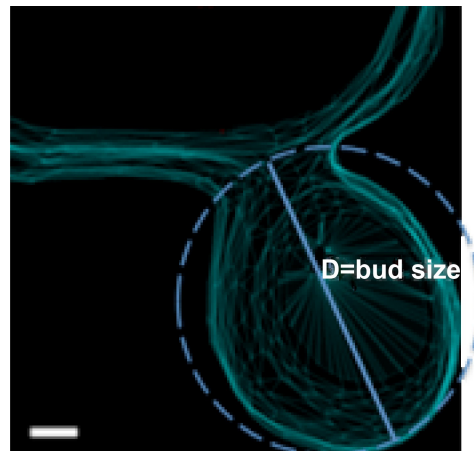Figure S1.   **Diagram to demonstrate how bud sizes were measured.** A sphere is fit from the tip of the bud to the base of the bud. The diameter of the sphere fit (D) equals the bud size. Bar, 200 nm.
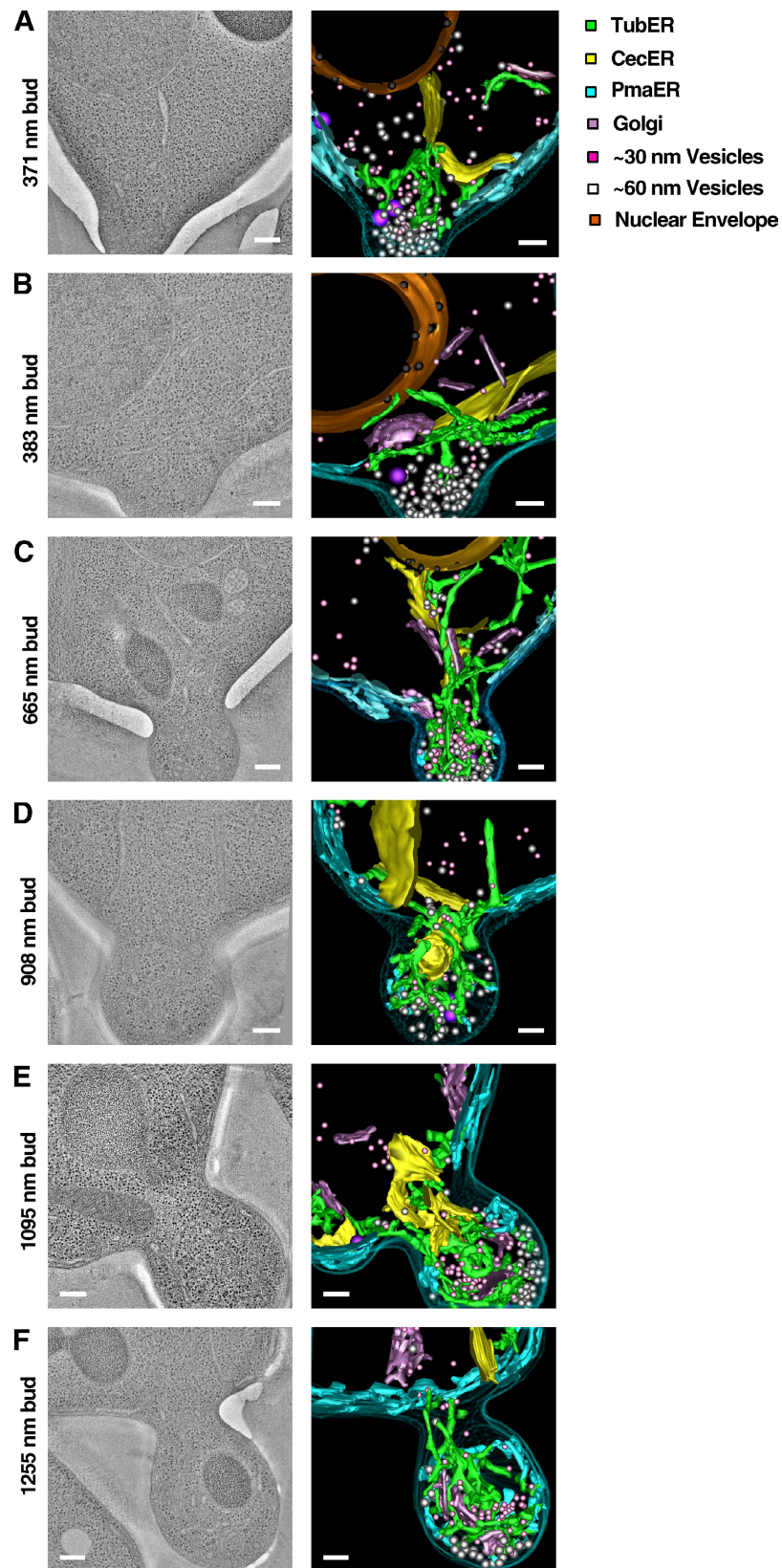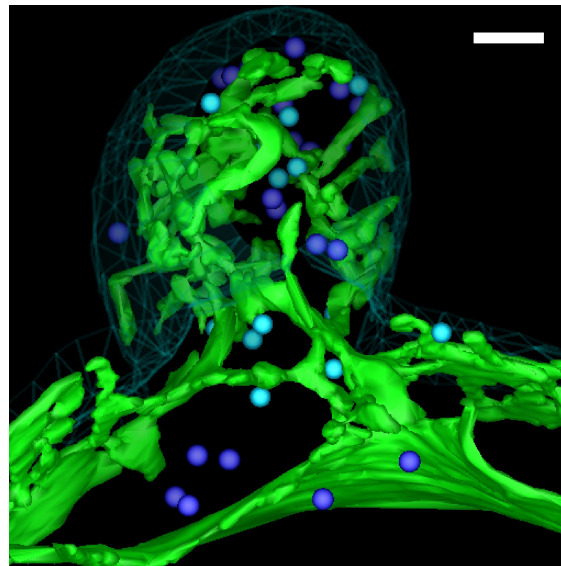
Figure S2.   ER, Golgi, and vesicle distribution during inheritance. (A–F) 2D tomographs derived from a 200-nm-thick section (left) and 3D models (right) derived from multiple serial sections show ER domain organization, Golgi, the NE, and vesicles in six wt budding yeast cells. The cells are ordered by increasing bud size. Bars, 200 nm.

