

Appendix to

Tubulin polyglutamylation differentially regulates microtubule-interacting proteins

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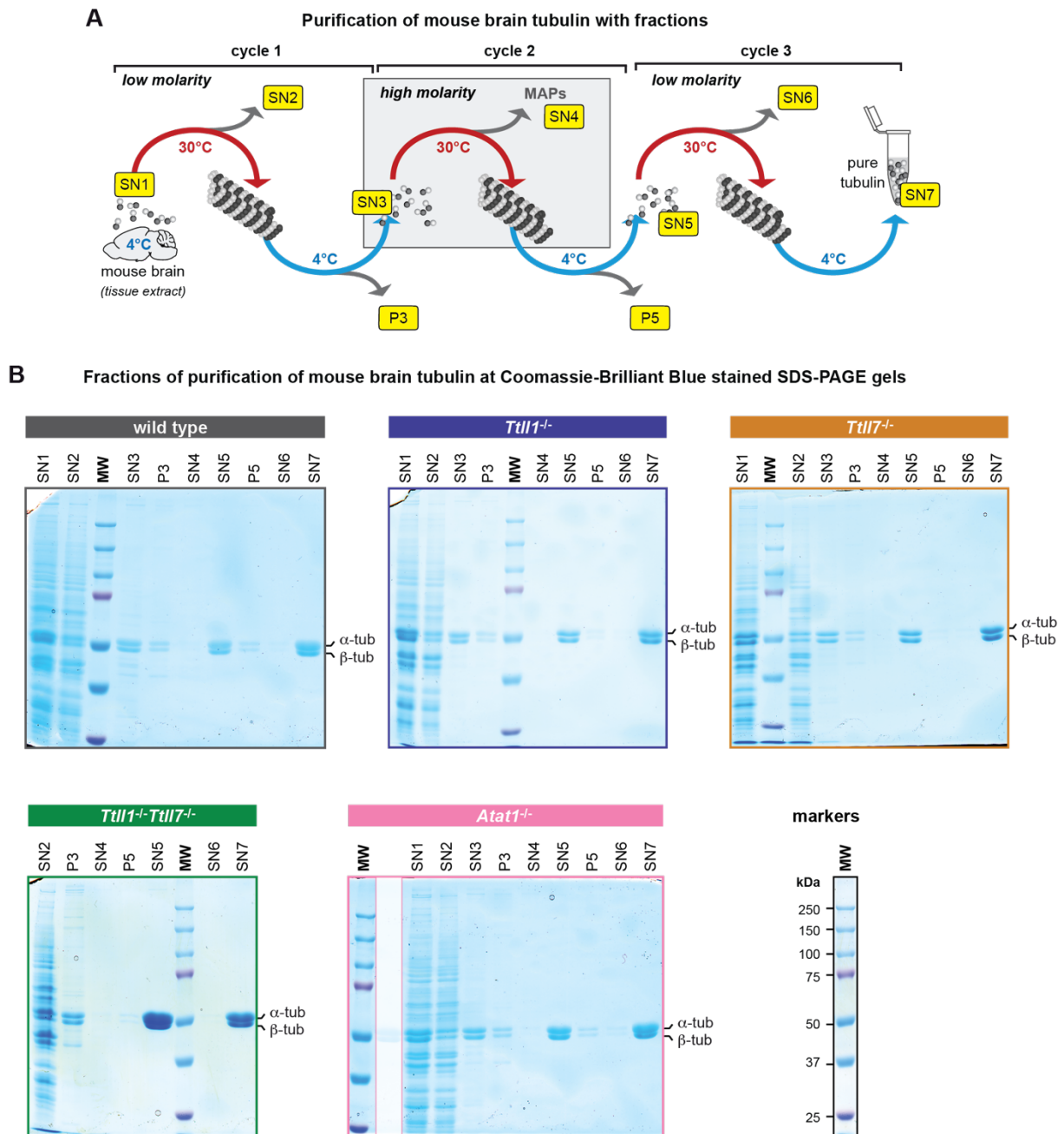
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Appendix Table S1. Primers and PCR conditions for genotyping.

