1 **SUPPLEMENTAL DATA**

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4 **Title: Rice GROWTH UNDER DROUGHT KINASE Is Required for Drought Tolerance**
5 **and Grain Yield under Normal and Drought Stress Conditions**

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18 Supplemental Figure S1. Number of spikelets in wild-type and mutant
19 plants under well-watered and drought stress conditions.

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22 **Supplemental Figure S2.** Kinase assays to identify substrate specificity and co-factor
23 requirement of GUDK.

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26 **Supplemental Figure S3.** *In vitro* autophosphorylation assays for GUDK.

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29 **Supplemental Figure S4.** Schematic representation of the workflow for identification of GUDK
30 targets.

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32 **Supplemental Table S1:** Primers used in the study

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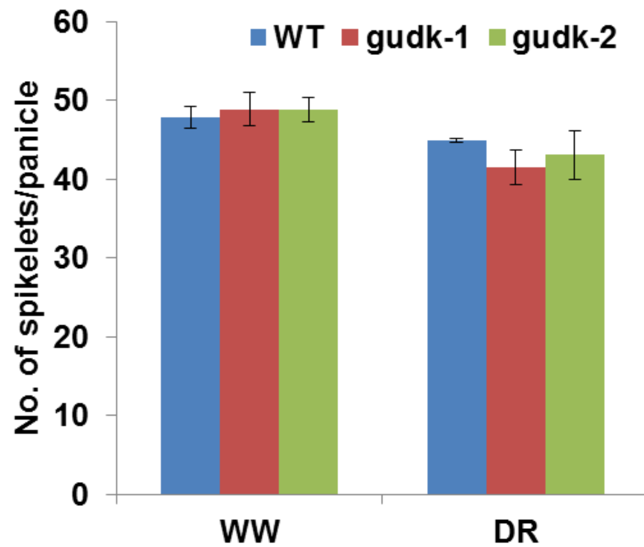
35 **Supplemental Fig. S1**



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37 **Supplemental Figure S1.** Number of spikelets in WT and mutant plants under well-watered and
38 drought stress conditions. Drought stress (DR) was applied to both wild-type (WT) and *gudk*
39 (*gudk-1* and *gudk-2*, two independent T-DNA insertion lines of *GROWTH UNDER DROUGHT*
40 *KINASE* gene) mutant plants at R3 stage by withholding irrigation until all the leaves roll and
41 wilt, followed by re-watering and maintaining the plants at well-watered (WW) condition until
42 physiological maturity. A set of plants were maintained under flooded condition as well-watered
43 plants. At physiological maturity panicles were harvested individually and number of
44 spikelets/panicle were counted. Values are means ± SE (n=6).
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45 **Supplemental Fig. S2**

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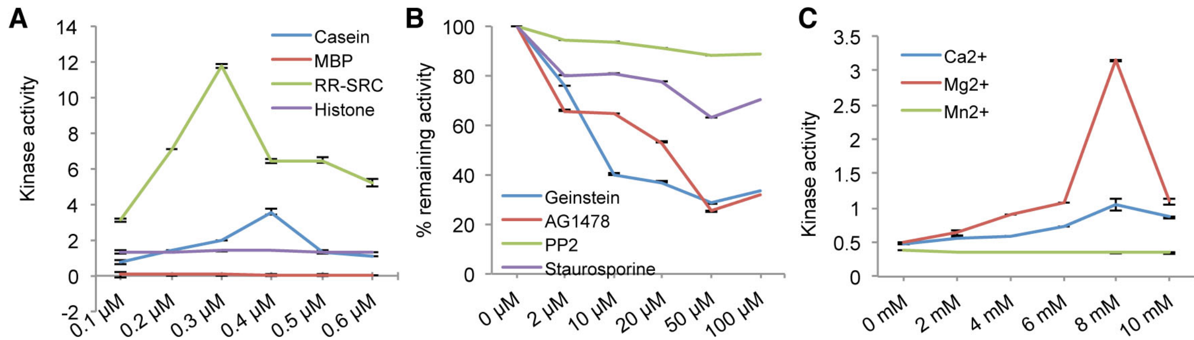

