Figure S1

(a) Phylogenetic tree showing the relationship between different WR isoforms.

(b) Diagram illustrating the gene structures of Gm08g227700.1, Gm08g227700.2, and Gm15g221600.1, indicating exon and intron positions.

(c) Gene sequences of Gm08g227700.1, Gm08g227700.2, Gm15g221600.1, with nucleotide positions indicated.
Figure S1. Phylogenetic (a) and gene structure analysis (b) and protein sequence alignment (c) of GmWRI1s.

(a) Unrooted phylogenetic tree of WRINKLED1s (WRI1) from various plants. The alignment was generated using Clustal and the unrooted phylogram was constructed with MEGA6 software using the neighbor-joining method, with bootstrap values based on 1000 replicates.
Figure S2. Schematic diagram of the GmWRI1s and AtWRI1 predicted protein sequence illustrating the locations of known domains.

(a) The two AP2 DNA binding domains are shown in red; three intrinsically disordered regions (IDR) are shown in green line. Two KIN10 phosphorylation sites are shown as blue bars. The 14-3-3 phosphopeptide binding site and BPM binding site colocalize to the same region represented by an orange line. The C-terminal PEST domain, residues with in IDR3, is represented as a purple line.

(b-e, o,p) Distribution of GUS activities in proGmWRI1a::GUS Arabidopsis plants.

(f-i, p,q) Distribution of GUS activities in proGmWRI1b::GUS Arabidopsis plants.

(j-m) Distribution of GUS activities in wild-type control plants.

Histochemical detection of GUS activity in different tissues, including seedling (b, f, j), young pod (c, g, k), leaf (d, h, l), inflorescences (e, i, m), flower (n, p), and old pod (o, q). Bars=500µm in (b), (f), (j) and (e, i, m), 1mm in pod, leaf and 50µm in flower.
Figure S3. Public data for expression of soybean GmWRI1s

(a) Expression patterns of GmWRI1 in different tissues of soybean plant. The public date are shown in phytozome (https://phytozome.jgi.doe.gov/). GmWRI1a (Glyma08g227700.1 in v10, Glyma08g24420 in v9), GmWRI1a’ (Glyma08g227700.2 in v10), GmWRI1b (Glyma15g221600.1 in v10, Glyma15g34770 in v9). (b) GmWRI1 mainly expressed in developing seeds. These data are retrieved from SoyKB (www. SoyKB.com)
Figure S4. Confirmation of transgenic *atwri1–1* (a) and wild-type (b) plants over-expressing *GmWRI1*s.
Figure S5

(a) Total TAG content (mg/g dry weight)

(b) Fatty acid composition (%)

(c) Total TAG content (mg/g dry weight)

(d) Fatty acid composition (%)

Legend:
- Col-0
- a-9
- a-13
- a-15
- b-3
- b-10
- b-11
- b'-6
- b'-12
- b'-15

Statistical significance indicated by asterisks:
- *: p < 0.05
- **: p < 0.01
- ***: p < 0.001
- ****: p < 0.0001

Figure S5. Functional expression of *GmWRIIa* and *b* in Arabidopsis thaliana seeds

(a) Total TAG contents in *GmWRII*s expressing Arabidopsis seeds.
(b) Fatty acid composition in TAGs from wild-type Arabidopsis seeds expressing *GmWRII*s.
(c) Total TAG contents in *GmWRII*-complemented Arabidopsis *wri1-1* mutant seeds.
(d) Fatty acid composition in TAGs from Arabidopsis *atwri1-1* mutant seeds expressing *GmWRII*s.

All data are expressed as mean ± SD from at least three biological duplicates. * P < 0.05 and ** P < 0.01 by Student’s *t* test for significant difference. T3 transgenic Arabidopsis seeds were used for analysis.
Figure S6. Proteins content of wild type, *atwrl* mutant and transgenic plant in mature seeds.
Figure S7. Quantitative PCR confirmation of gene regulation by GmWRI1 in soybean hairy roots.

