



**Supplementary Figure 4** IST1 activation in vivo.

**(a)** Ribbon diagram showing the location of the mutated IST1 Ile60, Leu166 and Ile169 residues within the interface between  $\alpha 2$  and the autoinhibitory helix  $\alpha 5$ .

**(b)** Quantification of the cytokinesis defects from panel **c** (below), and western blots showing partitioning of wild type and mutant IST1 proteins between the soluble state (upper blot) and the insoluble membrane-bound/assembled state (lower blot). Quantified percentages of insoluble proteins are shown below the blot.

**(c)** Immunofluorescence images showing the midbody phenotypes of cells following designated siRNA treatments and re-expression of wild type IST1 or IST1<sub>160D</sub> (final two panels). Microtubules (anti- $\alpha$ -Tubulin, grey) and nuclei (SYTOX green) were stained for reference, and yellow arrowheads highlight midbodies. Scale bars are 10  $\mu$ m.