

Final Report: Core Function Hypothesis for Wheat A0A3B6RKV1 — Histone Demethylase Activity (GO:0032452)

Executive Judgment

Verdict: Over-annotated. The seed hypothesis that A0A3B6RKV1 is a KDM5/JARID1-subfamily H3K4 lysine demethylase is incorrect in its subfamily assignment and substrate specificity. However, the broad GO term GO:0032452 (histone demethylase activity) is defensible by orthology to the experimentally characterized Arabidopsis JMJ22 (At5g06550), an H4R3me2 arginine demethylase. The correct specific annotation should be GO:0033749 (histone H4R3 demethylase activity), and all references to KDM5/JARID1 subfamily membership and H3K4 substrate specificity must be corrected to JMJD6 subfamily and H4R3me2 substrate.

Key caveats: 1. No direct experimental evidence exists for A0A3B6RKV1 itself; all functional inference is from orthology to AtJMJ22. 2. The JMJD6 family has debated dual activities (arginine demethylation vs. lysyl hydroxylation) in animals, though plant JMJ22 was experimentally shown to have arginine demethylase activity. 3. The UniProt "Similarity" field says "Belongs to the JARID1 histone demethylase family" — this likely propagated the misclassification, but it contradicts the more rigorous PANTHER and InterPro family-level classifications.

Summary

The wheat protein A0A3B6RKV1 (UniProt accession A0A3B6RKV1, *Triticum aestivum*, chromosome 7A) was proposed to possess histone demethylase activity (GO:0032452) as a core function, with the rationale that it belongs to the KDM5/JARID1 subfamily and catalyzes Fe(II)- and 2-oxoglutarate-dependent removal of methyl groups from histone H3K4. This investigation found that the KDM5/JARID1 classification is erroneous. Multiple independent lines of evidence — domain architecture, phylogenetic classification (PANTHER, InterPro), NCBI

orthology assignment, and sequence alignment — consistently place A0A3B6RKV1 in the **JMJD6 subfamily** of JmjC domain-containing proteins, orthologous to Arabidopsis JMJ22 (At5g06550).

JMJ22 has been experimentally demonstrated to function as a histone H4R3me2 arginine demethylase that promotes seed germination by removing repressive arginine methylation marks at GA biosynthesis loci (PMID: 22483719). The protein's domain architecture — an F-box domain (residues 89–135) and a JmjC domain (residues 285–445) within a compact 511-amino-acid protein — is fundamentally incompatible with KDM5/JARID1 proteins, which require JmjN, ARID, PHD finger, and FYR domains and are typically 800–1200+ amino acids in plants.

AlphaFold structural prediction confirms high-confidence folding of the JmjC domain (pLDDT 95.8) with a perfectly conserved Fe(II)-binding facial triad (His330-Asp332-His362). The protein exists as a complete homeologous triad on wheat chromosomes 7A, 7B (A0A3B6SQ95), and 7D (A0A3B6TWS8), all 511 amino acids with 96–98% sequence identity, indicating strong purifying selection on this enzymatic function. The UniProt "Similarity" annotation stating "Belongs to the JARID1 histone demethylase family" appears to be an automated annotation error that propagated the incorrect subfamily assignment.

Key Findings

Finding 1: A0A3B6RKV1 Is Orthologous to AtJMJ22, an H4R3me2 Arginine Demethylase — Not a KDM5/JARID1 H3K4 Lysine Demethylase

The most critical finding of this investigation is that the seed hypothesis misidentifies the protein's subfamily. A0A3B6RKV1 (511 amino acids) possesses only an F-box domain (residues 89–135) and a JmjC domain (residues 285–445). True KDM5/JARID1 family proteins in plants are substantially larger (800–1200+ amino acids) and contain a characteristic multi-domain architecture: JmjN, ARID (AT-rich interaction domain), one or more PHD (plant homeodomain) fingers, JmjC, and FYR (FY-rich) domains. A0A3B6RKV1 lacks all of these signature domains except JmjC, which is shared across all JmjC-containing demethylase subfamilies.

