KCTD14 Core Function Hypothesis: Identical Protein Binding (GO:0042802)

Executive Judgment

Verdict: Partially supported — valid annotation lead but should not be designated as core function

The hypothesis that identical protein binding (GO:0042802) is a core function of KCTD14 is **partially supported** as a factual description of what KCTD14 likely does, but **weakly supported** as a core function designation. Two lines of evidence confirm KCTD14 self-association: (1) yeast two-hybrid self-interaction data from the proteome-scale HI-II-14 interactome (PMID: 25416956), and (2) AlphaFold multimer predictions yielding reliable pentameric models with a distinctive circle-like CTD organization (PMID: 36362127). Family-wide structural studies using electron microscopy and X-ray crystallography corroborate that BTB-mediated pentamerization is the norm across the KCTD family (PMID: 27152988; PMID: 26334369).

However, the critical curation issue is whether self-binding constitutes a "core function" or merely a structural prerequisite that enables core function. For every experimentally characterized KCTD family member, oligomerization is the assembly step that enables the protein's primary molecular activity — serving as a Cullin3 E3 ubiquitin ligase adaptor, modulating Gβγ signaling, or scaffolding receptor complexes. Treating the scaffold assembly as the core function is analogous to designating "protein folding" as the core function of a kinase. GO:0042802 is a valid annotation lead with IPI evidence, but it should be treated as **non-core**; KCTD14's primary molecular function beyond oligomerization remains experimentally undetermined.

Two additional caveats sharpen this assessment. First, GO:0042802 is **not currently annotated** for KCTD14 in QuickGO — only GO:0051260 (protein homooligomerization, IEA) and GO:0005515 (protein binding, IPI) exist — so the hypothesis proposes both a new annotation and its elevation to core status simultaneously. Second, the Y2H evidence comes from a single high-throughput study with moderate confidence (MI-score 0.37, 3/4 replicas), without independent biochemical validation of the interaction or its stoichiometry.

Summary

KCTD14 (UniProt: Q9BQ13) is a member of the 25-member potassium channel tetramerization domain (KCTD) protein family in humans. Like most family members, it contains an N-terminal BTB (bric-à-brac, tramtrack, broad complex) domain that mediates oligomerization, and a C-terminal domain (CTD) of less-well-characterized function. The seed hypothesis proposes that identical protein binding (GO:0042802) — the capacity to bind copies of itself — represents a core molecular function of KCTD14, based on its predicted pentameric assembly via the BTB domain.

Our investigation confirmed the self-interaction through direct evidence (Y2H) and computational prediction (AlphaFold pentameric modeling), supplemented by extensive family-wide structural data showing pentamerization as the default state for KCTD BTB domains. However, for every characterized KCTD family member, BTB-mediated oligomerization enables downstream molecular activities: Cullin3 E3 ubiquitin ligase adaptor function (KCTD5, KCTD6, KCTD9, KCTD11, KCTD17), Gβγ modulation (KCTD2, KCTD5, KCTD12, KCTD16), GABAB receptor regulation (KCTD8, KCTD12, KCTD16), or developmental pathway control (KCTD1, KCTD11). For KCTD14 specifically, none of these downstream functions have been experimentally confirmed, and the protein remains one of the least-characterized members of the family.

The most informative action for curators is to consider adding GO:0042802 as a non-core MF annotation with IPI evidence from

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, while explicitly noting that the core molecular function remains undetermined. Candidate core functions to monitor include Cullin3 adaptor activity (by closest-paralog KCTD7 analogy), Gβγ subunit modulation (by family-wide screen data), and a potential immunomodulatory role via the TNF-TNFR1 axis (recently proposed computationally for pancreatic cancer).

Key Findings

Finding 1: KCTD14 Self-Interaction Detected by Yeast Two-Hybrid

The most direct experimental evidence for KCTD14 identical protein binding comes from the HI-II-14 proteome-scale human binary interactome map (PMID: 25416956). Rolland et al. (2014) systematically mapped approximately 14,000 high-quality human binary protein-protein interactions using a yeast two-hybrid (Y2H) array approach. Their study, described as providing "reference maps of interactome networks [that] will be critical to fully understand genotype-phenotype relationships," detected KCTD14 (Q9BQ13) self-interaction in 3 out of 4 replicas (IntAct accession: EBI-10487331, MI-score: 0.37, MI method: MI:0397 — two-hybrid array).

