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Supplemental Material to:

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Calmodulin protects Aurora B on the midbody to regulate the fidelity of cytokinesis

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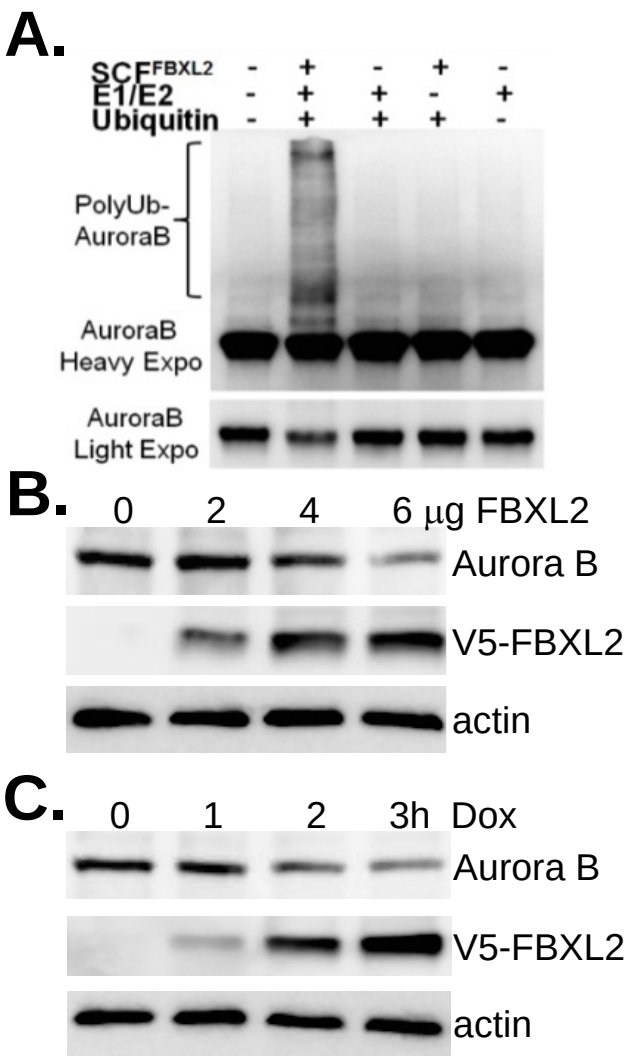
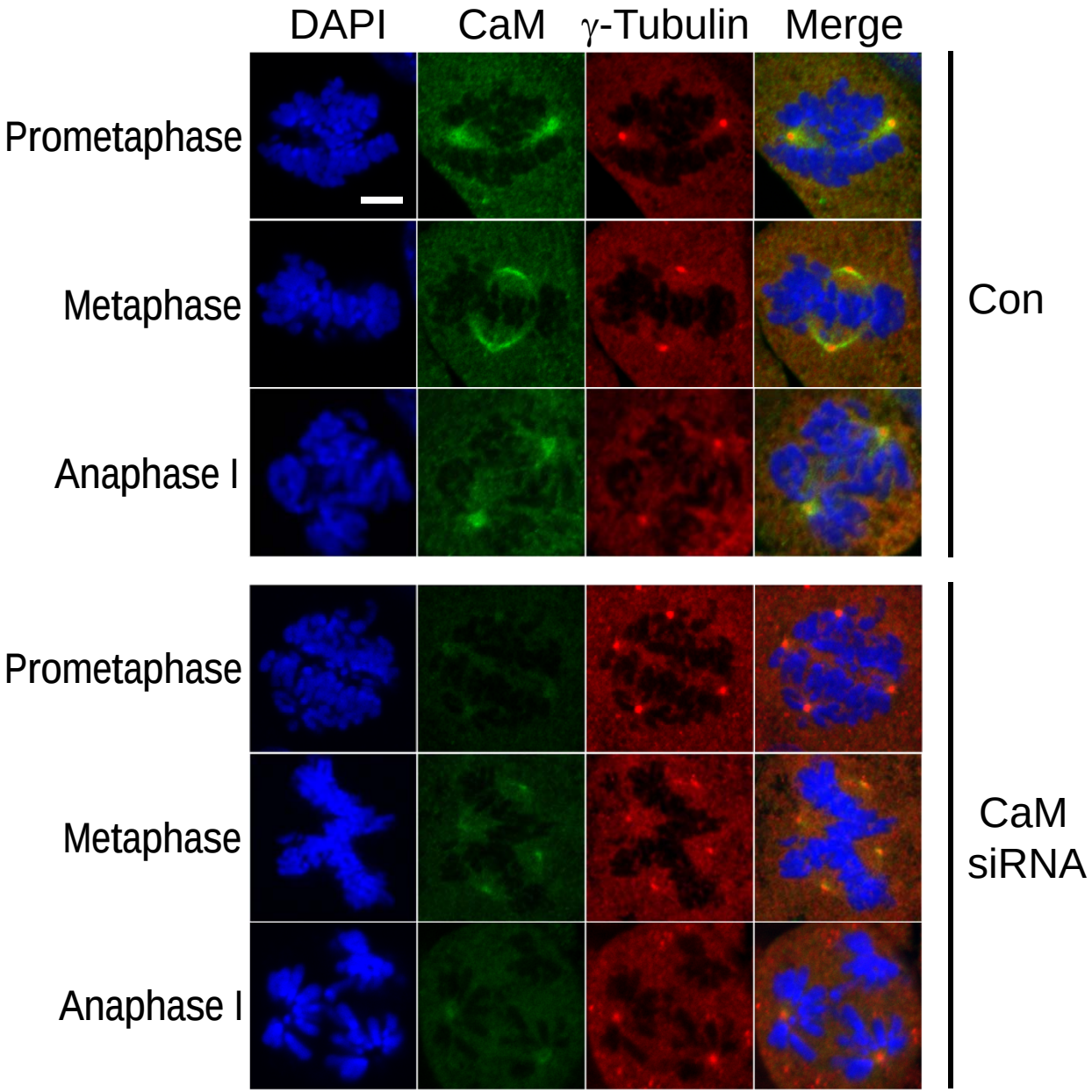
