

Supplementary Figures S1 to S4

A



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Result for species name : hg19 with job ID :1584693166

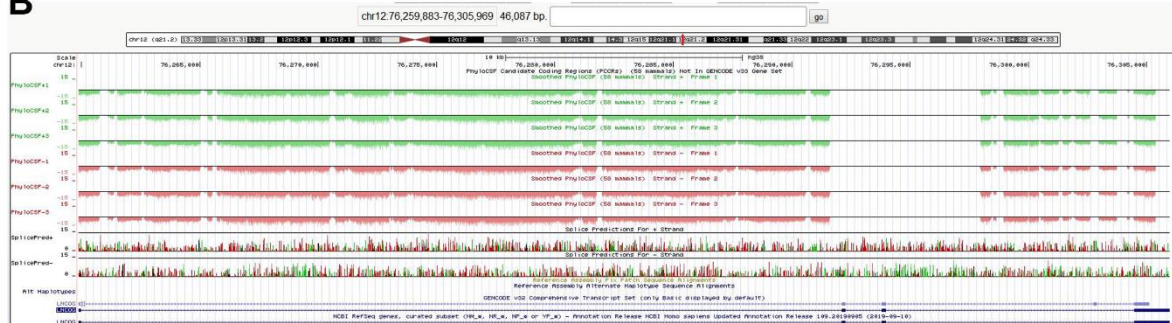
Data ID	Sequence Name	RNA Size	ORF Size	Fickett Score	Hexamer Score	Coding Probability	Coding Label
0	TCONS_00020478	2030	249	0.7665	0.00799296881268	0.044490685285902	no

This job has been stored with the job ID
[Download Table in tab delimited file \(.txt\)](#)

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B



C

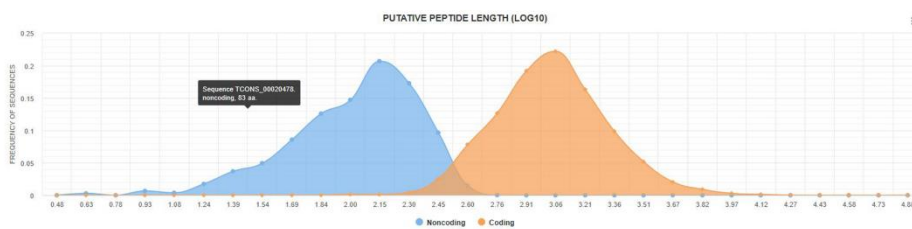
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Summary

Sequence TCONS_00020478 got Fickett score 0.30166 with a complete putative ORF 83 AA, a pI 6.00152587891, which, in total, classify it as a noncoding sequence with coding probability 0.0891574.

Details

PUTATIVE PEPTIDE
 >TCONS_00020478
 MKTTCVTFGAEDPGQRDSFGGPPVHCPCPHSVRRPTYDLGSSDQPAQGTSHQFQIGTPLENGLNSPQSHSQSPWNSGPRLSQ



D

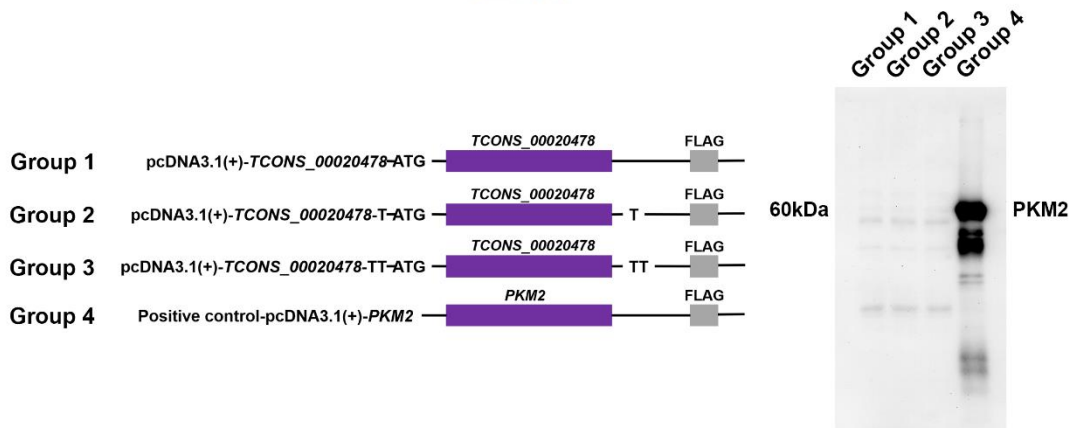


FIGURE S1

A to C, Prediction of the coding ability of *TCONS_00020478* using the online tools Coding Potential Assessment Tool, PhyloCSF, and Coding Potential calculator all showed little protein-coding possibility of *ROA*.

