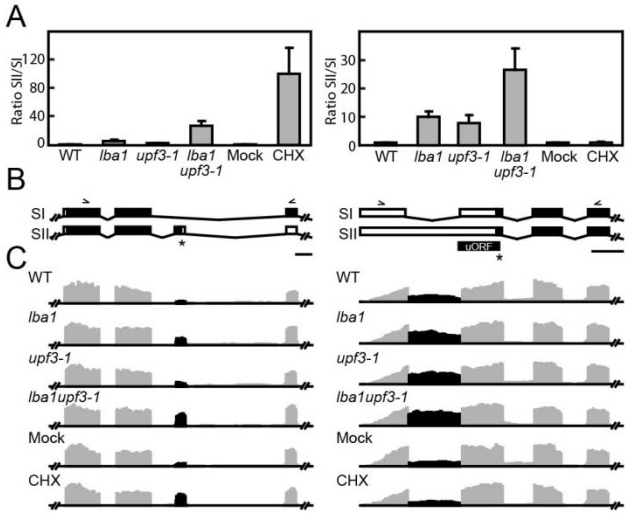
Supplementary Figures

Supplemental Figure 1. Quantitation of Co-Amplified PCR Products and Coverage Plots for NMD Candidate Genes Displayed in Figure 1.

(A) Ratio of splicing variants derived from genes *At5g53180* and *At4g36960* as determined by Bioanalyzer quantitation of co-amplified cDNA products (mean values +SD, n = 3).

(B) Gene models showing the alternatively spliced regions. For further details of gene models refer to legend of Figure 1.

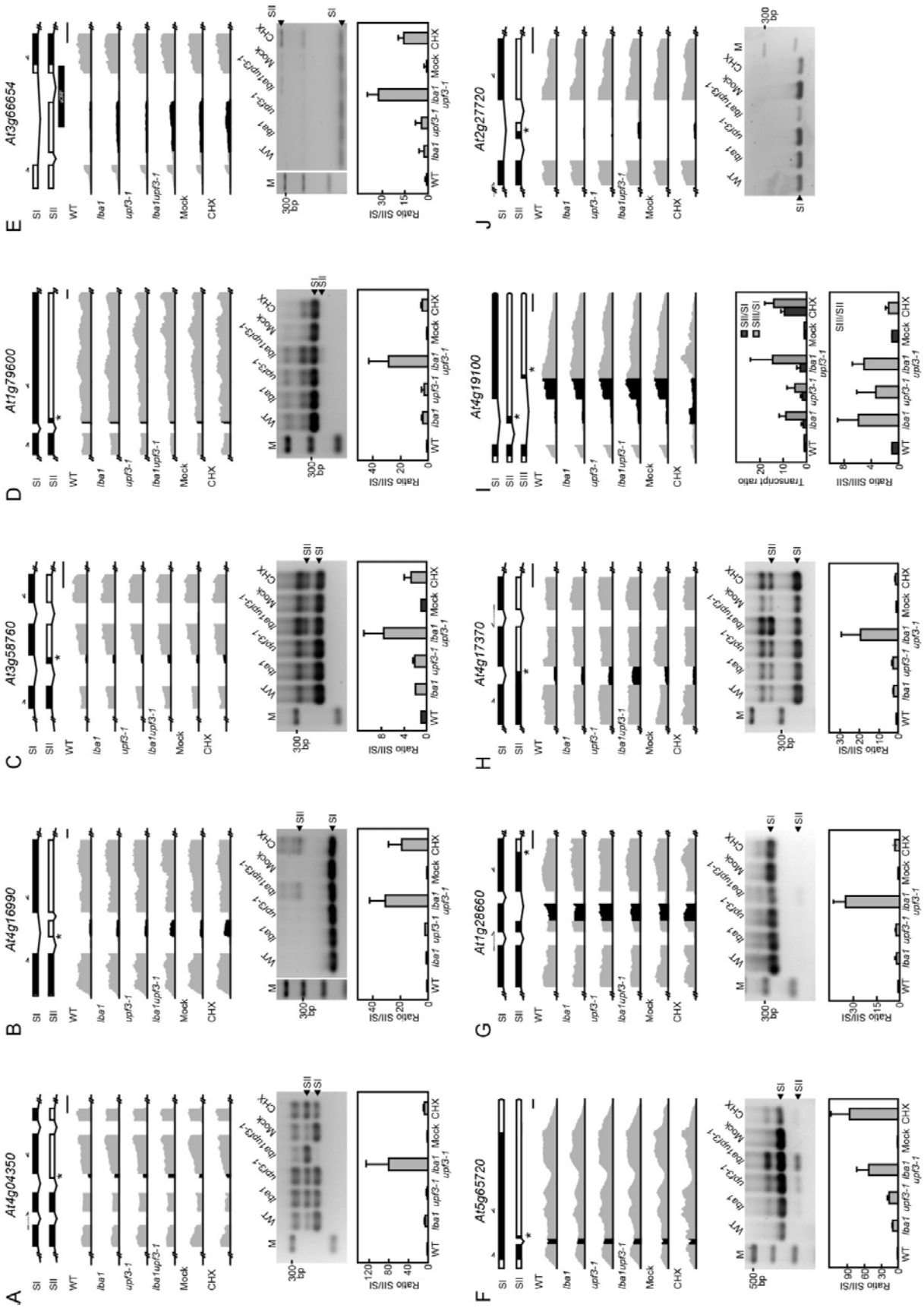
(C) Coverage plots showing aligned reads from RNA-seq data for the regions displayed in **(B)**. Reads in the altered regions are shown in black, other reads in gray.



Supplemental Figure 2. Gene Models, Coverage Plots, and Splice Ratio Analyses for AS Events Randomly Chosen for FDR Testing of Differential Testing *lba1 upf3-1* versus Wild Type.

(A-I) For each gene, partial gene model depicting the alternatively spliced region (top panel), representative coverage plots for control and NMD-impaired samples (middle panel), and, lower panel, either gel pictures showing coamplified RT-PCR products corresponding to splicing variants shown above and quantitation via Bioanalyzer **(A-H)**, or determination of splicing variant ratio via RT-qPCR **(I)**. Details of gene models as described in legend to Figure 1. Quantitative data are mean values of triplicates + SD, except CHX sample for *At1g28660* **(G)**, for which a duplicate has been analyzed (individual data points displayed as dots and mean value as bar). **(I)** Ratio determination based on RT-qPCR analysis of individual variants SI, SII, and SIII. This randomly selected AS event referred to splice forms SII and SIII, while SI corresponds to the representative gene model in the TAIR10 annotation. Besides the ratio SIII/SII, ratios for either isoform relative to SI were determined, revealing that both SII and SIII increase upon NMD impairment and thus are NMD targets.

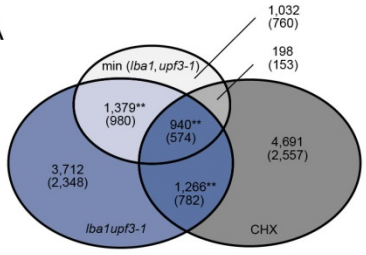
(J) RT-PCR aiming at coamplification of the two predicted splicing variants derived from *At2g27720* resulted only in a single band corresponding to SI.


A
At4g04350
B
At4g16990
C
At3g58760
D
At1g79600
E
At3g66654
F
At5g65720
G
At1g28660
H
At4g17370
I
At4g19100
J
At2g27720
SI
SII
SIII
WT
Iba1
upf3-1
Iba1upf3-1
Mock
CHX
M
bp
Ratio SII/SI
Ratio SIII/SII
Transcript ratio
SII/SI
SIII/SI

Supplemental Figure 3. Venn Diagrams and Numbers of AS Events Changed upon NMD Impairment Using Different Stringency Cutoffs.

(A-C) Size proportional Venn diagrams of significantly altered AS events and gene numbers (in parentheses, left panel) and tables with the corresponding event and gene numbers and subgroups (right panel). Depicted are comparisons of *lba1 upf3-1*, single mutants and CHX treatment with cutoff values of p <= 0.1 for all datasets **(A)**, FDR <= 0.1 for all datasets **(B)**, and of *lba1 upf3-1* and CHX treatment (FDR <= 0.1) and the two single mutants with p <= 0.1 (**C**, and Figure 3A). Asterisks provide information on statistical significance of the overlaps. Removed contradictory events had significant, opposite changes in two samples.

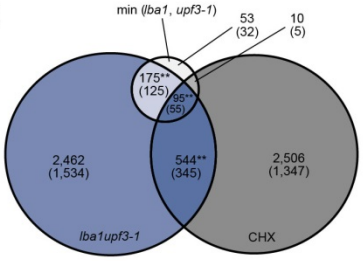
A



	#	% CHX	% *lba1upf3-1*	% min (*lba1, upf3-1*)
CHX	4,691	66.12		
lba1upf3-1	3,712		50.87	
min (*lba1, upf3-1*)	1,032			29.08
CHX & *lba1upf3-1*	1,266	17.84	17.35	
CHX & min (*lba1, upf3-1*)	198	2.79		5.58
***lba1upf3-1* & min (*lba1, upf3-1*)**	1,379		18.90	38.86
CHX & *lba1upf3-1* & min (*lba1, upf3-1*)	940	13.25	12.88	26.49
contradictory (removed)	476			
	13,694			

total:		**total overlapping:**	**#**	**%**
CHX	7,095	CHX	2,404	33.88
lba1upf3-1	7,297	*lba1upf3-1*	3,585	49.13
min (*lba1, upf3-1*)	3,549	min (*lba1, upf3-1*)	2,517	70.93

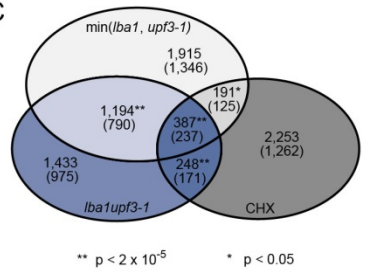
B



	#	% CHX	% *lba1upf3-1*	% min (*lba1, upf3-1*)
CHX	2,506	79.43		
lba1upf3-1	2,462		75.15	
min (*lba1, upf3-1*)	53			15.92
CHX & *lba1upf3-1*	544	17.24	16.61	
CHX & min (*lba1, upf3-1*)	10	0.32		3.00
***lba1upf3-1* & min (*lba1, upf3-1*)**	175		5.34	52.55
CHX & *lba1upf3-1* & min (*lba1, upf3-1*)	95	3.01	2.90	28.53
contradictory (removed)	86			
	5,931			

total:		**total overlapping:**	**#**	**%**
CHX	3,155	CHX	649	20.57
lba1upf3-1	3,276	*lba1upf3-1*	814	24.85
min (*lba1, upf3-1*)	333	min (*lba1, upf3-1*)	280	84.08

