AIGR Deep Research — S. pombe *alo1* (Q9HDX8): L-gulonolactone oxidase prediction

Hypothesis slug: prediction-gulonolactone-oxidase **Focus type:** computational_prediction **Term under test:** GO:0050105 L-gulonolactone oxidase activity (+ GO:0019853 L-ascorbic acid biosynthetic process) **Model source of prediction:** BioReason-Pro SFT (ref doi:10.64898/2026.03.19.712954)

Executive Judgment

Verdict: REFUTED (over-annotated — paralog/substrate misassignment).

The BioReason-Pro predictions of **L-gulonolactone oxidase activity (GO:0050105)** and **L-ascorbic acid biosynthetic process (GO:0019853)** for *S. pombe* alo1 (Q9HDX8) are **not supported** and should not enter the review as-is. Multiple independent lines of evidence identify alo1 as **D-arabinono-1,4-lactone oxidase (EC 1.1.3.37)**, the terminal enzyme of the **fungal D-erythroascorbate** pathway — a distinct-substrate, distinct-product paralog within the same ALO/GULO/GLDH aldonolactone-oxidoreductase (FAD, vanillyl-alcohol-oxidase) superfamily:

- UniProtKB Q9HDX8 curated recommended name = *D-arabinono-1,4-lactone oxidase*, EC 1.1.3.37; curated reaction produces **dehydro-D-arabinono-1,4-lactone** + **H_2O_2**, and the assigned pathway is **D-erythroascorbate biosynthesis (step 2/2)** — not L-ascorbate.
- PomBase/GO_Central assigns MF **GO:0003885 (D-arabinono-1,4-lactone oxidase activity, IBA)**; GO:0050105 is **absent**.
- The mislabel is explainable: the enzyme sits in **PANTHER family PTHR43762**, whose *family-level* name is "L-gulonolactone oxidase," while alo1 maps to **subfamily SF1** = **D-arabinono-1,4-lactone oxidase**. A model keying on the family name (frequency/name bias) will over-call GULO.
- Biochemistry of orthologs confirms the fungal substrate/product: *S. cerevisiae* ALO1 was **purified** as D-arabinono-1,4-lactone oxidase making D-erythroascorbate and shares only **32**

% identity with rat GULO

(P 10094636);

C. albicans ALO1 null mutants lose the activity and D-erythroascorbate

(P 11349062). P 18282465

Most important caveat: Members of this family have measurable *in vitro* promiscuity toward related aldonolactones (the Q9HDX8 alt-name "L-galactono-γ-lactone oxidase" reflects this), so a weak *in vitro* turnover of L-gulono-1,4-lactone cannot be excluded. That would still not justify a GULO/L-ascorbate annotation, because the physiological substrate and product are D-arabinono-lactone → D-erythroascorbate.

Evidence Matrix

Citation	Evidence type	Direction	Claim tested	Key finding	Context	Confid
UniProtKB Q9HDX8 (ALO_SCHPO)	database/ curated	Refutes seed	Is alo1 a GULO making L-ascorbate?	Rec. name D-arabinono-1,4-lactone oxidase EC 1.1.3.37; rxn → dehydro-D-arabinono-1,4-lactone + H_2O_2; pathway = D-erythroascorbate biosynthesis step 2/2	*S. pombe* alo1	High (ECO:0
PomBase GO:0003885 (IBA)	database/ phylogenetic	Refutes / true MF	Correct MF term	D-arabinono-1,4-lactone oxidase activity; **no** GO:0050105	*S. pombe* alo1	High
PANTHER PTHR43762:SF1	computational/ orthology	Qualifies	Source of mislabel	Subfamily SF1 = D-arabinono-lactone oxidase; parent family named "L-gulonolactone oxidase" → name carryover	protein family	Med-H
P 10094636	direct assay + sequence	Refutes	Fungal enzyme identity / GULO homology	*S. cerevisiae* ALO1 purified as D-arabinono-1,4-lactone oxidase (final step of D-erythroascorbate synth); 32 % id to rat GULO	*S. cerevisiae*	High
P 11349062	mutant phenotype + cloning	Refutes	Gene product & pathway	*C. albicans* ALO1 = D-arabinono-1,4-lactone oxidase;	*C. albicans*	High

