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The mechanism by which MALAT1/CREG1 regulates premature rupture of fetal membrane through autophagy mediated differentiation of amniotic fibroblasts

Background

Premature rupture of fetal membrane (PROM) is one of the main causes of premature delivery. The amniotic membrane plays a major role in bearing weight, and amniotic fibroblasts play an important role. The purpose of this study was to explore the scientific problems associated with amniotic membrane repair by intervening with fibroblasts to provide evidence for the clinical treatment of PROM.

Methods

This research group conducted experiments on fetal membrane tissue via single-cell sequencing, Sirius staining, fluorescence staining and Raman spectroscopy to explore changes in fetal membrane structure and verified key targets and pathways in clinical tissues and primary fibroblasts through WB, PCR, RNA Pulldown, RIP and molecular docking experiments.

Results

The fetal membrane structure in the PROM group was obviously damaged, and the amniotic fibroblasts were activated and autophagy was activated, and the activated autophagy promoted the activation of fibroblasts. The expression of Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1) was significantly increased in amniotic fibroblasts. RNA PULL DOWN and molecular docking results suggested that MALAT1 binds to human E1A promoter repressor 1 (CREG1) and promotes autophagy.

Conclusions

By interacting with CREG1, MALAT1 can increase the expression of CREG1, regulate the expression of autophagy-related molecules, mediate the differentiation of amniotic fibroblasts into myofibroblasts, partic ipate in amniotic repair, and promote the repair of PROM fetal membrane tissue.

Keywords: Premature rupture of fetal membrane; Metastasis-associated lung adenocarcinoma transcript 1; Autophagy Fibroblasts

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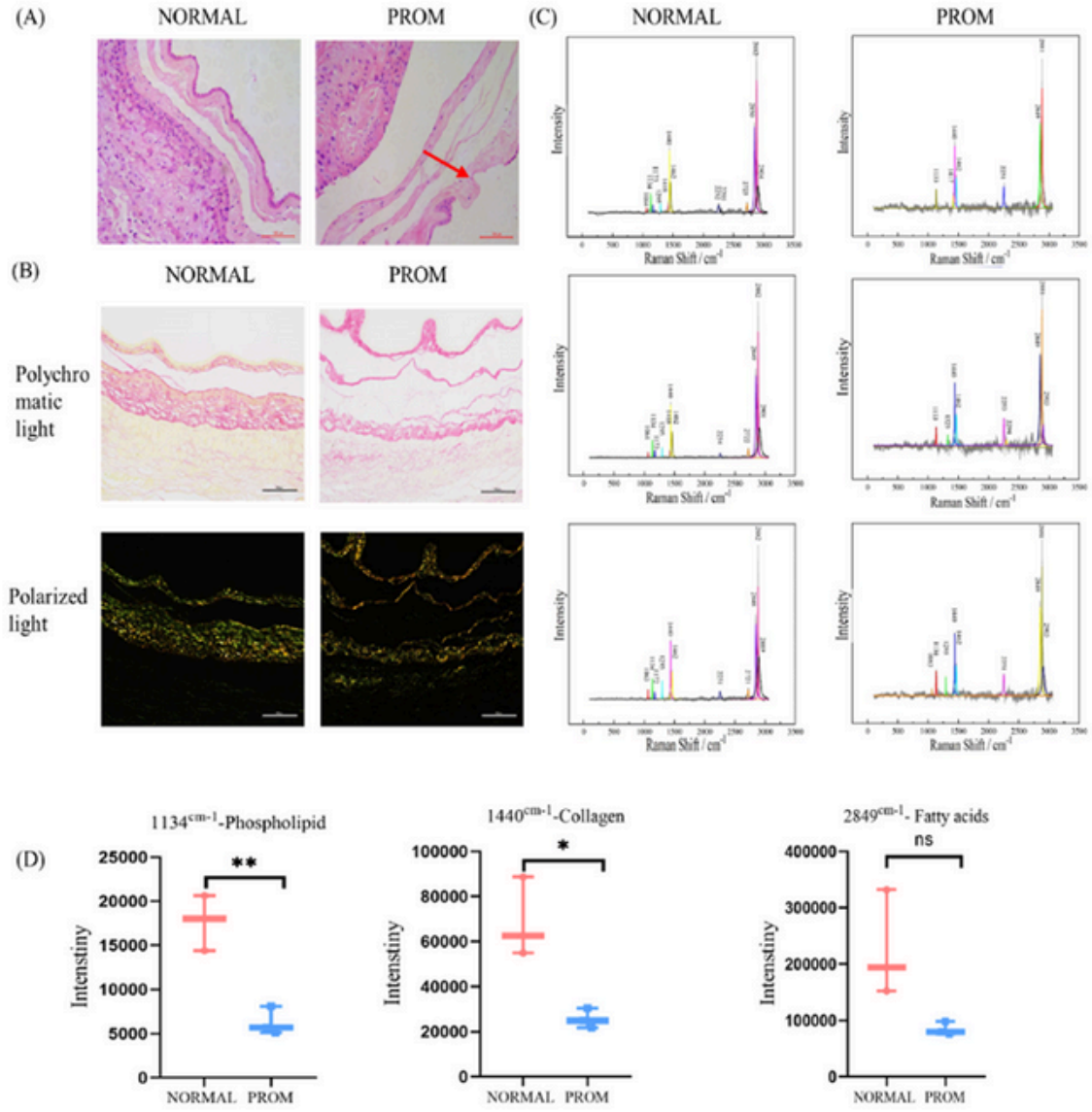