C



** $p < 2 \times 10^{-5}$ * $p < 0.05$

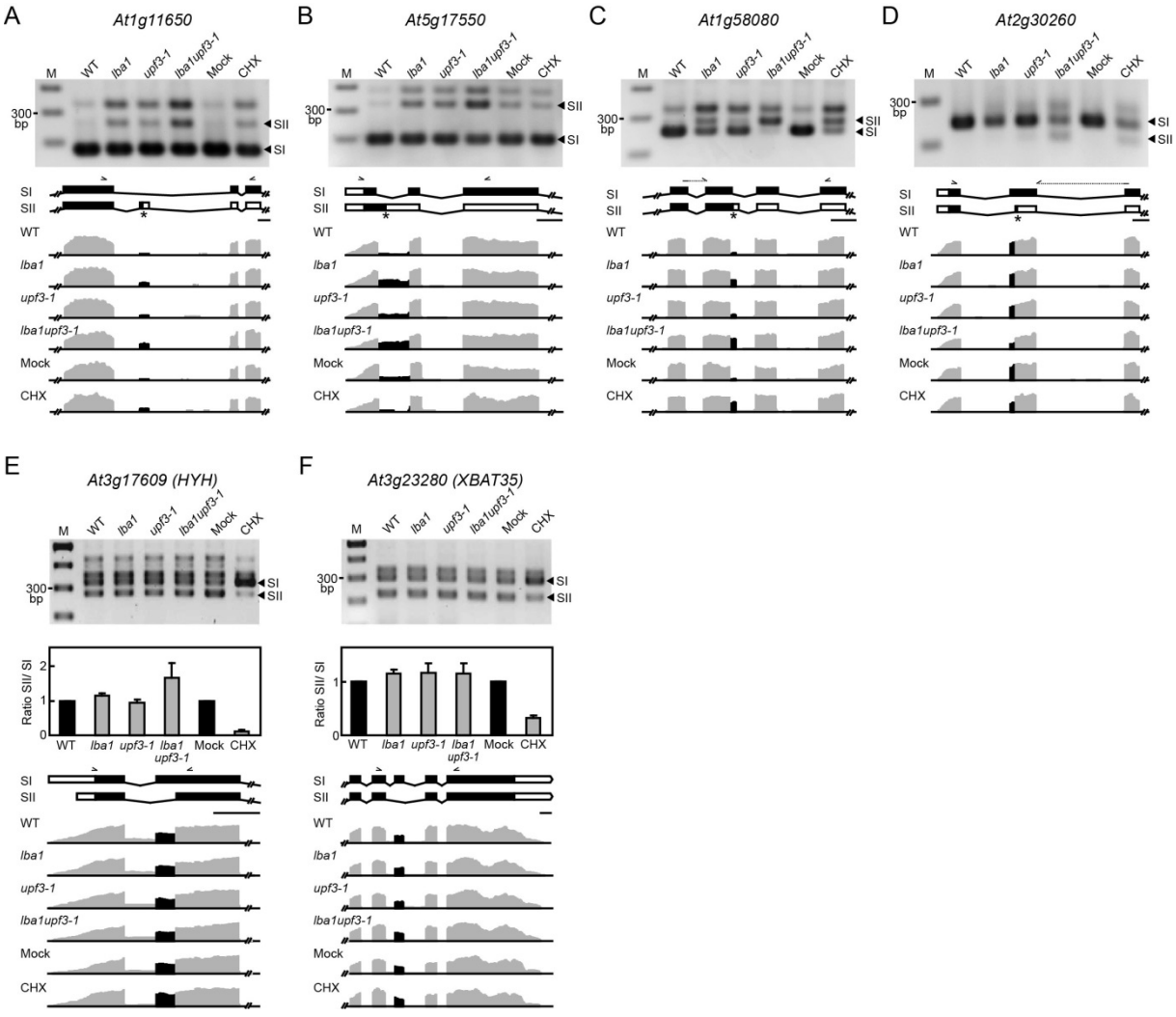
	#	% CHX	% *lba1upf3-1*	% min (*lba1, upf3-1*)
CHX	2,253	73.17		
lba1upf3-1	1,433		43.93	
min (*lba1, upf3-1*)	1,915			51.94
CHX & *lba1upf3-1*	248	8.05	7.60	
CHX & min (*lba1, upf3-1*)	191	6.20		5.18
***lba1upf3-1* & min (*lba1, upf3-1*)**	1,194		36.60	32.38
CHX & *lba1upf3-1* & min (*lba1, upf3-1*)	387	12.57	11.86	10.50
contradictory (removed)	173			
	7,794			

total:		**total overlapping:**	**#**	**%**
CHX	3,079	CHX	826	26.83
lba1upf3-1	3,262	*lba1upf3-1*	1,829	56.07
min (*lba1, upf3-1*)	3,687	min (*lba1, upf3-1*)	1,772	48.06

Supplemental Figure 4. Gel Pictures, Gene Models, and Complete Sets of Coverage Plots for the AS Events Shown in Figure 3D and Control Events not Linked to NMD.

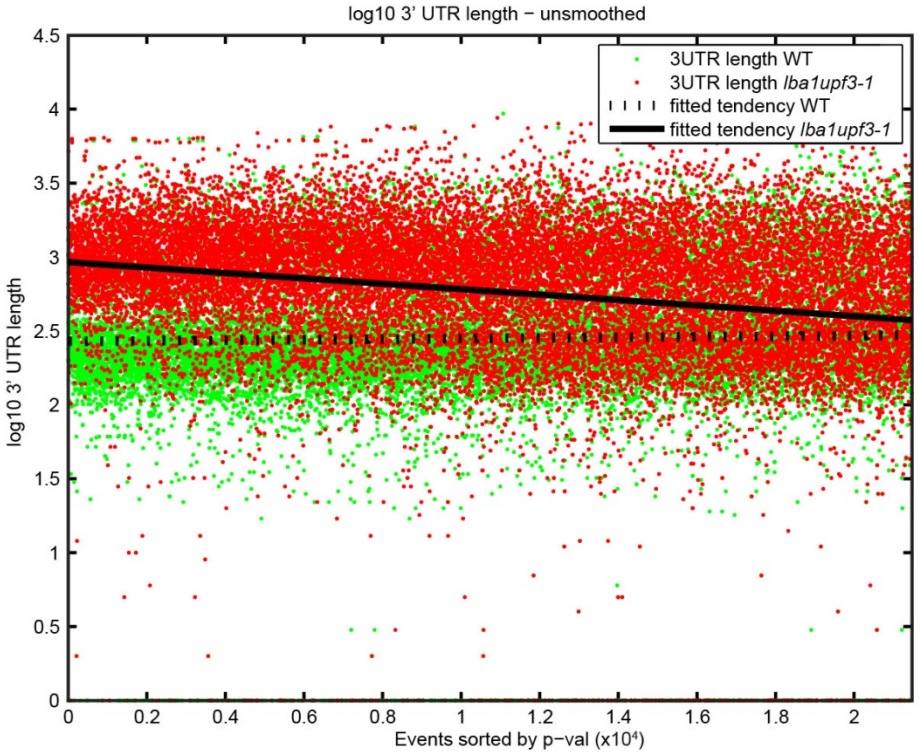
(A-D) Alternative splicing events shown in Figure 3D. Upper panel shows representative gel pictures of RT-PCR products for *At1g11650* **(A)**, *At5g17550* **(B)**, *At1g58080* **(C)**, and *At2g30260* **(D)** used for quantitation (Figure 3D). "M" indicates DNA ladder in 100 bp increments. Bands corresponding to splicing variants SI and SII are indicated and the corresponding partial gene models are displayed below gel pictures. Details of gene models as described in legend to Figure 1. Lower panel shows coverage plots derived from RNA-Seq data for representative samples.

(E, F) Alternative splicing events of genes *At3g17609* and *At3g23280* leading to two major splice forms, both of which have been shown to encode a protein (Sibout et al., 2006; Carvalho et al., 2012), and thus are not expected to be linked to NMD. Setup and details of display as described for **(A-D)**.



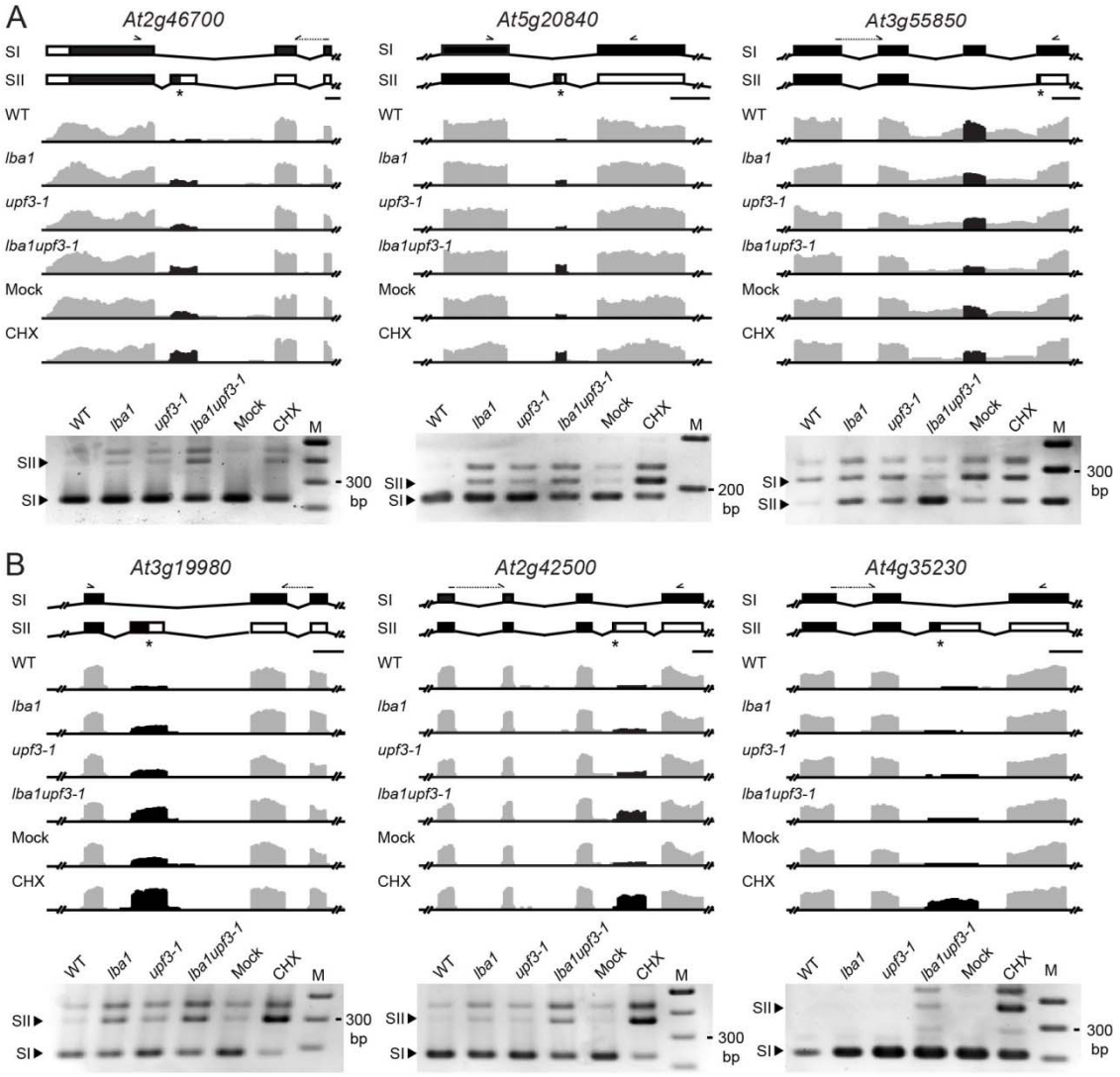
Supplemental Figure 5. Correlation of 3' UTR Lengths in Double Mutant and Wild Type Isoforms with the Predicted Significance of Differential Transcript Expression.

All events tested as differentially expressed for wild type vs. *lba1 upf3-1* having a p-value < 1 were sorted by p-value and displayed along the x-axis. Red and green dots show 3' UTR lengths of *lba1 upf3-1* and wild type isoforms, respectively. Linear regression lines of the 3' UTR representing the tendency along the significance axis are depicted in black solid and dashed for the double mutant and wild type isoforms, respectively.



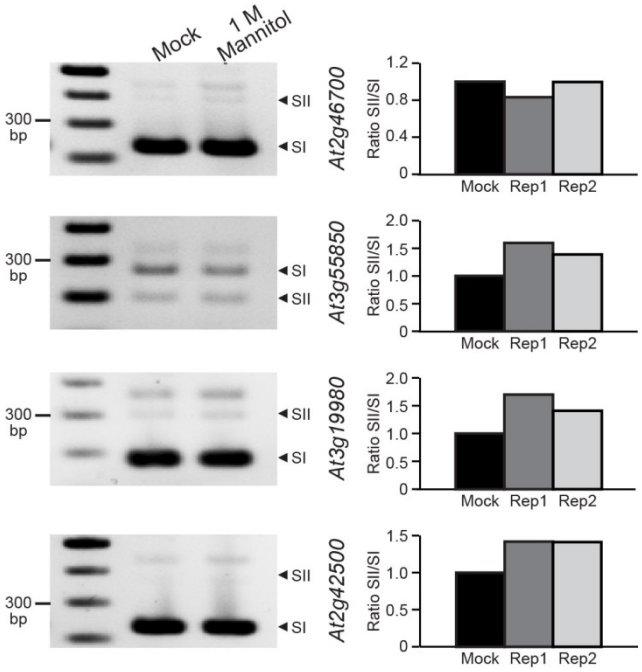
Supplemental Figure 6. Gene Models, Coverage Plots, and Representative Gel Pictures for the AS Events Shown in Figures 5B and 5C.

