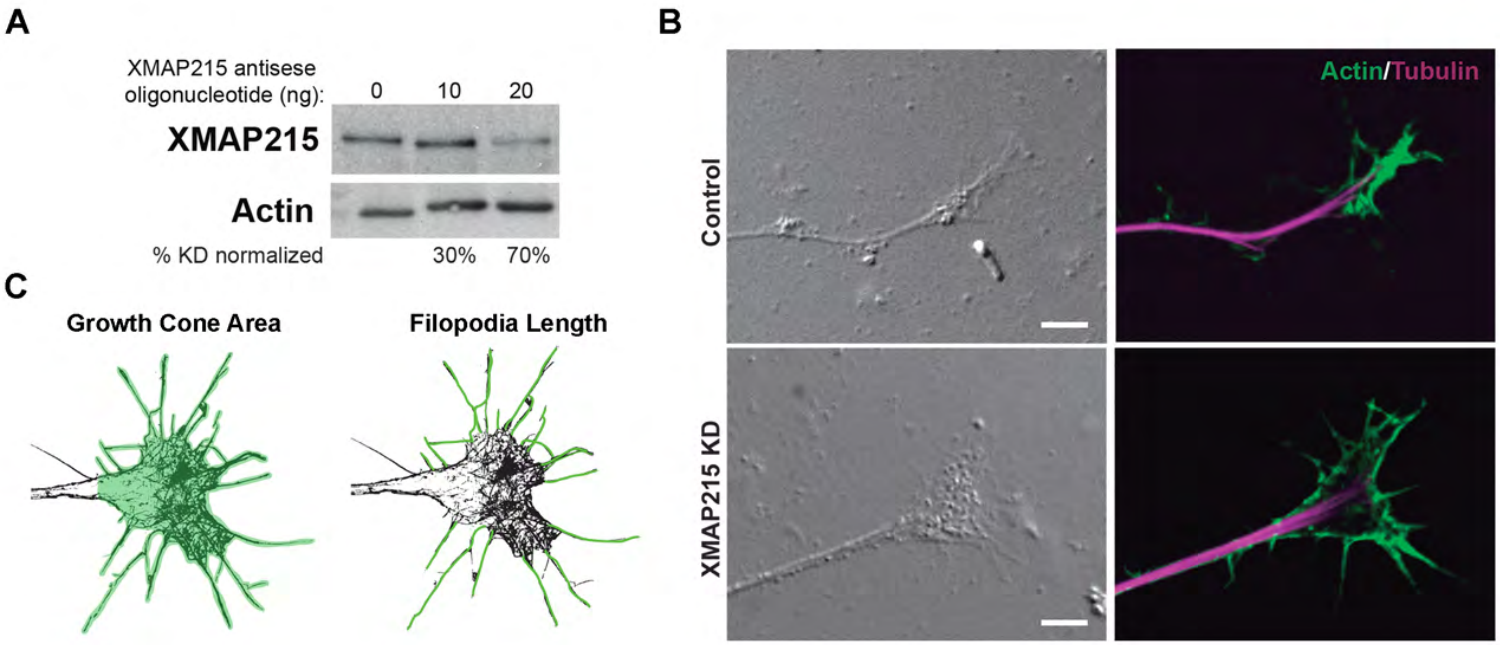


Figure S1

Figure S1. A. Western blot showing increasing levels of XMAP215 knockdown with increasing levels of antisense oligonucleotide. Actin was used as a loading control. **B.** DIC images (left) and actin and tubulin merge images (right) of growth cones from control and XMAP215 KD neural explants. Scale bar: 5 µm. **C.** Diagrams illustrating the measurements taken to analyze either growth cone area (left), or filopodia length (right) following XMAP215 KD, rescue, or OE. Structures analyzed are highlighted in green. Growth cone measurements correspond to data shown in Fig. 1A. Average filopodia length corresponds to data shown in Fig. 1C.

