Expanded View Figures

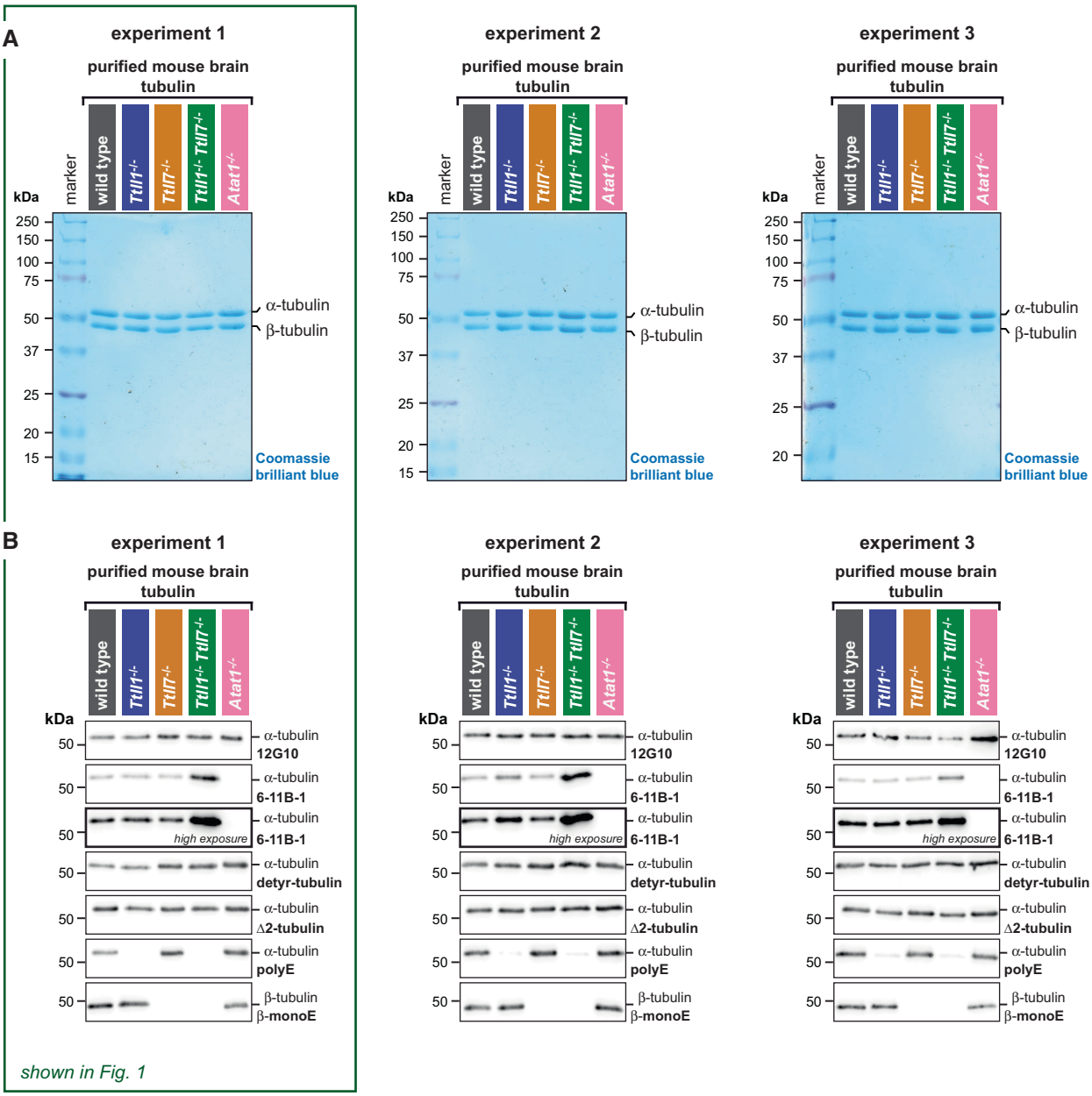

Figure EV1. Analyses of three independent sets of purified tubulin (complement to Fig 1D and E).

A Direct comparison of SDS-PAGE analysis of purified tubulin from brains of all mouse models in three independent experiments, stained with Coomassie brilliant blue. Alpha- and β-tubulin are separated using a specific SDS-PAGE protocol (Souphron *et al*, 2019). Experiment 1 is also shown in Fig 1D.

B Direct comparison of immunoblot analyses of all purified tubulin variants from three independent experiments. Experiment 1 is also shown in Fig 1E. The molecular weight in (A) and (B) is indicated in kDa. Source data are available.

