**Supplementary Information**

# MAC5, an RNA-binding protein, protects pri-miRNAs from SERRATE-dependent exoribonuclease activities

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**Figures S1-S10**

**Table S1**

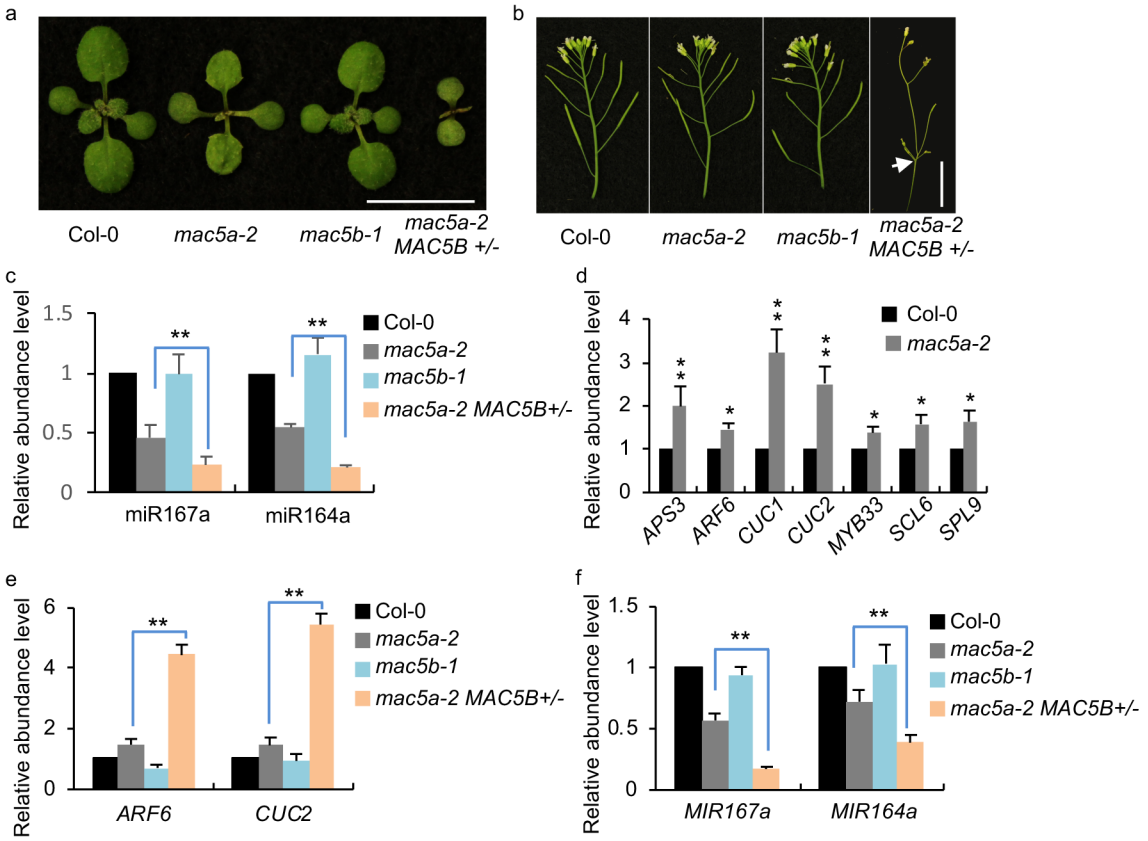


**Figure S1.** ***MAC5A*** **and** ***MAC5B*** **act redundantly in miRNA accumulation**. **a** and **b**, Phenotypes of 14d-old seedlings (**a**) and inflorescences (**b**). Bar = 1 cm. White arrow indicates the clustered inflorescence architecture. **c**, The abundance of miR167a and miR164a in the indicated plants detected by Quantitative RT-PCR (qRT-PCR). The values in Col-0 were set as 1. ** P<0.01 by student's *t* test compared with *mac5a-2* value. **d,** The abundance of miRNA targets in the Col-0 and *mac5a-2*. The values in Col-0 were set as 1. * P<0.05 and ** P<0.01 by student's *t* test compared with Col-0 value. **e**, The abundance of *ARF6* and *CUC2* in the indicated plants detected by qRT-PCR. The values in Col-0 were set as 1. ** P<0.01 by student's *t* test compared with *mac5a-2* value. **f**, The abundance of pri-miR167a and pri-miR164a in the indicated plants detected by qRT-PCR. The values in Col-0 were set as 1. ** P<0.01 by student's *t* test compared with *mac5a-2* value. The values in Col-0 were set as 1. ** P<0.01 by student's *t* test compared with *mac5a-2* value.

