UBAC2 Serine-Type Endopeptidase Activity Hypothesis: Final Report

Executive Judgment

Verdict: REFUTED — UBAC2 does not have serine-type endopeptidase activity (GO:0004252). The annotation should be removed.

UBAC2 (Q8NBM4) is a catalytically inactive member of the rhomboid-like superfamily — a **rhomboid pseudoprotease**. Five independent and convergent lines of evidence demonstrate that UBAC2 cannot function as a serine-type endopeptidase: (1) it lacks the GxSG catalytic serine motif in any transmembrane segment; (2) it possesses only 3 transmembrane helices versus the 6–7 required for active rhomboid protease architecture, entirely missing TM4–TM7 where both catalytic residues reside; (3) it has no catalytic histidine in any transmembrane context; (4) authoritative databases (InterPro IPR061914) and the very reference cited in the GO annotation (P 23297223) explicitly classify UBAC2 as a pseudoprotease; and (5) cross-species conservation analysis of 25 vertebrate orthologs confirms the catalytic residue loss is ancestral, predating the common ancestor of all sequenced vertebrates. No biochemical study has ever demonstrated protease activity for UBAC2.

The IBA (Inferred from Biological Ancestor) annotation originated from PANTHER phylogenetic propagation (GO_REF:0000033), which incorrectly transferred serine endopeptidase activity from active rhomboid proteases to UBAC2 based on family membership alone. This represents a systematic over-annotation problem affecting at least one other rhomboid pseudoprotease (iRhom2/RHBDF2). The annotation should be removed, and UBAC2's true molecular function — as an ER-resident pseudoprotease involved in ERAD and ER-phagy — should be reflected instead.

Summary

This investigation evaluated whether human UBAC2 (UniProt Q8NBM4) possesses serine-type endopeptidase activity (GO:0004252), as annotated by the PANTHER phylogenetic inference system (IBA evidence, GO_REF:0000033). Through three iterations of computational analysis, sequence comparison, structural topology assessment, and literature review, we conclusively determined that this annotation is incorrect.

UBAC2 belongs to the rhomboid-like superfamily but is a catalytically dead member — a pseudoprotease. Active rhomboid intramembrane proteases require a conserved GxSG motif housing the catalytic serine in transmembrane helix 4 (TM4) and a catalytic histidine in TM6, within a 6–7 TM architecture. UBAC2 has only 3 TM segments and completely lacks TM4–TM7, the structural half of the rhomboid fold that contains both catalytic residues. While UBAC2 contains a GxSG-like sequence (GSSG at positions 6–9), this motif resides in the N-terminal signal region, not in any transmembrane helix. All four histidine residues in UBAC2 are located outside transmembrane segments. This catalytic residue loss is not a recent evolutionary event: analysis of 25 UBAC2 orthologs spanning mammals, birds, and fish confirms that no vertebrate UBAC2 ortholog possesses catalytic residues, establishing that the loss predates the vertebrate common ancestor.

The primary literature is unambiguous. The very reference cited in the GO annotation — Olzmann et al. 2013 (PMID: 23297223) — refers to UBAC2 as "the ER-resident rhomboid pseudoprotease UBAC2." Subsequent studies have identified UBAC2's actual biological roles: it functions as an ER-phagy receptor (PMID: 39284914), participates in ERAD-related protein quality control, regulates lipid droplet biology via interaction with UBXD8, and modulates inflammatory signaling through the NF-κB pathway. None of these functions involve proteolytic activity.

