### Supplementary information

### Figure S1. Cantharidin specifically inhibits PP2A activity.

A. Wild type Col-0 plants were treated with mock, 1  $\mu$ M flg22 or 50  $\mu$ M cantharidin. Pictures of treated leaves were taken 4 days post-infiltration.

B. BAK1-associated PP2A activity is inhibited by cantharidin. PP2A activity in Col-0 seedlings was measured by colorimetry on protein extracts enriched with anti-BAK1 and supplemented with mock or 50  $\mu$ M cantharidin. PP2A activity relative to background detected in non-enriched protein extracts (control) is presented as average of two biological repeats ± SE. Similar enrichment of BAK1 protein (bottom panel) was assessed by immunoblot in the same samples.

#### Figure S2. *PP2A* gene expression profile in response to biotic stresses.

A. Data were extracted from the publicly available micro-array database (eFP browser; <u>http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi?dataSource=Biotic Stress</u>) and presented as ratio normalized to respective mock treated sample. Flg22: infiltration of 1 μM bacterial-derived elicitor flg22; HrpZ: infiltration of 10 μM bacterial-derived HrpZ; LPS: infiltration of 100 μg/ml bacterial-derived lipopolysaccharide; NPP1: infiltration of 1 μM oomycete-derived GST-NPP1; hrcC: infiltration of *Pseudomonas syringae* pv. *tomato* DC3000 *hrcC*<sup>-</sup> at 10<sup>8</sup> cfu.mL<sup>-1</sup>; DC3000: infiltration of *Pseudomonas syringae* pv. *tomato* DC3000 at 10<sup>8</sup> cfu.mL<sup>-1</sup>; Psp: infiltration of *Pseudomonas syringae* pv. *phaseolicola* at 10<sup>8</sup> cfu.mL<sup>-1</sup>. Red and green colors indicate respectively increased or reduced gene expression compared to mock treated sample.

B. The phylogenetic tree of PP2A-B' subunits was obtained following MUSCLE alignment of protein sequences by neighbor-joining method. Number on each branch indicates number of substitution per site.

### Figure S3. Genetic characterization of the *pp2a* mutants used in this study.

A. Identification number and references for each T-DNA insertion line used in this study.

B. *PP2A* gene expression in corresponding *pp2a* mutants was assessed by qRT-PCR with primers designed after the T-DNA insertion for each lines. Normalized values are presented relative to *PP2A* gene expression in Col-0.

### Figure S4. Overexpression of PP2A suppresses PAMP-triggered ROS burst.

ROS production in response to increasing elf18 concentration was measured in 5 week-old *rcn1-1* mutant and *rcn1-1/RCN1<sub>pro</sub>:RCN1-YFP* complemented line compared to respective wild type Ws-4. Values presented are average of three biological repeats  $\pm$  SE.

### Figure S5. *pp2a* mutants do not show sign of constitutive immune responses.

A. Pictures of Col-0, *pp2a-a1*, *pp2a-c4*, *pp2a-b'* $\eta$  and *pp2a-b'* $\zeta$  plants grown on soil at 21 °C for 4 weeks. Bar represents 1 cm.

B. Accumulation of marker gene transcripts *FRK1* (At2g19190) and *NHL10* (At2g35980) was assessed by qRT-PCR in un-elicited *pp2a-a1*, *pp2a-c4*, *pp2a-b'q* and *pp2a-b'ζ* seedlings. Values presented are average of three biological repeats  $\pm$  SE compared with Col-0 samples.

### Figure S6. Enhanced BR responsiveness in *pp2a* mutants.

A-B. Accumulation of marker gene transcripts *CPD* (A) and *SAUR-AC1* (B) was assessed by qRT-PCR in Col-0, *pp2a-a1* and *pp2a-c4* seedlings 3 h after treatment with mock or 1  $\mu$ M brassinolide (BL). Prior to treatment all seedlings were incubated for 16 h with 2,5  $\mu$ M brassinazole. Values presented are average of three biological repeats ± SE compared with mock-treated samples.

## Figure S7. PP2A activity but not its association with BAK1 is regulated by PAMPs.

A. PP2A/BAK1 interaction was detected by co-immunoprecipitation in *rcn1-1* or *rcn1-*  $1/RCN1_{pro}$ :RCN1-YFP protein extract enriched on GFP-Trap beads. Seedlings were treated with 100 nM elf18 for the indicated time.

