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Protocol

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Supplementary Information

Comprehensive multiscale analysis of lactate metabolic dynamics *in vitro* and *in vivo* using highly responsive biosensors

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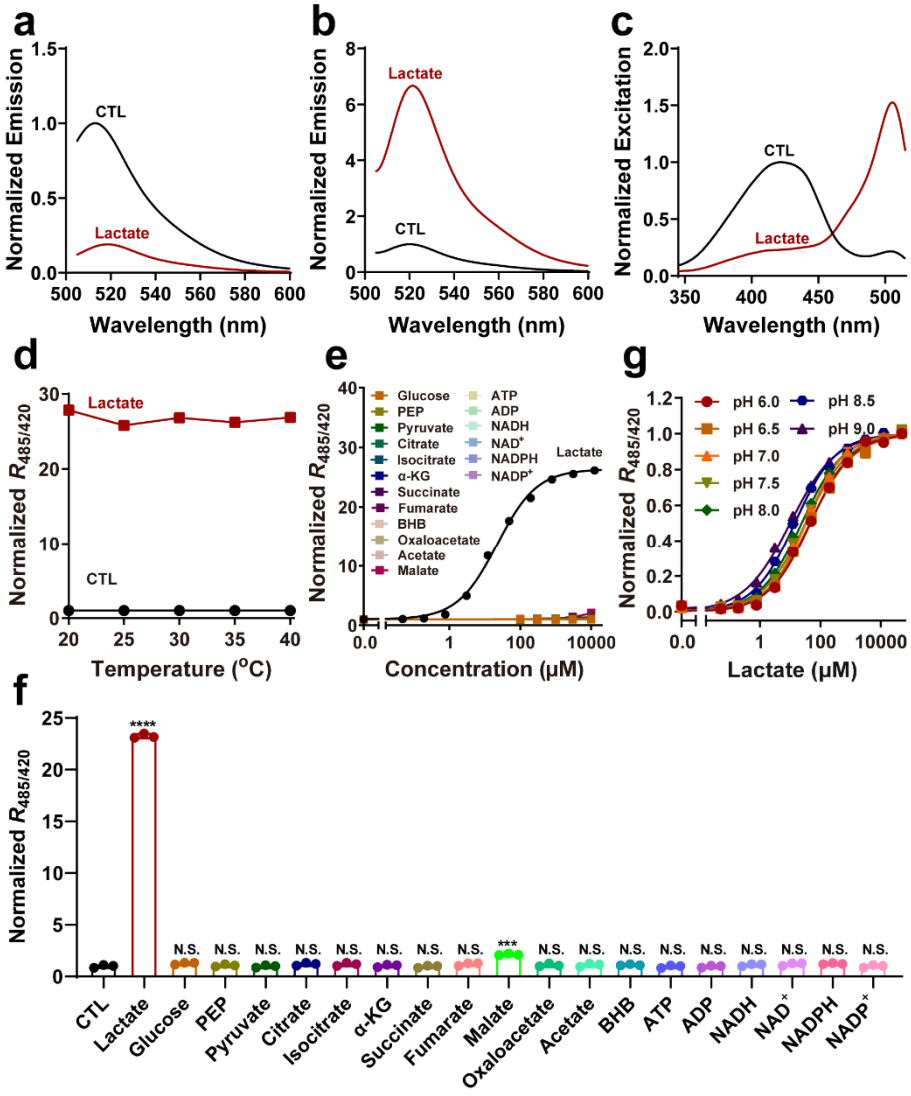
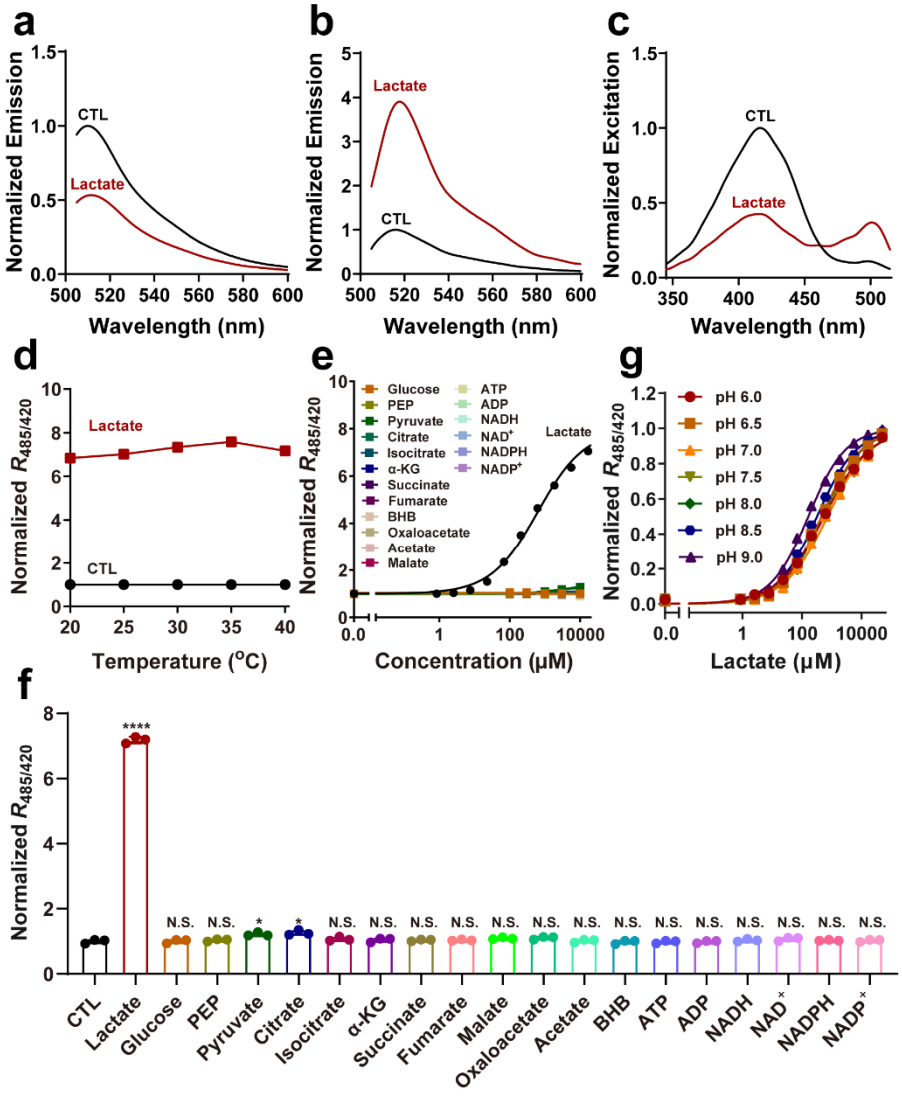