D, An in vitro expression assay confirmed that *TCONS_00020478* had no protein-coding ability.

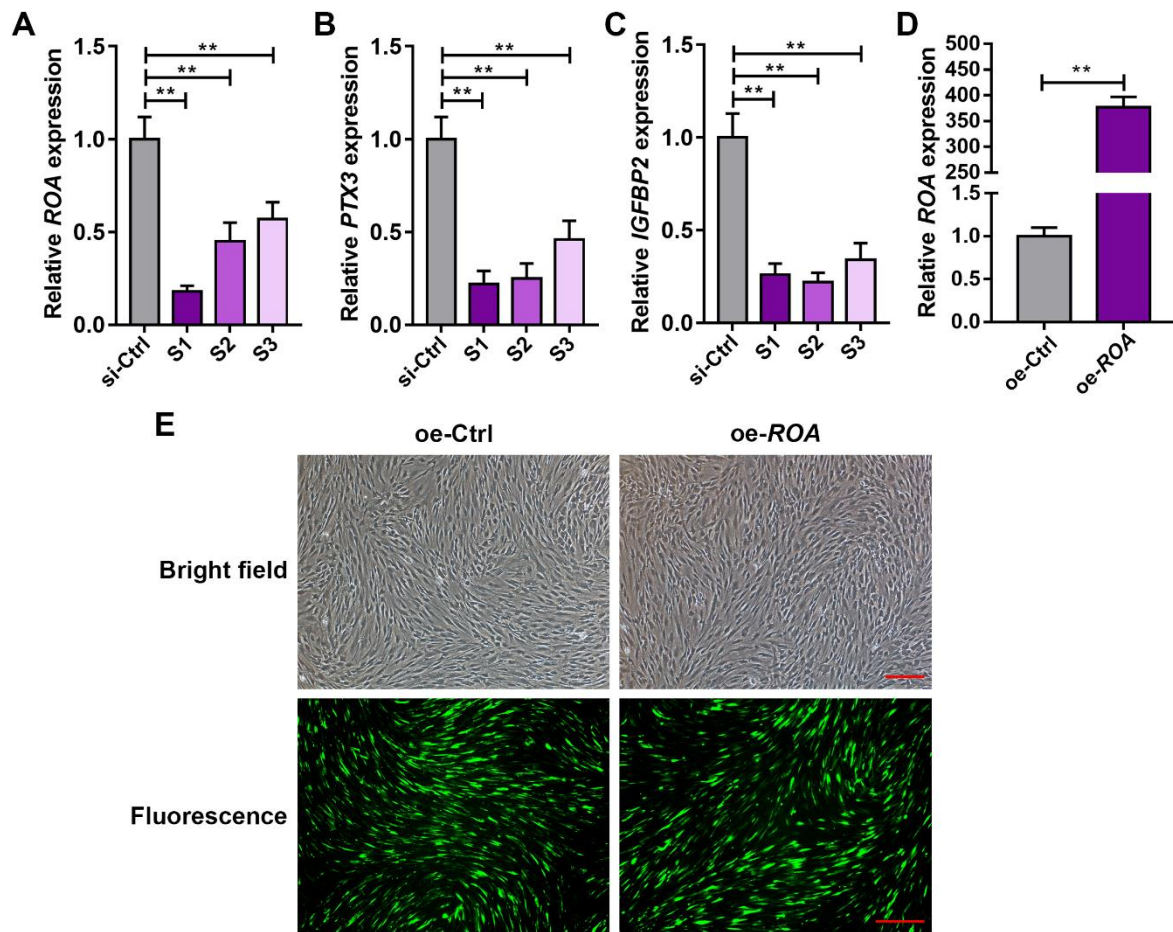


FIGURE S2

A to C, Knockdown efficiency of siRNA sequences designed for *ROA*, *PTX3* and *IGFBP2* detected by qRT-PCR on day 3 after transfection. The ones with the best interfering efficiency were chosen for the following experiments.

