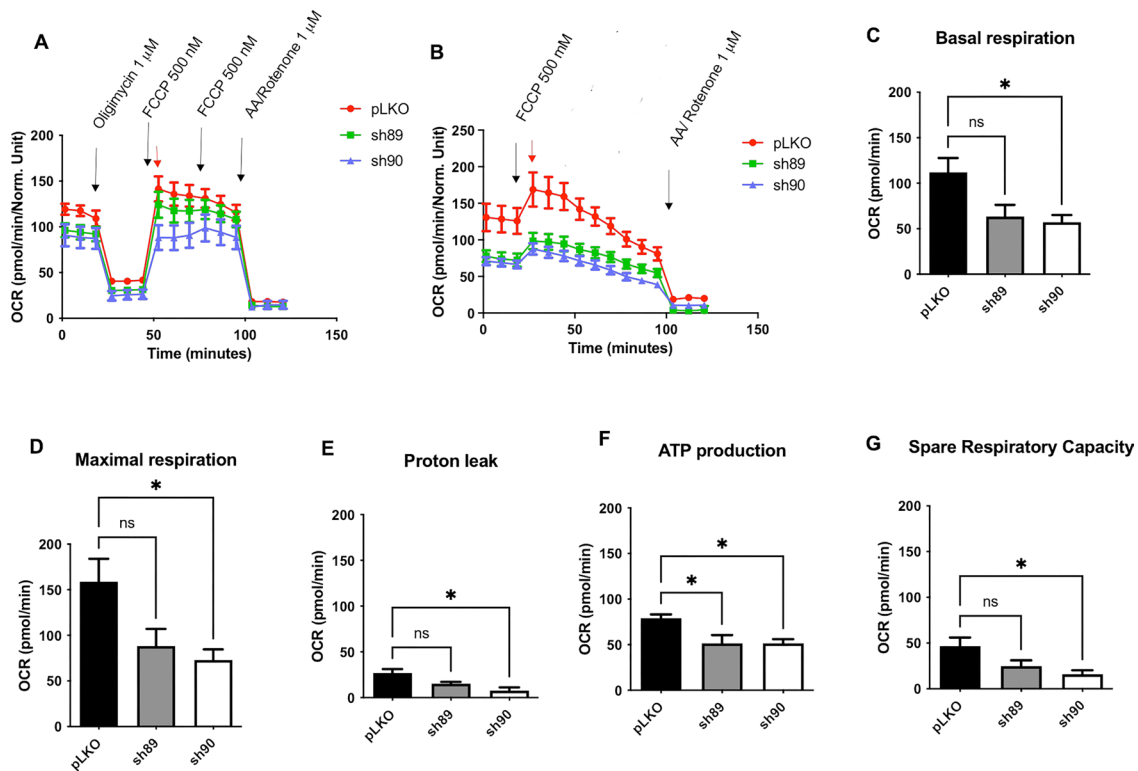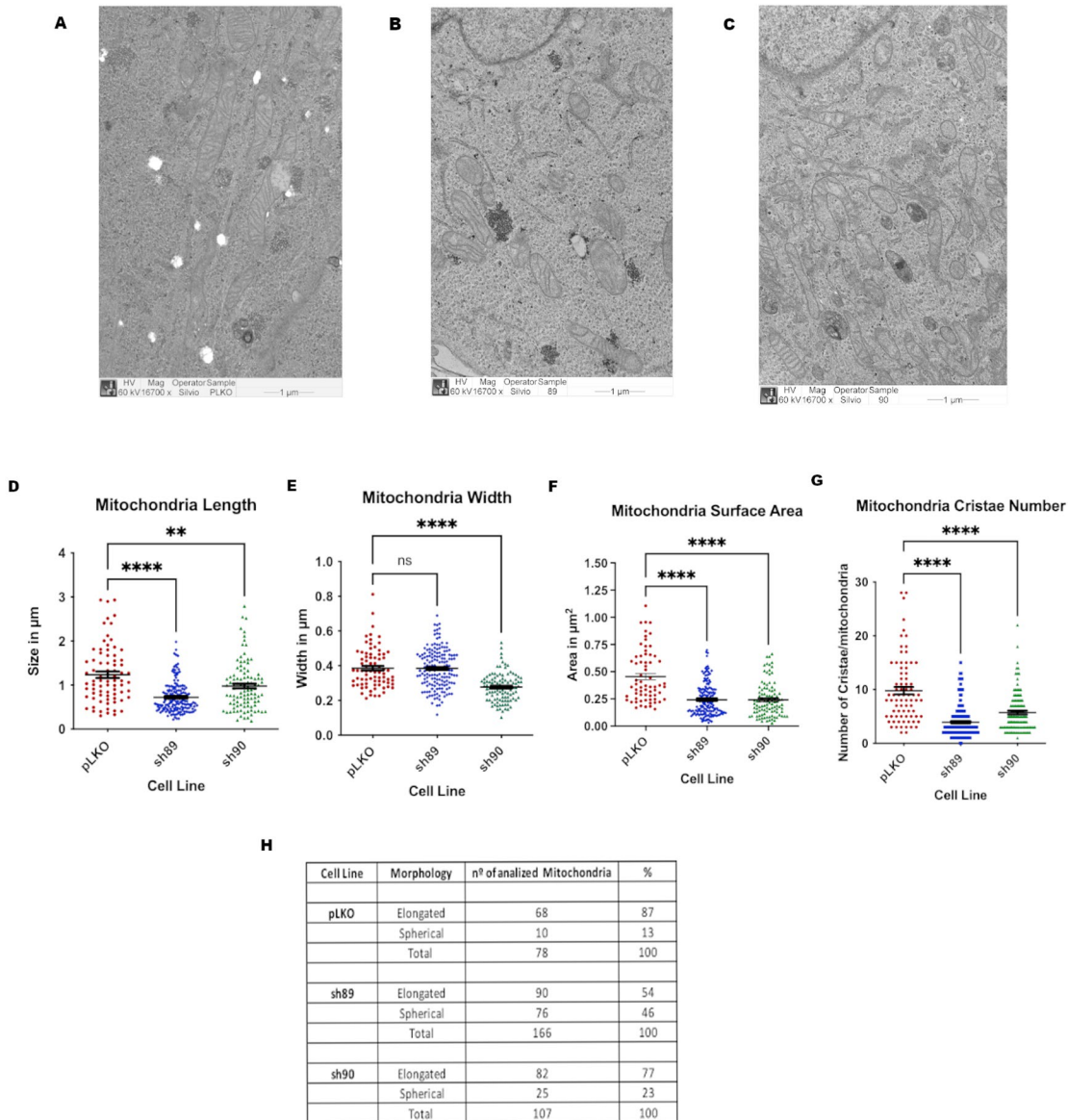— NEK10

50
— β-Tub

250
150
100
75
50
Ponceau S

Supplemental Figure S1 - Genetic characterization of NEK10 knockout cells. A) NEK10 gene sequencing and gene alignment of Parental and NEK10 KO HAP1 cells (from Horizon web site). NEK10 KO cells have a 35 bp deletion in exon 4. B) Location of diagnostic oprimer in NEK10 exon 4. C) 10% polyacrylamide gel of PCR amplification of parental and NEK10 KO DNA using the primers depicted in panel B. Lane 1 shows the molecular marker DNA – (100 bp Low Ladder Thermo Scientific). Amplicons of NEK10 exon 4 are approximately 150 bp for the Parental line, but 35 bp shorter for the NEK10 KO cells. Each lane shows a different combination of oligonucleotide primers (Forwards F1 and F2 with Reverse B1 and B2). Non-tempate controls showed no amplification (not shown). D) Western blot of homogenates of HAP1 control and NEK10 KO lines. An antibody generated in goats by Santa Cruz Biotechnology (cat#sc-103067 NEK10 (D-17)) was used to detect the 133 KD band, which was absent in the KO line.