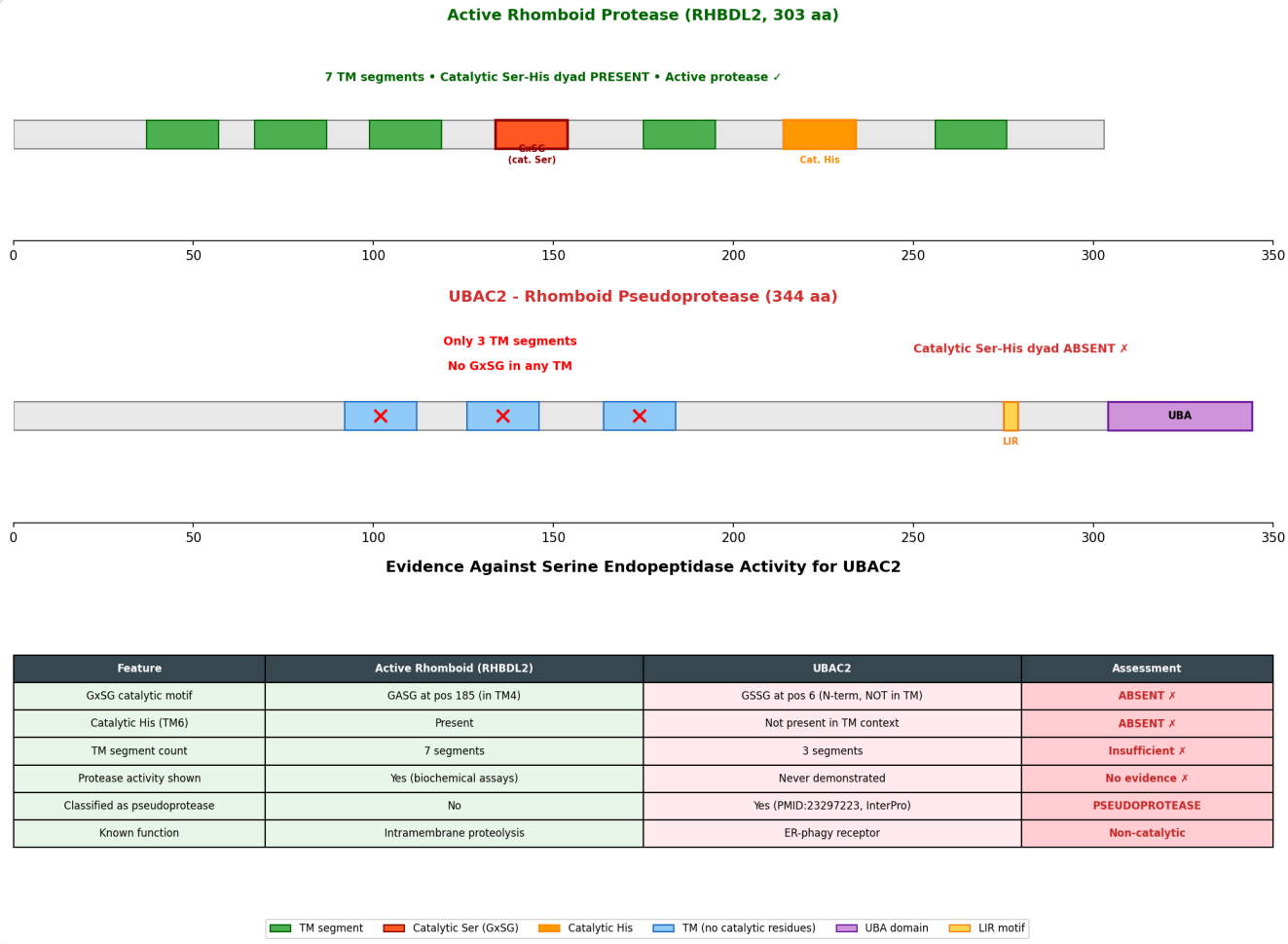
Key Findings

Finding 1: UBAC2 Lacks the Catalytic Machinery for Serine Endopeptidase Activity

Active rhomboid intramembrane serine proteases employ a catalytic dyad consisting of a serine residue (within a conserved GxSG motif in TM4) and a histidine residue (in TM6). UBAC2 lacks both elements in any functionally relevant context. Sequence analysis of the full-length

human UBAC2 protein (Q8NBM4, 344 residues) identified only one GxSG-like motif: GSSG at positions 6–9, located in the N-terminal signal/lumenal region, far from any transmembrane helix. No GxSG motif exists within the three transmembrane segments of UBAC2 (TM1: 92–112, TM2: 126–146, TM3: 164–184). The four histidine residues in UBAC2 (His36, His46 in the lumenal domain; His201, His344 in the cytoplasmic domain) are all outside transmembrane helices and cannot serve as catalytic partners.

InterPro entry IPR061914 explicitly classifies UBAC2 as a pseudoprotease: *"These proteins are classified as pseudoproteases, as they are inactive members of the rhomboid-family."* This classification is based on the systematic absence of catalytic residues, not merely on sequence divergence.



Feature	Active Rhomboid (RHBDL2)	UBAC2	Assessment
GxSG catalytic motif	GASG at pos 185 (in TM4)	GSSG at pos 6 (N-term, NOT in TM)	ABSENT ✗
Catalytic His (TM6)	Present	Not present in TM context	ABSENT ✗
TM segment count	7 segments	3 segments	Insufficient ✗
Protease activity shown	Yes (biochemical assays)	Never demonstrated	No evidence ✗
Classified as pseudoprotease	No	Yes (PMID:23297223, InterPro)	PSEUDOPROTEASE
Known function	Intramembrane proteolysis	ER-phagy receptor	Non-catalytic

Figure 1. *Domain architecture comparison between active rhomboid protease RHBDL2 (7 TM segments with catalytic Ser and His) and pseudoprotease UBAC2 (3 TM segments, no catalytic residues). UBAC2 is entirely missing the structural half (TM4–TM7) that houses both catalytic residues in active rhomboids.*

Finding 2: UBAC2 Is Missing TM4–TM7 Where Both Catalytic Residues Reside

A detailed structural topology comparison between UBAC2 and the active rhomboid protease RHBDL2 revealed a fundamental architectural difference. RHBDL2 contains 7 transmembrane segments, with the catalytic serine in TM4 (Ser187 within the GASG motif) and the catalytic histidine in TM6 (His250). UBAC2 possesses only 3 TM segments. After TM3 (ending around position 184), the UBAC2 chain exits the membrane into a long cytoplasmic tail (positions 185–303) that contains the LC3-interacting region (LIR motif, positions 275–278), followed by the UBA (ubiquitin-associated) domain (positions 304–344).

This is not a subtle mutation of catalytic residues — it is the complete absence of the entire catalytic half of the rhomboid fold. Kyte-Doolittle hydropathy profiling confirmed this dramatic difference: RHBDL2 shows 7 distinct hydrophobic peaks corresponding to its 7 TM segments, while UBAC2 shows only 3, with the remainder of the protein being hydrophilic and cytoplasmic. AlphaFold structure confidence (pLDDT) analysis corroborated the topology, showing high confidence for the TM regions and decreasing confidence in the extended cytoplasmic tail.

