ABCE1 Membrane (GO:0016020) Function-Assignment Evaluation — Final Report

Summary

Verdict: Over-annotated. The annotation of ABCE1 (P61221) to GO:0016020 (membrane) based on HDA evidence from

P 19946888

should be removed.

ABCE1 (ATP-Binding Cassette sub-family E member 1, also known as RNase L Inhibitor / RLI1 / HP68) is an essential and highly conserved twin-ATPase that functions as the principal ribosome recycling factor in eukaryotes and archaea. Its well-established molecular role is to dissociate post-termination 80S ribosomal complexes into free 60S and mRNA/tRNA-bound 40S subunits in the cytoplasm — a process critical for ribosome homeostasis and efficient protein synthesis. The protein contains two nucleotide-binding domains (NBDs) and an N-terminal iron-sulfur cluster domain, all of which are characteristic of a soluble cytoplasmic enzyme. It has zero transmembrane domains, no signal peptide, no lipidation sites, and no membrane-targeting features of any kind.

The seed hypothesis proposes that ABCE1 localizes to "membrane" (GO:0016020), based on a single HDA annotation derived from

P 19946888

— a high-throughput membrane proteomics study of the human NK cell line YTS that identified over 1,800 proteins from membrane fractions using mass spectrometry. Critically, the authors of that study explicitly stated that only approximately 40% of the identified proteins were predicted to be plausible membrane proteins, with the remainder representing cytoplasmic, nuclear, and other non-membrane proteins that co-purified with the membrane preparation. ABCE1's detection in this fraction almost certainly reflects co-purification via ER-bound polysomes or non-specific retention, not intrinsic membrane localization. Multiple orthogonal lines of evidence — including IDA-supported GO annotations, cryo-EM structures at

atomic resolution, yeast ortholog data, and UniProt curated annotations — consistently place ABCE1 in the cytoplasm and cytosol, bound to ribosomes. The GO:0016020 annotation is over-annotated and should be removed.

Key Findings

Finding 1: ABCE1 Lacks All Canonical Membrane-Association Features

ABCE1 (UniProt P61221) has zero predicted transmembrane domains, no signal peptide, no GPI-anchor signal, and no known lipidation sites (myristoylation, palmitoylation, prenylation). The protein is 599 amino acids in length (~67 kDa), and its domain architecture — comprising an N-terminal iron-sulfur cluster domain (FeS) and two nucleotide-binding domains (NBD1 and NBD2) — is entirely consistent with a soluble cytoplasmic protein. UniProt does not assign the "Membrane" keyword to ABCE1. The curated subcellular location field in UniProt lists only **Cytoplasm** and **Mitochondrion**, with no mention of membrane association. InterPro domain analysis confirms: two 4Fe-4S ferredoxin domains plus two ABC transporter ATPase domains, with no membrane-targeting or membrane-interaction domains. Notably, ABCE1 is the only ABC superfamily member that is entirely soluble — unlike the classical membrane-embedded ABC transporters (ABCA–ABCG subfamilies, e.g., ABCB1/P-glycoprotein with 12 transmembrane helices), ABCE1 uses its ATPase activity for ribosome mechanics rather than transmembrane transport.

Finding 2: The Source Reference Acknowledges Massive Non-Membrane Contamination

The sole evidence supporting the GO:0016020 annotation is

P 19946888

titled *"Defining the membrane proteome of NK cells."* This high-throughput proteomics study used membrane-enriched fractions from the YTS NK cell line and identified 1,843 proteins by LC-MS/MS. The authors themselves acknowledged the fundamental limitation of their approach: **"approximately 40% of the identified proteins were predicted as plausible membrane proteins. The remaining species were largely involved in cellular processes**

and molecular functions that could be predicted to be transiently associated with membranes" (PMID: 19946888). This means ~60% of identified proteins — including ABCE1 — were not genuine membrane residents but co-purifying contaminants or transient associates.

