Supporting Information

Comparative *In Vitro* Studies of MR Imaging Probes for Metabotropic Glutamate Subtype-5 Receptor Targeting

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Table of Contents

1. Supplementary Figures

Figure S1. Plot of proton relaxivity (20 MHz, 25°C) versus molecular weight for mono-aqua polyaminocarboxylate-**[Gd.DOTA]** derivatives. The correlation coefficient, R^2, is 0.984. All data except for L^1, L^2, and L^3 are taken from Caravan et al.[1]

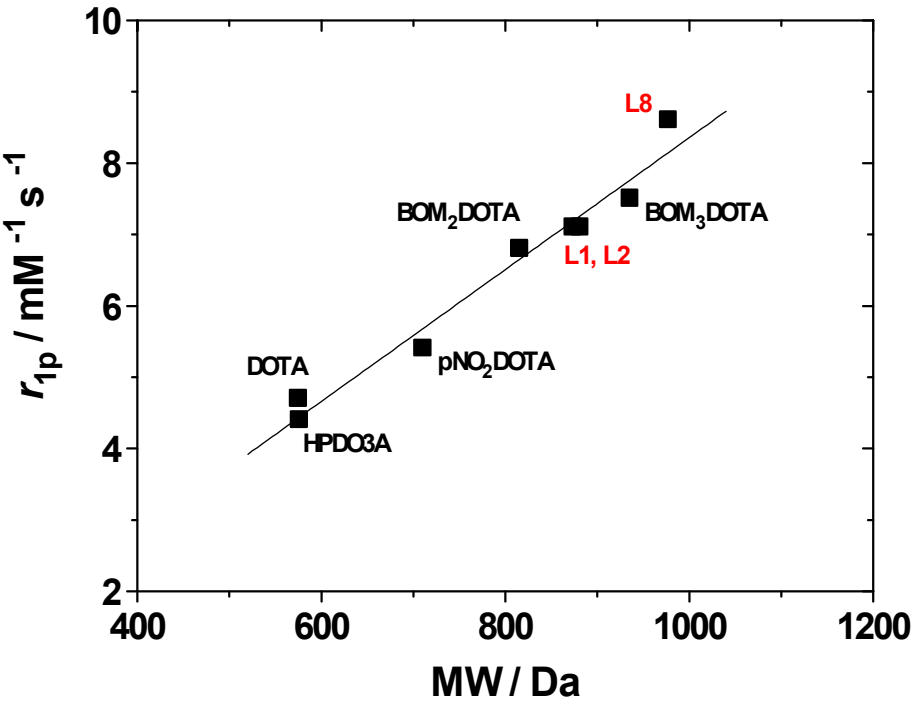
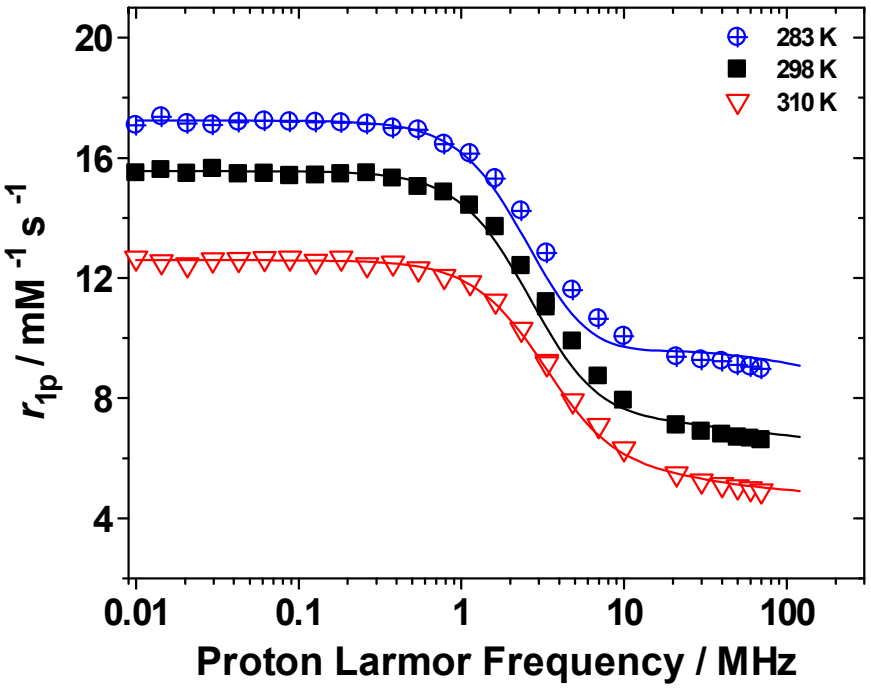


Figure S2. NMRD profile for **[Gd.L^1]** and (Table) fitted data. The lines show the fit to the experimental data points, using the data shown in the Table.



