Supplementary Methods

Cell culture, transfections and chemical treatment. The 293, 293T, 786-O, 769-P, RCC4, A498, UMRC6, Caki1, TK-10 and ACHN cell lines were grown as described previously. Cultured cells plated overnight were transiently transfected using Lipofectamine 2000 (Invitrogen) per manufacturer instructions. Lithium chloride (Sigma) and 6-bromoindirubin-3'-oxime (BIO) (Calbiochem) were used to inhibit GSK-3β activity. Human recombinant Wnt-3a and DKK-1 dissolved in PBS + 0.1% bovine serum albumin were obtained from R & D Systems.

Constructs. Flag-tagged Jade-1 wild-type and Jade-1 lacking both PHDs (Jade-1 dd) in pFlag-CMV2 vector have been described previously. Wild-type human Myc-tagged β-catenin was cloned into Xenopus expression vector pCS2+. Myc-tagged ubiquitin in pCMV2 vector was a gift from Z.-X. Xiao (Boston University). Constitutively active (CA) β-catenin S33A (amino acid substitution at GSK-3β phosphorylation site) in pCS2+MMBcmyc-tag vector and β-catenin delN (aa 131-781) in pGEX2T were kindly provided by R. Kemler (Max-Planck Institute for Immunobiology, Germany). Human β-catenin C and N terminus deletion constructs (delC and delN) in pBAT expression vector were generous gifts from W. Birchmeier (Max-Delbruck-Center for Molecular Medicine, Germany). TOP-Flash and FOP-Flash constructs were gifts from B. Vogelstein (Johns Hopkins University). β-TrCP wild type and dominant negative (DN) in pcDNA3.1 myc/His vector were gifts from R. Benarous (Institute Pasteur, France). HA-tagged β-TrCP wild-type was a gift from S. C. Sun (Penn State University). GSK-3β shRNA plasmid (pKD-GSK3β-v1) was purchased from Millipore-Upstate. Additional information about the above constructs can be obtained from the authors.

Human wild-type Jade-1 and Jade-1 dd were cloned into the Xho I site of the pCS2+ vector for Xenopus experiments. Human VHL wild-type and VHL del96-122 were cloned into pCS2MT+ at the Eco RI and Xba I sites. Jade-1 shRNA plasmid was
generated by targeting *Jade-1* coding sequence from nucleotide 1363 to 1385 (5’-AAGTTGAAGGAAGGTCAACTT -3’) using a duplex oligonucleotide synthesized by OligoEngine

5’-GATCCCCGTTGAAGGAAGGTCAACTTCAAGAGAGTTGACCTTCCCTCTTCAACTTT
TTA -3’ and 5’-

AGCTTAAAAAGTTGAAGGAAGGTCAACTCTCTTGAAGTTGACCTTCTCTTCAACGGG-3’. The duplex oligonucleotide was cloned into the pSuper vector (OligoEngine) using Bgl II and Hind III sites. The *Jade-1* silencing system in GIPZ shRNAmir lentiviral vector was purchased from Open Biosystems. The two silencing constructs target different parts of the *Jade-1* 3’ untranslated region.

**J1Sh1**: Targeting exclusively the *Jade-1* short version, nucleotide 3043-3063

TGCTGTGGAGCTGAGCGCGGCCTGAGAGTGAAAGTTCTTATAGTGAAGCCACAGAT
GTATAAGACTTTCACTTCTACGGCATGCCTACTGCCTCGGA

**J1sh2**: Targeting exclusively the *Jade-1* short version, nucleotide 1937-1957

TGCTGTGGAGCTGAGCGAGCTGGTAAACTCATTGTACATTAGTGAAGCCACAGAT
GTAATGTCAAATGAGTTTCACCAGCCTGCCTACTGCCTCGGA

**Antibodies**. Mouse monoclonal β-catenin, E-cadherin and pVHL antibodies were from BD Transduction Laboratories. Mouse monoclonal Myc-tag, N-cadherin and polyclonal β-catenin against a C-terminal antigen, polyclonal GSK-3β and phospho-β-catenin (detecting phosphorylated serine 33, 37 and threonine 41) antibodies were from Cell Signaling Technologies. Polyclonal Axin2 and LEF1 antibodies were from Cell Signaling Technologies. Monoclonal ubiquitin, β-actin, α-tubulin and Flag-tag antibodies were from Sigma. Rabbit polyclonal fibrillarin antibody was from Abcam. Polyclonal Jade-1 antibody has been described. Polyclonal pVHL (FL181) antibody from Santa Cruz Biotechnology was used for pVHL immunoprecipitation and immunoblotting17.
Immunoprecipitated pVHL was detected using a secondary antibody that detects only intact immunoglobulin (Sigma, Clone RG-16). Mouse monoclonal antibody for endogenous c-Myc (sc-40) was from Santa Cruz Biotechnology. Mouse monoclonal cyclin D1 antibody was from Neomarker. Goat anti-mouse and anti-rabbit HRP conjugated secondary antibodies were from Bio-Rad for immunofluorescence. Alexa Fluor 594 donkey anti-mouse and Oregon green 488 goat anti-rabbit as secondary antibodies were obtained from Invitrogen-Molecular Probes.

