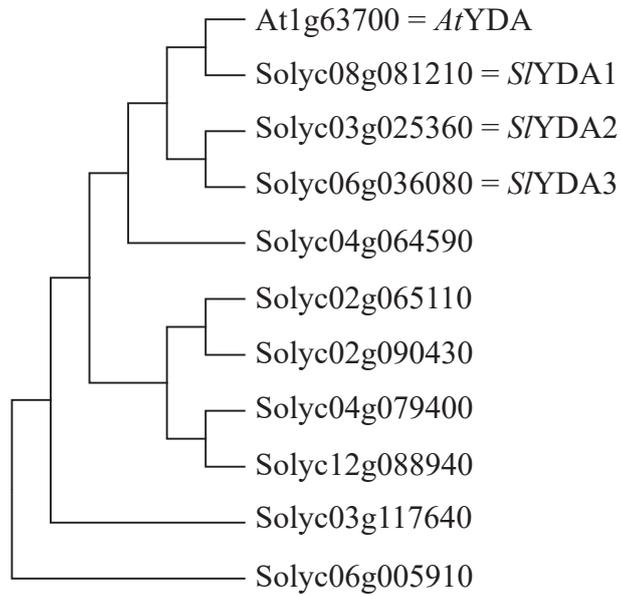


Supplementary Figure 1



Supplementary Figure 1. Phylogenetic analysis of *AtYDA* and tomato *SlYDA*-like MAPKKKs. Phylogenetic analysis using the Neighbor-Joining method based on the full-length protein sequence of *Arabidopsis thaliana AtYDA1* (At1g63700) and putative orthologs from *Solanum lycopersicum SlYDA1* (Solyc08g081210), *SlYDA2* (Solyc03g025360), *SlYDA3* (Solyc06g036080). The tree also includes 6 members of the MAP3K family from *Solanum lycopersicum* (Solyc04g064590, Solyc20g065110, Solyc02g090430, Solyc04g079400, Solyc12g088940 and Solyc03g117640). Solyc06g005910 (coding for a tubulin β -chain) is used as an outlier.

Supplementary Figure 2

(A)

