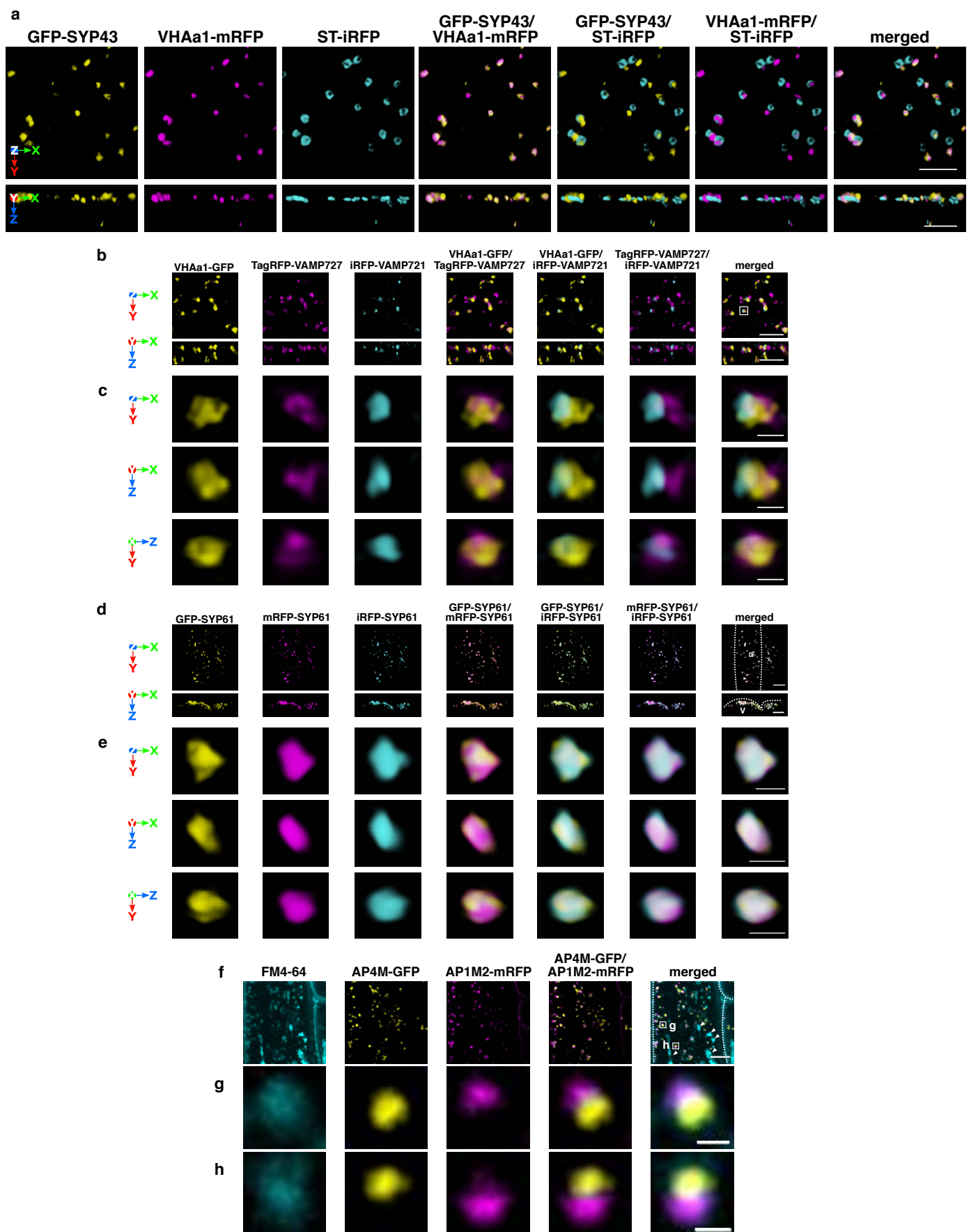


Supplementary Information

Cargo sorting zones in the *trans*-Golgi network visualized by super-resolution confocal live imaging microscopy in plants

