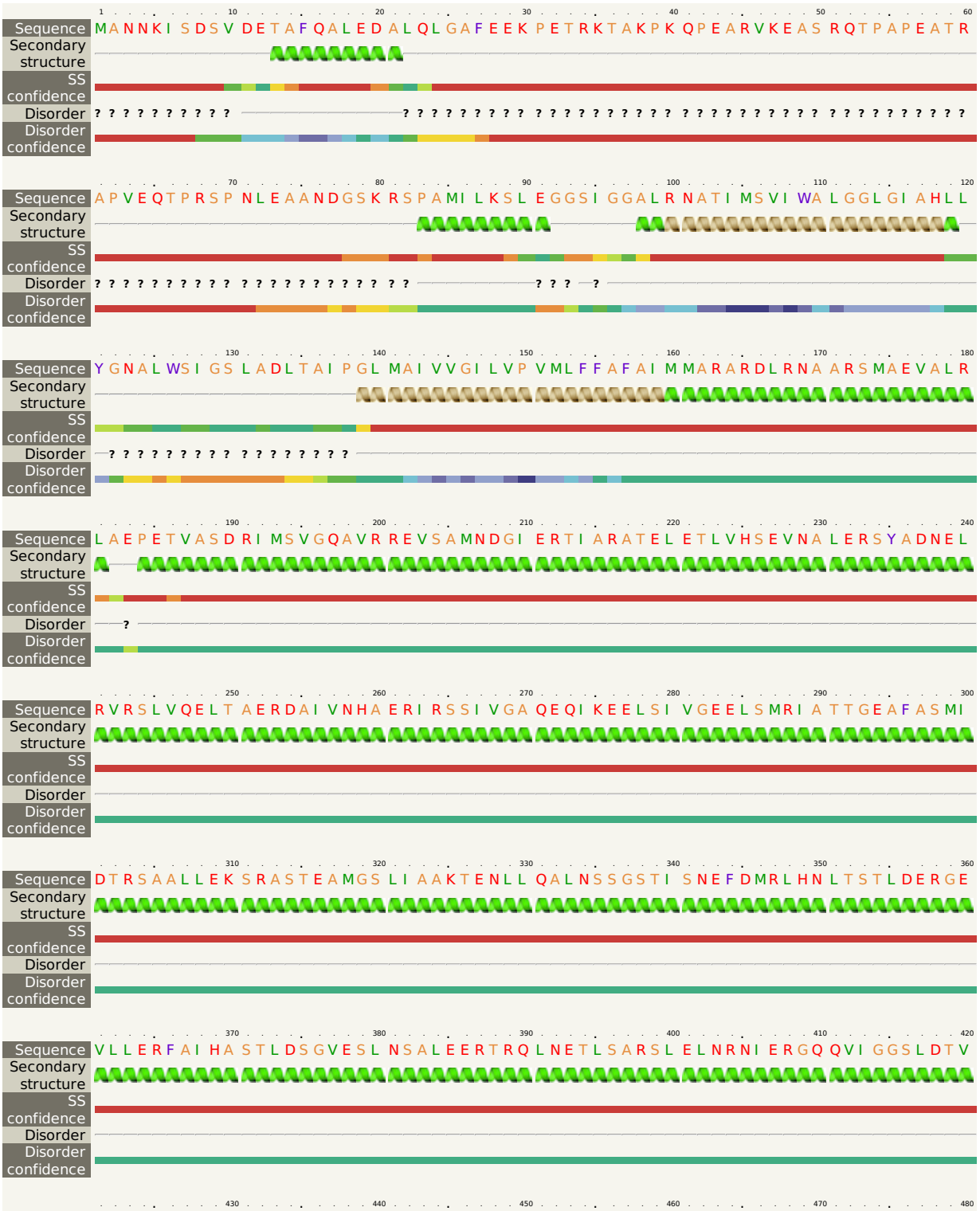
Supporting Information Appendix

Figure S1. **Secondary structure predictions from PHYRE (1)**. A) *Agrobacterium tumefaciens* protein GPR. B) *Caulobacter crescentus* protein TipN.

A.

