SUPPLEMENTARY MATERIAL

Oligonucleotide Array-QC-Labeling and sample processing. High density oligonucleotide Affymetrix U133A chips, representing approximately 22,000 genes and 1,000 ESTs, were used in this study according to manufacturer's protocols. U133A microarray data from human OEAs will be published elsewhere (Cho laboratory, manuscript in preparation). RNA quality control was performed prior to labeling: 25 to 50 nanograms of total RNA was run on a RNA 6000 Nano Assay (Agilent, Palo Alto, CA) using a Bioanlyzer 2100. 2 μg of total RNA was then labeled according to protocols recommended by manufacturers. Briefly, after reverse transcription with an oligo-(dT)24-T7 (Genset), double stranded cDNA was generated with the Superscript double stranded cDNA synthesis custom kit (Invitrogen Life Technologies, Carlsbad, CA). In an in vitro transcription step (IVT) with T7 RNA polymerase (MessageAmp aRNA kit from Ambion, Austin, TX), the cDNA was linearly amplified and labeled with biotinylated nucleotides (Enzo Diagnostics, Farmingdale, NY). 10 ug of labeled and fragmented cRNA was then hybridized onto a human genome U133A expression array (Affymetrix) for 16 hours at 45^0C. Post-hybridization fluorescent staining was processed according to the manufacturer's instruction (Affymetrix). Finally, chips were scanned with a Hewlett Packard argon-ion laser confocal scanner. The image was quantified using Microarray Suite 5.1 (MAS 5.1, Affymetrix) with the default parameters for the statistical algorithm.

RT-PCR. Total RNA from cells was isolated using Qiagen RNAeasy Kits according to manufacturer instructions. Total RNA from frozen tissues was isolated using Trizol (Invitrogen). RT-PCR was performed using Superscript One-Step RT-PCR (Invitrogen). Primers used for our studies were: human *FGF20*, forward 5'-

AGGTGTGGACAGTGGTCTC-3' and reverse 5'-TTTCCAAATCCAGTCTCT CAAG-3'; mouse *Fgf20*, forward 5'-GGCAGGATCACAGTCTCTTCG-3' and reverse 5'-AGTTCCATCCTTGTTAAGTGC-3'; rat *Fgf20*, forward 5'-ATCACAGCCTCTTCGGTATCC-3' and reverse 5'-TCCAGTGT ACACCAGTAGGTC-3'; human *DKK1*, forward 5'-CAGTAATTCTTCTAGGCTTCA C-3' and reverse 5'-CAAGAGATCCTT GCGTTCTAGGAC-3'; human *LMO2*, forward 5'-GAGGACTGCCTGAGCTGCGAC-3' and reverse 5'-TATCATCCCATTGATCTTAGTC-3'; human *ETV5*, forward 5'-TTA GAGACGCTGAAAGCACC-3' and reverse 5'-AAGCCTCTTGAAGTTGACTGAG-3'; human *RBP1*, forward 5'-CTCCAGTCACTCCCGAAATG-3' and reverse 5'-GGACAT TTTTGCCTCATGCT-3'; human *GAPDH*, forward 5'-CAACAGCCTCAAGATCATCAGC-3' and reverse 5'-CTCCCCAGCAGTGAGGGTCTCTC-3';mouse *Actin*, forward 5"-TCATGAAGTGTGACGTTGACATCCGT-3' and reverse 5"-CTTAGAAGCATTGGTGCACGATG-3'; rat *GAPDH*, forward 5'-CAATGGCACAGTCAAGGCTGAG-3' and reverse 5'-GGTCTGGGATGGAATTGTGAG-3'. All primer pairs amplified correct size product with an annealing temperature of 55^0C. Amplification of *FGF20* usually required between 36 and 40 cycles, *GAPDH* between 18 and 22 cycles. All other genes required between 30 and 34 cycles. All products were verified by DNA sequencing**.** 0.2 to 0.5ug of total RNA was routinely used in RT-PCR.

ChIP assays. Chromatin fixation and immunoprecipitation were performed as previously described (Orlando *et al,* 2000; Weinmann A.S. *et al,* 2001) with some modifications.

