Supporting Information:

Functional diversification in bacterial rhodopsins: a new class of light-driven Cl$^-$ pumps in marine flavobacteria.

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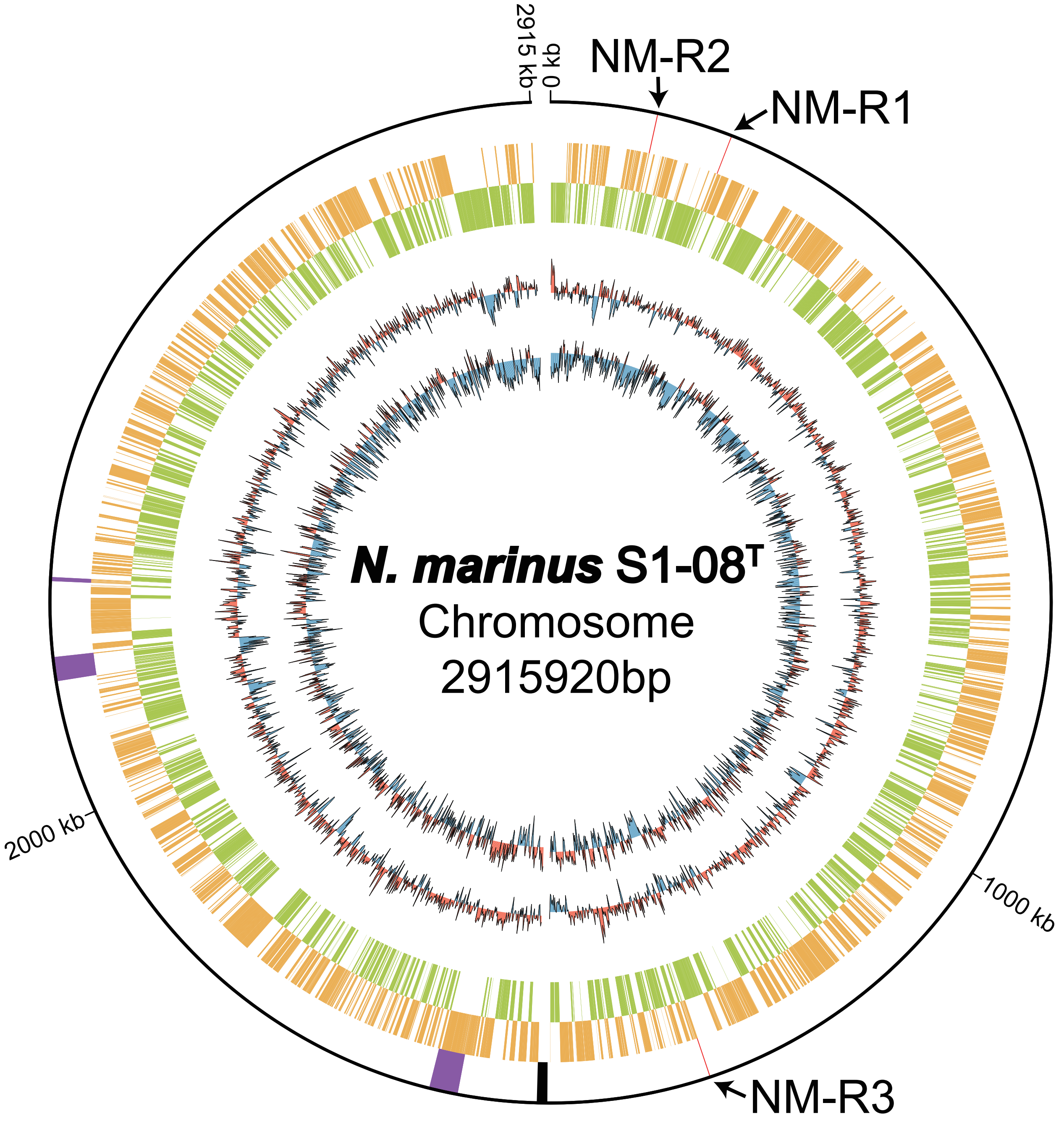

Figure S1.

