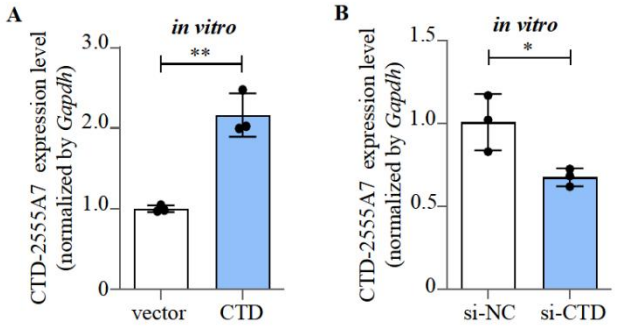
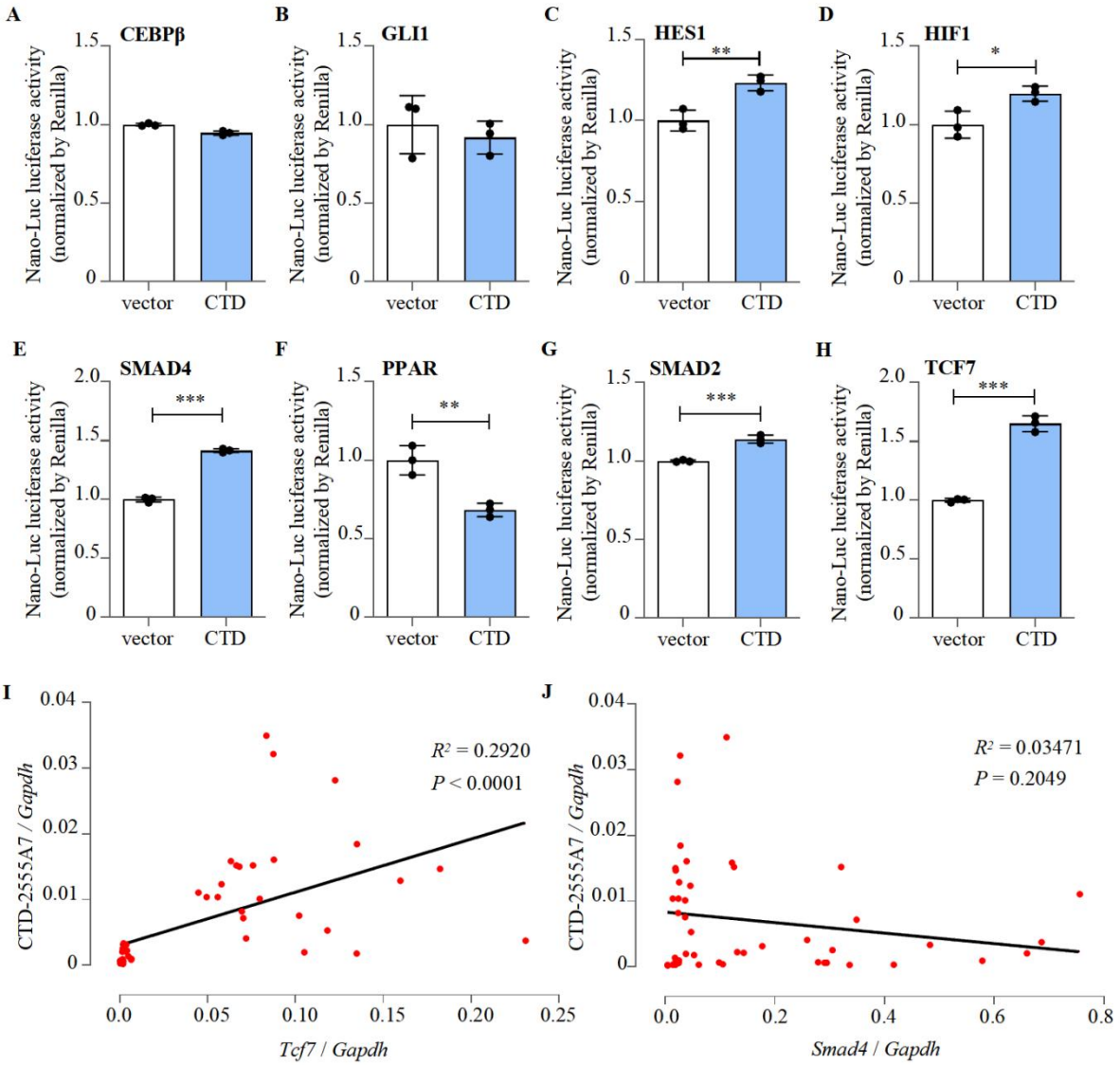


Supplemental Figure 1. Effects of CTD-2555A7.2 over-expression plasmid and CTD-2555A7.2 siRNA

A. CTD-2555A7.2 expression levels of hMSCs treated with CTD-2555A7.2 over-expression plasmid (compared with pCDNA3.1 plasmid), as detected by RT-PCR (mean ± SD, $^{**}P < 0.01$, n = 3). vector: pCDNA3.1 plasmid. CTD: plasmid containing CTD-2555A7.2 full length.

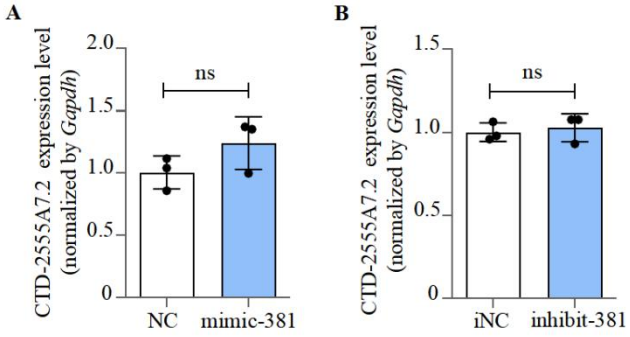
B. CTD-2555A7.2 expression levels of hMSCs treated with CTD-2555A7.2 siRNA (compared with negative control siRNA, mean ± SD, $^{*}P < 0.05$, n = 3). si-NC: negative control siRNA. si-CTD: CTD-2555A7.2 siRNA.



Supplemental Figure 2. Screening of CTD-2555A7.2 downstream signaling pathway

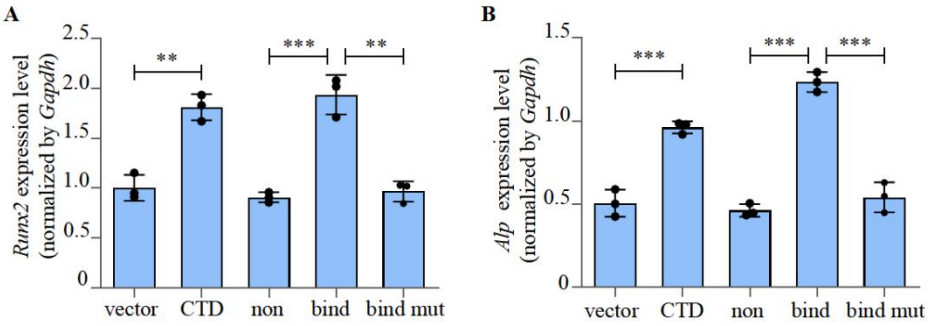
A-H. CEBPβ/ GLI1/ HES1/ HIF1/ SMAD4/ PPAR/ SMAD2/ TCF7 activities of hMSCs treated with CTD-2555A7.2 over-exprssion plasmid (compared with pCDNA3.1 plasmid), as detected by luciferase reporter assay (mean ± S.D., **P* < 0.05, ***P* < 0.01, ****P* < 0.001, n = 3). vector: pCDNA3.1 plasmid. CTD: CTD-2555A7.2 over-exprssion plasmid.

I-J. Correlation analysis between CTD-2555A7.2 level and *Tcf7* or *Smad4* mRNA levels in bone tissues from osteoporosis patients, as detected by RT-PCR.