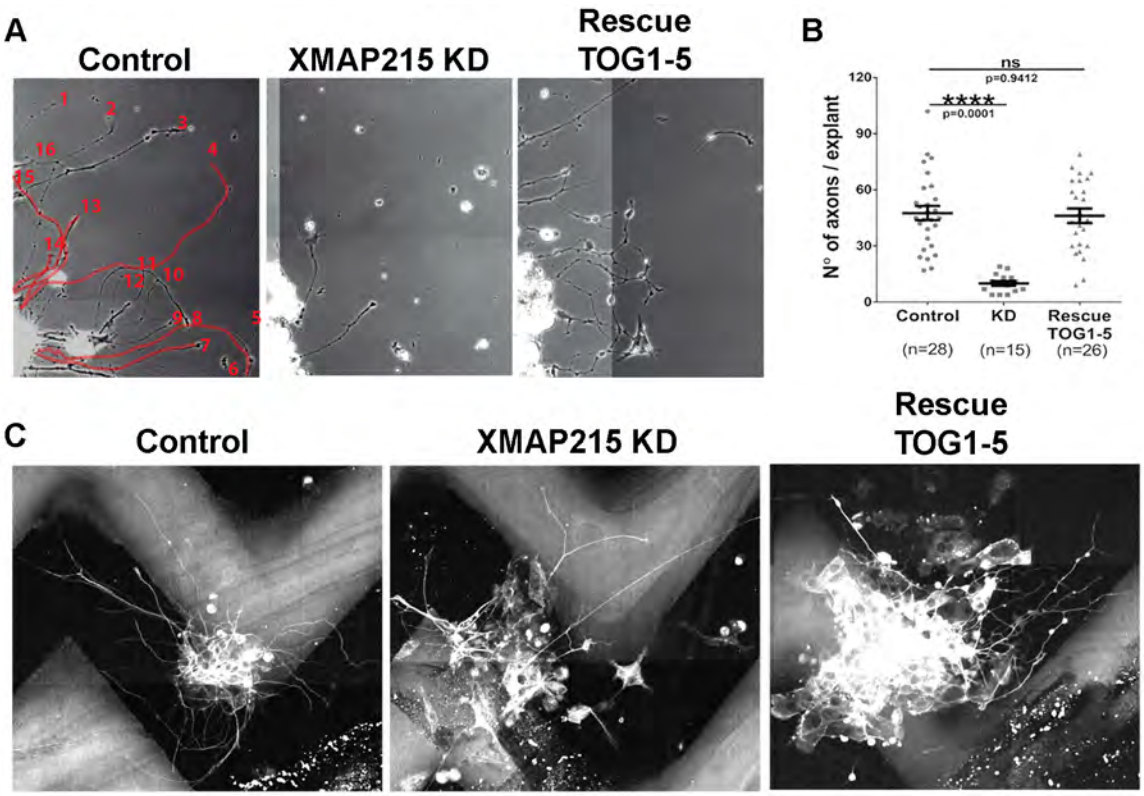


Figure S2

Supplemental Figure 2. (A-B) TOG1-5 rescues the axon outgrowth parameters affected by XMAP215 KD. **A.** Phase contrast images of axons from control, XMAP215 KD and XMAP215 KD rescued by TOG1-5 neural explants. Red numbers are shown as an example of axons considered for number of axon per explants quantifications. Only the axons with a clear end or growth cone were considered. Red lines denote the axon length, with only axons starting from the spinal cord explants, that are clearly separated from other axons and with a clear end or growth cone, were considered. **B.** XMAP215 KD decreases the number of axons per explant and can be rescued by TOG1-5 mRNA. **C.** Representative images from control, XMAP215 KD and XMAP215 KD rescued by TOG1-5 neural explants. Explants expressing LifeAct (F-actin marker). Grey stripes correspond to repellent EphrinA5-coated surface. Measurements taken of axons that were present and crossed onto repellent ephrin stripes per explant.

