# Supplemental Materials

***Molecular Biology of the Cell***

Jao et al.

## Supplemental Figure Legends

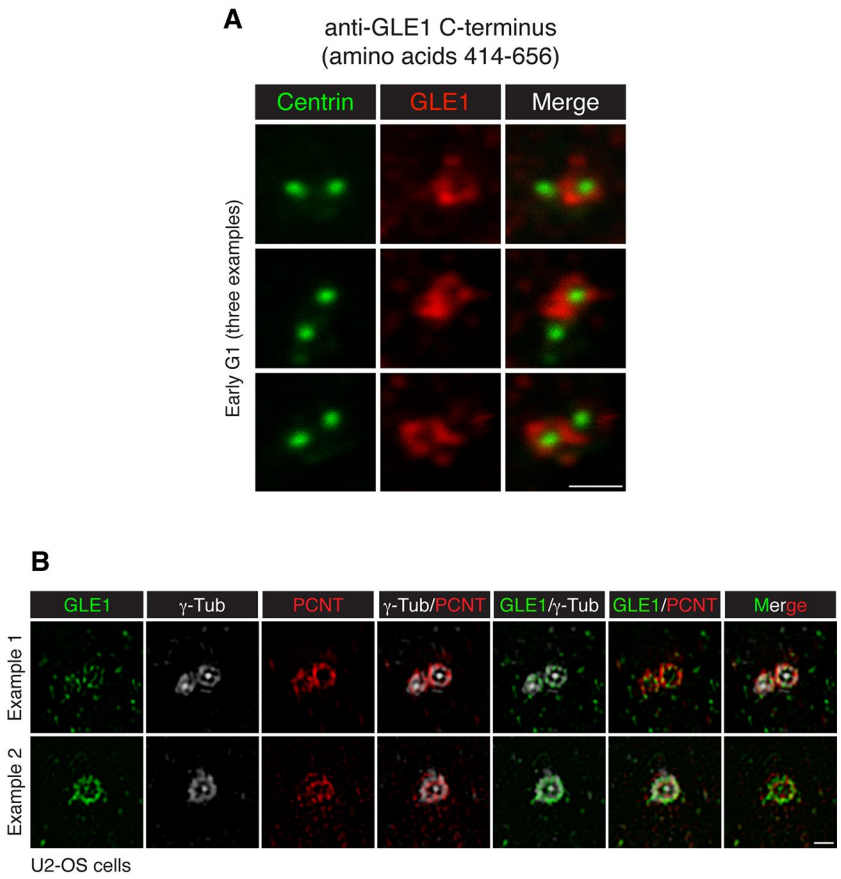


**Figure S1.** Validation of Gle1 enrichment around the mother centriole using a different antibody and 3D-SIM microscopy (related to Fig 2). (A) Confocal images of CETN-GFP RPE-1 cells stained with a rabbit antibody against the carboxy-terminal domain of human Gle1. Figure 3 used a guinea pig antibody raised against the amino-terminal domain of human Gle1. Note that only one of the centrioles was surrounded by the anti-Gle1 signals. (B) 3D-SIM images of interphase human U-2 OS cells co-stained with antibodies to Gle1, γ-tubulin (γ-Tub), and PCNT. Note that all three proteins form toroid structures, decorating the outer layer of the PCM. γ-Tub also occupies the centriolar core as previously reported. Scale bars: A: 1 µm; B: 0.5 µm.

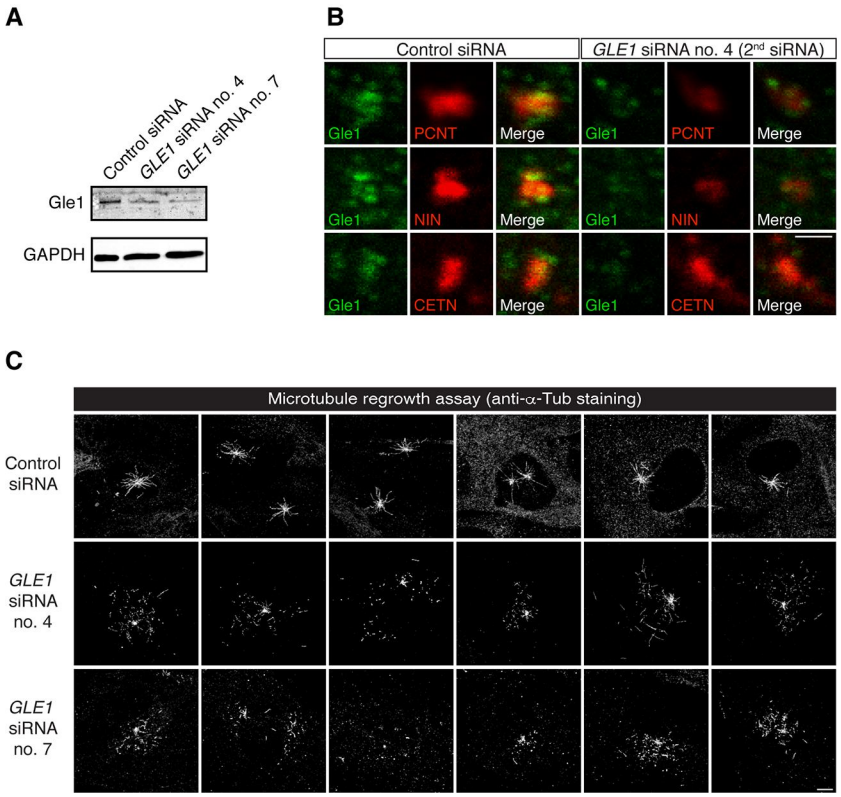


**Figure S2.Validation of *GLE1* siRNA knockdown phenotypes by two siRNAs against different regions of human *GLE1* (related to Fig 3).** (A) Total cell lysates of scrambled control siRNA-, *GLE1* siRNA no. 4 (Hs_GLE1L_4 FlexiTube siRNA, Qiagen)-, or *GLE1* siRNA no. 7 (Hs_GLE1L_7 FlexiTube siRNA, Qiagen)-transfected cells were analyzed by immunoblotting for Gle1. GAPDH served as a loading control. (B) Human RPE-1 cells transfected with scrambled control siRNA or*GLE1* siRNA no. 4 were processed for indirect immunofluorescence microscopy with antibodies against Gle1 as well as to PCNT (top row), NIN (middle row), or CETN (bottom row). (C) Human RPE-1 cells transfected with scrambled control siRNA, *GLE1* siRNA no. 4, or *GLE1* siRNA no. 7, were subjected to a MT regrowth assay, where the cells were fixed at 6 minutes after re-warming to 30°C and stained for α-tubulin (α-Tub). Note that similar reduction in Gle1 levels, defects in PCNT and NIN recruitment, and defects in MT organization (e.g., ectopic cytoplasmic MT nucleation, 6 examples per condition) were observed using the two different *GLE1* siRNAs. Scale bars: B: 1 µm; C: 5 µm.