

## Supplementary Methods

**Recombinant plasmid construction and plant transformation.** A 3.5 kb of genomic DNA fragment containing the *AtSNX1* gene was amplified by PCR (Pyrobest, Takara) using specific Gateway primers (for exact sequences, see Table below) and recombined in pDONR207 (Invitrogen, Carlsbad, CA). This fragment was then subcloned in the pMDC107 vector for C-terminal GFP6 fusion<sup>5</sup>, and in the pGIInK::mRFP<sup>6</sup> vector for C-terminal mRFP fusion. cDNAs corresponding to *RABF1*, *RABF2b* and *TLG2a* were amplified by RT-PCR and recombined in pDONR207 (for primer sequences, see Table below). They were then subcloned into pMDC43 for N-terminal GFP<sub>6</sub> fusion or into pMDC83 for C-terminal GFP<sub>6</sub> fusion. These constructs as well as *ERD2-GFP* and *ST-GFP* containing vectors were introduced into Columbia accession or *snx1-1* by the *Agrobacterium*-mediated floral dip method<sup>7</sup> and transformants were selectively grown on MS medium with kanamycin (50 µg/mL) for the pGIInK::mRFP or hygromycin (30 µg/mL) for other constructs. For each line, plants with a unique T-DNA insertion were chosen for further experiments and at least ten independent transformants were analyzed in segregating T2 populations.

**Statistical analysis of results.** *P* values indicated for measurement of root length, lateral root density and gravitropic response were obtained using a two-sided Student's test assuming unequal variances. All calculations were performed using Microsoft<sup>®</sup> Excel 2003. All error bars in graphs indicate standard errors of the means.

## Primer sequences used in this study

Primer sequences	Use
AttB1-ATAAAAGCAGAAAGTGAG	Gateway cloning of the <i>At5g06140 locus</i> and intergenic genomic DNA region
AttB2-AGACAGAATAAGAAGCTTC	
AttB1-TAATGGGATGTGCTTCTTCT	Gateway cloning of <i>RABF1</i> cDNA
AttB2-ATGACGAAGGAGCAGGACG	
AttB1-TAATGGCTGCAGCTGGAAAC	Gateway cloning of <i>RABF2b</i> cDNA
AttB2-CTAAGCACAACAAGATGAGCT	
AttB1-TAATGGCGACGAGGAATCGTACGTTGCTG	Gateway cloning of <i>TLG2a</i> cDNA
AttB2-ACAAGAATATTTCCCTTGAG	
GCATCCGTATCTGTCAGTCTCCGTC	<i>AtSNX1</i> Full length cDNA amplification (RT-PCR)
GGGAAGAAGGGATCTCCAAGCATCCGCG	

## Supplementary references

1. Campbell, R. E. et al. A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 7877-7882 (2002).
2. Ulmasov, T., Murfett, J., Hagen, G. & Guilfoyle, T. J. Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* **9**, 1963-1971 (1997).
3. Grebe, M. et al. *Arabidopsis* sterol endocytosis involves actin-mediated trafficking via ARA6-positive early endosomes. *Curr. Biol.* **13**, 1378-1387 (2003).
4. Dharmasiri, S. et al. AXR4 is required for localization of the auxin influx facilitator AUX1. *Science* **312**, 1218-1220 (2006).
5. Curtis, M. D. & Grossniklaus, U. A gateway cloning vector set for high-throughput functional analysis of genes *in planta*. *Plant Physiol.* **133**, 462-469 (2003).
6. Rotman, N. et al. A novel class of MYB factors controls sperm-cell formation in plants. *Curr. Biol.* **15**, 244-248 (2005).
7. Clough, S. J. & Bent, A. F. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735-743 (1998).