LEGENDS TO SUPPLEMENTARY FIGURES

FIGURE S1. **Controls for any nonspecific effects of Pak1 adenovirus infection on cardiomyocyte microtubules.** Immunoblots of free and polymerized tubulin, with a GAPDH loading control, were prepared from cultured quiescent adult feline cardiomyocytes infected 48 hr earlier with either no virus, or AdβGal, an adenovirus encoding bacterial β-galactosidase, or AdKDPak1, an adenovirus encoding kinase-dead Pak1-K299R, or AdPak1, an adenovirus encoding constitutively active Pak1, each at a MOI of ~1. A monoclonal anti-β-tubulin antibody (clone DM-1B; Abcam) was used for the upper two immunoblots, and a monoclonal anti-GAPDH antibody (clone 6C5; Upstate Biotech) was used for the bottom immunoblot. The only difference among the four groups of cells, an increase in polymerized tubulin, is seen solely in the AdPak1-infected cells.

FIGURE S2. **Microtubule network density in cardiomyocytes from mice overexpressing PP2A or PP1 after okadaic acid exposure.** These cardiomyocytes were isolated from the hearts of the two strains of transgenic mice and than treated for 8 hr with the specified concentrations of okadaic acid before being fixed for confocal microscopy. The antibody used for the confocal micrographs was a monoclonal anti-β-tubulin antibody (clone DM-1B; Abcam); each micrograph is a single 0.1 μm confocal section taken at the level of the nuclei. The mean pixel intensity (white level) within the boundary of each cardiomyocyte was determined for 5 cells in each strain at each okadaic acid concentration, and these mean data ± SEM are shown in the graphs.

FIGURE S3. **Free and polymerized tubulin and microtubule stability.** *A* & *B*, Free and polymerized tubulin in the hearts of control and colchicine-treated mice. These immunoblots were prepared from myocardial homogenates from the same groups of vehicle-treated mice (baseline) and colchicine-treated mice used for the micrographs in Fig. 8 by using a monoclonal anti-β-tubulin antibody (clone DM-1B; Abcam). For the loading control a monoclonal anti-GAPDH antibody (clone 6C5; Upstate Biotech) was used in the same samples as those used for free tubulin. For Panel *A*, for this and two other sets of immunoblots the densitometric ratio of MAP4/Control signals in the baseline groups was 4.45 ± 0.77 for free tubulin and 3.99 ± 0.68 for polymerized tubulin; in the colchicine-treated groups it was 2.09 ± 0.22 for free tubulin and 5.34 ± 0.69 for polymerized tubulin. This ratio for PP2A/Control in the baseline groups was 4.91 ± 0.88 for free tubulin and 4.53 ± 0.81 for polymerized tubulin; in the colchicine-treated groups it was 2.00 ± 0.20 for free tubulin and 6.48 ± 0.98 for polymerized tubulin. For Panel *B* the densitometric ratio of MAP4/Control signals in the baseline groups was 1.87 ± 0.13 for free tubulin and 3.31 ± 0.77 for polymerized tubulin; in the colchicine-treated groups it was 1.19 ± 0.77 for free tubulin and 3.64 ± 0.25 for polymerized tubulin. This ratio for PP1/Control in the baseline groups was 2.02 ± 0.12 for free tubulin and 3.15 ± 0.04 for polymerized tubulin; in the colchicine-treated groups it was 1.00 ± 0.16 for free tubulin and 3.76 ± 0.26 for polymerized tubulin.

FIGURE S4. **MAP homology in the region of the first KXGS-containing pseudorepeat of the microtubule-binding domain.** Sites where the amino acid sequences are not 100% conserved are identified in red. The conserved serine of interest in the underlined first KXGS repeat is identified in green. From above down, the amino acid sequences are from: 1) human Tau, full-length, 441 amino acids (GenBank™ accession number AAC04279.1); 2) human MAP4, isoform 1, 1152 amino acids (GenBank™ accession number NM_002375.4); 3) feline MAP4, 1135 amino acids (GenBank™ accession number EU921827.1); 4) murine MAP4, 1125 amino acids (GenBank™ accession number M72414.1); 5) bovine MAP4, 1072 amino acids (GenBank™ accession number NM_174105.2).

