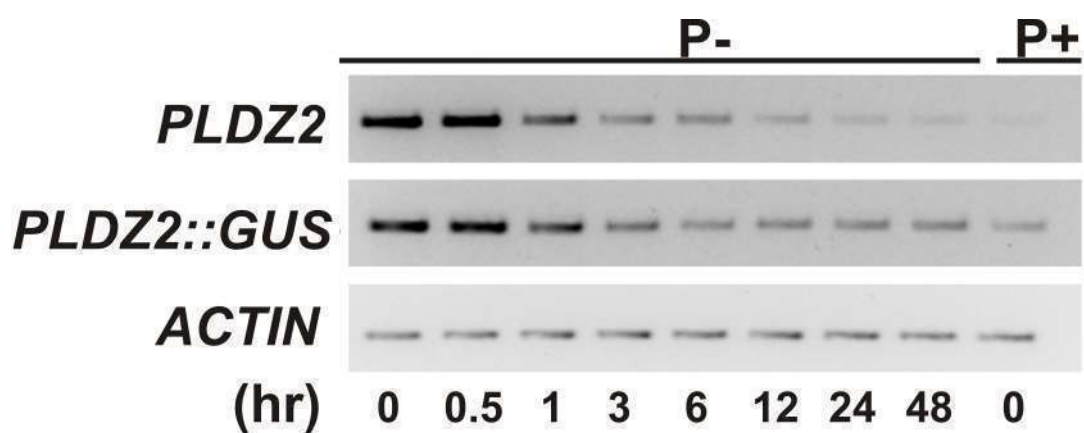


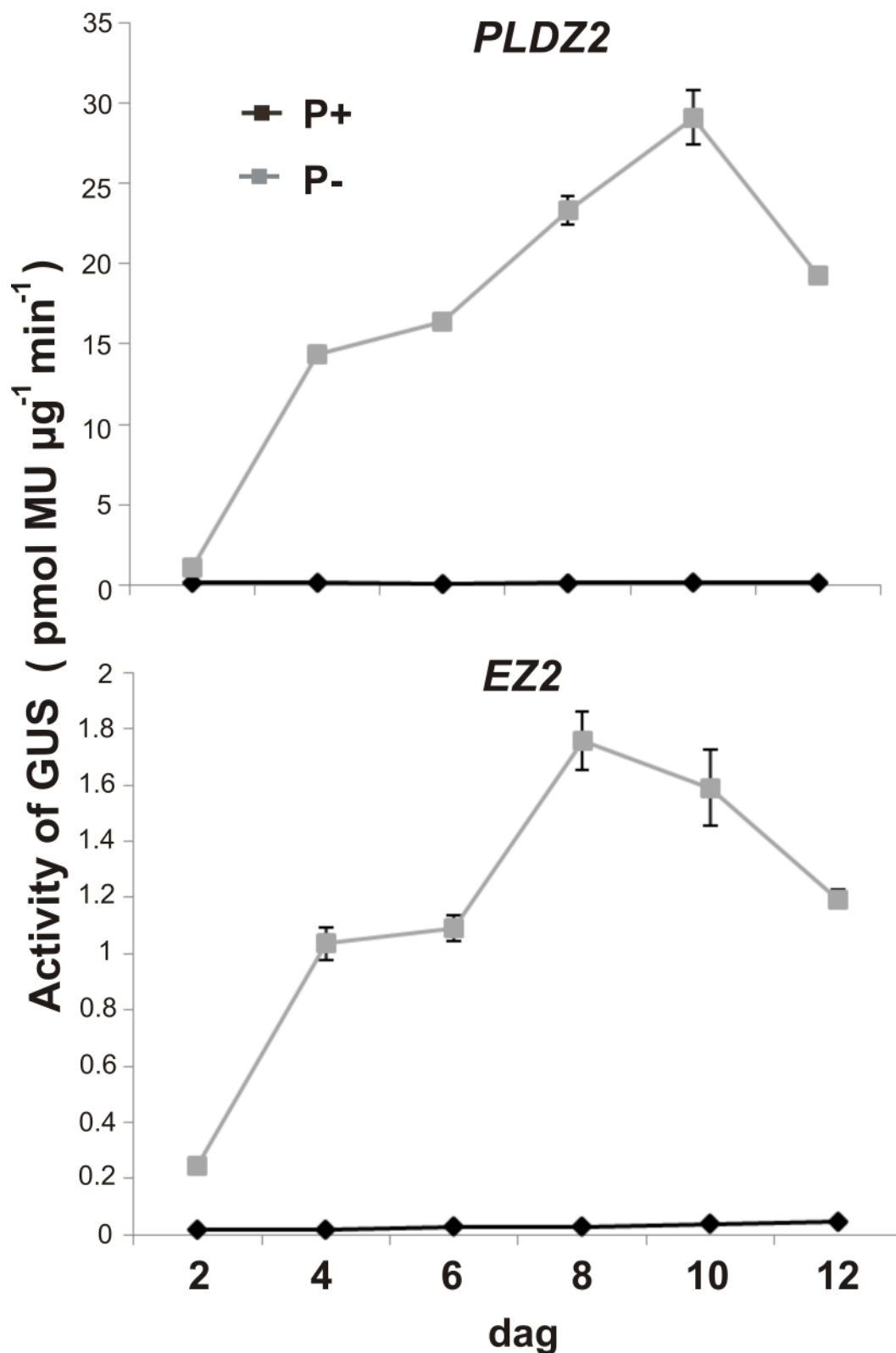
SUPPLEMENTARY MATERIAL

Functional analysis of the Arabidopsis PLDZ2 promoter reveals an evolutionarily conserved low-Pi- responsive transcriptional enhancer element

