SUPPLEMENTAL METHODS

Immunohistochemistry

The study was approved by the Roswell Park Cancer Institute Institutional Review Board. Patients included in the study were diagnosed with melanoma at Roswell Park Cancer Institute (RPCI). Primary cutaneous melanoma specimens were studied using whole sections to assess the entire tumor architecture. Metastatic melanoma specimens were evaluated using TMAs, since the overall specimen architecture is not a requirement for accurate assessment. Formalin fixed and paraffin-embedded cutaneous and metastatic melanoma tissues were processed at the Pathology Core Facility (Roswell Park Cancer Institute). Positive and negative control slides were included with every immunochemistry run. The FOXQ1 antibodies (ab51340, Abcam) were visualized with the Novocastra (Newcastle,UK) PowerVision kit, followed by Fast Red (Thermo Scientific). Human tissue specimens were scored for intensity and percentage of stained cells by a board-certified pathologist. An IHC H-score was calculated as a product of these parameters.

RNA-seq assay

The sequencing libraries are prepared with the TruSeq Stranded Total RNA kit (Illumina Inc), from 1ug total RNA. Following manufacturer's instructions, the first step depletes rRNA from total RNA. After ribosomal depletion, the remaining RNA is purified, fragmented and primed for cDNA synthesis. Fragmented RNA is then reverse transcribed into first strand cDNA using random primers. The next step removes the RNA template and synthesizes a replacement strand, incorporating dUTP in place of dTTP to generate ds cDNA. AMPure XP beads (Beckman Coulter) are used to separate the ds cDNA from the second strand reaction mix resulting in blunt-ended cDNA. A single 'A' nucleotide is

then added to the 3' ends of the blunt fragments. Multiple indexing adapters, containing a single 'T' nucleotide on the 3' end of the adapter, are ligated to the ends of the ds cDNA, preparing them for hybridization onto a flow cell. Adapter ligated libraries are amplified by PCR, purified using Ampure XP beads, and validated for appropriate size on a 4200 TapeStation D1000 Screentape (Agilent Technologies, Inc.). The libraries are quantitated using KAPA Biosystems qPCR kit, and are pooled together in an equimolar fashion, following experimental design criteria. Each pool is denatured and diluted to 16pM for On-Board Cluster Generation and sequencing on a HiSeq2500 sequencer using the 50 single end cluster kit and rapid mode SBS reagents following the manufacturer's recommended protocol (Illumina Inc.).

Raw reads that passed the Illumina RTA quality filter were first pre-processed by using 1) FASTQC for sequencing base quality control and 2) Cutadapt to remove adapter sequences. The remaining reads were mapped to the human reference genome (hg19) and RefSeq annotation database using Tophat(48). A second round of quality control was performed to identify potential RNASeq library preparation problems by examining mapped BAM files using RSeQC(49) The number of reads aligning to each gene was calculated using HTSeq(50). Differentially expressed genes were identified using DESeq2(51), a variance-analysis package developed to infer statistically significant differences in RNA-seq data

Antibodies and other reagents

FOXQ1 (K-12) sc-47597, (C-9) sc-166265, MITF (Clone 5, Thermofisher, MA5-14146), TYR (C-19) sc-7833 GAPDH (Thermofisher, PA1-987), N-cadherin (CST, D4R1H-13116), β-Catenin (CST, D10A8-8480), CREB (CST, 48H2-9197), TLE1/2/3/4 (CST, 4681S),

FLAG (Sigma, F3165) LaminA (SantaCruz, sc-56137). Forskolin (LC labs) was prepared fresh in DMSO, 5-Aza-2′-deoxycytidine (A3656 Sigma-Aldrich), doxycycline (D3447 Sigma-Aldrich).

Expression Vectors

FOXQ1 ORF was PCR amplified from NHM cDNA and cloned into the lentiviral expression vector pLV-puro in frame with an N-terminus flag-tag.

Lentiviral expression vector containing beta-catenin cDNA (S33Y)-pcw107-V5 was a gift from Drs. David Sabatini & Kris Wood (Addgene plasmid #64616), CREB cDNA was purchased from DNASU repository (DNASU, HsCD00441528), TLE1-4 was purchased from OriGene (SC111039, RC224066, RC220536, RC202474), Lentiviral vector containing N-cadherin was purchased from Addgene (Plasmid #38153). shRNA targeting CREB was purchased from OriGene (RC210577), shRNAs targeting human and mouse FOXQ1, MITF, pLVp-puro and pLKO-1.puro lentiviral control vectors were purchased from Sigma-Aldrich (Sigma, St. Louis, MO). pLKO.1 puro shRNA beta-catenin was a gift from Dr. Robert Weinberg (Addgene plasmid #18803). pLenti-CMV-Puro-LUC was purchased from Addgene (w168-1-7477). pHAGE TRE dCas9-KRAB was a gift from Drs. Rene Maehr & Scot Wolfe (Addgene plasmid # 50917).