FIGURE S5. **Concordant changes in tubulin and MAP4 in the myocardial microtubule fraction from mice overexpressing PP2Acα or PP1cα.** These immunoblots were prepared under the conditions used to prepare the middle immunoblots in Figs. 6 & 7. That is, as described in the Experimental Procedures, after the myocardial samples were homogenized under conditions which stabilize the microtubules, and the tubulin heterodimers had then been removed, these immunoblots were prepared from the remaining sample under the same conditions used for the corresponding middle immunoblots in Figs. 6 & 7. Equal loading was used for all lanes of all four of these blots. The antibodies used were a monoclonal anti-β-tubulin antibody (clone DM-1B; Abcam) and our anti-MAP4 antibody (24).

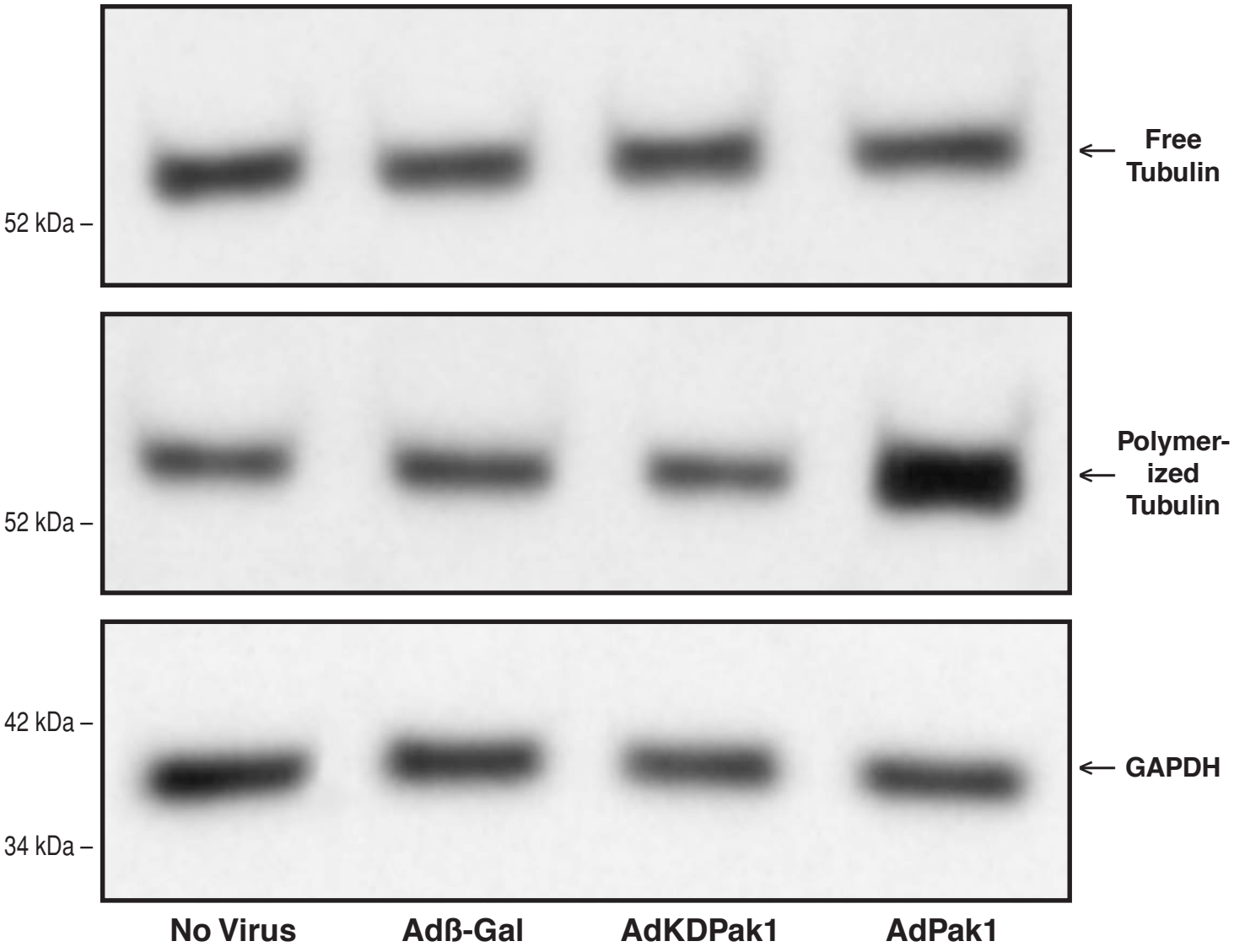
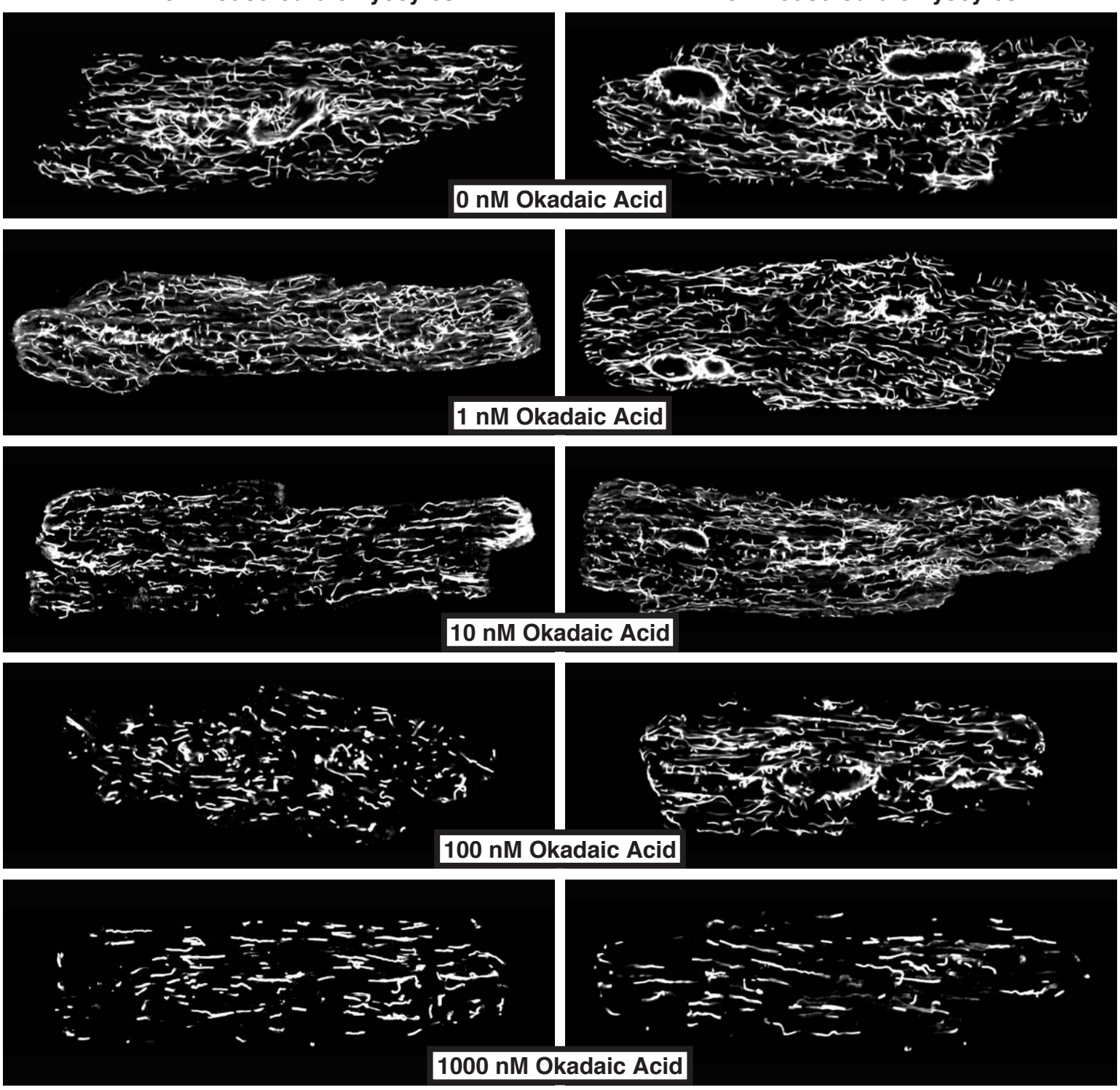
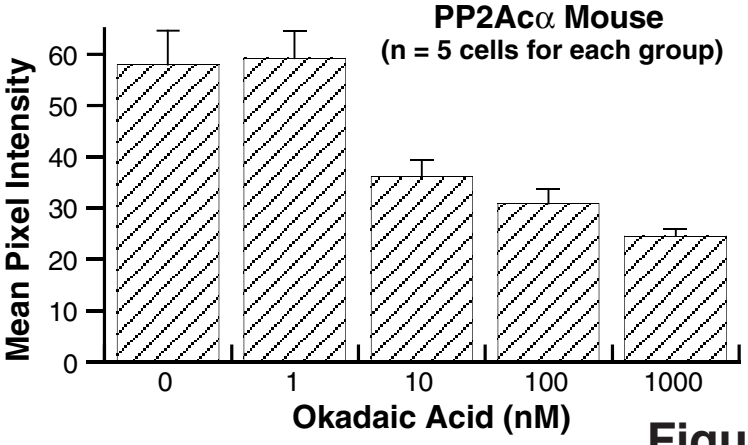