**Yeast two-hybrid screen.** An adult human kidney cDNA library in pB42AD (Clontech) was screened against human Jade-1 in the LexA-expressing, inducible yeast expression vector pGilda (Clontech) per manufacturer instructions. Full-length Jade-1 and other truncations of Jade-1, such as Jade-1 del1, del2, dd and dC were cloned into pGilda vector using Eco RI and Sal I sites. Only Jade-1 dd did not autoactivate transcription. Therefore, Jade-1 dd was used as bait. Two x 10^7 pB42AD library clones were initially screened by growth in deficient medium and X-gal staining. Positive clones were rescued using the Zymoprep kit (Zymo Research) per manufacturer instructions. Nine strong candidate interactors were isolated, including β-catenin.

**Immunoblotting and immunoprecipitation.** Cells were lysed in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 30 mM EDTA, 0.5% Triton X-100 with complete protease inhibitor (Roche). Immunoblotting and immunoprecipitation were performed as described previously.

**Immunofluorescence.** Cells were grown in Chamber slides (Lab-Tek) and fixed with HEPES 20 mM, pH 7.4, NaCl 140 mM, CaCl_2 2 mM, MgCl_2 2 mM with 2% formaldehyde and 0.2% glutaraldehyde for 15 min. Slides were blocked with 1% BSA in PBS and 0.2%
Triton X-100. Cells were incubated with primary antibody and washed with PBS. Slides were blocked in 1% BSA, 0.2% Triton X–100 in PBS, then incubated with secondary antibody. Slides were washed with PBS, mounted with Gelvatol and evaluated by fluorescence microscopy followed by confocal laser scanning microscopy (Perkin-Elmer Ultraview). As controls, one set of slides was incubated with primary antibody alone and another set with secondary antibody alone.

Co-localization was performed in cells having comparable expression of the endogenous proteins. All cells were screened. Randomly selected representative cells were analyzed. At least 15 confocal images comprising one Z-stack were generated for each cell, and co-localization was performed using the entire Z-stack. Images were background subtracted from a randomly chosen region of interest. For the endogenous proteins, the profile plots were generated using NIH ImageJ 1.37a WCIF. For over-expressed Jade-1 and β-catenin, randomly selected cells having comparable expression of both the constructs were screened. Randomly selected cells were used for confocal analysis. Images were background subtracted from a randomly chosen region of interest. The scatter frequency plots representing the grade of co-localization were generated with NIH ImageJ 1.37a WCIF.

**Cellular fractionation.** Cells were Dounce (Kontes) homogenized in 250 mM sucrose, 10 mM HEPES, pH 7.4, 2.5 mM MgCl₂, 0.5 mM EDTA, 100 μM Na₃VO₄, 100 μM PMSF and complete protease inhibitor (Roche). After two centrifugations at 1,000 x g for 10 min at 4 °C, the anucleated supernatant was centrifuged at 100,000 x g at 4 °C for 30 min. The supernatant (S100 soluble fraction) was removed. The nuclear pellet from the first spin was resuspended in 50 mM HEPES, pH 7.4, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 100 μM Na₃VO₄, 100 μM PMSF and complete protease inhibitor (Roche) and lysed by three cycles of freeze and thaw. The nuclear fraction was cleared by centrifugation.
**Digitonin extraction.** Cells washed with ice-cold PBS were covered with buffer containing 120 mM KCl, 5 mM KH$_2$PO$_4$, 10 mM HEPES, pH 7.4, 2 mM EGTA, 0.15 mg/ml digitonin (Sigma) and gently rocked on ice for 15 min. The gently aspirated supernatant was labeled as digitonin extract. The remaining cells were scraped from the dish and lysed for 30 min on ice in RIPA buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 5 mM EDTA. The supernatant was obtained after centrifugation for 30 min at 4 °C.

