File S1

Supporting Results and Discussion

Strain creation and genotype testing

3000 bp of upstream sequence from the ATG of *vvd* was fused to *wc*-1 which was tagged with the V5 tag followed by 500 bp of the *vvd* 3' UTR. This cassette was linked to the *bar* gene (which confers resistance to ignite) and was targeted by transformation to the *csr-1* locus (BARDIYA and SHIU 2007) in WT and *Δvvd* strains, creating strains WC-1 WT and WC-1 Δ*vvd* respectively (Figure S1A). A construct containing 3000 bp of the *vvd* promoter was fused to *gfp* followed by 500 bp of the *vvd* 3' UTR. This cassette was linked to the *bar* gene and was targeted by transformation to the *csr-1* locus in the *Δvvd* strain creating strain GFP (Figure S1A). 3000 bp of the *vvd* promoter was fused to the *gh-5-1* gene followed by the *gfp* gene linked to the His^6 tag, with 500 bp of the *vvd* 3' UTR on the 3' end. This cassette was targeted by transformation to the *csr-1* locus in the *Δvvd* strain creating strain GH5-1 (Figure S1A). Genotyping of the *csr-1* locus was achieved by allelic-specific nested PCR (primers listed in Table S1). The control strain (74A) shows two bands, the 350 bp band representing the intact locus of *csr-1* while the 750 bp band represents the presence of the *actin* locus, a positive control (Figure S1B). As expected, strains WC-1 WT, WC-1 Δ*vvd*, GFP and GH5-1 strains all show only the 750 bp band, indicating that *csr-1* is no longer present and the strains are homokaryon for the cassette of interest at the *csr-1* locus as expected with this type of transformation (Figure S1B) (BARDIYA and SHIU 2007).

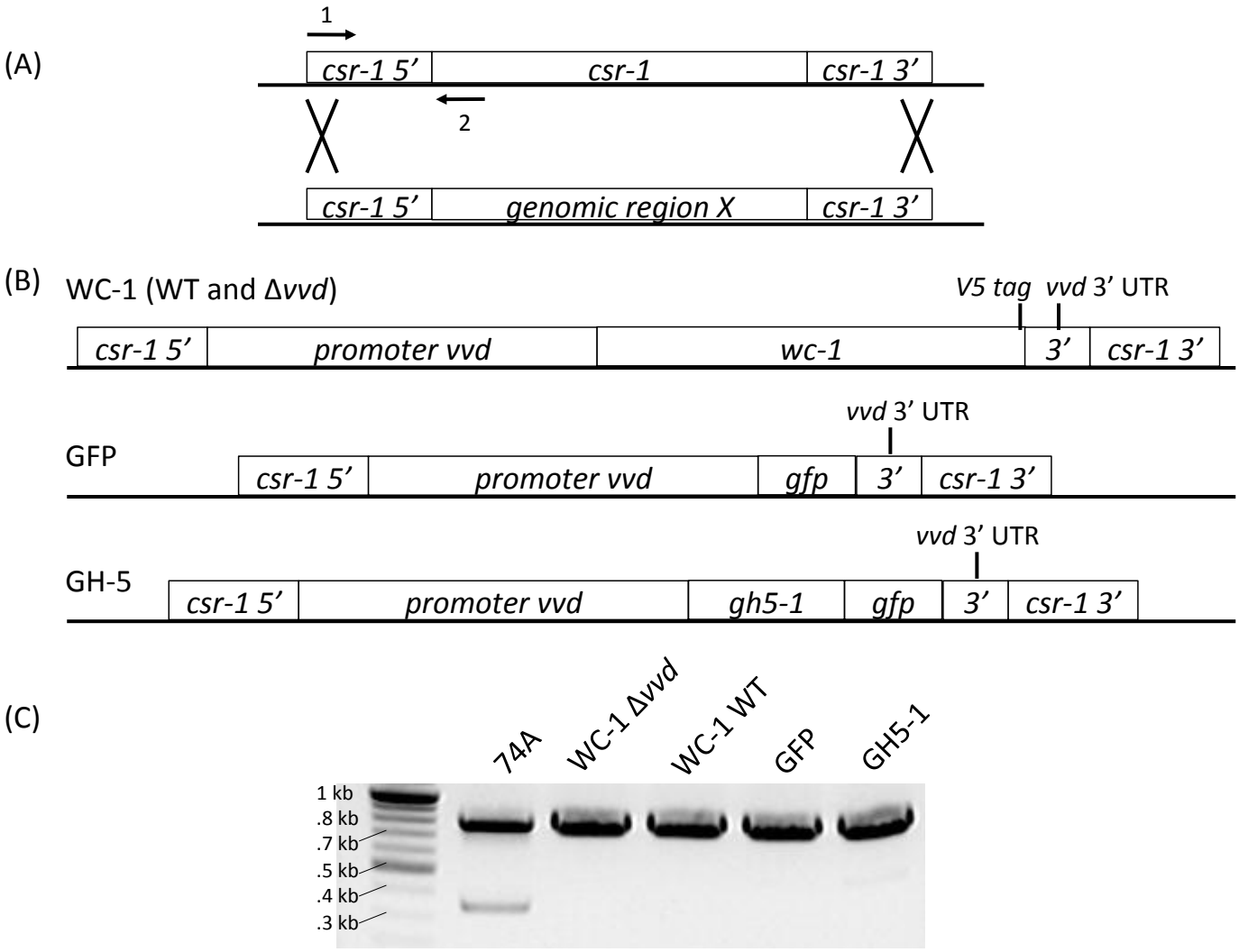

Figure S1 Constructs used to examine *vvd* promoter-driven gene expression. (**A**) Constructs bearing either *wc-1, gfp* or *gh5-1* were transformed into a WT or *Δvvd* strain at the *csr-1* locus (BARDIYA and SHIU 2007). The arrows represent primers used in the PCR genotyping analysis in (**B**). Representation of constructs designed to test expression from the *vvd* promoter. Cassette containing *gh5-1* is based off of the design from Sun et. al. using the *vvd* promoter in place of the *ccg-1* promoter (SUN *et al.* 2011). (**C**) Genomic DNA samples from *wt*, *Δvvd wc-1*^{v5}, *wt wc-1*^{v5}, *gfp* and *gh5-1* strains were subjected to PCR analysis to examine the *csr-1* locus. Bands at 350 bp represent the presence of the *csr-1* locus while bands at 750 bp represent the presence of the *actin* locus (+ control).

BARDIYA, N., and P. K. SHIU, 2007 Cyclosporin A-resistance based gene placement system for *Neurospora crassa*. Fungal Genet Biol **44:** 307-314.

SUN, J., C. M. PHILLIPS, C. T. ANDERSON, W. T. BEESON, M. A. MARLETTA *et al.*, 2011 Expression and characterization of the *Neurospora crassa* endoglucanase GH5-1. Protein Expr Purif **75:** 147-154.

Table S1 Vectors and Primers

PVVD-GH-5:

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTG
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PVVD-WC-1:
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PRIMERS:
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WC-1 REVERSE
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