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Secondary structure
SS confidence
Disorder
Disorder confidence
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G Q E M L E N A L E S R T Q A F S D A I G Q R T A E I T D A F T N S H A K I D T V L A E R S N A L F G A L S A S Q D R F
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D E A L A S R S L A I T G S V S G T A E H L A A M L D E R A A A I N S V V A D V E R R L T E T L E T R A A A I T G A V S
670 680 690 700 710 720
G I E D R I S D T L E S R T A A L H D V V S G A E S R I A D T L D G R T A A L S S A I S G V E E R I A D T M D S R T L S
730 740 750 760 770 780
L D M T F A N V E E R L S E T L D N R T S A L T G I V A S A E E K I A G A L D S R T A T F G D V V A G A E T R I A E T L
790 800 810 820 830 840
D G R T A A L N A V V S G A E E R I A D A L D S R T M A L D M T F S G A E E K I A E A L D T R T A A L G E L V A S A E T
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910 920 930 940 950 960
V A G V E D R I A G A L D S R T A A L S G I V S G A E E R I A E A L D S R T L A L D M T I S G V E E R I A E A M D A R A


SS confidence
Disorder
Disorder confidence
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Secondary structure
SS confidence
Disorder
Disorder confidence
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Secondary structure
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Secondary structure
SS confidence
Disorder
Disorder confidence
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Secondary structure
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Disorder
