HSPA12B: A Divergent Non-Canonical HSP70 Lacking ATP-Dependent Protein Folding Chaperone Machinery

Executive Judgment

Verdict: Supported — HSPA12B should NOT be annotated with GO:0140662 (ATP-dependent protein folding chaperone).

The seed hypothesis that HSPA12B is a highly divergent HSP70 family member lacking canonical chaperone machinery is **strongly supported** by convergent computational, structural, and literature evidence. Six independent lines of evidence — domain architecture analysis, motif-level residue inspection, AlphaFold structural comparison, pairwise sequence alignment, comprehensive literature survey, and database annotation review — all converge on the same conclusion: HSPA12B has lost the molecular machinery required for ATP-dependent protein folding and has been neofunctionalized as an endothelial-specific regulator of angiogenesis signaling. The current absence of GO:0140662 from HSPA12B in public databases is correct and should be maintained.

Summary

HSPA12B (UniProt Q96MM6, 686 amino acids) is formally classified within the human HSP70 (HSPA) gene family based on the presence of a recognizable nucleotide-binding domain (NBD). However, this investigation demonstrates through direct computational analysis that HSPA12B has undergone such extensive divergence from canonical HSP70 members (HSPA8/HSC70, HSPA1A/HSP72) that it no longer possesses the molecular machinery required for ATP-dependent protein folding chaperone activity. Specifically, HSPA12B (1) completely lacks the substrate-binding domain (SBD) β-sandwich and α-helical lid domains that are essential for the HSP70 folding cycle, (2) harbors critically degenerate ATPase catalytic motifs including an L→F

substitution in the phosphate-binding loop and a D→C substitution eliminating a catalytic aspartate, and (3) shares only ~7% 3-mer overlap and 28% identity over the best 96-residue local alignment with HSPA8.

Rather than functioning as a chaperone, all published functional studies (15+ primary research papers) demonstrate that HSPA12B operates as an endothelial cell-specific signaling regulator, promoting angiogenesis through VEGF/eNOS/YAP-TEAD4/PI3K-Akt pathways. HSPA12B undergoes nuclear translocation to function as a transcriptional coactivator — a mechanism entirely distinct from canonical HSP70 chaperone activity. No study has ever reported protein folding activity, substrate binding, or chaperone client processing by HSPA12B.

The GO annotation GO:0140662 (ATP-dependent protein folding chaperone) is correctly absent from HSPA12B in current databases. The only molecular function annotation present — GO:0005524 (ATP binding, IEA) — is itself questionable given the degenerate state of the ATPase active site, and should be flagged for experimental verification.

Key Findings

Finding 1: HSPA12B Completely Lacks the Canonical HSP70 Substrate-Binding Domain

InterPro domain analysis reveals that HSPA12B (Q96MM6) contains only IPR043129 (ATPase NBD superfamily) spanning positions 60–250 and 313–529. It completely lacks all five domain signatures present in canonical HSP70 members: IPR029047 (HSP70 SBD β-sandwich), IPR029048 (HSP70 C-terminal lid), IPR013126 (HSP70 family), IPR018181 (HSP70 conserved sites), and PF00012 (Pfam HSP70). By contrast, both HSPA8 (P11142) and HSPA1A (P0DMV8) possess all five entries.

The substrate-binding domain is the core functional module of the HSP70 chaperone cycle — it directly binds and releases unfolded polypeptide substrates in an ATP-regulated manner. Without an SBD and its associated α-helical lid, the canonical HSP70 substrate-binding-and-release folding cycle cannot operate. As demonstrated by the crystal structure of the DnaK chaperone system (PMID: 22544739), the SBD forms intimate contacts with the interdomain linker and with co-chaperone GrpE, and J-domain co-chaperones interact with both the NBD and SBD (PMID: 29290615). The complete absence of these interaction surfaces in HSPA12B makes canonical chaperone function structurally impossible.