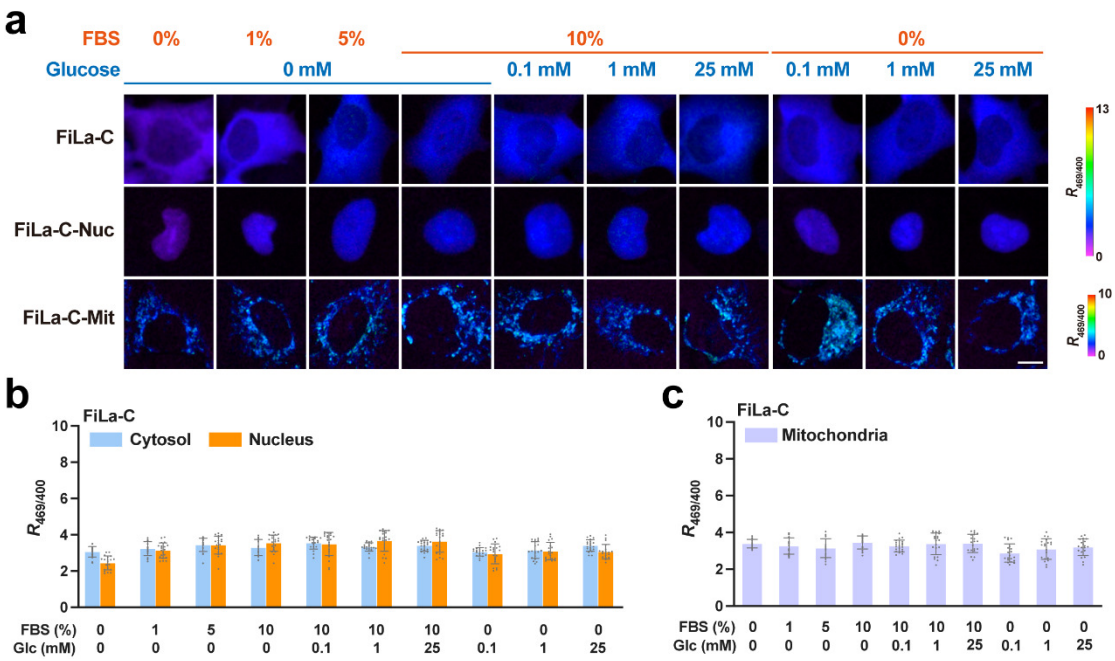
Supplementary Fig. 1 Characterization of lactate biosensor FiLa-H.

