



Supplementary Materials

Do DEEPER ROOTING 1 homologs regulate the lateral root slope angle in cucumber (*Cucumis sativus*)?

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The following Supplementary Material is available for this article:

	1	10	20	30	40	59	Section 1
AtqSOR1 (Kitomi et al., 2020; PNAS) AT1G17400.1_ATNGR1/DRO3/LAZY2	(1)	MKFFGWMQNKLNGDHNRNSTSSASSHHVQEPREEFSDWPHELLAIGTFGTTSNSVSEN					
	(1)	MKFFGWMQNKLNGDHNRNSTSSASSHHVQEPREEFSDWPHELLAIGTFGTTSNSVSEN					
	60	70	80	90	100	118	Section 2
AtqSOR1 (Kitomi et al., 2020; PNAS) AT1G17400.1_ATNGR1/DRO3/LAZY2	(60)	E SKNVHEEIEAEKKCTAQSEQEEFPSSSVNLEDFTPEEVGKLQELMKLLSRTKKRKSD					
	(60)	E SKNVHEEIEAEKKCTAQSEQEEFPSSSVNLEDFTPEEVGKLQELMKLLSRTKKRKSD					
	119	130	140	150	160	177	Section 3
AtqSOR1 (Kitomi et al., 2020; PNAS) AT1G17400.1_ATNGR1/DRO3/LAZY2	(119)	VNRELMKNLPLDRFLNCPSLSSLEVDRRISNALSAVVDSSSEENKEEDMERTINVILGRCKE					
	(119)	VNRELMKNLPLDRFLNCPSLSSLEVDRRISNALSAVVDSSSEENKEEDMERTINVILGRCKE					
	178	190	200	210	220	236	Section 4
AtqSOR1 (Kitomi et al., 2020; PNAS) AT1G17400.1_ATNGR1/DRO3/LAZY2	(178)	I SIESKNNKKRDISKNSVSYLFKKIFVCADGISTAPSPSLRDTLQESRMKELLKMMHL					
	(178)	I SIESKNNKKRDISKNSVSYLFKKIFVCADGISTAPSPSLRDTLQESRMKELLKMMHL					
	237	250	260	270	280	295	Section 5
AtqSOR1 (Kitomi et al., 2020; PNAS) AT1G17400.1_ATNGR1/DRO3/LAZY2	(237)	K KINAQASSKPTSLTTKRYLQDKKQLSLKSEEEEETSERRSSSDGYKWKTDSDFIVLEI					
	(237)	K KINAQASSKPTSLTTKRYLQDKKQLSLKSEEEEETSERRSSSDGYKWKTDSDFIVLEI					

Figure S1. Alignment of AtqSOR1 (Kitomi et al., 2020) and AtNGR1/AtDRO3/AtLAZY2 (gene ID: AT1G17400.1) proteins. The sequences have 100% identity between each other.

Kitomi, Y., Hanzawa, E., Kuya, N., Inoue, H., Hara, N., Kawai, S., et al. (2020). Root angle modifications by the DRO1 homolog improve rice yields in saline paddy fields. *Proc. Natl. Acad. Sci. USA* 117(35), 21242–21250. doi: 10.1073/pnas.2005911117

