

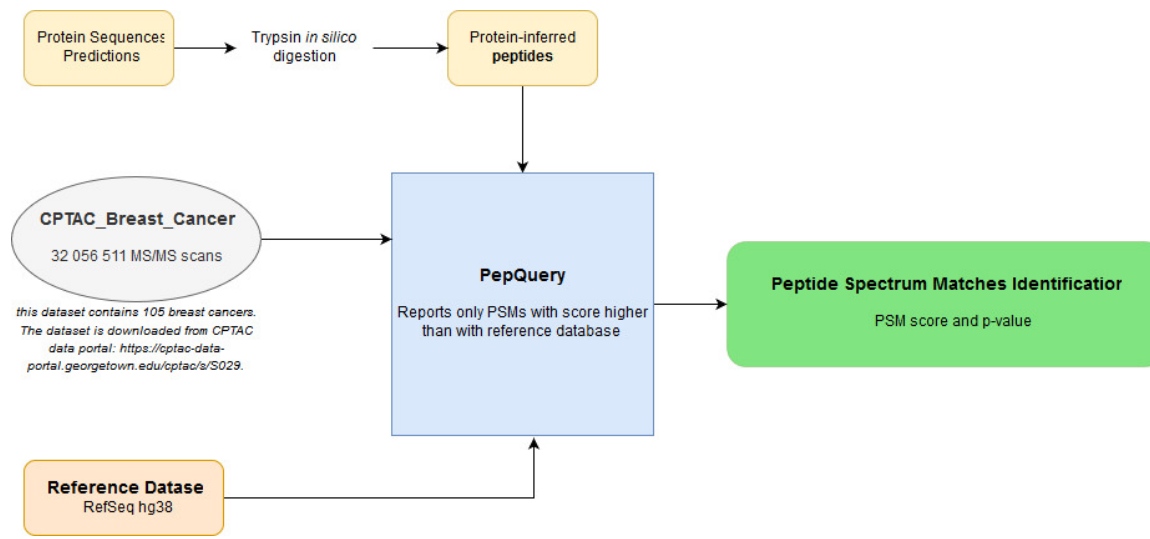


Figure S1. Using the PepQuery tool to interrogate spectrums from published proteome datasets.

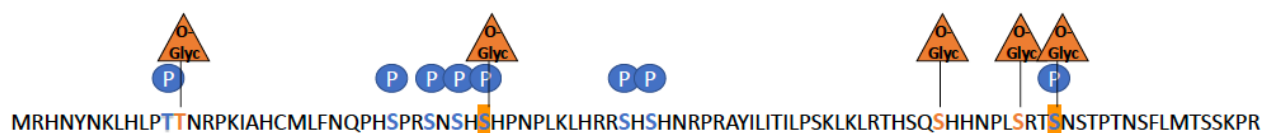


Figure S2. Putative post-translational modifications of MTALTND4. O-Glyc: putative O-glycosylation; P: putative phosphorylation. PTM prediction were done using webserver reviewed by [75], only scores with 80% probabilities were retained.

