

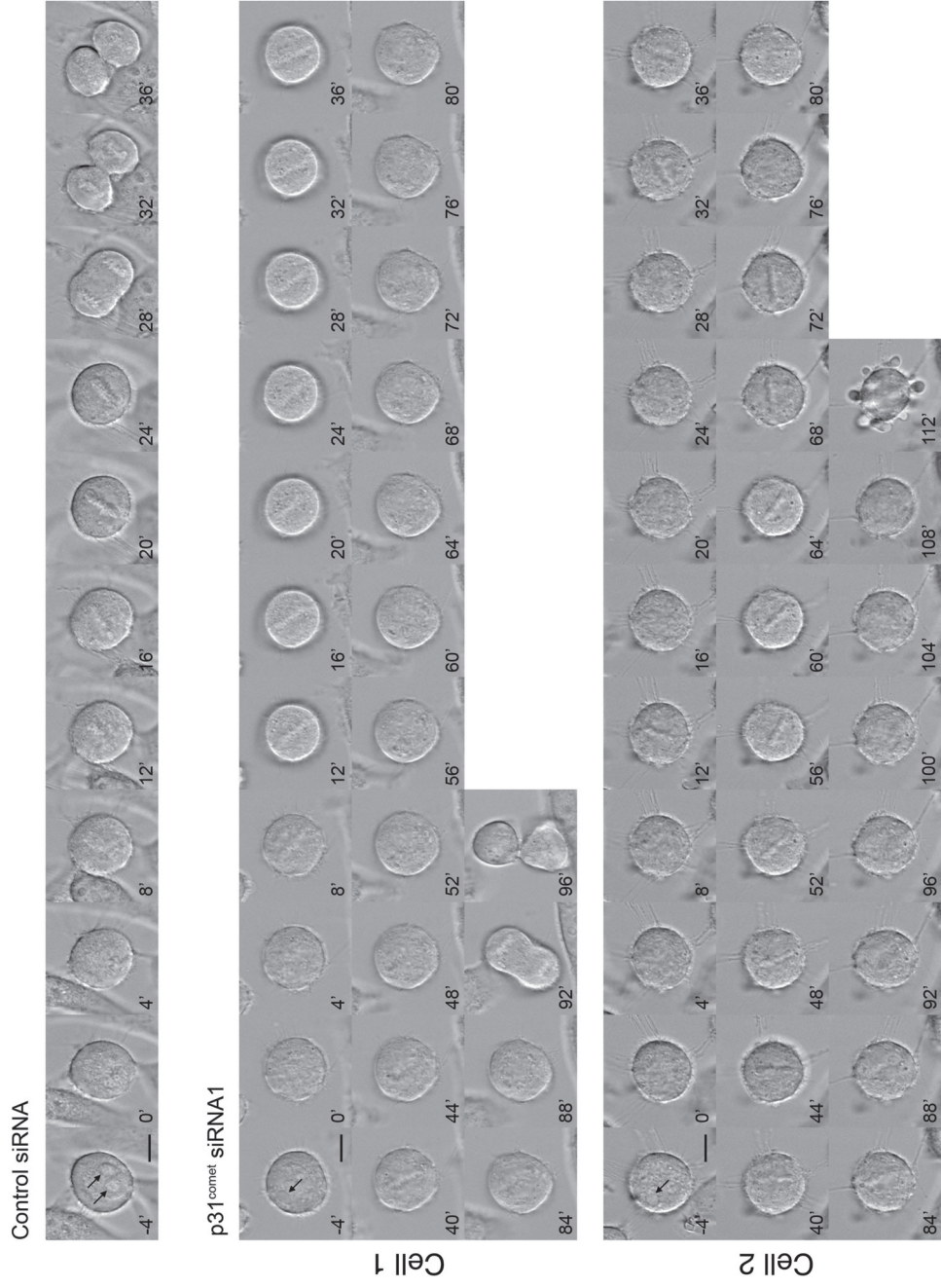
**Supplemental Figure Legends**

**FIGURE S1: p31<sup>comet</sup> is required for proper mitotic progression.** Differential interference contrast (DIC) images of representative cells transfected with control siRNA or p31<sup>comet</sup> siRNA1 at the indicated time points. NEBD is defined as time 0. Cell 1 transfected with p31<sup>comet</sup> siRNA1 experienced a metaphase delay, but eventually completed cell division. Cell 2 transfected with p31<sup>comet</sup> siRNA1 also experienced a metaphase delay, but underwent mitotic cell death. Arrowheads indicate nucleolus. Scale bar, 5  $\mu$ m.

