AIGR Gene Hypothesis Deep Research — LysB (Q08694, DROME)

Focus type: computational_prediction · **Hypothesis slug:** prediction-chitinase-immune **Term under evaluation:** chitinase activity (GO:0004568) **Reference context:** doi:10.64898/2026.03.19.712954

Summary

The BioReason-Pro SFT model predicts that *Drosophila melanogaster* LysB (UniProt **Q08694**) has **chitinase activity (GO:0004568)** and two immune roles — **negative regulation of innate immune response (GO:0045824)** and **defense response to Gram-negative bacterium (GO:0050829)**. Independent evaluation from sequence classification, domain architecture, a custom catalytic-site alignment, primary expression literature, and the current GO annotation record indicates that all three predictions are either **wrong** or **unsupported by the physiological evidence**. The overall verdict is **REFUTED / OVER-ANNOTATED**.

LysB is a **glycoside hydrolase family 22 (GH22) C-type lysozyme (muramidase, EC 3.2.1.17)** — not a chitinase (EC 3.2.1.14; GH18/GH19). Its molecular function is peptidoglycan hydrolysis. The "chitinase" prediction is best explained as a **fold/activity misassignment** arising from the well-documented, weak chito-oligosaccharide side-activity that C-type lysozymes possess, together with the general confusion between lysozyme and chitinase glycosidase folds. Physiologically, the Drosophila *LysB–E* cluster comprises **constitutive, midgut-restricted, acidic digestive lysozymes** that are transcriptionally **repressed** (not induced) by bacterial infection and are **not expressed in fat body or hemocytes** — the opposite of what an induced humoral immune effector or an immune *regulator* would look like. This makes **GO:0045824** unsupported and biologically implausible for LysB, and it substantially weakens the case for **GO:0050829**, which exists in the GO record only as an ISS "by similarity" carry-over with no experimental basis.

For a curator, the actionable conclusion is: **do not add GO:0004568 or GO:0045824**; treat the existing **GO:0050829** as low-confidence (ISS-only, conflicting with digestive biology) and consider flagging it for review or "non-core" status; and **retain the well-supported core terms — GO:0003796 (lysozyme activity, MF)** and **GO:0005576 (extracellular region, CC)** — ideally complemented by a BP term reflecting **digestion / bacteriophagy** rather than immunity.

Executive Judgment

Verdict: REFUTED / OVER-ANNOTATED.

All three predicted terms fail independent evaluation:

- **GO:0004568 (chitinase activity):** Refuted. LysB is a GH22 muramidase with a conserved lysozyme catalytic dyad and no GH18/GH19 chitinase domain or active-site motif. The only "chitinase-like" property is the weak chitodextrin side-activity intrinsic to C-type lysozymes, which is captured by the canonical lysozyme catalytic-activity description and does not warrant a chitinase MF term.
- **GO:0045824 (negative regulation of innate immune response):** Refuted/unsupported. No experimental or credible computational basis; contradicts the constitutive, infection-repressed, midgut-restricted expression profile.
- **GO:0050829 (defense response to Gram-negative bacterium):** Weakly supported at best; present only as a single ISS "by similarity" annotation, in tension with the digestive role.

The most important caveat is that a minority of insect c-type lysozymes are genuinely dual-function (digestive + mild immune effector), so a low-level bactericidal activity for LysB cannot be fully excluded — but even the strongest reading of that literature would never justify a *negative regulator of immunity* assignment, and the Drosophila-specific expression data argue against an immune-effector role.

Key Findings

Finding 1 — LysB is a GH22 C-type lysozyme (EC 3.2.1.17), not a chitinase

UniProt Q08694 carries the recommended name **"Lysozyme B"**, EC number **3.2.1.17** (lysozyme/muramidase), and the family SIMILARITY statement **"Belongs to the glycosyl hydrolase 22 family."** The domain architecture is **exclusively** C-type lysozyme with no chitinase signature whatsoever:

Resource	Identifier	Assignment
InterPro	IPR001916 / IPR000974	Glycoside hydrolase family 22 / C-type lysozyme
Pfam	PF00062	Lys (lysozyme)
CDD	cd16899	LYZ_C_invert (invertebrate C-type lysozyme)
PANTHER	PTHR11407	LYSOZYME C
SUPFAM	SSF53955	Lysozyme-like superfamily

No GH18 or GH19 chitinase domain (e.g., Pfam PF00704 / Glyco_hydro_18, or Glyco_hydro_19) is present. The GO molecular-function annotation for Q08694 is **GO:0003796 lysozyme activity**, not GO:0004568 chitinase activity.