(A, B) Tested candidates belong to the GO terms “signaling” **(A)** and “posttranslational protein modification” **(B)**. Display details as described in previous legends.



Supplemental Figure 7. Gel Pictures and Quantification of Transcript Variants for AS Events Shown in Figure 6 under Osmotic Stress Conditions.

RT-PCR analysis and quantification as described in legend to Figure 6. Two biological replicates (Rep1 and Rep2) have been analyzed and ratios of SII/SI are normalized to the corresponding mock samples.



Supplemental Figure 8. Determination of Full-Length Sequences for Two of the Identified, Transcribed Intergenic Regions using RACE.

An intronic sequence is depicted in yellow, the longest putative ORF for each of the two intergenic regions is marked in red (predicted start and stop codons are in bold) and experimentally determined 5' and 3' ends are boxed in blue. Based on the RACE procedure, transcripts from both intergenic regions contain a 5' cap structure and a polyA tail. Furthermore, transcripts from both loci are derived from the minus strand.

Intergenic region 869

5′ – ACGGT AGGAAGACAA AGTGGACCAT AA**ATG**GCTAC AAAGATATCA CATTTAGTTT
CTCTTCTTTT GTCTTTACTT CTTCTACTTC TATTTATCTC CTCTCAAGTT GGATTCACAG
AAGCCAAACG CGACGAACGT TAGTATTTGC ACTCATTTTT TATTTATTGT CATGGTGCAA
ATGTTCAGAT GTATATTTTC AATTGATTCT TTGATTGTAT ATTTAATGGT TGCAAATTAT
TTAATGAAAC AATATCTTCA ATATTGTTAT CAGGTAAAAA GATGTCGTCT CCACCAATTC
CATCTCCACT AATTCCGTCT CCACCAATTC CTCCTCCTCC CCCTCGTTTT TATGTACCTC
CTTCAAAATC CCGCAGAGGA AAAGGCCCA**T** **AA**ACAAATTG AGTAAAAGGC TCATCAAGAA
AAATTATTAA TCATATATGG TTTCATACAT GTATTTGTAC TCACTTTAAT AATTCTATTT
GATTATATAA ATAAATGATT GGTTTTTGC – 3′

Intergenic region 881

5′ – ATGAC AAAACTGAAC TAAACCGGTT ACATATTGAA ATTCTAATAT CTGATCATAG
AACACAATTG AAGACGCCGT CCAATGTGAG CAGATTCTCG TCGGAACAGG ACATCCAATG
AACCTGGGCG ACGTGAGCTG A**ATG**ATGATG ATTTTCCAAG ATCCGCTGTC ATCCCACCAC
TATATAGCCG TTTTGAGAAG GAAGAAGATA TATGATATTG TCATTGTCGA GGAGATACTT
GTTCGATCG**T** **GA**GAGAGAGA AATACTAAGG AGTAAAAAAT AGACCTTCGA TGGTGGCTCA
GCTCGCCGAA CCGGAGTCTA CCTCCCCTAT CGACAAAGAT TCCATGAAAT CTTATGAATC
TTCGGTCCCT TGATCAAATT CTTGTCTCTG TTATTTTCAT CTTAGATCTC CGTCTTCTAC
TATGTATTAC TACACTGCCG GTAGAACTCT TATCGTCGGT GTGAGACTAA AGCTTCTTTT
CTCCACTAAT ACAACATATT ACAGTTGAAG AACTATGAGT CTTTAAATCC TCATCATCAT
CCAAATCGAC GGAAACCCCG ACCGAAGAAA CAAAGAGTTA TAATCAGAAA ATAAATAAAA
TTTGAGAGTA TGACCGTGAA TAAAATTTAC GCACTGCAAC TTTTTCTCGA CTTAATTATT
TTATTATCTA TTTATGAATT TTAGTTAAGA TCTTATTTGC ATTTGTGAAT TTTTTTTTTT
TTTTTATGAG – 3′

Supplemental Figure 9. DNA Oligonucleotides Used for Detection of Splicing Variants and Splicing Isoform Sequences as Revealed by Sequencing.

Oligonucleotides

Oligonucleotides for genotyping of *Arabidopsis* mutants

Gene	mutant	comment	Primer name	Primer sequence	Fragment sizes
UPF1	*lba1*	CAPS PCR (*Xba*I)	DNA1	GAGGCTGTTTACTAAGAACTTTTG	338 bp (WT), 299 bp (*lba1*)
			DNA2	AAACTCTCGAACCTACAGGTTGCCA GTGTTGATTCTTTTCTAG	
UPF3	*upf3-1*	WT allele	DNA3	CCAGGTACATTATGCTCACAG	353 bp
			DNA4	TTGAACTTTCTTTTTCCACATAC	
		Mutant allele	DNA3	CCAGGTACATTATGCTCACAG	603 bp
			DNA5	TGGTTCACGTAGTGGGCCATCG	

Oligonucleotides for co-amplification

Gene	Alt. event	Primer name	Primer sequence	Fragment sizes
At5g53180	14429	DNA6	ATGGGAATGCACTGAAGAGG	381 bp (SI), 439 bp (SII)
		DNA7	TGGTATCCGGCTGTCTTCTC	
At4g36960	7059	DNA8	CAGAGAAAGGGTCTTGCCAG	268 bp (SI), 440 bp (SII)
		DNA9	CAAGTCTCCAAATTTTGACATG	
At1g11650	10271	DNA10	GTGCTATGACGGAGATGAATGG	174 bp (SI), 261 bp (SII)
		DNA11	CATCTAATCCACCAACAAAAACAGT	
At5g17550	9364	DNA12	TGAGGAAGAGAGACTCGAGAATA	194 bp (SI), 321 bp (SII)
		DNA13	GTAACCCCATTCCAAGACCT	
At1g58080	16583	DNA14	CTGTCACCTGTCTATTGCGATT	253 bp (SI), 280 bp (SII)
		DNA15	ACCACTACTCACAAGATCCAAAAT	
At2g30260	30373	DNA16	CACCGAATCAATCAATCTACATC	208 bp (SI), 243 bp (SII)
		DNA17	TTGCATATTGTAAGCGCATTG	
At4g04350	34651	DNA18	AGGTTTGGCATATCAGGCT	237 bp (SI), 260 bp (SII)
		DNA19	CTTCCTATCCAGTTTCTTTGCATT	
At4g16990	12996	DNA20	AAGCGAAAATGGATGCGAG	162 bp (SI), 339 bp (SII)
		DNA21	TTCAATCCCGACGAAGTCAC	
At3g58760	32604	DNA22	GGCTCCAGAGGTTTATAGGAAT	237 bp (SI), 270 bp (SII)
		DNA23	CTTTTTGATGCTTCTTTGTCCC	
At1g79600	28255	DNA24	CTCGTCTCGGACCTACTTTT	284 bp (SI), 256 bp (SII)
		DNA25	AGGGCGTTGAACTTTGACA	
At3g66654	12107	DNA26	CAATCTCCACGATTCCTCTCTT	65 bp (SI), 317 bp (SII)
		DNA27	GAAAGCAAACGAGGGATACAAC	

At5g65720	9015	DNA28	CAGAACCCTAACCAAACCACAC	355 bp (SPI), 278 bp (SPII)
		DNA29	CATATTGTTCGCCTCCGTTG	
At1g28660	16281	DNA30	TGCCTCATCTTCTCGTGATTG	263 bp (SI), 168 bp (SII)
		DNA31	CTTCTGTCGCGTTCTGATATT	
At4g17370	6670	DNA32	AAGGCAGAGACTTTGGTAATG	251 bp (SI), 331 bp (SII)
		DNA33	ATCAATTATATCAGGCACCTTTCC	
At2g27720	11513	DNA34	CCATCAACACTTCCGCAAAA	177 bp (SI), 237 bp (SII)
		DNA35	CAGCCAAGTCTTTCCCTTT	
At3g17609	---	DNA36	TGTCTCTCCAACGACCCAAT	321 bp (SI), 279 bp (SII)
		DNA37	ATTCCAAATCACTCACATACACTTTC	
At3g23280	---	DNA38	TGTGGAAAGCAAACTTGGAAG	296 bp (SI), 224 bp (SII)
		DNA39	ATCTTCTGATGGTGGTGGTG	
At2g46700	12039	DNA40	GTCATACTTGTTCTGGCCGT	227 bp (SI), 393 bp (SII)
		DNA41	TCCACCATCACACAACTCCA	
At5g20840	14231	DNA42	AGTGTCAAAGAGTGATATGATTGC	184 bp (SI), 210 bp (SII)
		DNA43	ACTCTCATGGTCGCATATATTCT	
At3g55850	12806	DNA44	TTGCCACTCTCAAGGGTT	268 bp (SI), 195 bp (SII)
		DNA45	CATGATGCAGAGGGCAATT	
At3g19980	12227	DNA46	AATATTACACTTTTGCGCGGAA	177 bp (SI), 293 bp (SII)
		DNA47	ACCGTGAACACATAGAACTGT	
At2g42500	11984	DNA48	GAAACCATGAAAGTCGTCAGATT	227 bp (SI), 360 bp (SII)
		DNA49	ATGGGGCACTTCTTGAACT	
At4g35230	13461	DNA50	CTAAGAAATGGAAGAGTGACACC	206 bp (SI), 367 bp (SII)
		DNA51	CATTGAGAGGCGAGTTCGAC	

Oligonucleotides for RT-qPCR

Gene	Transcript variant	Primer name	Primer sequence	Fragment sizes
At5g53180 Alt_event 14429	SI	DNA52	TTGATGTCTTGCATCTGGTATTC	161 bp
		DNA53	ACCTAGGGATGCTTCTTCCAT	
	SII	DNA54	TTCAGTGGCAGCCTAATGC	156 bp
		DNA55	TCCATCGAGGGCAAGTTT	
	total	DNA56	GCAATCCACAGCAATACCAC	109 bp
		DNA57	CCACCCATTCCCATAGAGC	
At4g36960 Alt_event 7059	SI	DNA58	AGAGATTTCAAAAGGAGGAAGAGG	95 bp
		DNA59	GAAACCCTAACACACTCAAAAAG	
	SII	DNA60	GAAAGAGATGTGCTTGTGGTTTG	107 bp
		DNA59	GAAACCCTAACACACTCAAAAAG	
	total	DNA61	ATGAAATCTGTGGACAAGAGGTAG	113 bp
		DNA62	ACCAAAATATTCAGGACGAGAAG	