AtYDA	1	-----MP--WWSKSS--KDEKKKTNKESIIDAFNRKLGFASE	32
SlYDA1	1	-----MRSWWGKSSSKDVRRKSTIKESFIDIIINRKLKIIFTT	35
SlYDA2	1	-----MPSWWKSS--KEAKKKPTIKESFIDTLHRKFKKSPA	33
SlYDA3	1	MRSLLRNELVYACDATSNQLKAPEKMPSSWWGKSS--K-AKKKATIKESFIDSLHRKFKKSPA	57
AtYDA	33	DRSSCRSRKSRRRRDELIVSEFGAISRLPSSRSPPSPSTRVSRCSQSFARSPFAVPLPRPIVRR	92
SlYDA1	36	EKSSGKSGSSRRRRKDTNSVKGSQSRV--SRSPSPST-----EMLKSAITLSDNQ	70
SlYDA2	34	VKSPGKSGGSRRHNSDTASEKGSLSQAQSRASSPSKHVSRCSQSFARPLAQPPLPLPGVRR	93
SlYDA3	58	AKSPKSGGSRRHNEIASEKGSLSQAQSRSSSPSKNVSRCSQSFARALAQPLPLPLGLPR	117
AtYDA	93	-HVTSTDSGMNGSORPGLDANLKPS-WLPLPKPHGATSIPDNTGAEPDFATASVSSGSSV	150
SlYDA1	71	-----GSIILVTGEVSEPSLITLPLMPRHLPHGPTAAGVDRDLPPTASVSCDSS	119
SlYDA2	94	ANVGRSDSGISPSAKSRVEKASKPFLPLPKPACIRHRLDPTDDTGEIVFASISSECSI	153
SlYDA3	118	ASVVRADSGISQSAKPRIGKSKLISLFLPLPKPACIRHRLDPTADADGETVFAISSECSI	177
AtYDA	151	-GDIPLSDS-LLSPLASDCENGNRTPVN----ISSRDOSMH-SNKNSAEMFKPVP--NKNR	201
SlYDA1	120	DSDDLTDSSRFISPOTSDYENCSRTALNSPSSLKOKVOSPIASNASSGEMLSATITLSDNQ	179
SlYDA2	154	ESDDPITDSRORSPLATDYETCSRTAAGSPSSLVKQDOSAV-GQISLKEMTRPVSLSPSRN	212
SlYDA3	178	ESDDPITDSRORSPLTFDYETGNRTPLGSPPRLAVKQDOSAV-GQTSIKIATELVNLSPSGH	236
AtYDA	202	ILSASPRRRPLGTHV-KNLIQLPORDLVLCSAPDSLISPSRSPMRSFIPDOVSNHGLLIS	260
SlYDA1	180	AIPTSPRORILRSHVPPGLOIPHG-ASYAPDSSMSPPSRSPMRVFGHEITVMNPGFWLW	238
SlYDA2	213	RAYPDLPSLSSHV-TTLOVPPG-AFCAPDSSMSPPSRSPMRAAASEOVTSSTLWAG	270
SlYDA3	237	VSSRSPKRRPLNSHL-SSIQLPFHG-TLCSVPDSSISSPSRNPMKAAGCEQVSSSTFWAG	294
AtYDA	261	KPYSIDVSLIGSGQCSSPGSGYNSGNNSIIGDMATQLEFWPQSRCSPECSEVPSPRMTSPGP	320
SlYDA1	239	KPHGEITIFLIGSGHCSSPGSGONGSHNSIIGDMLAOPFWPHSRCSPECSPVPSPRMTSPGP	298
SlYDA2	271	RAYPDLPSLIGSGHCSSPGSGONGSHNSMGGDMGOLFQPCRGSPSEYSPITPSPRMTSPGP	330
SlYDA3	295	KTYPDLPLLIGSGHCSSPGSGONGSHNSMGGDMVGLQFWQPSRGSPSEYSPITPSPRMTSPGP	354
AtYDA	321	SSRIHSGAVTPIHPRAGGSTTGSPTIRRLDDNROOSSHRLPLPPLILISNTCPFSPTYSAATS	380
SlYDA1	299	GSRHSGAVTPIHPRAGGTLAESSASLIDNGKQOSSHRLPLPPLISIPHSSITFSLSCSM--T	356
SlYDA2	331	SSRIHSGAVTPIHPRAGGAGELQTSWPDDGKAOSHPLPLPPLITISNSSPFSSHSNSVATS	390
SlYDA3	355	SSRIHSGAVTPIHPKAGGAGELQTNWPDDAKPESHPLPRPPLAISNSSPFSSHSNSVATS	414
AtYDA	381	PSVPRSPARAEATVSPGSPWKKGRILLGMCSEFGHVYLVGFENSESGEMCAMKEVTILCSDDPKS	440
SlYDA1	357	PATPRSPGRITGNPPSPGPRWKKGRILIGSGTFGHVYLVGFENSESGEMCAMKEVTILCSDDPKS	416
SlYDA2	391	PSVPRSPGRAENLASPGSPWKKGRILLGRCTFGHVYLVGFENSESGEMCAMKEVTILCSDDPKS	450
SlYDA3	415	PSVPRSPGRAENLSPPGSPWKKGRILLGRCTFGHVYLVGFENSESGEMCAMKEVTILCSDDPKS	474