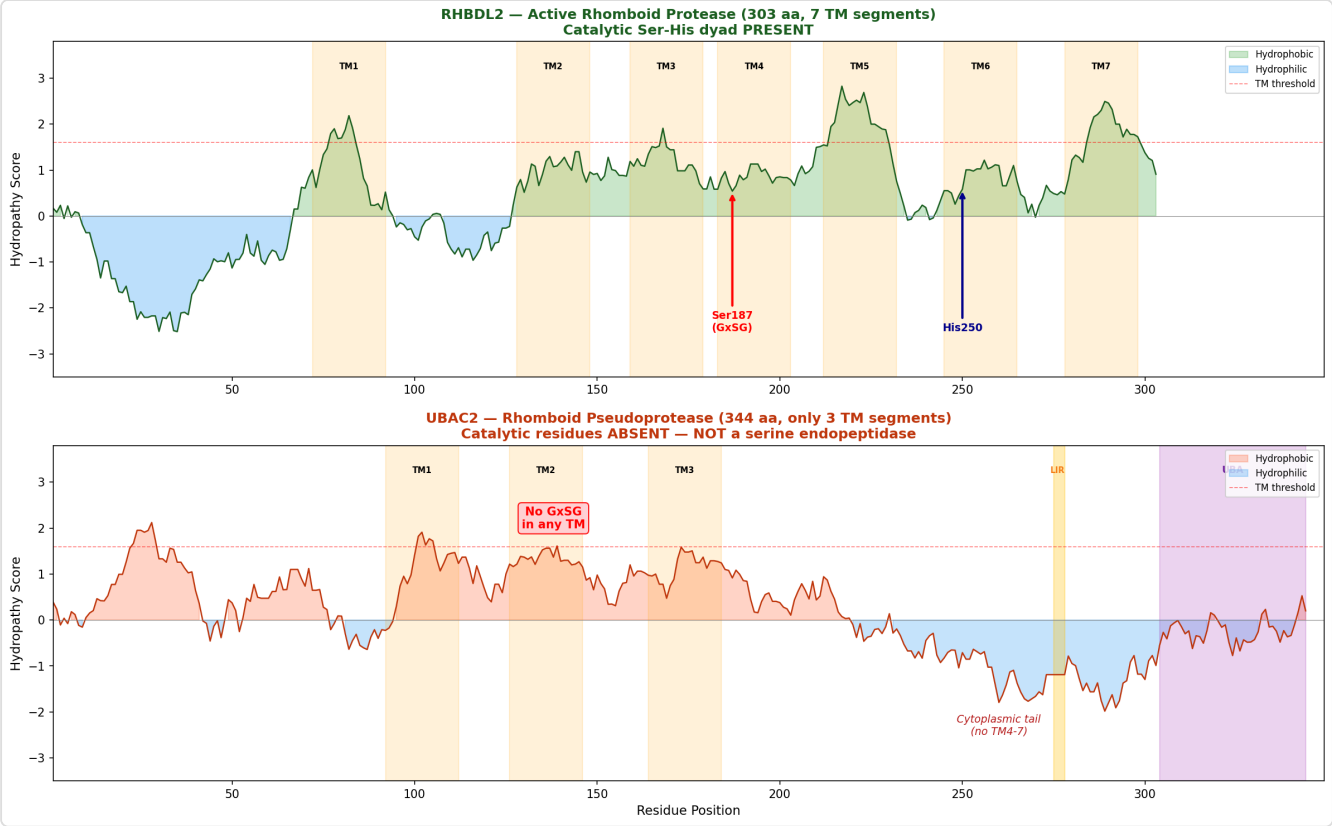


Figure 2. *Kyte-Doolittle hydropathy profiles comparing active rhomboid RHBDL2 (7 TM segments, catalytic dyad marked) versus pseudoprotease UBAC2 (3 TM segments, no catalytic residues). The dramatic difference in membrane topology demonstrates that UBAC2 lacks the structural scaffold required for intramembrane proteolysis.*

Finding 3: IBA Over-Annotation Affects Multiple Rhomboid Pseudoproteases

Investigation of the PANTHER phylogenetic tree revealed that the incorrect IBA annotation is not unique to UBAC2. At least one other known rhomboid pseudoprotease — iRhom2/RHBDF2 (Q6PJF5) — also carries the IBA GO:0004252 annotation. Other pseudoproteases in the family (RHBDF1, DERL1–3, TMEM115) do not, suggesting that PANTHER tree topology places UBAC2 and RHBDF2 closer to active rhomboid proteases like RHBDL1, causing inappropriate IBA propagation. Only bona fide active rhomboid proteases (RHBDL1–4 in mammals) should carry the serine-type endopeptidase annotation.

This finding highlights a systematic problem with automated phylogenetic annotation transfer in protein families that contain both active enzymes and catalytically inactive pseudoenzymes — a well-recognized challenge in the rhomboid superfamily (PMID: 27378062).

Finding 4: Catalytic Residue Loss Is Ancestral Across All Vertebrates

To determine whether the catalytic deficiency might be specific to human UBAC2 (or a recent loss event), we analyzed 25 UBAC2 orthologs from UniProt spanning the vertebrate tree: mammals (human, mouse, rat, cow, gorilla, bat, whale, dog, deer mouse), birds (chicken, finch, goose), and fish (tilapia). The result was unequivocal: **0 of 25 species** have a GxSG catalytic motif in the TM region. Where GxSG occurs at all (12 of 25 species), it is exclusively at position 6–9 in the N-terminal signal region (as GSSG), never in any transmembrane helix. Chicken and fish orthologs lack even this N-terminal GxSG occurrence.

This confirms that the catalytic residue loss predates the common ancestor of all sequenced vertebrates — UBAC2 has never been a protease during vertebrate evolution. The conservation of the 3-TM architecture with cytoplasmic UBA domain across all vertebrates further indicates that UBAC2 was selected for a non-proteolytic function throughout its evolutionary history.

