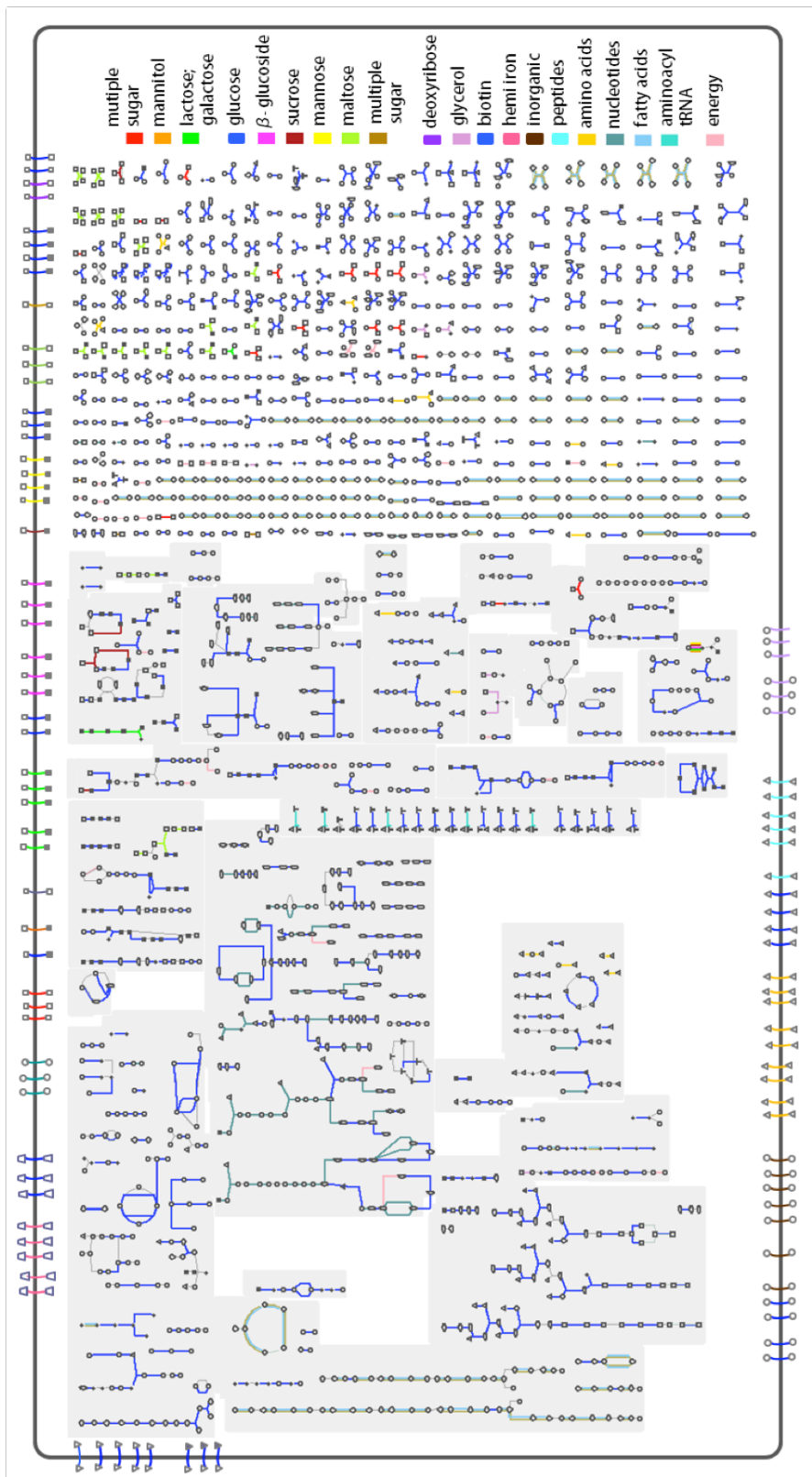
