Figure S1

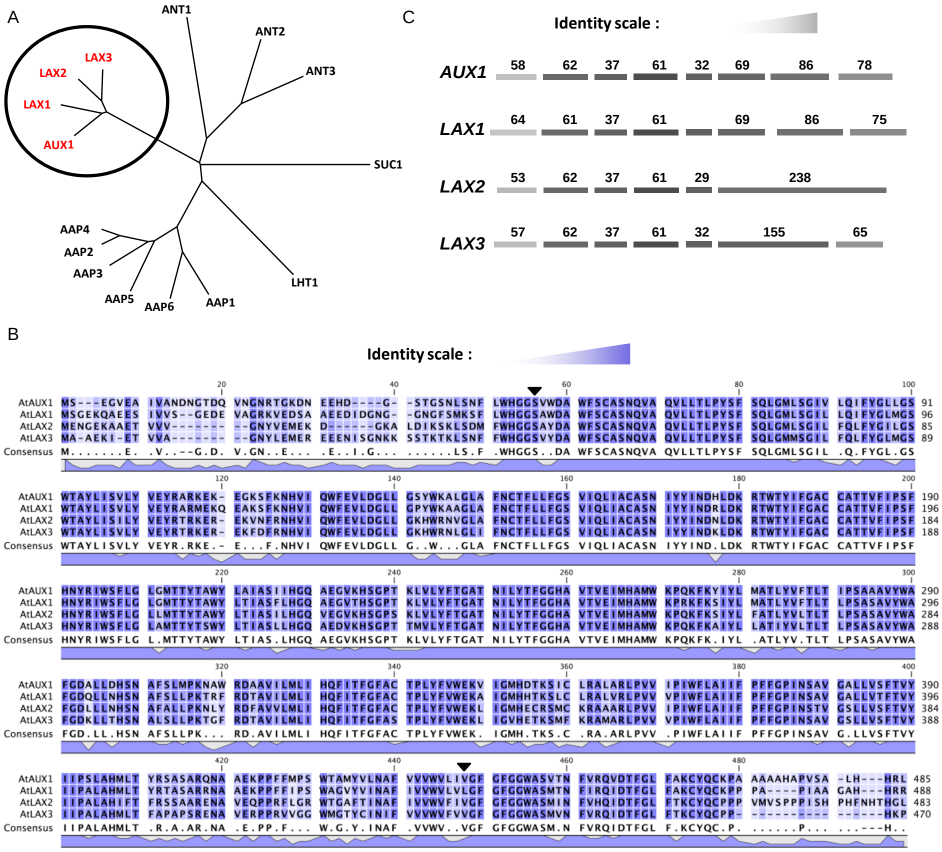

Figure S1. The *AUX/LAX* genes represent a highly conserved family of auxin influx transporters.

(A) Phylogenetic tree of the AUX/LAX protein (encircled) and members of the auxin amino acid permeases family (AAAP).

The AAAP family comprises the aromatic and neutral amino acid transporters (ANT1-3), the amino acid pereases (AAP1-6), the lysine-histidine transporter (LHT1) and AUX/LAX auxin transport proteins (AUX1, LAX1-3). The tree was generated from a Clustal X alignment (29) and rooted with the sucrose transporter (SUC1).

(B) Protein sequence alignment of AUX1, LAX1, LAX2 and LAX3.

Sequence alignments and trees were generated with CLC Free Workbench 4.0.1 (CLC bio A/S). Level of identity is given as a blue scale. Arrowheads indicate the boundaries of the conserved trans-membrane amino acid transporter domain.

(C) Comparative exon structures of AUX/LAX genes.

AUX/LAX genes exhibit very similar gene structures. The number above each exon corresponds to the number of amino acid encoded by the exon. Introns are not represented. Level of identity is given as a grey scale.

