Supporting Information

Supporting Figure Legends

Fig. S1. Vector exchange experiments

(a) pTrc-S16 plasmid with the gene encoding essential ribosomal protein S16 cloned by the XhoI and SpeI sites of pTrc99A. (b) ΔS16 deletion strain was electro-transformed with pTrc-S16 plasmid, recovered overnight in 2YT + 1mM IPTG and plated on LB agar containing 100ug/ml ampicillin, 1mM IPTG, and 5% sucrose for counter selection against the *sacB* marker. Surviving cells should contain the pTrc-S16, but have lost pCDSSara-S16. (c) Single colonies were picked from the counter selection plate and streaked on LB agar plates containing 100ug/ml ampicillin + 1mM IPTG, 100ug/ml ampicillin, or 50ug/ml spectinomycin + 0.2% arabinose. Efficiency of plasmid exchange was observed to be 100% among 27 colonies examined.

Fig. S2. Deletion strategies.

(a) Complete replacement of the gene. The target gene was completely replaced in-frame by a CAT gene (Cm^r). (b) Partial replacement with preservation of the downstream Shine-Dalgarno (SD) sequence. When the SD sequence for the downstream gene in the same operon is embedded in the target gene, the corresponding region was preserved. Sequences of the genomic SD-containing regions are shown underneath the diagram. Gene names are indicated in parentheses, and the coding regions, including stop codons, are in capital letters. The CAT gene, SD sequences, and downstream gene are colored in green, orange, and cyan, respectively. The original stop codon for the target gene is underlined. (c) Partial replacement of the 3' portion of the target gene. The 3' portion of the target gene was replaced by a CAT gene expression cassette containing a TGA stop codon in-frame for the 5' fragment, a SD sequence, and a CAT gene. (d) Partial replacement of the 5' portion of the target gene. The 5' portion of the target gene was replaced by a CAT gene followed by a SD sequence and an ATG start codon for the 3' fragment of the target gene.

Fig. S3. PCR analysis of genomic recombination.

(a) A schematic view of the genomic region targeted for recombination. Black arrows represent genomic sites that anneal primers used in this analysis. (b–d) Agarose gel electrophoresis images of PCR products with primer pairs *a* and *c* (b), *b* and *d* (c), and *a* and *d* (d) shown in panel A on all deletion mutants. D, deletion mutant; C, DH10B control; M, size marker.

Fig. S4. PCR analysis of knockout mutants deprived of a complementing plasmid.

(a) Design of primers to analyze the presence of the genomic recombination and the absence of the target gene. Black arrows represent genomic sites that anneal to primers used in this analysis. Each primer pair is numbered as indicated. (b) Agarose gel electrophoresis images of PCR products with primer pairs 1-4 on all knockout mutants lacking a complementing plasmid.

Fig. S5. Quantitative mass spectrometry analysis of ribosomal protein truncations.

(a) Relative abundance of all 30S proteins found within 70S ribosomes isolated from ΔL5 cells expressing (L5Δ70-83) in green, ΔL27 cells expressing (L27NΔ16) in red, ΔL27 cells expressing (L27NΔ20) in blue, and ΔS19 cells expressing (S19CΔ16) in yellow. (b) Relative abundance of all 50S proteins found within 70S ribosomes isolated from ΔL5 cells expressing (L5Δ70-83) in green, ΔL27 cells expressing (L27NΔ16) in red, ΔL27 cells expressing (L27NΔ20) in blue, and ΔS19 cells expressing (S19CΔ16) in yellow.

