

Supplemental Figures to

An RBPJ-*Drosophila* model reveals dependence of RBPJ protein stability on the formation of transcription-regulator complexes

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The supplemental Figures contain

Figure S1: Sequence comparison between fly Su(H) and murine RBPJ protein,

Figure S2: Substitution of murine *RBPJ* for *Su(H)* in the fly by genome engineering,

Figure S3: Defective adult genitalia rotation in *RBPJ^{wt}* males,

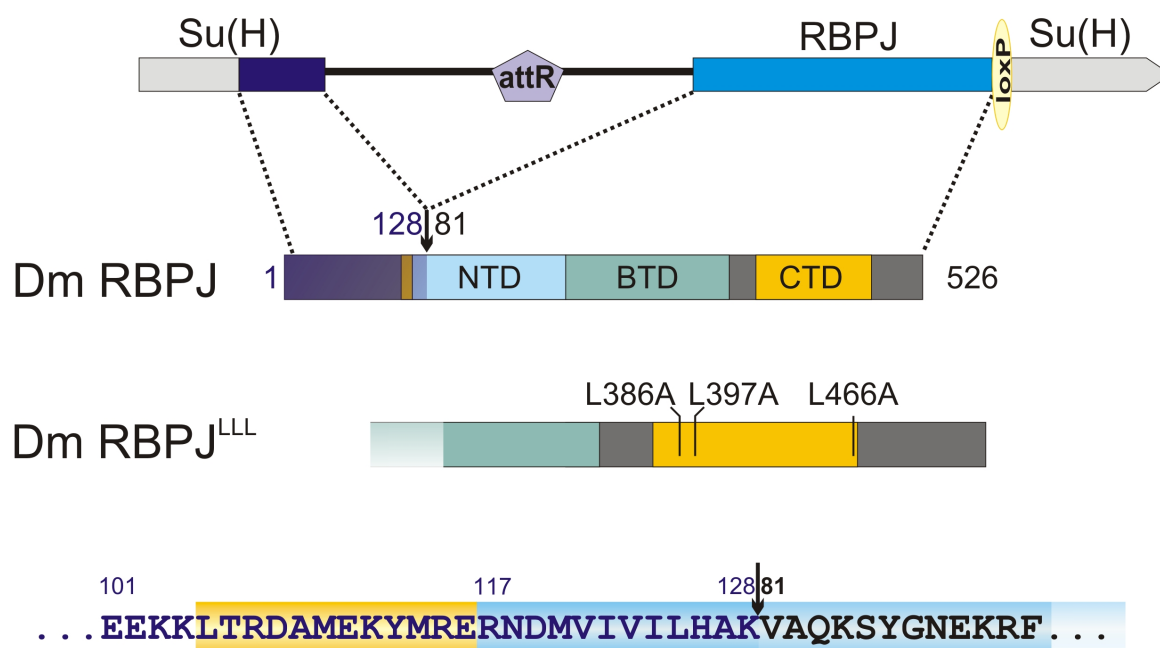
Figure S4: Uncropped blots used for RBPJ protein quantification.

