

Supplemental Materials

Molecular Biology of the Cell

Neto et al.

Supplemental Figure Legends.

Supplemental Figure 1. Over-expression of Sx16- Δ TM.

Panel A. HeLa cells were infected with Sx16- Δ TM, Sx12- Δ TM or not infected, and incubated for 48h. Thereafter, cell lysates were prepared and immunoblotted with anti-Sx16. Data from a representative experiment is shown. Levels of Sx16- Δ TM expression varied between 40-90% of endogenous Sx16. The approximate positions of molecular weight markers is shown at the left of the figure. **Panel B.** HeLa cells were infected with Sx16- Δ TM, Sx12- Δ TM or not infected, and incubated for 48h. Thereafter cells were stained with anti-Sx16 or anti-Sx12, as indicated. Note that in virally infected cells, >95% of cells expressed the transgene. Note that the same detector gain/offset and laser power was used for both control (non-infected cells) and Sx16- Δ TM-infected cells, stained with anti-Sx16; under these conditions, endogenous Sx16 is not detected (but compare Figure 1B).

Supplemental Figure 2. Arf6 localisation to the midbody is not impaired by Sx16- Δ TM expression.

HeLa cells were infected with Sx16- Δ TM or Sx12- Δ TM, and incubated for 48h. Thereafter cells were stained with anti-Sx16 (pseudo-coloured blue) or anti-Arf6 (pseudo-coloured green). The characteristic localisation of Arf6 to the midbody is observed in both Sx16- Δ TM or Sx12- Δ TM positive cells. Similar data were observed in three experiments of this type.

Supplemental Figure 3. Distribution of ALIX, Cep55 and Sx16.

Panel A shows that ALIX, Tsg101 and Cep55 populate similar buoyant density vesicles as Sx16, but are largely distinct from TfR-positive membranes. HeLa cells in telophase were harvested, and membranes in a post-nuclear supernatant were fractionated on the basis of density using iodixanol gradient analysis. Shown are representative immunoblots of the indicated proteins. Denser membranes are at the bottom of the tube. The experiment was repeated a further twice with qualitatively similar results. **Panel B** shows an immunofluorescence analysis of HeLa cells stained with either anti-

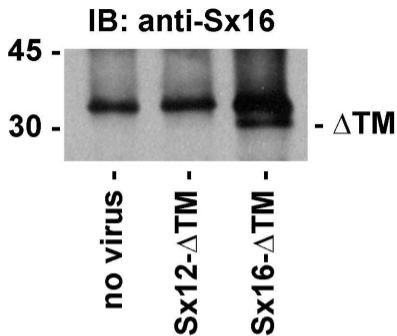
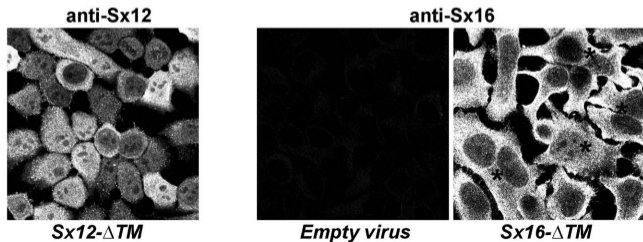
Sx16 (green) or anti-ALIX or anti-Cep55 (red), with expanded areas of intercellular bridge staining shown. Data from a typical experiment is shown.

Supplemental Figure 4. ALIX knockdown does not impair Exocyst localisation at the midbody.

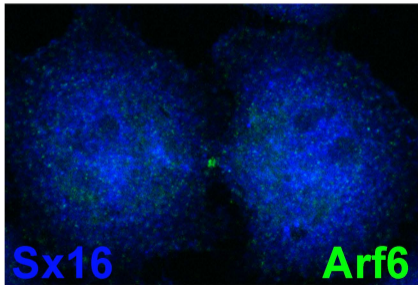
HeLa cells were treated with siRNAs designed to knockdown GAPDH or ALIX, or scrambled siRNA as indicated. 'Lipo' refers to cells treated with transfection reagent in the absence of siRNA duplexes, and 'Mock' refers to cells exposed to neither transfection reagents nor siRNA duplexes. **Panel A** shows a typical immunoblot of HeLa lysates 48h after transfection, probed with anti-ALIX or anti-GAPDH as indicated. **Panel B** shows typical immunofluorescence analysis of the distribution of ALIX in scrambled siRNA control or ALIX knockdown cells. Panel C shows immunofluorescence analysis of Sec5 and Sec 15 in cells in telophase after treatment with scrambled siRNA duplexes or siRNA targeting ALIX. The * indicates a characteristic midbody ring-like structure for the Exocyst components (see also Figure 3). Data from a representative experiment is shown, repeated three times (or more) with similar results.

