Supporting Information

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SI Methods

Peptide Synthesis and Characterization. Peptides were synthesized manually or automated (Tetras Thuramed) on 4-methylbenzhydrylamine (MBHA) rink amid resin using standard Fmoc-based solid-phase peptide synthesis (1). Amino acid coupling was performed using 4 equivalents of Fmoc-protected amino acids, 3.8 equivalents of 5-Chloro-1-[bis(dimethylamino)methylene]-1H-benzotriazolium 3-oxide hexafluorophosphate, and 8 equivalents of *N*,*N*-diisopropyl ethylamine in *N*-methyl-2-pyrrolidinone (equivalents relative to initial loading of MBHA rink amid resin). Chemical cross-linking of nonnatural amino acids (Fig. S1*C*; purchased from Okeanos Tech) was performed using 0.5 equivalents of Grubbs I catalyst in dichloroethane (5 mM) three times for 2 h. After olefin-metathesis, peptides were subjected to a final round of deprotection (10 min 20% piperidine in *N,N*-dimethylformamide) and capping by standard methods with Ac_2O, FITC, or Biotin (1). After cleavage, crude peptides were dissolved in 2:1 water/acetonitrile and purified by reverse-phase HPLC (solvent A: water + 0.1% TFA; solvent B: acetonitrile + 0.1% TFA; flow rate: 4 mL min^{-1}) using a Zorbax C18 reverse-phase column (9.4 × 250 mm, pore size 80 Å, particle size 3.5 μm; Agilent). Pure product fractions were combined, concentrated in a speed vac, and lyophilized. Compound identification and purity was assessed by coupled liquid chromatography/mass spectrometry (HPLC/ESI; solvent A: water + 0.1% TFA; solvent B: acetonitrile + 0.1% TFA; flow rate: 0.5 mL min^{-1}) using a Zorbax C18 reverse-phase column (2.1 × 150 mm, pore size 80 Å, particle size 3.5 μm; Agilent). Table S1 provides calculated and found masses (*m*/*z*), and Fig. S2 shows high-resolution mass spectroscopy (HRMS) data and HPLC traces of peptides used in this study. Peptides were quantified by their UV absorbance. FITC-labeled peptides were measured in pH 8.8 phosphate buffer (ε_{494} = 77,000 $M^{-1}cm^{-1}$). For non-FITC–labeled peptides the tryptophan absorbance (ε_{280} = 5600 M^{-1} cm^{-1} per tryptophan) in pH 7.0 phosphate buffer was used.

Phage Display Selection. The selection followed standard procedures (2). Oligonucletides encoding randomized Axin peptides (Fig. S3*A*) were synthesized, pooled, and inserted into pUC19 gp3 phagemid vector. The resulting plasmids were electroporated into XL1 Blue *Escherichia coli* competent cells to create the phage display library. To screen for β-catenin binders, 100 pmol biotinylated β-catenin were immobilized on streptavidin-coated ELISA plates, and the phage library was then applied into the wells for capturing the β-catenin binding phage. After incubation at 4 °C for 8 h, unbound phages were removed by extensive washing with Tris-buffered saline [TBS; 150 mM NaCl, 20 mM Tris (pH 7.4)] + 0.1% Tween 20. The remaining phages were absorbed by XL1 Blue *E. coli* and amplified. After two and three rounds of panning, individual phage clones were obtained, and the phagemid DNA was sequenced to determine the selected sequences (Fig. S3*B*).

Protein Expression and Purification. BL21 cells harboring pGEX-TEV β-catenin expression plasmid of full-length or 134–665 human β-catenin were cultured at 37 °C until OD_{600} = 0.8 and were then induced with 100 mM isopropyl-β-D-thiogalactopyranosid (IPTG). Cells were cultured for another 12 h at 20 °C. Cell pellets were collected by centrifugation. After sonication in 300 mM NaCl, 20 mM Tris (pH 8.8) buffer, and centrifugation, soluble GST–β-catenin fusion proteins from cell lysate were first purified by glutathione Sepharose resin, followed by monoQ ion exchange purification in 20 mM Tris buffer (pH 8.8), 2 mM DTT, and 2% glycerol with salt gradient from 100 mM to 1 M NaCl. For proteins used in phage screening and X-ray crystallography, GST tag was removed by TEV protease cleavage before ion exchange. GST-human E-cadherin(731-882) was expressed and phosphorylated according to published protocols (3).

Circular Dichroism. A circular dichroism spectrometer (Jasco J-710) and a quartz cuvettete (path length: 0.1 cm) were used to determine α-helicities. Peptides were dissolved into deionized water to a final concentration of 50 μM. Absorbance was measured between 190 and 260 nm. The percentage helicity was calculated from the absorbance at 222 nm using helical models as previously reported (4).

Fluorescence Polarization Assays. Binding assays were performed in triplicates by incubating 10 nM fluorescein-labeled peptides with 2.16-fold dilutions of β-catenin in 300 mM NaCl, 25 mM Tris (pH 8.8), 2 mM DTT, and 2% glycerol (total volume = 50 μL). Incubations were made in 384-well, black flat-bottom plates (Corning) and equilibrated for 45 min. Polarization was measured on a Spectramax-M5 plate reader with λ(ex) = 485 nm and λ(em) = 525 nm. Dissociation constants (K_d) were determined by nonlinear regression analysis of dose–response curves using Prism software (GraphPad).

