SUPPLEMENTARY DISCUSSION

Mutations in the IST1 α5-core interface inhibit cytokinesis and redistribute the protein to the insoluble/membrane-bound fraction

Truncated ESCRT-III proteins that lack autoinhibition typically redistribute, together with other ESCRT-III subunits, into "trapped" membrane-bound complexes. Such constructs are typically inactive and, indeed, often dominantly inhibit normal ESCRT-III functions (e.g., refs. [2,3]). As discussed in the main text, mutations that destabilize the interface between the autoinhibitory α5 helix and the core can constitutively activate CHMP3 proteins for assembly and inhibition of HIV-1 budding. We therefore tested whether analogous mutations in IST1 would also inhibit abscission and redistribute IST1 into the insoluble membrane-bound/assembled fraction of cell lysates.

We performed analyses of IST1 abscission functions by depleting endogenous IST1[4] and then testing the ability of siRNA-resistant IST1 constructs to rescue the midbody arrest (Supplemental Fig. 4). Three different IST1 mutant proteins were tested in these experiments ($IST1_{I60D}$, $IST1_{I169D}$, and the double mutant $IST1_{I60D, L166D}$). Each of these proteins harbored a different mutation that was predicted to disrupt the interface between the autoinhibitory α5 helix and the core α2 helix (see Supplemental Fig. 4a). As expected, siRNA depletion of endogenous IST1 increased the percentage of HeLa cells with visible midbodies from 4±1% to 20±3% (Supplemental Fig. 4b, compare lanes 1 and 2 and Supplemental Fig. 4c, panels 1 and 2). The midbody arrest defect was largely (though not entirely) rescued by re-expression of a wild type, siRNA-resistant IST1 construct (8±2%, lane/panel 3,

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