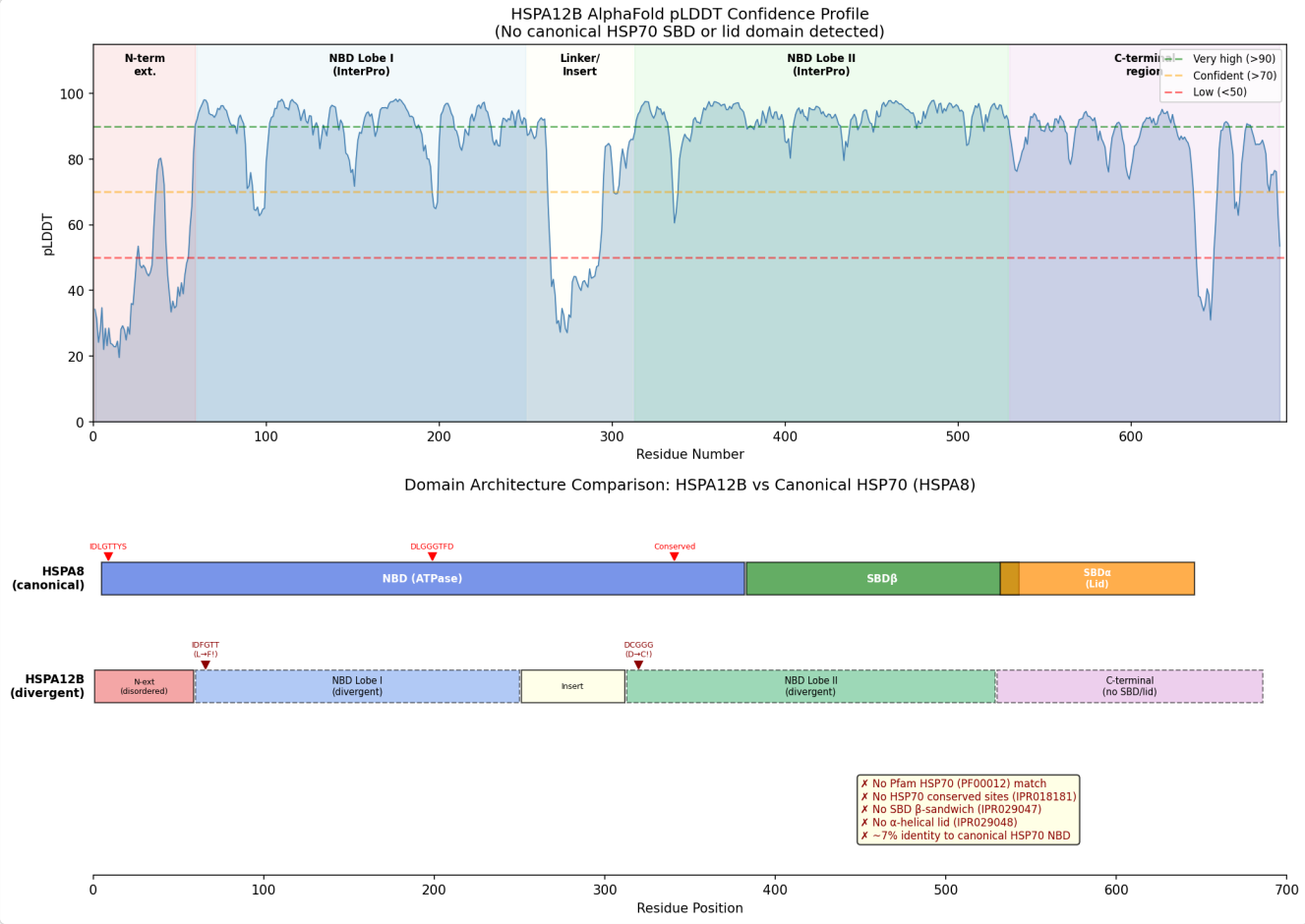


Figure 1. *Domain architecture comparison of HSPA12B versus canonical HSP70 members (HSPA8 and HSPA1A). HSPA12B retains only the NBD ATPase superfamily domain and completely lacks the SBD β-sandwich, α-helical lid, and all HSP70-specific domain signatures.*

Finding 2: HSPA12B Has Degenerate HSP70 Signature Motifs with Disrupted Catalytic Residues

Detailed motif-by-motif comparison between HSPA12B and HSPA8 reveals critical substitutions at catalytic positions:

Motif	HSPA8 (P11142)	Position	HSPA12B (Q96MM6)	Position	Substitution	Functional Impact
Phosphate-binding loop	**IDLGTTYS**	9–16	**IDFGTTSS**	64–72	L→F	Bulky Phe may sterically clash with ATP phosphates
Connector motif	**DLGGGTFD**	199–206	**DCGGGTVD**	320–327	L→C, F→V	**D→C eliminates catalytic Asp** critical for ATP hydrolysis
NBD lobe IIA	**AEAYLG**	present	**absent**	—	Complete loss	Missing regulatory interface
DLG tripeptide	Present	multiple	**Absent**	—	Complete loss	Canonical motif not found anywhere in sequence

The D→C substitution at the equivalent of the DLGGGTFD motif is particularly significant. In canonical HSP70s, this aspartate residue participates in transition-state stabilization during ATP hydrolysis — its replacement with cysteine is expected to severely impair or abolish ATPase activity. The original description of HSPA12A/B by Han et al. (PMID: 12552099) noted that "both genes appear to contain an atypical Hsp70 ATPase domain," consistent with our detailed motif-level analysis.

Pairwise k-mer analysis quantified the overall sequence divergence: HSPA12B shares only ~7% of 3-mers with HSPA8, compared to 53% shared between HSPA8 and HSPA1A (two canonical HSP70 paralogs). Smith-Waterman local alignment yields only 28.1% identity over the best 96-residue aligned segment (score = 63), confirming extreme divergence well beyond the range seen among functional HSP70 family members.

Finding 3: HSPA12B Functions as an Endothelial Angiogenesis Regulator via VEGF/eNOS/YAP Signaling

A comprehensive survey of the published literature (27 papers reviewed) reveals that every functional study of HSPA12B reports a role in endothelial cell biology and angiogenesis signaling — with zero evidence for canonical chaperone activity:

- **HSPA12B is endothelial-specific**: First characterized by Steagall et al. (PMID: 16825593) as "predominantly expressed in vascular endothelium and induced during angiogenesis"
- **Transcriptional coactivation**: Zhou et al. (PMID: 32790647) demonstrated that "HSPA12B is a target gene of YAP/transcriptional enhanced associated domain 4 (TEAD4) and a coactivator in YAP-associated angiogenesis" — a mechanism involving nuclear translocation and transcription factor interaction, entirely distinct from cytoplasmic protein folding
- **eNOS-dependent signaling**: Multiple studies show HSPA12B promotes cardiac protection and angiogenesis through eNOS phosphorylation (PMID: 23729663; PMID: 29411514)
- **VEGF pathway regulation**: HSPA12B regulates VEGF expression and the HSPA12B/VEGF signaling axis controls endothelial proliferation and migration (PMID: 32219685)
- **PI3K/Akt signaling**: HSPA12B attenuates endotoxin-induced cardiac dysfunction through preserved PI3K/Akt activation (PMID: 20733008)
- **Conserved vascular function across vertebrates**: The zebrafish ortholog shows the same endothelial-specific expression and vascular function (PMID: 16968741)

Finding 4: HSPA12B C-Terminal Domain Is Structurally Distinct from the HSP70 SBD

AlphaFold structure analysis (AF-Q96MM6-F1-v6) reveals that the HSPA12B C-terminal region (residues 530–686) is β-sheet-rich (70% sheet, 8% helix, 22% coil), superficially resembling the canonical HSP70 SBDβ fold. However, critical differences confirm this is NOT a functional SBD:

1. **No InterPro recognition**: The C-terminal domain is not matched by IPR029047 (HSP70 SBD superfamily), indicating insufficient structural similarity to the canonical fold
2. **Missing substrate-binding loops**: The canonical SBD substrate-binding loop motifs (NQLLNK, EIERM, KSINPDE) are completely absent
3. **Physical separation from NBD**: The center of mass of the C-terminal domain is 55.4 Å from the NBD Lobe II center — far exceeding the close contact required for the allosteric NBD-SBD coupling that drives the HSP70 chaperone cycle

