

Supplementary data

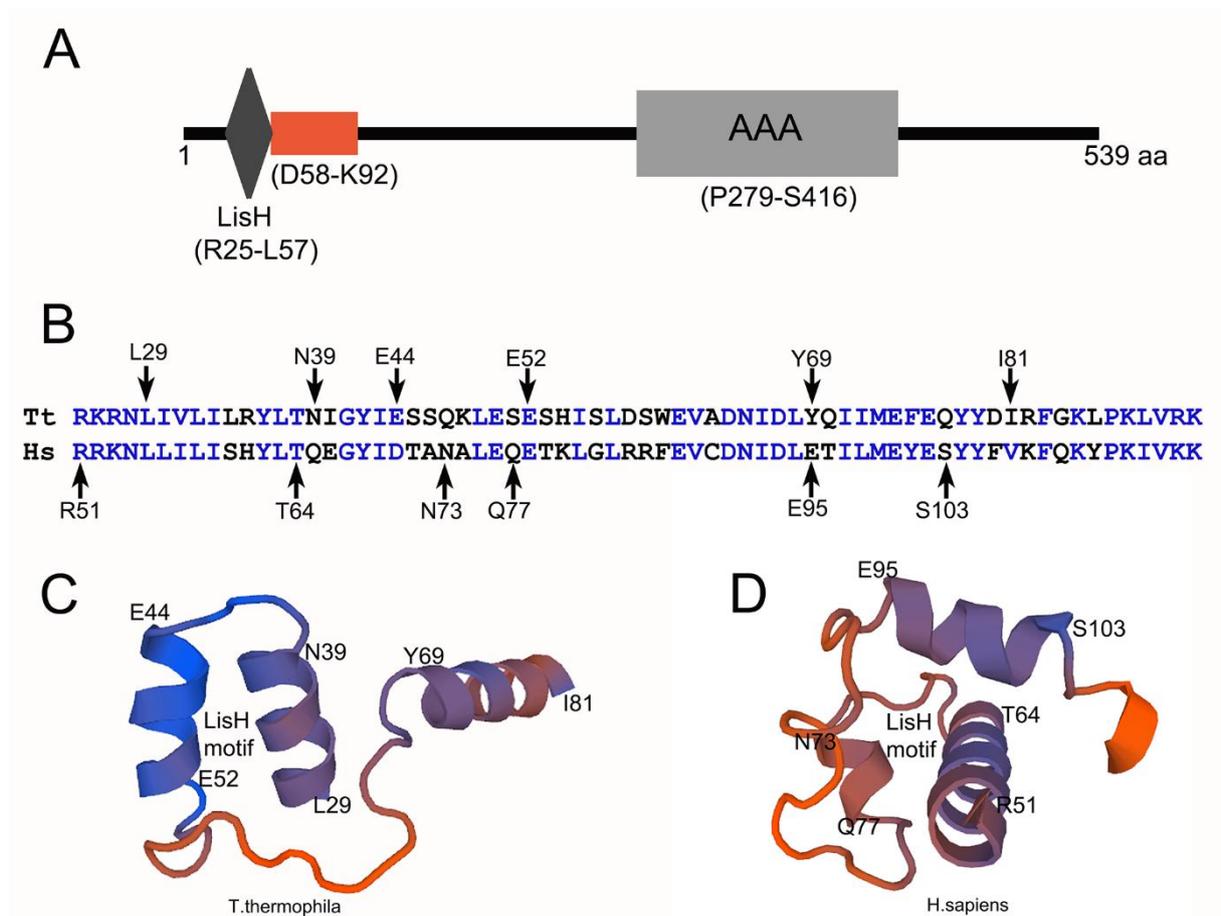


Figure S1. The analyses of the LisH and CTLH domains in human Katnal2 and *T. thermophila* ortholog, Kat2. (a) Schematic representation of domains in *T. thermophila* Kat2: LisH domain (black rhombus), CTLH (orange rectangle) and AAA domain (grey rectangle). The position of first and last amino acids of the domains are indicated. (b, c) An alignment and a 3D model of an N-terminal fragment of the *Tetrahymena* Kat2 (XP_001007193, TTHERM_00414230) and human Katnal2 (XP_005258414.1) containing a LisH domain and an adjacent CTLH as predicted, using an automated protein structure homology-modelling server (<https://swissmodel.expasy.org/>). Numbers indicate the initial and final (margin) amino acids of the predicted helices. Identical or similar amino acid residues in the corresponding aligned fragments of the *Tetrahymena* and human proteins are shown in blue.

10 20 30 40 50 60 70

TrMSYL LSKVRSEGESRMNEEKKIADPKRNLIVLILRYLTNIGYIESSQKLESESHISLDSWEVAD
 InMLAKIKSESESRMNEEKKIHDRKRNILVILILRYLANIGYIDSCSKISTESHIGLDQWDVAD
 PtMLNKIKIDSESRLESEKQIKDRNRNVIILIQRYLVNSGYIDSATKLGTESNLTINQYDAAD
 SlMLMKYKAEGESRLQEEKRITERRRNILLVIVQRHLINCGYIDAANALTRCNLGLDRWEVAD
 AtMATDE
 OsMTGADE
 PpMSEDA
 CrMSEL AAL KALSKAKES EKKRIQERRRNLIVLILRHLADHG YTD TYERLCTESNLSLQKVDVAD
 VcMSEL AAL KAIKSKAKDS EKKRLQERRRNLIVLILRHLADHG YVD TYEKLCTETNINLQKVDVAD
 McMAAL ASMKINTMVKDAEAAKAKERKRNAILLLRYLRDNGYVDSAEVLQRESLAPGTVDAAD
 Lm MRHLQPLSNTAAPAGSSLAHMKTQQLAREEEKLRARVKGVIIVLVEQFLLEQGYHQTALHALQRESRISLTQFSAAD
 PsMSLMLQLKAQNESRLAEEKQLIDRRRNVLMMLQHC TENG YLQTA EKLQQAAGVALSKFEVVE
 AlMSLTIQLKAANDSRLTEKKTIEERRNILLIAQFCREYGYLQTA EKLQQAEGGLAFSKFDVVE
 HvMSRELNYQHIKAYNEARVSEEHRT EQKKKNLLILVLFHLYEEGYLDSMRCLAEAGDNLKFFQLCD
 TaMTHELSYMQIKTANQVREAEQKIEQRKKSLLVILQYLND EGYVQSAKCLTESSTLNKFFQVCD
 SpMMESLNYAAIKTSQVREAEQKSEQRKKNLLILMLHLLGEGYSEAKALESENLCFNKFEVCD
 HsMELSYQTLKFTHQAREACEMRTEARRKNLLILISHYLTQEGYIDANALEQETKLGRRFEVCD
 GgMELSCQVLRARQAREADELRTAARRKNLLILILHYLMEEGYMDAANSLEQETKLGRRFEVCD
 XtMELSYQALRVASQNRAREELRTEARRKNLLILIMHYLQEGYMDAANSLEQETKISLRRFEVCD
 CiMALELSYQSIKTANSVRESEDI RQETRRKNLLVLMLSHLSDN GYIEAAAALKEKANSISLNRWQVCD
 ShMAELSYCMMSRANQARESEEQRSTARKKNLLILVMHYLSEGYVESAMSLSKETNLDFFKFEVCD
 ZnMAFEISYS SLKIAHQVRES EKKAL EKKQLL YLVLQYLKDEGYLDTAQSF SNEAHLT-NQYQVCD
 MbMTEELSYIKIKTANEARLAEAEARTE TLKRNIVLMLHLLQMSYVEAAAALAEAGV SGRFTVCD
 TvMLMTEHEISYMQTHCKSQAKDYS EDEKKRTRDGLVLIYNHLLKQLGYVNSAEHLAQEAGIGPDRFELCD
 Hs_Lis1 QDELNRAIADYLR SNGYEEAYSVFKEAEALDV
 Hs_muskelin EQEAI RLC LKHFRQHNYTEAFESLQKKT KIAL
 Tr_Kat2 RKRNLIVLILRYLTNIGYIESSQKLESESHISL
 consensus L - I - YL - GY- EA - VF - E -