mouse strain	PCR N°	PCR primers	PCR product size	annealing		elongation		N° of cycles
				time	temp	time	temp	
<i>Ttll1</i> ^{-/-}	PCR1	ACTCGTTCTGTGGCACCCCTGGC; TGTGGTCCGCTCAGGTGCCTCC	WT: 330 bp	15 s	62°C	20 s	72°C	38
	PCR2	CAATGTGCTTGGCGGTTTCAGGATCCC; GAACTCGACACCACCTGCAACCAACC	KO: 520 bp (WT: 1,500 bp)					
<i>Ttll7</i> ^{-/-}	PCR1	CGACCGAGAACCTAGCTACTGCTCATT; CGCTATGAAATAACCCTGATGCTGAAG	WT: 320 bp					
	PCR2	TTCCTCATGACTTCACACTCCTCTGTG; CGCTATGAAATAACCCTGATGCTGAAG	KO: 330 bp; (WT: 1,000 bp)					
<i>Atat1</i> ^{-/-}	PCR1	CCTCTCCCACTATTGTCTCTCATTATTG; GCAGGTGTACATGCAGATAGAGTACTC	WT: 230 bp					
	PCR2	TATGCCCTTGATGGTGTGTCCCTG; GCAGGTGTACATGCAGATAGAGTACTC	KO: 370 bp					

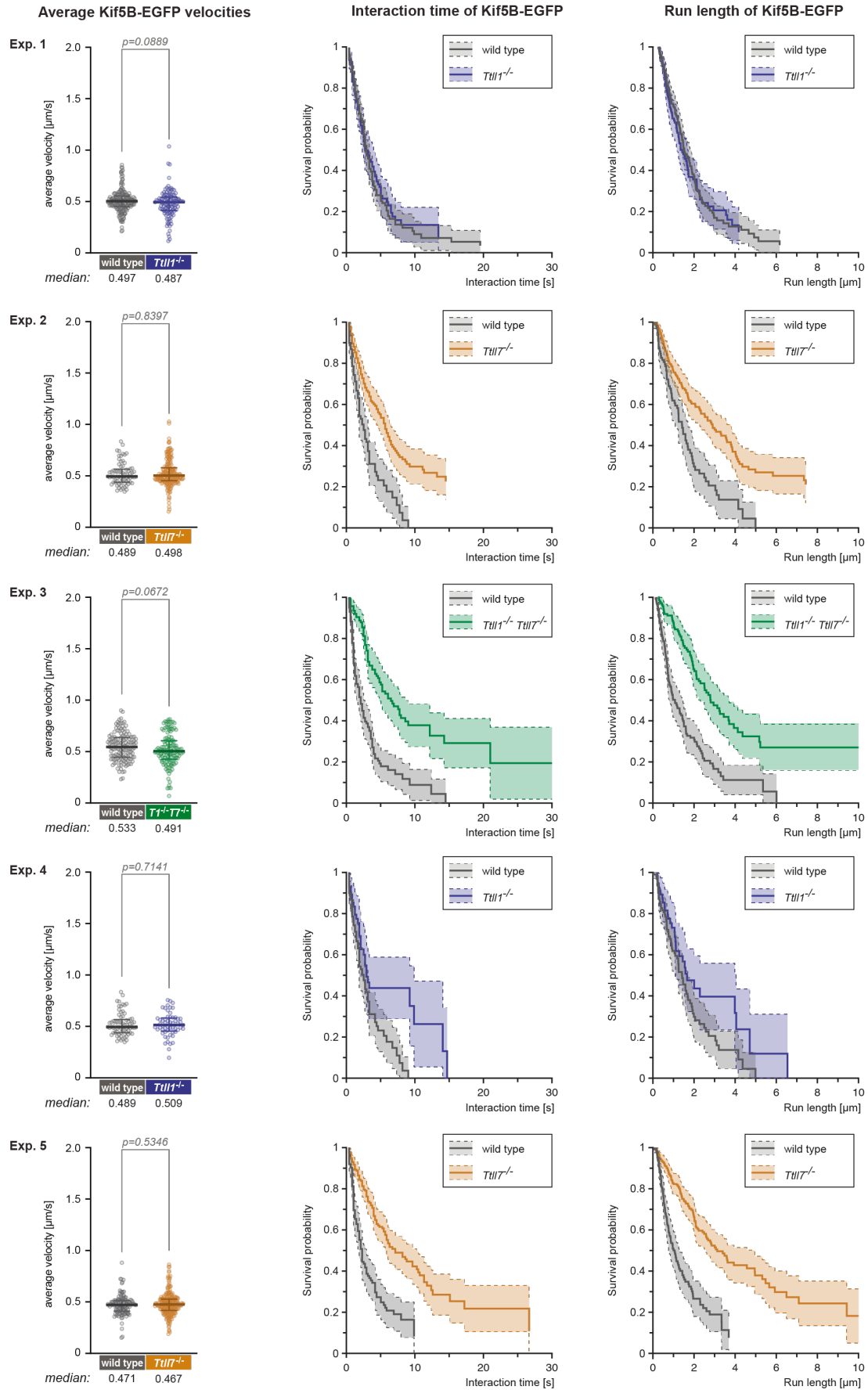
Appendix Table S2. Antibodies for immuno blot analyses

