**SUPPLEMENTAL MATERIAL**

# The pro-angiogenesis effect of miR33a-5p/Ets-1/DKK1 signaling in ox-LDL induced HUVECs

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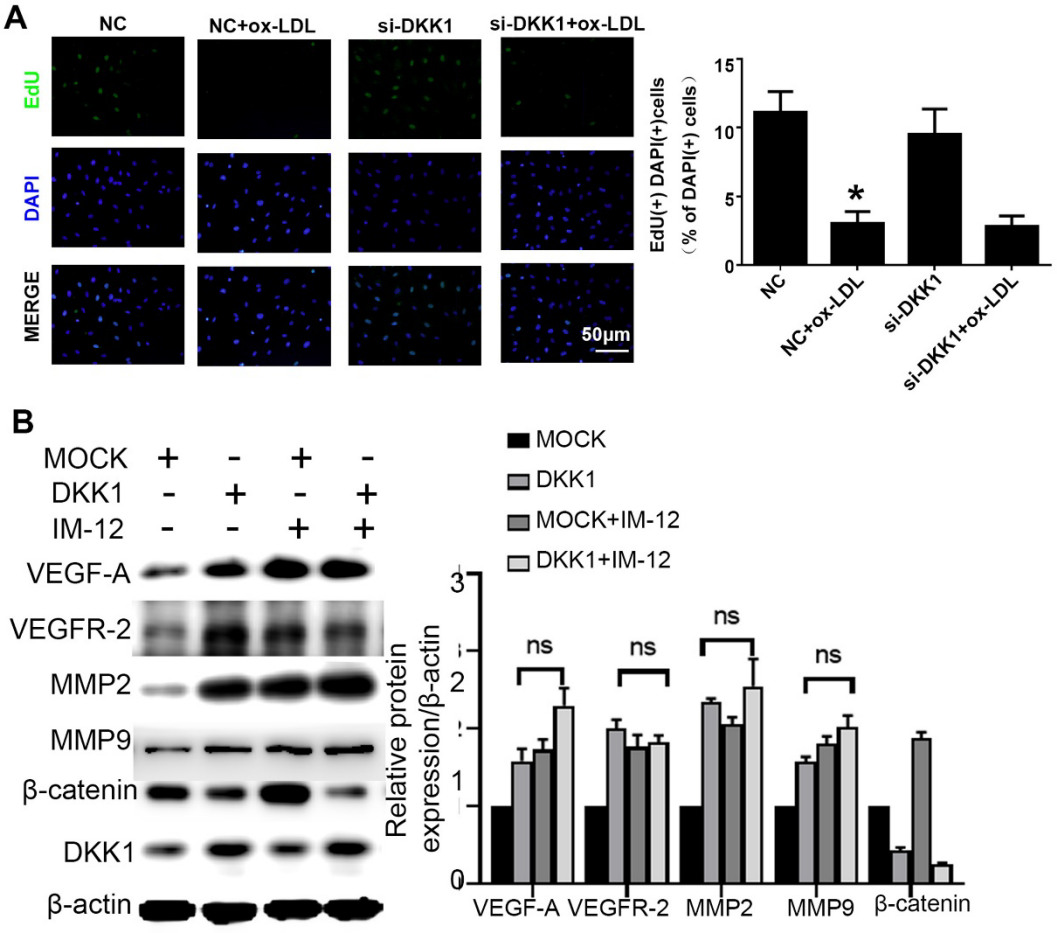


**Figure S1. DKK1 did not participate in the proliferation of ox-LDL-treated HUVECs, and DKK1-induced angiogenesis did not occur via the canonical Wnt/β-catenin pathway. (A)** HUVECs were transiently transfected with negative control (NC) and DKK1 siRNA (si-DKK1) for 24h and then treated with 150 μg/ml ox-LDL for 6h. The percentage of Edu (+) cells was calculated and quantified. Bars indicate 50 μm**. (B)** Cells were pretreated with DMSO or IM-12 (3 μM) for 1 h and then transfected with lenti-DKK1. Western blot to quantify the VEGF-A, VEGFR-2, MMP2, MMP9 and β-catenin protein levels. The data are shown as the mean±SEM. n=6. **P*<0.05 vs.NC.

