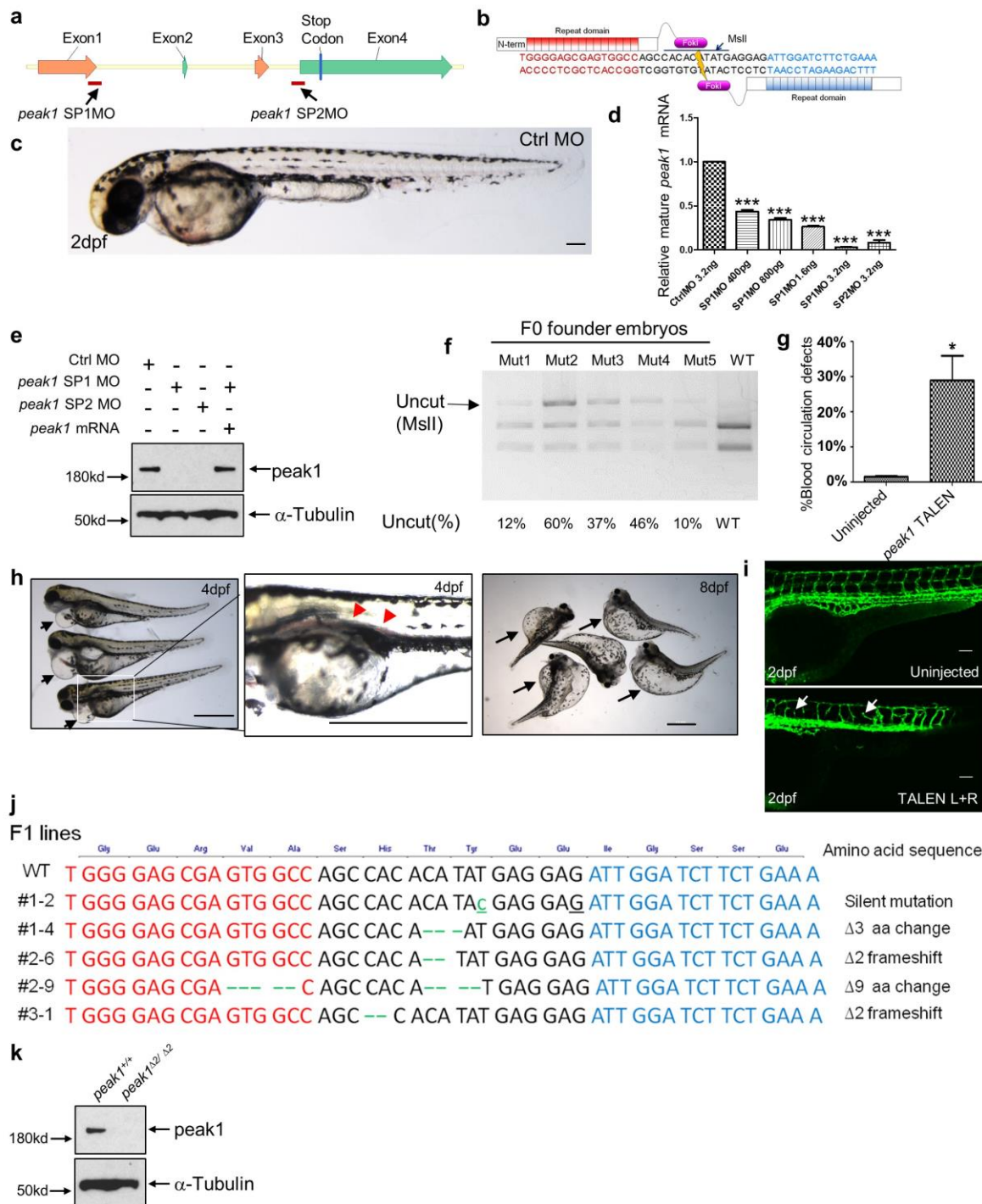


**b**

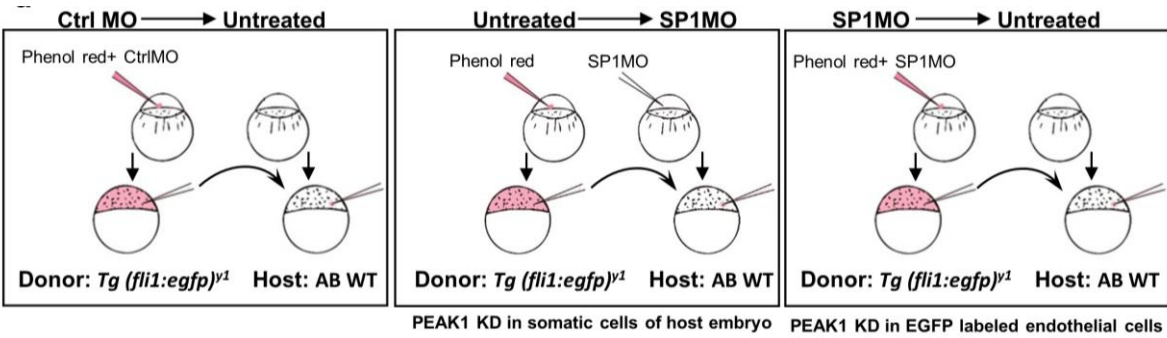
Section	Human PEAK1	Mouse Peak1	Zebrafish peak1	Consensus
Section 1	(1) MSACNFFTEVHVKGECKNCFKPKSLQLPDPPEKAPITGHVKTNAHNSHNR	(1) MSACNFFTEVHVKGECKNCFKPKSLQLPDPPEKAPITGHVKTNAHNSHNR	(1) MSACNFFTEVHVKGECKNCFKPKSLQLPDPPEKAPITGHVKTNAHNSHNR	(1) MSACNFFTEVHVKGECKNCFKPKSLQLPDPPEKAPITGHVKTNAHNSHNR
Section 2	(55) 55 60 70 80 90 108	(55) 55 60 70 80 90 108	(55) 55 60 70 80 90 108	(55) 55 60 70 80 90 108
Section 3	(109) 109 120 130 140 150 162	(109) 109 120 130 140 150 162	(109) 109 120 130 140 150 162	(109) 109 120 130 140 150 162
Section 4	(163) 163 170 180 190 200 216	(163) 163 170 180 190 200 216	(163) 163 170 180 190 200 216	(163) 163 170 180 190 200 216
Section 5	(217) 217 230 240 250 260 270	(217) 217 230 240 250 260 270	(217) 217 230 240 250 260 270	(217) 217 230 240 250 260 270
Section 6	(271) 271 280 290 300 310 324	(271) 271 280 290 300 310 324	(271) 271 280 290 300 310 324	(271) 271 280 290 300 310 324
Section 7	(325) 325 330 340 350 360 378	(325) 325 330 340 350 360 378	(325) 325 330 340 350 360 378	(325) 325 330 340 350 360 378
Section 8	(379) 379 390 400 410 420 432	(379) 379 390 400 410 420 432	(379) 379 390 400 410 420 432	(379) 379 390 400 410 420 432
Section 9	(433) 433 440 450 460 470 486	(433) 433 440 450 460 470 486	(433) 433 440 450 460 470 486	(433) 433 440 450 460 470 486
Section 10	(487) 487 500 510 520 530 540	(487) 487 500 510 520 530 540	(487) 487 500 510 520 530 540	(487) 487 500 510 520 530 540
Section 11	(541) 541 550 560 570 580 594	(541) 541 550 560 570 580 594	(541) 541 550 560 570 580 594	(541) 541 550 560 570 580 594