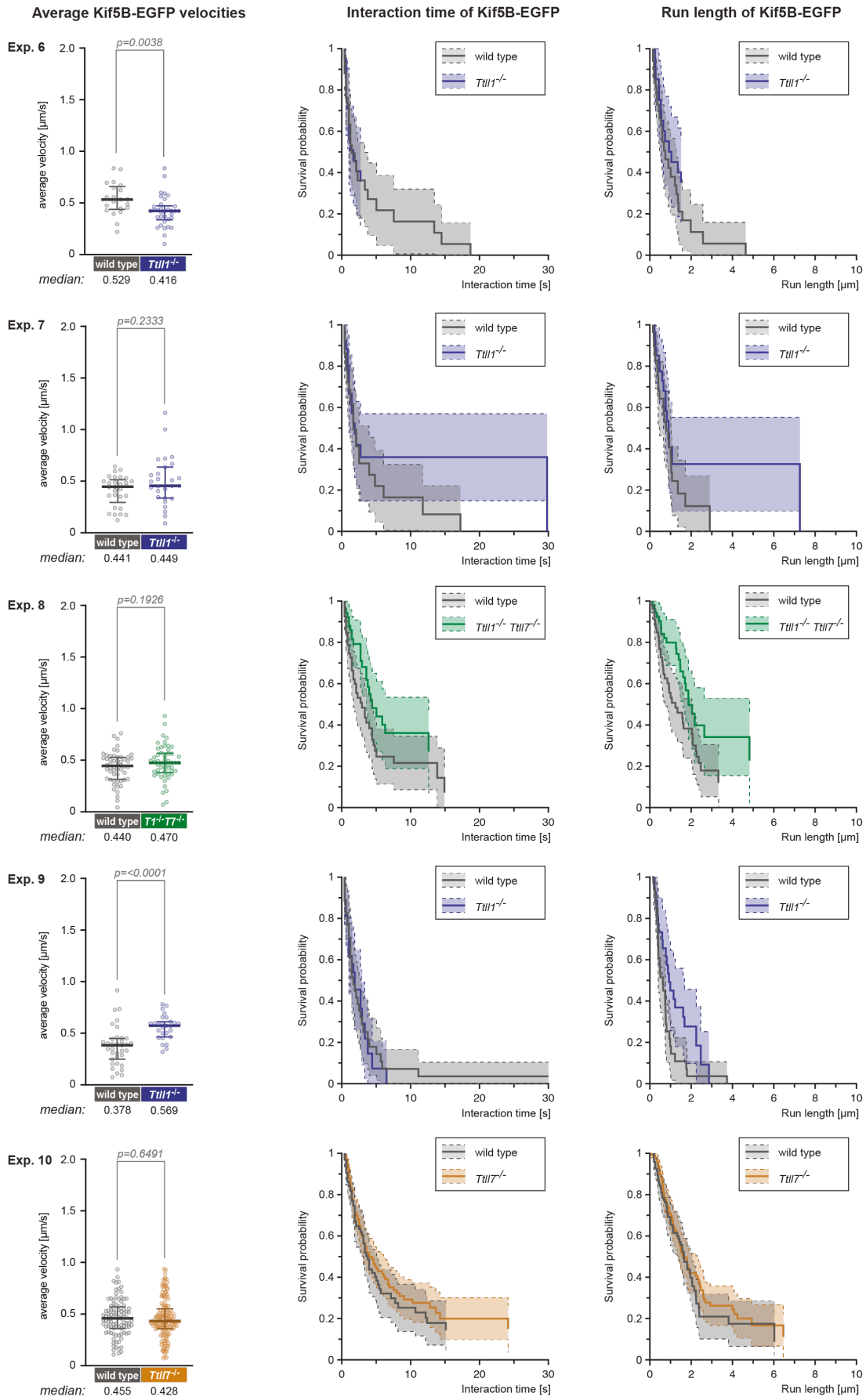
Antibody	Reference	Description	Dilution
12G10	Developed by J. Frankel and M. Nelson, obtained from the Developmental Studies Hybridoma Bank, developed under the auspices of the NICHD, and maintained by the University of Iowa	α -tubulin, mouse monoclonal	1:5,000
polyE	AdipoGen #AG-25B-0030	polyglutamylate chains longer than 3E with C-terminal E, rabbit polyclonal	1:5,000
β -monoE	AdipoGen #AG-25B-0039	β 2-tubulin C-terminal peptide sequence with 1E-branch at E435, rabbit polyclonal	1:5,000
anti-tubulin, detyrosinated	Sigma-Aldrich #AB3201	Peptide corresponding to detyrosinated C-terminus of α -tubulin (-CGEEEGEE), rabbit polyclonal	1:1,000
anti- Δ 2-tubulin	Millipore #AB3203	α -tubulin C-terminus lacking the last two amino acids, rabbit polyclonal	1:5,000
6-11B-1 (acetyl- α -tubulin)	Sigma-Aldrich #T6793	K40 acetylation on α -tubulin, mouse monoclonal IgG2b	1:2,000
HRP-anti-mouse	Bethyl #A90-516P	HRP-conjugated goat anti mouse	1:10,000
HRP-anti-rabbit	Bethyl #A120-201P	HRP-conjugated goat anti rabbit	1:10,000

