Appendix

LUBAC assembles a signaling platform at mitochondria for signal amplification and transport of NF-κB to the nucleus

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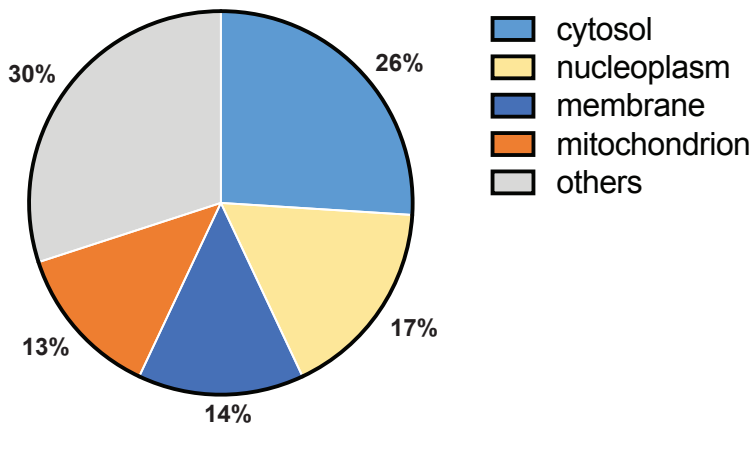
Table of contents:

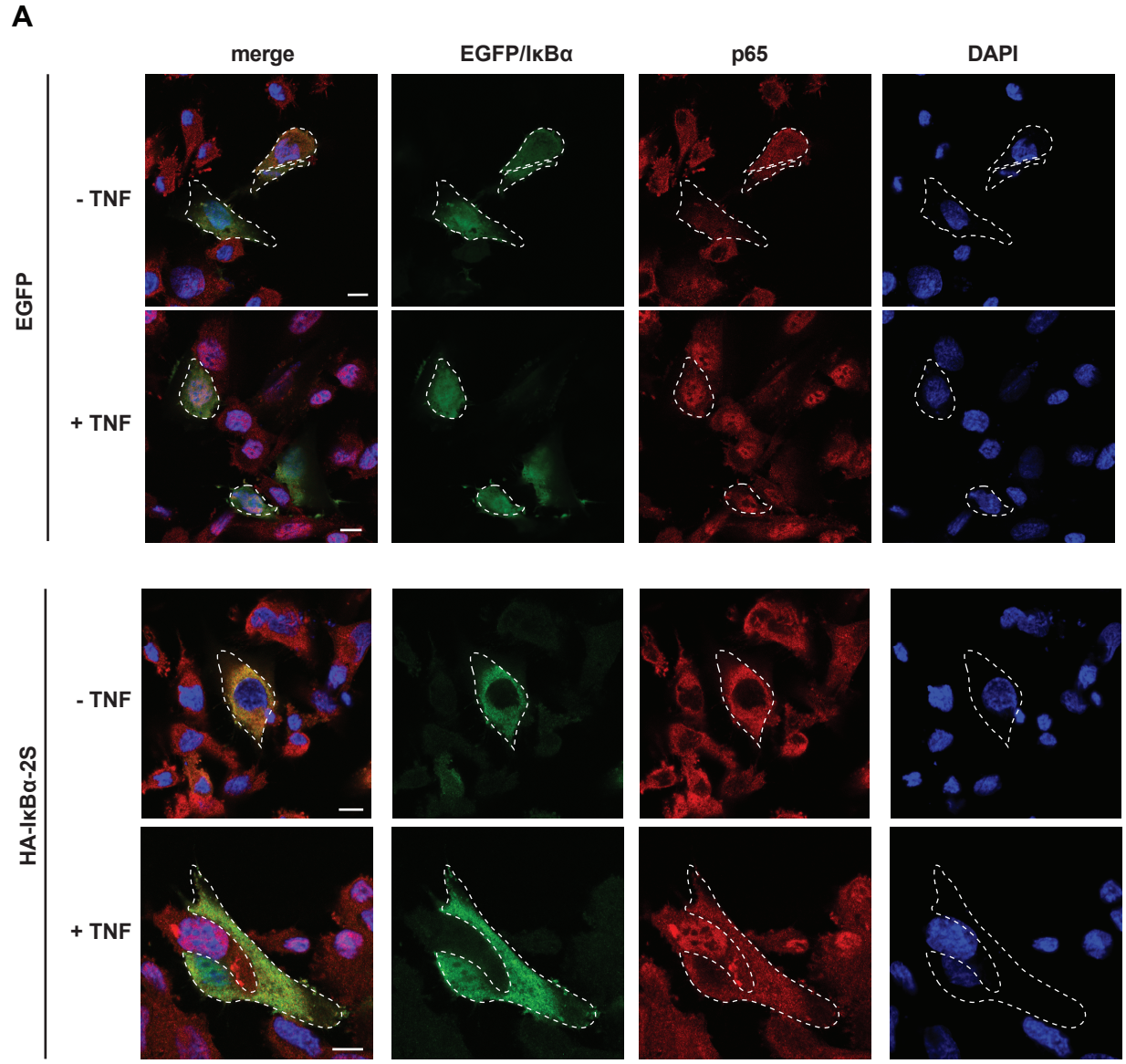
Appendix Figure S1: page 2
Appendix Figure S2: page 3
Appendix Figure Legends: page 4

