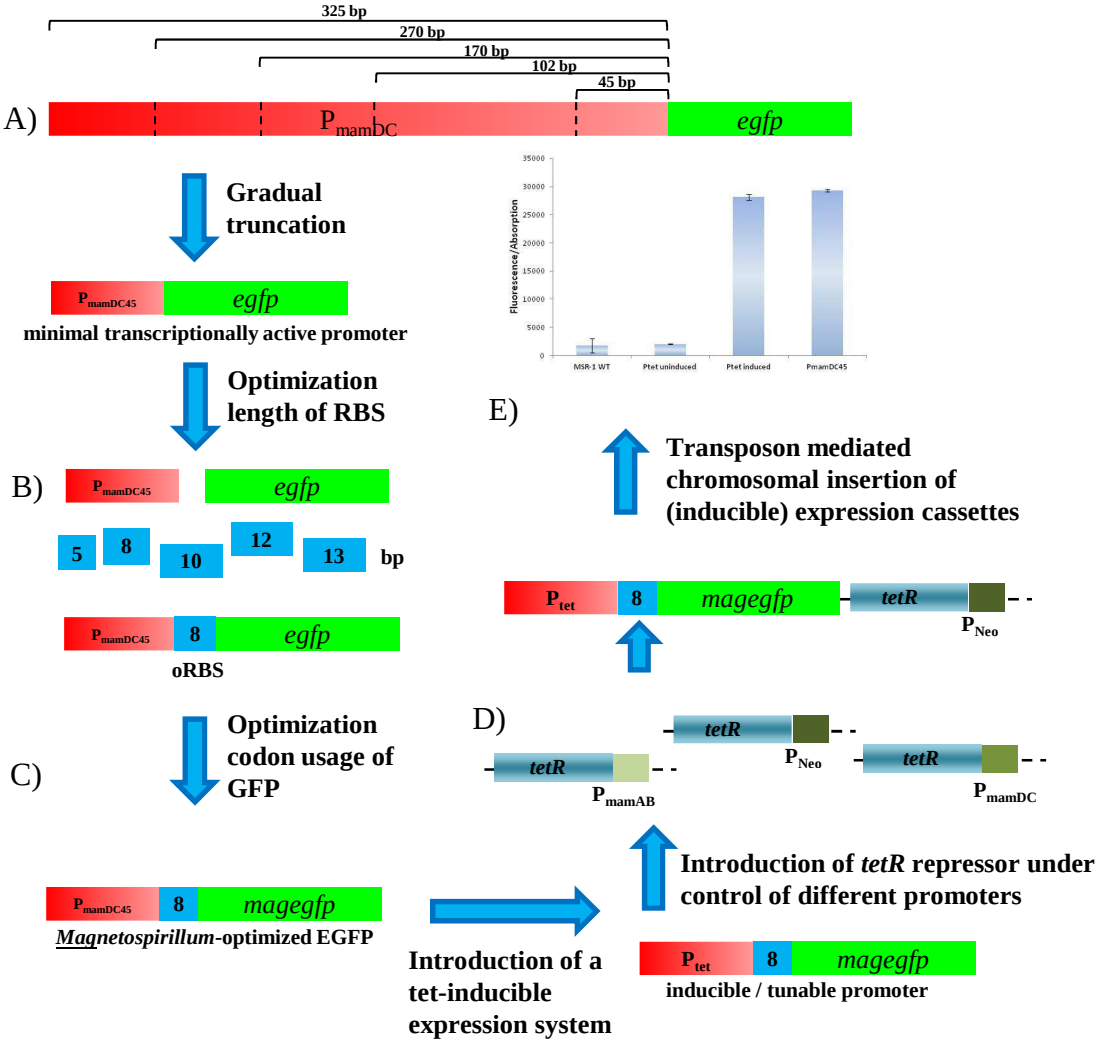
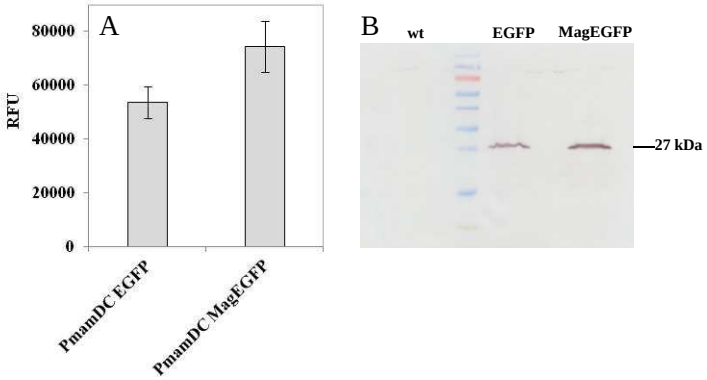
Optimization of gene expression cassette for *M. gryphiswaldense*

