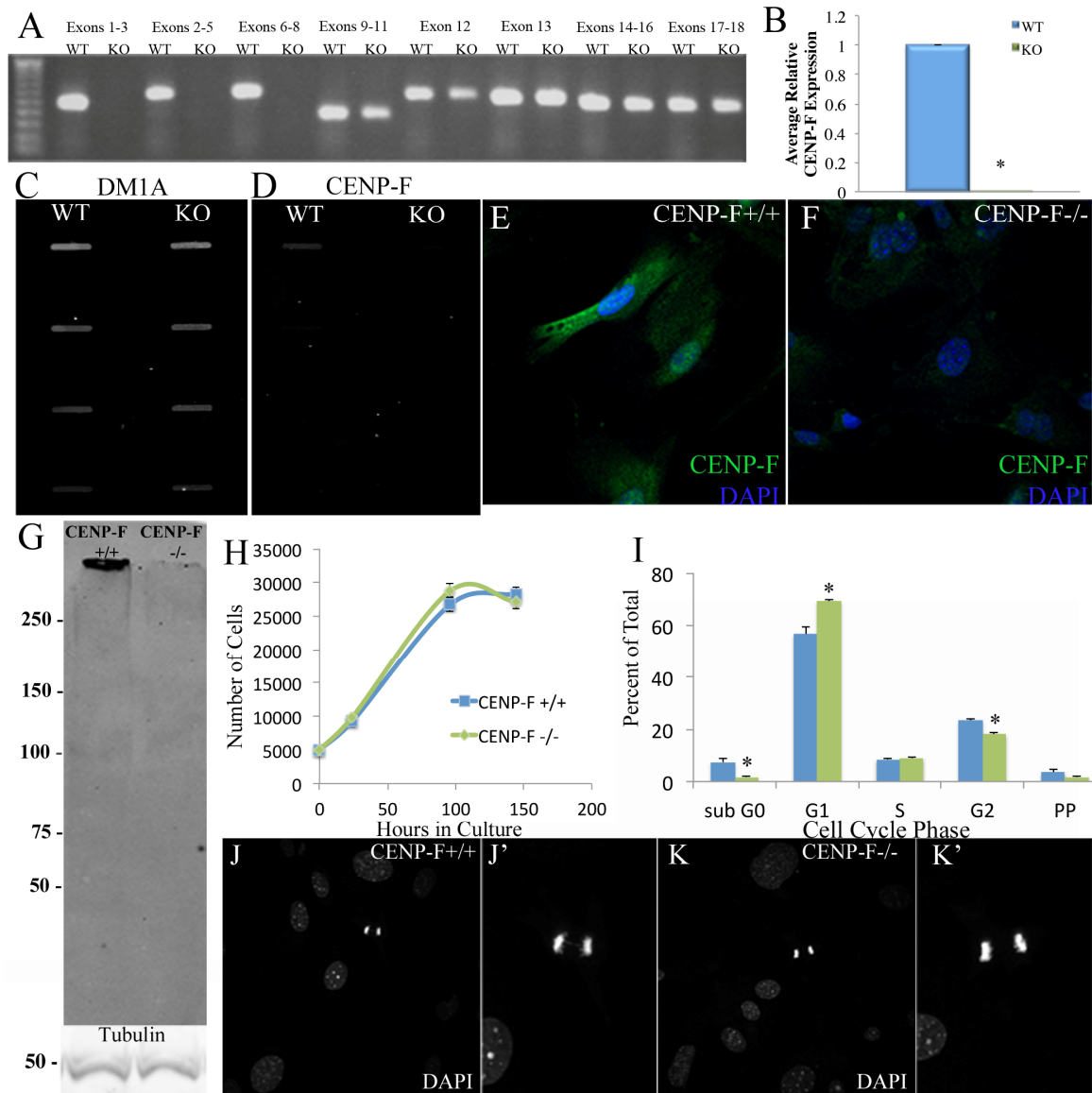


# Supplemental Materials

*Molecular Biology of the Cell*

Pfaltzgraff et al.



**Supplemental Figure 1.** Confirmation of *CENP-F* model in MEFs. PCR showing mRNA loss of recombined region in *CENP-F*<sup>-/-</sup> MEFs (A). rt-PCR data demonstrating the average relative level of *CENP-F* mRNA between *CENP-F*<sup>+/+</sup> and *CENP-F*<sup>-/-</sup> MEFs (B). The relative amount of mRNA was determined by comparison to HPRT. Error bars represent SEM. *CENP-F* forward primer: ATCGGCAGATGTTTCTCTGAA, *CENP-F* reverse primer: CGTATGTGGAAGCTCGGTGTG. n=6, \*p<0.001. Slot blots of MEF cell extracts (C, D). Decreasing amounts of protein from MEF Cell extracts were bound to a

membrane using a slot blot apparatus. B) Slot blot labeled with DM1A demonstrating the amount of alpha-tubulin protein in the WT and KO samples providing the internal control for equivalent protein loading. C) Slot blot labeled with anti-CENP-F antibody (ELDA6) demonstrating the low amount of CENP-F present in WT MEFs and the absence of CENP-F in KO MEFs. MEFs labeled with anti-CENP-F antibody (ab5) demonstrating loss of CENP-F protein in *CENP-F*<sup>-/-</sup> MEFs (E,F). Western blot of MEF cell lysates blotted with anti-CENP-F D10 from Tim Yen and alpha tubulin (G). GTT assay quantifying cell number over time measured in hours (H). No statistical difference in cell number between cell lines,  $p=0.07$ .  $n=9$ . Error Bars represent SEM. Fluorescent activated cell sorting of *CENP-F*<sup>+/+</sup> and *CENP-F*<sup>-/-</sup> MEFs (I). Data represent the percentage of each flow sorted population that falls within different phases of the cell cycle based on DNA content. \*  $p<0.01$ . Error Bars represent SEM. While there are slight differences, no difference in the portion of cells in S phase or in polyploid cells was observed,  $n=3$ . Micrographs of MEFs undergoing cell division (J-K'). Chromosomes in *CENP-F*<sup>-/-</sup> MEFs segregate.