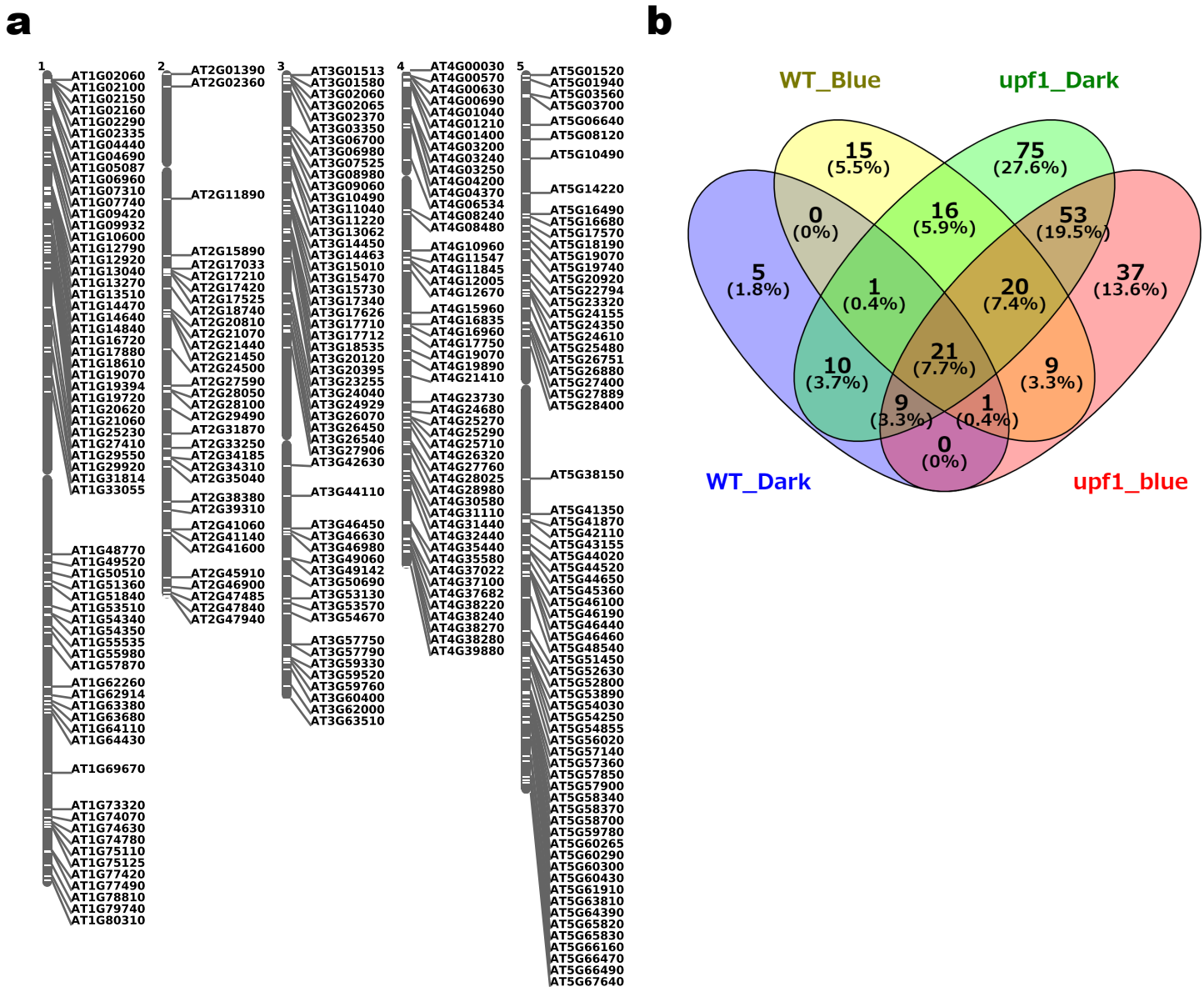
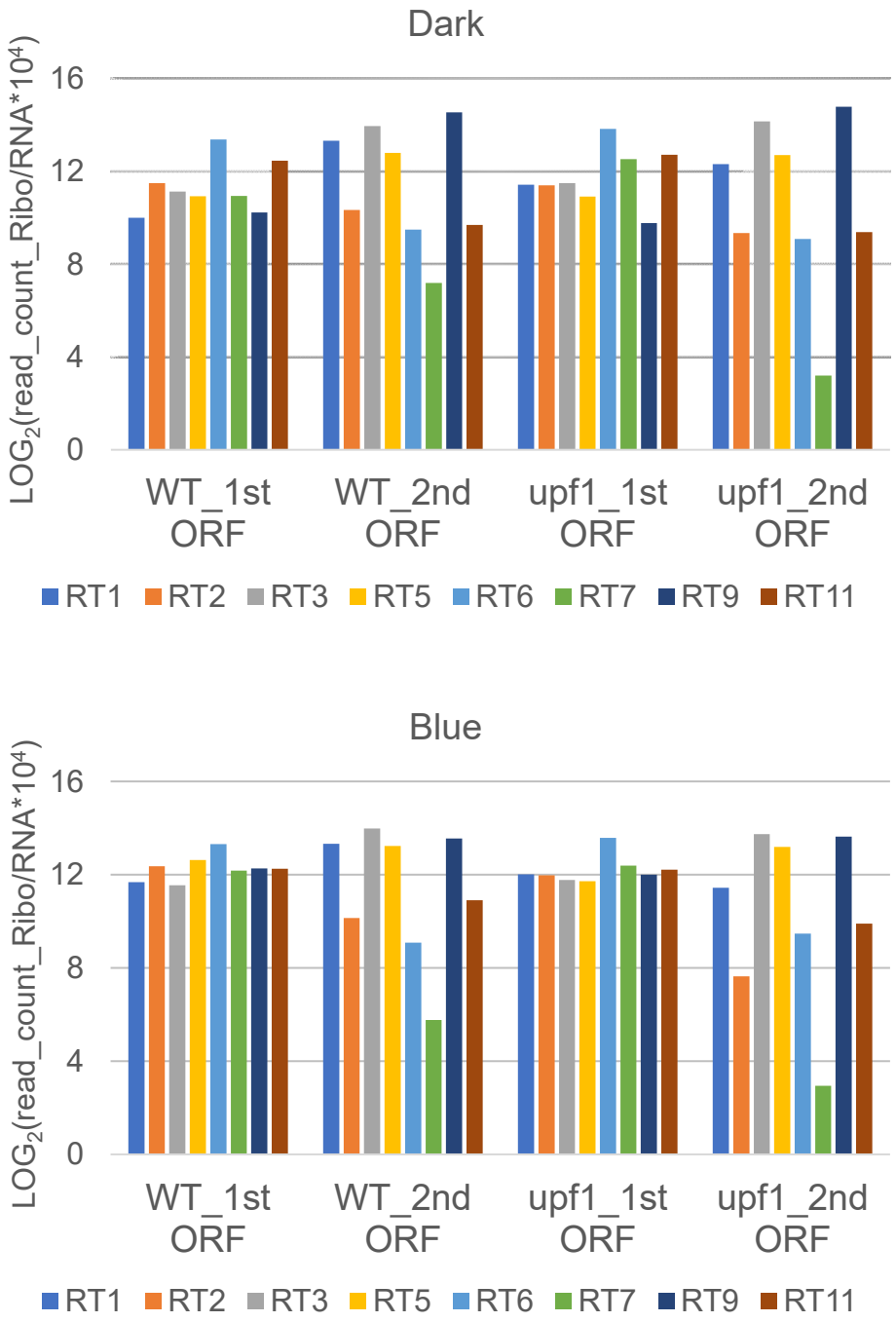
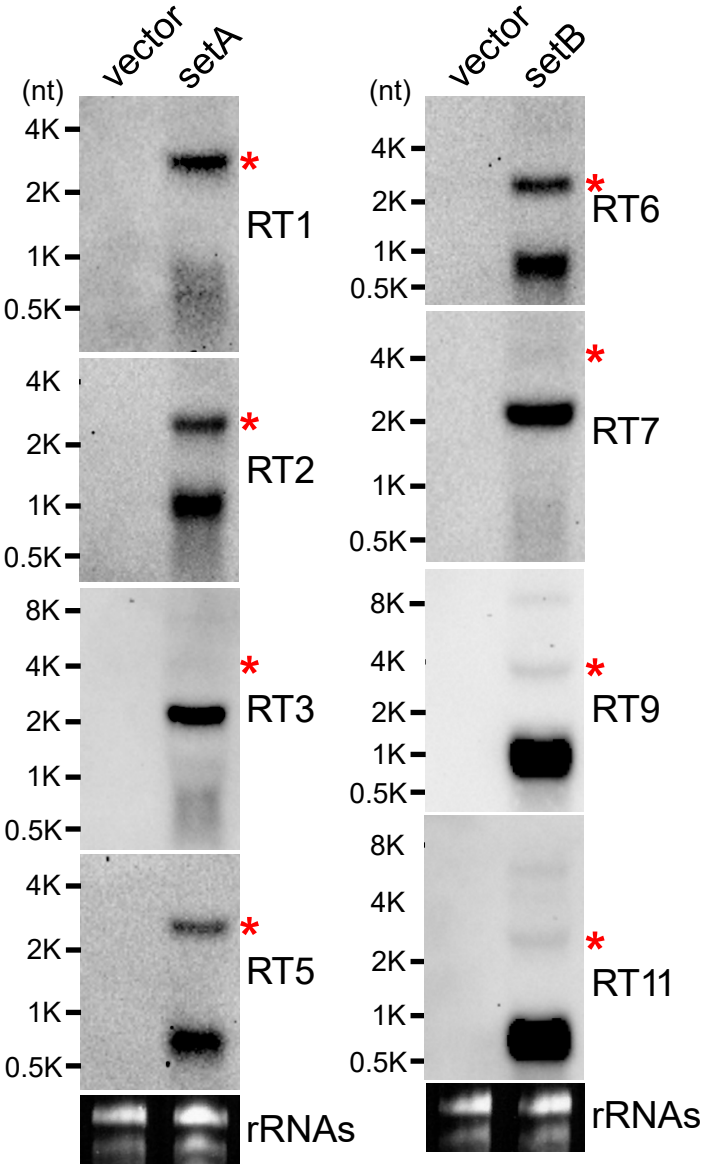