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49 **Supplemental Figure S2. Kinase assays to identify substrate specificity and co-factor**
50 **requirement of GUDK. (A)** Substrate specificity of GUDK. Kinase assays were performed in
51 with various concentrations of artificial substrates RR-SRC, histone III-S, MBP and casein. (**B**)
52 Effect of different kinase inhibitors on the activity of GUDK. Influence of different inhibitors on
53 the GUDK activity was studied by kinase assays in presence of various concentrations of
54 artificial inhibitors. (**C**) Effect of various concentrations of Ca^{2+}, Mg^{2+} and Mn^{2+} on the activity
55 of GUDK. Kinase assays were performed using RR-SRC as substrate in presence of various
56 concentrations of divalent cations. Each data points represent the mean ± SE of three
57 independent experiments. The kinase activity is expressed as the reciprocal of luminescence.

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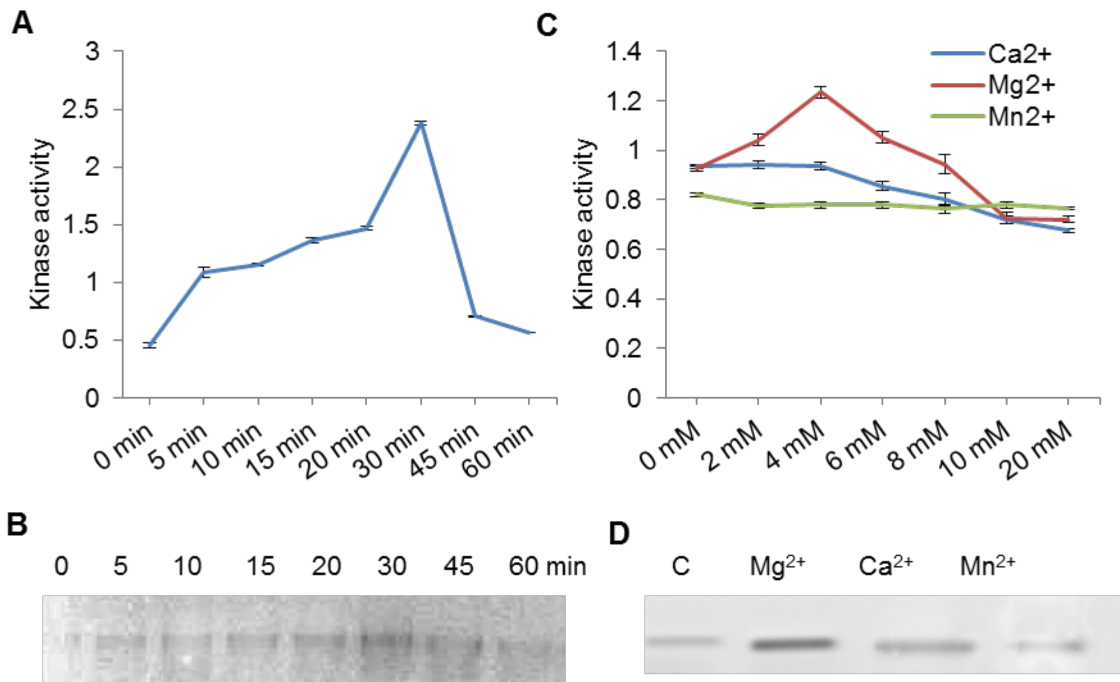
58 **Supplemental Fig. S3**