Source data are available online for this figure.

Figure EV2. Additional assays on Tau-microtubule interactions (complement to Fig 2).

A Quantification of the integrated intensity of Tau-meGFP on GMPCPP microtubules shown for the single assays that were combined in Fig 2C after normalisation to the median values measured on wild-type microtubules. Three independent sets of experiments were performed with independently purified tubulin samples. Each data point represents the quantification of one microtubule. Median values and interquartile ranges are shown for each scatter plot. Mann–Whitney test, *P*-values displayed.

B Representative still images from a time lapse of Tau-meGFP binding to GMPCPP microtubules. The images are false coloured to better reveal the intensity evolution over time. Scale bars 5 μm.

C Raw integrated intensity values (A.U. × 10^4) of Tau-meGFP binding to GMPCPP wild-type and *Ttll1*$^{-/-}$*Ttll7*$^{-/-}$ microtubules over a 150 s time interval (data points every 10 s). For each type 5 microtubules from the same assay were analysed.

D Saturation curves from individual experiments with microtubules from independently purified tubulin samples. Increasing concentration Tau-mCherry binding to GMPCPP wild-type and *Ttll1*$^{-/-}$*Ttll7*$^{-/-}$ microtubules were quantified in one assay with each of the shown concentrations per experiment. Each data point is a mean (±standard deviation) of the raw integrated intensity values of single microtubules for the respective concentration (A.U. × 10^4). The derived dissociation constants K_d and R^2 coefficient from the nonlinear fit for each microtubule type are indicated below the plots. The means of the three independent experiments shown here are combined in Fig 2E.

E Quantification of normalised integrated intensity of Tau-meGFP fluorescent intensity on Taxol-stabilised microtubules. Data of individual assays were normalised to mean values from wild-type microtubules, and then combined as in Fig 2C. Each data point represents a single microtubule measurement from one experiment (n = 57–80 microtubules). Scatter plots with mean values (A.U.) and standard deviation. Student's *t*-test, *P*-values are shown.