ACP1b:Glyma15g098500 (Glyma15g10520)
PDCT1a:Glyma08g213100 (Glyma08g22750)
ABCPb:Glyma09g214500 (Glyma09g34770)
PKP2a:Glyma09g126300 (Glyma09g23150)
Annexin-like protein RJ4(NAX4)
NAX4a:Glyma13g199500 (Glyma13g26960)
NAX4b:Glyma11g153800 (Glyma11g21480)
WAT1-1: Glyma19g173800 (Glyma19g35720)
WAT1-2: Glyma04g251000 (Glyma04g43000)
FABG1:3-oxoacyl-[acyl-carrier-protein] reductase 1
FABG1a:Glyma11g248000 (Glyma11g37320)
BC: Biotin carboxylase 1
BC1b:Glyma08g027600 (Glyma08g03120)
DLD1: Dihydrolipoyl dehydrogenase 1
DLD1b:Glyma17g032300 (Glyma17g03560)
DLD1c:Glyma15g143100 (Glyma15g15310)
PDHE2: Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenases
PDHE2-5:Glyma10g215400 (Glyma10g35960)
PDHE2-4:Glyma.20g176300 (Glyma20g24830)
FAAH: Fatty acid amide hydrolase
FAAH:Glyma08g003100 (Glyma08g00535)

All data are expressed as mean ± SD from at least three biological duplicates. * P < 0.05 and ** P < 0.01 by Student’s t test for significant difference.
Figure S8

(a) Nod factors → NFR1/NFR5 → SYMRK → Ca\(^{2+}\) spiking → CCaMK → NSP1/NSP2 → NIN → Nodule organogenesis

(b) Bar graphs showing expression levels of proteins.

(c) Bar graphs showing expression levels of proteins.

Additionally, a flowchart illustrating the processes:

Nod factors → NFR1/NFR5 → SYMRK → Ca\(^{2+}\) spiking → CCaMK → NSP1/NSP2 → NIN → Nodule organogenesis

Nin factors

Nodule organogenesis

Bacterial infection

Cytokinin signaling

SYMRK

CCaMK

LHK1

NSP1/NSP2

NIN

(c)
Figure S8. Effects of GmWR11a and b overexpression on soybean nodulation and glycolysis and lipid metabolism.
(a) Schematic of nodulation signal pathway.
(b) qRT-PCR verification of expression levels of early nodulation genes.
(c) qRT-PCR verification of expression levels of glycolysis and lipid metabolism gene.
All data are expressed as mean ± SD from at least three biological duplicates. * P < 0.05 and ** P < 0.01 by Student’s t test for significant difference.
Figure S9

(a) Expression Level (Ratio to ACTIN) GmWRI1a and GmWRI1b.

(b) Total free fatty acid content (mg/g dry weight).

(c) TAG fatty acid content (dry weight mg/g).

(d) MGDG and PC content (dry weight nmol/mg).

(mg/g dry weight)
Figure S9. Accumulation of lipids and expression patterns of soybean lipid synthesis–related genes at soybean nodule developmental stages.

(a) qRT-PCR analysis of GmWRII expression.
(b) Contents of free fatty acids in soybean nodules of GmWRIIa or b-overexpressing and GmWRIIRNAi hairy roots, as compared with these nodules from GUS-expressing hairy roots (as a control).
(c) Fatty acid contents in TAGs and MAGs from nodules overexpressing GmWRIIa and GmWRIIb.
(d) Total MGDG and PC contents in the nodules overexpressing GmWRIIa and GmWRIIb.
(e) Up-regulation of PC and MGDG synthesis genes lysophosphatidylcholine acyltransferase 1 (LPCAT1)(Glyma17g14070) and LPCAT2(Glyma05g03510) and Type B MGDG synthase 2a (MGD2a) (Glyma17g11720) and GmMGD2b (Glyma13g23150) in GmWRIIb OE hairy roots as compared with GUS control.
(f) qRT-PCR verification of expression levels of lipid transporter gene GmSTR2b (Glyma09G202400).
Figure S10

