

Table S1. Protein constructs employed in the *in vitro* studies

Construct	Organism	Protein	Residues	Residue exchanges/deletions
SRPK79D	<i>D. melanogaster</i>	SRPK79D isoform F	1-869	
SRPK79D ^{Core}	<i>D. melanogaster</i>	SRPK79D isoform F	327-869	
SRPK79D ^{CoreΔdock}	<i>D. melanogaster</i>	SRPK79D isoform F	327-869	D758A, D774A, E781A, K825A
SRPK79D ^{Core-dead}	<i>D. melanogaster</i>	SRPK79D isoform F	327-869	K376M
SRPK79D ^{CoreΔlinker1}	<i>D. melanogaster</i>	SRPK79D isoform F	327-869	Δ570-696
SRPK79D ^{CoreΔlinker2}	<i>D. melanogaster</i>	SRPK79D isoform F	327-869	Δ492-696
SRPK79D ¹⁻³⁴⁰	<i>D. melanogaster</i>	SRPK79D isoform F	1-340	
BRP-170	<i>D. melanogaster</i>	Bruchpilot isoform I	1-1397	
BRP-190	<i>D. melanogaster</i>	Bruchpilot isoform G	1-1786	
BRP-190 ^{Δ1-152}	<i>D. melanogaster</i>	Bruchpilot isoform G	153-1786	
BRP-190 ¹⁻¹⁵²	<i>D. melanogaster</i>	Bruchpilot isoform G	1-152	
BRP-190 ³⁶⁻¹⁵²	<i>D. melanogaster</i>	Bruchpilot isoform G	36-152	
BRP-190 ^{1-(Δ107-122)152}	<i>D. melanogaster</i>	Bruchpilot isoform G	1-152	Δ107-122
BRP-190 ^{1-152,6SD}	<i>D. melanogaster</i>	Bruchpilot isoform G	1-152	S32D, S34D, S71D, S73D, S90D, S118D
SRPK1	<i>Homo sapiens</i>	SRSF protein kinase 1	1-655	
SRPK1 ^{CoreΔlinker}	<i>H. sapiens</i>	SRSF protein kinase 1	58-655	Δ256-473
SRPK2 ^{Core}	<i>Mus musculus</i>	SRSF protein kinase 2 isoform c	49-682	
Cast2 ¹⁻¹⁶³	<i>Rattus norvegicus</i>	Cast2a	1-163	
Cast2 ¹⁻³⁵³	<i>R. norvegicus</i>	Cast2a	1-353	
Cast1	<i>R. norvegicus</i>	Cast1	1-957	

Table S2. Peptide sequences of the BRP-190¹⁻¹⁵² SPOT membrane

No.	Peptides	Length	Residues
1	MSRDDYNPVTSSGVRSPGRVRRLLQE	25	1-25
2	PVTSSGVRSPGRVRRLLQELPTVDRS	25	8-32
3	RSPGRVRRLLQELPTVDRSPSRDYGA	25	15-39
4	RLQELPTVDRSPSRDYGAPRGSPLA	25	22-46
5	VDRSPSRDYGAPRGSPLAMGSPYYR	25	29-53
6	DYGAPRGSPLAMGSPYYRDMDEPTS	25	36-60
7	SPLAMGSPYYRDMDEPTSPAGAGHH	25	43-67
8	PYYRDMDEPTSPAGAGHHRSRASR	25	50-74
9	EPTSPAGAGHHRSRASRPPMAHAM	25	57-81
10	AGHHRSRASRPPMAHAMDYPRTY	25	64-88
11	SASRPPMAHAMDYPRTYQSLDRGG	25	71-95
12	AHAMDYPRTYQSLDRGGLVDPHDR	25	78-102
13	RTRYQSLDRGGLVDPHDREFIPIRE	25	85-109
14	DRGGLVDPHDREFIPIREPRDRSRD	25	92-116
15	PHDREFIPIREPRDRSRDRSLERGL	25	99-123
16	PIREPRDRSRDRSLERGLYLEDELY	25	106-130
17	RSRDRSLERGLYLEDELYGRSARQS	25	113-137
18	ERGLYLEDELYGRSARQSPSAMGGY	25	120-144
19	DELYGRSARQSPSAMGGYNTGMGPT	25	127-151
20	ARQSPSAMGGYNTGMGPTS	19	134-152

