Supplementary Material for
Somatic mutations in *SVIL* in cerebral aneurysms

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eMethods

RT-qPCR

To detect the change of SVIL expression due to somatic mutations, mRNA was extracted from available aneurysm tissue in 4 patients with variants in the *SVIL* gene using AllPrep DNA/RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The middle cerebral artery obtained from a patient without an aneurysm undergoing epilepsy surgery was used as control. cDNA was synthesized using 1 μg of total RNA with the Maxima cDNA Synthesis Kit for RT-qPCR (Thermo Scientific). RT-qPCR was performed using SYBR Green Master Mix (Applied Biosystems) on the AB step one plus real-time PCR system (Applied Biosystems). The primer sequences for amplification were: SVIL sense 5'- GGAAGAGTCAAGCAGAGATG -3' and antisense 5'- AAGAGAAGGTCGAGGAACTC -3', KLF4 sense 5'-CTGCGGCAAAACCTAC-3' and antisense 5'-GAATTTCCATCCACA-3', MMP2 sense 5'-CCAAGGTCAATGTCAGG-3' and antisense 5'-GCACCCATTTACACC-3', SM22α sense 5'-ATCCTATGGCATGAG-3' and antisense 5'-CAGGCTGTTCACCAA-3', PDGF sense 5'-CGATCCGCTCCTTTG-3' and antisense 5'-TCCAACTCGGCCCC-3', α-SMA sense 5'-TGACGCTGAAGTATCCG-3' and antisense 5'-ACCAAGGTCTCCAGGA-3', MMP9 sense 5'-TGAATCAGCTGGCTT-3' and antisense 5'-ACCTTCCAGTAGGGG-3', and GAPDH sense 5'-CCCAGAATATCATCC-3' and antisense 5'-CTGCTTCACCACCTTC-3'. All samples were amplified in technical triplicates.

Western blot

vSMCs were lysed in RIPA Lysis and Extraction Buffer (Thermo Scientific) and protein concentration was measured using a BCA assay (Pierce). Total protein (30μg per well) was separated by SDS-PAGE and transferred onto a PDVF membrane. After blocking with 5% skim milk solution for an hour, the membranes were incubated with antibodies: anti-SVIL, anti-KLF4, anti-MMP2, anti-PDGF, anti-α-SMA, anti-MMP9, anti-ROCK, anti-LIMK (Cell Signaling Technology). The membrane was visualized using ImageQuant LAS 500 (GE Healthcare).

Immunocytochemistry

Scrambled sgRNA-treated or SVIL sgRNA-treated vSMCs were seeded onto glass coverslips, and the cells were treated with Y27632 (20 μM) or PBS. The cells were then fixed, permeabilized, and blocked. They were incubated with antibody (Alexa Fluor 488-conjugated phalloidin (to visualize F-actin) overnight at 4 °C in the dark. Images were taken on a Leica TCS-SP5 confocal laser scanning microscope (CLSM).