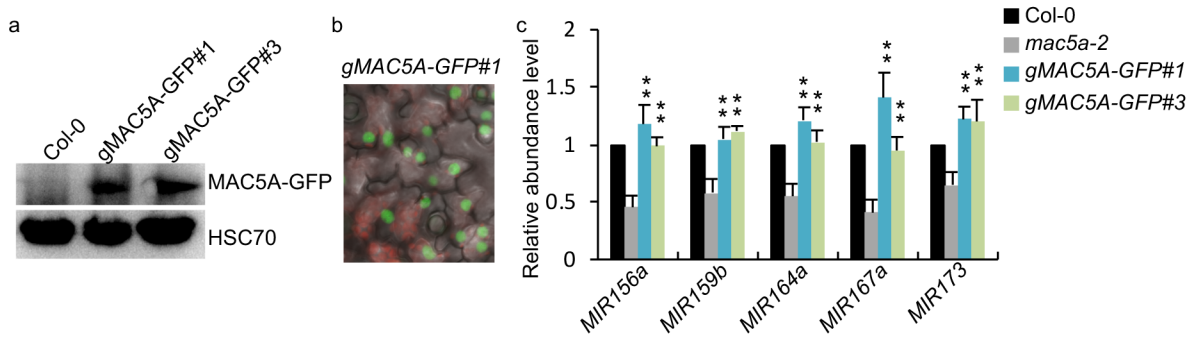


**Figure S2. Complementation of *mac5a*. a**, MAC5A-GFP protein expression in two transgenic lines in the *mac5a-2* background detected by western blot. HSC70 serves as the loading control. **b**, The MAC5-GFP protein in the transgenic plants harboring *gMAC5A-GFP* detected by confocal microscopy. **c**, Detection of pri-miRNA expression levels in the complementary lines by qRT-PCR. The values in Col-0 were set as 1. ** P<0.01 by student's *t* test compared with *mac5a-2* value.

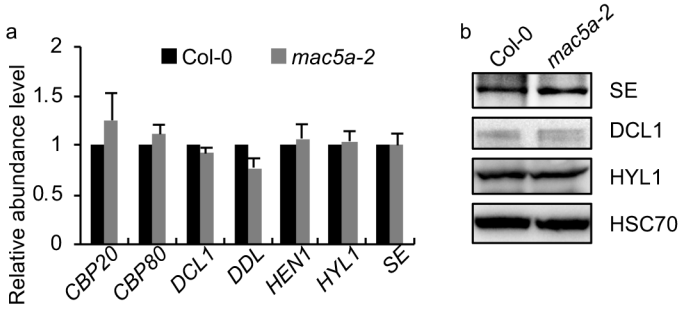


**Figure S3. MAC5A does not influence the expression of several genes required for miRNA biogenesis**. **a**, The transcript levels of genes involved in miRNA biogenesis detected by qRT-PCR. **b**, The protein levels of SE, DCL1, and HYL1. HSC70 serves as the loading control.

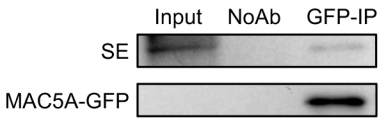


**Figure S4. The interaction between MAC5A and SE in Arabidopsis**. Total proteins were extracted in transgenic plants harboring *gMAC5A-GFP*. IP was performed using anti-GFP antibodies. After IP, SE and MAC5A-GFP proteins were detected by immunoblot using anti-SE and anti-GFP antibodies, respectively. NoAb means no antibody negative control.

