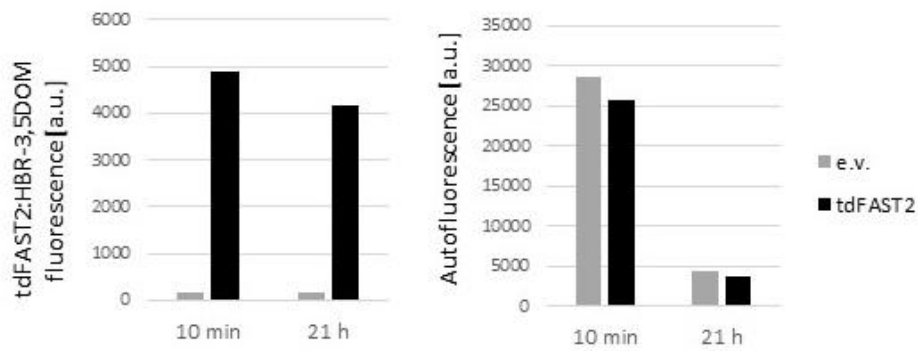


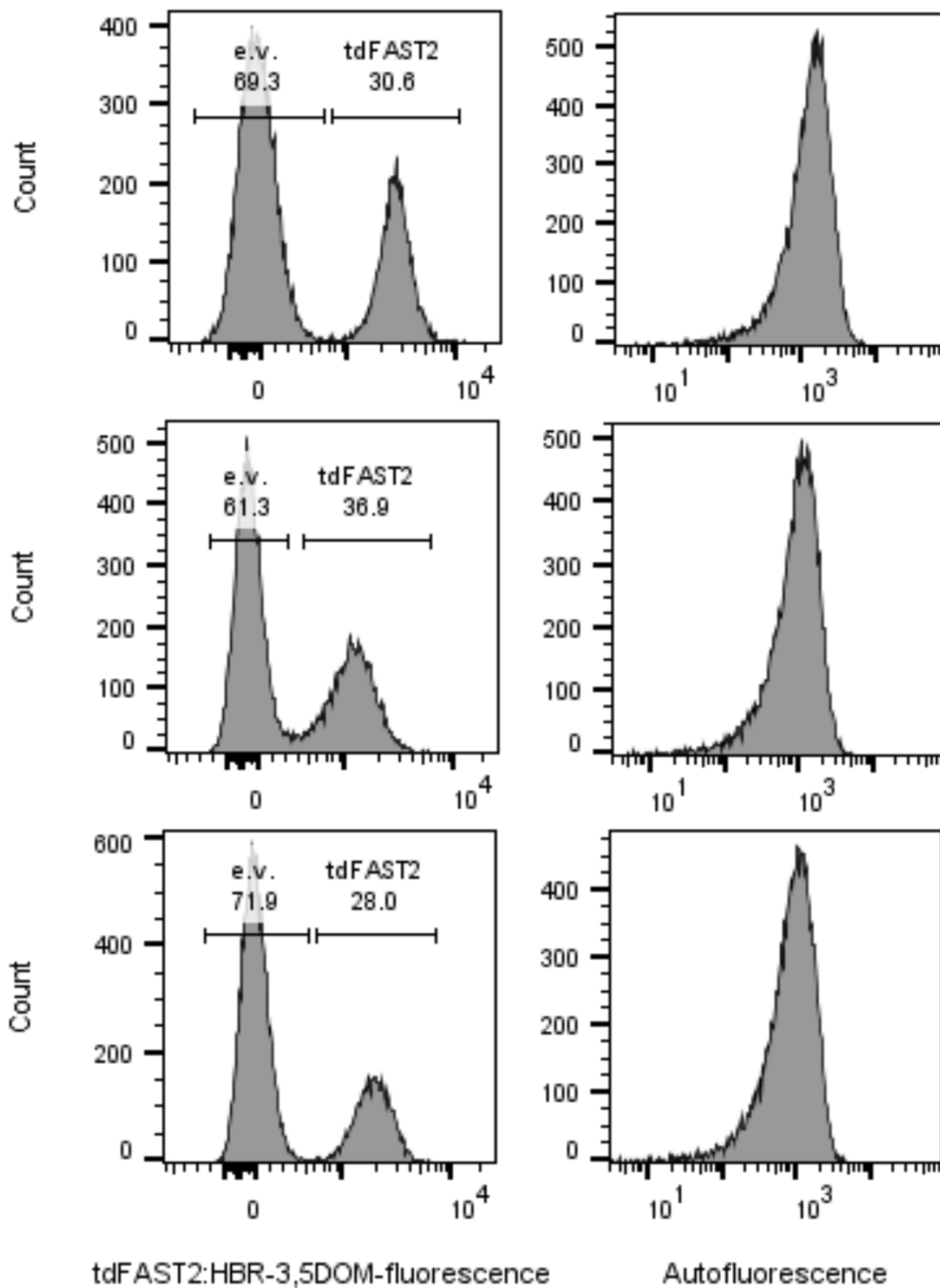
Supplementary Figure 1. tdFAST2-expressing methanogens show a specific fluorescent when the fluorogen HMBR is present.

Fluorescence of *M. acetivorans* and *M. maripaludis* cells is shown. **a)** Fluorescence spectrum upon excitation at 480 nm. **b)** Fluorescence at 540 nm when different excitation wavelengths are applied. Cells expressing tdFAST2 (tdFAST2) or control cells carrying an empty vector construct (e.v.) were analysed in presence (w/ fluorogen) or absence (w/o fluorogen) of HMBR. Used cells were in the stationary growth phase. Mean values and standard deviation of duplicates are shown.



Supplementary Figure 2. tdFAST2:fluorogen fluorescence can be detected in methanogens after 21 h exposure to oxygen.

M. acetivorans cells expressing tdFAST2 or harbouring an empty vector construct (e.v.) were analyzed in the presence of 5 μ M HBR-3,5DOM using a microplate reader. tdFAST2:HBR-3,5DOM fluorescence (λ Ex = 515 nm / λ Em = 590 nm) and autofluorescence (λ Ex = 420 nm / λ Em = 480 nm) was measured after storing the plate under aerobic conditions in the dark for 10 min and 21h.



Supplementary Figure 3. Flow cytometry provides reproducible results.

M. acetivorans cultures expressing tdFAST2 or harbouring an empty vector (e.v.) construct were mixed three times (top row, middle row, bottom row) from different cultures on different days in a ratio of 1:2 and analyzed with flow cytometry in the presence of 5 mM HBR-3,5DOM. Histograms show the HBR-3,5DOM-fluorescence and autofluorescence.

Supplementary File 1
Synthesized sequences

Synthesized fragment encoding codon-optimized tdFAST2 for expression in *M. acetivorans*

CATATGGAACACGTCGCGTTTGGCTCGGAAGATATTGAGAACACGTTAGCTAAAAATGGAT
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underlined = tdFAST2 protein-encoding sequence

CATATG = *NdeI* restriction site

GGATCC = *BamHI* site

Synthesized fragment encoding codon-optimized tdFAST2 for expression in *M. maripaludis*

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underlined = tdFAST2 protein-encoding sequence

bold = terminator sequence

ACTAGT = *SpeI* restriction site

CTGCAG = *PstI* site

Supplementary File 2

Plasmid sequences

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