Supplemental Information

Exosomal lncRNA-H19 promotes osteogenesis and angiogenesis through mediating Angpt1/Tie2-NO signaling in CBS- heterozygous mice

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Running title: Role of the exosome in bone formation and vascularization in CBS-heterozygous mice.

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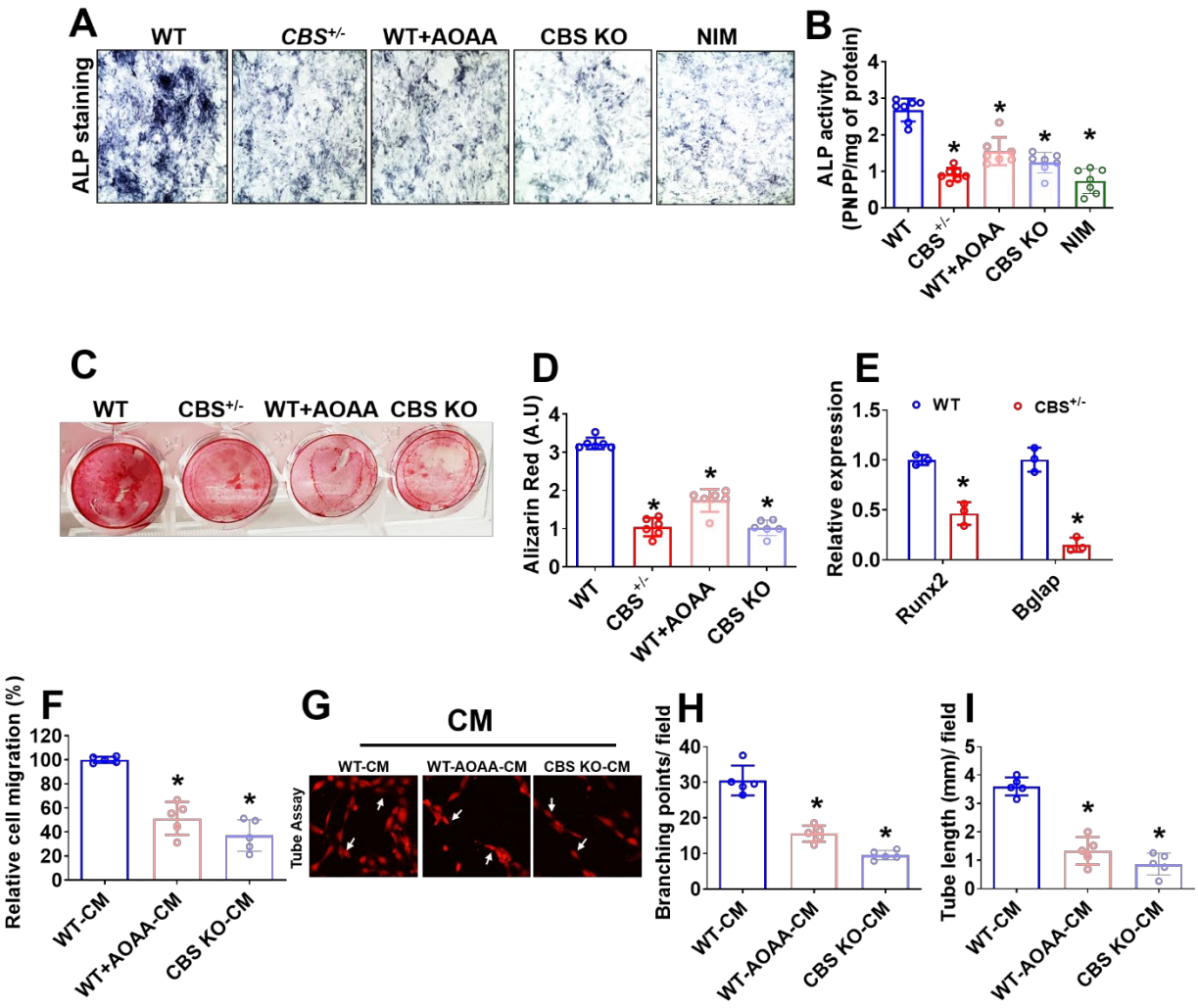
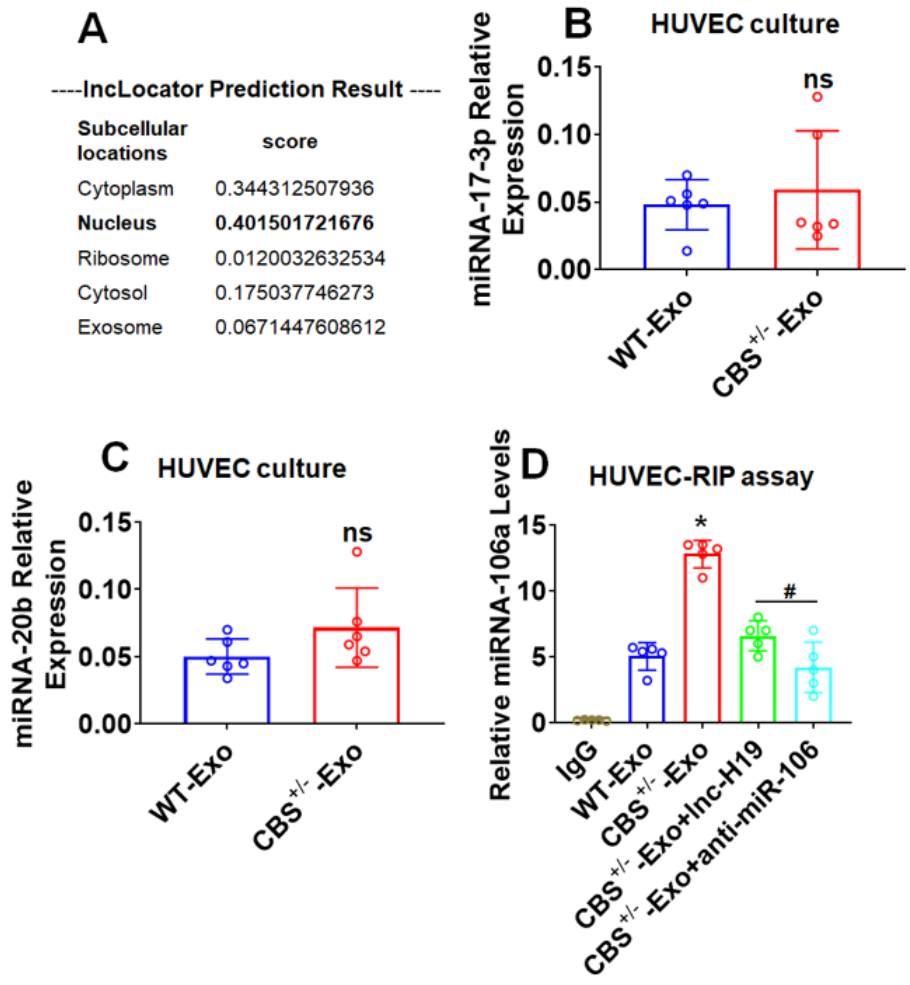
Supplementary Figure S1.



