Figure S1

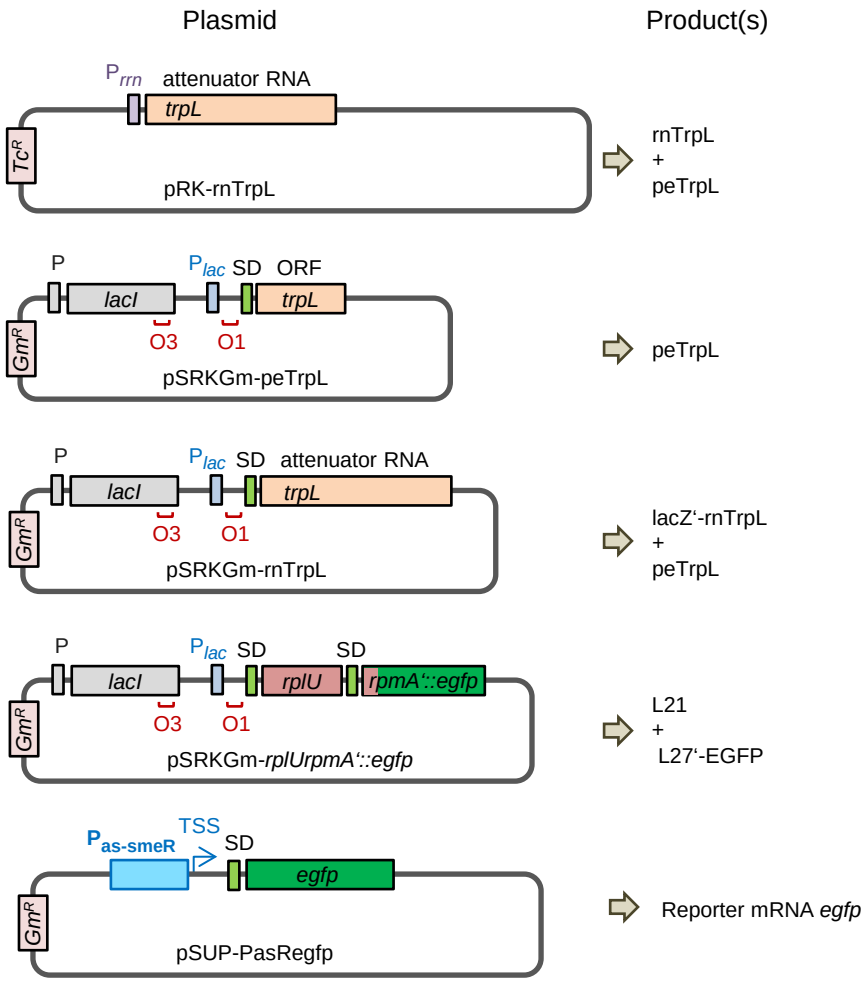


Figure S1. Plasmids for overexpression of rnTrpL and for *egfp* reporter. Schematic representations of the indicated plasmids. In addition to the here shown pSRKGm plasmids with a Gm resistance gene, pSRKTc plasmids with a Tc resistance gene were also used for induced rnTrpL and/or peTrpL expression. The *rplUrpmA'::egfp* fusion contains the first three *rpmA* codons fused to the third *egfp* codon. In pSUP-PasRegfp, the genomic region containing the promoter of the asRNA As-smeR and the putative two first nucleotides if the asRNA were fused to a sequence containing a standard Shine-Dalgarno ribosome binding site, followed by the *egfp* gene. Mutations in the constructs are not shown here. P, promoter; O, operator; SD, Shine-Dalgarno sequence. Relevant genes, gene products and regulatory elements are indicated.