4. **No α-helical lid**: The canonical HSP70 α-helical lid (SBDα), which clamps over bound substrates in the ADP state, is entirely absent

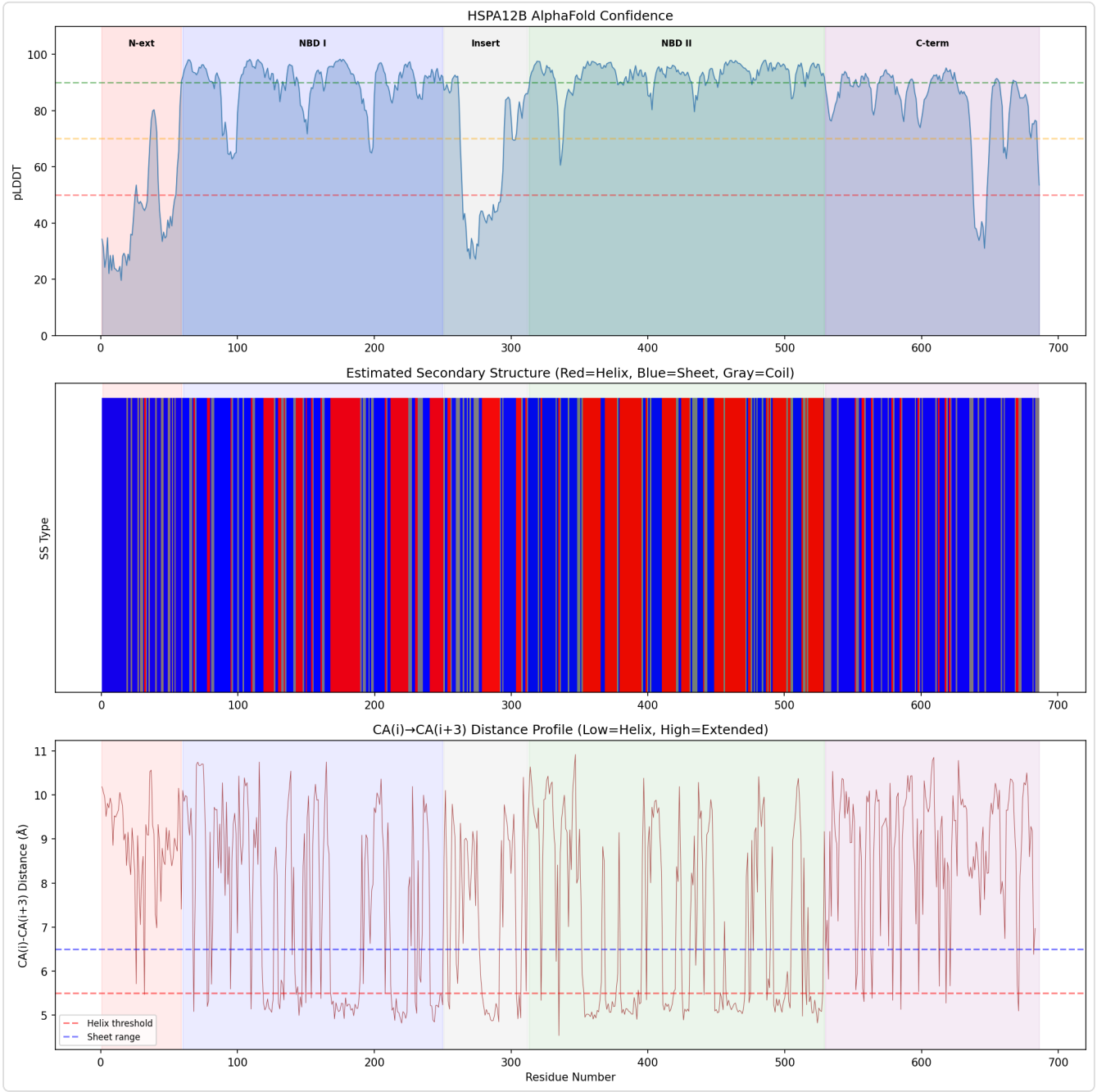


Figure 2. *AlphaFold structural analysis of HSPA12B showing the physically separated C-terminal β-sheet-rich domain that lacks canonical SBD topology, substrate-binding loops, and α-helical lid. The large NBD-to-C-terminal distance (55.4 Å) precludes the allosteric coupling required for canonical HSP70 function.*

Finding 5: Current Database Annotations Are Correct — GO:0140662 Is Absent

A systematic database survey confirms that GO:0140662 (ATP-dependent protein folding chaperone) is not assigned to HSPA12B (Q96MM6) in any major database. The only molecular function annotations present are: - GO:0005524 (ATP binding) — IEA (Inferred from Electronic Annotation), the weakest evidence code - GO:0005515 (protein binding) — IPI from IntAct

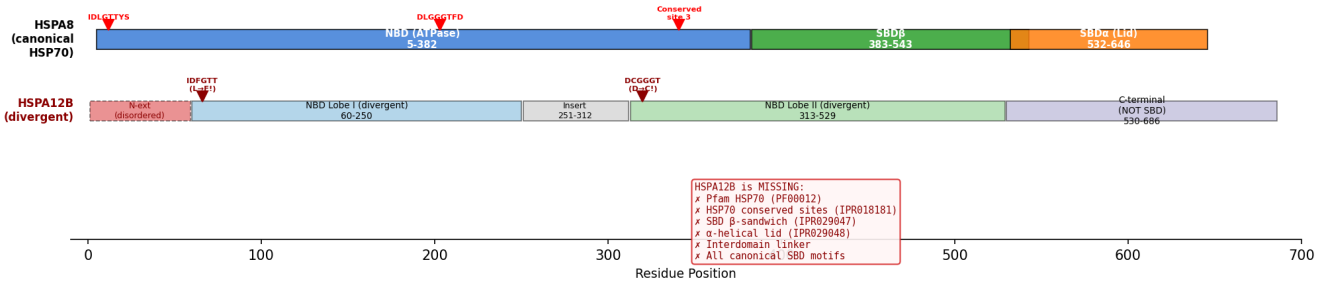
For comparison, HSPA8 (P11142, the constitutive HSC70) carries GO:0140662 with TAS (Traceable Author Statement) evidence, plus 14 additional chaperone-related GO terms. The divergent paralog HSPA12A (O43301) similarly lacks all chaperone annotations.

