Supplemental Figure 1. Multiplexed CRISPRi identifies known essential genes. A) Line chart depicting Log2FC (final pool/initial pool) of essential and non-essential gene knockdowns across a 24 hour growth assay. B) Individual growth assays across 16 hours for each of these 4 strains depicting growth defects for essential gene knockdowns. C) Volcano plot of padj and Log2FC values for the pool of ~500 knockdown strains. Guides with a significant padj value and a Log2FC < -1 are depicted in red. Guides targeting known essential genes are depicted as large circles; known non-essential genes are depicted as small circles. All guides targeted to a select number of known essential genes (rplB, cysE, aspS) are shown, depicting that some genes are identified by multiple guides. D) Log2FC and padj values for guide hits to gene aspS are depicted alongside an individual growth curve for an aspS gene knockdown strain. Similar growth curves for cysE and rplB (and others) can be found in our previously published data.

Supplemental Figure 2. GC content but not guide position is significantly correlated with guide performance. (A) sgRNAs with GC content > 50% are consistent with more positive log2FCs, indicating worsening performance of sgRNAs with higher GC content. (B) No significant difference in overall log2FC of sgRNAs was observed based on their position in the gene, as measured by distance into the gene from the transcriptional start site.

Supplemental Figure 3. Scramble sgRNAs behave distinctly within the CRISPRi screen. (A) Distribution of the log2FC values for guides targeting putative essential and non-essential genes and scramble sgRNAs demonstrates aberrant behavior of strains containing scramble sgRNAs. Consequently, we deem estimation of size factors for based on scrambled sgRNAs inappropriate.

Supplemental Figure 4. Median Log2FC for genes is generally consistent between conditions of a media type. Heatmaps of the median log2FC of genes across conditions grouped by media type (a= minimal, b=defined, c=rich). Hierarchical clustering was generated based on Euclidean distances.

Supplemental Figure 5. Guides targeting the putative promoter region or gene body of putative essential genes across conditions. The putative promoter region of an essential gene may be identified based on log2FC of guides upstream of the gene body. Plotted here are the log2FCs of guides targeting an essential gene just upstream of the gene body (shown in red) and guides targeting the gene body (shown in blue); each dot represents log2FC of the guide in one condition.

Supplemental Figure 6. A) Single S. epidermidis cells captured in droplets. Single cells can be visualized in droplets at the start of the droplet-based CRISPRi screen. Note that most droplets are empty, ensuring that most cells are captured singly within a droplet. B) Multiple S. epidermidis cells growing within a droplet in TSB. After 20 hours of growth, multiple S. epidermidis cells can be visualized multiplying within a droplet in the plain TSB (RM) condition. Note that individual cells can be visualized independently and do not demonstrate a clumping phenotype. C) Multiple S. epidermidis cells growing within a droplet in Acidic-TSB. After 20 hours of growth, multiple S. epidermidis cells can be visualized multiplying within a droplet in the Acidic TSB (RM) condition. Note the clumped phenotype.

Supplemental Figure 7. Oligo design for CRISPRi cloning. Oligos containing unique sgRNA sequences were ordered as ssDNA oligos and amplified via PCR. This figure depicts the oligo sequence (additional provided in Supplemental Tables), with an example sgRNA sequence and the location of the PCR sites for amplification and the BSAI sites for digestion.
Supplemental Table 1. Guides designed by a customized version of our GuideFinder script for large-scale CRISPRi targeting.

Supplemental Table 2. High confidence putative essential gene hits identified by CRISPRi screening

Supplemental Table 3. Medium confidence putative essential gene hits identified by CRISPRi screening

Supplemental Table 4. Low confidence putative essential gene hits identified by CRISPRi screening

Supplemental Table 5. RM-Salt-specific essential genes identified by CRISPRi screening

Supplemental Table 6. RM-Ciprofloxacin-specific essential genes identified by CRISPRi screening

Supplemental Table 7. RM-Mupirocin-specific essential genes identified by CRISPRi screening

Supplemental Table 8. RM-Urea-specific essential genes identified by CRISPRi screening

Supplemental Table 9. DM-Acidic-specific essential genes identified by CRISPRi screening

Supplemental Table 10. RM-Glycerol-specific essential genes identified by CRISPRi screening

Supplemental Table 11. RM-Sucrose-specific essential genes identified by CRISPRi screening

Supplemental Table 12. RM-Acidic-specific essential genes identified by CRISPRi screening

Supplemental Table 13. RM-H202-specific essential genes identified by CRISPRi screening

Supplemental Table 14. RM-HOCl-specific essential genes identified by CRISPRi screening

Supplemental Table 15. MM-HighGlucoseHighAA-specific essential genes identified by CRISPRi screening

