## **Description of Additional Supplementary Files**

File Name: Supplementary Data 1

Description: List of 135 genes not represented in E. coli Dubseq library

File Name: Supplementary Table 2

Description: List of protein coding genes with details on number of Dub-seq fragments covering the gene, and if the gene is essential (according to the Keio library, PMID: 16738554), has RB-TnSeq data

(PMID: 29769716) and has Dub-seq data (this work)

File Name: Supplementary Data 3

Description: Filtered gene scores for reliable effects in Dub-seq dataset and if they have

representative data in RB-TnSeq mutant library (PMID:29769716)

File Name: Supplementary Data 4

Description: List of genes whose high dosage is known to yield

positive fitness effects

File Name: Supplementary Data 5

Description: Novel gene-function associations with fitness score >=4; hypothesis and general notes

File Name: Supplementary Data 6

Description: List of primers used in this work

File Name: Supplementary Data 7

Description: List of plasmids used in this work

File Name: Supplementary Data 8

Description: List of strains used in this work