Supplementary material

Plant host and microbe specific gene expression in the mutualistic root endophytic fungus *Serendipita vermifera*

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Supplementary methods

Cloning: To clone *SvCHIT-CBM5 and SiCHIT-CBM5* without signal peptide into the empty vector (Fig. S5) for expression in *E. coli*, both sequences were amplified from cDNA using the program and primers listed in Table S1+2. PCR products run on 1 % agarose gels. Bands were extracted and purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel). The concentration of the PCR product was adjusted to a three-fold molar excess of plasmid. The plasmid was linearized with restriction enzyme KpnI and cloning was performed via Gibson Assembly (Table S3). The reactions incubated at 50 °C for 15 min. The resultant Plasmids as pictured in Fig. S6+7.

Table S1: PCR program for the amplification of *Sv*CHIT-CBM5 and *Si*CHIT-CBM5 including the individual steps, temperature, and duration

Step	Temperature (°C)	Time (mm:ss)
Initial denaturation	95	00:30
Denaturation	95	00:20
Primer annealing	62	00:20
Extension	72	00:30 per Kb
Final extension	72	02:00
Hold	4	~

Table S2: Primer list for the amplification of *Sv*CHIT-CBM5 and *Si*CHIT-CBM5 listing all primer-names and sequences

Name	Sequence
*Sv*CHIT-CBM5 (Forward)	ATCACCATCACCATCACGGATCCGCATGCG AGCTCGGTACCACTCCCGTCATGCAACGAC
*Sv*CHIT -CBM5 (Reverse)	CTCAGCTAATTAAGCTTGGCTGCAGGTCGA CCCGGGGTACCTCAGCAGGCTCGCACCT
*Si*CHIT -CBM5 (Forward)	ATCACCATCACCATCACGGATCCGCATGCG AGCTCGGTACCACGCCCGCCATGATGC
*Si*CHIT-CBM5 (Reverse)	CTCAGCTAATTAAGCTTGGCTGCAGGTCGA CCCGGGGTACCTCAGCACGACTTGAGTTGA TTCCAC

Table S3: Gibson Assembly mix showing the components and amounts, that vary depending on the Plasmid concentration and fragment length and was calculated via the formula below.

Component	Amount
Plasmid	X µL
PCR fragment	Y µL
2 x Gibson Master Mix	10 µL
Deionized H_2O	10 – (X+Y) µL

$$\frac{Plasmid\ concentration\ in\ ng/\mu L * 1000}{length\ of\ fragment\ in\ bp * 650\ kDa} = pmol/\mu L$$

Transformation of *E. coli*: 10 µL plasmid were added to competent *E. coli* cells (Mach1) and incubated for 20 min on ice. Plasmid uptake was stimulated by a 45 sec incubation at 42 °C and subsequent incubation on ice for 2 min. The cells regenerated in 500 µL LS-LB medium at 37 °C for at least 30 min under continuous shaking, where then centrifuged for 30 sec at 11,000 rpm. The cells were resuspended in 20 µL LS-LB medium, plated on carbenicillin containing LS-LB agar plates and incubated at 37 °C overnight. Colony PCR components and program are listed in Table S4.

Table S4: Colony PCR Components

Component	**Amount for 25 µL reaction**
10 x Thermo pol buffer	2.5 µL
10 mM dNTPs	0.5 µL
Forward primer	0.5 µL
Reverse primer	0.5 µL
Taq DNA polymerase	0.125 µL
H_2O	To 25 µL

Step	**Temperature (°C)**	**Time (mm:ss)**
Initial denaturation	98	00:30
Denaturation	98	00:30
Primer annealing	45-68	00:30
Extension	68	01:00 per kB
Final extension	68	05:00
Hold	4	~

PCR products were run on 1 % agarose gels to confirm successful transformation of cloned plasmids.

Mini- and Maxi plasmid DNA preparation: Mini- and Maxi plasmid DNA preparations were performed from either 4 mL or 300 mL bacteria cultures grown in LS-LB medium containing the appropriate antibiotics after incubation at 37°C and 180 rpm using either the NucleoSpin^{R} Plasmid kit (Macherey & Nagel) or the NucleoBond^{R} Xtra Maxi kit (Macherey & Nagel). Plasmid concentration was measured via nanodrop.

