

Supplementary Material

Table S1. Putative SUMOylation sites in published proteins involved in *Entamoeba histolytica* phagocytosis.

Function	Protein	Number access	Position	Peptide/sequence	Score	Site	Reference
Retromer	Vacuolar sorting protein 29	EHI_025270	3 - 7	*****ML VLVIG DFHVPHR	64.072	SUMO Interaction	
			56 - 60	LRTLARE VHVVK GDFDEM Q	65.27	SUMO Interaction	[1]
			60	AREVHVV K GDFDEM Q	44.952	Sumoylation	
			110 - 114	QRQLDVD ILITG HTHKLET	60.6	SUMO Interaction	
			173	TLVDGSV K CERVDFN	45.097	Sumoylation	
	Vacuolar sorting protein 26	EHI_062490	9-13	AFLFGTP IQIDI LLDNDHE	72.761	SUMO interaction	
			40	TEIPIY M KNEDVNGK	30.527	Sumoylation	
			145 - 149	GLSKEQE IWVIN YQDEPTK	74.151	SUMO interaction	
			238	LMDGAPV K GESMPIR	43.592	Sumoylation	
			342	PQQSEEP K EEIKEPV	21.38	Sumoylation	[1]
			362	QSIQEEP K EEIKEEK	21.38	Sumoylation	
			366	EEPKEE I EEKKEEP	45.815	Sumoylation	
			374	EEKKEEP K EEVKEE I	21.902	Sumoylation	
			378	EEPKEEV K EEIKEEP	42.644	Sumoylation	

			382	EEVKEE I KEEPKEEV	47.766	Sumoylation	
			386	EEIKEEP K EEVKEP	21.902	Sumoylation	
			390	EEPKEEV K EKP KAID	39.231	Sumoylation	
			406	SSFIQDD K QDDDNLF	37.443	Sumoylation	
Vacuolar protein sorting 35	EHI_002990	462 - 466	86	TPLFMFL K DEYEGSL	25.295	Sumoylation	
			309	KVLVNFI K SEETTL	49.647	Sumoylation	
			476	QDQPDD I KEEEIGVD	48.626	Sumoylation	SUMO interaction [1]
			556	AISKMVA K LEHYVLA	17.505	Sumoylation	
			35	TSVMQYY K FSATVRT	25.383	Sumoylation	
			47 - 51	VRTYADG I ILAS LVGTIPI	56.467	SUMO interaction	[2]
ESCRT	EHI_178530	67 - 71	67 - 71	YRGSQFC L PLCI MYPYDYP	56.376	SUMO interaction	
			283	TLTPLNT K *****	38.49	Sumoylation	
			78	MISGERV K SDDPDKE	45.994	Sumoylation	
			128 - 132	EALQEAV I LPIK FPQLFTD	59.356	SUMO interaction	[2]
Vesicular trafficking	EHI_118900	231	231	TEASRRV K TEFLVQM	41.814	Sumoylation	
			82 - 86	YYRGSQG I LIVY DVSNFDS	63.59	SUMO Interaction	[3]
			105	TYWIGEL K KENVDGI	28.197	Sumoylation	
			83-87	FRGTDGC V LVCS VTSIESF	60.125	SUMO interaction	[4]
Actin related proteins	EHI_001870	120	PFVAVAN K SDLDPIE	39.109	Sumoylation		
			51 - 55	KEVIFKP V VIVR VPSEPSG	61.242	SUMO interaction	[5]
	Actin related protein 2/3 complex, subunit 4	EHI_030820					

