Supplemental Materials

Molecular Biology of the Cell

Hookway et al.

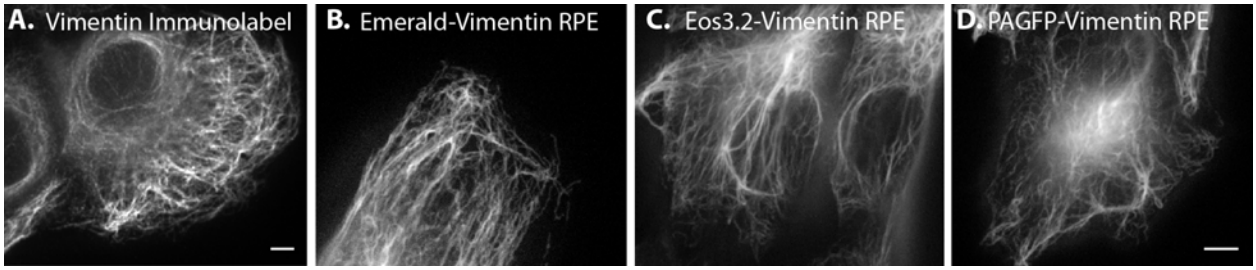

Figure S1. Tagged vimentin fusion proteins do not change vimentin network organization. Confocal images of RPE cell immunolabeled for vimentin (A.), expressing Emerald-vimentin (B.), expressing Eos3.2-vimentin (C.), and expressing PA-GFP-vimentin (D.) show that expression of probes produced normal networks and did not induce bundling or aggregation of vimentin. (Scale bars, 5 µm)

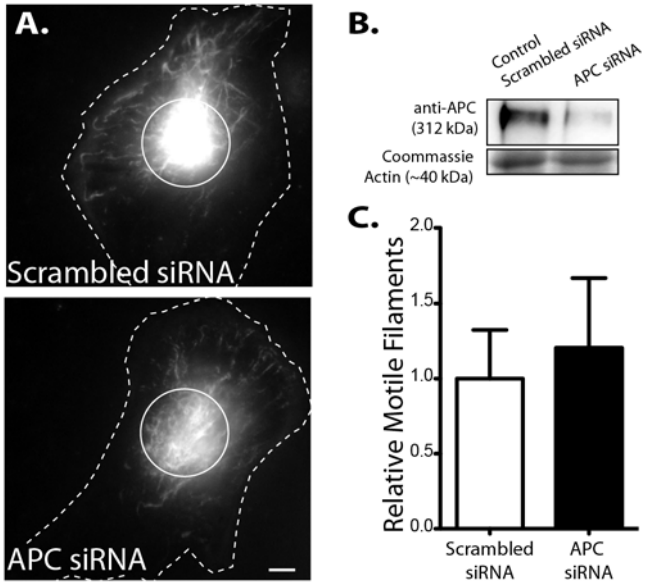

Figure S2. APC depletion does not affect vimentin IF transport. A. Similar amounts of transported filaments were observed in scrambled siRNA-transfected (control) RPE cells and APC-siRNA-transfected cells. (Scale bar, 5 µm) B. Western blot of cell lysates reveals APC siRNA treatment was sufficient to deplete cells of APC protein. Top portion of gel was cut and blotted for APC, bottom was Coomassie-stained for loading control. C. Quantification of filament motility shows no difference between scrambled-siRNA controls (n = 17) and APC-depleted (n = 17) cells. 95% confidence interval represented by error bars.