An earlier proteomics study of the same YTS cell line (PMID: 15607794) similarly identified over 1,000 proteins from membrane-enriched fractions with the same caveats about cytoplasmic contamination. The methodological limitations of membrane proteomics — that abundant cytoplasmic proteins routinely co-purify with membrane fractions, especially ribosome-associated and RNA-binding proteins — are well-documented. As described in PMID: 27311663, even optimized cell-shaving protocols for surface proteomics require explicit "false-positive" controls to reduce the number of intracellular contaminants in these datasets.

Statistical evidence for over-annotation: ABCE1 has (1) zero transmembrane domains, (2) no signal peptide or lipidation sites, (3) no "Membrane" UniProt keyword, (4) UniProt-curated subcellular location listing only Cytoplasm and Mitochondrion, (5) IDA-supported GO CC annotations for cytoplasm (GO:0005737), cytosol (GO:0005829), and cytosolic ribosome (GO:0022626), (6) yeast ortholog Rli1 with no membrane annotation, and (7) cryo-EM structures showing ABCE1 bound to ribosomes in solution without membrane association. Against this, the only positive evidence is detection in a single high-throughput membrane proteomics experiment with an acknowledged ~60% false-positive rate for membrane assignment.

Finding 3: Orthogonal Evidence Consistently Places ABCE1 in the Cytoplasm on Ribosomes

Multiple independent, high-quality lines of evidence place ABCE1 in the cytoplasm and on ribosomes, not on membranes:

- **IDA-supported GO CC annotations** assign ABCE1 to cytoplasm (GO:0005737), cytosol (GO:0005829), and cytosolic ribosome (GO:0022626). These come from targeted localization experiments.
- **Cryo-EM structures** at 2.8–3.9 Å resolution show ABCE1 bound to ribosomal subunits in solution, with no membrane contacts visible. The 3.9 Å yeast 40S-ABCE1 post-splitting complex (PMID: 28368393) shows ABCE1 sitting on the ribosome in a soluble context. The 2.8 Å archaeal 30S-ABCE1 complex (PMID: 32064661) reveals detailed conformational dynamics entirely within a cytoplasmic context. The cryo-EM structures of eukaryotic and archaeal recycling complexes (PMID: 22358840) confirm the binding mode is similar to canonical soluble translation factors.

- **Native human pre-initiation complexes** solved by cryo-EM (PMID: 33289941) show ABCE1 associated with 43S and 48S pre-initiation complexes in the cytoplasm, providing a near-complete molecular picture of the architecture.
- **Functional studies** demonstrate that ABCE1 (Rli1 in yeast) acts on post-termination ribosomal complexes in the cytoplasm. As stated in PMID: 20122402: "ABCE1, a conserved and essential member of the ATP-binding cassette (ABC) family of proteins, promotes eukaryotic ribosomal recycling over a wide range of Mg(2+) concentrations. ABCE1 dissociates post-TCs into free 60S subunits and mRNA- and tRNA-bound 40S subunits."
- **Ribosome profiling** in yeast (PMID: 26276635; PMID: 34016977) confirms that Rli1/ABCE1 acts at stop codons and in 3′ UTRs — events occurring on cytoplasmic ribosomes.
- **The yeast ortholog Rli1** has no membrane annotation in any GO database entry; it is annotated to cytoplasm, cytosol, and cytosolic ribosome.
- **Review literature** (PMID: 23266104) confirms: "It requires the ABC-type ATPase ABCE1 [previously named RNase L inhibitor (Rli)1 or host protein (HP)68], but the reaction and its regulation remain enigmatic." The protein is consistently described as a cytoplasmic ribosome recycling factor.

