Focused Curation Report — *Dictyostelium discoideum* tipA (UniProt Q94489)

Hypothesis evaluated: *TipA is a functional PP2C-family (PPM), metal-dependent protein serine/threonine phosphatase.* **Focus type:** function_assignment · **Slug:** tipa-pp2c-phosphatase **Date:** 2026-07-12 · **Iterations:** 1–3

Executive Judgment

Verdict: PARTIALLY SUPPORTED (family/fold supported; catalytic "functional phosphatase" claim UNRESOLVED — over-annotation risk if asserted with experimental confidence).

- The **domain/fold assignment is well supported.** Q94489 is annotated by multiple orthogonal signature databases as a PPM/PP2C-type phosphatase domain (InterPro IPR001932/IPR053287, Gene3D 3.60.40.10, SUPFAM SSF81606, PANTHER PTHR21586 "TIPA"; UniProt domain 388–706). AlphaFold models this region as a confident PP2C fold (domain mean pLDDT ≈ 81).
- The **"functional / catalytically active phosphatase" part is not demonstrated.** There is **no biochemical evidence** — no phosphatase assay, no metal-dependence test, and no substrate has ever been reported for TipA. The only functional study (Stege et al. 1999, P 10402673) is a developmental-genetic phenotype (tip formation, cell sorting), not an enzymatic characterization.
- A **conserved-motif red flag:** the invariant PP2C metal-1/general-acid signature **[VY]-D-G-H-x-G ("DGH")** — present in human PPM1A and in the *Dictyostelium* functional PP2C **Spalten/SpnA** — is **absent** from TipA (the equivalent position reads **ADGC**, His→Cys).
- **Counter-nuance from structure:** despite the linear-motif loss, the AlphaFold model assembles a **spatial cluster of aspartate carboxylates (D413, D430, D576, D642, D646) plus His645** into a pocket resembling a PP2C bimetal active site. So a *divergent* but real

metal-dependent active site remains plausible; this is not a clearly collapsed pseudophosphatase.

- **Family-wide catalytic-motif loss (over-annotation signal, added Iteration 2):** across the entire TIPA-specific PP2C-like family (InterPro IPR053287, n=100; Dictyostelium, Drosophila CG9801, Caenorhabditis, Trichinella, cnidarians, molluscs, Entamoeba), the canonical DGHxG motif is present in **0%** and a bare DGH in only **7%**, versus **79%** in the canonical PP2C family (IPR001932). The TIPA family is a divergent lineage that systematically lacks the invariant catalytic His, so **family/domain membership cannot by itself justify a "functional phosphatase" call** — this is a textbook paralog/frequency-bias over-annotation scenario. TipA's His645 is a *Dictyostelium-specific* substitution (family consensus has Ser here), i.e., a speculative species-specific candidate general acid, not a conserved catalytic signature.
- **Metal binding vs catalysis (Iteration 3):** the metal-coordinating **aspartate scaffold is conserved family-wide** (N-terminal motif 80%, C-terminal 86%, both 69% of 100 members) while the **catalytic general-acid His is not** (7%). This cleanly separates a plausibly retained *metal-binding* capacity from an *unsupported catalytic* capacity — the molecular signature of a metal-binding but catalytically divergent/pseudo-PP2C.

Bottom line for the curator: the PP2C **domain membership** can be annotated (computational/ISS-level), and **metal ion binding (GO:0046872)** is the best-supported computed inference. A **protein Ser/Thr phosphatase activity term should NOT be asserted with experimental evidence**; catalytic activity is unproven and the canonical catalytic His motif is degenerate across the whole TIPA lineage. Treat "functional metal-dependent Ser/Thr phosphatase" as a hypothesis to be tested, not an established fact.

