

Supplementary Note

1. Hereditary hypotrichosis simplex (HHS)

The hair follicle is a complex organ which periodically regenerates in the form of the hair cycle. Recent advances in molecular genetics have enabled the identification of numerous genes that are expressed in the hair follicle¹. Disruption of some of these genes underlies different types of hereditary hypotrichosis (HH).

HH can be largely divided into syndromic and non-syndromic forms. In syndromic forms of HH, hypotrichosis appears as a part of the disease. For example, mutations in the P-cadherin gene (*CDH3*) are known to cause not only hypotrichosis, but also weak eyesight due to macular dystrophy of the retina (Hypotrichosis with Juvenile Macular Dystrophy; HJMD; OMIM 601553)^{2,3}. Some cases with *CDH3* mutations also show severe digit anomalies (Ectodermal dysplasia, Ectrodactyly, and Macular dystrophy; EEM syndrome; OMIM 225280)^{3,4}.

In non-syndromic forms of HH, hypotrichosis is the only clinical finding observed in affected individuals. For example, Marie Unna hypotrichosis (OMIM 146550) is an autosomal dominant disorder characterized by coarse, wiry and twisted hair shaft, and was recently reported to be caused by mutations in the 5'-regulatory region of the hairless gene (*HR*) on chromosome 8p21 (ref. 5). In addition, monilethrix is characterized by a specific hair shaft anomaly known as a moniliform hair. This disease can show either an autosomal dominant (OMIM 158000) or recessive (OMIM 252200) inheritance pattern, and several causative genes have been identified to date⁶⁻¹¹.

A rare form of non-syndromic hereditary hypotrichosis without a characteristic hair shaft anomalies is known as hereditary hypotrichosis simplex (HHS; OMIM 146520/605389)^{12,13}. Affected individuals with HHS typically show normal hair at birth, but hair loss and thinning of the hair shaft start during early childhood and progress with age. HHS can be largely divided into two forms: the scalp-limited form and the generalized form, in which all body hair is affected¹⁴. Histologically, HHS is characterized by progressive hair follicle miniaturization, which is a typical feature of androgenetic alopecia^{12,15}. HHS is known to be

inherited as either an autosomal dominant (ADHHS)¹²⁻¹⁷ or autosomal recessive (ARHHS)¹⁸ trait. Mutations in corneodesmosin (*CDSN*) gene were identified in several families with the scalp-limited form of ADHHS¹⁶. In addition, we (A.B., S.B., A.S.) previously analyzed an Italian family with a generalized form of ADHHS and found linkage of the family to a 9.8 Mb interval on chromosome 18p11.32-p11.23 (ref. 17), in which the *APCDD1* gene resides. Most recently, mutations in the *P2RY5* and *Lipase H* genes were reported to underlie ARHHS^{18,19} (with autosomal recessive woolly hair)^{20,21}.

Whereas mutations in the *CDSN* gene underlie scalp-limited form of ADHHS¹⁶, the mutation in the *APCDD1* gene that we identified in this study results in a generalized form of ADHHS. *CDSN* is predominantly expressed in the inner root sheath (IRS) of the hair follicle¹⁶. In contrast, we have shown that *APCDD1* is expressed in the matrix, the hair shaft, and dermal papilla of the hair follicle, but not in the IRS (Fig. 1f-j). In addition, promoter sequences of the *CDSN* gene do not have potential LEF1/TCF1 binding sites based on the analysis with VISTA tools (<http://genome.lbl.gov/vista/index.shtml>; data not shown), suggesting *CDSN* is not a direct Wnt target gene. Unlike the ligand-receptor relationship between *P2RY5* and *Lipase H* underlying ARHHS¹⁸, we found no obvious connection between *CDSN* and *APCDD1* genes, though an indirect relationship may emerge in the future.

2. Clinical features of Pakistani families with HHS

We identified two Pakistani families, HHS1 and HHS2, with typical clinical features with HHS (Fig. 1a-d and Supplementary Fig. 1a-l). The pedigrees of both families show clear autosomal dominant inheritance (Supplementary Fig. 1p). All affected individuals had normal scalp hair density at birth, and the hair loss gradually progressed with age, beginning around 2-5 years old (Fig. 1a-d and Supplementary Fig. 1a-l). The hair grows slowly and stops growing after a few inches. Some affected individuals show light-colored or hypopigmented hair shafts (Supplementary Fig. 1a, c). In most cases, body hair, axillary and pubic hair are also sparse (Supplementary Fig. 1k). Eyebrows, eyelashes, and beard hairs are

not affected. Under light microscopy, the bulb portion of the plucked hair shows dystrophic features (Fig. 1c) and is miniaturized (Fig. 1d). The hair shaft is thin and without any characteristic anomalies, and the distal ends appear tapered (Supplementary Fig. 1l). Affected individuals in both families show normal teeth, nails, and sweating, and do not show keratosis pilaris. There was no familial history of either neurologic abnormalities or a high prevalence of cancers. Based on these clinical findings, we diagnosed both families with the generalized form of ADHHS. We initially excluded the *CDSN* gene in both families by linkage analysis (data not shown).

3. Wnt signaling and human hair disorders

To our knowledge, our findings represent the first involvement of a Wnt inhibitor in a human hair disorder. The Wnt gene, *WNT10A*, has been previously associated with an ectodermal dysplasia of which sparse hair is an occasional feature. Odonto-onycho-dermal dysplasia (OODD; OMIM 257980) is a rare autosomal recessive ectodermal dysplasia characterized by hypodontia, hyponychia and palmoplantar keratoderma, as well as a variable feature of occasional dry and sparse hair (hypotrichosis). It has been associated exclusively with mutations in the *WNT10A* gene²². Recent findings suggest that Schöpf-Schulz-Passarge syndrome (OMIM 224750), phenotypically similar to OODD, may also be associated with *WNT10A* mutations²³. While mutations in *WNT10A* cause a spectrum of ectodermal abnormalities in OODD, the predominant sites of the phenotype are the teeth and nails, with the hair being variably involved^{22,23}. In contrast, mutations in *APCDD1*, a Wnt inhibitor, appears to affect the hair exclusively, with sparing of the teeth and nails.

4. Potential role for *APCDD1* in multifactorial hair diseases

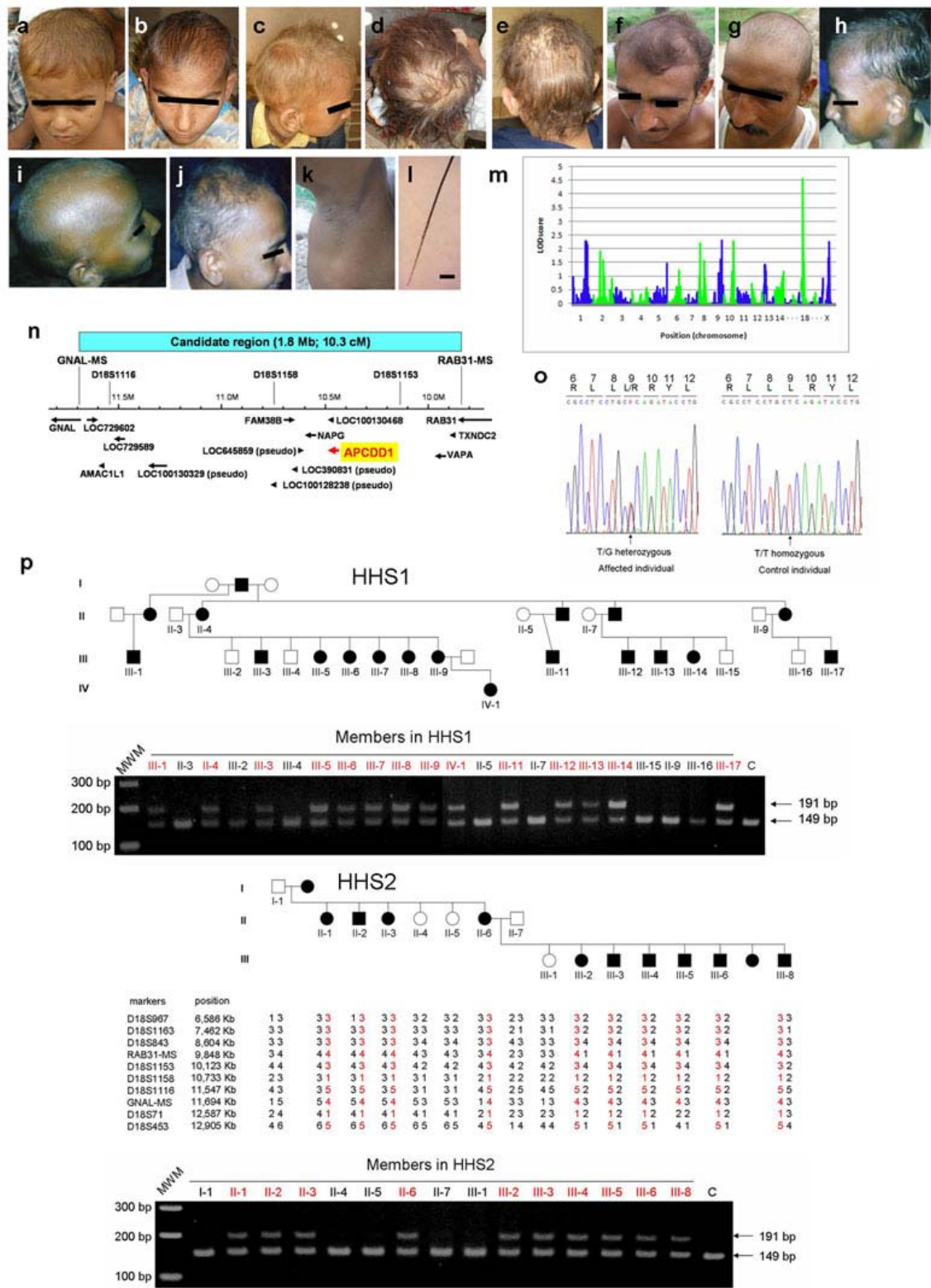
Recently performed genome-wide family-based linkage studies for Alopecia Areata (AA) and Androgenic Alopecia (AGA) have suggested the presence of disease loci on chromosome 18p. AA is one of the most common

