Sh 1 10 20 3050 60 70 80 90 100110120 130 đû Arabidopsis MSQPSYILATASYDHTIRFWEAETGRCYRTIQYPDSHYNRLEITPDKHYLAAACNPHIRLFDYNSNSPQPYNTYDSHTNNYHAYGFQCDAKWMYSGSEDGTYKINDLRAPG--CQKEYESYAA MPHPSYYLATAGYDHTIKFWEATTGRCYRTLOYTDSOYNKLEYTPDKOYLAAAGNPHIRLYDYNSNHPOPYLTYESHNNNYTAYGFOCDGKWMYSGSEDGTYKIHDLRAPG--COREYESRAA Physcomitrella MAQPSYILATASYDHSIKFWEAKSGRCYRTLQHTESHINRLEITPDKRFLAAAGNPHIRLFDINSNSNHPYISYDSHTSNYHAYGFHCDGNNHYSGSEDGTYRINDLRTAT--CQREYESRAA Oryza Chlanydononas MRSSSYVLASAGYDHTIRFNEATSGCCYRTLQYAESQVNKLEITADKTRIAAAGNPQIRYFDYNSPDPNPLLSYDGHAGNYTAYGFHKDGKNNFTGGEDGTVRYNDTRSPY--CQRTYESRAA MSYILYSAGYDHTIRFMEALTGYCSRTIQHSDSQVNRLEITNDKKLLATAGHQNYRLYDIRTTNPNPVASFEGHRGNYTSYSFQQDNRMMYTSSEDGTIKYMDYRSPS--IPRNYKHNAP Saccharomyces NNTSPGTYGSDPYILATAGYDHTYRFHQAHSGICTRTYDHQDSQYNALEYTPDRSHIAAAGYQHIRHYDLNSNNPNPIISYDGYNKNIASYGFHEDGRWHYTGGEDCTARIHDLRSRNLQCORIFQYNAP Hono Consensus .w...sY!LatAgYDHt!rFW#A.sG.CyRT.Qh.#Sq!N.LE!TpDk..1AaAgnph!R1%D.nsn.pnPv.s%#gh..N!taYgFh.Dg.WHytgsEDgTvr!WD1Rsp...cqr.%es.Aa 131 170 180 190 200 210 220 230 240 250 260 150160 140 VNTVYLHPNQTELISGDQNGNIRVHDLRANSCSCELVPEVDTAVRSLTVHHDGTHVVAANNRGTCYVHRLLRGK-QTHTEFEPLHKLQAHNGHILKCLLSPA----NKYLATASSDKTVKIHNVDG-FKL Arabidopsis VNTYYLHPNOTELISGDONGNIRYHDLOSNSCSCELYPEYDTAYRSLTYMHDGSLYYAANNNGTCYYHRLLRGT-OMMTNFEPLHKLOAHDRYVLKCLLSPEYCEPNKYLATTSSDHTYKIHNIFD-FSL Physconitrella VNTVVLHPNQKELISGDQNGNIRVHDLAANSCSCELVPEVDTAVRSLTVHHDGSNVVAANNRGTCYVHRLLKGT-QTITCFEPLHKLQAHDGYILKCLLSPEFCDPNRVLATASSDHTVKIHNVDG-FKL Oryza Chlanudomonas VNSVYLHPNQGELISGDQTGHIRVHDLTASACSCELVPEIGTAVRSLTVALDGTHIVAANNNGTCYVHRMMRGA-SLTTHFEPLHKLKAHSNIILKCLISPD----CQQLATTSADKTVKLHNLDG-FTL Saccharonyces VNEVYIHPNQGELISCDRDGNIRINDLGENQCTHQLTPEDDTSLQSLSHASDGSHLAAANTKGNCYVNEMPNHT-DA-SHLKPYTKFRAHSTYITRILLSSD----VKHLATCSADHTARVNSIDDDFKL INCYCLHPNQAELIYGDQSGAIHINDLKTDH-NEQLIPEPEYSITSAHIDPDASYMARYNSTGNCYYNNLTGGIGDEYTQLIPKTKIPAHTRYALQCRFSPD----STLLATCSADQTCKINRTSN-FSL Hono Consensus !N, Yv1HPNQ, ELIsgDq, GnIr ! WDL, ., n, csc #LvPE, dtavrSltv., Dgsn, vAaNn, GtCYYWr\$, .g, ..., t, feP1hK1, AH, .yi1kc11Spd, ..., LAT, SaD, TvkiWn, d, .F, L 261 270 280 290 310 320 330 335 300 +----| ----TGHQRHYHDCYFSYDGEFLYTASSDMTARLHSMPAGKEVKYYQGHHKATYCCALHD Arabidopsis EKYL. -----KGHSRWYWDCVFSYDGAFLYTASSDHTARLWELSTGEAIRYYQGHQKATYCCALHDGTDPSAY Physconitrella ARTL Oruza EKTL -----YGHQRWYWDCYFSYDGAYLITASSDTTARLWTMSTGEAIRYYTSHHKPYYCCALHDGAESAPS -YGHTRHYNDCYFSYDAAYLYTASSDATARLNDLGSSEAIRTYSGHHKAYYCCALNDSAIEGRD Chlanydononas DRTL ---DGHQRHYHDCAFSADSAYLYTASSDHYYRLHDLSTREIYRQYGGHHKGAYCYALNDY Saccharomyces ETTL Hono MTELSIKSGNPGESSRGHNHGCAFSGDSQYIYTASSDNLARLHCVETGEIKREYGGHQKAYVCLAFNDSYLG