AtYDA	441	RESAQQOLGOEISVLSRLRHPNIVVOYYGSETVDDKLYIYLEYVSGGSYKLLQEYGOEFGEN	500
SlYDA1	417	RESAQQOLGOEISLRLRHPNIVVOYYGSETVDDKLYIYLEYVSGGSYKLLQEYGOEFGEL	476
SlYDA2	451	KESAKOLAQEIALLSRLRHPNIVRYYGTEETVDDKLYIYLEYVSGGSYKLLQEYGAFFGEA	510
SlYDA3	475	KESVKQLTQEISSLRHPNIVVOYYGSEMVVDDKLYIYLEYVSGGSYKLLQEYGAFFGET	534
AtYDA	501	AIRNSYTOOILSGLAYLHAKNTVHRDIKGANILVDPNGRIVKLVADFGMAKHITGOSCPILSEFK	560
SlYDA1	477	AIQSYTOOILSGLAYLHAKNTVHRDIKGANILVDPNGRIVKLVADFGMAKHITGHYCPLSEFK	536
SlYDA2	511	AIRSYTOOILSGLAFVHAKNTVHRDIKGANILVDPNGRIKLVADFGMAKHITGOSCPILSEFK	570
SlYDA3	535	TIRSYTOOILSGLAYLHAKNTVHRDIKGANILVDPNGRIKLVADFGMAKHITGOSCPILSEFK	594
AtYDA	561	GSPYWMapeVIKNSNGCNLAVDIWSLGTVLEMATTKPPWSOYEGVPAAMFKIGNSKELPD	620
SlYDA1	537	GSPYWMapeVIKNSNGCNLAVDIWSLGTVLEMATTKPPWSOYEGVAAMFKIGNSKELVPA	596
SlYDA2	571	GSPYWMapeVIKNSNGCNLAVDIWSLGTVLEMATSKPPWSOYEGVAAMFKIGNSKELPT	630
SlYDA3	595	GSPYWMapeVIKNTSGCNLAVDVWSLGTVLEMATSKPPWSOYEGVAAMFKIGNSKELPT	654
AtYDA	621	LPFDHLSSEEGKDFVRKCLORNPANRPTAAQLLDHAFVARNVMPMERPIVSGEPAEAMNVASS	680
SlYDA1	597	LPYHLSDDKDKDFVROCLORNPVLRPTASQLLKHFPVKSTAPMER-----	640
SlYDA2	631	LPFDLSDEAKDFVRKCLORNPVLRPTAAQLLDHFPVKVAVATLEKPNISP-PADPPCAGAN	689
SlYDA3	655	LPFDLSDEGKDFVRKCLORNPVLRPTAAQLLDHFPVKDAAPLEKQNMFPSTFDLPCVAAS	714
AtYDA	681	TMRSLDIGHARSLPCLDSEED-ATNYOOKGLKHGSGFSSISOSPRNMSOPTISPVG--SPTFH	737
SlYDA1	641	---FTGIGHLKDPFCVGSEEVAVHHEPRSSIFEPGFSDVPVPR--SCPVSPVGIESPVYH	695
SlYDA2	690	GVKSLGIGOARNIPTSESERLATHSSRVSKSNFHC-SDISITRNI SCPVSPIG--SPLLH	746
SlYDA3	715	GIKL LGTGSARNYPTPDSERLATHSSRAKSKFHC-SDIHTPKNISCPVSPIG--SPL--	769
AtYDA	738	SHSP-HISGRSPSPISSEPHALSGSSTPLTICGGGAIPEHHROROTTVNFLEHGIGSSRSRSP	796
SlYDA1	696	SQSPKHMGRILSPSTISSPRAVSGSSTPLSGGGGAVPLSNPIMPT--TSSSEDMGTSRSPK	752
SlYDA2	747	PRSPHNLNRLSPSPISSPITMSGSSTPLSGGTGAIPEHHLNOSV--YLQEAAPLQSPY	804
SlYDA3	770	PRSPHNLNGRMSPSPISSPINTSGSSTPLSGGNQVLPFRHINOSV--YLQEAARTVPNSPY	827
AtYDA	797	SGGNFYT-NSFFQEPSROODRSRSSPRTPPHFVWDNNGSIO----PGYNWNKDNQPVLS	851
SlYDA1	753	AQSCFY--PDAYTSHGLKSDMSREAPPYNGFFGENEGGHAQSGVNGOPY--OGOSVLAN	808
SlYDA2	805	MNGFSYWDPDVLRGPPSGSHAFRELASSONDALGKQFGRTT---GELY--DGOSVLAN	858
SlYDA3	828	MNGFSYWDPDVLRGSPSGSHAFRELASVEYDALGKQFGRLA----TGELC--NGOSALAN	881
AtYDA	852	HVSOQLLSEHLKI-KSLDIRPGFSTPGSTNRGP	883
SlYDA1	809	RVAOQLLRDQVKLSPSFDLNGSPVFSWDN-GV	840
SlYDA2	859	RVSOQLLRDHVKLVPSLDLNPCCPLDGRG-GEA	890
SlYDA3	882	RVSOQLLRDHVKSISPVDLNPCCPLDGRPG-GT	913