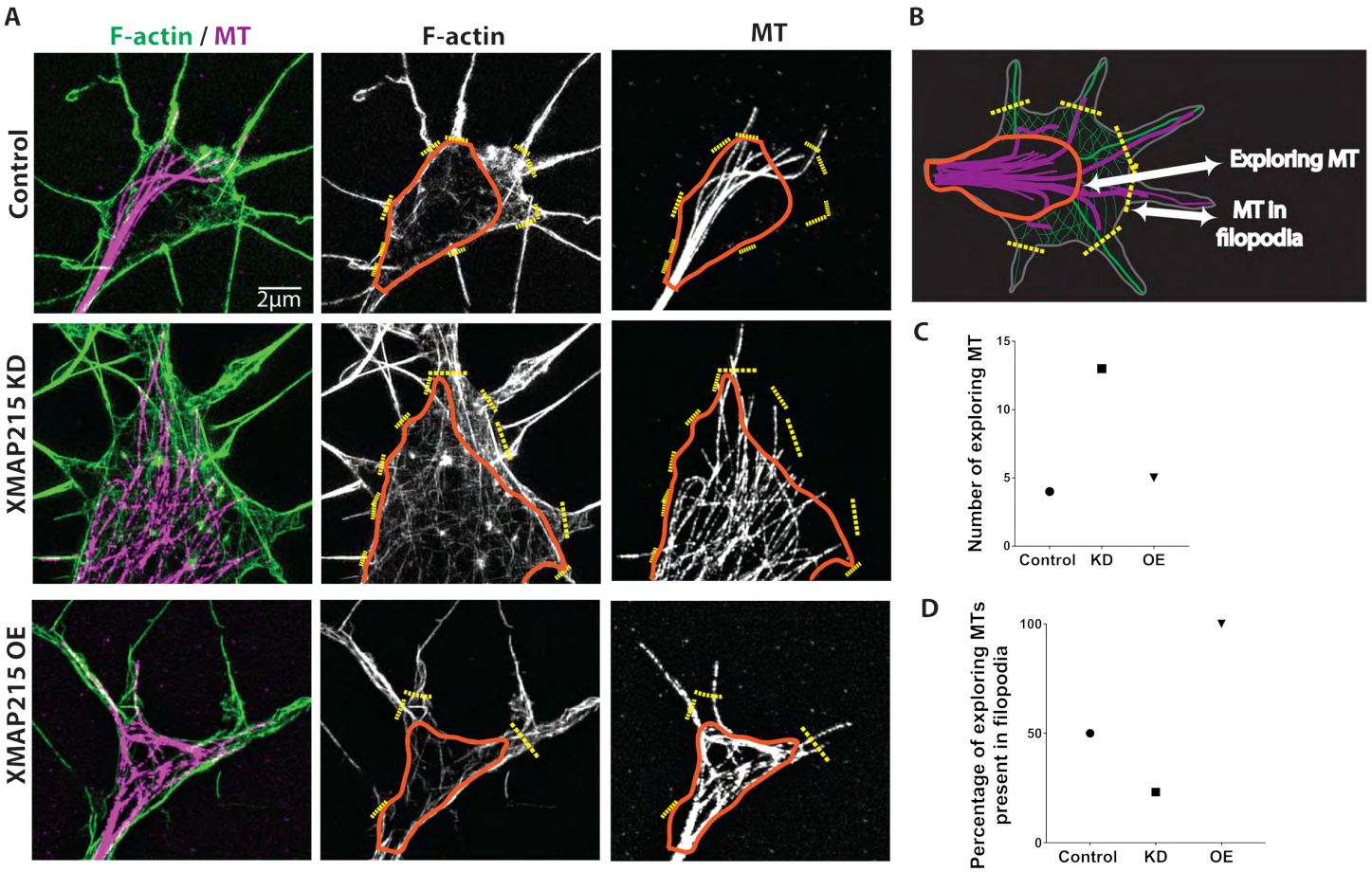


Figure S3

Figure S3. Example of the measurements taken of MT and actin-stained SIM imaging, corresponding to data displayed in Figure 3. A. Representative images of control, XMAP215 KD and XMAP215 OE growth cone stained for F-actin, MT and merge. Orange lines delineate the central domain, the beginning of the actin mesh is considered as the limit of the central domain, and the yellow dotted lines denotes the beginning of the filopodia. **B.** Growth cone cartoon showing that MTs that progress out of the central domain (orange line) are considered "exploring MTs", and the ones progressing beyond the doted yellow lines are considered "MTs present in the filopodia". **C-D.** Quantification of the number of exploring MTs (C), and percentage of exploring MTs present in filopodia (D) of the example in A.

