**Supplementary Materials and Methods**

***Hematoxylin Eosin staining, and immunohistochemistry***

For hematoxylin and eosin staining, teratoma tissue sections were deparaffinized with two 10 min washes with xylene, and were step-wise rehydrated in alcohol. Sections were washed briefly in dH2O, then stained in Harris hematoxylin solution for 1 min, and washed with H2O for 5 min. Slides were then immersed in 0.1% acid alcohol for 10s followed by a 1 min wash under tap water. Sections were then immersed in 0.2% ammonia water for 10s intervals until desired level of blue stain was reached. Sections were then washed with H2O for 5 min, followed by a brief wash in 95% alcohol. Tissue sections were then counterstained with eosin Y solution for 2 min, followed by step-wise dehydration and clearing in ethanol and xylene, respectively. Slides were mounted with Permount mounting medium (Fischer Scientific SP15-100).

For tumor immunohistochemistry, slides were deparaffinized, treated with EDTA and incubated in anti-HMB45 antibody (Abcam, #787), to detect gp100, at a dilution of 1:50 at 37oC for 15 min. For SMC immunohistochemistry, cells were seeded to confluency on Lab-Tek chamber slides (ThermoFisher) and fixed with 2% formalin in PBS for 20 min. Cells were then washed three times and permeabilized using 0.3% Triton X-100 in PBS for 10 min. After washing twice, the cells were incubated in anti-HMB45 at a dilution of 1:25 for 16 h. Following incubation with anti-HMB45 primary antibodies, all slides (tumor and SMC) were then incubated in HRP-conjugated anti-rabbit secondary antibodies (Roche) for 15 min and counterstained using hematoxylin. All slides were stained and scanned using the VENTANA Benchmark Ultra automated slide staining system (Roche).

***Cell staining, imaging, and high content imaging quantification***

Alkaline phosphatase staining was performed using Burstone’s staining protocol. The cells were fixed in 4% formaldehyde for 15 min, and were stained with a mixture of Naphthol AS-MX-PO4 (Sigma), N, N dimethylformamide (Fisher Scientific, Fair Lawn, New Jersey, USA), Tris HCl (Sigma), and Red violet LB salt (Sigma) for 45 min. For immunofluorescence staining, cells were fixed in 4% formaldehyde in PBS for 20 min, permeabilized by 0.1% Triton X-100 (Sigma) for 10 min at room temperature and blocked with 10% goat serum or 2% BSA for 30 min. Subsequently, the cells were incubated with the primary antibodies overnight at 4°C. Primary antibodies used include: SSEA4 (1:500, EMD Millipore, MAB4304), TRA-1-60 (EMD Millipore, MAB4360), α-SMOOTH MUSCLE ACTIN (SMA, 1:100, Dako Cytomation, Glostrup, Denmark, #M0851), βIII-TUBULIN (1:400, Sigma, #T8660), GATA4 (1:300, Covance, #PRB-278P), Calponin (Cedarlane, #M3556), TSC2 (Cell Signaling, #4308), GD3 (Mel-1; Covance, #SIG-38110), ER-a (1:200, Thermo Scientific, MA3-310), PR (1:100, Santa Cruz, sc-166169) and Melanoma gp100 (HMB45; 1:100, Abcam, ab137062). Secondary antibodies used were conjugated with either Alexa 488 or Alexa 555 (Typically 1:1000, goat-anti-mouse (A11001, A21424) and goat-anti-rabbit [A11034, A21429]), which were incubated with the cells for 1 hr at room temperature in the dark.

Images were captured on a Zeiss Fluorescence microscope or on a Thermo Scientific ArrayScan VTI HCS (High Content Screening) Reader. Quantification of immunofluorescence staining was performed using the HCS Studio software (version 6.5.0).

***Protein and mRNA analysis***

Western blots analysis was performed as previously described(1). The following antibodies and dilution factors were used: P-S6 (1:1000, Cell Signaling #2211), P-S6K (1:1000, Cell Signaling #9205), S6K(1:1000, Cell Signaling #9202)*,* HIF1α (1:1000, Abcam ab51608), VEGF-D (1:2000, Abcam ab155208), Enolase (1:1000, Santa Cruz #7455), LC3B (1:1000, Cell Signaling #2775), β-actin (1:10000, Cell Signaling #3700)*,* B-Catenin(1:1000, Santa Cruz #7963)*,* andTubulin (1:1000, Abcam ab131205). Immunoreactive proteins were exposed to anti-rabbit, anti-mouse or anti-goat horseradish peroxidase-conjugated secondary antibodies (Jackson Laboratories). Antigen-antibody complexes were detected using Immobilon ECL Western Chemiluminescent HRP Substrate (ThermoFisher) and recorded with a VersaDoc imaging system (Bio-Rad).