Competition Fluorescence Polarization Assays. Competitive fluorescence polarization assays were performed by incubating 2.25-fold dilutions of aStAx-35 or aStAx-41R with a preequilibrated (30 min) mixture of 90 nM GST–β-catenin with fStAx-35 or fStAx-35R (10 nM). Polarization measurements and calculations were performed as above for fluorescence polarization assays.

Surface Plasmon Resonance. A Biacore T100 instrument (Biacore-GE) was used for surface plasmon resonance (SPR)-binding measurements to investigate binding between GST-tagged β-catenin and aStAx peptides. Initially, anti-GST IgG was immobilized on a CM5 Biacore chip by amine coupling with EDC [*N*-ethyl-*N*′-(3-dimethylamino propyl) carbodiimide] activation and NHS (*N*-hydroxysuccinimide) coupling. GST-tagged β-catenin was exchanged into binding buffer (Tris buffer + 0.01% P-20) before immobilization. Lyophilized peptides were dissolved in the same buffer and passed over the GST–β-catenin functionalized chip as well as a GST-only functionalized reference. Measurements were performed in triplicates with threefold dilutions (for aStAx-35: 1.37 nM to 1 μM; for aStAx-35R: 41.1 nM to 10 μM).

Pull-Down Assays and Western Blotting. For competition pull-down experiments with purified full-length β-catenin, 0.5 μM β-catenin and the required amount of acetylated stapled Axin (StAx) peptide (0.1, 0.5, 2.5 μM) in buffer [300 mM NaCl, 25 mM Tris (pH 8.8), 2 mM DTT, and 2% glycerol] were incubated for 30 min at 4 °C. Then, glutathione-labeled agarose beads with immobilized GST-tagged β-catenin binding domains (CBD) of T-cell factor (TCF) 4 (amino acids 1–52) were added and incubated for 2.5 h at 4 °C (final volume 200 μL). Beads were washed repeatedly with incubation buffer before elution of bound proteins by heating in SDS gel loading buffer at 95 °C for 10 min. Competition pull-down experiments with GST-tagged CBD of E-cadherin were performed analogously. For pull-down of β-catenin from cellular lysates, DLD1 cells were lysed in TBS [150 mM NaCl and 20 mM Tris (pH 7.4)] with 1 mM PMSF and 1% Triton X-100 at 4 °C for 30 min, followed by removal of insoluble debris by centrifugation at 13,000 × *g*. Lysate (400 μL) was incubated with 125 nM biotinylated StAx peptide at 4 °C for 4 h. Then, streptavidin-labeled

magnetic beads (M280; Invitrogen) were added at room temperature for 45 min. Beads were washed repeatedly with TBS + 0.01% Tween 20 before the elution of bound proteins by heating in SDS gel loading buffer at 95 °C for 10 min. Western blotting was performed following standard methods and using antibodies specific for β-catenin (129353; EMD), Axin1 (A0481; Sigma), and tubulin (Cell Signaling).

Confocal Fluorescence Microscopy. To investigate intracellular penetration, DLD1 cells were seeded in chamber slides (LAB-TEK) and incubated in 500 μL media (RPMI 1640) supplemented with 10% FBS and 7.5 μM fStAx peptide for 24 h at 37 °C. Then cells were washed twice with PBS and fixed with 4% paraformaldehyde in PBS. Finally, mounting media with DAPI was used to attach No 1.5 cover slides. Confocal fluorescence microscopy was performed with an LSM 700 inverted confocal microscope [DAPI: λ(ex) = 420 nm, fluorescein: λ(ex) = 492 nm]. All images (Fig. S6) were recorded with the same instrument settings.

X-Ray Crystallography and Structure Determination. β-Catenin (residues 134–665, 0.4 mg mL^{-1}) in 1 mL 300 mM NaCl, 25 mM Tris (pH 8.8), 2 mM DTT, and 2% glycerol was incubated with a twofold excess of aStAx-35 in 70 μL H_2O. After buffer exchange with 150 mM NaCl, 20 mM Tris (pH 8.5), 0.5 mM DTT, and 2% glycerol, the protein/peptide complex solution was concentrated to 1.2 mg ml^{-1}. Crystals of the β-catenin/aStAx-35 complex were grown at 25 °C using the hanging drop vapor diffusion method, with the drop consisting of a 1.67:1 ratio of the protein/peptide complex solution and a reservoir solution of 1.4–2.0 M NaCl, 100 mM Bis·Tris (pH 6.2), 100 mM KH_2PO_4, and 100 mM NaH_2PO_4. Before data collection, the crystals were briefly soaked in mother liquor containing increasing percentages of glycerol from 5% to 30% and then flash-frozen in liquid nitrogen. Data for the β-catenin/aStAx-35 structure were collected on beamline 24-IDC at the NE-CAT at the Advanced Photon Source, Argonne National Laboratory. Data were processed with HKL2000 and merged with SCALEPACK (5). The crystals have one protein:peptide complex in the asymmetric unit and belong to the orthorhombic space group $P2_12_12_1$. Molecular replacement, using the unliganded β-catenin monomer (PDB ID 1QZ7) (6) as a search model, was performed using PHASER (7) as part of the CCP4 program suite (8). $2F_o$-F_c and F_o-F_c maps showed clear density for the aStAx-35 peptide. The peptide sequence was fitted to the electron density using the program COOT (9) and the model refined in CNS (10) and PHENIX (11). Crystallographic statistics for the β-catenin/StAx35 structure are presented in Table S2. The coordinates of the structure have been deposited in the Protein Data Bank (ID code 4DJS).

