



Research Article

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Proteomic landscape of hydatidiform mole reveals molecular evolution and immune evasion in recurrence

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Abstract: Hydatidiform mole (HM) is a gestational trophoblastic disease causing abnormal trophoblastic proliferation and pregnancy loss, often accompanied by a significant psychological burden. To elucidate the molecular basis and recurrence mechanisms of HM, we performed high-throughput proteomic profiling of FFPE villous tissues from 8 sporadic HM cases (HMS), 6 primary HM from recurrent cases (HMRP), 13 recurrent HM cases (HMR), and 8 normal villus cases (NV). A total of 2,959 differentially expressed proteins were identified, revealing immune dysregulation and metabolic reprogramming in HM. CD55 and ITGAM emerged as central network hubs of HM, while the recurrence-associated signature indicated altered extracellular matrix composition and the suppression of key transcriptional regulators (YAP1, STK3, POU2F1). Proteomic trajectory analysis from HMS to HMR highlighted the persistent downregulation of neutrophil degranulation and the upregulation of the immune checkpoint CD276 (B7-H3). These findings delineate the proteomic evolution and immune landscape of HM, offering molecular insights for recurrence risk assessment and potential targeted therapies for improved clinical management.

Key words: Hydatidiform mole; Recurrent HM; Proteomics; Immune microenvironment; Metabolic reprogramming; Recurrence risk

1 Introduction

A hydatidiform mole (HM) is a form of gestational trophoblastic disease characterized by abnormal embryonic development and excessive trophoblastic proliferation. Beyond its reproductive consequences, HM often imposes profound psychological distress, particularly in women with recurrent disease (Lurain, 2010). The global incidence of HM ranges from 0.57 to 2 per 1000 pregnancies, with up to 15% of cases progressing to gestational trophoblastic neoplasia (Kalogiannidis et al., 2018; Chawla et al., 2023). Recurrent HM, defined as two or more consecutive molar pregnancies in the same patient, carries an elevated risk of malignancy (Ogilvie et al., 2009; Hafezi et al., 2020). A prior molar pregnancy increases the recurrence risk nearly tenfold, reaching up to 23% after two episodes (Sebire et al., 2003; Savage et al., 2013). Despite its clinical and psychological burden, the molecular basis of HM pathogenesis and recurrence remains incompletely understood.

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Previous studies regarding HM have mainly focused on genetic and transcriptomic alterations. A defining feature of this condition is aberrant genomic imprinting resulting from androgenetic origins, while the silencing of p57KIP2 has been implicated in its development (Frost and Moore, 2010; Takahashi et al., 2019). At the RNA level, miR-30a, miR-196b and miR-21 have been shown to modulate trophoblastic proliferation (Wang et al., 2017; Guo et al., 2019; Guo et al., 2020). However, as the direct executors of cellular function, proteins more accurately reflect biological activity and disease dynamics (Bai et al., 2021; Zhu et al., 2025). Proteomic profiling thus provides a unique opportunity to capture functional molecular alterations and reveal key drivers that may not be evident at the transcriptomic level.

The molecular mechanisms underlying HM recurrence remain particularly elusive. Although prior molar pregnancy and mutations in NLRP7 or KHDC3L are recognized risk factors (Savage, et al., 2013; Moein-Vaziri et al., 2018), reliable biomarkers predicting the recurrence risk at initial diagnosis are lacking. Comprehensive proteomic characterization offers the potential to identify recurrence-associated molecular signatures and pathways that could inform prognostic stratification and precision therapy.

The maternal-fetal interface represents a finely tuned immune environment essential for successful pregnancy. The dysregulation of local immunity contributes to HM pathogenesis and persistence. Previous studies have reported elevated immune cell infiltration and altered cytokine signaling in HM tissues, suggesting an immune-evasive microenvironment (Nagymanyoki et al., 2007; Hussein et al., 2009; King et al., 2019; Dridi et al., 2022; Lakatos et al., 2022). However, the immune proteome architecture of HM and its recurrent form has not been systematically defined.

Herein, we performed the high-throughput proteomic profiling of Formalin-Fixed Paraffin-Embedded (FFPE) villous tissues from sporadic HM (HMS), recurrent HM (HMR), primary HM from recurrent cases (HMRP), and normal villus (NV) controls. We delineated the proteomic landscape of HM, identified dysregulated metabolic and immune pathways, and defined molecular signatures predictive of recurrence. By reconstructing proteomic trajectories from HMS to HMR, we were able to reveal dynamic alterations associated with recurrence, including the persistent suppression of neutrophil degranulation and upregulation of the immune checkpoint CD276 (B7-H3). Together, our findings provide a comprehensive proteomic framework for understanding HM pathogenesis and recurrence, offering new molecular insights for risk stratification and potential therapeutic intervention.

2 Results

2.1 Clinical characteristics and global proteomic profiling

To characterize the proteomic landscape of HM and its recurrent forms, we analyzed FFPE villous tissues from four groups: 8 sporadic HM (HMS), 13 recurrent HM (HMR), 6 corresponding primary HM samples from recurrent cases (HMRP), and 8 normal villous (NV) controls. The clinical characteristics of all patients are summarized in Table S1. All samples underwent high-throughput proteomic profiling as outlined in Fig. 1a. Principal component analysis (PCA) revealed a clear separation between the HM and NV groups, indicating distinct global proteomic signatures associated with disease state (Fig. 1b). Across all samples, approximately 4,000 to 5,000 proteins were identified per specimen, yielding over 8,000 unique protein species in total (Figs. 1c and 1e). Of these, 5,786 proteins were consistently detected across all four groups (Fig. 1d). Although the protein identification quality appeared slightly higher in NV samples than in HM samples, the difference was not statistically significant. Density distribution plots confirmed the normal distribution of protein intensities within each group, suggesting minimal bias in protein detection (Fig. 1f). The peptide lengths and peptide count distributions further supported the robustness and consistency of the mass spectrometry (MS) data (Figs. S1a and S1b). Intra-group correlation analysis demonstrated strong molecular similarity within HM samples (HMS, HMRP, and HMR), in contrast to the greater heterogeneity observed among NV controls (Fig. S1c). This lower

correlation in NV tissues likely reflects a broader biological variability in normal pregnancies, whereas the consistent clustering within HM groups suggests shared, disease-specific proteomic alterations.