At4g19100 Alt_event 35151	SI	DNA63	CGTAGATCTTCTTCCAAGCTTGA	118 bp
		DNA64	CTAAGCGGTTAATGCAAGTAATG	
	SII	DNA65	CGTAGATCTTCTTCCAAGTTTGATT	101 bp
		DNA66	ATGTAACAAGAGATCGGAAACG	
	SIII	DNA67	AGATCTTCTTCCAAGCACCAT	104 bp
		DNA68	GTTTCCGGGTTTTGGCTTTT	
	total	DNA69	CTTCTTTGGTACGGCTTTAGC	103 bp
		DNA70	CTTCTTAGCTTCGTTCCAGC	
At2g27720 Alt_event 11513	SI	DNA71	GAAGGTTGTTGCTGCGTTTT	101 bp
		DNA72	CTGTCTCAGCACCAACTGA	
	SII	DNA73	CATTTCCAGCAAACATATACAATTCC	111 bp
		DNA72	CTGTCTCAGCACCAACTGA	
	total	DNA74	GGTTGCTTCTGCTACATCTGGA	119 bp
		DNA75	ACAAACTGAAACCCATGTCATC	
Intergenic region 286	total	DNA76	TCTTCGCATTCATTACCGGTG	102 bp
		DNA77	GAGGAGACATGAAAGAGCACTT	
Intergenic region 650	total	DNA78	TTCACCTCTCGATAATCACATATTTTTG	98 bp
		DNA79	ACCAGAAAAAAAGAAAGAGATTCCAAG	
Intergenic region 881	total	DNA80	ATGGAATCTTTGTCGATAGGGG	106 bp
		DNA81	TGTTCGATCGTGAGAGAGAGA	
Intergenic region 869	total	DNA82	AGGTACATAAAAACGAGGGGG	101 bp
		DNA83	CCAAACGCGACGAACGTAA	
Intergenic region 471	total	DNA84	CAGGGATATGTGTGCAGCT	95 bp
		DNA85	AAGGTATTTACCAAACGAGCG	
At2g33051	total	DNA86	AGATTTTATGACGCGACGAC	92 bp
		DNA87	TGCTTATGAAATCACCTGGCA	
At1g56612 Alt_event 2380	SI	DNA88	ATCCGTGGATAAGGAAGAAGAGA	98 bp
		DNA89	CCTTGCTGGATAGGAAGATTT	
	SII	DNA88	ATCCGTGGATAAGGAAGAAGAGA	112 bp
		DNA90	AGTTGGTCCTATCCTGTAATTCC	
	total	DNA91	GGGAATCAGCAGCTCCT	108 bp
		DNA92	GTTCTGTCTGTCTTTGTTCGC	
At1g13320	total	DNA93	GGTAATAACTGCATCTAAAGACAGAGTTCC	102 bp
		DNA94	CCACAACCGCTTGGTCG	

Oligonucleotides for RLM-RACE

Intergenic region	Transcript end	Primer description	Primer name	Primer sequence
Intergenic region 869	5 prime	GSP	DNA95	CGTTCGTCGCGTTTGGCTTCTGT
		GSP nested	DNA96	CCATTTATGGTCCACTTTGTCTTCCT
	3 prime	GSP	DNA97	CGTCTCCACCAATTCCATCTCCACTA
		GSP nested	DNA98	CCCGCAGAGGAAAAGGCCCATAAA
Intergenic region 881	5 prime	GSP	DNA99	GGGATGACAGCGGATCTTGGAAAA
		GSP nested	DNA100	GGGATGACAGCGGATCTTGGAAAA
	3 prime	GSP	DNA101	TCCGCTGTCATCCCACCACTATAT
		GSP nested	DNA102	CATCATCCAAATCGACGGAAACCC

Sequences

Primer binding sites are marked in bold letters; alternative regions are underlined.

At4g36960

Primer: DNA8/DNA9

Alternative splicing event: 7059

SI

CAGAGAAAGG GTCTTGCCAG TTTAGGGCAT CCTTGTGGGT GAGAGAGCGA TTGAGAGATT
TCAAAAGGAG GAAGAGGAAG ATACGAGTGT GAGAGAGATT TAGAGGGAGA GAAGAAAGAG
AGATTCTTTT TGAGTGTGTT AGGGTTTCAT TTCCTCTGCA GATCTACATT TATCTCTTCT
CATGGAACGG AAGCTTGTGG TTTTGGGAAT CCCGTGGGAT ATTGATTCCG ATGGGCTTAA
GGATTA**CATG TCAAAATTTG GAGACTTG**

SII

CAGAGAAAGG GTCTTGCCAG TTTAGGGCAT CCTTGTGGGT GAGAGAGCGA TTGAGAGATT
TCAAAAGGTC AGTGTGTGAG TTTTCGAATT TTCACAATGA ATCTAAAAAA AGAAGATAAA
TTTAGTTTCA TTTGTTTCTT AATCGGAGAG AAATTTGTAT GCCCCAAAAA CAAAAATGAT
GAATTGGTGT TTGATGATGA TAATTGTGTG TGAGAAAGAG ATGTGCTTGT GGTTTGCAGG
AGGAAGAGGA AGATACGAGT GTGAGAGAGA TTTAGAGGGA GAGAAGAAAG AGAGATTCCT
TTTGAGTGTG TTAGGGTTTC ATTTCCTCTG CAGATCTACA TTTATCTCTT CTCATGGAAC
GGAAGCTTGT GGTTTTGGGA ATCCCGTGGG ATATTGATTC CGATGGGCTT AAGGATTA**CA**
TGTCAAAATT TGGAGACTTG

At1g11650

Primer: DNA10/DNA11

Alternative splicing event: 10271

SI

GTGCTATGAC GGAGATGAAT GGCGTTCCTT GTTCTACTAG ACCTATGAGA ATTGGTCCCG
CTGCTAGCAA GAAAGGTGTA ACTGGTCAAA GAGATTCATA CCAGAGCTCT GCTGCAGGGG
TAACAACTGA TAATGATCCA AATAACACA**A CTGTTTTTGT TGGTGGATTA GATG**

SII

GTGCTATGAC GGAGATGAAT GGCGTTCCTT GTTCTACTAG ACCTATGAGA ATTGGTCCCG
CTGCTAGCAA GAAAGGTGTA ACTGGTCAAA GAGGGGAGTC AAGAGGCTCT CTATCATCTT
GAGGCCTGGA ACTGATATTC AGCTGAAATG GTTATCTCCT TATTGAGTGC CCATGCTTAG
ATTCATACCA GAGCTCTGCT GCAGGGGTAA CAACTGATAA TGATCCAAAT AACACA**ACTG**
TTTTTGTTGG TGGATTAGAT G

At5g17550

Primer: DNA12/DNA13

Alternative splicing event: 9364

SI

TGAGGAAGAG AGACTCGAGA ATATGGCCAA CGATACTCAC ACCGATGACT TAGACGAGCT
TCTCGATAGT GCCTTGGATG ATTTCAAGGA TCTTAATCTT ACTCAAAGAA ATGGAGGGGT
TAAGAAAGAA GAGGGTGATA AAAAAGAGAC AGAGTCATTG CCAAGTGGGG TTCA**AGGTCT**
TGGAATGGGG TTAC

SII

TGAGGAAGAG AGACTCGAGA ATATGGCCAA CGATACTCAC ACCGATGACT TAGACGAGCT
TCTCGATAGT AATAATTTCC TAACGAATTT TAATTTGATT TGATAACATG TTCACCAATT
CTAGGTGATT GGTGAAAAAC GTTTTTGTTC TTGTGGGTTC GTTTTCTATA ATTTAGAATT
CTGGGTCTTT CTCAGGTGCC TTGGATGATT TCAAGGATCT TAATCTTACT CAAAGAAATG
GAGGGGTTAA GAAAGAAGAG GGTGATAAAA AAGAGACAGA GTCATTGCCA AGTGGGGTTC
A**AGGTCTTGG AATGGGGTTA C**

At1g58080

Primer: DNA14/DNA15

Alternative splicing event: 16583

SI

CTGTCACCTG TCTATTGCGA TTCCAAACTA TGGGATATTT GAGAATATAA ATTCTCTGAA
GGAGCTAGCG CAAATGCCCC AATGGAGTGG AGAGAGACCC TTACGCTTAG CTACTGGCTT
CACTTATCTC GGCCCCAAAT TTATGAAAGA AAATGGCATA AAGCATGTGG TGTTTTCAAC
TGCAGACGGA GCACTGGAGG CAGCTCCAGC GATGGGGATA GCTGATGCC**A TTTTGGATCT**
TGTGAGTAGT GGT

SII

CTGTCACCTG TCTATTGCGA TTCCAAACTA TGGGATATTT GAGAATATAA ATTCTCTGAA
GGAGCTAGCG CAAATGCCCC AATGGAGTGA AGAGAGACCC TTACGCTTAG CTACTGGCTT
CACTTATGTA TGATGAGTTA CAGATTCTTC TTTGCTCGGC CCCAAATTTA TGAAAGAAAA
TGGCATAAAG CATGTGGTGT TTTCAACTGC AGACGGAGCA CTGGAGGCAG CTCCAGCGAT
GGGGATAGCT GATGCC**ATTT TGGATCTTGT GAGTAGTGGT**

At2g30260

Primer: DNA16/DNA17

Alternative splicing event: 30373

SI

CACCGAATCA ATCAATCTAC ATCCAAAACC TCAACGAAAG GATCAAGAAA GAGGAATTGA
AGAGATCTCT TTACTGTTTG TTCTCTCAGT TTGGGAGGAT ACTTGATGTG GTTGCTTTGA
AGACTCCGAA GCTCCGAGGA CAAGCTTGGG TTACTTTTAG TGAAGTCACT GCTGCTGGTC
ATGCTGTTCG TCAGATGCAA AATTTTCCCT TCTATGATAA ACC**AATGCGC TTACAATATG**
CAA

SII

CACCGAATCA ATCAATCTAC ATCCAAAACC TCAACGAAAG GATCAAGAAA GAGGTTTGGG
AGGATACTTG ATGTGGTTGC TTTGAAGACT CCGAAGCTCC GAGGACAAGC TTGGGTTACT
TTTAGTGAAG TCACTGCTGC TGGTCATGCT GTTCGTCAGA TGCAAAATTT TCCCTTCTAT
GATAAAC**CAA TGCGCTTACA ATATGCAA**

At4g04350

Primer: DNA18/DNA19

Alternative splicing event: 34651

SI

AGGTTTGGCA TATCAGGCTG AAGTACCGGT CAATTGGTGC CCGGCTCTTG GTACTGTTTT
GGCCAATGAA GAAGTGGTGG ATGGTGTTAG TGAGCGTGGT GGCCACCCGG TTATAAGAAA
GCCGATGAGG CAATGGATGC TGAAGATTAC TGCGTACGCT GATCGTCTTC TAGAAGATTT
GGACGAGCTT GAGTGGCCTG AAAGTATAAA GGA**AATGCAA AGAAACTGGA TAGGAAG**

SII

AGGTTTGGCA TATCAGGCTG AAGTACCGGT CAATTGGTGC CCGGCTCTTG GTACTGTTTT
GGCCAATGAA GAAGTGGTGG ATGGTGTTAG TGAGCGTGGT GGCCACCCGG TTATAAGAAA
GTGACATTGT TTATTACCAA GCAGCCGATG AGGCAATGGA TGCTGAAGAT TACTGCGTAC
GCTGATCGTC TTCTAGAAGA TTTGGACGAG CTTGAGTGGC CTGAAAGTAT AAAGGA**AATG**
CAAAGAAACT GGATAGGAAG

At4g16990

Primer: DNA20/DNA21

Alternative splicing event: 12996

SI

AAGCGAAAAT GGATGCGAGC TCTAGCAGAG GTAGCACATC TAGCCGGAGA AGATCTTCGG
AACTGGCGTA GCGAAGCAGA AATGCTTGAA AATATCGCCA AGGATGTTTC AAACAAACTC
TTCCCCCCAT CAAATAATTT CA**GTGACTTC GTCGGGATTG AA**

SII

AAGCGAAAAT GGATGCGAGC TCTAGCAGAG GTAGCACATC TAGCCGGAGA AGATCTTCGG
AACTGATAAG TTTCAGGTGG GTAAACTTCC GATAAGTGAT TGTCGGAACA GTGTGTATGA
GGATTAAACA CAGAGAAAGA TCGTGTGGTG ATTGTAGTGT CGGTAAACAA GTTTTTAAAG
CCTCCCAGCT GTAATGTATC AACCGTCGGA AAGGGGTGGG TCCCACGAGC CTGAGTAAAG
ACGCGTAGCG AAGCAGAAAT GCTTGAAAAT ATCGCCAAGG ATGTTTCAAA CAAACTCTTC
CCCCCATCAA ATAATTTCA**G TGACTTCGTC GGGATTGAA**