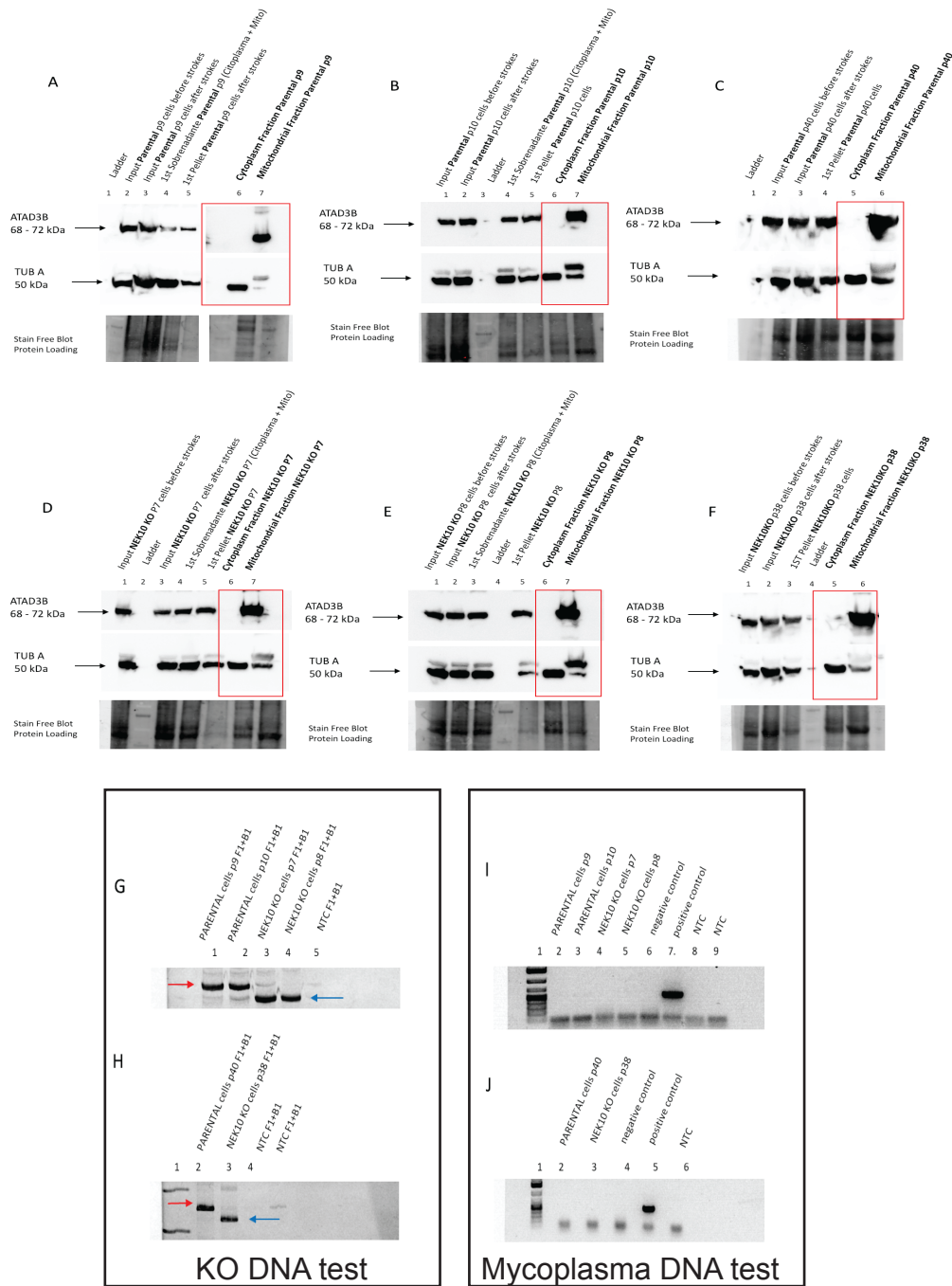
Supplemental Figure S2 – Mitochondrial respiration analysis of control (pLKO), and NEK10-knockdown cells (sh89 e sh90). The graphs in figures A and B show the OCR (oxygen consumption rate) during mitochondrial respiration. The pLKO cells are shown in red, sh89 cells in green; sh90 cells in the blue line. The black arrows show the time when the inhibitors were added with their respective concentration. The red arrow indicates maximal respiration. C – The graph shows the Basal Respiration of pLKO, sh89, and sh90 cells. In D-Maximal respiration. E- Proton Leak. F- ATP production linked to OCR. G- Spare Respiratory Capacity. All the graphs show the results of 4 independent experiments (n=4) in quadruplicate (4 different cell passages). The data was obtained with Seahorse XFe24 Analyzer – Agilent equipment and analyzed at Seahorse Wave desktop software 35. The statistical analyses were performed with GraphPad Prism 47 and used a one-way ANOVA test followed by the post hoc Dunnett test, where * = p <0.05. The graphs are shown in Mean ± SD.

