Supplementary material

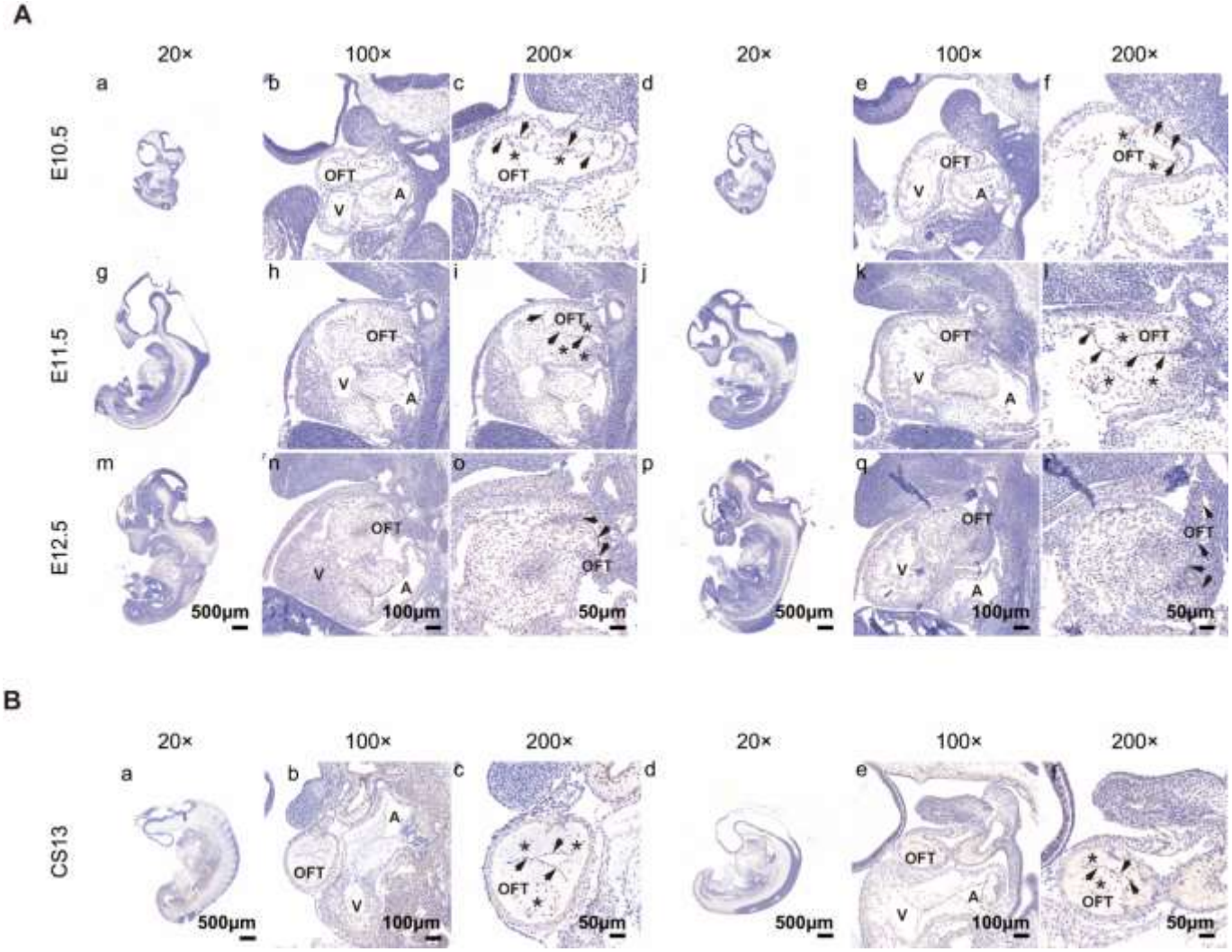

Figure S1. Immunohistochemical staining for SOX7 in OFT during embryonic heart development.