causes of hair loss in humans with a lifetime risk of nearly 2%. It affects individuals of both genders and all ages and ethnic backgrounds. Hair loss is non-scarring and includes a broad range of phenotypes that are united by the presence of a lymphocytic infiltrate around the hair follicle. The first genome-wide linkage study performed for AA identified several potential loci, including one located on chromosome 18p11.31 (ref. 24). Subsequent fine-mapping performed for this study identified significant linkage (maxLOD=3.93) at 18p11.23 and defined a -2LOD interval that spanned from 3.4Mb to 12.9Mb and includes the *APCDD1* locus²⁴. AGA is another highly prevalent complex disease that causes common male or female pattern hair loss in humans. Individuals affected with AGA share some clinical features with the patients we report here to carry the *APCDD1* mutation, such as miniaturized hair follicles and the distribution of the hair loss regions. An AGA genome-wide linkage study identified several loci, one of which is located on chromosome 18p11-q23, in a region containing *APCDD1* (ref. 25). Based on the location of *APCDD1* in or near these linkage intervals, together with its expression in the hair follicle, suggest consideration of *APCDD1* as a potential candidate gene.

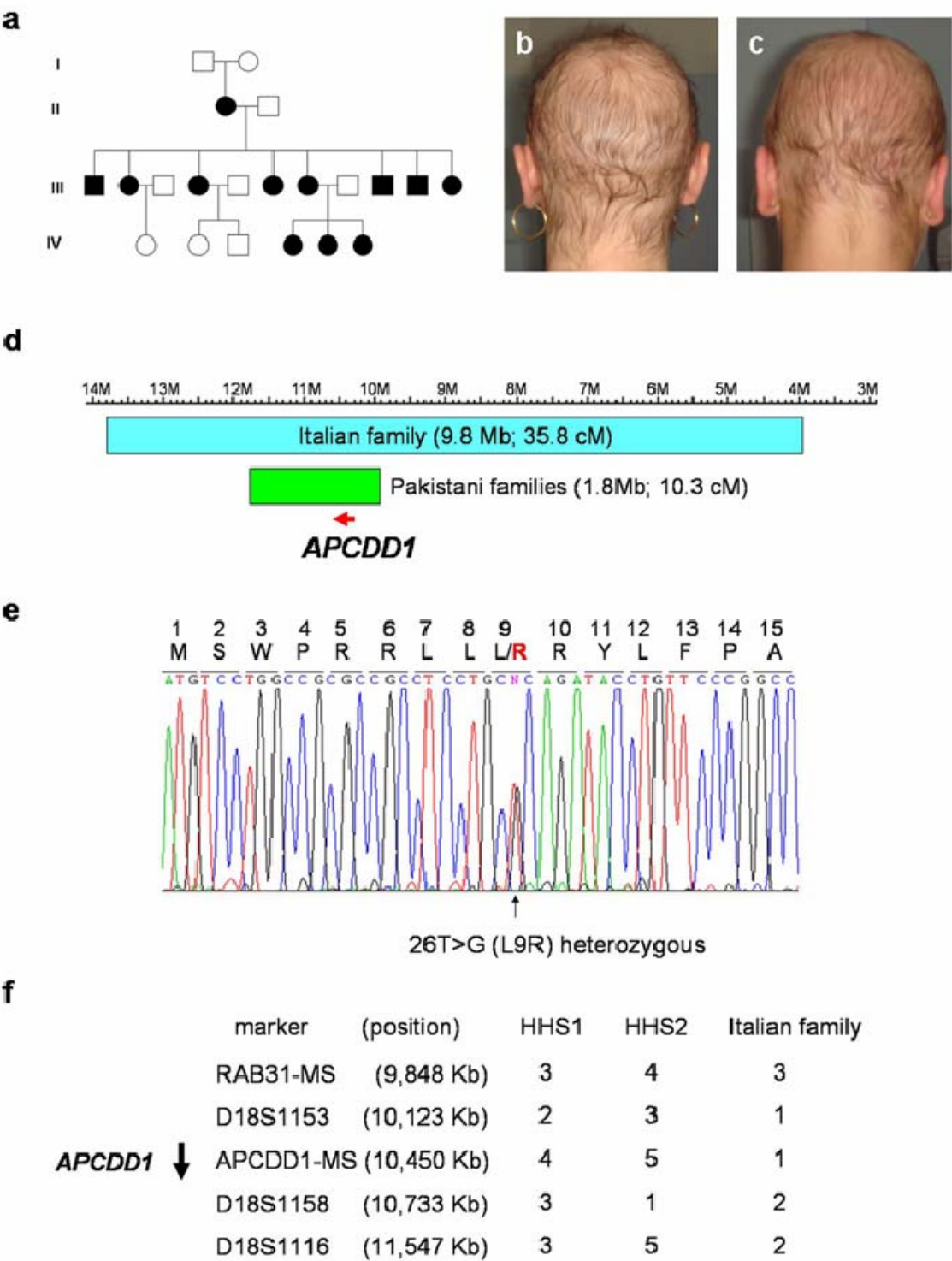
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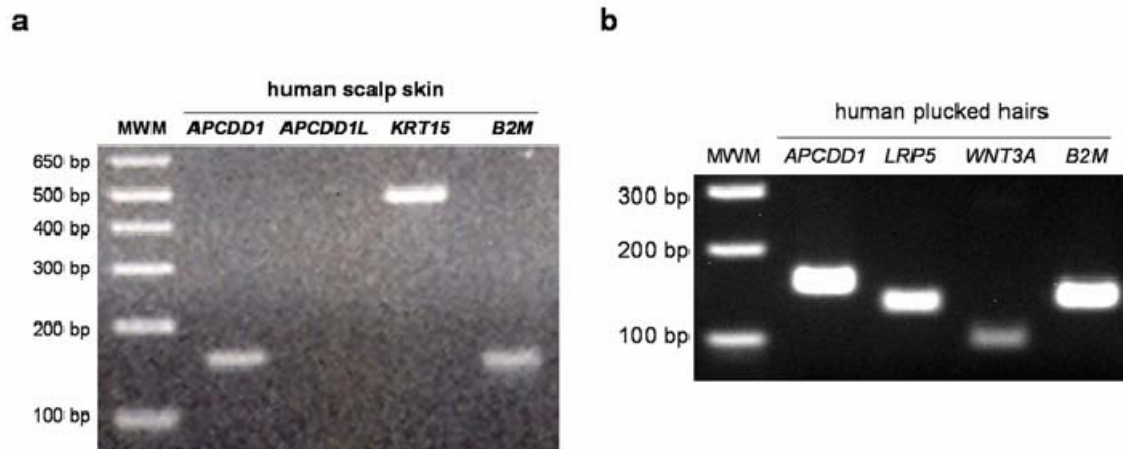
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Supplementary Figure 1. Clinical appearance of Pakistani families with HHS, and identification of the mutation L9R in the *APCDD1* gene in the families HHS1 and HHS2. **a-l**, Clinical features of HHS. Affected individuals in the families HHS1 (**a-g**, **k**, **l**) and HHS2 (**h-j**). The age of each individual is 2 (**a**), 6 (**b**), 10 (**c**), 3 (**d**), 28 (**e**), 20 (**f**), 28 (**g**), 8 (**h**), 9 (**i**), 12 (**j**), and 20 (**k**) years old, respectively. Hair shafts of affected individuals show tapered distal ends (**l**). Scale bar: 100 μ m (**l**). **m**, Results of autozygosity mapping. The maximum LOD score was obtained for a region on chromosome 18 (**m**). **n**, Schematic representation of the candidate region harboring the HHS gene. **o**, Heterozygous mutation 26T>G (Leu9Arg) in *APCDD1* gene of both families HHS1 and HHS2. **p**, Results of haplotype analysis and screening assays for the mutation. The disease-related haplotype and affected individuals are colored in red (**p**). The PCR product from wild-type allele, 191 bp in size, was digested into 149 bp and 42 bp fragments, while that from the mutant allele was undigested (**p**). The 42 bp fragment was not shown. MWM, molecular weight markers; C, control individual (**p**).



Supplementary Figure 2. An Italian family with HHS. a, Pedigree of the family. **b,c**, Clinical appearance of affected individuals. Scale bars: 100 μ m. **d**, Candidate region for the Italian family that was previously defined¹⁷. The region found in Pakistani families, as well as the position of *APCDD1* gene, are also shown. **e**, Identification of the heterozygous 26T>G (Leu9Arg) mutation in the *APCDD1* gene in the Italian family. **f**, Comparison of haplotypes between three families with an identical point mutation in the *APCDD1* gene. The marker APCDD1-MS is located within intron 1 of the *APCDD1* gene, which is only 5 Kb distant from the position of the mutation. Note that the three families had a distinct disease-related haplotype, suggesting that the mutation arose independently in each family, and that nucleotide 26 of the *APCDD1* gene may be a mutational hotspot.

