

OzTracs: Optical Osmolality Reporters Engineered from Mechanosensitive Ion Channels

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SUPPLEMENTAL FIGURES

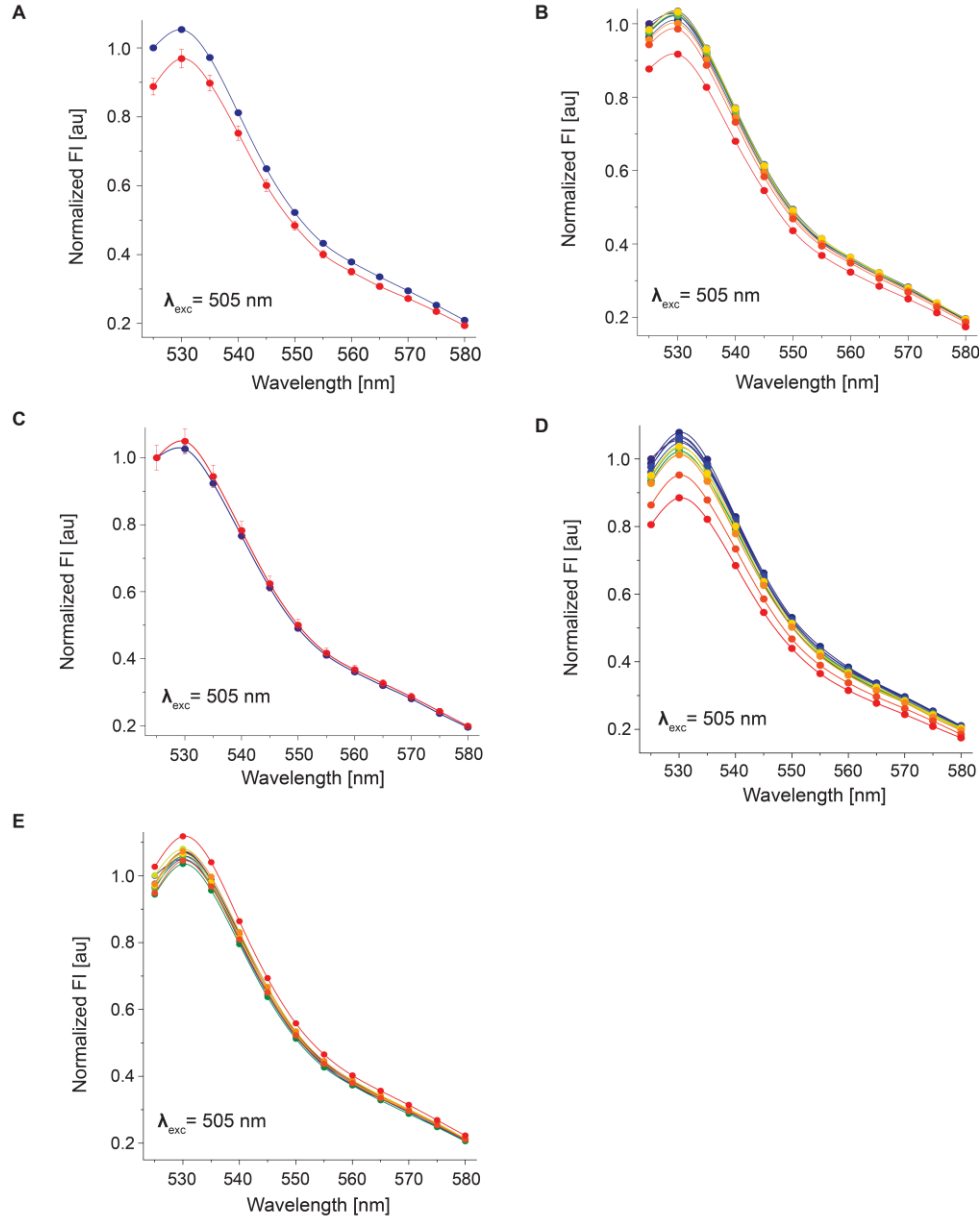


Figure S1. Acceptor fluorophore emission spectra under direct excitation (AxAm). **(A)** Emission spectra of yeast cells expressing OzTrac-MscL-36 exposed to buffer with (blue) or without NaCl (red) at 505 nm excitation. Control for Figure 2A. **(B)** Concentration-dependent effects of NaCl treatment on yeast expressing OzTrac-MscL-36 at 505 nm excitation. Control for Figure 2C. **(C)** Emission spectra of yeast cells expressing OzTrac-MSL10-34 exposed to buffer with (blue) or without NaCl (red) at 505 nm excitation. Control for Figure 3A. **(D)** Concentration-dependent effects of NaCl treatment on yeast expressing OzTrac-MSL10-34 at 505 nm excitation. Control for Figure 4A. **(E)** Concentration-dependent effects of glycerol treatment on yeast expressing OzTrac-MSL10-34 at 505 nm excitation. Control for Figure 4C.

