SUPPLEMENTAL MATERIAL FIGURE LEGENDS

Supplemental Figure 1: Hook2 localization at the centrosome, the basal body and the Golgi apparatus but not on early or late endosomes. (A) 7-days cultured ARPE19 cells were fixed in PFA and permeabilized with Saponin. Cells were then co-stained with Abs raised against Hook2 and proteins localized either on early endosomes (EEA1, upper panels) and late endosomes (lamp-1, lower panels). Arrows indicate separate markers. Bar = 10 μm. Insert magnification, x15. (B) ARPE19 cells were transfected with Myc-tagged C-term (from aa 641 to 719) or N-term (from aa 1 to 533) parts of Hook2, fixed in PFA and permeabilized with Tx100. Cells were then co-stained with Abs raised against Myc (left panels) and TGN46 (middle panels). Bar = 10 μm. Insert magnification, x15. (C) Newborn CD1 mouse heads were fixed in PFA, incubated in sucrose, embedded in OCT and frozen on dry ice. OCT embedded cryosections of the mouse heads were Tx100 permeabilized and stained with Abs for the indicated proteins. Arrowheads indicate the base of the cilia where Hook2 accumulates with colocalizing markers. C, cilia. Bar = 10 μm. Inserts magnification, x2.

Supplemental Figure 2: Hook2 depletion does not interfere with the mother centriole maturation. Control and Hook2 siRNA transiently transfected cells were fixed and permeabilized in methanol after 7 days in culture and further compared by immunofluorescence and confocal microscopy analysis for the localization of Cenexin (A) or CEP170 (B) to the mother centriole. Bar = 10 μm. Insert magnification, x15. Arrows indicate colocalizing markers.

Supplemental Figure 3: ARPE19 Hook2 depleted clones are not ciliated. (A) ShRNAs corresponding to the siRNAs H1 and H2, flanked with the sequence for GFP expression, were transfected twice at 4-days interval to transiently decrease Hook2 production in ARPE19 cells. After 7 days in culture, Hook2 shRNA transfected cells were fixed in PFA and compared to control shRNA treated cells for their production of Hook2 as well as their ability to promote ciliogenesis by immunofluorescence and confocal microscopy analysis. Arrowheads point at control cells that express the control shRNA, produce Hook2 and develop a primary cilium. Arrows indicate cells that express Hook2 shRNA, have a decreased Hook2 level of expression and consequently do not develop a primary cilium. Asterisks indicate untransfected cells that display a primary cilium and normal Hook2 expression and localization. Bars = 10 μm. (B) Before the second shRNA transfection, part of the cells was cultured for 4 weeks in the presence of Geneticin antibiotics to select shRNA::GFP-producing clones. 6 clones were obtained and tested by WB analysis for Hook2 production and by immunofluorescence and confocal analysis for ciliogenesis. A quantification of the results is shown in (B). Error bars represent the standard deviation, n=3. Clones 2.3 and 2.5 were extensively characterized and further used as representative control and Hook2 depleted cells, respectively. (C) Control (clone 2.3) and Hook2 depleted (clone 2.5) stable clones were assayed for Hook2 production by WB analysis of total cell lysates. MW markers are indicated on the left in kDa. (D) 2.3 and 2.5 clones were tested for primary cilium formation after 7 days in culture by immunofluorescence and confocal microscopy analysis. Bar = 10 μm; insert magnification, x15. Asterisks indicate cilia in the control clone 2.3.

