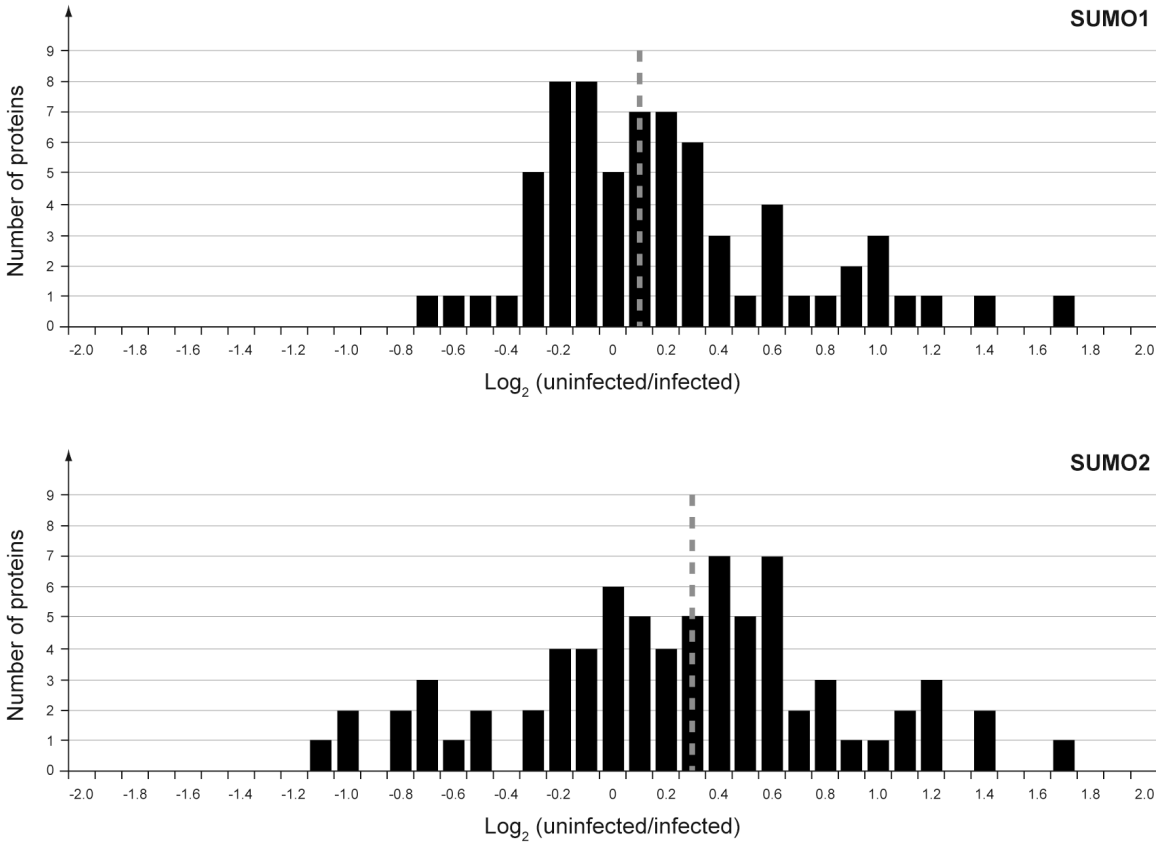
SUPPLEMENTARY FIGURES AND LEGENDS

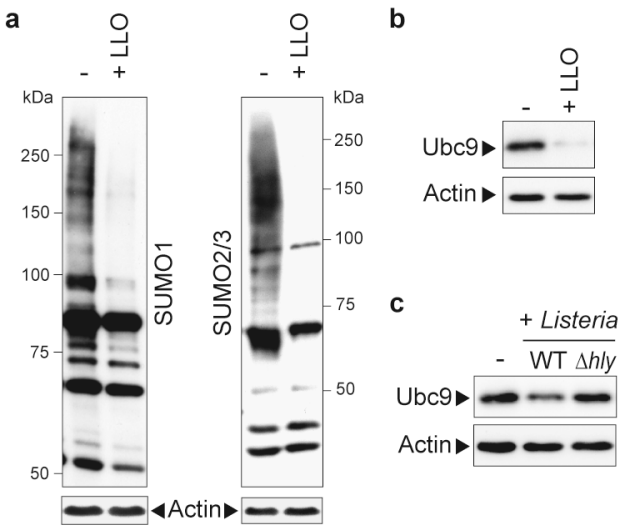


Supplementary Figure 1 : Proteomics analysis of SILAC-labeled SUMOylated proteins from uninfected cells and cells infected with *L. monocytogenes*.

