Combined haploinsufficiency and purifying selection drive retention of *RPL36a* paralogs in Arabidopsis
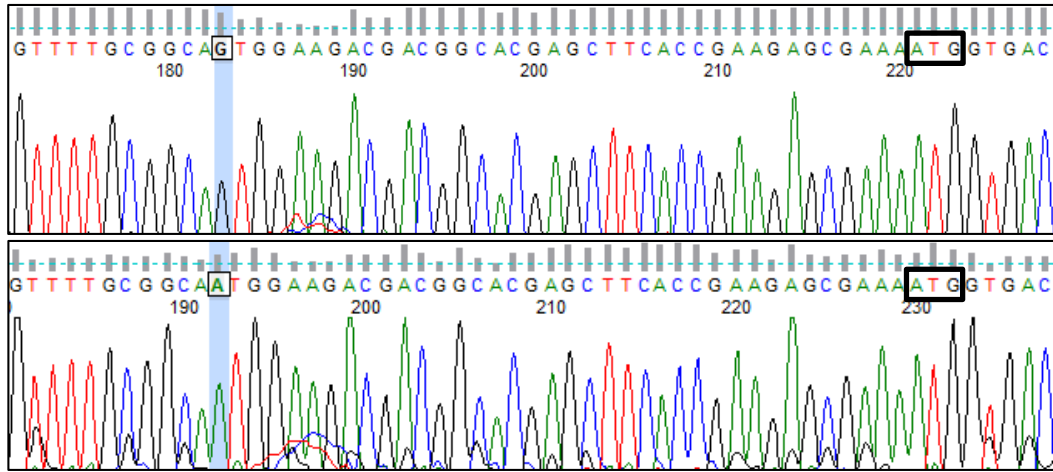
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Supplementary Information

L*er*

api2

Supplementary Figure S1.- The *api2* mutation creates an out-of-frame ATG codon upstream of the wild-type translation start site of *API2* transcripts. Electropherograms showing the 5'-UTR of the *API2* gene from L*er* and *api2/api2* plants. The G→A *api2* point mutation is highlighted in blue. The wild-type *API2* translation start codon is boxed in black.

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        M   V   N   I   P   K   T   K   N   T   Y   C   K   N   K   E   C   K   K   H
API2    ATGGTGAACATTCCGAAAACTAAGAACACTTACTGCAAGAACAAGGAGTGCAAGAAGCAT
RPL36aA ATGGTGAACATTCCAAAGACAAAGAACACTTACTGTAAGAACAAGGAATGCAAAAAGCAT
        M   V   N   I   P   K   T   K   N   T   Y   C   K   N   K   E   C   K   K   H

        T   L   H   K   V   T   Q   Y   K   K   G   K   D   S   L   A   A   Q   G   K
API2    ACCTTGCACAAGGTGACGCAGTATAAGAAAGGTAAAGATAGCCTTGCTGCCCAAGGAAAG
RPL36aA ACTTTGCACAAGGTTACCCAATACAAGAAGGGTAAAGACAGTCTTGCTGCTCAAGGAAAG
        T   L   H   K   V   T   Q   Y   K   K   G   K   D   S   L   A   A   Q   G   K

        R   R   Y   D   R   K   Q   S   G   Y   G   G   Q   T   K   P   V   F   H   K
API2    CGTCGTTATGATCGTAAACAATCTGGTTATGGAGGTCAGACCAAGCCCGTCTTCCACAAG
RPL36aA CGTCGTTATGACCGTAAACAATCTGGTTATGGTGGTCAGACTAAGCCTGTCTTCCACAAA
        R   R   Y   D   R   K   Q   S   G   Y   G   G   Q   T   K   P   V   F   H   K

        K   A   K   T   T   K   K   I   V   L   R   L   Q   C   Q   S   C   K   H   F
API2    AAGGCTAAGACAACTAAGAAGATTGTGCTGAGGCTTCAATGCCAAAGCTGCAAGCATTTC
RPL36aA AAGGCTAAGACCACGAAGAAGATTGTTTTGAGGCTTCAGTGTCAAAGCTGCAAGCACTTT
        K   A   K   T   T   K   K   I   V   L   R   L   Q   C   Q   S   C   K   H   F

