

Expression of aspartyl protease and C3HC4-type RING zinc finger genes are responsive to ascorbic acid in *Arabidopsis thaliana*

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Supplementary Table S1 – see .xls file

Supplementary Table S2.

Number	Affymetrix probe	AGI Number	Description	Fold
1	256525_at	AT1G66180	aspartyl protease family protein	5.38
2	259681_at	AT1G77760	nitrate reductase 1 (NR1)	5.33
3	246919_at	AT5G25460	expressed protein	4.16
4	245451_at	AT4G16890	disease resistance protein (TIR-NBS-LRR class), putative	4.44
5	261927_at	AT1G22500	zinc finger (C3HC4-type RING finger) family protein	4.28
6	254743_at	AT4G13420	potassium transporter (HAK5)	28.41
7	259839_at	AT1G52190	proton-dependent oligopeptide transport (POT) family protein	4.4
8	250045_at	AT5G17700	MATE efflux family protein	6.11
9	262373_at	AT1G73120	expressed protein	20.21
10	250277_at	AT5G12940	leucine-rich repeat family protein	4.06
11	255177_at	AT4G08040	1-aminocyclopropane-1-carboxylate synthase, putative / ACC synthase, putative	4.51
12	252011_at	AT3G52720	carbonic anhydrase family protein	4.43
13	262045_at	AT1G80240	expressed protein	7.76
14	262628_at	AT1G06490	glycosyl transferase family 48 protein	4.14

Fig. S1. Effect of L-GalL supplementation on total AsA concentration in the AsA deficient *Arabidopsis* mutant *vtc2-1*. Excised leaves from *vtc2-1* plants were fed with 5 mM L-GalL or water (control) in the light ($80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Left panel, detached *vtc2-1* leaves fed with water. Right panel, *vtc2-1* leaves fed with 5 mM L-GalL. Data are the mean values \pm SD from three independent experiments. Values that are significantly different from control according to Student's *t*-test are indicated ; ^a, $p < 0.01$; ^b, $p < 0.001$.

Fig. S2. Effect of DHA feeding on AsA concentration and transcript levels of two L-GalL responsive genes in *vtc2-1* mutant. Excised leaves from plants were fed with 5 mM DHA in the light ($80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 3 and 6 h. Total AsA concentration in leaves (A). Relative transcript levels of genes At1g66180 and At1g22500 (B). The transcript levels were normalized against *Actin-2*. Data are the mean values \pm SD of three independent experiments. Values that are significantly different from control according to Student's *t*-test are indicated; ^a, $p < 0.05$; ^b, $p < 0.01$; ^c, $p < 0.001$.

Fig. S3. Effect of DHA feeding on AsA concentration and transcript levels of two L-GalL responsive genes in *Arabidopsis thaliana* wild type. Excised leaves from plants were fed with 5 mM DHA in the light ($80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 3 and 6 h. Total AsA concentration in leaves (A). Relative transcript levels of genes At1g66180 and At1g22500 (B). The transcript levels were normalized against *Actin-2*. Data are the mean values \pm SD of three independent experiments. Values that are significantly different from control according to Student's *t*-test are indicated; ^a, $p < 0.05$; ^b, $p < 0.01$; ^c, $p < 0.001$.

Fig. S4. GO analysis of 337 genes whose transcript levels were increased 2-fold or more by L-GalL.

Fig. S5. Expression pattern over a range of experimental treatments of L-GalL-responsive genes. Eight genes shown by qRT-PCR to be induced by L-GalL treatment of *Arabidopsis thaliana vtc2-1* leaves (A). Top 50 L-GalL-induced genes from Affymetrix GeneChip analysis (B). Gene expression patterns were determined with the Genevestigator tool (<https://www.genevestigator.com/gv/index.jsp>).

Fig. S6. Transcripts levels of reference genes in each group of samples. To evaluate the

most stable reference gene for our experiments, qPCR was performed using specific primers of the selected reference genes (“Materials and Methods”). All the cDNA templates were prepared with the same concentration to detect the stability of candidate reference genes. The axis of “Quantity” indicates the raw data. (A) Transcript levels of 3 reference genes over a 24 h time course in *vtc2-1* following feeding with 5 mM L-GalL; (B) Transcript levels of 3 reference genes in *vtc2-1* following feeding with H₂O, 5 mM D-Glc, L-GalL and AsA in the dark; (C) Transcript levels of 3 reference genes in WT following the cycle of 48 h light, 48 h dark and 48 h light; (D) Transcript levels of 3 reference genes in WT following feeding with H₂O, 5 mM D-Glc, L-GalL and AsA in the light.