Fig. S2

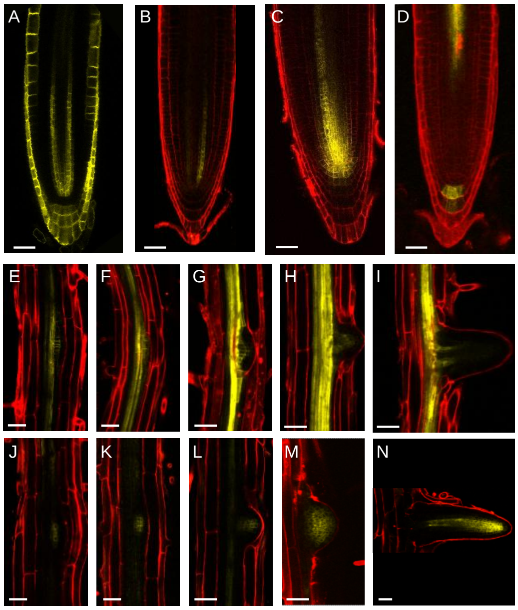

Figure S2. ***AUX/LAX* genes exhibit complementary expression patterns.**
(A-D) Expression profile of *ProAUX1:AUX1-YFP* (A), *ProLAX1:LAX1-VENUS* (B), *ProLAX2:LAX2-VENUS* (C) and *ProLAX3:LAX3-VENUS* (D) in the primary root apex.
(E-N) Expression profile of *ProLAX1:LAX1-VENUS* (E-I) and *ProLAX2:LAX2-VENUS* (J-N) during lateral root primordium development.
Scale bars 50 µm (A-D); 40 µm (E-N).

Figure S3

A

TFBS	AUX1	LAX1	LAX2	LAX3
Canonical AuxRE TGTCTC (GAGACA)	0	3	1	1
Aux-RR CORE (*Nicotiana tabacum*) GGTCCAT (ATGGACC)	0	1	0	1
Aux-RR CORE (*Pisum sativum*) GGTCAAT (ATTGACC)	0	0	0	2
Aux-RR CORE (*Pisum sativum*) AAGGAC (GTCCTT)	0	0	0	1
TGA-element (*Brassica oleracea*) AACGAC (GTCGTT)	0	0	0	1
CATATGGMSAUR (*Glycine max*) CATATG (CATATG)	1	1	0	2
NTBBF1ARROLB (*Agrobacterium*) ACTTTA (TAAAGT)	1	0	1	2
TOTAL	**2**	**5**	**2**	**10**

B

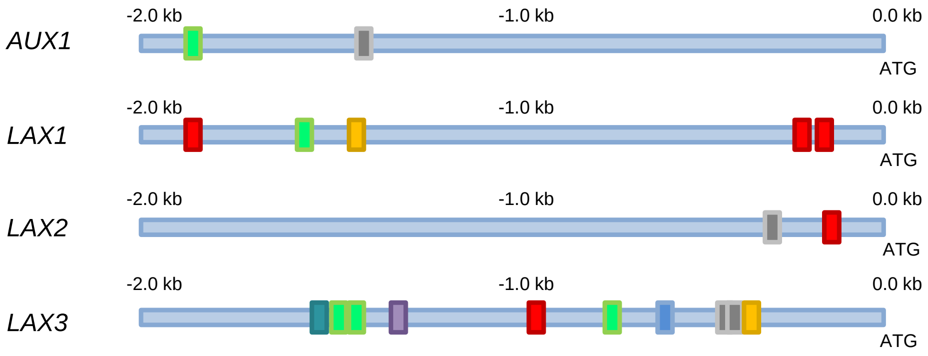

Figure S3. Promoter analysis of *AUX/LAX* genes.

A. Two-kilobase upstream sequences of *AUX/LAX* genes were analysed for putative canonical auxin response elements (AUXRE), auxin responsive region (AuxRR) and auxin related transcription factor binding sites (TFBS).

