Huang et al., https://doi.org/10.1083/jcb.201706103

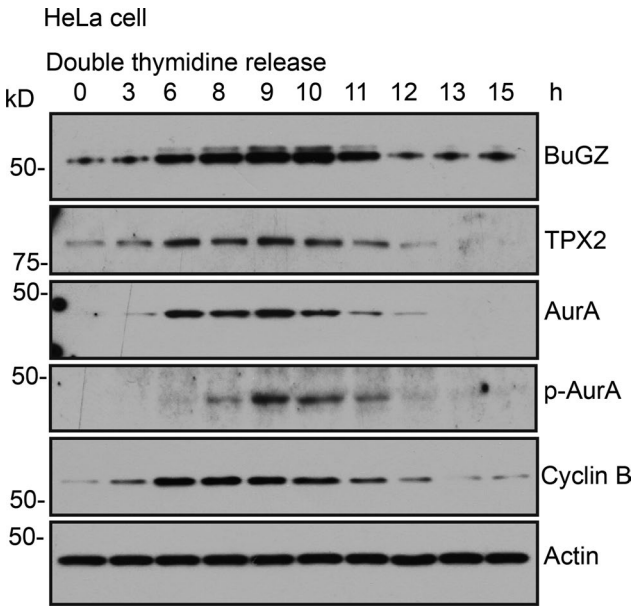

Figure S1. **Validation of cell synchronization.** After releasing from the double thymidine block, the cells were collected at the indicated time, and cell lysates were analyzed by Western blotting with the indicated antibodies.

