EPSTI1 in interferon signaling and proteostasis: Model-specific functions and clinical associations (Review)

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Abstract. Epithelial-stromal interaction protein 1 (EPSTI1) is an interferon-responsive protein with experimentally supported functions in cancer, immune regulation and tissue remodeling. This review examines EPSTI1 regulation, cellular functions and disease-associated expression, with particular emphasis on valosin-containing protein (VCP/p97)-associated proteostasis. In breast cancer models, EPSTI1 promotes NF-κB activation and invasive behavior, accompanied by reduced abundance of NF-κB inhibitor α and interactions with VCP-containing complexes. In mouse myogenic models, EPSTI1 facilitates VCP-dependent proteasomal clearance of interferon-γ-activated STAT1 and supports skeletal muscle regeneration. Functional studies in macrophages and B cells further implicate EPSTI1 in inflammatory responses and immune-cell activation. Clinical investigations have identified EPSTI1 expression within interferon-associated disease signatures, highlighting its potential relevance to immune-related disorders and patient stratification. Model heterogeneity and limited independent mechanistic validation constrain cross-model interpretations. Direct substrate recruitment by EPSTI1 and its added value beyond existing clinical markers remain to be established. By distinguishing signal activation from activated-protein clearance and considering cellular source and treatment context, this review provides a more critical interpretation of the available evidence.

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Abbreviations: EPSTI1, epithelial-stromal interaction protein 1; VCP, valosin-containing protein; IFN, interferon; ISG, interferon-stimulated gene; JAK, Janus kinase; STAT1, signal transducer and activator of transcription 1; NF-κB, nuclear factor-κB; IκBα, NF-κB inhibitor α; ISRE, interferon-stimulated response element; GAS, gamma-activated sequence; IRF, interferon regulatory factor; PKR, protein kinase R; EMT, epithelial-mesenchymal transition; SLE, systemic lupus erythematosus; TLR, Toll-like receptor; HLA, human leukocyte antigen; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; m^6A, N^6-methyladenosine

Key words: EPSTI1, interferon-stimulated genes, VCP/p97, NF-κB, STAT1, proteostasis, autoimmunity, cancer

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1. Introduction

The appropriate initiation and resolution of inflammatory responses are essential for maintaining tissue homeostasis (1-3). Interferons help coordinate these responses by inducing interferon-stimulated genes (ISGs) involved in host defense and immune regulation (4-10). Functional studies have examined epithelial-stromal interaction protein 1 (EPSTI1) in tumor and immune cells and in skeletal muscle regeneration (11-14).

EPSTI1 was first identified as a transcript induced by epithelial-stromal interactions in breast cancer (15). Its interferon-responsive expression was subsequently demonstrated in epithelial and immune models (11,16,17). Initial sequence analysis predicted three coiled-coil regions in human EPSTI1 (15). In mouse EPSTI1, deletion analysis implicated residues 71-180 in association with valosin-containing protein (VCP/p97) and residues 181-314 in association with signal transducer and activator of transcription 1 (STAT1)-containing complexes (14). This mapping supports a role for these regions in complex association, but does not establish direct-binding interfaces; the coiled-coil regions remain sequence-based predictions. Apoptosis-related interactions with caspase-8 and breast cancer anti-estrogen resistance protein 3 (BCAR3) are discussed separately in Section 5.

EPSTI1 has been detected in both the nucleus and cytoplasm. In human breast cancer cells, immunostaining showed predominantly nuclear localization after interferon-α (IFN-α) stimulation or fibroblast coculture (17). IFN-γ treatment also increased the nuclear localization of EPSTI1 in mouse myoblasts (14).

VCP/p97 is an AAA+ ATPase involved in ubiquitin-dependent protein extraction, remodeling and proteasomal degradation (18,19). Its association with EPSTI1 has been investigated in breast cancer and muscle regeneration models (13,14). In breast cancer models, EPSTI1 overexpression increased nuclear factor-κB (NF-κB) activity and cell invasion and reduced the abundance of NF-κB inhibitor α (IκBα) (13). In mouse myogenic cells, EPSTI1 facilitated VCP-dependent proteasomal clearance of IFN-γ-activated STAT1, while *Epsti1*-deficient mice showed impaired muscle regeneration (14).

Studies in immune cells have identified additional functions of EPSTI1. *Epsti1* deficiency reduced proinflammatory gene induction in mouse bone marrow-derived macrophages (11). In a study of primary Sjögren's syndrome (pSS), EPSTI1 silencing reduced CpG-stimulated B-cell proliferation and immunoglobulin production (12). These findings extend the functional evidence to macrophage inflammatory responses and B-cell activation.

This review interprets EPSTI1 findings in relation to the molecular process, cellular source and measurement timing. It compares measures of STAT1 activation with the clearance of activated STAT1, cancer-cell phenotypes with immune-associated tissue expression and baseline prognostic associations with observations made during treatment. These comparisons help distinguish findings that address different biological questions from mechanistic links that remain untested. By integrating molecular studies with findings from cancer, autoimmune diseases and tissue remodeling, this review provides a framework for understanding the biological functions of EPSTI1 across distinct experimental and clinical contexts.

2. Literature scope and evidence appraisal

This narrative review examines EPSTI1 regulation, molecular function and disease-associated expression in cellular, animal and human studies. PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Web of Science (https://www.webofscience.com/) were searched using the terms 'EPSTI1', 'epithelial stromal interaction 1' and 'epithelial-stromal interaction 1'; the last search was performed on October 25th, 2026. Targeted searches for 'VCP', 'p97' and 'valosin-containing protein' supplemented the assessment of proteostatic mechanisms. The core EPSTI1 search did not require VCP-related terms. Reference lists of relevant publications were also examined.

Peer-reviewed original studies of EPSTI1 expression, regulation, molecular associations, functional perturbation or clinical outcomes were included. EPSTI1-specific perturbation studies informed functional and mechanistic interpretations, whereas expression-only studies informed the assessment of disease associations and cellular context. The broader interferon and VCP/p97 literature provided biological background. Studies of circEPSTI1 were considered separately and were not used to infer EPSTI1 protein function unless that function was directly examined.

The review distinguishes four categories of evidence. Mechanistic evidence combines EPSTI1 perturbation with experiments testing a defined molecular step, without necessarily resolving the mechanism of an entire pathway. Functional evidence demonstrates changes in signaling or phenotype after EPSTI1 gain or loss of function without resolving the underlying mechanism. Correlative evidence links EPSTI1 expression to disease, clinical outcomes or cellular signatures without establishing causality. Hypothesis-generating interpretations draw on related biology to propose roles where EPSTI1 function has not been directly tested. Individual findings from the same study may fall into different categories (13,14,20-23). These categories indicate what can be inferred from a finding; they are not numerical scores of study quality.

Confidence in each finding is assessed separately from its evidence category, considering perturbation specificity, appropriate controls, rescue experiments, complementary assays, biological replication and independent validation. Co-immunoprecipitation supports association within a complex rather than direct pairwise binding, and association with VCP/p97 is distinguished from functional dependence on it. Clinical findings are evaluated in relation to cellular composition, relevant confounders and validation in independent cohorts (24). Multiple assays within a publication and repeated analyses of the same public dataset are not considered independent replication. These criteria guide a qualitative appraisal rather than a formal risk-of-bias score.

Findings are compared across species, cell types, stimuli, interventions, sampling times and measured endpoints. Apparent discrepancies are first examined for differences in the biological process and endpoint being measured. Changes in STAT1 phosphorylation, accumulation of phosphorylated STAT1 and transcriptional output are interpreted within their experimental context rather than assumed to reflect the same underlying mechanism. When comparable studies report different results, the relative support for each interpretation was assessed using perturbation specificity, the adequacy of controls, rescue evidence and independent validation. Findings supported by complementary approaches receive greater interpretive weight for the claim being evaluated. Unresolved differences are retained and discussed within the relevant experimental context. Table I

Table I. EPSTI1 findings across biological contexts, classified by evidence category.

