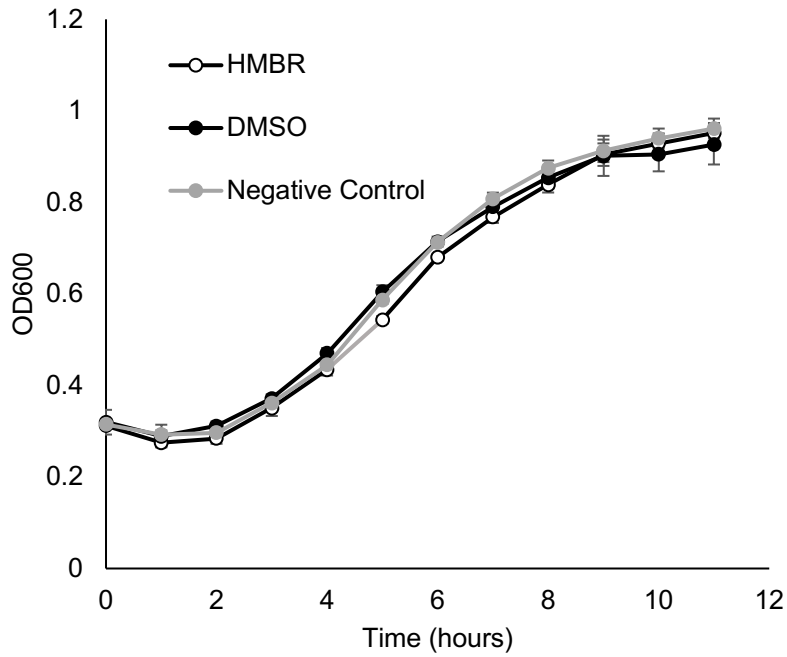
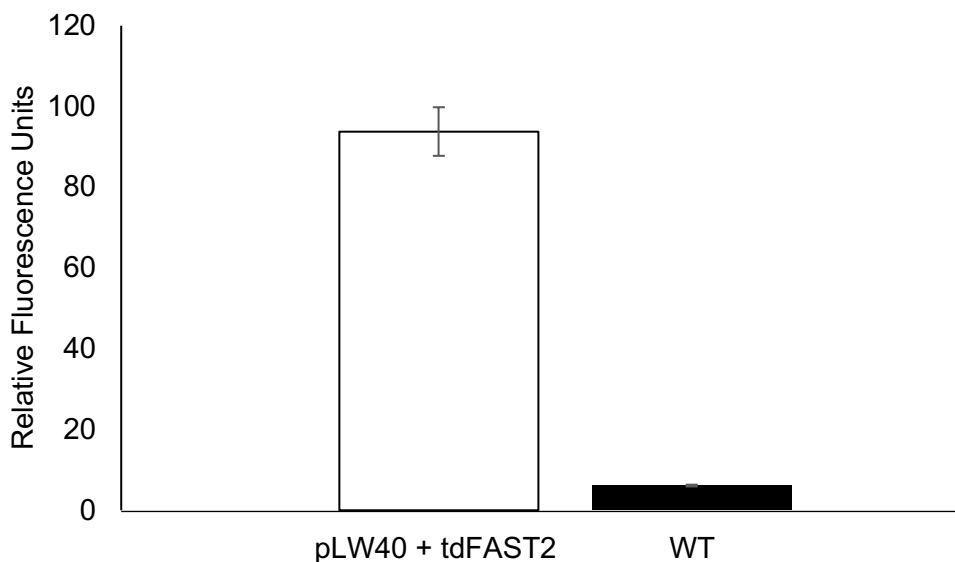


**Figure S1.** Autofluorescence of individual cells grown with either H<sub>2</sub> (grey lines) or formate (black lines) as the electron donor. Formate grown cultures tend to reach stationary phase around an OD<sub>600</sub> of 0.5 to 0.6, and H<sub>2</sub> grown cultures tend to reach stationary phase around an OD<sub>600</sub> of 0.9 to 1.0.



**Figure S2.** Growth of *M. maripaludis* expressing FAST1 after a 30-minute exposure to 20  $\mu$ M HMBR. Data are averages and standard deviations of triplicate cultures.



