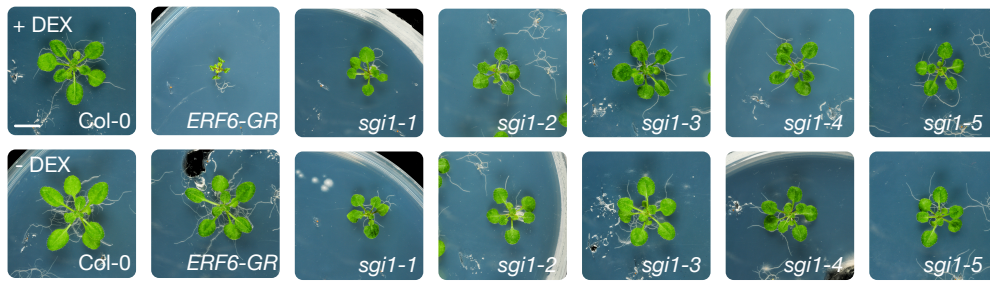


In the format provided by the authors and unedited.

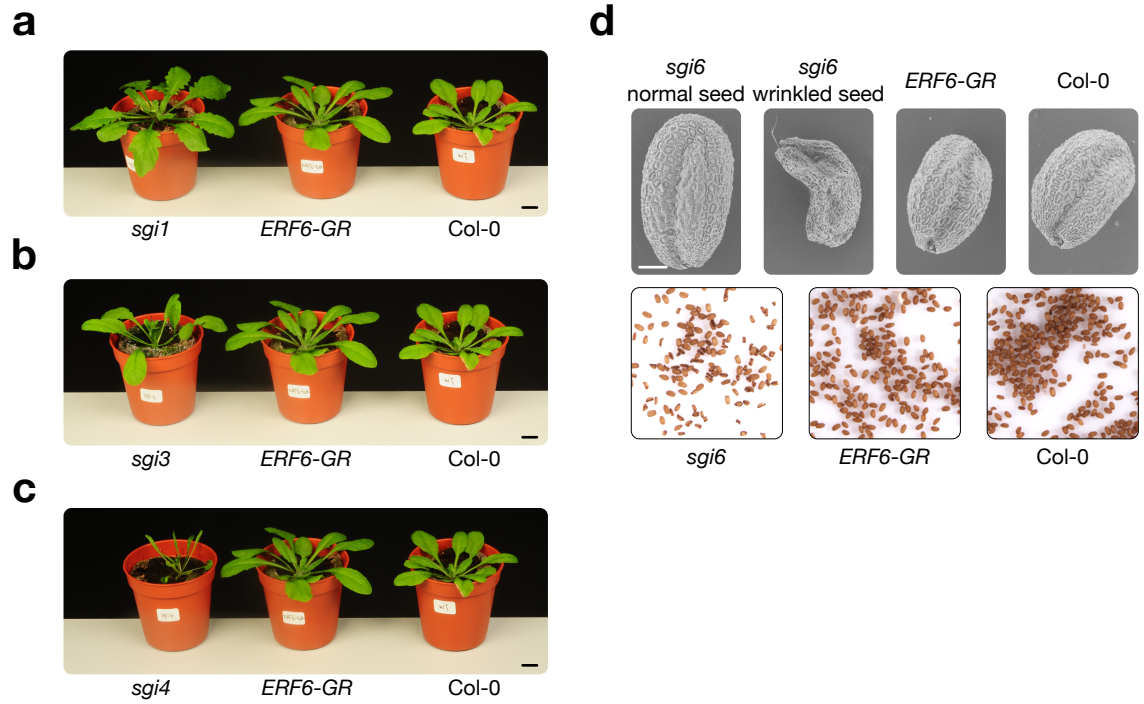
A genetics screen highlights emerging roles for CPL3, RST1 and URT1 in RNA metabolism and silencing

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Nathalie Gonzalez^{1,2,4,5}, Marieke Dubois ^{1,2,5} and Dirk Inzé ^{1,2,5*}