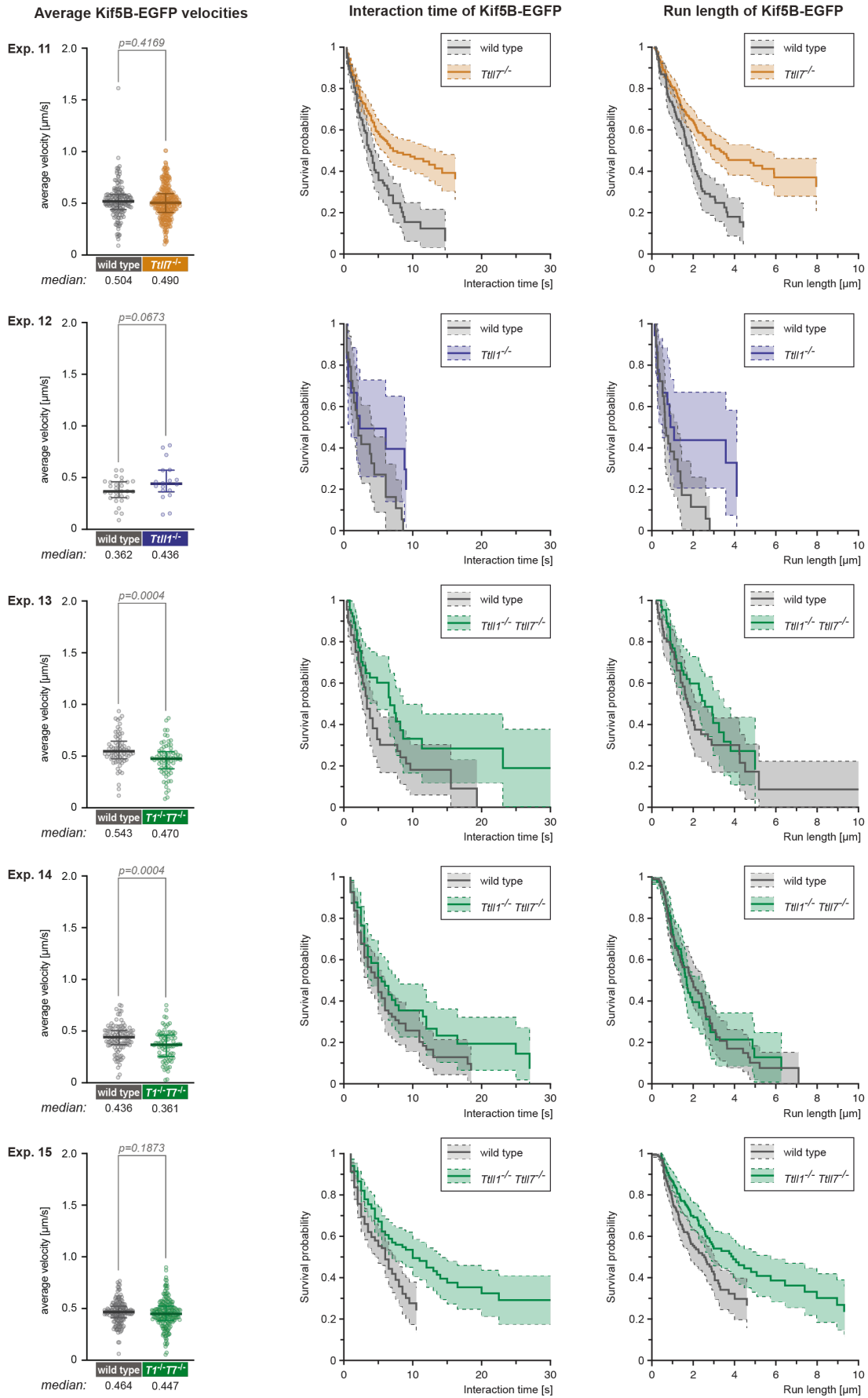


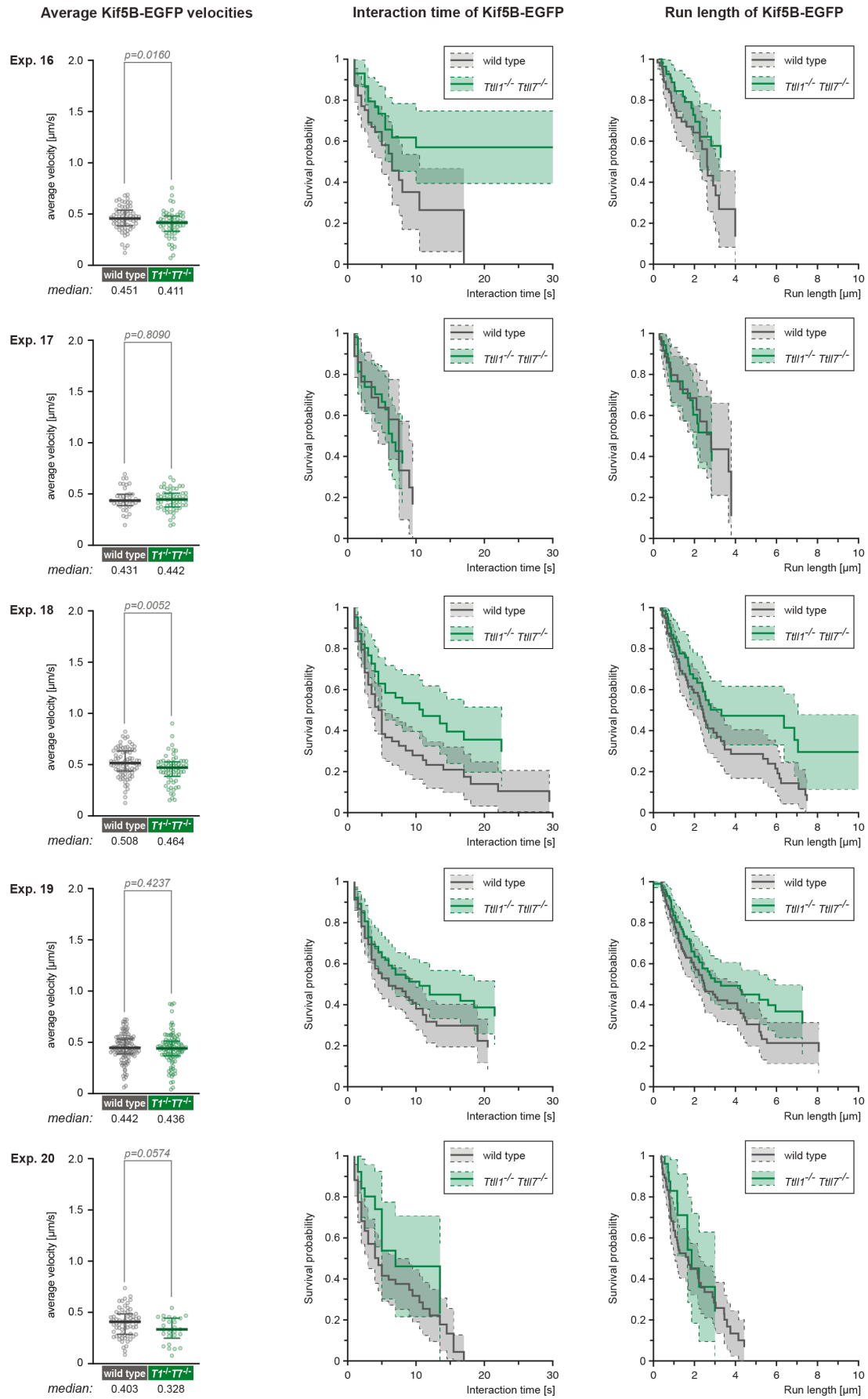
Appendix Figure S1. Analyses of intermediate protein samples during tubulin purification (complement to Fig 1; EV1).