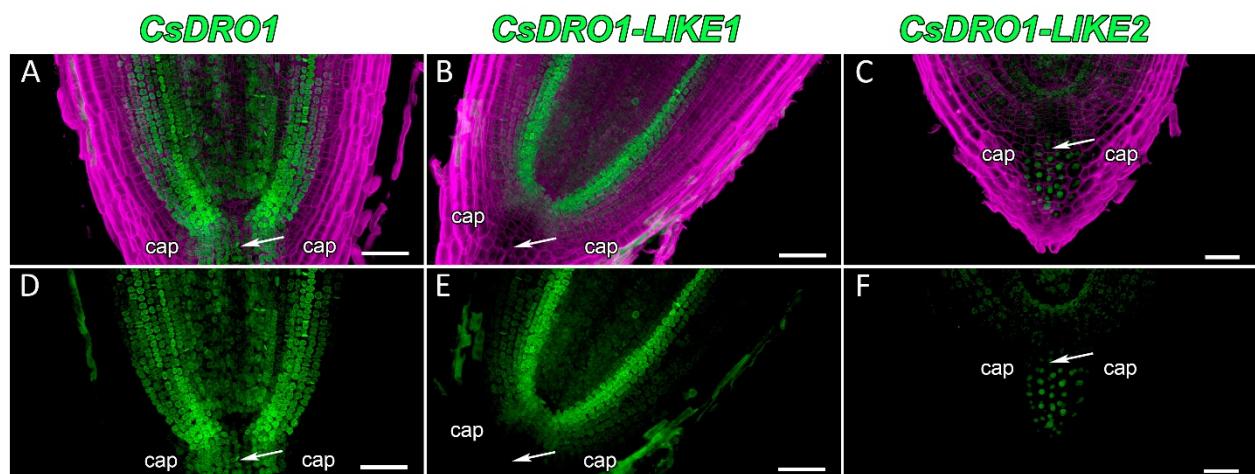


Figure S2. Localization of *CsDRO1*, *CsDRO1-LIKE1*, and *CsDRO1-LIKE2* expression in the root cap of *Cucumis sativus* (*pCsDRO::mNeonGreen-H2B*). Confocal laser scanning microscopy of longitudinal vibratome sections. Expression of *CsDRO1* (A,D) was active in the root cap columella (white arrow). (B,E) Activity of *pCsDRO1L1* was not observed in the root cap columella (white arrow). (C,F) Expression of *CsDRO1L2* was maintained in the apical part of the root cap columella (white arrow). Green channel, fluorescence of mNeonGreen; magenta channel, SR2200-stained cell walls. Maximum intensity projection of z-series: of 12 optical sections, 8 μm in depth. Scale bars – (A,B,D,E), 100 μm; (C,F), 50 μm.

Figure S3. CsDRO1 (biallelic homozygous mutations)

