Supplementary Material:

Increased flexibility in the use of exogenous lipoic acid by *Staphylococcus aureus*

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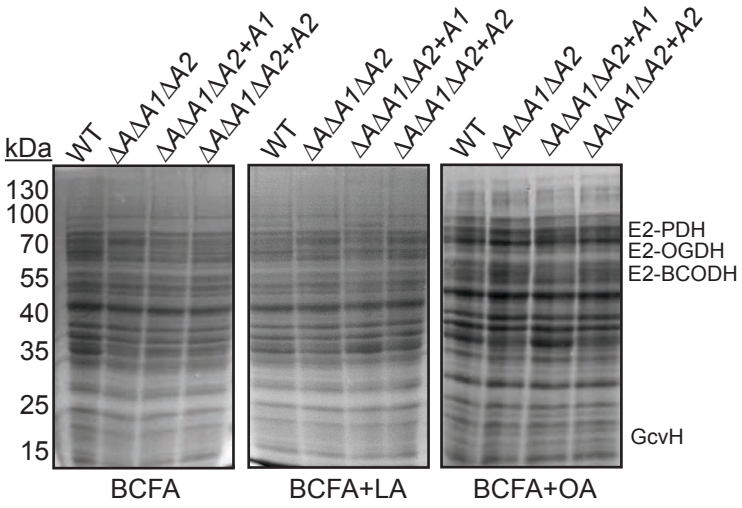

Figure S1. Loading controls related to Figure 2. Coomassie blue stained SDS-PAGE gels of whole cell lysates derived from the indicated strains grown in RPMI supplemented with branched chain carboxylic acids (10 mM isobutyric acid, 9 mM 2-methylbutyric acid, 9 mM isovaleric acid, and 10 mM sodium acetate - BCFA) in order to bypass the requirement of lipoic acid for replication with or without 5 μM lipoic acid (LA) and 150 μM octanoic acid (OA). Strain designations are as follows: Wildtype (WT), Δ*lipA* Δ*lplA1* Δ*lplA2* (Δ*A* Δ*A1* Δ*A2*), Δ*lipA* Δ*lplA1* Δ *lplA2* + *lplA1* (Δ*A* Δ*A1* Δ*A2* + *A1*), and Δ*lipA* Δ*lplA1* Δ*lplA2* + *lplA2* (Δ*A* Δ*A1* Δ*A2* + *A2*).