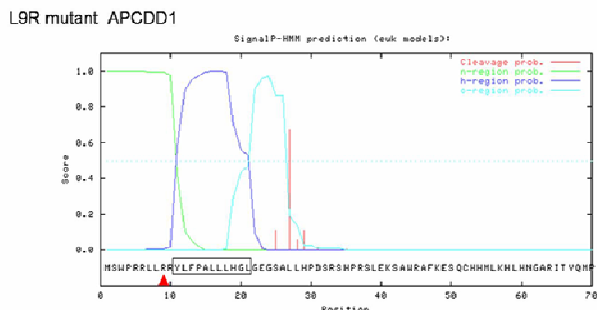


Supplementary Figure 3. *APCDD1* mRNA is expressed in human scalp skin.

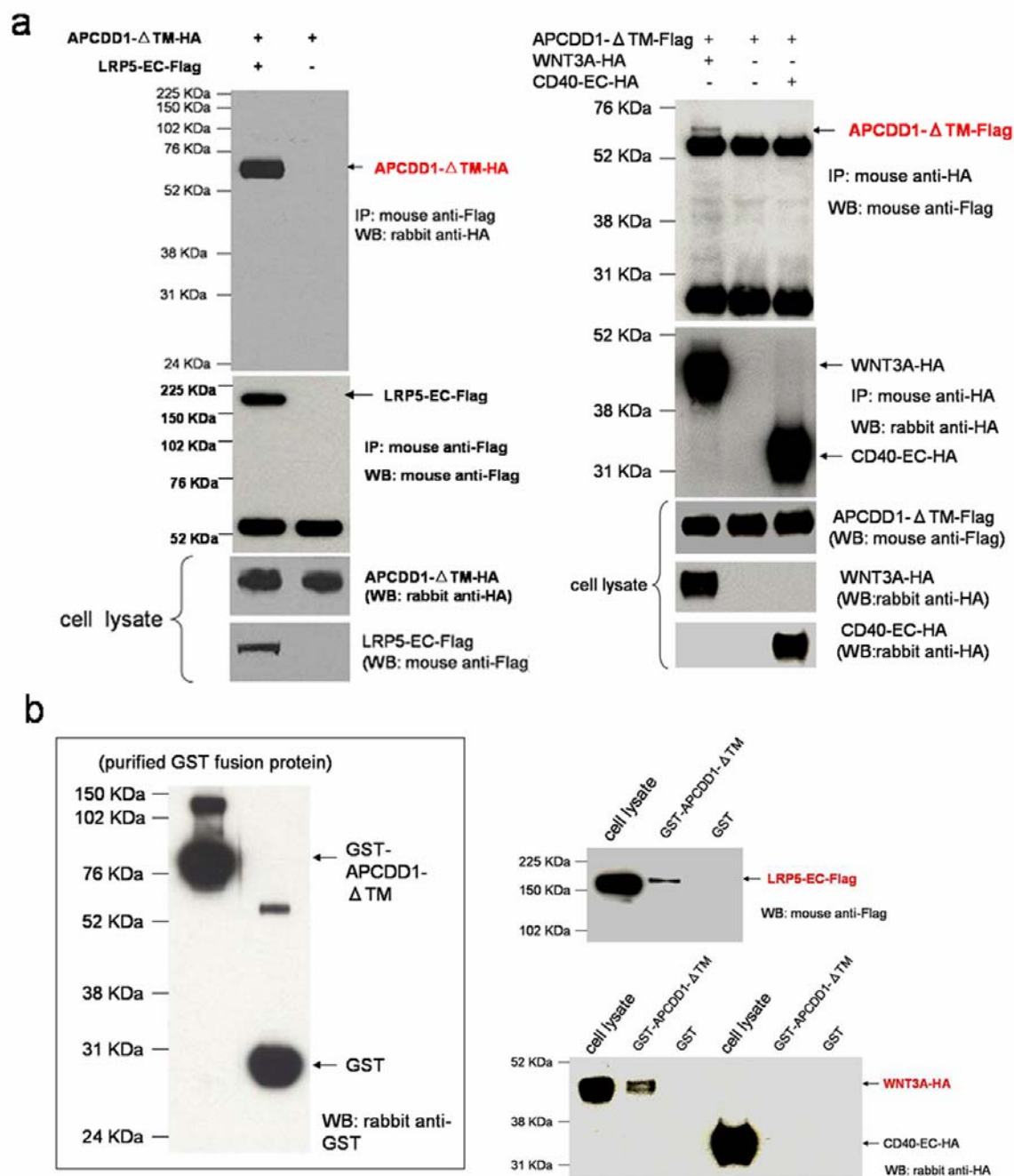
a, RT-PCR amplification of *APCDD1* mRNA from human scalp skin. Note that *APCDD1* mRNA was amplified, while its homologue *APCDD1L* mRNA was not. **b**, RT-PCR using total RNA from human plucked hairs shows the expression of *LRP5* and *WNT3A* in human hair follicles. MWM, molecular weight markers (**a**, **b**).

b

C

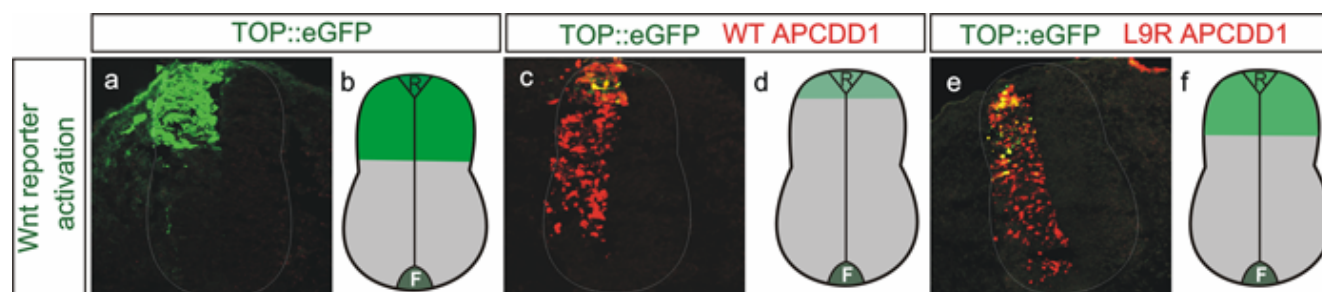


Supplementary Figure 4. Multiple amino acid sequence alignment of APCDD1 proteins between different species, and prediction of the signal peptide for APCDD1 protein. **a**, The N-terminal signal peptide and the C-terminal transmembrane sequences are marked in red and black, respectively. The conserved residues among 6 species are indicated by asterisks. The amino acid residue Leu9 is indicated in blue. Highly conserved cysteine residues are highlighted in yellow. **b**, Multiple amino-acid sequence alignment of the APCDD1 signal peptide sequences between different species. Residues that are conserved among at least five species are colored yellow. The Leu9 is colored in red. The accession numbers of GenBank or Ensembl databases for the respective APCDD1 proteins are: *Homo sapiens*, NP_694545; *Equus caballus*, ENSECAP00000009668; *Canis familiaris*, XP_537333; *Mus musculus*, NP_573500; *Myotis lucifugus*, ENSMLUP00000001735; *Gallus gallus*, ENSGALP00000001313; *Pelodiscus sinensis*, BAD74115; *Xenopus tropicalis*, *Xenopus laevis*, BAE02564; ENSXETP000000056413; *Danio rerio*, ENSDARP000000081410; *Ciona intestinalis*, ENSCINP000000022338. **c**, The N-terminal signal peptide sequences of the wild type (top) and the Leu9Arg mutant (bottom) APCDD1 protein was analyzed using the SignalP-HMM program (version 3.0; www.cbs.dtu.dk/services/SignalP/). The predicted hydrophobic core sequences are boxed. Amino acid position 9 is indicated by red arrowheads. Note that several other autosomal dominant diseases, such as familial hypocalciuric hypercalcemia (OMIM 145980)²⁶ and antithrombin III deficiency (OMIM 107300)²⁷ are also caused by substitutions in a leucine residue within the signal peptide.