**Figure S3.** *M. maripaludis* expressing FAST is fluorescent upon HBR-3,5DOM addition. Fluorescence intensities of *M. maripaludis* strains cultivated in McCas medium with H<sub>2</sub> as the electron donor for growth. HBR-3,5DOM was added to a final concentration of 20  $\mu$ M. Relative fluorescence units were determined by normalizing emission readings from a microplate reader against baseline autofluorescence of the sample without fluorogen. Values were also normalized to the OD<sub>600</sub> of the culture. Data are averages and standard deviations of triplicate measurements.

<b>Table S1: Strains, plasmids, and primers used in study</b>		
<b>Strain</b>	<b>Description</b>	<b>Reference</b>
KC13	<i>M. maripaludis</i> strain JJ $\Delta$ upt (MMJJ_RS02980)	1
KC90	KC13 expressing <i>FAST1</i> on pLW40neo	This study
KC91	KC13 expressing <i>fruA</i> tagged with FAST1 on the C-terminus	This study
KC92	KC13 expressing <i>fruA</i> tagged with FAST1 on the N-terminus	This study
KC93	KC13 expressing <i>fdh1A</i> tagged with NFAST at the C-terminus and <i>fdh1B</i> tagged with CFAST at the C-terminus	This study
KC94	KC13 expressing <i>fdh1A</i> tagged with NFAST at the C-terminus and <i>mtd</i> tagged with CFAST at the C-terminus	This study
KC95	KC13 expressing <i>mtd</i> tagged with NFAST at the C-terminus and <i>fdh1B</i> tagged with CFAST at the C-terminus	This study
KC96	KC13 expressing tdFAST2 on pLW40neo	This study
KC97	KC13 expressing <i>flaI</i> tagged with tdFAST2 on pLW40neo	This study
<b>Plasmids</b>		<b>Reference</b>
pCRuptneo	pCRprtneo plasmid containing the <i>upt</i> gene	2
pLW40	Replicative expression vector containing puromycin and ampicillin resistance genes	3
pLW40neo	pLW40 with neomycin resistance cassettes	3
	<b>Sequence (5' - 3')</b>	<b>Notes</b>
<b>Primers used to generate KC90</b>		
MM_co_YFAST-F-Nsi	AAAAATGCATGGAACACGTTGCATTCCGG	NsiI
MM_co_YFAST_R-Asc	TTTTGGCGCGCCTTAAATCTTTTAACGA AAAC	AscI
<b>Primers used to generate KC91</b>		
Fru_YF_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CATATTGCACAAACCGCAACAAACCAG	NotI
Fru-YF-us-Rg	CCGAATGCAACGTGTTCCATGAATTCTC TTATTTCAACAACTTTTTTAGTCTG	
YFAST_F	ATGGAACACGTTGCATTCCGGATC	
YFAST_R	TTAAACTCTTTTAACGAAAACCCAG	
Fru_YF_ds_Fg	GTTTTCGTTAAAAGAGTTTAATAAATTCC TGAAAAGGAAAGTCTTG	

Fru_YF_ds_Rg	CTATAGGGCGAATTGGGCCCTCTAGAA ACTTTAAAGTCTATTTACAGAG	Xbal
<b>Primers used to generate KC92</b>		
Fru_YF_N_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CAAGGAACTATCGGAAGAAGCCTTGAA G	NotI
Fru_YF_N_us_Rg	CTGATCCGAATGCAACGTGTTCCATTTT ATTCACCTCCAAGGGTAATATGC	
YFAST_Fr_N_Fg	ATGGAACACGTTGCATTCCGATCAG	
YFAST_Fr_N_Rg	TGATCCTCCTCCTCCTGATCCTCCTCCT CCTGAAACTCTTTTAACGAAAACCCAGT ATG	
Fru-YF-N-ds-Fg	TCAGGAGGAGGAGGATCAGGAGGAGG AGGATCAGTGGCAGAACCTGTAACATC AG	
Fru-YF-N-ds-Rg	CTATAGGGCGAATTGGGCCCTCTAGAG TGATGTTGGTTCTCATTCCACCAAC	Xbal
<b>Primers used to generate KC93</b>		
Fdh1B_CF_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CCTGAAAAAGGTAAGCAGTTACTTG	NotI
Fdh1B_CF_us_Rg	CCTCCTGATCCTCCTCCTCCTGATTGAG TTGGGCATGACCCTCCAAG	
CFAST_10_F	TCAGGAGGAGGAGGATCAGGAGGAGG AGGATCAGGAGACTCATACTGGGTTTTC G	
Fdh1B_CFAST_10_R	CAGAACTAAAAAATTAATAATAAATAA TTAATAATAATTTATTATCTTTAACGAA AACCCAG	
Fdh1B_CF_ds_Fg	TAAATTATTTATTAATTATTTATTTTTAA TTTTTTAGTTCTG	Xbal
Fdh1B_CF_ds_Rg	TATAGGGCGAATTGGGCCCTCTAGAAC ATAATTCATGTTTTAGGATATGAAG	
Fdh1A_NF_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CAAAGACTTGGTGTAGATGGACT	NotI
Fdh1A_NF_us_Rg	CTCCTCCTGATCCTCCTCCTCCTGATAT TTTTCCACCTTTGCAGCACATA	
NFAST_Fg	TCAGGAGGAGGAGGATCAGGAGGAGG AGGATCAATGGAACACGTTGCATTCCG ATC	
NFAST_Rg	TGATAATGCTTTTTTCATGTG	
Fdh1A_NF_ds_Fg	CACATGAAAAAAGCATTATCATAATTTTC CCAAAATCGGGATTTTAAACGAG	
Fdh1A_NF_ds_Rg	TATAGGGCGAATTGGGCCCTCTAGAA CGATTTCTTCTTTTACAACG	Xbal