HeLa cells transfected with His-tagged SUMO expression vectors (either SUMO1 or SUMO2 in two independent experiments) were cultivated in “heavy” or “light” DMEM arginine SILAC medium containing $^{13}C_6$ or $^{12}C_6$ arginine respectively. Cells cultured in the “heavy” medium were infected with a super invasive strain of *L. monocytogenes* (BUG1641[1]), whereas cells cultured in the “light” medium served as a control population. After 1 hour of infection, cells were harvested, mixed and lysed in buffer A (50 mM Na_2HPO_4/NaH_2PO_4, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS, 0.05% Tween 20, 10 mM imidazole, 20 mM N-ethylmaleimide). His-tagged SUMOylated proteins were then loaded on Ni-NTA magnetic agarose beads (QIAGEN), washed with buffer B (50 mM Na_2HPO_4/NaH_2PO_4, pH 8.0, 150 mM NaCl, 0.5% Na-deoxycholate, 0.1% SDS, 0.05% Tween 20, 20 mM imidazole, 20 mM N-ethylmaleimide) and eluted with buffer C (50 mM Na_2HPO_4/NaH_2PO_4, pH 8.0, 150 mM NaCl, 0.5% Na-deoxycholate, 0.1% SDS, 250 mM

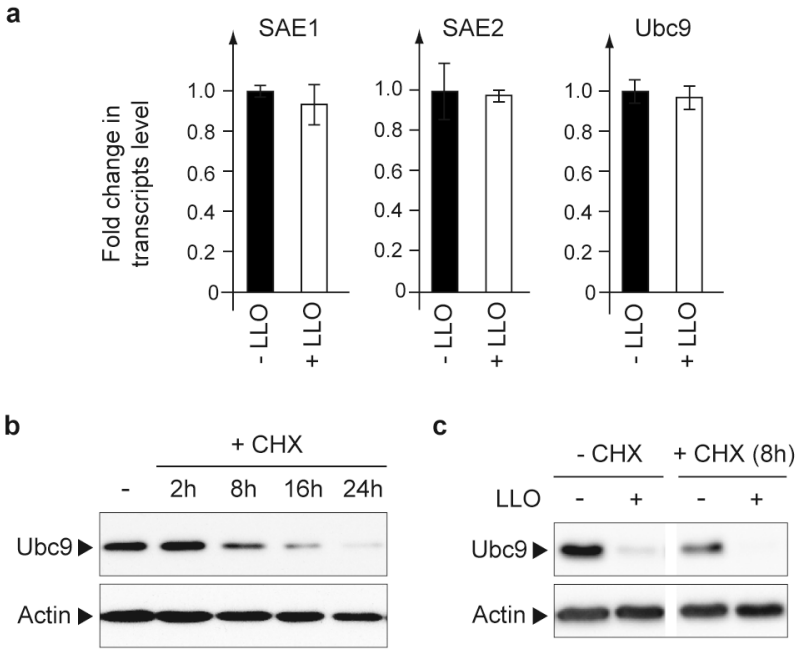
imidazole, 20 mM N-ethylmaleimide). The eluted protein mixture was dialyzed against a buffer composed of 50 mM Na_2HPO_4/NaH_2PO_4, pH 8.0, 150 mM NaCl and proteins were digested overnight with trypsin. For both analyses, the peptide mixtures were separated on RP-HPLC and the collected fractions were analysed by LC-MS/MS on an Agilent XCT ion trap instrument. Mascot searches were performed in the human Swiss-Prot and TrEMBL databases and identified peptides ending on arginine residues were quantified manually by calculating their light/heavy (=uninfected/infected) ratio from the MS peptide envelope intensities. As such, based on the average ratio of their peptides, 69 and 73 proteins were quantified in the SUMO-1 and SUMO-2 analysis respectively.

Histograms correspond to the distribution of the log_2 values of the uninfected/infected ratios of all quantified proteins. In both analyses, most proteins carried positive log_2 unifected/infected ratios. This is reflected by the log_2 median of all protein ratios which was found to be 0.16 and 0.33 for the SUMO1 and SUMO2 analysis respectively, indicating that less SUMOylated proteins were isolated form *L. monocytogenes* infected cells compared to uninfected cells.



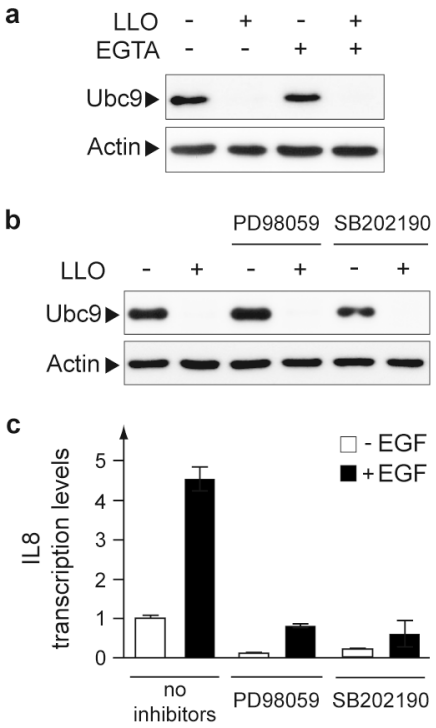
Supplementary Figure 2 : Effect of *Listeria* infection and LLO treatment on Jeg3 cells.