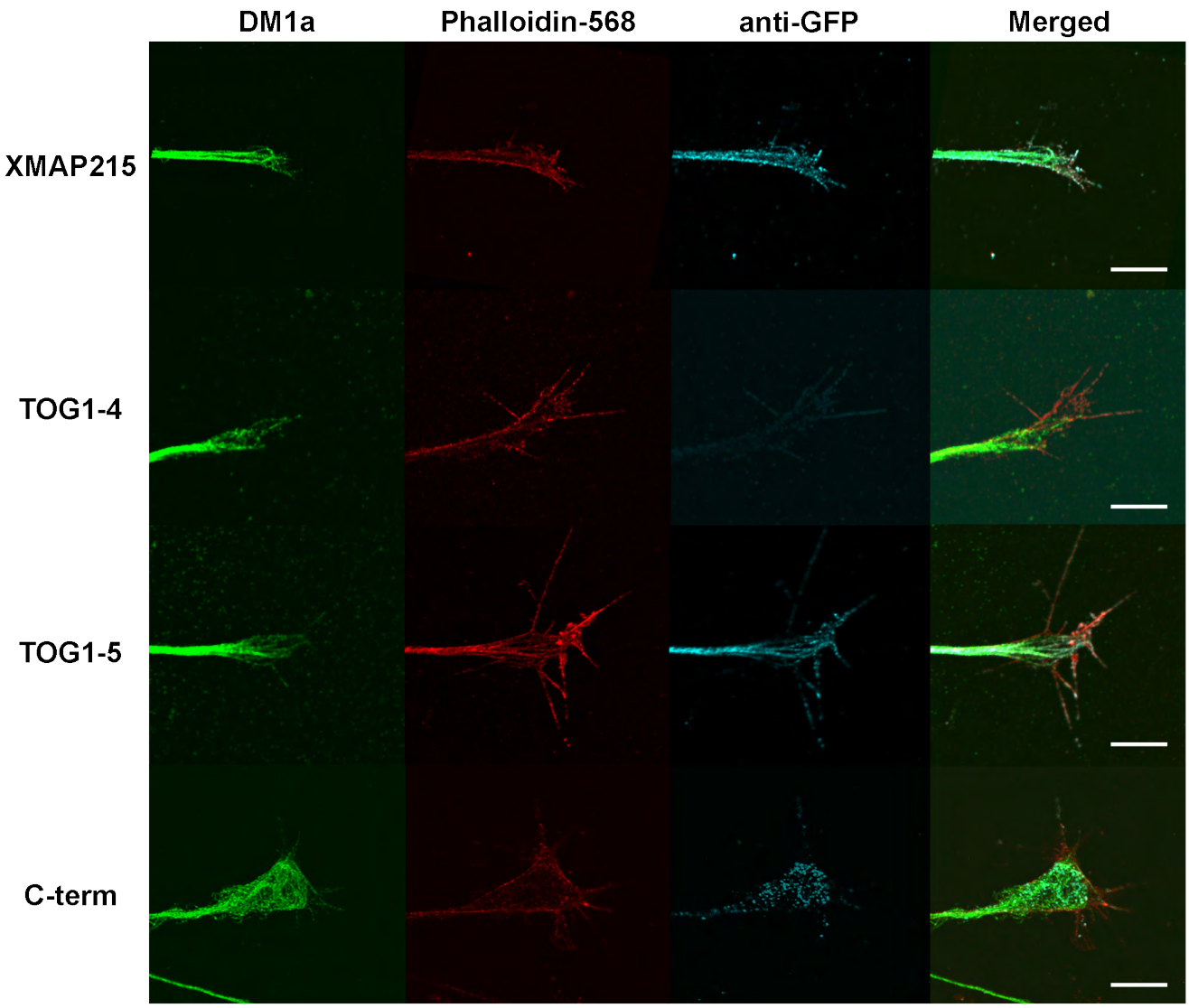
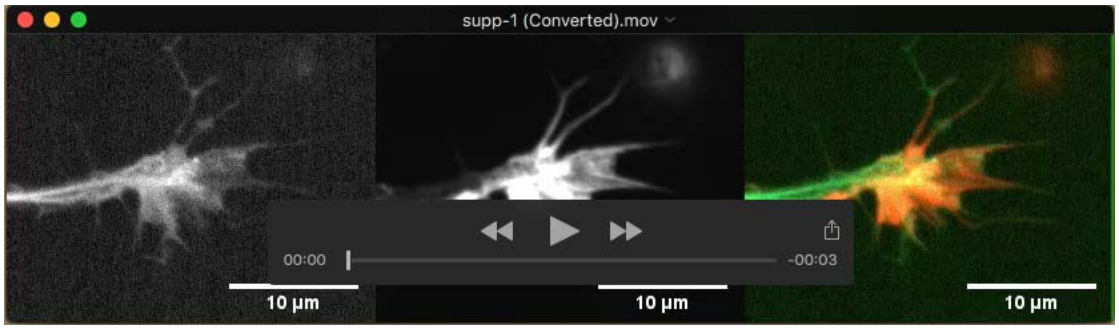
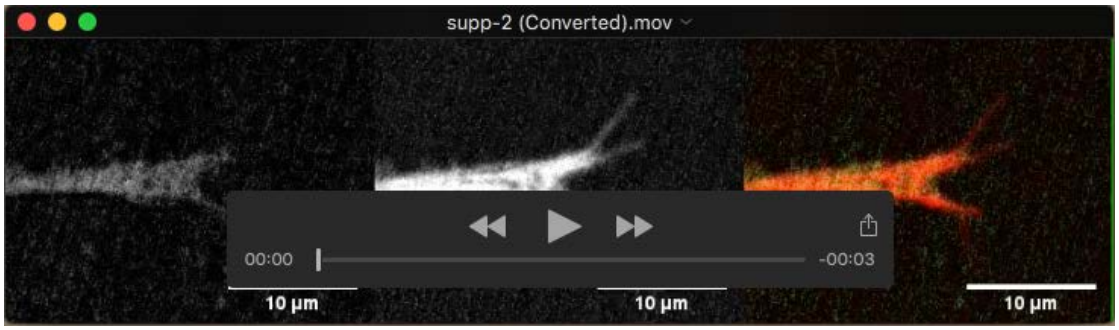