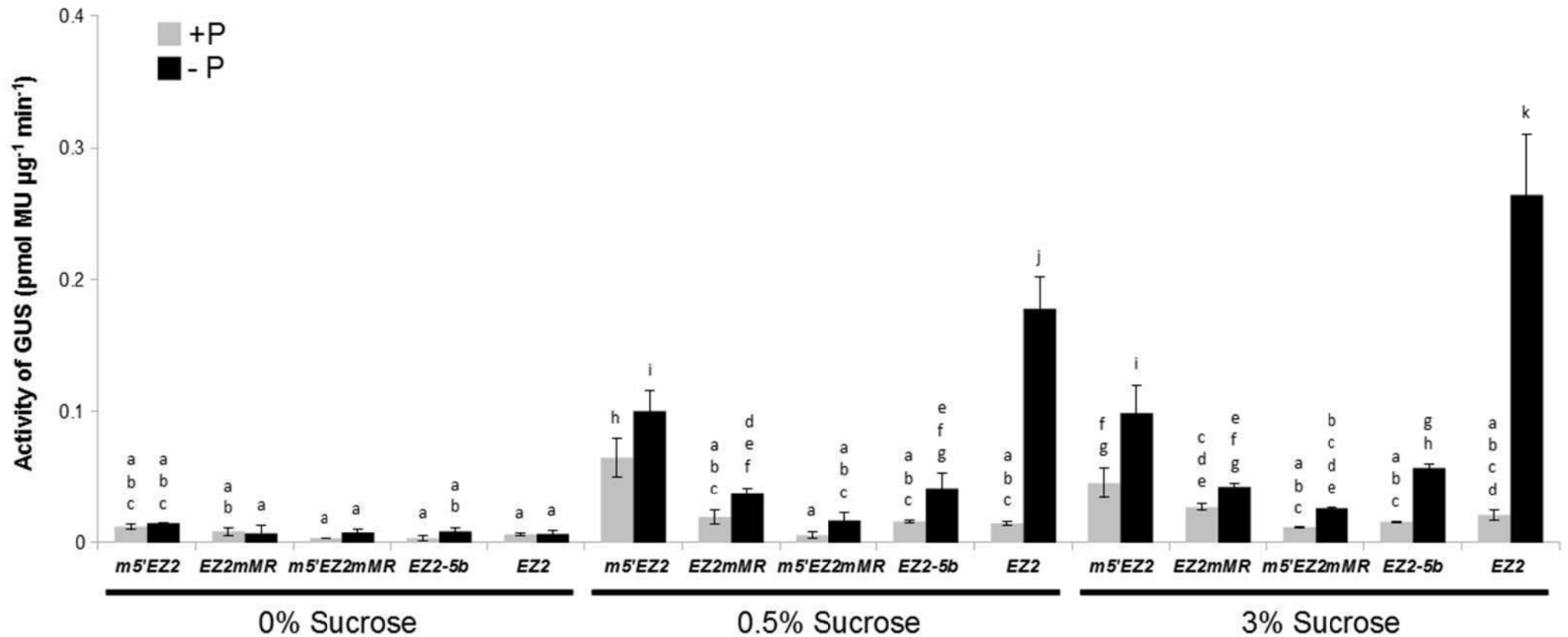
Araceli Oropeza-Aburto, Alfredo Cruz-Ramírez, Gustavo J. Acevedo-Hernández, Claudia-Anahí Pérez-Torres, Juan Caballero-Pérez and Luis Herrera Estrella