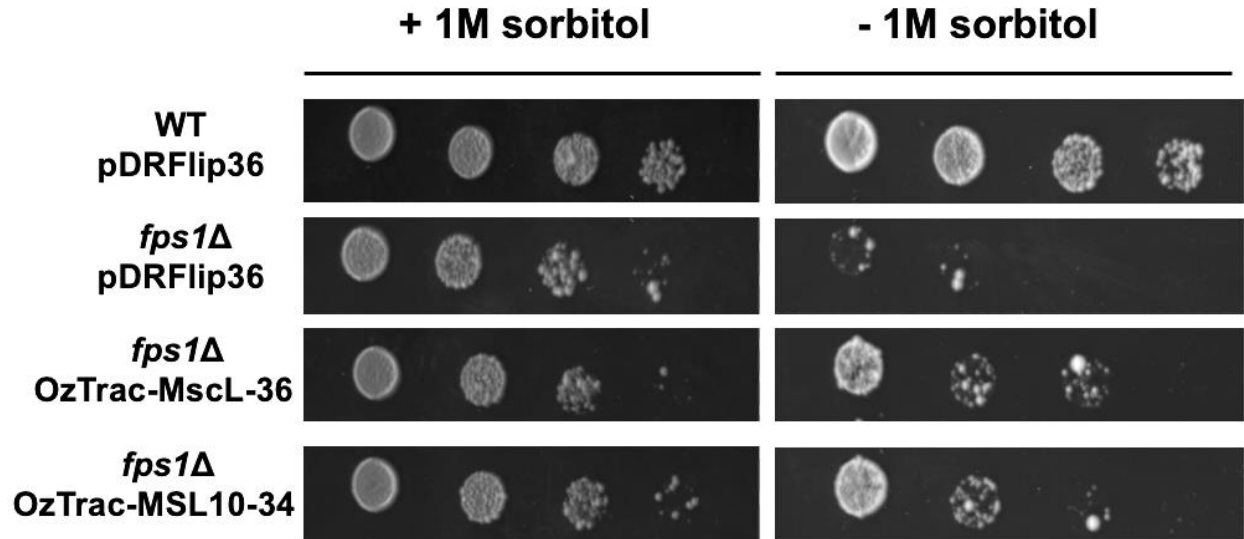


Figure S2. Partial suppression of the growth phenotype of the yeast plasma membrane aquaglyceroporin mutant *fps1Δ*. of expression of pDRFLIP36 (vector alone), OzTrac-MscL-36 or OzTrac-MSL10-34 in the yeast plasma membrane aquaglyceroporin mutant *fps1Δ* (BY4743). Cells were grown in liquid medium with 1 M sorbitol and then 1:10 serial dilution of yeast cultures were pipetted onto agar plates with (left panel) or without sorbitol (right panel) to expose cells to hypoosmotic conditions.

SUPPLEMENTAL TABLES

Table S1. Fluorophore pairs in pDRFLIP destination vectors and observed DxAm/DxDm ratios when treated with 1 M NaCl. Higher values correspond to more intense red coloration.

pDRFLIP #	FRET donor	FRET acceptor	DxAm/DxDm Response to 1 M NaCl	
			<i>EcMscL</i>	<i>AtMSL10</i>
30	mCer	AFPt9	1.28	1.37
32	t7CFPt9	AFPt9	0.97	1.36
34	t7TFPt9	AFPt9	ND	1.65
35	TFPt9	AFPt9	ND	1.50
36	Cer	AFPt9	1.45	ND
37	Cer	Cit	1.30	ND
38	sCer	Cit	0.90	1.00
39	t7sCFPt9	sAFPt9	1.03	ND
42	mCer	Cit	ND	1.52
43	sCer	sAFPt9	1.20	ND
48	mTrq2	AFPt9	1.28	ND
49	t7mTrq2t9	AFPt9	1.20	1.40
50	t7sTrq2t9	sAFPt9	ND	1.20
51	sTrq2	sAFPt9	ND	1.05

At - *A. thaliana*, *Ec* - *E. coli*, ND - not determined.

Table S2. Candidate proteins for a FRET membrane tension sensor.

Candidate sensory protein (gene identifier)	Ref.
<i>EcMscL</i> (JW3252) mechanosensitive channel of large conductance	40
<i>AtMSL10</i> (At5g12080) mechanosensitive channel of small conductance-like 10	5
<i>AtAHK1</i> (At2g17820) protein histidine kinase 1	41
<i>AtOSCA1.1</i> (At4g04340) hyperosmolality-gated calcium-permeable channel 1.1	42

At - *A. thaliana*, *Ec* - *E. coli*, Ref – reference