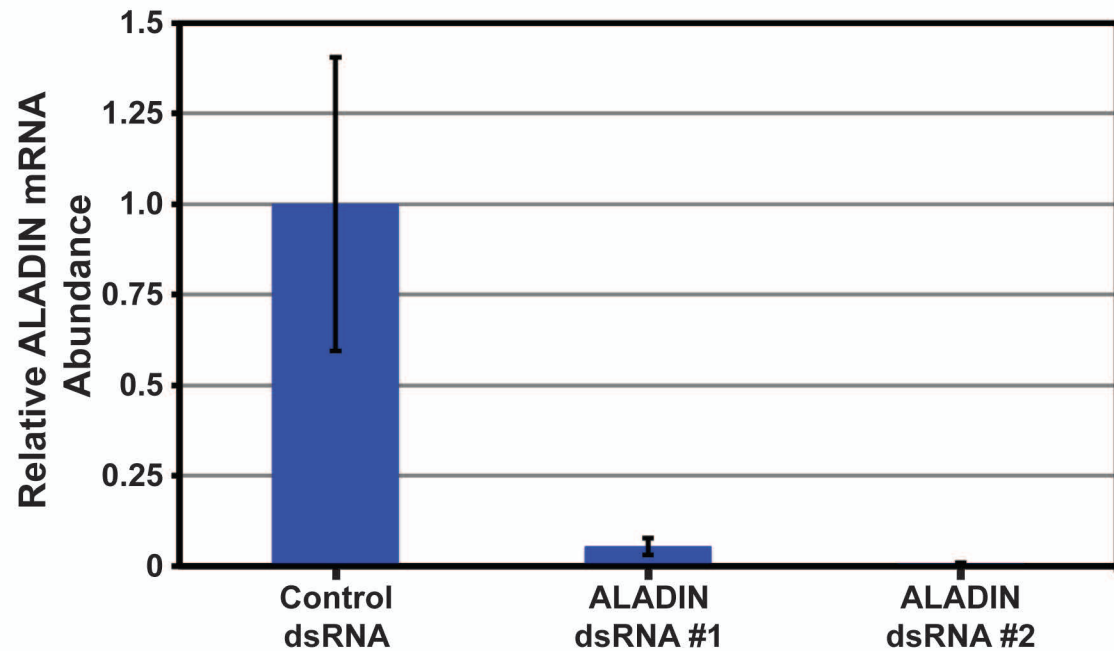
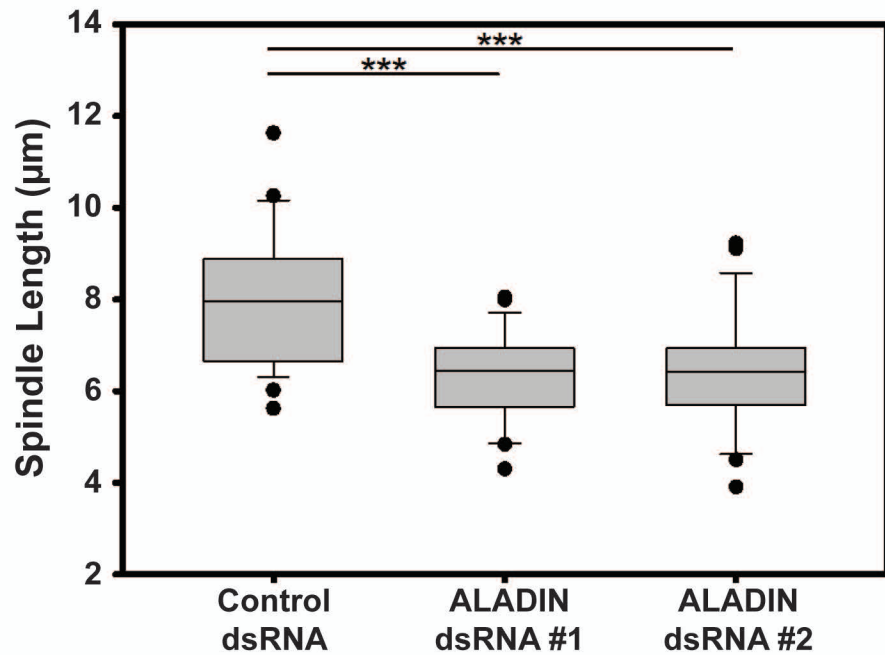


