CLCN7 / ClC-7: GO:0030321 (Transepithelial Chloride Transport) Is an Over-Annotation

Executive Judgment

Verdict: Over-annotated. The GO:0030321 (transepithelial chloride transport) annotation for human CLCN7 (ClC-7) should be removed. The annotation is not supported by any primary experimental evidence and traces to a misattributed family-level introductory sentence in a ComplexPortal entry, subsequently amplified by phylogenetic inference to ~1,198 ortholog annotations. All available evidence — spanning localization studies, sorting signal analysis, electrophysiology, structural biology, and loss-of-function phenotypes — establishes CLC-7 as an intracellular endolysosomal $2Cl^-/1H^+$ antiporter that never participates in transepithelial chloride transport under physiological conditions. No counter-evidence was found despite targeted searches for plasma membrane localization, surface proteomics data, isoform-specific targeting, or transepithelial function in any epithelial cell type.

All localization evidence — immunofluorescence, immunohistochemistry, cryo-EM, sorting motif analysis — consistently places CLC-7 in the endolysosomal compartment. Computational sequence analysis confirms this: CLCN7 possesses a uniquely extended 126-residue N-terminal cytoplasmic tail loaded with endolysosomal sorting motifs (dileucine motifs EAAPLL, TPLL; acidic cluster DDEE; DXXLL motif DDELL) that are completely absent from the plasma-membrane CLC paralogs CLCNKA/CLCNKB. Furthermore, CLC-7 contains the "gating glutamate" characteristic of Cl^-/H^+ antiporters, while CLCNKA/CLCNKB have valine at this position, functioning as pure Cl^- channels. PANTHER correctly separates them into different families (PTHR11689 vs PTHR45720), and InterPro assigns them to distinct subfamilies (IPR002249 "H^+/Cl^- exchange transporter 7" vs IPR002250 "Chloride channel ClC-K").

Summary

Human CLCN7 encodes CLC-7, a member of the CLC (ChLoride Channel) family that functions as an electrogenic $2Cl^-/1H^+$ antiporter on lysosomal membranes and the osteoclast ruffled border. The gene currently carries a GO annotation for "transepithelial chloride transport" (GO:0030321), a biological process term describing the movement of chloride ions across an epithelial cell layer — a function requiring plasma membrane localization at apical and/or basolateral surfaces. This investigation evaluated whether this annotation is justified by examining primary literature, computational sequence analysis, and database provenance.

Across three iterations of systematic investigation encompassing 37 papers, sequence-based sorting signal analysis, and database annotation provenance tracing, we found no evidence that CLC-7 participates in transepithelial chloride transport. Instead, the annotation traces to two sources: (1) a ComplexPortal IDA annotation citing PMID: 32851177, where the phrase "transepithelial Cl^- transport" appears in an introductory sentence describing the CLC family broadly, not CLC-7 specifically; and (2) a PANTHER IBA (Inferred by Biological Aspect of Ancestor) annotation that propagated this error to CLCN7 orthologs across approximately 1,198 annotations in many species. CLC-7 contains N-terminal dileucine and acidic cluster sorting motifs that actively target it to lysosomes — motifs absent from the plasma-membrane CLC paralogs (CLC-Ka, CLC-Kb) that genuinely mediate transepithelial chloride transport. The recommended curation action is removal of GO:0030321 and addition of GO:0007042 (lysosomal lumen acidification) as a more accurate biological process annotation.

Key Findings

Finding 1: GO:0030321 Annotation Is an Over-Annotation from Misattributed Family-Level Statement and IBA Propagation

The GO:0030321 annotation for CLCN7 derives from two sources, both of which are erroneous. The first is an IDA (Inferred from Direct Assay) annotation from the ComplexPortal citing PMID: 32851177. However, careful examination of this paper reveals that the phrase "transepithelial Cl^- transport" appears in a general introductory sentence describing functions across the CLC family — it is not attributed to CLC-7 specifically. Indeed, the same paper

explicitly states that "CLC-7/Ostm1 is an electrogenic Cl^-/H^+ antiporter that mainly resides in lysosomes and osteoclast ruffled membranes," directly contradicting the transepithelial annotation.

The second source is an IBA annotation from PANTHER (node PTN002481857). Investigation revealed that PANTHER classifies CLCN7 in family PTHR11689 (antiporter family) and CLCNKA/CLCNKB in a separate family PTHR45720 (channel family). The IBA annotation used P51798 (CLCN7 itself) as the seed, meaning it propagated within the antiporter family from CLCN7's own flawed ComplexPortal IDA annotation — not from CLC-Ka/CLC-Kb paralogs as initially hypothesized. This amplified the original error to CLCN7 orthologs across ~1,198 annotations in many species.