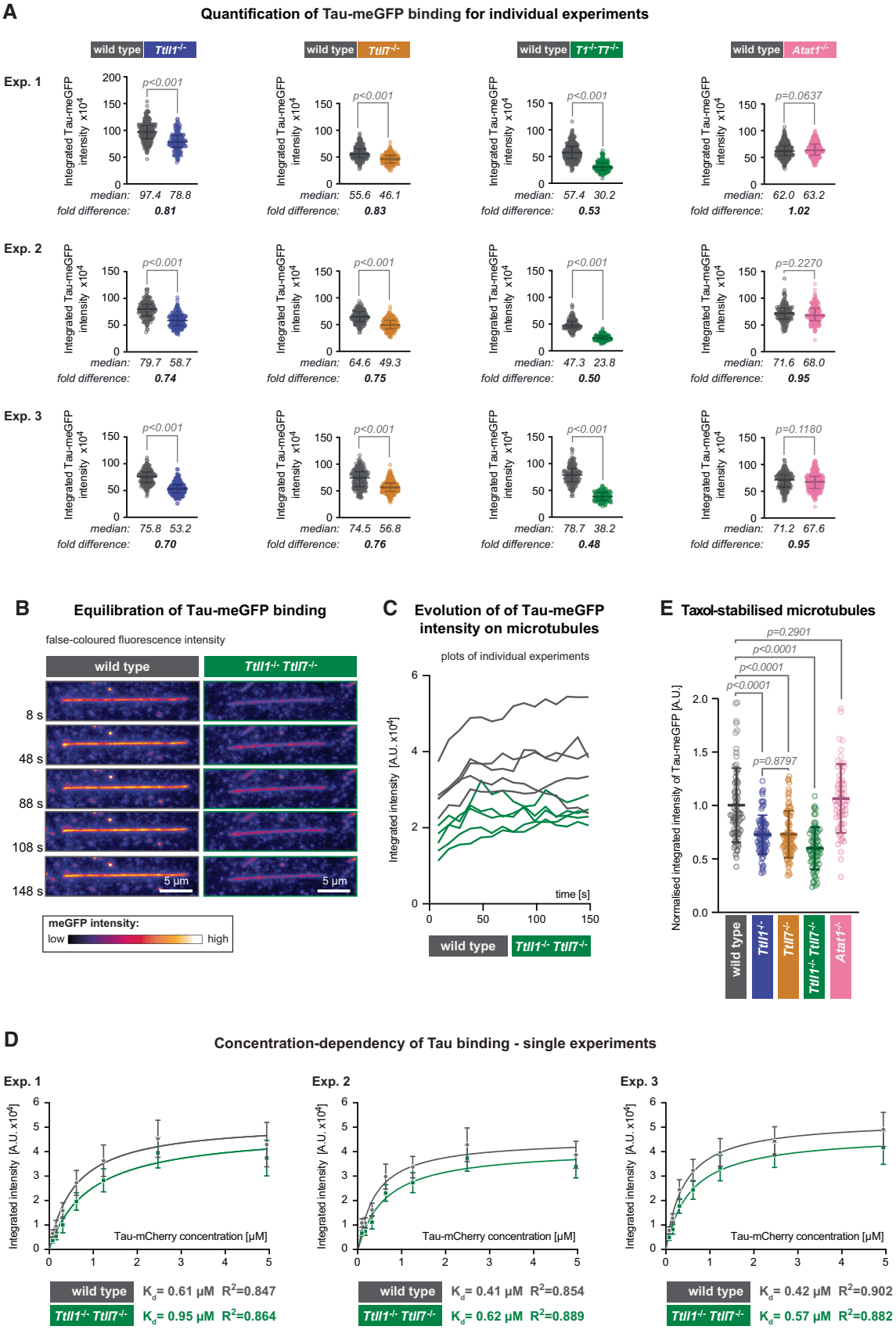

Figure EV2.