A

Accession	Proteine Name	Gene Name	$\bar{X}$ iBAQ
Q96BN8	Ubiquitin thioesterase otulin	OTULIN	19.52
Q96L92	Sorting nexin-27	SNX27	18.75
O15347	High mobility group protein B3	HMGB3	16.69
Q16718*	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	NDUFA5	15.28
Q9Y3D3*	28S ribosomal protein S16	MRPS16	15.25
O14828	Secretory carrier-associated membrane protein 3	SCAMP3	15.23
O00264*	Membrane-associated progesterone receptor component 1	PGRMC1	15.21
P40616	ADP-ribosylation factor-like protein 1	ARL1	15.19
P62495	Eukaryotic peptide chain release factor subunit 1	ETF1	15.14
P61421	V-type proton ATPase subunit d 1	ATP6V0D1	14.91
P20618	Proteasome subunit beta type-1	PSMB1	14.64
Q9HB71	Calcyclin-binding protein	CACYBP	14.58
P56556*	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6	NDUFA6	14.51
P51570	Galactokinase	GALK1	14.44
P08243	Asparagine synthetase [glutamine-hydrolyzing]	ASNS	14.39
Q9BTV4	Transmembrane protein 43	TMEM43	14.38
O15173	Membrane-associated progesterone receptor component 2	PGRMC2	14.27
P30040	Endoplasmic reticulum resident protein 29	ERP29	14.01
P00387*	NADH-cytochrome b5 reductase 3	CYB5R3	13.98
Q9Y3B4	Splicing factor 3B subunit 6	SF3B6	13.96
Q96EP0	E3 ubiquitin-protein ligase RNF31	RNF31	13.89
Q9HD45	Transmembrane 9 superfamily member 3	TM9SF3	13.64
P82664*	28S ribosomal protein S10	MRPS10	13.45
Q9UHI6	Probable ATP-dependent RNA helicase DDX20	DDX20	13.44
P50995	Annexin A11	ANXA11	13.41
O95470	Sphingosine-1-phosphate lyase 1	SGPL1	13.21
P46977	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A	STT3A	13.13
O00505	Importin subunit alpha-4	KPNA3	13.09
P31153	S-adenosylmethionine synthase isoform type-2	MAT2A	13.04
P28331*	NADH-ubiquinone oxidoreductase 75 kDa subunit	NDUFS1	12.93
O60841	Eukaryotic translation initiation factor 5B	EIF5B	12.91
O14734	Acyl-coenzyme A thioesterase 8	ACOT8	12.89
Q8NF37	Lysophosphatidylcholine acyltransferase 1	LPCAT1	12.83
P36507	Dual specificity mitogen-activated protein kinase kinase 2	MAP2K2	12.79
Q8IXH7	Negative elongation factor C/D	NELFCD	12.75
P82663*	28S ribosomal protein S25	MRPS25	12.66
Q01581	Hydroxymethylglutaryl-CoA synthase	HMGCS1	12.65
P48730	Casein kinase I isoform delta	CSNK1D	12.63
Q86W42	THO complex subunit 6 homolog	THOC6	12.62
Q9NRK6*	ATP-binding cassette sub-family B member 10	ABCB10	12.50
Q9H5Q4*	Dimethyladenosine transferase 2	TFB2M	12.49
P04350	Tubulin beta-4A chain	TUBB4A	12.32
Q96EY7*	Pentatricopeptide repeat domain-containing protein 3	PTCD3	12.16
Q96DV4*	39S ribosomal protein L38	MRPL38	12.15
Q9BUQ8	Probable ATP-dependent RNA helicase DDX23	DDX23	12.11
Q8N163	Cell cycle and apoptosis regulator protein 2	CCAR2	12.04
Q8WWY3	U4/U6 small nuclear ribonucleoprotein Prp31	PRPF31	11.90
P23921	Ribonucleoside-diphosphate reductase large subunit	RRM1	11.59
Q9Y6N5*	Sulfide:quinone oxidoreductase	SQRDL	11.52
Q06210	Glutamine--fructose-6-phosphate aminotransferase	GFPT1	11.49
Q05397	Focal adhesion kinase 1	PTK2	11.33
P25685	DnaJ homolog subfamily B member 1	DNAJB1	11.26
Q969Z0*	FAST kinase domain-containing protein 4	TBRG4	11.21
Q3SY69*	Mitochondrial 10-formyltetrahydrofolate dehydrogenase	ALDH1L2	11.10
O60701	UDP-glucose 6-dehydrogenase	UGDH	11.09
O94826*	Mitochondrial import receptor subunit TOM70	TOMM70A	10.89
Q13085	Acetyl-CoA carboxylase 1	ACACA	10.26
O75122	CLIP-associating protein 2	CLASP2	10.06
Q6P0Q8	Microtubule-associated serine/threonine-protein kinase 2	MAST2	9.90
Q29RF7	Sister chromatid cohesion protein PDS5 homolog A	PDS5A	9.82

