Supplementary Figure Legends

Supplemental Figure S1. Giant vacuoles with I-pores: percentages with a single I-pore or multiple I-pores

Overall, of the total 477 giant vacuoles (GVs) observed with at least one I-pore, 397 (83.2%) had a single I-pore, and 80 (16.8%) had more than one I-pore. 13.2% had two I-pores, 2.7% had three I-pores, 0.4% had 4 I-pores, and only 2 GVs of the 477 GVs observed with I-pores had 6 I-pores (0.4%). The proportions of GVs with a single I-pore or multiple I-pores were similar when analyzed by flow-type area; however, the range in non-flow area was 1-3 I-pores/GV, whereas high- and low-flow had ranges of 1-6 I-pores/GV.

Supplemental Figure S2. Surface area and maximal cross-sectional area of 180 3D-reconstructed giant vacuoles

A: Median surface area of reconstructed giant vacuoles (GVs) with I-pores (35.62 μm^2, IQR: 79.27 – 205.11) was significantly larger than GVs without I-pores (78.16 μm^2, IQR: 32.99 – 169.02, $P \leq 0.01$). **B:** Median surface area of reconstructed GVs with multiple I-pores (201.82 μm^2, IQR: 134.92 – 317.01) was significantly larger than GVs with a single I-pore (93.90 μm^2, IQR: 66.60 – 167.61; $P \leq 0.01$). **C:** Median surface area of GV Types I-IV: **Type I:** no basal opening or I-pore; **Type II:** basal opening, no I-pore; **Type III:** with I-pore, no basal opening; **Type IV:** both basal opening and I-pore. Median surface area of Type I GVs was 36.98 μm^2 (IQR: 20.73 – 74.30), Type II was 93.86 μm^2 (IQR: 39.75 – 178.62), Type III was 58.01 μm^2 (IQR: 45.30 – 137.84), and Type IV was 150.56 μm^2 (IQR: 84.46 – 210.56). Median surface area of Type IV GVs was significantly larger than Types I and II (both $P \leq 0.01$). **D:** Median maximal cross-sectional area (CSA) of reconstructed GVs with I-pores (21.29 μm^2, IQR: 12.44 – 37.34) was significantly larger compared to GVs without I-pores (13.66 μm^2, IQR: 4.82 – 28.77; $P \leq 0.01$). **E:** Median maximal CSA of GVs with multiple I-pores (25.87 μm^2, IQR: 19.01 – 47.44) was significantly larger than GVs with a single I-pore (17.49 μm^2, IQR: 11.52 – 31.61; $P = 0.02$). **F:** Median maximal CSA of Type I GVS was 8.56 μm^2 (IQR: 3.76 – 18.46), Type II was 15.96 μm^2 (IQR: 6.63 – 32.92), Type III was 16.11 μm^2 (IQR: 7.21 – 26.36), Type IV was 22.71 μm^2 (IQR: 14.02 – 37.70). Type IV GV maximal CSA was significantly larger than Type I ($P \leq 0.01$) and Type II ($P = 0.04$) GVs.

Supplemental Figure S3. Shapes of 180 3D-reconstruced giant vacuoles

A: Example of a 3D-reconstructed giant vacuole (GV). **B:** SBF-SEM cross section through the GV shown in (**A**). **C:** Example of a 3D-reconstructed collapsed GV. **D:** SBF-SEM cross section through the GV shown in (**C**). **E:** Similar percentages of round and collapsed GVs had I-pores (49.7%, 52.9%) or not (50.3%, 47.1%, respectively). **F:** Box plots showing the distributions of volumes of round vs. collapsed GVs. Median volume of round GVs (85.15 um3, IQR: 32.21 – 202.88; $n = 163$) was not significantly smaller than collapsed GVs (139.13 um3, IQR: 40.86 – 233.72; $n = 17$; $P > 0.05$). *Whiskers*: 1.5 interquartile range. X = mean.