Fig. 1. Structural anomaly of premature rupture of membranes.
(A) Representative images of HE staining of fetal membrane tissue, and red arrows indicate defects, 20X. (B) Representative images of Sirius staining of fetal membrane tissue, 20X. (C) Raman spectrogram of amniotic tissue. (D) Statistical Raman spectra of amniotic tissue (phospholipids, collagen, and fatty acids).

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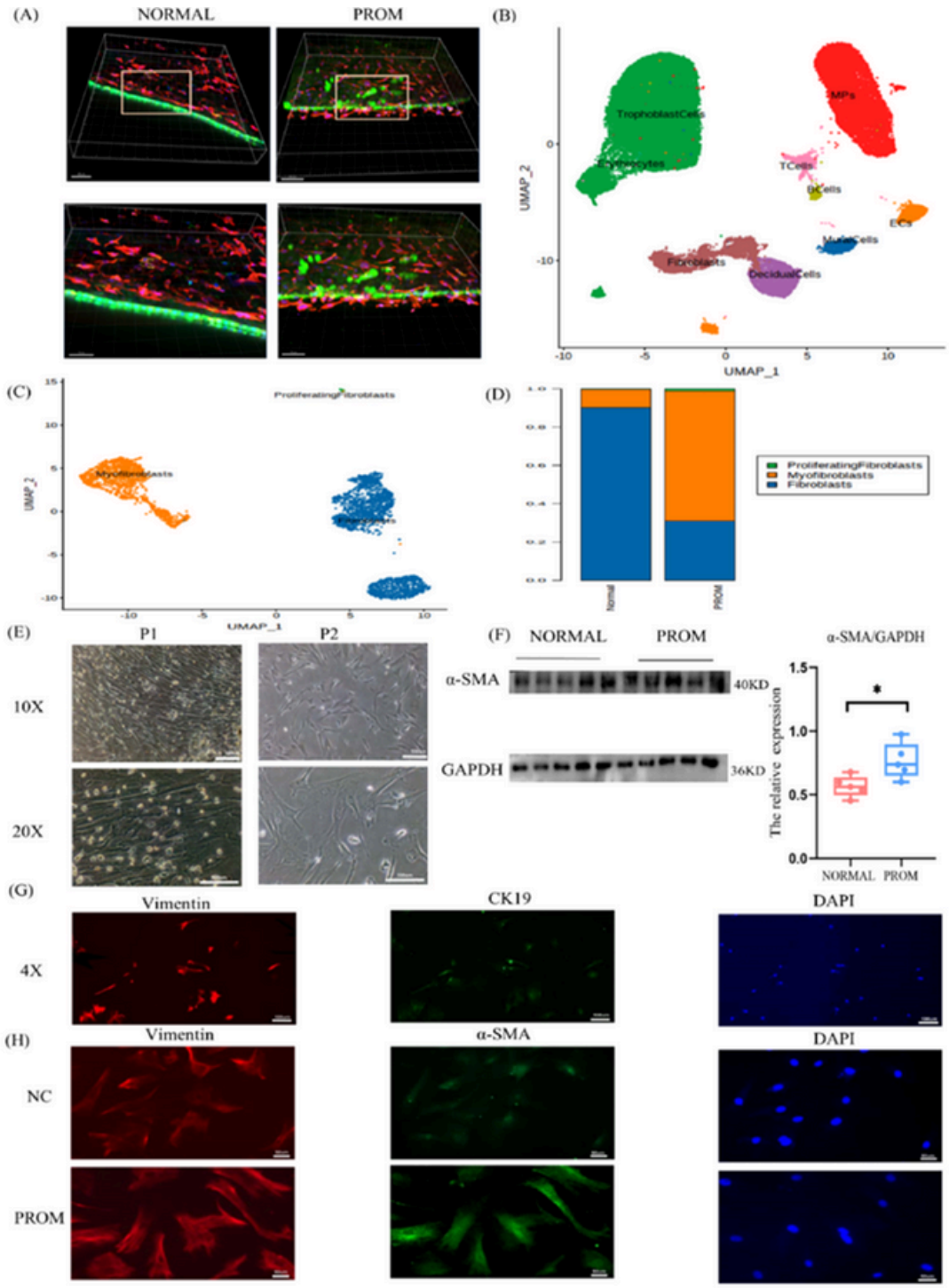

