Examination of MO targeting
To determine efficacy, reverse transcription was performed on 1.0 µg of total RNA isolated from WT-, and MO- injected embryos at 24 h after fertilization (hpf) by using oligo(dT) primers. The following cycling parameters were used for PCR: 35 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min; and 72°C for 10 min. The following primers were used for zNgBR MO-efficacy experiments: control primer pairs F:TGGCCGTGGTGGACTTATTATTGC/R:ATACACCAGACCACCAGATTGGCA; exon2-exon4 primer pairs F:TGATGGAGGAGATCCTGAAACAGC/R:TTGCACAGTCCAAAC TTCAGCAC. The following primers were used for zNogo-B MO-efficacy experiments: control primer pairs F: ACCATTAACCAGAGCCAGAAG/ R: ACCAATGAGCAGAAGAGTTAC; exon1-exon2 primer pairs F:AAGAGAAGCCAACAGTAGCG/R:ACAGACCAGCTCCAAACAC.

Real-time RT-PCR
Total RNA from zebrafish at 24 hpf was isolated by using the RNeasy kit (Qiagen, Valencia, CA). Reverse transcription was then performed by using 100 ng of RNA and iScript cDNA synthesis kit (Bio-Rad). Real-time PCR analysis was done with MyiQ detection system using the iQ SYBR Green Supermixes (Bio-Rad). The zNgBR or zNogo-B mRNA levels were normalized by house keeping gene beta-actin and were presented as relative expression levels as compared to WT controls. We used the following primers: for MO2-modified zNgBR, forward (TATTGCCAATCTGGTGTTGCTGGTG) and reverse (CTTAGTGGCTCCCTGCTTTATTGTACC); for MO3-modified zNgBR, forward (GGGCAGCTCCAAATATTCAGTGGA) and reverse (GTAATGAGTCCAGCACACTGACA); for zNogo-B, forward (ATATAAATGTAACTCTTCGTGCTCATTGGAG) and reverse (TACGTAGGAGAGGACGCTGATGAT); for zebrafish beta-actin (NM_131031), forward (AATCCCAAAGCCAACAGAGA) and reverse (CACACCATCACCAGAGTCCA).
Figure S1. Alignments of NgBR and Nogo-B zebrafish ortholog. (A) Alignment of zebrafish NgBR (NM_001002356) with human (NM_138459) and mouse (NM_030250). Green rectangle box indicates the transmembrane region of NgBR. (B) Alignment of zebrafish Nogo-B (NM_001079912) with human (NM_153828) and mouse (NM_194052). Green rectangle box indicates the crucial active domain of Nogo-B.

Figure S2. (A) NgBR transcripts at the different time points of development were determined by RT-PCR. (B) Nogo-B transcripts at the different time points of development were determined by RT-PCR. (C) A montage of NgBR whole-mount in situ staining is shown at 3hpf and 12hpf, respectively. (D) transverse section images of NgBR whole-mount in situ staining (WISH) (shown as blue color) and fli1a-GFP immunostaining (shown as red color) at 24hpf. NC: notochord; DA: dorsal aorta.

Figure S3. NgBR and Nogo-B splice MO targeting (A) The exon-intron genomic structure of NgBR is shown. MO1 targets the ATG translation start codon. MO2 and MO3 target the Exon 2 and Exon 3, respectively, the predicted cytoplasmic domains of NgBR. Total RNA was extracted from uninjected (lane 1 and lane 4), MO2 (lane 2 and lane 5) and MO3 (lane 3 and lane 6) injected embryos at 24 hpf, and RT-PCR was performed as described in methods. PCR1 (lane 1-3) was performed to show the equal amount RNA using primers derived from Exon1. PCR2 (lane 4-6) was carried out to show the splicing products after MO injection using primers derived from Exon 2 and Exon 4, respectively. The grey arrow indicates common untargeted bands; the black arrow indicates the alternative transcripts that were generated in MO-targeted embryos. (B) The exon-intron genomic structure of Nogo-B is shown. MO1 targets the ATG translation start codon. MO2 targets the boundary region of Exon 1 and Intron 1, the known active domain of Nogo-B. Total RNA was extracted from uninjected...
(lane 1 and lane 3), MO2 (lane 2 and lane 4) injected embryos at 24 hpf, and RT-PCR was performed as described in methods. PCR1 (lane 1-2) was performed to show the equal amount RNA using primers derived from Exon1. PCR2 (lane 3-4) was carried out to show the splicing products after MO injection using primers derived from Exon 1 and Exon 2, respectively. The grey arrow indicates common untargeted bands; the black arrow and the black arrowhead indicate the alternative transcripts that were generated in MO-targeted embryos. (C) zNgBR morpholinos decrease the wild-type transcripts of zNgBR in zebrafish determined by real-time RT-PCR. WT: uninjected embryos; MO2/MO3: zNgBR splice MOs; (D) zNogo-B morpholinos decrease the wild-type transcripts of zNogo-B in zebrafish determined by real-time RT-PCR. WT: uninjected embryos; MO2: zNogo-B splice MO.

