Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Supplementary Table 1

Characteristics of mglB-derived glucose sensors

Clone	Mutation	**Dissociation constant, K_d (μM)**
gT112	truncation	0.5
gT112M37	truncation, L238M	284
gT112M44	truncation, Q45L, K223R	1.1
gT112M179	truncation, K113N	159
gT112M183	truncation, D154H	53
gT112M192	truncation, D50E, K58I, S229C	0.3

Supplementary Table 2

Characteristics of PdhR-derived pyruvate sensors

	Mutation	Dissociation constant, K_d (μM)
pT162	Truncation	410
pT162M57	Truncation, D92A	n.a.
pT162M60	Truncation , A217V	n.a.
pT162M61	Truncation, D92A	710
pT162M100	Truncation, A112T	2500
pT162M104	Truncation, S77N	390
pT162M115	Truncation, V188D, V198M, S245T	n.a.
pT162M156	Truncation, S83G, N163K	70
pT162M176	Truncation, C172Y, D92G	330

Supplementary Table 3

Characteristics of TbpA-derived thiamine sensors

Clone	Mutation	**Dissociation constant, K_d (μM)**
TbpA	-	1.30
tM51	K19N, S218P	1.90
tM110	P158S, G35R	0.17
tM152	S91N, D121N, Q134H, K228N	0.06
tM172	Q196S, N279D	1.20

Supplementary Table 4

List of primers used in this work

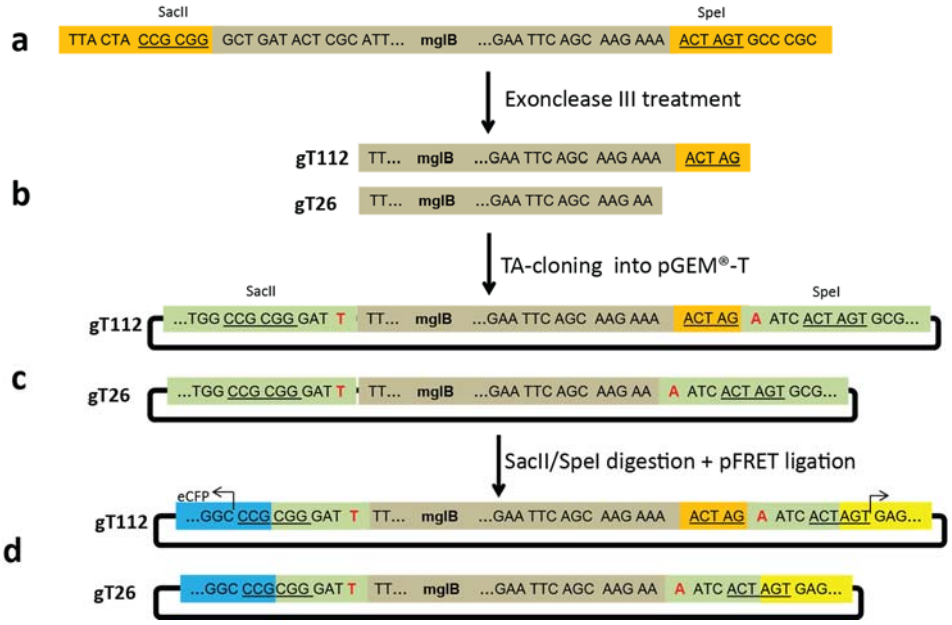
	Forward Primer (5' - 3')	Reverse Primer (5' - 3')
Addition of SacII site to pGWF1*	GTCGACGGTACCCCGCGGATGCAAAATCGGCTGACC	GCACCCCGCCGACTAGTGGACAGGGTGGCGG-3
Removal of 3 residues from the N-terminus of Venus in pGWF1	ATCAACACTAGTGAGCTGTTCACCGGGGTGGT	GTGACAGGGCCCTTACTTGTACAGCTCGTCCA
Cloning of mglB into pFRET and generation of truncation library	TTACTACCGCGGTGCTGATACTCGCATTGGTGT	GCGGGCACTAGTTTTCTTGCTGAATTCAGCCA
Cloning of TbpA into pFRET and generation of truncation library	TCGGTCCCGCGGTAAACCCGTTCTGACTGTTTATAC	ATAGATACTAGTACGGCTGACGGCGCGTTGCCATTC
Cloning of PdhR into pFRET and generation of truncation library	ATAATACCGCGGTatgGCCTACAGCAAAATCCGCC	ACGCGCACTAGTATTCTTTCGTTGCTCCAGACGAC
Cloning of pyruvate sensor constructs into pcDNA^{TM}3.1(+)	ATACTAGGTACCATGGCTAGCATGACTGGTG	GGACGAGCTGTACAAGTAAGAATTCGATCTG
Generation of mutation libraries	TCGAGGTGAGCAAGGGCGAGGAG	GGATCACTCTCGGCATGGACGAGC