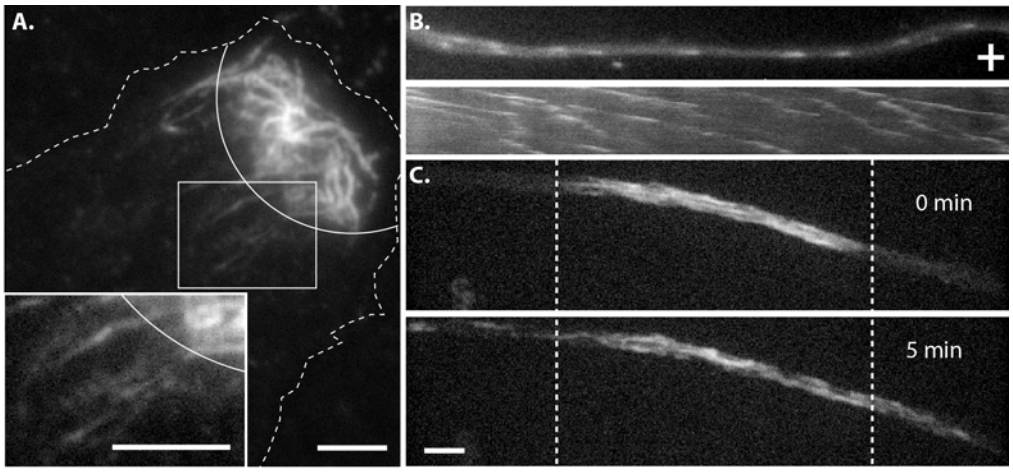

Figure S3. Vimentin filament transport occurs in anterograde and retrograde directions. A. RPE cell three minutes after photoconversion at the cell periphery (region marked with semi-circle). Inset shows many filaments have moved outside of the region towards the cell center. (Scale bars, 5 µm) B. Microtubule plus-tip labeling by GFP-EB3 in CAD neurite (top). Kymograph of neurite over 1 min demonstrates uniform polarity of microtubules (bottom). B. PA-GFP-vimentin expressing CAD cell immediately after conversion (top) and after 3 minutes (bottom) shows that activated filaments are transported in both directions. (Scale bar, 5 µm) Corresponds to Video 6.

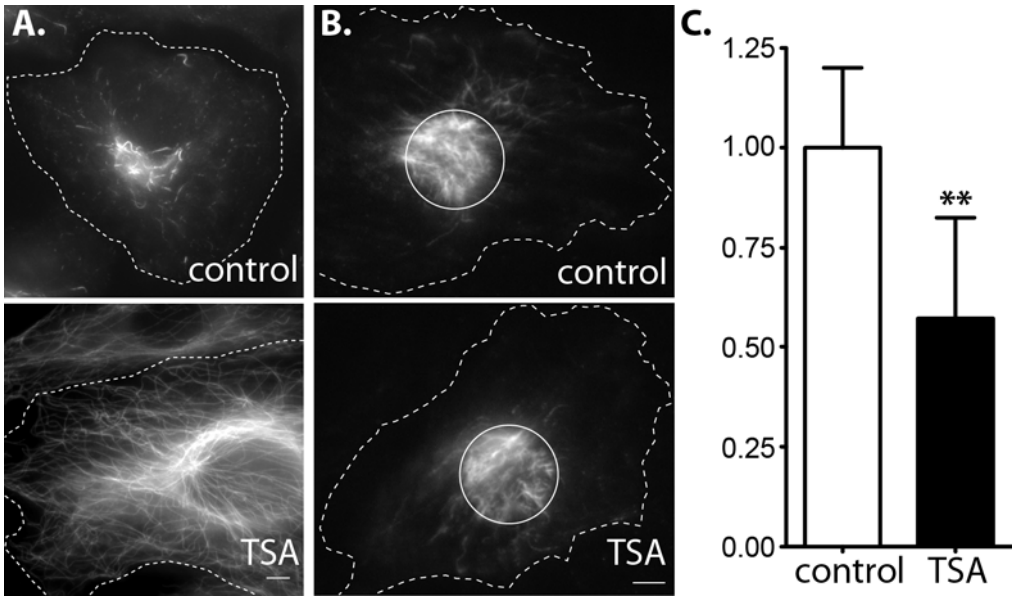

Figure S4. Increasing acetylated tubulin using the HDAC inhibitor TSA reduces filament transport. A. RPE cells labeled for acetylated tubulin in untreated control (top) and after ~16hrs TSA incubation (bottom) show that TSA increases microtubule acetylation. (Scale bar, 5 µm) Representative cells at three minutes after photoconversion in area marked with circle. (Scale bar, 5 µm) B. Quantification of filament transport. Error bars show 95% confidence interval.