Figure S4. NgBR MO blocks ISV formation. (A) Whole-mount flk1- in situ staining (blue) was performed to localize the ISV at 24hpf embryos. Upper panels are the images of whole embryos at 24hpf and bottom panels are magnified images of the trunk region, showing WT (uninjected), Ctrl MO (8 ng control MO), MO1 (8 ng ATG MO) and MO3 (8ng splice MO)-injected embryos. Asterisk indicates location of ISV. (B) Quantification of embryos with ISV defects (missing more than 5 ISV). n: embryo number in each group. Anterior is to the left. WT: uninjected embryos; Ctrl MO: control MO-injected embryos; MO1: ATG-MO-injected embryos; MO3: splice MO-injected embryos.

Figure S5. (A) Effects of NgBR siRNA treatment on expression of other neuronal guidance molecules involved in angiogenesis such as Semaphorin 3e, Robo-4, Plexin B2 and Plexin D1 in HUVEC cells. The protein expression of Semaphorin 3e, Robo4, Plexin B2 and Plexin D1 was determined by Western blot analysis as described in methods. Rabbit polyclonal antibodies for these proteins were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). (B) Expression of NgBR coding cDNA in NgBR siRNA-treated HUVEC was determined by Western blot analysis. NS: non-silencing
siRNA; siNgBR: siRNA targeting NgBR; Vector: transfected with pIRES-neo empty vector; NgBR: transfected with pIRES-neo vector carrying NgBR coding-region cDNA. siRNA: 10 nM. (C) Expression of myrAkt, NgBR, Nogo-B and AmNogo-B proteins in these mRNA injected zebrafish embryos. Total protein was isolated from whole zebrafish embryos at 24 hpf. The protein expression of myrAkt, NgBR-HA, Nogo-B-myc and AmNogo-B-myc was determined by Western blot analysis using anti-Akt, HA and myc-tag antibodies, respectively (Cell Signaling).

Figure S6. Effects of Nogo-B knockdown and increasing free cholesterol levels on endothelial cell migration. (A) Effects of Nogo-B knockdown on VEGF/AmNogo-B-induced endothelial cell migration. Nogo-B in HUVEC was knocked down using siRNA (10nM). Cell migration was examined in modified Boyden chambers in response to VEGF (100 ng/ml) or AmNogo-B (100 nM) (n=3). NS: non-silencing siRNA as a negative control. Expression of Nogo-B in HUVEC was determined by Western blot analysis. (B) Effects of U18666A on VEGF-induced endothelial cell migration. HUVEC were treated with U18666A (Sigma) at the concentration of 1 and 10 µM for overnight (18 h). Cell migration was examined in modified Boyden chambers in response to VEGF (100 ng/ml). * P <0.05, v.s. without U18666A treatment and without VEGF stimulation; # P <0.05, v.s. without U18666A treatment and with VEGF stimulation. (C) Effects of imipramine on VEGF-induced endothelial cell migration. HUVEC were treated with imipramine (Sigma) at the concentration of 3 and 30 µM for overnight (18 h). Cell migration was examined in modified Boyden chambers in response to VEGF (100 ng/ml).
Figure S1

A

Human NgBR:
 MTGLYELVWRLHALCLCLMLTGTGLRVRFGTWNWIIWRRCRAAASAHLVA 50
 Mouse NgBR:
 MTGLYELVWRLHALCLCLMLTGTGLRVRFGTWNWIIWRRCRAAASAHLVA 50
 Zebrafish NgBR:
 MSLXEMWRLHALLVLQRAVYANFVH--------HWR--------WKLAVVDELL 42

Human NgBR:
 PLGFTLRLPKPAVGRNRRHRHRPRGGS-------CLAAAHHRMRWNADCRSLEK 96
 Mouse NgBR:
 PLGFTLRLPKPAVGRNRRHRHRPRGGS-------CLAAAHHRMRWNADCRSLEK 96
 Zebrafish NgBR:
 PLALGFHNQKTKG----------PKGR---------TSRVRMGDGRTELK 76

Human NgBR:
 LPHVHLGVEITEVQBPSPFSDDTTSLWVCMAVGISYSIVHDQGIRKNNS 146
 Mouse NgBR:
 LPHVHLGVEITEVQBPSPFSDDTTSLWVCMAVGISYSIVHDQGIRKNNS 146
 Zebrafish NgBR:
 LPLLVGLLTEEB1--------YTIDANLVWVMAVGISYSIVHDQGVFKRNS 124

Human NgBR:
 RLMEILKQQELGLDCSKYSPEFAN--------SNKDQQVNLCHLAVKLSP 194
 Mouse NgBR:
 RLMEILKQQELGLDCSKYSPEFAN--------SNKDQQVNLCHLAVKLSP 194
 Zebrafish NgBR:
 RLMEILKQQELGLMGKSYSVEILKNGTNKOEHOVLSCQSMKVLSPD 174

