PNAS
www.pnas.org

Supplementary Information for

MRI demonstrates glutamine antagonist mediated reversal of cerebral malaria pathology in mice

Brittany A. Riggle, Sanhita Sinharay, William Schreiber-Stainthorp, Jeeva P. Munasinghe, Dragan Maric, Eva Prchalova, Barbara S. Slusher, Jonathan D. Powell, Louis H. Miller, Susan K. Pierce, Dima A. Hammoud*

Dima A. Hammoud
Email: hammoudd@mail.nih.gov

This PDF file includes:

Figs. S1 to S6
Table S1
Captions for movies S1 to S2

Other supplementary materials for this manuscript include the following:

Movies S1 to S2

www.pnas.org/cgi/doi/10.1073/pnas.1812909115

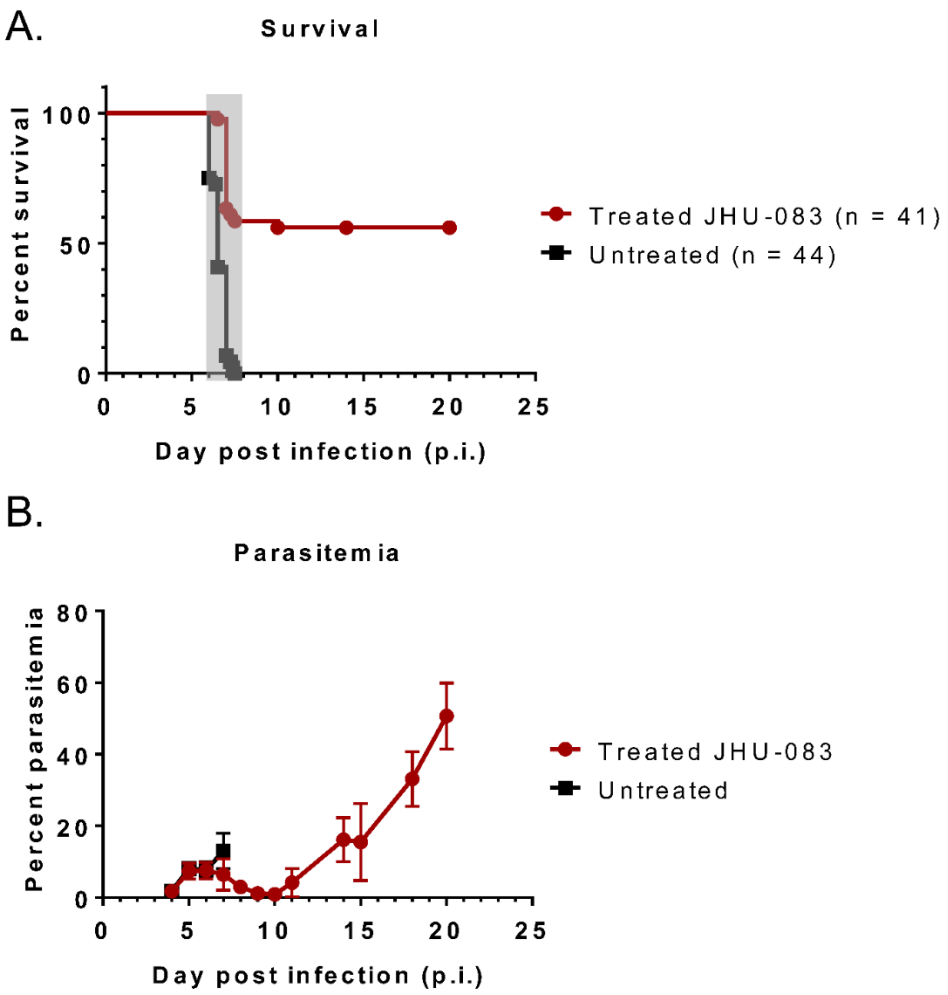

Fig. S1. Drug efficacy studies for JHU-083. (*A*) Survival curve for *P. berghei* ANKA infected animals treated with JHU-083 (red) initiated at 6 am on day 6 p.i. or untreated animals (grey box indicates time range of clinical symptoms) and (*B*) corresponding parasitemia over the same time range. The results shown are combined from four independent experiments. Animals in the treated group received an initial treatment at 6 am with a second treatment at 6 pm on day 6 p.i. third and fourth treatments were given at 6 am on day 8 and 10 p.i., respectively.