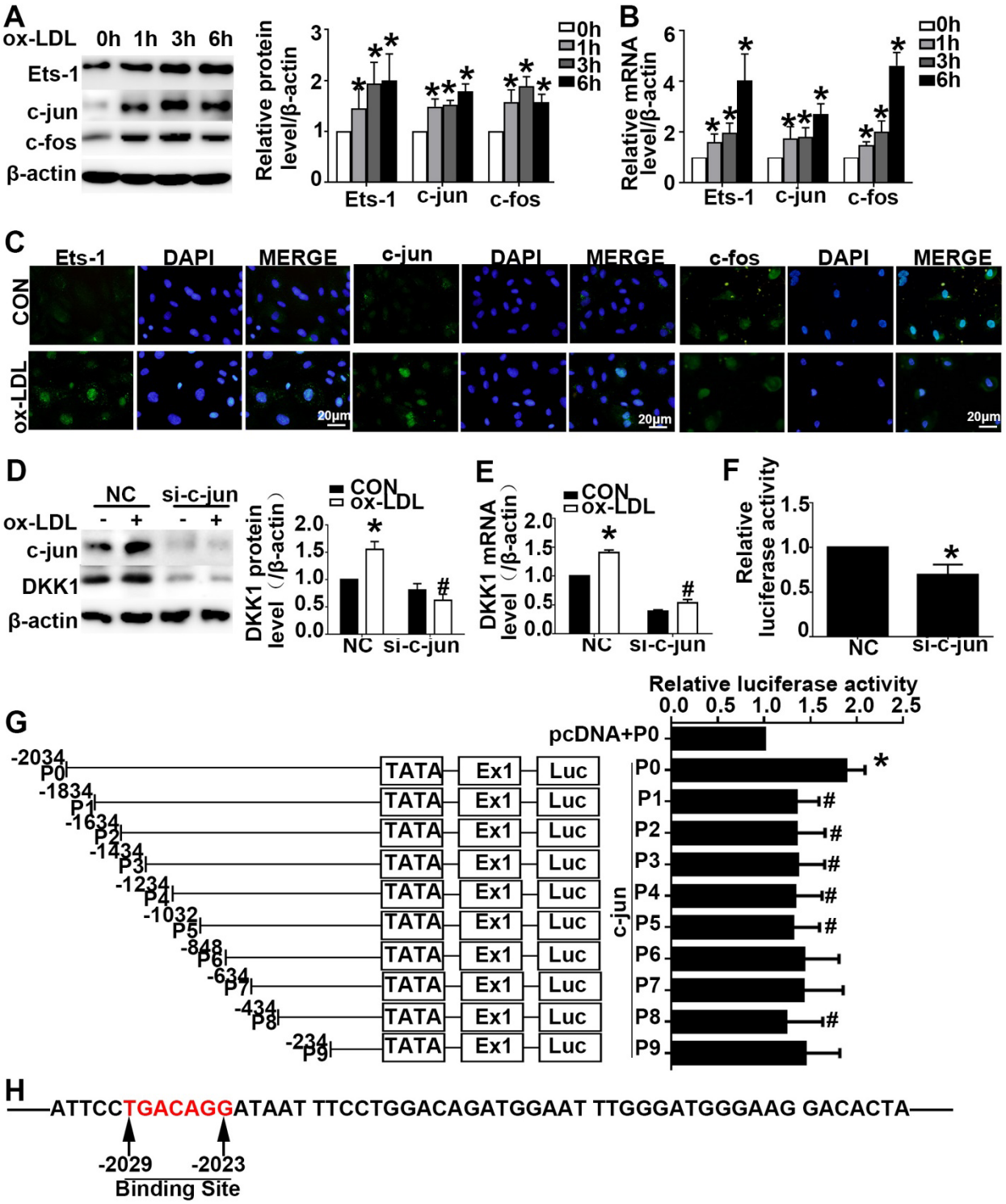


**Figure S2. c-jun promotes the expression of DKK1 by binding with a key positive regulatory region of the human DKK1 promoter. (A-B)** HUVECs treated with ox-LDL (150 μg/ml) for various lengths of time (0h, 1h, 3h, and 6h). Western blot and qRT-PCR to quantify the Ets-1, c-jun or c-fos protein and mRNA levels. **(C)** The immunofluorescence localization of Ets-1, c-jun or c-fos in HUVECs treated with ox-LDL (150 μg/ml) for 6h. Bars indicate 20 μm**. (D-E)** HUVECs were transiently transfected with NC or c-jun siRNA (si-c-jun) for 24h and then treated with 150 μg/ml ox-LDL for 6h. Western Blot and qRT-PCR were used to quantify the DKK1 protein and mRNA levels**. (F-G)** The luciferase activities were analyzed: **(F)** Full-length DKK1 promoter in 293T cells transfected with c-jun siRNA. **(G)** Rough characterization of the DKK1 promoter using a serial fragment from -2034 to -234bp. pcDNA3.0+P0, pcDNA3.0-c-jun +P0-P9 were transfected into 293T cells. **(H)** The prediction of c-jun binding sites in this region (-2034 ~ -1834bp) of the DKK1 promoter: Binding site (-2029 to -2023bp). The data are shown as the mean±SEM. n=6. **P*<0.05 vs. the untreated group or NC; #*P*<0.05 vs. NC+ox-LDL or P0+ PCDNA3.1-c-jun.

