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Supplemental Information

A Fluorescent Reporter of AMPK Activity and Cellular Energy Stress

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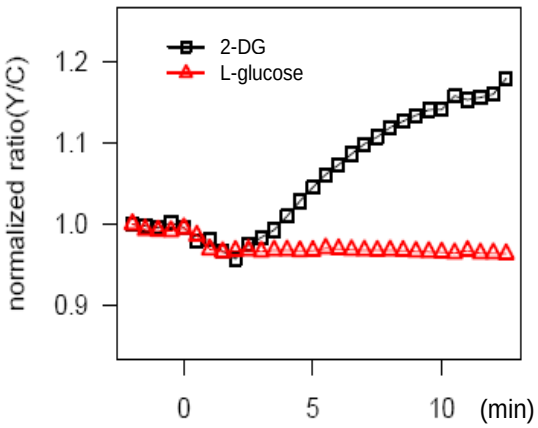
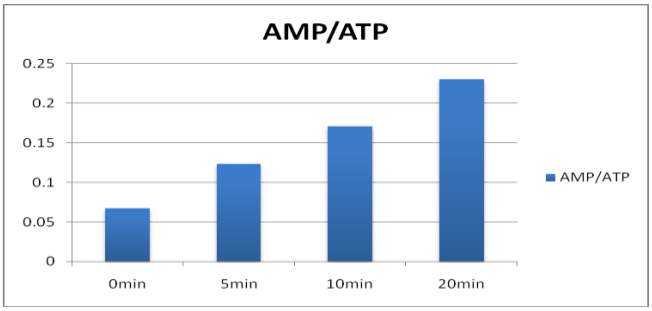
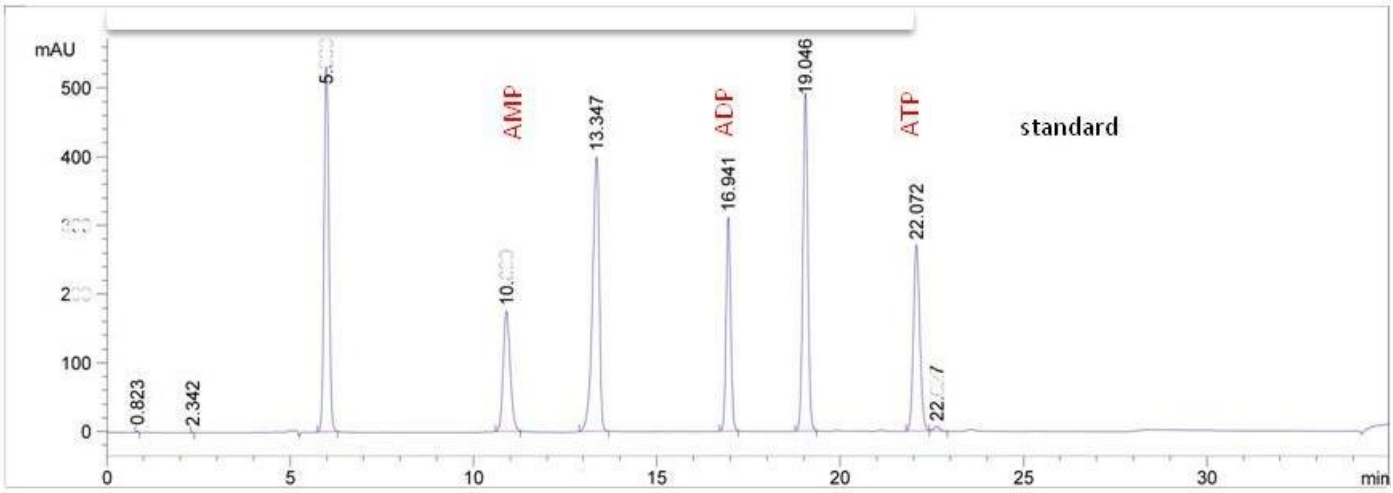