A) Schematic overview of the tubulin purification pipeline from mouse brains using three cycles of temperature-induced polymerisation-depolymerisation as in Fig 1C; EV1A. The different fractions analysed in (B) are indicated. **B)** Representative examples of protein samples from different purification steps of tubulin from the five different PTM variants used in the current study. Annotation of one representative molecular weight markers (MW) is shown apart as markers are loaded at varying positions on different gels.





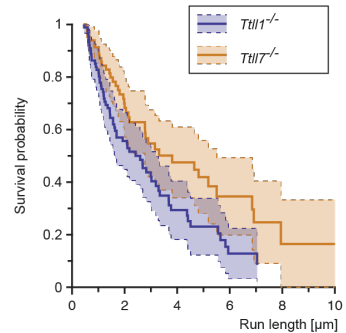
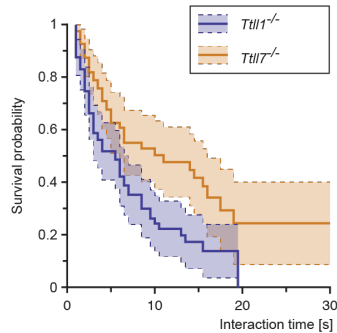
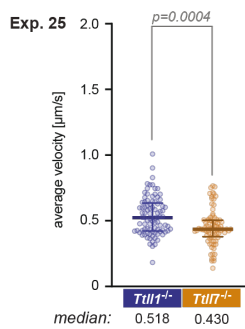
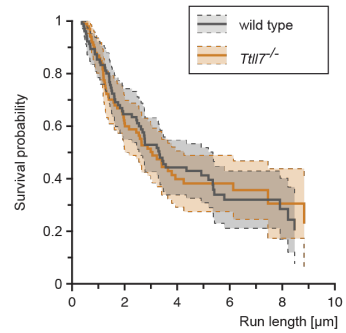
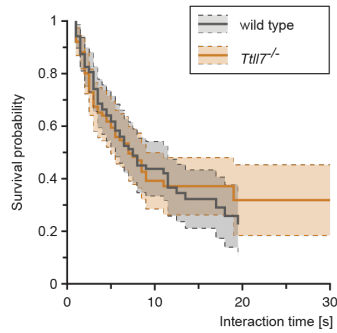
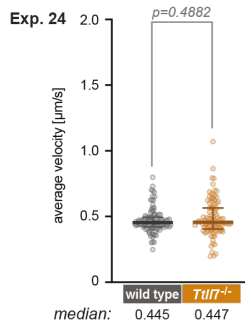
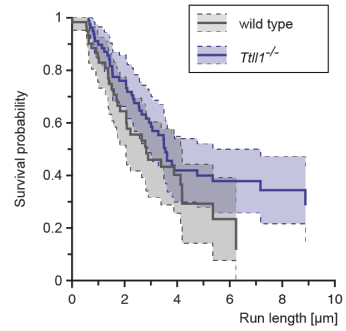
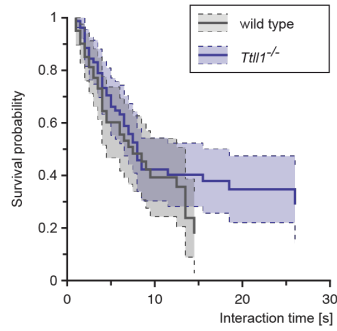
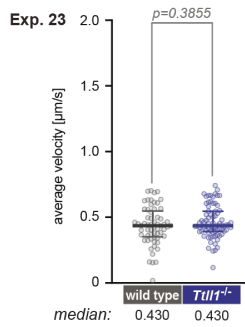
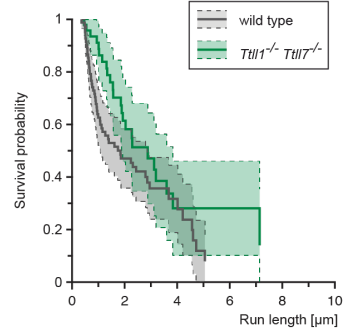
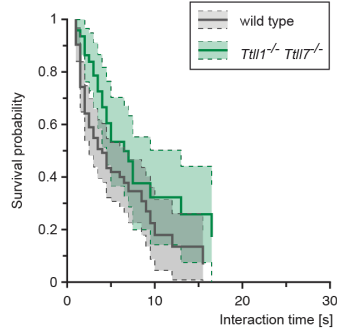
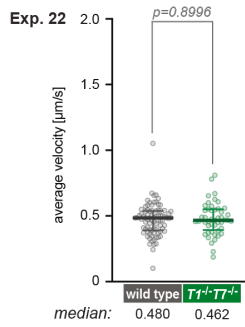
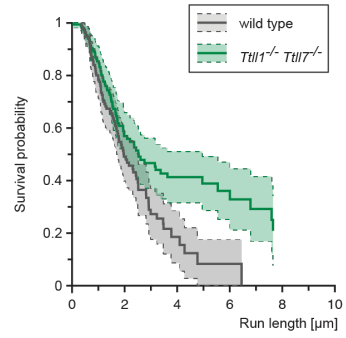
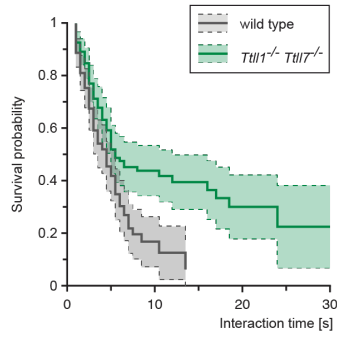
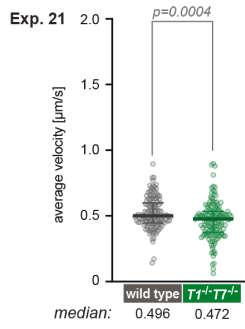