NCBI Gene identifies the Arabidopsis ortholog of wheat LOC123148885 (encoding A0A3B6RKV1) as At5g06550, which corresponds to JMJ22. PANTHER classifies both proteins in family PTHR12480 (JMJD6 arginine demethylases/lysyl-hydroxylases), subfamily SF21. InterPro independently classifies both under IPR050910 (JMJD6 family arginine demethylases/lysyl-

hydroxylases). JmjC domain identity between A0A3B6RKV1 and AtJMJ22 is approximately 77% at the full-length alignment level and ~88.5% in conserved blocks — far exceeding typical cross-subfamily identity.

Crucially, AtJMJ22 has been experimentally characterized as an H4R3me2 arginine demethylase, not an H3K4 lysine demethylase. Cho et al. (2012) demonstrated that JMJ20 and JMJ22 "act redundantly as positive regulators of seed germination" by removing "repressive histone arginine methylations at GA3ox1/GA3ox2," thereby increasing gibberellic acid levels and promoting germination under phytochrome B activation (PMID: 22483719).

Finding 2: Current GO Annotation Status — GO:0032452 Absent from A0A3B6RKV1, Present on AtJMJ22 but Without Specific H4R3 Term

QuickGO shows that A0A3B6RKV1 currently carries zero annotations for GO:0032452 (histone demethylase activity). The seed hypothesis therefore proposes a new annotation rather than evaluating an existing one. The Arabidopsis ortholog AtJMJ22 (Q67XX3) does carry GO:0032452 with IGI (Inferred from Genetic Interaction) evidence, based on PMID: 22483719 and assigned by TAIR, citing genetic interaction with AT5G63080.

Notably, even AtJMJ22 does not carry the more specific GO:0033749 (histone H4R3 demethylase activity), despite experimental evidence supporting H4R3me2 as its substrate. This represents an annotation gap in the reference organism. AtJMJ22 also does not carry GO:0032453 (histone H3K4 demethylase activity), which is consistent with the experimental evidence showing it is not an H3K4 demethylase.

Finding 3: UniProt JARID1 Family Annotation Is an Automated Error Contradicted by PANTHER/InterPro

The UniProt "Similarity" field for A0A3B6RKV1 and its orthologs (including A0A453STL0 from *Oryza sativa*, A0A8R7R6R6 from *Zea mays*, and Q67XX3/AtJMJ22 from *Arabidopsis thaliana*) states: "Belongs to the JARID1 histone demethylase family." This annotation contradicts the PANTHER (PTHR12480, JMJD6 family) and InterPro (IPR050910, JMJD6 family) classifications. NCBI Gene describes the wheat locus LOC123148885 simply as "F-box protein At5g06550," avoiding the JARID1 subfamily claim. This discrepancy likely arose from automated annotation pipelines that classified any JmjC-containing protein with histone demethylase activity into the JARID1 family without checking domain architecture. True wheat KDM5/JARID1 proteins do exist as separate, much larger proteins (940+ amino acids) in distinct PANTHER subfamilies.

Finding 4: AlphaFold Structure and Sequence Alignment Confirm Catalytic Competence and JMJD6 Orthology

AlphaFold predictions for A0A3B6RKV1 show an overall mean pLDDT of 84.5, with the JmjC domain achieving 95.8 and the F-box domain achieving 93.8 — both indicating high-confidence structural predictions. The Arabidopsis ortholog AtJMJD2 achieves a comparable JmjC pLDDT of 95.6.

Needleman-Wunsch alignment of the full proteins yields 62.3% identity (311/499 aligned positions), while the JmjC domain alone shows 77.0% identity (124/161 aligned positions). The Fe(II)-binding catalytic facial triad is perfectly conserved: A0A3B6RKV1 has His330-Val-Asp332...His362 with the flanking context SSFHVDPNS, while AtJMJD2 has His324-Ile-Asp326...His356 with context SSFHIDPNS. The conservative Val/Ile substitution at the non-catalytic position within this motif does not affect metal coordination. This conservation strongly supports maintained enzymatic activity in the wheat protein.

Finding 5: Complete Homeologous Triad on Wheat Chromosomes 7A/7B/7D

A0A3B6RKV1 exists as part of a complete homeologous triad in hexaploid wheat (*Triticum aestivum*, AABBDD genome): the A-genome copy on chromosome 7A (A0A3B6RKV1), B-genome copy on 7B (A0A3B6SQ95), and D-genome copy on 7D (A0A3B6TWS8). All three homeologs are exactly 511 amino acids and share the identical domain architecture (F-box [89–135] + JmjC [285–445]) and PANTHER SF21 classification.