Biological context and model (reference)	EPSTI1-specific finding	Evidence category	VCP/p97 evidence	Validation and scope of inference
Breast cancer-Human mammary cell lines and mouse xenografts; 293ft cells for complex identification and VCP truncation analysis (13)	EPSTI1 knockdown reduced invasion; overexpression increased invasion and NF-κB activity and reduced IκBα abundance. Orthotopic tumors showed local invasion, while tail-vein injection of EPSTI1-overexpressing cells resulted in enhanced experimental lung colonization.	Functional: IκBα abundance, NF-κB activation, invasion and xenograft phenotypes. Support for VCP-associated pathway.	Association: Co-IP/MS and truncation mapping. Functional test: Interference using VCP-N187.	EPSTI1 gain- and loss-of-function experiments in multiple cell lines; VCP-N187 restored IκBα abundance and attenuated invasion. VCP involvement was tested in cells, but remains to be established in xenografts. Direct substrate recruitment was not demonstrated.
Human OSCC (HSC3-M3, HSC4) and LSCC (LK-2, EBC-1) cell lines (70)	EPSTI1 knockdown in OSCC and overexpression in LSCC both reduced proliferation and induced G1 arrest, with changes in EMT-associated markers. Migration was reduced in the OSCC cells tested and in EBC-1 cells.	Functional: Proliferation, cell cycle and EMT-associated phenotypes.	Association and dependence: Not assessed.	Cell-line perturbations, cell cycle assays and analyses of EMT markers support model-specific effects. Differences in the direction of manipulation and the limited number of independent clones restrict comparisons across cancers; no *in vivo* validation was reported.
Skeletal muscle regeneration-Mouse myoblasts and muscle-injury models; 293T cells for EPSTI1 deletion mapping (14)	*Epsti1* depletion increased activated STAT1 abundance and reduced its association with VCP. *Epsti1*-deficient mice showed impaired muscle regeneration.	Mechanistic: Cellular STAT1 clearance. Functional: Muscle regeneration in mice.	Association: Co-IP and deletion analysis. Dependence: Pharmacological VCP inhibition in cells.	Concordant findings in knockdown cells, knockout primary myoblasts and mice; proteasome inhibition supported the turnover model. Whole-body knockout does not isolate muscle-cell-specific effects; the cellular pathway was not directly tested *in vivo*.
Macrophage activation-Mouse bone marrow-derived macrophages and mice (11)	*Epsti1* deficiency reduced inflammatory gene induction and STAT1/p65 activation, with increased alternative-activation markers and fewer peritoneal M1 macrophages.	Functional, with evidence of signaling-pathway involvement.	Association and dependence: Not established.	Genetic deficiency and complementary signaling and marker assays support the phenotype. The cellular responses and peritoneal macrophage findings were observed under the conditions examined in that study.
Primary Sjögren's syndrome: B cells from patients and controls, with *ex vivo* stimulation (12)	EPSTI1 silencing reduced CpG-stimulated proliferation and IgG/IgM production. Silencing and overexpression produced reciprocal changes in p-p65 and IκBα.	Functional: B-cell responses. Correlative: Patient expression.	Association and dependence: Not established.	Confirmation of patient expression findings, reciprocal perturbations and IκBα-related pharmacological intervention provide complementary support. *Ex vivo* B-cell responses do not establish disease modification *in vivo*.
Dermatomyositis: Patient blood and muscle samples and primary human myoblasts (22)	Patient samples showed higher EPSTI1 expression. In IFN-β-stimulated myoblasts, EPSTI1 knockdown reduced HLA-A expression and CXCL10/CXCL11 secretion.	Functional: Myoblast responses Correlative: Patient expression.	Association and dependence: Not assessed.	Patient datasets from the investigators and public sources, tissue confirmation and complementary myoblast assays support the findings. HLA-A abundance and chemokine secretion are measured endpoints, not direct demonstrations of antigen presentation or disease modification.

Table I. Continued.

Biological context and model (reference)	EPSTI1-specific finding	Evidence category	VCP/p97 evidence	Validation and scope of inference
Systemic lupus erythematosus GEO datasets and patient PBMCs (21)	Higher EPSTI1 expression was associated with SLE, disease activity and T-cell-related transcripts. ROC analyses assessed diagnostic discrimination; no EPSTI1 perturbation was reported.	Correlative.	Association and dependence: Not established.	Analysis of public data was supplemented by RT-qPCR confirmation in patient samples. Validation of expression does not establish an effector mechanism or prospective diagnostic utility beyond established IFN signatures.
Osteoclast differentiation RAW264.7 cells and patient femoral specimens (20)	EPSTI1 knockdown reduced osteoclast differentiation and resorption, with changes in PKR/NF-κB signaling. Patient osteoclasts showed increased EPSTI1 expression and associated pathway markers.	Functional: Osteoclast phenotype. Correlative: Tissue markers.	Association and dependence: Not established.	Differentiation, molecular and resorption assays provide complementary cellular evidence, alongside human tissue observations. Osteoclast-specific causality and therapeutic efficacy were not established *in vivo*.
Osteogenic differentiation: Human bone marrow mesenchymal stem cells (44)	METTL14/YTHDF3-associated m^{6}A regulation was reported to reduce EPSTI1 expression. EPSTI1 knockdown enhanced osteogenic differentiation; overexpression inhibited differentiation and partially counteracted the pro-osteogenic effects associated with METTL14.	Functional: Osteogenic differentiation following EPSTI1 perturbation	Association and dependence: Not established.	RNA-association and m^{6}A-enrichment assays, reciprocal perturbations and partial phenotypic reversal support a cellular regulatory relationship. A conserved interferon-dependent effector mechanism and therapeutic efficacy *in vivo* were not established.
Colorectal cancer: Translation and invasion Human CRC cell lines; complementary organoid analyses (43)	KSR1/ERK regulated *EPSTI1* mRNA translation. EPSTI1 knockdown reduced EMT-like features, migration and invasion; re-expression in KSR1-deficient cells restored several phenotypes.	Mechanistic: Translational regulation. Functional: EMT/invasion.	Association and dependence: Not assessed.	Experiments in multiple cell lines, translation assays and downstream re-expression support the relationship. Organoid observations did not involve direct EPSTI1 perturbation; metastasis *in vivo* was not demonstrated.
Colorectal cancer: Cell-line drug response; Human CRC cell lines (32)	EPSTI1 depletion reduced viability and increased apoptosis. Sensitization of HCT116 cells was statistically supported for irinotecan; Caco-2 experiments showed reciprocal effects of knockdown and overexpression on drug sensitivity.	Functional.	Association and dependence: Not assessed.	Complementary viability and apoptosis assays, experiments in additional cell lines and partial reversal with a caspase inhibitor support the phenotype. The interaction with fluorouracil in HCT116 cells was not significant; pharmacological reversal is distinct from rescue by EPSTI1 re-expression.

Table I. Continued.

Biological context and model (reference)	EPSTI1-specific finding	Evidence category	VCP/p97 evidence	Validation and scope of inference
Colorectal cancer: Clinical and organoid associations; Patient-derived tumor organoids and patient datasets (32)	Epithelial ISG programs were associated with lower organoid chemosensitivity. EPSTI1-positive epithelial cells persisted after chemotherapy in tumors with incomplete pathological responses; pretreatment epithelial EPSTI1 expression did not distinguish complete from partial responders.	Correlative.	Association and dependence: Not assessed.	Integrates organoid drug-response profiling with patient transcriptomic analyses. These associations do not demonstrate that EPSTI1 perturbation changes drug response in organoids or patients, or establish clinical predictive utility.
Stage III colon cancer prognosis; Retrospective public cohorts and single-cell data (23)	Higher EPSTI1 expression was associated with longer relapse-free survival and immune-infiltration features. No EPSTI1-specific functional intervention was reported.	Correlative.	Association and dependence: Not assessed.	Analyses of multiple cohorts and single-cell data support an association between expression and outcome. Cox analyses were univariable. Different pooled HRs are reported in the abstract and main text; neither estimate establishes an adjusted prognostic effect or a protective cellular function.
Clear cell renal cell carcinoma prognosis; Institutional surgical cohort (n=221) and TCGA data (n=533) (80)	Tumor EPSTI1 protein and mRNA expression exceeded that in non-tumor tissues. Higher EPSTI1 protein expression was associated with poorer survival and remained associated with poorer overall survival after multivariable adjustment.	Correlative: Multivariable-adjusted prognostic association.	Association and dependence: Not assessed.	Institutional IHC and TCGA transcriptomic analyses provide complementary expression evidence; multivariable Cox analysis supports an adjusted prognostic association. The retrospective design and lack of EPSTI1 perturbation limit causal and clinical inference.
Vascular inflammatory responses; Human endothelial cells and monocyte adhesion assays (40)	EPSTI1 overexpression increased monocyte-endothelial adhesion and VCAM-1/ICAM-1 expression; EPSTI1 knockdown reduced LPS-associated adhesion responses.	Mechanistic: Adhesion-molecule dependence. Functional: Adhesion.	Association and dependence: Not established.	Reciprocal EPSTI1 perturbation and VCAM-1/ICAM-1 knockdown support the cellular pathway. The adhesion assays do not establish that targeting EPSTI1 modifies atherosclerosis *in vivo*.

Table I. Continued.

Biological context and model (reference)	EPSTI1-specific finding	Evidence category	VCP/p97 evidence	Validation and scope of inference
Host antiviral response; Human hepatoma-cell HCV models (16)	EPSTI1 gain- and loss-of-function experiments supported a contribution to IL-28A-associated antiviral responses, with PKR-linked pathway-dependence evidence.	Mechanistic: PKR dependence. Functional: Antiviral response.	Association and dependence: Not assessed.	Complementary perturbations and pathway-dependence experiments support a model-specific host-response function. The findings do not establish a broad antiviral role or efficacy in animals or patients.