Figure 1

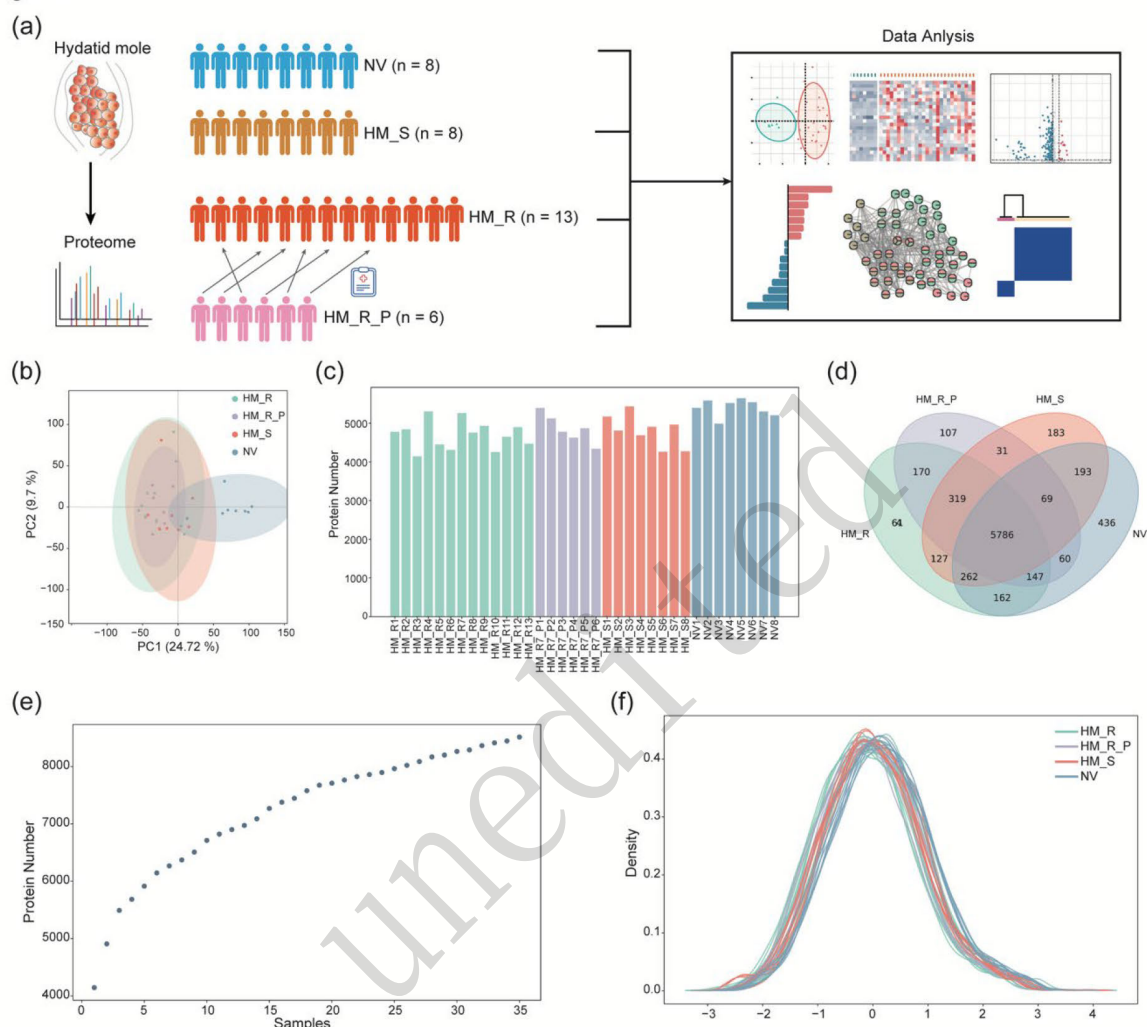


Fig. 1 Proteomic analysis workflow and qualitative protein identification. (a) Flowchart illustrating the study design, sample inclusion, and omics data analysis workflow. (b) Principal component analysis (PCA) plot displaying the proteomic analysis results after dimensionality reduction across all samples. (c) Bar plot showing the number of protein molecules identified in each individual sample. (d) Venn diagram depicting the overlap of identified protein molecules across different sample groups. (e). Cumulative identification curve representing the total number of proteins cumulatively identified across all samples. (f) Density distribution curves illustrating the density distribution of identified proteins in each sample.

2.2 Comparative analysis of proteomic profiles between HM and NV

To delineate the global proteomic alterations underlying HM, we compared the protein expression profiles between HM tissues (including HMS, HMR and HMRP) and NV. PCA revealed a clear segregation of HM

from NV, indicating distinct molecular phenotypes (Fig. 2a). Differential expression analysis identified 2,959 significantly altered proteins, comprising 522 upregulated and 2,437 downregulated ones in HM relative to NV (Fig. 2b). Pathway enrichment analysis showed that upregulated proteins were predominantly involved in complement and coagulation cascades, immune response, and neutrophil degranulation (Figs. 2c and 2d), indicating extensive immune dysregulation. Protein-protein interaction (PPI) network mapping of these pathways identified CD55 and ITGAM as central hub proteins (Fig. 2e). CD55, a key complement regulator, accelerates C3 convertase decay and protects trophoblasts from complement-mediated lysis (Lublin and Atkinson, 1989; Nicholson-Weller, 1992). Its marked upregulation in HM likely contributes to immune evasion by shielding abnormal trophoblasts from maternal immune attack. Similarly, increased ITGAM expression may enhance neutrophil degranulation and further promote immune imbalance (Ding et al., 2025). In contrast, pathways related to cellular metabolism, including ferroptosis, fatty acid β -oxidation, carbon metabolism, and glutathione metabolism, were significantly downregulated in HM (Figs. 2c and 2d, Fig. S2a). These findings suggest a metabolic reprogramming that favors the proliferation and survival of trophoblastic cells. The suppression of ferroptosis, a form of iron-dependent cell death, may provide a selective survival advantage by reducing oxidative stress and immune-mediated clearance (Jiang et al., 2021). Together, these changes reflect a metabolic environment optimized for biosynthesis and sustained trophoblast expansion. Immunohistochemical validation confirmed the elevated expression of CD55 and ITGAM in HM compared with NV (Fig. 2f, Fig. S2b).