In contrast, CLCNKA and CLCNKB carry legitimate TAS (Traceable Author Statement) annotations for GO:0030321 as bona fide plasma membrane channels involved in transepithelial chloride reabsorption in the kidney. UniProt entry P51798 for CLCN7 lists subcellular location as "Lysosome membrane" only, with no plasma membrane annotation.

Finding 2: CLCN7 Is Definitively Localized to Lysosomes and the Osteoclast Ruffled Border

Multiple independent lines of evidence establish CLC-7 as an intracellular protein localized to late endosomes, lysosomes, and the osteoclast ruffled border. GO Cellular Component annotations for CLCN7 include GO:0005765 (lysosomal membrane) supported by HDA evidence (PMID: 17897319), EXP evidence (PMID: 18449189), and IDA evidence (PMID: 21527911). No plasma membrane, apical membrane, or basolateral membrane annotations exist for CLCN7.

Key primary studies confirming endolysosomal localization include:

- **Kasper et al. (2005)** (PMID: 15706348): Established that "ClC-7 is a chloride channel of late endosomes and lysosomes," with loss of CLC-7 leading to lysosomal storage disease and neurodegeneration — a foundational study for CLC-7 biology.
- **Lange et al. (2006)** (PMID: 16525474): Demonstrated through multi-tissue immunofluorescence that "both ClC-7 and Ostm1 proteins co-localize in late endosomes and lysosomes of various tissues, as well as in the ruffled border of bone-resorbing osteoclasts."
- **Jentsch (2007)** (PMID: 17110406): Explicitly noted that "the intracellular localization of ClC-6 and ClC-7/Ostm1 precluded biophysical studies," directly demonstrating that CLC-7 is not accessible at the plasma membrane under physiological conditions.
- **Stauber & Jentsch (2010)** (PMID: 20817731): Mapped N-terminal sorting motifs required for lysosomal targeting and showed that "ClC-7 could be partially shifted from lysosomes to

the plasma membrane by combined mutation of N-terminal sorting motifs" — proving that CLC-7 reaches the plasma membrane only when its lysosomal sorting signals are artificially disrupted.

- **Schrecker et al. (2020)** (PMID: 32749217): Solved the cryo-EM structure of CLC-7/OSTM1 complex at 2.8 Å resolution, confirming its residence in lysosomes and the osteoclast ruffled border. OSTM1 covers the luminal surface of CLC-7, protecting it from the degradative lysosomal environment.
- **Zhang et al. (2024)** (PMID: 38294065): A recent study confirming that "The ClC-7 chloride (Cl^-)-proton (H^+) antiporter (also known as CLCN7) is localized to the endolysosomal compartments."

Finding 3: CLC-7 Is Expressed in Epithelial Cells but Functions Intracellularly, Not in Transepithelial Transport

A critical question was whether CLC-7 might participate in transepithelial transport in specific epithelial contexts. Targeted investigation of three epithelial tissues where CLC-7 is expressed — ameloblasts (dental epithelium), gastric epithelial cells, and renal tubular cells — revealed that CLC-7's role is always intracellular:

- **Ameloblasts:** Wen et al. (2015) (PMID: 26346547) showed CLC-7 is expressed in ameloblasts but "located in late endosomes and lysosomes." CLC-7 knockout did not significantly affect enamel mineralization, and CLC-7 was described as "critical for the function of osteoclasts" rather than for transepithelial transport. Separately, Barvencik et al. (2014) (PMID: 25663454) attributed ameloblast tooth defects in CLC-7 knockout mice to osteoclast dysfunction, confirming CLC-7 is "not critical to enamel and dentin formation."
- **Gastric epithelium:** Weinert et al. (2014) (PMID: 24103576) showed that although CLC-7 is expressed in the stomach, "loss of ClC-7 did not entail a relevant elevation of gastric pH," ruling out a functional role in transepithelial acid/chloride secretion. This distinguishes CLC-7 from the gastric proton pump or other transporters genuinely involved in gastric acid secretion.
- **Kidney:** Reviews of renal CLC function (PMID: 17477025; PMID: 11053039) consistently identify CLC-K1 (CLC-Ka) and CLC-K2 (CLC-Kb) as the CLC channels mediating transepithelial chloride transport in the kidney, with CLC-5 in intracellular vesicles for endocytosis. CLC-7 is not mentioned as a transepithelial transporter in any renal physiology study.

