Haemoglobin modulates salicylate and jasmonate/ethylene-mediated resistance mechanisms against pathogens

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Supplementary Figure 1: Arabidopsis rosettes used to assess trace gas emissions following pathogen attack.

Arabidopsis rosettes of Col-0 and the Glb line photographed prior to measurement of trrace gas measurements using either the Quantum Casade Laser (for NO) or laser photoacoustic detector (for ethylene). Bar = 1 cm

Supplementary Figure 2: The Quantum Cascade Laser (QCL)-based sensor adapted for Nitric Oxide detection.

A quantum cascade laser (QCL) emitting around 1900 cm^{-1} wavelength passes through a multi-pass cell where the NO released by the biological samples is transported with a carrier flow of air via gas tubing to the multi-pass cell at a flow rate of 1 L/h. The intensity of the laser (I0) is strongly attenuated due to the NO absorption of the light in the multi-pass cell (effective path length L = 76 m), following the Beer–Lambert law. The detected signal (I) depends of the laser intensity (I0), the absorption path length (L) and the molar absorption coefficient (ε) of NO at this wavelength. The NO concentration is calculated by measuring the attenuation of the light (I/I0).

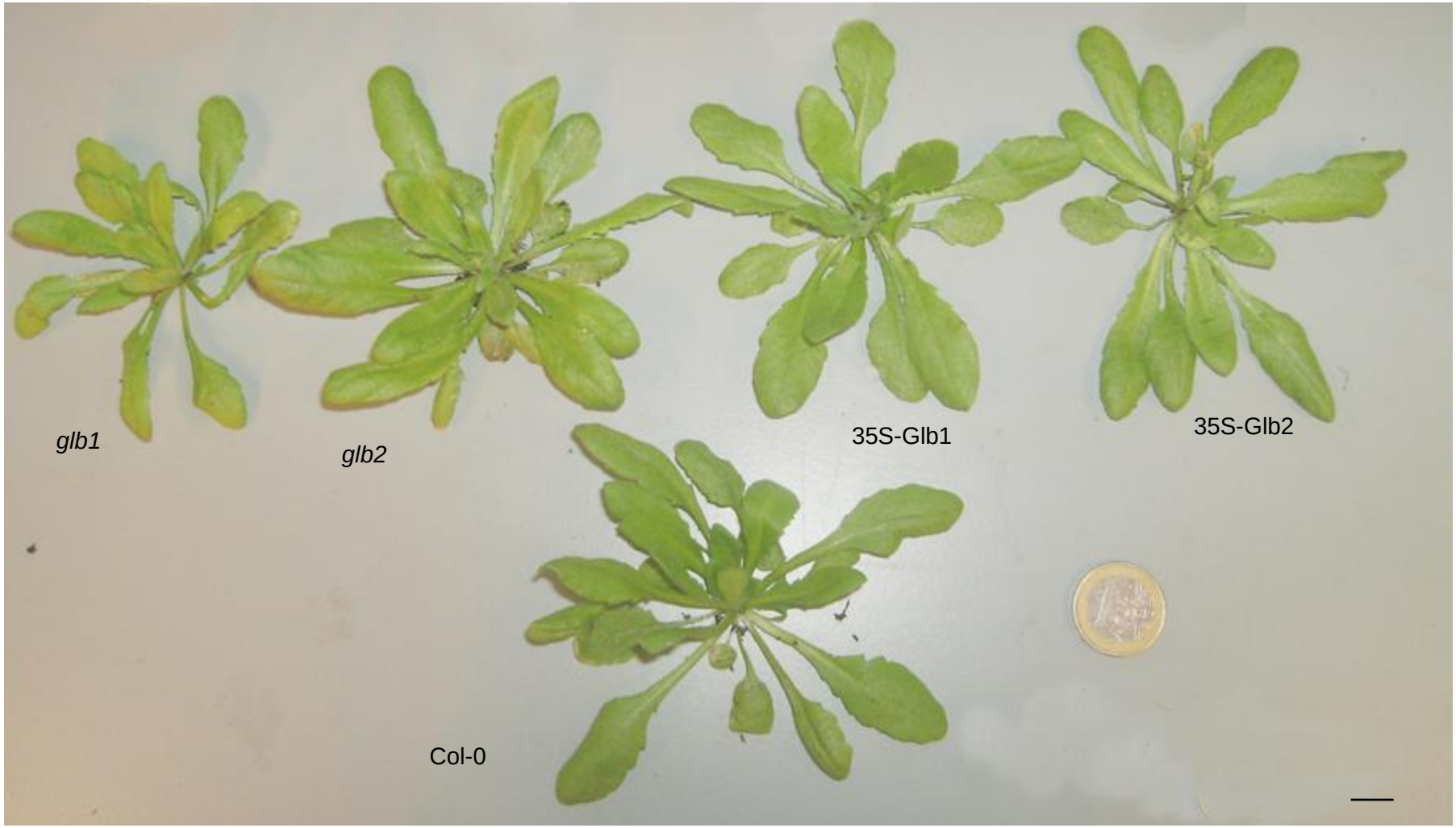
Supplementary Figure 3: Glb1 and Glb2 expression in the over-expression lines.