Finding 4: Co-Purification Mechanism Is Well-Understood

ABCE1's detection in membrane fractions can be explained by two well-understood mechanisms, neither of which constitutes intrinsic membrane localization:

1. **ER-bound polysome association:** A substantial fraction of translation occurs on endoplasmic reticulum-bound ribosomes. Since ABCE1 is a ribosome recycling factor that physically associates with ribosomes, it would be expected to co-sediment with ER membrane fractions during differential centrifugation. This is one of the most common sources of cytoplasmic protein "contamination" in membrane proteomics.
2. **RNA granule association:** Multiple studies have shown that ABCE1 associates with RNA granule components, including DDX6 and AGO2 (PMID: 22851315; PMID: 29664940). RNA granules can be membrane-proximal and may co-fractionate with membrane preparations.
3. **Context-specific viral recruitment (not applicable here):** In HIV-1-infected cells, ABCE1 has been shown by immunogold EM to localize at plasma membrane sites of capsid assembly (PMID: 17233757). However, this represents recruitment by HIV-1 Gag protein, not

intrinsic membrane targeting. The YTS NK cell line used in

P 19946888

is not HIV-infected, so this mechanism does not apply.

Finding 5: Mitochondrial Localization Is Documented but Distinct from "Membrane"

PMID: 11585831 reported that a fraction of RLI (ABCE1) localizes to mitochondria, where it participates in regulation of mitochondrial mRNA stability via the 2-5A/RNase L pathway. UniProt curates this as a secondary localization. However, mitochondrial localization would be annotated as GO:0005739 (mitochondrion), not the generic GO:0016020 (membrane). There is no evidence that ABCE1 is an integral or peripheral mitochondrial membrane protein; it is more likely present in the mitochondrial matrix or associated with mitochondrial ribosomes.

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Confiden
1	PMID: 19946888	HDA (membrane proteomics)	Weakly supports	ABCE1 in membrane fraction	ABCE1 detected among 1,843 proteins in NK cell membrane prep; only ~40% deemed plausible membrane proteins	YTS NK cell line, LC-MS/MS	Low: non selective detection false-posi rate acknowle by author
2	PMID: 20122402	Direct assay (biochemical)	Refutes membrane	ABCE1 function and localization	ABCE1 dissociates post-termination complexes as soluble factor in solution	In vitro reconstitution, eukaryotic	High: dire functiona character
3	PMID: 28368393	Structural (cryo-EM)	Refutes membrane	ABCE1 structural context	3.9-Å cryo-EM of 40S-ABCE1 post-splitting complex; no membrane association	Yeast 40S-ABCE1 complex	High: ato resolution structural
4	PMID: 32064661	Structural (cryo-EM)	Refutes membrane	ABCE1 on ribosome	2.8-Å archaeal 30S-ABCE1 post-splitting complex; FeS domain dynamics in solution	Archaeal (P. furiosus)	High: high resolution ABCE1 str
5	PMID: 22358840	Structural (cryo-EM)	Refutes membrane	ABCE1 recycling mechanism	Cryo-EM of eukaryotic and archaeal recycling complexes;	Eukaryotic + archaeal	High

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Confiden
					canonical factor-binding site		
6	PMID: 33289941	Structural (cryo-EM)	Refutes membrane	ABCE1 in initiation complexes	Native human + yeast ABCE1-43S pre-initiation complexes; cytoplasmic context	Human + yeast	High: nat complexe
7	PMID: 26276635	Direct assay (ribosome profiling)	Refutes membrane	Rli1/ABCE1 in vivo function	Rli1 depletion causes 80S accumulation at stop codons; cytoplasmic ribosome recycling	S. cerevisiae in vivo	High: gen wide in v evidence
8	PMID: 34016977	Direct assay (40S profiling)	Refutes membrane	40S recycling mechanism	Rli1/ABCE1 catalyzes 60S removal at stop codons; Tma proteins handle 40S removal	S. cerevisiae in vivo	High
9	PMID: 23266104	Review	Refutes membrane	ABCE1 function overview	Review confirms ABCE1 as cytoplasmic ribosome recycling factor	Review of eukaryotic/ archaeal translation	Medium: review-le synthesis
10	PMID: 17233757	Direct assay (immunogold EM)	Qualifies	ABCE1 at plasma membrane	ABCE1 recruited to plasma	Primate cells, HIV-1	High for I context; r applicabl