a, Decrease in SUMO1 and SUMO2/3-conjugated protein levels in Jeg3 cells (placental epithelial cells) incubated with 3 nM LLO for 20 min. b, Decrease in Ubc9 level in Jeg3 cells incubated with 3 nM LLO for 20 min. c, Ubc9 level is decreased in Jeg3 cells infected for 3 hours with *L. monocytogenes* (WT) but not in cells infected with a Δ*hly* mutant.



Supplementary Figure 3 : LLO acts on Ubc9 at the post-translational level.

a, The global transcriptional response of HeLa cells incubated with LLO for 20 min was analysed using human genome Affymetrix chips designed to quantify the expression level of 47,000 transcripts[2]. As highlighted in the figure, SAE1, SAE2 and Ubc9 genes were not differentially expressed in HeLa cells incubated with LLO compared to untreated cells (n=3; error bars, s.d.). b, Immunoblot analysis of HeLa cells treated with 100 μM cycloheximide (CHX), showing that Ubc9 is a relatively stable protein with a half-life >8 hours. c, Immunoblot analysis of HeLa cells pretreated with 100 μM DMSO (-CHX) or 100 μM cycloheximide (+CHX) for 8 hours, and incubated with 3 nM LLO for 20 min. Inhibition of cellular protein synthesis by cycloheximide does not prevent the decrease in Ubc9 level triggered by LLO.



Supplementary Figure 4 : LLO-mediated degradation of Ubc9 is independent of calcium influxes and MAPK activation.

a, Immunoblot analysis of HeLa cells incubated with 3 nM LLO for 20 min in control medium or in medium supplemented with 20 mM EGTA, a calcium chelator blocking Ca^{2+} influx induced by LLO pores. Blockage of calcium influx from extracellular medium does not prevent Ubc9 degradation upon treatment with LLO. b, Immunoblot analysis of HeLa cells pretreated for 1 hour with 50 μM PD98059 (an inhibitor of MEK1/2 kinases) or 10 μM SB202190 (an inhibitor of p38 MAPK) and incubated with 3 nM LLO for 20 min. Inhibition of MAPK activities does not prevent Ubc9 degradation upon treatment with LLO. c, Control of MAPK inhibitor activities by analysis of IL8 expression, a MAPK-regulated gene[3]. HeLa cells were treated with MAPK inhibitors (PD98059 and SB202190, as above) and then incubated with 50 µg/mL EGF for 30 minutes. RNA was isolated using the RNeasy kit (Qiagen), and cDNA was synthesized using 1 µg RNA in an RT reaction (iscript cDNA synthesis kit, Biorad). Quantitative PCR specific for IL8 (using 5'-ACTTTCAGAGACAGCAGAGCAC-3' and 5'-CCAGCTTGGAAGTCATGTTTAC-3' primers) or GAPDH, an internal control gene (using 5'-ACTCACCCTGCCCTCAATATC-3' and 5'-AGACAGTGTGCCTTTCATTCC-3' primers), were performed on a myIQ cycler (Biorad). Ratios of IL8/GAPDH transcription levels are indicated (means and standard deviations from qPCR duplicates).

SUPPLEMENTARY TABLES

Supplementary Table 1 : List of quantified proteins from the SILAC/SUMO1 experiment.