N-terminal
 Kinase domain
 C-terminal

(B)

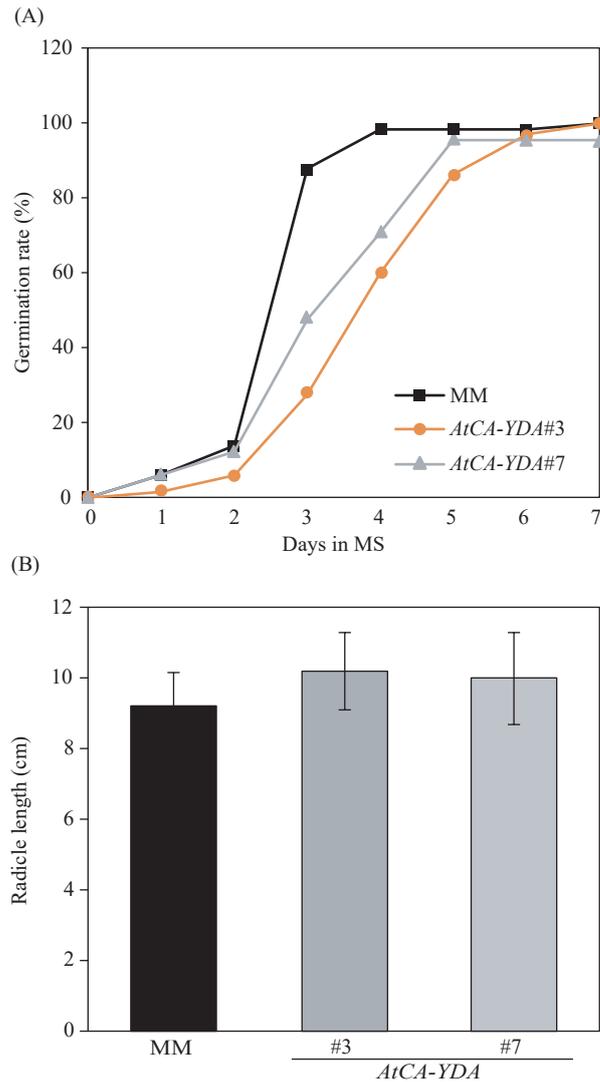
IDENTITY				
At1g63700	100%			
<i>Sl</i>YDA1	60.71%	100%		
<i>Sl</i>YDA2	59.22%	60.83%	100%	
<i>Sl</i>YDA3	57.19%	59.64%	81.79%	100%
	At1g63700	<i>Sl</i>YDA1	<i>Sl</i>YDA2	<i>Sl</i>YDA3

(C)

SIMILARITY				
At1g63700	100%			
<i>Sl</i>YDA1	67.5%	100%		
<i>Sl</i>YDA2	67.15%	66.9%	100%	
<i>Sl</i>YDA3	65.23%	65.95%	85.5%	100%
	At1g63700	<i>Sl</i>YDA1	<i>Sl</i>YDA2	<i>Sl</i>YDA3

