Suppl. Fig. 1. *Effects of postsynaptic receptors and glial glutamate transporter blockers on NAD(P)H and oxygen responses to synaptic stimulation.*
Shaffer collaterals stimulation 10 Hz, 10 s. Cocktail of blockers consists of NBQX (10 μM), AP-5 (40 μM), gabazine (10 μM), E4CPG (500 μM).

Suppl. Fig. 2. *Variations of metabolic and electrophysiological parameters following exchange of glucose for pyruvate.*
A. Reversible decrease of LFPs induced by a single pulse Shaffer collaterals stimulation in pyruvate-ACSF. Red trace - standard 5mM ACSF, blue – 10mM pyruvate-ACSF, orange - standard ACSF after pyruvate-ACSF washout. Arrow depicts the presynaptic volley. **B.** Decrease of LFPs in pyruvate-ACSF still persists in DPCPX (100 nM) and therefore is not induced by activation of adenosine receptors. **C.** Averaged LFP integrals in response to the stimulation train in glucose-ACSF and pyruvate-ACSF. In each experiment, the integral values have been normalized to the first response in glucose-ACSF.

Suppl. Fig. 3. *Effect of pyruvate on NAD(P)H transients does not depend on stimulation intensity.* In red – 5 mM glucose-ACSF, in blue – 10 mM pyruvate-ACSF.

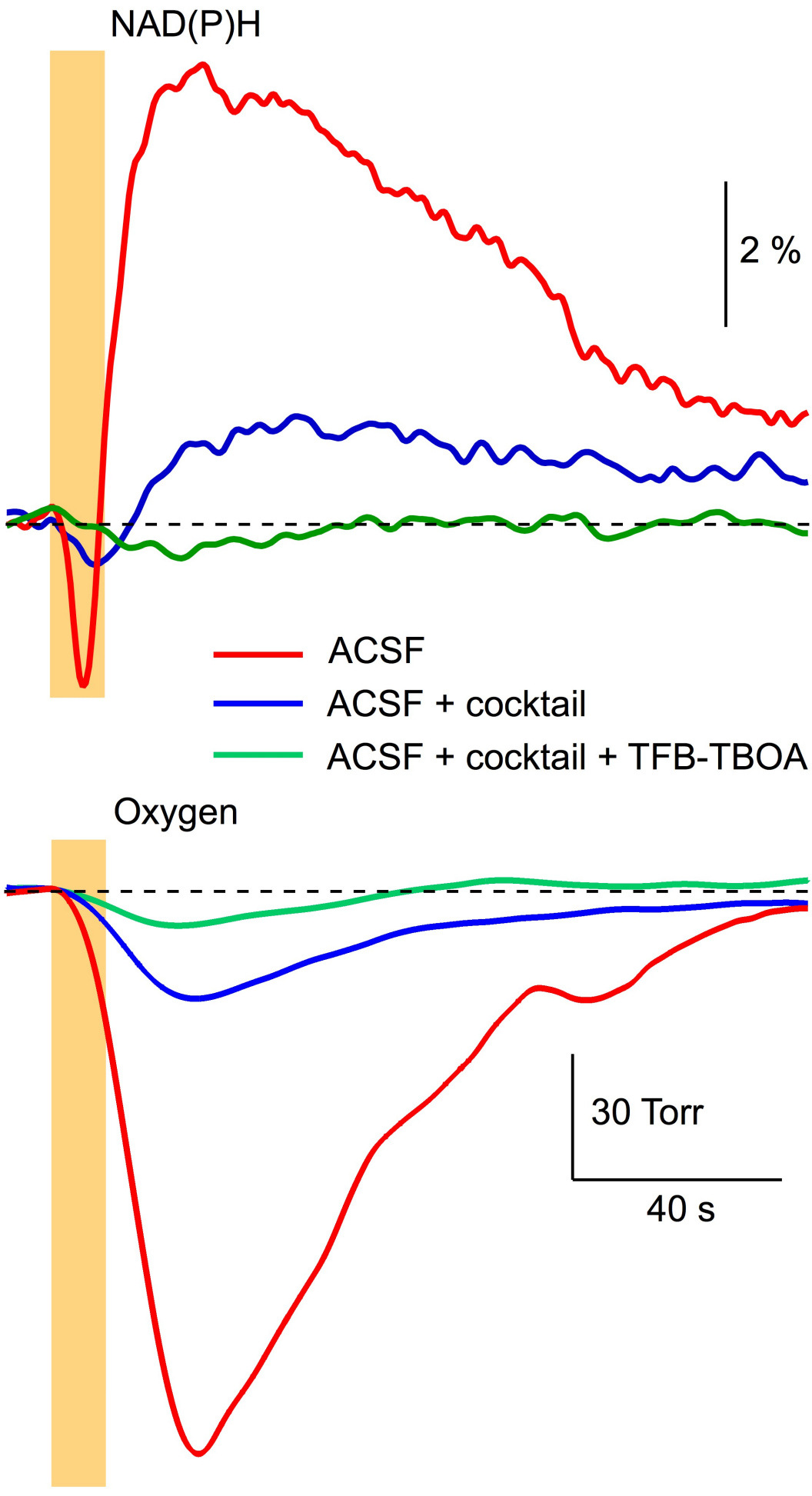
Suppl. Fig. 4. *NAD(P)H and oxygen transients in 5 mM glucose- and 10 mM pyruvate-ACSF in response to a prolonged (30 s, 10 Hz) synaptic stimulation.*
Note, that the baseline fluorescences may be different for glucose and pyruvate (see Fig. 4) and are aligned on the figure.