Luciferase Reporter Gene Assays. For TOPFLASH reporter assay, cells cultured in 96-well plates were transfected with 100 ng TOPFLASH plasmid and 2.5 ng CMV-RL plasmid. Twelve hours after transfection, cells were treated with various concentrations of fStAx peptides in the presence of 200 ng mL^{-1} Wnt3a for another 24 h. For alternative pathways, reporter constructs were purchased from SABiosciences. Cells were stimulated by overexpression of ΔEGFΔLNR-NOTCH1(27,42) or with 200 ng mL^{-1} Shh, TGF-β, and BMP4, respectively. Luciferase activity was measured using Dual-Glo luciferase assay kit.

Quantitative RT-PCR. Total RNA from cells was extracted using TriZol reagent (Invitrogen) following the manufacturer's instruction. The purified RNA was treated with DNaseI (Invitrogen) to remove contaminated genomic DNA and reverse transcribed into cDNA using MMLV-RT (Invitrogen). Quantitative PCR reactions from cDNA were performed using the QuantiTect SYBR PCR kit (Qiagen) following the manufacturer's instruction.

Cell Viability Assays. Cells were plated in 48-well cell culture plates with seeding density 2,500 cells per well. Twenty-four hours after settling, aStAx peptides or DMSO vehicle alone in RPMI 1640 culture medium with 10% FBS were added to the cells and incubated for the specified period. After treatment courses, viable cell titers were measured using Cell-titer Glo assay (Promega).

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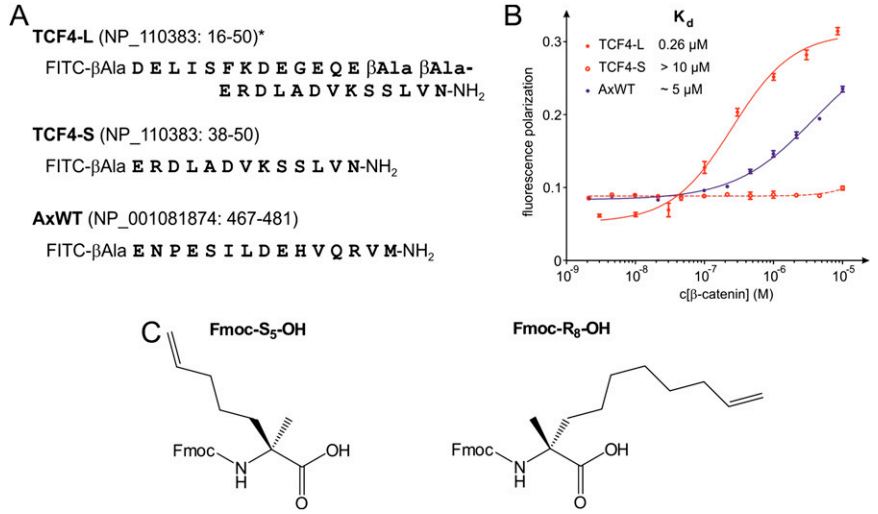

Fig. S1. (*A*) Peptide sequences with National Center for Biotechnology Information accession and covered amino acids (*amino acids 29–37 were replace by two β-alanines bridging the gap between α-helix and extended region). (*B*) Fluorescence polarization assays using fluorescein-labeled peptides and N-terminally GST-tagged human β-catenin with corresponding dissociation constants (K_d). (*C*) Structures of Fmoc-protected nonnatural amino acids: (*S*)-*N*-Fmoc-2-(4′-pentenyl)alanine (Fmoc-S_5-OH) and (*R*)-*N*-Fmoc-2-(7′-octenyl)alanine (Fmoc-R_8-OH).