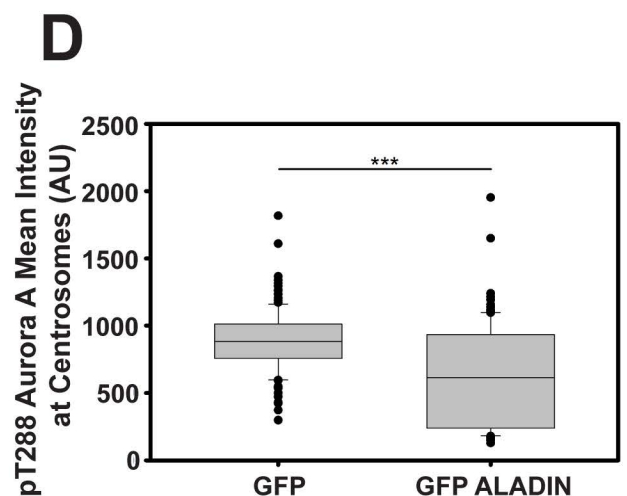
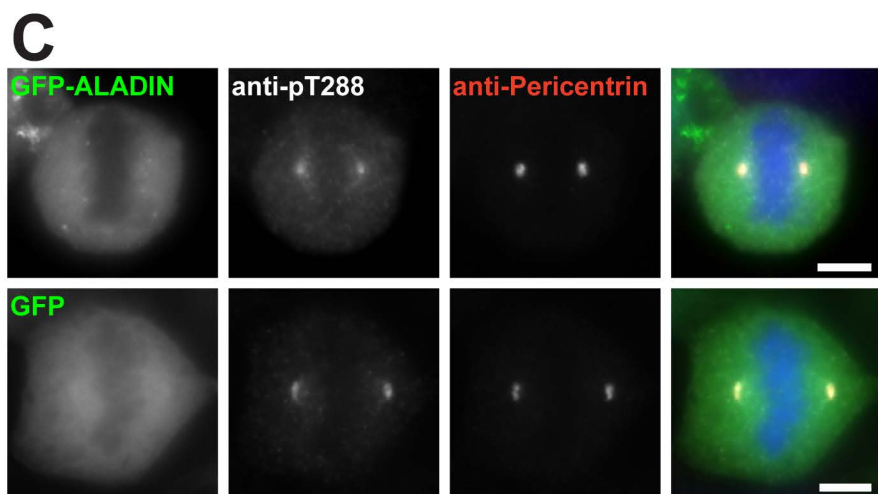
