1 SUPPLEMENTARY INFORMATION



2 3 4 Supplementary Figure 1. Temporal dependence of the mean intracellular fluorescence of the glucose analogue 2-NBDG averaged over 60 individual E. coli at the top of each dead-end 5 bacteria-hosting channel (red squares, position 1) or below such cells (black circles, position 6 2). Bacteria at position 2 are closer to the main microfluidic chamber i.e. the 2-NBDG source, 7 whereas bacteria at position 1 are screened by one cell. Statistical comparisons are reported in 8 Table S1. Inset: bright-field microscopy image illustrating bacteria in positions 1 and 2, scale 9 bar: 5 µm.

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Time (s)	t ratio	df	<i>p</i> -value
0			
100	1,26	58	0,21
200	1,60	58	0,11
300	0,52	58	0,60
400	0,18	58	0,86
500	0,12	58	0,91
600	0,01	58	0,99
700	0,11	58	0,92
800	0,07	58	0,94
900	0,11	58	0,91

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Supplementary Table 1. Statistical comparisons of 2-NBDG accumulation in E. coli at the

14 top of each dead-end bacteria-hosting channel or below such cells (positions 1 and 2 in Fig.

15 S1, respectively).

	Optimal grow	th conditions	Nutrient depletion		
T	Mean	CV	Mean	CV	
lime (s)	fluorescence	fluorescence	fluorescence	fluorescence	
	(a.u.)	(%)	(a.u.)	(%)	
0	0	0	0	0	
100	17	48	22	61	
200	37	90	40	49	
300	250	46	417	58	
400	435	35	546	58	
500	533	33	647	55	
600	613	32	692	57	
700	685	32	762	55	
800	699	33	769	58	
900	733	32	740	60	
1000	730	34	683	54	
1100	761	32	663	59	
1200	580	46	458	54	
1300	350	47	293	61	
1400	231	49	208	64	
1500	156	52	150	67	
1600	112	52	113	69	
1700	83	55	91	67	
1800	63	55	76	69	
1900	53	53	64	64	
2000	42	47	51	64	
	Salt de	pletion	Nutrient and	salt depletion	
Time (s)	Salt de Mean	pletion CV	Nutrient and Mean	salt depletion CV	
Time (s)	Salt de Mean fluorescence	pletion CV fluorescence	Nutrient and Mean fluorescence	salt depletion CV fluorescence	
Time (s)	Salt de Mean fluorescence (a.u.)	pletion CV fluorescence (%)	Nutrient and Mean fluorescence (a.u.)	salt depletion CV fluorescence (%)	
Time (s)	Salt de Mean fluorescence (a.u.) 0	pletion CV fluorescence (%) 0	Nutrient and Mean fluorescence (a.u.) 0	salt depletion CV fluorescence (%) 0	
Time (s)	Salt de Mean fluorescence (a.u.) 0 12	pletion CV fluorescence (%) 0 86	Nutrient and Mean fluorescence (a.u.) 0 12	salt depletion CV fluorescence (%) 0 86	
Time (s) 0 100 200	Salt de Mean fluorescence (a.u.) 0 12 13	pletion CV fluorescence (%) 0 86 84	Nutrient and Mean fluorescence (a.u.) 0 12 16	salt depletion CV fluorescence (%) 0 86 90	
Time (s) 0 100 200 300	Salt de Mean fluorescence (a.u.) 0 12 13 148	pletion CV fluorescence (%) 0 86 84 70	Nutrient and Mean fluorescence (a.u.) 0 12 16 722	salt depletion CV fluorescence (%) 0 86 90 43	
0 100 200 300 400	Salt de Mean fluorescence (a.u.) 0 12 13 148 262	pletion CV fluorescence (%) 0 86 84 70 45	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208	salt depletion CV fluorescence (%) 0 86 90 43 42	
0 100 200 300 400 500	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298	pletion CV fluorescence (%) 0 86 84 70 45 43	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507	salt depletion CV fluorescence (%) 0 86 90 43 42 40	
0 100 200 300 400 500 600	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332	pletion CV fluorescence (%) 0 86 84 70 45 43 39	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38	
0 100 200 300 400 500 600 700	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326	pletion CV fluorescence (%) 0 86 84 70 45 43 39 41	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39	
0 100 200 300 400 500 600 700 800	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317	pletion CV fluorescence (%) 0 86 84 70 45 43 39 41 43	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38	
0 100 200 300 400 500 600 700 800 900	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373	pletion CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1676 1714	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 38 38	
0 100 200 300 400 500 600 700 800 900 1000	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373 393	pletion CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 41 43 39 43	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 38 38 38 39	
0 100 200 300 400 500 600 700 800 900 1000 1100	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373 393 429	pletion CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 41 43 39 41 39 43 38	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 39 38 38 39 38 38 39 38	
0 100 200 300 400 500 600 700 800 900 1000 1100 1200	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373 393 429 328	pletion CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 41 43 39 41 43 39 41 43 39 41 43 39 43 43 38 45	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 39 38 38 39 38 46	
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373 393 429 328 155	pletion CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 41 43 39 41 43 39 41 43 39 41 43 39 41 43 39 43 38 45 38	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146 582	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 38 39 38 38 39 38 46 55	
Time (s) 0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373 393 429 328 155 83	CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 43 39 43 39 43 39 43 39 43 39 43 38 45 38 50	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146 582 341	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 39 38 39 38 39 38 46 55 64	
Time (s) 0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 298 332 326 317 373 393 429 328 155 83 44	CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 43 39 43 39 43 39 43 39 43 39 43 38 45 38 50 98	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146 582 341 186	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 39 38 38 39 38 38 46 55 64 55	
Time (s) 0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 298 332 326 317 373 393 429 328 155 83 44 44 34	CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 43 39 43 39 43 39 43 39 43 39 43 38 45 38 50 98 118	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146 582 341 186 124	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 38 39 38 38 39 38 38 46 55 64 55 64	
Time (s) 0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 298 332 326 317 373 393 429 328 155 83 429 328 155 83 44 44 34	CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 43 39 43 39 43 39 43 39 43 39 43 38 50 98 118 128	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146 582 341 186 124 82	salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 38 39 38 38 39 38 46 55 64 55 64 55 60 79	
Time (s) 0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373 393 429 328 155 83 429 328 155 83 44 44 34 23 25	CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 43 39 43 39 43 38 50 98 118 128 143	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146 582 341 186 124 82 53	Salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 39 38 64 55 64 55 60 79 70	
Time (s) 0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900	Salt de Mean fluorescence (a.u.) 0 12 13 148 262 298 332 326 317 373 393 429 328 155 83 429 328 155 83 44 44 34 23 25 29	CV fluorescence (%) 0 86 84 70 45 43 39 41 43 39 43 39 43 39 43 38 50 98 118 128 143 151	Nutrient and Mean fluorescence (a.u.) 0 12 16 722 1208 1507 1669 1648 1676 1714 1583 1670 1146 582 341 186 124 82 53 40	Salt depletion CV fluorescence (%) 0 86 90 43 42 40 38 39 38 39 38 64 55 60 79 70 62	

