Supplementary Figures:

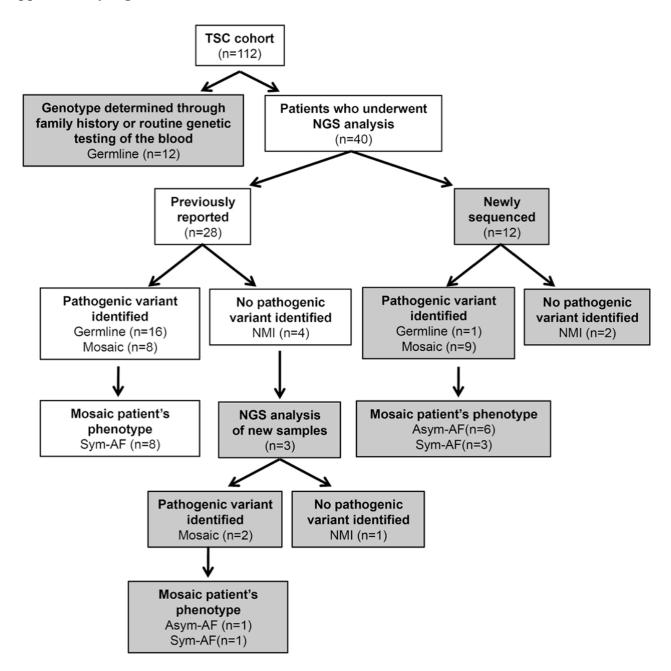


Figure S1. Flow Chart of Patients with Tuberous Sclerosis Complex Included in This Study. Our cohort consisted for 112 patients who met diagnostic criteria for TSC. There were 52 patients included in this study consisting of 29 with germline TSC, 12 with Sym-AF mosaicism, 7 with Asym-AF mosaicism, and 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.

		GERMLINE														MOSAIC																		
		Family History	Mucocutaneous Findings									Internal Findings							>	(1	Mucocutaneous Findings						Internal Findings							
			Major					Minor (m)			Major m							stor	%	Major				Minor (m)			Major m				m			
#d	Gene		AF	FCP	MΗ	UF	SP	DP	OF	Confetti	Tuber	SEN	SEGA	LAM	AML	RH	# <u></u>	Gene	Family History	VAF Blood (%)	AF	FCP	НМ	UF	SP	DP	OF	Confetti	Tuber	SEN	SEGA	LAM	AML	RH
P60	U	+											Г			U	P04	TSC2	-	U														
P09	TSC2	-															P12	TSC2	-	U														
P28	TSC2	-													&	U	P03	TSC2	-	U														U
P08	TSC2	-															P19	TSC2	-	19														
P01	TSC2	-															P21	TSC2	-	10														
P61	U	+															P14	TSC2	-	9.6														
P13		+															P05	TSC2	-	7.0														
P43	TSC2	+															P10	TSC2	-	6												П		
		-															P33	TSC2	-	5.1	х													
P26	TSC2	-	Х														P32	TSC2	-	4.0														
P22	TSC2	+															P30	TSC2	-	1.5														
P62	U	+														U	P25	TSC2	-	1.2	х													
P63	U	+														U	P40	TSC2	-	0.5	х													
P64	U	+														U	P20	TSC2	-	0.4	х													
P07	TSC2	-														U	P11	TSC2	-	0														U
P15	TSC2	-															P29	TSC2	-	0	х													
P02	TSC2	+															P39	TSC2	-	0														
P37	TSC2	+															P49	TSC2	-	0	х													
P27	TSC2	-													&		P31	TSC2	-	0	Х											T		
P36	TSC2	+														U																_		
P65	TSC1	-																																
P67	U	+		П					U																									_
P17	TSC1	+											Г				1							K	ΕY									i
P50	TSC1	-							Г							U						Present, insufficient number												
P48	U	+														U	TSC Finding			:	Abs						ent	i	Present					
P35	TSC2	+														U																		
P66	U	+									U		U			U																		
P68	TSC1	-											U																					
P69	U	+											U			U																		

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 \geq 3, HM \geq 3, UF \geq 2, DP \geq 3, and OF \geq 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.

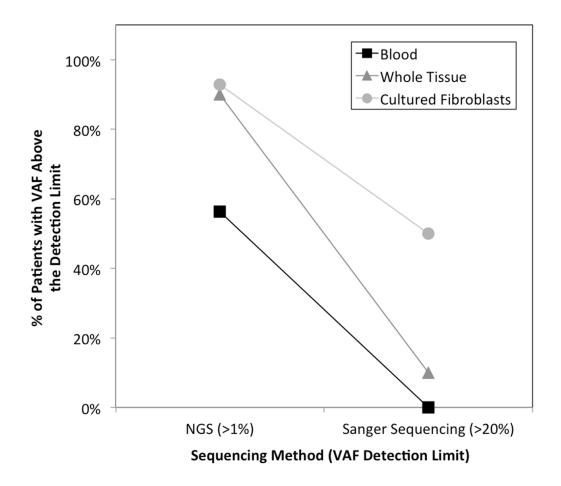


Figure S3. Sensitivity of Mosaic Variant Allele Fraction (VAF) Detection in the Blood, Skin Tumor Whole Tissue, and Skin Tumor Cultured Fibroblast Samples. Patients with mosaicism underwent NGS of the blood (n=16 patients), and skin tumor samples processed as whole tissue (n=10) or cultured fibroblasts (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).