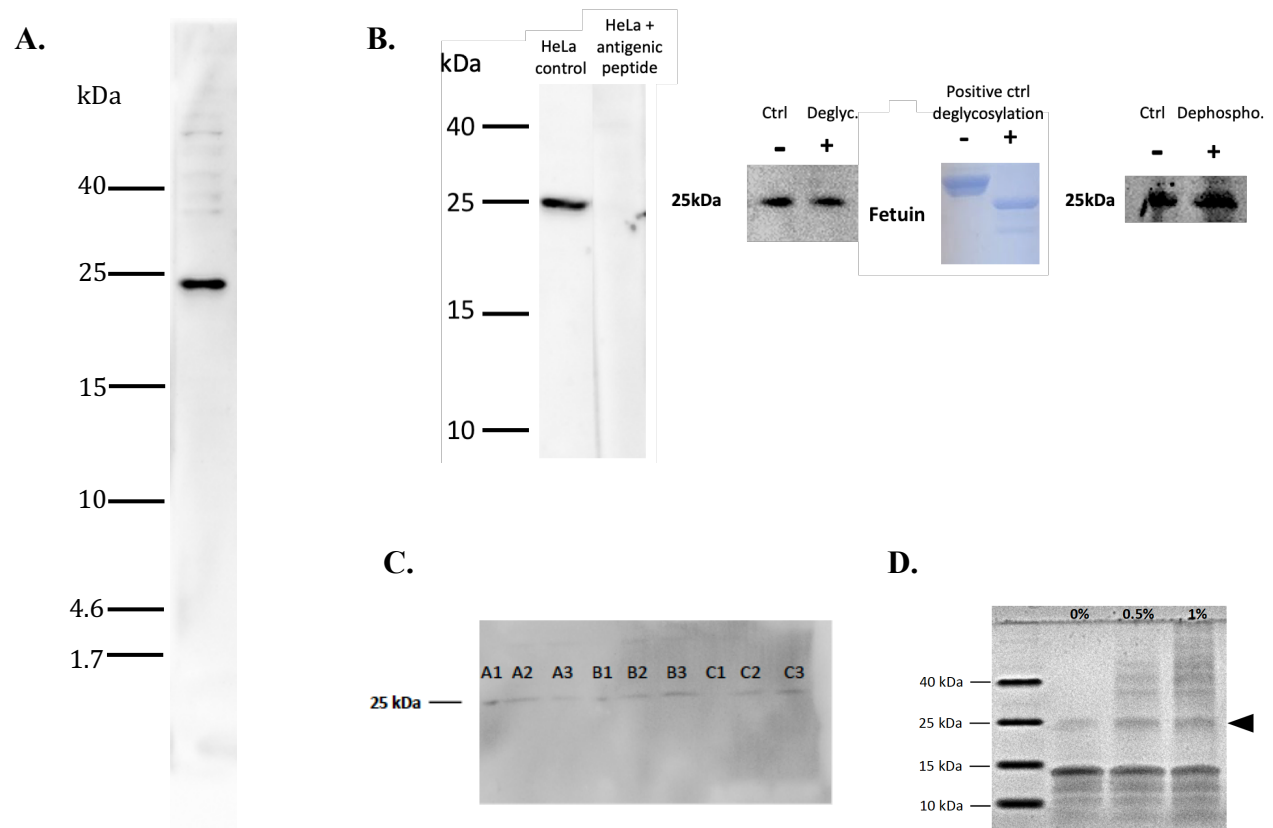


Figure S3. Specificity of the anti-MTALTND4 antibody and detection of putative post-translational modifications (PTMs) of MTALTND4. **A.** Complete western blot of the antibody on a cell lysate (Fig. 2B). **B.** Specificity of anti-MTALTND4 and PTMs. Deglyc: deglycosylation (whole cell lysates were treated with a complete protein deglycosylation mix II kit [NEB, Cat# P6044] following the manufacturer's protocol: (-) non-treated cells, (+) treated cells and fetuin positive control are shown); Dephosphos: dephosphorylation (whole cell lysates were treated with a lambda protein phosphatase [NEB, P0753S] following the manufacturer's protocol: (-) non-treated cells and (+) treated cells are shown). **C.** Apparent molecular weight of MTALTND4 exposed to different concentrations of denaturing agent β -Mercaptoéthanol and different heating times at 95°C. A = 5 minutes, B = 30 minutes and C = 3 hours. 1 = 0% β -Mercaptoethanol, 2 = 5% β -Mercaptoethanol and 3 = 10% β -Mercaptoethanol. **D.** Self-association of MTALTND4: 3 μ g of synthetic peptide were incubated at 30 °C for 10 min in the presence of 0%, 0.5% and 1% paraformaldehyde, quenched with LSB, separated by tricine-SDS-PAGE and visualized with Coomassie Imperial Protein stain. Arrowheads indicate main cross-linked products.

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Query: None Query ID: lcl|Query_3741 Length: 99

>Homo sapiens mitochondrion, complete genome
Sequence ID: NC_012920.1 Length: 16569
Range 1: 11557 to 11853

Score:152 bits(383), Expect:3e-42,
Method:Compositional matrix adjust.,
Identities:98/99(99%), Positives:99/99(100%), Gaps:0/99(0%)

Query 1      MRHNYNKLHLPTTNRPKIAHCMLFnqphsprsnshshnpLKLHRRSHSHNRPRAYILIT 60
             MRHNYNKLHLPTTNRPKIAHC+LFNQPHSPRSNSHSHPNPLKLHRRSHSHNRPRAYILIT
Sbjct 11557 MRHNYNKLHLPTTNRPKIAHCILFNQPHSPRSNSHSHPNPLKLHRRSHSHNRPRAYILIT 11736