Critically, the mechanistic origin of the model's error is visible in the enzyme's own catalytic-activity description. The canonical C-type lysozyme reaction hydrolyzes the β-1,4 linkage between *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) in **peptidoglycan** — "**and between N-acetyl-D-glucosamine residues in chitodextrins.**" This weak activity on chito-oligosaccharides is an intrinsic, well-known side-property of C-type lysozymes and is almost certainly the textual/feature signal that led a machine-learning model to over-predict full **endochitinase** activity. A weak in-vitro side-activity on short chitodextrins is **not** equivalent to biological chitinase activity (processive hydrolysis of insoluble chitin polymer), which is carried out by structurally distinct GH18/GH19 enzymes.

This finding is anchored in the review-level statement that **"Lysozymes from family 22 of glycoside hydrolases are usually part of the defense system against bacteria"** (PMID: 17825580), confirming that C-type/digestive insect lysozymes belong to GH22 — a family distinct from the chitinase families.

Finding 2 — Drosophila LysB is a constitutive midgut digestive lysozyme, not an induced immune effector

The defining expression study of the Drosophila lysozyme locus (PMID: 8159165, Daffre et al. 1994) reports that **"four closely related genes, LysB, C, D and E, are all strongly expressed in the midgut of larvae and adults"** and that **"None of the genes is expressed in the fat body or haemocytes."** In insects, the fat body and hemocytes are the principal tissues of the systemic humoral immune response; the complete absence of LysB expression there is direct evidence against a humoral/immune-effector role. The same work notes that all of these genes except LysP encode **acidic** proteins "highly reminiscent" of the acidic digestive lysozymes of mammalian foregut fermenters — a hallmark of enzymes optimized for acidic gut lumen conditions and bacteriolytic digestion.

An independent study of the same locus (PMID: 1588905, Kylsten et al. 1992) found that lysozyme transcription **decreases** after bacterial injection and concluded that these genes **"have been recruited for the digestion of bacteria present in fermenting food."** A gene whose transcription is *repressed* by infection and whose product acts in the gut lumen to digest dietary bacteria is behaving as a **digestive enzyme**, not as an inducible immune effector and certainly not as a *negative regulator* of the innate immune response.

Consistent with the primary literature, UniProt Q08694 states in its FUNCTION field that LysB is **"Unlikely to play an active role in the humoral immune defense. May have a function in the digestion of bacteria in the food,"** and its TISSUE SPECIFICITY field localizes expression to the midgut with peak expression in the 3rd larval instar.

The digestive-lysozyme model is reinforced by comparative work across arthropods: housefly (*Musca domestica*) digestive lysozymes MdL1/MdL2 are acidic-optimum midgut enzymes (PMID: 19099150), the first crystal structure of a digestive lysozyme was solved from housefly (PMID: 17825580), and synanthropic mites use acidic digestive lysozymes to exploit bacteria as a food source, with maximal activity at the acidic pH of the gut (PMID: 18357505). These establish "acidic midgut digestive lysozyme" as a recurrent, well-characterized functional class into which Drosophila LysB clearly falls.

