SUPPLEMENTARY INFORMATION

MUTATION IN *SHOC2* PROMOTES ABERRANT PROTEIN *N*-MYRISTOYLATION AND UNDERLIES NOONAN-LIKE SYNDROME WITH LOOSE ANAGEN HAIR

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Supplementary Table 1. Leading Noonan syndrome disease candidates predicted by mammalian protein-protein interaction network analysis.

Protein name	Links for the node	Total links in background	Links to seed	Total links in subnetwork	z-score
One node/Two links subnetwork					
SHOC2	4	11184	2	22	22.48034
NRAS	25	11184	2	22	8.80567
CRKL	34	11184	2	22	7.48228
JAK1	39	11184	2	22	6.95066
INSR	41	11184	2	22	6.76514
JAK2	60	11184	2	22	5.48344
BCL2	68	11184	2	22	5.10772
PRKCA	91	11184	2	22	4.30827
EGFR	106	11184	2	22	3.92713
Two nodes/Three links subnetwork					
SHOC2	4	11184	2	223	6.86825
RASSF2	2	11184	1	223	4.85658
SPRY1	3	11184	1	223	3.88303
FRS2	11	11184	2	223	3.84067
SLAMF6	4	11184	1	223	3.29149
APBB1IP	4	11184	1	223	3.29149
GRAP	5	11184	1	223	2.88021
HRAS	5	11184	1	223	2.88021
RIT2	7	11184	1	223	2.32640
NRAS	25	11184	2	223	2.14823
RRAS	8	11184	1	223	2.12572

Supplementary Table 2. Clinical features of *SHOC2* mutation-positive patients.

Patient	N02	27518	N11	26841	N16	N10	N01	27925
Sex	F	M	M	F	F	M	M	M
Age (years)	0.1	1.6	2.7	5.8	6.0	7.2	7.5	7.9
Short stature (<3rd centile)	na	+	+	+	+	+	+	+
GH deficiency	nd	nd	nd	+	nd	+	+	nd
Craniofacial anomalies								
- *Macrocephaly*	-	+	+	+	+	+	+	+
- *Prominent forehead*	+	+	+	+	+	-	+	+
- *Bitemporal narrowing*	-	-	+	-	+	-	-	-
- *Hypertelorism*	+	+	+	+	+	+	+	+
- *Palpebral ptosis*	+	+	+	+	+	+	+	+
- *Low-set/posteriorly rotated ears*	+	+	+	+	+	+	+	+
Congenital heart defect								
- *Pulmonic stenosis*	-	+	+	-	+	-	-	+
- *Hypertrophic cardiomyopathy*	-	+	-	-	+	-	-	-
- *Mitral/tricuspid dysplasia*	+	nd	-	-	-	+	-	-
- *Atrial/ventricular septal defect*	-	ASD	-	-	ASD	VSD	-	-
Skin/ectodermal anomalies								
- *Dark skin*	na	-	-	-	+	+	+	-
- *Hyperkeratosis*	-	-	-	-	KP	-	-	-
- *Sparse/absent scalp hair*	+	+	+	+	+	+	+	+
- *Loose anagen hair*	nd	nd	nd	+	nd	nd	+	+
- *Eczema*	-	+	+	-	+	-	-	-
Skeletal abnormalities								
- *Short/webbed neck*	+	+	+	-	+	+	+	-
- *Pectus anomalies*	+	+	+	+	+	-	+	-
Undescended testes	na	-	-	na	na	-	-	-
CNS involvement								
- *Mental retardation*	na	+	+	+	+	+	+	+
- *Hyperactivity*	na	nd	-	-	+	+	+	+
Ocular anomalies								
- *Strabismus*	-	-	-	+	+	-	+	-
- *Refraction errors*	-	-	-	-	-	-	-	-
Coagulation defects	-	-	+	-	nd	-	-	-
Hypernasal/hoarse voice	-	-	-	-	+	-	-	+

Supplementary Table 2. Clinical features of *SHOC2* mutation-positive patients. (continued)