Supplemental Table 16. MM-LowGlucoseHighAA-specific essential genes identified by CRISPRi screening

Supplemental Table 17. MM-HighGlucoseLowAA-specific essential genes identified by CRISPRi screening

Supplemental Table 18. MM-LowGlucoseLowAA-specific essential genes identified by CRISPRi screening

Supplemental Table 19. RM-42C-specific essential genes identified by CRISPRi screening

Supplemental Table 20. RM-Alkaline-specific essential genes identified by CRISPRi screening

Supplemental Table 21. RM-Vancomycin-specific essential genes identified by CRISPRi screening

Supplemental Table 22. DM-HighIron-specific essential genes identified by CRISPRi screening

Supplemental Table 23. DM-specific essential genes identified by CRISPRi screening

Supplemental Table 24. DM-LowIron-specific essential genes identified by CRISPRi screening

Supplemental Table 25. DM-30C-specific essential genes identified by CRISPRi screening

Supplemental Table 26. DM-Antiobiotic-specific essential genes identified by CRISPRi screening

Supplemental Table 27. RM-Antiobiotic-specific essential genes identified by CRISPRi screening

Supplemental Table 28. RM-specific essential genes identified by CRISPRi screening

Supplemental Table 29. Genes sensitive to targeting on both strand under anaerobic growth. These gene hits were identified as resulting in a growth deficit when targeted on either strand in the gene body during growth in anaerobic rich media.

Supplemental Table 30. RM-Acidic_vs_RM_Log2FC data

Supplemental Table 31. RM-Mupirocin_vs_RM_Log2FC data

Supplemental Table 32. RM-Alkaline_vs_RM_Log2FC data

Supplemental Table 33. RM-Glycerol_vs_RM_Log2FC data

Supplemental Table 34. RM-H202_vs_RM_Log2FC data

Supplemental Table 35. RM-HOCl_vs_RM_Log2FC data

Supplemental Table 36. RM-Urea_vs_RM_Log2FC data

Supplemental Table 37. RM-Vancomycin_vs_RM_Log2FC data

Supplemental Table 38. RM-Antiobiotic_vs_RM_Log2FC data

Supplemental Table 39. RM-Sucrose_vs_RM_Log2FC data

Supplemental Table 40. DM-LowIron_vs_RM_Log2FC data

Supplemental Table 41. DM_vs_RM_Log2FC data

Supplemental Table 42. DM-Antiobiotic_vs_RM_Log2FC data

Supplemental Table 43. MM-HighGlucoseHighAA_vs_RM_Log2FC data

Supplemental Table 44. MM-LowGlucoseHighAA_vs_RM_Log2FC data

Supplemental Table 45. MM-HighGlucoseLowAA_vs_RM_Log2FC data

Supplemental Table 46. MM-LowGlucoseLowAA_vs_RM_Log2FC data

Supplemental Table 47. MM-High AA, high glucose_vs_MM-High AA, low glucose_Log2FC data
Supplemental Table 48. MM-Low AA, low glucose vs MM-Low AA, high glucose Log2FC data
Supplemental Table 49. MM-Low AA, high glucose vs MM-High AA, high glucose Log2FC data
Supplemental Table 50. MM-Low AA, low glucose vs MM-High AA, low glucose Log2FC data
Supplemental Table 51. DM-Anaerobic vs DM_Log2FC data
Supplemental Table 52. DM-LowIron vs DM_Log2FC data
Supplemental Table 53. Key for RNA-sequencing data linking file names, condition type, Log2FC interpretation and justification.
Supplemental Table 54. Genes enriched in all three osmolarity conditions; Log2FC cutoff of >1.0. Log2FC values, padj values, and gene annotation included in this file.
Supplemental Table 55. Genes enriched in all three osmolarity conditions; Log2FC cutoff of >0.5. Log2FC values, padj values, and gene annotation included in this file.
Supplemental Table 56. RNA-sequencing identifies pathways involved in S. epidermidis plasticity across diverse environments. All significant KEGG pathways up and down regulated in each condition, grouped by media type. RM samples are compared to RM baseline media, DM samples are compared to DM baseline media and MM samples are compared to high amino acid (1.0% CAA), high glucose (1.0%) minimal media. (+) represent significantly upregulated KEGG pathways; (-) represent significantly downregulated pathways.
Supplemental Table 57. Droplet-based CRISPRi acid-specific gene hits.
Supplemental Table 58. Batch-based CRISPRi acid-specific gene hits.
Supplemental Table 59. All CRISPRi calculated Log2FC and FDR-adjusted p-values for each guide at each time point for every condition.
Supplemental Table 60. Oligo cloning design
Supplemental Table 61. Sequencing primers used in this study