

**P029** Regulation of Tsg101 expression by the Steadiness Box: a role of Tsg101-associated ligase

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As part of the endosomal sorting complex required for transport (ESCRT) machinery, Tsg101 is essential for endosomal sorting, membrane receptor degradation and the final stages of cytokinesis. Depletion or overproduction of the protein can cause disruption of these vital processes and results in severe consequences for the cell. Tsg101 expression is thus controlled posttranslationally within a narrow range and this autoregulation has been mapped to the C-terminus of the protein. Here we elucidate further the mechanisms of this regulation and describe a novel function of Tsg101-associated ligase (Tal) in mediating this control. We show that Tal polyubiquitinates lysine residues in the C-terminus of uncomplexed Tsg101, resulting in proteasomal degradation. However, accessibility to these lysines is prevented by the presence of the other ESCRT-I proteins. We show that VPS28 is a limiting factor, and consequently Tsg101 expression surplus to ESCRT-I function is vulnerable to degradation. The role of Tal in the regulation of Tsg101 steady-state control is highlighted when Tsg101 is overexpressed; however, our data also suggest that additional ligases regulate Tsg101 expression under normal conditions. Lastly, we demonstrate that while the C-terminal lysines are targets for polyubiquitination, they are not required for any additional function necessary for ESCRT activity.