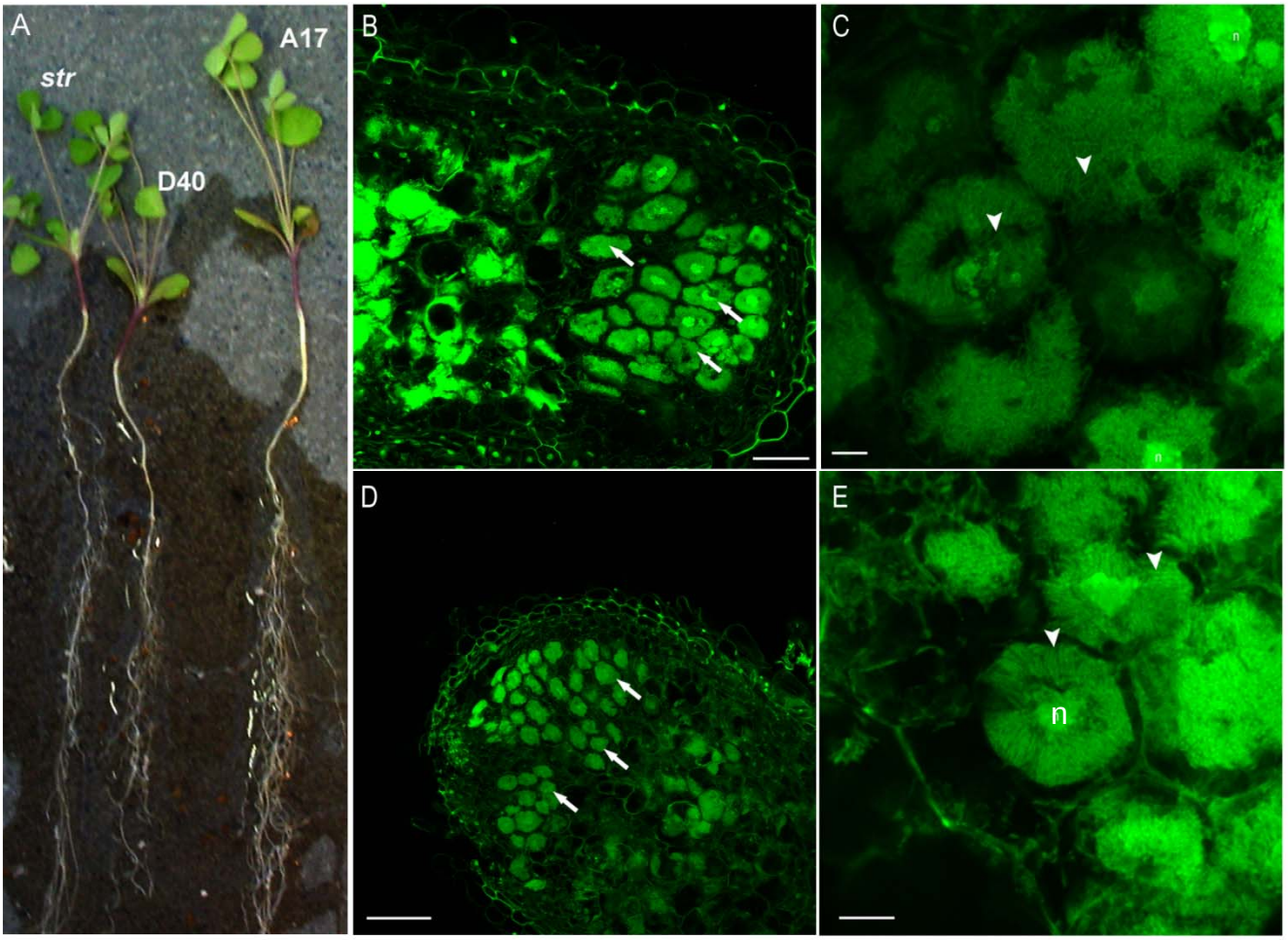
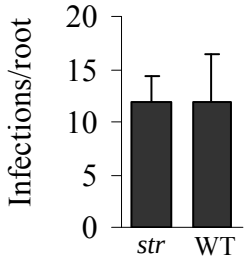
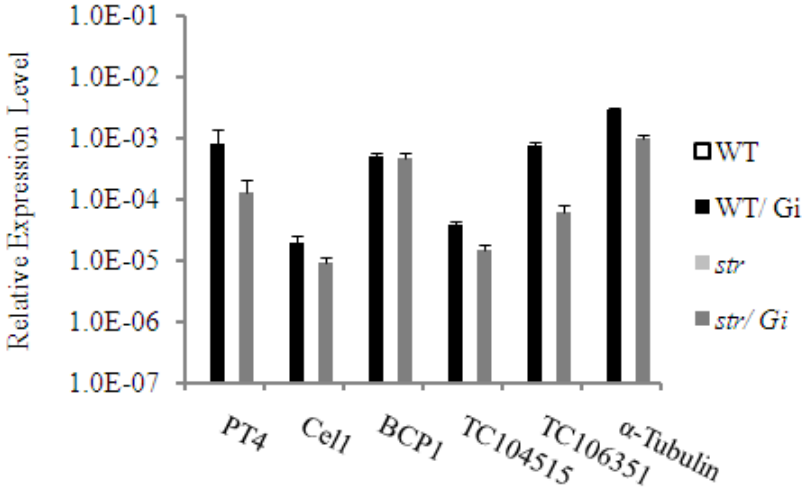