B. BAK1-associated PP2A activity is attenuated by flg22. PP2A activity (top) was measured by colorimetry on Col-0 protein extract enriched with anti-BAK1 antibodies

over indicated time following 100 nM flg22 treatment. PP2A activity is presented relative to the maximum activity detected in untreated seedlings. Ligand-dependent FLS2/BAK1 dimerization (bottom) was detected by co-immunoprecipitation in the same samples.

Table S1. Primers used to genotype *pp2a-b* mutant lines.

Table S2. Primers used for quantitative PCR studies.

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		fl	<u>g22</u>	H	rpZ	LPS NPP1		<u>hrcC</u>			DC3000		Psp					
Name	AGI	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h	2 h	6 h	24 h	2 h	6 h	24 h	2 h	6 h	24 h
A1	At1g25490	0.9	1.0	1.0	1.1	1.1	0.9	0.9	1.1	0.9	1.1	0.9	1.0	0.9	0.9	0.8	1.1	0.8
A2	At3g25800	0.9	1.3	0.9	1.4	1.0	1.0	0.9	1.2	1.2	1.1	1.3	0.9	1.1	1.5	0.9	1.4	1.3
A3	At1g13320	0.9	1.0	1.0	0.9	1.0	1.0	1.0	0.9	1.0	1.2	1.0	1.0	1.1	0.9	0.9	1.1	1.0
Βα	At1g51690	1.1	0.9	0.9	0.8	1.2	1.2	0.6	0.9	1.3	0.9	0.9	1.1	1.1	1.1	1.0	1.0	1.0
вβ	At1g17720	0.8	1.3	0.8	1.2	1.1	1.1	0.8	1.3	1.2	1.0	1.2	1.4	0.8	1.3	1.1	1.0	1.2
Β'α	At5g03470	0.7	1.2	1.3	1.0	1.1	1.1	0.8	0.8	0.9	1.1	0.8	0.9	1.3	1.1	0.9	0.7	0.8
в'β	At3g09880	0.9	1.0	0.9	1.0	1.0	1.0	0.8	1.2	1.1	0.9	0.8	1.1	1.1	1.2	1.1	0.9	0.9
Β'γ	At4g15415	0.9	1.5	0.9	1.2	1.0	0.9	0.9	1.1	1.0	1.2	0.8	0.8	0.9	0.6	0.8	1.1	0.9
в'δ	At3g26030	0.8	1.0	0.7	1.1	0.9	1.0	0.7	0.9	1.1	1.0	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Β'ε	At3g54930	0.6	0.6	0.8	0.3	0.9	1.2	0.7	0.8	0.3	0.6	0.4	1.7	0.5	1.1	0.3	0.7	1.3
Β'η	At3g26020	2.1	1.3	2.0	2.3	1.1	0.8	1.6	2.0	1.6	1.1	1.2	1.5	0.8	1.3	1.7	2.2	1.1
в'ζ	At3g21650	2.0	1.1	2.1	2.1	0.9	1.0	2.0	2.1	1.2	1.0	0.9	1.2	0.8	0.8	1.6	1.8	0.9
В'к	At5g25510	1.0	1.2	0.9	1.2	0.9	1.0	0.8	1.0	1.2	1.0	1.1	1.3	0.8	1.0	1.0	1.1	1.0
в'θ	At1g13460	1.1	1.1	1.1	1.0	0.9	0.9	0.8	1.1	1.6	1.3	1.1	1.3	0.6	0.8	1.1	1.1	1.1
Β"α	At5g44090	1.3	1.1	1.3	1.7	1.1	0.9	1.3	1.3	1.6	0.9	1.2	1.7	0.9	2.0	1.5	1.0	1.2
в"β	At1g03960	1.1	0.9	1.1	0.8	0.9	0.9	0.8	1.2	1.0	1.3	0.8	0.9	1.2	0.7	1.1	1.4	0.6
Β"γ	At1g54450	1.3	0.7	1.4	0.7	0.8	0.7	0.6	1.3	1.0	1.0	0.7	1.2	1.1	0.8	1.2	1.1	0.8
в"δ	At5g28900									n. d								
Β"ε	At5g28850									n. d								
(B) TAP46	At5g53000	1.0	1.9	1.2	1.9	1.0	1.2	0.9	1.4	1.6	1.1	1.3	1.7	0.9	1.2	1.6	1.4	1.3
(B) TON2	At5g18580	0.9	1.2	0.8	1.3	0.9	0.9	0.9	1.2	1.1	1.1	1.0	1.1	1.1	1.1	1.0	1.0	1.0
C1	At1g59830	0.9	1.1	0.9	1.1	0.9	0.9	0.9	0.9	1.0	1.1	0.9	0.9	1.8	1.2	0.8	1.0	0.9
C2	At1g10430	0.9	1.1	0.9	1.3	1.0	1.0	0.8	1.3	1.0	1.0	1.0	1.1	1.2	1.0	1.1	1.2	1.1
C3	At2g42500									n. d								
C4	At3g58500									n. d								
C5	At1g69960	1.4	1.3	1.4	1.6	1.0	1.0	1.1	1.2	1.2	1.3	1.0	1.1	0.9	0.9	1.1	1.5	1.1