Suppl. Fig. 5. A. Intracellular calcium influx during 10 Hz, 10 s synaptic stimulation is similar in 5 mM glucose- and 10 mM pyruvate-ACSF. **B.** Antioxidant Tempol (2 mM) does not change the effects of pyruvate on NAD(P)H and FAD transients. Black bar indicates synaptic stimulation. Note, that the baseline fluorescences may be different for glucose and pyruvate (see Fig. 4) and are aligned on the figure.

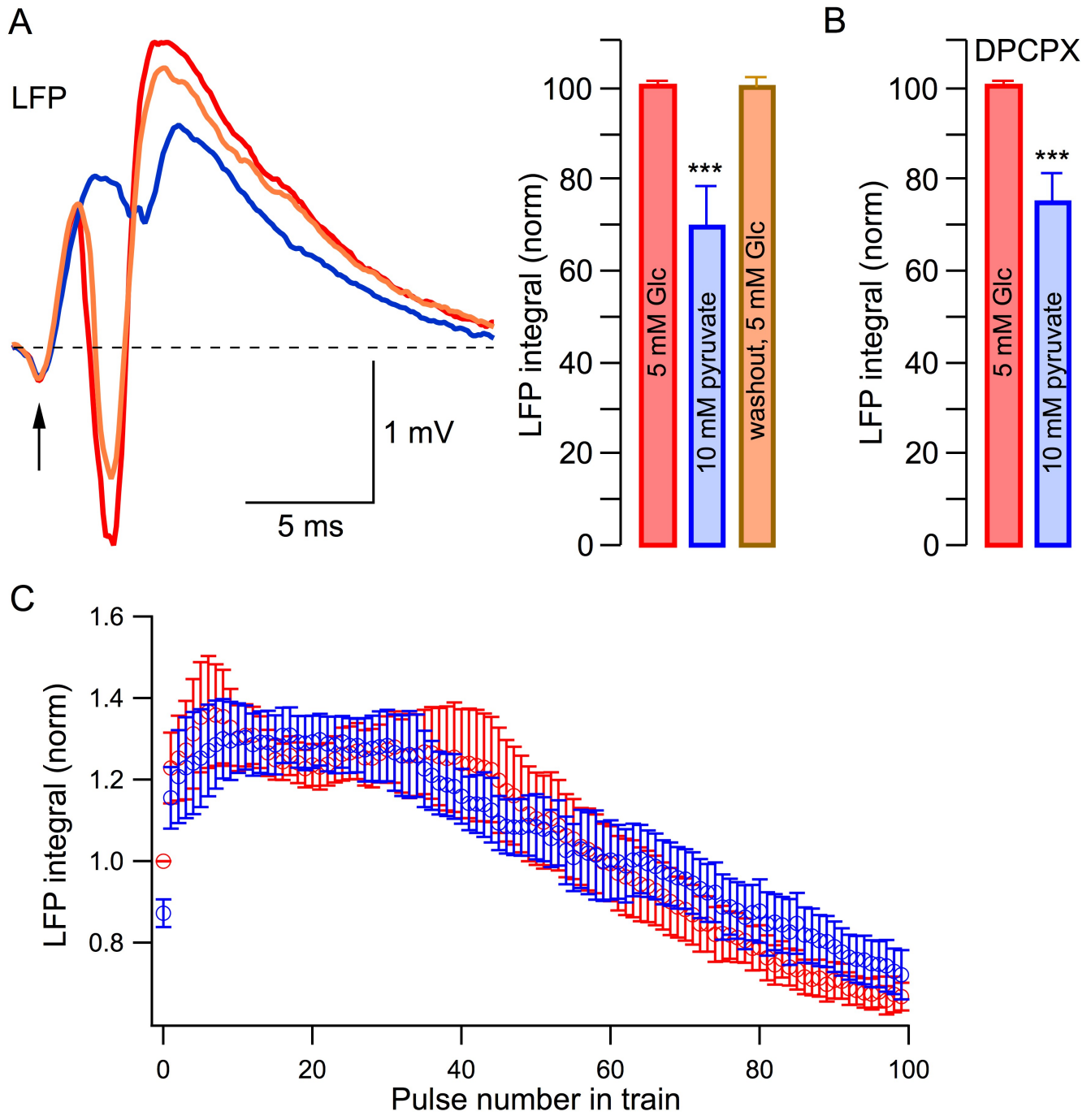
Suppl. Fig. 6. *FAD fluorescence depends on the glucose and pyruvate content in ACSF.* **A.** Example of long lasting FAD fluorescence recordings in a slice of P44 mouse. Black arrows indicate stimulations of Shaffer collaterals (10 Hz, 10 s). The FAD baseline fluorescence decreased when pyruvate was supplemented to glucose or completely replaced glucose in ACSF (-13.3 ± 4.2 %, n=3). FAD responses to the Shaffer collaterals train stimulation are modified by pyruvate in the same manner as in the case of short lasting recordings: pyruvate decreased undershoot amplitude (-0.8±0.3% vs -4.2 ± 0.8 % in 5 mM glucose-ACSF, n=6, $p<0.001$) without significant change of the oxidative dip (see **B**).

Suppl. Fig. 7. *Supplementing glucose in ACSF with lactate results in a strong change in NAD(P)H response to synaptic stimulation.* Black bar indicates synaptic stimulation. Note, that the baseline fluorescences may be different for glucose and lactate (see Fig. 4) and are aligned on the figure.

