# 1 Supplementary Information

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# A multi-factor trafficking site on spliceosome remodeling enzyme, BRR2, recruits C9ORF78 to regulate alternative splicing

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7	A	lexandra Bergfort <sup>1,§</sup> , Marco Preußner <sup>2</sup> , Benno Kuropka <sup>3,4</sup> , İbrahim Avşar Ilik <sup>5</sup> , Tarek Hilal <sup>1,4,6</sup> ,
8	G	ert Weber <sup>7</sup> , Christian Freund³, Tuğçe Aktaş⁵, Florian Heyd², Markus C. Wahl¹. <sup>,,</sup> *
9		
10	1	Freie Universität Berlin, Institute of Chemistry and Biochemistry, Laboratory of Structural
11		Biochemistry, Takustrasse 6, D-14195 Berlin, Germany
12	2	Freie Universität Berlin, Institute of Chemistry and Biochemistry, Laboratory of RNA
13		Biochemistry, Takustrasse 6, D-14195 Berlin, Germany
14	3	Freie Universität Berlin, Institute of Chemistry and Biochemistry, Laboratory of Protein
15		Biochemistry, Thielallee 63, D-14195, Berlin, Germany
16	4	Freie Universität Berlin, Institute of Chemistry and Biochemistry, Core Facility BioSupraMol,
17		Thielallee 63, D-14195, Berlin, Germany
18	5	Max Planck Institute for Molecular Genetics, Ihnestr. 63, D-14195 Berlin, Germany
19	6	Freie Universität Berlin, Institute of Chemistry and Biochemistry, Research Center of
20		Electron Microscopy and Core Facility BioSupraMol, Fabeckstr. 36a, 14195 Berlin,
21		Germany
22	7	Helmholtz-Zentrum Berlin für Materialien und Energie, Macromolecular Crystallography,
23		Albert-Einstein-Straße 15, D-12489 Berlin, Germany
24		
25	§	Present address: Yale University, Molecular Biophysics and Biochemistry, 333 Cedar
26		Street, New Haven, CT 06510, USA

27 \* Correspondence to: markus.wahl@fu-berlin.de

#### **Supplementary Tables**

#### Supplementary Table 1: CryoEM data collection and refinement statistics

Complex	BRR2 <sup>HR</sup> -PRPF8 <sup>Jab1</sup> - C9ORF78	BRR2 <sup>HR</sup> - FBP21 <sup>200-376</sup>
Data collection		
Microscope	FEI Titan Kri	os G3i
Voltage [keV]	300	
Camera	Falcon 3	EC
Magnification	120,000	96,000
Pixel size [Å/px]	0.657	0.832
Total electron exposure [e <sup>-</sup> /Å <sup>2</sup> ]	40	40
Exposure rate [e <sup>-</sup> /px/s]	0.6	0.7
No. of frames collected during exposure	33	33
Defocus range [µm]	0.8 -1.8	0.8 -1.8
Automation software	EPU2.10	EPU2.09
Micrographs collected	5,160	1,986
Micrographs used	4,986	1,877
Data processing		
Total extracted particles	936,716	787,610
Refined particles	542,565	271,493
Final particles	370,493	57,854
Point-group or helical symmetry parameters	C1	Ć1
Global resolution [Å]	2.72	3.30
FSC0,143 unmasked/masked [Å]	3.1 / 2.7	3.7 / 3.0
Local resolution range [Å]	30.0 - 2.2	24.6 - 2.4
Map sharpening B factor [Å <sup>2</sup> ] / (B factor range)	-97	-120.3
Map sharpening method	Local B-factor	Local B-factor
Model refinement	L	1
CC mask	0.89	0.86
CC volume	0.89	0.85
Model		
Non-H atoms	16 485	14 054
Protein residues	2.046	1.746
DMOD1	_,	.,
RMSD <sup>1</sup>	0.004	0.005
Bond lengths [A]	0.004	0.005
	0.364	0.602
Ramachandran plot		
Favored [%]	97.84	97.18
Allowed [%]	2.16	2.82
Outliers [%]	0	0
Model quality <sup>2</sup>		
Clash score	7,93	10.12
Rotamer outliers [%]	0.93	0.96
Overall score	1.47	1.68
Data availability		
FMDB ID	13046	13045
PDB ID	70S2	70S1

RMSD, root-mean-square deviation from ideal geometry
 Assessed using MolProbity<sup>67</sup>