	283 K	298 K	310 K
r_{1p}^{20} (mM^{-1} s^{-1})	9.4	7.09	5.5
Δ^2 (s^{-2};×10^{19})	1.42	1.42	1.42
τ_V (ps)	27.9	20.0	17.6
τ_R (ps)	178	135	96
τ_M (ns)*	200	100	90
q*	1	1	1
r (Å)*	3.0	3.0	3.0
a (Å)*	4.0	4.0	4.0
D (cm^{2}s^{-1};×10^{-5})*	1.30	2.24	3.10

* fixed in the fitting

Figure S3. Temperature dependence of the relaxivity of **[Gd.L^1]** in water at 20 MHz (using the best fit parameters from NMRD**).**

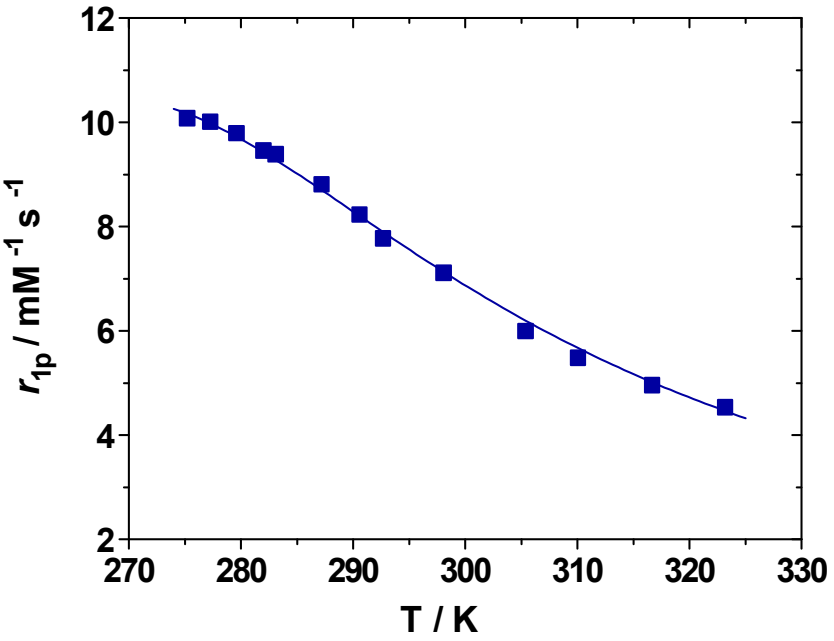
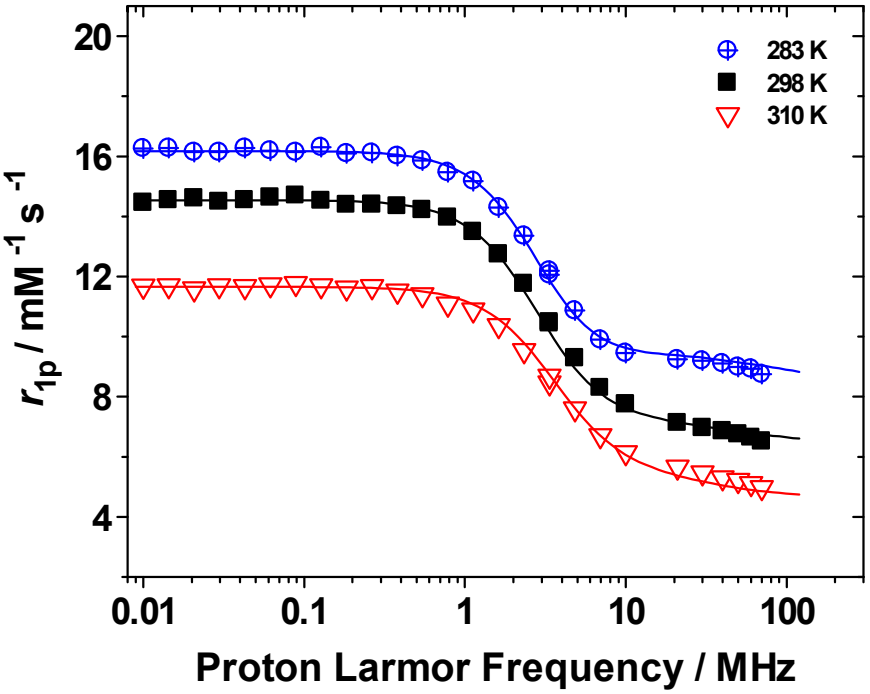


Figure S4. Relaxation behaviour of **[Gd.L^2]** and (Table) fitted data. The lines show the fit to the experimental data points.



	283 K	298 K	310 K
r_{1p}^{20} (mM^{-1} s^{-1})	9.2	7.1	5.6
Δ^2 (s^{-2};×10^{19})	1.4	1.4	1.4
τ_V (ps)	32.5	24.5	23.0
τ_R (ps)	170	132	91
τ_M (ns)*	200	120	80
q	1	1	1
r (Å)*	3.0	3.0	3.0
a (Å)*	4.0	4.0	4.0
D (cm^{2}s^{-1};×10^{-5})*	1.30	2.24	3.10

* fixed in the fitting

Figure S5. Temperature dependence of the relaxivity of **[Gd.L^2]** in water at 20 MHz (using the best fit parameters from NMRD).