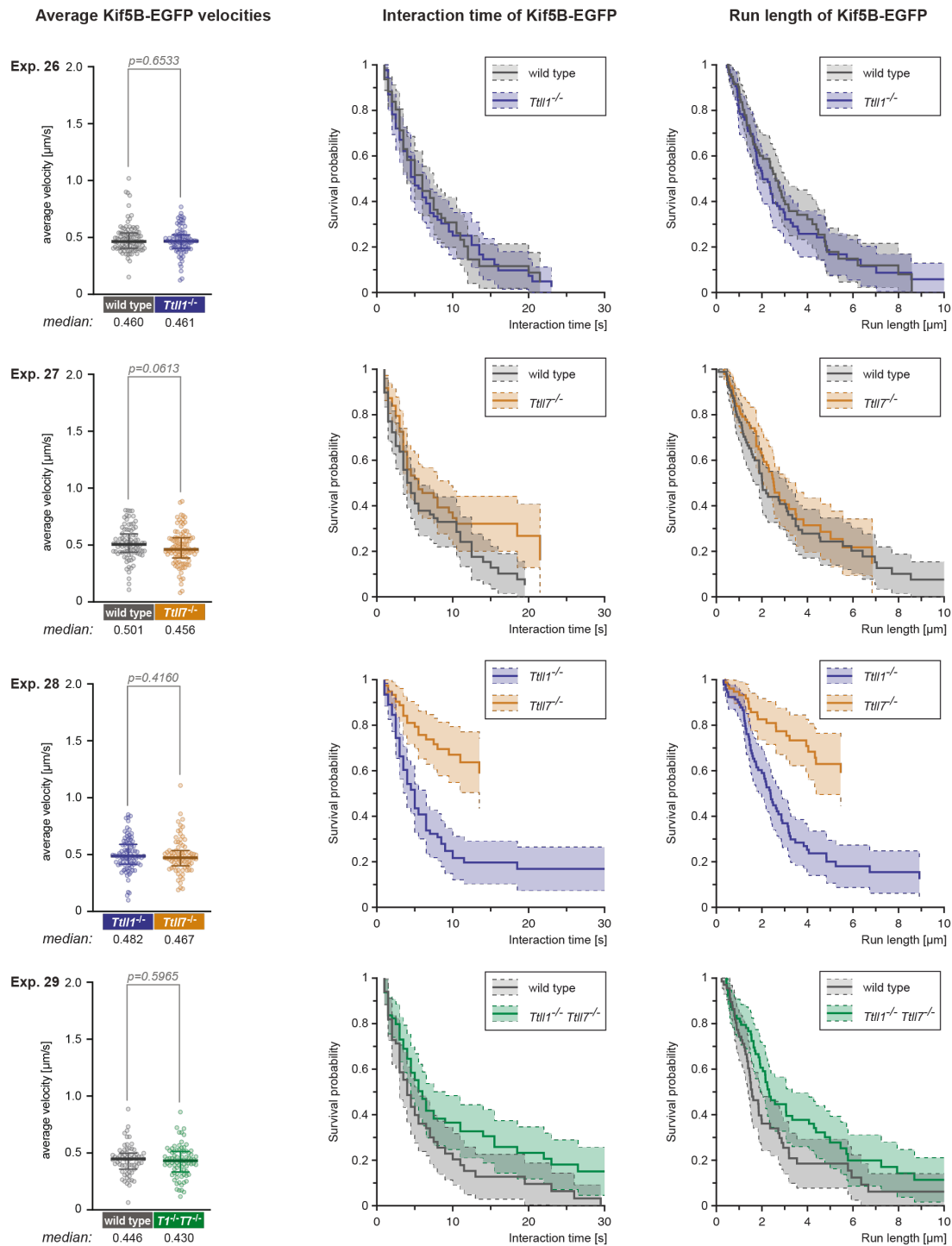


Average Kif5B-EGFP velocities

Interaction time of Kif5B-EGFP

Run length of Kif5B-EGFP





Appendix Figure S2. Independent Kinesin-1 experiments (complement to Fig 4C,D,E).

A-F Representation of individual experiments to determine Kif5B-GFP motility. First column: Scatter plots show average velocities ($\mu\text{m/s}$) exhibited by single Kif5b-EGFP molecules on the different microtubule types. Medians with interquartile ranges shown, Mann-Whitney test, p-values displayed. Second and third column: Survival probability (Kaplan-Meier plots, taking varying microtubule lengths into account; see Methods) of the interaction time and run length of Kif5b-EGFP molecules on different microtubule types.