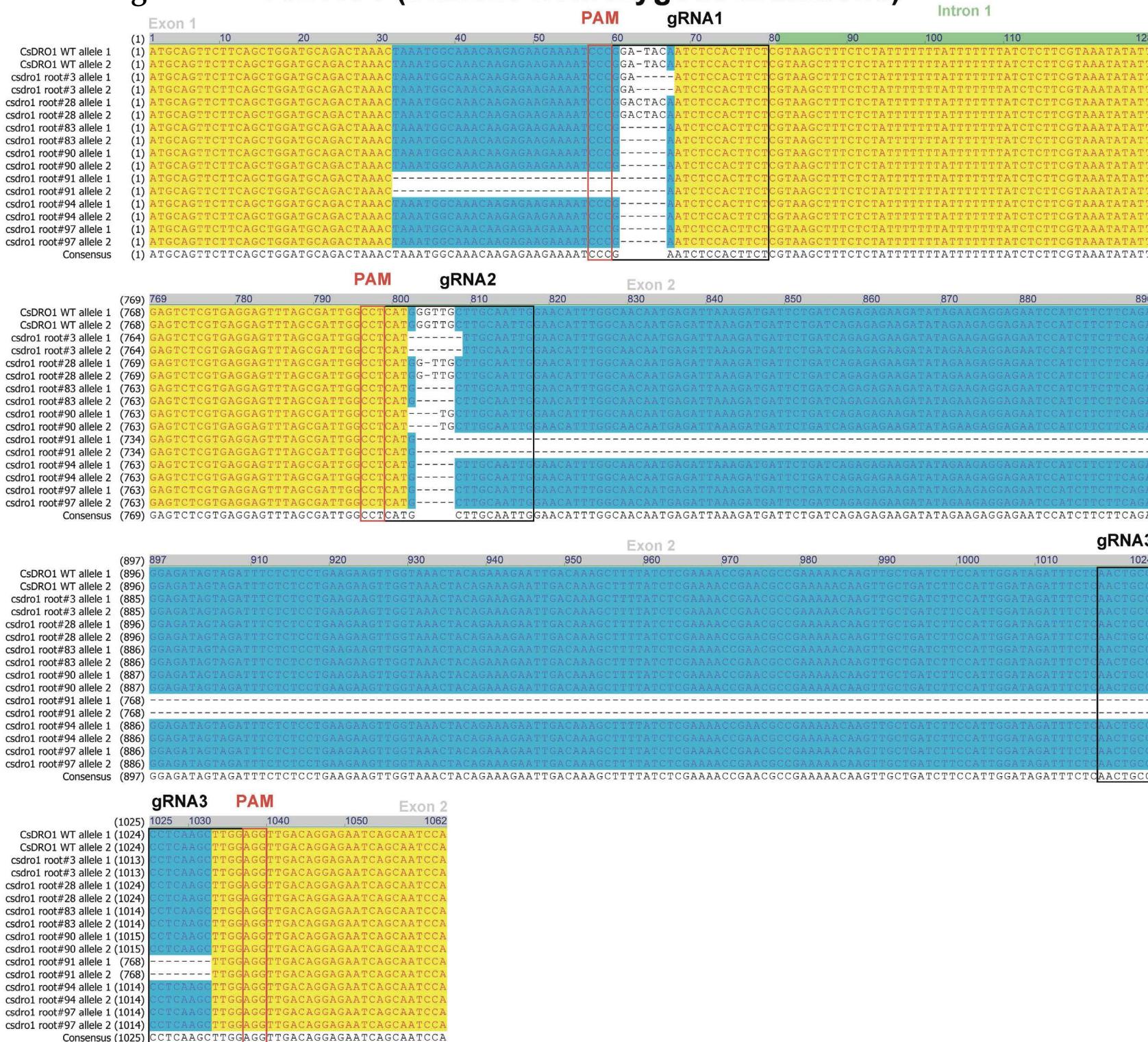


Figure S4. CsDRO1-LIKE1 (biallelic heterozygous mutations)

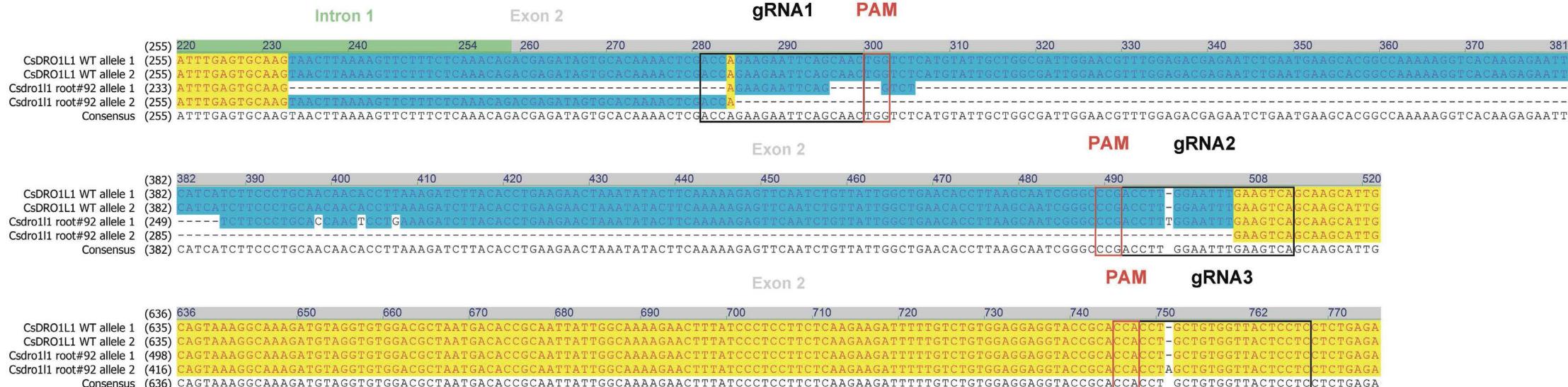


Figure S5. CsDRO1-LIKE1 (biallelic homozygous mutations)