(**A**) Distribution of the endogenous Sox7 protein during OFT development at mouse E10.5 (a-f), E11.5 (g-l) and E12.5 (m-r). Strong nuclear staining for Sox7 is seen in the endocardial cells and mesenchymal cells of OFT. (**B**) Distribution of the endogenous SOX7 protein in OFT at human Carnegie Stage 13 (CS13).SOX7 expression in the OFT is limited to the endocardial cells and mesenchymal cells. Images of the other two embryos at each stageare shown. Arrowheads, endocardial cells; asterisks, mesenchymal cells. OFT, outflow tract; A, atrium; V, ventricle. Scale bars=500 μm A (a, d, g, j, m, p) and B (a, d); Scale bars=100 μm A (b, e, h, k, n, q) and B (b, e); Scale bars=50 μm A (c, f, i, l, o, r) and B (c, f).

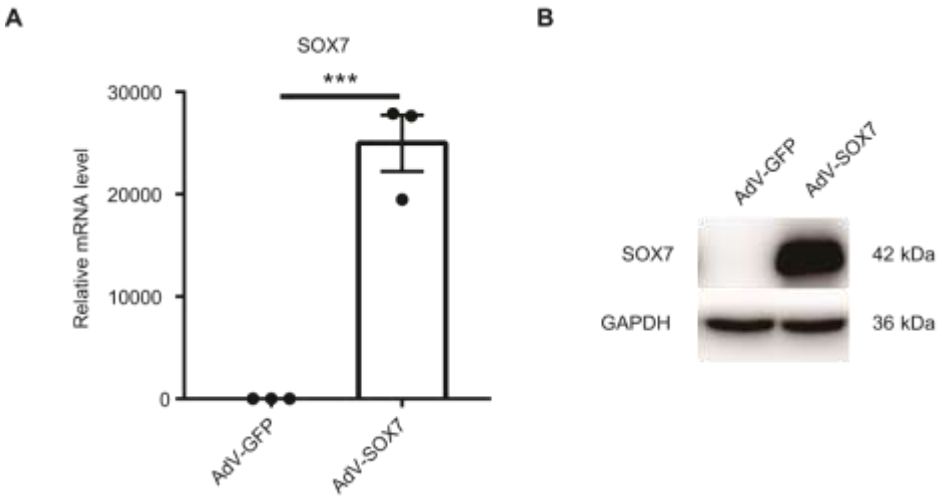

Figure S2. Verification of SOX7 overexpression.

(**A**) Quantitative real-time PCR analysis of the mRNA expression levels of SOX7 in AdV-GFP and AdV-SOX7 infected HUVECs. Results were normalized to reference gene GAPDH. RNA levels in control (AdV-GFP) are set as 1. Data are shown as the mean ± SEM, two-tailed unpaired T test was used for statistical calculation, n=3independent experiments, ***p<0.001 vs AdV-GFP. (**B**) Representative immunoblots of SOX7 in HUVECs transfected with AdV-GFP and AdV-SOX7. GAPDH was used as internal control. Repeat at least 3 independent experiments.

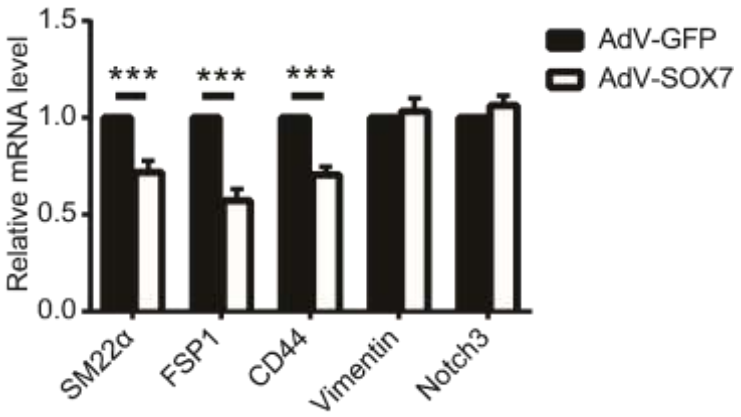

Figure S3.Analysis of EndMT marker expression by qRT-PCR in ECs isolated from OFT explants infected with AdV-GFP or AdV-SOX7.