52 kDa –
Free Tubulin
52 kDa –
Polymer-ized Tubulin
42 kDa –
34 kDa –
GAPDH
No Virus
Adß-Gal
AdKDPak1
AdPak1

Figure S1


PP2Acα Mouse Cardiomyocytes
PP1Acα Mouse Cardiomyocytes
0 nM Okadaic Acid
1 nM Okadaic Acid
10 nM Okadaic Acid
100 nM Okadaic Acid
1000 nM Okadaic Acid


PP2Acα Mouse
(n = 5 cells for each group)
Mean Pixel Intensity
0
10
20
30
40
50
60
0
1
10
100
1000
Okadaic Acid (nM)

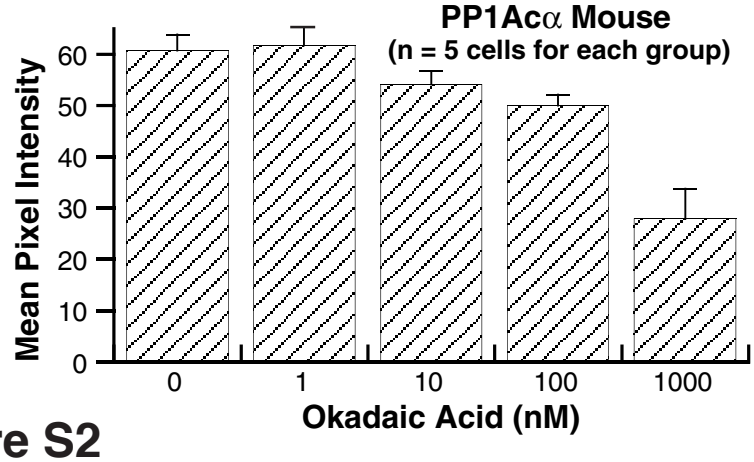

PP1Acα Mouse
(n = 5 cells for each group)
Mean Pixel Intensity
0
10
20
30
40
50
60
0
1
10
100
1000
Okadaic Acid (nM)

