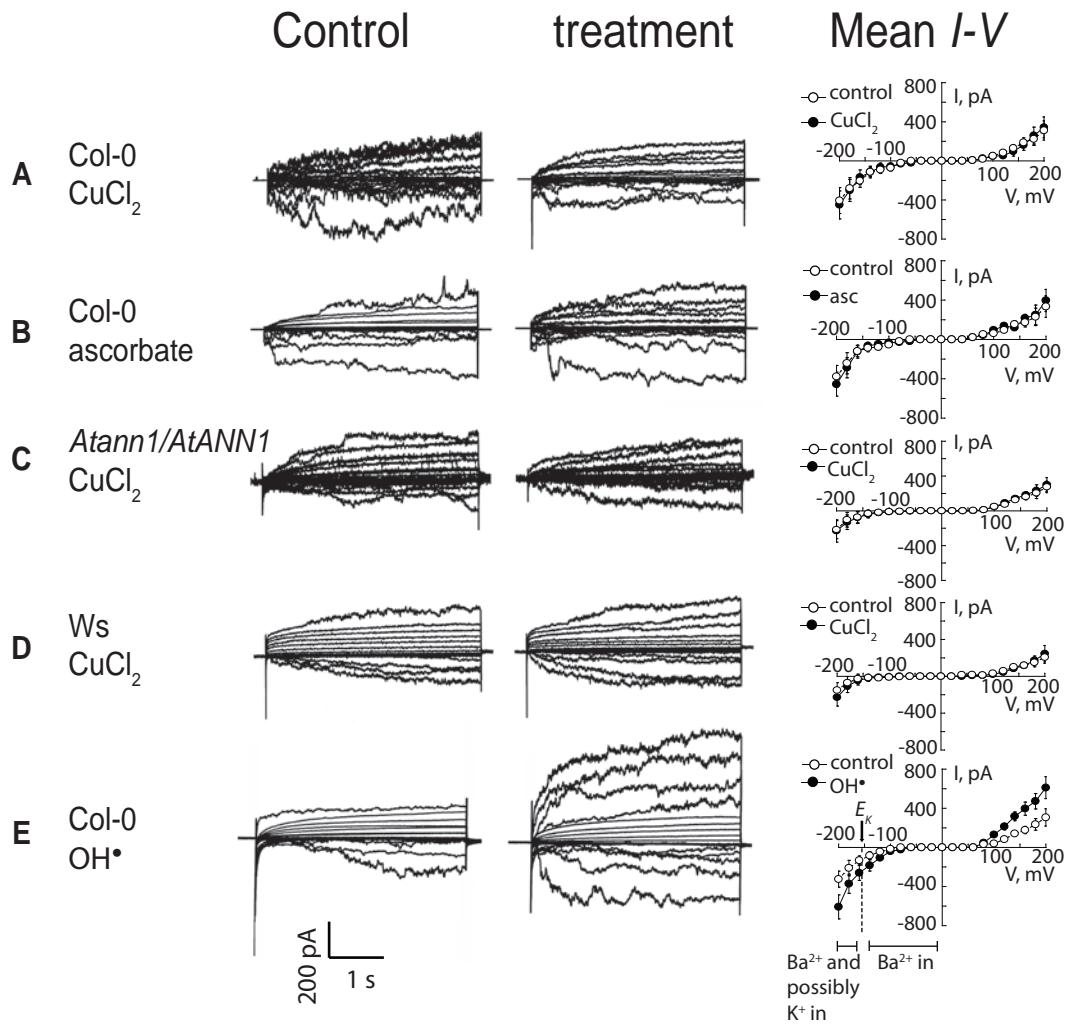


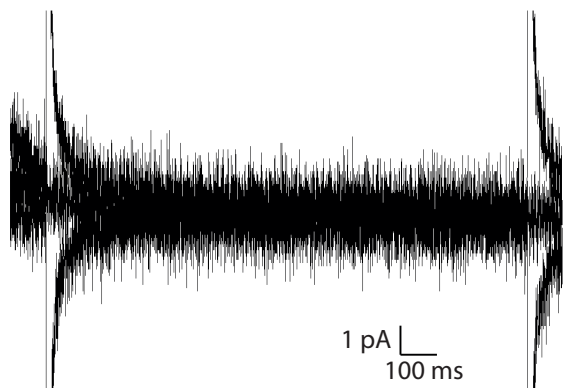
Figure S1



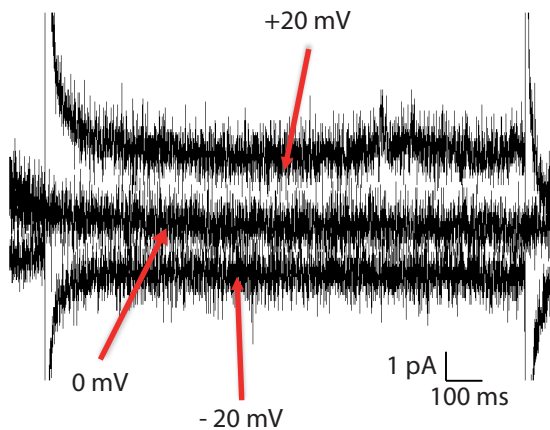
Supplemental Figure 1. Copper or ascorbate alone do not elicit plasma membrane currents; copper and ascorbate together elicit a barium-permeable inward conductance. Whole cell patch clamp recordings from epidermal protoplast plasma membrane. Recording conditions as in Figure 1 unless otherwise stated. **(A)** Col-0. Left, control currents (I) elicited by step voltage (V) changes, from a representative protoplast. Centre, I after exposure to extracellular 1 mM CuCl_2 . Right, mean \pm S.E.M. $I-V$ relationships for control (open circles) and test exposure ($n = 3$). **(B)** Col-0 response to 1 mM ascorbate ($n = 3$). **(C)** *ann1/ANN1* response to 1 mM CuCl_2 ($n = 3$). **(D)** WS response to 1 mM CuCl_2 ($n = 3$). **(E)** Col-0 response to extracellular OH^\bullet generated by 1 mM copper and 1 mM ascorbic acid with BaCl_2 replacing CaCl_2 in the bath solution ($n = 3$). Negative current to the left of the equilibrium potential for K^+ (E_K) can be carried by Ba^{2+} (as a proxy for Ca^{2+}) with a possible K^+ component. Negative current to the right of E_K is carried by Ba^{2+} .

