

Figure S1. GFP-tagging of Trs85 does not impair growth.

Colonies of the indicated genotypes were cultured on synthetic complete medium for 3 days at 37°C

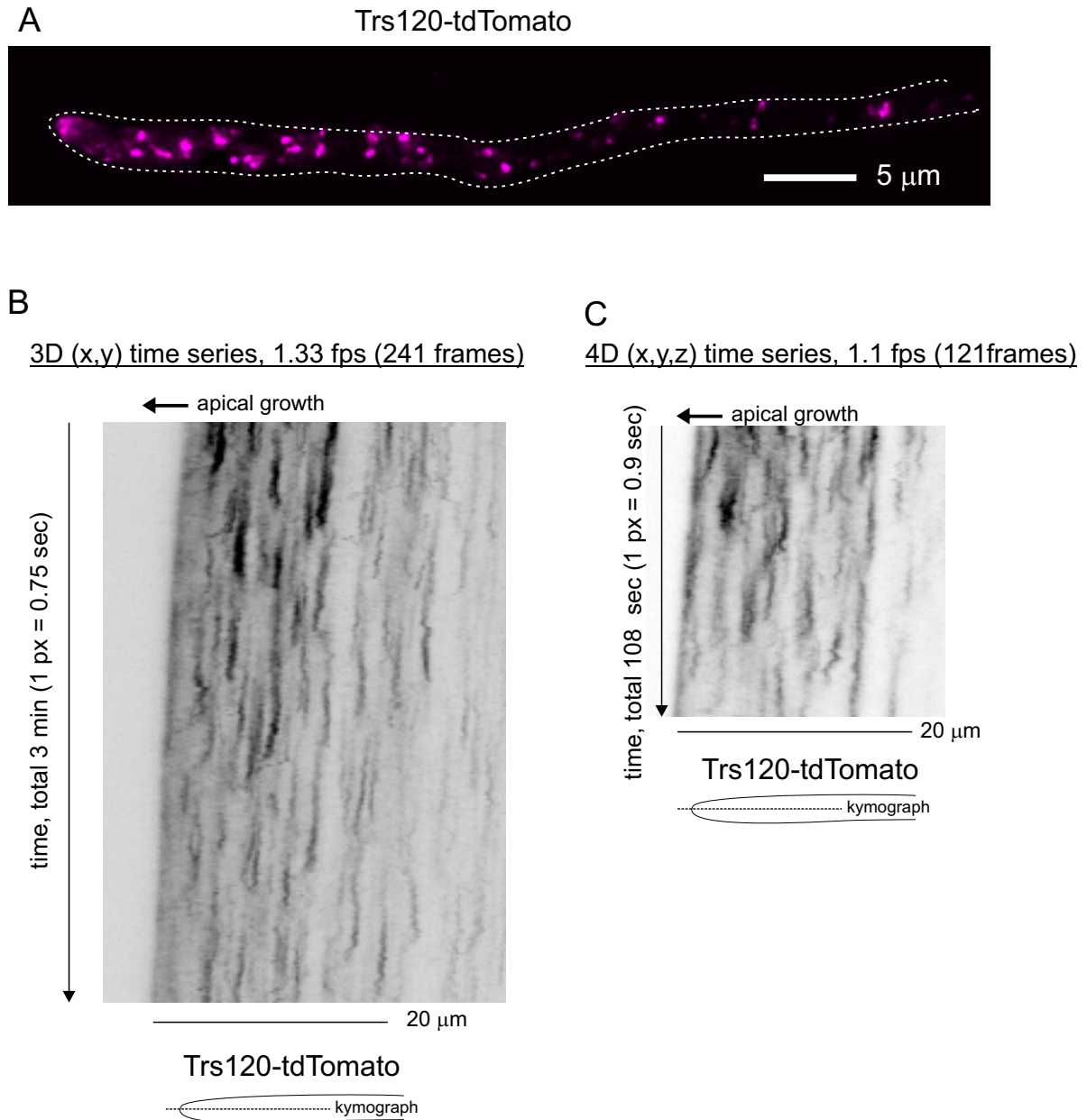


Figure S2. Transient recruitment of TRAPP^{II} to TGN cisternae as determined with Trs120-tdT

(A) Maximal intensity projection of a deconvolved Z-stack of a cell expressing Trs120-tdT. (B)(C) Kymographs derived from 3D and 4D movies illustrating that Trs120-tdT behaves as Trs120-GFPX3 over time. The 3D movie in (B) is 3 min- and 241 frames-long and was acquired with 2x2 binning.

