



**Supplemental Figure 1.** Influences on the phosphorylation pattern of flagellum proteins by ATP treatment of isolated flagella. Cells were grown in a LD cycle and then released to dim light (LL) according to Methods. Cells were harvested at LL29 and flagella were isolated and resuspended in a HMD buffer containing phosphatase inhibitors and Nonidet NP-40 according to [11]. The flagella extract was incubated at 25°C for 10 min in the absence (-) or presence (+) of 1 mM ATP according to [12]. Proteins from the flagellum matrix/membrane/axoneme fraction were separated by 9% SDS-PAGE (25 µg per lane) along with a molecular mass standard and immunoblotted with anti-phosphoSer (A) and anti-phosphoThr (B) antibodies, respectively, according to Methods.

**Supplemental Table 1.** Newly identified phosphopeptides or phosphorylation sites of already known phosphopeptides in CKI-7 treated cells.

Protein-ID		AA / MW	Phosphopeptide along with the number of the first amino acid within its protein	z	Xcorr	Function and/or homologies of predicted proteins determined by NCBI BLASTp
Vs2	Vs3					
<b>Phosphoproteins only present in CKI-7 treated 137c cells</b>						
166046	114692	409 AA / 45.7 kDa	234-GEPNISYpICSR 230-VLVKGEPNISYpICSR -> VLVKGEPNISpYICSR	2 2 2	2.62 2.64 2.62	ref XP_001690881.1 , gb AAT40314.1  glycogen synthase kinase 3 [Chlamydomonas reinhardtii], 0.0; Ser/Thr protein kinase domain, 6.91e-79
167565	186414	776 AA / 82.9 kDa	454-GGDSpAGPSGVGDVDLR	2	3.51	ref XP_001701617.1 , gb EDP06592.1 , kinesin-like protein [Chlamydomonas reinhardtii], 0.0; Kinesin motor domain, KIF9-like subgroup, 1.85e-140
165299	81483	600 AA / 64.5 kDa	148-HLYGGLIMSASpHNPGGPENDFGIK -> HLYGGLIMSASHNPGGPENDFGIK	3 3	4.46 4.4	ref XP_001697991.1 ,gb EDO99576.1  phosphoglucomutase [Chlamydomonas reinhardtii], 0.0
170435	182635	204 AA / 21.4 kDa	32-RQPTpDLIAFSAK	2	3.43	ref XP_001698630.1 , gb ABC02022.1  radial spoke protein 11 [Chlamydomonas reinhardtii], 8e-115; RIIalpha domain [32]
169475	182408	390 AA / 42.5 kDa	95-VLVHIEEQSPDIGQGVHGMoGTpK -> VLVHIEEQSPDIGQGVHGMGTpK	3 3	6.26 5.17	ref XP_001696661.1 , gb EDP08638.1  S-Adenosylmethionine synthetase [Chlamydomonas reinhardtii], 0.0

171076	191309 <sup>a</sup>	1083 AA / 114.1 kDa	295-YELDDDGNLASpFSR	2	2.67	ref XP_001694913.1 , gb EDP02065.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP139
169308 <sup>b</sup>	34449 <sup>a</sup>	425 AA / 45.8 kDa <sup>c</sup>	37-DALTWNDERPSTpPDDIKK -> DALTWNDERPSpTPDDIKK	3 3	4.51 3.6	ref XP_001695261.1 , gb EDP01969.1  flagellar associated protein [Chlamydomonas reinhardtii], 2e-101; found in the flagellar proteome as FAP21
165688	143938	551 AA / 60.4 kDa	20-VNDPNAPEQALIPTSpPR -> VNDPNAPEQALIPTpSPR	2 2	3.72 3.17	ref XP_001703474.1 , gb EDP06156.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP56
169508	142494	1264 AA / 125.2 kDa	1087-QSSpAEAAEVAEAEAAAAAAEAQR -> QSpSAEAAEVAEAEAAAAAAEAQR	2 2	4.85 4.62	ref XP_001696434.1 ,gb EDP08411.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP75
157003	147732	522 AA / 59 kDa	129-SpADAPAAGSVpVDWTLpQR	2	2.82	ref XP_001692676.1 , gb EDP03695.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP98
154693	190617	496 AA / 52.6 kDa	97-AFSKTPGGGYWISQR	2	2.76	ref XP_001693237.1 , gb EDP03263.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP129

153321 <sup>b</sup>	193605 <sup>a</sup>	809 AA / 99.5 kDa <sup>c</sup>	20-RRTpDDVINPLDITEAELK	3	5.46	ref XP_001699413.1 , gb EDO98659.1 , flagellar associated protein [Chlamydomonas reinhardtii], 1e-56; found in the flagellar proteome as FAP165
165288	192970	457 AA / 49.7 kDa	277-GPFAGTSTYATEYRPRTPAPAYER -> GPFAGTSTYATEYRPRTPAPAYpER	3 3	4.87 4.57	ref XP_001698000.1 , gb EDO99585.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP236
167668	141178	359 AA / 41.2 kDa	30-LVGGTPGKSSLVDAK	2	3.28	ref XP_001701817.1 , gb EDP06792.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP241
169099	193154 <sup>d</sup>	996 AA / 106.1 kDa	888-TPDTNPLLLASpAPPTpPR	2	3.14	ref XP_001698225.1 , gb EDO99510.1  flagellar associated protein [Chlamydomonas reinhardtii], 1e-180; found in the flagellar proteome as FAP243; annotated as FAP183 in Vs3