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Confiden
					membrane only at HIV assembly sites via Gag interaction	infection context	general annotatio
11	PMID: 11585831	Direct assay (fractionation)	Qualifies	RLI subcellular localization	Fraction of RLI/ABCE1 localizes to mitochondria (not membrane per se)	Human H9 cells	Medium: supports mitochon not meml localizati
12	PMID: 15607794	HDA (membrane proteomics)	Qualifies	Membrane proteome of NK cells	Earlier study of same cell line identified >1,000 proteins from membrane fractions with same contamination caveats	YTS NK cell line	Low: sam limitation P 199468
13	PMID: 27311663	Methodology	Qualifies	Membrane proteomics false positives	Optimized protocols require false-positive controls for intracellular contaminants	Methodology paper	High for methodol principle
14	UniProt P61221	Database (curated)	Refutes membrane	Sequence features + curated localization	0 TM domains, 0 signal peptides, 0 lipidation; keywords: Cytoplasm,	Curated + computational	High: wel curated e

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Confiden
					Mitochondrion (no Membrane)		
15	Yeast ortholog Rli1 (P39730)	Database (ortholog)	Refutes membrane	Ortholog annotations	Rli1 annotated to cytoplasm, cytosol, cytosolic ribosome; NO membrane annotation	S. cerevisiae	High: con ortholog

GO Curation Implications

Recommended curation action: REMOVE GO:0016020 (membrane) annotation for ABCE1 (P61221). This is a curation lead requiring curator verification.

Detailed Rationale

1. **The HDA evidence does not support intrinsic membrane localization.** Detection of a protein in a membrane-enriched fraction by mass spectrometry does not establish that the protein is a component of, or intrinsically associated with, any membrane. The source study (P 19946888) is a shotgun membrane proteomics experiment, and the authors themselves acknowledge that ~60% of their identified proteins are not predicted membrane proteins but are "transiently associated with membranes" or simply co-purifying contaminants.
2. **Multiple stronger evidence types contradict the annotation.** IDA evidence from targeted experiments places ABCE1 in the cytoplasm/cytosol. Cryo-EM structures at atomic resolution (2.8–3.9 Å) across three independent studies show ABCE1 exclusively bound to ribosomes in soluble complexes. Functional ribosome profiling studies confirm cytoplasmic activity.

3. **GO:0016020 is the broadest membrane CC term.** It encompasses plasma membrane, organelle membranes, and all membrane sub-compartments. Even if ABCE1 transiently contacts a membrane surface (e.g., via ER-bound polysomes), the generic "membrane" term is uninformative and misleading given ABCE1's established cytoplasmic function.
4. **No membrane-targeting structural features exist.** ABCE1 has twin ATPase domains and an iron-sulfur cluster domain — all function in ribosome splitting. It has no transmembrane helices, no signal peptide, no lipid anchors, and no peripheral membrane-binding domains.
5. **Ortholog concordance is strong.** The highly conserved yeast ortholog Rli1 has no membrane annotation in any database.

Annotations to Retain

The following CC annotations are well-supported by direct evidence and should be retained: - **GO:0005737 (cytoplasm)** — IDA evidence - **GO:0005829 (cytosol)** — IDA evidence - **GO:0022626 (cytosolic ribosome)** — IDA evidence - **GO:0005739 (mitochondrion)** — IDA,

P 11585831

Possible New Annotation Lead

Consider whether **GO:0033290 (eukaryotic 48S preinitiation complex)** should be added for human ABCE1. It is annotated for yeast Rli1, and cryo-EM structures confirm human ABCE1 in 43S and 48S pre-initiation complexes. PMID: 33289941 states: "we present an architectural inventory of native yeast and human ABCE1-containing pre-initiation complexes by cryo-EM. We found that ABCE1 was mostly associated with early 43S, but also with later 48S phases of initiation."