This Y2H self-interaction constitutes the experimental basis for any potential GO:0042802 annotation. The evidence is genuine but has important limitations: Y2H operates in yeast, not in human cells; it detects binary interactions between fusion proteins that may not reflect full-length protein behavior or native oligomeric context; and the MI-score of 0.37 is moderate, consistent with high-throughput detection rather than focused biochemical validation. Notably, the self-interaction data exists in IntAct but has been annotated to the broader GO:0005515 (protein binding) rather than the more specific GO:0042802 (identical protein binding), suggesting GO curators have not yet propagated this evidence to the more specific term.

Finding 2: AlphaFold Predicts KCTD14 Pentameric Assembly with Distinctive CTD Organization

Esposito et al. (2022) applied AlphaFold multimer predictions to model oligomeric states of all KCTD family members (PMID: 36362127). Their approach "led to the identification of reliable three-dimensional models for the pentameric states of KCNRG, KCTD6, KCTD4, KCTD7, KCTD9, and KCTD14 and possibly for KCTD11 and KCTD21 that are involved in key biological processes and that were previously uncharacterized from a structural point of view." Critically, the study revealed a distinctive structural signature for the KCTD7/14 subclade: "the structure of the related proteins KCTD7 and KCTD14, although pentameric, appears to be characterized by a different organization of the CTD region, with the five chains forming a circle-like structure with a large cavity." This contrasts with the propeller-like CTD arrangement seen in other KCTD family members.

The AlphaFold monomer model for KCTD14 (AF-Q9BQ13-F1) has a mean pLDDT of 85.88, indicating high overall confidence. The pentameric prediction provides specific structural context for the self-interaction — predicting not just that KCTD14 self-associates but that it does so as a specific pentameric ring with an unusual CTD architecture. However, these remain computational predictions; no crystal structure, cryo-EM structure, or solution biophysics data exist for KCTD14 itself.

Finding 3: KCTD Family BTB Domains Prevalently Form Pentamers — Structural Evidence from Related Members

The structural basis for KCTD self-interaction is robustly established at the family level through converging experimental approaches:

- **Electron microscopy:** Smaldone et al. (2016) examined BTB domains from 7 KCTD members spanning 5 major phylogenetic clades and found that their "present electron microscopy data highlight the occurrence of well-defined pentameric states for all domains" (PMID: 27152988).
- **X-ray crystallography:** Ji et al. (2016) reported pentameric crystal structures of the KCTD1 and KCTD9 BTB domains and demonstrated that Cul3 binding affinity varies — "KCTD proteins 5, 6, 9 and 17 bind to Cul3 with high affinity, while the KCTD proteins 1 and 16 do not have detectable binding" (PMID: 26334369). Pinkas et al. (2017) added crystal structures for additional members, noting that "Members of the potassium channel tetramerization domain (KCTD) family are soluble non-channel proteins that commonly function as Cullin3 (Cul3)-dependent E3 ligases" (PMID: 28963344).
- **Molecular dynamics simulations:** Balasco et al. (2019) demonstrated that "KCTD-BTB domains are stable in the simulation timescales, even in their monomeric forms" and that open pentameric states relate to functional roles (PMID: 31370201).

One important exception is KCTD11, which forms tetramers rather than pentamers (PMID: 21237243), demonstrating that the oligomeric state is not invariant across the family and cannot be automatically assumed for unstudied members.

Finding 4: GO:0042802 Is Not Currently Annotated for KCTD14 in QuickGO

Examination of the current GO annotation status for Q9BQ13 revealed that GO:0042802 (identical protein binding) is **not currently assigned**. The existing annotations are:

GO Term	GO ID	Evidence Code	Source
protein homooligomerization	GO:0051260	IEA	InterPro IPR003131
protein binding	GO:0005515	IPI	IntAct (PMIDs: 25416956, 28514442, 29892012, 33961781)

The IPI-supported GO:0005515 annotation encompasses interactions with STK16, TCF4, and ACSF3 in addition to the self-interaction. The Y2H self-interaction data from

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exists in IntAct but has not been propagated to GO:0042802. This means the seed hypothesis proposes both a new annotation and its elevation to core status — a step requiring stronger justification than is currently available.

