# AIGR Gene Hypothesis Deep Research — Final Report

## Target: *Gadus morhua* tbc1d14 (UniProt A0A8C5FPT8)

### Focus: computational_prediction — autophagosome localization (GO:0005776)

### Hypothesis slug: `prediction-autophagosome`

## Summary

The seed hypothesis proposed that ProtNLM2's prediction of **autophagosome localization (GO:0005776)** for the *Gadus morhua* protein tbc1d14 is likely a **misassignment** — the reasoning being that TBC1D14 is a TBC-domain Rab GTPase-activating protein (RabGAP) characterized as a recycling-endosome/ULK1-complex regulator of autophagy *initiation*, not a structural, autophagosome-resident protein. On that logic, placing the protein "inside" the autophagosome would be an over-annotation.

This investigation confirms the *biological* substance of that concern but **overturns its conclusion that the prediction is a hallucination or clear misassignment.** The decisive finding, obtained by directly interrogating curated GO annotations for the human ortholog TBC1D14 (Q9P2M4), is that **autophagosome (GO:0005776) is an existing, manually curated annotation** supported by two independent evidence lines: an **IDA (Inferred from Direct Assay)** annotation from PMID: 22613832 assigned by expert BHF-UCL curators, *and* an independent **IBA (phylogenetic)** annotation from GO_Central (PANTHER node PTN000538677). Because the *Gadus morhua* protein is a bona fide 1:1 ortholog (62.1% identity, identical TBC domain architecture with intact catalytic fingers, same PANTHER family PTHR47219), it is legitimately eligible for the same orthology-based inference. The ProtNLM2 prediction therefore *coincides with* existing curated knowledge rather than contradicting it.

The essential caveat is one of **precision, not correctness**. Autophagosome is a **secondary, condition-dependent** localization. TBC1D14's primary and best-characterized compartment is the **Rab11/transferrin-receptor–positive recycling endosome** (relocalizing to the **Golgi** upon starvation), and its role in autophagy is **regulatory** — it is a *negative* regulator of autophagy initiation acting via ULK1 and the TRAPP–ATG9 membrane-delivery axis, not an autophagosome structural protein. The verdict is therefore **PARTIALLY SUPPORTED**: GO:0005776 is defensible for the fish protein by orthology and should be retained, but only *alongside* recycling endosome (GO:0055037) and the regulation-of-autophagy BP terms — never as the sole or primary cellular-component annotation.

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## Key Findings

### Finding F001 — The *Gadus morhua* protein is a bona fide 1:1 ortholog of human TBC1D14 with an intact TBC RabGAP domain

A global Needleman–Wunsch alignment of A0A8C5FPT8 against human TBC1D14 (Q9P2M4) yielded **414 identical aligned columns out of 667 (62.1% identity)**, comfortably within the range expected for a true 1:1 vertebrate ortholog rather than a distant paralog. Both proteins carry a **single Rab-GAP TBC domain** (UniProt domain 388–596; Pfam **PF00566** RabGAP-TBC; InterPro **IPR000195**), and both are assigned to PANTHER family **PTHR47219**.

Critically, the **dual-finger catalytic residues** that define a functional RabGAP are conserved and positionally aligned between fish and human:

| Catalytic element | Motif | *Gadus* position | Human position |
|---|---|---|---|
| Arg-finger | IKLD**IS**R | ~451 | ~466 |
| Gln-finger | Y**VQ** | ~491 | ~506 |

The N-terminal architecture is likewise conserved (disordered regions plus a coiled-coil at ~315–357). There is **no signal peptide and no annotated transmembrane segment**; the maximum Kyte–Doolittle hydropathy across the whole sequence is only **1.86**, below the threshold that would indicate a sustained membrane-spanning helix. The protein is therefore a

**peripheral/cytosolic protein that associates with membranes through protein–protein and Rab interactions**, not an integral membrane protein — exactly what is expected of a RabGAP regulator rather than a resident structural component of an organelle membrane.