Supplemental Figure 5. Oncomine profiles of Sx16 expression in human cancers.

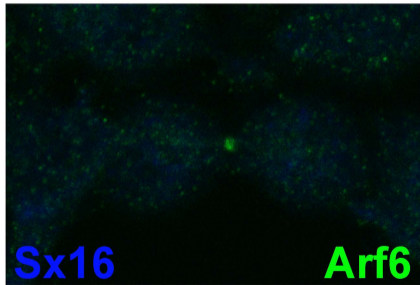
Expression of Sx16 in human cancers was explored using the Oncomine database of array experiments. Shown are data for control and cancer groups (light and dark boxes, respectively). Significantly elevated levels of Sx16 transcript were observed in colon, renal oncocytoma, Wilms tumour, colon adenocarcinoma and colorectal groups (among others). See www.oncomime.org for details.

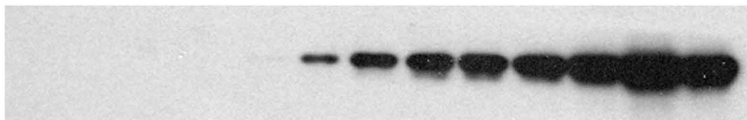
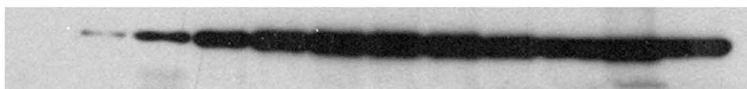
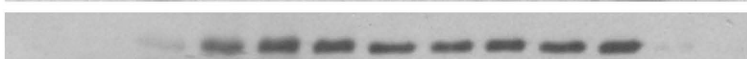
A**B**

Sx16- Δ TM



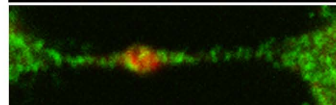
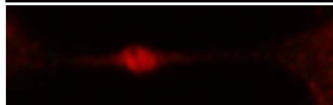
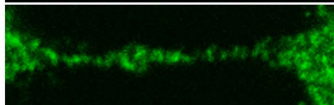
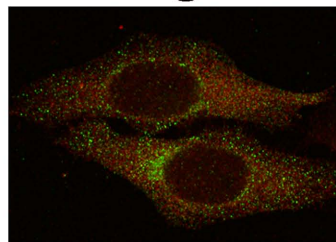
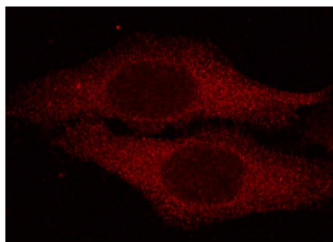
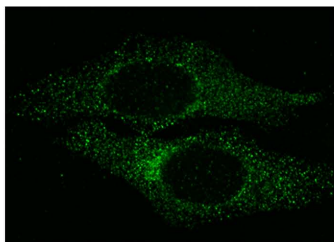
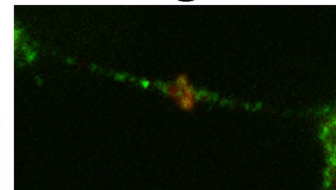
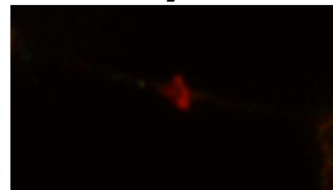
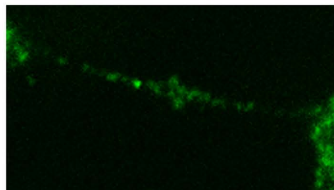
Sx12- Δ TM