# 34 Supplementary Table 2: PCR primers

C9ORF78 (RT-qPCR)						
Forward	5'-TCCTTCGTGCCTACCAACAT-3'					
Reverse	5'-GCTTCTCCGTGTCACCTACT-3'					
GAPDH (RT-qPCR)						
Forward	5'-CTTCGCTCTCTGCTCCTCCTGTTCG-3'					
Reverse	5'-ACCAGGCGCCCAATACGACCAAAT-3'					
PTBP2 (radioactive RT-PC	PTBP2 (radioactive RT-PCR)					
Forward	5'-CTGAGTCCTTTGGCCATTCC-3'					
Reverse 5'-CACGCTGCACATCTCCATAA-3'						
SMARCA4 (radioactive RT-PCR)						
Forward	5'-AAACTCTCCCCTAACCCACC-3'					
Reverse	5'-TGGATGAAGACCTCGCTGAG-3'					
C1ORF131 (radioactive RT-PCR)						
Forward	5'-ACAAGTAGAAGTGGTAGAATTTCAC-3'					
Reverse	5'-TCCACATCTCTCTCCAAAACAC-3'					

## 36 Supplementary Figures

#### а



#### С





## **b** kDa



#### Band 1

#### Protein sequence coverage: 39%

Matched	peptides	shown	in	bold	red.	

1	MPVVRKIFRR	RRGDSESEED	EQDSEEVRLK	LEETREVQNL	RERPNGVSAV
51	ALLVGERVQE	ETTLVDDPFQ	MKTGGMVDMR	KLKERGKDKI	SEEEDLHLGT
101	SFSAETNRRD	EDADMMKYIE	TELKKRKGIV	EHEEQKVKPK	NAEDCLYELP
151	ENIRVSSAKK	TEEMLSNQML	SGIPEVDLGI	DAKIKNIIST	EDAKARLLAE
201	QQNKKRDSET	SEVPTNMAVN	YVQHNRFYHE	ELNAPIRRNK	EEPKARPLRV
251	GDTEKPEPER	SPPNRKRPAN	EKATDDYHYE	KFKKMNRRY	

#### Band 2

Protein sequence coverage: 8%

Match	ned peptides	shown in bo	ld red.		
1	MPVVRKIFRR	RRGDSESEED	EQDSEEVRLK	LEETREVONL	
51	ALLVGERVOR	FTTLUDDPFO	METGOMUDME	RUKERGKDET	

DI	MULAGERARE	CITRADDELA	NUT GOMA DNU	UDVEROVDVI	2FFFDTUD01	
101	SFSAETNRRD	EDADMMKYIE	TELKKRKGIV	EHEEQKVKPK	NAEDCLYELP	
151	ENIRVSSAKK	TEEMLSNQML	SGIPEVDLGI	DAKIKNIIST	EDAKARLLAE	
201	QQNKKKDSET	SEVETNMAVN	YVQHNRFYHE	ELNAPIRRNK	EEPKARPLRV	

RKRPNGVSAV

C 251 GDTEKPEPER SPPNRKRPAN EKATDDYHYE KFKKMNRRY



#### 39 Supplementary Figure 1: Protein interaction studies in vitro

a, SDS-PAGE gels showing elution fractions from analytical SEC, monitoring interaction of
GST-C9ORF78 with BRR2 truncation variants (BRR2<sup>HR</sup>, BRR2<sup>NC</sup>, BRR2<sup>CC</sup>). Elution direction
is indicated by an arrow. The same elution fractions from runs under identical conditions are
shown. Protein bands are identified on the right. M, molecular mass marker. In the second
panel, upper and lower regions of the same gels were spliced together. Dotted line, splice
position. Independent experiments were conducted twice with similar results.