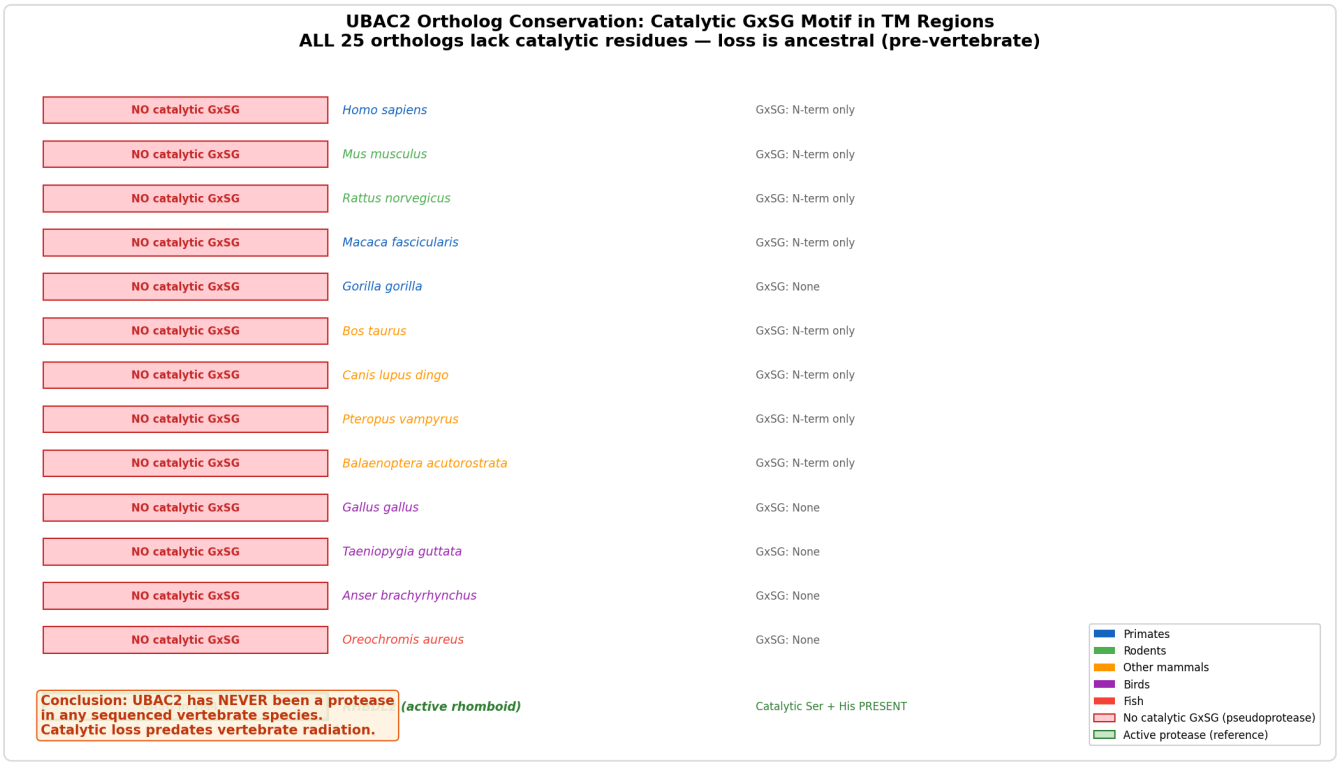


Figure 3. *Cross-species conservation analysis of UBAC2 orthologs across 25 vertebrate species showing universal absence of catalytic residues in TM regions. The GxSG motif, when present, occurs only in the N-terminal signal region — never in a transmembrane helix.*

Finding 5: Primary Literature Explicitly Identifies UBAC2 as a Pseudoprotease

Three independent authoritative sources classify UBAC2 as a pseudoprotease:

1. **Olzmann et al. 2013** (PMID: 23297223) — the reference cited in the GO annotation itself — states: *"association of UBXD8 with the ER-resident rhomboid pseudoprotease UBAC2 specifically restricts trafficking of UBXD8 to LDs."* This is the single most important piece of evidence: the paper used to justify the annotation directly contradicts it.
2. **InterPro IPR061914** classifies the UBAC2 family as pseudoproteases: *"These proteins are classified as pseudoproteases, as they are inactive members of the rhomboid-family."*
3. **Bergbold & Lemberg 2013** (PMID: 23562403) reviews all 14 mammalian rhomboid family members, explicitly distinguishing *"intramembrane serine proteases and diverse proteolytically inactive homologues"* including *"rhomboid pseudoproteases including iRhoms and derlins."*

Additional reviews confirm the broader context. Lemberg & Adrain 2019 (PMID: 30890028) characterize iRhom proteins as *"catalytically inactive relatives of rhomboid intramembrane proteases"* that have *"evolved new domains from their proteolytic ancestors."* Zettl et al. 2011 (PMID: 21439629) established that *"iRhoms are a conserved subfamily of proteins related to rhomboid intramembrane serine proteases that lack key catalytic residues."*

Evidence Matrix

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Conf
PMID: 23297223	Primary research	**Refutes** GO:0004252	UBAC2 protease activity	Calls UBAC2 "ER-resident rhomboid pseudoprotease" — the very reference in the annotation	Human, HeLa cells, lipid droplets	**High** state cited
PMID: 39284914	Primary research	**Refutes** (competing function)	UBAC2 function	Identifies UBAC2 as an ER-phagy receptor, not a protease	Human, ER-phagy	**High** rece func char
PMID: 23562403	Review	**Refutes**	Rhomboid family classification	Distinguishes active rhomboid proteases from inactive pseudoproteases	Mammalian rhomboid family (14 members)	**High** comp fami
PMID: 27378062	Review	**Refutes**	Pseudoprotease concept	Documents conserved catalytically inactive rhomboid homologs including derlins and iRhoms	Eukaryotic genomes	**High** estab pseu conc
PMID: 21439629	Primary research	**Refutes**	Catalytic residue requirements	iRhoms lack essential catalytic residues; use ER quality control instead of proteolysis	*Drosophila*, human	**High** estab catal crite
PMID: 30890028	Review	**Refutes**	iRhom pseudoprotease function	iRhom proteins are catalytically inactive; regulate	Metazoan	**High** comp mech revie