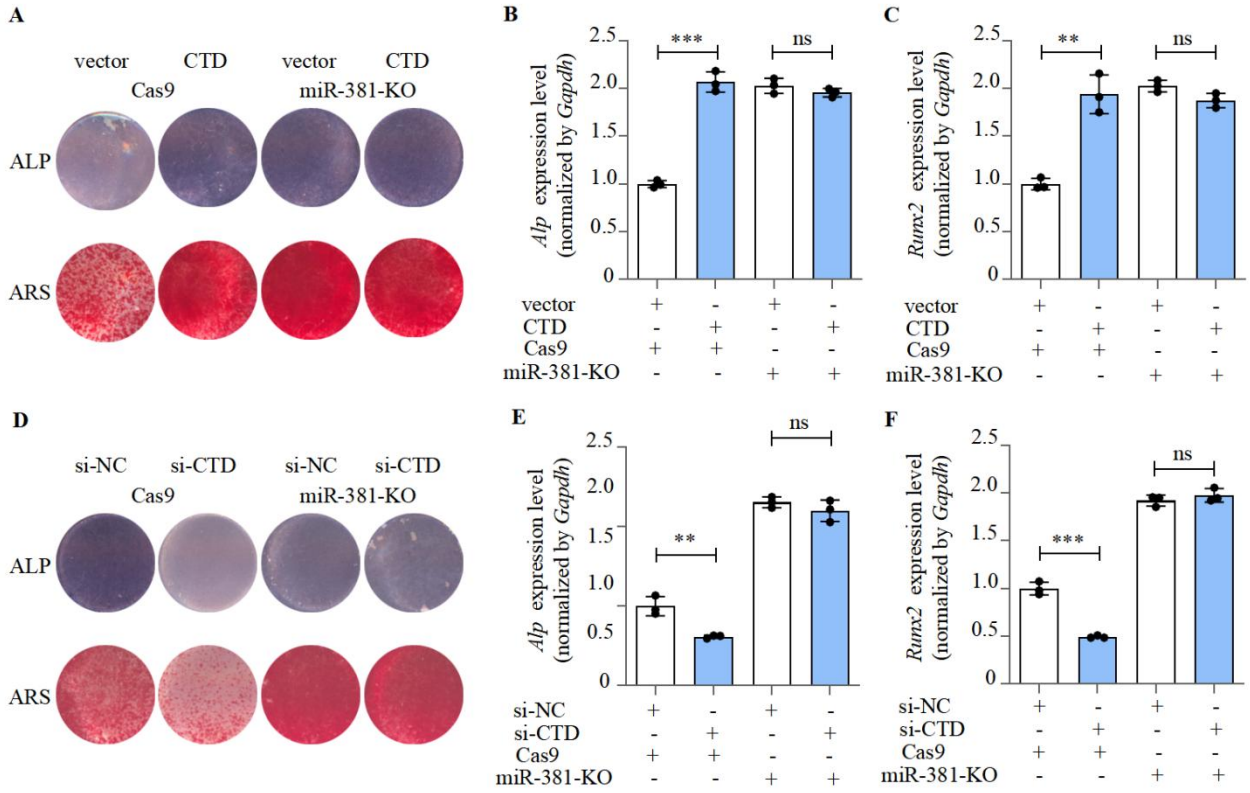
Supplementary Figure 3. The regulation effect of miR-381-3p on CTD-2555A7.2

A-B. CTD-2555A7.2 expression levels of hMSCs treated with mimic-381-3p (left) or inhibitor-381-3p (right), as detected by RT-PCR (mean ± SD, n = 3). NC: mimic-NC. mimic-381: mimic-381-3p. iNC: inhibitor-NC. inhibit-381: inhibitor-381-3p.



Supplementary Figure 4. CTD-2555A7.2 binding region promoted osteogenic differentiation genes

A-B. *Runx2* and *Alp* expression levels of hMSCs treated with CTD-2555A7.2 expression plasmids of different regions, as detected by RT-PCR (mean ± SD, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3). vec: pCDNA3.1 plasmid. CTD: plasmid containing CTD-2555A7.2 full length. non: plasmid containing CTD-2555A7.2 non-binding region. bind: plasmid containing CTD-2555A7.2 binding region. bind mut: plasmid containing CTD-2555A7.2 mutant binding region.



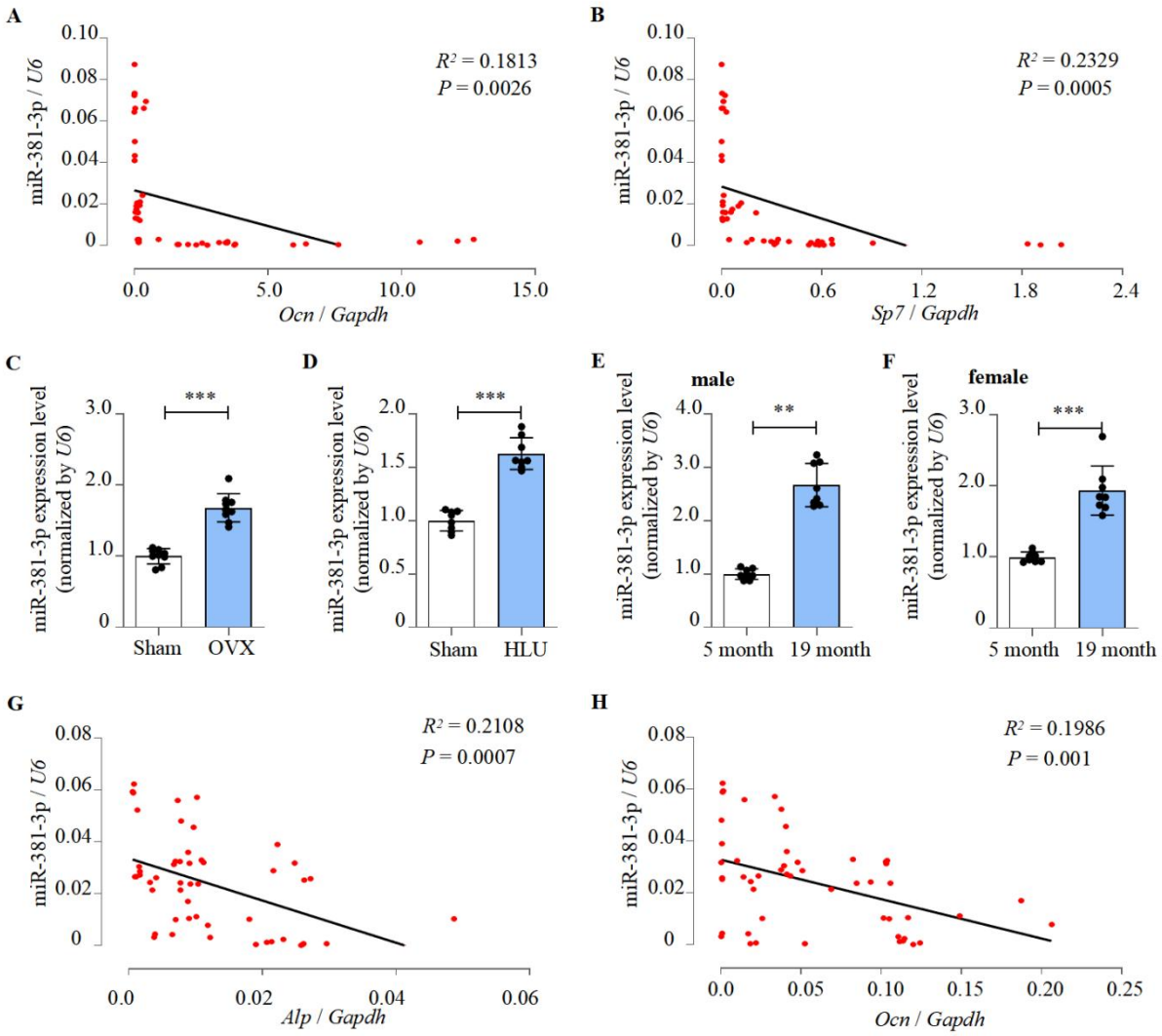
Supplementary Figure 5. CTD-2555A7.2 promoted osteogenic differentiation through miR-381-3p

A. ALP and Alizarin Red staining of miR-381-3p knock out hMSCs treated with CTD-2555A7.2 over-expression plasmids, as detected by ALP staining and Alizarin Red staining.

B-C. *Alp* and *Runx2* expression levels of miR-381-3p knock out hMSCs treated with CTD-2555A7.2 over-expression plasmids, as detected by RT-PCR (mean ± SD, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3).

D. ALP and Alizarin Red staining of miR-381-3p knock out hMSCs treated with CTD-2555A7.2 siRNA.

E-F. *Alp* and *Runx2* expression levels of miR-381-3p knock out hMSCs treated with CTD-2555A7.2 siRNA (mean ± SD, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3).



