Supplementary information

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Supplementary Figure S1. HMG20b is required for the completion of cell division. (a) Western blot showing depletion of HMG20b in HeLa cells transfected with HMG20b siRNA. β-Actin is shown as a loading control. (b) Western blot showing depletion of HMG20b in HeLa cells transfected with control siRNA, and the individual HMG20b siRNAs #2 and #4, respectively. (c) Frequency of failure to complete cell division after transfection with the individual HMG20b siRNAs #2 and #4. Experiments were carried out as described in the main text. (d) Box-and-whisker plot showing time taken from anaphase onset to completion of cell division measured from time-lapse images after transfection with the individual HMG20b siRNAs #2 and #4. The median value is shown; the bottom and top of the box represent the 25^{th} and 75^{th} percentile respectively. Experiments were carried out as described in the main text.

Supplementary Figure S2. Increased interaction between BRCA2 and HMG20b during mitosis. Extracts were prepared from cells enriched in early mitosis (by nocodazole exposure, lanes 2 and 6) or in cytokinesis (by Purvalanol A treatment after nocodazole exposure, lanes 3 and 7), and immunoprecipitated with anti-BRCA2 antibody. HMG20b or RAD51 co-immunoprecipitating with BRCA2 were detected by western blotting. A lower exposure of the blot (lower panel) shows BRCA2 in the immunoprecipitates. Cyclin B levels mark cell cycle progression.

Supplementary Figure S3. GST-BRCA2 fragments (whose span is shown in Figure 2) were incubated with 293T extracts expressing Flag-HMG20b and the bound proteins were analyzed by western blotting ('GST pull-down assay'). (a)

B2-4 fragment binds to HMG20b. A longer exposure of the blot (compare with Figure 2b) shows that the B2-4 fragment binds to endogenous HMG20b as well as Flag-HMG20b. (b) Coomassie Blue staining of purified GST-BRCA2 fragments 1-9 used in Figure 2b. (c) Coomassie Blue staining of purified GST-B2-4 sub-fragments used in Figure 2c. These results show the sample loading controls for the experiments in Figure 2b-c.

Supplementary Figure S4. BRC5 over-expression inhibits the completion of cell division. (a) Two additional, independently-derived clones of HeLa TetOn cells expressing Myc-BRC5 (5.8 and 5.12) were analysed for bi- or multinucleated cell formation after treatment with Doxycycline for the indicated time. The mean ± SEM from 3 independent experiments is shown. Over 500 cells were enumerated in each sample. (b) Time taken from anaphase onset to the completion of cell division was measured by time-lapse imaging and plotted in a box-and-whisker graph. The median value is shown; the bottom and top of the box represent the 25^{th} and 75^{th} percentile respectively. (c) A western blot showing the expression of Myc-BRC5 in each clone after treatment with Doxycycline. β-Actin is shown as a loading control.

Supplementary Figure S5. Defects in cell division provoked by BRCA2 depletion. (a) Western blot showing depletion of BRCA2 in HeLa (Kyoto) cells transfected with individual BRCA2 siRNA sequences. Two siRNA sequences used by Lekomtsev et al 2010 (Qiagen #6, and Invitrogen #7) are boxed. GAPDH is shown as a loading control. (b) Frequency of failure to complete cell division after transfection with individual BRCA2 siRNA sequences. Cells that did not complete cytokinesis by the end of the observation period (6 hrs), or formed bi- or multi-nucleated cells, were classified as having failed to complete cell division. One-way ANOVA by the Kruskal-Wallis test was used for the statistical analysis. (c) Box-and-whisker plot showing the time taken from anaphase onset to the

completion of cell division measured from time-lapse images after transfection with individual BRCA2 siRNA sequences. The median value is shown; the bottom and top of the box represent the 25th and 75th percentile respectively. A gap was introduced into the Y-axis to enable comparison. Each BRCA2 siRNA was compared with control (Luciferase) siRNA using 1 way ANOVA, Dunnett's multiple comparison test.