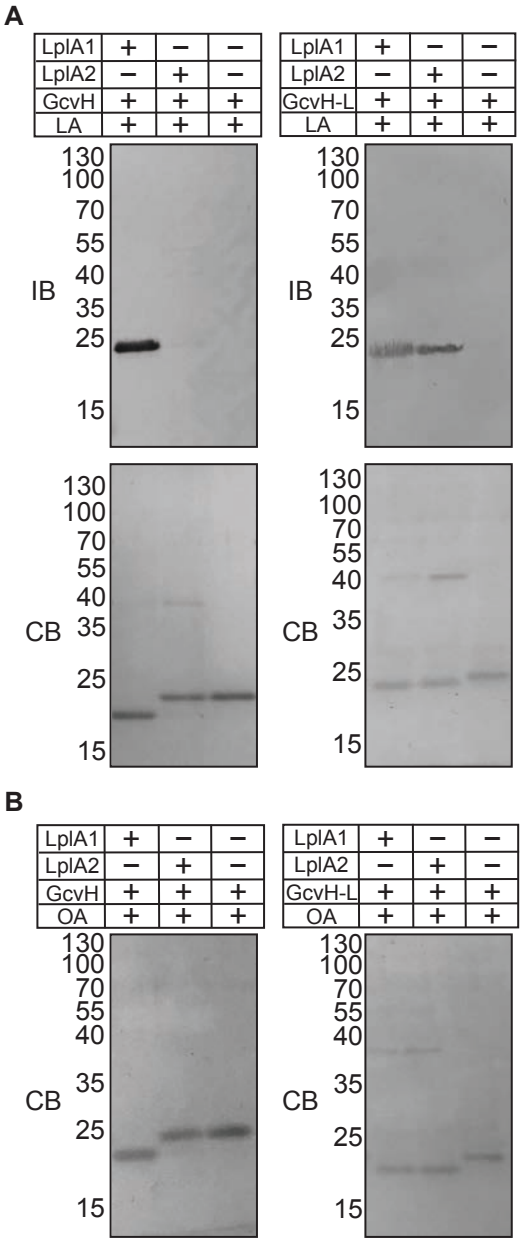

Figure S2. Original uncropped images related to Figure 3. (**A-B**) LplA1 and LplA2 attachment of (**A**) lipoic acid (2.4 mM) and (**B**) octanoic acid (2.4 mM) to GcvH and GcvH-L. Lipoylation was assessed by conducting an immunoblot (IB) with rabbit α-lipoic acid antibody. Parallel 12% SDS PAGE gels were stained with GelCode Blue (CB). Octanoylation was visualized as a shift in apparent molecular weight after resolving proteins on a 12% SDS PAGE gel and staining with GelCode Blue (CB).

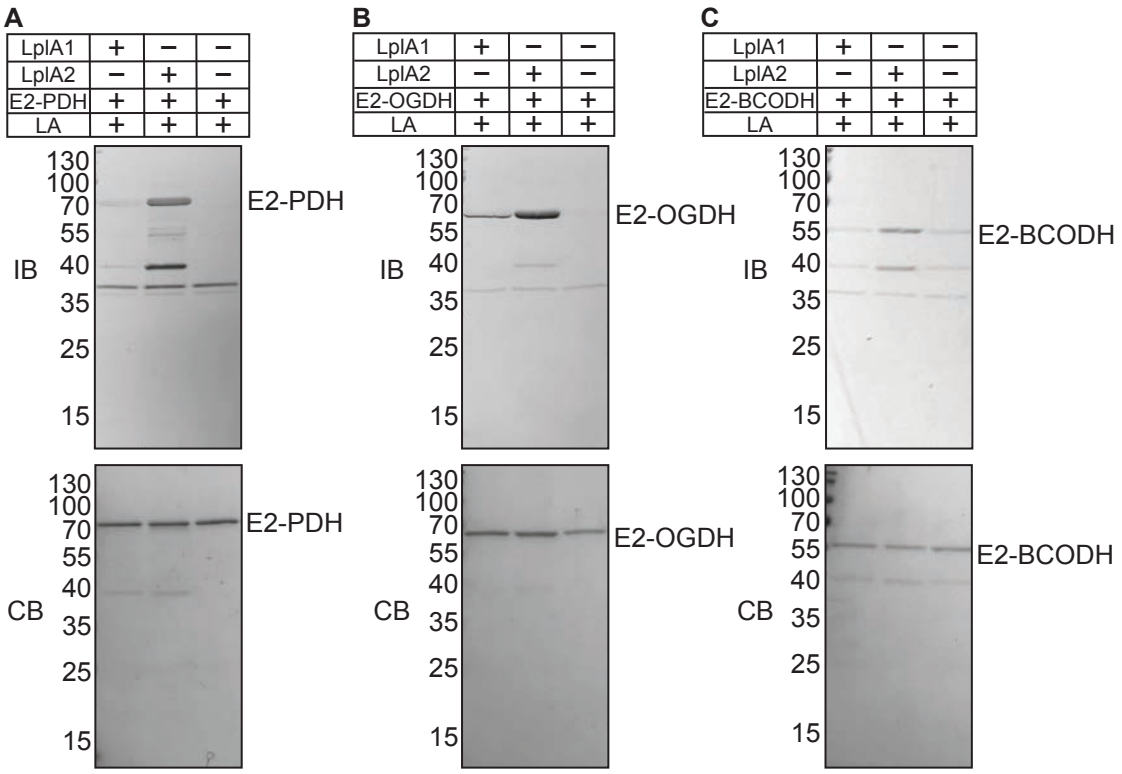

Figure S3. Original uncropped images related to Figure 4. (**A-C**) LplA1 and LplA2 attachment of lipoic acid (2.4 mM) to (**A**) E2-PDH, (**B**) E2-OGDH, and (**C**) E2-BCODH. Lipoylation was assessed by conducting immunoblots (IB) with rabbit α-lipoic acid antibody. Parallel 12% SDS PAGE gels were stained with GelCode Blue (CB).

