

## Supplementary Material

# Physalin H, physalin B, and isophysalin B suppress the quorum-sensing function of *Staphylococcus aureus* by binding to AgrA

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## 1 SUPPLEMENTARY TABLES

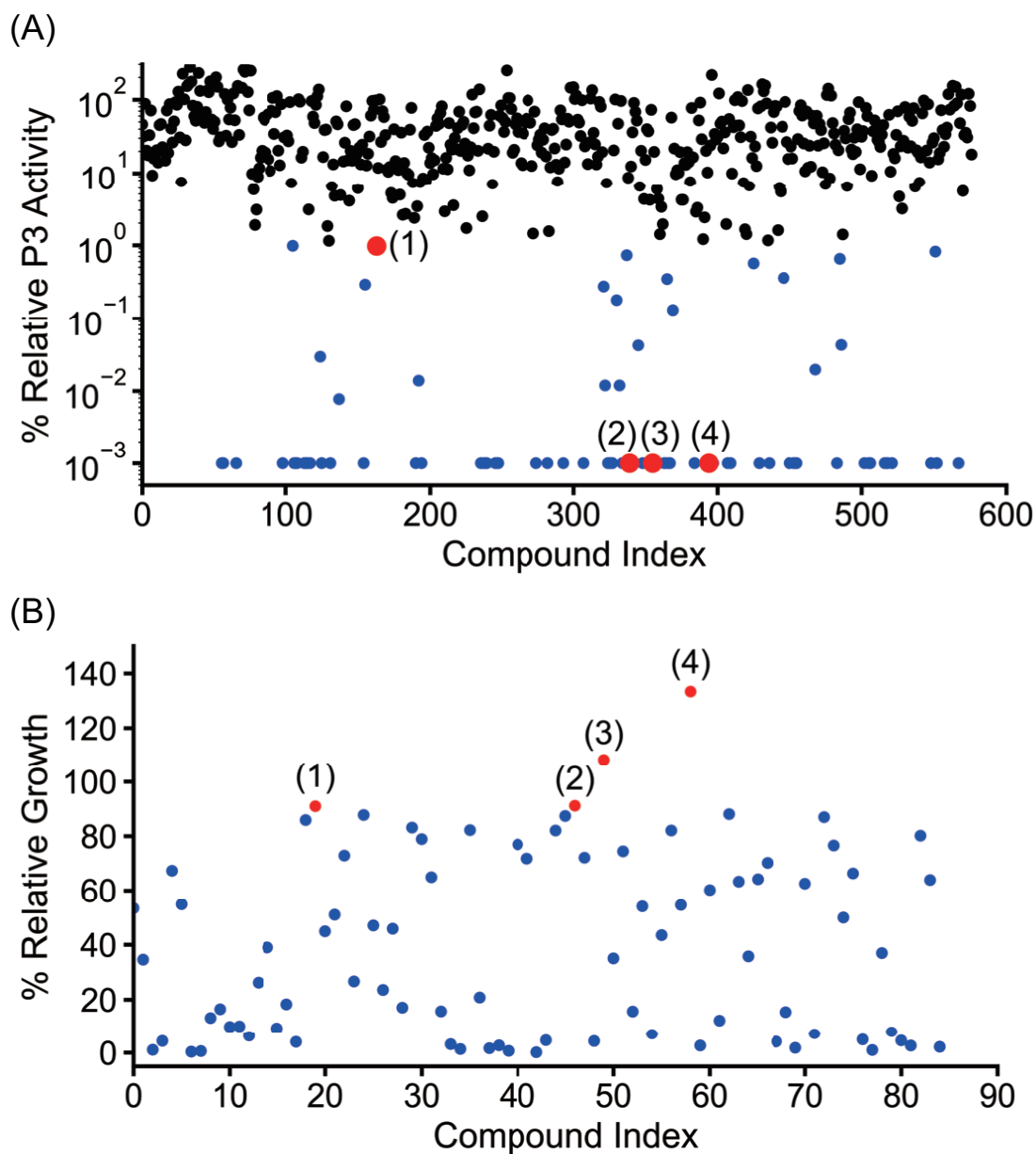
**Table S1.** Oligonucleotide sequences used in this study