■ **All ER domains**

■ **Vesicles that are not in close proximity to the ER**

■ **Vesicles that are contacting or within 10nm of the ER**

Figure S3. **Vesicles in close proximity or bound to the ER.** Modeled vesicles are shown in light blue that are within 10 nm of the ER membrane. Vesicles in purple are not in close proximity to the ER membrane. Bar, 200 nm.

A

Thickness of PmaER (n=162 pts)

mean = 30.3

B

Diameter of TubER (n=82 pts)
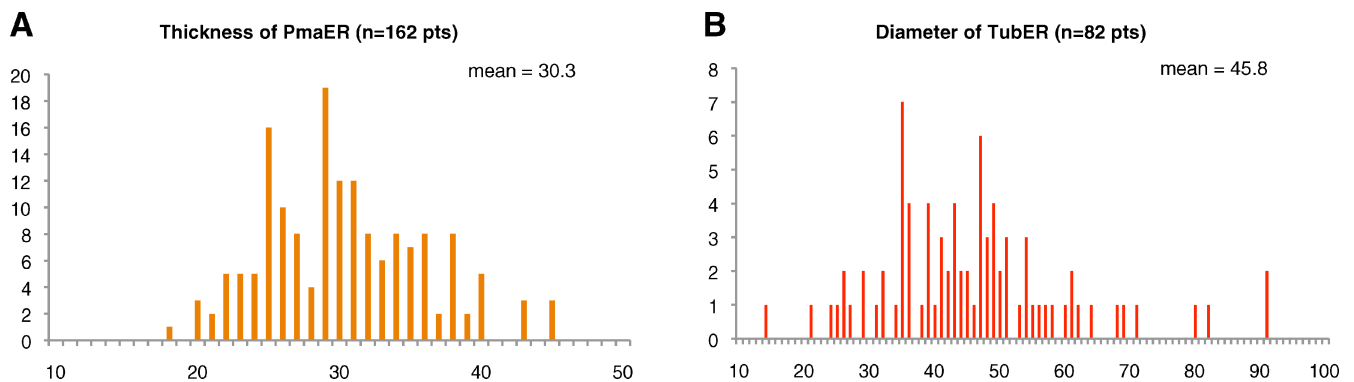
mean = 45.8