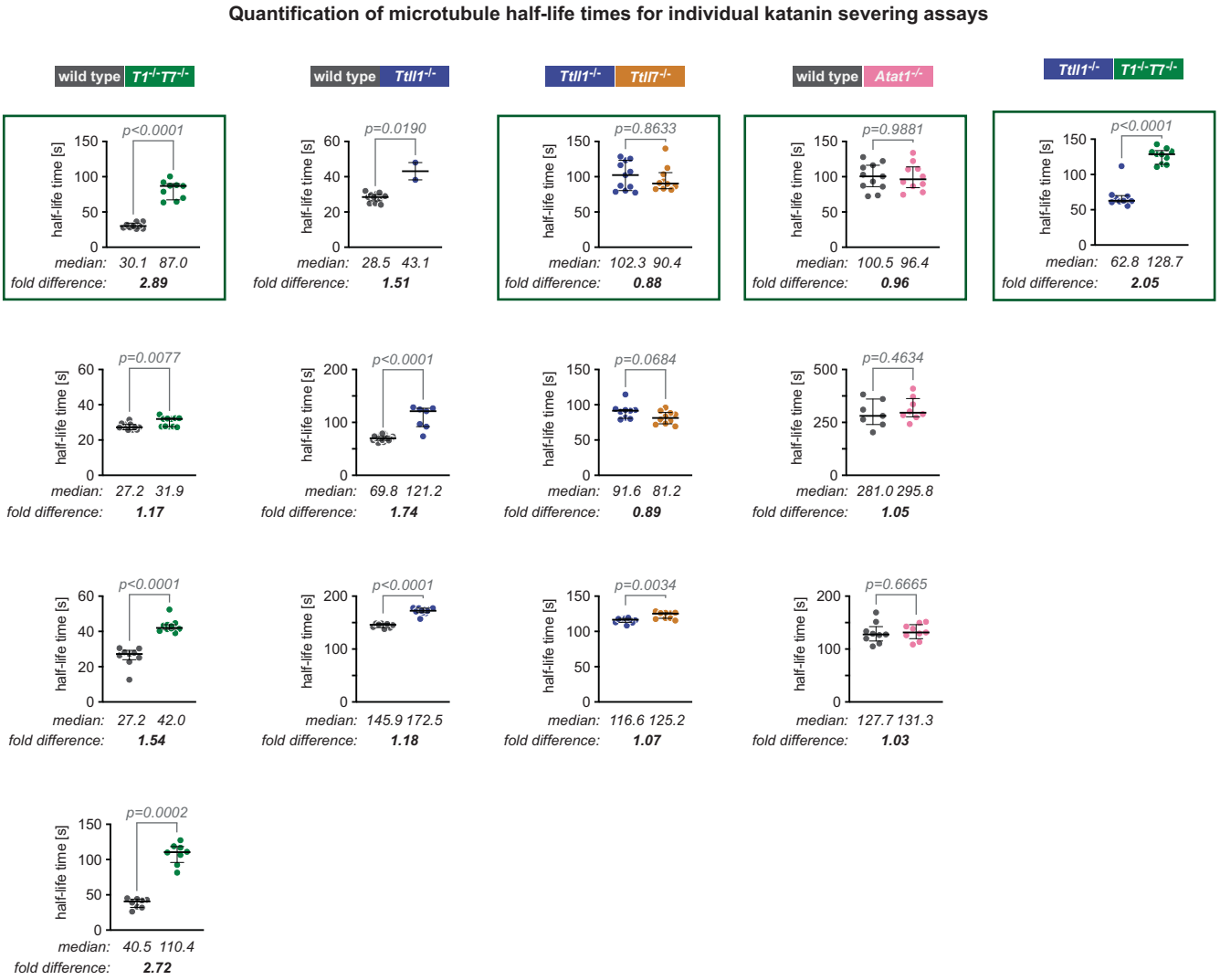

Figure EV3. Individual katanin severing assays (complement to Fig 3).

Representation of all individual quantifications of microtubule length half-life times in the presence of 100 nM p60/p80 katanin separately for all 14 experiments. As shown in Fig 3A, two types of microtubules were always compared in the same chamber to mitigate the variability of katanin and microtubule concentrations in different measurement channels and, thus, ensure comparability between the samples. Each data point represents the half-life time of one microtubule determined as shown for representative examples in Fig 3B, with bars representing medians and interquartile ranges. Mann–Whitney test, *P*-values are shown. Selected plots (green frames) are shown in Fig 3D. To adjust all assays for a comparative analysis shown in Fig 3E, values were normalised to wild-type microtubules. To normalise assays not including this type of microtubules, we used the weighted median fold difference between wild-type and *Ttll1*$^{-/-}$ microtubules from three independent experiments (second column) which provided the factor 1.45. This factor was used to normalise values in experiments omitting wild-type, but including *Ttll1*$^{-/-}$ microtubules.

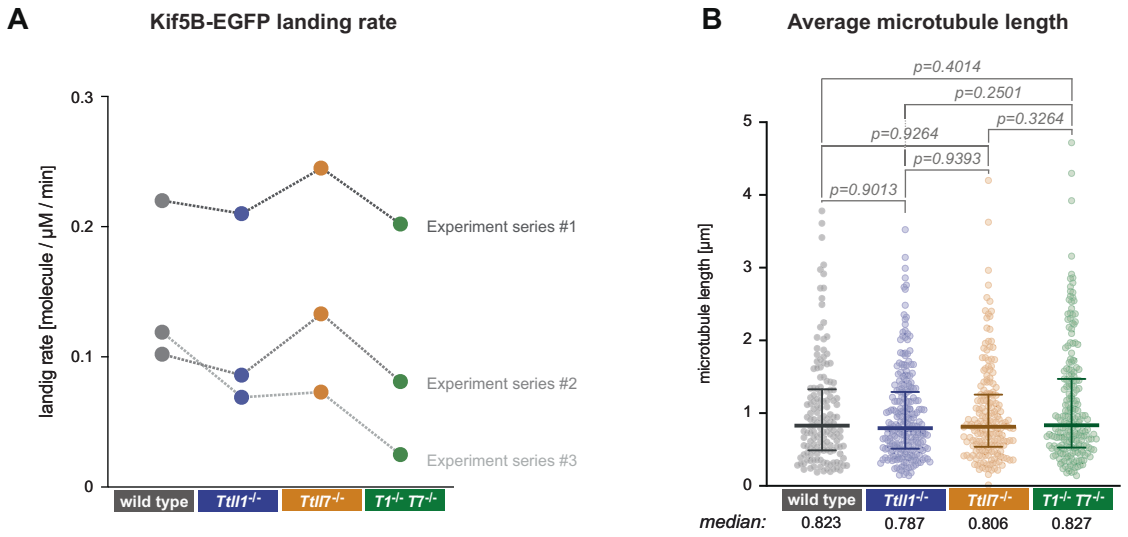

Figure EV4. Additional parameters for kinesin-1 motility assays (complement to Fig 4).

A Comparison of the Kif5B-EGFP landing rates in three independent sets of experiments. Landing rates were calculated by summing up the total number of single-molecule Kif5B-EGFP landing events on microtubules relative to the total (summed up) length of microtubules in the field of view and the observation time. Note that landing rates differ between different sets of experiments, but are mostly similar for different PTM subtypes of microtubules within all experimental sets.

B Median microtubule length (each data point represents one microtubule) determined from all microtubules observed in three independent sets of experiments. Medians with interquartile ranges shown, Mann–Whitney test and *P*-values displayed.