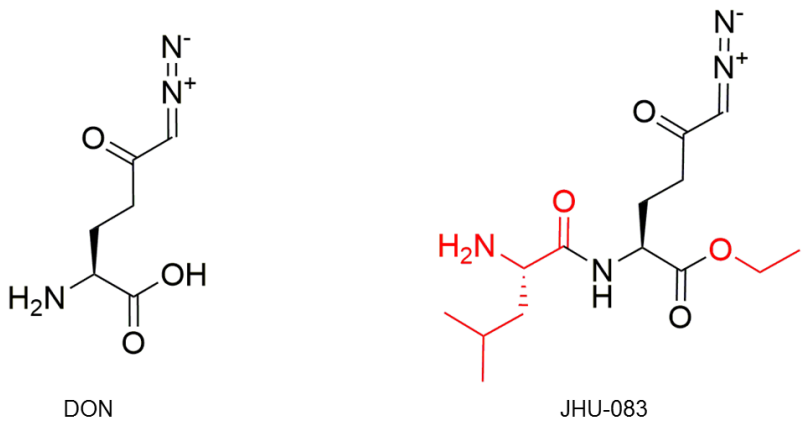

Fig. S2. Chemical structure of DON and JHU-083. The structure of 6-diazo-5-oxo-L-norleucine (DON) is shown on the left. The pro-drug form of DON used in this study, ethyl 2-(2-amino-4-methylpentanamido)-6-diazo-5-oxohexanoate, here named JHU-083, is shown on the right with the protecting groups indicated in red.