To further characterize the immune landscape, xCell deconvolution analysis was performed to estimate immune and stromal cell abundances between HM and NV samples. Both immune and stromal components were markedly increased in HM (Figs. S2c and S2d), consistent with a restructured and immunologically active microenvironment. Notably, fibroblast enrichment suggested enhanced extracellular matrix deposition and remodeling, potentially providing structural and metabolic support to HM. The upregulation of immature dendritic cells (iDCs) indicates a shift toward an immune-tolerant state, whereas reduced macrophage scores may reflect impaired clearance of aberrant cells (Fig. S2c). Collectively, these results demonstrate that HM is characterized by profound immune dysregulation and metabolic reprogramming. Trophoblastic proliferation and immune evasion are likely cooperatively driven by the concurrent activation of complement pathways and the suppression of metabolic homeostasis, defining the molecular pathology of HM.

Figure 2

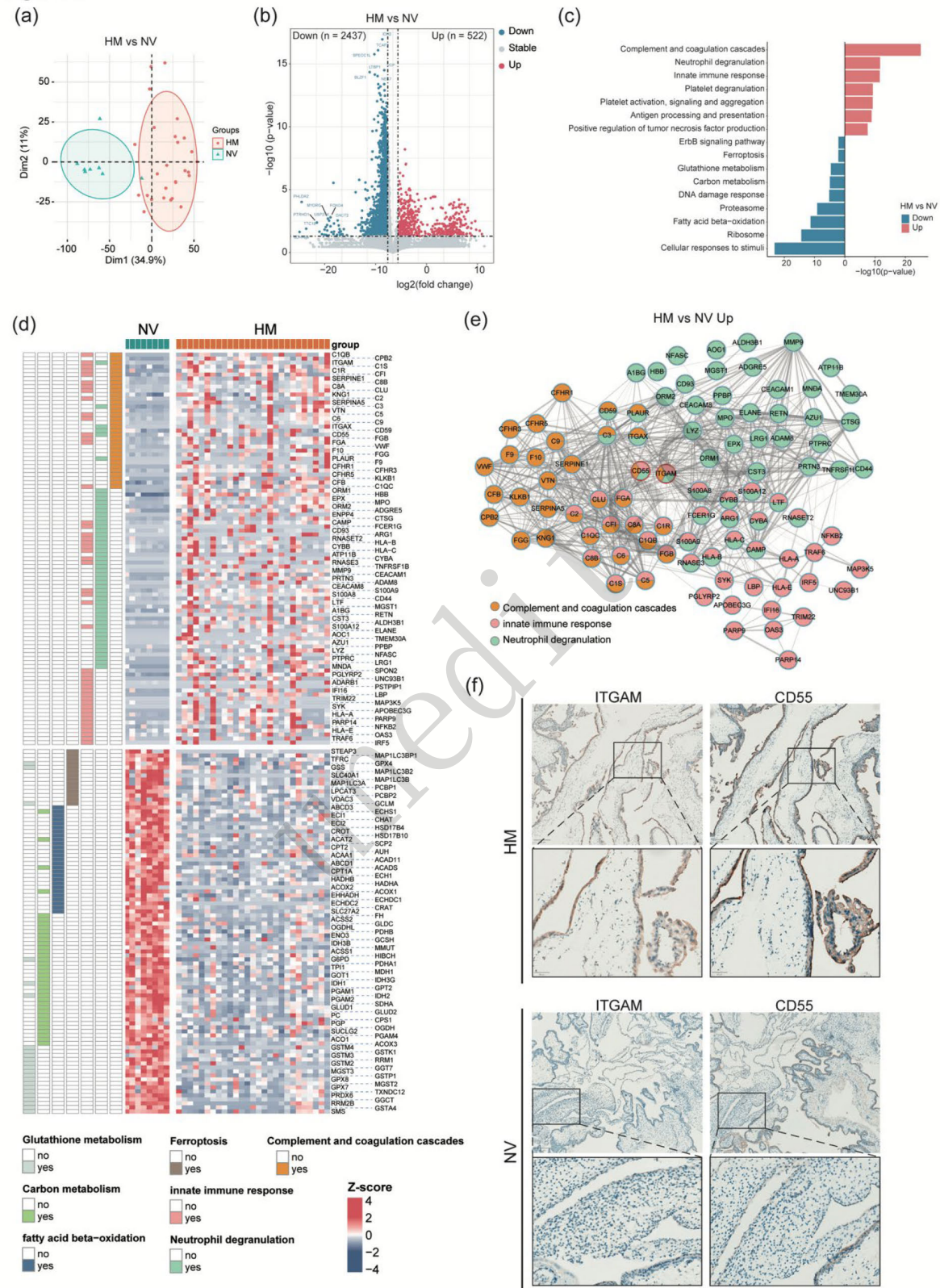


Fig. 2 Comparative analysis of proteomic profiles between HM and NV. (a) PCA plot displaying the dimensionality reduction results for HM and NV samples. (b) Volcano plot illustrating the number of differentially expressed proteins between HM and NV groups. A two-sided Student's t-test was used to assess the mean differences between groups. Proteins with a fold change (FC) ≥ 2 or ≤ 0.5 and a p-value ≤ 0.05 were considered significantly differentially expressed. (c) Bar plot showing the pathways enriched for the differentially expressed proteins in HM versus NV. (d) Heatmap depicting the expression patterns of proteins involved in key pathways across HM and NV samples. (e) PPI network of proteins within key pathways significantly enriched in HM. (f) IHC staining for CD55 and ITGAM in HM and NV tissues. Scale bars: 200 μm (main images) and 50 μm (higher magnification).

2.3 Molecular features in high-risk HM for recurrence

To identify molecular features associated with HM recurrence, we compared the proteomic profiles between primary HM samples from patients who later developed recurrence (HMRP) and sporadic HM samples without recurrence (HMS) (Fig. 3a). Differential expression analysis revealed 215 significantly altered proteins, including 15 upregulated and 200 downregulated ones in HMRP relative to HMS (Fig. 3). The predominance of downregulated proteins suggests that the loss of specific molecular functions may contribute to recurrence susceptibility. Pathway enrichment analysis demonstrated that downregulated proteins were enriched in endoplasmic reticulum function, Hippo signaling, collagen-containing extracellular matrix (ECM), hypoxia response, and keratinization (Figs. 3c and 3d, Fig. S3a). Conversely, upregulated proteins were associated with protein tyrosine kinase binding, negative regulation of T cell receptor signaling, cell-surface interactions at the vascular wall, and fibronectin matrix formation (Fig. 3c, Fig. S3a). These findings highlight profound alterations in ECM composition and cell-ECM communication in high-risk HM. Compared with low-risk HMS, high-risk HMRP samples displayed reduced collagen content and elevated fibronectin expression, indicative of enhanced adhesion dynamics and increased migratory potential. The concurrent attenuation of hypoxia-responsive pathways and the enrichment of cell-vasculature interaction signatures may also suggest improved oxygen availability within recurrent-prone lesions. In addition, the downregulation of keratin-associated proteins points to a more dedifferentiated cellular phenotype in HMRP.