SDS-PAGE and Western Blot: *E. coli* crude extracts were measured by Bradford assay and equal protein concentrations were adjusted. The supernatant was boiled in SDS loading dye for 5 min at 95 °C. 20 µL of sample were loaded on 10 x SDS-Gels and run at 190 V for 50 min. Proteins were blotted on PVDF membranes with transfer buffer for 1 h at 17 V. The membrane was blocked in TBS-T-BSA overnight at 4 °C and next the HisProbe-HRP conjugate was added (1:20000 dilution) was added in TBS-T-BSA and incubated 1 h at RT. Next, the membrane was washed 3 times in TBS-T and 3 times in TBS (each washing step 5 min). Developing solution was added to detect chemiluminescence

Supplementary figures

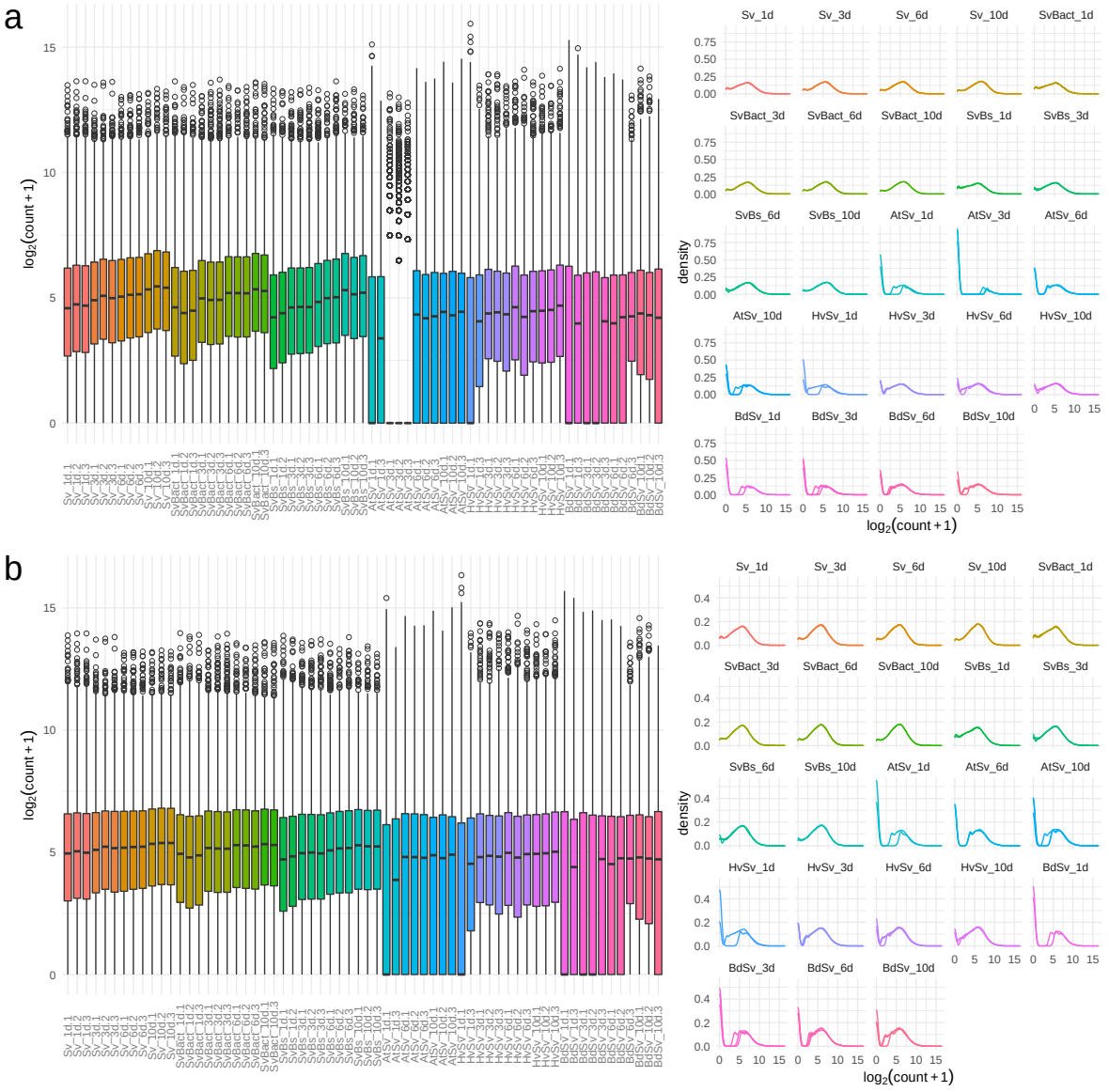


Figure S1: ***Distribution and density of the normalised log2 transformed read counts.*** *At: Arabidopsis thaliana*; *Bd: Brachypodium distachyon*; *Hv: Hordeum vulgare*; *Sv: Serendipita vermifera*; *Bs: Bipolaris sorokiniana*; Bact: bacteria; d: days a) Before removal of the inconsistent samles *AtSv*_3d. b) After removal of *AtSv*_3d. A set of data excluding *AtSv*_3d was used for co-expression and DEG analyses.