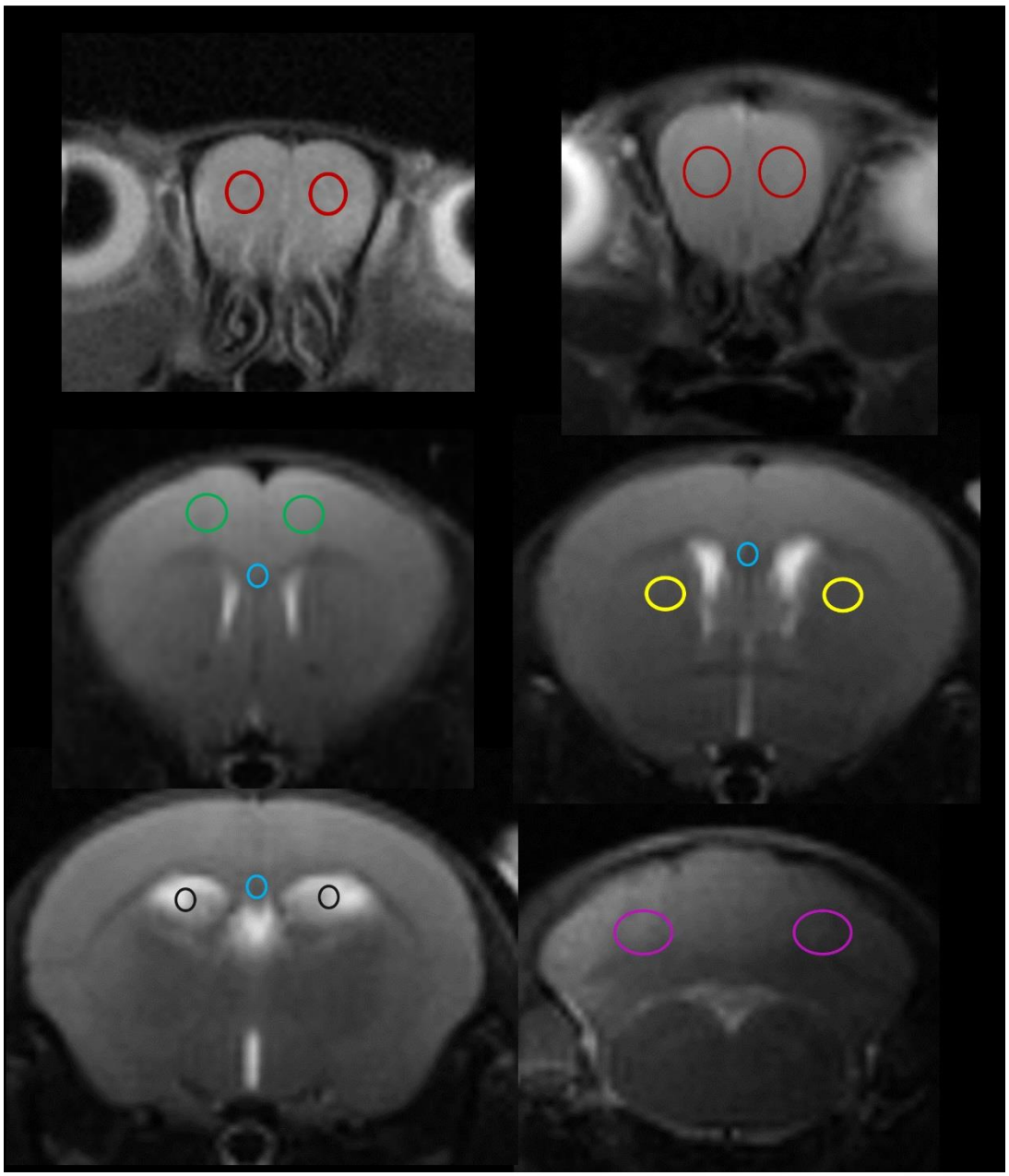

Fig. S3. Regions of interest used in quantification. Regions of interest in the olfactory bulbs (red circles), cortex (green circles), corpus callosum (blue circles), ventricles (black circles), striatum (yellow circles), and cerebellum (purple circles) superimposed on T_2-weighted images of an uninfected mouse.

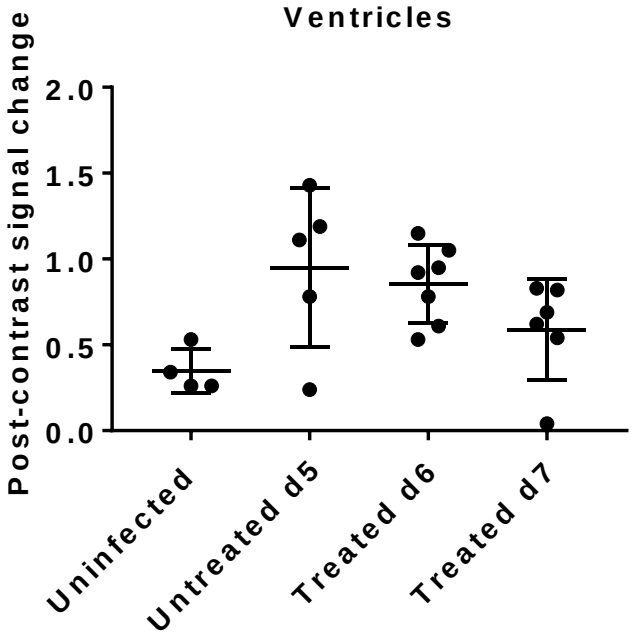

Fig. S4. Percent change in signal between pre- and post-contrast in the ventricles. Percent change in signal within the ventricles after administration of contrast agent, Magnevist®, in uninfected mice (n = 4), infected untreated mice (day 5 p.i., n = 5), infected treated mice (day 6 p.i., n = 7), and infected multi-treated mice (day 7 p.i., n = 6) (Kruskal-Wallis, $p = 0.051$).