Pairwise sequence identity is extremely high: 7A–7B = 97.3%, 7A–7D = 96.9%, 7B–7D = 98.2%. Only 19 positions out of 511 are variable (96.3% overall conservation), with variations concentrated in the N-terminal disordered region (11.4% variable). Functional domains are highly conserved: F-box 2.1% variable, linker 2.9%, JmjC 1.8%, C-terminus 1.5%. The catalytic facial triad (His330, Asp332, His362) is perfectly conserved across all three homeologs. This extreme conservation across all three subgenomes indicates strong purifying selection, consistent with an essential, non-redundant enzymatic function.

Accession	Chromosome	Length	PANTHER SF	Identity to 7A
A0A3B6RKV1	7A	511 aa	SF21	—
A0A3B6SQ95	7B	511 aa	SF21	97.3%
A0A3B6TWS8	7D	511 aa	SF21	96.9%

Wheat also possesses 6 longer JMJ21-like paralogs (808–946 amino acids) on chromosomes 2A/2B/2D and 5A/5B/5D in different PANTHER subfamilies (SF34, SF35), confirming that A0A3B6RKV1 is distinct from other JmjC family members in wheat and is specifically the JMJ22 ortholog.

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
1	PMID: 22483719	Direct assay / mutant phenotype	Qualifies — supports H4R3me2 demethylase, refutes H3K4 demethylase	AtJMJ22 enzymatic activity	JMJ20/JMJ22 remove repressive histone arginine methylations at GA3ox1/GA3ox2 to promote seed germination	<i>A. thaliana</i> , seed germination, phyB signaling
2	PANTHER PTHR12480:SF21	Computational (phylogenetic)	Refutes KDM5; supports JMJD6	Subfamily identity	A0A3B6RKV1 classified as JMJD6 family, SF21	Cross-species classification
3	InterPro IPR050910	Computational (domain)	Refutes KDM5; supports JMJD6	Family identity	JMJD6 family assignment	Automated domain analysis
4	UniProt A0A3B6RKV1	Database annotation	Conflicts (erroneous)	JARID1 family membership	UniProt Similarity field says "JARID1"	Automated annotation
5	NCBI Gene LOC123148885	Database (orthology)	Supports JMJ22 orthology	Gene identity	Ortholog identified as At5g06550 = JMJ22	Cross-species orthology
6	QuickGO (A0A3B6RKV1)	Database	Qualifies	Current annotation status	A0A3B6RKV1 has 0 annotations for GO:0032452	Current GO database state (June 2026)

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
7	QuickGO (AtJMJ22)	Database	Supports GO:0032452 broadly	Histone demethylase annotation transferability	AtJMJ22 has GO:0032452 with IGI evidence; lacks specific GO:0033749	<i>A. thaliana</i> experimental annotation
8	AlphaFold AF-A0A3B6RKV1-F1	Structural prediction	Supports catalytic competence	Protein is structurally competent	JmjC pLDDT 95.8; catalytic triad intact	AlphaFold prediction
9	Sequence alignment	Computational (sequence)	Supports orthology	JmjC domain conservation	77% identity to AtJMJ22 JmjC domain; catalytic triad context SSFH[VI]DPNS conserved	Global alignment
10	Domain architecture	Computational (domain)	Refutes KDM5/JARID1	Domain composition	F-box + JmjC only (511 aa); lacks JmjN, ARID, PHD, FYR	All KDM5/JARID1 require multi-domain architecture
11	Homeolog triad analysis	Computational (genomic)	Supports functional importance	Selective constraint	7A/7B/7D copies 96–98% identical; catalytic triad invariant	<i>T. aestivum</i> hexaploid genome
12	PMID: 26059336	Computational (evolutionary)	Supports subfamily distinction	JMJD6 as distinct from KDM5	14 JmjC subfamilies defined; JMJD6 shared by plants, animals, fungi; KDM5 separate	Eukaryote-wide phylogenomic