Section 12	(595) 595 600 610 620 630 648	(595) 595 600 610 620 630 648	(595) 595 600 610 620 630 648	(595) 595 600 610 620 630 648
Section 13	(649) 649 660 670 680 690 702	(649) 649 660 670 680 690 702	(649) 649 660 670 680 690 702	(649) 649 660 670 680 690 702
Section 14	(703) 703 710 720 730 740 756	(703) 703 710 720 730 740 756	(703) 703 710 720 730 740 756	(703) 703 710 720 730 740 756
Section 15	(757) 757 770 780 790 800 810	(757) 757 770 780 790 800 810	(757) 757 770 780 790 800 810	(757) 757 770 780 790 800 810
Section 16	(811) 811 820 830 840 850 864	(811) 811 820 830 840 850 864	(811) 811 820 830 840 850 864	(811) 811 820 830 840 850 864
Section 17	(865) 865 870 880 890 900 918	(865) 865 870 880 890 900 918	(865) 865 870 880 890 900 918	(865) 865 870 880 890 900 918
Section 18	(919) 919 930 940 950 960 972	(919) 919 930 940 950 960 972	(919) 919 930 940 950 960 972	(919) 919 930 940 950 960 972
Section 19	(973) 973 980 990 1000 1010 1026	(973) 973 980 990 1000 1010 1026	(973) 973 980 990 1000 1010 1026	(973) 973 980 990 1000 1010 1026
Section 20	(1027) 1027 1040 1050 1060 1070 1080	(1027) 1027 1040 1050 1060 1070 1080	(1027) 1027 1040 1050 1060 1070 1080	(1027) 1027 1040 1050 1060 1070 1080
Section 21	(1081) 1081 1090 1100 1110 1120 1134	(1081) 1081 1090 1100 1110 1120 1134	(1081) 1081 1090 1100 1110 1120 1134	(1081) 1081 1090 1100 1110 1120 1134
Section 22	(1135) 1135 1140 1150 1160 1170 1188	(1135) 1135 1140 1150 1160 1170 1188	(1135) 1135 1140 1150 1160 1170 1188	(1135) 1135 1140 1150 1160 1170 1188
Section 23	(1189) 1189 1200 1210 1220 1230 1242	(1189) 1189 1200 1210 1220 1230 1242	(1189) 1189 1200 1210 1220 1230 1242	(1189) 1189 1200 1210 1220 1230 1242