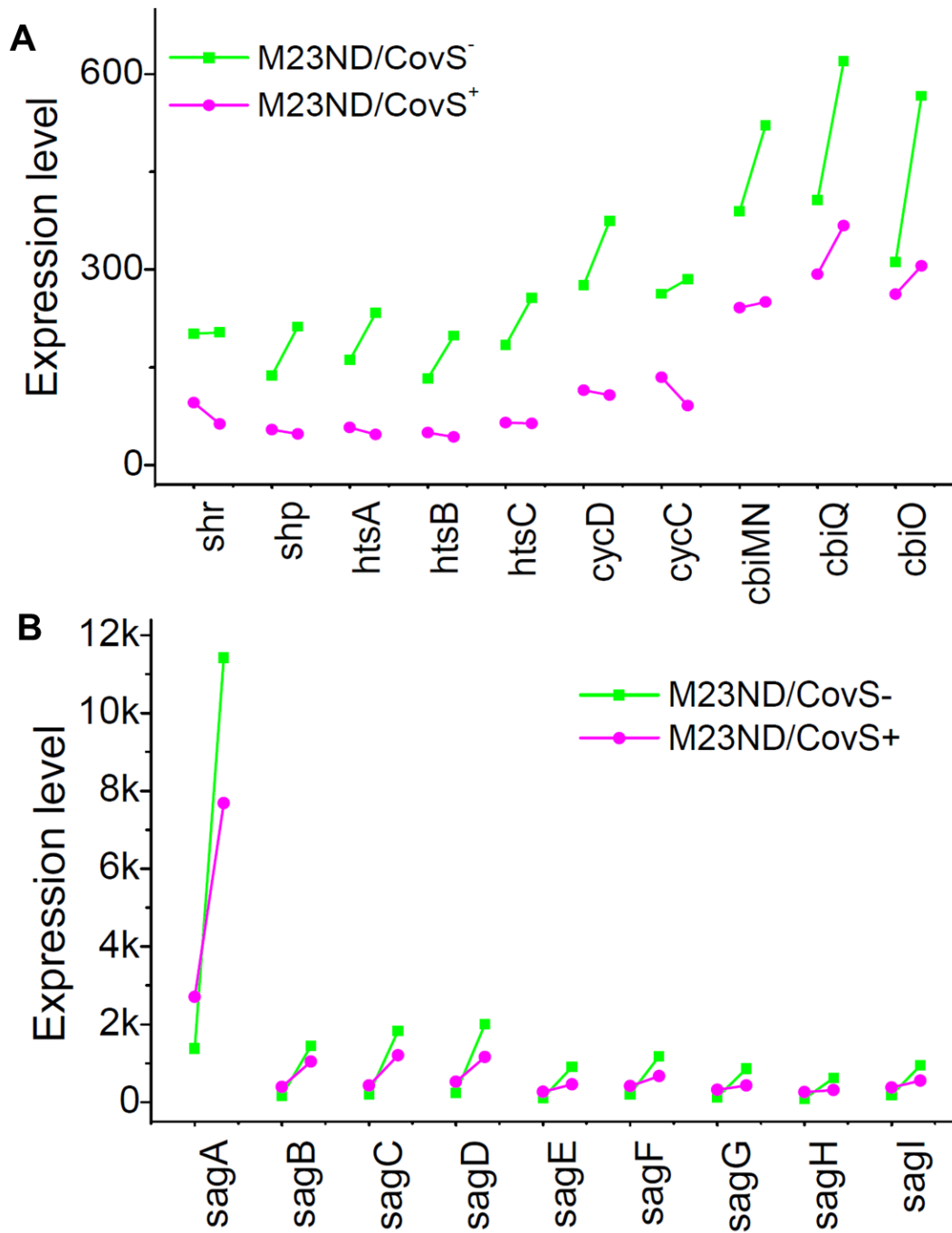