* using pGWF1 containing the gene TreR (described in Peroza et. al., Anal Biochem., 2015)

Supplementary Figure 1

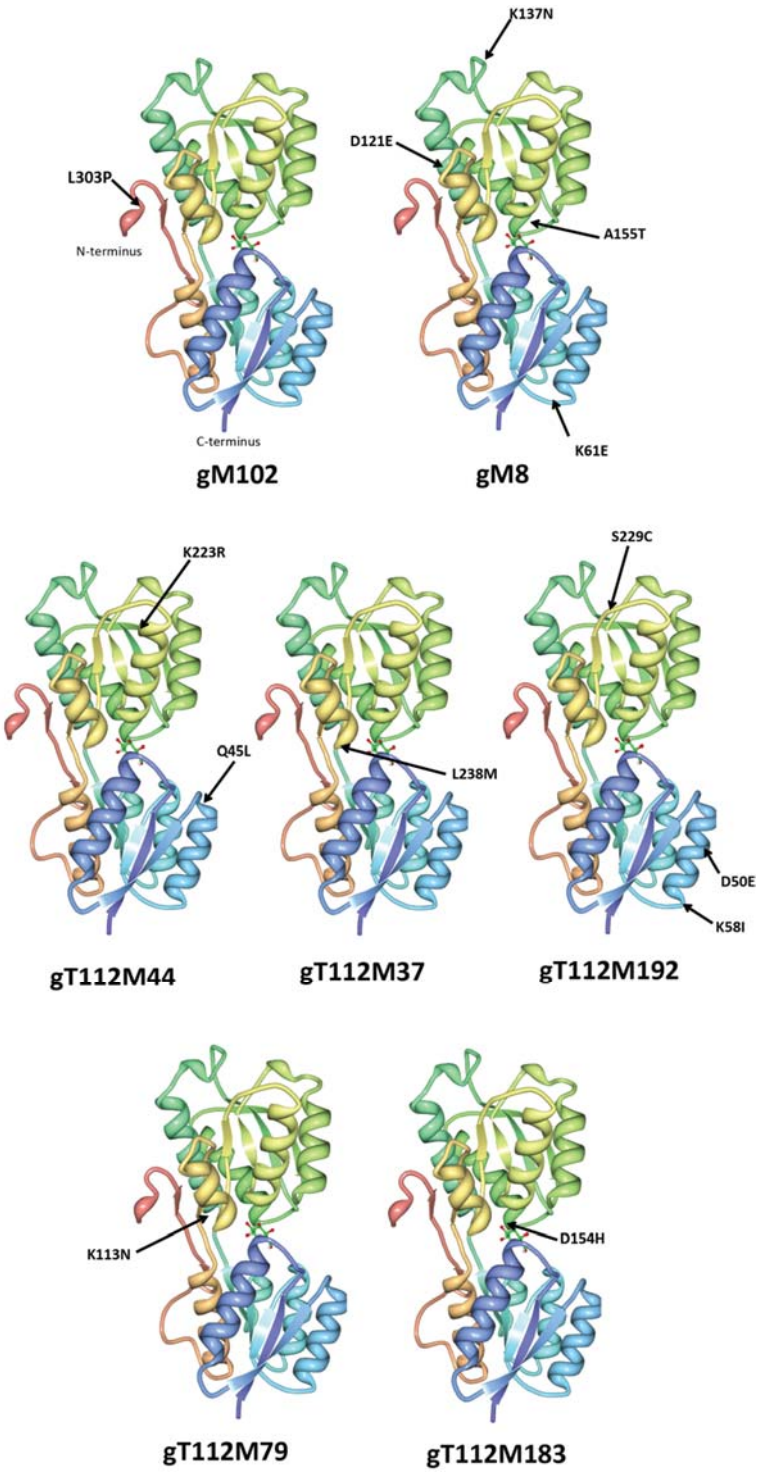
Detailed information about the generation of truncated versions of the mglB

(**a**) Sequence of the PCR product indicating the restriction sites SacII and SpeI and additional nucleotides introduced by the primers used for amplification (shaded in orange). (**b**) Sequences of the truncated versions of mglB found in clones 2B4 and 1C2 after treatment with exonuclease III and mung bean nuclease. (**c**) Product of the ligation of the digested amplicons into the pGEM®-T vector FRET. The nucleotides adenine and the thiamine that were added by TA-cloning are indicated in red. The restriction sites SacII and SpeI from pGEM®-T are underlined (**d**) Product of the subcloning of the truncated versions of mglB into pFRET using the SacII and SpeI sites.



