Appendix for

# Plant YTHDF proteins are direct effectors of antiviral immunity against an m$^6$A-containing RNA virus

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**Appendix Table S1. List of *Arabidopsis thaliana* mutant lines employed in this work.** All the lines are in the Col-0 ecotype.

| ID | Genotype | Methodology | Ref * |
|---|---|---|---|
| **Single KO mutants** | | | |
| ***ect2-1*** | SALK_002225 | T-DNA insertion | 1 |
| ***ect3-1*** | SALK_077502 | T-DNA insertion | 1 |
| ***ect4-2*** | GK_241H02 | T-DNA insertion | 1 |
| ***ect5-1*** | SALK_131549 | T-DNA insertion | this work |
| ***ect2-3*** | GK_132F02 | T-DNA insertion | 1 |
| ***ect3-2*** | GABIseq_487H12 | T-DNA insertion | 1 |
| ***rdr6-11*** | C → T (805), nonsense | T-DNA insertion | 3 |
| ***rdr6-12*** | 7 bp del. (997-1003), frameshift | Fast neutron | 3 |
| ***sgs3-11*** | G → A (2283), splice site | EMS | 3 |
| ***dcl2-1*** | SALK-064627 | T-DNA insertion | 4 |
| ***dcl4-4*** | Premature stop codon | EMS mutagenesis | 5 |
| ***alkbh9b-3*** | SALK_015591 | T-DNA insertion | 6 |
| **Double and triple KO mutants** | | | |
| ***de23*** | *ect2-1/ect3-1* | Genetic cross | 1 |
| ***Gde23*** | *ect2-3/ect3-2* | Genetic cross | 1 |
| ***de24*** | *ect2-1/ect4-2* | Genetic cross | 1 |
| ***de34*** | *ect3-1/ect4-2* | Genetic cross | 1 |
| ***te234*** | *ect2-1/ect3-1/ect4-2* | Genetic cross | 1 |
| ***de25*** | *ect2-1/ect5-2* | CRISPR/Cas9-deletion of *ECT5* in *ect2-1* | this work |
| ***ect2-1/ect5-3*** | *ect2-1/ect5-3* | CRISPR/Cas9-deletion of *ECT5* in *ect2-1* | this work |
| ***te235*** | *ect2-1/ect3-1/ect5-4* | CRISPR/Cas9-deletion of *ECT5* in *de23* | this work |
| ***ect2-1/ect3-1/ect5-5*** | *ect2-1/ect3-1/ect5-5* | CRISPR/Cas9-deletion of *ECT5* in *de23* | this work |
| ***alkbh9b/te235*** | *alkbh9b-3/ect2-1/ect3-1/ect5-4* | Genetic cross | this work |
| ***rdr6/sgs3*** | *rdr6-11/sgs3-11* | Genetic cross | 3 |
| ***rdr6/de23*** **(2 lines)** | *rdr6-12/ect2-1/ect3-1* | Genetic cross | this work |
| **Transgenic lines** | | | |
| ***de23/ECT2-mCh*** **(2 lines)** | *ect2-1/ect3-1 ECT2p:ECT2-mCherry-ECT2t* | USER cloning & Agrob. transformation | 1 |
| ***de23/ECT2$^{W464A}$-mCh*** **(2 lines)** | *ect2-1/ect3-1 ECT2p:ECT2$^{W464A}$-mCherry-ECT2t* | USER cloning & Agrob. transformation | 1 |
| ***ect2-1*****/ECT2-FLAG-ADAR (5 lines)** | *ect2-1 ECT2p:ECT2-FLAG-ADAR-ECT2t* | USER cloning & Agrob. transformation | 2 |
| **FLAG-ADAR (5 lines)** | *ECT2p: FLAG-ADAR-ECT2t* | USER cloning & Agrob. transformation | 2 |
| ***te234/ECT2-mCh*** **(3 lines)** | *ect2-1/ect3-1/ect4-2 ECT2p:ECT2-mCherry-ECT2t* | USER cloning & Agrob. transformation | 1 |
| ***te234/ECT2$^{\Delta 2}$-mCh*** **(3 lines)** | *ect2-1/ect3-1/ect4-2 ECT2p:ECT2$^{\Delta 2}$-mCherry-ECT2t* | USER cloning & Agrob. transformation | this work |

* References: [1]Arribas-Hernández *et al*, 2018; [2]Arribas-Hernández *et al*, 2021; [3]Peragine *et al*, 2004; [4]Xie *et al*, 2004; [5]Dunoyer *et al*, 2005, [6]Martínez-Pérez *et al*, 2017.