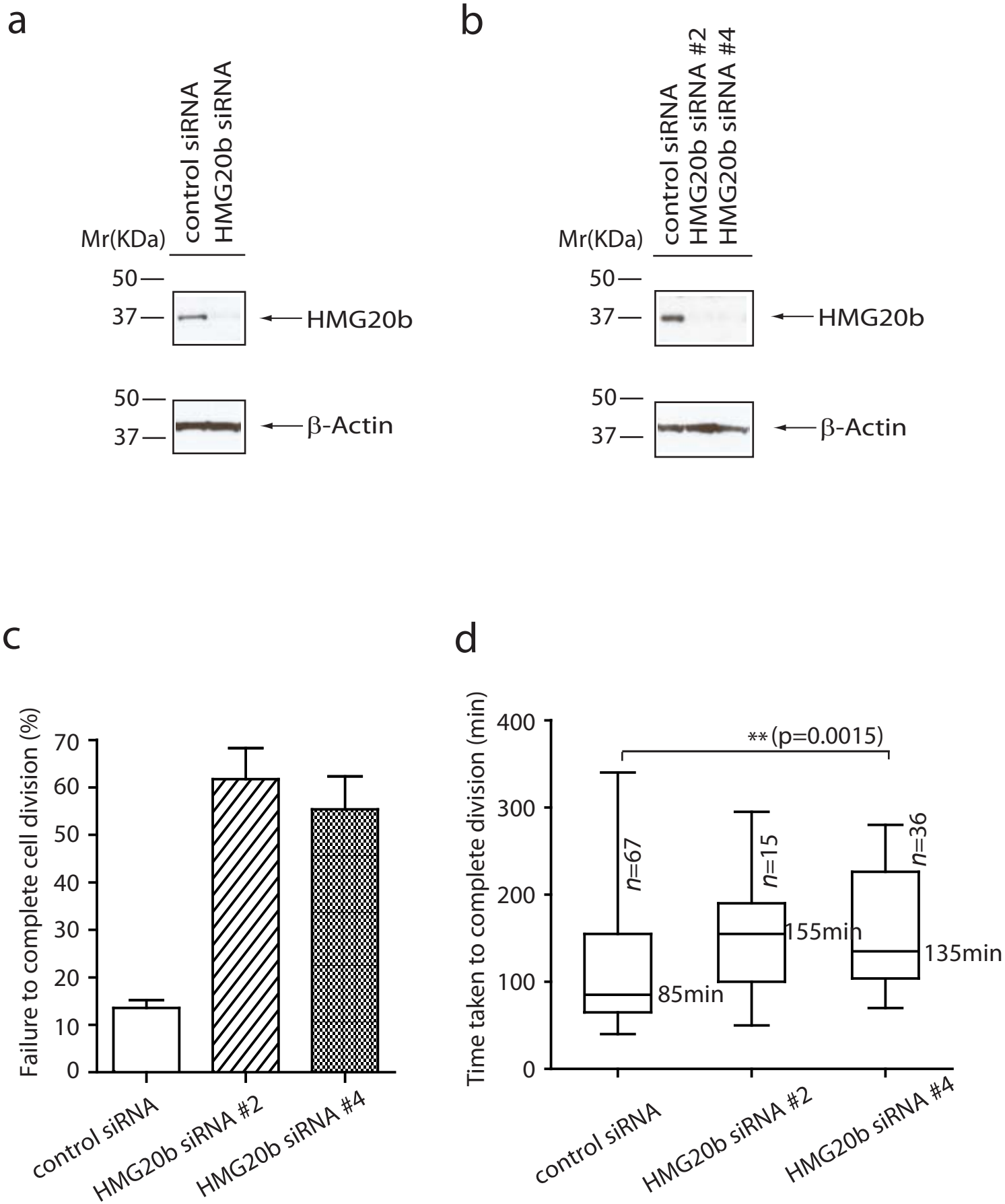
Supplementary Figure S6. Anti-BRCA2 staining at the cytokinetic midbody is reduced by BRCA2 depletion. Mean intensity of BRCA2 immunostaining with mouse mAb 5.23 (Millipore) was measured in control and BRCA2 siRNA transfected cells. The mean ± SEM is shown for (a) interphase nuclei and (b) late anaphase midbodies. (c) Representative images showing anti-BRCA2 (green) nuclear staining in interphase cells (upper 2 rows) and a late anaphase midbody (lower 2 rows), after exposure to control or BRCA2 siRNA sequences. Midbody staining is marked with yellow arrows. Aurora B (in red) was co-stained as a midbody marker. Scale bars are 10 μm (upper 2 rows) and 5μm (lower 2 rows), respectively.