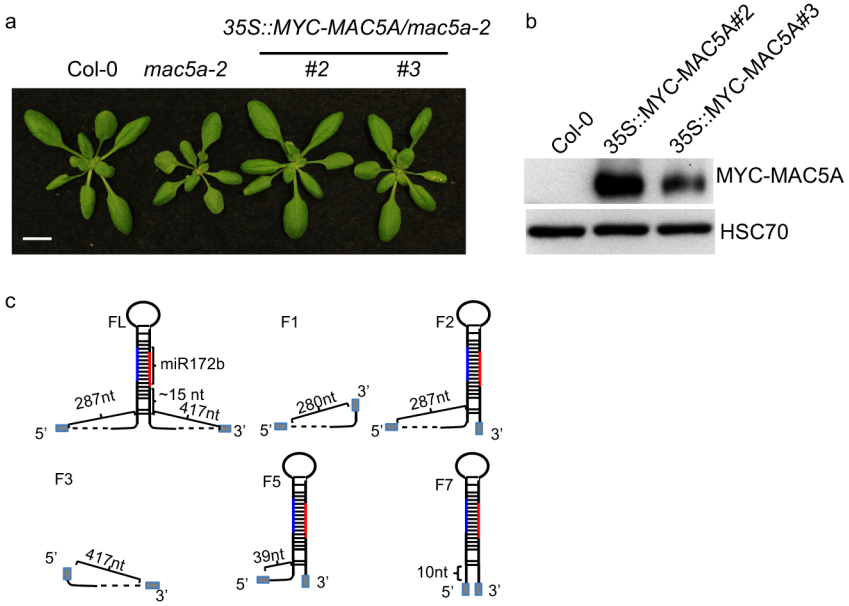


**Figure S5. The interaction between MAC5A with *MIR172b*. a**, Phenotype of 4-week old seedlings of transgenic plants harboring *p35S::MYC-MAC5A* in the *mac5a-2* background. Bar: 1 cm. **b**, MYC-MAC5A protein expression in the transgenic *mac5a-2* harboring *p35S::MYC-MAC5A*. HSC70 serves as the loading control. **c,** Diagrams of various *MIR172b* transcripts used for RNA-binding assay. Gray boxes indicate the sequence from vectors. miR172b is shown in red; miR172b* is shown in blue.