EhABPH (actin-binding protein)	EHI_122800	422	RRSSVSL KPEHMKS I	26.543	Sumoylation	[6]
		523 - 527	TEEIEGK VGIVI EIDEESE	63.088	SUMO interaction	
		586	KQETKEV KEEEKTQR	44.223	Sumoylation	
		674 - 678	QLTSKGN VIHLA CKNEEEL	62.786	SUMO interaction	
		698	ACTPNNL KGEEADEE	24.332	Sumoylation	
		753 - 757	NRTDSTP ILKIY LKDIENI	61.532	SUMO interaction	
		787	KGNKHEF KTEECKAW	18.512	Sumoylation	
		795	TEECKAW KEEIMERK	24.947	Sumoylation	
		930	VILLTHG KDDRETDM	37.458	Sumoylation	
		973 - 977	QDKEEPE IIRIY RIVHNEK	61.19	SUMO interaction	
		1236 - 1240	KIFKGKF IVHLG GYSEYYD	63.428	SUMO interaction	
		1297 - 1301	VFKTIKG IEVIE GKDSNEE	67.442	SUMO interaction	
		1426	ERGLKEV KIEQENE E	42.552	Sumoylation	
		1467 - 1471	PVCQKKE IELIT QVIDGTF	60.742	SUMO interaction	
		1486	NEIDPAL KNEISVEG	25.939	Sumoylation	
EhNCABP166	EHI_093850	51 - 55	LKDLQDG IILSR YLQIETG	60.206	SUMO interaction	
		111 - 115	MNGEPKP ITLM FYLMYRF	61.066	SUMO interaction	
		129 - 133	FRLNDTP VRIID FSKWIKC	73.317	SUMO interaction	
		152	NNPLFDL KTEFSDGI	28.859	Sumoylation	[7]
		340	LEAMELV KEESSTPI	45.139	Sumoylation	

	455	ELIIQQQL K DEIIQLT	25.181	Sumoylation
	503 - 507	IQKKEQE IIDI K KKNEETI	79.148	SUMO interaction
	514 - 518	KKKNEET IQLIQ KEMEKER	64.693	SUMO interaction
	585	QQELNK I KEENNEYK	54.425	Sumoylation
	645	EIKEKEV K ELQKIIIE	38.869	Sumoylation
	721	MKELERIK G EIEVLK	41.473	Sumoylation
	724 - 728	LERIKGE IEVL K IKETGDS	61.956	SUMO interaction
	749 - 753	IEELKKQ IEII K NDNEKER	61.44	SUMO interaction
	877 - 881	INELNKS IIE LK EEWNKKE	59.355	SUMO interaction
	881	NKSIIEL K EEWNKKE	26.249	Sumoylation
	923	NENLKKV K EEIEKKT	43.876	Sumoylation
	1016	ENENEII K KENKKKE	49.683	Sumoylation
	1058 - 1062	ERELGTK IKL IIE MIKNEKD	60.142	SUMO interaction
	1065	IKLIEMI K NEKDIME	45.305	Sumoylation
	1076	DIMEKDF K KEVDNKN	18.489	Sumoylation
	1100 - 1104	IEKKKND ITL II QKNDEDK	66.441	SUMO interaction
	1129	NQEIEK I KSEKNDVQ	47.32	Sumoylation
	1168	ENEKLQ I KEEHENNI	43.513	Sumoylation
	1228	TKKYEEV K GQIDGAH	40.95	Sumoylation
	1242	HQEVENI K KETEQQI	42.417	Sumoylation
	1253	EQQINQM K KECDEM Q	29.943	Sumoylation
	1267	QKTTFEA K EELRVQV	16.67	Sumoylation

