

Supplements

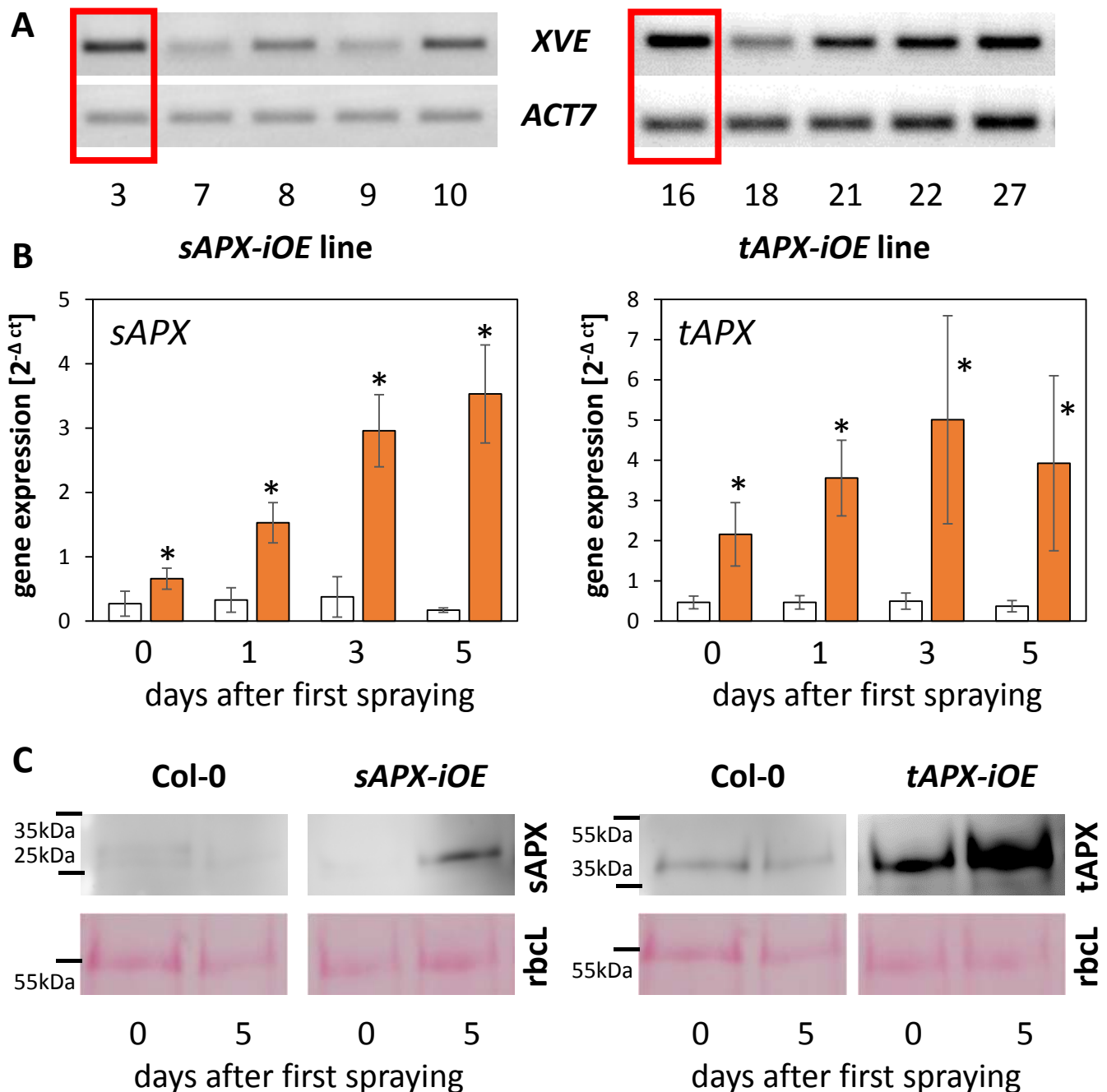
Cold-priming of chloroplast ROS signalling is developmentally regulated and is locally controlled at the thylakoid membrane

Jörn van Buer, Andreas Prescher, Margarete Baier

Supplement 1

Cold-priming of chloroplast ROS signalling is developmentally regulated and is locally controlled at the thylakoid membrane