Evidence categories: Mechanistic=EPSTI1-specific perturbation combined with experiments testing the stated molecular relationship; functional=an EPSTI1-dependent signaling or phenotypic effect; correlative=an expression-based association; hypothesis-generating=a proposed role for EPSTI1 without direct functional testing in the specified setting. These categories apply to individual findings rather than entire disease contexts, and they do not constitute numerical quality scores. VCP/p97 evidence: Complex association is distinguished from functional dependence. Co-IP does not establish direct pairwise binding. 'Not assessed' indicates that no relevant VCP/p97 assay was identified in the reviewed full text; 'Not established' indicates that the available evidence is insufficient; it does not exclude a biological role. Validation: The table describes the validation performed in each cited study. Multiple assays, models or cohorts within a study provide complementary support; independent replication by other research groups is considered separately. Scope: The table summarizes selected original studies. Expression-based observations in neural and metabolic settings are discussed separately in Section 8. Reference numbers correspond to those in the manuscript bibliography. co-IP, co-immunoprecipitation; CRC, colorectal cancer; EMT, epithelial-mesenchymal transition; EPSTI1, epithelial-stromal interaction protein 1; GEO, Gene Expression Omnibus; HCV, hepatitis C virus; HLA-A, major histocompatibility complex class I A; ICAM-1, intercellular adhesion molecule 1; IFN, interferon; IgG/IgM, immunoglobulin G/M; IL-28A, interferon-λ2; VCP, valosin-containing protein; IκBα, NF-κB inhibitor α; KSR1, kinase suppressor of Ras 1; LPS, lipopolysaccharide; METTL14, methyltransferase-like 14; MS, mass spectrometry; NF-κB, nuclear factor-B; PBMC, peripheral blood mononuclear cell; PKR, protein kinase R; ROC, receiver operating characteristic; STAT1, signal transducer and activator of transcription 1; VCAM-1, vascular cell adhesion molecule 1; ISG, interferon-stimulated gene; HR, hazard ratio; IHC, immunohistochemistry; LSCC, lung squamous cell carcinoma; OSCC, oral squamous cell carcinoma; TCGA, The Cancer Genome Atlas; ERK, extracellular signal-regulated kinase; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; YTHDF3, YTH domain-containing family protein 3; CpG, cytosine-phosphate-guanine; m^6A, N^6-methyladenosine; SLE, systemic lupus erythematosus; VCP-N187, the N-terminal fragment of VCP comprising amino acid residues 1-187.

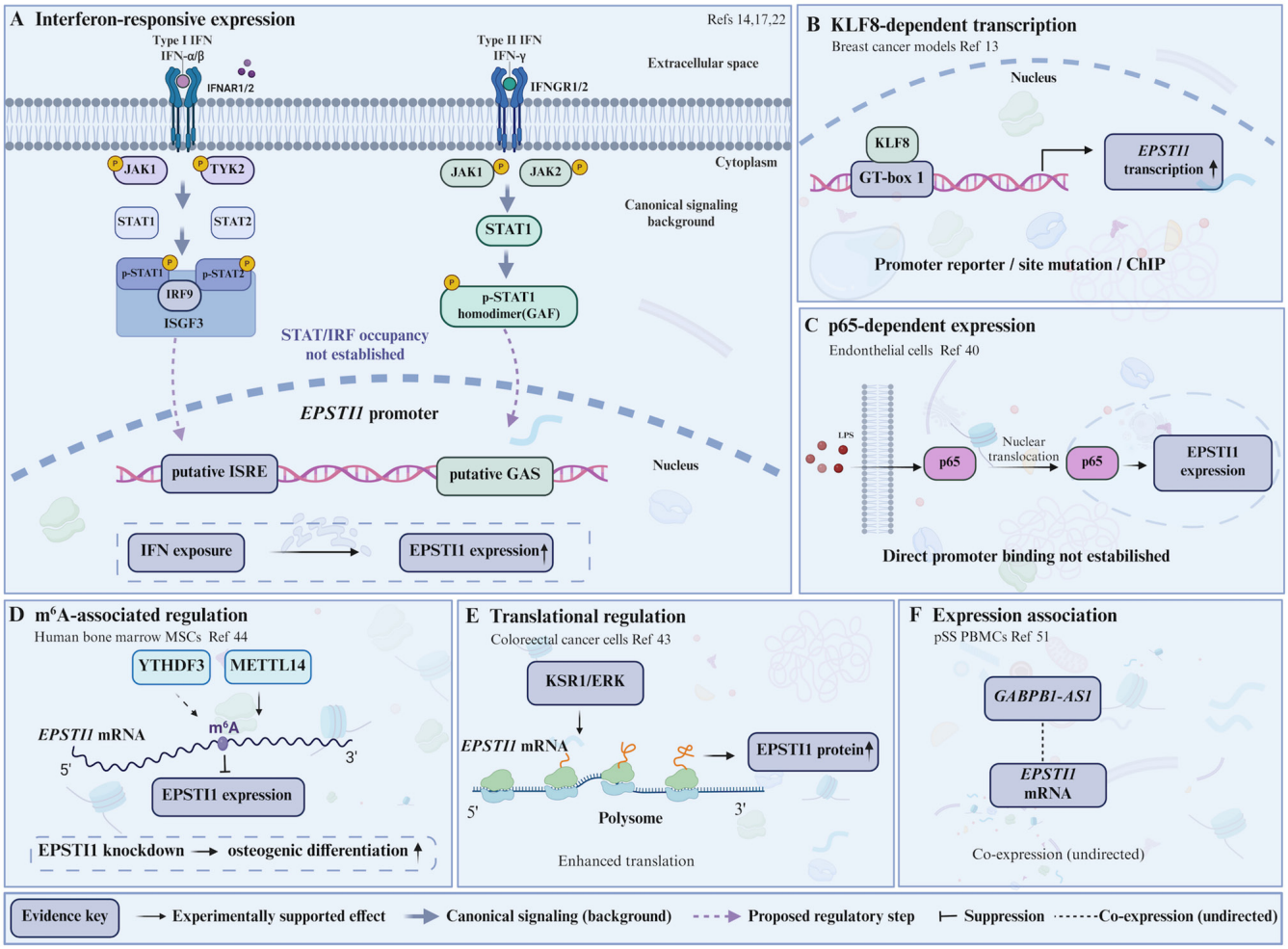


Figure 1. Regulation of EPSTI1 expression. (A) Interferon exposure increases EPSTI1 expression; STAT/IRF occupancy at candidate promoter elements remains unconfirmed. (B) KLF8-dependent transcription is supported by promoter assays, mutation of the relevant promoter site and chromatin immunoprecipitation. (C) p65 contributes to basal and LPS-induced EPSTI1 expression in endothelial cells. (D) METTL14-associated m^{6}A regulation involving the m^{6}A reader YTHDF3 was reported to reduce EPSTI1 expression in hBMSCs, in which EPSTI1 depletion enhanced osteogenic differentiation. (E) KSR1/ERK supports *EPSTI1* translation in colorectal cancer cells. (F) *GABPB1-AS1* and *EPSTI1* are co-expressed in PBMCs from patients with pSS. Solid arrows indicate effects tested in the specified models; gray arrows show canonical signaling pathways as background; dashed arrows indicate proposed regulatory steps or m^{6}A-reader association; an undirected dashed line denotes co-expression; and a T-bar denotes suppression. The figure was created with BioRender.com. EPSTI1, epithelial-stromal interaction protein 1; ERK, extracellular signal-regulated kinase; hBMSCs, human bone marrow mesenchymal stem cells; KLF8, Krüppel-like factor 8; KSR1, kinase suppressor of Ras 1; LPS, lipopolysaccharide; m^{6}A, N^6-methyladenosine; METTL14, methyltransferase-like 14; STAT, signal transducer and activator of transcription; YTHDF3, YTH domain-containing family protein 3; ChIP, chromatin immunoprecipitation; GAF, gamma-activated factor; ISGF3, interferon-stimulated gene factor 3; *GABPB*1-AS1, GABPB1 antisense RNA 1; GAS, gamma-activated sequence; IFN, interferon; IFNAR1/2, interferon-α/β receptor subunits 1 and 2; IFNGR1/2, interferon-γ receptor subunits 1 and 2; IRF9, interferon regulatory factor 9; ISRE, interferon-stimulated response element; JAK1/2, Janus kinases 1 and 2; mRNA, messenger RNA; MSCs, mesenchymal stem cells; P, phosphate group; p-STAT1/2, phosphorylated STAT1/2; TYK2, tyrosine kinase 2; PBMCs, peripheral blood mononuclear cells; pSS, primary Sjögren's syndrome.

summarizes the models, principal findings, evidence categories and key limitations of selected studies.

3. Regulation of EPSTI1 expression

Fig. 1 summarizes experimentally supported and proposed mechanisms controlling EPSTI1 expression, distinguishing transcriptional and post-transcriptional regulation from expression-based associations.

Interferon-responsive induction. Interferon-stimulated EPSTI1 expression has been demonstrated in several cellular models (14,17,22), consistent with its classification as an ISG (5,25). IFN-α increased *EPSTI1* mRNA and EPSTI1 protein abundance in human breast cancer cells, while IFN-β and IFN-γ induced its expression in human and mouse myoblasts, respectively (14,17,22). Induction by IL-28A (IFN-λ2) has also been reported in hepatoma cells, extending these observations to type III interferons (16).