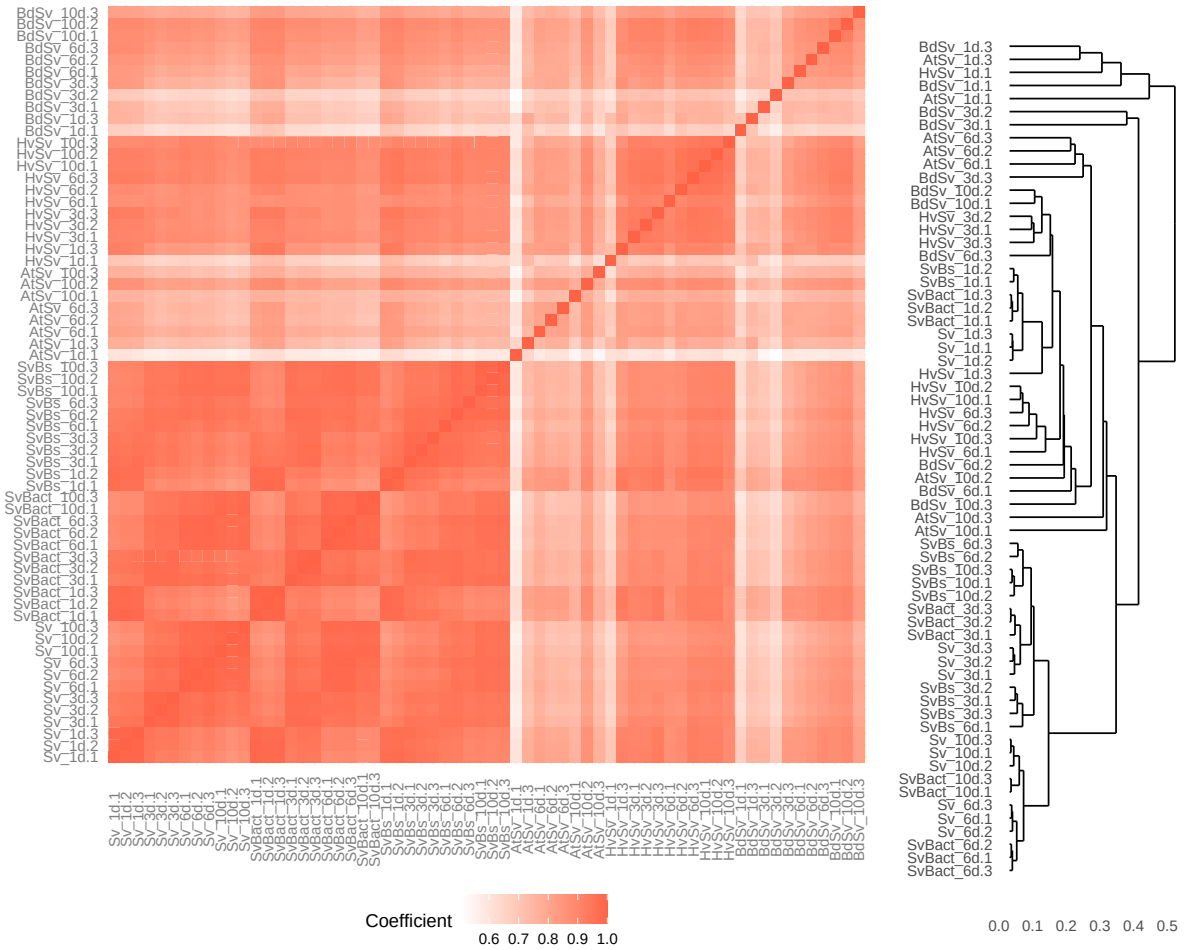


Figure S2: Correlations of RNA-seq Samples. Left part: Adjacent patrix of the Spearman correlation coefficients. Right part: Hierarchical clustering of biological replicates based on distances of transcriptomic similarities. *At: Arabidopsis thaliana; Bd: Brachypodium distachyon; Hv: Hordeum vulgare; Sv: Serendipita vermifera; Bs: Bipolaris sorokiniana*; Bact: bacteria; d: days.