Cell migration assay

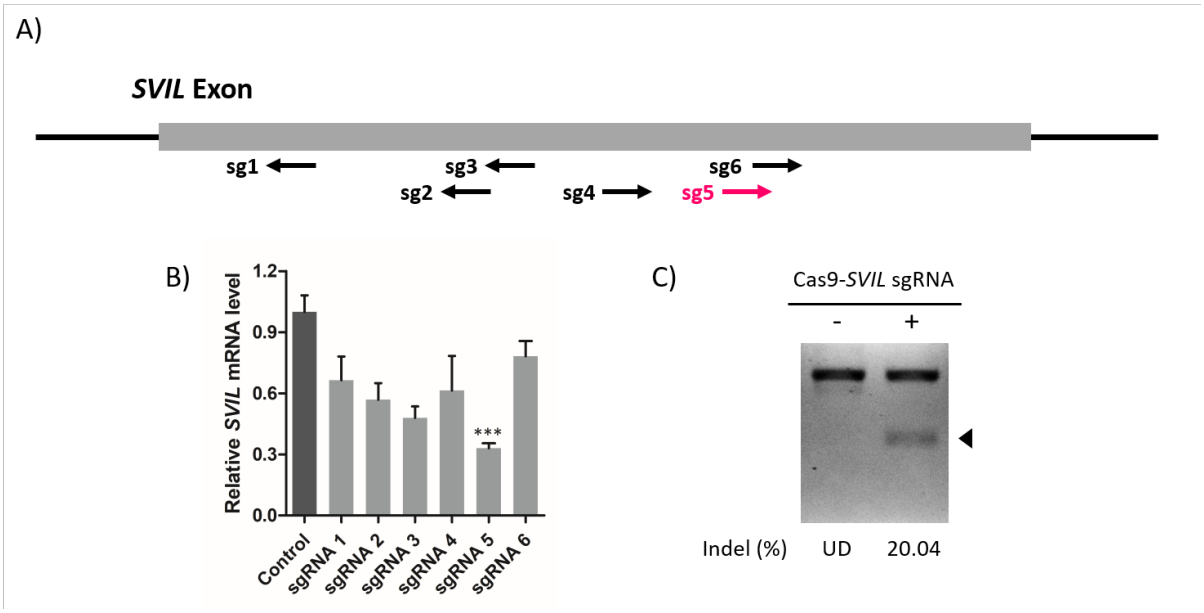
Cell migration was evaluated by the wound healing assay. Scrambled sgRNA-treated or SVIL sgRNA-treated vSMCs were seeded to a well with an insert to make the wound pre-casted, and then incubated with Y27632 (20 μM) or PBS for 24-48 h. The cells were cultured until a monolayer forms, then the inserts were removed to generate a wound field. The cells were monitored for migration into the wound field.

RNA sequencing and analysis

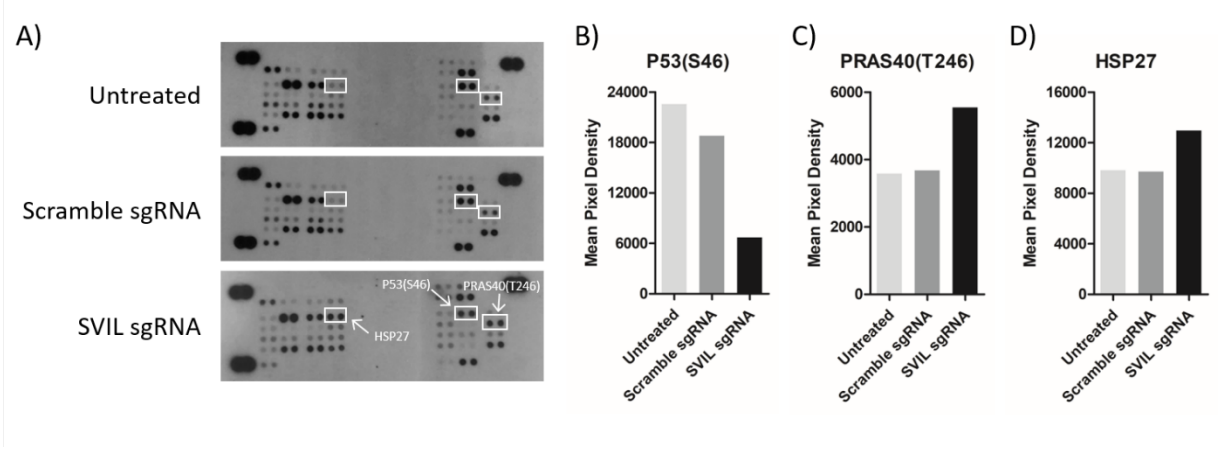
RNA was extracted using the AllPrep DNA/RNA kit (Qiagen) according to the manufacturer's protocol. RNA sequencing was performed at the Center for Cancer Computational Biology at the Dana Farber Cancer Institute. Library construction was performed using NEBNext Ultra RNA. Sequencing was performed twice per sample on HiSeq2000 using a 50-cycle paired-end protocol at 4 samples per lane or NextSeq 500 using a 75-cycle paired-end protocol at 10 samples per flowcell for a target of ~40 million reads per sample.