Figure S2

A. Control current at 0, -20 and +20 mV

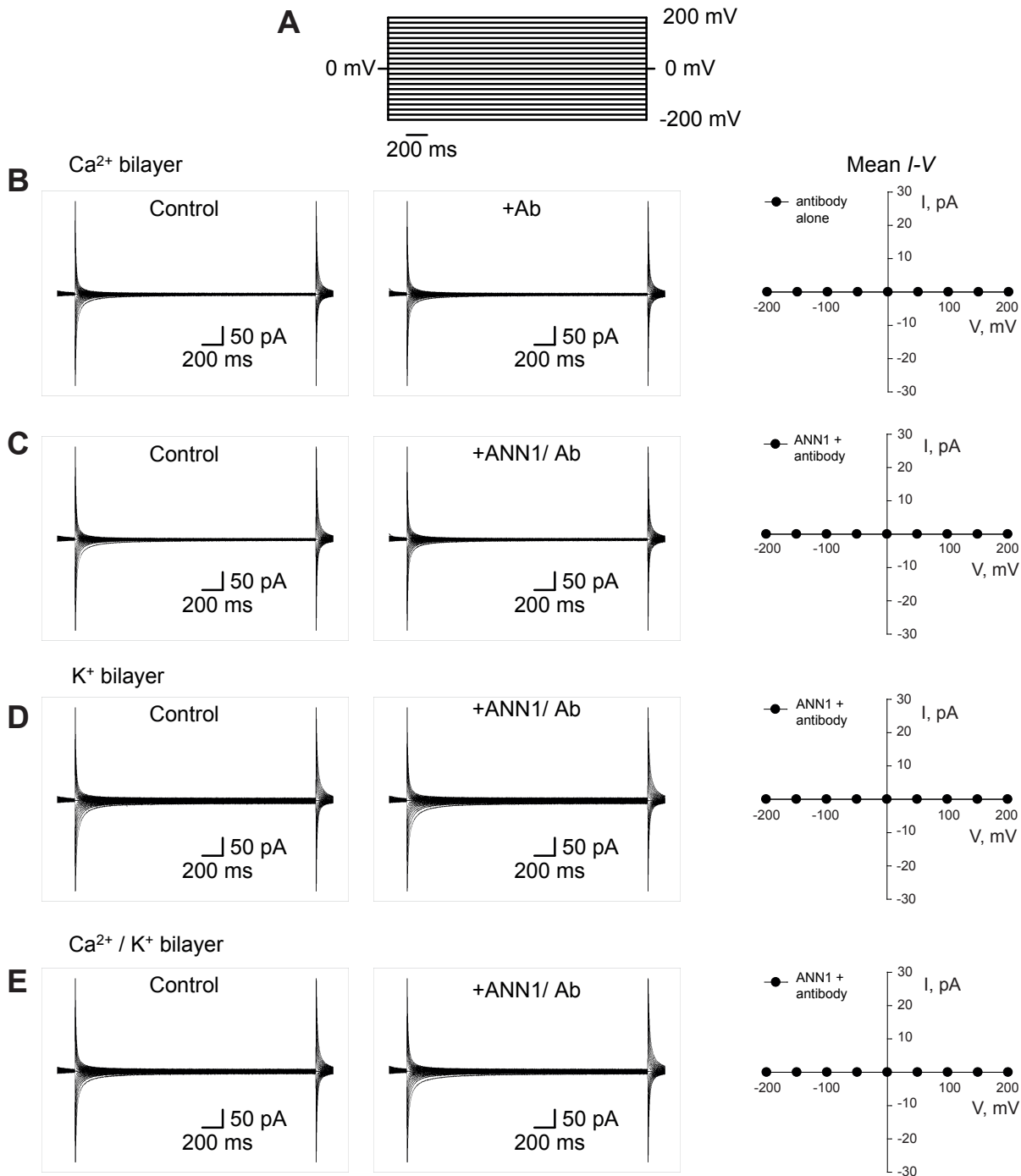


B. OH[•]-activated current at 0, -20 and +20 mV



Supplemental Figure 2. Expanded planar lipid bilayer traces. Current traces are those shown in Figure 7C for control (**A**) and in the presence of hydroxyl radicals (**B**). There are measurable increases in current magnitude evoked by radicals even at the lowest voltages applied (± 20 mV).

Figure S3



Supplemental Figure 3. Immunoprecipitated ANN1 does not support a conductance in planar lipid bilayers.

The ANN1 preparation was incubated for 30 minutes with anti-ANN1 peptide antibody in a 2:1 ratio. Antibody (Ab) alone was used as a control. **(A)**. Schematic representation of the voltage pulse protocol used in planar lipid bilayer studies. **(B)**. Representative current traces and mean *I-V* relationships. The *cis* chamber contained 1 mM Ca²⁺, the *trans* contained 200 mM Ca²⁺, both pH 6. Left panel, OH⁻ were generated in the *trans* chamber by Cu-Asc. Centre panel, OH⁻ were generated in the *trans* chamber by Cu-Asc, the *cis* chamber contained Ab. Right panel, *I-V* relationship for the PLB with Ab and OH⁻. **(C)**. Ionic conditions as **(B)** but in the centre panel, immunoprecipitated ANN1 (ANN1/Ab) was in the *cis* chamber and OH⁻ were generated in the *trans* chamber. Right panel, *I-V* relationship for ANN1/Ab. **(D)**. *Cis* contained 200 mM KCl; *trans* 50 mM KCl; both pH 6. Left panel, OH⁻ were generated in the *trans* chamber. Centre panel, OH⁻ were generated in the *trans* chamber, ANN1/Ab was in the *cis* chamber. Right