

**Interaction of the MAGUK family member Acvrinp1 and the cytoplasmic domain of the Notch ligand Delta1**

Sabine Pfister, Gerhard K. H. Przemeck, Josef-Karl Gerber, Johannes Beckers, Jerzy Adamski and Martin Hrabé de Angelis\*

GSF-National Research Center for Environment and Health, Institute of Experimental Genetics, Ingolstaedter Landstr. 1, D-85764 Neuherberg, Germany

\* Author for correspondence

Tel.: +49-89-3187-3302

Fax: +49-89-3187-3500

Email: [hrabe@gsf.de](mailto:hrabe@gsf.de)

Running title: Interaction of Acvrinp1 and Dll1

**SUMMARY**

The evolutionary conserved Notch signal transduction pathway regulates cell fate and cellular differentiation in various tissues and has essential functions in embryonic patterning and tumorigenesis. Cell-cell signaling by the Notch pathway is mediated by the interaction of the transmembrane receptor Notch with its ligands Delta or Jagged presented on adjacent cells. Whereas signal transduction to Notch expressing cells has been described, it is yet unclear whether Delta-dependent signaling may also exist within the Delta expressing cell. Here, we report on the identification of Acvrinp1, a MAGUK family member, interacting with the intracellular domain of Delta1 (Dll1). We confirmed the interaction between Dll1 and Acvrinp1 by pull-down experiments *in vitro* and in a mammalian two-hybrid system *in vivo*. We delimited the fourth PDZ domain of Acvrinp1 and the PDZ-binding domain of Dll1 as major interacting domains. *In situ* expression analyses in mouse embryos revealed that *Dll1* and *Acvrinp1* show partly overlapping but distinct expression patterns, for example, in the central nervous system and the vibrissae buds. Further, we found that expression of *Acvrinp1* is altered in *Dll1* loss-of-function mouse embryos.

**Keywords:** Delta, Notch, signal transduction, yeast two-hybrid screen, PDZ domain

**Abbreviations:** ADAM, a disintegrin and metalloprotease domain; Nlc, Notch intracellular domain; Su(H)/CBF1, suppressor of hairless/*Candida glabrata* centromere binding factor 1; Eph, Ephrin receptor; Dll1, Delta-like 1; PDZ, PSD-95/Dlg/ZO-1; NLS, nuclear localization signal; MAGUK, membrane associated guanylate kinase; GUK, guanylate kinase; SH3, Src-homology 3; Acvrip1, activin receptor interacting protein 1; S-SCAM, synaptic scaffolding molecule; GST, glutathione S-transferase; RKE, rat kidney epithelial; HEK, human embryonic kidney

The evolutionary conserved Delta-Notch cell-cell signal transduction pathway is required for various cell-fate decisions in vertebrates and invertebrates.<sup>1; 2</sup> During vertebrate embryogenesis Delta-Notch signaling plays a crucial role, for example, in left-right axis formation, somitogenesis, neurogenesis and the development of several organs.<sup>3; 4; 5; 6; 7</sup> Cell-cell signaling in this pathway involves the binding of the ligands Delta or Serrate (Jagged in vertebrates) to the Notch receptor expressed on neighboring cells. After binding of the ligand the Notch protein is cleaved extracellularly by an ADAM metalloprotease and subsequently in its transmembrane domain by a presenilin-dependent  $\gamma$ -secretase.<sup>8; 9; 10</sup> This regulated processing leads to nuclear translocation of the Notch intracellular part (NIC) involving Su(H)/CBF1 and activation of target genes.<sup>11; 12</sup> Recent findings on the ephrin/Eph pathway, also mediated by membrane-bound receptors and ligands, revealed that at least in some developmental contexts signaling is bidirectional.<sup>13</sup> In the classical view Notch signaling is unidirectional. It remains open whether the ligand (Delta, Serrate) may mediate intrinsic signaling also into the ligand-expressing cell.

To identify functional domains, we analyzed the evolutionary conservation of targeting signals and binding motifs in the cytoplasmic part of Dll1 (GenBank accession number **NM\_007865**) and its homologues in various vertebrate species. As has been previously described, a consensus X-T/S/Y-X-V/L/I (one letter amino acid code) PDZ-binding motif (PSD-95/Dlg/ZO-1)<sup>14</sup> is present at the intracellular C-terminus of Dll1.<sup>15</sup> Furthermore, we identified a signal for nuclear localization (NLS) at amino acids 688-691 (RKRK) of the mouse Dll1 sequence. Both motifs are conserved among Delta homologues from different vertebrate species, suggesting that these domains may be functionally important (Fig. 1).