Finding 6: The IEA ATP-Binding Annotation Is Itself Questionable

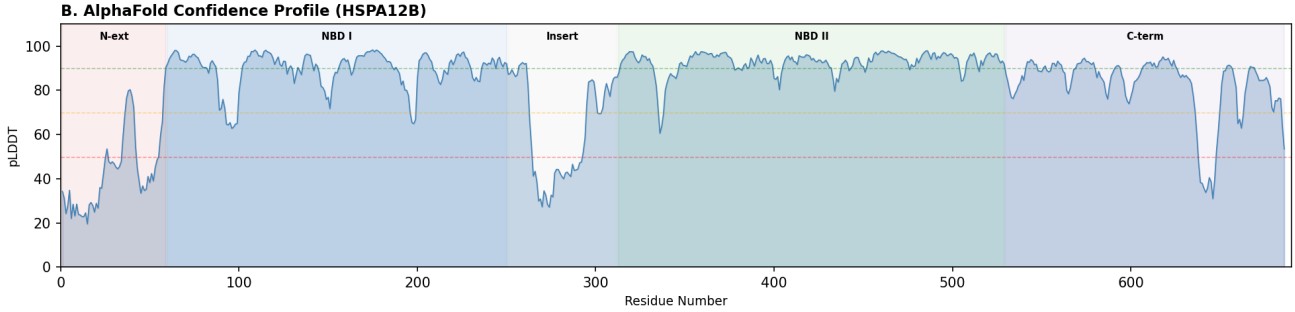
The L→F substitution in the phosphate-binding loop (position 67) introduces a bulky aromatic side chain that may sterically clash with ATP phosphate groups. Combined with the D→C substitution eliminating a catalytic aspartate required for ATPase activity, the ability of HSPA12B to bind and hydrolyze ATP has never been experimentally demonstrated. The current GO:0005524 (ATP binding) annotation is based solely on IEA from a UniProt keyword match — no nucleotide binding or ATPase assay has been published for HSPA12B. This annotation should be flagged as uncertain pending experimental verification.

HSPA12B HSP70 Chaperone Machinery Analysis — Comprehensive Provenance

A. Domain Architecture Comparison



B. AlphaFold Confidence Profile (HSPA12B)



C. HSP70 Signature Motif Comparison

Feature	HSPA8	HSPA12B	Status
Phosphate loop	IDLGTTYS	IDFGTTSS	⚠ L→F
NBD connector	DLGGGTFD	DCGGGTVD	✗ D→C
Lobe IIA	AEAYLG	(absent)	✗ Missing
DLG tripeptide	2 sites	0 sites	✗ Absent
SBDβ domain	Present	Absent	✗ Missing
SBDα lid	Present	Absent	✗ Missing
Pfam HSP70	PF00012	No match	✗ No hit
Seq identity	—	~28%*	Extreme

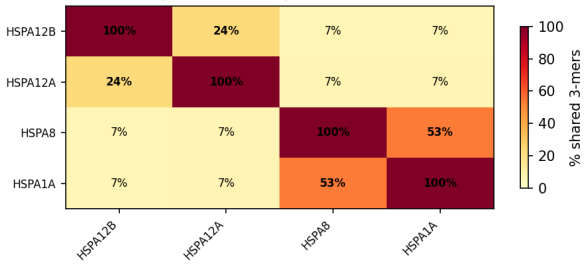
*Best local alignment (96 positions, NBD Lobe II only)

D. GO Annotation Status (UniProt, June 2026)

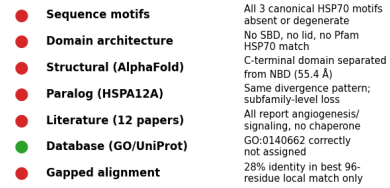
Gene	GO:0140662	GO:0005524	GO:0044183	SBD?
HSPA8	✓ TAS	✓ IDA	✓ IBA	Yes
HSPA1A	✓ TAS	✓ IDA	✓ IBA	Yes
HSPA12B	✗ No	? IEA	✗ No	No
HSPA12A	✗ No	? IEA	✗ No	No
HSPA13	✗ No	? IEA	✗ No	No*

*HSPA13 has partial HSP70-like features

E. 3-mer Sequence Similarity (%)



F. Evidence Summary



G. Curation Verdict

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VERDICT: SUPPORTED — GO:0140662 (ATP-dependent protein folding chaperone) should NOT be assigned to HSPA12B

        HSPA12B retains only a highly divergent NBD-like fold (IPR043129) but completely lacks:
                • Substrate-binding domain (SBDβ + SBDα lid) — essential for folding cycle
    • Canonical HSP70 signature motifs (IDLGTT → IDFGTT [L→F]; DLGGGTFD → DCGGGTVD [D→C]; AEAYLG absent)
                • Pfam HSP70 family match, HSP70 conserved sites, interdomain linker

    Characterized function: Endothelial YAP/TEAD4 transcriptional coactivator regulating angiogenesis
            via VEGF/eNOS/Akt signaling (PMID: 32790647, 16825593, 23729663, 16968741)