Fig. 2. Analysis and verification of single-cell RNA sequencing data.
(A) Representative images of fluorescent confocal 3D imaging of amniotic tissue showing structural abnormalities of amniotic cells in the PROM group. Green: CK19 (epithelial cell marker); red: vimentin (fibroblast marker); blue: DAPI. (B) Nine cell subtypes were obtained by cluster analysis of fetal membrane cells. (C) UMAP of fibroblast clusters from the NORMAL group and PROM group, 3 subpopulations with a total of 3023 cells were obtained by detection clustering. They are fibroblasts, myofibroblasts and proliferating fibroblasts. (D) The bar chart shows the proportion of fibroblast clusters in the NORMAL and PROM groups. (E) Representative images of the morphology of primary amniotic fibroblasts. (F) WB results showing the expression of the α-SMA in the NORMAL and PROM groups. (G) Representative images of cellular immunofluorescence, 4X. Red: vimentin; green: CK19; blue: DAPI. (H) Representative images of cellular immunofluorescence, 20X. Red: vimentin; green: α-SMA; blue: DAPI.

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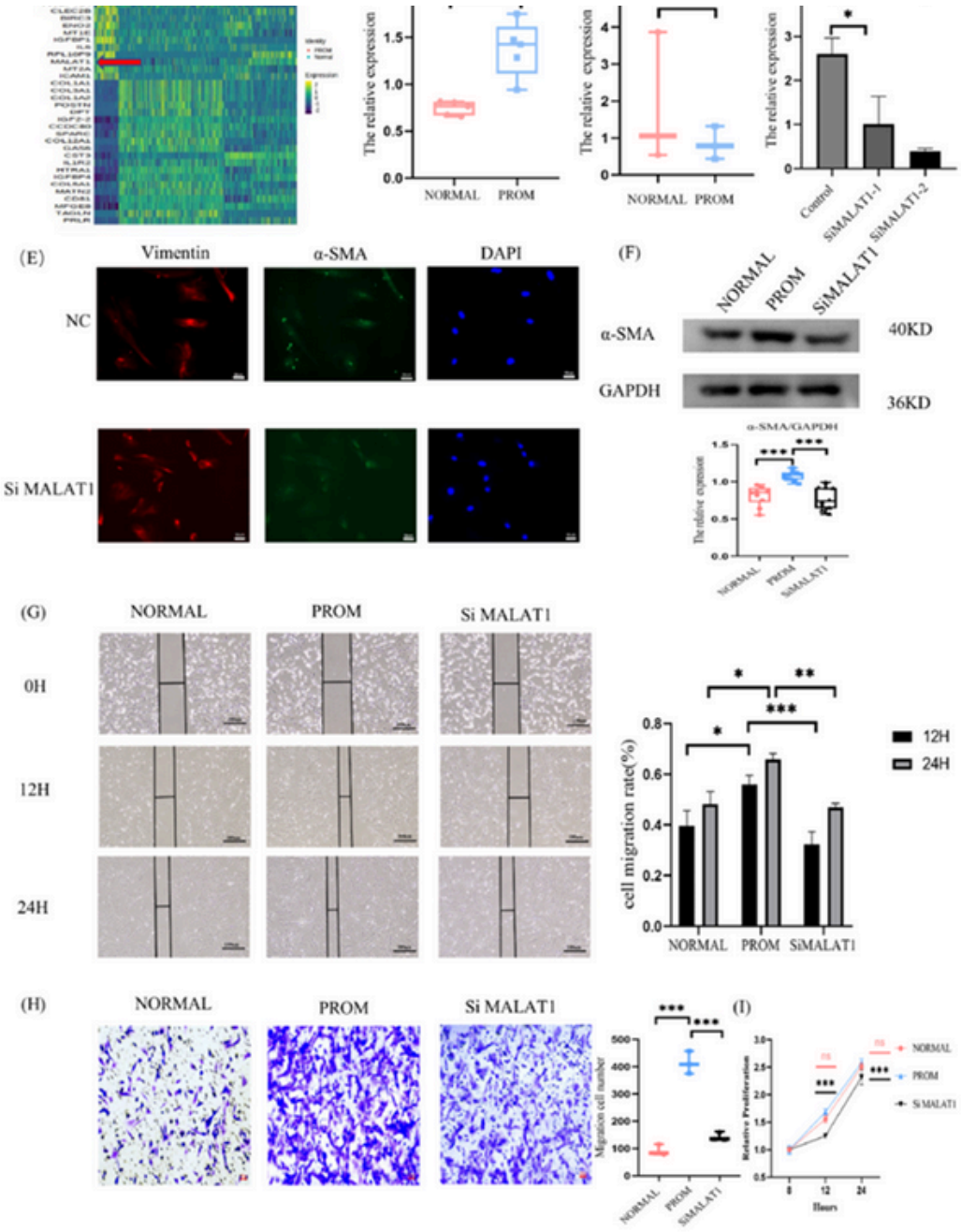

