Supplementary Figures:

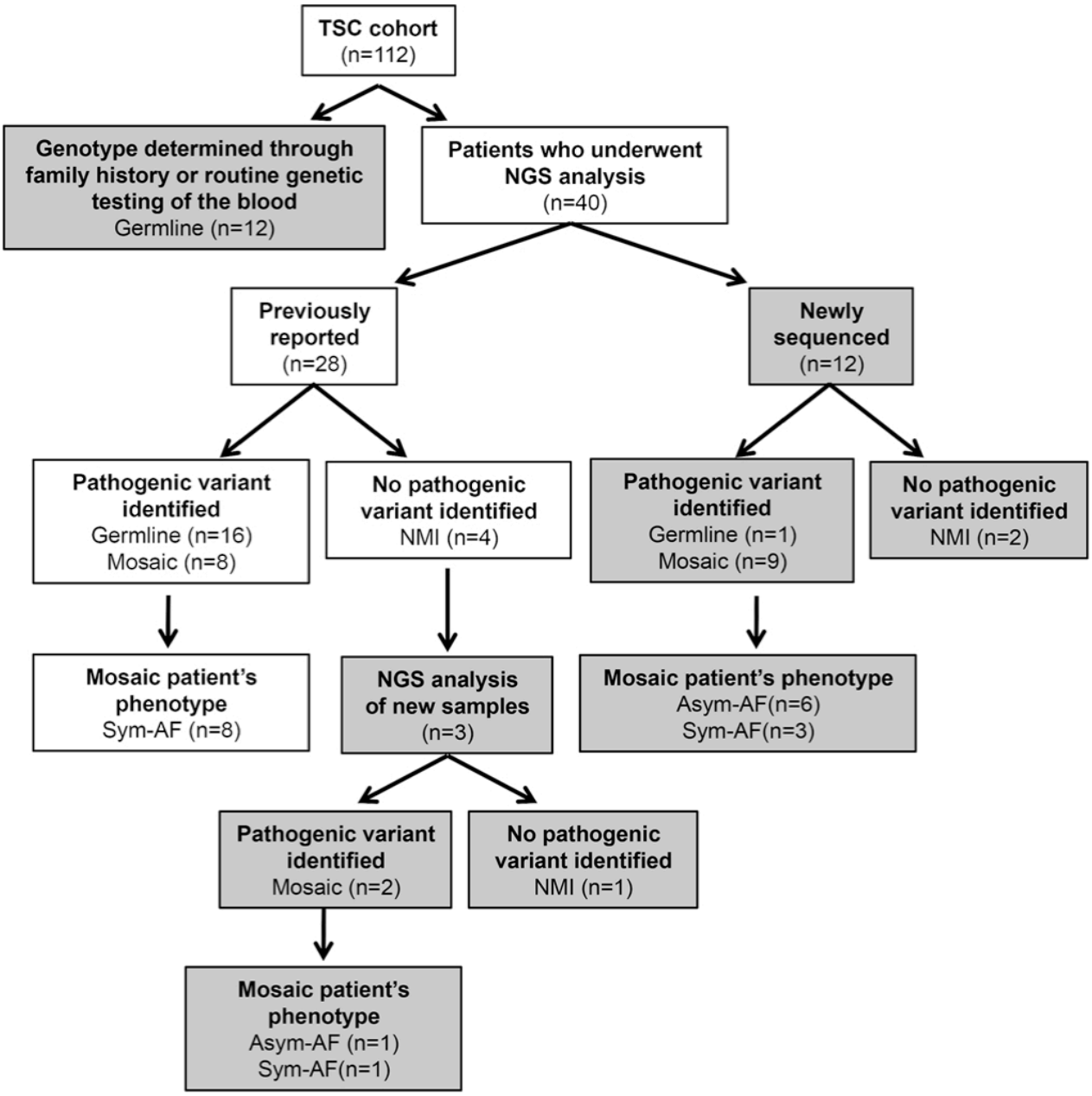

Figure S1. Flow Chart of Patients with Tuberous Sclerosis Complex Included in This Study. Our cohort consisted for 112 patients who met diagnostic criteria for TSC. There were 52 patients included in this study consisting of 29 with germline TSC, 12 with Sym-AF mosaicism, 7 with Asym-AF mosaicism, and 4 with NMI. Shaded in grey are the newly sequenced patients whose genetic findings have not been previously reported. Three of four patients previously reported as NMI had new samples analyzed, and of these, two (P14, P20) had newly identified mosaic variants. Abbreviations used: TSC, Tuberous Sclerosis Complex; NGS, Next-generation Sequencing; NMI, No Mutation Identified.