Disorder confidence
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Secondary structure
SS confidence
Disorder
Disorder confidence
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Secondary structure
SS confidence
Disorder
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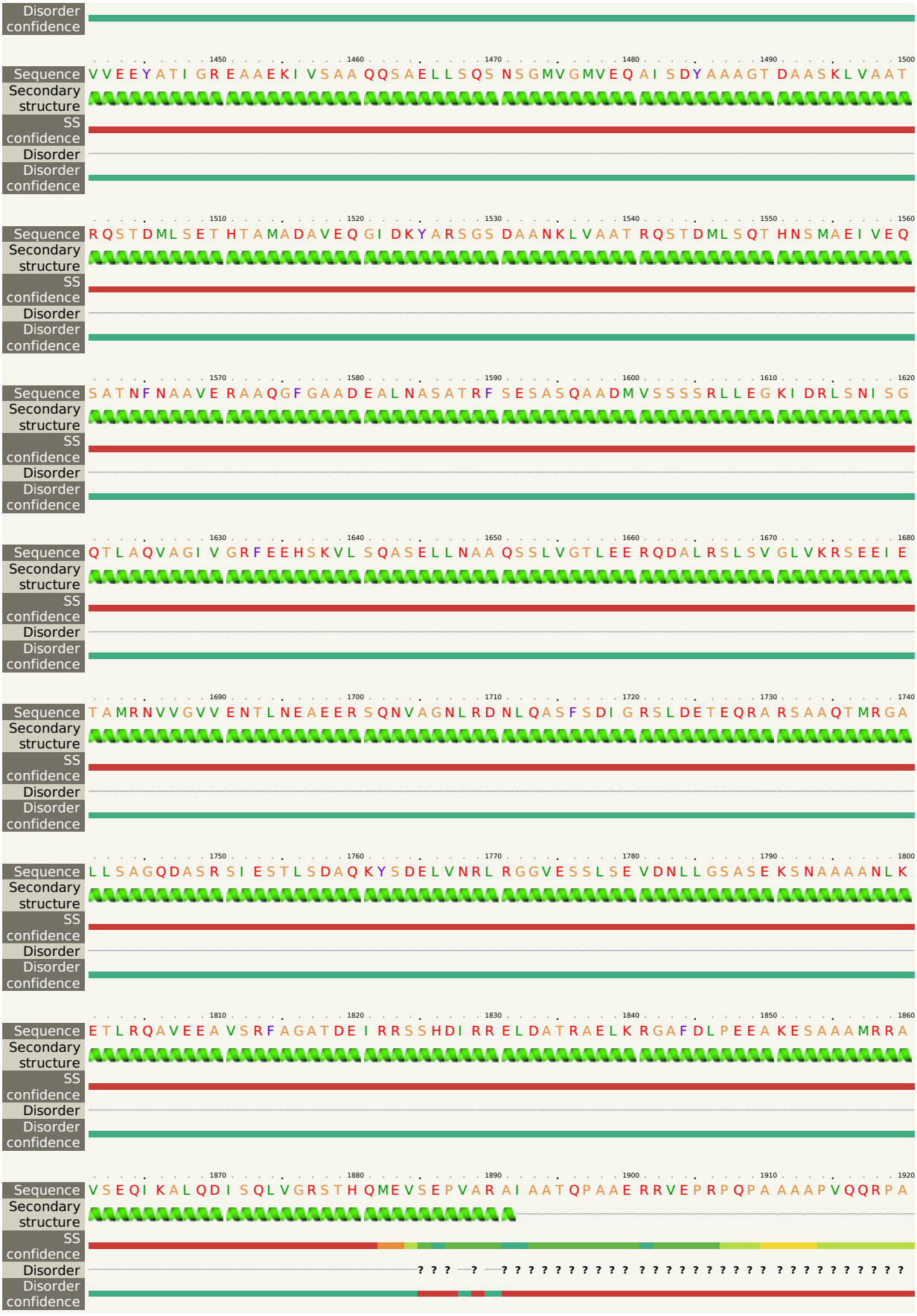
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SS confidence
Disorder
Disorder confidence
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Secondary structure
SS confidence
Disorder
Disorder confidence
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Disorder
Disorder confidence
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SS confidence
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Secondary structure
SS confidence
Disorder
Disorder confidence
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Secondary structure
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Disorder
Disorder confidence
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Secondary structure
SS confidence
Disorder
Disorder confidence
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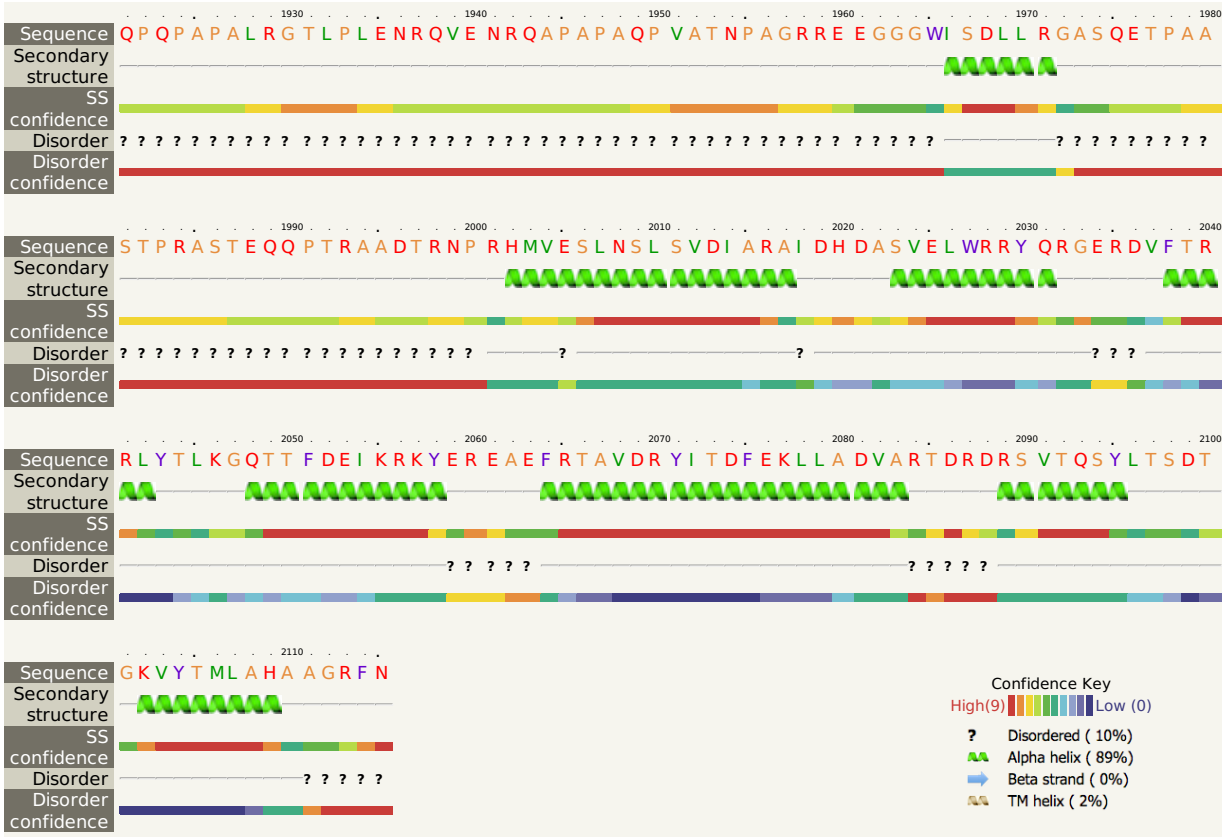
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S T P R A S T E Q Q P T R A A D T R N P R H M V E S L N S L S V D I A R A I D H D A S V E L W R R Y Q R G E R D V F T R
R L Y T L K G Q T T F D E I K R K Y E R E A E F R T A V D R Y I T D F E K L L A D V A R T D R D R S V T Q S Y L T S D T
G K V Y T M L A H A A G R F N
Confidence Key
High(9)
Low (0)
? Disordered (10%)
Alpha helix (89%)
Beta strand (0%)
TM helix (2%)

B.