Supplemental Figure S1. Comparison of the expression in response to Pi availability of *PLDZ2* and *PLDZ2::GUS*. Semiquantitative RT-PCR analysis of *PLDZ2* and *PLDZ2::GUS* gene expression in wild type and transgenic *Arabidopsis*. Plants were grown in P- limiting medium for 7 days, and transferred to P+ liquid medium for 30 min, 60 min, 3 h, 6h, 12h, 24h and 48h. Controls of *PLDZ2* and *PLDZ2::GUS* expression for seedlings grown continuously under P+ conditions are shown.



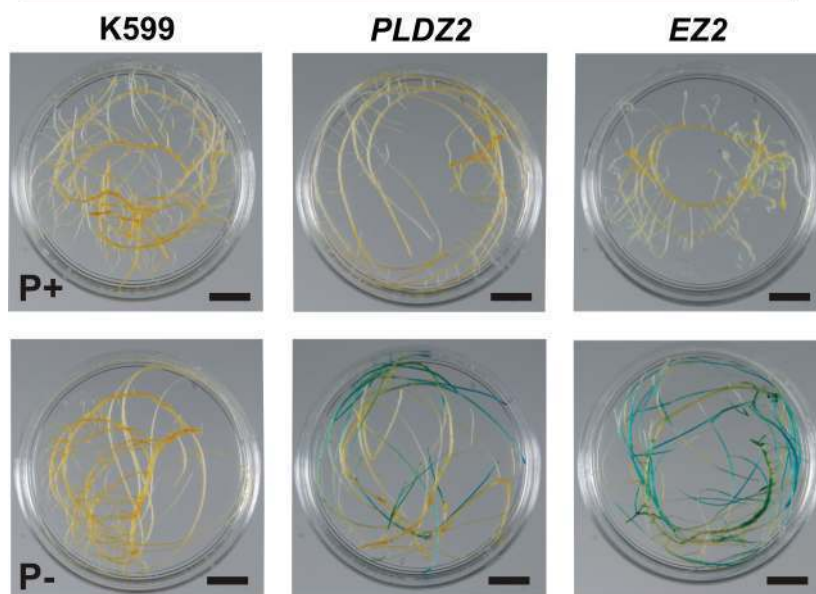
Supplemental Figure 2. Temporal expression profiles of the *PLDZ2::GUS* and *EZ2::GUS*. Seedling of *Arabidopsis* carrying the *PLDZ2::GUS* and *EZ2::GUS* were grown in MS P+ or P- and harvested after 2, 4, 6, 8, 10 and 12 days for fluorometric GUS assay.



