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SUPPLEMENTARY INFORMATION

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Analysis of redox landscapes and dynamics in living cells and in vivo using genetically encoded fluorescent sensors

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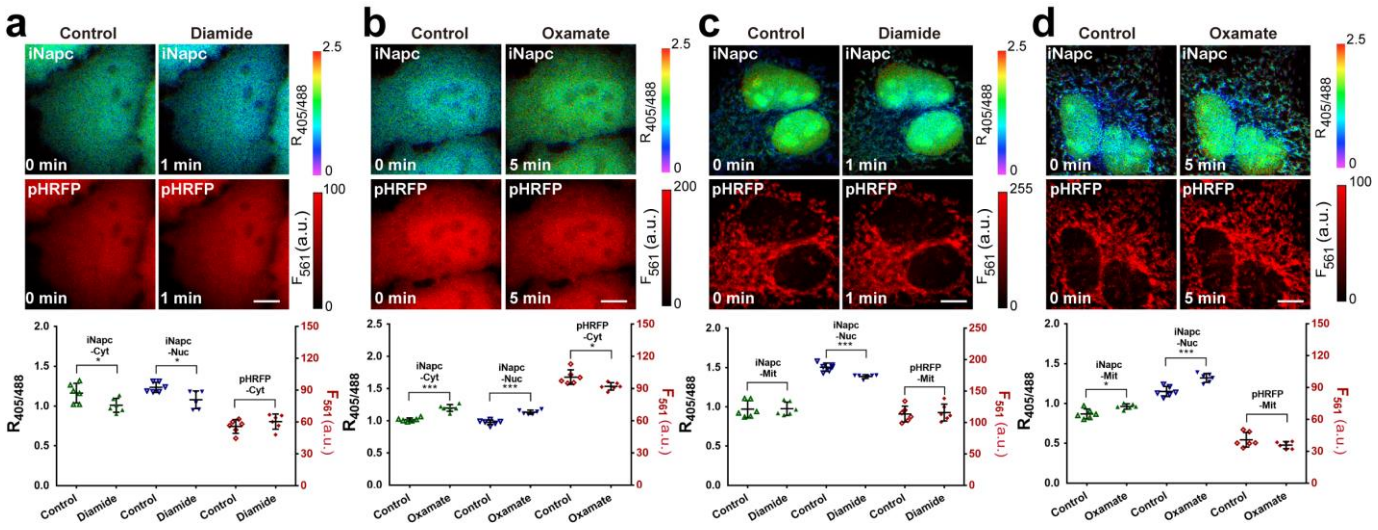
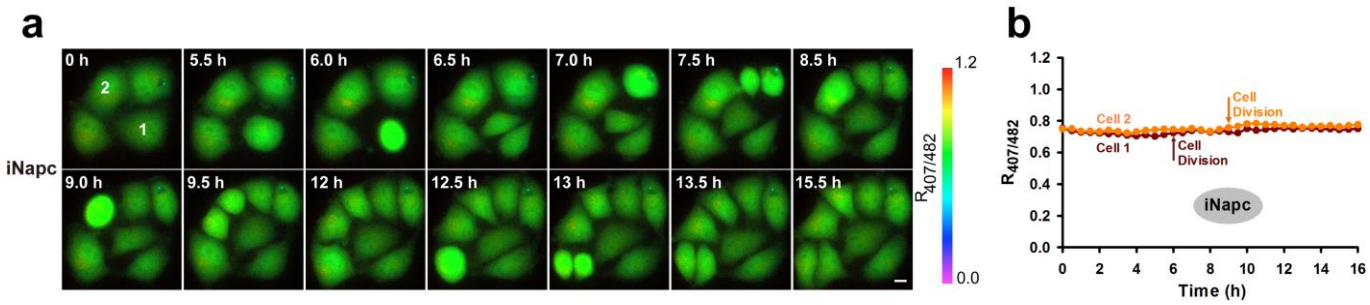


Supplementary Figure 1

Effect of diamide or oxamate treatment on subcellular pH.