80 90 100 110 120 130 140 150

Tr NIDL YQIIMEFEQYYDIRFGKLPKLVKRVND QKKK MP SFPKIS
 In NIDL YQIVTEFEHYEELKFGKYIKLVKRVTS E KN PASFPKIT
 Pt NMDL YMIFCFEYQFYEMKFMKPPKLVKVVDDGQ S TPGLPRIIP
 Sl NIDL FYIVQDFEYEMKFKQRKPTMVRKKSFN VDPKKQ RVNLPKIN
 At PSQTRWSFLFEKFTFYDAKFG R KKLPEE DVSNKDQ PEDG
 Os PSITRWTFEDFEVYVEVRLGI RREP GG DEDGDG DGGGGGRGYA PLGS
 Pp AALTRWLFEDFKQAYDAKFG R KNSFSS SVGRGRWGR SEGQ
 Cr NVDL LRILQEFEE SYELKFGKRPKVVRRLVEE VGMQRIAGVQV DGRGG
 Vc NIDL IRILQEFEE SYELKFGKRPKVVRRLVEE MQRIAGVAV DGRGA
 Mc NVHLERIFRELEEHHIQRFGRAPKLFRRAGET LEIDAGGAAGTATGRP GQRGVPLGR
 Lm NIDL LRVVQYEEYAFKCCQRAPKLYRVRGSS DDADGVVGGVGS ES GERMLSRARK GAPAPRAA
 Ps NIDL LRIVQYEDDFQEKIFGKRPKLVRRRLSPD GQDRARSANDKKATAAAKRARR NDYTS PHMTEA
 Al NIDL MRIVQEFEE YEEIKFGRRPKLVRRRISE EQDKCKSLASKRAARIKY SPQIAPEA
 Hv NVDL QTI LQY EYES YYYVVKFNKYPKIKKKVQEV TSNKSPVHKS QKS KS SFLPSIL
 Ta NVDL P TILQY EYES YYYVVKFNKYPKIKKKVSAE KQAQFHS QNS SISKNG RSNPLPKIP
 Sp NVDL ETILMEYES YYYVVKFKYKPKVTKKISAE AINNKNKGNKK TSS TPIPKIP
 Hs NIDL ETILMEYES YYYVVKFKYKPKVTKKISSDT AENNL PQRSGKTRR MMNDS CQNLPKIN
 Gg NVDL ETILMEYES YYYVVKFKYKPKITRKLIDT AENKQLGTGGRQR AVSSQNLPRIK
 Xt NVDL ETILMEYES YYYIKFKYKPKITKKALDH DSRVQSKPRSAGKLR AGSNS TQGLPRIA
 Ci NVDL DNLQY EYES YYYIKFKYKPKITKKLASA DVNKARAHRT SIPSSDHS TSLPRIA
 Sh NVDL ETVLLQY EYES YYYIKFKYKPKITKRLSDK LLQCEKKRHGSS IKT S SLPRL
 Zn NIDL GTIVQDFEYTYYYVRFQYKPKICKVTVVE ENRR NILKMPNLE
 Mb NIDL ISIVQEFEFAYKIKFGGRAPKIKKQAPT SAELRPGRLQAKKAANKMRQSASPASTGAGS TGGGSP LPRVE
 Tv NISFQRIIVDFEKAYEQRYGVKPKIAKVPVKG KKTTP TSLGN IYDIPAVIEKAKN NAGIDGHPKPV
 Hs_Lis1
 Hs_muskelin
 Tr_Kat2
 consensus

160 170 180 190 200 210 220 230

Tr TPTKASSTSSNIKQELQNNNSNSVNSNLNTGSS TQRVGS QLGLLPKKNL AQNANKVKVSNSS LDEKGVQDQNTLN
 In QN NKVLQSANSQKIQQENNIQSTKQIQKRNTINTSITNE - - QQINSTPQ
 Pt SS GKS GSNNS SNNQNAAGSNKKNAAQSKKEDNNSKDAKNEPD - S
 Sl SA QSNQSRDNGQNGSNS VPIQSNVAAVGGQMNKPGQT IYNGKAPLAKQKS QNGKGEESKADTP
 At SS NGNNGDVNNNS SPTFN
 Os GS AGSTRPSAAHANGGADLAVFEQFRIS
 Pp EG NRSGGTYEFGARRYSK
 Cr VL AGMSREDVAAAQ
 Vc VL SAGSGGGGGGGGAGMSREDVAAAQ
 Mc DANSNVNNAAAAAGRGYGS
 Lm TS SSSPPLSSVSGKPPPHLRPTNLARALSHVQANGDSGANGDKRAVALNGDTS
 Ps RV ENNVAMRTIHNNASHAATQAVSARS SKDASPPPEDDS
 Al QV ENSVAFKLLHQMNS TKLHNVTLS TGVKNE
 Hv SP TSNHDSKRCSS SKYPSQACPSLNSLTKSKVSKEDLTKELSDHANPLEVCG
 Ta PS RRP SDDTAAREPRDNNQNRSL SASSVSTKKNSTNKESVSTPEAKVESDLG
 Sp HA PD
 Hs QQ RPRS KTTAGKTGDTKSLNKEHPNQEVVDNTRLESA
 Gg QQ PMQQPS SKTSLGNTLTKSP TKE SPRQNNES T VTL EQS
 Xt QQ TVLHRPVS GSYFRTHAQKAL SRENSKQENGGNSPREAS
 Ci NG NQSAPIGVRRRPGSGDGKKKATQRQNSDL SKS S TDS TGENVTVKAA
 Sh NR GNSIHF EQNHS SNILNSEENS SVTKNRACNGLAVQGRQDPLVQTSGRIVKS GISPNV
 Zn NE IQDTTKPRTNLQENQRENKLVHPK
 Mb SP PGIGSGS GAGSGGS GGGRRRPESTMRRQGNSS GNGAGATLASAANGVEPLPPS TRS GGRVVRTPD
 Tv KK AKSSDTKSEKSS
 Hs_Lis1
 Hs_muskelin
 Tr_Kat2
 consensus

	240	250	260	270	280	290	300	310
<i>Tr</i>	MEIQGQSALG		AKKNEEESTENN					FFDVRVL
<i>In</i>	IEIQGQSFAFQ		KTK.EEEQTQN					FFDVRVL
<i>Pt</i>	LEIQGTGVVQQ		KQQNEDANHKD					WFDPRVL
<i>Sl</i>	FVLEGNKIKM		LKKEEVDQVEE					YFENRII
<i>At</i>	QDQNT		ALANGNVI					REKP.KK
<i>Os</i>	LERKV		ELRNGAI					EAGPPQK
<i>Pp</i>	VPREG		GLNGVUVV					EKKGWQ
<i>Cy</i>	LQGLCVAGQ		GNGSDSDSDPDN					FFERRVL
<i>Vc</i>	LHGLHVAGQAAHLSANRGGAGQPPGGGGSGSDSDPEN							FFERRVL
<i>Md</i>	VAGKTSASTSERAGSRAK		TPASQRGAAPDL SVS GGGARRRGA					GAAADVS
<i>Lm</i>	ALGLSIQGESAAHTS		AREGEKGRDRNGRDGEGSDDAEDPLGS					LMSRRIL
<i>Ps</i>	AVDLGLVGQKPAQAAR		NGKAGVAKKAQPADQQQEE					SIERLIL
<i>Al</i>	NADLELVGQKPT		KQKVHSDKSNAEVD					SVEDRLI
<i>Hv</i>	VSGLISLNQKYC		KEKQKLVDNCNS ILNNTLKEMTLCDAE					ISNTRLI
<i>Ta</i>	LTGVAVQSTSSSTSGK		QQKLSKTTDSTTNLSNPKVKS IANTRKSDPY					DPSERLI
<i>Sp</i>	HSGNASANISGRKRPTS		SVAQRRQSGNGPRKSTEGARQSPQVNGHG					DFLDRIL
<i>Hs</i>	NFGLIHISRIRKDSGEENA		HPRRGQIDFQGLL TDAIKGATSELALNTFDHNP					DPSERLI
<i>Gg</i>	DFGLSISAINRS GGGGEGP		HPRRGQVDFHGMIQH.VKVPNGIGLSSLTGDP					DPSERLI
<i>Xt</i>	EIGLNVSAISKTS GEGG		QTRRRQVDFRSMIQDTIKGASQEIALLNSLNCNP					DPSERLI
<i>Ci</i>	DLGLTVSSVAIRDKKK		ERNRKEIVDVRSMIQNDAIRGASNDIMT					NQDRMV
<i>Sh</i>	PNGSSVTSLSNHLDP TQNS		FLSSSTRQNFPRQITDYRAIINQETRLPLEENQLSE					DPQERIL
<i>Zn</i>	SSGKASSISVQKTFKSNNSLS		DLNMLVPLTPEGFNTIQVNSAQNNVQS					KSEKLL
<i>Mb</i>	ATGGSDNGILPDL		LAVQATSVAQPAGGRAGTKSRPQVIDVKS QLADAVQHATREALDGYNHDDKLL					PPDIQLM
<i>Tv</i>	EKGKSDDPLQSD		DLLSINATP IVAQPPKPKKSKKEEITDETPLPV					PPDIQLM
<i>Hs_Lis1</i>								
<i>Hs_muskelin</i>								
<i>Tr_Kat2</i>								
<i>consensus</i>								