<b>Primers used to generate KC94</b>		
Fdh1A_NF_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CAAAGACTTGGTGTAGATGGACT	NotI
Fdh1A_NF_us_Rg	CTCCTCCTGATCCTCCTCCTCCTGATAT TTTTCCACCTTTGCAGCACATA	
NFAST_Fg	TCAGGAGGAGGAGGATCAGGAGGAGG AGGATCAATGGAACACGTTGCATTCGG ATC	
NFAST_Rg	TGATAATGCTTTTTTTCATGTG	
Fdh1A_NF_ds_Fg	CACATGAAAAAAGCATTATCATAATTTTC CCAAAATCGGGATTTTAAACGAG	
Fdh1A_NF_ds_Rg	TATAGGGCGAATTGGGCCCTCTAGAAT CGATTTCTTCTTTTACAACG	XbaI
Mtd_CF_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CGATACGTTTTAATCAAATGCGAC	NotI
Mtd_CF_us_Rg	CTCCTGATCCTCCTCCTCCTGATTCTGG TTTTGTCATTAATTTACATTTG	
CFAST_10_F	TCAGGAGGAGGAGGATCAGGAGGAGG AGGATCAGGAGACTCATACTGGGTTTTTC G	
Mtd_CFAST_10_R	GGTAAATTTTTTAATTTAAATGTAATTT ATTATCTTTTAAACGAAAACCCAGTATGA G	
Mtd_CF_ds_Fg	TAAATTTACATTTAAATTAATAAATTTAC C	
Mtd_CF_ds_Rg	TATAGGGCGAATTGGGCCCTCTAGATTA ATTGGAGTATGGTTTGCAATAG	XbaI
<b>Primers used to generate KC95</b>		
Mtd_NF_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CGATACGTTTTAATCAAATGCGAC	
Mtd_NF_us_Rg	CTCCTGATCCTCCTCCTCCTGATTCTGG TTTTGTCATTAATTTAC	
NFAST_Fg	TCAGGAGGAGGAGGATCAGGAGGAGG AGGATCAATGGAACACGTTGCATTCGG ATC	
NFAST_Rg	TGATAATGCTTTTTTTCATGTG	
Mtd_NF_ds_Fg	CACATGAAAAAAGCATTATCATAAATTTA CATTTAAATTAATAAATTTACC	
Mtd-NF-ds-Rg	TATAGGGCGAATTGGGCCCTCTAGATTA ATTGGAGTATGGTTTGCAATAG	
Fdh1A_NF_us_Fg	TGCAGATATCCATCACACTGGCGGCCG CAAAGACTTGGTGTAGATGGACT	NotI
Fdh1A_NF_us_Rg	CTCCTCCTGATCCTCCTCCTCCTGATAT TTTTCCACCTTTGCAGCACATA	