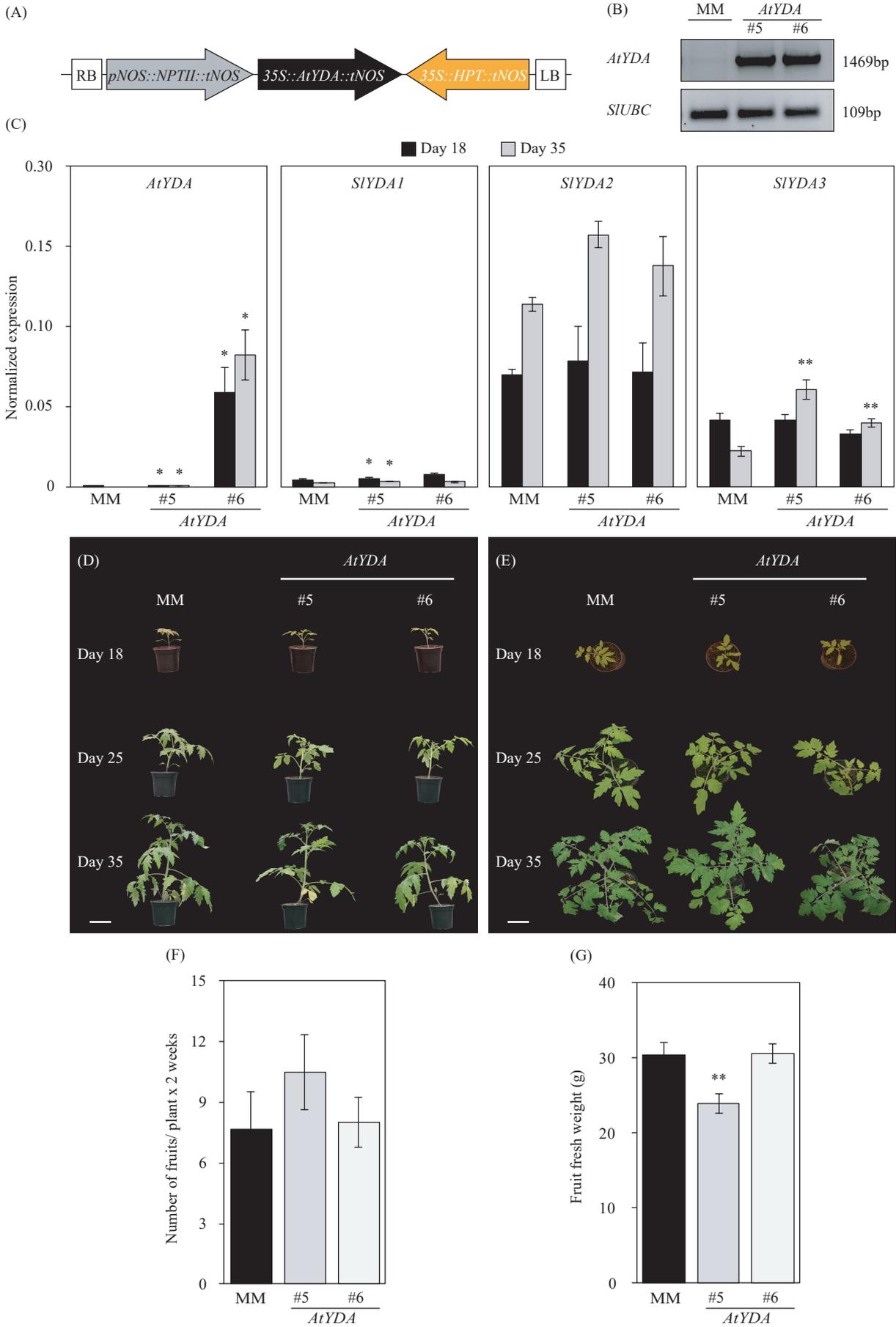
Supplementary Figure 2. Protein sequence alignment of *At*YDA and putative orthologs from *Solanum lycopersicum* *Sl*YDA1 (Soly08g081210), *Sl*YDA2 (Soly03g025360) and *Sl*YDA3 (Soly06g036080). (A) Full length protein multiple sequence alignment by MUSCLE using MEGA. (B) Identity and (C) similarity results between the protein sequences using Sequence Identity And Similarity (SIAS) tool and BLOSUM62 matrix (imed.med.ucm.es).