Briefly, formaldehyde was added directly to cell culture media of log phase adherent $3x10^7$ 293Top cells for 10 minutes at room temperature. Cross-linking was quenched by the addition of glycine to a final concentration of 0.125M. Cells were washed three times with PBS, scraped off the plates in small amount of PBS, centrifuged, and the pellet was washed once with PBS containing protease inhibitors (Complete, Roche). Cells were then resuspended in 1 ml lysis buffer (5mM PIPES pH 8, 85mMKCl, 0.5% NP40 and protease inhibitors). incubated on ice for 10 minutes, and dounced with twenty strokes to release nuclei. Cell lysates were centrifuged at 5,000 rpm for 5 minutes at 4ºC, and the pelleted nuclei were resuspended in 1 ml nuclei lysis buffer (50mM Tri-HCl pH8, 10mM EDTA, 1% SDS and protease inhibitors), centrifuged and resuspended again in 1 ml nuclei lysis buffer, and incubated on ice for 10 minutes. Prior to sonication, 0.2 grams of glass beads was added to the sample, and the sample was sonicated to obtain approximately 1 Kb chromatin fragments. After sonication, the sample was centrifuged at top speed for 10 minutes at 4ºC in an Eppendorf centrifuge. The supernatant was diluted 1:10 with IP dilution buffer (50mM Tris-HCL pH8, 2mMEDTA, 200mM NaCl, 1% Triton-X-100 and proteinase inhibitors). 400 ul of 50% slurry of salmon sperm-coated Protein A-agarose beads was added for 30 minutes on ice and then centrifuged to pre-clear chromatin and prevent non-specific binding. Then, either 3ul polyclonal rabbit anti- ß-catenin antibody (Santa Cruz Antibodies) or polyclonal rabbit anti-K-ras (Santa Cruz Antibodies) to 1ml of pre-cleared chromatin and incubated overnight at 4ºC with gentle agitation. After addition of 40ul of salmon sperm-coated Protein A-agarose beads and incubation for 2 hours at 4ºC, beads were washed twice with 50mM Tris-HCl pH 8, 2mM EDTA, 0.2% Sarkosyl and twice with 100mM Tris-HCl, 500mM LiCl, 1% NP40 and 1%

deoxycholate. Immunoprecipitated chromatin was eluted from beads by the addition of 50 mM NaHCO3, 1% SDS for 5 minutes at room temperature. Reversal of crosslinking before PCR amplification has been previously described. Primers used to amplify precipitated samples: human *FGF20* promoter, forward 5'-CTT TCATGTGTGTCTGGGCAGGTC-3' and reverse5'-CTTAAACCGGTCTCCTCTCAAC-3'; human *DKK1* promoter, forward 5'-GGCCACTTTGATCTCACGCGTC-3' and reverse 5'-CTG GGAACTTGGGTGCCCTTGCC-3'; human *CCND1* promoter, forward 5'-CCTCCC GCTCCCATTCTCTGCC-3' and reverse 5'-GCGGATGGT TTCCACTTCGCAG-3'; human *GAPDH* promoter, forward 5'-CAGACCACAGTCCATGCCATCAC-3' and reverse 5'-CCA TGAGGTCCACCACCCTGTTGC-3'; pOT promoter, forward 5'-CGATAGTACTAACATACGCTC-3' and reverse 5'-TTATGCAGTTGCTCTCCAGCG-3'.

Expression constructs, *luciferase* reporter constructs and assays. DNA fragments encoding ICAT and the E-cadherin intracellular domain were cloned into pcDNA3 (Invitrogen) under the control of the cytomegalovirus (CMV) promoter. The plasmid expressing ß-cateninS37A under the control of the CMV (pMH- ß-cateninS37A) has been previously described (Zorn et al.1999). pDKK1-*luciferase* and pFGF20-*luciferase* reporters were constructed by inserting PCR-generated fragments containing regions 5' to the translational start sites of the human *Dkk1* gene (-1068 to+43) and the human *FGF20* gene (-888 to +43) into the Kpn1 and Xma1 sites of the pGL3basic vector (Promega). 5' deletions of pDKK1-*luciferase* were also generated using PCR. Putative TCF binding