A
A17
str
D40
B
C
D
E
n

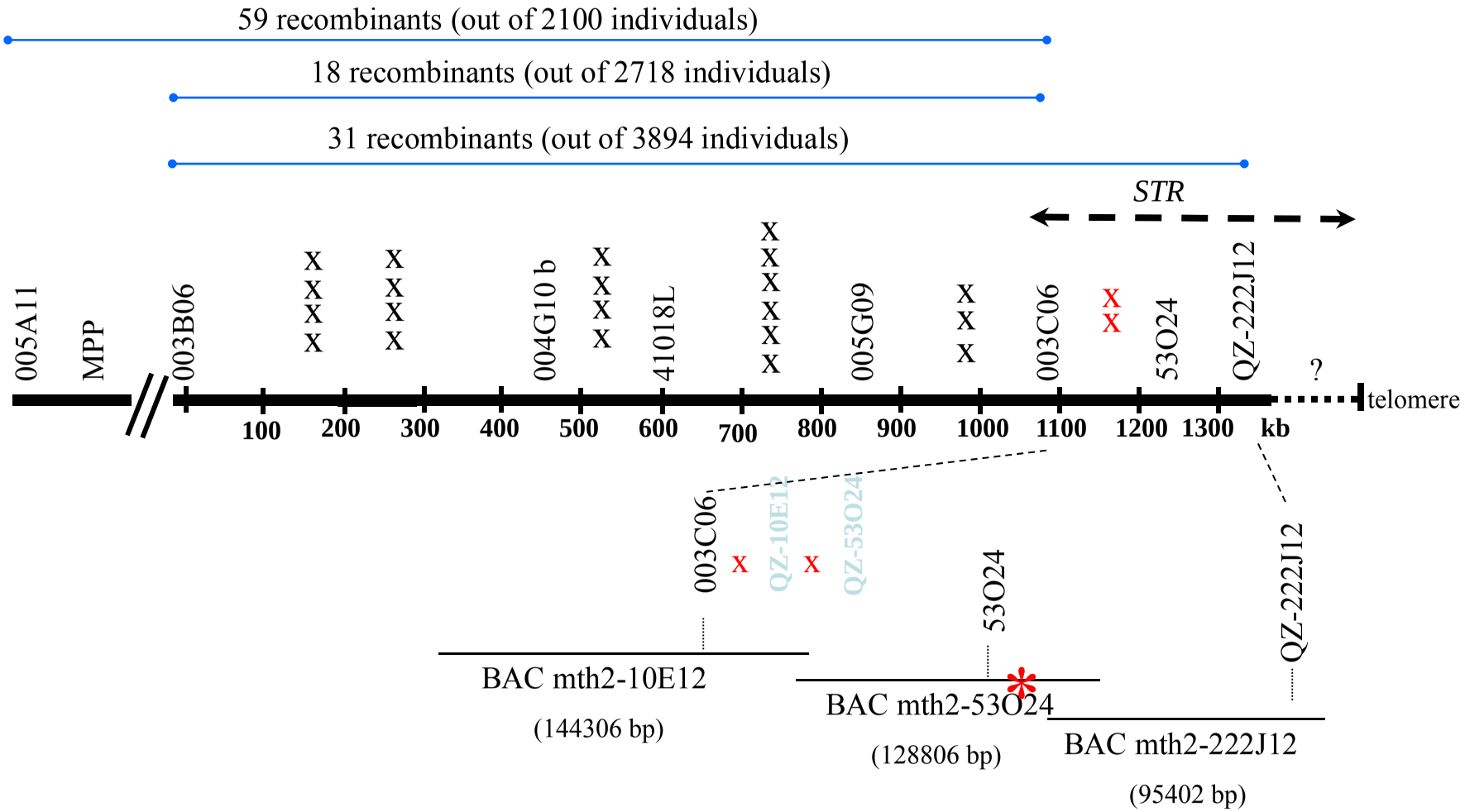
F


Infections/root
20
15
10
5
0
str
WT

G

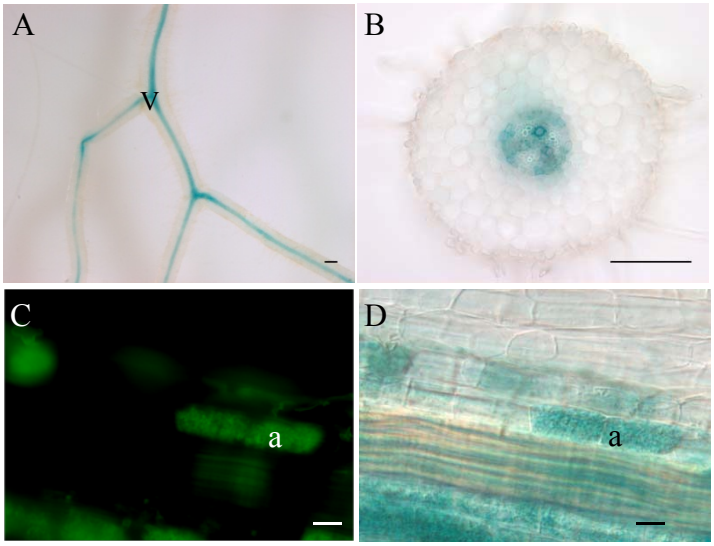

Relative Expression Level
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
1.0E-06
1.0E-07
PT4
Cel1
BCP1
TC104515
TC106351
α-Tubulin
WT
WT/ Gi
str
str/ Gi

Supplemental Figure 1. Symbiotic phenotypes of *str* and A17
(A-E) Nodulation phenotypes (F-G) mycorrhizal phenotypes
(A) *str* (left), D40 (middle) and wild type (right) plants at 21 dpi with *S. meliloti*. Nodule numbers did not differ between *str* and wild type (data shown in Table 1). Note that the plants were grown for 21 days in the absence of any added nitrogen and the shoots of both *str* and A17 are equivalently green suggesting that in both lines nitrogen-fixation is occurring. D40 is an AM symbiosis mutant (M.J. Harrison, unpublished) which was included in the experiment and could not be readily removed from this image.
(B -E) Longitudinal sections through nodules from wild type roots (B and C) and *str* roots (D and E) stained with stained with SYTO 13. (B and D) Arrows point to cells within the nodule harboring *S. meliloti* bacteroids. (C and E) Higher magnification images show that the bacteroids (indicated by arrowheads) have elongated rod shapes that are typical of the terminally differentiated bacteroids found in *M. truncatula* /*S. meliloti* nodules. Bacteroids in wild type (C) and *str* (E) did not differ in appearance. Bars equal 50 μm (B and D) and 10 μm (C and E). Plant cell nucleus, n.