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48 **Supplementary Figure 6. miR-381-3p negatively correlated with osteoporosis**

49 A-B. Correlation analysis between miR-381-3p level and *Ocn*/*Sp7* level in bone tissues from

50 osteoporosis patients, as detected by RT-PCR.

51 C. Expression levels of miR-381-3p in primary BMSCs of ovariectomized (OVX) and control

52 C57BL/6 mice, as detected by RT-PCR (mean ± S.D., ***$P < 0.001$, n = 9).

53 D. Expression levels of miR-381-3p in primary BMSCs of hind limb unloaded (HLU) and control

54 C57BL/6 mice (mean ± S.D., ***$P < 0.001$, n = 8).

55 E-F. Expression levels of miR-381-3p in primary BMSCs of 19 month old and 5 month old male (left)

56 or female (right) C57BL/6 mice (mean ± S.D., **$P < 0.01$, ***$P < 0.001$, n = 8).

57 G-H. Correlation analysis between miR-381-3p level and *Alp* or *Ocn* mRNA levels in C57BL/6 mice,

58 as detected by RT-PCR.

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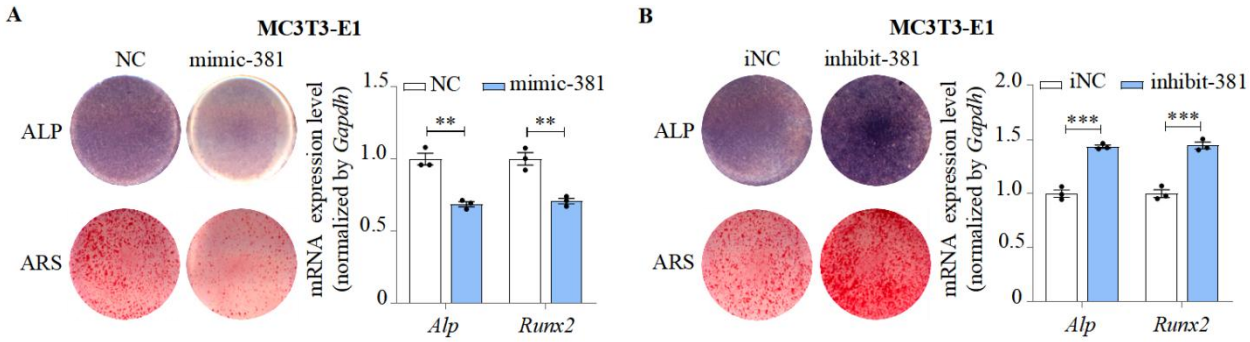
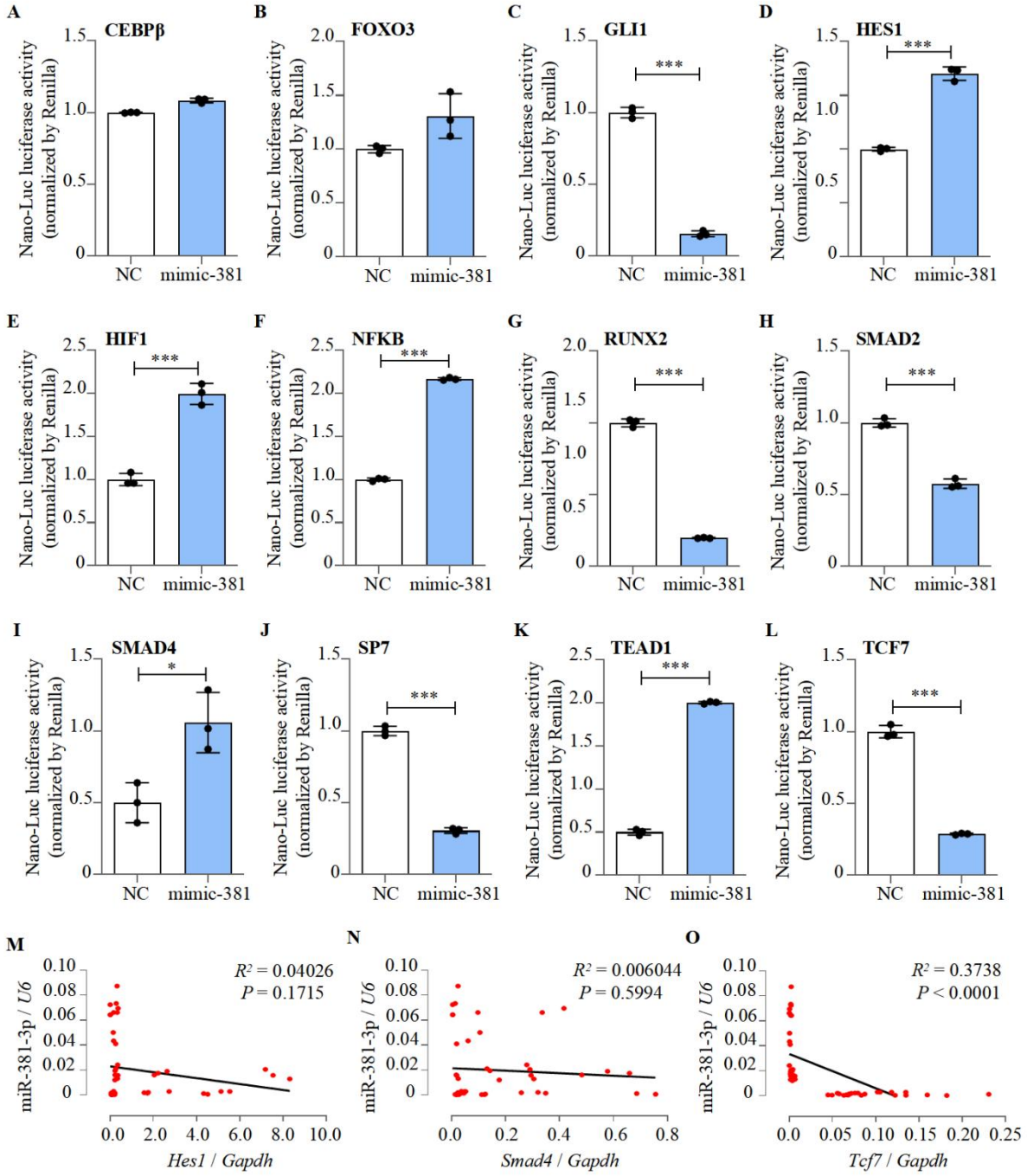


Supplementary Figure 7. miR-381-3p inhibited osteogenic differentiation in MC3T3-E1 cells

A. ALP and Alizarin Red staining of MC3T3-E1 cells treated with mimic-381-3p (compared to mimic-NC), as detected by ALP staining and Alizarin Red staining (left), and *Alp*/*Runx2* expression levels of MC3T3-E1 cells treated with mimic-381-3p, as detected by RT-PCR (right, mean ± S.D., $^{**}P < 0.01$, n = 3).