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
1	PMID: 25416956 (Rolland et al. 2014)	Direct assay (Y2H)	**Supports**	KCTD14 binds itself	Self-interaction detected in 3/4 replicas; MI-score 0.37	Human proteins i yeast Y2H II-14 systemati screen
2	PMID: 36362127 (Esposito et al. 2022)	Computational (AlphaFold)	**Supports**	KCTD14 forms pentamers	Reliable pentameric model with distinctive circle-like CTD	In silico (AlphaFol multimer
3	PMID: 27152988 (Smaldone et al. 2016)	Structural/ evolutionary (EM)	**Supports** (by analogy)	KCTD BTB domains pentamerize	Pentameric states for all 7 tested BTB domains across 5 clades	In vitro E recombin BTB doma
4	PMID: 26334369 (Ji et al. 2016)	Structural (X-ray, cryo-EM)	**Supports** (by analogy)	KCTD proteins form pentameric rings	Crystal structures of KCTD1 and KCTD9 BTB pentamers; Cul3 binding varies	In vitro
5	PMID: 28963344 (Pinkas et al. 2017)	Structural (X-ray)	**Supports** (by analogy)	KCTDs are pentameric Cul3 adaptors	Crystal structures confirm pentameric assemblies; KCTDs commonly function as Cul3 E3 ligases	In vitro
6	PMID: 31370201 (Balasco et al. 2019)	Computational (MD)	**Supports** (by analogy)	BTB domain stability in oligomeric states	MD simulations show stability in various oligomeric states including pentamers	In silico
7	PMID: 36736897 (Barthet/	Direct assay (co-IP, BRET)	**Qualifies**	KCTDs interact with Gβγ	Nearly all 25 KCTDs interact with Gβγ; functional	HEK293 c family-wi screen

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
	Sloan et al. 2023)				consequence on cAMP signaling	
8	PMID: 37762619 (Bhatt/Liao et al. 2023)	Direct assay (co-IP, BRET)	**Qualifies**	KCTDs form hetero-oligomers	KCTD5 forms hetero-oligomeric complexes with various family members	HEK293 c
9	PMID: 22748208 (Staropoli et al. 2012)	Direct assay (paralog KCTD7)	**Qualifies**	KCTD7-Cullin3 interaction	KCTD7 R184C mutation abrogated Cullin3 interaction	Human K in cell-ba assays
10	PMID: 30295347 (Metz et al. 2018)	Direct assay (paralog KCTD7)	**Qualifies**	KCTD7 disease mechanism	KCTD7 mutations impair Cul3 binding and autophagy	Patient fibroblast yeast
11	PMID: 41080575 (Liang et al. 2025)	Computational/ functional	**Competing**	KCTD14 immunomodulatory function	KCTD14 implicated as immunomodulatory oncogene via TNF-TNFR1 axis in PC	Computat + scRNA-s
12	PMID: 21237243 (Dementieva et al. 2011)	Structural (biophysical)	**Qualifies**	All KCTDs pentamerize	KCTD11 forms tetramers, not pentamers	In vitro (g filtration, scattering
13	QuickGO/ UniProt (database)	Database/ computational	**Qualifies**	Current annotation status	GO:0042802 not annotated despite	Database records 2026-07

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
					Y2H evidence in IntAct	

GO Curation Implications

Current Annotation Status

KCTD14 (Q9BQ13) has a minimal GO annotation profile:

- **GO:0051260** (protein homooligomerization, BP) — IEA from InterPro IPR003131. Appropriately assigned based on the BTB domain, which is the canonical oligomerization motif for this family.
- **GO:0005515** (protein binding, MF) — IPI from IntAct, supported by multiple PMIDs. This covers both the self-interaction and heteromeric interactions with STK16, TCF4, and ACSF3.
- **GO:0042802** (identical protein binding, MF) — **Not currently annotated**, despite the Y2H self-interaction evidence existing in IntAct.

Assessment of GO:0042802 as Core Function

The proposed annotation GO:0042802 is technically justifiable as an MF annotation but should NOT be treated as a core function. The reasoning is threefold:

1. **As a GO MF annotation:** GO:0042802 could be validly assigned based on the Y2H self-interaction from PMID: 25416956, using IPI evidence code. This would represent a more specific annotation than the currently assigned GO:0005515 and would accurately capture the observed self-interaction. This is a legitimate annotation lead.
2. **As a core function:** Identical protein binding is a *prerequisite activity* shared across virtually all KCTD family members. It enables the protein's actual downstream function but is not itself the endpoint activity. For characterized family members, the core molecular function is typically Cullin3 E3 ubiquitin ligase adaptor activity, Gβγ binding and GPCR signal modulation, or receptor scaffolding. Designating self-binding as the core function for KCTD14 is premature and mechanistically superficial.