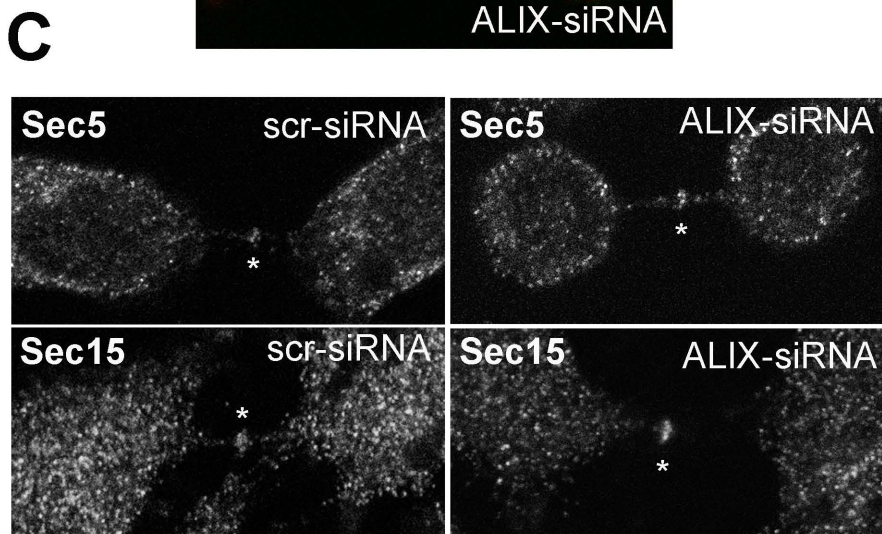
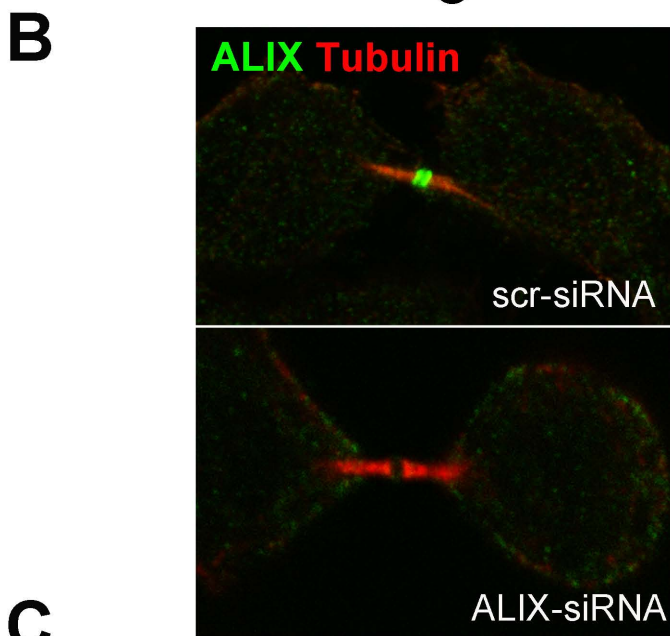
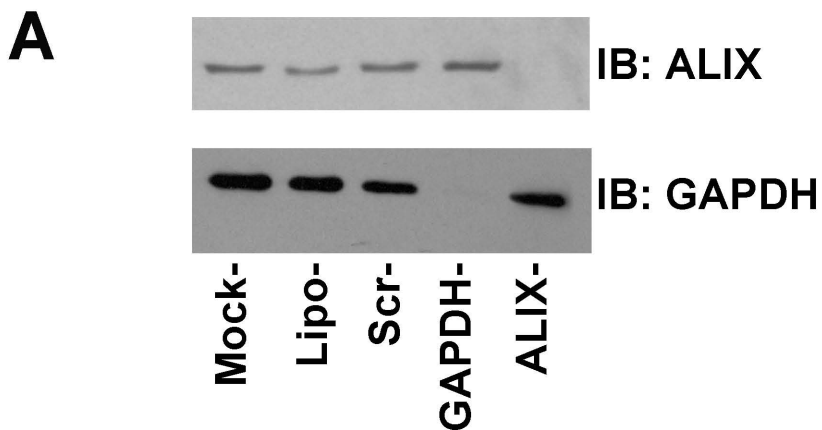