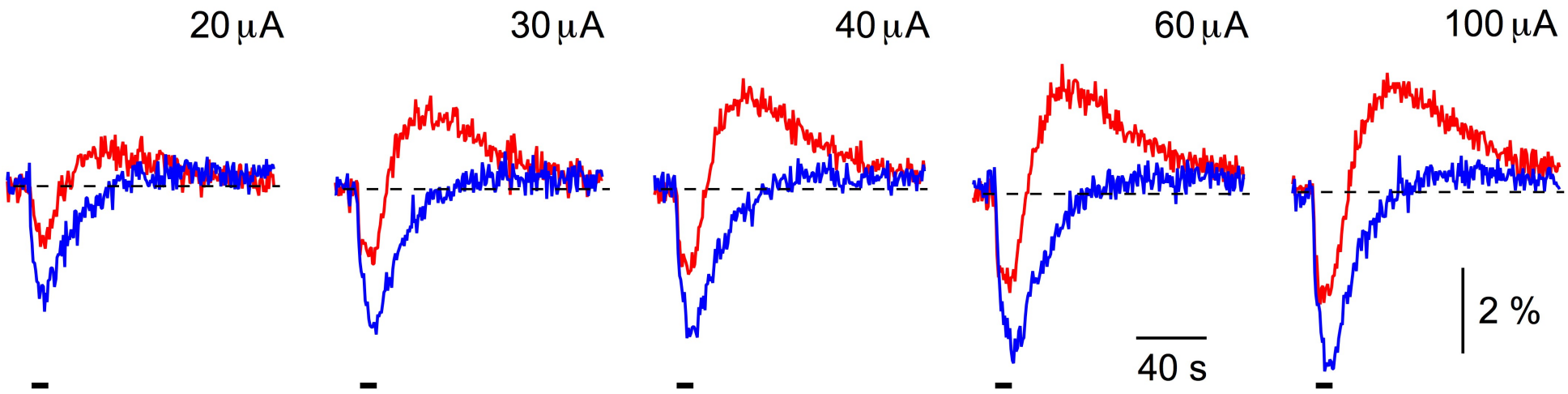
Suppl. Fig. 1


NAD(P)H
2 %
ACSF
ACSF + cocktail
ACSF + cocktail + TFB-TBOA
Oxygen
30 Torr
40 s

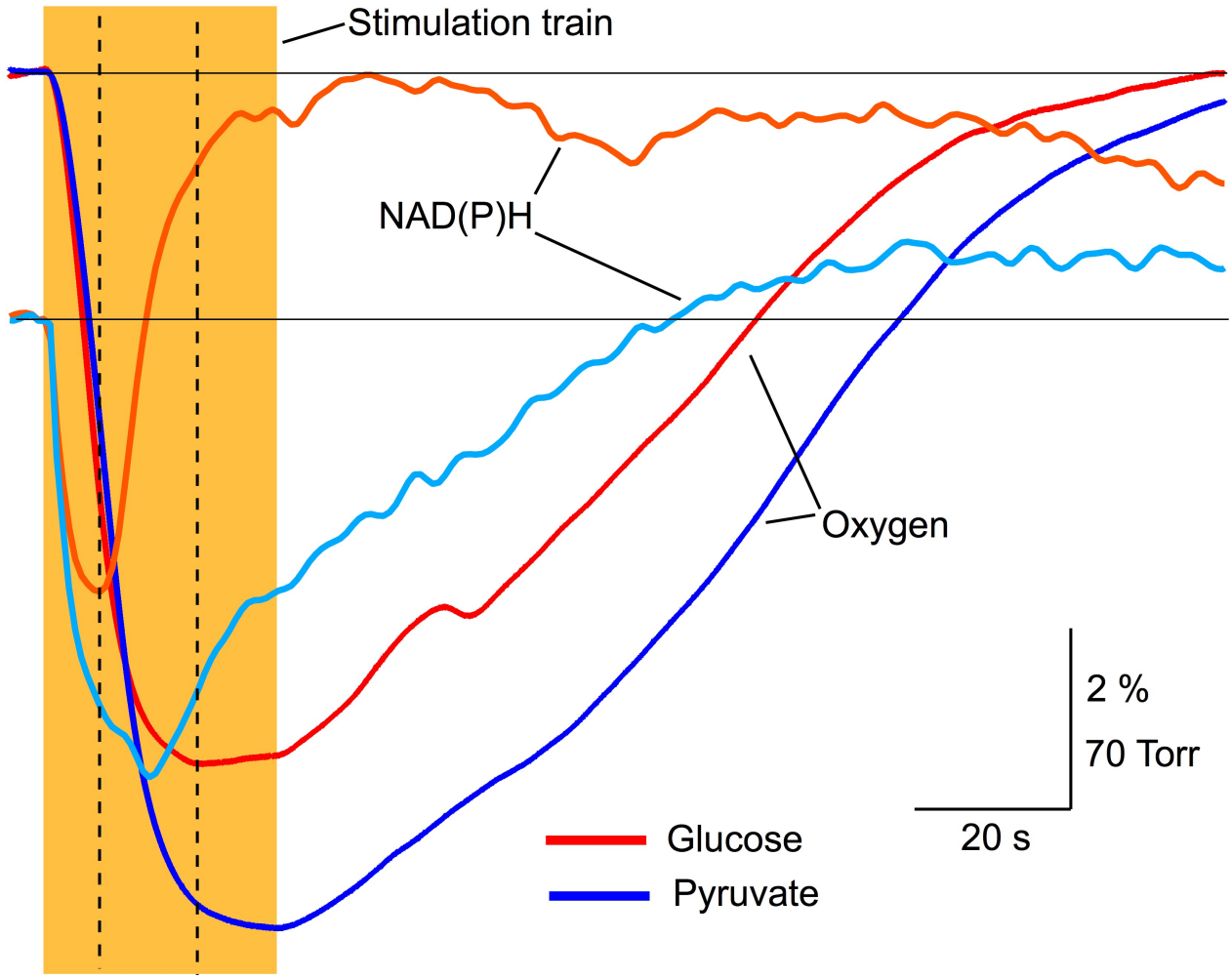
Suppl. Figure 2



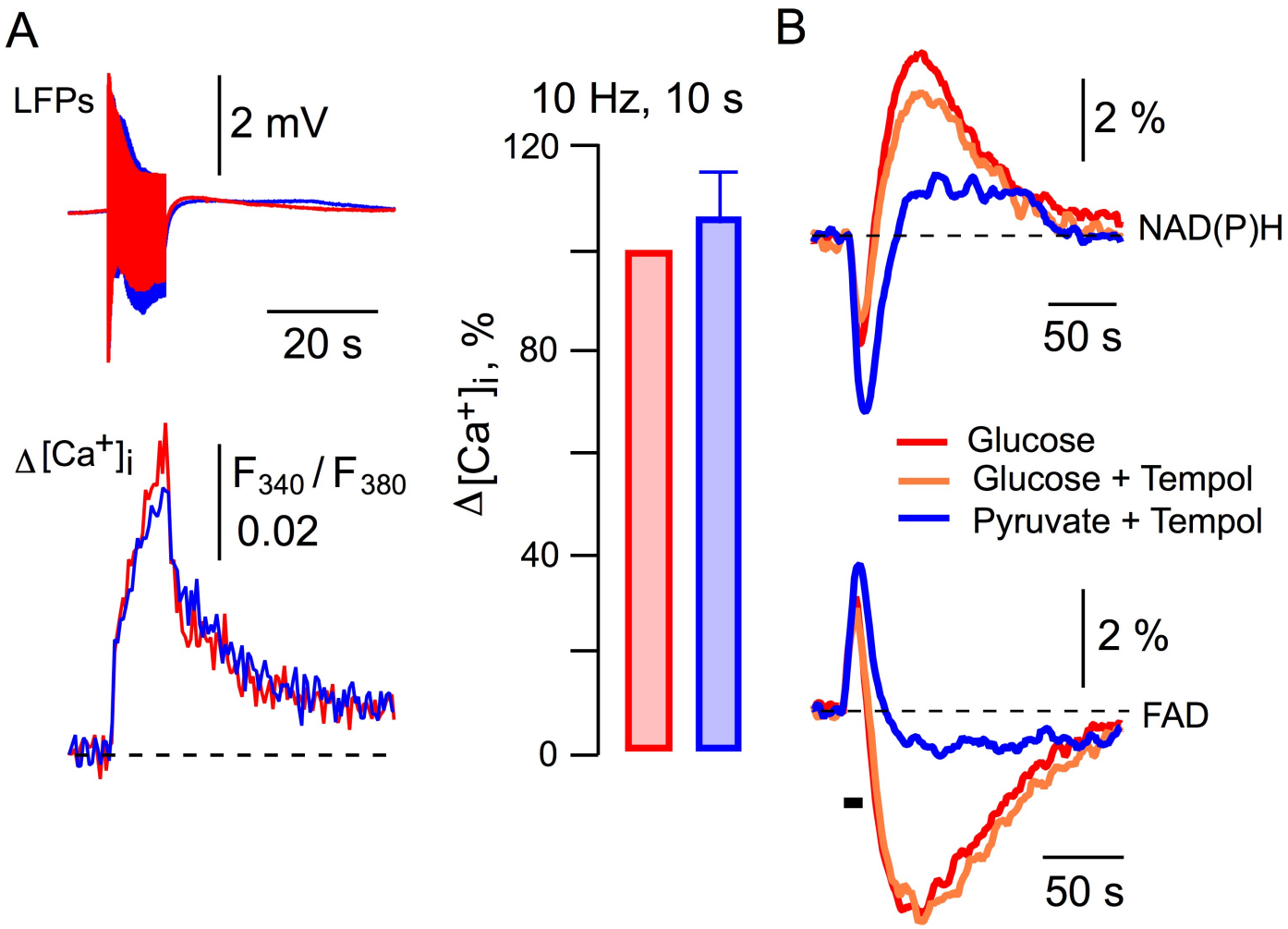
Suppl. Figure 3



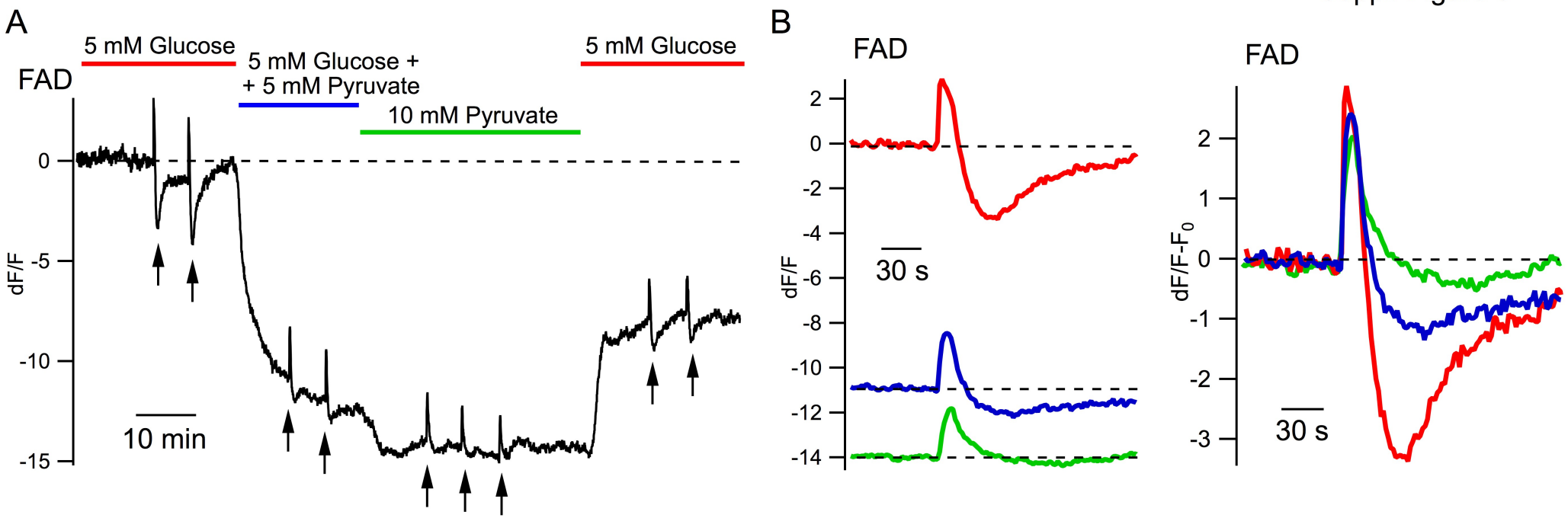
Suppl. Figure 4



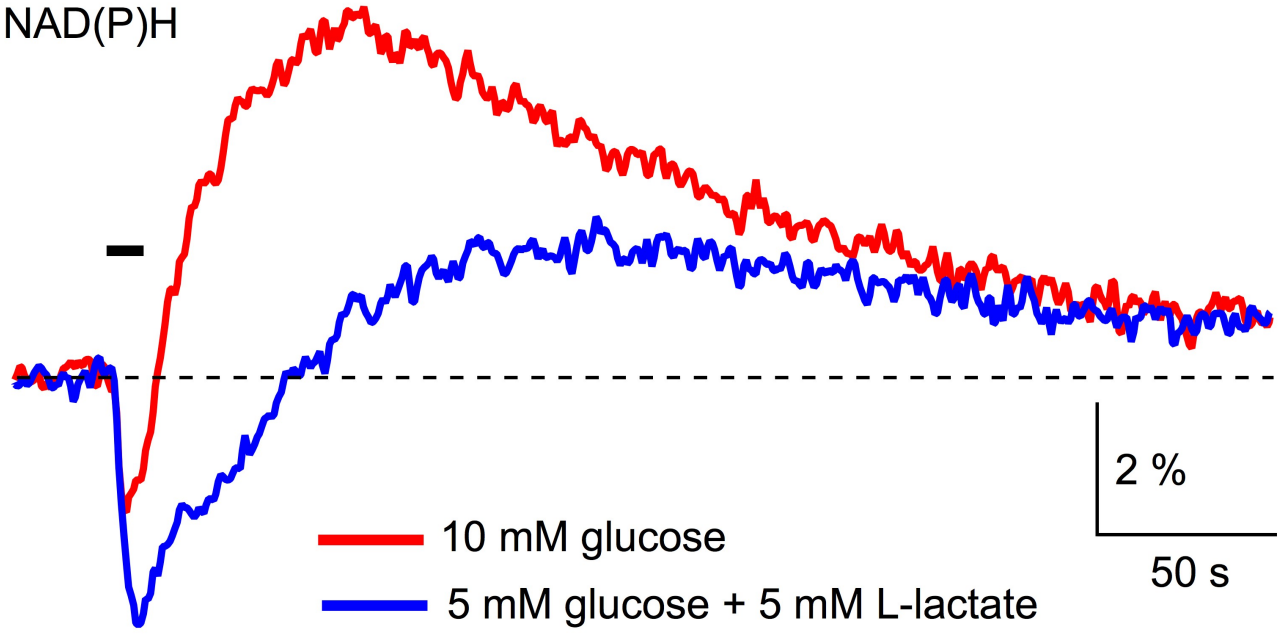
Suppl. Figure 5



Suppl. Figure 6



Suppl. Figure 7



Supplementary materials

Methods

Intracellular pH fluorescence imaging.