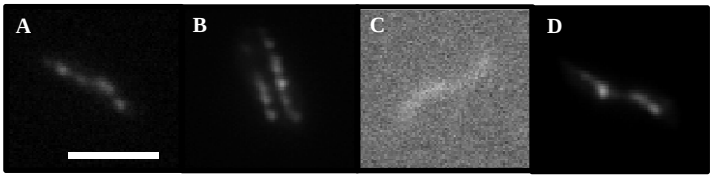


Suppl. Fig. S1: Optimization strategy of (inducible) expression cassette.

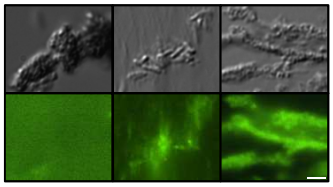
Suppl. Fig S2: Sequence alignment of non-optimzed *egfp* and *Magnetospirillum*-optimized *magegfp* using CLUSTALW (version 2.1, http://www.ebi.ac.uk/Tools/msa/clustalw2/). Sequence similarity is 89 %, start and stop codon are over-lined.



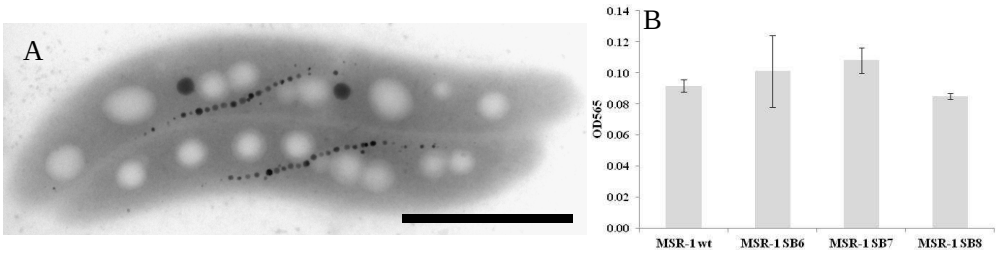
Suppl. Fig S3: (A) Fluorescence of *Magnetospirillum*-optimized (MagEGFP) versus non-optimized GFP (EGFP) expressed from the $P_{mamDC45}$ promoter. Fluorescence was normalized to the cell density and described as relative fluorescence units (RFU). Error bars represent standard deviations, calculated from three independent experiments. (B) Western blot of whole *M. gryphiswaldense* cells expressing EGFP or MagEGFP from the control of the $P_{mamDC45}$. (Mag)EGFP was detected using rabbit αGFP IgG as primary, and goat anti-rabbit IgG alkaline phosphatase antibodies as secondary antibody. PageRuler™ Prestained Protein Ladder from fermentas was used as a standard.



