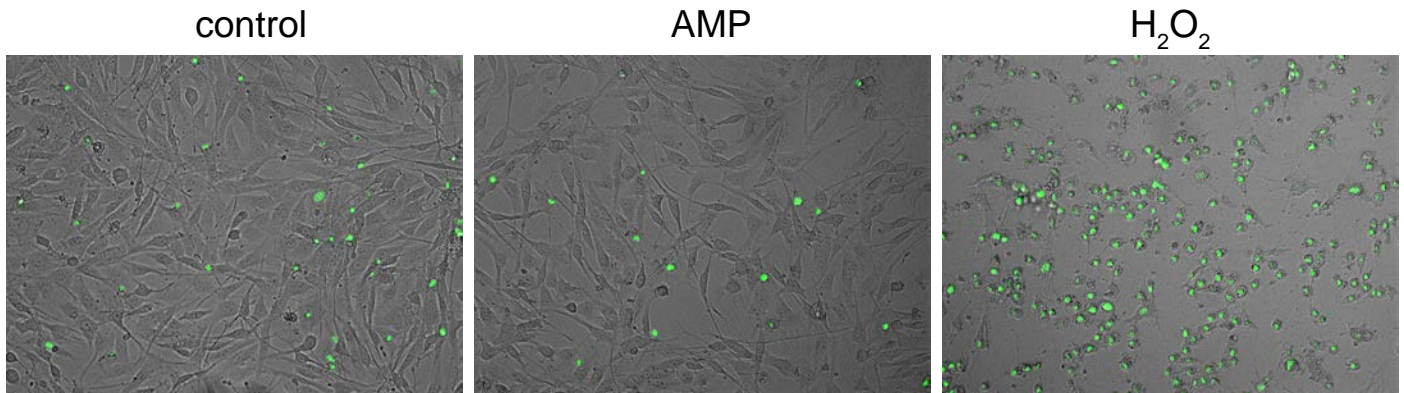
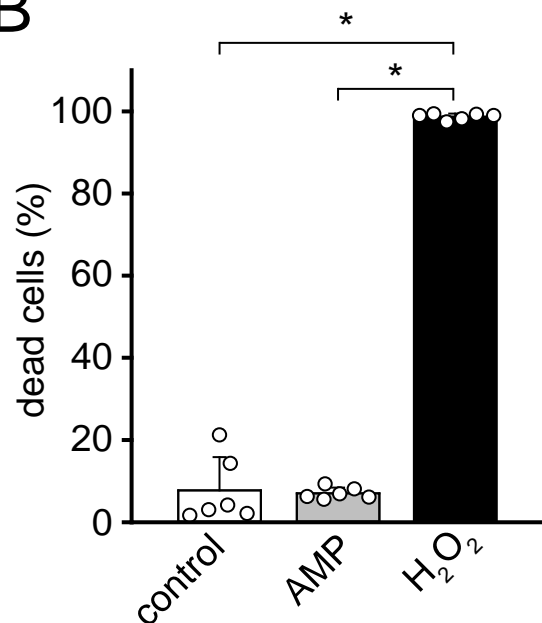