Section 24	(1243) 1243 1250 1260 1270 1280 1296	(1243) 1243 1250 1260 1270 1280 1296	(1243) 1243 1250 1260 1270 1280 1296	(1243) 1243 1250 1260 1270 1280 1296
Section 25	(1297) 1297 1310 1320 1330 1340 1350	(1297) 1297 1310 1320 1330 1340 1350	(1297) 1297 1310 1320 1330 1340 1350	(1297) 1297 1310 1320 1330 1340 1350
Section 26	(1351) 1351 1360 1370 1380 1390 1404	(1351) 1351 1360 1370 1380 1390 1404	(1351) 1351 1360 1370 1380 1390 1404	(1351) 1351 1360 1370 1380 1390 1404
Section 27	(1405) 1405 1410 1420 1430 1440 1458	(1405) 1405 1410 1420 1430 1440 1458	(1405) 1405 1410 1420 1430 1440 1458	(1405) 1405 1410 1420 1430 1440 1458
Section 28	(1459) 1459 1470 1480 1490 1500 1512	(1459) 1459 1470 1480 1490 1500 1512	(1459) 1459 1470 1480 1490 1500 1512	(1459) 1459 1470 1480 1490 1500 1512
Section 29	(1513) 1513 1520 1530 1540 1550 1566	(1513) 1513 1520 1530 1540 1550 1566	(1513) 1513 1520 1530 1540 1550 1566	(1513) 1513 1520 1530 1540 1550 1566
Section 30	(1567) 1567 1580 1590 1600 1610 1620	(1567) 1567 1580 1590 1600 1610 1620	(1567) 1567 1580 1590 1600 1610 1620	(1567) 1567 1580 1590 1600 1610 1620
Section 31	(1621) 1621 1630 1640 1650 1660 1674	(1621) 1621 1630 1640 1650 1660 1674	(1621) 1621 1630 1640 1650 1660 1674	(1621) 1621 1630 1640 1650 1660 1674
Section 32	(1675) 1675 1680 1690 1700 1710 1728	(1675) 1675 1680 1690 1700 1710 1728	(1675) 1675 1680 1690 1700 1710 1728	(1675) 1675 1680 1690 1700 1710 1728
Section 33	(1729) 1729 1740 1750 1760 1770 1782	(1729) 1729 1740 1750 1760 1770 1782	(1729) 1729 1740 1750 1760 1770 1782	(1729) 1729 1740 1750 1760 1770 1782
Section 34	(1783) 1783 1796	(1783) 1783 1796	(1783) 1783 1796	(1783) 1783 1796

