A NMDA-receptor calcium influx assay sensitive to stimulation by glutamate and glycine/D-serine

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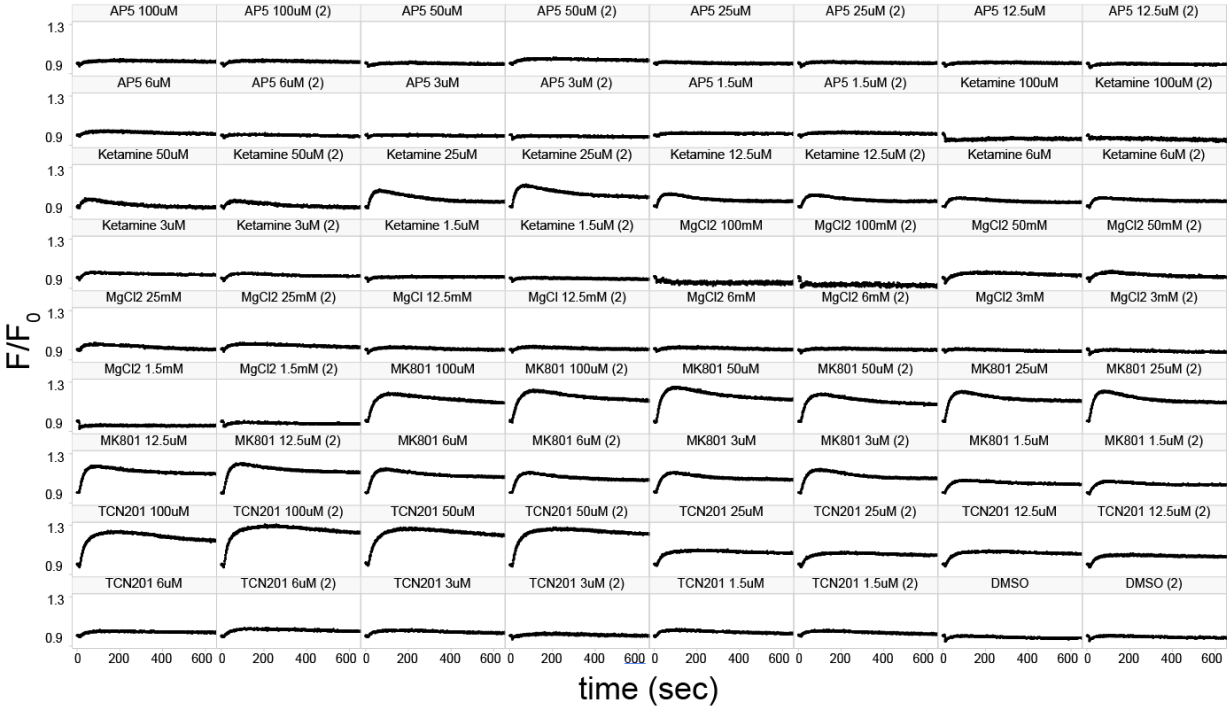
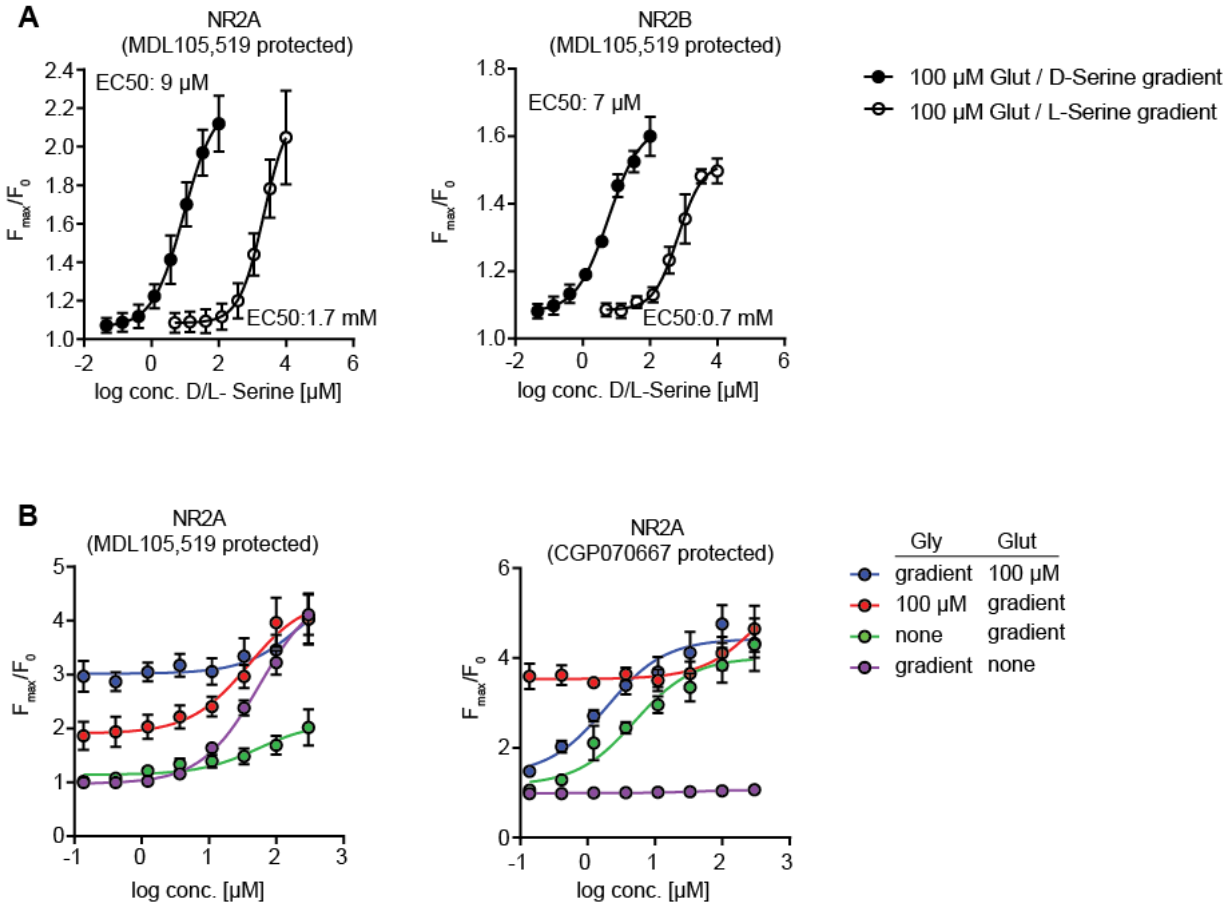
Supplementary Figure 1



