

## Supplementary Material

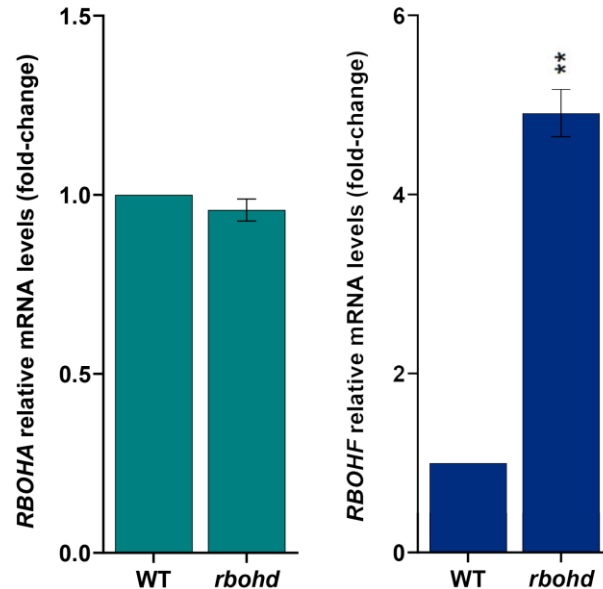
### Distinct role of AtCuAO $\beta$ - and RBOHD-driven H<sub>2</sub>O<sub>2</sub> production in wound-induced local and systemic leaf-to-leaf and root-to-leaf stomatal closure

Ilaria Fraudentali, Chiara Pedalino, Riccardo D'Incà, Paraskevi Tavladoraki, Riccardo Angelini, Alessandra Cona \*

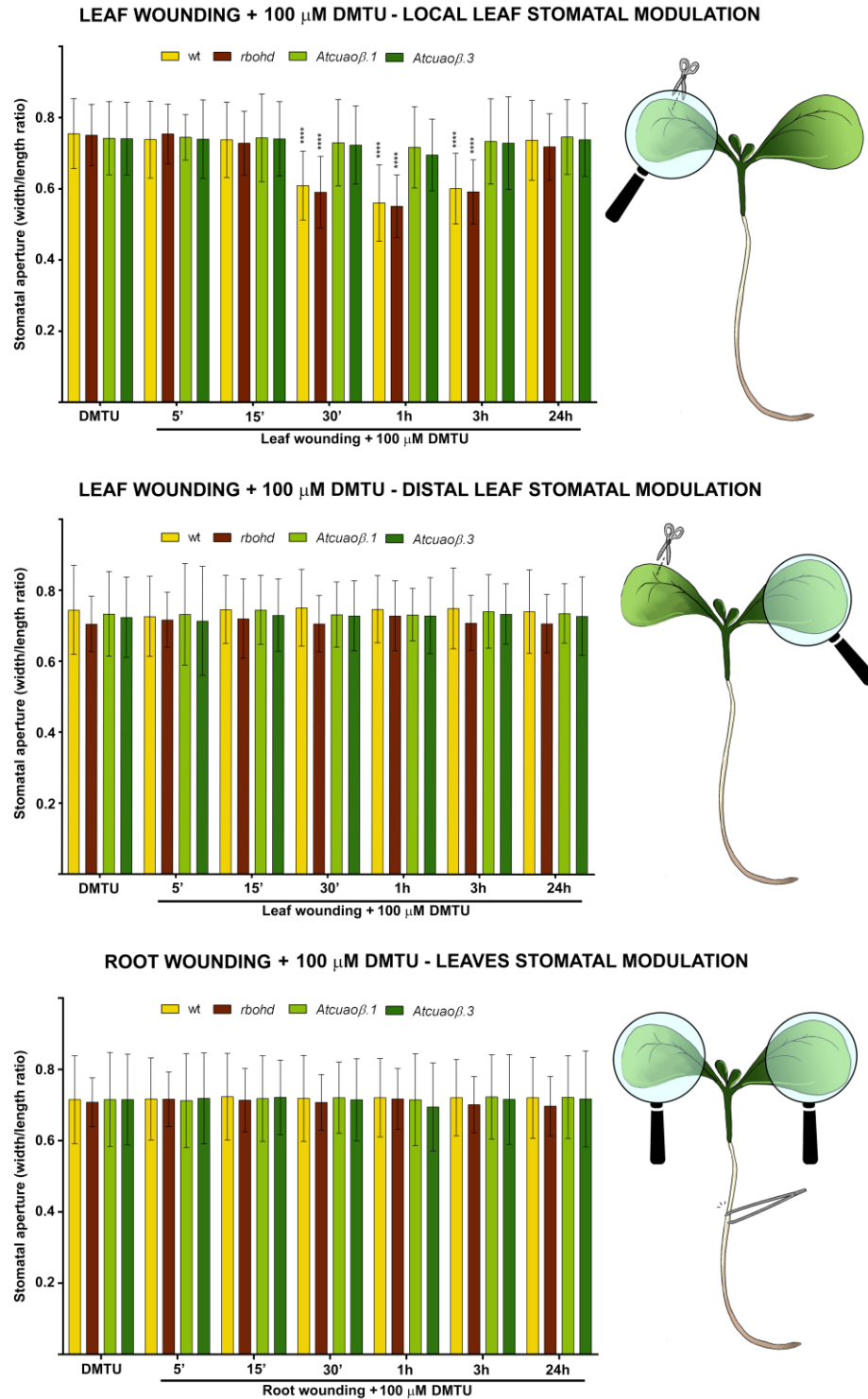
\* Correspondence:

alessandra.cona@uniroma3.it

#### 1 Supplementary Figures

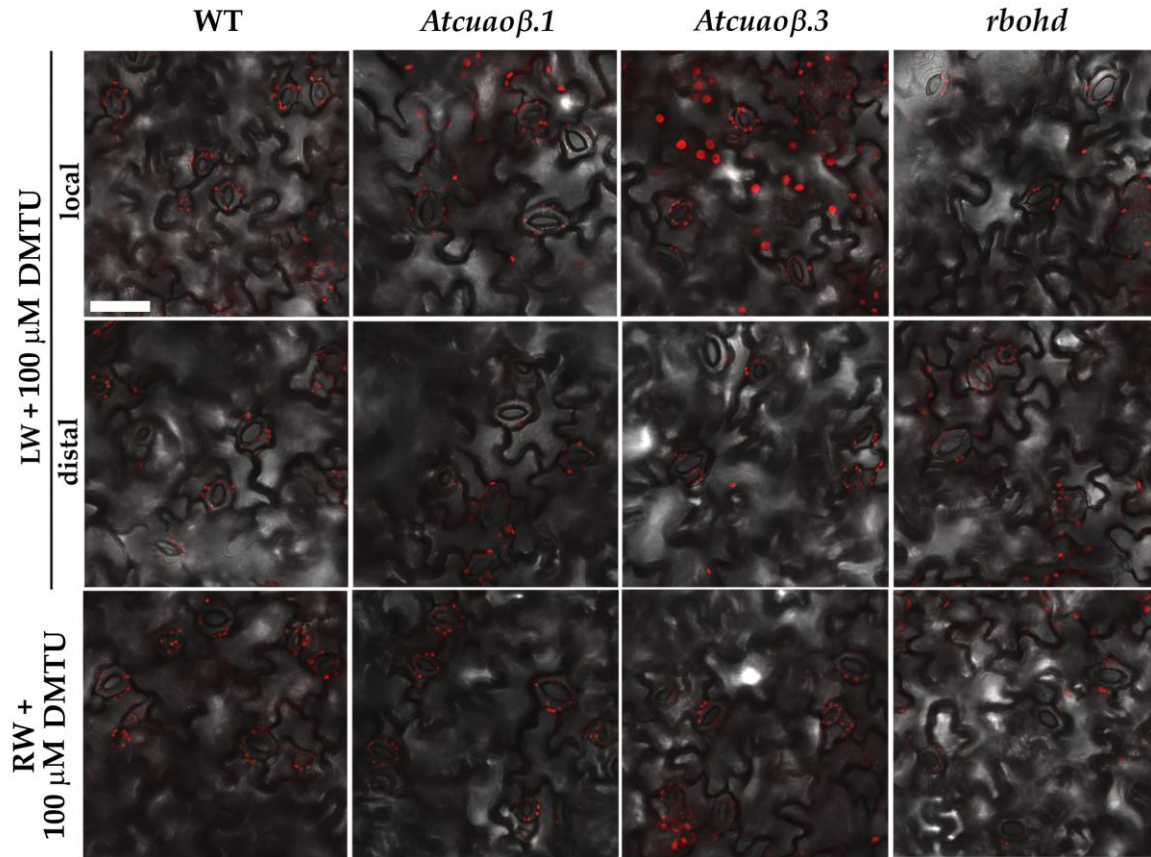


**Supplementary Figure 1.** Analysis of *RBOHA* and *RBOHF* constitutive expression by RT-qPCR in 7-day-old WT and *rbohD* seedlings. The reported values of fold-inductions in *rbohD* are relative to WT, with the value for WT assumed to be one. Data are the result of three biological replicates, each with three technical replicates (mean values  $\pm$  SD;  $n = 3$ ). The significance levels between relative mRNA levels are reported only when  $p \leq 0.05$ . \*\*,  $p$  levels  $\leq 0.01$ .



**Supplementary Figure 2.** Effect of 100  $\mu$ M DMTU combined with cotyledonary-leaf wounding or root wounding on stomatal pore modulation of 7-day-old WT, *Atcuaob*, and *rbohD* mutant seedlings. Cotyledonary-leaf or root was injured and seedlings were incubated in presence of 100  $\mu$ M DMTU for 5, 15, 30 min, 1, 3 and 24 h. After cotyledonary-leaf wounding, analyses of stomatal pore modulation

were carried out separately measuring stomata in wounded cotyledonary-leaves (local effect, top panel) and stomata in distal unwounded cotyledonary-leaves (distal effect, middle panel). After root wounding, analyses of stomatal pore modulation were carried out measuring stomata randomly chosen from both cotyledonary-leaves of each seedling (distal effect, bottom panel). Mean values  $\pm$  SD ( $n = 15$ ) are reported. Significance levels between unwounded and wounded DMTU treated seedlings of each genotype are reported.  $p$  levels have been calculated with one-way ANOVA analysis;  $p$  levels  $> 0,05$ ; \*\*\*\*  $p$  levels  $\leq 0,0001$ . If not shown, the statistical difference is not significant.



**Supplementary Figure 3.** Effect of 100  $\mu$ M DMTU combined with cotyledonary-leaf wounding or root wounding on ROS production in guard cells of 7-day-old seedling. *In situ* ROS detection by LSCM analyses after CM-H<sub>2</sub>DCFDA staining were carried out in cotyledonary-leaves of WT, *Atcuaob.1*, *Atcuaob.3*, and *rbohD* seedlings. Stomata of wounded cotyledons (LW local), distal unwounded cotyledons (LW distal) and cotyledons from root wounded seedlings (RW) were observed 1 h after the injury combined with DMTU treatment. Micrographs are representative of those obtained from five independent experiments, each time analyzing cotyledonary leaves from five seedlings per genotype and treatment. Bar = 50  $\mu$ m. DMTU, *N,N*<sup>1</sup>-dimethylthiourea; LW, leaf wounding; RW, root wounding.