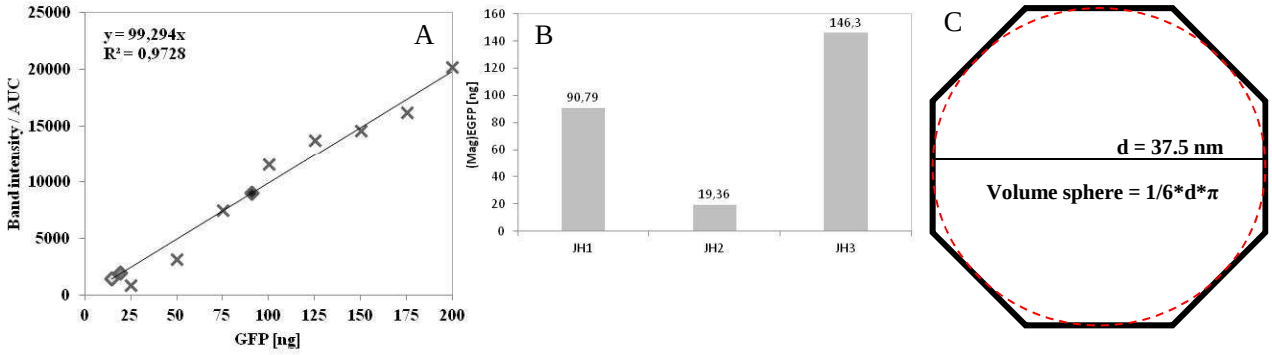
Suppl. Fig. S4: Fluorescence micrographs of *M. gryphiswaldense ΔC* strains carrying chromosomal insertions of (A) $P_{mamDC45}$-*mamC-magegfp* (JH1) (B) P_{tet}-*mamC-magegfp* (JH2) induced with 70 ng ml^{-1} Atet, (C) uninduced and (D) $P_{mamDC45}$-*mamC-egfp-magegfp* (JH3) expression cassettes. White bar corresponds to 2 µm.



Suppl. Fig. S5: Fluorescence and DIC micrographs of isolated magnetosomes from *M. gryphiswaldense* MSR-1 *ΔC* (A) JH2 (induced) (B) JH1 and (C) JH3. White bar corresponds to 2 µm.



Suppl. Fig. S6: A) Transmission electron micrographs of *M. gryphiswaldense* SB6, expressing MagEGFP chromosomally from $P_{mamDC45}$. The black scale bar represents 200 nm. B) Optical density (OD_{565}) after overnight growth of strains SB6, SB7 and SB8 in comparison to wt. Error bars represent standard deviations calculated from triplicate cultures, experiment was repeated three times, data is from one representative experiment.



Suppl. Fig. S7: (A) Quantitative Western blot of GFP standard curve (marked by "x") and MM samples from strains JH1 (black square), JH2 (grey square) and JH3 (light grey square, 10x diluted). (B) Corresponding GFP protein concentrations of strains JH1, JH2 and JH3. (C) Schematic drawing of approximation of magnetosome size.