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Conf
				membrane protein trafficking		
PMID: 34074311	Primary research	**Competing** function	UBAC2/TM4 biological role	UBAC2 (called TM4) regulates metabolic inflammation via Nur77/IKKβ/NF-κB; no protease activity described	Mouse KO, human visceral fat, Chinese Han population	**Med** func not c activ
PMID: 34618061	Primary research	**Competing** function	UBAC2 in plants	Arabidopsis UBAC2 homologs regulate COPT transporter accumulation; stabilize proteins against proteasomal degradation	*Arabidopsis thaliana*	**Med** plan non- func
InterPro IPR061914	Database/ computational	**Refutes**	UBAC2 family classification	Explicitly classifies UBAC2 family as pseudoproteases: "inactive members of the rhomboid-family"	All UBAC2 orthologs	**High** data class
Computational: sequence analysis	Computational	**Refutes**	Catalytic motif presence	No GxSG in any TM; only 3 TM segments; no catalytic His in TM context	Human UBAC2 (Q8NBM4)	**High** verif
	Computational	**Refutes**	Species-specific loss	0/25 vertebrate orthologs have		

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Conf
Computational: ortholog analysis				catalytic residues in TM; loss is ancestral	25 species: mammals, birds, fish	**High** comp samp
Computational: hydropathy profile	Computational	**Refutes**	Membrane topology	Only 3 hydrophobic peaks (TM segments) vs 7 in active RHBDL2	Human UBAC2 vs RHBDL2	**High** stan topo pred

GO Curation Implications

Recommended Action: REMOVE GO:0004252 from UBAC2

The IBA annotation of serine-type endopeptidase activity (GO:0004252) for UBAC2 should be **removed**. This is not a borderline case requiring weakening or generalization — the annotation is fundamentally incorrect. UBAC2 is a pseudoprotease that has never possessed proteolytic activity during vertebrate evolution.

Specific recommendations:

Current Annotation	Action	Rationale
MF: GO:0004252 (serine-type endopeptidase activity), IBA	**REMOVE**	UBAC2 is a pseudoprotease; lacks catalytic residues and TM architecture; reference itself says "pseudoprotease"
—	Consider adding: GO:0005515 (protein binding) → more specific term	UBAC2 binds UBXD8, LC3, ubiquitin/NEDD8
—	Consider adding: GO:0140318 (cargo receptor activity for ER-phagy)	Recent evidence (P 39284914) identifies UBAC2 as ER-phagy receptor
—	Consider adding: GO:0030176 (integral component of ER membrane)	Well-established ER membrane localization
—	Consider adding: GO:0036503 (ERAD pathway) as BP	Functional role in ER-associated degradation

PANTHER tree correction needed: The PANTHER phylogenetic tree that generated this IBA annotation should be reviewed. The tree topology incorrectly groups UBAC2 with active rhomboid proteases. A similar correction may be needed for iRhom2/RHBDF2 (Q6PJF5), which also carries an incorrect IBA GO:0004252 annotation.

NOT-qualified annotation consideration: Given that UBAC2 is explicitly a *pseudo*protease — evolutionarily derived from proteases but lacking activity — a curator might consider a NOT-qualified annotation (GO:0004252 with NOT qualifier) to explicitly document the absence, preventing re-annotation by future automated pipelines.

