## SUPPLEMENTAL INFORMATION, Manosalva et al.

**Figure S1.** Backcross (BC<sub>3</sub>) rice line BC116 contains the chr 8 QTL harboring the *OsGLP8* gene cluster from the resistant parent SHZ-2. Confirmation that BC116 contains the QTL region and that these alleles were from the SHZ-2 resistant parent are from: (**A**) Single Feature Polymorphism (SFP) analysis and Single Sequence Repeat (SSR) marker analysis. RM547 locates on chr 8 between 5586058 – 5586291. (**B**) TILLING analysis for two chr 8 *OsGLP* family members, *OsGLP8-8* and *OsGLP8-9* (located on chr 8 between 5,242,446-5,254,949). Template DNA used in TILLING analyses were from BC116, BC116 + SHZ-2 (116/S), and BC116 + TXZ-13 (116/T). Double band (arrow) in 116/T is indicative of heteroduplex formation between the two different alleles from SHZ-2 and TXZ-13. M, molecular marker.



**Figure S2.** RNAi silencing vector pTSi-1 and the *OsGLP* RNAi construct. (**A**) Vector pTSi-1 contains the HBT35S promoter, which includes a minimal CaM35S enhancer plus the 5' UTR of maize *CDDPK* gene (1). The inverted *NOS* carry the antisense sequence of the *Agrobacterium NOS* terminator plus an 81 bp fragment of the 3' end of the *GFP* gene. A pair of *XcmI* sites is used to generate "T" 3' extensions for cloning PCR products. (**B**) RNAi silencing construct pTSi-*OsGLP* was constructed by inserting an antisense 500 bp PCR product of *OsGLP8-3* into the pTSi-1 RNAi vector. *NheI*-digested pTSi-*OsGLP* vector was inserted into *XbaI*-digested pCambia 1305 binary vector.



**Figure S3.** Silencing patterns of the *OsGLP* in independent  $T_0$  and  $T_1$  transgenic plants. RT-PCR was performed using gene-specific primers for each *OsGLP* gene family member (SI Table 3) in uninoculated  $T_0$  (**A**) and  $T_1$  (**B**) transgenic plants. After amplification, band intensities were quantified and compared with the wild type band (WT). *EF1-a* and ubiquitin genes were amplified as internal controls; hygromycin was the selection marker. Seed from  $T_0$  lines 10 and 24 were advanced to  $T_1$ . For  $T_1$  analysis, RNA was extracted from the third youngest leaf of 2-wk-old uninoculated  $T_1$  transgenic and Kitaake wild type plants (WT). (**C**) RT-PCR using gene specific primers revealed silencing for the *OsGLP* gene members on chr 12 in the cDNA samples from  $T_1$  plants used in (**B**) 1 Kb ladder (L) and DNA amplification controls.



**Figure S4**. Disease phenotypes of Kitaake and Nipponbare after *Magnoporthe oryzae (Mo)* inoculation. (A) Spot inoculation of rice detached leaves of Kitaake (resistant) and Nipponbare (susceptible) with *Mo* isolate Che86061. (B) Spray inoculation of Kitaake and Nipponbare plants with *Mo* isolate Che86061.



**Figure S5.** *OsGLP*-silenced  $T_1$  plants show higher levels of sheath blight disease. (A) Sheath blight disease scores of  $T_1$  RNAi transgenic plants assessed using the sheath blight disease index (2). Kitaake (WT, resistant control ) and Lemont (LEM, susceptible control). (B) Sheath blight disease phenotypes on  $T_1$  silenced plants with different levels of gene suppression.



**Table S1.** Disease ratings for Sanhuangzhan 2 (SHZ-2, donor resistant parent), BC116 (backcross line harboring QTL from SHZ-2) and commercial cultivar Texianzhan 13 (TXZ-13, recurrent susceptible parent) after 7 yr of field evaluation in a rice blast nursery in Yangjiang, Guangdong, China.

Year	Disease Scale <sup>a</sup>			t-value <sup>b</sup>	Significance	
	SHZ-2	BC116	TXZ-13	t-value	Significance	
2001	0.87±0.35	5.40±1.35	9.00±0.00	-11.0227	0.0000	
2002	$0.67 \pm 0.49$	4.73±1.83	8.33±0.98	-8.08845	0.0000	
2003	$0.67 \pm 0.49$	4.87±1.77	$9.00 \pm 0.00$	-9.05741	0.0000	
2004	$1.67 \pm 0.98$	$4.02 \pm 1.01$	$6.07 \pm 1.49$	-3.5000	0.0035	
2005	$0.87 \pm 0.74$	$2.87 \pm 1.63$	$8.60 \pm 0.83$	-12.1276	0.0000	
2006	$0.87 \pm 0.74$	$6.60 \pm 0.83$	$8.70 \pm 0.83$	-5.9161	0.0000	
2007	$0.73\pm0.46$	$2.73 \pm 1.28$	$7.80 \pm 1.01$	-11.7671	0.0000	

<sup>a</sup> Panicle blast symptoms were evaluated on each rice line using IRRI Standard Evaluation System for Rice (<u>http://www.knowledgebank.irri.org/ses/SES.htm</u>). Data are averages of disease for the two crops for each year (mean ± standard error).

<sup>b</sup> t-test between BC116 and TXZ-13.

Table S2. Gene members	of the barley germi	in-like protein	(HvGER)	subfamilies	used f	or the
phylogenetic analysis of th	ne OsGER subfamil	lies in rice.				