Supporting Figures

Fig. S1

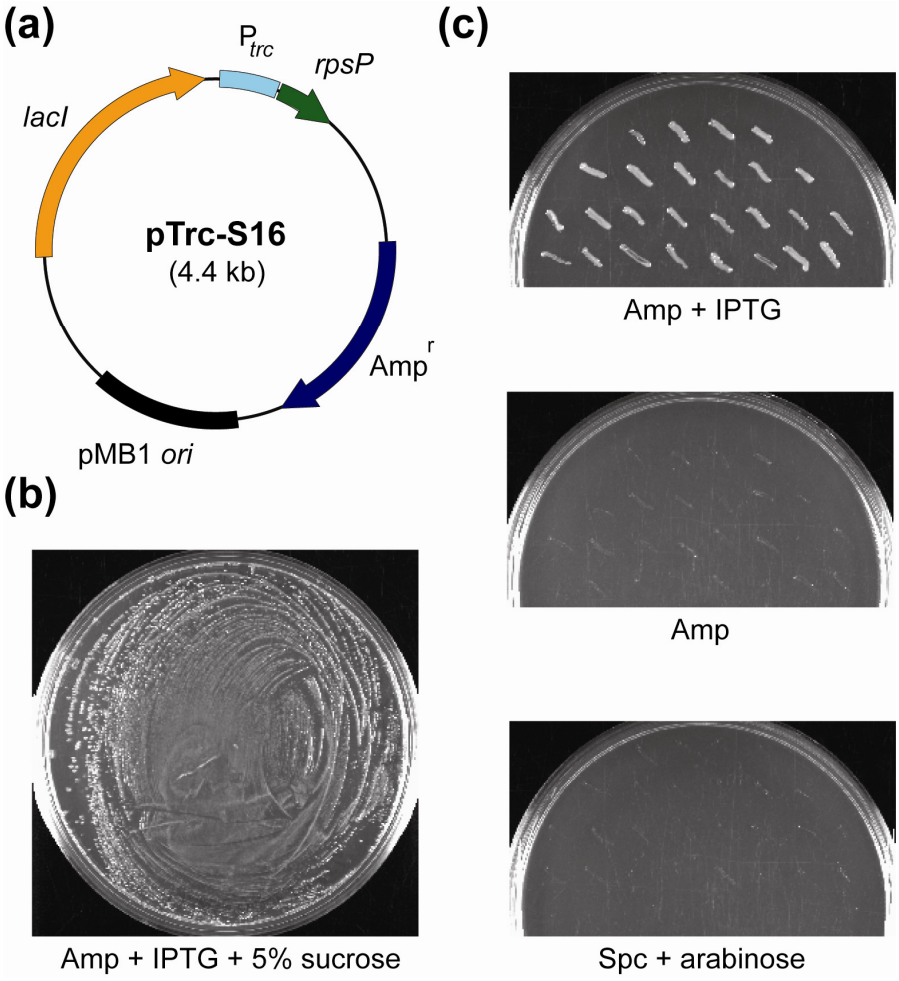

Fig. S2

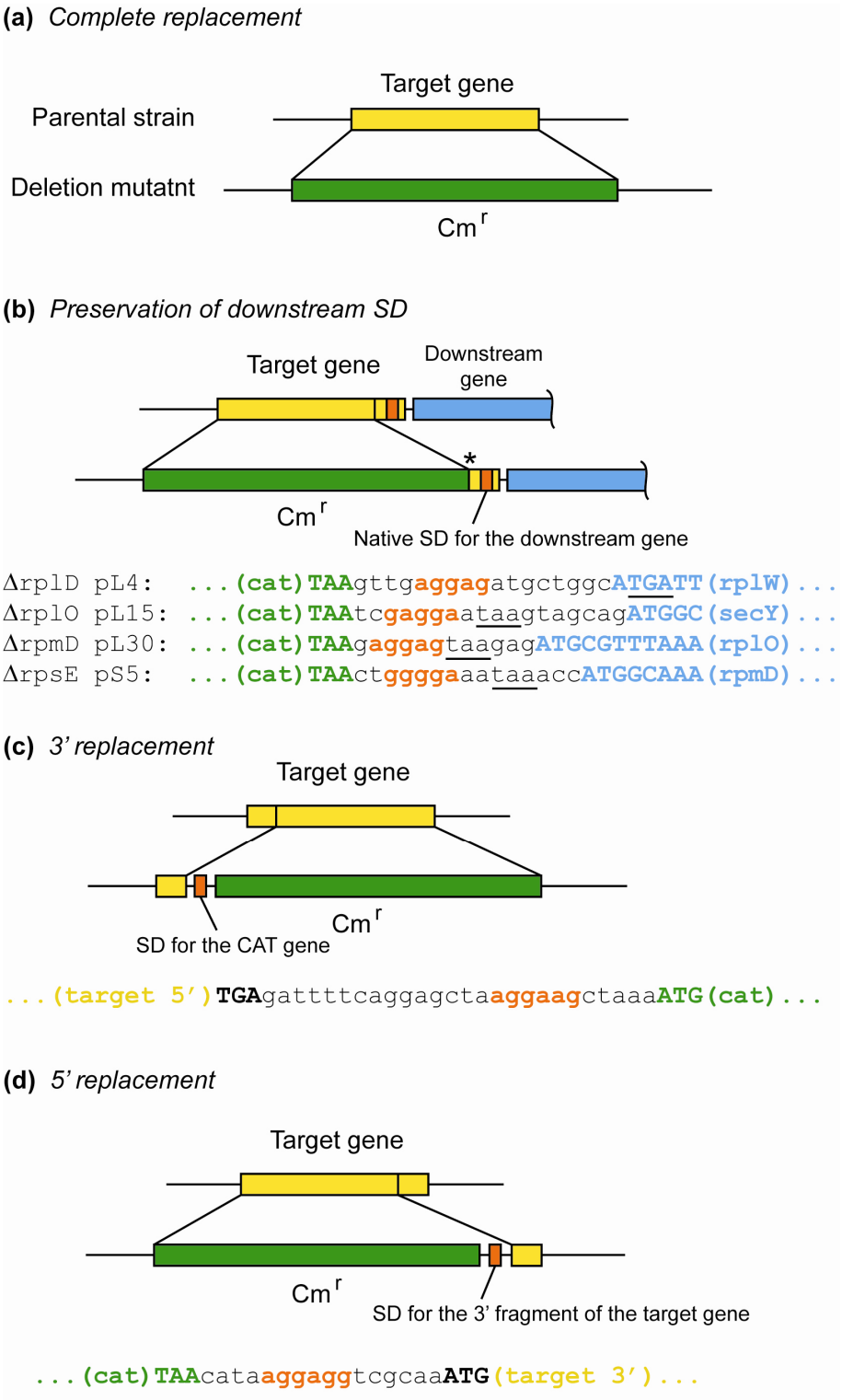
(a) Complete replacement
Target gene
Parental strain
Deletion mutatnt
Cm r
(b) Preservation of downstream SD
Target gene
Downstream gene
*
Cm r
Native SD for the downstream gene
ΔrplD pL4: ...(cat)TAAgttgaggagatgctggcATGATT(rplW)...
ΔrplO pL15: ...(cat)TAAtcgaggaataagtagcagATGGC(secY)...
ΔrpmD pL30: ...(cat)TAAgaggagtaagagATGCGTTTAAA(rplO)...
ΔrpsE pS5: ...(cat)TAActgggaaataaaccATGGCAAA(rpmD)...
(c) 3' replacement
Target gene
Cm r
SD for the CAT gene
...(target 5')TGAgattttcaggagctaaggaagctaaaATG(cat)...
(d) 5' replacement
Target gene
Cm r
SD for the 3' fragment of the target gene
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Fig. S3