(F) The average number of fungal infection events per root in *str* and in wild type (WT) 14 days post-inoculation with *Gigaspora gigantea*. Data shown are the average of 10 root systems per line each inoculated with 4 *Gigaspora gigantea* spores. For this experiment, the number of spores was reduced to a minimum to enhance the possibility of revealing a difference in the early phase of the symbiosis. There is no significant difference between the number of infection events in wild type (WT) and *str*.
(G) Quantitative RT-PCR analysis of mycorrhiza-induced genes in *M. truncatula* wild type and *str* roots colonized with *G. intraradices*. Genes analyzed are molecular markers of arbuscule development and AM symbiosis (*PT4*, *Cel1*, *BCP1*, Cysteine-rich anti-fungal protein (TC104515), α-amylase/subtilisin inhibitor (TC106351)(Harrison et al., 2002; Hohnjec et al., 2005; Liu et al., 2007). α-tubulin is a *G. intraradices* gene. The RNA samples are from wild-type mock-inoculated roots (WT), wild-type roots colonized by *G. intraradices* (WT/Gi), *str* mock-inoculated roots (*str*); *str* roots colonized by *G. intraradices (str/*Gi*)*. Data shown are from three biological replicates. Roots were harvested at 7 days after contact with *G. intraradices* spores. The values represent transcript levels of each gene expressed relative to the transcript levels of *M. truncatula* EF-1α. Error bars represent standard deviation (n=3 independent biological replicates).
Note that these genes are all expressed only in mycorrhizal roots so there are no values for wild type and *str* mock-inoculated control roots (white bars and light grey bars).



