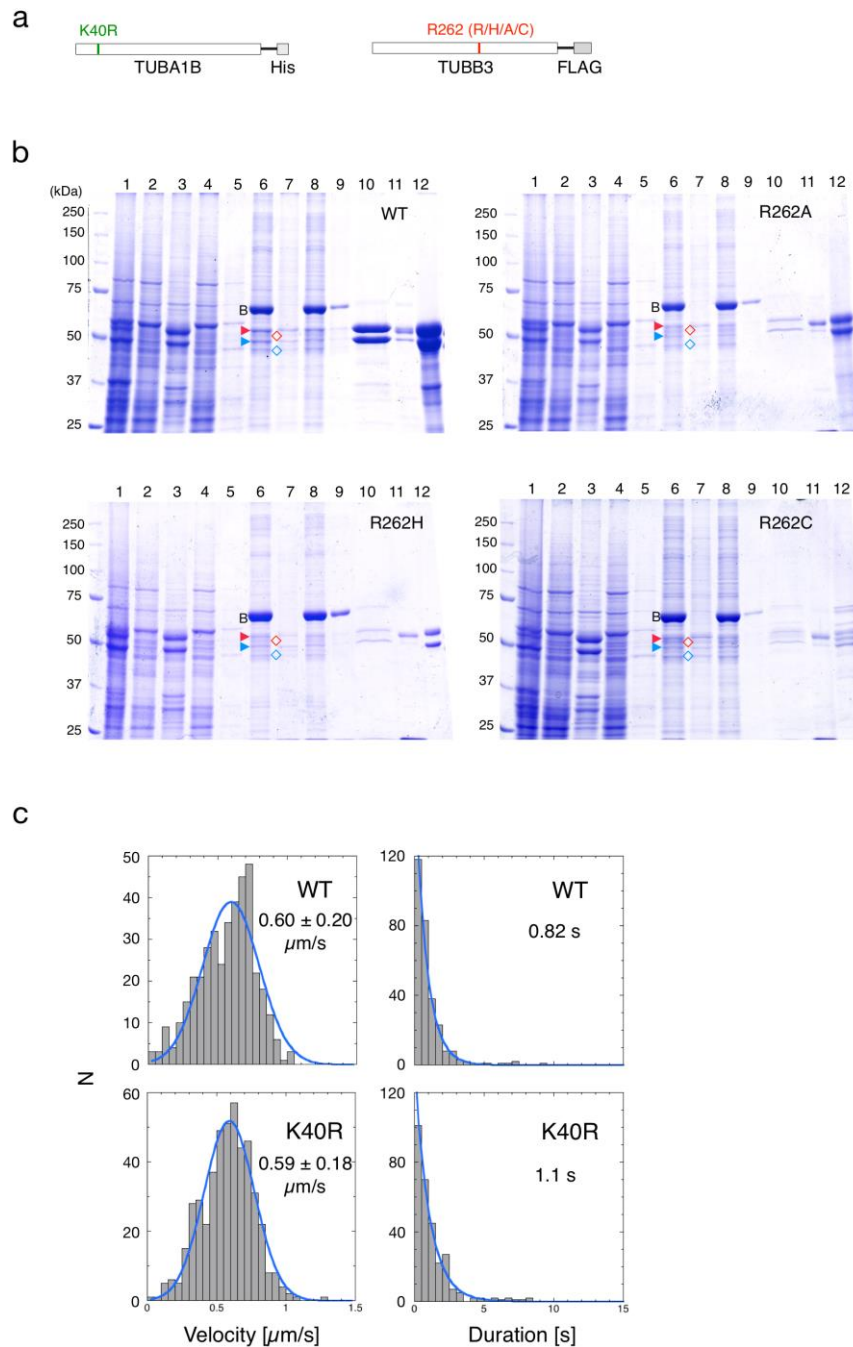
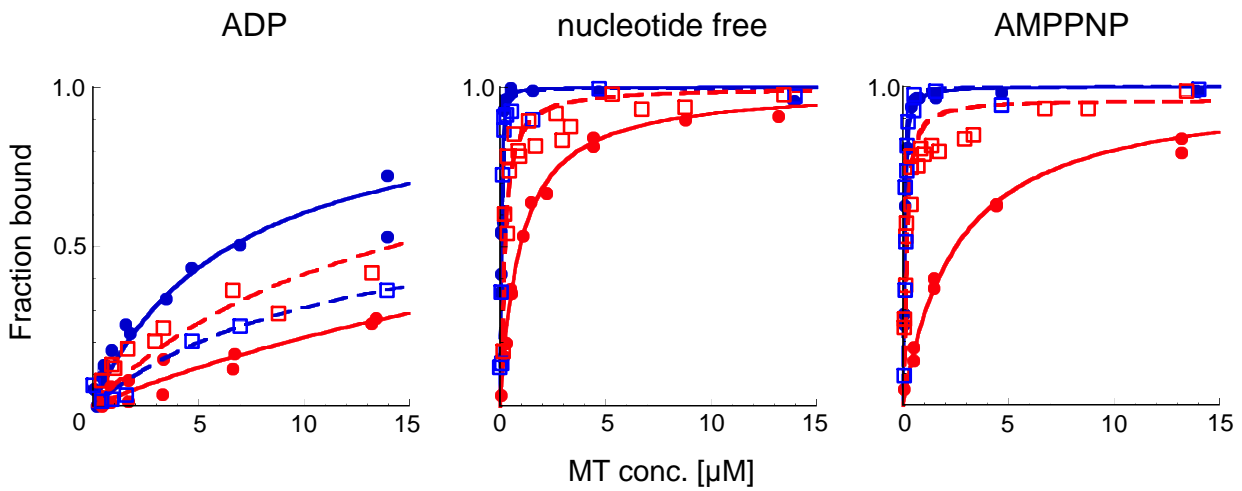