Cell Line	Morphology	nº of analized Mitochondria	%
pLKO	Elongated	68	87
	Spherical	10	13
	Total	78	100
sh89	Elongated	90	54
	Spherical	76	46
	Total	166	100
sh90	Elongated	82	77
	Spherical	25	23
	Total	107	100

Supplemental Figure S3 – Morphology analysis of the control (pLKO), and NEK10-depleted cells (sh89 e sh90) by Transmission Electron Microscopy (TEM). In A are shown the representative picture of the mitochondria of the pLKO; B - sh89; and C - sh90 cells. A total of 78 mitochondria from pLKO cells, 166 mitochondria from sh89 cells, and 107 mitochondria from sh90 cells were analyzed. The scale bar is 1µm. In total, 15 images were taken for pLKO cells; 17 images for sh89 cells; and 18 images for sh90 cells. In D – The graph shows the analysis of the mitochondrial length (Size in µm); E- mitochondrial width (Width in µm); F- mitochondrial area (Area in µm2); and G - number of mitochondrial cristae per mitochondrion. The mitochondrion was classified based on their mitochondrial morphology: spherical (<6 µm), or elongated (> = 6 µm) [11] (Figure H). Mitochondrial morphology analysis was performed using Image J2 software [33]. The statistical analyses were performed with GraphPad Prism [47], and the one-way ANOVA tests followed Tukey's test was employed, where p-value <0.05 = *, p-value <0.01 = **, p-value < 0.001 ***, and p-value < 0.0001= ****. The graphs are shown in Mean with SD.
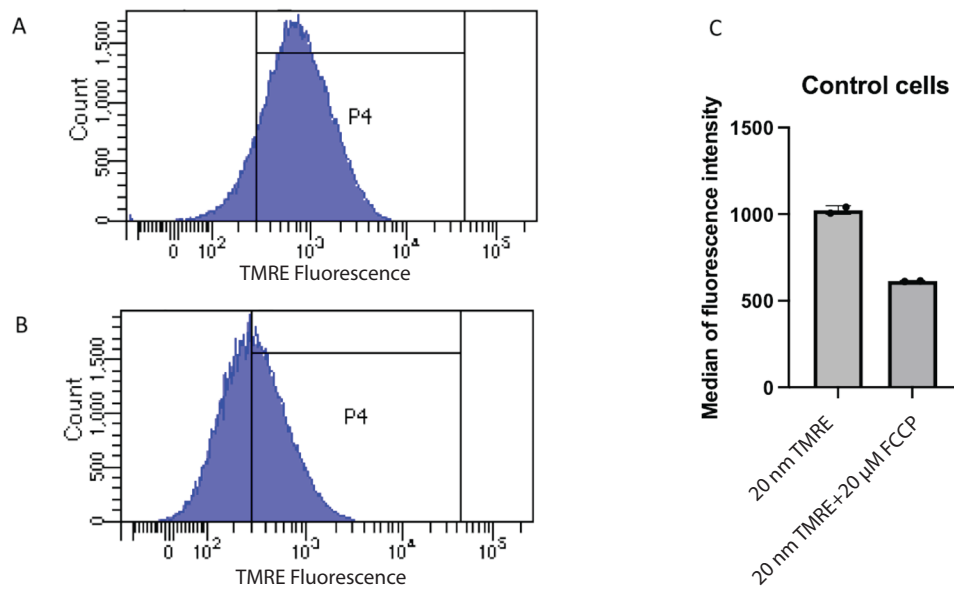
Supplemental Figure S4. FCCP reduces fluorescence of TMRM/TMRE in the concentrations used (20 nM). HEK293T cells were incubated with TMRE 20 nM for 30 minutes and analyzed in an BD FACSAria TM flow analyzer (panel A). FCCP was added at 20 µM for mitochondrial depolarization before measurements (panel B). Quantification shown in panel C.