Fig. 3. MALAT1 promotes fibroblast transformation.
(A) Heat maps showed the top 20 up-regulated and down-regulated differentially expressed genes in NORMAL and PROM composed fibrocytes. (B) Q-PCR results indicating that the expression of MALAT1 in amniotic tissue was significantly increased, n = 5. (C) Q-PCR results indicating that there was no significant difference in the expression of MALAT1 in chorionic tissue, n = 3. (D) Q-PCR was used to detect the mRNA expression of cells after MALAT1 knockout, n = 3. (E) Representative

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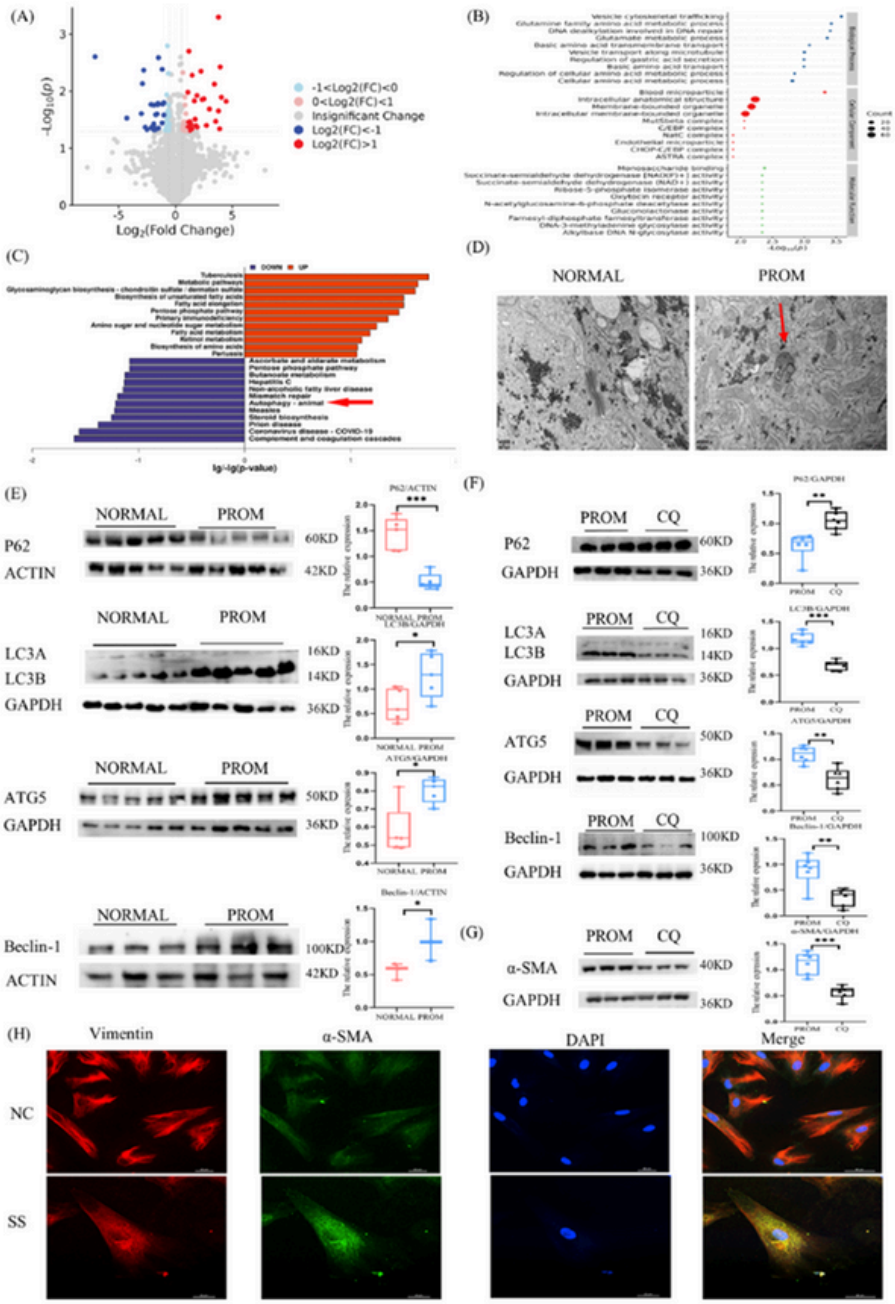

