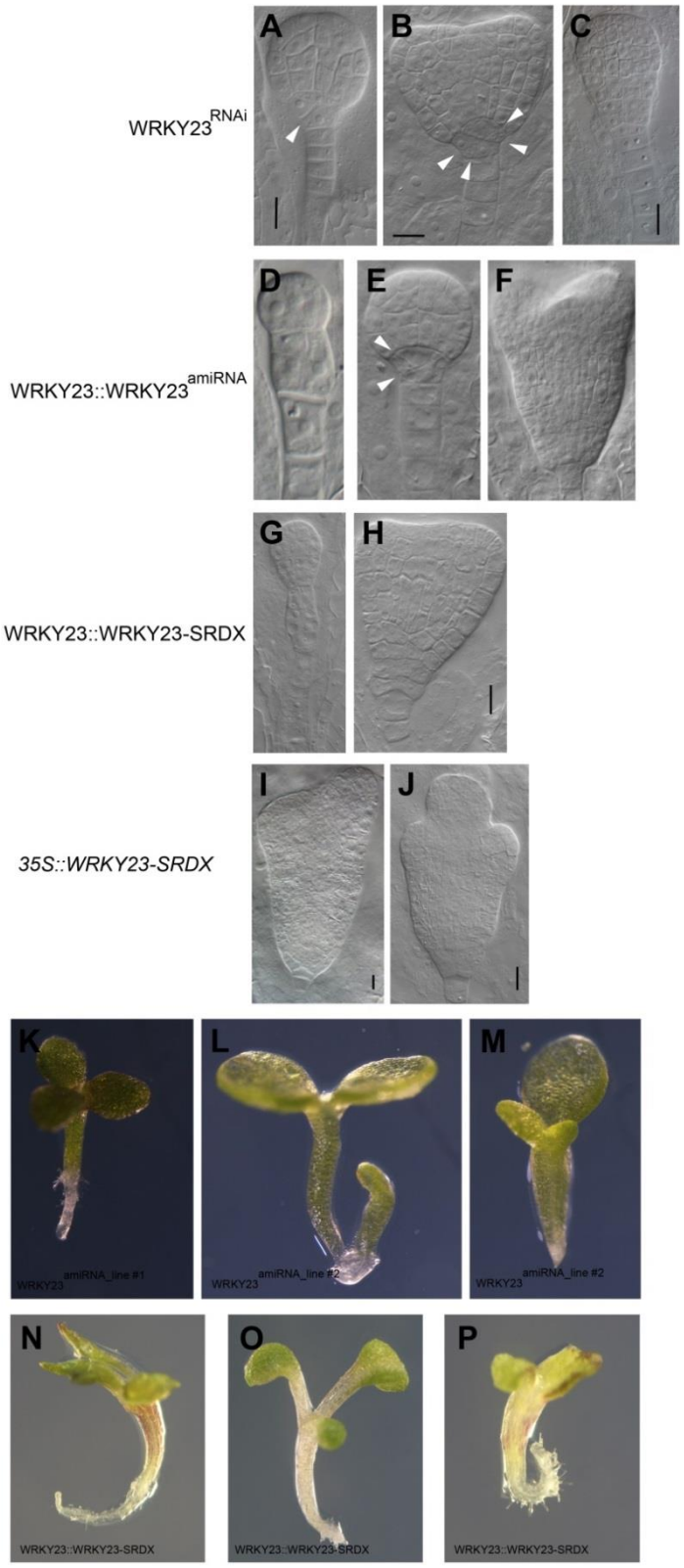

Supplemental Figure S1. Embryo phenotypes in WRKY23^{RNAi} (A – C), WRKY23::WRKY23^{amiRNA} (D – F), WRKY23::WRKY23-SRDX (G – H), and 35S::WRKY23-SRDX (I – J). Scale bar, 10 µm. Arrowheads point to irregular hypophysis divisions. (K-L) Seedling phenotpyes for independent WRKY23::WRKY23^{amiRNA} lines. (N-P) Seedling phenotypes for WRKY23::WRKY23-SRDX phenotypes.