**Generation of Jade-1 silenced cell lines.** Lentiviral constructs were co-transfected in 293T packaging cells along with packaging, envelope and Rev vectors using Lipofectamine 2000 per manufacturer instructions. Medium containing active viral particle collected after 48 h was centrifuged and stored at -80 °C. For lentiviral infection, the cells were seeded at 50-60% confluence. The cells were treated overnight with the medium containing active viral particles along with hexadimethrine bromide (Sigma), a cationic polymer, to increase the efficiency of infection. Puromycin (Sigma) selection was initiated after 24 h. The cells were harvested after 4 days to examine the effect on Jade-1 protein.

**GST purification.** BL21 chemically competent *E. coli* (Invitrogen) were transformed with pGEX-2T β-catenin or pGEX-6P-1 Jade-1 constructs. Protein expression was induced in early log state (OD$_{600}$ of 0.6-0.8) by isopropyl-β-D-thiogalactopyranoside (IPTG) (Invitrogen). Bacteria were lysed with lysozyme (American Bioanalytical) and 1% Triton X-100, followed by brief sonication. GST-tagged proteins were purified by passing over a glutathione Sepharose™ 4B bead slurry (GE Healthcare).

**GST pull-down assay.** Jade-1 or β-catenin cleaved from GST-Jade-1 and GST-β-catenin glutathione Sepharose™ beads using Prescision protease or thrombin (GE Healthcare), respectively, were applied to GST-β-catenin or GST-Jade-1 containing glutathione Sepharose™ beads for 2 h at 4 °C in 50 mM Tris-HCl, pH 7.6, 150 mM
NaCl, 30 mM EDTA, 0.5% Triton X-100. Beads extensively washed with the same buffer containing 300 mM NaCl were boiled in Laemmli buffer (Boston Bioproducts).

**In vitro phosphorylation of β-catenin.** GST-β-catenin beads washed twice with kinase buffer containing 25 mM HEPES, pH 7.4, 10 mM MgCl₂, 1 mM EDTA and 0.1 M DTT. Then GST-β-catenin beads suspended in kinase buffer were incubated with 1 mM ATP (Sigma) and 1 unit of recombinant CK1 and GSK-3β (New England Biolabs) for 30 mins at 30 °C.

**Tcf/β-catenin-responsive luciferase reporter assay.** The 293T cells seeded in 6-well plates were cotransfected with the TOP-Flash or FOP-Flash constructs (0.6 μg), Flag-tagged Jade-1 (full-length or dd) (1.5 μg), β-catenin (wild-type or S33A) (1.5 μg) and pRL-SV40 Renilla luciferase vector (5 ng) (to normalize for transfection efficiency) using Lipofectamine 2000 per manufacturer instructions (Invitrogen). After 48 h of transfection, luciferase assays were performed using the Dual Luciferase kit® (Promega).

**In vivo ubiquitination assay.** The 293T cells were transiently transfected and treated with 10 μM proteasome inhibitor MG132 (Boston Biochem) for 10-12 h before harvesting in lysis buffer containing 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 30 mM EDTA, 0.5% Triton X-100 with complete protease inhibitor (Roche). For immunoprecipitation, cell lysates were mixed with 1 μg β-catenin antibody overnight at 4 °C. Protein complexes were pelleted with protein A-agarose beads (Santa Cruz), washed in lysis buffer, eluted with Laemmli buffer (Boston Bioproducts), separated by SDS-PAGE and immunoblotted. Thirty μg cell extracts (5%) were probed to determine inputs.

**Ex vivo ubiquitination of β-catenin using HeLa cell S100 fraction.** A HeLa S100 Conjugation kit (Boston Biochem) was used per manufacturer instructions. Briefly, 2.8 μM GST-β-catenin on glutathione-Sepharose™ beads was incubated with 200 μg HeLa S100 fraction (pretreated with 200 μM MG132 and 100 μM ubiquitin aldehyde for 15 min
at RT), 750 nM Jade-1, 600 μM Myc-tagged ubiquitin and 5 μl of energy regenerating solution (ERS) in 50 mM HEPES, pH 7.6, for 90 min at 37 °C. β-catenin ubiquitination was detected by immunoblot after cleaving β-catenin from GST beads using 1 unit thrombin for 2 h at RT. Reactions without ubiquitin and ERS served as negative controls.

**VHL silencing with siRNA oligonucleotide.** For VHL silencing, SMARTpool® VHL siRNA oligonucleotides (Dharmacon RNAi technologies) were transfected into cultured cells per manufacturer protocol using DHARMAFECT, and cells were harvested after 72 h.

**Synthesis of capped mRNA.** The vectors were linearized with Not I restriction enzyme, treated with Proteinase K (Sigma) and extracted with phenol. Linearized plasmid DNA (1 μg/μl) in RNAse-free water was used for *in vitro* capped mRNA synthesis using the mMMessage mMachine® SP6 kit (Ambion) according to manufacturer instructions.