Detection of Glb1 and Glb2 protein accumulation in protein extracts from mature rosettes of Col-0, 35S-GLB1 and 35S-GLB2. Note the over-accumulation of GLB1 in 35S-GLB1 and GLB2 in 35S-GLB2 lines.

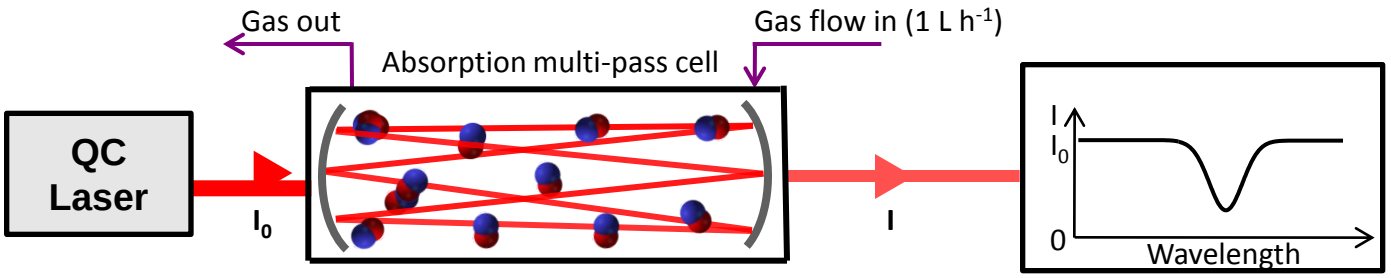
Supplementary Figure 4: Nitric oxide, salicylic acid and ethylene production and from Arabidopsis lines with modified hemoglobin expression on inoculation with *Pseudomonas syringae* pv. *tomato.*

(A) NO production was determined from *Arabidopsis* Col-0 and the hemoglobin (Hb) *GLB11* RNAi suppressed Arabidopsis line *glb1* and the CaMV 35S- *GLB11* over-expression line, following inoculation with virulent *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 strains. NO was determined using a Quantum Cascade Laser system. (**B)** Salicylic acid accumulation at 48 h post inoculation of *Arabidopsis* Col-0, *glb1* and *35S-GLB1* with *Pst* (grey bar) or mock-inoculated with 10 mM $MgCl_2$ (White bar). Results are given as mean μmol SA (n = 6) per g fresh weight (g fwt) ± SE. Statistical comparison was made between inoculated Col-0 with *glb1* or *35S-GLB1* plants and also between mock-inoculated and *glb1* or *35S-GLB1* plants. Levels of significant are indicated: NS = no significant difference; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. (**C)** Ethylene production was determined using laser photoacoustic detection (LPAD) from *Arabidopsis* Col-0 and *glb1* and *35S-GLB11* following inoculation *Pst*.

Supplementary Figure 1 .



