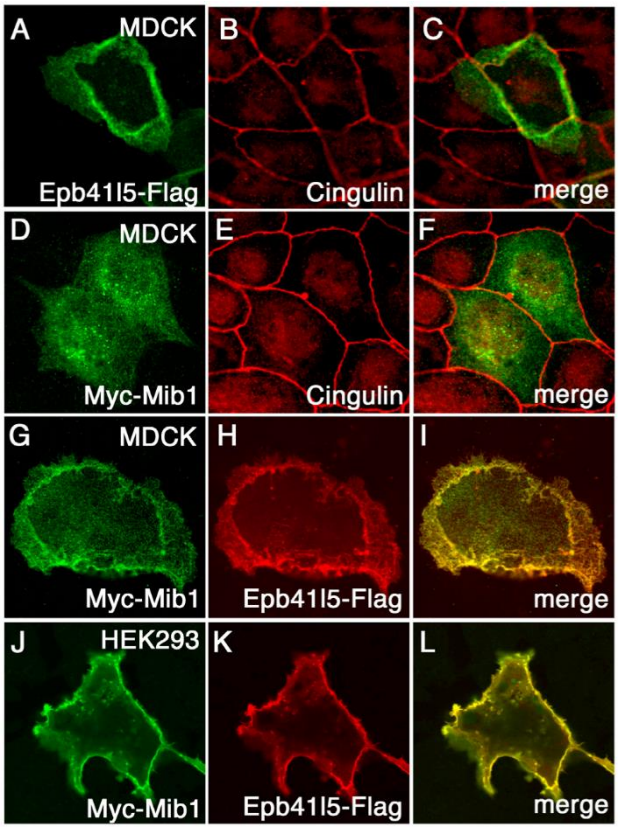

Fig.S1 Epb41l5 changes subcellular distribution of Mib1

(A-F) Epb41l5 is a membrane-associated protein and Mib1 is a cytoplasmic protein. Flag-Epb41l5 or Myc-Mib1 was transfected in MDCK cells and their cellular distribution was examined. Cell-to cell junction is labeled by Tight Junction protein, Cingulin. (G-I) Epb41l5 changes cellular distribution of Mib1 from the cytoplasm to the basolateral membrane in MDCK cells. (J-L) Epb41l5 changes cellular distribution of Mib1 from the cytoplasm to the plasma membrane in HEK293 cells.