Figure S2

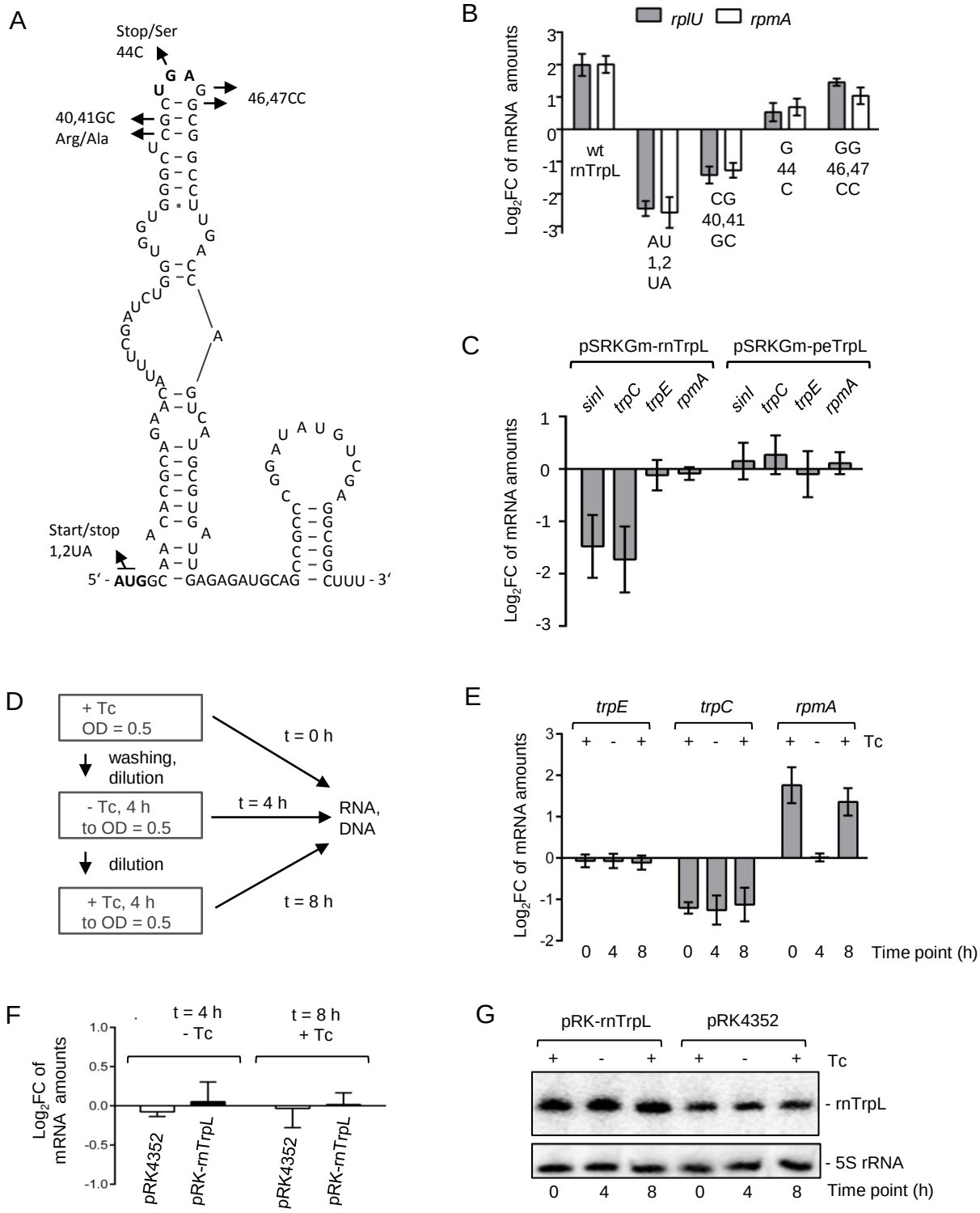

A
Stop/Ser
44C
46,47CC
40,41GC
Arg/Ala
Start/stop
1,2UA
5' - AUGGC – GAGAGAUGCAG – CUUU - 3'
B
rplU
rpmA
Log2FC of mRNA amounts
wt
rnTrpL
AU
1,2
UA
CG
40,41
GC
G
44
C
GG
46,47
CC
C
pSRKGm-rnTrpL
pSRKGm-peTrpL
sinI
trpC
trpE
rpmA
Log2FC of mRNA amounts
D
+ Tc
OD = 0.5
washing,
dilution
- Tc, 4 h
to OD = 0.5
dilution
+ Tc, 4 h
to OD = 0.5
t = 0 h
t = 4 h
t = 8 h
RNA,
DNA
E
trpE
trpC
rpmA
Tc
Log2FC of mRNA amounts
Time point (h)
F
t = 4 h
- Tc
t = 8 h
+ Tc
Log2FC of
mRNA amounts
pRK4352
pRK-rnTrpL
pRK4352
pRK-rnTrpL
G
pRK-rnTrpL
pRK4352
Tc
- rnTrpL
- 5S rRNA
Time point (h)