Evidence Matrix

#	Citation	Evidence type	Supports/ Refutes/ Qualifies	Claim tested	Key finding	Context
1	UniProt Q94489; InterPro IPR001932/ IPR053287; SUPFAM SSF81606; Gene3D 3.60.40.10; PANTHER PTHR21586	Structural/ evolutionary (database)	Supports (domain)	TipA contains a PP2C/PPM domain	PPM-type phosphatase domain at 388–706; PANTHER "TIPA" family	Sequence/ profile
2	This work — direct motif scan	Computational	Refutes/ Qualifies (catalysis)	Canonical PP2C catalytic motif conserved?	Invariant **DGH** (metal-1 Asp + general-acid His) present in PPM1A ("VYDGHAGS") and Spalten O15743 ("VYDGHGGT") but **absent in TipA** ("VADGCNWG", His→Cys)	Q94489 vs P35813 vs O15743
3	This work — AlphaFold AF-Q94489-F1 (v6) geometry	Computational (structural)	Qualifies/ Supports (catalysis plausible)	Does an active-site pocket assemble in 3D?	Domain pLDDT ≈ 81; Asp413/430/576/642/646 + His645 cluster within ~4–6 Å into a PP2C-like pocket	Predicted structure
4	Stege, Laub, Loomis 1999 (P 10402673)	Mutant phenotype	Supports BP/CC; silent on MF	What does TipA do biologically?	tipA-null: defective cell sorting, tip formation on mounds, reduced prespore/prestalk gene expression; cell-autonomous; acts in parallel with tipB/C/D	*D. discoideum* developme

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5	Aubry & Firtel 1998 (P 9585512)	Direct assay (paralog control)	Competing/ Qualifies	Is there a bona fide *Dictyostelium* developmental PP2C?	Spalten (SpnA) encodes a **functional PP2C** (demonstrated), essential for cell-type differentiation; PP2C domain is the effector	*D. discoideum* developmer
5b	This work — InterPro family motif census	Computational (evolutionary)	Qualifies/ Refutes (family-based inference)	Does TIPA-family membership imply catalytic residues?	Canonical DGHxG in **0/100** TIPA-family (IPR053287) vs **79/100 (DGH)** in canonical PP2C (IPR001932); family shares divergent motifs "ADG[VC]NWG" and "(T/I)SDG[IV]xDN" but lacks the general-acid His	100 cross-species family members
5c	This work — metal-Asp scaffold census (Iteration 3)	Computational (evolutionary)	Qualifies (metal binding yes, catalysis no)	Are the metal-coordinating aspartates conserved family-wide?	Metal-Asp scaffolds retained: N-term [AG]DG[VCA]N[WF] **80/100**, C-term SDG-Asp **86/100**, both **69/100**; but catalytic His only **7/100**. TipA has both scaffolds + rare His645	100 TIPA-family members
6	Das, Helps, Cohen, Barford 1996 (P 9003755)	Structural (reference)	Orientation	What residues make PP2C catalytic?	PP2C is Mn^{2+}/Mg^{2+}-dependent; a **binuclear metal centre** with metal-bound water provides nucleophile/general acid; requires conserved Asp ligands	Human PP2Cα

GO Curation Implications (leads — require curator verification)

- **Molecular Function.** Do **not** assign an experimentally-supported protein Ser/Thr phosphatase MF term. Options, in order of conservatism:
- Acceptable as **computational/ISS or IBA** (from PANTHER PTHR21586 "TIPA"): `GO:0004722` protein serine/threonine phosphatase activity **or** the more generic `GO:0016791` phosphatase activity — **with an ISS/IEA evidence code and a caveat note** that the canonical catalytic His motif is degenerate and activity is unassayed.
- `GO:0046872` metal ion binding is the **best-supported computed inference** (the metal-coordinating aspartate scaffold is conserved family-wide: N-term 80%, C-term 86%, both 69%), but is still only sequence-level (ISS/IEA) — no metal-binding assay exists.
- If the review's prior action asserted an **experimental** MF phosphatase term, that is **too strong** → recommend downgrading to computational or removing pending assay.
- **Biological Process (retain).** `GO:0031154` culmination involved in sorocarp development (IMP) and `GO:0030587` sorocarp development (HMP) are supported by Stege et al. 1999 (P 10402673). Keep.
- **Cellular Component (retain).** `GO:0005737` cytoplasm (IDA, dictyBase). Keep.
- **Net:** the seed hypothesis, if used to add an experimentally-weighted MF phosphatase annotation, is **too strong**; a computational MF annotation with an explicit "atypical/unverified active site" caveat is defensible.

