**Supplementary Materials and Methods**

**Immunofluorescence**

For histological analysis, tissues were fixed in 4% paraformaldehyde overnight at 4°C and embedded in paraffin wax for sectioning. The sections (4 µm) were stained with hematoxylin and eosin (H&E) or Periodic acid–Schiff (PAS)/Hematoxylin (H). For immunohistochemistry, paraffin-embedded sections were rehydrated, and the antigenic epitopes were exposed using Tris/ethylenediaminetetraacetic acid (EDTA) buffer. Sections were incubated in blocking solution (5% BSA, 5% goat serum or horse serum, and 0.5% Tween-20 in PBS) at room temperature for 4 hr and then incubated with primary antibodies to Aimp2 (1:200; Proteintech), BrdU (1:200; Abcam), Ki67 (1:200; Leica Biosystems), GFP (1:200; Abcam), chromogranin A (1:200; Santa Cruz), lysozyme (1:200; DAKO), cleaved Caspase 3 (1:200; Cell signaling) and Cd44 (1:200; BD Biosciences). Cell death was assessed by TdT-mediated dUTP nick end labelling (TUNEL) of paraffin-embedded sections of the respective genotypes using the TUNEL cell death detection kit (Roche). For immunocytochemistry, HCT116 cells plated on cover slides washed with PBS at room temperature, fixed in 4% paraformaldehyde for 60 minutes, and blocked with 5% BSA in PBS at room temperature for 4 hr. Samples were incubated with primary antibodies to HA (Santa Cruz) and GFP (Abcam) overnight at 4°C. Specific binding was detected with an Alexa 488-conjugated (green) and/or Alexa 594-conjugated (red) antibody (Molecular Probes). Immunofluorescence was detected using an Observer Z1 fluorescent microscope (Zeiss) equipped with a SPOT Flex camera or with an LSM710 confocal system (Zeiss).

**Intestinal epithelium and crypt isolation**

The small intestine was removed, and the fat/mesentery was dissected away. After a flush with cold PBS, a fragment (4 cm) of the ileum from *Aimp2* mutant or control littermates was rinsed again with cold PBS. The fragments were cut into 3- to 5-mm pieces and placed into 50-ml conical tubes that were then filled with 30 ml of cold PBS containing 20 mM EDTA. The samples were incubated for 30 min at 4°C with intermittent shaking. The samples were then washed with 10 ml of cold PBS. The first supernatant consisted of debris and a few villi. The second to fifth supernatants contained the majority of the intestinal epithelium, including the villi and crypts. The isolated intestinal epithelium was centrifuged 200 × *g* for 5 min at 4°C to separate the villi and crypts from single cells, and the resulting pellets were used for immunoblotting and qRT-PCR analysis. For villus fraction, the second and third supernatants were centrifuged at 200 × *g* for 5 min and used for qRT-PCR analysis. For crypt isolation, the fourth and fifth supernatants were passed through a 70-µm cell strainer (BD Biosciences) to remove residual villous material. Isolated crypts were centrifuged at 200 × *g* for 5 min and used for qRT-PCR analysis and organoid culture.

***In vitro* binding assay**

*In vitro* binding assay was performed as described previously ([1](#_ENREF_1)). GST and GST-AIMP2 were expressed in the BL-21strain. Proteins were induced with 1 mM IPTG and cultured at 20°C overnight. Cells were pelleted and lysed with cold PBS buffer containing 1% Triton X-100, 0.5% *N*-laurylsarcosine, 1 mM dithiothreitol, 2 mM EDTA, and 300 µM phenylmethylsulfonyl fluoride. The lysates were centrifuged for 20 min at 12,000 rpm, and the supernatants were mixed with Glutathione Sepharose™ 4B beads (GE Healthcare) for 6 hr at 4°C with agitation. The samples were then washed 3 times with the cold PBS buffer. Proteins were eluted from the Sepharose beads and separated by SDS-PAGE, and binding partners were identified with immunoblotting.

**Organoid culture**

Freshly isolated crypts were pelleted and mixed with 30 µl of Matrigel (BD Biosciences). After Matrigel polymerization, advanced DMEM/F12 (Invitrogen) containing 50 ng/ml EGF (Peprotech), 250 ng/ml R-spondin 1 (R&D systems), and 100 ng/ml Noggin (Peprotech) was added. After culture for 7 days, organoids were removed from the Matrigel, dissociated mechanically, and transferred into fresh Matrigel and medium (split ratio 1:6) under the indicated growth conditions. Growth of the organoids was measured daily using stereomicroscopy, and the formation of organoids was scored. The proliferation rate was determined by counting the number of BrdU-positive vs. DAPI-positive cells. The organoids were incubated with 20 µM BrdU for 1 hr at 37°C before fixation. For organoid size measurement, morphometric software (INS Industry), SPOT software (version 5.1; Diagnostic Instruments), and Photoshop CS6 (Adobe) were used.