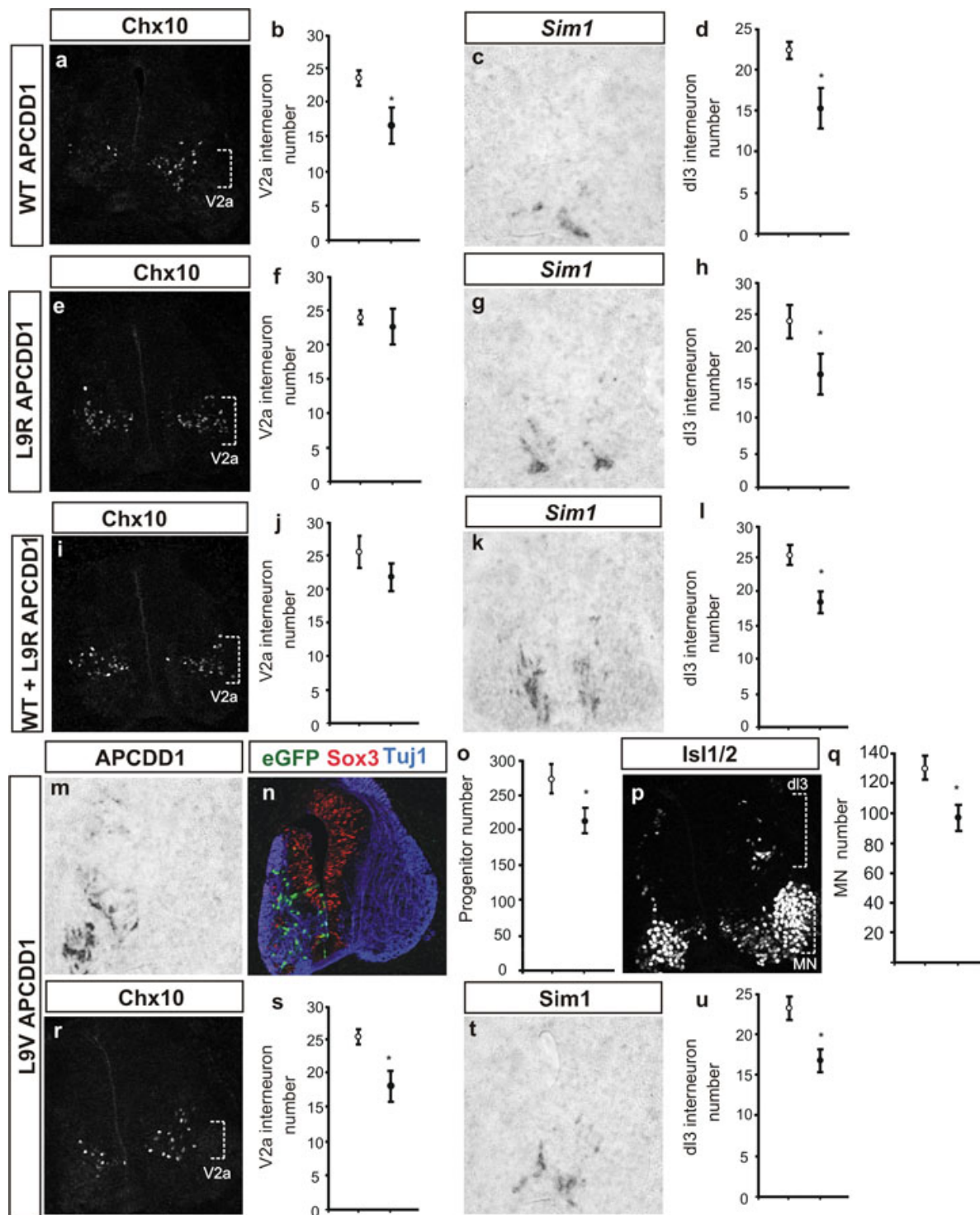
Supplementary Figure 5. APCDD1 is able to interact with LRP5 and WNT3A *in vitro*. **a**, Co-immunoprecipitation assays in HEK293T cells. The HA-tagged extracellular domain of APCDD1 protein (APCDD1- Δ TM-HA) was co-immunoprecipitated with the Flag-tagged extracellular domain of LRP5 (LRP5-EC-Flag; left panel). Flag-tagged extracellular domain of APCDD1 protein (APCDD1- Δ TM-Flag) was co-immunoprecipitated with the HA-tagged

WNT3A (WNT3A-HA), but not with the HA-tagged extracellular domain of CD40 (CD40-EC-HA; right panel). **b**, GST-pulldown assays. N-terminal GST fusion protein for the extracellular domain of APCDD1 (GST-APCDD1- Δ TM) was generated in bacteria, and was purified with glutathione-Sepharose beads (left panel). The purified GST-APCDD1- Δ TM was incubated with lysates of HEK293T cells overexpressing LRP5-EC-Flag, WNT3A-HA, or CD40-EC-HA, and was analyzed by western blots with mouse monoclonal anti-Flag-M2 (1:1,000; Sigma) or rabbit polyclonal anti-HA (1:4,000; Abcam) antibodies. The GST-APCDD1- Δ TM showed an affinity with LRP5-EC-Flag and WNT3A-HA, but not with CD40-EC-HA (right panels). CD40 is a Wnt signaling-unrelated single-pass transmembrane protein, and was used as a negative control (**a**, **b**).



Supplementary Figure 6. Overexpression of Wt-APCDD1, but not L9R mutant, blocks the activation of the Wnt reporter TOP::eGFP in the chick spinal cord.

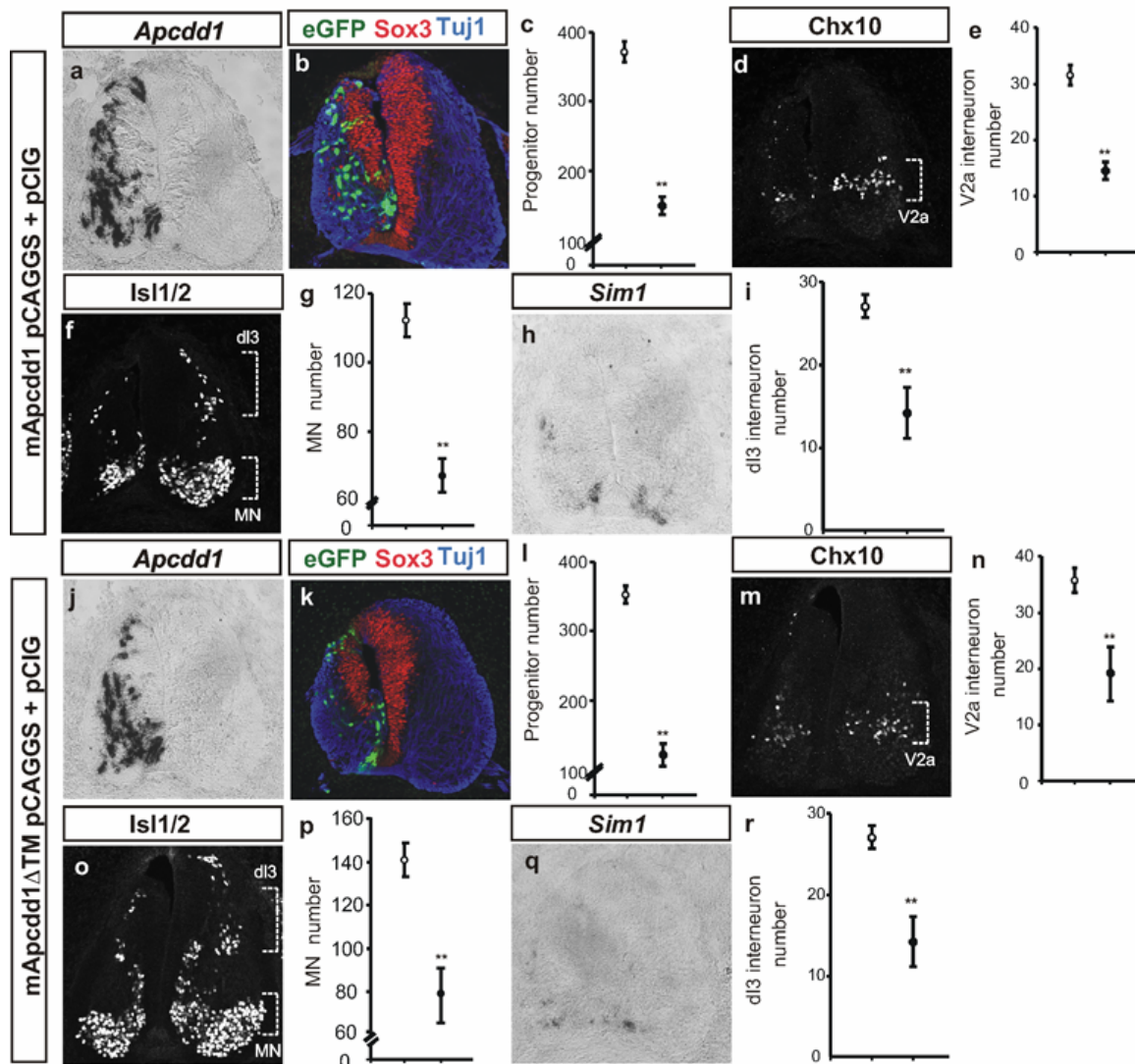
a-f Immunostaining for eGFP and APCDD1 in chick neural tubes electroporated with the Wnt reporter TOP::eGFP alone (**a**), or together with Wt (**c**), or L9R (**e**) APCDD1. Note that high eGFP levels in the dorsal and intermediate spinal cord from the Wnt reporter were inhibited strongly by the Wt APCDD1 and very weakly by L9R. This effect is illustrated in the diagrams (**b**, **d**, **f**).



Supplementary Figure 7. Overexpression of Wt- or L9V-, but not L9R-APCDD1 inhibits progenitor proliferation and generation of neurons in the chick spinal cord.