Species	Accession	Gene	Protein	Length
Dm	1	<u>MKSYSQFNLNAAAPPAIAYETTVVNPNGSPLDPHQQQQQQSQDMPHFGLP</u>	50	
Mm	1	-----MPSGFPQSPRTSPRAR-----PKTRIT	22	
Dm	51	<u>GPQPSSQQQQQQQLQVHHQQQQQQQQQQQQQHQQQMOMSLLPGPYRPHI</u>	100	
Mm	23	GALP-----MDYSEGLSAEERPAHAPS-----AGKFGERP	52	
Dm	101	<u>EEKKLTRDAMEKYMRENDMVIVILHAKVAQKSYGNEKRFFCPCPPCIYLF</u>	150	
Mm	53	PPKRLTREAMRNYLKERGDQTVLILHAK <u>VAQKSYGNEKRFFCPCPPCVYLM</u>	102	
Dm	151	GSGWRRRYEEMLQQGEGEQAQLCAFIGIGSSDQDMQQLDLNGKQYCAAK	200	
Mm	103	<u>GSGWKKKKEQMERDGCSEQESQPCAFIGIGNSDQEMQQLNLEGKNYCTAK</u>	152	
Dm	201	TLFISDSDKRKHFM LSVKMFYGNHGDIGVFNSKRIKVISKPSKKKQSLKN	250	
Mm	153	<u>TLYISDSDKRKHFM LSVKMFYGNSDDIGVF LSKRIKVISKPSKKKQSLKN</u>	202	
Dm	251	ADLCIASGTNVALFNRLRSQTVSTRYLHVENGHFHASSTQWGAFTIHLDD	300	
Mm	203	<u>ADLCIASG TKVALFNRLRSQTVSTRYLHVEGGNFHASSQQWGA FYIHLDD</u>	252	
Dm	301	DNESESEEFQVRDGYIHYGATVKLVCSVTGMALPRLIIRKVDKQMALLEA	350	
Mm	253	<u>DDESEGE EFTVRDGYIHYGQTVKLVCSVTGMALPRLIIRKVDKQTALLDA</u>	302	
Dm	351	DDPVSQLHKCAFYMKDTRMYLCLSQEKIIQFQATPCPKEPNKEMINDGA	400	
Mm	303	<u>DDPVSQLHKCAFYLK DTERMYLCLSQERI IQFQATPCPKEQNKEMINDGA</u>	352	
Dm	401	CWTIISTDKAEYQFYEGMGPVASPVTPVPPIVNSINLNGGGDVAMLELSGD	450	
Mm	353	<u>SWTIISTDKAEYTFYEGMGPVLAPVTPVPV VESLQLNGGGDVAMLELTGQ</u>	402	
Dm	451	NFTPHLQVWFGDVEAETMYRCTETLLCVVPEISQFRGEWLWVRQPTQVPI	500	
Mm	403	<u>NFTPNLRVWFGDVEAETMYRCGESMLCVVPDISAFREGWRWVRQPVPV</u>	452	
Dm	501	SLVRNDGIIYATGTFTTYTPEPGPRPHCNTQAEDVMRARQN-----	541	
Mm	453	<u>TLVRNDGVIYSTSLTTYTPEPGPRPHCSA-AGAILRANSSQVPSNESNT</u>	501	
Dm	542	NNNNNITSISNNNNSNAGSPAAGGGLQQQQQQHQALPSISEVQWNHSGSGLS	594	
Mm	502	<u>NSEGNYTNASTNSTSVTSSTATVVS</u>	526	

