hsTUBB3 (63)	MRETVHTOAC	20 OCGNOTGAKE	WEVISDEHCT	40	DLOLERISVV	60 VNFASSHKVV		80 CTMDSVPSCA
mmTUBB3 (β3)	MREIVHIOAG	OCGNOIGAKF	WEVISDENGI	DPSGNYVGDS	DLOLERISVY	YNEASSHKYV	PRAILVDLEP	GTMDSVRSGA
ggTUB4B (β3)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPSGNYVGDS	DLQLERISVY	YNEASSHKYV	PRAILVDLEP	GTMDSVRSGA
hsTUBB1 (β1)	MREIVHIQIG	QCGNQIGAKF	WEMIGEEHGI	DLAGSDRGAS	ALQLERISVY	YNEA<mark>YGR</mark>KYV	PRAVLVDLEP	GTMDS <mark>IRSSK</mark>
mmTUBB1 (β1)	MREIVHIQ <mark>I</mark> G	QCGNQIGAKF	WEVI <mark>GE</mark> EHGI	DCAGSYCGTS	ALQLERISVY	YNEA <mark>YGK</mark> KYV	PRA <mark>V</mark> LVDLEP	GTMDSIRSSR
ggTUB6B (β1)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPTGSYHGDS	DLQLERINVY	YNEAAGNKYV	PRAILVDLEP	GTMDSVRSGP
mmTLIBB2B (B2B)	MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPTGSYHGDS	DLQLERINVY	YNEATGNKYV	PRAILVDLEP	GTMDSVRSGP
aaTUBB1 (62B)	MREIVHIQAG	OCGNOIGARF	WEVISDENGI	DPTGTYHGDS	DLOLERINVY	VNEATGGKYV	PRAVLVDLEP	GTMDSVRSGP
hsTUBB4 (β4A)	MREIVHLOAG	OCGNOIGAKF	WEVISDEHGI	DPTGTYHGDS	DLOLERINVY	YNEATGGNYV	PRAVLVDLEP	GTMDSVRSGP
scTUB2 (β)	MREIIHISTG	QCGNQIGAA F	WETICGEHGL	DFNGTYHGHD	DIQKERLNVY	FNEASSGKWV	PRSINVDLEP	GTIDAVRNSA
boTLIDD2 (02)	EQUI EDDDNE	100		120	DRECENCOOL	140	COMOCONOMI	160 T T CKUPEEND
mmTUBB3 (B3)	FGHLFRPDNF	TEGOSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKECENCDCL	OGFOLTHSLG	GGTGSGMGTL	LISKVREEIP
gqTUB4B (β3)	FGHLFRPDNF	IFGOSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKECENCDCL	OGFOLTHSLG	GGTGSGMGTL	LISKVREEYP
hsTUBB1 (β1)	LGALFQPDSF	VHGNSGAGNN	WAKGHYTEGA	ELIENVLEVV	RHESESCDCL	QGFQIVHSLG	GGTGSGMGTL	LMNKIREEYP
mmTUBB1 (β1)	LGVLFQPDSF	VHGNSGAGNN	WAKGHYTEGA	ELIENVMDVV	RRESESCDCL	QGFQIVHSLG	GGTGSGMGTL	LMNKIREEYP
ggTUB6B (β1)	FGQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKESESCDCL	QGFQLTHSLG	GGTGSGMGTL	LISKIREEYP
mmTLIBB2B (B2B)	FGQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKESESCOCL	OCFOLTHSLG	GGTGSGMGTL	LISKIREEYP
aaTUBB1 (62B)	FROTFRPDNF	VFGOSGAGNN	WAKGHYTEGA	ELVDSVLDVV	RKEAESCDCL	OGFOLTHSLG	GGTGSGMGTL	LISKIREEYP
hsTUBB4 (β4A)	FGQIFRPDNF	VFGQSGAGNN	WAKGHYTEGA	ELVDAVLDVV	RKEAESCDCL	QGFQLTHSLG	GGTGSGMGTL	LISKIREEFP
scTUB2 (β)	IGNLFRPDNY	IFGQS <mark>S</mark> AGNV	WAKGHYTEGA	ELVDSVMDVI	RREAEGCDSL	QGFQ <mark>I</mark> THSLG	GGTGSGMGTL	LISK <mark>I</mark> REE <mark>F</mark> P
boTLIDD2 (02)		180	EDVNATT CTU	200 OT VENUE	CTDNEAT VDT	220 CEDULKI AUD	TWODI NUT VO	
mmTLIBB3 (B3)	DRIMNTFSVV	PSPKVSDTVV	EPINATLSIN	OLVENTDETT	CIDNEALYDI	CFRTLKLATP	TYGDLNHLVS	ATMSGVIISL
gqTUB4B (β3)	DRIMNTFSVV	PSPKVSDTVV	EPYNATLSIH	QLVENTDETY	CIDNEALYDI	CFRTLKLATP	TYGDLNHLVS	ATMSGVTTSL
hsTUBB1 (β1)	DRIMNSFSVM	PSPKVSDTVV	EPYNA <mark>V</mark> LSIH	QLIENADACF	CIDNEALYDI	CFRTLKLTTP	TYGDLNHLVS	LTMSGITTSL
mmTUBB1 (β1)	DRILNSFSVM	PSPKVSDTVV	EPYNAVLSIH	QLIENTDACF	CIDNEALYDI	CFRTLRLTP	TYGDLNHLVS	LTMSGITTSL
ggTUB6B (β1)	DRIMNTFSVM	PSPKVSDTVV	EPYNATVSVH	QLVENTDETY	CIDNEALYDI	CFRTLKLTTP	TYGDLNHLVS	ATMSGVTTCL
hsTUBB2B (β2B)	DRIMNTFSVM	PSPKVSDTVV	EPYNATLSVH EDVNATT SVH	QLVENTDETY OT VENTDETY	CIDNEALYDI	CFRTLKLTTP	TYGDLNHLVS	ATMSGVTTCL
aaTUBB1 (62B)	DRIMNTFSVM	PSPKVSDTVV	EPYNATLSVI	OLVENTDETY	CIDNEALYDI	CFRTLKLTTP	TYGDLNHLVS	ATMSGVTTCL
hsTUBB4 (β4A)	DRIMNTFSVV	PSPKVSDTVV	EPYNATLSVH	QLVENTDETY	CIDNEALYDI	CFRTLKLTTP	TYGDLNHLVS	ATMSGVTTCL
scTUB2 (β)	DR <mark>M</mark> MATFSVL	PSPK <mark>T</mark> SDTVV	EPYNATLS <mark>V</mark> H	QLVEHSDETF	CIDNEALYDI	CQRTLKLNQP	SYGDLNNLVS	SVMSGVTTSL
beTUBB3 (83)	REPCOLNADI.	260 RKLAVNMVDF	DRIHEEMDCE	280		300	AACDDRHCRV	320
