SUPPORTING INFORMATION

SUPP. MATERIALS AND METHODS

Reagents and Antibodies

EGF was obtained from BD Bioscience. Cycloheximide was purchased from ENZO. Specific proteins were detected using the following primary antibodies: EGFR, RAF-1(S338), ERK1/2, phospho-ERK1/2, MEK1/2, GAPDH (Cell Signaling); SHOC2 (Genetex, Proteintech), tRFP (Evrogen); GST, HA, M-Ras, RAF-1, phospho-MEK1/2 (Santa Cruz) and PP1 (Millipore).

Expression Plasmids

SHOC2-tRFP and GST-SHOC2 expressing constructs were described previously [Galperin, Abdelmoti et al. 2012, Jeoung and Galperin 2014]. 3xHA-MRAS was purchased from Missouri S&T cDNA Resource Center (www.cdna.org). To generate the plasmids expressing either SHOC2(M173I)-tRFP or GST-SHOC2(M173I), a point mutations in the SHOC2-tRFP or GST-SHOC2 construct were introduced using a QuickChange site-directed mutagenesis kit according to the manufacturer's directions (Stratagene). The following primers were used: 5'-AAGAAGCTGCGGATACTTGATTTACGG-3' and 5'-CCGTAAATCAAGTATCCGCAGCTTCTT-3'. All constructs was verified by dideoxynucleotide sequencing.

Cell Culture and DNA Transfections

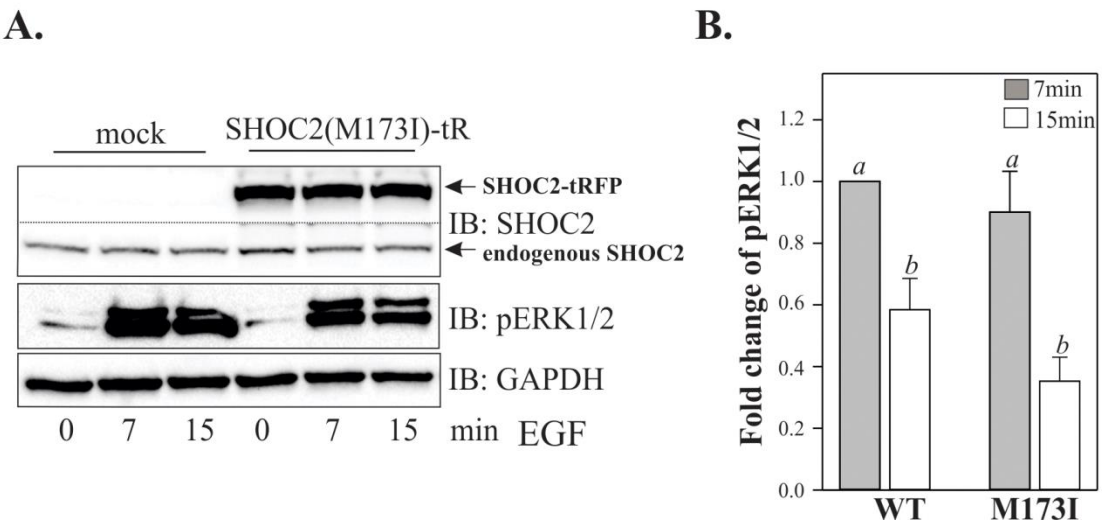
293FT cells (Invitrogen) and Cos-1 (ATCC), and stable cell lines (derivatives of Cos-1 cells) were grown in Dulbecco Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) supplemented with, MEM-NEAA, Penicillin, Streptomycin, and L-Glutamate (Invitrogen). Cos1-LV1 cells expressing SHOC2-tRFP and SHOC2 shRNA were described previously [Jeoung and Galperin 2014]. The transfections of DNA constructs were performed using PEI (Neo Transduction Laboratories, Lexington, KY) or *Trans*IT® (Mirus Bio LLC) reagents. Expression of tagRFP-fused proteins was confirmed by Western blotting as described below.