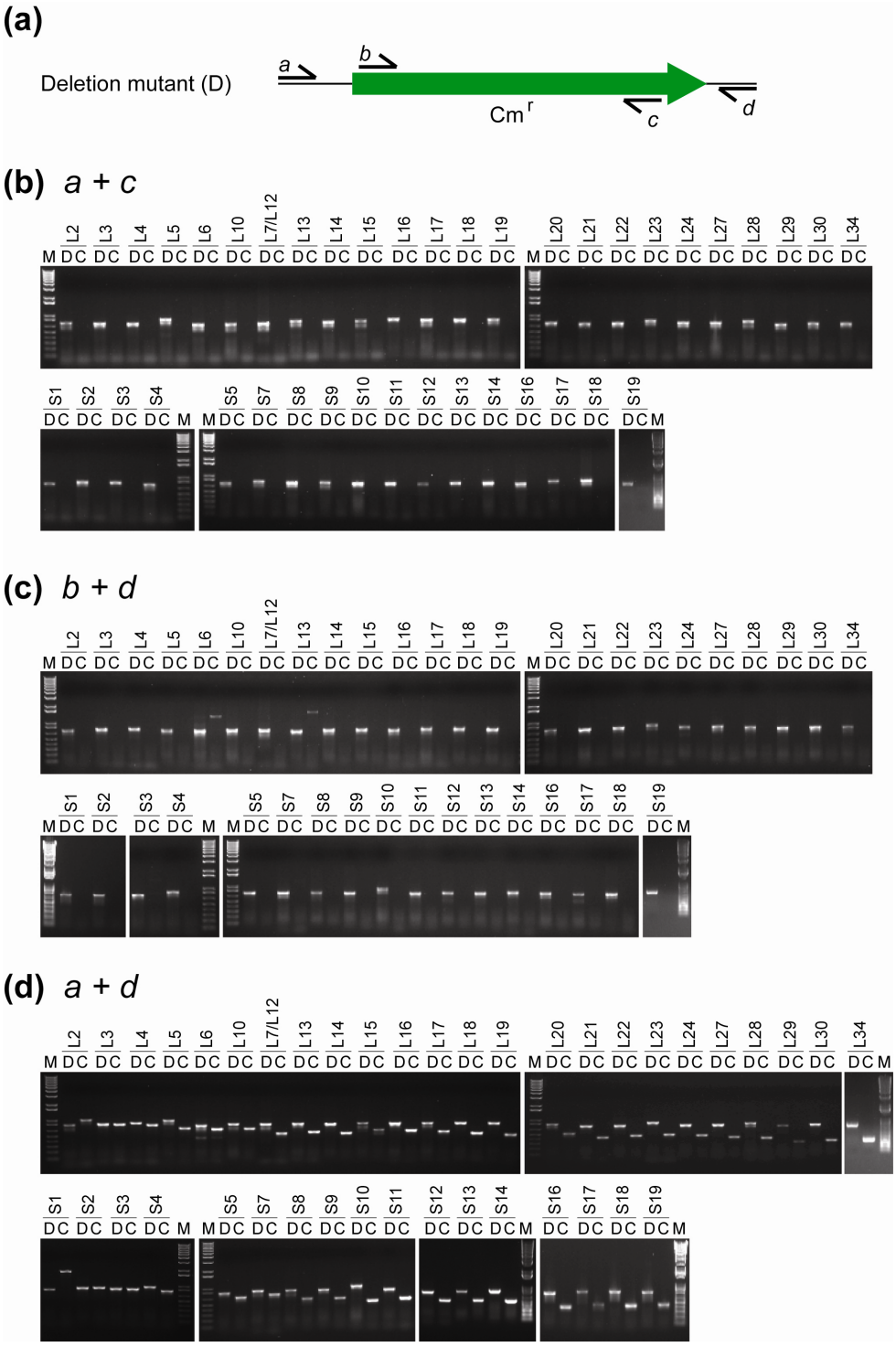

(a)
Deletion mutant (D)
a
b
Cm r
c
d
(b) a + c
L2 L3 L4 L5 L6 L10 L7/L12 L13 L14 L15 L16 L17 L18 L19 L20 L21 L22 L23 L24 L27 L28 L29 L30 L34
M DC DC DC DC DC DC DC DC DC DC DC DC DC DC DC DC M DC DC DC DC DC DC DC DC DC DC DC
S1 S2 S3 S4 S5 S7 S8 S9 S10 S11 S12 S13 S14 S16 S17 S18 S19
DC DC DC DC M M DC DC DC DC DC DC DC DC DC DC DC DC DC DC M
(c) b + d
L2 L3 L4 L5 L6 L10 L7/L12 L13 L14 L15 L16 L17 L18 L19 L20 L21 L22 L23 L24 L27 L28 L29 L30 L34
M DC DC DC DC DC DC DC DC DC DC DC DC DC DC DC DC M DC DC DC DC DC DC DC DC DC DC DC
S1 S2 S3 S4 S5 S7 S8 S9 S10 S11 S12 S13 S14 S16 S17 S18 S19
M DC DC DC DC M M DC DC DC DC DC DC DC DC DC DC DC DC DC DC M
(d) a + d
L2 L3 L4 L5 L6 L10 L7/L12 L13 L14 L15 L16 L17 L18 L19 L20 L21 L22 L23 L24 L27 L28 L29 L30 L34
M DC DC DC DC DC DC DC DC DC DC DC DC DC DC DC DC M DC DC DC DC DC DC DC DC DC DC DC M
S1 S2 S3 S4 S5 S7 S8 S9 S10 S11 S12 S13 S14 S16 S17 S18 S19
DC DC DC DC M M DC DC DC DC DC DC DC DC DC DC DC DC DC DC DC M