Supplementary Figure 3



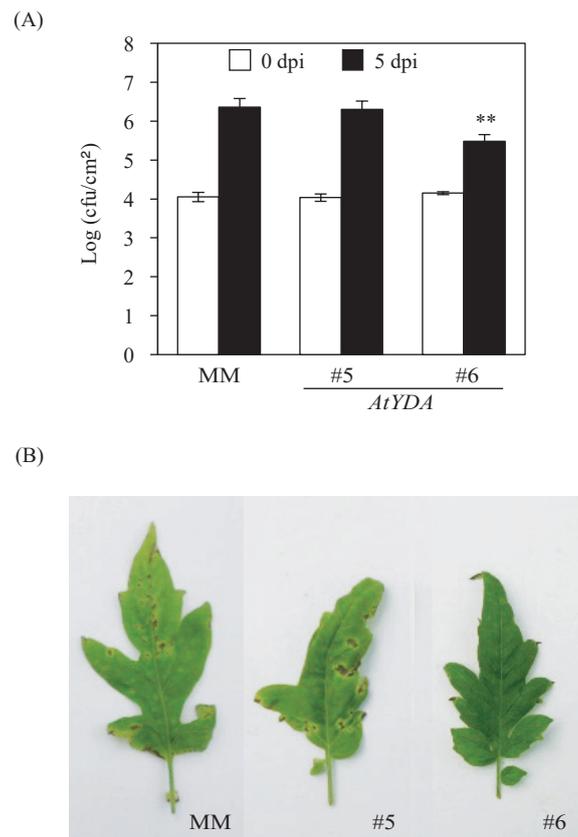
Supplementary Figure 3. Transgenic overexpression of *AtCA-YDA* in tomato does not have a significant effect on root development. **(A)** Germination rate of the different tomato lines grown in MS media in dark conditions. **(B)** Measurements of radicle lengths of the indicated lines 7 days after germination in MS media. Data shown are average ($n=8$) \pm SE. Asterisks indicate statistical differences with respect to MM plants (Student's *t*-test; * p -value < 0.05). These assays were performed at least three times with similar results.

Supplementary Figure 4



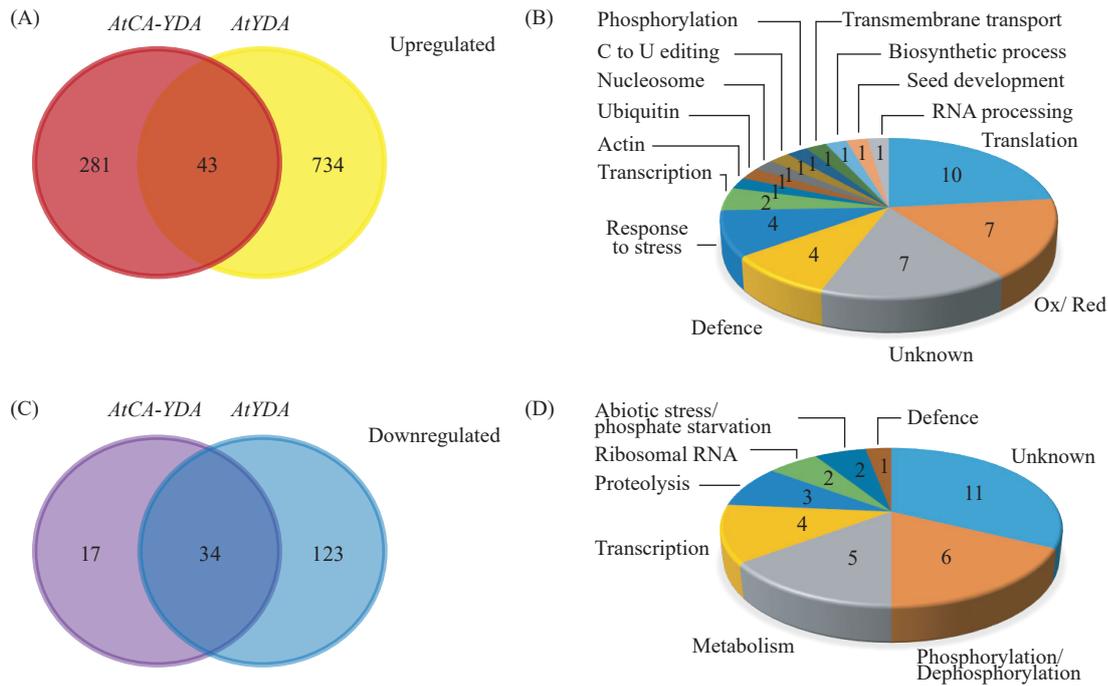
Supplementary Figure 4. Transgenic over expression of *AtYDA* in tomato does not affect tomato development. **(A)** Scheme of construction used to transform tomato plants with *AtYDA* using pGWB2 vector, driven by the *CaMV 35S* promoter (35S) and nopaline synthase terminator (*tNOS*). RB, right border sequence of T-DNA; LB, left border sequence of T-DNA; *NPTII*, aminoglycoside phosphotransferase from Tn5 driven by nopaline synthase promoter (*pNOS*) and nopaline synthase terminator (*tNOS*); *HPT*, hypoxanthine-guanine