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
13	PMID: 26152513	Computational (evolutionary)	Supports JMJD6 classification	Group-V JMJD6 substrate associations	JMJD6 found in all plant species; associated with H3R2, H4R3 demethylation and H4 hydroxylation	Green lineage evolution
14	PMID: 36092409	Computational / expression	Supports broadly	Wheat JmjC family context	Genome-wide identification of JmjC genes in wheat; subfamily classification including JMJD6	<i>T. aestivum</i> , drought stress
15	PMID: 20684070	Structural (crystallography)	Qualifies	JMJD6 enzymatic mechanism	Crystal structure shows JMJD6 catalyzes C-5 lysyl hydroxylation rather than Nε-demethylation	Animal JMJD6
16	PMID: 22189873	Direct assay	Qualifies	JMJD6 substrate specificity	Animal Jmjd6 showed lysyl-hydroxylase but not arginine demethylase activity in vitro	Mouse/human JMJD6

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
17	PMID: 24349476	Direct assay / mutant	Supports H4R3 biology	H4R3sme2 function in plants	SKB1/PRMT5- catalyzed H4R3sme2 regulates SAM maintenance via CRN repression	<i>A. thaliana</i> , shoot apical meristem
18	PMID: 23943859	Direct assay / mutant	Supports H4R3 biology	H4R3sme2 function in plants	CAU1/PRMT5/ SKB1 mediates H4R3sme2 to regulate CAS expression and stomatal closure	<i>A. thaliana</i> , stomata, drought

GO Curation Implications

Current Annotation State

- A0A3B6RKV1 does **not** currently carry GO:0032452 in QuickGO (as of June 2026).
- Existing annotations include: GO:0000987 (cis-regulatory region sequence-specific DNA binding, IBA), GO:0046872 (metal ion binding, IEA), GO:0005634 (nucleus, IBA/IEA).

Recommended Curation Actions (Leads Requiring Curator Verification)

1. **GO:0033749 (histone H4R3 demethylase activity): Recommended as the most specific and accurate MF term**, based on orthology to AtJMJ22 which demethylates H4R3me2 ([P 22483719](#)).
Evidence code: ISS (Inferred from Sequence or Structural Similarity) with AtJMJ22 (Q67XX3) as the reference. This term is a child of GO:0032452.

2. **GO:0032452 (histone demethylase activity): Supportable as IBA** from AtJMJ22 (Q67XX3), which carries this term with IGI evidence. May be retained as a broader parent-level annotation alongside GO:0033749.
3. **GO:0032453 (histone H3K4 demethylase activity): Should NOT be annotated.** No evidence supports H3K4 lysine demethylation for this protein or its ortholog. The seed hypothesis's implied H3K4 specificity is refuted.
4. **GO:0040029 (epigenetic regulation of gene expression): Supportable as IBA** from AtJMJ22 which has IDA evidence.
5. **GO:0005634 (nucleus): Already annotated (IBA).** Retain — consistent with chromatin-associated function.
6. **Subfamily designation:** Must be corrected from "KDM5/JARID1" to "JMJD6" throughout the review.

GO Decision Table

GO Term	Current Status	Proposed Action	Evidence Basis	Confidence
GO:0032452 (histone demethylase activity)	Not annotated	Supportable (IBA) but prefer more specific term	AtJMJ22 orthology, IGI evidence	Medium
GO:0033749 (histone H4R3 demethylase activity)	Not annotated	Recommended as specific MF (ISS)	AtJMJ22 demethylates H4R3me2 (P 22483719)	Medium–High
GO:0032453 (histone H3K4 demethylase activity)	Not annotated	Do NOT annotate	KDM5 misclassification refuted	High confidence to exclude
GO:0040029 (epigenetic regulation of gene expression)	Not annotated	Supportable (IBA)	AtJMJ22 has IDA	Medium
GO:0005634 (nucleus)	Annotated (IBA)	Retain	Consistent evidence	High
GO:0009845 (seed germination)	Not annotated	Consider adding (IBA) with caution	AtJMJ22 demonstrated; conservation in wheat unknown	Low–Medium

Note: Even the reference organism AtJMJ22 lacks GO:0033749, despite experimental evidence. This is an upstream annotation gap that should be flagged to TAIR curators.

Mechanistic Model / Interpretation

Direct Gene-Product Activity (Inferred from Orthology)

The immediate molecular function of A0A3B6RKV1, inferred from orthology to AtJMJ22, is the **Fe(II)- and 2-oxoglutarate-dependent oxidative removal of methyl groups from histone H4 arginine 3 (H4R3me2)**. This is an arginine demethylation reaction, mechanistically distinct from the lysine demethylation performed by KDM5/JARID1 enzymes, although both reaction types use the same JmjC catalytic mechanism (2-oxoglutarate-dependent dioxygenase chemistry).

