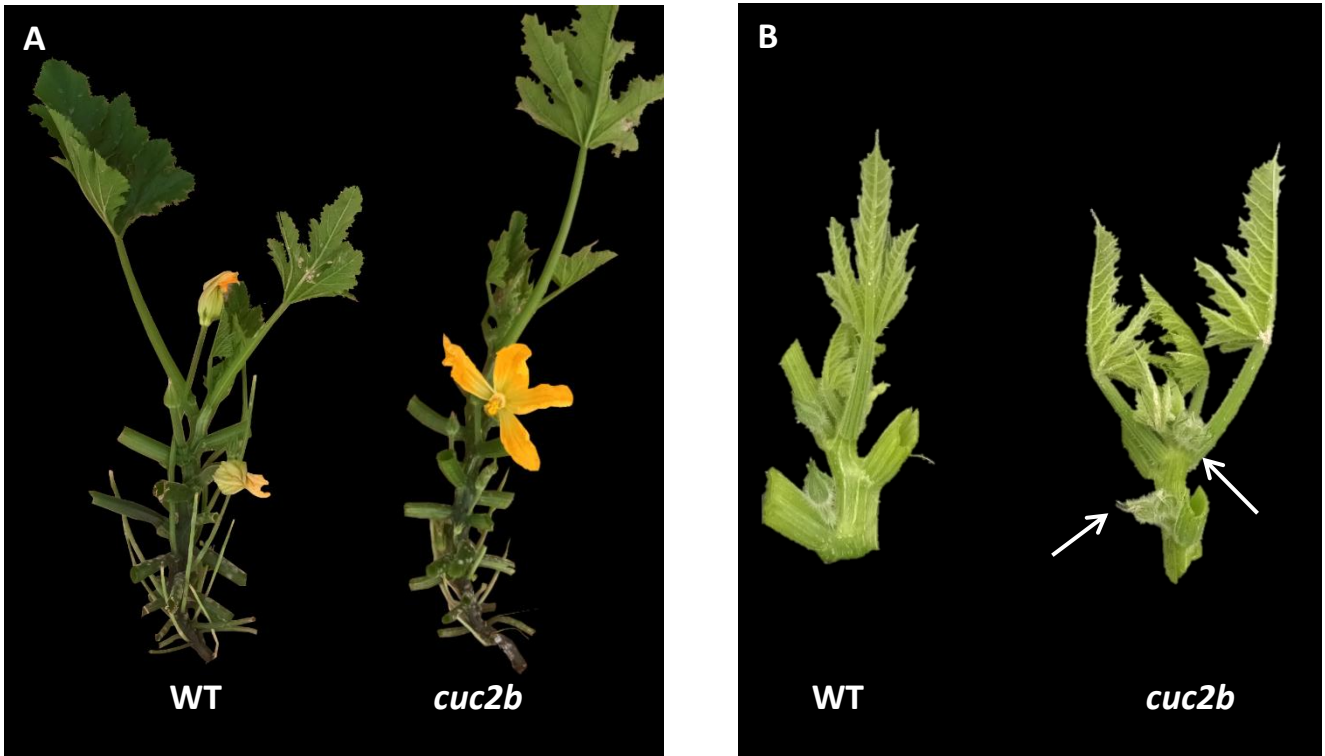


Supplementary Table S1. List of the CUC genes and proteins used in alignments and phylogenetic analysis.

Species	GeneID	Gene name
	111810386	<i>CpCUC1A</i>
	111803201	<i>CpCUC1B</i>
	111789288	<i>CpCUC2A</i>
<i>Cucurbita pepo</i>	111797633	<i>CpCUC2B</i>
	111805279	<i>CpCUC3A</i>
	111789055	<i>CpCUC3B1</i>
	111799080	<i>CpCUC3B2</i>
<i>Cucurbita maxima</i>	111476901	<i>CmaxCUC2</i>
<i>Cucurbita moschata</i>	111442545	<i>CmosCUC2</i>
<i>Cucumis sativus</i>	101205357	<i>CsCUC2</i>
<i>Cucumis melo</i>	103501784	<i>CmCUC2</i>
	820748	<i>AtCUC1</i>
<i>Arabidopsis thaliana</i>	835478	<i>AtCUC2</i>
	843975	<i>AtCUC3</i>
<i>Solanum lycopersicum</i>	100301934	<i>SIGOB</i>
<i>Capsicum annuum</i>	107877396	<i>CaCUC2</i>
<i>Prunus persica</i>	18779165	<i>PpCUC2</i>
<i>Vitis vinifera</i>	100246914	<i>VvCUC2</i>
<i>Medicago truncatula</i>	25487304	<i>MtCUC2</i>
<i>Carica papaya</i>	110808876	<i>CpaCUC2</i>
<i>Malus domestica</i>	103419033	<i>MdCUC2</i>

Supplementary Table S2: Primers used for gene expression and mRNA degradation analysis by qPCR.