		20		40		60		80
hsTUBB3 (β3)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPSGNYVGDG	DLQLERISVY	YNEASSHKYV	PRAILLVDLEP	GTMDSVRSRGA
mmTUBB3 (β3)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPSGNYVGDG	DLQLERISVY	YNEASSHKYV	PRAILLVDLEP	GTMDSVRSRGA
ggTUB4B (β3)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPSGNYVGDG	DLQLERISVY	YNEASSHKYV	PRAILLVDLEP	GTMDSVRSRGA
hsTUBB1 (β1)	MREIVHIQIG	QCGNQIGAKF	WEMIGEEHGI	DLAGSDRGAS	ALQLERISVY	YNEAYGRKYV	PRAILLVDLEP	GTMDSIRSSK
mmTUBB1 (β1)	MREIVHIQIG	QCGNQIGAKF	WEVIGEEHGI	DCAGSYCGTS	ALQLERISVY	YNEAYGKKYV	PRAILLVDLEP	GTMDSIRSSR
ggTUB6B (β1)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPGTSYHGDS	DLQLERINVY	YNEAAGNKYV	PRAILLVDLEP	GTMDSVRSRGP
hsTUBB2B (β2B)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPGTSYHGDS	DLQLERINVY	YNEATGNKYV	PRAILLVDLEP	GTMDSVRSRGP
mmTUBB2B (β2B)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPGTSYHGDS	DLQLERINVY	YNEATGNKYV	PRAILLVDLEP	GTMDSVRSRGP
ggTUBB1 (β2B)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPGTSYHGDS	DLQLERINVY	YNEATGNKYV	PRAILLVDLEP	GTMDSVRSRGP
hsTUBB4 (β4A)	MREIVHLQAG	QCGNQIGAKF	WEVISDEHGI	DPGTYHGDS	DLQLERINVY	YNEATGGNYV	PRAILLVDLEP	GTMDSVRSRGP
scTUB2 (β)	MREIITHISTG	QCGNQIGAAF	WETICGEHGL	DFNGTYHGHD	DIQKERLNVY	FNEASSGKRW	PRRSINVDLEP	GTIDAVRNSA
		100		120		140		160
hsTUBB3 (β3)	FGHLFRPDNF	IFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKECENCDCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKVREEYYP
mmTUBB3 (β3)	FGHLFRPDNF	IFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKECENCDCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKVREEYYP
ggTUB4B (β3)	FGHLFRPDNF	IFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKECENCDCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKVREEYYP
hsTUBB1 (β1)	LGALFPDPSF	VHGNSGAGNN	WAKGHYTEGA	ELIENVLEVV	RHESESCDCL	QGFQIVHSLG	GGTGS GMGTL	LMNKIREEYYP
mmTUBB1 (β1)	LGALFPDPSF	VHGNSGAGNN	WAKGHYTEGA	ELIENVMDVV	RHESESCDCL	QGFQIVHSLG	GGTGS GMGTL	LMNKIREEYYP
ggTUB6B (β1)	FGQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RHESESCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKIREEYYP
hsTUBB2B (β2B)	FGQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RHESESCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKIREEYYP
mmTUBB2B (β2B)	FGQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RHESESCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKIREEYYP
ggTUBB1 (β2B)	FRQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RHESESCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKIREEYYP
hsTUBB4 (β4A)	FRQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDAVLDVV	RHESESCDCL	QGFQLTHSLG	GGTGS GMGTL	LISKIREEYYP
scTUB2 (β)	IGNLFRPDNY	IFGQSSAGNV	WAKGHYTEGA	ELVDSVMDVI	RREAECDL	QGFQIVHSLG	GGTGS GMGTL	LISKIREEYYP
		180		200		220		240
hsTUBB3 (β3)	DRIMNTFSVV	PSPKVS DTVV	EPYNATLSIH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTSSL
mmTUBB3 (β3)	DRIMNTFSVV	PSPKVS DTVV	EPYNATLSIH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTSSL
ggTUB4B (β3)	DRIMNTFSVV	PSPKVS DTVV	EPYNATLSIH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTSSL
hsTUBB1 (β1)	DRIMNTFSVM	PSPKVS DTVV	EPYNAVLSIH	QLIENADACF	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	LTMSGITSSL
mmTUBB1 (β1)	DRILNFSVM	PSPKVS DTVV	EPYNAVLSIH	QLIENADACF	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	LTMSGITSSL
ggTUB6B (β1)	DRIMNTFSVM	PSPKVS DTVV	EPYNATLSVH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTCL
hsTUBB2B (β2B)	DRIMNTFSVM	PSPKVS DTVV	EPYNATLSVH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTCL
mmTUBB2B (β2B)	DRIMNTFSVM	PSPKVS DTVV	EPYNATLSVH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTCL
ggTUBB1 (β2B)	DRIMNTFSVV	PSPKVS DTVV	EPYNATLSVH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTCL
hsTUBB4 (β4A)	DRIMNTFSVV	PSPKVS DTVV	EPYNATLSVH	QLVENTDETY	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	ATMSGVTTCL
