Supplemental Dataset

Cerebral cavernous malformations arise independent of the Heart of Glass receptor

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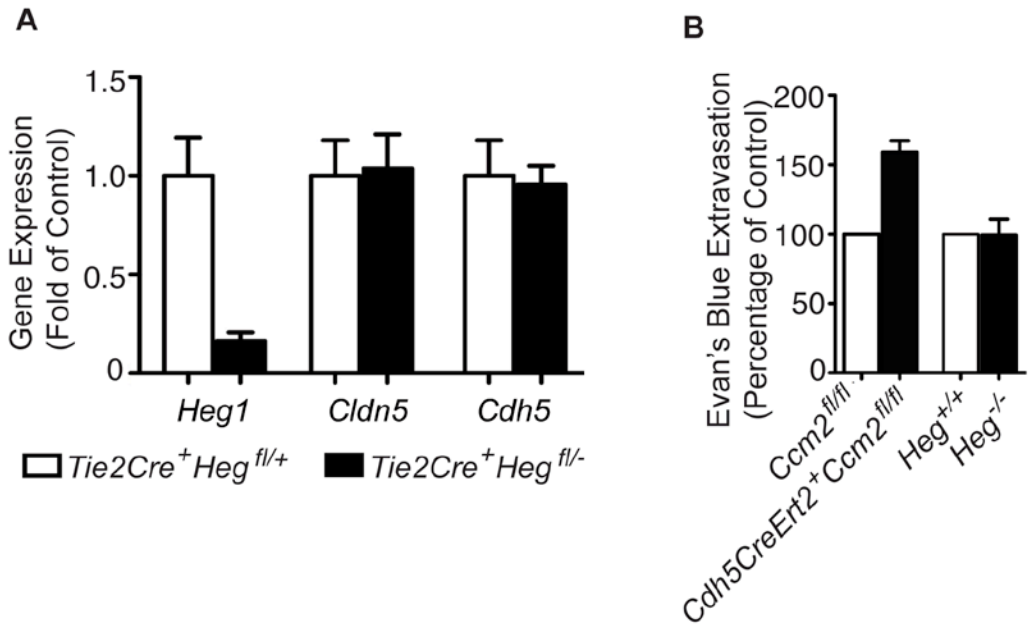
Supplemental Figure I. *Heg* deficiency does not alter endothelial junction protein expression in endothelial barrier function in vivo. (A) *Heg*-deficiency does not alter the expression of junction proteins in freshly isolated lung endothelial cells as measured by quantitive PCR. (B) Vascular permeability is increased in adult mice with *Ccm2* deficiency (Cdh5-CreERT2;$Ccm2^{fl/fl}$), but *Heg* deficiency ($Heg^{-/-}$) as measured by extravasated Evans blue dye.



Supplemental Table I. Primer sequences

Genomic DNA PCR / sequence Primers

exons	forward primer	reverse primer
1	GCCTCACTCCGCTCACTT	CTCAGTCCAGCCAACTGC
2	GCAATGGCACGTTCTTGG	GGCACAATGGCTCAGAAT
3	TCTGATACTTACCTCCAGTGAGC	GCTGGCTATGCACATTGATT
4	GAGAGTGCAGGCATGTGTC	GATGGAACCAAGAACCCTGA
5b*	CAAGGGCAACAGACATCTCA	GCAAACACTATCCCAAATTCC
6(a)	CAATGTCTTTATTCAGAGTGCTCA	GCCAGTGACCAGAGATGCT
6(b)	CCACAAGCACCAACTTAGCA	CTGATCCAGACGCAGATACC
7-8	GGCCTTTCAGGGCAAGTA	TGCAAAGTAGGGTGTTCTATCTC
9	GAGATGTGCAAACACACAGC	GTGAAGAGCCCAGGAGACAC
10-11	AGGACACCACTTCTGTGCAA	TCTTTGACTTAACACCCATGC
12	AGGTTTGAAGTGGAGAAGGTT	GTCACGCCTAAGCAAAGACA
13-14	TAGGGTGGCTGGCGTTTAAT	GGTGTGAACTGAGGGAAGTG
15	AGGCAGGGTGGTGACTGTA	AAAGGAACGGGATTGGAAC
16	TGTAGAAGAGTCTAAATCTGCCCTAT	AAATCAGAGGTTCATGCCACT
17	AGTTGCCTTCTGCATGGTT	CTTCCTGCTTGCCACTCA

cDNA PCR / sequence Primers

exons	forward primer	reverse primer
2-4	CAGCTCTGACTTCCCAGAGT	GGCCAGTGGAGTTGTTAAGC
3-6	CCATCCAGCAGTTCAGAGTT	CCACGCACTTGACCGTAG
5-6	GACCAACAGCACAACATCTG	CATCCACAATCACTCGGTTC
6-11	CAGCCACAAGCACCAACTTA	GCAGAGGACTTGCAGGAGTT
8-17	CACAGCCATGTGCAACAATA	CTTCCTGCTTGCCACTCA