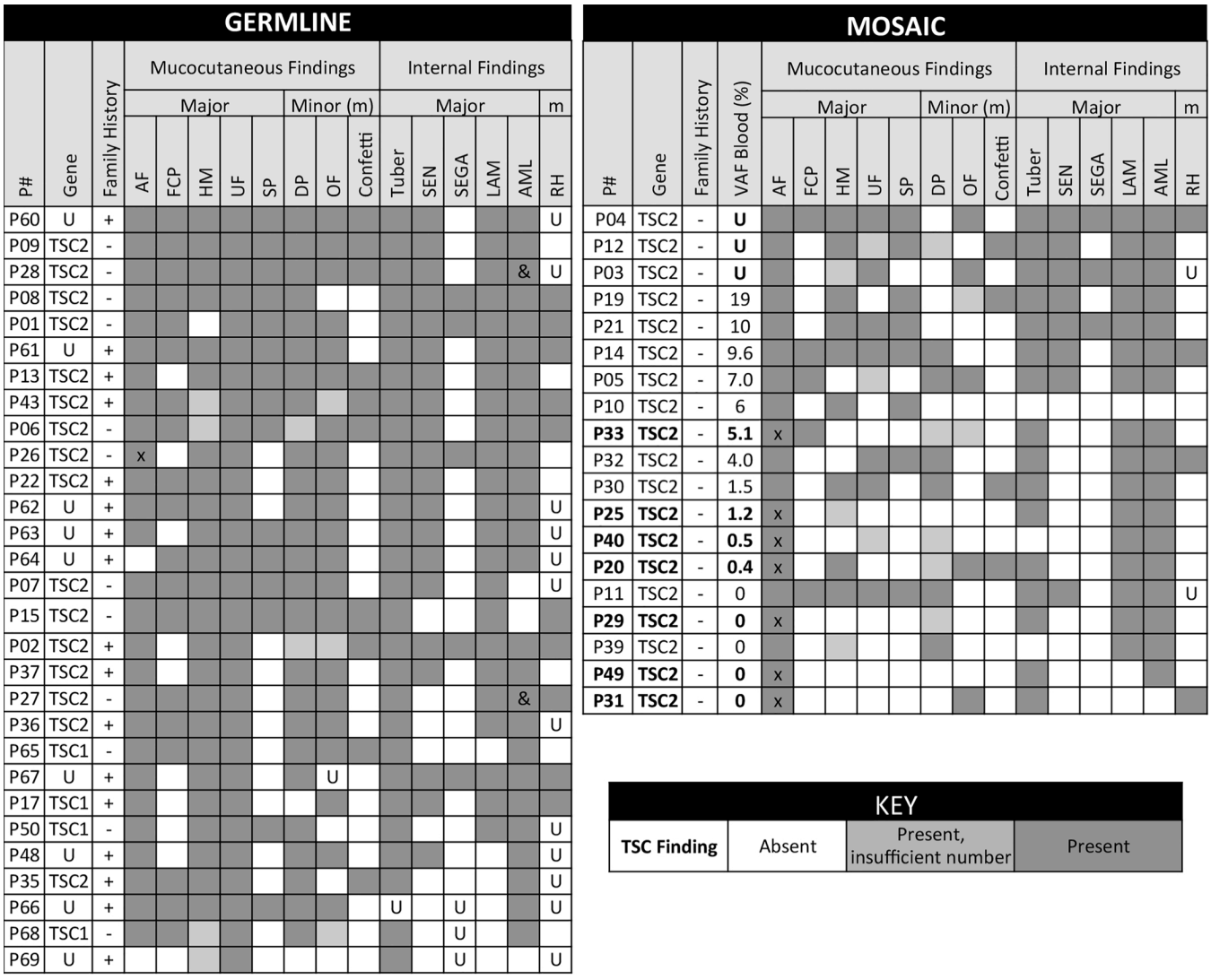

Figure S2. Clinical Findings of Tuberous Sclerosis Complex in Patients with Asym-AF Mosaicism, Sym-AF Mosaicism, or Germline Disease. Patients were first sorted from highest to lowest variant allele fraction in the blood, and then from the highest to lowest sum of total findings (major & minor mucocutaneous and major internal). Three patients with mosaicism who did not have their blood tested are located at the top of the mosaic group. Patients with mosaic TSC often exhibited TSC findings that were present but insufficient in number to meet diagnostic criteria (AF≥3, HM≥3, UF≥2, DP≥3, and OF≥2). Symbols and abbreviations used: X = asymmetric angiofibromas; U = unknown; & = polycystic kidney disease; AF, Angiofibroma; FCP, Fibrous Cephalic Plaque; HM, Hypomelanotic Macule; Ungual Fibroma, UF; SP, Shagreen Patch; DP, Dental Pitting; OF, Oral Fibroma; SEN, Subependymal Nodule; SEGA, Subependymal Giant Cell Astrocytoma; LAM, Lymphangioleiomyomatosis; AML, Angiomyolipoma; RH, Retinal Hamartoma.