Supplementary Figure 2



Beer Lambert law

$I = I_0 \times \exp(-\varepsilon \times C \times L)$

I = transmitted laser intensity

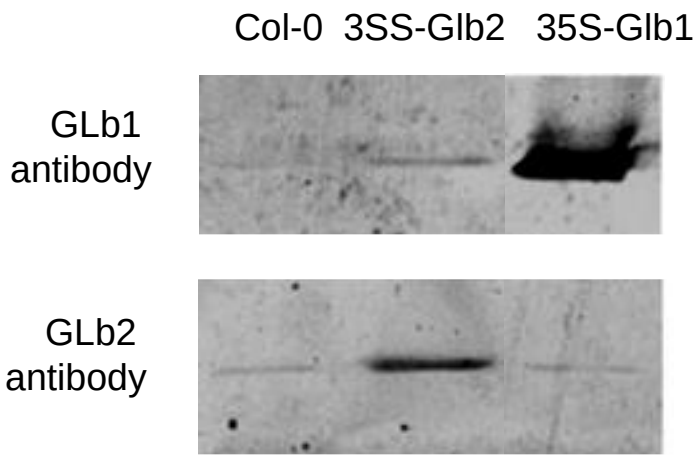
I_0 = incident laser intensity

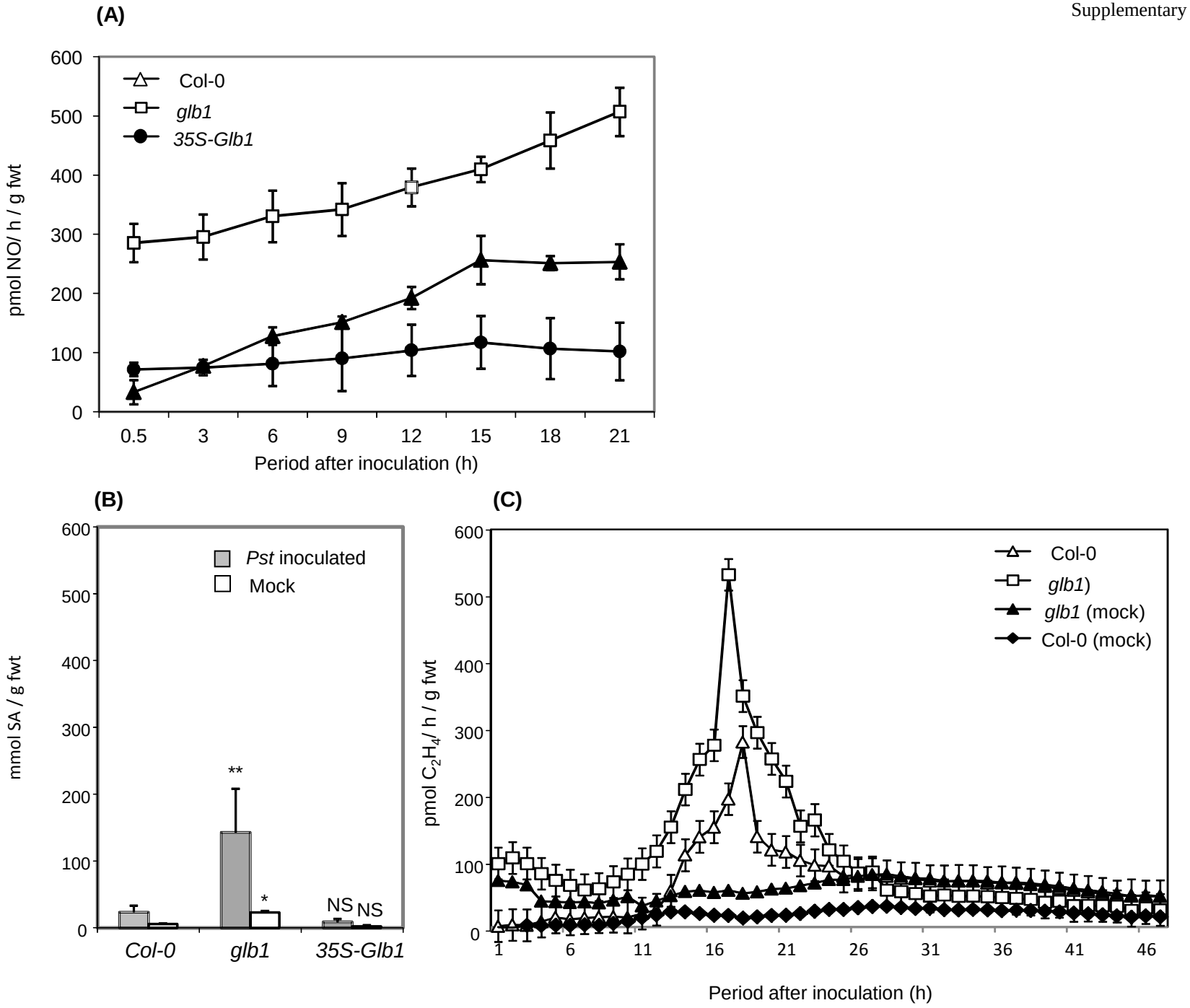
ε = molar absorption coefficient

L = absorption length

C = NO concentration

Supplementary Figure 3.




(A)
Col-0
glb1
35S-Glb1
pmol NO/ h / g fwt
Period after inoculation (h)
(B)
Pst inoculated
Mock
mmol SA / g fwt
**
*
NS
NS
Col-0
glb1
35S-Glb1
(C)
Col-0
glb1)
glb1 (mock)
Col-0 (mock)
pmol C2H4/ h / g fwt
Period after inoculation (h)