Suppl. Tab. S1: Plasmids used in this study

Plasmid name	Description	Source or reference
pJET1.2/blunt	Cloning vector; Amp^R	Fermentas
pBBR-MCS2	Mobilizable broad-host-range vector; Km^R	Kovach, M. E., *et al*,. 1995
pBAM1	Km^R , Amp^R, oriR6K, *tnpA*	Martinez-Garcia, E., *et al.*, 2011
p11AAGJZC	Amp^R,ColE1 ori, oRBS, *magegfp*	GeneArt® (Invitrogen), life technologies, Darmstadt
pAP150	pBBR-MCS2, with $P_{mamDC45}$, *egfp*, terminator-fragment; Km^R	A. Pollithy (unpublished)
pAP158	pBBR-MCS2, with P_{tet}, *egfp*, terminator-fragment,P_{mamAB}-TetR; Km^R	A. Pollithy (unpublished)
pAP159	pBBR-MCS2, with P_{tet}, *egfp*, terminator-fragment,P_{mamDC}-TetR; Km^R	A. Pollithy (unpublished)
pAP160	pBBR-MCS2, with P_{tet}, *egfp*, terminator-fragment,P_{Neo}-TetR; Km^R	A. Pollithy (unpublished)
pAP161	pBBR-MCS2, with $P_{mamDC325}$, *egfp*, terminator-fragment; Km^R	A. Pollithy (unpublished)
pAP162	pBBR-MCS2, with $P_{mamDC102}$, *egfp*, terminator-fragment; Km^R	A. Pollithy (unpublished)
pAP163	pBBR-MCS2, with $P_{mamDC170}$, *egfp*, terminator-fragment; Km^R	A. Pollithy (unpublished)
pAP164	pBBR-MCS2, with $P_{mamDC270}$, *egfp*, terminator-fragment; Km^R	A. Pollithy (unpublished)
pLYJ97	pBBR-MCS2 with *gusA*	Li, Y., *et al.*, 2012
pSB1	pBBR-MCS2, with $P_{mamDC45}$, *magegfp*, terminator-fragment; Km^R	this study
pSB6	pBAM1 with $P_{mamDC45}$, *magegfp*, Km^R, Amp^R	this study
pSB7	pBAM1 with P_{tet}, *magegfp*, P_{Neo}-TetR, Km^R, Amp^R	this study
pSB8	pBAM1 with P_{tet}, *gusA*, P_{Neo}-TetR, Km^R, Amp^R	this study
pJH1	pBAM1 with $P_{mamDC45}$, *mamC-magegfp*, Km^R, Amp^R	this study
pJH2	pBAM1 with P_{tet}, *mamC-magegfp*, P_{Neo}-TetR, Km^R, Amp^R	this study
pJH3	pBAM1 with $P_{mamDC45}$, *mamC-magegfp-egfp*, Km^R, Amp^R	this study

Suppl. Tab. S2: Strains used in this study

Strain	Description	Source or reference
Escherichia coli		
DH5α	F⁻ *sup*E44 Δ*lac*U169 (Φ 80 *lacZ*DM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1	
WM3064	*thrB1004 pro thi rpsL hsdS lacZΔM15 RP4-1360 Δ(araBAD)567 ΔdapA1341::[erm pir]*	W. Metcalf, unpublished
BW29427	DAP auxotroph derivative of *E. coli* strain B2155	K. Datsenko and B. L. Wanner, unpublished
Magnetospirillum gryphiswaldense		
M. gryphiswaldense MSR-1 R3/S1	Rif^R, Sm^R spontaneous mutant, lab strain	D. Schultheiss, *et al.*, 2003
M. gryphiswaldense Δ*C*	*ΔmamC*	A. Scheffel, *et al.*, 2007
M. gryphiswaldense (pAP150)	Km^R, conjugated with pAP150	A. Pollithy, unpublished
M. gryphiswaldense (pAP160)	Km^R, conjugated with pAP160	A. Pollithy, unpublished
M. gryphiswaldense (pSB1)	Km^R, conjugated with pSB1	this study
M. gryphiswaldense MSR-1 SB6	Km^R, transposon mutant with inserted *magegfp* from $P_{mamDC45}$	this study
M. gryphiswaldense MSR-1 SB7	Km^R, transposon mutant with inserted *magegfp* from P_{tet}	this study
M. gryphiswaldense MSR-1 SB8	Km^R, transposon mutant with inserted *gusA* from P_{tet}	this study
M. gryphiswaldense MSR-1 JH1	Km^R, transposon mutant with inserted *mamC-magegfp* from $P_{mamDC45}$	this study
M. gryphiswaldense MSR-1 JH2	Km^R, transposon mutant with inserted *mamC-magegfp* from P_{tet}	this study
M. gryphiswaldense MSR-1 JH3	Km^R, transposon mutant with inserted *mamC-magegfp-egfp* from $P_{mamDC45}$	this study