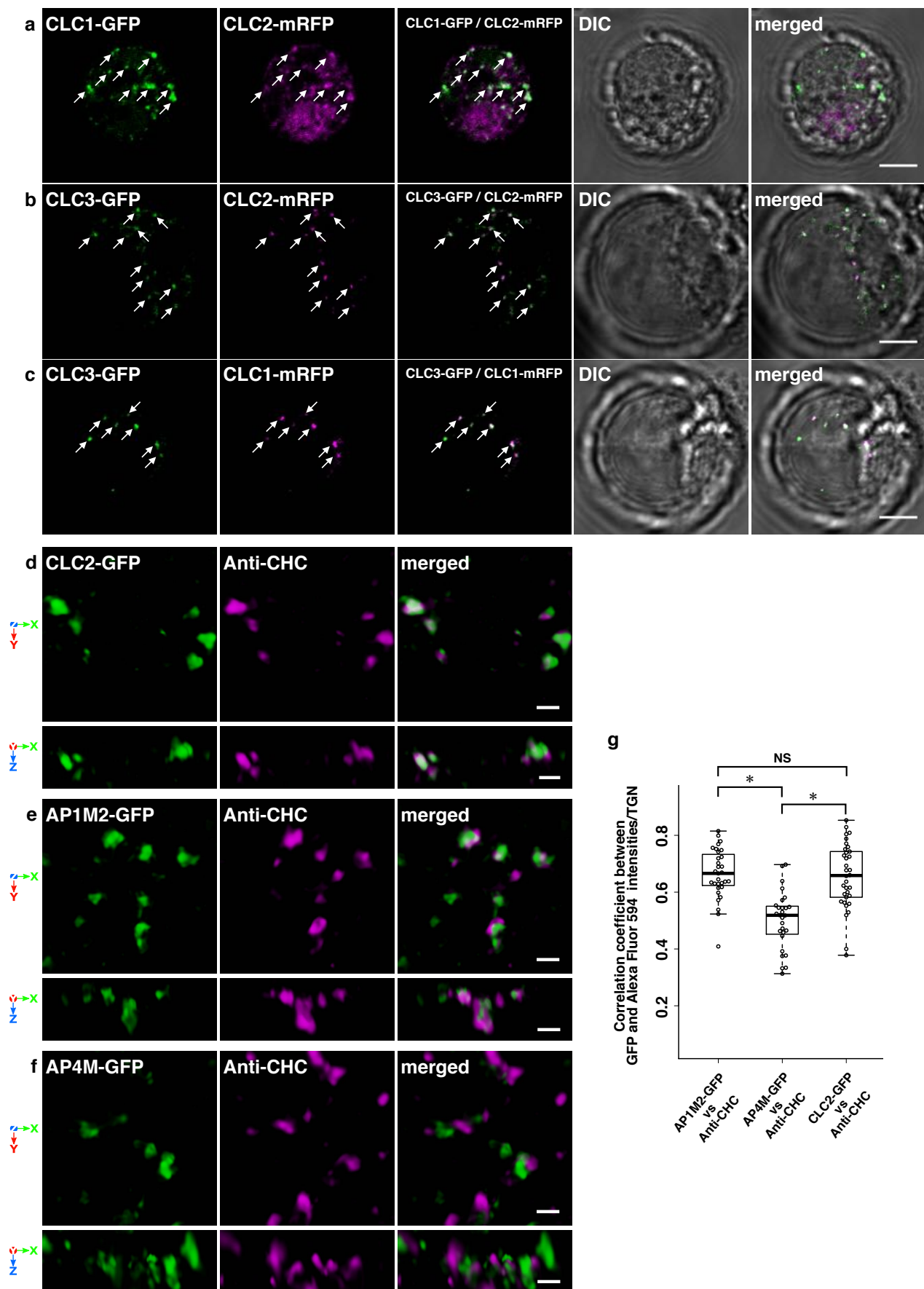
Yutaro Shimizu, Junpei Takagi, Emi Ito, Yoko Ito, Kazuo Ebine, Yamato Komatsu, Yumi Goto, Mayuko Sato, Kiminori Toyooka, Takashi Ueda, Kazuo Kurokawa, *Tomohiro Uemura, and *Akihiko Nakano.



Supplementary Fig. 1 Triple-color imaging of the TGN.

a–d 3-color SCLIM imaging of root epidermal cells in the elongation zone of Arabidopsis expressing GFP-SYP43 × VHAa1-mRFP × ST-iRFP (**a**), Arabidopsis expressing VHAa1-GFP × TagRFP-VAMP727 × iRFP-VAMP721 (**b, c**), or GFP-SYP61 × mRFP-SYP61 × iRFP-SYP61 (**d, e**). **a, b, d** 3D images. **c, e** Multi-angle magnified 3D images of the boxed area in **a** and **c**, respectively.