Gene	Primer	Sequence
<i>CpACO1A</i>	F	CATAGAGTGATGACTCAGACAAGC
	R	CCATTGGACCCAAATTAGCA
<i>CpACS11A</i>	F	CGTCGTCTTAAGGCCTTTG
	R	GGTGTACCTAATTTAACGCAAC
<i>CpACO2B</i>	F	GGAGGGAGAGGAAGATAAGG
	R	TGGGTTATTGGAAAATGGAG
<i>CpWIP1B</i>	F	TCCCTACTCCATGCTTCAC
	R	TCCTCCTCATTCAACAAC
<i>CpACS27A</i>	F	CCAATACGGACGGTGAA
	R	GGAGAAGCTGAAGAAGGAAG
<i>CpCUC1A_mir164</i>	F	GGATCCCGAACCGAGTTCCT
	R	GACCGAGGGGGAGAGATCAG
<i>CpCUC1B_mir164</i>	F	GCCGACACCAGTATCAGCAC
	R	AGACGGACAGATTAGGACCGC
<i>CpCUC2A_mir164</i>	F	GCCACTTCTCGACTCGTCACTT
	R	GTTTCGGTGGAAAGCGGGTAG
<i>CpCUC2B_mir164</i>	F	GCCGCTGCTAGATTCGTCTT
	R	GAGGGGGAGTGGCAAATCG
<i>CpCUC2B_3'</i>	F	TACGATTTTGCCACTCCCCC
	R	AGGACATGTTGCCATGGTGG
<i>CpEF1α</i>	F	CGTCAAGAAGAAATAAGCCA
	R	CTACTACGAGAGAGAGAGCCG
<i>CpACT</i>	F	CCTCTCAATCCCAAAGCTA
	R	CGGCCTGGATAGCAACATA



Supplementary Figure S1. Effects of the *cuc2b* mutation on vegetative development. **(A)** WT and *cuc2b* complete plant with removed old leaves and flowers, showing the main stem. The absence of lateral branches and unaltered apical dominance is observed. **(B)** Detail of the apical shoot of WT and *cuc2b* plants. White arrows indicate double flowers.

CpCUC2B LYSAPVAATAVSGN-ITDRDSCSYNS-----P--REHVSCFST-TAGGSFNIPS 265
CpCUC2A LYSAPATISTASGN-IPDRDRSYNS-----PAAREHVSCFST-TAGGSLNIPN 263
CpCUC1B A-----D-----PSEHELHVTCFSTNNVNY----- 225
CpCUC1A PYLSAAADTSISTSAFANREGSSLDD-----QREHELHVTCFSTNNLSYPAAS-- 249
CmaxCUC2 LYSAPVAATAVSGN-ITDRESFSYNS-----P--REHVSCFST-TTGGSFNIPS 265
CmosCUC2 LYSAPVAATAVSGN-ITDRDSCSYNS-----P--REHVSCFST-TTGGSFNIPS 266
CsCUC2 LYSAPATGTAASGN-ITDRDSCSYNS-----PTAREHVSCFSTTTAGGSFNMPN 268
CmCUC2 LYSAPATGTAASGN-ITDRDSCSYNS-----PTAREHVSCFSTTTAGGSFNIPS 267
CaCUC2 PYSTTATSSSVVIS--A-----RDH-----SFKKEHVSCFSTTATNSATIDPH 253
AtCUC2 TTLGY-----TDSSCSYDSRSTN--TTVTASAIITEHVSCFSTVPTTTTALGLD 274
CpaCUC2 TASTT-----AA-ATSLDSCSYDGHFSFSTSAASAAAAASSEHVSCFSTIAAAAAAGSS 260
MtCUC2 PYTAASTASFNGGQ-MCNYNS-SNNN-----TDLKEHVSCFSTTTSTTNNVSVH 276
PpCUC2 PYQNMN-----TAG-LTDRDSCSYDS-----PIPKEHVSCFSTNP-NSGFNLSS 257
VvCUC2 PYAGTSA-----AA-VNDRESCSYDGGESSNN---SNARDQHVVPWFSTIAAAAAAAN 259
MdCUC2 PYQPIS-----AAG-----DGCSYDS-----PIPREHVSCFSTNPSNNGFNLPA 252