Figure S2. Effects of rnTrpL and Tc on mRNA levels. A) Secondary structure of the sRNA rnTrpL (drawn according to Melior et al., 2019) with indicated mutations. B), C), and E) show qRT-PCR and F) shows qPCR results (means and standard deviations from three independent experiments, each performed in duplicates). **B)** Constitutive rnTrpL overproduction in strain 2011 (pRK-rnTrpL), grown with Tc, increased the *rplUrpmA* mRNA level (in comparison to the EVC). The strong effect of the start to stop codon mutation in *trpL* suggested a function for peTrpL in the regulation of *rplUrpmA*. **C)** Influence of the induced rnTrpL and/or peTrpL overproduction on the levels of the indicated mRNAs. Strain 2011 harboring one of the indicated plasmids was used; the cultures were grown with gentamycin (Gm). The mRNA levels, 10 min post induction with IPTG, were compared to those before induction. In contrast to our expectations, no effect on *rpmA* was detected, although the levels of the control mRNAs *trpC* and *sinI* were decreased (Baumgardt et al., 2016; Melior et al., 2019), confirming the functionality of the sRNA lacZ'-rnTrpL in this experiment. Panels D) to G) show an experiment addressing the effect of Tc in strain 2011 (pRK-rnTrpL) and the EVC 2011 (pRK4352). **D)** Scheme of the experiment. **E)** The levels of the indicated mRNAs in the overexpressing strain were compared to the levels in the EVC. RNA samples isolated at the indicated time points (see panel D) were used. **F)** Plasmid level per chromosome was not changed during the experiment. 4 h – Tc, relative plasmid level at time point 4 h was compared to the level at time point 0 h; 8 h + Tc, relative plasmid level at time point 8 h was compared to the level at time point 0 h**. G)** The steady-state level of rnTrpL was not changed during the Tc-removal experiment, as shown by Northern blot hybridization. Used plasmid and time point of RNA isolation are indicated. The membrane was re-probed with a 5S rRNA specific probe (loading control) .

Figure S3

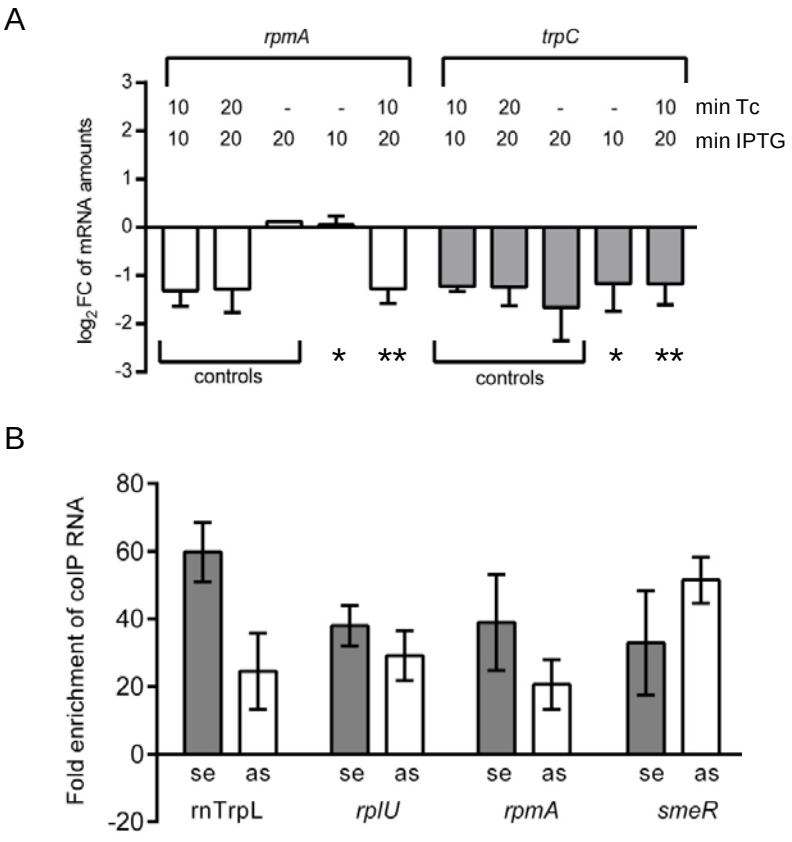


Figure S3. Effect of Tc on *rpmA* downregulation by rnTrpL and CoIP of antisense RNAs with 3xFLAG-peTrpL. A) Tetracycline is not necessary for downregulation of *trpC* upon rnTrpL induction, but is crucial for the downregulation of *rpmA***.** qRT-PCR analysis of the indicated mRNAs in strain 2011*ΔtrpL* (pSRKGm-rnTrpL, pRK4352). For detailed information, see Fig. 1F and 1G in the main text. **B)** Enrichment of sense (se) and antisense (as) RNAs in the 3xFLAG-peTrpL CoIP samples, which were obtained upon washing with Tc in the buffer (see Fig. 3B). The enrichment was calculated in comparison to the control, mock CoIP conducted with the peTrpL-containing lysate. Strand-specific qRT-PCR analysis of the indicated RNAs was performed. Shown are the results from three independent experiments, each performed in duplicates (means with standard deviations are indicated).