sites were mutated using QuickChange Site Directed Mutagenesis Kit (Stratagene). Mutating oligos used: DKK1 promoter TCF site 1, 5'-CCCATCCCGGCcgTGTTGTCTCCCTCC-3', TCF site 2, 5'-CCTCCCAGCGCgcTGAAATCCCATCC-3'; *FGF20* promoter TCF site1, 5'-CAAAGCTCACATCAcgtACGGTTGCACGG-3', TCF site 2, 5'-GTTAAACTGTCAAAGCaTcGATTTTTCTTTCATG-3'. Mutations are represented in lower case letters and the TCF sites are underlined. Luciferase assays on reporter constructs to measure ß-catenin transcriptional activity were performed in triplicate. Briefly, 293 cells were plated in 12 well dishes and transfected with pDKK1-*luciferase* or pFGF20-*luciferase* reporters using calcium phosphate. Cells were co-transfected with a CMV-driven *Renilla luciferase* construct to normalize for variations in transfection efficiencies. We routinely used 0.1ug of the *luciferase* reporter construct, 0.025ug of the CMV-*Renilla* construct, and 0.3ug of effector plasmid. pCDNA was used to keep amounts of DNA constant in the transfection assays. After 48 hours of incubation, transfected cells were washed with PBS and lyzed in 200ul dual luciferase lysis buffer (Promega). Luciferase activity was measured using the dual luciferase assay kit according to Promega's instructions.

Western Analysis. Protein lysates were resolved in 4-20% polyacrylamide gels containing SDS and transferred to nitrocellulose. After incubating with mouse monoclonal antibodies against V5-tag (Invitrogen, 1:5,000) or γ-tubulin (Sigma 1:5,000), the reaction was visualized by peroxidase-conjugated secondary antibodies (Roche, 1:10,000) and a chemiluminescent substrate (Pierce, SuperSignal West Femto).

***Xenopus* embryo manipulations and RNA transcription.** RNA for DN Xtcf-3, VP16

Xtcf-3 (Vonica et al. 2000), TVGR (Darken et al. 2001), and Xombi/VegT (Lustig, 1996) were prepared with an mMessage mMachine in vitro SP6 transcription kit from Ambion. RNA injections were performed in 10 nl volume as indicated. Cap assays were performed on animal caps dissected at stage 9.5. For the cycloheximide and dexamethasone treatment experiment, embryos were injected at the 2-cell stage in both blastomeres with 20 pg *TVGR* RNA, animal caps were cut at stage 9, treated with cycloheximide (5 μg/ml) for 30 minutes prior to additon of dexamethasone (10 μM) for another 30 minutes collected for RT-PCR processing. RT-PCR analysis was performed as described (Wilson et al. 1994), using DNA amplified from ornithine decarboxylase (*ODC*) RNA as loading control. RT-PCR primers for X*FGF-20* were sense 5'-GGCTCCTCTGGCCGACGTGGG-3' and antisense 5'-CGACCC CTCGAATGCTAACC-3', and for *Xdkk-1* sense 5'-ACAAGTACCAACCTCTGGATG C-3' and antisense 5'-ACAGGGACACAAATTCCGTTG C-3'.

l*uciferase* reporter assays in *Xenopus*. Reporter plasmids were injected alone or with *VP16 Xtcf-3* RNA as indicated. Embryos were lysed and luciferase activity measured with the Luciferase Assay kit (Promega Corp., Madison, WI) as described (Vonica et al.2000).

siFGF20 constructs

The siRNA expression vector pSilencerHygro (Ambion) was used to clone DNA encoding double stranded inhibitory RNA hairpins derived from the following FGF-20 mRNA sequences: GTCGGTGCCTTCTTGGGCG (siFGF20.A), GAGAACTGGTATAATACCT (siFGF20.B) and GAGAAC**C**G**A**TATAATACCT (siFGF20.C; mutated bases are shown in bold).

Soft agar assays

RK3E-S33YA cells were transfected using Fugene (Roche) with siRNA expression constructs. Stable transfectants were selected with 200ug/ml hygromycin for 10 days and pooled. Assays of colony formation in soft agar were performed essentially as described previously (Freshney *et al,* 1994.). Briefly, 1ml underlayers of 0.8% agar medium were prepared in 35-mm dishes by combining equal volumes of 1.6% Noble agar and 2x DMEM with 20% fetal bovine serum (Gibco). The cells were passaged once in the absence of hygromycin before performing assays. 36 hours later, plates were trypsinized and 10^5 cells were plated in 0.4% agar medium in triplicates. The surface was kept wet by addition of a small amount of growth medium. After 2 to 3 weeks, dishes were stained with methylene blue and colonies were photographed and counted.