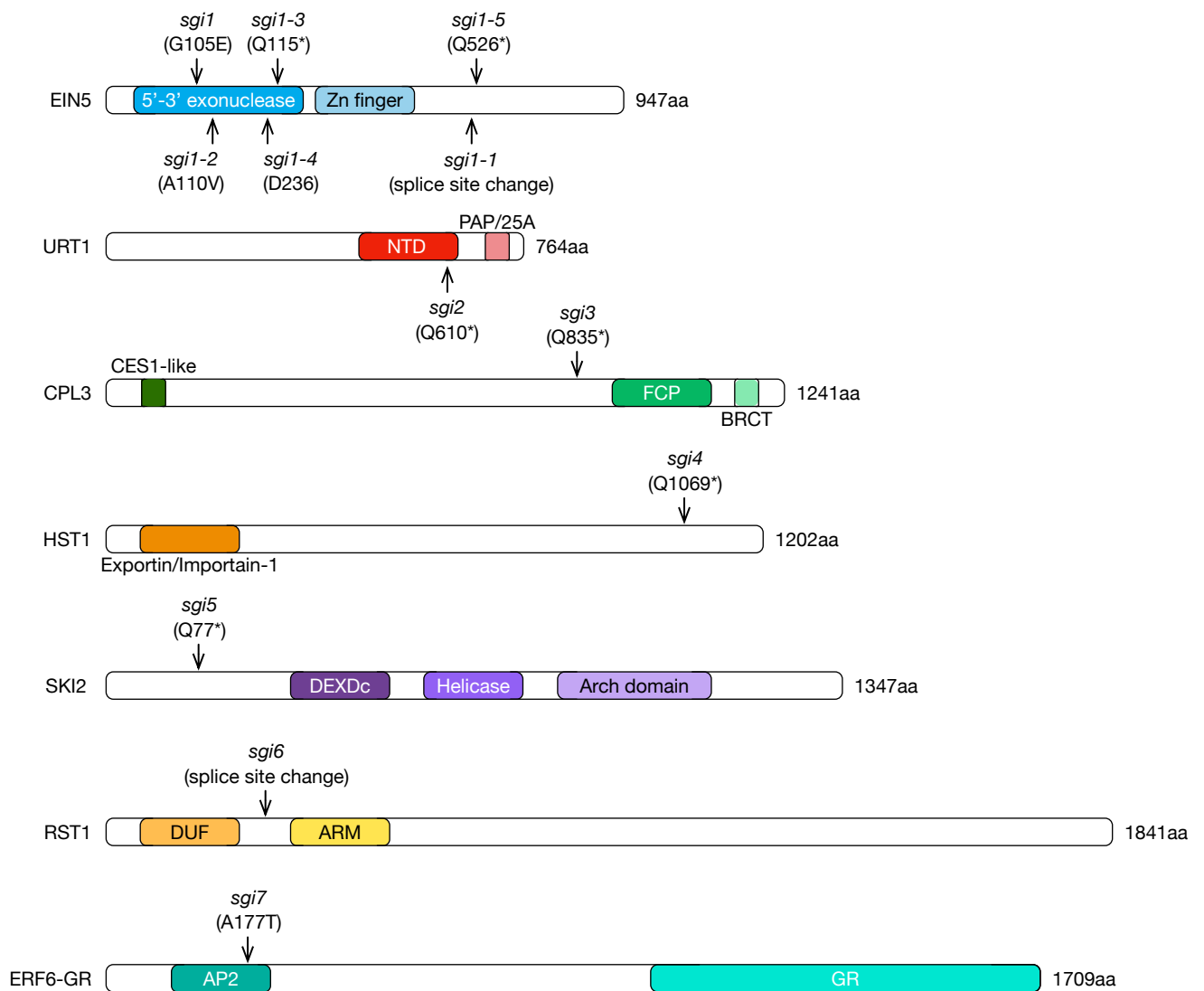
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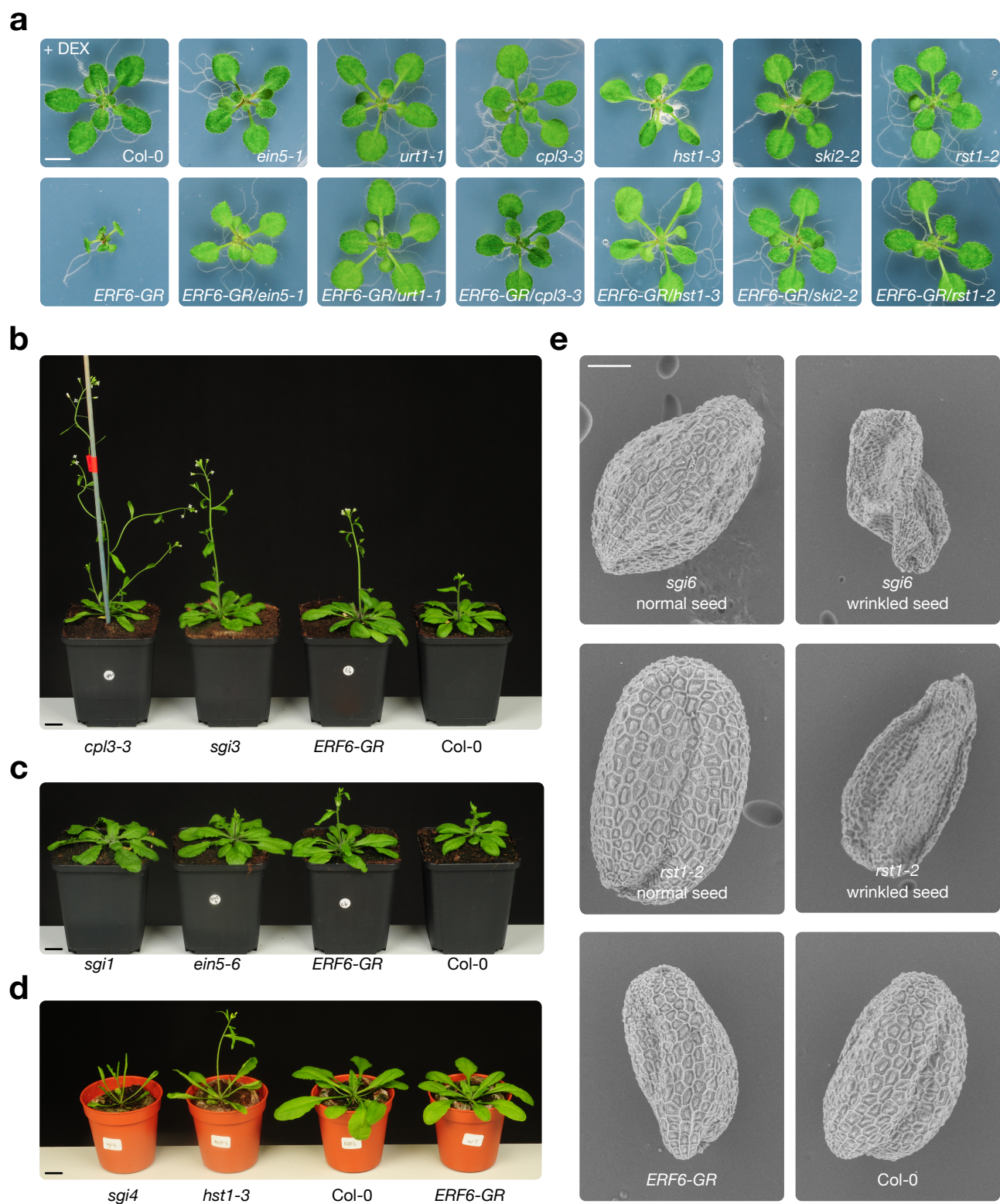
Supplementary Figure 1 | Five additional alleles of *sgi1*. The plants were sown on medium with (upper panel) or without DEX treatment (lower panel). Scale bar = 1 cm. The experiment was performed 3 times with similar results.



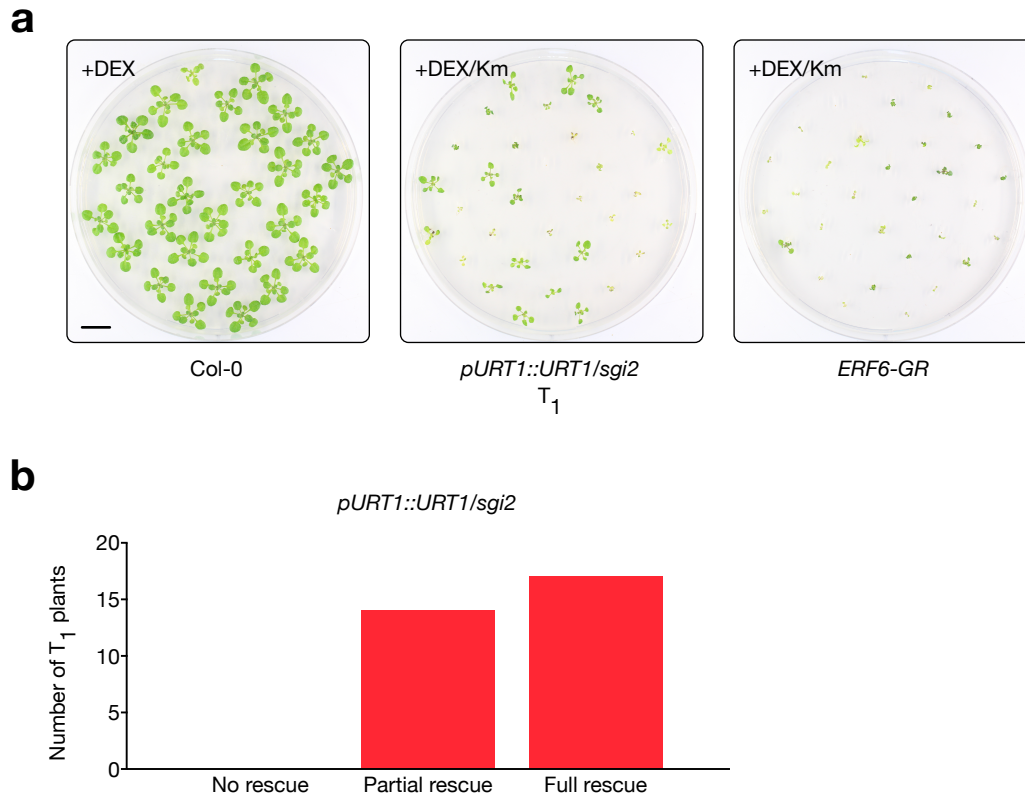
Supplementary Figure 2 | Additional phenotypes observed for *sgi1*, *sgi3*, and *sgi4* rosettes and for *sgi6* seeds. (a-c) Morphology of *sgi1* (a), *sgi3* (b) and *sgi4* (c), grown in soil. Scale bars = 1cm. (d) Mature seeds of *sgi6*. Scale bar = 100 μ m. (a-d) The experiment was performed 3 times with similar results.