Fig. S4

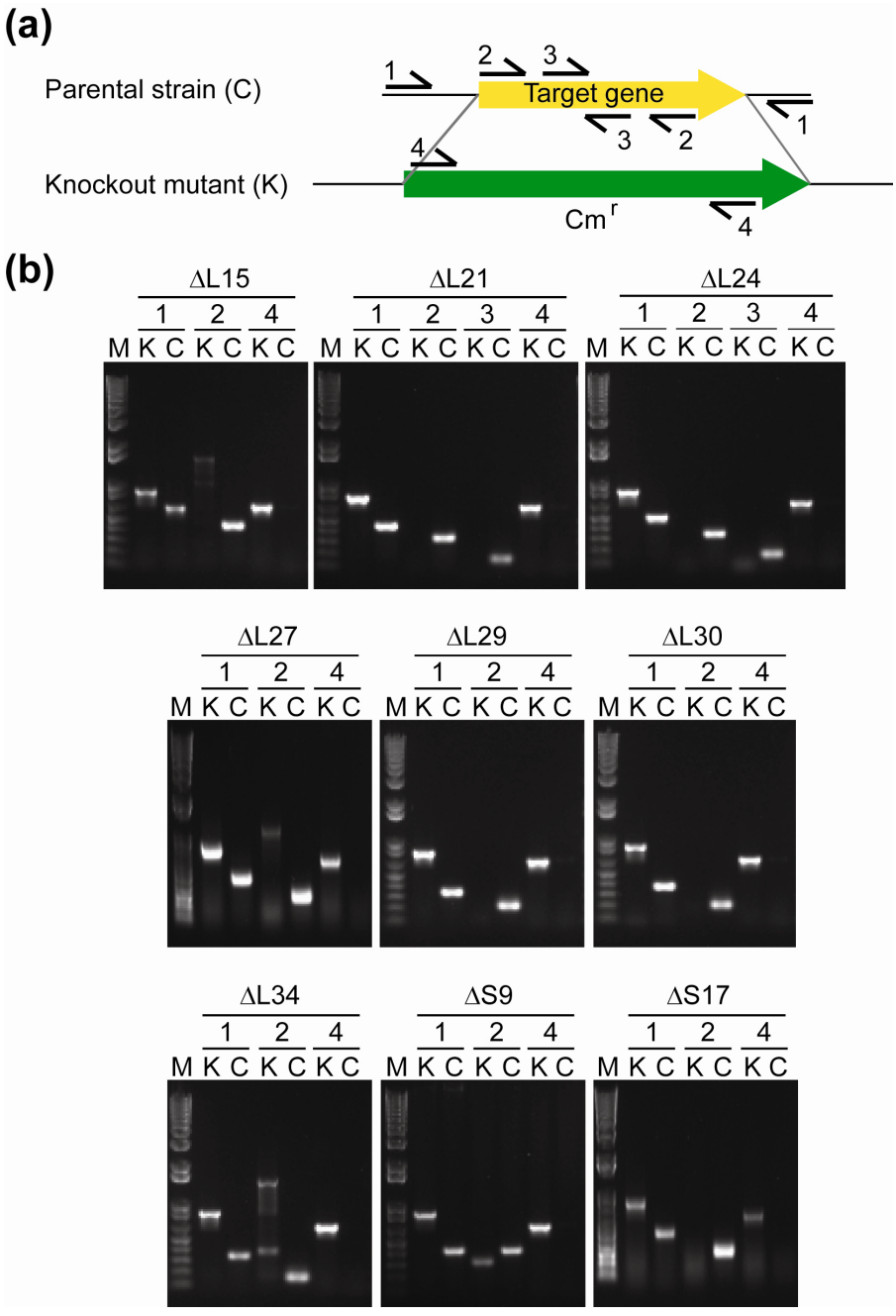
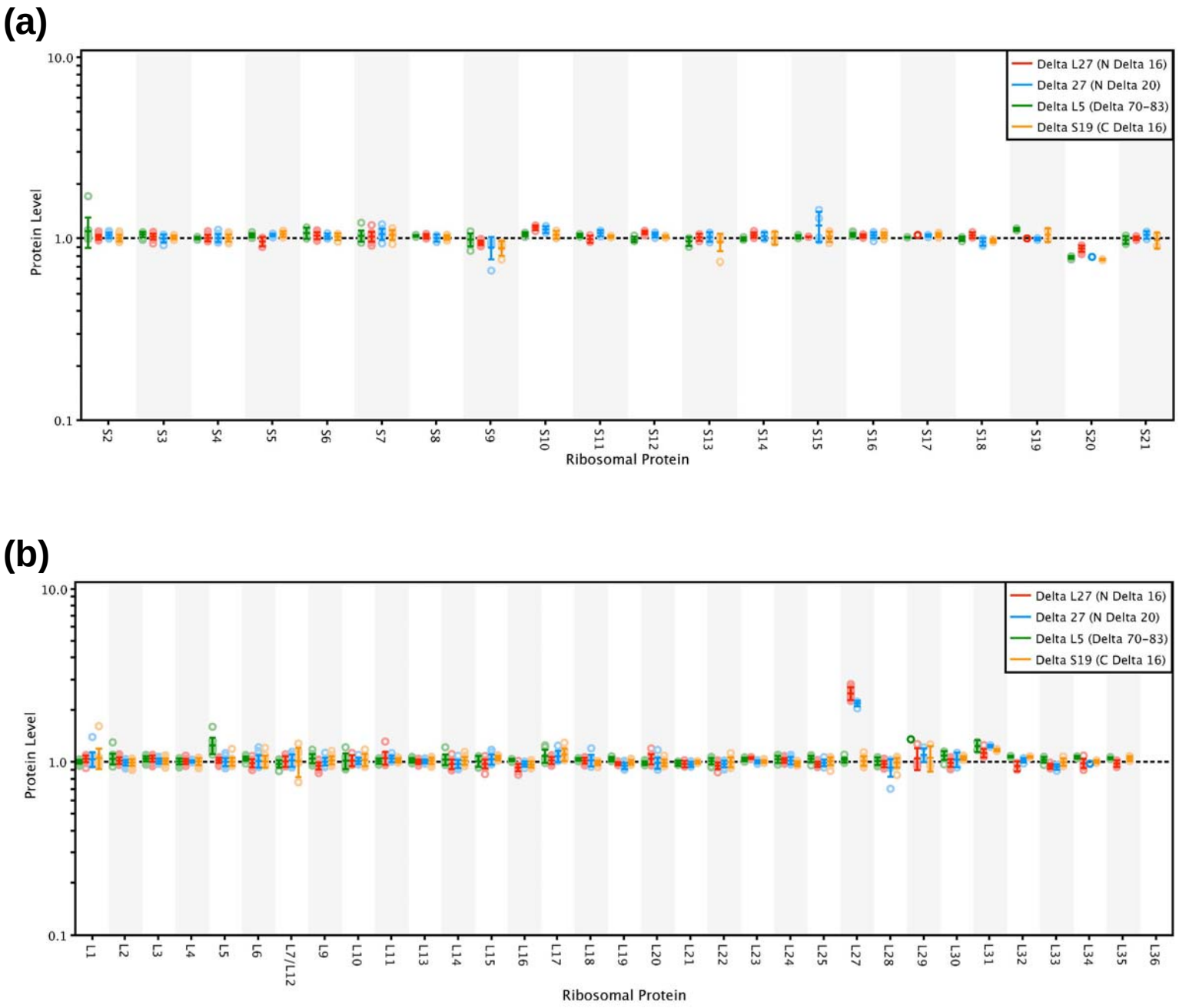

(a)
Parental strain (C)
Knockout mutant (K)
Target gene
Cm r
1
2
3
4
(b)
ΔL15
ΔL21
ΔL24
ΔL27
ΔL29
ΔL30
ΔL34
ΔS9
ΔS17
M K C K C K C

Fig. S5


(a)
Delta L27 (N Delta 16)
Delta 27 (N Delta 20)
Delta L5 (Delta 70–83)
Delta S19 (C Delta 16)
Protein Level
10.0
1.0
0.1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
S13
S14
S15
S16
S17
S18
S19
S20
S21
Ribosomal Protein
(b)
Delta L27 (N Delta 16)
Delta 27 (N Delta 20)
Delta L5 (Delta 70–83)
Delta S19 (C Delta 16)
Protein Level
10.0
1.0
0.1
L1
L2
L3
L4
L5
L6
L7/L12
L9
L10
L11
L13
L14
L15
L16
L17
L18
L19
L20
L21
L22
L23
L24
L25
L27
L28
L29
L30
L31
L32
L33
L34
L35
L36
Ribosomal Protein