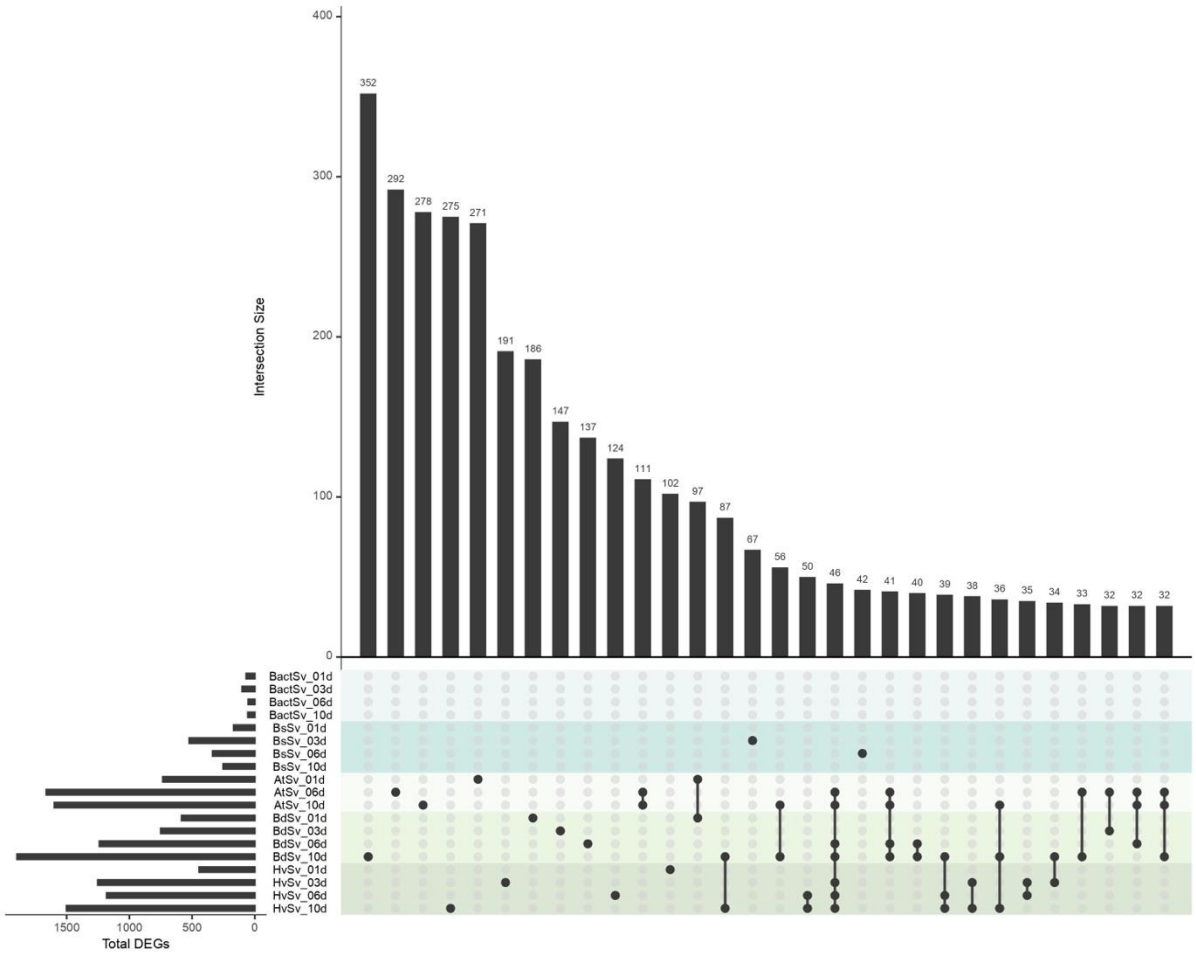


Figure S3: Differentially expressed genes (DEGs) of *Sv* in response to different interaction partners and timepoints. UpSetplot of significantly differentially expressed genes (DEGs) (FDR-adjusted $p < 0.05$) compared to control (fungus alone). Dotted lines indicate a combination