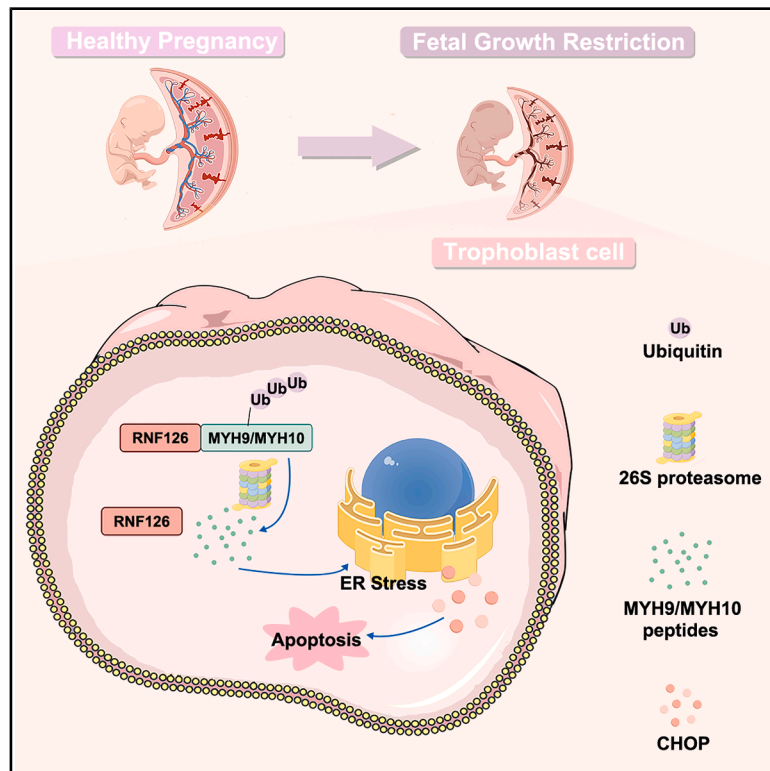


RNF126 mediates fetal growth restriction via ubiquitination-dependent degradation of the MYH9/MYH10 complex

Graphical abstract



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In brief

Therapy; Molecular interaction; Molecular network

Highlights

- Placental RNF126 cKO mice link elevated RNF126 to fetal growth restriction *in vivo*
- RNF126 ubiquitinates MYH9/MYH10, causing ER stress and trophoblast dysfunction
- RNF126 binds MYH9 and MYH10 via distinct domains, promoting their ubiquitination
- RNF126 shows dual potential: diagnostic biomarker and therapeutic target for FGR



Article

RNF126 mediates fetal growth restriction via ubiquitination-dependent degradation of the MYH9/MYH10 complex

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SUMMARY

Fetal growth restriction (FGR) remains a major contributor to neonatal morbidity and mortality worldwide, with limited effective diagnostic and therapeutic options. To better understand its molecular mechanism, we performed integrated multi-omics analyses of placental tissues from FGR pregnancies and normal controls, identifying Ring Finger Protein 126 (RNF126), an E3 ubiquitin ligase, as a key regulator of FGR and a potential biomarker distinguishing FGR from small-for-gestational-age (SGA) fetuses. Placenta-specific RNF126 conditional knockout (cKO) mice demonstrated a causal role of elevated placental RNF126 in FGR development *in vivo*. Functional studies revealed that RNF126 induced endoplasmic reticulum (ER) stress and apoptosis in trophoblasts. Mechanistically, RNF126 promoted a ubiquitin-proteasome-mediated degradation of the MYH9/MYH10 complex, thereby exacerbating ER stress and impairing trophoblast function, with lysine 833 (K833) of MYH9 identified as a critical ubiquitination site. Collectively, these findings elucidate an RNF126-mediated pathogenic mechanism in FGR and highlight RNF126 as a promising biomarker and therapeutic target.

INTRODUCTION

Fetal growth restriction (FGR) is a major cause of neonatal morbidity and mortality, ranking as the second leading cause of perinatal death worldwide.¹ Beyond its immediate perinatal consequences, FGR poses long-term health risks, including neurodevelopmental delays, cognitive impairment, and increased susceptibility to cardiovascular disease.² Epidemiological studies estimate that FGR affects approximately 5–10% of pregnancies globally,¹ with a reported incidence of 6.39% in China.³ Despite its clinical significance, the underlying pathogenesis of FGR remains poorly understood, and no effective early screening or targeted therapeutic strategies are currently available. Increasing evidence suggests that abnormal placental development and function play a central role in the occurrence of FGR,⁴ with trophoblast dysfunction recognized as a key contributor to placental insufficiency.⁵

Post-translational modifications (PTMs) are covalent chemical changes to proteins after translation that regulate conformation, activity, subcellular localization, and interactions, thereby influencing diverse biological processes and disease progression.^{6–8} Among them, ubiquitination plays a crucial role in maintaining intracellular protein homeostasis by modulating protein degradation and function.⁹ Preliminary studies re-

vealed that ubiquitination levels in placentas from fetuses with FGR were significantly elevated compared to the normal pregnancy (NP) group, suggesting a potential link between abnormal ubiquitination and FGR pathogenesis (Figures 1B and 1C). However, the role of ubiquitination in FGR remains largely unexplored. To address this, we performed integrated 4D label-free quantitative proteomics and ubiquitin-modified proteomics on human placental tissues from FGR and NP. This analysis identified the E3 ubiquitin ligase RNF126 as significantly dysregulated, implicating it as a potential key regulator in FGR-related ubiquitination processes and warranting further mechanistic investigation.

RNF126 is an RING E3 ubiquitin ligase that mediates the ubiquitination process via the ubiquitin-proteasome system. It is related to protein degradation, signal transduction, DNA damage repair, and cell cycle functions, all of which are involved in embryonic development.^{10–12} While there are no reports on the impact of abnormal RNF126 expression on trophoblast cell function and pregnancy-related disorders, a study published in *Nature* in 2010 highlighted the essential role of the E3 ubiquitin ligase RNF12, a member of the RING family, in the development of extraembryonic trophoblast.¹³ Hence, we hypothesize that aberrant expression of RNF126 may affect trophoblast cell function, leading to the occurrence of FGR and potentially providing



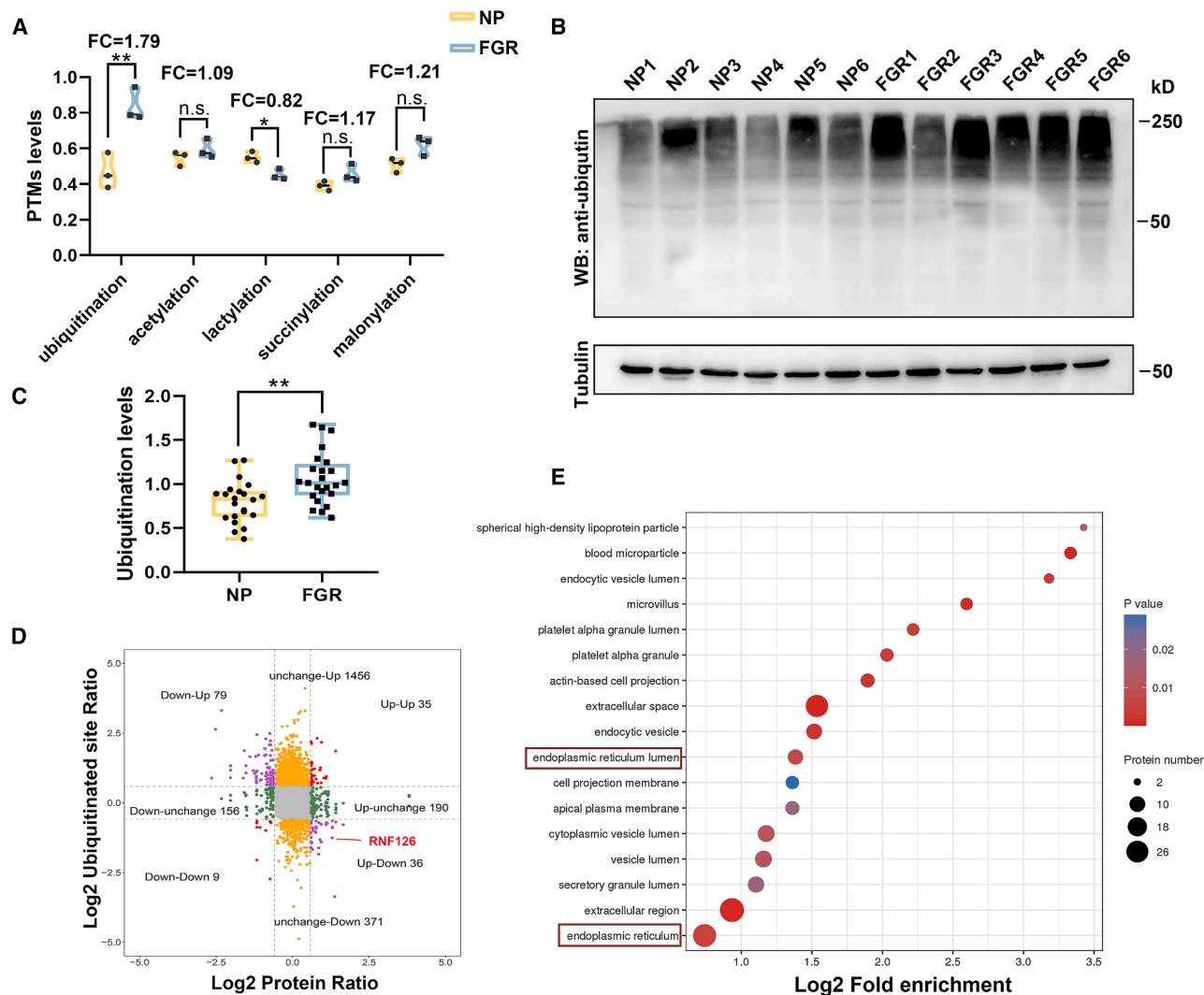


Figure 1. Multi-omics profiling identifies RNF126 as a candidate regulator in FGR placentas

(A) Violin plot shows the expression levels of five post-translational modifications in placental tissues from NP and FGR groups ($n = 3$ placentas per group). (B and C) Representative western blot and corresponding quantification of protein ubiquitination in NP ($n = 22$ placentas) and FGR ($n = 24$ placentas) placentas. (D) The cross-linking analysis diagram of the DEPs (FGR/NP |Log2 protein ratio| > 0.58) between ubiquitin-modified proteomics and quantitative proteomics (NP, $n = 5$ placentas; and FGR, $n = 5$ placentas). (E) GO enrichment analysis of cellular component on the DEPs (FGR/NP |Log2 protein ratio| > 0.58, $p < 0.05$) in the two groups. Violin plot in A shows all individual points with the median indicated. Boxplot in C represents median and IQR, whiskers indicate min-max. * $p < 0.05$ and ** $p < 0.01$, n.s. not significant.

an avenue for investigating FGR and other pregnancy-related disorders.