Cell proliferation analysis

Cells ($5x10^3$ cells) were seeded onto 96 well pates and grown for 24-72hrs followed by fixation and staining in 0.5% Methylene blue in water/methanol (50:50) for ~1 hour. The plates were rinsed using ddH20 and placed in an inverted position overnight to dry. The

stain was solubilized in 200μL of 1% SDS in PBS and the optical density was determined at 650nm using a fluorimeter (SpectraMax).

Chromatin Immunoprecipitation (ChIP)

Cultured cells (approximately $5x10^7$) were fixed with 1% formaldehyde at room temperature for 15min, followed by addition of glycine (0.125M) for 5 min. Next, cells were washed with ice-cold phosphate buffered saline, pelleted, and re-suspended in ice-cold cell lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl at pH 8.1, containing protease inhibitors) (EZ-ChIP kit, Millipore, CA, USA). The samples were then incubated on ice for approximately 20min, followed by sonication (12x for 30s each at 30s intervals) with a Microson (Misonix Inc., USA). Next, the samples were centrifuged at 15,000rpm at 4°C for 10min. A control aliquot (whole-cell extract) was saved, and supernatants were diluted 10-fold in ChIP dilution buffer (16.7 mM Tris-HCl (pH 8.1), 167 mM NaCl, 1.1% Triton X-100, 0.01%SDS, 1.2 mM EDTA protease inhibitor) (EZ-ChIP kit, Millipore, CA, USA). A two-hour incubation on a rotating platform at 4°C with protein A/G beads was used to remove non-specific background.

The pre-cleared chromatin material was incubated with anti-FLAG-conjugated beads (approximately 25μl slurry per 1 x 10^6 cells) on a rotating platform overnight at 4°C, followed by magnetic separation. The beads were sequentially washed and the precipitated material was de-crosslinked and treated according to the EZ-ChIP kit instructions (Millipore, CA, USA). The DNA was recovered by column purification and quantified using QUBIT ® DNA HS assay kit (Invitrogen).

Immunoprecipitation

Nuclear extracts were prepared by lysing cells using buffer containing 10mM Hepes, pH 7.9,1.5mM $MgCl_2$10mM KCl, 0.5mM DTT, followed by centrifugation at 1500g. The pellets were resuspended in the buffer containing 1% NP-40, 50mM Hepes-NaOH, pH 7.9, 300mM NaCl, 1% (v/v) NP-40 (tergitol), 10mM $MgCl_2$, 15% Glycerol followed by centrifugation at 12000g for 10min and overnight incubation of the supernatants with either FLAG-specific magnetic beads, IgG, or antibodies specific to the protein of interest at 4°C. If needed, agarose A/G beads were added and the material was incubated for additional 4hrs. The beads (Flag or A/G) were washed three times in the solution containing 25mM Hepes, pH 7.9, 300mM NaCl, 0.2% NP-40.

Generation of LUC containing cells

The lentiviral vector containing a plasmid pLenti-CMV-Puro (Addgene, Plasmid #17477) encoding the *firefly* luciferase gene was used to transduce cells at approximately 40%-60% confluency. The plate was incubated at 37°C and the media was changed the following day. Two days after infection, puromycin selection was initiated. After 5 days of maintenance in puromycin containing media, cells were seeded onto a 96 well plate (~1 cell/well) for clonogenic selection. Approximately 7 days later cells were analyzed using IVIS bioluminescence imaging. High and consistent luciferase expressing cells were selected and serially expanded in puromycin containing media. Several frozen aliquots were prepared from these early passage clones. No differences in luminescence intensity or variability were observed for cells grown with or with continued puro-selection. All cells were maintained in DMEM media supplemented with 10% FBS, 1% glutamine, and 1% streptomycin/penicillin.