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CpCUC2B --Y-----DFAT-PPPLLAVDPSPRFHRNMGLSAFPSLRSLQENLQ-LPFFYSPVN 313
CpCUC2A --Y-----DFAP-LPPLA-AADPSTRFHRNIGLSAFPSLRSLQENLQ-LPFFYSPAN 310
CpCUC1B -----PAGGGSDLSPSVFNNLSFQENLQ-APFFSP-VG 257
CpCUC1A -----F-----PAGGGPNLSVSAFPNLRSIQENLQ-APFFSP-VS 282
CmaxCUC2 --Y-----DFAT-PPPLLAVDPSPRFHRNMGLSAFPSLRSLQENLQ-LPFFYSPVN 313
CmosCUC2 --Y-----DFAT-PPPLLAVDPSPRFHRNMGLSAFPSLRSLQENLQ-LPFFYSPVN 314
CsCUC2 --Y-----DFAT-PSPLL-AADPSPRFHRNIGLSAFPSLRSLQENLQ-LPFFYSPVN 315
CmCUC2 --Y-----DFAT-PSPLL-AADPSPRFHRNIGLSAFPSLRSLQENLQ-LPFFYSPVN 314
CaCUC2 --S-----VFDI-SNTLH-----ALPFTHYGRNSTFPSLTSLHENLQ-LPLFSGGA- 295
AtCUC2 VN-----SFSRLPPLGDFDPPFRFVSR-NVSTQSNFRSFQENFNQPPYFGSSA 324
CpaCUC2 ASSTFDGFPPLQPP-QPQMYAGYDSFTRFPRSVGLSGFPSLRSLQENLQ-LPFFSSST 318
MtCUC2 --NNFNNSNGSFDLVS PSMNATMDPFARFQRNVGVSAFP SLRSLQDNLQ-LPFFSSAA 333
PpCUC2 --S-----CFDL-AH-----AQPPPQPSFGGVSAFP SLRSLQENLQ-LPFFSPMS 299
VvCUC2 SFNAHH--QPPFDL-APPSIIGSIDPSRFPRNGAVPAFPNLRSIQENLH-LPFFSQVA 315
MdCUC2 --S-----CFDL-AH-----PPPQPPNFGGVSAFP SLRSLQENLQ-LPFFSPMP 294

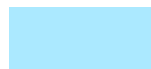
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CpCUC2B LPSPPPLHGHNMSSSADVACNSAGNNG---VSD-----EPRSMNSTEL-DCM 359
CpCUC2A LPSTTAVH--HG-NISSADIGACSSAGNWA---VSD-----ETRFMNSTEL-DCM 353
CpCUC1B PPLVHLLT-----DAELVCGG---DGPAP-----DSRLVGSADMAAYM 294
CpCUC1A PPLVHLLS-----GAADVDCG---GAPSPE-----DSRTIGSADLGAYM 319
CmaxCUC2 LPSPPPLHGHNIPSSTDVVACNSAGNWA---VSD-----EPRSMNSTEL-DCM 359
CmosCUC2 LPSPPPLHGHNMSSSADVAACNSAGNWA---VSD-----EPRSMNSTEL-DCM 360
CsCUC2 LCSPPVP---VL-NNGN-DVAGCSSGGNNG---VSD-----EPKVMNSTEL-DCM 356
CmCUC2 LCSPPVP---AL-NNGNNDVAACSSGGNNG---VAD-----EPKVMNSTEL-DCM 356
CaCUC2 -----SAMHGGFSNSIGNWSVPE-----TQKVEQSEL-DCM 325
AtCUC2 STMTSSVN-----LPSFQGGGVSGVNYWLPATAEE-----NESKVGVLHAGL-DCI 370
CpaCUC2 SSPSASIP-----PPLFQGGGGAFFSWPFM--PDE---GG---GKLCMGASEL-DCM 361
MtCUC2 AAQPPFPGGD-----ILGGWMPSEEQRVVVDSSGGGLNMGGGLGSSEL-DCM 378
PpCUC2 GHQPVHVGSSGGGGSTIDLGLSSAGSWPTPPHQE-----EPRTVGPTTEL-DCM 349
VvCUC2 PPIPSSGD-----PSTEMGITNSAGNWPAPENQKM---DN---GRLPMGATEL-DCM 360
MdCUC2 AHPPVHVGSVAGGGSSVDFT----GNWP---PVSE-----EARAVGPTTEL-DCM 336