B. Diagrammatic representation of TFBS in *AUX/LAX* promoters.

Figure S4

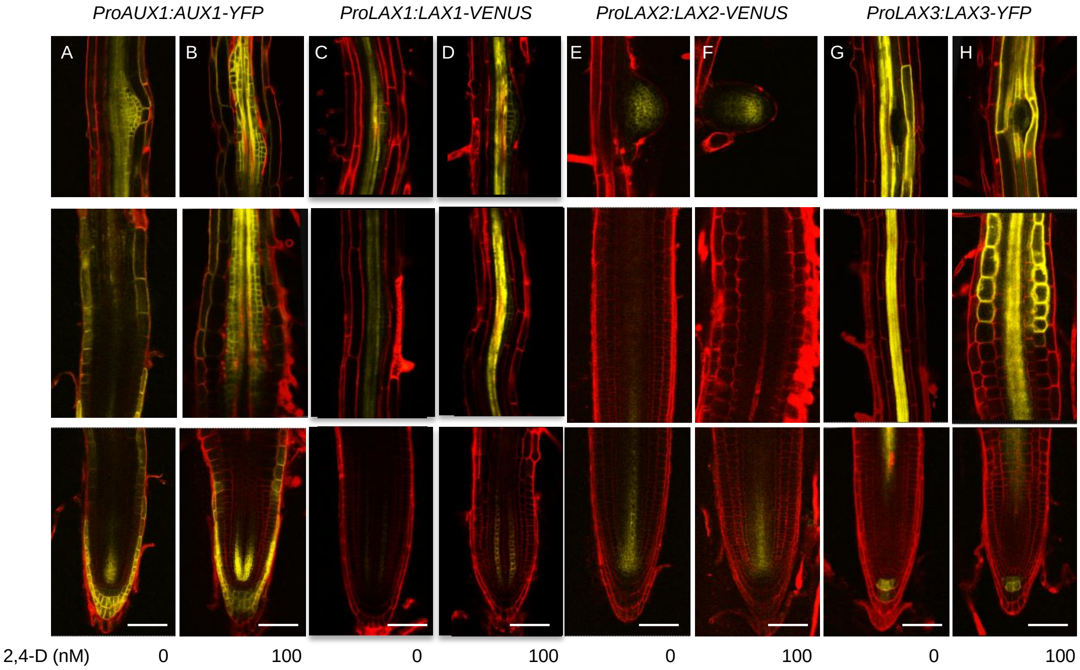

Figure S4. ***LAX1* and *LAX3* genes are induced by auxin.**
Expression profile of *ProAUX1:AUX1-YFP* (A,B), *ProLAX1:LAX1-VENUS* (C,D), *ProLAX2:LAX2-VENUS* (E,F) and *ProLAX3:LAX3-VENUS* (G,H) in the absence and presence of 100 nM 2,4-D.
Scale bars, 50 µm.

Figure S5

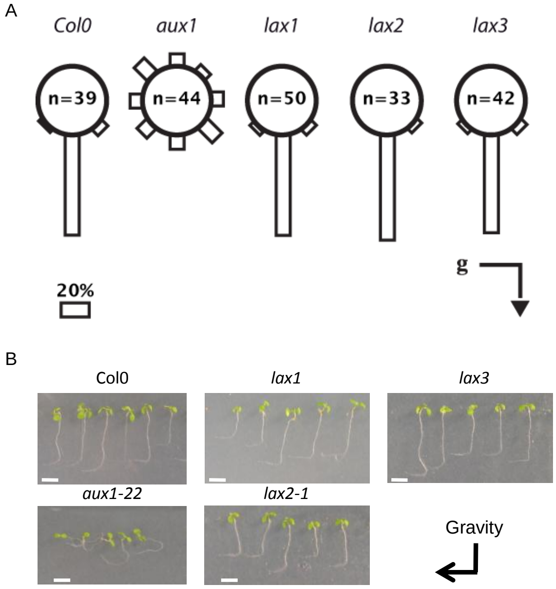

Figure S5. *lax1*, *lax2* and *lax3* mutants exhibit normal gravitropic response.
Wheel diagrams (A) and images (B) showing gravitropic responses of wild-type (Col0), *aux1-22*, *lax1*, *lax2-1* and *lax3* seedlings. Plates were rotated at 90° and root angles were determined 3, 6, 9 and 12 h after gravistimulation and grouped into 8 classes of 45°(A). Bars sizes represent percentage of plants according to scale. Photographs (A) were taken 24 hrs after the gravistimulus (n=20).

Figure S6

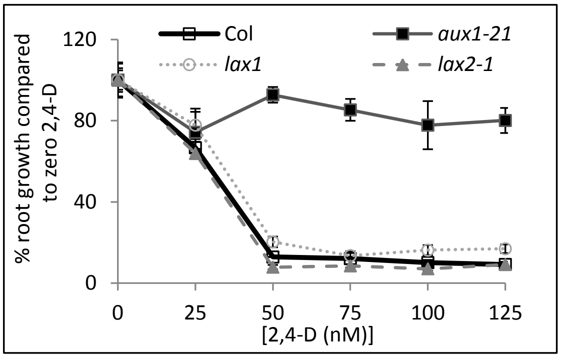

Figure S6. *lax1* and *lax2* mutants exhibit a normal response to 2,4-D.
Growth responses of wild-type (Col0), *aux1-21*, *lax1* and *lax2-1* grown at various concentrations of 2,4-D and root growth expressed as percentage of zero control (n=25). Error bars represent standard error.