Figure S4

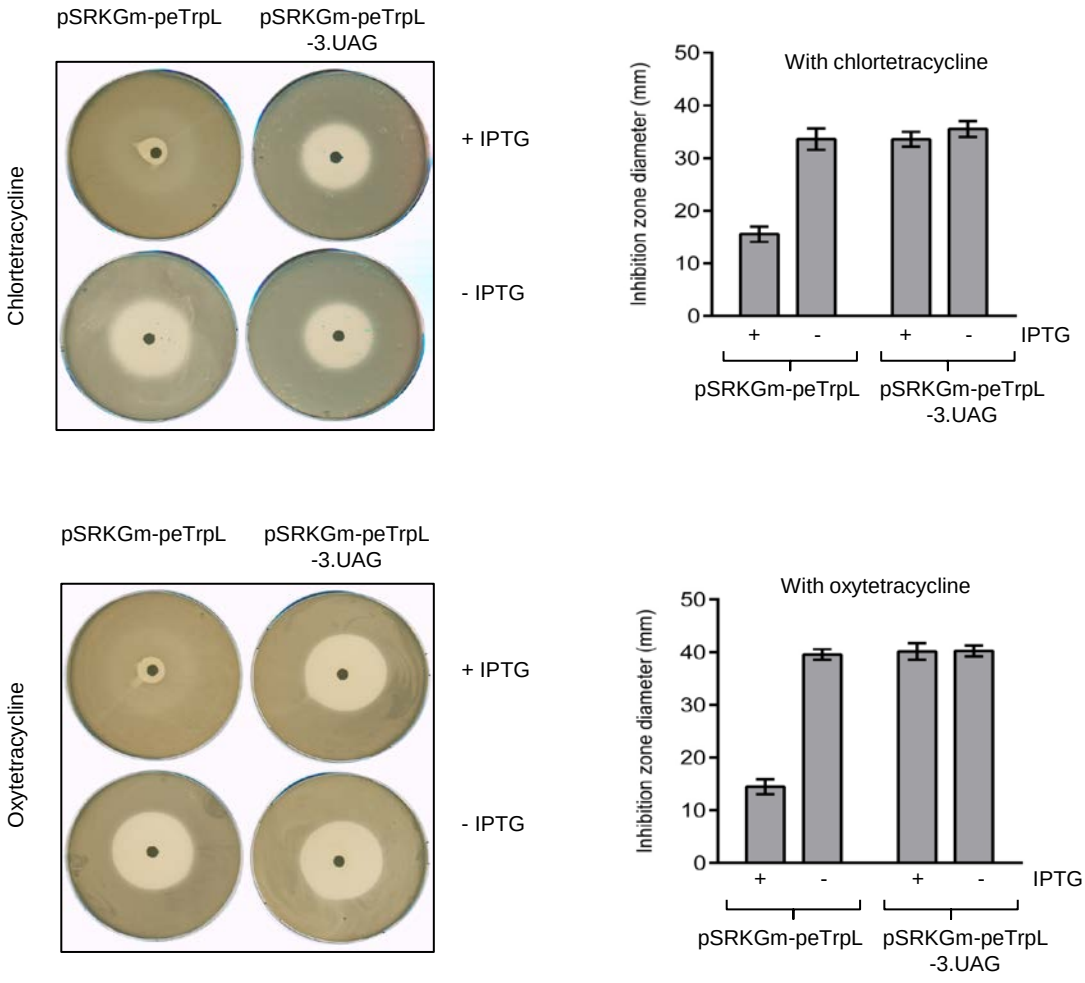


Figure S4. Increased resistance to natural tetracyclines upon induced peTrpL overproduction in strain *S. meliloti* 2011*ΔtrpL*. Representative plates with zones of growth inhibition caused by the centrally applied tetracycline antibiotics. Used antibiotics, plasmids and presence of IPTG in the growth media are indicated. On the right side, graphic representations of the results are shown. Data from three independent experiments were evaluated. Shown are means and error bars depicting the standard deviations.