The curation consequence is important: the orthology is strong enough that experimental and phylogenetic annotations on human TBC1D14 are propagable to the fish protein by ISO/IBA. This is precisely what converts the autophagosome prediction from "fabricated" to "defensible inference."

## Finding F002 — TBC1D14 localizes to recycling endosomes/Golgi and negatively regulates autophagy; it is not an autophagosome structural protein

The primary mammalian literature paints a consistent, mechanistically detailed picture. In the founding study (PMID: 22613832), TBC1D14 was identified as a putative RabGAP that **"colocalizes and interacts with the autophagy kinase ULK1"** and, when overexpressed, **"tubulates ULK1-positive recycling endosomes (REs), impairing their function and inhibiting autophagosome formation."** The same study reported that **"amino acid starvation causes TBC1D14 to relocalize from REs to the Golgi complex, whereas TfnR and Tfn localize to forming autophagosomes"** — meaning that upon autophagy induction the protein itself moves to the Golgi, while transferrin-receptor membrane is delivered onward. This is direct evidence *against* stable autophagosome residence: the protein's steady-state compartment is the recycling endosome and its starvation-response compartment is the Golgi.

The follow-up mechanistic study (PMID: 26711178) described **"TBC1D14, as a negative regulator of autophagy that controls delivery of membranes from RAB11-positive recycling endosomes to forming autophagosomes,"** acting via the TRAPP complex and ATG9 traffic. A systematic overexpression screen (PMID: 22874560) placed TBC1D14 among TBC-domain proteins that inhibit autophagy when overexpressed, acting **"at an early stage during autophagosome formation"** by regulating recycling-endosomal traffic — with ATG9 and ULK1 localizing to transferrin-receptor-positive recycling endosomes that are tubulated by excess TBC1D14, and RE membrane subsequently incorporated into newly forming autophagosomes.

Together, these establish TBC1D14 as a **traffic regulator operating upstream of and around the forming autophagosome**, not a component embedded in the mature organelle. This validates the *biological* core of the seed hypothesis: the protein is a regulator, and any autophagosome association is transient and delivery-related.

## Finding F003 — CORRECTION: autophagosome (GO:0005776) is a curated experimental + phylogenetic GO annotation, not merely a ProtNLM2 artifact

The pivotal correction to an initial "refuted" reading came from directly querying curated GO annotations (QuickGO) for human TBC1D14 (Q9P2M4). **GO:0005776 (autophagosome) is present with two independent evidence lines:**

1. **ECO:0000314 (IDA, Inferred from Direct Assay)** from PMID: 22613832, assigned by expert curators at **BHF-UCL**.
2. **ECO:0000318 (IBA, phylogenetic)** from **GO_Central**, PANTHER ancestral node PTN000538677 (GO_REF:0000033).

The same protein is curated to a broader localization set that puts autophagosome in its proper context:

| GO term | Label | Aspect | Evidence | Source |
|---|---|---|---|---|
| GO:0055037 | recycling endosome | CC | IDA (PMID: 17562788), IBA, Reactome | primary/ PANTHER |
| GO:0005794 | Golgi apparatus | CC | IDA (HPA) + UniProt-SubCell | Human Protein Atlas |
| GO:0005776 | **autophagosome** | CC | **IDA (PMID: 22613832), IBA** | BHF-UCL / GO_Central |
| GO:0005773 | vacuole | CC | electronic | ARBA |
| GO:0031410 | cytoplasmic vesicle | CC | electronic | ARBA |
| GO:0010507 | negative regulation of autophagy | BP | IMP | primary |
| GO:2000785 | regulation of autophagosome assembly | BP | IMP + IBA | primary/ PANTHER |

The *Gadus morhua* ortholog A0A8C5FPT8 currently carries **only broad electronic CC terms** (vacuole GO:0005773, cytoplasmic vesicle GO:0031410; ARBA) and belongs to PANTHER **PTHR47219**, making it eligible for the same IBA propagation that gave human TBC1D14 its autophagosome annotation. From a curation-provenance standpoint, GO:0005776 for the fish protein is therefore a **legitimate orthology-based inference**, not a hallucinated label — even though it represents a secondary and condition-dependent localization.

