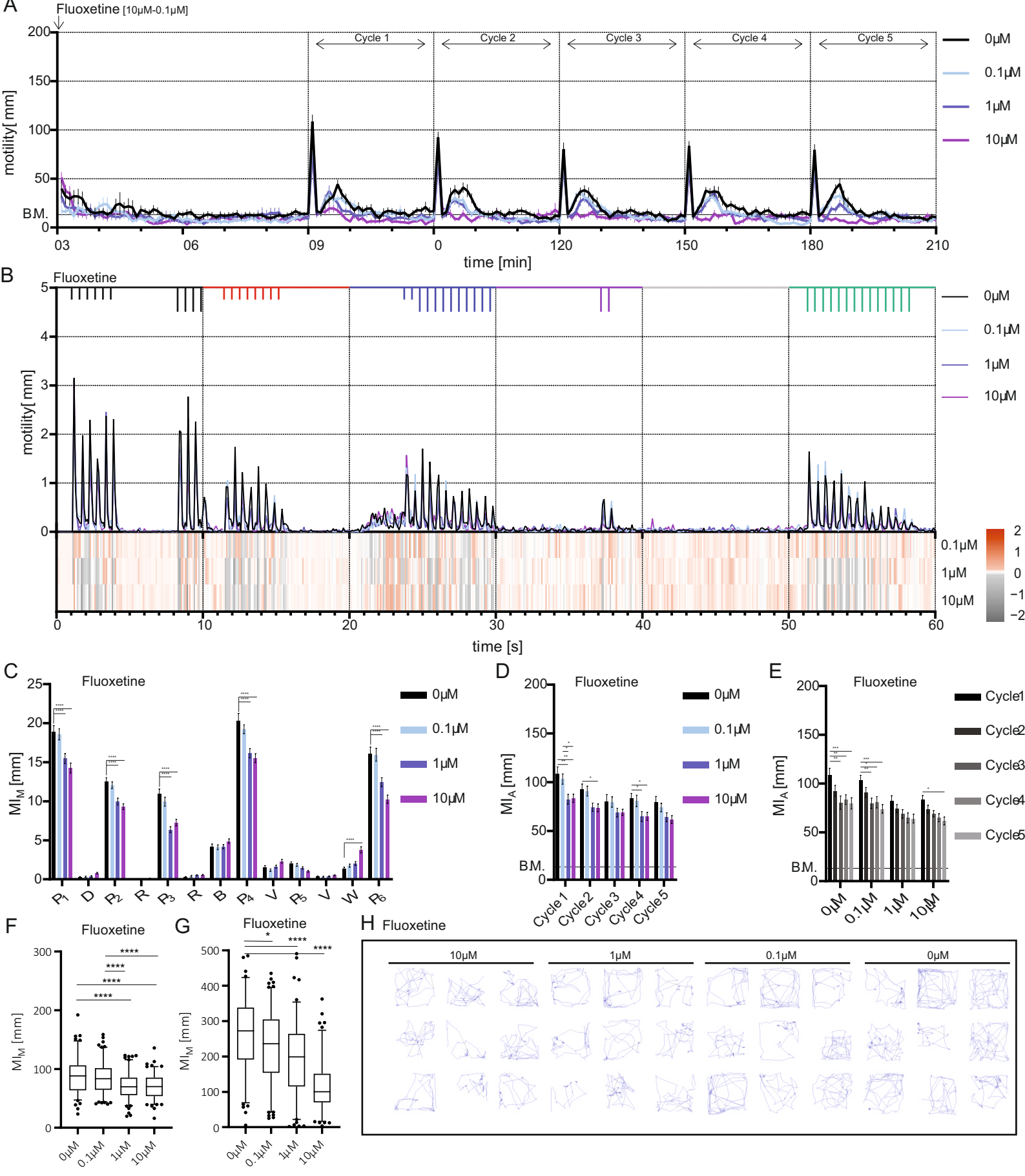
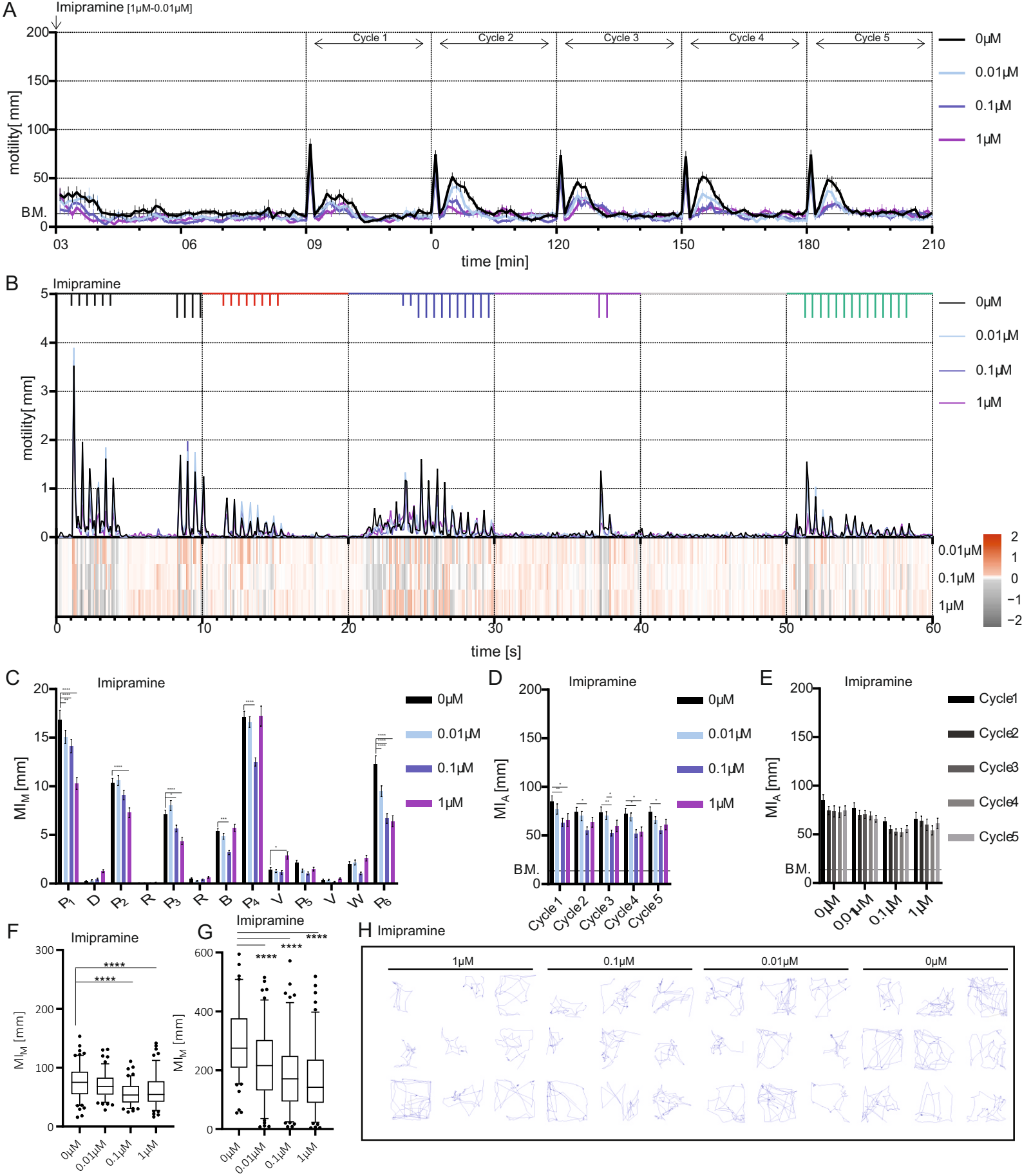