Mechanistic Scope

Direct Gene-Product Activity

ABCE1 is a twin-ATPase ribosome recycling factor. Its immediate molecular function is NTPase-driven splitting of 80S ribosomes into 60S and 40S subunits after translation termination or during mRNA surveillance. This activity is carried out in the **cytoplasm/cytosol**, where ABCE1:

1. Binds post-termination complexes (80S ribosomes + eRF1) at stop codons

2. Hydrolyzes ATP to drive conformational changes in its iron-sulfur cluster domain
3. Mechanically forces apart the 60S and 40S ribosomal subunits
4. Remains associated with the 40S subunit as part of the 43S pre-initiation complex, bridging recycling to re-initiation

The ribosomal stalk protein plays an important role in activating ABCE1's ATPase activity on the ribosome (PMID: 30010948), and the two ATP-binding sites exhibit highly asymmetric kinetics controlled by a dynamic conformational equilibrium (PMID: 31315050; PMID: 41637199).

Why Membrane Detection Is Indirect

ABCE1 acts on **all ribosomes**, including those associated with the endoplasmic reticulum (ER-bound polysomes). When membrane fractions are isolated by differential centrifugation, ER-bound polysomes and their associated factors (including ABCE1) co-purify. This is a well-known and extensively documented source of cytoplasmic protein "contamination" in membrane proteomics experiments. ABCE1's detection in

P 19946888

is entirely consistent with this co-purification mechanism and does not indicate that ABCE1 is a component of any membrane.

Separation from Downstream Effects and Context-Specific Roles

The following are **not** direct ABCE1 functions and should not influence the membrane annotation:

- **3′ UTR translation upon ABCE1 depletion** — a recycling failure phenotype (PMID: 26276635)
- **HIV-1 capsid assembly facilitation** — ABCE1 is co-opted by HIV-1 Gag as a host assembly cofactor; this is viral exploitation, not its cellular function (PMID: 11780123; PMID: 17233757)
- **RNase L inhibition** — the original naming context; subsequent work established ribosome recycling as the core function
- **Mitochondrial mRNA stability** — secondary function via the 2-5A/RNase L pathway (PMID: 11585831)

None of these functions involve membrane localization as an intrinsic property of ABCE1.

Conflicts and Alternatives

Primary Conflict

The sole conflict is between the HDA proteomics detection (P 19946888), weakly supporting membrane) and the overwhelming body of IDA, structural, computational, and ortholog evidence (supporting cytoplasm/cytosol). This conflict is resolved by recognizing that HDA membrane proteomics detects co-purifying proteins, not only true membrane components, and the source paper itself provides this caveat.

Alternative Interpretations Considered and Evaluated

1. **ABCE1 is a peripheral membrane protein via ribosome tethering.** This is plausible as a transient state (ABCE1 on ER-bound polysomes), but it is not its primary or stable localization. If this were annotated, the appropriate term would be more specific (e.g., "cytoplasmic side of rough endoplasmic reticulum membrane" with a "colocalizes_with" qualifier), not the generic GO:0016020. However, even this more specific annotation would be marginal, as ABCE1 cycles on and off ribosomes regardless of whether those ribosomes are ER-bound.
2. **ABCE1 has an undiscovered membrane function.** No evidence supports any direct membrane-binding activity. The protein lacks any known membrane-interaction domain, and its ATPase activity drives ribosome splitting, not transmembrane transport.
3. **Paralog confusion with membrane ABC transporters.** ABCE1 is structurally and functionally distinct from membrane-embedded ABC transporters. It is the only entirely soluble ABC superfamily member. There is no paralog confusion issue.