| Primers        | Sequence (5'—3')                                                                                          |
|----------------|-----------------------------------------------------------------------------------------------------------|
| BamHI-AgrA-F   | ACGGATCCGATAATAGCGTTGAAACGATTG                                                                            |
| HindIII-AgrA-R | AATTAAGCTTTTATATTTTTTTAAACGTTTCTCACC                                                                      |
| gyrB-F         | CGCAGGCGATTTTACCATTA                                                                                      |
| gyrB-R         | GCTTTCGCTAGATCAAAGTCG                                                                                     |
| RNAIII-F       | GCCATCCCAACTTAATAACCA                                                                                     |
| RNAIII-R       | TGTTGTTTACGATAGCTTACATGC                                                                                  |
| agrA-F         | TGATAATCCTTATGAGGTGCTTGA                                                                                  |
| agrA-R         | CACTGTGACTCGTAACGAAAATAAT                                                                                 |
| hla-F          | CCCGGTATATGGCAATCAAC                                                                                      |
| hla-R          | GGTAGTCATCACGAACCTCGT                                                                                     |
| psma-F         | ATCATCGCTGGCATCA                                                                                          |
| psma-R         | AAGACCTCCTTTGTTTGTTATG                                                                                    |
| Cy5-agrP3-F    | AAAACATGCTAAAAGCATTATTTTCCAATTTTCTTAACTAGTCGTTTT<br>TTATTCTTAACTGTAAATTTTTTTATGTTAAAATATTAAATACAAATTAC    |
| Cy5-agrP3-R    | GTAATTTGTATTTAATATTTTAAACATAAAAAAATTTACAGTTAAGAATAAA<br>AAACGACTAGTTAAGAAAAATTGGAAAATAAATGCTTTTAGCATGTTTT |
| Cy5-LytTR-F    | AAATACTTAACTGTAAAT                                                                                        |
| Cy5-LytTR-R    | ATTTAACAGTTAAGTATTT                                                                                       |

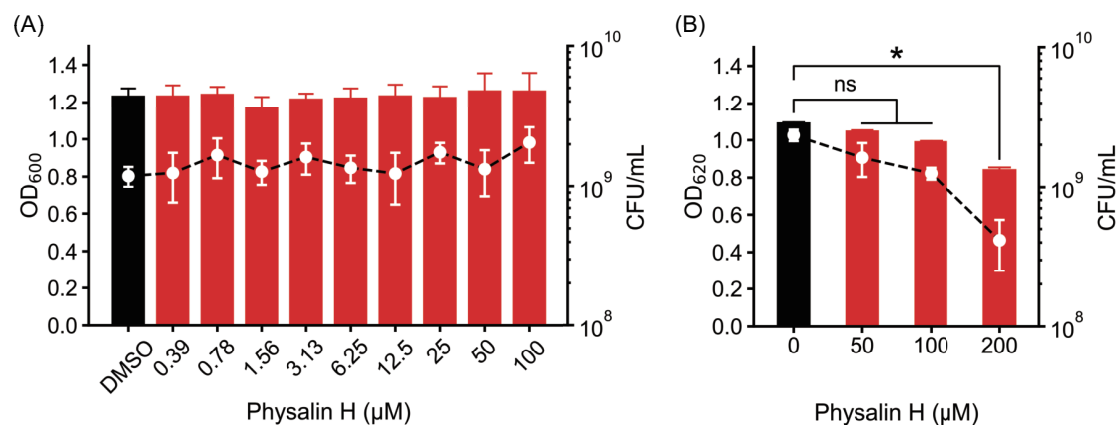
**Table S2.** Physicochemical properties of physalins used in this study

| PS | Compound name                                                       | MW     | Fraction Csp3 | H-bond acceptors | Molar Refractivity | TPSA   | Mol-LogP |
|----|---------------------------------------------------------------------|--------|---------------|------------------|--------------------|--------|----------|
| 1  | physalin H                                                          | 562.99 | 0.79          | 10               | 131.25             | 145.66 | 0.96     |
| 2  | physalin F                                                          | 526.53 | 0.79          | 10               | 124.27             | 137.96 | 0.76     |
| 3  | physalin B                                                          | 510.53 | 0.71          | 9                | 124.78             | 125.43 | 1.5      |
| 4  | isophysalin B                                                       | 510.53 | 0.71          | 9                | 124.78             | 125.43 | 1.5      |
| 5  | 5 $\alpha$ -hydroperoxy<br>-6,7-didehydro-5,6-<br>dihydrophysalin B | 542.53 | 0.71          | 11               | 127.48             | 154.89 | 1.02     |
| 6  | physalin K                                                          | 558.53 | 0.79          | 12               | 126.51             | 164.12 | -0.32    |
| 7  | physalin D                                                          | 544.55 | 0.79          | 11               | 127.62             | 165.89 | -0.27    |
| 8  | physalin XII                                                        | 558.53 | 0.75          | 12               | 127.82             | 182.96 | -1.09    |
| 9  | physalin G                                                          | 526.53 | 0.71          | 10               | 125.95             | 145.66 | 0.52     |