In order to isolate proteins binding to the cytoplasmic domain of Dll1 (Dll1cyto), we used the GAL4-based yeast two-hybrid system MATCHMAKER 3 (Clontech). Approximately  $2.3 \times 10^8$  independent clones of a cDNA library of 11 days old mouse embryos (Clontech) were screened with the intracellular part of Dll1 (amino acids 569-722) as bait. After positive selection the obtained clones were divided into 16 groups

coding for identical proteins according to restriction fragment analysis and sequence determination. Two proteins isolated in the two-hybrid screen are members of the membrane associated guanylate kinase (MAGUK) family. MAGUK proteins consist of an inactive guanylate kinase (GUK) domain, WW or Src-homology 3 (SH3) domains and several PDZ domains. Each of these modules mediates interactions with different proteins.<sup>16</sup> Activin receptor interacting protein 1 (Acvrinp1; GenBank accession number **NM\_015823**) is one of the PDZ proteins isolated. The isolated *Acvrinp1* clones showed strong induction of reporter genes in yeast, which indicates that the protein is a true interactor of Dll1. The obtained clones contained nucleotides 2195-3076 of the *Acvrinp1* coding sequence corresponding to PDZ4 and the N-terminal part of PDZ5 of the protein, followed by 18 additional nucleotides that did not belong to the *Acvrinp1* sequence (Fig. 2a). Synaptic scaffolding molecule (S-SCAM) from rat was the first *Acvrinp1* homologue that had been previously identified.<sup>17</sup> It has been reported that S-SCAM assembles various components at synaptic junctions to maintain the structure of synapses.<sup>17</sup> Our analyses of Delta1 mutant embryos demonstrated that Dll1 plays a crucial role during neurogenesis (unpublished results). Therefore, *Acvrinp1* was selected for further investigations.

To gather independent evidence for the interaction between Dll1 and *Acvrinp1*, a direct *in vitro* assay was performed using an [<sup>35</sup>S]-methionine labeled *Acvrinp1* probe and an affinity purified GSTDll1cyto fusion protein. As expected, *Acvrinp1* bound to GSTDll1cyto but not to GST alone (Fig. 2b). This result confirms the interaction between *Acvrinp1* and Dll1cyto found in the yeast two-hybrid screen. To delimit the region of *Acvrinp1* that is interacting with Dll1cyto, a series of deletion mutants of *Acvrinp1* was tested in the *in vitro* system (Fig. 2b). It was evident that all mutants containing PDZ4 of *Acvrinp1* were able to bind to the intracellular part of Dll1, whereas all proteins lacking this domain displayed no interaction. In agreement with the protein part obtained in the yeast two-hybrid screen (Fig. 2a) we suggest that PDZ4 of *Acvrinp1* is a key domain for binding to Dll1. To confirm that the PDZ-binding motif of Dll1 is essential for binding to *Acvrinp1*, we deleted the last four C-terminal amino acids of

Dll1cyto and tested this mutant for interaction with Acvrinp1 (Fig. 2c). As expected, no positive signal could be detected. Our results indicate that the association of Dll1 and Acvrinp1 is most likely mediated by binding of the C-terminus of Dll1 to the fourth PDZ domain of Acvrinp1.

To verify the results obtained in yeast and *in vitro*, we tested the protein interaction between Dll1 and Acvrinp1 using a mammalian two-hybrid system. The cytoplasmic part of Dll1 was expressed as a fusion protein with the transcription activation domain of VP16, whereas Acvrinp1 was present as fusion protein with the DNA-binding domain of Gal4. Both proteins were transiently expressed in the HeLa cell line and tested for interaction with the help of a luciferase reporter gene. As negative control we used the co-expression of VP16 and Gal4 that should show no activation of the luciferase reporter gene. As expected, we observed specific interaction of Acvrinp1 and the intracellular domain of Dll1. Respectively, the relative luciferase activity was increased nearly 12 fold compared to the negative control, whereas cells transfected with Acvrinp1 or Dll1cyto alone exhibit no significant activation of luciferase expression (Fig. 3). Transfections with the positive control proteins MyoD and Id showed 83 fold activation, which confirms the functionality of the system. These data suggest that Dll1 and Acvrinp1 specifically interact with each other in mammalian cells.