	320	330	340	350	360	370	380
<i>Tr</i>	KGMPDFG	DVQELKELAAAYLQRDILVENPNVKFKD	IVGLDDAKRLLKEAVQIPLKYPHFFTG				IIEPWRGVL
<i>In</i>	KGMPDFG	DVQELKELAAAYLQRDIVVENPNCKFKD	IVGLEDAKRLLEAVL IPLKYPHFFTG				IIEPWRGVL
<i>Pt</i>	KGLPDYS	DVPEFQQLAAAYLQRDICEENPNVKFSD	IAGLDQAKRLLKEAVL VPLKYPHFFTG				IIEPWRGVL
<i>Sl</i>	KPLPDYS	FNPELKEALATIQREIINENPNVRFHD	IVGLEDAKRLLEAVL MPLKYPHFFTG				IIEPWRGVL
<i>At</i>	SMFPFF	ESAETRTLAESLSDRIIRGNPNIKWES	IKGLENAKRLLEAVVMP IKYPTYFNG				LLTPWKGIL
<i>Os</i>	SLPSPF	ESAEMENLAETLLRDIIRGSPDVKWES	IKGLENAKRLLEAVVMP IKYPKYFKG				LLTPWKGIL
<i>Pp</i>	KPLPVF	NSLETTILAEINMQDIVKGDMDVSWDT	IKGLENAKRLLEAVVMP IKYPPYFTG				LLTPWKGIL
<i>Cy</i>	KPLPQ	LQGELELGAATRDIIFTDSPNVRWED	IAGLDSAKRLIKEAVVMP IKYPLFTG				LLAPWKGVL
<i>Vc</i>	KPLPQ	LQGELELGAATRDIIFTDSPNVRWED	IAGLDQAKRLIKEAVVMP IKYPLFTG				LLAPWKGVL
<i>Md</i>	GPCPPAAS TL D LGAGDLNDLAEVIRRD	IHWGNPNVWESVAGLDDAKRLLKEAVVMP	IRYPELFRG				LLAPWKGVL
<i>Lm</i>	KPLPPF	PTSELSLAATILREILDVDPVVRWRD	IADLENAKHLREAVVMP VKYPLFQG				LLRPWKGIL
<i>Ps</i>	KPLPSFA	HDLRLPLAETITREIFQKMPDVRWDD	IVGLEHTKRLLEAVVMP LKYPQLFQG				LLSPWGTGIL
<i>Al</i>	KPLPVL	HDSDLRPLAETISREIFQQPNVVKWDD	IVGLEETKRLLEAVVMP LRYPPQIFKG				LLSPWGTGIL
<i>Hv</i>	KPLSGYTG	FTGEFRELAAIVSRDIYLENPNVHWDD	IIGLDSAKRLVKEAVVYP IKYPLFTG				LLSPWKGIL
<i>Ta</i>	KPLSTMIG	YSNEMKELVGIISREIYLNHPNVRWDD	IIGLEKPKIKLVKESVYYP IKYPLFTG				LLSPWKGIL
<i>Sp</i>	KPLGGYAG	YSLWEWELAQNISKDIYLNHPNVRWDD	IIGLDAKRLVKEAVVYP IKYPLFTG				LLSPWKGIL
<i>Hs</i>	KPLSAFAG	MNSEMRELAAVVSRDIYLNHPNVRWDD	IIGLDAKRLVKEAVVYP IKYPLFTG				LLSPWKGIL
<i>Gg</i>	KPLSAFAG	MNGEMRELAAVVSRDIYLNHPNVRWDD	IIGLDAKRLVKEAVVYP IKYPLFTG				LLSPWKGIL
<i>Xt</i>	KPVGAFAG	GNSEMRELAAVVSRDIYLNHPNVRWDD	IIGLDAKRLVKEAVVYP IKYPLFTG				LLSPWKGIL
<i>Ci</i>	KPLGGVVG	FNHEMRELAATVISRDIYLDHPNVKWS	IVGLDHAKSLVKEAVVYP IKYPLFTG				LLTPWKGIL
<i>Sh</i>	KPLGSYL G	YTGWEWELALITSRDIFLQPNVVRWDD	IIGLSSAKRLVKEAVVYP IKYPLFAG				LLSPWKGIL
<i>Zn</i>	KPLSGCVT	YSTEWKFAEAVISKEICATDLNVHWDD	IMGLEEAKRLLEAVVYP IKYPLFTG				VLAPWKGVL
<i>Mb</i>	KPIAGFG	YTGQMRDLANVISRDIYSQPNVVRWDD	IIGLDAKRLVKEAVVYP IKYPLFTG				LLSPWKGIL
<i>Tv</i>	KEVLPQ	LRADFGDLTDVLAARDIFTANTGMTWSD	IVGLDGAKRVLREAVVMP LKYPQLFEGGKLLRPWKGVL				LLRPWKGVL
<i>Hs_Lis1</i>							
<i>Hs_muskelin</i>							
<i>Tr_Kat2</i>							
<i>consensus</i>							

	400	410	420	430	440	450	460
<i>Tr</i>	LYGPPGTGKTMLAKAVATECG	TTTTFNISASSVSKWRGSESEKL	IRVLFELARHYQPSTIFLDELDS	IMSKRGGDN			
<i>In</i>	LYGPPGTGKTMLAKAVATECG	TTTTFNISASSVSKWRGSESEKL	IRVLFELARHYQPSTIFLDELDS	IMSKRGGQ			
<i>Pt</i>	LYGPPGTGKTMLAKAVATECRT	TTTTFNQASSVSKWRGSESEKL	IRVLFELARHYEPSTIFIDEMDS	IMGQRGSAGN			
<i>Sl</i>	LYGPPGTGKTMLAKAVATECRT	TTTTFNMSASTVSKWRGSESEKL	VRLLFELARHFQPS TIFIDEIDS	IMSSRTSTG			
<i>At</i>	LYGPPGTGKTMLAKAVATECNT	TTTTFNISASSVSKWRGSESEKL	IRVLFELARHHAPSTIFLDEIDAI	ISQRGGERS			
<i>Os</i>	LYGPPGTGKTMLAKAVATECK	TTTTFNISASSVSKWRGSESEKL	VKVLFELARHHAPSTIFLDEIDAI	ISQRG.EARS			
<i>Pp</i>	LYGPPGTGKTMLAKAVATECK	TTTTFNISASSVSKWRGSESEKL	VKVLFELARHFAPSTIFLDEIDAL	ISTRG.EGSS			
<i>Cy</i>	LYGPPGTGKTLLAKAVATECRT	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHYHAPSTVFLDEIDAL	MAARGGEG			
<i>Vc</i>	LYGPPGTGKTLLAKAVATECRT	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHYHAPSTVFLDEIDAL	MAARGGEG			
<i>Md</i>	LYGPPGTGKTMLAKAVATECD	TTTTFNVSSTVSKWRGSESEKL	VRVLFELARHHAPSTVFMDEIDAL	MSARGGPGGGG			
<i>Lm</i>	LYGPPGTGKTLLAKAVATECRT	TTTTFNIAASSVSKWRGSESEKL	VRMLFDLAVHYAPSTIFIDEIDS	LSMSARS.DG			
<i>Ps</i>	LYGPPGTGKTMLAKAVATECRT	TTTTFNISASSVSKWRGSESEKL	IRMLFELARHHAPSTIFLDEIDS	IMGQRD.SGG			
<i>Al</i>	LYGPPGTGKTMLAKAVATECK	TTTTFNISASSVSKWRGSESEKL	IRILFELARHYHAPSTIFLDEIDS	IMGQRD.SSGG			
<i>Hv</i>	LYGPPGTGKTLLAKAVATECNT	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHFAPSTIFLDELDS	IMGQRG.SVDGGN			
<i>Ta</i>	LYGPPGTGKTMLAKAVATECNT	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHHAPSTIFLDEIES	LMGQRGSAGIS			
<i>Sp</i>	LYGPPGTGKTLLAKAVATECNT	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHFAPSTIFLDEIES	VMGQRGGGNN			
<i>Hs</i>	LYGPPGTGKTLLAKAVATECK	TTTTFNISASTVSKWRGSESEKL	VRVLFELARHYHAPSTIFLDEIES	VMSQRGT.SGG			
<i>Gg</i>	LYGPPGTGKTLLAKAVATECNT	TTTTFNISASTVSKWRGSESEKL	VRVLFELARHYHAPSTIFLDEIES	VMSQRGT.SGG			
<i>Xt</i>	LYGPPGTGKTLLAKAVATECNT	TTTTFNISASTVSKWRGSESEKL	VRVLFELARHYHAPSTIFLDEIES	VMSQRGTGPGG			
<i>Ci</i>	LYGPPGTGKTMLAKAVATECNT	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHFAPSTIFLDEIES	VMSQRGSGPGG			
<i>Sh</i>	LYGPPGTGKTLLAKAVATECK	TTTTFNISASTVSKWRGSESEKL	VRVLFELARHFAPSTIFLDELDS	LSMSQRG.SL.SGYG			
<i>Zn</i>	LYGPPGTGKTLLAKAVATECK	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHYHAPSTIFLDELDA	LSHRDAS			
<i>Mb</i>	LYGPPGTGKTMLAKAVATECQ	TTTTFNISASSVSKWRGSESEKL	VRVLFELARHYHAPSTIFLDELDS	IMSTRDGGGKRR			
<i>Tv</i>	LYGPPGTGKTLLAKAVAGEG	TTTTFNISASTVSKWRGSESEKL	IRVLFELARHFAPSTIFIDEMDS	IMSKRS.EE			
<i>Hs_Lis1</i>							
<i>Hs_muskelin</i>							
<i>Tr_Kat2</i>							
<i>consensus</i>							

