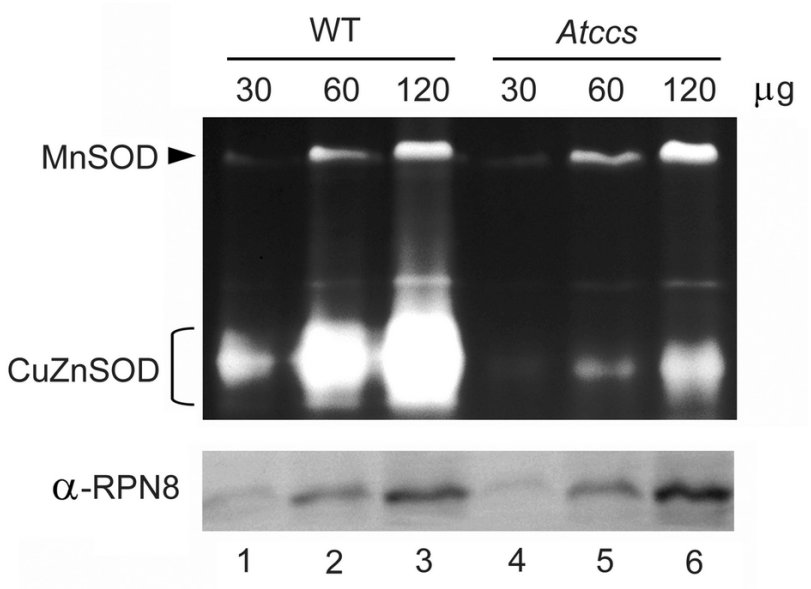
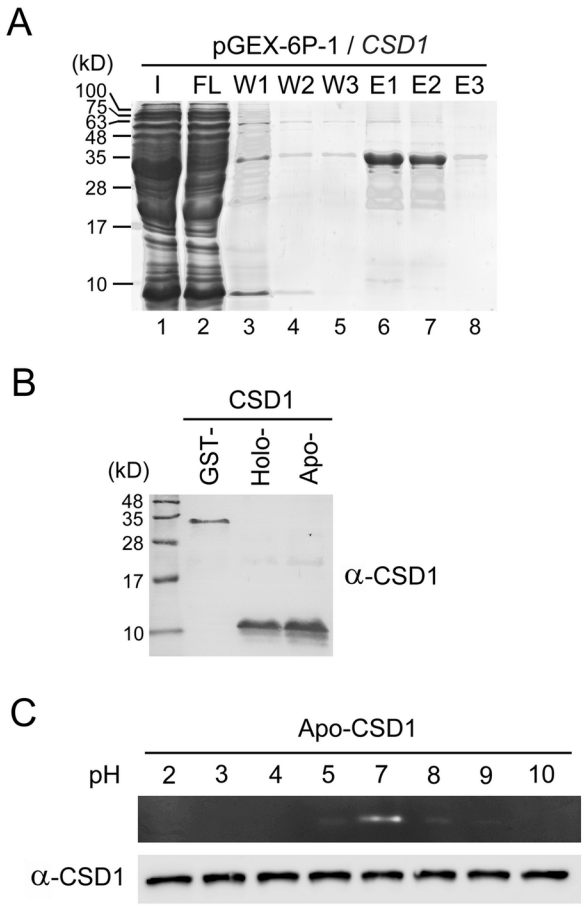
Supplemental Table SI. Primers used in this study. The mutation sites of *CSD1* are underlined.

Construct	Primer Set	Sequence (5′ to 3′)
Full length CSD1 (and RT-PCR)	CSD1-Fw-*Nco*I	TCTCCATGGCGAAAGGAGTTGCAGTTTT
	CSD1-Rv	TTAGCCCTGGAGACCAATGATGC
$CSD1_{AA}$ mutant	G141A/V143A-Fw	AACGCAGGCGCCCGTGCTGCTTGCGGC
	G141A/V143A-Rv	GCCGCAAGCAGCACGGGCGCCTGCGTT
$CSD1_{SL}$ mutant	G141S/V143L-Fw	AACGCAGGCTCCCGTCTTGCTTGCGGC
	G141S/V143L-Rv	GCCGCAAGCAAGACGGGAGCCTGCGTT
$CSD1_{PP}$ mutant	G141P/V143P-Fw	AACGCAGGCCCCCGTCCTGCTTGCGGC
	G141P/V143P-Rv	GCCGCAAGCAGGACGGGGGCCTGCGTT
TP_{CSD2} for CSD1	TP-CSD2-Fw-*EcoR*I	CAGAATTCATGGCTGCCACCAACACAATCCT
	TP-CSD2-Rv-*Nco*I	CACCATGGGAAGCACTGCAACAGCCTTCT
TP_{CSD2}-CSD1	CSD1-Fw-*Nco*I	TCTCCATGGCGAAAGGAGTTGCAGTTTT
	CSD1-Rv	TTAGCCCTGGAGACCAATGATGC
Full length CSD1-YFP	CSD1-Fw-*Nco*I	TCTCCATGGCGAAAGGAGTTGCAGTTTT
	CSD1ns-Rv-*Sal*I	CAGTCGACCCGCCCTGGAGACCAATGATG
TP_{CSD2}-CSD1-YFP	CSD1-Fw-*Nco*I	TCTCCATGGCGAAAGGAGTTGCAGTTTT
	CSD1ns-Rv-*Sal*I	CAGTCGACCCGCCCTGGAGACCAATGATG
GST-CSD1	CSD1-Fw-*EcoR*I	CAGAATTCATGGCGAAAGGAGTTGCAGT
	CSD1-Rv	TTAGCCCTGGAGACCAATGATGC
Full length CSD2 (and RT-PCR)	CSD2-Fw-*Nco*I	TCTCCATGGCTGCCACCAACACAATCCT
	CSD2-Rv	TTAGAGCGGCGTCAAGCCAATCA