The protein's F-box domain (residues 89–135) suggests possible integration with SCF ubiquitin ligase complexes, which could provide an additional regulatory layer or facilitate substrate targeting. This F-box + JmjC combination is distinctive and not found in KDM5/JARID1 proteins.

Mechanistic Model (inferred from AtJMJ22 orthology):

Environmental Signal (e.g., light/phyB activation)



Derepression of JMJ22
(= A0A3B6RKV1 in wheat)

← (SOMNUS represses JMJ22 when phyB inactive)



H4R3me2 demethylation at
target gene loci
(e.g., GA3ox1/GA3ox2)

← Direct enzymatic activity
(Fe²⁺ + 2-OG dependent)



Transcriptional activation
of target genes

← Downstream consequence



Increased GA levels →
Seed germination

← Phenotypic outcome

Downstream vs. Direct Effects

The seed hypothesis mentions "regulate gene expression epigenetically" (GO:0040029). This is a downstream consequence of the direct enzymatic activity (histone demethylation), not the primary molecular function itself. While appropriate as a BP annotation, it should not be conflated with the MF annotation. Similarly, seed germination is a developmental outcome, not a molecular function.

H4R3sme2 as an Active Epigenetic Mark in Plants

The H4R3sme2 mark that JMJ22-type proteins remove is deposited by PRMT5/SKB1 (PMID: 24349476; PMID: 23943859). In Arabidopsis, PRMT5-mediated H4R3sme2 is a repressive mark involved in: - Shoot apical meristem maintenance via CRN repression (PMID: 24349476) - Stomatal closure via CAS regulation (PMID: 23943859) - Seed germination control via GA3ox1/GA3ox2 (PMID: 22483719)

This establishes H4R3sme2 as a biologically significant regulatory mark in plants, supporting the relevance of JMJ22-type demethylases as functional epigenetic regulators.

Caveat: JMJD6 Bifunctionality

In animals, JMJD6 has been shown to possess dual activity — both arginine demethylase and lysyl-hydroxylase functions (PMID: 20684070; PMID: 28587176). One study found that animal Jmjd6 showed lysyl-hydroxylase but not arginine demethylase activity in vitro (PMID: 22189873). However, the plant ortholog AtJMJ22 has been experimentally demonstrated to function as an arginine demethylase in vivo, and plant JMJD6 subfamily members classified in Group-V are associated with H3R2, H4R3 demethylation and H4 hydroxylation (PMID: 26152513). Whether A0A3B6RKV1 also possesses lysyl-hydroxylase activity remains an open question.

Evidence Base

Primary Experimental Evidence (Ortholog)

Cho et al. (2012) — "Control of seed germination by light-induced histone arginine demethylation activity" (PMID: 22483719)

This is the single most important paper for this hypothesis. The authors demonstrated that Arabidopsis JMJ20 and JMJ22 "act redundantly as positive regulators of seed germination. When PHYB is inactive, JMJ20/JMJ22 are directly repressed by the zinc-finger protein SOMNUS. However, upon PHYB activation, JMJ20/JMJ22 are derepressed, resulting in increased gibberellic acid levels through the removal of repressive histone arginine methylations at GA3ox1/GA3ox2, which in turn promotes seed germination." This establishes JMJ22 as an H4R3me2 arginine demethylase with a defined biological role, and since A0A3B6RKV1 is the wheat ortholog of JMJ22 (NCBI Gene orthology, 77% JmjC identity), this evidence directly informs the expected function of A0A3B6RKV1.

Evolutionary and Phylogenomic Context

Lei and Liu (2015) — *"Expansion and Functional Divergence of Jumonji C-Containing Histone Demethylases"* (PMID: 26059336)

Defined 14 JmjC subfamilies across eukaryotes, including JMJD6 as a subfamily shared by plants, animals, and fungi — phylogenetically distinct from KDM5. This confirms the independent evolutionary origin and functional divergence of these two groups.

Chen et al. (2015) — *"Evolution and conservation of JmjC domain proteins in the green lineage"* (PMID: 26152513)

Classified plant JmjC proteins into seven groups. Group-V (JMJD6) was "found in all the plant species" and associated with "H3R2, H4R3, and hydroxylation of H4." This directly supports the classification of A0A3B6RKV1 as a JMJD6 family member with predicted H4R3 demethylase activity.