FIGURE LEGENDS

Figure 1S. Analysis of levels of *FGF20* mRNA by RT-PCR in human colon tumors. MSKCC refers to RNA isolated from tumors and normal colon mucosa specimens obtained from Memorial Sloan-Kettering Cancer Center. Samples obtained from Ambion represent normal mucosa and tumor specimens from the same patient. RT-PCR were obtained using 200ng of input RNA at 55o annealing temperature for a total of 40 cycles.

Gene symbol	Gene name	Fold Change	P value	Function
FGF20	Fibroblast growth factor 20	17.7	0.002	Signal transduction; cell-cell signalling
DKK1	Dickkopf (Xenopus laevis) homolog 1	15.5	<0.001	Extracellular Wnt signaling antagonist
LY6G6D	Lymphocyte antigen 6 complex, locus G6D (MEGT1)	9.4	0.011	Unknown
BIK	BCL2-interacting killer	9.2	0.002	Apoptotic program; induction of apoptosis
EST	GenBank acc.# AK022120	9.1	0.013	Unknowm
ETV5	Ets variant gene 5	6.2	0.006	Transcription factor
WNT11	Wingless-type MMTV integration site, member 11	5.3	0.006	Signal transduction; cell-cell signalling; embryogenesis and morphogenesis
SLC2A3	Solute carrier family 2, member 3	5.2	0.026	Glucose transport; carbohydrate metabolism
SERPIND1	Serine proteinase inhibitor, clade D, member 1	5	0.049	Plasma glycoprotein; proteinase inhibitor
SLC7A8	Solute carrier family 7, member 8	4.9	0.006	Cationic amino acid transporter
ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	4.7	0.001	Cell motility; G-protein linked receptor signalling pathway; transcription factor binding
DUSP6	Dual specificity phosphatase 6	4.6	0.023	Apoptosis; MAPKKK cascade; cell cycle control; inactivation of MAPK
LMO2	LIM domain only 2 (rhombotin-like 1)	4.2	<0.001	Oncogenesis; developmental processes
SLC2A14	Solute carrier family 2, member 14	3.8	0.028	Carbohydrate transport
ARHGAP26	GTPase regulator associated with FAK	3.8	0.028	Neurogenesis; cell growth/maintenance
GAD1	Glutamate decarboxylase 1 (brain, 67kD)	3.6	0.002	Synaptic transmission; glutamate decarboxylation
FUT1	Fucosyltransferase 1	3.5	0.035	Carbohydrate metabolism
QPTC	Glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	3.5	<0.001	Protein modification
RBP1	Cellular Retinol-binding protein 1	3.3	0.024	Retinoid binding; vitamin A metabolism
ABCB1	MDR/TAP member 1	3.1	0.001	Drug resistance; small molecule transport
STC1	stanniocalcin	3	0.027	Calcium ion homeostasis, cell surface receptor, cell-cell signaling
CHODL	chondrolectin	3	0.01	Heterophilic cell adhesion
SERPINI1	neuroserpin	2.9	0.023	Central nervous system development; peripheral nervous system development
EEF1A2	translation elongation factor 1 alpha 2	2.8	0.02	Protein biosynthesis; translational elongation
TNC	tenascin C	2.8	0.007	Cell adhesion
ENC1	BTB-like domain, ectodermal neural cortex	2.7	0.002	Development; neurogenesis