scTUB2 (β)	DRIMNTFSVI	PSPKVS DTVV	EPYNATLSVH	QLVHSDDEF	CIDNEALYDI	CFRTLKLTATP	TYGDLNLHLS	SVMSGVTTSSL
		260		280		300		320
hsTUBB3 (β3)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTARGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
mmTUBB3 (β3)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTARGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
ggTUB4B (β3)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTARGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
hsTUBB1 (β1)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTAQGSQQ	YRALSVALET	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
mmTUBB1 (β1)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTAQGSQQ	YRALSVALET	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
ggTUB6B (β1)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTSRGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
hsTUBB2B (β2B)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTSRGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
mmTUBB2B (β2B)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTSRGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
ggTUBB1 (β2B)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTSRGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
hsTUBB4 (β4A)	RFPGQLNADL	RKLAVNMVVF	PRLHFFMPGF	APLTSRGSQQ	YRALTVPELT	QQMFDAKNMM	AACDRPHGRY	LTVAIVFRGR
scTUB2 (β)	RYPGQLNSDL	RKLAVNLVVF	PRLHFFMVGY	APLTAIGSQS	FRSLTVPELT	QQMFDAKNMM	AAADPRNGRY	LTVAIVFRGR
		340		360		380		400
hsTUBB3 (β3)	MSMKEVDEQM	LAIQSKNSSY	FVEWIPNNVK	VAVCDIPPRG	LKMSSTFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
mmTUBB3 (β3)	MSMKEVDEQM	LAIQSKNSSY	FVEWIPNNVK	VAVCDIPPRG	LKMSSTFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
ggTUB4B (β3)	MSMKEVDEQM	LAIQSKNSSY	FVEWIPNNVK	VAVCDIPPRG	LKMSSTFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
hsTUBB1 (β1)	MSTKEVDQQL	LSVQTRNSSC	FVEWIPNNVK	VAVCDIPPRG	LKMSSTFFIGN	NTAIQELFNR	VSEHFSAMFK	RKAFVHWYTS
mmTUBB1 (β1)	MSTKEVDQQL	LSVQTRNSSC	FVEWIPNNVK	VAVCDIPPRG	LKMSSTFFIGN	NTAIQELFNR	VSEHFSAMFK	RKAFVHWYTS
ggTUB6B (β1)	MSMKEVDEQM	LNVQNKNSY	FVEWIPNNVK	TAVCDIPPRG	LKMSATFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
hsTUBB2B (β2B)	MSMKEVDEQM	LNVQNKNSY	FVEWIPNNVK	TAVCDIPPRG	LKMSATFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
mmTUBB2B (β2B)	MSMKEVDEQM	LNVQNKNSY	FVEWIPNNVK	TAVCDIPPRG	LKMSATFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
ggTUBB1 (β2B)	MSMKEVDEQM	LNVQNKNSY	FVEWIPNNVK	TAVCDIPPRG	LKMSATFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
hsTUBB4 (β4A)	MSMKEVDEQM	LSVQSKNSY	FVEWIPNNVK	TAVCDIPPRG	LKMAATFFIGN	STAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
scTUB2 (β)	VSVKEVEDEM	HKVQSKNSDY	FVEWIPNNVQ	TAVCSVAPQG	LDMAATFIAN	STSIQELFKR	VGDQFSAMFK	RKAFLHWYTS
		420		440				
hsTUBB3 (β3)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE EEGEMYE	DDDEESEAQG	PK-----		
mmTUBB3 (β3)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE EEGEMYE	DDDEESEAQG	PK-----		
ggTUB4B (β3)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE EEGEMYE	DDDEESEQ-G	AK-----		
hsTUBB1 (β1)	EGMDINEFGE	AENNIHDLVS	EYQQFQDARA	VLEEDDEVTE	EAEMEPEDKG	H-----		
mmTUBB1 (β1)	EGMDISEFGE	AESDIHDLVS	EYQQFQDVRA	GLEDESEEDVE	EAEEVAEDKD	H-----		
ggTUB6B (β1)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE QGFEFE	EGEEDEA---	-----		
hsTUBB2B (β2B)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE QGFEFE	EGEEDEA---	-----		
mmTUBB2B (β2B)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE QGFEFE	EGEEDEA---	-----		
ggTUBB1 (β2B)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE EEGFEFE	EAEEEA---	-----		
hsTUBB4 (β4A)	EGMDEMEFTE	AESNMNDLVS	EYQQYQD--A	TAE E-GEFE	EAEEVA---	-----		
scTUB2 (β)	EGMDELEFSE	AESNMNDLVS	EYQQYQE--A	TVEDEDEVE	NGDFGAPQNG	DEPTTENFE		