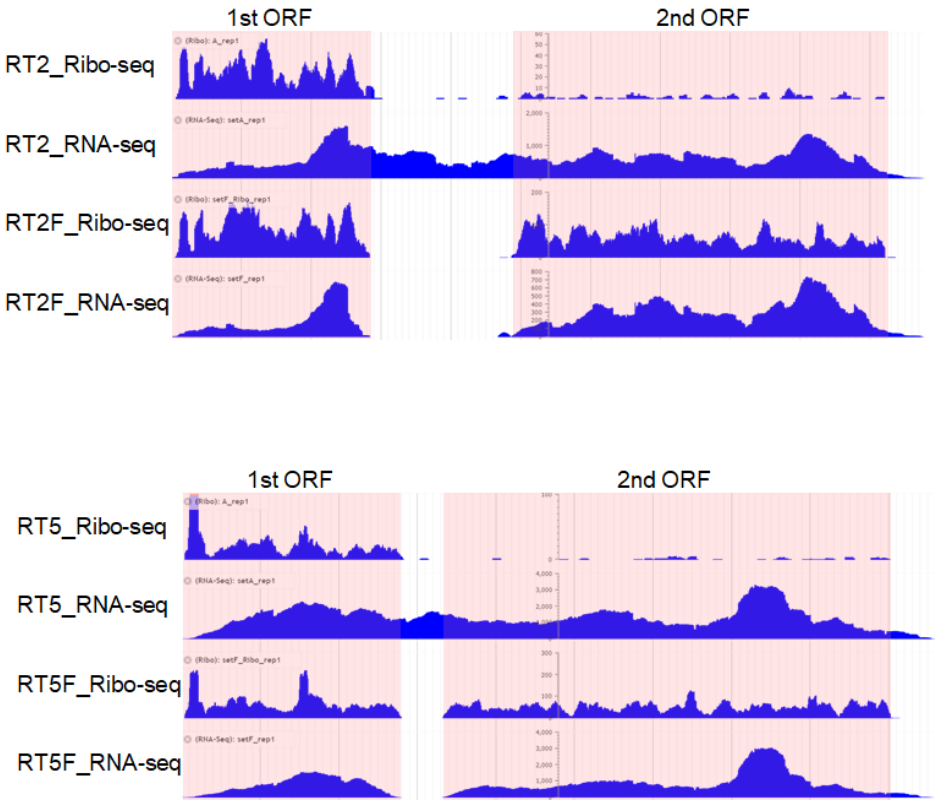
Supplementary Fig. 1 **a** Chromosomal distribution of the 271 RT loci. **b** Overlap of the RT loci detected under the four conditions tested.



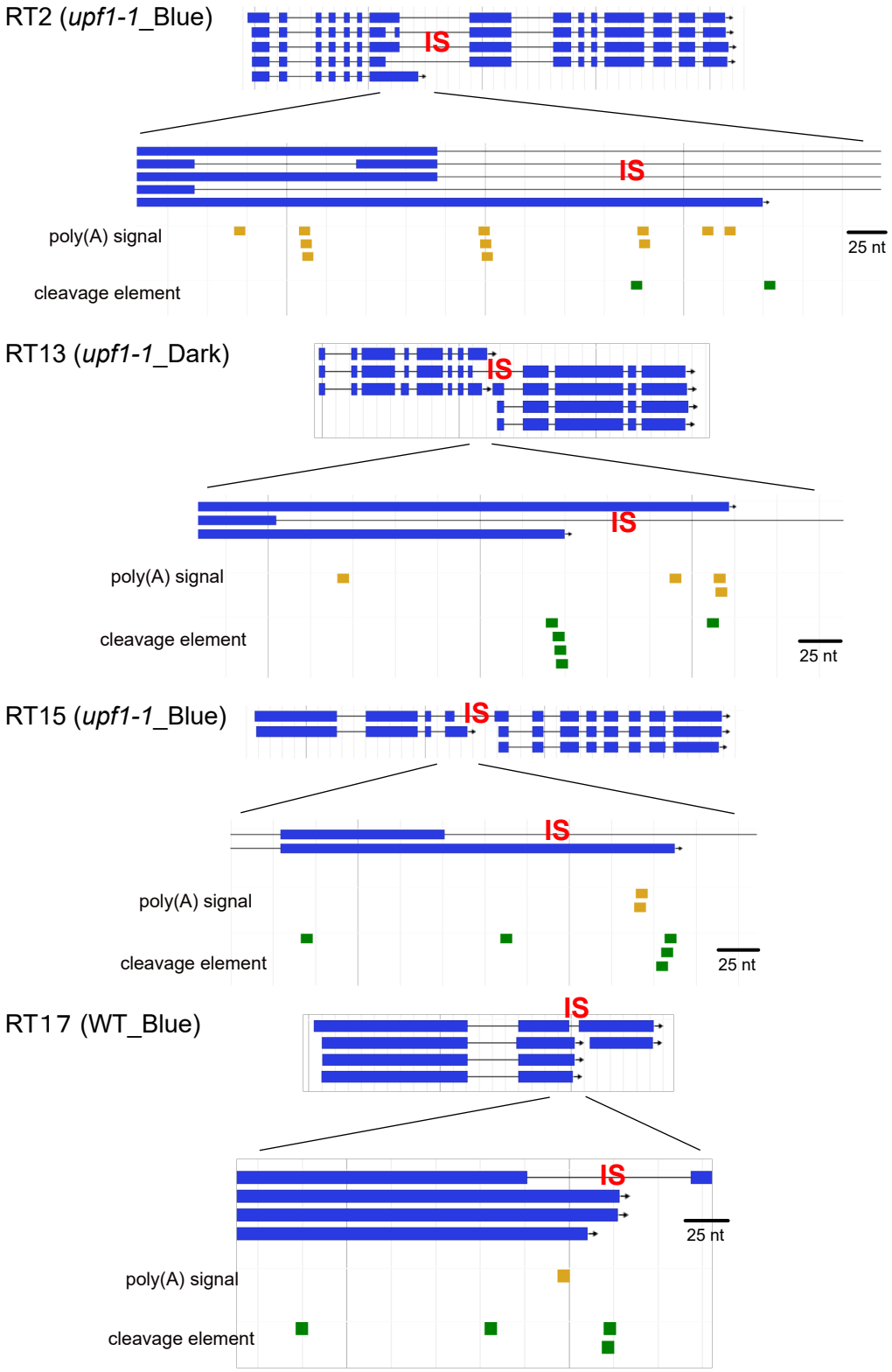
Supplementary Fig. 2 Ribo-seq analysis for the first and second genes of RT1, RT2, RT3, RT5, RT6, RT7, RT9 and RT11 in *Arabidopsis*. Translation efficiency of each ORF is shown.



