

Figure S1. Hirst et al.

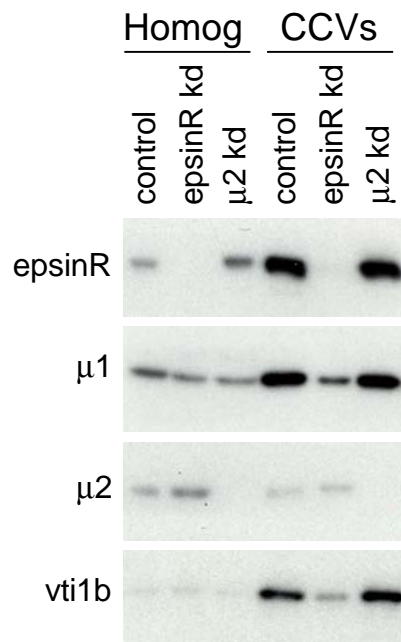


Figure S2. Hirst et al.

LEGENDS FOR SUPPLEMENTAL FIGURES

Figure S1. Cathepsin D sorting by control cells, μ 1-depleted cells, and epsinR-depleted cells. The cells were pulse labeled for 15 min with ^{35}S and chased for 2.5 hr, after which cell-associated (C) and secreted (S) cathepsin D were immunoprecipitated and analysed by SDS-PAGE, as previously described (Hirst et al., 2003). Precursor (P) and mature (M) bands are indicated on the autoradiograph. The experiment was done in duplicate, the bands were quantified using a phosphorimager, and missorting was defined as secreted cathepsin D precursor relative to secreted precursor plus mature cell-associated cathepsin D. The values of the duplicate experiments were: control, 64% and 68% missorted; μ 1 knockdown, 84% and 86% missorted; and epsinR knockdown, 48% and 54% missorted. Thus, even when we deplete epsinR to undetectable levels, cathepsin D sorting is not impaired.

Figure S2. Effects of epsinR and AP-2 depletion on CCV composition. Total homogenates and CCV-enriched fractions were prepared from control cells, epsinR-depleted cells, and μ 2-depleted cells, and Western blots were probed with the indicated antibodies. As also shown in Figure 7, epsinR depletion causes a reduction in the amounts of μ 1 and vti1b associated with CCVs. However, μ 2 depletion does not affect the amount of either μ 1 or vti1b.