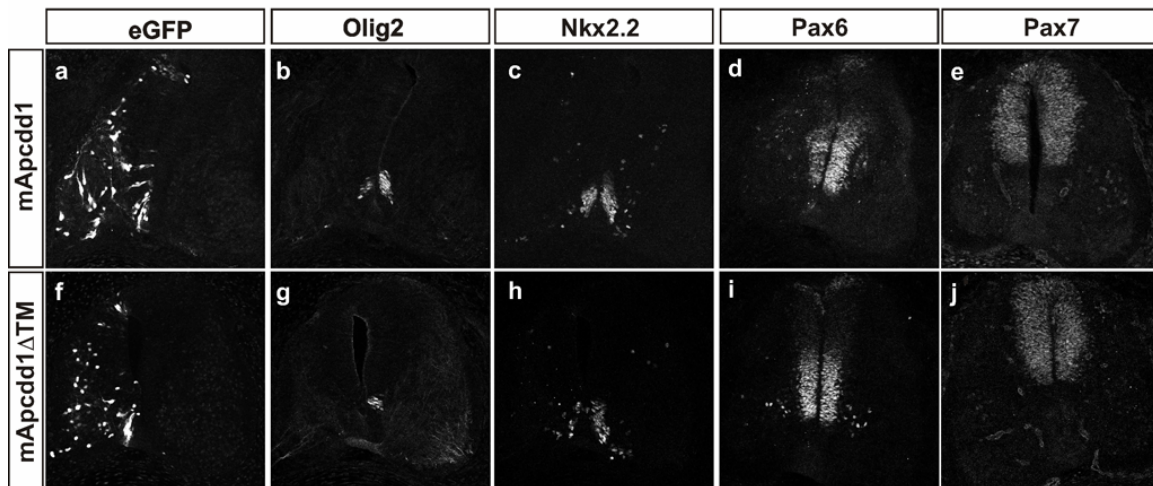
Immunofluorescence for Chx10⁺ (V2a ventral interneurons; **a, e, i**), or *in situ* for *Sim1*⁺ (**c, g, k**; V3 interneurons) shows that these neuronal populations are only reduced in the Wt APCDD1, but not L9R or both isoform electroporation. Plots of Chx10⁺ V2a interneurons (**b, f, j**) and Isl1/2⁺ dl3 interneurons (**d, h, l**) in control (open circles) and transfected embryos (closed circles) show that V2a interneurons are reduced in WT-APCDD1 transefction. Transfection of L9V APCDD1 is revealed by *in situ* hybridization for human *APCDD1* (**m**) and eGFP immunofluorescence (**n**, green). Immunofluorescence for Sox3 labels neural progenitors in red and Tuj1 labels neurons in blue (**n**). Note that misexpression of L9V APCDD1 reduces the number of Sox3⁺ progenitors and neurons. The inhibition of progenitor proliferation was more effective in the ventral than dorsal spinal cord, because of lower Wnt/ β -catenin levels in this region.

Immunofluorescence for Chx10⁺ (V2a ventral interneurons; **r**), Isl1/2⁺ (**p**; MNs - ventral Isl1/2⁺ cells; dl3 interneurons - dorsal Isl1/2⁺ cells) or *in situ* for *Sim1*⁺ (**t**; V3 interneurons) shows that these neurons are reduced in the L9V APCDD1 electroporation. Plots of Sox3⁺ progenitor numbers (**o**), Chx10⁺ V2a interneuron number (**s**), Isl1/2⁺ MN number (**q**) or Isl1/2⁺ dl3 interneurons (**u**) in control (open circles) and transfected embryos (closed circles). Bars represent mean $\pm$ s.e.m. (n=40 embryos for WT-APCDD1, n=35 embryos for L9R-APCDD1, n=28 embryos for WT- and L9R-APCDD1, and n=15 embryos for L9V-APCDD1, Student's t-test, *p<0.05).

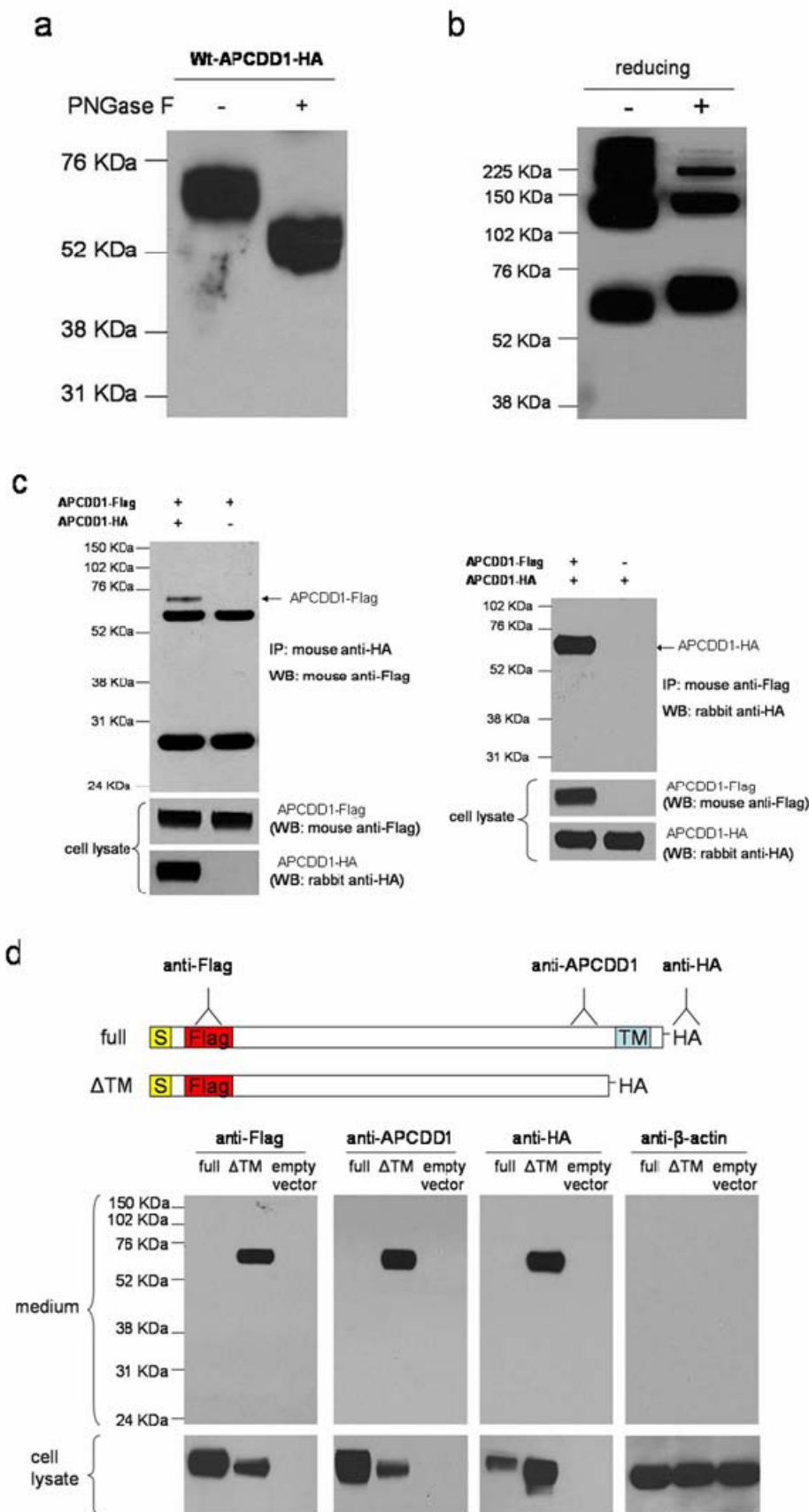


Supplementary Figure 8. Overexpression of mouse *Apcdd1* or *Apcdd1* Δ TM inhibits progenitor proliferation and generation of neurons in the chick spinal cord. Transfection of mouse *Apcdd1* or *Apcdd1* Δ TM is revealed by immunofluorescence for eGFP (**b, k**; green) and *in situ* hybridization for *mApcdd1* (**a, j**). Sox3 labels neural progenitors in red and Tuj1 labels neurons in blue (**b, k**). Note that misexpression of either mApcdd1 or mApcdd1 Δ TM, reduces by 2-fold the number of Sox3⁺ neural progenitors and neurons. The reduction in progenitor proliferation was more pronounced in the ventral than dorsal spinal cord, with lower levels of the Wnt/ β -catenin signaling. Immunofluorescence for Chx10⁺ (V2a ventral interneurons; **d, m**), *Isl1/2*⁺ (**f, o**; MNs - ventral *Isl1/2*⁺ cells; dl3