Supplementary Fig. 3 Northern blot analysis for transiently expressed RTs in *Nicotiana benthamiana* leaves. Red asterisks indicate deduced positions of full-length RTs.



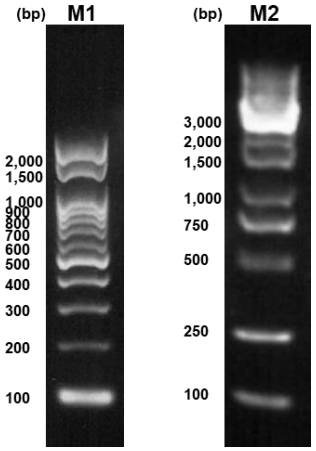
Supplementary Fig. 4 Genome browser view of RT2 and RT5 loci. Each ORF is highlighted by pink color.



Supplementary Fig. 5 Examples of positions of polyadenylation signal-like and cleavage element-like hexamers in IS introns. Polyadenylation signal-like hexamers; AAUAAA, AUAAAA, UAAUAA, AAAUAA, AUAAAU, AUAAUA, AUAUAU, UAUAUA, AAUAAU, UAUAAA. Cleavage element-like hexamers; UUUUUA, UUUCUU, UGUUUA, UUGUUU, UUCUUA, UUUCUA, UUUUCA, UUCUUU, UUAUUA, UUUUCU. They were selected with reference to Loke *et al.* (2005) Plant Physiol. 138: 1457-1468.

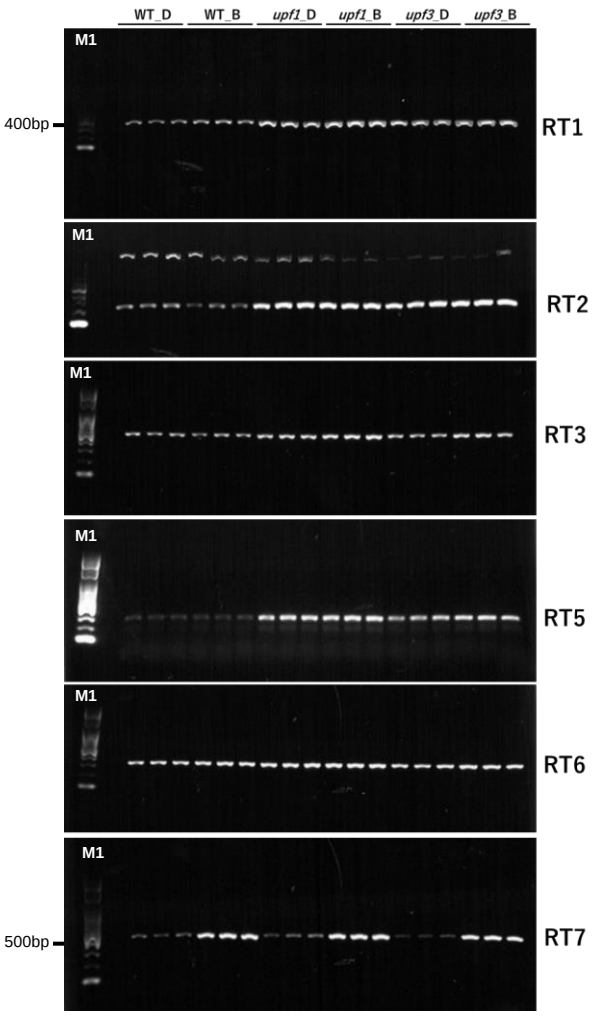
Supplementary Figure 6 Uncropped data.

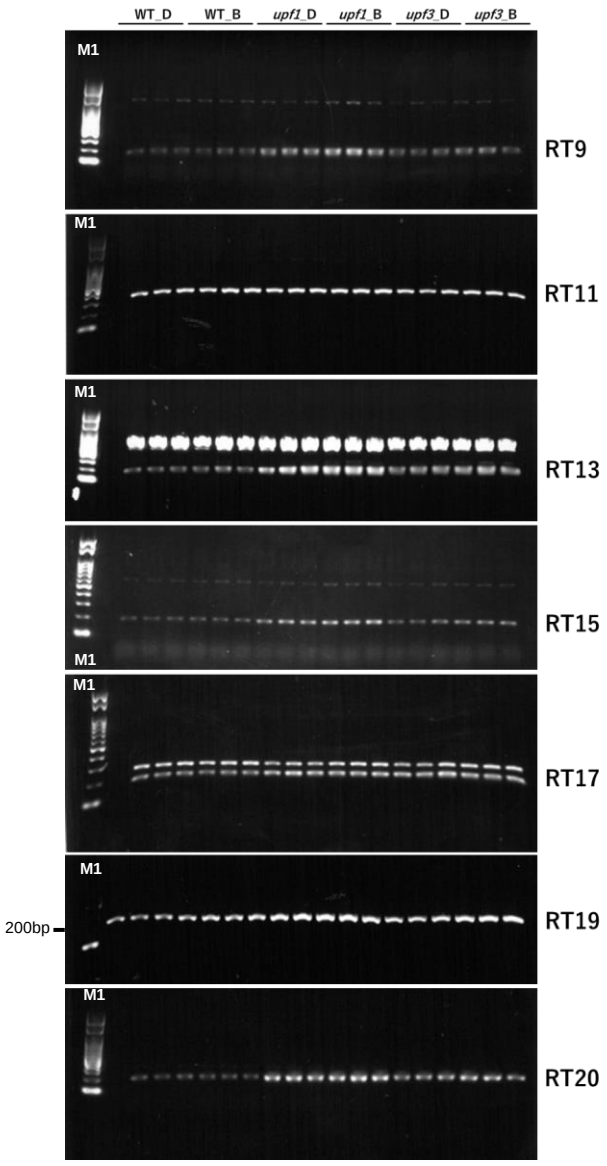
Used markers (M1 and M2) for agarose gel electrophoresis