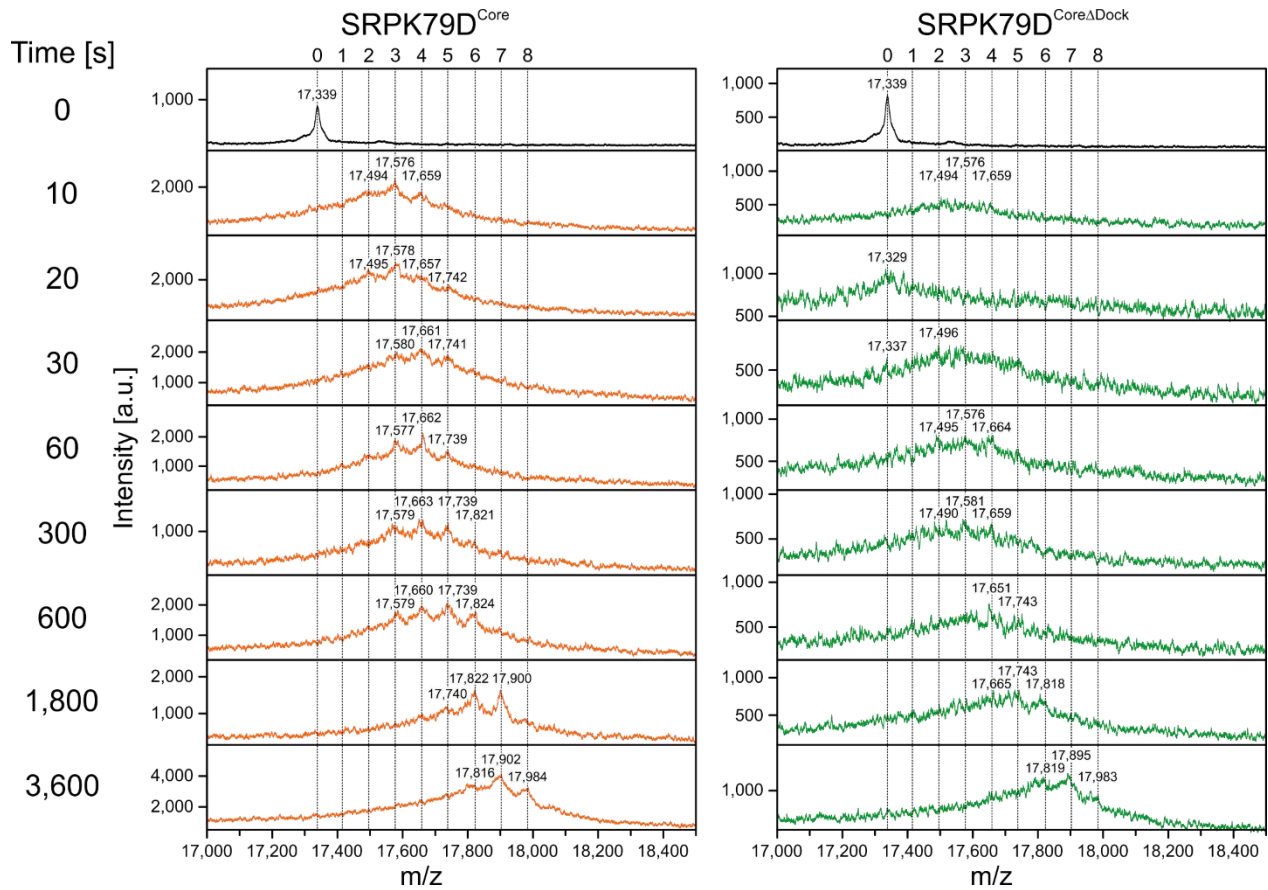


Fig. S1. Intact protein mass analysis of phosphorylated BRP-190¹⁻¹⁵² in a time course experiment. BRP-190¹⁻¹⁵² (unphosphorylated, black) was phosphorylated by SRPK79D^{Core} (orange) or SRPK79D^{CoreΔdock} (green). Reactions were stopped by adding SDS sample buffer at selected time points (left) and analyzed by MALDI-TOF-MS. Dashed lines indicate expected m/z values for BRP-190¹⁻¹⁵² carrying the indicated number of phosphorylated residues. SRPK79D^{Core} phosphorylates BRP-190¹⁻¹⁵² faster than SRPK79D^{CoreΔdock}, as indicated by higher intensity peaks for phosphorylated BRP-190¹⁻¹⁵² at all time points. After one hour, both kinase variants had added up to eight phosphates to BRP-190¹⁻¹⁵².