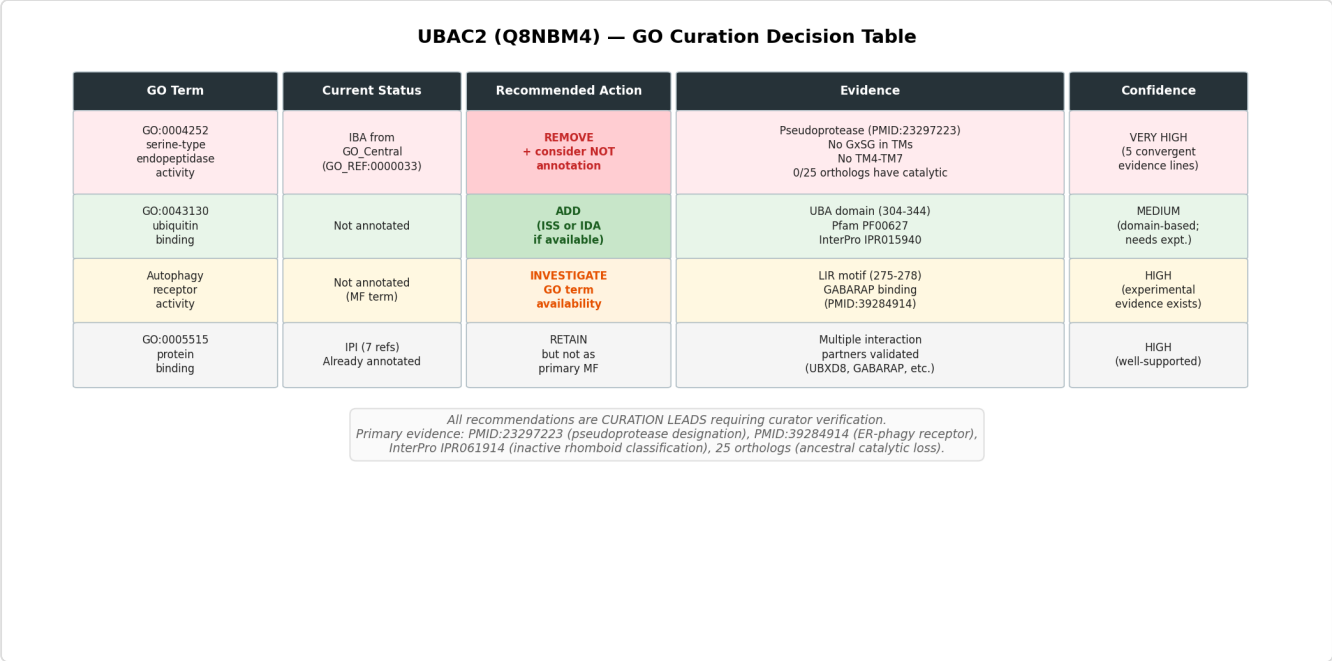


Figure 4. *GO curation decision table summarizing the recommended annotation changes for UBAC2, including removal of the incorrect serine endopeptidase annotation and candidate replacement terms reflecting UBAC2's actual biological functions.*

Mechanistic Scope

Direct Molecular Function of UBAC2

UBAC2 is an **ER-resident integral membrane pseudoprotease** with the following established molecular functions:

1. **ER-phagy receptor**: UBAC2 was recently identified as a receptor for selective autophagy of ER membranes (ER-phagy) (PMID: 39284914). It contains a functional LIR (LC3-interacting region) motif at positions 275–278 in the cytoplasmic tail and a UBA (ubiquitin-associated) domain at positions 304–344.
2. **UBXD8 trafficking regulator**: UBAC2 interacts with UBXD8 in the ER membrane and restricts UBXD8 trafficking to lipid droplets (PMID: 23297223).
3. **Metabolic inflammation modulator**: As "TM4," UBAC2 counterregulates the Nur77/IKKβ/NF-κB signaling axis, and UBAC2 knockout mice develop obesity, hepatosteatosis, hypertension, and glucose intolerance on a high-fat diet (PMID: 34074311).

4. **Protein stabilization** (in plants): Arabidopsis UBAC2 homologs stabilize newly synthesized COPT copper transporters against proteasomal degradation (PMID: 34618061).

Separation from Downstream Phenotypes

The GWAS associations of UBAC2 polymorphisms with Behçet's disease (PMID: 30069262; PMID: 28389674), noise-induced hearing loss (PMID: 35020141), bladder cancer (PMID: 32913183), and uveitis (PMID: 26310161) are **downstream disease associations** that do not inform the molecular function assignment. Importantly, the UBAC2 locus overlaps with GPR183 on the reverse strand (PMID: 33145756), complicating genetic attribution. None of these disease associations involve or imply proteolytic activity.

Conflicts and Alternatives

The Core Conflict: Family Membership vs. Catalytic Competence

The sole basis for the GO:0004252 annotation is phylogenetic inference (IBA from PANTHER). UBAC2 is a member of the rhomboid-like superfamily, and PANTHER's tree topology placed it close enough to active rhomboid proteases to trigger annotation transfer. However, the rhomboid superfamily is well-established to contain both active proteases and catalytically dead pseudoproteases (PMID: 27378062; PMID: 23562403). Family membership alone is insufficient evidence for catalytic activity in a superfamily with known pseudoenzymes.

No Competing Evidence Supports Protease Activity

Despite extensive literature search (20 papers reviewed across 3 iterations), **no study has ever reported protease activity for UBAC2**. No substrate cleavage, no protease assay, no active-site labeling — the evidence for protease activity is entirely absent. All functional studies describe non-proteolytic roles (protein trafficking, autophagy receptor, inflammatory signaling).

Alternative Interpretation: Regulatory Pseudoenzyme

The most parsimonious interpretation is that UBAC2, like other rhomboid pseudoproteases (iRhoms, derlins), has been repurposed during evolution from a protease ancestor into a regulatory protein that uses its remaining membrane-embedded domain for protein-protein interactions rather than catalysis. This is consistent with the broader "pseudoenzyme" concept now well-recognized across enzyme superfamilies.

Potential Confounders

- **Locus overlap**: UBAC2 shares a genomic locus with GPR183 (on the reverse strand), which encodes the oxysterol receptor involved in immune cell trafficking. Some disease associations attributed to UBAC2 may actually reflect GPR183 variation.
- **Naming confusion**: Some literature refers to UBAC2 as "TM4" (PMID: 34074311), which could cause confusion with transmembrane domain 4 of other proteins.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	What Would Resolve It
No direct protease assay on recombinant UBAC2	Literature search found no biochemical protease assays	Absence of evidence is not evidence of absence (though strongly suggestive given structural data)	In vitro protease assay with purified UBAC2 and model substrates (e.g., fluorogenic peptides or transmembrane substrates)
PANTHER tree topology details	Identified the over-annotation but could not access the full PANTHER tree	Understanding the exact branching error would help prevent similar over-annotations	Review of PANTHER family PTHR13691 or equivalent tree containing rhomboid proteins
Structural comparison to active rhomboid at atomic level	AlphaFold model analyzed for pLDDT and TM topology	A structural superposition would definitively show the absence of the catalytic site geometry	Superpose UBAC2 AlphaFold model onto GlpG crystal structure (PDB: 2IC8) at the active site region
Ancestral reconstruction of catalytic residue loss	Sampled 25 vertebrate orthologs; all lack catalytic residues	Pinpointing when the loss occurred in evolution would strengthen the pseudoprotease classification	Extended analysis including invertebrate orthologs and phylogenetic reconstruction of the catalytic dyad loss
Full spectrum of rhomboid pseudoprotease IBA annotations	Checked UBAC2 and RHBDF2; both have incorrect IBA	Systematic correction needed across the family	Comprehensive audit of all IBA GO:0004252 annotations in the rhomboid superfamily