Supplementary Movie 1. Time-lapse imaging of a GFP-H2B expressing HeLa cell transfected with control siRNA shows normal cell division.

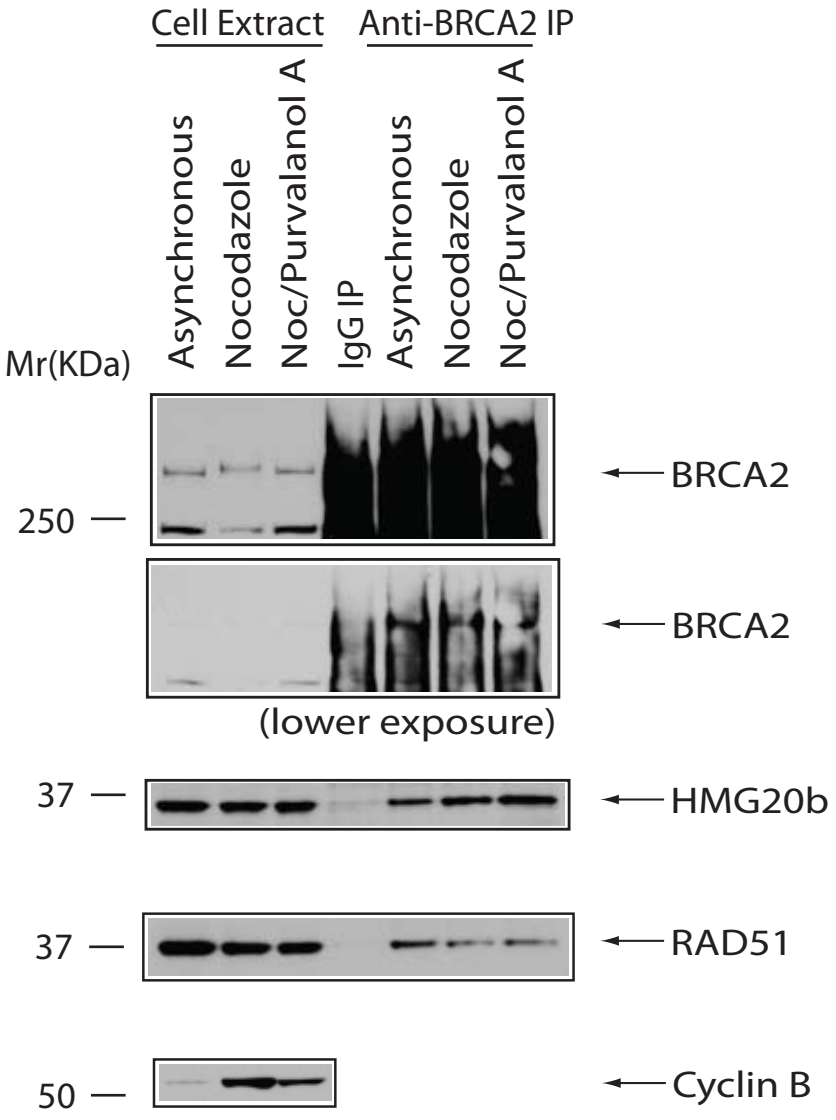
Supplementary Movie 2. Time-lapse imaging of an HMG20b-depleted HeLa cell (expressing GFP-H2B) which undergoes furrow ingression but experiences rapid oscillation of nuclei between emerging daughter cells and finally forms a bi-nucleate cell.