(a and b) Fluorescence imaging (top) and fluorescence changes (bottom, n=6 cells) in HeLa cells simultaneously expressing cytosol-localized iNapc and cytosol-localized pHRFP in response to 200 μM diamide (a) or 2 mM oxamate (b).

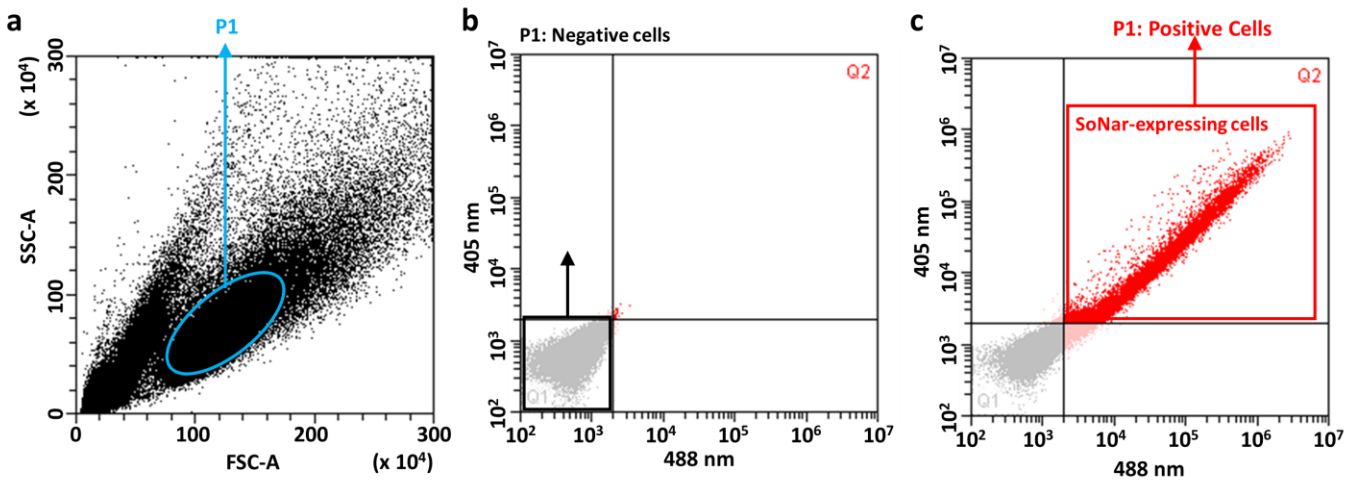
(c and d) Fluorescence imaging (top) and fluorescence changes (bottom, n=6 cells) in HeLa cells simultaneously expressing mitochondria-localized iNapc, nuclear-localized iNapc, and mitochondrial-localized pHRFP in response to 200 μM diamide (c) or 2 mM oxamate (d). Data are the mean ± s.d. All p values were obtained using unpaired two-tailed Student's t test. *$p < 0.05$, ***$p < 0.001$. Scale bars, 10 μm.



Supplementary Figure 2

pH fluorescence imaging during the cell cycle.