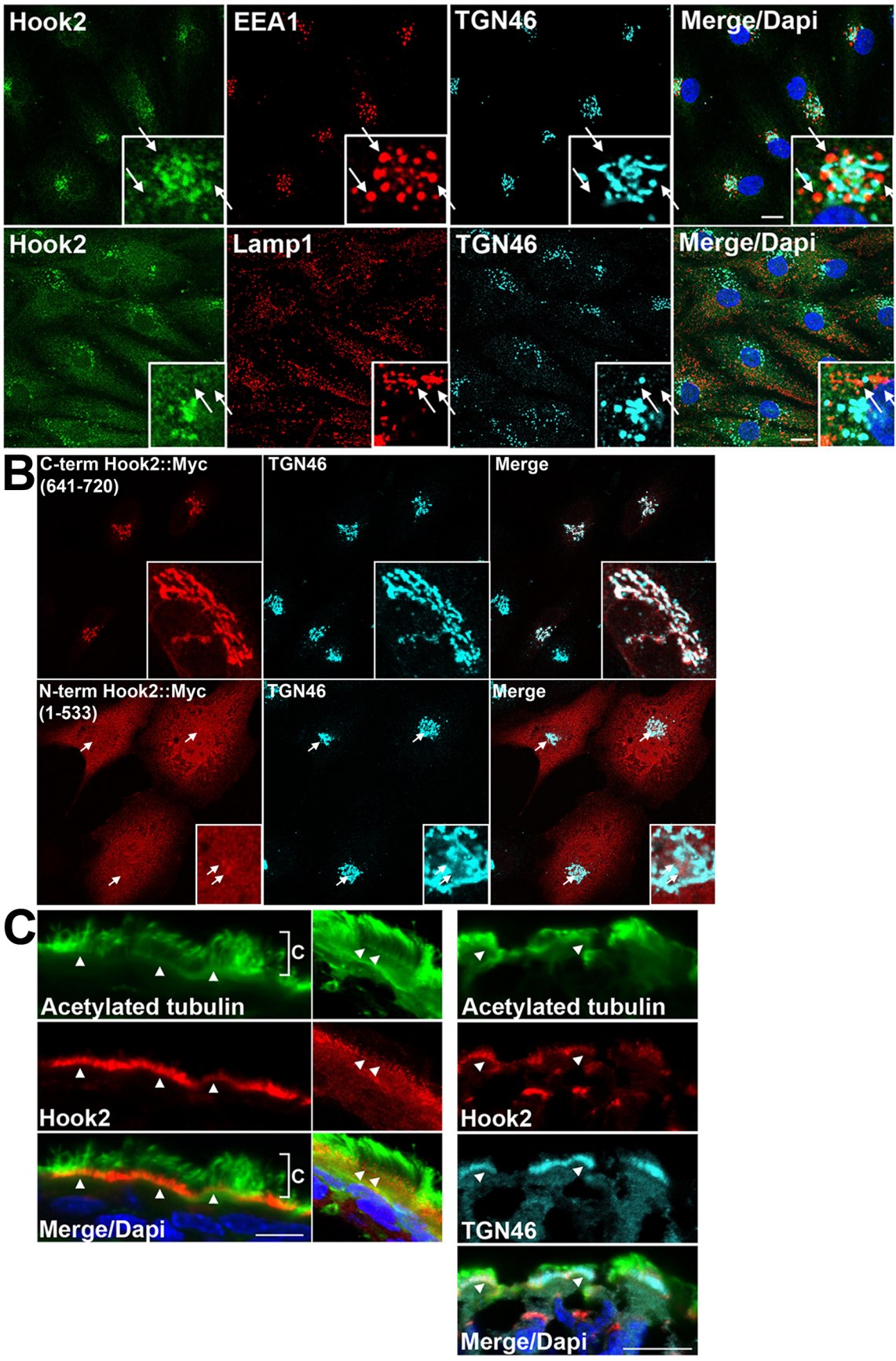
Supplemental Figure 4: Unlike Hook1 and Hook3, PCM1 phenocopies Hook2 depletion. (A, B) SiRNA treated cells were fixed in methanol after 7 days in culture and compared by immunofluorescence and confocal microscopy analysis for centrosomal localization of Cenexin (A, γ-tubulin and Cenexin) and CEP170 (B, γ-tubulin and CEP170) as well as TGN46 staining. Bar = 10 μm. Insert magnification, x15. Note that Hook3 siRNA induces a decrease of Cenexin expression that might be a consequence of Golgi breakdown.

Supplemental Figure 5: (A) ARPE19 cells transiently transfected with Hook2 siRNA were further transfected with either a control cDNA, mouse Hook2 cDNA, GFP or GFP::Rab8a and compared to control cells (Control siRNA+control cDNA). After 7 days, cells were processed for WB analysis (A) and quantification (B) of PCM1 and Hook2 production in the total cell lysates as compared to Actin as a loading control. MW markers are indicated on the left in kDa, n=2.