3. **Comparison with paralogs:** For KCTD7 (the closest paralog), disease-causing mutations disrupt Cullin3 binding and autophagy-lysosome function — not oligomerization per se (PMID: 30295347; PMID: 22748208). This strongly suggests that the core function of the KCTD7/14 subclade lies downstream of self-assembly.

Recommended Curation Actions (Leads for Curator Verification)

- **Retain** GO:0051260 (protein homooligomerization, BP) — well-supported by domain architecture and family-wide evidence
- **Consider adding** GO:0042802 (identical protein binding, MF) with IPI evidence from PMID: 25416956 — but flag as non-core
- **Do not designate** GO:0042802 as core MF — the true core molecular function is undetermined
- **Monitor** Cullin3 adaptor activity, Gβγ modulation, and TNF-TNFR1 axis involvement as candidate core functions pending experimental data

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Mechanistic Scope

Direct Gene-Product Activity

The immediate molecular activity under evaluation is **self-binding** — the ability of KCTD14 monomers to associate into homo-oligomeric (likely pentameric) complexes via the BTB domain. This is a well-supported structural property, but it is mechanistically **upstream** of the protein's biological role. The assembly hierarchy can be represented as:

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KCTD14 monomer
    ↓ BTB domain-mediated self-association
Pentameric BTB ring (predicted)
    ↓ CTD region forms circle-like structure with large cavity
Full-length pentameric assembly
    ↓ [UNKNOWN — no experimental data for KCTD14]
    ├── Cullin3 binding? → E3 ubiquitin ligase complex → substrate ubiquitination
    ├── Gβγ binding? → GPCR signal modulation → cAMP pathway effects
    ├── TNF-TNFR1 axis? → immunomodulation (computational evidence only)
    └── Novel function? → unknown biological process
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What Self-Binding Enables — Family Analogies

For characterized KCTD family members, the pentameric BTB ring serves as a platform for three main classes of molecular activity:

KCTD Member(s)	Downstream Function	Core MF	Evidence Level
KCTD5, KCTD6, KCTD9, KCTD11, KCTD17	Cullin3 E3 ubiquitin ligase adaptor	Ubiquitin ligase complex component	Crystal structures, biochemistry
KCTD2, KCTD5, KCTD12, KCTD16	Gβγ binding → GPCR modulation	G protein modulator	Co-IP, BRET, functional assays
KCTD8, KCTD12, KCTD12b, KCTD16	GABAB receptor regulation	Receptor auxiliary subunit	Co-IP, electrophysiology
KCTD7 (closest paralog)	Cul3-dependent autophagy-lysosome regulation	Cul3 adaptor	Genetics, cell biology
KCTD14	**Unknown**	**Unknown**	—

Separation of Direct Activity from Downstream Phenotypes

KCTD14 has been identified in several transcriptomic and computational disease studies, but these represent expression-level associations that do not inform molecular function:

- **Dengue infection:** KCTD14 upregulated in dengue patients regardless of severity (PMID: 39397194; PMID: 35220956) — transcriptomic correlation, not MF evidence
- **Pancreatic cancer:** KCTD14 identified as potential immunomodulatory oncogene via TNF-TNFR1 axis (PMID: 41080575) — computational/scRNA-seq inference, not validated
- **Ovarian cancer:** KCTD14 included in DNA methylation-related prognostic signature (PMID: 36978087) — statistical association only
- **Diabetes:** KCTD14 transcript altered in diabetic rat colon (PMID: 34806320) — rat model, low fold-change

None of these disease-context findings should influence GO MF annotation for KCTD14.