RNA-seq indicated that increased RNF126 expression in trophoblast cells activates ER stress-related pathways. Recent studies have shown that ER stress inhibits trophoblast proliferation, promotes apoptosis, and ultimately disrupts placental development, thereby impairing fetal growth.¹⁴ ER stress, caused by the abnormal accumulation of unfolded or misfolded proteins within the endoplasmic reticulum (ER), has been recognized as a contributing factor in various human diseases, including cancer, neurodegenerative disorders, and placental pathologies.^{15–18} The ER transmits information about protein folding status to the nucleus and cytoplasm

by activating the unfolded protein response (UPR) pathway, which is mainly mediated by three signaling pathways: protein kinase RNA (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring kinase 1 (IRE1). This adaptive response aims to restore protein homeostasis; however, prolonged or severe ER stress can trigger apoptosis.¹⁹ The proapoptotic UPR involves (1) the PERK-elf2 α branch, which triggers the translation of ATF4, leading to the activation of the proapoptotic factors CHOP and GADD34, and (2) the IRE1 α branch, which contributes to caspase 2-dependent or caspase 8-dependent apoptosis through RIDD or the activation of TRAF2-JUN N-terminal kinase (JNK) signaling.^{20–22}

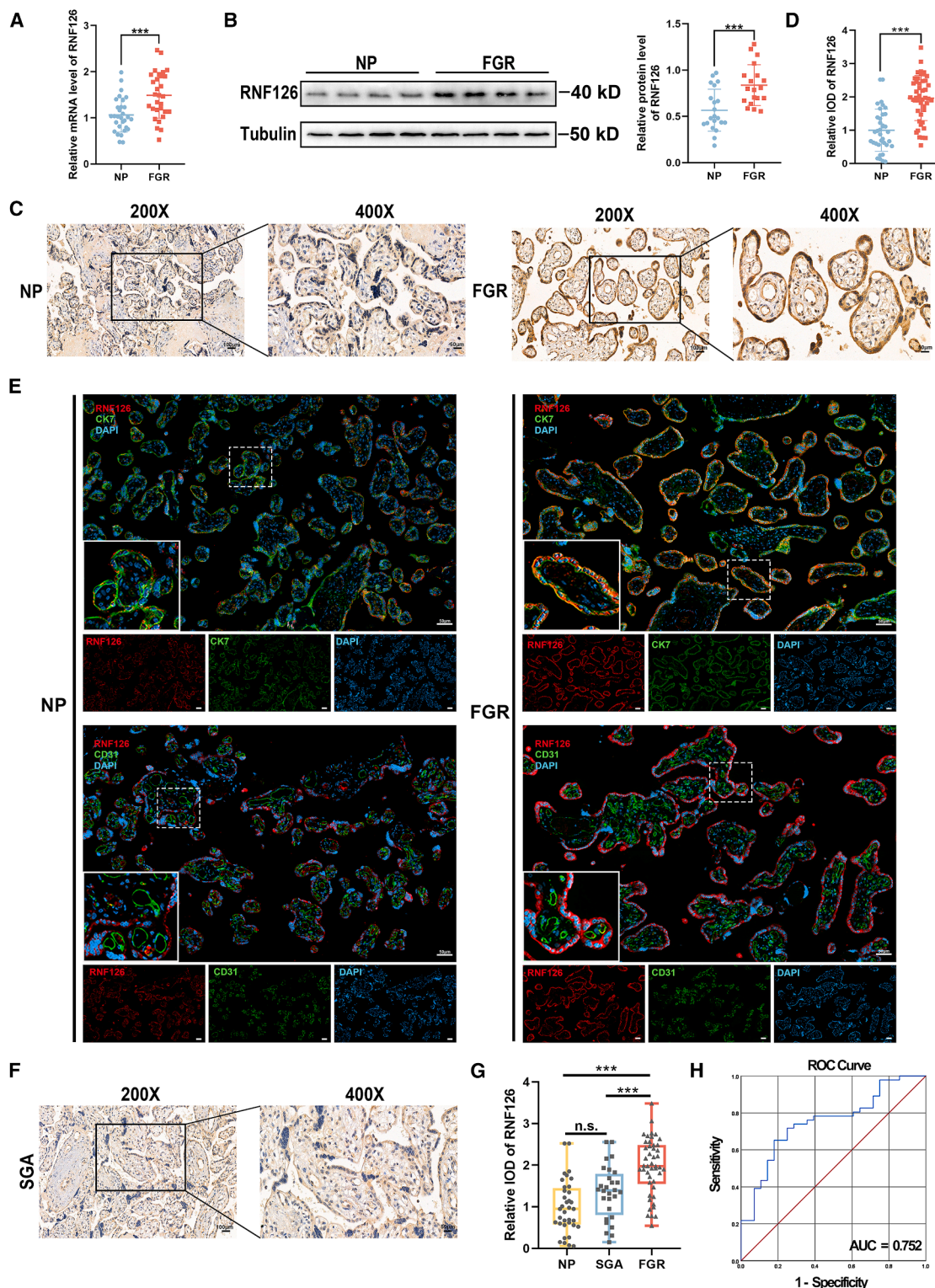


Figure 2. RNF126 is upregulated in FGR placentas, localized to trophoblasts, and distinguishes FGR from SGA

(A) RT-qPCR analysis of RNF126 mRNA expression in placental tissues from NP and FGR groups ($n = 30$ placentas per group).

(B) Representative western blot and corresponding quantification of RNF126 protein levels in placentas from NP ($n = 21$ placentas) and FGR ($n = 19$ placentas).

(C and D) Representative IHC staining and quantitative analysis of RNF126 expression in placentas from NP ($n = 36$ placentas) and FGR ($n = 46$ placentas). Scale bars, 100 μ m and 50 μ m in the enlarged image.

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To explore potential RNF126-interacting proteins in trophoblast cells, we constructed a GST-RNF126 fusion protein and performed a GST pull-down assay combined with mass spectrometry analysis. This approach preliminarily identified non-muscle myosin IIA (NM IIA, encoded by MYH9) and non-muscle myosin IIB (NM IIB, encoded by MYH10) as candidate binding partners of RNF126. MYH9 and MYH10 are structural proteins that encode components of the non-muscle myosin II (NM II) family, which plays critical roles in cytoskeletal organization and intracellular transport.²³ Recent evidence suggests that these two proteins can interact to form a complex involved in regulating diverse cellular processes, including proliferation, apoptosis, and ROS homeostasis, as well as mitigating ER Stress.^{24,25} Moreover, loss of MYH9 or MYH10 has been associated with impaired placental and fetal development in mouse models.^{26–28} However, the functions and mechanisms of MYH9 and MYH10 in FGR and placental trophoblasts are still unknown.

E74-like ETS transcription factor 5 (ELF5) is an epithelial-specific member of the ETS family that plays key roles in terminal keratinocyte differentiation and the regulation of epithelial gene expression in glandular tissues, including the salivary and prostate glands.²⁹ In the placenta, Elf5-Cre transgene activity is specifically restricted to the trophoblast lineage, including spongiotrophoblasts, trophoblast giant cells, and labyrinthine trophoblasts.³⁰ This lineage-specific expression makes Elf5-Cre mice a robust and widely accepted model for trophoblast-specific gene deletion during placental development.

In this study, we performed integrated multi-omics analyses of human placental tissues from pregnancies complicated by FGR and identified the E3 ubiquitin ligase RNF126 as a critical regulator in the pathogenesis of FGR, as well as a potential biomarker for distinguishing FGR from small-for-gestational-age (SGA). Mechanistically, RNF126 promotes ER stress and apoptosis in trophoblasts by facilitating the ubiquitin-proteasome-dependent degradation of the MYH9/MYH10 complex. These findings uncover a previously unrecognized pathogenic mechanism in FGR and highlight RNF126 as a promising diagnostic biomarker and therapeutic target for its clinical management.

RESULTS

Multi-omics profiling identifies RNF126 as a candidate regulator

In preliminary experiments, Coomassie brilliant blue staining confirmed comparable total protein levels in human placental lysates from NP ($n = 3$) and FGR ($n = 3$) samples (Figure S1A). Using equal protein loading, we then assessed multiple PTMs, including ubiquitination, acetylation, lactylation, succinylation,

and malonylation (Figures S1B–S1F). Among these, ubiquitination exhibited the greatest fold increase ($FC = 1.79$) and statistical significance ($p < 0.01$) in FGR tissues (Figure 1A). Therefore, we focused subsequent analyses on ubiquitination. To validate this finding, we expanded the cohort (NP, $n = 22$; FGR, $n = 24$), which confirmed a robust and consistent elevation of ubiquitination in FGR placentas ($p < 0.01$, Figures 1B and 1C).

Based on the above results, we proceeded with a multi-omics analysis, combining quantitative proteomics with ubiquitin-modified proteomics, using 10 human placental samples (5 NP and 5 FGR). Characteristics of the collected samples are shown in Table S1. Integrated analysis of ubiquitin-modified and quantitative proteomic datasets revealed proteins with inverse correlations between expression and ubiquitination levels, suggesting dual regulation of protein stability or activity in FGR. Among these candidates, RNF126 was the only differentially expressed E3 ubiquitin ligase ($FC > 1.5$, Figure 1D), whereas no E1 or E2 enzymes exhibited significant changes. Given that aberrant E3 ligase activity often underlies dysregulated ubiquitination and has been implicated in developmental and neurodegenerative disorders,³¹ RNF126 was prioritized for further investigation. RNF126 is involved in key cellular processes such as protein degradation, DNA damage repair, signal transduction, and cell cycle regulation. Data from the Human Protein Atlas (<https://www.proteinatlas.org/>) show relatively high expression of RNF126 in placental tissue, yet its role in pregnancy-related disorders or trophoblast biology has not been reported, indicating potential significance in placental physiology and pathology. GO enrichment analysis of cellular components based on the differentially expressed proteins (DEPs) between the two placental groups revealed significant enrichment in several ER-related pathways, including the “endoplasmic reticulum lumen” ($p < 0.01$, Figure 1E).