Figure S5

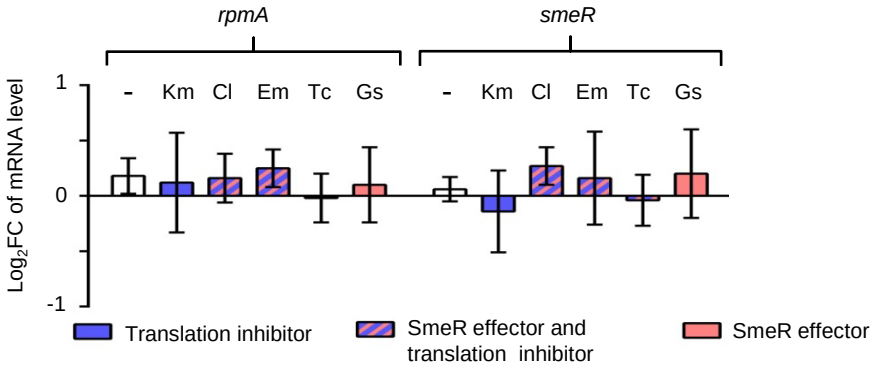


Figure S5. Analysis of the effect of antimicrobial substances in strain 2011*ΔtrpL*. The indicated antimicrobial substances were added to the cultures at subinhibitory concentrations (for details, see Fig. 6). Before and 10 min after addition of an antimicrobial substance, RNA was isolated and changes in the mRNA levels were determined by qRT-PCR. *trpE* mRNA was analyzed as a negative control. The antbiotics Km, Cl, Em and Tc are inhibitors of translation; Cl, Em, Tc and the plant flavonoid genistein (Gs) are effectors of SmeR, a repressor of the multidrug efflux pump SmeAB. The flavonoid Gs is an SmeR effector, but does not affect translation (indicated by colors). The results show that in the deletion mutant *S. meliloti* 2011*ΔtrpL,* the levels of *rpmA* and *smeR* mRNAs are not changed upon exposure to antimicrobial substances. Data from three independent experiments were evaluated. Shown are means and error bars depicting the standard deviations.

