**Key resources table**

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| **REAGENT or RESOURCE** | **SOURCE** | **IDENTIFIER** |
| **Antibodies** | | |
| rabbit anti-detyrosinated tubulin | EMD Millipore | Cat#: AB3201 |
| mouse anti-alpha tubulin | Sigma | Cat#: T-9026 |
| Tau R1 | provided by Dr. N. Kanaan | N/A |
| rabbit anti-ßIII-tubulin | BioLegend | Cat#: 802001 |
| mouse anti-acetylated tubulin | Sigma | Cat#: T6793 |
| rat anti-tyrosinated tubulin | EMD Millipore | Cat#: MAB1864 |
| mouse anti-GAPDH | Abcam | Cat#: ab8245 |
| goat anti-rabbit AlexaFluor-488 | Thermo Scientific | Cat#: A32731 |
| goat anti-mouse AlexaFluor-555 | Thermo Scientific | Cat#: A21422 |
| goat anti-rat AlexaFluor-647 | Thermo Scientific | Cat#: A21244 |
| IRDye 800CW Goat anti-Rabbit IgG | LI-COR | Cat#: 926-32211 |
| IRDye 680CW Goat anti-Mouse IgG | LI-COR | Cat#: 926-68070 |
| **Biological samples** |  |  |
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| **Chemicals, peptides, and recombinant proteins** | | |
| PFA | Electron Microscopy Sciences | Cat#: 19202 |
| Euthasol-III | VEDCO | Cat#: 50989056912 |
| Phosphatase inhibitor | Thermo Scientific | Cat#: A32957 |
| Protease inhibitor | Thermo Scientific | Cat#: A32953 |
| Nocodazole |  |  |
| Sucrose | Fisher Bioreagents | Cat#: BP220-1 |
| M-1 Shandon embedding matrix | Epredia | Cat#: 1310 |
| DAPI | Thermo Scientific | Cat#: D1306 |
| RIPA lysis buffer | Thermo Scientific | Cat#: 89901 |
| Lipofectamine 2000 | Thermo Scientific | Cat#: 11668019 |
| TBS (10X) | Bio-Rad | Cat#: 1706435 |
| 6X protein sample loading buffer | Boston BioProducts | Cat#: BP-111R-25 |
| Intercept TBS blocking buffer | LI-COr | Cat#: 927-60001 |
| Tris/Glycine/SDS (TGS) Buffer (10X) | Bio-Rad | Cat#: 1610772 |
| Triton X-100 | Sigma-Aldrich | Cat#: X100 |
| DMSO | Sigma-Aldrich | Cat#: D5879-100ML |
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| **Critical commercial assays** | | |
| BCA Protein Assay Kit | Thermo Scientific | Cat#: 23228 |
| **Deposited data** | | |
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| **Experimental models: Cell lines** | | |
| RFL-6 | ATCC | CCL-192 |
| **Experimental models: Organisms/strains** | | |
| Mouse - C57BL/6J | The Jackson Laboratory | Strain #:000664,  RRID: IMSR\_JAX:000664 |
| **Oligonucleotides** | | |
| MAP6\_N\_delStop\_F:  5'-ATTGAGAGCTCCCCTCACTCACCCCTTGAC-3' | IDT | N/A |
| MAP6\_N\_delStop\_R:  5'-GTCAAGGGGTGAGTGAGGGGAGCTCTCAAT-3' | IDT | N/A |
| Tau ASO | Ionis Pharmaceuticals | N/A |
| Control ASO | Ionis Pharmaceuticals | N/A |
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| **Recombinant DNA** | | |
| human alpha tubulin pEGFP-Tub | ClonTech |  |
| mCherry-3R\_Tau | provided by Dr. I. Gozes | N/A |
| mCherry-4R\_Tau | provided by Dr. I. Gozes | N/A |
| eMAP6-EGFP | provided by Dr. A. Andrieux | N/A |
| nMAP6-EGFP | provided by Dr. A. Andrieux | N/A |
| **Software and algorithms** | | |
| ImageJ | ImageJ | https://imagej.net/ |
| Diffraction PSF 3D | ImageJ | https://imagej.net/plugins/diffraction-psf-3d |
| Parallel Spectral Deconvolution | ImageJ | https://imagej.net/plugins/parallel-spectral-deconvolution |
| create\_spectrum.jru v1 | ImageJ | https://research.stowers.org/imagejplugins/Jay\_Plugins.jar |
| combine\_all\_trajectories.jru v1 | ImageJ | https://research.stowers.org/imagejplugins/Jay\_Plugins.jar |
| normalize\_trajectories.jru v1 | ImageJ | https://research.stowers.org/imagejplugins/Jay\_Plugins.jar |
| batch\_FRAP\_fit.jru v1 | ImageJ | https://research.stowers.org/imagejplugins/Jay\_Plugins.jar |
| average\_trajectories.jru v1 | ImageJ | https://research.stowers.org/imagejplugins/Jay\_Plugins.jar |
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| **Other** | | |
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