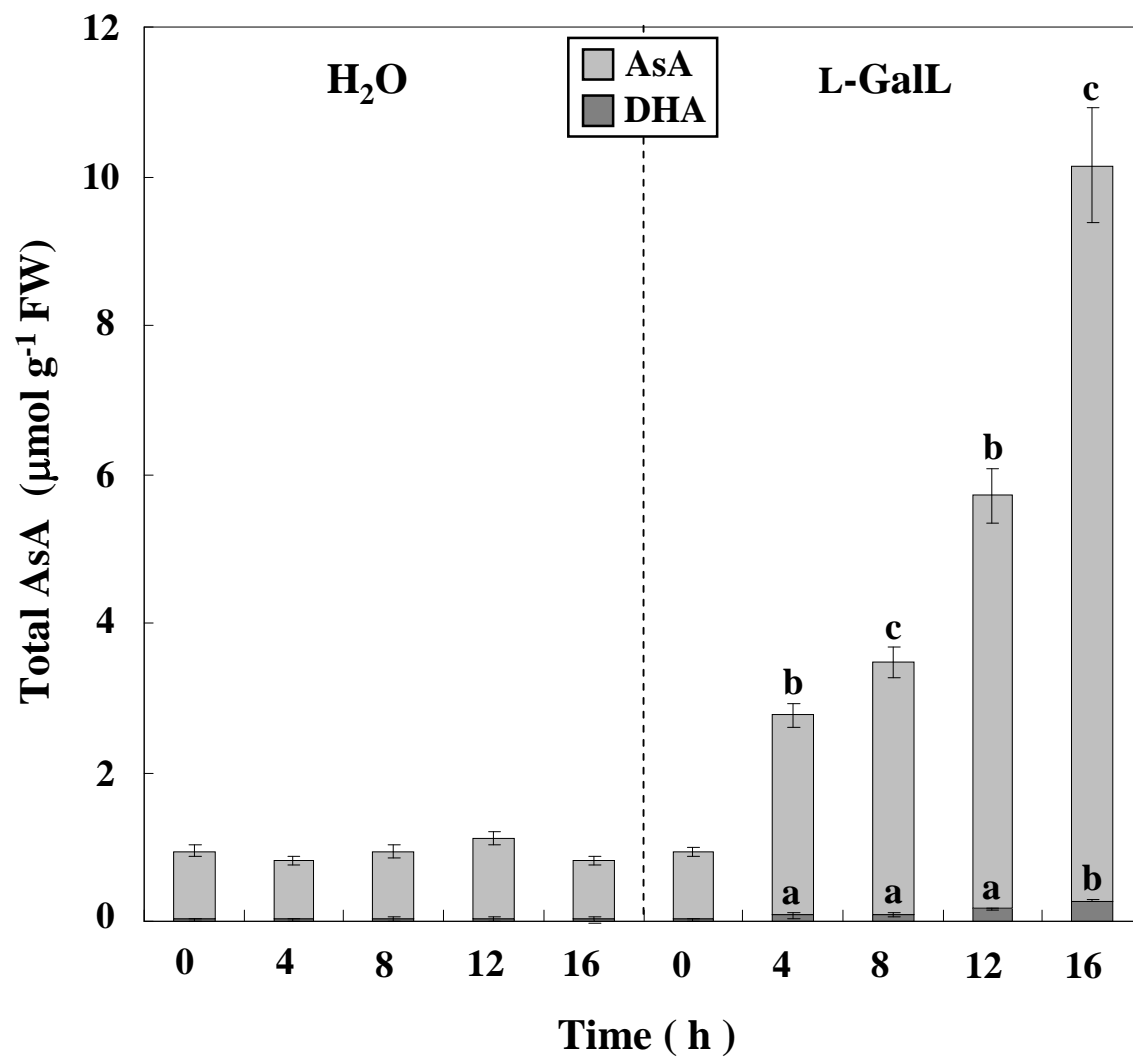


Figure S1 Gao et al.,

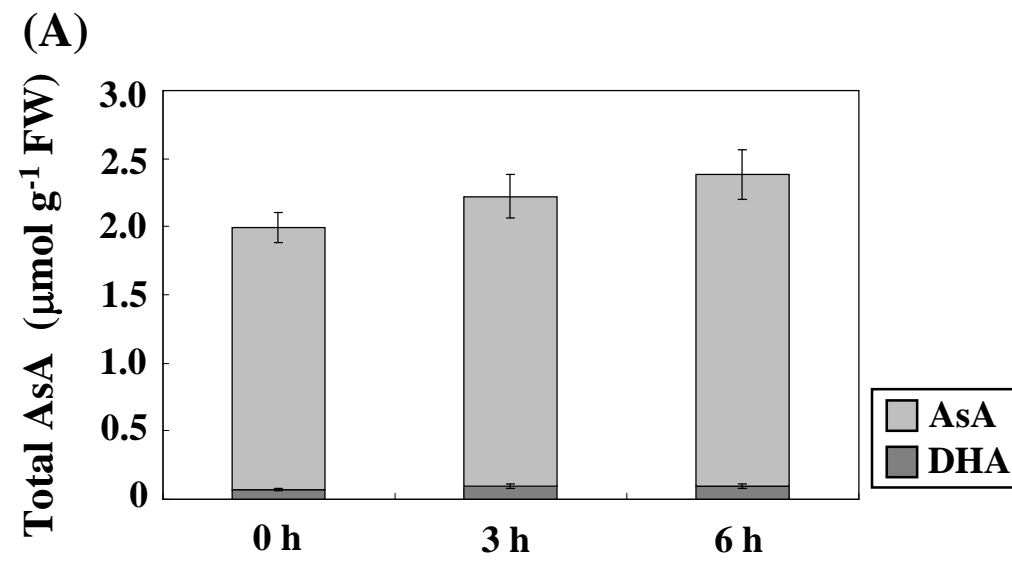


Figure S2A Gao et al.,

(B)