To examine whether Dll1 and Acvrinp1 also have overlapping domains of expression in the organism in which they could potentially interact during embryogenesis, we performed whole mount *in situ* hybridizations on mouse embryos from day 8.5 to day 12.5 of gestation (E8.5 to E12.5). During this period *Dll1* mRNA is expressed in a dynamic pattern, for example, in the paraxial mesoderm, somites, and subsets of cells in the nervous system (Fig. 4d-f).<sup>4; 18; 19; 20</sup> At E8.5 *Acvrinp1* expression was not detectable by *in situ* hybridization and only weak signals were found in presumptive neural tissue at E9.5 (Fig. 4o and data not shown). At later stages, *Acvrinp1* was expressed in a distinct pattern in developing neural tissues, pharyngeal arches, the genitalia, and in parts of the developing facial region (Fig. 4a-c, m). The

expression of *Acvrinp1* in the developing central nervous system is in some areas similar to the expression of *Dll1* (Fig. 4d-f). Cross sections through the neural tube at E11.5 and E12.5 reveal that both *Acvrinp1* and *Dll1* are expressed in the mantle layer of the dorsal neural tube (Fig. 4g, h, j, k). The overlapping expression domain includes the region where neural crest cells emerge from the neural tube. Other regions of overlapping expression domains with *Dll1* include the vibrissae primordia, where both genes are expressed in the dermal condensations underlying the epidermis (Fig. 4i, l). To gain further insight into the relationship of both genes we analyzed *Acvrinp1* expression in *Dll1* null-mutant embryos. *Acvrinp1* expression was found to be upregulated in mutant embryos (Fig. 4m, n). At E10.5 high transcript levels were detected in regions, which normally express *Acvrinp1* at E11.5 in wild-type embryos (compare Fig. 4n with 4a). Premature *Acvrinp1* expression was also found at E9.5 in a region where ventral motoneurons emerge (Fig. 4o, p). These findings are consistent with our observation of premature formation of neuronal progenitor cells in *Dll1* mutant embryos (unpublished results).

The fact that we confirmed the interaction of Acvrinp1 and Dll1 *in vitro* by pull-down assays and *in vivo* in a mammalian two-hybrid system strongly supports the specificity of this interaction. In addition, the identification of a PDZ binding motif at the C-terminus of Dll1 is in agreement with an interaction of Dll1 and the PDZ domain containing protein Acvrinp1. Proteins with PDZ domains coordinate the assembly of multiprotein-signaling complexes at specific subcellular sites.<sup>16</sup> The identification of such a scaffolding molecule as intracellular Dll1 binding protein leads us to coin the hypothesis that Dll1 might mediate an intrinsic signal transduction pathway by binding to the PDZ protein Acvrinp1. Recent investigations support this idea. Neoplastic transformation of RKE cells expressing human Jagged 1 involves a PDZ protein dependent signaling into the Jagged 1 expressing cell.<sup>15</sup> Furthermore, it has been suggested that Dll1 is a target of the metalloprotease Kuzbanian and a presenilin-dependent  $\gamma$ -secretase resulting in the generation of a soluble intracellular form.<sup>21; 22</sup> In *Drosophila* S2 and Kc167 cells such a Delta cytoplasmic cleavage product showed

prominent nuclear localization.<sup>21</sup> In addition, a Delta1-Gal4VP16 fusion protein expressed in HEK293 cells activated transcription of a luciferase reporter gene.<sup>22</sup> These data, together with our identification of a nuclear localization signal (NLS) in the intracellular domain, indicate that *Dll1* may be able to translocate into the nucleus eventually regulating expression of target genes. Taking into consideration the described function of MAGUK proteins in the assembly of intracellular multi-protein signaling complexes, it seems reasonable to implicate that the interaction of *Dll1* and *Acvrin1* may be a trigger for the intrinsic signal. Our expression analysis revealed that *Acvrin1* is partly co-expressed with *Dll1*, for example, in the developing central nervous system and the vibrissae primordia during mouse embryogenesis. The overlapping expression domains of *Acvrin1* and *Dll1* include the region of neural crest cell emergence in the dorsal neural tube and the dermal condensations in the vibrissae follicles. It has been described that *Dll1* has essential functions in the migration and differentiation of neural crest cells and the morphogenesis of vibrissae.<sup>5; 23</sup> Thus, it is conceivable that regional and temporal co-expression might lead to modulation of *Dll1* function. Comparing expression patterns of *Acvrin1* in wild type and *Dll1* loss-of-function (*Dll1<sup>lacZ</sup>*) mouse embryos, we found upregulation of *Acvrin1* transcription. This observation shows that *Dll1* is necessary for the proper expression of *Acvrin1*. Further studies on the proposed intrinsic signaling activity of Delta will help to understand the complexity of Delta-Notch signal transduction, Delta function and the interpretation of Delta mutant phenotypes.