a-**c**, Emission spectra (a, b) and excitation spectra (c) of purified FiLa-H in the control condition (black), and saturated lactate (dark red), normalized to the peak intensity in the control condition. Excitation wavelength was fixed at 420 nm (a) or 490 nm (b), respectively. The excitation spectrum of FiLa-H recorded at an emission wavelength of 530 nm has maxima around 420 and 500 nm. **d**, Fluorescence response of FiLa-H to 50 mM lactate at various temperatures (n=3). **e**, Fluorescence response of FiLa-H at the indicated concentrations of lactate or other metabolites. Data are normalized to the initial value (n=3). PEP, phosphoenolpyruvate; α-KG, α-ketoglutarate; BHB, β-hydroxybutyrate. **f**, Fluorescence response of FiLa-H to lactate or other metabolites at 10 mM (n=3). **g**, Lactate titration curves of FiLa-H at the indicated pH. Data are the mean ± SEM (d-g). All *p* values were obtained using unpaired two-tailed Student's t test. ***$p < 0.001$, ****$p < 0.0001$, N.S., not significant.



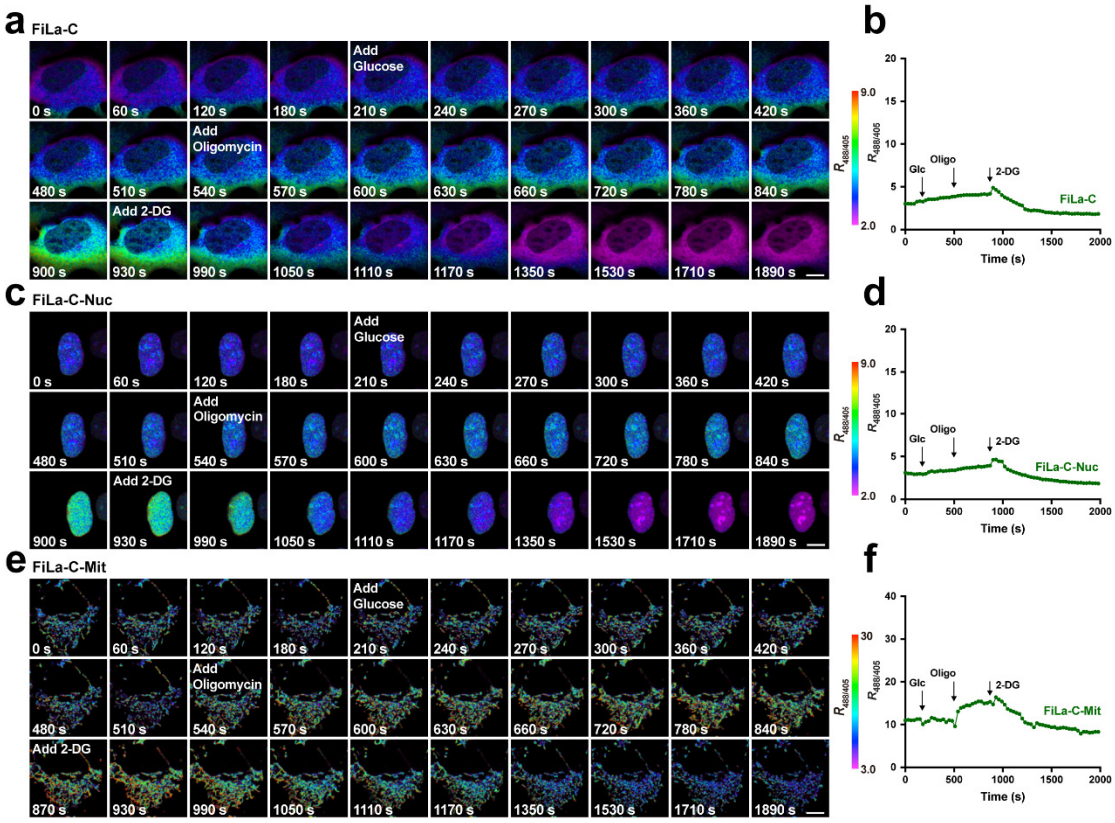
Supplementary Fig. 2 Characterization of lactate biosensor FiLa-L.