Patient	N08	10424	N04	34644	N15	N07	N06	N05
Sex	M	F	M	F	M	F	F	M
Age (years)	8.0	8.3	8.3	9.5	9.6	10.8	11.2	12.0
Short stature (<3rd centile)	+	+	+	+	+	+	+	+
GH deficiency	+	+	-	-	+	+	+	-
Craniofacial anomalies								
- *Macrocephaly*	+	+	+	+	+	-	+	+
- *Prominent forehead*	+	+	+	+	+	+	+	-
- *Bitemporal narrowing*	-	-	-	-	-	-	-	-
- *Hypertelorism*	+	+	+	+	+	+	+	+
- *Palpebral ptosis*	+	+	-	+	+	-	+	-
- *Low-set/posteriorly rotated ears*	+	+	+	-	+	+	+	-
Congenital heart defect								
- *Pulmonic stenosis*	+	-	-	+	-	-	-	+
- *Hypertrophic cardiomyopathy*	-	-	-	-	+	-	-	-
- *Mitral/tricuspid dysplasia*	-	-	-	-	-	+	-	-
- *Atrial/ventricular septal defect*	-	-	-	ASD	-	ASD	ASD	ASD
Skin/ectodermal anomalies								
- *Dark skin*	+	+	+	+	+	+	+	+
- *Hyperkeratosis*	-	-	ICH	-	-	-	ICH	-
- *Sparse/absent scalp hair*	+	+	+	+	+	+	+	+
- *Loose anagen hair*	+	nd	+	nd	+	nd	+	+
- *Eczema*	+	-	-	-	+	+	-	-
Skeletal abnormalities								
- *Short/webbed neck*	-	-	+	+	+	-	+	+
- *Pectus anomalies*	+	+	-	+	+	+	+	+
Undescended testes	-	na	-	na	+	na	na	-
CNS involvement								
- *Mental retardation*	-	+	+	+	+	+	+	-
- *Hyperactivity*	-	-	+	-	+	+	+	+
Ocular anomalies								
- *Strabismus*	-	+	-	+	+	-	+	-
- *Refraction errors*	HYP, AST	-	-	HYP	-	-	-	MYO
Coagulation defects	nd	-	-	+	-	+	-	+
Hypernasal/hoarse voice	+	-	+	-	-	-	+	-

Supplementary Table 2. Clinical features of *SHOC2* mutation-positive patients. (continued)

Patient	32696	N03	N14	20179	22407	N13	N17	33526	N09
Sex	M	M	F	M	M	M	M	M	M
Age (years)	12.9	13.0	13.1	13.1	14.2	17.2	22.6	36.5	39.7
Short stature (<3rd centile)	+	+	+	+	+	+	+	+	+
GH deficiency	nd	nd	+	nd	+	+	+	nd	nd
Craniofacial anomalies									
- *Macrocephaly*	+	+	+	+	+	+	+	+	+
- *Prominent forehead*	+	+	+	+	+	+	+	+	+
- *Bitemporal narrowing*	-	-	-	-	-	-	-	-	-
- *Hypertelorism*	+	+	-	+	+	+	+	-	+
- *Palpebral ptosis*	+	+	+	+	+	+	+	+	+
- *Low-set/posteriorly rotated ears*	+	+	-	+	+	+	+	+	+
Congenital heart defect									
- *Pulmonic stenosis*	+	-	-	-	-	-	+	-	-
- *Hypertrophic cardiomyopathy*	-	+	-	+	-	-	-	-	-
- *Mitral/tricuspid dysplasia*	+	+	-	+	-	+	-	+	-
- *Atrial/ventricular septal defect*	VSD	-	-	-	ASD	-	-	-	ASD
Skin/ectodermal anomalies									
- *Dark skin*	-	-	+	-	+	+	+	-	+
- *Hyperkeratosis*	-	KP	-	KP	-	KP	KP	-	KP
- *Sparse/absent scalp hair*	+	+	+	+	+	+	+	+	+
- *Loose anagen hair*	nd	+	+	nd	nd	+	+	nd	nd
- *Eczema*	-	+	+	-	-	-	-	-	-
Skeletal abnormalities									
- *Short/webbed neck*	+	+	+	+	-	-	+	+	-
- *Pectus anomalies*	+	+	+	+	-	+	-	+	+
Undescended testes	+	+	na	+	-	-	+	+	-
CNS involvement									
- *Mental retardation*	-	+	+	+	+	-	+	+	+
- *Hyperactivity*	-	-	+	nd	nd	+	+	-	+
Ocular anomalies									
- *Strabismus*	-	+	+	-	+	+	+	+	-
- *Refraction errors*	HYP	-	MYO	-	HYP	MYO, AST	AST	-	-
Coagulation defects	-	+	-	-	-	-	-	+	-
Hypernasal/hoarse voice	-	+	-	-	-	-	-	-	-

F: female; M: male; na: not applicable; nd: no data.

Cardiac anomalies: ASD, atrial septal defects; VSD, ventricular septal defects.