SIII

AAGCGAAAAT GGATGCGAGC TCTAGCAGAG GTAGCACATC TAGCCGGAGA AGATCTTCGG
AACTGGTGGG TAAACTTCCG ATAAGTGATT GTCGGAACAG TGTGTATGAG GATTAAACAC
AGAGAAAGAT CGTGTGGTGA TTGTAGTGTC GGTAAACAAG TTTTTAAAGC CTCCCAGCTG
TAATGTATCA ACCGTCGGAA AGGGGTGGGT CCCACGAGCC TGAGTAAAGA CGCGTAGCGA
AGCAGAAATG CTTGAAAATA TCGCCAAGGA TGTTTCAAAC AAACTCTTCC CCCCATCAAA
TAATTTCA**GT GACTTCGTCG GGATTGAA**

At3g58760

Primer: DNA22/DNA23

Alternative splicing event: 32604

SI

GGCTCCAGAG GTTTATAGGA ATGAAGAGTA TGATACAAAA GTAGATGTAT TCTCTTTCGC
TTTAATCTTA CAAGAGATGA TAGAAGGTTG TGAACCATTT CATGAGATAG AAGACCGCGA
AGTTCCTAAA GCATATATTG AAGATGAACG TCCACCATTC AATGCTCCAA CAAAATCATA
TCCTTTCGGG TTACAAGAGC TAATCCAGGA TTGTT**GGGAC AAAGAAGCAT CAAAAAG**

SII

GGCTCCAGAG GTTTATAGGA ATGAAGAGTA TGATACAAAA GTAGATGTAT TCTCTTTCGC
TTTAATCTTA CAAGAGAATG TTGTTTTGTG GGTTAACAAA ACCTAACAGA TGATAGAAGG
TTGTGAACCA TTTCATGAGA TAGAAGACCG CGAAGTTCCT AAAGCATATA TTGAAGATGA
ACGTCCACCA TTCAATGCTC CAACAAGATC ATATCCTTTC GGGTTACAAG AGCTAATCCA
GGATTGTT**GG GACAAAGAAG CATCAAAAAG**

At1g79600

Primer: DNA 24/DNA25

Alternative splicing event: 28255

SI

CTCGTCTCGG ACCTACTTTT GTTAAATTGG GTCAAGGTTT GTCCACCCGA CCCGACCTCT
GTCCACCCGA TTACCTTGAA GAACTTGCAG AGCTTCAGGA TGCTTTGCCA ACCTTCCCTG
ATGCAGAGGC ATTTGCTTGC ATTGAGAGAG AGTTGGATTT GTCGCTAGAG ACCATTTTCT
CGTCTGTATC TCCTGAGCCA ATCGCAGCAG CTAGTCTTGG CCAGGTTTAC AAAGCTCAGC
TGAGGTATTC AGGTCAGGTT GTTG**CTGTCA AAGTTCAACG CCCT**

SIII

CTCGTCTCGG ACCTACTTTT GTTAAATTGG GTCAAGGTTT GTCCACCCGA CCCGACCTCT
GTCCACCCGA TTACCTTGAA GAACTTGCAG AGCTTCAGAG GCATTTGCTT GCATTGAGAG
AGAGTTGGAT TTGTCGCTAG AGACCATTTT CTCGTCTGTA TCTCCTGAGC CAATCGCAGC
AGCTAGTCTT GGCCAGGTTT ACAAAGCTCA GCTGAGGTAT TCAGGTCAGG TTGTTG**CTGT**
CAAAGTTCAA CGCCCT

SIV

CTCGTCTCGG ACCTACTTTT GTTAAATTGG GTCAAGGTTT GTCCACCCGA CCCGACCTCT
GTCCACCCGA TTACCTTGAA GAACTTGCAG AGCTTCAGTG TCTAAGTAGG ATGCTTTGCC
AACCTTCCCT GATGCAGAGG CATTTGCTTG CATTGAGAGA GAGTTGGATT TGTCGCTAGA
GACCATTTTC TCGTCTGTAT CTCCTGAGCC AATCGCAGCA GCTAGTCTTG GCCAGGTTTA
CAAAGCTCAG CTGAGGTATT CAGGTCAGGT TGTTG**CTGTC AAAGTTCAAC GCCCT**

At3g66654

Primer: DNA26/DNA27

Alternative splicing event: 12107

SI

CAATCTCCAC GATTCCTCTC TTCTCTCTAA CTTCCAGAGA CAAG**TTGTAT CCCTCGTTTG**
CTTTC

SII

CAATCTCCAC GATTCCTCTC TTCTCTCTAA CTTCCAGGTC TTTGGATTTC TATAGAAAGA
AAGATCTGTT TATTATTTGA TTGGAACATG ATAAGCTGCT CCGTTTCTCA TATCCACTTT
TGTTCAATTT ATTTCAATTT GATCGATAAT TGGAAAAGTA CTGGATCTGG TGTATCGGAA
GTTACCGGTC AAGCTATGTA TGAACCCTTG TGATATTTTC ACATGCGTTT ATTCATTCAA
ATATGCTAGA TTTTTAATCT GGTCAATACG TGATATCAGG GTTTCTTCAG TTGGAGACAA
G**TTGTATCCC TCGTTTGCTT TC**

SIII

CAATCTCCAC GATTCCTCTC TTCTCTCTAA CTTCCAGTAC TGGATCTGGT GTATCGGAAG
TTACCGGTCA AGCTATGTAT GAACCCTTGT GATATTTTCA CATGCGTTTA TTCATTCAAA
TATGCTAGAT TTTTAATCTG GTCAATACGT GATATCAGGG TTTCTTCAGT TGGAGACAAG
TTGTATCCCT CGTTTGCTTT C

SIV

CAATCTCCAC GATTCCTCTC TTCTCTCTAA CTTCCAGGTA CGTTTTTCGA GAACCCAATG
AAGCAATCAA AGTCCTTGGA ATTCATTTGA TTTTACTTTC AGGTCTTTGG ATTTCTATAG
AAAGAAAGAT CTGTTTATTA TTTGATTGGA ACATGATAAG CTGCTCCGTT TCTCATATCC
ACTTTTGTTC AATTTATTTC AATTTGATCG ATAATTGGAA AAGTACTGGA TCTGGTGTAT
CGGAAGTTAC CGGTCAAGCT ATGTATGAAC CCTTGTGATA TTTTCACATG CGTTTATTCA
TTCAAATATG CTAGATTTTT AATCTGGTCA ATACGTGATA TCAGGGTTTC TTCAGTTGGA
GACAAG**TTGT ATCCCTCGTT TGCTTTC**

At5g65720

Primer: DNA28/DNA29

Alternative splicing event: 9015

SI

CAGAACCCTA ACCAAACCAC ACGGCACTTT TTCCCGGTGT CGCTACTTAT CAACCGCCGC
TGCTGCGACG GAGGTGAATT ACGAGGATGA ATCGATTATG ATGAAAGGAG TTCGAATTTC
AGGTAGACCT CTTTACTTAG ATATGCAAGC GACGACTCCG ATTGATCCTA GAGTATTCGA
TGCGATGAAT GCTTCACAGA TCCATGAGTA TGGGAATCCT CACTCGCGAA CGCATCTCTA
CGGCTGGGAA GCTGAGAACG CCGTCGAGAA CGCACGAAAC CAGGTCGCGA AACTGATCGA
AGCTTCACCG AAGGAGATCG TATTCGTGTC CGGTG**CAACG GAGGCGAACA ATATG**

SII

CAGAACCCTA ACCAAACCAC ACGGCACTTT TTCCCGGTGT CGCTACTTAT CAACCGCCGC
TGCTGCGACG GAGGTGAATT ACGAGGATGA ATCGATTATG ATGAAAGGAG TTCGAATTTC
AGATCCATGA GTATGGGAAT CCTCACTCAC GAACGCATCT CTACGGTTGG GAAGCTGAGA
ACGCCGTCGA GAACGCACGA AACCAGGTCG CGAAACTGAT CGAAGCTTCA CCGAAGGAGA
TCGTATTCGT GTCCGGTGC**A ACGGAGCCGA ACAATATG**

At1g28660

Primer: DNA30/DNA31

Alternative splicing event: 16281

SI

TGCCTCATCT TCTCGTGATT GCAGAGAGAT GCTTGGAGAC TCGCTGATAC TCATGGGAGA
GATTGGAGGT AACGACTTTT TTTACCCATC CTCCGAAGGC AAAAGTATCA ATGAAACCAA
ACTACAGGAT TTGATCATCA AAGCTATTTC TTCTGCAATT GTGGATTTGA TCGCTTTAGG
GGGCAAAACA TTTTTGGTAC CCGGAGGCTT CCCAGCAGGA TGTTCCGCAG CGTGTCTTAC
TC**AATATCAG AACGCGACAG AAG**

SII

TGCCTCATCT TCTCGTGATT GCAGAGAGAT GCTTGGAGAC TCGCTGATAC TCATGGGAGA
GATTGGAGGA TTTGATCGCT TTAGGGGGCA AAACATTTTT GGTACCCGGA GGCTTCCCAG
CAGGATGTTC CGCAGCGTGT CTTACTC**AAT ATCAGAACGC GACAGAAG**

At4g17370

Primer: DNA32/DNA33

Alternative splicing event: 6670

SI

AAGGCAGAGA CTTTGGTAAT GTCAAGATGG TAGCAATCCG AGAACATAGA TTCCCTTTCT
TGGTTAAGGT GAACAACTGG AATAGATTCA ATGTGAACAC TGGAGGGACC TTAGTGGAGA
AATGCTGCCA CTTCTTTGAT CTAATGAGGC TCTTTGCCGG TGCAAATCCT GTCTGTGTGA
TGGCTTCTGG AGGCATGGAT GTGAACCACA AGGATGAAGT TTATGGTGG**A AAGGTGCCTG**
ATATAATTGA T

SII

AAGGCAGAGA CTTTGGTAAT GTCAAGATGG TAGCAATCCG AGAACATAGA TTCCCTTTCT
TGGTTAAGGT AGGCTTAAAC AAGATGGGAC TATGTGTAGT GTTTACGCTT CACATTTTTA
TATGATTGCT GAGAGAACAC ATCACTAGGT GAACAACTGG AATAGATTCA ATGTGAACAC
TGGAGGGACC TTAGTGGAGA AATGCTGCCA CTTCTTTGAT CTAATGAGGC TCTTTGCCGG
TGCAAATCCT GTCTGTGTGA TGGCTTCTGG AGGCATGGAT GTGAACCACA AGGATGAAGT
TTATGGT**GGA AAGGTGCCTG ATATAATTGA T**

At2g46700

Primer: DNA40/DNA41

Alternative splicing event: 12039

SI

GTCATACTTG TTCTGGCCGT GGTAAGAAGG GAGATATTAA GGATCATCCT ATTGCTGTCA
AGATCATCTC CAAGGCTAAG ATGACAACAG CAATTGCAAT TGAAGATGTT CGCAGGGAGG
TGAAGTTACT GAAATCATTA TCTGGACACA AATATTTAAT CAAATATTAT GATGCATGTG
AGGACGCCAA TAACGTATAC ATAGTCA**TGG AGTTGTGTGA TGGTGGA**

At5g20840

Primer: DNA42/DNA43

Alternative splicing event: 14231

SI

AGTGTCAAAG AGTGATATGA TTGCATTGCA ACATTCTAGC GTGCTTTGCA ACACTGCAAA
TTTAAGAGAC GAGAACAGGT ACAAGAGACT CCTGTGTATG GTGGACCTTA CGAAAGACTT
CTTTTTCAGC TATTCTTACA ATATAATGCG AAGTTTCCAG A**AGAATATAT GCGACCATGA**
GAGT

SII

AGTGTCAAAG AGTGATATGA TTGCATTGCA ACATTCTAGC GTGCTTTGCA ACACTGCAAA
TTTAAGAGAC GAGAACAGCG AGAGTGCACG GCTTTAGCAA CATTGTACAA GAGACTCCTG
TGTATGGTGG ACCTTACGAA AGACTTCTTT TTCAGCTATT CTTACAATAT AATGCGAAGT
TTCCAGA**AGA ATATATGCGA CCATGAGAGT**