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61 **Supplemental Figure S3. *In vitro* autophosphorylation assays for GUDK.** (A) Kinetic
62 analysis of GUDK autophosphorylation by kinase assay. The assay was carried out with GUDK
63 alone and the luminescence was measured at different time points. Each data point represents the
64 means ± SE of three independent assays. Kinase activity is expressed as the reciprocal of
65 luminescence. (B) Autophosphorylation of GUDK detected by immunoblot analysis using
66 phosphotyrosine antibody. The samples were collected at different time points and 1 µg of the
67 protein was separated in 10% SDS-PAGE, subsequently transferred to the PVDF membrane and
68 immunoblotted with phosphotyrosine antibody. (C) Kinase assays showing the effects of various
69 concentrations of divalent cations on autophosphorylation of GUDK. Each data point represents
70 the means ± SE of three independent assays. Kinase activity is expressed as the reciprocal of
71 luminescence. (D) Effect of different divalent cations on autophosphorylation of GUDK detected
72 by immunoblot analysis using phosphotyrosine antibody. C – indicates control without any
73 cations.
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79 **Supplemental Fig. S4**

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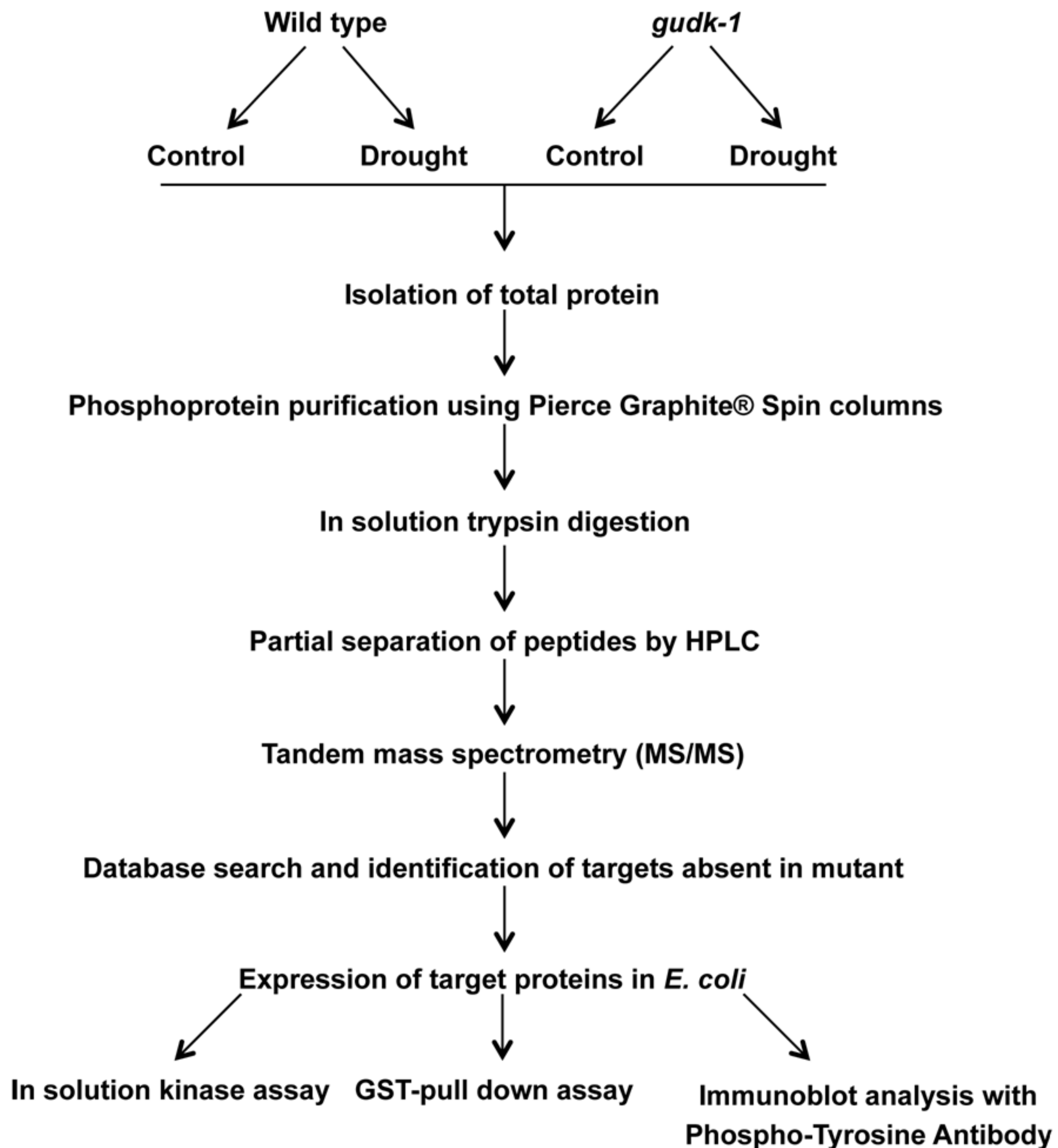