**b**, Like **a**, monitoring simultaneous interaction of PRPF8<sup>Jab1 $\Delta$ C</sup> and C9ORF78 with BRR2<sup>HR</sup> (upper panels), and interaction of C9ORF78 with PRPF8<sup>Jab1 $\Delta$ C</sup> in the absence of BRR2<sup>HR</sup> (lower panels). Elution direction is indicated by an arrow. The same elution fractions from runs under identical conditions are shown. Protein bands are identified on the right. M, molecular mass marker. In the first, second and fourth panel, different regions of the same gels were spliced together. Dotted lines, splice positions. Independent experiments were conducted twice with similar results. Source data for **a** and **b** are provided as a Source Data file.

c, Left, SDS-PAGE gels showing elution fractions from analytical SEC after digest of the BRR2<sup>HR</sup>-C9ORF78 complex with elastase. Elution direction is indicated by an arrow. Protein bands are identified on the right. M, molecular mass marker. IS, injected sample. Gel bands subjected to mass spectrometric analysis are numbered and highlighted by red boxes. Right, C9ORF78 peptides and sequence coverage identified in the analyzed gel bands by mass spectrometry. Independent experiments were conducted twice with similar results.

d, C9ORF78 peptide SPOT arrays incubated with His-tagged BRR2 constructs and probed by
anti-His antibody. Peptides 1 and 2 (red boxes) are highly reactive with BRR2<sup>FL</sup>, BRR2<sup>HR</sup> and
BRR2<sup>CC</sup>, but not with BRR2<sup>NC</sup>. Sequences for peptides 1 and 2 are indicated in the lower left
box. The conserved BRR2-interacting F8-R9 motif, contained in these peptides, is shown in
bold and underlined. Positively charged residues, blue; negatively charged residues, red.



- 66 Supplementary Figure 2: CryoEM analysis of BRR2<sup>HR</sup>-PRP8<sup>Jab1</sup>-C9ORF78 and BRR2<sup>HR</sup>-
- 67 **FBP21**<sup>200-376</sup> **complexes**

a, Representative cryoEM micrographs of the BRR2<sup>HR</sup>-PRP8<sup>Jab1</sup>-C9ORF78 complex. Scale
 bar, 50 nm. 5,160 micrographs were recorded from the same sample, particle images were
 picked from 4,986 high-quality micrographs.

b, 2D class averages of BRR2<sup>HR</sup>-PRP8<sup>Jab1</sup>-C9ORF78 particle images after reference-free
 classification.

**c**, Viewing direction distribution plot of the particle images used for the final reconstruction of

the BRR2<sup>HR</sup>-PRP8<sup>Jab1</sup>-C9ORF78 cryoEM map as obtained during NU refinement with cryoSPARC.

d, Global resolution estimation for the BRR2<sup>HR</sup>-PRP8<sup>Jab1</sup>-C9ORF78 cryoEM map by gold standard Fourier shell correlation (FSC). Dashed line, FSC<sub>0.143</sub>.

**e**, Local resolution estimation as determined with cryoSPARC, ranging from 2.2 Å to 5.4 Å for

the BRR2<sup>HR</sup>-PRP8<sup>Jab1</sup>-C9ORF78 cryoEM map.

**f**, Representative cryoEM micrographs of the BRR2<sup>HR</sup>-FBP21<sup>200-376</sup> complex. Scale bar, 50 nm.

1,986 micrographs were recorded from the same sample, particle images were picked from

82 1,877 high-quality micrographs.

g, 2D class averages of BRR2<sup>HR</sup>-FBP21<sup>200-376</sup> particle images after reference-free
 classification.

**h**, Viewing direction distribution plot of the particle images used for the final reconstruction of

the BRR2<sup>HR</sup>-FBP21<sup>200-376</sup> cryoEM map as obtained during NU refinement with cryoSPARC.

i, Global resolution estimation for the BRR2<sup>HR</sup>-FBP21<sup>200-376</sup> cryoEM map by gold-standard

88 Fourier shell correlation (FSC). Dashed line, FSC<sub>0.143</sub>.

j, Local resolution estimation as determined with cryoSPARC, ranging from 2.4 Å to 6.4 Å for
 the BRR2<sup>HR</sup>-FBP21<sup>200-376</sup> cryoEM map.