Accession	Start	End	Identified peptide	# spectra	peptide ratio (light/ heavy)	protein ratio (light/ heavy)	Protein name	isoforms
Q04446	566	576	QFHLTDDDLLR	1	0.71	0.71	1,4-alpha-glucan-branching enzyme	
P62263	129	141	IEDVTPIPSDSTR	1	2.10	2.10	40S ribosomal protein S14	
P23396	19	27	AELNEFLTR	1	1.03	1.03	40S ribosomal protein S3	
P62701	192	198	IGVITNR	1	0.97	0.97	40S ribosomal protein S4, X isoform	
P46776	95	105	TGAAPIIDVVR	1	0.96	0.96	60S ribosomal protein L27a	
O43707	67	79	AGTQIENIDEDFR	1	0.99	0.99	Alpha-actinin-4	
P08243	50	63	LAVVDPLFGMQPIR	1	1.22	1.22	Asparagine synthetase [glutamine-hydrolyzing]	
P14868	412	421	QSNSYDMFMR	1	2.68	2.68	Aspartyl-tRNA synthetase, cytoplasmic	
P25705	134	149	TGAIVDVPVGEELLGR	1	1.52	1.52	ATP synthase subunit alpha, mitochondrial precursor	
Q86XP3	548	560	DIPVLVATDVAAR	1	0.81	0.81	ATP-dependent RNA helicase DDX42	
P11586	761	772	TDTESELDLISR	2	0.86	0.86	C-1-tetrahydrofolate synthase, cytoplasmic	
O75534	65	77	VGDDVEFEVSSDR	2	1.13	1.13	Cold shock domain-containing protein E1	
P17812	467	477	LYGDADYLEER	1	1.79	1.79	CTP synthase 1	
Q07065	229	240	DFTSLENTVEER	1	1.01	1.17	Cytoskeleton-associated protein 4	
	455	473	LEGLGSSEADQDGLASTVR	2	1.34			
P49736	665	677	DTVDPVQDEMLAR	1	2.25	2.25	DNA replication licensing factor MCM2	
P11387	559	567	QPEDDLFDR	2	0.89	0.89	DNA topoisomerase 1	
P31689	351	372	EVEETDEMDQVELVDFDPNQER	1	1.12	1.12	DnaJ homolog subfamily A member 1	
Q9UBS4	207	217	TLEVEIEPGVR	1	0.92	0.92	DnaJ homolog subfamily B member 11 precursor	
P68104	256	266	IGGIGTVPVGR	1	1.31	1.31	Elongation factor 1-alpha 1	Q05639, Q5VTE0
P49411	92	102	YEEIDNAPEER	2	2.14	1.56	Elongation factor Tu, mitochondrial precursor	
	316	327	AEAGDNLGALVR	2	0.98			
P60842	325	334	VLITTDLLAR	1	1.25	1.25	Eukaryotic initiation factor 4A-I	Q14240
P05198	277	288	VVTDTDETELAR	1	1.30	1.30	Eukaryotic translation initiation factor 2 subunit 1	
P47756	182	195	SGSGTMNLGGSLTR	1	2.14	2.14	F-actin-capping protein subunit beta	
P21333	64	76	IANLQTDLSDGLR	1	1.00	1.12	Filamin-A	
	428	437	GTVEPQLEAR	1	1.01			
	1020	1032	VEPGLGADNSVVR	1	0.95			
	1072	1087	AFGPGLQGGSAGSPAR	2	1.51			
P04075	44	56	LQSIGTENTEENR	1	1.17	1.17	Fructose-bisphosphate aldolase A	
Q06210	164	176	ESQDTSFTTLVER	1	1.00	1.00	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	
P00367	125	136	DDGSWEVIEGYR	1	0.91	0.91	Glutamate dehydrogenase 1, mitochondrial precursor	P49448
P08107	37	49	TTPSYVAFTDTER	1	0.82	0.82	Heat shock 70 kDa protein 1	P11142, P17066, P34931, P48741, P54652
P08238	73	82	IDIIPNPQER	1	1.00	1.00	Heat shock protein HSP 90-beta	Q58FF7
P61978	423	433	IDEPLEGSEDR	1	1.17	1.17	Heterogeneous nuclear ribonucleoprotein K	
Q04695	322	334	ASLEGNLAETENR	1	3.45	3.45	Keratin, type I cytoskeletal 17	
P08729	215	226	VDALNDEINFLR	1	0.94	0.74	Keratin, type II cytoskeletal 7	
	332	342	LEAAIAEAEER	2	0.53			
P05787	329	341	ASLEAAIADAEQR	2	1.24	1.44	Keratin, type II cytoskeletal 8	
	353	362	LSELEAALQR	1	1.65			
P02545	12	25	SGAQASSTPLSPTR	2	1.26	1.33	Lamin-A/C	
	33	41	EDLQELNDR	2	1.53			
	51	60	SLETENAGLR	2	1.07			
	63	72	ITESEEVVSR	1	1.55			
	79	89	AAYEAELGDAR	1	1.40			
	157	166	TLEGELHDLR	1	0.94			
	209	216	NIYSEELR	1	1.99			
	241	249	LADALQELR	1	1.41			
	281	296	NSNLVGAAHEELQQSR	3	1.37			
	379	386	LLEGEEER	1	0.58			P20700, Q03252
	472	482	QNGDDPLLTYR	10	1.31			
	528	541	TALINSTGEEVAMR	4	1.65			
	628	644	SVGGSGGGSFGDNLVTR	2	1.23			
P35579	1878	1888	QLEEAEEEAQR	1	0.88	0.88	Myosin-9	
Q14697	115	127	VPDVLVADPPIAR	1	1.02	1.02	Neutral alpha-glucosidase AB precursor	
Q15233	76	91	LFVGNLPPDITEEEMR	9	1.17	1.15	Non-POU domain-containing octamer-binding protein	
	127	135	VELDNMPLR	2	1.33			
	257	270	FAQPGSFEYEYAMR	2	1.06			
	294	304	EKLEMEMEAAR	1	1.11			
	384	398	MGQMAMGGAMGINNR	4	1.07			
Q8WX93	477	488	QGSEIQDSPDFR	1	1.58	1.41	Palladin	
	1240	1252	LQNTGVADGYPVR	1	1.25			