IFN-α/β and IFN-γ activate Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling, leading to the phosphorylation and nuclear translocation of STAT family transcription factors (26,27). STAT and interferon regulatory factor (IRF) complexes, together with the regulatory elements they recognize, form part of the general transcriptional framework governing ISG expression (28-31). Pharmacological evidence implicating this pathway in EPSTI1 expression comes from HCT116 colorectal cancer cells, in which ruxolitinib reduced EPSTI1 expression and attenuated its chemotherapy-induced upregulation (32). Together, these

findings support the involvement of interferon-responsive signaling in EPSTI1 induction. The precise contributions of STAT/IRF occupancy and candidate ISRE and GAS elements to EPSTI1 transcription warrant further investigation.

Cell context and transcriptional regulation. Cellular responses to interferons vary with receptor availability, chromatin state, signaling history and cytokine crosstalk (29,33-38). These general features provide context for interpreting differences in EPSTI1 expression across experimental systems. Evidence for EPSTI1-specific regulatory mechanisms, however, comes from studies that directly manipulate its upstream regulators.

One defined example is transcriptional activation by Krüppel-like factor 8 (KLF8) in breast cancer models. KLF8 overexpression increased EPSTI1 expression, whereas KLF8 knockdown reduced it. *EPSTI1* promoter-reporter assays, mutation of the required GT-box 1 site and chromatin immunoprecipitation supported direct activation of the EPSTI1 promoter by KLF8 (13). These experiments establish a promoter-level mechanism in the cellular models examined.

Inflammatory inputs and NF-κB-associated regulation. NF-κB integrates inflammatory inputs through stimulus-dependent activation and crosstalk with other signaling pathways (28,39). In human umbilical vein endothelial cells, lipopolysaccharide (LPS) increased EPSTI1 expression. p65 overexpression increased *EPSTI1* mRNA and EPSTI1 protein abundance, whereas p65 depletion lowered basal expression and attenuated LPS-induced upregulation (40). These findings support p65-dependent regulation of EPSTI1 expression in endothelial cells, but do not establish direct p65 binding to the *EPSTI1* promoter.

EPSTI1 has also been implicated in the amplification of inflammatory signaling in selected cellular contexts (11-13,22). In breast cancer and pSS B-cell models, EPSTI1 perturbation altered IκBα abundance and NF-κB activation (12,13). Together, these studies reveal regulatory connections between EPSTI1 and NF-κB signaling in endothelial cells, breast cancer cells and B cells, providing a basis for investigating potential feedback regulation within defined cellular contexts.

Post-transcriptional regulation. Post-transcriptional mechanisms, including RNA modifications, provide an additional layer of control over immune and antiviral gene expression (41,42). EPSTI1-specific regulation has been examined in distinct cellular models. In colorectal cancer cells, polysome profiling and perturbation of kinase suppressor of Ras 1 (KSR1) and extracellular signal-regulated kinase (ERK) signaling showed that KSR1 and ERK promoted *EPSTI1* mRNA translation, identifying translational control as a determinant of EPSTI1 protein abundance (43).

A separate mechanism involving N^6-methyladenosine (m^6A) has been reported in human bone marrow mesenchymal stem cells (hBMSCs) (44). In that study, methyltransferase-like 14 (METTL14) and the m^6A reader YTH domain-containing family protein 3 (YTHDF3) suppressed EPSTI1 expression. An RNA immunoprecipitation assay was used to assess associations with *EPSTI1* mRNA, whereas a methylated RNA immunoprecipitation assay was utilized to assess its m^6A enrichment. EPSTI1 knockdown enhanced osteogenic differentiation, whereas overexpression inhibited it. Concomitant EPSTI1 overexpression partially reversed the pro-osteogenic effects of METTL14 overexpression (44). These findings identify m^6A-associated regulation as an additional mechanism controlling EPSTI1 expression and link this regulatory process to osteogenic differentiation in hBMSCs.

Disease-associated expression patterns. Interferon-associated transcriptional programs are prominent in several autoimmune diseases (45,46), with distinct signatures reported across myositis subtypes (47). Increased EPSTI1 expression has been reported in interferon-active conditions, including pSS, dermatomyositis and systemic lupus erythematosus (12,21,22,48). These patient findings identify disease settings in which EPSTI1 expression is altered and provide a clinical context for the experimental studies.

Noncoding RNAs also participate in interferon- and infection-associated gene networks (49,50). In peripheral blood mononuclear cells from patients with pSS, *GABPB1-AS1* was reported to be upregulated and co-expressed with interferon-associated mRNAs, including interferon induced protein 44 (*IFI44*), *IFI44L*, *IFI6* and *EPSTI1* (51). The evidence supports co-expression of *GABPB1-AS1* and *EPSTI1*, but does not establish a regulatory relationship or its direction.

Cellular composition also matters when interpreting tissue-level expression. Single-cell analyses of stage III colon cancer identified macrophages and dendritic cells as major EPSTI1-expressing populations (23), while EPSTI1-containing expression signatures have been associated with immune features and clinical outcomes in lung adenocarcinoma (52). These observations demonstrate the importance of cellular composition in interpreting disease-associated EPSTI1 expression and provide a foundation for investigating its functions in specific immune and tumor-cell populations.

4. EPSTI1 and VCP/p97-associated proteostatic signaling

Fig. 2 presents the experimental findings discussed below as separate relationships observed in specific models.

Overview of VCP/p97-mediated proteostasis. VCP/p97 is a highly conserved AAA+ ATPase and a central component of ubiquitin-dependent protein quality control (18,53). As a ubiquitin-directed segregase/extractase, it uses ATP hydrolysis to extract ubiquitinated substrates from membranes, chromatin and macromolecular protein complexes, enabling their remodeling, recycling or delivery to the proteasome for degradation (18,54,55). This activity supports diverse proteostatic and stress-response pathways, including endoplasmic reticulum-associated degradation, mitochondrial quality control, autophagy-related proteostasis, DNA damage responses and the regulation of inflammatory signaling (53,54).

VCP/p97 cofactors and adaptor proteins coordinate substrate recognition and processing by influencing client engagement, subcellular localization and ATPase organization (53,56-59). A study on Fas associated factor family member 2 showed that its ubiquitin-binding helix-ubiquitin regulatory X module enhanced ubiquitinated-substrate engagement, p97 ATPase activity and substrate unfolding, facilitating membrane-protein extraction (60). These findings illustrate an