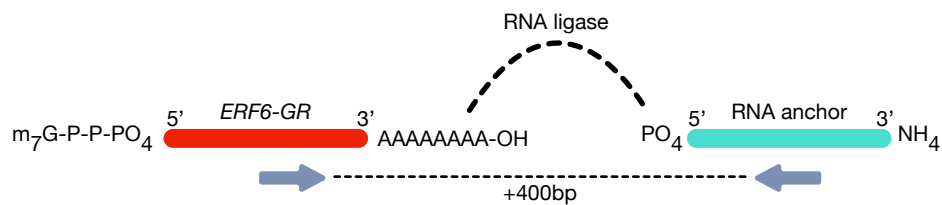
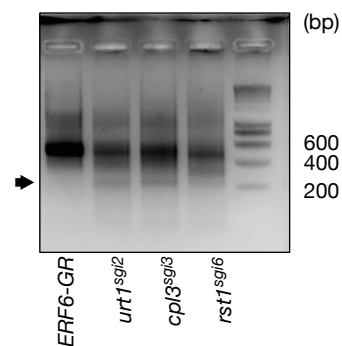
Supplementary Figure 3 | Position of mutations in the causative genes of the EMS mutants. NTD, nucleotidyl-transferase domain; PAP/25A, poly(A) polymerase associated domain; FCP, Fcp1-like phosphatase domain; BRCT, BRCA1 C-terminal domain; DEXDc, dead-like helicases superfamily domain; Arch domain, rRNA processing arch domain; DUF, domain of unknown function. ARM, armadillo repeat.



Supplementary Figure 4 | Phenotypic similarity between EMS mutants and publicly available mutants of candidate causative genes. (a) Phenotype of publicly available mutants of the causative genes identified in *sgi1* to *sgi6* crossed with the *ERF6-GR* line. Plants were grown on medium supplemented with 5 μ M DEX. Scale bar = 0.5 cm. (b-d) Comparison of *sgi3* and *cpl3-3* (b), *sgi1* and *ein5-6* (c), and *sgi4* and *hst1-3* (d). Scale bars = 1 cm. (e) Mature seeds of *rst1-2* and *sgi6*. Scale bar = 100 μ m. (a-e) The experiment was performed 3 times with similar results.

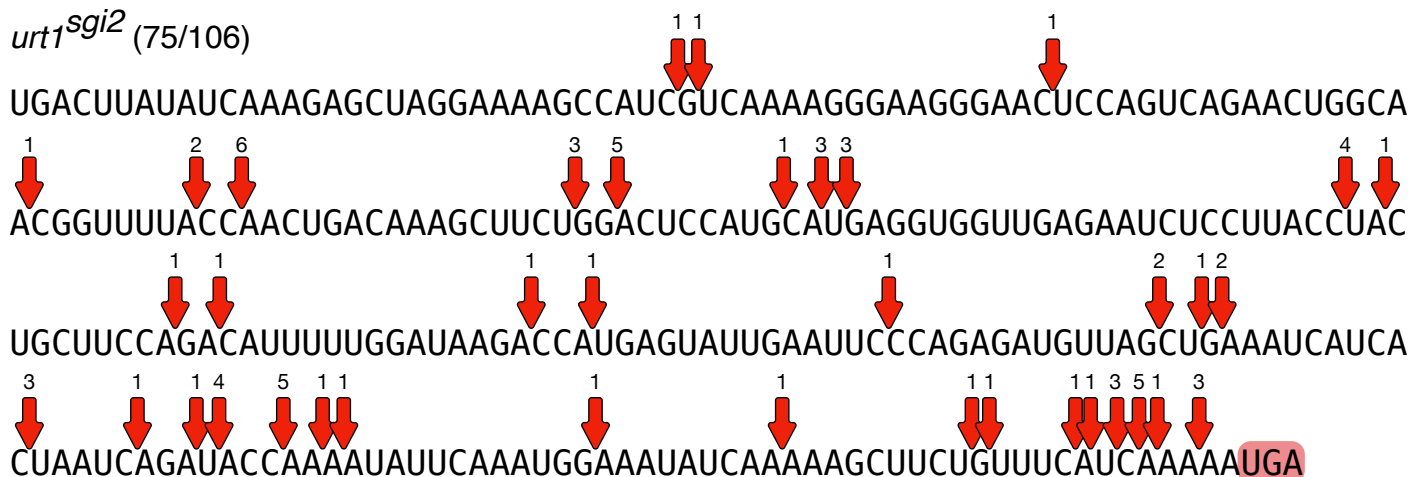


Supplementary Figure 5 | Additional evidence for *URT1* as the causative gene in *sgi2*. (a) Phenotype of 31 T_1 plants of the *sgi2* mutant transformed with *pURT1::URT1*. Plants were grown on medium supplemented with 5 μ M DEX and Kanamycin. Scale bar = 0.5 cm. (b) Quantification of the number of plants in the middle panel of (a) showing partial, or full rescue of the dwarfed *ERF6-GR* phenotype. The experiment was performed 3 times with similar results.