Suppplementary Movie 3. Time-lapse imaging of Myc-BRC5 transfected HeLa TetOn cell clone 5.10 before Doxycycline-induced expression of Myc-BRC5 shows normal cell division.

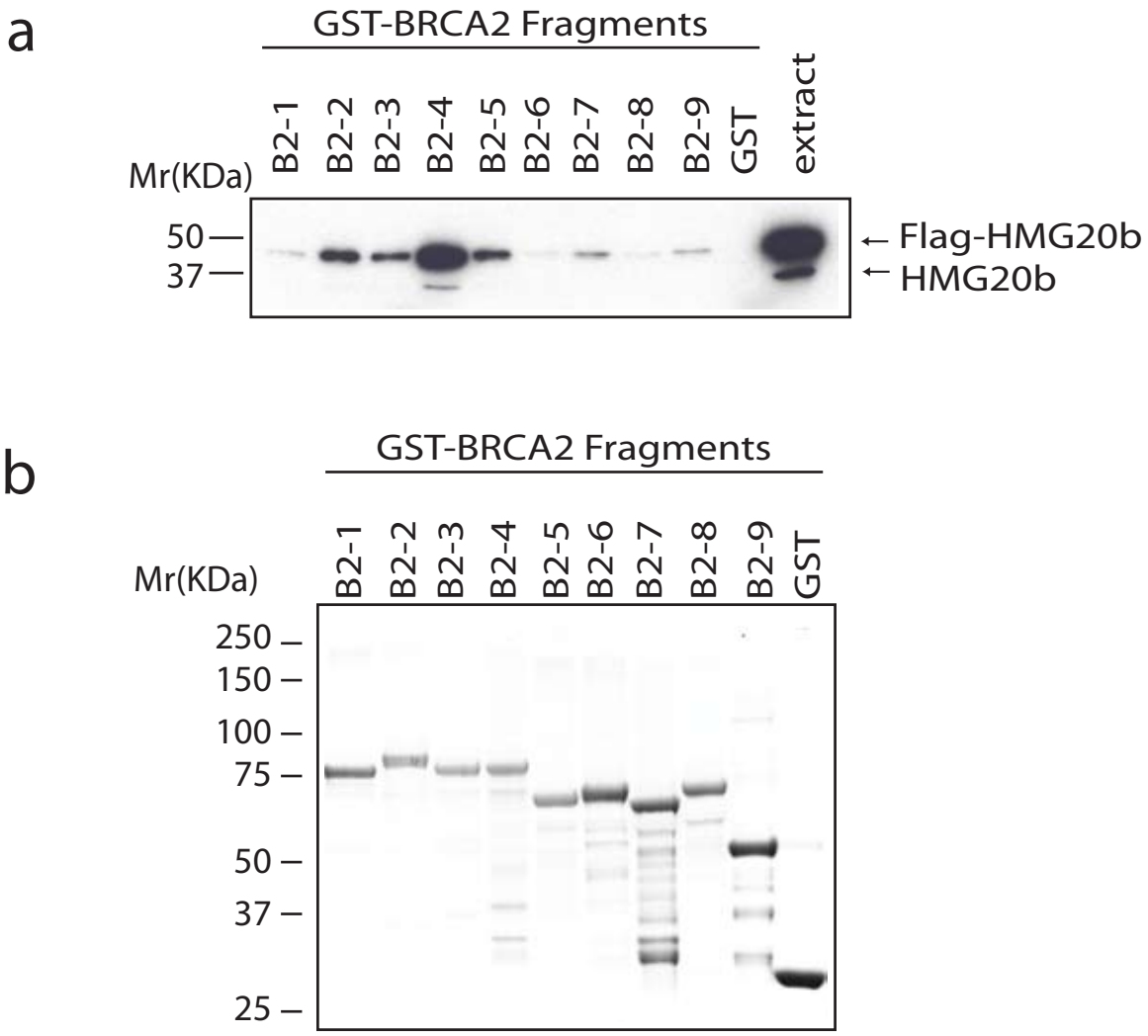
Supplementary Movie 4. Time-lapse imaging of Doxycycline-treated HeLa TetOn clone 5.10 expressing Myc-BRC5 shows a delay in cell division.