**Acknowledgements:**

We would like to thank Veronika Leiss for performing whole mount *in situ* hybridizations and Marion Schieweg for excellent technical assistance. We thank Kunihiro Tsuchida (University of Tokushima, Japan) for the kind gift of the Acvrinp1 cDNA.

**REFERENCES**

1. Artavanis-Tsakonas, S., Matsuno, K. & Fortini, M. E. (1995). Notch signaling. *Science* 268, 225-32.
2. Artavanis-Tsakonas, S., Rand, M. D. & Lake, R. J. (1999). Notch signaling: cell fate control and signal integration in development. *Science* 284, 770-6.
3. Przemeck, G. K., Heinzmann, U., Beckers, J. & Hrabé de Angelis, M. (2003). Node and midline defects are associated with left-right development in Delta1 mutant embryos. *Development* 130, 3-13.
4. Hrabé de Angelis, M., McIntyre, J., 2nd & Gossler, A. (1997). Maintenance of somite borders in mice requires the Delta homologue Dll1. *Nature* 386, 717-21.
5. De Bellard, M. E., Ching, W., Gossler, A. & Bronner-Fraser, M. (2002). Disruption of segmental neural crest migration and ephrin expression in Delta-1 null mice. *Dev Biol* 249, 121-30, doi:10.1006/dbio.2002.0756
6. de la Pompa, J. L., Wakeham, A., Correia, K. M., Samper, E., Brown, S., Aguilera, R. J., Nakano, T., Honjo, T., Mak, T. W., Rossant, J. & Conlon, R. A. (1997). Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development* 124, 1139-48.
7. Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D. J., Honjo, T., Hrabé de Angelis, M., Lendahl, U. & Edlund, H. (1999). Notch signalling controls pancreatic cell differentiation. *Nature* 400, 877-81.
8. Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cumano, A., Roux, P., Black, R. A. & Israel, A. (2000). A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol Cell* 5, 207-16.
9. Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Tian, X., Pan, D. J., Ray, W. J. & Kopan, R. (2000). A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. *Mol Cell* 5, 197-206.

10. De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A. & Kopan, R. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* 398, 518-22.
11. Baron, M. (2003). An overview of the Notch signalling pathway. *Semin Cell Dev Biol* 14, 113-9, doi:10.1016/S1084-9521(02)00179-9
12. Portin, P. (2002). General outlines of the molecular genetics of the Notch signalling pathway in *Drosophila melanogaster*: a review. *Hereditas* 136, 89-96.
13. Kullander, K. & Klein, R. (2002). Mechanisms and functions of Eph and ephrin signalling. *Nat Rev Mol Cell Biol* 3, 475-86.
14. Songyang, Z., Fanning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chishti, A. H., Crompton, A., Chan, A. C., Anderson, J. M. & Cantley, L. C. (1997). Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* 275, 73-7.
15. Ascano, J. M., Beverly, L. J. & Capobianco, A. J. (2003). The C-terminal PDZ-Ligand of JAGGED1 is essential for cellular transformation. *J Biol Chem* 278, 8771-9.
16. Fanning, A. S. & Anderson, J. M. (1999). PDZ domains: fundamental building blocks in the organization of protein complexes at the plasma membrane. *J Clin Invest* 103, 767-72.
17. Hirao, K., Hata, Y., Ide, N., Takeuchi, M., Irie, M., Yao, I., Deguchi, M., Toyoda, A., Sudhof, T. C. & Takai, Y. (1998). A novel multiple PDZ domain-containing molecule interacting with N-methyl-D-aspartate receptors and neuronal cell adhesion proteins. *J Biol Chem* 273, 21105-10.
18. Bettenhausen, B., Hrabé de Angelis, M., Simon, D., Guenet, J. L. & Gossler, A. (1995). Transient and restricted expression during mouse embryogenesis of Dll1, a murine gene closely related to *Drosophila* Delta. *Development* 121, 2407-18.