Sequence
Secondary structure
SS confidence
Disorder
Disorder confidence
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Sequence
Secondary structure
SS confidence
Disorder
Disorder confidence
Confidence Key
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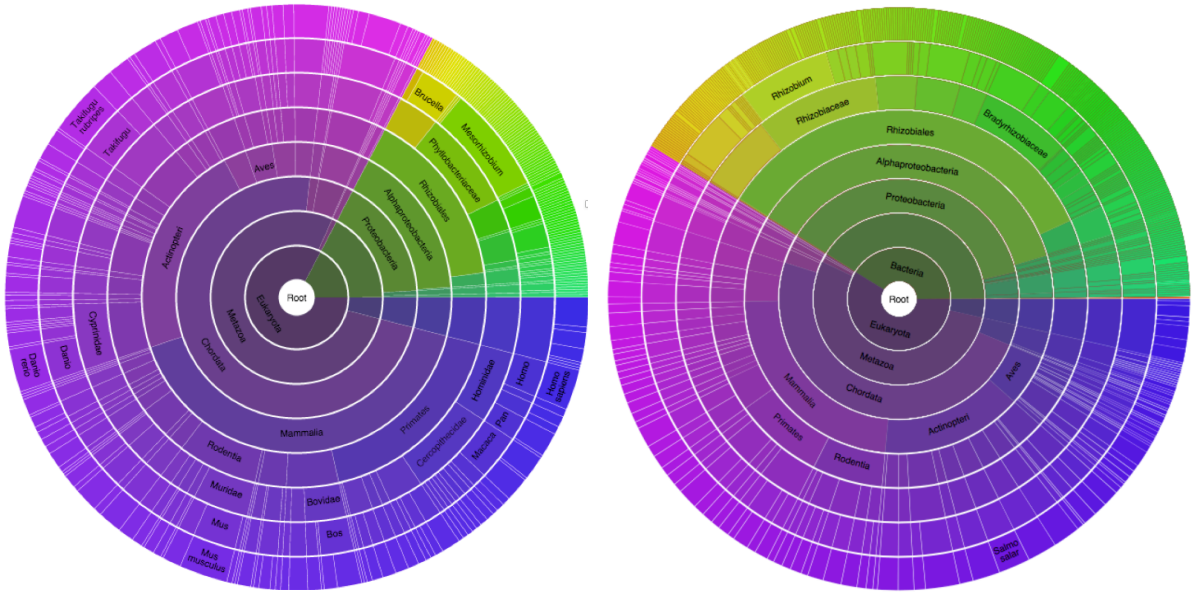

September, 2015 October, 2018

Figure S2. Change in species distribution of apolipoproteins (Pfam, PF01442, (2)) between Sept., 2015, and October, 2018.

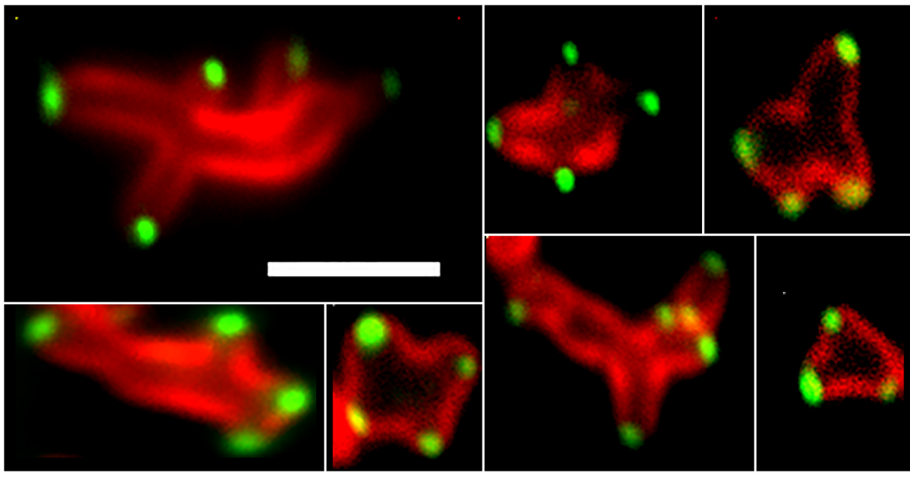

Figure S3. Expression of GPR-GFP *in trans* to WT GPR results in formation of ectopic poles. Cells are counterstained with FM4-64. Scale bar = 3μm.