hsTUBB3 (β3) mmTUBB3 (β3)	RFPGQLNADL RFPGOLNADL	260 RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF	280 APLTARGSQQ APLTARGSOO	YRALTVPELT YRALTVPELT	300 QQMFDAKNMM OOMFDAKNMM	AACDPRHGRY AACDPRHGRY	320 LTVATVFRGR LTVATVFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3)	RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF	280 APLTARGSQQ APLTARGSQQ APLT <mark>R</mark> RGSQQ	YRALTVPELT YRALTVPELT YRALTVPELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM	AACDPRHGRY AACDPRHGRY AACDPRHGRY	320 LTVATVFRGR LTVATVFRGR LTVATVFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF	280 APLTARGSQQ APLTARGSQQ APLTRRGSQQ APLTAQGSQQ	YRALTVPELT YRALTVPELT YRALTVPELT YRAL <mark>S</mark> VAELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM QQMFDA <mark>R</mark> N <mark>T</mark> M	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDLRRGRY	320 LTVATVFRGR LTVATVFRGR LTVATVFRGR LTVA <mark>CI</mark> FRG <mark>K</mark>
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB1 (β1)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF	280 APLTARGSQQ APLTARGSQQ APLTRRGSQQ APLTAQGSQQ APLTAQGSQQ	YRALTVPELT YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM QQMFDARNIM QQMFDARNIM	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDLRRGRY AACDPRRGRY	320 LTVATVFRGR LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB1 (β1) ggTUB6B (β1) baTUB2B (β29)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF PKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF DRLHFFMPGF	280 APLTARGSQQ APLTARGSQQ APLTRGSQQ APLTAQGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM QQMFDARNIM QQMFDSKNMM QQMFDSKNMM	AACDPRHGRY AACDPRHGRY AACDLRRGRY AACDLRRGRY AACDPRHGRY AACDPRHGRY	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB1 (β1) ggTUB6B (β1) hsTUBB2B (β2B) mmTUBB2B (β2B)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF	APLTARGSQQ APLTARGSQQ APLTRGSQQ APLTAQGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM QQMFDARNIM QQMFDSKNMM QQMFDSKNMM	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB1 (β1) ggTUB6B (β1) hsTUBB2B (β2B) mmTUBB2B (β2B) gqTUBB1 (β2B)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPDLT	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACEPGHGRY	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAIFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB1 (β1) ggTUB6B (β1) hsTUBB2B (β2B) ggTUBB1 (β2B) hsTUBB4 (β4A)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACEPGHGRY AACEPGHGRY	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUBB2B (β2B) mmTUBB2B (β2B) ggTUBB1 (β2B) hsTUBB4 (β4A) scTUB2 (β)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTARGSQQ	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT FRSLTVPELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUBB2B (β2B) mmTUBB2B (β2B) ggTUBB1 (β2B) hsTUBB4 (β4A) scTUB2 (β)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGY	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTARGSQS	YRALTVPELT YRALSVAELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT FRSLTVPELT	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM	AACDPRHGRY AACDPRHGRY AACDLRGRY AACDLRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRNGRY	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUB22B (β2B) mmTUB22B (β2B) ggTUBB1 (β2A) scTUB2 (β) hsTUBB4 (β4A) scTUB2 (β)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RYPGQLNSDL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNVPF RKLAVNSF LAIOSKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY	280 APLTARGSQQ APLTRGSQQ APLTAQGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTAIGSQS VAVCDIPPRG	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT FRSLTVPELT FRSLTVPELT	300 QOMFDAKNMM QOMFDAKNMM QOMFDARNTM QOMFDARNTM QOMFDARNTM QOMFDSKNMM QOMFDSKNMM QOMFDAKNMM QOMFDAKNMM QOMFDAKNMM STAIOELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRNGRY	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAFFRGK 400 RKAFLHWYTG
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUB82B (β2B) mmTUB82B (β2B) ggTUBB1 (β2B) hsTUBB4 (β4A) scTUB2 (β) hsTUBB3 (β3) mmTUBB3 (β3)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RVPGQLNSDL MSMKEVDEQM	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF LAIQSKNSSY LAIQSKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQS S360 VAVCDIPPRG VAVCDIPPRG	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT FRSLTVPELT LKMSSTFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDARNIM QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY ISEQFTAMFR ISEQFTAMFR	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAFFRGK 4000 RKAFLHWYTG RKAFLHWYTG