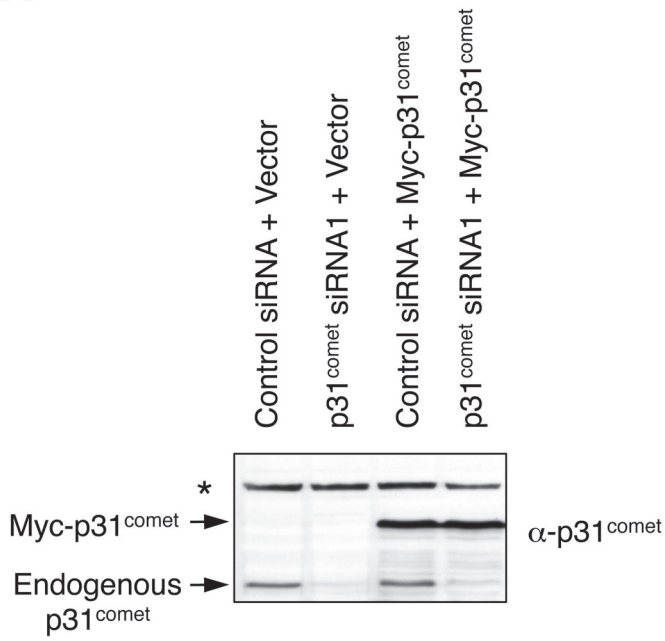
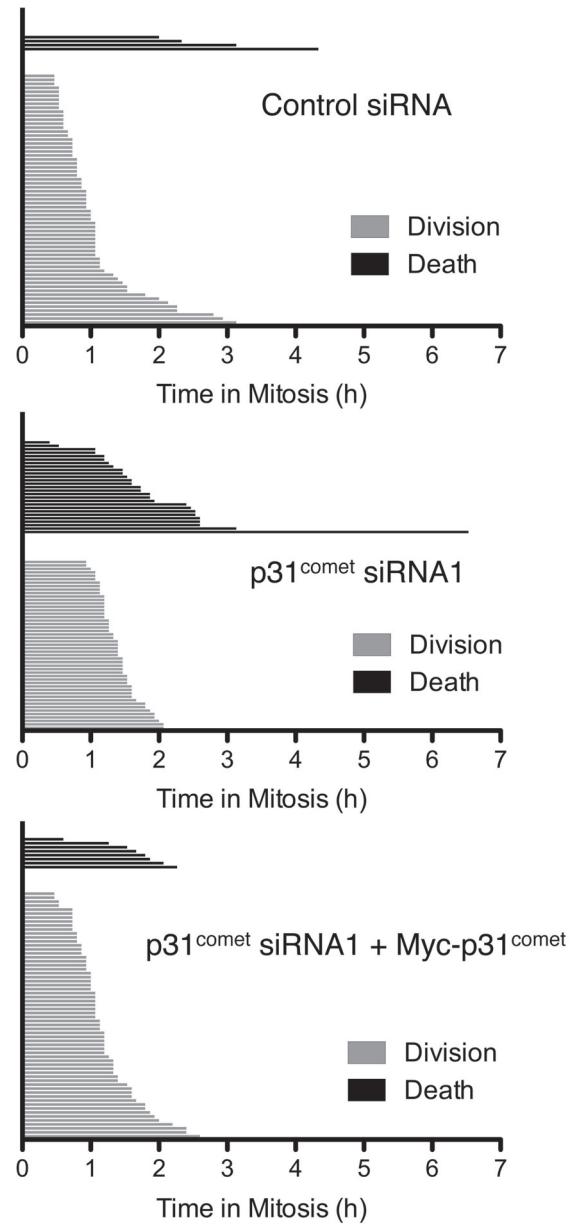
**FIGURE S2: Ectopic expression of siRNA-resistant Myc-p31<sup>comet</sup> rescues the mitotic delay and death phenotypes of p31<sup>comet</sup> RNAi cells.** (A) HeLa Tet-on cells were transfected with either empty vector or siRNA1-resistant Myc-p31<sup>comet</sup> plasmid. 6 hrs after plasmid transfection, cells were transfected with either 5 nM control siRNA or p31<sup>comet</sup> siRNA1. Lysates of cells at 48 hrs after siRNA transfection were blotted with anti-p31<sup>comet</sup>. The endogenous and Myc-p31<sup>comet</sup> bands are labeled. A cross-reacting band (indicated by an asterisk) is used as the loading control. (B) HeLa Tet-on cells seeded onto a 4-well chambered coverglass were transfected with either empty vector or siRNA1-resistant Myc-p31<sup>comet</sup> plasmid. 6 hrs after plasmid transfection, cells were transfected with either control siRNA or p31<sup>comet</sup> siRNA1. 48 hrs after siRNA transfection, live-cell imaging was performed with a DeltaVision microscope. The mitotic duration and fate (death or division) of each cell were plotted in waterfall plots.