RNF126 is upregulated in FGR placentas, localized to trophoblasts, and distinguishes FGR from SGA

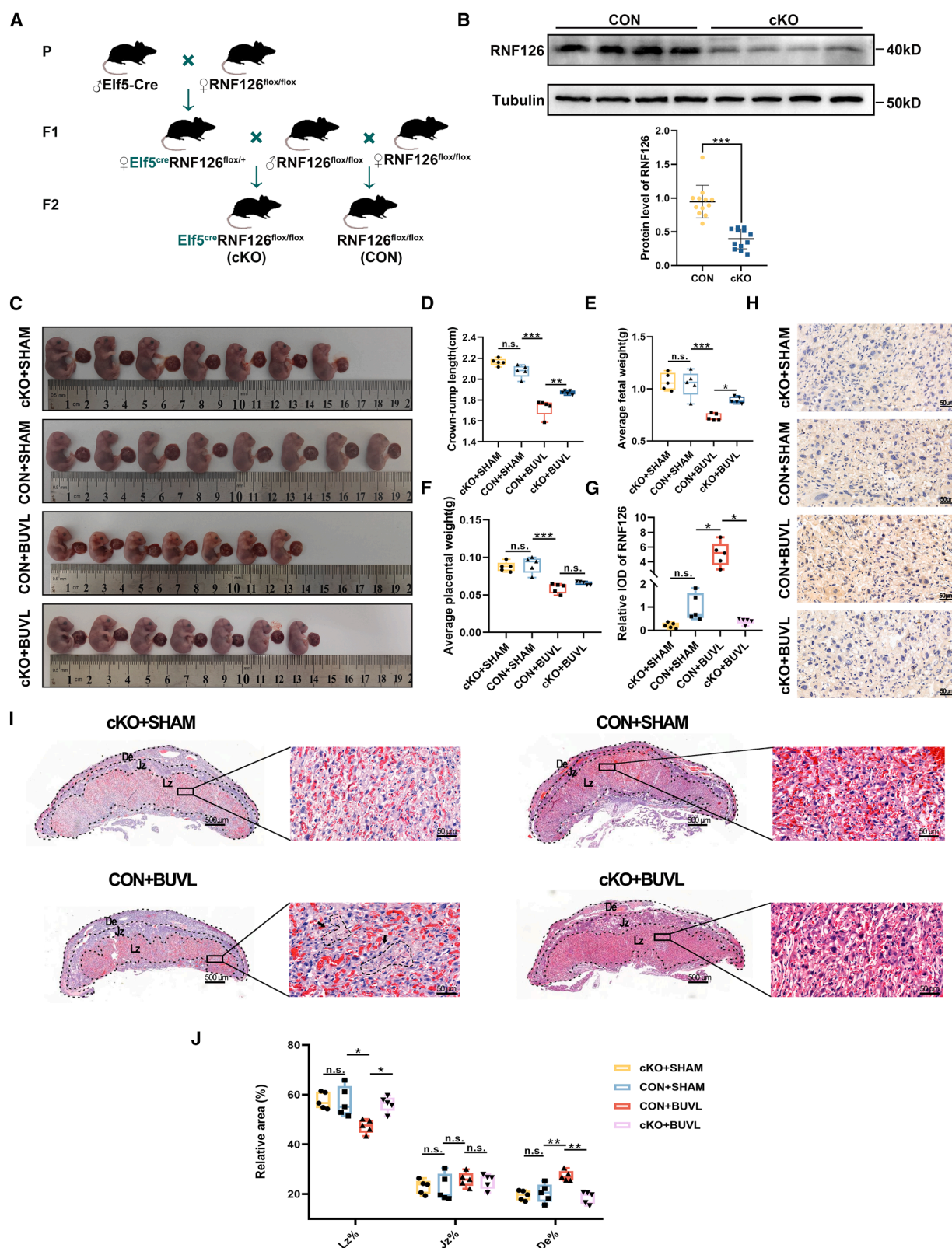
We further collected human placental samples from 36 normal pregnancies and 46 FGR pregnancies, which were promptly processed. The baseline characteristics of the collected placental clinical samples are listed in Table S2. RT-qPCR analysis revealed a significant upregulation of RNF126 mRNA levels in FGR placentas compared to controls ($p < 0.001$, Figure 2A). The increased protein levels of RNF126 in FGR placentas were confirmed through western blot ($p < 0.001$, Figure 2B) and IHC ($p < 0.001$, Figures 2C and 2D). Correlation analysis revealed a negative association between RNF126 expression and neonatal birth weight, whereas placental diameter and weight were excluded from the analysis as they represent estimated rather than direct clinical measures (Table S3). Cytokeratin 7 (CK7), broadly expressed across all trophoblast subtypes, is widely used to distinguish trophoblasts from other placental cell

(E) Representative images of immunofluorescence co-staining of RNF126 and CK7 or CD31 in the NP and FGR placentas ($n = 3$ placentas per group). Scale bars, 50 μm .

(F) Representative IHC staining in SGA ($n = 28$ placentas) placental tissues. Scale bars, 100 μm and 50 μm in the enlarged image.

(G) Quantitative analysis of RNF126 expression in placentas from NP ($n = 36$ placentas), SGA ($n = 28$ placentas), and FGR ($n = 46$ placentas).

(H) ROC curve analysis of RNF126 for distinguishing FGR from SGA. Data in A, B, and D are presented as mean $\pm$ SD. Boxplot in G represents median and IQR, whiskers indicate min-max. *** $p < 0.001$, n.s. not significant.



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types.^{32,33} To determine the cellular localization of RNF126, we performed multiplex immunofluorescence staining with CK7-marked trophoblast cells and CD31-marked vascular endothelial cells (Figure 2E). RNF126 co-localized predominantly with CK7-positive cells, indicating its expression in trophoblasts. These findings suggest that RNF126 may contribute to the development of FGR by modulating trophoblast cell function.

Given the clinical challenge of distinguishing SGA from FGR, we next examined whether RNF126 could serve as a discriminatory biomarker. IHC analysis of 28 paraffin-embedded SGA placentas revealed RNF126 expression levels comparable to those of the NP group but significantly lower than those of the FGR group ($p < 0.001$, Figures 2F and 2G). ROC curve analysis further demonstrated that RNF126 effectively differentiated FGR from SGA (AUC = 0.752; Figure 2H). Collectively, these results identify RNF126 as a potential diagnostic marker for FGR and a biomarker capable of differentiating FGR from SGA.

Placenta-specific RNF126 conditional knockout rescues FGR phenotypes

Following the breeding strategy outlined in Figure 3A, the protein levels of RNF126 in the mouse placentas of the cKO group were significantly decreased compared to those in the CON group ($p < 0.001$, Figure 3B). To generate experimental cohorts, $Elf5^{cre}RNF126^{fl/fl}$ and $RNF126^{fl/fl}$ females were mated with $RNF126^{fl/fl}$ males, producing $Elf5^{cre}RNF126^{fl/fl}$ (cKO) and $RNF126^{fl/fl}$ (CON) fetuses. At gestational day (GD) 17, pregnant dams underwent bilateral uterine vessel ligation (BUVL) under isoflurane anesthesia, whereas control animals received sham surgery (SHAM). Fetuses and placentas were collected at GD18.5, and placental genotypes were confirmed (Figure S2). BUVL induced an FGR phenotype in the CON+BUVL group, which was alleviated in cKO+BUVL fetuses (Figure 3C). Comparison among the cKO+SHAM, CON+SHAM, CON+BUVL, and cKO+BUVL groups revealed that placenta-specific knockout of RNF126 reversed the reductions in average crown-rump length ($p < 0.01$, Figure 3D) and average fetal weight ($p < 0.05$, Figure 3E) observed in the FGR model, while having no significant effect on average placental weight (Figure 3F). IHC staining confirmed RNF126 expression patterns across the four genetically defined groups (Figures 3G and 3H). H&E staining showed infarctions in the labyrinth zones (Lz) of placentas in the CON+BUVL group (Figure 3I), which were mitigated by RNF126 deletion. Quantitative analysis further demonstrated that RNF126 knockout restored the proportions of the Lz ($p < 0.05$) and decidua areas ($p < 0.001$) (Figure 3J), indicating improved placental structure in FGR.

ER stress mediates the effect of RNF126 on the trophoblast's function

To investigate the underlying mechanisms, we established stable RNF126 overexpression and knockdown models in BeWo cells, which serve as a model of human villous trophoblast, via lentiviral transduction (Figures 4A and S3A). RNF126 overexpression markedly reduced BeWo cell proliferation ($p < 0.01$) and increased apoptosis ($p < 0.001$), whereas RNF126 depletion produced the opposite effects (Figures 4B, 4C, S3B, and S3C). These *in vitro* findings parallel the enhanced trophoblast apoptosis observed in FGR placentas, supporting a pathogenic role for RNF126 in trophoblast dysfunction.