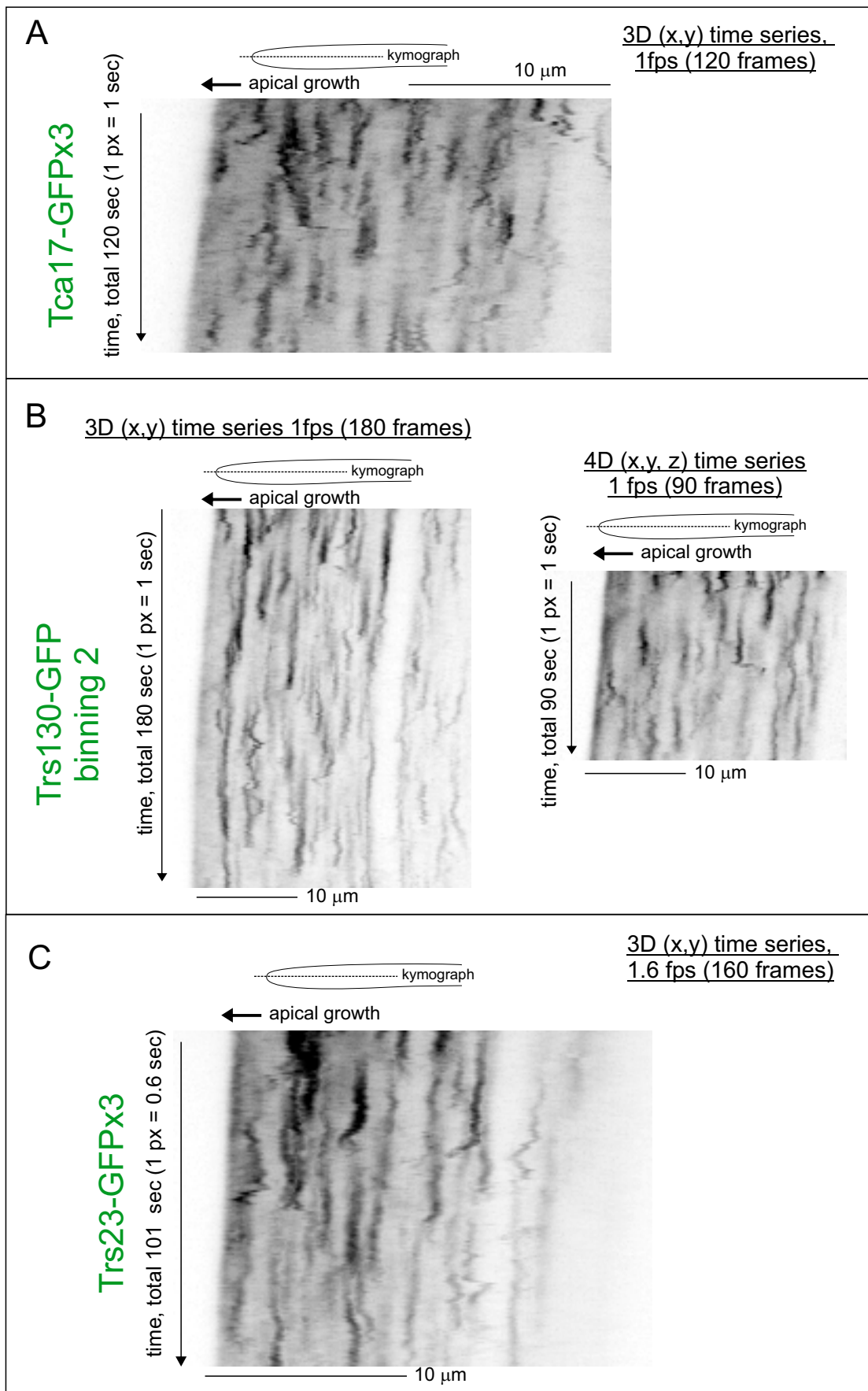


Figure S3. Transient recruitment of other TRAPP subunits to TGN cisternae 3D or 4D movies, as indicated, of strains expressing: (A) Tca17-GFPx3. (B) Trs130-GFP. (C) Trs23-GFPx3. Acquired with the specified conditions.

