

Figure S1 Vasculature of plants infected with the *E. festucae* $\Delta sakA$ mutant

A. Light micrographs of perennial ryegrass blade tissue showing branching between the vasculature of plants infected with wild-type *E. festucae* (WT) and the $\Delta sakA$ mutant. Bar = 100 µm. **B**. Quantification of vasculature branching in plants infected with the WT and $\Delta sakA$ mutant strains. 1 cm sections of blade tissue were taken from multiple tillers on multiple plants and the number of branches within each section counted. n = 24 and 22 for the WT and $\Delta sakA$ strains respectively. Statistical significance in comparison to the WT strain was determined using the Student's *t*-test (*t* = 8.23, df = 39.4 and *p* = 4.3 x 10⁻¹⁰).



Figure S2 Fluorescence micrographs of FM4-64 stained *E. festucae* vacuoles FM4-64 selectively stains the vacuolar membrane red and can be observed by fluorescence microscopy. No difference is seen between vacuoles at the hyphal tips in wild-type (WT) or the $\Delta sakA$ mutant. Vacuoles in regions distant from the tip are highly variable in size but no obvious difference is observed between WT and the $\Delta sakA$ mutant. Bar = 5 µm.



Figure S3 Differentially expressed fungal and plant genes organised by catalytic activity ontology

Organisation of *E. festucae* (**A**) and *L. perenne* genes (**B**) differentially expressed between the wild-type and $\Delta sakA$ mutant-infected samples by catalytic activity GO category. Bars show the number of genes within each category that are up- or downregulated in the $\Delta sakA$ mutant-infected sample relative to the WT-infected sample. Categories are: cyclase activity (GO:0009975), deaminase activity (GO:0019239), demethylase activity (GO:0032451), glycogen debranching enzyme (GDE) activity (GO:0004133), hydrolase activity (GO:0016787), isomerase activity (GO:0016853), ligase activity (GO:0016874), lipoic acid (LA) synthase activity (GO:0017140), lyase activity (GO:0016829), non-ribosomal peptide synthetase activity (NRPS; non-GO category), Mo-molybdopterin cofactor sulfurase activity (Mo sulfurase; GO:0008265), Mo-molybdopterin synthase activity (Mo synthase; GO:0030366), oxidoreductase activity (oxidoR; GO:0016491), polyketide synthase activity (PKS; GO:0016740).



Differential expression of fungal and plant oxidoreductase genes Figure S4 Organisation of *E. festucae* (A) and *L. perenne* oxidoreductase genes (B) differentially expressed between the wild-type and $\Delta sakA$ mutant-infected samples by GO category. Bars show the number of genes within each category that are up- or down-regulated in the $\Delta sakA$ mutant-infected sample relative to the WT-infected sample. Categories are oxidoreductase activity acting on: aldehyde or oxo groups of donors (GO:0016903), CH or CH2 groups (GO:0016725), CH-CH group of donors (GO:0016627), CH-NH group of donors (GO:0016645), CH-NH2 group of donors (GO:0016638), CH-OH group of donors (GO:0016614), diphenols and related substances as donors (GO:0016679), iron-sulfur proteins as donors (Fe-S; GO:0016730), oxidising metal ions (GO:0016722), NADH or NADPH (GO:0016651), other nitrogenous compounds as donors (N-groups; GO:0016661), paired donors with incorporation or reduction of molecular oxygen (GO:0016705), single donors with incorporation of molecular oxygen (GO:0016701), sulfur group of donors (S-groups; GO:0016667), superoxide radicals as acceptor (GO:0016721), dioxygenase activity (GO:0051213), fatty acid alpha-hydroxylase activity (FAH; GO:0080132), monooxygenase activity (MO; GO:0004497), phytanoyl-CoA dioxygenase activity (PHYD; GO:0016705) and unclassified oxidoreductase activity.



Figure S5 Differential expression of fungal and plant transferase genes Organisation of *E. festucae* (A) and *L. perenne* transferase genes (B) differentially expressed between the wild-type and $\Delta sakA$ mutant-infected samples by GO category. Bars show the number of genes within each category that are up- or down-regulated in the $\Delta sakA$ mutant-infected sample relative to the WT-infected sample. Categories are transferase activity transferring: one-carbon groups (GO:0016741), acyl groups (GO:0016746), aldehyde or ketonic groups (AH/ketonic; GO:0016744), alkyl or aryl (other than methyl) groups (GO:0016765), glycosyl groups (GO:0016757), nitrogenous groups (N-groups; GO:0016769), phosphorus-containing groups (P-groups; GO:0016772), and sulfur-containing groups (S-groups; GO:0016782).



Figure S6 Putative gene clusters down-regulated in Δ *sakA* **mutant association A**. Fold changes in expression of genes in a putative nitrogen metabolism cluster displaying significantly reduced expression in Δ *sakA* mutant-infected sample relative to the wild-type strain-infected sample. GTP, GTP-binding protein; Hyp, hypothetical protein; GOOX, gluco-oligosaccharide oxidase; MFS, MFS amine transporter; NmrA, NmrA-like transcriptional regulator; NRPS, non-ribosomal peptide synthetase. Where the difference in expression between the two samples is non-significant (fold change <1 or p<0.05), the fold change is displayed as 0. **B**. Fold changes in expression of genes in a putative Nc25-associated cluster displaying significantly reduced expression in Δ *sakA* mutant-infected sample relative to the wild-type strain-infected sample. EF200-EF202, hypothetical proteins; Nc25, highly expressed novel endophyte gene; *kexB*, kexin-encoding gene.



Figure S7 Putative gene clusters up-regulated in $\Delta sakA$ mutant association A. Fold changes in expression of genes in a putative sugar metabolism cluster displaying significantly increased expression in $\Delta sakA$ mutant-infected sample relative to the wild-type strain-infected sample. TF, transcription factor; GL, glucosidase; MFS, major facilitator superfamily sugar transporter; DHO, dihydroorotase; Hyp, hypothetical protein; P450, cytochrome P450 monooxygenase. Where the difference in expression between the two samples is non-significant (fold change<1 or p<0.05), the fold change is displayed as 0. B. Fold changes in expression of genes in a putative cell integrity-associated cluster displaying significantly increased expression in $\Delta sakA$ mutant-infected sample relative to the wild-type strain-infected sample. RlmA, RlmA-like transcription factor; CAP, cell adhesion protein; FR, fumarate reductase; EfU, E. festucae unique gene; MFS, major facilitator superfamily monosaccharide transporter. C. Fold changes in expression of genes in a putative digestive cluster displaying significantly increased expression in $\Delta sakA$ mutant-infected sample relative to the wild-type strain-infected sample. AP, aminopeptidase; EL, extracellular lipase; EfU, *E. festucae* unique gene; MP, membrane protein; Hyp, hypothetical protein.



Figure S8 Differential expression of perennial ryegrass hydrolase genes Organisation of *L. perenne* hydrolase genes differentially expressed between the wild-type and $\Delta sakA$ mutant-infected samples by GO category. Bars show the number of genes within each category that are up- or down-regulated in the $\Delta sakA$ mutantinfected sample relative to the WT-infected sample. Categories are: hydrolases acting on acid anhydrides (GO:0016817), acid halide bonds (GO:0016824), carbon-nitrogen (but not peptide) bonds (C-N; GO:0016810), carbon-sulfur bonds (C-S; GO:0046508), ester bonds (GO:0016788), ether bonds (GO:0016801), glycosyl bonds (GO:0016798); peptidase activity (GO:0008233); deacetylase activity (GO:0019213) and unclassified hydrolase activity.