Quantitative real-time PCR analysis of the mRNA expression levels of mesenchymal markers (SM22α, FSP1 and Vimentin), mesenchymal stem cell marker (CD44) and EndMT transcriptional factor (Notch3) in ECs isolated from OFT explants infected with AdV-GFP or AdV-SOX7. Results were normalized to reference gene β-actin. RNA levels in control (AdV-GFP) are set as 1. Data are shown as the mean ± SEM, two-tailed unpaired T test was used for statistical calculation for each marker, n=7 independent samples per group, ***$p<0.001$ vs AdV-GFP.

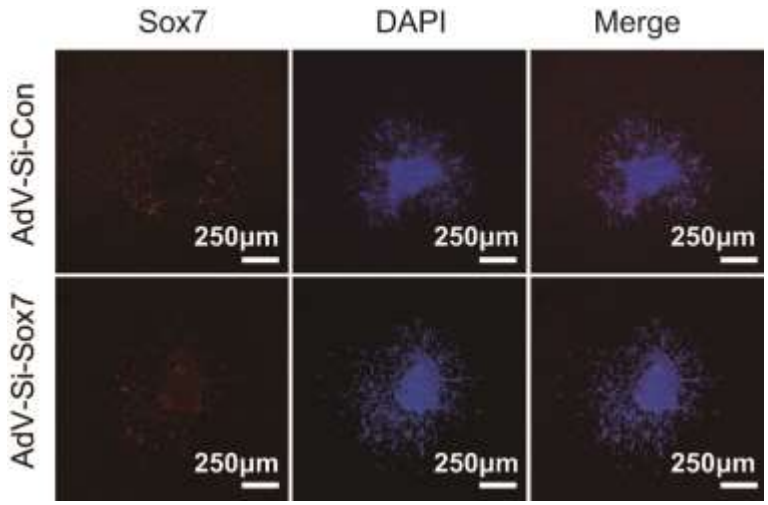

Figure S4. Verification of Sox7 knockdown.

Representative photomicrographs of OFT explants infected with GFP labeled adenovirus encoding siRNA control (AdV-Si-Con) or siRNA Sox7 (AdV-Si-Sox7). Sox7 (red), the nuclei are stained with DAPI (blue), scale bars=250 μm. Repeat 3 independent experiments.

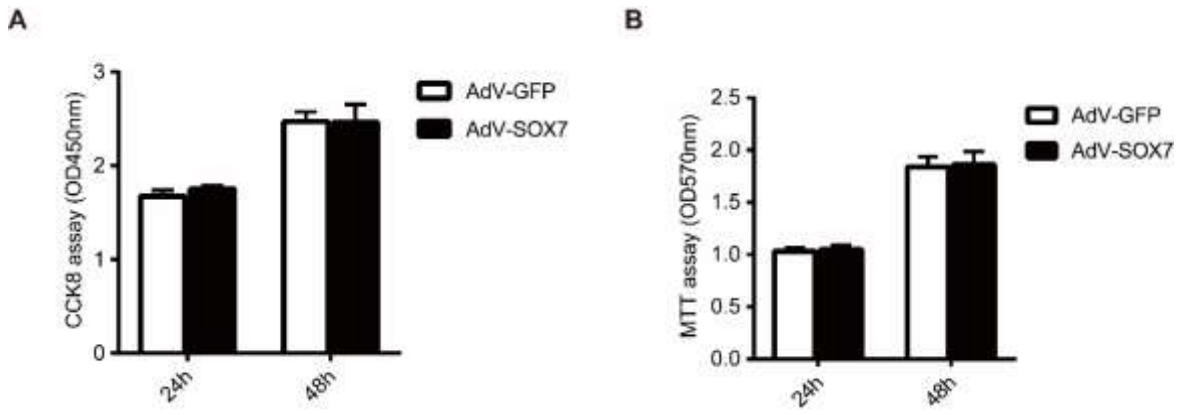

Figure S5. Effects of SOX7 on cell proliferation and viability in HUVECs.

(**A** and **B**) Cell proliferation and viability of HUVEC infected with AdV-Con or AdV-SOX7 for 24 h, 48h by CCK-8 assay and MTT assay. Data are shown as the mean ± SEM, two-tailed unpaired T test was used for statistical calculation for each time point, n= 3 independent experiments, each consisting of 6 different wells. *$p<0.05$ vs AdV-GFP group.

