positive control). In contrast, none of the mutant IST1 proteins rescued the midbody arrest to any significant extent, and the percentages of HeLa cells with visible midbodies ranged from 17-22%, which was not appreciably different from IST1-depleted cells treated with the empty vector control. We therefore conclude that disruption of the interface between the autoinhibitory α5 helix and the core α2 helix inactivates IST1 abscission functions.

ESCRT-III proteins cycle between soluble and membrane-bound/assembled states, and ESCRT-III partitioning assays have been used to detect enrichment of post-translational modifications in the assembled state[5], disassembly of membrane-bound ESCRT-III complexes by VPS4 enzymes[5-7], altered protein interactions in assembled vs. soluble states[8], and release of ESCRT-III autoinhibition by C-terminal protein truncations[3]. We employed the partitioning assay to test whether the α5-α2 interface mutants caused IST1 to be enriched in the insoluble membrane-bound/assembled fraction of cell extracts, as would be expected if this mutation constitutively activates IST1. As shown in the western blots in Supplemental Fig. 4b, both endogenous and exogenous wild type IST1 proteins partitioned between the soluble and insoluble fractions, but the bulk of these proteins were in the soluble fraction (lanes 1 and 3, compare upper and lower panels, 42% and 30% insoluble, respectively). In contrast, all three of the α5-α2 mutations dramatically repartitioned IST1 into the insoluble fraction (lane 4-6, 85-95% insoluble). Thus, mutations predicated to disrupt binding of the autoinhibitory helix to the core repartitioned IST1 toward the membrane-bound/assembled state *in vivo*.

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