D, Overexpression efficiency of *ROA* detected by qRT-PCR on day 3 after Lentivirus transfection.

E, Bright field and fluorescent images showing *ROA* was evenly overexpressed among cells and did not have an apparent influence on cell viability or morphology. Scale bar = 150 μ m.

The results are presented as the mean \pm SD (n = 18, three independent experiments, each with six different samples). **, p<0.01, as determined by ANOVA.

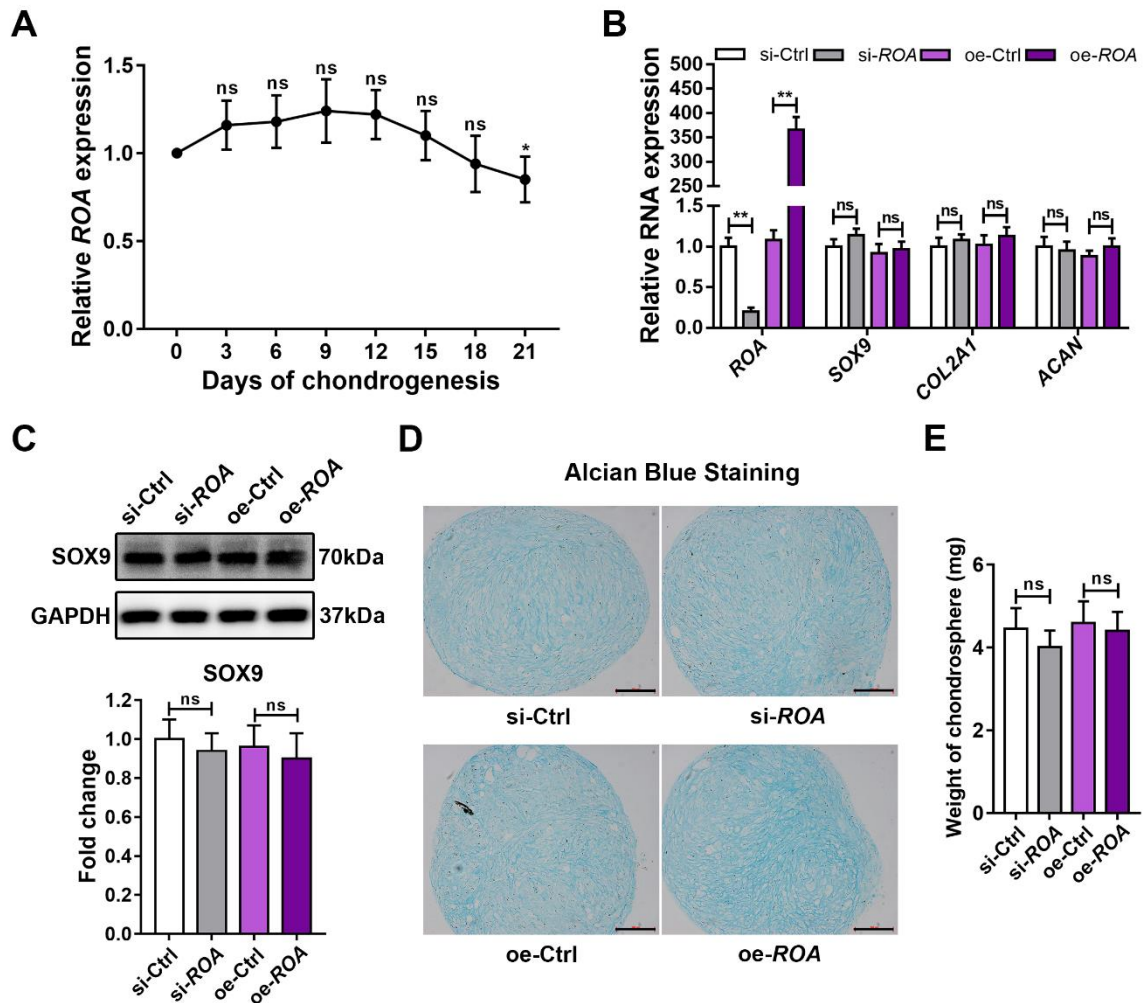


FIGURE S3

A, Dynamic *ROA* expression during MSC chondrogenesis detected by qRT-PCR. *, $p < 0.05$, ns, not significant, compared to day 0.