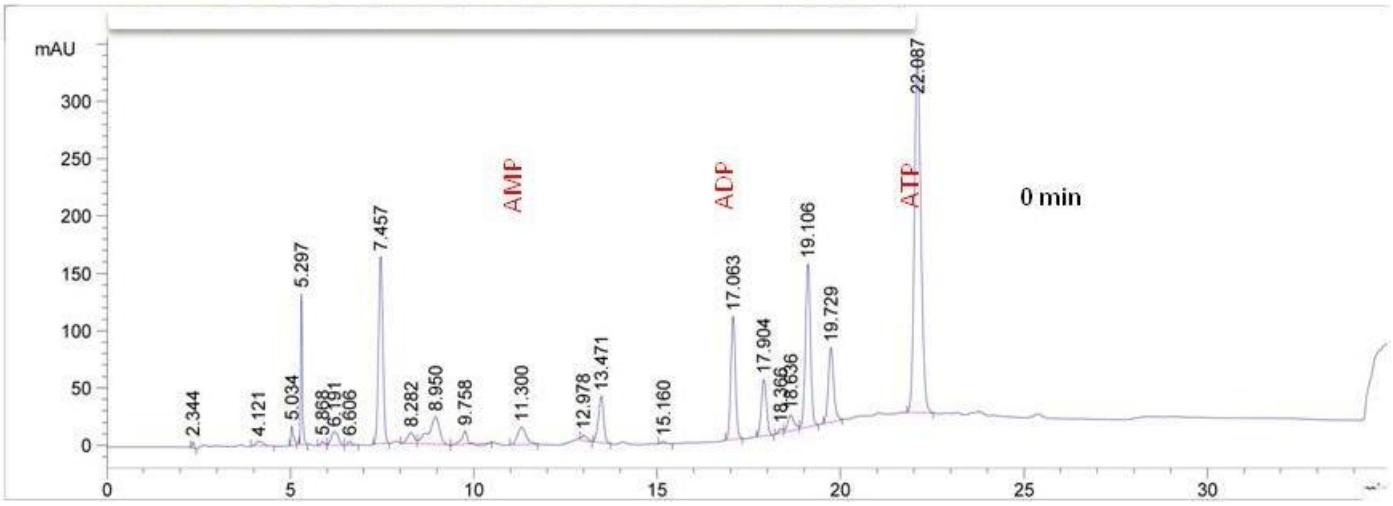
Figure S1. Non-metabolized L-glucose also caused a small decline of FRET ratio without later increase of signal, Related to the Results
COS-7 cells expressing AMPKAR were treated with either 20 mM 2-DG (n=30) or 20 mM L-glucose (n=14).

