Supporting Information

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SI Materials and Methods

Immunoblots and ChIP. Immunoblots were performed as previously described using the SuperSignal Femto West Substrate (Thermo Fisher Scientific) and an LAS-4000 Mini Image Analyzer (FUJIFILM) (1). ChIP and the subsequent quantitative RT-PCR (RT-qPCR) were performed as previously described (2, 3). ChIP was performed with antibodies against K9-acetylated HISTONE3 (H3K9Ac; ab10812; Abcam) with the GFP Vector Fusion Aid Kit (Axorra) for ARF2:GFP and anti–c-Myc agarose (Sigma) for ARF7:MYC. Fold enrichment of each genomic DNA fragment after ChIP and qPCR was calculated by normalizing the amount of target DNA fragment against a genomic fragment of *ACTIN8* as an internal control. Then, the value of the AUXIN RESPONSE FACTOR (ARF) transgenic line input was normalized against the value obtained with the respective loss-of-function mutant as a negative control using the following equation: $2^{(Ct_{transgene\ input} - Ct_{transgene\ ChIP})} / 2^{(Ct_{mutant} - Ct_{mutant\ ChIP})}$. DNA enrichments of each fragment were determined by RT-qPCR in three independent biological replicates.

Microarray Analysis. Microarray analysis was performed using total RNA extracted from 10-d-old seedlings grown in 150 μmol m^{-2} s^{-1} white light in long-day conditions (16/8 h at 21 °C/18 °C) using the NucleoSpin RNA Plant Kit (Machery-Nagel). Plant material was collected in the middle of the 16-h light period; 500 ng total RNA were prepared and labeled with Cy3 using the Quick Amp Labeling Protocol (Agilent Technologies). Three biological replicate samples were prepared for each genotype, and *Arabidopsis* arrays (V4, design ID 21169; Agilent Technologies) were hybridized at 65 °C for 17 h in rotating hybridization chambers (Agilent Technologies). Subsequently, the arrays were washed and scanned using an Agilent Microarray Scanner (Agilent Technologies). Total RNA and probe quality were controlled with a Bioanalyzer 2100 (Agilent Technologies). Raw data were extracted using the Feature Extraction software v.10.7.3.1 (Agilent Technologies). Raw data files were imported into GeneSpring GX (v. 12) and normalized choosing the scale-to-median and baseline-to-median options. Data were then filtered using the fold change algorithm (twofold change) and subsequently subjected to an ANOVA analysis ($P < 0.05$) (Dataset S1). Microarray data were deposited in the Gene Expression Omnibus (GSE35730).

1. Richter R, Behringer C, Müller IK, Schwechheimer C (2010) The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. *Genes Dev* 24(18):2093–2104.
2. Oh E, et al. (2009) Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in Arabidopsis. *Plant Cell* 21(2):403–419.
3. Fode B, Gatz C (2009) Chromatin immunoprecipitation experiments to investigate in vivo binding of Arabidopsis transcription factors to target sequences. *Methods Mol Biol* 479:261–272.

