

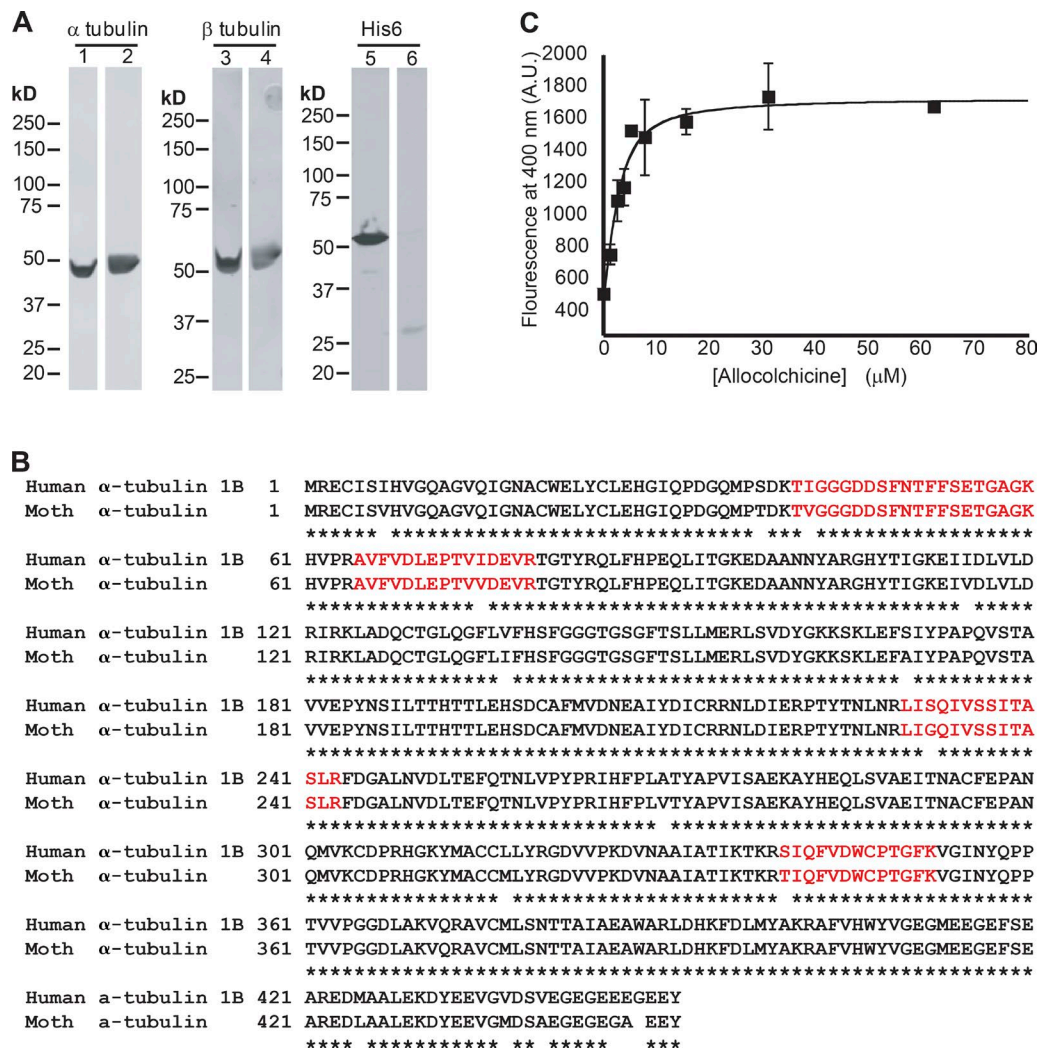
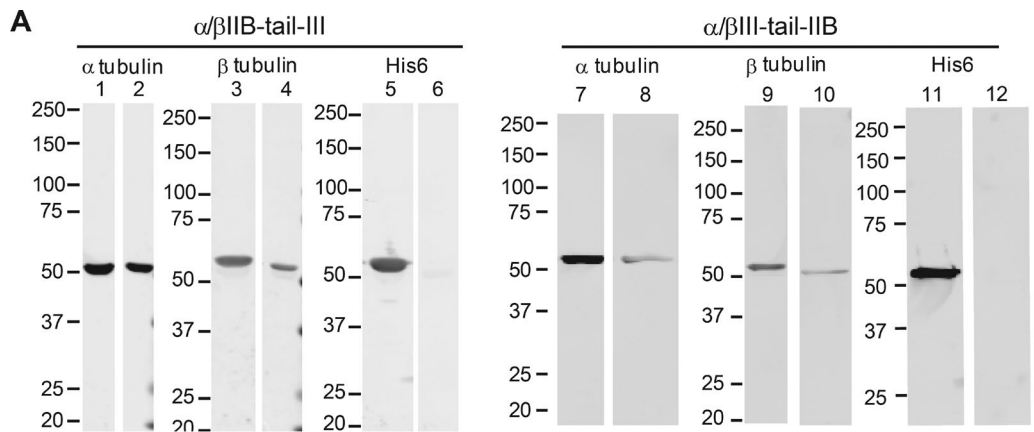
Pamula et al., <http://www.jcb.org/cgi/content/full/jcb.201603050/DC1>