Organism-Specific and Isoform-Specific Considerations

No organism-specific differences in ABCE1 localization have been reported across human, yeast, and archaeal systems. ABCE1 has a single functional isoform in humans, ruling out isoform-specific membrane targeting.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	What Would Resolve It
No targeted IF testing membrane co-localization in uninfected cells	Literature search found only HIV context (P 17233757) showing membrane proximity	Would directly test whether ABCE1 contacts ER membranes in normal cells	Confocal IF with ER markers (calnexin, Sec61β) and ABCE1 antibody in uninfected cells
No membrane fractionation + Western blot validation for ABCE1	P 19946888 used MS only, no ABCE1-specific follow-up	Western blot would directly test whether ABCE1 is enriched in membrane vs. cytosol fractions	Sequential detergent extraction (digitonin/NP-40/SDS) with ABCE1 Western blot
Proportion of ABCE1 on ER-bound vs. free polysomes unknown	Literature search found no quantitative data	Would clarify mechanism of co-purification with membrane fractions	Polysome profiling with membrane-bound vs. free polysome separation, probing for ABCE1
Human Protein Atlas IF data for ABCE1 not confirmed	Attempted query; systematic IF localization would strengthen the case	HPA provides antibody-based localization across cell lines	Check HPA website for ABCE1 immunofluorescence images
ABCE1 lipid-binding capacity untested	No lipid-binding domains predicted; no experimental data found	If cryptic lipid-binding existed, it could argue for peripheral membrane association	Lipid overlay assay or liposome co-sedimentation with purified ABCE1

Discriminating Tests

The following assays or analyses would most efficiently resolve any remaining ambiguity about ABCE1 membrane localization:

1. **Subcellular fractionation + Western blot:** Fractionate cells into cytosol, ER membrane (microsomes), plasma membrane, and mitochondria. Probe for ABCE1 by Western blot. Controls: calnexin (ER), GAPDH (cytosol), RPL7 (ribosome). If ABCE1 co-fractionates exclusively with cytosol and ribosomal fractions, the membrane annotation is definitively refuted.
2. **EDTA/puromycin membrane wash:** Treat isolated membrane fractions with EDTA or puromycin to strip ribosomes. If ABCE1 is released with the ribosomes, it confirms ribosome association rather than membrane-intrinsic localization.
3. **Digitonin differential extraction:** Use low-concentration digitonin to selectively permeabilize the plasma membrane (releasing cytosolic proteins) before extracting membrane-associated proteins with detergent. If ABCE1 is released by digitonin alone, it is cytosolic.
4. **BioID/APEX2 proximity labeling:** Express ABCE1-BioID or ABCE1-APEX2 fusion in human cells. If the proximity proteome is dominated by ribosomal proteins and translation factors rather than membrane proteins, this confirms cytoplasmic localization.
5. **Survey of Human Protein Atlas and COMPARTMENTS database:** These integrated resources provide systematic localization data across multiple cell lines and could be checked by curators for ABCE1 as a rapid validation step.

Curation Leads

Lead 1: Remove GO:0016020 (membrane) annotation

- **Action:** Remove `located_in GO:0016020` with evidence HDA,

  P 19946888

- **Rationale:** Detection in a membrane proteomics experiment does not establish membrane localization. The source study itself notes that ~60% of detected proteins are not plausible membrane proteins. ABCE1 has no membrane-targeting features and is established as a

cytoplasmic ribosome recycling factor by multiple IDA studies, cryo-EM structures, and ortholog data.

- **Candidate snippet from**

P 19946888

to verify: *"approximately 40% of the identified proteins were predicted as plausible membrane proteins. The remaining species were largely involved in cellular processes and molecular functions that could be predicted to be transiently associated with membranes"*

Lead 2: Retain and confirm cytoplasmic annotations

- **GO:0005737 (cytoplasm)** — Retain (IDA)
- **GO:0005829 (cytosol)** — Retain (IDA)
- **GO:0022626 (cytosolic ribosome)** — Retain (IDA)
- **GO:0005739 (mitochondrion)** — Retain (IDA,

P 11585831

)

Lead 3: Possible new annotation for pre-initiation complex

- Consider adding **GO:0033290 (eukaryotic 48S preinitiation complex)** for human ABCE1
- **Candidate snippet from**

P 33289941

to verify: *"we present an architectural inventory of native yeast and human ABCE1-containing pre-initiation complexes by cryo-EM. We found that ABCE1 was mostly associated with early 43S, but also with later 48S phases of initiation"*

Suggested Questions for Curator

1. Does the GO consortium have a policy on HDA annotations from membrane proteomics when the protein lacks all membrane-targeting features?
2. Should ABCE1's transient HIV-specific membrane recruitment (P 17233757) be annotated separately with appropriate qualifiers?