**Supplementary FigS1.** *peak1* structure shows high conservation across species. **(a)** Comparison of amino acid homology of PEAK1 in human, mouse, and zebrafish. The percentage of identity amino acids of PEAK1 proteins in all species is 44.5%. The percentage of consensus amino acids is 92.0%. **(b)** Complete amino acid sequence homologies shown for human, mouse and zebrafish PEAK1. Yellow back= identical sequences. Blue back= identical sequences in two species. Green back= amino acid consensus sequence.

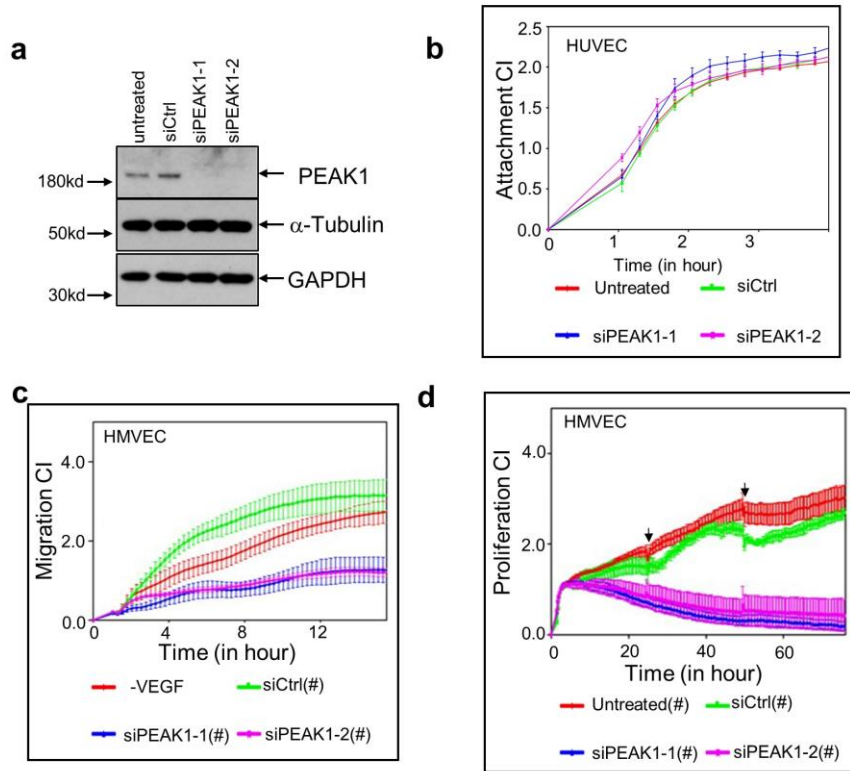


**Supplementary FigS2.** *peak1* is required for vascular development in zebrafish embryos. (a) Schematic showing design of splicing morpholino to target the zebrafish *peak1* gene. (b) Schematic showing the design of TALEN used to target the zebrafish *peak1* gene. (c) Bright field image show normal pericardial cavity and circulation of Ctrl MO injected zebrafish embryos. Scale bar = 50  $\mu$ m. (d) Bar graph represents relative *peak1* mRNA level versus *actn1* from 30 hpf

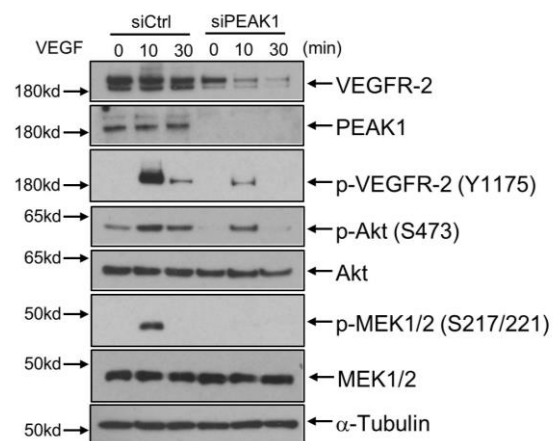
embryos treated with Ctrl MO, SP1MO or SP2MO and analyzed by qPCR. (e) Western blot analyses for indicated proteins of 30 hpf embryos treated as in Figure 1c. (f) Genotyping of zebrafish *peak1* TALEN F0 founder embryos. Primers TALEN test F+R and restriction enzyme MslII were used. Uncut (%), the percentage of uncut band out of the total DNA. (g) Bar graph represents percentage of animals with blood circulation defects caused by injection of zebrafish *peak1* TALEN mRNAs. (h) Bright field images of zebrafish embryos showing severe pericardial edema (arrows) and blood circulation defects (arrowheads) caused by *peak1* TALEN mRNAs at indicated dpf. Scale bar = 1 mm. (i) Confocal fluorescence images of the tail vasculature of *Tg(fli1:egfp)<sup>y1</sup>* embryos treated with *peak1* TALEN mRNAs were captured at 2dpf. Arrows point to mosaic stunted and disrupted ISV vessels. Scale bar = 50  $\mu$ m. (j) DNA sequencing results of F1 TALEN mutant lines. (k) *peak1 $\Delta^2/\Delta^2$*  TALEN homozygous mutants were incrossed and progeny embryos were lysed at 2dpf and analyzed by western blotting with indicated antibodies. All data are representative of at least three independent experiments. \*\*\*, P<0.001; \*, P<0.05; N.S., not significant.