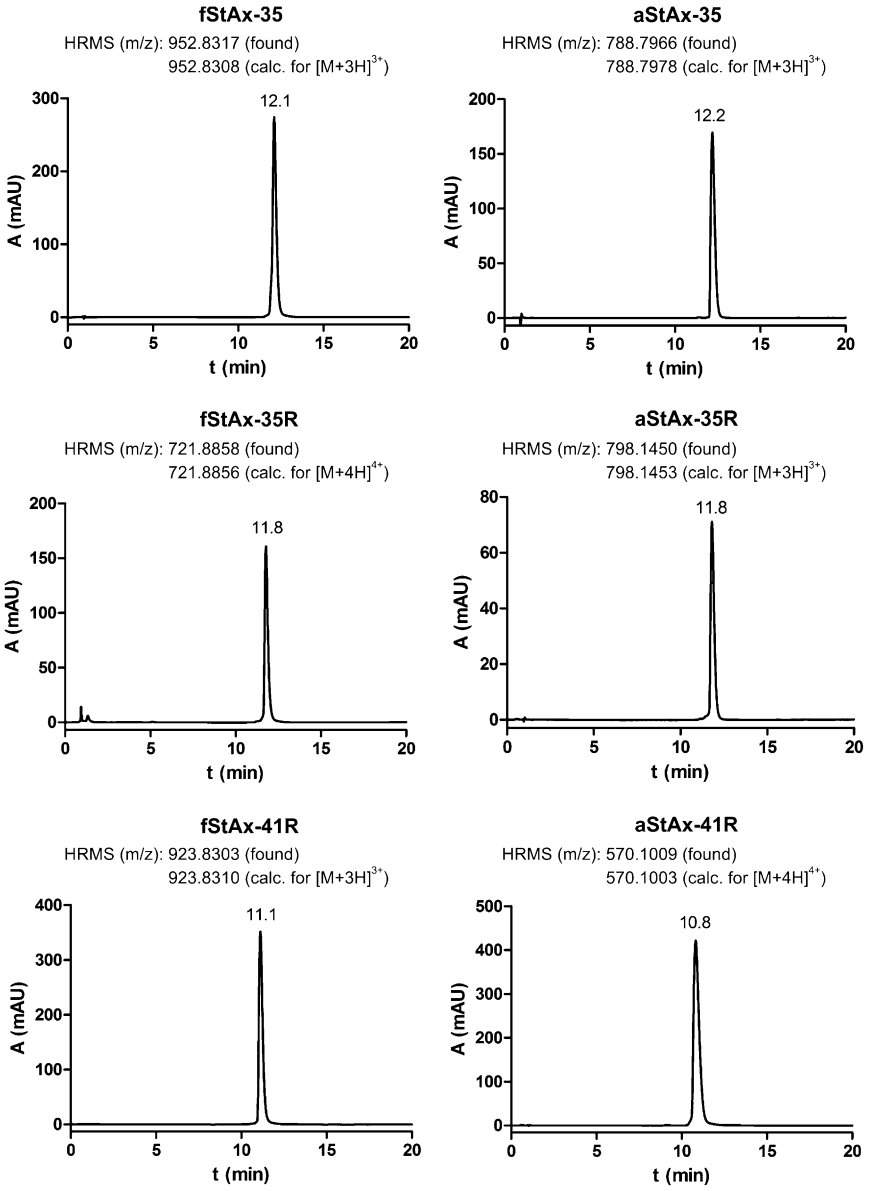

Fig. S2. Found masses generated by HRMS with calculated exact masses for protonated structures (+3H or +4H). HPLC traces of purified stapled peptides (gradient for fStAx-peptides: 10–70% B in 20 min; gradient for aStAx-peptides: 10–60% B in 20 min) measured at 280 nm.

A **phage library**

XXXX SILD EHVQ RVMR
XXPE XXLD EHVQ RVMR
XXPE SIXX EHVQ RVMR
XXPE SILD XXVQ RVMR
XXPE SILD EHXX RVMR
XXPE SILD EHVQ XXMR
XXPE SILD EHVQ RVXX

ENXX XXLD EHVQ RVMR
ENXX SIXX EHVQ RVMR
ENXX SILD XXVQ RVMR
ENXX SILD EHXX RVMR
ENXX SILD EHVQ XXMR
ENXX SILD EHVQ RVXX

ENPE XXXX EHVQ RVMR
ENPE XXLD XXVQ RVMR
ENPE XXLD EHXX RVMR
ENPE XXLD EHVQ XXMR
ENPE XXLD EHVQ RVXX

ENPE SIXX XXVQ RVMR
ENPE SIXX EHXX RVMR
ENPE SIXX EHVQ XXMR
ENPE SIXX EHVQ RVXX

ENPE SILD XXXX RVMR
ENPE SILD XXVQ XXMR
ENPE SILD XXVQ RVXX

ENPE SILD EHXX XXMR
ENPE SILD EHXX RVXX

ENPE SILD EHVQ XXXX

B **results**

1	RWPE	SILD	EHWE	RVMR
2	VSPE	SILD	EHVQ	RVWG
3	EWPE	SILD	EHWH	RVMR
4	RLPE	SILD	EHVQ	RVWP
5	ENLQ	SILD	EHVQ	RWMR
6	ENPE	SILD	EHFD	KWMR
9	ENPE	SILD	QHWV	RWMR
10	ENSW	SILD	EHVQ	RVWG
11	RYPE	SILD	EHVQ	RWMR
12	QKPE	SILD	EHVQ	RVWP
13	TWPE	SILD	EHWE	RVMR
14	ENWQ	SILD	EHMQ	RVFR
15	RLPE	SILD	EHVQ	RWMR
16	QVPE	SILD	EHVQ	RVWG
17	ENPE	SILD	EHVQ	RWMR
18	ENPE	SILD	EHVQ	RWMR
19	ENSQ	SILD	EHVQ	RVWG
20	ENWQ	SILD	EHVQ	RVWR
21	RYPE	SILD	EHVQ	RWMR
22	KNPE	SILD	EHVQ	RWMR
23	ENPE	SILD	EHWE	RVMR
24	ENAQ	SILD	EHVQ	RWMR
25	ENPE	SILD	EHMQ	RVFE
26	ENSE	SILD	EHVQ	RVWG
27	ENPE	SILD	EHLQ	RVWQ
28	ENFQ	SILD	EHVQ	RWMR
29	RLPE	SILD	EHVQ	RVWR
30	WYPE	SILD	EHLL	RVMR
31	ENPE	SILD	EHIL	RVEK
32	WVPE	SILD	EHVQ	RVWG
33	LQPE	SILD	EHVQ	RVWH