Supplementary Figure 1 Protection of NMDAR-expressing cells with MDL105,519, but not MK801 or Ketamine, facilitates NMDAR-activity in functional assay

NMDAR-mediated calcium flux in HEK293 cells transduced with baculovirus. NR1/2A-transduced HEK293 cells were treated with the indicated compounds. 16h later, cells were washed three times, loaded with Calcium6 dye and NMDAR-mediated calcium flux was measured after stimulation with 100 µM glycine/glutamate. Data represents the raw traces of the fluorescence ratio (fluorescence/baseline fluorescence, F/F0) of one representative experiment. The summary of the data across multiple experiments is shown in Figure 2A.

Supplementary Figure 2

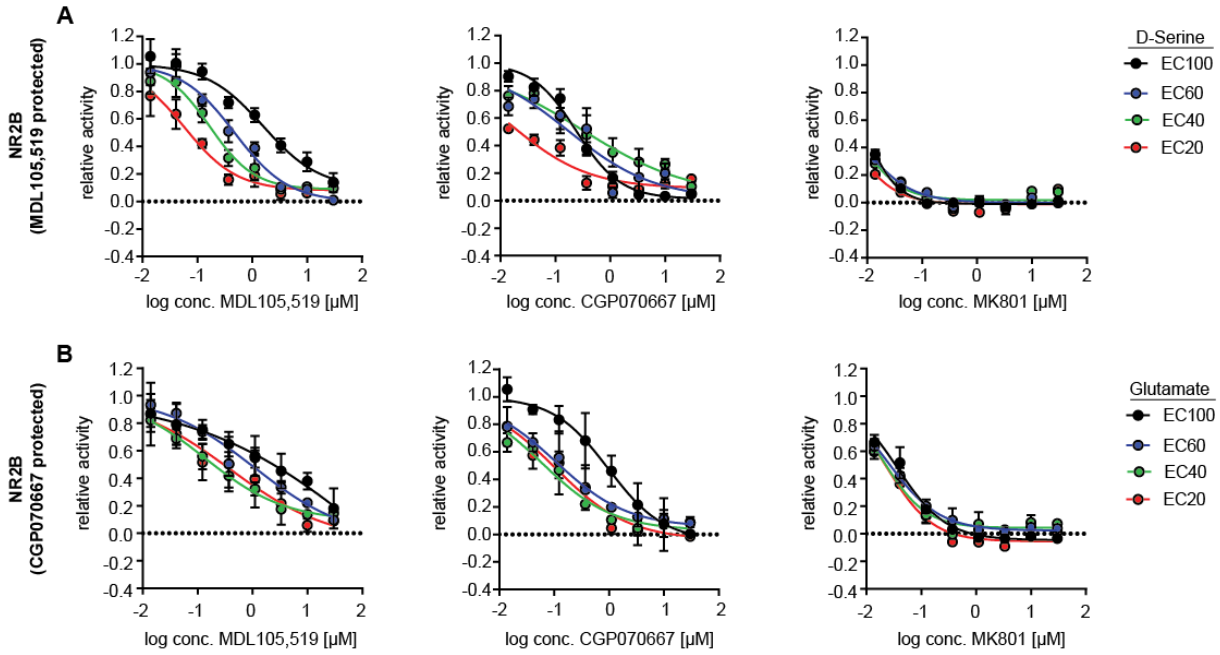


Supplementary Figure 2 Baculovirus mediated expression of NMDAR recapitulates expected NMDAR biology

(A) Sensitivity of NMDAR-mediated calcium flux to D-serine and L-serine. HEK293 cells were transduced with baculovirus encoding NR1 and NR2A or NR2B in the presence of MDL105,519 and NMDAR-mediated calcium flux was measured as described in the presence of varying amounts of D-serine or L-serine and saturating concentration of glutamate. Data represents the mean of the maximal fluorescence ratio ± SEM of four experiments.

(B) HEK293 cells stably expressing NR1 and NR2A under the control of a tetracycline-inducible promotor were seeded and induced with tetracycline in the presence of MDL105.519 or CGP070667, and NMDAR-mediated calcium flux was measured as previously described after addition of the indicated concentration of glycine and glutamate. Data represents the mean of the maximal fluorescence ratio ± SEM of three experiments.

Supplementary Figure 3



Supplementary Figure 3 Characterization of the mode of action of NMDAR (NR2B) inhibitors

(A-B) Sensitivity of NR1/2B mediated calcium flux to inhibition by MDL105,519 (first column), CGP070667 (middle column) or MK801 (last column) at different ligand concentrations. HEK293 cells were transduced with baculovirus encoding NR1 and NR2B in the presence of MDL105,519 (A) or CGP070667 (B), and NMDAR-mediated calcium flux was measured as previously described in the presence of the indicated ligand concentrations. The integral of the maximal fluorescence ratio was normalized to the activity measured in the absence of inhibitor (DMSO only) at the respective ligand concentration. Data represents the mean ± SEM of three experiments.

Sequence of pFastBac1-CMV/GW-DEST

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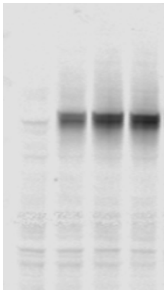
Western blot images

NR1 (full blot image)



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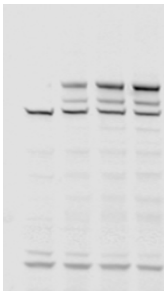
NR2A (full blot image)



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