: : : :

CpCUC2B WSY----- 362
CpCUC2A WSY----- 356
CpCUC1B WTF----- 297
CpCUC1A WTF----- 322
CmaxCUC2 WSY----- 362
CmosCUC2 WSY----- 363
CsCUC2 WSY----- 359
CmCUC2 WSY----- 359
CaCUC2 WTY----- 328
AtCUC2 WNY----- 373
CpaCUC2 WSF----- 364
MtCUC2 WNY----- 381
PpCUC2 WTYKTECL 358
VvCUC2 WSY----- 363
MdCUC2 WNYKTECL 345

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NAC domain

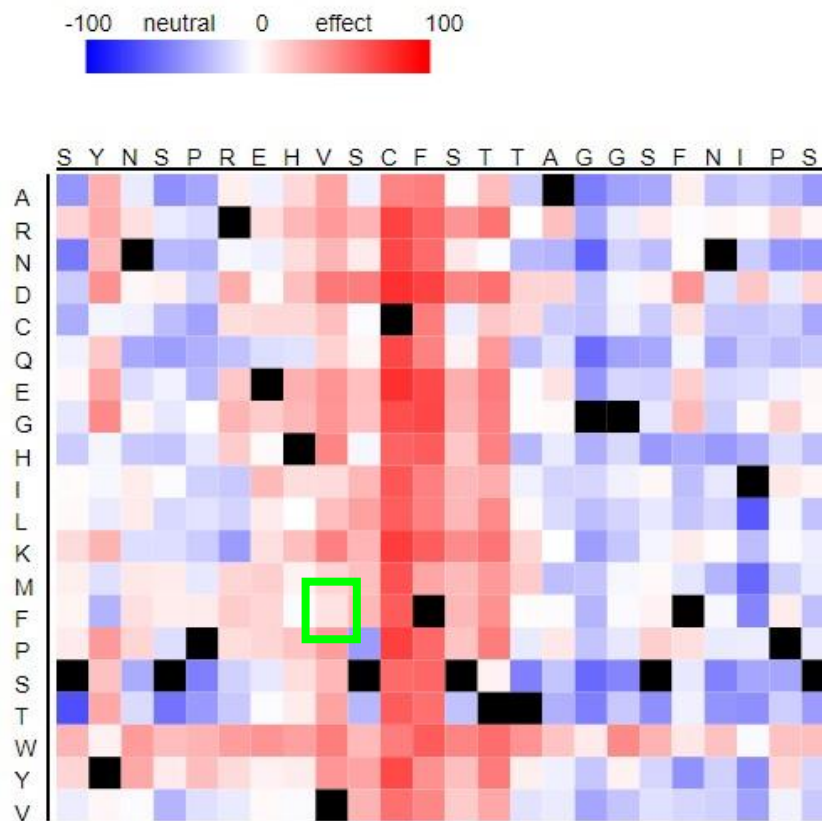


miR164 binding site



cuc2b mutation effect: S251F

Supplementary Figure S2: Multiple alignment of *CpCUC2* amino acid sequences with homologous *CUC2* proteins from diverse species. Blue boxes indicate the conserved NAC domain, yellow box the amino acid sequence of the miR164 binding site. The amino acid transition cause by the *cuc2b* mutation is highlighted in red.

