Legends to supplemental material

The following videos correspond to the images shown in figures 1 – 5 as mentioned and show trafficking of GFP-COL1A1 to the Golgi with addition of asc/biotin (as does Video 2 for Figure S1 at a higher frame rate), as well as the influence of nocodazole on this process.

Video 1: Ascorbate- and biotin-dependent trafficking of GFP-COL1A1

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 21 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Image stills are shown in Fig. 1D. Acquisition at 1 frame every 30 seconds over a period of 49 mins with a playback rate of 12 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Video 2: Transport of GFP-COL1A1 to the Golgi occurs without the use of large carriers.

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 18.5 hours post-co-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Accumulation and filling of the Golgi with GFP-COL1A1 occurs without visible large structures in the proximity of the Golgi. Small GFP-positive puncta can be observed traversing in the direction towards the Golgi (highlighted by arrow heads). A total of n = 3 was acquired. Acquisition at 1 frame every second over a period of 30 mins with a playback rate of 12 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin and a scale bar of 1 µm. Corresponding image stills are shown in Fig. S1.

Video 3: GFP-COL1A1 transport to the Golgi in RPE1 cells

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 23 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Image stills are shown in Fig. 3A - B. Cells fixed after live imaging and labelled for giantin are shown in Fig. 4Aii. Acquisition at 1 frame every 20 seconds over a period of 52 mins with a playback rate of 6 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Video 4: GFP-COL1A1 transport to the Golgi in RPE1 cells

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 18 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; expressed from a bicistronic vector; hence, all ST-Cherry positive cells also express the ER hook). Image stills are shown in Fig. 3C - D. Acquisition at 1 frame every 30 seconds over a period of 19 mins with a display rate of 6 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Video 5i: GFP-COL1A1 transport from the ER to the Golgi, prior to fixation and co-labelling with Giantin

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 18 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Image stills are shown in Fig. 4Ai. Acquisition at 1 frame every 30 seconds over a period of 12 mins with a display rate of 6 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Video 5ii: GFP-COL1A1 transport from the ER to the Golgi, prior to fixation and co-labelling with Hsp47

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 22 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Image stills are shown in Fig. 4B. Acquisition at 1 frame every 40 seconds over a period of 45 mins with a display rate of 6 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Video 5iii: GFP-COL1A1 transport from the ER to the Golgi, prior to fixation and co-labelling with Sec31A

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 24 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Image stills are shown in Fig. 4Di. Acquisition at 1 frame every 30 seconds over a period of 8 mins with a display rate of 6 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Video 5iv: GFP-COL1A1 transport from the ER to the Golgi, prior to fixation and co-labelling with antibodies of interest

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 20.5 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Image stills are shown in Fig. 4Dii. Acquisition at 1 frame every 15 seconds over a period of 17 mins with a display rate of 6 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Video 6: GFP-COL1A1 transport to the Golgi is independent of microtubules

Confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 18 hours post-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Image stills are shown in Fig. 5A - B. Acquisition at 1 frame every 25 seconds over a period of 90 mins with a display rate of 3 frames per second. Time stamp shows time after addition of asc/biotin with a final concentration of 500 $\mu g.ml^{-1}$ ascorbate and 400 µM biotin and incubation with 5 nocodazole for 60 mins. At timepoint 0 GFP-COL1A1 and ST-Cherry show distinct localisation. For each set of live imaging experiment 3 cells from the same dish were imaged simultaneously. A total of n=3 sets was acquired. Scale bar indicates 10 µm.

Supplemental Figure S1: Transport of GFP-COL1A1 to the Golgi occurs without the use of large carriers.

Image stills from confocal live cell imaging of RPE-1 stably expressing GFP-COL1A1 (green; GFP-COL1A1-RPE) 18.5 hours post-co-transfection with the *trans*-Golgi marker ST-Cherry (red; utilising the RUSH system). Timepoints indicate mins after addition of asc/biotin. **A:** Shows a whole cell imaged at 1 frame every second (derived from Video 2) with corresponding enlargements with channels for GFP-COL1A1 and ST-Cherry in greyscale, as well as the overlay image below. Accumulation and filling of the Golgi with GFP-COL1A1 occurs without visible large structures in the proximity of the Golgi. **B:** Selected image stills from A highlighting a GFP-positive structure moving towards the Golgi. A total of n = 3 was acquired. Scale bars = 10 µm and 1 µm (in enlargements).