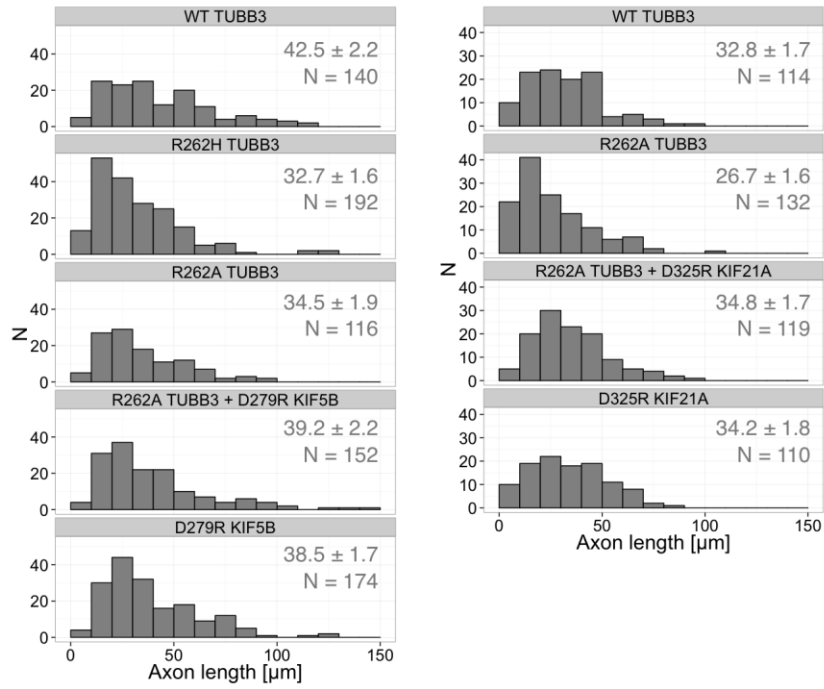
Supplementary Figure 1. Sequence comparison of β-tubulin isotypes in vertebrates and yeast. The sequences of β3 isotypes are highly conserved in vertebrates, showing only three amino acid residue differences between humans and chickens (coloured in yellow). In contrast, the sequence of human β3-tubulin differs from that of human β1, β2B, β4A-tubulin and yeast β-tubulin at 100, 34, 32 and 123 residues, respectively (coloured in pink). For each isotype, the sequence was obtained from UniProt (<http://www.uniprot.org/>): AAH03021 (human β3, hsTUBB3), AAH88749 (mouse β3, mmTUBB3), AAA49119 (chicken β3, ggTUB4B), AAH33679 (human β1, hsTUBB1), AAI47699 (mouse β1, mmTUBB1), AAA49124 (chicken β1, ggTUB6B), AAH63610 (human β2B, hsTUBB2B), BAE40722 (mouse β2B, mmTUBB2B), CAA23687 (chicken β2B, ggTUBB1), AAH13683 (human β4A, hsTUBB4), DAA12403 (yeast β, scTUB2). Amino acid sequences were aligned by clustalX.