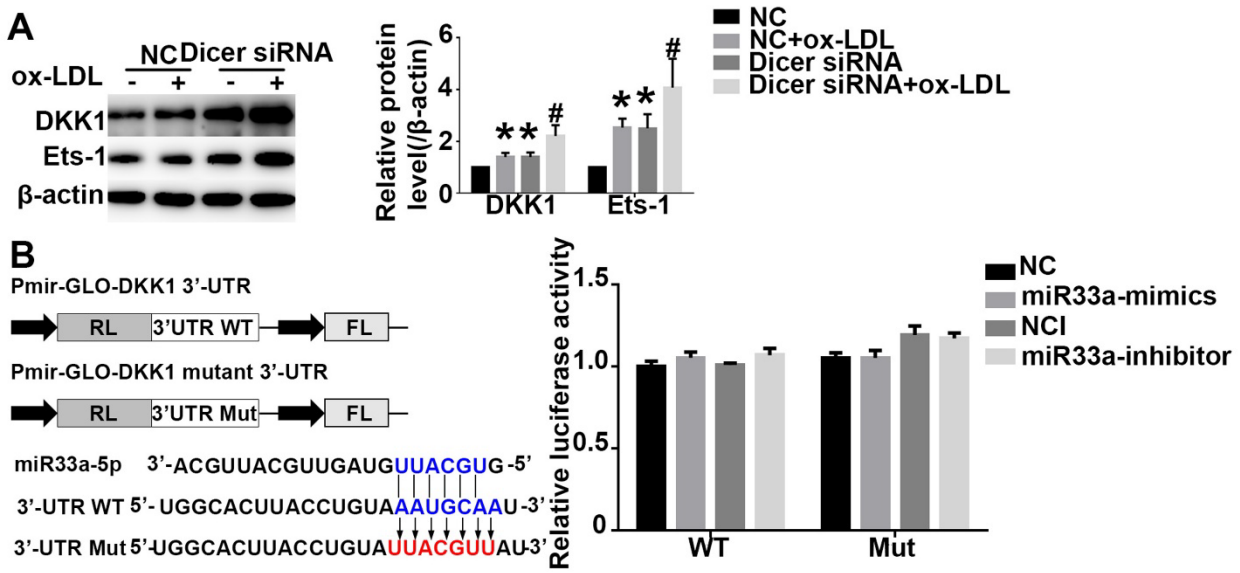


**Figure S3. MiRNA participated in the upregulation of Ets-1 and DKK1 in ox-LDL-induced HUVECs, but miR33a-5p did not directly targeted DKK1. (A)** HUVECs were transiently transfected with NC or Dicer siRNA for 24h and then treated with 150 μg/ml ox-LDL for 6h. Western blotting was used to quantify the Ets-1 and DKK1 protein levels. **(B)** Possible binding sites for miR33a-5p in the DKK1 3'-UTR, as predicted. A miR target reporter luciferase assay was performed after the miR33a-5p mimic and inhibitor were delivered to 293T cells. The results were normalized to data obtained from an assay with Renilla luciferase. The data are presented as the mean ± SEM. n=6. **P*<0.05 vs. the NC group or the control group; #*P*<0.05 vs. NC+ ox-LDL or P0+ PCDNA3.1-c-jun.

