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Supplemental Data

SMOC1 Is Essential for Ocular and Limb Development in Humans and Mice

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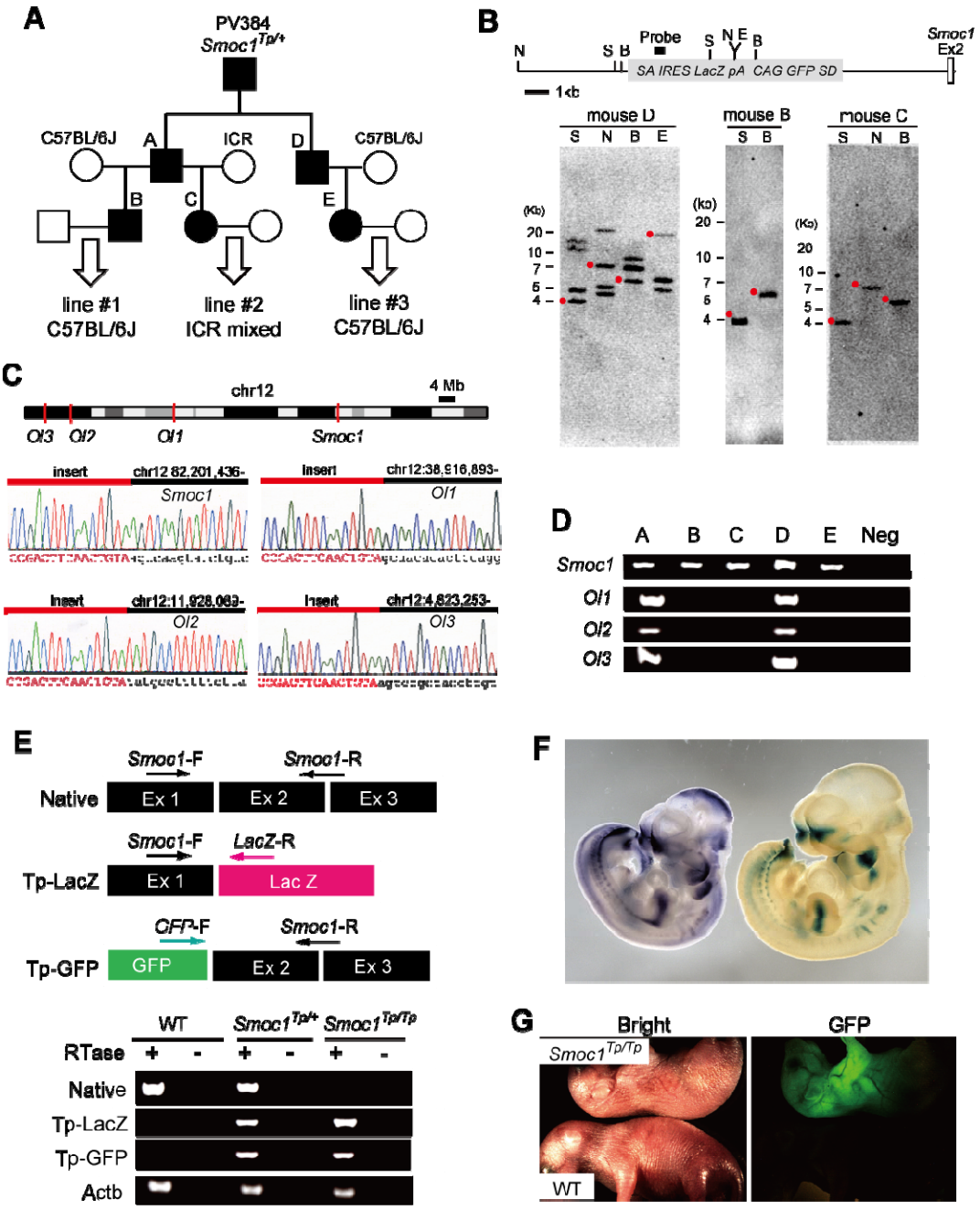