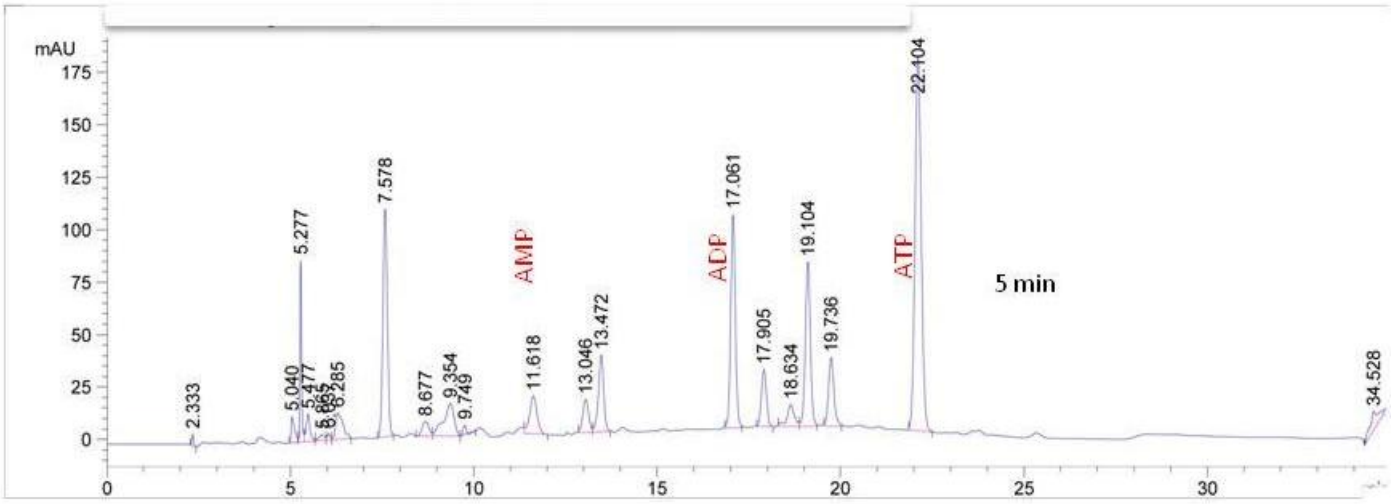
A

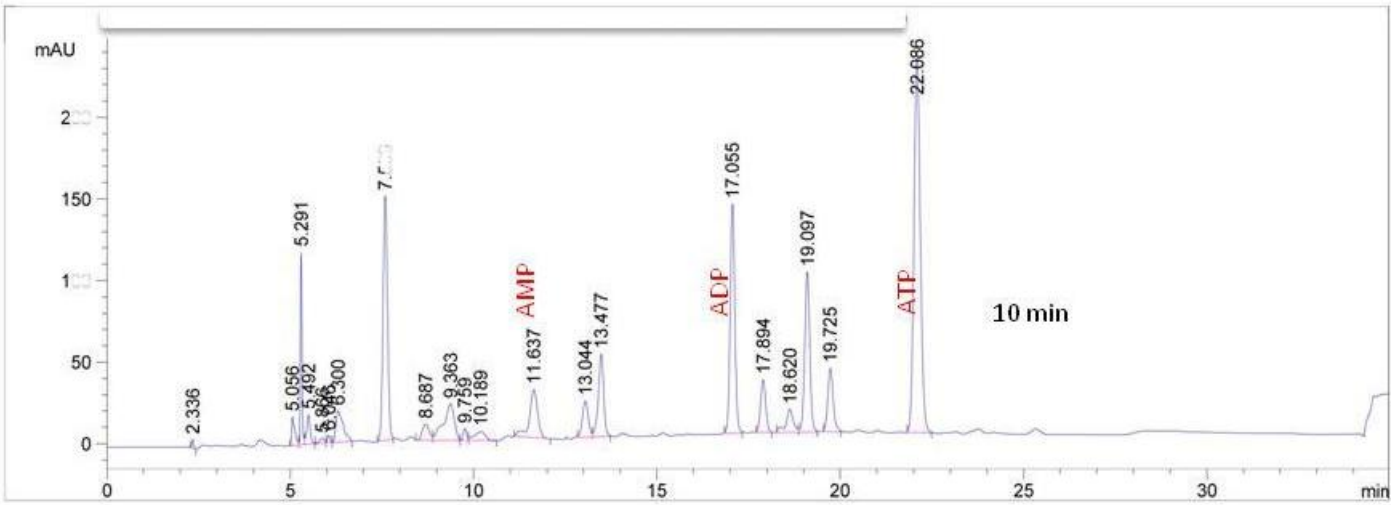

AMP/ATP
0.25
0.2
0.15
0.1
0.05
0
0min
5min
10min
20min
AMP/ATP

B


mAU
500
400
100
0
0.823
2.342
AMP
13.347
ADP
16.941
19.046
ATP
22.072
standard
0
5
10
15
20
25
30
min


mAU
300
250
200
150
100
50
0
2.344
4.121
5.034
5.297
7.457
8.282
8.950
9.758
11.300
12.978
13.471
15.160
AMP
ADP
17.063
17.904
19.106
19.729
ATP
22.087
0 min
0
5
10
15
20
25
30


mAU
175
150
125
100
75
50
25
0
2.333
5.040
5.277
7.578
8.677
9.354
9.749
11.618
AMP
13.046
13.472
17.061
ADP
17.905
18.634
19.104
19.736
22.104
ATP
34.528
5 min
0
5
10
15
20
25
30


mAU
2.336
5.056
5.291
8.687
9.363
9.759
10.189
11.637
AMP
13.044
13.477
17.055
ADP
17.894
18.620
19.097
19.725
22.086
ATP
10 min
0
5
10
15
20
25
30
min