Figure S6

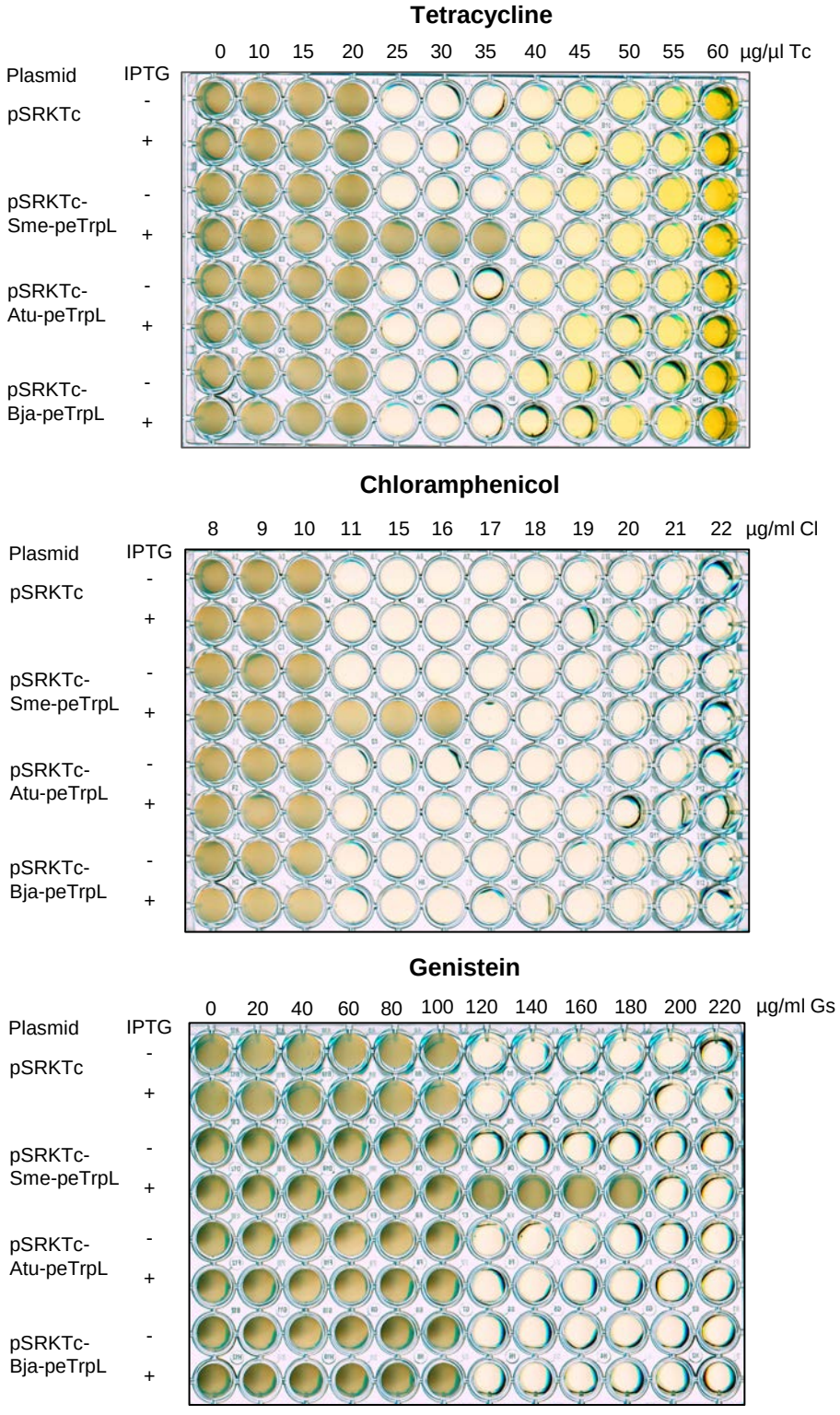
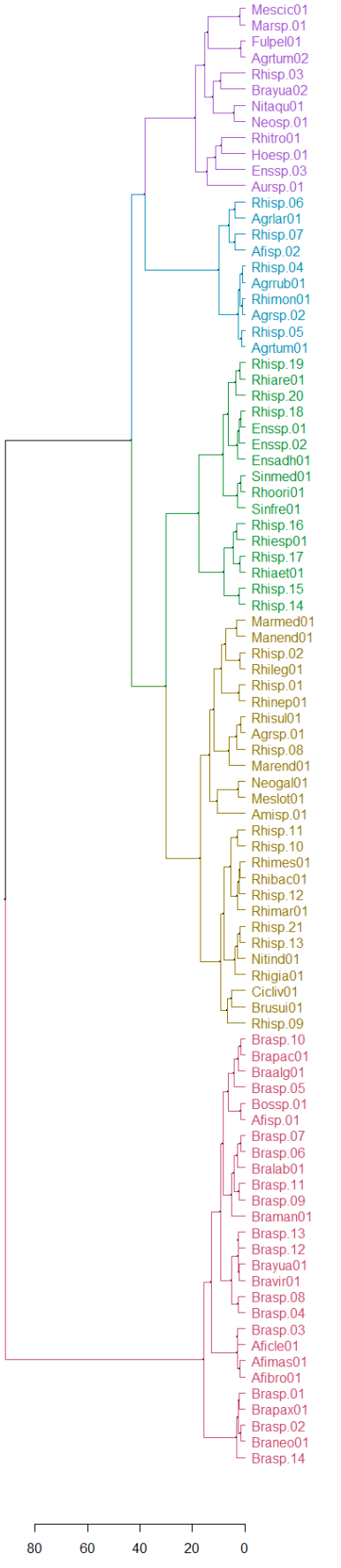
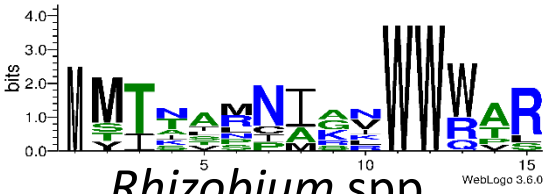


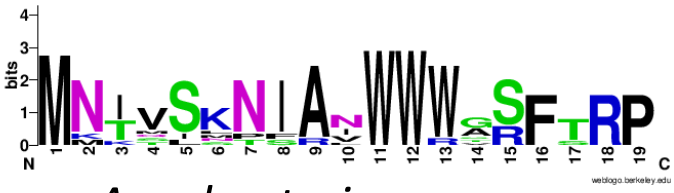
Figure S6. Growth of *S. meliloti* 2011*ΔtrpL* containing the indicated plasmids, in media containing Tc, Cl or Gs at the indicated concentrations. Cultures were diluted to an OD_{600} of 0.1 and grown for 60 h in 96-well plates. Presence of IPTG in the medium is indicated. Shown is one representative result of two independent experiments.