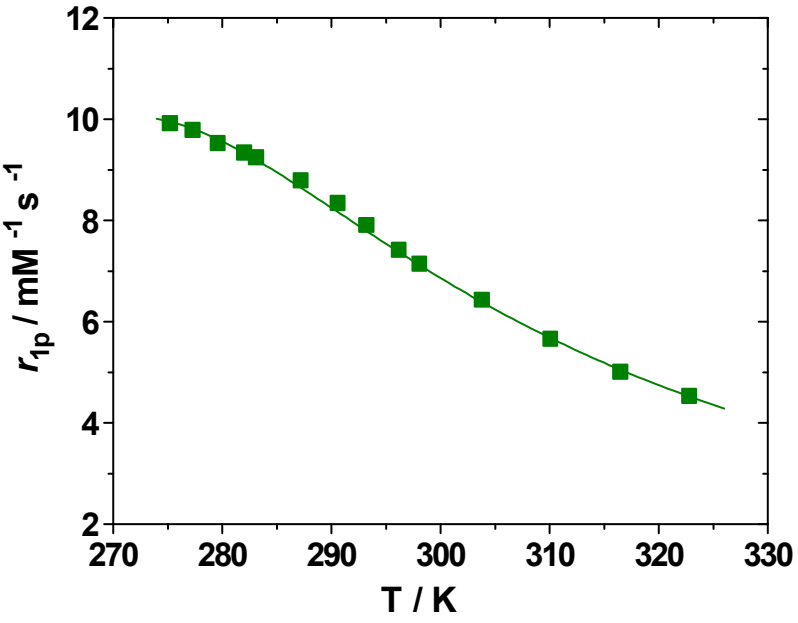
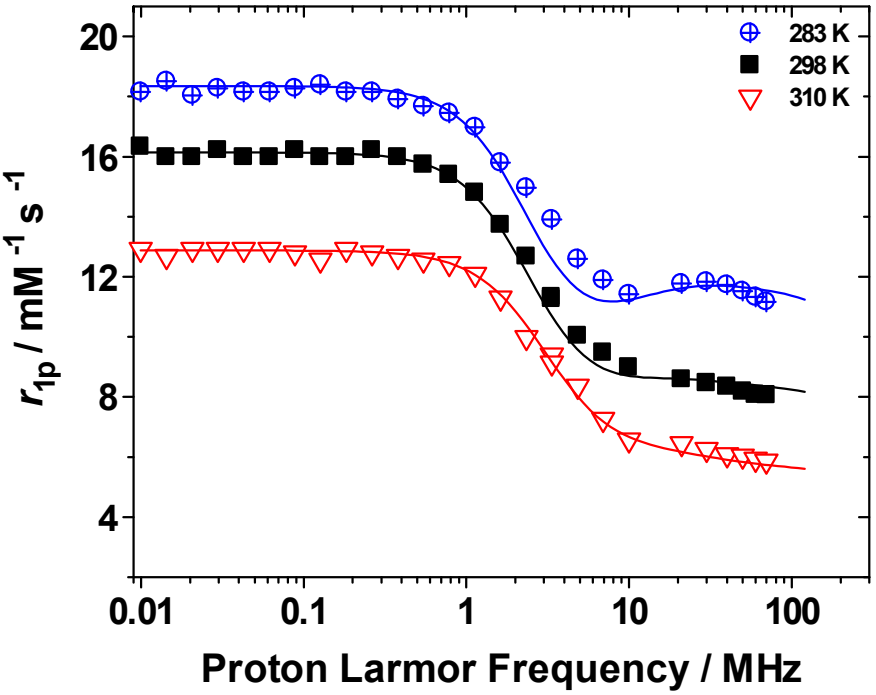


Figure S6. Relaxation behaviour of **[Gd.L^{8}]** and (Table) fitted data. The lines show the fit to the experimental data points.



	283 K	298 K	310 K
r_{1p}^{20} (mM^{-1} s^{-1})	11.8	8.6	6.5
Δ^2 (s^{-2};×10^{19})	1.5	1.5	1.5
τ_V (ps)	29.6	24.6	23.0
τ_R (ps)	245	177	115
τ_M (ns)*	160	100	80
q	1	1	1
r (Å)*	3.0	3.0	3.0
a (Å)*	4.0	4.0	4.0
D (cm^{2}s^{-1};×10^{-5})*	1.30	2.24	3.10

* fixed in the fitting

Figure S7. Temperature dependence of the relaxivity of **[Gd.L^{8}]** in water at 20 MHz (using the best fit parameters from NMRD analysis).

