

Description of Additional Supplementary Files

- 1
- 2
- 3 **Supplementary Data 1.** DNA constructs; Plasmids utilized in promoter-transcriptomics experiments;
- 4 Promoter sfGFP plasmids; MAGE & DIVERGE oligonucleotides; MASC PCR primers; SynOMICS deletion
- 5 cassettes; SynOMICS genome-targeting CRISPR/Cas9 guide RNAs; Sequence of additionally recoded
- 6 genes; Strains and plasmids used in this study.
- 7 **Supplementary Data 2.** Fitness and Biolog datasets.
- 8 **Supplementary Data 3.** Source data for RNA-seq and Ribo-seq experiments.
- 9 **Supplementary Data 4.** DNA synthesis errors in synthetic genomic segments of Ec_Syn57.
- 10 **Supplementary Data 5.** Identified mutations in *E. coli* strains containing the genome of Ec_Syn57.
- 11 **Supplementary Data 6.** LC-MS/MS identified cryptic ORFs in *E. coli* MDS42 and Syn61.
- 12 **Supplementary Data 7.** Detailed description of troubleshooting changes in Ec_Syn57.