of conditions. *At: Arabidopsis thaliana*; *Bd: Brachypodium distachyon*; *Hv: Hordeum vulgare*; *Sv: Serendipita vermifera*; *Bs: Bipolaris sorokiniana*; Bact: bacteria; dpi: days post inoculation

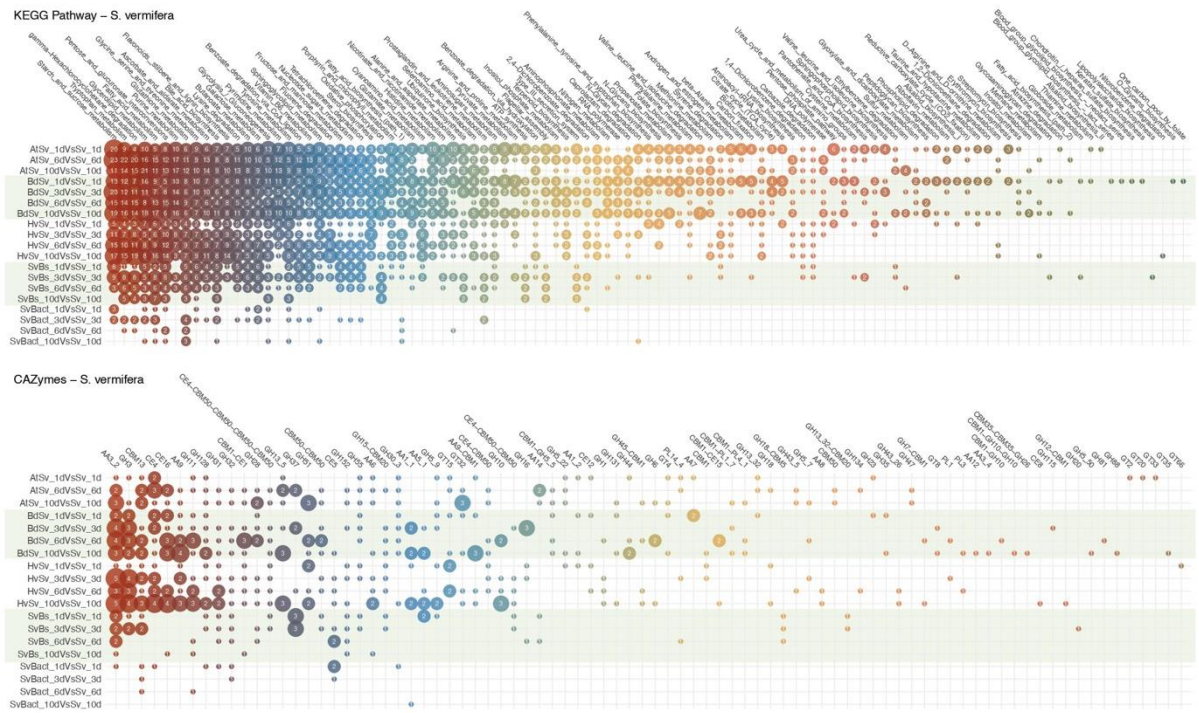
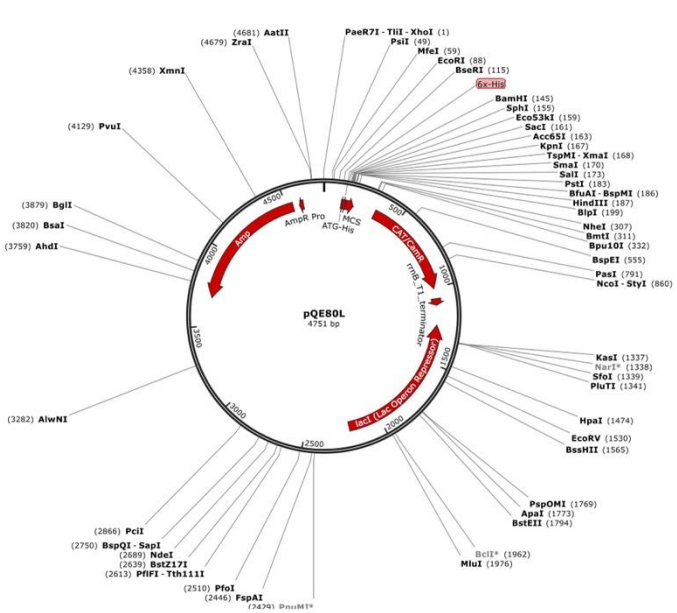
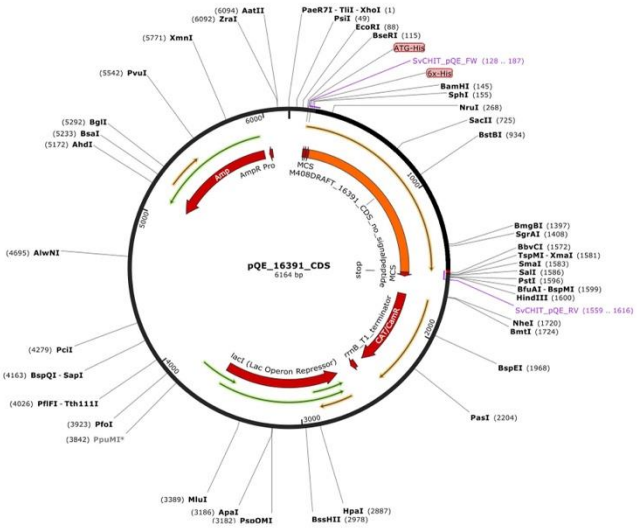