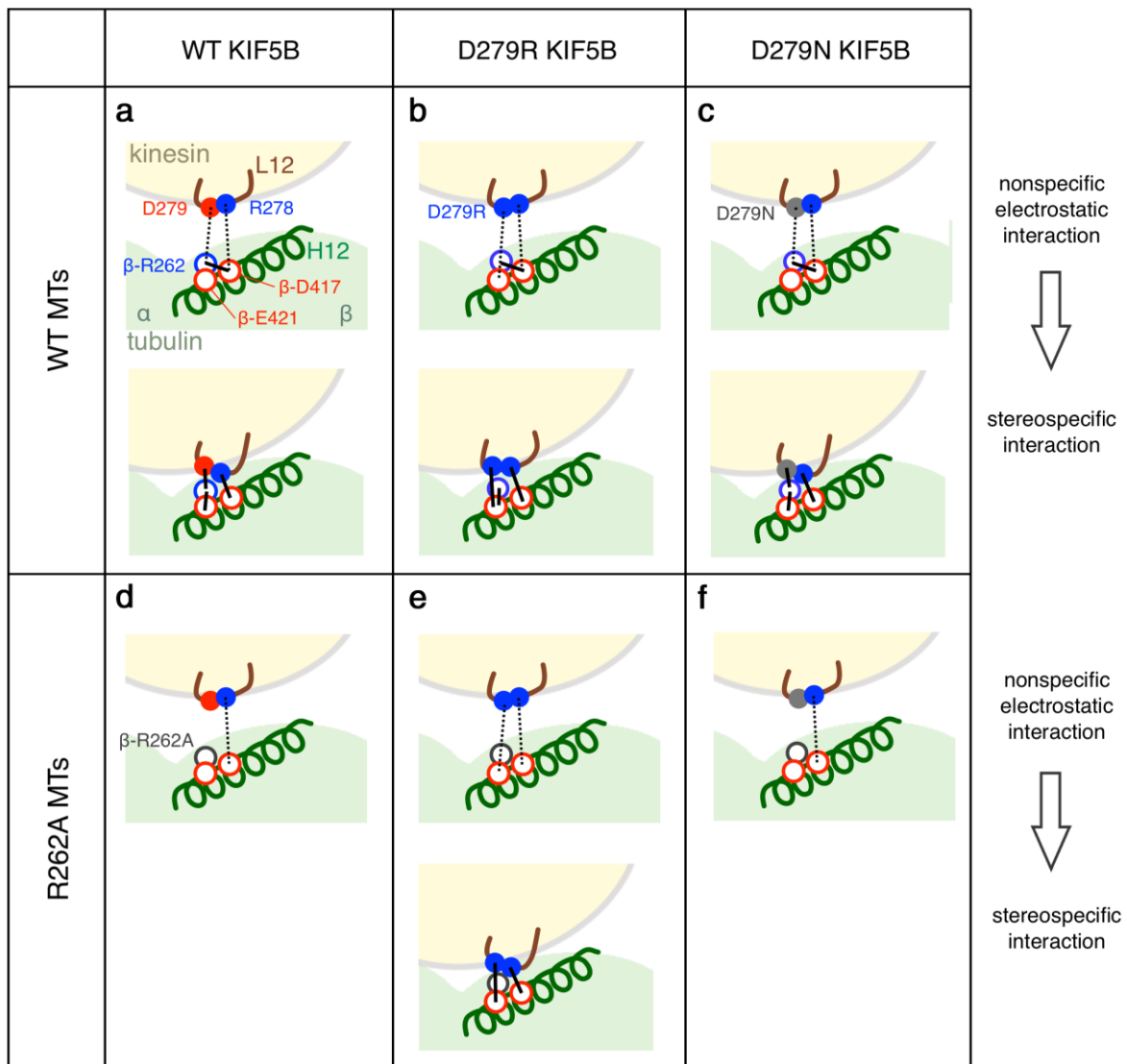
Supplementary Figure 2. Design and preparation of tubulin mutants. (a) Design of α - and β -tubulin constructs. These constructs are cloned into a single baculovirus vector. (b) SDS PAGE showing each preparation step of WT and R262A, R262H and R262C tubulin. Lanes: (1) Cell lysate, (2) soluble fraction and (3) precipitate after centrifugation, (4) His-affinity column flow-through, (5) His-affinity column wash, (6) His-affinity column eluent (supplemented with 2 mg ml^{-1} BSA), (7) the fraction that remained attached to the His-affinity column, (8) FLAG-affinity column flow-through, (9) FLAG-affinity column wash, (10) FLAG-affinity column eluent, (11) the fraction that remained attached to the FLAG-affinity column, and (12) after polymerization and centrifugation. \blacktriangleright and \blacktriangleleft , recombinant α - and β -tubulin; \diamond and \diamond , endogenous α - and β -tubulin; B, BSA. (c) The effect of residue substitution at α -Lys40 residue on single-molecule motility of KIF5B. To prepare recombinant tubulin with a single post-translational modification state¹, the Lys40 of α -tubulin was substituted by Arg and treated as WT. The velocity and duration of α -K40R MTs was only slightly different from that of inherent WT, in which 10–30 % of α -tubulin is acetylated.