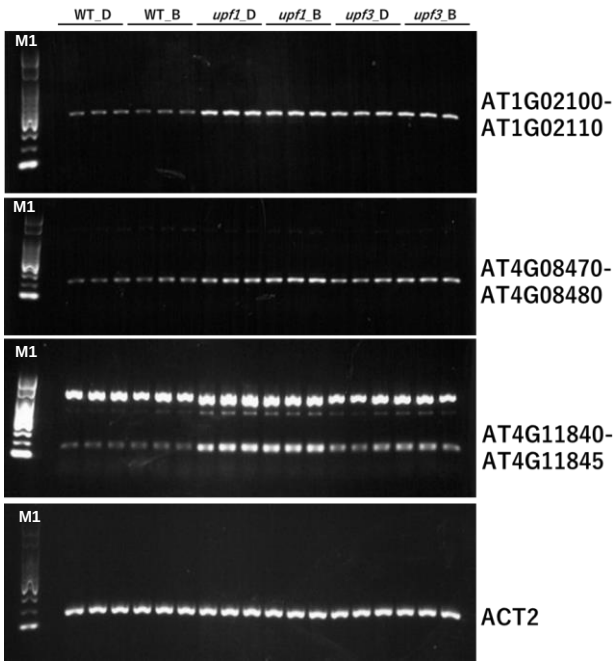
Marker (M1 or M2) is indicated on each image.