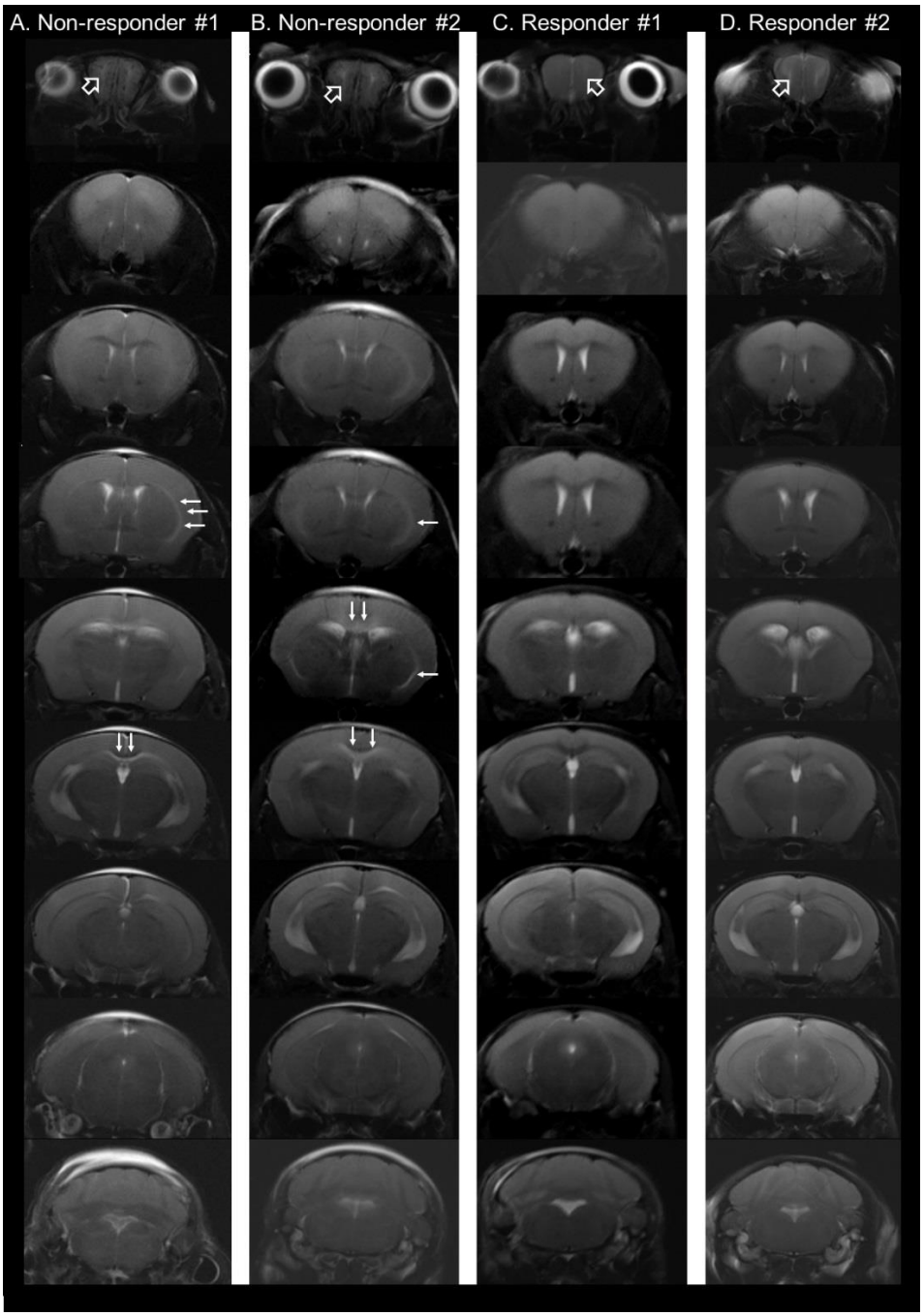

Fig. S5. Coronal T_2-weighted images of four infected animals that were treated on day 6 p.i. Two animals (*A* and *B*) did not survive past day 6 p.i. and showed extensive high signal intensity suggestive of vasogenic edema in the olfactory bulbs as well as the corpus callosum and external capsules (solid white arrows). Two animals (*C* and *D*) responded to treatment and those showed less severe edematous changes that mainly presented in the olfactory bulbs.

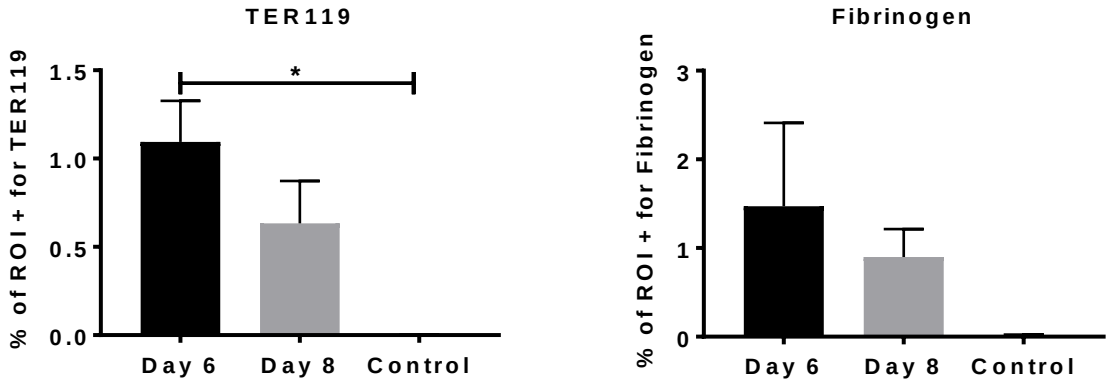

Fig. S6. Quantification of immunohistochemical staining of mouse brains. Quantification of IHC staining for red blood cells (TER119) and protein leakage (fibrinogen) in uninfected (n = 3), infected untreated (day 6 p.i., n = 3), and infected treated (day 8 p.i., n = 3) animals. There is a significant increase between day 6 p.i. and control animals (Dunn's post-hoc analysis). Kruskal-Wallis test: TER119, $p = 0.0036$; fibrinogen, $p = 0.050$. Error bars represent mean ± SD. Dunn's post-hoc analysis: $*p < 0.05$.

Table S1. Summary of animals imaged including sample numbers and types of MR sequences utilized for the cross-sectional and longitudinal studies.

MR image weighting	Cohort	Total # animals	Animal subsets		
			Uninfected	Infected	Responders/ Non-responders
T_1	Cross-sectional	23	4	19	
	Longitudinal	7	0	7	7/0
T_2†	Cross-sectional*	14	4	10	
	Longitudinal	6	0	6	4/2
DWI†	Cross-sectional*	13	4	9	
	Longitudinal	6	0	6	4/2

*4 animals from the longitudinal study (day 6 only) were also used in cross-sectional analysis
†the same animals were used for T_2 and DWI experiments

Additional Supplementary Materials:

Movie S1. Longitudinal study of clinical disease progression of ECM. Disease progression is presented in a representative 6-week-old, female, C57Bl/6 mouse infected with *Pb* ANKA and followed over days 4-7 post infection.

Movie S2. Longitudinal study of clinical disease progression of ECM with treatment. Disease progression is presented in a representative 6-week-old, female, C57Bl/6 mouse infected with *Pb* ANKA and receiving treatment starting on day 6 post infection in the morning. The video follows the animal over days 4-8 post infection.