Materials and methods

1 Vector construction

For the construction of pW501, the pKSE401 vector (Xing et al., 2014) was chosen as the backbone. The single guide RNA was removed by *Hind*III digestion and re-ligation. Then the *Cas9* gene was removed by *Xba*I and *Sac*I digestion, and replaced with DsRed by Gibson Assembly using the primer pair DsRed-F-XbaI/ DsRed-R-SacI.

For the construction of vectors with DRs, the *UBQ10* promoter and *Hsp* terminator were amplified with UBQ10p-F/UBQ10p-R and HspT-F/HspT-R, respectively, from the *Arabidopsis thaliana* Col-0 genome DNA. These fragments were introduced into the pW501 vector digested with *Hind*III by Gibson Assembly. For the expression of *WUS*, a DNA fragment containing the *Nos* promoter, *WUS* coding sequence and *CaMV* terminator was synthesized (**Supplemental sequences**). Then the *WUS* expressing cassette was amplified with the primer pair WUS-F/WUS-R and inserted into pW501 by Gibson Assembly. Other DRs were amplified with the corresponding primers and inserted into the *Kpn*I site between the *UBQ10* promoter and *Hsp* terminator.

For the coding optimization of *AtGRF5*, the codons rarely used in *Arabidopsis*, were replaced with synonymous codons with high usage frequencies. Two *Bsa*I sites in the *AtGRF5* sequence were also removed. The final sequence fragment (listed in **Supplemental sequences**) was synthesized at Sangon Biotech.

For the genome editing vector, the GRF5 expression cassette was amplified with primers ZHW512-F/ZHW512-R and inserted into the *EcoR*I site of the CRISPR/Cas9 genome editing vector pHSE401, generating the pZHW512 vector. Then two spacers targeting *ClPDS* were added by PCR with primers ClaPDS-F/ClaPDS-R using the pCBC-DT1T2 plasmid (Xing et al.,

2014) as the template, and the PCR product was inserted into the pZHW512 vector digested with *Bsa*I by Gibson Assembly.

All of the primers are listed in **Supplemental Tab. 1.**

2 Watermelon transformation

The genetic transformation was conducted as previously described with slight modifications (Tian et al., 2017). In brief, surface-sterilized watermelon seeds were sown on Murashige and Skoog (MS) solid medium for 3 days in the dark. Then the middle parts of the cotyledons without embryo were cut into 1.5 × 1.5 mm pieces as explants. *A. tumefaciens* strains harboring the indicated binary vectors were co-cultivated with the cotyledon fragments in the dark for 3 days on MS solid medium containing 1.5 mg/L 6-BA. Then the cotyledon fragments were transferred onto selective induction medium containing 2 mg/L 6-BA, 0.2 mg/L IAA, 50 mg/L Kan, and 100 mg/L Timentin. The regenerated adventitious buds were excised and transferred onto elongation medium containing 0.1 mg/L 6-BA, 0.01 mg/L NAA, 100 mg/L Timentin, and 50 mg/L Kan. Plants with full leaves and stems were transferred to rooting medium containing 1mg/L IBA and 100 mg/L Timentin. Positive transgenic events were detected using the hand-held dual-wavelength fluorescent protein excitation light source LUYOR-3415RG (Luyor Corporation, Shanghai, China) or Zeiss SteREO Discovery.V20.

3 Detection of mutations

Genomic DNA was extracted from the transgenic watermelon plants using the CTAB method. Detection of the transgene was performed with the primers Cas9-F2/Cas9-R2 and DsRed-F3/ DsRed-R3. The PCR products with the primer pair PDS-T2-F/PDS-T2-R are used as the internal control. The target regions were amplified with two pairs of primers PDS-T1-F/PDS-T1-R and

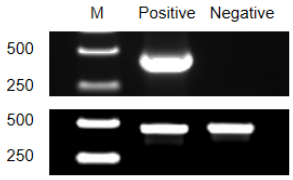
PDS-T2-F/PDS-T2-R. PCR products were sent for Sanger sequencing at Sangon Biotech to determine the types of mutation. The mutation types are decoded by the DSDecodeM program (http://skl.scau.edu.cn/dsdecode/). The primers used are listed in Supplementary Tab. 1.

Supplemental References

Tian, S., Jiang, L., Gao, Q., Zhang, J., Zong, M., Zhang, H., Ren, Y., Guo, S., Gong, G., Liu, F.*, et al.* (2017). Efficient CRISPR/Cas9-based gene knockout in watermelon. Plant Cell Rep *36*, 399-406.

Xing, H.L., Dong, L., Wang, Z.P., Zhang, H.Y., Han, C.Y., Liu, B., Wang, X.C., and Chen, Q.J. (2014). A CRISPR/Cas9 toolkit for multiplex genome editing in plants. Bmc Plant Biol *14*, 327.