O75400	777	789	EPAFEDITLESER	2	1.25	1.25	Pre-mRNA-processing factor 40 homolog A	
P17844	392	403	APILIATDVASR	1	1.07	1.07	Probable ATP-dependent RNA helicase DDX5	Q92841
P49756	639	649	FEDEDSDDVPR	1	1.59	1.59	Probable RNA-binding protein 25	
P29590	412	424	DPIDVDLPEEAER	1	1.25	1.25	Probable transcription factor PML	
Q02809	431	441	SEDYVDIVQGR	1	1.05	1.05	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 precursor	
P07737	76	89	DSLLQDGEFSMDLR	5	0.98	0.98	Profilin-1	
P49257	351	360	QLDMILDEQR	3	1.53	1.99	Protein ERGIC-53 precursor	
	449	456	DIDNLVQR	1	2.45			
Q15436	534	544	AETEEGPDVLR	1	1.23	1.23	Protein transport protein Sec23A	
Q9P035	133	146	LESEGSPETLTNLR	1	1.12	1.12	Protein tyrosine phosphatase-like protein PTPLAD1	
O43143	235	243	YMTDGMLLR	1	1.64	1.34	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	O60231, Q14562
	244	253	EAMNDPLLER	1	1.21			
	492	504	TEMQDNTYPEILR	1	1.18			
A6NKZ8	170	179	FPGQLNADLR	1	1.17	1.17	Putative tubulin beta chain-like protein ENSP00000290377	A6NNZ2, P04350, P07437, P68371, Q13509, Q13885, Q3ZCM7, Q99867, Q9BUF5, Q9BVA1, Q9H4B7
P08559	46	58	LEEGPPVTTVLTR	1	0.92	0.92	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial precursor	
P14618	33	43	LDIDSPPITAR	1	1.51	1.65	Pyruvate kinase isozymes M1/M2	
	93	106	TATESFASDPILYR	2	1.45			
	368	376	GDYPLEAVR	2	2.58			
	476	489	DPVQEAWAEDVDLR	1	1.06			
P46060	192	205	LENDGATALAEAFR	1	0.83	0.83	Ran GTPase-activating protein 1	
Q96EV2	786	798	TETEFPDEDEETR	1	1.55	1.55	RNA-binding protein 33	
P63165	55	63	QGVPMNSLR	10	0.54	0.65	Small ubiquitin-related modifier 1 precursor	
	64	70	FLFEGQR	4	0.76			
Q13126	12	29	IGIIGGTGLDDPEILEGR	1	1.30	1.15	S-methyl-5'-thioadenosine phosphorylase	
	272	282	NMAQFSVLLPR	1	1.00			
Q9Y6M7	138	148	FEEDVEDGGDR	1	2.46	2.46	Sodium bicarbonate cotransporter 3	
Q15637	228	240	QGIETPEDQNDLR	3	1.07	1.07	Splicing factor 1	
Q13435	112	123	VGEPVALSEEER	1	0.91	0.91	Splicing factor 3B subunit 2	
P23246	33	44	SPPPGMGLNQNR	1	1.38	1.06	Splicing factor, proline- and glutamine-rich	
	350	358	AELDDTPMR	2	1.03			
	400	407	AVVIVDDR	1	0.96			Q15233
	667	681	FGQGGAGPVGGQGPR	1	0.86			
Q9UBT2	108	119	QFILVMNALDNR	1	0.83	0.83	SUMO-activating enzyme subunit 2	
Q9BS26	271	285	EDTESLEIFQNEVAR	2	1.93	1.93	Thioredoxin domain-containing protein 4 precursor	
P52657	43	51	AINAALAQR	1	0.85	0.85	Transcription initiation factor IIA subunit 2	
P25490	110	122	EEVVGGDDSDGLR	4	0.95	0.95	Transcriptional repressor protein YY1	
P29401	284	302	ILATPPQEDAPSVDIANIR	1	0.88	0.88	Transketolase	
P68363	65	79	AVFVDLEPTVIDEVR	1	1.14	2.08	Tubulin alpha-1B chain	Q71U36, Q9BQE3
	113	121	EIIDLVLDR	1	3.03			Q71U36, Q9BQE3
P07437	63	77	AILVDLEPGTMDSVR	1	0.94	0.94	Tubulin beta chain	Q13509, Q13885, Q9BVA1
Q9Y3I0	322	329	SSMTFLTR	1	1.21	1.21	UPF0027 protein C22orf28	
P08670	105	113	VELQELNDR	1	1.22	1.00	Vimentin	P17661
	197	207	EEAENTLQSFR	2	0.79			
Q8NEW0	92	102	DNDAFSYGYVR	1	0.93	0.93	Zinc transporter 7	

Proteins are ordered alphabetically by protein name and then by increasing start position of the identified peptide. Columns from left to right contain the Uniprot accession number, the start and end position of the identified peptide in the protein sequence, the identified peptide, the number of MS/MS spectra by which the peptide was identified, the average light/heavy (=uninfected/infected) peptide ratio, the average light/heavy (=uninfected/infected) protein ratio, the protein name and possible protein isoforms indicating protein accessions in which the same peptide sequence was found.