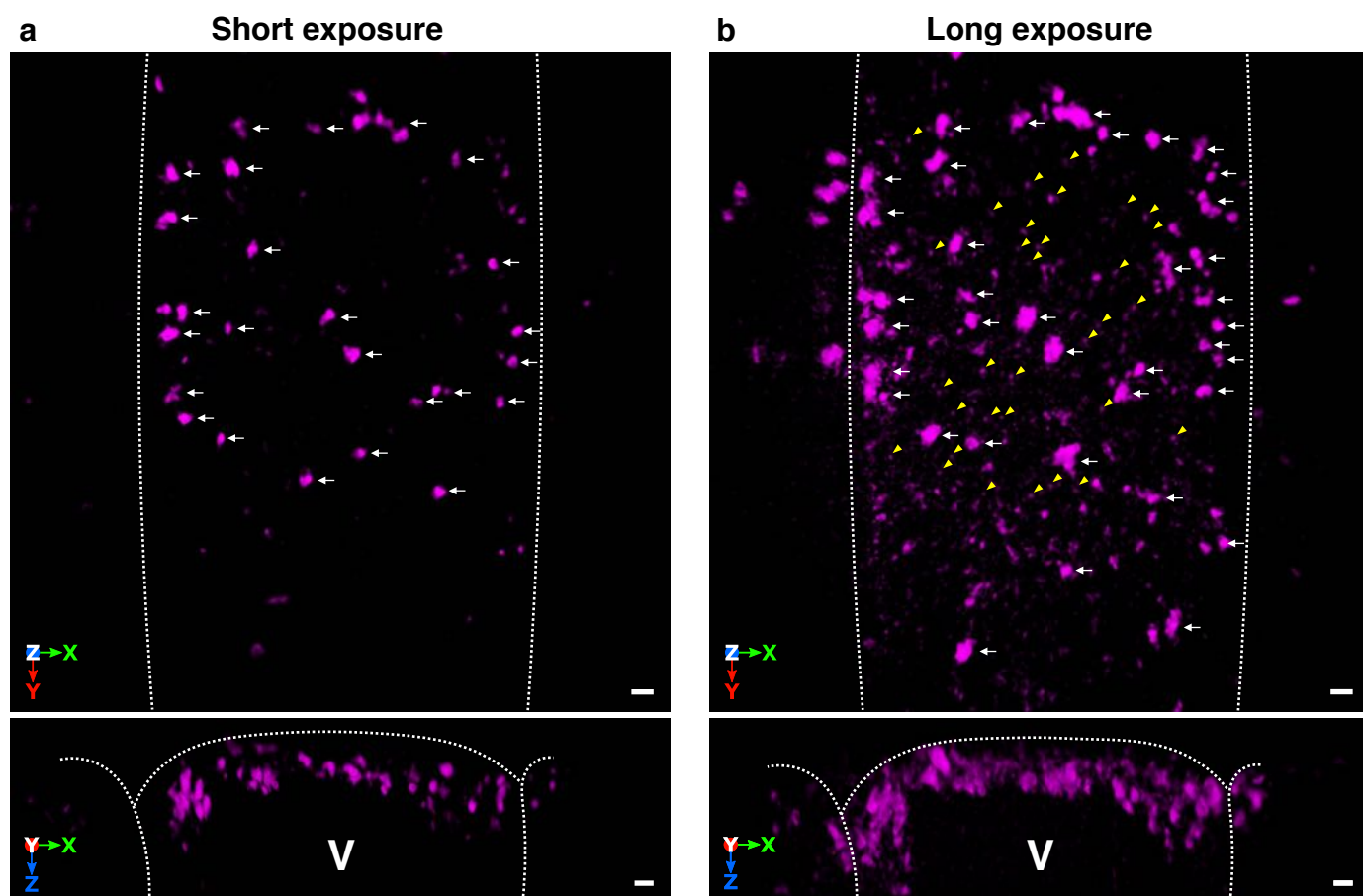
f–h Confocal images of FM4-64 × AP4M-GFP × AP1M2-mRFP under conventional CLSM with a high-resolution objective lens after 6 min of FM4-64 uptake. **f** Low-magnification images. **g, h** High-magnification images of the boxed area in **f**. Dashed lines indicate cell edges. Arrowheads show strong fluorescence signals of FM4-64 from the plasma membrane/cell wall. V, vacuole area. Scale bars = 5 μm (**a, b, d, f**); 1 μm (**c, e, g, h**). The experiments were repeated two times (**a–c, f–h**) and five times (**d, e**) with similar results, and micrographs from representative experiments are presented.