QMPSF primers

exons	forward primer	reverse primer
1	GCGGCTCCCGATAACTTTT	GGCGAGGCCATGGTGAC
2	TCAGATGCTGCCTGGAAACA	CTCTTTGGAGGACACTGTTGAAA
3	GAAAGTCTTCACCTGCCATCCA	TCCCATCTCCGAGGCTGTT
4	CCTCAGTCCTTCTTTCACCCTCT	GGAATCATTTTGCCAACGCA
5	CTTACAGCCTTTGGAGAACATCA	GGGTGCGACTGACCTAGACAC
6	CATTACAGAATCAAAGTCACCAAGC	CTTGTGGCTGGAAGGGTTTG

7	AGCTGCCTTTACATTTCCTGTG	GGCTGGTGTTGTCTGCGAC
8	ATGTGAATGAGTGCCTGTCGA	CGGAATAAAGTCCGAAATAGACTAA
9	GAGAACTTTTCTTAATACAACTGTGGAA	GCAGTCCCCTCTTGGAATAAAAT
10	ATCCGATCTACAGTTCACGCCT	CACCCATGTTAGAACAGCCTCC
11	TGCAAACAACCTTTTCCCTG	CAAGAGCTGGCAGACCTCAG
12	AGTCCCGAATGTGACAAAGACAC	TGGTCCATCTTGTTGAACTGAAA
13-14	ATAGGCTTGAAAATGAAACCTGC	CATAATGGTCCACTTTTGTACACTTAC
15	AGGCATCGCACTGATTGTTACC	TTGTGAACCATCTCAAGTATCCTGTA
16	CACAAGAATGGGGCCGAGA	CCCTGGGGTCATCCTGACA
17	TCCTCGCTCACAAGAATGGG	TCCTTTACTTCTCAGAGGAACCG

Supplemental Table II. Frequency of CCM formation in the mice lacking HEG and CCM2.

Genotypes	n	% with CCM
TieERT2^{+}Ccm2$^{fl/fl}$ (P18-21)	4	100
TieERT2^{+}Ccm2$^{fl/fl}$ (3-7mo)	3	100
Heg$^{-/-}$ (P18-21)	20	0
Heg$^{-/-}$ (3-8 mo)	23	0
Heg$^{-/-}$ (9-20 mo)	11	0
TieERT2^{+}Heg$^{fl/fl(-)}$Ccm2$^{fl/+}$ (P18-21)	16	0
TieERT2^{+}Heg$^{fl/fl(-)}$Ccm2$^{fl/fl}$ (P18-21)	6	100

Supplemental Table III. Patients and biological materials

Family number	Sporadic/Familial	RNA source
C036	Familial	LCL
C046	Familial	LCL
C053	Sporadic	LCL
C056	Sporadic	LCL
C057	Sporadic	LCL
C064	Sporadic	LCL
C080	Sporadic	LCL
C082	Sporadic	LCL
C083	Sporadic	LCL
C099	Sporadic	LCL
C108	Sporadic	LCL
C129	Sporadic	None
C130	Sporadic	LCL
C133	Sporadic	LCL
C140	Sporadic	None
C142	Sporadic	LCL
C153	Familial	PBMC
C155	Sporadic	None
C157	Sporadic	None
C169	Sporadic	None
C183	Sporadic	PBMC

LCL : Lymphoblastoid cell line ; PBMC : Peripheral blood mononuclear cell

Supplemental Table IV. SNPs in *HEG1* gene detected in patients

SNP		Allele frequency in CCM patients (%)	SNP present in dbSNP137	MAF[1] in 1000g[2] (%)	MAF in EVS[3] (%)
c.69_70insATG / p.L23_P24insM		21.4	No	No	16,8
c.92 G>A ; p.R31Q	rs186106404	9.5	Yes	5.3	No
c.303 A>C ; p.R101S	rs62265663	23.8	Yes	15.6	No
c.423 C>T ; p.G141=	rs2860440	47.6	Yes	48.8	46
c.615A>G ; p.S205=	rs4404487	45.2	Yes	26,6	35
c.780 G>T ; p.P260=	rs2333041	52.4	Yes	49.2	48.7
c.913T>C ; p.S305P	rs2981546	47.6	Yes	47.6	45.8
c.940A>G ; p.N314D	rs150448589	2.4	Yes	0.1	0.3
c.996C>T ; p.A332=	rs6438874	52.4	Yes	49.8	48,2
c.1587 G>A ; p.S529=	rs59232004	47.6	Yes	48.9	46
c.1805T>C ; p.F602S	rs6790837	47.6	Yes	44.4	42.4
c.1975_1992dup ; p.S667_S672dup	rs376430192	7.1	Yes	No	0.2*
c.2734A>T ; p.T912S	rs78680419	7.1	Yes	26.1	15.9
c.2938G>C ; p.V980L	rs10804567	42.8	Yes	27.4	37.5
c.3116T>C ; p.M1039T	rs6438869	35.7	Yes	35.7	37
c.3882G>A ; p.P1294=	rs2270778	26.2	Yes	29.6	33

[1] MAF : Minor Allele Frequency
[2] 1000 genome data base
[3] Exome Variant Server database
* insertion at the same position of 5 serines. This polymorphism is in a polySer repeat.