panel, *I-V* relationship for ANN1/Ab. **(E)**. *Cis* chamber held 200 mM K⁺, 1 mM CaCl₂ and the *trans* held 1 mM K⁺, 200 mM CaCl₂, both pH 6. Left panel, OH⁻ were generated in the *trans* chamber. Centre panel, OH⁻ were generated in the *trans* chamber, ANN1/Ab was in the *cis* chamber. Right panel, *I-V* relationship for ANN1/Ab. Observations were made for 2 hours. For all data sets, *n* = 4 except **(E)**, where *n* = 3.

Supplemental Table 1. Root plasma membrane currents. Mean \pm SEM currents (in pA) are reported for epidermal protoplasts ($n = 6$) and root hair apical spheroplasts ($n = 6$) of the genotypes used in this study, determined using whole cell mode of patch clamp electrophysiology. *ann1/ANN1* denotes the complemented *ann1* mutant. Values were determined under control conditions and at maximal response to the generation of OH \cdot by 1 mM Cu-Asc addition. Current recorded at a membrane potential of -200 mV (inside negative) was inward and denoted by a negative value. That at +200 mV was outward and denoted by a positive value.

	-200 mV (Ca_{in})		200 mV (K_{out})	
Epidermis	Control	OH \cdot	Control	OH \cdot
WT (Col-0)	-181 \pm 56	-487 \pm 123	280 \pm 102	829 \pm 156
<i>ann1</i>	-142 \pm 58	-160 \pm 43	263 \pm 108	249 \pm 106
<i>ann1/ANN1</i>	-231 \pm 78	-569 \pm 133	298 \pm 128	847 \pm 148
WT (WS)	-191 \pm 88	-562 \pm 94	246 \pm 122	840 \pm 164
<i>gork</i>	-222 \pm 99	-527 \pm 128	265 \pm 84	811 \pm 144
Root hair apex	Control	OH \cdot	Control	OH \cdot
WT (Col-0)	-53 \pm 38	-280 \pm 73	185 \pm 68	511 \pm 106
<i>ann1</i>	-17 \pm 4	-10 \pm 3	231 \pm 66	249 \pm 56