Circular genome map of S1-08^T. The positions of NM-R1, NM-R2 and NM-R3 are indicated by arrows. From outside to inside: rhodopsin genes (red), putative HGT regions (purple) and gap region (black); genes on leading strand (yellow); genes on lagging strand (green); GC content; GC-skew.

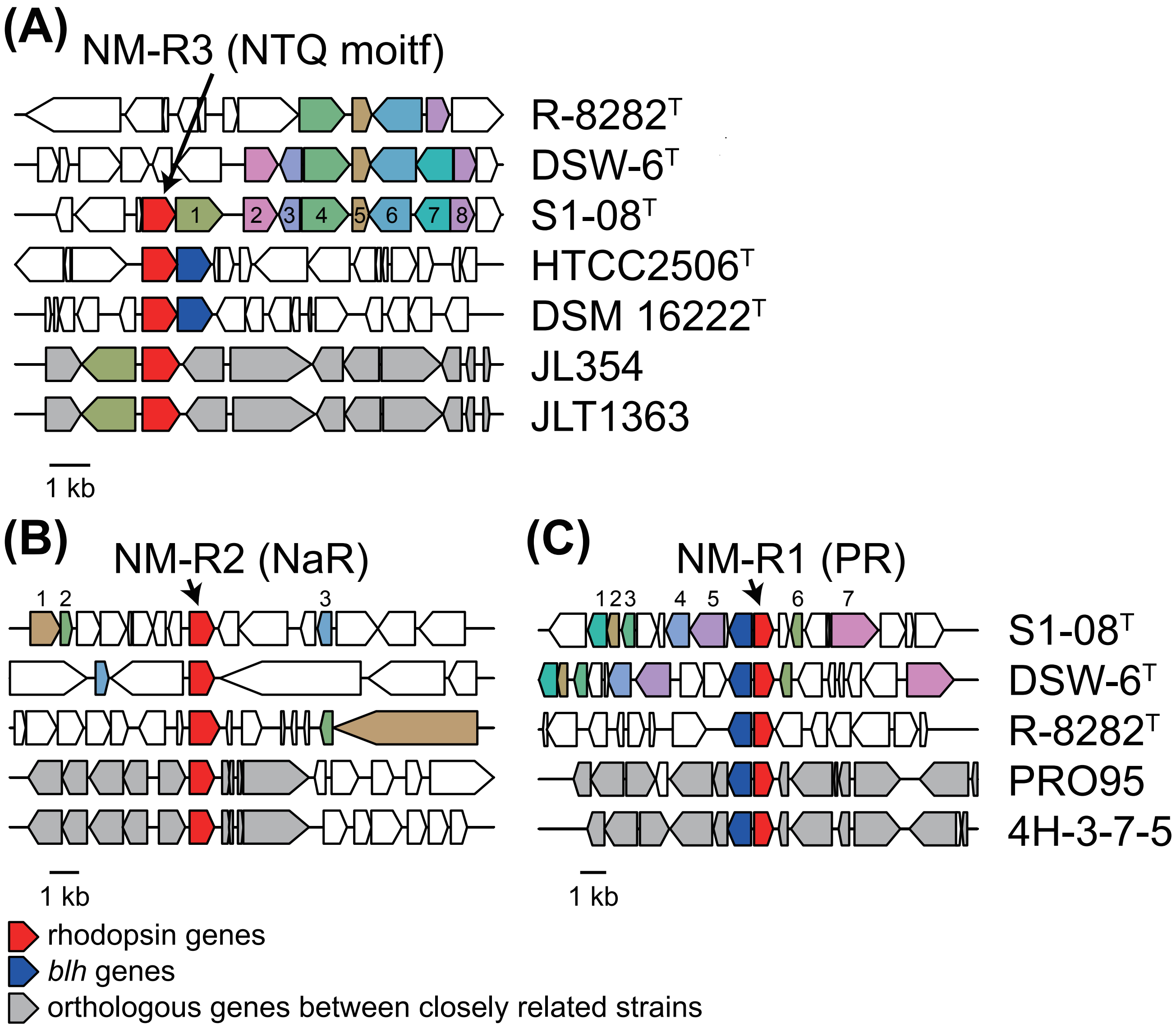

Figure S2.