Supplementary Fig. 2 Subcellular localization of fluorescent protein-tagged CLCs or endogenous CHCs.

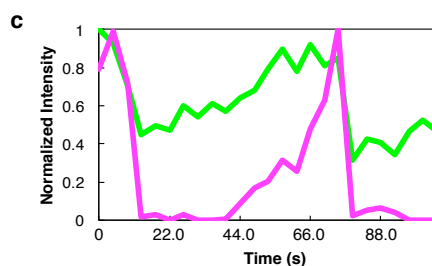
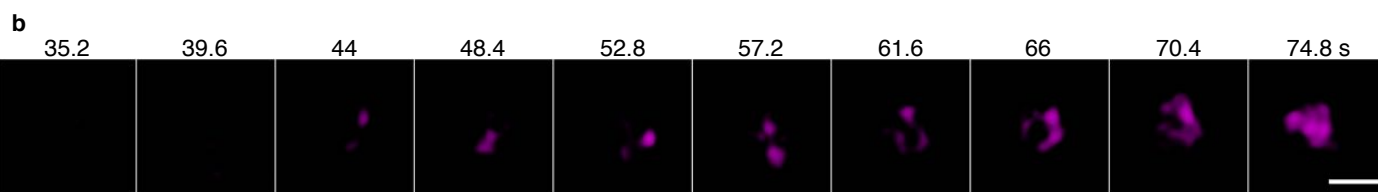
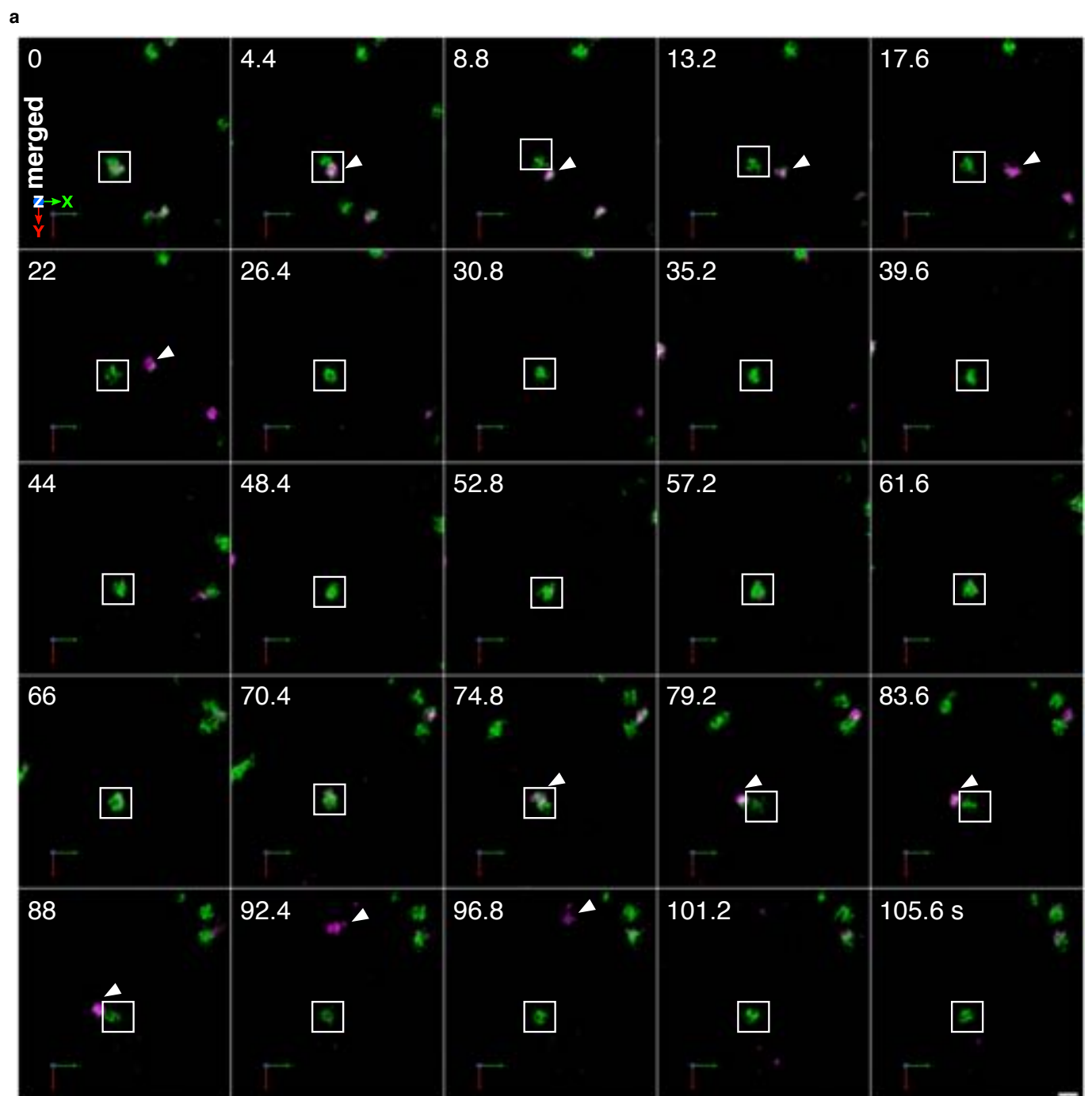
a–c Confocal images of CLC1-GFP and CLC2-mRFP (**a**), CLC3-GFP and CLC2-mRFP (**b**), or CLC3-GFP and CLC1-mRFP (**c**) transiently expressed in the protoplasts of Arabidopsis suspension cultured cells.

