Supplemental Material

Evaluation of SK-N-SH Cells as a Model for NMDA Receptor Induced Toxicity

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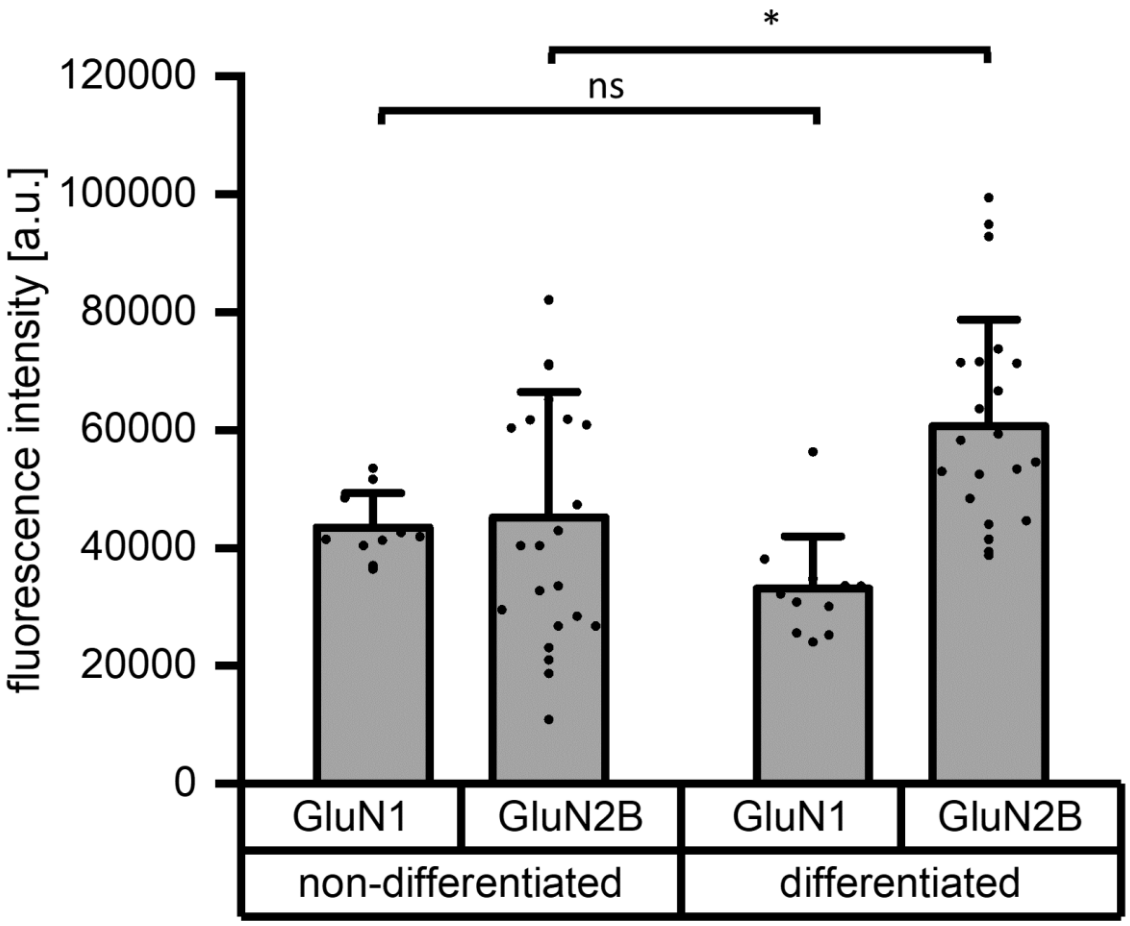
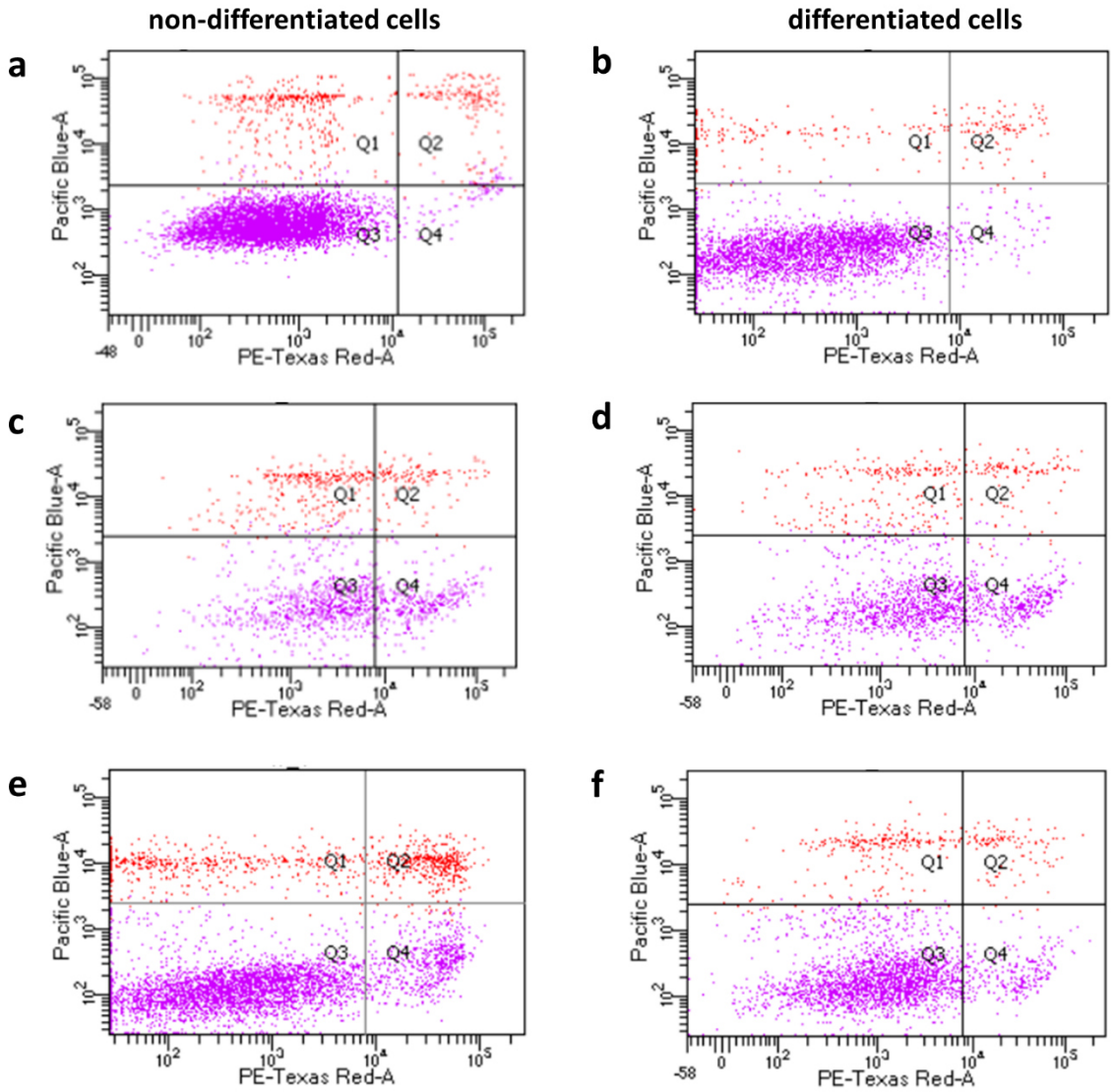
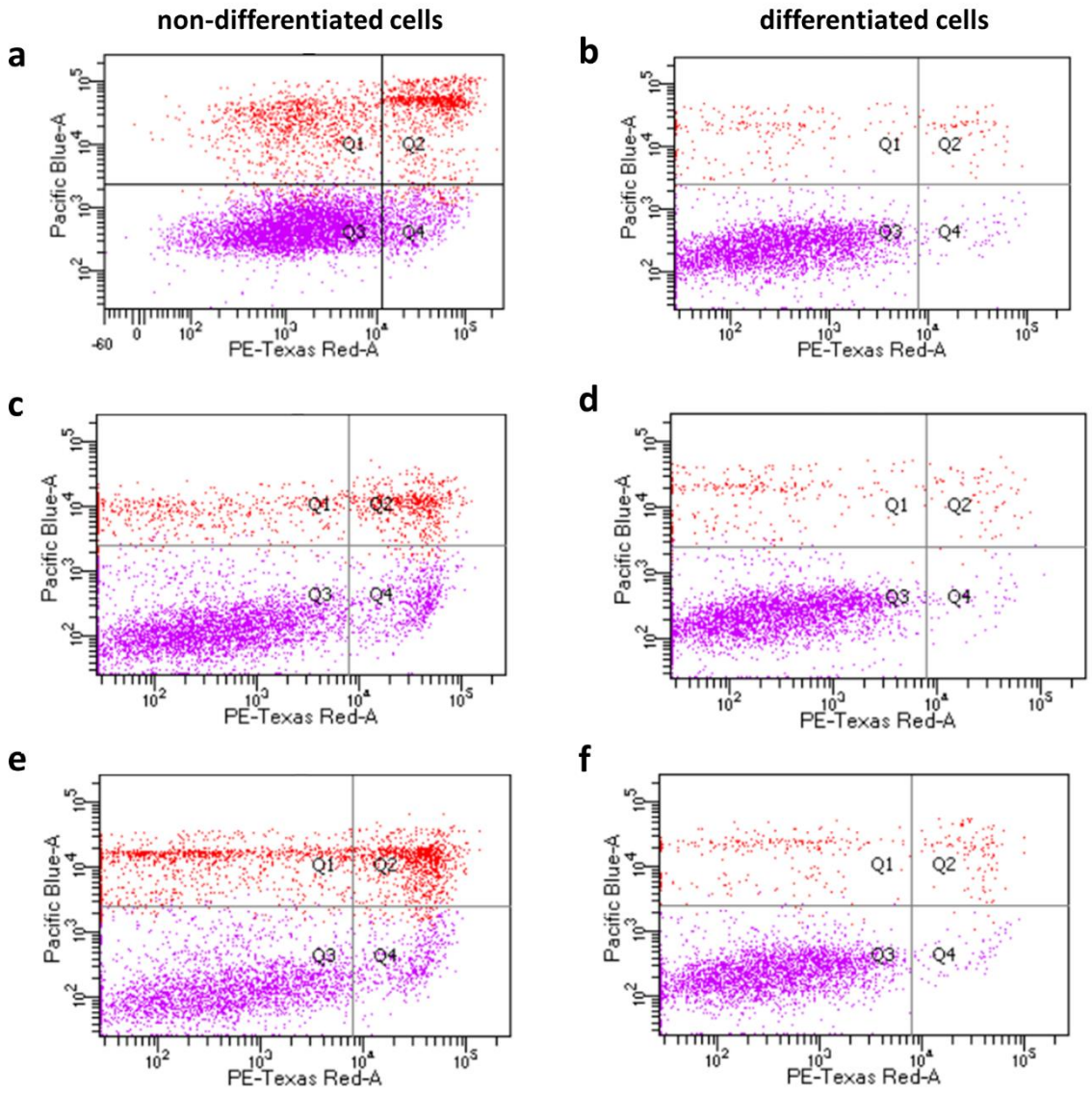


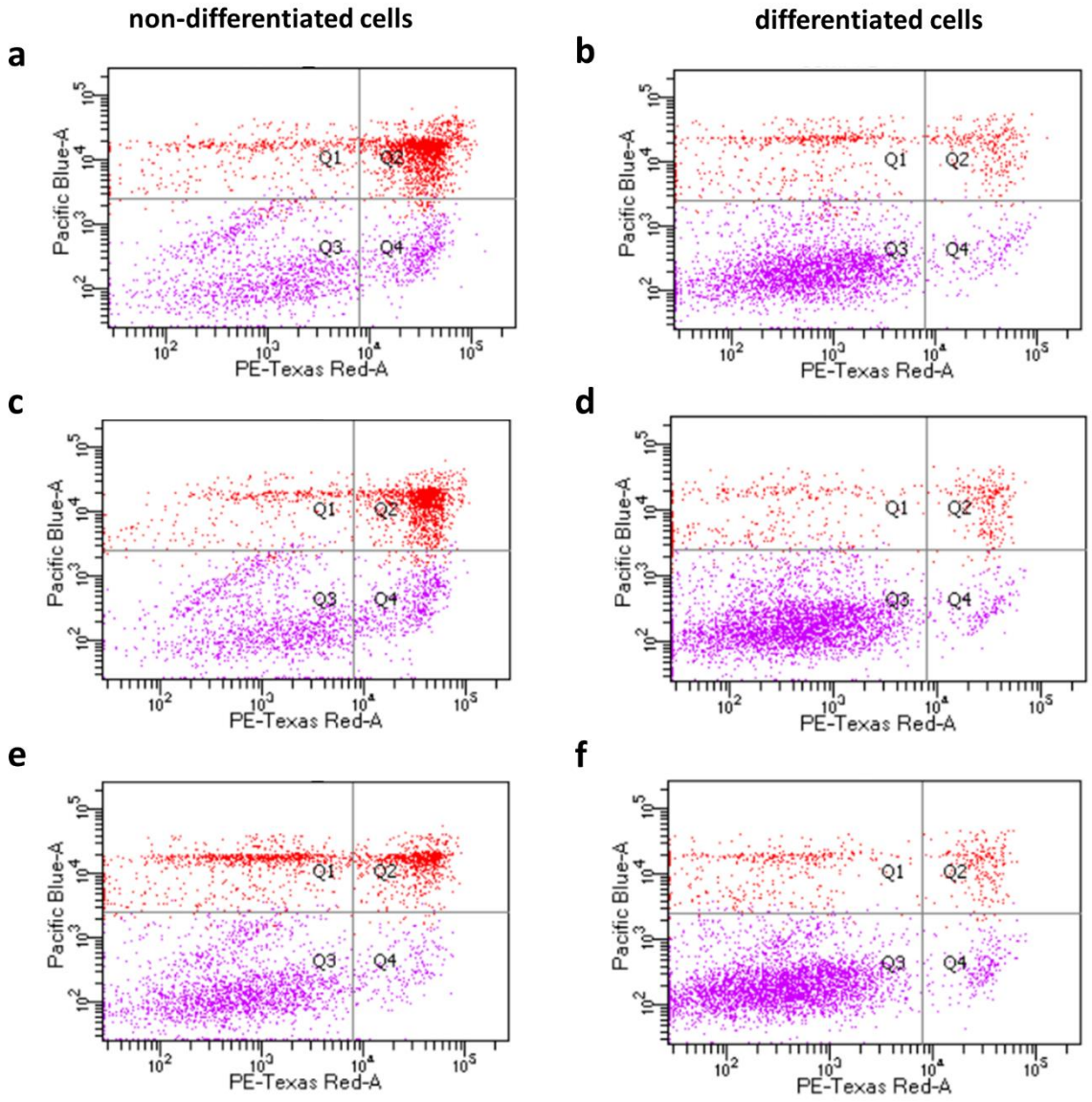
SI Figure 1 | Quantification of immunofluorescence for NMDA receptor forming subunits. Non-differentiated and differentiated cells were detected via GluN1 and GluN2B specific fluorescent-coupled antibodies and