Finding 3 — Sequence analysis confirms the C-type lysozyme catalytic dyad and the absence of a chitinase active site

To test the classification directly rather than relying on database labels, an in-run global alignment (Needleman–Wunsch, implemented in NumPy) was performed between the **mature LysB sequence** (122 aa, after removing the signal peptide, residues 1–18) and **hen egg-white lysozyme (HEWL)**, the archetypal GH22 C-type lysozyme:

Metric	Result
Identity to HEWL	**41.3%** (50/121 aligned positions)
HEWL catalytic Glu35	**Conserved** (→ Glu in LysB)
HEWL catalytic Asp52	**Conserved** (→ Asp in LysB)
Cysteine count	**8** (4 disulfides), matching HEWL's C-type scaffold
GH18 chitinase catalytic motif (DxxDxDxE)	**Absent**
Enzyme classification	EC 3.2.1.17 (lysozyme), **not** EC 3.2.1.14 (chitinase)

The conservation of **both** HEWL catalytic residues (the Glu general acid and the Asp that stabilizes/participates in catalysis) together with the eight-cysteine (four-disulfide) C-type lysozyme fold is diagnostic of a functional GH22 muramidase. The **absence** of the GH18 chitinase catalytic signature (the DxxDxDxE motif that defines the GH18 active site) is direct negative evidence against the chitinase prediction: LysB does not possess the active-site chemistry required for a genuine chitinase.

Finding 4 — The current GO record contains no chitinase or immune-regulation term; the sole defense term is ISS-only

A QuickGO retrieval (performed in-run) returned **6 annotations** for Q08694 spanning **three distinct terms**:

GO term	Aspect	Evidence in the record
GO:0003796 lysozyme activity	MF	Supported four independent ways: ISS from P00697; **IBA** from GO_Central (PANTHER PTN004256961, including HEWL P00698); **IEA** from InterPro IPR000974 / EC 3.2.1.17; and ISS with reference P 8159165
GO:0050829 defense response to Gram-negative bacterium	BP	**Single ISS** annotation, by-similarity to UniProtKB P00697 (GO_REF:0000024, FlyBase). No experimental evidence.
GO:0005576 extracellular region	CC	ISS

Two facts are decisive for the curation decision:

1. **GO:0004568 (chitinase activity) is entirely absent** from the current annotation set, and **GO:0045824 (negative regulation of innate immune response) is entirely absent.** Both predicted terms would therefore be **new additions**, and neither is justified by any experimental or well-founded computational evidence.
2. The one immune-adjacent term that does exist — **GO:0050829** — rests on a **single ISS "by similarity"** annotation with no experimental support, and it is in tension with the digestive, infection-repressed expression profile documented in Finding 2. It should be treated as low-confidence, not as corroboration of the seed hypothesis.

Mechanistic Model / Interpretation

The seed hypothesis effectively bundles two distinct claims, and both fail for the same underlying reason — **conflation of superficially related categories**:

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BioReason-Pro prediction                Reality (this analysis)
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MF: chitinase activity      ──►  GH22 C-type lysozyme (muramidase, EC 3.2.1.17)
    (GO:0004568)                  · Glu35/Asp52 dyad conserved (41% id to HEWL)
                                  · 8 Cys / 4 disulfides, lysozyme fold
                                  · NO GH18/GH19 chitinase domain or motif
                                  · "chitinase" = weak chitodextrin SIDE-activity
                                    intrinsic to C-type lysozymes → misread as
                                    full endochitinase

BP: neg. reg. innate        ──►  Constitutive DIGESTIVE lysozyme
    immune response               · midgut-restricted; NOT in fat body/hemocytes
    (GO:0045824)                  · transcription REPRESSED by infection
BP: defense vs Gram-neg.          · "recruited for digestion of bacteria in food"
    (GO:0050829, ISS-only)        · immune terms = digestive-vs-immune conflation
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Molecular axis (lysozyme vs chitinase). Both lysozymes and chitinases are glycoside hydrolases that cleave β-1,4 glycosidic bonds in GlcNAc-containing polymers, so they share a substrate chemistry motif. But they are **different enzyme families with different folds and active sites**: GH22 (lysozyme) versus GH18/GH19 (chitinase). C-type lysozymes have a documented, weak ability to hydrolyze short **chitodextrins** — this is even written into the UniProt/Rhea catalytic-activity string for Q08694. A model trained to associate GlcNAc-hydrolysis language with "chitinase" will over-predict GO:0004568 for a lysozyme. The direct evidence (conserved lysozyme dyad, correct fold, absent chitinase motif) refutes a genuine chitinase assignment.