Fig. 4. Relationship between autophagy and fibroblast transformation.
(A) For the volcano map of the SiRNA vs NC group, the horizontal coordinate is the logarithmic transformation of FC, and the vertical coordinate is the negative logarithmic transformation of the p value. The red dots represent the significantly highly expressed proteins, and the darker the color is the higher the upregulation ratio. The blue dots represent significantly lower protein expression, and the darker the color is the greater the downregulation ratio. The gray dots represent non-differentially expressed proteins. (B) GO functional enrichment bubble diagram, showing the top 10 terms significantly enriched with differentially expressed under the three branches of BP, MF and CC. The horizontal coordinate is the negative logarithmic transformation of the enrichment significance p-value, and the vertical coordinate is the GO term. Each circle represents a term, and the size of the circle represents the count. (C) The KEGG pathway with the top 12 enrichment significance is represented by logarithmic (downregulated) and negative logarithmic (upregulated) transformation of p value at the horizontal coordinate, with blue columns representing downregulated protein enrichment pathways and red columns representing upregulated protein enrichment pathways (arrows indicating autophagy pathways). (D) Autophagosomes (arrow indicating autophagosomes) were observed in the TEM of amniotic tissue from patients with PROM. (E) WB results showing the expression of autophagy-related molecules P62, LC3B, ATG5 (n = 5) and Beclin-1 in amniotic tissue (n = 3). (F) WB results showing the expression of the autophagy-related molecules P62, LC3B, ATG5 and Beclin-1 in the PROM and CQ groups, n = 6. (G) WB results showing α-SMA expression in the PROM and CQ groups, n = 6. (H) Immunofluorescence staining of cells cultured in serum-free medium (SS), 40X. Red: vimentin; green: α-SMA; blue: DAPI.