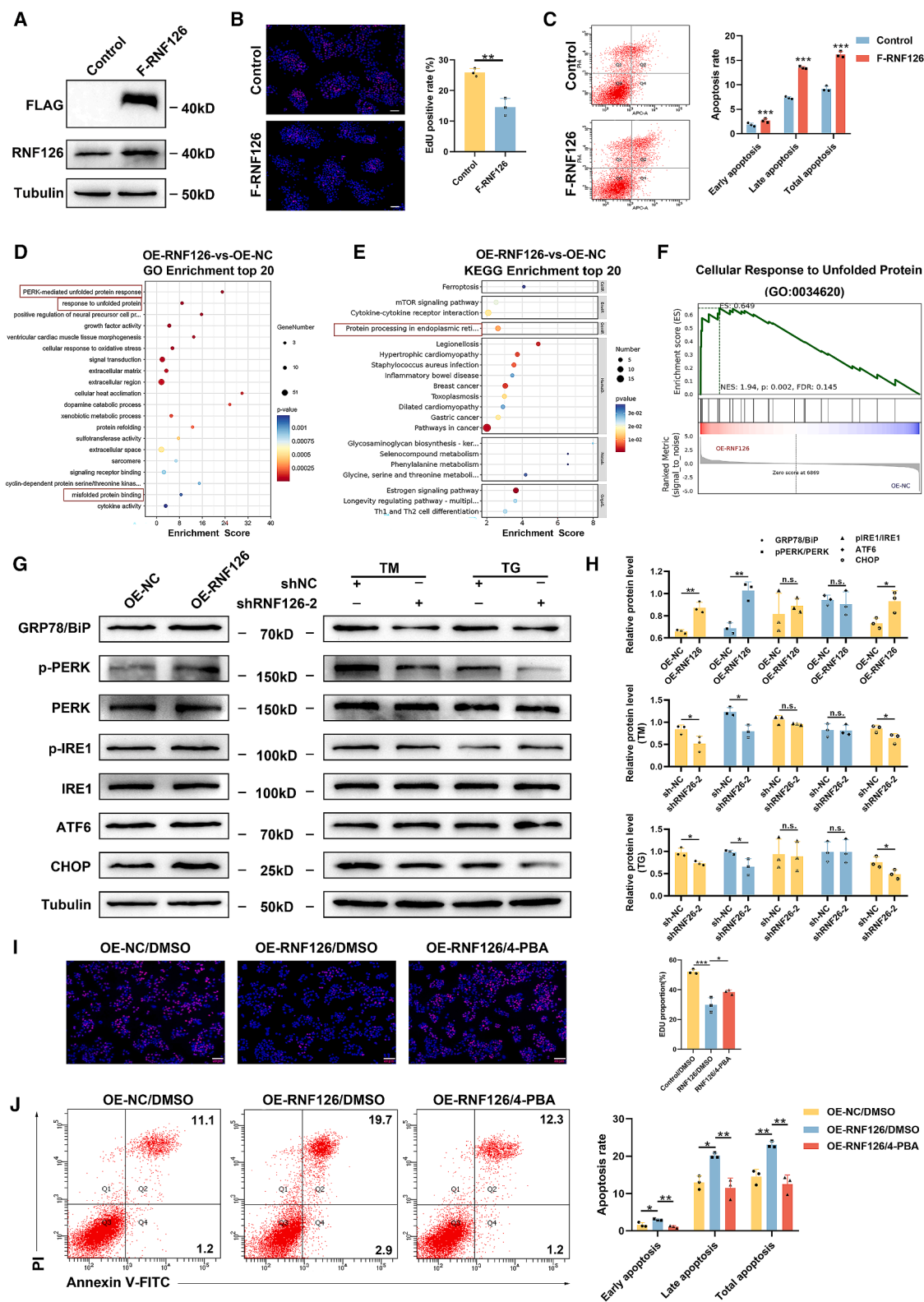
To explore the downstream mechanisms of RNF126, we performed RNA sequencing in BeWo cells with stable RNF126 overexpression (OE-RNF126) or empty vector (OE-NC). This analysis identified 332 differentially expressed genes ($|\log_2 \text{Fold-Change}| > 0.58$, $p < 0.05$), and subsequent GO, KEGG, and GSEA analyses revealed a notable enrichment of ER stress-related pathways, including “PERK-mediated unfolded protein response” (Figures 4D–4F). To validate these transcriptomic findings at the protein level, western blot analysis was conducted to examine key ER stress markers. Stable RNF126 overexpression and knockdown efficiency in BeWo cells were confirmed (Figure S6A). RNF126 overexpression increased, while its knockdown under tunicamycin (TM)- or thapsigargin (TG)-induced ER stress decreased, the levels of GRP78/BiP, pPERK, and CHOP (Figures 4G and 4H). These findings demonstrate that RNF126 disrupts ER homeostasis, specifically by activating ER stress via the PERK pathway. We then tested whether alleviating ER stress could reverse the effects of RNF126. Treatment with 4-phenylbutyric acid (4-PBA), a chemical chaperone known to reduce ER stress and CHOP expression,^{34,35} significantly restored proliferation ($p < 0.05$) and decreased apoptosis ($p < 0.01$) in OE-RNF126 cells, as measured by EdU incorporation (Figure 4I) and flow cytometry (Figure 4J). Optimal concentrations of TM, TG, and 4-PBA were determined through concentration gradient experiments, using GRP78/BiP expression as a positive control (Figures S4A–S4D). Together, these results suggest that RNF126 may inhibit proliferation and promote apoptosis in BeWo cells by activating ER stress, primarily through the PERK-CHOP pathway.

RNF126 interacts with MYH9 and MYH10, promoting their ubiquitin-mediated degradation

To explore how RNF126 induces ER stress in BeWo cells, GST pull-down assays followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) were performed to

Figure 3. RNF126 knockout in the placenta rescues FGR phenotypes

(A) Breeding strategy for generating RNF126 cKO mice.
(B) RNF126 protein levels in placentas from control (CON) and cKO mice ($n = 12$ placentas from 4 litters per group).
(C) Representative images of fetuses and placentas from each group ($n = 5$ litters per group).
(D–F) Boxplots of average crown-rump length (D), average fetal weight (E) and average placental weight (F) ($n = 5$ litters per group).
(G and H) Representative IHC staining images and quantification of RNF126 expression in mice placentas from each group ($n = 5$ placentas from 5 independent litters per group; three fields were analyzed per placenta). Scale bars, 50 μm .
(I and J) Representative H&E staining images of midsagittal placental sections and corresponding quantitative analysis of the percentage areas of decidua (De), junctional zone (Jz), and labyrinth zone (Lz) in each group ($n = 5$ placentas from 5 independent litters per group). Scale bars, 500 μm and 50 μm in the enlarged image. Typical lesions are marked with a dotted line. Data in B are presented as mean $\pm$ SD. Boxplots in D, E, F, G, J represent median and IQR, whiskers indicate min-max. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, n.s. not significant.



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identify potential RNF126 substrates (Figure 5A). MYH9 and MYH10, both showing significantly increased ubiquitination modification in the prior ubiquitin-modified proteomic analysis, were identified as the top two potential interacting roles (Figures 5B and 5C). The top 10 potential interacting proteins were listed in Table S8. Western blot analysis revealed reduced protein levels of MYH9 and MYH10 upon RNF126 overexpression in BeWo cells, while their mRNA levels remained unchanged (Figures 5D and 5E), suggesting post-translational regulation. Co-IP confirmed physical interactions between RNF126 and these proteins (Figures 5F and 5G), which were further supported by colocalization in immunofluorescence and 3D reconstruction imaging (Figure 5H). Cycloheximide (CHX) chase assays showed that RNF126 overexpression (Figure S6B) accelerated the degradation of endogenous MYH9 and MYH10 (Figures 5I and 5J). This effect was blocked by the proteasome inhibitor MG132 but not by the lysosome inhibitor CQ, indicating proteasome-dependent degradation (Figure 5K). Furthermore, RNF126 overexpression significantly enhanced the ubiquitination of MYH9 and MYH10, as confirmed by denaturing-IP assays (Figure 5L).

RNF126 induces ER stress and dysfunction in trophoblast cells by ubiquitinating and degrading the MYH9/MYH10 complex

Given that we found that RNF126 binds to MYH9 and MYH10, we next examined whether this regulation is unidirectional. Overexpression of MYH9 or MYH10 in BeWo cells, followed by western blot, revealed that each protein upregulated RNF126 and also enhanced the expression of the other (Figure 6A), suggesting the three may form a functional complex *in vivo*. Notably, co-overexpression of MYH9 (1 μ g) and MYH10 (1 μ g) in RNF126-overexpressing cells (Figure S6C) more effectively restored ER stress-related marker expression than individual overexpression at equivalent total plasmid amounts (2 μ g) (Figure 6B). Functional assays showed that this co-overexpression increased proliferation ($p < 0.05$; EdU assay, Figure 6C) and reduced apoptosis ($p < 0.05$; flow cytometry, Figure 6D) compared with OE-RNF126 cells alone. Next, molecular docking analysis was performed to predict the interaction domains among RNF126, MYH9, and MYH10. RNF126 primarily bound to the coiled-coil domains of both MYH9 and MYH10 (Figure S5A), and multiple binding sites were detected across different domains of MYH9 and MYH10 (Figure S5B). To further investigate, we generated a series of RNF126 truncated mutants (Figure 6E). Co-IP exper-

iments were performed in BeWo cells co-expressing full-length MYH9 and MYH10 with different deletion mutants of Flag-tagged RNF126 (Figure 6F). Deletion of the zinc finger domain markedly impaired RNF126-MYH9 interaction, whereas the deletion of the RING domain significantly reduced RNF126-MYH10 binding, indicating that RNF126 can simultaneously recognize and bind both proteins, thereby facilitating their ubiquitination as a complex. Consistent with our ubiquitin-modified proteomic data, MYH9 in FGR placentas exhibited increased ubiquitination at lysine residues K821 and K833, while MYH10 showed enhanced ubiquitination at K859 (Figure 6G). To dissect RNF126-mediated ubiquitination, these sites were mutated and analyzed in *in vitro* ubiquitination assays (Figure 6H). Mutation of MYH9 K833 markedly reduced RNF126-induced ubiquitination in BeWo cells, whereas the MYH10 K859 mutation had no significant effect, suggesting that MYH10 ubiquitination is mediated through lysine residues other than K859.