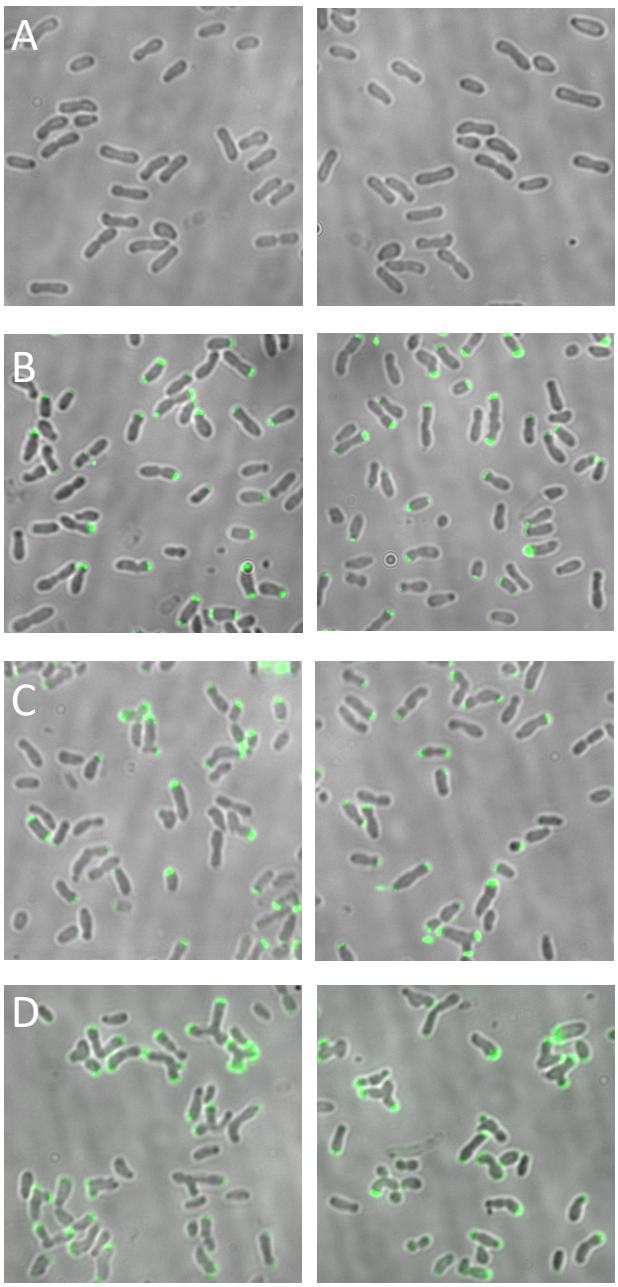

Figure S4. Induction of GFP-GPR in WT *Agrobacterium tumefaciens* with increasing concentrations of IPTG. Ectopic poles are formed at higher concentrations of IPTG. Cells were grown for 24 hours prior to imaging in the IPTG concentration indicated. A) 0mM IPTG, B) 0.25mM IPTG, C) 0.5mM IPTG, D) 1.0mM IPTG.

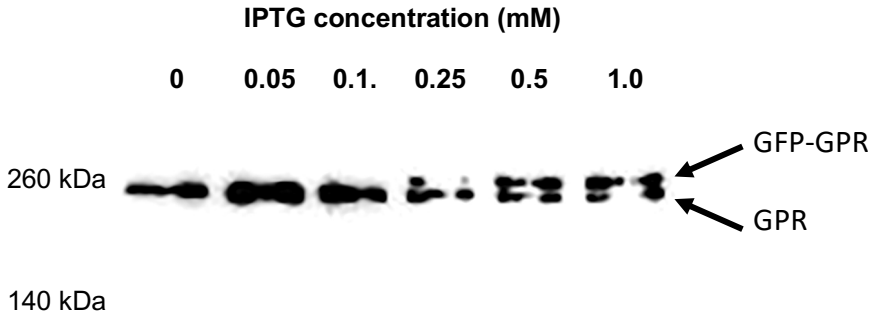

Figure S5. Western blot to detect WT GPR or GFP-GPR. Different concentrations of IPTG were used to induce GPR-GFP expression, cell extracts were separated by SDS-gel electrophoresis, and GPR was detected following Western blotting using antibodies to GPR.

