**Supplemental Digital Content 4**

**Measurements of the HepG2 cells extracellular L-lactate levels (lactate released to the culture medium)**

For these experiments, the cells were grown in 24-well cell culture flasks and treated with fentanyl for 1 hour (2 ng/ml), or pretreated with 5-HD at 50 µM for 30 minutes, followed by incubation with fentanyl at 2 ng/ml or medium alone for an additional hour (n=6 per condition). Lactate concentrations in the cell culture supernatants were detected using a 96-well fluorescence-based assay kit from Cayman Chemical Company (Ann Harbor, Mich., USA) according to the manufacturer’s instructions. The kit contains: L-lactate standard, assay buffer (50 mM potassium phosphate, pH 7.5), lactate dehydrogenase cofactor mixture, lactate fluorescent substrate, metaphosphoric acid and potassium carbonate. The assay is based on the oxidation of lactate to pyruvate (catalyzed by lactate dehydrogenase) and the reduction of NAD+ to NADH. NADH reacts with the fluorescent substrate and produces a fluorescent product.

Briefly, after treatment, 200 µl of the extracellular media were collected, deproteinated by addition of 200 µl of 0.5 M metaphosphoric acid, vortexed, placed on ice for 5 min and centrifuged at 10,000 x g (10 min, 4°C). The resulting supernatants were neutralized by the addition of equal amount of 5 M potassium carbonate solution and centrifuged again at 10,000 x g (10 min). 20 µl of resulting deproteinated samples (diluted 1:20) and lactate standards were added to the wells of a 96-well plate containing 100 µl of assay buffer (50 mM potassium phosphate, pH 7.5), 20 µl of reconstituted lactate dehydrogenase cofactor mixture and 20 µl of lactate fluorescent substrate. The reactions were initiated by adding 40 µL of lactate enzyme mixture and the plates were incubated at room temperature for 20 min. The fluorescent product was analyzed on a fluorometer counter (Tecan Infinite M1000 plate reader,Tecan, Männedorf, Switzerland) with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. L-lactate concentrations of the samples were calculated using the L-lactate standards using the equation below:

L-lactate = [Corrected fluorescence – (y-intercept)] ÷ Slpope of the lactate standard curve x sample dilution