fluorescence intensities were quantified. Data were statistical evaluated by one-way-ANOVA followed by post hoc mean comparison Tukey test. P-values are indicated by ns (not significant) for $p > 0.05$ and * for $p < 0.05$.



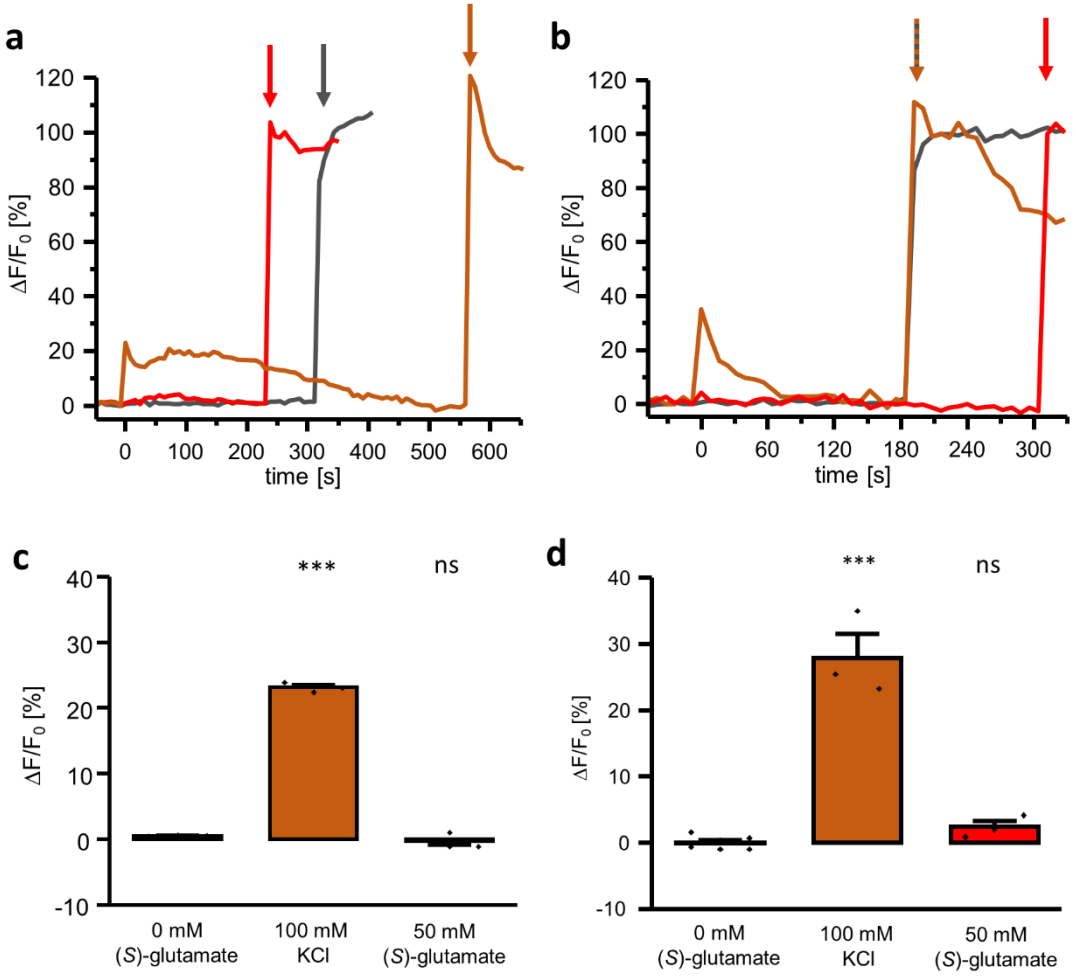
SI Figure 2 | Flow cytometric analysis of (non-)differentiated SK-N-SH cells without stimulation via (*S*)-glutamate. Cells were analyzed via flow cytometric analysis after incubation with no compound (**a**, **b**), 100 µM ketamine (**c**, **d**) and 10 µM WMS14-10 (**e**, **f**). Magenta marked signals are defined as viable cells, red marked signals as non-viable cells.



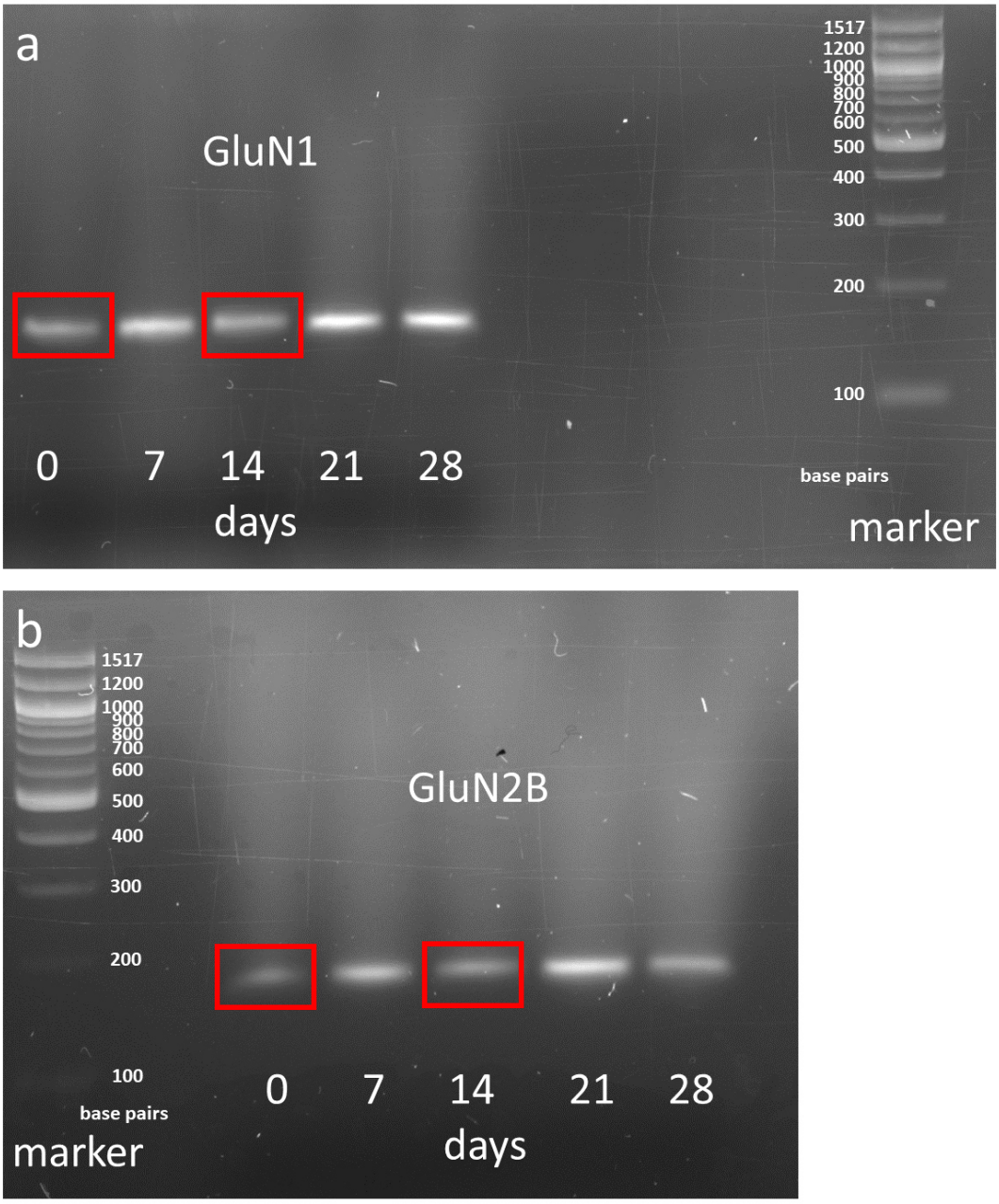
SI Figure 3 | Flow cytometric analysis (FACS) of (non-)differentiated SK-N-SH cells in presence of (*S*)-glutamate. FACS analysis after incubation with 5 mM (**a**, **b**), 10 mM (**c**, **d**) and 25 mM (**e**, **f**) (*S*)-glutamate. Magenta marked signals are defined as viable cells, red marked signals as non-viable cells.