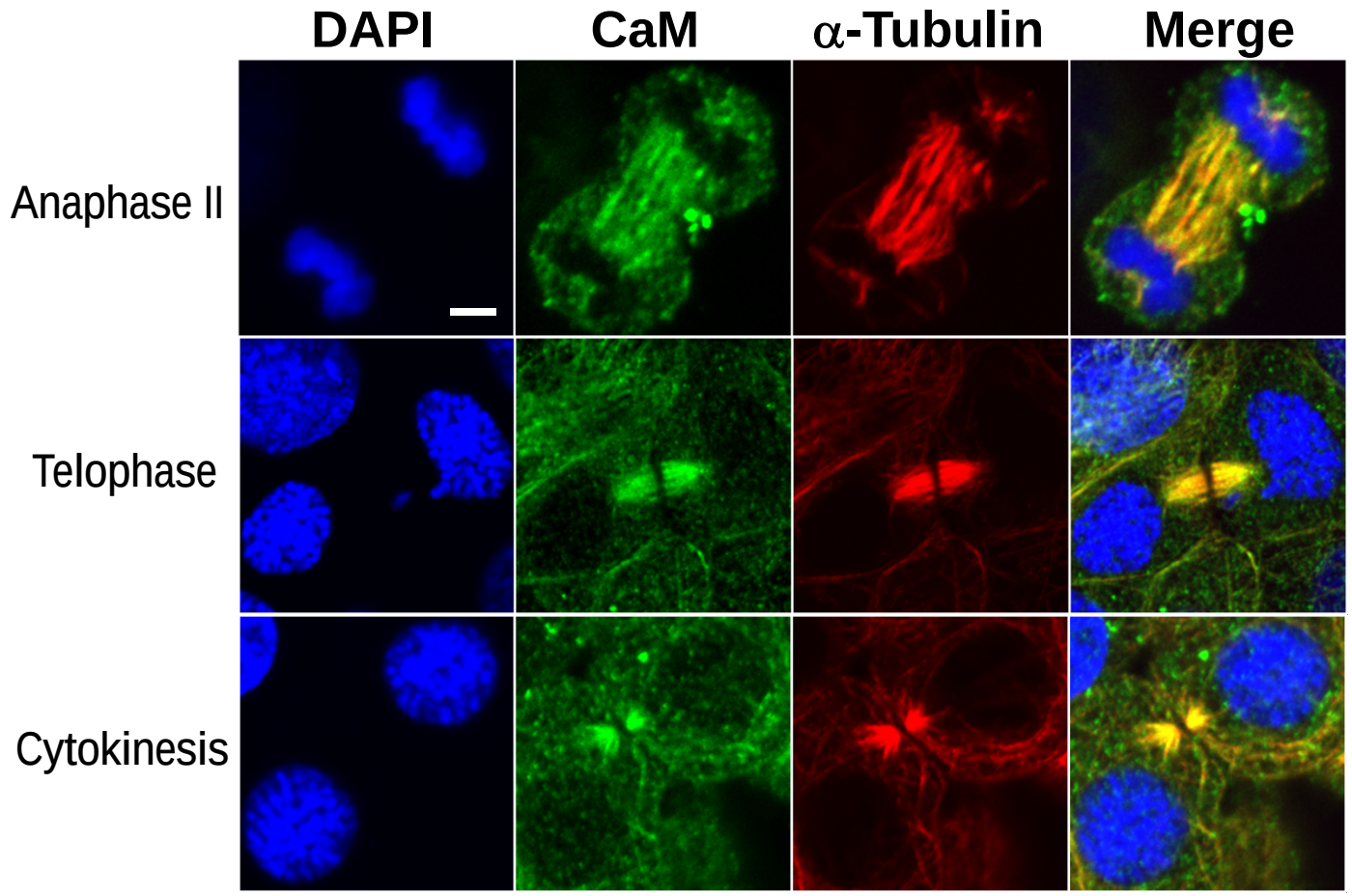


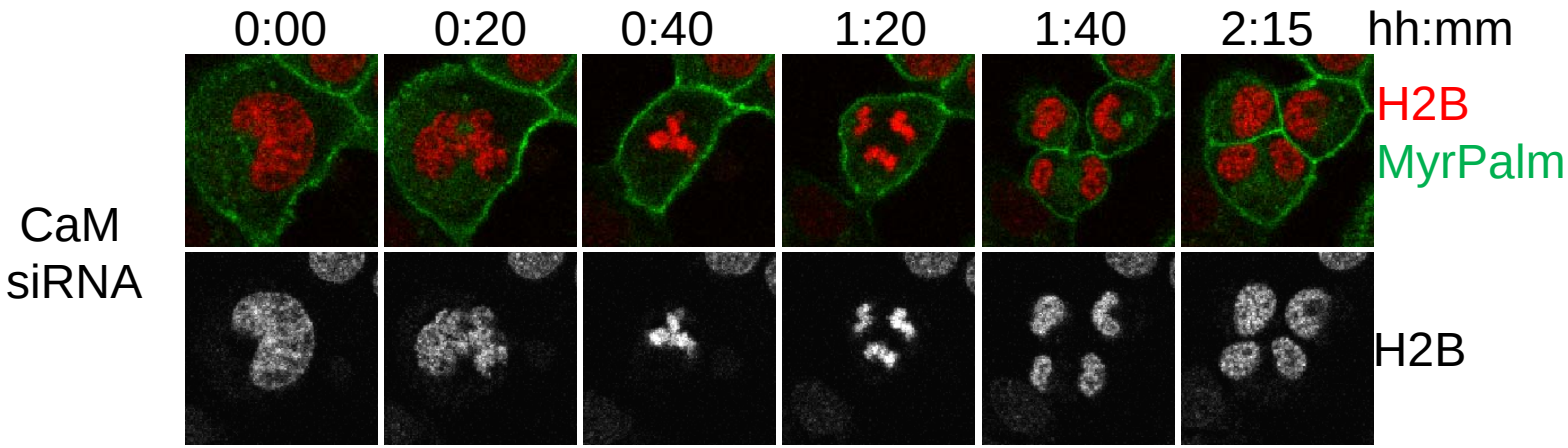
Supplementary Figure 1. A. *In vitro* ubiquitination assays. Purified SCF complexes were incubated with V5-Aurora B and full complement of ubiquitination reaction components. **B.** MLE cells were transfected with increasing amount of FBXL2 plasmid. Cells were collected and cell lysates were analyzed for V5-FBXL2, Aurora B and β-actin immunoblotting. **C.** MLE cells were transfected with an inducible FBXL2 plasmid under control of exogenous doxycycline. Cells were treated with doxycycline for various times, cells were then collected and cell lysates were analyzed for V5-FBXL2, Aurora B and β-actin by immunoblotting.