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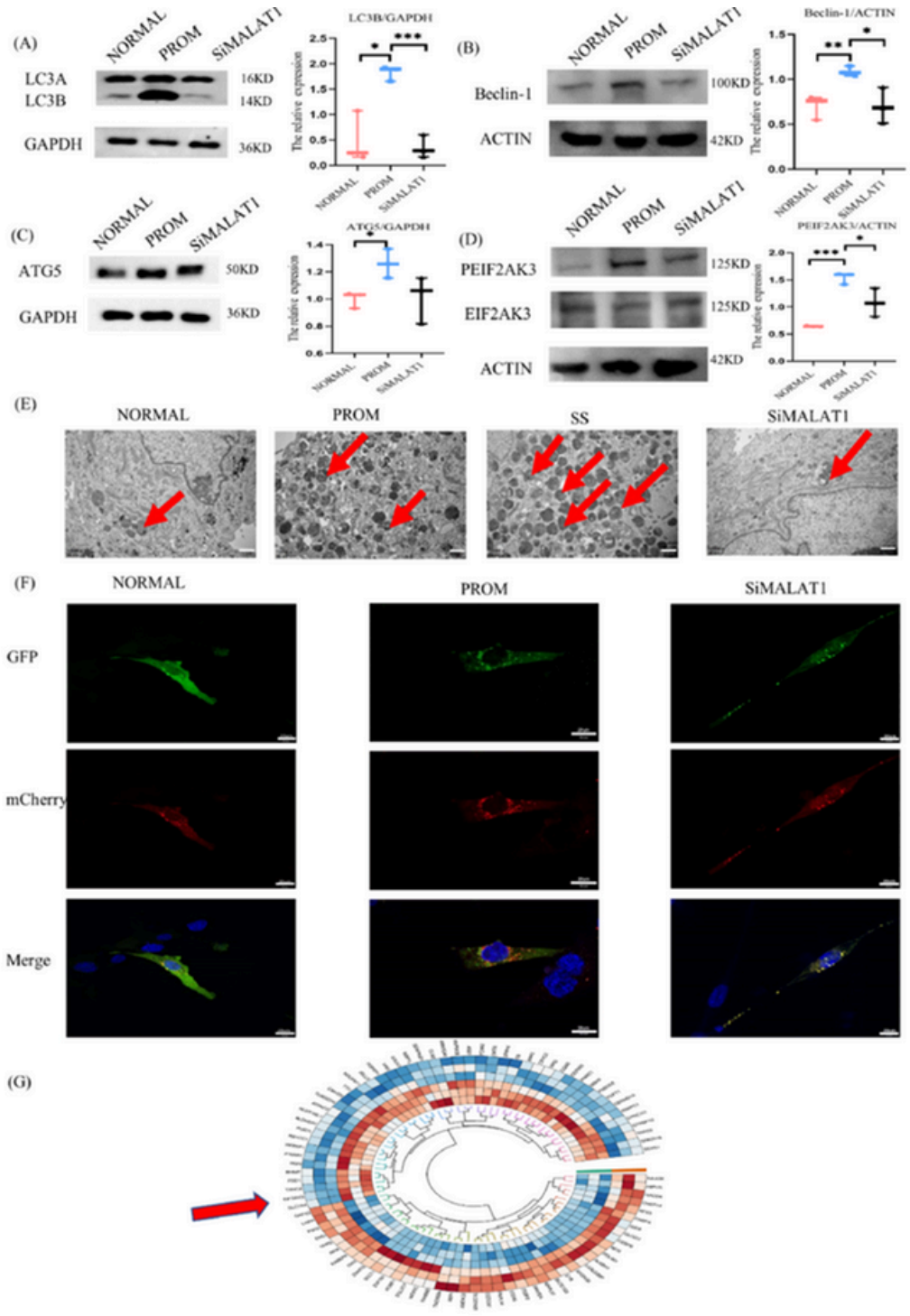

Fig. 5. Effect of MALAT1 on autophagy in amniotic fibroblasts
(A) WB results showing the expression of LC3 in cells,n = 3. (B) WB results showing the expression of Beclin-1 in cells, n = 3. (C) WB results showing the expression of ATG5 in cells, n = 3. (D) WB results showing the expression of PEIF2AK3 in cells, n = 3. (E) Representative TEM image of a fibroblast with arrows indicating autophagy lysosomes. (F) Detection of autophagy flow in the NORMAL, PROM and SiMALAT1 groups, 60X. (G) Circular clustering heatmap of differentially expressed proteins. Red indicates a relatively high relative expression level, blue indicates a relatively low relative expression level, and the red arrow indicates EIF2AK3.