	Intron 1	Exon 2		
			gRNA1	PAM
(255) CsDRO1L1 WT allele 1	255	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAAATTTCAGC	A
CsDRO1L1 WT allele 2	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAAATTTCAGC	A
Csdro1l1 root#20 allele 1	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAA-----	A
Csdro1l1 root#20 allele 2	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAA-----	A
Csdro1l1 root#38 allele 1	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAA-----	A
Csdro1l1 root#38 allele 2	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAA-----	A
Csdro1l1 root#44 allele 1	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAA-----	A
Csdro1l1 root#44 allele 2	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAA-----	A
Csdro1l1 root#63 allele 1	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAAATT-----	A
Csdro1l1 root#63 allele 2	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAAATT-----	A
Csdro1l1 root#86 allele 1	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAAATT-----	A
Csdro1l1 root#90 allele 1	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAAATT-----	A
Csdro1l1 root#90 allele 2	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAAATT-----	A
Consensus	(255)	255	ACAGACGAGATAGTCACAAAACCTCACCAGAAGAA	AACTGCTCATGTATTGCTGGCATTGGAACCTTGGAGACGAGAATCTGAATGAAGCACGGCCAAAAGGTACAAGAGAATT
			PAM	gRNA2
(382) CsDRO1L1 WT allele 1	382	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
CsDRO1L1 WT allele 2	(382)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#20 allele 1	(375)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#20 allele 2	(375)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#38 allele 1	(376)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#38 allele 2	(376)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#44 allele 1	(376)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#63 allele 1	(376)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#63 allele 2	(377)	382	-----	-
Csdro1l1 root#63 allele 2	(377)	382	-----	-
Csdro1l1 root#86 allele 1	(376)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#86 allele 2	(376)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	-
Csdro1l1 root#90 allele 1	(291)	382	-----	-
Csdro1l1 root#90 allele 2	(291)	382	-----	-
Consensus	(382)	382	CATCATCTCCCTGCAACAACACCTTAAAGATCTACACCTGAAGAACTAAATACTTC	AAAAAGAGTTCAATCTGTATTGGCTAACACCTTAAGCAATCGGGCCCGACCTTGGAAATTGAAAG
gRNA2			Exon 2	
(509) CsDRO1L1 WT allele 1	509	509	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
CsDRO1L1 WT allele 2	(508)	508	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#20 allele 1	(502)	502	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#20 allele 2	(502)	502	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#38 allele 1	(503)	503	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#38 allele 2	(503)	503	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#44 allele 1	(472)	503	-----	-
Csdro1l1 root#44 allele 2	(472)	503	-----	-
Csdro1l1 root#63 allele 1	(377)	503	-----	-
Csdro1l1 root#63 allele 2	(377)	503	-----	-
Csdro1l1 root#86 allele 1	(503)	503	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#86 allele 2	(503)	503	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#90 allele 1	(303)	503	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Csdro1l1 root#90 allele 2	(303)	503	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	-
Consensus	(509)	509	TGCAAGCATTGTCATCAAATATACTTCTAACAGACAATTAACTTTGGATCTGAACACAAACAGAGAACAT	TTGGCTAACACCTTAAGCAATCGGGCCCGACCTTGGAAATTGAAAG
PAM			gRNA3	
(636) CsDRO1L1 WT allele 1	636	636	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
CsDRO1L1 WT allele 2	(635)	635	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#20 allele 1	(629)	629	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#20 allele 2	(629)	629	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#38 allele 1	(630)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#38 allele 2	(630)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#44 allele 1	(597)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#44 allele 2	(597)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#63 allele 1	(384)	630	-----	-
Csdro1l1 root#63 allele 2	(384)	630	-----	-
Csdro1l1 root#86 allele 1	(630)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#86 allele 2	(630)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#90 allele 1	(430)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Csdro1l1 root#90 allele 2	(430)	630	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	-
Consensus	(636)	636	CAGTAAGGCAAAGATGAGGTGGACGCTAATGACACCGCAATTATGGCAAGAACACTTT	TTGGCTAACACCTTAAGCAATCGGGCCACCCACTTGGAAATTGAAAG

Figure S6. **CsDRO1-LIKE2** (biallelic heterozygous mutations)

Figure S7. **CsDRO1-LIKE2** (biallelic homozygous mutations)