19. Beckers, J., Clark, A., Wunsch, K., Hrabé de Angelis, M. & Gossler, A. (1999). Expression of the mouse Delta1 gene during organogenesis and fetal development. *Mech Dev* 84, 165-8, doi:10.1016/S0925-4773(99)00065-9
20. Morrison, A., Hodgetts, C., Gossler, A., Hrabé de Angelis, M. & Lewis, J. (1999). Expression of Delta1 and Serrate1 (Jagged1) in the mouse inner ear. *Mech Dev* 84, 169-72, doi:10.1016/S0925-4773(99)00066-0
21. Bland, C. E., Kimberly, P. & Rand, M. D. (2003). Notch-induced proteolysis and nuclear localization of the Delta ligand. *J Biol Chem* 278, 13607-10.
22. Ikeuchi, T. & Sisodia, S. S. (2003). The Notch ligands, Delta1 and Jagged2, are substrates for Presenilin-dependent "gamma -secretase" cleavage. *J Biol Chem* 278, 7751-4.
23. Favier, B., Fliniaux, I., Thelu, J., Viallet, J. P., Demarchez, M., Jahoda, C. A. & Dhouailly, D. (2000). Localisation of members of the Notch system and the differentiation of vibrissa hair follicles: receptors, ligands, and Fringe modulators. *Dev Dyn* 218, 426-37.
24. Gloeckner, C. J., Mayerhofer, P. U., Landgraf, P., Muntau, A. C., Holzinger, A., Gerber, J. K., Kammerer, S., Adamski, J. & Roscher, A. A. (2000). Human adrenoleukodystrophy protein and related peroxisomal ABC transporters interact with the peroxisomal assembly protein PEX19p. *Biochem Biophys Res Commun* 271, 144-50, doi:10.1006/bbrc.2000.2572
25. Spörle, R. & Schughart, K. (1998). Paradox segmentation along inter- and intrasomitic borderlines is followed by dysmorphology of the axial skeleton in the open brain (opb) mouse mutant. *Dev Genet* 22, 359-73.

**FIGURE LEGENDS**

**Fig. 1. Amino-acid sequence comparison of intracellular domains of Delta1 homologues from various vertebrate species.** Identical amino acids are boxed. Similar residues are shaded grey. Gaps that were introduced for the alignment are shown as lines. Amino-acid positions are indicated on the left. The predicted nuclear localization signal is shaded blue, the PDZ-binding motif is shaded green.

**Fig. 2. Interaction of Dll1cyto and Acvrinp1 *in vitro*.** (a) Schematic illustration of Acvrinp1 and the Acvrinp1-deletion mutants. GUK domains are illustrated as grey boxes, WW domains as white boxes and PDZ domains as black boxes. The additional amino acids of the original two-hybrid positive clone are shown as dotted line. In the right panel interaction (+) or no interaction (-) with Dll1cyto is indicated. (b) Interaction of Dll1cyto and Acvrinp1 or Acvrinp1-deletion mutants as tested by GST pull-down assays. For the GST pull-down assays a previously described protocol was used.<sup>24</sup> Acvrinp1 and the deletion mutants containing PDZ1-5 (PDZ1-5; amino acids 243-1113), PDZ4-5 (PDZ4-5; amino acids 738-1113) and the mutant with deletion of PDZ5 ( $\Delta$ PDZ5; amino acids 1-949) were bound by GSTDll1cyto (amino acids 569-722) but not by GST alone. The mutants containing the guanylate kinase and WW domains (GUKWW; amino acids 1-236), PDZ5 (PDZ5; amino acids 955-1113) and the mutant lacking PDZ 4-5 ( $\Delta$ PDZ4-5; amino acids 1-733) displayed no interaction with GSTDll1cyto and GST. Acvrinp1 probes used for the GST pull-down assays are shown in the left lanes (20% input). Multiple bands represent alternative methionine translation products. Molecular mass markers are shown in kDa on the left. (c) Interaction of Dll1cyto with deletion of the PDZ-binding domain and Acvrinp1. No interaction could be detected between Acvrinp1 and GST (Lane 2) and between Acvrinp1 and a GSTDll1cyto fusion protein with deleted PDZ-binding domain (GSTDll1 $\Delta$ PDZ-BD;

amino acids 569-718) (Lane 4). As positive control the interaction between Acvrinp1 and Dll1cyto is shown in Lane 3.

**Fig. 3. Interaction of Dll1cyto and Acvrinp1 in HeLa cells analyzed by mammalian two-hybrid system.** The mammalian two-hybrid assay was performed using the CheckMate Mammalian Two-Hybrid System (Promega) according to manufacturer's protocol. pBINDAcvrinp1 coding for an Acvrinp1 fusion protein with the DNA-binding domain of Gal4, and pACTDll1cyto coding for a Dll1cyto fusion protein with the transcription activation domain of VP16, were co-transfected with the reporter plasmid pG5-luc into human HeLa cells. After 48 hours the cells were lysed. Quantification of the luciferase reporter genes was done using the Dual-Luciferase Reporter Assay System (Promega). Interaction levels are shown on the left and right as relative luciferase activities of cell lysates. Values given are the mean of triplicate measurements and are normalized to the Renilla luciferase activity of the pBIND vectors. The value obtained with the negative control was set to 1. Relative luciferase activities of Acvrinp1, Dll1cyto or both are analyzed as indicated. As negative and positive controls co-transfections of VP16 and Gal4 or MyoD and Id are shown.

