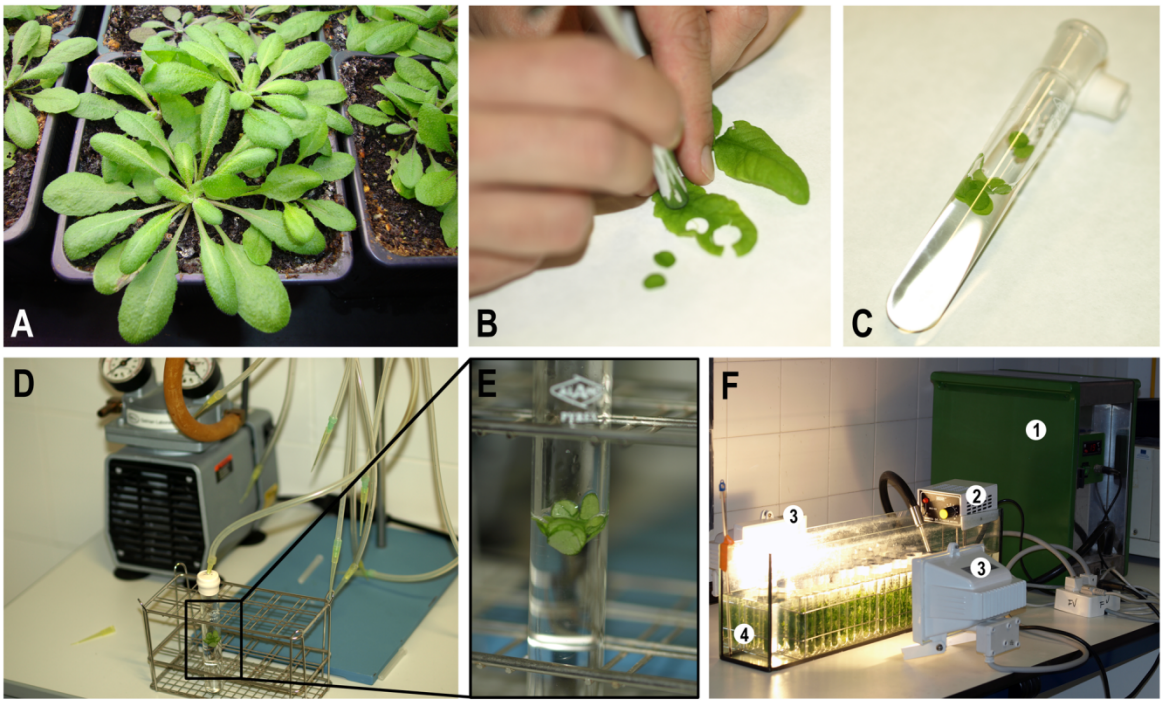
SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS INFORMATION

Plant material

Arabidopsis thaliana seedlings of wild type (Col-0), *cad2-1*, *rax1-1* and *cad1-3* genotypes germinated in square Petri dishes for 5 days, were transplanted in a perlite-peat (1:3) mixture and grown for 1 month in a short-days light regime. Leaves were cut in 1 cm diameter disks, placed in 15 mL glass tubes, sealed with silicone septum stoppers, and infiltrated under vacuum with deionised water (control), 3 or 30 µM $CdCl_2$ or $HgCl_2$ solutions (analytical grade ≥ 99.0 %). The disks were incubated at 20 ºC with continuous illumination for 24 and 48 hours (Fig. S1).