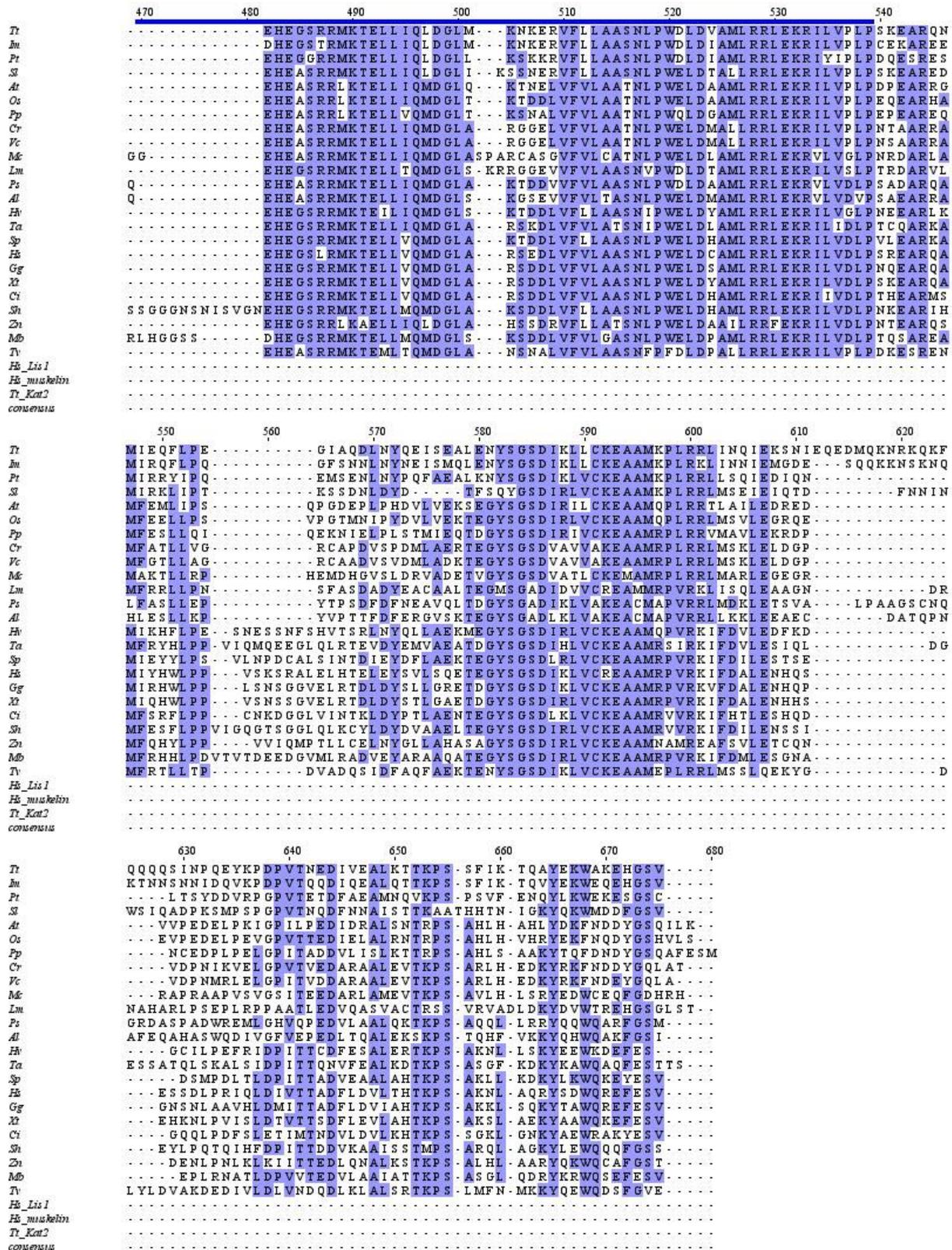


Figure S2. A multiple alignment of Katnal2 ortholog sequences. Al (*Albugo laibachii* Nc14, CCA18838), At (*Arabidopsis thaliana*, NP_973600), Cr (*Chlamydomonas reinhardtii*, XP_001698289.1, corrected), Ci (*Ciona intestinalis*, XP_002130824.1), Gg (*Gallus gallus*, XP_414699.3), Hs (*Homo sapiens*, XP_005258414.1), Hs Miller-Dieker Lissencephaly protein (Lis1) (*Homo sapiens*, AAL34972.1), Hs muskelin (*Homo sapiens*, AAF06698.1), Hv (*Hydra vulgaris*, XP_012554311.1), Im (*Ichthyophthirius multifiliis*, XP_004036585.1), Lm (*Leishmania major* strain Friedlin, XP_001681840.1), Mc (*Micromonas commoda*, XP_002501840.1), Mb

(*Monosiga brevicollis* MX1, XP_001748555.1), Os (*Oryza sativa*, XP_015622246.1), Pt (*Paramecium tetraurelia*, XP_001443881.1), Pp (*Physcomitrella patens*, XP_001761938.1), Ps (*Phytophthora sojae*, XP_009514542.1), Sl (*Stylonychia lemnae*, CDW81059), Sh (*Schistosoma haematobium*, XP_012794975.1, corrected) Sp (*Strongylocentrotus purpuratus*, XP_783887.3), Tt (*Tetrahymena thermophila*, XP_001007193, TTHERM_00414230), Tv (*Trichomonas vaginalis*, XP_001319230.1), Ta (*Trichoplax adhaerens*, XP_002110399.1), Vc (*Volvox carteri f. nagariensis*, XP_002956158.1, corrected), Xt (*Xenopus (Silurana) tropicalis*, NP_001090643.1), Zn (*Zootermopsis nevadensis*, KDR07071.1). An N-terminal fragment and a part of the weakly conserved region of Katnal2 from *Chlamydomonas reinhardtii* (missing in XP_001698289.1 prediction) were reconstructed based on the analysis of the genomic DNA sequence from *C. reinhardtii* v 4.0 database, chromosome 10, contig 53).

The 14 N-terminal amino acids of *Schistosoma haematobium* Katnal2 ortholog were identified about 100 bp upstream of the predicted ATG during genomic DNA analysis using NCBI database. The 95 N-terminal highly conserved amino acids of *Volvox carteri f. nagariensis* were predicted based on the data from blast search of the *Volvox* genomic DNA fragment obtained from NCBI against human proteome.

