### Delta-like1 Lysine 613 regulates Notch signaling

### Liguo Zhang, Ryan C. Widau, B. Paul Herring, Patricia J. Gallagher\*

#### **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 DII1, HEK293 cells were transfected with wild type DII1. 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 DII1. Comparison of lane 2 to lane 4 reveals that allowing endocytosis before treatment with MesNa, protects a fraction of the biotinylated DII1 inside the cells.

# **Supplemental Figure S1**

mouse human rat	501 HRYVCECARGY	GGPNCOFLLPEL	PPGPAVVDL	SERHMESQGGPFF TEKLE-GQGGPFF -DLIVAAQGGSFF	PWYAVC	549
	Transmembrane do	main 🖳	K575			
mouse human rat	550 AGVVLVLLLL	GCAAVVVCVRLK GCAAVVVCVRLR	LOKHRPPAD	PCGGETETMNNLÆ PCRGETETMNNLÆ PCGGETETMNNLÆ	ANCORE	599 599 591
	K600	K613 K617K618	K629	K633	K648	
mouse human rat	600 KDVSVSIIGAT 600 KDISVSIIGAT 592 KDVSVSIIGAT	() I KNTNKKADFH	GDHSADKNG	FŘVRYPTVDYNLY FKARYPAVDYNLY FKARYPTVDYNL	/QDLKG	649 649 641
	K6	60 K664	K675	K689		
mouse human rat	650 DEATVRDTHSK 650 DDTAVRDAHSK 642 DEATVRDAHSK	RDTKCQPQGSSG	EEKGTPTTL	RGGE I PDRÅRPES RGGEASERKRPDS RGGEVPDRKRPES	GCSTS	698 699 690
	K699 K702	K713				
mouse human rat	700 KDTKYQSVYVI	SAEKDECVIATE SEEKDECVIATE SAEKDECVIATE	V 723			

## **Supplemental Figure S2**