		1309	ERTIEKL K NERNEKE	26.905	Sumoylation	
		1354	VMPKEVL K IDNTMLY	37.908	Sumoylation	
Myosin heavy chains 1	EHI_140720	7-11	*MNKEEA I SHIQ SAIKEYC	67.784	SUMO interaction	[8]
		69	QNENA K LKNELQTKE	26.214	Sumoylation	
		183	ELADV K IKLDTTQQE	36.796	Sumoylation	
		366 - 370	KQKGDEE I IKLH DDLAEQA	62.69	SUMO interaction	
		463	AMD K ELMKEEK K QKE	30.951	Sumoylation	
		468	LMKEE K KL K QEIQALK	26.856	Sumoylation	
		711	DKEETIA K LEKDKNT	16.977	Sumoylation	
		787	RLDIVDL K GELESKE	25.353	Sumoylation	
		934 - 938	VADLENQ L E I I K DSIEEKD	62.341	SUMO interaction	
		994	KSKYDQL K SDNLMV L	38.597	Sumoylation	
		998 - 1002	DQLKSDN L M V LS EKEDIEE	61.696	SUMO interaction	
		1015	EEELSSV K EMTKME	44.303	Sumoylation	
		1134	DDDYVRMK K ADNDKIR	38.706	Sumoylation	
Cholesterol 1 trafficking	Phagosome- associated TMK96 (PATMK)	17 - 21	YILLFLS I E I VG EYCDWNK	67.129	SUMO interaction	[9]
		49 - 53	FNYNK TG V FI I R NRETETM	61.818	SUMO interaction	
		100 - 104	KELNTSF I VLIN DTTINGD	62.47	SUMO interaction	
		222	PSVLTIL K QEVVNST	27.292	SUMOylation	
		483	NQTRQCV K TDIHQE I	37.543	SUMOylation	
		818 - 822	SQCSQCS I GY I V NYNGTRC	60.948	SUMO interaction	

		951 - 955	YELTVSP ITIIL KKNEGCI	75.893	SUMO interaction		
		1082 - 1086	AVINKNN VSIIT EYAPYGS	61.53	SUMO interaction		
		1143 - 1147	RDIKLAN VLIIS LEDEMEI	67.864	SUMO interaction		
		1254 - 1258	ISLKIKS LLDLM WCQNPEN	61.691	SUMO interaction		
EhNPC1	EHI_080220	325 - 329	IGFIIMG IIVVG FPMEGVI	64.822	SUMO interaction		
		391 - 395	DIIDSAK VSILG QEESCEE	66.998	SUMO interaction		
		431 - 435	FLWKFKW IILTV VILCCIV	63.514	SUMO interaction		
		504 - 508	NQVLTQP LIVQL QQMIDEI	60.057	SUMO interaction	[10]	
		758 - 762	LAIGVDN IFILT NTIDEQP	66.525	SUMO interaction		
		1133	MNSIFDV KTFPYAYH	40.095	Sumoylation		
		1150 - 1154	YFQQYFN IVDLC VMDVCLA	60.804	SUMO interaction		
		1191 - 1195	LCVLMCV IDLIG IMYLWGV	60.091	SUMO interaction		
	EhNPC2a	EHI_068260	104	LPGPISIK FEMEIIPN	42.327	Sumoylation	[10]
Kinases	EhNPC2b	EHI_188770	143 - 146	SCSEFGP VLVK* *****	59.366	SUMO interaction	[10]
			146	EFGPVLV K*****	37.397	Sumoylation	
	Phosphatidylinositol phosphate kinase	EHI_153770	49	GKTLPELK NEKYIFK	25.986	Sumoylation	[11]
	p21-activated kinase 8 protein kinase	EHI_103610	151	FVIKVIP KREEKILC	21.671	Sumoylation	
			103 - 107	TLCEQPS ILLLM QNESDFN	62.875	SUMO interaction	[12]