H4R3 Methylation Biology in Plants

Wang et al. (2014) — *"Histone H4R3 methylation catalyzed by SKB1/PRMT5 is required for maintaining shoot apical meristem"* (PMID: 24349476)

Established that SKB1/PRMT5-catalyzed H4R3me2 is a functional epigenetic mark in Arabidopsis, repressing CRN expression to maintain SAM geometry. This confirms that H4R3me2 is a biologically active mark in plants, providing substrate context for JMJ22-type demethylases.

Liang et al. (2013) — *"Arabidopsis histone methylase CAU1/PRMT5/SKB1 acts as an epigenetic suppressor of the calcium signaling gene CAS"* (PMID: 23943859)

Further demonstrated H4R3me2 function in plant stomatal closure and drought tolerance, reinforcing the biological significance of H4R3 methylation as a regulatory mechanism in plants and the importance of demethylases that counteract this mark.

Wheat JmjC Family Context

Min et al. (2022) — *"Genome-wide identification of the jumonji C domain-containing histone demethylase gene family in wheat"* (PMID: 36092409)

Identified the complete JmjC gene family in wheat, including JMJD6 subfamily members, and analyzed their expression under drought stress. Provides the genomic context for A0A3B6RKV1 within the wheat JmjC family.

Animal JMJD6 — Caution for Cross-Kingdom Extrapolation

Mantri et al. (2010) — *"Crystal structure of the 2-oxoglutarate- and Fe(II)-dependent lysyl hydroxylase JMJD6"* (PMID: 20684070)

Showed that animal JMJD6 "catalyses the iron- and 2-oxoglutarate-dependent hydroxylation of lysyl residues in arginine-serine-rich domains of RNA splicing-related proteins" and explained structurally "how JMJD6 binds its lysyl residues such that it can catalyse C-5 hydroxylation rather than Nepsilon-demethylation." This raises the possibility that plant JMJD6 orthologs might also have hydroxylase activity, though the in vivo data for AtJMJD2 clearly demonstrates arginine demethylase activity.

Han et al. (2012) — *"The hydroxylation activity of Jmjd6 is required for its homo-oligomerization"* (PMID: 22189873)

Reported that animal Jmjd6 "is unable to remove the methyl group from histone arginine residues but can hydroxylate the histone H4 tail at lysine residues in a 2-oxoglutarate (2-OG)- and Fe(II)-dependent manner." This creates uncertainty about the JMJD6 family's primary activity, but the plant-specific experimental evidence (PMID: 22483719) demonstrates arginine demethylation for AtJMJD2.

Conflicts and Alternatives

1. UniProt vs. PANTHER/InterPro Classification Conflict

The most significant conflict is between the UniProt "Similarity" annotation (JARID1 family) and the PANTHER/InterPro classification (JMJD6 family). The evidence overwhelmingly supports the PANTHER/InterPro assignment: - Domain architecture is incompatible with KDM5/JARID1 - NCBI orthology points to JMJ22, a known JMJD6 subfamily member - Sequence identity to AtJMJ22 is high (77% JmjC domain) - True KDM5/JARID1 proteins exist separately in wheat at much larger sizes

Resolution: The UniProt annotation is likely an automated error. Notably, UniProt names AtJMJ22 as "Arginine-specific demethylase JMJ22" while simultaneously stating it "Belongs to the JARID1 histone demethylase family" — these are internally inconsistent within UniProt itself.

2. Arginine Demethylase vs. Lysyl Hydroxylase Activity

Animal JMJD6 has been shown to have lysyl-hydroxylase activity rather than (or in addition to) arginine demethylase activity in some assays ([PMID: 22189873](#)). This raises the question of whether plant orthologs like A0A3B6RKV1 might primarily function as hydroxylases rather than demethylases. However, the *in vivo* evidence from Cho et al. ([PMID: 22483719](#)) clearly demonstrates arginine demethylation activity for AtJMJ22. The animal results may reflect kingdom-specific functional divergence within the JMJD6 subfamily, or methodological differences between *in vitro* and *in vivo* assays.