Endogenously tagged Bet3 fusion proteins

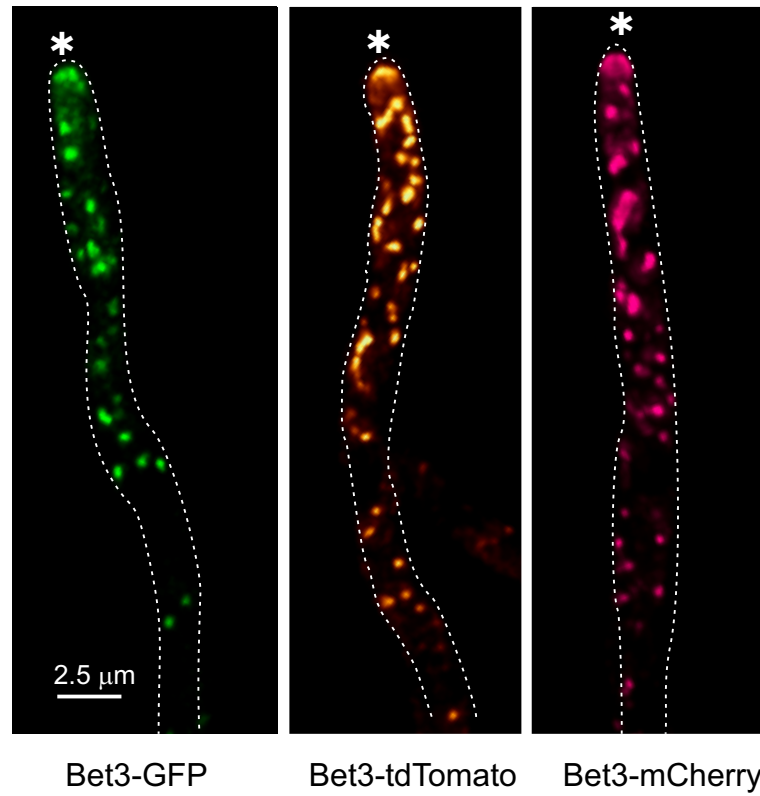


Figure S4. Bet3 localization with different fluorescent protein tags
Images are maximal intensity projections of deconvolved Z-stacks. Asterisks indicate the SPKs