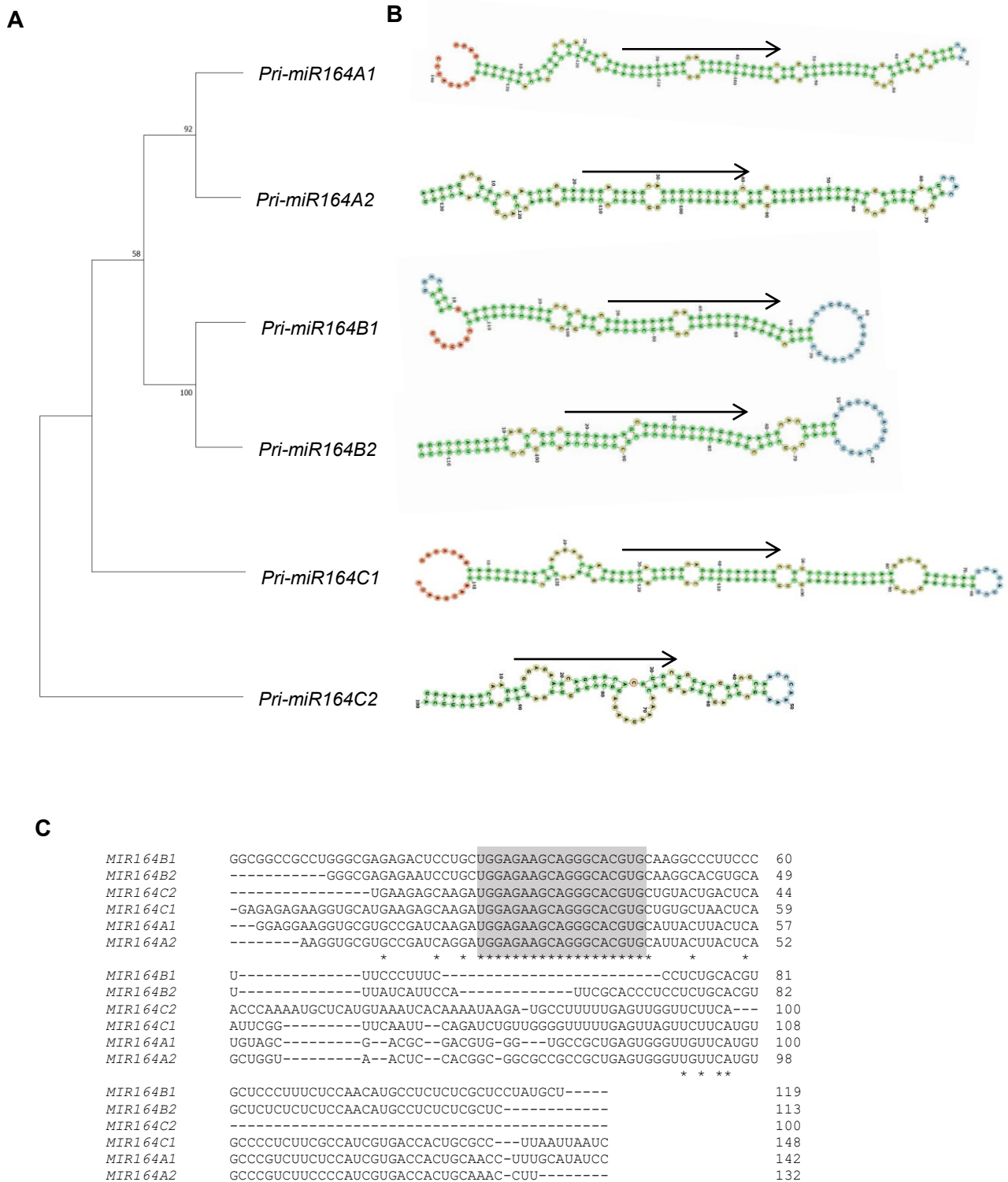


Supplementary Figure S3: Protein stability analysis of the S251F change in CUC2 protein. Heat map obtained by SNAP2 tool suggest a reduced effect of the mutation in the protein function.