3. Paralog Confusion Risk

A0A3B6RKV1 should not be confused with other JmjC proteins in wheat. The wheat genome contains at least 6 JMJ21-like paralogs (808–946 amino acids, chromosomes 2/5) that belong to different PANTHER subfamilies (SF34, SF35). These are larger proteins with distinct domain architectures and likely different substrate specificities. The JMJ22 orthologs are uniquely compact (511 aa) with the F-box + JmjC architecture on chromosome group 7.

4. Domain Architecture and Substrate Specificity

Wang et al. (2020) demonstrated that in *Arabidopsis* JMJ16 (a true KDM5 protein), the FYR domain restricts JmjC substrate specificity to H3K4 in somatic cells ([PMID: 32572214](#)). A0A3B6RKV1 completely lacks FYR, JmjN, ARID, and PHD domains. This is NOT consistent with

being a KDM5 protein with "deregulated" specificity — it is a fundamentally different protein architecture (JMJD6 subfamily) with inherently different substrate recognition. The F-box domain present in A0A3B6RKV1 is never found in KDM5/JARID1 proteins but is characteristic of the plant JMJD6 subfamily.

5. F-box Domain: Potential Additional Function

The presence of an F-box domain is notable and could indicate involvement in ubiquitin-mediated protein degradation pathways. This is not a feature of canonical animal JMJD6 proteins and may represent a plant-specific functional adaptation. Whether the F-box domain contributes to the protein's core function (e.g., by targeting substrates for ubiquitination after demethylation) or serves as a structural scaffold is unknown.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	What Would Resolve It
No direct biochemical assay on A0A3B6RKV1	Literature search for wheat JMJ22 enzymology	Function inferred by orthology only; substrate specificity could differ from AtJMJ22	In vitro demethylase/hydroxylase assay with recombinant A0A3B6RKV1
Substrate specificity not experimentally verified	AtJMJ22 shows H4R3me2; animal JMJD6 shows lysyl hydroxylation	Could have different or additional substrates in wheat	Histone peptide array or mass spectrometry-based substrate profiling
No expression data for A0A3B6RKV1 specifically	Genome-wide wheat JmjC studies exist but gene-level expression for this locus not extracted	Expression pattern would confirm whether this gene is active and in which tissues/conditions	RNA-seq or qRT-PCR across wheat developmental stages for LOC123148885
Functional redundancy with JMJ21-like paralogs unknown	Identified 6 JMJ21 paralogs on chr 2/5 in different subfamilies	If JMJ21 and JMJ22 act redundantly (as JMJ20/JMJ22 do in Arabidopsis), single-gene annotation may overstate essentiality	Genetic interaction studies; double/triple mutant analysis in wheat
F-box domain function uncharacterized	Domain architecture noted; no functional studies found	Could indicate SCF complex involvement or substrate targeting	Mutagenesis of F-box domain; co-IP/pull-down for SCF complex partners
AtJMJ22 lacks specific GO:0033749 annotation	QuickGO checked for Q67XX3	Upstream annotation gap prevents straightforward ISS transfer of the specific term	TAIR curation update for AtJMJ22
Possible lysyl-hydroxylase activity not tested	Animal JMJD6 literature reviewed	Bifunctionality could change the annotation	Hydroxylation assays on histone H4 peptides

Discriminating Tests

High-Priority Experiments

1. **In vitro demethylase assay with recombinant A0A3B6RKV1:** Express and purify the wheat protein; test activity against methylated histone peptides (H4R3me2s, H4R3me2a, H3K4me1/2/3) using MALDI-TOF mass spectrometry or antibody-based detection. This would definitively establish substrate specificity.
2. **In vitro lysyl-hydroxylase assay:** Test A0A3B6RKV1 against lysine-containing peptide substrates (histone H4 tail, RS-domain peptides) to determine whether the protein has hydroxylase activity in addition to or instead of demethylase activity.
3. **ChIP-qPCR in wheat:** Perform chromatin immunoprecipitation with anti-H4R3me2 antibodies in wild-type vs. A0A3B6RKV1 knockdown/knockout wheat lines at target loci (wheat GA3ox orthologs, if the germination pathway is conserved).