B

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p65
DAPI
EGFP
- TNF
+ TNF
HA-IκBα-2S
- TNF
+ TNF

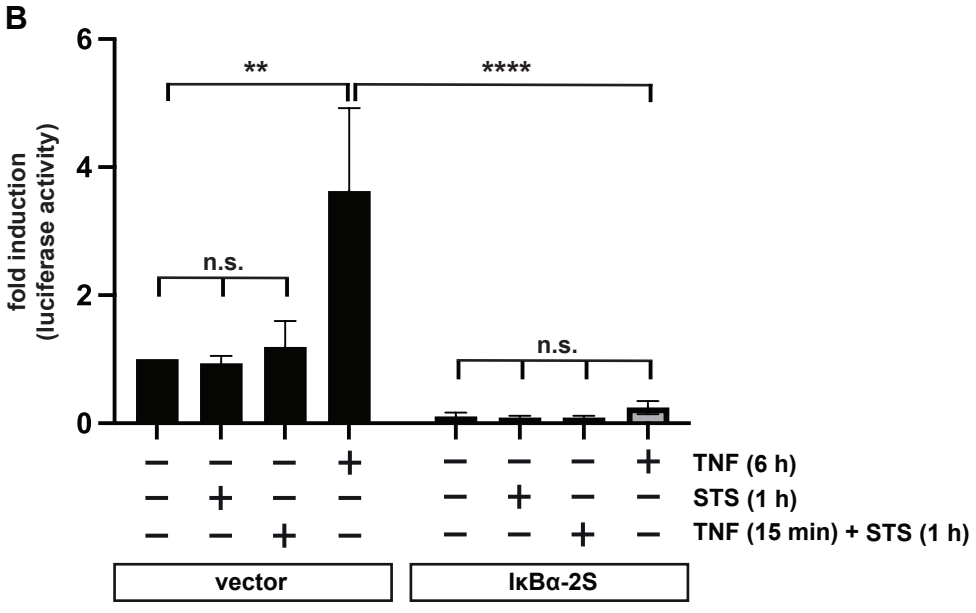
B
fold induction
(luciferase activity)
6
4
2
0
**
****
n.s.
n.s.
TNF (6 h)
STS (1 h)
TNF (15 min) + STS (1 h)
vector
IκBα-2S

Figure legends

Figure S1. Identification of OTULIN-interacting proteins by mass spectrometry

A. OTULIN-interacting proteins. Proteins identified by co-immunoprecipitation coupled to mass spectrometry analysis. Only proteins that were not identified in the control and that were reliably identified in both OTULIN co-immunoprecipitations by at least two unique peptides are listed according to their abundance (iBAQ-value). Mitochondrial proteins are marked with an asterisk.

B. Gene Ontology cellular compartment (GOCC) characterization of OTULIN-interacting proteins. The GOCC classifications for each of the identified proteins were performed using DAVID.

Figure S2. The NF-κB super suppressor IκBα-2S prevents NF-κB activation.

A. IκBα-2S interferes with p65 translocation upon TNF stimulation. Representative images of p65 immunofluorescence staining in SH-SY5Y cells expressing HA-tagged IκBα-2S or EGFP as a control. The cells were treated with or without TNF (25 ng/ml, 15 min) before fixation, and analyzed by immunocytochemistry and confocal microscopy using antibodies against p65 (red) and HA (green). Cell nuclei were stained by DAPI (blue).

B. IκBα-2S blocks TNF-induced NF-κB transcriptional activity. SH-SY5Y cells were transfected with an NF-κB luciferase reporter construct and HA-tagged IκBα-2S or vector (pcDNA3.1) as a control. 24 hours after transfection, the cells were treated with TNF (25 ng/ml) and/or STS (5 μM) for the indicated time before harvesting. Luciferase activity was quantified in cell lysates with luciferase activity in untreated control cells set to 1. Data represent the mean with standard deviation. Dunnett's multiple comparisons test, n=3 independent experiments, $^{**}p < 0.01$.