SUPPLEMENTARY INFORMATION

Figure S1. Complete Western blot of CRISPR M18A KO Rat2 cells (A) Shown is the full Western blot of seven CRISPR-generated M18A KO Rat2 cell lines that was cropped to generate lanes 6 and 7 in Fig. 1, Panel D. The red boxes outline the specific lanes that were cropped.

Figure S2. Additional analyses confirm that abrogation of M18Aα expression does not alter Golgi morphology (A and B) Representative examples of HeLa cells expressing non-targeting shRNA (NT HeLa, A1 and A2) and HeLa cells expressing M18A shRNA (M18A KD HeLa, B1, B2) that were fixed and stained with the trans-Golgi marker α-VTi1a (green) and the nuclear marker DAPI (blue). White boxes in A1 and B1 correspond to A2 and B2, respectively. Scale bar, 20 μm in A1 and 10 μm in A2. Cells were imaged in a single z-plane using Zeiss Airyscan technology. These images indicate that the organization of the trans-Golgi network is qualitatively similar in control and M18A KD cells. (C and D) Representative example of a HeLa cell expressing non-targeting shRNA (NT HeLa, C1-C3) and a HeLa cell expressing M18A shRNA (M18A KD HeLa, D1-D3) that were fixed and stained with the cis Golgi marker α-GM130 (green), the nuclear marker DAPI (blue), and Phalloidin 568 (red). Z-stacks were acquired with Zeiss Airyscan technology in 0.189 μm steps. Shown are maximum z-projections from an overhead view (C1 and D1), 3D surface-rendered images from an overhead view (C2 and D2), and 3D surface-rendered images from an orthogonal view (C3 and D3). Scale bar, 5 μm. These reconstructions show that the 3D morphology of the Golgi in M18A KD cells is often indistinguishable from that in WT cells. (E) Comparison of Golgi height in WT Rat2 cells (grey) and M18A KO Rat2 cells (red). Lines indicate mean +/- standard deviation. P value is as indicated. n.s. indicates a lack of significance. This data was extrapolated from Figure 5G.

Video S1. Exogenous M18Aα does not localize to the Golgi

A HeLa cell expressing EGFP-M18Aα (green, left) and Halo-Mann II-JF646 (red, middle) was imaged every 30 sec in two focal planes, one near the bottom of the cell (top panels), and the other in the middle of the cell (3.7 μm from coverslip, bottom panels) on a Zeiss Airyscan microscope. The playback rate is 10 frames per second. Scale bar, 5 μm. Time in min:sec.

Video S2. The morphology of the Golgi varies continuously within single cells

Rat2 cells expressing Mann II-mEmerald (green) and mCherry-H2B (red) were imaged every 10 min on an Essen Biosciences Incucyte microscope equipped with a 20X objective. The playback rate is 10 frames per second. Scale bar, 20 μm. Time in hours:min.

Video S3. Lat A treatment causes lateral reduction and vertical extension of the Golgi and nucleus

A Rat2 cell expressing Emerald-Mannosidase-II (green) and mCherry-H2B (red) and treated with 2 μM Lat A was imaged in 3D using 0.5 μm steps acquired every 10 seconds on a Zeiss Airyscan microscope. Shown are a maximum intensity projection (left panel), an overhead reconstruction (middle panel), and an orthogonal reconstruction (right panel). The playback rate is 30 frames per second. Scale bar, 5 μm. Time in min:sec.

Video S4. Actin disassembly causes MTOC movement that parallels Golgi reconfiguration

A Rat2 cell expressing Mann II-mCherry (red) and GFP-tubulin (green) was imaged in 3D using 1 μm steps acquired every 30 seconds on a Zeiss Airyscan microscope. Frames 1-50 show a maximum intensity projection during 2 μM Lat A treatment (when indicated) and washout. Frames 51-100 show a corresponding orthogonal view of the same cell. The playback rate is 15 frames per second. Scale bar, 5 μm. Time in min:sec.