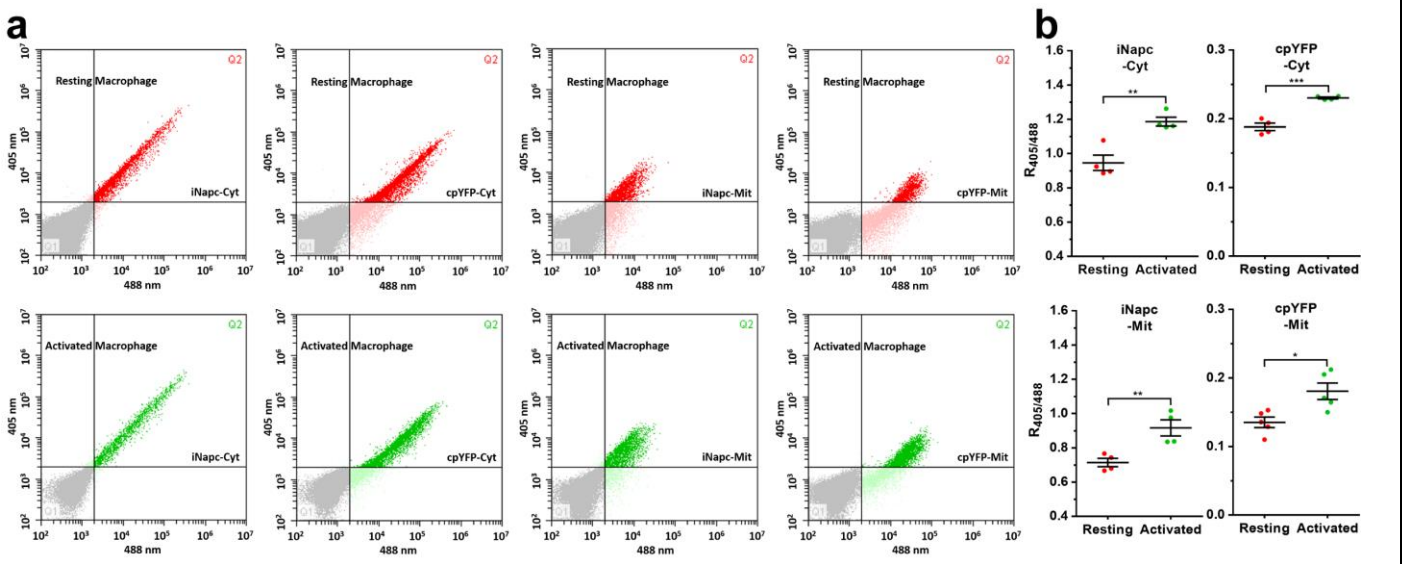
(a and b) Fluorescence images (a) and quantification (b) of pH dynamics during cell division. Scale bars, 10 μm.



Supplementary Figure 3

Example gating strategy.

(a) Sample gating strategy for forward and side scatter (FSC/SSC). (b and c) Sample gating strategy for negative cells (b) and positive cells (sensor-expressing cells, c).

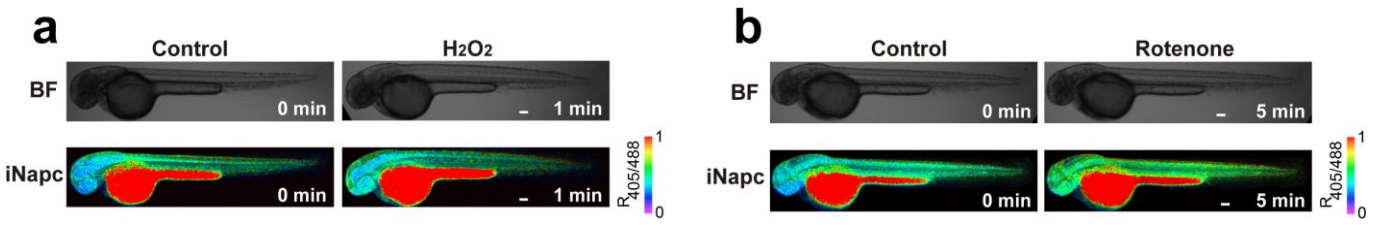


Supplementary Figure 4

Flow cytometry analysis of the pH state in resting and activated mouse macrophages.

(a) Cytosolic or mitochondrial pH detection in resting or activated RAW264.7 mouse macrophages by flow cytometry.

(b) Quantitative data for cytosolic or mitochondrial pH sensor fluorescence were obtained from three or more independent detections by flow cytometry. Data are the mean ± s.e.m. All *p* values were obtained using unpaired two-tailed Student's t test. **p*< 0.05, ***p*< 0.01, ****p*< 0.001.



Supplementary Figure 5

pH fluorescence imaging of zebrafish larvae.

(a and b) *In vivo* fluorescence imaging of zebrafish larvae expressing iNapc in response to 50 mM H_2O_2 (a) or 5 µM rotenone (b).