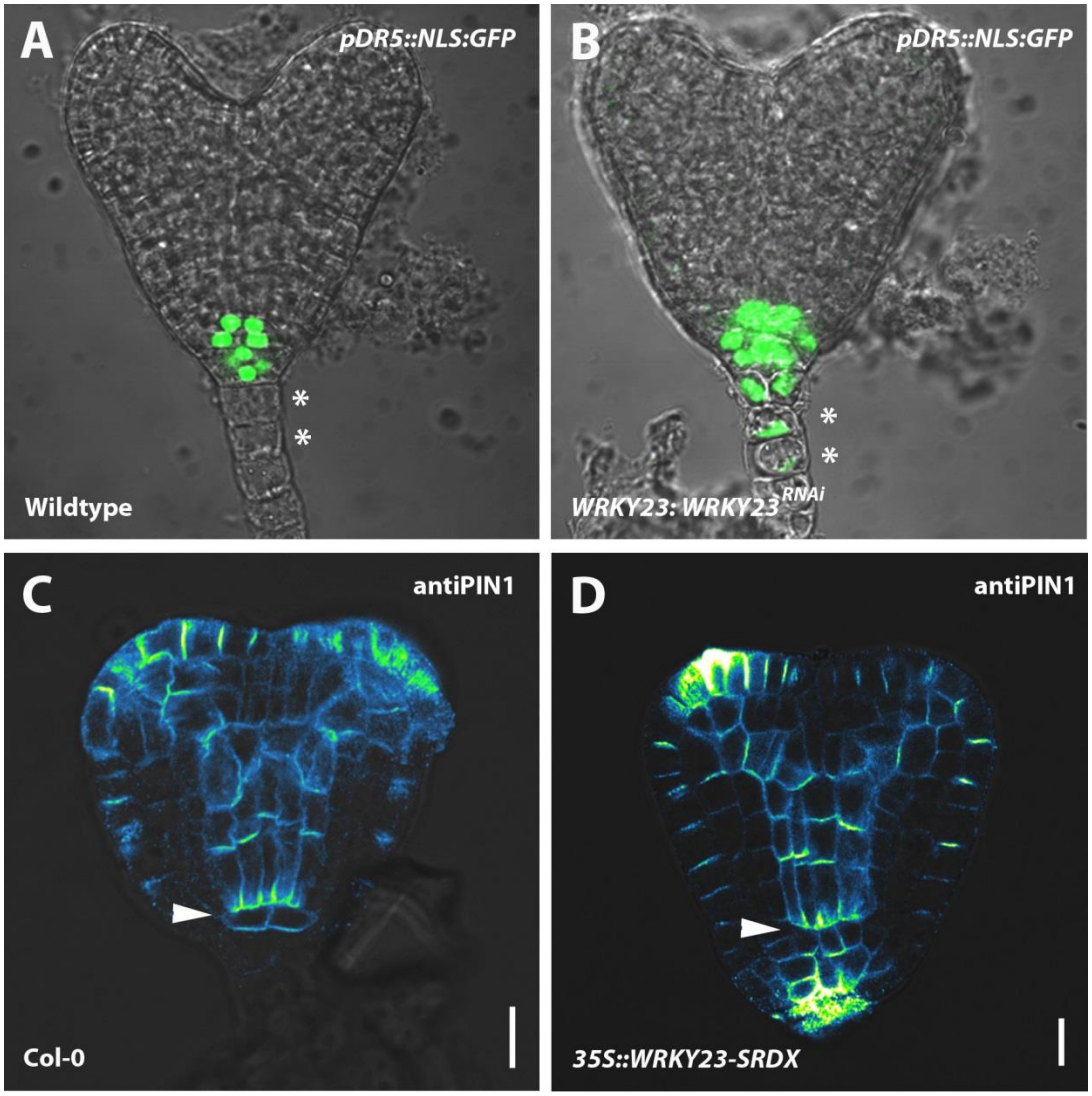

Figure S2. Auxin response and transport in lines with reduced WRKY23 levels or activity. (A-B) pDR5::NLS:GFP in wildtype (A) and WRKY23::WRKY23^{RNAi}. (C-D) PIN localisation in wildtype (C) and 35S::WRKY23-SRDX using PIN1 antibody (antiPIN1).