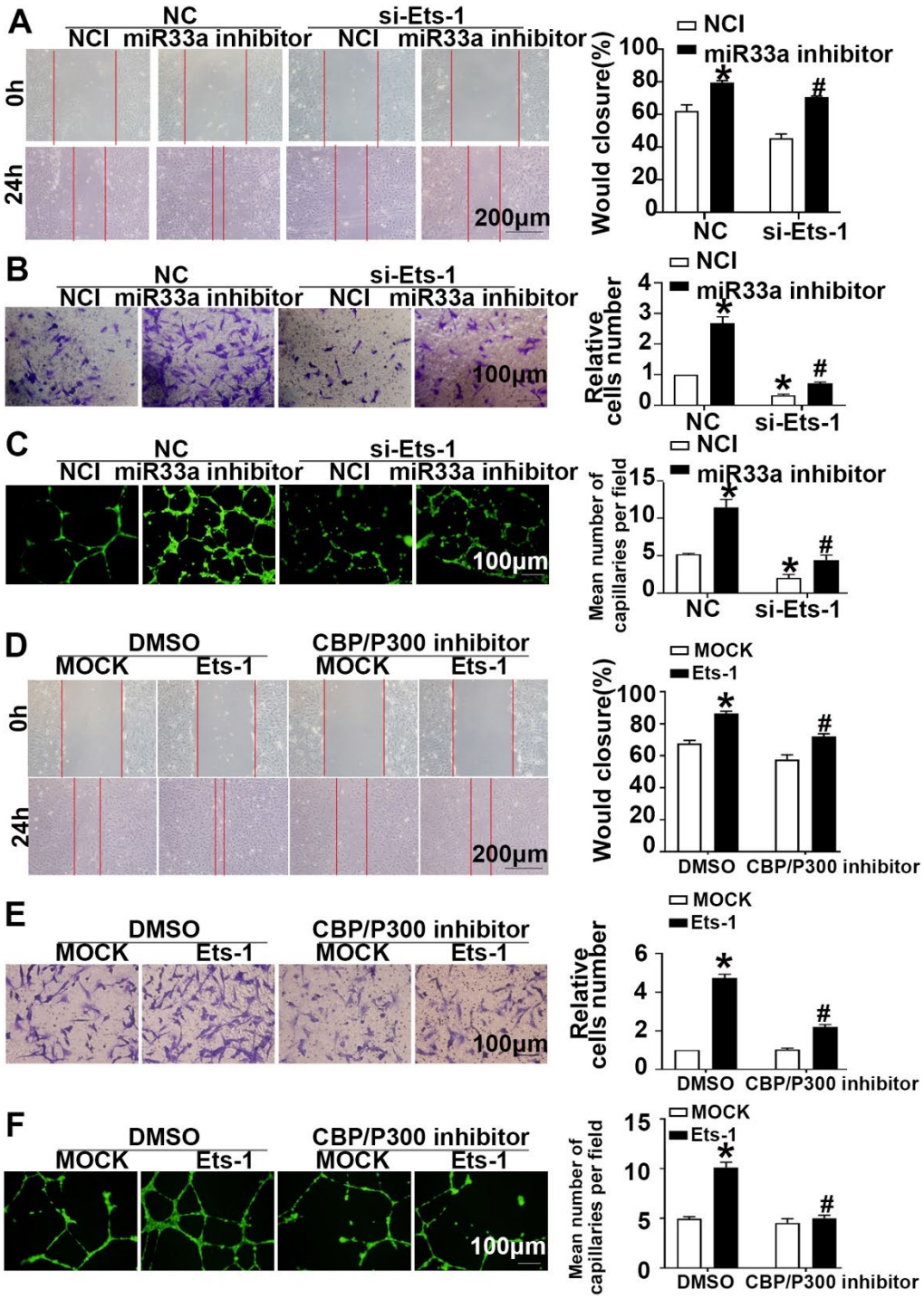


**Figure S4. miR33a-5p/Ets-1 participated in the angiogenic effects in HUVECs via assistance of CBP/P300.**

**(A-C)** HUVECs were cotransfected with an miR33a inhibitor and Ets-1 siRNA. **(D-F)** HUVECs were pretreated with DMSO or a CBP/P300 inhibitor (25 μM) for 1h after transfection with lenti-Ets-1(Ets-1). Representative images and quantification of cell migration in Transwell assays. The cell counts on the bottom of the Transwell are shown here: **(A, D)** Representative images and quantification of cell migration in Transwell assays. The cell counts on the bottom of the Transwell are shown here. Bars indicate 200 μm**. (B, E)** Representative images and quantification of cell migration in the in vitro scratch wound assay (relative to 0 h) were obtained at 0h and 24h post-wounding. Bars indicate 100 μm**. (C, F)** Representative image and quantification of the tube length (% of control). Bars indicate 100 μm**.** The data are presented as the mean±SEM. n=6. **P*<0.05 vs. the NCI group or the DMSO group; #*P*<0.05 vs. the miR33a-5p inhibitor or the DMSO+Ets-1 group.

**Major Resources Table**

**Animals (in vivo studies)**

| Species | Vendor or Source | Background Strain | Sex |
|---|---|---|---|
| ApoE-/- mice | Beijing HFK Bioscience Co.,Ltd. | C57BL/6J | male |