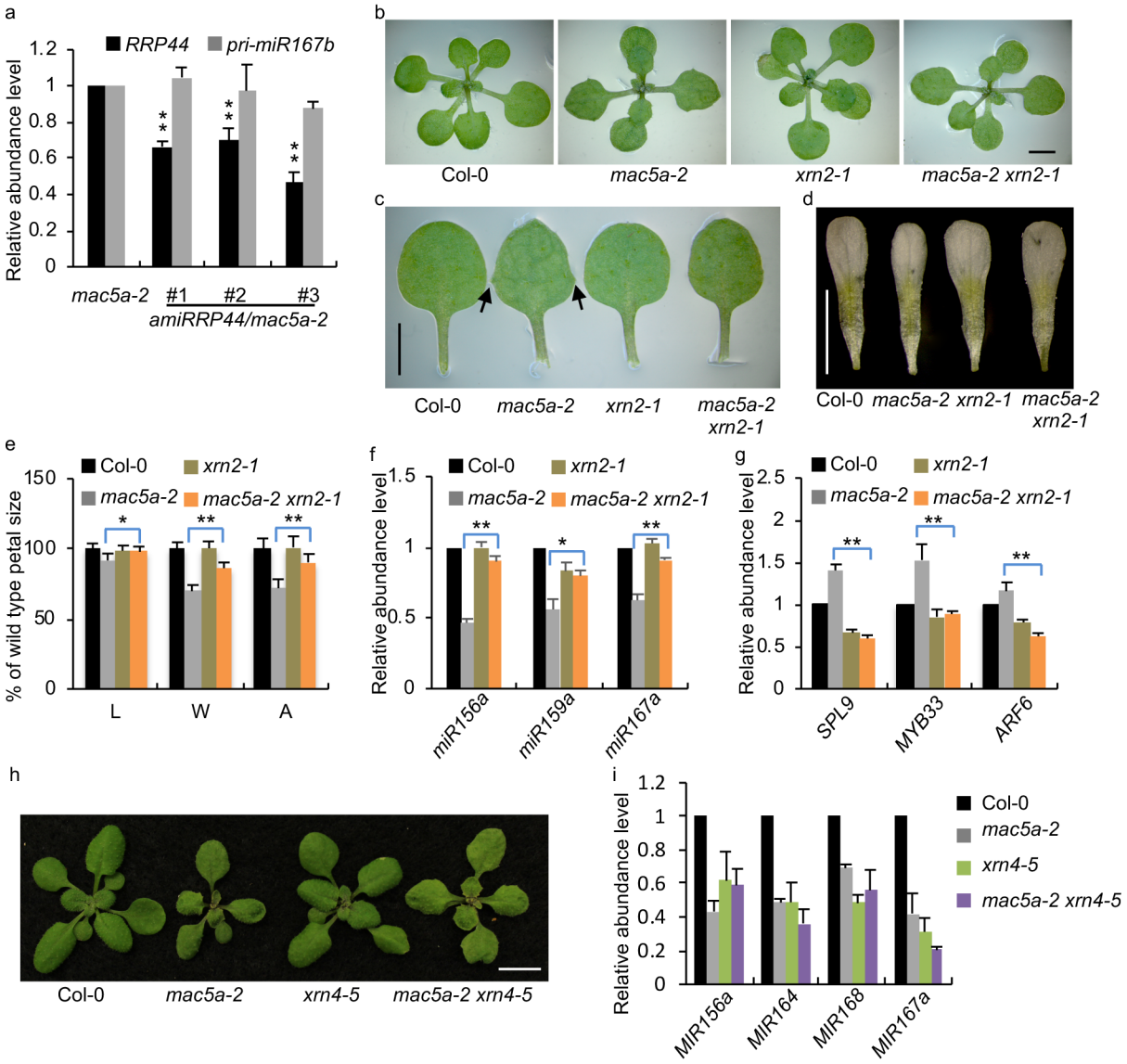


**Figure S6. MAC5 protects pri-miRNAs from 5'-to-3' degradation of pri-miRNAs. a,** qRT-PCR analysis of the mRNA level of *RRP44* and *pri-miR167b* in the three transgenic lines. **b-d**, Phenotypes of 14d-old seedlings **(b),** first-pair true leaves (c), and petals (d) in the indicated plants. *mac5a-2* displays the leaf tooth phenotype in the margin marked by black arrows that is suppressed in *mac5a-2 xrn2-1* double mutant. **e**, Statistic analysis of petal length (L), petal width (W), and petal area (A) in the indicated plants. The values of Col-0 were set as 100. **f** and **g**, Relative abundance levels of mature miR156a, miR159a and miR167a and their targets *SPL9*, *MYB33* and *ARF6* detected by qRT-PCR in the indicated plants. **h**, Phenotype of 3-week old plants. **i**, Relative abundance levels of pri-miRNA in the indicated plants. * P<0.05 and ** P<0.01 by student's *t* test compared with *mac5a-2* value. Bars: 2 mm in **b-d,** 1 cm in h.

