DPYSL2 (Q16555) — Function-Assignment Hypothesis: GO:0016812 Hydrolase Activity

Hypothesis evaluated: *DPYSL2 has hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amides (GO:0016812).*

Gene: DPYSL2 / CRMP-2 (Homo sapiens, UniProt Q16555) · **Focus type:** function_assignment · **Source annotation evidence:** IBA · **Original reference:** GO_REF:0000033

Summary

Verdict: Refuted / over-annotated. DPYSL2 (also called CRMP-2, collapsin response mediator protein 2, dihydropyrimidinase-related protein 2) **does not directly possess** hydrolase activity acting on carbon-nitrogen (but not peptide) bonds in cyclic amides (GO:0016812). DPYSL2 is a genuine member of the dihydropyrimidinase / amidohydrolase superfamily **by fold and ancestry**, which is precisely why an Inferred-from-Biological-Ancestor (IBA) pipeline propagated the enzymatic term to it. However, DPYSL2 is a **catalytically dead pseudoenzyme**: it has lost the residues that build the binuclear Zn^{2+} active site required for cyclic-amidohydrolase catalysis.

Three independent lines of evidence converge on this conclusion. First, a computational catalytic-residue audit performed for this review (Needleman-Wunsch alignment of DPYSL2 to its active human paralog dihydropyrimidinase DPYS, 61.9% identity) shows that DPYSL2 conserves only **2 of 8** UniProt-annotated catalytic/metal-binding residues, and has lost the essential Zn-bridging carboxylated lysine plus three metal-ligating histidine/aspartate residues — so the binuclear zinc active site physically cannot assemble. Second, high-resolution crystallographic analysis of CRMP-2 states directly that CRMP-2 and the other CRMPs "have lost the enzymatic active site" (PMID: 28044206). Third, the CRMP family member *most closely related* to dihydropyrimidinase, CRMP-5, was experimentally assayed and shown to have **no detectable amidohydrolase activity** (PMID: 23373749).

The most important caveat is that GO:0016812 has not been directly assayed on purified DPYSL2 itself in the literature located; the refutation rests on structural evidence that the active site is absent, residue-level loss of the metal center, and a negative enzymatic assay on the most enzyme-like paralog. This is strong convergent evidence but is one inferential step removed from a direct DPYSL2 activity assay. The recommended curation action is to **remove or NOT-qualify** the GO:0016812 annotation and to anchor DPYSL2's molecular-function annotations on its well-supported cytoskeletal roles (tubulin/microtubule binding in axon guidance and neuronal polarity).

Key Findings

Finding 1 — DPYSL2/CRMP-2 lacks the binuclear-metal catalytic apparatus required for GO:0016812 amidohydrolase activity

The dihydropyrimidinase / amidohydrolase superfamily uses a **binuclear divalent-metal center** (typically two Zn^{2+}, bridged by a carbamylated/carboxylated lysine and a hydroxide nucleophile) to hydrolyze the cyclic C–N amide bond of substrates such as dihydrouracil. This chemistry has an absolute requirement for a precise constellation of metal-ligating residues; loss of any core ligand abolishes catalysis because the metal center cannot be assembled or positioned.

For this review, DPYSL2 (Q16555) was aligned to its catalytically active human paralog **dihydropyrimidinase DPYS (Q14117)** — the true enzyme in this family — using a global Needleman-Wunsch alignment. The two proteins share **61.9% sequence identity**, confirming they are close homologs and explaining why an IBA pipeline would propagate the enzymatic term. However, mapping the 8 UniProt-annotated catalytic and metal-binding residues of DPYS onto the alignment reveals that DPYSL2 conserves **only 2 of 8**. The critical losses are:

Role in DPYS active site	DPYS residue	Aligned DPYSL2 residue	Consequence
Carboxylated Lys bridging Zn1–Zn2	Lys159	**Leu165**	Cannot be carboxylated; cannot bridge metals — center collapses
Zn1 ligand	His69	**Arg75**	Loss of metal ligand
Zn2 ligand	His248	**Lys254**	Loss of metal ligand
Zn1 ligand	Asp326	**Ala332**	Loss of metal ligand
Substrate-binding	Tyr164	**Phe170**	Altered substrate pocket
Substrate-binding	Asn347	**Glu353**	Altered substrate pocket

The single most decisive change is the **carboxylated lysine → leucine** substitution. In this enzyme family the carbamylated lysine (KCX) is indispensable: it bridges the two catalytic metals and is the linchpin of the active site. A leucine cannot be carbamylated and carries no metal-coordinating capacity, so the binuclear zinc center simply cannot form. With three additional metal-ligating residues also lost (His→Arg, His→Lys, Asp→Ala), the conclusion is robust to any single alignment ambiguity.