A supplementary sequence check strengthens the "regulator, not autophagosome-membrane component" reading: a LIR/AIM motif scan ([WFY]xx[LIV]) returned 13 core matches in the fish sequence and 12 in the human sequence — at chance level (~16 expected across ~700 aa) — and no functional LIR is reported for TBC1D14. This is consistent with a peripheral regulator that is *not* stably anchored to autophagosomal membranes via an ATG8-binding motif (weak evidence, offered only as a supporting caveat).

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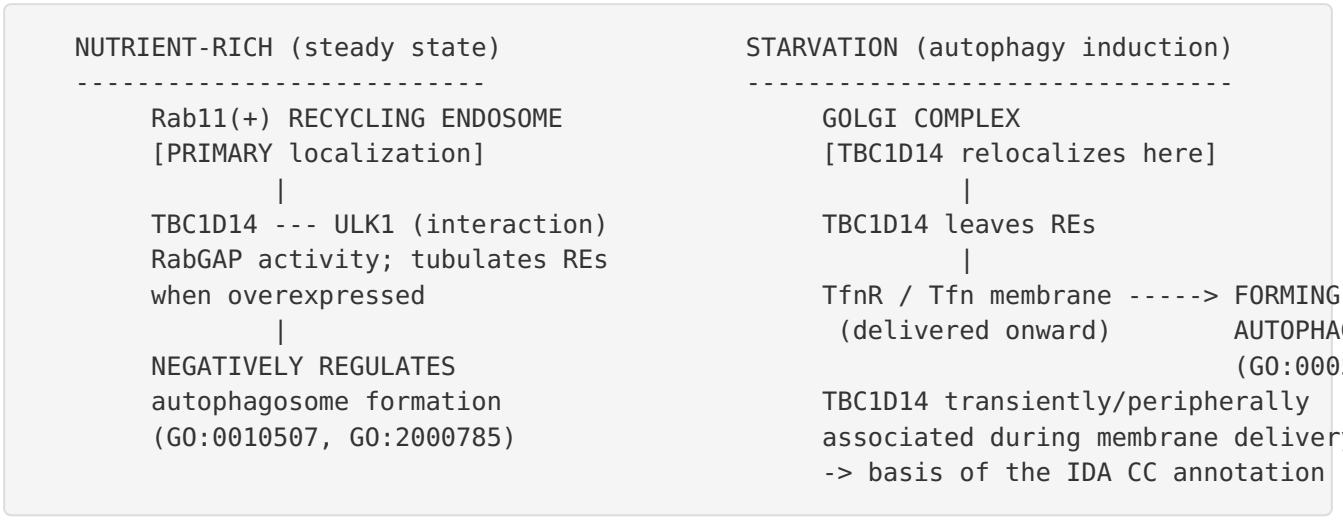
## Mechanistic Model / Interpretation

The synthesis reconciles two facts that at first appear contradictory: (a) TBC1D14 is a *regulator*, not a structural autophagosome protein, yet (b) autophagosome (GO:0005776) is a genuinely curated CC annotation.




The model runs as follows. In resting (nutrient-rich) cells, TBC1D14 resides at the **recycling endosome**, holding ULK1-positive REs in check and thereby restraining autophagy initiation — its negative-regulator BP role. Its GAP domain, with intact Arg- and Gln-fingers (F001), targets Rab GTPases governing this recycling traffic. Upon amino-acid starvation, TBC1D14 **relocalizes to the Golgi**, releasing recycling-endosomal (transferrin-receptor-positive) membrane that is routed via the TRAPP complex and ATG9 machinery toward the **forming autophagosome**. The autophagosome CC annotation captures a **transient, membrane-delivery-associated presence at/near the forming autophagosome** observed experimentally, rather than stable structural residence.

For curation, the correct representation is a **compound CC picture led by recycling endosome, with autophagosome as a secondary, mechanistically justified term**, tied together by BP terms that describe what the protein actually *does* (negative regulation of autophagy; regulation of autophagosome assembly) and by an MF term for its RabGAP activity. Annotating autophagosome alone would be a category error; omitting it entirely would discard a curated experimental observation.