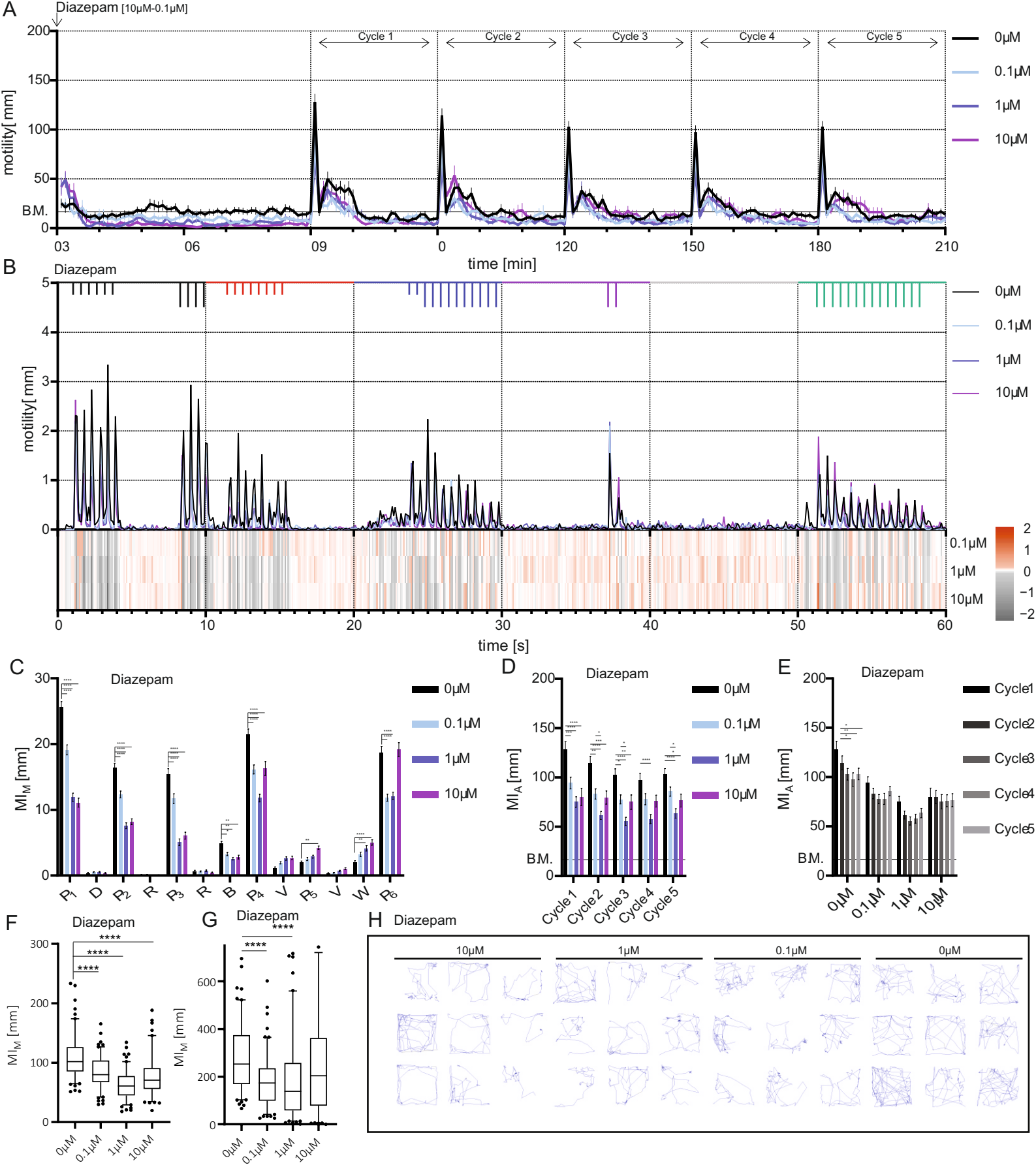