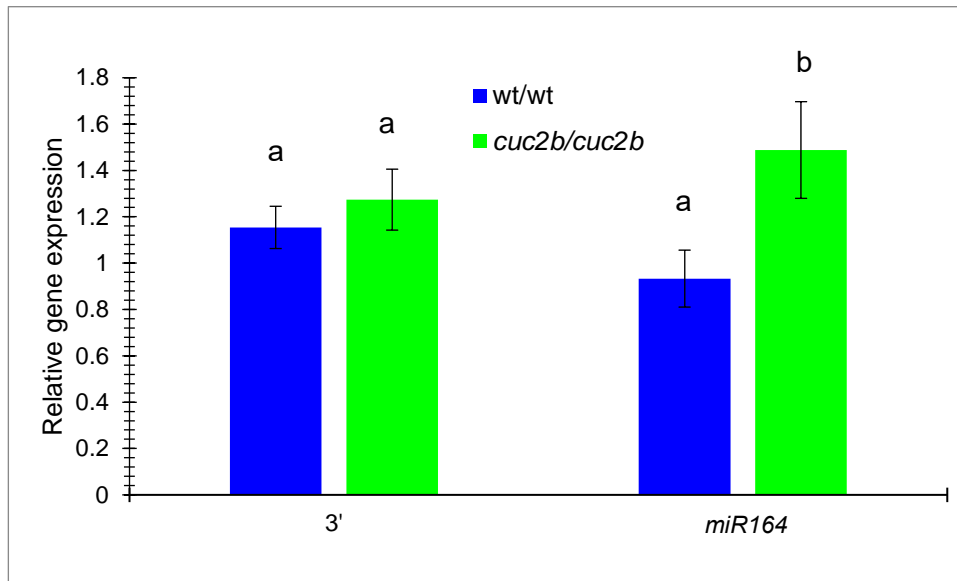
Consensus	UGGAGAAGCAGGGCACGUGCA	
<i>CpmiR164</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>CmmiR164a</i>	UGGAGAAGCAGGGCACGUGC U	21
<i>CmmiR164b</i>	UGGAGA G GCAGGGCAC A UGC U	21
<i>CmmiR164c</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>CmmiR164d</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>AtmiR164a</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>AtmiR164b</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>AtmiR164c</i>	UGGAGAAGCAGGGCACGUGC G	21
<i>CpamiR164a</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>CpamiR164b</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>CpamiR164c</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>CpamiR164d</i>	UGGAGAAG GGG GCACGUGCA	21
<i>CpamiR164e</i>	UGGAGAAG GGG GCACGUGCA	21
<i>MtmiR164a</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>MtmiR164b</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>MtmiR164c</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>MtmiR164d</i>	UGGAGAAGCAGGGCAC A UGC U	21
<i>PpmiR164a</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>PpmiR164b</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>PpmiR164c</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>PpmiR164d</i>	UGGAGAAGCAGGGCAC A UGC U	21
<i>VvmiR164a</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>VvmiR164b</i>	UGGAGAAGCAGGGCAC A UGC U	21
<i>VvmiR164c</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>VvmiR164d</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>SlmiR164a</i>	UGGAGAAGCAGGGCACGUGCA	21
<i>SlmiR164b</i>	UGGAGAAGCAGGGCACGUGCA	21

