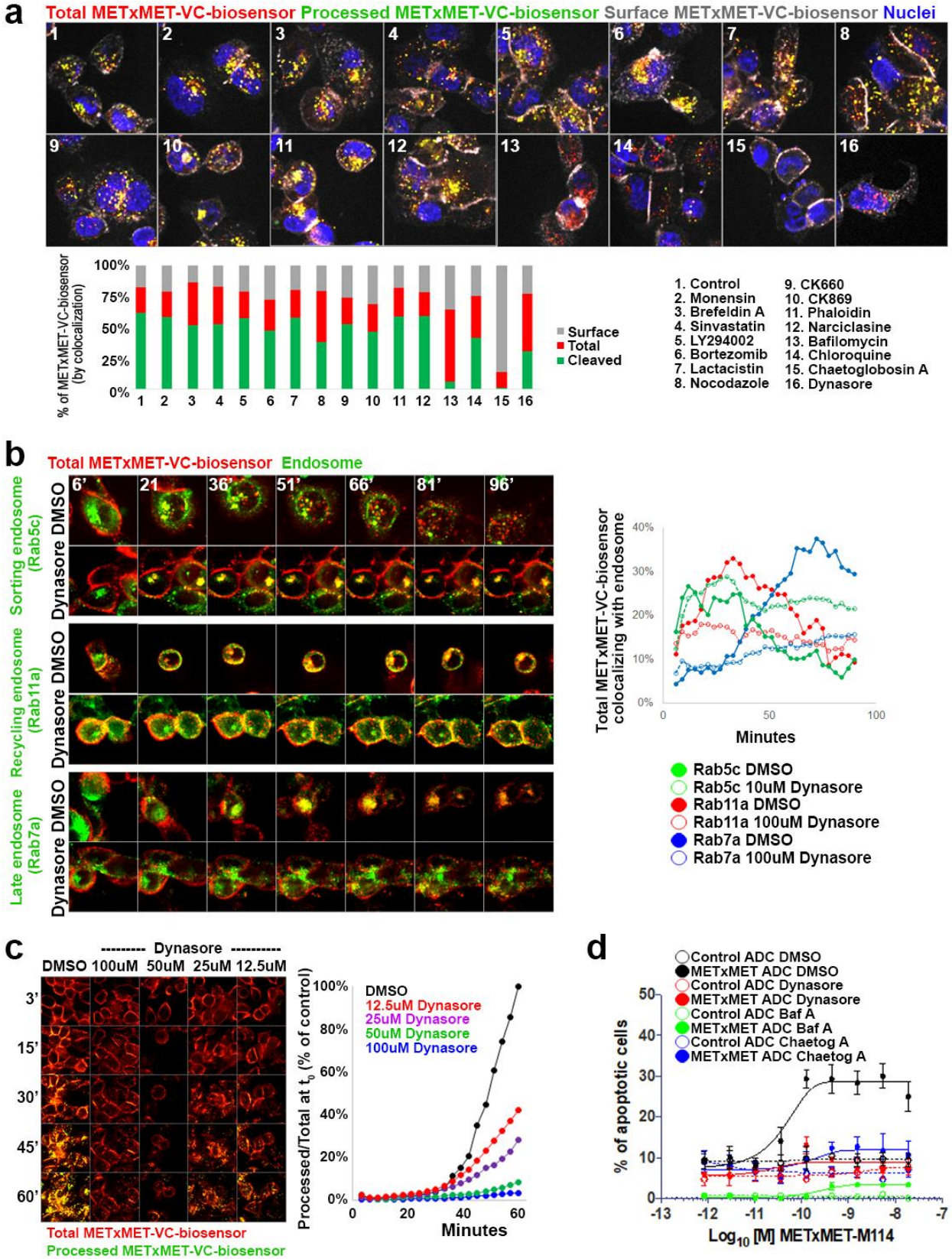
Supplementary fig 6



Supplementary Fig 6. Dynasore disrupts trans-endosomal trafficking, inhibits processing and killing efficacy of METxMET-VC-ADC.

(a) METxMET-VC-biosensor pre-bound at 4C to the surface of EBC1 cells was internalized at 37C for 60 minutes and subsequently stained with anti-human-AF488 Fab. Cells were treated with the indicated inhibitors during the binding and internalization times (see concentrations on methods). A triple MCC analysis (see methods) was used to quantify the three fractions of METxMET-VC-biosensor: surface/total (gray), internalized/total (red) and internalized/processed (green). Representative images (top) and quantification in nine confocal fields/condition (bottom). **(b)** METxMET-VC-biosensor was pre-bound on ice to the surface of EBC1 cells stably expressing Rab5c-GFP, Rab11a-GFP or Rab7a-GFP and internalization was recorded in real time with confocal live imaging. Trafficking to sorting, recycling or late endosomes was quantified by the colocalization of intact METxMET-VC-biosensor (red) with Rab5c-GFP, Rab11a-GFP or Rab7a-GFP, respectively (green), using MCC. Representative images (left) and quantification (right) from 5 ROIs/condition containing 1-5 cells each. **(c)** METxMET-VC-biosensor was pre-bound to the surface of EBC1 cells and internalization was recorded in real time with confocal live imaging. 100uM dynasore completely blocked METxMET-VC-biosensor cleavage, as quantified by the mean intensity fluorescence of AF568 (green) over that of AF647 (red) at t_0 and normalized to control. Representative images (left) and quantification in nine confocal fields/condition (right). **(d)** EBC1/Cas9 cells were treated for 24 hours with the indicated concentrations of METxMET-VC-ADC or Control-VC-ADC, the indicated inhibitors and the apoptotic cell marker caspase 3/7 dye. Percentage of apoptotic cells was determined as the ratio of caspase 3/7 positive cells over Hoechst positive cells using high content microscopy and a segmentation algorithm.