C

Phage-start
FITC-βAla **E N P E S I L D E H V Q R V M R**-NH_2

Phage1
FITC-βAla **R W** P E S I L D E H **W E** R V M R-NH_2

Phage4
FITC-βAla **R L** P E S I L D E H V Q R V **W P**-NH_2

Phage17/18
FITC-βAla **E N P E S I L D E H V Q R V W** R-NH_2

D

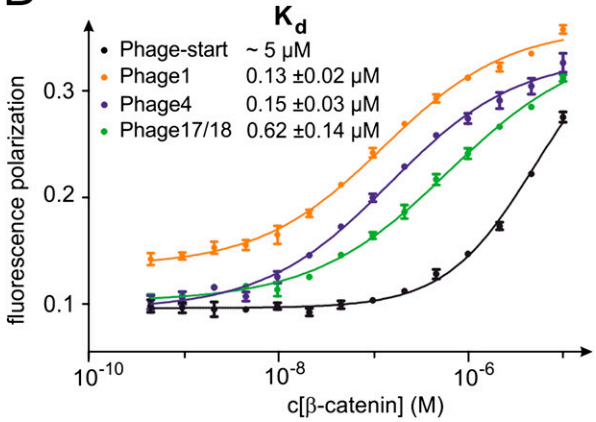

Fig. S3. (*A*) Phage display library, with X as randomized position. (*B*) Sequences found in picked colonies after two (entries 1–16) and three (entries 17–33) selection cycles. Sequences of entries 17 and 18 are identical. (*C*) Fluorescein-labeled peptides including the starting sequence and three sequences resulting from phage display (entries 1, 4, and 17/18 in *B*). (*D*) Fluorescence polarization assay with fluorescein-labeled peptides and N-terminally GST-tagged human β-catenin (with corresponding K_ds). Peptides derived from phage display selection (Phage1, -4, and -17/18) provide increased binding affinity to β-catenin.