Supplemental Figure 2. Diagram to illustrate mapping of *STR* on chromosome 4

The number of recombinants found within a given region (indicated by the blue lines) is shown at the top of the diagram. A detailed location of the recombination events is shown only for a selection of the recombinants (indicated by X). Recombinants indicated by a red X were further mapped with new markers (shown in green). This placed *STR* in a 230 kb region covered by 3 BACs (mth2-10E12, mth2-53024 and mth2-222J12). The distance between BAC mth2-222J12 and the telomere is unknown. *STR* is indicated by a red asterisk.



Supplemental Figure 3. Histochemical staining for GUS activity in *M. truncatula* roots expressing a *STR2* promoter–*UidA* fusion construct.
(A) and (B) GUS staining in mock inoculated roots reveal gene expression in the vascular tissue (V). Bars equal 100 µm.
(C) and (D) GUS staining in roots colonized with *Glomus versiforme.* (C) Fluorescent microscopy image and (D) corresponding bright field image showing GUS expression in cortical cells with arbuscules (a). *G. versiforme* is stained with WGA-Alexafluor 488 which fluoresces green. Bars equal 10 µm.

A

```
STR   993 ATATTGAGTATCTCCTAGATGTTATAACTGAATACGATCAAGCGACGGTT
STR2 1048 CAATTGAGAATCTCATTGATGTGATACAAGAGTATGATCAG---------

STR  1043 GGACTTGACCCTCTTGTCCAATACCAACATGATGGCCATAAACCTGACCC
STR2 1089 ---TGTGATTTTGTTG----GTGTTGAGGTGCTAGC--TGAGTTTGCACG

STR  1093 AGCAGCCATGACCCCTGTTCCTAAACCTCCAAGAACACCTTACAGAAGGA
STR2 1130 TACCGGAATGAAACCACCTCTT-----TTGAGTGA-----TATGGAGGAA

STR  1143 ACACCCCAGCATCTAAACACATGATTAGCCTGCGCAGCCAAGGATTTACT
STR2 1170 ATAAT------TTCATATAC-TAATTCCATTGCAC---------------

STR  1193 GCTGGTACCCCACAACCTGATTCTTCACAGTTTGGCCTTGATGATGATGA
STR2 1198 -------CTTCACCATCT---CCTTTGCATAGAGGATCTAAATATGAGGA

STR  1243 CAATGATGATGACGAAAATTTTGATAACTCCCTTGAAAGGAGAAGTGTTC
STR2 1238 --------------AAAATCTCAAGATTTCTCTT---------ATTCATC

STR  1293 AAACATCAAGAAACATTGTGACCAGTGGTGTTTATCCACGTTTAGCTTCT
STR2 1265 ACAAATTAGCAGAA------------GGAGTTTGA--ATGATGAGTTTGA

STR  1343 CAGTTCTACCAAGATTTCTCAGCCAAAGATTTCTCTGTCTGGCTTTACAA
STR2 1301 TCATAGTATTAGAAGTCCATATAATAACACACCAATGTC----------A

STR  1393 TGGTGTAGTAGGAACCCCACGTCGCCCACCATCATGGACTCCGGCAAGAA
STR2 1341 TGGAGTGCTAGTAATAGTGCA------GCTTTCTTGAAATTC----ACAC
```

B

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STR  2293 TGAATTCAAGAACAATCGCGGCTGCTACTCAGGAAACAAAGCAGAT-TTA
STR2 2198 TGAGTATCAGACTAATGAAACTTTTGGATCAAATGATGGTGTATCTATTA

STR  2342 TC--CCCCGGACCTTTAGGAGATGTTAAGCCCAGCAAACACCACAATGCC
STR2 2248 CTGGTTTTTGATATTTTGAAAAGTCTTCATATTGGTACTGAAGAGATTA--

STR  2390 AGTCTTCCGCTTAACTGCTTATTAGGGGAAGATGTCTTGTCC-ACAATGG
STR2 2296 AGAAAAGGAACAATGTGCTTATAATGTTAGGTTGGGCTGTTCTATATAGG

STR  2439 ATATTACAATGGAAAGTCTATGGTATGACATCTTAATCCTGCTAGCTTGG
STR2 2346 ATTTTATTTTACATTATTCTTCGTTTTGCATCAAAA-------AATCAAA

STR  2489 GGTGTTCTTTACAGGTTCTTCTTCTACTTGGTTTTGAGATTCTACTCCAA
STR2 2389 GGT-CCTAGTAGAGGAAAAATGTGTGATAAAGTTAACAATTTAATTTCTT

STR  2539 AAATGAAA-GAAAATGAATAATTTACCAACCCCAATTTCTTTGGATAACC
STR2 2438 TTGTATAATGCAAACATATTGTTTG--GATATTTGTTGCTTC----AATG