Skin/ectodermal anomalies: ICH, Ichthyosis; KP, keratosis pilaris.

Ocular anomalies: AST, astigmatism; HYP, hypermetropia; MYO, myopia.

Supplementary Table 3. Vulval precursor cell induction in *C. elegans* strains expressing the wild-type or mutant SHOC2 protein.

Genotype	Transgene	*N*	Muv[a]	*N*	Induction of vulval fate VPC						Average
					P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	
let-60^{gof}	none	135	68.1	39	30.8	41.0	100	100	100	30.8	4.0
let-60^{gof};*sur-8*^{rf}	none	302	7.9	85	2.4	5.9	100	100	100	14.1	3.2
let-60^{gof}; *sur-8*^{rf}	*SHOC2*^{wt}	271	19.6[b]	45	4.4	35.6[c]	100	100	100	37.8	3.8[b]
let-60^{gof};*sur-8*^{rf}	*SHOC2*^{S2G}	104	10.6	29	3.4	3.4	100	100	100	3.4	3.1[d]
wild type	none	>100	0								
let-60^{gof}	none	135	68.1								
let-60^{gof}	*SHOC2*^{wt}	72	61.1								
let-60^{gof}	*SHOC2*^{S2G}	132	65.9								

Strains: *let-60*^{gof} indicates *let-60*(*n1046*), *sur-8*^{rf} indicates *sur-8*(*ku167*).

SHOC2^{wt} indicates *hsp16.2::SHOC2*^{wt}*::V5*, *SHOC2*^{S2G} indicates *hsp16.2::SHOC2*^{S2G}*::V5*.

N indicates the number of animals scored, VPC indicates vulval precursor cell.

Animals were heat-shocked at early L3 stage. Induction of vulval fate is expressed as the percent of individual VPCs (P3.p to P8.p) dividing more than one time. Average refers to the average number of VPCs induced per animal.

P values were calculated using t-Student test for average VPC induction and z statistics for all other proportions.

[a]Muv is expressed as the percent of animals with ectopic pseudovulvae.

[b]Significantly different from the *let-60*^{gof}; *sur-8*^{rf} strain ($P < 0.001$).

[c]Significantly different from the *let-60*^{gof}; *sur-8*^{rf} strain ($P < 0.005$).

[d]Significantly different from the *let-60*^{gof};*sur-8*^{rf} strain expressing the *SHOC2*^{wt} transgene ($P < 0.001$).

Supplementary Table 4. Phenotypes in *C. elegans* resulting from expression of the *SHOC2^{wt}*, *SHOC2^{S2G}* or *myr::SHOC^{wt}* transgene.

Genotype	Transgene	*N*	Pvl	*N*	Egl	*N*	Bag
Wild type	none	56	1.8	25	19.2	20	5.0
Wild type	*SHOC2^{wt}*	44	0	25	16.2	20	5.0
Wild type	*SHOC2^{S2G}*	96	17.7[a]	25	26.3[a]	20	55.0[d]
Wild type	*myr::SHOC^{wt}*	49	36.7[b]	25	25.5[c]	20	45.0[e]

Animals were heat-shocked at early L3 stage. *SHOC2^{wt}* indicates *hsp16.2::SHOC2^{wt}::V5*, *SHOC2^{S2G}* indicates *hsp16.2::SHOC2^{S2G}::V5*, *myr::SHOC2^{wt}* indicates *hsp16.2::myr::SHOC2^{wt}::V5*.

N indicates the number of animals scored. Pvl is the percent of animals with a protruding vulva. Egl is the average number of eggs per worm contained in the uterus. Bag is the percent of bag-of-worms animals 6 days post fertilization.

[a-e]Significantly different from *SHOC2^{wt}* ([a]P <0.01; [b]P <0.0001; [c]P <0.05; [d]P <0.005; [e]P <0.02).

Supplementary Table 5. Phenotypes observed in *C. elegans let-23*^{rf} mutants after expression of the wild-type or mutant *SHOC2* transgene.