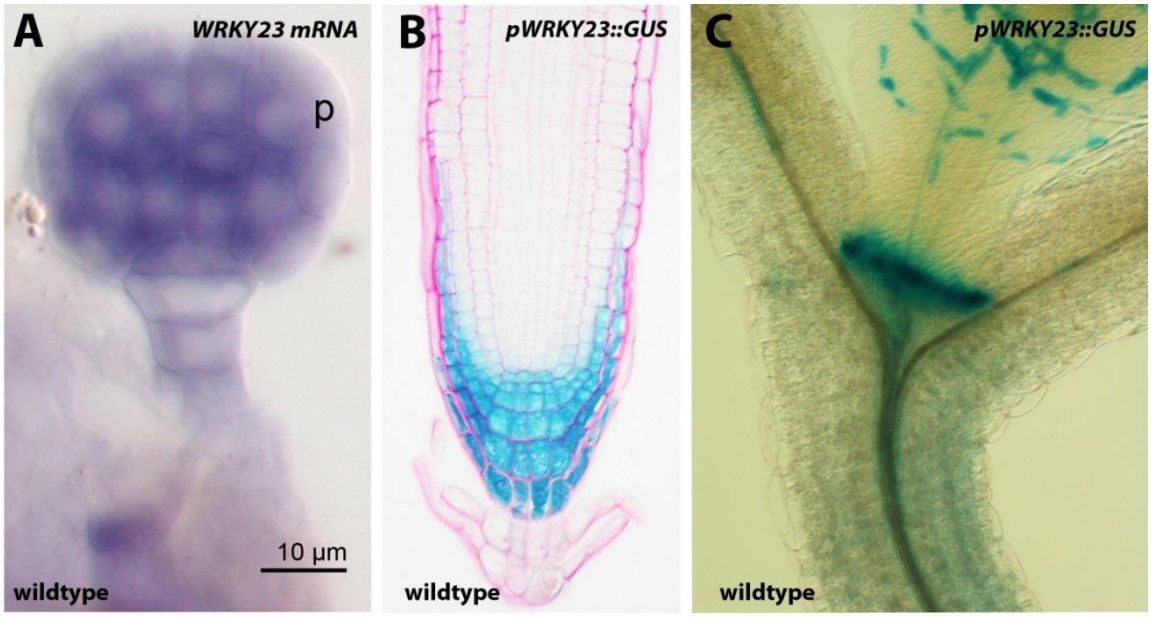

Figure S3. *WRKY23* expression in (A) late globular embryo using mRNA in situ and (B-C) p*WRKY23::GUS* in 5-day old seedling root tip (B) and shoot apical meristem (C).