(a)



(b)

Agrobacterium strain	Number of explants	Positive transformants	Efficiency (%)
GV3101	114	1	0.88
EHA105	109	1	0.92
LBA4404	60	0	0

(c)

Agrobacterium strain	Number of explants	Positive transformants	Efficiency (%)
GV3101	126	36	28.57
EHA105	116	8	6.90
LBA4404	76	0	0

(d)

Agrobacterium strain	pW501 (Control)			pW502 (AtGRF5)		
	Number of explants	Positive transformants	Efficiency (%)	Number of explants	Positive transformants	Efficiency (%)
GV3101	119	9	7.56	111	23	20.72
EHA105	111	11	9.90	111	14	12.61
LBA4404	113	0	0	112	0	0

Fig. S1 Verification of transgenic plants and the effect of different *Agrobacterium* strains on watermelon transformation. (a). PCR verification of the transgenic lines. M, marker. Left number indicate the size of markers. Positive and negative indicate DsRed florescent positive and negative plants. The PCR product of 424 bp corresponding to the DsRed sequence is shown in the upper panel. ClPDS is used as the internal control. (b). The transformation efficiency of watermelon cultivar WW150 using different *Agrobacterium tumefaciens* strains. carrying the pW501 plasmid. (c). The transformation efficiencies of watermelon cultivar WW150 obtained using different *Agrobacterium* strains carrying the pW502 plasmid. (d). AtGRF5-mediated transformation of a second watermelon cultivar 83166.

Table S1 List of primers used in this study.

Primers	Sequence
DsRed-F-XbaI	aacgatactcgagtaatctagaatggcctcctccgagaacgtc
DsRed-R-SacI	tacgaacgaaagctctgagctcctacaggaacaggtggtggcg
UBQ10p-F	aacgacggccagtgccactagtgatcaggatattcttgtttaag
UBQ10p-R	tcatatggtaccctgttaatcagaaaaactcaga
HspT-F	ACAGGGTACCatatgaagatgaagatgaaatat
HspT-R	tgttgacctgcaggcatgccttatctttaatcatattcca
WUS-F	aaaacgacggccagtgcccctagggaaccgcaacgttgaaggagcca
WUS-R	aacaagaatatcctgatcactagttttgatcttgaaagat
BBM -F	tgagtttttctgattaacagatggccactgtgaacaactggctc
BBM -R	atttcatcttcatcttcatatttaagtgtcgttccagacactgaa
GRF5-F	gtttttctgattaacagatgatgagtctaagtggaagtagcggga
GRF5-R	catcttcatcttcatatttagctaccagtgtcgagtcttgagtg
GRF4-F	GTTTTTCTGATTAACAGatggcgatgccgtatgcctctctt
GRF4-R	gcatGGCAGCGGCCGCgtacatctcgccggcgaacagcat
GIF1-F	cGCGGCCGCTGCCatgcagcagcaacacctgatgcaga
GIF1-R	catcttcatcttcatatctagctgccttcctcctcggtgcc
ipt-F	gtttttctgattaacagatggatcttagacttatttt
ipt-R	atcttcatcttcatatttaacacattccaaaaggaggtc
ZHW512-F	ccaattgattgacaacgaattcGATCAGGATATTCTTGTTTA
ZHW512-R	aacagctatgacatgattacCCTAGGcttatctttaatcatattcc
ClaPDS-F	agagtcgaagtagtgattgaatggagaacagcatctcggttttagagctagaaatagc
ClaPDS-R	ctatttctagctctaaaacggcaccactctagcggcatcaatctcttagtcgactctac
PDS-T1-F	ATGAATTTCGGGAAATTGGGTG
PDS-T1-R	TTTGGGTCACAAGACGGTCTTC
PDS-T2-F	GGATTGGAGCTAAGATTTAGTTG
PDS-T2-R	CAATGGTTTACTGGGACGTGCA
Cas9-R2	CCTCACATCGTAAACCTTGTAGTCCC
Cas9-F2	TCTACCTGTACTACCTCCAGAATGGC
DsRed-F3	GGCTCCAAGGTGTACGTGAAG
DsRed-R3	TGTAGTCCTCGTTGTGGGAGG

Supplementary Sequences

The complete coding sequence for different versions of *GRF5*, and the synthetic *WUS* expressing cascades.