**Phosphoproteins found in CKI-7 treated cells with additional phosphopeptide(s) in comparison to 137c<sup>e</sup>**

171763	132719	552 AA / 62.2 kDa	274-ITKGSpWNVGYNK	2	3.56	ref XP_001701436.1 , gb EDO97433.1  flagellar outer dynein arm-docking complex protein 2 (ODA1; ODA-DC2) [Chlamydomonas reinhardtii], 0.0
157568	36026 <sup>a</sup>	1329 AA / 138 kDa	38-AHRISpGGGDGALAASGFADATVK	3	5.16	ref XP_001699049.1 , gb EDO99103.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP33; in Vs3 annotated as ANK17 with ankyrin repeats, 3e-15

156262 156264 <sup>f</sup>	16685 <sup>a</sup>	2324 AA / 244.9 kDa	1767-ALASQTTHLLDDDES <sup>p</sup> DDEEDEDGAAAAAASK -> ALASQT <sup>p</sup> HLLDDDES <sup>p</sup> DDEEDEDGAAAAAASK -> ALASQT <sup>p</sup> HLLDDDES <sup>p</sup> DDEEDEDGAAAAAASK -> ALAS <sup>p</sup> QTTHLLDDDES <sup>p</sup> DDEEDEDGAAAAAASK	3 3 3 3	6.45 4.9 4.85 4.75	ref XP_001696012.1 , gb EDP00961.1  flagellar associated protein [Chlamydomonas reinhardtii], 2e-61; annotated as FAP49 (156262) and FAP154 (156264) in Vs2; both models were fused to FAP154 in Vs3
153201	146112	1301 AA / 133.9 kDa	433-AADDPFLVELTPDGIPIEEAT <sup>p</sup> TCDLRR -> AADDPFLVELTPDGIPIEEAT <sup>p</sup> CDLRR	3 3	4.03 4.02	ref XP_001692474.1 , gb EDP03952.1  flagellar associated protein [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP217

#### Phosphoproteins found in CKI-7 treated cells with the same peptide (Boesger et al. 2009) but with additional phosphorylation site(s)<sup>g</sup>

166958	189266 <sup>d</sup>	1156 AA / 124.6 kDa	1137- <u>Sp</u> AGSGSTAPLLIDPST <sup>p</sup> PR ->SAGS <sup>p</sup> SpGSTAPLLIDP <u>Sp</u> TPR ->SpAGSGSTAPLLIDP <u>Sp</u> TPR ->SAGSGS <u>Sp</u> TAPLLIDPST <sup>p</sup> PR ->SAGSGS <u>Sp</u> TAPLLIDP <u>Sp</u> TPR ->SAGS <sup>p</sup> GS <u>Tp</u> APLLIDPSTPR ->SAGSGS <u>Tp</u> APLLIDPST <sup>p</sup> PR ->SAGSGS <u>Tp</u> APLLIDP <u>Sp</u> TPR	2 2 2 2 2 2 2	3.66 3.55 3.47 3.09 2.88 2.80 2.79 2.58	ref XP_001692057.1 , gb EDP04547.1  plasma membrane calcium- transporting ATPase [Chlamydomonas reinhardtii], 0.0; found in the flagellar proteome as FAP39
168935	58843 <sup>a</sup>	585 AA / 63.3 kDa	151-SRPPY <sup>p</sup> TDYVST <sup>p</sup> R ->SRPPY <sup>p</sup> TDY <sup>p</sup> VST <sup>p</sup> R ->SRPPY <sup>p</sup> TDYVST <sup>p</sup> R	2 2 2	3.20 2.64 2.53	ref XP_001697865.1 , gb EDO99748.1  mitogen-activated protein kinase 7 [Chlamydomonas reinhardtii], 5e-178

AA, number of amino acids according to [4]. MW, molecular weight according to (Pazour et al., 2005). z, charge. Sp, phosphorylated Ser or Dehydroalanine. Tp, phosphorylated Thr or Methyldehydroalanine. Yp, phosphorylated Tyr. Mo, oxidized Met. Function and/or homologies of depicted proteins are given as determined by NCBI BLASTp along with their conserved domains and their e-values. If a peptide is marked with an arrow, the peptide above shows identical or overlapping sequences, but the phosphorylation sites may be different. <sup>a</sup> Sequence of Vs3 model differs significantly from sequence of Vs2 model, peptides were present in both. <sup>b</sup> Vs2 model contains unidentified amino acids. <sup>c</sup> AA, MW and the position of the first amino acids of the peptides were calculated according to the Vs3 model. <sup>d</sup> Sequence of Vs3 model differs significantly from sequence of Vs2 model, peptide is only present in Vs2 model. <sup>e</sup> Only additional phosphopeptides are shown. Former identified peptides are listed in Table S1 in [11]. <sup>f</sup> Vs2 models were combined to one model in Vs3. <sup>g</sup> Only phosphopeptides containing novel phosphorylation sites are shown. Novel phosphorylation sites are underlined.