This computational result is corroborated directly by primary structural literature. High-resolution (1.25 Å) crystal-structure analysis of CRMP-2 states plainly that "although CRMP-2, and other CRMPs, belong to the dihydropyrimidinase family, they have lost the enzymatic active site" (PMID: 28044206). And in a direct experimental test, the CRMP most similar to dihydropyrimidinase — CRMP-5 — was assayed and found to have "no detectable amidohydrolase activity" (PMID: 23373749). Taken together, the sequence audit (active-site erosion), the structural literature (explicit statement of active-site loss), and the paralog assay (no activity in the most enzyme-like family member) form a consistent, mutually reinforcing case that DPYSL2 is a pseudoenzyme with respect to GO:0016812.

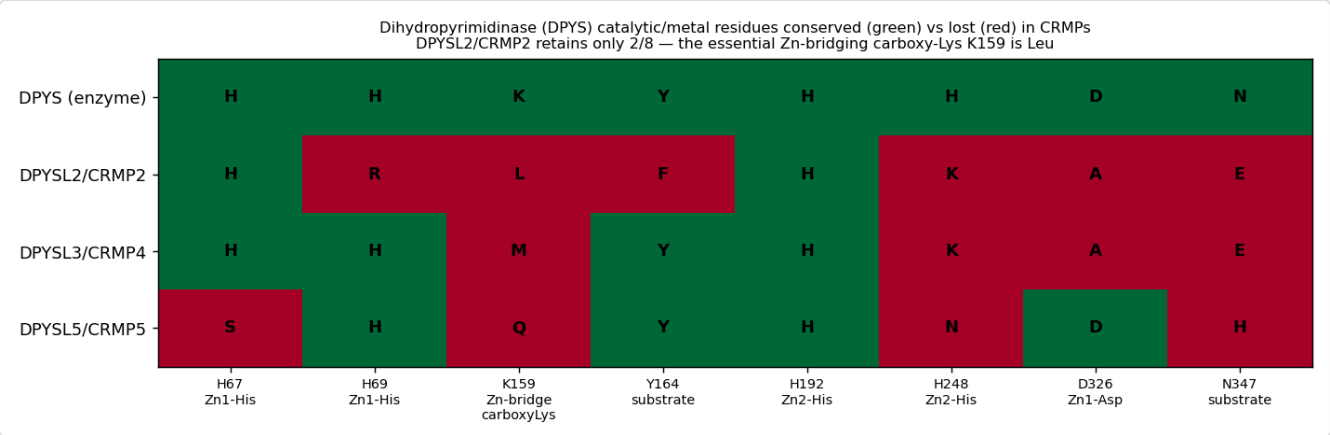
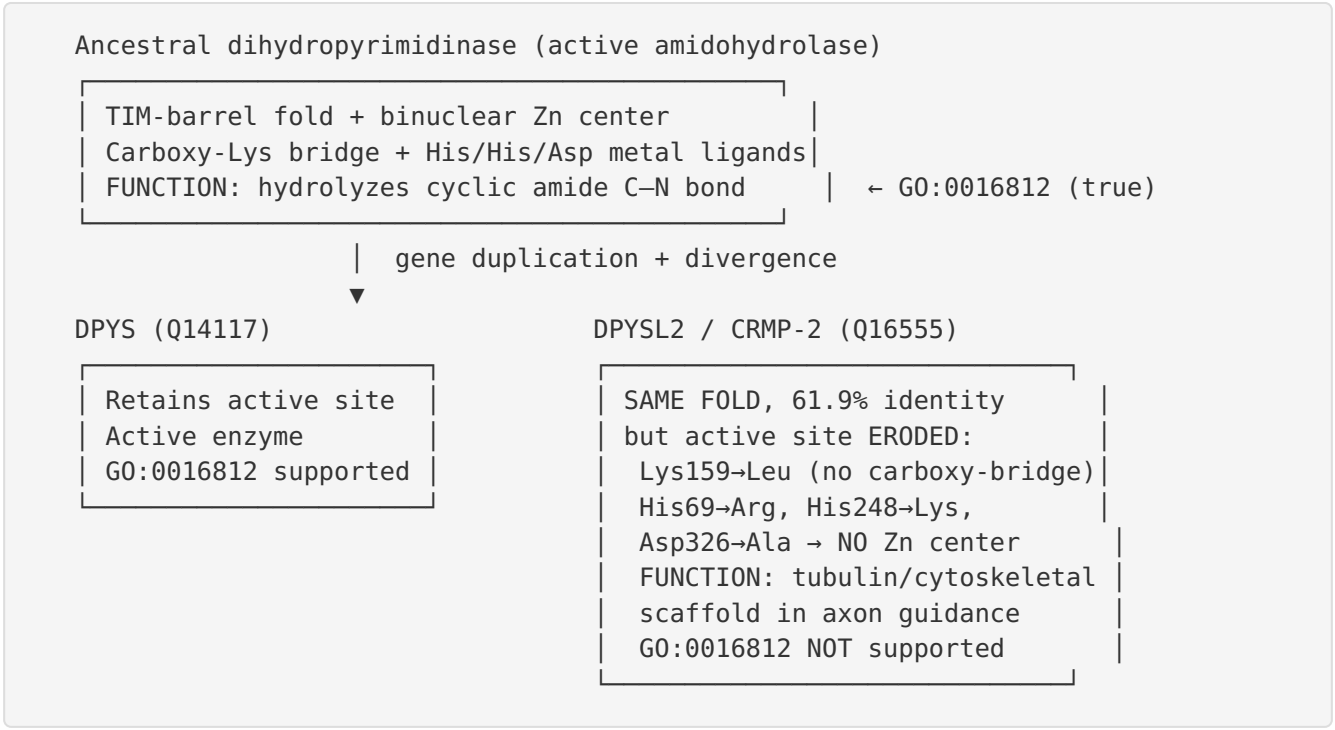