**Antibodies**

| Target antigen | Vendor or Source | Catalog # | Working concentration |
|---|---|---|---|
| β-actin | Cell Signaling Technology | 3700 | WB(diluted1:1000) |
| DKK1 | Abcam | ab109416 | WB( diluted 1:500); IHC(diluted1:100) |
| P300 | Abcam | ab14984 | WB( diluted 1:500); |
| CBP | Abcam | ab2832 | WB( diluted 1:500); IP(diluted1:50) |
| c-fos | Abcam | ab214672 | WB( diluted 1:1000); IF(diluted1:200) |
| c-jun | Abcam | ab32137 | WB( diluted 1:2000); IF(diluted1:200) |
| Ets-1 | Abcam | ab96478(mice antibody) | WB( diluted 1:1000); IF(diluted1:400); ChIP (diluted 1:50) |
| Ets-1 | Cell Signaling Technology | 14069(rabbit antibody) | WB(diluted1:1000) ;IP (diluted 1:50) |
| IgG1 | Cell Signaling Technology | 3900 | IP (diluted 1:50) |
| CD31 | Abcam | ab281583 | IHC(diluted1:50) |
| VEGF-A | Abcam | ab52917 | WB( diluted 1:10000); IHC(diluted1:250) |
| VEGFR-2 | Abcam | ab39638 | WB( diluted 1:1000); IHC(diluted1:100) |
| MMP2 | Abcam | ab37150 | WB( diluted 1:1000); IHC(diluted1:50) |
| MMP9 | Abcam | ab38898 | WB( diluted 1:1000); IHC(diluted1:50) |
| PI3K | Cell Signaling Technology | 4249 | WB(diluted1:1000) |
| CKAP4 | Abcam | ab152154 | WB(diluted1:10000) |
| β-catenin | Cell Signaling Technology | 8480 | WB(diluted1:1000) |