**FIG S1** The expression levels of *nga* and *speB*, along with their respective inhibitor targets, *ifs* and *spi*, for CovS<sup>+</sup> and CovS<sup>-</sup> at LP and SP. (A, B). The schematic representation of the genomic location of *nga* and its inhibitor, *ifs*; and *speB* and its inhibitor, *spi*. The four genes *nga*, *ifs*, *speB*, and *spi* are highlighted in orange. (C, D) The growth-dependent and phenotype-dependent expression of the inhibitors are highly coordinated with their inhibiting target genes with ~15-fold (*ifs*) and ~118-fold (*spi*) changes, corresponding to 12-fold and 91-fold changes in *nga* and *speB*, respectively. The expression levels are shown in RPKM.



**FIG S2 Overview of the metabolic network of *S. pyogenes* M23ND.** The connecting lines represent the gene reactions and the nodes represent the metabolites. The CovS regulated nutritional genes are indicated by different colors and all other genes are indicated by blue. The different shapes of nodes represent different metabolites: squares for carbohydrates; downward triangles for cofactors; diamonds for proteins; upward triangles for amino acids; vertical ellipses for purines; horizontal ellipses for pyrimidines; trapezoids for heme

iron; filled symbols for phosphorylated metabolites; T for tRNAs; circles for other metabolites. The black solid frame depicts the bacterial cell membrane, and transporters are shown as located across the membrane.



**FIG S3 Expression profile of genes associated with heme iron utilization and hemolysin.** (A) Expression of genes in *sia* operon for heme iron utilization. (B) Expression of genes in *sagA* operon for hemolysin. The expression levels of the gene members in the operons are shown for CovS<sup>+</sup> and CovS<sup>-</sup> (vertical direction) at LP and SP growth (horizontal direction). The genes in *sia* operon exhibit robust upregulation in CovS<sup>-</sup> compared to CovS<sup>+</sup>, indicating the importance of the operon for virulence of GAS. The genes in *sagA* operon are coordinately upregulated at SP compared to LP growth. At SP growth, *sagA* operon is shown to be more activated by CovS<sup>-</sup> compared to CovS<sup>+</sup>. The expression levels are shown as RPKM.