Physiological axis (digestive vs immune). Insect lysozymes occupy two functionally divergent niches: (i) **inducible immune-effector** lysozymes expressed in fat body/hemolymph and up-regulated by infection, and (ii) **constitutive digestive** lysozymes expressed in an acidic midgut and used to lyse and digest dietary/gut bacteria (the "bacteriophagy" strategy shared with ruminants, foregut-fermenting mammals, and acaridid mites). Drosophila LysB belongs unambiguously to class (ii): midgut-restricted, acidic, constitutive, and infection-**repressed**. A negative *regulator* of innate immunity (GO:0045824) would be a signaling/regulatory role entirely unlike a secreted gut hydrolase; nothing in the evidence supports it, and the model likely generated it by over-generalizing "lysozyme → immunity" and then inverting it to fit a repression signal.

The coherent model is therefore: **LysB is a secreted, extracellular (GO:0005576), acidic midgut muramidase (GO:0003796) whose biological process is the digestion of bacteria in food (bacteriophagy), not immune defense or immune regulation.**

Evidence Matrix

#	Citation	Evidence type	Direction	Claim tested	Key finding	Context
1	UniProt Q08694 (database)	Review/ database	**Refutes** chitinase; **supports** lysozyme	Is LysB a chitinase or a lysozyme?	Recommended name "Lysozyme B", EC 3.2.1.17, GH22 family; MF = GO:0003796 lysozyme activity	*D. melanogaster* protein record
2	InterPro/ Pfam/ CDD/ PANTHER/ SUPFAM (database)	Structural/ evolutionary (computational)	**Refutes** chitinase	Does LysB carry a chitinase domain?	Exclusively C-type lysozyme domains (IPR001916/000974, PF00062, cd16899, PTHR11407, SSF53955); no GH18/GH19	Domain architecture
3	PMID: 17825580	Structural/ review	**Refutes** chitinase; supports digestive lysozyme	Are family-22 lysozymes a defense/ digestive class distinct from chitinases?	"Lysozymes from family 22 of glycoside hydrolases are usually part of the defense system against bacteria"; first digestive-lysozyme crystal structure	Housefly digestive lysozyme
4	In-run alignment (this run)	Computational	**Refutes** chitinase	Does LysB have a lysozyme dyad and lack a chitinase motif?	41.3% id to HEWL; Glu35/Asp52 conserved; 8 Cys; GH18 DxxDxDxE motif absent	Mature LysB vs HEWL
5	PMID: 8159165	Localization / expression	**Refutes** immune roles;	Where is LysB expressed?	"LysB, C, D and E... strongly expressed in the midgut"; "None... expressed	Larval & adult midgut

#	Citation	Evidence type	Direction	Claim tested	Key finding	Context
			supports digestive		in the fat body or haemocytes"	
6	PMID: 1588905	Expression / mutant-adjacent	**Refutes** immune induction; supports digestive	Is LysB induced or repressed by infection?	Transcription decreases after bacterial injection; "recruited for the digestion of bacteria present in fermenting food"	Drosophila gut
7	QuickGO for Q08694 (database)	Review/ database	**Refutes** both predicted terms	Do current GO annotations include chitinase/ immune-reg?	No GO:0004568 and no GO:0045824; GO:0050829 is single ISS by-similarity; core = GO:0003796 + GO:0005576	GO annotation record
8	PMID: 19099150	Direct assay (ortholog)	**Qualifies/ supports** digestive	Do insect gut lysozymes act as acidic digestive enzymes?	MdL1/MdL2 housefly midgut lysozymes, acidic pH optimum (~4.8)	Housefly midgut
9	PMID: 18357505	Direct assay (comparative)	**Qualifies/ supports** digestive	Is bacteriophagy a general digestive-lysozyme strategy?	Acidic digestive lysozymes let mites use bacteria as food; gut-pH activity optimum	Acaridid mites
10	PMID: 19161941	Direct assay (ortholog)	**Competing/ qualifies**	Can an insect c-type lysozyme have immune activity?	Housefly MdLys mildly up-regulated by bacteria; recombinant protein inhibits	Housefly midgut/fat body

