Table S1. Amino acid sequence comparison of MbnT in *Methylocystis* sp. SB2 with 30 identified TonB-dependent transporters in *Methylosinus trichosporium* OB3b

Gene locus tag[1]	Identity (%)	E value
10210 (MbnT2)	***57.36***	***0***
12822	32.56	3.00E-14
12005	29.52	9.00E-11
12471	29.32	2.00E-07
11307	28.10	1.00E-05
11588	27.91	8.00E-09
13835	27.88	1.00E-03
10030	27.64	2.00E-05
13035	27.40	8.00E-08
11125	26.83	1.00E-06
11295	26.47	6.00E-06
11411	26.17	1.10E-03
11829	25.96	6.00E-03
14481	25.85	3.00E-09
10151	25.83	5.00E-04
13641	25.59	3.00E-05
20043	25.12	2.00E-05
20011	25.10	2.00E-06
13651 (MbnT1)	***25.00***	***3.00E-04***
10208	24.92	2.00E-10
13988	24.69	2.00E-04
10117	24.50	1.50E-03
12052	24.27	1.00E-05
12284	23.50	5.00E-04
12266	23.19	2.00E-04
10250	23.03	4.00E-04
20138	22.33	4.00E-05
13925	21.77	2.00E-06
13850	21.74	3.00E-04
14264	21.28	9.00E-05

[1]gene locus tag according to the genome of *Methylosinus trichosporium* OB3b (WGS ADVE02.2)

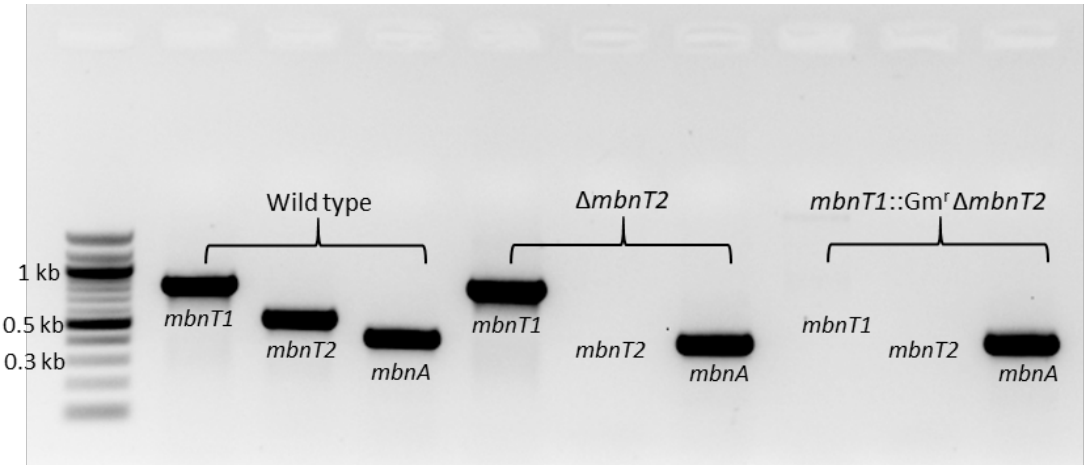

Fig. S1 Verification of the targeted gene deletion in the mutants by PCR with genomic DNAs extracted from wild type *M. trichosporium* OB3b and the constructed mutants shown in the figure.

0 µM Cu	1 µM Cu	1 µM Cu + 5 µM MB-OB3b	1 µM Cu + 5 µM MB-SB2

Fig. S2 Naphthalene assay for sMMO activity in *M. trichosporium* Δ*mbnT2* mutant cells. Naphthalene assay was performed using actively grown cells under the indicated conditions as described earlier (1).

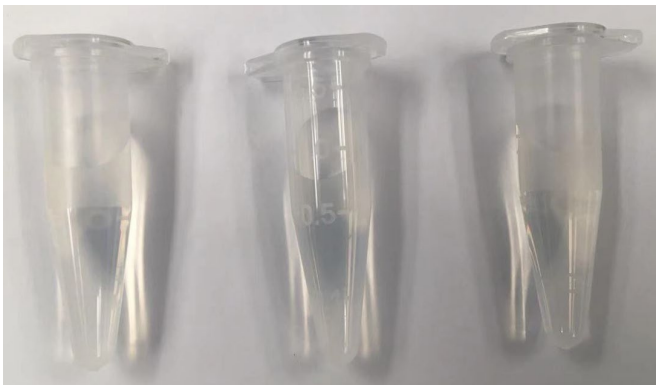

Fig. S3 Naphthalene assay for sMMO activity in *M. trichosporium mbnT1*::Gm^r Δ*mbnT2* mutant cells grown without copper. The assay was performed with *M. trichosporium mbnT1*::Gm^r Δ*mbnT2* culture after three consecutive transfer in NMS medium without addition of copper.