(a) Root Nodule 1
Nodule 2

GmRGL1 (Glyma.10G190200)
GmRGL2 (Glyma.20G200500)
GmRGL3 (Glyma.06G213100)
GmRGL4 (Glyma.04G150500)
GmRAD1a (Glyma.15G213300)
GmRAD1b (Glyma.05G045300)
GmRAD1c (Glyma.17G127500)
GmMIG1 (Glyma.09G036500)
GmGPAT5a (Glyma.09G036500)
GmGPAT5b (Glyma.14G067200)
GmGPAT4a (Glyma.03G008300)
GmGPAT4b (Glyma.07G069700)
GmGPAT6a (Glyma.03G078600)
GmGPAT6b (Glyma.07G146800)
GmFatAa (Glyma.18G167300)
GmFatBa (Glyma.04G197400)
GmFatBb (Glyma.06G168100)
GmSTRL1 (Glyma.20G242000)
GmSTRL2 (Glyma.19G176000)
GmSTRL3 (Glyma.03G175300)
GmAUX1 (Glyma.18G198400)
GmARF (Glyma.01g103500)
GmRGL4 (Glyma.04G151600)
GmRGL3 (Glyma.06G211300)
GmRGL2 (Glyma.20G012300)
GmRGL1 (Glyma.05G012300)
GmMIG1 (Glyma.09G036500)
GmGPAT5a (Glyma.09G036500)
GmGPAT5b (Glyma.14G067200)
GmGPAT4a (Glyma.03G008300)
GmGPAT4b (Glyma.07G069700)
GmGPAT6a (Glyma.03G078600)
GmGPAT6b (Glyma.07G146800)
GmFatAa (Glyma.18G167300)
GmFatBa (Glyma.04G197400)
GmFatBb (Glyma.06G168100)
GmSTRL1 (Glyma.20G242000)
GmSTRL2 (Glyma.19G176000)
GmSTRL3 (Glyma.03G175300)
GmAUX1 (Glyma.18G198400)
GmARF (Glyma.01g103500)
GmRGL4 (Glyma.04G151600)
GmRGL3 (Glyma.06G211300)
GmRGL2 (Glyma.20G012300)
GmAUX1 (Glyma.18G198400)
GmARF (Glyma.01g103500)

(b) WT FRI1b.OE

Fat B
Fat M
Fat A

(c) Fat

Glyma.04G151600
Glyma.06G211300
Glyma.05G012300
Glyma.17G120400
FatB(AT1G08510.1)
Glyma.04G197400
Glyma.06G168100
Glyma.08G349200
FatA(AT4G13050.1)
Glyma.08G349200
GmFatAa (Glyma.18G167300)
Figure S10. Identification and transcriptional analysis of FatB, GPAT, GRAS transcription factors RAM1 and ABCG transporter STR1/2.

(a) Heatmap analysis of soybean homologue genes for Medicago RAM1, RAM2, WRI1, STR, and FatM.

(b) Expression of ortholog genes (g) for *M. truncatula* RAM1, RAM2, WRI1, STR1/2, and FatM in root and nodule and genes with WRI1-binding cis-elements related to auxin modification, transport, signaling, or 2-MAG synthesis or transport in WT and *GmWRI1bOE* line.

(c) Phylogenetic identification of soybean FatB and FatA genes homology to Medicago and Lotus or other fatty acyl binding protein thioesterase (Fat).

(d) Phylogenetic identification of soybean GRAS genes homology to Medicago and Lotus or other GPATs.

(e) Phylogenetic identification of soybean GPAT genes homology to Medicago and Lotus or other GPATs.

(f) Phylogenetic identification of soybean ABCG transporter genes homology to Medicago and Lotus or other STR1/2 or lipid transporters ABCG11, ABCG26.

The alignment was generated using Clustal W and the unrooted phylogram was constructed with MEGA6 software using the neighbor-joining method.
Supplemental figure S11. The heat map analysis of tissue expression patterns of ortholog genes (a), other homology genes (b) for M. truncatula RAM1, RAM2, WRI1, STR1/2, and FatM. (c) is the tissue expression of ABCG11, ABCG26, RGL, RAD, and STR-like (STRL).
Supplementary figure 12. The phylogenic tree (a) and heat map analysis (b) in roots and nodules of CUS (cutin synthase). The alignment was generated using Clustal and the unrooted phylogram was constructed with MEGA6 software using the neighbor-joining method. The heat map was made by MeV software.
Figure S13. Expression level of GmGH3, GmPIN, and Auxin-responsive protein SAUR genes in GmWRI1b overexpression (GmWRI1bOE) hairy roots, as compared with control (GUS) hairy roots.
Figure S14. Expression level of jasmonate biosynthesis and abscisic acid biosynthesis and catabolic genes in *GmWRI1b* overexpression (*GmWRI1bOE*) hairy roots, as compared with control (*GUS*) hairy roots.
Figure S15. Proposed functions for GmWRI1s in soybean nodulation

**Root**
- KIN10 phosphorylation

**GmWRI1**
- Translation, Dimerization, Interaction with partners, …
- Alternative splicing forms
- Degradation

**Up-stream regulators**
- RY

**Nodule**
- Symbiosomes
- N2
- NH3
- Endomembrane expansion, Rhizobial Proliferation, …
- Nitrogen fixation
- Organic acids, amino acids, …
- Starch degradation, Glycolysis
- Fatty acid biosynthesis
- Membrane lipid, 2-MAG synthesis
- Cutin monomers, Adaptation to various environments
- Modify auxin, JA signaling, ...
- Target genes

**Symbiosomes**
- nodule organogenesis
- Adaptation to various environments

**Figure S15**