Figure S1. **Purification of recombinant  $\alpha$ / $\beta$ IIIB tubulin heterodimers.** (A) Full blots corresponding to those shown in Fig. 1 C, showing western blot analyses of proteins eluted from nickel-affinity (lanes 1, 3, and 5) and TOG-affinity (lanes 2, 4, and 6) chromatography. Antibodies against  $\alpha$  tubulin,  $\beta$  tubulin, and C-terminal hexahistidine tag are indicated. (B) Alignment of protein sequences from human  $\alpha$  tubulin 1B (NP\_006073.2) and  $\alpha$  tubulin from insect cells (ABU94679.1). Peptide fragments that were used to estimate the relative amounts of human and insect  $\alpha$  tubulin are labeled in red. (C) Equilibrium binding curve for  $\alpha$ / $\beta$ IIIB with allocolchicine. The data from three independent experiments were averaged and fitted to a curve, weighed by the SD.  $K_d = 1.8 \pm 0.42 \mu\text{M}$  ( $n = 3$ , mean  $\pm$  SD). A.U., arbitrary units.



**B**  
Percent Identity Matrix - created by Clustal2.1

		$\beta$ VI	$\beta$ V	$\beta$ III	$\beta$ IVA	$\beta$ IIA	$\beta$ IIB	$\beta$ IVB	$\beta$ I
1: $\beta$ VI	NM_030773.3	100.00	78.03	77.33	78.60	78.43	78.65	78.43	78.60
2: $\beta$ V	NM_032525.2	78.03	100.00	92.38	91.89	90.34	90.56	90.79	90.77
3: $\beta$ III	NM_006086.3	77.33	92.38	100.00	91.67	91.46	91.69	92.81	92.57
4: $\beta$ IVA	NM_006087.3	78.60	91.89	91.67	100.00	94.59	95.05	97.97	96.61
5: $\beta$ IIA	NM_001069.2	78.43	90.34	91.46	94.59	100.00	99.55	96.40	95.05
6: $\beta$ IIB	NM_178012.4	78.65	90.56	91.69	95.05	99.55	100.00	96.85	95.50
7: $\beta$ IVB	NM_006088.5	78.43	90.79	92.81	97.97	96.40	96.85	100.00	97.52
8: $\beta$ I	NM_178014.3	78.60	90.77	92.57	96.61	95.05	95.50	97.52	100.00

Figure S2. **Purification of chimeric  $\beta$  tubulin heterodimers and tubulin isotype identity matrix.** (A) Full blots corresponding to those shown in Fig. 4 D showing western blot analyses of proteins eluted from nickel-affinity (lanes 1, 3, 5, 7, 9, and 11) and TOG-affinity (lanes 2, 4, 6, 8, 10, and 12) chromatography. (B) Percent identity matrix for human  $\beta$  tubulin isotypes.