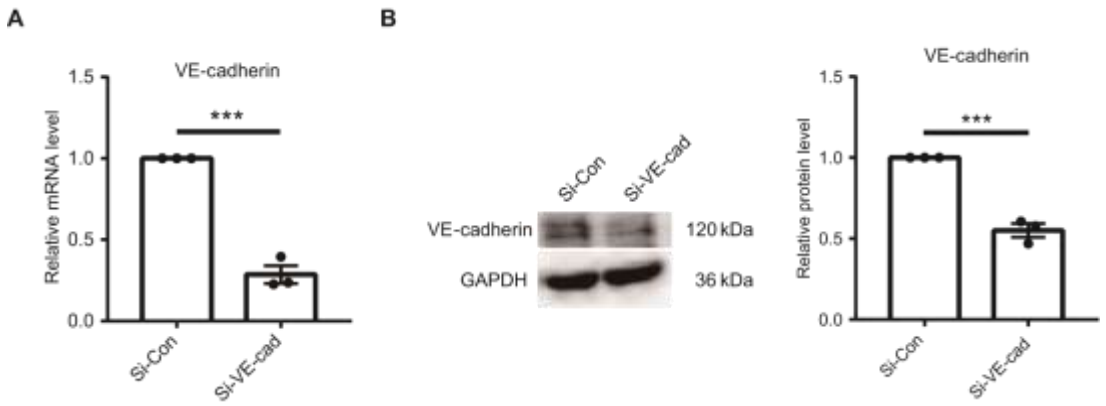

Figure S6. Verification of VE-cadherin knockdown

(**A**) Quantitative real-time PCR analysis of the mRNA expression levels of VE-cadherin in Si-Con and Si-VE-cad transfected HUVECs. Results were normalized to reference gene GAPDH. RNA levels in control (Si-Con) are set as 1. Data are shown as the mean ± SEM, two-tailed unpaired T test was used for statistical calculation, n=3 independent experiments, ***p<0.001 vs Si-Con. (**B**) (*Left*) Representative immunoblots of VE-cadherin in HUVECs transfected with Si-Con and Si-VE-cad. GAPDH was used as internal control. (*Right*), The band density of VE-cadherin on the Western blot of three independent protein samples was digitally quantified by ImageJ software. Data shown are the mean ± SEM, two-tailed unpaired T test was used for statistical calculation, n=3 per group, ***p<0.001 vs Si-Con.

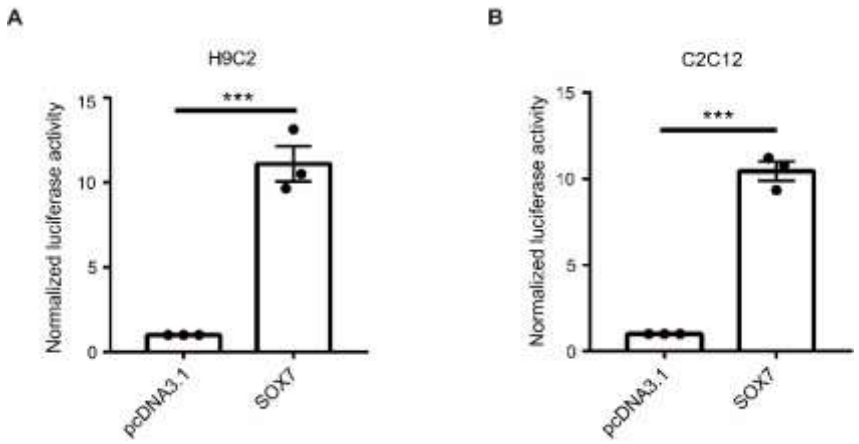

Figure S7. SOX7 increases the activity of the *VE-cadherin* promoter in H9C2 and C2C12 cell lines.

(**A**and**B**) Cells were transfected with the *VE-cadherin* promoter luciferase reporter in the presence of control pcDNA3.1 or SOX7 (pcDNA3.1-SOX7) expression vector. The activity of the promoter constructs is expressed relative to that of the control pcDNA3.1 for each cell line. Data are shown as the mean ± SEM, two-tailed unpaired T test was used for statistical calculation, n=3 independent experimentsfor H9C2 andC2C12, ***$p<0.001$ vs. pcDNA3.1.