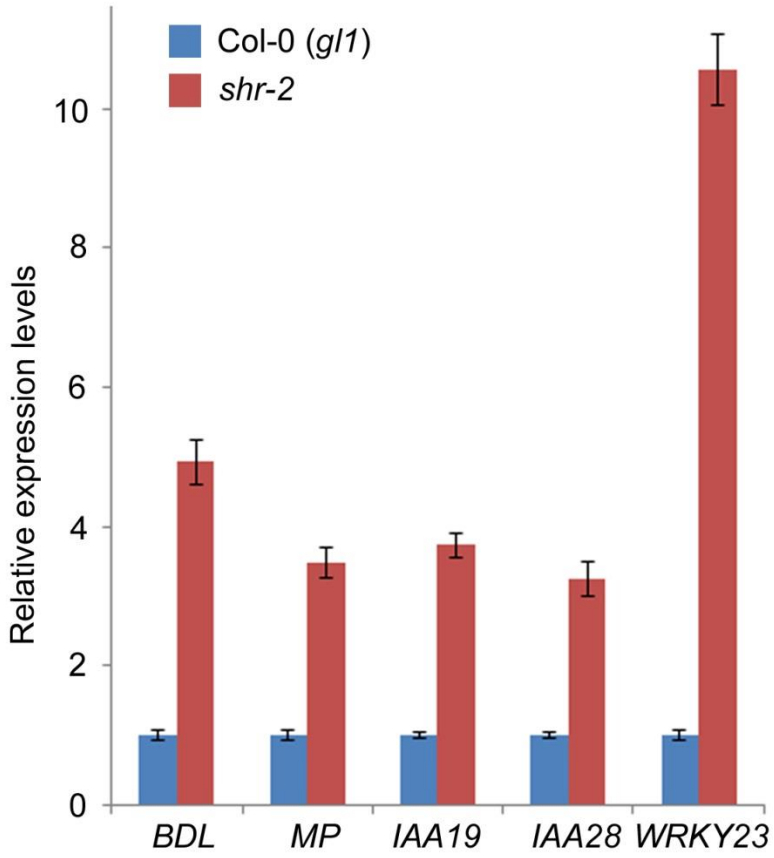

Figure S4. Expression level of *WRKY23* and auxin responsive genes in 3-day old *shr-2* root tips. Standard error is indicated.