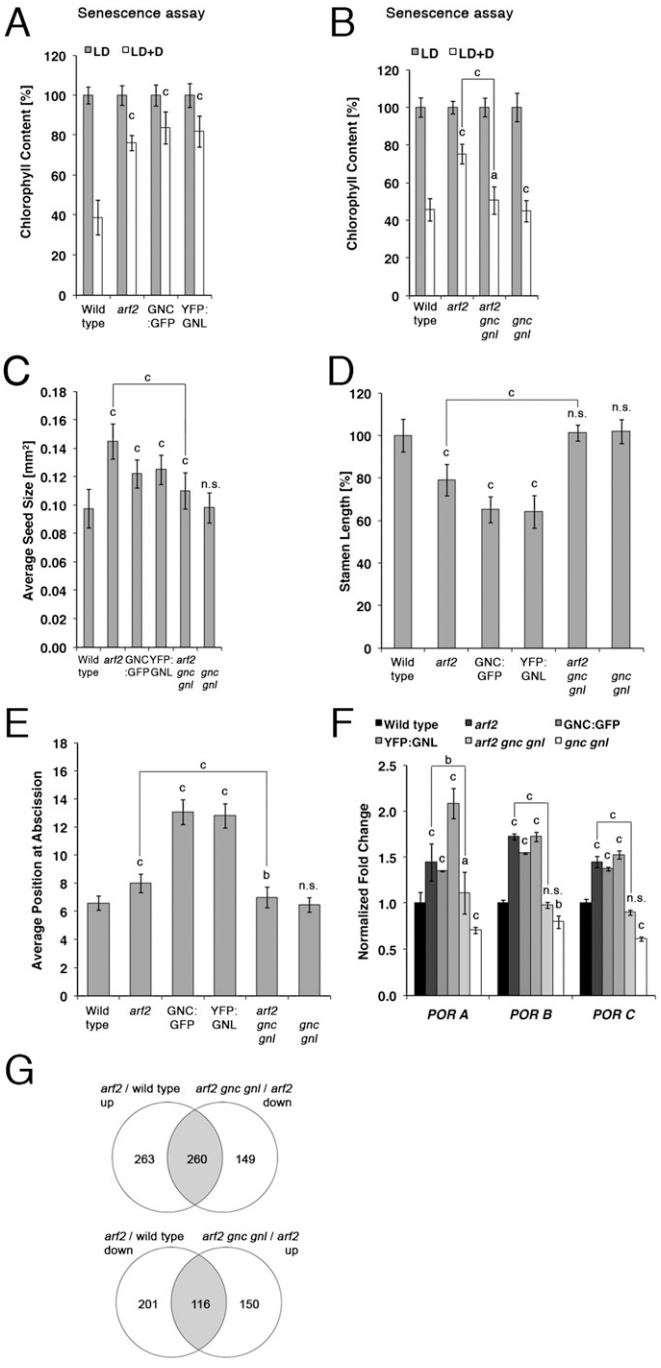

Fig. S1. Genetic interaction between *ARF2* and *GATA, NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED* (*GNC*) and *GNC-LIKE* (*GNL*)—analysis of additional phenotypes. (*A* and *B*) Relative chlorophyll content of detached leaves before and after transfer to the dark as a quantitative read out for leaf senescence. LD, long day-grown plants; LD+D, long day-grown plants transferred to the dark. (*C*) Quantification of seed size. (*D*) Stamen length at floral stage 15. (*E*) Average position of floral organ abscission. (*F*) *PROTOCHLOROPHYLLIDE OXIDOREDUCTASE A*–*C* (*POR A*–*C*) gene expression as detected by RT-qPCR in 10-d-old seedlings. (*G*) Venn diagrams indicating the number of differentially expressed up- and down-regulated genes between WT and *arf2* (*arf2*/wild type) as well as *arf2* and *arf2 gnc gnl* (*arf2 gnc gnl*/*arf2*) as presented in Table S1. The average and SE of three replicate measurements are shown in *A*–*F*. Student *t* test: a = $P \leq 0.05$; b = $P \leq 0.01$; c = $P \leq 0.001$; n.s., not significant.