1. Arribas-Hernández L, Bressendorff S, Hansen MH, Poulsen C, Erdmann S & Brodersen P (2018) An m $^{6}$ A-YTH Module Controls Developmental Timing and Morphogenesis in Arabidopsis. *Plant Cell* 30: 952–967

2. Arribas-Hernández L, Rennie S, Köster T, Porcelli C, Lewinski M, Staiger D, Andersson R & Brodersen P (2021) Principles of mRNA targeting via the Arabidopsis m6A-binding protein ECT2. *Elife* 10: e72375

3. Peragine A, Yoshikawa M, Wu G, Albrecht HL & Poethig RS (2004) SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. *Genes Dev* 18: 2368–2379

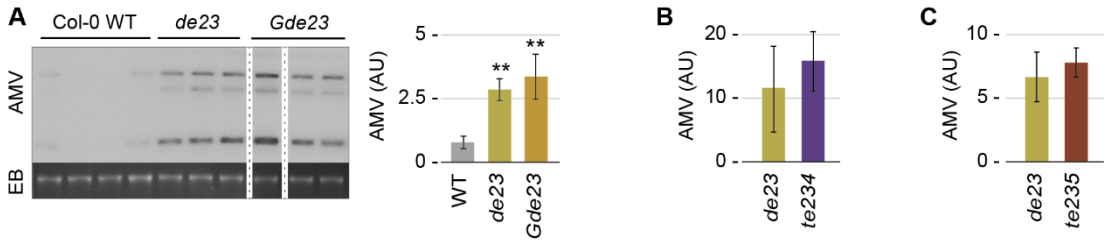
4. Xie Z, Johansen LK, Gustafson AM, Kasschau KD, Lellis AD, Zilberman D, Jacobsen SE & Carrington JC (2004) Genetic and functional diversification of small RNA pathways in plants. PLoS Biol 2: E104

5. Dunoyer P, Himber C & Voinnet O (2005) DICER-LIKE 4 is required for RNA interference and produces the 21-nucleotide small interfering RNA component of the plant cell-to-cell silencing signal. *Nat Genet* 37: 1356–61

6. Martínez-Pérez M, Aparicio F, López-Gresa MP, Bellés JM, Sánchez-Navarro JA & Pallás V (2017) Arabidopsis m6A demethylase activity modulates viral infection of a plant virus and the m6A abundance in its genomic RNAs. Proc Natl Acad Sci U S A 114: 10755–10760