Since transcriptional regulators are pivotal in maintaining trophoblastic identity, we next examined the key transcription factors and co-regulators among the differentially expressed proteins. Notably, the Hippo pathway effector YAP1 and its upstream kinase STK3 were both downregulated in HMRP, consistent with reduced Hippo signaling activity. The transcription factor POU2F1, which regulates genes involved in cell cycle progression, stress response, and embryonic development (Li et al., 2024), was also significantly decreased (Fig. 3e). Immunohistochemical validation confirmed the markedly lower expression of YAP1, STK3 and POU2F1 in HMRP compared with HMS (Fig. 3f, Fig. S3b). Collectively, these results identify a recurrence-associated proteomic signature defined by altered ECM organization and the suppression of the YAP1–STK3–POU2F1 axis. This molecular profile may promote trophoblastic dedifferentiation and microenvironmental remodeling, thereby predisposing HM to recurrence.

Figure 3

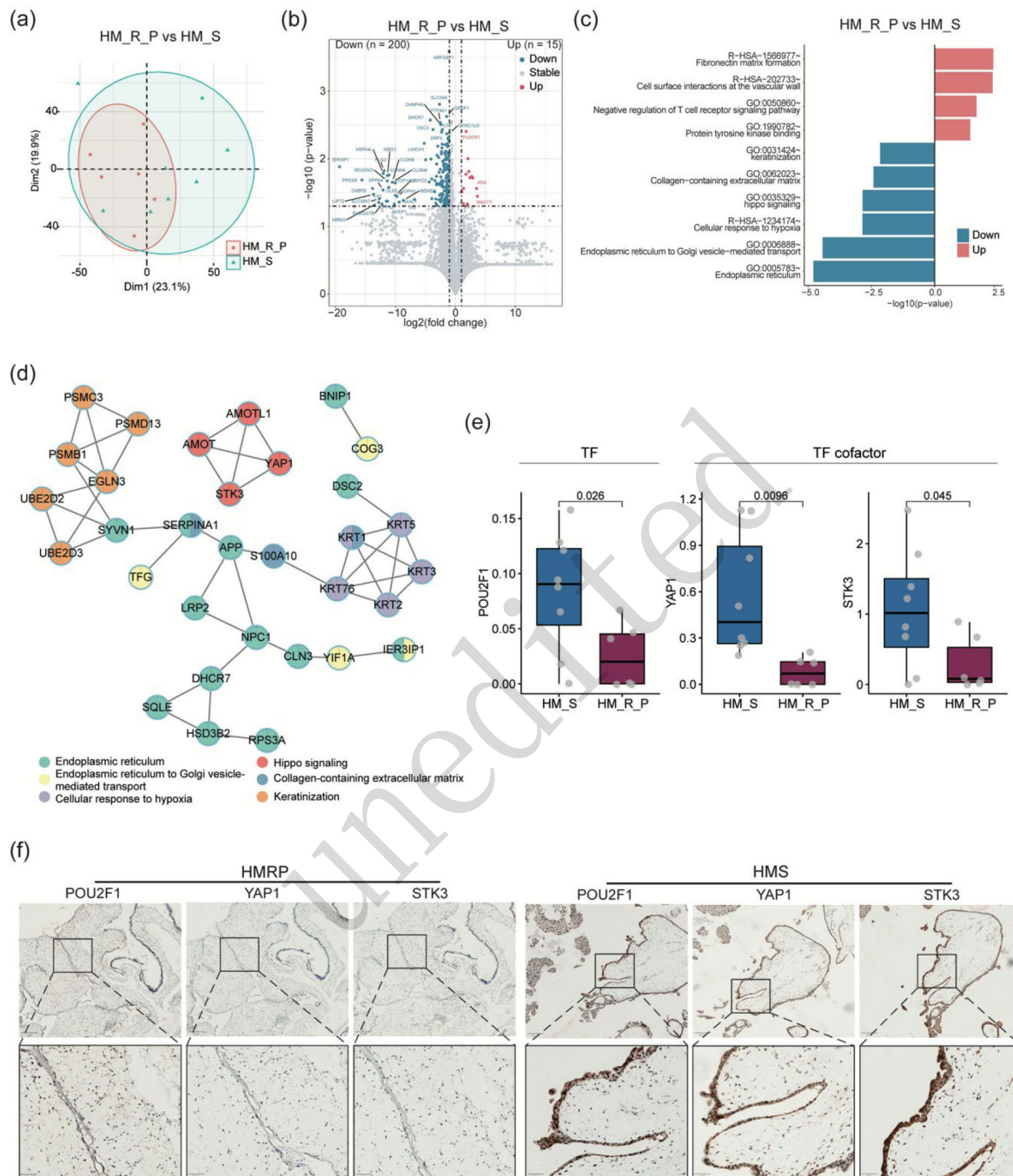


Fig. 3 Molecular signatures for stratifying recurrence risk in HM. (a) PCA plot of the proteomic data for HMRP and HMS groups after dimensionality reduction. (b) Volcano plot identifying proteins with significant differential expression between HMRP and HMS. Proteins with a fold change (FC) ≥ 2 or ≤ 0.5 and $p \leq 0.05$ were considered significant. (c) Bar plot displaying the pathways enriched for the differentially expressed proteins in HMRP versus HMS. (d) PPI network of proteins involved in key pathways that are significantly downregulated in HMRP. (e) Box plot comparing the expression levels of

the significantly differentially expressed transcription factor (POU2F1) and transcriptional co-factors (YAP1 and STK3) between HMS and HMRP. (f) IHC staining for POU2F1, YAP1 and STK3 in HMS and HMRP tissues. Scale bars: 200 μ m (main images) and 50 μ m (higher magnification).