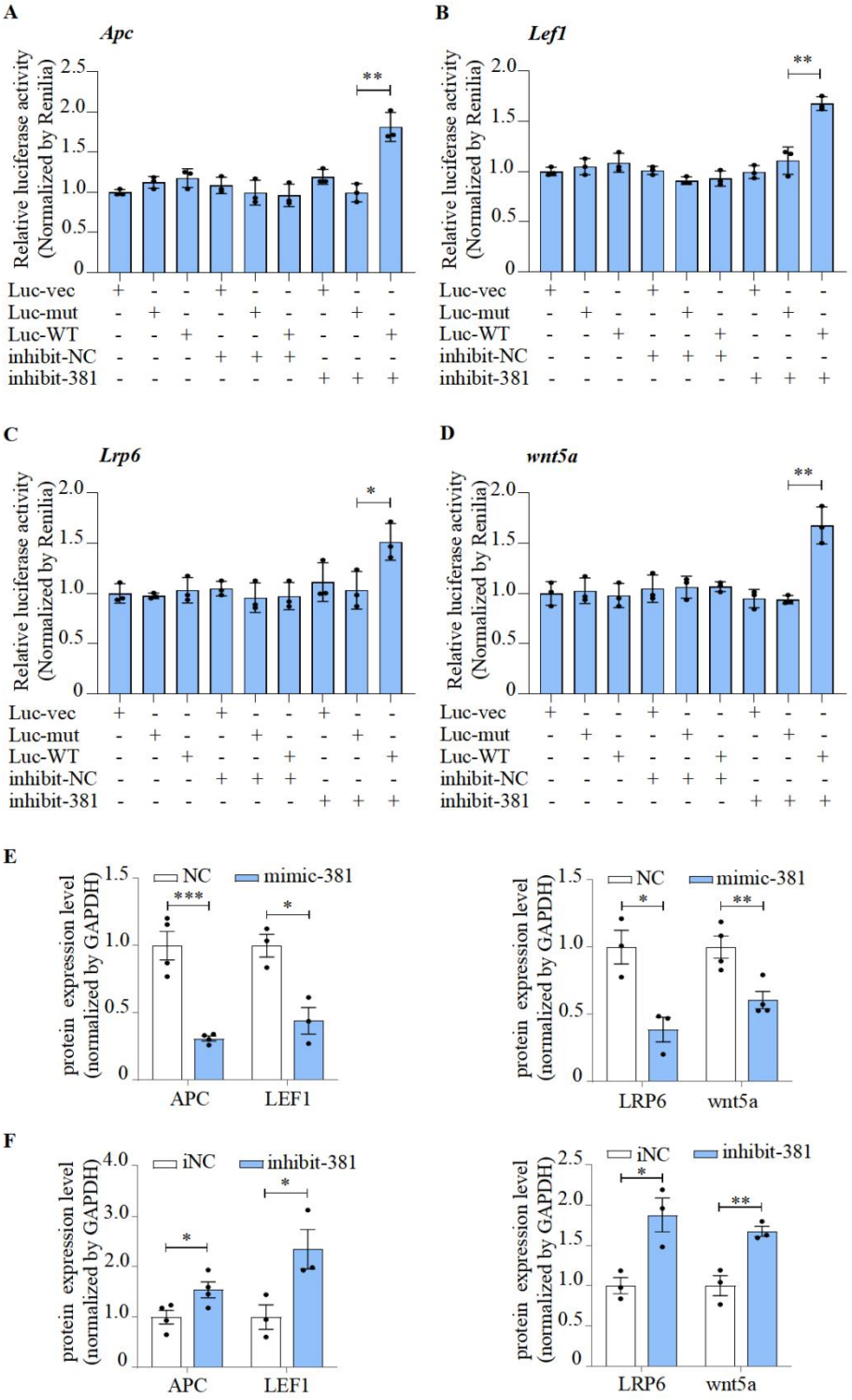
B. ALP and Alizarin Red staining (left), and *Alp*/*Runx2* expression levels (right) of MC3T3-E1 cells treated with inhibitor-381-3p (mean ± S.D., $^{***}P < 0.001$, n = 3).



Supplementary Figure 8. miR-381-3p inhibited Wnt signaling pathway

A-L. CEBPβ/ FOXO3/ GLI1/ HES1/ HIF1/ NFKB/ RUNX2/ SMAD2/ SMAD4/ SP7/ TEAD1/ TCF7 activities of hMSCs treated with mimic-381-3p (compared with mimic-NC), as detected by luciferase reporter assay (mean ± S.D., *$P < 0.05$, ***$P < 0.001$, n = 3).

M-O. Correlation analysis between miR-381-3p level and *Hes1*, *Smad4* or *Tcf7* mRNA levels in femur tissues from C57BL/6 mice, as detected by RT-PCR.

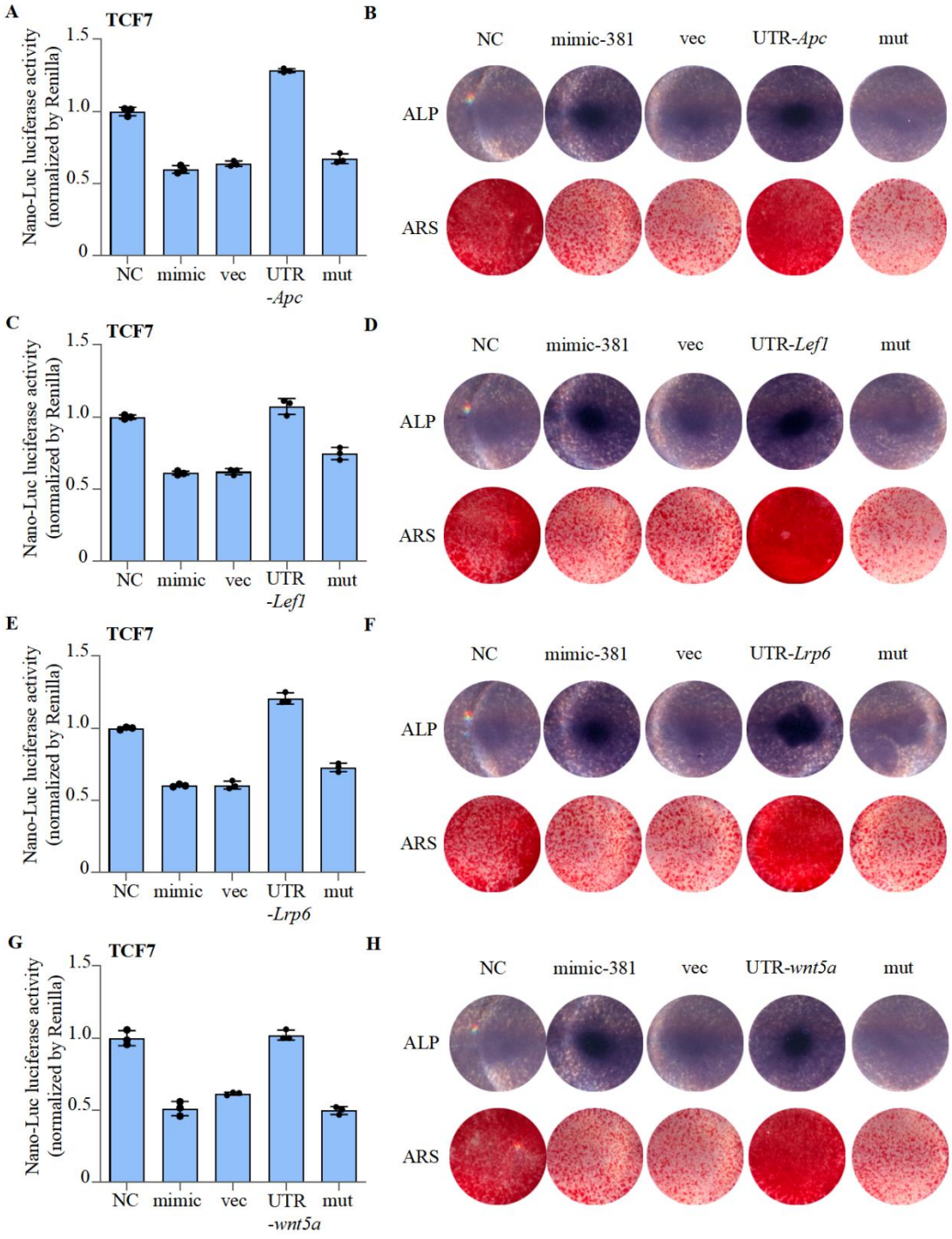


Supplementary Figure 9. miR-381-3p targeted four genes of Wnt signaling pathway

A-D. Binding effect of miR-381-3p and 3′UTR sequences of *Apc*, *Lef1*, *Lrp6*, and *wnt5a*, as detected by luciferase reporter assay (mean ± S.D., n = 3). Luc-vec: empty luciferase reporter plasmid. Luc-mut: luciferase reporter plasmid containing mutant 3′UTR. Luc-WT: luciferase reporter plasmid containing wild-type 3′UTR. inhibit-NC: inhibitor-NC. inhibit-381: inhibitor-381-3p.