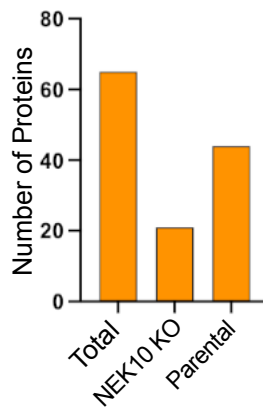
Supplemental Figure S5- Composition analysis of mitochondrial fractions of Parental cells and NEK10 KO cells, NEK10 knockout, and PCR mycoplasma analysis. In A – Mitochondrial fractionation of Parental cells passage 9 (p9); B- Mitochondrial fractionation of Parental cells passage 10 (p10) and C- Mitochondrial fractionation of Parental cells passage 40 (p40). In D – Mitochondrial fractionation of NEK10 KO cells passage 7 (p7); E- Mitochondrial fractionation of NEK10 KO cells passage 8 (p8) and F- Mitochondrial fractionation of NEK10 KO cells passage 38 (p38). Western blots were analyzed with anti-ATAD3 antibodies (1:1000) in the upper panels and anti-Tubulin A (1:1000) in the middle panels, and total protein analysis was detected by the Stain-Free Blot technique Bio-Rad. The molecular protein marker used in the blotting westerns was Thermo Scientific Spectra™ Multicolor Broad Range Protein Ladder. Figures G and H are shown a 12% polyacrylamide with amplified bands of the Parental cells (p9, p10, and p40) of approximately 150 bp (indicated by the red arrow) and of the NEK10 KO cells (p7, p9, and p38) of approximately 115 bp (indicated by the blue arrow). In I and J, the 1% agarose gel shows the negative mycoplasma test of the Parental cells (p9, p10, and p40) and of the NEK10 KO cells (p7, p9, and p38).
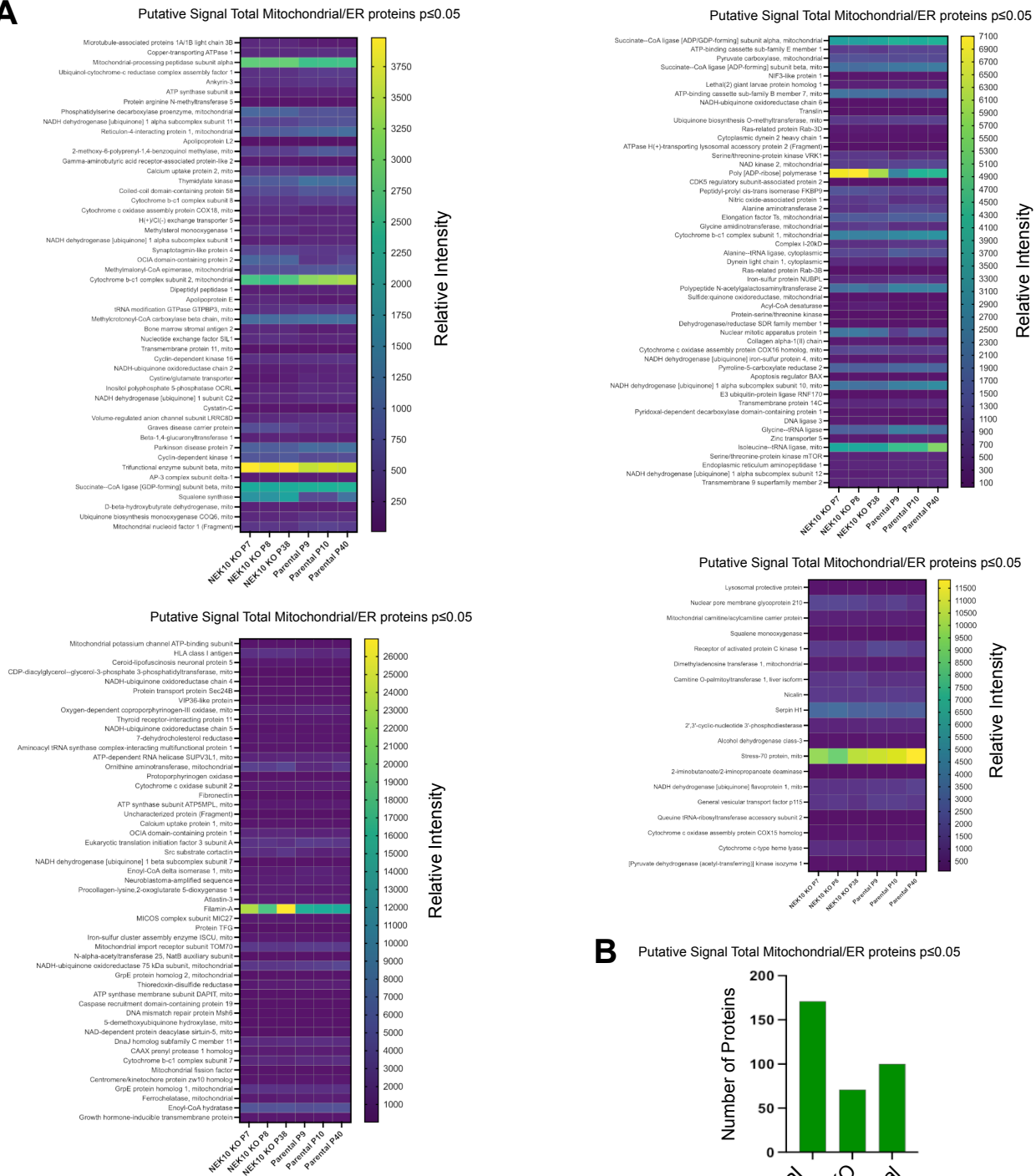
A

B
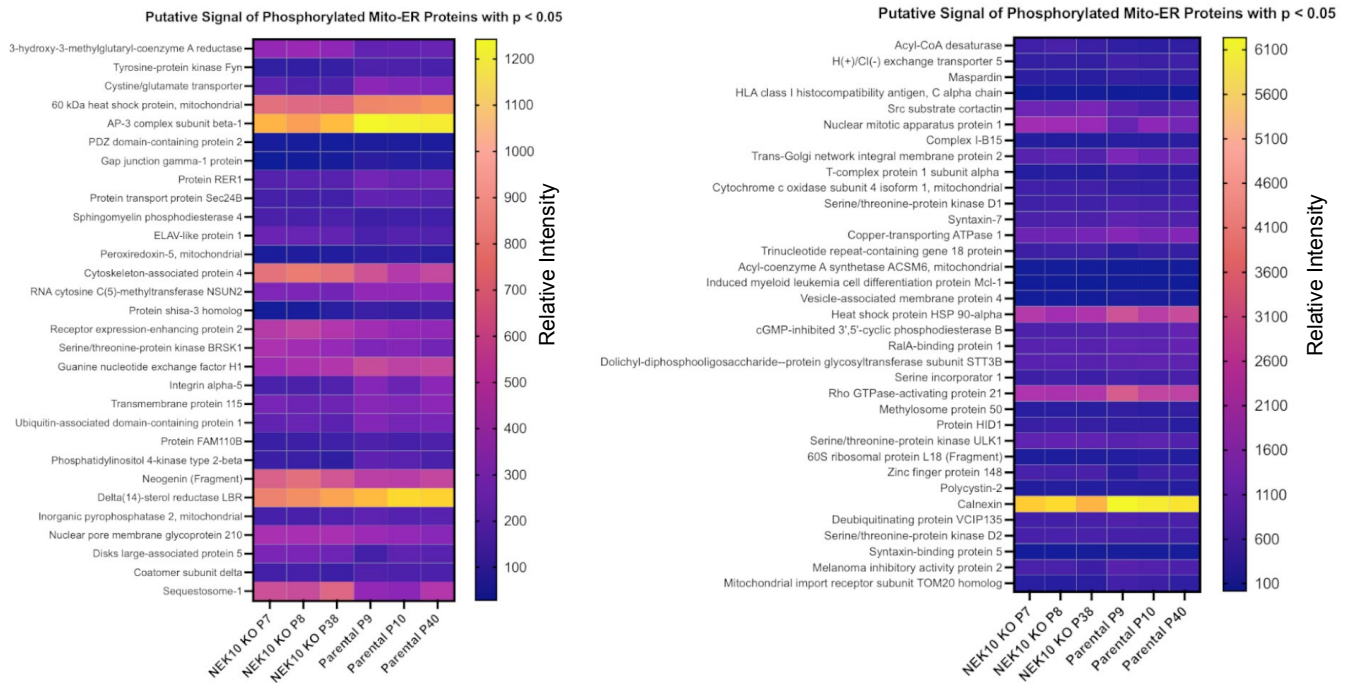
Supplemental Figure S6 - Differential putative signal of phosphorylated mitochondrial and ER proteins from Parental and NEK10 KO cells. A) Heat map of the 65 phosphorylated putative signals of mitochondrial and ER proteins from NEK10 KO (p7, p8, p38) and Parental cells (p9, p10, p40). The putative signal found for each phosphorylated protein, with a p-value < 0.05, is shown. B) Significantly different phosphorylation. 44 (68%) of a total of 65 proteins have a putative phosphorylation signal higher in Parental cells than in NEK10 KO cells. And 21 proteins (32%) have a higher putative phosphorylation signal in NEK10 KO cells compared to Parental cells.

**Supplemental Figure S7. Putative signal of mitochondrial/ER-related proteins altered in NEK 10 KO cells.**
All significantly altered propteins (p<0.05) in NEK10 KO (relative to parental) crude mitochondria are shown.
**Each NEK10 KO passage was normalized to its respective Parental control signal.**

Gene Symbol	Accession	Ratio (KO/Parent)	p-value