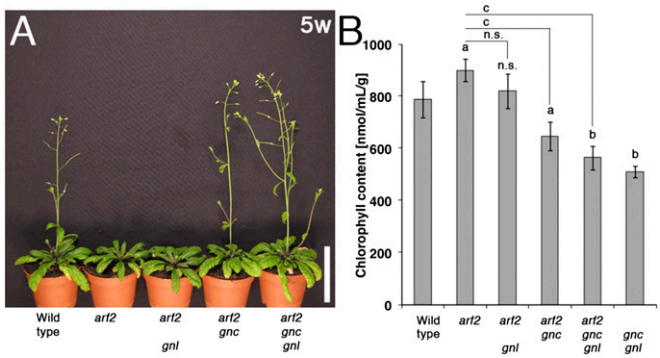

Fig. S2. Genetic interaction between *ARF2*, *GNC*, and *GNL*. (*A*) Representative photographs of 5-wk-old (5w) WT and *arf2* mutant as well as *arf2 gnc*, *arf2 gnl*, and *arf2 gnc gnl* mutant plants grown in long-day conditions. (Scale bar: 5 cm.) (*B*) Absolute chlorophyll content determined from 10-d-old light-grown seedlings of the genotypes shown in *A*. Student *t* test: a = $P \leq 0.1$; b = $P \leq 0.05$; c = $P \leq 0.01$; n.s., not significant.

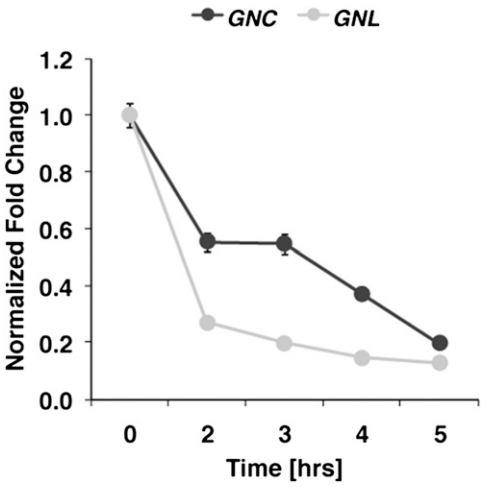

Fig. S3. Gene expression analysis of GNC and GNL after auxin treatment. *GNC* and *GNL* gene expression as detected by RT-qPCR in 10-d-old seedlings after treatment with 5 μM 2,4D. The fold change relative to transcript levels at time point 0 h is shown.

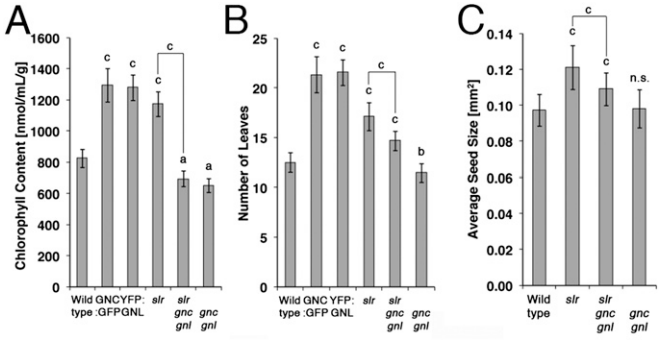

Fig. S4. Genetic interaction between *SOLITARY ROOT* (*SLR*) and *GNC* and *GNL*—analysis of additional phenotypes. (*A*) Absolute chlorophyll content of 10-d-old light-grown seedlings. (*B*) Flowering time analysis. Number of leaves until bolting. (C) Quantification of seed size. The average and SE of three replicate measurements are shown. Student *t* test: a = $P \leq 0.05$; b = $P \leq 0.01$; c = $P \leq 0.001$; n.s., not significant. CBB, Coomassie Brilliant Blue (loading control).

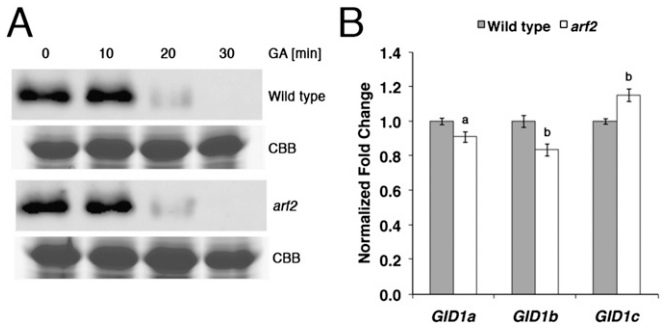

Fig. S5. The gibberellin (GA) response pathway is functional in *arf2* mutants. (*A*) Representative immunoblots with anti-REPRESSOR-OF-*ga1-3* (anti-RGA) of total protein extracts (45 μg) prepared from 10-d-old light-grown WT and *arf2* seedlings that had been treated with GA3 (100 μM). (*B*) RT-qPCR detection of *GID1a–GID1c* transcripts from 10-d-old light-grown WT and *arf2* mutant seedlings. Student *t* test: a = $P < 0.05$; b = $P < 0.01$.