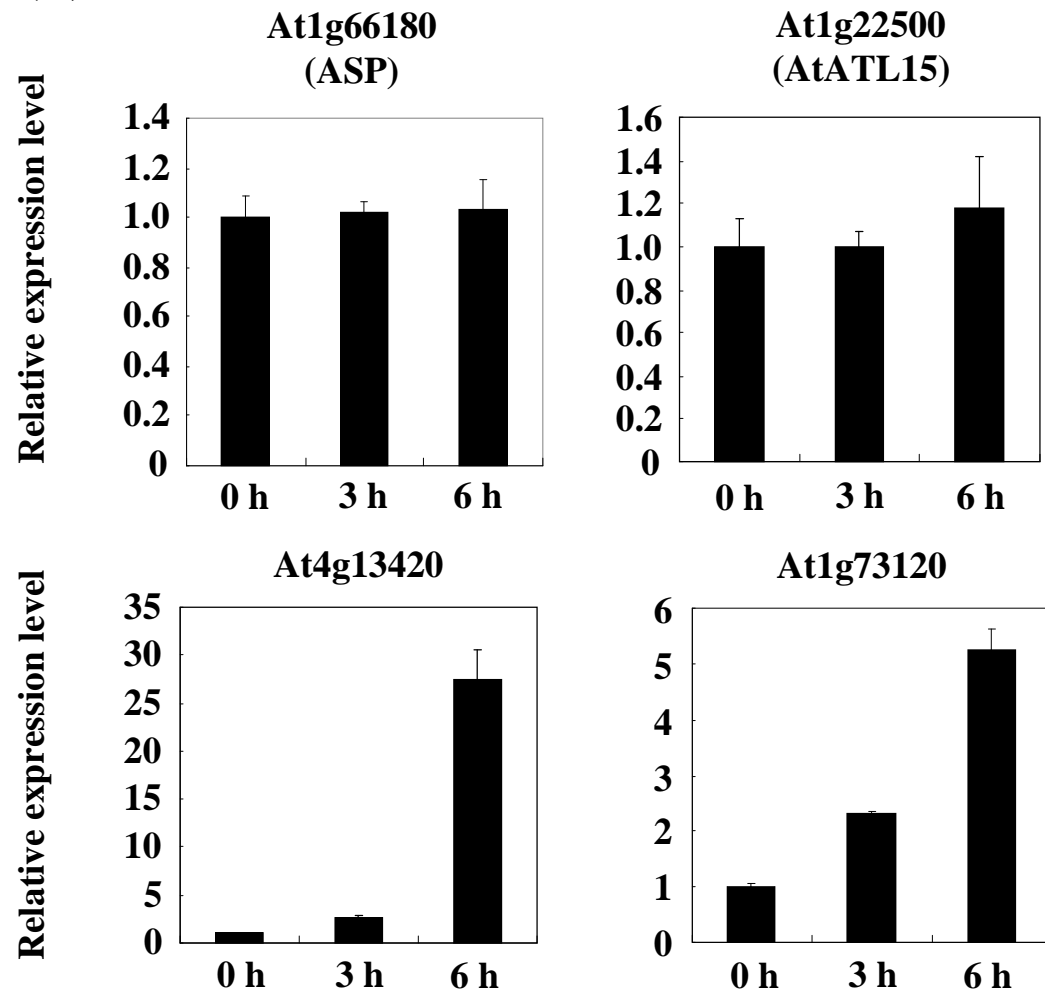


Figure S2B Gao et al.,

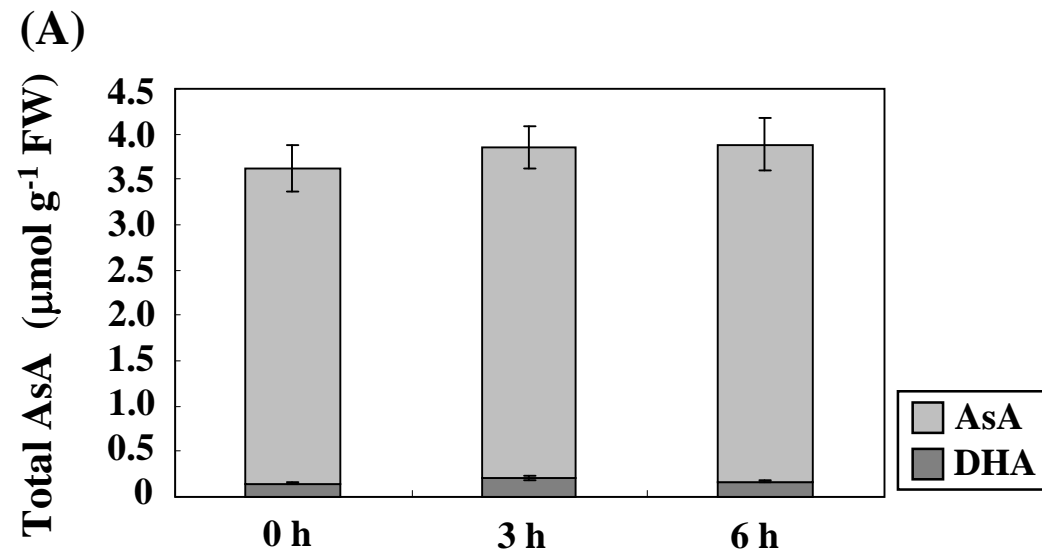


Figure S3A Gao et al.,

(B)