Supplemental Figure S3. Effect of sucrose on the low-Pi responsiveness of mutant versions of *EZ2*.

Fluorometric determination of GUS activity in transgenic seedlings harboring the *GUS* reporter gene under the control of the *EZ2* enhancer element and its mutant derivatives where deletions and substitutions were introduced. The region upstream of P1BS4 (*m5'EZ2*), the spacer sequence between P1BS4 and P1BS3 (*EZ2mMR*) or both the upstream and spacer sequences (*m5'EZ2mMR*) were replaced by random DNA sequences and the distance between the P1BS sites was reduced by 5 bp (*EZ2-5b*). Six days-old seedlings grown in P+ solid medium were transferred into P- liquid medium supplemented with different concentrations of sucrose (0%, 0.5% and 3%). Seedlings were harvested after 72 h of treatment for fluorometric GUS assay. Error bars represent SD of eight replicates. Different letters on the bars indicate significant differences at 95% confidence level, employing statistical ANOVA and Tukey analyses.

***A. rhizogenes* K599**



Supplemental Figure S4. Expression of *EZ2::GUS* in transgenic bean roots. Bean hairy roots transformed with *Agrobacterium rhizogenes* containing the *PLDZ2::GUS* or *EZ2::GUS* constructs. Hairy roots grown in solid P+ media were transferred to P+ or P- liquid media for 6 days and then subjected to GUS histochemical staining assays.

Supplemental Table S1. Sequence of oligonucleotides used to produce deletion versions of the *PLD22* promoter, *EZ2* enhancer and primers used for Real Time and RT-PCR analysis

Sequence of oligonucleotides used to produce deletion versions of the *PLD22* promoter

Name	SEQUENCE
PLD22 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGGAGTACCATTACAAAAAC 3'
PLD22 Δ1 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGTATTTCGCAATTGCTTTAG 3'
PLD22 Δ2 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGATGGAAGGTAGTATATATG 3'
PLD22 Δ3 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGACAGATTAGTTTAGTAAC 3'
PLD22 Δ4 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGTTATGGTTTACTTAAGG 3'
PLD22 Δ5 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGAATTAATAAGTGTGTTG 3'
PLD22 Δ6 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGTATCTGGCAAAGCACC 3'
PLD22 Δ7 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGTCGTCTGATGCTTGCAT 3'
PLD22 Δ8 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAACAGAGCTCTTGCCAATTTG 3'
PLD22 Δ9 FWD	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTAACAGGTAGACGGGAG 3'
PLD22 REV	5' GGGGACCACCTTGTACAAGAAAGCTGGTACCGTCGACATCGATTAAC 3'