MAP1LC3B	Q9GZQ8	1.896750524	8.425E-05
ATP7A	Q04656	0.876484822	0.000103
PMPCA	Q10713	1.093680399	0.0001752
UQCC1	Q9NVA1	0.752057688	0.0005114
ANK3	Q12955	0.872760801	0.0006727
MT-ATP6	P00846	0.818612245	0.0007649
PRMT5	O14744	0.763243243	0.0008858
PISD	Q9UG56	1.35160366	0.0009494
NDUFA11	Q86Y39	0.79533129	0.0010876
RTN4IP1	Q8WWV3	0.790507005	0.0010884
APOL2	Q9BQE5	1.8125	0.0012098
COQ5	Q5HYK3	0.639601079	0.0012133
GABARAPL2	P60520	1.478971963	0.0016325
MICU2	Q8IYU8	1.24074393	0.0016719
DTYMK	P23919	0.742454136	0.0017567
MIX23	C9JQ41	0.798353909	0.0017773
UQCRQ	O14949	0.825229035	0.0018405
COX18	Q8N8Q8	1.510895472	0.0018741
CLCN5	P51795	0.791392065	0.0020817
MSMO1	Q15800	1.534944181	0.0021489
NDUFA1	O15239	0.745527113	0.0022267
SYTL4	Q96C24	1.322241406	0.0022308
OCIAD2	Q56VL3	2.116402116	0.0023634
MCEE	Q96PE7	0.880968651	0.0027022
UQCRC2	P22695	0.819374135	0.0027047
CTSC	P53634	0.74586269	0.0028152
APOE	P02649	1.918116976	0.0029204
GTPBP3	Q969Y2	0.777683002	0.0029319
MCCC2	Q9HCC0	0.933081427	0.003059
BST2	Q10589	1.650746269	0.0031486
SIL1	Q9H173	1.403764706	0.0040973
TMEM11	P17152	0.807971014	0.0041418
CDK16	Q00536	0.895788789	0.0042373
MT-ND2	P03891	0.72949301	0.0043884
SLC7A11	Q9UPY5	0.508732978	0.0043932
OCRL	Q01968	0.720838124	0.004405
NDUFC2	O95298	0.794526902	0.0044322
CST3	P01034	1.494922139	0.0047487
LRRC8D	Q7L1W4	0.829951691	0.0047643
SLC25A16	P16260	1.700980392	0.004872
B4GAT1	O43505	1.218878813	0.005197
PARK7	Q99497	0.821196581	0.0058193
CDK1	P06493	1.220389015	0.0061841
HADHB	P55084	1.082789575	0.0062973
AP3D1	O14617	0.912780269	0.0064598
SUCLG2	Q96I99	0.942527588	0.0069314
FDFT1	P37268	2.298319672	0.0070343
BDH1	Q02338	1.199078139	0.0072738
COQ6	Q9Y2Z9	0.773358787	0.0076194
UQCC2	V5IRT4	0.745494741	0.0076643
SUCLG1	P53597	0.880879629	0.0079255
ABCE1	P61221	0.797233837	0.0082222
PC	P11498	1.107594022	0.0093414
SUCLA2	A0A2R8Y6Y7	0.879236496	0.0098128
NIF3L1	Q9GZT8	0.830544747	0.0100185
LLGL1	Q15334	0.921229492	0.0101264
ABCB7	O75027	1.155276785	0.0103018
MT-ND6	P03923	0.737964656	0.010419
TSN	Q15631	0.808966074	0.0104996
COQ3	Q9NZJ6	0.834023073	0.010562
RAB3D	O95716	1.357790428	0.010614
DYNC2H1	Q8NCM8	1.277268094	0.0107298
ATP6AP2	A0A1B0GTB0	1.360764755	0.0107494
VRK1	Q99986	1.443961885	0.0113724
NADK2	Q4G0N4	0.830400658	0.0113819
PARP1	P09874	1.773443089	0.01157
CDK5RAP2	Q96SN8	0.735040191	0.0116329
FKBP9	O95302	1.272814793	0.0121078