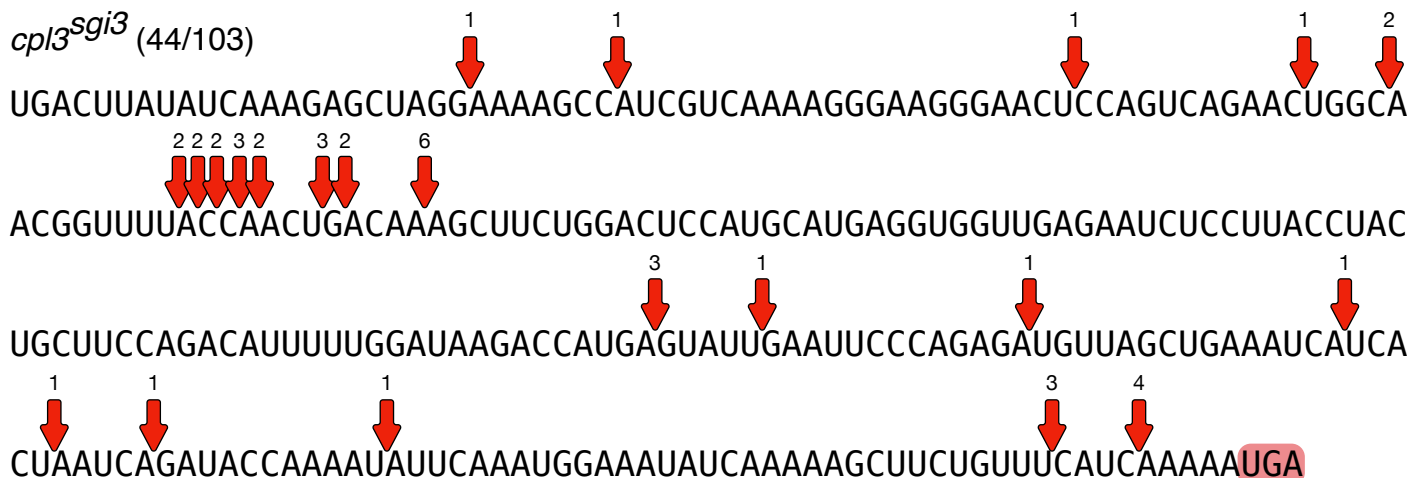
a**b**

Supplementary Figure 6 | Schematic representation of 3' RACE-PCR of *ERF6-GR* mRNA in EMS mutants. (a) 3' RACE-PCR workflow. The RNA adapter was first ligated to the 3' end of *ERF6-GR* transcripts. 400 bp *ERF6-GR* 3' fragments were amplified using nested PCR with a GR-specific forward primer and a reverse primer complementary to the RNA anchor sequence. (b) Inverted SYBR-safe stained 2% gel with *ERF6-GR* 3' fragments determined by 3' RACE-PCR. The arrow represents the bands lower than 400 bp in *urt1^{sgl2}*, *cpl3^{sgl3}*, and *rst1^{sgl6}* lanes. The experiment was performed 3 times with similar results.

urt1^{sgi2} (75/106)



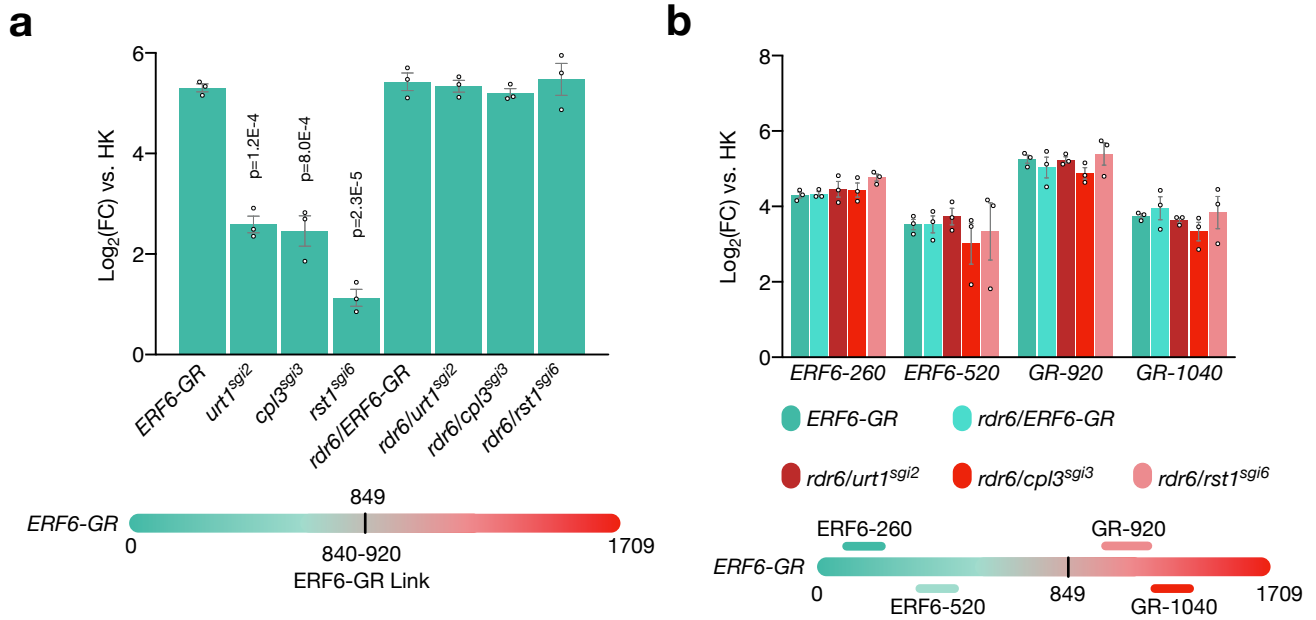
cpl3^{sgi3} (44/103)



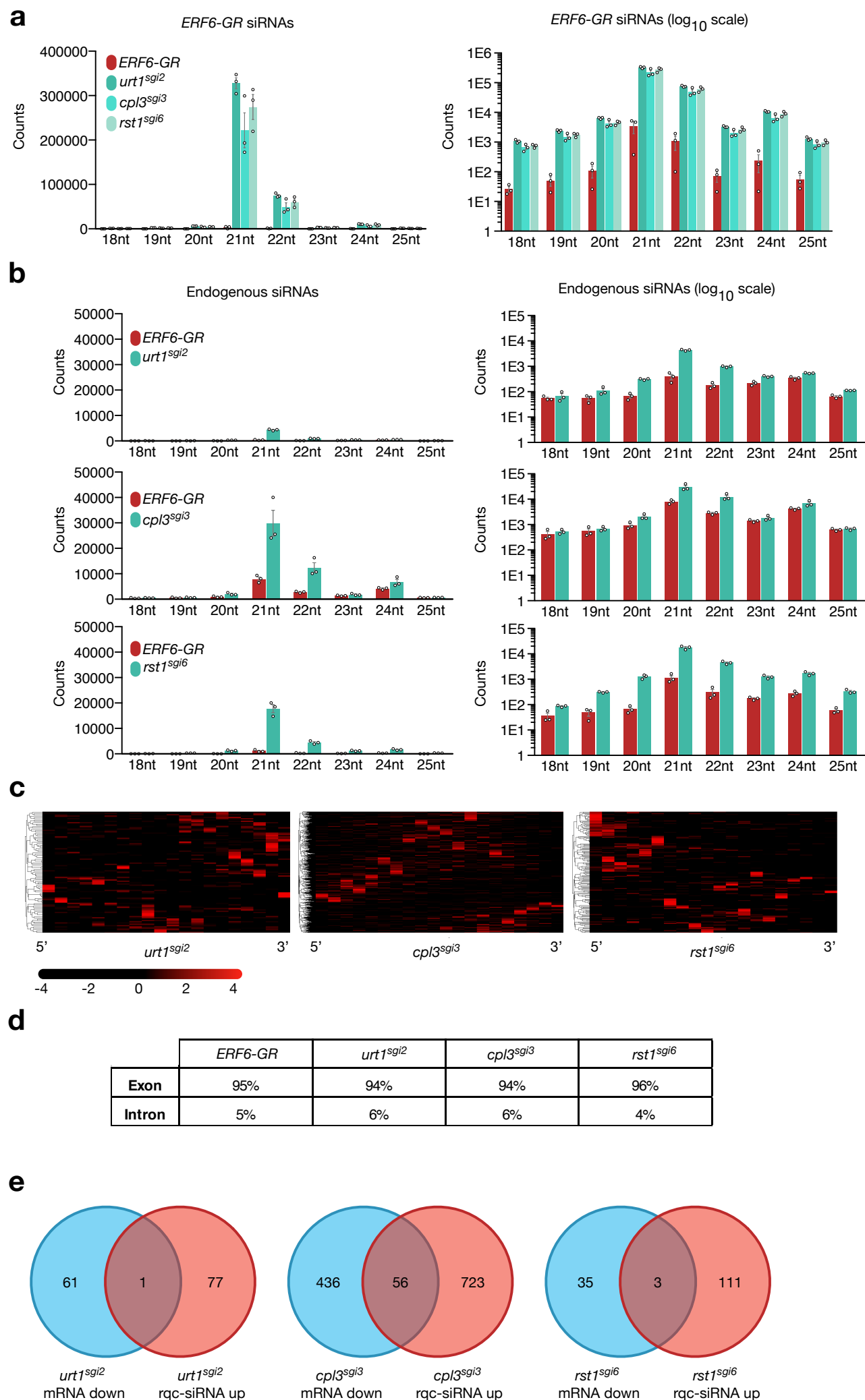
rst1^{sgi6} (44/85)