2.4 Proteomic dynamics across HM recurrence trajectory

To systematically explore the proteomic alterations underlying HM recurrence, we analyzed protein expression across low-risk (HMS), primary HM from recurrent cases (HMRP), and recurrent HM (HMR) tissues. PCA revealed that HMRP and HMR clustered closely, whereas both were clearly separated from HMS, suggesting progressive molecular divergence during recurrence (Fig. 4a). Pairwise comparisons confirmed that HMRP and HMR shared a large set of commonly upregulated and downregulated proteins relative to HMS (Fig. 4b). Differential expression analysis identified 73 upregulated and 176 downregulated proteins in HMR versus HMS (Fig. 4c). Pathway enrichment analysis indicated that upregulated proteins in HMR were primarily involved in energy metabolism, including mitochondrial function, respiratory electron transport, and L-lactate dehydrogenase activity, reflecting an increased metabolic demand in recurrent HM (Figs. 4d and 4f). PPI network analysis of these pathways revealed that several proteins play roles in multiple pathways (Fig. S4a). Conversely, downregulated proteins were associated with the endoplasmic reticulum and Golgi apparatus functions, suggesting altered protein processing and trafficking. Protein domain enrichment of the upregulated proteins further supported a shift toward enhanced energy metabolism (Fig. S4b).

To model the dynamic proteomic changes along the recurrence trajectory, we considered HMRP as an intermediate state between HMS and HMR, consistent with the PCA distribution Fig. 4a. Pseudotemporal expression profiling using Mfuzz grouped proteins into three clusters based on expression trends across the trajectory (Fig. 4g). Cluster 1 exhibited an initial downregulation from HMS to HMRP followed by stabilization. Cluster 2 demonstrated sustained downregulation, associated with RNA metabolism, protein metabolism, viral infection, and notably, neutrophil degranulation. Cluster 3 displayed transient upregulation from HMS to HMRP before declining in HMR, including pathways such as IL-7 signaling, platelet activation, and mRNA splicing. Of particular interest, neutrophil degranulation was progressively and persistently downregulated throughout disease progression, implicating a potential protective role of neutrophils in preventing HM recurrence. These findings suggest that recurrent HM exhibits coordinated metabolic reprogramming coupled with sustained immune modulation, providing an insight into mechanisms that facilitate disease progression.

Figure 4

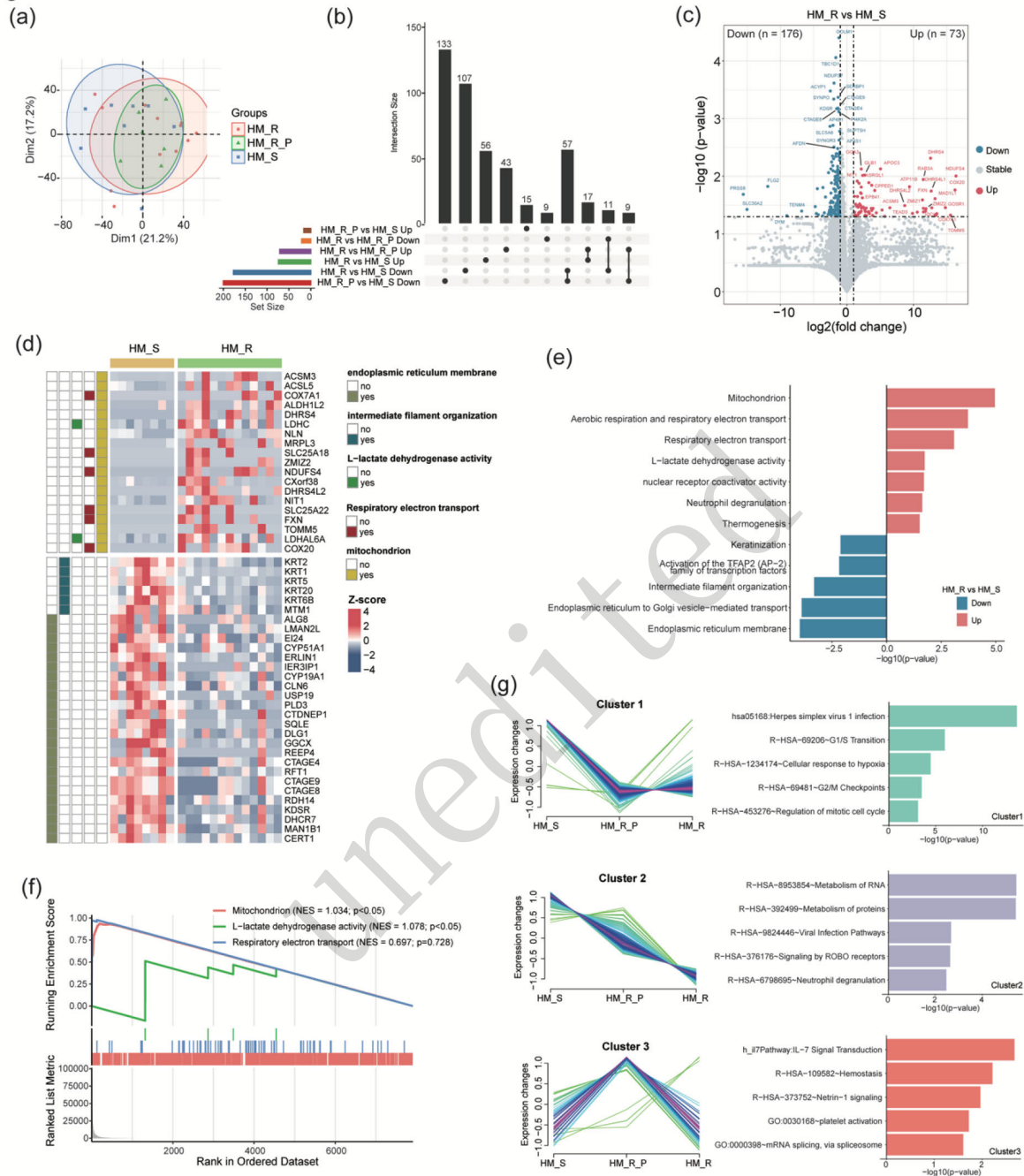


Fig. 4 Proteomic dynamics across HM recurrence trajectory. (a) PCA plot of the proteomic data for the HMR, HMRP and HMS groups after dimensionality reduction. (b) UpSet plot illustrating the intersections of differentially expressed protein molecules from the pairwise comparisons among the HMR, HMRP and HMS groups. (c) Volcano plot identifying proteins with significant differential expression between HM_R and HM_S. Proteins with a fold change (FC) ≥ 2 or ≤ 0.5 and $p \leq 0.05$ were considered significant. (d) Heatmap depicting the expression patterns of proteins within key pathways in HMR versus HMS. (e) Bar plot showing the pathway enrichment results for proteins differentially expressed between HMR and HMS. (f) Gene Set Enrichment Analysis (GSEA) plot for key pathways

significantly upregulated in HMR. (g) Mfuzz pseudotemporal analysis of HMS, HMRP and HMR, dividing proteins into three distinct clusters based on their expression trends. The pathway enrichment results for the proteins in each cluster are displayed on the right.