Immunoprecipitation and Western blot analysis

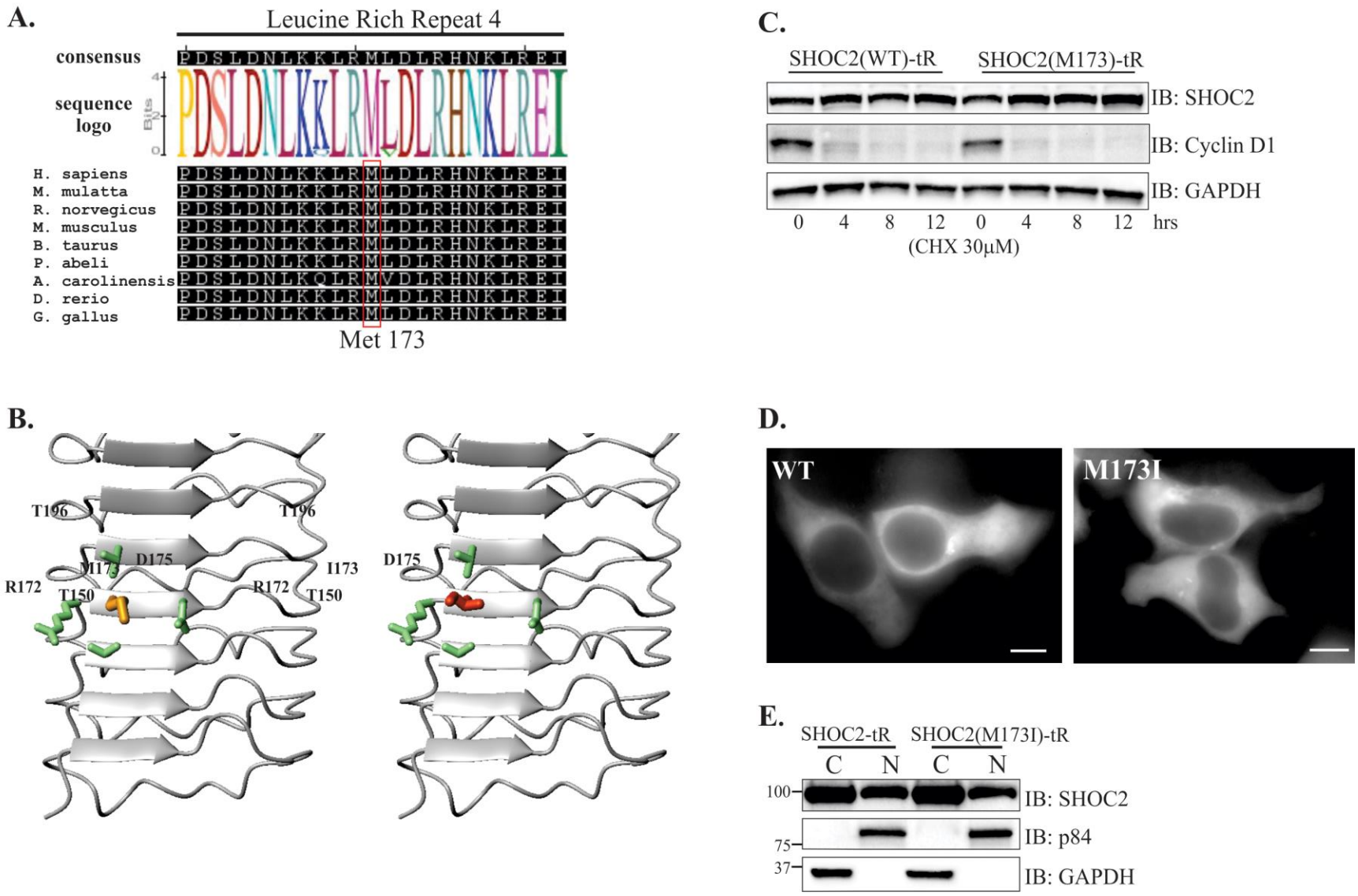
Immunoprecipitation was performed as described in [Galperin, Abdelmoti et al. 2012]. Immunoprecipitates and aliquots of cell lysates were denatured in the sample buffer at 95 °C, resolved by electrophoresis, and probed by Western blotting with various antibodies followed by the chemiluminescence detection as described previously [Jeoung, Abdelmoti et al. 2013]. Proteins transferred from SDS-PAGE gels to nitrocellulose membranes were visualized using the ChemiDoc analysis system (Bio-Rad). The quantifications were performed using densitometry analysis mode of the Image Lab software (Bio-Rad, Inc).