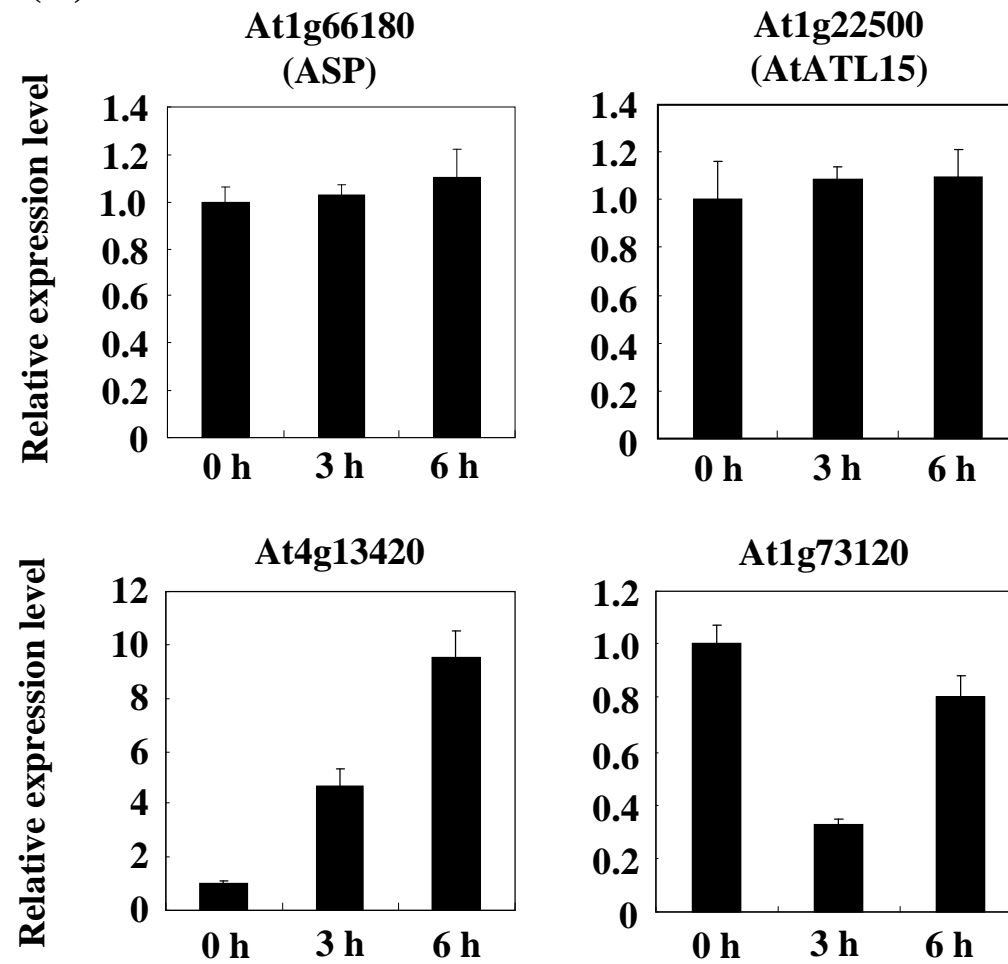
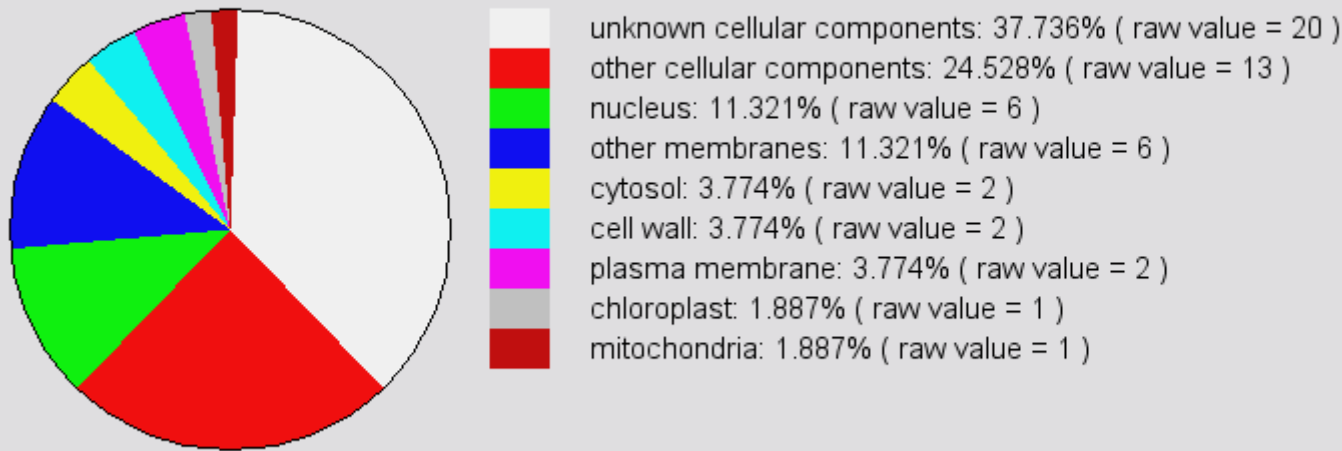