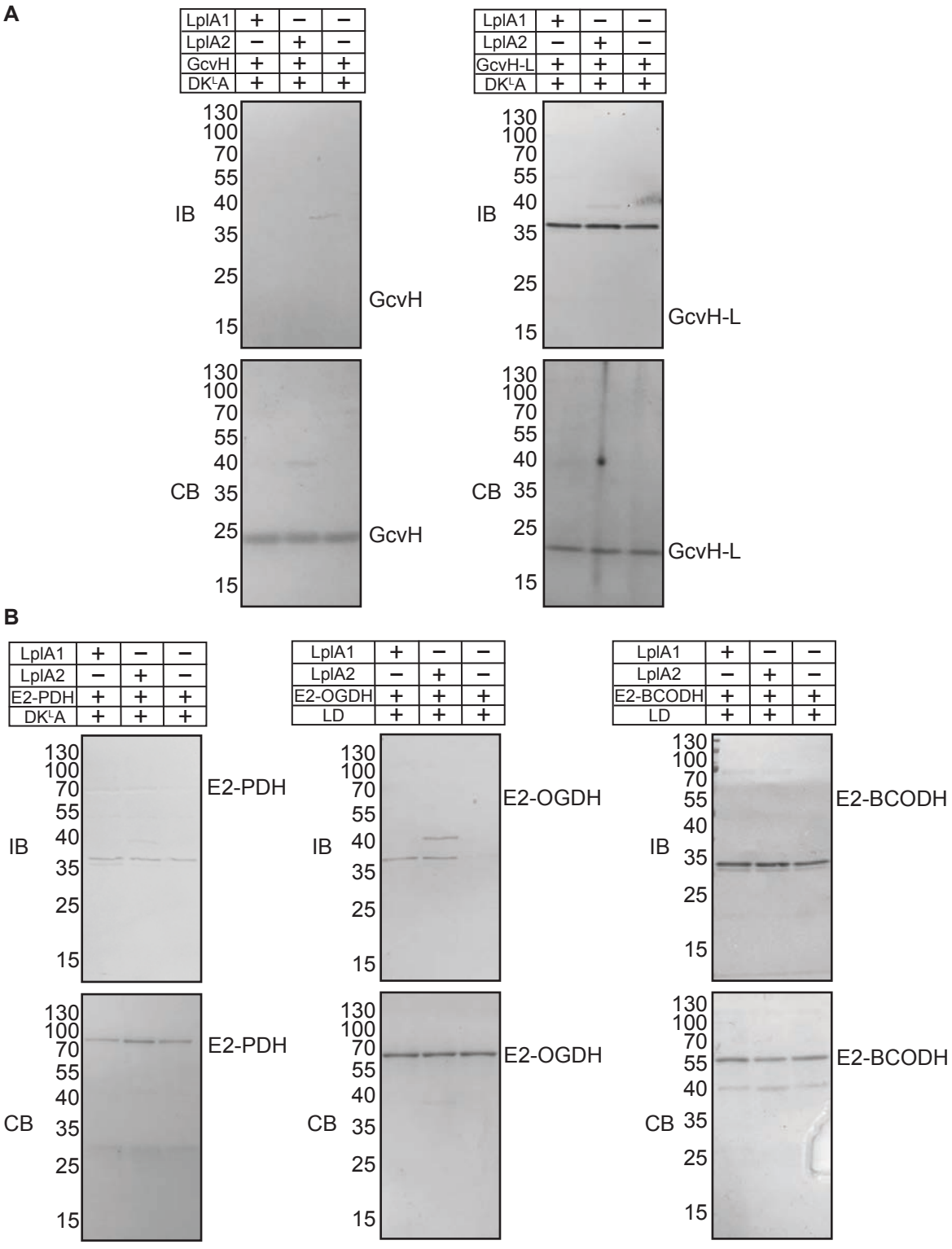

Figure S4. Original uncropped images related to Figure 6A. LplA1 and LplA2 attachment of DK^{L}A tripeptide-derived lipoic acid (2.4 mM) to (**A**) GcvH and GcvH-L, or (**B**) E2-PDH, E2-OGDH, and E2-BCODH. Lipoylation was assessed by conducting immunoblots (IB) with rabbit α-lipoic acid antibody. Parallel 12% SDS PAGE gels were stained with GelCode Blue (CB).

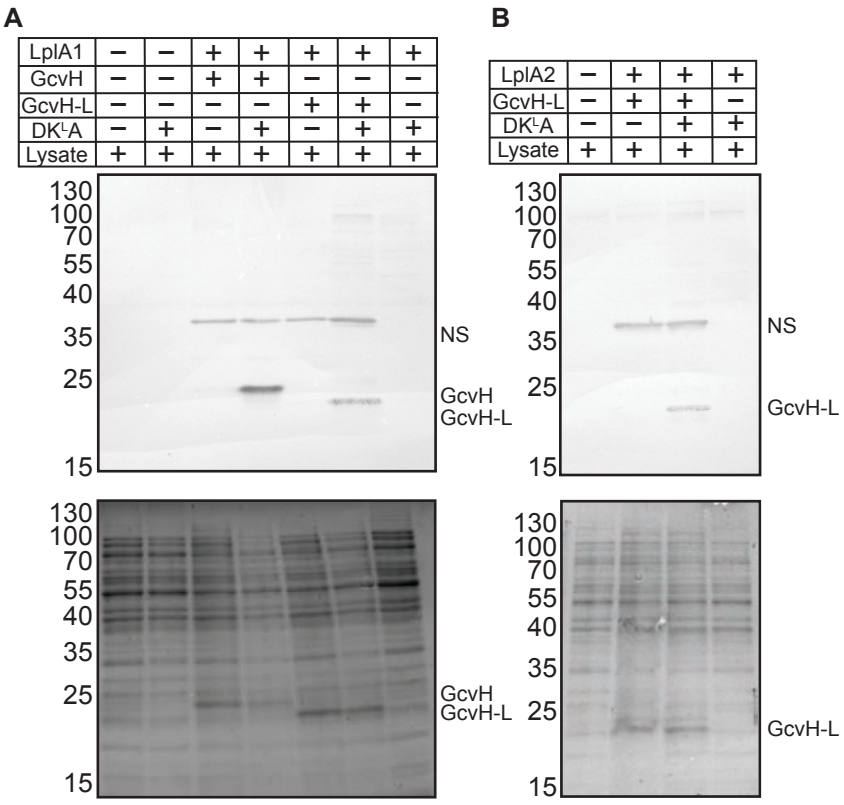

Figure S5. Original uncropped images related to Figure 6B-C. (**A**) LplA1 attachment of $DK^{L}A$ tripeptide-derived lipoic acid (2.4 mM) to GcvH and GcvH-L, or (**B**) LplA2 attachment of $DK^{L}A$ tripeptide-derived lipoic acid (2.4 mM) to GcvH-L in the presence of crude S. aureus lysates derived from a Δ*lipA* Δ*lipM* Δ*lipL* Δ*lplA1* Δ*lplA2* mutant. Lipoylation was assessed by conducting immunoblots (IB) with rabbit α-lipoic acid antibody. Parallel 12% SDS PAGE gels were stained with GelCode Blue (CB). (NS) Non-specific, reactive band in samples containing H proteins. The positions of GcvH and GcvH-L are indicated.