2.5 Key upregulated molecules in recurrent HM

To uncover the critical proteins associated with HM recurrence, we constructed a PPI network of proteins upregulated in HMR. Central hub proteins included FXN, AOC3, GZMB, and RAB33B, highlighting their potential functional significance (Fig. 5a). Notably, several immune-related proteins, including GZMA, GZMB, and the immune checkpoint molecule CD276, were significantly upregulated in HMR (Fig. 5b). CD276, a well-characterized immune checkpoint associated with poor prognosis, exhibited marked elevation in recurrent HM, implicating it as a potential mediator of immune evasion during disease progression. MCODE clustering via Metascape identified a highly connected module comprising ALDH1L2, ECHDC1, DHRS4, DHRS4L2, and ALDH1A3, suggesting coordinated metabolic regulation in recurrent HM (Fig. 5c). In addition, multiple transcription factors (ZMIZ2, ZMIZ1, TEAD3) and transcriptional co-factors (TERF2IP, NELFE, CHTOP, NCOA7) were enriched among upregulated proteins (Figs. 5d and 5e). TEAD3, a key effector of Hippo signaling, forms transcriptional complexes with YAP/TAZ, while ZMIZ2 and ZMIZ1 modulate sex hormone receptor activity and may indirectly promote trophoblast proliferation through hormonal signaling pathways (Lomelí, 2022; Masliantsev et al., 2025). Collectively, these findings highlight recurrent HM as a molecularly reprogrammed state characterized by coordinated metabolic, transcriptional and immune regulatory alterations, with CD276 representing a potential therapeutic target.

Figure 5

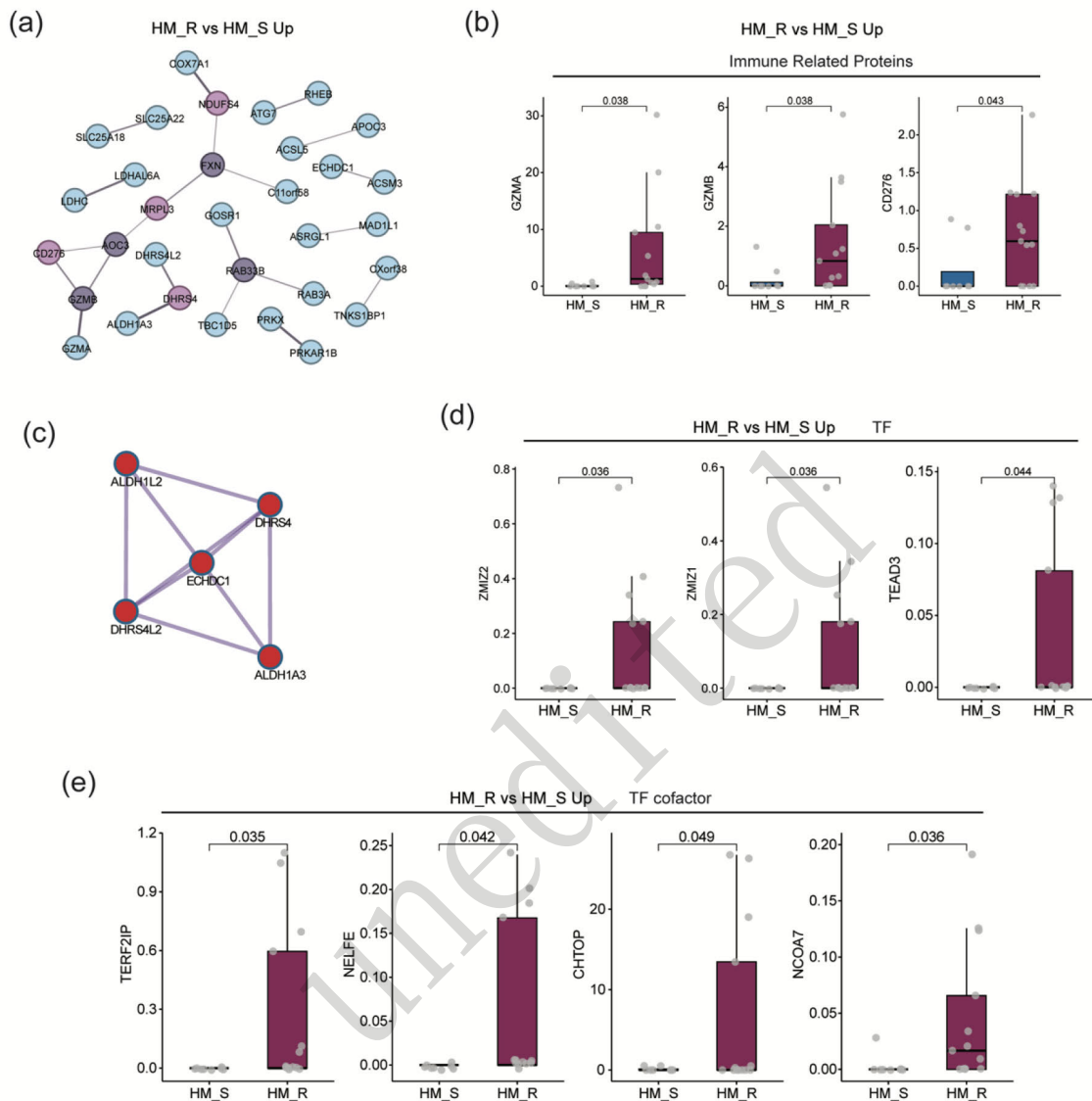


Fig. 5 Key molecule upregulated in HMR. (a) PPI network of proteins that are significantly upregulated in HMR compared to HMS. (b) Box plots showing the expression levels of significantly upregulated immune-related proteins in HMR. (c) Network diagram showing the clustering results of the MCODE module for upregulated proteins in HMR. (d) Box plots displaying the expression levels of significantly upregulated transcription factors in HMR. (e) Box plots illustrating the expression levels of significantly upregulated transcriptional co-factors in HMR.