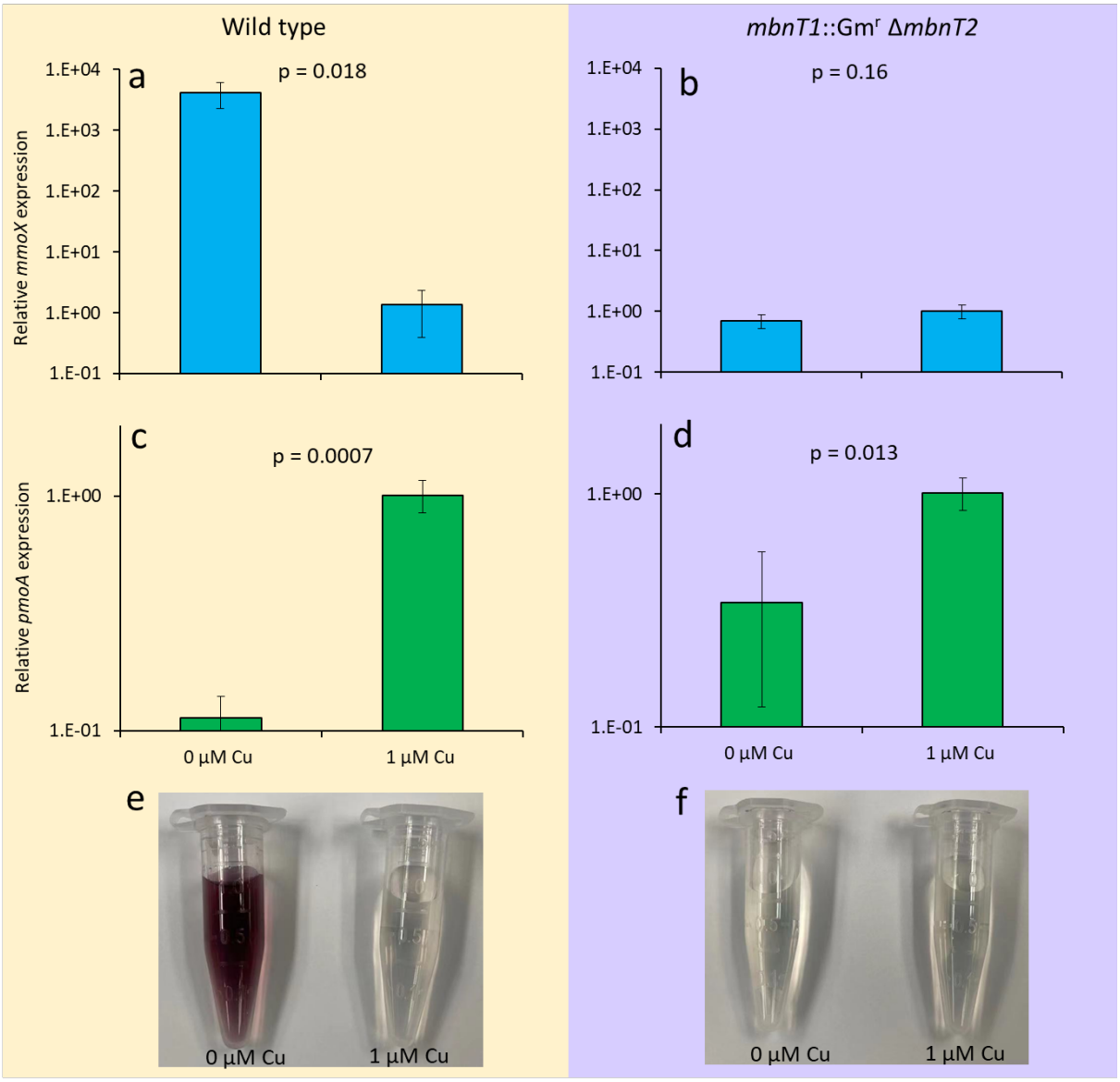

Fig. S4 RT-qPCR analysis of the relative expression of *mmoX* (a-b) and *pmoA* (c-d), and naphthalene assay for sMMO activity (e-f) in *M. trichosporium* OB3b wild type (left panel) *mbnT1*::Gm^r Δ*mbnT2* mutant (right panel) grown in 0.5% methanol with and without copper. Error bars indicate standard deviations from triplicate biological cultures. T-test was performed for variance analysis between the growth conditions.