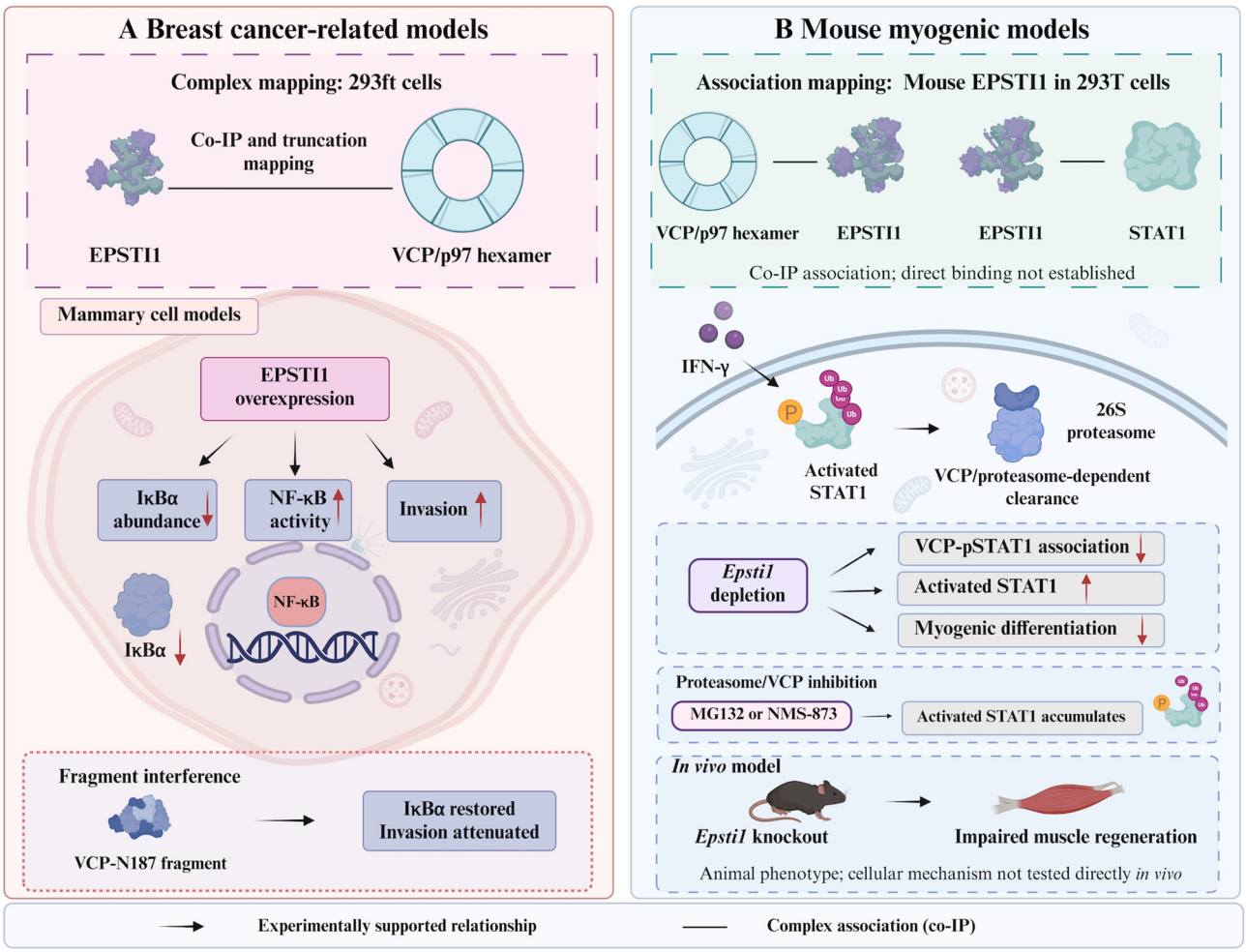


Figure 2. EPSTI1-associated proteostatic signaling in breast cancer and myogenic models. The panels summarize distinct experimental systems. (A) In breast cancer-related models, EPSTI1 overexpression lowers IκBα abundance and increases NF-κB activity and invasion. Interference using VCP-N187 restores IκBα abundance and attenuates invasion. Co-immunoprecipitation and truncation analysis support an association between EPSTI1 and VCP within a complex, but do not establish direct substrate delivery. (B) In mouse myogenic models, *Epsti1* depletion reduces VCP-pSTAT1 association and increases activated STAT1 abundance. Inhibitor experiments support VCP- and proteasome-dependent clearance. Whole-body *Epsti1* deficiency impairs skeletal muscle regeneration. Solid arrows indicate effects tested in the specified models; lines without arrowheads denote separate complex associations supported by co-immunoprecipitation. The separate co-immunoprecipitation associations do not imply direct binding or a ternary complex. The figure was created with BioRender.com. EPSTI1, epithelial-stromal interaction protein 1; IκBα, NF-κB inhibitor α; NF-κB, nuclear factor-κB; pSTAT1, phosphorylated STAT1; STAT1, signal transducer and activator of transcription 1; VCP, valosin-containing protein; co-IP, co-immunoprecipitation; IFN, interferon; P, phosphate group; Ub, ubiquitin; VCP-N187, the N-terminal fragment of VCP comprising amino acid residues 1-187.

established p97 cofactor mechanism; EPSTI1-specific complex associations are considered separately in the next section.

Evidence for EPSTI1-VCP/p97 complex association. EPSTI1 associates with VCP/p97-containing complexes in selected experimental models (13,14). In a breast cancer study, co-immunoprecipitation followed by mass spectrometry identified VCP as an EPSTI1-associated protein in 293ft cells, and truncation analysis implicated the N-terminal region of VCP (residues 1-187) in the association (13). In a muscle regeneration study, deletion of mouse EPSTI1 residues 71-180 abolished co-immunoprecipitation with VCP, whereas deletion of residues 181-314 reduced STAT1 association in 293T cells (14). These mapping experiments identify regions that contribute to a complex association under the conditions tested, but do not define a direct pairwise binding interface. The observed effects could also reflect changes in protein conformation or localization caused by the deletions.

EPSTI1-dependent changes in IκBα abundance and NF-κB activation. Canonical NF-κB activation typically involves stimulus-induced IκBα phosphorylation, ubiquitination and degradation, allowing NF-κB dimers to enter the nucleus and induce genes involved in inflammation, survival, migration and immune-cell recruitment (61-64).

An independent study of TNF-induced signaling showed that VCP/p97 facilitates the release of RelA from ubiquitinated IκBα and promotes efficient IκBα degradation (65). This establishes a role for VCP/p97 in IκBα processing, but does not identify EPSTI1 as the factor that recruits IκBα to this machinery.

The EPSTI1-specific evidence comes from breast cancer models. EPSTI1 overexpression reduced IκBα abundance and increased NF-κB nuclear accumulation and reporter activity. Co-expression of the VCP N-terminal fragment comprising residues 1-187 restored IκBα abundance and attenuated EPSTI1-induced invasion (13). Together, these findings provide

functional evidence linking EPSTI1 to VCP-associated regulation of IκBα abundance, NF-κB activation and invasive phenotypes in breast cancer models. The precise molecular steps connecting EPSTI1-VCP association to IκBα processing remain an important direction for further investigation.

EPSTI1-associated STAT1 clearance and IFN-γ signal resolution. A distinct role for EPSTI1 has been reported in mouse models of skeletal muscle regeneration (14). IFN-γ activates the JAK-STAT1 pathway, leading to STAT1 phosphorylation, dimerization, nuclear translocation and transcriptional induction of interferon-responsive genes (66,67). Although transient STAT1 activation is essential for host defense and immune regulation, prolonged STAT1 signaling can sustain inflammatory gene expression and interfere with tissue repair (14,66).

In mouse myoblasts, *Epsti1* depletion increased phosphorylated STAT1 (pSTAT1) abundance and reduced its co-immunoprecipitation with VCP. K48-linked polyubiquitin signals increased in pSTAT1 immunoprecipitates, consistent with impaired clearance. Experiments with the proteasome inhibitor MG132 and the VCP inhibitor NMS-873 further supported proteasome- and VCP-dependent clearance of activated STAT1 (14). Proteasome inhibition also increased nuclear pSTAT1 abundance. *Epsti1* depletion increased mRNA levels of IRF1 and class II major histocompatibility complex transactivator and reduced myogenin mRNA levels (14).

The study also reported impaired regeneration in *Epsti1*-deficient mice, as discussed in Section 7 (14).

Substrate engagement and mechanistic questions. The breast cancer and myogenic studies examine different molecular steps. The breast cancer study links EPSTI1 overexpression and interference using a VCP fragment to altered IκBα abundance and invasion (13), whereas the myogenic study supports VCP- and proteasome-dependent clearance of activated STAT1 (14). The findings allow comparison of VCP-associated signaling effects, but do not demonstrate direct EPSTI1-mediated substrate delivery or a switch between IκBα and STAT1 recruitment. Neither study identifies an EPSTI1-specific E3 ubiquitin ligase, a mechanism of ubiquitin-chain selection or intrinsic ubiquitin-modifying activity of EPSTI1.

STAT1 measurements also need to be interpreted in relation to the signaling process examined. *Epsti1* deficiency reduced STAT1 phosphorylation and nuclear localization in stimulated mouse bone marrow-derived macrophages (11), but increased activated STAT1 abundance in mouse myoblasts, consistent with impaired clearance (14). An increase in pSTAT1 abundance cannot, by itself, distinguish increased phosphorylation from reduced dephosphorylation or degradation. The studies also differ in stimuli, sampling times and perturbation strategies. These findings therefore distinguish questions of signal activation from those of signal persistence, without establishing that cell lineage alone explains the contrasting observations.

The evidence therefore supports model-specific effects on signaling and activated STAT1 clearance. Further studies examining VCP/p97 involvement in macrophages and the molecular steps linking EPSTI1-associated complexes to substrate processing may help clarify these context-dependent mechanisms. Table II outlines experimental approaches to address these questions.

5. EPSTI1 in cancer

Breast cancer: Epithelial plasticity, invasion and cell survival. Early breast cancer studies first linked EPSTI1 to epithelial-stromal interactions; subsequent work examined its roles in epithelial plasticity, invasion, inflammatory signaling and apoptosis (13,15,17). These endpoints capture distinct aspects of cancer-cell behavior and warrant separate interpretation.

In mammary epithelial and breast cancer models, EPSTI1 perturbation altered migration, cell spreading in three-dimensional collagen and epithelial-mesenchymal transition (EMT)-associated features (17). In patient tumor samples, EPSTI1 expression was also associated with poorer differentiation, complementing these experimental observations (17).

In a separate study, EPSTI1 knockdown reduced invasion, whereas inducible EPSTI1 expression promoted local invasion into adjacent mammary fat tissue and experimental lung colonization after intravenous injection (13). The increase in local invasion was not accompanied by a corresponding increase in orthotopic tumor growth rate. These results distinguish effects on invasion behavior from effects on primary tumor growth, while the intravenous assay specifically assesses the colonization stage of metastasis. Additional functional evidence comes from experiments in which EPSTI1 re-expression partially reversed the effects of microRNA-654-5p on growth, invasion and apoptosis in the breast cancer cell lines tested (68). The associated VCP/IκBα/NF-κB findings are discussed in Section 4 (13).

Functional studies also implicate EPSTI1 in the regulation of apoptosis in breast cancer cells. Pairwise yeast two-hybrid assays identified interactions of EPSTI1 with caspase-8 and BCAR3. In MCF-7 and MDA-MB-231 cells, EPSTI1 silencing increased poly(ADP-ribose) polymerase (PARP) cleavage. Conversely, overexpression reduced TNF-related apoptosis-inducing ligand-induced PARP cleavage but did not significantly restore cell viability. Other apoptotic readouts varied between cell lines, and the silencing experiments included IFN-α stimulation (69). These findings support an anti-apoptotic contribution of EPSTI1 in the models examined, although it remains unclear whether the identified protein interactions are required for this effect.