Genotype	Transgene	*N*	Bag
wild type	none	24	0
let-23^{rf}	none	93	84.9
let-23^{rf}	*SHOC2*^{wt}	92	85.9
let-23^{rf}	*SHOC2*^{S2G}	93	95.7[a]

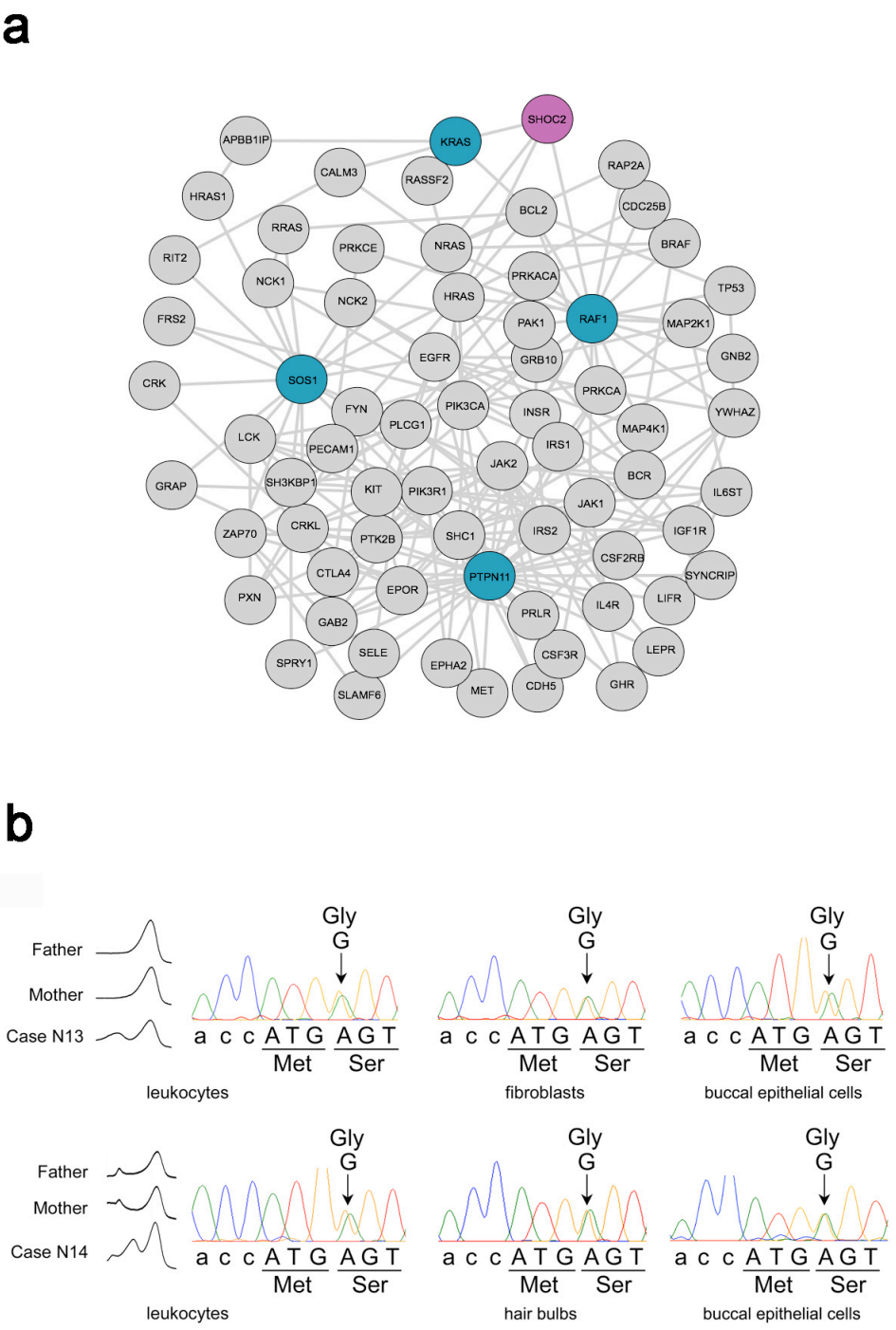
let-23^{rf} indicates *let-23(sy1)*, *SHOC2*^{wt} indicates *hsp16.2::SHOC2*^{wt}*::V5*, *SHOC2*^{S2G} indicates *hsp16.2::SHOC2*^{S2G}*::V5*.

Worms were heat-shocked at early L3 stage.