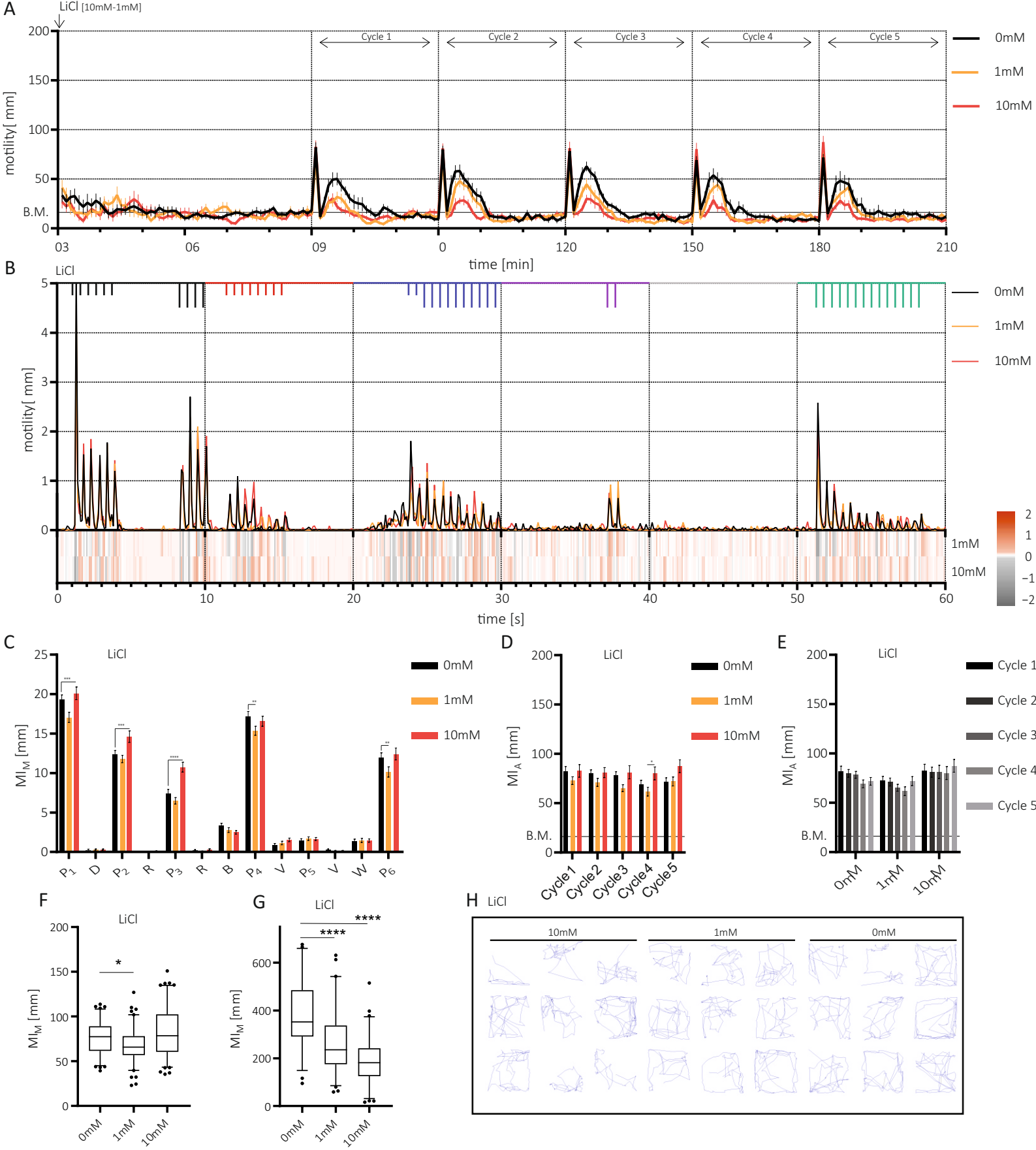
Supplementary figure 1 - Fluoxetine



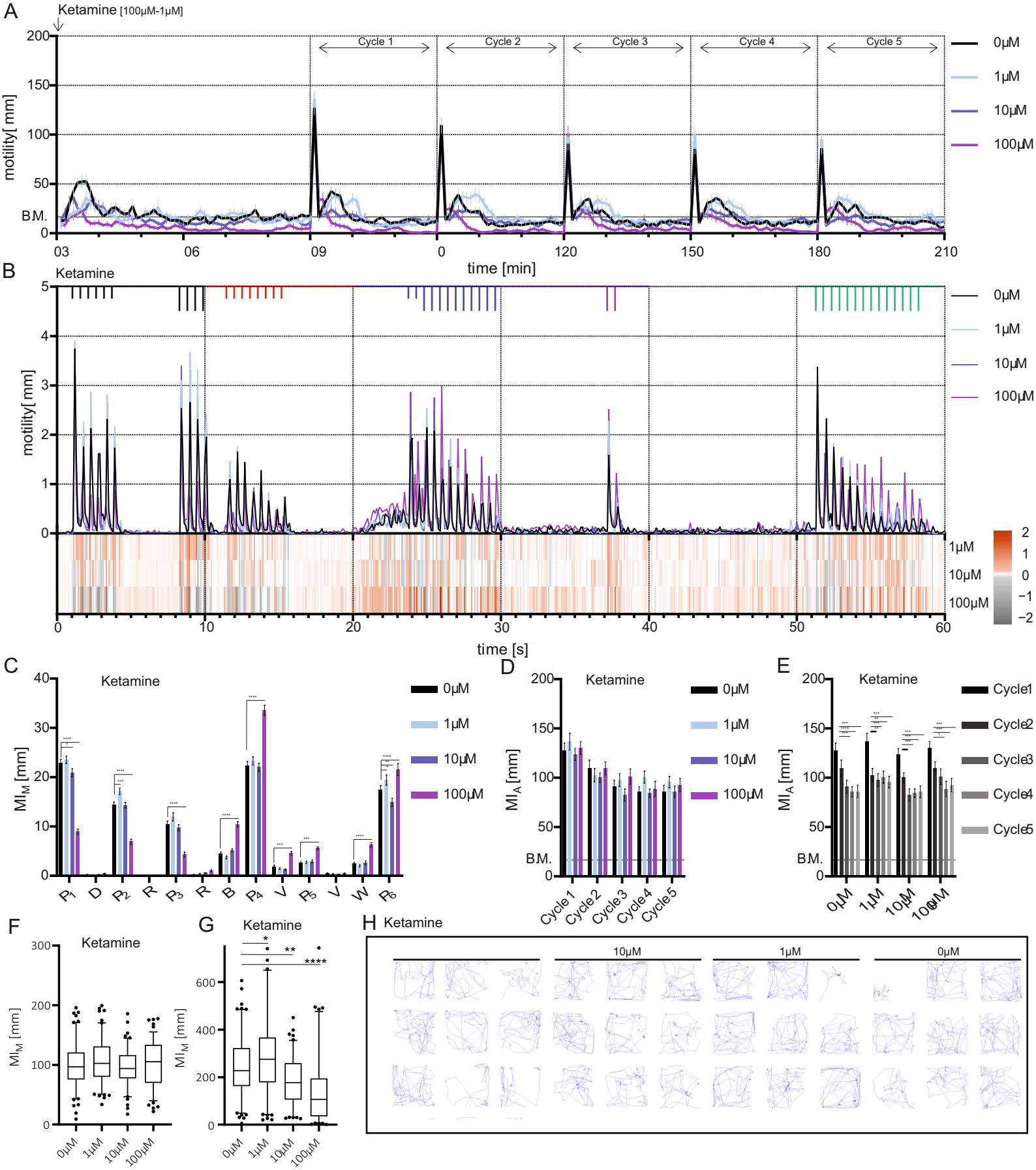
Supplementary figure 2 - Imipramine



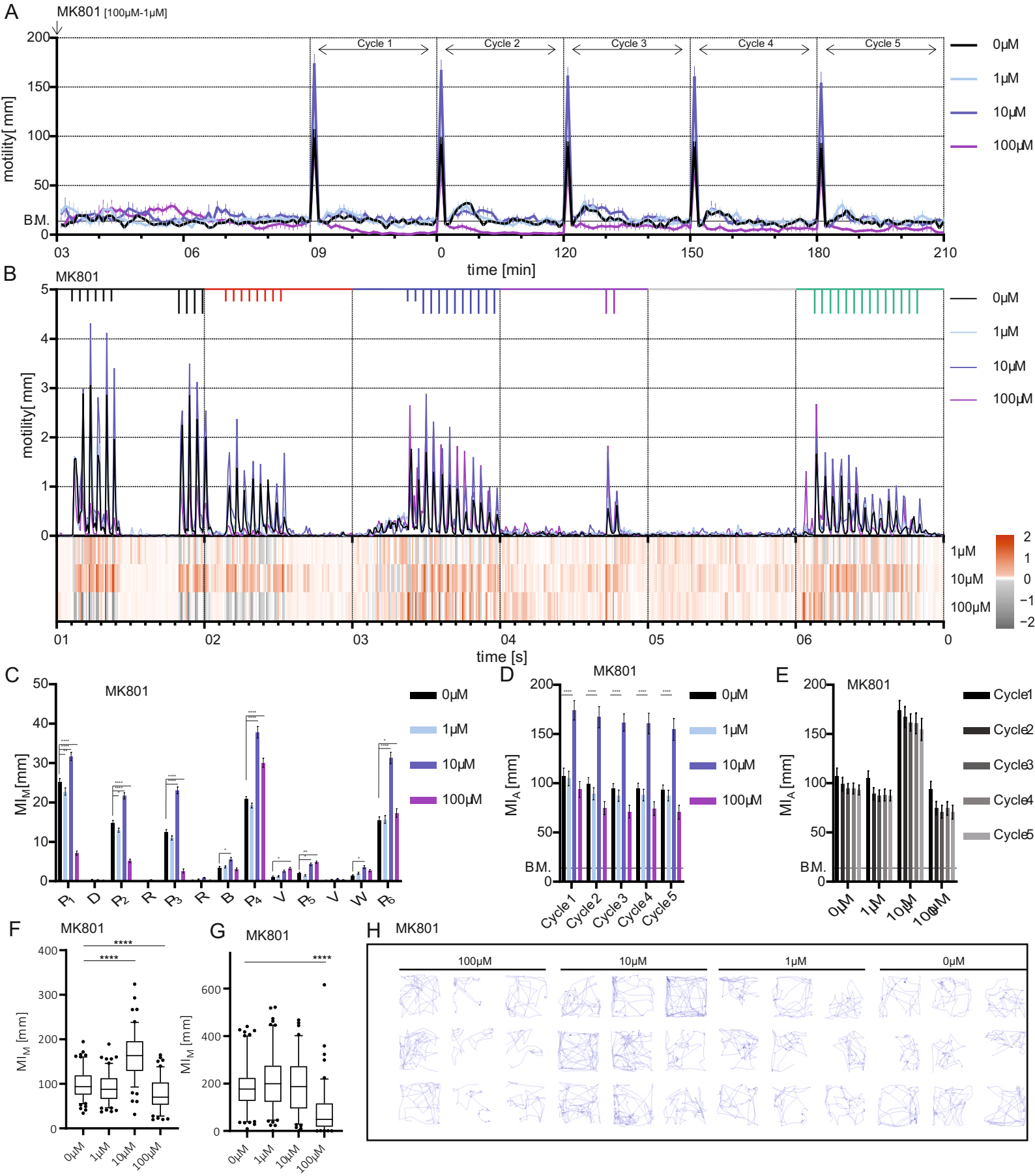
Supplementary figure 3 - Diazepam



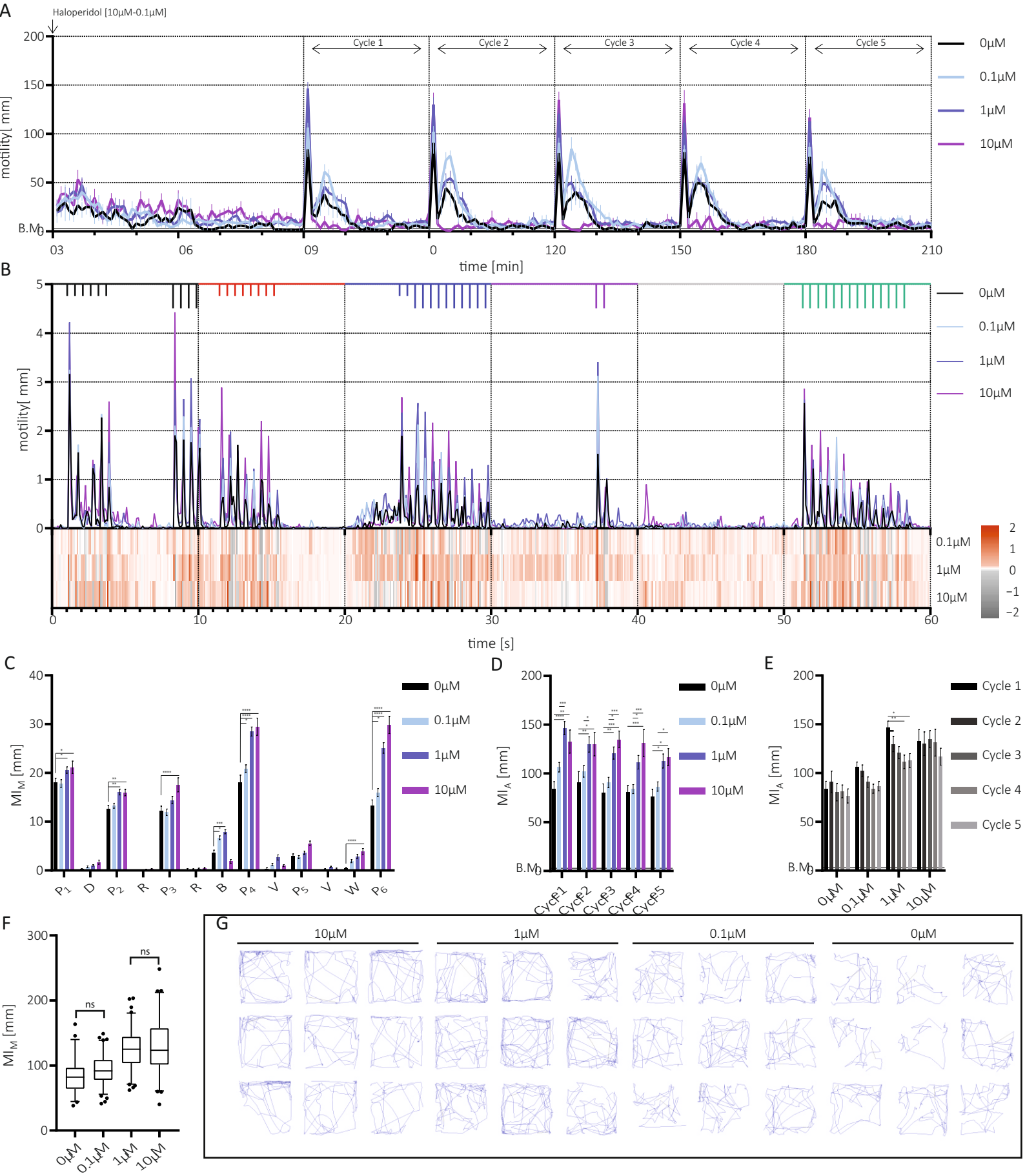
Supplementary figure 4 - Lithium



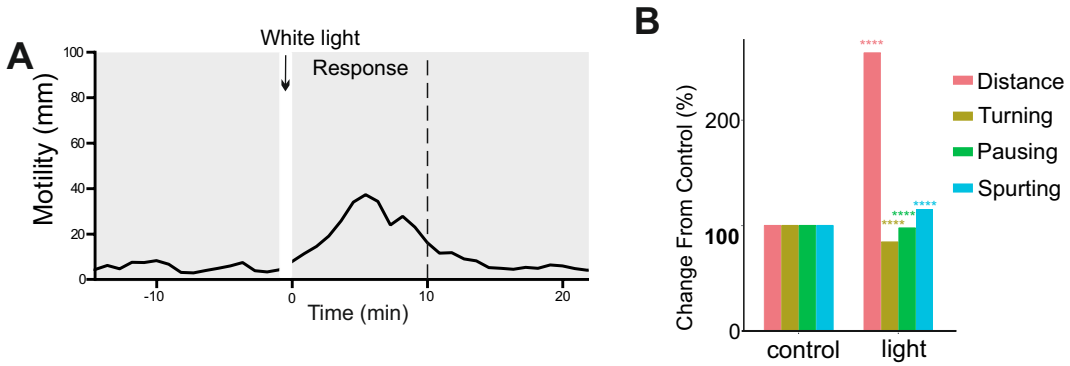
Supplementary figure 5 - Ketamine



Supplementary figure 6 - MK801



Supplementary figure 7 - Haloperidol



Supplementary figue 8

MATERIALS AND METHODS SUPPLEMENTARY

