SUPPLEMENTAL INFORMATION

Regulation of Angiogenesis by Histone Chaperone HIRA-Mediated Incorporation of Lysine 56-Acetylated Histone H3.3 at Chromatin Domains of Endothelial Genes

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Supplemental Table 1: *Oligonucleotides used for RT-PCR and ChIP analyses.*

Primers used for RT-PCR Analysis

Genes	Forward sequence (5'-3')	Reverse sequence (5'-3')
VEGFR1	CGAAGAAAAAGAAAGCGGAATC	TGGGTATGTCAGTGTGCATCTCTA
HIRA	CAGGAGGATGACGAGAAGGA	ACTGTTTGACCACCGCACAC
CAF1-p150	GCACCTTCCTCAGTGACCTAAA	CACCTTGCTGCTCTCCACA
CBP	GGTCAATAGTTTACCTGCTTTTCCT	CCTGTTGCAATTGCTTGTGT
Sphk1	GGCTCTGCAGCTCTTCCA	CTCCCTGGCATGGTTCTTC
Ereg	CATGGCCAGTGCATCTACCT	AGTGCTCACATCGCAGACC
Fgf1	CGAAGTGTATATAAAGGGTACGGAGA	GAACAGACATTCCTCATTTGGTG
PlGF	GCCGATAAAGACAGCCAACA	TGAGAAAATGTCATCTCCACATAGAA
CCl2	CAGCAGGTGTCCCAAAGAAG	TTCCGATCCAGGTTTTTAATGT
Efnb2	TTTCAAGAATTCAGCCCTAACC	TATCCAGGCCCTCCAAAGA
Plxdc1	CAACCATAACTACTACGTGTCCC	AGGTCCACCCACAGATCCTG
Pdgfa	GAGGAAGCCGAGATACCCC	TGCTGTGGATCTGACTTCGAG
Cxcl1	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
Cxcl5	TCCAGCTCGCCATTCATGC	TTGCGGCTATGACTGAGGAAG
Polr2a	CGAATCCGCATCATGAACAG	TGCATCGCAGGAAGACATCA
Rpl27	GGACGCAAAGCCGTCATC	GGTCAATTCCAGCCACCA
Gapdh	TGCCCCCATGTTTGTGATG	TGTGGTCATGAGCCCTTCC

Primers used for ChIP Analysis (Promoter regions)

Genes	Forward sequence (5'-3')	Reverse sequence (5'-3')
VEGFR1	TGACGTCACTGGAAGGAGGTG	AGGACCTCGCTGAAGTGCA
Sphk1	ACCTCCCAATCCTTCCAGAG	CGGGAATGGACAAGAACAAG
Ereg	GGGGGTGGCATTAGGAAA	CCTTTATAAGTCTGGGGAGGTG
Angpt1	CAGCAAGCACACCCGAAC	CCTTAGCAGATGCACGGAAA
Fgf1	TGCAGACTGTGAAGAGCTAGAGG	CCACATCAGTGCACAACAAA
PlGF	CATCTCCAAGGCAGATGTTG	CGGAGCTAGGCGTTCATTAG
CCl2	GCAGCCAGAAGTGCAGAGAG	TCAGTGAGAGTTGGCTGGTG
Efnb2	GTGCTCTCCTGACTGCTTAGTG	CTGGAGCGCAGAGATAAAGG
Pdgfa	AGACAGGTGATCGGTGGTG	CGTCGCTGGCTTTAGGAG
Plxdc1	ACACCAGGAAGCCACAGAAG	CACCACCCATCCCAAGAG
Cxcl1	TGGAGTTTCGAGCATAAAAGG	TGGAGTGCTGGAACTGGTTAG
Cxcl5	CCTGCCTGAAGGAAGAGAGA	TGGAGGAGGTGTGGAGATTG
Tie-2	TGAAGGGCAAGATGGATAGG	GCAGATTAGGATGGGAAAGG
Polr2a	GCGAATCTATAAAGGGCGTCACT	TCGGCGCTTCTGAGGAGA
Rpl27	GGGGACTACAGCAATGAAGG	GAGGGAGGAGCGAGGATC
Necdin	GGTCCTGCTCTGATCCGAAG	GGGTCGCTCAGGTCCTTACTT

Primers used for measuring H3acK56 incorporation at other regions of mouse *Vegfr1* locus is available on request.