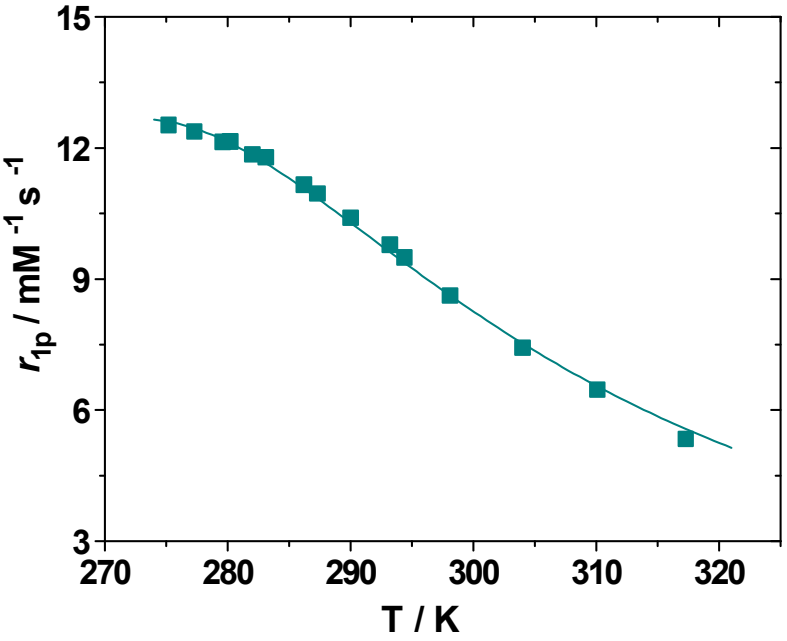


Figure S8 Experimental NMRD profiles for 0.15 mM solutions of the complexes **[Gd.L^1]** and **[Gd.L^2]** in the presence of HSA (61 mg/mL) at 298 K. Under these conditions, the molar fractions of the bound complexes are 0.81 (**[Gd.L^1]**) and 0.72 (**[Gd.L^2]**) respectively.

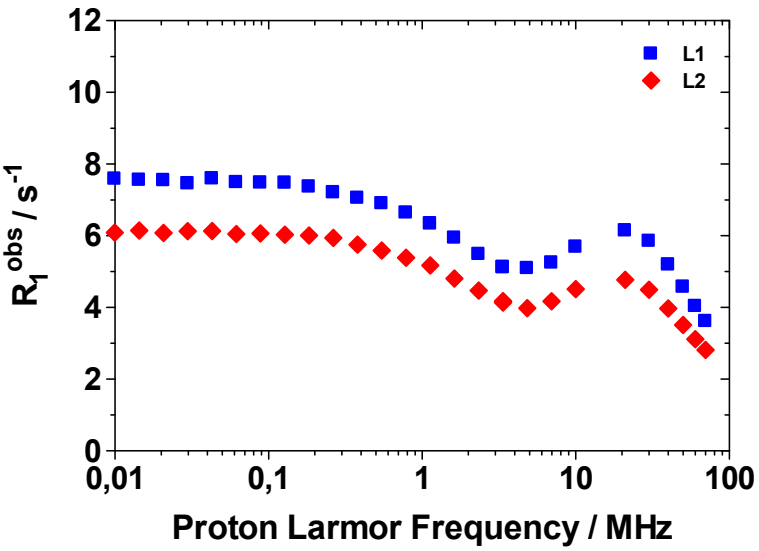
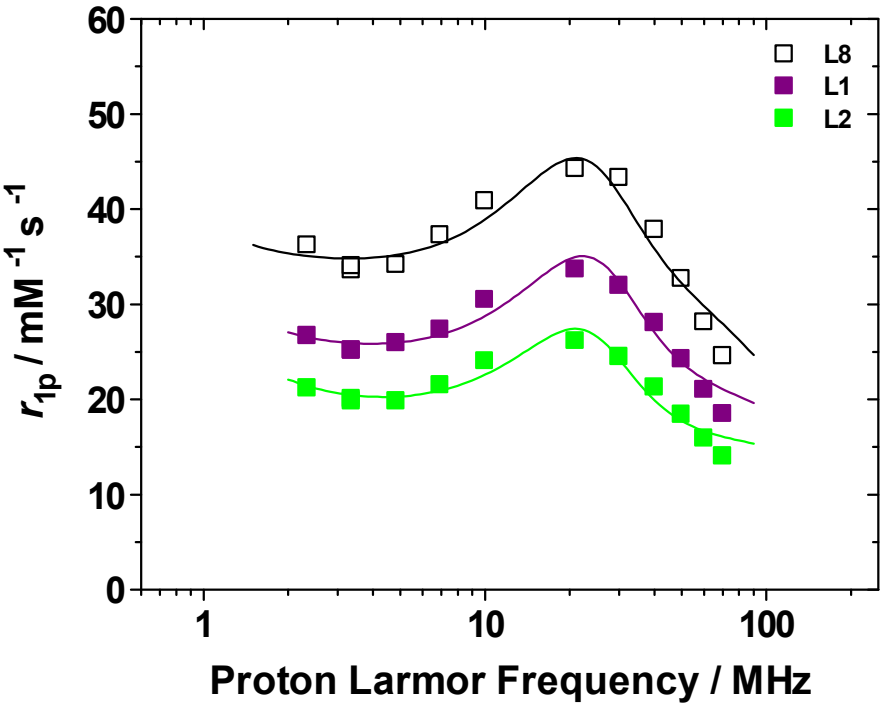


Figure S9. Comparison of the high field NMRD profiles of the HSA-bound complexes of the Gd complexes of $\mathbf{L^1}$, $\mathbf{L^2}$ and $\mathbf{L^8}$ (298 K).