Sequence of oligonucleotides used to produce *EZ2* enhancer versions

Name	SEQUENCE
EZ2	5' CGGGGTACCGAATTAATAAGTGTGTTGGGAATATTCTGATCTGGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
EZ2 REV	5' GCGAAGCTTA 3'
EZ2P1B54(2X)	5' CGGGGTACCGAATTAATAAGTGTGTTGGGAATATTCTGATCTGGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
EZ2P1B53(2X)	5' CGGGGTACCGAATTAATAAGTGTGTTGGGGAATATTCTGATCTGGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
EZ2mP1B54	5' CGGGGTACCGAATTAATAAGTGTGTTGGAGCGCGCTGATCTGGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
EZ2mP1B53	5' CGGGGTACCGAATTAATAAGTGTGTTGGGAATATTCTGATCTGGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
EZ2mP1B54-3	5' CGGGGTACCGAATTAATAAGTGTGTTGGAGCGCGCTGATCTGGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
m5'EZ2	5' CGGGGTACCGAATTAATAAGTGTGTTGGGAATATTCTGATCTGGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
EZ2mMR	5' CGGGGTACCGAATTAATAAGTGTGTTGGGAATATTCCAGCTCATTCTGATCTGGGGGCCAGGATATTCTTAAGCTTCGC 3'
m5'EZ2mMR	5' CGGGGTACCGAATTAATAAGTGTGTTGGGAATATTCCAGCTCATTCTGATCTGGGGGCCAGGATATTCTTAAGCTTCGC 3'
EZ2-5b	5' CGGGGTACCGAATTAATAAGTGTGTTGGGAATATTCTGCAAAGCACCAAAAAATTGGGATATTCTTAAGCTTCGC 3'
EZ2, EZ2P1B54(2X), EZ2P1B53(2X), EZ2mP1B54, EZ2mP1B53, EZ2mP1B54-3, EZ2mMR, EZ2 -5b (RECOMBINATION FORWARD)	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTACGGTACCGAATTAATAAGTGTG 3'
m5'EZ2, m5'EZ2mMR (RECOMBINATION FORWARD)	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTACGGTACCGTACCGATCCGCTCGCAG 3'
REV 46S (RECOMBINATION REVERSE)	5' GGGGACCACCTTGTACAAGAAAGCTGGTACCTGAATTCAGATCTCTAGA 3'

REAL TIME and RT-PCR OLIGONUCLEOTIDES

Name	SEQUENCE
PLD22-RT FWD	5' CGAATGAACATATACGCTCAAGAGCTG 3'
PLD22-RT REV	5' AGCAGTGAAGTAGAACTCGACTTGAGG 3'
ATPT2-RT FWD	5' GCTTATTGTTGGGTGTAG 3'
ATPT2-RT REV	5' CTAACATTTGGGACCGTTC 3'
IPS1-RT FWD	5' CTTCAATGTTTGGGTTGTACC 3'
IPS1-RT REV	5' GCACTGGTCTGACTATTCTC 3'
ACTINA FWD	5' GAATTGCTCTGTTGCTCCTCAC 3'
ACTINA REV	5' TGCAATCCAGCCTCCACCATAC 3'
UBQ4 FWD	5' GACGGACGCACACTTGCTGACTAC 3'
UBQ4 REV	5' TCCTGGATCTTGCCTTGACATTG 3'
GUS FWD	5' TGCTGTGCGCTTTAACCTCT 3'
GUS REV	5' GGCACAGCACATCAAGAGA 3'

Supplemental Table S2. GUS activity of *PLDZ2* promoter and deletion derivatives.

GUS activity ($\mu\text{moles MU } \mu\text{g protein}^{-1} \text{ min}^{-1}$)		
Construct	P+	P-
<i>PLDZ2::GUS</i>	0.04 \pm 0.003	42.5 \pm 2.76
	0.07 \pm 0.005	39.72 \pm 2.31
	0.09 \pm 0.003	110.94 \pm 9.72
<i>PLDZ2 Δ1::GUS</i>	0.03 \pm 0.003	50.10 \pm 0.62
	0.05 \pm 0.001	29.68 \pm 0.55
	0.14 \pm 0.008	21.10 \pm 0.75
<i>PLDZ2 Δ2::GUS</i>	0.007 \pm 0.004	32.19 \pm 2.52
	0.09 \pm 0.006	12.83 \pm 0.97
	0.02 \pm 0.002	59.51 \pm 4.23
<i>PLDZ2 Δ3::GUS</i>	0.02 \pm 0.001	29.18 \pm 1.38
	0.01 \pm 0.002	94.12 \pm 2.78
	0.02 \pm 0.002	63.40 \pm 4.25
<i>PLDZ2 Δ4::GUS</i>	0.04 \pm 0.001	6.51 \pm 0.11
	0.02 \pm 0.0003	3.59 \pm 0.04
	0.06 \pm 0.003	4.96 \pm 0.34
<i>PLDZ2 Δ5::GUS</i>	0.06 \pm 0.005	11.14 \pm 1.07
	0.02 \pm 0.001	5.86 \pm 0.59
	0.03 \pm 0.001	4.87 \pm 0.25
<i>PLDZ2 Δ6::GUS</i>	0.02 \pm 0.001	1.1 \pm 0.05
	0.02 \pm 0.01	0.57 \pm 0.03
	0.13 \pm 0.006	3.72 \pm 0.14
<i>PLDZ2 Δ7::GUS</i>	0.01 \pm 0.003	0.04 \pm 0.002
	0.04 \pm 0.003	0.07 \pm 0.004
	0.02 \pm 0.002	0.05 \pm 0.004
<i>PLDZ2 Δ8::GUS</i>	0.02 \pm 0.004	0.11 \pm 0.01
	0.01 \pm 0.001	0.04 \pm 0.002
	0.01 \pm 0.001	0.05 \pm 0.002
<i>PLDZ2 Δ9::GUS</i>	0.01 \pm 0.002	0.1 \pm 0.007
	0.01 \pm 0.002	0.09 \pm 0.006
	0.01 \pm 0.005	0.03 \pm 0.002