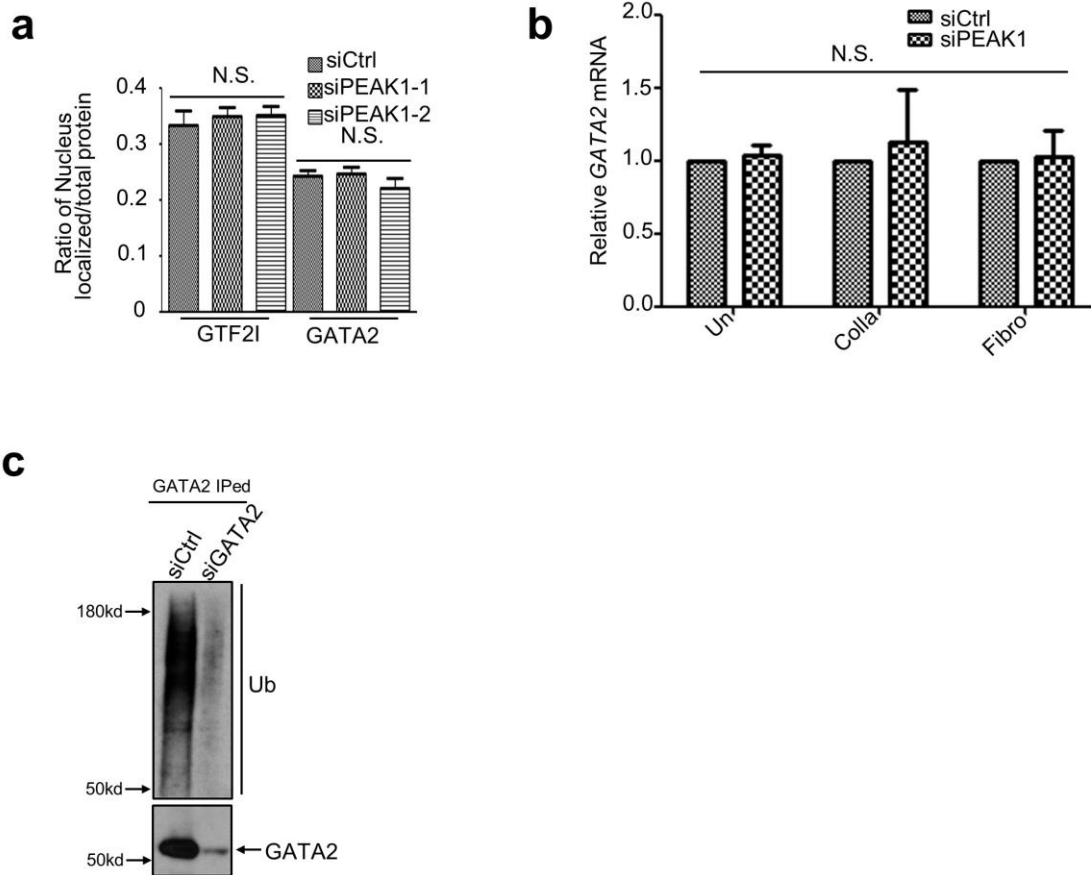
**Supplementary FigS3.** Schematic illustration showing transplantation of endothelial precursor cells from donor *Tg(fli1:egfp)<sup>Y1</sup>* into AB host embryos at the sphere stage. Donor or host embryos were treated with indicated MOs at the one-cell stage.



**Supplementary FigS4.** PEAK1 is required for VEGF induced proliferation, migration and morphogenesis of human ECs *in vitro*. **(a)** HUVECs were treated with either siCtrl, siPEAK1-1, or siPEAK1-2. Cells were then lysed and western blotted for the indicated proteins. **(b)** Real time cell attachment kinetics of HUVECs using the xCelligence E16 plate system. Cells were treated as in Figure3E. **(c)** Real-time migration kinetics of HMVECs treated with either siCtrl or two different siRNAs to PEAK1 (siPEAK1-1, siPEAK1-2) in the absence (-VEGF group) or presence of VEGF (# labeled); CI = Cell Index; Mean  $\pm$  SEM of quadruplicate wells. **(d)** Real-time proliferation kinetics of HMVECs treated as in (c) in the presence VEGF. Arrows indicate the time point of adding supplemental VEGF. Mean  $\pm$  SEM of quadruplicate wells.