Figure S2

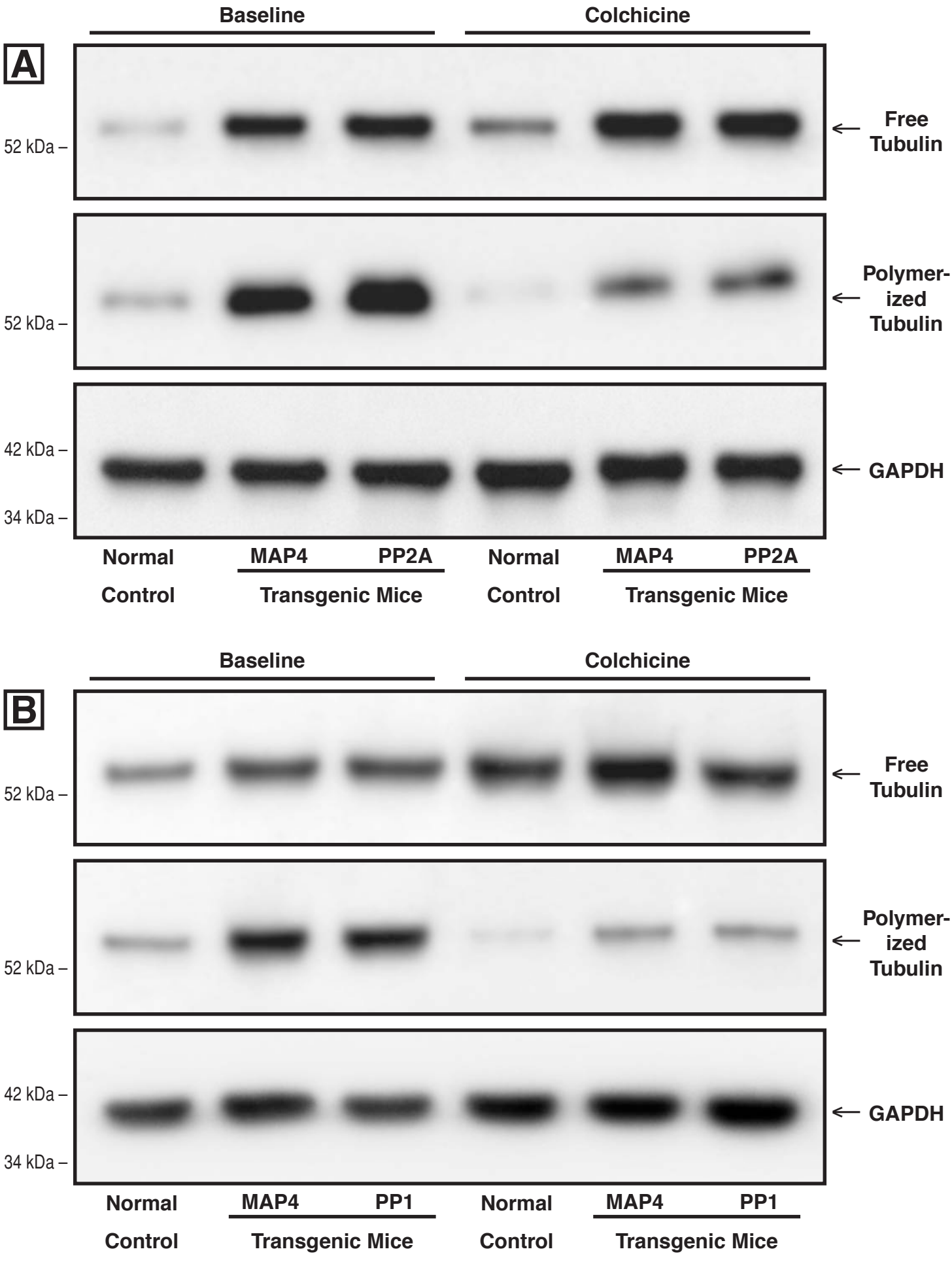

A
Baseline
Colchicine
52 kDa –
Free Tubulin
52 kDa –
Polymer-ized Tubulin
42 kDa –
34 kDa –
GAPDH
Normal Control
MAP4
PP2A
Transgenic Mice
Normal Control
MAP4
PP2A
Transgenic Mice
B
Baseline
Colchicine
52 kDa –
Free Tubulin
52 kDa –
Polymer-ized Tubulin
42 kDa –
34 kDa –
GAPDH
Normal Control
MAP4
PP1
Transgenic Mice
Normal Control
MAP4
PP1
Transgenic Mice

Figure S3

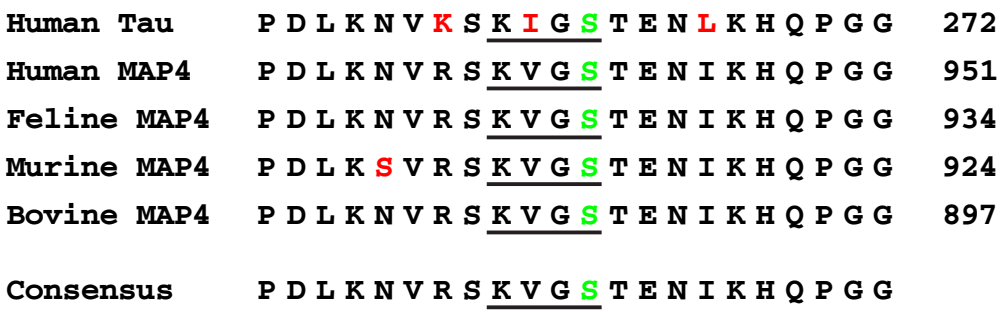

Human Tau P D L K N V K S K I G S T E N L K H Q P G G 272