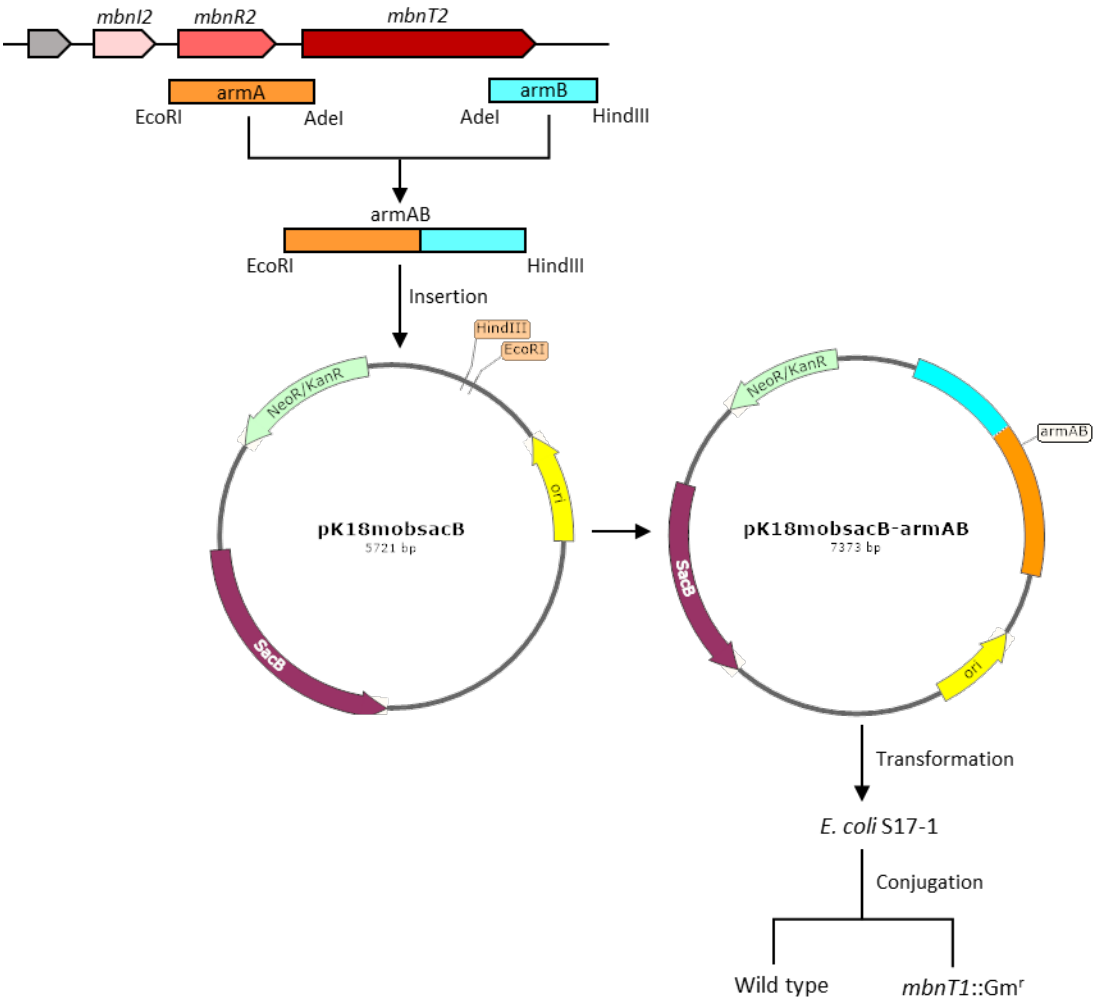

Fig. S5 Schematic representation of the *M. trichosporium* mutant construction.

References

1. Farhan Ul-Haque M, Kalidass B, Vorobev A, Baral BS, DiSpirito AA, Semrau JD. 2015. Methanobactin from *Methylocystis* sp. strain SB2 affects gene expression and methane monooxygenase activity in *Methylosinus trichosporium* OB3b. Appl Environ Microbiol 81:2466-2473.
2. Semrau JD, DiSpirito AA, Obulisamy PK, Kang-Yun CS. 2020. Methanobactin from methanotrophs: genetics, structure, function and potential applications. FEMS Microbiol Lett 367:fnaa045.