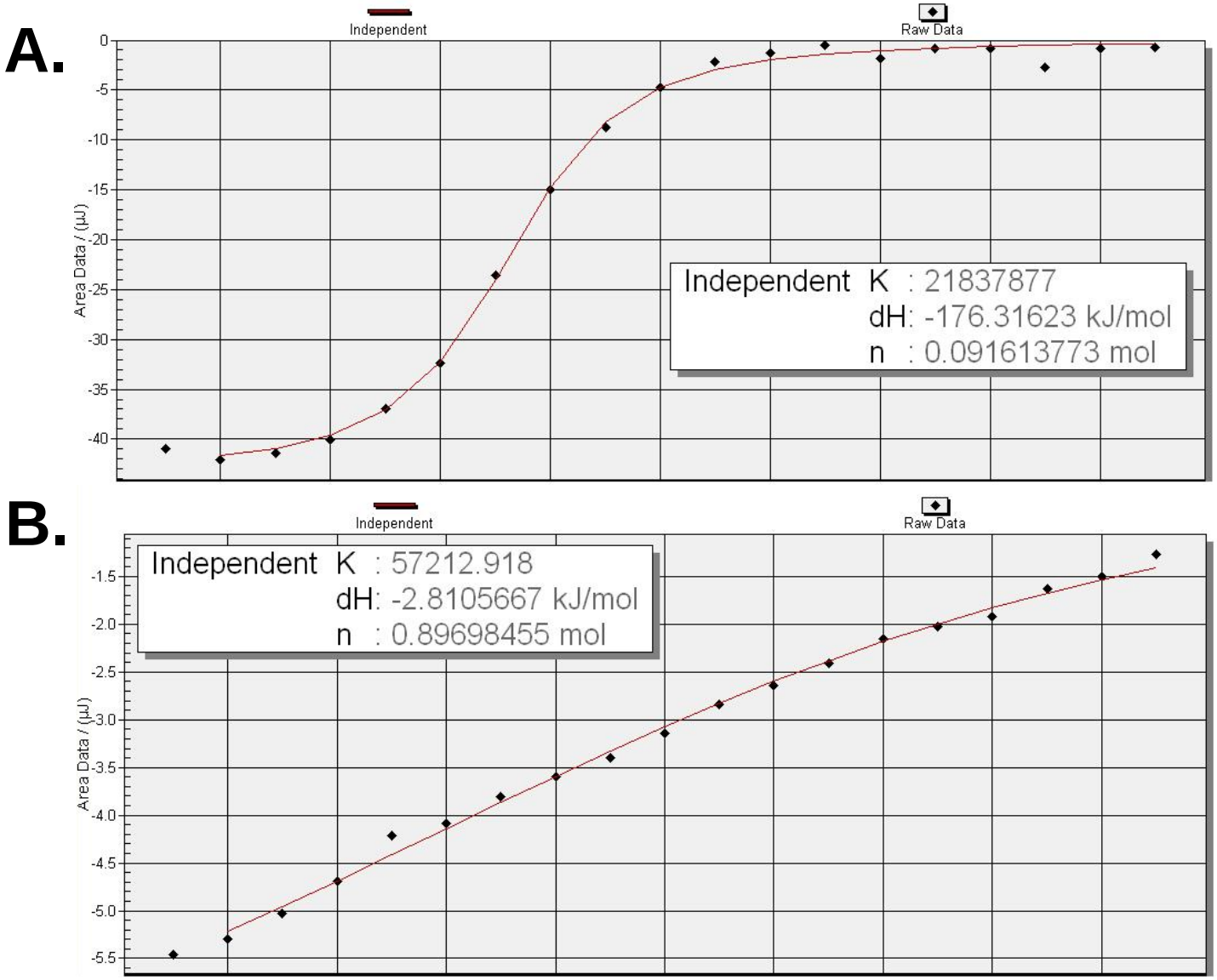
Supplementary Figure 2. MLE cells were transfected with either con RNA or CaM siRNA for 48h. Cells were then washed with PBS and fixed with 4% paraformaldehyde for 20 min. Cells were co-immunostained for CaM and γ-Tubulin as centrosome marker. Nuclei were counterstained using DAPI. Green: CaM, Red: γ-Tubulin, Blue: DAPI. White scale bar indicates 2 μm.