2.6 Immune remodeling in recurrent HM

To characterize immune alterations during HM recurrence, we performed pathway enrichment analysis on proteins differentially expressed across HM groups (ANOVA, $p \leq 0.05$). Immune- and inflammation-related pathways were predominantly downregulated in HMRP and HMR relative to HMS, indicating a broad

suppression of immune signaling during recurrence (Fig. 6a). Xcell deconvolution further revealed specific shifts in immune and stromal populations: dendritic cells (DCs), Th2 cells, smooth muscle cells, and lymphatic endothelial cells were significantly enriched in HMR compared with HMRP, whereas mast cells were depleted (Fig. 6b). These findings delineate an immunosuppressive microenvironment in recurrent HM, characterized by the global attenuation of immune pathways and the selective remodeling of cellular constituents. Unsupervised clustering of the 13 HMR patients based on proteomic profiles revealed two distinct subgroups: Cluster 1 (C1; HMR1, HMR6, HMR11) and Cluster 2 (C2; remaining 10 samples) (Fig. 6c). C2 exhibited elevated immune cell scores, along with higher fibroblasts and stromal scores compared with C1 (Figs. 6d and 6e), suggesting intrinsic heterogeneity in recurrent HM. Although the elevated immune cell scores in C2 (the predominant cluster comprising 10 of 13 HMR patients) may seem at odds with the concurrent downregulation of immune pathways in the HMR group (Fig. 6a), it is important to note that xCell scores are designed to reflect cellular abundance rather than functional status (Aran et al., 2017). Importantly, a high abundance of immune cells does not necessarily equate to immune activation. Thus, the coexistence of increased immune, fibroblast and stromal cell infiltration and suppressed immune pathway activity points to an immunosuppressive microenvironment in C2 HMR patients. Additionally, the analysis of the immune checkpoint VISR across the HMS, HMRP, HMR, and NV groups demonstrated significantly reduced expression in HM samples relative to NV controls (Fig. 6f). Overall, these results highlight that recurrent HM is associated with immunosuppressive remodeling, accompanied by heterogeneity in immune activity across patients, which may have implications for targeted immunotherapies.

Figure 6

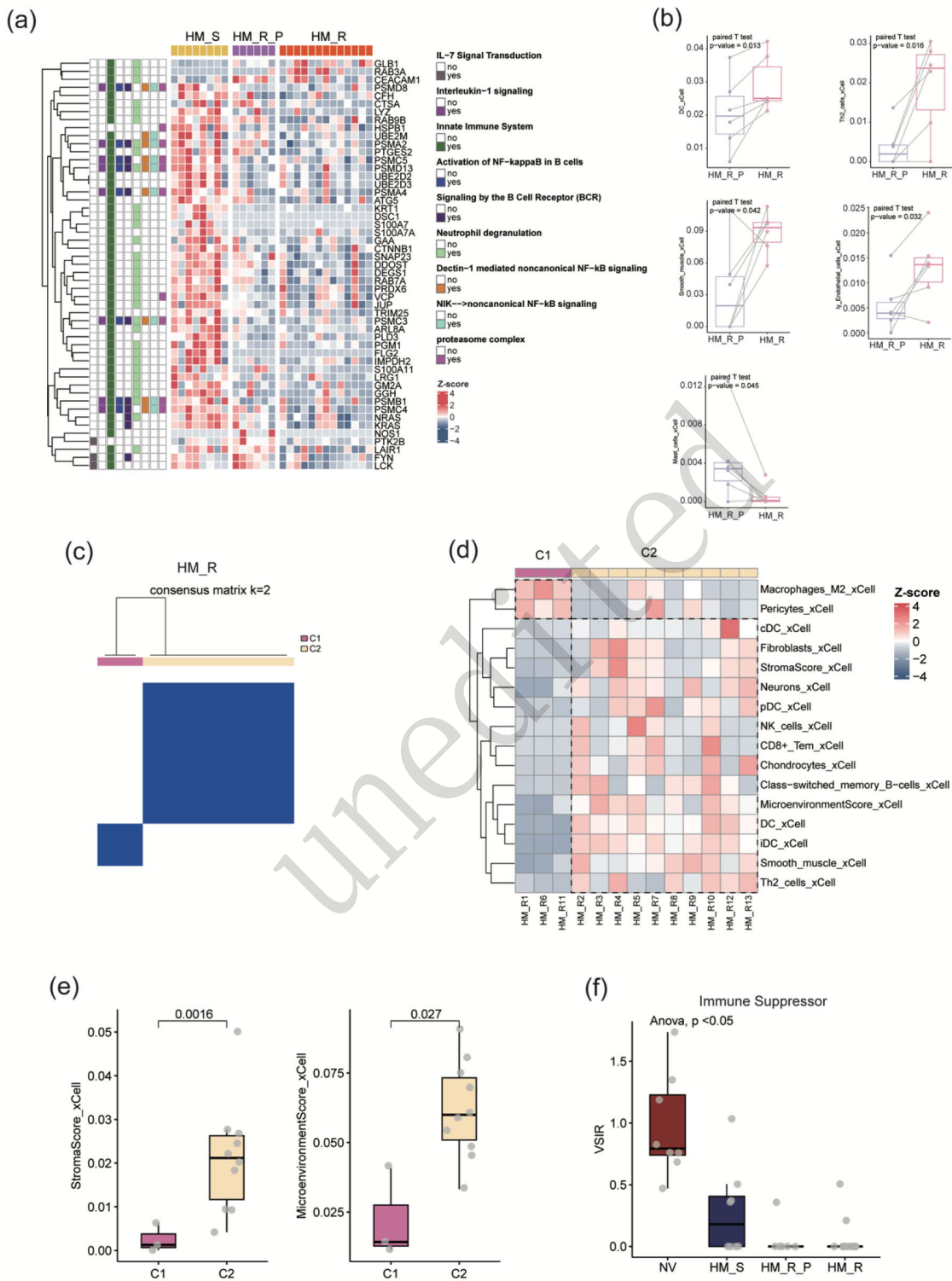


Fig. 6 Immune microenvironment landscape in HM. (a) Heatmap displaying the expression levels of significantly differential immune-related proteins across the NV, HMS, HMRP, and HMR groups. (b)

Box plots comparing the significantly altered xCell scores between paired HMRP and HMR samples. A paired Student's t-test was used to assess the mean differences. (c) Unsupervised consensus clustering of HMR samples was performed using ConsensusClusterPlus. The consensus matrix illustrates the resulting cluster assignments for HMR samples. (d) Heatmap of immune cell score disparities between different HMR clusters. (e) Box plots showing the differences in stromal score and microenvironmental score between the distinct HMR clusters. (f) Box plot comparing the expression of the immune co-inhibitory molecule VISTA across different sample groups.