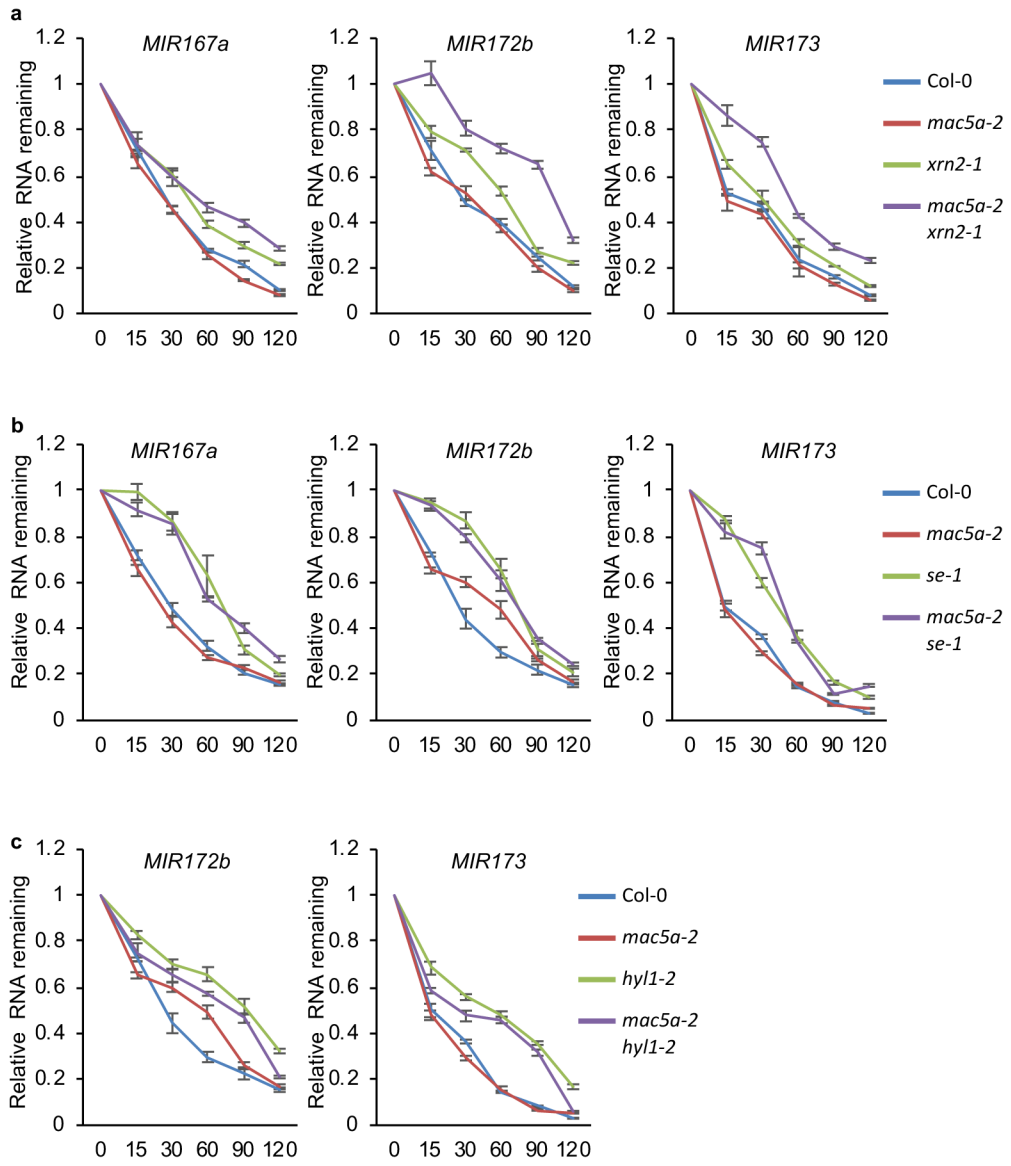


**Figure S7. RNA decay analyses to determine the RNA stability. a**, pri-miRNA half-life analysis in Col-0, *mac5a-2*, *xrn2-1* and *mac5a-2 xrn2-1*. **b,** pri-miRNA half-life analysis in Col-0, *mac5a-2*, *se-1*, and *mac5a-2 se-1*. **c.** pri-miRNA half-life analysis in Col-0, *mac5a-2*, *hyl1-2* and *mac5a-2 hyl1-2*. 7-day old seedlings were treated with 0.6 mM 3'-deoxyadenosine (Cordycepin, Sigma) at various times (0, 15, 30, 60, 90 and 120 minutes). qRT-PCR was performed to examine RNA abundances in the indicated plants. The values were normalized to the internal control (EIF4a) that is not affected by Cordycepin reagent. Values of time 0 were set as 1. Error bars indicate standard deviation of three technical replications.

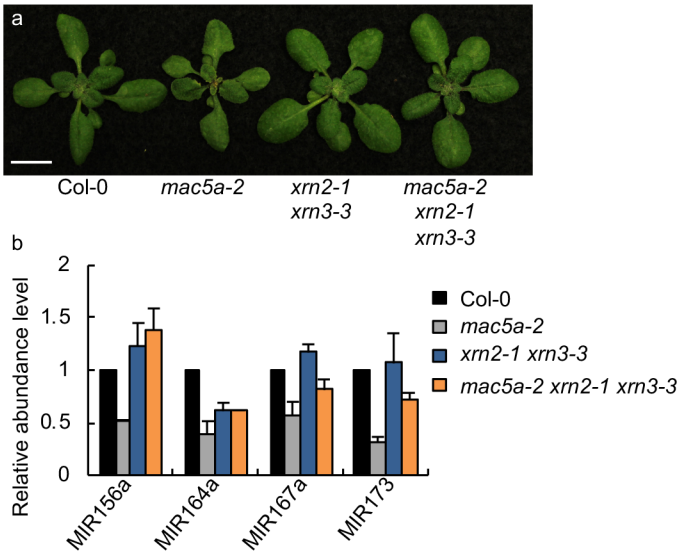


**Figure S8. Genetic analysis of *mac5a-2*, *xrn2-1*, and *xrn3-3*. a**, Phenotypes of 4-week old plants. Bar: 1 cm. **b**, qRT-PCR analysis of the abundance of pri-miRNAs. pri-miRNA levels were normalized to those of UBQ5 and the values in wild-type were set as 1. Error bars indicate SD of three replicates.