For quantitative RT-PCR, total RNA was extracted using a NucleoSpin RNA kit (Macherey-Nagel) according to the manufacturer’s instructions. 1 μg of total RNA was used for Oligo (dT)20-primed reverse transcription. Quantitative Real-Time PCR was performed with LightCycler 480 SYBR Green I Master (Roche). Reactions were carried out on the LightCycler 480 Real-Time PCR System (Roche). After initial denaturation at 95°C for 5 min, we performed 45 cycles of 95°C for 10s, 60°C for 10s, and 72°C for 10s, followed by 95°C for 5s, 65°C for 1 min, and a hold at 95°C. Triplicate amplifications were carried out for each target gene. We performed quantification using the comparative Ct method, normalized to the housekeeping gene GAPDH, and presented as a percentage of biological controls. Primer sequences are as follows (sequences written 5’ to 3’):

*EF1*α*:* Forward (CCAGGCAAGGCAAGTCAAGCCA); Reverse (AGGGCCAGGCTCATAGCACACAT)

*OCT4:* Forward (TCAGCCAAACGACCATCTGCCG); Reverse (AGCAAGGGCCGCAGCTTACA)

*NANONG:* Forward (ACGCAGAAGGCCTCAGCACCTA); Reverse (AGGTTCCCAGTCGGGTTCACCA)

*SOX2:* Forward (TACAGCATGTCCTACTCGCAG); Reverse (GAGGAAGAGGTAACCACAGGG)

*GAPDH:* Forward (AGCACCAGGTGGTCTCCTCTGA); Reverse (AATGCCAGCCCCAGCGTCAA)

***Cell size analysis***

Cell diameter measurements (Fig. 3C) were obtained of SMC suspension cultures following disassociation with 0.05% trypsin using the automated Cellometer Auto 2000 cell viability counter (Nexcelom Biosciences). Data reported are the average cell diameter of at least 100 cells for each cell line and replicate. Cell area measurements of lentivirus-transduced SMCs (Supplementary Fig. S6) were obtained by analysis of phase contrast images using the freehand selection tracing and measurement analysis tools in ImageJ. The reported data are the average cell area of at least 50 cells for each cell line and replicate.

***Genotyping analyses***

DNA was extracted from fibroblast-derived iPSCs using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany). Regions containing the known parental *TSC2* mutations were amplified using PCR with the following conditions: denaturation at 95°C for 7 min, followed by 39 cycles of 95°C for 30s, 58°C for 30s, and 72°C for 1 min, and final extension at 72°C for 7 min. Primer sequences can be found in (Supplementary Fig. S5C). PCR products were digested by site-specific restriction enzymes as indicated (Supplementary Fig. S5A&B). Digestion products were examined via electrophoresis (3% agarose gel) at 80 mV for 60 min.

Next generation sequencing was performed to search for mutations in *TSC1* and *TSC2* at high sensitivity as reportedly previously(2). Briefly, DNA was captured using Agilent SureSelect Target Enrichment with a set of oligonucleotide probes covering the entire genomic extent of *TSC1* and *TSC2,* and including exonic coverage of 49 mTOR pathway related genes, including *MTOR* and *RHEB*. After library preparation, sequencing was performed on the Illumina HiSeq 2500. Read data were processed using both standard GATK and custom tools as described(2). A read depth of >500x was achieved for the non-repetitive regions of *TSC1* and *TSC2.* After computational analysis, all potential variants were reviewed using the Integrated Genomics Viewer. SNP allele frequencies were also examined to search for evidence of loss of heterozygosity.

**Supplementary References**

1. Goldberg AA, Joung K-B, Mansuri A, Kang Y, Echavarria R, Nikolajev L, et al. Oncogenic effects of urotensin-II in cells lacking tuberous sclerosis complex-2. Oncotarget. 2016;7:61152–65.

2. Tyburczy ME, Dies KA, Glass J, Camposano S, Chekaluk Y, Thorner AR, et al. Mosaic and Intronic Mutations in TSC1/TSC2 Explain the Majority of TSC Patients with No Mutation Identified by Conventional Testing. Korf BR, editor. PLoS Genetics. Public Library of Science; 2015;11:e1005637.