Note that the alignment also shows the amino acid sequence of a LisH domain identified in human Lis1 (AAA02881.1), human muskelin (AAF06698.1) and *Tetrahymena* Kat2 proteins and a consensus within the LisH domain.

Color lines above the alignment indicate position of: a LisH (red), a CTLH (green) and AAA domain (navy blue) as determined using SMART program. The conserved amino acids were marked using Jalview 2.11.0 program.

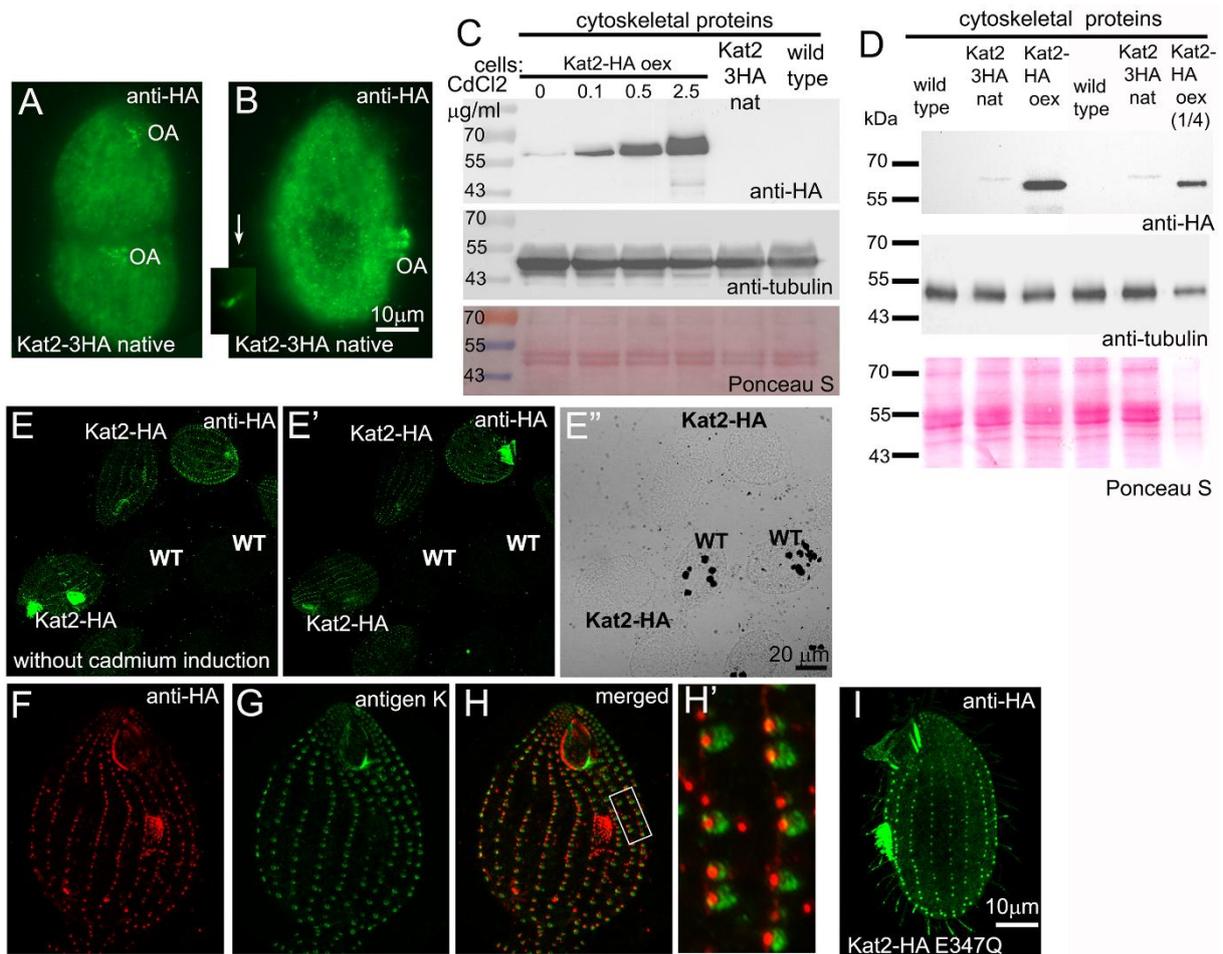


Figure S3. A localization of Kat2-3HA expressed under the control of the native gene promoter. (a, b) Immunofluorescence confocal images showing presence of Kat2-3HA in short growing cilia of the oral apparatus (a) and oral and somatic cilia (b), (c-d) A Western blot analysis of the levels of Kat2 in the cytoskeletal fraction isolated from cells expressing HA tagged Kat2 either at its native level (nat) or under the control of cadmium dependent *MTT1* promoter (cells were grown with or without (- Cd) cadmium). Tubulin staining (c,d middle panel) and total protein staining with Ponceau S (c,d lower panel) are shown as loading controls. (d) Note that the native level of Kat2 is significantly lower than in cells harboring *MTT1-KAT2-HA* transgene grown in a medium without cadmium (d). Please note that Kat2-3HA expressed under the control of the native promoter migrates more slowly in the gel than the overexpressed Kat2-HA, perhaps due to its posttranslational modification. (e-e'') Immunofluorescence confocal images of the mixed population of wild-type cells (WT, cells fed with India ink and thus containing dark food vacuoles) and cells carrying *MTT1-KAT2-HA* transgene grown in a medium without cadmium stained with anti-HA antibodies to compare the intensity of the HA signal. Note that Kat2-HA localizes both, in cilia and near basal bodies. (f-h'') Immunofluorescence confocal images of Kat2-HA overexpressing cells stained with anti-HA (f) and anti-K antigen (g) antibodies. (h) Merged image. (h') Magnification of the part of the cell (h) marked with a white rectangle. Note that a K antigen is less abundant near new (proximal) basal body of the pair. (i) Immunofluorescence confocal image showing a localization of the Kat2-HA (E347Q) with a mutation in the Walker B motif of the AAA catalytic domain. Bar=10 μm .

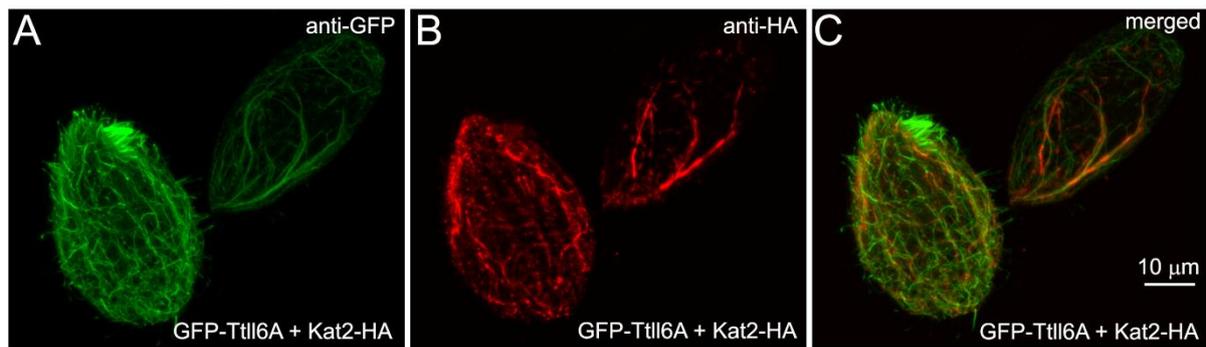


Figure S4. Partial co-localization of the overexpressed GFP-Ttl6A glutamylase and Kat2-HA. *Tetrahymena* cells were grown in SPP medium supplemented with 2.5 μg/ml cadmium chloride for 4 h. Please note that the cell to the right is imaged at the median level. Note also that both GFP-Ttl6A and Kat2-HA highly decorate bundles of subcortical and cytoplasmic microtubules (please compare to Janke et al., 2005, Wloga et al., 2010 [23,24]). (a) GFP-Ttl6A, (b) Kat2-HA, (c) merged image.