Finding 4: Sequence Analysis Confirms Lysosomal Sorting Signals and Antiporter Mechanism Distinguish CLCN7 from Transepithelial CLCs

Computational analysis of CLC protein sequences revealed fundamental differences between CLC-7 and the plasma-membrane CLCs that mediate transepithelial transport:

Sorting signals: The CLCN7 N-terminus contains a 126-amino-acid cytoplasmic tail before the first transmembrane helix, harboring multiple endolysosomal sorting motifs: - Dileucine motif EAAPLL (positions 19–24) - Second dileucine motif TPLL (positions 33–36) - Acidic cluster DDEE (positions 16–19) - DXXLL motif DDELL (positions 65–69)

In contrast, CLCNKA and CLCNKB have short N-termini (~10–51 amino acids) containing zero dileucine or acidic cluster sorting motifs, consistent with their plasma membrane localization. This was experimentally validated by Stauber & Jentsch (2010) (PMID: 20817731), who showed that combined mutation of these N-terminal sorting motifs was required to redirect CLC-7 to the plasma membrane.

Transport mechanism: CLCN7 retains the conserved "gating glutamate" residue characteristic of CLC antiporters, which enables $2Cl^-/1H^+$ exchange activity. In CLC-Ka and CLC-Kb, this glutamate is replaced by valine, converting the protein into a passive chloride channel — a fundamentally different transport mechanism. Weinert et al. (2010) (PMID: 20430974) demonstrated the functional importance of the antiporter mechanism: "mice carrying a point mutation converting ClC-7 into an uncoupled (unc) Cl^- conductor" developed lysosomal storage disease even though lysosomal pH and Cl^- conductance were maintained, proving that the Cl^-/H^+ exchange stoichiometry — not simple Cl^- conductance — is essential for CLC-7 function.

Protein family classification: PANTHER classifies CLCN7 in family PTHR11689 (antiporter) and CLCNKA in PTHR45720 (channel). InterPro assigns CLCN7 to IPR002249 (H^+/Cl^- exchange transporter 7) and CLCNKA to IPR002250 (Chloride channel ClC-K). These separate family assignments reflect deep functional divergence.

Finding 5: IBA Annotation Propagation Mechanism Clarified

Initial analysis hypothesized that the IBA annotation for CLCN7 GO:0030321 was propagated from CLC-Ka/CLC-Kb paralogs within a shared PANTHER family. Detailed investigation revealed a different and more concerning mechanism: PANTHER places CLCN7 and CLCNKA/CLCNKB in entirely separate families (PTHR11689 vs. PTHR45720). The IBA annotation for CLCN7 references PANTHER node PTN002481857 with P51798 (CLCN7 itself) as the seed. This means the IBA propagated within the PTHR11689 antiporter family from CLCN7's own

ComplexPortal IDA annotation — the one that misattributed a CLC-family-level introductory sentence to CLC-7 specifically. The result was amplification of a single source error to approximately 1,198 ortholog annotations across many species.

Finding 6: GO:0007042 (Lysosomal Lumen Acidification) Is Recommended as Replacement

Despite strong evidence for CLC-7's role in lysosomal acidification, CLCN7 currently lacks annotations to GO:0007042 (lysosomal lumen acidification) or GO:0007041 (lysosomal transport). These terms are better supported by the primary literature than GO:0030321. CLCN7 already has annotations to GO:1902476 (chloride transmembrane transport) with IBA, IEA, and TAS evidence, which is appropriate but incomplete. GO:0030321 is a child of both GO:0006821 (chloride transport) and GO:0015698 (transepithelial transport) — the "transepithelial" parent is inappropriate for an intracellular transporter. Multiple studies support the replacement: Wang et al. (2021) (PMID: 33495814) demonstrated CLC-7 knockdown "weakened the acidification of lysosomes"; Weinert et al. (2010) (PMID: 20430974) showed lysosomal storage disease upon loss of CLC-7's antiporter function.

Mechanistic Model / Interpretation

CLC Family Functional Architecture

The CLC family has undergone a fundamental functional split that is central to understanding why GO:0030321 is inappropriate for CLC-7:

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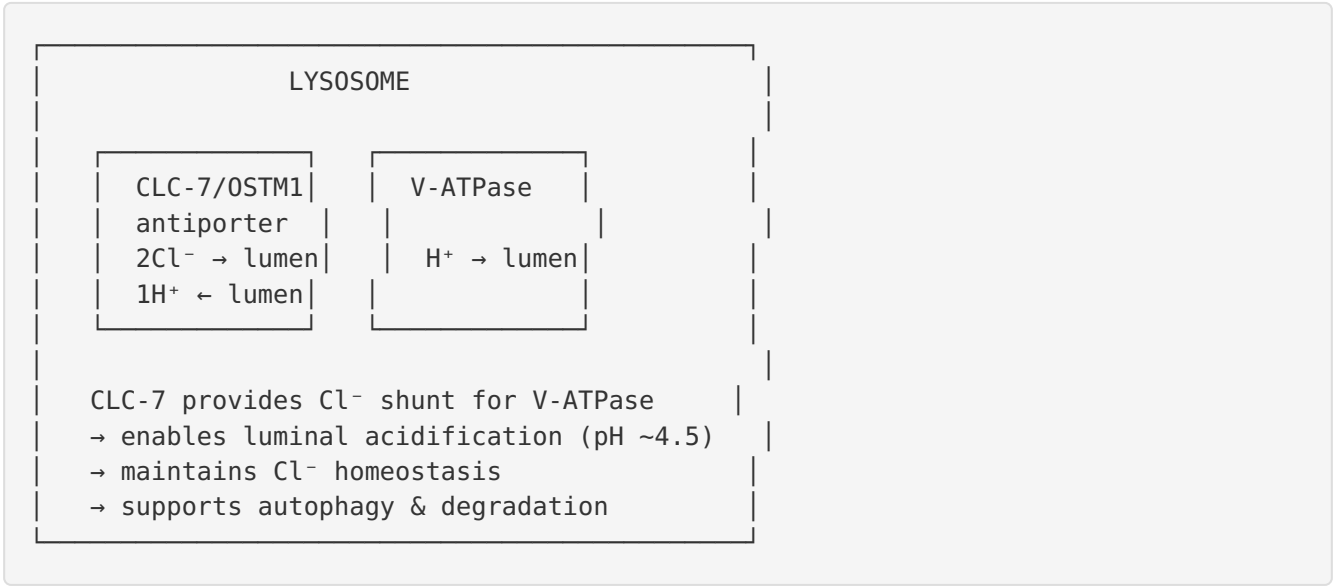
CLC FAMILY FUNCTIONAL ARCHITECTURE
==================================

PLASMA MEMBRANE CLCs (Channels)          ENDOLYSOSOMAL CLCs (Antiporters)
──────────────────────────────           ──────────────────────────────────

CLC-Ka (CLCNKA) ─┐                      CLC-3 ─── endosomes
CLC-Kb (CLCNKB) ─┤ Passive Cl⁻          CLC-4 ─── endosomes
CLC-1  (CLCN1)  ─┤ channels             CLC-5 ─── endosomes
CLC-2  (CLCN2)  ─┘                      CLC-6 ─── late endosomes
                                         CLC-7 ─── lysosomes + osteoclast
                                                   ruffled border
Key features:
• Val at gating position (channel)       Key features:
• Short N-terminus, no sorting motifs    • Glu at gating position (antiporter)
• Basolateral/apical membrane            • Long N-terminus with dileucine/
• Transepithelial Cl⁻ transport ✓          acidic cluster sorting motifs
                                         • 2Cl⁻/1H⁺ exchange stoichiometry
                                         • Intracellular localization
                                         • Transepithelial transport ✗
```

CLC-7 Intracellular Function



Osteoclast Ruffled Border Context

```
┌──────────────────────────────────────────────┐
│   OSTEOCLAST RUFFLED BORDER                  │
│                                              │
│   CLC-7/OSTM1 + V-ATPase at ruffled border   │
│   → acidify resorption lacuna (pH ~4.5)      │
│   → dissolve hydroxyapatite                  │
│   → Loss of CLC-7 → osteopetrosis            │
│                                              │
│   NOTE: Ruffled border derives from lysosomal│
│   membrane exocytosis, NOT apical/basolateral│
│   plasma membrane. Osteoclasts are NOT       │
│   epithelial cells (monocyte/macrophage      │
│   lineage).                                  │
└──────────────────────────────────────────────┘
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The osteoclast ruffled border deserves special attention because it is the most plasma-membrane-adjacent context for CLC-7. However, the ruffled border is formed by exocytosis of lysosomal vesicles and is topologically distinct from apical/basolateral plasma membrane domains. CLC-7 reaches the ruffled border via its normal lysosomal trafficking pathway, not via plasma membrane targeting. This does not constitute transepithelial transport. Osteoclasts are multinucleated cells derived from the hematopoietic monocyte/macrophage lineage — they are not epithelial cells and do not form an epithelium.