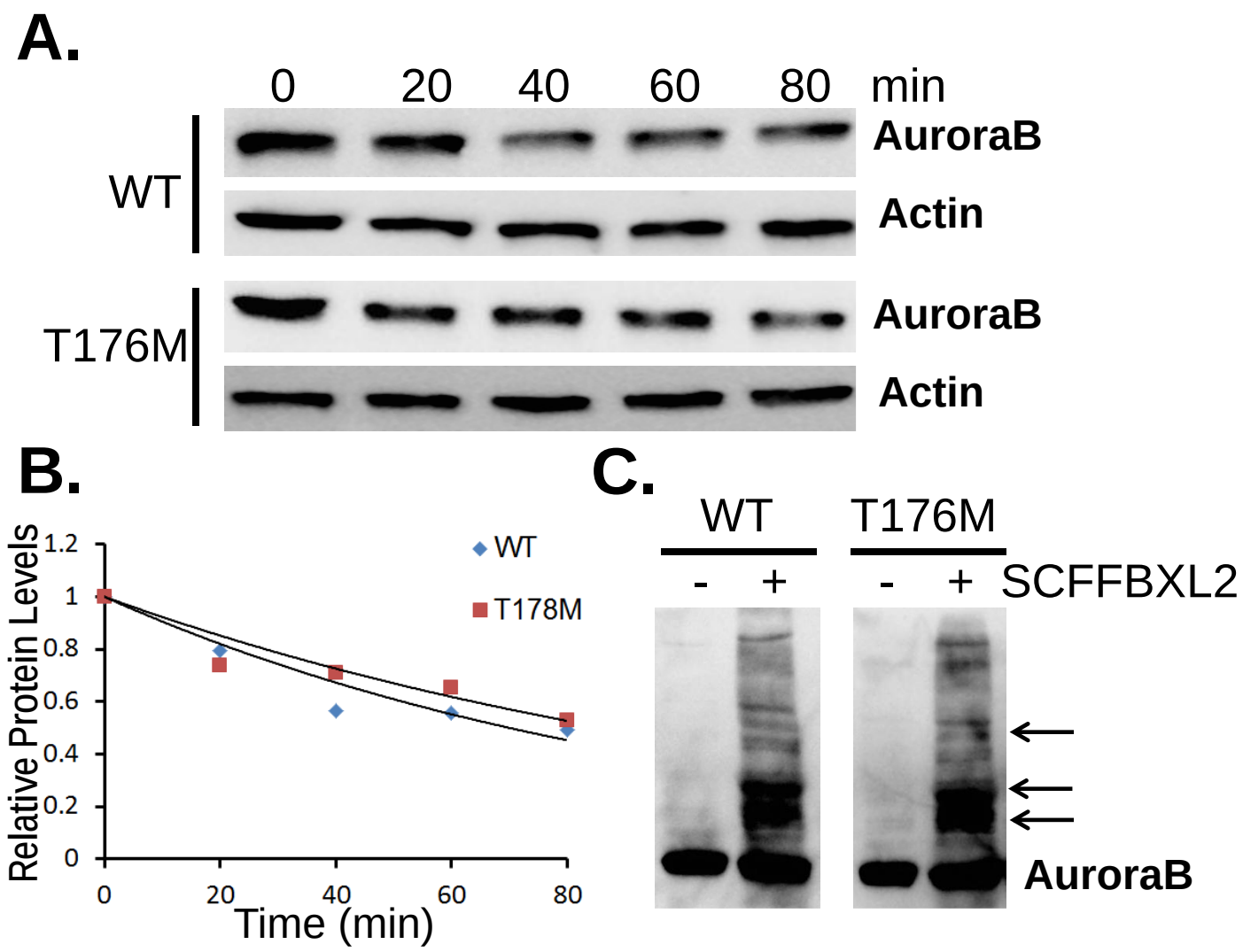
Supplementary Figure 3. MLE cells were plated for 48h. Cells were then washed with PBS and fixed with 4% paraformaldehyde for 20 min. Cells were co-immunostained for CaM and α-Tubulin. Nuclei were counterstained using DAPI. Green: CaM, Red: α-Tubulin, Blue: DAPI. White scale bar indicates 2 μm.



Supplementary Figure 4. Expression of CaM siRNA leads to multi-polar spindle formation (0:40) and binucleate cell formation in MLE cells expressing H2B-mCherry and MyrPalm-mEGFP.



Supplementary Figure 5. A. ITC binding analysis of CaM and Calmidazolium *in vitro* (n=2 experiments). **B.** ITC binding analysis of AuroraB peptide (LQKSRTFEDQR) encoding a CaM-binding motif binding with Calmidazolium saturated CaM *in vitro* (n=2 experiments).



Supplementary Figure 6. A. AuroraB protein half-life determination after expression of WT V5-AuroraB, point mutant (*n=2* experiments). **B.** Endogenous AuroraB protein levels were quantitated by imageJ software. The levels of AuroraB are expressed as the percent of the initial levels present at time zero. **C.** *In vitro* ubiquitination assays. Purified SCF complex were incubated with AuroraB WT or point mutant and the full complement of ubiquitination reaction components.