Delta-like1 Lysine 613 regulates Notch signaling

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Supplemental Figures

Figure S1. An alignment of the mouse, human and rat Dll1 transmembrane and intracellular domain residues. Numbering above the alignment indicates the conserved lysine residues (indicated by *), which were mutated in these studies.

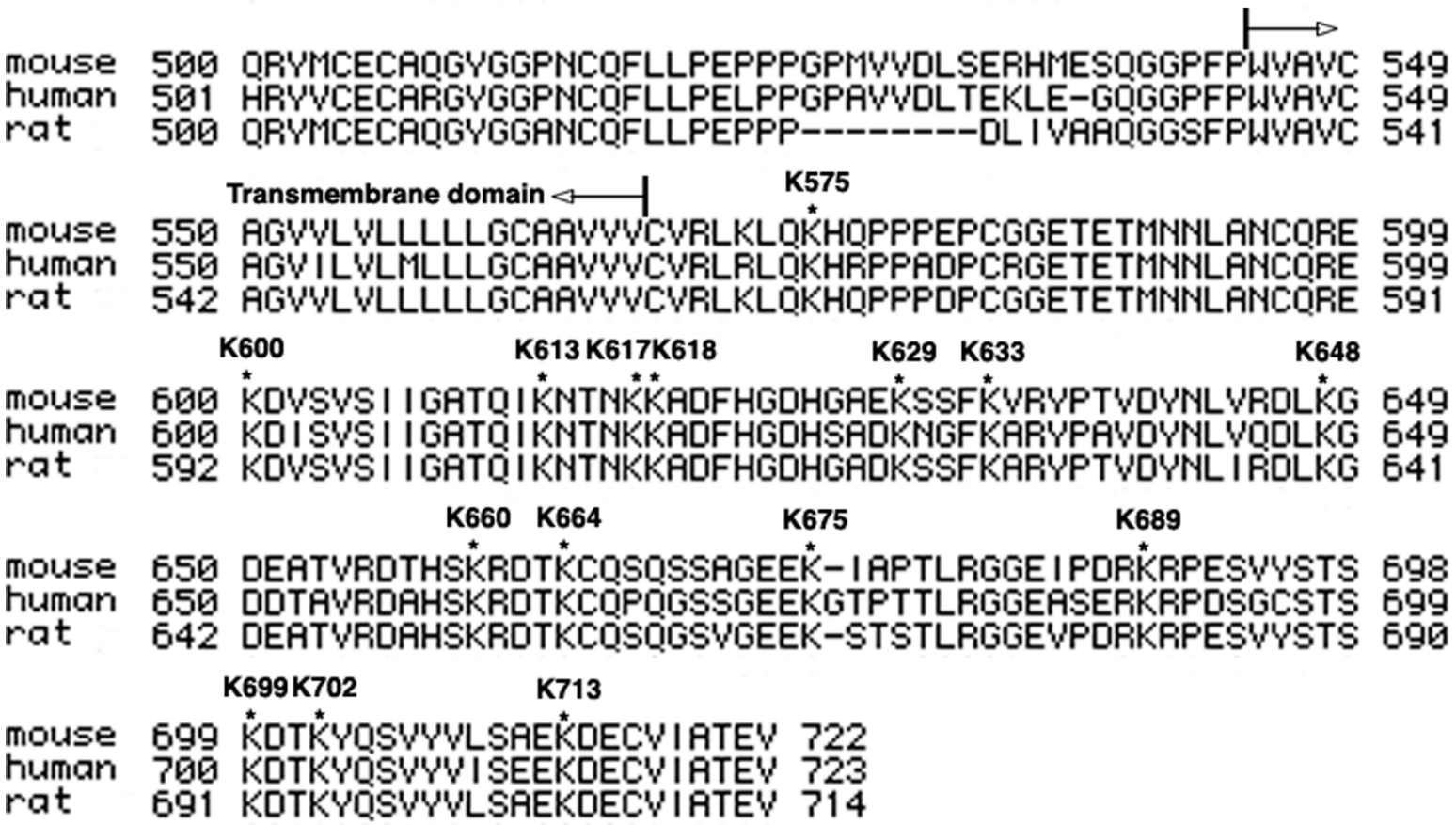
Figure S2.

A, Schematic representation of the endocytosis assay used to determine the internalized, biotinylated fraction of Dll1 present in cells. HEK293 cells were transfected with wild type Dll1, and 36 h later cells were treated as indicated in the figure. Induction of endocytosis of biotinylated surface protein was initiated by incubation of cells at 37°C for 30 min and MesNa treatment was used to strip biotin from biotinylated surface proteins (see “Materials and Methods”). Biotinylated Dll1 was detected in streptavidin fractions using anti-Delta antibody and western blotting.

B, To determine the time required to maximally biotinylate Dll1, HEK293 cells were transfected with wild type Dll1 and at 36 h cells were biotinylated at 37°C for the indicated times before lysis and fractionation. Western blotting of the streptavadin fraction revealed that within 30 min (lane 2), the pool of Dll1 present in the transfected cells is maximally labeled. The decrease in biotinylated Dll1 at 45 and 60 min reflects degradation or turnover of the biotinylated Dll1.

C, To determine if treatment with MesNa efficiently removes biotin from biotinylated Dll1, HEK293 cells were transfected with wild type Dll1. At 36 h the transfected cells were biotinylated at 4°C for 30 min and then either treated with MesNa (lane 3) or returned to 37°C for 30 min to allow endocytosis (lane 4) and then returned to 4°C before stripping with MesNa. Comparison of lane 2 to lane 3 confirms that treatment with MesNa removes all of the biotin label on the Dll1. Comparison of lane 2 to lane 4 reveals that allowing endocytosis before treatment with MesNa, protects a fraction of the biotinylated Dll1 inside the cells.

Supplemental Figure S1



Supplemental Figure S2