Genomic flanking regions of NM-R3 (A), NM-R2 (B) and NM-R1 genes (C). The positions of each rhodopsin and *blh* gene (15,15’ -β-carotene dioxygenase) are indicated in red and blue, respectively. Gray colors show orthologous genes between closely related strains. Each orthologous gene is indicated in same color.

A. 1 hypothetical protein (DUF2254); 2 Hydrolase (HAD superfamily); 3 hypothetical protein; 4 TPR domain protein; 5 hypothetical protein; 6 N-acetylglucosaminyltransferase; 7 hypothetical protein; 8 Crossover junction endodeoxyribonuclease RuvC.

B. 1 Sensory transduction histidine kinase; 2 Two-component response regulator; 3 hypothetical protein.

C. 1 Alkyl hydroperoxide reductase and/or thiol-specific antioxidant family (AhpC/TSA) protein; 2 hypothetical protein; 3hypothetical protein; 4 Methylenetetrahydrofolate dehydrogenase (NADP$^+$) / Methenyltetrahydrofolate cyclohydrolase; 5 Signal recognition particle, subunit Ffh SRP54; 6 FKBP-type peptidyl-prolyl cis-trans isomerase SlyD; 7 GTP-binding protein TypA/BipA.

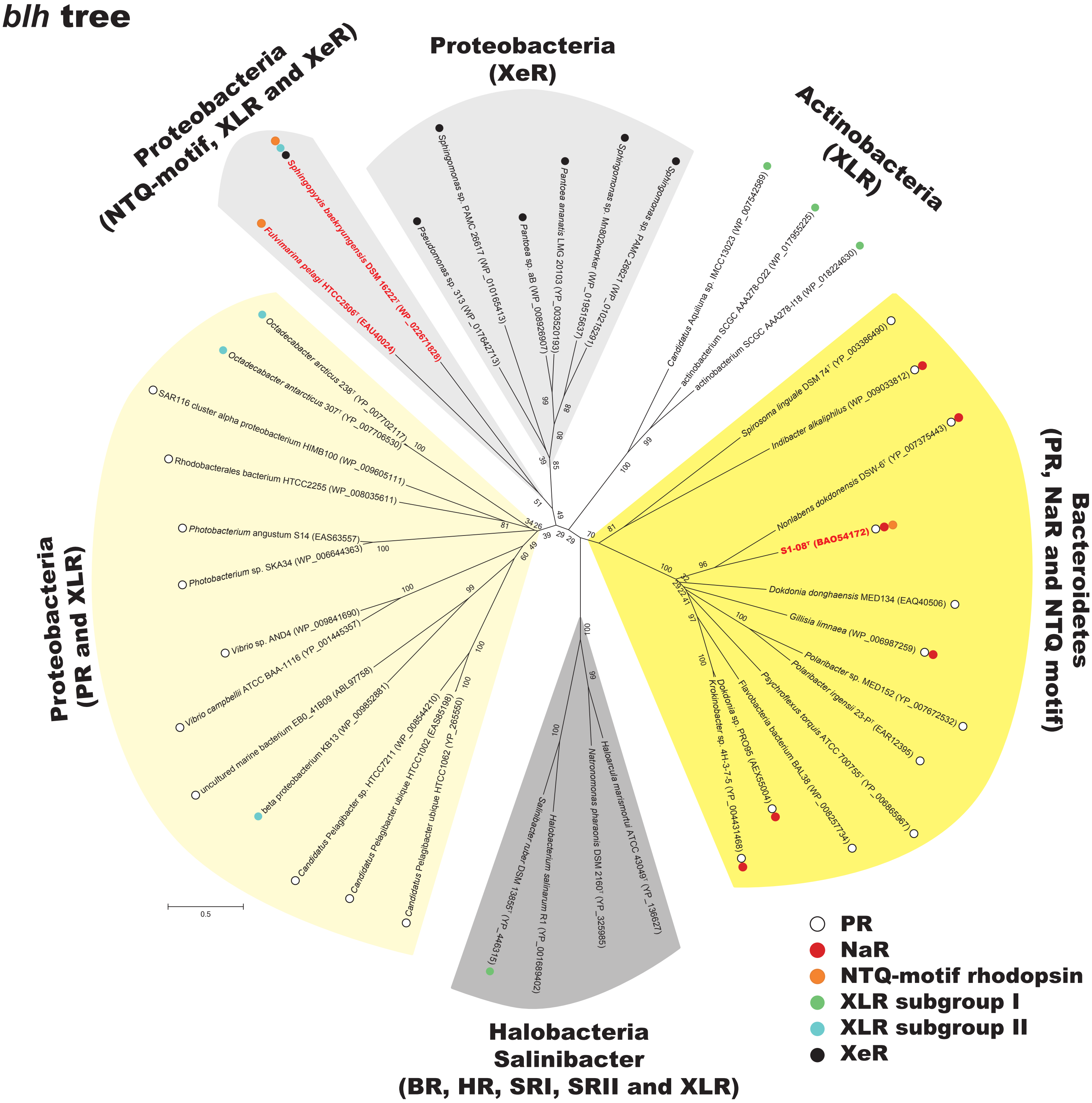

Figure S3.

Phylogenetic tree of *blh* amino acid sequences in rhodopsin gene containing strains. The rhodopsin type found in each strain is indicated by colored circles. NTQ motif rhodopsin gene containing strains are indicated by red characters. XLR is classified into XLR subgroup I and subgroup II according to the criteria reported by Vollmers *et al*. [1]. Abbreviations: PR, Proteorhodopsin; NaR, Sodium-pumping rhodopsin; XLR, Xanthorhodopsin-like rhodopsin; XeR, Xenorhodopsin [2]; BR, Bacteriorhodopsin; HR, Halorhodopsin; SR I, Sensory rhodopsin I; SRII, Sensory rhodopsin II.