Functional evidence in other cancer-cell models. Functional evidence also extends to colorectal cancer. KSR1/ERK-dependent *EPSTI1* translation was linked to cadherin switching and EMT-like phenotypes in colorectal cancer cells. EPSTI1 depletion reduced migration, matrix invasion and anchorage-independent growth, whereas re-expression in KSR1-deficient cells restored several of these phenotypes. These findings support a functional role for EPSTI1 downstream of KSR1/ERK (43).

A separate colorectal cancer study examined the contribution of EPSTI1 to cell survival and chemotherapy responses. EPSTI1 depletion reduced viability and increased apoptosis. In HCT116 cells, knockdown increased sensitivity to irinotecan, whereas the interaction between knockdown and fluorouracil

Table II. Key unresolved questions and proposed experimental approaches.

Priority question	Evidence and significance	Proposed approach
What is EPSTI1's molecular role in VCP/p97-associated complexes?	Complex association and deletion mapping in breast cancer and myogenic models provide a basis for characterizing the molecular relationship (13,14).	Confirm endogenous association, test binding between purified proteins and map candidate interfaces by mutagenesis. Assess function in rescue experiments while controlling for mutant abundance, localization and integrity.
Which molecular steps link EPSTI1 to reduced IκBα abundance and the clearance of activated STAT1?	Breast cancer studies report altered IκBα abundance, whereas myogenic experiments support VCP-dependent proteasomal turnover of activated STAT1 (13,14).	Measure protein turnover and ubiquitination following EPSTI1 perturbation; test VCP/p97 and proteasome dependence separately. Use EPSTI1 mutants with reduced VCP association to distinguish effects on substrate engagement from those on subsequent processing.
How do EPSTI1-dependent functions differ across cellular contexts?	Macrophage readouts associated with STAT1 activation, myogenic VCP/STAT1 turnover, breast cancer IκBα/NF-κB findings and osteoclast PKR/NF-κB findings concern distinct signaling processes and experimental models (11,13,14,20).	Combine gain- and loss-of-function studies and rescue experiments with time-resolved pathway readouts to test VCP/p97 or PKR dependence within each model. Replicate key effects in relevant primary cells and independent laboratories.
Which cell populations contribute to disease-associated EPSTI1 expression and function?	Patient expression studies and selected cellular perturbations highlight the need to distinguish changes in cell abundance from EPSTI1 induction and function within individual cell populations (12,21-23).	Combine single-cell or spatial profiling with protein localization and cell-composition analysis. Test EPSTI1-dependent effects in relevant human cell populations using perturbation and rescue.
Does EPSTI1 improve clinical assessment beyond established interferon signatures?	Expression associations and multigene scores highlight the need to test added value beyond existing ISG measures and clinical variables (21,23,89,117).	Compare prespecified, otherwise equivalent models with and without *EPSTI1* in the same cohorts; assess discrimination, calibration and clinical net benefit, with external validation. Distinguish pretreatment prediction from on-treatment monitoring; use treatment comparisons to evaluate treatment-specific benefit.
Can EPSTI1 be modulated selectively while preserving normal tissue function?	EPSTI1 perturbation supports preclinical investigation, while the muscle regeneration findings highlight the need to assess tissue tolerance (14). CB-5083 inhibits PDE6 as an off-target effect (120).	Establish target engagement and EPSTI1-dependent efficacy for candidate interventions. Compare effects with broad VCP/p97 inhibition, assess immune and repair outcomes and distinguish VCP-related proteostatic effects from compound-specific off-target toxicity.

The evidence column summarizes published findings, whereas the proposed approaches identify research priorities. Co-immunoprecipitation supports complex association, whereas direct binding requires separate evidence. EPSTI1, epithelial-stromal interaction protein 1; ISG, interferon-stimulated gene; IκBα, NF-κB inhibitor α; NF-κB, nuclear factor-κB; PDE6, phosphodiesterase-6; PKR, protein kinase R; STAT1, signal transducer and activator of transcription 1; VCP, valosin-containing protein.

treatment was not statistically significant. In Caco-2 cells, knockdown increased sensitivity to both drugs, whereas overexpression reduced sensitivity. Exogenous IFN-γ induced ISGs but did not reproduce the reduction in chemosensitivity under the conditions tested (32). Thus, EPSTI1 contributed to cell survival and drug responses in these models, but interferon-induced ISG expression alone did not reproduce the observed chemotherapy-response state.

Responses to EPSTI1 perturbation differed between oral and lung squamous cell carcinoma models. In oral squamous cell carcinoma cells, EPSTI1 knockdown reduced proliferation and migration, increased E-cadherin expression and decreased N-cadherin expression. In lung squamous cell carcinoma cells, EPSTI1 overexpression reduced proliferation; reduced migration was also documented in EBC-1 cells (70). These findings argue against a uniformly growth-promoting role for EPSTI1. Because the studies used different perturbations and cell backgrounds, the contrasting responses cannot be attributed to cancer type alone.

Cellular sources of EPSTI1 expression and immune-cell function. Tumors contain malignant cells, stromal cells, endothelial cells, myeloid populations, lymphocytes and other microenvironmental compartments (71). Interpretation of EPSTI1 expression in tumor specimens requires attention to both cellular source and cell state. In bulk transcriptomic datasets, high EPSTI1 expression may reflect malignant epithelial cells, interferon-activated stromal cells, infiltrating immune cells or a mixture of these populations (72,73), rather than a uniform tumor-cell-intrinsic signal.

In stage III colon cancer, EPSTI1 expression correlated with immune-infiltration features, and single-cell analyses identified macrophages and dendritic cells as major EPSTI1-expressing populations (23). In lung adenocarcinoma, a *CXCL13/EPSTI1/CDK1* expression signature was enriched in non-epithelial populations, particularly proliferating T cells (52). These immune-associated expression patterns differ from the cancer-cell-intrinsic functions examined in perturbation studies. Functional evidence also comes from primary mouse $CD8^+$ T cells; in wild-type cells, *Epsti1* knockdown increased IκBα abundance and reduced the proportions of cells positive for CD69, IFN-γ or TNF-α after T-cell receptor (TCR) stimulation (74). This response differed from the enhanced activation observed with *Tbc1d10c* deficiency in the same study. The EPSTI1-specific experiments therefore address T-cell activation, whereas the *in vivo* antitumor phenotype was demonstrated following *Tbc1d10c* loss (74).

Interferon-related immune programs have also been incorporated into multigene signatures of response to checkpoint inhibitors (75). More recently, *EPSTI1* was included in an 11-gene signature derived from melanoma-infiltrating immune cells and evaluated in external immunotherapy datasets (76). Complementary single-cell and spatial approaches can help identify the cellular sources of these signals; applications and integration strategies have been described in breast cancer and colorectal cancer (77-79).

Prognostic and treatment-associated findings. Across five retrospective cohorts comprising 547 patients with stage III colon cancer, higher EPSTI1 expression was associated with longer relapse-free survival (23). The main text and Fig. 4C of the original study report a pooled hazard ratio of 0.81 (95% confidence interval, 0.71-0.91), whereas its abstract reports 0.79 (95% confidence interval, 0.65-0.96). Despite the small discrepancy between the reported estimates, both indicate a consistent association between higher EPSTI1 expression and longer relapse-free survival. These findings support the prognostic relevance of EPSTI1 in stage III colon cancer, providing a basis for further evaluation in multivariable models (23).

A different association was reported in clear cell renal cell carcinoma. The retrospective study included an institutional surgical cohort of 221 patients and The Cancer Genome Atlas (TCGA) data from 533 patients. In the institutional cohort, higher EPSTI1 protein expression remained associated with poorer overall survival after multivariable adjustment (80). This finding offers a clinical comparison with the favorable relapse-free survival association reported in stage III colon cancer, although the studies differ in cancer type, expression measurement and outcome.

Treatment context also affects interpretation. In lung adenocarcinoma, a higher C-X-C motif chemokine ligand (*CXCL*)*13*/*EPSTI1*/cyclin-dependent kinase (*CDK*)*1* signature score was associated with poorer overall survival in treatment-naive cohorts, but with longer overall survival in the Stand Up To Cancer-Mark Foundation cohort treated with immune checkpoint inhibitors (52). The contrasting survival associations highlight the potential importance of treatment context in interpreting the *CXCL13/EPSTI1/CDK1* signature and provide a rationale for evaluating its predictive performance in appropriately designed treatment cohorts.