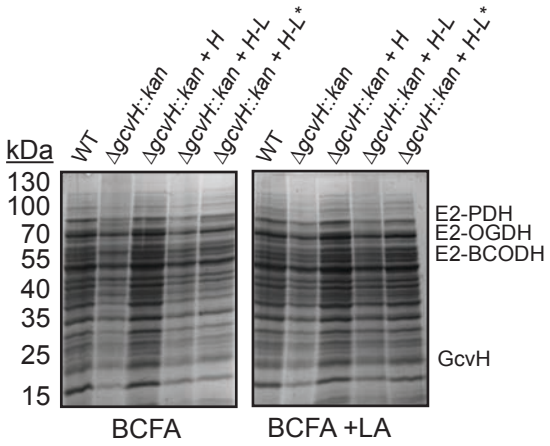

Figure S6. Loading controls related to Figure 7. Coomassie blue stained SDS-PAGE gels of whole cell lysates derived from the indicated strains grown in RPMI supplemented with branched chain carboxylic acids (10 mM isobutyric acid, 9 mM 2-methylbutyric acid, 9 mM isovaleric acid, and 10 mM sodium acetate - BCFA) in order to bypass the requirement of lipoic acid for replication with or without lipoic acid (LA).

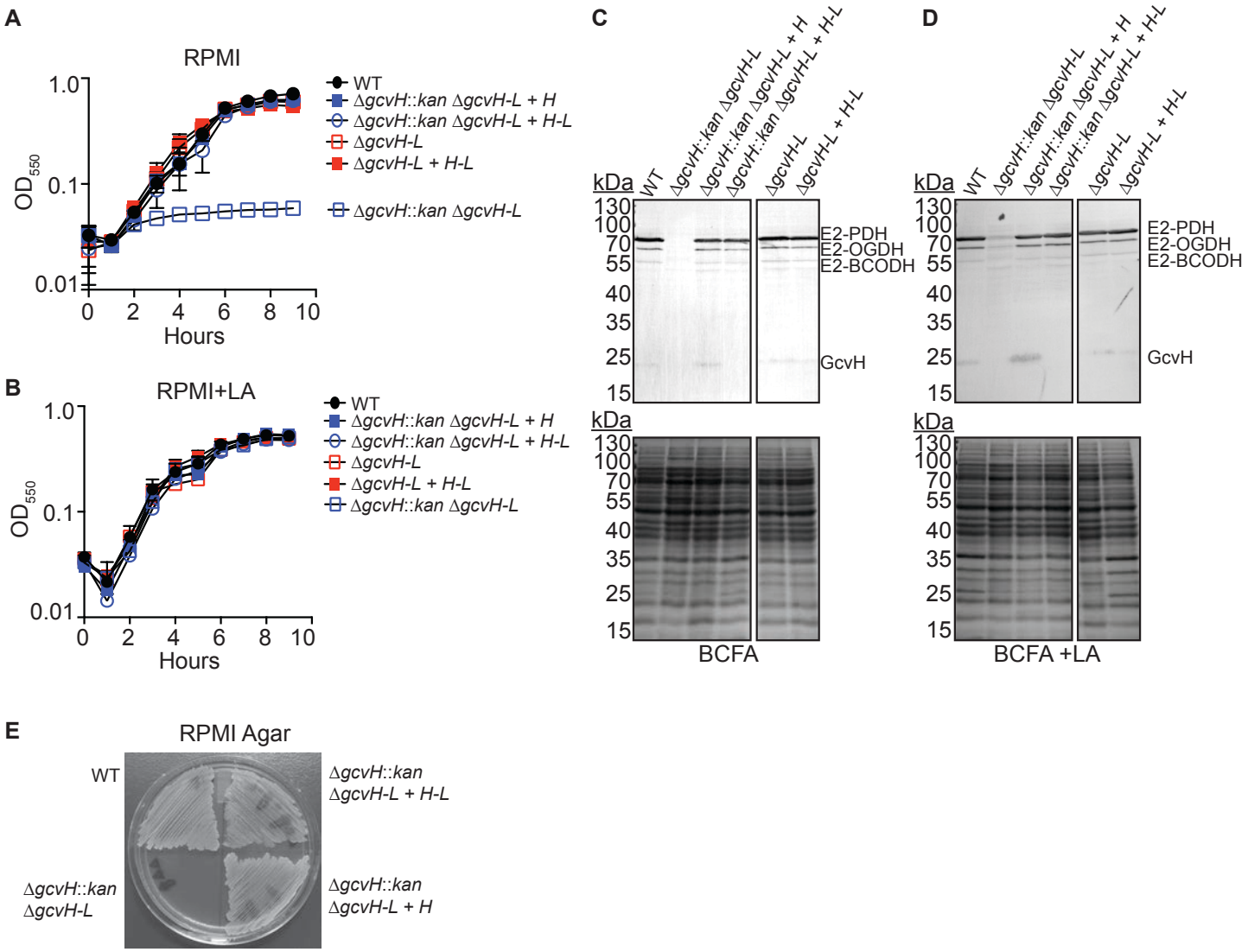

Figure S7. GcvH and GcvH-L both participate in lipoyl relay to E2 subunits. (A-B) Growth (OD550) of the indicated strains in (A) RPMI and (B) RPMI + 5 μM lipoic acid (LA). (C-D) α-lipoic acid immunoblot of whole cell lysates derived from the indicated strains grown in RPMI supplemented with branched chain carboxylic acids (10 mM isobutyric acid, 9 mM 2-methylbutyric acid, 9 mM isovaleric acid, and 10 mM sodium acetate - BCFA) in order to bypass the requirement