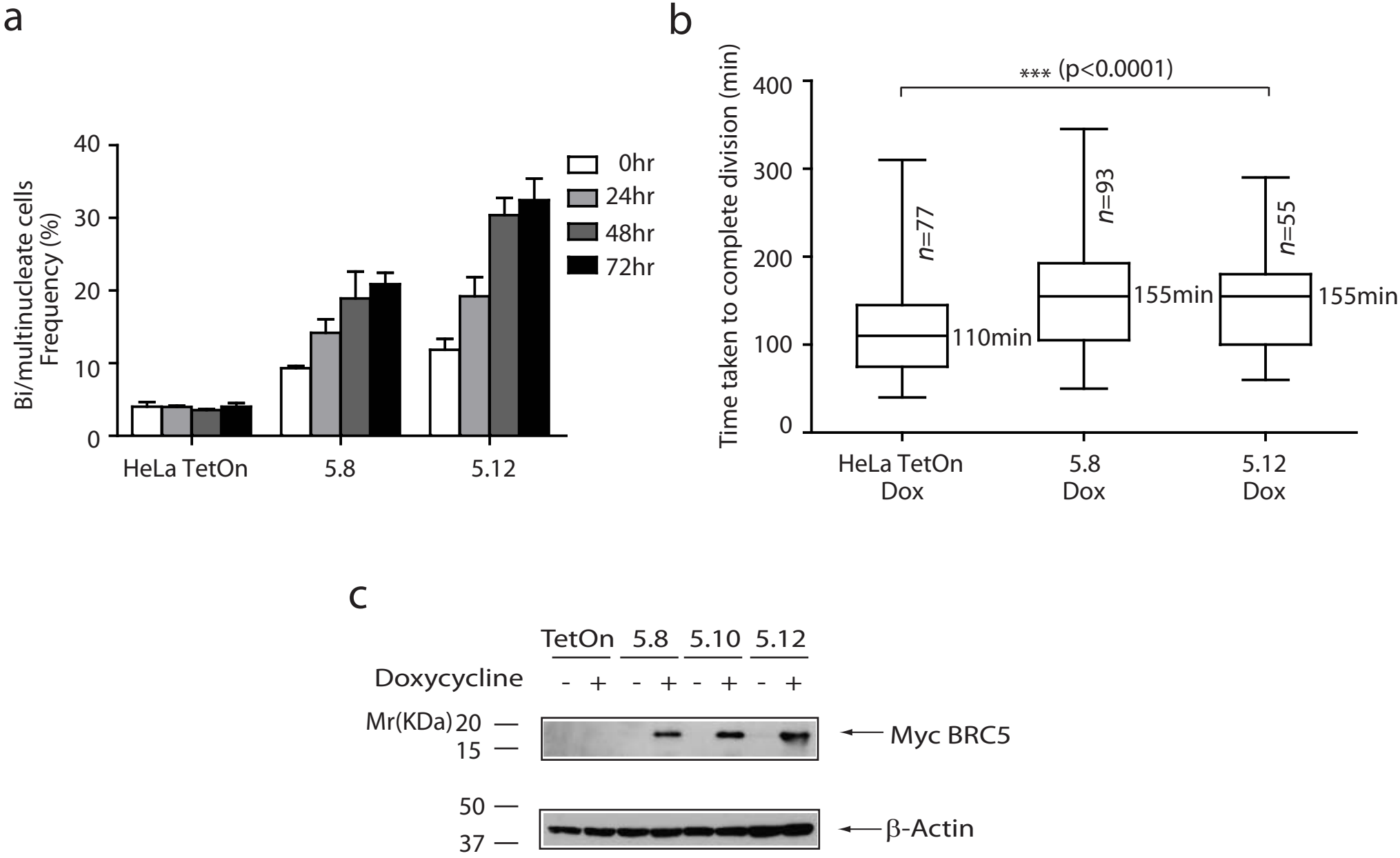
Supplementary Figure S1, Lee



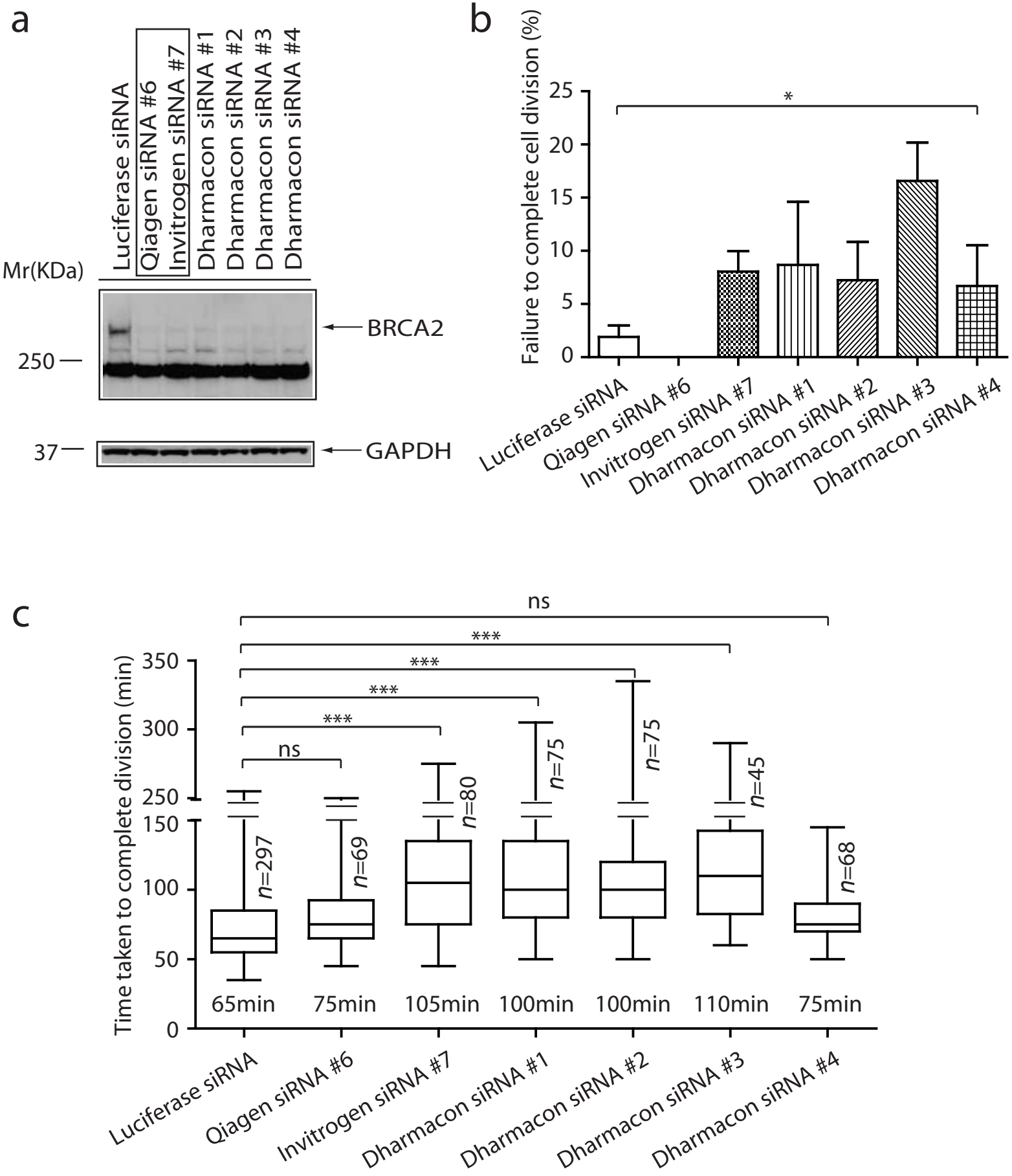