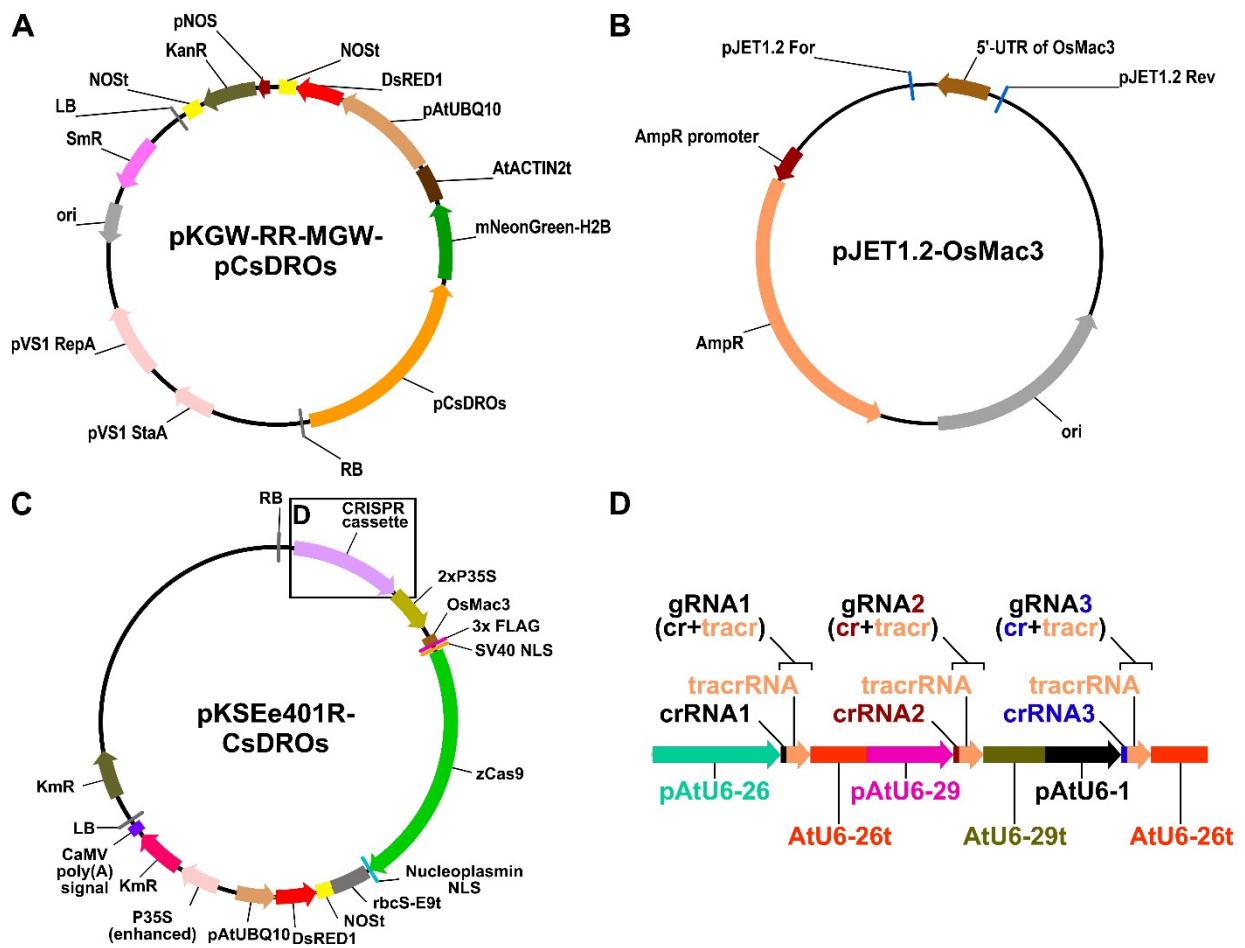


Figure S8. Maps of vectors used in this study. (A) Map of the pKGW-RR-MGW binary vector containing the *H2B-mNeonGreen* reporter under control of *CsDRO1*, *CsDRO1-LIKE1*, or *CsDRO1-LIKE2* promoter (*pCsDROs*). (B) Map of the pJET1.2 plasmid harboring the 158-bp 5'-UTR fragment of *OsMac3*. (C) Common map of the binary plasmids for genome editing, pKSEe401R-*CsDRO1*, pKSEe401R-*CsDRO1-LIKE1*, and pKSEe401R-*CsDRO1-LIKE2* (pKSEe401R-*CsDROs*). (D) General scheme of CRISPR cassette consisting of three guide RNA (gRNA)-expressing domains for targeting of each gene *CsDRO1*, *CsDRO1-LIKE1*, or *CsDRO1-LIKE2*. One domain comprises the promoter of the Arabidopsis *U6* small nucleolar RNA (*snoRNA*) gene (*pAtU6-26*, *pAtU6-29*, or *pAtU6-1*), the gRNA sequence (gRNA1, gRNA2, or gRNA3), and the Arabidopsis *U6* *snoRNA* transcription terminator (*AtU6-26t* or *pAtU6-29t*). Each gRNA sequence, in turn, was designed to contain two fused sequences, the 19-bp target specific *crisprRNA* (crRNA1, crRNA2, or crRNA3) and the conservative 80-bp *trans*-activating crRNA (tracrRNA). Both pKGW-RR-MGW-*pCsDROs* (A) and pKSEe401R-*CsDROs* (C) binary vectors carried the *pAtUBQ10-DsRED1-NOST* screening cassette (Limpens et al., 2004) in the backbone.