Query 61     ILPSKCLKLRTHSQSHHNPLSRTSNSTPTNSFLMTSSKPR 99
             ILPSKCLKLRTHSQSHHNPLSRTSNSTPTNSFLMTSSKPR
Sbjct 11737 ILPSKCLKLRTHSQSHHNPLSRTSNSTPTNSFLMTSSKPR 11853

>Homo sapiens chromosome 5, GRCh38.p13 Primary Assembly
Sequence ID: NC_000005.10 Length: 181538259
Range 1: 134926946 to 134927239

Score:132 bits(331), Expect:3e-35,
Method:Compositional matrix adjust.,
Identities:87/98(89%), Positives:91/98(92%), Gaps:0/98(0%)

Query 1      MRHNYNKLHLPTTNRPKIAHCMLFnqphsprsnshshnpLKLHRRSHSHNRPRAYILIT 60
             +RHNYNKLHLP TNRPKIAHC+LFNQPHSPRSNSH HPNPLKLHRRSHSH+RPR YILIT
Sbjct 134927239 VRHNYNKLHLPATNRPKIAHCILFNQPHSPRSNSHHPNPLKLHRRSHSHSRPTYILIT 134927060

Query 61     ILPSKCLKLRTHSQSHHNPLSRTSNSTPTNSFLMTSSKPR 98
             ILPSKCLKLRT+ QSHHN LSRTSN TPTNS LMTSSKPR
Sbjct 134927059 ILPSKCLKLRTYPQSHHNSLSRTSNPTPTNSLLMTSSKPR 134926946

>Homo sapiens chromosome 2, GRCh38.p13 Primary Assembly
Sequence ID: NC_000002.12 Length: 242193529
Range 1: 120214413 to 120214703

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Method:Compositional matrix adjust.,
Identities:49/97(51%), Positives:68/97(70%), Gaps:0/97(0%)

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             MR+ Y+KL+L TTN+ KI +C+L ++P+S +S+ HP PLKL+R SH +N PR Y ++
Sbjct 120214413 MRNGYDKLYLSTTNQSKITYCLLLHKPYSTCYHSPHPKPLKLYRCSHPYNCPRTYFILI 120214592

Query 61     ILPSKCLKLRTHSQSHHNPLSRTSNSTPTNSFLMTSSKPR 97
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Sbjct 120214593 ILPSKFELRANYPNHYTPRPSNTASTNSLLMTSSKPR 120214703

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Figure S4. Comparison of MTALTND4 peptide encoded in mitochondrial DNA (mtDNA) and hypothetically in nuclear DNA that have been transferred from mtDNA (NUMT) through evolution. Only sequences with >50% identities and complete without stop codons are shown.