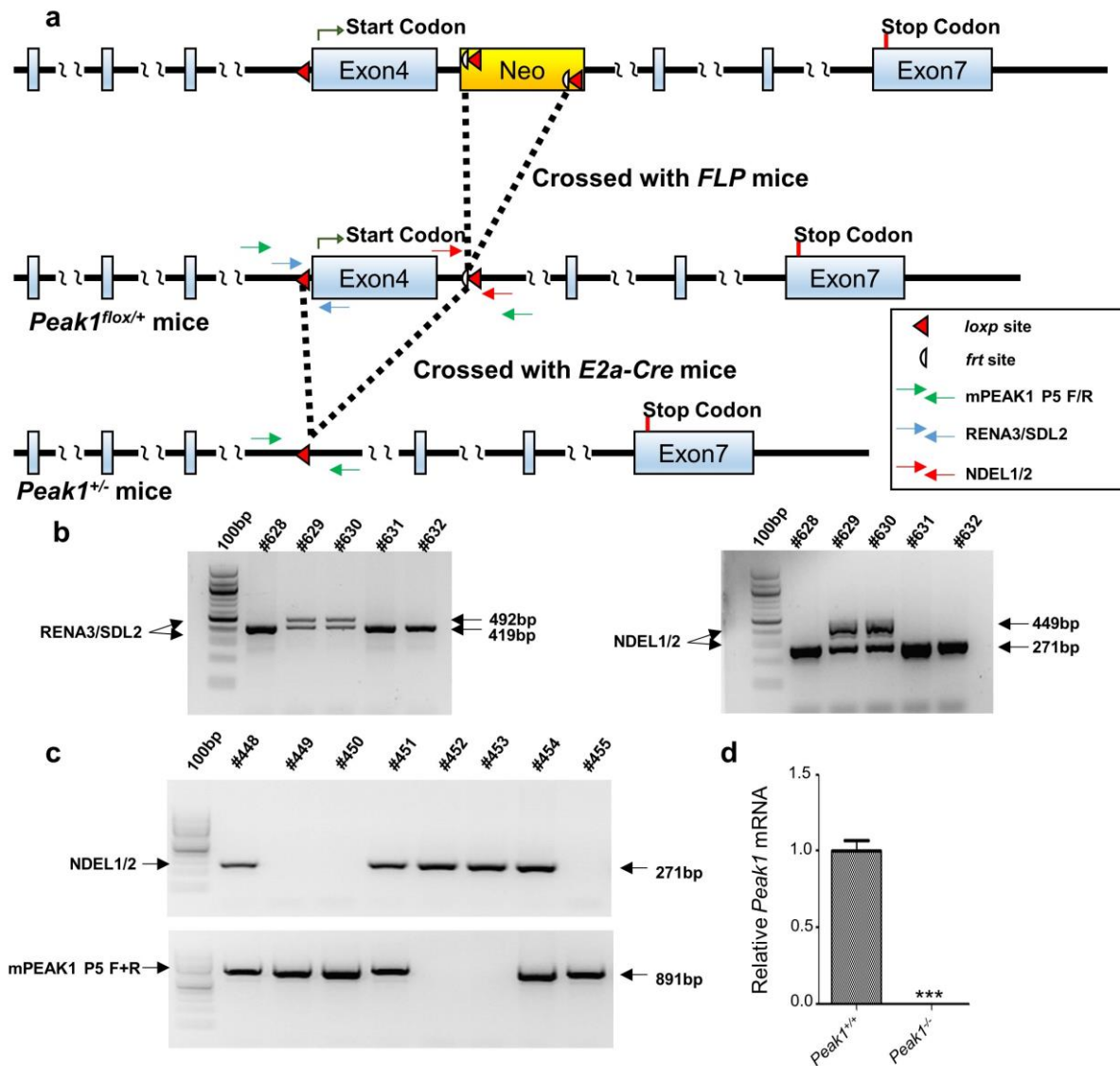


**Supplementary FigS5.** Western blot analysis of HMVECs treated with siRNAs with indicated antibodies after 12 hours of starvation with or without VEGF stimulation.