Figure S1. Characterization of PV384 (*Smoc1* Mutant) Mice

(A) Schematic representation of PV384 mouse lines. Mice heterozygous for a *Smoc1* gene-trap insertion are indicated by filled symbols. (B) Southern hybridization analysis of PV384 mice. (Top) A partial restriction map (N, *Nde*I; S, *Sac*I; Bg, *Bgl*II; E, *Eco*RI) and the position of the probe for Southern hybridization are indicated. SA, splice acceptor; IRES, internal ribosome entry site; pA, poly(A); GFP, green fluorescent protein; SD, splice donor. (Bottom) Southern hybridization of the probe on the genomic DNA of mice D, B and C. While mouse D showed three to four bands, mice B and C showed only one band corresponding to the DNA fragment containing the *Smoc1* locus (red dots). (C) Identification of the other three loci containing gene-trap insertions (*Ol1* to *Ol3*). Mouse chromosome 12 ideogram and the four loci are indicated with red bars (top). Electropherogram of flanking genomic sequences are shown (bottom). (D) PCR genotyping to detect gene-trap insertions at four different loci. Note that the three lines (#1 to #3) were derived from mice (B, C and E) which had a single insertion at the *Smoc1* locus. Neg, no template PCR. (E) Confirmation by RT-PCR of native *Smoc1*, promoter-trapped (Tp-LacZ) and poly(A)-trapped (Tp-GFP) transcripts. The native *Smoc1* transcript was detected in WT and $Smoc1^{Tp/+}$ embryos, but was undetected in $Smoc1^{Tp/Tp}$ embryos, indicating that $Smoc1^{Tp/Tp}$ is null for *Smoc1*. Promoter-trapped and poly(A)-trapped transcripts were detected in both $Smoc1^{Tp/+}$ and $Smoc1^{Tp/Tp}$ mice. *β-actin* (*Actb*) was used as an internal control. (F) LacZ staining of heterozygous embryos (right) shows a similar pattern to that of *Smoc1* expression (left) in the limbs, optic nerve, pharyngeal arches and somites. β-galactosidase activity in whole embryos was detected as previously described (Hogan, B.L., Beddington, R., Constantini, F. & Lacy, E. *Manipulating the Mouse Embryo: A Laboratory Manual, 2nd edn.*, (Cold Spring Harbor Laboratory Press, New York, 1994). (G) Comparable bright-field and fluorescence photographs of GFP-positive (top, $Smoc1^{Tp/Tp}$) and GFP-negative (bottom, WT) newborns

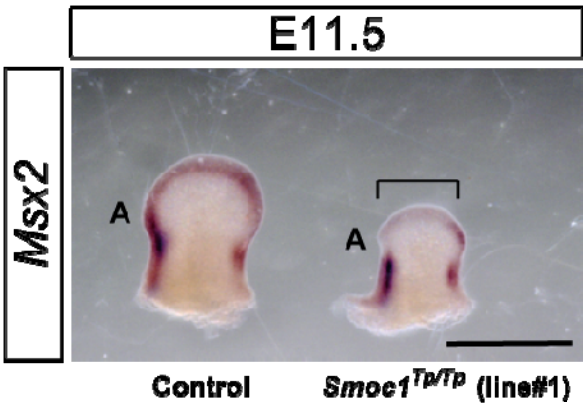

Figure S2. Reduced Expression of *Msx2* in Hindlimbs of *Smoc1* Mutant Mice

Whole mount *in situ* hybridization at E11.5. Dorsal view of the right hindlimbs is presented. Anterior side is indicated by A. Expression of *Msx2* was reduced in progressive zone of hindlimbs of *Smoc1*$^{Tp/Tp}$ mice (bracket). Scale bar, 1 mm.

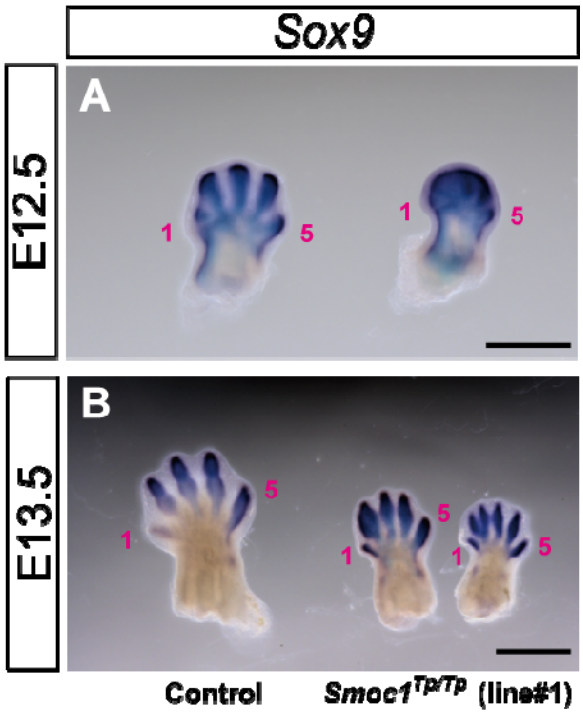

Figure S3. Delayed and Altered Expression of *Sox9* in Hindlimbs of *Smoc1* Mutant Mice

Whole mount *in situ* hybridization of right hindlimbs at E12.5 (A) and E13.5 (B). Future digit identities are indicated by numbers 1 (thumb, anterior) and 5 (little finger, posterior). (A) Expression of *Sox9* at E12.5 was delayed in hindlimbs of *Smoc1*$^{Tp/Tp}$ mice compared with that of control mice, suggesting a delay of limb development. (B) Comparted with control mice (left), expression of *Sox9* in hindlimbs of *Smoc1*$^{Tp/Tp}$ mice at E13.5 showed abnormally thick cartilage condensation in future digit 2 (middle) or extra numbers of cartilage condensation (right), suggesting limb patterning defects. Scale bar, 1 mm.