A

Human	MRHNYNKLHLPTTNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHSQSHHNPLS	80
Chimpanzee	MRHNNHKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRNYPHNRPRTYILITILPSKLLRTHPQSHHNSLP	80
Bonobo	MRHNNHKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRTYILITILPSKLLRTHPQSHHNSLP	80
Gorilla	MGYNHKLHLPTATNRPKIAHCMLLN*PHSPCSSYPYPNPPLALERRSCSYNCPRTYIIIIILPSKLLRTHPQSHHNSLS	80
Orangutan	MRHNNHKLHLPTTNRPKITHRMLLSQPHSACSSYPHSNPPLALERRNHHPNCWTHILPTVILPSKLLRTHPQSHHNSLS	80
Zebrafish	MRSHNRISLSTTNRLKIANCLFISRPGYTSRRNRFDSPNPLIYRSNYFNRRPRTNILRPIILSHI*VWTNS*PDHNPRS	80
Bear	MGNHNYKLYLPTPNRFKVPNCLFLC*SHSPSHCSSINPNPMKLYRSNSLNRRPRTNILLTILSCKLQLWNP*PNYDPA	80
Lion	MRNSHDKFYLPSTPDGPEILNRMLISKPHSPNCSCCTNPNALIYRSYSPYNNRSRTNLLSTILSCKLQLRTP*PNNNSST	80
Dog	MRHNNHKLHLPPSNRSKIIPNCMLLS*SYGTGYRSGSYNTMLYRCSSNNCPWFNLNTILLSQLQLRTP*PYNSRT	80
Rat	MRHNYN*LNLLPTPNRSKIINCLLISKPYSPSHSHYNDTMALERSYNTNRRPRNLTLILPSKHQLRTPNSQPNYNS	80
Mouse	MRNNYN*LNLLPTPNRFKITNCLLLS*PHSTCYCINHNPNMSMLERSNNINRRPWPHIITPILPSKLLRTHPQSHHNSLP	80
Horse	VRDNH*FHLPTATNRPKIETCYMLLCQPHSPNRSRPHNTMLYRSYSPNNRRPRYIINTILPSKLLRTHPQSHHNSLP	80
Naked Mole Rat	MRNSYNELHLPTANRPKITPYCLLHQPHSPCNHSPHSNTMLERSHNLNRRPRPHIIPILPSKLLRTHPQSHHNSLP	80
Dolphin	MCNNHMQFYLPSTNRPKITNRMLLRQPHSTRHSCPYNPPLALERSYRLNRCPRPYILHTILPSKLLRTHPQSHHNSLP	80
Cow	MRHNYNQLNLPPSNGPKITHRMLLCKPHSTRYRSHYPDTLALERSNRPYDCRPHILHTILPSKLLRTHPQSHHNSLP	80
Human	RTSNSTPTNSFLMTSSKPR-----	99
Chimpanzee	RTSNSTPTNSLLMTSPKPR-----	99
Bonobo	RTSNSTPTNSLLMTSPKPRQPRTPHH-----	107
Gorilla	RTPNPTPTNSPLMTSGKPRQPRTPHH-----	107
Orangutan	RPSNPTPTNSPLMTSSKPR-----	99
Zebrafish	RTPDSTATNSMVIYC-----	96
Bear	RTDTSPTYSGLMTTGKPH-----	99
Lion	RPNHPPNSCLMTTSGPREGPAPYH-----	107
Dog	RTSNSPSPNSLMTTISKPHKSGSPNN-----	107
Rat	RTNNLSIDSNMTTISKLSQPTTTPN-----	107
Mouse	RTSNGLPYSHMTTDSKSS-----	99
Horse	RTSNTSPYSLMTTISQNPQGPFSQH-----	107
Naked Mole Rat	RTTNHPSPSALMTPS-----	96
Dolphin	RTNLFTSNLYMTTISMLNKPCTSYH-----	107
Cow	RPTNAPSTNSHMTTISKSNQLSSTPNQLNWRITCSNVNLFMI	123

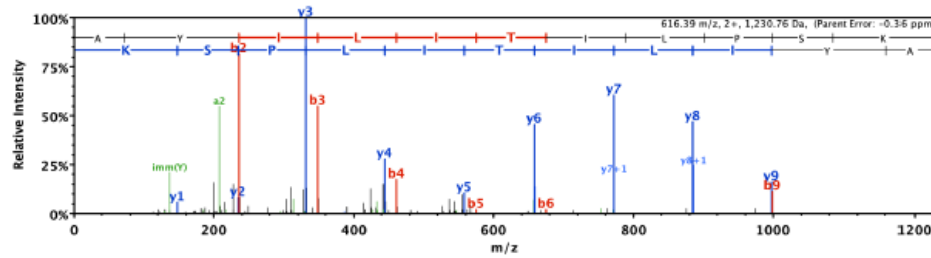
G, A, V, L, I
F, Y, W
C, M
S, T
R, H, E
D, E
N, Q
P

B

neanderthal_Mezmaisk	MGHNYNKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHPQSHHNSLP	80
neanderthal_GoyetQ57	MGHNYNKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHPQSHHNSLP	80
neanderthal_DC1227	MGHNYNKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHPQSHHNSLP	80
neanderthal_LesCotte	MGHNYNKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHPQSHHNSLP	80
neanderthal_Denisova	MRHNYNKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHPQSHHNSLP	80
heidelbergensis	MRHNYNKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHPQSHHNSLP	80
ModernHuman	MRHNYNKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRSHSHNRPRAYILITILPSKLLRTHPQSHHNSLP	80
Chimpanzee	MRHNNHKLHLPTATNRPKIAHCMLFQPHSPRSNSHSHPNPLALERRNYPHNRPRTYILITILPSKLLRTHPQSHHNSLP	80
neanderthal_Mezmaisk	RTSNSTPTNSFLMTSSKPR	99
neanderthal_GoyetQ57	RTSNSTPTNSFLMTSSKPR	99
neanderthal_DC1227	RTSNSTPTNSFLMTSSKPR	99
neanderthal_LesCotte	RTSNSTPTNSFLMTSSKPR	99
neanderthal_Denisova	RTSNSTPTNSFLMTSSKPR	99
heidelbergensis	RTSNSTPTNSFLMTSSKPR	99
ModernHuman	RTSNSTPTNSFLMTSSKPR	99
Chimpanzee	RTSNSTPTNSLLMTSPKPR	99