Supplementary Figure S2, Lee



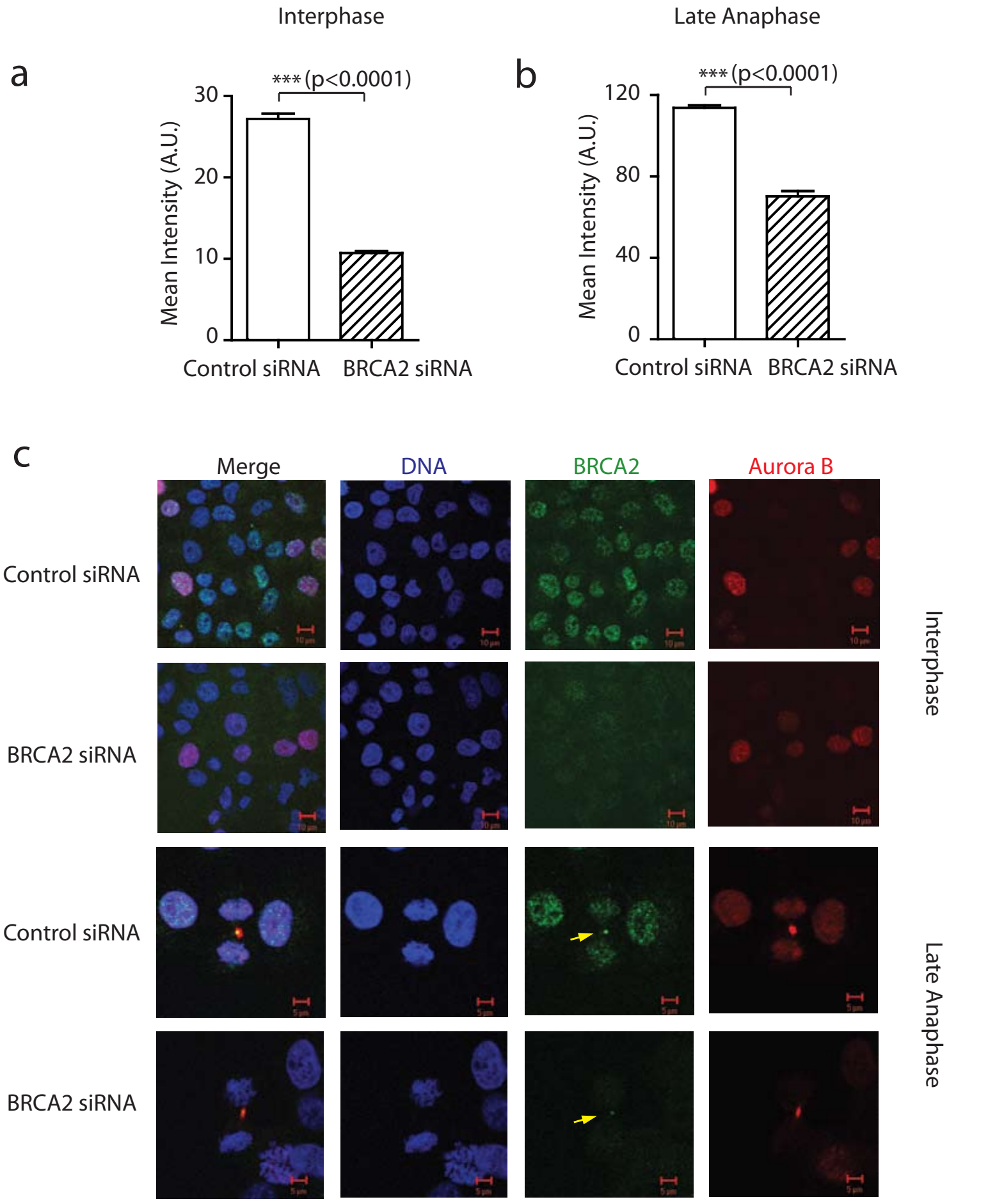
Supplementary Figure S3, Lee



Supplementary Figure S4, Lee



Supplementary Figure S5, Lee



Supplementary Figure 6, Lee