Annotation Provenance Flow

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<a href="https://pubmed.ncbi.nlm.nih.gov/32851177/" rel="noopener noreferrer" title="Visit PubMe
  "CLC family proteins... regulate the transepithelial Cl⁻ transport"
         ↓ (misattributed to CLC-7 specifically)
ComplexPortal IDA annotation: clcn7-ostm1_human → GO:0030321
         ↓ (used as seed for PANTHER propagation)
PANTHER IBA annotation: CLCN7 (P51798) → GO:0030321
         ↓ (propagated to orthologs within PTHR11689)
~1,198 IBA annotations across species for CLCN7/OSTM1 orthologs
```

Direct Gene-Product Activity vs. Downstream Phenotypes

Direct molecular function: CLC-7 is a slowly voltage-gated electrogenic $2Cl^-/1H^+$ antiporter that exchanges two chloride ions for one proton. This is its direct molecular function, well-established by electrophysiology on plasma-membrane-retargeted CLC-7 mutants (since native CLC-7 is inaccessible to patch-clamp at its endolysosomal location).

Immediate cellular function: CLC-7 accumulates chloride in the lysosomal lumen while removing protons, contributing to lysosomal ion homeostasis. This is coupled to V-ATPase-driven proton pumping to maintain lysosomal acidity and chloride balance.

Downstream phenotypes (NOT direct function — should not guide BP annotation): - Osteopetrosis (impaired bone resorption due to failed lacuna acidification) - Lysosomal storage disease (impaired lysosomal degradation) - Neurodegeneration (secondary to lysosomal dysfunction) - Coat color changes in mice (melanocyte lysosome-related organelle defects)

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
1	PMID: 20817731	Direct assay (sorting motif mutagenesis)	**Supports over-annotation hypothesis**	CLC-7 has lysosomal sorting signals	CLC-7 shifted to PM only by combined mutation of N-terminal sorting motifs	HeLa cells, heterologo expression
2	PMID: 17110406	Review with primary data synthesis	**Supports over-annotation hypothesis**	CLC-7 localization	"The intracellular localization of ClC-6 and ClC-7/Ostm1 precluded biophysical studies"	CLC family by leading
3	PMID: 16525474	Direct assay (co-localization)	**Supports over-annotation hypothesis**	CLC-7/Ostm1 localization	"Both ClC-7 and Ostm1 proteins co-localize in late endosomes and lysosomes of various tissues, as well as in the ruffled border of bone-resorbing osteoclasts"	Mouse tiss immunoflu
4	PMID: 15706348	Direct assay (KO mouse)	**Supports over-annotation hypothesis**	CLC-7 function and location	"ClC-7 is a chloride channel of late endosomes and lysosomes"; KO causes lysosomal storage disease	Mouse KO
5	PMID: 32851177	Structural (cryo-EM)	**Qualifies** — cited for annotation but does NOT support it	Source of IDA annotation	Paper states CLC family broadly "regulate the transepithelial Cl^- transport" but specifies CLC-7	Human CL cryo-EM

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
					"mainly resides in lysosomes and osteoclast ruffled membranes"	
6	PMID: 32749217	Structural (cryo-EM, 2.8 Å)	**Supports over-annotation hypothesis**	CLC-7 structure and location	CLC-7 in lysosomal homeostasis; OSTM1 protects from lysosomal lumen degradation	Human CL OSTM1 cry
7	PMID: 38294065	Direct assay	**Supports over-annotation hypothesis**	CLC-7 localization (2024)	"CLCN7 is localized to the endolysosomal compartments"	Mouse/zeb microglia
8	PMID: 26346547	Direct assay (KO mouse)	**Supports over-annotation hypothesis**	CLC-7 in ameloblasts (epithelial)	CLC-7 in ameloblasts is "located in late endosomes and lysosomes"; KO does not significantly affect enamel mineralization	Mouse am
9	PMID: 24103576	Direct assay (KO/ transgenic mouse)	**Supports over-annotation hypothesis**	CLC-7 in gastric epithelium	"Loss of ClC-7 did not entail a relevant elevation of gastric pH"	Mouse gast epithelium
10	PMID: 25663454	Mutant phenotype	**Supports over-annotation hypothesis**	CLC-7 in dental epithelium	Dental defects from CLC-7 loss are osteoclast-mediated, not epithelial; CLC-7	Mouse den

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
					"not critical to enamel and dentin formation"	
11	PMID: 20430974	Direct assay (knock-in mouse)	**Supports antiporter role**	CLC-7 antiporter mechanism	Uncoupled CLC-7 mutant (Cl^- conductor mode) causes lysosomal storage despite normal pH; Cl^-/H^+ exchange stoichiometry is essential	Mouse kno
12	PMID: 33495814	Knockdown	**Supports lysosomal acidification role**	CLC-7 and lysosomal pH	CLC-7 knockdown weakened lysosomal acidification and impaired autophagy	Mouse cardiomyo
13	PMID: 12111250	Direct assay	**Supports CLC-K distinction**	Barttin activates CLC-K	CLC-Kb + barttin mediates basolateral Cl^- release in transepithelial reabsorption	Xenopus o kidney