SIII

AGTGTCAAAG AGTGATATGA TTGCATTGCA ACATTCTAGC GTGCTTTGCA ACACTGCAAA
TTTAAGAGAC GAGAACAGGT ATTTTGATAT ATCTTTGGCT ACATTATGCT TTTTTAATTT
ATATATATTT ATTGCAATCC CATTGCTGAA GAAATGTTCT GTTTTTCGTT TCTGTTTGGT
CGTTTCTGTT TGGTGAAAAG CGAGAGTGCA CGGCTTTAGC AACATTGTAC CATTCCATCC
ATTATATGTA CTGAAGTCAT TAAACTCTTG CGTTTCTCTA TCTGATGTTT CCCACTTGTT
GATAAATAGG TACAAGAGAC TCCTGTGTAT GGTGGACCTT ACGAAAGACT TCTTTTTCAG
CTATTCTTAC AATATAATGC GAAGTTTCCA GA**AGAATATA TGCGACCATG AGAGT**

SIV

AGTGTCAAAG AGTGATATGA TTGCATTGCA ACATTCTAGC GTGCTTTGCA ACACTGCAAA
TTTAAGAGAC GAGAACAGGT ATTTTGATAT ATCTTTGGCT ACATTATGCT TTTTTAATTT
ATATATATTT ATTGCAATCC CATTGCTGAA GAAATGTTCT GTTTTTCGTT TCTGTTTGGT
CGTTTCTGTT TGGTGAAAAG CGAGAGTGCA CGGCTTTAGC AACATCGTAC AAGAGACTCC
TGTGTATGGT GGACCTTACG AAAGACTTCT TTTTCAGCTA TTCTTACAAT ATAATGCGAA
GTTTCCAGA**A GAATATATGC GACCATGAGA GT**

SV

AGTGTCAAAG AGTGATATGA TTGCATTGCA ACATTCTAGC GTGCTTTGCA ACACTGCAAA
TTTAAGGGAC GAGAACAGCG AGAGTGCACG GCTTTAGCAA CATTGTACCA TTCCATCCAT
TATATGTACT GAAGTCATTA AACTCTTGCG TTTCTCTATC TGATGTTTCC CACTTGTTGA
TAAATAGGTA CAAGAGACTC CTGTGTATGG TGGACCTTAC GAAAGACTTC TTTTTCAGCT
ATTCTTACAA TATAATGCGA AGTTTCCAGA **AGAATATATG CGACCATGAG AGT**

At3g55850

Primer: DNA44/DNA45

Alternative splicing event: 12806

SI

TTGCCACTCT CAAGGGTTTT ATAGGAGATG GAACTATGGA GGTGAATCTG GAGGGGAAGA
TTGTAGTGCC TGGACTTATT GATTCACATG TTCATCTGAT TTCTGGGGGA TTACAGATGG
CCCAAGTTGG ACTTCGTGGA GTGAGTCAAA AAGATGAGTT TTGTAAAATG GTGAAGGACG
CTGTGCAGAA TGCAAAAGAA GGCTCATGGA TTTTGGGTGG TGGCTGGAAC AATGATTTCT
GGGGAGGAG**A ATTGCCCTCT GCATCATG**

SII

TTGCCACTCT CAAGGGTTTT ATAGGAGATG GAACTATGGA GGTGAATCTG GAGGGGAAGA
TTGTAGTGCC TGGACTTATT GATTCACATG TTCATCTGAT TTCTGGGGGA TTACAGATGC
AAAAGAAGGC TCATGGATTT TGGGTGGTGG CTGGAACAAT GATTTCTGGG GAGGAG**AATT
GCCCTCTGCA TCATG**

SIII

TTGCCACTCT CAAGGGTTTT ATAGGAGATG GAACTATGGA GGTGAATCTG GAGGGGAAGA
TTGTAGTGCC TGGACTTATT GATTCACATG TTCATCTGAT TTCTGGGGGA TTACAGGTGA
TAATGGCATA GTGCAAACTT ATTTCTGTGT TCTTTCTTCT TGCAATGATA TGTAATTATG
TATATTGTTA AGTTAACCTG AGATTAGATG ATTACATGCC ATTGTTTGAT CAGGTTTAGA
TATGAAGAAG TATACTATAC TCTCGTTTAC CTTATATCTT TTCCTTTTGC TTTTAATGTC
TTATCGCTTG AGCATAGATG GCCCAAGTTG GACTTCGTGG AGTGAGTCAA AAAGATGAGT
TTTGTAAAAT GGTGAAGGAC GCTGTGCAGA ATGCAAAAGA AGGCTCATGG ATTTTGGGTG
GTGGCTGGAA CAATGATTTC TGGGGAGGAG **AATTGCCCTC TGCATCATG**

At3g19980

Primer: DNA46/DNA47

Alternative splicing event: 12227

SI

AATATTACAC TTTTGCGCGG AAATCATGAA AGTAGGCAGC TAACGCAGGT GTATGGTTTC
TATGACGAAT GCCAGAGGAA GTATGGTAAC GCTAATGCGT GGCGATATTG CACAGATGTT
TTTGACTATC TTACCCTGTC AGCTATTATA GATGGC**ACAG TTCTATGTGT TCACGGT**

SII

AATATTACAC TTTTGCGCGG AAATCATGAA AGTAGGCAGC TAACGCAGGG TTTTGAAAGT
TCTGATTTTG TACTACATGT GGCAATGTCA TGTCAGATGT AGACATCGAT GTTAGCTAAG
ATGCATTTTA TGTGAATCAA GTATTGGAAA GCTAATTTCA GAAGGTGTAT GGTTTCTATG
ACGAATGCCA GAGGAAGTAT GGTAACGCTA ATGCGTGGCG ATATTGCACA GATGTTTTTG
ACTATCTTAC CCTGTCAGCT ATTATAGATG GC**ACAGTTCT ATGTGTTCAC GGT**

At2g42500

Primer: DNA48/DNA49

Alternative splicing event: 11984

SII

GAAACCATGA AAGTCGTCAG ATTACTCAGG TTTATGGATT TTATGATGAA TGTCTACGAA
AGTACGGCAA CGCAAATGTT TGGAAAATCT TTACAGACCT CTTCGACTAT TTTCCTCTGA
CAGCCTTGTA TTATTTGGAA CCAAAGTAGT ACTTTTGTAC TGTGCTGTCG TATTTCTTTT
GCTAGTGTCA CATCAACTGC AAGACGTTAG TATTCTGAAA AATGCAAAGA ATGGAGATCT
GTTATATATG GAAGATTTCA TGTTGAGTCA GAAATATTTT GCCTTCATGG TGGATTATCT
CCATCTATCG AGACCCTTGA CAACATAAGG AATTTTGATC G**AGTTCAAGA AGTGCCCCAT**

At4g35230

Primer: DNA50/DNA51

Alternative splicing event: 13461

SI

CTAAGAAATG GAAGAGTGAC ACCTGAAAGT GTTACGTATA GCTTTGGAAC TGTCCTTCTG
GATTTGCTTA GCGGAAAACA CATCCCTCCA AGCCATGCTC TCGATATGAT ACGAGGCAAG
AATATTATTC TGTTGATGGA TTCACACCTC GAAGGAAAGT TCTCAACAGA AGAGGCTACT
GTAGTG**GTCG AACTCGCCTC TCAATG**

SII

CTAAGAAATG GAAGAGTGAC ACCTGAAAGT GTTACGTATA GCTTTGGAAC TGTCCTTCTG
GATTTGCTTA GCGGAAAACA CATCCCTCCA AGCCATTTAT GTGAAGCTCT CGTAGTAGGT
CATTGACCAT GGTTTCTAGA AACTGTATAA TACTGCTCTG TCATCATCTC TCTTGAAATT
AGCTAAGATA TGTGCCACGC TCTTGTTCTT TAACCACTGT CTATCAGATT TAGCTCTCAA
TATGTATTAT GGGCAAGGCT CTCGATATGA TACGAGGCAA GAATATTATT CTGTTGATGG
ATTCACACCT CGAAGGAAAG TTCTCAACAG AAGAGGCTAC TGTAGTG**GTC GAACTCGCCT**
CTCAATG

Supplemental Table 1. Numbers of Alternative Splicing Events and Corresponding Gene Fractions as Depicted in Figure 3.

	lba1upf3-1		CHX		*lba1upf3-1* + CHX		*lba1upf3-1*+ SM-CHX		all NMD events		all AS events		*lba1upf3-1* or CHX	
	events	%	events	%	events	%	events	%	events	%	events	%	events	%
	3,361	100	3,238	100	641	100	1,231	100	1,872	100	41,491	100	5,878	100
# events with significant changes in opposite directions (excluded in Venn diagrams in Fig. 3 and S3)	99		159		6		37							
exon_skip	**533**	**15.9**	**758**	**23.4**	**221**	**34.5**	**154**	**12.5**	**375**	**20.0**	**4,400**	**10.6**	**1,056**	**18.0**
up	366	68.7	726	95.8	211	95.5	75	48.7	286	76.3				
down	167	31.3	32	4.2	10	4.5	79	51.3	89	23.7				
intron_ret	**767**	**22.8**	**1,574**	**48.6**	**184**	**28.7**	**196**	**15.9**	**380**	**20.3**	**10,139**	**24.4**	**2,124**	**36.1**
up	267	34.8	175	11.1	18	9.8	85	43.4	103	27.1				
down	500	65.2	1,399	88.9	166	90.2	111	56.6	277	72.9				
alt 5' ss	**685**	**20.4**	**356**	**11.0**	**84**	**13.1**	**279**	**22.7**	**363**	**19.4**	**8,946**	**21.6**	**940**	**16.0**
up	432	63.1	218	61.2	63	75.0	176	63.1	239	65.8				
down	253	36.9	138	38.8	21	25.0	103	36.9	124	34.2				
alt 3' ss	**1,376**	**40.9**	**550**	**17.0**	**152**	**23.7**	**602**	**48.9**	**754**	**40.3**	**18,006**	**43.4**	**1,758**	**29.9**
up	674	49.0	254	46.2	81	53.3	281	46.7	362	48.0				
down	702	51.0	296	53.8	71	46.7	321	53.3	392	52.0				
genes	1,905		1,660		390		802		1,126		11,711		3,004	
protein coding multi-exon genes	1,851		1,598		371		780		1,091		11,393		2,915	
fraction protein coding multi-exon genes	8.5		7.3		1.7		3.6		5.0		52.2		13.3	

Supplemental Table 2. Types of AS Events Detected in Different Samples.

Total numbers and fractions of AS events detected in various samples **(A)**, mutually exclusively detected in indicated comparisons **(B)**, and uniquely found in indicated control and NMD-impaired samples **(C)**.

(A)

Event type	WT		*lba1*		*upf3-1*		*lba1upf3-1*		Mock		CHX	
intron_retention	6022	29,5%	7097	26,8%	6949	27,1%	7715	24,5%	6917	27,9%	6918	26,1%
exon_skip	1433	7,0%	2077	7,8%	2081	8,1%	2800	8,9%	2236	9,0%	2638	9,9%
alt_3prime	8945	43,9%	11883	44,8%	11446	44,7%	14328	45,6%	10476	42,2%	11222	42,3%
alt_5prime	3980	19,5%	5441	20,5%	5158	20,1%	6597	21,0%	5168	20,8%	5739	21,6%
Total	20380	100,0%	26498	100,0%	25634	100,0%	31440	100,0%	24797	100,0%	26517	100,0%

(B) Mutually exclusive

Event type	*lba1*_not_WT		*up3-1*_not_WT		single_mutants _not_WT		*lba1upf3-1*_not_WT		Mock_not_CHX	
intron_retention	1650	19,4%	1545	19,6%	2198	19,6%	2470	18,2%	1981	23,4%
exon_skip	883	10,4%	844	10,7%	1209	10,8%	1566	11,6%	1126	13,3%
alt_3prime	4022	47,4%	3744	47,5%	5264	46,8%	6376	47,0%	3476	41,1%
alt_5prime	1936	22,8%	1741	22,1%	2567	22,8%	3144	23,2%	1872	22,1%
Total	8491	100,0%	7874	100,0%	11238	100,0%	13556	100,0%	8455	100,0%

(C) Unique to experiment

Event type	WT		*lba1*		*upf3-1*		*lba1upf3-1*		Mock		CHX	
intron_retention	41	23,6%	76	16,7%	69	16,8%	262	11,3%	104	16,7%	633	23,8%
exon_skip	13	7,5%	50	11,0%	52	12,7%	291	12,5%	110	17,7%	593	22,3%
alt_3prime	69	39,7%	214	47,0%	194	47,3%	1149	49,4%	238	38,3%	814	30,6%
alt_5prime	51	29,3%	115	25,3%	95	23,2%	623	26,8%	169	27,2%	616	23,2%
Total	174	100,0%	455	100,0%	410	100,0%	2325	100,0%	621	100,0%	2656	100,0%

Supplemental Table 3. Comparison of Previously Published Analysis of Coupled Alternative Splicing-NMD from Kalyna et al. (2012) with RNA-Seq Data from this Work.