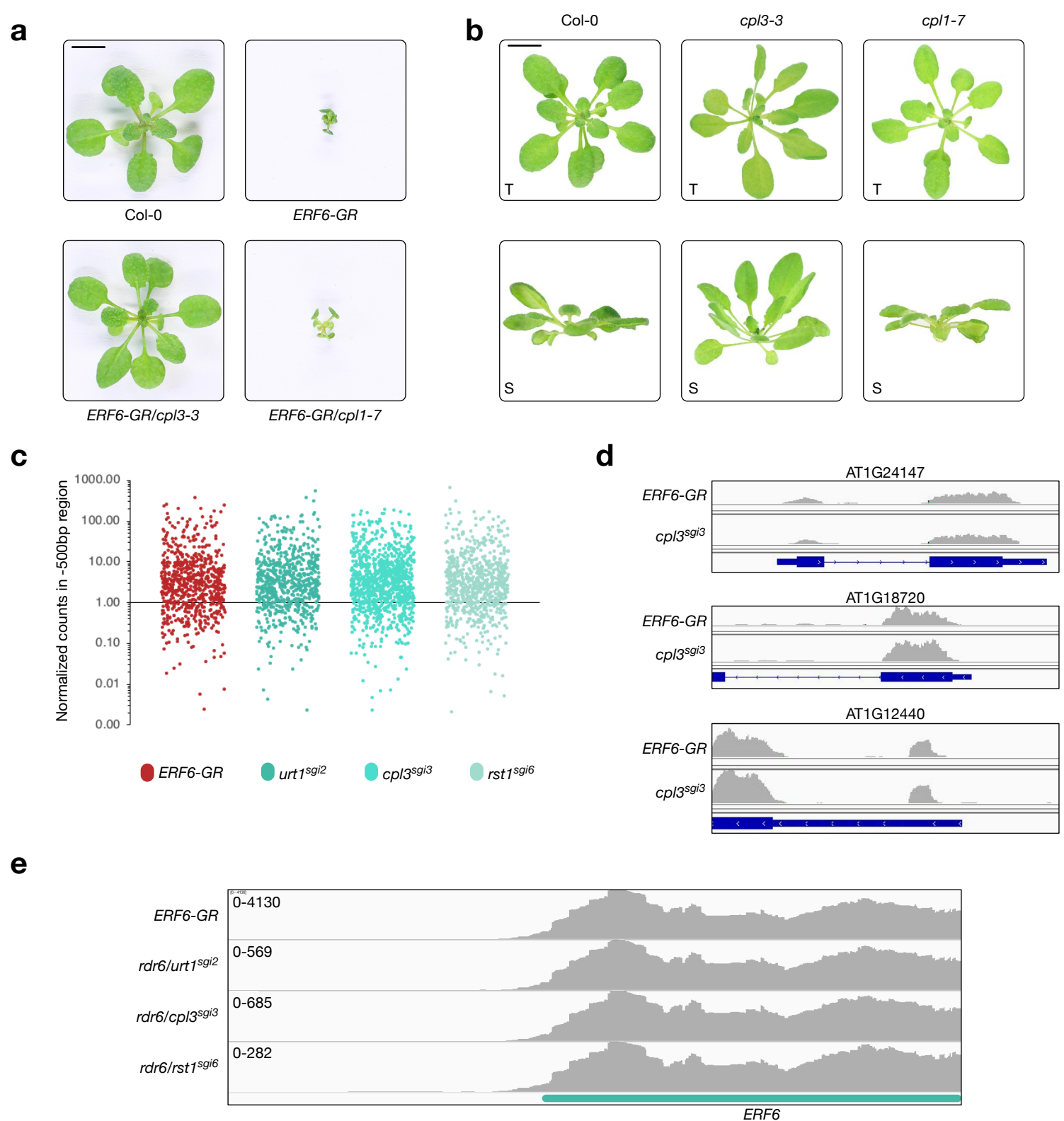
Supplementary Figure 7 | Truncation sites in the ERF6-GR transcripts in *urt1^{sgi2}*, *cpl3^{sgi3}*, and *rst1^{sgi6}*. The arrows indicate the truncation sites. The number above the arrows represents the number of clones containing transcripts truncated at this site.



Supplementary Figure 8 | Quantification of *ERF6-GR* transcript level in the *rdr6-11/sgi* double mutants. (a) The *ERF6-GR* level was measured with a primer located at the linker region between ERF6 and the GR domain, in the single *sgi* mutants and the *rdr6-11/sgi* double mutants. (b) The *ERF6-GR* level was measured with primers located along the *ERF6-GR* transcript, as indicated below the graph. (a-b) Values are averages of the Log₂ (Fold Change (FC)) of three independent biological replicates, normalized to the expression level of housekeeping (HK) genes. Error bars indicate SE (n=3 biological repeats). Statistical difference was tested by two-sided Students t-test. P-values are indicated in the graph whenever the difference with the *ERF6-GR* control line was significant.



Supplementary Figure 9 | Characteristics of siRNAs of protein-coding genes in *ERF6-GR*, *urt1sgl2*, *cpl3sgl3*, and *rst1sgl6*. (a) Number of the 18-25nt siRNAs derived from the *ERF6-GR* transgene in *ERF6-GR*, *urt1sgl2*, *cpl3sgl3*, and *rst1sgl6*. Left panel: linear scale, right panel: $\log_{10}$ scale. (b) Number of 18-25nt DE siRNAs ($\text{FDR} \leq 0.05$) derived from the endogenous genes in *ERF6-GR*, *urt1sgl2*, *cpl3sgl3*, and *rst1sgl6*, respectively. Left panel: linear scale, right panel: $\log_{10}$ scale. (a,b) Values indicate the averages of 3 independent biological replicates. Error bars represent SE ($n=3$). (c) Heatmap of the distribution of rqc-siRNAs along the DE endogenous genes in *urt1sgl2*, *cpl3sgl3*, and *rst1sgl6*. (d) Percentage of siRNAs derived from exons and introns in the *ERF6-GR* line and in *urt1sgl2*, *cpl3sgl3*, and *rst1sgl6*. (e) Overlap between the endogenous genes producing significantly more rqc-siRNAs ($\text{FDR} \leq 0.05$, Students t-test corrected for multiple testing (BH), using DEseq2) and genes down-regulated ($\text{FDR} \leq 0.05$, pre-defined contrasts upon applying a Generalized Linear Model, EdgeR) in *urt1sgl2* ($p=0.10$; Chi-Square test), *cpl3sgl3* ($p=1.55\text{E-}19$; Chi-Square test), and *rst1sgl6* ($p=4.57\text{E-}10$; Chi-Square test).