B, Relative expression of chondrogenesis-related genes detected by qRT-PCR on day 3 of MSC adipogenesis after *ROA* knockdown/overexpression.

C, Western blot of SOX9 on day 7. Data were normalized to GAPDH.

D, Alcian blue staining on day 21. Scale bar = 150 μ m.

E, Quantification of chondrosphere weight on day 21.

The results are presented as the mean \pm SD ($n = 18$, three independent experiments, each with six different samples). **, $p < 0.01$; ns, not significant, as determined by ANOVA.

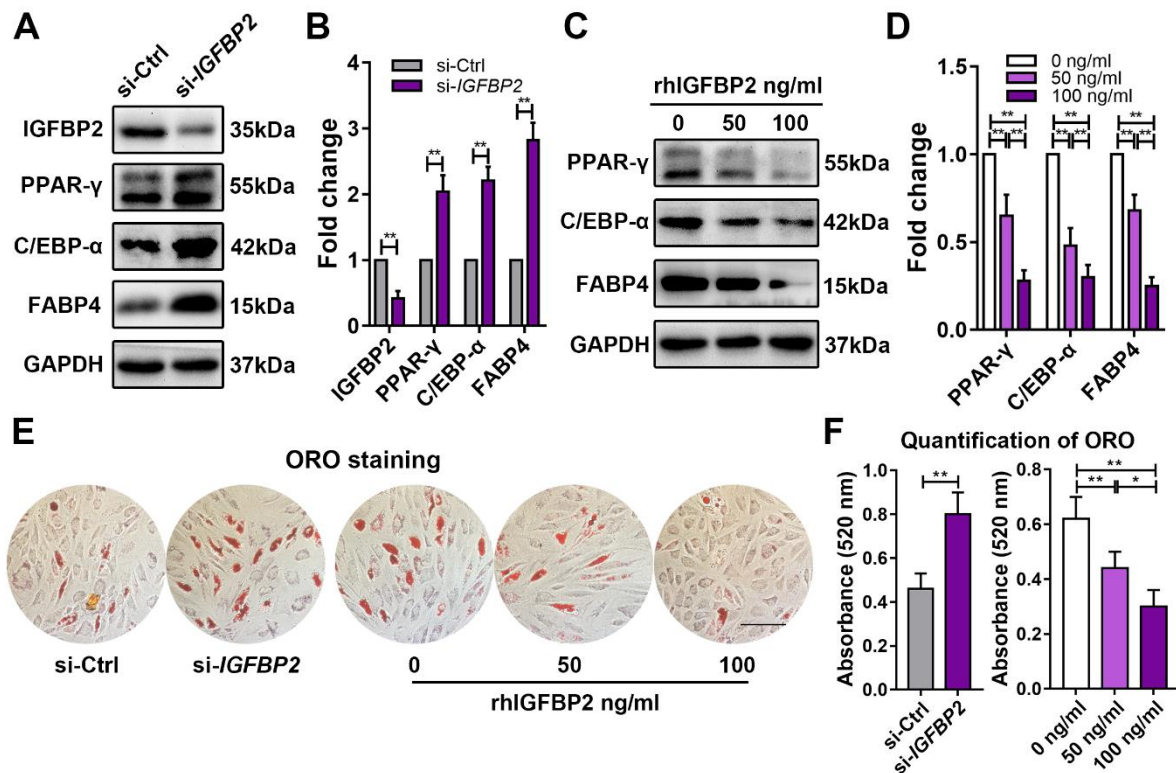


FIGURE S4

A and B, Western blot of IGFBP2 and adipogenic markers on day 5 of MSC adipogenesis after *IGFBP2* knockdown. Data were normalized to GAPDH.

C and D, Western blot of adipogenic markers on day 5 of MSC adipogenesis after recombinant human IGFBP2 (rhIGFBP2) stimulation. Data were normalized to GAPDH.

E and F, MSC adipogenesis evaluated by ORO staining and quantification on day 12 after *IGFBP2* knockdown or rhIGFBP2 stimulation. Scale bar = 150 μ m.

The results are presented as the mean \pm SD (n = 18, three independent experiments with six different samples). *, p<0.05; **, p<0.01, as determined by Student's t test or ANOVA.