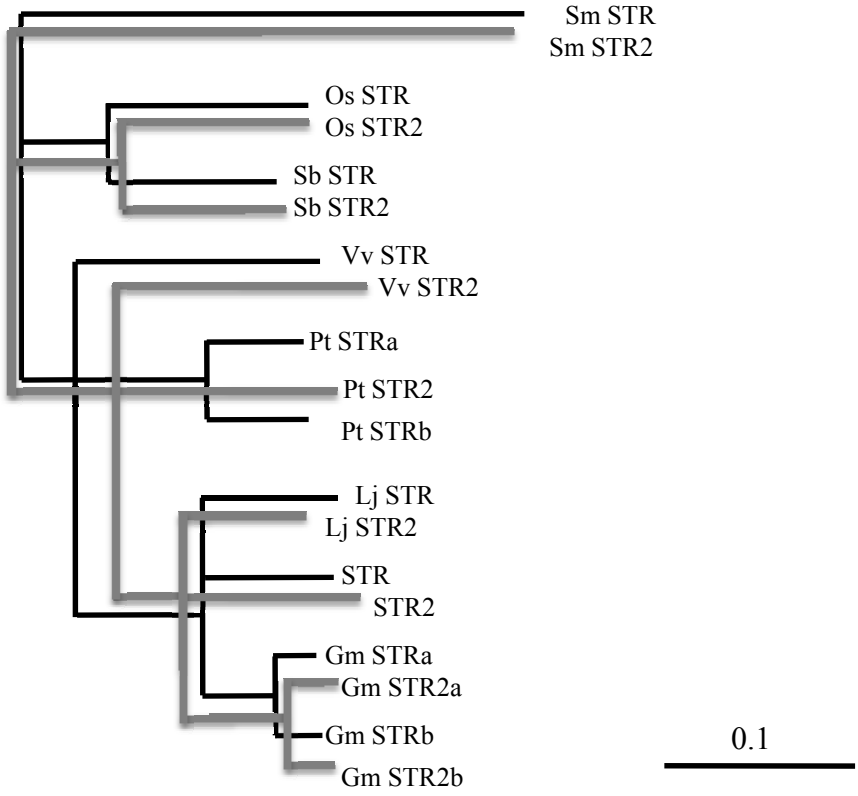
STR  2588 TATTACTTAAATAGCCAAGGCCACAGTGATCACATTATTTCTTGTATTAT
STR2 2482 TATTG--TATTTGAACAAGGTT--TATAAATTGAGTGATCATTGTATTGT
```

Supplemental Figure 4. Alignment of *STR* and *STR2* nucleotide sequences in the regions targeted for RNA interference.

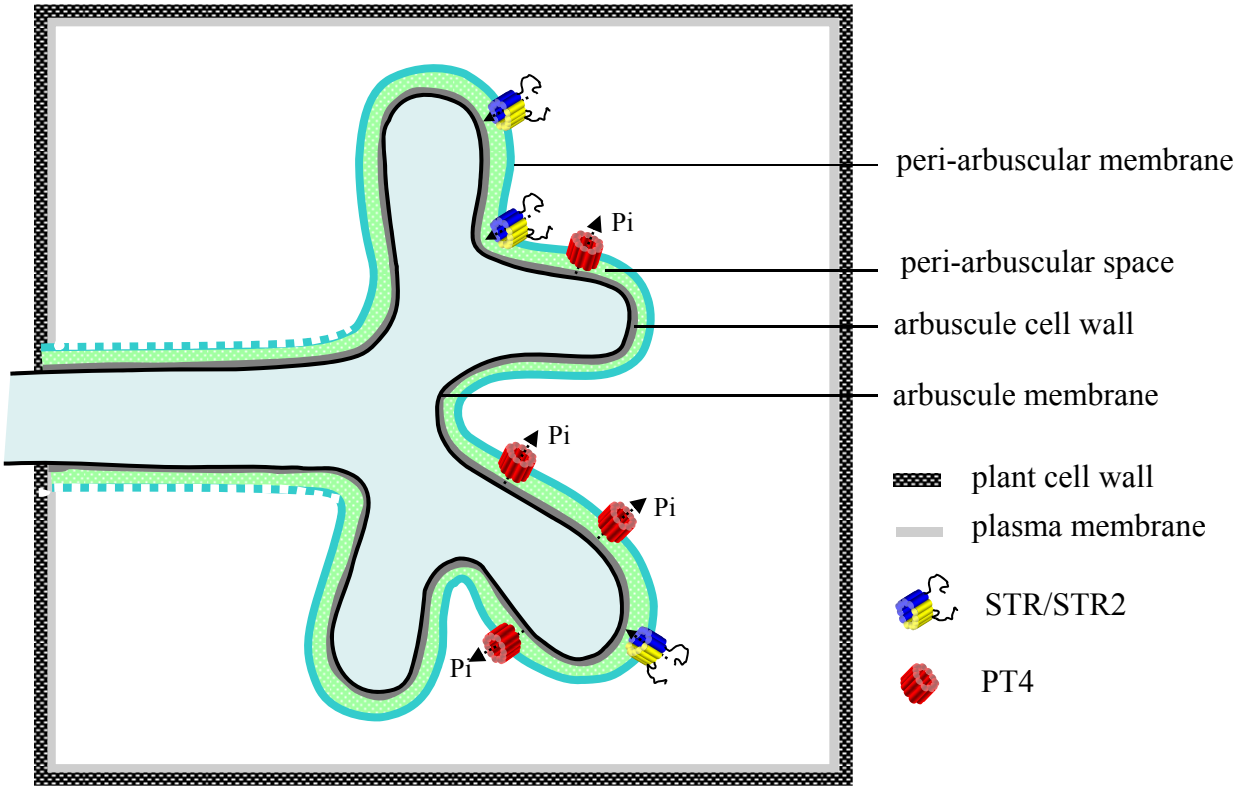
(A) Alignment of sequences for *STR2* RNAi-1.

(B) Alignment of sequences for *STR2* RNAi-2.

No significant DNA sequence similarity between these two genes and no regions of identity greater than 7 nucleotides. The regions from *STR2* corresponding to the RNAi constructs are underlined.



Supplemental Figure 5. Phylogenetic trees of STR and STR2 orthologs.
Phylogenetic trees of STR (black lines) and STR2 (grey shadowed lines) orthologs were constructed separately as described in materials and methods. Gm (*Glycine max*), Sb (*Sorghum bicolor)*, Os (*Oryzae sativa)*, Pt (*Populus trichocarpa)*, Vv (*Vitis vinifera)*, Lj (*Lotus japonicus)*, Sm (*Selaginella moellendorffii)*. SmSTR and SmSTR2 were used as outgroups, respectively. Branches with a bootstrap value lower than 980 were collapsed. Scale bar represents 0.1 amino acid substitutions per site. Accession numbers for these sequences are shown in Supplemental Table 4.



Supplemental Figure 6. Diagram illustrating the location of STR/STR2 on the peri-arbuscular membrane in a cortical cell containing an arbuscule. STR/STR2 transports an unknown molecule(s) into the peri-arbuscular space. PT4, a mycorrhizal specific phosphate transporter is located on the same membrane as STR/STR2.

Supplemental References:

Harrison, M.J., Dewbre, G.R., and Liu, J. (2002). A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell **14,** 2413-2429.

Hohnjec, N., Vieweg, M.F., Pühler, A., Becker, A., and Küster, H. (2005). Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different Glomus fungi provide insights into the genetic program activated during arbuscular mycorrhiza. Plant Physiol. **137,** 1283-1301.

Liu, J., Maldonado-Mendoza, I.E., Lopez-Meyer, M., Cheung, F., Town, C.D., and Harrison M, J. (2007). The arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. The Plant Journal **50,** 529-544.

Supplemental Data. Zhang et al. (2010). Plant Cell 10.1105/tpc.110.074955

Supplemental Tables

Supplemental Table 1. *str* is linked to 4 markers at the on chromosome 4