Conflicts and Alternatives

1. Hetero-Oligomerization Complicates "Identical Protein Binding"

Bhatt et al. (2023) demonstrated that KCTD5 forms hetero-oligomeric complexes with various KCTD family members, with "different regions on KCTD5 responsible for uniquely contributing to interactions with other KCTD proteins" (PMID: 37762619). The authors concluded that "KCTD hetero-oligomeric interactions may occur throughout the KCTD family." If KCTD14 preferentially forms hetero-oligomers in vivo (e.g., with KCTD7 or other family members), then GO:0042802 (identical protein binding) would be an incomplete or misleading annotation. The Y2H self-interaction assay cannot distinguish between a protein that exclusively forms homo-oligomers and one that can also form hetero-oligomers.

2. Cullin3 Adaptor Activity as the True Core Function

The closest paralog, KCTD7, has well-established Cullin3 interaction: Staropoli et al. (2012) showed that the R184C mutation "abrogated interaction with cullin-3, a ubiquitin-ligase component and known KCTD7 interactor" (PMID: 22748208), and Metz et al. (2018) demonstrated that KCTD7 mutations impair autophagy through Cul3-dependent pathways (PMID: 30295347). If KCTD14 similarly functions as a Cul3 adaptor, then its core MF would be related to ubiquitin ligase activity, not self-binding.

However, not all KCTDs bind Cul3. Ji et al. (2016) found that "KCTD proteins 1 and 16 do not have detectable binding" to Cul3 despite having functional BTB domains (PMID: 26334369). Whether KCTD14 falls in the Cul3-binding or non-binding subgroup has not been tested.

3. Gβγ Modulation as an Alternative Core Function

The family-wide screen by Sloan/Barthet et al. (2023) demonstrated that "nearly all the 25 KCTD proteins interact with Gβγ" with functional consequences for adenylyl cyclase-cAMP signaling (PMID: 36736897). KCTD14 was included in this screen. If Gβγ binding proves to be KCTD14's primary signaling role, the core MF would shift toward G-protein modulation rather than self-binding.

4. Paralog Confusion Risk with KCTD7

KCTD14 and KCTD7 form a distinct subclade with shared structural features (circle-like CTD, large cavity) (PMID: 36362127). KCTD7 is well-characterized as a disease gene (progressive myoclonic epilepsy EPM3/CLN14) with Cul3 interaction and autophagy-lysosome involvement.

There is a risk of inappropriately transferring KCTD7 annotations to KCTD14 without direct experimental evidence. KCTD14 may have distinct substrates, tissue expression, and biological roles despite sharing structural organization with KCTD7.

5. Oligomeric State Exceptions

KCTD11 forms tetramers rather than pentamers (PMID: 21237243), showing that the oligomeric state cannot be automatically assumed for untested members. While the AlphaFold prediction for KCTD14 specifically indicates a pentameric state, this has not been experimentally validated.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	Resolving Evidence
No direct biochemical characterization of KCTD14	PubMed literature, UniProt, IntAct	Cannot confirm oligomeric state, binding affinity, or stoichiometry	Recombinant expression + SEC-MALS, analytical ultracentrifugation, or native MS
No experimental structure	PDB search (no entries); AlphaFold DB (monomer model, pLDDT 85.88)	Pentameric prediction is computational only; circle-like CTD cavity is unverified	X-ray crystallography or cryo-EM of KCTD14 BTB domain and/or full-length protein
Cullin3 binding status unknown	Literature on KCTD-Cul3 interactions; KCTD14 not tested in any study	Determines whether E3 ligase adaptor is a candidate core function	Co-IP or ITC of KCTD14 with Cul3 N-terminal domain
Gβγ binding specifics unknown	Family-wide screen (PMID: 36736897) included KCTD14 but individual data not reported in detail	If confirmed, would suggest GPCR modulation as core function	Focused BRET/co-IP with domain deletions
Substrate identity unknown	No data available	If KCTD14 is a Cul3 adaptor, the substrate defines its specific biological role	Proximity labeling (BioID/TurboID) or ubiquitin remnant profiling
In vivo oligomeric state unknown	Y2H detects binary interactions only	Actual stoichiometry in human cells may differ from predictions	BRET titration, cross-linking MS, or fluorescence fluctuation spectroscopy
Hetero-oligomerization partners unknown	KCTD5 hetero-oligomers studied (PMID: 37762619); KCTD14 not specifically tested	If KCTD14 preferentially hetero-oligomerizes, GO:0042802 may be secondary	Co-IP screen with all KCTD family members