a-**c**, Emission spectra (a, b) and excitation spectra (c) of purified FiLa-L in the control condition (black), and saturated lactate (dark red), normalized to the peak intensity in the control condition. Excitation wavelength was fixed at 420 nm (a) or 490 nm (b), respectively. The excitation spectrum of FiLa-L recorded at an emission wavelength of 540 nm has maxima around 420 and 500 nm. **d**, Fluorescence response of FiLa-L to 50 mM lactate at various temperatures (n=3). **e**, Fluorescence response of FiLa-L at the indicated concentrations of lactate or other metabolites. Data are normalized to the initial value (n=3). PEP, phosphoenolpyruvate; α-KG, α-ketoglutarate; BHB, β-hydroxybutyrate. **f**, Fluorescence response of FiLa-L to lactate or other metabolites at 10 mM (n=3). **g**, Lactate titration curves of FiLa-L at the indicated pH. Data are the mean ± SEM (d-g). All *p* values were obtained using unpaired two-tailed Student's t test. **p* < 0.05, *****p* < 0.0001, N.S., not significant.



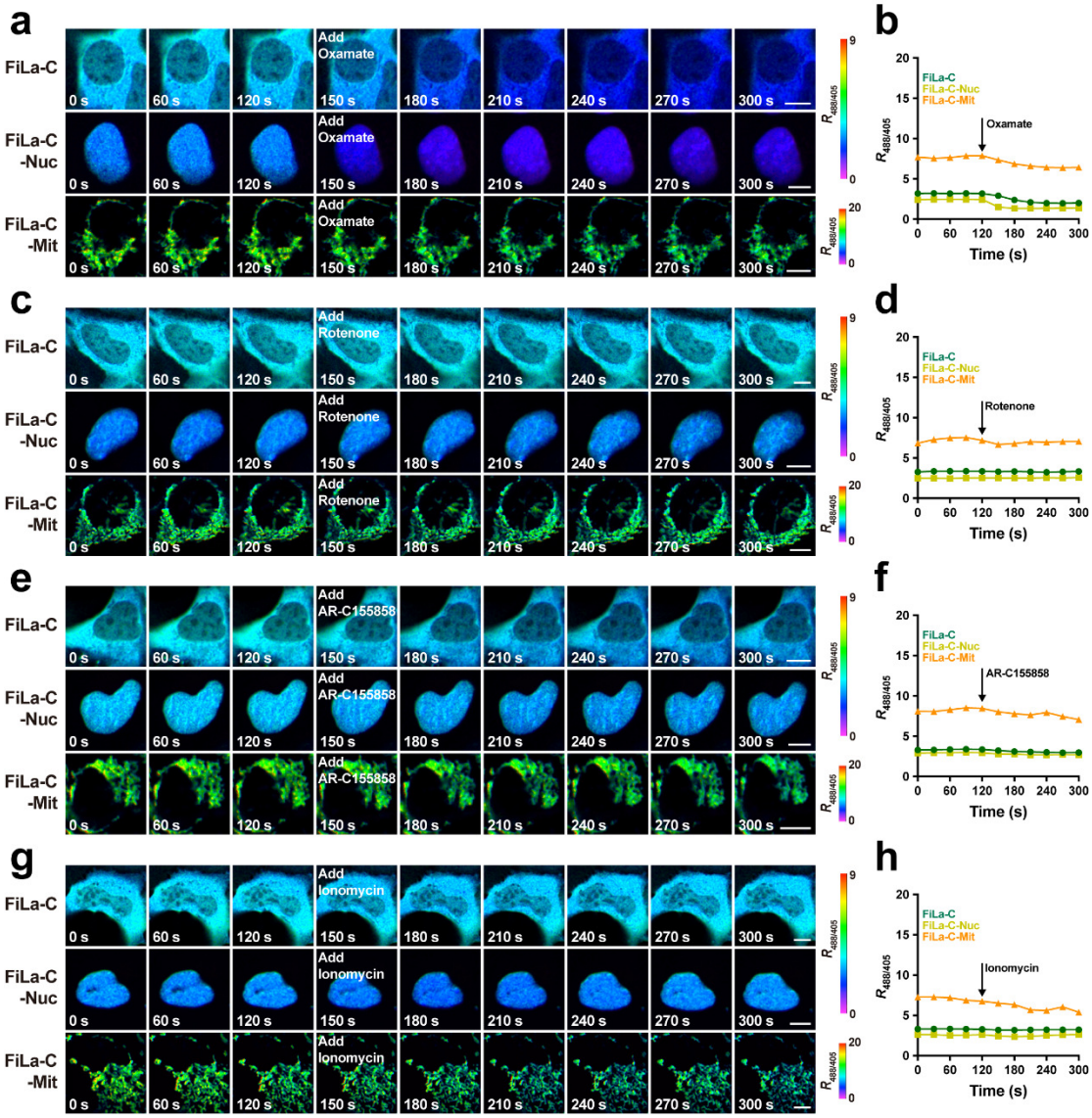
Supplementary Fig. 3 Fluorescence response of FiLa-C in live cells to different nutritional conditions.

