Fig. S1



Fig. S1. Mutant strains are confirmed by diagnostic PCR. All of PHA-related genes are deleted from the genome of wild-type symbiont *Burkholderia*. Red arrows indicate DNA band of each wild-type and mutants.



Fig. S2. Genomic information of PHArelated genes in *Burkholderia* RPE75. (A) Gene loci of PHA-related genes in *Burkholderia* RPE75. (B) Domain analysis of PHA-related genes. All PhaP family proteins have Phasin2 domain,

PhaR has one DNA-binding domain with two PHB accumulation regulatory domain, and PhaC has PHB polymerase domain.



Fig. S3. Phylogenetic analysis of Phasin family proteins identified from *Burkholderia* sp. RPE75 and its othologs. The amino acid sequences of Phasin family proteins were obtained from GenBank (<u>https://www.ncbi.nlm.nih.gov/</u>) and the phylogenetic analysis was performed by Clustal X2 and MEGA7 programs: (A) PhaP family proteins, (B) PhaR proteins, and (C) PhaC proteins.



Fig. S4. *In vitro* PHA production by wild-type and PHA-related gene-depleted mutants of the symbiont *Burkholderia*. (Fig. S3-i-viii) Images of PHA-derived fluorescence intensities under the fluorescence microscope. Phase-contrast images (left) and fluorescent images (right) of *Burkholderia* cells cultured in YG medium are

stained with Nile blue A: (i) Wild-type, (ii) $\Delta phaP1$, (iii) $\Delta phaP2$, (iv) $\Delta phaP3$, (v) $\Delta phaP4$, (vi) $\Delta phaR$, (vii) $\Delta phaC$, and (viii) $\Delta phaR/phaR$ complemented *Burkholderia* (Scale bars, 0.5 µm).



Fig. S5. Bacterial titers of wild-type and mutant *Burkholderia* symbionts colonized in the M4 region of the third instar (A) and fourth instar nymph (B).

Fig. S6

(A)







Fig. S6. In vivo PHA production by wild-type and PHA-related genesdepleted mutants of the symbiont *Burkholderia* colonized in the midgut of adult female host insects. (A) Images of PHA-derived fluorescence intensities under the fluorescence microscope. Phase-contrast images (left) and fluorescent images (right) of *Burkholderia* cells: (A-i) Wild-type, (Aii) $\Delta phaP1$, (A-iii) $\Delta phaP2$, (A-iv) $\Delta phaP3$, (A-v) $\Delta phaP4$, (A-vi) $\Delta phaR$ and (A-vii) $\Delta phaC$ and (A-viii) $\Delta phaR/phaR$ complemented *Burkholderia*. (B) Flow cytometric histograms of PHA-derived fluorescence from *Burkholderia* cells. The color and name of lines are shown on the left. The

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shifted lines are indicated using arrows (green: $\Delta phaP3$, blue: $\Delta phaR$ and