In vivo expression levels of MYH9, MYH10, and CHOP

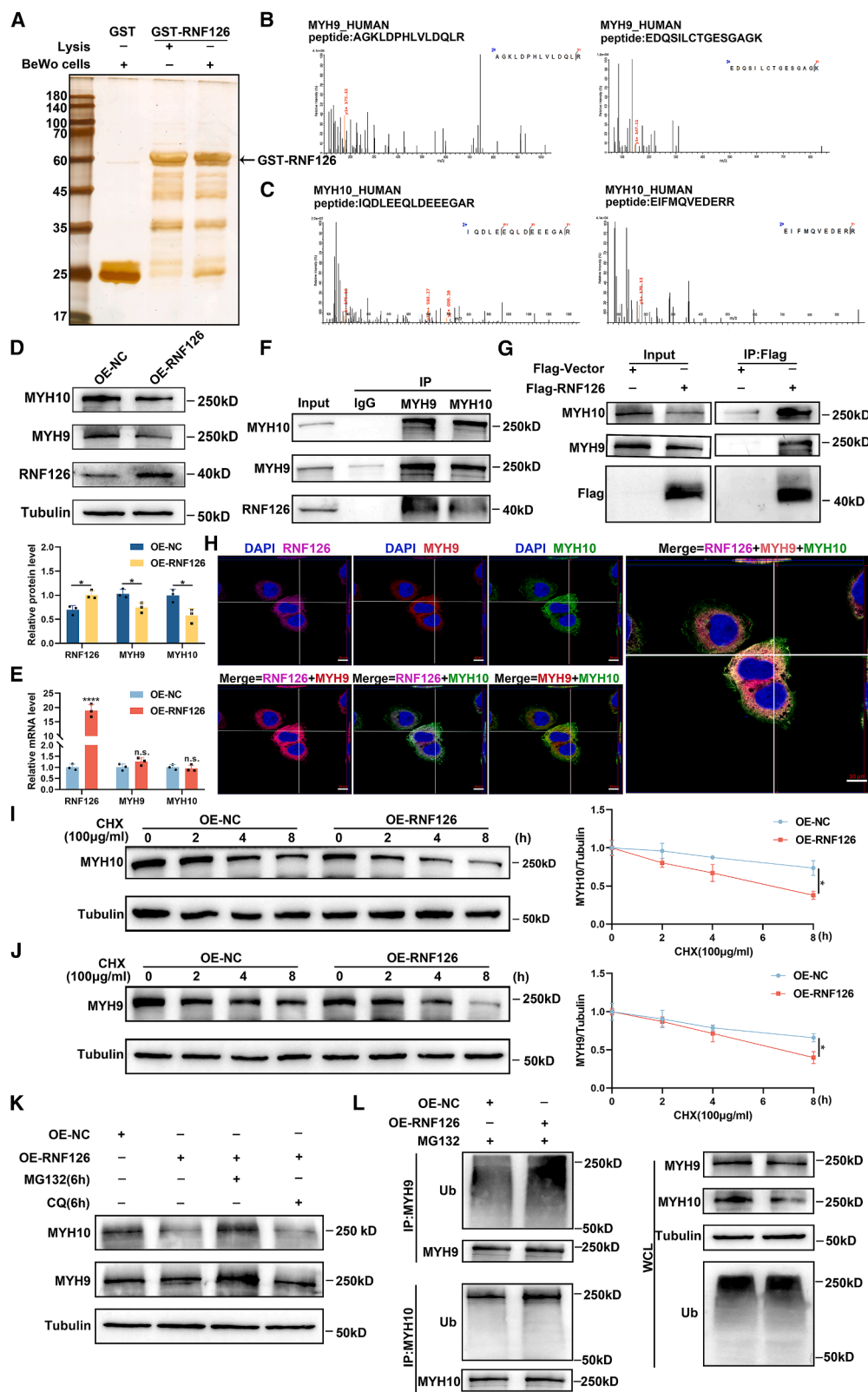
To extend our *in vitro* mechanistic findings, we next examined the relationship among MYH9, MYH10, CHOP, and RNF126 *in vivo*. IHC staining was performed in placentas from four groups of genetically modified mouse models, as well as human placentas from FGR and control pregnancies. In the CON+BUVL (FGR model) group, CHOP expression was markedly elevated ($p < 0.01$), while MYH9 ($p < 0.01$) and MYH10 ($p < 0.001$) levels tended to decrease (Figure 7A). Importantly, these changes were reversed in the placenta-specific RNF126 knockout group. Consistently, placentas from patients with FGR showed decreased MYH9 ($p < 0.001$) and MYH10 ($p < 0.05$) expression alongside elevated CHOP levels ($p < 0.01$) (Figure 7B). These results provide *in vivo* support for the mechanistic link between RNF126 and the MYH9/MYH10-ER stress axis observed *in vitro*.

DISCUSSION

Our study identifies RNF126 as a critical regulator of placental dysfunction in FGR. RNF126 was significantly upregulated in FGR placentas, localized primarily to trophoblasts. Transcriptional and protein interaction analyses suggest that RNF126 promotes trophoblast ER stress through interactions with MYH9 and MYH10, thereby driving the pathological cascade leading to growth restriction. These findings not only provide mechanistic insight but also highlight RNF126 as a potential biomarker

Figure 4. ER Stress mediates the effect of RNF126 on trophoblast function

(A) Western blot confirms stable overexpression of Flag-RNF126 (F-RNF126) in BeWo cells. Representative blot is shown from three independent experiments. (B and C) Stable expression of RNF126 inhibited proliferation (B) and promoted apoptosis (C) in BeWo cells. Data are shown as representative images and quantitative analyses ($n = 3$ independent experiments). Scale bars, 50 μ m. (D and E) The GO and KEGG enrichment analysis plot of the differentially expressed genes (DEGs; $|\text{Log}_2 \text{FoldChange}| > 0.58$, $p < 0.05$) between two groups. (F) Gene set enrichment analysis of RNA-seq data shows the enrichment of unfolded-protein-response-signaling related genes in OE-RNF126 cells compared to OE-NC cells. (G and H) Representative western blots and quantification show increased GRP78/BiP, pPERK, and CHOP with RNF126 overexpression, and reduced levels upon RNF126 knockdown under TM- or TG-induced ER stress for 24h ($n = 3$ independent experiments). (I and J) Representative images and quantification show that 24h treatment with 4-PBA (1 mM) reverses RNF126 overexpression-induced changes in cell proliferation (I) and apoptosis (J) ($n = 3$ independent experiments). Scale bars, 50 μ m. Data in B, C, H, I, and J are presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, n.s. not significant.



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and therapeutic target. Given the clinical challenge of distinguishing FGR from constitutionally small but healthy SGA cases, RNF126 expression may offer a molecular tool for more precise diagnosis, and targeting its activity or downstream pathways could inform therapeutic strategies.

Aberrant ubiquitination, often driven by mutations or dysregulation of E3 ubiquitin ligases, has been linked to adverse pregnancy outcomes such as miscarriage, preeclampsia, and FGR.^{31,36–38} Among the DEPs identified by our multi-omics analysis, RNF126, a RING-type E3 ligase, emerges as a central mediator of protein ubiquitination and regulates fundamental processes including protein degradation, signal transduction, DNA damage repair, and cell cycle. Dysregulated RNF126 expression has also been reported in several cancers^{39,40} and is crucial for embryonic development,^{12,39,41} underscoring its broad biological significance.

When misfolded proteins accumulate beyond a critical threshold, cells activate several adaptive pathways to restore proteostasis, including the UPR, ER-associated degradation (ERAD), and autophagy.⁴² Among these mechanisms, the UPR is the principal pathway. It transmits stress signals from the ER to the cytoplasm and nucleus, enhances the cellular protein-folding capacity, and, when stress cannot be resolved, initiates apoptosis.¹⁹ Excessive activation of this pathway has been implicated in various diseases, including FGR.¹⁴ In our study, the treatment of BeWo cells with the ER stress inhibitor 4-PBA effectively attenuated UPR activation and restored the expression of the ER chaperone GRP78/Bip while reducing apoptosis. Consistent with these *in vitro* findings, the FGR mouse model induced by BUVL exhibited marked upregulation of CHOP downstream of ATF4. Increased CHOP expression promoted trophoblast apoptosis, leading to placental structural damage and contributing to the development of FGR.

MYH9 and MYH10 encode non-muscle myosin II heavy chains that contribute to cytoskeletal organization, cell division and proliferation, adhesion and migration, and mitochondrial homeostasis.^{43–46} Their abnormal expression or dysfunction has been linked to impaired embryonic development and neurodevelopmental disorders.^{26–28} Recent evidence indicates that MYH9 and MYH10 can form a functional complex that regulates diverse cellular processes, including proliferation, apoptosis, reactive oxygen species homeostasis, and plays a protective role against

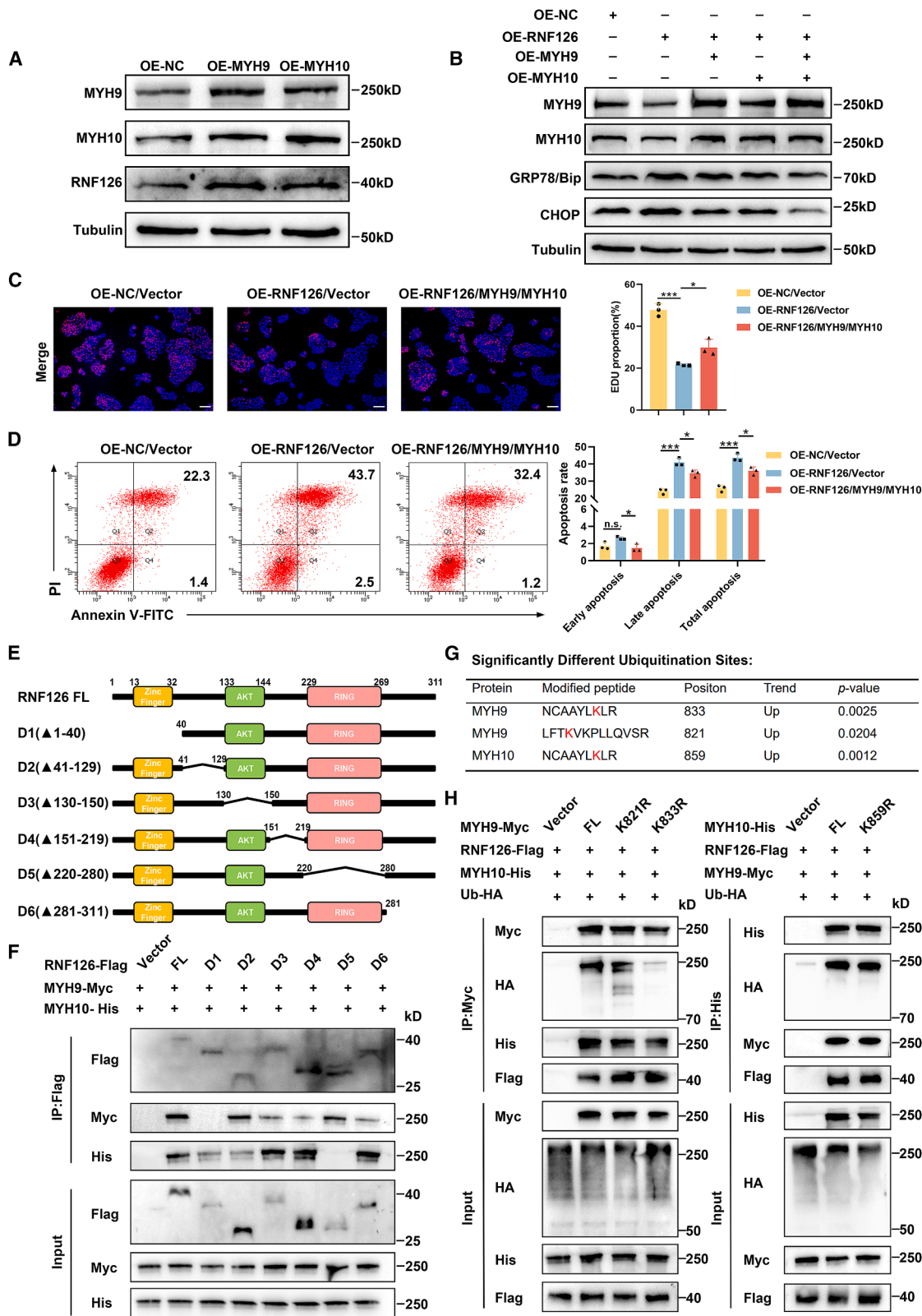
ER stress.^{24,25} In our ubiquitin-modified proteomic analysis, both MYH9 and MYH10 exhibited increased ubiquitination sites, suggesting enhanced PTM. This finding led us to hypothesize that RNF126 may promote ubiquitin-mediated degradation of the MYH9/MYH10 complex, thereby triggering ER stress in trophoblasts. Supporting this hypothesis, co-transfection of MYH9 and MYH10 restored ER homeostasis and improved cell function more effectively than the transfection of either protein alone at an equivalent total plasmid dose, consistent with a synergistic effect when both proteins form a complex. Furthermore, IHC analysis of placentas from patients with FGR and from the BUVL-induced FGR mouse model confirmed a marked decrease in MYH9 and MYH10 expression, reinforcing the *in vivo* relevance of the RNF126–MYH9/MYH10 axis in the pathogenesis of FGR.