a-c, Fluorescence images (a) and quantification (b, c, n=20) of FiLa-C expressed in cytosol (top), nucleus (middle) or mitochondria (bottom) of HEK-293. Cells were cultured in different levels of glucose (0, 0.1, 1 and 25 mM) and fetal bovine serum (FBS) (0, 1%, 5% and 10%). Scale bars, 10 μm. Data are presented as the mean ± SD (b, c).



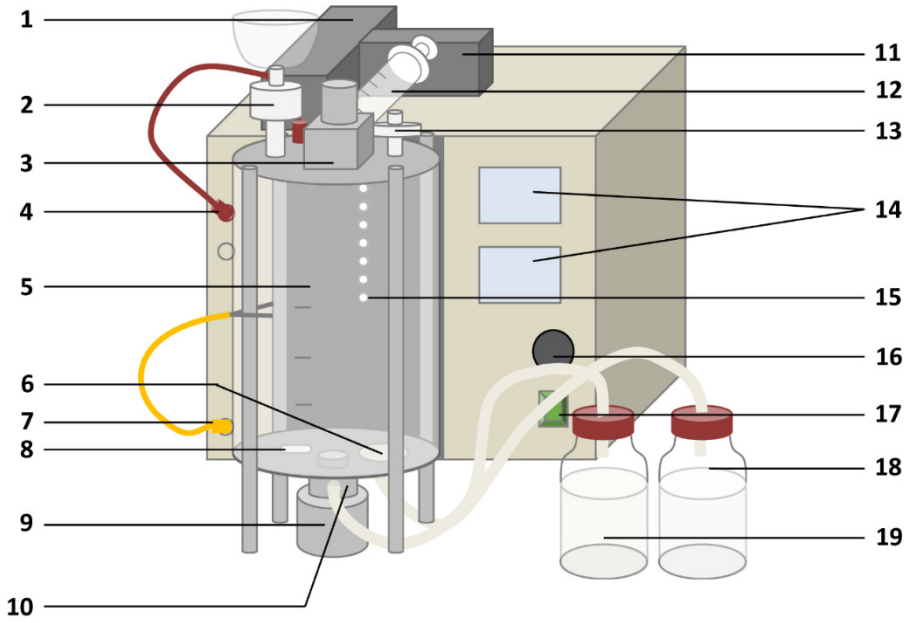
Supplementary Fig. 4 Fluorescence response of FiLa-C in live cells to three chemicals.

a-f, Fluorescence images (a, c, e) and quantification (b, d, f) of FiLa-C expressed in cytosol (a, b), nucleus (c, d) or mitochondria (e, f) of HEK-293. Cells were treated with 1 mM glucose, 1 μM oligomycin and 50 mM 2-DG successively at the indicated time. Scale bars, 10 μm.