Table provides information on gene identifier, primer pairs to analyze corresponding events in Kalyna et al (2012), corresponding AS event number from this work as well as minimum (Min) p-value for testing *lba1 upf3-1* versus WT and CHX versus mock treatment. "n.d." indicates that AS event was not detected in our analysis. The list includes AS events that were significantly changed in *upf1-5*, *upf3-1*, and upon CHX treatment **(A)**, in the two mutants, but not upon CHX treatment **(B)**, and AS events that were not changing upon NMD impairment **(C)** in the work from Kalyna et al. (2012).

				Min p-value		
	Gene	Primer pair in Kalyna et al.	Corresponding event RNA-seq	*lba1 upf3-1*	CHX	Comment
(A)	*At2g37340*	21	30628	0.00	0.00	
	At3g53270	30	12779-12782	0.12 - 0.38	0.35 – 1.00	
	At5g13730	44	40030	0.13	0.52	
	At5g43910	50	40714	0.19	1.00	
	At2g04790	72	17116	0.25	1.00	
	At1g72050	110	10632	0.00	0.04	
	At2g18300	121	n.d.			
	At2g46790	125	17687	0.03	0.11	
	At3g12250	143	18573	0.50	0.24	
	At1g07350	193	10781	0.00	0.06	
	At3g49430	194	12344	0.00	0.00	Cassette exon 4 nt shorter than described in Kalyna et al.
	At2g21660	206	17874	0.00	0.07	
	At4g25500	219	13065	0.00	0.00	
	At3g55460	220	12395	0.00	0.00	
	At1g15200	224	10892	0.11	0.00	Cassette exon detected in our analysis is shorter
	At1g07830	237	14708	0.03	0.90	
	At1g02090	241	25993	0.02	1.00	
	At5g09230	244	21563	0.00	0.04	
	At5g46110	245	22040	0.00	0.00	
	At5g63120	282	23388	0.14	0.15	
	At3g19840	285	18710	0.11	0.58	
	At5g09880	298	14139	0.07	0.00	
	At5g09790	301	alt 5' ss: 22650	0.13	1.00	
			alt 3' ss: 39881	0.21	1.00	
	At5g65060	309	39440	0.27	0.43	
	At5g65070	310	n.d.			
	At5g59950	327	8944	0.05	0.00	
	At3g29160	344	33679	0.00	0.75	
	At1g49730	345	27243	0.38	1.00	
	At5g35410	370	38353	0.13	0.20	
	At3g20270	375	---			Splicing variant assignment based on description in Kalyna et al. unclear

	At3g62190	378	n.d.			
	At4g02200	384	20195	0.00	0.93	
	At2g26150	393	11480	0.00	0.17	
(B)	*At1g49950*	106	n.d.			
	At1g77080	109	25739	0.00	0.32	
	At3g51880	141	19904	0.01	0.26	
	At1g79880	200	28283	1.00	0.00	
	At4g35785	229	13464	0.06	0.33	
	At1g37150	251	n.d.			
	At5g43270	324	23091	0.57	0.20	
	At1g76460	372	28021	0.00	1.00	
	At4g36960	374	7092	0.88	0.06	
(C)	*At2g43410*	314	4357	1.00	1.00	Transcript variant not expected to be NMD-sensitive; in frame intron retention
	At3g06510	360	4588	0.54	1.00	Transcript variant not expected to be NMD-sensitive; in frame intron retention
	At1g01060	305	1449	1.00	0.00	Transcript variant not expected to be NMD-sensitive; uORFs in all variants
	At2g47890	325	3723	1.00	1.00	Transcript variant not expected to be NMD-sensitive; C-terminal change
	At1g27770	353	1994	0.00	0.01	Cryptic intron
	At4g13850	361	n.d.			Cryptic intron
	At3g16800	332	31646	0.41	0.70	Transcript variant not expected to be NMD-sensitive; alt 3'ss, in frame introduction of 18 nt

Supplemental Table 4. Impact of AS on the Presence of Protein Domains for Candidate Genes from GO Terms “Signaling” and “Posttranslational Protein Modification”.

Table provides for the selected candidate genes ID, name, and TAIR description. Furthermore, domains detected by PROSITE (Sigrist et al., 2012) in SPI-encoded proteins and the position of the PTC in the corresponding SPII-derived, hypothetical protein variants are given.

Gene ID	Name	TAIR description	Domain position	Domain name	Position of PTC in protein variant resulting from SPII
At2g46700	CRK3	CDPK-related kinase; involved in protein phosphorylation, N-terminal protein myristoylation	aa 143-405:	Protein kinase domain	Stop codon at position aa 199
			aa 446-481:	EF-hand	
			aa 523-558:	EF-hand	
At5g20840		Phosphoinositide phosphatase	aa 162-551:	SAC phosphatase domain	Stop codon at position aa 163
At3g55850	LAF3	Long after far red; involved in regulation of mitotic cell cycle; hydrolase activity	---	---	Stop codon at position aa 161
At3g19980	ATFYPP3	Flower-specific, phytochrome-associated protein phosphatase 3; negative regulation of flower development, embryo development ending in seed dormancy; subunit of Ser/Thr protein phosphatase 2A	aa 107-112:	Ser/Thr phosphatase	Stop codon at position aa 136
At2g42500	PP2A-3	Protein phosphatase 2A-3; encodes one of the catalytic subunits of protein phosphatase 2A; Ser/Thr phosphatase activity	aa 118-123:	Ser/Thr phosphatase	Stop codon at position aa 169
At4g35230	BSK1	Brassinosteroid-signaling kinase 1; involved in protein phosphorylation, N-terminal protein myristoylation, brassinosteroid mediated signaling pathway, response to abscisic acid stimulus	aa 76-331:	Protein kinase domain	Stop codon at position 282

Supplemental Experimental Methods

For adapter ligation, indices 10 and 13 were used. Final PCR enrichment was performed with half the sample volume running 14 PCR cycles. Resulting libraries were either directly subjected to Illumina sequencing (Illumina, San Diego, USA) or gel-purified. In the latter case, samples were separated on a 2% agarose TAE gel and fragments in the size range of 250 to 350 bp were extracted from the gel using MinElute columns (Qiagen).

Cluster generation was performed according to the manufacturer's instructions using a Cluster Station (Illumina) or cBot (Illumina) with reagents from TruSeq Single Read Cluster Generation Kits v5 (Illumina) or cBot Truseq Single Read Cluster Generation Kit v5 (Illumina), respectively. For sequencing, one lane per sample was used. About 800,000 raw clusters per mm2 on the flow-cell surface were generated with 8 to 10pM solutions of denatured DNA. Sequencing was carried out according to the manufacturer's recommended protocols on a Genome Analyzer GAIIx (Illumina) using the TruSeq SBS Sequencing Kit v5 (36 cycle) (Illumina). Sequencing control software was SCSversion2.8 and RTA1.8.7. Sequencing runs were performed at 100 bp cycles.

Supplemental Computational Methods

Pre-processing of the Illumina raw data and alignment

The sequencing reads were converted to fastq file format using the SHORE pipeline version 4.1 (Ossowski et al., 2008) with standard settings under the application profile mRNA and disabled Illumina filter. The reads were subsequently aligned to the TAIR10 reference genome sequence of *A. thaliana* using the PALMapper spliced alignment tool in its most recent version 0.5 (Jean et al., 2010; http://raetschlab.org/suppl/palmapper). The spliced alignment was performed against the reference genome with the ability to use annotated as well as to discover new splice junctions. Junction information from the TAIR10 annotation was used for alignment. A detailed list of command line parameters is available below, the genome index as well as splice site predictions are available at:
http://www.raetschlab.org/suppl/nmd.

To increase sensitivity, unmappable reads were allowed to be trimmed down to a length of at least 40 nts.

PALMapper command line parameters:

```
-M 6 -G 1 -E 6 -l 15 -L 25 -K 8 -C 35 -I 25000 -NI 2
-SA 100 -CT 50 -a -S
-seed-hit-cancel-threshold 10000
-report-map-read
-report-spliced-read
-report-map-region
-report-splice-sites 0.9
-filter-max-mismatches 0
```

```
-filter-max-gaps 0
-filter-splice-region 5
-qpalma-use-map-max-len 1000
-f bamn -threads 2 -polytrim 40
-qpalma-prb-offset-fix
-min-spliced-segment-len 15
-junction-remapping-coverage 5
-junction-remapping-min-spliced-segment-len 15
-junction-remapping <JUNCTION_GFF>
-score-annotated-splice-sites <JUNCTION_GFF>
-qpalma-indel-penalty 1
```

Alignment post-processing

The alignments were sorted and indexed with SAMtools (Li et al., 2009). For further disambiguation, multiply-mapped reads were resolved using the MMR tool from the RNAgeeq toolbox that globally smoothes the alignment coverage based on a local variance minimization. The implementation is available from http://www.bioweb.me/MMR.

Augmentation of the annotation and construction of a splicing graph

Preliminary to the annotation of alternative events, the TAIR10 annotation was transformed into a splicing graph representation, with each vertex of the graph representing an exon and each edge representing an intron connecting two exons. Based on the read evidence from the combination of all alignments, this splicing graph was augmented using the software SplAdder. For example, if the RNA-Seq alignments show a high number of reads that map to two exons that are not adjacent within any annotated transcript isoform (and thus have no connecting edge in the splicing graph), a new edge would be added to the splicing graph to represent a newly identified intron. Similar criteria are applied to add intron retentions or alternative exon ends to the splicing graph. SplAdder was used with the following confidence criteria, putting restrictions on the RNA-Seq alignments used for augmentation:

Maximum intron length	20,000
Minimum segment length for spliced alignments	25
Maximum number of edit operations per alignment	0
Minimum splice junction support	2
Minimum intron retention coverage	10
Minimum relative covered position in intron retention	0.9

Minimum relative coverage in in intron retention regions	0.2
Maximum relative coverage in in intron retention regions	1.2

The remaining parameters were left in their respective default configuration. The software SplAdder is available by the authors upon request. Future versions will be integrated into the RNA-geeq toolbox (http://www.bioweb.me/rnageeq).

Extraction and filtering of alternative splicing events

With the splicing graph defined and augmented with SplAdder, it is straightforward to define and extract alternative splicing events. We distinguish four different AS events: exon skip (ES), intron retention (IR), alternative five prime site (A5), and alternative three prime site (A3). All events are defined on the splicing graph as follows: ES are all cycles in the graph that have length three. IR are pairs of nodes where one node start coincides with a third node's start and the other node's stop coincides with the same third node's stop. A3 (A5) are all node pairs, that share a common start (stop) and are both connected to a common third node. To clean the list of potential AS events from duplicates, all AS events were made unique on their inner event coordinates, keeping the event with the longest flanking region, e.g. if two ES events existed that contain the same cassette exon and the same introns but differ in length of the flanking exons, the longer ES event was kept for processing. An analogous procedure was applied to the remaining AS event types.