Figure S3B Gao et al.,

Functional Categorization by annotation for : GO Cellular Component



Functional Categorization by annotation for : GO Biological Process

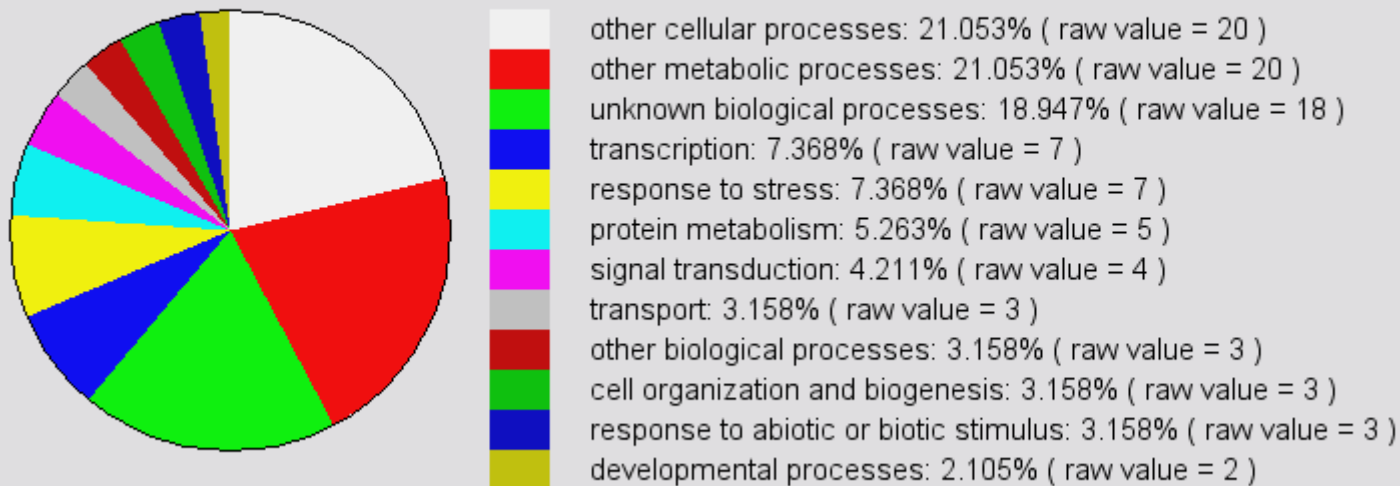


Figure S4-1
Gao et al.,

Functional Categorization by annotation for : GO Molecular Function

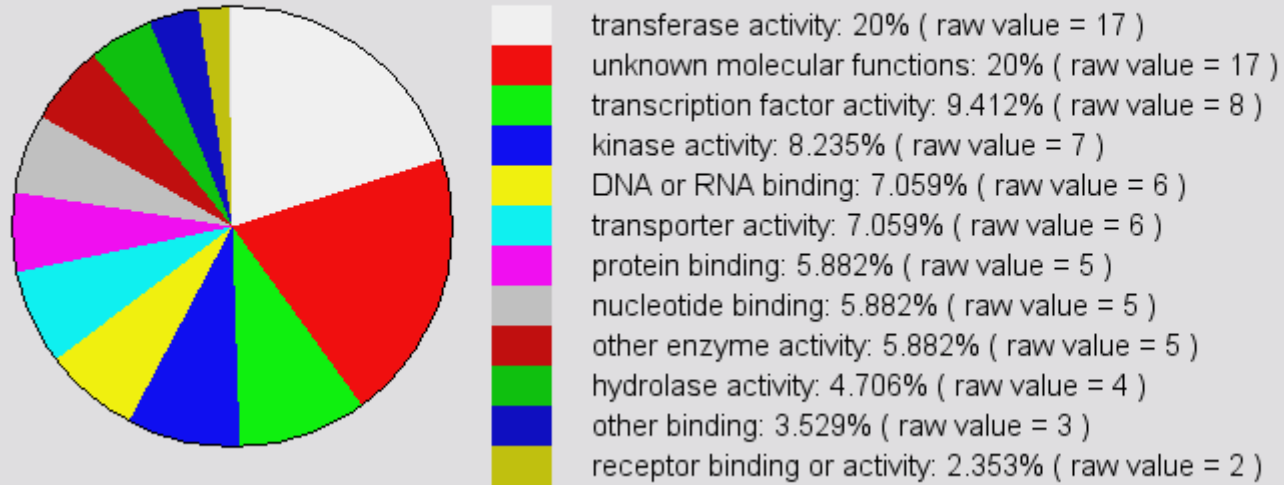


Figure S4-2
Gao et al.,

a)