phosphoribosyltransferase gene driven by 35S promoter and *tNOS* terminator. **(B)** Detection by PCR of the transgene in the indicated Moneymaker (MM) and *AtYDA* #5 and *AtYDA* #6 transgenic lines. PCR amplified *SIUBC* was used as equal DNA loading control. Of note *AtYDA* #5 does not harbour the transgene and it is included as control. **(C)** Expression of *AtYDA*, *SIYDA1* (*Solyc08g081210*), *SIYDA2* (*Solyc03g025360*) and *SIYDA3* (*Solyc06g036080*) in *S. lycopersicum* MM and transgenic *AtYDA* plants (lines #5 and #6) at 18 and 35 days after sowing. Shown are the averages of three independent experiments (n=3) ± SE of normalized expression. Asterisks indicate statistical significance with respect to MM plants (Student's *t*-test; * *p*-value < 0.05; ** *p*-value < 0.01). **(D-E)** Morphology of the indicated genotypes at 18, 25 and 35 days after sowing. Bar = 10 cm. **(F)** Number of fruits collected per plant from the indicated lines (n=12). **(G)** Fresh weight (in g) of the tomato fruits produced by the transgenic lines (#5 n=92; #6 n=72) and the Moneymaker (n=58) plants (MM). Student's *t*-test; * *p*-value < 0.05; ** *p*-value < 0.01.