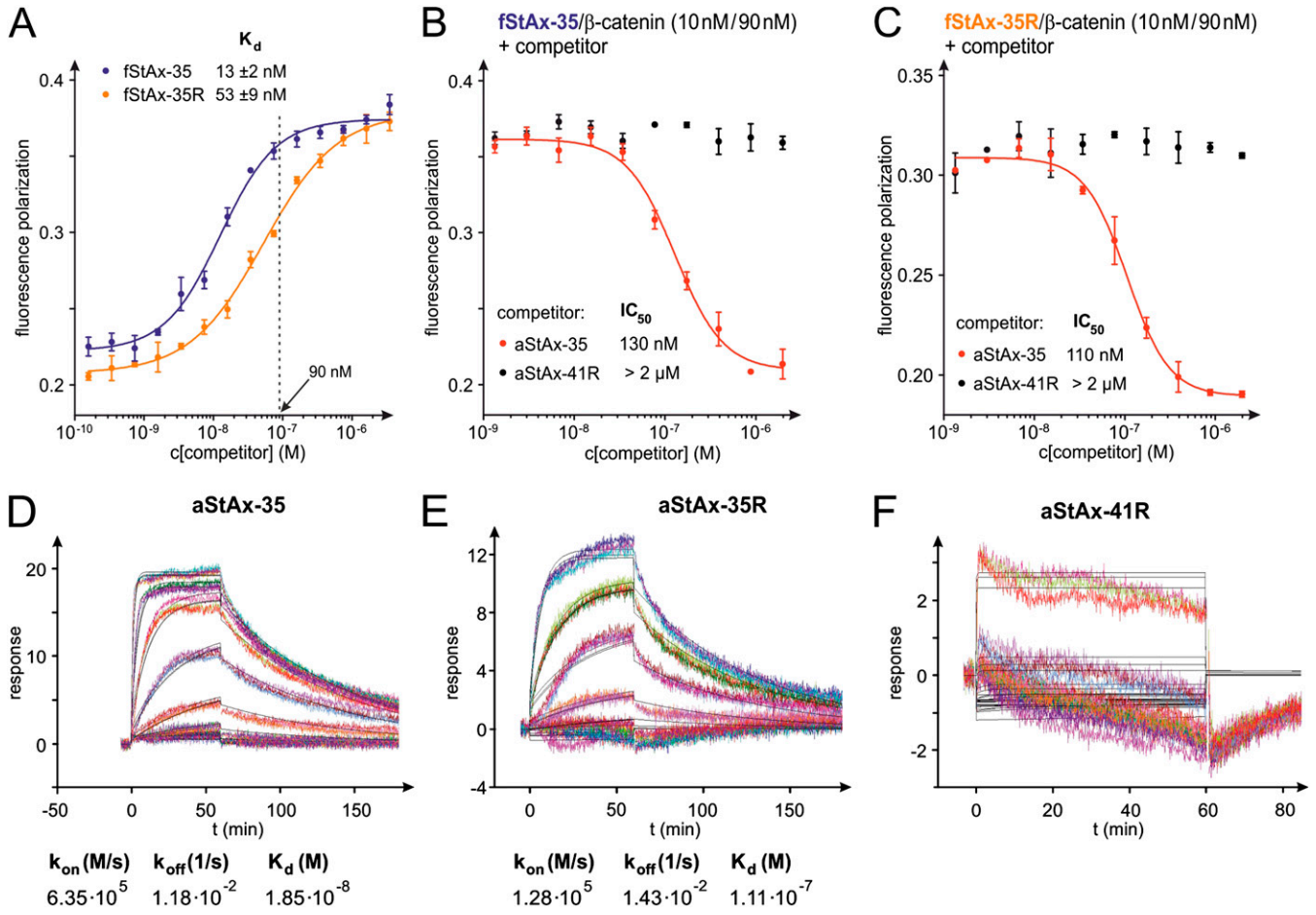

Fig. S4. (*A*) Fluorescence polarization (FP) assay with fluorescein-labeled peptides and N-terminally GST-tagged human β-catenin. (*B* and *C*) competition FP assay with increasing amounts of competing peptide (aStAx-35: red; aStAx-41R: black). The decrease of polarization with increasing amounts of aStAx-35 (red) verifies competition for β-catenin binding, therefore indicating the reversibility of fStAx-35 and -35R binding. Negative control aStAx-41R did not compete for β-catenin binding (black). Surface plasma resonance: aStAx-35 (*D*), aStAx-35R (*E*), and aStAx-41R (*F*) binding to immobilized β-catenin was measured. Colored curves represent sensogram data, and the black curve denotes fit to kinetic model. Binding constants are shown (k_{on} = association rate; k_{off} = dissociation rate). For aStAx-35 and -35R reversible binding to β-catenin with K_ds in the range of those obtained from fluorescence polarization experiments were observed. Negative control aStAx-41R did not show affinity for β-catenin.

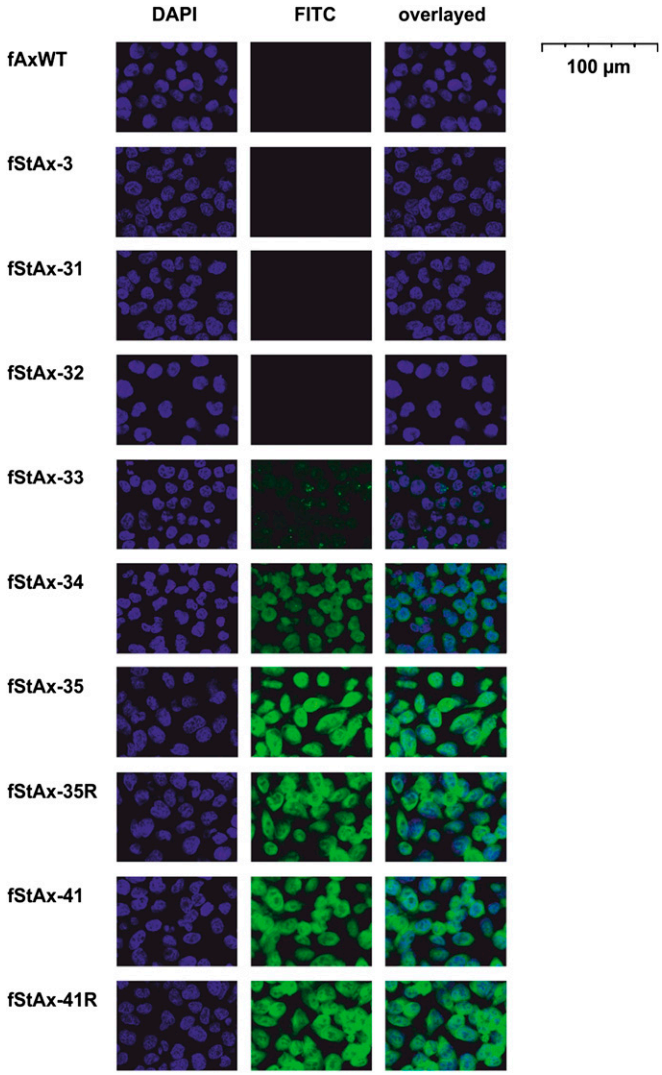

Fig. S5. Analysis of DLD1 cellular uptake of fStAx peptides (7.5 µM, 24 h) by confocal fluorescence microscopy. Images of nuclear DAPI fluorescence (blue), fluorescein fluorescence (FITC), and overlaid DAPI and FITC fluorescence.