Supplementary Figure 10 | Comparison of *CPL1* and *CPL3*. (a) Phenotype of *ERF6-GR/cpl1-7* and *ERF6-GR/cpl3-3* upon 5 μ M DEX treatment. (b) Top-view (T) and side-view (S) phenotype of *cpl1-7* and *cpl3-3*. Scale bar = 1cm. (c) Genome-wide detection of transcripts showing >20 counts in the 500bp upstream of the start codon. For each transcript, represented by a dot, the counts in the 500bp upstream region were normalized to the expression level of the corresponding transcript. (d) Visualization of the reads along the transcript and the 500bp upstream region of three genes that showed 5'-extension in the *cpl1-7* mutant⁴⁷. (e) Visualization of the reads along the 5' region of the *ERF6-GR* transcript and the 500bp upstream region. Please note that the scale was adapted for each line in order to normalize to the *ERF6-GR* expression level. (a,b,d,e) The experiment was performed 3 times with similar results.

Supplementary Table 1. Candidate causative genes in the 7 non-allelic EMS mutants.

Mutant	Candidate Gene ID	Gene model description	Ori.AA	Mut.AA	Mutated position
<i>sgi1</i>	AT1G54490	ETHYLENE INSENSITIVE 5. It is involved in the ethylene response. XRN4 does not appear to regulate ethylene signaling via an RNA-INDUCED SILENCING COMPLEX-based RNA silencing mechanism but acts by independent means.	G	E	105AA
<i>sgi2</i>	AT2G32140	Transmembrane receptor	G	E	309AA
	AT2G32230	Encodes a protein-only RNase P that is involved in the 5' cleavage of the precursor tRNAs and is able to cleave tRNA-like structures involved in the maturation of plant mitochondrial mRNAs.	R	K	335AA
	AT2G36350	Protein kinase superfamily protein	L	S	776AA
	AT2G45620	Nucleotidyltransferase family protein	Q	*	610AA
<i>sgi3</i>	AT2G31810	ACT domain-containing small subunit of acetolactate synthase protein	A	T	215AA
	AT2G33540	C-terminal domain phosphatase-like 3	Q	*	835AA
	AT2G32620	Encodes a gene similar to cellulose synthase	G	D	662AA
<i>sgi4</i>	AT3G05040	Encodes member of importin/exportin family. Involved in timing of shoot maturation. Involved in miRNA transport.	R	*	1202AA
	AT3G18970	Encodes a pentatricopeptide repeat protein (PPR) protein involved in mitochondrial mRNA editing.	T	I	110AA
	AT3G05060	SAR DNA-binding protein, putative, strong similarity to SAR DNA-binding protein-1 (Pisum sativum)	P	L	351AA
<i>sgi5</i>	AT3G43610	Spc97 / Spc98 family of spindle pole body (SBP) component	E	K	921AA
	AT3G46960	The gene encodes a DExDH box RNA helicase, involved in the regulation of K+ deprivation stress response. SKI2 encodes the RNA helicase subunit of the SKI complex.	Q	*	74AA
	AT3G49370	Calcium-dependent protein kinase (CDPK) family protein	P	L	84AA
<i>sgi6</i>	AT3G27670	A novel protein, did not show high similarity to any protein of known function; reveals a novel genetic connection between lipid synthesis and embryo development.	splice site change		-
<i>sgi7</i>		-			

Supplementary Table 4. Common differentially expressed protein-coding genes in *urt1^{sgl2}*, *cpl3^{sgl3}*, and *rst1^{sgl6}*.

Gene ID	Short name	Log ₂ FC in			Gene Description
		<i>urt1^{sgl2}</i>	<i>cpl3^{sgl3}</i>	<i>rst1^{sgl6}</i>	
AT4G27800	PPH1	1.506	1.249	1.204	Chloroplast protein phosphatase TAP38/PPH1 is required for efficient dephosphorylation of the LHCII antenna and state transition from state 2 to state 1.
AT4G17490	ERF6	-2.762	-2.642	-3.718	Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ERF6). The protein contains one AP2 domain. It is involved in the response to reactive oxygen species and light stress.
AT5G19280	RAG1	7.329	6.702	6.559	kinase associated protein phosphatase composed of three domains: an amino-terminal signal anchor, a kinase interaction (KI) domain, and a type 2C protein phosphatase catalytic region
AT5G19210	-	4.870	4.805	4.669	P-loop containing nucleoside triphosphate hydrolases superfamily protein;(source:Araport11)
AT5G63260	-	6.358	6.355	7.358	Zinc finger C-x8-C-x5-C-x3-H type family protein;(source:Araport11)
AT5G20300	TOC90	8.199	7.852	9.286	Encodes Toc90, part of the TOC (translocon at the outer chloroplast membrane) machinery involved in the import of nucleus-encoded proteins into the chloroplast.
AT5G55700	BAM4	6.669	6.400	6.502	In vitro assay indicates no beta-amylase activity of BAM4. However mutation in BAM4 impairs starch breakdown. BAM4 may play a regulatory role.
AT3G56410	-	8.033	8.281	9.795	hypothetical protein (DUF3133);(source:Araport11)
AT5G64390	HEN4	8.030	5.653	7.857	Encodes a K homology (KH) domain-containing, putative RNA binding protein that interacts with HUA1, a CCCH zinc finger RNA binding protein in the nucleus. HEN4 acts redundantly with HUA1 and HUA2 in the specification of floral organ identity in the third whorl.
AT1G06960	U2BL	7.172	7.049	6.520	RNA-binding (RRM/RBD/RNP motifs) family protein;(source:Araport11)
AT1G66540	-	3.637	3.114	3.001	Cytochrome P450 superfamily protein;(source:Araport11)
AT5G12250	TUB6	1.549	1.842	2.069	Encodes a beta-tubulin. Expression of TUB6 has been shown to decrease in response to cold treatment.
AT3G04720	ATPR4	-1.018	-0.820	-1.240	Encodes a protein similar to the antifungal chitin-binding protein hevein from rubber tree latex. mRNA levels increase in response to ethylene and turnip crinkle virus infection. The mRNA is cell-to-cell mobile.
AT3G07160	GSL10	0.342	0.247	0.421	Encodes GSL10, a member of the Glucan Synthase-Like (GSL) family believed to be involved in the synthesis of the cell wall component callose. GSL10 is required for male gametophyte development and plant growth.
AT3G44630	-	7.536	7.743	7.876	Disease resistance protein (TIR-NBS-LRR class) family;(source:Araport11)
AT5G26910	TRM8	-0.088	-5.801	-6.919	Encodes a member of the TRM superfamily, that plays a role in preprophase band formation during plant cell division and controls the robustness of the orientation of that cell division.
AT3G11560	-	6.984	7.160	8.133	LETM1-like protein;(source:Araport11)
AT2G30880	SWAP70	6.876	5.898	6.088	Pleckstrin homology (PH) domain-containing protein;(source:Araport11)
AT3G14590	NTMC2T6.2	7.095	7.072	6.552	Calcium-dependent lipid-binding (CaLB domain) family protein;(source:Araport11)
AT5G63050	EMB2759	7.330	7.667	8.580	embryo defective 2759;(source:Araport11)
AT5G53570	RABGAP2.2	0.658	0.647	0.736	Ypt/Rab-GAP domain of gyp1p superfamily protein;(source:Araport11)
AT2G18860	-	3.668	2.978	3.538	Syntaxin/t-SNARE family protein;(source:Araport11)
AT3G23370	-	6.998	7.486	6.807	RNA-binding (RRM/RBD/RNP motifs) family protein;(source:Araport11)
AT3G49110	PRX33	-0.746	-0.655	-0.713	Class III peroxidase Perx33. Expressed in roots. Located in the cell wall. Involved in cell elongation. Expression activated by light. May play a role in generating H2O2 during defense response. The mRNA is cell-to-cell mobile.
AT1G80910	CCZ1B	-0.635	-0.644	-0.711	Vacuolar fusion CCZ1-like protein (DUF1712);(source:Araport11)
AT1G12140	FMO GS-OX5	8.418	6.461	7.130	Belongs to the flavin-monooxygenase (FMO) family, encodes a glucosinolate S-oxygenase that catalyzes the conversion of methylthioalkyl glucosinolates to methylsulfinylalkyl glucosinolates
AT1G15125	-	-2.523	-1.993	-2.428	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein;(source:Araport11)