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## Α

Gene	Name	Background	Line #	Reference
At1g59830	c1	Col-0	SALK_102599	Ballesteros et al., 2013
At1g10430	c2	Col-0	Ws insertion backcrossed into Col-0	Ballesteros et al., 2013
At2g42500	с3	Col-0	SAIL_182_A02	Ballesteros et al., 2013
At3g58500	c4	Col-0	SALK_035009	Ballesteros et al., 2013
At1g69960	с5	Col-0	SALK_013178	Ballesteros et al., 2013
At1g25490	a1 (rcn1-6)	Col-0	SALK_059903	Skottke <i>et al.</i> , 2011
At1g25490	rcn1-1	Ws-4		Deruere <i>et al</i> ., 1999
At3g25800	a2 (pp2aa2-2)	Col-0	SALK_017541	Michniewicz et al., 2007
At1g13320	a3 (pp2aa3-1)	Col-0	SALK_014113	Michniewicz et al., 2007
At3g26020	b'η	Col-0	SALK_039168	this study
At3g21650	bζ	Col-0	SALK_107944C	this study
At5g44090	b"a	Col-0	SALK_135978	this study











### Α





pp2a-a1



pp2a-c4





Col-0

В



pp2a-b'η

pp2a-b'ζ





Table S1. Primers used to genotype *pp2a-b* mutant lines.

Gene	Name	Line #	LB primer (5'>3')	RB primer (5'->3')
At3g26020	b'η	SALK_039168	GTATGTCGAAACGATATGGCC	CAACAACAACAACAACGG
At3g21650	b'ζ	SALK_107944	CACTCGTCGAAAAGAACTTGG	CCGAATCTCTTTATCGGGAAG
At5g44090	b"a	SALK_135978	GGCATAGAAGCGTCAAACAAG	ACTCTTCTCCCAGTGGCTTTC

### Table S2. Primers used for quantitative PCR studies.

Gene	Name	Forward primer (5'>3')	Reverse primer (5'->3')
At1g25490	A1	CCAAACTCCTCTGCGAGGCGC	AGCTCGGAGCCCTTTGCATGC
At3g25800	A2	TGCGTGCGGTGTCTCTTCTTG	AGCTCCACAAGCCCAGGACG
At1g13320	A3	CGTGGCCAAAATGATGCAATCTCTC	TAGCTCCACCAAGCATGGCCGTA
At3g21650	Βζ	ACGGGAAAGGACATGGCAACGG	GACCCTGTGGACTCAGAGCTGC
At3g26020	Β'η	TGAACAGTCTGCATTTTCAGGCCA	CTCTCTCTGCAACACCTAGAGCTGA
At5g44090	В"а	GGCGTTTGAAACCCGTGACCC	GCAAATCGGTCCCATTCCGTCA
At1g59830	C1	ACAGATGTGGAAACATGGCCGCA	TGCGCGTGGTATCGGGTTCG
At1g10430	C2	TACCGGTGTGGAAACATGGCTGC	CGAGGAGCTGGATCGAACTGGA
At2g42500	С3	AAGTGCCCCATGAAGGGCCG	CCGGCACCCCGAGGAGAGAT
At3g58500	C4	GCACCGAGGAGAGGAGAGCCA	CCGGAAGCTGCAGGAGGAGC
At1g69960	C5	CGTTGTGGCAACATGGCTGCG	TGCGAGTGGTTTCGGGTTCGAC
At2g19190	FRK1	ATCTTCGCTTGGAGCTTCTC	TGCAGCGCAAGGACTAGAG
At2g35980	NHL10	TTCCTGTCCGTAACCCAAAC	CCCTCGTAGTAGGCATGAGC
At5g15400	U-box	TGCGCTGCCAGATAATACACTATT	TGCTGCCCAACATCAGGTT