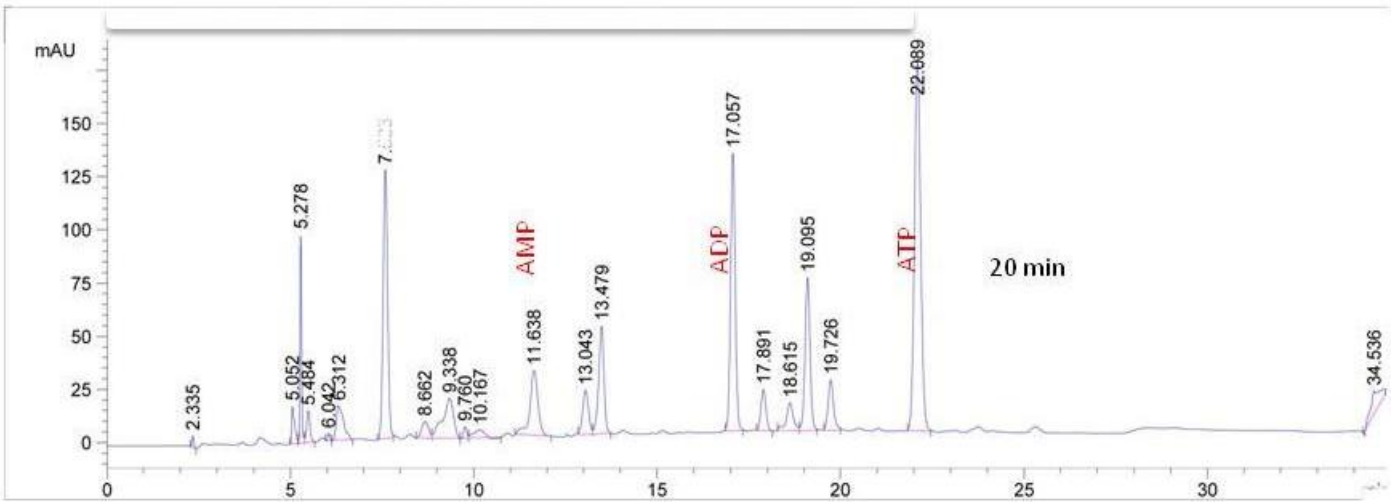

mAU
150
125
100
75
50
25
0
2.335
5.052
5.278
6.042
6.312
8.662
9.338
9.760
10.167
11.638
AMP
13.043
13.479
17.057
ADP
17.891
18.615
19.095
19.726
22.089
ATP
34.536
20 min
0
5
10
15
20
25
30

Figure S2. Intracellular AMP:ATP ratio correlates the FRET signal of the AMPK reporter, Related to the Results

COS7 cells were treated with or without 2-DG (20mM) for the indicated time and cellular ATP and AMP levels were determined by HPLC. (A) Relative quantities of cellular ATP and AMP at each time points. (B) The number at the peak indicates the retention time of the labeled nucleotides.