Supplemental Table 2: *Fold Changes in mRNA expression of angiogenic genes in starved YSECs and HUVECs after they were treated with FGF2/EGF for 3 h. Genes that are induced in both cell types are underlined.*

YSEC		HUVEC	
Gene	Fold Change	Gene	Fold Change
Ereg	48.24697771	*FGFR3*	21.52786484
Sphk1	35.67067637	***PLXDC1***	12.25784391
Lep	27.09710212	*VEGFC*	10.26835613
Vegfr1	16.27365794	*IL8*	9.516514907
Ctgf	12.15743242	***FGF1***	8.388037628
Pgf	11.17936754	*BAI1*	6.492316502
Ccl2	10.59643285	***EREG***	6.291652706
Thbs1	9.657613092	***CXCL1***	4.834773236
Cxcl1	7.469784729	***SPHK1***	4.147830627
Tgfa	4.936767379	*JAG1*	3.856877523
Efnb2	4.164567473	***CXCL5***	3.610000312
Fgf1	3.515833297	***VEGFR1***	3.565337851
Cxcl5	2.807839582	***PDGFA***	3.313218463
Csf3	2.793098916	*CXCL6*	3.256976552
Plxdc1	2.788892466	*IL6*	2.881526591
Pdgfa	2.546456944	***EFNB2***	2.600622765
Tgfb1	2.40105203	*CXCL3*	2.31679972
Mmp19	2.054612983	*HPSE*	2.282687445
Itgav	2.029278206	*IFNA1*	1.92283282
Plau	2.009820512	*TGFA*	1.879349429
Col18a1	1.902856367	*ANGPT2*	1.827705129
Gna13	1.808716964	*THBS2*	1.636976185
Lect1	1.807839582	*IGF1*	1.486926498
Mmp9	1.807839582	*ID1*	1.36986463
Pecam1	1.807839582	*CXCL9*	1.345957945
Plg	1.807839582	*TGFB1*	1.293278813
Tnf	1.807839582	*TYMP*	1.290681769
Itgb3	1.776905142	*CXCL10*	1.276535212
Angpt2	1.691691696	*TIMP1*	1.264207548
Mapk14	1.485621569	*S1PR1*	1.255039989
Nrp1	1.368852186	*ITGB3*	1.22300757
Stab1	1.357325436	*IL1B*	1.154712211
Tgfbr1	1.302846093	*CCL2*	1.151179336
Nrp2	1.271707311	*MDK*	1.120259789
Lama5	1.211812646	*TEK*	1.117777727
Kdr	1.141843246	*ENG*	1.058068721
Eng	1.130426089	*ID3*	1.047778187
Timp2	1.067966702	*ITGAV*	1.023728628
Ptgs1	1.015835408	*PF4*	0.98762368
Tnfaip2	0.983479351	*PROK2*	0.975716096
Efna1	0.936380416	*PTGS1*	0.942544954
Tmprss6	0.887037294	*ANPEP*	0.914042615
Egf	0.79640085	*COL18A1*	0.901708692
Epas1	0.736862156	*NRP1*	0.870570678
Tgfb2	0.713045808	*THBS1*	0.870389666
Vegfa	0.705034943	*HIF1A*	0.860849733
Npr1	0.66853114	*CDH5*	0.85787142
Fgf2	0.659510104	*EPHB4*	0.854311041
Jag1	0.617909161	*PECAM1*	0.846882271
Smad5	0.5942602	*Akt1*	0.818942084
Vegfb	0.545657153	*B2M*	0.817184267
Igf1	0.541024813	*TIMP2*	0.788910385

Angpt1	0.500127093	*PLAU*	0.786344461
Mdk	0.4847009	*ANGPTL4*	0.781562598
Vegfc	0.469783083	*TGFB2*	0.774444272
Hif1a	0.466861546	*LECT1*	0.749672992
Fgfr3	0.463090693	*MMP2*	0.740685671
Mmp2	0.460115087	*HAND2*	0.729830492
Ephb4	0.432468412	*IFNG*	0.729830492
Fzd5	0.40826185	*PLG*	0.729830492
Figf	0.380763387	*TIMP3*	0.729830492
Tnfsf12	0.373988721	*ANGPT1*	0.700197266
Tgfb3	0.348074171	*TNFAIP2*	0.699178794
Serpinf1	0.16807379	*STAB1*	0.688788678
Bai1	No Significant expression	*VEGFA*	0.657030994
Anpep	No Significant expression	*FGF2*	0.63289292
Col4a3	No Significant expression	*COL4A3*	0.626954796
S1pr1	No Significant expression	*NRP2*	0.603168643
Timp1	No Significant expression	*LAMA5*	0.586024682
Tymp	No Significant expression	*LEP*	0.540300279
Il1b	No Significant expression	*NOTCH4*	0.530871035
Hand2	No Significant expression	*EGF*	0.527167499
Ccl11	No Significant expression	*KDR*	0.444554849
Cdh5	No Significant expression	*TGFBR1*	0.435617354
Cxcl2	No Significant expression	*EFNA3*	0.389294356
F2	No Significant expression	*SERPINF1*	0.358695943
Fgf6	No Significant expression	*FIGF*	0.322737547
Hgf	No Significant expression	*PGF*	0.309311705
Ifng	No Significant expression	*MMP9*	0.307026127
Il6	No Significant expression	*ANGPTL3*	0.282012953
Tbx1	No Significant expression	*TNF*	0.235538261
Tbx4	No Significant expression	*EFNA1*	0.079221363
Tek	No Significant expression	*HGF*	No Significant expression
Thbs2	No Significant expression	*IFNB1*	No Significant expression
		CCL11	No Significant expression