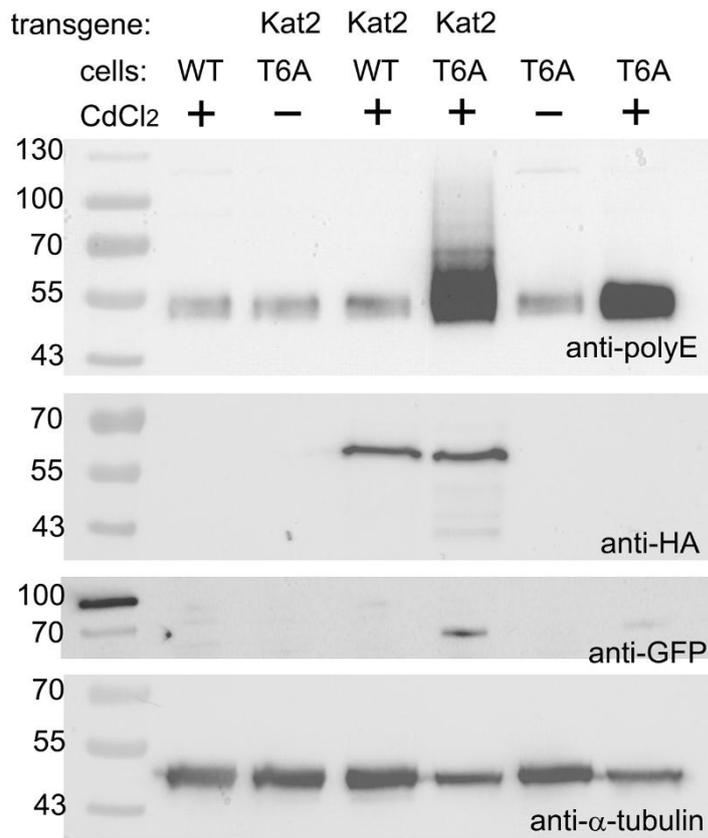


Figure S5. Comparative Western blot analysis of the level of tubulin glutamylation in cells co-overexpressing Kat2-HA and tubulin glutamylase, GFP-Ttl6A. Cytoskeletal fraction was isolated from wild-type cells, cells carrying transgenes enabling expression of either Kat2-HA or GFP-Ttl6A glutamylase, and cells co-overexpressing both proteins. Cells were either non-induced (CdCl₂ "-") or induced ("+") with 2.5 μ g/ml cadmium chloride for 4 h. To detect Kat2-HA and GFP-Ttl6A, 20 μ g of cytoskeletal proteins was loaded. To detect α -tubulin and glutamylated tubulin, 5 μ g of cytoskeletal proteins was loaded.

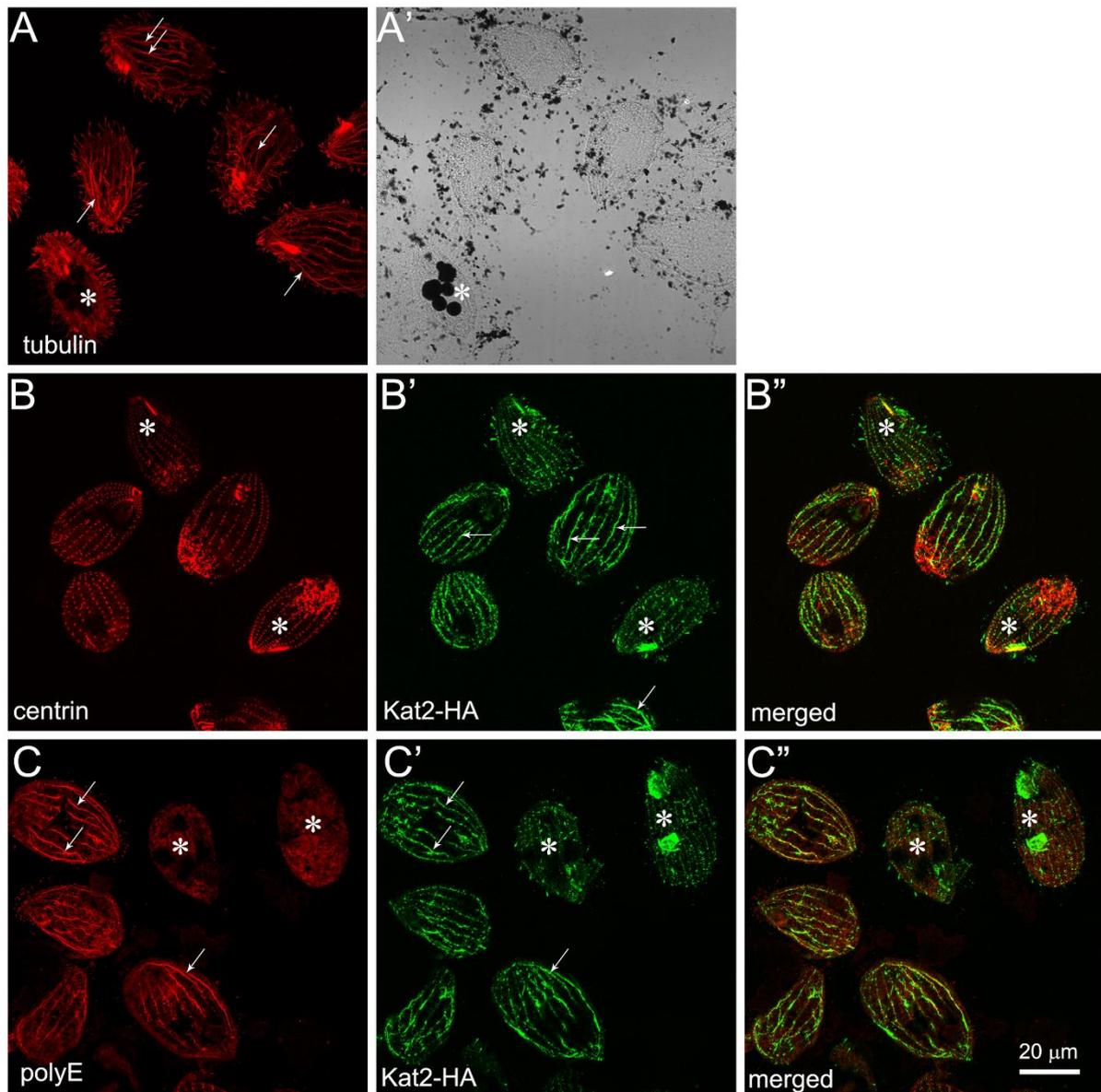


Figure S6. The elevated level of tubulin glutamylation in cells co-overexpressing Kat2-HA and GFP-Ttll6A alters Kat2-HA localization. Immunofluorescence confocal images of the mixed population of cells overexpressing Kat2-HA alone (indicated by the presence of dark food vacuoles and by white star) or co-overexpressing Kat2-HA and GFP-Ttll6A and thus having highly glutamylated microtubules. (a-a') Cells stained with 12G10, anti- α -tubulin antibodies to visualize cilia; note a presence of the bundles of cortical and subcortical microtubules (some are indicated by white arrows) in cells overexpressing GFP-Ttll6A glutamylase. (a') A corresponding phase contrast image showing which cell has food vacuoles (left lower corner). (b-c'') Immunofluorescence confocal images of cells stained with either anti-centrin antibodies (b) to visualize basal bodies or polyE antibodies (c) to show presence of the glutamylated microtubules and co-stained with anti-HA antibodies (b', c') to localize Kat2-HA. (b'', c'') Merged images. The centrin localization (b) is not altered in cells with normal and elevated levels of tubulin glutamylation. Please note a presence of bundles of highly glutamylated cortical and subcortical microtubules (some are indicated by white arrows) in cells overexpressing GFP-Ttll6A glutamylase (c) and re-localization of Kat2-HA in these cells (c'). Note also that in GFP-Ttll6A overexpressing cells Kat2-HA is no longer visible in cilia but instead co-localizes with bundles of microtubules (some are indicated by white arrows). Bar=20 μ m.