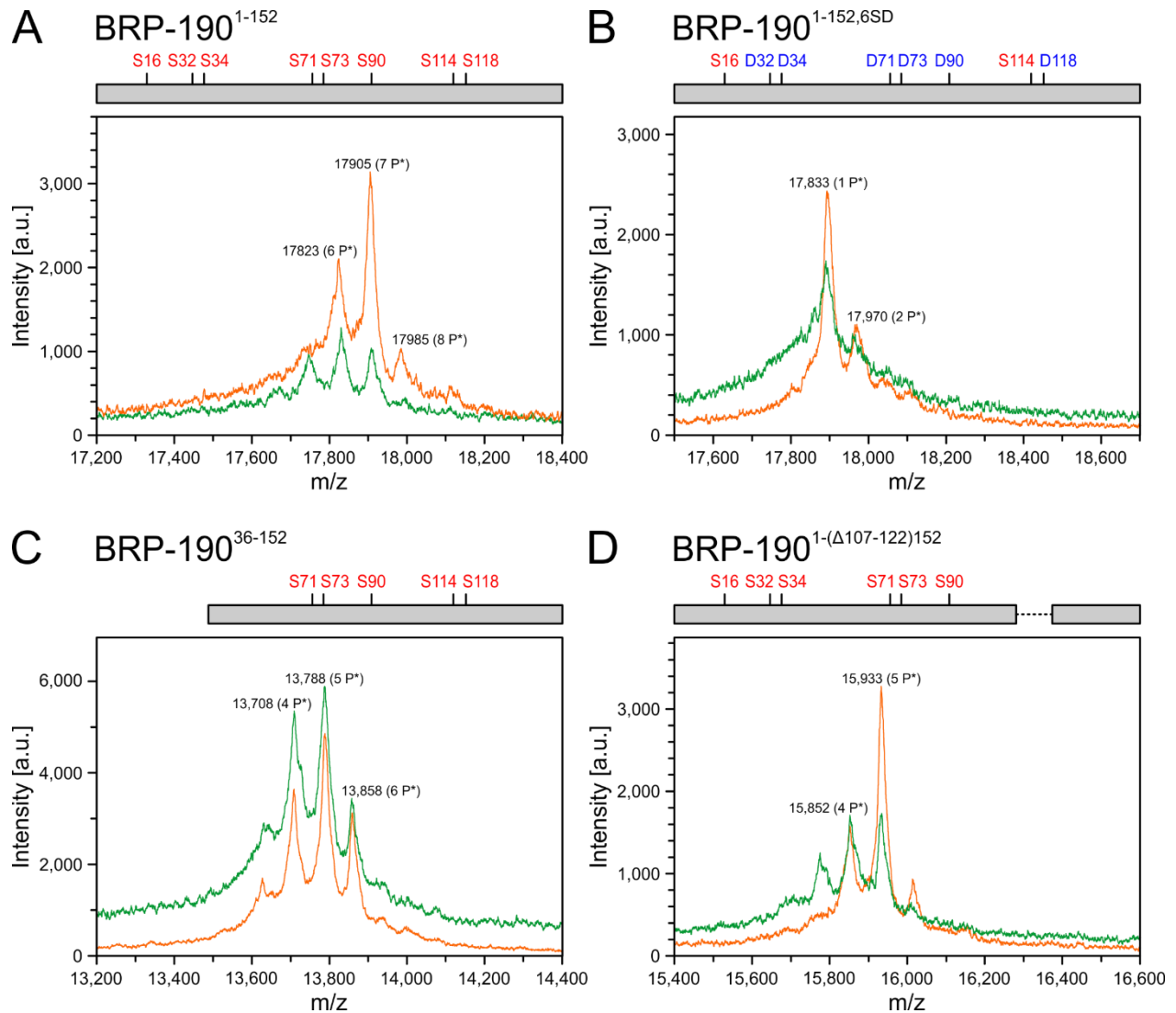


Fig. S2. Mass analysis of phosphorylated BRP-190 fragments. (A-D) Phosphorylation was carried out by SRPK79D^{Core} (orange) and SRPK79D^{CoreΔdock} (green). (A) Both kinases added about seven phosphates to BRP-190¹⁻¹⁵². (B) BRP-190^{1-152,6SD} (phospho-mimetic aspartate residues at positions 32, 34, 71, 73, 90 and 118) was phosphorylated predominantly at one site by both kinase variants. (C) BRP-190³⁶⁻¹⁵² was phosphorylated predominantly at five sites, suggesting that only two predominant phosphorylation sites are located within residues 1-35 (presumably S32 and S34). (D) BRP-190^{1-(Δ107-122)152} (deletion of one SRPK binding motif identified in the peptide SPOT analysis; Fig. 5E) was phosphorylated predominantly at five sites. Together, these results suggest a more complete phosphorylation of S114 compared to S16.