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB48 (β3) hsTUBB1 (β1) ggTUB68 (β1) hsTUB828 (β2B) mmTUB828 (β2B) ggTUB81 (β2B) hsTUB84 (β4A) scTUB2 (β) hsTUBB3 (β3) mmTUBB3 (β3) ggTUB48 (β3)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL MSMKEVDEQM MSMKEVDEQM	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY FVEWIPNNVK	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTAIGSQS VAVCDIPPRG VAVCDIPPRG	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT FRSLTVPELT LKMSSTFIGN LKMSSTFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY ISEQFTAMFR ISEQFTAMFR	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUBB2B (β2B) mmTUBB2B (β2B) hsTUBB4 (β4A) scTUB2 (β) hsTUBB3 (β3) ggTUBB4 (β4) hsTUBB3 (β3)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL MSMKEVDEQM MSMKEVDEQM MSMKEVDEQM	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF LALQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY FVEWIPNNVK FVEWIPNNVK	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTPRG VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT FRSLTVPELT LKMSSTFIGN LKMSSTFIGN LSMAATFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM QQMFDARNIM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR STAIQELFKR NTAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACEPGHGRY AACEPGHGRY AACPCFAMFR ISEQFTAMFR ISEQFTAMFR VSEHFSAMFK	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR KAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB1 (β1) ggTUB6B (β1) hsTUBB28 (β2B) ggTUBB1 (β2B) hsTUBB2 (β2B) hsTUBB4 (β4A) scTUB2 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB1 (β1)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RYPGQLNADL MSMKEVDEQM MSMKEVDEQM MSTKEVDQQL MSTKEVDQQL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNLVPF 340 LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK	280 APLTARGSQQ APLTARGSQQ APLTAQGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTAIGSQS VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG	YRALTVPELT YRALSVAELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT FRSLTVPELT FRSLTVPELT LKMSSTFIGN LKMSSTFIGN LSMAATFIGN LMMAATFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR STAIQELFKR NTAIQELFKR NTAIQELFKR NTAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPCHGRY AACEPGHGRY AACPCHGRY AACPCHGRY AACPCHAMFR ISEQFTAMFR ISEQFTAMFR VSEHFSAMFK VSEHFSAMFK	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR CTVAAFFRGK 400 RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFVHWYTS RKAFVHWYTS
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUBB2B (β2B) ggTUB82B (β2B) ggTUB81 (β2B) hsTUBB2 (β2B) hsTUBB3 (β3) ggTUB4B (β3) hsTUBB3 (β1) mmTUBB1 (β1) ggTUB82B (β2B)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL MSMKEVDEQM MSMKEVDEQM MSTKEVDQQL MSTKEVDQQL MSTKEVDQQL MSTKEVDQQL	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNLVPF 340 LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTAGSQS VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG TAVCDIPPRG TAVCDIPPRG	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT KRALTVPELT FRSLTVPELT LKMSSTFIGN LKMSSTFIGN LSMAATFIGN LKMSATFIGN LKMSATFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR STAIQELFKR NTAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY ISEQFTAMFR ISEQFTAMFR VSEHFSAMFK VSEHFSAMFK ISEQFTAMFR	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR CTVAAFFRGK 400 RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTS RKAFLHWYTS RKAFLHWYTS