**Dose-reponse curve analysis**

All curve fitting was done in GraphPad Prism. Each point shown in Wnt3a dose-response curve assay represents the mean of measure­ments from triplicate wells, with error bars representing the SEM. The experi­ment was repeated at least three times. The solid lines represent nonlinear curve fits of the data to a sigmoidal (variable-slope) equation. Curve fits were calculated in GraphPad Prism using the log (agonist) versus response or normalized response as appropriate.

**Immunoblotting and qRT-PCR analysis**

Immunoblotting was performed as described previously ([2](#_ENREF_2)). Primary antibodies to AIMP2 (Proteintech), active -catenin (Upstate), AXIN2 (Abcam), NKD1 (Abcam), DVL1 (Santa Cruz), TRAF2 (Santa Cruz), p53 (Cell Signaling), phospho-p53 (ser15) (Cell Signaling), anti-GFP (Abcam), anti-HA (Santa Cruz), anti-Myc (Cell Signaling) and -actin (Sigma) were utilized to detect each protein. Protein bands were detected with enhanced chemiluminescence (Amersham Pharmacia Biotech), and analyzed by ImageJ software (NIH). For qRT-PCR analysis, total RNA was isolated from samples using TRIzol reagent (Life Technologies), and complementary DNA synthesis was performed according to the manufacturer’s instructions (Omniscript kit; Qiagen). The data were normalized to -actin.

**qRT-PCR primer information**

*Actin*: 5’- AAGGAAGGCTGGAAAAGAGC -3’ 5’- AAATCGTGCGTGACATCAAA -3’

*Bmp2*: 5’-GAAGTTCCTCCACGGCTTCT-3’ 5’-AGATCTGTACCGCAGGCACT-3’

*Smad6*: 5’- GTGCTCCCAGTACGCCAC -3’ 5’- ACCAACTCCCTCATCACTGC -3’

*Ephb3*: 5’- AAGAGACTCTCATGGACACGAAAT-3’ 5’-ACTTCCCGCCGCCAGATG-3’

*Sox9*: 5’- CGGCGGACCCTGAGATTGC -3’ 5’-CTGGAGGCTGCTGAACGAGAG -3’

*Aimp2*: 5’-GGTTTGCGTTGATCACAATG-3’ 5’-AGTTGAAGGCAGCAGTCGAT-3’

*Gob5*: 5’- TCTTGTGTAGATGCCATCATTTTT -3’ 5’- CCAATGTCACAGCCCTCATA -3’

*Chga*: 5’- GTCTCCAGACACTCAGGGCT -3’ 5’- ATGACAAAAGGGGACACCAA -3’

*Mmp7*: 5’- CAGACTTACCTCGGATCGTAGTGG-3’

5’- GTTCACTCCTGCGTCCTCACC-3’

*Lyz*: 5’-ATGGAATGGCTGGCTACTATGGAG-3’

5’-CTCACCACCCTCTTTGCACATTG-3’

*Lgr5*: 5’-GAGTCAACCCAAGCCTTAGT-3’ 5’-CATGGGACAAATGCAACTGA-3’

*Olfm4*: 5’- GCCACTTTCCAATTTCAC-3’ 5’- GAGCCTCTTCTCATACAC-3’

*Msi1*: 5’-GATGCCTTCATGCTGGGTAT-3’ 5’-AATTCGGGGAACTGGTAGGT-3’

*C-myc*: 5’-ACGGAGTCGTAGTCGAGGTC-3’ 5’-AGAGCTCCTCGAGCTGTTTG-3’

*Cd44*: 5’-TCCACATGGAATACACCTGC-3’ 5’-CAAGTTTTGGTGGCACACAG-3’

*Axin2*: 5’-TGCATCTCTCTCTGGAGCTG-3’ 5’-ACAGCGAGTTATCCAGCGAC-3’

*Nkd1*: 5’-GTAATGTCCTCACGGGTCACTT-3’ 5’- AGAATGGAGAGACTGAGCGAAC -3’

*Puma:* 5’-TGTCGATGCTGCTCTTCTTG-3’ 5’-GTGTGGAGGAGGAGGAGTGG-3’