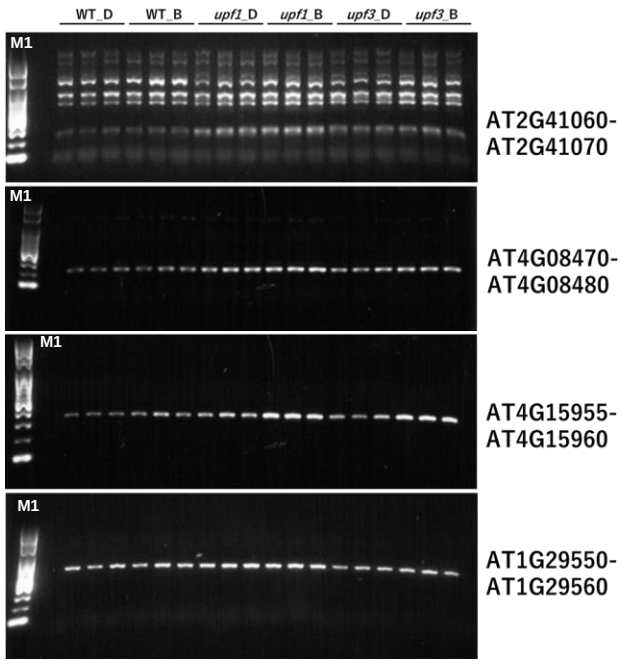
Unprocessed data for Fig. 2



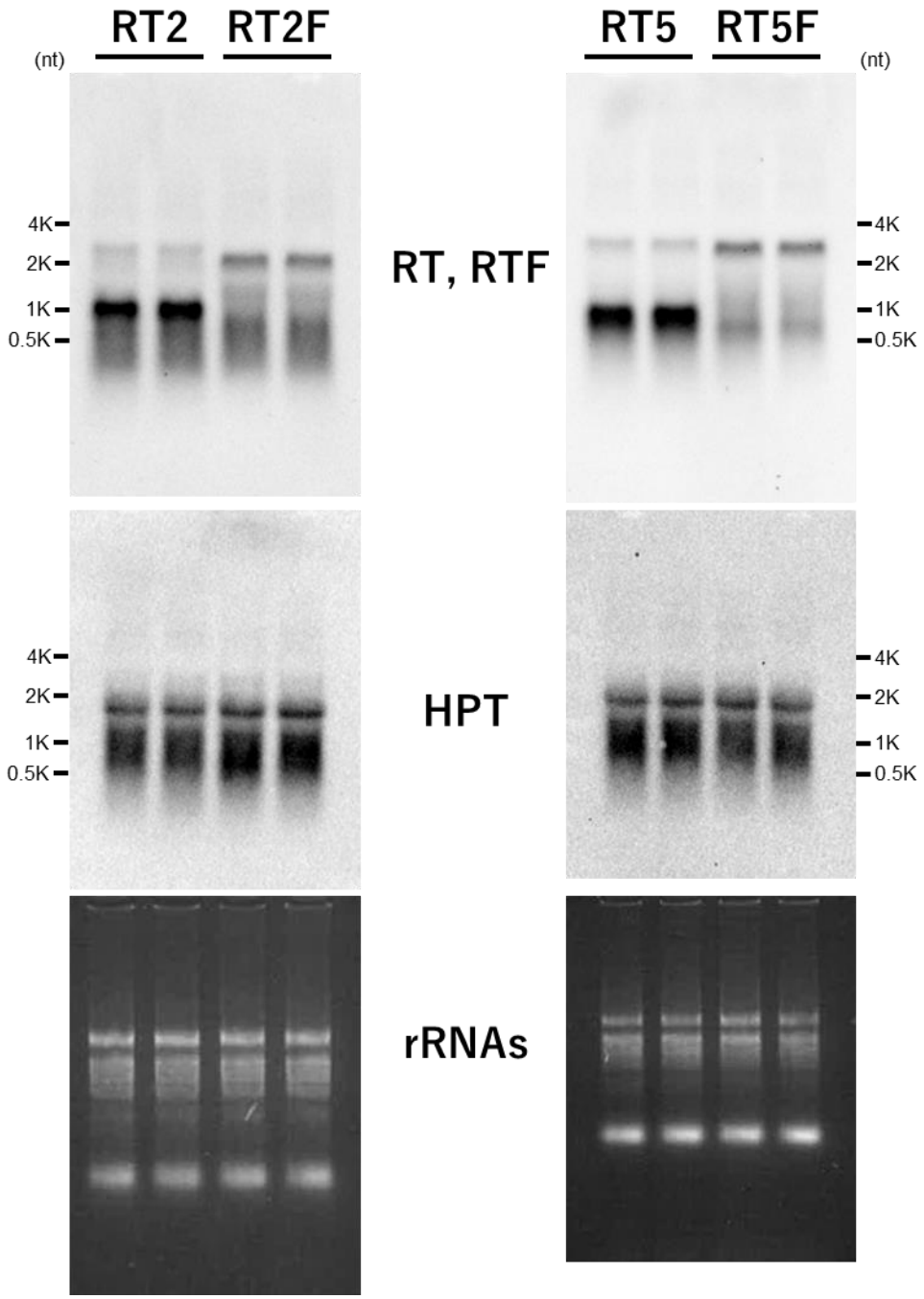


Unprocessed data for Fig. 2

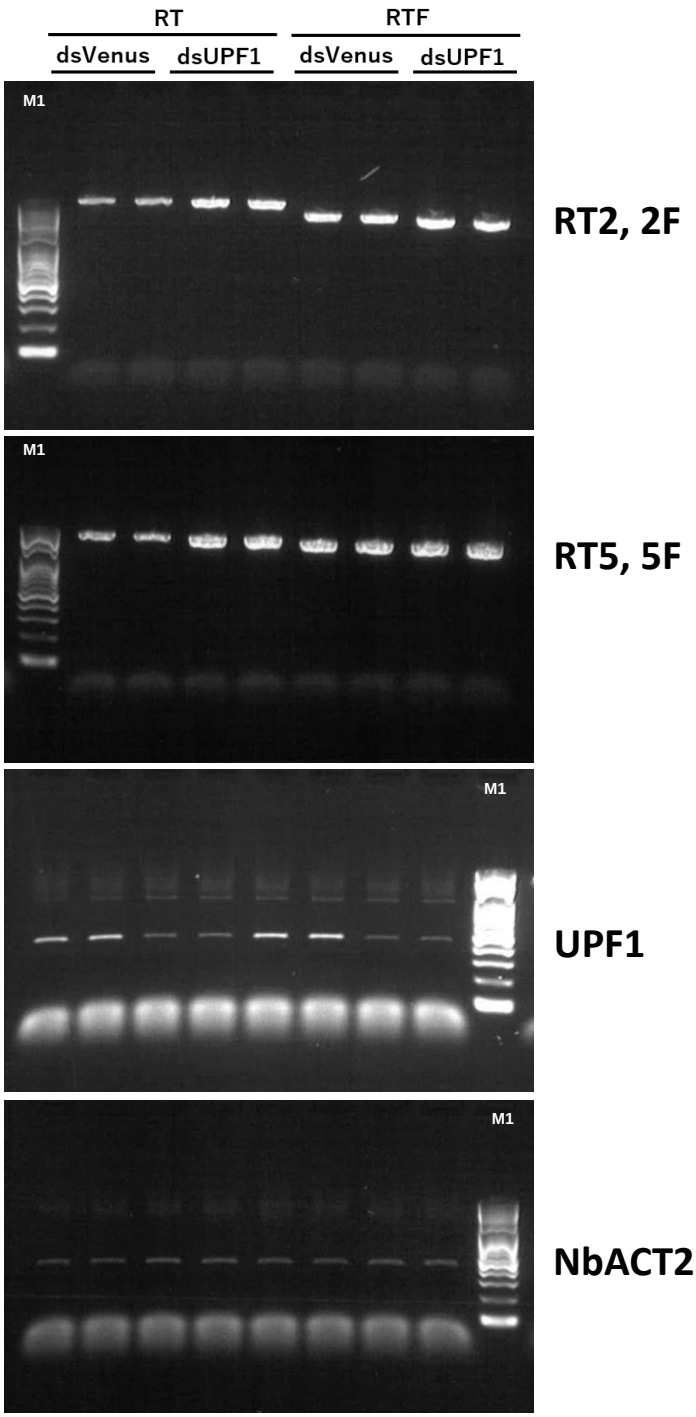




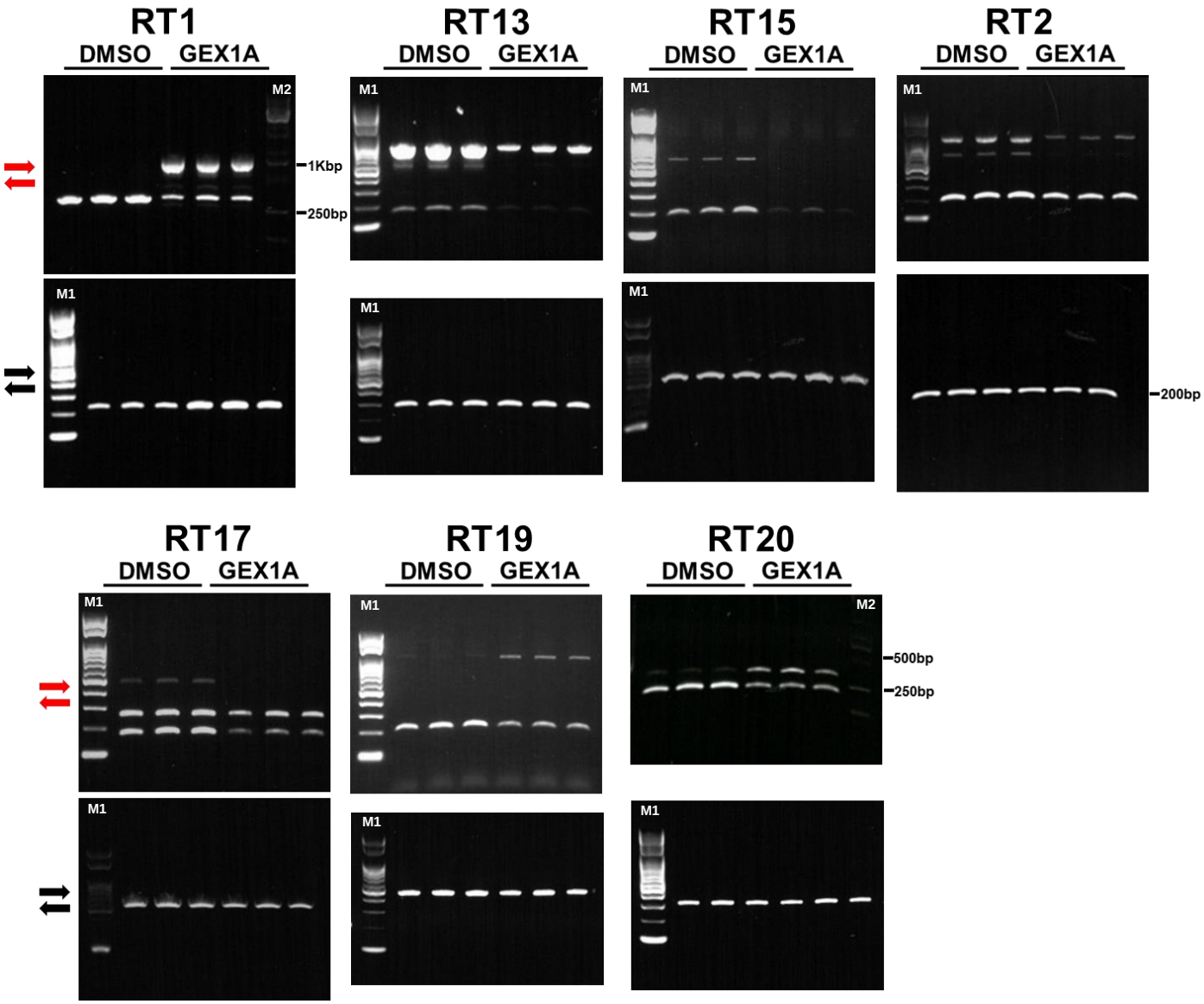
Unprocessed data for Fig. 3c