NOA1	Q9NC60	1.359159095	0.0121424
GPT2	Q8TD30	0.683488896	0.0122001
TSFM	P43897	0.892999979	0.0129092
GATM	P50440	1.430699593	0.0131649
UQCRC1	P31930	0.862318434	0.013178
NDUFS7	F5H5N1	0.833272595	0.0132898
AARS1	P49588	0.734503501	0.0134156
DYNLL1	P63167	1.140525141	0.0134924
RAB3B	P20337	1.434664247	0.0135877
NUBPL	Q8TB37	0.705690185	0.0135962
GALNT2	Q10471	0.788749088	0.0138681
SQOR	Q9Y6N5	1.441088898	0.0142112
SCD	O00767	2.380105018	0.0142781
BCKDK	H3BS02	1.669776119	0.0146127
DHRS1	Q96LJ7	1.407354001	0.0147935
NUMA1	A0A087WY61	1.788034703	0.0150632
COL2A1	P02458	1.513370119	0.0153484
SYNJ2BP-COX16	A0A087WYV9	1.255399629	0.0155042
NDUFS4	O43181	0.768454039	0.01604531
PYCR2	Q96C36	0.895993384	0.01616341
BAX	Q07812	1.091957706	0.01621322
NDUFA10	O95299	0.784537452	0.01685225
RNF170	Q96K19	0.845929171	0.01697735
TMEM14C	Q9P0S9	0.904122279	0.01784726
PDXDC1	Q6P996	1.302038012	0.01817852
LIG3	P49916	1.718463445	0.01870091
GARS1	P41250	0.721143545	0.01875204
SLC30A5	Q8TAD4	0.648037326	0.0192248
IARS2	Q9NSE4	0.760929729	0.02001179
MTOR	P42345	0.81509434	0.02114274
ERAP1	Q9NZ08	0.933338556	0.02126
NDUFA12	Q9UI09	0.788216426	0.02198759
TM9SF2	Q99805	0.834173792	0.02301564
ABCB8	Q9NUT2	1.083612814	0.02428979
HLA-A	Q53Z42	1.330183265	0.02437287
CLN5	O75503	1.114444991	0.02446412
PGS1	Q32NB8	1.456483126	0.02514707
MT-ND4	P03905	0.736647999	0.02583178
SEC24B	O95487	0.826273885	0.02633618
LMAN2L	Q9H0V9	0.87846168	0.02650217
CPOX	P36551	0.874981385	0.02653792
TRIP11	Q15643	0.877745311	0.02700614
MT-ND5	P03915	0.756728275	0.02782088
DHCR7	Q9UBM7	1.154406356	0.0279679
AIMP1	Q12904	0.863011768	0.02822622
SUPV3L1	Q8IYB8	0.887411788	0.02844017
OAT	P04181	1.720669201	0.0292547
PPOX	P50336	0.754415195	0.02940751
MT-CO2	P00403	0.856852216	0.02947087
FN1	P02751	0.595776031	0.03059181
ATP5MJ	A0A0A0MTQ5	0.844018447	0.03073883
	F5H5T6	1.380514706	0.03150694
MICU1	A0A0U1RRK1	1.141864814	0.03171816
OCIAD1	Q9NX40	1.247997366	0.03217916
EIF3A	Q14152	0.799429905	0.03237866
CTTN	Q14247	1.619933749	0.03248963
NDUFB7	P17568	0.870799448	0.03294738
ECI1	P42126	0.705161252	0.03385329
NBAS	A2RRP1	0.887094786	0.0340257
PLOD1	Q02809	1.193676424	0.03434599
ATL3	Q6DD88	0.873181759	0.03443278
FLNA	P21333	1.483308629	0.03459456
APOOL	Q6UXV4	1.13953115	0.03504888
TFG	Q92734	0.789585547	0.03510912
ISCU	Q9H1K1	1.104905345	0.03517402
TOMM70	O94826	0.888693726	0.03528466
NAA25	Q14CX7	0.844070962	0.03560931
NDUFS1	P28331	0.760253326	0.03609751