Table S1. Phenotypes of *SMOC1/Smoc1* Mutations in Humans and Mice

	A-II-2	A-II-3	C-II-3	X-II-1	***Smoc1*** $^{Tp/Tp}$ **mice**
origin	Okinawa, Japan	Okinawa, Japan	Lebanon	Turkey	
consanguinity	–	–	**+**	**+**	
sex	male	female	male	female	
ocular abnormality	**+**	**+**	**+**	**+**	+
anophthalmia	bilateral	bilateral	bilateral	bilateral	small eye
loss of optic nerve (CT)	bilateral	bilateral	bilateral	nc	+ (aplasia/hypoplasia)
loss of optic tract (CT)	+	+	–	nc	ND
upper limb abnormality	**+**	**+**	**+**	+[a]	+
syndactyly	–	–	–	+	+
metacarpal synostosis	4th and 5th fingers	4th and 5th fingers	–	4th and 5th fingers	–
hypoplasia	–	–	–	5th finger	–
coalition of capitate and hamate	–	–	–	+	–
clinodactyly	**+**	–	**+**	+	–
camptodactyly	**+**	–	**+**	–	–
simian crease	**+**	**+**	–	+	ND
lower limb abnormality	**+**	**+**	**+**	**+**[b]	+
oligodactyly / syndactyly / polydactyly	bilateral oligodactyly	bilateral oligodactyly	bilateral syndactyly	bilateral oligodactyly	syndactyly
metatarsal synostosis	**+**	**+**	–	–	+
bowed tibia	**+**	+ (mild)	–	–	+
hypoplastic fibula	**+**	+ (mild)	–	+	+
abnormal cleavage between toes	1st and 2nd toes	1st and 2nd toes	1st and 2nd toes	–	–
dermal syndactyly	2nd and 3rd toes	2nd and 3rd toes	2nd to 5th toes	–	+
pes valgus	**+**	–	–	–	+
other					
congenital malformation of palate	–	–	–	+ (high arched)	+ (cleft palate, in line#2)
failure to thrive	**+**	**+**	+	+	+ (growth retardation)
developmental retardation	DQ=10	DQ=15	+	–	ND
cryptorchidism	right		nc		ND
sacral dimple	nc	nc	nc	+	ND
SMOC1 **mutation**	**c.718C>T**	**c.718C>T**	**c.664+1G>A**	**c.378+1G>A**	**gene trapping**

CT, computed tomograpy; DQ, developmental quotient nc, not confirmed ND, not determined

a, 5th metacarpal in the left hand is absent

b, distal phalanges of the 4th toe on both feet are absent

Table S2. Marker Primers for Chromosome 14 Mapping

Marker	Forward (5' > 3')	Reverse (5' >3')	Fluorescence	Product size (bp)
D14S70	ATCAATTTGCTAGTTTGGCA	AGCTAATGACTTAGACACGTTGT	VIC	214
D14S288	AGCTAGACTCTGCCATAAACA	TGGAGACAGGAACAACACAC	NED	203
D14S276	TGCTTTACCAAGTGCATCAC	AGCTCAGAATCTAGGCCCT	NED	90
Ch14-STS1	GCCCTGGAGCATCTTGTAGT	GTTTCAGGTTTGGCCATGAG	FAM	162
D14S63	GGCCAGGTTTCAATCAGTTT	GCCAGAGAGCCACACTGTAT	VIC	205
AFMA346YG1	AAGAGACTGACATAGCCAGTT	CCGAGATACAAACATGGA	NED	112
Ch14-STS2	TTTTCATATTTTTGAGAGTTTTAGG/	GCTGGCGAAAAGACAAGATT	NED	288
AFM114YH10	TGTTCTAGTTGATGTGAGACTT	TATTTGAGGACCTGCTGTAA	FAM	216
AFMA064ZH5	TGGATTGTTTGCTCTCAGAT	TAATGTCACTGCCTGGGA	FAM	261
AFMB315YF5	CTGGGCAGTGACTCTAGGAGAC	GGGAATACAGTGTCCAATGACC	VIC	196
Ch14-STS3	TGCTTCAAACCTTGCCTCTT	CCCTGCTTTGTCACCTCTTC	VIC	243
CHLC.GGAA4A12	GCCGAAAGAAAGAAAAAAGG	CGAATGCATACTTGCTGTTG	VIC	120
D14S258	TCACTGCATCTGGAAGCAC	CTAACTAAATGGCGAGCATTGAG	FAM	176
AFMA336YC5	AGATTTTGGATGTATCAGGC	CAGAAGCAATAGGATGGATG	NED	168
Ch14-STS5	TTATGCAACCATAGCCTTTGC	GAGGTTGAGCAAGACCCTGT	NED	201
Ch14-STS6	CCCACATCCAACACTGAGAA	CCTTCCCTCTGTGTCCTCAC	VIC	215
Ch14-STS7	CTCCCTTGATGTGTGAAGCA	TTTTCAACACCACCACCAGA	NED	218
AFM295ZD5	TTGCTTTCACTCCCCATT	TGCACTTGAAGATTCAGATAAGG	FAM	152
Ch14-STS4	GGCCAACATGATGAAACCC	AAGGCTCAGCAAGAAGAAACTC	FAM	355
AFM184XA5	GACTGAGGCTCAAGGATTGC	CTTCCACTAATGGCGAGGAA	VIC	250
D14S74	CCTGTACCACTACCTGAGTTGAGT	CTTTGGCTGCCCGAAA	VIC	304

