SUPPLEMENTARY MATERIALS AND METHODS

DNA Library Preparation, Sequencing, and Variant Analysis at GENEWIZ:

Atypical leiomyoma genomic DNA from 20 cases underwent targeted sequencing of the *FH* genomic locus at GENEWIZ (South Plainfield, NJ). Please see the Main Text Materials and Methods for genomic DNA preparation methods. The genomic DNA was sent to GENEWIZ in a de-identified fashion. The following library preparation, sequencing, and analysis methods were provided directly by the GENEWIZ Bioinformatics and Next Generation Sequencing teams (South Plainfield, NJ) and may represent a standard pipeline utilized there; and thus we have included these GENEWIZ methods verbatim here. Quotation marks surround the two below paragraphs provided verbatim from GENEWIZ.

"Genomic DNA samples were quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Enrichment probes were designed against the *FH* genomic region and synthesized through Twist Biosciences (South San Francisco, CA, USA). Library preparation was performed according to the manufacturer's guidelines. Briefly, the genomic DNA was fragmented by acoustic shearing with a Covaris S220 instrument. Fragmented DNAs were cleaned up and end repaired, as well as adenylated at the 3'ends. Adapters were ligated to the DNA fragments, and adapter-ligated DNA fragments were enriched with limited cycle PCR. Adapter-ligated DNA fragments were validated using Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified using a Qubit 2.0 Fluorometer. Adapter-ligated DNA fragments were hybridized with biotinylated baits. The hybrid DNAs were captured by streptavidin-coated binding beads. After extensive washing, the captured DNAs were amplified and indexed with Illumina indexing primers. Post-capture DNA libraries were validated using Agilent TapeStation and quantified using a Qubit 2.0 Fluorometer and Real-Time PCR (Applied Biosystems, Carlsbad, CA, USA). Illumina reagents and kits for DNA library sequencing cluster generation and sequencing were used for DNA sequencing. Post-capture DNA libraries were

multiplexed in equal molar mass, and pooled DNA libraries were clustered on one lane of a flow cell, using the cBOT from Illumina. After clustering, the flow cell was loaded on the Illumina HiSeq instrument according to the manufacturer's instructions. The samples were sequenced using a 2x150 paired-end (PE) configuration. Image analysis and base calling was conducted by the HiSeq Control Software (HCS 2.0) on the HiSeq instrument.

The raw binary base call (BCL) files generated by the Illumina HiSeq sequencer were converted to FASTQ files for each sample using bclfastq. Sequencing adapters and low quality bases in raw reads were trimmed using Trimmomatic 0.39. Cleaned reads were then aligned to the GRCh37 reference genome using Sentieon 202112.01. Alignments were then sorted and PCR/Optical duplicates were marked. Somatic SNVs (single nucleotide variants) and small INDELs (insertion-deletions) were called by using Sentieon 202112.01 (TNseq algorithm). The VCF files generated by the pipeline were then normalized (left alignment of INDELs and splitting multiallelic sites into multiple sites) using bcftools 1.13. Overlapped transcripts were identified for each variant, and the effects of the variants on the transcripts were predicted by Ensembl VEP 104."