Markers on Chromosome 4	002B07	002D07	003F10	005D04	004H02	005A11	MPP	005H08	41018L	005G09
Genetic location (CM)	7.4	11..8	41.4	48.1	51.8	59.2	61.8*	61.1	61.8	61.1*
Recombinants (1)	16/19	17/19	6/18	5/18	5/18	1/24	0/24	0/24	0/24	0/24

Initial mapping was carried out on a population of 122 F2 individuals containing 24 plants homozygous for the *str* allele.
* marker position relocated based on our mapping data.
(1) The proportion of plants showing recombination between the marker and *str*

Supplemental Table 2. Sequence confirmation that a wild type *STR* transgene is expressed in the complemented *str* roots

Constructs transformed in *str*	Number of plants with transgenic roots	Number of plants showing wild type phenotype	Wild type *STR* allele detected by sequencing cDNA
STR in pCambia2301	23	23	15/15
pCambia2301	11	0	0/8

Supplemental Table 3.	**Primer sequences**		
Primer set	**Forward primer sequence 5' - 3'**	**Reverse primer sequence 5' - 3'**	**PCR purpose**
QZ-10E12	GTGAAGACTTTGCGGTGGAT	AAGCATCTGAACGGTGAAGG	SSR marker at the end of chromosome 4
QZ-53O24	GGTGTCTTTGGCTTAGTCTGTAAC	CTTCTTCAAACTCATCCGGCTC	SSR marker at the end of chromosome 4
QZ-222J12	ATCAGGGCCTATGTGGTTGTGGTA	GAAACCCATGCAATGCCTAGGGAAA	SSR marker at the end of chromosome 4
7D10	TCGCAATAATCGGAATGTGA	CCTGGGGATGGAAAGAAAAG	PCR product labeled for BAC library screen
STR	ATGAAGAAGCACGGTAAGCATTG	ACTTCACACTCACCTTTCGGG	Expression *of STR*
STR2	CCTGTTAGTTTCACTGGAGGACTTG	GCCCTAATCTGAAATCAGCAGCA	Expression *of STR2*
ABCG10-Q	CATCTTGTGGAAGCTACCGC	GCAGTGAAGTTTGTGTTGAGACC	Real time QPCR for Mt_ABCG10
ABCG13-Q	AATGTGAGTTTCCAAGCCAGAC	GGATCGTGGATGATGTCTGTG	Real time QPCR for Mt_ABCG13
STR-pro	GAGTAAGCTTTGCGTAGTAGAGCAA TCACTTCTTATG	GAGTTCTAGACTTTGATTTATATCCCC GTAGTGGTTGT	*STR* promoter GUS fusion construct
STR2-pro	GAGATCTAGAGTAGTGCATAAGAT GAAACACTGGACA	TGTGAAGCTTTTTCTTGAGGTGCAAGT TAAGTTTTG	*STR2* promoter GUS fusion construct
STR2-RNAi1	GGGACAAGTTTGTACAAAAAAGCA GGCTGTGATACAAGAGTATGATCAG TGTGA	GGGACCACTTTGTACAAGAAAGCTGG GTTGCACTATTACTAGCACTCCATGA C	*STR2* RNAi-1 construct
STR2-RNAi2	GGGACAAGTTTGTACAAAAAAGCA GGCTCTTTTGGATCAAATGATGGTG TATCT	GGGACCACTTTGTACAAGAAAGCTGG GTCCTTGTTCAAATACAATACATTGA AGC	*STR2* RNAi-2 construct
STR2-Q	GCAAGTGGGAGTCTTAAAGGA	GCCCTAATCTGAAATCAGCAG	*STR2* real time QPCR for STR2 RNAi materials
