Supplementary Materials

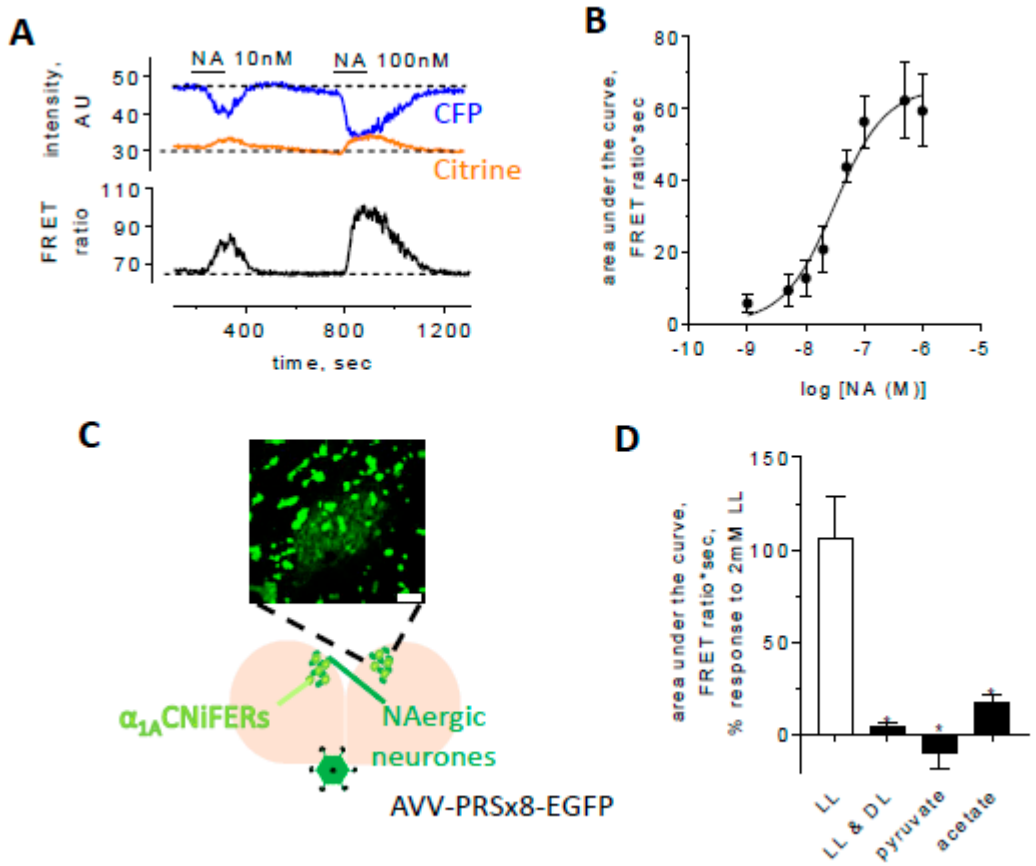

Figure S1. L-lactate evokes noradrenaline release from organotypic brain slices cultures as confirmed by using α1 CNiFER cells. (**A**) Representative traces for responses of α1 CNiFER cells evoked by 10nM and 100nM noradrenaline (NA) in orange (F1) and cyan (F2) fluorescence (top) and their FRET ratio (F1/F2*100; below). Area under the curve of the FRET ratio corresponds to the amount of NA released. (**B**) Concentration-response curve for α1 CNiFER cells in response to NA, EC_{50} = 31±1.75nM. Data are represented as mean±SEM (*n* = 4 for each data point). (**C**) Schematic of the recording set up: organotypic brain slice cultures containing locus coeruleus were transduced with AVV-PRSx8-EGFP to visualise noradrenergic neurones. $4x10^5$ of α1 CNiFER cells were placed on the cell culture insert 24hrs before imaging. Response to L-lactate (LL), its analogues and other metabolites was calculated by measuring the area under the curve of the FRET ratio for α1 CNiFERs plated onto locus coeruleus (LC) area. Inset–confocal stack image, scale bar 100µm. (**D**) 2mM LL evokes NA release from organotypic brain slices which is abolished by pre-incubation with 200µm DL. Neither 2mM pyruvate nor 2mM acetate were able to stimulate NA release. *$p < 0.05$ vs. 2mM LL, one-way ANOVA with Dunnett's multiple comparison as a post-hoc test. Data are represented as mean ± SEM.