Unprocessed data for Fig. 3e

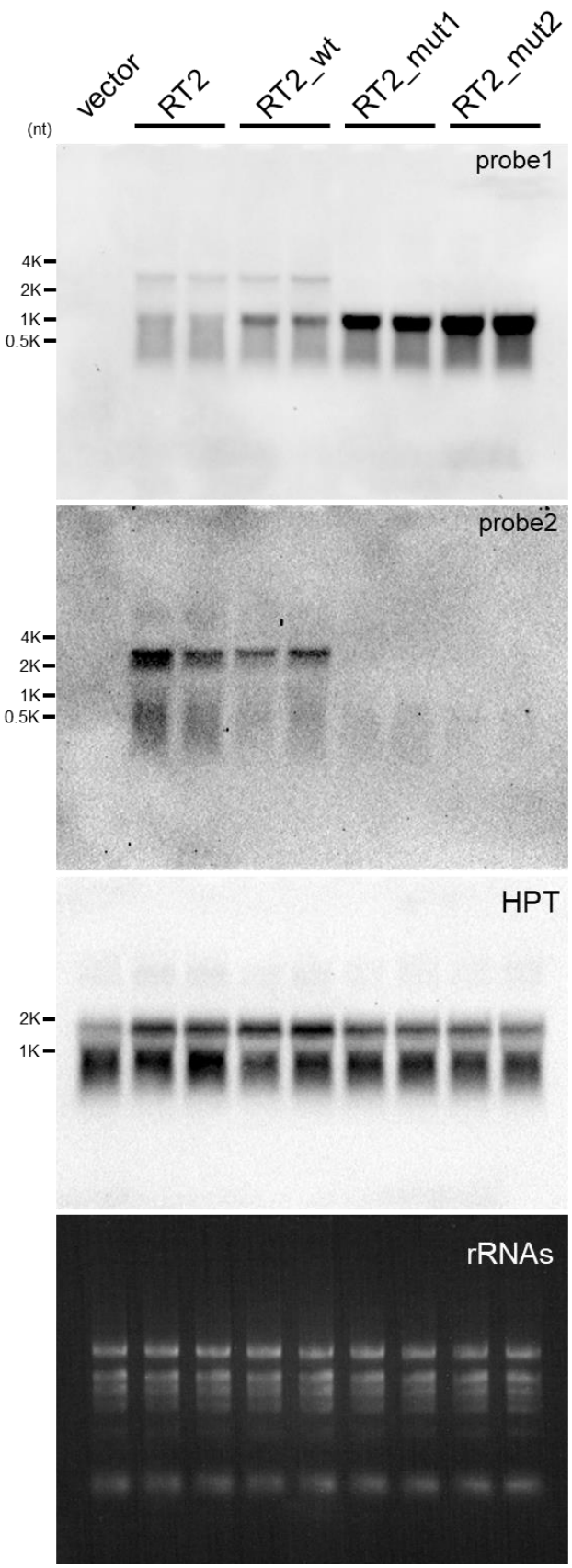


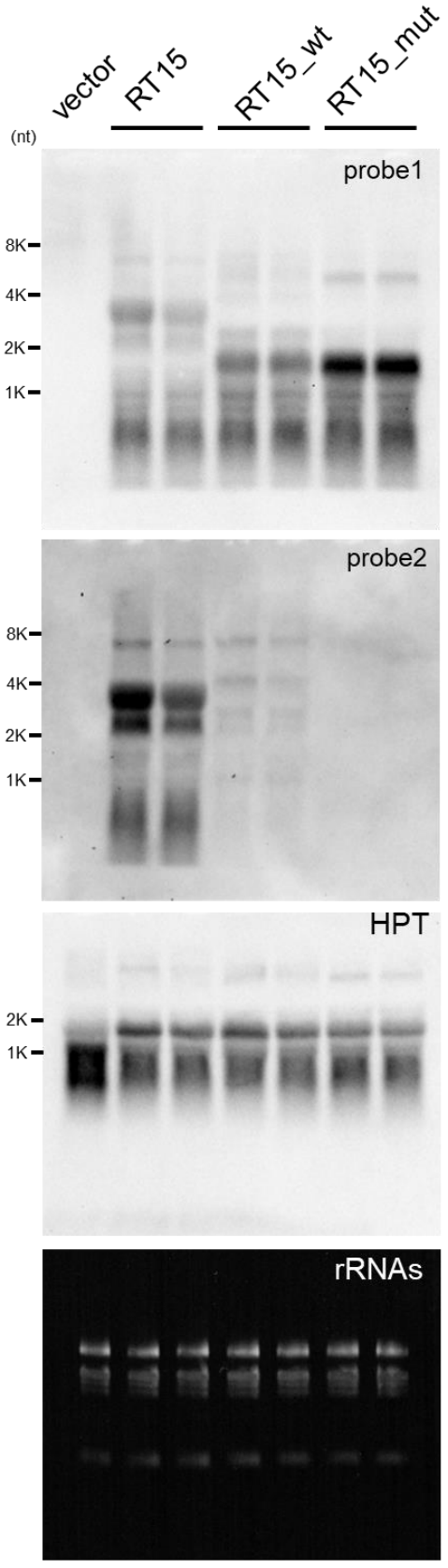
Unprocessed data for Fig. 4



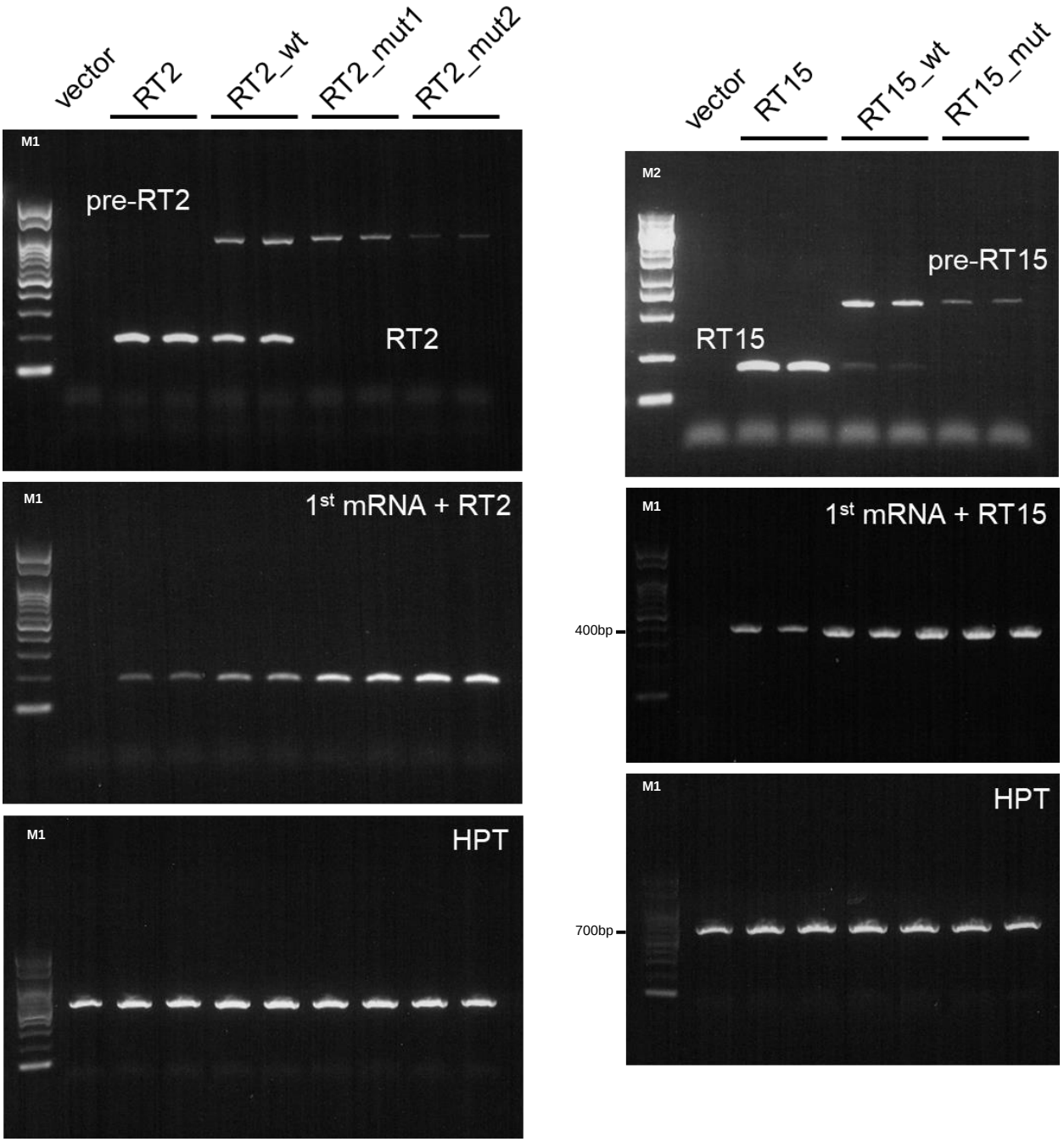
To know the bp length for the lower panel in RT2, the same DMSO sample was loaded with the M1 marker.