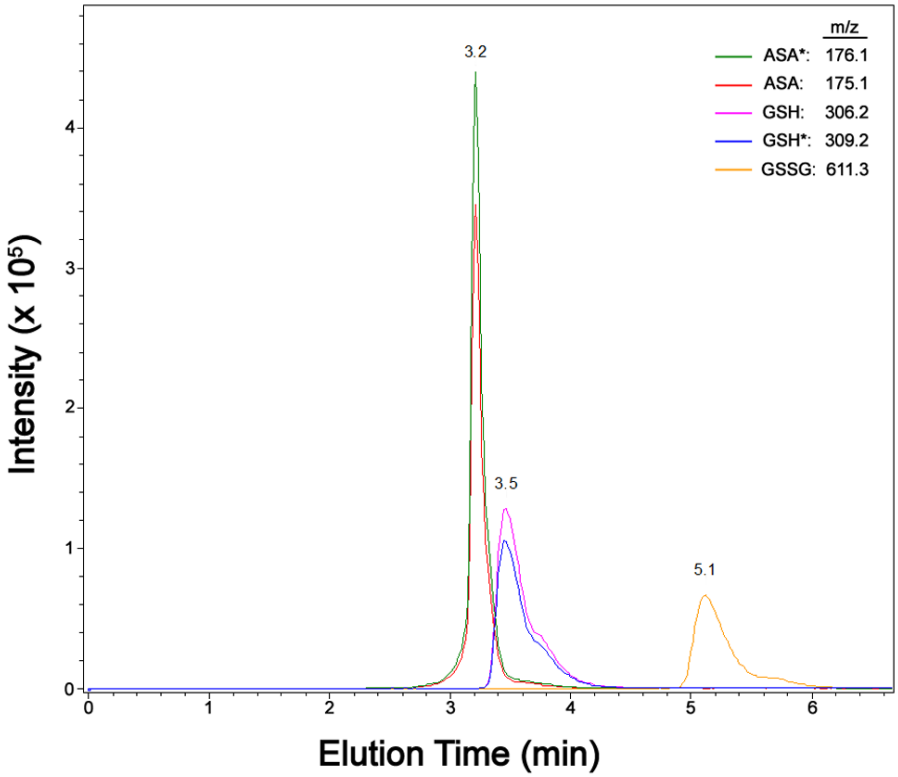


Supplementary Fig. 1. A, *Arabidopsis* were grown in a perlite-peat (1:3) mixture for 1 month in short-days light regime (8/16 h light/darkness at 25/18ºC respectively). **B,** Leaves were cut in 1 cm diameter disks. **C,** Disks were introduced in 15 mL glass tubes that were sealed with silicone septum stoppers. **D,** Leaf disks were infiltrated under vacuum with deionized water (dH_2O), 3 or 30 µM $CdCl_2$ or $HgCl_2$. **E,** Detail of infiltrated disks. **F,** Incubation of disks at 20 ºC, temperature that was controlled by using a cooling finger device (**1**), under illumination with halogen lamps for 24 and 48 h (**3**). A water rotary pump (**2**) was used to homogenize the temperature of the bath (**4**).

Chromatography and mass-spectrometry conditions

Chromatographic separation was carried out by injecting 20 µL aliquots of standard solutions and samples extracts spiked with the internal standards in a Mediterranea SEA18 column (3 µm, 150 x 2.1 mm; Teknokroma, Sant Cugat del Vallès, Spain). Samples were eluted with a gradient mobile phase built with 0.1% formic acid in Milli-Q water (solvent A) and 0.1% (v/v) formic acid in acetonitrile (solvent B) and a flow rate of 250 µL/min. The gradient program started increasing linearly the concentration of solvent B from 2% to 10% B for 5 min. Then, to wash the column, this proportion was increased linearly to 50% B for 1 min, this solvent composition was maintained for 3 min, and returned linearly to initial conditions in 2 min. The column was then allowed to stabilise for 9 min at the initial conditions before the next injection. Autosampler and column temperatures were 6 and 30°C, respectively. The total exit flow from the column was loaded into the ESI interface of the MS(TOF) apparatus from the HPLC using a 125 µm i.d. PEEK tube (Upchurch Scientific, Oak Harbor, WA). The MS(TOF) operated in negative ion mode at -500 and 3000 V of endplate and spray tip voltages, respectively. The orifice voltage was set at -90 V to acquire spectra in the mass-to-charge ratio (*m/z*) ranges of 50-1000. The nebulizer gas (N_2) pressure, drying gas (N_2) flow rate and drying gas temperature were 2.7 bar, 8.5 L min^{-1} and 200 °C.