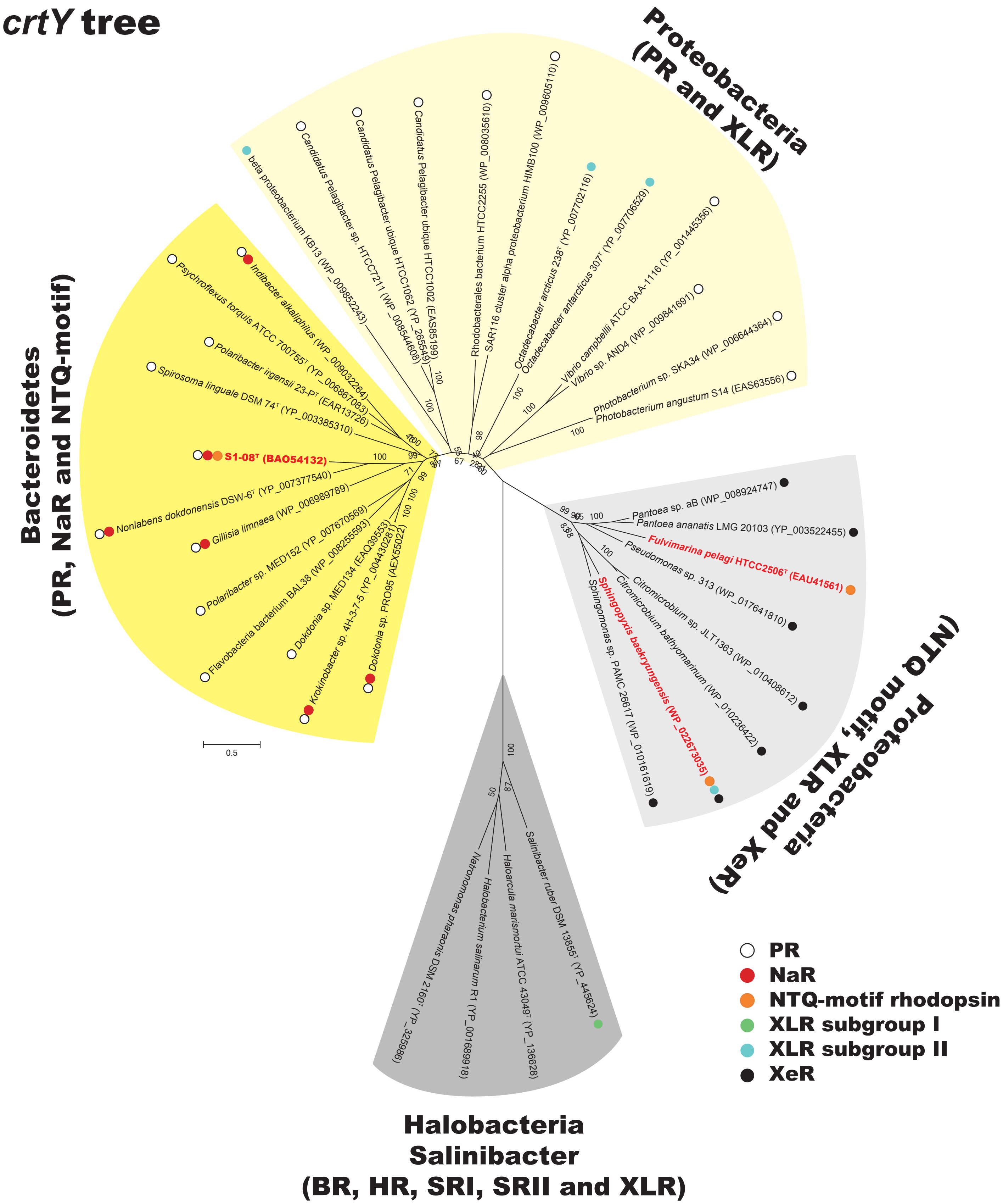

Figure S4.

Phylogenetic tree of *crtY* amino acid sequences in rhodopsin gene containing strains. The rhodopsin type found in each strain is indicated by colored circles. Strains that also contain the NTQ motif rhodopsins are indicated by red characters.