The relaxivity decreases from **[Gd.L^8]** to **[Gd.L^2]** following the corresponding increase in local mobility (rotation) of the probe



	[Gd.L^1]	[Gd.L^2]	[Gd.L^8]
$^{20}r^b_{1p}$ (mM^{-1} s^{-1})	33.7	26.1	43.1
Δ^2 (s^{-2};×10^{18})	5.7	5.4	4.8
τ_V (ps)	11	13	10
τ_{RL} (ns)	0.70	0.45	1.25
τ_{RG} (ns)*	100	100	100
S^2	0.17	0.11	0.20
τ_M (ns)*	100	100	100
q	1	1	1
r (Å)*	3.0	3.0	3.0

*, fixed in the fitting

Figure S10. Cytotoxicity of **[Gd.L$^{1-8}$]**.

Metabolic activity (XTT) and cell number (H33342) were analyzed after treating primary astrocytes with 200µM of compounds [Gd.L$^{1-8}$] for 24 hours as described before.[5] The calcium ionophore A23187 (10µM, 24h) served as a positive control for cell death. Metabolic activity was also normalized to cell number (XTT/H33342; main article Figure **7**). All data are given as percent of control (untreated cells). Values are means ± SEM of up to four experiments with 4-6 replicates each.***P<0.001, *P<0.05 vs. untreated cells (control). ANOVA with Bonferroni's multiple comparison post test.

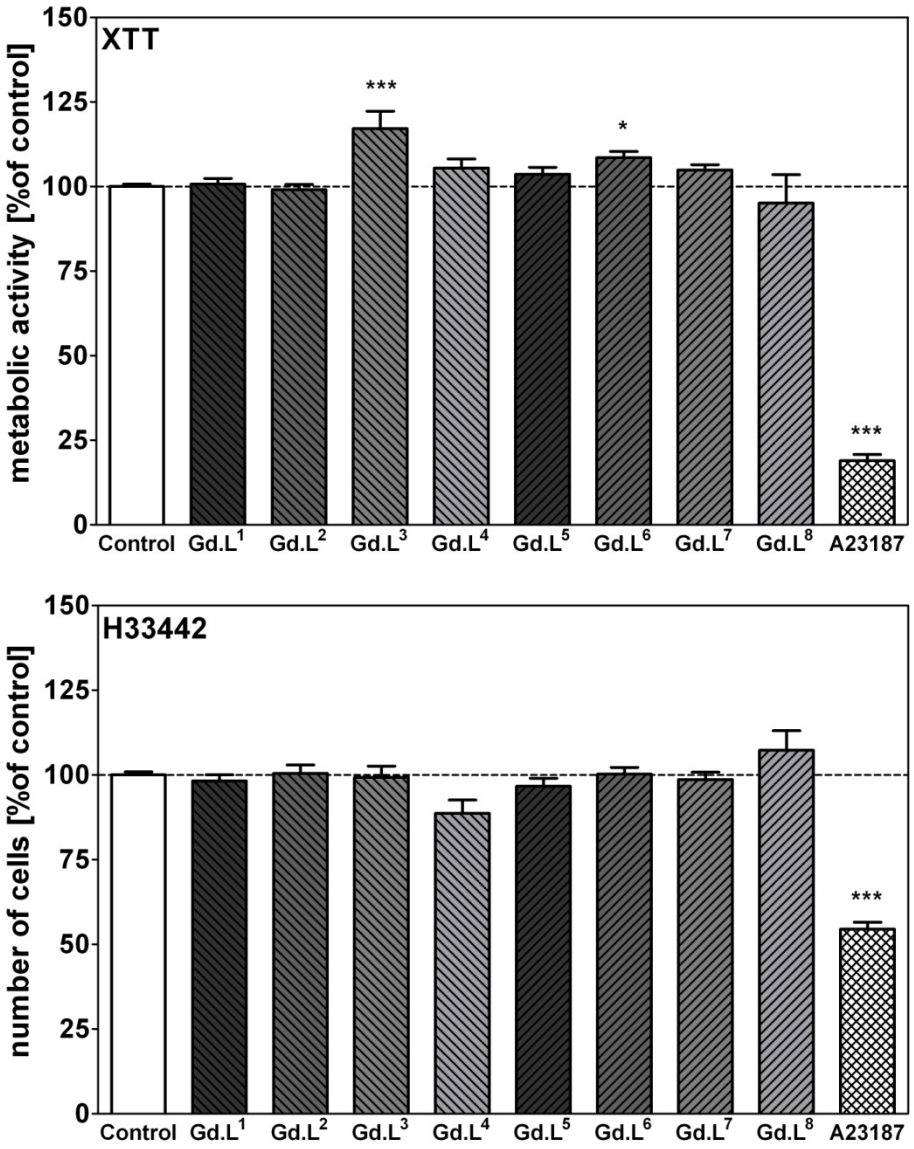
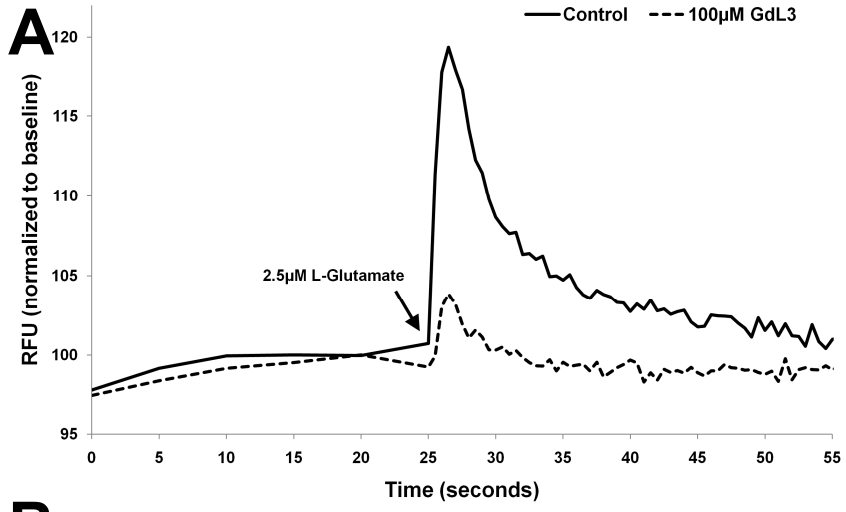