Figure S5. Multiple MTALTND4 peptide sequence alignments. **A.** In 15 mammal species: human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*), gorilla (*Gorilla gorilla*), orangutan (*Pongo pygmaeus*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), naked mole rat (*Heterocephalus glaber*), dog (*Canis lupus familiaris*), cow (*Bos taurus*), zebrafish (*Danio rerio*), lion (*Panthera leo*), bear (*Ursus arctos*), horse (*Equus caballus*), dolphin (*Tursiops truncatus*). **B.** In the genus *Homo* (including chimpanzee): *Homo heidelbergensis* (Sima de los Huesos; KF683087.1); *Homo sapiens neanderthalensis* (Denisova 15; MK033602.1); *Homo sapiens neanderthalensis* (Les cottes; MG025536.1); *Homo sapiens neanderthalensis* (Mezmaiskaya 2; MG025537.1); *Homo sapiens neanderthalensis* (DC1227; KU131206.2); *Homo sapiens neanderthalensis* (GoyetQ57-2; KX198088.1); modern human (*Homo sapiens*), chimpanzee (*Pan troglodytes*). *Indicates a stop codon.

AYILITILPSK



Mass spectrum of the 657.99 m/z precursor ion. The x-axis represents m/z from 0 to 1750, and the y-axis represents relative intensity from 0% to 100%. The spectrum shows a series of peaks labeled with b and y fragment ions. The base peak is at m/z 754.12, labeled y13+2H. Other significant peaks include y13 at 1498.12 and y12 at 1356.12. The x-axis ranges from 0 to 1750 m/z, and the y-axis ranges from 0% to 100% relative intensity.

Figure S6. Endogenous MTALTND4 detected in HeLa cells by mass spectrometry. Unique mass spectrometry MTALTND4-derived peptides detected in HeLa cells lysates. Probabilities = 100%.

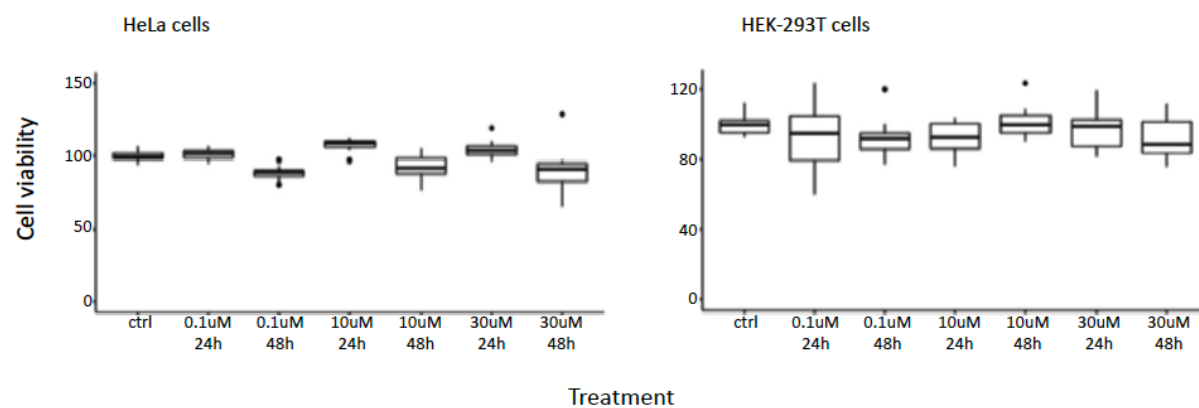


Figure S7. Effect of MTALTND4 on cell viability. HeLa and HEK-293T cells were cultured in low glucose DMEM with 0.1 μ M, 10 μ M or 30 μ M MTALTND4 peptide or water (control) for 24h or 48h and assessed for cell viability using the alamarBlue assay (n=3 for each cell line).