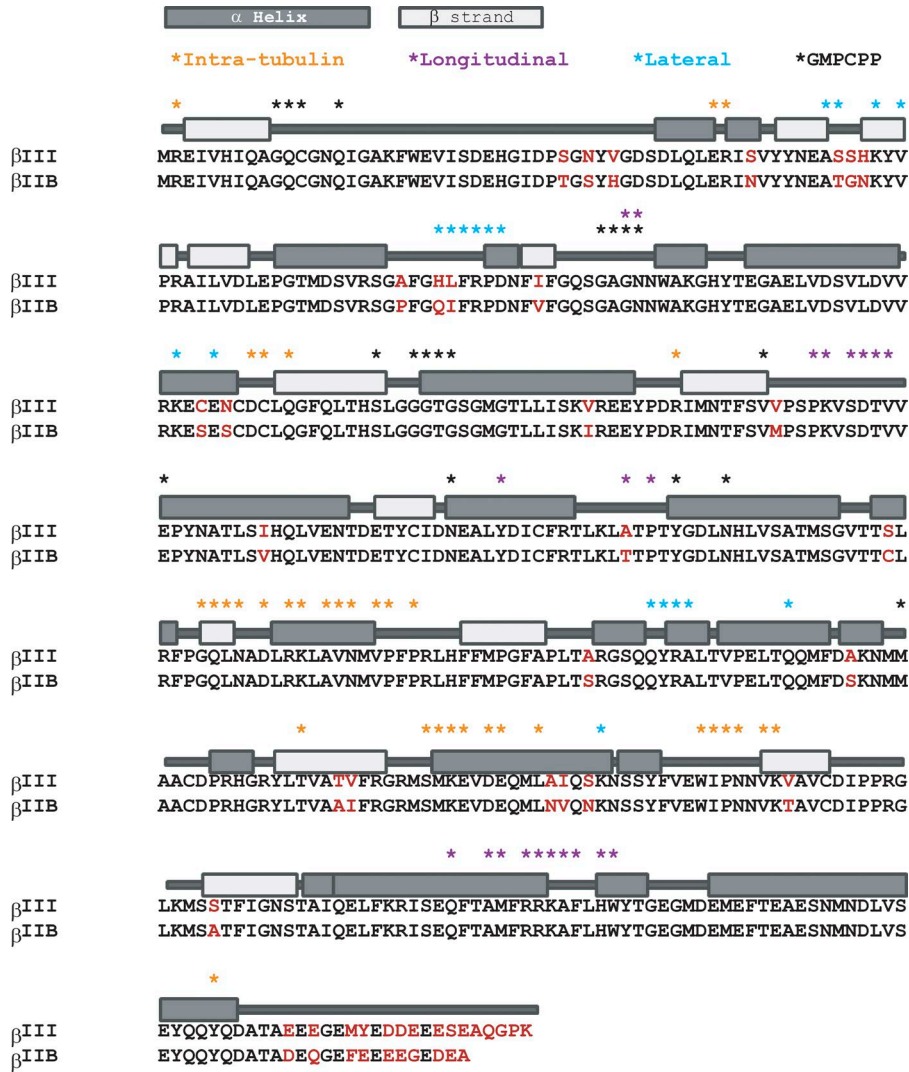


Figure S3.  $\beta$  tubulin isotypes IIB and III alignment and a secondary structure topology map. Alignment of  $\beta$ III and  $\beta$ IIB amino acid sequence. Secondary structure topology map corresponds to features derived from PDB listing 3J6E. Residues on  $\beta$  tubulin within 3 Å of intra-tubulin (orange stars), longitudinal (purple stars), or lateral (blue stars) contacts or the GMPCPP binding site (black stars) are indicated. Residues that differ between isotypes  $\beta$ III and  $\beta$ IIB are labeled in red.