Figure S4: Feature count of *Sv* upregulated genes (>2 log2 FC) in dipartite interactions that are associated with KEGG pathways (upper graph) or that contain a CAZyme domain (lower graph) per timepoint. *At: Arabidopsis thaliana. Bd: Brachypodium distachyon. Hv: Hordeum vulgare. Sv: Serendipita vermifera. Bs: Bipolaris sorokiniana.* Bact: *bacterial SynCom*



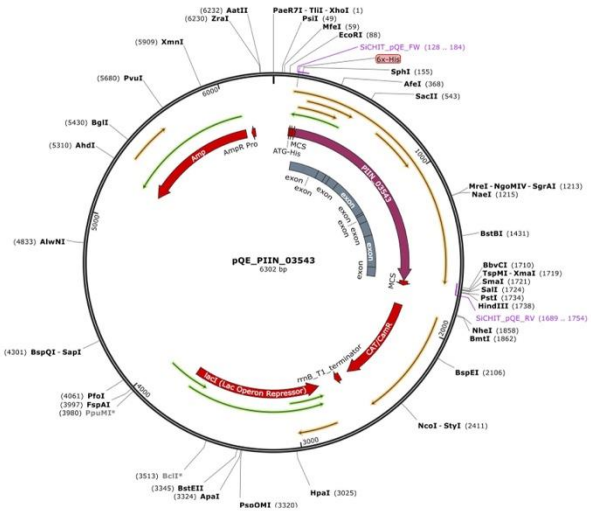
PQE80L-Empty

Figure S5: Empty vector plasmid for the expression in *E. coli*



PQE80L-*Sv*CHIT-CBM5

Figure S6: Plasmid for the expression of *Sv*CHIT-CBM5 in *E. coli*



PQE80L-*Si*CHIT-CBM5

Figure S7: Plasmid for the expression of *Si*CHIT-CBM5 in *E. coli*