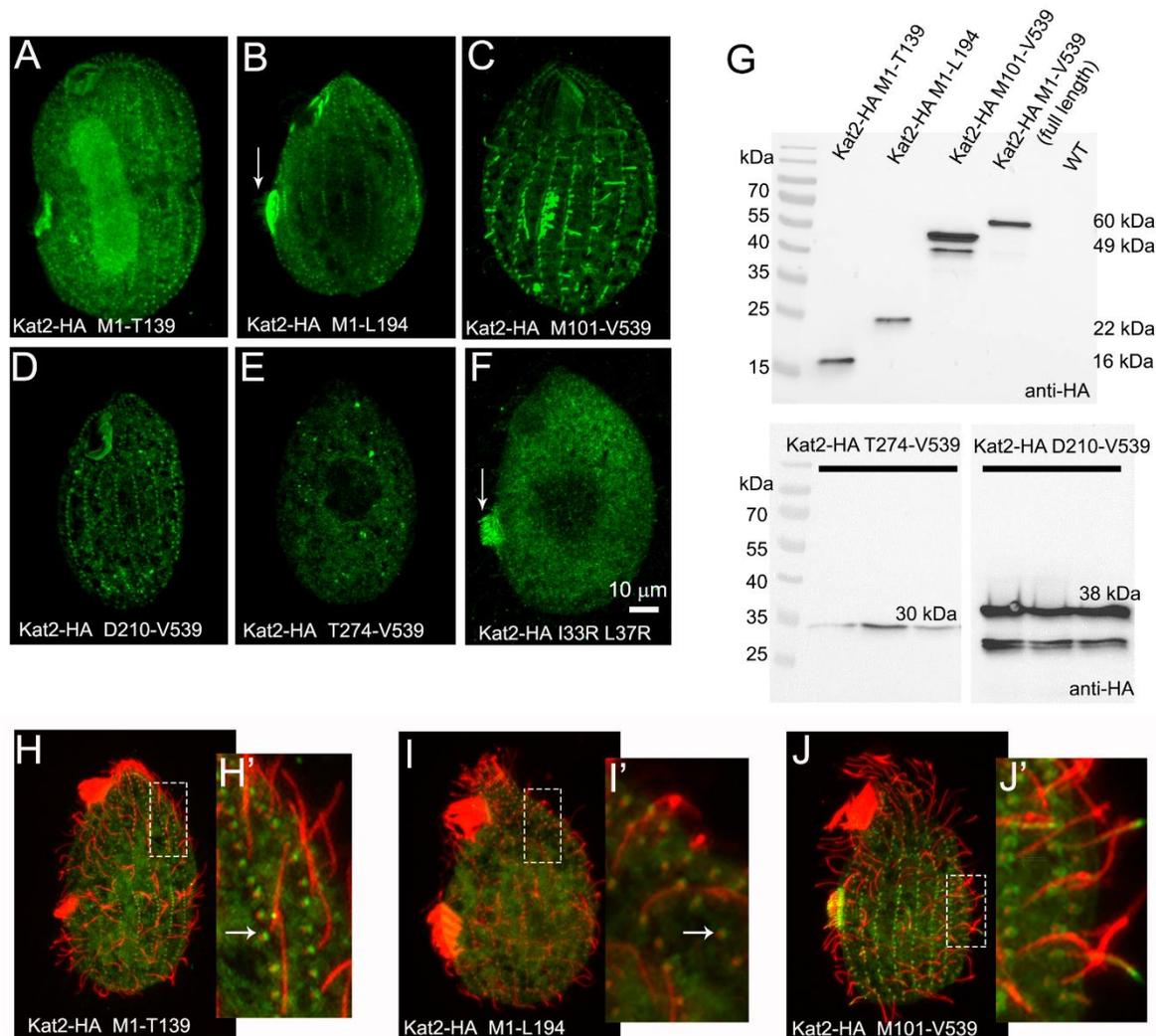


Figure S7. Immunolocalization of Kat2-HA truncations. (a-f) Immunofluorescence confocal images of *Tetrahymena* cells carrying a transgene enabling overexpression of the C-terminally HA-tagged truncated variants of Kat2: (a) M1-T139, (b) M1-L194, (c) M101-V539, (d) D210-V539 or (e) T274-V539. (f) Cell overexpressing Kat2-HA with double mutation within a LisH motif (I33R, L37R); note a presence of the protein in growing cilia (white arrow); a presence of the mutated Kat2 near the basal bodies is obscured by the overexpressed protein accumulated in the cytoplasm. Before fixation, cells were induced for 3 h with 2.5 $\mu\text{g/ml}$ CdCl₂. Note that the truncations containing LisH and CTLH localize near basal bodies (a, b), while a lack of these fragments causes partial Kat2 redistribution (c). Bar = 10 μm . (g) A Western blot analysis of the cytoskeletal fraction isolated from cells overexpressing Kat2 truncations. Numbers to the right (upper panel) or above the bands (lower panel) indicate the calculated molecular mass of the Kat2 truncation. (h-j') Immunofluorescence confocal images of *Tetrahymena* cells overexpressing (h-h') Kat2-HA M1-T139, (i-i') Kat2-HA M1-L194, (j-j') Kat2-HA M101-V539, double labeled with anti-HA and GT335 antibodies recognizing glutamylated tubulin in cilia and basal bodies. (h', i', j') Magnification of the part of the cell marked with a white rectangle (h, i, j).

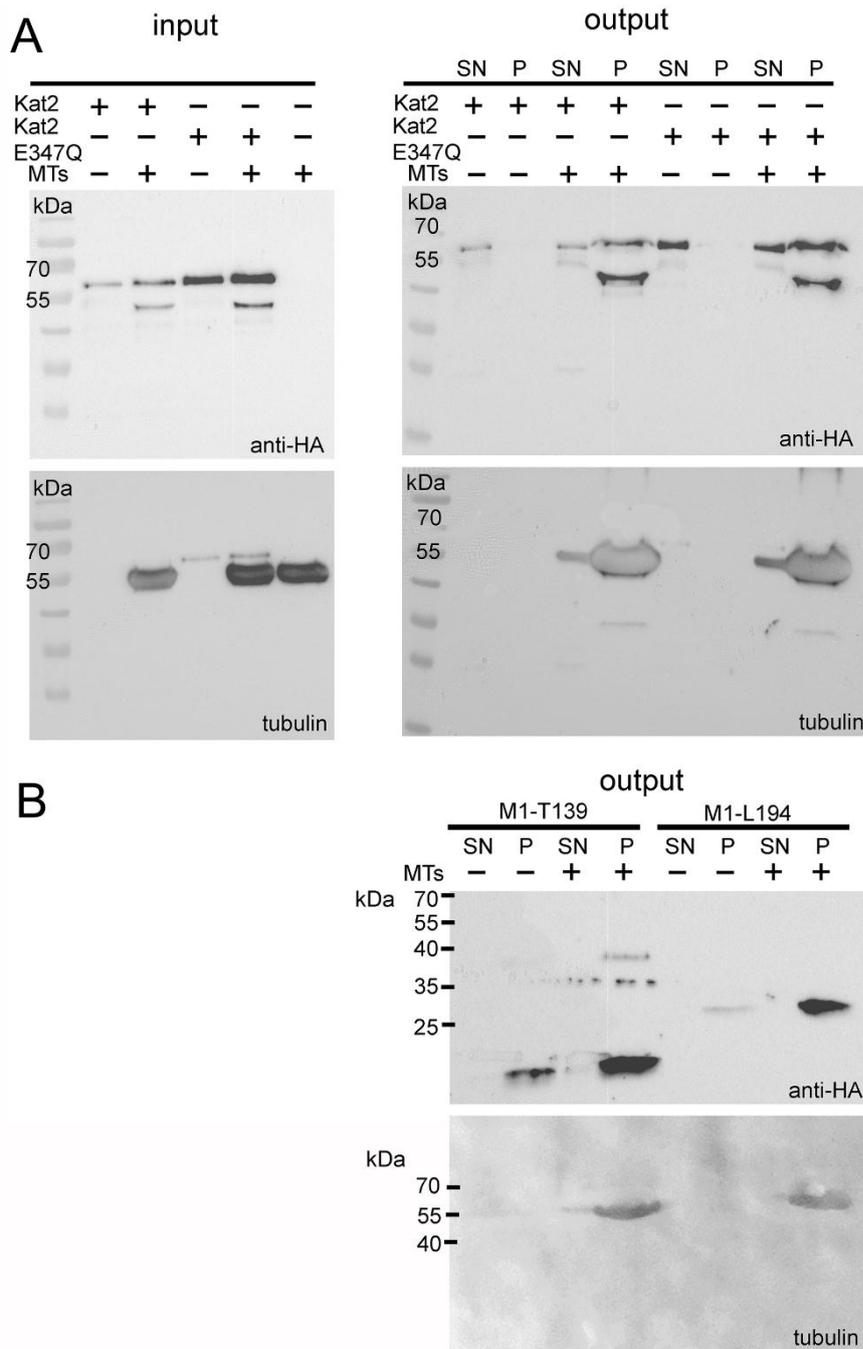


Figure S8. An in vitro microtubule binding assay. **(a, b)** A Western blot analysis of the Kat2-HA or Kat2-HA E347Q isolated from the cytosolic fraction of the overexpressing cells and incubated with in vitro polymerized microtubules. The panel to the left shows proteins just after mixing (input) while the panel to the right shows the same samples after 30 min. of incubation. Note that after incubation with microtubules both Kat2-HA and Kat2-HA E347Q were in the pellet fraction suggesting binding to microtubules **(a)** while some Kat2-HA M1-T139 and Kat2-HA M1-L194 pelleted even without microtubules **(b)**.