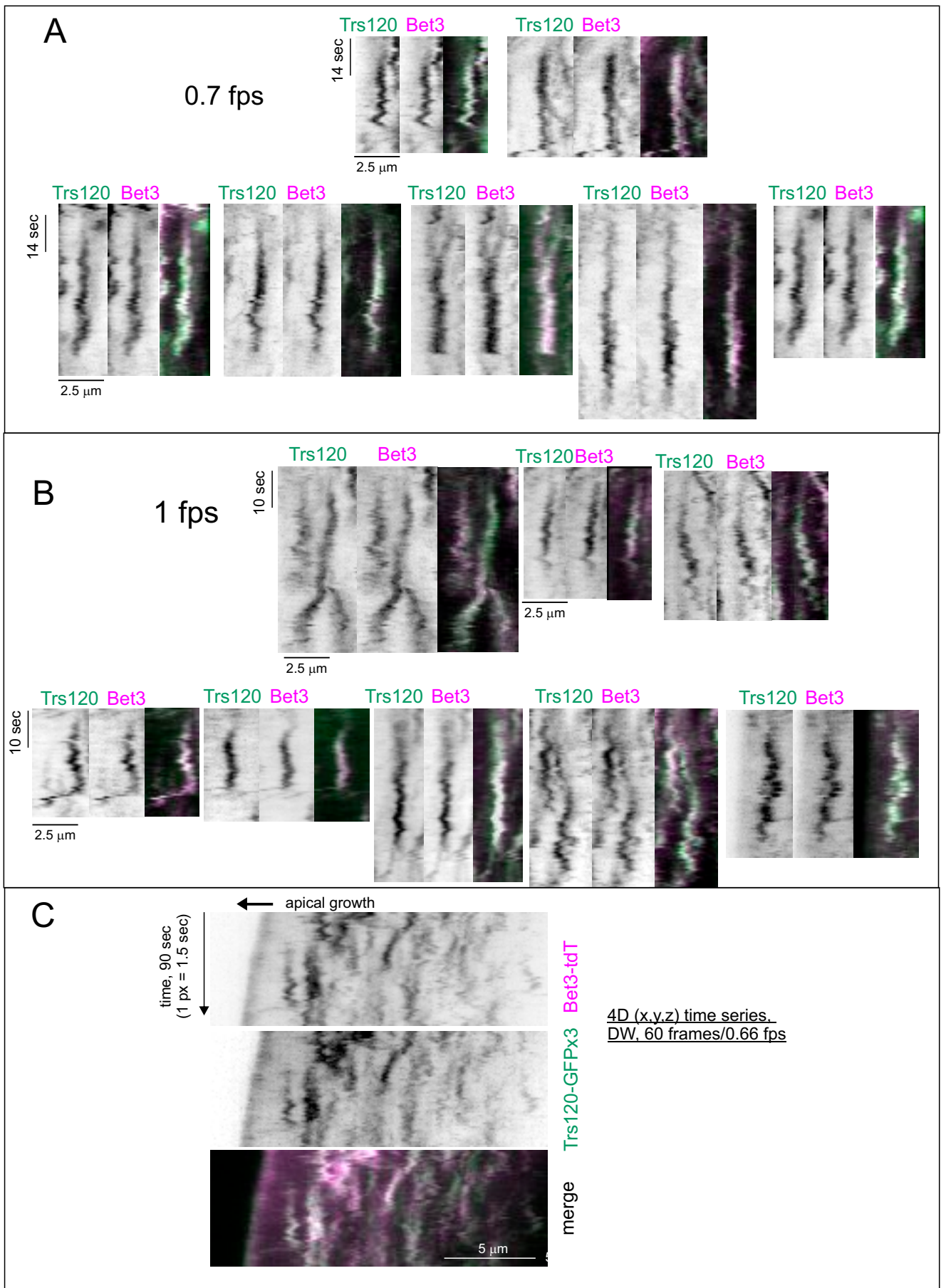


Figure S5. Trs120 and Bet3 strictly colocalize continuously on TGN cisternae. (A) Multiple examples showing that Trs120-GFPx3 and Bet3-tdT simultaneously accumulate at, and dissipate from maturing TGN cisternae. Kymographs from which these examples were extracted were obtained from 3D movies of the two channels simultaneously acquired with a beam splitter at a Tr of 0.7 fps. (B) As above, but using 3D movies with a Tr of 1 fps. (C) Overall view of a kymograph obtained from a 4D movie acquired with a beam splitter at a Tr of 0.66 fps. Note the clear example of the maturing cisterna closest to the SPK.