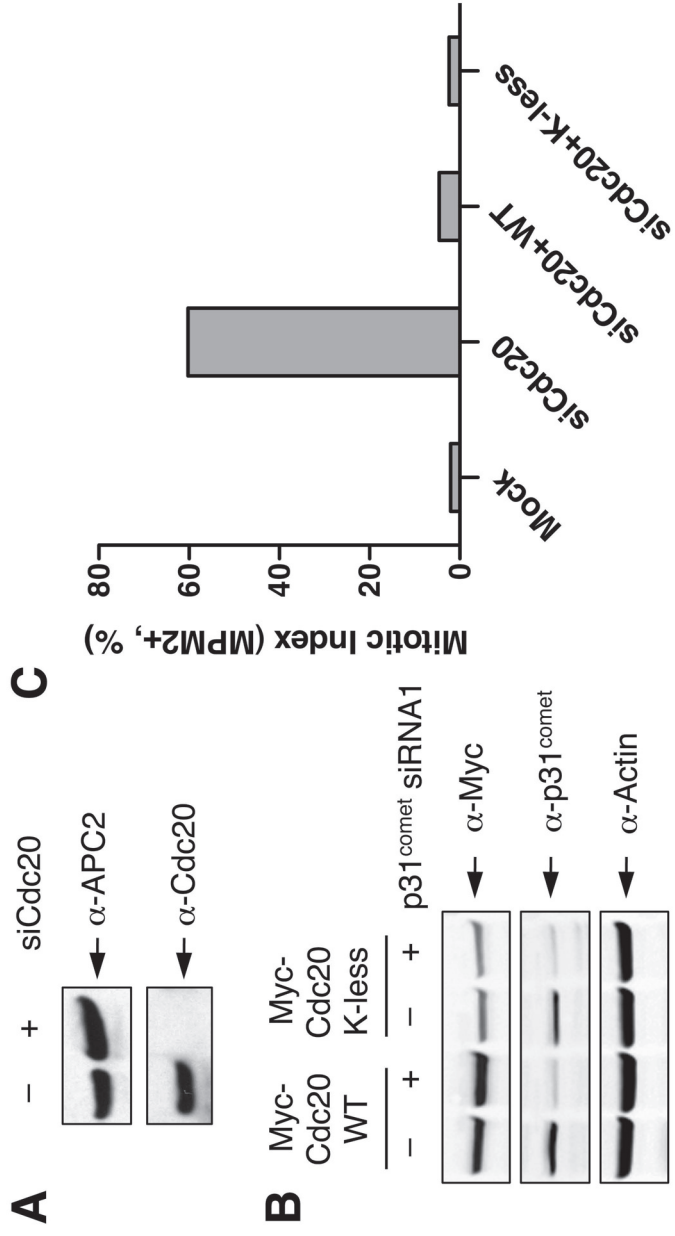
**Figure S3: Construction of stable HeLa Tet-on cell lines expressing Myc-Cdc20 WT or K-less.** (A) Lysates of HeLa Tet-on cells mock transfected or transfected with siCdc20 were

blotted with anti-Cdc20 and anti-APC2 (loading control). (B) HeLa Tet-on cells expressing Myc-Cdc20 WT (clone 11) or K-less (clone 9) were mock transfected or transfected with p31<sup>comet</sup> siRNA1. Lysates were blotted with the indicated antibodies. Note that Myc-Cdc20 WT was expressed at higher levels than Myc-Cdc20 K-less.



**Figure S1**

**A****B****Figure S2**



**Figure S3**