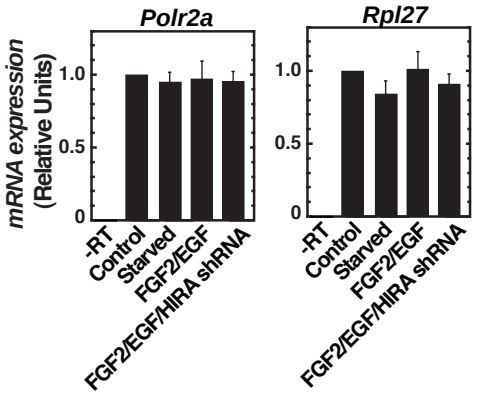

Figure S1. YSECs were infected with lentiviral vectors expressing HIRA shRNA, starved from growth factors for over night, treated with FGF2/EGF containing medium for 3h, and mRNA expression of constitutive genes ***Polr2a*** and ***Rpl27*** were measured. The plot shows relative mRNA expression levels in HIRAkd YSECS when they are starved and treated with FGF2/EGF with respect to the uninfected control YSECs.

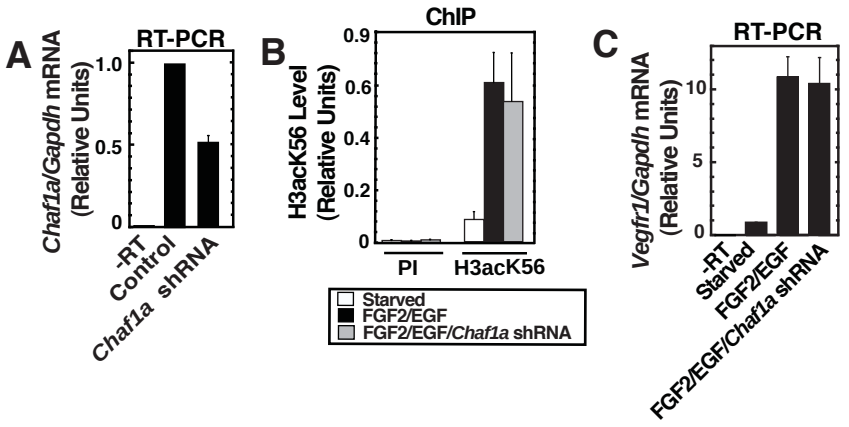

Figure S2. A, YSECs were infected with lentiviral particles expressing shRNA against the CAF1p150 (*Chaf1a*) and RT-PCR analysis were performed to determine mRNA expression with respect to the control, uninfected,YSECs. B, and C, ChIP and RT-PCR analysis, respectively, showing knockdown of CAF1p150 expression does not inhibit FGF2/EGF-induced H3acK56 incorporation and transcription at the *Vegfr1* locus.

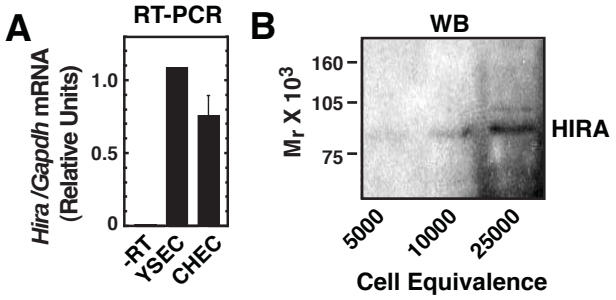

Figure S3. A, RT-PCR analysis showing Hira mRNA expression in mouse choroidal endothelial cells (CHEC) with respect to that in YSECs. B, Western blot analysis with increasing concentrations of CHEC extracts showing HIRA protein expression.