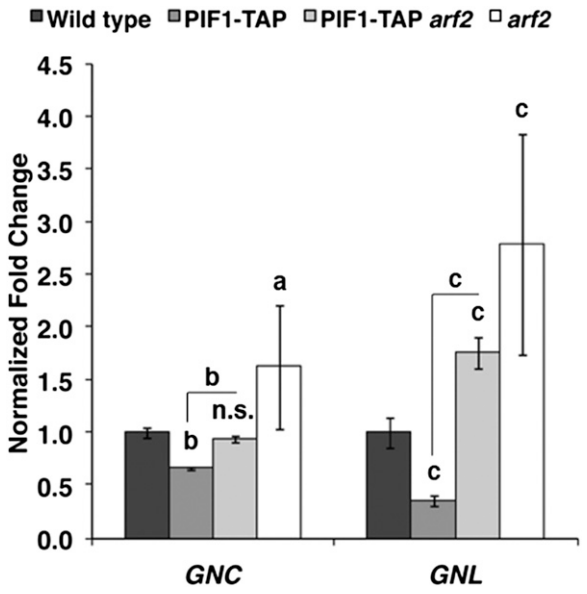

Fig. S6. Independent regulation of *GNC* and *GNL* transcription by ARF2 and PHYTOCHROME INTERACTING FACTOR 1 (PIF1). RT-qPCR amplification of *GNC* and *GNL* transcripts from 10-d-old seedlings. Student *t* test: a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$; n.s., not significant.

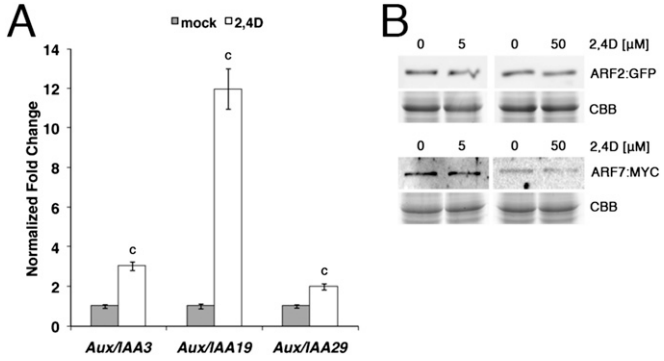

Fig. S7. Auxin has no effect on ARF7:MYC and ARF2:GFP protein abundance. (*A*) RT-qPCR analysis of the transcript analysis of three representative auxin-regulated genes after 30 min 2,4D treatment (5 μM) reveals the activation of the auxin response machinery by the auxin treatment. Control experiment for the experiment is shown in *B*. Fold change relative to mock-treated WT levels. (*B*) Anti-GFP and anti-MYC immunoblots to detect ARF2:GFP and ARF7:MYC from total protein extracts (30 μg) prepared from 30 min mock- and 2,4D-treated seedlings. Student *t* test: c = $P < 0.001$. CBB, Coomassie Brilliant Blue (loading control); IAA, indole-3-acetic acid.

