



**Fig. S1. (A)** U2OS cells transiently expressing RFP, survivin-RFP or survivin<sup>F61A,L64A</sup>-RFP, and LC3-GFP were treated with RAP (200 nM) and CQ (120  $\mu$ M) for 2h then imaged live. Mean number of GFP-LC3 puncta per cell was assessed per condition,  $n > 35$  cells, 3 independent experiments. All error bars indicate SEM. **\*\*** $P < 0.005$ , **\*\*\*\*** $P < 0.0001$ . **(B)** Living U2OS cells stably expressing GFP or survivin-GFP were incubated with Lysotracker Red to highlight acidic compartments, and CQ (120  $\mu$ M), for 2h. Mean number of acidic puncta per cell was measured,  $n > 30$  cells, 3 independent experiments. Error bars indicate SEM. **\*\*** $P < 0.005$ , **\*\*\*\*** $P < 0.0001$ . **(C)** Representative images of live U2OS cells transiently expressing RFP, survivin-RFP or survivin<sup>F61A,L64A</sup>-RFP and LC3-GFP treated with RAP (200 nM) and CQ (120  $\mu$ M) for 2h.  $n > 35$  cells, 3 independent experiments. Scale bar 15  $\mu$ m.