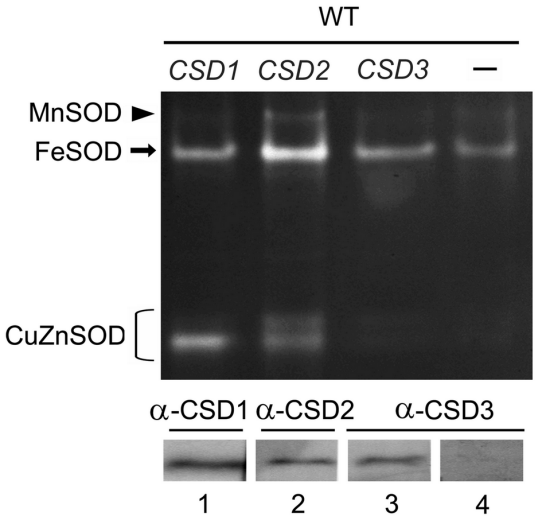
Full length CSD2-YFP	CSD2-Fw-*Nco*I	TCTCCATGGCTGCCACCAACACAATCCT
	CSD2ns-Rv-*Sal*I	CAGTCGACCCGAGCGGCGTCAAGCCAATCA
ΔTP-CSD2	ΔTP-CSD2-Fw-*Hind*III	CAAAGCTTATGTCCGCGGCGAAGAAGGCT
	CSD2-Rv	TTAGAGCGGCGTCAAGCCAATCA
ΔTP-CSD2-YFP	ΔTP-CSD2-Fw-*Hind*III	CAAAGCTTATGTCCGCGGCGAAGAAGGCT
	CSD2ns-Rv-*Sal*I	CAGTCGACCCGAGCGGCGTCAAGCCAATCA
Full length CSD3 (and RT-PCR)	CSD3-Fw-*Eco*RI	TCTGAATTCATGGAAGCTCCTAGAGGAAATCT
	CSD3-Rv-*Eco*RI	TCTGAATTCCTATAGTTTAGCATCCGCAGATG
GFP-full length CSD3	CSD3-Fw-*Sma*I	CACCCGGGCCATGGAAGCTCCTAGAGGAAAT
	CSD3-Rv-*Eco*RI	TCTGAATTCCTATAGTTTAGCATCCGCAGATG
CSD3-ΔAKL	CSD3-Fw-*Eco*RI	TCTGAATTCATGGAAGCTCCTAGAGGAAATCT
	CSD3-Rv-ΔAKL	CACTAATCCGCAGATGATTGAAGTCC
GFP-CSD3-ΔAKL	CSD3-Fw-*Sma*I	CACCCGGGCCATGGAAGCTCCTAGAGGAAAT
	CSD3-Rv-ΔAKL	CACTAATCCGCAGATGATTGAAGTCC
ROXY1	ROXY1-Fw-*Sma*I	CACCCGGGATGCAATACCAGACAGAATC
	ROXY1-Rv-*BamH*I	CAGGATCCTCAGAGCCAGAGAGCG
GRXcp	GRXcp-Fw-*Sma*I	CACCCGGGATGGCTCTCCGATCTGTCAAA
	GRXcp-Rv-*BamH*I	CAGGATCCTCAAGAGCACATAGCTTTCTCCA



Supplemental Figure S1. Residual CSD activities analyzed in Arabidopsis *Atccs*. It has been reported previously that Arabidopsis with various SOD activity profiles among different tissues (Chu et al., 2005). The level of CSD activity was high in flowers and siliques but low in rosette leaves and cauline leaves. In this figure, cellular extracts of 45-d-old Arabidopsis wild-type (WT) and *Atccs* flowers were analyzed for SOD activity with different protein amounts as indicated. RPN8 was a loading control. Here, the *Atccs* mutant clearly showed residual CSD activity (lanes 5 and 6; about 6% to 30% of the WT levels), which indicates a CCS-independent CSD activation pathway in Arabidopsis. The percentage of CCS-independent activity represents the mean of at least 4 replicates and was calculated relative to the WT activity.



Supplemental Figure S2. Affinity purification of CSD1 and its activity under various pH treatments. A, *CSD1* gene was cloned into the pGEX-6P-1 vector for fusion with a GST tag. Samples at each step underwent SDS-PAGE and Coomassie Brilliant Blue staining. I, IPTG induced cellular extract. F, flow through extract. W1 to W3, wash with buffer. E1 to E3, GST-tagged protein elution. B, Fifty nanograms each of purified GST-, and GST-removed Holo- and Apo-CSD1 was analyzed by immunoblotting. C, The activity of Apo-CSD1 (870 ng) incubated with 0.1 μM $CuSO_4$ and 20 μM $ZnSO_4$ under various pH treatments as indicated. Buffers for each pH were: 0.2 M Na_2HPO_4 and 0.1 M citrate for pH 2 to 7, and 0.2 M glycine-NaOH for pH 9 and 10. A replicate of 50-ng Apo-CSD1 was subjected to the same treatments, then analyzed for protein level by immunoblotting.



Supplemental Figure S3. Characterization of individual CSD activity in Arabidopsis protoplasts. *CSD1*, *CSD2* and *CSD3* gene was overexpressed in WT protoplasts, then activity (top) and protein level (bottom) were analyzed. Lane 4 contains protoplast extracts without transformation used as a control.