In colorectal cancer, epithelial ISG activity in patient-derived tumor organoids was associated with reduced chemosensitivity. In the patient transcriptomic dataset analyzed, EPSTI1-positive epithelial cells persisted after chemotherapy in tumors with incomplete pathological responses, whereas pretreatment expression did not distinguish complete from partial responders (32). The same study also reported reduced epithelial and stromal EPSTI1 protein staining in paired post-treatment resection specimens. Differences in treatment context, sampling interval and measurement method complicate direct comparison with the transcriptomic observations (32). Together, these findings connect EPSTI1-associated epithelial states with chemotherapy responses and complement the functional evidence from cell-line perturbation studies, supporting further investigation of EPSTI1 in treatment-response biology.

Cancer-cell function, tissue-level prognosis and prediction of treatment response therefore require separate interpretation. EPSTI1-dependent invasion in breast cancer models (13) can coexist with favorable relapse-free survival in immune-rich, EPSTI1-high colon tumors (23). Cellular source offers one possible explanation, although the findings do not show that immune-cell EPSTI1 causes the favorable outcome. Similarly, the renal protein-expression association was obtained with a different assay and an overall-survival endpoint (80); it does not, by itself, establish a cancer-specific reversal of EPSTI1 function.

These studies support interpreting high EPSTI1 expression in the context of its cellular source, measurement time and clinical endpoint, rather than assigning it a uniform clinical meaning.

6. EPSTI1 in autoimmune and interferon-driven inflammatory diseases

EPSTI1 as part of interferon signatures. EPSTI1 is upregulated alongside other ISGs in several autoimmune diseases, as part of the broader interferon-associated transcriptional response (12,21,22). Dysregulated type I interferon signaling is a shared feature of pSS, dermatomyositis and systemic lupus erythematosus (SLE) (46,81,82). Biomarker and cellular effector roles may coexist, but require different evidence: Expression studies identify interferon-associated states, whereas EPSTI1 perturbation tests its contribution to a defined biological response. The following sections compare these forms of evidence across diseases and cellular compartments.

Dermatomyositis. Type I interferon activation is a prominent feature of dermatomyositis (DM), with interferon-responsive transcriptional programs detected in blood and muscle (83-85). A study of EPSTI1 in DM reported increased expression in patient peripheral blood mononuclear cells (PBMCs) and muscle tissue, supported by public transcriptomic datasets and additional patient samples. PBMC EPSTI1 expression was inversely associated with manual muscle testing of eight muscle groups (MMT8) scores, linking higher expression to reduced muscle strength in the cohort studied (22). In contrast to PBMC expression, muscle *EPSTI1* mRNA was not significantly associated with MMT8 in the same study (22).

Functional experiments were conducted in commercially sourced primary human skeletal muscle myoblasts. Under IFN-β stimulation, EPSTI1 knockdown reduced HLA-A protein expression and the secretion of CXCL10 and CXCL11, supporting a contribution to the inflammatory responses in muscle cells, with class I HLA expression and chemokine secretion as the measured outputs (22). These cellular findings provide a rationale for investigating EPSTI1 in DM, but do not directly demonstrate antigen presentation, leukocyte recruitment or muscle injury *in vivo*.

The DM and regeneration studies address different biological processes. The DM-related experiments measured IFN-β-induced human leukocyte antigen (HLA)-A expression and chemokine secretion in human myoblasts (22), whereas the regeneration study linked EPSTI1 to the clearance of activated STAT1 in mouse myogenic cells and to effective repair *in vivo* (14). Reduced inflammatory outputs after knockdown in the DM-related model therefore do not establish improved muscle regeneration. The studies also differ in species, interferon subtype and differentiation conditions, so the contrasting findings cannot be attributed to disease state alone. Current evidence supports distinct functions in these experimental systems, rather than a demonstrated switch between pathogenic and protective actions within the same muscle system.

SLE. In SLE, *EPSTI1* has mainly been studied as an interferon-associated transcript in peripheral immune cells (21,86). An analysis of Gene Expression Omnibus datasets, supplemented by reverse transcription-quantitative PCR validation in patient PBMCs, identified increased EPSTI1 expression and a positive correlation with SLE Disease Activity Index scores (21). This single-transcript association should be distinguished from findings for broader interferon signatures, whose associations with global disease activity have varied across cohorts (87).

EPSTI1 has also been included in multigene assessments of SLE immunophenotypes (88). In a belimumab-treated cohort, a five-gene score that included *EPSTI1* and was based on changes in ISG expression during the first 3 months of treatment was associated with a subsequent clinical response. The reported predictive performance applies to this composite score, not to EPSTI1 alone (89). Cross-sectional serum analyses across cutaneous and systemic lupus phenotypes provide additional biomarker evidence (90). These studies examine expression and clinical associations; they do not test whether EPSTI1 perturbation alters lupus-associated immune dysfunction or tissue injury.

pSS. Studies in pSS provide a functional link between EPSTI1 and B-cell activation (91,92). In a study reporting elevated EPSTI1 expression in peripheral B cells from patients, EPSTI1 silencing reduced CpG-stimulated proliferation and immunoglobulin G (IgG) and IgM production. Silencing also decreased p65 phosphorylation and increased IκBα abundance, whereas EPSTI1 overexpression produced the opposite changes. These findings support a contribution to Toll-like receptor 9-responsive NF-κB signaling and B-cell activation (12). The immunoglobulin measurements reflect total IgG and IgM production, rather than antigen-specific autoantibody responses.

A separate pSS study illustrates the importance of cell type and functional endpoint. Although EPSTI1 expression was elevated in naive $CD4^+$ T cells from patients, overexpression in naive $CD4^+$ T cells from healthy donors did not enhance differentiation into follicular helper T cells positive for programmed cell death protein 1 (93). This result is compatible with the B-cell findings, but limits their extension to other lymphocyte responses.

Subsequent transcriptomic studies have also identified EPSTI1 as a candidate biomarker (48,94,95). A 2026 study confirmed increased PBMC expression in an additional patient cohort, with its single-cell analysis identifying monocytes and dendritic cells as the predominant EPSTI1-expressing populations. These findings extend the cellular context of EPSTI1-associated expression beyond B cells (96).

Comparative interpretation of autoimmune evidence. Rheumatoid arthritis (RA) combines interferon-associated immune dysregulation with synovial inflammation and bone erosion (97-100). A study of early RA identified increased EPSTI1 expression in B cells from patients with a high interferon signature, together with reduced DNA methylation at associated loci. Increased EPSTI1 expression was also confirmed in PBMCs from an independent cohort, supporting its association with an interferon-active state in patients (101).

Other experimental studies have demonstrated EPSTI1-dependent osteoclast differentiation and bone-resorption phenotypes in RAW264.7 cells (20), as well as increased monocyte adhesion following upregulation of endothelial vascular cellular adhesion molecule 1 and intercellular adhesion molecule 1 (40,102). These findings provide biological context for inflammatory bone loss and leukocyte recruitment,

but disease-specific studies are needed to establish their contribution to RA synovitis and joint erosion.

The functional studies identify distinct cellular responses: CpG-responsive B-cell activation in pSS and IFN-β-induced HLA-A expression and chemokine production in myoblasts studied in relation to DM. SLE studies primarily support an interferon-associated biomarker interpretation (12,21,22). These differences reflect the cell types, stimuli and endpoints examined; they do not establish a mechanism shared across autoimmune diseases.

Additional patient cohorts strengthen the expression evidence, whereas the specific B-cell and myoblast mechanisms remain supported largely by individual functional studies. Confirmation of expression is therefore distinct from independent replication of an EPSTI1-dependent cellular effect. Reanalysis of shared public datasets and computational 'virtual knockout' analyses likewise do not constitute independent experimental validation (96). Independent perturbation and rescue studies linked to disease-relevant immune responses or tissue injury would strengthen the interpretation of EPSTI1 as a pathogenic effector.

7. EPSTI1 in tissue repair and musculoskeletal remodeling

Skeletal muscle regeneration and VCP/p97-associated STAT1 clearance. Skeletal muscle repair requires coordination between inflammatory signaling and myogenic differentiation. The effects of IFN-γ depend on the responding cell population and stage of repair, as illustrated by studies of endogenous IFN-γ and its cooperation with TNF-α during macrophage transitions (103-105). Whole-body *Epsti1*-knockout mice showed impaired regeneration after muscle injury, delayed myofiber maturation and increased inflammatory gene expression (14). The complementary experiments in C2C12 mouse myoblasts supporting VCP-associated clearance of activated STAT1 are discussed in Section 4.

These findings support VCP-dependent proteasomal clearance of activated STAT1 as a cellular mechanism associated with myogenic progression. The animal experiments establish a contribution of *Epsti1* to regeneration, while the myoblast experiments help explain its regulation of STAT1 signaling.

Protein kinase R (PKR)/NF-κB-associated osteoclastogenesis and bone resorption. NF-κB regulates osteoclast differentiation, and independent studies have implicated PKR in inflammatory osteoclast formation and bone destruction (106-108). These studies provide the signaling context for interpreting the EPSTI1-specific findings.

In RAW264.7 cells, EPSTI1 knockdown reduced osteoclast differentiation and resorptive activity, with pathway analyses implicating PKR phosphorylation and NF-κB signaling. Increased EPSTI1 expression was also observed in acid phosphatase 5-positive osteoclasts in femoral sections from patients with osteoporosis. The cell experiments support a functional contribution to osteoclastogenesis, while the patient samples provide complementary evidence of expression and pathway associations (20).