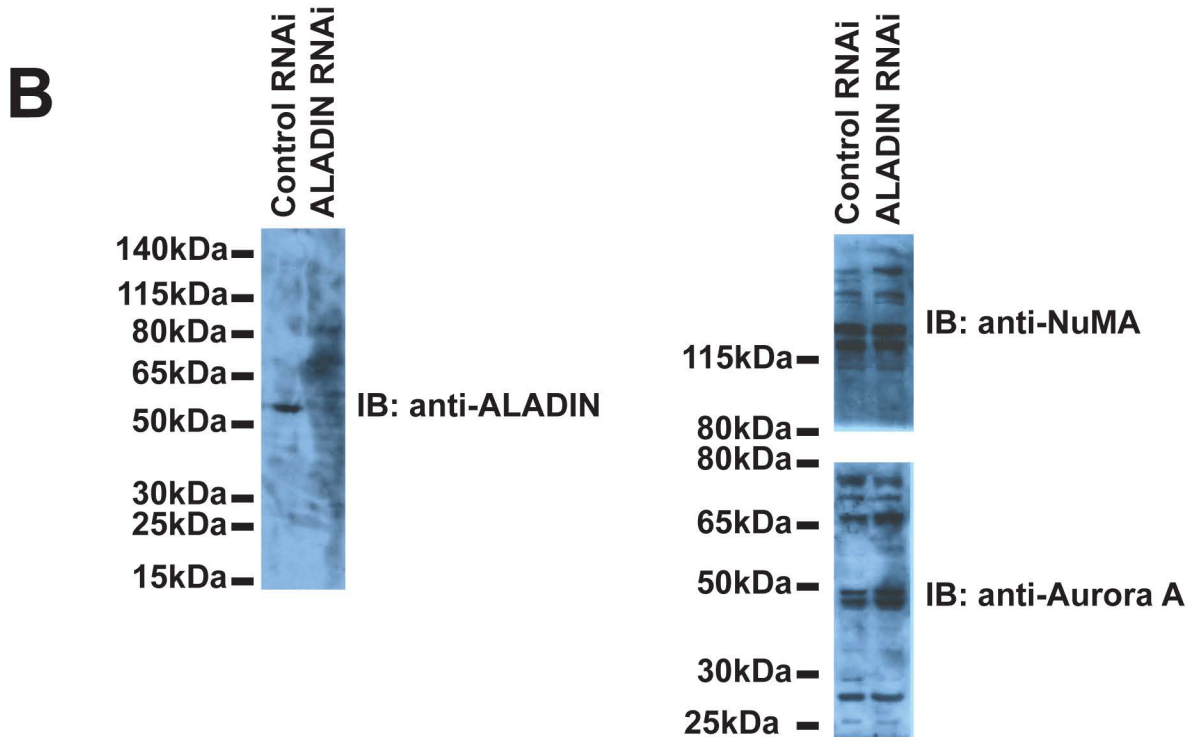
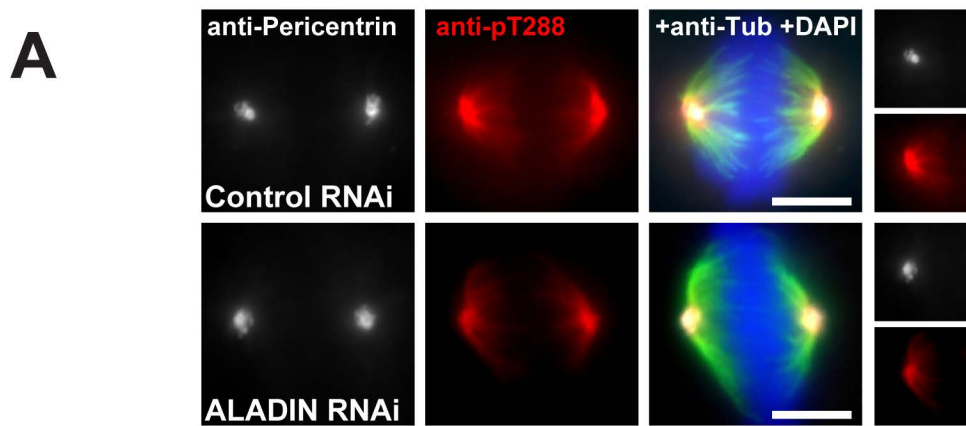
# Supplemental Materials