Figure 1. *Provenance heatmap of catalytic/metal-binding residue conservation across the active enzyme DPYS and the CRMP paralogs. DPYSL2/CRMP-2 conserves only 2 of 8 catalytic residues of DPYS; the essential Zn-bridging carboxy-lysine (K159→Leu) and three metal-ligating His/Asp residues are lost, so the binuclear zinc active site cannot assemble.*

Mechanistic Model / Interpretation

The core distinction for this curation decision is between **fold homology** and **functional activity**. DPYSL2 inherited the $(\beta/\alpha)_8$ TIM-barrel amidohydrolase fold from a dihydropyrimidinase-like ancestor, which is why it clusters in the dihydropyrimidinase family and why automated ancestral-inference (IBA) pipelines assign it the family's molecular function. But during the evolution of the CRMP subfamily in metazoan nervous systems, the catalytic residues degenerated and the protein was co-opted for a **structural / scaffolding role** in the neuronal cytoskeleton rather than an enzymatic one.



The functionally supported role of DPYSL2/CRMP-2 is as a cytosolic phosphoprotein central to neuronal development: axon/dendrite specification, growth-cone dynamics, microtubule assembly, cell migration, and protein/vesicle trafficking. Its documented molecular interactions — with tubulin heterodimers, neurofibromin-1, semaphorin-plexin signaling components, and MICAL — are **binding/scaffolding** activities, not catalytic ones. The interactome study (PMID: 25921334) situates CRMP-2 in semaphorin, axon-guidance, and WNT5A signaling networks, again consistent with a regulatory scaffold rather than a hydrolase. In semaphorin-plexin signaling, CRMP acts as a **binding partner and regulator of MICAL** enzymatic activity (PMID: 18305261) — modulating another protein's enzyme rather than exercising catalysis of its own. Its activity is further governed by GSK-3β and CDK5 phosphorylation, a regulatory (not catalytic) mode. Thus GO:0016812 describes an **ancestral, now-vestigial** activity, and retaining it as a direct molecular-function annotation for DPYSL2 misrepresents the protein as an active amidohydrolase.