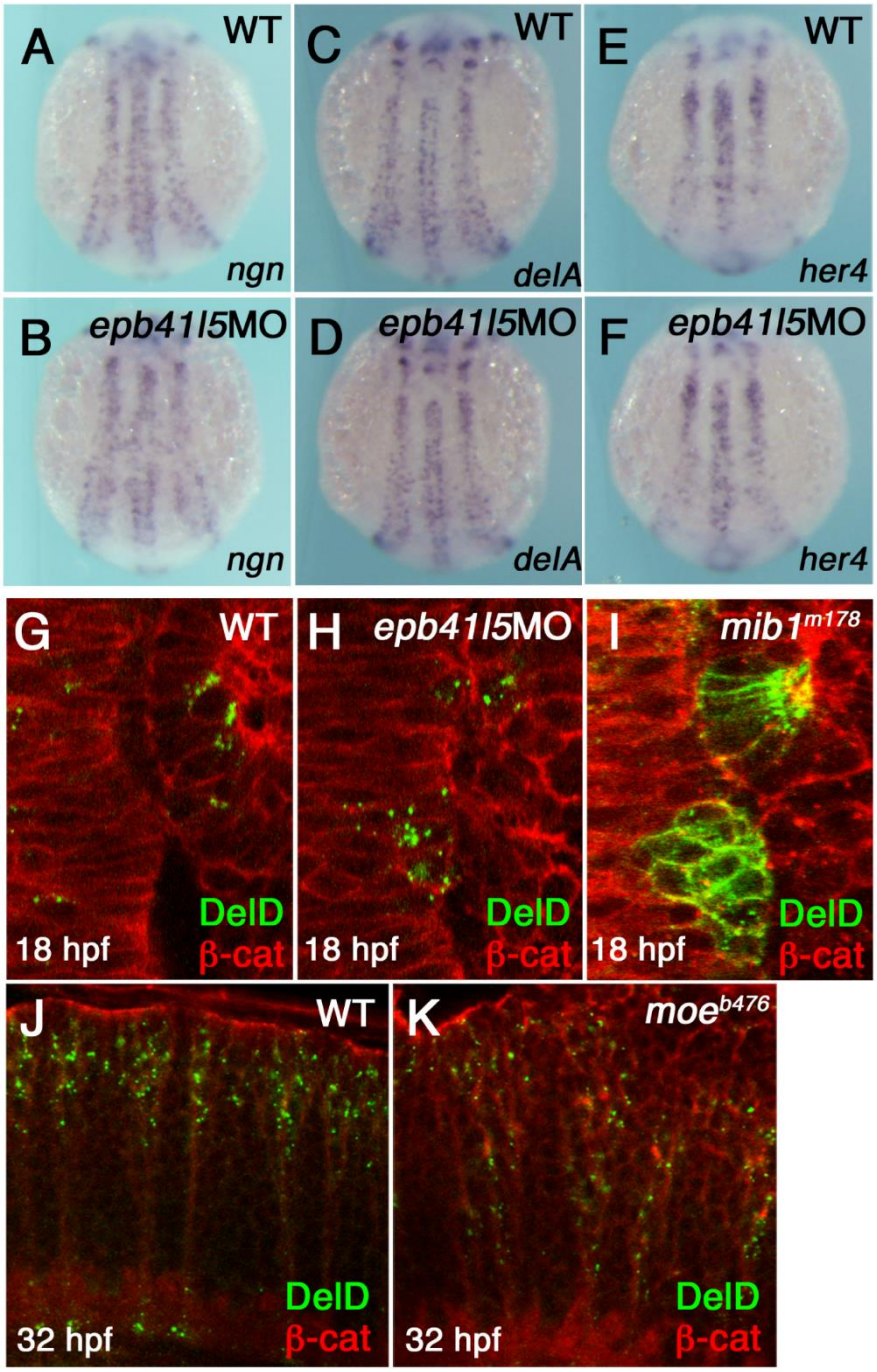

Fig.S2 Epb41l5 does not modify Notch signaling or subcellular distribution of DeltaD in the neuroepithelium.

(A-F) Reduced expression of *epb41l5* does not change fate specification of neurons mediated by Notch-mediated lateral inhibition. *ngn*: bHLH proneural transcription factor, *delta*: Notch ligand, *her4*: direct outcome of Notch signaling. 3-somite stage embryos. (G-I) Reduced expression of Epb41l5 does not change subcellular distribution of DeltaD in the hindbrain neuroepithelium. Dorsal views of the 4th rhombomere and the otic placode in 10-somite stage embryos. DeltaD is mainly localized in cytoplasmic puncta in wild-type embryos. DeltaD is mainly localized on the plasma membrane in *mib1*^{m178} mutants, confirming that Mib1 facilitates endocytosis of DeltaD in neuroepithelial cells. DeltaD is mainly localized in cytoplasmic puncta in *epb41l5* deficient embryos, suggesting that Epb41l5 is not essential for Mib1-mediated endocytosis of Delta. (J,K) Loss of Epb41l5 does not change subcellular distribution of DeltaD in the hindbrain in *epb41l5* mutant *moe*^{b476}. Lateral view of the 4th rhombomere at 32 hpf.