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## 94 Supplementary Figure 3: CryoEM data refinement.

a, Reconstruction of the BRR2<sup>HR</sup>-PRPF8<sup>Jab1</sup>-C9ORF78 cryoEM map. 936,716 particle images
were picked from 4,986 micrographs and subjected to reference-free 2D classification.
836,442 particle images were selected for heterogeneous 3D refinement into 3 classes. The
best appearing class, consisting of 545,045 particle images, was selected for re-extraction with

a box size of 384 px, Fourier-cropped to 192 px (pixel size 1.314 Å). Homogeneous NU 99 refinement yielded a reconstruction at 2.79 Å resolution that could be improved to 2.71 Å 100 101 resolution by CTF refinement. To further improve the reconstruction, local motion correction was applied and particle images were re-extracted at full spatial resolution with a box size of 102 384 px (0.657 Å/px). NU refinement yielded a reconstruction at 2.64 Å resolution that showed 103 only fragmented density for C9ORF78. 3D variability analysis using a generous mask covering 104 105 the N-terminal part of C9ORF78 was applied to remove sub-stoichiometric complexes. 370,493 particle images were selected for final NU refinement yielding a reconstruction at 2.76 106 Å resolution. 107

b, Reconstruction of the BRR2<sup>HR</sup>-FBP21<sup>200-376</sup> cryoEM map. 787,610 particle images were 108 109 picked from 1,877 micrographs and subjected to reference-free 2D classification. 349,113 particle images were selected for heterogeneous 3D refinement into 3 classes. Two classes 110 were merged, the 271,494 particle images were re-extracted with a box size of 336 px, Fourier-111 cropped to 168 px (pixel size 1.664 Å). Homogeneous refinement yielded a reconstruction of 112 113 3.47 Å that was subjected to another round of heterogeneous 3D refinement. 165,229 particle images were selected for local motion correction and un-binned re-extraction (box size 336 px. 114 0.832 Å/px), which yielded a map at a resolution of 3.0 Å. 3D variability analysis using a mask 115 around the FBP21<sup>200-376</sup>-binding region was applied to select the final 57,854 particle images 116 for NU refinement, yielding a reconstruction at 3.30 Å resolution. 117



## 121 Supplementary Figure 4: Details of cryoEM maps.

a-d, CryoEM maps as shown in Fig. 1c,d of a BRR2<sup>HR</sup>-PRPF8<sup>Jab1</sup>-C9ORF78 complex (a, b)
 and of a BRR2<sup>HR</sup>-FBP21<sup>200-376</sup> complex (c, d), contoured at > 10 RMSD and covering the
 C9ORF78 or FBP21<sup>200-376</sup> molecules, respectively. BRR2 is shown as a cartoon, C9ORF78 or
 FBP21<sup>200-376</sup> are shown as sticks. The close-up views (b, d) zoom in on the common BRR2 interacting motifs (C9ORF78, F8-R9; FBP21<sup>200-376</sup>, L369-R370).





Supplementary Figure 5: C9ORF78 has a moderate inhibitory effect on the BRR2 helicase activity. 

**a**, Exemplary non-denaturing PAGE, monitoring BRR2<sup>HR</sup>-PRPF8<sup>Jab1</sup>-mediated U4/U6 unwinding in the absence (black outline) or presence (orange outline) C9ORF78. Bands are identified on the right with numbers of nucleotides (nts). U4\*, radio-labeled U4 snRNA.

**b**, Quantification of data shown in **a**. Data points represent means  $\pm$  SD; n = 3 technical replicates. Data were fit to a single exponential equation (fraction unwound = A (1 - exp[-  $k_u$ t])); A, amplitude of the reaction;  $k_u$ , apparent first-order rate constant of unwinding; t, time).

138 **c**, Change of unwinding rate of the indicated BRR2 variants alone or in complex with 139 PRPF8<sup>Jab1 $\Delta$ C</sup> or PRPF8<sup>Jab1</sup> in the absence (black bars) or presence (orange bars) C9ORF78. 140 Gray bar, control with GST instead of C9ORF78 added. Bars indicate means ± SD obtained 141 from curve fits to all n = 3 technical replicates, each for ten time points. Source data for **b** and 142 **c** are provided as a Source Data file.



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#### 146 Supplementary Figure 6: Features of alternative exons regulated by C9ORF78.

147 **a**, Scheme for alternative exons.

**b-f**, Features of skipped exons regulated by C9ORF78. In each panel, unregulated (UR) exons quantified by rMATS (black; n = 122,362) are compared to alternative exons showing C9ORF78 KD-induced skipping (red; n = 196) or C9ORF78 KD-induced inclusion (blue; n =194). Horizontal lines, medians; whiskers, minimum and maximum values; boxes, upper and lower quartile. Statistical significance was determined by unpaired, two-sided Student's t-tests (\*\*, p < 0.01; \*\*\*\*, p < 0.0001; ns, not significant).

