Supplementary Data Set S12. Comparison of Lp19 endophyte effects on the grass transcriptome with Fl1 endophyte effects reported by Dupont et al. (Dupont et al., 2015)

Dupont et al. (Dupont et al., 2015) assessed the effect of the endophyte Fl1 on *the L. perenne* transcriptome by mapping reads to a set of 42,083 representative *L. perenne* ORFs, extracted from the same de novo assembly used in our work, using DEGseq to identify statistically significant (FDR q <0.05) changes, but considering only ≥2 fold changes in gene expression to be biologically significant. They inferred plant-wide effects from the analysis of "pseudostem" samples, a mixture of LES and sheaths.

To compare our results with theirs we initially identified, in select categories from their Supplementary Tables, ORFs for which our read distributions indicated a significant (FDR q <0.05) Lp19 endophyte effect in one or several tissues. If the expression change, averaged across all these significant effects, was more than 2-fold, we considered the effect to meet Dupont and coworkers' criteria of a biologically significant effect. If such an effect indicated Lp19-induced upregulation, and if Fl1-induced ≥2-fold upregulation had been reported by Dupont et al., we considered the Lp19 effect to be consistent with the Fl1 effect. If the effect indicated Lp19-induced ≥ 2-fold downregulation and Fl1-induced ≥ 2-fold upregulation had been reported by Dupont et al. (or vice versa) we considered the Lp19 effect to be opposite to the Fl1 effect.

Based on these criteria, Lp19 affected fewer ORFs than Fl1 and there were about as many effects common to both as there were opposing effects (Supplementary Table S3).

Using the same approach and criteria, we also carried out a comparison for all ORFs for which Dupont et al. (Dupont et al., 2015) reported significant Fl1 endophyte effects and assessed how often Lp19 induced either the same or opposite effects (Supplementary Table S4). Only for a small percentage of ORFs for which expression was affected by Fl1 was it also affected by Lp19 - and if so, opposing effects were almost as common as instances in which both endophytes increased or both decreased expression. In addition, using the above criteria, Lp19 affected 2/3 fewer ORFs than Fl1. We also tested if the correlation between Lp19 effects and Fl1 effects would improve if we only considered ORFs for which a significant (FDR P <0.05) ≥2-fold Lp19 endophyte effect occurred in all 7 tissues. It did in that consistent effects outnumbered opposing effects 10:1. However, only 0.6% of Fl1 effects had matching LP19 effects observable in all tissues (Supplementary Table S4).

Not all of our 42 (3 x 2 x 7) tissue samples were sequenced as deeply as the four (2 x 2) samples analyzed by Dupont et al. (Dupont et al., 2015). In addition in this comparative analysis we only considered read counts against the representative ORFs used for mapping by Dupont et al. This would not explain why, if an ORF was affected by both Fl1 and Lp19, opposing effects were almost as common as instances in which both endophytes increased or both decreased expression. However, lower

depth of sequencing in this work could explain why Lp19 appeared to affect fewer plant ORFs than Fl1.

We therefore investigated how strongly the depth of sequencing might have affected our ability to reproduce effects reported by Dupont et al. (Dupont et al., 2015). There was no indication that the number of reads we mapped to ORFs affected by Fl1 infection was a strong determinant of the probability of finding Lp19 effects meeting Dupont et al.'s criteria for significance (FDR q <0.05; ≥ 2x change) in such ORFs (Supplementary Fig. S5): The frequency of such Lp19 effects in Fl1-affected ORFs was highest if we had mapped 801- 1600 reads to these ORFs. Across all Fl1-affected ORFs to which we mapped ≥ 801 reads the probability of finding Lp19 effects was 17%. Only below 200 reads per ORF did our ability to detect an Lp19 effect fall below this average, and only to 24% of ORFs with Fl1 effects (FDR P <0.05; ≥ 2x change) had we mapped fewer than 200 reads.

Thus our ability to detect Lp19 effects (FDR P <0.05; ≥ 2x effect) should have been largely unaffected in the remaining 76% of ORFs for which Fl1 effects had been reported by Dupont and coworkers.

Literature Cited

Dupont, P.-Y., Eaton, C.J., Wargent, J.J., Fechtner, S., Solomon, P., Schmid, J., Day, R.C., Scott, B., and Cox, M.P. 2015. Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. New Phytol. 208:1227–1240.