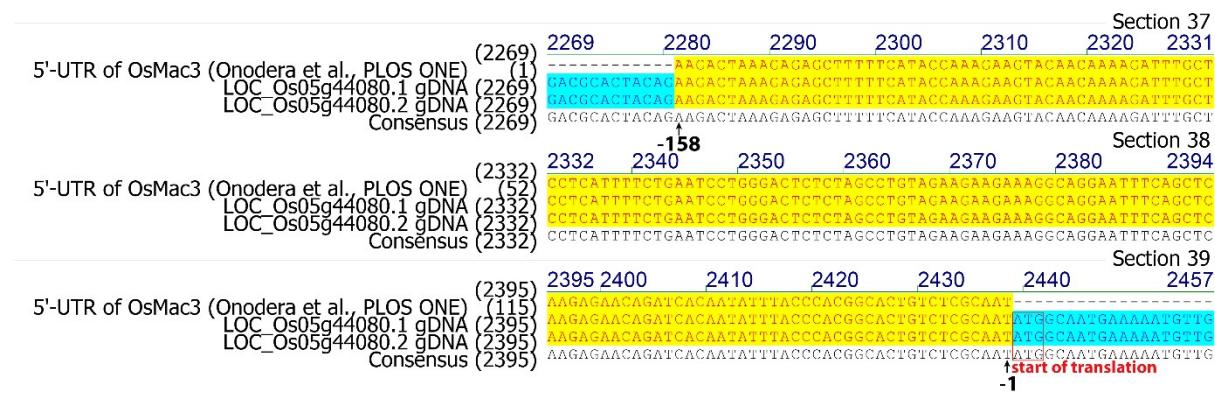


Figure S9. Fragment of the nucleotide sequence alignment between 5'-UTRs of LOC_Os05g44080.1, LOC_Os05g44080.2 (*Oryza sativa* v7.0 based on Phytozome v13), and 158-bp 5'-UTR fragment (from -158 to -1 b.p. before the ATG) of *OsMac3* from Onodera et al. (2018). The yellow color indicates the similar area between all sequences analyzed. Arrows with numbers indicate the position of the site relative to the translation start, ATG (marked with a red rectangle).

- Limpens, E., Ramos, J., Franken, C., Raz, V., Compaan, B., Franssen, H., et al. (2004). RNA interference in *Agrobacterium rhizogenes*-transformed roots of *Arabidopsis* and *Medicago truncatula*. *J. Exp. Bot.* 55(399), 983-992. doi: 10.1093/jxb/erh122
- Onodera, H., Shingu, S., Ohnuma, M., Horie, T., Kihira, M., Kusano, H., et al. (2018). Establishment of a conditional TALEN system using the translational enhancer dMac3 and an inducible promoter activated by glucocorticoid treatment to increase the frequency of targeted mutagenesis in plants. *PLOS ONE* 13(12), 1-13. doi: 10.1371/journal.pone.0208959

Table S1. List of primers used in this study.