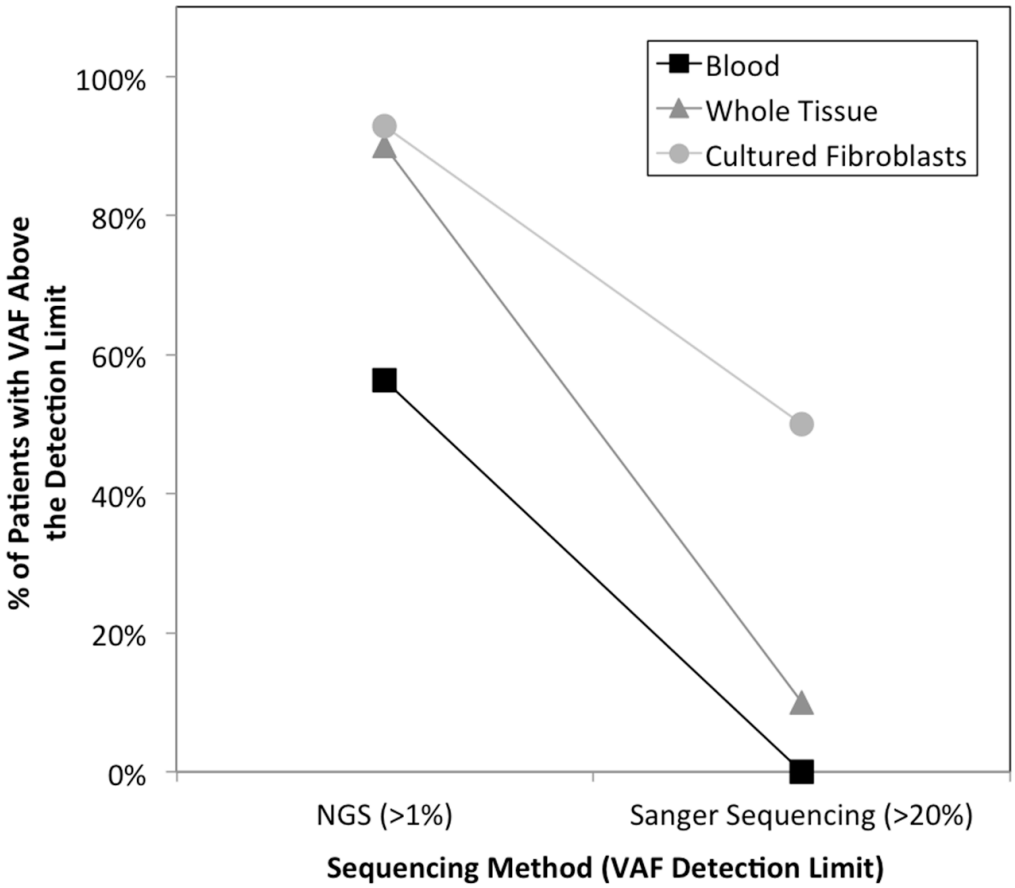

Figure S3. Sensitivity of Mosaic Variant Allele Fraction (VAF) Detection in the Blood, Skin Tumor Whole Tissue, and Skin Tumor Cultured Fibroblast Samples. Patients with mosaicism underwent NGS of the blood (n=16 patients), and skin tumor samples processed as whole tissue (n=10) or cultured fibroblasts (n=14). These samples include our newly analyzed and previously published samples. NGS of the blood identified a VAF >1% in 9/16 (56%) of patients with mosaicism, and none were above the detection limit for Sanger sequencing (VAF>20%). 9/10 patients with whole tissue samples analyzed had a VAF >1%, and only 1/10 (10%) had a VAF > 20%. There were 13/14 (93%) patients with cultured fibroblast samples with a VAF >1%, and 7/14 (50%) > 20%. Patients had a VAF > 1% more often in cultured fibroblast samples than in the blood (p=0.040). There was no difference in detection rate (VAF >1%) between whole tissue and cultured fibroblast samples or blood (p=1.0, p=0.10). Patients were more likely to have a VAF >20% in cultured fibroblasts than the blood (p=0.002), but there was no difference in detection rates between whole tissue and the blood or cultured fibroblasts (p=0.39, p=0.08).