Mechanistic Scope

- **Immediate molecular function under test:** metal-dependent hydrolysis of phosphoserine/phosphothreonine on protein substrates by a PPM/PP2C catalytic domain. This requires a binuclear Mn^{2+}/Mg^{2+} centre coordinated by conserved aspartates plus a general acid (P 9003755).

- **What the evidence actually shows:** TipA *has* the PP2C fold and can *geometrically* assemble a candidate metal-binding pocket, but **no dephosphorylation, metal dependence, or substrate** has been measured.
- **Downstream/indirect layers (not the MF):** the tipA-null phenotype (cell sorting, tip formation, delayed prespore/prestalk differentiation, aggregate morphogenesis) is a **developmental outcome**, not direct evidence of catalytic activity. Attributing the phenotype to phosphatase catalysis is inference, not demonstration.

Conflicts and Alternatives

1. **Paralog confusion (important).** The validated *Dictyostelium* developmental **PP2C is Spalten/SpnA**
   (P 9585512 **),**
   not TipA. Spalten retains the intact DGH catalytic motif; TipA does not. A curator should ensure phosphatase evidence is not being transferred from Spalten (or the broader PP2C family) onto TipA by homology/family inference.
2. **Pseudophosphatase possibility.** Loss of the invariant general-acid His (DGH→DGC) is a classic hallmark of degenerate/pseudo-phosphatases. TipA could function as a **non-catalytic scaffold/adaptor** whose PP2C-like fold binds phospho-proteins without turning them over.
3. **Divergent-but-active possibility.** The AlphaFold carboxylate+His cluster leaves open that TipA is a *sequence-atypical* active phosphatase (His role relocated to His645). This cannot be resolved without an assay.
4. **Prediction-quality caveats.** Family-level annotation (PANTHER "TIPA") is prone to frequency/paralog bias; AlphaFold does not model catalysis; the linear-motif call ignores 3D. Each tool, alone, is weak — hence the "unresolved" MF verdict.
5. **Family-wide degeneracy quantified (Iteration 2).** The catalytic His loss is not idiosyncratic to TipA but is shared by the whole TIPA/IPR053287 lineage (0/100 with DGHxG vs 79/100 in canonical PP2C). This means any "functional phosphatase" annotation propagated from family/domain membership is unsupported by conserved catalytic residues; if the review's assignment derives from InterPro2GO/PANTHER family inference, that is the specific over-annotation to flag.

Knowledge Gaps

Gap	What was checked	Why it matters	What would resolve it
No enzyme activity data	PubMed searches returned **no** biochemical assay for TipA	"Functional phosphatase" is the crux of the hypothesis	In vitro phosphatase assay (pNPP + protein/ phosphopeptide substrate) ± Mn^{2+}/Mg^{2+}
Catalytic-residue identity	Motif scan (reliable) + AF geometry (suggestive); pairwise alignment was **unreliable** (failed even on the functional Spalten control)	Determines active vs pseudo-phosphatase	Structure-guided HMM/MSA of PPM family with metal modeling; site-directed mutagenesis of candidate Asp/His
Substrate & physiological target	None known	Needed to link catalysis to the tip/sorting phenotype	Phosphoproteomics of tipA-null vs WT; substrate trapping
Metal dependence	Not tested	Defines "metal-dependent" claim	Activity assay ± EDTA / with Mn^{2+}, Mg^{2+} titration
Is His645 the general acid?	Inferred from AF cluster only	Distinguishes divergent-active from dead	H645A / D642A / D646A catalytic mutants + activity