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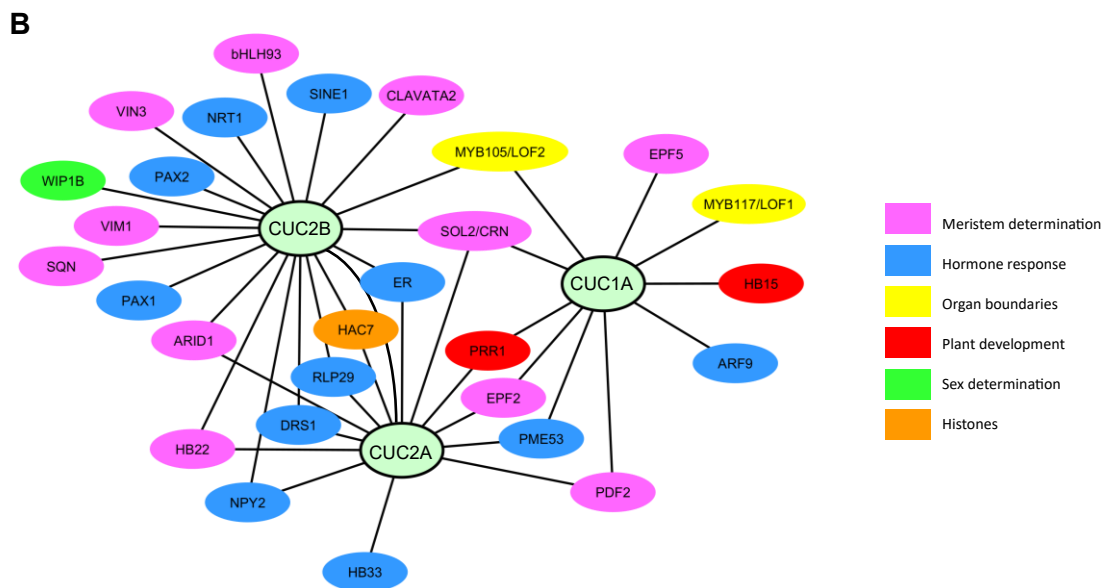
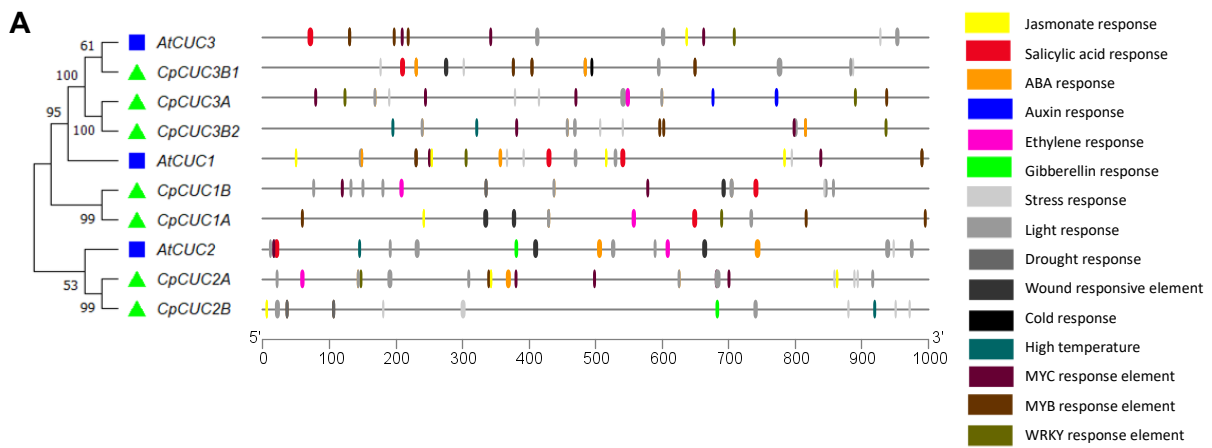
Supplementary Figure S4: Multiple alignment of *CpmiR164* mature sequence with homologous *miR164* from diverse plant species.