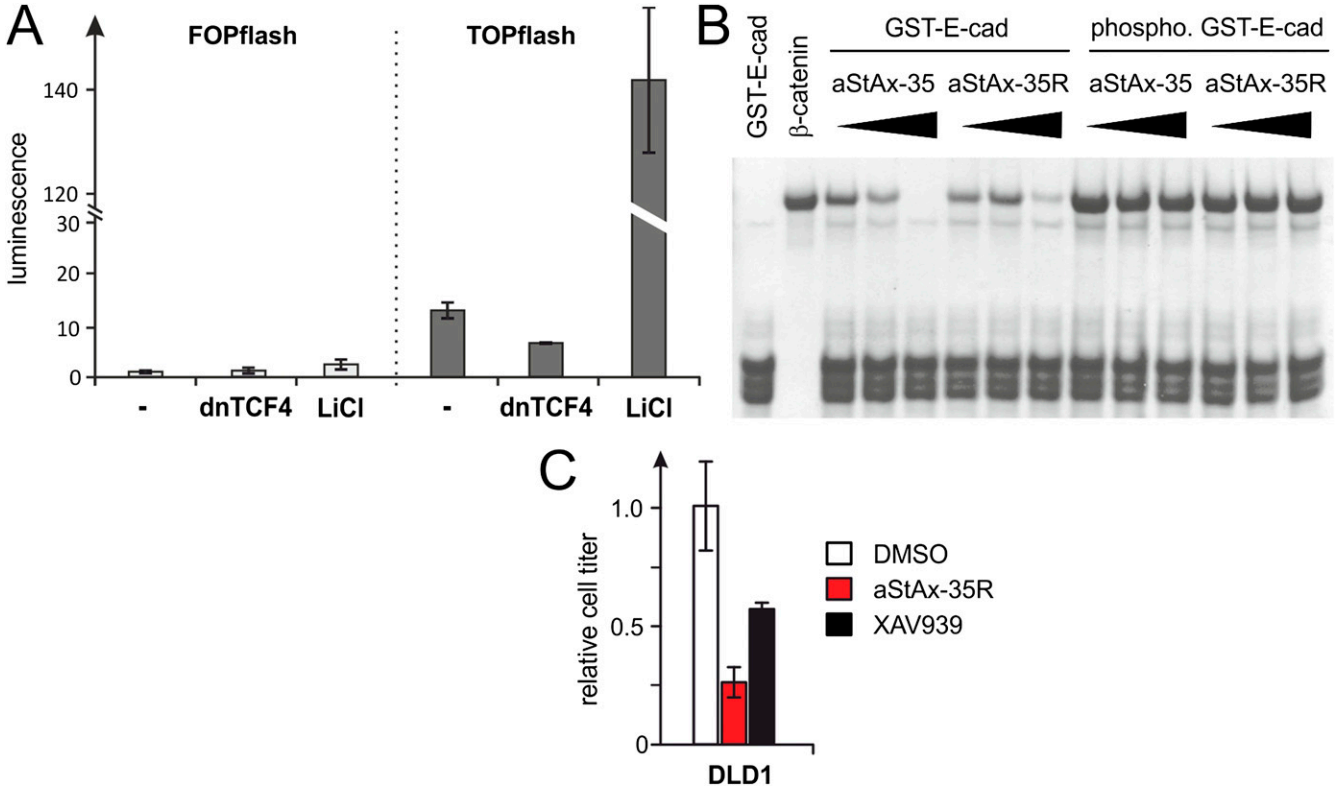

Fig. S6. (*A*) Only TOPflash but not negative control FOPflash showed significant signal change upon pathway inhibition, indicating that TOPflash luciferase activity is a TCF-mediated event (dnTCF4: dominant negative TCF4 fragment). (*B*) In vitro competition of acetylated StAx (aStAx) peptides (0.1, 0.5, 2.5 μM) with bead-immobilized GST–E-cadherin(731-882) (phosphorylated and nonphosphorylated) for β-catenin (0.5 μM). After washing, bound proteins were resolved by gel electrophoresis (Coomassie). Acitve peptides aStAx-35 and -35R do not inhibit the binding of β-catenin to phosphorylated E-cadherin (*Right*). In a control pull-down with unphosphorylated E-cadherin (known to have lower affinity for β-catenin) competition for β-catenin binding was observed (*Left*). (*C*) Proliferation of DLD1 cells was blocked by aStAx-35R (10 μM) and XAV939 (10 μM). Cells were treated for 5 d, and viability was analyzed by measuring the cellular ATP level.

Table S1. List of peptides (with C-terminal amide) used in this study with their molecular formula (MF), calculated molecular masses (*m/z*) for charged ions ($[M+2H]^{2+}$/$[M+3H]^{3+}$/$[M+4H]^{4+}$), and found masses