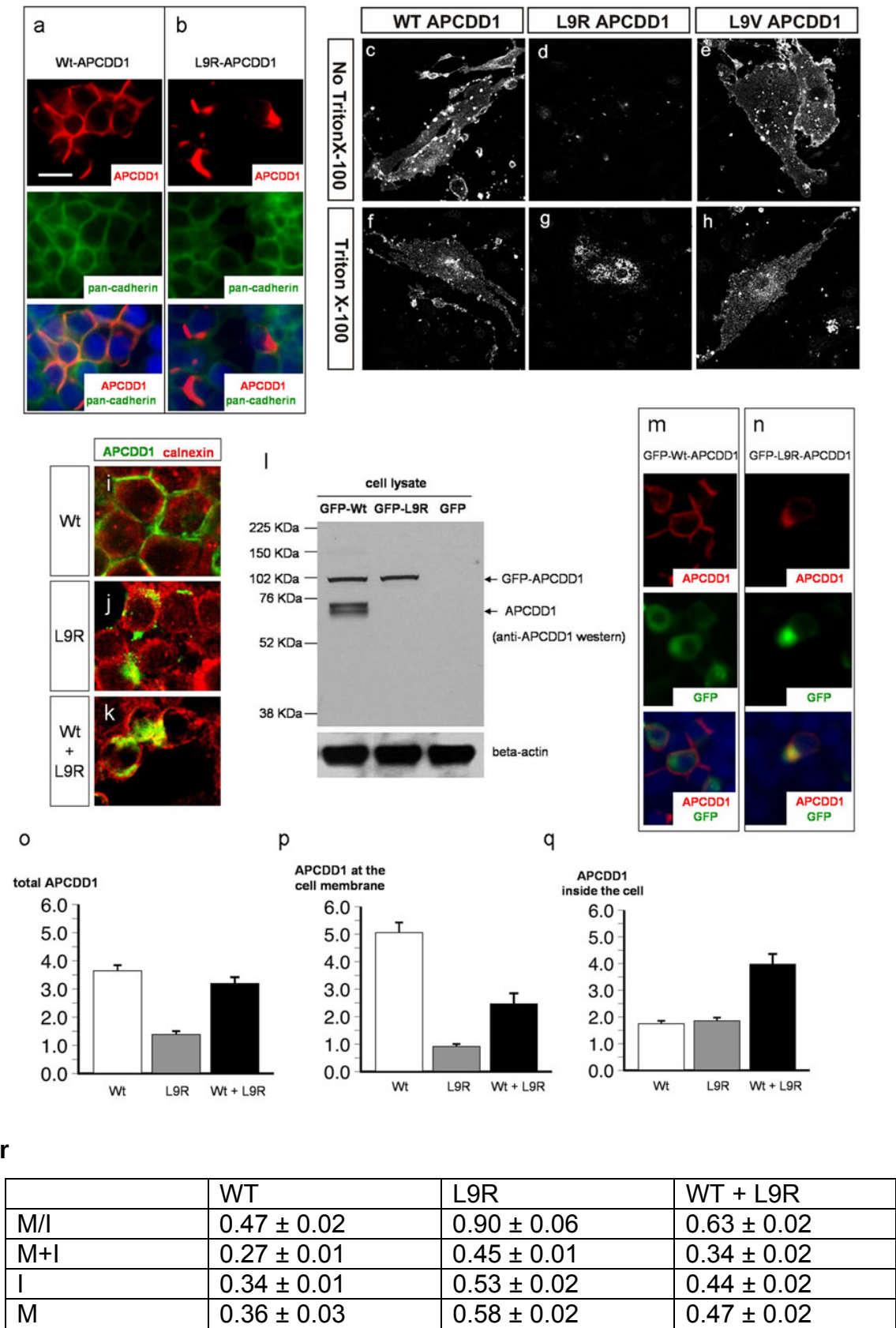
interneurons - dorsal $Isl1/2^+$ cells) or *in situ* for $Sim1^+$ (**h, q**; V3 interneurons) reveals that these neuronal populations are also reduced. Plots of $Sox3^+$ progenitor numbers (**c, l**), $Chx10^+$ V2a interneuron number (**e, n**), $Isl1/2^+$ MN number (**g, p**) or $Isl1/2^+$ dl3 interneurons (**i, r**) in control (open circles) and transfected embryos (closed circles). Bars represent mean $\pm$ s.e.m. (n=20 embryos transfected with mApccdd1; n=16 embryos transfected with mApccdd1 Δ TM; Student's t-test; **p<0.01). Note the similarity in the inhibition of neural progenitor number and ventral and dorsal interneuron and motor neurons between mApccdd1 and mApccdd1 Δ TM.



Supplementary Figure 9. Misexpression of either mouse *Apccdd1* or *mApccdd1ΔTM* isoforms reduces the expression of several progenitor domain markers in the chick spinal cord. **a-e**, Immunohistochemistry for eGFP (**a**), Olig2 (**b**), Nkx2.2 (**c**), Pax 6 (**d**) and Pax7 (**e**) in *mApccdd1*-transfected chick spinal cord (stage 22). Note that misexpression of *mApccdd1* in the ventral spinal cord reduces the domain of Olig2 (pMN) and to a much lesser extent that of Nkx2.2 (p3). In addition it slightly represses the expression of Pax6 (**d**), but not Pax7 (**e**). **f-j**, Immunohistochemistry for eGFP (**f**), Olig2 (**g**), Nkx2.2 (**h**), Pax 6 (**i**) and Pax7 (**j**) in *mApccdd1ΔTM*-transfected chick spinal cord (stage 22). Note that *mApccdd1ΔTM* also represses the expression of Olig2 (pMN) and to a lesser degree that of Nkx2.2 (p3) in the ventral spinal cord.

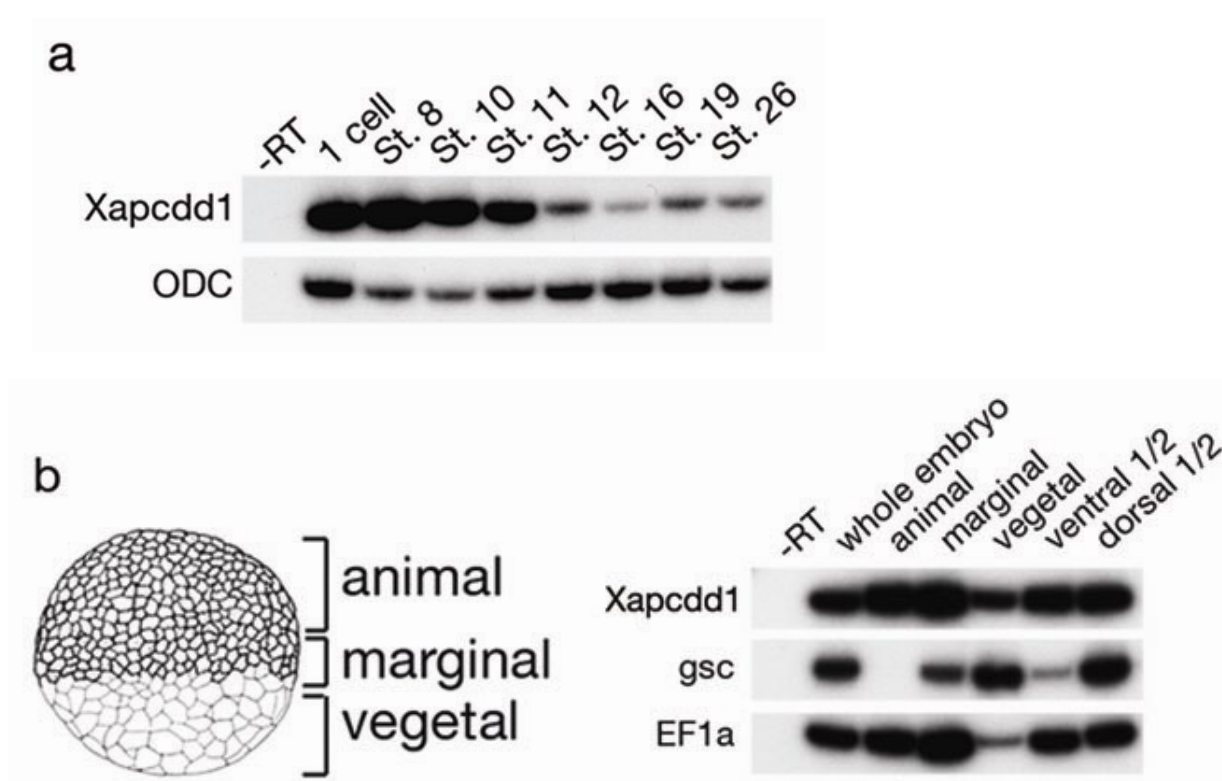


Supplementary Figure 10. Characterization of the APCDD1 protein. **a**, Cell lysates from HA-tagged wild-type APCDD1-expressing HEK293T cells were treated with N-glycosylase (PNGase F). The 68 KDa fragment was clearly digested into a 53 KDa fragment with PNGase F, suggesting that the APCDD1 protein undergoes N-glycosylation. **b**, Equal amounts of the cell lysate from HA-tagged wild-type APCDD1-expressing HEK293T cells were separated by 10% SDS PAGE under either non-reducing (-) or reducing (+) conditions. The intensity of the 130 KDa fragment markedly increased under non-reducing conditions. **c**, Co-immunoprecipitation (Co-IP) assays between Flag-tagged APCDD1 (APCDD1-Flag) and HA-tagged APCDD1 (APCDD1-HA) proteins. APCDD1-Flag protein is co-immunoprecipitated with APCDD1-HA protein (left panel), and APCDD1-HA protein is co-immunoprecipitated with APCDD1-Flag protein (right panel). These results demonstrate homodimerization of the APCDD1 protein. **d**, We transfected HEK293T cells with a full-length APCDD1 expression construct containing a Flag-tag just downstream of the signal peptide and an HA-tag at the C-terminus, and analyzed cell lysates and supernatants by western blotting. An expression construct for a truncated APCDD1 lacking the transmembrane domain (APCDD1- Δ TM) was also transfected as a positive control. S: signal peptide. TM: transmembrane domain. Western blots with anti-Flag, anti-APCDD1 and anti-Flag antibodies detected a strong fragment, around 63 KDa in size, in the medium of APCDD1- Δ TM-expressing cells, while no fragments were detected in medium of full-length APCDD1-expressing cells. β -actin was used as a normalization control for the cell lysate, and also used to show that the cell lysate did not contaminate the medium.