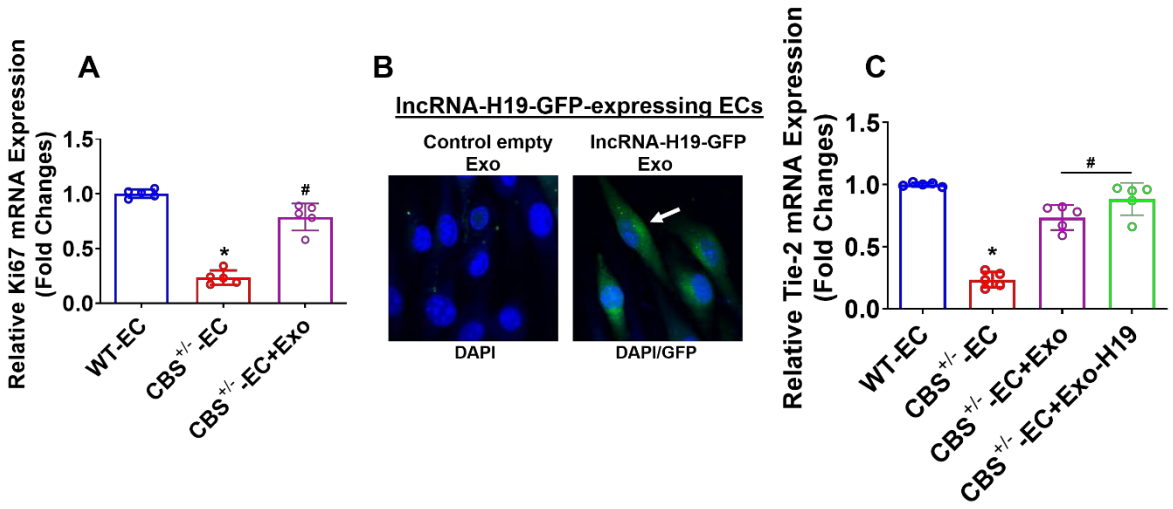
Supplementary Figure S1. Osteogenesis of BMMSCs in the different experimental mice. (A-D) BMMSCs were cultured under osteogenic induction medium (OIM) and non-induction medium (NIM) in different experimental conditions. Osteogenic differentiation was confirmed by both ALP on Day 7 (A-B) and ARS staining on day 21 (C-D) respectively. The scale bar represents 200 μm. (E) Expression profiles of the osteogenic markers, Runx2, and Bglap were examined by qPCR analysis. (F) ECs migration was tested using Trans-well migration assay. (G-I) 3D-matrigel tube formation assay of ECs was performed. The photographs (X20) were taken in five random fields. The scale bar represents 200 μm. The tube length and branching point/field were quantified by using AngioQuant image software. Results were repeated at least three times. All data are expressed as mean±SEM. n=5-7 mice for all groups. *p< 0.05 compared with the WT.