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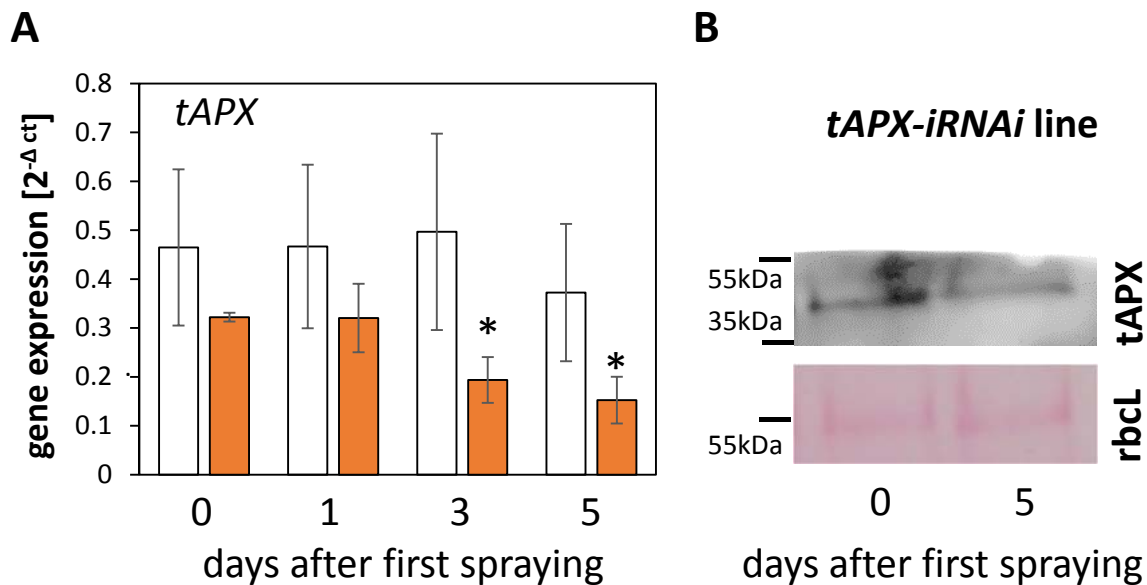


Supplement 1: Selection and pre-testing of the *sAPX-iOE* and *tAPX-iOE* lines. (A) Screening of independent, homozygous transgenic lines for the expression intensity of the transactivator *XVE*. The lines used for subsequent experiments are boxed red. (B) Transcript levels of *sAPX* and *tAPX* in the over-expression lines after spraying with estradiol at day 0 and 3 (orange) relative to the transcript levels in parallel grown Col-0 plants (white). Asterisks label transcript levels significantly higher than the levels in Col-0 plants (t-Test $p < 0.05$; $n = 4$). (C) *sAPX* and *tAPX* protein amounts in the same iOE-lines 0 and 5 days after the first spraying. Gel loading was controlled by Ponceau S staining of the Western blot membrane. The strongest band, representing the large subunit of RUBISCO, *rbcL*, is shown.