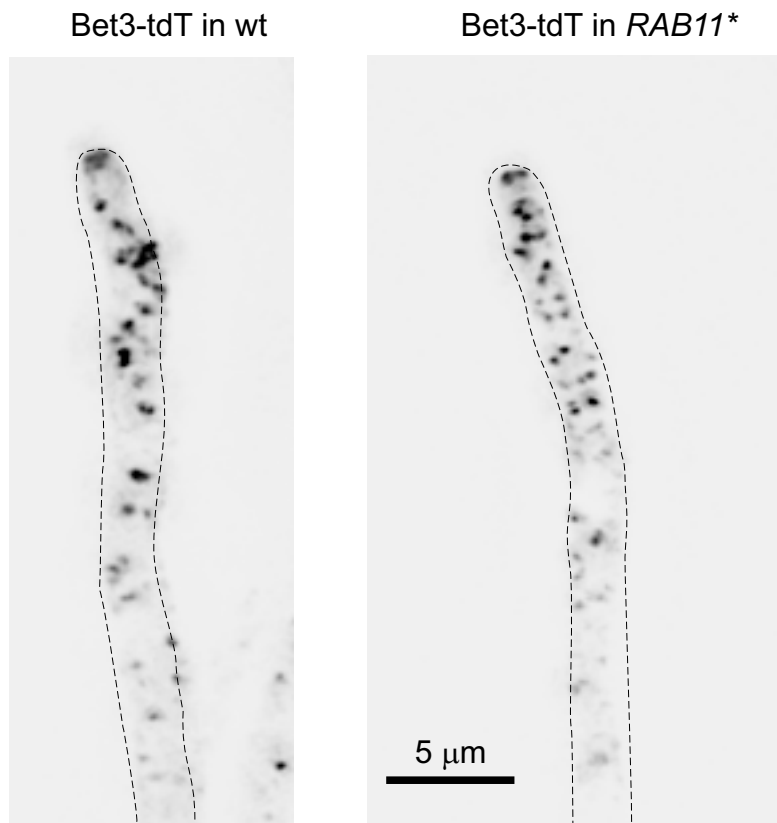
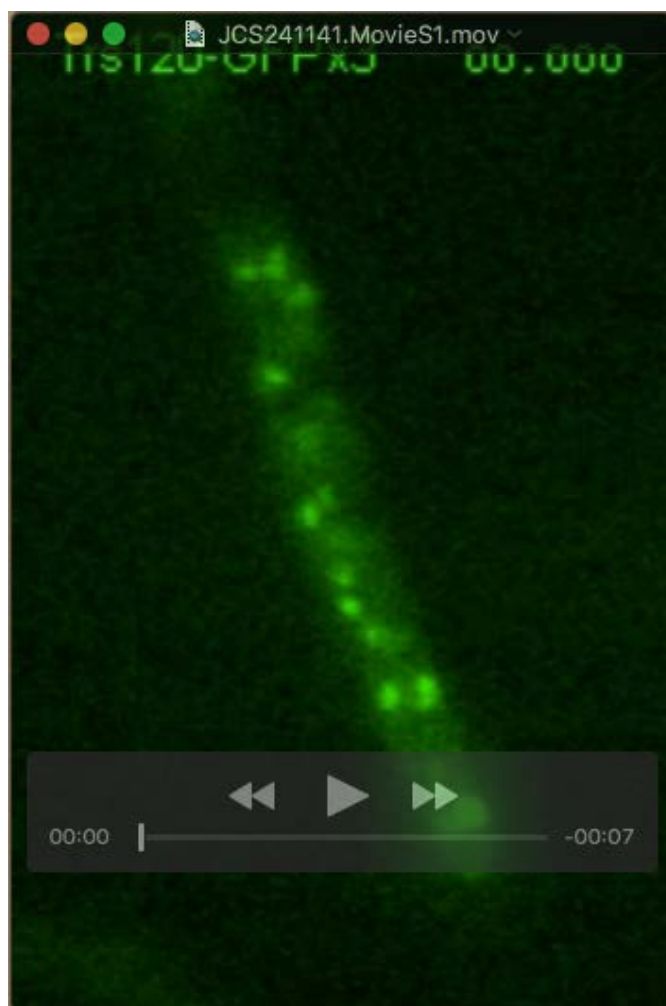


Figure S6. Bet3-tdT localization to the TGN is not altered by RAB11*. Images are maximal intensity projections of deconvolved z-stacks.