*Perp;* 5’-GGCGAAGAACGAGAGAATGAA-3’ 5’-GCTGCAGCCACGCTTTTC-3’

*Bax;* 5’- CCCCAGTTGAAGTTGCCATC-3’ 5’-GTTTCATCCAGGATCGAGCAG-3’

*Tnfa:* 5’-ACCCTGGTATGAGCCCATATAC-3’ 5’-ACACCCATTCCCTTCACAGAG-3’

*A20:* 5’-GTTGTCCCATTCGTCATTCC-3’ 5’-AAACCAATGGTGATGGAAACTG-3’

*Ikba:* 5’-GCCACTTTCCACTTATAATGTC-3’ 5’-GTAACCTACCAAGGCTACTC-3’

*AXIN2*: 5’-CAAACTCATCGCTTGCTTTTT-3’ 5’-CACTTACTTTTTCTGTGGGGAAG-3’

*NKD1*: 5’- GCTGAGCGTGTCTCTCAACA -3’ 5’- AGGAGTGGATCGGGAGACAG -3’

**Supplementary Figure Legends**

**Supplementary Figure 1. Analysis of polyposis and tumor initiation in *Aimp2*+/-*:Apc*Min/+ intestine**

(A) Graph of polyp numbers in Male and female *ApcMin/+* (Male n=11, Female n=12) and *Aimp2+/-*:*ApcMin/+* (Male n=12, Female n=12), analyzed in whole intestinal tracks from 20-week-oldlittermates. (B) Representative images of methylene blue staining in the colons of 20-week-old *ApcMin/+* and *Aimp2+/-*:*ApcMin/+* littermates. (C) Representative H&E staining of ACF from 6-week-old *ApcMin/+* and *Aimp2+/-*:*ApcMin/+* littermates. Scale bar, 100 µm. (D) Representative images of H&E staining in the large intestines of 7-week-old *ApcMin/+* and *Aimp2+/-*:*ApcMin/+* littermates. Polyps are circled with black dashed lines. Student *t*-test was applied, and data are the mean ± standard error of the mean (SEM). \**P* < 0.01.

**Supplementary Figure 2. *Aimp2+/-* mice exhibit increased villi length, crypt depth, and colonic epithelial cell proliferation**

(A-B) Quantification of villi length (A) and crypt depth (B) in *Aimp2+/+* (n = 3) and *Aimp2+/-* littermates (n = 3). Graph presents the villi length and crypt depth in different regions of the intestine (Duo: duodenum, Jej: jejunum, and Ile: ileum). (C) Positional counting of BrdU+ cells in the ileal crypts of 20-week-old *Aimp2+/+* (n = 3) and *Aimp2+/-* (n = 3). Mice were sacrificed 2 hr post BrdU injection. (D) Immunohistochemistry with AIMP2 antibody on sections of the adult (20-week-old) colon. (E) Representative images of Ki67 immunohistochemistry in the colonic crypts of 20-week-old *Aimp2+/+* and *Aimp2+/-* littermates. (F) Quantification of Ki67+ cells in *Aimp2+/+* (n = 5) and *Aimp2+/-* (n = 5) colonic crypts. At least 30 well-oriented crypts were analyzed on 3–4 slides for each mouse. (G) Representative images of TUNEL staining in intestinal sections (ileum) from 6-week-old *Aimp2+/+* and *Aimp2+/-* littermates. (H) Quantification of TUNEL+ cells in *Aimp2+/+* (n = 5) and *Aimp2+/-* villi (n = 5). (I) Representative PAS/H staining in intestinal sections (ileum) from 6-week-old *Aimp2+/+* and *Aimp2+/-* littermates. (J) Quantification of PAS+ cells in *Aimp2+/+* (n = 3) and *Aimp2+/-* villi (n = 3). (K) Representative images of immunohistochemistry with an anti-chromogranin A antibody in intestinal sections (ileum) from 6-week-old *Aimp2+/+* and *Aimp2+/-* littermates. (L) Quantification of chromogranin A+ cells in *Aimp2+/+* (n = 3) and *Aimp2+/-* villi (n = 3). (H, J, L) The cells were counted in at least 30 well-oriented villi for each mouse. Scale bar, 50 µm. Student *t*-test was performed, and data were presented as the mean ± standard error of the mean. \**P* < 0.01.