Gap	What Was Checked	Why It Matters	Resolving Evidence
Tissue/cell expression context	Disease transcriptomics mention KCTD14 in dengue, cancer, diabetes	Expression pattern constrains which functions are physiologically relevant	GTEx/HPA queries, single-cell atlas data

Discriminating Tests

Priority 1: Confirm Self-Interaction and Determine Stoichiometry

- **Recombinant KCTD14 BTB domain expression** followed by size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) to determine native oligomeric state
- **Analytical ultracentrifugation** (AUC) to confirm or refute the pentameric stoichiometry predicted by AlphaFold
- **Co-immunoprecipitation with differentially epitope-tagged constructs** in human cells (e.g., FLAG-KCTD14 + HA-KCTD14) to validate Y2H self-interaction in a mammalian context

Priority 2: Test Cullin3 Adaptor Activity

- **Co-immunoprecipitation or pull-down** of KCTD14 with the Cul3 N-terminal domain, following the approach used for KCTD5, KCTD9, and KCTD7
- **Isothermal titration calorimetry** (ITC) to measure binding affinity, as performed for KCTD5-Cul3 (Kd = 59 nM; PMID: 24747150)
- If Cul3 binding confirmed: **in vitro ubiquitination assay** with candidate substrates

Priority 3: Characterize Gβγ Interaction

- **Focused BRET assay** with KCTD14 and Gβγ subunits, including agonist-dependence testing
- **Domain deletion mapping** to identify whether the BTB, CTD, or both regions mediate Gβγ binding
- **Functional readout:** adenylyl cyclase 5 sensitization assay to test whether KCTD14 blunts Gβγ-mediated cAMP signaling, as shown for other KCTDs (PMID: 36736897)

Priority 4: Structural Determination

- **Cryo-EM** of full-length KCTD14 pentamer to verify the distinctive circle-like CTD cavity predicted by AlphaFold
- **X-ray crystallography** of the BTB domain to compare with other family members
- These would definitively resolve the oligomeric state and provide a structural basis for understanding specificity

Priority 5: Identify Biological Context and Substrates

- **BioID/TurboID proximity labeling** with KCTD14 as bait in relevant cell types to identify the in vivo interactome
- **CRISPR knockout** followed by transcriptomic and proteomic profiling to identify downstream effects
- **Tissue expression profiling** using GTEx, Human Protein Atlas, and single-cell atlas data to identify primary sites of function

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Curation Leads

All items below are leads requiring curator verification.

Lead 1: Add GO:0042802 as Non-Core MF Annotation

- **Action:** Consider annotating GO:0042802 (identical protein binding) for Q9BQ13 with IPI evidence code
- **Reference:** PMID: 25416956 (Rolland et al. 2014)
- **Snippet to verify:** "Just as reference genome sequences revolutionized human genetics, reference maps of interactome networks will be critical to fully understand genotype-phenotype relationships. Here, we describe a systematic map of ~14,000 high-quality human binary protein-protein interactions."
- **IntAct accession:** EBI-10487331 (KCTD14 self-interaction, Q9BQ13-Q9BQ13)
- **Caveat:** Y2H detects binary interactions; does not confirm oligomeric stoichiometry. Should be flagged as non-core function.

Lead 2: Retain GO:0051260 but Consider Evidence Upgrade

- **Action:** Retain GO:0051260 (protein homooligomerization) and consider whether AlphaFold pentamer predictions warrant ISS or IC evidence upgrade from IEA
- **Reference:** PMID: 36362127 (Esposito et al. 2022)
- **Snippet to verify:** "our approach led to the identification of reliable three-dimensional models for the pentameric states of KCNRG, KCTD6, KCTD4, KCTD7, KCTD9, and KCTD14 and possibly for KCTD11 and KCTD21 that are involved in key biological processes and that were previously uncharacterized from a structural point of view"
- **Question for curator:** Does the combination of Y2H self-interaction + AlphaFold pentamer prediction + family-wide EM data warrant upgrading from IEA to a manually assigned evidence code?

Lead 3: Do Not Designate Self-Binding as Core Function

- **Rationale:** For every experimentally characterized KCTD family member, BTB-mediated oligomerization enables the protein's downstream molecular activity — it is the assembly step, not the functional output. The KCTD7/14 subclade data specifically suggest Cullin3 adaptor activity as the likely core function. Until KCTD14's primary molecular activity is determined, the core MF slot should remain open.
- **Suggested action:** Annotate GO:0042802 but note it as a structural/assembly property rather than core function. Add a curator note that the primary molecular function beyond oligomerization is experimentally undetermined.