**Appendix Table S2.** List of oligonucleotides used in this work.

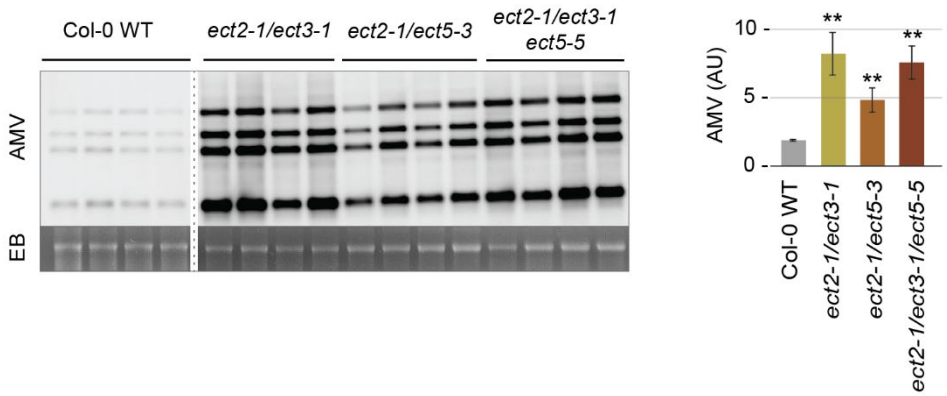
| | | |
|---|---|---|
| qPCR | ECT5_qPCR_F | AGGCAGCAAAGAAGCAGTCA |
| | ECT5_qPCR_R | CCAGACCCGGTCAGACGTAA |
| | MTA_qPCR_F | CGGATCCACCATGGGACATT |
| | MTA_qPCR_R | AAGCTCCAAACATTCACGGC |
| | MTB_qPCR_F | GTACTGTGTTTCAGCGTTCC |
| | MTB_qPCR_R | TTCTGAGTCGAACCATAAGGAG |
| | VIR_qPCR_F | ACGCAAGTCCAGCCTTACTATCAC |
| | VIR_qPCR_R | CGGTCACTTAATAGAGCCTGAATGG |
| *ect5* genotyping | LPect5-1 | TGGACACGGTTCTTTCTCATC |
| | RPect5-1 | TGATCAAATCAGGTTGGCTTC |
| | LB | ATTTTGCCGATTTCGGAAC |
| | ECT5_F | GGTCTCcCATGGCAACGACTCAATCACAC |
| | ECT5_R | GGTCTCgCTAGTTGGATTGACCAGAAGAG |
| CRISPR/Cas9 RNA guides | sgRNA_ECT5.1F | ATTGAAGTGTCCGGCAAAATCAC |
| | sgRNA_ECT5.1R | AAACGTGATTTTGCCGGACACTT |
| | sgRNA_ECT5.2F | ATTGTGAAAACCCCACAGGCGAA |
| | sgRNA_ECT5.2R | AAACTTCGCCTGTGGGGTTTTCA |
| | sgRNA_ECT5.3F | ATTGGGCAAGGGACTTGCTGCAG |
| | sgRNA_ECT5.3R | AAACCTGCAGCAAGTCCCTTGCC |
| CRISPR/Cas9 genotyping | C9_ECT5g_F | GCAGACTGTCCCGGCAAAC |
| | C9_ECT5g_R | CGGGCTGAGTTGGAGAAGTAA |
| | C9(LA)_ECT5-F | CTATGTTGGGGAGCCAGG |
| | C9(LA)_ECT5-R | GCTGGTAATACGGTGATGC |




**Appendix Figure S1. Susceptibility of *ect2/ect3, ect2/ect3/ect4* and *ect2/ect3/ect5* mutants to AMV infection.**

A. RNA blot analysis of AMV systemic infection at 6 days post-inoculation in mutants with two allele combinations of *ect2/ect3*, *de23*(*ect2-1/ect3-1*) and *Gde23* (*ect2-3/ect3-2*), compared to wild type plants. Left, blot displaying AMV RNAs 1-4; right, quantification histogram. Dashed lines indicate non-contiguous samples that are analyzed on the same membrane. Ethidium bromide staining of rRNAs (EB) was used as RNA loading control.

B, C**.** Quantification of three independent experiments comparing AMV systemic infection levels in *de23* and *te234* (B) or *te235* (C) by RNA blot analysis. The height of the bars represents the average, for the 3 experiments, of the means over four samples in each experiment. In all cases, error bars indicate standard deviation, and asterisks (**) show $p < 0.01$ relative to wild type applying Student's t-test. AU, arbitrary units.




**Appendix Figure S2. AMV susceptibility in independent *ect2/ect5* and *ect2/ect3/ect5* transgenic lines.**

RNA blot of AMV systemic infection at 6 days post-inoculation in *de23*, *ect2-1/ect5-3* and *ect2-1/ect3-1/ect5-5* lines compared to wild type. Left, RNA blot displaying AMV RNAs 1-4; right, quantification histogram. Error bars indicate standard deviation, and asterisks (**) indicate $p < 0.01$ relative to wild type applying Student's t-test. Ethidium bromide staining of rRNAs (EB) was used as RNA loading control. AU, arbitrary units.