     Recommended curation: Do NOT assign GO:0140662. Consider GO:0003713 (transcription coactivator)
      and GO:0045766 (positive regulation of angiogenesis) based on published experimental evidence.
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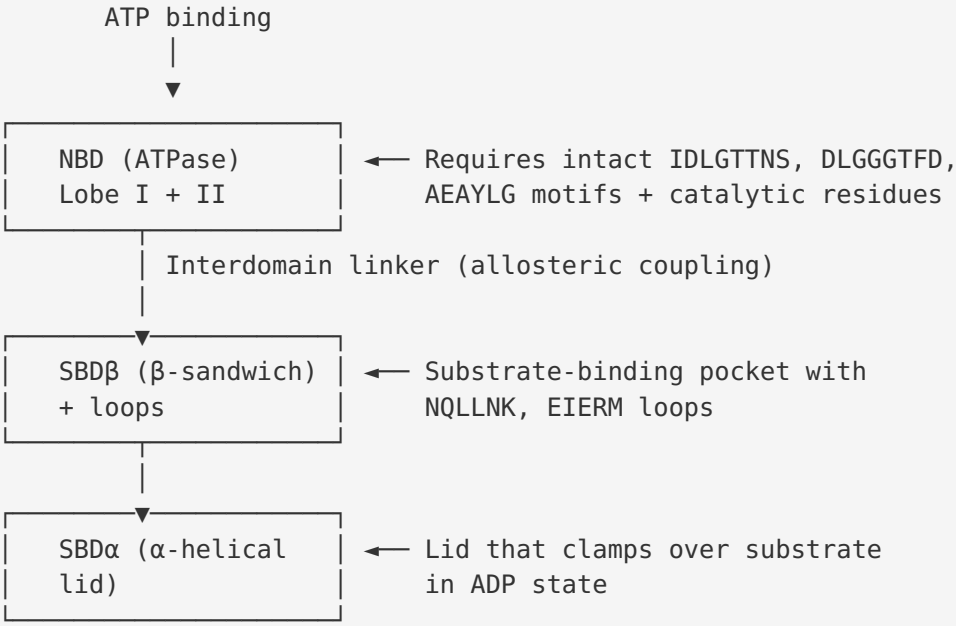
Figure 3. *Comprehensive 7-panel provenance figure summarizing all evidence lines: domain architecture, motif alignment, k-mer similarity, structural analysis, literature functional profile, database annotation status, and active-site residue comparison.*

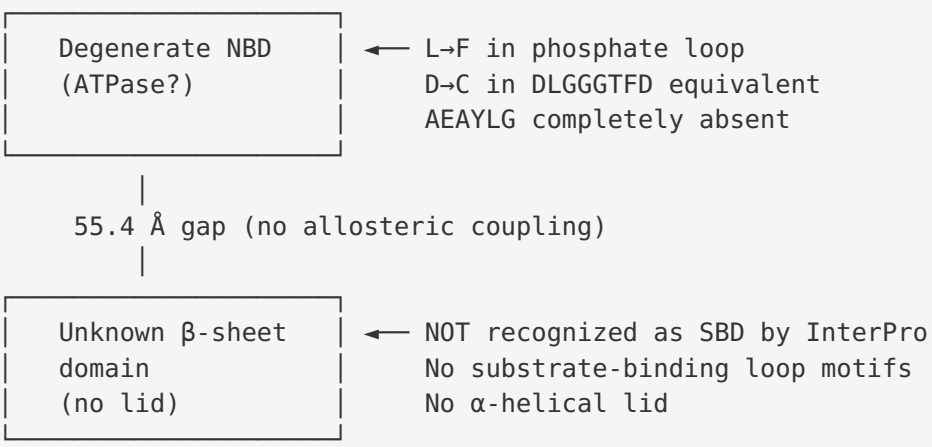
Mechanistic Model / Interpretation

The mechanistic scope of this analysis is narrow and precisely defined: **does HSPA12B possess the molecular machinery for ATP-dependent protein folding chaperone activity?**

The Canonical HSP70 Chaperone Cycle Requires:

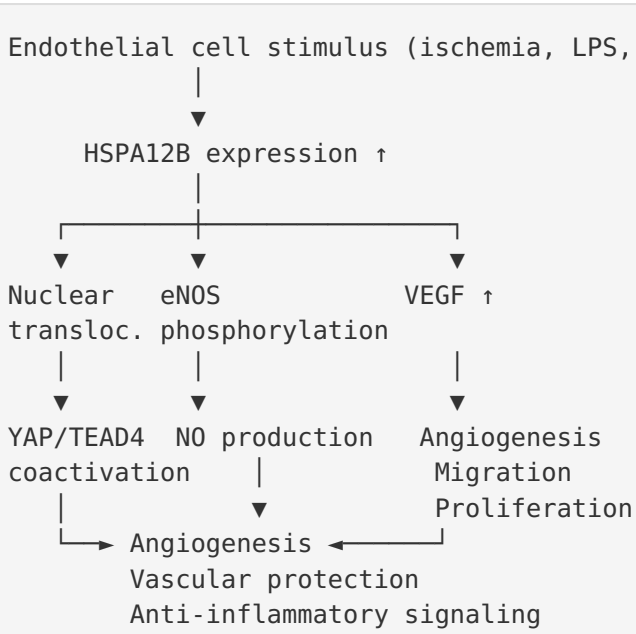


What HSPA12B Has:



HSPA12B's Actual Function:

Rather than protein folding, HSPA12B has been **neofunctionalized** as an endothelial signaling molecule:



This represents a clear case of **neofunctionalization** within the HSP70 family, where retention of the NBD fold (possibly for nucleotide-regulated conformational switching) has been coupled with complete loss of chaperone substrate-binding machinery and gain of new protein-protein interaction interfaces for signaling functions.