Behavioural battery test - 7 days post fertilization (dpf) zebrafish larvae were transferred to a 96-well plate and drug dosages were administered following which the plate was directly transferred to the DanioVision^{TM} device (Noldus Information Technology, BV, Wageningen, Netherlands) and acclimatized for 1 h in the dark at 28.5 °C. Larvae then underwent the test battery consisting of 5 cycles of a 60 s string of acoustic/vibratory (tapping) and light stimuli programmed using Ethovision13 XT software (Noldus Information Technology, BV, Wageningen, Netherlands). The 60 s stimuli were as follows; high (H) and low (L) intensity patterns (1.6 & 4.2 watt): 1 s pause, 6x L-tap (0.5 s break between individual taps), 4 s pause, 4x H-tap, 1 s pause, 8x L-tap, 8 s pause, 2x L- tap, 10x H- tap, 7 s pause, 2x H-tap, 13 s pause, 14x H-tap, 3 s pause. Visual stimuli evoked using red (635 nm, 192 Lm), blue (470 nm, 240 Lm), purple (red and blue together) and white LEDs, switched colour at 10 s intervals in the following order: darkness, red, blue, purple (red & blue), white, and sequential flickering light of all colours sequentially at 0.1 to 0.2 s intervals. After the stimuli period there was a 29-minute intermission period. This 30 min sequence was repeated 4 more times. Digital video tracking was performed using 60 fps and exported as csv file for analysis using customized software in R.

Stress response assay - 5 or 7 dpf larvae (as indicated) were transferred to a 48-well plate with 1 fish/well and acclimatized for 1 h following which they were exposed to stress: either with white light illumination (10,000 Lux) for 50 s or addition of 100 mM NaCl. Larvae were tracked for a further 30 min following stressor in the dark using infrared light. NaCl experiments were performed using ambient level (500 Lux) white light.