Read counting and test for differential transcript expression

For read counting and differential testing, we used scripts implemented in the differential testing toolbox rDiff (Drewe et al., 2013, previous version in Stegle et al., 2010). To do this, each alternative event is decomposed into different sets of exonic segments supporting either one or the other isoform of the event; expression in segments that are the same in both isoforms are used for normalization but not for differential testing. Each aligned read was assigned to one of the differential segment sets resulting in a read count for each of the two isoforms. After library size normalization, a p-value for differential isoform expression was computed with a Negative Binomial test (Anders and Huber, 2010, Drewe et al., 2013). The variance function for the Negative Binomial Test describing the biological variance of the experiment was previously estimated on the two replicates of the double mutant alignments. As the replicates for each condition were sequenced in two separate sequencing runs, for each condition one replicate was taken from the first and a second one from the second sequencing run, addressing for possible (artifactual) differences between the single sequencing runs. To correct for multiple testing bias, the p-values were transformed into False Discovery Rate (FDR) values using the method of Benjamini and Hochberg (Benjamini and Hochberg, 1995). To compute a direction of the tested difference, the normalized counts for the tested condition were compared. If the counts in the second condition in a test setting condition 1 vs. condition 2 were higher, the event was called an UP event and a DOWN event otherwise. The p-value of the respective other direction was set to 1 in each case. Exonic and intronic counts were interpreted such that UP and DOWN can be read as the accumulation and depletion of the longer of both event isoforms, respectively.

Test for differential gene expression

The differential gene expression was analyzed using the DESeq package for the R Bioconductor Suite (Anders and Huber, 2010). An alignment was counted for the expression of a gene, if it overlapped to at least one exonic position of this gene. To address for biological variance, the same replicates as for the differential isoform expression test have been used in DESeq.

Calling expressed genes

Following the procedure used and described in Gan et al. (2011), we assumed that the number of reads per gene observed by chance follow a Poisson distribution with the probability of no mapped read per gene $e^{-\lambda}$ with a fixed rate λ. Based on this model, we computed for each gene a p-value of observing the respective count value by chance. After correction for multiple testing (Benjamini and Hochberg, 1995), we chose a maximum q-value of 0.05 as cutoff for calling a gene “expressed”.

Ranking of the event list

A common list of all events was ranked by the FDR value for the differential transcript expression test WT vs. *lba1 upf3-1*. For the ranking, the minimum over both directions of the test was used. To identify significantly differential events, an FDR threshold of 0.1 (10%) was applied. See Supplemental Figure 2 online for an experimental validation of the estimated false discovery rate.

The list was further analyzed by incorporating the test results of WT vs. both single mutants individually. Then, an event was considered significantly differentially expressed and consistent with the experiment design, if it showed both an FDR less than 0.1 and one of the tests WT vs. single mutant had a p-value less than 0.1 in the same direction as the test of WT vs. *lba1 upf3-1*. A similar combination for the test control vs. Cycloheximide (CHX) treated plants has been computed and the same FDR threshold of 0.1 was applied. Both rankings were combined into a common list “All NMD”.

Venn visualization and permutation test

The events showing significant test results in several tests were visualized in Venn diagrams. All Venn diagrams were drawn in scale to the set sizes they represent. As UP and DOWN events have been combined in the diagrams, contradictory events have been removed. An event was counted as contradictory, if the different test directions resulted in different overlap assignments. To evaluate if significantly more events could be counted in the overlaps of the different tests than expected, we performed a permutation test on the ranked list, randomly permuting the p-values in both lists and counting the events in the overlap. The permutations have been repeated 50,000 times, resulting in a minimum p-value of 0.00002 ($2x10^{-5}$).

Identification of expressed intergenic loci

We generated initial regions by searching for areas in the genome that were continuously covered by read alignments. This coverage was not only defined on exonic alignment positions but also on positions spanned by spliced reads. To initially define the regions, we did not filter the reads.

We then trimmed regions from both ends until we found read coverage greater or equal to 2. We further discarded a region if it was located within 500 nts distance of any TAIR10 annotated gene or was overlapping a repetitive region with more than 5% of its positions.

Differential expression of new loci

For the remaining regions we counted the number of reads without mismatches for each of the six sets "WT", "*lba1*", "*upf3-1*", "*lba1 upf3-1*", "Mock", and "CHX", merging the respective replicates.

For each region we performed 8 directional binomial tests for increased and decreased number of read alignments in 3 WT vs. mutant pairs and "Mock" vs. "CHX". The Null hypothesis of the test was independent random assignment of reads to the different sets with a probability according to the differing total numbers of read alignments in the two sets.

We reported regions as significant, if the Bonferroni corrected p-values were smaller than 0.01.

Transcript identification

For each region we ran MITIE (Behr et al., 2013) to infer transcripts. While intron boundaries are very accurately defined by spliced reads the transcription start and end sites are difficult to detect directly from RNA-seq data. We identified potential transcript boundaries based on a binomial test. The test statistic compared read-start- and read-end-counts in consecutive fixed size windows to detect transcript starts and ends, respectively. We performed the test in a sliding window approach along the complete locus. MITIE then identified potentially overlapping transcripts using the RNA-seq alignments based on mixed integer optimization. Thereby, it selects transcript such that the read coverage can be well explained in a quantitative way. Due to the very low read coverage near the true transcript ends, this approach tends to slightly underestimate the transcript length.

Visualization in GBrowse

All alignments and annotation tracks have been visualized within a customized version of GBrowse2 (http://gbrowse.cbio.mskcc.org/gb/gbrowse/NMD2013/). Additional information display for splice junction support counts and differential testing was made available through customized GBrowse plugins. The respective tracks can be chosen via the register “Select Tracks”.

NMD feature analysis

To analyze for characteristic NMD features, the events have been incorporated into the representative gene models defined by the TAIR10 genome annotation. An event was integrated into the respective representative isoform, if its inner event coordinates were overlapping with at least one position to the annotated isoform. Here, the inner event coordinates describe the first exon-intron boundary after the start and the last intron-exon boundary before the end of the event. If an event was located within an intergenic region, fell completely into an intron, or event start and stop were located in different introns spanning one or several exons, the event was discarded from integration. However, only a small percentage of events had to be excluded following these criteria.

When the event could be integrated into the representative isoform, the two event isoforms were extended by the remaining exons of the representative isoform, generating two long event isoforms. Note that neither of the two long event isoforms has to be identical to the representative isoform nor to any other annotated isoform. However, many of the created isoforms have a corresponding annotated isoform.

An event was further excluded from analysis, if CDS information was not available in the annotation or both isoforms lost the annotated CDS start site. Again, only a small fraction of events had to be excluded based on that filter criterion. An overview of the number of events excluded by certain filtering criteria is provided in Supplemental Data Set 2A online.

Subsequent, each event was assigned one of the three CDS-type groups: 5' UTR, CDS, or 3' UTR. If the inner event coordinates (as defined above) overlapped to one of the UTRs it was assigned to this UTR. No event overlapped both UTRs. The remaining events were assigned to CDS.

The two isoforms of each event were assigned one of the labels "Control" and "delta (Δ) NMD" based on the direction of the differential test, defining the longer isoform showing an UP behavior in the test as Δ NMD, as an accumulation upon NMD impairment was expected.

Based on the CDS-type assigned to the events, following NMD features have been evaluated:

5' UTR:

- existence of upstream open reading frame (uORF)
- uORF > 35 amino acids
- uORF overlapping annotated start codon

CDS:

- existence of premature termination codon (PTC) causing the 3' UTR to be longer than 347 nts
- splice junction more than 50 nts downstream of stop codon

3' UTR:

- 3' UTR longer than 347 nts
- splice junction more than 50 nts downstream of stop codon

To assess PTCs, we searched for the first in frame stop codon, starting at the annotated CDS start. A comparison to the annotated stop revealed the PTC. Further, we searched for upstream open reading frames (uORFs) by searching the longest ORF starting in the annotated 5' UTR in a different reading frame than the annotated CDS start.

The assessment of lengths of UTRs, uORFs and isoforms as well as the distance calculation of downstream splice junctions is straightforward.

Event combination analysis

A possible drawback of the single event integration strategy above is the possibility, that combinations of events correct the reading frame shift and abolish the PTC introduced by a single event. To evaluate the extent of such "rescue" events, we took all significantly altered events per gene and created all possible combinations that could be integrated into the representative isoform. A

combination was deemed possible, if the alternative exonic positions of the two events were not overlapping and could thus be combined in a single isoform. The events have been integrated in a way such that the tested direction of accumulation was consistent. The generated isoforms have been analyzed in the same manner as the single integration events.

3' UTR length tendency estimation

For analyzing the trend of the 3' UTR length in the NMD targeted isoforms vs. the WT isoforms based on the predicted significance, all events overlapping to CDS or 3' UTR were taken into account. Based on the p-values of the directed test WT vs. *Iba1 upf3-1*, all event isoforms were assigned one of the labels "Control" and "Δ NMD", asserting two different labels per event. Subsequently, the events were sorted by ascending p-value. Correlations of the 3' UTR lengths of the events were computed as rank correlation coefficients after Spearman. Significance of the correlation was inferred using the "corr" function provided in the MATLAB statistics toolbox. To visualize the tendency of 3' UTR lengths based on the p-values, the UTR lengths were smoothed by a sliding window approach, replacing each 3' UTR length with the geometric mean of its 50 successors and predecessors. All computations were done for both the "Control" and the "Δ NMD" isoforms sets independently.

Supplemental References

Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. Genome Biol **11**, R106.

Behr, J., Kahles, A., Zhong, Y., Sreedharan, V.T., Drewe, P., and Rätsch, G. (2013). MITIE: Simultaneous RNA-Seq-based transcript identification and quantification in multiple samples. Bioinformatics, doi: 10.1093/bioinformatics/btt442.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological) **57**, 289-300.

Carvalho, S.D., Saraiva, R., Maia, T.M., Abreu, I.A., and Duque, P. (2012). XBAT35, a Novel Arabidopsis RING E3 Ligase Exhibiting Dual Targeting of Its Splice Isoforms, Is Involved in Ethylene-Mediated Regulation of Apical Hook Curvature. Mol Plant **5**, 1295-309.

Drewe, P., Stegle, O., Hartmann, L., Kahles, A., Bohnert, R., Wachter, A., Borgwardt, K., and Rätsch, G. (2013). Accurate detection of differential RNA processing. Nucleic Acids Res **41**, 5189-5198.

Gan, X., Stegle, O., Behr, J., Steffen, J.G., Drewe, P., Hildebrand, K.L., Lyngsoe, R., Schultheiss, S.J., Osborne, E.J., Sreedharan, V.T. et al. (2011). Multiple reference genomes and transcriptomes for Arabidopsis thaliana. Nature **477,** 419-423.

Jean, G., Kahles, A., Sreedharan, V.T., De Bona, F., and Rätsch, G. (2010). RNA-Seq read alignments with PALMapper. Curr. Protoc. Bioinformatics **32**: 11.6.1-11.6.37.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics **25**, 2078-2079.

Ossowski, S., Schneeberger, K., Clark, R.M., Lanz, C., Warthmann, N., and Weigel, D. (2008). Sequencing of natural strains of Arabidopsis thaliana with short reads. Genome Res **18**, 2024-2033.

Sibout, R., Sukumar, P., Hettiarachchi, C., Holm, M., Muday, G.K., and Hardtke, C.S. (2006). Opposite root growth phenotypes of hy5 versus hy5 hyh mutants correlate with increased constitutive auxin signaling. PLoS Genet **2,** e202.

Sigrist, C.J.A., de Castro, E., Cerutti, L., Cuche, B.A., Hulo, N., Bridge, A., Bougueleret, L., Xenarios, I. (2012) New and continuing developments at PROSITE. Nucleic Acids Res **41**, D344-7.

Stegle, O., Drewe, P., Bohnert, R., Borgwardt, K.M., and Rätsch, G. (2010). Statistical Tests for Detecting Differential RNA-Transcript Expression from Read Counts. Nat. Preced. 10.1038/npre.2010.4437.1.