Peptide	Sequence	MF	Calculated *m/z*	Found *m/z*
TCF4-L	FITC-βAla-DELISFKDEGEQE-βAla-βAla-ERDLADVKSSLVN	$C_{155}H_{228}N_{38}O_{57}S$	1784.9/1190.3/893.0	1190.0/892.7
TCF4-S	FITC-βAla-ERDLADVKSSLVN	$C_{84}H_{121}N_{21}O_{28}S$	953.5/636.0/477.2	953.3/635.9
AxWT	FITC-βAla-ENPESILDEHVQRVM	$C_{99}H_{139}N_{25}O_{32}S_2$	1128.7/752.8/564.9	1128.2/752.6
Phage-start	FITC-βAla-ENPESILDEHVQRVMR	$C_{105}H_{151}N_{29}O_{33}S_2$	1206.8/804.9/603.9	1206.5/804.5
Phage1	FITC-βAla-RWPESILDEHWERVMR	$C_{119}H_{160}N_{32}O_{31}S_2$	1300.4/867.3/650.7	1299.8/866.9
Phage4	FITC-βAla-RLPESILDEHVQRVWP	$C_{113}H_{157}N_{29}O_{30}S$	1217.9/812.2/609.4	1217.3/812.0
Phage17/18	FITC-βAla-ENPESILDEHVQRWMR	$C_{111}H_{152}N_{30}O_{33}S_2$	1250.4/833.9/625.7	1250.3/833.6
fStAx-1	FITC-βAla-ENPE-R8-ILDEHV-S5-RVM	$C_{108}H_{154}N_{24}O_{30}S_2$	1167.3/778.6/584.2	1166.9/778.1
fStAx-2	FITC-βAla-ENPESILD-S5-HVQ-S5-VM	$C_{102}H_{142}N_{22}O_{30}S_2$	1111.3/741.2/556.1	1110.8/740.9
fStAx-3	FITC-βAla-ENPE-S5-ILD-S5-HVQRVM	$C_{105}H_{149}N_{25}O_{29}S_2$	1145.8/764.2/573.4	1145.3/763.7
fStAx-31	FITC-βAla-PE-S5-ILD-S5-HVQRVM	$C_{96}H_{136}N_{22}O_{24}S_2$	1024.2/683.1/512.6	1023.8/682.7
fStAx-32	FITC-PEG_1-PQ-S5-ILD-S5-HVRRVMR	$C_{106}H_{159}N_{29}O_{25}S_2$	1152.9/768.9/576.9	1152.5/768.5/576.8
fStAx-33	FITC-PEG_1-PQ-S5-ILD-S5-HVRRVWR	$C_{112}H_{160}N_{30}O_{25}S$	1180.4/787.3/590.7	1179.8/786.8/590.6
fStAx-34	FITC-PEG_1-RWPQ-S5-ILD-S5-HVRRVWR	$C_{129}H_{182}N_{36}O_{27}S$	1351.6/901.4/676.3	1351.1/901.1/676.1
fStAx-35	FITC-PEG_1-RRWPQ-S5-ILD-S5-HVRRVWR	$C_{135}H_{194}N_{40}O_{28}S$	1429.7/953.4/715.3	1429.1/953.0/715.1
fStAx-35R	FITC-PEG_1-RRWPR-S5-ILD-S5-HVRRVWR	$C_{136}H_{198}N_{42}O_{27}S$	1443.7/962.8/722.4	1443.2/962.3/722.0
fStAx-40	FITC-PEG_1-RRWPQ-S5-ILH-S5-DVRRVWR	$C_{135}H_{194}N_{40}O_{28}S$	1429.7/953.4/715.3	1429.1/953.0/715.1
fStAx-41	FITC-PEG_1-RRWPQ-S5-ILH-S5-DVRRVAR	$C_{127}H_{189}N_{39}O_{28}S$	1372.1/915.1/686.6	1371.5/914.9/686.3
fStAx-41R	FITC-PEG_1-RRWPR-S5-ILH-S5-DVRRVAR	$C_{128}H_{193}N_{41}O_{27}S$	1386.1/924.4/693.6	1385.6/924.2/693.5
aStAx-35	Ac-RRWPQ-S5-ILD-S5-HVRRVWR	$C_{110}H_{174}N_{38}O_{21}$	1183.4/789.3/592.2	1183.1/788.9/592.1
aStAx-35R	Ac-RRWPR-S5-ILD-S5-HVRRVWR	$C_{111}H_{178}N_{40}O_{20}$	1197.4/798.6/599.2	1196.9/798.2/599.0
aStAx-41R	Ac-RRWPR-S5-ILH-S5-DVRRVAR	$C_{103}H_{173}N_{39}O_{20}$	1139.9/760.3/570.4	1139.6/760.1/570.2
bStAx-35	Biotin-PEG_1-RRWPQ-S5-ILD-S5-HVRRVWR	$C_{124}H_{197}N_{41}O_{25}S$	1348.1/899.1/674.6	1347.5/898.7674.3
bStAx-40	Biotin-PEG_1-RRWPQ-S5-ILH-S5-DVRRVWR	$C_{124}H_{197}N_{41}O_{25}S$	1348.1/899.1/674.6	1347.5/898.7/674.3
bStAx-41	Biotin-PEG_1-RRWPQ-S5-ILH-S5-DVRRVAR	$C_{118}H_{185}N_{37}O_{24}S$	1233.0/822.3/617.0	1290.2/860.3/645.5

FITC, fluorescein-label generated by reaction of FITC with the primary amine; PEG_1, [2-(2-aminoethoxy)ethoxy]acetyl.

Table S2. Data collection and refinement statistics

Parameter	β-catenin/aStAx-35
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	63.1, 74.8, 135.7
α, β, γ (°)	90.0, 90.0, 90.0
Resolution (Å)	50.0–3.0 (3.1–3.0)
R_{sym} (%)	7.0 (51.2)
$I/\sigma I$	23.8 (2.4)
Completeness (%)	99.9 (99.8)
Refinement	
Resolution (Å)	50.0–3.0
No. reflections	12,929
R_{work}/R_{free} (%)	26.3/29.1
No. atoms	4,008
rmsd	
Bond lengths (Å)	0.002
Bond angles (°)	0.487
Ramachandran statistics	
Favored (%)	95.7
Allowed (%)	4.3
Outlier (%)	0.0

Data were collected from a single crystal. Values in parentheses are for the highest-resolution shell ($R_{sym} = \Sigma|I-<I>|/\Sigma I$, where I is the integrated intensity of a given reflection; $R_{work} = \Sigma|F(obs)-F(calc)|/\Sigma F(obs)$, where F(obs) and F(calc) are observed and calculated structure factor amplitudes, respectively; $R_{free} = \Sigma|F(obs)-F(calc)|/\Sigma F(obs)$, calculated using 10% of the data omitted from the refinement).