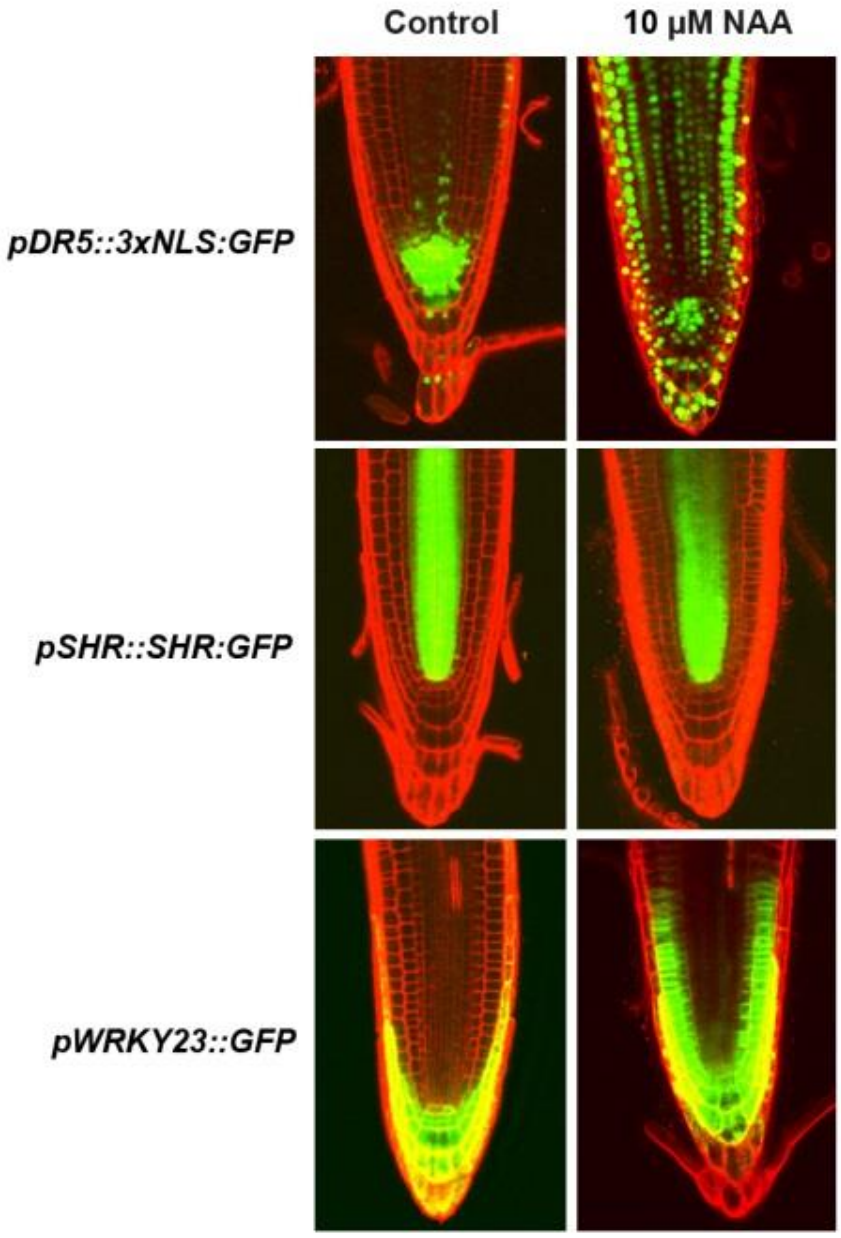

Figure S5. Tissue-specific induction of *WRKY23* by 10 µM NAA for 4 hours.

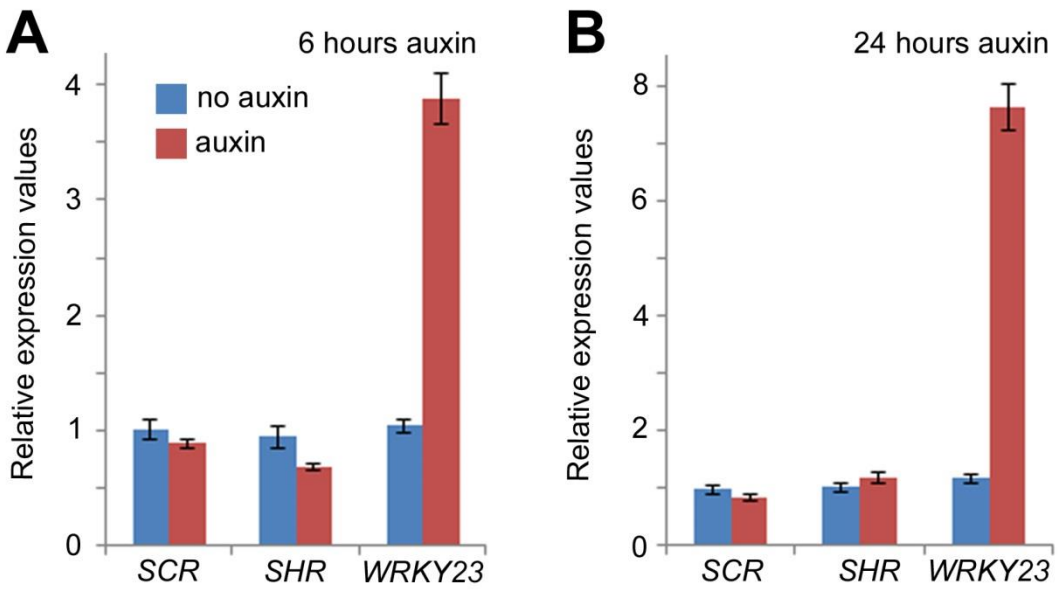

Figure S6. Auxin treatment (6 hours or 24 hours of 10 µM NAA) on 6-day old wildtype (Col-0) seedlings. Experiment performed in triplicate. Standard error is indicated.