Lead 4: Monitor Emerging Functional Evidence

- **Gβγ binding:** PMID: 36736897 — "we report that nearly all the 25 KCTD proteins interact with Gβγ" — family-wide screen suggesting alternative core function
- **TNF-TNFR1 axis:** PMID: 41080575 — "highlights KCTD14 as a novel immunomodulatory oncogene acting through the TNF-TNFR1 axis" — computational evidence in pancreatic cancer
- **Autophagy/lysosome connection:** If KCTD14 shares functional parallels with KCTD7 (PMID: 30295347), autophagy-related GO terms may become relevant

Lead 5: Candidate GO Terms for Future Consideration

If downstream function is experimentally established, consider:

Candidate GO Term	GO ID	Ontology	Condition
Cul3-RING ubiquitin ligase complex	GO:0031463	CC	If Cul3 binding confirmed
ubiquitin protein ligase binding	GO:0031625	MF	If Cul3 binding confirmed
Gβγ-subunit complex binding	GO:0002058 (or related)	MF	If Gβγ interaction confirmed as primary activity
ubiquitin-dependent protein catabolic process	GO:0006511	BP	If E3 ligase substrate adaptor role confirmed

Lead 6: Relationship Between GO:0042802 and GO:0051260

- **GO:0042802** (identical protein binding) is an MF term describing the binding activity itself
- **GO:0051260** (protein homooligomerization) is a BP term describing the process outcome
- These are complementary, not redundant — if GO:0042802 is added as MF, it should complement rather than replace GO:0051260
- For a gene with minimal experimental characterization, the BP term GO:0051260 may be more informative because it specifies the *outcome* (homo-oligomeric assembly) rather than just the binding activity

Evidence Base: Key Literature

Primary Evidence for KCTD14

Rolland et al. (2014) — *A proteome-scale map of the human interactome network.* PMID: 25416956 The HI-II-14 interactome provides the only direct experimental evidence for KCTD14 self-interaction. This systematic Y2H study detected KCTD14 self-binding in 3 of 4 replicas (MI-score 0.37). This is the foundation for any potential GO:0042802 annotation and represents the most important primary evidence for this hypothesis.

Esposito et al. (2022) — *AlphaFold Predictions Provide Insights into the Structural Features of the Functional Oligomers of All Members of the KCTD Family.* PMID: 36362127 Used AlphaFold multimer to predict oligomeric states for all KCTD members. Identified reliable pentameric models for KCTD14 and revealed its distinctive circle-like CTD architecture shared with KCTD7. This is the most detailed structural information available for KCTD14, albeit computational.

Family-Wide Structural Evidence

Smaldone et al. (2016) — *The BTB domains of the potassium channel tetramerization domain proteins prevalently assume pentameric states.* PMID: 27152988 EM data establishing pentamerization as the norm for KCTD BTB domains across multiple phylogenetic clades.

Ji et al. (2016) — *Structural Insights into KCTD Protein Assembly and Cullin3 Recognition.* PMID: 26334369 Crystal structures of KCTD1 and KCTD9 BTB pentamers. Crucially demonstrated that Cul3 binding affinity varies across the family — KCTD1 and KCTD16 do not detectably bind Cul3, challenging the assumption that all KCTDs are Cul3 adaptors.

Pinkas et al. (2017) — *Structural complexity in the KCTD family of Cullin3-dependent E3 ubiquitin ligases.* PMID: 28963344 Additional crystal structures establishing that KCTDs "commonly function as Cullin3 (Cul3)-dependent E3 ligases." Provides the structural framework for understanding KCTD oligomerization as a means to Cul3 binding.

Balasco et al. (2019) — *The Structural Versatility of the BTB Domains of KCTD Proteins.* PMID: 31370201 MD simulations showing BTB domain stability across oligomeric states and demonstrating that open pentameric conformations relate to functional roles, not structural instability.

Closest Paralog KCTD7

Staropoli et al. (2012) — *A homozygous mutation in KCTD7 links neuronal ceroid lipofuscinosis to the ubiquitin-proteasome system.* PMID: 22748208 Demonstrated that KCTD7 mutations can abrogate Cullin3 interaction, linking the KCTD7/14 subclade to the ubiquitin-proteasome system. The R184C mutation "abrogated interaction with cullin-3."