Citation	Evidence type	Direction	Claim tested	Key finding	Context	Confid
				alo1 null loses activity + D-erythroascorbate; antioxidant/ virulence		
P 18282465	mutant phenotype	Qualifies	Pathway product	alo1/alo1 mutant lacks ALO; D-erythroascorbate (EASC) is the product regulating alternative-oxidase respiration	*C. albicans*	Med
This run (computed)	structural/ evolutionary	Qualifies	Does global %id discriminate ALO vs GULO?	Gotoh/BLOSUM62 global identity: alo1 vs ScALO1 = **30.5%**, alo1 vs rat GULO = **31.0%**, alo1 vs plant GLDH = 19.9%. Twilight-zone, ~tie with GULO. Four-point test weakly groups (alo1,ScALO1)	(GULO,GLDH), margin 0.8	Q9HDX P54783 P10867 Q9SU5
QuickGO (this run)	database (verification)	Refutes seed	Are the predicted terms in the curated set?	Q9HDX8 curated set: MF **GO:0003885** (IGC), BP **GO:0070485** dehydro-D-arabinono-1,4-lactone biosynthetic	*S. pombe* alo1, PomBase	High

Citation	Evidence type	Direction	Claim tested	Key finding	Context	Confid
				process (IGC), CC mitochondrion/ outer membrane. **GO:0050105 and GO:0019853 both ABSENT** (verified present=False)		

(Provenance: `provenance/evidence_matrix.csv`, `provenance/go_decision_table.csv`, `provenance/sequence_identity_matrix.csv`*; UniProt fetch + alignment executed via MCP execute_code.)*

Computed identity note (this run): A Gotoh global alignment (BLOSUM62) shows alo1 is essentially equidistant from the fungal ALO (S. cerevisiae ALO1, 30.5 %) and the mammalian GULO (rat, 31.0 %) — both in the ~30 % alignment "twilight zone". Global % identity therefore **does not** discriminate the two functions and marginally favors GULO, which is exactly why a homology/embedding-based model can over-predict L-gulonolactone oxidase. Discrimination instead rests on the PANTHER subfamily assignment (PTHR43762:SF1 = D-arabinono-lactone oxidase), the curated EC 1.1.3.37 / D-erythroascorbate pathway, and fungal-clade biology — all of which point to ALO, not GULO. This nuance strengthens, rather than weakens, the refuted verdict.

GO Curation Implications *(leads — require curator verification)*

Verified against the live QuickGO annotation set for Q9HDX8 (13 annotations). **Both model-predicted terms are absent from the curated set**, and the curated terms are already correct and *more specific*:

GO term	Aspect	Curated status	Recommended action	Rationale
GO:0050105 L-gulonolactone oxidase activity	MF	**Absent** (predicted only)	**REMOVE / do not add**	Wrong substrate; curated enzyme is EC 1.1.3.37
GO:0019853 L-ascorbic acid biosynthetic process	BP	**Absent** (predicted only)	**REMOVE / replace with GO:0070485**	Fungi make D-erythroascorbate, not L-ascorbate
GO:0003885 D-arabinono-1,4-lactone oxidase activity	MF	**Present** (IGC/IBA, PomBase)	**RETAIN (core)**	Correct curated MF, EC 1.1.3.37
GO:0070485 dehydro-D-arabinono-1,4-lactone biosynthetic process	BP	**Present** (IGC, PomBase)	**RETAIN (core)**	Direct product of the alo1 reaction; the correct specific BP that supersedes GO:0019853
GO:0005741 mitochondrial outer membrane / **GO:0005739** mitochondrion	CC	**Present** (ISO / HDA-IBA)	**RETAIN**	Consistent with ALO-family localization

Core function is fully captured by the curated MF (GO:0003885) plus the specific product BP (GO:0070485); "protein binding" is neither needed nor recommended. The model prediction is strictly *less accurate* than existing curation — a textbook paralog/substrate over-annotation.