**TABLE S1 Primers and probes for Q-RT-PCR of selected genes.**

| <b>Primer</b>  | <b>Sequence (5'→3')</b>    | <b>Sequence position<sup>#</sup><br/>(Length)</b> |
|----------------|----------------------------|---|
| <i>cfa-F</i>   | CGCACTCAGTTTTAGCTGATGATGC  | 68-340 (273)                                      |
| <i>cfa-R</i>   | CTGATAATGCTTCTACTCTTGCCCC  |   |
| <i>parE-F</i>  | GTTCAACAGATGCTACGGGATTGC   | 101-358 (258)                                     |
| <i>parE-R</i>  | GCAAACCACCTGACGTTTTGTAGC   |   |
| <i>inlA-F</i>  | CAGATCAGGGAATCGTTGTTGACC   | 257-466 (210)                                     |
| <i>inlA-R</i>  | CTTCAGCCACAATATCCGCTGGG    |   |
| <i>htpA-F</i>  | CGATGGATCAAATCAGTGCTGAAG   | 170-488 (319)                                     |
| <i>htpA-R</i>  | GTTCCTTTGGCTACTTGCTCAGC    |   |
| <i>ifs-F</i>   | GGGAGATATTTCTGATCCAGAAG    | 3-338 (336)                                       |
| <i>ifs-R</i>   | CTTTCGACCATATCAAGCATAG     |   |
| <i>hypo0-F</i> | GGAGGAAGCACTTGTTTAGCAGATG  | 97-398 (302)                                      |
| <i>hypo0-R</i> | CCATCTTGACTGCCTTGGCTAAATG  |   |
| <i>htsA-F</i>  | GTGTGAATCAGCACCTAAAACGGC   | 59-384 (326)                                      |
| <i>htsA-R</i>  | CTGGTACATGCCCTCAACACTTCG   |   |
| <i>shr-F</i>   | CAGTCACAACCTCGTGACAACAGTAG | 94-488 (395)                                      |
| <i>shr-R</i>   | GTGCCTTGGTTTTGTGGCACTGGT   |   |
| <i>shp-F</i>   | GCAGATAGAAGATAGTGGAGGGGAG  | 99-284 (186)                                      |
| <i>shp-R</i>   | CTTCCAGTTCCTCCAGGTTGTATCC  |   |
| <i>mtlA-F</i>  | CATTGGTGCATTTATTGCCTGGGG   | 75-328 (254)                                      |
| <i>mtlA-R</i>  | CACCAAGTGGTCCCATTGCCATTG   |   |
| <i>gatC-F</i>  | CTATTCAGTTGGGATCAGGTGTGG   | 47-346 (306)                                      |
| <i>gatC-R</i>  | CAATATTAACCACCAGCATCACCAGC |   |
| <i>ptsD-F</i>  | GTCTTTTGGCGCTCATGGACGATG   | 321-52 (270)                                      |
| <i>ptsD-R</i>  | CGAAGCCTTAACAGCAGTGATAGC   |   |
| <i>atpE-F</i>  | CCTATTTTCGCATTGGCTTTGGCC   | 7-190 (184)                                       |
| <i>atpE-R</i>  | GAATGAAGGCCATAACAAGGGTAAC  |   |
| <i>atpB-F</i>  | GACCCTACTTGCTGTTTGTATTGTC  | 54-354 (301)                                      |
| <i>atpB-R</i>  | GTCAAAAGCCAGATTGGCTGTTGG   |   |
| <i>plr-F</i>   | GACGGTACTGAAACAGTTATCTC    | 241-655 (234)                                     |
| <i>plr-R</i>   | GATAGCTTTAGCAGCACCAGTTG    |   |

# Numbering is from the position of start codon of the specific gene. Length refers to the amplicon length.

**TABLE S2 Sequencing statistics of the RNA samples.**

| Strain/Condition       | <i>covS</i> <sup>-</sup> |           |           |           | <i>covS</i> <sup>+</sup> |           |           |           |
|------------------------|--------------------------|-----------|-----------|-----------|--------------------------|-----------|-----------|-----------|
|                        | LP1                      | LP2       | SP1       | SP2       | LP1                      | LP2       | SP1       | SP2       |
| <b># of Reads</b>      | 5,847,068                | 9,700,716 | 4,190,672 | 6,087,132 | 6,918,530                | 6,112,658 | 6,739,006 | 5,084,304 |
| <b>Yields</b>          | 468Mbp                   | 776Mbp    | 335Mbp    | 487Mbp    | 553Mbp                   | 489Mbp    | 539Mbp    | 407Mbp    |
| <b>Non-redundant</b>   | 3,461,470                | 5,625,656 | 2,327,868 | 3,206,068 | 4,039,690                | 3,766,152 | 3,589,934 | 2,533,308 |
| <b>Yields</b>          | 277Mbp                   | 450Mbp    | 186Mbp    | 256Mbp    | 323Mbp                   | 301Mbp    | 287Mbp    | 203Mbp    |
| <b>% non-redundant</b> | 59.20                    | 57.99     | 55.55     | 52.67     | 58.39                    | 61.61     | 53.27     | 49.83     |
| <b>Total mapped</b>    | 3,305,756                | 4,900,081 | 2,252,833 | 3,099,328 | 3,840,394                | 3,570,483 | 3,149,839 | 2,435,677 |
| <b>% mapped</b>        | 95.50                    | 87.10     | 96.78     | 96.67     | 95.07                    | 94.80     | 87.74     | 96.15     |
| <b>Unique mapped</b>   | 3,111,550                | 4,573,396 | 2,085,780 | 2,759,400 | 3,668,997                | 3,412,414 | 2,904,766 | 2,248,705 |
| <b>% Unique mapped</b> | 89.89                    | 81.30     | 89.60     | 86.07     | 90.82                    | 90.61     | 80.91     | 88.77     |