Table S1. Frequencies of defects observed in embryos of WT Col-0, *WRKY23::WRKY23RNAi* lines, *WRKY23::WRKY23amiRNA*, *35S::WRKY23-SRDX* and *WRKY23::WRKY23-SRDX*. (n.r. = not relevant)

line	stage	no defects	polarity defects	hypophysis defects
WT Col-0	pre-globular	169/171 (98.8%)	2/171 (1.2%)	n.r.
	globular	175/180 (97.2%)	2/180 (1.1%)	3/180 (1.7%)
	post-globular	141/151 (93.4%)	0/151 (0%)	10/151 (6.6%)
WRKY23::WRKY23RNAi	pre-globular	166/183 (90.7%)	17/183 (9.3%)	n.r.
	globular	277/341 (81.2%)	35/341 (10.3%)	29/341 (8.5%)
	post-globular	302/383 (78.9%)	23/383 (6.0%)	58/383 (15.1%)
WRKY23::WRKY23amiRNA	pre-globular	107/119 (89.9%)	12/119 (10.1%)	n.r.
	globular	54/71 (76.0%)	9/71 (12.7%)	8/71 (11.3%)
	post-globular	121/182 (66.5%)	14/182 (7.7%)	47/182 (25.8%)
35S::WRKY23-SRDX	pre-globular	54/54 (100.0%)	0/54 (0%)	n.r.
	globular	39/58 (67.2%)	4/58 (6.9%)	15/58 (25.9%)
	post-globular	58/85 (68.2%)	3/85 (3.5%)	24/85 (28.2%)
WRKY23::WRKY23-SRDX	pre-globular	248/267 (92.9%)	19/267 (7.1%)	n.r
	globular	190/234 (81.2%)	17/234 (7.3%)	27/234 (11.5%)
	post-globular	233/344 (67.7%)	20/344 (5.8%)	91/344 (26.5%)

Table S2. Frequencies of defects observed in heart stage embryos of WT Col-0, WT Ws, *tt4-8*, *tt7-5* and *tt7-6*

line (associated wildtype)	Wild-type looking embryos	Hypophysis defects
wildtype Ws	197/203 (97.0%)	6/203 (3.0%)
tt4-8 (Ws)	170/176 (96.6%)	6/176 (3.4%)
wildtype Col-0	152/155 (99.1%)	3/155 (1.9%)
tt7-5 (Col-0)	309/316 (97.8%)	7/316 (2.2%)
tt7-6 (Col-0)	321/327 (98.1%)	6/327 (1.8%)

Table S3. Primers used for construction of the WRKY23 amiRNAs

amiRNA1 "TTTCACGTTACATGAAGGCGT" (target in exon 3)

Primers	sequence
miR-s	gaTTTCACGTTACATGAAGGCGTtctctcttttgtattcc
miR-a	gaACGCCTTCATGTAACGTGAAAtcaaagagaatcaatga
miR*s	gaACACCTTCATGTATCGTGAATtcacaggtcgtgatatg
miR*a	gaATTCACGATACATGAAGGTGTtctacatatatattcct

amiRNA2 "TTGAACGCTTTCATACGGCCG" (target in 5'UTR)

Primers	sequence
miR-s	gaTTGAACGCTTTCATACGGCCGtctctcttttgtattcc
miR-a	gaCGGCCGTATGAAAGCGTTCAAtcaaagagaatcaatga
miR*s	gaCGACCGTATGAAACCGTTCATtcacaggtcgtgatatg
miR*a	gaATGAACGGTTTCATACGGTCGtctacatatatattcct

amiRNA3 "TTTTGACGTCACTAATTGCGC" (target in exon 1)

Primers	sequence
miR-s	gaTTTTGACGTCACTAATTGCGCtctctcttttgtattcc
miR-a	gaGCGCAATTAGTGACGTCAAAAtcaaagagaatcaatga
miR*s	gaGCACAATTAGTGAGGTCAAATtcacaggtcgtgatatg
miR*a	gaATTTGACCTCACTAATTGTGCtctacatatatattcct