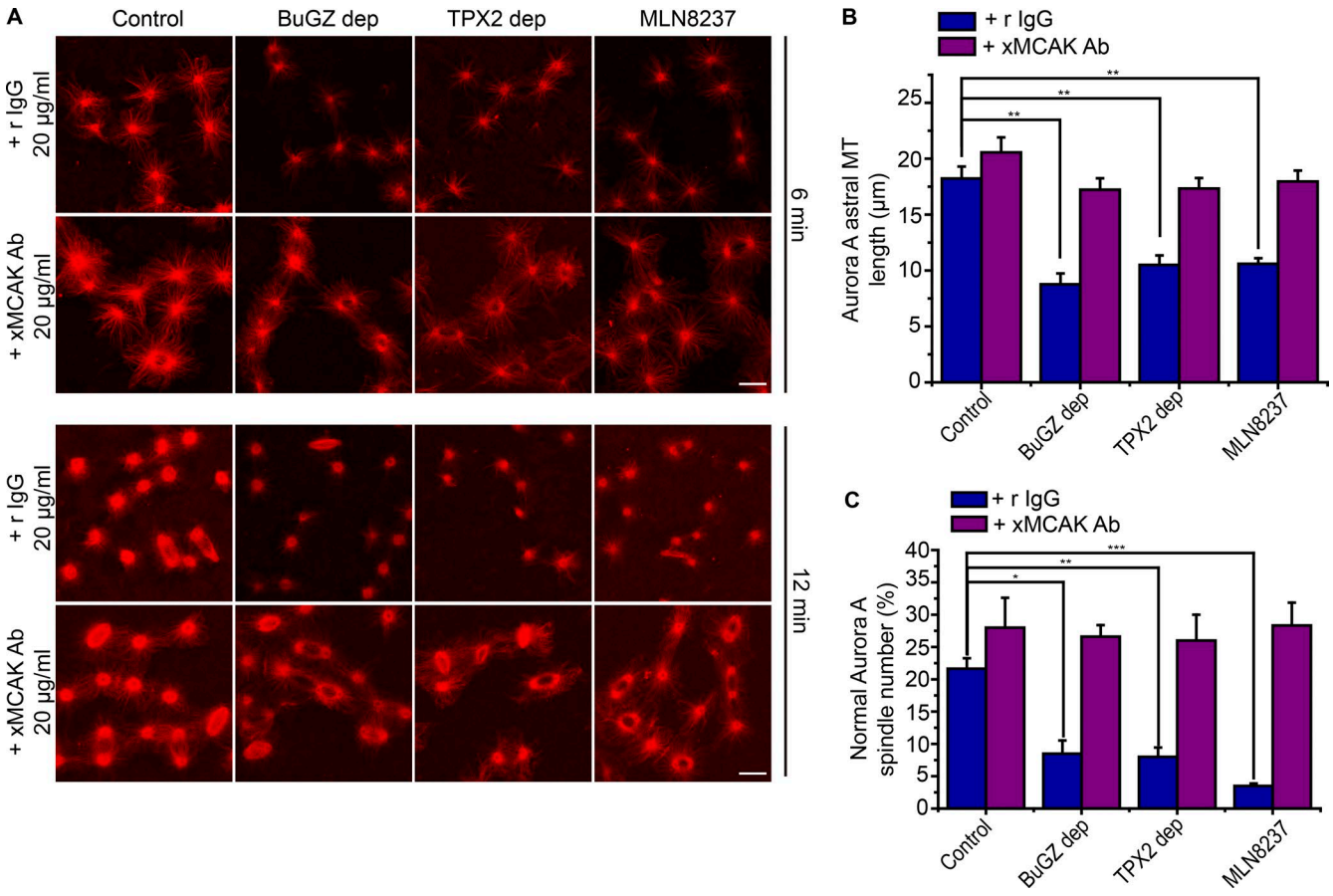

Figure S2. **xMCAK inhibition counteracts the effect of xBuGZ depletion in egg extracts.** (A) XEE was mock depleted (control), was depleted (dep) of xBuGZ or xTPX2, or was treated with AurA inhibitor MLN8237 and then used for an AurA bead assay in the presence of unimmunized control IgG or a functional blocking xMCAK antibody (Ab). Bars, 10 µm. (B and C) The lengths of MT asters or numbers of bipolar spindles were quantified as described in Fig. 2. Error bars show SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. r IgG, rabbit polyclonal IgG.

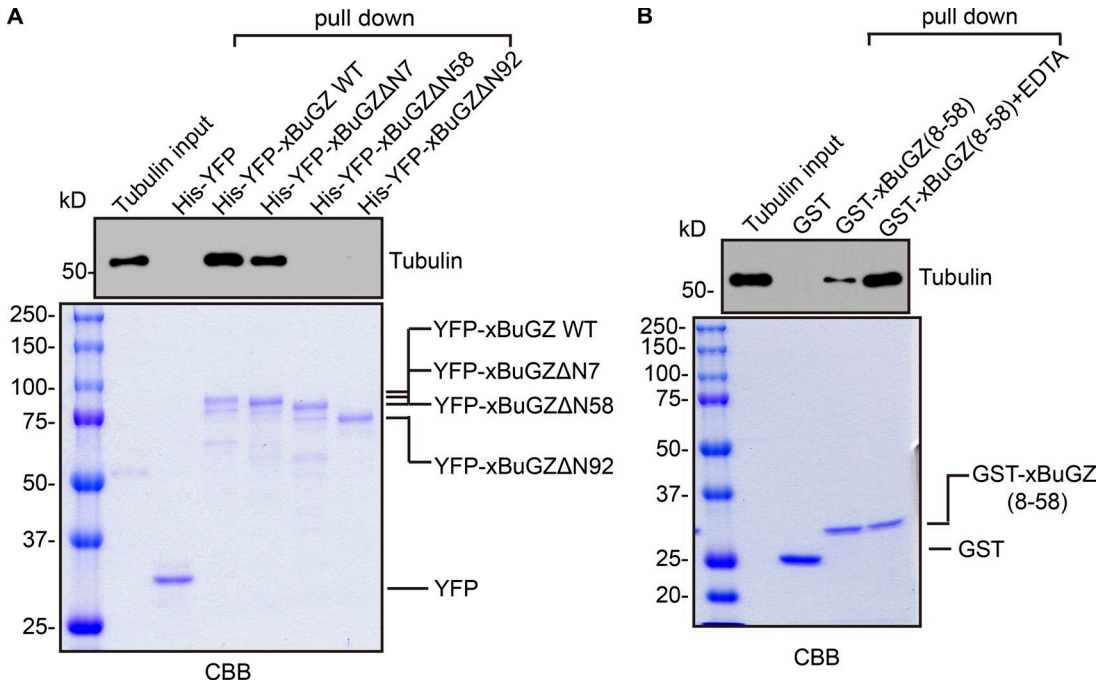

Figure S3. **Tubulin binding to BuGZ involves the first 58 amino acids, including the zinc finger sequence, and is reduced in the presence of EDTA.** (A) Purified tubulin was incubated with purified proteins as indicated followed by pulldown using antibody to YFP as well as Western blotting and Coomassie Brilliant blue (CBB) staining. (B) Purified tubulin was incubated with purified GST or GST-xBuGZ(8–58) in the presence or absence of EDTA. For Western blotting, 0.1% of total reaction and 10% of total pulldowns were analyzed. For Coomassie blue staining, 1% of the reaction and 10% of total pulldowns were analyzed.

Table S1 is a separate Excel file showing lists of protein expression constructs (including primers used in their construction) and antibodies used in this study.