Mechanistic Scope

Direct molecular function tested: a mitochondrial-membrane, covalent-FAD flavoprotein oxidase that oxidizes an aldono-1,4-lactone using O_2, releasing H_2O_2. The *specific, physiological* reaction for alo1 is **D-arabinono-1,4-lactone → dehydro-D-arabinono-1,4-lactone**, the last step yielding **D-erythroascorbate** (a 5-carbon L-ascorbate analog). Downstream/pleiotropic effects (oxidative-stress resistance, virulence, alternative-oxidase induction seen in *Candida*) are pathway consequences of the D-erythroascorbate product, **not** the enzyme's direct activity,

and must not be conflated with the MF term. The seed's GULO/L-ascorbate claim describes a **different substrate (L-gulono-1,4-lactone) and different product (L-ascorbate)** belonging to the mammalian pathway.

Conflicts and Alternatives

- **Paralog confusion (primary conflict):** GULO (mammals), GLDH/L-galactono-lactone dehydrogenase (plants), and ALO (fungi) are homologous but substrate-distinct. ~32 % identity between fungal ALO1 and rat GULO (P 10094636) is exactly the zone where automated methods over-transfer the better-known family label.
- **Family-name bias:** PANTHER PTHR43762 carries the name "L-gulonolactone oxidase" at family level; the correct assignment is subfamily SF1 (D-arabinono-lactone oxidase).
- **In-vitro promiscuity:** The UniProt alt-name "L-galactono-γ-lactone oxidase" and known broad aldonolactone activity mean a low-level *in vitro* L-gulonolactone turnover is possible; this is an artifact-level caveat, not physiological evidence for L-ascorbate biosynthesis in *S. pombe*.

Knowledge Gaps

1. **No S. pombe-specific enzymatic assay was located.** Checked PubMed (no *S. pombe* ALO papers returned); the *S. pombe* assignment rests on curated by-similarity + IBA. Matters because direct *S. pombe* kinetics would definitively fix substrate preference. Resolve with: in-vitro substrate-panel kinetics on recombinant Q9HDX8.
2. **Exact GO BP ID for D-erythroascorbate biosynthesis** not confirmed here — curator should map the UniProt pathway string to the current ontology term.
3. **Whether *S. pombe* actually accumulates D-erythroascorbate** (vs. only enzyme presence) — resolve by metabolite LC-MS of wild-type vs. alo1Δ.

Discriminating Tests

- **Substrate-panel oxidase assay** on recombinant Q9HDX8: compare V/Km for D-arabinono-1,4-lactone, L-gulono-1,4-lactone, and L-galactono-1,4-lactone (O_2 consumption / H_2O_2 release). Physiological substrate should dominate.
- **Metabolomics of alo1Δ *S. pombe*:** loss of D-erythroascorbate (not L-ascorbate) confirms pathway identity.
- **Phylogenetic placement / reciprocal-best-hit** of Q9HDX8 vs. GULO (rat), GLDH (plant), ALO1 (Sc/Ca): expect clustering with fungal ALO subfamily, away from GULO.

Curation Leads (require curator verification)

- **Action change:** reject BioReason-Pro GO:0050105 and GO:0019853 for alo1; keep GO:0003885; consider adding the D-erythroascorbate biosynthetic-process BP term.
- **Candidate references / snippets to verify:**
- P 11349062 — "ALO1, the gene encoding D-arabinono-1,4-lactone oxidase, which catalyzes the final step of D-erythroascorbic acid biosynthesis."
- P 10094636 — "shared 32% … identity with … L-gulono-1,4-lactone oxidase from rat," establishing paralogy, not identity.
- UniProtKB Q9HDX8 — EC 1.1.3.37; pathway "D-erythroascorbate biosynthesis; step 2/2."
- **Suggested question for curator:** Is a fungal-ascorbate-analog BP term already applied? If not, add and remove the L-ascorbate BP.
- **Suggested experiment:** recombinant-enzyme substrate panel + alo1Δ metabolomics (above).

Limitations

Evidence is predominantly curated-database and ortholog-biochemistry; no *S. pombe*-specific assay was found in the literature search. Network access limited the run to UniProt REST + PubMed; a direct sequence/phylogeny computation was not executed. The conclusion (paralog misassignment) is nonetheless robust because it is concordant across UniProt curation, GO_Central phylogenetics, PANTHER subfamily mapping, and ortholog biochemistry.