Table S1: Strains used in this work

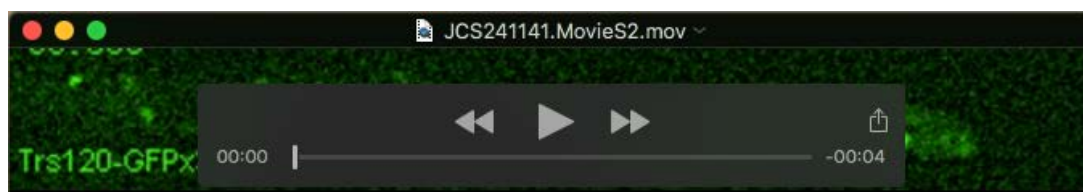
| Strain code | Genotype |
|-------------|---|
| MAD3921 | <i>trs120::gfp::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD4397 | <i>pyrG89; trs85::gfp::pyrG^{Af} pyroA4 nkuAΔ::bar</i> |
| MAD5319 | <i>pyrG89; pyroA4 nkuAΔ::bar; riboB2</i> |
| MAD5321 | <i>pyroA4 nkuAΔ::bar; riboB2</i> |
| MAD5736 | <i>pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD5743 | <i>pyrG89 sec7::gfp::pyrG^{Af} pyroA4 nkuAΔ::bar</i> |
| MAD6305 | <i>pyrG89; pyroA4 nkuAΔ::bar; bet5::gfp::pyrG^{Af}</i> |
| MAD6317 | <i>pyrG89; bet5::mcherry::pyrG^{Af} pyroA4 nkuAΔ::bar</i> |
| MAD6381 | <i>pyrG89; pyroA4 nkuAΔ::bar; bet3::gfp::pyrG^{Af}</i> |
| MAD6420 | <i>trs20::gfp::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD6439 | <i>pyrG89; trs23::gfp::pyrG^{Af} pyroA4 nkuAΔ::bar</i> |
| MAD6443 | <i>trs31::gfp::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD6461 | <i>pyrG89?; trs85Δ::pyrG^{Af} nkuAΔ::bar?; riboB2</i> |
| MAD6610 | <i>trs33::gfp::pyrG^{Af} pyrG89 pabaA1; nkuAΔ::bar</i> |
| MAD6635 | <i>[rab11^P::rabE::rab11^P::mcherry::rab11::pyrG^{Af}] pyrG89 pabaA1; nkuAΔ::bar</i> |
| MAD6639 | <i>pyrG89 pabaA1; nkuAΔ::bar; bet5-3x-gfp::pyrG^{Af}</i> |
| MAD6648 | <i>trs31::3x-gfp::pyrG^{Af} pabaA1 pyrG89; nkuAΔ::bar</i> |
| MAD6672 | <i>trs120::3x-gfp::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD6726 | <i>pabaA1 pyrG89; trs23::3x-gfp::pyrG^{Af} nkuAΔ::bar</i> |
| MAD6760 | <i>trs120::3x-gfp::pyrG^{Af} pyrG89; nkuAΔ::bar; [rab11^P::rab11::rab11^P::mcherry::rab11::pyrG^{Af}]</i> |
| MAD6780 | <i>pabaA1 pyrG89; tca17::gfp::pyrG^{Af}; nkuAΔ::bar</i> |
| MAD6783 | <i>pabaA1 pyrG89; nkuAΔ::bar; bet3::mcherry::pyrG^{Af}</i> |
| MAD6807 | <i>trs120::tdTomato::pyrG^{Af} pabaA1 pyrG89; nkuAΔ::bar</i> |
| MAD6826 | <i>pabaA1 pyrG89; nkuAΔ::bar; bet3::tdTomato::pyrG^{Af}</i> |
| MAD6835 | <i>pyrG89; pyroA4 nkuAΔ::bar; trs65::gfp::pyrG^{Af}</i> |
| MAD6837 | <i>pyrG89; pyroA4 nkuAΔ::bar; trs130::gfp::pyrG^{Af}</i> |
| MAD6865 | <i>pyrG89; tca17::3x-gfp::pyrG^{Af}; pyroA4 nkuAΔ::bar</i> |
| MAD6867 | <i>trs120::3x-gfp::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar; bet3::tdTomato::pyrG^{Af}</i> |
| MAD6971 | <i>trs120Δ::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar; bet3::tdTomato::pyrG^{Af}; rab11^{D125E}</i> |
| MAD6973 | <i>trs120Δ::pyrG^{Af} pyrG89; trs23::3x-gfp::pyrG^{Af} pyroA4 nkuAΔ::bar; rab11^{D125E}</i> |
| MAD6982 | <i>sec7::gfp::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar; bet3::tdTomato::pyrG^{Af}</i> |
| MAD7025 | <i>pyrG89?; pyroA4::[pyroA[*]- gpd^{mini}::gfp::sedV] nkuAΔ::bar?; pantoB100; bet3::tdTomato::pyrG^{Af}</i> |
| MAD7027 | <i>trs120::tdTomato::pyrG^{Af} sec7::gfp::pyrG^{Af} pabaA1 pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD7042 | <i>sec7::tdTomato::pyrG^{Af} pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD7044 | <i>pyrG89; trs85::mcherry::pyrG^{Af} pyroA4 nkuAΔ::bar</i> |
| MAD7056 | <i>trs120::3x-gfp::pyrG^{Af} sec7::tdTomato::pyrG^{Af} pabaA1 pyrG89; pyroA4 nkuAΔ::bar</i> |
| MAD7067 | <i>pabaA1 pyrG89; trs85::3x-gfp::pyrG^{Af} nkuAΔ::bar</i> |
| MAD7405 | <i>pyrG89 trs120Δ::pyrG^{Af}; trs85::3x-gfp::pyrG^{Af} pyroA4 nkuAΔ::bar; rab11^{D125E}</i> |
| MAD7416 | <i>pyrG89; nkuAΔ::bar; bet3::tdTomato::pyrG^{Af}; rab11^{D125E}</i> |