#	Citation	Evidence type	Direction	Claim tested	Key finding	Context
					G− and G+ bacteria	

Evidence Base (literature summary)

Paper	PMID	Role in this evaluation
The lysozyme locus in Drosophila melanogaster: an expanded gene family adapted for expression in the digestive tract	8159165	**Primary, decisive.** LysB–E strongly midgut-expressed; none in fat body/hemocytes; acidic, digestive-lysozyme-like.
The lysozyme locus in Drosophila melanogaster: different genes are expressed in midgut and salivary glands	1588905	**Primary, decisive.** Transcription decreases after bacterial injection; recruited for digestion of dietary bacteria.
The crystal structure of a lysozyme c from housefly Musca domestica, the first structure of a digestive lysozyme	17825580	Confirms GH22 family placement and digestive-lysozyme structural class.
Cloning, purification and comparative characterization of two digestive lysozymes from Musca domestica larvae	19099150	Ortholog evidence: acidic-optimum midgut digestive lysozymes.
Digestive function of lysozyme in synanthropic acaridid mites enables utilization of bacteria as a food source	18357505	Comparative evidence: acidic digestive lysozyme = bacteriophagy strategy.
Molecular characterization and expression analysis of a chicken-type lysozyme gene from housefly (Musca domestica)	19161941	Competing/qualifying: some insect c-type lysozymes are dual digestive/immune — but MdLys (fat-body-expressed, infection-induced) differs from LysB.

GO Curation Implications

Lead (requires curator verification):

1. **GO:0004568 (chitinase activity, MF) — DO NOT ADD.** The prediction is a fold/activity misassignment. LysB is a GH22 muramidase with a conserved lysozyme catalytic dyad and no GH18/GH19 chitinase domain or active-site motif. The only "chitinase-like" property is

the intrinsic weak chitodextrin side-activity of C-type lysozymes, which is already captured by the lysozyme catalytic-activity description and does not warrant a chitinase MF term (in vitro-only, side-activity, not the biological function).

2. **GO:0045824 (negative regulation of innate immune response, BP) — DO NOT ADD.** No experimental or credible computational support. The term is biologically implausible for a secreted digestive hydrolase and contradicts the constitutive, infection-repressed, midgut-restricted expression profile.
3. **GO:0050829 (defense response to Gram-negative bacterium, BP) — FLAG / treat as non-core.** Present in the record only as a **single ISS "by similarity"** to HEWL-type P00697, with no experimental basis, and in tension with digestive biology. Recommend curator review; at minimum it should not be treated as core function, and it should not be cited as corroboration of the immune hypothesis.
4. **GO:0003796 (lysozyme activity, MF) — RETAIN (core).** Well supported by four independent evidence lines (ISS, IBA, IEA/InterPro-EC, and ISS to P 8159165) and by the in-run catalytic-dyad analysis. This is the correct primary molecular function.
5. **GO:0005576 (extracellular region, CC) — RETAIN.** Consistent with a secreted gut lysozyme.
6. **Consider ADDING a digestion-focused BP term** (curator lead): a term reflecting **digestion / catabolism of bacterial cell wall in the gut / bacteriophagy** would more accurately capture the biological process than any immune term. Candidate directions include a peptidoglycan catabolic process or digestion term; the exact choice should be curator-verified against GO structure and the P 8159165 / P 1588905 evidence.

Aspect summary: MF = lysozyme activity (retain), not chitinase (reject). BP = digestion/bacteriophagy (add, curator-verify), not immune regulation (reject) and not defense-response as core (downgrade). CC = extracellular region (retain).

Mechanistic Scope

The immediate molecular function under test is **glycoside-hydrolase activity on a GlcNAc-containing polymer**. The direct gene-product activity of LysB is **muramidase/lysozyme activity** — hydrolysis of the MurNAc–GlcNAc β-1,4 bond in bacterial peptidoglycan — as established by family classification, domain content, the conserved Glu/Asp catalytic dyad, and EC 3.2.1.17. This is distinct from **chitinase activity**, which requires the GH18/GH19 fold and active site that LysB lacks.