Supplementary Figure S2.



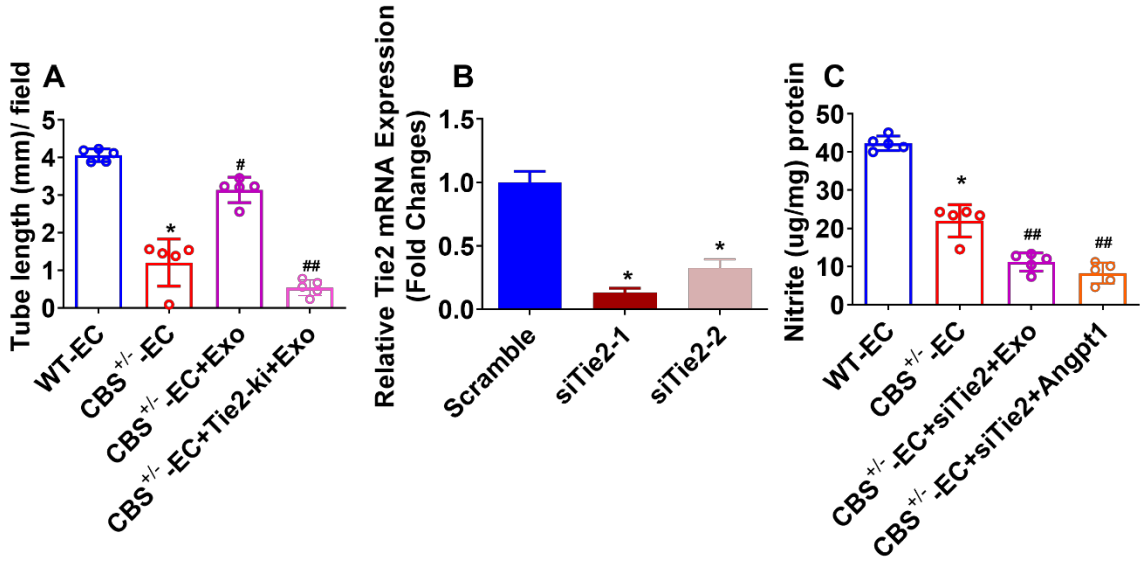
Supplementary Figure S2. miRNA transcript expression of miR-17-3p family members and RNA immunoprecipitation in HUVEC culture. (A) Prediction of lncRNAH19 cellular location using lncLocator Database (http://www.csbio.sjtu.edu.cn/bioinf/ lncLocator/) (B-C) miR-17-3p family members: miR-17-3p and miR-20b were analyzed in HUVEC culture under CBS$^{+/-}$-Exo treatment by qPCR. (D) HUVEC culture was transfected with synthetic lncH19 or anti-miR-106a before the Exo treatment. RNA immunoprecipitation assay was performed to assess the physical interaction of H19 with the RISC complex by using a specific antibody against Ago2 protein. Results were repeated at least three times. All data are expressed as mean±SEM. n=5-6 mice for all groups. *p< 0.05 compared with the WT-Exo and #p< 0.05 compared with the CBS$^{+/-}$-Exo.

Supplementary Figure S3.