Metz et al. (2018) — *KCTD7 deficiency defines a distinct neurodegenerative disorder with a conserved autophagy-lysosome defect.* PMID: 30295347) Showed that KCTD7 mutations cause impaired autophagy through Cul3-dependent mechanisms, establishing that the core function of KCTD7 is Cul3-dependent — not merely self-binding.

Family-Wide Functional Studies

Sloan/Barthet et al. (2023) — *Multiple potassium channel tetramerization domain (KCTD) family members interact with Gβγ, with effects on cAMP signaling.* PMID: 36736897 Family-wide screen showing nearly all KCTDs interact with Gβγ, with functional consequences for cAMP signaling. Raises the possibility that Gβγ modulation, not self-binding, is a more informative core function annotation for KCTD14.

Bhatt/Liao et al. (2023) — *KCTD5 Forms Hetero-Oligomeric Complexes with Various Members of the KCTD Protein Family.* PMID: 37762619 Demonstrated that KCTD hetero-oligomerization is widespread, suggesting KCTD14 may form mixed complexes in vivo — complicating the exclusive "identical protein binding" annotation.

Disease Context (Not MF Evidence)

Liang et al. (2025) — *Dendritic cell-related gene signature in pancreatic cancer stratifies patient subtypes and implicates a KCTD14-TNF signaling axis.* PMID: 41080575 Computational/scRNA-seq study proposing KCTD14 as an immunomodulatory oncogene acting through TNF-TNFR1 in pancreatic cancer. While not direct MF evidence, this is the most specific functional claim about KCTD14 in the current literature and warrants monitoring.

Limitations

1. **No direct biochemical characterization of KCTD14 exists.** All functional inferences are based on family analogy, one Y2H result, and computational predictions. KCTD14 remains one of the least-characterized members of the KCTD family.
2. **The Y2H evidence is from a single high-throughput study.** While the HI-II-14 interactome is well-validated at the dataset level, the individual KCTD14 self-interaction has not been independently confirmed by co-immunoprecipitation, BRET, or other orthogonal methods.
3. **AlphaFold predictions are not experimental evidence.** The pentameric model and circle-like CTD cavity, while plausible and consistent with family trends, require experimental validation.

4. **Family-wide functional data cannot be directly transferred to KCTD14.** The KCTD family shows considerable functional diversity: some are Cul3 adaptors, some are GABAB receptor modulators, some bind Gβγ, some do not bind Cul3 at all, and substrate specificity varies widely. Shared BTB domain architecture does not guarantee shared molecular function.
5. **Disease-context transcriptomic associations are not MF evidence.** The recurring identification of KCTD14 in dengue, cancer, and diabetes transcriptomic studies reflects expression changes, not molecular function.
6. **The closely related KCTD7 has strong disease genetics, but even KCTD7's molecular function is incompletely characterized.** While KCTD7 binds Cullin3 and regulates autophagy, the specific substrates and detailed mechanism remain under investigation, limiting knowledge transfer to KCTD14.
7. **This analysis could not programmatically access certain bioinformatics resources** (e.g., live QuickGO API queries, tissue expression data from GTEx/HPA, PDB structure search). Annotation status and expression data were derived from literature and database records available in the knowledge base.

Conclusion

The seed hypothesis that identical protein binding (GO:0042802) is a core function of KCTD14 is **partially supported as a factual claim** about what KCTD14 likely does — it almost certainly self-associates via its BTB domain to form homo-oligomeric assemblies — but is **not justified as a core function designation**. Self-binding is a structural prerequisite shared across the entire KCTD family that enables downstream molecular activities (Cullin3 adaptor function, Gβγ modulation, receptor scaffolding), which are the true core functions for characterized family members. For KCTD14, the downstream molecular function remains experimentally undetermined.

The recommended curation approach is to consider adding GO:0042802 as a non-core MF annotation with IPI evidence from the Y2H self-interaction data (PMID: 25416956), while clearly noting that the core molecular function of KCTD14 awaits experimental characterization. The most promising leads for future core function identification are Cullin3 adaptor activity (by KCTD7 analogy), Gβγ modulation (by family-wide screen data), and the computationally proposed TNF-TNFR1 axis involvement.