Primary cortical neuron cultures, transfection, transduction and imaging

- Primary neuronal cultures were prepared from cortices of E17-19 Wistar rat embryos, as previously described [29, 38].
- For multi-electrode array (MEA) recordings, $1.143 * 10^5 \mathrm{cells/cm}^2$ were plated onto coated 24 well plates (M384-tMEA-24W; Axion Biosystems, Atlanta, GA, USA).
- Neurons were transfected or transduced at DIV8-9 with 3–4 µg of DNA using the calcium phosphate method, or 150 ug lentivirus, respectively [39].
    - DNA constructs encoding pre-miR-186, a miR-186-5p inhibitor, or a scramble control

Multielectrode array recordings and data analysis

- Simultaneous extracellular recordings from 16 extracellular electrodes were collected from DIV17 neurons, using the Maestro MEA system (Axion Biosystems).
- Data were digitized at 12.5 kHz and analysed using Axion Integrated Studio software.
    - Network activity was detected using the Envelope algorithm.
- Multielectrode array recordings were obtained from cortical neuronal cultures (prepared as described previously) plated in M384-tMEA-24 well plates (Axion Biosystems, Atlanta, GA, USA). Simultaneous recordings from 16 extracellular electrodes per well were obtained using the Maestro microelectrode array system (Axion Biosystems) at a constant temperature of 37°C and in an atmosphere of 5% CO2/95% air, for 5 min (300 sec).
- Neurons in experimental wells were treated with dexamethasone (250 nM, DIV7-17) and transduced with lentivirus encoding a scrambled sequence or a miR-186-5p inhibitor at DIV9. Extracellular recordings were obtained at DIV17. Data were sampled at 12.5 kHz and digitized and analysed using Axion Integrated Studio software (Axion Biosystems). Spike events were detected using an adaptive spike detection threshold of 5.5 SD of noise for each electrode.
    - Single-electrode bursts were defined as at least 5 consecutive spikes with inter-spike intervals shorter than 100 ms.
    - Network bursts were identified using the envelop algorithm with a threshold factor of 1.25, a minimum inter-burst interval of 100 ms, at least 75% of burst inclusion and having a minimum of 35% of electrodes simultaneously active.