Human MAP4 P D L K N V R S K V G S T E N I K H Q P G G 951
Feline MAP4 P D L K N V R S K V G S T E N I K H Q P G G 934
Murine MAP4 P D L K S V R S K V G S T E N I K H Q P G G 924
Bovine MAP4 P D L K N V R S K V G S T E N I K H Q P G G 897
Consensus P D L K N V R S K V G S T E N I K H Q P G G

Figure S4

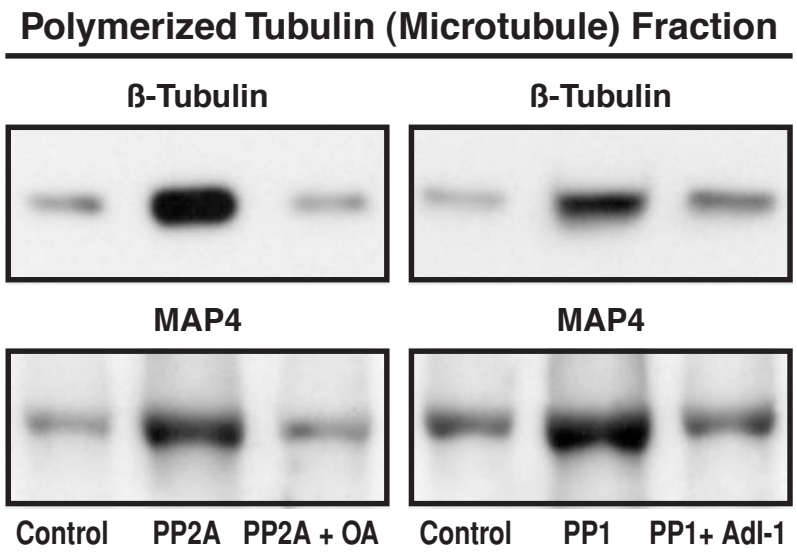

Polymerized Tubulin (Microtubule) Fraction
ß-Tubulin
ß-Tubulin
MAP4
MAP4
Control
PP2A
PP2A + OA
Control
PP1
PP1+ Adl-1

Figure S5