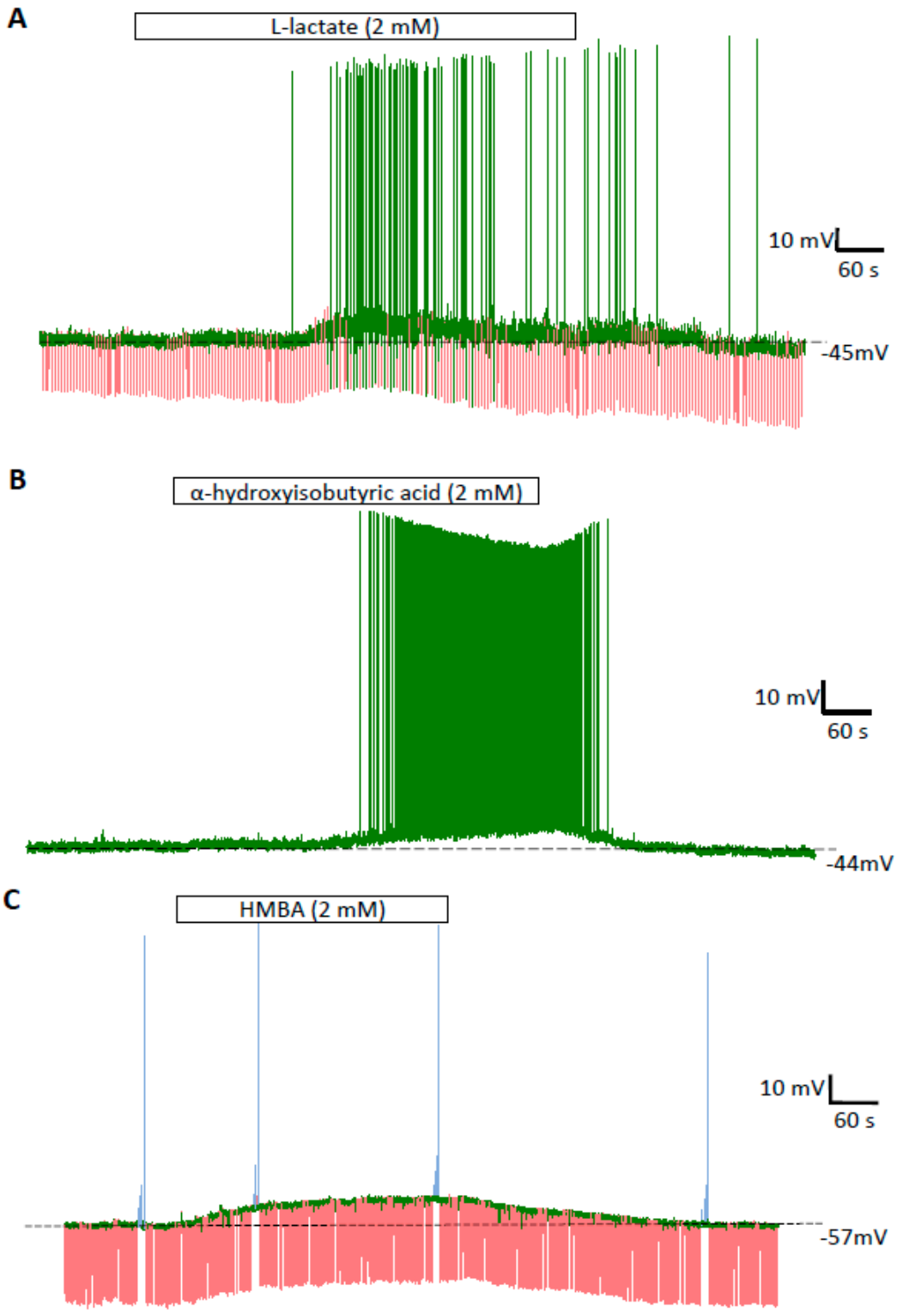

Figure S2. L-lactate and analogues excite noradrenergic LC neurones. Representative current clamp recordings from noradrenergic neurones of locus coeruleus in organotypic brain slice cultures show transient depolarisations in response to L-lactate (LL; **A**), or its analogues α-hydroxyisobutyric acid (**B**) and 2-hydroxy-3-methyl-butyric acid (HMBA; **C**). To visualize noradrenergic neurones the cultures were transduced with an adenoviral vector (AVV-PRSx8-eGFP) which drives expression of green fluorescent protein specifically in noradrenergic

neurons. Dotted lines indicate membrane potential of the neurone. Red downward voltage deviations mark responses to application of −0.06 nA square current pulses for monitoring series and input resistance. Blue upward voltage deviations show response to application of positive square current pulses (increments of 0.01 nA) to determine action potential threshold in silent neurones. No adjustment for junctional potential has been made.