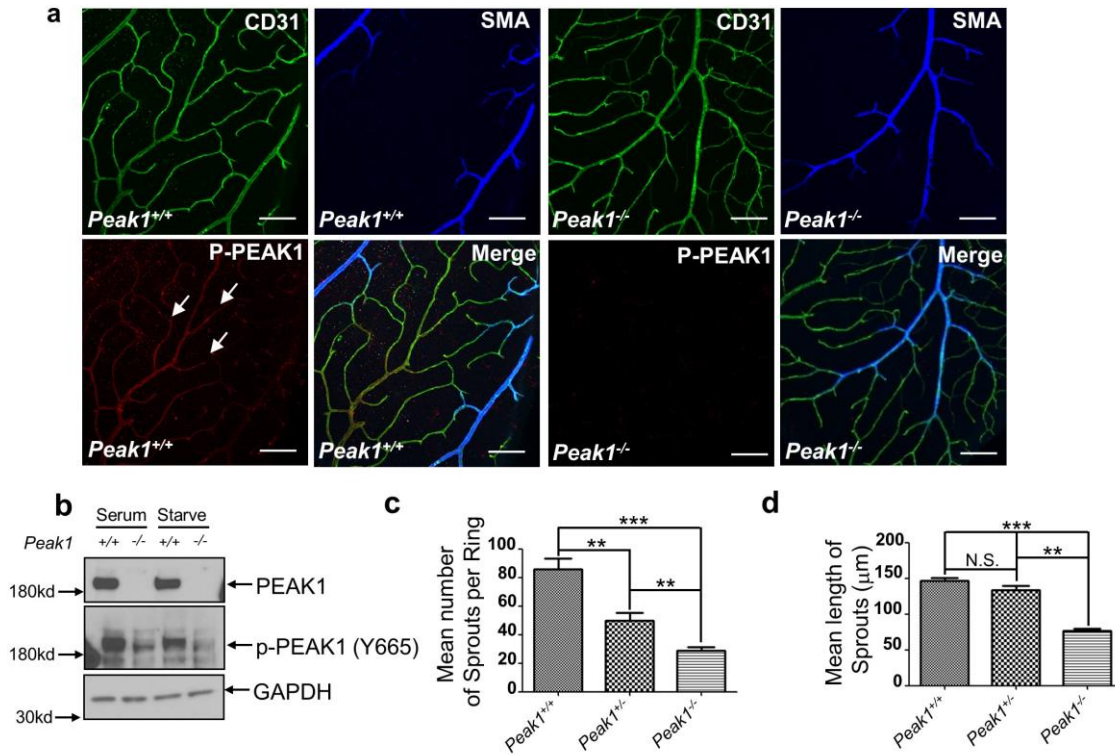


**Supplementary FigS6.** *PEAK1* regulates *VEGFR2* transcription through *GATA2*. **(a)** Bar graph shows the Manders' coefficient value of GTF2I or GATA2 fluorescent signal versus DAPI nuclear stain of HUVECs cultured on collagen I coated plates and treated with siRNAs. Mean  $\pm$  SEM; n=10. **(b)** Bar graph represents relative *GATA2* mRNA expression level versus *HPRT1* in HUVECs treated as in Figure 7C; Un, uncoated plate; Colla, collagen coated; Fibro, fibronectin coated. **(c)** HUVECs were treated as in Figure 7H. Ubiquitination of IPed GATA2 protein was analyzed by WB. All data are representative of at least three independent experiments. N.S., not significant.