A subsequent study incorporated EPSTI1 small interfering (si)RNA into a composite hydrogel containing RADA16 (a 16-amino-acid self-assembling peptide) and gelatin methacrylate and reported reduced bone resorption and improved repair of femoral defects in ovariectomized rats, extending the evidence to a local bone-repair intervention. Because the formulation also contains a component intended to promote bone formation, its overall repair benefit cannot be attributed exclusively to EPSTI1 silencing (109).

Mechanistic comparison across muscle and bone models. The effects of EPSTI1 depletion differ across three musculoskeletal models. In mouse myogenic cells, depletion increased activated STAT1 abundance and impaired differentiation; whole-body deficiency also impaired regeneration (14). In RAW264.7 osteoclast models, knockdown reduced differentiation and resorption, with PKR/NF-κB signaling implicated (20). In hBMSCs, a METTL14/YTHDF3 study found that EPSTI1 knockdown enhanced osteogenic differentiation, whereas EPSTI1 overexpression inhibited it (44).

The bone-cell findings suggest complementary directions for investigating EPSTI1 in skeletal remodeling, as its depletion reduced osteoclast resorption in one model and enhanced osteogenic differentiation in another. By contrast, *Epsti1* deficiency impaired myogenic progression. These distinct effects highlight the importance of tissue and cellular context. The involvement of VCP/STAT1 signaling in myogenic cells, PKR/NF-κB signaling in osteoclasts and METTL14/YTHDF3-associated regulation in hBMSCs provides several mechanistic entry points for understanding EPSTI1 function across musculoskeletal tissues (14,20,44).

8. Additional EPSTI1-associated contexts

Expression-based observations in neural and metabolic settings. In Alzheimer's disease, *EPSTI1* was part of a female-biased, interferon-associated transcriptional signature in postmortem brain tissue. Related expression changes were observed in the hippocampi of female amyloid precursor protein/presenilin 1 double-transgenic mice and in mouse microglia following innate immune stimulation (110). Analysis of human adipose transcriptomes identified *EPSTI1* as a candidate component of an interferon-enriched network inversely associated with insulin sensitivity in the Caucasian subgroup analyzed in the original study (111). These expression-based observations extend the association of EPSTI1 with interferon-related transcriptional programs to neural and metabolic settings and identify additional biological contexts for functional investigation.

Antiviral responses and inflammation induced by microbial stimuli. Infection-related studies provide functional evidence in selected experimental systems. A hepatitis C study implicated EPSTI1 in IL-28A/IFN-λ2-associated antiviral activity in hepatoma-derived cell models (16). An independent overexpression study subsequently reported an inhibitory effect in a Sindbis virus model, but no comparable effect in an HIV-1 reporter assay. These findings support antiviral activity under specific experimental conditions rather than a uniform effect across viruses (112).

In a separate study, EPSTI1 silencing reduced apoptosis and inflammatory cytokine production and lowered the p-p65/p65 and p-IκBα/IκBα ratios in LPS-treated WI-38 lung fibroblasts.

These experiments assessed inflammatory injury induced by a bacterial component, rather than pathogen infection or clearance (113). The inflammatory and antiviral findings therefore address distinct biological outcomes.

9. Biological and translational implications of EPSTI1

Biomarker associations and clinical utility. EPSTI1 has been investigated as a marker of interferon-associated disease states and immune context (21-23,114). A 2026 study of Sjögren's disease reported areas under the receiver operating characteristic curve (AUCs) of 0.871 and 0.874 for a 14-gene model in two external datasets, alongside a separately reported AUC of 0.844 for *EPSTI1* alone (96). These values describe different models and are not interchangeable. The two external datasets also differed in tissue source: Whole blood in GSE84844 and parotid tissue in GSE40611. Their performance therefore does not constitute equivalent validation of a blood-based diagnostic test (96). These findings support the potential diagnostic relevance of EPSTI1 in Sjögren's disease and provide a basis for further comparison with clinical features, serological markers and established interferon signatures, including evaluation across other interferon-active diseases.

Established multigene signatures offer useful benchmarks for clinical utility. A predefined four-gene signature comprising *IFI27, IFI44, IFI44L* and radical S-adenosyl methionine domain containing 2 was used for stratification and treatment-response analyses in randomized anifrolumab trials (115). In a phase II randomized trial of RSLV-132 in pSS, a three-gene IFN signature comprising HECT and RLD domain containing E3 ubiquitin protein ligase 5, cytidine/uridine monophosphate kinase 2 and *EPSTI1* was used to identify IFN-positive patients for enrollment (116). This provides an example of an EPSTI1-containing signature used for patient selection, although the trial did not separately establish the incremental value of EPSTI1 or its ability to predict treatment benefit. Guidance from the European Alliance of Associations for Rheumatology also emphasizes that interferon assays measure different aspects of pathway activity and lack a universal reference standard (117). Meaningful comparisons therefore require the same clinical endpoint and comparable samples and populations; AUCs from different studies cannot establish superiority. When EPSTI1 is included in a signature, its incremental contribution should be tested by comparing otherwise equivalent models with and without the gene. Agreement between analytical platforms is distinct from added clinical value (118).

The belimumab score discussed in Section 6 is based on expression changes after treatment initiation (89), whereas the colorectal dataset in Section 5 did not distinguish response groups on the basis of pretreatment epithelial EPSTI1 expression (32). These examples distinguish on-treatment monitoring from baseline treatment selection. Establishing treatment-specific benefit also requires an appropriate treatment comparison; an association within a treated cohort alone is insufficient.

Preclinical modulation of EPSTI1. Studies involving EPSTI1 perturbation in colorectal cancer cells and local siRNA delivery in bone-repair models provide an experimental basis for investigating EPSTI1-directed interventions (32,109). These approaches demonstrate that modulation of EPSTI1 expression can influence cellular phenotypes and contribute to the evaluation of therapeutic strategies in selected preclinical settings.

The development of EPSTI1-selective pharmacological approaches remains an important research direction. An *in silico* study evaluated paeoniflorin as a potential therapeutic candidate in Sjögren's disease, including molecular docking with EPSTI1 and other candidate targets (48). Separately, inhibition of upstream JAK signaling was shown to reduce EPSTI1 expression in colorectal cancer cells (32). These findings provide different starting points for therapeutic investigation, with direct target engagement, selectivity and efficacy remaining important priorities for future studies.

VCP/p97 inhibition: Selectivity and toxicity. Broad VCP/p97 inhibition differs from selective targeting of EPSTI1. The development of VCP/p97 inhibitors illustrates the need to distinguish compound-specific liabilities from the consequences of inhibiting a protein involved in multiple proteostatic pathways (18,53,119). The clinical development of CB-5083 was halted following visual adverse effects, including photophobia and dyschromatopsia. Subsequent biochemical and retinal electrophysiology studies identified potent, reversible inhibition of phosphodiesterase-6 (PDE6), supporting an off-target explanation for these symptoms (120). In preclinical comparisons, CB-5339 retained VCP inhibitory potency while showing less PDE6 inhibition and lower retinal exposure (121). These improvements address a compound-specific liability, but do not confer selectivity for EPSTI1-associated complexes.

In a comparative oncology study in tumor-bearing dogs, CB-5339 was associated predominantly with gastrointestinal adverse effects, with severe liver enzyme abnormalities also reported (122). These animal findings do not establish human tolerability. Because VCP inhibition affects multiple proteostatic pathways, its efficacy and toxicity cannot be attributed specifically to EPSTI1.

Physiological functions and tissue-specific considerations. The muscle and bone findings discussed in Section 7 illustrate the different consequences of constitutive whole-body deficiency and local RNA delivery (14,109). These findings highlight the importance of tissue-specific strategies that preserve physiological immune and regenerative functions when developing EPSTI1-directed interventions.

Table II brings together the remaining questions about mechanism, added clinical value and selective intervention.

10. Conclusions

EPSTI1 has emerged as more than an interferon-responsive marker in selected experimental systems. Functional studies demonstrate its involvement in inflammatory signaling, immune-cell activation, cancer-cell behavior and tissue remodeling. In particular, findings from breast cancer and myogenic models have linked EPSTI1 to VCP/p97-associated signaling processes, including changes in IκBα abundance and the clearance of activated STAT1. Clinical investigations further associate EPSTI1 with interferon-driven diseases and cancer-related outcomes. Causal evidence in human

disease remains limited, and the contribution of EPSTI1 to VCP/p97 substrate recruitment and specificity requires further molecular characterization. Its clinical applications remain investigational, with added biomarker value and the efficacy and safety of EPSTI1-directed interventions requiring validation. Together, these findings provide a foundation for investigating how interferon-responsive expression relates to protein regulation and cellular behavior.

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Authors' contributions

LB and WY conceived and supervised the review. SL, RL and PH conducted the literature search, synthesized the evidence, developed the conceptual framework, designed the figures and tables, and drafted and revised the manuscript. XL and AD contributed to figure preparation and critically revised the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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