- 154 **b**, Lengths of the upstream introns.
- 155 **c**, Lengths of the alternative exons; \*\*, p = 0.0027; \*\*\*\*, p < 0.0001.
- 156 **d**, Lengths of the downstream introns.
- **e**, Strengths of the 5'-splice sites of the alternative exons (5'-ss); \*\*, p = 0.0054; \*\*\*\*, p < 0.0001.

**f**, Strengths of the 3'-splice sites of the alternative exons (3'-ss).



Supplementary Figure 7: Features of alternative 3'-ss regulated by C9ORF78. 162

**a**, Scheme for alternative 3'-ss usage. 163

b-h, Features of alternative 3'-ss regulated by C9ORF78. In each panel, all alternative 3'-ss 164 quantified by rMATS (black; n = 6,160) are compared to alternative 3'-ss showing C9ORF78 165

- 166 KD-induced skipping (red; n = 89) and alternative 3'-ss showing C9ORF78 KD-induced
- 167 inclusion (blue; n = 16). Horizontal lines, medians; whiskers, minimum and maximum values;
- boxes, upper and lower quartile. Statistical significance was determined by unpaired, two-sided
- 169 Student's t-tests (\*, p < 0.05; \*\*, p < 0.01; \*\*\*\*, p < 0.0001; ns, not significant).
- 170 **b**, Lengths of the upstream introns.
- **c**, Distances between the upstream 3'-ss and the downstream 3'-ss (3'-ss gap); \*\*, p = 0.0084;
- 172 \*\*\*\*, p < 0.0001.
- **d**, Lengths of the exons containing the alternative 3'-ss; \*\*, p = 0.0095.
- **e**, Strengths of the 5'-splice sites of the upstream introns (upstream 5'-ss).
- 175 **f**, Strengths of the upstream 3'-splice sites; \*, p = 0.0176.
- 176 **g**, Strengths of the downstream 3'-splice sites.
- 177 **h**, Strengths of the 5'-splice sites of the downstream introns (downstream 5'ss).
- 178 Upstream 3'-ss requiring C9ORF78 for usage are characterized by a very short distance to the
- alternative downstream 3'-ss (c), a comparatively long exon that contains the alternative 3'-ss
- 180 (d), and they are comparatively weak (f).