To check the modifications in the method described by Rellán-Álvarez *et al.*[24], basically regarding to the type of column and chromatographic conditions, we run mixtures of ASA, GSH and GSSG with internal standards, obtaining a sufficient separation of the analytes: ASA/ASA* co-eluted at 3.2 min, GSH/GSH* co-eluted at 3.5 min, and GSSG had a longer retention time of 5.1 min (Fig. S2). Concentrations of GSH, GSSG and ASA were always quantified by external calibration with internal standardization.



Supplementary Fig. 2. Typical chromatogram of a mixture of standards: 100 µM ASA*, 100 µM ASA, 100 µM GSH, 75 µM GSH*, 25 µM GSSG. Numbers on top of the peaks represent retention times in minutes.

SUPPLEMENTARY RESULTS

SUPPLEMENTARY TABLE 1

Concentration of biothiols (nmol/g FW) in leaf disks of *Arabidopsis* infiltrated with 0, 3 and 30 µM Cd or Hg and incubated for 24 and 48 h was analyzed by conventional HPLC and postcolumn derivatization with Ellman's reagent. The quantification of the concentration was performed by the addition of an internal standard N-acetylcysteine and relative to the weight of sample (100 mg). These results are shown graphically in Figure 2 using spheres with different colour for each detected biothiols, which concentrations are represented by different diameters.

Sup. Table 1. Concentration of biothiols (nmol g-1 FW) in disks of Wild type (Col-0), cad2-1, rax1-1 and cad1-3 A. thaliana leaves treated with 0, 3 and 30 µM Cd or Hg for 24 and 48 h. Different letters denote significant differences between treatments and genotypes at $p < 0.05$ (except Cys 24 h $P < 0.1$). n.d. (not detected).

