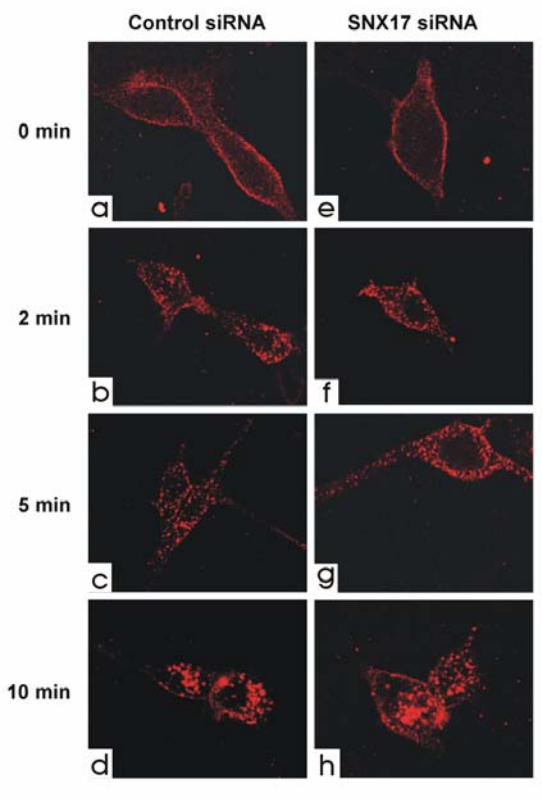
Supplementary Figure 2; van Kerkhof et al



Supplementary Figure 2: Snx17 is not involved in LRP endocytosis. U87 cells were transfected with control siRNA (panel a-d) or Snx17 siRNA (panel e-h). Forty-eight hour after transfection, Cy3-α2M (2 nM) was incubated with cells for 2 h at 4°C. Fluorescent ligands were visualized by confocal microscopy without (a, e) or with 37°C incubation for 2 min (b, f), 5 min (c, g), or 10 min (d, h). As seen in panels a and e, LRP ligand was localized to the cell surface after binding on ice. After 2 min incubation at 37°C, cell surface label diminished and intracellular vesicular staining appeared both in Snx17 silenced (panel f) and Snx17 expressing cells (panel b). Intracellular staining became more clear after 5 min (panel c and g) or 10 min (panel d and h) internalization, independent of Snx17 expression. The effectiveness of Snx17 siRNA was confirmed by anti-Snx17 antibody staining (not shown).