

Title: Supplementary Data 1

Description: CAD Microfluidic device. Description: CAD layout of the microfluidic device used in this study. 3.

Title: Supplementary Data 2

Description: (d)Tn-Seq Fitness of *S. pneumoniae* 19F in 1% Agarose. Description: (droplet) Tn-Seq data of *S. pneumoniae* Taiwan-19F grown in 1% agarose droplets. 4.

Title: Supplementary Data 3

Description: (d)Tn-Seq Fitness of *S. pneumoniae* TIGR4 in AGP. Description: (droplet) Tn-Seq data of *S. pneumoniae* TIGR4 grown in droplets and liquid media with AGP as the main carbon source. 5.

Title: Supplementary Data 4.

Description: (d)Tn-Seq Fitness of *S. pneumoniae* TIGR4 in Elastase. Description: (droplet) Tn-Seq data of *S. pneumoniae* TIGR4 exposed in droplets and liquid media to the protease Elastase. 6.

Title: Supplementary Data 5.

Description: Adapters and primers. Description: All adapters and primers used in this study. 7.

Title: Supplementary Data 6.

Description: Strains and Plasmids. Description: All bacterial strains and plasmids used in this study.