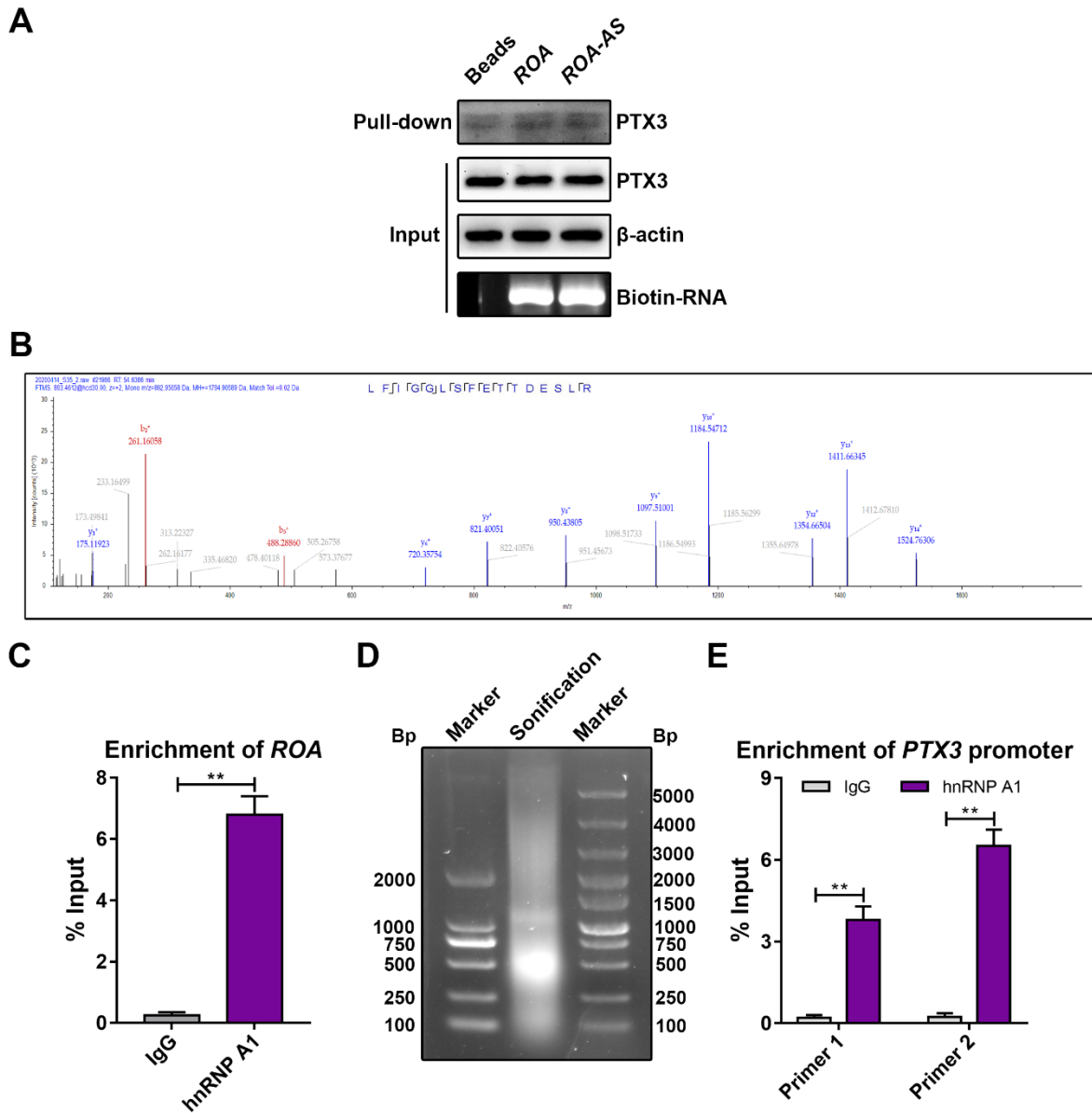


FIGURE S5

A, Western blotting showing PTX3 was not enriched by pull-down with biotinylated *ROA* compared to *ROA-AS* or empty magnetic beads control.

B, The peptide sequence of hnRNP A1 was detected in the pull-down proteins by mass spectrometry. Red peaks indicate secondary fragments close to the N-terminal and blue to the C-terminal.

C, Quantification of the RIP assay.

D, Agarose gel electrophoresis of ChIP sonification product. Most fragments were of 200~1000 bp in length.

E, Quantification of the ChIP assay.