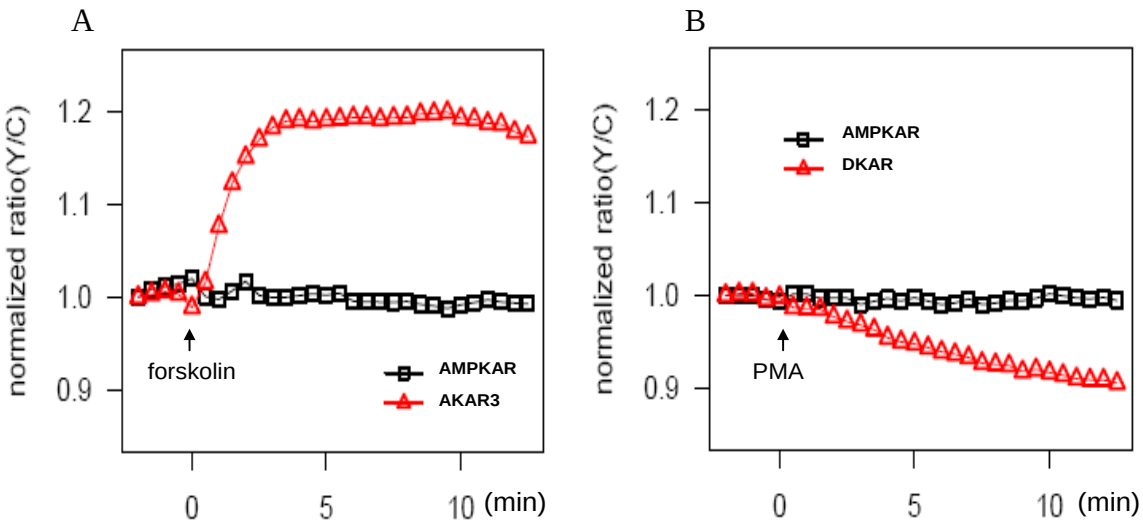


Figure S3. The AMPKAR FRET response is specific to AMPK activation, Related to the Results

AMPKAR does not report (**A**) forskolin induced PKA activation, or (**B**) PMA mediated PKD activation. (A) COS-7 cells were transfected with AMPKAR (n=21) or protein kinase A reporters (AKAR3) (n=6), and then were treated with either 50 μM forskolin. Forskolin caused an increase in the AKAR3 FRET signal but had no effect on the AMPKAR FRET signal. (B) COS-7 cells were transfected with AMPKAR (n=7) or protein kinase D (DKAR) reporters (n=5), and then were treated with 200 nM PMA. PMA caused a decrease in the DKAR FRET signal (which reflected increase of PKD activity) but had no effect on the AMPKAR FRET signal.

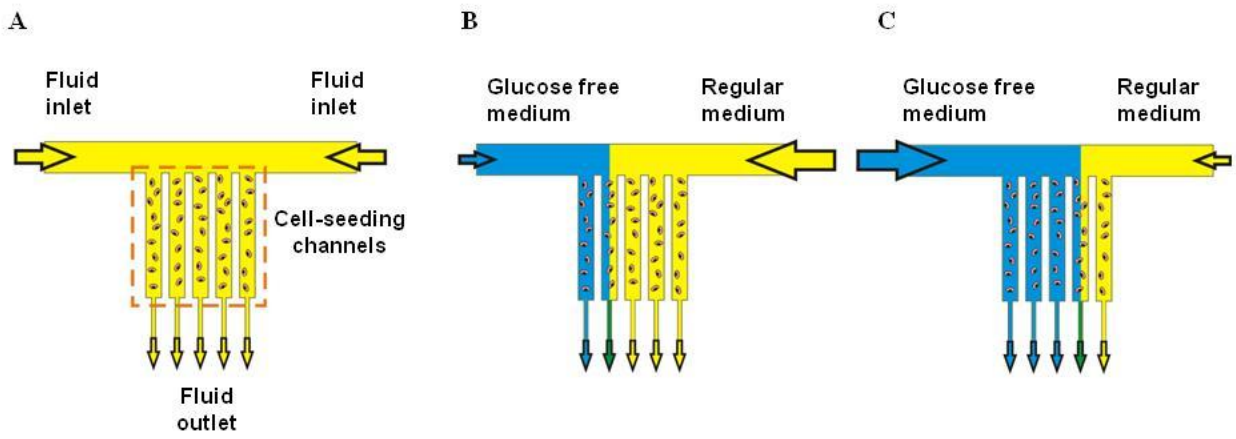


Figure S4. Schematics of the microfluidic device design and operation, Related to the Results and Experimental Procedures

(**A**) The device contains two inlets and cell-seeding channels. Glucose-free and regular medium are introduced from the two inlets and the position of the fluid interface is determined by the relative hydrostatic pressure from both inlets.

(**B**) The hydrostatic pressure is adjusted to change the position of the fluid interface, and thus expose the cells on the 3rd channel (from the left) to regular medium.

(C) Expose the cells on the 3rd channel (from the left) to glucose free medium. Cells on the 5th channel were constantly exposed to regular medium and used as a control.