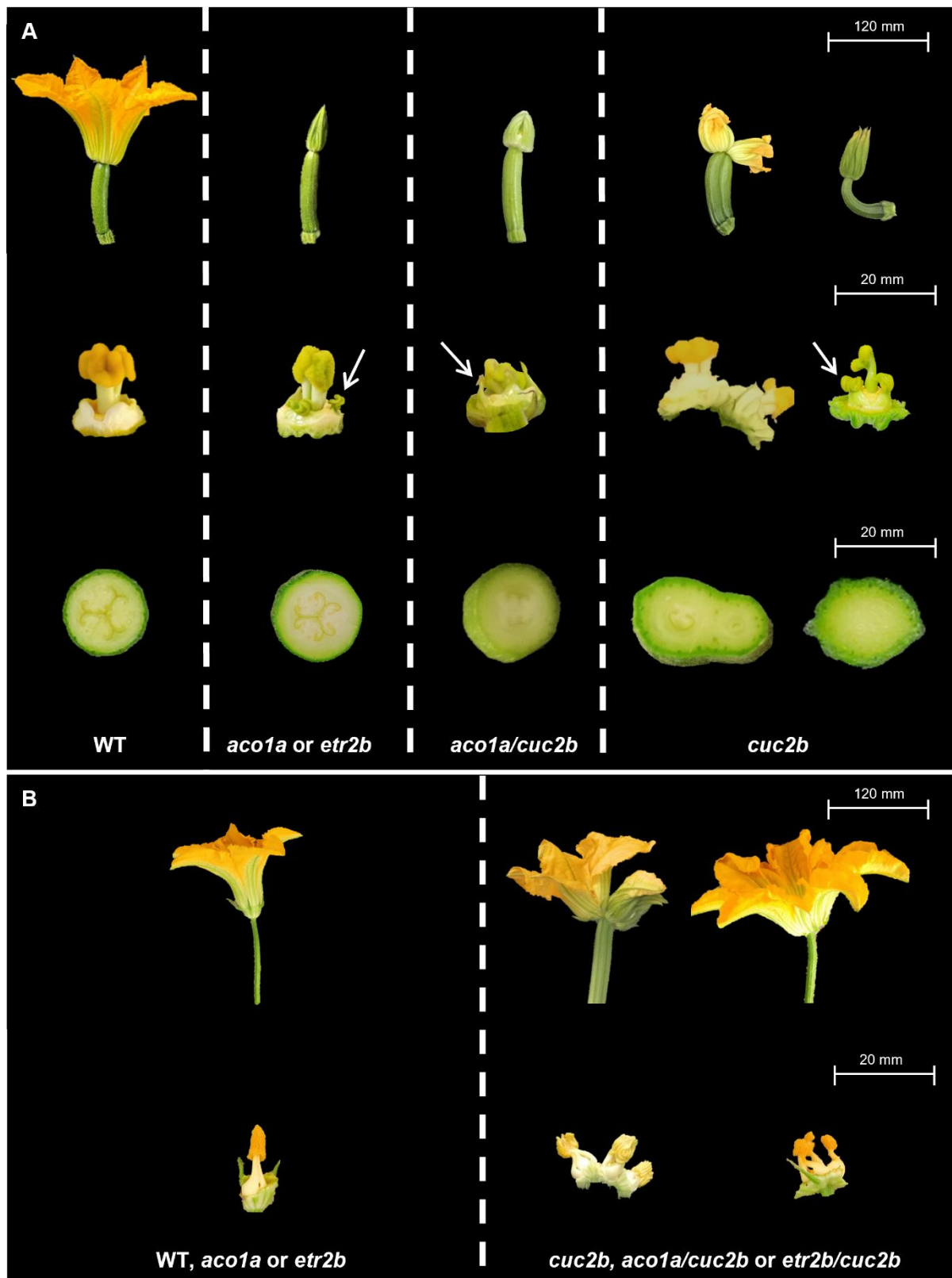
Supplementary Figure S5: Loci encoding *miR164* in *C. pepo*. (A) Phylogenetic tree and (B) secondary structure of six *pri-miRNA164* encoded by different genes in *C. pepo*. Black arrows indicate the position of *miR164* mature sequence. (C) Multiple alignment of the *MIR164* transcripts of *C. pepo*. Grey shadow shows the mature *miR164*.



Supplementary Figure S6: qRT-PCR of *CpCUC2B* gene in the apical shoots of WT and *cuc2b* plants after female flowering. Left: Relative gene expression obtained with primers designed in the 3' region of the gene. Right: Relative gene expression obtained with primers designed in the flanking regions of *miR164* binding site. Different letters indicate significant differences between samples for each pair of primers (ANOVA, $p \leq 0.05$); nd, no detectable expression.



Supplementary Figure S7. *CUC* genes regulation network. (A) Cis-element analysis of *CUC* genes promoters. Elements related to hormones, stresses, and other transcription factors are shown. **(B)** Gene regulatory network of *CUC* transcription factors predicted by ARACNE algorithm.



Supplementary Figure S8. Phenotypes of single and double mutants of *etr2b*, *aco1a* and *cuc2b*. **(A)** Pistillate flowers of each genotype. Up, complete pistillate flower; middle, detail of style and stigma of pistillate flowers; and down, transverse sections of ovaries. WT female flowers develop style and stigma, while bisexual *aco1a* and *etr2b* flowers develops both carpels and stamens. The double mutant *aco1a/cuc2b* produces bisexual flowers with stamens and defective carpels. Only 2.4% of the total flowers of *cuc2b* were bisexual, developing stamens and defective carpels, and an ovary with no endocarp and placental tissue. The double mutant *etr2b/cuc2b* only produces male flowers. White arrows indicate stamens of pistillate flowers. **(B)** Male flowers of the different genotypes. WT, *aco1a* and *etr2b* plants developed regular male flowers. The single *cuc2b* and the double *aco1a/cuc2b* and *etr2b/cuc2b* mutants develop single or double male flowers with increased number of floral organs.