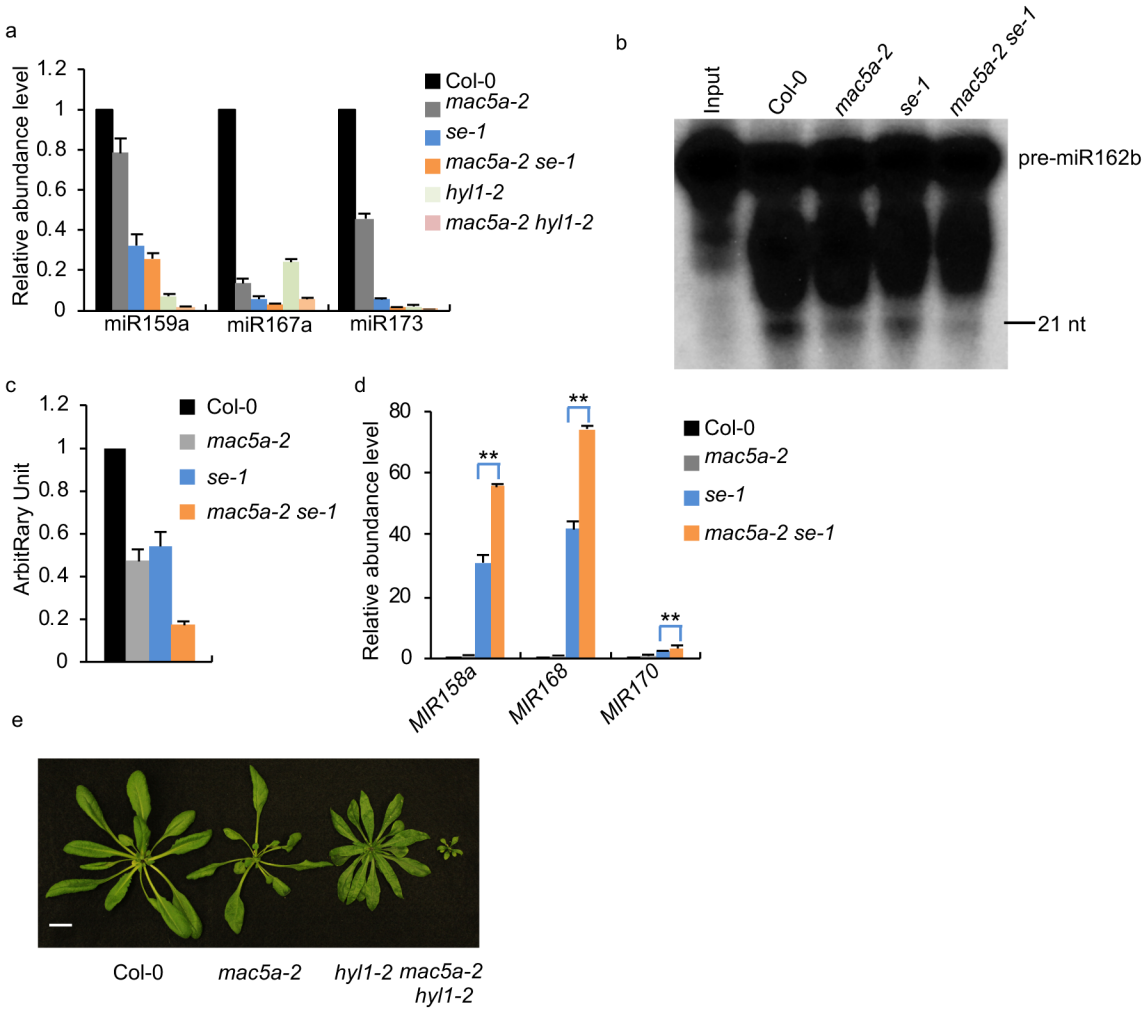


**Figure S9. MAC5A, SE, and HYL1 synergistically regulate miRNA processing and plant development**. **a**, miRNA abundance detected by qRT-PCR in the indicated plants. **b**, pre-miR162b processing assay using protein extracts from the indicated plants. **c**, Quantification of miRNA production (the value in Col-0 was set as 1). **d**, qRT-PCR analysis of pri-miRNA abundance in Col-0, *mac5a-2*, *se-1*, and *mac5a-2 se-1*. **e**, 30d-old seedlings of Col-0, *mac5a*, *hyl1*, and *mac5a hyl1*. Bar: 1 cm.

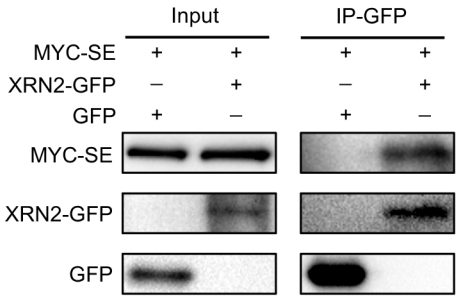


**Figure S10. Co-IP shows the interactions between SE and XRN2.** XRN2-GFP and GFP were co-expressed with MYC-SE in *N. Benthamiana*. IP was performed using anti-GFP antibodies. MYC-SE, XRN2-GFP, and GFP were detected by immunoblot.