*Molecular Biology of the Cell*

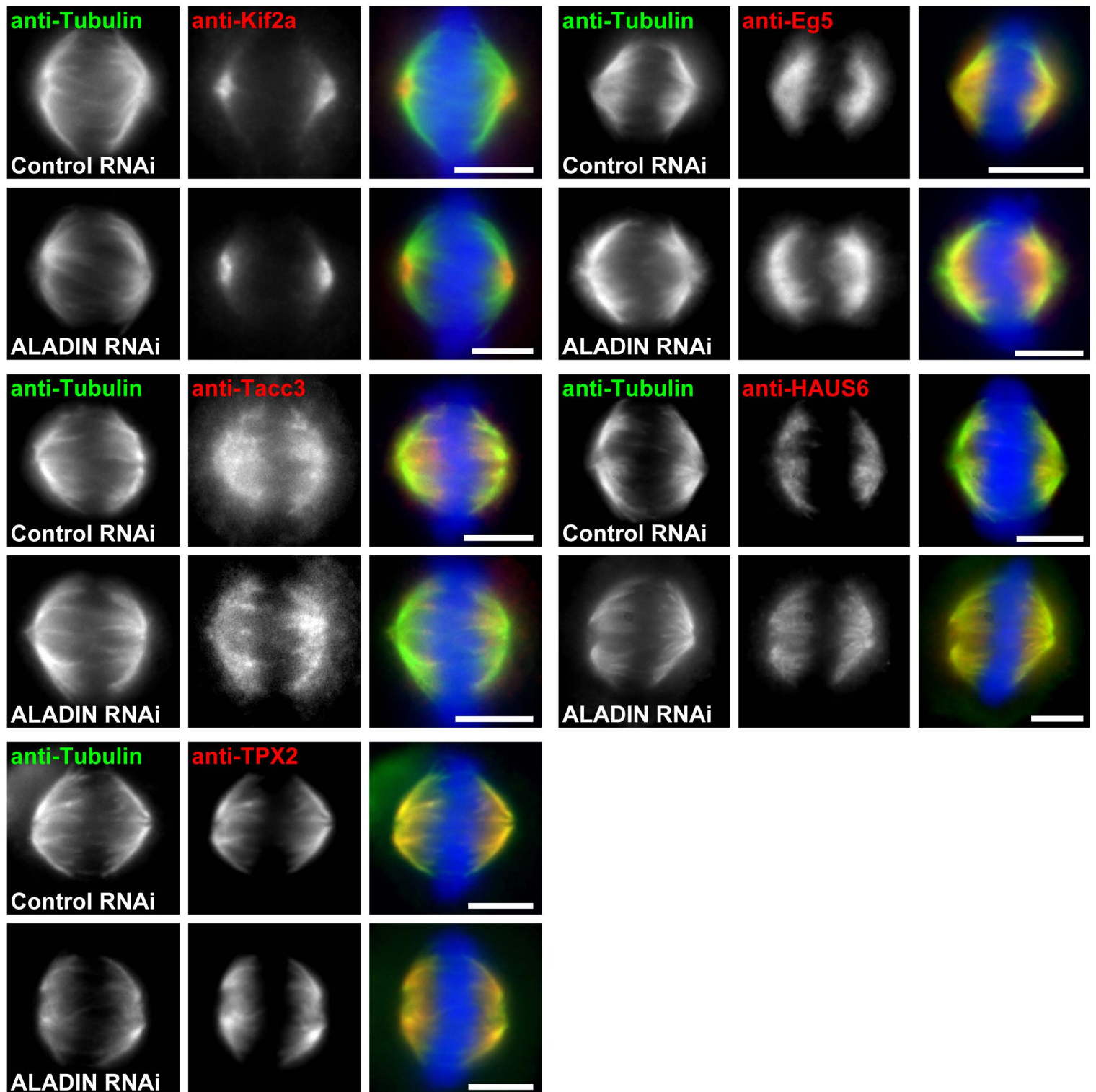
Carvalho et al.