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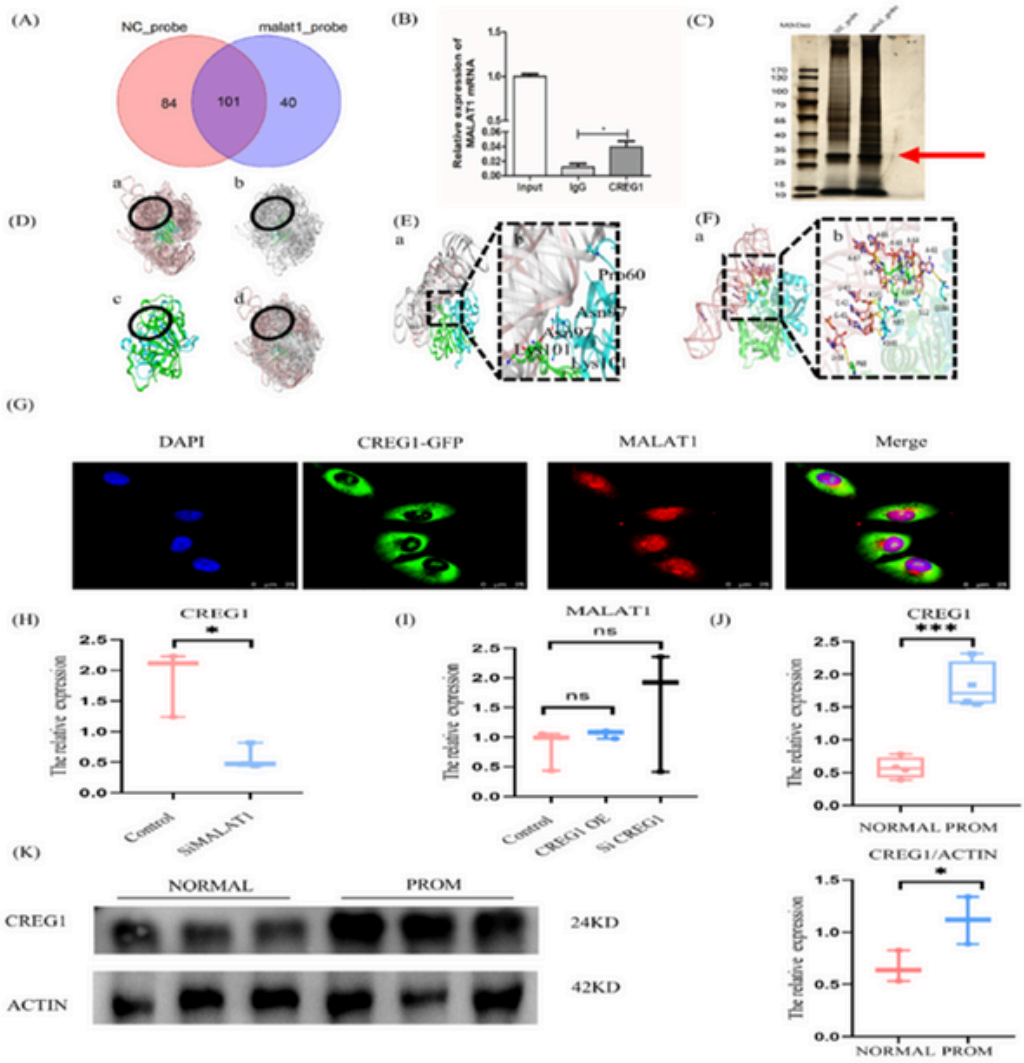

Fig. 6. Combination of MALAT1 and CREG1.
(A) Venn diagram of enriched proteins in the NC and MALAT1 probe groups. (B) The combination of MALAT1 and CREG1 was confirmed by a RIP assay. (C) RNA PULL DOWN protein silver stain map. (D) Location distribution of the docking results of the MALAT1 conformation and CREG1 dimer (a: MOE docking results; b: HDOCK docking results; c: CREG1 protein dimer; d: MOE, HDOCK docking results and CREG1 protein dimer). (E) The 7 conformations of the conformation concentration region (a: CREG1 protein dimer A and B chains are shown in green and blue, respectively. The conformation of the MOE docking results is light pink, and the HDOCK docking results are gray white. b: Local view of 5 interacting high frequency amino acids and 7 lncRNA conformations of the CREG1 protein dimer). (F) CREG1 and lncRNA interaction modes (a: In the overall view, the A and B chains of the CREG1 protein dimer are shown in green and blue cartoons respectively; LncRNAs are shown in light pink cartoons, amino acids and nucleotides are shown in stick patterns, carbon atoms are in the same color as in cartoon patterns, oxygen atoms are in red; and nitrogen atoms are in blue. Phosphorus atoms are orange. b: local view). (G) Images of MALAT1 and CREG1 fluorescence in situ hybridization. (H) The expression of CREG1 in cells after siMALAT1 was detected by qPCR, n = 3. (I) MALAT1 expression in CREG1-overexpressing and CREG1-knockdown cells was detected by qPCR, n = 3. (J) The expression of CREG1 in human amniotic tissue was detected by qPCR, n = 5. (K) WB results showing the expression of CREG1 in human amniotic tissue, n = 3.

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