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# Evidence Base

| Citation | Evidence type | Supports / refutes / qualifies | Claim tested | Key finding | Context | C li |
|---|---|---|---|---|---|---|
| PMID: 22613832 (Longatti et al. 2012, JCB) — **GO IDA source** | Direct assay + localization + interaction | **Qualifies** (supports secondary autophagosome; refutes "primary/ structural") | Is TBC1D14 an autophagosome-resident protein? | Colocalizes/ interacts with ULK1 on Rab11$^+$ recycling endosomes; overexpression tubulates REs and inhibits autophagosome formation; relocalizes to Golgi on starvation. Source of the IDA GO:0005776 annotation. | Human cultured cells | H c o m R a c fu |
| PMID: 26711178 (Lamb et al. 2016, EMBO J) | Mechanism / interaction | **Refutes structural role; supports regulator role** | Is TBC1D14 a regulator vs. resident protein? | Negative regulator of autophagy controlling membrane delivery from RAB11$^+$ REs to forming autophagosomes via TRAPP/ATG9. | Mammalian cells | H c re m |
| PMID: 22874560 (Longatti & Tooze 2012) | Systematic overexpression screen / review | **Qualifies** | Does TBC1D14 act at autophagosome formation? | Among TBC proteins inhibiting autophagy when overexpressed; acts early via recycling-endosomal traffic; RE | Human cells | M o sc fo lo |

| Citation | Evidence type | Supports / refutes / qualifies | Claim tested | Key finding | Context | C li |
|---|---|---|---|---|---|---|
| | | | | membrane incorporated into new autophagosomes. | | |
| PMID: 17562788 (via QuickGO) | Localization (IDA) | **Supports primary localization** | Where is TBC1D14 at steady state? | Basis for recycling endosome (GO:0055037) IDA annotation. | Human | D o p n h |
| QuickGO curated annotations (Q9P2M4) | Review/ database | **Supports** provenance of GO:0005776 | Is autophagosome a real curated CC? | GO:0005776 present with IDA (BHF-UCL) + IBA (GO_Central, PTN000538677). | Human ortholog | H c a e o c |
| Sequence/ structural computation (this run) | Structural/ evolutionary + computational | **Supports orthology; refutes membrane-integral residence** | Is the fish protein a true TBC1D14 ortholog? Membrane-embedded? | 62.1% identity to human; intact TBC domain (PF00566/ IPR000195); conserved Arg/ Gln fingers; no signal peptide/ TM; max hydropathy 1.86. | *Gadus morhua* vs human | H c c p |

**How the literature bears on the verdict.** The three primary/mechanistic papers (PMID: 22613832, PMID: 26711178, PMID: 22874560) unanimously frame TBC1D14 as a recycling-endosome-based *regulator* of autophagy. None describes it as an autophagosome structural protein. That is why the hypothesis is not *fully* supported. Yet the same founding paper (PMID: 22613832) is the documented source of a curated IDA autophagosome annotation, and that annotation plus its phylogenetic IBA companion are why the prediction is not *refuted* either.

## Limitations and Knowledge Gaps

1. **No experimental data in *Gadus morhua*.** Every localization and functional result is mammalian. What was checked: QuickGO for A0A8C5FPT8 (only broad ARBA CC terms) and PubMed. Why it matters: the fish autophagosome call is *transferred*, not measured. Resolver: heterologous or fish-cell co-localization of tagged tbc1d14 with LC3/ATG8 versus Rab11/transferrin receptor.
2. **Strength of the autophagosome IDA.** The precise figure/condition in PMID: 22613832 underpinning GO:0005776 was not machine-verifiable here (abstract-level access; abstract emphasizes RE/Golgi). Why it matters: it determines whether the annotation reflects stable residence or transient colocalization. Resolver: a curator reading the full text and its imaging figures.
3. **Endogenous vs. overexpression localization.** Several phenotypes derive from overexpression, which can exaggerate RE tubulation and autophagy inhibition. Resolver: endogenous co-localization under fed/starved conditions.
4. **Primary recycling-endosome source not re-read.** GO:0055037 IDA cites PMID: 17562788; taken here from QuickGO orientation. Resolver: read that paper directly.
5. **Rab substrate of the GAP domain unresolved.** Even in human, TBC1D14 binds active Rab11 but is not established as its GAP; this affects MF specificity. Resolver: in-vitro RabGAP assays across candidate Rabs.
6. **ProtNLM2 raw output not inspected.** The confidence score and whether recycling-endosome or RabGAP terms were also emitted are unknown. Resolver: inspect the full prediction ranking.