**DNA/cDNA Clones**

| Clone Name | Sequence |
|---|---|
| β-actin primer | Forward: CGTGCGTGACATTAAGGAGA<br>Reverse: CACCTTCACCGTTCCAGTTT |
| DKK1 primer | Forward: ATAGCACCTTGGATGGGTATTCC<br>Reverse: CTGATGACCGGAGACAAACAG |
| Ets-1 primer | Forward:TACACAGGCAGTGGACCAATC<br>Reverse: CCCCGCTGTCTTGTGGATG |
| c-fos primer | Forward: CACTCCAAGCGGAGACAGAC<br>Reverse: AGGTCATCAGGGATCTTGCAG |
| c-jun primer | Forward: TCCAAGTGCCGAAAAAGGAAG<br>Reverse: CGAGTTCTGAGCTTTCAAGGT |
| U6 primer | Forward: CAGCACATATACTAAAATTGGAAGG<br>Reverse: ACGAATTTGCGTGTCATCC |

| | |
|---|---|
| miR33a-5p primer | Forward: CCTCATAAGCGGTGCATTGTA<br>Reverse:TATGCTTGTTCTCGTCTCTGTGTC |
| biotin-DKK1-S | TGACAGGATAATTTCCTGGACAGATGGAATTTGGGATGGGAAGGA |
| DKK1-S | TGACAGGATAATTTCCTGGACAGATGGAATTTGGGATGGGAAGGA |
| DKK1-A | TCCTTCCCATCCCAAATTCCATCTGTCCAGGAAATTATCCTGTCA |
| DKK1-MUT1-S | TGTAGCTGCACGTTCCTGGACAGATGGAATTTGGGATGGGAAGGA |
| DKK1-MUT1-A | TCCTTCCCATCCCAAATTCCATCTGTCCAGGAACGTGCAGCTACA |
| DKK1-MUT2-S | TGACAGGATAATTTCCTGGACAGGCTCGCATTGGGATGGGAAGGA |
| DKK1-MUT2-A | TCCTTCCCATCCCAATGCGAGCCTGTCCAGGAAATTATCCTGTCA |
| DKK1-MUT3-S | TGACAGGATGGTTTCCTGGACAGATGGAATTTGGGAGCTGCACGA |
| DKK1-MUT3-A | TCGTGCAGCTCCCAAATTCCATCTGTCCAGGAAATTATCCTGTCA |
| NC | Sense：UUCUCCGAACGUGUCACGUTT<br>Antisense：ACGUGACACGUUCGGAGAATT |
| c-fos siRNA | Sense: CUGUCAACGCGCAGGACUUTT<br>Antisense: AAGUCCUGCGCGUUGACAGTT |
| c-jun siRNA | Sense: CUGAUAAUCCAGUCCAGCATT<br>Antisense: UGCUGGACUGGAUUAUCAGTT |
| Ets-1 siRNA | Sense: AUGAUGUCUCAAGCAUUAAdTdT<br>Antisense: UUAAUGCUUGAGACAUCAUdTdT |
| CKAP4 siRNA | Sense:GGAAUGAUCUGGAUAGGUUTT<br>Antisense:AACCUAUCCAGAUCAUUCCTT |
| has-miR33a-5p-mimics | Sense: GUGCAUUGUAGUUGCAUUGCA<br>Antisense: CAAUGCAACUACAAUGCACUU |
| NCI | CAGUACUUUUGUGUAGUACAA |
| has- miR33a-5p-inhibitor | UGCAAUGCAACUACAAUGCAC |

**Cultured Cells and reagents**

| **Name** | **Source and working concentration** |
|---|---|
| HUVECs | ScienCell |
| HEK-293T | American Type Culture Collection |
| ox-LDL | Yiyuan Biotechnologies(Guangzhou, China), 150 μg/ml |
| CBP/P300 inhibitor | Abcam，ab142163,2ug/ml |
| IM-12 | Selleck, S7566，3 μM |
| 740 Y-P | Selleck, S7865, 50 μg/ml |