Animal studies using a subcutaneous xenograft model

Cells (1-5x10^6) were inoculated subcutaneously in both flanks of 4-6-week old female SCID mice (Strain: C.B-Igh-1bIcrTac-Prkdcscid/Ros, bred and maintained by the in house transgenic mouse facility at RPCI; n=5 per group). Mice were monitored daily until the experiment reached its endpoint. Tumor burden was measured using calipers and volume was determined using the formula V= 0.5 (length) x (width^2). Mice were euthanized when tumor volume reached 2cm^3, when a tumor became ulcerated, or if the mice displayed any morbidity. While the animals were not randomized after the injections, the animals were coded to blind the investigator until the experimental endpoint was reached.

IVIS imaging

Approximately 1x10^6 cells stably expressing *firefly* luciferase were injected via the tail vein into 4-6 week old female SCID mice. Approximately 2-4 weeks later bioluminescence imaging was performed using the IVIS^{TM} imaging platform for assessment of tumor burden. During each imaging session, mice were anesthetized using isoflurane, and intraperitoneally injected with D-luciferin (150mg/kg). Mice were positioned uniformly within the imaging platform. Imaging was performed in 2min intervals starting 4mins post injection for 3-4 intervals. Total flux (p s^{-1}) in the regions of interest was measured. Image acquisition and bioluminescence imaging data analysis were done with Living Image 4.0 Software (PerkinElmer). The signal intensity of tumor burdens expressed as total photons/s/cm^2 (p/s/cm^2/sr) was normalized and data were represented as fold change in tumor burden.

Luciferase assay

The *CDH2* promoter region (-2200:500bp) was synthesized using GeneArt synthesis (Thermo Fisher Scientific, GeneArt) followed by sub-cloning into a pGL3-basic firefly luciferase vector (Promega, Madison, WI, USA) generating pGL3-CDH2. The obtained constructs were mixed with pRLSV40 plasmid expressing the *Renilla* luciferase gene (Promega). Cells were transfected in triplicates with the plasmid mixtures, using SuperFect reagent (Qiagen). Approximately 48hrs after transfection, *Firefly* luciferase and *Renilla* signals were detected according to manufacturer's instructions via Dual-Luciferase Assay Kit (Promega). *Firefly* luciferase signals were normalized by corresponding *Renilla* signals to control for transfection efficiency.

TOP-FLASH/FOP-FLASH reporter assay

The reporter assay consisted of a lentiviral-TOP-dGFP-reporter, a gift from Dr. Tannishtha Reya (Addgene plasmid # 14715)(48) (harboring three ß-catenin binding sites upstream of the TCF promoter) and a M51 Super 8x FOP-Flash (TOP-Flash mutant)(49), a gift from Dr. Randall Moon (Addgene plasmid # 12457) (harboring three mutant ß-catenin binding sites). The latter was used as a control for the background luciferase signal due to non-specific binding. Cells were transfected with reporter constructs including plasmid expressing the *Renilla* luciferase gene (Promega) and harvested 24-48hrs later followed by measurement of luciferase activities using dual luciferase reporter assay system as per manufacturer's instructions (Dual Glo Reporter Assay, Promega).

Matrigel invasion assay

Cells (1-5x10^3) were seeded in serum free media onto a polycarbonate 8µm-Matrigel coated inserts (BD Bioscience, San Jose, CA). Serum containing media was added to the

wells below the inserts. Cells were incubated for 16-18hrs at 37°C in 5% atmospheric CO_2. After completion of the assay, cells present in the top chamber were wiped off with wet cotton swabs. Cells on the underside of the insert were fixed and stained using Diff-quick stain (VWR Scientific, West Chester, PA). The inserts were allowed to air dry for 2-4hrs. The inserts were placed on standard glass slides and invaded/migrated cells were imaged at five random fields at 20X magnification followed by determination of invasion index (ratio of migration/invasion).

METHODS REFERENCES

1 Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics (Oxford, England)* **25**, 1105-1111, doi:10.1093/bioinformatics/btp120 (2009).

2 Wang, L., Wang, S. & Li, W. RSeQC: quality control of RNA-seq experiments. *Bioinformatics (Oxford, England)* **28**, 2184-2185, doi:10.1093/bioinformatics/bts356 (2012).

3 Anders, S., Pyl, P. T. & Huber, W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics (Oxford, England)* **31**, 166-169, doi:10.1093/bioinformatics/btu638 (2015).

4 Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology* **15**, 550, doi:10.1186/s13059-014-0550-8 (2014).

5 Reya, T. *et al.* A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**, 409-414, doi:10.1038/nature01593 (2003).

6 Veeman, M. T., Slusarski, D. C., Kaykas, A., Louie, S. H. & Moon, R. T. Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Current biology : CB* **13**, 680-685 (2003).