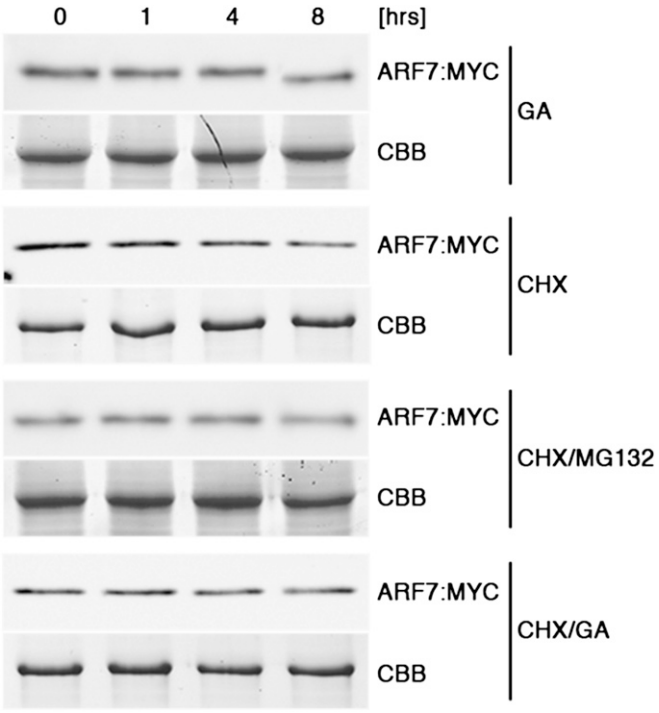

Fig. S8. Protein behavior of ARF7:MYC in response to GA. ARF7:MYC immunoblot analyses of 10-d-old light-grown seedlings treated with 10 μM GA3, 50 μM cycloheximide (CHX), and 100 μM MG132 (Z-Leu-Leu-Leu-al) as specified. CBB, Coomassie Brilliant Blue (loading control).

Table S1. Positions of auxin response elements in the *GNC*, *GNL*, and *ARF2* promoters

Name	Sequence	Position	Strand
GNC promoter			
Ia	TGT.CTN	−23 to −19	+
Ib	TGT.CTC	−135 to −130	+
IIa	TGT.CTC	−232 to −227	−
IIb	TGT.CTN	−269 to −265	+
IIIa	TGT.CTC	−694 to −689	+
IIIb	TGT.CTC	−777 to −772	+
GNL promoter			
I	TGT.CTN	−492 to −488	−
IIa	TGT.CTN	−637 to −633	−
IIb	TGT.CTN	−710 to −706	+
III	TGT.CTC	−935 to −930	+
ARF2 promoter			
I	TGT.CTN	−137 to −133	−
IIa	TGT.CTC	−387 to −382	−
IIb	TGT.CTC	−401 to −396	−
IIIa	TGT.CTN	−647 to −643	+
IIIb	TGT.CTN	−798 to −794	+