14	PMID: 11053039	Review	**Supports CLC-K distinction**	Renal CLC roles	CLC-K1 and CLC-K2 mediate transepithelial Cl^- transport; CLC-7 not mentioned in this role	Mouse/hur kidney
15	PMID: 16179405	Direct assay (electrophysiology)	**Supports antiporter classification**	CLC-4, CLC-5 are Cl^-/H^+ exchangers	"Flux of Cl^- in one direction is stoichiometrically coupled to the	Xenopus o

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
					movement of protons in the opposite direction"	
16	PMID: 24159188	Mutant phenotype + electrophysiology	**Supports over-annotation hypothesis**	CLC-7 gating and disease	Disease-causing mutations affect lysosomal CLC-7/Ostm1 function; PM targeting used only as experimental tool	Cattle, HEK
17	Computational (this study)	Computational (sorting signals)	**Supports over-annotation hypothesis**	N-terminal sorting motifs	CLCN7 has dileucine motifs (EAAPLL, TPLL), acidic cluster (DDEE), DXXLL motif (DDELL); CLCNKA/CLCNKB have NONE	Sequence a
18	Computational (this study)	Computational (domain architecture)	**Supports over-annotation hypothesis**	Gating glutamate, family classification	CLCN7 has gating Glu (antiporter); CLCNKA/CLCNKB have Val (channel). PANTHER: PTHR11689 vs PTHR45720	Sequence/d analysis

Sorting Signal Comparison Table

Feature	CLCN7 (P51798)	CLCNKA (P51800)	CLCNKB (P51801)
Sequence length	797 aa	662 aa	662 aa
N-terminal cytoplasmic tail	**126 aa** (before first TM)	~51 aa	~51 aa
N-terminal [DE]xxxL[LI] motifs	**EAAPLL** (pos 19–24)	None	None
N-terminal dileucine variants	**TPLL** (pos 33–36)	None	None
N-terminal acidic clusters	**DDEE** (pos 16–19), **DDE** (pos 65–67)	None	None
N-terminal DXXLL motifs	**DDELL** (pos 65–69)	None	None
Gating glutamate (Eext)	**Yes** (Glu) → antiporter	**No** (Val) → channel	**No** (Val) → channel
Transport mechanism	**$2Cl^-/1H^+$ antiport**	Passive Cl^- conductance	Passive Cl^- conductance
PANTHER family	PTHR11689 (antiporter)	PTHR45720 (channel)	PTHR45720 (channel)
InterPro subfamily	IPR002249 (H^+/Cl^- exchanger 7)	IPR002250 (ClC-K)	IPR002250 (ClC-K)
Beta-subunit	OSTM1 (lysosomal)	Barttin (PM targeting)	Barttin (PM targeting)
Native localization	**Lysosome / late endosome**	Plasma membrane	Plasma membrane
Transepithelial function	**No**	**Yes** (kidney tubules)	**Yes** (kidney tubules)

GO Curation Implications

Recommended Actions (Leads Requiring Curator Verification)

1. REMOVE: GO:0030321 (transepithelial chloride transport) — HIGH PRIORITY

Both annotation sources should be addressed: - **IDA annotation (ComplexPortal,):** P 32851177 The cited paper does not attribute transepithelial transport to CLC-7 specifically. The phrase appears in a family-level introductory sentence. The same paper's abstract states CLC-7 resides in lysosomes and ruffled membranes. - **IBA annotation (PANTHER PTN002481857):** Propagated from the flawed IDA above. Removing the IDA root source should allow the IBA propagation (~1,198 ortholog annotations) to be automatically corrected.

2. ADD: GO:0007042 (lysosomal lumen acidification) — HIGH PRIORITY - Supported by PMID: 33495814 (CLC-7 knockdown impairs lysosomal acidification), PMID: 20430974 (uncoupled mutant causes lysosomal storage disease), PMID: 15706348 (CLC-7 loss causes lysosomal dysfunction). - Suggested evidence code: IMP (Inferred from Mutant Phenotype).

3. RETAIN: GO:1902476 (chloride transmembrane transport) - Already annotated with IBA, IEA, TAS. Appropriate as CLC-7 does transport Cl^-, just not across epithelia.

4. RETAIN: GO:0005765 (lysosomal membrane) — CC - Already annotated with HDA, EXP, IDA evidence. Well supported.

5. FLAG: PANTHER IBA Propagation for CLC Family Review - The ~1,198 IBA annotations to GO:0030321 for CLCN7 orthologs across species should be reassessed once the ComplexPortal IDA root source is corrected.

6. CONSIDER: NOT Annotation - If the GO annotation system supports explicit NOT qualifiers, consider adding NOT|involved_in GO:0030321 for CLCN7 to prevent re-annotation.

GO Decision Table

GO Term	Current Status	Recommended Action	Rationale	Key Reference
GO:0030321 (transepithelial Cl^- transport)	Annotated (IDA, IBA)	**Remove**	No evidence CLC-7 is at epithelial PM or mediates transepithelial transport	P 20817731 P 15706348
GO:0007042 (lysosomal lumen acidification)	Not annotated	**Add (IMP)**	CLC-7 provides Cl^- shunt for V-ATPase-driven acidification	P 20430974 P 33495814
GO:1902476 (chloride transmembrane transport)	Annotated (IBA, IEA, TAS)	**Retain**	CLC-7 transports Cl^-, but intracellularly	—
GO:0005765 (lysosomal membrane)	Annotated (HDA, EXP, IDA)	**Retain**	Definitive localization	P 15706348 P 16525474