PLK2	serum-inducible kinase	2.7	0.006	Protein amino acid phosphorylation; cell cycle; positive regulation of I-kappaB kinase/NF-kappaB cascade
IRS1	Insulin receptor substrate 1	2.6	0.002	Signal transduction
TBX3	T-box 3	2.5	<0.001	skeletal development; regulation of transcription from Pol II promoter; morphogenesis
MSX2	msh homeobox homolog 2	2.5	<0.001	skeletal development; regulation of transcription, DNA-dependent; development
CXCR4	Chemokine receptor CXCR4	2.5	0.011	Activation of MAPK; apoptosis; chemotaxis; inflammatory response
MSX1	msh homeo box homolog 1	2.5	0.001	skeletal development /// regulation of transcription, DNA-dependent /// development
CDH1	E-cadherin	2.4	0.024	Cell adhesion; homophilic cell adhesion
	clone MGC:4655 IMAGE:3530459	2.4	0.017	Protein amino acid glycosylation
RHOB	ras homolog gene family, member B	2.3	0.011	Rho protein signal transduction /// cell growth and/or maintenance
IBRDC3		2.3	0.002	Unknown
CD9	CD9 antigen	2.3	0.006	cell motility; cell adhesion
HOXA1	homeo box A1	2.3	0.001	Regulation of transcription, DNA-dependent; development
CCND1	Cyclin D1	2.3	0.02	regulation of cell cycle; cell growth and/or maintenance
MYB	v-myb	2.3	<0.001	Regulation of transcription, DNA-dependent; cell growth and/or maintenance
EFEMP1	fibulin-like extracellular matrix 1	2.3	0.009	Visual perception
THBD	Thrombomudulin	2.2	0.04	Blood coagulation
DNCI1	dynein	2.2	<0.001	motor activity
	EST	2.2	0.02	Unknown
CALCB	calcitonin-related polypeptide	2.2	0.0003	Calcium ion homeostasis; signal transduction
PPM2C	pyruvate dehydrogenase phosphatase	2.1	0.025	Protein amino acid dephosphorylation
C9orf3	hypetherical protein	2.1	0.005	Proteolysis and peptidolysis
LBH	ortholog of mouse limb-bud and heart gene	2.1	0.046	
GPR49	G-protein couple recepto 49	2.1	0.006	G-protein coupled receptor protein signaling pathway
PKP2	plakophilin 2	2.1	<0.001	Cell-cell adhesion
KRT18	keratin 18	2	0.005	
NPTX1	neuronal pentraxin 1	2	0.001	

KAL1	Kallmann syndrome 1	2	0.004	Cell motility /// chemotaxis /// cell adhesion /// axon guidance
ESPRED2	Similar to sprouty homolog 3	2	0.005	Development; regulation of signal transduction
BAMBI	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	2	0.003	
FLJ20152	hypothetical protein FLJ20152	2	0.033	Unknown
AASS	aminoadipate-semialdehyde synthase	2	0.015	Electron transport
LEF1	lymphoid enhancer-binding factor 1	2	0.003	Regulation of transcription, DNA-dependent /// Wnt receptor signaling pathway
SLCA5	solute carrier family 7, member 5	2	0.004	Amino acid metabolism; amino acid transport
SOX13	SRY (sex determining region Y)-box 13	2	0.017	Regulation of transcription, DNA-dependent; morphogenesis
F12	coagulation factor XII (Hageman factor)	2	0.011	Proteolysis and peptidolysis
FZD7	frizzled homolog 7	2	0.009	G-protein coupled receptor protein signaling pathway; frizzled and Wnt signaling pathway; development
BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	-2	0.031	Regulation of transcription, DNA-dependent
MSH5	mutS homolog 5 (E. coli)	-2	0.011	DNA metabolism; mismatch repair
CELSR1	G-2 and S-phase expressed 1	-2	0.004	homophilic cell adhesion; development
N2N	similar to NOTCH2 protein	-2	0.026	Unnown
KIAA0590	KIAA0590 gene product	-2.1	0.006	Unknown
BASP1	brain abundant, membrane signal protein 1	-2.1	0.005	Cytoskeleton; plasma membrane
ISYNA1	myo-inisitol 1-phosphate synthase	-2.2	<0.001	Myo-inositol biosynthesis /// phospholipid biosynthesis
	unknown	-2.2	0.025	
FLNA	filamen A alpha	-2.3	0.004	Cell motility; cell surface receptor linked signal transduction; neurogenesis
LOC283232	hypothetical protein LOC283232	-2.4	0.016	Unknown
SNAI2	snail homolog 2 (Drosophila)	-2.4	<0.001	Negative regulation of transcription from Pol II promoter; development
IGFBP5	insulin-like growth factor binding protein 5	-2.8	0.002	Regulation of cell growth; signal transduction
SEC6L1	SEC6-like 1 (S. cerevisiae)	-2.9	0.02	Intracellular protein transport; exocytosis
C10orf10	decidual protein induced by progesterone	-3.0	0.04	Unknown
ZNF492	zinc finger protein 492	-9.9	0.01	Regulation of transcription

Table IS. List of genes regulated >2-fold by mutant ß-catenin in 293Top cells.