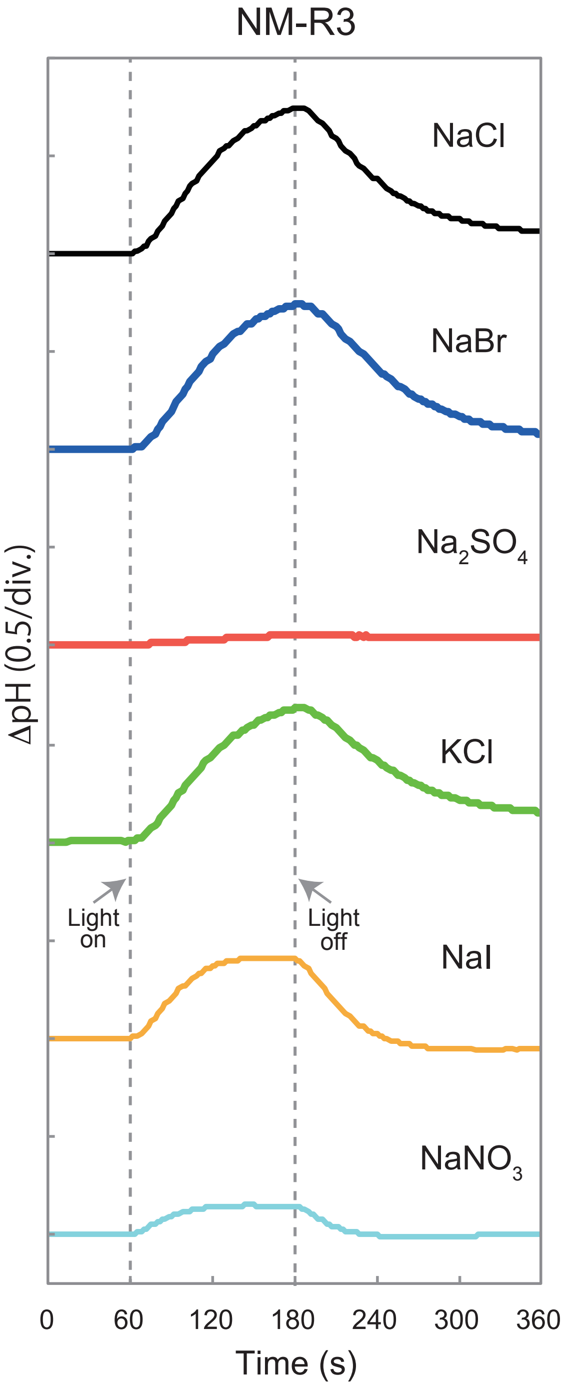

Figure S5.

Light-induced pH changes of *E. coli* cell suspensions expressing NM-R3 in different salt solutions (100 mM). Experimental details are described in the methods.

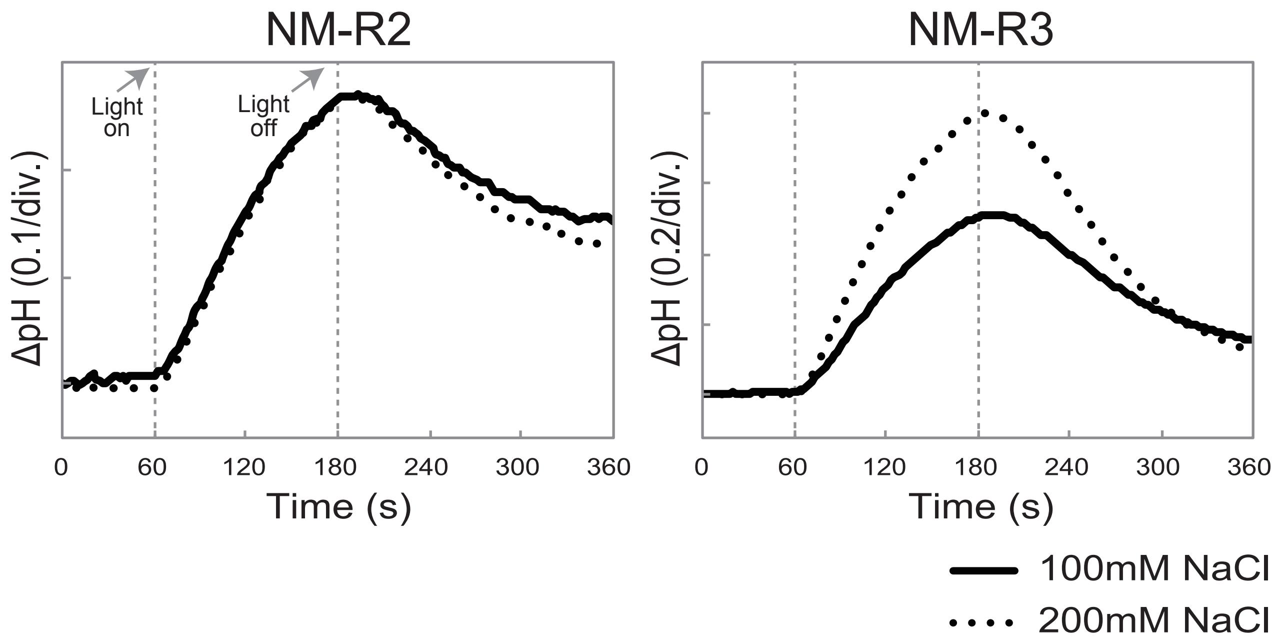

Figure S6.