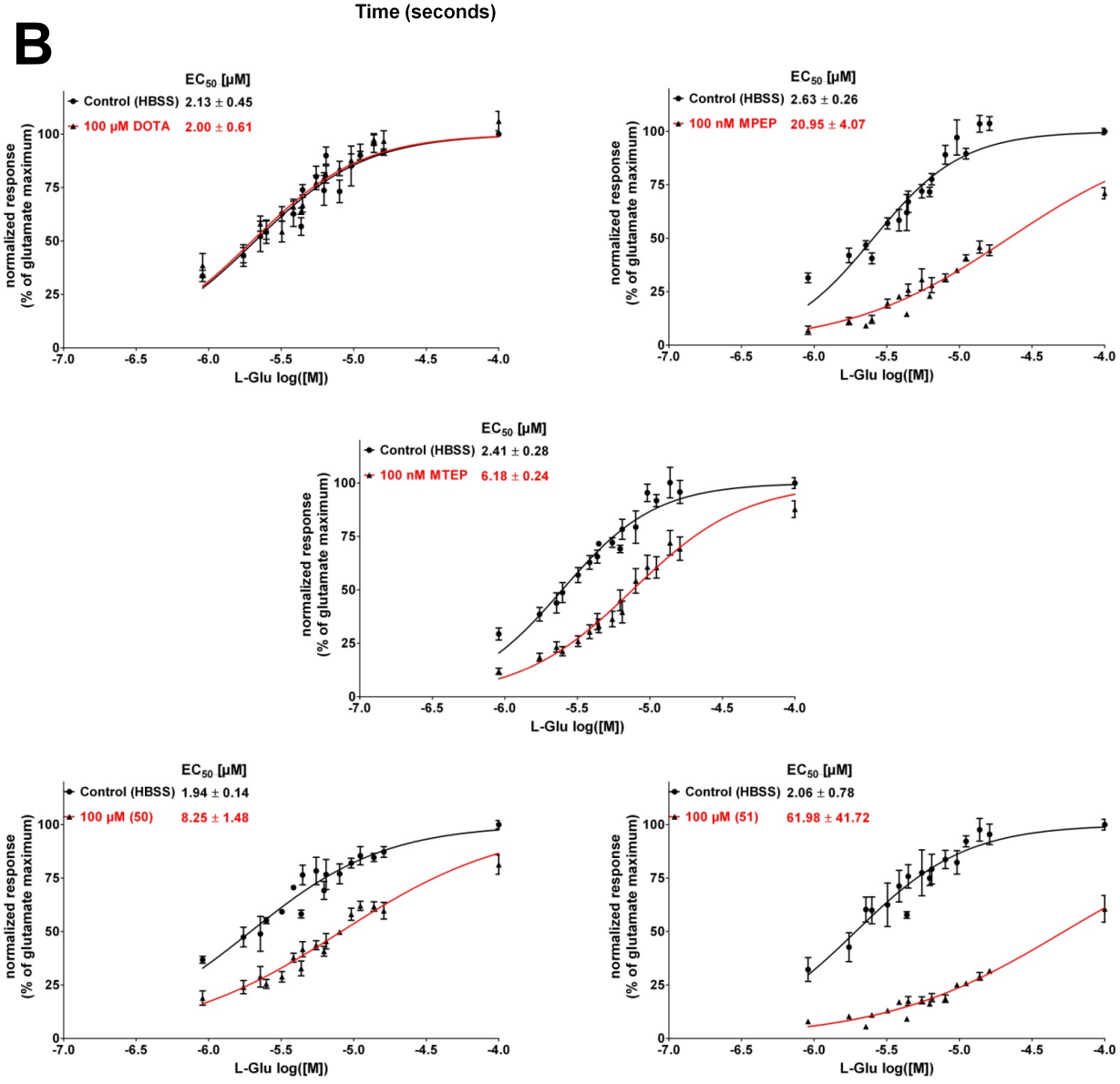
Figure S11. Influence of **[Gd.DOTA]**, **[Gd.L$^{1-8}$]** (100 µM each) or MPEP, MTEP (100 nM each) on L-glutamate induced calcium transients (antagonistic effect) after pre-treatment with the respective compound.