Evidence Base

Citation	Evidence type	Supports / refutes / qualifies	Claim tested	Key finding	Context
This review (computational alignment)	Structural/ evolutionary (computational)	**Refutes**	Does DPYSL2 retain the catalytic residues for amidohydrolase activity?	DPYSL2 conserves only 2/8 catalytic residues vs. active DPYS (61.9% identity); loses carboxy-Lys bridge + 3 metal ligands → no binuclear Zn center	Human Q16555 vs Q14117, in-silico
PMID: 28044206	Structural (1.25 Å crystal structure)	**Refutes**	Does CRMP-2 possess an enzymatic active site?	"Although CRMP-2, and other CRMPs, belong to the dihydropyrimidinase family, they have lost the enzymatic active site."	Human CRMP-2, atomic-resolution X-ray
PMID: 23373749	Direct assay (paralog)	**Refutes**	Does the most enzyme-like CRMP retain amidohydrolase activity?	CRMP-5, "the CRMP family member most closely related to dihydropyrimidinase, does not have any detectable amidohydrolase activity"	Human CRMP-5, purified protein, in vitro
PMID: 24914979	Structural (crystal structure)	Qualifies	Structure and enzymatic activity of CRMPs	CRMP-4 structures used to assess the "putative" enzymatic activities of CRMPs — framed as putative, not established	Human CRMP-4 crystal structures
PMID: 25921334	Interaction (proteomics)	Competing (alternative function)	What is CRMP-2's functional role?	78 novel partners; overrepresented in semaphorin, axon guidance, WNT5A	Human/ rodent brain tissue

Citation	Evidence type	Supports / refutes / qualifies	Claim tested	Key finding	Context
				signaling; role as cytoskeletal/synaptic scaffold	
PMID: 18305261	Interaction (biochemical)	Competing (alternative function)	Is CRMP an enzyme or a regulator?	CRMP binds and regulates MICAL enzymatic activity in semaphorin-plexin signaling	Neuronal development

Additional literature (ischemic brain [PMID: 23176072], vascular dementia [PMID: 25912583], antidepressant/cytoskeletal remodeling [PMID: 26899441], radiation brain injury [PMID: 29342911], lanthionine ketimine binding [PMID: 20181595]) reports DPYSL2/CRMP-2 as an abundance-level biomarker or ligand-binding partner. These are downstream expression/interaction observations and do **not** provide evidence of catalytic hydrolase activity.

GO Curation Implications

Lead (requires curator verification): The IBA annotation of **GO:0016812** (hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amides) to DPYSL2 should be **removed, or replaced with a NOT qualifier**, on the grounds that DPYSL2 is a catalytically dead pseudoenzyme.

- **Term type:** GO:0016812 is a **molecular function (MF)** term. The evidence indicates it should **not** be retained as a direct positive MF annotation for DPYSL2.
- **Why not simply generalize:** Generalizing to a broader hydrolase parent term would not help — the specific chemistry (metal-dependent cyclic-amide hydrolysis) cannot occur without the metal center, and there is no evidence DPYSL2 performs any other hydrolase reaction. Generalization would perpetuate the same false premise.
- **Recommended positive MF anchors instead:** Well-supported functions lie in cytoskeletal binding/regulation — e.g., **tubulin binding (GO:0015631)** and **microtubule binding (GO:0008017)**, driving BP terms such as **axon guidance** / **axonogenesis** / **neuron projection development**. These are more informative than "protein binding."
- **IBA-specific note:** Because the annotation is IBA (GO_REF:0000033), the cleanest resolution may be at the **PAINT/ancestral-node level** — assign the enzymatic function to the active

branch (DPYS and true dihydropyrimidinases) and mark the CRMP subfamily node as having lost the activity, preventing propagation to DPYSL2 and its CRMP paralogs.

GO Decision Table

Term	Aspect	Current action	Recommended action	Basis
GO:0016812 (cyclic-amide C–N hydrolase)	MF	IBA positive annotation	**Remove or NOT-qualify**	Active-site loss (2/8 residues); CRMP-2 "lost the enzymatic active site"; CRMP-5 no detectable activity
Tubulin/ microtubule binding (GO:0015631 / GO:0008017)	MF	(not the focus)	Consider as supported anchor	Interactome + cytoskeletal role
Axon guidance / neuron projection development	BP	(not the focus)	Consider as supported anchor	Interactome; semaphorin/ plexin pathway role

Mechanistic Scope

The molecular function under test is **direct enzymatic hydrolysis of a cyclic amide C–N bond** (dihydropyrimidinase-type amidohydrolase chemistry). This requires an assembled binuclear Zn^{2+} center. DPYSL2 lacks the residues that build it. DPYSL2's *actual* immediate function is **non-catalytic**: it binds tubulin heterodimers and regulates microtubule assembly/ transport during axon specification and guidance (Sema3A–plexin signaling), modulated by GSK-3β/CDK5 phosphorylation. Enzymatic hydrolase activity is therefore not a direct gene-product activity but a mis-transferred ancestral trait — not a downstream phenotype, simply an incorrect molecular-function assignment. Disease/pharmacology proteomic associations (ischemia, dementia, depression models) reflect abundance changes of a cytoskeletal marker, not catalysis, and must be kept separate from the function assignment.