Everything in the immune framing of the seed hypothesis is either **downstream/indirect** or **absent**: - "Defense response to Gram-negative bacterium" is an inferred phenotype-level consequence, imported by similarity, not a demonstrated function of LysB. - "Negative regulation of innate immune response" is a regulatory/signaling process category with no mechanistic link to a secreted digestive hydrolase; there is no evidence LysB participates in immune signaling. - The digestive role (lysing dietary bacteria in the acidic midgut) is the direct physiological deployment of the muramidase activity — a **nutritional/digestive** process, not an immune-defense process, even though the underlying chemistry (bacteriolysis) is shared with immune lysozymes.

Conflicts and Alternatives

- **Paralog / family carry-over confusion.** The Drosophila lysozyme locus contains a tandem cluster (LysB–E, plus LysP, LysS, etc.). Annotations and predictions can bleed across paralogs and across the broader "lysozyme/glycosidase" family. The GO:0050829 ISS to HEWL (P00697) is a concrete example of **defense-response carry-over** from a canonical immune lysozyme to a digestive one.
- **Dual-function orthologs are real but do not rescue the immune prediction.** Housefly MdLys (PMID: 19161941) is expressed in midgut *and* fat body, is mildly infection-induced, and its recombinant protein inhibits bacteria — i.e., some insect c-type lysozymes are genuinely dual digestive/immune. This is a legitimate **competing consideration**. However, it does **not** apply to Drosophila LysB, which — unlike MdLys — is **not** expressed in fat body/hemocytes and is **repressed** (not induced) by infection (PMID: 8159165; PMID: 1588905). Even the strongest immune interpretation would be a mild bactericidal *effector* activity, never *negative regulation* of the immune response (GO:0045824).

- **Weak in-vitro chitodextrin activity vs biological chitinase.** C-type lysozymes can slowly hydrolyze short chito-oligosaccharides. Treating this in-vitro side-activity as a biological chitinase function would be an over-annotation; the physiological substrate is peptidoglycan.
- **Organism/tissue specificity.** Evidence supporting digestion comes partly from orthologs (housefly, mites). While the Drosophila-specific expression data are decisive against the immune-effector/regulator interpretation, direct enzymatic assays of purified Q08694 protein are not in the reviewed literature set (see Knowledge Gaps).

Limitations and Knowledge Gaps

1. **No direct enzymatic assay of purified LysB (Q08694).** Checked: the primary literature reviewed here characterizes the *locus* expression (P 8159165 P 1588905) and orthologous digestive lysozymes (housefly, mites), but not a purified recombinant LysB kinetic assay. This matters because the definitive discriminator between lysozyme and chitinase, and between digestive and immune roles, is a direct activity/pH-profile assay on the LysB protein. *Resolution:* recombinant expression + muramidase vs chitinase activity assays and an acidic-pH-optimum profile.
2. **Alignment used a single reference (HEWL).** The in-run NW alignment (41% identity, conserved dyad) is robust for classification but is a custom implementation against one archetype. *Resolution:* a multiple-sequence alignment across GH22 members and a structural superposition (e.g., AlphaFold model vs HEWL and vs a GH18 chitinase) would strengthen the fold assignment. Structural tools (Phenix/superpose) were available but not required given the concordant sequence/domain evidence.
3. **GO:0050829 provenance.** It is ISS-by-similarity to P00697; the exact rationale and whether FlyBase intends it as core is not fully resolved from the record alone. *Resolution:* curator inspection of GO_REF:0000024 and the FlyBase gene report.
4. **No in-run structural confidence metrics (pLDDT/PAE).** An AlphaFold confidence check was not run; it would add independent support for the lysozyme fold but is not necessary to reach the verdict.

5. **Reference doi:10.64898/2026.03.19.712954** (the source of the BioReason-Pro prediction) was not independently retrieved; the evaluation treats the prediction as stated in the seed context.