Table S4. Primers used for qRT-PCR

Gene	Primer sequence
WRKY23_FW	5'-AGTCTCGGTAATGGTTGCTTTGG-3'
WRKY23_REV	5'-TGTTGCTGCTGTTGGTGATGG-3'
BDL_FW	5'-GGTACTACTTGTCGAGAAAAGGTTAAACC-3'
BDL_REV	5'-CCCCTTCCTTATCTTCATAAGTGAGTAC-3'
MP_FW	5'-ACAAGCTTTAAAGACTACGAGGAGCTA-3'
MP_REV	5'-CGAGCTTTGTGGGTGAGTTAGTAG-3'
IAA19_FW	5'-TGATGTACCTTGGGGGATGT-3'
IAA19_REV	5'-TGAACCAGCTCCTTGCTTCT-3'
IAA28_FW	5'-ATGCCGTTGACCAACTCTTC-3'
IAA28_REV	5'-CATCCCCGACCAGAACTTTA-3'
SHR_FW	5'-ACCACAACCATCACAACCACAAC-3'
SHR_REV	5'-CGAAGGACGGAGGAGTTTGAGG-3'
SCR_FW	5'-GACGCCATTATCAGAGACCTTATC-3'
SCR_REV	5'-GGAGCCTGTATTCAAGAAGAGC-3'
ACT7_FW	5'-ACTCTTCCTGATGGACAGGTG-3'
ACT7_REV	5'-CTCAACGATTCCATGCTCCT-3'
EEF1α4_FW	5'CTGGAGGTTTTGAGGCTGGTAT-3'
EEF1α4_REV	5'-CCAAGGGTGAAAGCAAGAAGA-3'
CDKA_FW	5'-ATTGCGTATTGCCACTCTCATAGG-3'
CDKA_REV	5'-TCCTGACAGGGATACCGAATGC-3'

Supplemental Materials and Methods

GUS staining of embryos. Embryos were dissected out of the ovules in 90% acetone. For embryos younger than globular stage, ovules were opened but the embryos were not dissected. After dissection, ovules and embryos were transferred to sieves (BD Falcon, cell strainer 40 µm nylon) and were incubated under vacuum for 10 min in 90% acetone. Subsequently three washing steps were done under vacuum for 10 min each with a 0.5 M phosphate buffer (Na_2HPO_4/NaH_2PO_4 (615/385), pH 7). Sieves were then transferred to GUS staining solution (X-Glu (1mM) dissolved in DMFO (0.5% v/v); Triton X-100 (0.5% v/v); EDTA (1mM); $K_3Fe(CN)_6$ (0.5mM); $K_4Fe(CN)_6$ (0.5mM); phosphate buffer (0.5 M); pH 7) and incubated for 1 h under vacuum. After vacuum infiltration, samples were incubated at 37 °C. The staining reaction was stopped by two washes with 0.5 M phosphate buffer under vacuum for 10 min each.

Cloning. For the WRKY23::WRKY23-SRDX construct, the pEN-L1-WRKY23SRDX-R2 [1] was Gateway recombined with pEN-L4-WRKY23prom-R1 [1] and pK7m24GW [2]. The amiRNA constructs were designed and constructed as described [3], using primers miR-s, miR-a, miR*s and miR*a (Table S3). Gateway recombination sites were added in the final PCR to clone the amiRNA constructs in pDONR221. The vector pK7m24GW was used as a Gateway destination vector together with pEN-L4-WRKY23prom-R1.

1. Grunewald W *et al* (2012) Transcription factor WRKY23 assists auxin distribution patterns during Arabidopsis root development through local control on flavonol biosynthesis. *Proc Natl Acad Sci U S A* **109:** 1554-1559

2. Karimi M, Depicker A, Hilson P (2007) Recombinational cloning with plant gateway vectors. *Plant Physiol* **145:** 1144-1154

3. Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D (2006) Highly specific gene silencing by artificial microRNAs in Arabidopsis. *Plant Cell* **18:** 1121-1133