Supplementary Figure 3. Equilibrium dissociation constants of the KIF5B-MT complex. Binding of 0.1 μM single-headed HK349 KIF5B with varying concentrations of MTs. The fraction of HK349 in the MT pellet was plotted as a function of the total MT concentration. ●, WT KIF5B-WT MT; ●, WT KIF5B-R262A MT; □, D279R KIF5B-WT MT; □, D279R KIF5B-R262A MT. For each condition, measurements are repeated 2–3 times and all data from multiple rounds of measurements are plotted in the graph. The curves are the best fit to hyperbola with dissociation constant (K_d) values given in Supplementary Table 2 and Fig. 3f.



Supplementary Figure 4. Distribution of axon lengths when WT and mutant TUBB3, and/or mutant KIF5B/KIF21A were expressed in mouse cortex neurons. Data from two independent sets of experiment are shown. Numbers indicate mean \pm s.e.m. lengths in μm .



Supplemental Figure 5. Possible models for interactions between the L12 loop in KIF5B and the area near the residue β -R262 in TUBB3. Red, blue, and grey filled circles represent basic, acidic and neutral residues in KIF5B, respectively, whereas red, blue and grey unfilled circles represent basic, acidic and neutral residues in TUBB3, respectively. The dotted line indicates electrostatic attraction. Hydrogen bonds within tubulin molecule (indicated by thick lines) were predicted by using PyMOL, based on the simulated structure of $\alpha\beta$ -tubulin dimer with or without β -R262A mutation (PDB: 1JFF)².