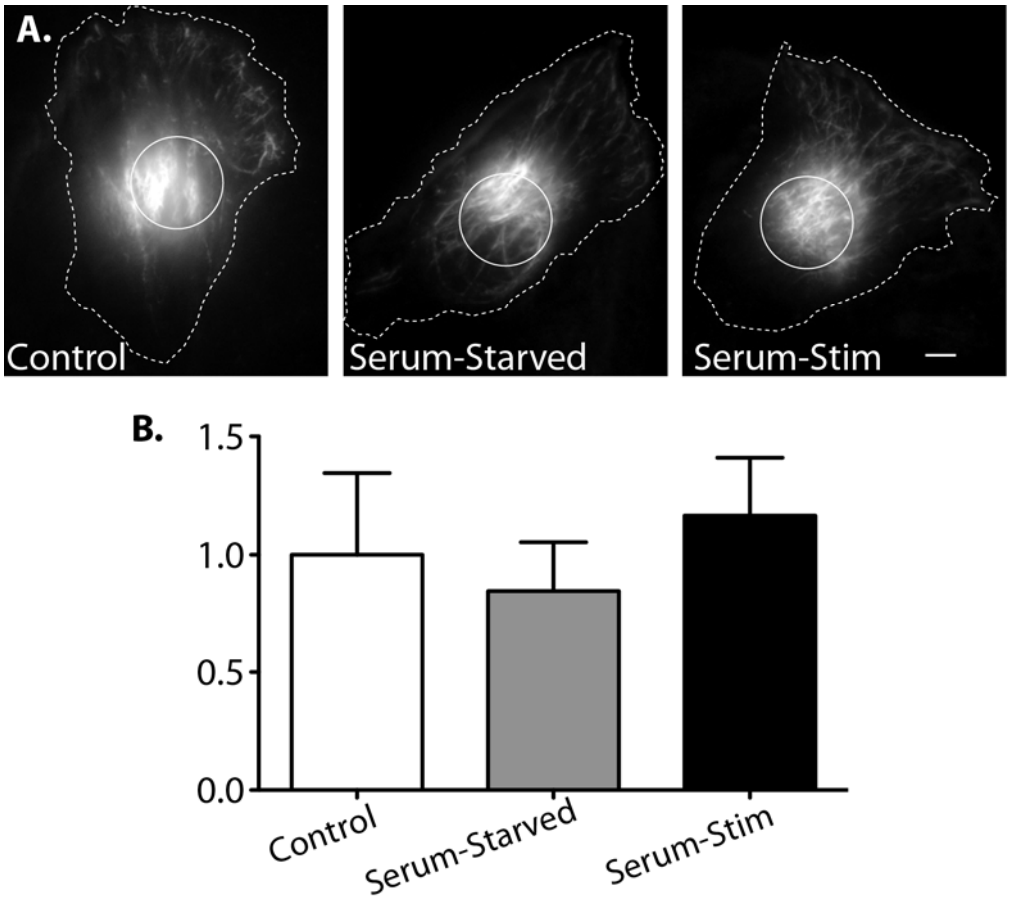

Figure S5. Serum starvation and serum stimulation do not affect filament motility. A. Representative cells three minutes after photoconversion in area marked with circle shows little difference in transport. (Scale bar = 5 µm) B. Quantification of filament transport between groups. Error bars show 95% confidence interval.

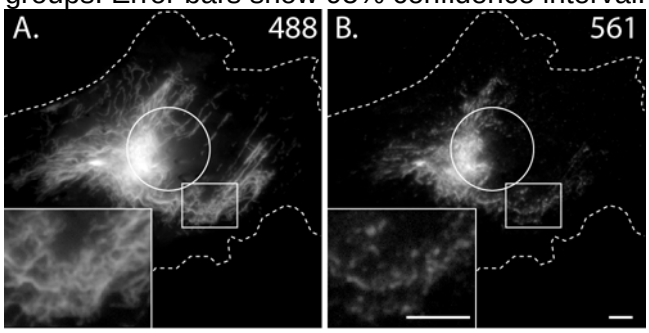

Figure S6. Severing and annealing occurs independently of microtubules. Nocodazole-treated Eos3.2-vimentin expressing SW13 cell 17 hours after it was photoconverted in the region marked with a circle in 488 and 561 channels (A. and B. respectively). Note that converted vimentin appears as segments along filaments in B. even in the absence of microtubules. (Scale bars, 5 µm)

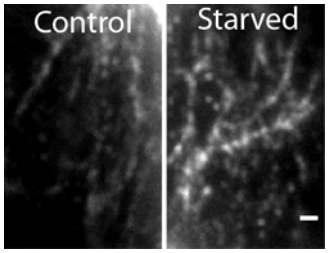

Figure S7. Severing and re-annealing persists even in serum-starved cells. Segmented filaments mEos3.2-vimentin RPE cells ~20 hours after a subset of vimentin filaments were photoconverted (as in figure 7) can be seen in both controls and cells starved of serum for 72 hours before conversion. (TIRFM, Scale bar = 1 µm)