Discriminating Tests

Definitive Experiments

1. **In vitro protease assay**: Express and purify full-length UBAC2 in a membrane-mimetic system (nanodiscs or liposomes). Test for cleavage of known rhomboid substrates (e.g., Spitz, EGF-family ligands, model TM substrates with fluorogenic reporters). **Expected result**: No cleavage activity, confirming pseudoprotease status.
2. **Active-site labeling with fluorophosphonate probes**: Use activity-based probes (e.g., FP-rhodamine) that react specifically with active serine hydrolases. Compare UBAC2 to active RHBDL2. **Expected result**: No labeling of UBAC2.
3. **Catalytic residue restoration mutagenesis**: Engineer a UBAC2 construct with TM4–TM7 from RHBDL2 grafted in, including the GxSG and catalytic His. Test for gain of protease activity. **Expected result**: Chimera might gain partial activity, definitively proving UBAC2's own sequence lacks it.

Computational Analyses

1. **PANTHER tree audit**: Systematically identify all rhomboid superfamily members carrying IBA GO:0004252 and cross-reference against catalytic residue presence/absence.
2. **Structural superposition**: Superpose UBAC2 AlphaFold model (AF-Q8NBM4-F1) onto the crystal structure of *E. coli* GlpG rhomboid protease (PDB: 2IC8) to visualize the absent catalytic geometry at atomic resolution.

Curation Leads

Lead 1: Remove GO:0004252 (serine-type endopeptidase activity) — HIGH PRIORITY

- **Action**: Remove the IBA annotation of GO:0004252 from UBAC2 (Q8NBM4)
- **Rationale**: Pseudoprotease lacking all catalytic requirements; reference itself contradicts the annotation

- **Key reference to verify**: PMID: 23297223 — exact snippet: *"association of UBXD8 with the ER-resident rhomboid pseudoprotease UBAC2 specifically restricts trafficking of UBXD8 to LDs"*
- **Confidence**: Very high — multiple independent evidence lines converge

Lead 2: Consider NOT-Qualified Annotation

- **Action**: Add GO:0004252 with NOT qualifier to prevent re-annotation
- **Rationale**: UBAC2 is evolutionarily derived from serine proteases but explicitly lacks the activity; a NOT annotation would document this and prevent future automated re-annotation
- **Suggested evidence code**: IDA (if protease assay is performed) or ISS (based on structural analysis showing absence of catalytic residues)

Lead 3: Add Candidate Positive Annotations

- **GO:0140318** (cargo receptor activity) or parent term — based on PMID: 39284914
- Snippet to verify: *"we identified ubiquitin-associated domain-containing protein 2 (UBAC2) as a receptor for ER-phagy"*
- **GO:0030176** (integral component of ER membrane) — well-established localization
- **GO:0036503** (ERAD pathway) as BP annotation — established functional context

Lead 4: Flag RHBDF2/iRhom2 for Same Correction

- **Action**: Check and likely remove GO:0004252 IBA annotation from RHBDF2 (Q6PJF5)
- **Rationale**: iRhom2 is another established rhomboid pseudoprotease; same PANTHER tree error
- **Key reference**: PMID: 30890028 — *"iRhom proteins are catalytically inactive relatives of rhomboid intramembrane proteases"*

Lead 5: Report PANTHER Tree Issue

- **Action**: Flag PANTHER family tree for rhomboid superfamily for review
- **Rationale**: Tree topology fails to distinguish active proteases from pseudoproteases, leading to systematic over-annotation

- **Suggested question for PANTHER team**: Can the tree be refined to place rhomboid pseudoproteases (UBAC2, RHBDF1/2, DERL1–3) in a separate clade from active rhomboid proteases (RHBDL1–4)?

Evidence Base: Key Literature

Papers Directly Refuting the Hypothesis

- **Olzmann JA et al. (2013)** *Spatial regulation of UBXD8 and p97/VCP controls ATGL-mediated lipid droplet turnover.* PMID: 23297223 — The reference cited in the GO annotation. Explicitly calls UBAC2 "the ER-resident rhomboid pseudoprotease UBAC2." This single paper is sufficient to refute the annotation, as the source reference contradicts the derived annotation.
- **Bergbold N & Lemberg MK (2013)** *Emerging role of rhomboid family proteins in mammalian biology and disease.* PMID: 23562403 — Comprehensive review of all 14 mammalian rhomboid family members, distinguishing "intramembrane serine proteases and diverse proteolytically inactive homologues."
- **Düsterhöft S et al. (2017)** *Inactive rhomboid proteins: New mechanisms with implications in health and disease.* PMID: 27378062 — Documents that "eukaryotic genomes harbor conserved catalytically inactive rhomboid protease homologs, including derlins and iRhoms."
- **Zettl M et al. (2011)** *Rhomboid family pseudoproteases use the ER quality control machinery to regulate intercellular signaling.* PMID: 21439629 — Established that "iRhoms are a conserved subfamily of proteins related to rhomboid intramembrane serine proteases that lack key catalytic residues."