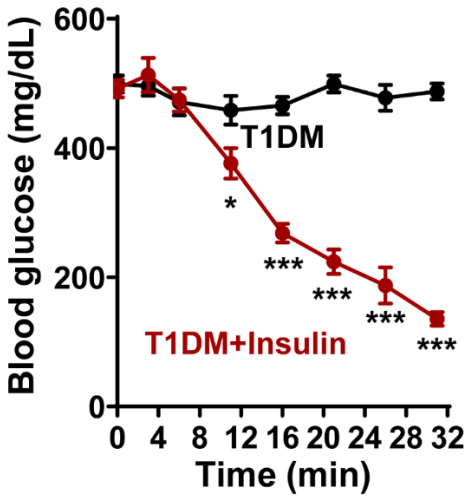
Supplementary Fig. 5 Fluorescence response of FiLa-C in live cells to four chemicals.

a-**h,** Fluorescence images (a, c, e, g) and quantification (b, d, f, h) of FiLa-C expressed in the cytosol (top), nucleus (middle) or mitochondria (bottom) of HEK-293 cells. Cells were treated with 10 mM oxamate (a, b), 10 µM rotenone (c, d), 2 µM AR-C155858 (e, f) or 2 µM ionomycin (g, h). Scale bars, 10 µm.



Supplementary Fig. 6 Schematic representation of the Encapsulator B-395 Pro system.

1 Liquid flow regulating valve, **2** Liquid filter, **3** Bead producing unit (with a nozzle), **4** EDU (Voltage outlet), **5** Glass cylinder of reaction vessel, **6** Filter of drain line, **7** Plug for grounding wire, **8** Magnetic stirrer bead, **9** Magnetic stirrer, **10** Harvesting valve, **11** Syringe pump, **12** Vibration unit, **13** Air filter, **14** Control panel (upper for vibration frequency & electrode, lower for syringe pump, magnetic stirrer control & pressure indication), **15** Stroboscope lamp, **16** Pressure regulating valve, **17** Mains switch, **18** Bead collecting bottle, **19** Liquid waste bottle. The reaction unit includes 2, 3, 5, 6, 10, 13, 18, 19. The control unit includes 1, 4, 7, 8, 9, 11, 12, 14, 15, 16, 17.



Supplementary Fig. 7 Blood glucose monitoring of T1DM mice by insulin therapy.

Insulin (1.25 units/kg) was injected via intraperitoneal injection and blood glucose was measured (n=5 mice). Data are the mean ± SEM. All *p* values were obtained using unpaired two-tailed Student's t test. **p* < 0.05, ****p* < 0.001. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of East China University of Science and Technology.

Tai Chi athletes					Rugby athletes				
No.	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	No.	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)
1	20	180	72	22.2	1	22	187	93	26.6
2	20	170	65	22.5	2	17	184	65	19.2
3	20	163	55	20.7	3	17	185	70	20.5
4	20	180	85	26.2	4	20	178	75	23.7
5	21	184	95	28.1	5	17	183	63	18.8
6	22	179	75	23.4	6	17	177	74	23.6
7	21	187	72	20.6	7	16	191	88	24.1
8	20	173	60	20.0	8	16	180	68	21.0
9	22	175	65	21.2	9	16	183	69	20.6
10	22	180	75	23.1	10	15	171	55	18.8

Supplementary Fig. 8 The clinical characteristics of 10 Tai Chi athletes and 10 Rugby athletes.

All procedures related to human research subjects were approved by the School of Exercise and Health, Shanghai University of Sport and were conducted in strict accordance with institutional guidelines.