

Supplementary Information

Supplementary Table 1. Targeted genes and gRNA sequences of the editing experiment.

Target gene	gRNA name	Protospacer sequence	Position
Niben101Scf04551g02001.1 (<i>NbXT2</i>)	gXT2	GCTCTGATTGCACAATGGAA	+1628
Niben101Scf01950g02001.1 (<i>SPL1950</i>)	gSPL	ATGGACATAACAGGCGTCGA	+562
Niben101Scf00366g0008.1 (<i>SPL9294</i>)			+553

Supplementary Table 2. List of plasmids used in this work. Sequence information is available at <http://www.gbcloning.upv.es/> by entering the GB Number.

GB Number	Name
GB0107	pDGB1 α 2:SF
GB0108	pDGB3 α 1:P35S:p19:TNOS
GB0639	pDGB2 α 2:P35S:hCas9:TNOS
GB1116	pDGB3 α 1_PNOS:Luciferase:TNOS-SF-P35S:Renilla:TNOS-P35S:p19:TNOS
GB1119	pDGB3 α 1_P35S:Luciferase:TNOS-SF-P35S:Renilla:TNOS-P35S:p19:TNOS
GB1160	pDGB1 Ω 1_SIDFR:Luc:TNos-SF-35S:Renilla:TNos-35S:P19:Tnos
GB1770	pDGB3 α 1_U6-26:gXT2:psgRNA
GB2049	pDGB3 α 1_U6-26-4gRNA Pnos-F6x2_35s-dCas9:EDLL-Tnos-U6-26-5gRNA Pnos scf F6x2 - 35s-Ms2:VPR-Tnos
GB2513	pDGB3 α 1_dCas9EDLL-Ms2:VPR SF -gRNA DFR -150 2.1
GB3333	pUPD2_SV40-AcrIIA4 (B3-B4-B5)
GB3659	pUPD2_SV40-AcrIIA4 (B3-B4)
GB3660	pUPD2_AcrIIA4 (B3-B4)
GB3661	pUPD2_AcrIIA4 (B3-B4-B5)
GB3663	pUPD2_mAID (N-tag) B2
GB3664	pUPD2_mAID (C-tag) B5
GB3344	pDGB3 α 1:P35S:SV40-AcrIIA4:TNOS
GB3666	pDGB3 α 1_P35S:mAID:SV40-AcrIIA4:TNOS
GB3667	pDGB3 α 1_P35S:SV40-AcrIIA4:mAID:TNOS
GB3668	pDGB3 α 1_P35S:mAID:AcrIIA4:TNOS
GB3669	pDGB3 α 1:p35S:AcrIIA4:mAID:TNOS
GB3670	pDGB3 α 1_P35S:K2:SV40-AcrIIA4:TNOS
GB3671	pDGB3 α 1_P35S:K2:AcrIIA4:TNOS
GB3701	pDGB3 α 1:U6-26:gSPL1.6:psgRNA

Supplementary Table 3. List of primers used in this work.

Gene identifier	Primer Sequence (5'→ 3')
NbXT2	TGCACGGTTGTCCGAGTTTG
	TTACTTGTGAATTGCTCTCTGGT
SPL1950	AAATGTTCAATCCCTGGACGAC
	CAAAACGGACAGCGACATGGT
SPL9294	GCCCAGGTTTGAATGCATTAGGG
	GAAAACGGACAACGACATGGT
NbDFR	TTCATCTGCGCATCCCATCA
	TCCCTACTGAGTTTAAAGGTATCGA
NbAN2	GGAAAAGTTGCAGACTGAGGTG
	ACCCGCAATAAGTGACCATCTG
NbF-box	TTGGAAACTCTCTCCCCACTTG
	GCTCATTGTTGGATGGGTACCT

The tables provided below show the *Agrobacterium* cultures co-infiltrated in the same mix for each experiment.

Construct	Plant 1	Plant 2	Plant 3	Plant 4
35S:SV40-AcrIIA4:TNos (GB3344)	+	-	+	-
35s:hCas9:tNos (GB0639)	+	+	+	+
U6-26:gXT2:psgRNA (GB1770)	+	+	-	-
U6-26:gSPL1.6:psgRNA (GB3701)	-	-	+	+
pSF (GB0107)	-	+	-	+
35s:P19:TNos (GB0108)	+	+	+	+

Construct	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
35S:SV40-AcrIIA4:Tnos (GB3344)	+	-	-	-	-
SIDFR:Luc:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos (GB1160)	+	+	+	-	-
dCas9EDLL-Ms2:VPR SF -gRNA DFR -150 2.1 (GB2513)	+	+	-	-	-
U6-26-4gRNA Pnos-F6x2_35s-dCas9:EDLL-Tnos-U6-26-5gRNA Pnos scf F6x2 - 35s-Ms2:VPR-Tnos (GB2049)	-	-	+	-	-
pSF (GB0107)	-	+	+	-	-
PNos:Luciferase:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos-SF (GB1116)	-	-	-	+	-

[illegible]

Time-course assay (Figure 2B)

Construct	Plant 1 (day 1)	Plant 2 (day 2)	Plant 3 (day 3)	Plant 4 (day 4)	Plant 5	Plant 6	Plant 7	Plant 8
35S:SV40-AcrIIA4:Tnos (GB3344)	+	+	+	+	-	-	-	-
SIDFR:Luc:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos (GB1160)	+	+	+	+	+	+	-	-
dCas9EDLL-Ms2:VPR SF -gRNA DFR -150 2.1 (GB2513)	+	+	+	+	+	-	-	-
U6-26-4gRNA Pnos-F6x2_35s-dCas9:EDLL-Tnos-U6-26-5gRNA Pnos scf F6x2 - 35s-Ms2:VPR-Tnos (GB2049)	-	-	-	-	-	+	-	-
pSF (GB0107)	-	-	-	-	+	+	-	-
PNos:Luciferase:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos-SF (GB1116)	-	-	-	-	-	-	+	-