Unprocessed data for Fig. 5a





Unprocessed data for Fig. 5b



Supplementary Table 1. List of primers used for plasmid construction.	
Primer name	Sequence 5' to 3'
RT2_HiFi_F	TCATTTGGAGAGAACACGGGGGACT GATGCTCATCCATTAGAGGAGGA
RT2_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC GAAAACCCAATGACAAACCAGA
RT1_HiFi_F	TCATTTGGAGAGAACACGGGGGACT GGTTTAGTTTCTGATCTGTTAAAG
RT1_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC ATCAAAACCATATCAACGTTCA
RT3_HiFi_F	TCATTTGGAGAGAACACGGGGGACT ACACTCAAGAGGACGAGCCATTA
RT3_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC CGAAGTTTCAATGTGATTGCAGT
RT5_HiFi_F	TCATTTGGAGAGAACACGGGGGACT ACCACTAAAGTTTAGGGACATA
RT5_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC AGTACCAAACCGATCTGTATTG
RT6_HiFi_F	TCATTTGGAGAGAACACGGGGGACT AACCTAACCATGGAAATTGCA
RT6_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC AGATGTATTTCTGTGATTTAC
RT7_HiFi_F	TCATTTGGAGAGAACACGGGGGACT TCCACCACAATCCCTCTCTC
RT7_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC GAGGAAGCAGTAGTCTTTGA
RT9_HiFi_F	TCATTTGGAGAGAACACGGGGGACT ATAAGAAAAAACCAGAATTTTG
RT9_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC GGTGCTGAAAAGAATGTATAC
RT11_HiFi_F	TCATTTGGAGAGAACACGGGGGACT CTGCTCTTCTTCCTCTTGTTG
RT11_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC GGTAAATCTAAGGACATTGA
RT15_HiFi_F	TCATTTGGAGAGAACACGGGGGACT

	GAAGTTCTTCTTCCTCATCG
RT15_HiFi_R	GAACGATCGGGGAAATTCGAGCTGC AGACCGAAATTTCATTGACT
RT2_wt_IS_intron_F	GTCTGATTCGGGTTATGAACACAAG GTACCTTCTATTAAGCTTAGCAA
RT2_wt_IS_intron_R	TCCGTGACAGAGGTGACTGTGACAT CTGTAGAGAGAGAAGATAGGGTA
RT2_mut1_IS_intron_F	GTCTGATTCGGGTTATGAACACAAG CTCCCTTCTATTAAGCTTAGCAA
RT2_mut1_IS_intron_R	TCCGTGACAGAGGTGACTGTGACAT CCCTAGAGAGAGAAGATAGGGTA
RT2_5'exon_R	CTTGTGTTCATAACCCGAATCAGAC
RT2_3'exon_F	ATGTCACAGTCACCTCTGTCACGGA
RT2_mut2_minor_IS_intron_F	TTCGAGATTTTTGATCTACGTTAAG CTCACGACTTCTGTGGACTTATC
RT2_mut2_minor_IS_intron_R	GCCTTCTCAGACCAAGTATACAGTT CCCGTTAGGGTGGTGGTTATGAT
RT2_5'exon2_R	CTTAACGTAGATCAAAAATCTCGAA
RT2_3'exon2_F	AACTGTATACTTGGTCTGAGAAGGC
RT15_wt_IS_intron_F	TATATGGAAGGCACTAAATCTTCAG GTACTATTATTTAGTTTTCGG
RT15_wt_IS_intron_R	GTCAAAAGATATTTGACCCGCCCGT CTTGGACAGATTGGATTAAA
RT15_mut1_IS_intron_F	TATATGGAAGGCACTAAATCTTCAG CTCCTATTATTTAGTTTTCGG
RT15_mut1_IS_intron_R	GTCAAAAGATATTTGACCCGCCCGT CCCGGACAGATTGGATTAAA
RT15_5'exon_R	CTGAAGATTTAGTGCCTTCCATATA
RT15_3'exon_F	ACGGGCGGGTCAAATATCTTTTG
RT2_1st_R	ACGTAGATCAAAAATCTCGAACC
RT2_2nd_F	TAGGTTCGAGATTTTTGATCTACGT ATGATGATGATGAGAGGTGGT
RT5_1st_R	ATTAGGAATTTCAGGGATTGCATTG
RT5_2nd_F	CAATGCAATCCCTGAAATTCCTAAT ATGGCGGAACTAAAGCTATC

dsUPF1_F	CACCTCATTTCTGCTAAGGCAGAGAGTG
dsUPF1_R	AGACGCAACTCATTATCTTCCTTAG

Supplementary Table 2. List of primers used for RT-PCR (Figs. 2 and 5).	
Primer_name	Sequence 5' to 3'
AT2G41060-AT2G41070_F	GTCCTTACATGGGTCGTTAGATTAG
AT2G41060-AT2G41070_R	AGGCTCTTCAATGTTTCCTCTAATAG
AT2G01390-AT2G01400_F	TCTTGTTGCTTCTGCACGTTTCCGAG
AT2G01390-AT2G01400_R	GAAAGTGAGATCAAAACAGGTGTCCA
AT4G11840-AT4G11845_F	GGGACTTAGCTTTCCGATCCAAAGA
AT4G11840-AT4G11845_R	CAAACGGCACGAGTTGCTTGTCAAG
AT4G08470-AT4G08480_F	GTTTATGAGATATTAGACCCCATAGA
AT4G08470-AT4G08480_R	TTAGGGATAAGATACTCTCTGGATTG
AT4G25280-AT4G25290_F	TTCGTAAGCCTGAATCCGTAATGTC
AT4G25280-AT4G25290_R	TCAGAATCGAGCAAGAAATATCGTC
AT1G02100-AT1G02110_F	GATGATTGATTGCACAAAGTTAGAAAG
AT1G02100-AT1G02110_R	ACAGCATCCTCACTGTCGAGCTT
AT4G15955-AT4G15960_F	TCAACACATTCACAACTTCTTTCAC
AT4G15955-AT4G15960_R	GCTTTTCTCGGCGACGTGCATA
AT4G19880-AT4G19890_F	TGTCGCCTGTTCAGTAGATTCTAAAG
AT4G19880-AT4G19890_R	GTTGCTAATGATCGTGGAATAAGACA
AT3G26539-	TGATCATTACCTTCAGCCACCATGAATG

AT3G26540_F	
AT3G26539-AT3G26540_R	CAATGTCCATGGAGGCCACGATATAAG
AT1G29550-AT1G29560_F	CGTCGATATACTGTATGAGATTCAAC
AT1G29550-AT1G29560_R	CTCCAGCGTCTGCCATTAAAGTTGAGA
AT4G00030-AT4G00040_F	GGACTGAATCATTTGTATAATTTGTGATGC
AT4G00030-AT4G00040_R	ACCAACATCTTTGTGAGTTCACAATG
AT5G67630-AT5G67640_F	CTAATTAGGGTTCAAATTATCACAGAC
AT5G67630-AT5G67640_R	CATATTCGATTATCGTCTTCGTGCGA
AT4G03250-AT4G03260_F	GACGATGAGACTGACGAATCTAGC
AT4G03250-AT4G03260_R	ACTTTGCAGAAGCGGCCAGAGTGA
AT5G24610-AT5G24620_F	GTTACTCCTTAACTGTGAGAAGAAG
AT5G24610-AT5G24620_R	AGACTGTCCCACATCAAGCCTGAAC
AT1G10600-AT1G10610_F	CTGTATACTTGGTCTGAGAAGGCTG
AT1G10600-AT1G10610_R	ATATACGATCGTACAAGACAATGGTA
AT5G57140-AT5G57150_F	GGAAACTTCCGACTCATTTCTGAAG
AT5G57140-AT5G57150_R	ACTGTCTCTAGTTCGTTTCGTTGGTC
AT3G42630-AT3G42640_F	CTAGCTACATGCCACATCTTTGCTG
AT3G42630-AT3G42640_R	TCATCCCACGAGAATTCAGTCGCCA