**Table S1: DNA oligos used in this study.**

| Name | Sequence | Application |
|---|---|---|
| **Primers for genotyping** | | |
| salk_142085LP | ATGGACATTCAGCACCTCTTG | *mac5a-2* |
| salk_142085RP | TCTAAGCTATTTGGTTCCCGC | |
| GK_419F11LP | CAGCTTCAACACTAAGAAAC | *mac5b-1* |
| GK_419F11RP | TAGAGTGTGGATCGAAACGG | |
| se-1F | TGCCCCTTTTCTGTTGTCTC | *se-1* |
| se-1R | GGGTAATGGACTCATAGCACAGA | |
| hyl1-2F | AGTTCTCCCAGCGCTAATCTC | *hyl1-2* |
| hyl1-2R | TTCTTGGAAATTGGATTGCAG | |
| salk_041148 LP | CTTGAGATCTGATGCGAAAGG | *xrn2-1* |
| salk_041148 RP | GTCATCTCGTATCCGAGGAGG | |
| xrn3-3 LP | GCCTTCGATTTCAACAGGC | *xrn3-3* |
| xrn3-3 RP | GAAATCGAACACAAATCCG | |
| xrn4-5 LP | GTTTCTTGGTTGTTGCAGCTC | *xrn4-5* |
| xrn4-5 RP | TCATGACGAATTCCTTTGAGG | |
| LBa1 | TGGTTCACGTAGTGGGCCATCG | T-DNA |
| LB1 | GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC | T-DNA |
| **Primers for constructs** | | |
| proMAC5A-1F | CACCCGGTTCCAATGTCACCGGCAG | MAC5A genomic DNA |
| MAC5Acds-1R | CTGAGACGAACCAGTAGCTGTA | MAC5A genomic or CDS sequence |
| MAC5Acds-1F | CACC ATGGCTCACAGAATACTGAGA | MAC5A CDS sequence |
| XRN2cds-1F | CACCATGGGAGTTCCGTCGTTCTAC | XRN2 CDS sequence |
| XRN2cds-1R | AGCTGTTTTGGGAGGAGCTCC | XRN2 CDS sequence |
| MAC5A-2F(Sal1) | ACGCGTCGACATGGCTCACAGAATACTGAGA | BiFC |
| MAC5A-2R(Pst1) | AACTGCAG CTACTGAGACGAACCAGTAGC | BiFC |
| MAC5A-3F(Not1) | ATGCGGCCGCATGGCTCACAGAATACTGAGA | MBP-MAC5A |
| MAC5A-3R(Sal1) | GCGTCGACCTACTGAGACGAACCAGTAGC | MBP-MAC5A |
| XRN2cds-1F | CACC ATGGGAGTTCCGTCGTTCTAC | XRN2-GFP |
| XRN2cds-1R | AGCTGTTTTGGGAGGAGCTCC | XRN2-GFP |
| **Primers for stem-loop PCR** | | |
| miR159a stemloop RT | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGC CAACTAGAGC | stem-loop PCR |
| miR159a stemloop forward | CGGCGGTTTGGATTGAAGGGA | stem-loop PCR |
| miR161 stemloop RT | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGC CAACACCCCG | stem-loop PCR |
| miR161 Forward | CGGCGG TGAAAGTGACTACAT | stem-loop PCR |
| miR167a stem-loop RT | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGC CA ACTAGATC | stem-loop PCR |
| miR167a Forward | GGCGTC TGAAGCTGCCAGCAT | stem-loop PCR |
| miR169i stem-loop RT | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACCAGGCA | stem-loop PCR |
| miR169i forward | GGCGTC TAGCCAAGGATGACT | stem-loop PCR |
| miR173 stem-loop RT | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCA ACGTGATT | stem-loop PCR |
| miR173 forward | GTTGGC TTCGCTTGCAGAGAG | stem-loop PCR |
| U6 stem-loop RT | GTGCAGGGTCCGAGGTTTTGGACCATTTCTCGAT | stem-loop PCR |