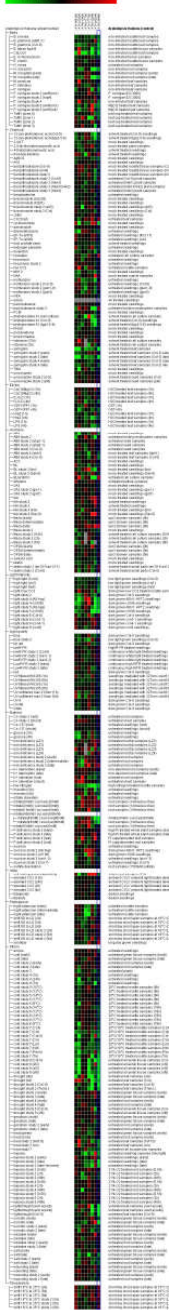


Fig. S5a Gao et al.

b)

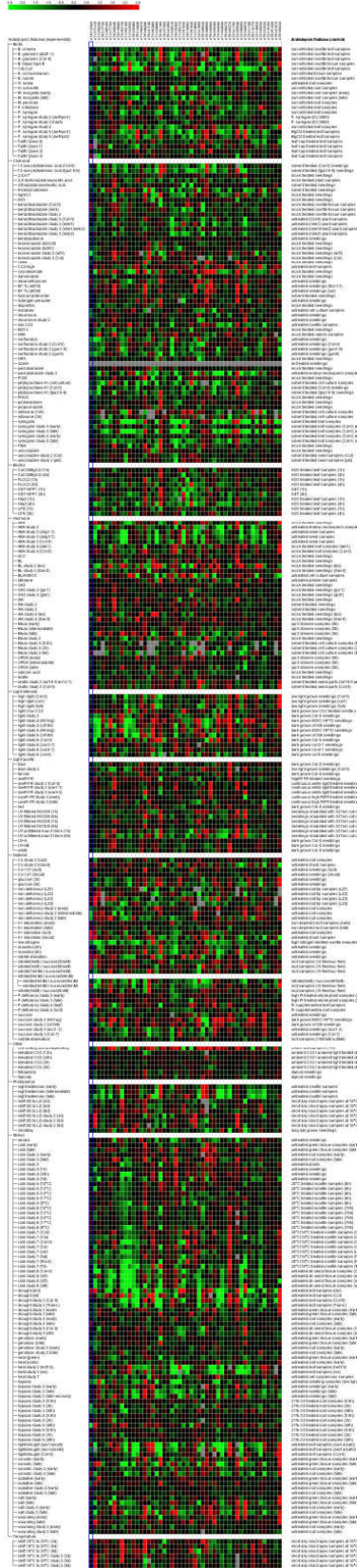


Fig. S5b Gao et al.