E-F. Quantification results corresponding to Figure4 A-B (mean ± S.D., n > 3).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

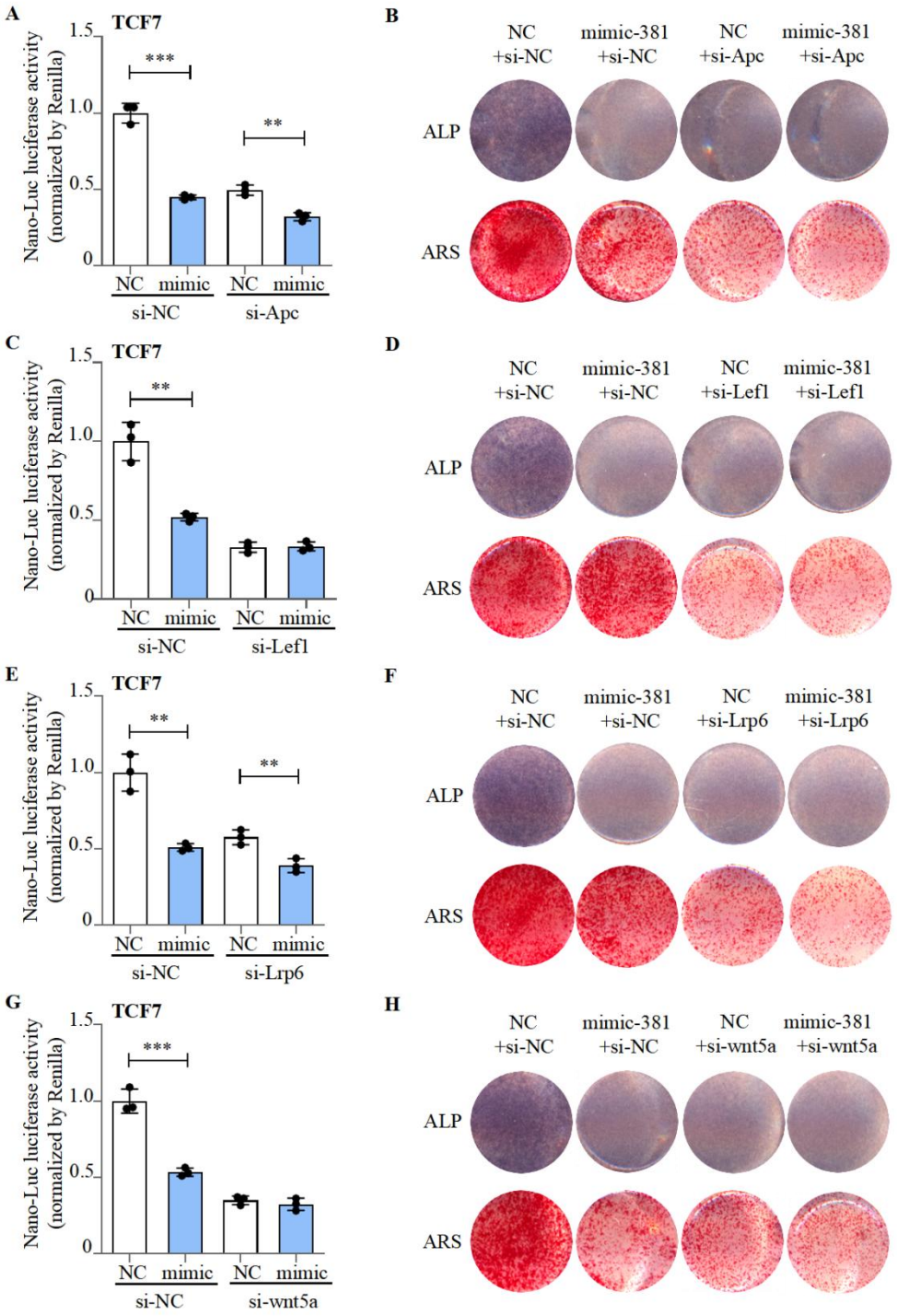


Supplementary Figure 10. miR-381-3p regulated four target genes by combining with their 3′UTR sequence

A, C, E, G. TCF7 activities of hMSCs after transfected with TCF7 luciferase reporter plasmid and treated with mimic-381-3p and 3′UTR sequences of *Apc*, *Lef1*, *Lrp6*, and *wnt5a*, respectively, as detected by luciferase reporter assay (n = 3). NC: mimic-NC. mimic: mimic-381-3p. vec: cells treated with mimic-381-3p and empty luciferase reporter plasmid. UTR: cells treated with

mimic-381-3p and luciferase reporter plasmid containing 3′UTR sequences of *Apc*, *Lef1*, *Lrp6*, and *wnt5a*, respectively. mut: cells treated with mimic-381-3p and luciferase reporter plasmid containing mutant 3′UTR sequences of *Apc*, *Lef1*, *Lrp6*, and *wnt5a*, respectively.

B, D, F, H. ALP and Alizarin Red staining of hMSCs treated with mimic-381-3p and 3′UTR sequences of *Apc*, *Lef1*, *Lrp6*, and *wnt5a*, respectively, as detected by ALP staining and Alizarin Red staining.



Supplementary Figure 11. Inhibiting the target genes of miR-381-3p can depress the sensitivity of osteogenic differentiation to miR-381-3p

A, C, E, G. TCF7 activities of hMSCs after transfected with TCF7 luciferase reporter plasmid and treated with mimic-381-3p and siRNA of *Apc*, *Lef1*, *Lrp6*, and *wnt5a*, respectively, as detected by luciferase reporter assay (mean ± S.D., $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3).

B, D, F, H. ALP and Alizarin Red staining of hMSCs treated with mimic-381-3p and siRNA of *Apc*,

107 *Lef1*, *Lrp6*, and *wnt5a*, respectively, as detected by ALP staining and Alizarin Red staining.

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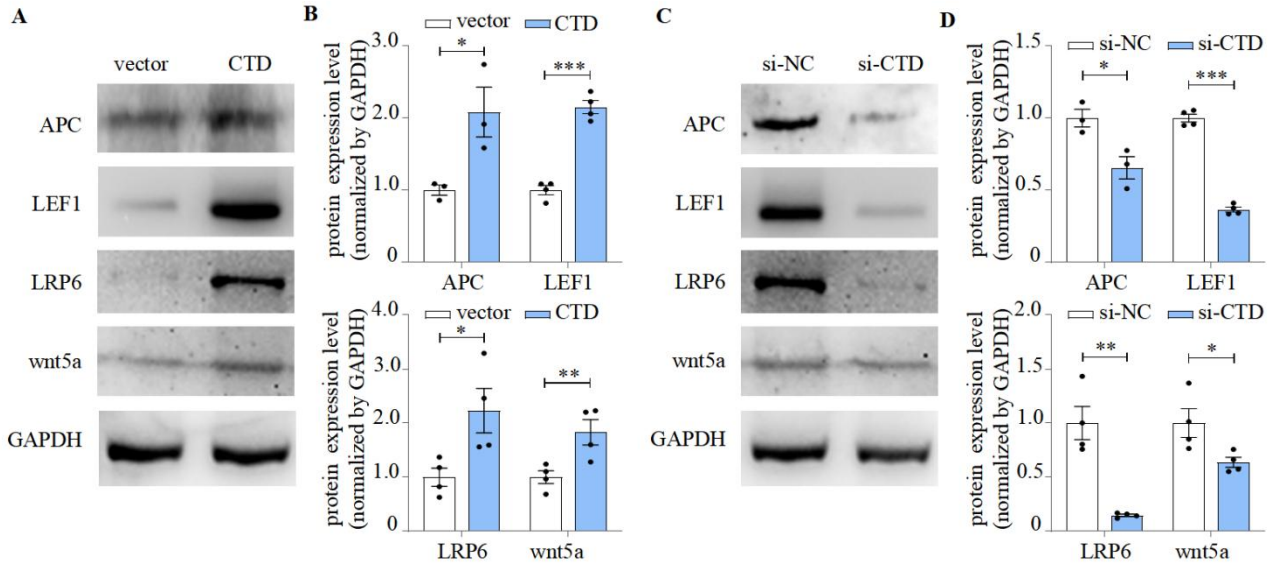
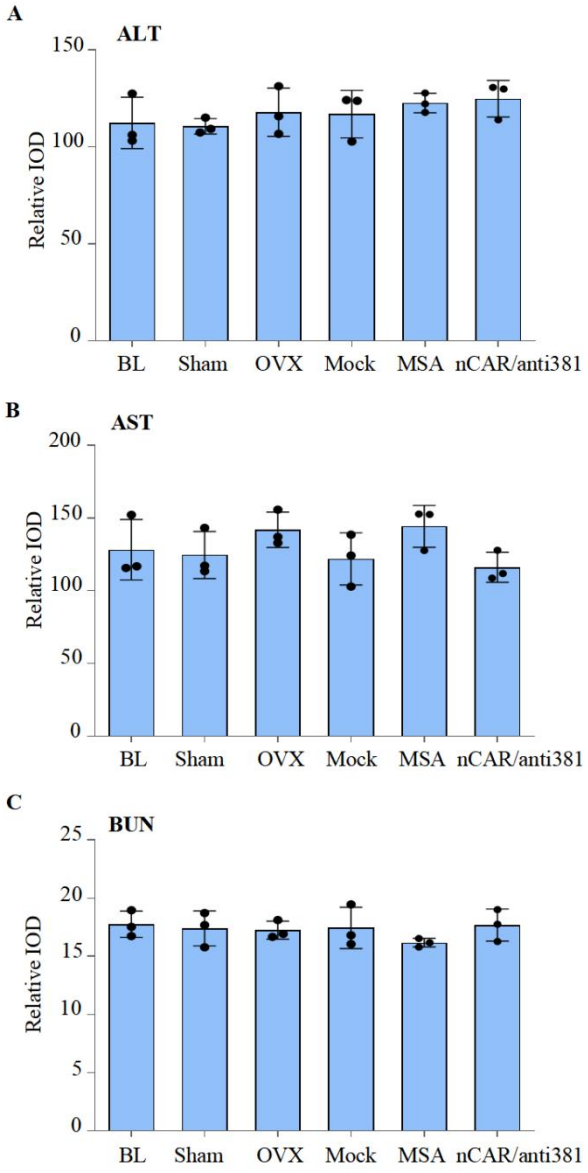