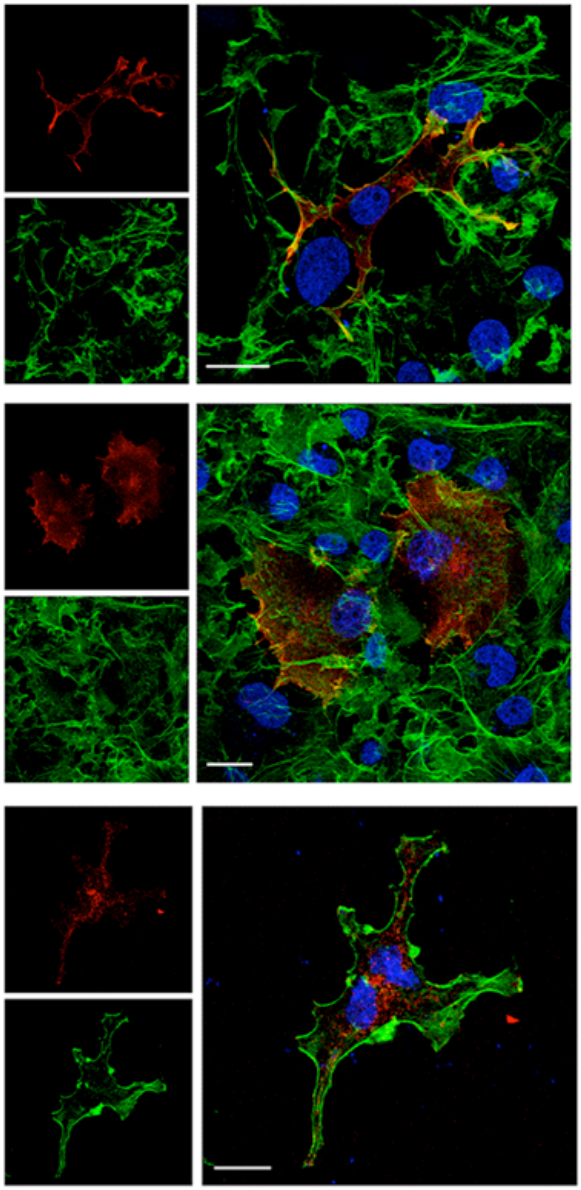
N indicates the number of animals scored. Bag is the percent of animals that become a "bag-of-worm" 4 days post-fertilization.

[a]Significantly different from the *let-23*^{rf} strain and the *let-23*^{rf} strain expressing *SHOC2*^{wt} (P <0.05 in both comparisons).

Supplementary Figure 1. *SHOC2* is mutated in Noonan-like syndrome with loose anagen hair. (a) Mammalian protein-protein interaction network analysis identified *SHOC2* as the best disease gene candidate for Noonan syndrome. The network was constructed by connecting proteins known to be mutated in Noonan syndrome (cyan) through two nodes/three links using the human interactome. SHOC2 is shown in magenta. Connections indicate protein-protein interactions. Leading candidates and Z scores are reported in Supplementary Table 1. (b) Germline origin of the disease-causing 4A>G missense change in *SHOC2*. DHPLC profiles showing the *de novo* origin of the 4A>G nucleotide change in two affected subjects, and electropherograms documenting the heterozygous condition for this mutation at codon 2 in peripheral leukocytes, skin fibroblasts, buccal epithelial cells and/or hair bulb cells from these individuals.



Supplementary Figure 2. Myristoylation is required for membrane targeting of the SHOC2^{S2G} mutant. V5-tagged SHOC2^{S2G} is membrane-targeted in untreated Cos-1 cells (top), while membrane targeting is progressively lost in cells treated with an NMT inhibitor (200 μM, middle; 300 μM, bottom). Confocal laser scanning microscopy was performed using anti-V5 monoclonal antibody, followed by Alexa Fluor-594 goat anti-mouse antibody (red), while actin cytoskeleton was detected by Alexa Fluor 488-phalloidin (green). Nuclei are visualized by DAPI staining (blue). Co-localization areas were detected in yellow. Images represent single optical sections representative of > 50 transfected cells observed in each experiment. Bars indicate 20 μm.



Supplementary Figure 3. Subcellular localization of the V5-tagged SHOC2^{S2A} proteins expressed in Cos-1 and Neuro2A cells, basally and following EGF stimulation. SHOC2^{wt} and SHOC2^{S2A} are uniformly spread in the cytoplasm and nucleus in starved cells (upper left and lower left, respectively; panel a: Cos-1 cells, panel b: Neuro2A cells), and are restricted to the nucleus following stimulation (upper right and lower right, respectively; panel a: Cos-1 cells, panel b: Neuro2A cells). Confocal laser scanning microscopy was performed using anti-V5 monoclonal antibody, followed by Alexa Fluor-594 goat anti-mouse antibody (red), while actin cytoskeleton was detected by Alexa Fluor 488-phalloidin (green). Nuclei are visualized by DAPI staining (blue). Images represent single optical sections representative of > 50 transfected cells observed in each experiment. Bars indicate 20 μm (Cos-1 cells) or 10 μm (Neuro2A cells).