H. sapiens M. mulatta P. troglotydes B. taurus C. lupus M. musculus G. gallus X. tropicalis D. rerio D. melanogaster A. thaliana O. sativa	MSDAEET MSE	K A E A T T K V L S E E P Q E K V F	. МР V V R K I . МР V V R K I . МР V V R K I . МР V T G K S . М R I T G K S R A . МР G G R N . МР G G R K . МР G G R K . МР G G K K . МР G G K K . МР C G K K C K . МР Р К K K C K K C K . МР Р К K C K K C K	10 FRRRG.DS FRRRRG.DS FRRRRRA.DS FRRRRRA.DS FRRRRRA.DS FRRRRRASDS FRRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRRRKESS FRKRNLEAT	E       S       E       D       S       E       E       D       E       E       E       D       D       N       K       A	20 EDEQDSEEVR EDEQDSEEVR EDEQDSEEVR EDEQDSEEVR EDEQDSEEVR EDEQLSEEVR EDEQLAEEVR EALEVTQEVR CAELEVTQU QEQETEADIL AISEEEKRR DDDDARR	30       40         L K L EE T RE V Q N L R K         K L EE T RE V Q N L R K         L K L EE T RE V Q N L R K         L K L EE T RE V Q N L R K         L K L EE T RE V Q N L R K         L K L EE T RE V Q N L R K         L K L EE T RE V Q N L R K         L K L EE T RE V Q N L R K         L K L EE A K E V Q S L R K         I K L EE A K E V Q S L R K         I K L EE A K E V Q S L R K         J K L DE A K E R Q K L R K         J K L DE I K E R Q K L R N         A K L EE T K F L Q K L R E         V A L EE T K F L Q K L R E
H. sapiens M. mulatta P. troglotydes B. taurus C. lupus M. musculus G. gallus X. tropicalis D. rerio D. melanogaster A. thaliana O. sativa	RPPNGVSA RPPNGVSA RPPNGVSA RPPNGVSA RPPNGVSA RPPNGVSA RPPNGVSA RCPNGVSI RCPNGVSI RCPNGVSI RCPNGVSI RCPNGVSI RCPNGVSI RCPNGVSI RCPNGVSI	50 VALLVGEK VALLVGEK VALLVGEK AALLVGEK AALLVGEK AALLVGEK AALLVGEK AALLVGEK AALLVGEK AALLVGEK AALLVGEK AALLVGEK AALSS AAAA SS	60 Y QE. ETTLV Y QE. ETTLV Y QE. ETTLV QE. ETTLV QE. ETTLV QE. EXTLV PE. EVIMA PL. EAELE AP EEELTV GKV	70 DDPFQMKT DDPFQMKT DDPFQMKT DDPFQMKT DDPFQMAT DDPFKKNQS DDPFKLKS DDPFKLKS CDPFKLKT KDPFNIKT KDPFNIKT ASPRGRGG	80 3 G M V D M K K L K 3 G M V D M K K L K 3 G M V D M K K L K 3 G M V D M K K L K 3 G M V D M K K L K 3 G M V D M K K L K 3 G M V D M K K L K 3 G V V D M K K L K 3 G V V D M K K L K 3 G V V M M Q A L K 3 G G	90 ERGK. DKI SE ERGK. DKI SE ERGK. DKI SE ERGK. DKI SE ERGK. DKI SE ERGK. DRI SE DRSR. DRI GE DRSR. DRL GE DRSR. DAL O AGKLKAAVED . EKTETEGE . GLAAGGDAE	100 E E D L H L GT S F S A E T E E D L H L GT S F S A E T E E D L H L GT S F S A E T E E D L H L GT S F S A E T E E D L H L GT S F S A E T E E D L N L GT S F S A E T E E D L N L GT S F S A E T E N D L N L GT S F S A E T A Y D V G I GT Q F S A E T A Y D V G I GT Q F S A E T K E E L V L Q D T F A Q E T K E D L V L Q D T F A Q E T
H. sapiens M. mulatta P. troglotydes B. taurus C. lupus M. musculus G. gallus X. tropicalis D. rerio D. melanogaster A. gambiae A. thaliana O. sativa	110 NRRDEDA NRRDEDA NRRDEDA NRRDEDA NRRDEDA NRRDEDA NRRDEDA NRRDEDA NRRDEDA NRRDEDA NKRDEDA NKRDEDA NKRDEDA NKRDEDA NKRDEDA	120 D M M K Y I E T E D M M K Y I E T E E M M K Y I E E E M M K Y I E E E M M K Y I E E N M K Y I E E	13 GIV KKRKGIV LKKRKGIV LKKRKGIV LKKRKGIV LKKRKGIV LKKRKGIV LKKRKGIV LKKKKGGV LKKKKGGV LKKKKGGV LKKKKGK LGKRKGRN LAKKRGK	30         E H E E Q K V         E H E E Q K V         E H E E Q K V         E H E E Q K V         E H E E Q K V         E H E E Q K V         E H E E Q K V         E H E E Q K V         E Q E Q K V         E Q E Q K V         Q E Q D N Q A E Q         I D D A E E V E         V D V K D K	140		160 NI RVSSAKKTEEML NI RVSSAKKTEEML NI RVSSAKKTEEML NI RVSSAKKTEEML NI RVSSAKKTEEML NI RVSSAKKTEEML SI KVSSAKKTEEML NI RVSSAKKTEEML NI RVSSAKKTEEML HL RQSSSAKKTEEML HL RQSSSAKKTEEML HL KVKKRSSEES HL KVRKKNSE
H. sapiens M. mulatta P. troglotydes B. taurus C. lupus M. musculus G. gallus X. tropicalis D. rerio D. melanogaster A. gambiae A. thaliana O. sativa	170 SNQMLSG	18 1 P E V DL G I 1 P E V DL	A K K N I I S K K N I I S A K K K K N I I S A K K K K N I I S A K K K K K I I S A K K K K K I S A K K K K K K K K K K K K K K K K K K K	190 T E D A K A R L I T E E A K A K L I T E E A K K K I T E A A K . K M	200 A E Q Q N K K K E A E Q Q N K K K E Q Q A K N K K E Q E Q R R L M G R F Q E K R L A G K T	210 SETSFVPTNM SETSFVPTNM SETSFVPTNM SETSFVPTNM SETSFVPTNM SETSFVPTNM SETSFVPTNM SETSFVPTNM SETSFVPTNM SGPSGFVPTNM GPSGFVPTNM SGPSGFVPTNM KSEFSIPSSY KSDANIPSSY	220 A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y V QHN R F Y H E E A V N Y QHN R F Y H E E A V N Y QHN R F Y H E E A V N Y QHN R F Y H E E A V N Y QHN R F Y H E E A V N F MQH R R Y R I D N S A D Y F Q R G K D Y A E K N A D F F H R G K D Y T E K
H. sapiens M. mulatta P. troglotydes B. taurus C. lupus M. musculus G. gallus X. tropicalis D. rerio D. melanogaster A. gambiae A. thaliana O. sativa	L N A P I RR L N A P V RR L N A P V RR L N A P V RR Q N T P Q RR N S D Q RK R N S D Q RK R R R E L R R E	240 NKEEPKAR. NKEEPKAR. NKEEPKAR. NKEEPKAR. NKEEPKAR. NKEEPKPR. NKEEPKPR. KREEPKPR. KREECHRGQ. HPELYKDQG	250 PLRVGDT PLRVGDT PLRVGDT PLRVGDT PLRVGDT PLRVGDT PLRVGDT CRVGDT CRVGDT CRVGDT CRVGDT CRCC CGPQAD CCCC CCCCC CCCCCCCCCCCCCCCCCCCCCCC	260 E K P E P E R. E K P A P E A. K P A P E A. K P A P E A. K P A S T S S S G K S MG. GI	S P P N.         R K R P A           S P P N.         R K R P A           S P P N.         R K R P A           S P P N.         R K R P A           S P P N.         R K R P A           S P P N.         R K R P A           S P P N.         R K R P P           S P P N.         R K R P P           S P P N.         R K R P P S           S P P N.         R K R P P S           S P P N.         R K R P P N S           S A Q H Q T N D R V         A A D S G K S           S N N N A D S G A G F         N H P D G A G A G F	270 NEKATDDYHY NEKATDDYHY NEKATDDYHY NEKATDDYHY NEKATDDYHY NEKATDDYHY NEKATDDYHY NEKATDDYHY SKEKATDDYHY SKEKATDDYHY SVKRATDDYHY SKEKATDDYHY SKEATDDYHY SKEATDDYHY SKEATDDYHY	280 E K F K K MN R R Y E K F K K Q R R R Y D K F K K Q Y R R H E R F R K R E R N R V M R R E R F R K R E K F R V M R R