Supplemental movies



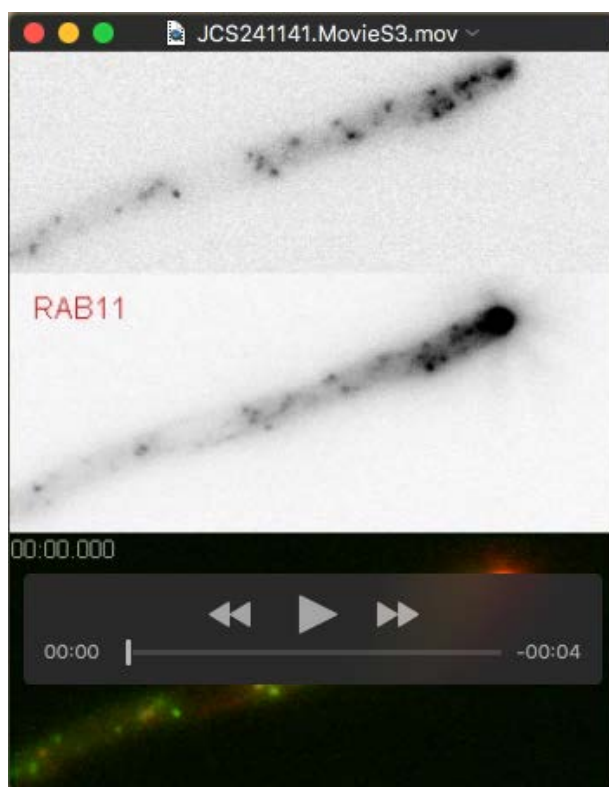
Movie 1: 3D movie of Trs120-GFPx3

Consisting of 210 middle planes acquired every 0.5 sec and reproduced at 30 fps. Time stamp in sec.msec.



Movie 2: 4D movie of Trs120-GFPx3.

Consisting of 120 frames; each frame is the maximal intensity projection of a 2 μ m-deep Z-stack acquired every 1.5 sec and reproduced at 30 fps. Time stamp in sec.msec.