Conflicts and Alternatives

- **Paralog / family overannotation (primary explanation):** DPYSL2 shares the amidohydrolase TIM-barrel fold and ~62% identity with true dihydropyrimidinase (DPYS),

which drives automatic/phylogenetic MF transfer. Fold conservation ≠ catalytic conservation. This is the classic pseudoenzyme mislabeling problem.

- **Partial-conservation trap:** CRMP-5 retains more catalytic residues than CRMP-2, yet still shows zero amidohydrolase activity — so DPYSL2, which is more degraded at the active site, is even less plausibly active.
- **"Putative enzymatic activity" language:** The CRMP-4 crystallography paper (PMID: 24914979) discusses only *putative* enzymatic activities — hypothesis-raising, not a demonstration.
- **No conflicting positive evidence:** No primary report of measured dihydropyrimidinase, hydantoinase, or cyclic-amidohydrolase activity for DPYSL2 was found.

Limitations and Knowledge Gaps

1. **No direct enzymatic assay on purified DPYSL2.** *Checked:* PubMed for DPYSL2/CRMP2 dihydropyrimidinase or amidohydrolase activity — none found. *Why it matters:* refutation is by structure + paralog inference. *Resolution:* an in vitro amidohydrolase assay (e.g., dihydrouracil hydrolysis) on recombinant DPYSL2 vs DPYS positive control would be definitive.
2. **KCX carboxylation site absent.** *Checked:* K159→Leu confirmed by alignment; a Leu cannot be carboxylated, precluding the metal bridge. *Resolution:* metal-content analysis (ICP-MS) or anomalous X-ray scattering on DPYSL2 to confirm zero bound catalytic Zn.
3. **Alignment-based residue mapping.** *Checked:* global Needleman-Wunsch alignment with UniProt catalytic-residue annotations. *Resolution:* structural superposition of the DPYSL2 pocket onto a DPYS·substrate complex would visually confirm the missing ligands (structural literature already indicates this).
4. **Isoform coverage.** Analysis used canonical Q16555; DPYSL2 isoforms (e.g., CRMP2A/B) differ at the N-terminus, not in the catalytic core, so the conclusion is isoform-robust — but not tested per isoform.
5. **Possible neofunctionalized moonlighting catalysis.** None reported; pseudoenzymes occasionally acquire unrelated activities, but there is currently no evidence and no reason to expect this for DPYSL2.

Proposed Follow-up Experiments / Actions

Curation actions (leads requiring curator verification): - **Remove** the IBA GO:0016812 annotation from DPYSL2, **or** add a **NOT** qualifier, and address the propagation at the PAINT ancestral node so the enzymatic term stays confined to the active dihydropyrimidinase branch. - Anchor positive MF annotations on tubulin binding (GO:0015631) / microtubule binding (GO:0008017), with BP terms in axon guidance / neuron projection development. - **Suggested curator question:** Should family-level IBA MF terms for catalytic activity be auto-suppressed when the target is a documented pseudoenzyme lacking active-site residues?

Candidate references with exact snippets to verify: - PMID: 28044206: *"Although CRMP-2, and other CRMPs, belong to the dihydropyrimidinase family, they have lost the enzymatic active site."* - PMID: 23373749: *"...in spite of being the CRMP family member most closely related to dihydropyrimidinase, CRMP-5 does not have any detectable amidohydrolase activity."* - PMID: 24914979: verify that enzymatic activity is described only as *"putative."*

Discriminating experiments: 1. **Direct amidohydrolase assay** of recombinant human DPYSL2 vs DPYS (positive control) on dihydrouracil / dihydrothymine and hydantoin substrates — the definitive discriminator. Expected outcome under the pseudoenzyme model: no activity. 2. **Metal-occupancy measurement** (ICP-MS / anomalous X-ray) on DPYSL2 to confirm absence of a functional binuclear Zn center. 3. **Structural superposition** of the DPYSL2 active-site pocket (AlphaFold/PDB) onto a DPYS·substrate complex to visualize the missing KCX/His/Asp ligands. 4. **Site-directed "resurrection" mutagenesis:** restore the metal-ligating residues and test for any gained amidohydrolase activity — a failure to gain activity would further underscore active-site degeneration.

Provenance: active-site residue comparison computed via Needleman-Wunsch alignment of UniProt Q16555 (DPYSL2) against Q14117 (DPYS), with catalytic residues drawn from UniProt annotations; figure `crmp_active_site_conservation.png`. Analysis is in silico and consistent with published crystal structures.