Supplementary Table 1. The numerical analysis of all cells and positive cells.

	iNap1-Cyt				SoNar-Cyt		
	Positive	All Events	Positive Percentage (%)		Positive	All Events	Positive Percentage (%)
Resting	835	50000	1.67	Resting	7884	100000	7.88
	835	50000	1.67		7730	100000	7.73
	754	50000	1.51		8211	100000	8.21
Activated	1683	50000	3.37		4076	100000	4.08
	1490	50000	2.98	Activated	2760	100000	2.76
	1613	50000	3.23		1777	68052	2.61
					3853	96427	4.00
					3089	100000	3.09
	roGFP1-Cyt				HyPer-Cyt		
	Positive	All Events	Positive Percentage (%)		Positive	All Events	Positive Percentage (%)
Resting	2278	100000	2.28	Resting	1025	100000	1.03
	2245	100000	2.25		976	100000	0.98
	2221	100000	2.22		999	100000	1.00
	681	50000	1.36		5817	100000	5.82
Activated	1282	100000	1.28		5748	100000	5.75
	1542	100000	1.54		5670	100000	5.67
	1479	100000	1.48		6098	100000	6.10
	481	50000	0.96	Activated	1372	100000	1.37
					1407	100000	1.41
					1336	100000	1.34
					5441	100000	5.44
					5072	100000	5.07
					4640	100000	4.64
					5090	100000	5.09
	iNap3-Mit				Frex-Mit		
	Positive	All Events	Positive Percentage (%)		Positive	All Events	Positive Percentage (%)
Resting	2700	100000	2.70	Resting	716	100000	0.72
	3100	100000	3.10		818	100000	0.82
	2610	100000	2.61		496	100000	0.50
	3083	100000	3.08		890	100000	0.89
Activated	3134	89984	3.48		700	100000	0.70
	3142	97811	3.21	Activated	1220	49739	2.45
	3120	77246	4.04		1617	64015	2.53
	3116	88844	3.51		1071	77915	1.37
					1129	60665	1.86

					1150	82274	1.40
	roGFP1-Mit				HyPer-Mit		
	Positive	All Events	Positive Percentage (%)		Positive	All Events	Positive Percentage (%)
Resting	7671	88182	8.70	Resting	2547	95385	2.67
	6557	61765	10.62		2383	91044	2.62
	6170	63818	9.67		2122	71043	2.99
	6608	60780	10.87		3249	100000	3.25
Activated	5098	77811	6.55		3493	100000	3.49
	5334	83305	6.40	Activated	3104	70306	4.41
	5223	86465	6.04		2374	57691	4.12
	5253	91349	5.75		2629	70957	3.71
					3147	100000	3.15
					2932	100000	2.93
	iNapc-Cyt				cpYFP-Cyt		
	Positive	All Events	Positive Percentage (%)		Positive	All Events	Positive Percentage (%)
Resting	703	50000	1.41	Resting	4428	100000	4.43
	750	50000	1.50		4457	100000	4.46
	738	50000	1.48		4446	100000	4.45
	2925	100000	2.93		4450	100000	4.45
Activated	1263	50000	2.53	Activated	3232	100000	3.23
	1217	50000	2.43		3627	100000	3.63
	1346	50000	2.69		3426	100000	3.43
	1772	100000	1.77		3436	100000	3.44
	iNapc-Mit				cpYFP-Mit		
	Positive	All Events	Positive Percentage (%)		Positive	All Events	Positive Percentage (%)
Resting	2019	100000	2.02	Resting	2101	98159	2.14
	2042	100000	2.04		1769	91440	1.93
	2001	100000	2.00		2046	92747	2.21
	2046	100000	2.05		1859	86712	2.14
Activated	2324	100000	2.32		1927	100000	1.93
	1938	100000	1.94	Activated	2009	87981	2.28
	2336	81963	2.85		1811	94156	1.92
	1937	93250	2.08		1981	79906	2.48
					1953	90729	2.15
					1399	80750	1.73

Note : Quantitative data for cytosolic or mitochondrial sensor fluorescence were obtained from three or more independent assessments by flow cytometry.