STR-Q	TTCCAATGATGCAGTCCCA	TGGTTATGACTGCAAATGTGAG	*STR* real time QPCR for STR2 RNAi materials
Cel1	GGTGAAATTGTGGCAGCACT	TCAACAGCGGTGTAGGTTCC	Real time QPCR for core Mt mycorrhizal gene expression
PT4	GGATTCTTTTGCACGTTCTTGG	CCTGTCATTTGGTGTTGCAGTG	Real time QPCR for core Mt mycorrhizal gene expression

α-Tubulin	TGTCCAACCGGTTTTAAAGT	AAAGCACGTTTGGCGTACAT	Real time QPCR for core Mt mycorrhizal gene expression
BCP1	AAGGTTGTCACCAACACGAA	TGCAATCTACCTTGCCATTT	Real time QPCR for core Mt mycorrhizal gene expression
TC104515	TGCCTCGTCTTTCACCTTATTT	CCTCTCTTTCTCTGCATTGGTC	Real time QPCR for core Mt mycorrhizal gene expression
TC100720	TACTTTTTCTGTATCCATATTTTGTTG	TGAAAAGAAACAGAAATCCCAGA	Real time QPCR for core Mt mycorrhizal gene expression
EF-1α	TGACAGGCGATCTGGTAAGG	TCAGCGAAGGTCTCAACCAC	Real time QPCR for core Mt mycorrhizal gene expression
TC106351	TCAAATTCACACCATTTGCTC	CCTTCTTCCACTCTTGGTGTC	Real time QPCR for core Mt mycorrhizal gene expression

1.SSR and CAPs markers

All SSR markers were amplified with the following PCR conditions: 94°C for 4 min, followed by 38 cycles at 94°C for 30 s, 55°C for 40 s, and 72°C for 40 s, followed by 72°C for 10 min. Reactions were performed in 10 µl volume containing 1 µl of genomic DNA, 1.5 mM $MgCl_2$, 500 µM dNTP, 1 U of Taq polymerase, and 500 µM of each primer. PCR products were run on a 3% agarose gel (1X TAE buffer) and stained with ethidium bromide. For CAPS markers, PCR products were digested with the appropriate restriction enzymes for 1 hour.

2. RT-PCR and Q RT-PCR

Real time PCR analysis was carried out essentially as described by Liu et al., (2007) with the following modifications. Primers were designed using an online real-time PCR primer design tool (http://www.idtdna.com/Scitools/Applications/RealTimePCR/). PCR reactions were carried out in a total volume of 10 µl which contained 5 µl 2x SYBR Green PCR master mix, 150 nM of each primer, and 20 ng of cDNA template. The PCR program was as follows: 95°C for 5 min, 40 cycles of 95°C for 30 sec, 56°C for 30 sec and 72°C for 30 sec. Dissociation curve analysis was performed, with the thermal cycle of 95°C for 15 sec, 60°C for 15 sec and 95°C for 15 sec, to exclude non-specific reactions. The data were analyzed with Applied Biosystems SDS 2.2 software. The *M. truncatula* elongation factor 1-alpha (EF-1α) gene was included to enable normalization of expression levels of all genes assayed.