**Fig. 4. Comparison of *Acvrinp1* and *Dll1* gene expression.** For embryo collection C3HeB/FeJ mice and mice carrying a *Dll1<sup>lacZ</sup>* knock-in allele<sup>4</sup> were used. *Dll1<sup>lacZ</sup>* mice were maintained on a mixed 129Sv;C56BL/6J background. Sense and antisense *Acvrinp1* (nucleotides 727-3339) and *Dll1*<sup>18</sup> RNA probes were generated using DIG RNA labeling mix (Roche) according to manufacturer's instructions. Whole mount *in situ* hybridization was carried out as previously described.<sup>25</sup> The embryos were stained with BM purple AP substrate (Roche) and fixed afterwards in 4% PFA in PBS. Stained embryos were cryo-sectioned with 35µm. a-f, m, n show whole mount *in situ* hybridizations. g, h, j, k, o, p show cryo-sections at the level of the forelimb bud and i, l cryo-sections of vibrissae buds of whole mount *in situ* hybridized embryos. (a) At E11.5 *Acvrinp1* is expressed in a distinct pattern in the developing nervous system, the

pharyngeal arches and in the facial region. **(b, c)** At E12.5 *Acvrinp1* transcripts are detected in the nervous system, the developing limbs, the vibrissae buds and in the genital bud. **(d-f)** *Dll1* is expressed in a distinct pattern in the nervous system, the presomitic mesoderm, newly formed somites and in the vibrissae buds. **(g)** At E11.5 *Acvrinp1* is expressed in the mantle layer of the entire neural tube with stronger expression in the dorsal half. **(h)** At E12.5 *Acvrinp1* transcripts are present in the dorsal half of the neural tube including the uppermost neural crest region. **(i)** In the vibrissae buds *Acvrinp1* is expressed both in the epidermal layer and in the dermal condensations. **(j)** At E11.5 *Dll1* is present throughout the entire neural tube with a stronger expression in the dorsal part including the mantle layer. **(k)** At E12.5 high transcript levels of *Dll1* exist in the dorsal most part of the neural tube. **(l)** In the vibrissae buds *Dll1* transcripts are present at high levels in the dermal condensations. **(m, n)** In *Dll1* null-mutant embryos at E10.5 *Acvrinp1* expression is upregulated, for example, in the neural tube. **(o, p)** In the neural tube of *Dll1* null-mutants at E9.5 premature *Acvrinp1* expression is present in the region of developing motorneurons. Since *Dll1* null-mutants are haemorrhagic, blood cells are visible in the lumen of the neural tube. Abbreviations: dc, dermal condensations; ep, epidermal layer; gb, genital bud; lb, limb; ln, lumen of the neural tube; ml, mantle layer of the neural tube; mn; region of developing motorneurons; nc, region of neural crest cell emergence; ns, newly formed somites; nt, neural tube; pa, pharyngeal arches; pm; presomitic mesoderm; vb, vibrissae bud.