Figure S7

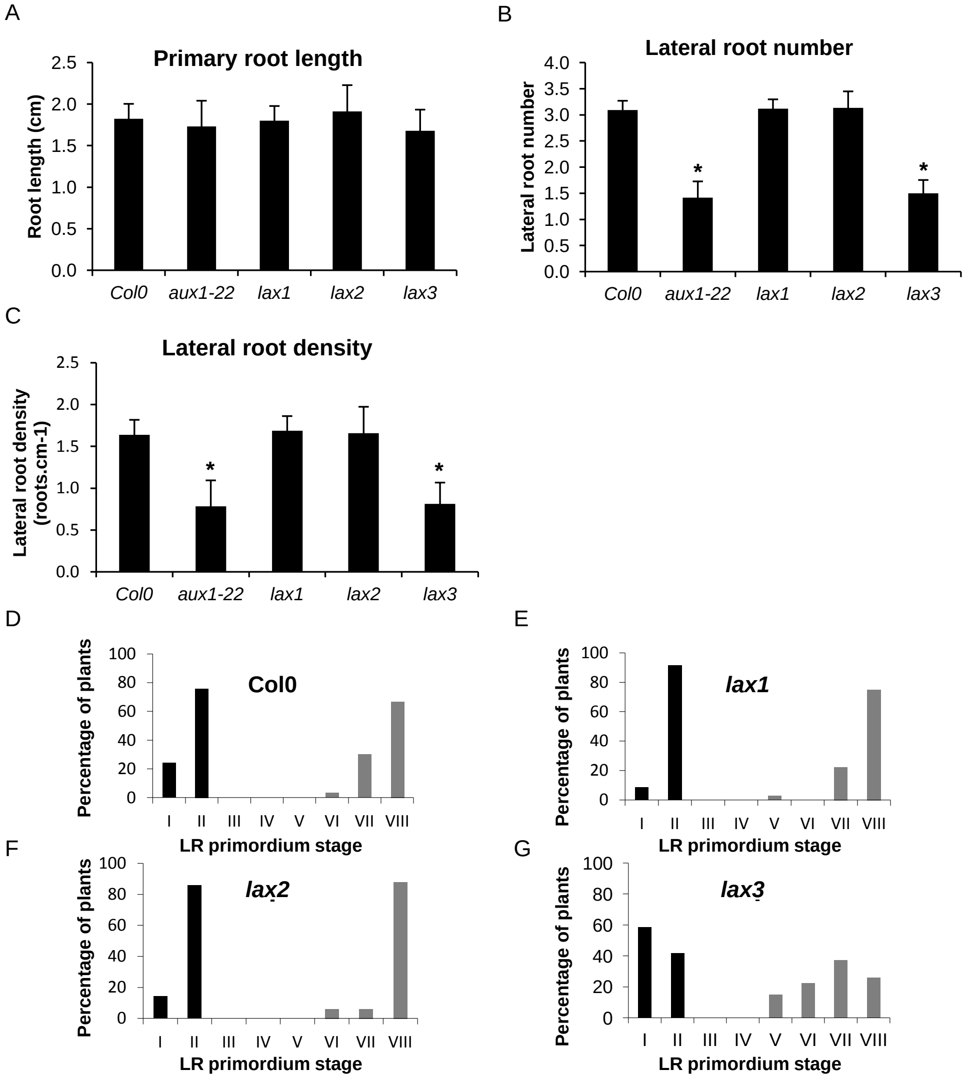

Figure S7. ***lax1*** **and** ***lax2*** **mutants exhibit normal root growth and lateral root growth.**
(A) Primary root length of 6-day-old *aux1-22*, *lax1*, *lax2* and *lax3* mutants (n=20).
(B) Lateral root number of 6-day-old *aux1-22*, *lax1*, *lax2* and *lax3* mutants (n=20).
(C) Lateral root density of 6-day-old *aux1-22*, *lax1*, *lax2* and *lax3* mutants (n=20)
(D-G) Lateral root emergence phenotype of wild-type (Col0), *lax1*, *lax2* and *lax3*. Lateral root formation was induced by gravistimulation and lateral root primordium stages were determined after 18 h (black bars) and 42 h (grey bars).
Error bars represent standard error. Asterisks represent statistically significant difference (Student's *t* test, P<0.01).