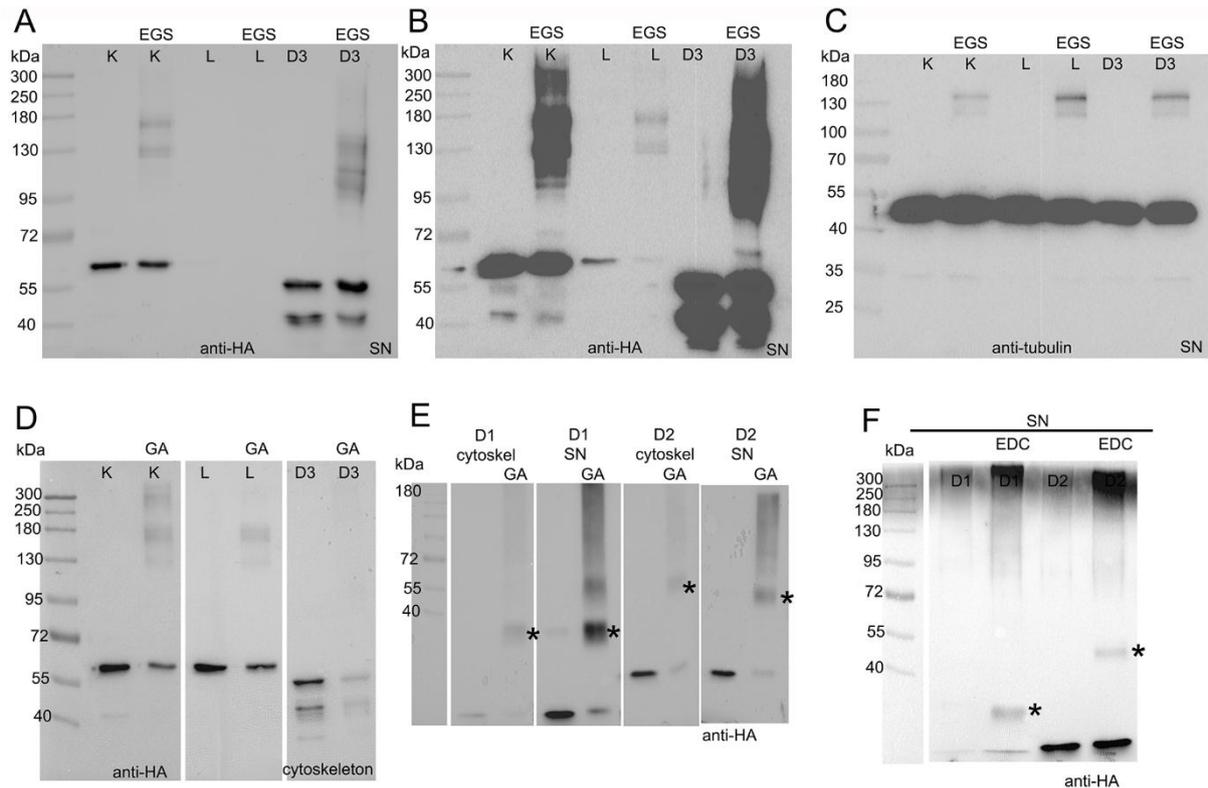


Figure S9. LisH domain plays a role in the formation of Kat2 complexes. **(a-c)** A Western blot-based identification of the HA-positive **(a, b)** and tubulin-positive **(c)** in vivo-formed and EGS-stabilized complexes in supernatant (SN) isolated from *Tetrahymena* cells overexpressing for 4 h Kat2-HA (K), Kat2-HA I33R L37R (L), or Kat2-HA M101-V539 (D3). **(b)** The same as **(a)** but with longer exposure to detected Kat2-HA I33R L37R. **(a, b)** Note that complexes formed by Kat2-HA M101-V539 are smaller compared to those formed in cells overexpressing a full-length protein while tubulin-positive complexes are of similar size in all analyzed samples. Note also that Kat2-HA I33R L37R may form complexes although the level of the mutated protein is significantly lower in the cell. **(d-f)** The Western blot-based identification of the HA-positive complexes stabilized in vitro by 0.02% glutaraldehyde **(d, e)** or EDC **(f)**. Stars in **(e)** and **(f)** indicate bands of the size corresponding to the molecular mass of the dimer formed by Kat2 truncation; Kat2 M1-T139 (D1) and Kat2 M1- L194 (D2).

Table S1

Primers used to amplify fragments of the genomic DNA. The nucleotide sequence of the introduced restriction site is underlined. The ATG or TGA are in bold. In red are marked changes introduced in the nucleotide sequence.

Amplified fragment	Forward primer	Reverse primer
KAT2 ORF	AATT <u>ACGCGT</u> T ATGAGTTATCTACTATCAAAAGTCCGC	AATT <u>GGATCC</u> AACTGAACCATGTTTCCTTAGCC
KAT2 M1-T139	AATT <u>ACGCGT</u> T ATGAGTTATCTACTATCAAAAGTCCGC	AATT <u>GGATCC</u> TGTTGTAT TTAAAT TGCTGT TCACG
KAT2 M1-L194	AATT <u>ACGCGT</u> T ATGAGTTATCTACTATCAAAAGTCCGC	AATT <u>GGATCC</u> TACAAGAGCTGATTAACCCTAAATTC
KAT2 M101-V539	AATT ACGCGT T ATG CCATCTTTTCCGAAGATATC	AATT <u>GGATCC</u> AACTGAACCATGTTTCCTTAGCC
KAT2 D210-V539	AATT ACGCGT T ATG GAT GTT AGA GTA TTA A AAGGAA TGC	AATT <u>GGATCC</u> AACTGAACCATGTTTCCTTAGCC
KAT2 T274-V539	AATT ACGCGT T ATG ACT GGA ATA TTA GAA C CTGG	AATT <u>GGATCC</u> AACTGAACCATGTTTCCTTAGCC
LisH domain mutagenesis	GCTT <u>CGT</u> TTGAGATAT <u>CGT</u> TACAAACATTGG G TATATAGAATCATCTT	TGT <u>ACG</u> ATATCTCAA <u>ACG</u> AAGCACTAT TAAGTTTCTTTTACGATCA
E347Q mutagenesis	ATTTTCTTAGAT <u>CAGCTG</u> GATTCTATTATGT CG	CGACATAATAGAATCC <u>CAGCTG</u> ATCTAAGAAAA T
Native locus ORF	AATT <u>ACGCGT</u> T ATGAGTTATCTACTATCAAAAGTCCGC	AATT <u>GGATCC</u> AACTGAACCATGTTTCCTTAGCC
Native locus 3'UTR	AATT <u>CTGCAG</u> CTATTTTAAGATACCTTAGAAAAGCAC	AATT <u>CTCGAG</u> GATCCAGTATAAACTCAAACCG
GRL4 fragment	AATT <u>CCGCGG</u> TT GAA GCT GAT TAA GGC AAG AA	AATT <u>AGATCT</u> ACTTAGGAGCACTCAAACTTCA
GRL3 fragment	AATT <u>GATATC</u> TGC TGG ATA AAG TGC TGG TAG	AATT <u>CTCGAG</u> GTTAATGTTTCAACAGCAGGAAC

Table S2

Proteins that co-immunoprecipitate with Kat2-HA. X/Y – number of total and specific peptides identified. In experiment 1 and 2 total immunoprecipitate was analyzed. In experiment 3, the immunoprecipitate was run on the 8% SDS-PAGE gel and silver stained bands were excised and analyzed.

protein	TGD number	Exp1	Exp 2	Exp2-Ctr	Exp 3
Kat2	TTHERM_00414230	54/26	35/20	0	204/55
α -tubulin ATU1	TTHERM_00558620	6/4	1/1	0	23/16
β -tubulin BTU1	TTHERM_00348510	15/13	9/9	0	26/19