Supplemental Figure 1: Alignment of plants, yeast and human LST8 protein sequences. Predicted turns and loops are, respectively, shown on top in red and blue. Each WD 40 repeat contains four β strands (Sa to Sd) which are only shown for the beginning and the end of the sequences. Conserved residues are shown in red.

...tL.......gh,rHvHdCvFSvD,a%1!TASSD,taRLH,1,tge,.r,Y,gHhKavYCcA1nD,.....

Consensus



Supplemental Figure 2 : Expression of the Arabidopsis *LST8* genes in various organs. The expression level is correlated to the intensity of the color. Data are from the Genevestigator website (genevestigator.com).



Supplemental Figure 3 : GUS staining of a transformed Arabidopsis plants carrying a pLst8:*GUS* construct containing 1kb of *LST8-1* promoter. Reconstitution of a stained inflorescence.



Supplemental Figure 4 : Position of the T-DNA insertions in the *lst8-1* mutants. LB indicates the left borders of the inserted T-DNAs. *lst8-1-1*: Salk collection *lst8-1-2*: Sail collection

Supplemental Data. Moreau et al. Plant Cell. (2012). 10.1105/tpc.111.091306



Supplemental Figure 5 : Mutations in *LST8-1* gene affect stem and flower bud morphology and organization. (A) *lst8-1-1* mutant on the right fails to develop normal flowers compared to the WT on the left. Flowers formed by *lst8-1-1* mutant stay small and unexpanded (C, E) compared to WT ones (B, D). Moreover axillary buds in *lst8-1-1* mutant inflorescence appear larger and emerging stems are longer and more developed (C, E, black arrows) compared to WT (B, D).

Supplemental Data. Moreau et al. Plant Cell. (2012). 10.1105/tpc.111.091306 **BO BO Control** NaCl 50 mM Sucrose 6%

Supplemental Figure 8 : Germinating plantlets from the *lst8-1* mutant line display the same sensitivity to high sugar concentrations as a TOR RNAi line.

Seeds from the *lst8-1-1* mutant were sown in vitro on MS/2 medium and grown for 8 days in long day conditions with seeds from the TOR RNAi line 35-7 and a complemented *lst8-1-1* mutant. The *lst8-1-1* mutant was complemented by pLst8:Lst8 cDNA construct (see Methods for details).



Supplemental Figure 9 : Diurnal variations in sugar and starch contents during the transition from short days (SD) to long days (LD) in the wild-type (WT) and in the *lst8-1-1* mutant. Plants were first grown in controlled SD conditions and harvested at the beginning (morning, m) and end (night, n) of the day preceding the shift to LD. Plants were again harvested at day two after the start of LD conditions. Results are mean of at least three different samples ± SD



Supplemental Figure : Mapman representations of metabolite variations in *lst8-1-2* mutants using global GC-MS analysis. Leaves from the *lst8-1-2* mutants were collected from (A) short-day grown plants or (B) after transfer to long-day conditions for 12 days and relative metabolite accumulations were compared with WT plants grown in the same conditions. """.