[Gd.L$^{3, 5, 8}$] decrease L-glutamate induced calcium transients. Primary astrocytes were grown in poly-D-lysine coated 96 well plates for 10-15 days, changed to glutamine-free DMEM the day before the calcium-assay.

(A) Representative fluorescence recording (normalized to the average of 20 s baseline) of a control well vs. a well pre-treated with 100 µM **[Gd.L^{3}]** for 15 minutes showing the antagonistic effect of **[Gd.L^{3}]** on the L-glutamate induced calcium transient.

(B) Shift in the EC_{50} of L-glutamate to a higher concentration after pre-treatment of cortical primary astrocytes with 100 µM of **[Gd.DOTA]**, 100 µM **[Gd.L$^{1-8}$]**, [**50** and **51**] or 100 nM MPEP or MTEP. Curves were generated from at least three independent experiments. Data points represent means ± SEM.





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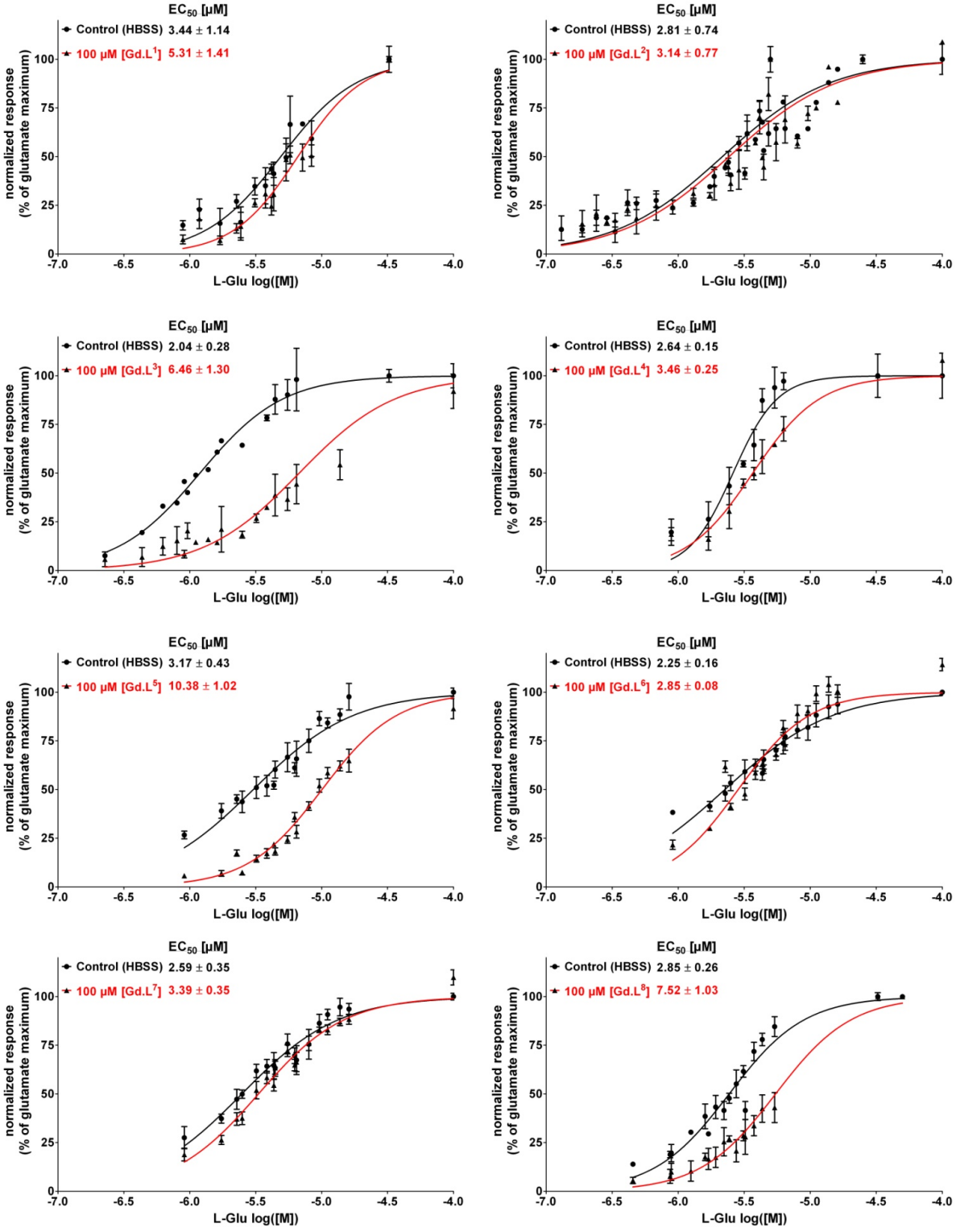
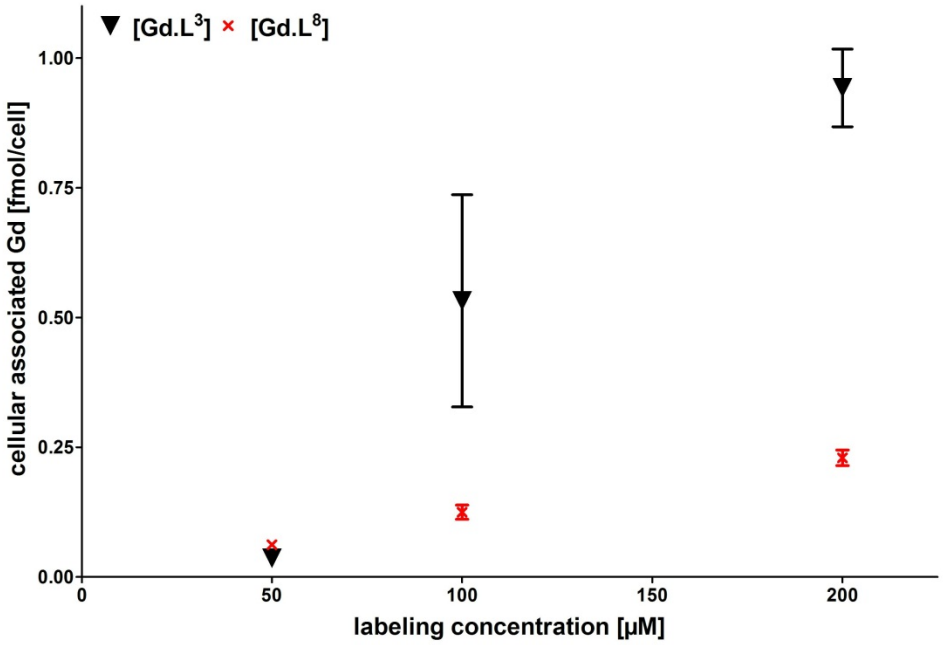