Figure S4

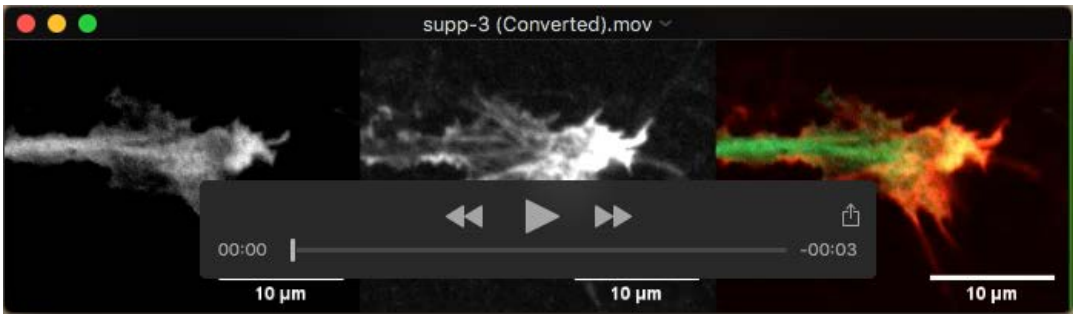
Figure S4. XMAP215 deletion constructs show distinct localizations with microtubules and F-actin in the growth cone. XMAP215 full-length or deletion mutants were expressed in *Xenopus* neural explants, fixed, and labelled with antibodies to GFP (labeling the XMAP215), microtubules, and actin. Representative images highlight microtubules (DM1a – green), Actin (phalloidin568 – red), XMAP215 deletion constructs (a-GFP - cyan), or a merged image of the three channels.



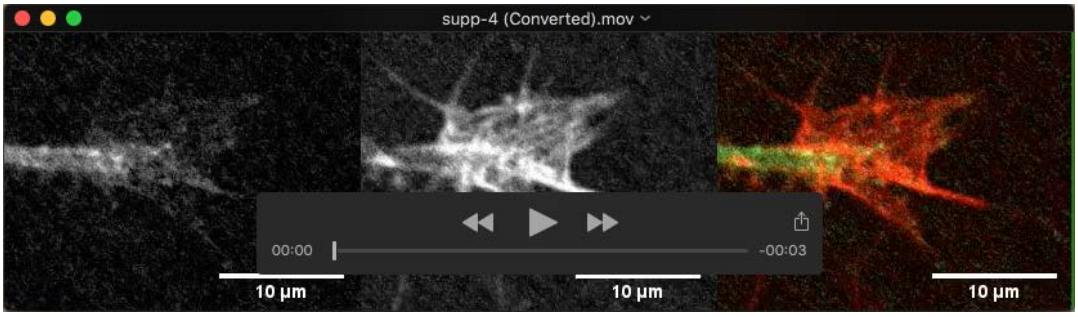
Movie 1. XMAP215-GFP-FL and mScarlet-Lifeact mRNAs were expressed in *Xenopus laevis* embryos. Representative confocal microscopy movie showing localization of GFP-tagged XMAP215 (green) and F-actin (red) in the growth cone.



Movie 2. TOG1-4-GFP and mScarlet-Lifeact mRNAs were expressed in *Xenopus laevis* embryos. Representative confocal microscopy movie showing localization of GFP-tagged XMAP215 TOG1-4 (green) and F-actin (red) in the growth cone.



Movie 3. TOG1-5-GFP and mScarlet-Lifeact mRNAs were expressed in *Xenopus laevis* embryos. Representative confocal microscopy movie showing localization of GFP-tagged XMAP215 TOG1-5 (green) and F-actin (red) in the growth cone.



Movie 4. XMAP215-Cterm-GFP and mScarlet-Lifeact mRNAs were expressed in *Xenopus laevis* embryos. Representative confocal microscopy movie showing localization of GFP-tagged XMAP215 C-terminus (green) and F-actin (red) in the growth cone.