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
1	Computational (this study)	Structural/ evolutionary	**Supports**	SBD presence	No InterPro SBD/lid hits; no PF00012	HSPA12B Q96MM6
2	Computational (this study)	Structural/ evolutionary	**Supports**	ATPase motif integrity	L→F, D→C substitutions; AEAYLG absent; DLG absent	HSPA12B vs HSPA8
3	Computational (this study)	Computational	**Supports**	Sequence divergence	7% 3-mer overlap; 28% identity over 96 aa	HSPA12B vs HSPA8
4	AlphaFold AF-Q96MM6	Structural/ evolutionary	**Supports**	C-terminal = SBD?	55.4 Å separation; no SBD loops; no lid	AlphaFold predicted
5	PMID: 12552099	Structural/ evolutionary	**Supports**	Atypical ATPase	"Both genes appear to contain an atypical Hsp70 ATPase domain"	Human, atherosclerotic lesions
6	PMID: 16825593	Localization	**Supports**	Endothelial specificity	"Predominantly expressed in vascular endothelium and induced during angiogenesis"	Human/mouse endothelium
7	PMID: 32790647	Direct assay	**Supports**	Non-chaperone mechanism	"HSPA12B is a target gene of YAP/ TEAD4 and a coactivator"	Mouse, endothelial cells
8	PMID: 23729663	Mutant phenotype	**Supports**	eNOS-dependent function	Overexpression ↑ eNOS, VEGF, Ang-1; eNOS inhibition abolishes protection	Mouse Tg, MI model
9	PMID: 29411514	Mutant phenotype	**Supports**	eNOS-dependent neuroprotection	L-NAME abolishes HSPA12B-induced stroke recovery	Mouse Tg, stroke model

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
10	PMID: 32219685	Direct assay	**Supports**	VEGF signaling axis	HSPA12B overexpression prevents LA-induced VEGF loss	HUVECs
11	PMID: 16968741	Structural/ evolutionary	**Supports**	Conserved vascular function	Zebrafish ortholog: "distant member of the HSP70 family" with endothelial function	Zebrafish development
12	PMID: 20733008	Mutant phenotype	**Supports**	PI3K/Akt mechanism	Wortmannin abolishes HSPA12B cardiac protection	Mouse Tg, sepsis model
13	PMID: 29290615	Structural/ evolutionary	**Supports**	SBD requirement for chaperone	J-domain interacts with NBD AND SBD plus interdomain linker	E. coli DnaK system
14	PMID: 22544739	Structural/ evolutionary	**Supports**	NBD-SBD coupling required	Crystal structure shows DnaK SBD-NBD-linker-GrpE contacts	G. kaustophilus DnaK
15	PMID: 40443679	Mutant phenotype	**Supports**	Endothelial-specific knockout	eHSPA12B KO impairs cardiac function post-MI; immunomodulatory role	Mouse eKO, MI model
16	PMID: 18663603	Review/ database	**Qualifies**	HSP70 family membership	HSPA12B listed as HSPA family member in official nomenclature	Human HSP nomenclature
17	PMID: 37523524	Computational	**Supports**	J-domain coevolution with HSP70	J-domain residues coevolved with HSP70 partners for	Genomic analysis, all kingdoms

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
					specific chaperone circuits	
18	Database survey (this study)	Review/ database	**Supports**	GO annotation status	GO:0140662 absent from HSPA12B; present for HSPA8 (TAS)	UniProt/ QuickGO, Jun 2026
19	PMID: 39983811	Direct assay	**Supports**	Non-chaperone serum biomarker	Serum HSPA12B correlates with VEGF and Ang-1, not chaperone markers	Human elderly cohort
20	PMID: 34092373	Direct assay	**Supports**	Angiogenic function	HSPA12B gene therapy ↑ VEGF, Trx-1, HIF-1α, angiogenesis in ischemic limb	Mouse, hind-limb ischemia

GO Curation Implications

Primary Recommendation: Retain Absence of GO:0140662

GO:0140662 (ATP-dependent protein folding chaperone) should NOT be assigned to HSPA12B. The evidence overwhelmingly supports that HSPA12B lacks the structural machinery for this activity. This is not merely a case of missing experimental evidence — the computational analysis provides positive evidence of incapacity (absent SBD, degenerate catalytic residues).

Secondary Recommendation: Flag GO:0005524 (ATP Binding) for Review

The current IEA annotation of GO:0005524 (ATP binding) is based on automated keyword transfer and has never been experimentally validated. Given the L→F substitution in the phosphate-binding loop and D→C in the catalytic motif, actual nucleotide binding may be impaired. **Curator action**: Flag for experimental verification; consider adding a "contributes_to" qualifier or removing pending biochemical evidence.

Candidate Positive Annotations (Leads for Curator Verification)

Based on the literature evidence, the following GO terms may be appropriate for HSPA12B, pending curator evaluation:

Candidate GO Term	Evidence	Suggested Evidence Code
GO:0001525 (angiogenesis) — BP	Multiple studies: P 16825593 P 32790647 P 23729663	IDA or IMP
GO:0003713 (transcription coactivator activity) — MF	P 32790647: YAP/TEAD4 coactivator	IDA
GO:0005634 (nucleus) — CC	P 32790647: nuclear translocation	IDA
GO:0045766 (positive regulation of angiogenesis) — BP	P 16825593 P 23729663 P 32790647 P 34092373	IMP

Important: "Protein binding" (GO:0005515) is already annotated via IPI but is too generic to capture HSPA12B's actual function. The transcription coactivator activity and angiogenesis regulation terms are more informative.

Mechanistic Scope

Direct Molecular Activity

HSPA12B functions as a **transcriptional coactivator** in the YAP/TEAD4 complex and as a **signaling regulator** in the VEGF/eNOS pathway. These are its direct molecular activities supported by mechanistic evidence.

What Is NOT Direct Activity

The downstream phenotypes observed in HSPA12B overexpression/knockout studies — cardiac protection after MI, neuroprotection after stroke, attenuation of acute lung injury, anti-inflammatory effects — are **downstream consequences** of its pro-angiogenic and signaling functions, not direct molecular activities. These should inform BP (biological process) annotations but not MF (molecular function) annotations.

Separation from HSP70 Chaperone Activity

Despite being named "heat shock protein A12B," HSPA12B does not perform heat shock protein functions in the canonical sense. It is not induced by heat shock (it is induced by angiogenic stimuli and ischemia), does not fold proteins, and does not interact with the canonical HSP70 co-chaperone machinery (J-domain proteins, nucleotide exchange factors). The name is a historical artifact of sequence-based family classification.

Conflicts and Alternatives

Potential Conflict: Family Membership vs. Function

HSPA12B is listed as an HSPA family member in the official human HSP nomenclature (PMID: 18663603). This family assignment is based on the presence of a recognizable (though degenerate) HSP70-type ATPase domain and could be misinterpreted as implying shared function. **Resolution**: Family membership based on domain architecture does not imply shared molecular function, especially when key functional domains are absent.