Discriminating Tests (most efficient first)

1. **In vitro phosphatase assay** of recombinant TipA (or its 388–706 domain) on a generic substrate (pNPP or phosphopeptide) with and without Mn^{2+}/Mg^{2+} — directly settles "functional metal-dependent phosphatase."
2. **Structure-guided catalytic mutagenesis** (candidate metal ligands D413/D430/D576/D642/D646 and His645) → test loss of activity and whether mutants fail to rescue the tipA-null developmental phenotype (links catalysis to biology).

3. **Rigorous PPM-family MSA/HMM with metal-site mapping** (e.g., against solved PP2C structures) to formally score active-site integrity rather than relying on a single linear motif.
4. **Phosphoproteomics** of tipA⁻ vs WT during aggregation/tip formation to identify candidate substrates (hyperphosphorylated targets).
5. **Comparative check of the PANTHER "TIPA" subfamily (PTHR21586, incl. Drosophila CG9801)** for conservation/loss of catalytic residues to see if the whole subfamily is degenerate.

Curation Leads (require curator verification)

- **Candidate references to cite/verify:**
- PMID **10402673** (Stege 1999) — snippet: *"null mutations in tipA showed a primary defect in cell sorting and the formation of tips on the developing mound"* → supports BP/CC only, **not** MF.
- PMID **9585512** (Aubry & Firtel 1998) — snippet: *"a carboxy-terminal domain that encodes a functional PP2C"* → the validated developmental PP2C is **Spalten**, useful as paralog control (do not transfer to TipA).
- PMID **9003755** (Das 1996) — snippet: *"Mn^{2+}- or Mg^{2+}-dependent protein Ser/Thr phosphatase … binds two manganese ions … provide a nucleophile and general acid"* → defines the catalytic requirements TipA has not been shown to meet.
- **Candidate GO actions:**
- Keep BP `GO:0031154`, `GO:0030587`; keep CC `GO:0005737`.
- If an MF phosphatase term exists with experimental evidence → **downgrade to ISS/IEA or remove** (too strong).
- If adding MF, use ISS/IBA `GO:0004722` (or `GO:0016791`) **with a caveat** that the catalytic His motif is atypical and activity is unverified. Avoid a bare "protein binding" fallback.
- **Suggested curator questions:**
- Is any experimental phosphatase evidence for TipA in the source, or is the assignment homology-only?
- Is the MF being transferred from Spalten/family membership (paralog carry-over)?
- **Suggested experiments:** see Discriminating Tests 1–2.

Provenance / Reproducibility

- UniProt Q94489 (759 aa; domain 388–706), P35813 (PPM1A), O15743 (Spalten/SpnA) fetched via UniProt REST.
- Active-site motif scan (`DG[HN].G`, `[ILVMFC]DG[ILVMFAWI]`): **DGH present in PPM1A@60 and Spalten@749; absent in TipA (ADGC@430)**.
- AlphaFold AF-Q94489-F1 v6: domain mean pLDDT 80.8; carboxylate cluster D413/D430/D576/D642/D646 + His645 within ~4–6 Å.
- InterPro family motif census (Iteration 2): IPR053287 (TIPA) n=100 → DGHxG 0%, DGH 7%; IPR001932 (canonical PP2C, reviewed) n=100 → DGH 79%. Conserved TIPA-family motifs: "ADG[VC]NWG" and "(T/I)SDG[IV]xDN"; His645 is a Dictyostelium-specific substitution of the family-consensus Ser.
- Pairwise BLOSUM62 global alignment was **explicitly judged unreliable** (failed on the functional Spalten control) and was therefore **not** used for residue-level conclusions.

Limitations: all sequence/structure analyses are computational; no wet-lab activity data exist. Conclusions distinguish direct results (motif presence/absence, fold confidence) from inference (catalytic competence).