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUB22 (β2B) mmTUB22B (β2B) ggTUB81 (β2B) hsTUB84 (β4A) scTUB2 (β) hsTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUBB28 (β2B) mmTUBB28 (β2B)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL MSMKEVDEQM MSMKEVDEQM MSTKEVDQQL MSTKEVDQQL MSTKEVDQQL MSMKEVDEQM	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNLVPF RKLAVNLVPF LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LSUQTRNSSC LSIQTRNSNC LSIQTRNSNC LNVQNKNSSY LNVQNKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK	280 APLTARGSQQ APLTRGSQQ APLTAQGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTAIGSQS VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG VAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT KRSSTFIGN LKMSSTFIGN LKMSSTFIGN LKMSATFIGN LKMSATFIGN LKMSATFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUB82B (β2B) mmTUB82B (β2B) mmTUB82B (β2B) hsTUBB4 (β4A) scTUB2 (β) hsTUBB3 (β3) ggTUB4B (β3) ggTUB4B (β3) hsTUBB1 (β1) mmTUBB2B (β2B) ggTUB6B (β1) hsTUBB2B (β2B) ggTUBB1 (β2B)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL MSMKEVDEQM MSMKEVDEQM MSTKEVDQQL MSMKEVDEQM MSMKEVDEQM	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LSVQTRNSCC LSIQTRNSCC LSIQTRNSCC LSIQTRNSCY LNVQNKNSSY LNVQNKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTRGSQQ APLTRQSQQ APLTRQSQQ APLTRQSQQ APLTSRGSQQ APLTPRG ACCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG	YRALTVPELT YRALSVAELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT KRSSTFIGN LKMSSTFIGN LKMSSTFIGN LKMSATFIGN LKMSATFIGN LKMSATFIGN LKMSATFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY SEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR KAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG
hsTUBB3 (β3) mmTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUB28 (β2B) mmTUB828 (β2B) msTUB828 (β2B) hsTUB84 (β4A) scTUB2 (β) hsTUBB3 (β3) ggTUB4B (β3) hsTUBB1 (β1) ggTUB6B (β1) hsTUBB28 (β2B) mmTUB828 (β2B) mmTUB828 (β2B) mmTUB828 (β2B) mmTUB828 (β2B) mmTUB828 (β2B) mmTUB828 (β2B) mmTUB828 (β2B) mmTUB828 (β2A)	RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL RFPGQLNADL MSMKEVDEQM MSMKEVDEQM MSMKEVDEQM MSMKEVDEQM MSMKEVDEQM MSMKEVDEQM	260 RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF RKLAVNMVPF LAIQSKNSSY LAIQSKNSSY LAIQSKNSSY LSVQTRNSCC LNVQNKNSSY LNVQNKNSSY LNVQNKNSSY LNVQNKNSSY LNVQNKNSSY LNVQNKNSSY	PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMPGF PRLHFFMVGY FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK FVEWIPNNVK	280 APLTARGSQQ APLTRGSQQ APLTRGSQQ APLTAQGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ APLTSRGSQQ TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG TAVCDIPPRG	YRALTVPELT YRALTVPELT YRALSVAELT YRALSVAELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT YRALTVPELT KASSTFIGN LKMSSTFIGN LKMSATFIGN LKMSATFIGN LKMSATFIGN LKMSATFIGN LKMSATFIGN	300 QQMFDAKNMM QQMFDAKNMM QQMFDARNTM QQMFDARNTM QQMFDSKNMM QQMFDSKNMM QQMFDSKNMM QQMFDAKNMM QQMFDAKNMM STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFTR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR STAIQELFKR	AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRGRY AACDPRGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY AACDPRHGRY ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR ISEQFTAMFR	320 LTVATVFRGR LTVATVFRGR LTVACIFRGK LTVACIFRGK LTVAAIFRGR LTVAAIFRGR LTVAAIFRGR LTVAAVFRGR LTVAAVFRGR LTVAAVFRGR KAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG RKAFLHWYTG
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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 (<u>http://www.uniprot.org/</u>): 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 eluent, (11) the fraction that remained attached to the FLAG-affinity column eluent, (11) the fraction that remained attached to the FLAG-affinity column, and (12) after polymerization and centrifugation. \blacktriangleright and \triangleright , 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 μ M 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 μ m.