Suppl. Tab. S3: Primers used in this study. Restriction sites indicated in bold

Primer name	Sequence	Restriction site
oEGFP BamHI Rev	cgaac**ggatcc**tcacttatacagctcg	BamHI
oEGFP HindIII Fw	cggctc**aagctt**aggagatcagcatatg	HindIII
pBam_pAP160 Fw	atc**gggaccc**cttccggctggctggttt	SanDI
pBam_ Tet w/o Term Rev	atc**gaattc**ggcggatttgtcctactca	EcoRI
pBam_pAP150 Fw	atc**gggaccc**ggatcctcacttatacagct	SanDI
pBam_DC w/o Term Rev	gc**gaattc**ctcgagctttttcgctttac	EcoRI
GusA BamHI Fw	gt**ggatcc**cccgggtcattgtttgcc	BamHI
GusA NdeI Rev	tt**catatg**ttacgtcctgtagaaa	NdeI
optGFP San/Bam Fw	ga**gggaccc***ggatcc*tcacttatacagctcgtcc	BamHI/SandDI
optGFP linker Rev	ggaggcggaggcggtggcggaggtggcgga**atcgat**atg gtgtcgaagggcga	ClaI
mamC ov linker Fw	cgccaccgcctccgcct**ccatgg**gccaattcttccctca	NcoI
mamC NdeI Rev	ta**catatg**agctttcaacttgcgcc	NdeI
optGFP2x Fw	agt**ggatcc**tcacttatacagctcgtcca	BamHI
optGFP2x Rev	ctgtgc**ctgcag**ggcgagatggtgtcgaagggcg	PstI
eGFP overl Fw	atctcgcc**ctgcag**gcacagcttgtacagctcgtccatgc	PstI
mamC_RBS Rev	cg**aagctt**aggagatcagcatatgagctttcaact	HindIII

Suppl. Tab. S4: Insertion sites of expression cassettes in *M. gryphiswaldense* strains

Strain	Gene	Putative function
MSR-1 SB6 K7	MGR_1519	hypothetical protein
MSR-1 SB6 K8	Inter region between MGR_1092 and MGR_2997	MGR_1092 - D-alanine-D-alanine ligase MGR_2997 - acyl carrier protein
MSR-1 SB7 K1	MGR_1581	sugar kinase, ribokinase family
MSR-1 SB7 K2	MGR_1519	hypothetical protein
MSR-1 SB8	MGR_1702	transposase IS3/IS911
MSR-1 JH1	MGR_3776	insertion element ISR1 from not characterized 10 kDa protein A3
MSR-1 JH2	MGR_3148	TorC, trimethylaminoxide (TMAO)-reductase I, cytochrom C subunit
MSR-1 JH3	MGR_612	hypothetical protein

References Supplements:

1. **Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, Peterson KM.** 1995. 4 New Derivatives of the Broad-Host-Range Cloning Vector pBBR1MCS, Carrying Different Antibiotic-Resistance Cassettes. Gene **166:**175-176.
2. **Martinez-Garcia E, Calles B, Arevalo-Rodriguez M, de Lorenzo V.** 2011. pBAM1: an all-synthetic genetic tool for analysis and construction of complex bacterial phenotypes. Bmc Microbiol **11**.
3. **Li YJ, Katzmann E, Borg S, Schüler D.** 2012. The Periplasmic Nitrate Reductase Nap Is Required for Anaerobic Growth and Involved in Redox Control of Magnetite Biomineralization in *Magnetospirillum gryphiswaldense*. J Bacteriol **194:**4847-4856.
4. **Schultheiss D, Schüler D.** 2003. Development of a genetic system for *Magnetospirillum gryphiswaldense*. Arch Microbiol **179:**89-94.
5. **Scheffel A, Gärdes A, Grünberg K, Wanner G, Schüler D.** 2008. The major magnetosome proteins MamGFDC are not essential for magnetite biomineralization in *Magnetospirillum gryphiswaldense* but regulate the size of magnetosome crystals. J Bacteriol **190:**377-386.