Figure S7





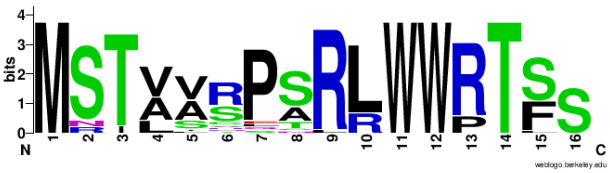
Rhizobium spp.



Agrobacterium spp.

Sinorhizobium spp.

Mesorhizobium spp.



Bradyrhizobium spp.

Figure S7. Analysis of putative peTrpL peptides from other Rhizobiales members revealed several groups of conserved leader peptides, generally consistent with taxonomy. For the complete list of species forming the groups shown by colors, see Table S1. Two major groups can be seen, one with two tryptophans and a consensus MSTvvrPsRL**WW**RTss present in *Bradyrhizobium* and related species (the red, bottom branch of the tree), and a larger group of four variants with slightly varying length and considerably varying sequence; clustering in this group is not very robust. The latter usually have three tryptophans within a loosely conserved context ni(a/s)(n/i/v)**WW**WAR. The comparison of the logos with the functional data in Fig. 7 yields a paradoxical observation that, despite functional conservation in *Sinorhizobium*, *Agrobacterium* and *Bradyrhizobium*, aside of the second tryptophan, the patterns of evolutionary and sequence conservation are markedly different.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial Strains		
Sinorhizobium meliloti 2011	Casse et al., 1979	N/A
Sinorhizobium meliloti 2011*ΔtrpL*	Melior et al., 2019	N/A
Sinorhizobium meliloti 2011*ΔtrpC*	Melior et al., 2019	N/A
Agrobacterium tumefaciens C58	Baumgardt et al., 2016	N/A
Bradyrhizobium japonicum USDA 110spc4	Regensburger and Hennecke, 1983	N/A
Escherichia coli DH5α	Thermo Fisher	
Escherichia coli S17-1	Simon et al., 1983	N/A
Chemicals		
Carbonyl-cyanide 3-chlorphenylhydrazone (CCCP)	Sigma Aldrich	Cat#C2759-100MG
Chloramphenicol	Sigma Aldrich	Cat#C-0378
Chlortetracycline hydrochloride	Sigma Aldrich	Cat#BCBM7261
Erythromycin	Sigma Aldrich	Cat#LRAA8967
Genistein	Sigma Aldrich	Cat#G6649-5MG
Kanamycin sulfate	Roth	Cat#T832.3
Luteolin	Sigma Aldrich	Cat#L9283-10MG
NileRed	Sigma Aldrich	Cat#063KJ730V
Oxytetracyline hydrochloride	Sigma Aldrich	Cat#107M4049
Streptomycine sulfate salt	Sigma Aldrich	Cat#SLBP6412
L-Tryptophane	Sigma Aldrich	Cat#BCBM5741V
Tetracycline Hydrochloride	Roth	Cat#HP63.2
FastDigest Restriction Endonucleases	Thermo Fisher	N/A
Denhardts Solution	Selfmade	Sambrook et al., 1989
[^{32}P]γ ATP	Hartmann Analytic	Cat#SRP-301
Critical Commercial Assays		
Anti FLAG® M2 Magnetic Beads	Sigma Aldrich	Cat#SLBT7133
RNAprotect Bacteria Reagent	Qiagen	Cat# 76506
Brilliant III Ultra Fast SYBR® Green QRT-PCR Mastermix	Agilent	Cat#600886
CloneJet PCR Cloning Kit	Thermo Fisher	Cat#K1232
E.Z.N.A. DNA Probe Purification Kit	Omega	Cat#D6538-02
GeneJET Gel Extraction Kit	Thermo Fisher	Cat#K0692
Phusion Polymerase	Thermo Fisher	Cat#F530L
Power SYBR® PCR Mastermix	Thermo Fisher	Cat#1705150
Pure Link™ RNase A	Thermo Fisher	Cat#12091021
Qiagen OneStep RT-PCR Kit	Qiagen	Cat#210212
RiboLock RNase inhibitor	Thermo Fisher	Cat#EO0381
RNeasy	Qiagen	Cat#74104
RNase-Free MicroSpin G-25 columns (GE Healthcare)	Sigma-Aldrich	Cat# GE27-5325-01
SIGMAFAST™ Protease Inhibitor Tablets	Sigma Aldrich	Cat#S8820-20TAB
SYBRGreen	Thermo Fisher	Cat#S7564
T4 DNA Ligase	NEB	Cat#M0202S
Turbo DNA*free*™ Kit	Thermo Fisher	Cat#AM1907
TRIzol	Ambion	Cat#14380401
Deposited Data		
Sequence Data; Genome-wide analysis of transcriptome of *S. meliloti* 2011 and 2011(pRK4352) strains by RNA-seq., and Analysis of coimmunoprecipitated RNA by RNA-seq	This work	GEO Series accession number GSE118689