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#### 184 Supplementary Figure 8: C9ORF78 conservation.

Multiple sequence alignment of C9ORF78 from different species, as indicated on the left. Residues are colored light to dark according to increasing level of conservation (54 % conservation, light yellow; 100 % conservation, orange). Residue numbering according to human C9ORF78 is indicated above the sequences. Secondary structure elements observed in the BRR2<sup>HR</sup>-PRPF8<sup>Jab1</sup>-C9ORF78 cryoEM structure are indicated below the sequences. The

- alignment was prepared with Homologene (NCBI) employing Clustal Omega<sup>75</sup> and shaded
- 191 with ALSCRIPT<sup>76</sup>.



194

#### 195 Supplementary Figure 9: Knock-down and over-expression.

196 RNA-seq data confirming KD of endogenous C9ORF78 and comparable levels of over-197 expression of siRNA-resistant C9ORF78<sup>wt</sup> and C9ORF78<sup>R41A</sup>. Horizontal lines, medians; 198 whiskers, minimum and maximum values (partially hidden due to the low variations); n = 3199 technical replicates. TPM, transcripts per million.



#### 203 Supplementary Figure 10: Comparison of siRNA KD and FLASH data.

a, Cumulative coverage plots obtained by deepTools, displaying RNAs that show changes in 204 alternative 3'-ss usage upon C9ORF78 KD and UV-crosslinks to C9ORF78 or GFP (negative 205 control; FLASH-derived sequence coverage of C9ORF78 splicing targets detected by rMATS 206

analysis). Heat maps are colored by normalized coverage average (n = 2 biologically independent experiments). Sequences of upstream exons as well as long and short exons harboring the alternative 3'-ss (scheme on the left) plus 50 base pairs upstream and downstream of the exons (illustrated below the heat maps) were analyzed. Top, targets with upstream 3'-ss more skipped upon C9ORF78 KD; bottom, targets with upstream 3'-ss more included upon C9ORF78 KD.

213 **b**, Like **a**, but for skipped exon events.