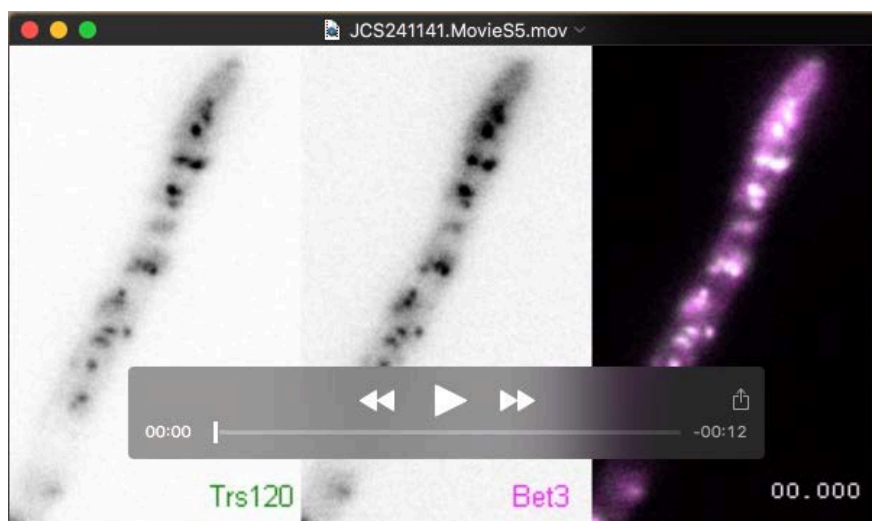
Movie 3: 4D movie of Trs120-GFPx3 and mCh-RAB11

Consisting of 62 frames; each frame is a maximal intensity projection of a Z-stack in which the two channels were simultaneously acquired with a beam splitter every 1.2 sec; the movie is reproduced at 15 fps. Time stamp in min:sec.msec.



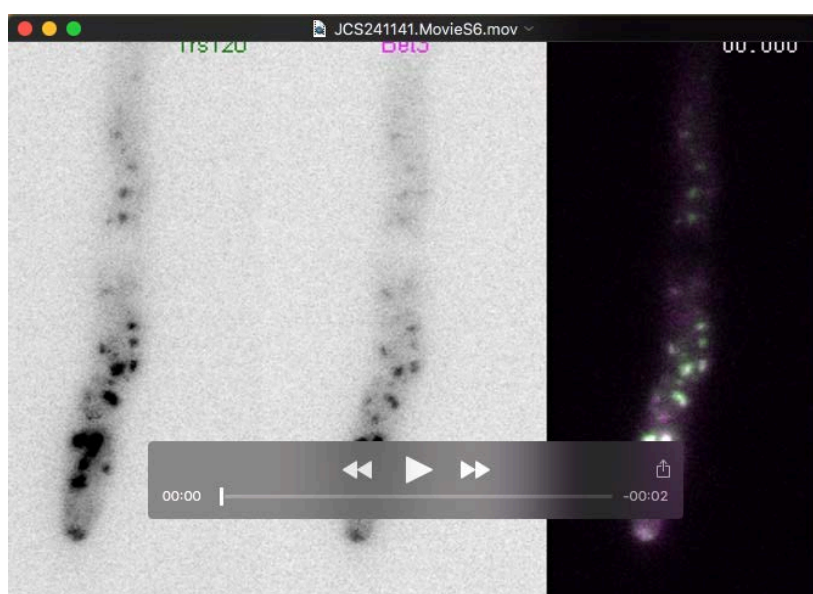
Movie 4: 4D movie of Bet3-tdTomato

Consisting of 120 frames; each frame is a maximal intensity projection of a Z-stack acquired every 1 sec. Reproduced at 15 fps. Time stamp in sec.msec.



Movie 5: 3D movie of Trs120-GFPx3 and Bet3-tdTomato

Consisting of 180 middle planes in which the two channels were simultaneously acquired with a beam splitter every 1 sec; the movie is reproduced at 30 fps. Time stamp in sec.msec.



Movie 6: 4D movie of Trs120-GFPx3 and Bet3-tdTomato

Consisting of 61 frames; each frame is a maximal intensity projection of a Z-stack. The GFP and tdT channels were simultaneously acquired every 1.5 sec. Reproduced at 30 fps. Time stamp in sec.msec.