Recombinant DNA		
pJet- rplUrpmA'-egfp	This work	N/A
pJet-rplU-CC222GG-rpmA'-egfp	This work	N/A
pJet-rplU-G228C-rpmA'-egfp	This work	N/A
pJet-PasRegfp	This work	N/A
pRK4352	Mank et al., 2012	N/A
pRK-rnTrpL (synonym pRK-RcsR1)	Baumgardt et al., 2016	N/A
pRK-rnTrpL-AU1,2UA	Melior et al., 2019	N/A
pRK-rnTrpL-CG40,41GC	Melior et al., 2019	N/A
pRK-rnTrpL-G44C (synonym pRK-RcsR1-G44C)	Baumgardt et al., 2016	N/A
pRK-rnTrpL-GG46,47CC	Melior et al., 2019	N/A
pSRKTc	Khan et al., 2008	N/A
pSRKGm	Khan et al., 2008	N/A
pSRKTc-rnTrpL	Melior et al., 2019	N/A
pSRKTc-rnTrpL-CG40,41GC	Melior et al., 2019	N/A
pSRKTc-rnTrpL-GG46,47CC	Melior et al., 2019	N/A
pSRKGm-rnTrpL	Melior et al., 2019	N/A
pSRKGm-peTrpL	This work	N/A
pSRKGm-peTrpL-3.UAG	This work	N/A
pSRKGm-peTrpL-N3A	This work	N/A
pSRKGm-peTrpL-T4A	This work	N/A
pSRKGm-peTrpL-Q5A	This work	N/A
pSRKGm-peTrpL-N6A	This work	N/A
pSRKGm-peTrpL-I7A	This work	N/A
pSRKGm-peTrpL-S8A	This work	N/A
pSRKGm-peTrpL-I9A	This work	N/A
pSRKGm-peTrpL-W10A	This work	N/A
pSRKGm-peTrpL-W11A	This work	N/A
pSRKGm-peTrpL-W12A	This work	N/A
pSRKGm-peTrpL-R14A	This work	N/A
pSRKGm-3×FLAG-peTrpL	This work	N/A
pSRKGm-rplUrpmA'-egfp	This work	N/A
pSRKGm-rplU-CC222GG-rpmA'-egfp	This work	N/A
pSRKGm-rplU-G228C-rpmA'-egfp	This work	N/A
pSUP202pol4	Fischer et al., 1993	N/A
pSUP-PasRegfp	This work	N/A
pRJ-MCS	Hahn et al., 2016	N/A
pRJ-Bja-peTrpL	This work	N/A
pRJ-Bja-rnTrpL	Melior et al., 2019	N/A
pSRKGm-Atu-peTrpL	This work	N/A
pSRKTc-Atu-rnTrpL	Melior et al., 2019	N/A
pK18mobsacB	Schäfer et al., 1994	N/A
pLK46	McIntosh et al., 2008	N/A
Software and Algorithms		
Biorad CFX Manager	Biorad	Version 3.0
Graph Pad Prism 6	Graph Pad	Version 6.07
Quantity One Basic	Biorad	Version 4.6.9
Tecan Icontrol	Tecan	Version 1.6
Other		
Biorad C1000 Thermal Cycler CFX96 Real Time System	Biorad	N/A
Biorad Personal Molecular Imager FX	Biorad	N/A
Rothi^{R}-Nylon_Plus (+ Charged Nylon Membrane)	Roth	Cat#K058.1
Glass beads	Roth	Cat#A553.1

Nunc Nunclon 96 Well Plate	Thermo Scientific™	Cat#AM10104
Semi-Dry Electro Blotter	Peqlab	Cat#028406
Retsch MM200 (Shacking Mill)	Retsch	N/A
Tecan infinity M200	Tecan	Cat# 168136

Table S2, Reagents and resources used in this work. N/A, not applicable