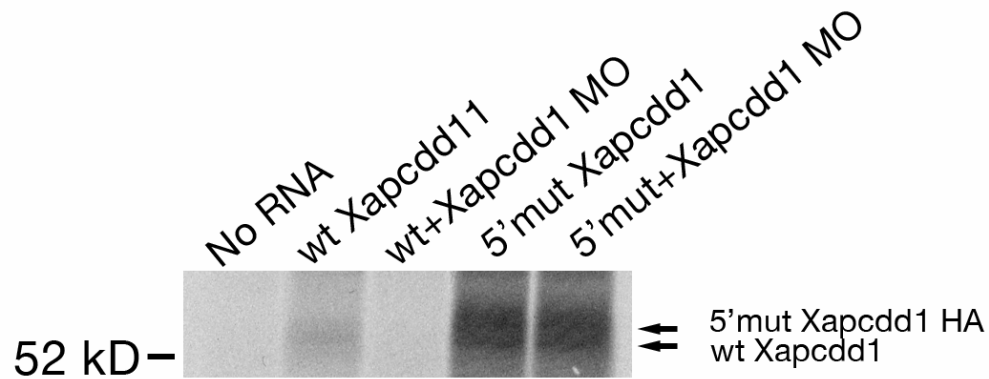


Supplementary Figure 11. The mutation L9R affects the co-translational processing of the mutant APCDD1. **a-k**, Immunofluorescence for APCDD1 on HEK293T cells (**a, b, i-k**) or Bend3.0 cells (**c-h**) transfected with Wt- (**a, c, f, i**), L9R- (**b, d, g, j**), L9V- (**e, h**), or co-transfected with Wt- and L9R-APCDD1 (**k**). Cell membrane was labeled with an anti-pan-cadherin antibody (**a, b**), and ER was stained with an anti-calnexin antibody (**i-k**). Scale bar: 20 μ m (**a**). Bend3.0 cells were either not permeabilized with TritonX-100 (**c-e**) to determine surface expression of APCDD1 or permeabilized (**f-h**) to detect total protein. Note that Wt- or L9V-APCDD1 localize to the plasma membrane (**a, c, f, e, h, i**), whereas the L9R-APCDD1 is retained in the ER (**b, d, g, j**). The majority of Wt-APCDD1 co-localizes with L9R-APCDD1 in the ER when co-transfected (**k**). **l-n**, N-terminal GFP-tagged APCDD1 proteins (GST-APCDD1) were overexpressed in HEK293T cells, and analyzed by western blot (**l**) and immunofluorescence (**m, n**) using a rabbit polyclonal anti-APCDD1 antibody. The western blot shows that GFP-Wt APCDD1 was cleaved, while GFP-L9R mutant was not (**l**). β -actin was used as a normalization control (**l**). GFP-Wt-APCDD1 protein is detected at the cell membrane (**m**), while the GFP-L9R-APCDD1 is retained in the cytoplasm (**n**). The bottom panels are merged images and counterstaining with DAPI is shown in blue (**a, b, m, n**). **o-q**, Analysis of the amount of APCDD1 protein in both (**o**), cell membrane (**p**) and intracellular (**q**) compartments from immunofluorescence in Fig. 2h-j. The expression of L9R-APCDD1 is significantly lower than that of Wt-APCDD1 (**o**). Note that co-transfection of Wt- and L9R-APCDD1 (Wt + L9R) decreased the membrane localized-APCDD1 protein (**p**), while it increased the expression of APCDD1 protein inside the cells (**q**). Data are represented as average $\pm$ 1 SEM ($n = 20$ cells per group). Wt versus L9R, $P < 3 \times 10^{-9}$; Wt versus Wt + L9R, $P = 0.58$; L9R versus Wt + L9R, $P < 3 \times 10^{-6}$ (**o**). Wt versus L9R, $P < 3 \times 10^{-9}$; Wt versus Wt + L9R, $P < 2 \times 10^{-5}$; L9R versus Wt + L9R, $P < 3 \times 10^{-5}$ (**p**). Wt versus L9R, $P = 0.99$; Wt versus Wt + L9R, $P < 9 \times 10^{-5}$; L9R versus Wt + L9R, $P < 3 \times 10^{-4}$ (**q**). The reported P -values are adjusted with the Bonferroni correction (**o-q**). **r**, Analysis of variability of expression of APCDD1. To assess the variability of APCDD1 expression, we measured noise in gene expression, defined as coefficient of variation (CV), in HEK293T cells transfected with wild-type

(WT)-APCDD1, L9R-APCDD1 or both. We found that at protein level WT-APCDD1 expression noise is ~ 27%, a mid-range value for a eukaryotic gene²⁸, and that L9R-APCDD1 has higher expression noise (45%), consistent with its lower expression level²⁹. When both WT- and L9R- APCDD1 are transfected, APCDD1 expression noise is more variable than WT, intermediate between WT and L9R, and in good agreement with the relative levels of WT and L9R, as expected from a weighted summation of independent (WT, L9R) noise levels³⁰. Notably, since WT and L9R APCDD1 proteins are likely to form not only homodimers (WT-WT, L9R-L9R) but also heterodimers (WT-L9R), the higher noise level of L9R is imparted not only to L9R-L9R homodimers, but also to WT-L9R heterodimers, both of which are expected to be non-functional. This suggests a molecular mechanism to explain the variability of dominant phenotypes, such that a low-level high-noise mutant protein directs the formation of high-noise heterodimers which are expressed at higher levels. Incomplete penetrance and phenotypic variability have received much attention and have recently been explained in part by noise at transcription level³¹. Our proposed molecular mechanism of heterodimerization between proteins with different noise levels may be an alternative way to generate incomplete penetrance. CV = standard deviation/mean. The CV values were obtained using a repeated jackknife procedure. M+I = total adjusted signal, M/I = ratio between signal in membrane and inside the cell, I = adjusted signal inside the cell signal, M= adjusted signal in the membrane. Data are represented as average $\pm$ SEM. Comparisons of noise levels are all significant in a 2-way heteroscedastic t-test. The Bonferroni correction has been applied to all *P*-values. All *P*-values are less than 0.0003.



Supplementary Figure 12. Timing (**a**) and localization (**b**, for stage 10) of *Xapcdd1* expression by RT-PCR. *gsc* is a dorsal (organizer) gene and *ODC* and *EF-1 α* are loading controls. Left panel in **b** shows a schematic representation of the three layers of blastula stage *Xenopus* embryos. Ectoderm and neural tissue derives from animal cells, mesoderm from marginal cells, and endoderm from vegetal cells.



Supplementary Figure 13. *Xenopus* apcdd1 MO specifically inhibit translation of wild type protein. In vitro translation for *Xenopus* Wt-apcdd1 and 5'mut-apcdd1-HA (1 μ g RNA) on the presence and absence of Xapcdd1-MO (1 μ g). The slight difference in molecular weight between wild type and 5'mut is due to the two HA tags in the mutant.

Supplementary Table 1. Phenotype of *Xenopus* *apcdd1* depletion

	Normal (%)	DAI 3-4 (%)	DAI 1-2 (%)	Total	P
Control	96	4	-	94	
Xapcdd1 MO	-	44	56	64	<0.0001
+Xapcdd1 RNA	-	87	3	46	<0.005*
+DNwnt8 RNA	-	100	-	60	<0.0001*

Embryos were injected in both dorsal blastomeres at 4 cell stage with 30 ng Xapcdd1 MO and scored at stage 40 for antero-posterior development (DAI index: 5) is normal, 0 lacks all recognizable anterior and dorsal structures³².

*: rescued embryos were compared to Xapcdd1-depleted embryos.

