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2 **Fig. S5. Identification of ligands sensed by GcbC's CACHE domain.** (A) Shown is a list of organic

3 acids that are color coordinated with the graph in panel B. The chemical structure of acetate,

4 pyruvate, succinate, fumarate, α -ketoglutarate, isocitrate, and citrate are shown. The number

5 of carbon atoms of each compound is listed on the right. (B) Quantitative analysis of biofilm

6 formation by WT *P. fluorescens* and the Δ 4DGC mutant strain in the presence of 0.4% acetate,

7 pyruvate, succinate, fumarate, α -ketoglutarate, and isocitrate. The empty vector control and a

8 plasmid carrying GcbC were introduced into the Δ 4DGC mutant strain. Biofilm assay data are

9 from six biological replicates (\pm SD). Linear models implemented in R (36) were used to identify

10 organic acid supplemented media whose properties significantly differed from the base

11 medium (K10T-1 minimal medium, see Materials and Methods) for both biofilm assay and B2H

12 assay experiments. The reduced biofilm levels for medium supplemented with α -ketoglutarate

1 is due to the poor growth of the strains in the presence of this compound (not shown). P values
2 of <0.05 were considered significant. P<0.05 (*); P<0.001 (***). (C) Biofilm formation by the
3 indicated strains \pm isocitrate. The Δ 4DGC mutant strain is used with WT GcbC and GcbC-R139E
4 variant introduced on plasmids. For panel C, experiments were performed in triplicate (\pm SD),
5 and horizontal black bars indicate a P value of <0.05(*) with a student's t-test comparing the
6 presence and absence of isocitrate amongst each strain.