vtc2.1 fed with L-GalL

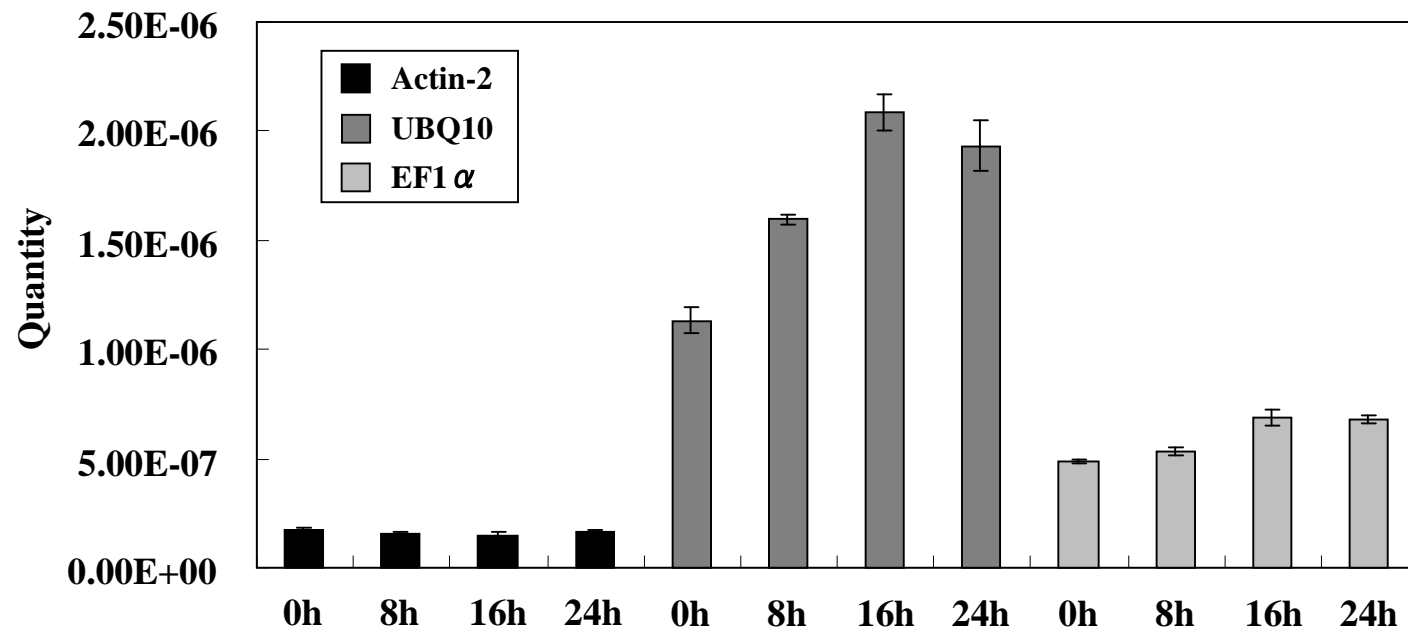


Figure S6A Gao et al.,

vtc2.1 fed with H₂O, D-Glu, L-GalL, AsA in the dark

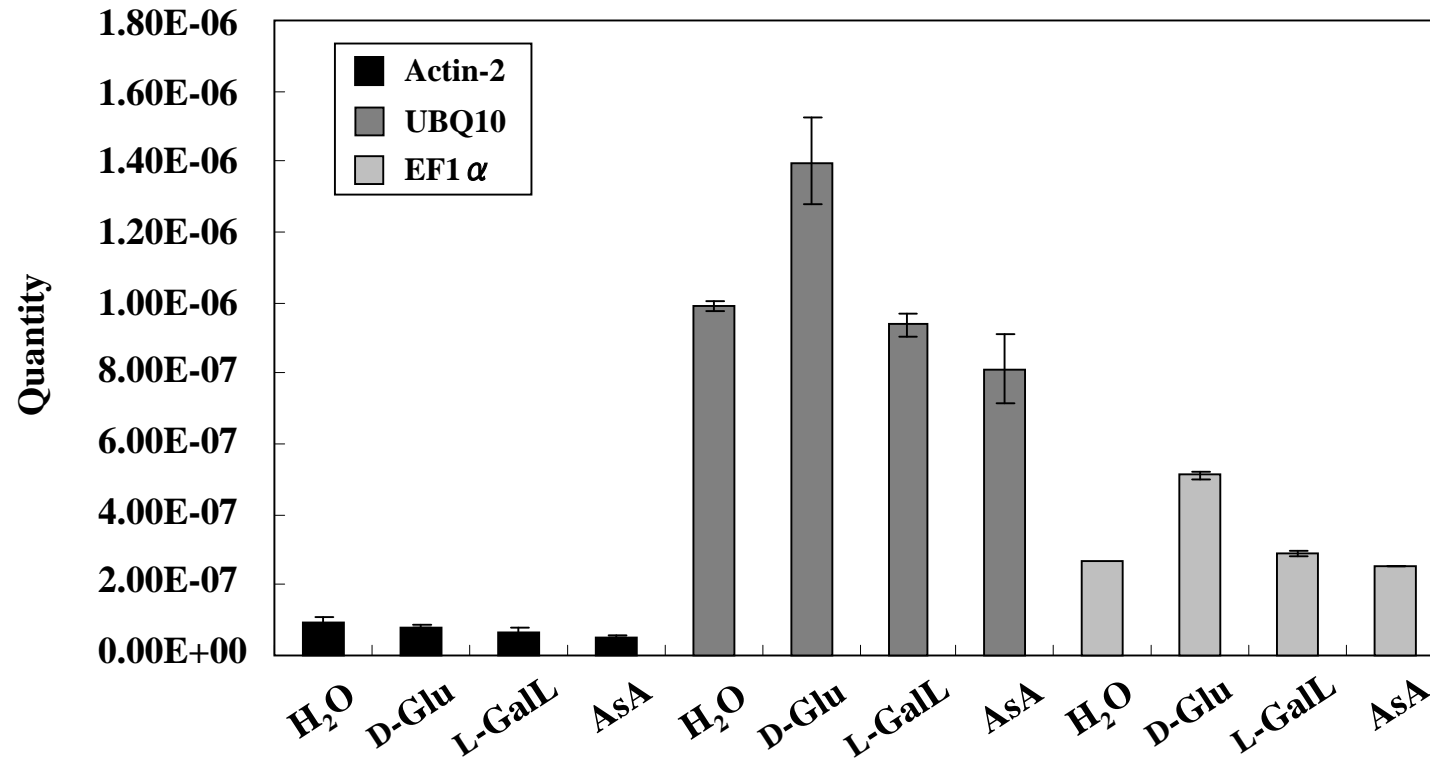


Figure S6B Gao et al.,

