



[A] Rarefaction curves of each sample. **[B]** Numbers of observed ASVs in the different compartments. **[C]** Numbers of estimated ASVs based on the Chao1 estimator. **[D]** Shannon index of the microbe diversity. The analysis was based on the table of normalized absolute abundance of ASVs. Letters denote statistical significance (p < 0.05, Turkey).



Decreased in *ibm1* (FDR< 0.05)

Figure S2. IBM1 dysfunction alters plant-associated microbiome (Related to Figure 1).

[A] The PCoA analysis of all ASVs separates the *ibm1* mutants from the wild type plants (Col-0) within the rhizosphere compartment. **[B]** Relative abundance (RA) of the top 10 bacteria phyla detected within the bulk soil (Soil), rhizosphere, and endosphere compartments. Statistical significance (FDR < 0.05) of phylum RA between the mutant and the wild type plants is indicated by asterisks in the table.





Enriched in *ibm1* (FDR< 0.05) Decreased in *ibm1* (FDR< 0.05)

Figure S3. Taxonomic structure of ASVs (Amplicon Sequence Variants) reveals the impacts of IBM1 dysfunction on root microbiome at family level (Related to Figure 1).

[A] Relative abundance (RA) of the top 20 bacteria families detected within the bulk soil (Soil), rhizosphere, and endosphere compartments. **[B]** A table summarizing statistical significance (FDR < 0.05 as indicated by asterisks) between the mutant and the wild type plants of family RA as showed in panel A.







Figure S5. IBM1 dysfunction activates defense-related responses in soil-grown Arabidopsis (Related to Figure 2).

[A] Gene expression levels of *EDS5*, *PAD4*, *PR1* and *PR5* in 2-week-old Col-0, *ibm1-1* and *ibm1-4* plants. Results of RT-qPCR are shown. Mean \pm SE, n = 3 biological replicates. * and ** indicate *p* < 0.05 and *p* < 0.01, respectively (Student's t-test). **[B]** A heatmap of DEGs (FC > 2, FDR < 0.05) involved in systemic acquired resistance (SAR). **[C]** A heatmap of DEGs involved in the flavonoid biosynthesis process.



Figure S6. IBM1 dysfunction causes autoimmunity in Arabidopsis grown in sterile medium (Related to Figure 2).

The differentially expressed genes (DEGs; fold change \geq 2, FDR \leq 0.05) that were up-regulated **[A]** and down-regulated **[B]** in *ibm1-1* compared to Col-0 were subject to the Gene Ontology (GO) analysis. The chord diagrams show the GO terms that link to their sub-classifications. The sub-

classifications are labeled with GO ID that can be queried together with their corresponding DEGs in Dataset S2 (Sheet 9 and 11). **[C]** A heatmap of DEGs involved in SA signaling or biosynthesis. **[D]** Gene expression levels of *PR1*, *PR4*, *PR5*, and *WRKY60* in 7-day-old Col-0 and *ibm1-1* plants grown in sterile half-strength MS medium. Results of RT-qPCR are shown. Mean \pm SE, n = 3 technical replicates. Two biological replicates were analyzed with similar results. ****** indicate p < 0.01 (Student's t-test). **[E]** A heatmap of DEGs involved in systemic acquired resistance (SAR). **[F]** A heatmap of DEGs involved in the flavonoid biosynthesis process. **[G]** A heatmap of DEGs involved in phytoalexin biosynthesis.





[A] IBM1 dysfunction decreases H3K9me2 level at the gene promoter region of *SIGMA FACTOR BINDING PROTEIN1* (*SIB1*) and increases the mRNA level of *SIB1*. Snapshots from ChIP-seq and RNA-seq are shown. The original ChIP-seq data were downloaded from DDBJ (DRA005154) as generated previously (24). The red box indicates the region with altered H3K9me2 levels. **[B]** IBM1 dysfunction increases H3K4me3 level at the gene body region of *DHYPRP1* and increases the mRNA level of *DHYPRP1*. **[C]** IBM1 dysfunction increases H3K4me3 level at the gene body region of *CRK45* and increases the mRNA level of *CRK45*.





[A] The composition of the hyper methylated and hypo methylated DMRs (differentially methylated regions) caused by IBM1 dysfunction. **[B]** The composition of differentially methylated cytosines in the CG, CHG, and CHH contexts. **[C]** IBM1 dysfunction decreases DNA methylation level at the promoter region of *RMG1* and increases the mRNA level of *RMG1*. Snapshots from whole genome bisulfite sequencing and RNA-seq are shown. The red box indicates the gene promoter region with altered CHH methylation levels. **[D]** IBM1 dysfunction decreases DNA methylation level at the promoter region of *WAKL10* and increases the mRNA level of *WAKL10*.



Figure S9. IBM1 dysfunction alters flg22-induced plant immune responses (Related to Figure 4). [A] Gene expression levels of the PTI marker gene *FRK1*. Seven-day-old *ibm1* mutants and wild type (Col-0) plants were dip-inoculated with 100 nM flg22 and harvested at the indicated time points. Results of RT-qPCR are shown. Mean \pm SE, n = 3 technical replicates. Two biological replicates were analyzed with similar results. ** indicate *p* < 0.01 (Student's t-test). [B] Measurements of ROS burst induced by flg22. Leaf discs from four-week-old plants elicited with 50 nM flg22. Mean \pm SE, n = 3 biological replicates, each consisting of 16 leaf discs.



Figure S10. Dysfunction of IBM1 and activation of defense impair plant growth-promotion triggered by GMVs (Related to Figure 4).

[A] IBM1 dysfunction impairs plant growth-promotion triggered by GB03-produced microbial volatiles (GMVs); the impairment can be rescued by a second mutation of *kyp* in the *ibm1* mutant. Images were taken at 7 days after treatment (DAT). Red-dotted lines indicate inner plastic partitions that divide the plate into four parts. **[B]** Quantification of total leaf area per seedling (TLA) of the plants at 7 DAT. Mean \pm SE, n = 15. All fold changes are associated with statistical significance of *p* < 0.01 (Student's t-test). **[C]** Exogenous application of SA and/or JA mimicked *ibm1* mutations in impairing GMV-triggered plant growth-promotion. Images were taken at 7 days after treatment (DAT). **[D]** Quantification of TLA of the SA/JA-treated plants at 7 DAT. Mean \pm SE, n = 15. All fold changes are associated with statistical significance of *p* < 0.01 (Student's t-test).



Figure S11. IBM1 has stronger impacts on plant-microbe interactions than the RNA-directed DNA methylation (RdDM) pathway (Related to Figure 4).

[A] IBM1 dysfunction (*ibm1-1*), but not defective RdDM (*nrpd1-3* and *nrpe1-11*), induces gene expression of all the examined defense regulators. Results of RT-qPCR are shown. Mean \pm SE, n = 3 biological replicates. * and ** indicate p < 0.05 and p < 0.01, respectively (Student's t-test). **[B]** The RdDM mutants *nrpd1-3* and *nrpe1-11* showed similar plant growth-promotion as wild type plants (Col-0) in response to GMVs. Images were taken at 6 days after treatment (DAT). Red-dotted lines indicate inner plastic partitions that divide the plate into three parts. **[C]** Quantification of total leaf area per seedling (TLA) of the plants at 6 DAT. Mean \pm SE, n = 15 seedlings in three biological replicates. All fold changes are associated with statistical significance of p < 0.01 (Student's t-test).