A**Sx16****Cep55****ALIX****Tsg101****TfR****Rab11**

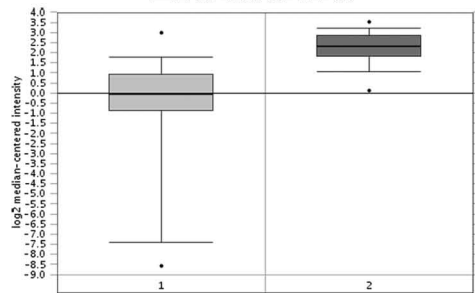
Bottom

Top

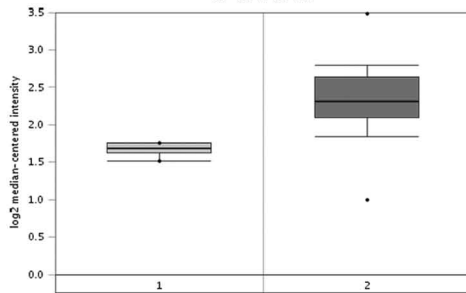
B**Sx16****ALIX****Merge****Sx16****Cep55****Merge**



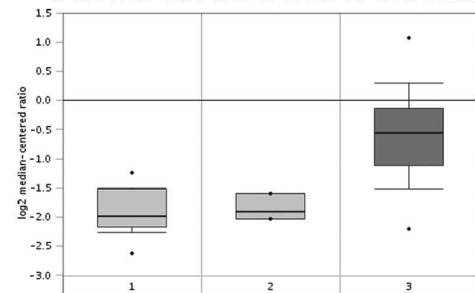
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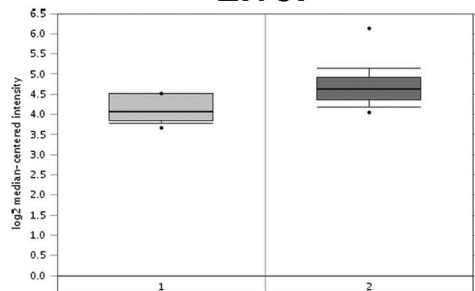
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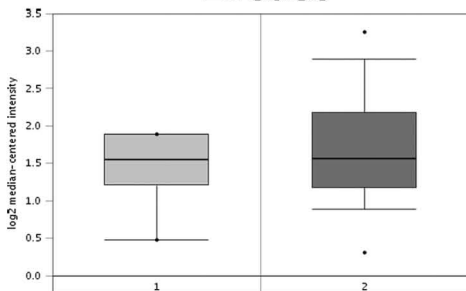
Colon adenocarcinoma



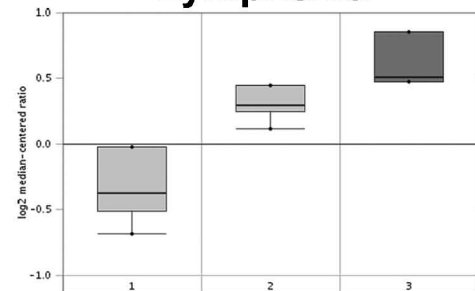
Liver



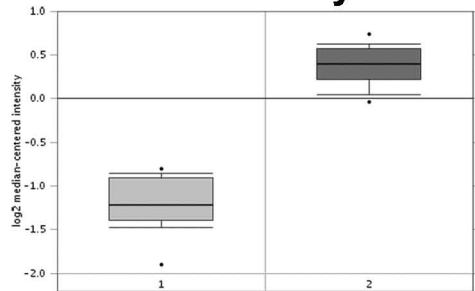
Breast



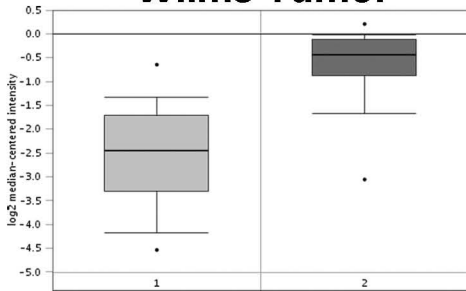
Lymphoma



Renal oncocytoma



Wilms Tumor



Colorectal