**Supplementary Figure 3. AIMP2 interacts with the DIX domain of DVL and modulate Wnt/-catenin signaling in an *Aimp2* gene dosage dependent manner**

(A) HEK293 cells were transfected with the indicated vector. Cell lysates were incubated with purified GST-Empty vector (EV) or GST-AIMP2 fusion protein *in vitro*. (B) HEK293 cells were transfected with different DVL plasmid constructs as indicated. Cell lysates were incubated with purified GST-EV or GST-AIMP2 fusion protein *in vitro* and analyzed by immunoblotting with anti-MYC and anti-HA antibodies. (C) HeLa cells were transfected with control or AIMP2 overexpression vectors, and the expression of Wnt/-catenin target genes were analyzed with qRT-PCR. HeLa cells were treated with Wnt3a for 12 hr. (D-E) The expression of *AIMP2* mRNA (D) and AIMP2 protein (E) was analyzed with qRT-PCR and immunoblotting to examine knockdown efficiency of siRNAs. (F) HeLa cells were transfected with scramble or AIMP2 siRNAs, and the expression of Wnt/-catenin target genes was analyzed with qRT-PCR. HeLa cells were treated with Wnt3a for 12 hr. (G-H) The expression of *Aimp2* mRNA (G) and AIMP2 protein (H) was analyzed with qRT-PCR and immunoblotting in *Aimp2+/+*, *Aimp2+/-*, and *Aimp2-/-* MEFs. (I-J) The expression of Wnt/-catenin target genes (I) and TOPflash activity (J) were measured in *Aimp2+/+*, *Aimp2+/-*, and *Aimp2-/-* MEFs treated with Wnt3a for 12 hr and 24 hr respectively. FOPflash activity was measured as control.(K) The expression of *Axin2* mRNA was analyzed with qRT-PCR analysis in *Aimp2+/+*, *Aimp2+/-*, and *Aimp2-/-* MEFs. The cells were pretreated with porcupine inhibitor (IWP4, Stemgent) for 24 hr, and stimulated with Wnt3a for 12 hr in the presence of IWP4. Student *t*-test (C) or ANOVA (F, I, J, K) was performed, and data were presented as the mean ± standard error of the mean. \**P* < 0.01. At least 3 independent experiments were performed.

**Supplementary Figure 4. The role of AIMP2 in intestinal Wnt signaling is dependent on  -catenin destruction complex.**

(A-C) The level of endogenous DVL1 protein was analyzed with immunoblotting in *Aimp2+/+*, *Aimp2+/-*, and *Aimp2-/-* MEFs (A), 6-week-old *Aimp2+/+*, *Aimp2+/-* intestinal epithelial cells (B), and postnatal day 0 *Aimp2+/+*, *Aimp2+/-*, and *Aimp2-/-* intestinal epithelial cells (C). (D-E) Immunoblotting (D) and mRNA expression (E) of whole cell extracts from 6-week-old *Aimp2+/+* and *Aimp2+/-* IECs. (E) The expression of known p53 and NF-B target genes reported in intestinal context ([3](#_ENREF_3),[4](#_ENREF_4)) (F-G) Immunoblotting (F) and mRNA expression (G) of whole cell extracts from postnatal day 0 *Aimp2+/+*, *Aimp2+/-*, and *Aimp2-/-* IECs (G) The expression of known p53 and NF-B target genes reported in intestinal context ([3](#_ENREF_3),[4](#_ENREF_4)) (H) HeLa cells were transfected with Empty vectors (EV) or AIMP2 overexpression vectors, and the expression of *AXIN2* was measured in the absence or presence of GSK-3 inhibitor (CHIR99021, Stemgent). (I) The expression of *Axin2* in *Aimp2+/+* and *Aimp2-/-* MEFs was analyzed in in the absence or presence of GSK-3 inhibitor (CHIR99021, Stemgent). Student *t*-test was performed, and data were presented as the mean ± standard error of the mean. At least 3 independent experiments were performed.

**Supplementary Figure 5. *Aimp2* gene dosage negatively correlates with organoid growth**

(A) The size of the organoids from *Aimp2+/+*, *Aimp2+/-***,** and*Aimp2-/-*micewas measured by morphometric analysis of stereomicroscopic images. Organoids were cultured for 3 days with increasing concentrations of R-spondin-1. At least 10 organoids were analyzed for each genotype. (B) Stereomicroscopic images of *Aimp2+/+*, *Aimp2+/-*,and*Aimp2-/-*intestinal organoids cultured with 25 ng/ml R-spondin-1. Images were obtained at 1, 3, and 5 days of culture as indicated. Scale bar, 50 µm. ANOVA was performed, and data were presented as the mean ± standard error of the mean. \**P* < 0.01. At least 3 independent experiments were performed.

**Reference**

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