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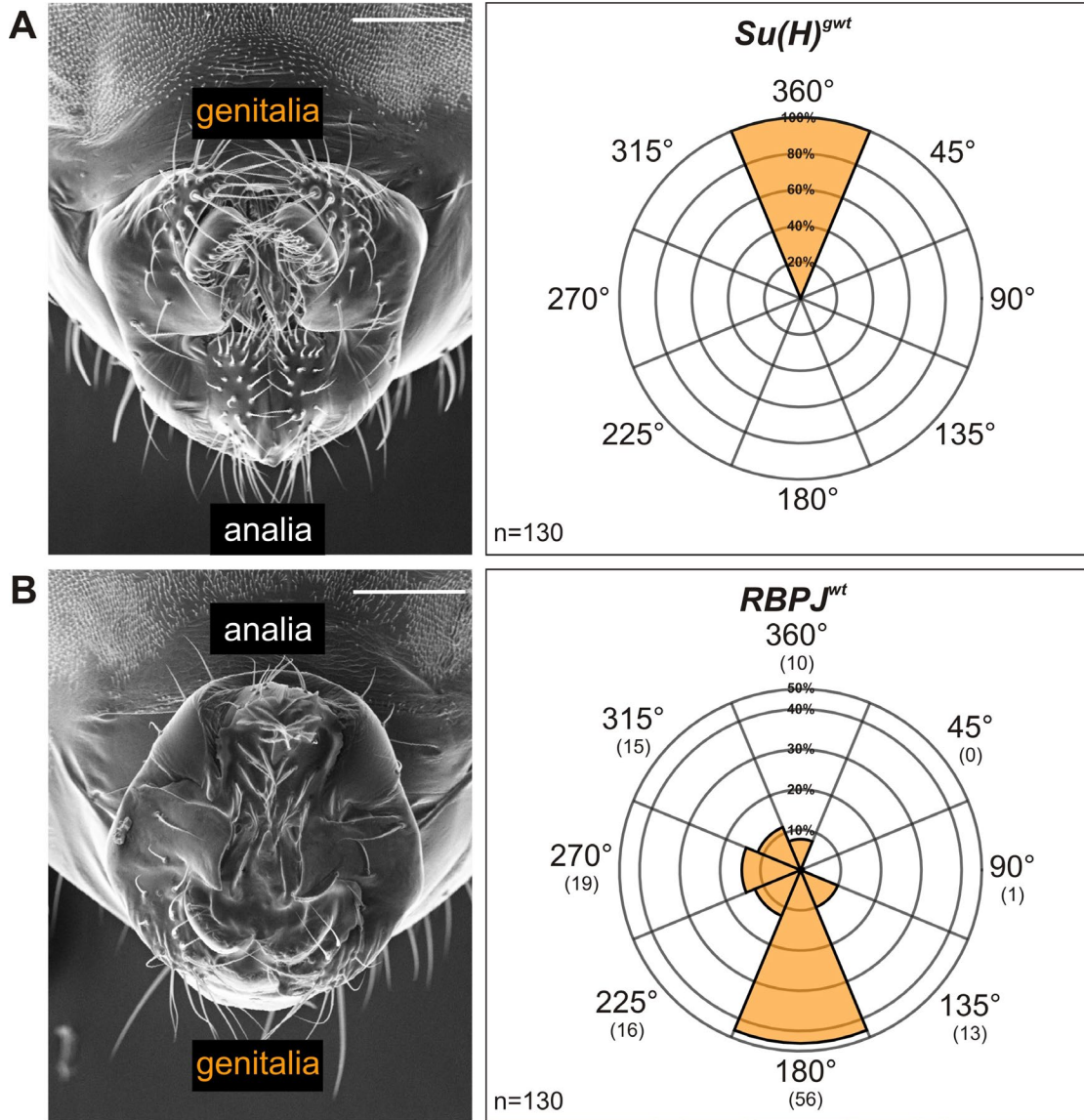
domain (see Fig. 1A), which is connected to the β -trefoil domain by a β strand (grey). Red color highlights the three leucine residues at position 434, 445 and 514 in Su(H) known to contact H in the repressor complex; their replacement by alanine prevents Su(H)-H repressor complex formation. The respective leucines in RBPJ at position 386, 397 and 466 are labeled in blue.

Figure S2 Substitution of murine *RBPJ* for *Su(H)* in the fly by genome engineering