Name	Sequence 5'-3'	Application
CsEF1a_qFor	ATGGGTAAGGAGAAGGTTCACATTAACATT	RT-qPCR analysis
CsEF1a_qRev	CGAACCTTCCACAAAGCAATATCAATT	
CsDRO1_qFor	CCATAAAAAACAAGAGTCTCGTGAGGAG	
CsDRO1_qRev	GCTTTGTCAATTCTTCTGTAGTTACCAA	
CsDRO1-LIKE1_qFor	GCAAAAGAACACATCAAATTCAATCAA	
CsDRO1-LIKE1_qRev	GTGCTTCATTCAAGATTCTCGTCTCC	
CsDRO1-LIKE2_qFor	GGATCATATAATTCTCAAACCCCTCAAGT	
CsDRO1-LIKE2_qRev	CAAGAGGCAAGGAAGCACTGGA	
pCsDRO1_For_XhoI	AA <u>ACTCGAGG</u> CATATCAATGGCTCAAATCCAAAAG	Promoter cloning into pENTRattL4attR1_BSAI
pCsDRO1_Rev_KpnI	AA <u>AGGTAC</u> CTAAATGAAAAAAAGAGAATCAAATCCC	
pCsDRO1-LIKE1_For_XhoI	AA <u>ACTCGAGG</u> GAGATGTTCTCCTCAAATCTTCTCGT	
pCsDRO1-LIKE1_Rev_KpnI	AA <u>AGGTAC</u> CTTGTCAAAAGACGGATTGGCAAC	
pCsDRO1-LIKE2_For_XhoI	AA <u>ACTCGAG</u> CTTGCTCAGAACATCACGTATATCA	
pCsDRO1-LIKE2_Rev_KpnI	AA <u>AGGTAC</u> CCCCAACTCAACAACTGAAACCCAAA	
OsMac3_For	AAGACTAAAGAGAGCTTTCATACCAAAG	Cloning of the <i>OsMac3</i> 5'-UTR fragment into pJET1.2 and sequencing
OsMac3_Rev	ATTGCGAGACAGTGCCGTGG	
OsMac3_For_Gi_XbaI	CATTTACGAACGATA <u>CTCGAGTAATCTAGA</u> AAGACTAAAG AGAGCTTTTCATACCAAAG	Fusion <i>OsMac3</i> with <i>zCas9</i> and cloning into pKSE401 via Gibson Assembly
OsMac3_Rev_Gi	CGTGGTCCTTGTAA <u>TCCCCGTCGTGGCCTTGTAA</u> ATCCATAT TGCAGACAGTGGCGTGG	
<i>zCas9</i> _For_Gi	CAATATITACCCACGGCACTGTCTCGCAAT <u>ATGGATTACAA</u> GGACCACGACGG	
<i>zCas9</i> _Rev_Gi_SacI	ACCGATGATA <u>CGAACGAAAGCTCTGAGCTCTCACTTCTTCT</u> TCTTCGCCTGCC	
<i>zCas9</i> _Rev1	CAACAGAGTTAGTCCCAATATCGAGG	Sequencing of the <i>OsMac3</i> - <i>zCas9</i> fusion after Gibson Assembly
<i>zCas9</i> _For2	ATGGATTACAAGGACCACGACGG	
<i>zCas9</i> _Rev2	CGAGACTTCGAGAGCCTAGCGGA	
<i>zCas9</i> _For3	CATCTCGGAAGAACGCTCGTGG	
<i>zCas9</i> _Rev3	ATGAATCTGGTGTGGATGCTGC	
<i>zCas9</i> _For4	CGATCAGTCGAAGAACGGCTACCG	
<i>zCas9</i> _Rev4	CTCATCTCTCGTGTCCAGGAAG	
<i>zCas9</i> _For5	ATCGTGGACCTCCTGTTCAAGACC	
<i>zCas9</i> _Rev5	CGTCGTAATCGCTGAGCCTGTTAATAT	
<i>zCas9</i> _For6	AAGCCTGAGAACATCGTCATTGAGAT	
<i>zCas9</i> _Rev6	TCCTCACATCGTAAACCTTGTAGTCCC	
<i>zCas9</i> _For7	GAAGGTCATCACCCCTGAAGTCAAAGC	
<i>zCas9</i> _Rev7	GCCAGCGGAAGGCCAGCA	
<i>zCas9</i> _For8	GGTCGACAGCCCCACTGTG	
<i>zCas9</i> _Rev8	AGAGCTCTCACTTCTTCTCGCCTGCC	
pAtUBQ10_For_EcoRI	A <u>AGAATTCCGGACGAAGTCCAGTAATAACGG</u>	Cloning of the <i>pAtUBQ10-DsRED1-NOS</i> cassette
NOS_Rev_EcoRI	A <u>AGAATT</u> CGATCTAGAACATAGATGACACCGCGC	