GRPEL2	Q8TAA5	0.759684607	0.03611999
TXNRD2	A0A182DWF2	0.840348384	0.03627015
ATP5MK	Q96IX5	0.86239539	0.03735858
CARD19	Q96LW7	1.212962963	0.03738221
MSH6	P52701	1.310607503	0.03826792
COQ7	H3BP28	0.814505979	0.03832307
SIRT5	Q9NXA8	0.882431828	0.03836766
DNAJC11	Q9NVH1	0.914288644	0.03850007
ZMPSTE24	O75844	0.874137931	0.03908396
UQCRB	P14927	0.841400656	0.04042972
MFF	Q9GZY8	1.196742031	0.04065759
ZW10	O43264	0.881993569	0.0407004
GRPEL1	Q9HAV7	1.115470974	0.04116106
FECH	P22830	0.838853031	0.04129133
HADHA	H0YFD6	1.070906313	0.04162355
GHITM	Q9H3K2	1.132198953	0.04169004
CTSA	P10619	1.108732222	0.0417012
NUP210	Q8TEM1	1.304665876	0.04192848
SLC25A20	O43772	1.158769426	0.04207959
SQLE	Q14534	1.495143331	0.0423004
RACK1	P63244	0.784912377	0.04235659
TFB1M	Q8WVM0	1.175881443	0.04262367
CPT1A	P50416	1.190184987	0.04295446
NCLN	Q969V3	0.940693797	0.04306423
SERPINH1	P50454	1.166245346	0.04327213
CNP	P09543	0.903667978	0.04493343
ADH5	P11766	0.873336772	0.04508755
HSPA9	P38646	0.865413628	0.04529779
RIDA	P52758	0.720510096	0.0454383
NDUFV1	G3V0I5	0.808518103	0.04577722
USO1	O60763	0.826526121	0.0460825
QTRT2	Q9H974	0.843821656	0.04639331
COX15	Q7KZN9	1.282993197	0.04713629
HCCS	P53701	1.198333333	0.04871752
PDK1	Q15118	0.808317268	0.04953672

Supplemental Figure S8. Putative signal of total mitochondrial and ER proteins from Parental and NEK10 KO cells. A) Heat map of 171 putative signals of mitochondrial and ER proteins from NEK10 KO (p7, p8, p38) and Parental cells (p9, p10, p40). The putative signal found for total protein, with a p-value < 0.05, is shown. B) 100 (58.4%) of a total of 171 proteins have a putative protein signal higher in Parental cells than in NEK10 KO cells. And 41.6% of these proteins (71 proteins) have a higher putative total protein signal in NEK10 KO cells compared to Parental cells.
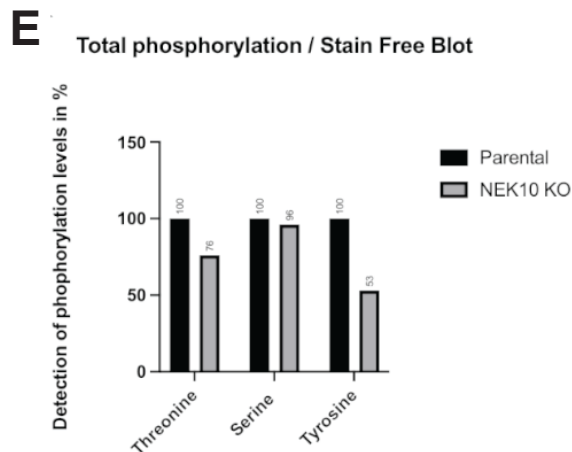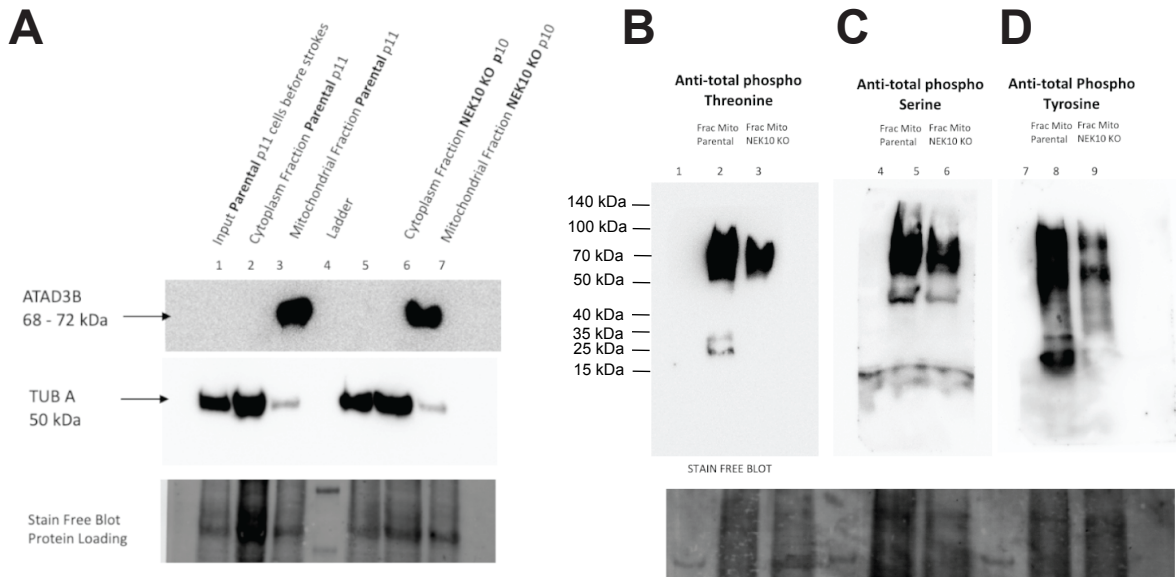
Supplemental Figure S9. Analyses of phosphoproteins. A) Crude mitochondrial fractionation of Parental cells passages p11 and NEK10 KO passage p10. The upper panel shows the western blotting analyzed with the anti-ATAD3 antibody. The middle panel shows western blotting analyzed with anti-Tubulin A antibody. Total protein analysis was detected by Bio-Rad Stain-Free Blot technique, lower panel. The protein molecular marker is Thermo Scientific Spectra™ Multicolor Broad Range Protein Ladder.
