



Figure S1. Selected mutants with symbiotic phenotype. Representative images of the *LORE1* mutants selected from the screening showing a Nod-, Fix- and hypernodulation phenotypes at 6 wpi with IRBG74. Scale, 1 cm. Images obtained at the same magnification.



Figure S2. Delayed and reduced nodulation in novel *LORE1* mutants. **A-E**, Number of pink nodules in Gifu and 20 *LORE1* mutants at 2-6 wpi with *M. loti* and IRBG74. Violin boxplots: center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range; points, individual data points. **F**, Shoot length in Gifu and 20 *LORE1* mutant at 6 wpi with *M. loti* and IRBG74. The number of plants tested is shown below the violin graphs.



Figure S3. Root length of 20 *LORE1* lines inoculated with IRBG74. Violin dot plots showing the root length at 6 wpi with IRBG74. Center line, median; box limits, upper and lower quartiles; whiskers, $1.5 \times$ interquartile range; points, individual data points. The number of tested plants is shown below the violin graphs. * *P*<0.05; ***P*<0.01; and *** *P*<0.001 Mann–Whitney *U*-test.



Figure S4. Shoot length of 20 LORE1 lines in nitrogen-repleted conditions. Violin dot plots showing the shoot length at 5 wpg of the selected mutants grown on plates with optimal nitrogen supply. Center line, median; box limits, upper and lower quartiles; whiskers, $1.5 \times$ interquartile range; points, individual data points. The number of tested plants is shown below the violin graphs. * *P*<0.05; ***P*<0.01; and *** *P*<0.001 Mann–Whitney *U*-test.



Figure S5. Heatmap expression of genes affected by *LORE1* insertions in the mutant collection. Expression pattern of the genes that showed *LORE1* insertions in 47 mutants selected in this study. The data was extracted from the Lotus Expression Atlas <u>https://lotus.au.dk/expat/</u>.





Quiescent center
 Trichoblasts
 Stele



Cortex
Atrichoblasts
Endodermis

Root tip Meristem Phloem

ATG9 LotjaGi5g1v0179800 0 1 2 3

Figure S6. Expression profile of sequences disrupted by *LORE1* elements. **A**, Heatmap of normalized expression of genes affected by *LORE1* insertions in the IM-S34 mutant. Data extracted from the Lotus Expression Atlas <u>https://lotus.au.dk/expat/</u>. **B**, UMAPs of *ATG9* expression in different root tissues at 10 dpi with *M. loti* and mock-treated (control). Collected from *Lotus japonicus* root single-cell database (Frank et al., 2023).