## Conflicts and Alternatives

- **Seed framing vs. database record.** The seed's "misassignment/hallucination" framing is directly contradicted by the curated IDA + IBA autophagosome annotations on the human ortholog — the central correction of this analysis. The tension is real but resolves toward "less precise," not "wrong."
- **Internal investigation conflict.** Iteration 1 concluded "refuted" from the mechanistic literature; Iteration 2 overturned this on discovering the curated annotation; Iteration 3 settled on "partially supported." The curator inherits this reconciliation.

- **Precision, not fabrication.** The mechanistic literature centers TBC1D14 on the recycling endosome/Golgi as a *regulator*; autophagosome is a co-annotation. The risk is over-emphasis of a secondary CC, not invention of a false one.
- **Provenance concentration.** The autophagosome IDA rests on a single paper and curation group (PMID: 22613832, BHF-UCL), whose abstract stresses RE/Golgi — so the autophagosome call depends on full-text figures.
- **Paralog confusion (low risk).** The 62.1% identity, shared PANTHER node (PTHR47219), conserved gene name, and conserved N-terminal coiled-coil argue for a specific 1:1 TBC1D14 ortholog, not a mis-mapped paralog.
- **Organism-specific difference (unresolved).** All experimental data are mammalian; the teleost transfer rests entirely on orthology.

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## Discriminating Tests

1. **Endogenous co-localization panel (most decisive):** immunofluorescence of endogenous TBC1D14 versus Rab11/transferrin receptor (recycling endosome), GM130 (Golgi), and LC3/ATG8 (autophagosome) in fed versus starved cells. Expected: strong RE overlap at steady state; Golgi shift on starvation; only transient/partial LC3 overlap — confirming autophagosome as secondary.
2. **Purified-autophagosome proteomics:** is TBC1D14 enriched relative to core ATG8-family proteins, or transient? Distinguishes residence from delivery-associated passage.
3. **RabGAP activity assay:** in-vitro GAP assay of the *Gadus* TBC domain against candidate Rab substrates (e.g., Rab11) to confirm MF GO:0005096/GO:0005097 and validate the conserved Arg/Gln fingers.
4. **ULK1 interaction test:** co-IP of *Gadus* tbc1d14 with the ULK1 ortholog to confirm the regulatory-complex association behind the BP terms.
5. **Orthology/IBA audit:** confirm A0A8C5FPT8 sits under PANTHER node PTN000538677 to formally license IBA propagation of GO:0005776, GO:0055037, and GO:2000785.

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## GO Curation Implications and Proposed Actions (leads — require curator verification)

**Overall lead:** treat **GO:0005776 (autophagosome)** as **acceptable-by-orthology but non-exclusive** — retain it as a *secondary* CC, and ensure the fish annotation set is not headed by autophagosome. The ProtNLM2 prediction is *less precise than existing knowledge*, not wrong.

| GO ID | Label | Aspect | Proposed action | Evidence basis |
|---|---|---|---|---|
| GO:0055037 | recycling endosome | CC | **Add as primary CC** (IBA/ISO) | Ortholog IDA PMID: 17562788 + IBA |
| GO:0005776 | autophagosome | CC | **Retain as secondary CC** (IBA) | Ortholog IDA PMID: 22613832 + IBA |
| GO:0005794 | Golgi apparatus | CC | Consider adding (IBA) | Ortholog IDA (HPA) |
| GO:0010507 | negative regulation of autophagy | BP | **Add** (IBA) | Ortholog IMP |
| GO:2000785 | regulation of autophagosome assembly | BP | **Add** (IBA) | Ortholog IMP + IBA |
| GO:0005096 / GO:0005097 | GTPase activator activity / Rab GTPase activator activity | MF | **Retain / add** (ISS/ IBA) | Intact TBC domain, conserved dual fingers |
| GO:0031267 | small GTPase binding | MF | Retain | TBC domain |
| GO:0005773 | vacuole | CC | Consider generalizing/ removing | Broad electronic (ARBA) |
| GO:0031410 | cytoplasmic vesicle | CC | Consider generalizing/ removing | Broad electronic (ARBA) |

