

# Supplemental Materials

*Molecular Biology of the Cell*

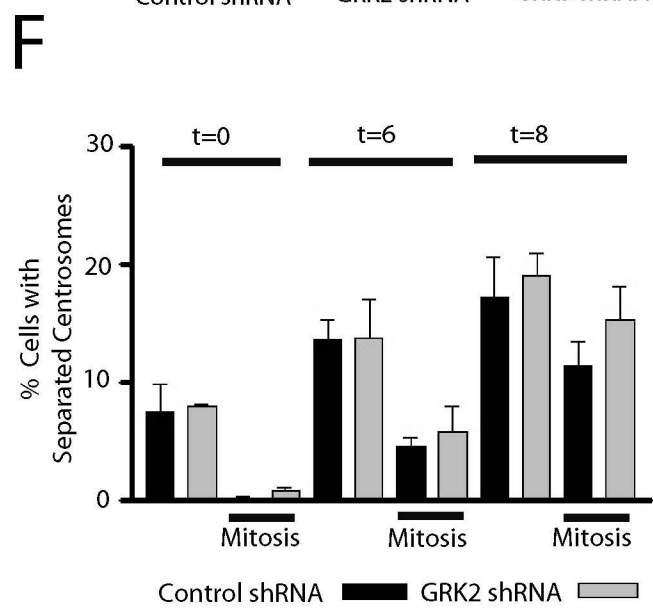
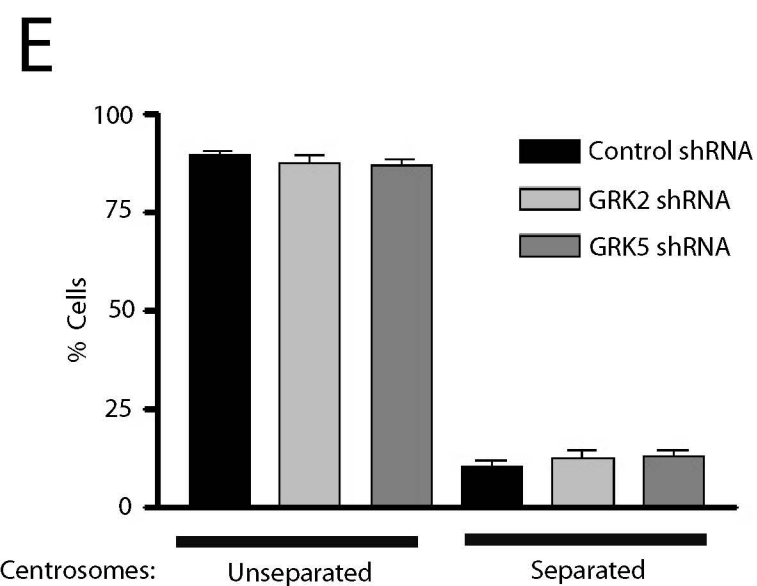
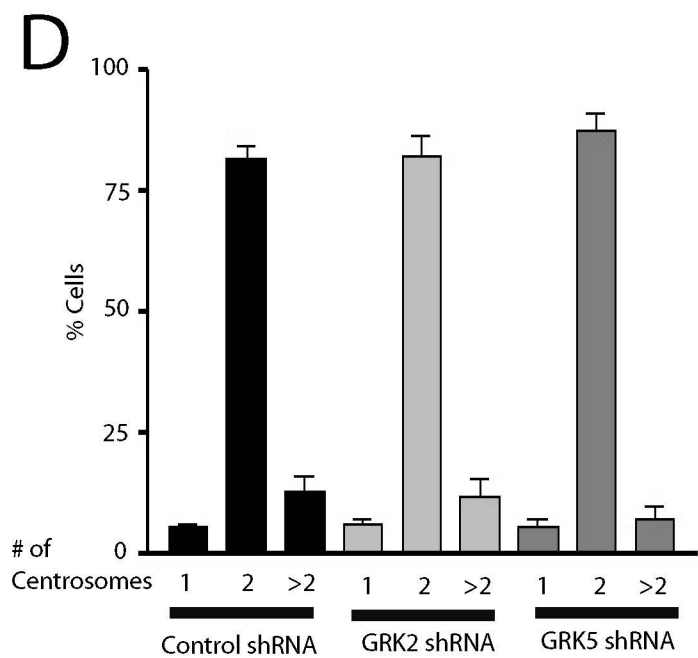
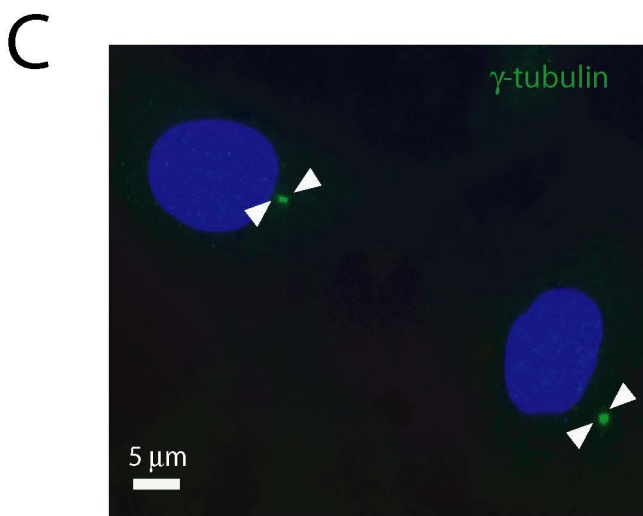
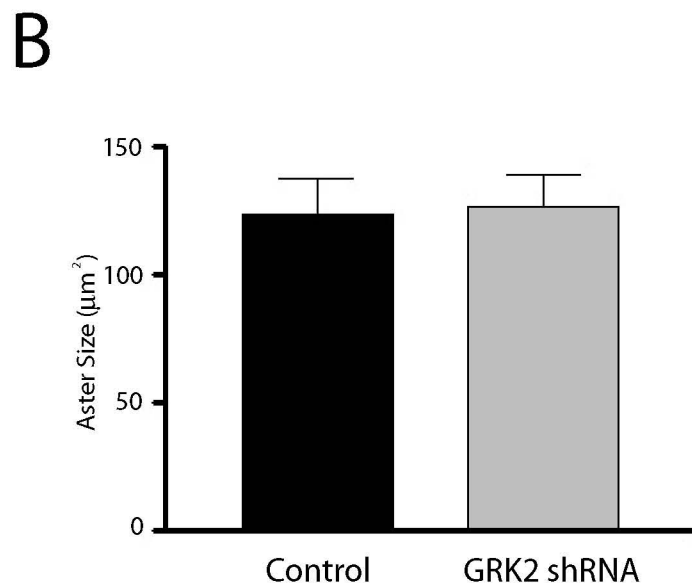
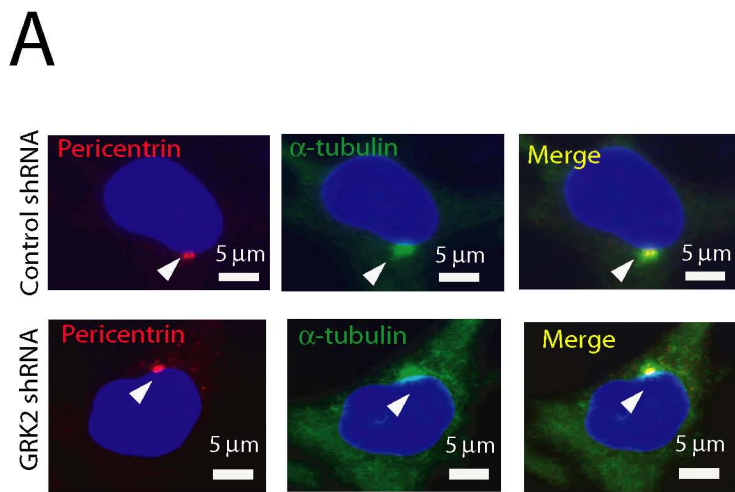
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**Supplemental Figure S1.** GRK2 does not regulate centrosome nucleation or duplication or cell cycle-specific centrosome separation. A. Representative nucleation of centrosomes in control shRNA cells. B. Depletion of GRK2 did not affect the microtubule nucleation capacity of the centrosome as determined by aster size ( $n > 75$  cells from 4 independent experiments). Only cells in interphase and with one or two centrosomes were utilized for analysis. C. Representative duplicated centrosomes stained for  $\gamma$ -tubulin. D. Quantification of cells with duplicated centrosomes ( $> 2$ ) in control, GRK2 or GRK5 shRNA cells after 48 hr hydroxyurea treatment ( $n = 6$ ). E. Quantitation of cells with separated centrosomes (centrosomes separated by greater than one centrosome diameter) in asynchronized cells ( $n = 4$ ). F. Quantitation of cells with separated centrosomes at times after double thymidine synchronization in control, GRK2 or GRK5 shRNA cells. Mitotic cells were determined as those cells with condensed DNA between separated centrosome pairs ( $n = 5$ ).

