Supplementary Material

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Supplementary figure 1. No gross differences in the number and morphological characteristics of neurons (red) and astrocytes (green), which were stained with MAP2 and GFAP antibodies, respectively. Dissociated hippocampal cultures were fixed at 20 DIV after being treated with vehicle (PBS), inactive or active form of heparinase I starting from day 11 in vitro. The scale bar is 100 µm, valid for A-C.



Supplementary figure 2. Chronic heparinase treatment has no effect on the time course of mEPSCs and mIPSCs. 10-90% rise time (left) and weighted decay time constant (right) of mEPSCs and mIPSCs for control (gray) and heparinase-treated primary hippocampal neurons (green). There is no significant difference between groups (n = 8 cells each; unpaired two-tailed Student's t test).



Supplementary figure 3. Heparinase upregulates GluA1 expression already on DIV 14/17. Mouse hippocampal neurons were treated with vehicle or heparinase (H1) on DIV 11, and DIV14. On DIV14/17 cells were lysed and expression of GluA1 or GluA2 subunits was analyzed by immunoblotting (IB). A. Representative Western blots. B. Data are shown as mean + SEM. There is a statistically significant increase in GluA1 expression on DIV 14/17 (P < 0.05, N = 3) upon heparinase treatment (paired t-test).



Supplementary figure 4. The effect of heparinase on phosphorylation of GluA1 at Serine 831.

Mouse hippocampal neurons were treated with vehicle or heparinase (H1) on DIV 11, 14 and 17. On DIV19 cells were lysed and 30 mg of extracts were subjected to immunoblotting (IB). (A) Membranes were probed with anti-P-GluA1 or anti-Tubulin antibodies and after stripping with an anti-GluA1. (B, C) Bars represent mean + SEM. P-GluR1 phosphorylation was calculated either as P-GluA1 to total GluA1 (B) or as P-GluA1 to Tubulin (C) ratio. There is a statistically significant increase in P-GluA1/Tubulin ratio (1.596±0.114, P<0.05; N = 4) but not in P-GluA1/GluA1 ratio (1.212±0.142, P=0.16; N=4) upon heparinase treatment (the values were normalized relative to the control for each Western blot, paired t-test was used for comparison).