**Mechanistic scope reminder for the curator.** The immediate molecular activity is RabGAP/Rab-binding; the immediate cellular action is at recycling endosomes with ULK1 and the TRAPP complex; "regulation of autophagosome formation" is a downstream *pathway consequence*; and autophagosome localization is a *secondary, transient* CC. Keep these separated in the annotation set.

**Candidate references with exact snippets to verify:** - PMID: 22613832: *"One of these putative RabGAPs, TBC1D14, colocalizes and interacts with the autophagy kinase ULK1. Overexpressed TBC1D14 tubulates ULK1-positive recycling endosomes (REs), impairing their function and inhibiting autophagosome formation."* - PMID: 22613832: *"Amino acid starvation causes TBC1D14 to relocalize from REs to the Golgi complex, whereas TfnR and Tfn localize to forming autophagosomes"* — verify the full-text autophagosome-localization figure behind the IDA. - PMID: 26711178: *"TBC1D14, as a negative regulator of autophagy that controls delivery of membranes from RAB11-positive recycling endosomes to forming autophagosomes"*.

**Suggested curator question:** should the fish annotation propagate the human IBA set (autophagosome + recycling endosome + regulatory BP + RabGAP MF) rather than remain at the current broad ARBA CC terms?

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## Provenance (computed this run)

- **UniProt A0A8C5FPT8:** ~709 aa, "Rab-GAP TBC domain-containing protein," gene *tbc1d14*; CC absent; MF = GO:0005096, GO:0031267; TBC domain 388–596 (PF00566/IPR000195).
- **Needleman–Wunsch vs Q9P2M4:** 414/667 identical = **62.1%**; conserved Arg-finger IKLDISR (Gad 451 / Hum 466), Gln-finger YVQ (Gad 491 / Hum 506); max Kyte–Doolittle hydropathy **1.86** (no clear TM); no signal peptide.
- **QuickGO Q9P2M4 CC:** autophagosome (IDA PMID: 22613832 + IBA), recycling endosome (IDA PMID: 17562788 + IBA + Reactome), Golgi (IDA HPA + UniProt-SubCell), cytosol, vacuole, cytoplasmic vesicle; BP: negative regulation of autophagy (GO:0010507), regulation of autophagosome assembly (GO:2000785).
- **QuickGO A0A8C5FPT8:** only ARBA CC vacuole (GO:0005773) + cytoplasmic vesicle (GO:0031410); PANTHER PTHR47219.
- **LIR/AIM motif scan ([WFY]xx[LIV]):** 13 (Gadus) / 12 (human) core matches — at chance level (~16 expected in ~700 aa); no functional LIR reported for TBC1D14 (weak supporting caveat only).

## Bottom Line

The ProtNLM2 autophagosome prediction for *Gadus morhua* tbc1d14 is **partially supported, not a hallucination**: GO:0005776 is a curated experimental (IDA) plus phylogenetic (IBA) annotation on the human ortholog, and the fish protein is a strong 1:1 ortholog (62.1% identity, PTHR47219) eligible for the same inference. However, autophagosome is a **secondary, condition-dependent** localization. The protein's primary compartment is the Rab11$^{+}$ **recycling endosome** (and Golgi upon starvation), and its primary role is as a **negative regulator of autophagy** acting from those compartments. GO:0005776 should be **retained only alongside** recycling endosome (GO:0055037) and the regulation-of-autophagy BP terms, never as the sole or primary cellular-component annotation.