Supplemental Table S3. GUS activity directed by the *PLDZ2* promoter, EZ2 enhancer element and derivative constructs

GUS activity (pmoles MU $\mu\text{g protein}^{-1} \text{min}^{-1}$)		
Construct	P+	P-
<i>PLDZ2::GUS</i>	0.006 \pm 0.002	36.7 \pm 6.43
	0.009 \pm 0.001	36.05 \pm 1.51
	0.1 \pm 0.004	35.55 \pm 2.19
<i>EZ2::GUS</i>	0.09 \pm 0.004	3.9 \pm 0.05
	0.03 \pm 0.002	3.45 \pm 0.02
	0.05 \pm 0.005	6.93 \pm 0.36
<i>EZ2P1BS4(2x)::GUS</i>	0.01 \pm 0.003	10.69 \pm 0.06
	0.60 \pm 0.02	24.76 \pm 1.23
	0.07 \pm 0.005	6.54 \pm 0.15
<i>EZ2P1BS3(2x)::GUS</i>	0.21 \pm 0.01	0.94 \pm 0.04
	0.04 \pm 0.003	0.83 \pm 0.01
	0.03 \pm 0.002	1.30 \pm 0.08
<i>EZ2mP1BS4::GUS</i>	0.009 \pm 0.001	0.06 \pm 0.002
	0.01 \pm 0.0005	0.09 \pm 0.007
	0.03 \pm 0.003	0.008 \pm 0.001
<i>EZ2mP1BS3::GUS</i>	0.07 \pm 0.003	0.15 \pm 0.003
	0.01 \pm 0.003	0.07 \pm 0.001
	0.01 \pm 0.001	0.06 \pm 0.002
<i>EZ2mP1BS4-3::GUS</i>	0.08 \pm 0.003	0.18 \pm 0.002
	0.19 \pm 0.007	0.24 \pm 0.002
	0.03 \pm 0.003	0.22 \pm 0.003
<i>m5'EZ2::GUS</i>	0.15 \pm 0.02	1.1 \pm 0.03
	0.01 \pm 0.001	1.02 \pm 0.05
	0.008 \pm 0.004	0.99 \pm 0.04
<i>EZ2mMR::GUS</i>	0.1 \pm 0.005	0.92 \pm 0.01
	0.06 \pm 0.006	0.50 \pm 0.02
	0.06 \pm 0.005	1.26 \pm 0.06
<i>m5'EZ2mMR::GUS</i>	0.02 \pm 0.007	0.04 \pm 0.005
	0.01 \pm 0.005	0.05 \pm 0.003
	0.02 \pm 0.001	0.02 \pm 0.002
<i>EZ2-5b::GUS</i>	0.03 \pm 0.001	1.9 \pm 0.05
	0.04 \pm 0.003	1.19 \pm 0.04
	0.11 \pm 0.008	0.83 \pm 0.01