17 18 Supplementary Table 2. Temporal dependence of the mean and coefficient of variation of 19 intracellular fluorescence of the glucose analogue 2-NBDG over at least 30 individual E. coli 20 21 22 23 24 25 (collated from biological triplicate) per environmental condition as detailed in Figure 2.

Time (s)	Nu	Nutrient depletion	
	t ratio	df	<i>p</i> -value
0			
100	2,07	112,00	0,04
200	0,45	112,00	0,65
300	4,96	112,00	<10 ⁻⁵
400	2,53	112,00	0,01
500	2,27	112,00	0,03
600	1,43	112,00	0,15
700	0,30	112,00	0,20
800	1,11	112,00	0,27
900	0,12	112,00	0,90
Time (s)	5	Salt depletion	on
	t ratio	df	<i>p</i> -value
0			
100	3,46	166,00	<10 ⁻³
200	6,43	166,00	<10 ⁻⁶
300	5,89	166,00	<10 ⁻⁶
400	7,94	166,00	<10 ⁻⁶
500	9,47	166,00	<10 ⁻⁶
600	10,74	166,00	<10 ⁻⁶
700	12,05	146,00	<10 ⁻⁶
800	12,35	146,00	<10 ⁻⁶
900	11,11	146,00	<10 ⁻⁶
	, ,		
Time (s)	Nutrien	t and salt d	epletion
	t ratio	df	p-value
0			1
100	3,39	120,00	<10 ⁻³
200	3,99	120,00	<10 ⁻⁴
300	11,91	120,00	<10 ⁻⁶
400	12,33	120,00	<10 ⁻⁶
500	13,14	120,00	<10 ⁻⁶
600	13,55	120,00	<10 ⁻⁶
700	11,89	110,00	<10 ⁻⁶
800	11,96	110,00	<10 ⁻⁶
900	11.60	110.00	<10 ⁻⁶
Statistical	comparie	ons of 2-1	NBDG ac

nutrient, salt or combined nutrient and salt depletion compared to optimal growth conditions.





Supplementary Figure 2. Total intracellular fluorescence of the glucose analogue 2-NBDG
in individual *E. coli* under optimal growth conditions and combined nutrient and salt depletion
(black and red bars, respectively) measured by flow cytometry after 900s bulk incubation in 2NBDG. Noteworthy, these measurements were not normalized by cell size.