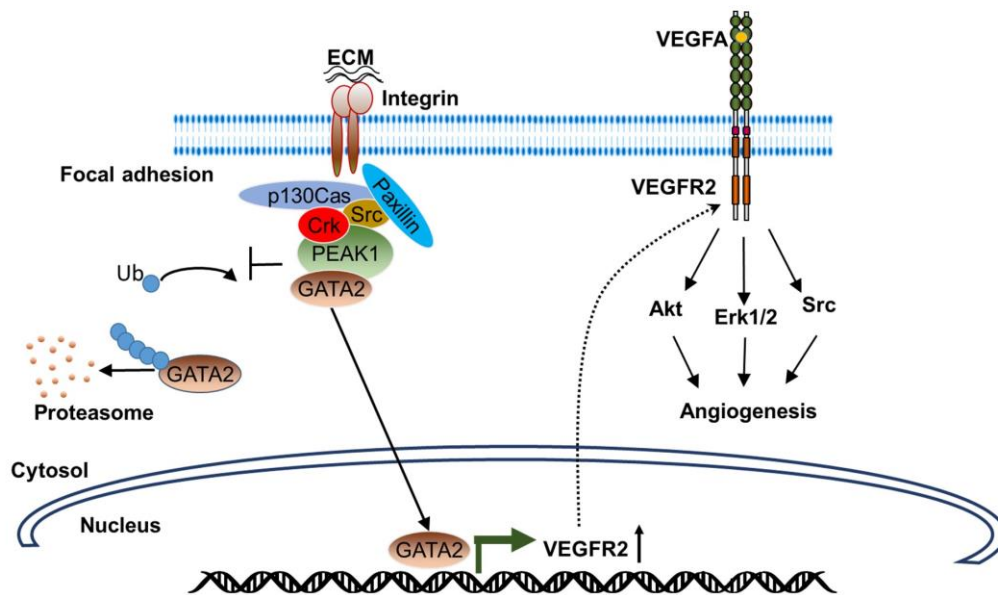




**Supplementary FigS7.** Generation of *Peak1<sup>flox/flox</sup>* mice and *Peak1<sup>-/-</sup>* mice. **(a)** Schematic showing the gene targeting strategy to generate *Peak1<sup>flox/+</sup>* mice and *Peak1<sup>-/-</sup>* mice. **(b)** Genotyping of *Peak1<sup>flox/+</sup>* for the distal loxP insertion with PCR primers RENA3/SDL2, and for the proximal loxP insertion with primers NDEL1/2. **(c)** Genotyping of *Peak1<sup>-/-</sup>* mice for the genomic deletion of the exon 4 of *Peak1* gene with primers NDEL1/2 and mPEAK1 P5 F+R. **(d)** Bar graph represents relative *Peak1* mRNA level versus *Hprt1* in *Peak1<sup>+/+</sup>* and *Peak1<sup>-/-</sup>* mice liver tissue. Mean  $\pm$  SEM;  $n=3$ . All data are representative of at least three independent experiments. \*\*\*,  $P<0.001$ .



**Supplementary FigS8.** (a) Confocal images of *Peak1<sup>-/-</sup>* and *Peak1<sup>+/+</sup>* adult mouse retinas stained with the indicated antibodies. CD31 (green). Phosphospecific Y665 PEAK1 (P-PEAK1, red). Smooth muscle actin (SMA, blue). Scale bar = 100 μm. (b) Western blot analysis of mouse embryonic fibroblast cells (MEFs) from *Peak1<sup>+/+</sup>* or *Peak1<sup>-/-</sup>* mice with the indicated antibodies. (c) Bar graph represents the mean number of sprouts per aortic ring from aortic explants treated as in Figure 8D; Mean ± SEM; *n*=10. (d) Bar graph represents the average length per sprout from aortic explants treated as in Figure 8d; Mean ± SEM; *n*≥286. \*\*\*, *P*<0.001; (\*\*) *P*<0.01; \*, *P*<0.05; N.S., not significant.



**Supplementary FigS9.** Working model of PEA1-mediated VEGFR2 expression in ECs. EC attachment to ECM mediates integrin dependent focal adhesion formation and the recruitment of its effector proteins Crk, Src, p130CAS, and paxillin. This complex promotes the interaction of PEA1 and a transcriptional factor GATA2, and increases GATA2 protein stability by inhibiting GATA2 ubiquitination and destruction by the proteasome. The increased total GATA2 protein levels drives increased VEGFR2 gene transcription as well as increased VEGFR2 protein expression and enhanced EC functions critical for angiogenesis.

**Supplementary Movie S1.** One-cell stage *Tg(fli:nls-egfp)* embryos were injected with Ctrl MO. Time lapse images of ISV ECs expressing nuclear GFP were taken with confocal microscope from 24hpf at 5-minutes intervals.

**Supplementary Movie S2.** One-cell stage *Tg(fli:nls-egfp)* embryos were injected with SP1MO to knockdown *peak1*. Time lapse images of ISV ECs expressing nuclear GFP were taken with confocal microscope from 24hpf at 5-minutes intervals.