			180 - 184	AVNGEIP L TILP FSDNVEI	65.13	SUMO interaction	
			312	QAHIEKPK K EPPEEE	21.484	Sumoylation	
EhC2PK	EHI_094060		67	KPKMRVA K LENTEQA	16.205	Sumoylation	[13]
			262 - 266	PGLKTGE V LVPQ EFTVTAK	59.917	SUMO interaction	
	EhCaBP1	EHI_120900	33	VSKKRA I KNEQLLQL	46.539	Sumoylation	[14]
Calcium related proteins	Cation/calcium exchanger	EHI_001770	46 - 50	VSFLINY L ELLY CYNSYWI	59.298	SUMO interaction	[15]
			57 - 61	YCYN SYW I VLVC MVIGIII	62.999	SUMO interaction	
			196 - 200	HSIIFIL I YILY VCFIGYI	60.578	SUMO interaction	
			336 - 340	PFILSIG I ILII HFTETQI	66.662	SUMO interaction	
Stress protein	Heat shock protein 70	EHI_002560	27	VAYFDVV K GEPVILQ	45.554	Sumoylation	
			230	IINDNY I KVESSGGD	48.879	Sumoylation	
			287	NKKLIR L KKEAERIK	26.186	Sumoylation	
			294	KKEAER I KIELSGKP	42.012	Sumoylation	
			300	IKIELSG K PDAELDL	36.684	Sumoylation	[16]
			366 - 370	KKGNVQL V LLIG GTCEMPR	61.124	SUMO interaction	
			423 - 427	NSICSKF I YDIV PTPIGIE	64.333	SUMO interaction	
Adhesin	Gal/GalNAc lectin heavy subunit	EHI_012270	206 - 210	TNTTSKC I DVH KNVNNTH	60.701	SUMO interaction	
			218 - 222	KNNVNNTH L AII L GITDSTV	60.45	SUMO interaction	
			255	GTIYYL K GDSYATD	37.476	Sumoylation	[17]
			270	NIKLKDL K YETLVKY	26.485	Sumoylation	

	437	PKAKPKA K KECPATC	16.996	Sumoylation
	552	VQEKVCV K TSPYIEM	41.585	Sumoylation
	970 - 974	IECKIQE IVITE KDGIKTT	60.791	SUMO interaction

The access number and sequence of proteins (already identified) that participate in *Entamoeba histolytica* phagocytosis were obtained from KEGG (<https://www.genome.jp/kegg>, 29/04/2021), AmoebaDB (<https://amoebadb.org/amoeba/app>, 29/04/2021) and NCBI (<https://www.ncbi.nlm.nih.gov>, 29/04/2021) databases. Then, these proteins were analysed using the GPS-SUMO 1.0 (<http://sumosp.biocuckoo.org>, 29/04/2021) program to predict SUMOylation sites.

The results displayed several hypothetical sites in the studied proteins, suggesting that this posttranslational modification could regulate their function during this virulence event.