Slices were transferred into a microchamber with 2 ml ACSF containing 7 μM/l of pH sensitive fluorescente probe SNARF-1 (Invitrogen, Life Technologies) and 0,15% of pluronic acid. Slices were incubated at room temperature with oxygenation for 1 h. After dye loading, slices were returned into the holding chamber and allowed to recover for additional 15-20 minutes prior to being used in experiments. For fluorescence monitoring, slices were placed in the submerged recording chamber and superfused (15 ml/min) with oxygenated ACSF at 32 °C. Excitation at various wavelengths was achieved using a 1-nm-bandwidth polychromatic light selector equipped with a 100 W (Polychrome II) xenon lamp (Till Photonics, Germany). Light intensity was attenuated using neutral density filters. A dichroic mirror (495 nm; Omega Optics, USA) was used to deflect light onto the samples. Fluorescence was visualized using the upright microscope (Axioskop, Carl Zeiss, Jena) equipped with a 60x water-immersion objective (n.a. 0.9; LumPlanFL, Olympus, USA). Fluorescent emitted light passed to a 16-bit (Andor iXon EM+; Andor Technology PLC, Northern Ireland) electron multiplying CCD digital camera system equipped with an image intensifier. All peripheral hardware control, image acquisition and image processing were achieved using iQ software (Andor Technology PLC, Northern Ireland). The average fluorescence intensity of each region of interest (ROI) was measured. Parameters of recording: exposure time 50-100 ms, acquisition rate 1 frame per