Figure S4. **Histogram of thickness/diameter measurements for mutant ER domains.** (A and B) A histogram showing the range and abundance of the thickness of the pmaER (n = 162 width measurements; A) and bud tubER diameters (n = 82 diameter measurements; B) in the mutant (mutant = $\Delta rtn1rtn2yop1$).
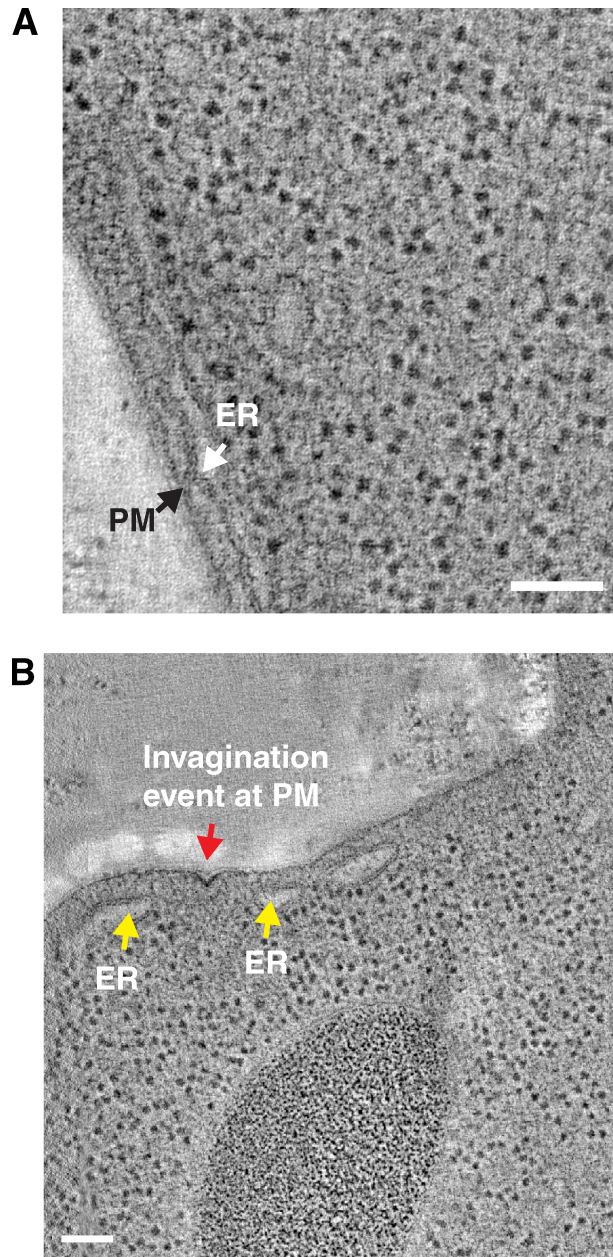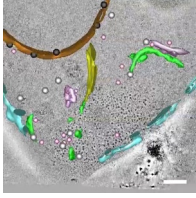
Figure S5. **Images of the PM and pmaER domains.** (A and B) A 2D image derived from a 200-nm-thick section that demonstrates a contact site between the PM and pmaER (white arrow) membrane bilayers (A) and an invagination event at the PM (red arrow) that is occurring at a region away from pmaER–PM contact (B). (B) The pmaER is marked by yellow arrows. Bars, 200 nm.
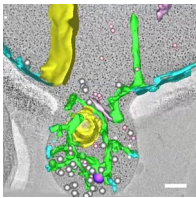
Video 1. **ER domains in 3D in a wt cell with a 371-nm bud (related to Fig. 2).** 3D reconstruction of a wt cell with a 371-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains are color coded as in Fig. 2. Bar, 200 nm.
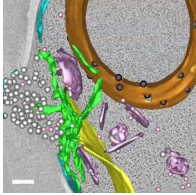
Video 2. **ER domains in 3D in a wt cell with a 383-nm bud (related to Fig. 2).** 3D reconstruction of a wt cell with a 383-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains are color coded as in Fig. 2. Bar, 200 nm.
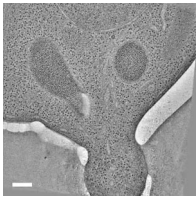
Video 3. **ER domains in 3D in a wt cell with a 665-nm bud (related to Fig. 2).** 3D reconstruction of a wt cell with a 665-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains color coded as in Fig. 2. Bar, 200 nm.
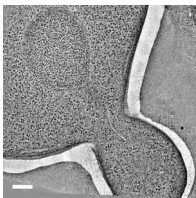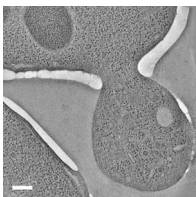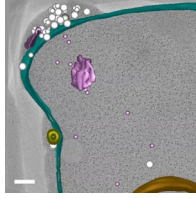

Video 4. **ER domains in 3D in a wt cell with a 908-nm bud (related to Fig. 2).** 3D reconstruction of a wt cell with a 908-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains are color coded as in Fig. 2. Bar, 200 nm.

Video 5. **ER domains in 3D in a wt cell with a 1,095-nm bud (related to Fig. 2).** 3D reconstruction of a wt cell with a 1,095-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains are color coded as in Fig. 2. Bar, 200 nm.

Video 6. **ER domains in 3D in a wt cell with a 1,255-nm bud (related to Fig. 2).** 3D reconstruction of a wt cell with a 1,255-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains are color coded as in Fig. 2. Bar, 200 nm.
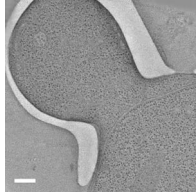
Video 7. **ER domains in 3D in a mutant cell with a 596-nm bud (related to Fig. 4).** 3D reconstruction of mutant cell with a 596-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains are color coded as in Fig. 4. Bar, 200 nm.

Video 8. **ER domains in 3D in a mutant cell with a 1,253-nm bud (related to Fig. 4).** 3D reconstruction of mutant cell with a 1,253-nm bud shows all ER domains, Golgi (purple), and vesicles (30 nm is shown in pink, and 60 nm is shown in white). Domains are color coded as in Fig. 4. Bar, 200 nm.