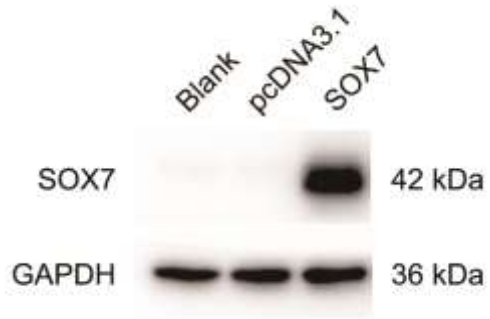

Figure S8. Verification of in vitro-translated SOX7 by reticulocyte lysates.

Representative immunoblots of SOX7 with in vitro-translated TNT blank protein, TNT pcDNA3.1 protein and TNT SOX7 protein. GAPDH was used as internal control. Repeat 3 independent experiments.

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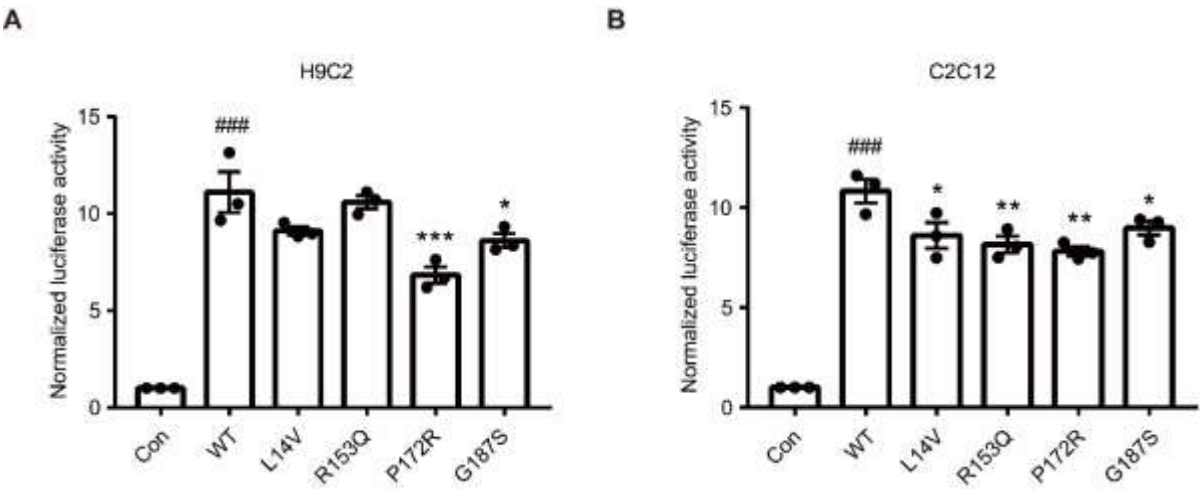

Figure S9. Effects of SOX7 mutations on transcriptional activation of downstream target gene *VE-cadherin*in H9C2 and C2C12 cells. Cotransfection of *VE-cadherin* promoter luciferase reporter with the WT and mutant SOX7 protein in H9C2 (**A**) and C2C12 cells (**B**). The luciferase activities were reported as fold increase relative to the activity of the reporter in the presence of an empty expression plasmid (Con). Data are shown as the mean ± SEM, n=3 independent experiments. Statistical significance was calculated by one-way ANOVA with Dunnett's*post hoc* test, *P<0.05 vs WT, **P<0.01 vs WT, ***P<0.001 vs WT. Two-tailed unpaired T test was used for statistical calculation , ###p<0.001 WT vs. Con.