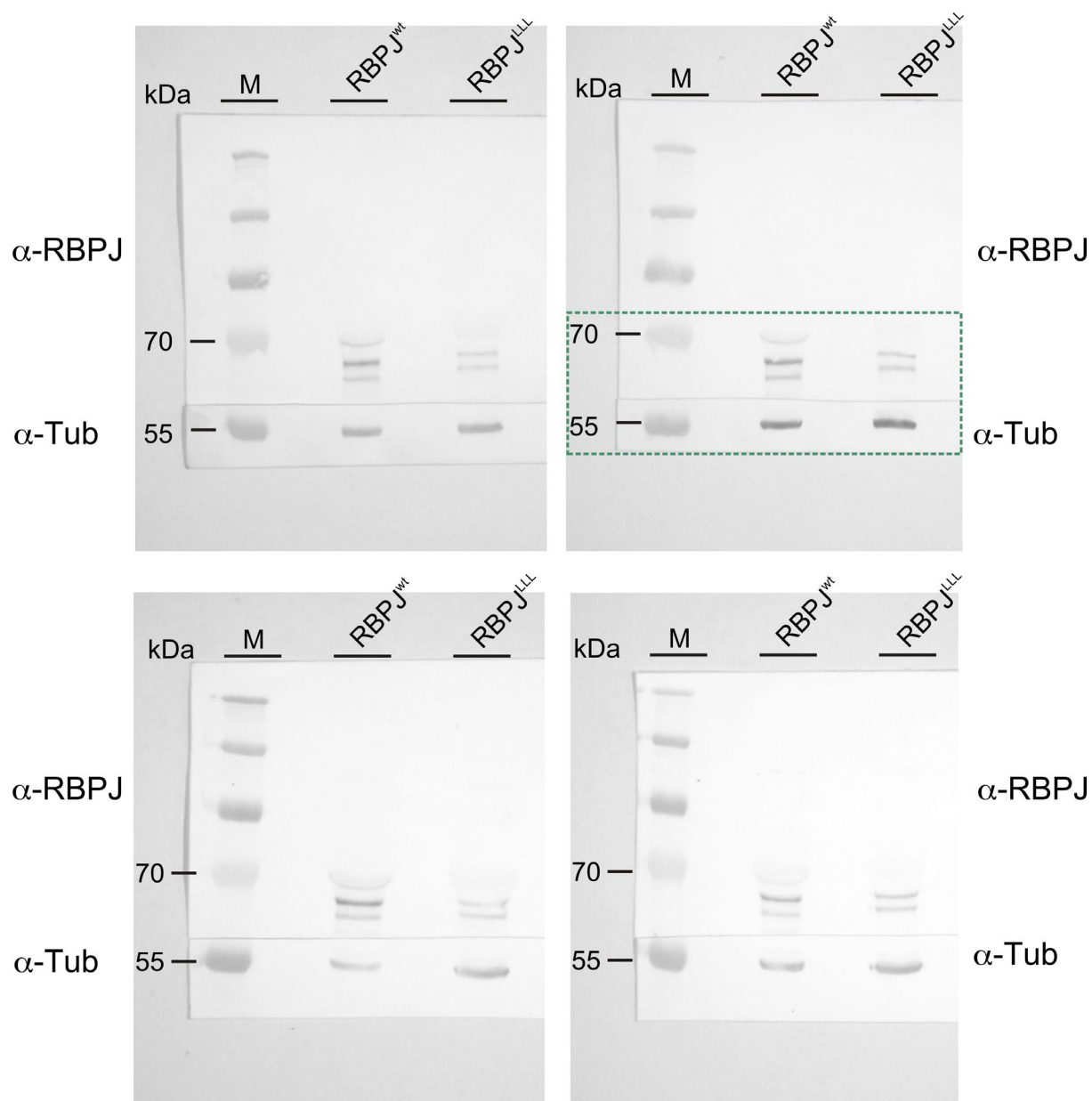
Subsequent to genome engineering, a gene fusion between *Su(H)* and *RBPJ* results in the formation of a fusion protein containing *RBPJ* from valine 81 fused to the N-terminal 128 amino acids of *Su(H)*. Hence α 1-helix and the start of NTD are derived from *Su(H)*, which is extremely similar to *RBPJ* in this part (58% identity and 79% similarity; see Figure S1 for a sequence comparison). The sequence of the fusion is depicted, color-coded as in Figure S1. *RBPJ*^{LLL} contains three leucine mutations at position 386, 397 and 466, which were replaced by alanine.

Figure S3: Defective adult genitalia rotation in $RBPJ^{wt}$ males



(A) $Su(H)^{wt}$ adult male genitalia. (B) Rotated genitalia are observed in $RBPJ^{wt}$ homozygous males with high frequency. Scanning electron micrographs show enlargements of the terminal abdomen with the genitalia and analia from $Su(H)^{wt}$ control (A) and a half rotated $RBPJ^{wt}$ (B). Most frequently, rotation stopped half way at an 180° angle, as shown in the chart (polarhistograph) (n=130). Scale bars: 100 μ m.

Figure S4 Uncropped blots used for RBPJ protein quantification



Original blots used for quantification of RBPJ levels in Figure 7C. The blot depicted in Figure 7C is framed. Blots were cut for parallel detection of RBPJ and Tubulin; the latter served as internal standard.