Figure S8

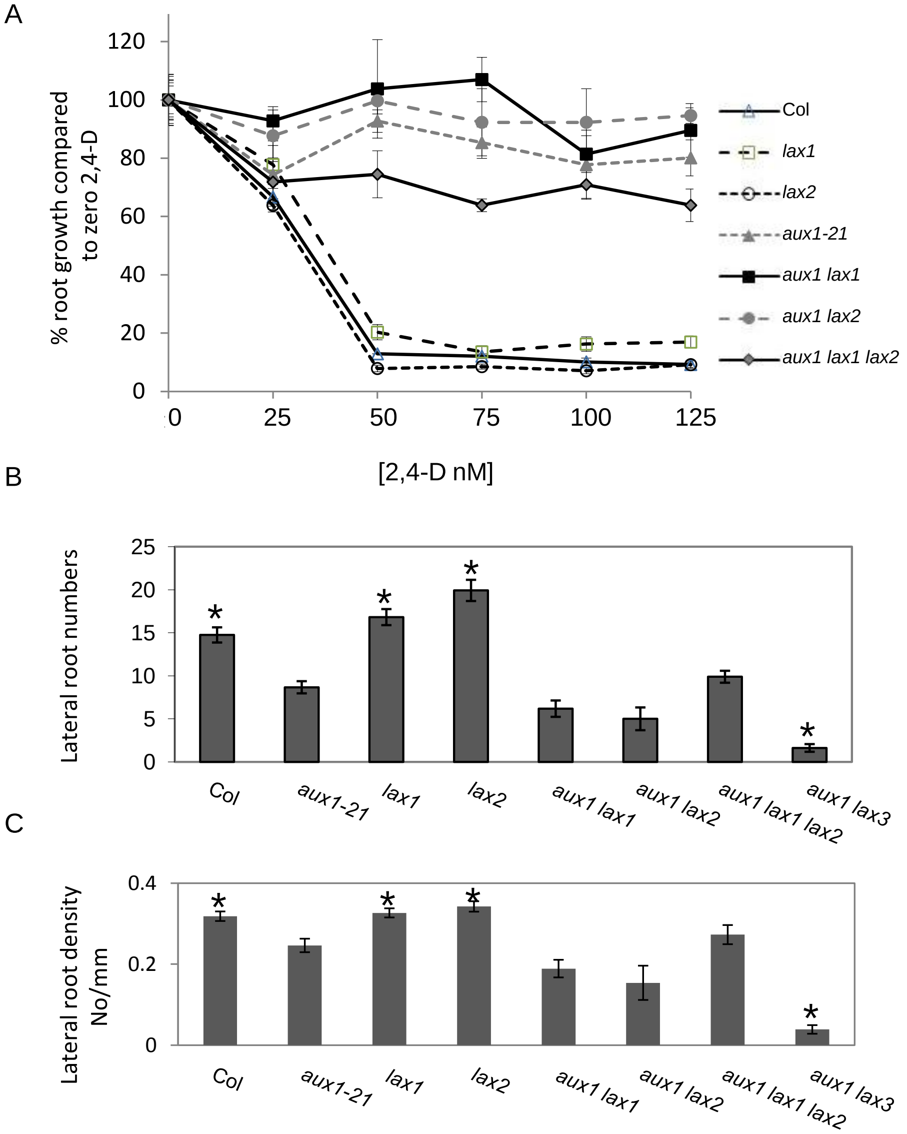

Figure S8. *lax1* and *lax2* mutants do not enhance *aux1* mutant phenotypes.

(A) Root growth responses of various a*ux/lax* mutants at various concentrations of 2,4-D. Root growth is expressed as percentage of zero control (n=20). Error bars represent standard error.

(B) Lateral root numbers in 10-day-old seedlings of various *aux/lax* mutants (n=20).

(C) Lateral root density in 10-day-old seedlings of various *aux/lax* mutants (n=20).

Error bars represent standard error. Asterisks represent statistically significant difference compared to *aux1-21* (Student's *t* test, P<0.05).

Figure S9

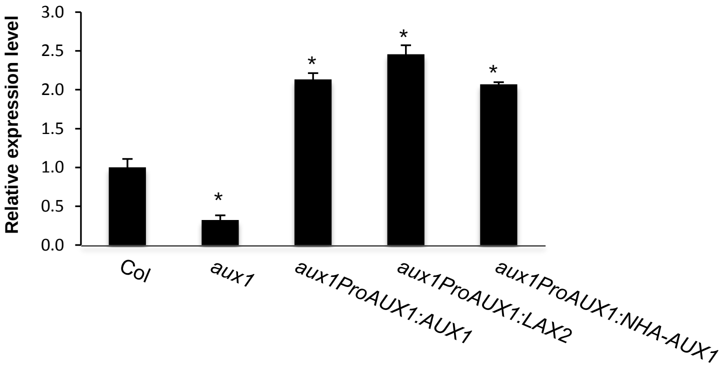

Figure S9. *ProAUX1:AUX1* and *ProAUX1:LAX2* seedlings exhibit comparable transgene expression levels.

Transgene expression levels were determined by quantitative RT-PCR in the wildtype (Col0), *aux1-22* and *aux1-22* complemented by *AUX1*, *LAX2* or *NHA-AUX1*. Error bars represent standard error. * indicates statistically significant difference in level of expression compared to the wildtype; n=3; Student's *t* test, P<0.01.