AT3G50685-AT3G50690_F	CAAAGGAAGAGTTTCGAGACCTTG
AT3G50685-AT3G50690_R	CTCTCACCAGTTAATTACCACACA
AT1G78800-AT1G78810_F	GCTTAATCATATTGTTTCTCTTCTACG
AT1G78800-AT1G78810_R	GACTTGACTGGTTTACGTTCTTCTG
AT4G03250-AT4G03260_1st_F	ATCAAAGTTGCATTCGCCAGATC
AT4G03250-AT4G03260_1st_R	CATGAGACTGATTGGTTTCAATATTG
AT3G42630-AT3G42640_1st_F	GACGATTCTCCTCTTGTCTTAACTG
AT3G42630-AT3G42640_1st_R	CAAGCATCGGAAAAGTTTATCAGG
AT2G01390-AT2G01400_1st_F	GTGTAGCTGCTTACACTGCGCTGA
AT2G01390-AT2G01400_1st_R	ACCAGATTACAACAGATTTGTAGC
AT4G11840-AT4G11845_1st_F	CTCTCGTTTCAAATGTGGACAACC
AT4G11840-AT4G11845_1st_R	CTGAGTGTAGCAATCGATTGCGGT
AT4G08470-AT4G08480_1st_F	GTTGAAGCCCTGTTTAGGATCAGA
AT4G08470-AT4G08480_1st_R	TAGATTCATCTACGGATTAGCGGA
AT3G26539-AT3G26540_1st_F	ATGGATGCTTGCATCAGCTTGAAG
AT3G26539-AT3G26540_1st_R	AGCTCCGATATGACTGCAGGAGA
AT1G10600-AT1G10610_1st_F	TACGGGATATTTAAGCTAACGGAC
AT1G10600-AT1G10610_1st_R	CTTAACGTAGATCAAAAATCTCG

AT5G57140-AT5G57150_1st_F	AGCCAGAGGATTCATGTTAATCC
AT5G57140-AT5G57150_1st_R	AGTTTCCCATAAGACTTGTTCGTC
AT4G00030-AT4G00040_1st_F	ATTCAATAGTGCGGTATTGAGAGG
AT4G00030-AT4G00040_1st_R	CAAATGATTCAGTCCAGTTATAAG
AT5G24610-AT5G24620_1st_F	TGGAGACGGAATGGAGTTGGTTTAG
AT5G24610-AT5G24620_1st_R	CATGTTTCGTTGATAGTTTTGAAC
AT4G19880-AT4G19890_1st_F	CAACCTTGTTACTGATTTGACTG
AT4G19880-AT4G19890_1st_R	CACTCTCCAGTCTTTAGAATCTAC
AT5G67630-AT5G67640_1st_F	GACTAATCGGTGACAATGATAAGAC
AT5G67630-AT5G67640_1st_R	TGAACCCTAATTAGGAATTTCAGG
AT4G25280-AT4G25290_1st_F	CACGATGAAATCTCAGAAGAGG
AT4G25280-AT4G25290_1st_R	ACTGCTTTCTGATGACCAAATC
AT3G42630-AT3G42640_1st_F	GAGTTTGTATACAACCGAATGAATC
AT3G42630-AT3G42640_1st_R	ATTGTGATAGTGAGCAAAGAGTGAC
AT3G50685-AT3G50690_1st_F	GATGAGTAATGGCCATAGTGAAAG
AT3G50685-AT3G50690_1st_R	ATCATCATCTTCATCCGCAAACTC
AT1G78800-AT1G78810_1st_F	CAACAGGTCATGTCGGTTGAAGA
AT1G78800-AT1G78810_1st_R	AGCCAAGTAGTTCACAGACAAAC

HPT_F (HPT_Rprobe_F)	ATGAAAAAGCCTGAACTCAC
HPT_R (HPT_Rprobe_R)	ATAATACGACTCACTATAGGGTCCATCACAGTTTGC CAGTG

Supplementary Table 3. List of primers used for probe construction.	
Primer_name	Sequence 5' to 3'
2-1Rprobe_F	CACGTTTCTCGGGTTCTAATC
2-1Rprobe_R	ATAATACGACTCACTATAGGG TGGCACCATTACCTGATACG
2-2Rprobe_F	GATTCATGGAGAAGTAGTGATG
2-2Rprobe_R	ATAATACGACTCACTATAGGG ATCCTTTTCCTTGTTCATTATC
15-1Rprobe_F	ATGATGAATATTTACGACGATG
15-1Rprobe_R	ATAATACGACTCACTATAGGG CGACTGAGCTATCTTCTTCAC
15-2Rprobe_F	GAGAGATTGATTGTTGATGCG
15-2Rprobe_R	ATAATACGACTCACTATAGGG ATGTATTGGCAAATGTTGATG
1-1Rprobe_F	GCGTGTTATCGGCACCGGAAC
1-1Rprobe_R	ATAATACGACTCACTATAGGG CAGCACAATGCAGCTGCCTC
3-1Rprobe_F	CCTAAGTCGAATCGATTCCTTC
3-1Rprobe_R	ATAATACGACTCACTATAGGG TCAATCTCCCAATCTCCGAG
5-1Rprobe_F	AAGCGTCGAGAATTTCACGAAG
5-1Rprobe_R	ATAATACGACTCACTATAGGG TCATTGTCACCGATTAGTCC
6-1Rprobe_F	CTCTCGCTGTCGGCATGTTC
6-1Rprobe_R	ATAATACGACTCACTATAGGG CAGTTCTTCCCTCTCAATAC
7-1Rprobe_F	TCTCCCGCATTTCCTGCATC
7-1Rprobe_R	ATAATACGACTCACTATAGGG GAGTACGCCAAGCAACAATC
9-1Rprobe_F	TCAGATTCAGGCGAAGCGAC
9-2Rprobe_R	ATAATACGACTCACTATAGGG TCTCTTTACGGCAGTGATA
11-1Rprobe_F	ATGGAGGTATTTGGAAAATCTC
11-1Rprobe_R	ATAATACGACTCACTATAGGG ACCAACTCCATTCCGTCTCC
HPT_Rprobe_F	ATGAAAAAGCCTGAACTCAC
HPT_Rprobe_R	ATAATACGACTCACTATAGGGTCCATCACAGTTTGCCAGTG

Supplementary Table 4. List of primers used for RT-PCR of RNAi assay.	
Primer_name	Sequence 5' to 3'
NbUPF1_F	ATTAACTGCTCAAGAGGAGGTTG
NbUPF1_R	CTAATTGGTCCACAGCAACATTAC
NbACT_F	ACTGATGAAGATACTCACAGAAAG
NbACT_R	GAGCTAATGCAGTAATTTCCTTG
AT1G10600-AT1G10610_Long_F	TACGGGATATTTAAGCTAACGGAC
AT1G10600-AT1G10610_Long_R	GAAAACCCAATGACAAACCAG
AT5G67630-AT5G67640_Long_F	GACTAATCGGTGACAATGATAAGAC
AT5G67630-AT5G67640_Long_R	CATAAATCCGAGTTCAACATC