Supplemental Figure 6: GFP::Rab8a-ARPE19 stable cell line depleted for Hook2 can undergo ciliogenesis. (A) A clonal ARPE19 cell line stably expressing GFP::Rab8a was depleted for Hook2 and compared to non-depleted control cells after 7 days in culture by WB for Hook2 and GFP::Rab8a expression using Abs against α–tubulin and GAPDH as loading controls. MW markers are indicated on the left in kDa. (B) Part of these cells were also fixed in PFA, permeabilized with Triton and

processed for immunofluorescence and confocal microscopy analysis after Hook2 and TGN46 staining to monitor Hook2 depletion and Golgi architecture. Note that ciliogenesis was not inhibited in ARPE19-GFP::Rab8a stable cell line that were Hook2 depleted. Bar = 10 μm. Insert magnification, x15. In (C), the number of GFP::Rab8a-ARPE19 cells with a cilium in each siRNA condition was quantified (n>60 cells for each condition). In (D), the volume of the Golgi apparatus was quantified in GFP::Rab8a control or Hook2 depleted cells and normalized to 100% for the control cells (n>62 cells for each condition).

Supplemental Movies 1-3: Hook2 localizes to the Golgi apparatus. 7-days cultured ARPE19 cells were sequentially fixed in PFA and permeabilized with Tx100. The cells were then co-stained with Abs raised against Hook2 (green), TGN (blue) and Giantin (Red, Movie 1), GM130 (Red, Movie 2) or Golgin-97 (Red, Movie 3). Confocal images of Z stacks were processed for 3-dimentional projections and movie imaging.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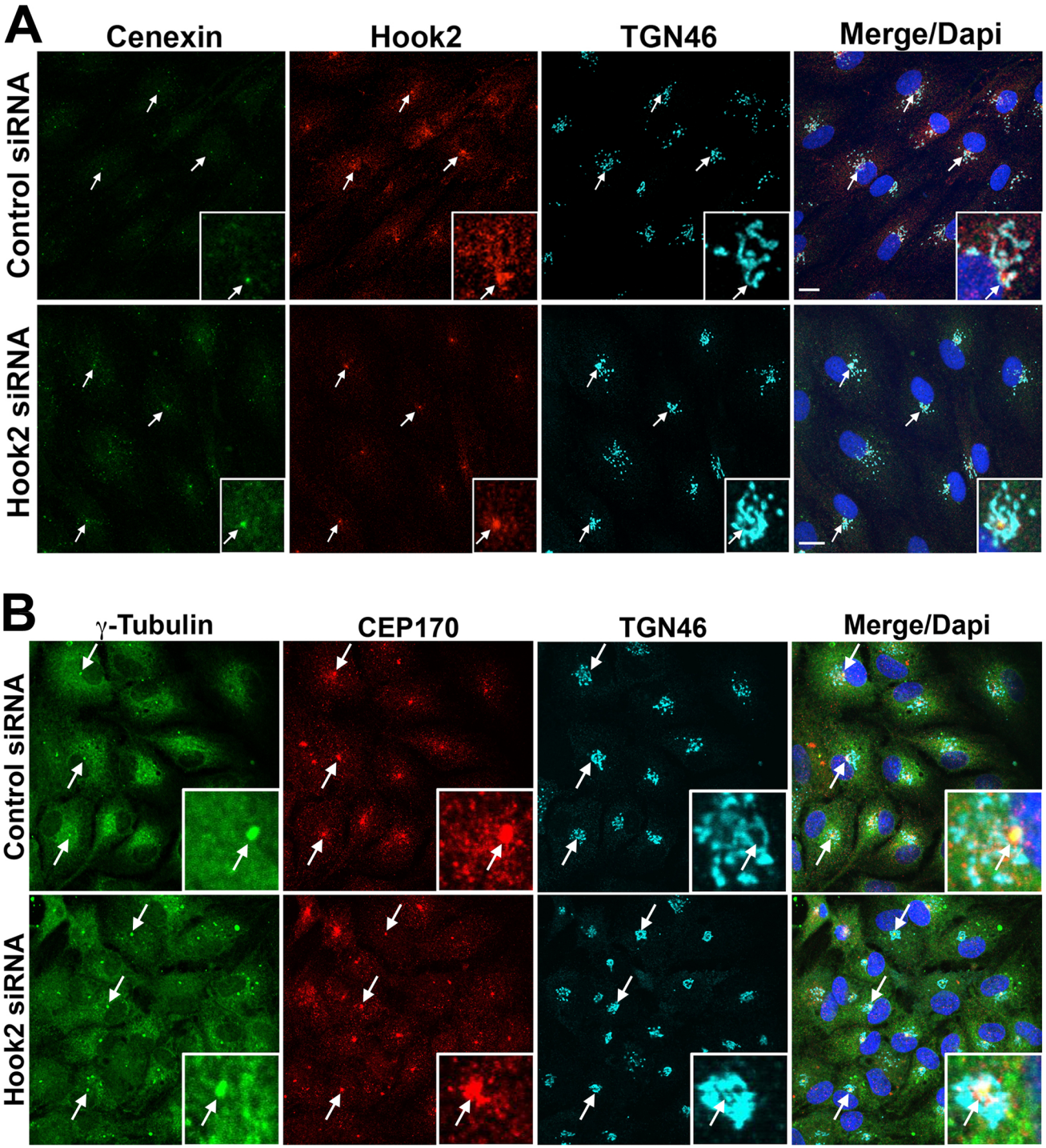
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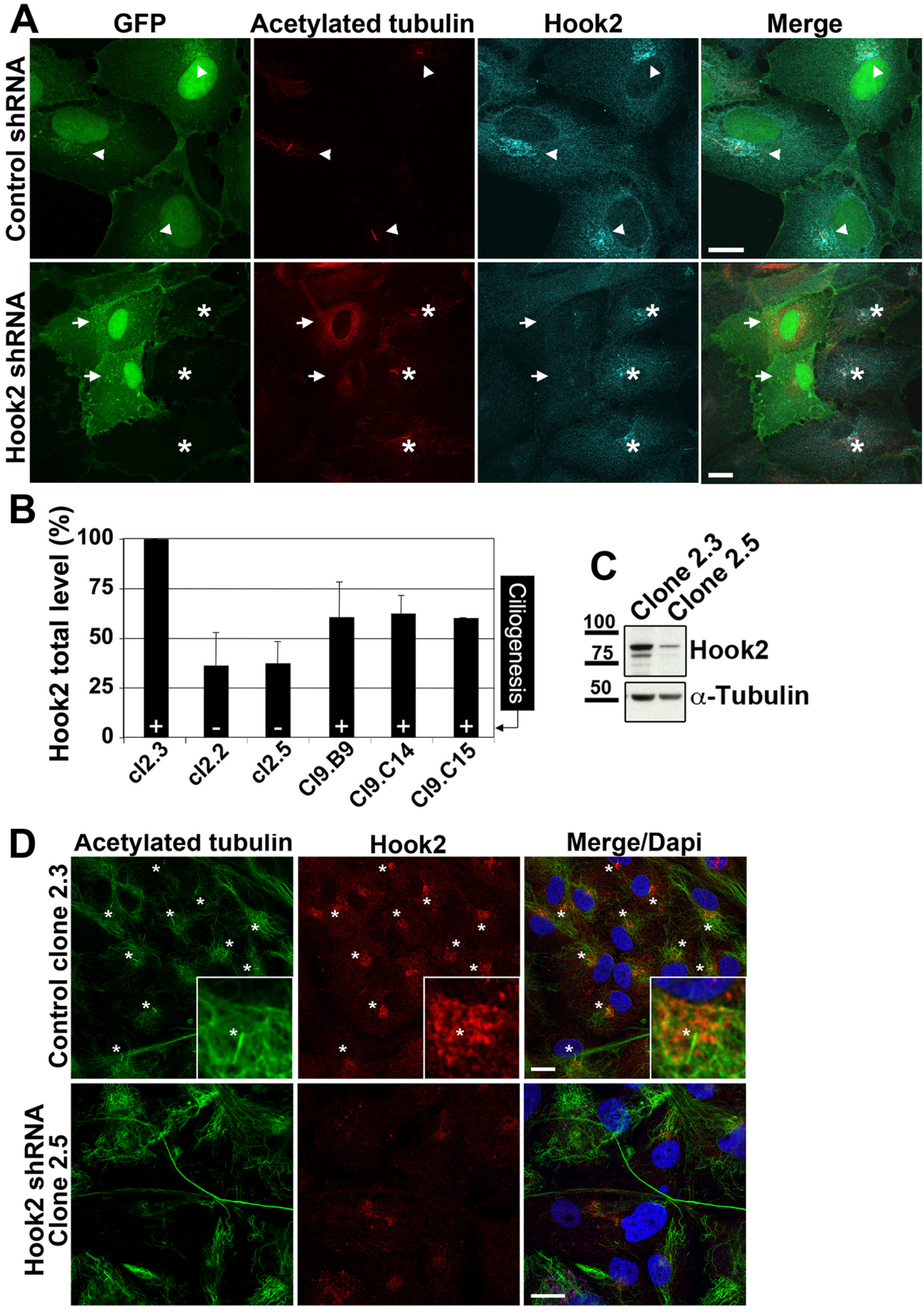