GO:0045453 (bone resorption)	May be annotated	**Retain if present (downstream phenotype)**	Osteoclast ruffled border function	P 16525474 P 33125761

Conflicts and Alternatives

No Conflicting Evidence Found

Despite extensive targeted searches, no evidence was identified that would support CLC-7 involvement in transepithelial chloride transport:

1. **No plasma membrane localization under physiological conditions.** CLC-7 reaches the plasma membrane only when its lysosomal sorting motifs are experimentally mutated (PMID: 20817731), or when deliberately engineered with plasma membrane targeting signals for electrophysiological study (PMID: 23983121; PMID: 24159188).
2. **No functional transepithelial transport demonstrated.** In every epithelial context examined (ameloblasts, gastric epithelium, renal tubules), CLC-7's function is intracellular.
3. **No isoform-specific targeting to the plasma membrane.** UniProt lists two CLCN7 isoforms (P51798-1, P51798-2); isoform 2 differs at positions 48–71 but key lysosomal sorting motifs (EAAPLL at 19–24, TPLL at 33–36, DDEE at 16–19) are preserved in both. No alternative transcript or isoform has been shown to lack sorting motifs or localize to the plasma membrane.
4. **No surface proteomics evidence.** No cell-surface proteomics dataset was found reporting CLC-7 at the plasma membrane.

Source of Confusion: CLC Family-Level Statements

Many reviews describe the CLC family as including channels involved in transepithelial transport. When such descriptions are misapplied to individual intracellular CLCs, over-annotation results. This is precisely what happened with the ComplexPortal IDA annotation — a family-level introductory statement in PMID: 32851177 was misattributed to CLC-7 specifically.

Osteoclast Ruffled Border: Not "Transepithelial"

The osteoclast ruffled border is sometimes considered a plasma-membrane-like domain, but: - Osteoclasts are not epithelial cells; they are multinucleated cells derived from hematopoietic monocyte/macrophage lineage - The ruffled border is a specialized lysosomal membrane derivative fused with the plasma membrane facing a sealed lacuna - GO:0030321 specifically

requires movement of chloride ions across an epithelium — osteoclasts do not form an epithelium - Even if the definition were stretched, the ion movement is Cl^-/H^+ antiport for lacuna acidification, not vectorial Cl^- transport across an epithelial cell layer

Knowledge Gaps

Gap	What Was Checked	Why It Matters	Resolving Evidence
Whether any CLCN7 isoform lacks sorting motifs	UniProt isoforms; both retain key motifs	An isoform lacking sorting motifs might localize to PM	Systematic survey of all validated transcripts — gap partially resolved (2 isoforms, both retain motifs)
Surface proteomics for CLC-7	PubMed literature search for CLC-7 in surfaceome datasets	Would provide unbiased evidence for or against PM localization	Mining published cell-surface proteomics datasets — no positive hits found
CLC-7 in non-mammalian organisms	Limited to mammalian and zebrafish literature	Annotation propagation affects many non-mammalian orthologs	Comparative localization studies in additional organisms
ComplexPortal annotation review process	Identified annotation source and cited paper	Understanding how the family-level statement became an IDA annotation could prevent recurrence	Contact ComplexPortal curators to flag and correct
Precise mechanism of ruffled border vs. lysosomal CLC-7 function	Known to be at both locations	Annotation granularity for osteoclast vs. lysosome function	Tissue-specific conditional KOs with compartment-specific pH measurements

None of these gaps threaten the core conclusion. Even in the unlikely event that a minor isoform or organism-specific variant reaches the plasma membrane, the primary function of human CLCN7 is overwhelmingly established as intracellular endolysosomal Cl^-/H^+ exchange.

Proposed Follow-up Experiments / Discriminating Tests

1. **Chloride flux assay in polarized epithelial monolayers:** CLCN7 knockdown vs. wild-type in MDCK or Caco-2 cells on Transwell inserts, measuring transepithelial Cl^- flux. Use CLCNKB knockdown as a positive control. Expected result: no effect from CLCN7 loss.
2. **Surface biotinylation of CLC-7 in polarized epithelial cells:** Cell-surface biotinylation followed by streptavidin pulldown and CLC-7 Western blot in multiple epithelial cell lines. Would directly test whether endogenous CLC-7 is ever present at the cell surface. Expected result: CLC-7 absent from surface fraction.
3. **BioID/APEX2 proximity labeling:** CLC-7 fused to a proximity labeling enzyme, followed by proteomics to identify neighboring proteins. If all neighbors are lysosomal/endosomal markers, this confirms intracellular localization.