NFAST_Fg	TCAGGAGGAGGAGGATCAGGAGGAGG AGGATCAATGGAACACGTTGCATTCCG ATC	
NFAST_Rg	TGATAATGCTTTTTTCATGTG	
Fdh1A_NF_ds_Fg	CACATGAAAAAAGCATTATCATAATTTTC CCAAAATCGGGATTTTAAACGAG	
Fdh1A_NF_ds_Rg	TATAGGGCGAATTGGGCCCTCTAGAAT CGATTTCTTCTTTTACAACG	Xbal
<b>Primers used to generate KC96</b>		
Tdf2_plw40_mm_fg_2	GATAACTAATAGGTGAAATGCATGGAAC ACGTTGCATTCCGGATCAGAAGACATC	Ascl
Tdf2_plw40_mm_rg	CCTCCTCCTGATCCTCCTCCAACCTTTTTAA CGAAAACCCAGTATGAGTCTCCTGATAAT	
Tdf2_plw40_mj_fg	GGAGGAGGATCAGGAGGAGGAGAACACG TTGCTTTTGGATCAGAAGATATTGAAA AT	
Tdf2_plw40_mj_rg	ACAGATCTCCTAGGCGCGCCTTAAACTC TTTTAACAAAAACC	Nsil
<b>Primers used to Generate KC97</b>		
Flal-tdf2-us-Fg	TGCAGATATCCATCACACTGGCGGCCG CAAGGAGCTGTTGCTTTCCAGGCAATG C	NotI
Flal-TDF2-us-Rg	CATAGATCCTCCTCCACCTGAACCTCCT CCTCCAGAAACCTGGAATGGCAGTCCT TCC	
tdf2-F	TCTGGAGGAGGAGGTTTCAGGTGGAGGA GGATCTATGGAACACGTTGCATTCGGAT CAGAAGACATC	
tdf2-R	TTAAACTCTTTTAAACAAAAACCCAATATG AATCTCCTGATAAA	
Flal-TDF2-ds-Fg	TTCATATTGGGTTTTTGTAAAAGAGTTT AATAAGGTGTTTCTTATGTTTTTTGATAT AC	
Flal-YF-ds-Rg	CTATAGGGCGAATTGGGCCCTCTAGATT AATTCCTGACCGCTGTCTATTG	Xbal

**Table S2.** Nucleotide sequences of FAST1 and tdFAST2 used in this study

>FAST1

ATGGAACACGTTGCATTCCGGATCAGAAGACATCGAAAACACATTAGCAAAAATGGACGACG  
GACAATTAGACGGATTAGCATTCCGGAGCAATCCAATTAGACGGAGACGGAAACATCTTACA  
ATACAACGCAGCAGAAGGAGACATCACAGGAAGAGACCCTAAACAAGTTATCGGAAAAAAC  
TTCTTCAAAGACGTTGCACCTGGAACAGACTCACCTGAATTCTACGGAAAATTCAAAGAAG  
GAGTTGCATCAGGAAACTTAAACACAATGTTCGAATGGATGATCCCTACATCAAGAGGACC  
TACAAAAGTTAAAGTTCACATGAAAAAAGCATTATCAGGAGACTCATACTGGGTTTTCGTTA  
AAAGAGTTTAA

>tdFAST2

ATGGAACACGTTGCATTCCGGATCAGAAGACATCGAAAACACATTAGCAAAAATGGACGACG  
GACAATTAGACGGATTAGCATTCCGGAGCAATCCAATTAGACGGAGACGGAAACATCTTACA  
ATACAACGCAGCAGAAGGAGACATCACAGGAAGAGACCCTAAACAAGTTATCGGAAAAAAC  
TTCTTCAAAGACGTTGCACCTGGAACAGACTCACCTGAATTCTACGGAAAATTCAAAGAAG  
GAGTTGCATCAGGAAACTTAAACACAATGTTCGAATGGATGATCCCTACATCAAGAGGACC  
TACAAAAGTTAAAATCCACATGAAAAAAGCATTATCAGGAGACTCATACTGGGTTTTCGTTA  
AAAGAGTTGGAGGAGGATCAGGAGGAGGAGAACACGTTGCTTTTGGATCAGAAGATATTG  
AAAATACATTAGCTAAAATGGATGATGGACAATTAGATGGATTAGCTTTTGGAGCTATTCAA  
TTAGATGGAGATGGAAATATTTTACAATATAATGCTGCTGAAGGAGATATTACAGGAAGAGA  
TCCAAAACAAGTTATTGGAAAAATTTTTTAAAGATGTTGCTCCAGGAACAGATTCACCAG  
AATTTTATGGAAAATTTAAAGAAGGAGTTGCTTCAGGAAATTTAAATACAATGTTTGAATGGA  
TGATTCCAACATCAAGAGGACCAACAAAAGTTAAAATTCACATGAAAAAAGCTTTATCAGGA  
GATTCATATTGGGTTTTTGTAAAAGAGTTTAA



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