Supplementary Table 2. Primers used in this study

primer name	position (bp)	forward primer (5' to 3')	reverse primer (5' to 3')	Annealing temperature (C°)	product size (bp)
Primers to amplify additional microsatellite markers					
RAB31-MS	9,847,609-9,847,756	GCAAATGAATACACTAAGTCCCA	CACCAGGCCTAGGCTAAAATG	55	148
APCDD1-MS	10,454,280-10,454,403	CCAGTGAGACCTTAAAAGCTTCA	TTGCTATACTTTAGGAGCCAGAA	55	124
GNAL-MS	11,693,739-11,693,854	GGCTTGGGTACAATAATGAGCT	CAGAGCTTCCTGCACTTCAC	55	116
Primers to amplify candidate genes from genomic DNA					
APCDD1 ex1		CGACGCGCCCTTTCAAGTCT	CGCCAAGGGGACAGTGTAG	61	668
APCDD1 ex2		CACTGTCTGCTGCAGAGGT	GGTCTTCCATAGTGGTGTGC	56	328
APCDD1 ex3		GAATCTCTTTCCCATACCTTCAG	TGGCAAGCCTTTAAGAAGGATC	56	659
APCDD1 ex4		CGGAAGCATGTGTGCACTG	AAGAGGTCTTTCCCTCAGC	56	481
APCDD1 ex5		GTCTAGTTAGAGTGTGGCCAG	TTCTCTGGCATTCAAGTGCATC	56	651
RAB31 ex6		CAAAGCAGGAGTGTGAAGCA	TGTGCTCACTCTTCTCAAAGTG	55	302
TXNDC2 ex1		GAGGGAAAACCAACTGTAACGT	CTACAACCTTCCCTTCTTGGTTC	55	323
TXNDC2 ex2		CAGACTTCATTCTGTATCTCGA	TACAAGCAGTGCCATTTGGACAT	59	1,802
VAPA ex1		AGCCTGGCCTCGTCTAGA	AAAGCAGCCGGGAAAGGGAA	55	335
VAPA ex2		GAAAGGCAGTGTTAGTAGCCAT	TATTTCCCTCCCTCCAGATG	55	339
VAPA ex3		GTTTAAGGCAATCCCAGACTT	ACTGCTAAACAACACCCACAGT	55	275
VAPA ex4		GTCCCGTGAGGTGAACTTA	GGAAGTGAAGCTCAACAGC	55	203
VAPA ex5		CAGTCATTCCCAATATCATGCAG	CACAATACCCTTATCTGCTAGG	55	299
VAPA ex6		ATCTGACTGCTGACATGTACTG	TGGCCTAATAACACTCATATGTC	55	383
VAPA ex7		CACTAATCTACTTTCCGTCCCTA	CGTGGGCCATACTACCAATG	55	350
NAPG ex1		TGTGCGCTGCACCAGCTT	GGAGTGTGACCCAGAGGAC	55	337
NAPG ex2		GACCTAGAAGGTCATTACAAGC	GCCTTATAGAAGCATATGAGTAAGT	55	249
NAPG ex3		CTGATTTGTTGCCAGTAGTCAAC	GCGAACCAGTGTGGACCTTA	55	527
NAPG ex4		TGGAACCAACTGGGTCAAGT	TTACAGTTAGGAGTAAGTCTTGGT	55	306
NAPG ex5		TATGTTGTGCATGAGCCATTG	CTTGCCTTACAGTAAGGAAGCT	55	261
NAPG ex6-8		CTGCACATGTGTCCAGAAC	TGGTGTGTCTACAGCTTATACC	55	869
NAPG ex9		AAGTACATTTACAGGGGACCT	AAGCTGTAGTGGCTTACTCTA	55	318
NAPG ex10-11		TCCCGTGGAAGTTACTATAGCA	GGTGAAATTCATGCAAGTGGTC	55	1,109
NAPG ex12		CCACCATGGTCATGAGGCAT	GGATATCCCTAATCTATTCCCAAG	55	405
FAM38B ex1		GCTCCACCACCCATCTTATG	CTGCCTTACGCAGATAACATTTG	55	299
FAM38B ex2-3		GAAGGTGGGACCTGACTGGA	CGTTGACTATTGACCCAAACCT	58	753
FAM38B ex4		GTTGGAATGATGAAGGGGAAATTC	GTTCCACAACGATTCCCACTG	55	395
FAM38B ex5		CAGTTAAGGAAGTCTTGAGCT	TGCCTTCATTGTGAGCAGAAGT	55	331
FAM38B ex6		CTCAGGATGCAGAGAAGAGC	TTCCCACCACCTCAGGCCAT	55	250
FAM38B ex7		AGTGCAATGGAACACTCAAGAC	CCCAACTCCATGTTTAGACTGG	55	389
FAM38B ex8		TGACCGTTGTGGACCCAATG	GAAGTTGCATTCTCATACACATG	55	351
FAM38B ex9		GAGAGTTTGTGACTGTAATAGGAAC	TGTCACACAGGGAGAGATAACT	55	371
FAM38B ex10		TTAGTAACCAGATTTTCCATCTG	CCCACTCTCAACTTTACATGATAC	55	345
FAM38B ex11		CTTCTACAGAATTTGAGGGAAAGT	TCCGTGCAACTAGAAATGCTTAG	55	455
AMAC1L1		CCACCTCAGGATAGTTCCAG	CCTCTGAATGGTTAGGTCCTTA	55	1239
GNAL ex1		CTGGGCGTTAGCAAGTGATC	CCCACAGTTTAAACGCTCTGTA	55	843
Primers used in RT-PCR experiments					
APCDD1-rtPCR		ATGCCACCCAGAGGATGTTT	GATGGTCAGGTCTGCCTTTG	56	155
KRT15-rtPCR		GGGTTTGGTGGTGGCTTTG	TCGTGGTTCTTCTCAGGTAGGC	56	474
B2M-rtPCR		CACAGCCCCAAGATAGTTAAGTG	GCATAAAGTGTAAGTGATAAGCATA	56	143
APCDD1L-rtPCR		GTGGAGGAGCTGTACCTGG	GGCAATGAGGCCACAGGCT	56	135
LRP5-rtPCR		GACCCAGCCCTTTGTTTTGAC	TGTGGACGTTGATGGTATTGGT	56	138
WNT3A-rtPCR		GCCCCACTCGGATACTTCTACT	GAGGAATACTGTGGCCCAACA	56	98
Primers used to generate the probes for in situ hybridization					
APCDD1-ISH		CCAGAGCAGGACTGGAAATG	CTATCTGCGGATGTTCCAATGC	60	1562

Supplementary Table 2 (continued)

Primers used for cloning into vectors				
vector name	description	forward primer (5' to 3')	reverse primer (5' to 3')	template
pCXN2.1-Wt-APCDD1	Full length wild type (Wt) human APCDD1	APCDD1-F-XhoI (AAAGCTCGAGCCAGAGCAGGACTGGAAATG)	APCDD1-R-NheI (AAAAGCTAGCCTATCTGCGGATGTTCCAATGC)	human scalp skin cDNA
pCXN2.1-Wt-APCDD1-HA	Full length Wt human APCDD1 with C-terminal HA tag	APCDD1-F-XhoI	APCDD1-R-HA-NheI (AAAAGCTAGCTCAGGCGTAGTCGGGCACGTCGTAGGGGTATCTGCGGATGTTCCAATGC)	pCXN2.1-Wt-APCDD1
pCXN2.1-Wt-APCDD1-Flag	Full length Wt human APCDD1 with C-terminal Flag tag	APCDD1-F-XhoI	APCDD1-R-Flag-NheI (AAAAGCTAGCTCACTTATCGTCGTCATCCTTGTAATCTCTGCGGATGTTCCAATGC)	pCXN2.1-Wt-APCDD1
pCXN2.1-L9R-APCDD1	Full length L9R mutant human APCDD1	APCDD1-L9R-F-XhoI (AAAGCTCGAGCCAGAGCAGGACTGGAAATGTCCTGGCCGCGCCGCTCTGCGCAGAT)	APCDD1-R-NheI	pCXN2.1-Wt-APCDD1
pCXN2.1-L9V-APCDD1	Full length L9V mutant human APCDD1	APCDD1-L9V-F-XhoI (AAAGCTCGAGCCAGAGCAGGACTGGAAATGTCCTGGCCGCGCCGCTCTGGTCAGAT)	APCDD1-R-NheI	pCXN2.1-Wt-APCDD1
pCXN2.1-APCDD1-ΔTM-HA	Extracellular domain of human APCDD1 (aa residues 1-486) with C-terminal HA tag	APCDD1-F-XhoI	APCDD1-ΔTM-R-HA-NheI (AAAAGCTAGCTCAGGCGTAGTCGGGCACGTCGTAGGGGTAGCCATACAGGCTGCTTCCACT)	pCXN2.1-Wt-APCDD1
pCXN2.1-APCDD1-ΔTM-Flag	Extracellular domain of human APCDD1 (aa residues 1-486) with C-terminal Flag tag	APCDD1-F-XhoI	APCDD1-ΔTM-R-Flag-NheI (AAAAGCTAGCTCACTTATCGTCGTCATCCTTGTAATCGCCATACAGGCTGCTTCCACT)	pCXN2.1-Wt-APCDD1
pGEX-4T-3-APCDD1-ΔTM	Extracellular domain of human APCDD1 (aa residues 28-486)	APCDD1-F-EcoRI (AAAAGAATTCCCTTCATCCAGACAGCAGGTC)	APCDD1-ΔTM-R-XhoI (AAAGCTCGAGTCAGCCATACAGGCTGCTTCCACT)	pCXN2.1-Wt-APCDD1
pCXN2.1-WNT3A	Full length human WNT3A	WNT3A-F-XhoI (AAAGCTCGAGCGGCGATGGCCCCACTCGGATACCT)	WNT3A-R-NheI (AAAAGCTAGCCTACTTGCAGGTGTGCACGTCGT)	human scalp skin cDNA
pCXN2.1-WNT3A-HA	Full length human WNT3A with C-terminal HA tag	WNT3A-F-XhoI	WNT3A-R-HA-NheI (AAAAGCTAGCTAGGCGTAGTCGGGCACGTCGTAGGGGTACTTGCAGGTGTGCACGTCGT)	pCXN2.1-WNT3A
pCXN2.1-CD40-EC-HA	Extracellular domain of human CD40 (aa residues 1-193) with C-terminal HA tag	CD40-F-XhoI (ATATCTCGAGCCTCGCTATGGTTCGTCTGCCT)	CD40-R-HA-NheI (ATATGCTAGCTAGGCGTAGTCGGGCACGTCGTAGGGGTATCTCAGCCGATCCTGGGGAC)	human thymus cDNA
pCXN2.1-LRP5-EC-Flag	Extracellular domain of human LRP5 (aa residues 1-1384) with C-terminal Flag tag	LRP5-F-EcoRI (AAAAGAATTCGGGACAACATGGAGGCAG)	LRP5-R-Flag-NheI (AAAAGCTAGCTACTTATCGTCGTCATCCTTGTAATCGCTGTGGGCCGGGCTGTCGTCTGA)	Expression vector for full length human LRP5
CS2+Xapcdd1 HA	Full length xenopus APCDD1 with C-terminal HA tag	Xapcdd1-F-ClaI (CCATCGATGGCCACCATGGGAAATCCCTGTTTCTACTGC)	Xapcdd1-R-SalI (CCACCGTCGACGCTTAGAATTTCCAAATGTAGCAAAG)	Full length cDNA clone of Xapcdd1
pCAGGS-mApcdd1	Full length mouse Apcdd1	Apcdd1F (GGGGACAGAGACGGACTACA)	Apcdd1R (CAAGGCATTCAAGTGCATC)	cDNA from mouse brain endothelial cells
pCAGGS-mApcdd1-ΔTM	Extracellular domain of mouse Apcdd1 (aa residues 1-486)	Apcdd1F	Apcdd1ΔTM-R (CTGCCCTGCCTGCCATACAGATGACCTTGACTGTC)	pCAGGS-mApcdd1