Supplementary Figure 12. CTD-2555A7.2 promoted four target genes of Wnt signaling pathway

A. APC, LEF1, LRP6 and wnt5a protein levels of hMSCs treated with CTD-2555A7.2 over-expression plasmid (compared with pCDNA3.1 plasmid), as detected by western blot.

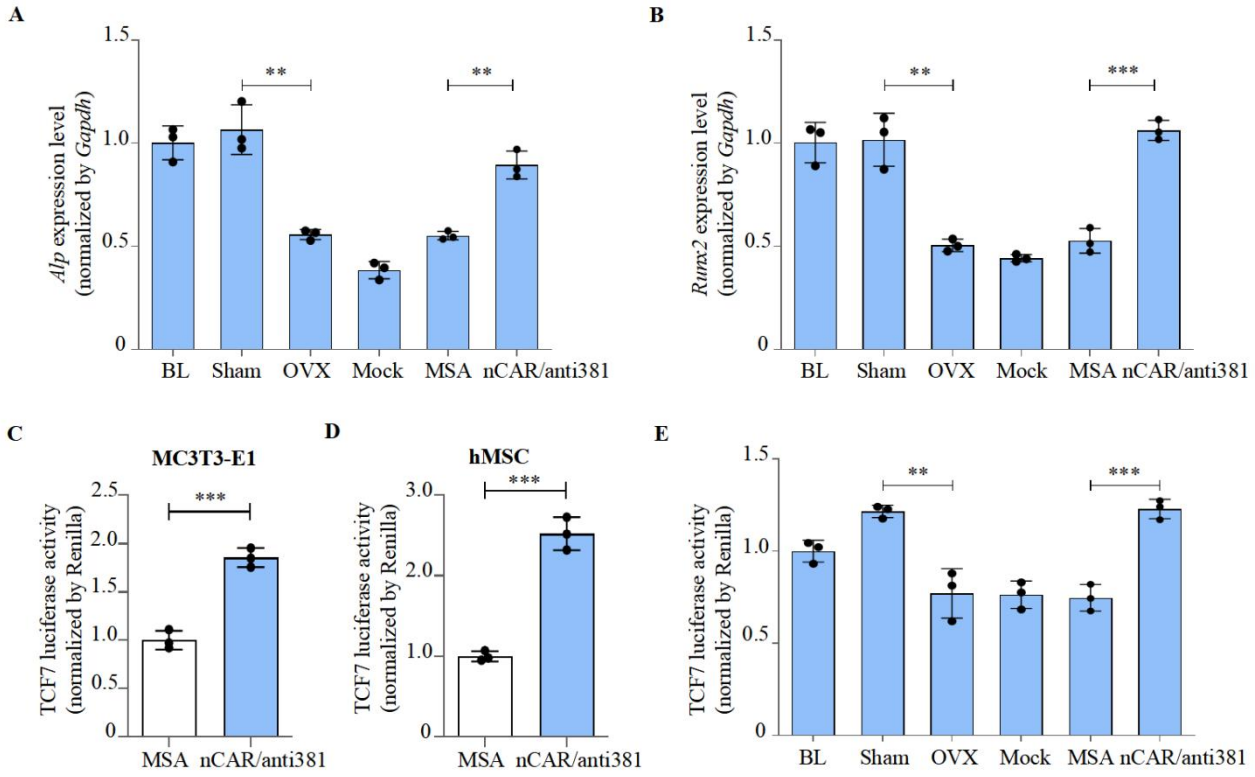
B. APC, LEF1, LRP6 and wnt5a protein levels of hMSCs treated with CTD-2555A7.2 over-expression plasmid. The quantification results with GAPDH as internal control (mean ± S.D., $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, n > 3).

C-D. APC, LEF1, LRP6 and wnt5a protein levels of hMSCs treated with CTD-2555A7.2 siRNA (mean ± S.D., $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, n > 3).



Supplementary Figure 13. Biochemical assays in blood serum of OVX mice after recombinant miR-381-3p inhibitor treatment

A-C. Alanine transaminase (ALT, A), Aspertate Aminotransferase (AST, B), and Blood Urea Nitrogen (BUN, C) levels in blood serum of OVX mice after recombinant miR-381-3p inhibitor treatment, as detected by ELISA (n = 3). BL (Baseline): sacrifice before RNA treatment. Sham: sham OVX operation group. OVX: OVX group. Mock: transfection reagent control group. MSA: empty recombinant tRNA treated group. nCAR/anti381: novel recombinant miR-381-3p inhibitor treated group.

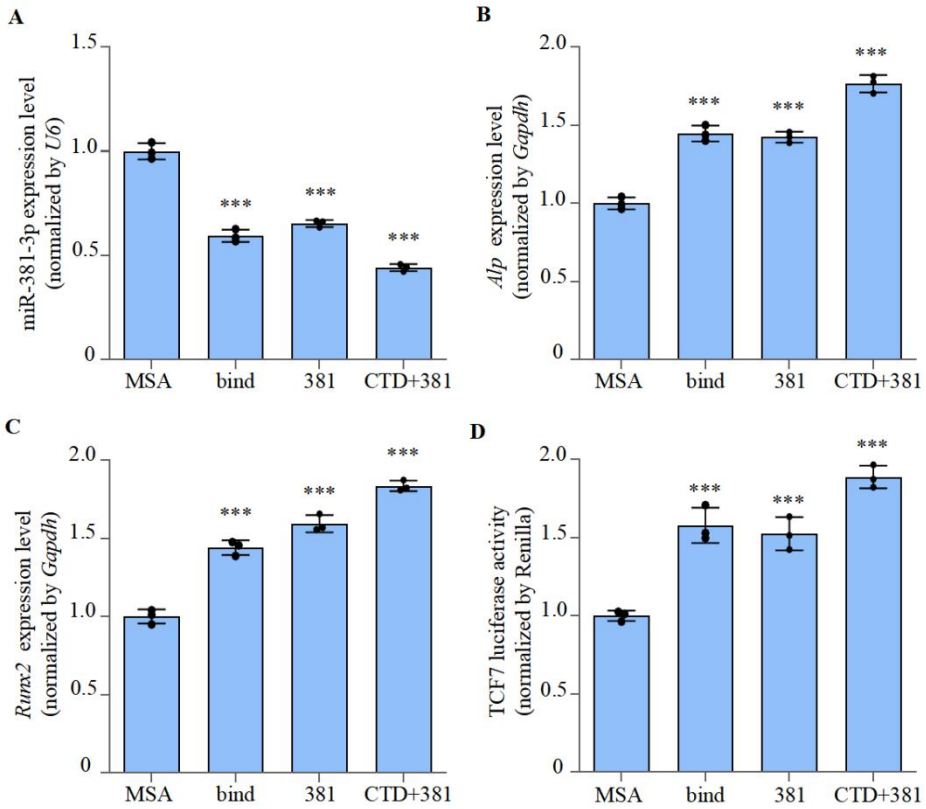


Supplementary Figure 14. Bioengineered recombinant miR-381-3p inhibitor enhanced osteogenic differentiation via Wnt signaling pathway

A-B. *Alp* or *Runx2* expression levels of primary BMSCs of OVX mice treated with recombinant miR-381-3p inhibitor, as detected by RT-PCR (mean ± S.D., $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3).

C-D. TCF7 activities of MC3T3-E1 (left) and hMSCs (right) cells treated with recombinant miR-381-3p inhibitor, as detected by luciferase reporter assay (mean ± S.D., $^{***}P < 0.001$, n = 3).

