GTTTTTGAAGGAAAATCACAGGAGGGACGCGGAAGCAAGATTGGTCTGAGAGGAAAATTGAGGAAG<mark>AGGG</mark> CAAAAACTTCCTTTTAGTGTCCTCCCTGCGCCTTCGTTCTAACCAGACTCTCCTTTTAACTCCTTCTCCC

GTTAATCGAGATGAAAATGGAGTTTGAAGTAATAGAGAGAACGTTGCGCAGATCGAGGAAGAACAGAGGA CAATTAGCTCTACTTTTACCTCAAACTTCATTATCTCTCTTGCAACGCGTCTAGCTCCTTCTTGTCTCCT

TAGGATCAACACAAGGATCTT<mark>GTGA</mark>AAATGAAAATGGCTGAGGAAATGAAGGACTATTTGTTAAATAAGA ATCCTAGTTGTGTTCCTAGAACACTTTTACTTTTACCGACTCCTTTACTTCCTGATAAACAATTTATTCT

TATAATATTATTTCT<mark>GAAATATTTC</mark>CATCTGACTCCTTTAATTTATACAAGCCTCCTTTTTTGTACATCT ATATTATAATAAAGACTTTATAAAGGTAGACTGAGGAAATTAAATATGTTCGGAGGAAAAAACATGTAGA

ATTTTCAGAAGATCCAAATAATTGTTTCTTCTATTTGTGTATTTTTGATATTTAAACGTAAATCTTTGGA TAAAAGTCTTCTAGGTTTATTAACAAAGAAGATAAACACATAAAAACTATAAATTTGCATTTAGAAACCT

GTTACAAATAAAAAG<mark>GAAATTC</mark>CACGCTGGCAATAAAATAAGC<mark>GAAAATTC</mark>CAC<mark>GTGA</mark>CATCTACCTGT CAATGTTTATTTTTTCCTTTAAGGTGCGACCGTTATTTTATTCGCTTTTAAGGTGCACTGTAGATGGACA

TTCTATGCAAAAAACGACGATAGTTCTCTATCT<mark>TTCCAGATGAATCTCCTTC</mark>CATATACAAAAGCAGTC AAGATACGTTTTTTTGCTGCTATCAAGAGATAGAAAGGTCTACTTAGAGGAAGGTATATGTTTTCGTCAG

ATGCCTCCTCGCTCTCGCAATTCACAAAGTATCCAAACATCTAAAGTTATCAATTTTACAACATTACC TACGGAGGAGCGAGAGAGCGTTAAGTGTTTCATAGGTTTGTAGATTTCAATAGTTAAAATGTTGTAATGG

GCTATAATCTGCTTGATTCTCTGCAAAAAGAGAAGACTTTTTACCGAGAAGAAGTCCTCTGGCTCATTGA CGATATTAGACGAACTAAGAGACGTTTTTCTCTTCTGAAAAATGGCTCTTCTTCAGGAGACCGAGTAACT

AGAAACTCAACGAAACAAACCCAGTTCTCATATATCGTTTTAAGGTAAATGATCGCGACAATCTTGTTCT TCTT<mark>TGAGTT</mark>GCTTTGTTTGGGTCAAGAGTATATAGCAAAATTCCATTTACTAGCGCCTGTTAGAACAAGA

TTTTTTTCAGGGATTACAAAAGCTAATCGAAG + AAAAAAAGTCCCTAATGTTTTCGATTAGCTTC -



Fig. S1. Nucleotide sequence of 1kb upstream (from ATG) promoter region of *AtClpB-C* gene. Both positive and negative strands are shown. Positions of various regulatory cis-elements noted in this sequence are marked.



Fig. S2. Analysis of Gus expression in 1kbAtClpB-Cpro:Gus plants under stress conditions. (A) Schematic representation of the 1kbAtClpB-Cpro:Gus construct. (B) Qualitative analysis of Gus in the 1kbPro-1, 1kbPro-2 and 1kbPro-3 seedlings. Histochemical Gus staining of the control [C] and heat stressed [HS; 38°C/2h] seedlings is shown. (C) Quantitative estimation of Gus activity by MUG assay. (D) Gus expression analysis in 1kbPro-1, 1kbPro-2 and 1kbPro-3 lines under heavy metal (arsenic and cadmium) stress. Graphs represent the quantitative estimation of Gus activity by MUG assays.



Fig. S3. (A) Schematic representation of 1kbAtClpB-Cpro and Δ AtClpB-Cpro fragments. Nucleotide region deleted from the 1kb promoter is shown. (B) Diagrammatic representation of Δ AtClpB-Cpro:Gus construct. (C) sqRT-PCR showing the comparison between transcript expression levels in 1kbPro and Δ pro seedlings under C and HS (38°C/15' and 30') conditions.



Fig. S4. Analysis of Gus expression in \triangle AtClpB-Cpro:Gus plants. (A) Qualitative (histochemical staining) and (B) quantitative estimation (MUG assay) of Gus expression in the \triangle pro-1, \triangle pro-2 and \triangle pro-3 lines under C and HS (38°C/2h) conditions. (C) Quantitative estimation of Gus in the \triangle pro-1, \triangle pro-2 and \triangle pro-3 lines under heavy metal (arsenic and cadmium) stress. (D) Histochemical staining for Gus expression analysis in \triangle AtClpB-Cpro:Gus plants under unstressed (control) conditions at different developmental stages. (a) inflorescence, (b) single flower, (c) anther showing microspores, (d) young silique, (e) mature imbibed seeds and (f) 10-d-old seedlings are shown.