Unresolved questions for future research include: (1) A key unanswered question is the identification of upstream regulators responsible for controlling RNF126 ubiquitination. Our ubiquitin-modified proteomic analysis revealed reduced ubiquitination of RNF126 in FGR placentas, suggesting that its regulatory machinery is altered under pathological conditions. Determining which E3 regulators, deubiquitinases, or signaling pathways modulate RNF126 ubiquitination may not only clarify its upstream control mechanisms but also reveal more accessible biomarkers or therapeutic targets for FGR. (2) The influence of the MYH9/MYH10 complex on ER stress remains unclear. Notably, our ubiquitin-modified proteomic analysis revealed an enrichment of vesicle-mediated transport pathways, suggesting that disruptions in intracellular trafficking may contribute to the observed ER stress phenotype. As core components of non-muscle myosin II, MYH9 and MYH10 play essential roles in vesicle transport and cargo trafficking.⁴⁷ Therefore, their reduced expression may impair vesicle dynamics, leading to inefficient protein transport and subsequent accumulation of misfolded or unfolded proteins within the ER.²⁴ This burden could in turn activate canonical ER stress pathways, offering a plausible mechanistic link between MYH9/MYH10 dysregulation and trophoblast dysfunction. Further studies are required to dissect this pathway and to determine whether vesicle-transport defects represent a primary driver of ER stress in FGR.

Despite growing recognition of ubiquitination as a key regulatory mechanism in placental biology, its role in FGR remains

Figure 5. RNF126 ubiquitinates MYH9 and MYH10, promoting their degradation

(A) GST and GST-RNF126 pull-down assays were conducted with BeWo cell lysates ($\approx 1 \times 10^7$ cells lysed per pull-down reaction; $n = 1$ independent experiment). (B and C) MYH9 and MYH10 peptides were identified by LC-MS/MS (using approximately 5–10 μ g of pull-down protein; $n = 1$ independent experiment). (D and E) Relative protein and mRNA level of RNF126, MYH9, and MYH10 in BeWo cells transfected with control or RNF126 overexpression vectors ($n = 3$ independent experiments). (F) Co-IP of MYH9 and MYH10 with RNF126 was conducted in BeWo cells. Representative blot is shown from three independent experiments. (G) Co-IP of Flag-RNF126 with MYH9 and MYH10 was conducted in BeWo cells. Representative blot is shown from three independent experiments. Whole cell lysate was prepared, and immunoprecipitation was performed with Flag beads. (H) Immunofluorescence co-staining of MYH9, MYH10, and RNF126 demonstrates colocalization (Magnification 63 $\times$ oil, Scale bars, 10 μ m, $n = 3$ independent experiments). (I and J) Representative western blot and densitometric analysis show time-dependent degradation of MYH9 and MYH10 in BeWo cells with or without RNF126 overexpression following cycloheximide (CHX) treatment (0, 2, 4, and 8 h) ($n = 3$ independent experiments). (K) MG132 (10 μ M), but not CQ (20 μ M), restores MYH9 and MYH10 protein levels in RNF126-overexpressing cells after 6 h of treatment. Representative blot is shown from three independent experiments. (L) RNF126 promotes ubiquitination-dependent proteasomal degradation of MYH9 and MYH10. Representative blot is shown from three independent experiments. The error bars represent mean $\pm$ SD. * $p < 0.05$ and **** $p < 0.0001$, n.s. not significant.



(legend on next page)

largely unexplored. A more comprehensive characterization of ubiquitination alterations will be essential for advancing our understanding of FGR pathogenesis. Such insights may not only clarify how aberrant protein turnover contributes to placental dysfunction but also uncover diagnostic markers and therapeutic targets, ultimately improving the clinical management of FGR.

From this study, we conclude that RNF126 promotes the degradation of the MYH9/MYH10 complex, triggering ER stress and subsequent activation of the PERK-CHOP pathway, ultimately leading to trophoblast apoptosis and FGR.

Limitations of the study

Several limitations of this study should be acknowledged. First, to ensure strict gestational age matching between patients with FGR and healthy controls, earlier gestational age samples were excluded, which may have led to the omission of biomarkers more relevant to severe FGR. Second, because of technical constraints, our *in vitro* experiments were limited to established cell lines and were not validated in primary trophoblasts, placental explants, or trophoblast organoids. In addition, although our study provides evidence supporting efficient trophoblast-specific deletion of RNF126 using the *Elf5*-Cre conditional knockout (cKO) model, we acknowledge limitations in the resolution of our validation strategy. Additional cell-type-specific or quantitative validation (e.g., cell sorting-based analysis) would further improve the assessment of deletion efficiency and specificity in placental tissues. Accordingly, the presence of residual RNF126 signal in placental lysates cannot be completely excluded, and its potential influence on the observed phenotypes cannot be fully ruled out. Finally, the utility of RNF126 in distinguishing FGR from SGA requires further investigation to account for potential confounding factors such as maternal comorbidities (e.g., hypertension and diabetes) and fetal sex.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Jing Li (lijing74@smu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The mass spectrometry data of quantitative proteomics and ubiquitin-modified proteomics, along with the profile of the

interacting proteins of RNF126, have been deposited to the ProteomeXchange Consortium (<https://proteomecentral.proteomexchange.org>) via the iProX partner repository^{48,49} with the dataset identifiers PXD059622 and PXD059526. The raw RNA-seq data have been submitted to the NCBI Gene Expression Omnibus (GEO) datasets with accession number < GSE285829 >.

- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of Guangdong Province of China (2024A1515012969 and 2025A1515012463) and the Clinical Research Fund of Nanfang Hospital, Southern Medical University (2025CR020). Graphics abstract created with [Figdraw.com](#).

AUTHOR CONTRIBUTIONS

L.L. and X.Y.: methodology, formal analysis, validation, visualization, writing – original draft, and writing – review and editing. J.L.: methodology, formal analysis, resources, and writing – review and editing. S.X., M.C., and C.L.: validation, formal analysis, and investigation. J.Z. and J.L.: conceptualization, resources, funding acquisition, supervision, and writing-review and editing.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS**
 - Ethics approval and consent to participate
 - Clinical human tissue specimens
 - Establishment of trophoblast-specific RNF126 cKO mice and FGR model
 - Cell culture
- **METHOD DETAILS**
 - Proteomics analysis
 - H&E staining and immunohistochemistry (IHC)
 - RNA sequencing (RNA-seq)
 - GST-pulldown
 - Lentiviruses and plasmid construction and transfection
 - Western blot analysis
 - Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis

Figure 6. RNF126 induces ER stress and trophoblast cell functional alterations by ubiquitinating and degrading the MYH9/MYH10 complex

(A) Western blot analysis shows changes in RNF126, MYH9, and MYH10 protein levels after separate overexpression of MYH9 or MYH10 in BeWo cells. Representative blot is shown from three independent experiments.

(B) Co-overexpression of MYH9 (1 μ g) and MYH10 (1 μ g) in RNF126-overexpressing BeWo cells reverses ER stress marker expression more effectively than individual overexpression (2 μ g each). Representative blot is shown from three independent experiments.

(C and D) The representative graphs and the corresponding quantified histograms demonstrate that co-transfection of MYH9 and MYH10 reverses the effects of cell proliferation and apoptosis caused by RNF126 overexpression ($n = 3$ independent experiments). Scale bars, 50 μ m.

(E) Schematic diagram of serial deletion mutants of Flag-tagged RNF126.

(F) Co-IP and western blot analysis show interactions between full-length MYH9-Myc, MYH10-His, and RNF126-Flag deletion mutants in BeWo cell lysates co-transfected with these constructs. Representative blot is shown from three independent experiments.

(G) Table listing significantly different ubiquitination sites on MYH9 and MYH10.

(H) Western blot analysis of ubiquitination levels of MYH9 and MYH10 in BeWo cells transfected with RNF126-Flag, Ub-HA, and full-length or mutant MYH9/MYH10 constructs. Representative blot is shown from three independent experiments. Data in C and D are presented as mean $\pm$ SD. * $p < 0.05$ and *** $p < 0.001$, n.s. not significant.