Table S2. List of primers used in this study

Primer name	Sequence (5′ to 3′)
ARF2 gLP	AGTACTGGCCACCGCATGGCATGCCA
ARF2 gRP	GCAGCATCATTCAAGTTGTTTCTCTG
GNL gLP	TATCTGATGGTGGTTCATCATCAAG
GNL gRP	ATGCTAGATCATCGAAATAGATATTG
GNC gLP	ACACTTATCACGCTGATCATCTCC
GNC gRP	TGTGGGAACACTTGTTGATAAGCTG
GA1 gLP	ACATTGTTGCATAGTTTGGAGGGGATGC
GA1 gRP	TGGAATATGTCCCATTCGAGCTGATGCTGAGC
UBC21 2step-LP	TCCTCTTAACTGCGACTCAGG
UBC21 2step-RP	GCGAGGCGTGTATACATTTG
ACT8 2step-LP	TCAGCACTTTCCAGCAGATG
ACT8 2step-RP	CTGTGGACAATGCCTGGAC
GNC 2step-LP	GCGTGATTAGGGTTTGTTCG
GNC 2step-RP	CTTTGCCGTATACCACATGC
GNL 2step-LP	CCATATCTCCCAACCTCTCG
GNL 2step-RP	TGGGCACCATTTGATCAC
GID1a 2step-LP	GCTGCGAGCGATGAAGTTA
GID1a 2step-RP	AACCCATGTATTGAGAGGAACC
GID1b 2step-LP	TTTTGCTGGGTTTTGGAGAC
GID1b 2step-RP	CCATGTGTTGAGTGGGACAA
GID1c 2step-LP	CTTTTTGCGTTTTGATTGGTAA
GID1c 2step-RP	CCATGTATTGAGAGGAACCACTG
POR A 2step-LP	CCCTTCAAGCTGCTTCTTTG
POR A 2step-RP	GATGCTGAAGCATTTAATTTTCC
POR B 2step-LP	CGAGAGCACATTCCTCTCTTC
POR B 2step-RP	GCTTGGATCACTCACCACCT
POR C 2step-LP	GGATTGAATGGGCAAAACAG
POR C 2step-RP	TGTAAGCCTTTGCTCCATCA
ARF2 2step-LP	ATGATGCTTGTTGGTGACGA
ARF2-2step-RP	GAAGATTTTGCGAACCATGC
ARF7 2step-LP	GCTCATATGCATGCTCCACA
ARF7-2step-RP	GCAATGCATCTCTGTCATATTTGT
GNC AuxRE-LP III	GATTTGTGTGGCTCTCACTCTCC
GNC AuxRE-RP III	AGAGGTTCACGTGAGTCCATGGC
GNC AuxRE-LP II	TCATTGGTATGACATCCGGACTTG
GNC AuxRE-RP II	TGAGACACGAGCCAAACACACATGG
GNC AuxRE-LP I	ATGAATATAATAGAGACCATGTG
GNC AuxRE-RP I	TGATGAGATAAACAAATGAAGAC
GNC H3K9 ChIP LP-Ha	GTGATTCTTCATGAATACAGT
GNC H3K9 ChIP RP-Ha	GATCCTTTTACTTGTTGTCT
GNC H3K9 ChIP LP-Hb	CGTCTATGATCTCTCTCAATC
GNC H3K9 ChIP RP-Hb	GACTTGATGATGATGATGATG
GNL AuxRE-LP III	ATCATGATATGTGTGGCTCTTG
GNL AuxRE-RP III	GAGGTTCACGTGAGTACATCTG
GNL AuxRE-LP II	AGATCTCAGCCGTTGTAGTTCG
GNL AuxRE-RP II	GGGCAGAGACCAGTGTTATATA
GNL AuxRE-LP I	CTTCATCAGTCTCTAAACATGGCC
GNL AuxRE-RP I	CTGATTTGGTGAAAGATACATA
GNL H3K9 ChIP LP-Ha	ACGAAATATTACAGATAGGA
GNL H3K9 ChIP RP-Ha	ATTCGAACTACAACGGCTGAGA
GNL H3K9 ChIP LP-Hb	CATAGTATAGCTTGGATCC
GNL H3K9 ChIP RP-Hb	GCTTGGTGATGAAAATGTTGT
ARF2 AuxRE ChIP-LP III	AGGATGACTTAAATTGCTATA
ARF2 AuxRE ChIP-RP III	CTATTACCGGTTGTAACCATTTCC
ARF2 AuxRE ChIP-LP II	TGAAGCTGAGTTTTGACTACCTCTG
ARF2 AuxRE ChIP-RP II	CTAGTCTAGGCTTTATCGGAGTC
ARF2 AuxRE ChIP-LP I	ATCGAGTAATGTCAAGGAGCTA
ARF2 AuxRE ChIP-RP I	AGCAGACGATATCAACAAGAA
Aux/IAA3 2step-LP	CAAAGATGGTGATTGGATGCT
Aux/IAA3 2step-RP	TGATCCTTAGTCTCTTGCACGTA
Aux/IAA19 2step-LP	CTATGATGATCTAGCCTTTGC
Aux/IAA19 2step-RP	AGAAACATCCCCCAAGGTACA
Aux/IAA29 2step-LP	CCGAGTCTTCAATAGTTTACGATG
Aux/IAA29 2step-RP	CGAATATGACGATGATGATAACTACC

Dataset S1. Differentially expressed genes in WT, *arf2*, and *arf2 gnc gnl* mutants as detected by microarray analysis

Dataset S1 (XLS)