Supplement 2

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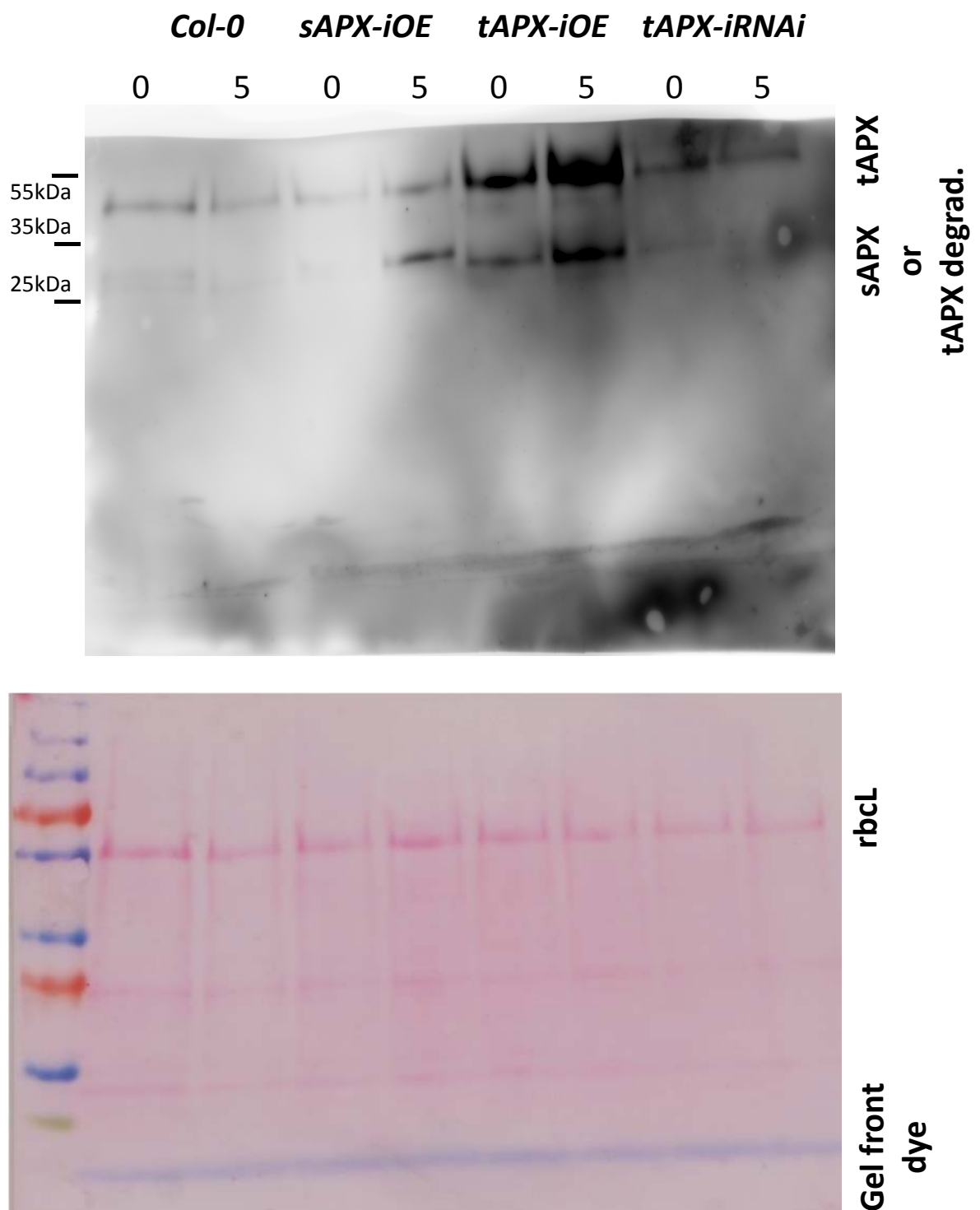


Supplement 2: Pre-testing of the *tAPX-iRNAi* line. (A) Transcript levels of *tAPX* in the inducible RNAi after spraying with estradiol at day 0 and 3 (orange) relative to the transcript levels in parallel grown Col-0 plants (white). Stars label transcript levels significantly higher than the levels in Col-0 plants (t-Test $p < 0.05$; $n = 4$). (B) *tAPX* protein amounts in the same RNAi-line 0 and 5 days after the first spraying. Gel loading was controlled by Ponceau S staining of the Western blot membrane. The strongest band, representing the large subunit of RUBISCO, *rbcl*, is shown.

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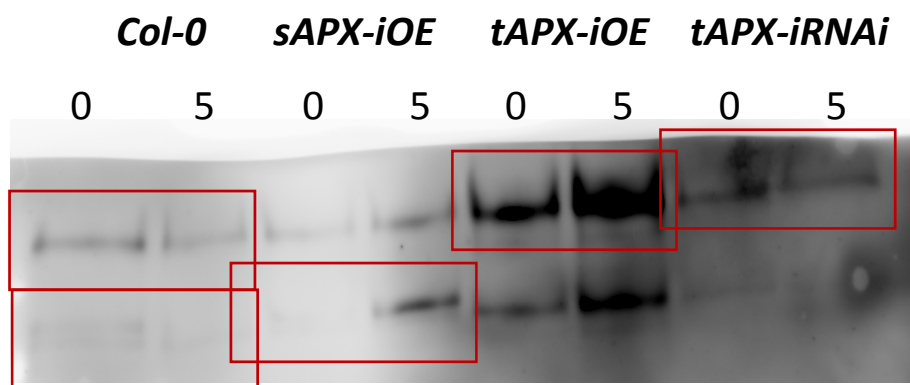
All Western Blot data and Ponceau S-staining data were taken from the same membrane, which was first stained with Ponceau-S and then treated with antibodies and the detection agent.



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The Western-Blot parts shown in Suppl. 1 and Suppl. 2 are boxed in red.



The lower band in the *sAPX-iOE* line is smaller than *sAPX* and has been interpreted as *tAPX* degradation product / artefact, which accumulates proportional with *tAPX*.

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From populations of independent transgenic lines, the information on the first 5 lines is shown in supplement 2. The ACT7- and XVE-PCRs were run for each iOE construct on the same gel in the same order.