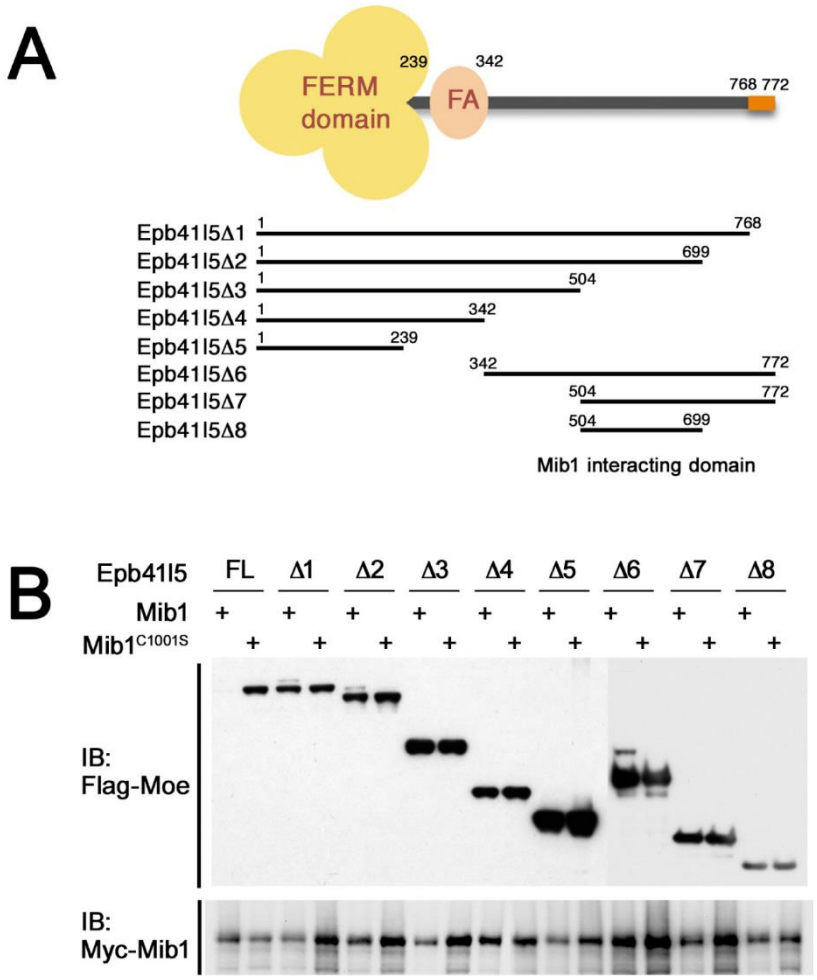

Fig.S3 The C-terminal PDZ binding domain is required for Mib1-mediated degradation of Epb41l5

(A) Schematic drawing of Epb41l5 truncated constructs. (B) The C-terminal PDZ binding domain is required for Mib1-mediated degradation of Epb41l5. Mib1 or Mib1^{C1001S}, which has a mutation in the Ring finger domain, was coexpressed with various mutant forms of Epb41l5. Deletion of four amino acids at the C-terminus of Epb41l5 is sufficient to prevent degradation of Epb41l5 by Mib1.

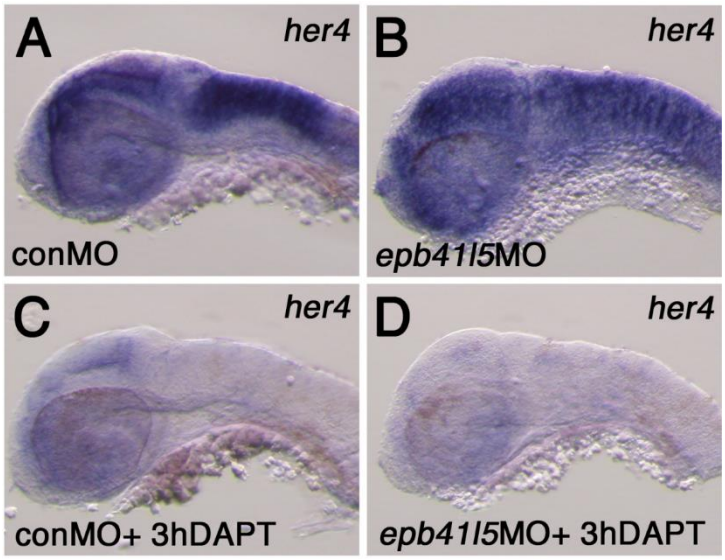

Fig.S4 Expression of *her4* in the hindbrain of *epb41l5* deficient embryos

(A,B) Reduced expression of *epb41l5* does not significantly change expression levels of *her4* in *epb41l5* morphants. Lateral view of the hindbrain at 32hpf. (C,D) DAPT treatment effectively reduces *her4* expression in *epb41l5* morphants as in control embryos. Lateral view of the hindbrain at 32hpf.

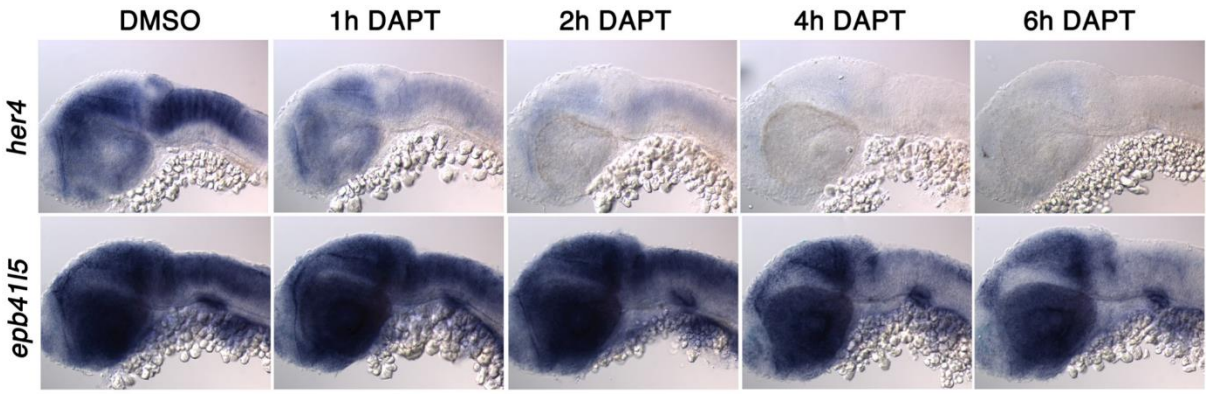

Fig.S5 Notch signaling changes the numbers of cells expressing *ebp41l5*

DAPT reduces expression of *epb41l5* in the hindbrain. Embryos were treated with DAPT for 1, 2, 4 and 6 hours. Lateral view of the hindbrain. 32 hpf