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Supplementary Figure 3. Temporal dependence of the mean intracellular fluorescence of 46 47 thioflavin T in individual E. coli in a) optimal growth conditions and b) under combined 48 nutrient and salt depletion. Lines are temporal dependences of the intracellular fluorescence of 49 individual bacteria collated from biological triplicate. Noteworthy, we measured thioflavin T 50 fluorescence as the mean fluorescent values of each pixel constituting each bacterium, thus 51 normalizing by cell size. Insets: corresponding fluorescence images at t=45min when the 52 intracellular ThT accumulation has reached saturation levels in individual bacteria. c) 53 Corresponding total intracellular fluorescence of thioflavin T under optimal growth conditions 54 or combined nutrient and salt depletion (black and red bars, respectively) measured by flow 55 cytometry after 45min bulk incubation in thioflavin T. Noteworthy, these measurements were 56 not normalized by cell size. Statistical comparisons are reported in Table S4.

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Time	Microfluidics-microscopy assay			ofluidics-microscopy assay Flow cyt		
(min)	t ratio	df	<i>p</i> -value	t ratio	df	<i>p</i> -value
0	3,78	94	<10 ⁻⁶			
12	7,98	94	<10 ⁻⁶			
22	5,56	94	<10 ⁻⁶			
37	6,01	94	<10 ⁻⁶			
42	6,23	94	<10 ⁻⁶	257,9	99578	<10 ⁻⁶

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60 **Supplementary Table 4.** Statistical comparisons of thioflavin T accumulation in *E. coli* in 61 optimal growth conditions and under combined nutrient and salt depletion as measured via

62 single-cell microfluidics-microscopy and flow cytometry.





Supplementary Figure 4. Distribution of total intracellular fluorescence of 2-NBDG under nutrient depletion alone or combined nutrient and salt depletion (green and red violins, respectively) using M9 minimal medium with limited (i.e. 0.1 g/L) a) glucose or b) ammonia. Measurements were performed on 50,000 bacteria for each environmental condition using flow cytometry after 900s bulk incubation in 2-NBDG. These measurements were not normalized by cell size. ****: p-value <0.0001.</p>





71 Supplementary Figure 5. Temporal dependence of the mean intracellular fluorescence of the 72 glucose analogue 2-NBDG in individual *E. coli* under **a**) optimal growth conditions, **b**) nutrient 73 depletion, c) salt depletion or d) combined nutrient and salt depletion during removal of 2-74 NBDG from the extracellular environment. Lines are temporal dependences of the intracellular 75 fluorescence of individual bacteria from biological triplicate. Symbols and error bars are the corresponding means and standard error of the means of such single-cell measurements. Means 76 and coefficient of variations of these single-cell values are reported in Table S2. These 77 78 measurements were normalized by cell size. Measurements were carried out on N=76, 38, 90 79 and 46 individual bacteria, in a)-d), respectively.

Comparisons with	Uptake rate			Degradation rate		
optimal growth	t ratio	df	<i>p-</i> value	t ratio	df	<i>p</i> -value
Nutrient depletion	3.88	62,0	0.0003	3.41	42	0.0015
Salt depletion	7.46	116,0	<0.0001	10.97	83	<0.0001
Nutrient and salt depletion	2.59	86,0	0.01	9.30	80	<0.0001

81 Supplementary Table 5. Statistical comparisons of the predicted 2-NBDG uptake and

83 degradation values under nutritional, salinity or combined nutritional and salinity depletion

- 84 compared to optimal growth conditions.
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Environment	Uptak	e rate	Degradation rate		
Linvironment	Median	CV (%)	Median	CV (%)	
Optimal growth conditions	-1,73	13	-2,35	3	
Nutrient depletion	-1,94	15	-2,31	9	
Salt depletion	-2,05	15	-2,12	9	
Nutrient and salt depletion	-1,52	19	-2,18	5	

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87 Supplementary Table 6. Median and coefficient of variation (CV) of the predicted uptake and

degradation rate values in optimal growth conditions, under nutritional, salinity or combined
 nutritional and salinity depletion.

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Supplementary Figure 6. Distribution of single-cell fluorescence after 900 s incubation in 2-NBDG for the parental strain (PS) and Δpgm deletion mutant under salt depletion or simultaneous nutrient and salt depletion. Dashed and dotted lines indicate the median and quartiles of each distribution, respectively. Under nutrient and salt depletion the Δpgm deletion mutant displayed significantly lower 2-NBDG accumulation compared to the parental strain (***). In contrast, under optimal growth conditions the Δpgm deletion mutant displayed 2-NBDG accumulation comparable to the parental strain.

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Supplementary Figure 7. Temporal dependence of the mean intracellular fluorescence of the glucose analogue 2-NBDG in *E. coli* cultured in optimal growth conditions without (circles) or with an additional 1h exposure to the supernatant collected from *E. coli* cultures under combined nutritional and salinity depletion (squares) before 2-NBDG accumulation measurements. Symbols and error bars are the means and standard error of the means over at least 20 single-cell measurements.



Supplementary Figure 8. Gating strategy for all flow cytometry experiments. Cells (red) were
 separated from background debris (black) by gating bacterial cells (red) using a plot of
 fluorescein isothiocyanate channel (FITC-A) against violet side scatter (Violet-SSC-A).