Supplementary Methods:

Next Generation Sequencing (NGS) analysis of *TSC1* and *TSC2*

Genomic DNA was isolated from skin tumors and control samples using DNeasy Blood & Tissue Kit (QIAGEN). DNA was isolated from whole blood samples using RBC Lysis Solution, Cell Lysis Solution, and Protein Precipitation Solution (QIAGEN).

High-depth, targeted sequencing analysis by NGS was performed in the Kwiatkowski laboratory at Brigham and Women's Hospital and the Center for Cancer Genome Discovery at Dana Farber Cancer Institute, as described previously. Briefly, hybrid capture (Agilent SureSelect platform, Santa Clara, CA, USA) was performed for the entire genomic extent of *TSC1* and *TSC2*, including 10 kilobases (kb) upstream and downstream of each gene; and the coding regions and intron-exon boundaries of 45 other mTOR pathway genes. 100 ng of DNA was used for library construction. DNA was fragmented (Covaris ultrasonication) to median size of 250 base pairs (bp) and purified using Agencourt AMPure XP beads (Beckman Coulter, IN, USA). The fragmented, size-selected DNA was ligated to standard adapters by automated library preparation (SPRIworks, Beckman Coulter, IN, USA), and subject to 10 cycles amplification, using barcoded specific primers for each library. Following hybrid capture, and additional limited amplification, libraries were quantified, pooled in equal mass, and sequenced on an Illumina HiSeq2500.

Sequencing results were demultiplexed using Picard tools, aligned to the hg19 reference sequence using the Burrows-Wheeler Aligner[1], and duplicate reads identified and marked using Picard tools. Alignments were refined using the Genome Analysis Toolkit (GATK)[2] at indel sites (https://software.broadinstitute.org/gatk/documentation/tooldocs/current/org_broadinstitute_gatk_tools_walkers_indels_IndelRealigner.php).

Variant analysis was performed using MuTect v1.1.4 run in paired mode, in comparison to a reference normal DNA sample, and annotated by Variant Effect Predictor (VEP)[3-5]. Sequence data was also analyzed using custom code in Unix, Python, and Matlab to identify low allele fraction *TSC1/TSC2* sequence variants; and large genomic deletions in *TSC1/TSC2*. Single nucleotide variants had to be seen in 3 reads minimum, at least one in each read direction, indels had to be seen in at least 2 reads, for further consideration.

Potential sequence variants were validated and analyzed in all available DNA samples from each subject using an amplicon NGS method on the Illumina platform as described previously[6] to enable read depths of 50,000-1,000,000x for each sample and region of interest. At least two control DNAs from normal subjects were included for each amplicon, and variant allele fraction (VAF) was determined using custom Python code.

Supplemental References

1. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009;25(14):1754-1760.
2. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20(9):1297-1303.
3. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet.* 2011;43(5):491-498.
4. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol.* 2013;31(3):213-219.
5. McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. *Bioinformatics.* 2010;26(16):2069-2070.
6. Tyburczy ME, Dies KA, Glass J, et al. Mosaic and Intronic Mutations in TSC1/TSC2 Explain the Majority of TSC Patients with No Mutation Identified by Conventional Testing. *PLoS Genet.* 2015;11(11):e1005637.