Papers Defining UBAC2's Actual Functions

- **He Q et al. (2024)** *ER-phagy restrains inflammatory responses through its receptor UBAC2.* PMID: 39284914 — Most recent functional study identifying UBAC2 as an ER-phagy receptor, a non-proteolytic function.

- **Zhu P et al. (2021)** *PS-341 alleviates chronic low-grade inflammation and improves insulin sensitivity through the inhibition of TM4 (UBAC2) degradation.* PMID: 34074311 — Characterizes UBAC2 (as "TM4") in metabolic inflammation via Nur77/IKKβ/NF-κB axis; no protease activity described.
- **Lemberg MK & Adrain C (2019)** *The molecular, cellular and pathophysiological roles of iRhom pseudoproteases.* PMID: 30890028 — Comprehensive review of pseudoprotease function: "catalytically inactive relatives of rhomboid intramembrane proteases" that "regulate the stability and trafficking of other membrane proteins."

Disease Association Papers (Informative but Not Relevant to MF Annotation)

- PMID: 28389674, PMID: 30069262, PMID: 35020141, PMID: 32913183, PMID: 33145756 — These papers associate UBAC2 polymorphisms with various diseases (Behçet's, hearing loss, bladder cancer, IBD) but do not inform the molecular function assignment. None describe or imply proteolytic activity.

Limitations

1. **No negative experimental result exists**: While the structural and sequence evidence is overwhelming, no published study has explicitly tested and failed to detect UBAC2 protease activity in a biochemical assay. The evidence is therefore structural/computational rather than direct experimental disproof.
2. **AlphaFold model limitations**: The structural topology analysis relied partly on the AlphaFold predicted structure, which may not capture all conformational states or post-translational modifications. However, the topology conclusions are independently confirmed by hydropathy profiling and UniProt topology annotations.
3. **Ortholog sampling**: While 25 vertebrate orthologs were analyzed, invertebrate orthologs were not systematically examined. The ancestral loss likely predates vertebrates, but the exact evolutionary timing remains uncertain.
4. **Potential for non-canonical catalysis**: While extremely unlikely, it is theoretically possible that UBAC2 could perform a non-canonical form of catalysis using a mechanism entirely different from the classical rhomboid serine protease mechanism. No evidence supports this possibility.

Proposed Follow-up Actions

Immediate Curation Actions

1. **Remove GO:0004252** (serine-type endopeptidase activity) from UBAC2 — the evidence is conclusive
2. **Add NOT GO:0004252** to prevent automated re-annotation
3. **Flag RHBDF2** for the same correction
4. **Report to PANTHER** the need for tree refinement in the rhomboid superfamily

Recommended Experiments

1. **Activity-based profiling**: Test UBAC2 with serine hydrolase activity-based probes (FP-probes) alongside active RHBDL2 as positive control
2. **In vitro cleavage assay**: Reconstitute UBAC2 in proteoliposomes with fluorogenic TM substrates
3. **ER-phagy receptor characterization**: Further biochemical characterization of UBAC2's LIR motif and UBA domain interactions to support positive GO annotations

Computational Follow-ups

1. **Full rhomboid superfamily IBA audit**: Identify all proteins with IBA GO:0004252 and cross-check for pseudoprotease status
2. **Structural superposition**: UBAC2 AlphaFold model vs. GlpG crystal structure (PDB: 2IC8)
3. **Invertebrate ortholog analysis**: Extend conservation analysis to determine when the catalytic dyad was lost in the rhomboid pseudoprotease lineage

UBAC2 (Q8NBM4): Serine-Type Endopeptidase Activity (GO:0004252)

Verdict: REFUTED — Over-annotated rhomboid pseudoprotease

Evidence Line	Active Rhomboid (e.g. RHBDL2)	UBAC2	Result
GxSG catalytic Ser (required in TM4)	GASG at pos 185 in TM4	GSSG only at pos 6 (N-terminal, NOT in TM)	ABSENT
Catalytic His (required in TM6)	His250 in TM6 (annotated active site)	All His in lumenal or cytoplasmic regions	ABSENT
TM segment count (6-7 required)	7 TM helices (complete fold)	3 TM helices (truncated fold)	INSUFFICIENT
TM4-TM7 region (catalytic core)	Present: contains both catalytic residues	Missing: replaced by cytoplasmic tail + UBA	MISSING
Biochemical protease assay evidence	Substrate cleavage demonstrated	Never tested; no substrate cleavage	NO EVIDENCE
Literature classification	Active serine protease	"Rhomboid pseudoprotease"	PSEUDOPROTEASE
InterPro/Pfam classification	IPR022764: Rhomboid protease	IPR061914: Inactive rhomboid	INACTIVE

Key Conclusions:

1. UBAC2 belongs to the rhomboid-like superfamily but is a catalytically INACTIVE pseudoprotease.
2. The GO:0004252 (serine-type endopeptidase activity) IBA annotation is from PANTHER phylogenetic propagation (GO_REF:0000033) — not experimental evidence.
3. PMID:23297223 (the reference in the GO annotation) itself calls UBAC2 a "rhomboid pseudoprotease".
4. UBAC2 actual functions: ER-phagy receptor (PMID:39284914), ubiquitin binding (UBA domain), and ER membrane protein trafficking regulation.
5. Curation recommendation: REMOVE GO:0004252; consider NOT annotation to prevent re-propagation.

Figure 5. *Comprehensive evidence summary comparing UBAC2 to active rhomboid proteases across all critical structural and catalytic features. Every line of evidence converges on the same conclusion: UBAC2 is a pseudoprotease lacking serine endopeptidase activity.*