Supplementary Figure 5



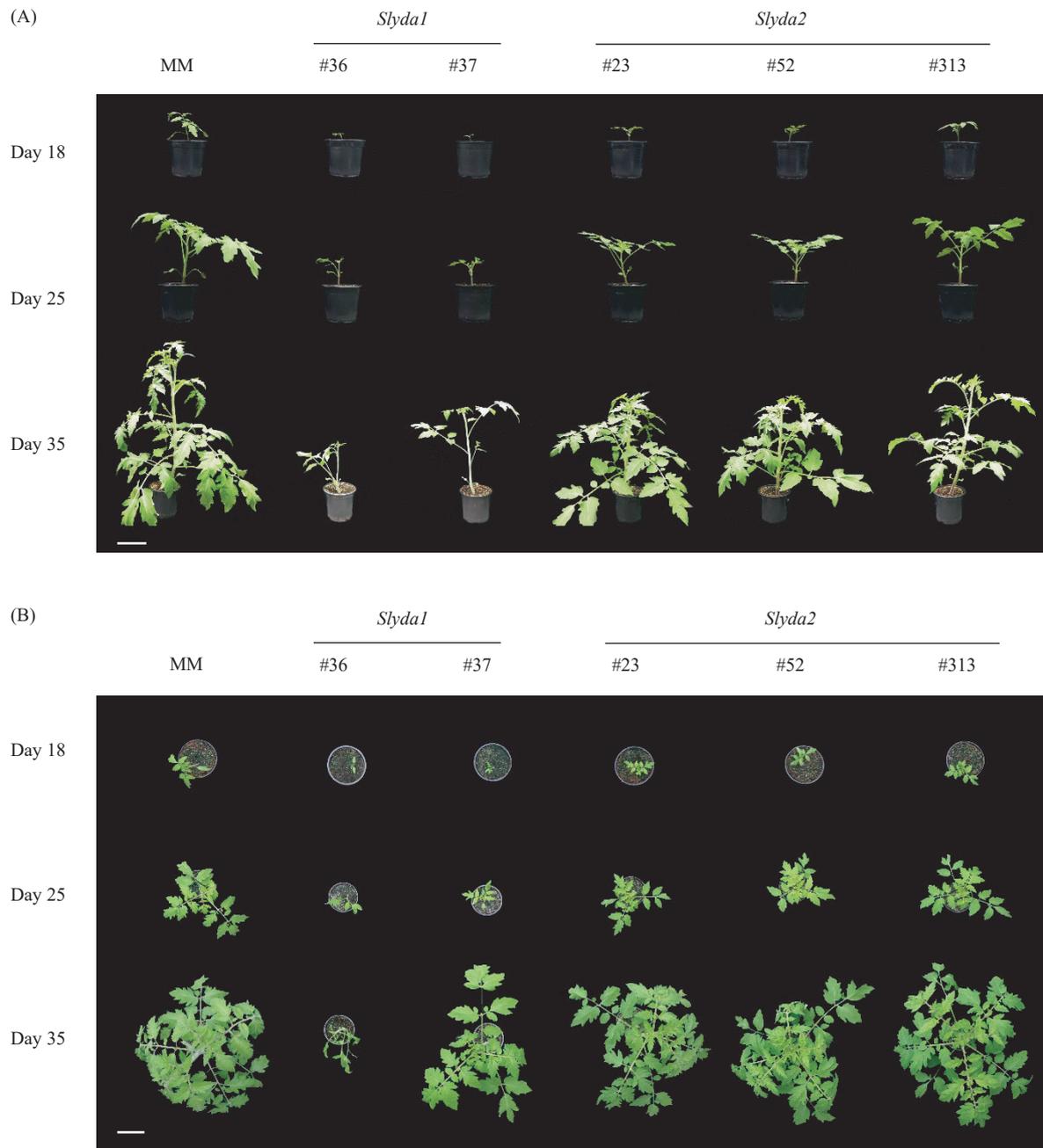
Supplementary Figure 5. Heterologous expression of *AtYDA* into tomato plants confers enhanced resistance to *Pseudomonas syringae* pv. tomato DC3000. **(A)** Quantification of bacterial growth at 0, and 5 days post-inoculation (dpi). Values represent the mean (n=16) \pm SE of three independent experiments. Asterisks indicate values statistically significant with respect to MM inoculated plants (Student's *t*-test; * *p*-value < 0.05; ** *p*-value < 0.01). **(B)** Macroscopic disease symptoms on tomato leaves inoculated with the bacterium at 8 dpi. This experiment has been performed at least three times with similar results.

Supplementary Figure 6



Supplementary Figure 6. Functional classification of differentially miss-regulated genes in *AtYDA* and *AtCA-YDA* overexpressing lines. **(A)** Venn diagram showing overlapping of genes commonly upregulated in *AtCA-YDA* lines (#3 and #7) and in *AtYDA* plants compared to MoneyMaker wild-type plants. Differential expression was defined as at least 2-fold change when compared with the non-transformed controls (MM). A minimal coverage value of 20 for each gene in RNAseq data was considered for selecting the genes. **(B)** Functional category classification of the 43 core genes up-regulated in *AtCA-YDA* and *AtYDA* tomato lines. BINGO pipeline was used to assign the categories. See Table S6 for further information on the 43 core genes up regulated. **(C)** Venn diagram showing the overlapping of genes downregulated in *AtCA-YDA* lines (#3 and #7) and *AtYDA* plants compared to MoneyMaker. **(D)** Functional category classification of the 34 down-regulated core genes found in the *AtCA-YDA* and *AtYDA* tomato lines using BINGO pipeline. Table S7 shows the 34 core genes downregulated.

Supplementary Figure 7



Supplementary Figure 7. Phenotypic characterization of leaf and stem development of tomato *Slyda1* and *Slyda2* mutants. **(A)** Frontal view of MM and CRISPR-edited tomato lines at 18, 25 and 35 days after sowing. Bar = 10 cm. **(B)** Aerial view of the indicated genotypes at 18, 25 and 35 days after sowing. Bar = 10 cm.