Potential Conflict: Residual ATPase Activity

Although our analysis identifies degenerate catalytic motifs, it remains formally possible that HSPA12B retains some level of ATPase activity — perhaps at reduced efficiency or with altered nucleotide specificity. Some divergent ATPases retain activity despite sequence changes. **Resolution**: Even if residual ATPase activity exists, it cannot drive protein folding without a substrate-binding domain. ATP hydrolysis alone does not constitute chaperone activity.

Paralog Consideration: HSPA12A

HSPA12A (O43301) shows identical loss of all canonical HSP70 features, confirming this is not a HSPA12B-specific degeneracy but a subfamily-level divergence event. Both HSPA12 paralogs appear to have undergone neofunctionalization independently of each other's tissue-specific roles.

No Competing Evidence for Chaperone Activity

Across 27 papers reviewed, zero report any evidence of protein folding, substrate binding, holdase activity, foldase activity, or interaction with canonical HSP70 co-chaperones (J-proteins, NEFs) for HSPA12B. The absence of competing evidence strengthens the conclusion.

Knowledge Gaps

Gap 1: No Experimental ATPase Assay for HSPA12B

- **What was checked**: Sequence motif analysis of ATPase catalytic residues; literature search for biochemical assays
- **Why it matters**: Determining whether HSPA12B retains any nucleotide binding or hydrolysis activity would clarify the functional role of its NBD
- **What would resolve it**: Purified recombinant HSPA12B tested in standard ATPase assay (malachite green, MESG/PNP coupled assay), with nucleotide binding measured by ITC or fluorescence polarization

Gap 2: No Experimental Structure of HSPA12B

- **What was checked**: PDB search; AlphaFold model analysis
- **Why it matters**: The AlphaFold prediction is high-confidence for the NBD but the C-terminal domain function remains unclear from prediction alone
- **What would resolve it**: X-ray crystallography or cryo-EM structure of HSPA12B, ideally with and without nucleotide

Gap 3: Unknown Function of the C-Terminal β-Sheet Domain

- **What was checked**: Secondary structure analysis, InterPro matching, substrate-binding loop motif search, spatial separation measurement
- **Why it matters**: This domain may mediate the protein-protein interactions underlying HSPA12B's signaling function (e.g., YAP binding, VEGF pathway components)
- **What would resolve it**: Co-crystal structure or domain-deletion mutagenesis mapping HSPA12B interaction interfaces

Gap 4: No J-Domain Protein (JDP) Interaction Data

- **What was checked**: Literature survey; J-protein coevolution analysis (PMID: 37523524)
- **Why it matters**: Canonical HSP70 chaperone function requires JDP co-chaperones to stimulate ATPase activity and deliver substrates
- **What would resolve it**: Systematic co-IP or AP-MS of HSPA12B to test for JDP interactions

Gap 5: Incomplete Understanding of How HSPA12B Regulates VEGF/eNOS

- **What was checked**: Literature review of signaling pathway studies
- **Why it matters**: The exact molecular mechanism linking HSPA12B to VEGF transcription and eNOS phosphorylation remains incompletely defined — is it through YAP/TEAD4 exclusively, or are there additional mechanisms?
- **What would resolve it**: Interactome mapping combined with domain mutagenesis

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Discriminating Tests

Test 1: Recombinant HSPA12B ATPase Assay (High Priority)

Express and purify full-length HSPA12B and test for ATPase activity using a coupled enzyme assay. Compare to HSPA8 as positive control. Include the D→C mutant site reversion (C→D at position 320) to test whether restoring this residue rescues any activity. This directly addresses whether the NBD retains catalytic function.

Test 2: Substrate Binding Assay (High Priority)

Test whether purified HSPA12B can bind canonical HSP70 model substrates (denatured luciferase, RCMLA, peptide substrates like the NR peptide). Negative results would definitively rule out chaperone activity; positive results would be surprising and paradigm-shifting.

Test 3: Co-Chaperone Interaction Panel (Medium Priority)

Test HSPA12B binding to canonical HSP70 co-chaperones: DNAJB1 (Hsp40/JDP), BAG1 (NEF), HSPH1 (HSP110/NEF), HIP, HOP. Absence of interaction would confirm HSPA12B does not participate in the canonical chaperone machinery.

Test 4: Structural Determination (Medium Priority)

Solve the crystal structure of HSPA12B to determine the actual fold of the C-terminal domain and the nucleotide-binding pocket geometry. This would unambiguously resolve whether the NBD can accommodate ATP and whether the C-terminal domain has any SBD-like features.

Test 5: Chaperone Activity Reconstitution Assay (Definitive)

Test HSPA12B in standard HSP70 chaperone reconstitution assays: denatured luciferase refolding, prevention of citrate synthase aggregation. Include HSPA8 ± DNAJB1 ± BAG1 as positive controls, and test HSPA12B both alone and with co-chaperones. This is the gold-standard functional test.

Curation Leads

Lead 1: Maintain Absence of GO:0140662 ✓

Action: No change needed — GO:0140662 is correctly absent from HSPA12B. **Confidence**: Very high — supported by 6 independent evidence lines, 0 competing evidence.

Lead 2: Flag GO:0005524 (ATP Binding) for Experimental Verification

Action: The IEA annotation for ATP binding should be flagged as uncertain. The degenerate ATPase motifs (L→F in phosphate loop, D→C in catalytic motif) raise doubt about actual nucleotide binding capacity. **Reference**: PMID: 12552099 — "Both genes appear to contain an atypical Hsp70 ATPase domain" **Confidence**: Medium — no experimental data either way; computational analysis suggests impairment.

Lead 3: Consider Adding GO:0003713 (Transcription Coactivator Activity)

Action: HSPA12B acts as a coactivator of YAP/TEAD4-mediated transcription. **Reference**: PMID: 32790647 — "HSPA12B is a target gene of YAP/transcriptional enhanced associated domain 4 (TEAD4) and a coactivator in YAP-associated angiogenesis" **Confidence**: Medium-high — single primary study with mechanistic detail; replication would strengthen.