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+
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Ciliogenesis
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cl2.2
cl2.5
Cl9.B9
Cl9.C14
Cl9.C15
C
Clone 2.3
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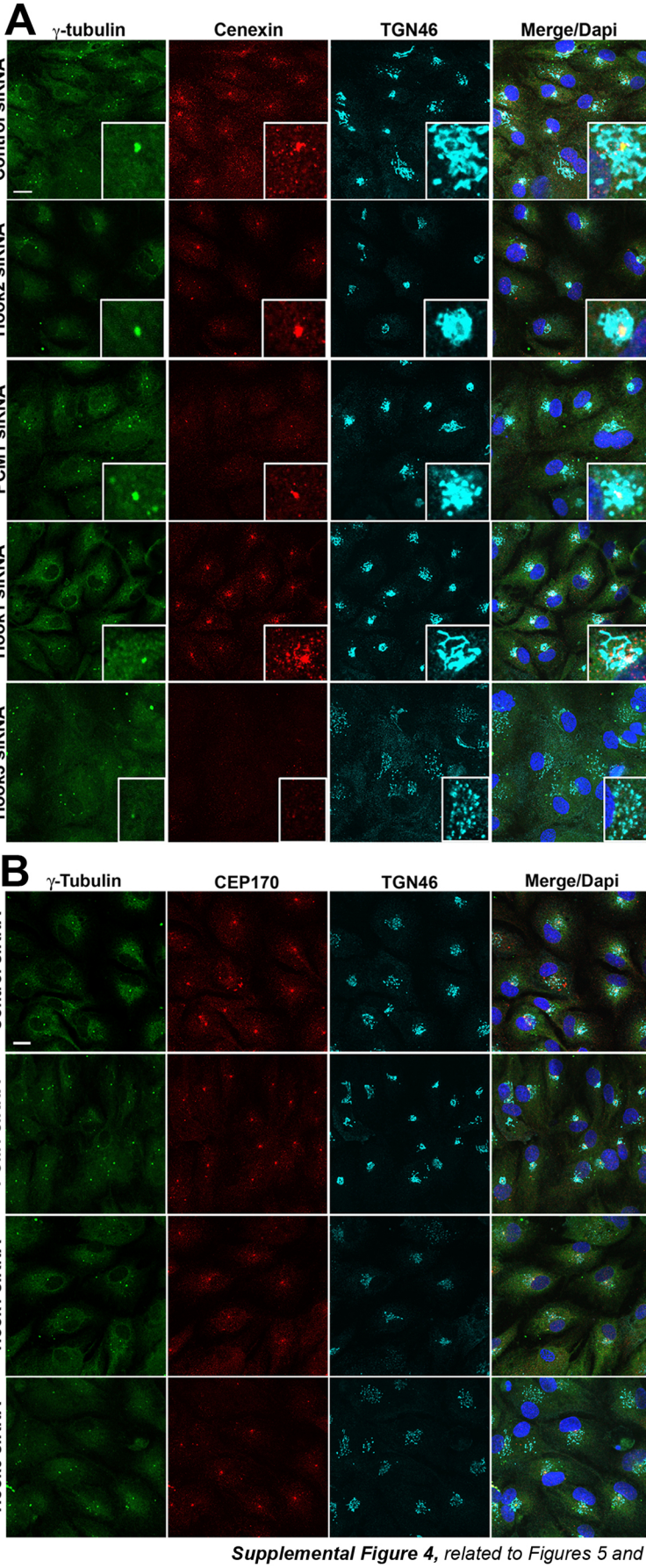
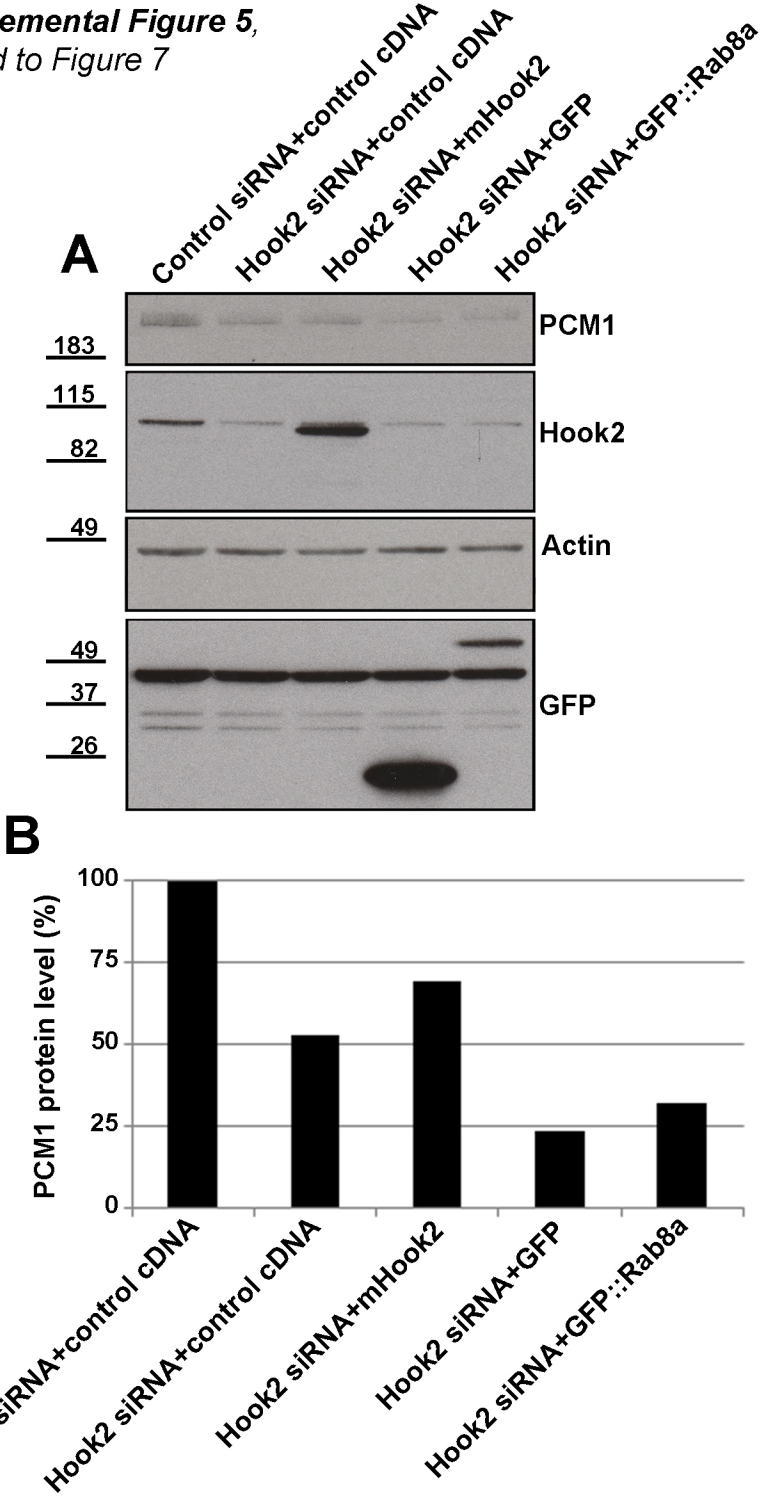


Supplemental Figure 4, *related to Figures 5 and 6*

Supplemental Figure 5,
related to Figure 7



***Supplemental Figure 6**,related to Figure 7*