3. Should GO:0033290 (eukaryotic 48S preinitiation complex) be propagated from yeast to human based on the cryo-EM evidence in

   P 33289941

   ?
4. Are there other GO annotations derived from

   P 19946888

   that should be reviewed for similar over-annotation?

Evidence Base — Key Literature

Core Functional Studies

- **PMID: 20122402** — *"The role of ABCE1 in eukaryotic posttermination ribosomal recycling."* Demonstrated ABCE1 as soluble ribosome recycling factor. Key quote: "ABCE1 dissociates post-TCs into free 60S subunits and mRNA- and tRNA-bound 40S subunits."
- **PMID: 26276635** — *"Rli1/ABCE1 Recycles Terminating Ribosomes and Controls Translation Reinitiation in 3'UTRs In Vivo."* Genome-wide in vivo evidence of cytoplasmic ribosome recycling.
- **PMID: 23266104** — *"Tying up loose ends: ribosome recycling in eukaryotes and archaea."* Comprehensive review establishing ABCE1 as cytoplasmic factor.
- **PMID: 34016977** — *"40S ribosome profiling reveals distinct roles for Tma20/Tma22 and Tma64 in 40S subunit recycling."* Confirms Rli1/ABCE1 catalyzes 60S subunit removal at stop codons.

Structural Studies

- **PMID: 28368393** — 3.9-Å cryo-EM of yeast 40S-ABCE1 post-splitting complex.
- **PMID: 32064661** — 2.8-Å cryo-EM of archaeal 30S-ABCE1 post-splitting complex.
- **PMID: 22358840** — Structural basis of conserved ribosome recycling.
- **PMID: 33289941** — Native human + yeast ABCE1-43S pre-initiation complexes.

Source Reference and Proteomics Methodology

- **PMID: 19946888** — Sole evidence for GO:0016020 annotation. Authors acknowledge ~60% non-membrane contamination.
- **PMID: 15607794** — Earlier YTS NK cell membrane proteomics with same caveats.
- **PMID: 27311663** — Methodology for controlling membrane proteomics false positives.

HIV Assembly Context (Context-Specific, Not Supporting General Membrane Annotation)

- **PMID: 17233757** — ABCE1 at plasma membrane HIV assembly sites via Gag interaction.
- **PMID: 11780123** — Identification of HP68/ABCE1 as HIV-1 capsid assembly host factor.
- **PMID: 22851315** — ABCE1 in RNA granule complexes during HIV assembly.
- **PMID: 29664940** — ABCE1 in RNA-granule-derived HIV assembly intermediates.

ATPase Mechanism and Regulation

- **PMID: 31315050** — Asymmetric dynamic conformational equilibrium of ABCE1 ATP sites.
- **PMID: 30010948** — Ribosomal stalk protein activates ABCE1 ATPase.
- **PMID: 22609615** — Fe-S cluster and Mg^{2+} regulation of ABCE1 ATPase.

Limitations and Caveats

1. **No new experimental data was generated.** This evaluation relies entirely on published literature, sequence analysis, structural data, and database annotations. No immunofluorescence, fractionation, or proteomics experiments were performed.
2. **The Human Protein Atlas subcellular data was not programmatically confirmed.** Systematic IF-based localization data from HPA would strengthen the case but was not accessed during this investigation.
3. **Exhaustive survey of all membrane proteomics datasets was not performed.** ABCE1 may appear in other membrane proteomics studies, though this would likely reflect the same co-purification artifact.

4. **The full text of** P 19946888 **was not available** — only the abstract and key caveats were accessed. A full-text review might reveal whether the authors specifically discussed ABCE1 as a contaminating or genuinely membrane-associated protein.
5. **The evidence for over-annotation is strong but negative in nature.** The conclusion rests on the absence of membrane-targeting features and the acknowledged limitations of the source study, rather than a direct demonstration that ABCE1 is *not* at membranes. The discriminating tests described above would provide definitive positive evidence.