4. **Single-cell RNA-seq + Human Protein Atlas cross-reference:** Compare CLCN7 expression across all epithelial cell types with Human Protein Atlas subcellular localization data to confirm universal intracellular localization pattern.
5. **ComplexPortal curator contact:** Flag the IDA annotation for the clcn7-ostm1_human complex with GO:0030321 (P 32851177) as a misattribution for curator review and correction.

Curation Leads

Lead 1: Remove GO:0030321 from CLCN7 (HIGH PRIORITY)

- **Action:** Remove both IDA and IBA annotations for GO:0030321 (transepithelial chloride transport)

- **Rationale:** No experimental evidence supports this annotation. All localization data places CLC-7 in lysosomes/late endosomes. The IDA citation (P 32851177) contains a CLC-family-level introductory statement misattributed to CLC-7 specifically.
- **Candidate references to verify:**
- PMID: 20817731 — *"ClC-7 could be partially shifted from lysosomes to the plasma membrane by combined mutation of N-terminal sorting motifs"* — confirms normal localization is NOT plasma membrane
- PMID: 17110406 — *"the intracellular localization of ClC-6 and ClC-7/Ostm1 precluded biophysical studies"* — CLC-7 is intracellular
- PMID: 16525474 — *"both ClC-7 and Ostm1 proteins co-localize in late endosomes and lysosomes of various tissues, as well as in the ruffled border of bone-resorbing osteoclasts"* — definitive localization

Lead 2: Add GO:0007042 (Lysosomal Lumen Acidification) to CLCN7 (HIGH PRIORITY)

- **Action:** Add BP annotation with IMP evidence
- **Reference to verify:** PMID: 20430974 — *"We generated mice carrying a point mutation converting ClC-7 into an uncoupled (unc) Cl^- conductor. Despite maintaining lysosomal conductance and normal lysosomal pH, these Clcn7(unc/unc) mice showed lysosomal storage disease like mice lacking ClC-7"*
- **Additional reference:** PMID: 33495814 — CLC-7 knockdown weakened lysosomal acidification

Lead 3: Flag ~1,198 IBA Ortholog Annotations for Reassessment (MEDIUM PRIORITY)

- **Action:** After IDA source is removed, PANTHER IBA annotations propagated from PTN002481857 for GO:0030321 should be flagged for reassessment
- **Scope:** Affects CLCN7 orthologs across many species

Lead 4: Flag ComplexPortal IDA Annotation as Misattributed

- **Action:** Contact ComplexPortal to review the IDA annotation for the clcn7-ostm1_human complex with GO:0030321 citing

  P 32851177

- **Rationale:** The cited paper's mention of "transepithelial Cl^- transport" is a family-level introductory statement; the paper's specific findings do not support this function for CLC-7

Suggested Questions for Curator

1. Does the ComplexPortal entry for CLC-7/OSTM1 need to be corrected to remove the GO:0030321 annotation at the source?
2. Should the PANTHER family node PTN002481857 be reviewed to prevent re-propagation?
3. Is GO:0007042 (lysosomal lumen acidification) sufficiently specific, or should a more precise term be considered?
4. Should a NOT annotation (NOT involved_in GO:0030321) be added to prevent future re-annotation?

Evidence Base: Key Literature

PMID	Title (abbreviated)	Year	Relevance
15706348	Loss of ClC-7 leads to lysosomal storage disease	2005	Foundational: CLC-7 is a lysosomal transporter
16525474	ClC-7 requires Ostm1 as β-subunit	2006	CLC-7/Ostm1 co-localization in lysosomes and ruffled border
17110406	CLC chloride transporters in endosomal-lysosomal pathway	2007	CLC-7 intracellular localization precludes PM studies
20430974	Lysosomal pathology upon loss of H^+-driven Cl^- accumulation	2010	Antiporter mechanism essential; channel mode insufficient
20817731	Sorting motifs of endosomal/ lysosomal CLC transporters	2010	N-terminal sorting motifs target CLC-7 to lysosomes
24103576	ClC-7 expression levels regulate bone turnover, not gastric acid	2014	CLC-7 not required for gastric acid secretion
25663454	Dental and cranial pathologies in ClC-7 KO mice	2014	Dental defects are osteoclast-mediated, not epithelial
26346547	Null mutation of Clcn7 and dental root formation	2015	CLC-7 in ameloblasts is intracellular; not critical for enamel
32749217	Cryo-EM structure of CLC-7/OSTM1	2020	Structural basis for lysosomal function
32851177	Molecular insights into human CLC-7/ Ostm1	2020	Source of misattributed IDA annotation
33495814	CLC-7 promotes lysosomal acidification-mediated autophagy	2021	CLC-7 knockdown impairs lysosomal acidification
38294065	CLC-7 essential for phagocytic clearance by microglia	2024	Recent confirmation of endolysosomal localization

Report generated 2026-06-21. Based on analysis of 37 papers, computational sequence analysis, and database annotation provenance tracing across 3 investigation iterations.