Human NgBR:
 DGGKADIRAAQDFQQLVAKQKRPTDLVDTLASSLS--SNGCPDDPLVSLNLK 243
 Mouse NgBR:
 DGGKADIRAAQDFQQLVAKQKRPTDLVDTLASSLS--SNGCPDDPLVSLNLK 243
 Zebrafish NgBR:
 DGGKADIRAAQDFQQLVAKQKRPTDLVDTLASSLS--SNGCPDDPLVSLNLK 243

Human NgBR:
 FGPVDSLGLFLPWHIRLTEIVSLPSHLNISYEDFSLQYACEQRLGK 293
 Mouse NgBR:
 FGPVDSLGLFLPWHIRLTEIVSLPSHLNISYEDFSLQYACEQRLGK 293
 Zebrafish NgBR:
 FGPVDSLGLFLPWHIRLTEIVSLPSHLNISYEDFSLQYACEQRLGK 293

B

Human Nogo-B1:
 MEDLDQSPVSSS-DSPPRPQOPFKYQVFVREPEDEEDEEDEEDEDEEDLD 49
 Mouse Nogo-B1:
 MEDLDQSPVSSS-DSPPRPQOPFKYQVFVREPEDEEDEEDEEDEDEEDLD 49
 Zebrafish Nogo-B1:
 MD-----DQISSSTTPSYDVHDDEGEEFRAEKOQANSMLDEDFSSESEQAHEYHVE 48

Human Nogo-B1:
 EELVLE--------RKPAAGLSAAVPVTAPAGAAGPMDFGNDVFPPAPRGPPLAA 97
 Mouse Nogo-B1:
 EELVLE--------RKPAAGLSAAVPVTAPAGAAGPMDFGNDVFPPAPRGPPLAA 97
 Zebrafish Nogo-B1:
 EEILVLEAGKALDERHRHSTMP-------REEPLLDDGE----EPFFPESTIKPE 93

Human Nogo-B1:
 PPVAPRQPSWDPSPVSSSTPVAPPSLASSAVAASPSSLKPEDDEPPAAPPDEPPAAPPD 147
 Mouse Nogo-B1:
 PPVAPRQPSWDPSPVSSSTPVAPPSLASSAVAASPSSLKPEDDEPPAAPPDEPPAAPPD 147
 Zebrafish Nogo-B1:
 PPVAPRQPSWDPSPVSSSTPVAPPSLASSAVAASPSSLKPEDDEPPAAPPDEPPAAPPD 147

Human Nogo-B1:
 PAPVQPQAEPWTPPPAPPAAPSTTPAPPAKPGRGSSGVVDLLYWRDIIK 197
 Mouse Nogo-B1:
 PAPVQPQAEPWTPPPAPPAAPSTTPAPPAKPGRGSSGVVDLLYWRDIIK 197
 Zebrafish Nogo-B1:
 PAPVQPQAEPWTPPPAPPAAPSTTPAPPAKPGRGSSGVVDLLYWRDIIK 197

Human Nogo-B1:
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 Mouse Nogo-B1:
 TGVFGAASNASSLSLTVFISVTAYALASSLTVIYFRKGVIQAIQK 247
 Zebrafish Nogo-B1:
 TGVFGAASNASSLSLTVFISVTAYALASSLTVIYFRKGVIQAIQK 247

Human Nogo-B1:
 SDEGHPFRAYLESEVAISELVDVQYSNASLGHVNCITIKELRLFVLDCLLV 297
 Mouse Nogo-B1:
 SDEGHPFRAYLESEVAISELVDVQYSNASLGHVNCITIKELRLFVLDCLLV 297
 Zebrafish Nogo-B1:
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Human Nogo-B1:
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 Mouse Nogo-B1:
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 Zebrafish Nogo-B1:
 DSGHFPKMYLDRPLALEMPHXYDSTLHVINTIKVLK 347

Human Nogo-B1:
 GLANKVNDMARIQAIPKGLKRKE 373
 Mouse Nogo-B1:
 GLANKVNDMARIQAIPKGLKRKE 373
 Zebrafish Nogo-B1:
 GLANKVNDMARIQAIPKGLKRKE 373
Figure S2

A

Fold of NgBR expression

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Fold of Nogo-B expression

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C

3hpf

12hpf

NgBR WISH

D

NgBR WISH  fli1a-GFP  Merge

NgBR WISH  fli1a-GFP  Merge
Figure S3

A. zNgBR

```
ATG  EX1
MO1  MO2  MO3
EX2  EX3  EX4  EX5
```

B. zNogo-B

```
ATG  EX1
MO1  MO2
EX2  EX3  EX4  EX5  EX6  EX7
```

C. Relative Expression of zNgBR

```
Relative Expression of zNgBR

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D. Relative Expression of zNogo-B

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Relative Expression of zNogo-B

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Figure S4

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B

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* * ****** ****** ****** ****** ****** ****

* * ****** ****** ****** ****** ****** ****

n=36 n=25
Figure S5

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C

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Figure S6

A

siRNA-NS

siRNA-Nogo-B

Migration (cells/field)

Nogo-B

Hsp90

siRNA-NS

siRNA-Nogo-B

B

U18666A

0 1 10 uM

-VEGF +VEGF

C

Imipramine

0 3 30 uM

-VEGF +VEGF