**TABLE S3 Fold changes and conservation of differentially regulated hypothetical genes at LP or SP**

| Gene ID   | Gene Symbol | covS+ vs. covS- |             |       |             | Conservation <sup>#</sup>                       |
|-----------|-------------|-----------------|-------------|-------|-------------|---|
|           |             | LP              |             | SP    |             |   |
|           |             | Fold            | FDR p-value | Fold  | FDR p-value |   |
| FE90_0104 | hypo0       | -49.9           | 0           | -15.6 | 0           | Unique to M23ND                                 |
| FE90_1770 | hypo1       | -49.8           | 0           | -7.5  | 0           | Conserved                                       |
| FE90_1362 | hypo2       | -15.6           | 0           | -14.4 | 0           | Conserved                                       |
| FE90_1535 | hypo4       | -21.4           | 0           | -22.4 | 0           | Average similarity < 50%                        |
| FE90_1726 | hypo6       | 1.8             | 0           | 10.3  | 0           | Conserved                                       |
| FE90_1729 | hypo7       | -5.6            | 0           | -3.6  | 0           | Conserved                                       |
| FE90_1733 | hypo8       | 1.3             | 0.559       | 90.1  | 0           | Conserved                                       |
| FE90_1734 | hypo9       | 1.9             | 0.091       | 52.2  | 0           | Conserved                                       |
| FE90_0391 | hypo10      | 1.3             | 0.003       | 1.7   | 0           | Encoded in 20% of M types of <i>S. pyogenes</i> |
| FE90_0381 | hypo11      | 1.3             | 0.020       | 2.1   | 0           | Conserved                                       |
| FE90_0500 | hypo13      | 1.4             | 0.014       | 2.4   | 0           | Conserved                                       |
| FE90_0648 | hypo14      | 9.9             | 1           | 3.0   | 0           | Encoded in 60% of M types of <i>S. pyogenes</i> |
| FE90_0676 | hypo15      | -1.6            | 0           | -1.2  | 0.064       | Conserved                                       |
| FE90_1011 | hypo16      | -2.4            | 0.024       | -2.4  | 0           | Conserved                                       |
| FE90_1015 | hypo17      | -2.1            | 0           | -3.0  | 0           | Conserved                                       |
| FE90_1083 | hypo18      | 2.0             | 0           | 1.5   | 0.000       | Conserved                                       |
| FE90_1390 | hypo19      | -4.4            | 0           | -2.1  | 0           | Conserved                                       |
| FE90_1524 | hypo20      | 1.3             | 0.033       | 1.9   | 0           | Conserved                                       |
| FE90_1532 | hypo21      | -9.2            | 0           | -8.1  | 0           | Average similarity < 50%                        |
| FE90_1673 | hypo22      | -2.6            | 0           | -4.3  | 0           | Conserved                                       |
| FE90_1830 | hypo24      | -1.2            | 0.163       | -2.1  | 0           | Conserved                                       |
| FE90_0569 | hypo25      | -1.7            | 0.024       | 1.8   | 0           | Conserved                                       |
| FE90_0571 | hypo26      | -1.0            | 0.952       | 2.7   | 0           | Conserved                                       |
| FE90_1664 | hypo27      | 1.1             | 0.631       | 3.5   | 0           | Conserved                                       |
| FE90_1666 | hypo28      | 1.1             | 0.409       | 2.2   | 0           | Conserved                                       |
| FE90_1442 | hypo29      | -2.5            | 0           | -2.7  | 0           | Conserved                                       |

<sup>#</sup> Conservation was assessed by comparing each gene to the homologs from other strains of *S. pyogenes* with known genome sequences at both nucleotide and amino acid level.