| | | |
|---|---|---|
| U6 forward | GGAACGATACAGAGAAGATTAGCA | stem-loop PCR |
| Universal | GTGCAGGGTCCGAGGT | stem-loop PCR |
| **Probe sequence for Northern blot** | | |
| miR156 | GTGCTCACTCTCTTCTGTCA | Northern blot |
| miR319/159 | GGG+AGC+TCC+CTT+CAG+TCC+AA | Northern blot |
| miR164 | TGCACGTGCCCTGCTTCTCCA | Northern blot |
| miR166 | GG+GGA+ATG+AAG+CCT+GGT+CCG+T | Northern blot |
| miR171 | G+ATA+TTG+GCG+CGG+CTC+AAT+CA | Northern blot |
| miR390 | G+GCG+CTA+TCC+CTC+CTG+AGC+TT | Northern blot |
| miR169 | T+CGG+CAA+GTC+ATC+CTT+GGC+TG | Northern blot |
| miR167 | T+AGA+TCA+TGT+TGG+CAG+TTT+CA | Northern blot |
| miR173 | G+TGA+TTT+CTC+TCT+CGA+AGC+GAA | Northern blot |
| U6 | TCATCCTTGCGCAGGGGCCA | Northern blot |
| **Primers for RT-PCR** | | |
| qAPS3-1F | GCGGCGGATTTGCCGAGAGT | RT-PCR |
| qAPS3-1R | CCCACGAAGAGGACTAGCCCAAC | RT-PCR |
| qARF6-1F | GGGGTCCTTTGGTAGGTCGC | RT-PCR |
| qARF6-1R | CGGCCAGGGGTCATCACCAA | RT-PCR |
| CUC1qF | CGCCTTGACGGCAAATTCTCTTAC | RT-PCR |
| CUC1qR | GATGATCGGAGCAATTGCAGAACC | RT-PCR |
| qCUC2-1F | GGAGGAGGAGGAGCAACTGTGAG | RT-PCR |
| qCUC2-1R | GAGTGAGACGGAGGAAGGAGAGC | RT-PCR |
| qSPL9-F | CAAGGTTCAGTTGGTGGAGGA | RT-PCR |
| qSPL9-R | TGAAGAAGCTCGCCATGTATTG | RT-PCR |
| DCL1 qF | CGTTGTTATGCGTTTCGACCTTGC | RT-PCR |
| DCL1 qR | AACGCTGCGTGAGATACATTTCCTC | RT-PCR |
| HYL1 qF | TTGCCTGGATTCTTCAATCGTAAGG | RT-PCR |
| HYL1 qR | TAGGTTCTTGCATAATCCCGTTTCG | RT-PCR |
| SE qF | CCACCGCCTCGTAGGGATTACA | RT-PCR |
| SE qR | CCACCATGGTCATACCCAAATCTTC | RT-PCR |
| DDLqF | ATGAGCCCCCAGAGGCTAGAAAAC | RT-PCR |
| DDLqR | CTGCAAGATGGGTGATCCGTAGGAA | RT-PCR |
| CBP20qF | ACCGGCCTATTCGTGTGGATTTTG | RT-PCR |
| CBP20qR | TGCCTTTGTGCTTCGAGTTCCTTC | RT-PCR |
| CBP80qF | TCTGGCAACTGCAACAGTATCCGTA | RT-PCR |
| CBP80qR | GGCAGCAGATGATAGCAATGTTTCG | RT-PCR |
| HEN1 qF | TTAGGATGACACCCCCTGATGCTG | RT-PCR |
| HEN1 qR | AAAAGCCGCCTCCATTCGTTCTTC | RT-PCR |
| MIR156a qF | AAGGGGGTCTTCTATCATCAGGA | RT-PCR |
| MIR156a qR | TGATTGGAATATGCCCTAAAGAGTG | RT-PCR |
| MIR156b qF | GAGAGATGGTGATTGAGGAATGC | RT-PCR |
| MIR156b qR | CAGAGATAGGCAACTGACAGAAAGAG | RT-PCR |
| MIR158a qF | GTGATGACGCCATTGCTCTTT | RT-PCR/RIP |
| MIR158a qR | TGTGACTTTAGATGCCCTTGTTCA | RT-PCR/RIP |
| MIR159a-F | TCAGGAGCTTTAACTTGCCCTTT | RT-PCR |
| MIR159a-R | CACGCTAAACATTGCTTCGGAAT | RT-PCR |
| MIR164a-F | CCCTCATGTGCTTGGAAATG | RT-PCR |
| MIR164a-R | GCAAATGAGACGGATTTCGTG | RT-PCR |
| pri-miR166a-F | GACTCTGGCTCGCTCTATTCA | RT-PCR |
| pri-miR166a-R | TGGTCCGAAGACGCTAAAAC | RT-PCR |
| MIR167a qF | TGTTGTGTTTCATGACGATGG | RT-PCR/RIP |
| MIR167a qR | AGCTCACAAAATCAGACTGAAGA | RT-PCR/RIP |
| MIR167b qF | TCCACAAGGGAACAAGTGAA | RT-PCR/RIP |

| | | |
|---|---|---|
| MIR167b qR | TTTCTTTCAATCGGCATGTG | RT-PCR/RIP |
| MIR172b-qF | GTAGGCGCAGCACCATTAAG | RT-PCR/RIP |
| MIR172b-qR | TTTGTAGCCGTCGATTGTTG | RT-PCR/RIP |
| MIR319a-F | | |
| MIR319a-R | | |
| GUS-F | CGATGTCACTCCGTATGTTATTG | RT-PCR |
| GUS-R | CAGTTCTTTCGGCTTGTTGC | RT-PCR |
| UBQ5-N | GGTGCTAAGAAGAGGAAGAAT | RT-PCR |
| UBQ5-C | CTCCTTCTTTCTGGTAAACGT | RT-PCR |
| **Primers for *in vitro* RNA binding** | | |
| T7miR162b-p3 | TAATACGACTCACTATAGGGAAAGAGTGAAGTCGCTGGAG | pri-miR162b Probe |
| miR162b-p4 | CATGAAGAGCAAGCAGCGCTGGATGC | pri-miR162b Probe |
| T7-premiR162bF | TAATACGACTCACTATAGGAGGCAGCGGTTCATCGATC | pre-miR162b Probe |
| premiR162bR | CTGGATGCAGAGGTTTATCGATC | pre-miR162b Probe |
| T7-premiR172bF | TAATACGACTCACTATAGGG GTCGTTGTTTGTAGGCGCAG | pre-miR172b Probe |
| premiR172bR | TTTGTAGCCGTCGATTGTTG | pre-miR172b Probe |
| UBQ5T7F | TAATACGACTCACTATAGGGATGCAGATCTTCGTGAAAAC C | ssRNA probe |
| UBQ R2 | GGATTCCTTCCTTGTCTTGGA | ssRNA probe |
| RNA/RNA blunt 1 | TAATACGACTCACTATAGGGCAACAACTGTCTCTTGGATCG TATATTGCCATTTATGTGTTGAGCCC | dsRNA probe |
| RNA/RNA blunt 2 | TAATACGACTCACTATAGGGCTCAACACATAAATGGCAAT ATACGATCCAAGAGACAGTTGTTGCCC | dsRNA probe |