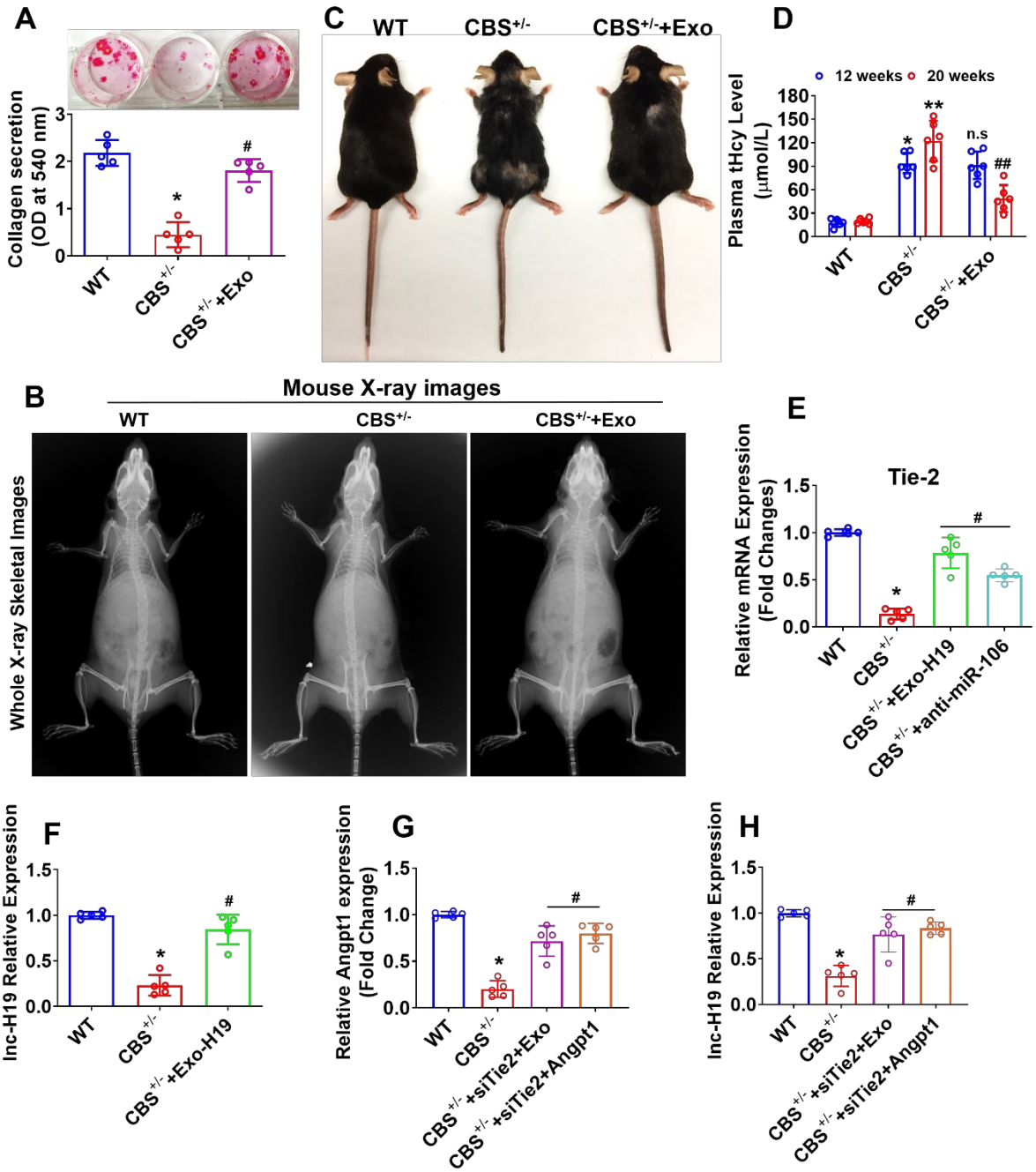
Supplementary Figure S3. mRNA transcript expression of proliferation marker Ki67 and effect of engineered Exo with lnc-H19 on Tie-2 expression in the endothelium. (A) Ki67 mRNA transcript expression by qPCR analysis. (B) We used Exo-Fect to transfect 5 µg of H19 (GFP-tagged) plasmid DNA into exosomes, and then added the plasmid-loaded exosomes to ECs. The cells were imaged after 48 h for detection of GFP protein signal. The appearance of GFP signal (plasmid cargo, white arrow) in ECs indicates successful cargo delivery. (C) Tie-2 mRNA transcript expression in ECs qPCR analysis. Results were repeated at least three times. All data are expressed as mean±SEM. n=5 mice for all groups. *p< 0.05 compared with the WT-EC and #p< 0.05 compared with the $CBS^{+/-}$-EC.

Supplementary Figure S4.



Supplementary Figure S4. The inhibition of Tie-2 expression reduces angiogenesis and NO metabolite level in the endothelium. (A) The effect of Tie-2 inhibition by potent Tie-2 inhibitor (1.3nM) reduces 3D-Matrigel angiogenesis. **(B)** qPCR assay was performed to confirm the efficacy of the siRNA1 and siRNA2 based Tie2 gene knockdown in endothelial culture. **(C)** NO metabolite nitrite was measured using the Griess assay method. Results were repeated at least three times. All data are expressed as mean±SEM. n=5 mice for all groups. *p< 0.05 compared with the WT-EC, #p< 0.05 compared with the $CBS^{+/-}$-EC, and ##p< 0.05 compared with the $CBS^{+/-}$-EC+Exo.

Supplementary Figure S5.