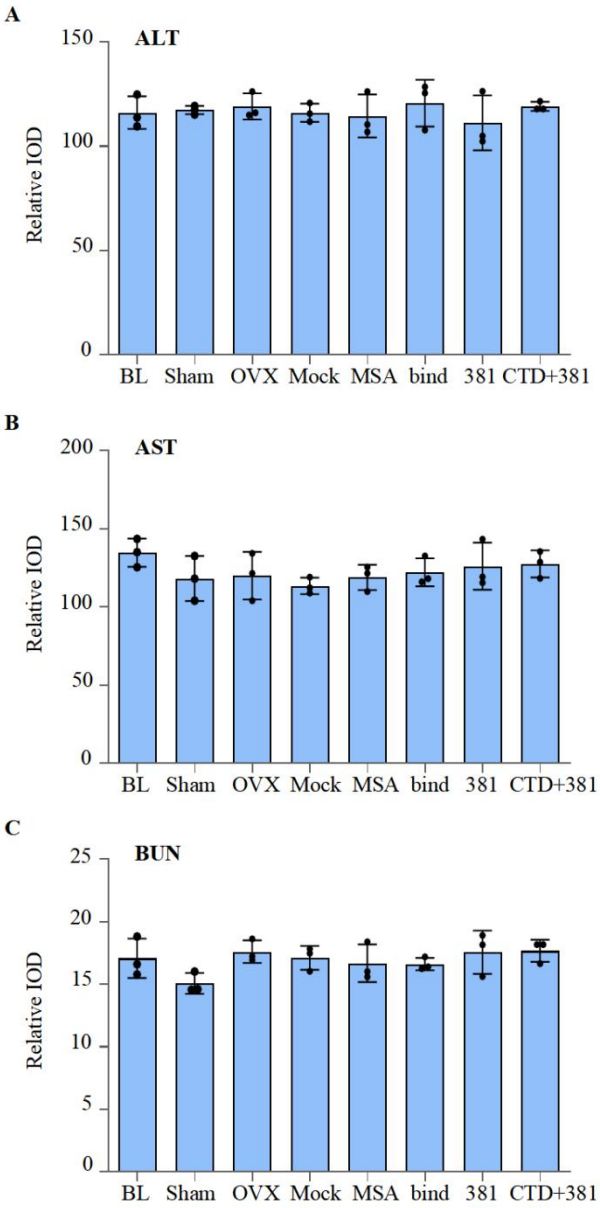
E. TCF7 activities of primary BMSCs of OVX mice treated with recombinant miR-381-3p inhibitor, as detected by luciferase reporter assay (mean ± S.D., $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3).



Supplementary Figure 15. Combination effect of CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor on hMSCs

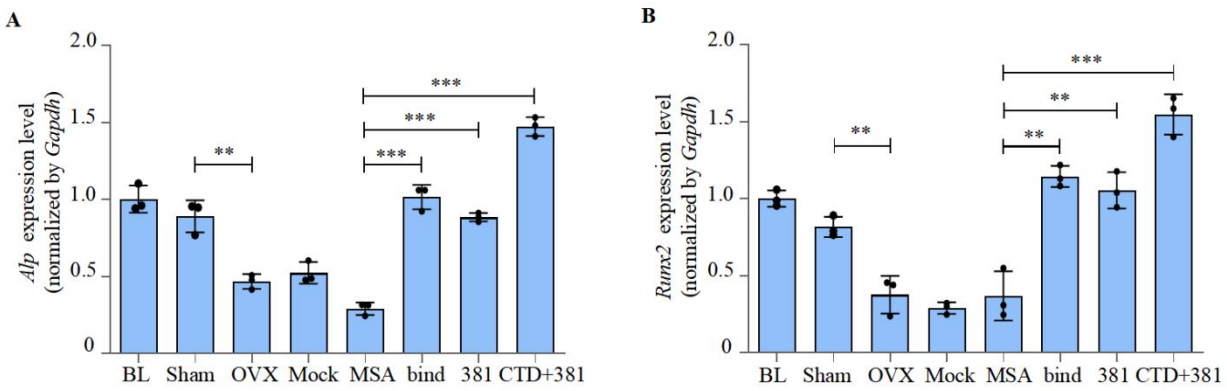
A-C. miR-381-3p, *Alp* and *Runx2* expression levels of hMSCs treated with CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor, as detected by RT-PCR (mean ± SD, ***$P < 0.001$, n = 3). MSA: empty recombinant tRNA as a negative control RNA sequence. bind: CTD-2555A7.2 binding sequence. 381: novel recombinant miR-381-3p inhibitor. CTD+381: Combination of CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor.

D. TCF7 activities of hMSCs treated with CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor, as detected by luciferase reporter assay (mean ± S.D., ***$P < 0.001$, n = 3).



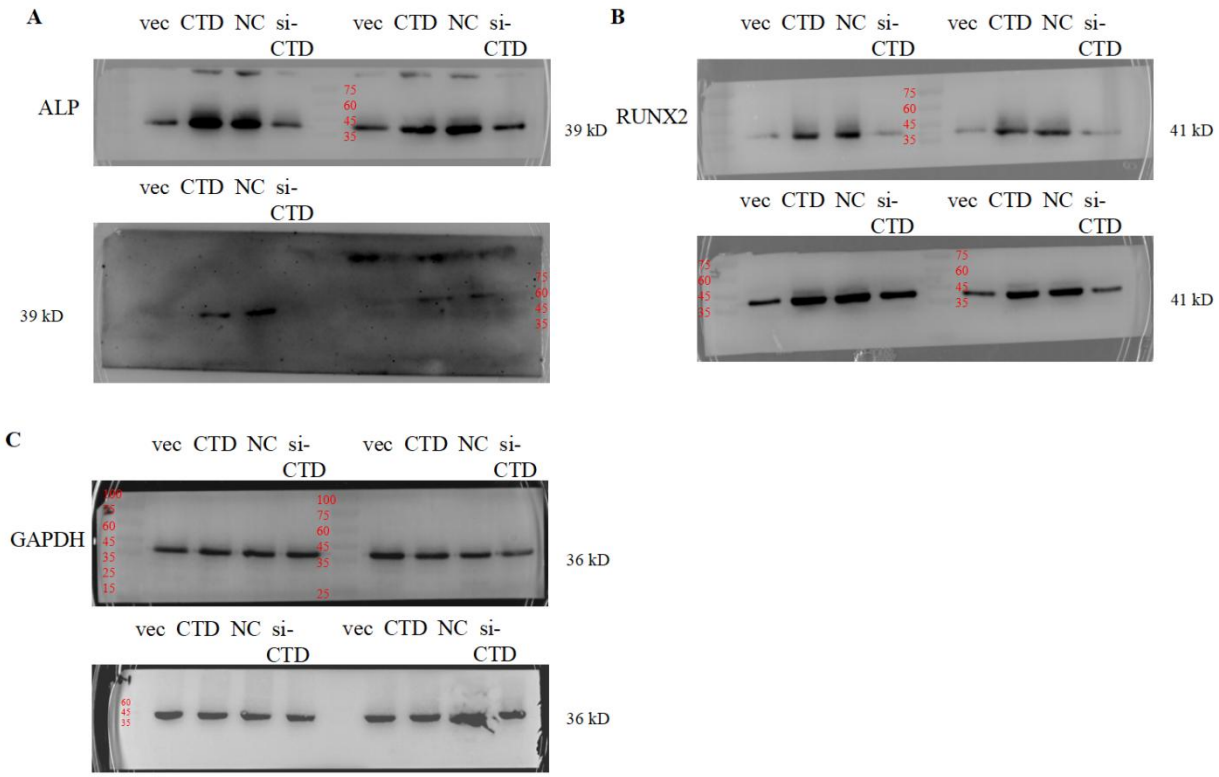
Supplementary Figure 16. Biochemical assays in blood serum of OVX mice after CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor treatment

A-C. Alanine transaminase (ALT, A), Aspertate Aminotransferase (AST, B), and Blood Urea Nitrogen (BUN, C) levels in blood serum of OVX mice after CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor treatment, as detected by ELISA (n = 3). BL (Baseline): sacrifice before RNA treatment. Sham: sham OVX operation group. OVX: OVX group. Mock: transfection reagent control group. MSA: empty recombinant tRNA treated group. bind: CTD-2555A7.2 binding sequence treated group. 381: novel recombinant miR-381-3p inhibitor treated group. CTD+381: CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor combined treated group.