Supplementary Table 2 : List of quantified proteins from the SILAC/SUMO2 experiment.

Accession	Start	End	Sequence	# spectra	peptide ratio (light/heavy)	protein ratio (light/heavy)	Protein name	isoforms
P23396	28	40	ELAEDGYSGVEVR	1	0.60	0.60	40S ribosomal protein S3	
P46776	95	105	TGAAPIIDVVR	4	1.29	1.29	60S ribosomal protein L27a	
P52209	120	136	GILFVGSGVSGGEEGAR	1	0.64	0.64	6-phosphogluconate dehydrogenase, decarboxylating	
P11021	186	197	DAGTIAGLNVMR	1	1.32	1.32	78 kDa glucose-regulated protein precursor	
P23526	21	34	ALDIAENEMPGLMR	1	1.56	1.56	Adenosylhomocysteinase	
O43488	51	61	MDAPASAAAVR	1	2.69	2.69	Aflatoxin B1 aldehyde reductase member 2	
O43707	240	255	MLDAEDIVNTARPDEK	1	1.55	1.55	Alpha-actinin-4	
A5A3E0	1060	1072	QEYDESGPSIVHR	2	1.13	1.13	ANKRD26-like family C member 1B	P60709, P63261, Q6S8J3, Q9BYX7
P14868	111	121	ESIVDVEGVVR	1	2.41	2.41	Aspartyl-tRNA synthetase, cytoplasmic	
Q92499	506	514	MDQAIIFCR	1	1.12	1.12	ATP-dependent RNA helicase DDX1	
Q86XP3	548	560	DIPVLVATDVAAR	1	1.32	1.38	ATP-dependent RNA helicase DDX42	
	606	615	DSNFAGDLVR	1	1.43			
P11586	505	517	TDPTTLTDEEINR	1	2.17	2.17	C-1-tetrahydrofolate synthase, cytoplasmic	
P48730	376	389	GAPVNISSSDLTGR	1	0.65	0.65	Casein kinase I isoform delta	
Q13409	92	112	SVSTPSEAGSQDSGDGAVGSR	1	2.00	2.00	Cytoplasmic dynein 1 intermediate chain 2	
Q07065	147	161	QREELGQGLQGVEQK	1	1.13	1.13	Cytoskeleton-associated protein 4	
O43175	76	90	AGTGVDNVDLEAATR	1	1.43	1.43	D-3-phosphoglycerate dehydrogenase	
P49736	665	677	DTVDPVQDEMLAR	2	2.76	2.76	DNA replication licensing factor MCM2	
P49411	92	102	YEEIDNAPEER	1	1.54	1.59	Elongation factor Tu, mitochondrial precursor	
	316	327	AEAGDNLGALVR	1	1.63			
P60842	178	190	MFVLDEADEMLSR	1	1.49	1.49	Eukaryotic initiation factor 4A-I	Q14240
P21333	64	76	IANLQTDLSDGLR	1	0.51	1.29	Filamin-A	
	1297	1312	VANPSGNLTETYVQDR	1	1.93			
	1453	1464	CSGPGLSPGMVR	1	1.42			
Q06210	346	359	EIFEQPESVVNTMR	1	1.62	1.62	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	
P00367	213	231	GFIGPGIDVPAPDMSTGER	1	1.71	1.71	Glutamate dehydrogenase 1, mitochondrial precursor	
P21266	74	82	ITQSNAILR	1	0.51	0.51	Glutathione S-transferase Mu 3	P28161, P46439
P08107	26	36	VEIIANDQGNR	1	0.81	0.91	Heat shock 70 kDa protein 1	P11021, P11142, P34931, P54652
	37	49	TTPSYVAFTDTER	1	1.01			P11142, P17066, P34931, P48741, P54652
P11142	160	171	DAGTIAGLNVLR	1	1.15	1.15	Heat shock cognate 71 kDa protein	
P61978	423	433	IDEPLEGSEDR	1	1.03	1.03	Heterogeneous nuclear ribonucleoprotein K	
O00470	181	190	MPIDLVIDDR	1	1.35	1.35	Homeobox protein Meis1	
P02533	224	232	VLDELTLAR	1	1.57	1.57	Keratin, type I cytoskeletal 14	P08727, P08779, P19012, Q04695
Q04695	31	41	LSGGLGAGSCR	1	1.25	1.34	Keratin, type I cytoskeletal 17	
	322	334	ASLEGNLAETENR	1	0.85			
	377	385	LEQEIATYR	1	1.93			P02533, P08727, P08779, P13646, P19012, P35900
P05787	329	341	ASLEAAIADAEQR	1	0.63	0.63	Keratin, type II cytoskeletal 8	
P02545	12	25	SGAQASSTPLSPTR	2	1.39	1.27	Lamin-A/C	
	29	41	LQEKEDLQELNDR	1	1.10			
	33	41	EDLQELNDR	6	1.14			
	51	60	SLETENAGLR	2	1.31			
	63	72	ITESEEVVSR	12	1.42			
	79	89	AAYEAELGDAR	2	1.27			
	124	133	EGDLIAAQAR	2	0.98			
	181	189	KQLQDEMLR	1	2.74			
	182	189	QLQDEMLR	3	1.21			
	209	216	NIYSEELR	1	0.90			
	241	249	LADALQELR	4	1.24			
	322	329	DLEDSLAR	1	1.41			
	379	386	LLEGEEER	1	1.11			P20700, Q03252
	472	482	QNGDDPLLTYR	9	1.24			