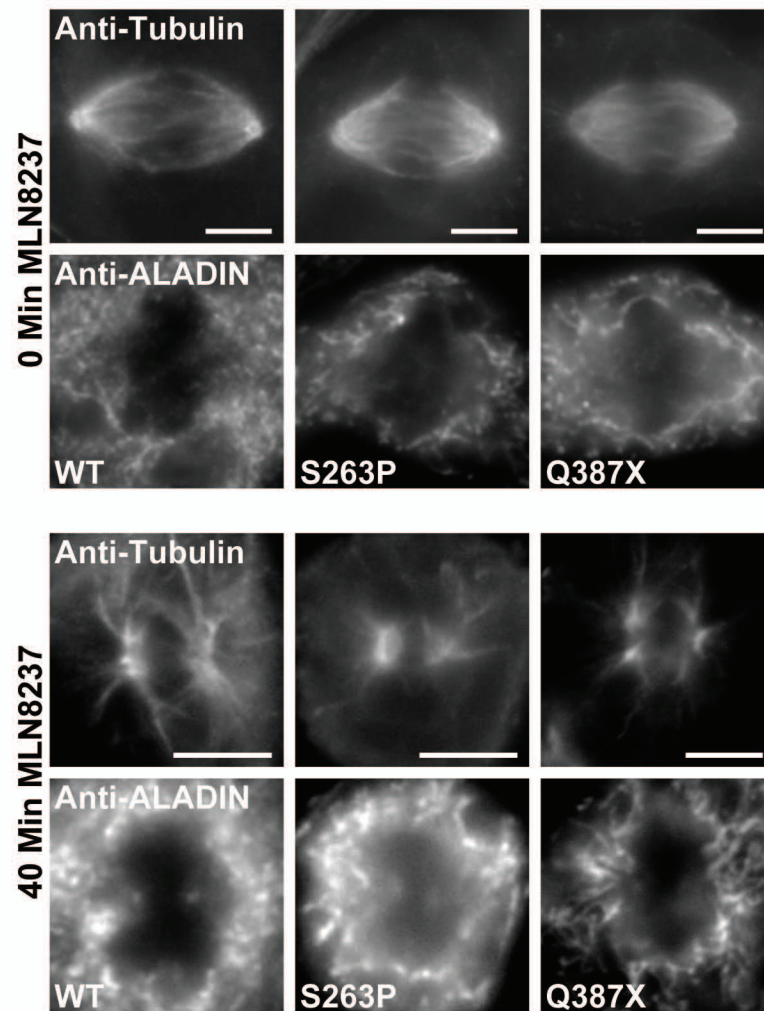
**Supplementary Figure 1. Two separate dsRNAs effectively deplete ALADIN mRNA and shorten mitotic spindles.** A) A schematic diagram of the ALADIN mRNA with the regions targeted by the dsRNAs shown. B) Spindle lengths were measured for cells treated with the dsRNAs noted ( $n \geq 24$  spindles per treatment.  $***p < 0.001$ ). C) The amount of ALADIN mRNA in the dsRNA treated cells was measured. ALADIN mRNA levels were normalized against Rpl32, and the amount relative to the control mean is shown. Error bars show  $\pm$  standard deviation.



**Supplementary Figure 2. ALADIN depletion or over expression reduces the levels of active pT288 Aurora A on centrosomes, but does not affect the amount of NuMA or Aurora A protein in cells.** A) Representative images of the cells treated with control and ALADIN specific siRNAs that are quantitated in Figure 6D. B) Whole cell lysates from cells treated with the indicated siRNAs were probed with an anti-ALADIN antibody before being cut, stripped, and probed with anti-NuMA (upper) and anti-Aurora A antibodies. We did not detect any reduction of NuMA or Aurora A in the cells depleted of ALADIN. C) Cells were transiently transfected for 48 hours with plasmids to overexpress GFP or GFP-ALADIN from a CMV promoter. Cells were then fixed and stained to visualize active Aurora A and the total amount of this protein that was coincident with pericentrin was measured for all GFP positive cells (D;  $n \geq 67$  spindles per condition). Scale Bars = 5  $\mu\text{m}$ . \*\*\* $p < 0.001$ .



**Supplementary Figure 3. The localizations of a subset of Aurora A substrates are affected by ALADIN depletion.** Cells treated with control and ALADIN specific siRNAs were fixed and stained with the indicated antibodies. Scale bar = 5  $\mu$ m.



**Supplementary Figure 4. The ALADIN Q387X mutant protein does not respond to Aurora A inhibition as the wild-type and S263P proteins do.** Fibroblasts expressing the given forms of ALADIN were untreated or treated with MLN8237 for 40 minutes to inhibit Aurora A, fixed, and then stained to visualize tubulin and ALADIN. Supplementary Figure 4. The ALADIN Q387X mutant protein does not respond to Aurora A inhibition as the wild-type and S263P proteins. Fibroblasts expressing the given forms of ALADIN were untreated or treated with MLN8237 for 40 minutes to inhibit Aurora A, fixed, and then stained to visualize tubulin and ALADIN. Scale bar = 5 $\mu$ m.