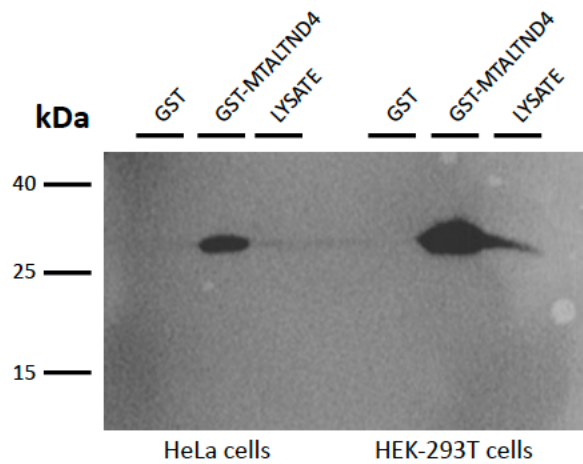


Figure S8. GST pull-down assay indicating that MTALTND4 and Complement component 1 Q interact. Western blot probed with anti-C1qbp antibodies. Lane 1: pull-down products from GST bound glutathione beads (HeLa cells). Lane 2: pull-down products from GST-MTALTND4-bound glutathione beads (HeLa cells). Lane 3: HeLa cells lysate. Lane 4: pull-down products from GST bound glutathione beads (HEK-293T cells). Lane 5: pull-down products from GST-MTALTND4-bound glutathione beads (HEK-293T cells). Lane 6: HEK-293T cells lysate.

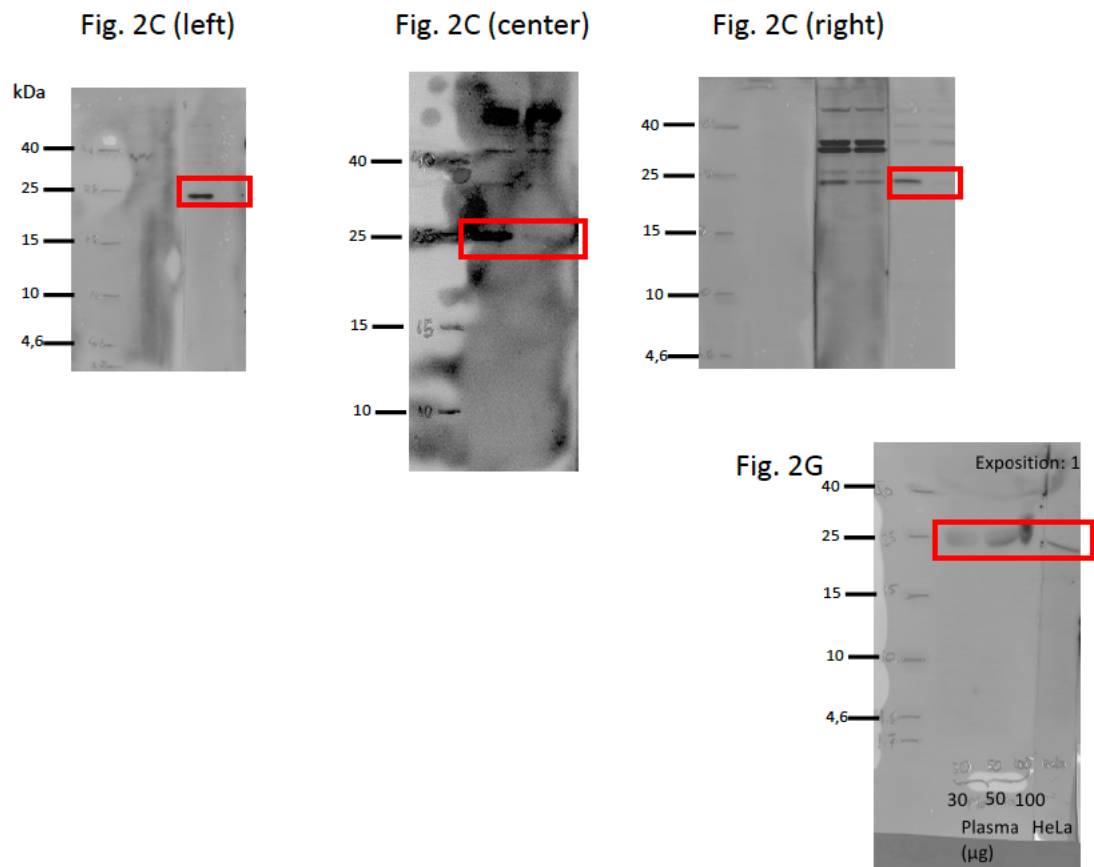


Figure S9. Original uncropped blots for MTALTND4.