of lipoic acid for replication with (C) or without (D) lipoic acid (LA). The positions of the four lipoyl proteins in *S. aureus* (E2-PDH, E2-OGDH, E2-BCODH, and GcvH) are indicated. Parallel SDS-PAGE gels were stained with GelCode Blue (lower panels). (E) Growth of the indicated strains on RPMI agar lacking free lipoic acid.

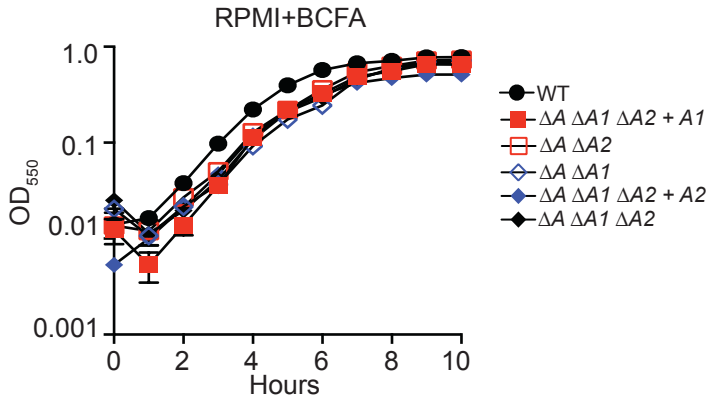

Figure S8. Supplementation of RPMI medium with BCFA restores growth of Δ*lipA* Δ*lplA1* and Δ*lipA* Δ*lplA1* Δ*lplA2* + *lplA2* strains in broth culture. Growth (OD550) of the indicated strains in RPMI medium supplemented with 10 mM isobutyric acid, 9 mM 2-methylbutyric acid, and 9 mM isovaleric acid. Strain designations are as follows: Wildtype (WT), Δ*lipA* Δ*lplA1* (Δ*A* Δ*A1*), Δ*lipA* Δ*lplA2* (Δ*A* Δ*A2*), Δ*lipA* Δ*lplA1* Δ*lplA2* (Δ*A* Δ*A1* Δ*A2*), Δ*lipA* Δ*lplA1* Δ*lplA2* + *lplA1* (Δ*A* Δ *A1* Δ*A2* + *A1*), and Δ*lipA* Δ*lplA1* Δ*lplA2* + *lplA2* (Δ*A* Δ*A1* Δ*A2* + *A2*).