Name	Sequence 5'-3'	Application
		into pKSEe401 and sequencing
M13_Rev	AACAGCTATGACCATG	Sequencing of the <i>pAtUBQ10-DsRED1-NOS</i> cassette after insertion into pKSEe401
pAtUBQ10_Rev	TGTTAACAGAAAAACTCAGATTAAATCTACAAATT	
DsRED1_For	ATGGCGCGCTCCAGAAG	
DsRED1_REV	CAGGAACAGGTGGTGGCGG	
CsDRO1_g1_DT1_BsaI_F	ATATAT <u>GGTCTCGATT</u> GagaagtggagattgtatccGTT	Assembly of pKSEe401R-CsDRO1
CsDRO1_g1_DT1_F0	TGagaagtggagattgtatcc <u>GTTTAGAGCTAGAAATAGC</u>	
DT0_BsaI_R2	ATATTATT <u>GGTCTCAATCTT</u> AGTCGACTCTACCAAT	
CsDRO1_g2_DT2_BsaI_F2	ATATTATT <u>GGTCTCAAGATT</u> GcaattgcaagcaacccatgGTT	Assembly of pKSEe401R-CsDRO1
CsDRO1_g2_DT2_F0	TGcaattgcaagcaacccatg <u>GTTTAGAGCTAGAAATAGC</u>	
CsDRO1_g3_DT3_R0	AAACcaagcttgaggggcagt <u>CAATCACTACTTCGCTCTAACCAT</u>	
CsDRO1_g3_DT3_BsaI_R	ATTATT <u>GGTCTCGAAC</u> CCaaagcttgagggcagt <u>C</u>	Assembly of pKSEe401R-CsDRO1-LIKE1
CsDRO1-LIKE1_g1_DT1_BsaI_F	ATATAT <u>GGTCTCGATT</u> GaccagaagaattcagcaacGTT	
CsDRO1-LIKE1_g1_DT1_F0	TGaccagaagaattcagcaac <u>GTTTAGAGCTAGAAATAGC</u>	
DT0_BsaI_R2	the same as for the pKSEe401R-CsDRO1 assembly	
CsDRO1-LIKE1_g2_DT2_BsaI_F2	ATATTATT <u>GGTCTCAAGATT</u> Gtgaattccaaattccagg <u>GTT</u>	
CsDRO1-LIKE1_g2_DT2_F0	TGtgaattccaaattccagg <u>GTTTAGAGCTAGAAATAGC</u>	
CsDRO1-LIKE1_g3_DT3_R0	AAACcctgctgttgtactc <u>CAATCACTACTTCGCTCTAACCAT</u>	
CsDRO1-LIKE1_g3_DT3_BsaI_R	ATTATT <u>GGTCTCGAAC</u> CCaaattccagg <u>GTT</u>	
CsDRO1-LIKE2_g1_DT1_BsaI_F	ATATAT <u>GGTCTCGATT</u> Gaaggactggattcttc <u>c</u> GT	Assembly of pKSEe401R-CsDRO1-LIKE2
CsDRO1-LIKE2_g1_DT1_F0	TGaggactggattcttc <u>c</u> GT <u>GTTTAGAGCTAGAAATAGC</u>	
DT0_BsaI_R2	the same as for the pKSEe401R-CsDRO1 assembly	
CsDRO1-LIKE2_g2_DT2_BsaI_F2	ATATTATT <u>GGTCTCAAGATT</u> Gcttagtaaccaagg <u>GTT</u>	
CsDRO1-LIKE2_g2_DT2_F0	TGcttagtaaccaagg <u>GTTTAGAGCTAGAAATAGC</u>	
CsDRO1-LIKE2_g3_DT3_R0	AAACattagaagagg <u>GGC</u> CAATCACTACTTCGCTCTAACCAT	Sequencing of the CRISPR-cassettes in pKSEe401R-DRO1, -CsDRO1-LIKE1, and -CsDRO1-LIKE2
CsDRO1-LIKE2_g3_DT3_BsaI_R	ATTATT <u>GGTCTCGAAC</u> Attagaagagg <u>GGC</u> C	
M13_For	GTA AAAACGACGCCAGT	
AtU6-26p_For	TGTCCCAGGATTAGAATGATTAGGC	
AtU6-29p_For	TTAACCAAACACTGCAGCCTGAC	PCR-amplification of the CsDRO1 ORF and sequencing to search genome editing events within it
AtU6-29p_Rev	AGCCCTCTCTTCGATCCATCAAC	
AtU6-29t_For	CGTGTTCAGCTCTCATGATCCTTG	
AtU6-1p_Rev	TATGCAAGTCTCACTCACACTCACG	
CsDRO1_5'-UTR _For	GTTCTGCTTCGGCATTCATCTTG	Additional primers for sequencing to search genome editing events within the CsDRO1 ORF
CsDRO1_3'-UTR _Rev	ATTTCAAAACAAGGGGTGGAGATTG	
CsDRO1_Intron1_For	TGTGCCCTGTGCCCTGTTCC	
CsDRO1_Intron2_Rev	TGATCTTAGGATTAAATAATGAATATGCACA	
CsDRO1_Exon2_For	CTTTATCTCGAAAACCGAACGCC	

Name	Sequence 5'-3'	Application
CsDRO1-LIKE1_5'-UTR_For	TCCCTGTTGCCAATCCGTCTT	PCR-amplification of the CsDRO1-LIKE1 ORF and sequencing to search genome editing events within it
CsDRO1-LIKE1_3'-UTR_Rev	CCAAGCGAGAAAATAATGTCTGTGAAC	
CsDRO1-LIKE2_5'-UTR_For	TTCTGAATAACCCCTTAATTTCGGTG	PCR-amplification of the CsDRO1-LIKE2 ORF and sequencing to search genome editing events within it
CsDRO1-LIKE2_3'-UTR_Rev	CTATGGCAATAGTTACCTCTCATCTTCATC	
CsDRO1-LIKE2_Exon2_Rev1	TTCTAATTGGGGACACCTTCAAG	Additional primers for sequencing to search genome editing events within the CsDRO1-LIKE2 ORF
CsDRO1-LIKE2_Exon2_For	TTGGAACCTTTGGCAACAATATTAACG	
CsDRO1-LIKE2_Exon2_Rev2	TTTGGTCTCTATTGGTCATCATCAAAGT	

Restriction enzyme sites in adaptors are underlined and given in **bold** style.

For long primers the primer sequences are separated from adaptors by the gray background.

The target specific *crispr*RNA sequences are given in small letters.