AT5G44420	PDF1.2	-4.249	-1.933	-4.258	Encodes an ethylene- and jasmonate-responsive plant defensin. mRNA levels are not responsive to salicylic acid treatment; although jasmonate and salicylic acid can act synergistically to enhance the expression of this gene.
AT4G39170	-	7.691	6.606	6.945	Sec14p-like phosphatidylinositol transfer family protein;(source:Araport11)
AT3G04030	MYR2	6.732	6.911	7.309	Homeodomain-like superfamily protein;(source:Araport11)
AT4G22340	CDS2	9.521	8.949	7.937	cytidinediphosphate diacylglycerol synthase 2;(source:Araport11)

Supplementary Table 5. Common protein-coding genes from which significantly more rqc-siRNAs are derived in *urt1^{sgl2}*, *cpl3^{sgl3}*, and *rst1^{sgl6}*.

Gene ID	Short name	Log ₂ FC in <i>urt1^{sgl2}</i>	Log ₂ FC in <i>cpl3^{sgl3}</i>	Log ₂ FC in <i>rst1^{sgl6}</i>	Gene Description
AT3G14530	-	5.497	5.018	3.675	Terpenoid synthases superfamily protein
AT3G23240	ERF1	3.869	2.442	6.672	Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ERF1). The protein contains one AP2 domain.
AT2G44840	ERF13	4.032	2.777	6.997	Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ERF13). The protein contains one AP2 domain.
AT5G47230	ERF5	4.290	3.066	7.324	Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ERF5). The protein contains one AP2 domain.
AT3G56360	-	1.367	1.209	1.422	Hypothetical protein
AT4G12820	KIB2	3.789	2.085	2.879	Member of a family of proteins containing an F-box domain at the N-terminal region and three kelch repeats at the C-terminal region. Involved in BR signaling.
AT4G17490	ERF6	6.274	5.016	8.586	Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ERF6). The protein contains one AP2 domain.
AT3G14550	GGPS3	5.498	5.021	3.675	Encodes a protein with geranylgeranyl pyrophosphate synthase activity involved in isoprenoid biosynthesis. The enzyme appears to be targeted to the chloroplast in epidermal cells and guard cells of leaves, and in etioplasts in roots.
AT5G47220	ERF2	3.775	2.418	6.648	Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ERF2). The protein contains one AP2 domain.

Supplementary Table 6. Primers used in this article.

Primers for genotyping:

<i>sgi1</i> -F	CGTACTTTTGATTACAATGCAG
<i>sgi1</i> -R	AGATTTGGCCCTCCATCTC
<i>sgi2</i> -F	GCATCAACAATGTATTGGCTGT
<i>sgi2</i> -R	TGAGATTGTTGCTTCACAGATG
<i>sgi3</i> -F	AAATAGGTGTTCCACATCTCCC
<i>sgi3</i> -R	AGGGTAAGGCAGAAGATTTGG
<i>sgi4</i> -F	GGACTCAACCATGAATGCTTCTG
<i>sgi4</i> -R	TGGAGAAGCGACCACCAAAGT
<i>sgi5</i> -F	CCATTTCTCCAACCTAGAGTCAG
<i>sgi5</i> -R	AAGGAATCAAAGGTTGCTCGTT
<i>sgi6</i> -F	ACGGAATAATGGGAACTGGAAG
<i>sgi6</i> -R	GAAATCAACTCCCTCGCAAACA
<i>ein5-1</i> -F	TTGACCAGTTAATGGGAAC
<i>ein5-1</i> -R	CAACCATGAACCTGCC
<i>ein5-6</i> -F	GGAGATATGTTAAGGTGTG
<i>ein5-6</i> -R	GAGCATTATCAAATCTGCATCC
<i>urt1-1</i> -LP	ACCTGTGGATGTCATTTTCGAC
<i>urt1-1</i> -RP	GTTTCCTCAATTTCCGCCTAG
<i>cpl3-1</i> -LP	ACTCGTTCCATGAACAGTTGG
<i>cpl3-1</i> -RP	GCACAGTGAAACCATGTGATG
<i>cpl3-sail</i> -LP	TGTTTTCTTGGGTTACCAAG
<i>cpl3-sail</i> -RP	TGTGCAGATTGCTGACTCTTG
<i>ski2-1</i> -LP	AGGTATCTCAATGTCCGGACC
<i>ski2-1</i> -RP	ACACAACCGTGGCACTTATTC
<i>rst1-1</i> -LP	GCGTGTCTAAGCCATCTTTG
<i>rst1-1</i> -RP	GCAAGGAAATAAGAGCAAGGG
<i>rst1</i> -Cas9-F	GTGCATTTGACACTGTTACC
<i>rst1</i> -Cas9-R	GATTAGGAATGGTGGGAGTA
<i>rdr6-11</i> -F	TACTGTCCCTGGCGATCTCT
<i>rdr6-11</i> -R	CCACCTCACACGTTTCCTCTT
<i>sgs3-11</i> -F	TATGAGCGAGAAGCTGCGTAGA
<i>sgs3-11</i> -R	ATACCTCTTTTCGGCAATCGTCA
<i>cpl1</i> -LP	GGGAATTGAATCAGCAACAAG
<i>cpl1</i> -RP	CAAGACTTGCATACCATTGGG