Both D279R and β -R262A mutations cause breaking of the salt bridge between D279 in KIF5B and R262 in TUBB3, which is formed in a WT-WT pair. However, the effect of each mutation on motility is different: While WT KIF5B was unable to move on β -R262A MT, D279R KIF5B was able to move on WT-MT (Fig. 3c, d, Table 1 in the main text). The result can be explained from the viewpoint of electrostatic interaction. In paired mutant WT KIF5B- β -R262A MT (d), it is difficult for residue D279 to find an alternative binding partner to replace β -R262A, because β -R262 is the only basic residue on an MT within an 8 Å distance from D279 (PDB: 4LNU). On the other hand, in paired mutant D279R KIF5B-WT MT (b), the D279R residue can easily find a binding partner, because the majority of kinesin-interacting tubulin residues are acidic. The local structure of the kinesin-MT interface³ indicates that the repulsion between D279R and β -R262 could well be compensated for by the salt bridges D279R- β -E421 and R278- β -D417, allowing the pair (D279R KIF5B-WT MT) to stereospecifically bind.

Supplementary Table 1. Comparison of the parameters of microtubule-activated kinesin ATPase in charged-to-alanine tubulin and kinesin mutants

mutant	relative ATPase parameter		protein	reference
	k_{cat} (fold wt)	$k_M(MT)$ (fold wt)		
WT-Tb	1.00 ± 0.03	1.00 ± 0.14	<i>Homo sapiens</i> TUBB3	This study ^a
β-Tb R262H	0.17 ± 0.03	9.71 ± 4.94		
β-Tb R262A	0.23 ± 0.01	10.3 ± 1.23		
WT-Tb	1.00 ± 0.03	1.00 ± 0.30	<i>S. cerevisiae</i> TUB2	Uchimura, et al, 2010 ^b
α-Tb E414A	0.20 ± 0.02	0.78 ± 0.04		
α-Tb E415A	0.80 ± 0.09	2.39 ± 0.22		
α-Tb E417A	0.82 ± 0.03	1.00 ± 0.35		
α-Tb E420A	0.94 ± 0.03	1.83 ± 0.39		
β-Tb E410A	1.21 ± 0.11	6.39 ± 1.70		
β-Tb D417A	1.18 ± 0.05	5.96 ± 0.87		
kinesin R278A	0.66 ± 0.30	15.5 ± 5.45	<i>Homo sapiens</i>	Woehlke, et al, 1997 ^c
kinesin D279A	0.78 ± 0.14	0.44 ± 0.21	KIF5B	

^a Values are mean ± errors of curve fitting

^b Values are mean ± s.d. of 4–6 independent measurements⁴

^c Values are mean ± s.d. of 2–5 independent measurements⁵

Supplementary Table 2. Equilibrium dissociation constants of the KIF5B-MT complex

microtubule	WT KIF5B			D279R KIF5B		
	ADP	nucleotide free	AMPPNP	ADP	nucleotide free	AMPPNP
WT	6.5 ± 0.7	$1.2 \pm 0.6 \times 10^{-2}$	$2.9 \pm 0.4 \times 10^{-2}$	$1.2 \pm 0.8 \times 10^1$	$1.2 \pm 0.6 \times 10^{-2}$	$1.0 \pm 0.4 \times 10^{-2}$
β -R262A	$3.6 \pm 0.3 \times 10^1$	$8.6 \pm 0.4 \times 10^{-1}$	2.5 ± 0.1	$1.4 \pm 0.2 \times 10^1$	$1.2 \pm 0.3 \times 10^{-1}$	$5.0 \pm 1.2 \times 10^{-2}$

Values are K_d in μM . Errors are those in curve fitting.

Supplementary Table 3. Summary of single-molecule motility assay of KIF21A on TUBB3 microtubules

microtubule	parameter	KIF21A	
		WT	D325R
WT	velocity [$\mu\text{m s}^{-1}$]	0.18 ± 0.32	0.24 ± 0.45
	duration [s]	7.70 ± 1.50	6.01 ± 1.74
	run length [μm]	1.56 ± 0.33	1.42 ± 0.41
β -R262H	velocity [$\mu\text{m s}^{-1}$]	UD	UD
	duration [s]	UD	UD
	run length [μm]	UD	UD
β -R262A	velocity [$\mu\text{m s}^{-1}$]	UD	0.16 ± 0.45
	duration [s]	UD	5.88 ± 0.89
	run length [μm]	UD	1.61 ± 0.73

Velocities, durations, and run lengths of the values in four independent experiments ($N > 60$ each, total $N > 240$; mean \pm s.d.). UD, undetected. Motility was measured using BG-549 labeled dimeric construct of KIF21A (KIF21A-552). Only the population of KIF21A molecules showing directional movements was analyzed.

Supplementary References

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