Table S3. Common Candidate Regions in Any Three of the Four Families

Chr	Physical position	size	SNP numbers	LOD scores					
				A	B	C	X	All families	3 families
5	44228425-45740067	1,511,643	17	0.852	1.164	-2.935	1.453	0.534	3.469
5	57974102-58367038	392,937	19	0.852	1.075	-0.474	1.453	2.907	3.380
5	61832737-62244988	412,252	13	0.852	1.150	-0.478	1.453	2.978	3.455
6	8431193-8722149	290,957	21	0.977	1.041	1.683	-0.847	2.854	3.701
6	25928376-27047713	1,119,338	10	0.977	1.176	1.790	-0.845	3.098	3.943
6	33478496-34613887	1,135,392	12	0.977	0.929	1.582	-0.843	2.645	3.488
6	123015089-123893054	877,966	17	0.977	1.177	-1.714	1.414	1.854	3.568
7	9174771-9431031	256,261	18	0.977	1.174	1.804	-6.996	-3.041	3.955
7	14738170-14997102	258,933	13	0.977	0.947	1.698	-0.845	2.776	3.621
10	16851432-17381572	530,141	18	0.977	1.183	-6.438	1.454	-2.825	3.613
10	17704372-24780906	7,076,535	151	0.977	1.183	-27.00	1.454	-23.40	3.613
10	28006811-28197289	190,479	6	0.977	1.183	-Inf	1.454	-Inf	3.613
10	28305685-28541472	235,788	14	0.977	1.183	1.829	1.454	5.442	
10	28633450-29361379	727,930	56	0.977	1.183	1.829	-3.742	0.247	3.988
11	48058313-48987539	929,227	6	-8.150	1.103	1.828	1.343	-3.876	4.275
12	43151728-43514937	363,210	22	-4.025	1.183	1.626	1.162	-0.054	3.971
14	68275342-71054478	2,779,137	63	0.977	-2.713	1.828	1.131	1.223	3.936
14	71220216-71295001	74,786	2	0.977	0.437	1.828	0.015	3.257	
14	71412340-71658253	245,914	6	0.977	0.437	1.828	-6.865	-3.623	3.243
15	58696863-58853363	156,501	10	-Inf	0.817	1.798	1.450	-Inf	4.065

Gray highlighted: previous candidate region on 10p12.33-p11.23 (Hamanoue, H. et al., Am J Med Genet A, 2009)
Green highlighted: the region analized in this study

Table S4. Analysis of the Splice Site Predictions of the Two Mutations

		ESEfinder3.0 (score)	NetGene2 (confidence)	HSF 2.4.1[a] (value)	SpliceView (score)	BDGP[a] (score)
c.378+1G>A	reference	11.9514	0.67	96.91	92	0.99
	mutation	<6.67 (under threshold)	under threshold	70.07	under threshold	<0.40 (under cutoff)
	assessment	***abolished***	***abolished***	***site broken***	***abolished***	***abolished***
c.664+1G>A	reference	9.8861	0.75	87.83	81	1.00
	mutation	<6.67 (under threshold)	under threshold	61	under threshold	<0.40 (under cutoff)
	assessment	***abolished***	***abolished***	***site broken***	***abolished***	***abolished***

[a]Human Splicing Finder Version 2.4.1

[b]Berkeley Drosophila Genome Project