Drug treatments – Larvae at the indicated ages were treated with increasing doses of drugs and compounds as follows: fluoxetine, diazepam, FR236924 and PMA (Phorbol 12-myristate 13-acetate) all from Tocris Bioscience, Abingdon, UK; ketamine, haloperidol, imipramine, LiCl, SP600125, SB590885, Bryostatin-1 and MK801 from MERCK Sigma Aldrich Solutions, Darmstadt, DE; R18 from Enzo Life Sciences distributed by AH Diagnostics, Helsinki, FI; SB216763, JNK-in-8 and AKTi from Selleck Chemicals LLC, Houston, Texas; bisindolylmaleimide I (BIM) from Santa Cruz Biotechnology Inc., Dallas, Texas and SC79 from R&D systems, McKinley Place NE, Minneapolis. Treatments were for 1h at 28.5 ^{o}C before commencing the startle battery. Control fish were treated with equivalent volume of carrier.

Hub analysis – 29 candidate hubs (Fig. 2H, I) that were significantly altered in *Jnk1-/-* mouse brain compared to wildtype brain and were part of the psychiatric disease associated genes according to the MetaCore^{TM} database schizophrenia gene list were selected to analyse as potential signalling hubs acting downstream of JNK1 in regulating depressive/anxiety-like behaviour. The selection criteria included a threshold for phosphoproteins that showed either ≥ 7 physical interactions, or > 3 high weight (weight > 0.04) physical interactions from the physical interaction network built with GeneMANIA from among the significantly altered phosphoproteins derived from the wildtype and *Jnk1-/-* mouse brain phosphoproteomes. 29 proteins fulfilled these criteria. To control for hub protein's inherent functional plasticity variations, 1000 control phosphoprotein networks of the same size as the *Jnk1-/-* psychiatric disease phosphoprotein network were randomly generated from the entire brain phosphoproteome dataset using GeneMANIA (v.3.4.1). The number of interactions of the 29 proteins from the significantly altered phosphoproteome network in *Jnk1-/-* mouse brain was each compared to the distribution of interaction count from 1000 randomly generated control networks to determine the significance of the hubs. Statistical significance for the hubs was

calculated using the hypergeometric test. P-values were adjusted using the Benjamini-Hochberg procedure.

Receiver Operating Characteristic (ROC) analysis - ROC curves were constructed to visualise the predictive proficiency of the machine learning models. Individual curves were plotted for each test drug from the test dataset, paired with a control drug that was either classified as a "positive control" or a "negative control". Each treatment drug is assigned with a "true" class label that reflected its known properties and molecular interactions. Drugs of the "antidepressant or anxiolytic" class were coupled with the "negative control" drug ketamine, which provided the "true" class label of "other". Conversely, drugs classified under the "other" class were paired with the "positive control" drug fluoxetine for the "true" class label of "Antidepressant or anxiolytic" for the purposes of ROC curve calculation. The construction of the ROC curve was facilitated by the 'pROC' package within the R statistical programming environment.

Statistics - All behavioural features were compared to control fish from the same experiment. For statistical testing, feature values were collected into a distribution, and this data according to drug dose was compared to respective control distributions using Wilcoxon signed-ranked test or one-way or two-way ANOVA with Tukey or Dunnett's multiple comparison test as indicated. Calculations and plots were made with R programming language or Graphpad.

Phosphoproteomics - The brain phosphoproteome data used for this study has been uploaded to the PRIDE database (www.ebi.ac.uk/pride/) to be made open access upon publishing. Reviewers can

access this data now using the following credentials: Username: reviewer44631@ebi.ac.uk; Password: qNDO1jC9.

Preparation of samples for mass spectrometry, SDS-PAGE and in-gel digestion – All the chemicals for digestion and mass spectrometry analysis were from Sigma-Aldrich (Stockholm, Sweden). Whole brain was isolated from WT and *Jnk1-/-* C57B6J mice [76] at embryonic day 15 (E15), post-natal day 0 (P0), post-natal day 21 (P21), and 8 months (Adult). We extracted brains from three mice for each age and genotype and snap froze them in liquid N_2 and then pulverized them in a Micro-dismembrator II (Braun Biotech International, Melsungen, Germany). We weighed the powder and stored aliquots at -80°C before use. SDS (1 %), supplemented with protease and phosphatase inhibitors (Sigma-Aldrich, St. Louis, USA), was added to the brain-powder. Mechanical disruption followed using a syringe twice. We centrifuged samples for 1 hour at 4°C and collected the supernatant. We quantified protein concentration using the Total Protein Kit, Micro Lowry, Peterson's Modification (Sigma-Aldrich, St. Louis, USA).

A total protein amount of 100 µg for each sample was separated on 12% Criterion gels (Bio-Rad Laboratories, Hercules, Ca, USA), following the manufacturer's instructions. We washed the gel in milliQ, stained for 30 min with GelCode (Bio-Rad Laboratories) and washed overnight in milliQ before manually slicing each lane into 5 equal slices. We de-stained gel slices by washing 3 times in 25 mM ammonium bicarbonate (AMBIC)/50% acetonitrile and dried in a vacuum centrifuge (Speedvac). Samples were reduced (10mM DTT/100 mM AMBIC, 1 h at 56°C), alkylated (55 mM iodoacetamide (IAA)/100 mM AMBIC, 45 min at room temperature in the dark), washed 2 times with 100 mM AMBIC and dehydrated with ACN before being dried in the Speedvac. We rehydrated slices with 12.5 µg/ml modified porcine trypsin (Promega, Madison, WI, USA) in 50

mM AMBIC and digested overnight at 37°C. Peptides were eluted 2 times in 75% ACN/5% FA, dried 1 h in the Speedvac and dissolved in 0.1% formic acid.