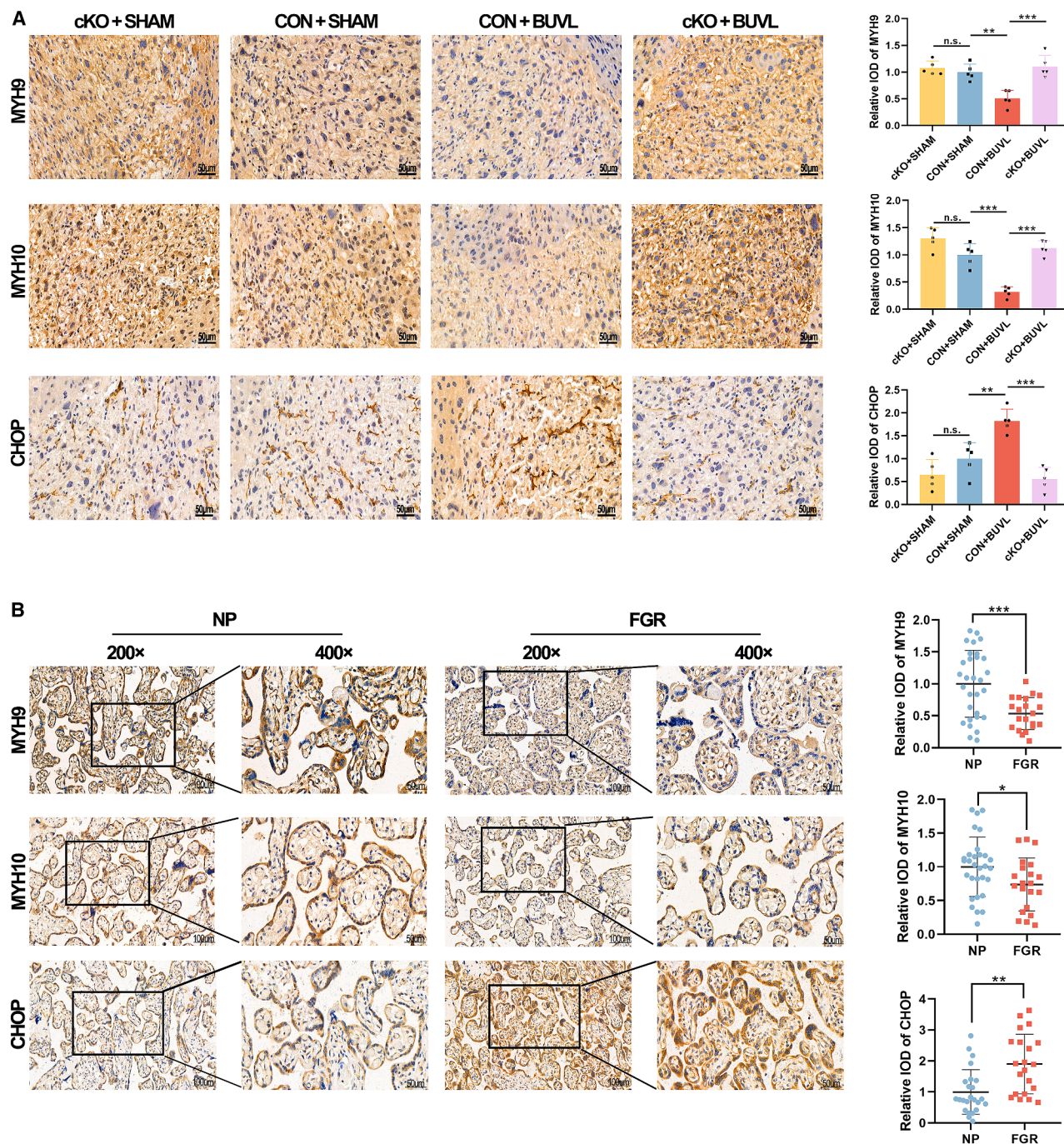


Figure 7. *In vivo* expression levels of MYH9, MYH10, and CHOP

(A) Representative IHC images and quantification of RNF126, MYH9, MYH10, and CHOP in mouse placentas from each group ($n = 5$ placentas from 5 independent litters per group; three fields were analyzed per placenta). Scale bars, 50 μm .

(B) Representative IHC images and quantification of MYH9, MYH10, and CHOP in placentas from NP ($n = 30$ placentas) and FGR ($n = 21$ placentas) pregnancies. Scale bars, 100 μm and 50 μm in the enlarged image. Data in A and B are presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, n.s. not significant.

- EdU assay
- Flow cytometry analysis
- Co-immunoprecipitation (Co-IP) assay
- Molecular docking
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2026.116285>.

Received: February 21, 2025

Revised: February 1, 2026

Accepted: May 21, 2026

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-ubiquitin	PTM BIO	Cat#PTM-1106RM, RRID: AB_3740869
Anti-lactyllysine	PTM BIO	Cat#PTM-1401RM, RRID: AB_2942013
Anti-acetyllysine	PTM BIO	Cat#PTM-101, RRID: AB_2940830
Anti-succinyllysine	PTM BIO	Cat#PTM-419, RRID: AB_2942099
Anti-malonyllysine	PTM BIO	Cat#PTM-902, RRID: AB_3665501
Anti-ubiquitin	HUABIO	Cat#ET1609-21, RRID: AB_3069833
RNF126	ABclonal	Cat#A20015, RRID: AB_2862919
RNF126	HUABIO	Cat#ER1915-92, RRID: AB_3740872
FLAG	Cell Signaling Technology	Cat#14793, RRID: AB_2572291
Tubulin	Abcam	Cat#Ab176560, RRID: AB_2860019
MYH9	Proteintech	Cat#11128-1-AP, RRID: AB_2147294
MYH10	Proteintech	Cat#19673-1-AP, RRID: AB_10858231
CK7	Proteintech	Cat#66483-1-Ig, RRID: AB_2881849
CD31	Abcam	Cat#ab76533, RRID: AB_1523298
GRP78/Bip	HUABIO	Cat#HA722202, RRID: AB_3718306
PERK	Abmart	Cat#TP52759, RRID: AB_3731312
p-PERK	ABclonal	Cat#AP1501, RRID: AB_2942102
IRE1	ABclonal	Cat#A21021, RRID: AB_3740874
p-IRE1	ABclonal	Cat#AP1442, RRID: AB_3683584
ATF6	HUABIO	Cat#EM1701-94, RRID: AB_3068817
CHOP	Abmart	Cat#T56694, RRID: AB_2936430
Myc	HUABIO	Cat#HA601081, RRID: AB_3071804
His	ABclonal	Cat#AE003, RRID: AB_2728734
HA	Abmart	Cat#M20003, RRID: AB_2864345
Anti-Rabbit	Proteintech	Cat#SA00001-2, RRID: AB_2722564
Anti-Mouse	Proteintech	Cat#SA00001-1, RRID: AB_2722565
Anti-IgG(H+L) omitted	Abmart	Cat#M21008, RRID: AB_3740875
Bacterial and virus strains		
Information on lentiviruses	See Table S5	N/A
Biological samples		
All human placental samples	Nanfang Hospital, Southern Medical University	N/A
Chemicals, peptides, and recombinant proteins		
Isoflurane	TargetMol	Cat#26675-46-7
TRIzol™ Reagent	Invitrogen	Cat#15596026
PVDF membranes	Bio-Rad	Cat#1620177
4% paraformaldehyde	Beyotime	Cat#P0099
Thapsigargin	Beyotime	Cat#Y263937
Tunicamycin Mixture	GLPBIO	Cat#GC16738
4-phenylbutyric acid	AbMole	Cat#1821-12-1
Lipomaster 3000	Invitrogen	Cat#L3000015
Matrigel	Corning	Cat#356230
Hematoxylin	Servicebio	Cat#G1004

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Polybrene	GeneChem	Cat#G1001
Protease and phosphatase inhibitors	NCM Biotech	Cat#P002
SDS loading buffer	ECOTOP	Cat#ED-8522
enhanced chemiluminescence (ECL) detection	NCM Biotech	Cat#P10100
Critical commercial assays		
ChamQ Universal SYBR qPCR Master Mix	Vazyme	Cat#Q711-02
HiScript Q RT SuperMix for qPCR (+g DNA wiper)	Vazyme	Cat#R123-01
Annexin V-FITC/PI staining kit	Elabscience	Cat#E-CK-A211-50t
Cell-Light™ EdU Apollo567 <i>In Vitro</i> Kit	RIBOBIO	Cat#C10310-1
Immunoprecipitation Kit with Protein A+G Magnetic Beads	Beyotime	Cat#P2179S
TaKaRa MiniBEST Universal Genomic DNA Extraction kit	TaKaRa	Cat#BS29001
DAB Kit	ZSGB-Bio	Cat#ZLI-9017
VAHTS Universal V6 RNA-seq Library Prep Kit	Vazyme	Cat#NR604-02
Annexin V-FITC/PI Apoptosis Kit	Elabscience	Cat#E-CK-A211
Deposited data		
The mass spectrometry data of quantitative proteomics and ubiquitin-modified proteomics	This paper	iProX: PXD059622
The profile of the interacting proteins of RNF126	This paper	iProX: PXD059526
The raw RNA-seq data	This paper	GEO: GSE285829
Experimental models: Cell lines		
Bewo	Procell	CL-0500
Experimental models: Organisms/strains		
Mouse: Elf5-cre; RNF126 ^{fl/fl} : C57BL/6J	Cyagen Biosciences	N/A
Mouse: RNF126 ^{fl/fl} : C57BL/6J	Cyagen Biosciences	N/A
Oligonucleotides		
shRNA interference target	See Table S6	N/A
Primers for RT-qPCR and PCR	See Table S7	N/A
Software and algorithms		
Image J	Image J	https://imagej.net/ij/index.html
GraphPad Prism 8	Graphpad	https://www.graphpad.com/resources
FlowJo software version 8	FlowJo	https://flowjo.com/previous-versions-flowjo
AlphaFold3	AlphaFold	https://alphafoldserver.com/
PyMol	PyMol	https://pymol.org/2/
IBM SPSS 26.0	SPSS	https://www.ibm.com/cn-zh/products/spss
GSEA software	GSEA	https://www.gsea-msigdb.org/gsea/msigdb/index.jsp

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Ethics approval and consent to participate

All animal experiments were performed in accordance with the guidelines and regulations approved by the Southern Medical University Experimental Animal Ethics Committee (SMUL202404006). For studies involving human participants, written informed

consent was obtained from all individuals prior to sample collection. The use of clinical materials and human placental tissues was approved by the Medical Ethics Committee of Nanfang Hospital of Southern Medical University (NFEC-2024-503).