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84 **Supplemental Figure S4. Schematic representation of the workflow for identification of**
85 **GUDK targets.** The wild-type and *gudk-1* mutant genotypes from well-watered or drought
86 treated plants were used for phosphoprotein extraction and comparative analysis using the flow-
87 diagram as shown to identify differentially expressed proteins. Putative identified GUDK-1
88 target proteins were used in subsequent validation assays of specific kinase activity.

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91 **Supplemental Table S1: Primers used in the study**

A. Primers used to identify *gudk* homozygous mutant lines	
T-DNA primer	GTCTGGACCGATGGCTGTGTAGAAG
Os03g08170 F	AAAGTTTATGCCAGCGAGTTG
Os03g08170 R	TGACGGCATGAGATTATTGC
B. Primers used for *GUDK* expression analysis	
Os03g08170 F	ATCAAGCGATGCCGGTAAA
Os03g08170 R	GTACGTCTGACATGCTCGTATT
C. Primers used for bacterial expression	
Os01g58420 F	ACGTGGATCCATGGCGCCCAGAGCAGCT
Os01g58420 R	ACGTGAATTCCTAGTTCTCTACCGGCGG
Os03g08170 F	ACGTGGATCCATGGGGAACTGCTTCGGCTCC
Os03g08170 R	ACGTGAATTCTCTCACTCGAGGAGAAGGGTA
D. Primers used for expression analysis of OsAP37 target genes	
Os07g34520 F	GCGCCAACTTCTACGACAGG
Os07g34520 R	CTTGGCTGAAGTCCAGAGTG
Os04g17660 F	TACTTGGACGTCAGGACAGA
Os04g17660 R	TGCTATGAGATCGGCAGATG
Os10g30850 F	CAGCATCAGGCAATTCTACG
Os10g30850 R	TCCAATCCTCGAAGCATCTC
Os08g32930 F	GGATCTTGGAGGTCCGATCT
Os08g32930 R	AGCTGTGTATCCGTGGAAGG
Os05g45450 F	ATTCCAAGAGGCTGACATCC
Os05g45450 R	TCGTTGGTCTGGTCGAACAT
E. Primers used for cloning promoters of OsAP37 for transactivation assay	
Os07g34520 F	ACGTTCTAGACCATCCGTCGCAGCCCATT
Os07g34520 R	ACGTGGATCCTGCTTCGCCTGCAGAAAACGC
Os04g17660 F	ACGTTCTAGATAGCACGGGTTTATAATC
Os04g17660 R	ACGTGGATCCATAAATATGATAGGAATGCAT
Os10g30850 F	ACGTAAGCTTCACTCCACTCCTCTCTCC
Os10g30850 R	ACGTGGATCCATCTAAAATTAGATAGAACATACA
Os08g32930 F	ACGTTCTAGACACAATTAAATTGGACCTCAC
Os08g32930 R	ACGTGGATCCGAGATTGGTTGATATTTTCCG
Os05g45450 F	ACGTTCTAGACGTCGCCCCAGAATTTCTC
Os05g45450 R	ACGTGGATCCTATTAGCGAGGATGAACAAGT

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