Light-induced pH changes of *E. coli* cell suspensions expressing NM-R2 or NM-R3 in 100 mM and 200 mM NaCl. Experimental details are described in the methods.

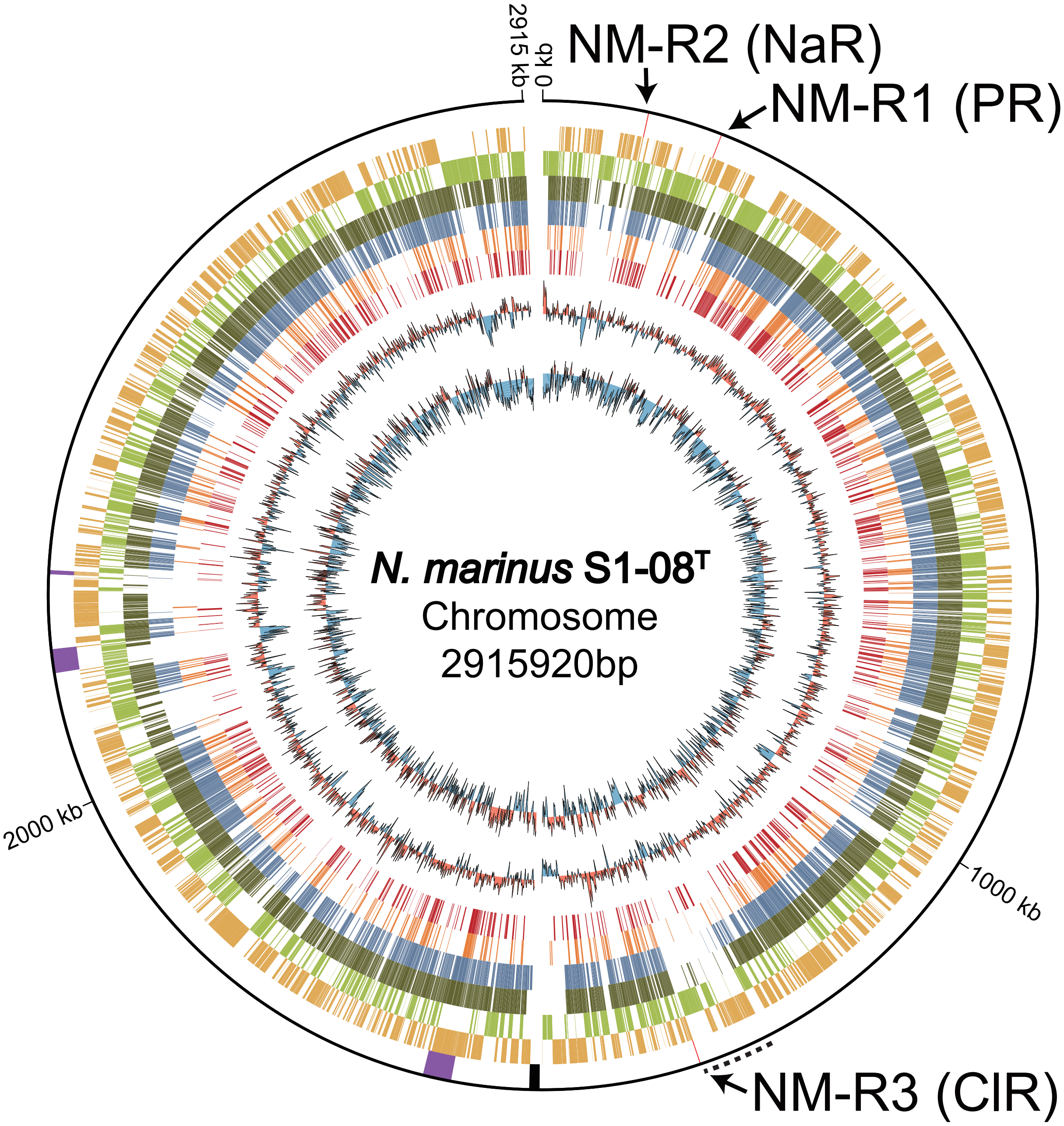

Figure S7.

Genome map of S1-08^T compared to other NaR or ClR containing bacteria. The positions of NM-R1, NM-R2 and NM-R3 are indicated by arrows. From outside to inside: rhodopsin genes (red), putative HGT regions (purple) and gap region (black); genes on leading strand (yellow); genes on lagging strand (green); orthologous gene between S1-08^T and DSW-6^T (dark green); Orthologous genes among NaR containing flavobacteria (S1-08^T, DSW-6^T, 4H-3-7-5, PRO95 and R-8282^T) (dark blue); orthologous genes among NaR containing bacteria (S1-08^T, DSW-6^T, 4H-3-7-5, PRO95, R-8282^T, LW1^T and DSM 17093^T) (orange); orthologous genes among ClR containing bacteria (S1-08^T, HTCC2506^T, JLT1363, JL354 and DSM 16222^T) (red); GC content; GC-skew.

Strain information used in this analysis: *Nonlabens marinus* S1-08^T, *Nonlabens dokdonensis* DSW-6^T, *Krokinobacter* sp. 4H-3-7-5, *Dokdonia* sp. PRO95, *Gillisia limnaea* R-8282^T, *Indibacter alkaliphilus* LW1^T, *Truepera radiovictrix* DSM 17093^T, *Fulvimarina pelagi* HTCC2506^T, *Citromicrobium* sp. JLT1363, *Citromicrobium* sp. JL354 and *Sphingopyxis baekryungensis* DSM 16222^T.