Supplementary Figure 2

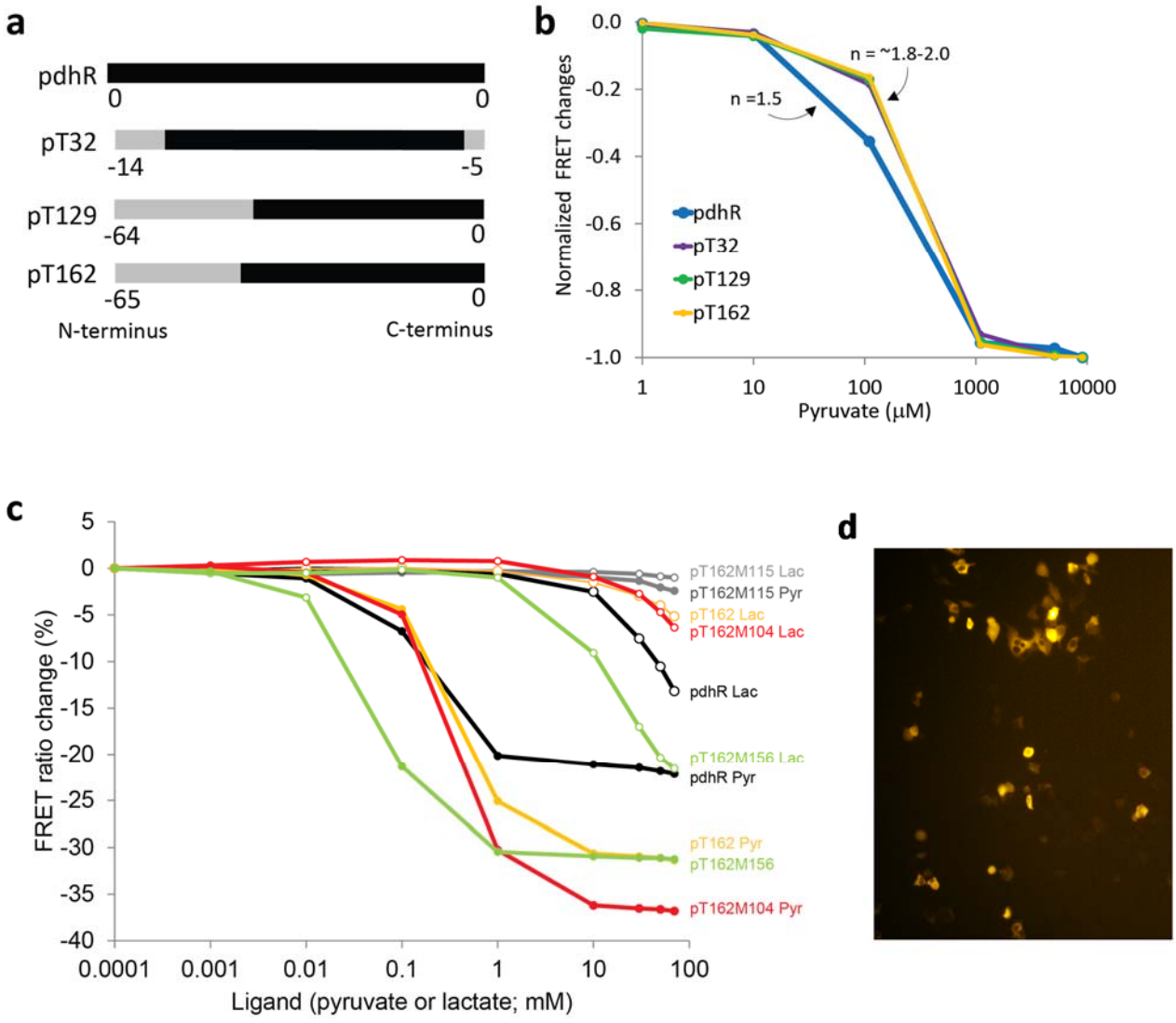
Mutations occurring the MglB derivatives. Structure images were generated using data reported by Borrok et al. (Protein Sci 16 (6), 1032 (2007)) and the Protein *Workshop Viewer* from protein data bank (www.pdb.org).



Supplementary Figure 3

Characterization of candidate pyruvate FRET sensors.

(**a**) Sequence of three derivatives obtained by truncation of PdhR with best in vitro FRET response to pyruvate upon insertion in pFRET. (**b**) Normalized titration plots of full-length and the three best truncated constructs. The estimated Hill coefficients *n* are indicated. **(c)** In vitro FRET response for candidate sensors for pyruvate (closed circles) and lactate (open circles) titration. The color is unique for each sensor. (**d**) Representative FRET-channel image of MCF-7 cells expressing the pyruvate sensor pT162M104.



Supplementary Figure 4

Mutations occurring the TbpA derivatives. Structure images were generated using data reported by Soriano et al. (Biochemistry-US 47 (5), 1346 (2008)) and the Protein *Workshop Viewer* from protein data bank (www.pdb.org).