        S   Q   R   P   I   K   R   C   K   H   F   E   I   G   G   D   K   K   G   K
API2    TCGCAACGCCCGATTAAGAGGTGCAAGCACTTTGAGATCGGTGGAGACAAGAAGGGAAAG
RPL36Aa TCGCAGCGTCCTATCAAGAGGTGCAAGCATTTCGAGATCGGTGGTGACAAGAAGGGAAAG
        S   Q   R   P   I   K   R   C   K   H   F   E   I   G   G   D   K   K   G   K

        G   T   S   L   F   *
API2    GGAACATCTCTCTTTTAA
RPL36Aa GGAACATCTCTGTTTTAA
        G   T   S   L   F   *
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Supplementary Figure S2.- Synonymous substitutions along the *API2* and *RPL36aA* coding sequences. Alignment of the *API2* and *RPL36aA* coding sequences with indication of the amino acids encoded by each codon. An asterisk indicates the stop codon. The alignment was obtained with the PAL2NAL program. Synonymous substitutions affecting the first or third base of a codon are highlighted in pink and green, respectively.

<div align="center">**Supplementary Table S1**.- Primer sets used in this work</div>

Purpose	Oligonucleotide names	Oligonucleotide sequences (5'→3')	
		Forward primer (F)	Reverse primer (R)
Linkage analysis	cer466314_F/R	CCGATTCTATTCCTCTAGGCT	GTTAAAAAATCTGGACTTATCAAG
	cer459267_F/R	AGCAGCTGCCCTTTCAACAG	AGGTTACTGATGCCCGAGAG
	cer453616_F/R	GAGCATTGGCATGTTCATTGTT	TTTGAGAGTTTCGTGTGGCCT
API2 sequencing	API2_F1/R1	CTGATTTGCAGCACAAAACG	CCACGGGCCACTGATTATTA
	API2_R2	TGATTTAGGAGAAGCAGGAAAT	
Topo/Gateway cloning	API2cds_F/R	CACCATGGTGAACATTCCGAAAACTA	AAAGAGAGATGTTCCCTTTCC
	RPL36aAcds_F/R	CACCATGGTGAACATTCCAAAGACAAA	AAACAGAGATGTTCCCTTTCCC
	API2pro_F/R	GGGGACCACTTTGTACAAGAAAGCTGGGT TTTCGCTCTTCGGTGAAGCTC	GGGGACAAGTTTGTACAAAAAAGCAGGCT TTGAGATTCAGAGAGCGTTAG
	RPL36aApro_F/R	GGGGACAAGTTTGTACAAAAAAGCAGGCT GATGAGGAACAAAGAAAGAGA	GGGGACCACTTTGTACAAGAAAGCTGGGT TTTCGCGTCTCTGATCTCCG
	M13_F/R	GTAAAACGACGGCCAG	CAGGAAACAGCTATGACCATGATT
qRT-PCR	18SrRNA_RTqPCR_F/R	AGGATCCATTGGAGGGCAAGT	CGACGTTTTTAACTGCAACAACT
	API2_RTqPCR_F/R	CCACAAGAAGGCTAAGACAACT	AAAGTGCTTGCACCTCTTAATC
	RPL36aA_RTqPCR_F/R	GACCACGAAGAAGATTGTTT	GAAATGCTTGCACCTCTTGATA
Genotyping of mutants, transgenic plants and constructs	LBb1.3_F[a]	ATTTTGCCGATTTCGGAAC	
	rpl36aa_F/R	GATGTTCCCTTTCCCTTCTTG	TGGTTCAAACGAAACCAAAAC
	35S-F[b]	GACAGTGGTCCCAAAGATG	
	GUS-R[c]		CACAAACGGTGATACGTACACT

[a-c]Each of these oligonucleotides was used together with: [a]rpl36aa_R to genotype the *rpl36aa* mutant; [b]API2cds_R and RPL36aAcds_R to genotype transgenic plants putatively carrying the *35S$_{pro}$:RPL36aB* and *35S$_{pro}$:RPL36aA* transgenes; [c]API2pro_R and RPL36aApro_R to genotype transgenic plants putatively carrying the *API2$_{pro}$:GUS* and *RPL36aA$_{pro}$:GUS* transgenes.