	528	541	TALINSTGEEVAMR	4	0.65			
	628	644	SVGGSGGGSFGDNLVTR	2	1.19			
Q14847	60	73	QSFTMVADTPENLR	1	0.61	0.61	LIM and SH3 domain protein 1	
P00338	158	169	VIGSGCNLDSAR	1	1.52	1.14	L-lactate dehydrogenase A chain	P07195, P07864, Q6ZMR3
	306	315	VTLTSEEEAR	3	0.77			
Q14697	192	206	DPAEGDGAQPEETPR	1	1.22	1.22	Neutral alpha-glucosidase AB precursor	
Q15233	76	91	LFVGNLPPDITEEEMR	4	1.10	1.67	Non-POU domain-containing octamer-binding protein	
	127	135	VELDNMPLR	1	1.17			
	457	468	AAPGAEFAPNKR	1	2.75			
Q7Z417	92	104	TGYGELNGNAGER	2	1.17	1.02	Nuclear fragile X mental retardation-interacting protein 2	
	110	122	NLSSDEATNPISR	1	0.87	1.02		
Q96ST3	156	167	SQSIDTPGVISR	1	0.53	0.53	Paired amphipathic helix protein Sin3a	
Q8WX93	477	488	QGSEIQDSPDFR	1	0.64	1.16	Palladin	
	1090	1098	LMVQAVNQR	1	1.84			
	1240	1252	LQNTGVADGYPVR	3	1.01			
Q8WXF1	105	115	YGEPSEVFINR	1	1.14	1.14	Paraspeckle component 1	
Q96HC4	306	324	ANNSQEPSPQLASSVASTR	1	2.38	2.38	PDZ and LIM domain protein 5	
Q8WUA2	48	61	DFIIQTGDPTGTGR	2	1.24	1.24	Peptidyl-prolyl cis-trans isomerase-like 4	
Q06830	141	151	QITVNDLPVGR	1	0.89	0.89	Peroxiredoxin-1	P32119
Q9NSD9	537	547	ASEGPAFFPGR	1	0.49	0.49	Phenylalanyl-tRNA synthetase beta chain	
O15067	528	541	ELSDPAGAIIYTSR	1	0.86	0.86	Phosphoribosylformylglycinamidine synthase	
O75400	777	789	EPAFEDITLESER	1	2.31	2.31	Pre-mRNA-processing factor 40 homolog A	
P07737	76	89	DSLLQDGEFSMDLR	3	0.90	0.90	Profilin-1	
P49257	351	360	QLDMILDEQR	4	1.49	1.82	Protein ERGIC-53 precursor	
	449	456	DIDNLVQR	1	2.15			
Q96RT1	1116	1125	TPPMMPGSQR	1	1.00	1.00	Protein LAP2	
P55735	44	54	NGGQILIADLR	1	2.05	2.05	Protein SEC13 homolog	
Q9P035	133	146	LESEGSPETLTNLR	3	0.98	0.98	Protein tyrosine phosphatase-like protein PTPLAD1	
O95070	251	265	TAALGPDSMGGPVPR	1	1.51	1.51	Protein YIF1A	
Q7L2E3	587	598	LVLMSATGDNER	1	1.75	1.75	Putative ATP-dependent RNA helicase DHX30	
O43143	146	152	FTDILVR	2	1.60	1.61	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	
	187	194	GVACTQPR	1	2.78			
	235	243	YMTDGMLLR	3	1.29			O60231, Q14562
	244	253	EAMNDPLLER	2	1.09			
	492	504	TEMQDNTYPEILR	3	1.29			
P11498	929	942	AEAEAQAEELSFPR	1	1.42	1.42	Pyruvate carboxylase, mitochondrial precursor	
P08559	46	58	LEEGPPVTTVLTR	3	0.82	0.82	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial precursor	
P14618	33	43	LDIDSPPITAR	2	1.38	1.35	Pyruvate kinase isozymes M1/M2	
	368	376	GDYPLEAVR	2	1.31			
P46060	44	55	EIEDFDSLEALR	1	1.02	1.02	Ran GTPase-activating protein 1	
Q96EV2	786	798	TETEFPDEDEETR	1	1.00	1.00	RNA-binding protein 33	
Q99985	56	65	ILLMDEDQDR	1	2.22	2.22	Semaphorin-3C precursor	
Q13126	100	116	EEIQPGDIVIIDQFIDR	2	0.88	0.88	S-methyl-5'-thioadenosine phosphorylase	
Q15637	228	240	QGIETPEDQNDLR	2	1.34	1.34	Splicing factor 1	
Q13435	112	123	VGEPVALSEEER	1	1.40	1.17	Splicing factor 3B subunit 2	
	126	140	LAQQQAALLMQQEER	1	0.95			
P23246	280	286	ANLSLLR	1	1.14	1.32	Splicing factor, proline- and glutamine-rich	
	400	407	AVVIVDDR	1	1.24			Q15233
	431	443	CSEGVFLLTTTPR	3	1.64			
	667	681	FGQGGAGPVGGQGPR	2	1.27			
Q9BS26	141	148	SDPIQEIR	1	0.70	0.98	Thioredoxin domain-containing protein 4 precursor	
	149	158	DLAEITTLDR	1	1.19			
	271	285	EDTESLEIFQNEVAR	1	1.05			
P52657	43	51	AINAALAQR	1	1.77	1.77	Transcription initiation factor IIA subunit 2	
P25490	110	122	EEVVGGDDSDGLR	1	1.31	1.31	Transcriptional repressor protein YY1	
Q9UNL2	9	22	QQSEEDLLLQDFSR	1	0.71	0.71	Translocon-associated protein subunit gamma	
Q14166	241	252	DFAYGETDPLIR	1	1.57	1.57	Tubulin--tyrosine ligase-like protein 12	
P08670	105	113	VELQELNDR	2	1.19	1.07	Vimentin	P17661
	197	207	EEAENTLQSFR	1	1.06			
	295	304	FADLSEAANR	1	0.96			
O75083	371	389	MTVDESGQLISCSMDDTVR	1	1.42	1.42	WD repeat-containing protein 1	
Q9BV38	402	410	VTELEDEVR	2	0.71	0.71	WD repeat-containing protein 18	
Q9Y6M5	204	213	LDPADPENPR	1	1.05	1.05	Zinc transporter 1	
Q8NEW0	340	349	LIVAPDADAR	1	0.69	0.69	Zinc transporter 7	