- [1] Y. S.-N. Kumiko Nakada-Tsukui and V. A. and T. Nozaki, "A Retromerlike Complex Is a Novel Rab7 Effector That Is Involved in the Transport of the Virulence Factor Cysteine Protease in the Enteric Protozoan Parasite *Entamoeba histolytica*," *Mol. Biol. Cell*, vol. 16, no. November, pp. 5356–5372, 2005, doi: 10.1091/mbc.E05.
- [2] I. Lpez-Reyes *et al.*, "Detection of the endosomal sorting complex required for transport in *entamoeba histolytica* and characterization of the EhVps4 protein," *J. Biomed. Biotechnol.*, vol. 2010, pp. 1–15, 2010, doi: 10.1155/2010/890674.
- [3] R. Javier-Reyna, S. Montaño, G. García-Rivera, M. A. Rodríguez, A. González-Robles, and E. Orozco, "EhRabB mobilises the EhCPADH complex through the actin cytoskeleton during phagocytosis of *Entamoeba histolytica*," *Cell. Microbiol.*, vol. 21, no. 10, 2019, doi: 10.1111/cmi.13071.
- [4] Y. Saito-Nakano, R. Wahyuni, K. Nakada-Tsukui, K. Tomii, and T. Nozaki, "Rab7D small GTPase is involved in phago-trogocytosis and cytoskeletal reorganization in the enteric protozoan *Entamoeba histolytica*," *Cell. Microbiol.*, vol. 23, no. 1, 2021, doi: 10.1111/cmi.13267.
- [5] M. Babuta, M. S. Mansuri, S. Bhattacharya, and A. Bhattacharya, "The *Entamoeba histolytica*, Arp2/3 Complex Is Recruited to Phagocytic Cups through an Atypical Kinase EhAK1," *PLoS Pathog.*, vol. 11, no. 12, pp. 1–27, 2015, doi: 10.1371/journal.ppat.1005310.
- [6] A. Sateriale, A. Vaithilingam, L. Donnelly, P. Miller, and C. D. Huston, "Feed-forward regulation of phagocytosis by *entamoeba histolytica*," *Infect. Immun.*, vol. 80, no. 12, pp. 4456–4462, 2012, doi: 10.1128/IAI.00671-12.
- [7] A. D. Campos-Parra, N. A. Hernández-Cuevas, R. Hernandez-Rivas, and M. Vargas, "EhNCABP166: A nucleocytoplasmic actin-binding protein from *Entamoeba histolytica*," *Mol. Biochem. Parasitol.*, vol. 172, no. 1, pp. 19–30, 2010, doi: 10.1016/j.molbiopara.2010.03.010.
- [8] H. Voigt, J. C. Olivo, P. Sansonetti, and N. Guillén, "Myosin IB from *Entamoeba histolytica* is involved in phagocytosis of human erythrocytes," *J. Cell Sci.*, vol. 112, no. 8, pp. 1191–1201, 1999.
- [9] D. R. Boettner *et al.*, "Entamoeba histolytica phagocytosis of human erythrocytes involves PATMK, a member of the transmembrane kinase family," *PLoS Pathog.*, vol. 4, no. 1, pp. 0122–0133, 2008, doi: 10.1371/journal.ppat.0040008.
- [10] J. Bolaños *et al.*, "EhNPC1 and EhNPC2 Proteins Participate in Trafficking of Exogenous Cholesterol in *Entamoeba histolytica* Trophozoites: Relevance for Phagocytosis," *PLoS Pathog.*, vol. 12, no. 12, pp. 1–29, 2016, doi: 10.1371/journal.ppat.1006089.
- [11] S. Sharma, S. Bhattacharya, and A. Bhattacharya, "PtdIns(4,5)P2 is generated by a novel phosphatidylinositol 4-phosphate 5-kinase in the protist parasite *Entamoeba histolytica*," *FEBS J.*, vol. 286, no. 11, pp. 2216–2234, 2019, doi: 10.1111/febs.14804.
- [12] E. Labruyère, C. Zimmer, V. Galy, J. C. Olivo-Marin, and N. Guillén, "EhPAK, a member of the p21-activated kinase family, is involved in the control of *Entamoeba histolytica* migration and phagocytosis," *J. Cell Sci.*, vol. 116, no. 1, pp. 61–71, 2003, doi: 10.1242/jcs.00190.
- [13] Somlata, S. Bhattacharya, and A. Bhattacharya, "A C2 domain protein kinase initiates phagocytosis in the protozoan parasite *Entamoeba histolytica*," *Nat. Commun.*, vol. 2, no. 1, pp. 210–230, 2011, doi: 10.1038/ncomms1199.
- [14] S. Kumar *et al.*, "Crystal structure and trimer-monomer transition of N-terminal domain of EhCaBP1 from *entamoeba histolytica*," *Biophys. J.*, vol. 98, no. 12, pp. 2933–2942, 2010, doi: 10.1016/j.bpj.2010.03.048.
- [15] M. Valle-Solis *et al.*, "A Calcium/Cation Exchanger Participates in the Programmed Cell Death and in vitro Virulence of *Entamoeba histolytica*," *Front. Cell. Infect. Microbiol.*, vol. 8, no. October, p. 342, 2018, doi: 10.3389/fcimb.2018.00342.
- [16] O. S. b and M. D. Stephan Ortner, Barbara Plaimauer, Marina Binder, Gerhard Wiedermann, "Humoral immune response against a 70-kilodalton heat shock protein of *Entamoeba histolytica* in a group of patients with invasive amoebiasis," *Trends Parasitol.*, vol. 54, pp. 175–183, 2021, doi: 10.1016/j.pt.2021.01.001.
- [17] B. J. Mann, "Structure and function of the *Entamoeba histolytica* Gal/GalNAc lectin," *Int. Rev. Cytol.*, vol. 216, pp. 59–80, 2002, doi: 10.1016/S0074-7696(02)16003-7.