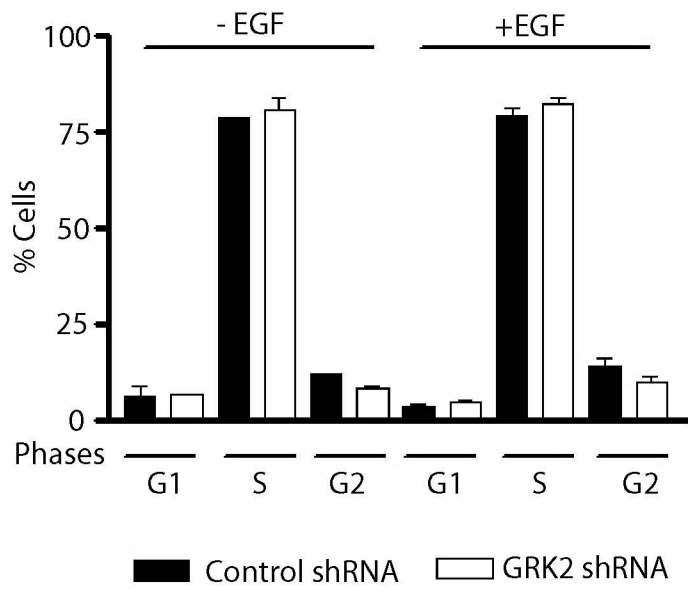
**Supplemental Figure S2.** EGF stimulation does not increase the rate of cell cycle progression. Control and GRK2 shRNA HeLa cells were subject to hydroxyurea block, released into serum free media with or without 100 ng/ml EGF for 30 min, collected and subjected to FACS analysis to examine cell cycle progression from at least three independent experiments.

**Supplemental Figure S3.** A. Examples of cells demonstrating separated duplicated centrosomes in S phase. Arrows indicate duplicated centrosomes in cells with no separation of duplicated centrosomes, duplicated centrosomes approximately one centrosome diameter apart ( $\sim 3 \mu\text{m}$ ), duplicated centrosomes whose separation is between 3-25  $\mu\text{m}$  and duplicated centrosomes fully separated ( $> 25 \mu\text{m}$ ). B-C. Histogram of the distances between duplicated centrosomes in control shRNA (B) or GRK2 shRNA (C) cells after vehicle or 100 ng/ml EGF treatment for 30 min. D. siRNA knockdown of GRK2 in HeLa cells, probed with anti-GRK2/3. E. Duplicated centrosome separation in control, GRK2 or GRK5 siRNA cells after 30 min treatment with 100 ng/ml EGF. \* indicates  $p < 0.05$ ,  $n = 4$ .

# Supplemental Figure S1



# Supplemental Figure S2



# Supplemental Figure S3