B) Analysis was performed with the anti-total phosphothreonine antibody. C) Analysis with anti-total phosphoserine antibody. D) Analysis with anti-total phosphotyrosine antibody. Lanes 2, 5, and 8 are the crude mitochondrial fractions of Parental cells. Lanes 3, 6, and 9 are the crude mitochondrial fractions of NEK10 KO cells. In the lower panel is shown the total protein analysis detected by the Bio-Rad Stain-Free Blot. The protein molecular marker used in the blotting westerns was Thermo Scientific Spectra™ Multicolor Broad Range Protein Ladder. E) The representative graph of total threonine, serine, and tyrosine phosphorylation is presented in percentage of control. The intensity of the bands was quantified with Image J2. The Stain Free Blot was used as a phosphorylation intensity normalizer.
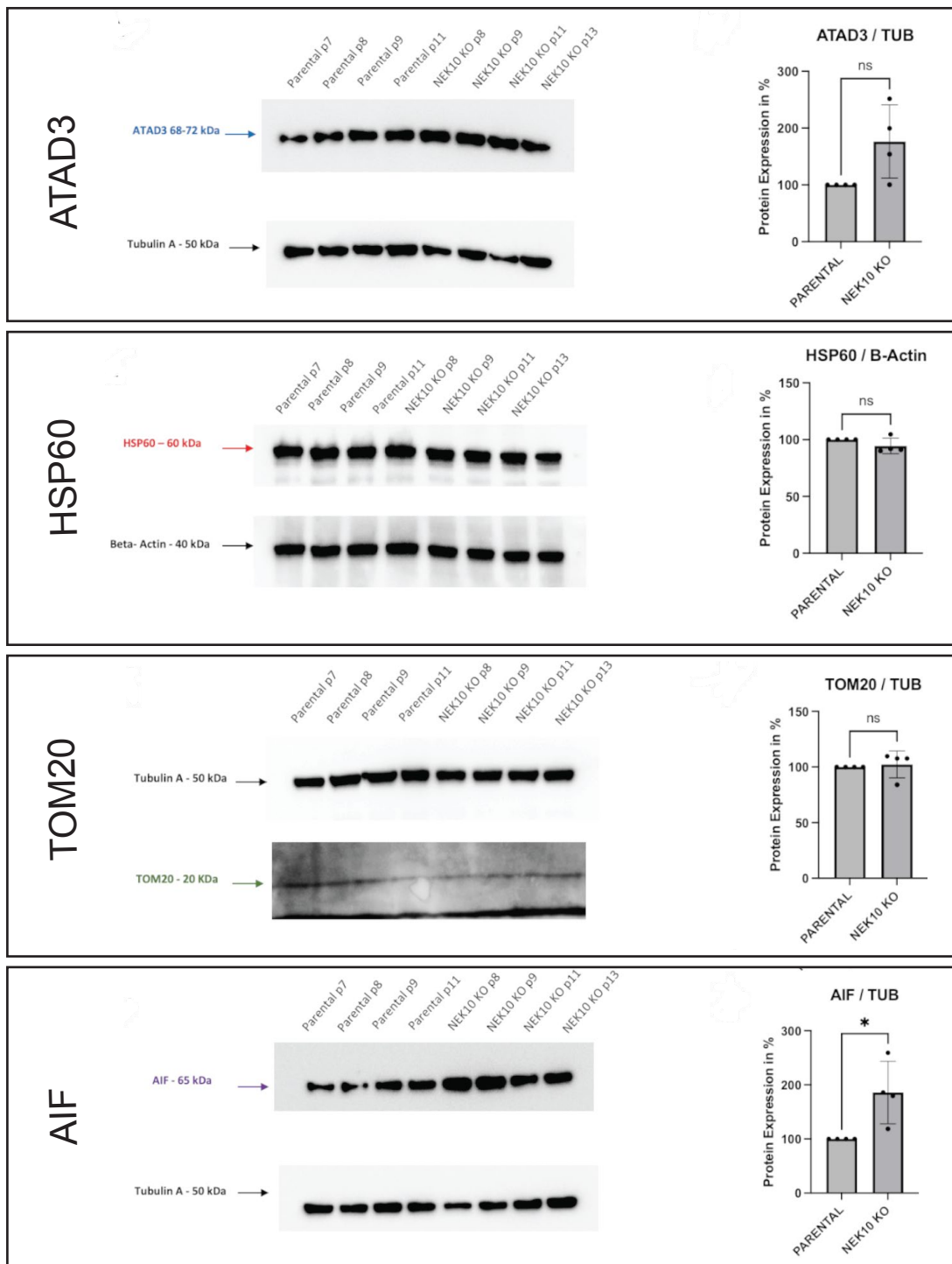
Supplemental Figure S10 – Mitochondrial proteins levels of Parental and NEK10 KO cells. The western blottings show the levels of ATAD3; HSP60; TOM20; AIF. The protein levels analysis in % is shown in graphs on the right of each blot, The Tubulin A or B-actin levels were used for protein levels normalization. The intensity of the bands was quantified with Image J2. The graphs show the result of n=4. The statistical analyses were performed with GraphPad Prism 4.7 employing paired t-test, where p-value <0.05 = *, and p-value <0.01 = **. The graphs are shown in Mean with SD.