Supplementary Figure S5. Exo effect on collagen formation and skeleton. (A) Sirius Red dye staining of the collagen matrix of BMMSCs culture treated with Exo at days 14. (B) X-ray images of the whole skeleton of experimental mice. (C) Representative view of 14-weeks-old WT, $CBS^{+/-}$ and $CBS^{+/-}$+Exo mice. (D) Total homocysteine (tHcy) was measured in plasma of experimental mice following transplantation of Exo for 8-weeks. (E) Tie-2 mRNA transcript expression was

studied under Exo-H19 treatment in BMMSCs by qPCR analysis. (F) H19 expression was performed in BMMSCs using qPCR analysis. (G-H) Angpt1 and H19 expression was quantified in BMMSCs culture using qPCR assay. All data are expressed as mean±SEM. n=5-7 mice for all groups. *p< 0.05 compared with the WT-BMMSC and #p< 0.05 compared with the $CBS^{+/-}$-BMMSC. Supplementary Figure S5a: Exo were treated at the concentration of 100 µg to obtain the data from an *in vitro* culture experiment. Supplementary Figure S5b, c, d: Exo were treated at the concentration of 100 µg to obtain the data from an *in vivo* experiments.

Supplementary Table S1: Sequences of PCR primers used for real-time quantitative PCR

Gene Name	**Primer sequence 5'-3'**
Runx2	FP: TTTAGGGCGCATTCCTCATC RP: TGTCCTTGTGGATTGAAAGGAC
Bglap	FP:GCGCTCTGTCTCTCTGACCT RP: ACCTTATTGCCCTCCTGCTT
Angpt1	FP: TGC AGC AAC CAG CGC CGA AA RP: CAG GGC AGT TCC CGT CGT GT
Tie-2	FP:GATTTTGGATTGTCCCGAGGTCAAG RP:CACCAATATCTGGGCAAATGATGG
Ki67	FP:AATCCAACTCAAGTAAACGGGG RP:TTGGCTTGCTTCCATCCTCA
GAPDH	FP: TGCACCACCAACTGCTTGC RP: GGCATGGACTGTAGTCAGAG
LncRNA-H19	FP: ATCGGTGCCTCAGCGTTCGG RP:CTGTCCTCGCCGTCACACCG
miRNA-106	FP:GATGCTCAAAAAGTGCTTACAGTGCA RP:TATGGTTGTTCTGCTCTCTGTCTC
miRNA-17-3p	FP:CCCGGGGAGCTCTAAAAATTGCACACA RP:GGGCCCACGCGTCTATCTTGCGCTCCTG
miRNA-20b	FP:GAGCTCTAAAAATTGCACACACT RP:CGTCTATCTTGCGCTCCTGAAAAACT
U6snRNA	FP:CTCGCTTCGGCAGCACA RP:AACGCTTCACGAATTTGCGT

Footnotes: Runt-related transcription factor 2 (RUNX2), bone gamma-carboxyglutamic acid-containing protein (Bglap), Angiopoietin 1 (Angpt1)

Supplementary Table S2: Primary antibodies used

Antibody	Clone	Dilution	Source	Cat. No
Western blot				
Rabbit Anti-RUNX2 antibody	polyclonal	1:250	Abcam	ab23981
Rabbit Anti-Osteocalcin antibody		1:250	Abcam	ab93876
Mouse anti-Tie-2 Antibody	(3A5)	1:200	Santacruz Biotechnology	sc-293414
Rabbit Phospho-Tie2 (Tyr992) Antibody	polyclonal	1:250	Cell signaling	#4221
Mouse anti-eNOS Antibody	Monoclonal	1:250	Abcam	Ab76198
Rabbit Phospho-eNOS (Ser1177) Antibody	polyclonal	1:250	Cell signaling	#9571
Rabbit Anti-CD63 antibody	polyclonal	1:200	Abcam	Ab134045
Rabbit Anti-CD9 antibody	Monoclonal	1:250	Abcam	Ab2215
Flow cytometry and immunofluorescence				
FITC Anti-mouse CD73 antibody	TY/11.8	1:200	Biolegend	127219
FITC Rat IgG1, κ Isotype Ctrl Antibody	RTK2071	1:200	Biolegend	400405
PE anti-mouse/human CD44 Antibody	IM7	1:200	Biolegend	103023
PE Rat IgG2b, κ Isotype Ctrl Antibody	RTK4530	1:200	Biolegend	400607