Primers for construction:

pURT1-F-GW	GGGGACAACCTTTGTATAGAAAAGTTGGAGCTTCCAAAGACGTAACCATT
pURT1-R-GW	GGGGACTGCTTTTTTTGTACAAACTTGTGGAGGAGGAGAGAAAGTGAAGA
URT1-F-GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCGGACGGTGGG
URT1-R-GW	GGGGACCACTTTGTACAAGAAAGCTGGGTATCAGTTGTGGCCTTGTCCA
RST1-Cas9-gRNA1	TTTTGGTCTCAATTGCGTGGCGGGATGAGCAACAAGTTTTAGAGCTAGAAATAGC
RST1-Cas9-gRNA2-Rev	TTTTGGTCTCAAACCCAGTTGCACACCGAGCAAACAATCACTACTTCGACTC

Primers for qRT-PCR:

AT1G13320-RT-F	TAACGTGGCCAAAATGATGC
AT1G13320-RT-R	GTTCTCCACAACCGCTTGGT
AT2G28390-RT-F	AACTCTATGCAGCATTTGATCCACT
AT2G28390-RT-R	TGATTGCATATCTTTATCGCCATC
ERF6-GR-260-RT-F	CCAATCGAATCCTCCTCGCGTTAC

ERF6-GR-260-RT-R	TTCGGTGGTGCGATCTTCAACG
ERF6-GR-520-RT-F	GCTAGAGCTTACGACAA
ERF6-GR-580-RT-R	TTCAAGCGGGAAATTCAG
ERF6-GR-720-RT-F	AGAGTTACGACGTTAGC
ERF6-GR-820-RT-R	TGGATAACCAAACGGTG
ERF6-GR-840-RT-F	CACCGTTTGGTTATCCA
ERF6-GR-920-RT-R	GATTTTCCGAAGTGTCTTG
ERF6-GR-920-RT-F	CAAGACACTTCGGAAAATC
ERF6-GR-1020-RT-R	GCTATCATATCCTGCATACAAC
ERF6-GR-1040-RT-F	TGGTCATCCAGGTGTAA
ERF6-GR-1140-RT-R	GCATGGAGAATTATGACCA
GFP-GR-1-RT-F	CGACGTAAACGGCCACAAGT
GFP-GR-1-RT-R	GGTCAGGGTGGTCACGAGGG
GFP-GR-2-RT-F	ACAAGCAGAAGAACGGCATCA
GFP-GR-2-RT-R	GCCGATGGGGGTGTTCTGCT
GFP-GR-3-RT-F	TGCTGGAGTTCGTGACCGCC
GFP-GR-3-RT-R	GGATTTTCCGAAGTGTCTTGAG
GFP-GR-4-RT-F	CCTTACTGCTTCTCTCCTCA
GFP-GR-4-RT-R	CCTTTTGACGATGGCTTTTCCT
AT3G54900-RT-R	TCCTCCTCCGTTACCAACAAGC
AT3G54900-RT-F	ACGAGTGCCGACGTTGGTTTAAG
AT2G26730-RT-F	TCGTCTGATGACCCGTCAAAGG
AT2G26730-RT-R	ATCGTGGTGGCGTCCTTGATTC
AT3G02020-RT-F	GTGTTGCATCCACTTCCATGCG
AT3G02020-RT-R	ATGCTGGTCAATACAGCCTTGC
AT1G78370-RT-F	CCGTTCTTCCCTTCCGATCCTTAC
AT1G78370-RT-R	CGTCGGTGAACCTTCTTGCCAC
AT3G23690_RT_F	GTTGGTTGTGACCCAAATTCA
AT3G23690_RT_R	GAGATTTACCGTTAACT
AT2G28080_RT_F	CACAAAGAAGGATCTTGTTG
AT2G28080_RT_R	GTCTGAACTAACAATGTCTGGT
AT5G49550_RT_F	AGAGTACGATGATATGGGT
AT5G49550_RT_R	TCCTACAAATTCATCCAAG
AT5G54940_RT_F	AAAGAGAACATTCACATTCTGA
AT5G54940_RT_R	CAAATCCTTGAGAATCCTC
AT1G50290_RT_F	TGCAGAATTACCTCAAGTATG
AT1G50290_RT_R	GAAAAATCTGAGTATTCCTCA
AT5G65860_RT_F	CCTGAAGTTTATAAGCGTAT
AT5G65860_RT_R	CTTGCCATCTTCCAAAAACA
AT5G14060_RT_F	GGGCAAACAAAACAGTGTTT
AT5G14060_RT_R	TTAAGGAGTGAAAAAGCGGC
AT4G17410_RT_F	CAGGGATACCGCGGTGGT
AT4G17410_RT_R	TCGCAGGGACAATGCTCA

Primers for 3'RACE-PCR:

RNA-adapter	CUAGAUGAGACCGUCGACAUGAAUUC
ERF6-GR-primer1	CAGGTTTGACGGCGATA
ERF6-GR-primer2	TGACTTATATCAAAGAGCTAGG
reverse-primer1	GAATTCATGTCGACGGTCTCA
reverse-primer2	CATGTCGACGGTCTCATCTAG

Primers for Northern blot:

ERF6-probe-F	ATGGCTACACCAAACGA
ERF6-probe-R	TTCAAGCGGGAAATTCAG
GR-probe-F	AGTGAAATGGGCAAAGGCGATAC
GR-probe-R	TGTCTGGAAGCAGTAGGT

Probes for sRNAs blot:

ERF6-GR-siRNAs-probe	TTTCTGAAGCCTGGTATCGCC
U6-probe	CTCGATTTATGCGTGTCATCCTTGC

Supplementary Tables

Supplementary Table 1. Candidate causative genes in the 7 non-allelic EMS mutants.

Supplementary Table 2. Quantification of rqc-siRNAs reads (CPM) arising from the protein-coding genes in *ERF6-GR*, *urt1^{sgi2}* (a), *cpl3^{sgi3}* (b), and *rst1^{sgi6}* (c). Three biological repeats for each genotype were performed. The DEseq tool (R) was used and a two-sided T-test with correction for multiple testing (BH) was performed to test for statistical significance.

Supplementary Table 3. Quantification of the mRNA of protein-coding genes differentially expressed in *urt1^{sgi2}* (a), *cpl3^{sgi3}* (b), and *rst1^{sgi6}* (c). Three biological repeats for each genotype were performed. A Generalized Linear Model was applied to the whole dataset and the statistical significance was tested by pre-defined contrasts (mutant vs. *ERF6-GR*) using the glmfit function in EdgeR (R).

Supplementary Table 4. Common differentially expressed protein-coding genes in *urt1^{sgi2}*, *cpl3^{sgi3}*, and *rst1^{sgi6}*.

Supplementary Table 5. Common protein-coding genes from which significantly more rqc-siRNAs are derived in *urt1^{sgi2}*, *cpl3^{sgi3}*, and *rst1^{sgi6}*.

Supplementary Table 6. Primers used in this article.