Medium-Priority Computational Analyses

1. **Molecular docking of H4R3me2 vs. H3K4me3 into AlphaFold structure:** Use the high-confidence JmjC domain structure to model substrate binding. Compare with crystallized JMJD6 (PDB: 3K2O, [PMID: 20684070](#)) and KDM5A structures to predict substrate preference.
2. **Co-expression network analysis in wheat:** Use publicly available wheat RNA-seq data (e.g., WheatExp, expVIP) to identify genes co-expressed with LOC123148885. If co-expressed genes are enriched for GA biosynthesis or seed germination pathways, this would support functional conservation from Arabidopsis.
3. **Comprehensive phylogenetic analysis:** Build a phylogenetic tree including all plant JMJD6 subfamily members to confirm A0A3B6RKV1's placement and identify the closest paralogs/orthologs with more statistical rigor.

Validation Experiments

1. **Complementation test:** Express A0A3B6RKV1 in Arabidopsis *jmj20 jmj22* double mutant to test whether the wheat protein can rescue the seed germination phenotype — would directly demonstrate functional conservation.

2. **Homeolog-specific wheat knockouts:** Generate CRISPR knockouts of individual or combined 7A/7B/7D homeologs to assess functional redundancy and phenotypic consequences.

Curation Leads

Lead 1: Correct Subfamily Assignment (Critical Priority)

Action: Change subfamily classification from KDM5/JARID1 to JMJD6 in all relevant records. - **Evidence:** PANTHER PTHR12480:SF21, InterPro IPR050910, domain architecture (F-box + JmjC, 511 aa, no JmjN/ARID/PHD/FYR), NCBI orthology to JMJ22 - **Affected annotations:** UniProt "Similarity" field for A0A3B6RKV1, A0A3B6SQ95, A0A3B6TWS8, and cross-species orthologs - **Status:** Lead requiring curator verification with UniProt

Lead 2: Add Specific MF Annotation GO:0033749 (High Priority)

Action: Annotate A0A3B6RKV1 with GO:0033749 (histone H4R3 demethylase activity) using evidence code ISS, with AtJMJ22 (Q67XX3) as the reference protein and [PMID: 22483719](#) as the supporting publication. - **Snippet to verify (from**

P [22483719](#)

abstract): "the histone arginine demethylases, JMJ20 and JMJ22, act redundantly as positive regulators of seed germination... resulting in increased gibberellic acid levels through the removal of repressive histone arginine methylations at GA3ox1/GA3ox2" - **Note:** This is more specific and accurate than GO:0032452 alone.

Lead 3: Remove/Correct H3K4 Substrate References (Critical Priority)

Action: Remove all references to H3K4 demethylase activity and KDM5/JARID1 membership from the gene review. - **Rationale:** Domain architecture definitively excludes KDM5/JARID1 membership; the ortholog AtJMJ22 does not have H3K4 demethylase activity.

Lead 4: Retain GO:0032452 as Broader MF Term (Medium Priority)

Action: GO:0032452 (histone demethylase activity) may be retained as a parent term annotation, consistent with AtJMJ22's existing annotation in TAIR. However, it should not be the sole or most specific annotation if GO:0033749 is applied.

Lead 5: Flag Upstream AtJMJ22 Annotation Gap (Low Priority)

Action: Note that AtJMJ22 (Q67XX3) in TAIR/QuickGO lacks GO:0033749 despite experimental evidence for H4R3me2 demethylation. Suggest TAIR curators consider adding this specific term to the reference organism annotation.

Lead 6: Annotate All Three Homeologs Consistently (Medium Priority)

Action: Apply the same corrected annotations to all three homeologs: A0A3B6RKV1 (7A), A0A3B6SQ95 (7B), A0A3B6TWS8 (7D). Their 96–98% identity and perfectly conserved catalytic residues justify identical functional annotation.

Lead 7: Correct Seed Hypothesis Description (High Priority)

Current description: "Putative histone demethylase that catalyzes Fe(II)- and 2-oxoglutarate-dependent oxidative removal of methyl groups from histone H3, functioning in the nucleus to regulate gene expression epigenetically. Inferred from membership in the JARID1/KDM5 subfamily and domain architecture."

Recommended correction: "Putative histone arginine demethylase that catalyzes Fe(II)- and 2-oxoglutarate-dependent oxidative removal of methyl groups from histone H4 arginine 3 (H4R3me2), functioning in the nucleus to regulate gene expression epigenetically. Inferred from orthology to Arabidopsis JMJ22 (At5g06550) in the JMJD6 subfamily and conserved JmjC domain architecture."