Table S1. General features of Flavobacteriaceae genomes containing multiple rhodopsin genes.

General features	*Nonlabens marinus* S1-08^T	*Nonlabens dokdonensis* DSW-6^T	*Krokinobacter* sp. 4H-3-7-5	*Gillisia limnaea* R-8282^T	*Dokdonia* sp. PRO95
Sequence size	2915920	3914632	3389993	3966857	3305193
Number of rhodopsin genes	3	2	2	2	2
Number of contigs	2	1	1	36	12
GC content (%)	39.7	35.3	37.3	37.6	37.4
Number of Coding Sequences	2790	3590	3101	3788	3090
TonB-dependent receptor	24	22	29	31	30
ABC transporters	50	58	48	62	47
tRNA's	36	42	42	44	36
Membrane proteins	763	1038	877	994	884
% of membrane proteins	27.3	28.9	28.3	26.2	28.6
COG [C] Energy production and conversion	98	107	112	127	109
COG [G] Carbohydrate transport and metabolism	82	99	103	141	100
COG [P] Inorganic ion transport and metabolism	89	94	113	135	108
COG [Q] Secondary metabolites biosynthesis, transport and catabolism	53	88	53	65	49

Supporting Information References

1. Vollmers J, *et al.* (2013) Poles apart: arctic and antarctic *Octadecabacter* strains share high genome plasticity and a new type of xanthorhodopsin. *PloS one* 8(5):e63422.
2. Ugalde JA, Podell S, Narasingarao P, & Allen EE (2011) Xenorhodopsins, an enigmatic new class of microbial rhodopsins horizontally transferred between archaea and bacteria. *Biology Direct* 6.