Name of gene	TIGR TC number <sup>a</sup>	Predicted protein size (number of aa)
HvOxOa	Y142203	224
HvOXOLP	X93171	229
HvGER1a	TC140112	224
HvGER1b	TC148017	215
HvGER1c	TC141021	223
HvGER1d	TC148015	228
HvGER2a	TC146914	212
HvGER2b	TC147369	212
HvGER3a	TC131410	226
HvGER3b	TC131415	227
HvGER3c	TC131413	226
HvGER4a	TC139505	228
HvGER4b	H011H17	229
HvGER4c	TC139504	211
HvGER4d	TC147149	229
HvGER4e	TC139503	229
HvGER5a	TC147527	216
HvGER5b	TC147526	216
HvGER6a	TC141367	219

<sup>a</sup> TC = Tentative Consensus sequences created by assembling ESTs into virtual transcripts. The TC annotation numbers corresponds to the *Hordeum vulgare* gene index (HvGI): <u>http://compbio.dfci.harvard.edu/tgi</u>

Gene	TIGR Locus ID <sup>a</sup>	Primer <sup>b</sup>	Sequence 5' - 3'	T <sub>A</sub> (°C) <sup>c</sup>
8-1	LOC Os08g08920	GLP1F	CAGGAAACACAAAGCATCTGATCAGG	57
	_ 0	GLP1R	CTTTGCAACACTATGGGACAATTA	
8-2	LOC Os08g08960	GLP2F	GAAACCATAAACACACAGGCATCTG	59
		GLP2R	ATGTAGTATACTGTCCTTGATGGTG	
8-3	LOC Os08g08970	GLP3F	CTCACCCAAAATAACGATAAACACAGGG	56
	_ 0	GLP3R	ACGCACAAAGGAACAATCAAG	
8-4	LOC Os08g08980	GLP4F	GGAGAAACCAACTCATAGTAGCTTAGC	52
		GLP4R	GCATCAATTGATTAGGGAGAATCAG	
8-5	LOC Os08g08990	GLP5F	GCAGAAATTAATCCAAAGCCGAAT	56
		GLP5R	GTAACACAAAAGCTAATACATTG	
8-6	LOC Os08g09000	GLP6F	CTTCCCATCAGAGAAAGATAGCAG	58
		GLP6R	GATTCACGGTATGCCAACAAAC	
8-7	LOC Os08g09010	GLP7F	CAAAGCCAAATGGCTTCACCATCTTC	58
		GLP7R	GCAGAATAGAAACTTATACATAGTATAA	
8-8	LOC Os08g09020	GLP8F	CAAATGGCTTCACCATCCTTCTGCCTA	59
	_ 0	GLP8R	CATAGAGTCCGTAAGCGGAC	
8-9	LOC Os08g09040	GLP9F	CAGTAGAGAAGATAGCAGAAACCC	57
	_ 0	GLP9R	GTATGCATAACAAGTACAAACTCC	
8-10	LOC Os08g09060	GLP10F	GCCAAGTAAATGGCTTCACCATC	52
		GLP10R	GTACAAACTCCATACCACTTATTTATG	
8-11	LOC Os08g09080	GLP11F	GCTAATTAAGAAGGGCATTAGAATGGC	59
		GLP11R	GTTATGTGCAGTTACAGAGATCCTGC	
8-12	LOC Os08g13440	GLP12F	GCTAGCTAACTACCAGAGAGAGATAC	59
		GLP12R	CCTCCCATACACAAAAGCAC	
12-1	LOC Os12g05840	GLP14F	ATCAACTATAGCTATACAAGAAT	53
		GLP14R	GTAGTGTTATACATTATTGATGCGT	
12-2	LOC Os12g05860	GLP15F	TAGACTACAGCTATACAAGAAGCAT	53
	_ 0	GLP15R	CTCTTTTTACTACCAATCACTAGTTTTG	
12-3	LOC Os12g05870	GLP16F	CAAGCTAGCATCGAGTAATACTTC	53
	_ 0	GLP16R	GATAACTAATTTTCAACAGATAAGCATC	
12-4	LOC Os12g0580	GLP17F	GACTACTTCTACAGGATCTGTAG	53
	_ •	GLP17R	CCTTTTTATACCAATCACTAATTCTGA	
EF1α	D63582	EF1a1F	AGCCTCGTTCAAATGGTGGT	57
		EF1a1R	TAGTGCACATTGCGAGCAGA	
Hygromycin	AF354046	HygroF1	GAGCCTGACCTATTGCATCTCC	54.5
		HygroR1	GGCCTCCAGAAGAAGATGTTGG	
CaMV35S	AY234331	CaMVR1	CGTGCTCCACCATGTTGGCAAGC	

 Table S3. Oligonucleotide primers used in the study.

<sup>a</sup> TIGR Rice Genome Annotation: <u>http://www.tigr.org/tdb/e2k1/osa1/</u>

 ${}^{b}F$  = forward primer, R = reverse primer

 $^{c}T_{A}$  = annealing temperature

## **References:**

- 1. Zhao B (2004) Isolation and characterization of the maize nonhost resistance gene *Rxo1* and the corresponding bacterial effector gene *avrRxo1* from *Xanthomonas oryzae* pv. *oryzicola*. *Doctorate thesis*.
- 2. Jia Y, *et al.* (2007) Rapid determination of rice cultivar responses to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. *Plant Dis* 91:485-489.