			Cys	GSH	PC_2	PC_3	PC_4	PC_5
24 hours	Control	**Col-0**	$38.10^{a} \pm 4.13$	$297.98^{fg} \pm 22.85$				
		cad2-1	$53.95^{bc} \pm 3.12$	$117.59^{abc} \pm 31.76$				
		rax1-1	$45.17^{ab} \pm 6.50$	$237.03^{ef} \pm 32.84$				
		cad1-3	$65.82^{cd} \pm 10.96$	$504.22^{h} \pm 25.57$				
	3 µM Cd	**Col-0**	$38.43^{a} \pm 4.02$	$330.73^{g} \pm 33.62$	$23.11^{a} \pm 8.51$	$8.76^{a} \pm 0.67$		
		cad2-1	$62.83^{cd} \pm 3,67$	$74.99^{ab} \pm 10.09$				
		rax1-1	$56.75^{bcd} \pm 12.09$	$226.96^{e} \pm 25.31$	$16.56^{a} \pm 1.35$			
		cad1-3	$43.36^{ab} \pm 6.15$	$476.64^{h} \pm 40.81$				
	30 µM Cd	**Col-0**	$40.79^{ab} \pm 10.09$	$150.59^{cd} \pm 7.21$	$37.32^{b} \pm 5.15$	$25.44^{b} \pm 3.21$	$13.34^{a} \pm 2.51$	
		cad2-1	$192.84^{f} \pm 11.77$	$57.36^{a} \pm 8.36$				
		rax1-1	$62.65^{cd} \pm 7.43$	$85.96^{ab} \pm 13.90$	$18.34^{a} \pm 2.48$	$12.77^{a} \pm 2.55$	$9.12^{a} \pm 1.12$	
		cad1-3	$103.83^{e} \pm 5.38$	$133.16^{bc} \pm 1.60$				
	3 µM Hg	**Col-0**	$38.91^{ab} \pm 3.55$	$306.79^{g} \pm 16.54$				
		cad2-1	$72.13^{d} \pm 4.30$	$115.58^{abc} \pm 13.68$				
		rax1-1	$61.30^{cd} \pm 9.70$	$239.41^{efg} \pm 1.44$				
		cad1-3	$52.40^{abc} \pm 5.01$	$527.38^{h} \pm 38.01$				
	30 µM Hg	**Col-0**	$47.09^{abc} \pm 5.15$	$209.88^{de} \pm 15.24$	$16.99^{a} \pm 2.47$	$10.53^{a} \pm 1.58$		
		cad2-1	$40.07^{ab} \pm 3.12$	$60.72^{ab} \pm 19.77$				
		rax1-1	$46.96^{abc} \pm 0.02$	$105.76^{abc} \pm 15.92$	$14.10^{a} \pm 1.33$			
		cad1-3	$72.67^{d} \pm 6.53$	$245.71^{ef} \pm 34.30$				
48 hours	Control	**Col-0**	$74.11^{ab} \pm 8.27$	$442.01^{ab} \pm 41.73$				
		cad2-1	$65.73^{a} \pm 17.34$	$163.66^{ef} \pm 25.35$				
		rax1-1	$57.44^{a} \pm 7.68$	$291.00^{c} \pm 33.06$				
		cad1-3	$90.25^{bc} \pm 15.07$	$419.64^{b} \pm 39.46$				
	3 µM Cd	**Col-0**	$58.60^{ab} \pm 17.66$	$398.38^{bd} \pm 34.80$	$24.16^{ab} \pm 0.98$	$32.27^{a} \pm 12.06$	$9.73^{a} \pm 2.08$	
		cad2-1	$141.00^{d} \pm 4.41$	$165.48^{e} \pm 19.79$				
		rax1-1	$77.56^{ab} \pm 5.24$	$189.06^{e} \pm 18.79$	$17.62^{a} \pm 1.26$			
		cad1-3	$89.55^{ab} \pm 7.08$	$335.29^{cd} \pm 19.66$				
	30 µM Cd	**Col-0**	$79.46^{ab} \pm 8.64$	$439.16^{ab} \pm 46.15$	$110.68^{d} \pm 1.97$	$104.44^{c} \pm 7.89$	$30.81^{b} \pm 4.72$	$5.93^{a} \pm 0.16$
		cad2-1	$113.25^{cd} \pm 24.13$	$99.52^{ef} \pm 16.03$	$24.25^{ab} \pm 9.05$	$17.72^{b} \pm 5.20$		
		rax1-1	$62.69^{ab} \pm 0.96$	$133.87^{e} \pm 29.97$	$34.86^{bc} \pm 4.05$	$22.90a^{b} \pm 1.15$	$8.36^{a} \pm 0.88$	
		cad1-3	$102.93^{cd} \pm 14.70$	$185.09^{e} \pm 69.97$				
	3 µM Hg	**Col-0**	$157.59^{d} \pm 20.68$	$407.99^{bd} \pm 7.61$				
		cad2-1	$125.80^{cd} \pm 22.54$	$178.93^{e} \pm 13.80$				
		rax1-1	$84.39^{ab} \pm 14.81$	$245.15^{ce} \pm 18.98$				
		cad1-3	$82.71^{ab} \pm 20.47$	$376.88^{bc} \pm 8.03$				
	30 µM Hg	**Col-0**	$71.28^{ab} \pm 10.49$	$494.73^{a} \pm 19.97$	$40.49^{c} \pm 6.19$	$11.80^{b} \pm 1.87$		
		cad2-1	n.d.	$42.54^{f} \pm 13.81$				
		rax1-1	$46.58^{a} \pm 8.36$	$148.87^{e} \pm 11.97$	$15.87^{a} \pm 0.71$	$11.00^{b} \pm 0.65$		
		cad1-3	$75.34^{ab} \pm 16.31$	$338.84^{cd} \pm 44.76$				