SI Figure 4 | Flow cytometric analysis of (non-)differentiated SK-N-SH in presence of 50 mM (*S*)-glutamate and NMDA receptor inhibitors. Cells were analyzed via flow cytometric analysis after incubation with 50 mM (*S*)-glutamate together with no NMDA receptor inhibitor (**a**, **b**), 100 µM ketamine (**c**, **d**) and 10 µM WMS14-10 (**e**, **f**). Magenta marked signals are defined as viable cells, red marked signals as non-viable cells.



SI Figure 5 | Intracellular Ca^{2+} recordings using (non-)differentiated SK-N-SH cells. Analysis of intracellular Ca^{2+} concentrations using calcium indicator Fluo-4-AM and non-differentiated (**a**) as well as differentiated (**b**) SK-N-SH cells after application of 50 mM (*S*)-glutamate (red), 100 mM KCl (brown) or control solution (grey). At a certain timepoint, marked with an arrow, 3 mM ionomycin was added.to every condition. **c, d** Evaluation of Ca^{2+} influx directly after application of different conditions at non-differentiated (**c**) and differentiated (**d**) SK-N-SH cells. Changes in fluorescence are shown as ΔF/F0 (%), with ΔF as change of the fluorescence relative to the mean basal fluorescence (F0) before application of ligands, normalized to the maximum peak of ionomycin as control. Data were statistical evaluated by one-way-ANOVA followed by post hoc mean comparison Tukey test. P-values are indicated by ns (not significant) for $p > 0.05$ and *** for $p < 0.001$.



SI Figure 6 | Native gel of RT-PCR products from whole cell RNA isolation after certain days of differentiation using GluN1 (a) and GluN2B (b) primer. Bands in red boxes are presented in Figure 1.