3 Discussion

Our study presents a comprehensive proteomic landscape of HM, elucidating the molecular alterations that underlie its pathogenesis, recurrence and immune microenvironmental remodeling. Through systematic proteomic and bioinformatic analyses, we delineated distinct signatures of sporadic and recurrent HM, identified key dysregulated pathways, and revealed dynamic molecular transitions along the recurrence trajectory. Moreover, molecular subtyping of recurrent HM uncovered two distinct patient clusters with divergent immune and stromal features, offering insights into intratumorally heterogeneity and potential therapeutic stratification.

A central finding of this study is the coexistence of immune dysregulation and metabolic reprogramming in HM. Pathways such as complement activation, coagulation cascades, and neutrophil degranulation were markedly upregulated, reflecting a pervasive yet dysregulated inflammatory milieu (Ding, et al., 2025). In parallel, the widespread downregulation of metabolic pathways, including fatty acid β -oxidation, glutathione metabolism, and ferroptosis, indicates a shift toward biosynthetic metabolism that may sustain trophoblastic proliferation and immune evasion (Lopaschuk et al., 2010; Esteves et al., 2021; Gasmi et al., 2024). Within this network, CD55 and ITGAM emerged as key regulatory hubs. Their overexpression, validated by immunohistochemistry, suggests their potential roles in protecting abnormal trophoblasts from complement-mediated lysis and modulating immune cell activation, respectively. Collectively, these findings highlight a tightly coupled immune-metabolic interplay that defines the molecular pathology of HM.

Comparison between HMRP and HMS further revealed a recurrence-associated proteomic signature characterized by altered ECM composition and the suppression of critical transcriptional regulators. The loss of collagen and enrichment of fibronectin suggest enhanced cell adhesion and motility (Kanchanawong and Calderwood, 2023; Niu et al., 2024), features associated with invasive potential. The concurrent downregulation of YAP1, STK3 and POU2F1 points to impaired Hippo signaling and trophoblastic dedifferentiation, implicating these molecules as potential biomarkers for recurrence risk. Trajectory analysis across HMS–HMRP–HMR delineated the dynamic proteomic evolution of HM recurrence. This analysis revealed the progressive enhancement of mitochondrial energy metabolism and the persistent downregulation of neutrophil degranulation, suggesting that diminished neutrophil activity may compromise the immune clearance of aberrant trophoblasts and facilitate recurrence. These findings underscore the significance of both metabolic adaptation and innate immune modulation in disease progression.

The immune microenvironment plays a pivotal role in HM recurrence. Pathway enrichment and xCell deconvolution analyses demonstrated a broad suppression of immune and inflammatory signaling in recurrent samples, accompanied by selective remodeling of immune and stromal cell populations. Notably, 13 HMR patients were divided into two subtypes based on the proteomic profiles, with C2 comprising the majority (10 patients) and exhibiting higher immune cell scores. This observation appears to contradict the downregulation of immune pathways shown in Figure 6A. However, it is crucial to note that xCell scores reflect cellular abundance rather than functional state, and a high abundance of immune cells does not indicate immune activation. Furthermore, our results align with the findings of Bagaev et al., who reported that enriched

fibroblast and stromal scores can suppress immune activation (Bagaev et al., 2021). Thus, we propose that despite the elevated immune cell infiltration, recurrent HM is characterized by an immunosuppressive microenvironment. The significant upregulation of the immune checkpoint CD276 (B7-H3) further supports a mechanism of immune evasion, positioning it as a promising therapeutic target (Getu et al., 2023). Moreover, unsupervised clustering revealed two molecular subtypes within recurrent HM: one with higher immune and stromal activity, suggesting that intrinsic microenvironmental heterogeneity may influence treatment response and prognosis.

Although our findings provide a deep understanding of the development and recurrence of HM, several limitations should be acknowledged. For instance, the retrospective nature of the study design may introduce inherent selection biases. Moreover, the relatively limited sample size of our cohort, particularly for the HMRP group, may restrict the statistical power and the generalizability of our findings. Future prospective, multicenter studies with larger cohorts should be undertaken to validate these proteomic signatures and further elucidate the underlying mechanisms of HM development and recurrence.

4 Conclusions

In summary, our integrated proteomic analysis delineates the molecular evolution of HM from primary to recurrent states, revealing coordinated immune suppression, metabolic adaptation and microenvironmental remodeling. These insights provide a valuable framework for recurrence risk stratification and offer potential molecular targets for personalized therapeutic intervention in gestational trophoblastic disease.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Acknowledgement

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Author contributions

Junfen XU and Weiguo LU conceived, supervised and administrated the study. ShanShan XU collected the samples and the clinical data. Mengyan TU and Xinru TU performed the bioinformatic data analysis and interpreted the results. Mengyan TU wrote the original draft. Mengyan TU and Hankai SHI performed the verification experiments and conducted the data analysis. Junfen XU and Weiguo LU revised the manuscript. All authors have full access to the data in this study, take responsibility for its integrity and security, and have read and approved the final manuscript.

Compliance with ethics guidelines

Mengyan TU, ShanShan XU, Xinru TU, Hankai SHI, Weiguo LU, and Junfen XU declare no conflicts of interest that pertain to this work.

This study was approved by the Ethics Committee of the Women's Hospital, Zhejiang University School of Medicine (IRB-20240038-R) and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Declaration on the use of generative AI tools

No generative AI tools were used in the preparation of this manuscript.

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Supplementary information:

Figs. S1–S4; Materials and methods