Columns from left to right contain the same information as Supplementary Table 1.

Supplementary Table 3 : Hemolytic activities and membrane binding efficiencies of LLO variants.

LLO	Hemolytic activity (HU/μg)[a]	Binding efficiency (%)[b]
WT	67 ± 17	100
C484A	19 ± 5	109 ± 4
Y206A	6 ± 1	93 ± 8
W492A	<1	104 ± 10

[a] Hemolytic activities of wild-type LLO and LLO variants were determined as previously described[4]. Briefly, sheep red blood cells (RBCs) were incubated with serial dilutions of wild-type LLO or LLO variants. After 1 hour incubation at 37°C, the percentage of RBCs lysis was estimated by measuring the OD_{540} of the cells supernatant. One hemolytic units (HU) corresponds to the quantity of toxin leading to the complete lysis of $1.5x10^8$ RBCs (means ± standard deviations from 3 independent experiments are indicated).

[b] Binding efficiencies of wild type LLO and LLO variants. 10^6 sheep red blood cells were incubated with 1 μg of wild type or mutant LLO for 30 min at room temperature. Cells were then lysed in 10 mM Na-phosphate buffer pH 7.4 and centrifuged 10 min at 1000 rpm. Supernatants containing red blood cell membranes were then ultracentrifuged 1 h at 200 000 rpm at 4°C to pellet cellular membranes. Pellets were then washed in 10 mM Na-phosphate buffer pH 7.4 and ultracentrifuged again 1 h at 200 000 rpm at 4°C. Finally, pellets were resuspended in Laemmli buffer, loaded for SDS-PAGE, and immunoblotted using a polyclonal rabbit anti-LLO antiserum. Binding efficiencies, corresponding to the proportion of LLO recovered in the pellet fractions, are indicated as percentage of wild-type LLO binding efficiency (means ± standard deviations from 3 independent experiments are indicated).

SUPPLEMENTARY REFERENCES

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