0.37

sample class - JL+01M sample class - JC_M sample class - JL+01C sample class - JC C sample class - JL+06C Sucrose 1755.4/352 Inositol U[galactinol] Xylose 1145.1/235 Mannose 1550.3/217 Trehalose 2415.4/204 Galactose Melibiose Digalactosylglycerol Galactinol Raffinose U2912.5/204(475) Maltitol Maltitol Malticol Arabinose U2750.0/494 Ribonate 2271.6/249 U3021.1/324 U2803.7/204(475) U2803.7/204(475) Theconate lactone1381.8/247 2104.1/259 U2692.5/361 C070.7/361 Malonate Theconate Malonate Threonate Sorbitol dikydroxybensoate Mannitol beta-lactate 2163.6/204 2183.6/204 Maltose Lactobionate Gluconate GABA dihydroxybutanoate Ethanolamine Erythritol Glucerate Glycerate U2721.2/204 U3378.6/361 GHB U3378.6/361 GHB Pyruwate Alanine Putrescine Glycine Betacyanoalanine Citrulline 1490.7/242 Asparagine Phosphate Aminomalonate Histidine Allantoin Ribose Malate Phenylalanine 2,4-di0Hbutanoate 0-Acetyl-serine Threonine beta-Alanine 1455.7/174 Serine 1455.7/174 Serine Citramalate Pyroglutamate 1690.4/243 Glutamine 1714.0/200 1714.0/200 UNKglutamine 2-0xoglutarate Methionine Isoleucine Valine Lyxoine Tyrosine Proline 1140.4/156 1250/150 Urate Aspartate Glutamate Glutamate Asparato Solutanato Tryptophan 2-Anino adipate Maleate Hexose-P 2514.8/273 (neohesperidine) Galactosylglycerol Succinate 2-ketogluconate Erythronate 2110.8/219 2172.2/290 2562.5/204 Galactouronate Gal acturonate 2-hydroxyglutarate 1,3-diaminopropane 1352.5/283 Amelaate Aselaate Suberate Glucopyranose Uracil Urea propylene glycol Aconitate Aconitate Glycolate 1521.6/276 Homoserine 1601.8/217 arabinofuranose Citrate Lactate Triethyl amine

Supplemental Figure 9:

Variations in metabolite levels in *lst8-1-2* mutants and WT plants exposed to long days. Samples and metabolites were clustered. The color scale shows

the concentrations ratios in log2.

JL+06M: lst8-1-2 mutant after 6 days of transfer to longdays conditions (triplicate)

JL+01M: same after one day of transfer to long-

days conditions (triplicate)

JC_M: lst8-1-2 mutant grown in short-days conditions JL+01C, JL+06C, JC_C: same as above for the WT control plants (triplicate).



Supplemental Figure 10 : Mapman representations of global metabolite analysis using GC-MS in *lst8-1* mutants.

Rosettes from the *lst8-1* mutants were collected after transfer to long-day conditions for 6 days and relative metabolite accumulation was compared with WT plants grown in the same conditions.

A: *lst8-1-1* mutant; B: *lst8-1-2* mutant.



Supplemental Figure 11: Mapman representations of global metabolite analysis using GC-MS in WT plants.

Leaves from the WT plants were collected after transfer to long-day conditions for 6 days and relative metabolite accumulation was compared with WT plants grown in short days.



Supplemental Figure 12: Changes in metabolite accumulations determined by GC-MS in the *lst8-1-2* mutant and in TOR inducible RNAi lines.

LST8 12LD: Leaves from the *lst8-1-2* mutant plants were collected after transfer to long-day conditions for 12 days and relative accumulations were compared for some metabolites with WT plants grown in the same conditions (see Figure 12 for details).

RNAi TOR: The two independent inducible TOR RNAi lines were induced with ethanol for 2 days and relative metabolite accumulation was compared with control plants grown in the same conditions (see Methods for details). Galactinol and raffinose were measured in dark-grown plantlets sown on ethanol. Ratio were obtained from the means of three independent samples for each of the two RNAi lines.