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 stererospecifically bind.

mutant	relative ATP	ase parameter	protein	reference	
	$k_{\rm cat}$ (fold wt)	$k_{\rm M}({ m MT})$ (fold wt)	_		
WT-Tb	1.00 ± 0.03	1.00 ± 0.14	11		
β-Tb R262H	0.17 ± 0.03	9.71 ± 4.94	TUBB3	This study ^a	
β-Tb R262A	0.23 ± 0.01	10.3 ± 1.23	TOBBS		
WT-Tb	1.00 ± 0.03	1.00 ± 0.30			
a-Tb E414A	0.20 ± 0.02	0.78 ± 0.04		Uchimura, et al, 2010 ^b	
α-Tb E415A	0.80 ± 0.09	2.39 ± 0.22	C		
a-Tb E417A	0.82 ± 0.03	1.00 ± 0.35	S. <i>cerevisiae</i> TUB2		
a-Tb E420A	0.94 ± 0.03	1.83 ± 0.39	1002		
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	Homo sapiens	Woehlke, et al,	
kinesin D279A	0.78 ± 0.14	0.44 ± 0.21	KIF5B	1997°	

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

^a Values are mean \pm errors of curve fitting

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

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

microtubule	WT KIF5B			D279R KIF5B			
	ADP	nucleotide free	AMPPNP	ADP	nucleotide free	AMPPNP	
WT	6.5 ± 0.7	$1.2\pm 0.6 \times \! 10^{-2}$	$2.9\pm0.4\times10^{-2}$	$1.2\pm0.8\times10^{1}$	$1.2\pm0.6\times10^{-2}$	$1.0\pm0.4\times10^{-2}$	
β-R262A	$3.6\pm0.3\times\!\!10^1$	$8.6\pm0.4\times10^{-1}$	2.5 ± 0.1	$1.4\pm0.2\times10^{1}$	$1.2\pm0.3\times10^{-1}$	$5.0\pm1.2\times10^{-2}$	

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

Values are K_d in μ M. Errors are those in curve fitting.

mianatuhula		KIF	KIF21A			
microtubule	parameter	WT	D325R			
WT	velocity [µm 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 [µm s ⁻¹]	UD	UD			
	duration [s]	UD	UD			
	run length [µm]	UD	UD			
β-R262A	velocity [µm s ⁻¹]	UD	0.16 ± 0.45			
	duration [s]	UD	5.88 ± 0.89			
	run length [µm]	UD	1.61 ± 0.73			

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

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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