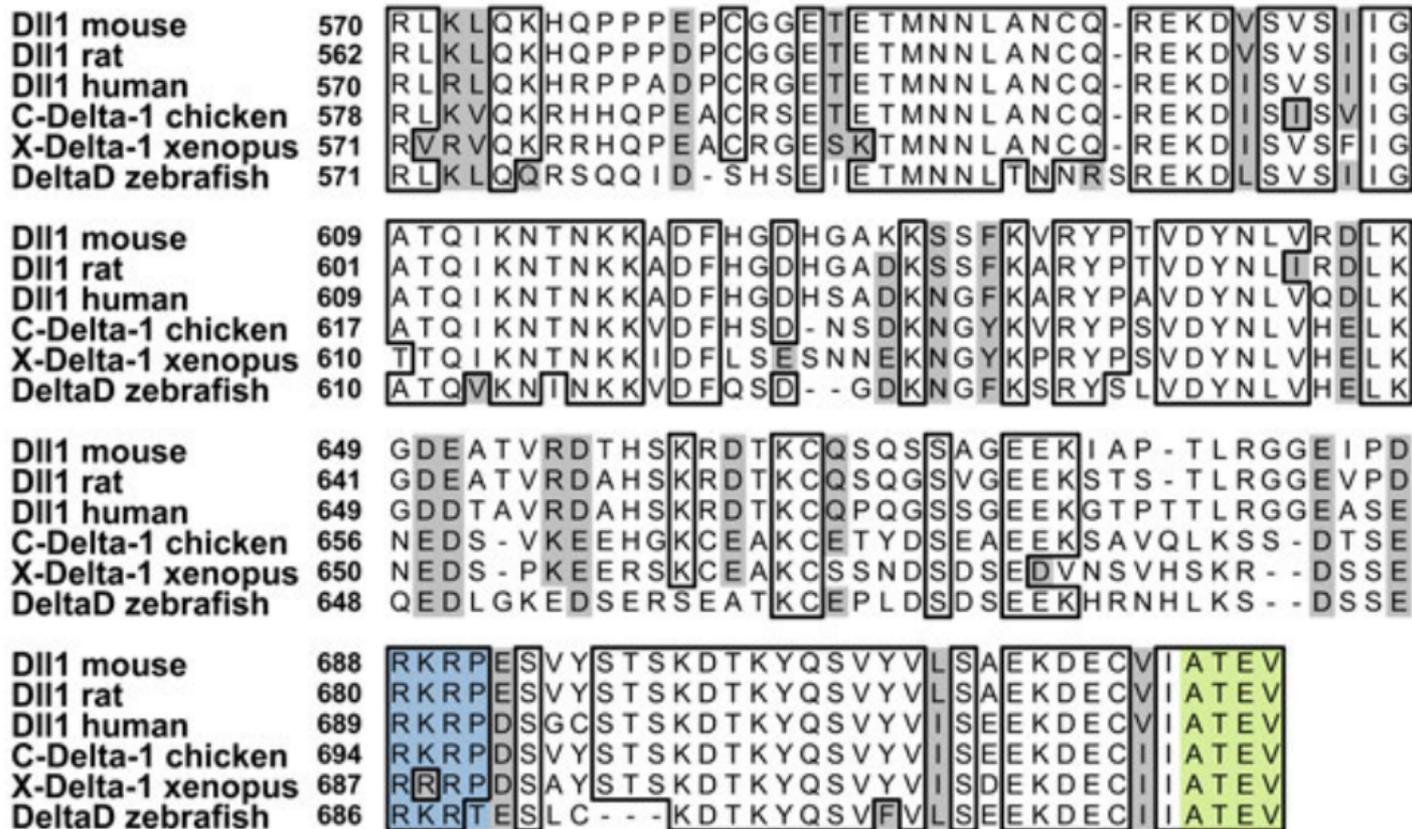
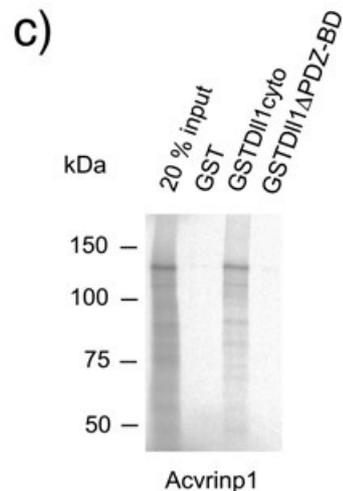
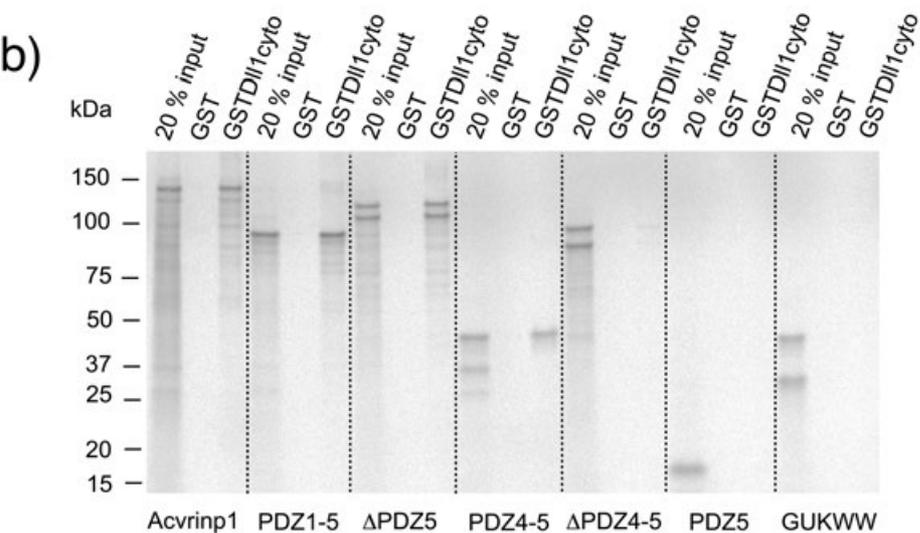
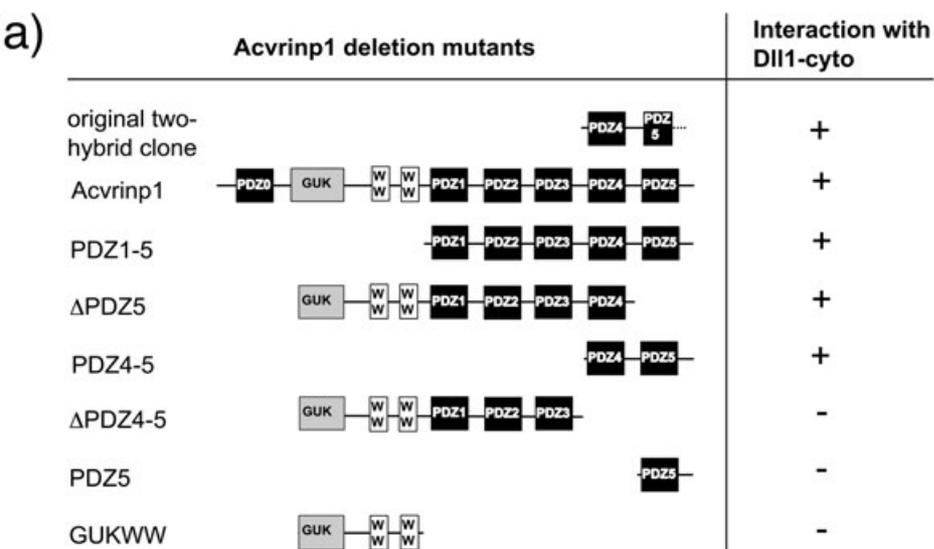