d–g 3D images of CLC2-GFP, AP1M2-GFP, and AP4M-GFP with anti-CHCs (Alexa 594) (**d**, **e**, and **f**, respectively) in the epidermal cells of the root elongation zone under SCLIM. **g** 3D colocalization analysis: $n = 31$, 28, and 34 TGNs for AP1M2-GFP vs Anti-CHC, AP4M-GFP vs Anti-CHC, and CLC2-GFP vs Anti-CHC, respectively, from 3 biological replicates. Two-sided Steel-Dwass test; $P = 8.3 \times 10^{-7}$ (Left: AP1M2-GFP $\times$ Anti-CHC versus AP4M-GFP $\times$ Anti-CHC), $P = 0.9$ (Top: AP1M2-GFP $\times$ Anti-CHC versus CLC2-GFP $\times$ Anti-CHC), and $P = 3.5 \times 10^{-6}$ (Right: AP4M-GFP $\times$ Anti-CHC versus CLC2-GFP $\times$ Anti-CHC); $*P < 0.01$, NS = nonsignificant. Boxes represent 25% and 75% quartiles, lines within the box represent the median, and whiskers represent the minimum and maximum values within 1.5x the interquartile range. Arrows indicate colocalized signals in endomembranes. Scale bars = 5 μm (**a–c**); 1 μm (**d–f**). The experiments were repeated three times with similar results, and micrographs from representative experiments are presented.



Supplementary Fig. 3 Fluorescent protein-tagged clathrin signal on the plasma membrane.

a, b Short exposure (**a**) and long exposure (**b**) 3D images of CLC2-mKO in the epidermal cell of the root elongation zone under SCLIM. Arrowheads indicate representative CLC2-mKO signals on the plasma membrane. Arrows indicate TGN-localized CLC2-mKO. Dashed lines show cell edges. V, vacuole area. Scale bars = 1 μm. The experiments were repeated five times with similar results, and micrographs from representative experiments are presented.



Supplementary Fig. 4 Accumulation and budding of clathrin in the TGN.

a 4D images of GFP-SYP61 and CLC2-mKO in the epidermal cell of the root elongation zone under SCLIM. Arrowheads indicate dissociation of the GI-TGN labeled with GFP-SYP61 and CLC2-mKO from the GA-TGN. **b** Magnified images of CLC2-mKO accumulation on the TGN shown in **a** (35.2 s – 74.8 s). Images are lined up every 4.4 s from left to right. Scale bars = 1 μ m. **c** Temporal fluorescence profile of boxed area in **a**. The experiment was repeated three times with similar results, and micrographs from representative experiments are presented.