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CCGGAGCGTCACCCAGTCCTACCTCACCTCCGATACCGGCAAGGTCTATACGATGCTTGCCCAT
GCGGCGGGCCGGTTTAATTGA

Figure S6. *gpr* deletion strategy. Atu1349 partial protein coding sequence in blue. Atu1348 (GPR) coding sequence in red. Intergenic region in black **bold**. Deleted sequence (underlined) comprises 238 bp upstream of the *gpr*-ATG and the first 2140 bp of the coding sequence for Atu1348 (total *gpr* protein coding sequence is 6345 bp).

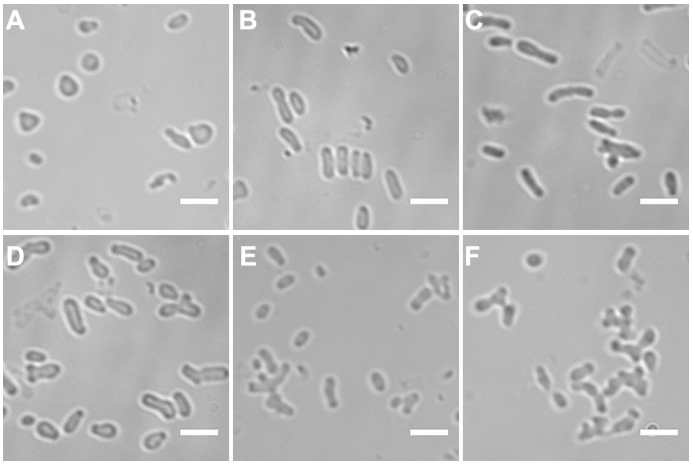

Figure S7. Δ*gpr* complementation. Unipolar growth and cell division were restored in Δ*gpr* following introduction of a plasmid carrying WT *gpr* under the control of the *lac* promoter (P_{lac}::*gpr* (pSRKGm)). Cells were grown for 24 hours prior to imaging in the IPTG concentration indicated. A) 0μm IPTG, B) 25μm IPTG, C) 50μm IPTG, D) 100μm IPTG, E) 250μm IPTG, and F) 500μm IPTG. Scale bar = 3μm.

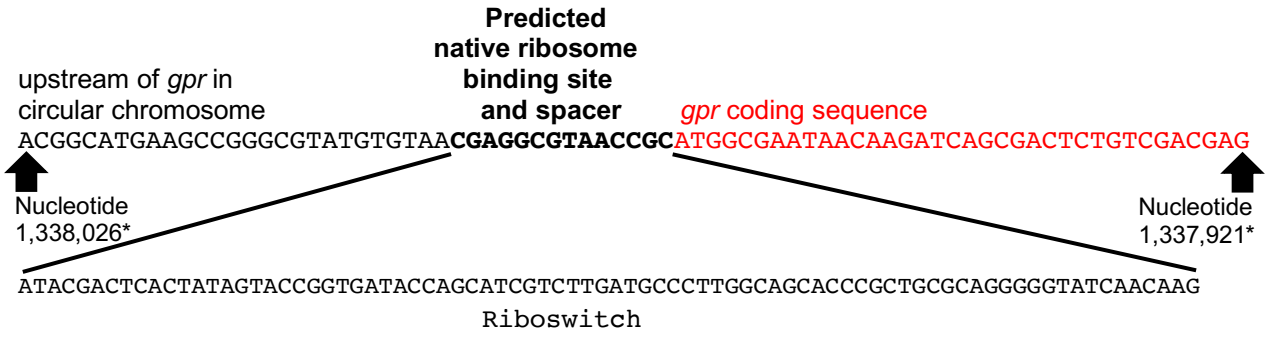

Figure S8. Riboswitch strategy for controlling GPR expression in circular chromosome. The predicted ribosome binding site and spacer (**bold**) were replaced by the riboswitch. *Nucleotide coordinates are for the circular chromosome of *Agrobacterium tumefaciens* (Accession AE007869.2, ncbi.nlm.nig.gov).