Clinical human tissue specimens

All human placental samples were collected from the Department of Obstetrics and Gynecology, Nanfang Hospital, Southern Medical University, with written informed consent obtained from all participants. The study was approved by the Medical Ethics Committee of Nanfang Hospital of Southern Medical University (NFEC-2024-503). All participants were women with singleton pregnancies and without other pregnancy-related complications. Participants had normal platelet counts, liver and kidney function, blood pressure, and blood glucose levels. Information regarding ancestry, race, and ethnicity was not collected. Definitions of fetal growth restriction (FGR) and small for gestational age (SGA) were based on the Delphi consensus criteria.⁵⁰ For multi-omics analyses, 10 placental samples (5 FGR and 5 NP) were analyzed, with clinical data listed in [Table S1](#). An additional 110 samples (46 FGR, 36 NP and 28SGA) were collected for further studies, and their baseline characteristics are summarized in [Table S2](#). [Table S3](#) details the clinicopathologic characteristics of the 46 FGR patients used for IHC analysis. Fetal sex was not considered during sample allocation and its potential influence on the results was not assessed.

Establishment of trophoblast-specific RNF126 cKO mice and FGR model

The *Elf5*-Cre mouse was engineered by inserting a P2A-Cre cassette into the stop codon site of the *Elf5* gene, ensuring that Cre expression mirrors the endogenous *Elf5* expression pattern. RNF126^{fl/fl} mice carry loxP sites flanking the RNF126 gene. *Elf5*-Cre mice were crossed with RNF126^{fl/fl} mice to generate trophoblast-specific RNF126 conditional knockout offspring via Cre-mediated recombination in placental trophoblast cells. Both *Elf5*-Cre and RNF126^{fl/fl} mice were on a C57BL/6J background and obtained from Cyagen Biosciences.

All mice were housed under specific pathogen-free (SPF) conditions with a 12 h light/dark cycle and *ad libitum* access to food and water. Timed pregnant females were generated by crossing genetically modified mice. Embryos were collected at embryonic day 18.5 (E18.5) for phenotypic analysis. Embryos were harvested without sex selection. Genotyping was performed by PCR using genomic DNA extracted from placental tissue according to the manufacturer's instructions (TaKaRa MiniBEST Universal Genomic DNA Extraction kit).

Several experimental models have been developed to induce FGR, including uterine vessel ligation,⁵¹ maternal nutrient restriction,⁵² dexamethasone exposure,⁵³ and hypoxia.⁵⁴ Among these, bilateral uterine vessel ligation most closely reproduces placental hypoperfusion observed in human FGR. Following the modified protocol of Mathilde Janot et al.,⁵¹ pregnant mice at GD 17 were anesthetized with isoflurane and subjected to midline laparotomy. The uterine vessels at the central part of each uterine horn were ligated to induce FGR, while controls received sham surgery without vessel ligation. Postoperative analgesia was provided with buprenorphine (0.05 mg/kg, subcutaneous). After surgery, mice were placed on a heating pad, monitored closely, and supplied with oxygen until full recovery. Cesarean section and euthanasia were performed at GD18.5.

Cell culture

BeWo cells (Procell, Wuhan, China), a human choriocarcinoma-derived trophoblast line commonly used as an *in vitro* model of human villous trophoblast,⁵⁵ were maintained in Ham's F-12 medium supplemented with 15% fetal bovine serum (Gibco, USA) at 37°C in 5% CO₂. The cell line was STR authenticated and tested negative for mycoplasma contamination.

METHOD DETAILS

Proteomics analysis

Placental tissues from patients with FGR (n=5) and normal pregnant women (n=5) were used for proteomics analysis. After protein extraction, enzymatic digestion, enrichment of modified peptides (specifically for ubiquitin-modified proteomics), and LC-MS/MS analysis to obtain Raw files, the subsequent steps are as follows: (1) Develop sample-specific protein databases based on sample origins and perform searches using the UniProt database; (2) Conduct quality control analyses at the peptide and protein levels based on search results; (3) Provide common functional annotations for identified proteins, including GO and KEGG annotations; (4) Perform quantitative analyses of proteins or modification sites; (5) Apply quantitative results for differential screening and generate statistical charts related to differential analyses; (6) Conduct functional classification statistical analyses on differential proteins or modified proteins; (7) Perform enrichment analyses using Fisher's exact test based on statistical results obtained from different classification methods.

H&E staining and immunohistochemistry (IHC)

Tissues fixed with 4% paraformaldehyde underwent standard procedures of paraffin embedding, sectioning, dewaxing, and hydration. The paraffin-embedded tissues were sectioned at 3 μm thickness using a paraffin microtome (Leica). After routine dewaxing and rehydration, they were stained with hematoxylin and eosin (H&E). The slides were further photographed under the microscope and analyzed for surface morphometry using Image J.

For IHC, the paraffin sections were immersed in citrate buffer (pH = 6.0) for heat-induced antigen retrieval and then incubated with primary antibodies overnight at 4°C. After being stained with DAB Kit (ZSGB-Bio, Beijing, China), the slides were then counterstained with hematoxylin, dehydrated, and sealed. Five random visual fields were chosen under the microscope, and their integrated optical density (IOD) was calculated using Image J. The details of the primary antibodies are listed in [Table S4](#).

RNA sequencing (RNA-seq)

Total RNA was extracted using the TRIzol reagent (Invitrogen, CA, USA) following the manufacturer's protocol. RNA purity and quantification were assessed using the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Subsequently, libraries were prepared using the VAHTS Universal V6 RNA-seq Library Prep Kit in accordance with the manufacturer's guidelines.

The libraries were sequenced on an Illumina NovaSeq 6000 platform, generating 150 bp paired-end reads. DESeq2⁵ was employed for the analysis of differential expression, with a q-value < 0.05 and a fold change > 1.5 or fold change < 0.67 established as the criteria for significantly DEGs. By utilizing the hypergeometric distribution, GO and KEGG pathway enrichment analyses of DEGs were conducted to identify significantly enriched terms using R (v 3.2.0). Additionally, gene set enrichment analysis (GSEA) was carried out using GSEA software.

GST-pulldown

Using GST-RNF126 protein as bait for BeWo cell lysate pull-down experiments, the resulting products were subjected to qualitative analysis using LC-MS/MS methods. The success of the pull-down experiment was determined by identifying GST-RNF126 in the results. The differential proteins obtained by subtracting the proteins of the control groups (GST+cell lysate and GST-RNF126+lysis buffer) from those of the experimental group (GST-RNF126+cell lysate) represent the binding proteins of RNF126.

Lentiviruses and plasmid construction and transfection

Detailed information on all lentiviruses and plasmids is provided in [Table S5](#), and the corresponding shRNA interference target sequences are listed in [Table S6](#). Polybrene (GeneChem, Shanghai, China) was used to transfect lentiviruses and Lipofectamine 3000 (Invitrogen, USA) was used to transfect plasmids in accordance with the manufacturer's guidelines.

Western blot analysis

For total protein extraction, human or mouse placental tissues and trophoblast cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors (NCM Biotech, Suzhou, China) on ice. Lysates were then denatured in SDS loading buffer (ECOTOP, Guangzhou, China) by heating for 5 min. Proteins were separated by SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad). After blocking with 5% non-fat milk, membranes were incubated overnight at 4 °C with primary antibodies, followed by a 1-hour incubation with the appropriate secondary antibodies at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL) detection (NCM Biotech, Suzhou, China). All experiments were repeated independently three times. Details of all antibodies used are listed in [Table S4](#).

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis

RNA was extracted by using a TRIzol reagent (Invitrogen, CA, USA) for reverse transcription with HiScript Q RT SuperMix for qPCR (Vazyme, Nanjing, China). The qRT-PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems, CA, USA) using ChamQ SYBR qPCR master mix (Vazyme, Nanjing, China). Relative quantification analysis was computed using the comparative $2^{-\Delta\Delta CT}$ method. All primers for qRT-PCR were listed in [Table S7](#).

EdU assay

The EdU Assay was performed using the Cell-Light™ EdU Apollo567 *In Vitro* Kit (RIBOBIO, Guangzhou, China) according to the manufacturer's instructions. Image acquisition using fluorescence microscopy and cell counting with Image J.

Flow cytometry analysis

After 48 h of transfection, the cells were analyzed for apoptosis using flow cytometry. Cell staining was performed using an Annexin V-FITC/PI Apoptosis Kit (Elabscience, Wuhan, China), and apoptosis levels were analyzed by the FlowJo software version 8 (FlowJo, Ashland, OR, United States). The percentages of early and late apoptotic cells were analyzed to evaluate the effect on apoptosis. All assays were performed using three independent replicates.

Co-immunoprecipitation (Co-IP) assay

The Co-IP experiment was performed using the Immunoprecipitation Kit with Protein A+G Magnetic Beads (Beyotime, Shanghai, China) according to the manufacturer's instructions. The details of the antibodies are listed in [Table S4](#).

Molecular docking

To investigate the protein-protein interactions between the human MYH9 (UniProt: P35579), MYH10 (UniProt: P35580), and RNF126 (UniProt: Q9BV68) proteins, docking was simulated using AlphaFold3 software, and the model with the highest confidence level was

selected from candidate complexes based on the docking score. All structural models were displayed using the PyMol software (<https://pymol.org/2/>).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed utilizing SPSS 26.0 software (IBM SPSS 26.0, SPSS Inc.). Graphical representations of the data were generated using the GraphPad Prism 8 software package (GraphPad Software, CA, USA). For comparisons between two groups with normally distributed data, a two-tailed Student's *t*-test was performed. For non-normally distributed data, the two-tailed Mann–Whitney *U* test was used. Comparisons among more than two groups were conducted using either a one-way ANOVA (with Bonferroni's post hoc test) for parametric data or the Kruskal–Wallis test for nonparametric data, as appropriate. Pearson's coefficient tests were performed to evaluate the statistical significance of the correlations between gene expression and clinical characteristics. Values are presented as the mean $\pm$ SD. *p* value < 0.05 was considered statistically significant.