WT time course

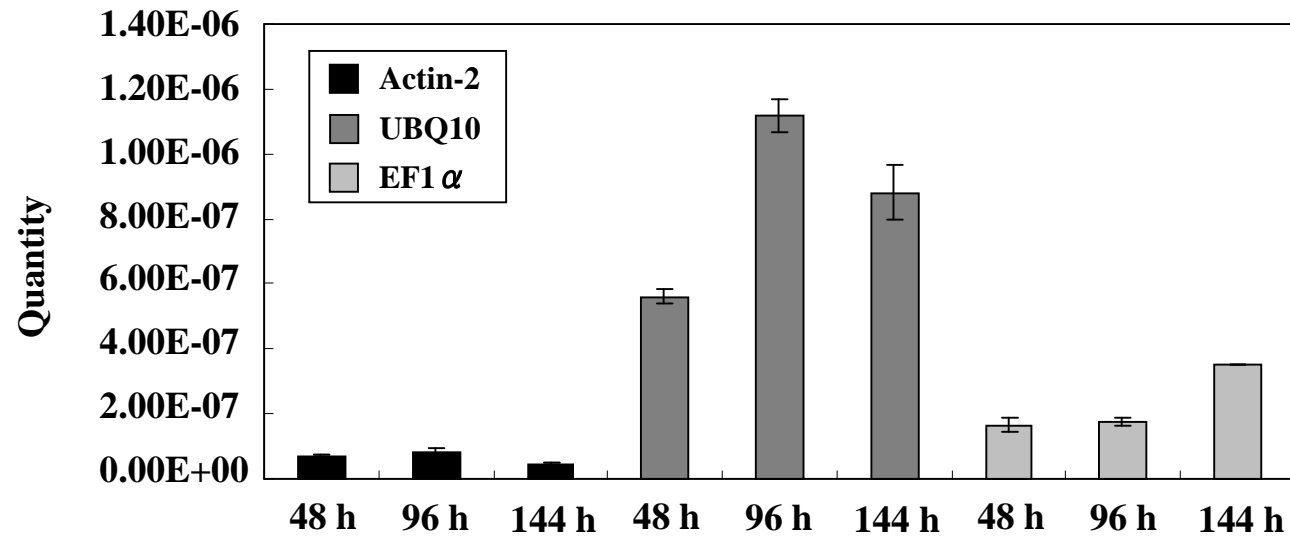


Figure S6C Gao et al.,

WT fed with H₂O, D-Glu, L-Gall, AsA in the light

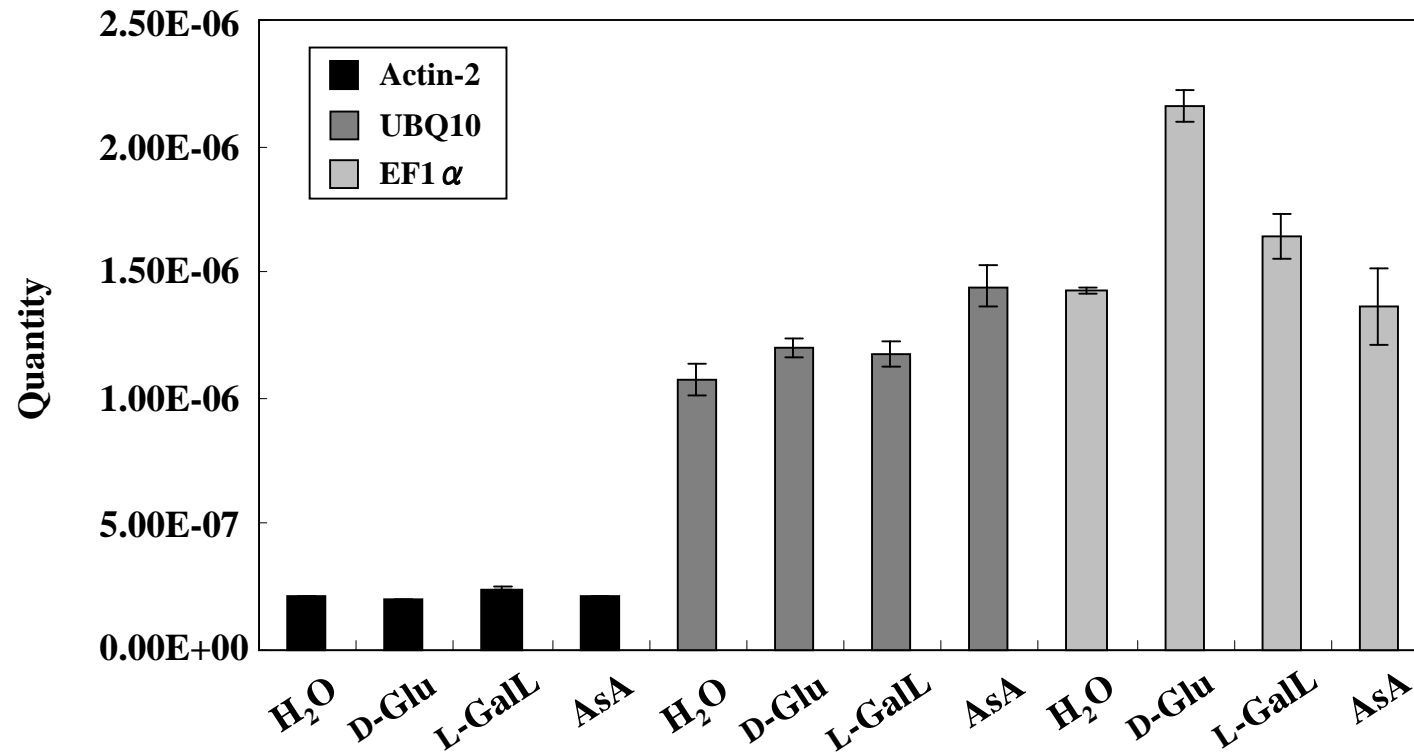


Figure S6D Gao et al.,