The raw reads were trimmed from both ends based on quality score and filtered by Trimmomatic (v0.39).[1] The quality-controlled reads were mapped to the Human reference genome (NCBI build 37/hg19) using STAR aligner (v2.7.3).[2] Read counts for genomic features were quantified using featureCounts (v2.0.0) with exon as the feature type.[3] To detect differentially expressed genes between aneurysm tissue and control vessels, DEseq2 was used for the gene counts with age and sex as covariates.[4] False discovery rate (FDR) was used to adjust for multiple testing, and FDR<0.05 was considered statistically significant. The log-CPM values of representative genes were visualized as bar plots with ggplot2.

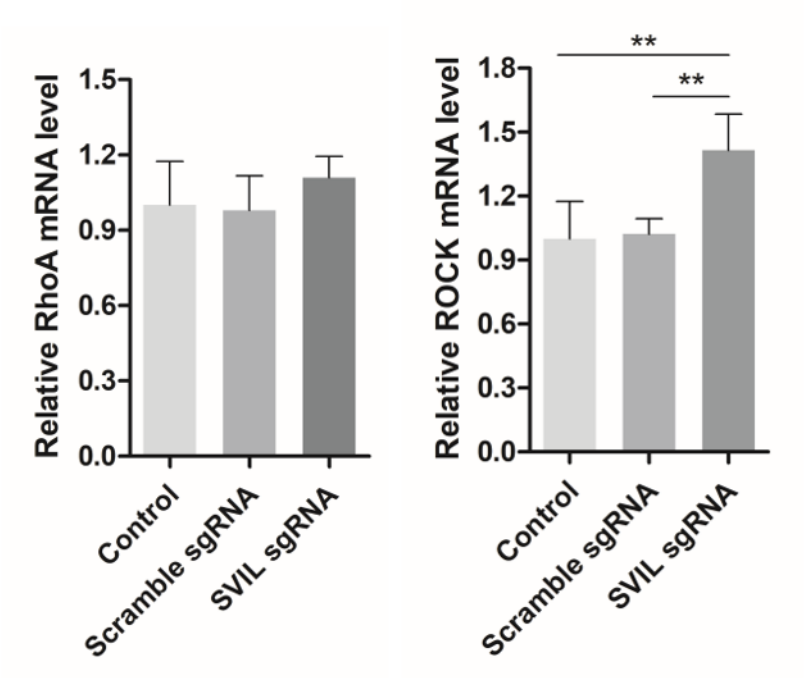
eFigure 1. Design of sgRNA for SVIL gene editing. A) Schematic of sgRNA design for the SVIL locus. B) The mRNA levels of SVIL using 6 different sgRNA sequences by RT-qPCR. C) Assessment of sgRNA 5 targeting of SVIL by T7E1 assay in vascular smooth muscle cells. Black arrows indicate the expected cleaved products after T7E1 cleavage assay. UD = undetectable. (n = 3, *** $p < 0.001$)



eFigure 2. SVIL deficiency regulated the phosphorylation of various kinases and proteins. A) The human Phospho-Kinase Array analysis of vSMCs using untreated, scramble sgRNA or SVIL sgRNA. Changes in phosphorylation for B) p53 at Ser46, B) PRAS40 at Thr246, and C) HSP27.



eFigure 3. SVIL knockdown regulates the RhoA/ROCK pathway. mRNA expression levels of RhoA and ROCK in SVIL-treated vSMCs by RT-qPCR. Error bars represent means ± S.D. (n = 3, **p < 0.01)



eTable 1. Sequences for designed oligonucleotides.

	Forward sequences	**Reverse sequences**
sgRNA 1	GATCGGGCTGTTCCGAAGAATCCTGG	AAAACCAGGATTCTTCGGAACAGCCC
sgRNA 2	GATCGGGATTCTTCGGAACAGCCACG	AAAACGTGGCTGTTCCGAAGAATCCC
sgRNA 3	GATCGGCGGGATAAATACTCGGAGTG	AAAACACTCCGAGTATTTATCCCGCC
sgRNA 4	GATCGTCCATGGTACCCGAACCATAG	AAAACTATGGTTCGGGTACCATGGAC
sgRNA 5	GATCGTGACGGCTCTTCCGACCCGGG	AAAACCCGGGTCGGAAGAGCCGTC AC
sgRNA 6	GATCGTAAATACTCGGAGTCGGCCTG	AAAACAGGCCGACTCCGAGTATTTAC

References

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3. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 2014; **30**(7): 923-30.
4. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; **15**(12): 550.