10 s, ×60 water immersion objective, binning 1×1. SNARF-1 was excited using dual-wavelength mode at 450 and 480 nm, and the emitted fluorescence signal was recorded at 630 nm. For pH estimation the ratio of fluorescence intensities at 450/488 was used. Calibration procedure was performed as described previously (Waseem *et al* 2010).

Ca^{2+} imaging.

Slices were transferred into microchamber with 2 ml ACSF containing 10 μM/l of Ca^{2+} sensitive fluorescente probe Fura-2 AM (Molecular Probes) and 0,15% of pluronic acid.

Slices were incubated at room temperature and oxygenation for 1 h. After dye loading, slices were returned into holding chamber and allowed for recovery for the additional 15-20 minutes prior being used in experiments. For monitoring fluorescence, slices were placed in the recording chamber and superfused (15 ml/min) with oxygenated ACSF at 32 °C. Excitation of Fura-2 AM at two wavelengths of 340 nm and 380 nm was achieved using a 1-nm-bandwidth polychromatic light selector equipped with a 100 W (Polychrome II) xenon lamp (Till Photonics, Germany). Light intensity was attenuated using neutral density filters. Fluorescent signals were recorded using a dichroic mirror 495 nm (Omega Optics, USA) and emission filter 500 nm LP (Chroma Technology Corporation, USA). Fluorescence was visualized using the upright microscope (Axioskop, Zeiss, Germany) equipped with a 60x water-immersion objective (n.a. 0.9; LumPlanFL, Olympus, USA). Fluorescent emitted light passed to 16-bit (Andor iXon EM+; Andor Technology PLC, Northern Ireland) electron multiplying charge-coupled device digital camera system equipped with an image intensifier. All peripheral hardware control, image acquisition and image processing were

achieved using iQ software (Andor Technology PLC, Northern Ireland). The average fluorescence intensities of each region of interest (ROI) at both wavelengths were measured and ratio values F_{340}/F_{380} were calculated to estimate the intracellular Ca^{2+} changes. The duration of excitation was 10 or 20 ms at the sampling interval of 500 ms.

ATP measurements.

After 2 h recovery at room temperature in the holding camera, slices were transferred to the dual-sided perfusion camera with two independent input-output inlets and constantly circulating, 95% O_2/5% CO_2 saturated ACSF. Slices were superfused with 10 mM glucose-ACFS for 20 min, then half of them was exposed to 5 mM glucose-ACSF and others to 10 mM pyruvate-ACSF for 40 min. Temperature was maintained at 32°C throughout the experiment.

At the end of incubation, slices were immediately frozen in liquid nitrogen and stored at -80°C until processing. Extracts for ATP measurement were prepared as follows: Cold (-9.6°C) 0.3 M prechlorioc acid, 1 mM EGTA (PCA-EGTA) solution was added to frozen slices (2 slices per tube). After brief vortexing, slices were placed in KCL/ice bath (-9.6°C) and sonicated using Hielscher UP50H at 80% power, 0.5 cycle for 2*30 sec. Obtained suspension was spun at 0°C, 16000*g, 5 min. The pellet was frozen and then used for total protein determination. The supernatant was neutralized with 2 M KOH, 0.4 M imidazole base and spun again at the same conditions. The resulting supernatant was stored at -80°C until use in ATP assay. ATP assay was done using BioVision Stay Brite ATP Bioluminiscence assay kit (#791-100) per manufacturer's instructions. ATP content in slices was normalized to total protein determined using Bio-Rad

protein assay (# 500-0006) in PCA precipitated pellets after their dissolution with 1M NaOH. Bovine serum albumin, fraction V, was used as a standard.