Temperature Dependent Degron assay (Figure 2D)

Construct	14°C				28°C			
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8
35S:K2:SV40-AcrIIA4:Tnos (GB3670) OD0.05	+	-	-	-	+	-	-	-
35S:K2:AcrIIA4:Tnos (GB3671) OD0.05	-	+	+	-	-	+	+	-
SIDFR:Luc:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos (GB1160)	+	+	+	-	+	+	+	-
dCas9EDLL-Ms2:VPR SF -gRNA DFR -150 2.1 (GB2513)	+	+	-	-	+	+	-	-
U6-26-4gRNA Pnos-F6x2_35s-dCas9:EDLL-Tnos-U6-26-5gRNA Pnos scf F6x2 - 35s-Ms2:VPR-Tnos (GB2049)	-	-	+	-	-	-	+	-
pSF (GB0040)	+	+	+	-	+	+	+	-
PNos:Luciferase:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos-SF (GB1116)	-	-	-	+	-	-	-	+

Auxin Inducible Degron assay (Figure 3B)

Construct	10μM IAA								0μM IAA								
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Plant 11	Plant 12	Plant 13	Plant 14	Plant 15	Plant 16	Plant 17
35S:SV40-AcrIIA4:Tnos (GB3344) OD0.05	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
35S:mAID:SV40-AcrIIA4:Tnos (GB3666) OD0.05	+	-		-		-	-	-	+	-		-		-	-	-	-
35S:SV40-AcrIIA4:mAID:Tnos (GB3667) OD0.05	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
35S:mAID:AcrIIA4:Tnos (GB3668) OD0.05	-	-	+	-	-	-		-	-	-	+	-	-	-	-	-	-
35S:AcrIIA4:mAID:Tnos (GB3669) OD0.05	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
SIDFR:Luc:TNos-SF-35S:Renilla:TNos-35S:P19:Tnos (GB1160)	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-
dCas9EDLL-Ms2:VPR SF -gRNA DFR -150 2.1 (GB2513)	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-
U6-26-4gRNA Pnos-F6x2_35s-dCas9:EDLL-Tnos-U6-26-5gRNA Pnos scf F6x2 - 35s-Ms2:VPR-Tnos (GB2049)	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
pSF (GB0107)	-	+	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-
PNos:Luciferase:TNos-SF-35S:Renilla:TNos-35S:P19:TNos-SF (GB1116)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-

Auxin Inducible Degron dose-response assay (Figure 3C)

Construct	Plant 1 (5 μ M)	Plant 2 (1 μ M)	Plant 3 (0.5 μ M)	Plant 4 (0.1 μ M)	Plant 5	Plant 6	Plant 7	Plant 8
35S:mAID:AcrIIA4:Tnos (GB3668) OD0.05	+	+	+	+	-	-	-	-
SIDFR:Luc:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos (GB1160)	+	+	+	+	+	+	-	-
dCas9EDLL-Ms2:VPR SF -gRNA DFR -150 2.1 (GB2513)	+	+	+	+	+	-	-	-
U6-26-4gRNA Pnos-F6x2_35s-dCas9:EDLL-Tnos-U6-26-5gRNA Pnos scf F6x2 - 35s-Ms2:VPR-Tnos (GB2049)	-	-	-	-	-	+	-	-
pSF (GB0107)	+	+	+	+	+	+	-	-
PNos:Luciferase:Tnos-SF-35S:Renilla:Tnos-35S:P19:Tnos-SF (GB1116)	-	-	-	-	-	-	+	-

Auxin Inducible Degron time-course assay (Figure 3D)

Construct	Plant 1 (0h)	Plant 2 (24h)	Plant 3 (48h)	Plant 4 (72h)	Plant 5 (24-72h)	Plant 6	Plant 7	Plant 8	Plant 9
35S:mAID:AcrlIA4:Tnos (GB3668) OD0.05	+	+	+	+	+	-	-	-	-
SIDFR:Luc:Tnos-SF- 35S:Renilla:Tnos- 35S:P19:Tnos (GB1160)	+	+	+	+	+	+	+	-	-
dCas9EDLL-Ms2:VPR SF -gRNA DFR -150 2.1 (GB2513)	+	+	+	+	+	+	-	-	-
U6-26-4gRNA Pnos- F6x2_35s-dCas9:EDLL- Tnos-U6-26-5gRNA Pnos scf F6x2 - 35s- Ms2:VPR-Tnos (GB2049)	-	-	-	-	-	-	+	-	-
pSF (GB0107)	+	+	+	+	+	+	+	-	-
PNos:Luciferase:Tnos- SF-35S:Renilla:Tnos- 35S:P19:Tnos-SF (GB1116)	-	-	-	-	-	-	-	+	-

Endogenous genes activation assay (Figure 4)

Construct	Plant 1	Plant 2	Plant 3	Plant 4
35S:SV40-AcrIIA4:Tnos (GB3344)	+	-	+	-
35s-Ms2:VPR-Tnos-35s- dCas9:EDLL-Tnos (GB2085)	+	+	+	+
U6-26-sgRNANbDFR -85,-198,- 268 F6x2 Multiplex (GB2170)	+	+	-	-
U6-26-sgRNANbAN2 -103,-175,- 196 F6x2 (GB2171)	-	-	+	+
pSF (GB0040)	-	+	-	+
35s:P19:Tnos (GB0108)	+	+	+	+