Table S1 Primers used for PCR, RT-PCR, EMSA and Site-directed mutagenesis

Primer Name	Sequence (5'-3')
PCR (promoter)	
VE-cadhein F	GGGGTACCCCACAAAGACATCATGGGATCACC
VE-cadherin R	CCAAGCTTTTCTGTTCCGTTGGACTGCCT
RT-PCR (human)	
VE-cadherin F	GTTCACGCATCGGTTGTTCAA
VE-cadherin R	CGCTTCCACCACGATCTCATA
PECAM1 F	ACCGTGACGGAATCCTTCTCT
PECAM1 R	GCTGGACTCCACTTTGCAC
α-SMA F	GCTATCCAGGCGGTGCTGTCTCTCT
α-SMA R	GCCAGCCAGATCCAGACGCATGATG
VIMENTIN F	GCAGGTGGACCAGCTAACCAACGA
VIMENTIN R	GCCAGAGACGCATTGTCAACATCCT
FN1 F	CGACGCCTCCACTGCCATTGAT
FN1 R	GCCAGTAATAGTAGCCTCTGTGACACC
SOX7 F	AAGATGCTGGGAAAGTCGTGGAA
SOX7 R	CGCTTGGCCTGCTTCTTCCT
GAPDH F	GAGTCCACTGGCGTCTTCACCACCAT
GAPDH R	GAGGCATTGCTGATGATCTTGAGGCTGTTG
RT-PCR (mouse)	
SM22α F	TGACGAGGAGCTGGAGGAGCGACTA
SM22α R	CAGGCTGTTCACCAATTTGCTCAGAATCAC
FSP1 F	ACTCAGGCAAAGAGGGTGACAAGTTCAAGC
FSP1 R	TGTCCCTGTTGCTGTCCAAGTTGCTCATCA
CD44 F	TCTTGGCATCTCTCCTGGCACTGGCTCTGA
CD44 R	GTCTTCCACCGTCCCATTGCCACCGTTGA
Vimentin F	GTGGATCAGCTCACCAACGACAAGG
Vimentin R	CAGGGTGCTTTCGGCTTCCTCTCT
Notch3 F	TGCTAGAGCGGATGCAGCCAAG
Notch3 R	TGGAGCGGTTCCTGATGAGAATCTG
β-actin F	GGCTGTATTCCCCTCCATCG

β-actin R	CCAGTTGGTAACAATGCCATGT
EMSA	
VE-cadherin biotin F	5’-Biotin-
VE-cadherin biotin R	CCCTCACAAAGGAACAATAACAGGAAACCATCCCAGGGGGAAG
VE-cadherin comp F	5’-Biotin-
VE-cadherin comp R	CTTCCCCCTGGGATGGTTTCCTGTTATTGTTCCTTTGTGAGGG
SiRNA	CCCTCACAAAGGAACAATAACAGGAAACCATCCCAGGGGGAAG
AdV-Si-SOX7 F	CTTCCCCCTGGGATGGTTTCCTGTTATTGTTCCTTTGTGAGGG
AdV-Si-SOX7 R	
Si-VE-cad F	GGATCGCAATGAATTTGATCATGATCAAATTCATTGCGATCC
Si-VE-cad R	GGATCGCAATGAATTTGATCATGATCAAATTCATTGCGATCC
	CCUCUGUCAUGUACCAAAUTT
	AUUUGGUACAUGACAGAGGTT
Site-directed mutagenesis	
c.40C>G:p.L14V F	CCTTGGCCCGAGGGT**G**TCGAGTGCC
c.40C>G:p.L14V R	GGCACTCGA**C**ACCCTCGGGCCAAGG
c.458G>A:p.R153Q F	AAGCGGCAGCC**A**GGGGGCGCTGG
c.458G>A:p.R153Q R	CCAGCGCCCCC**T**GGCTGCCGCTT
c.515C>G:p.P172R F	CACTGCCCTGC**G**CAGCCTCCGGG
c.515C>G:p.P172R R	CCCGGAGGCTG**C**GCAGGGCAGTG
c.559G>A:p.G187S F	CTGGTGGTGGC**A**GCGGCGGCACC
c.559G>A:p.G187S R	GGTGCCGCCGC**T**GCCACCACCAG

F, forward primers; R, reverse primers. Underlines represent restriction sites placed in the primers, GGTACC: *Kpn* Ⅰ , AAGCTT:*Hind Ⅲ*. VE-cadherin comp represents competition probe. Si-VE-cad: SiRNA VE-cadherin. Boldface indicates nucleotide changes in the oligo sequences.