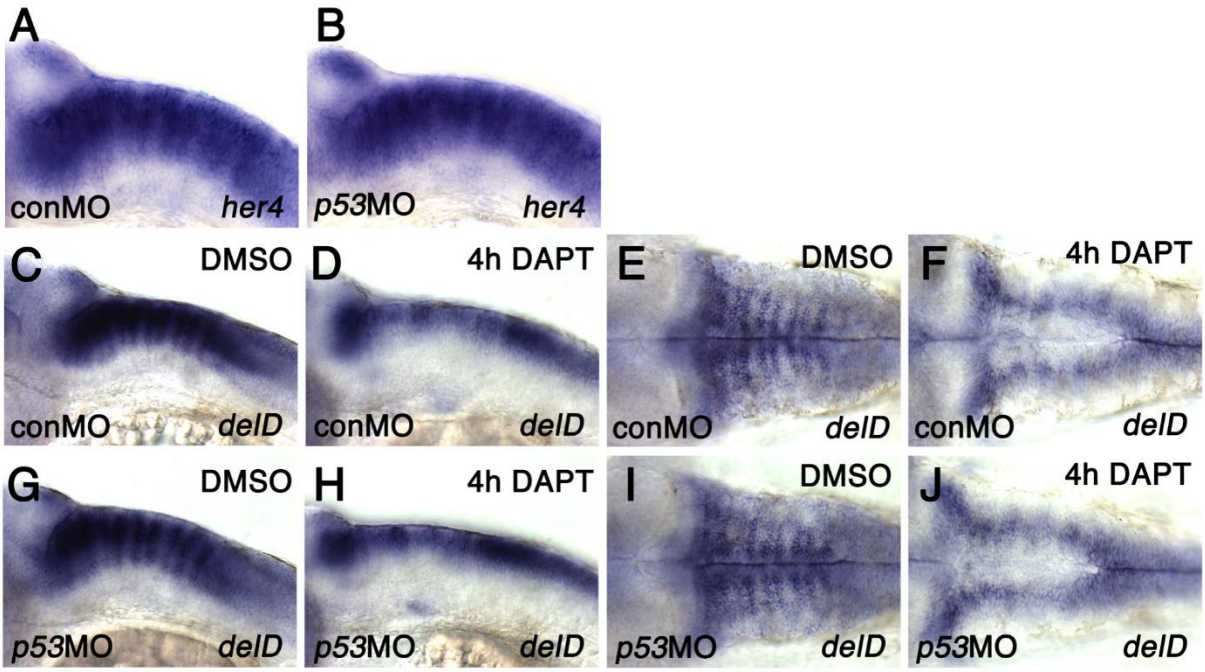

Fig.S6 Notch signaling changes the numbers of cells expressing *ebp41l5*

(A-B) p53 knockdown does not change expression of *her4*, a direct target of Notch signaling at 32 hpf. (C-J) p53 knockdown does not change expression of *deltaD* in DAPT-treated embryos. Lateral views of the hindbrain (C,D,G,H) and dorsal views of the hindbrain (E,F,I,J) at 32 hpf. After 4 hours of DAPT treatment, *deltaD* expression in the hindbrain paraboundary domains is downregulated and the expression is limited to the hindbrain rhombic lip, suggested that DAPT effectively inhibits Notch signaling and facilitates differentiation of DeltaD-positive NPCs determined to become neurons in *p53* deficient embryos.

Supplemental Table 1

gene	sequence	Amounts	References
epb41l5	GAGTCCGGCGTAGGAAGCTCAACAT	2 ng	Jensen et al., 2004
p53	GCGCCATTGCTTTGCAAGAATTG	2 ng	Robu et al., 2007
n-cadherin	TCTGTATAAAGAAACCGATAGAGTT	0.1 ng	Lele et al., 2002
random control 25-N		2 ng	Gene Tools

Supplemental Table 2

Protein	Catalog #	Host	Supplier	Dilution
DeltaD	ab73331	mouse	AbCam	1:400
EphA4		rabbit	Dr. David Wilkinson	1:1000
Crb2a	zs-4	mouse	Zebrafish International Resource Center	1:10
ZO-1	33-9100	mouse	Invitrogen	1:200
β-catenin	C2206	rabbit	Sigma	1:500
Cingulin	PA5-31654	rabbit	Invitrogen	1:1000
HuC	A-21271	mouse	Invitrogen	1:1000
Flag tag	F7425	rabbit	Sigma	1:1000
Flag tag	200471	mouse	Agilent Technologies	1:100
Myc tag	sc-789	mouse	Santa Cruz Biotechnology	1:500
HA tag	901501	mouse	BioLegend	1:1000