# Supplemental Figure 1

A



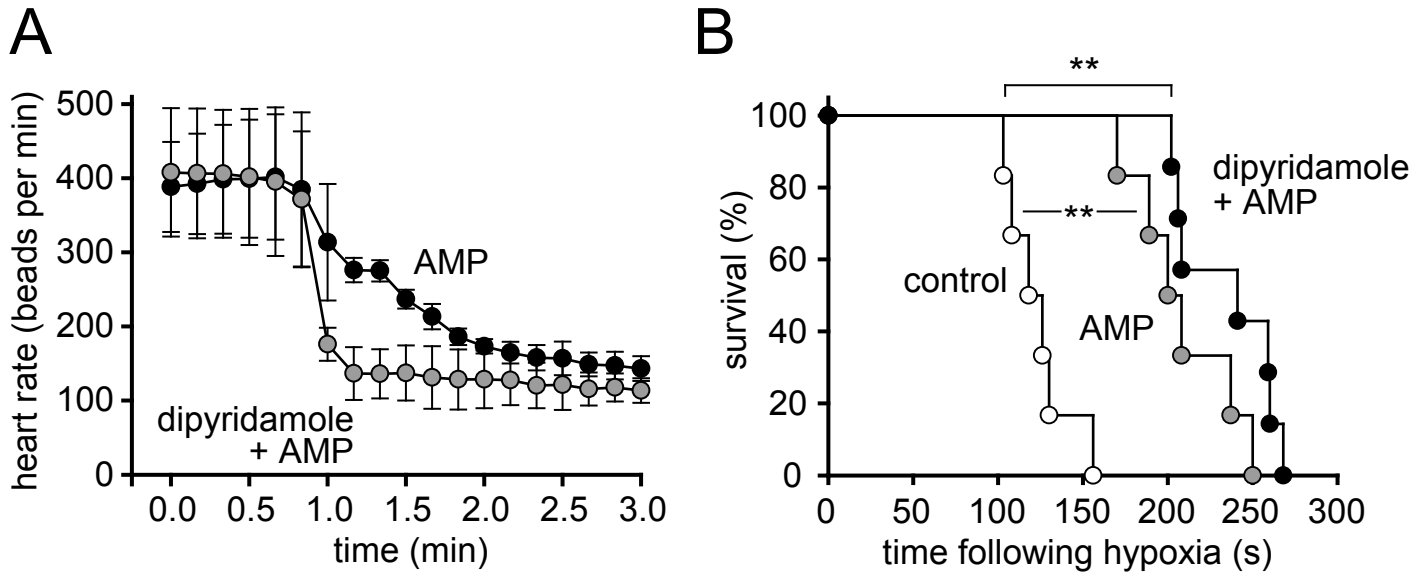
B



## Supplemental Digital Content 2 - Supplemental Figure 1. AMP does not affect cell viability.

**A-B**, SH-SY5Y cells were cultured for 16 h in the presence or absence of AMP (10 mM),  $H_2O_2$  (2 mM), or vehicle control. Then, cells were stained with SytoxGreen dye and the number of dead (SytoxGreen positive) cells was determined. **A**, Representative images of merged bright field and fluorescence images (20x objective, n=6). **B**, Averaged results of 6 independent experiments (mean + SD, n=6). \* $p < 0.05$ , Kruskal-Wallis test.

## Supplemental Figure 2



### Supplemental Digital Content 2 - Supplemental Figure 2. Dipyridamole tends to increase the hypometabolic effect of AMP.

**A**, C57BL/6J mice were anesthetized with isoflurane and treated with dipyridamole (1 mg/mouse *i.p.*) or saline. After 5 min, AMP (4 mg/g *i.p.*) was administered and the heart rate was monitored by ECG recording (mean  $\pm$  SD,  $n=3$  mice per group). **B**, C57BL/6J mice (control and AMP groups:  $n=6$ , dipyridamole + AMP group:  $n=7$ ) were anaesthetized with ketamine/xylazine and treated with AMP (4 mg/g *i.p.*) dipyridamole (1 mg/mouse *i.p.*) followed by AMP (4 mg/g, *i.p.*) after 2 min, or with normal saline (control). After 2 min, the mice were placed into a chamber containing  $<1\%$   $O_2$ . The mice were monitored and the time point when respiration ceased for more than 6 seconds was recorded;  $**p<0.01$  vs. control, Kaplan-Meier and log rank test.

## Supplemental Video Legends

### **Supplemental Digital Content 1 - Video 1. AMP renders mice indifferent to arousal.**

C57BL/6J mice were treated with AMP (0.5 mg/g body weight *i.p.*) and alertness and the response to physical stimulation were assessed.

### **Supplemental Digital Content 3 - Video 2. AMP stalls mitochondrial activity.**

SH-SY5Y cells were labeled with the mitochondrial membrane potential probe JC-1 and changes in red JC-1 fluorescence were recorded at a frame rate of 1 frame per second. Vehicle control, AMP (10 mM) or CCCP (1  $\mu$ M) were added 3 s after starting recording. Images were acquired using a 20x objective (NA 0.40).

### **Supplemental Digital Content 4 - Video 3. AMP and ATP have opposing effects on mitochondrial $\text{Ca}^{2+}$ uptake.**

Mitochondrial  $\text{Ca}^{2+}$  levels in SH-SY5Y cells expressing the mitochondrial  $\text{Ca}^{2+}$  sensor mito-CAR-GECO1 were recorded with a frame rate of 1 frame per second. Cells were exposed to ATP (10  $\mu$ M) or AMP (10 mM) 20 s after starting recording. Images were acquired using a 40x objective (NA 0.75).

### **Supplemental Digital Content 5 - Video 4. AMP and ATP have opposing effects on cytosolic $\text{Ca}^{2+}$ levels.**

SH-SY5Y cells were loaded with Fluo-4 and cytosolic  $\text{Ca}^{2+}$  levels were monitored with video fluorescence microscopy with a frame rate of 1 frame per second. ATP (100  $\mu$ M) or AMP (20 mM) were added 3 s after starting recording. Images were acquired using a 40x objective (NA 0.75).