Supplementary Figure 2 Effects of NMDA on cytoplasmic Ca\(^{2+}\) activity in hypothalamic cultures obtained from wild-type and NMDAR1 knockout mice. Representative recordings from fura–2 AM Ca\(^{2+}\) imaging experiments are shown. (a) Neurons in wild–type cultures respond both to NMDA (5 µM) and glutamate (10 µM, GLU) \((n = 56\) of 56). (b) Cells in NMDAR1 knockout cultures respond only to glutamate \((n = 62\) of 62). In Ca\(^{2+}\) imaging experiments, cells were incubated with fura–2 AM (5 µM, Invitrogen) for 30 min and examined using a Nikon microscope as described\(^{47}\). Conventional dual wavelength ratios (at 340 and 380 nm excitation) were obtained using a Sutter DG–4 filter changer and Axon Workbench software. Images were taken every 4 sec with SensiCam Digital CCD camera. Calcium standards (Invitrogen) were used to calibrate the imaging system\(^{47}\). Data were collected for each group from three independent cultures and analyzed with Igor Pro (WaveMetrics) and InStat (GraphPad) software. A neuron was considered as responding to a pharmacological agent (NMDA or glutamate) if, during the agent application, Ca\(^{2+}\) increased by ≥ 15 nM from the initial background level and if the level of Ca\(^{2+}\) decreased to the background after the agent was washed out.