Supplementary Table 1. Apolipoprotein domains (Family PF01442) in GPR identified by www.pfam.xfam.org (2). [a]amino acid number

Position in GPR[a]	Domain Length (amino acids)	e-value
290-450	161	$9.7x10^{-1}$
323-523	201	$3.6x10^{-1}$
416-612	197	$1.5x10^{-2}$
495-718	224	$7.3x10^{-3}$
625-824	201	$7.2x10^{-5}$
821-1008	188	$5.5x10^{-2}$
937-1126	190	$7x10^{-6}$
1115-1338	224	$1.9x10^{-4}$
1291-1439	149	13
1335-1542	208	$6.5x10^{-4}$
1583-1783	201	$5.3x10^{-4}$
1784-1878	95	290

Table S2. Strains and plasmids used in this study.

Strains	**Relevant genotype and features**	**Source**
XL1 Blue E. coli	cloning strain, endA1 gyrA96(nal^R) thi-1 recA1 relA1 lac glnV44 F'[::Tn10 proAB+ lacIq Δ(lacZ)M15] hsdR17(rKmK+), Tet^R	Lab stock
C58	wild-type A. tumefaciens strain C58	Lab stock
A185	C58 carrying pJZ251	This work
A187	C58 carrying pJZ253	This work
A202	C58 carrying pJZ253 and pJZ269	This work
A212	C58 with the native *gpr* ribosome binding (RBS) site substituted with a riboswitch, referred to as RS::*gpr* in text	This work
A248	C58 with a deletion of 2378bp including 238bp upstream of the *gpr* ATG and the first 2140bp of the *gpr* coding sequence, referred to as Δ*gpr* in text	This work
A239	A212 carrying pJZ253	This work
A253	A248 carrying pJZ280	This work
A256	A212 carrying pRG001	This work
RESJ001	C58 carrying pJZ253 and pTC077	This work
Plasmids	**Relevant genes and construction information**	**Source**
pSRKGm	Broad host-range, *lacI*, gentamycin^R	(3)
pSRKKm	Broad host-range, *lacI*, kanamycin^R	(3)
pBluescript II SK	Phagemid, carbenicillin^R	Stratagene
pJZ156	pBSKII+ with *sacB*, carbenicillin^R	(4)
pJZ210	pSRKGm with P$_{lac}$::*gfp*	This work
pJZ251	pSRKGm with P$_{lac}$::*gpr$_{At}$-gfp*	This work

pJZ253	pSRKGm with P_{lac}::*gfp-gpr*	This work
pJZ269	pSRKKm with P_{lac}::$popZ_{At}$-*rfp*	This work
pJZ274	pJZ156 carrying 2kb sequence homologous to C58 genomic DNA in which the native *gpr* RBS is replaced with a riboswitch (Fig. S8)	This work
pJZ280	pSRKGm with P_{lac}::*gpr*	This work
pJZ298	pJZ156 carrying 1kb homologous to C58 genomic DNA on either side of the sequence to be deleted (Fig. S6)	This work
pRG001	pSRKGm with P_{lac}::$popZ_{At}$-*gfp*	(5)
pTC077	pDW029 with P_{lac}::*ftsZ-rfp*, streptomycin/spectinomycin^R	(6)

SUPPLEMENTAL REFERENCES

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Supplemental Movies

Movie S1, S2, S3, S4. *Agrobacterium* cells expressing GFP-GPR display a ring of foci just below the growth pole. GFP-GPR localization in 4 cells shown by rotational animation of 3-dimensional renderings from images obtained with structured illumination superresolution microscopy.

Movie S5. *Agrobacterium* cells expressing GFP-GPR and PopZ-RFP display a ring of green foci just below the growth pole and a single red focus. Localization shown in rotational animation of 3-dimensional rendering from images obtained with structured illumination superresolution microscopy.

Movie S6. Time lapse of *Agrobacterium* cell expressing GFP-GPR shown in Fig. 4.

Movie S7. Time lapse of 2 *Agrobacterium* cells expressing GFP-GPR show variability in persistence of GPR at old pole.

Movie S8. Time lapse of Δ*gpr* cell in Fig. 5.

Movie S9. Δ*gpr* cell division produces quartet of cells.