Statistical analysis

Datasets were first tested for normality and homogeneity of variance. When appropriate, data were transformed before statistical analysis. Nontransformed data are depicted in all the figures. One-way ANOVA using SigmaStat 3.5 (Systat Software, Inc., Point Richmond, CA) was used to determine differences pERK1/2 activation levels. If differences were detected, Tukey's test was used to determine which means differed. The Student's *t*-test was used to determine differences in pRAF-1 activation.



Supp. Figure S1. Overexpression of SHOC2 M173I mutants in cells does not affect ERK1/2 activity. **A.** Cos1 cells were transiently transfected with SHOC2(M173)-tRFP truncated mutants and treated as in A. The lysates were probed by immuno-blotting (IB) for SHOC2, activated ERK1/2 (pERK1/2), and GAPDH (loading control). **B.** Multiple blots from the experiments exemplified in A were analyzed. Bars represent the mean values (±S.E., n = 3) of phosphorylated ERK1/2 activity normalized to total ERK1/2 in arbitrary units (pERK/ERK) (a, P = 0.1, b vs. c, P = 0.023).



Supp. Figure S2. Protein half-life and cellular distribution of the SHOC2(M173I)-tRFP mutant are not altered. **A.** Multiple alignment of the LRR4s of nine SHOC2 orthologues. The motif includes conserved sequence positions for the LRR4. Residue conservation color

scheme: residues boxed in black are identical, and those boxed is dark and light grey are conserved substitutions. **B.** Superimposed ribbon representation of the model structure of WT SHOC2 and the M173I mutant of SHOC2 with amino acid residues clustered around M173 highlighted. **C.** Cos-LV1 cells were transiently transfected with SHOC2-tRFP or the SHOC2(M173I)-tRFP mutant. Thirty-six hours post-transfection cells were treated with 30 μM Cycloheximide for indicated times at 37°C. The lysates were probed by immuno-blotting (IB) for SHOC2, Cyclin D1 and GAPDH (loading control). **D**. Cos-LV1 cells were transfected with SHOC2-tRFP and the SHOC2(M173I)-tRFP and imaged live. SHOC2-tRFP was detected using Cy3 (for tagRFP) filter channels for imaging of living cells. Scale bar, 10 μm. **E.** Cos-LV1 cells were transfected with full-length SHOC2-tRFP or the SHOC2(M173I)-tRFP mutant, and subjected to the cell fractionation. Cell lysates were immunoblotted (IB) with SHOC2 Abs to monitor expression of SHOC2, and p84 (nuclear fraction-N) Abs and GAPDH (cytosolic faction-C) to control for the purity of fractions. 30 μg of total lysate for each fraction was loaded for IB analysis. Results in each panel are representative of three independent experiments.

Supporting Information Legends

NP_031399.2- Homo sapiens, NP_001095413.1-Bos taurus, NP_062632.2-Mus musculus, NP_001013173.1-Rattus norvegicus, NP_001026407.1-Gallus gallus, NP_001038251.1-Danio rerio, NP_001026407.1-Gallus gallus, NP_001244517.1-Macaca mulatta, XP_003218575.1-Anolis carolinensis. Accession number of SHOC2 orthologues in databases (EMBL and NCBI).

Supp. Table S1. Clinical presentation of NS, CFC, NS/LAH Rasopathies and germ line mutation analysis of SHOC2

	NS	CFC	NS/LAH	PT 1	PT 2
Cardiac /ECG defects	90%	+	most	-	-
Short stature	~70%	most	most	-	-
GH deficiency	-	-	+	-	-
Hyperpigmented skin	CALS	+	~75%	-	-
Lymphatic dysplasia	+	+	ND	-	-
Anagen hair	-	-	+	-	-
Hyperkeratosis/eczema	-	+	~50%	-	-
Ptosis	+	+	+	+	-
Ocular anomalies	+	+	+	+	-
Macrocephaly	±	+	most	+	-
Abnormal nails	-	+	ND	+	+
Brain anomalies	rare	+	+	+	ND
High-arched palate	+	+	ND	-	+
Hyperactive/ADHD	±	+	~50%	+	-
Sparse, slow growing hair	rare	+	most	+	+
Developmental delay	25%-30%	+	+	+	+
Intellectual disability	Mild 1/3	most	most	?	Mild
Genes	PTPN11, SOS1, RAF1, KRAS, NRAS, BRAF, MAP2K1, CBL	BRAF, KRAS, MAP2K1/2	SHOC2	SHOC2	SHOC2