>AtGRF5

ATGATGAGTCTAAGTGGAAGTAGCGGGAGAACAATAGGAAGGCCTCCATT
TACACCAACACAATGGGAAGAACTGGAACATCAAGCTCTAATCTACAAGTA
CATGGTTTCTGGTGTTCCTGTCCCACCTGAACTCATCTTCTCCATTAGAAG
ATCATTGGACACTTCCTTGGTTTCTAGGCTCCTTCCTCACCAATCCCTTGG
ATGGGGGTGTTACCAGATGGGATTTGGGAGAAAACCAGATCCAGAGCCA
GGAAGATGCAGAAGAACAGATGGTAAGAAATGGAGATGCTCAAGAGAGG
CTTACCCAGATTCTAAGTACTGTGAAAAACACATGCACAGAGGAAGAAAC
CGTGCTAGAAAATCTCTTGATCAGAATCAGACAACAACAACTCCTTTAACA
TCACCATCTCTCTCATTCACCAACAACAACAACCCAAGTCCTACCTTGTCA
TCTTCTTCTTCATCTAATTCATCTTCTACTACTTATTCTGCTTCATCTTCATC
TATGGATGCTTACAGTAACAGTAATAGGTTTGGGCTTGGTGGAAGTAGTAG
TAACACTAGAGGTTATTTCAACAGCCATTCTCTTGATTATCCTTATCCTTCT
ACTTCACCTAAACAACAACAACAAACTCTTCATCATGCTTCCGCTTTGTCA
CTTCATCAAAATACTAATTCTACTTCTCAGTTCAATGTCTTAGCTTCTGCTA
CTGACCACAAAGACTTCAGGTACTTTCAAGGGATTGGGGAGAGAGTTGG
AGGAGTTGGGGAGAGAACGTTCTTTCCAGAAGCATCAAGATCATTTCAAG
ATTCTCCATACCATCATCACCAACAACCGTTAGCAACAGTGATGAATGATC
CGTACCACCACTGTAGTACTGATCATAATAAGATTGATCATCATCACACATA
CTCTTCATCTTCATCATCTCAACATCTCCATCACGACCATGATCATAGACA
GCAACAGTGTTTTGTTTTGGGTGCTGACATGTTCAACAAACCTACAAGAA
GTGTCCTTGCAAACTCATCAAGACAAGATCAAAATCAAGAAGAAGATGAG
AAAGATTCATCAGAGTCTTCAAAGAAGTCTCTACATCACTTCTTTGGTGAG
GACTGGGCACAGAACAAGAACAGTTCAGATTCTTGGCTTGACCTTTCTTC
CCACTCAAGACTCGACACTGGTAGCTAA

>AtGRF5 codon optimized

ATGATGAGTCTAAGTGGAAGTAGCGGGAGAACAATAGGAAGGCCTCCATT
TACACCAACACAATGGGAAGAACTGGAACATCAAGCTCTAATCTACAAGTA
CATGGTTTCTGGTGTTCCTGTCCCACCTGAACTCATCTTCTCCATTAGAAG
ATCATTGGACACTTCCTTGGTTTCTAGGCTCCTTCCTCACCAATCCCTTGG
ATGGGGGTGTTACCAGATGGGATTTGGGAGAAAACCAGATCCAGAGCCA
GGAAGATGCAGAAGAACAGATGGTAAGAAATGGAGATGCTCAAGAGAGG
CTTACCCAGATTCTAAGTACTGTGAAAAACACATGCACAGAGGAAGAAAC
CGTGCTAGAAAATCTCTTGATCAGAATCAGACAACAACAACTCCTTTAACA
TCACCATCTCTCTCATTCACCAACAACAACAACCCAAGTCCTACCTTGTCA
TCTTCTTCTTCATCTAATTCATCTTCTACTACTTATTCTGCTTCATCTTCATC
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TAACACTAGAGGTTATTTCAACAGCCATTCTCTTGATTATCCTTATCCTTCT
ACTTCACCTAAACAACAACAACAAACTCTTCATCATGCTTCCGCTTTGTCA
CTTCATCAAAATACTAATTCTACTTCTCAGTTCAATGTCTTAGCTTCTGCTA

CTGACCACAAAGACTTCAGGTACTTTCAAGGGATTGGGGAGAGAGTTGG
AGGAGTTGGGGAGAGAACGTTCTTTCCAGAAGCATCAAGATCATTTCAAG
ATTCTCCATACCATCATCACCAACAACCGTTAGCAACAGTGATGAATGATC
CGTACCACCACTGTAGTACTGATCATAATAAGATTGATCATCATCACACATA
CTCTTCATCTTCATCATCTCAACATCTCCATCACGACCATGATCATAGACA
GCAACAGTGTTTTGTTTTGGGTGCTGACATGTTCAACAAACCTACAAGAA
GTGTCCTTGCAAACTCATCAAGACAAGATCAAAATCAAGAAGAAGATGAG
AAAGATTCATCAGAGTCTTCAAAGAAGTCTCTACATCACTTCTTTGGTGAG
GACTGGGCACAGAACAAGAACAGTTCAGATTCTTGGCTTGACCTTTCTTC
CCACTCAAGACTCGACACTGGTAGCTAA

>WUS expressing cascade (Nos promoter-WUS-CaMV terminator)
GAACCGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGAGTTTAA
TGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCC
TAAGGTCACTATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCACTGAC
GTTCCATAAATTCCCCTCGGTATCCAATTAAAGCTAGCTTCCACCATGGAG
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GAGGCCGTTAGGGAAAAGATGCTAAGGCAGGGTTGGTTACGTTGACTCC
CCCGTAGGTTTGGTTTAAATATGATGAAGTGGACGGAAGGAAGGAGGAA
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CAAAACTCAAGAGGATAAAACCTCACCAAAATACGAAAGAGTTCTTAACTC
TAAAGATAAAAGATCTTTCAAGATCAAA