Discriminating Tests

The following would most efficiently separate the seed hypothesis from the digestive-lysozyme interpretation:

1. **Enzymatic assay of recombinant LysB.** Measure muramidase activity on *Micrococcus lysodeikticus* cell walls vs endochitinase activity on a fluorogenic chitin substrate (e.g., 4-MU-chitotrioside) and on insoluble/colloidal chitin. Prediction: strong acidic-pH muramidase activity, negligible/only weak short-chitodextrin activity, no processive chitin degradation. This directly refutes GO:0004568.
2. **pH–activity profile.** Digestive insect lysozymes have acidic optima (~4.8, cf. housefly MdL1/MdL2, PMID: 19099150). An acidic optimum supports a gut-digestive role.
3. **Infection-challenge transcriptomics.** RNA-seq/qPCR of LysB after septic injury vs oral bacterial feeding, in gut vs fat body. Prediction: no fat-body induction, repression in gut — inconsistent with an immune effector and with a negative regulator of immunity.
4. **Loss-of-function phenotype.** LysB knockdown/knockout scored for gut bacterial digestion/clearance and nutritional phenotype vs systemic immune-response readouts (AMP induction, survival to infection). Prediction: digestive/nutritional phenotype, not altered immune signaling.
5. **Structural superposition.** AlphaFold model of LysB superposed on HEWL (GH22) and on a GH18 chitinase (TIM-barrel). Prediction: RMSD-close to HEWL, fold-incompatible with the chitinase TIM-barrel.
6. **Comparative catalytic-residue mapping** across the Drosophila Lys cluster and canonical GH18/GH19 chitinases to confirm the absence of the chitinase catalytic glutamate context in all LysB-family members.

Proposed Follow-up Actions (Curation Leads)

All items are **leads requiring curator verification.**

Candidate action changes - **Reject** proposed GO:0004568 (chitinase activity) and GO:0045824 (negative regulation of innate immune response) — do not add. - **Downgrade / flag** GO:0050829 (defense response to Gram-negative bacterium) as ISS-only, non-core, conflicting with digestive expression data. - **Retain** GO:0003796 (lysozyme activity, MF) and GO:0005576 (extracellular region, CC) as core. - **Consider adding** a digestion/bacteriophagy BP term (curator to select the exact term; e.g., a peptidoglycan catabolic / digestion term).

Candidate references with snippets to verify - PMID: 8159165: "*four closely related genes, LysB, C, D and E, are all strongly expressed in the midgut of larvae and adults*" and "*None of the genes is expressed in the fat body or haemocytes.*" → supports digestive, refutes immune-effector. - PMID: 1588905: "*have been recruited for the digestion of bacteria present in fermenting food*" → supports digestive; note transcription decreases after bacterial injection. - PMID: 17825580: "*Lysozymes from family 22 of glycoside hydrolases are usually part of the defense system against bacteria*" → confirms GH22 family placement (distinct from chitinase families). - UniProt Q08694 FUNCTION: "*Unlikely to play an active role in the humoral immune defense. May have a function in the digestion of bacteria in the food.*"

Suggested curator questions - Should GO:0050829 remain given it is ISS-only and conflicts with midgut-digestive, infection-repressed biology? - Is there an appropriate GO BP term for gut bacteriophagy/digestion that should replace the immune framing? - Does FlyBase hold any direct enzymatic data on Q08694 that would confirm the acidic muramidase profile?

Suggested experiments — see Discriminating Tests above (recombinant muramidase-vs-chitinase assay; acidic pH profile; challenge transcriptomics; LOF phenotype; structural superposition).

Bottom Line

LysB (Q08694) is a **GH22 C-type digestive lysozyme (muramidase, EC 3.2.1.17)**. The BioReason-Pro predictions of **chitinase activity (GO:0004568)** and **negative regulation of innate immune response (GO:0045824)** are **refuted / over-annotated**: the chitinase call is a fold/side-activity misassignment, and the immune-regulation call conflates a constitutive, infection-repressed, midgut-restricted digestive enzyme with an immune regulator. The pre-

existing **GO:0050829** annotation is ISS-only and should be treated as low-confidence, non-core. Curators should retain **GO:0003796 (lysozyme activity)** and **GO:0005576 (extracellular region)** and consider adding a **digestion/bacteriophagy** BP term rather than any immune term.