Supplemental Figure 13: Frequency of functional classes in the genes down-regulated in *lst8-1* mutants grown in short-day conditions and compared to WT plants grown in short days. Results have been obtained from the BAR site (<u>http://bar.utoronto.ca</u>) using the classification SuperViewer. The input set has been bootstrapped 100 times to estimate the reliability of the data. The resulting mean values are shown together with standard deviations.



Supplemental Figure 14 : Changes in transcript abundance determined by either quantitative real-time RT-PCR (QPCR) or microarray hybridizations.

Leaves from the *lst8-1* mutant lines were collected after transfer to long-day conditions for 2 days and relative changes in transcript abundance were measured for the indicated genes (see Figure 13 for details). Gene-specific primers for RT-PCR amplification are the ones used for the mirco-arrays gene-specific tag (GST) amplification (see Methods for details and http://urgv.evry.inra.fr/projects/FLAGdb++ for the corresponding sequences).

FT: Flowering locus T

Elip1: Early light-induced protein 1

Apx1: Ascorbate peroxydase 1



Supplemental Figure 15: Frequency of functional classes in the genes down-regulated in *lst8-1* mutants after two days under long-day conditions when compared to WT plants grown in the same conditions.

Results have been obtained from the BAR site (<u>http://bar.utoronto.ca</u>) using the classification SuperViewer. The input set has been bootstrapped 100 times to estimate the reliability of the data. The resulting mean values are shown together with standard deviations.



Supplemental Figure 16: Frequency of functional classes in the genes up-regulated in *lst8-1* mutants after two days in long-day conditions when compared to WT plants grown in the same conditions.

Results have been obtained from the BAR site (<u>http://bar.utoronto.ca</u>) using the classification SuperViewer. The input set has been bootstrapped 100 times to estimate the reliability of the data. The resulting mean values are shown together with standard deviations.

LST8-1 (At3g18140)	SALK 02459	02459-F	AGG AGA GGA TGA AGA AAA CGG	
		02459-R	TTG CTT CCT TCT ACC TGC TTG	
LST8-1 (At3g18140)	SAIL 641D10	02459-R	TTG CTT CCT TCT ACC TGC TTG	Genotyping
		LST8 1-R	CAG TGG CTT TGT GAT GAC CTT	
T-DNA primer	SALK line	Lba-1	TGG TTC ACG TAG TGG GCC ATC G	
	SAILline	LB3-SAIL	TAG CAT CTG AAT TTC ATA ACC AAT CTC GAT ACA	
LST8-1 (At3g18140)		LST8-18140-5'	ATC GAT CGA AAA TGA GTC AGC	
		LST8-18140-3'	CTT ATT AAG GTT TTA ATC GTG A	NT-F CN
LSt8-2 (At2g22040)		LST8-22040-5'	ACA AAC CGG ATG ATA GTC CC	
		LST8-22040-3'	ACA GGA TTT AAT CAC GCA GT	RT-PCR
LST8-1 (At3g18140)		LST8-1 F	GTT TGG GAC TGC GTC TTC TC	Quanti tati ve RT
		LST8-18140-3'	CTT ATT AAG GTT TTA ATC GTG A	PCR

LST8-1	1kb promoter forward with <i>EcoRI</i> site	ATG AAT TCG ATA GAA CAG AGG TTA AGA AC
LST8-1	1kb promoter reverse with <i>Notl</i> site	ATT AGC GGC CGC TTT CGA TCG ATT TTA TCC CG
LST8-1	1 kb promoter forward with <i>BamHI</i> site	CGG GAT CCG ATA GAA CAG AGG TTA AGA AC
LST8-1	Gene 3' end with <i>Notl</i> site	TGC TGC GGC CGC AGG TTT TAA TCG TGA AGT GC
LST8-1	Start of coding sequence with Sall site	CGA AGT CGA CTA TGA GTC AGC CTT CT
LST8-1	Start of coding sequence with <i>EcoRI</i> site	AGG AAT TCA CCA TGA GTC AGC CT
LST8-1	End of coding sequence with <i>Notl</i> site	TGC TGC GGC CGC TTT AAA TCG TGA AG

Supplemental Table 1 : Primer sequences