3. 7D10 primers

Using a set of 7D10 primers (supplemental table 3), a DNA fragment corresponding to the BAC end sequence of mth2-7D10, was amplified with the following program: 95°C for 3 min, 30 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec.

Supplemental Table 4. Accession numbers of genes shown in Figure 4A.

Protein ID	Accession number	Gene Locus
AtABCG1	O80946	At2g39350
AtABCG2	Q9ZUT0	At2g37360
AtABCG3	Q9ZUU9	At2g28070
AtABCG4	Q9SW08	At4g25750
AtABCG5	Q9SIT6	At2g13610
AtABCG6	Q9FNB5	At5g13580
AtABCG7	Q9ZU35	At2g01320
AtABCG8	Q9FLX5	At5g52860
AtABCG9	Q9SZR9	At4g27420
AtABCG10	Q9MAH4	At1g53270
AtABCG11	Q8RXN0	At1g17840
AtABCG12	Q9C8K2	At1g51500
AtABCG13	Q9C8J8	At1g51460
AtABCG14	Q9C6W5	At1g31770
AtABCG15	Q8RWI9	At3g21090
AtABCG16	Q9M2V7	At3g55090
AtABCG17	Q9M2V6	At3g55100
AtABCG18	Q9M2V5	At3g55110
AtABCG19	Q9M3D6	At3g55130
AtABCG20	Q9LFG8	At3g53510
AtABCG21	Q7XA72	At3g25620
AtABCG22	NP_188746	At3g21090
AtABCG23	Q93YS4	At5g06530
AtABCG24	Q3E9B8	At5g19410
AtABCG25	Q9MAG3	At1g53390
AtABCG26	Q84TH5	At1g71960
AtABCG27	Q9LK50	At3g13220
AtABCG28	Q9FT51	At3g52310
AtABCG29	Q9FF46	At5g60740
STR	ACV73541	mth2-53O24
STR2	ACV73543	mth2-20E3
MtABCG3	AC146751*	mth2-60a22
MtABCG4	AC175537*	mth2-39i6
MtABCG5	AC174344*	mth2-167B8-1
MtABCG6	AC174344*	mth2-167B8-2
MtABCG7	AC174344*	mth2-167B8-3
MtABCG8	AC174344*	mth2-167B8-4
MtABCG9	AC174344*	mth2-167B8-5
MtABCG10	AC122164*	mth2-24H4
MtABCG11	AC146331*	mth2-7H18
MtABCG12	CT573506*	mth4-42D11
MtABCG13	AC186136*	mth2-5A14

MtABCG14	AC138131*	mth2-21H11
MtABCG15	AC148655*	mth2-61C11
Protein ID	**Accession number**	**Gene Locus**
MtABCG16	CU012051*	mth2-60M24
GmSTRa	ACUP01004567*	GLYMAchr_08_Cont4567
GmSTRb	ACUP01003232*	GLYMAchr_05_Cont3232
SbSTR	XP_002445326	SORBIDRAFT_07g009430
PtSTRa	XP_002318509	POPTRDRAFT_569718
PtSTRb	XP_002321525	POPTRDRAFT_807932
SmSTR		scaffold_146:124474..126912
VvSTR	XP_002278856	LOC100266434
OsSTR	NP_001063119	Os09g0401100
LjSTR	AP010316*	LjT49M15
SbSTR2	XP_002458373	SORBIDRAFT_03g032350
OsSTR2	BAD30878	OSJNBa0049I08
PtSTR2	XP_002310338	POPTRDRAFT_764936
SmSTR2		scaffold_11:1791209..1793756
GmSTR2a	ACUP01000026*	GLYMAchr_01_Cont26
GmSTR2b	ACUP01005538*	GLYMAchr_09_Cont5538
VvSTR2	XP_002272098	LOC100258701
LjSTR2	AP004579*	LjT11E23

*Gene has not been annotated. The accession number represents the BAC or contig where the gene is located.