(AT1G74320).. CATttgaatgccacacacacaaagataaatctctctgtacaagataaaacagatacttttagcgaatact tataacaacactgtgttttgtccaattcaggggaggttctacgatcaacccaatgcaacaattctatatgcttccgtttca taaaaattctgaatatcaatctaaacctatacaattacatccttactaatctagataaagtgtatacaataaaatgagagcaataaqqttcattaatctttatacttaqctcctccacaqqacqatatactttqaqactqacacaaaaacaaaataaqqaaqa ${\tt aagcttacgtttttgaaggaaaatcacaggagggacgcggaagcaagattggtctgagaggaaaattgaggaagaggggtt}$ aatcgagatgaaaatggagtttgaagtaatagagagaacgttgcgcagatcgaggaagaacagaggataggatcaacacaaggatettgtgaaaatgaaaatggetgaggaaatgaaggactatttgttAAATAAGATATAATATTATTATTGAAATATTTC CATCTGACTCCTTTAATTTATACAAGCCTCCTTTTTTGTACATCTATTTTCAGAAGATCCAAATAATTGTTTCTTCTATTT TTAATTCAAAGCGTTACAAATAAAAAAG<mark>GAAATTC</mark>CACGCTGGCAATAAAATAAGC<mark>GAAAATTC</mark>CACGTGACATCTACCTG TCGGATCAAAAAGAGTGGAATTGACATTTGTTTCTCTCACACTCTCTCGAATTCTCTGGTAGCTTCTAGTTCTATGCAA AAAAACGACGATAGTTCTCTATCT<mark>TTCCAGATGAATCTCCTTC</mark>Catatacaaaagcagtcatgcctcctcgctctctcgca ${\tt attcacaaagtatccaaacatctaaagttatcaattttacaacattaccgctataatctgcttgattctctgcaaaaagag$ aagactttttaccgagaagaagtcctctggctcattgaagaaactcaacgaaaccaaacccagttctcatatatcgttttaa \mathbf{g} gtaaatgatcgcgacaatcttgttctcatttgtgtgtttttgtgttttgtgattagggtttacaaaagatactgagattag ttttttttttttttttttttttttttttagggattacaaaagctaatcgaagATG ...(AT1G74310)

Fig. S5. Nucleotide sequence of 1,329-bp intergenic region between *AtClpB-C* and *AtCK2* genes. The four putative HSEs present between the TSSs of both the genes are marked in red. 5'UTRs (small, black letters), introns (small, green letters) and core promoter region (capital, black letters) are shown. ATG for both the genes is shown.





Fig. S6. Confirmation PCR to analyze the status of 4 individual mutant plants raised from *Salk_014505* T_3 seeds. Primers used are indicated by arrows on the above diagrammatic representation. Out of the four lines, line no. 1, 2, 3 are homozygous and 4 is heterozygous.



Fig. S7. Genes homologous to At1g74310 (*ClpB-C*) and At1g74320 (*AtCK2*) were identified in several plant genera covering different families across the angiosperm including both dicots and monocots in plant database Phytozome (<u>http://www.phytozome.net</u>) and looked for their genomic organization. The families and genera analyzed are represented in the above diagrammatic sketch. Details of the locus id of the homologous genes is provided separately in Table S1. Genera along with the family with conservation in the divergent organization of the two genes are marked by a yellow box. Distance between the TSSs of the two genes among the highlighted genera is mentioned in the Table S1.



of Fig. **S8.** Genomic organization the 16 heat up-regulated divergent gene pairs in Arabidopsis TSSs <1.5 analyzed (with intergenic region between kb), using the "Chromosome Map Tool" (http://www.arabidopsis.org/jsp/ChromosomeMap/tool.jsp) of The Arabidopsis Information Resource (TAIR) and modified for better representation. Positions of the divergent gene pairs with their locus id on the 5 chromosomes (numbered 1-5) of Arabidopsis are shown. The AtClpB-C (At1g74310) : AtCK2 (At1g74320) divergent gene pair is encircled. Functional annotation, fold up-regulation and distance between the gene pairs are provided in separately in Table S2.

Table S3. Primers used in the study.

	Primers	5' to 3' sequence	
1	Primers for 1kb promoter cloning		
	1kbpro_Rev	5' GGCCTCGAGCTTCGATTAGCTTTTGTAATC 3'	
2	Reverse primer for deletion analysis		
	ΔUTRpro_Rev	5' GGCCTCGAGTCACAAACACAAAAACACACA 3'	
3	Primer for bidirectional promoter cloning		
	1329pro_Fwd	5' CGGGATCCTTGAATGCCACACACAAAG 3'	
	1329pro_Rev	5' CGGGATCCCTTCGATTAGCTTTTGTAATC 3'	
4	sqRT-PCR primers for Gus		
	Gus_Fwd	5' CTACCGTACCTCGCATTACCC 3'	
	Gus_Rev	5' CGCTTTGGACATACCATCCG 3'	
5	sqRT-PCR primers for GFP		
	GFP_Fwd	5' CGGGATCCATGGTAGATCTGACTAGTAAAG 3'	
	GFP_Rev	5' GTGCCGCTTCATATGATCTGG 3'	
6	Primers for mutant zygosity analysis		
	Salk_LP	5' TCTTTTCAGTACCACCCATCG 3'	
	Salk_RP pROK2 LbP	5' GAGAACAAGATTGTCGCGATC 3' 5' GCGTGGACCGCTTGCTGCAACT 3'	
7	Primers for AtClpB-C Q-PCR analysis		
	At101_F At101 R	5' TCTTGAACAGGCTTGACGAGATT 3' 5' GAAGCCGAGCTACTTTCCTCAAC 3'	
8	Primers for AtCK O-PCR analysis		
-	Finiters for Alex Q-Fex analysis		
	AtCK_F	5' TCGAGCAGTATTGGTTAACAAAGC 3	
	AtCK_R	5' TCTTCTTGGGTTGGGTCCATAG 3'	