Figure S12. (A) Uptake of **[Gd.L^3]** and **[Gd.L^8]** into primary astrocytes (45 min, 37°C). After measuring cellular relaxation rates $R_{1,cell}$ the cell associated Gd^{III} content in the cell pellets was determined by ICP-MS. (B) Cellular relaxivites $r_{1,cell}$ of **[Gd.L^3]** and **[Gd.L^8]**, calculated from slopes of the linear regression line. The respective dashed lines display the 95% confidence line for both compounds. Values are mean ± SEM (n=2-4).

A



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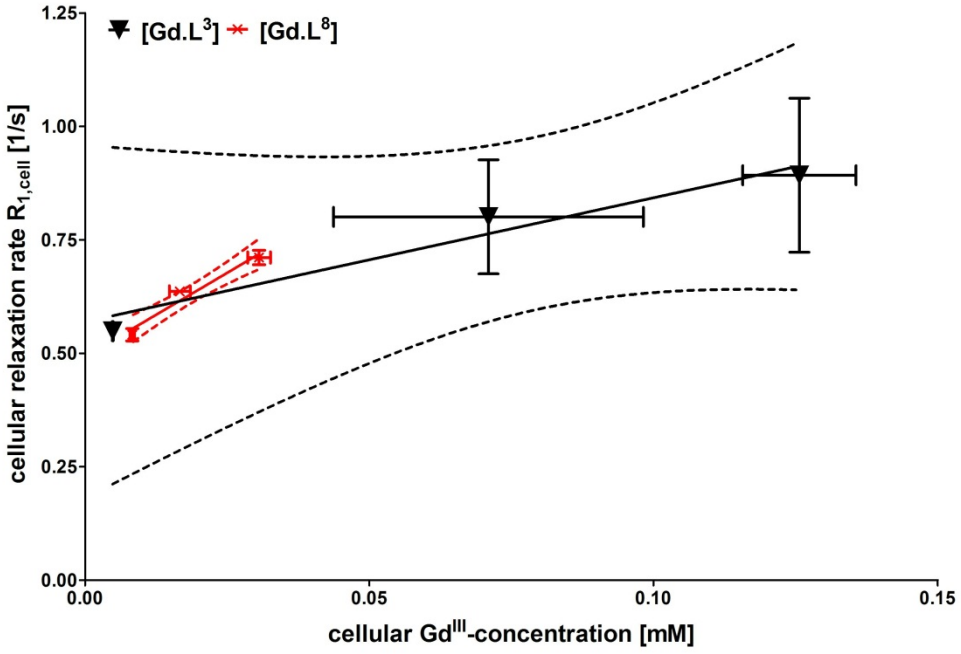

2. References

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