TiO_2 phospho-peptide enrichment – we incubated samples for 10 min with Buffer A (1 M GA, 80% ACN, 5% TFA) followed by incubation with the TiO2 Mag Sepharose (GE Healthcare Life Sciences) for 30 min in an Eppendorf Thermomixer at 800 rpm. Liquid was removed and washing steps were done using Buffer A and 2 times using Buffer B (80% ACN, 1% TFA). Phospho-peptides enriched were eluted 2 times using 100 mM ammonium hydroxide in water and dried 1h in the Speedvac.

LC-MS/MS analysis- Dried peptides were resuspended in 0.1% formic acid and separated using an Eksigent nano-LC 2D system (Eksigent, Dublin, CA, USA) consisting of a solvent degasser, a nano-flow pump and a cooled auto-sampler. Peptide concentrations were measured by Nanodrop (ThermoFisher, Stockholm) and the concentration adjusted to the same amount in all samples. Eight µl of sample was loaded and washed for 15 min onto a pre-column (Acclaim PepMap 100, C18, 3 µm particle size, 50 µm diameter, Thermo Fisher Scientific, Hägersten, Sweden) at a constant flow of 5 µl/min solvent B (0.1% FA in ACN). We ran three biological replicates and two technical repeats for each of the two genotypes at the four time points. Phosphopeptides were loaded into a RP analytical column (10 µm fused silica emitter, 75 µm ID column, 16 cm Pico Tip, New Objective) packed in-house with C18 material ReproSil-Pur and separated using an eighty-minute gradient from 3% to 40% solvent B at a flow rate of 300 nl/min. The gradient was followed by 20 min column washing with 90% ACN, 0.1% FA and 15 min re- equilibration with 3% solvent B. The HPLC system coupled to an LTQ-Orbitrap XL mass spectrometer (ThermoFisher Scientific, Bremen, Germany) operated in Data Dependent Acquisition mode. Spray voltage was set to 1.90

kV and the temperature of the heated capillary was set to 200 °C. The ten most intense ions from the survey scan performed by the Orbitrap were fragmented by collision-induced dissociation (CID) in the LTQ (normalized collision energy 35, parent mass selection window 0.5 Da, 30 ms activation time and minimum signal threshold for MS/MS scans set to 100). We excluded unassigned charge states and singly charged ions from fragmentation. The dynamic exclusion list was limited to a maximum of 500 masses with a retention time window of 2 minutes and a relative mass window of 10 ppm. The X-calibur software version 2.0.7 (Thermo Scientific) controlled the HPLC, mass spectrometer and data acquisition.

Shotgun data analysis from LTQ Orbitrap – The raw data was uploaded into Progenesis LC-MS (Waters) for analysis. After raw mass spectra were aligned and normalisation across all runs was carried out by total ion current in the analysis area manually defined for the reference HPLC for the time and mass ranges. We submitted the raw files from the mass spectrometer to Thermo Proteome Discoverer (version 1.0.43.0) for protein identification by Mascot. Cysteine carbamidomethylation was set as a fixed modification, methionine oxidation and phosphorylation of serine, threonine, tyrosine (STY) were set as variable modifications. Data were searched against Uniprot *Mus musculus* database containing 170.598 sequences (release date April 2014). The mass tolerance was set to 10 ppm for the precursor ion and 0.8 Da for the fragment ions. Trypsin was set as the protease and a maximum 2 of missed cleavages were allowed. We ran raw data using an FDR of 5% and a score cutoff of 20. We discarded proteins marked as contaminants, reverse hits and “non-unique peptides”. We selected phospho-peptides with a Mascot score higher than 20 and verified using the default Mascot Delta score of 0.1 for comparative analysis between groups.

Data merging and statistical analysis phosphoproteomics data – Downstream analysis of MS intensity outputs from Progenesis LC-MS (Waters) utilized a customized R (v.3.4.0) software pipeline. Phosphopeptide data were refined as follows: (i) all phosphopeptides from 5 gel slices were merged, (ii) identical phosphopeptides, i.e. those with identical sequence, UniProt identifier and number and position of phosphorylated residues, were merged by summing the intensities. (iii) If a peptide entry had >1 missing value across 3 replicates, all entries were removed. For quality control of data, a distribution plot of phosphopeptide intensities for each unique genotype at given ages was visualized using boxplot and histogram analysis, using ggplot2 [77] (Supplementary F1). "Phosphopeptide-centric" and phosphosite-centric analysis was performed as appropriate and is defined in figure legends. We used the Bioconductor package RankProd [78,79] for statistical analysis. We calculated mean ratios of phosphopeptide intensities [*Jnk1-/-*/WT] per entry (for both phosphopeptide-centric and protein-centric changes). Only phosphorylation intensity changes that passed a threshold $Ratio_{Jnk1\text{-}/\text{-}/WT} > 2$, with p-value <0.05 were included.