Fig.1



**Fig.2**

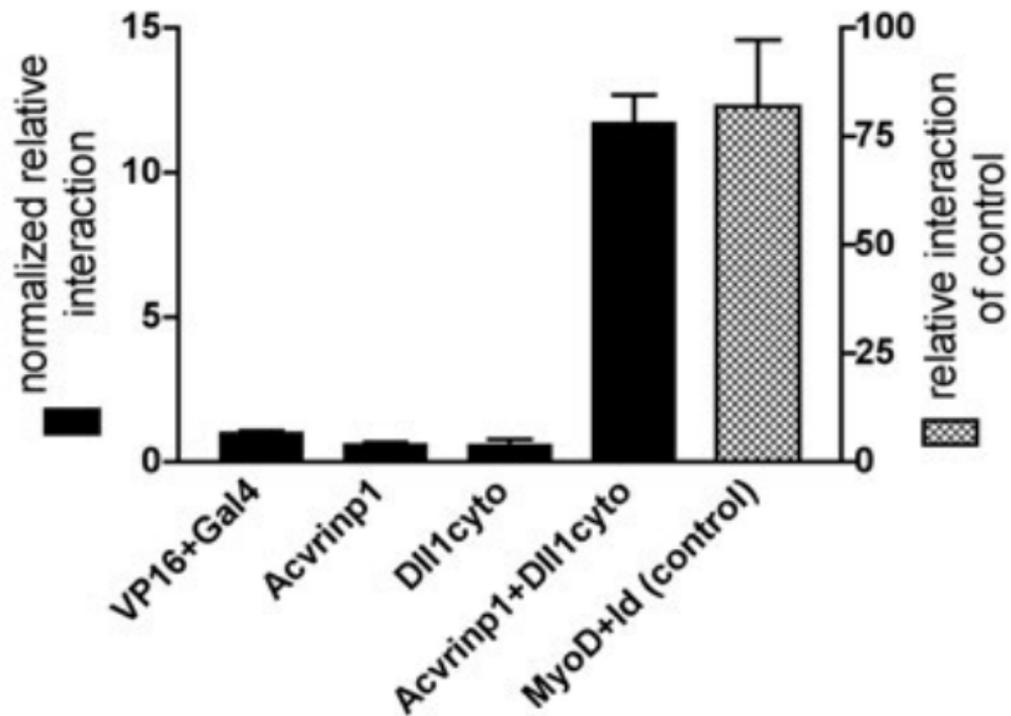


Fig.3