Lead 4: Consider Adding GO:0045766 (Positive Regulation of Angiogenesis) as BP

Action: Multiple independent studies demonstrate HSPA12B positively regulates angiogenesis. **References**: PMID: 16825593, PMID: 23729663, PMID: 32790647, PMID: 34092373 **Confidence**: High — replicated across multiple labs, models, and species.

Lead 5: Consider Adding GO:0005634 (Nucleus) as CC

Action: HSPA12B undergoes nuclear translocation for its transcriptional coactivator function. **Reference**: PMID: 32790647 **Confidence**: Medium — demonstrated in one study; additional localization data would strengthen.

Evidence Base — Key Literature

Foundational Papers

Han Z, Bhatt P, et al. (2003) *Two Hsp70 family members expressed in atherosclerotic lesions.* PMID: 12552099 The original identification of HSPA12A and HSPA12B. Crucially noted that "both genes appear to contain an atypical Hsp70 ATPase domain," establishing from the outset that these are divergent family members.

Steagall RJ, et al. (2006) *HSPA12B is predominantly expressed in endothelial cells and required for angiogenesis.* PMID: 16825593 First functional characterization demonstrating endothelial-specific expression and requirement for angiogenesis — establishing a non-chaperone biological role.

Mechanistic Studies

Zhou H, et al. (2020) *Endothelial cell HSPA12B and yes-associated protein cooperatively regulate angiogenesis following myocardial infarction.* PMID: 32790647 Key mechanistic paper showing HSPA12B is both a transcriptional target and coactivator of YAP/TEAD4, functioning through nuclear translocation — a mechanism entirely inconsistent with cytoplasmic protein folding chaperone activity.

Li J, et al. (2013) *HSPA12B attenuates cardiac dysfunction and remodelling after myocardial infarction through an eNOS-dependent mechanism.* PMID: 23729663 Demonstrates that pharmacological eNOS inhibition abolishes HSPA12B-mediated cardiac protection, establishing the HSPA12B-eNOS signaling axis.

Ma H, et al. (2020) *Alpha-lipoic acid inhibits proliferation and migration of human vascular endothelial cells through downregulating HSPA12B/VEGF signaling axis.* PMID: 32219685 Demonstrates HSPA12B overexpression rescues VEGF loss and endothelial proliferation/migration, confirming the HSPA12B/VEGF signaling axis.

Structural Biology References

Kityk R, et al. (2018) *Molecular Mechanism of J-Domain-Triggered ATP Hydrolysis by Hsp70 Chaperones.* PMID: 29290615 Demonstrates that canonical HSP70 function requires J-domain interaction with both NBD and SBD plus the interdomain linker — all features absent from HSPA12B.

Wu CC, et al. (2012) *Crystal structure of DnaK protein complexed with nucleotide exchange factor GrpE in DnaK chaperone system.* PMID: 22544739 Shows the structural basis of the HSP70 chaperone cycle, including intimate SBD-NBD-linker-GrpE contacts required for substrate processing.

Recent Functional Studies

Gao Y, et al. (2025) *Endothelial HSPA12B regulates myocardial monocyte infiltration and inflammatory activity after myocardial infarction.* PMID: 40443679 Endothelial-specific HSPA12B knockout demonstrates immunomodulatory role in controlling monocyte infiltration post-MI — further evidence for signaling rather than chaperone function.

Keshavarz M, et al. (2021) *Heat shock protein A12B gene therapy improves perfusion, promotes neovascularization, and decreases fibrosis in a murine model of hind limb ischemia.* PMID: 34092373 In vivo gene therapy demonstrating HSPA12B promotes angiogenesis through VEGF, Trx-1, and HIF-1α — confirming pro-angiogenic signaling function.

Limitations

1. **No experimental structure**: All structural conclusions are based on AlphaFold predictions and InterPro domain recognition. While AlphaFold is highly reliable for single-domain structures, the relative orientation of domains may be less accurate.
2. **Cannot rule out non-canonical ATPase activity**: While catalytic motifs are degenerate, some divergent ATPases retain activity. Without experimental biochemistry, residual ATPase activity cannot be definitively excluded.
3. **Literature bias toward vascular biology**: The research community studying HSPA12B is primarily focused on cardiovascular and vascular biology. It is possible (though unlikely given the structural evidence) that chaperone-like activity in other contexts has simply not been investigated.

4. **Negative evidence limitation**: The absence of evidence for chaperone activity is not proof of absence. However, when combined with positive structural evidence of missing machinery, the inference is strong.
5. **Single AlphaFold model**: Domain distance measurements come from a single predicted model. Domain flexibility in solution could differ from the predicted conformation.

Proposed Follow-up Experiments/Actions

Immediate Curation Actions (No Experiments Needed)

1. **Confirm**: Maintain absence of GO:0140662 from HSPA12B
2. **Review**: Flag GO:0005524 (ATP binding, IEA) for verification given degenerate ATPase motifs
3. **Evaluate**: Consider adding GO:0003713 (transcription coactivator activity) and GO:0045766 (positive regulation of angiogenesis) based on published evidence

Priority Experiments

1. **ATPase assay** of purified recombinant HSPA12B (addresses Gap 1)
2. **Substrate binding assay** with model HSP70 clients (addresses chaperone question definitively)
3. **Crystal structure** of HSPA12B (addresses Gaps 2 and 3)
4. **Domain deletion mutagenesis** mapping the YAP/TEAD4 interaction interface (addresses Gap 3)
5. **Systematic interactome** (BioID or AP-MS) to identify all HSPA12B binding partners (addresses Gap 4)

Computational Follow-ups

1. **Foldseek** structural search to identify the closest structural neighbors of the HSPA12B C-terminal domain
2. **Phylogenetic analysis** of HSPA12 orthologs across metazoa to date the neofunctionalization event

3. **Molecular dynamics** simulation of the HSPA12B NBD with ATP to assess binding pocket geometry

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