Supplementary Figure 17. Combination of CTD-2555A7.2 binding sequence and bioengineered recombinant miR-381-3p inhibitor enhanced osteogenic differentiation of OVX mice

A-B. Expression levels of *Alp* and *Runx2* in primary BMSCs of OVX mice treated with CTD-2555A7.2 binding sequence and recombinant miR-381-3p inhibitor, as detected by RT-PCR (mean ± S.D., $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3).



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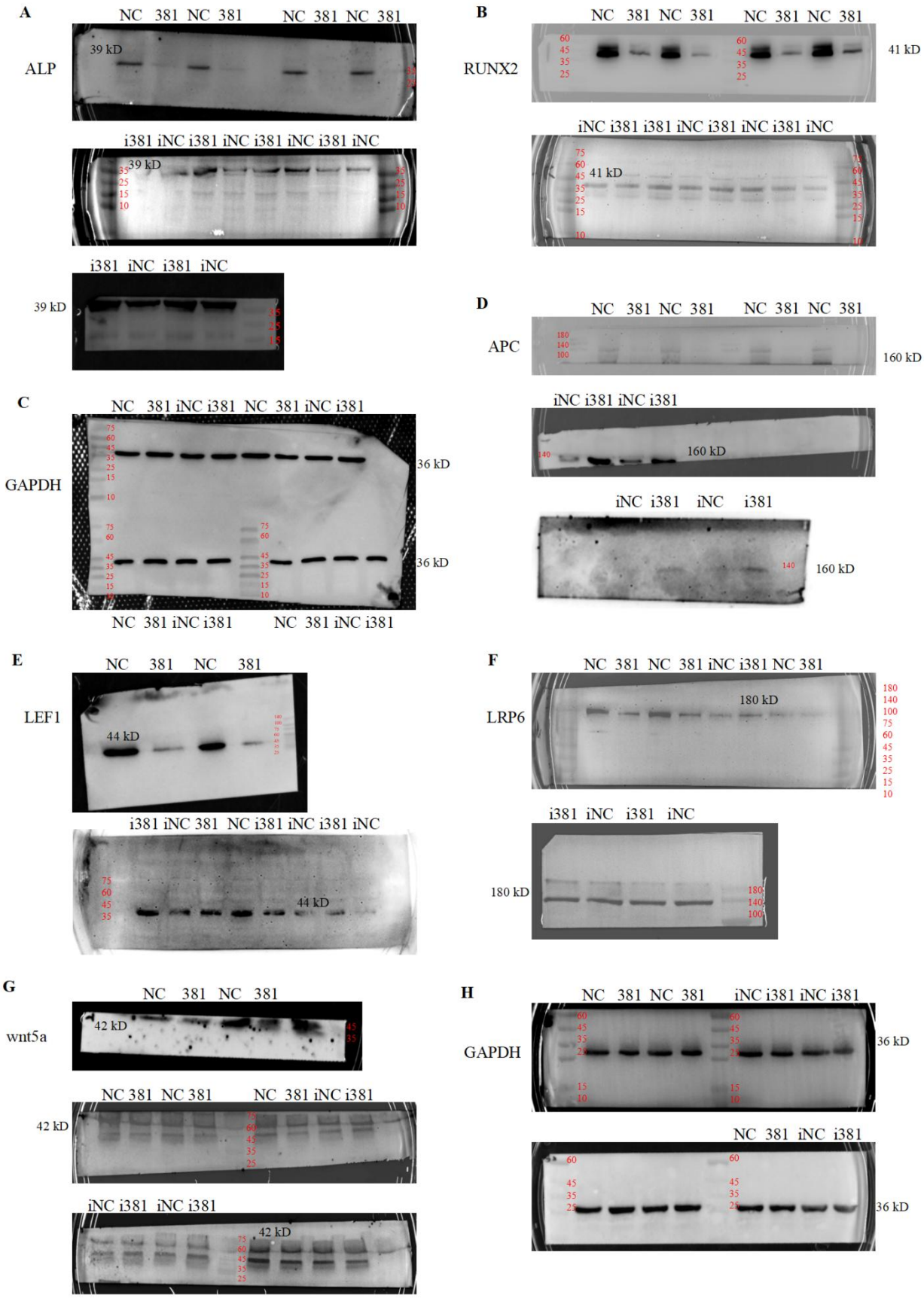
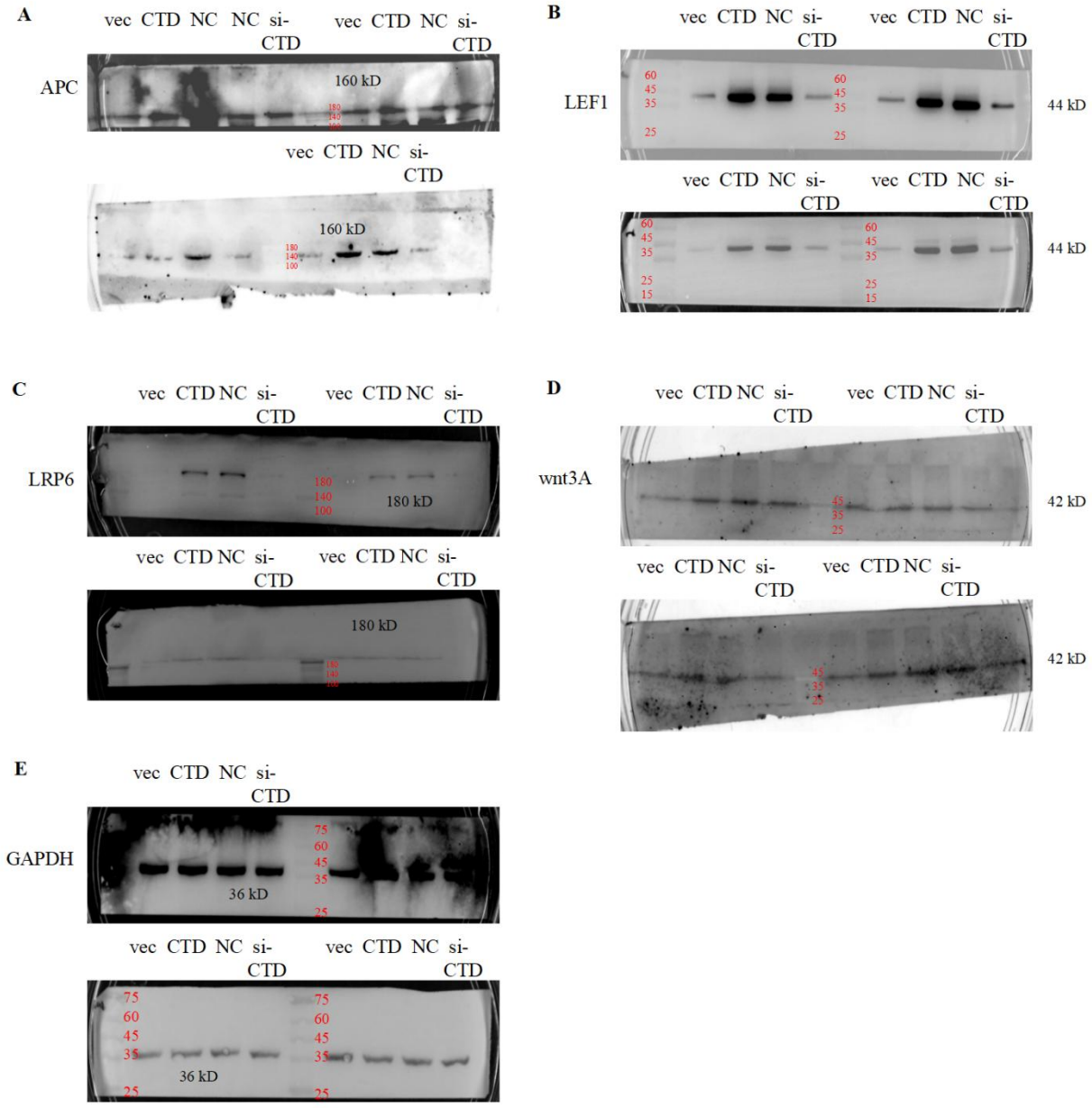
169 **Uncropped images of the original western blots in Figure 1 (H-I)**



Uncropped images of the original western blots in Figure 5 (C-D) and 6 (A-B)



Uncropped images of the original western blots in Figure 7 (A, C)