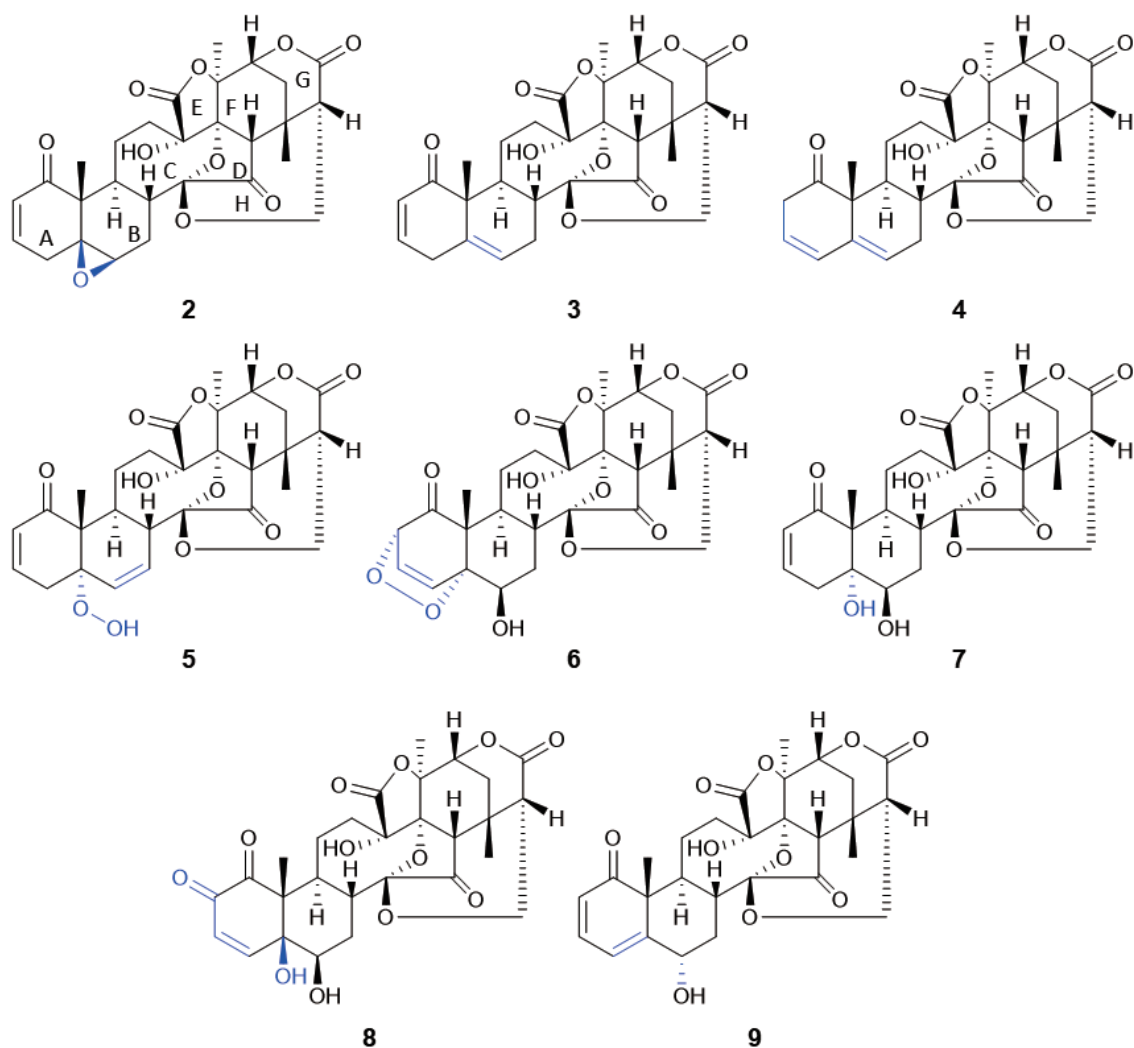
## 2 SUPPLEMENTARY FIGURES



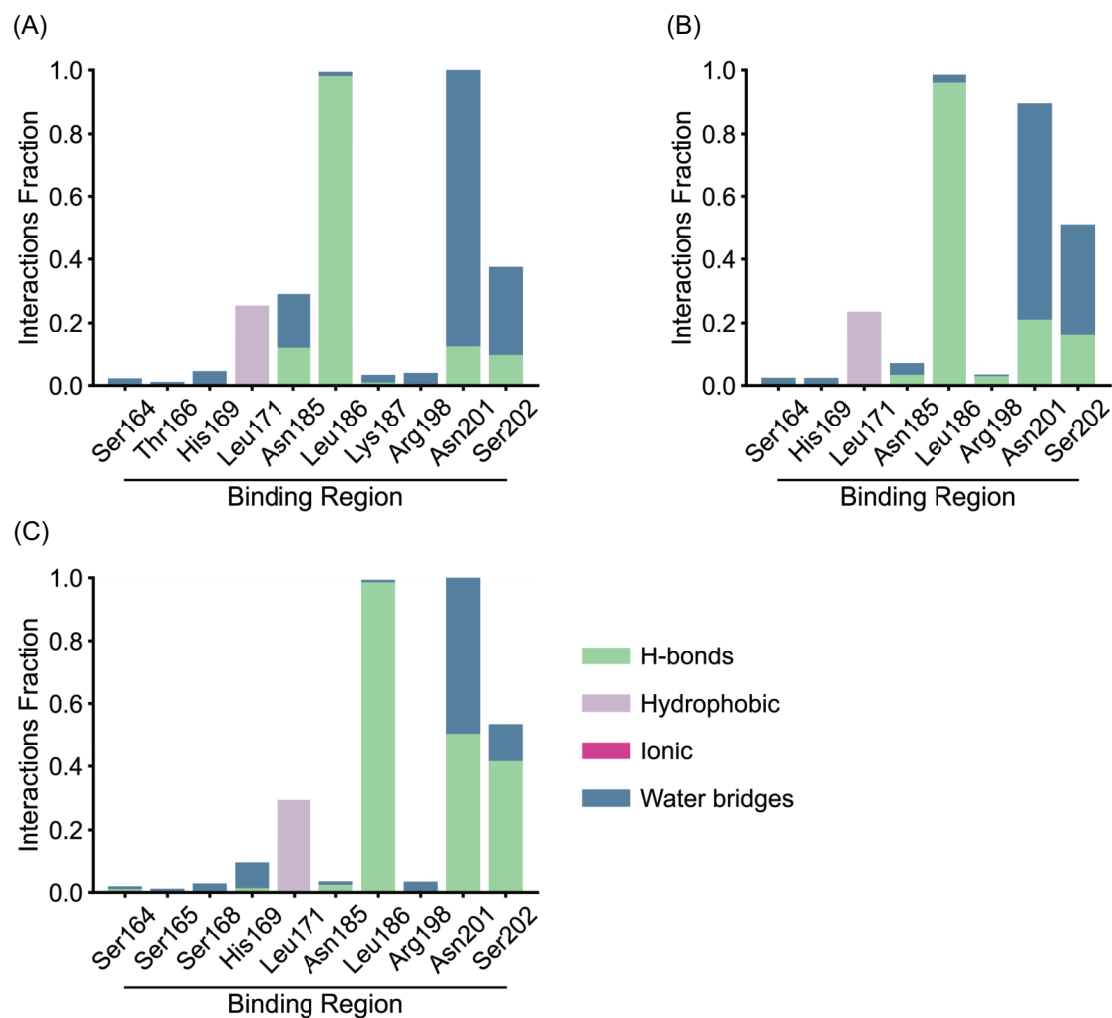
**Figure S1.** The result of screening of Agr-QS suppression compounds. **(A)** Relative P3 promoter activity in 24 hours of incubation for 577 compounds at screening. Relative P3 promoter activity above 1% is shown in black, less than 1% in blue. **(B)** Relative growth in 24 hours of incubation for 85 compounds at screening. The four hit compounds are shown in red. The four hit compounds (red) are (1) lasidiol *p*-methoxybenzoate, (2) physalin H, (3) ovatodiolide, and (4) ceanothic acid.



**Figure S2.** The correlation between OD (bar) and colony-forming unit (CFU) (white circle) in the presence of physalin H at various concentrations. **(A)** OD<sub>600</sub> and CFU of *agr::P3-luc* strain when cultured for 24 h added to media containing serial concentrations of physalin H. **(B)** OD<sub>620</sub> and CFU after 24 h of culture with LAC strain at  $2.5 \times 10^5$  CFU/mL and 50, 100, 200 μM physalin H and 1% DMSO (n=3). Data are presented as the mean  $\pm$  SD. \* $p < 0.05$ , ns, not significantly different, unpaired *t*-test.



**Figure S3.** The structures of physalins, PSs2–9, isolated from *Physalis minima*. The blue part in each structure indicates a different part from the structure of physalin H.



**Figure S4.** Interaction analysis with AgrA residues in molecular dynamics simulations of PS2 (A), PS3 (B), and PS4 (C).