

- Suppl. information -

***In vivo* nanoparticle-based T cell imaging can predict therapy response
towards adoptive T cell therapy in experimental glioma**

Jessica Hunger^{1,2}, Katharina Schregel¹, Berin Boztepe^{1,2}, Dennis Alexander Agardy^{2,3}, Verena Turco^{1,2,3}, Kianush Karimian-Jazi¹, Ina Weidenfeld¹, Yannik Streibel¹, Manuel Fischer¹, Volker Sturm¹, Rachel Santarella-Mellwig⁶, Michael Kilian^{2,3}, Kristine Jähne^{2,3}, Katharina Sahm^{2,3}, Wolfgang Wick^{4,5}, Lukas Bunse^{2,3}, Sabine Heiland¹, Theresa Bunse^{2,3}, Martin Bendszus¹, Michael Platten^{2,3,7} and Michael O. Breckwoldt^{1,2#}

¹Neuroradiology Department, University Hospital Heidelberg, 69120 Heidelberg, Germany

²Clinical Cooperation Unit Neuroimmunology and Brain Tumor Immunology, German Cancer Consortium (DKTK) within the German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany

³Department of Neurology, Medical Faculty Mannheim, Mannheim Center for Translational Neurosciences, Heidelberg University, Mannheim, Germany

⁴Clinical Cooperation Unit Neurooncology, DKTK within DKFZ, Heidelberg, Germany

⁵Department of Neurology, National Center for Tumor Diseases (NCT), Heidelberg University Hospital, Heidelberg, Germany

⁶European Molecular Biology Laboratory (EMBL), Heidelberg, 69117, Germany

⁷DKFZ-Hector Cancer Institute at the University Medicine Mannheim, Mannheim, 68167, Germany

Corresponding authors:

Michael Breckwoldt, MD, PhD, Tel +49 6221 5636436, michael.breckwoldt@med.uni-heidelberg.de

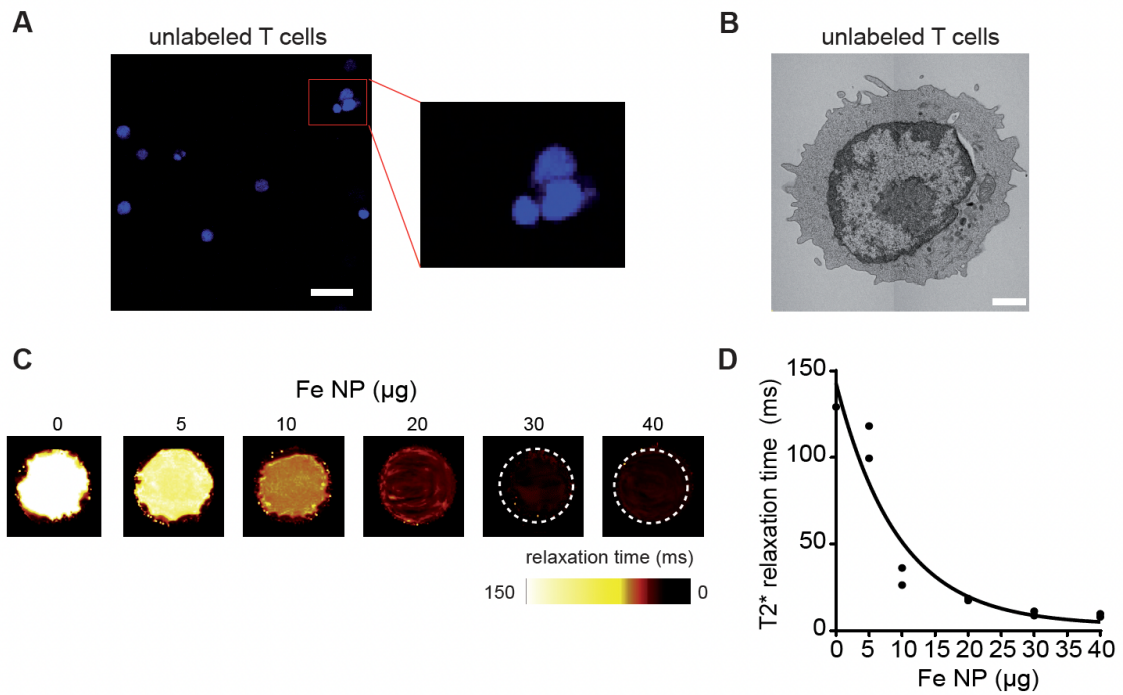


Figure S1

A: Confocal image of unloaded T cells as negative control **B:** TEM image of unlabeled primary murine T cell. **C:** T2* maps of 0 – 40 μg Fe of iron oxide NP **D:** Quantification of T2* maps of 0 – 40 μg Fe of iron oxide NP. Scale bars are 20 μm in confocal microscopy image and 500 nm in EM.

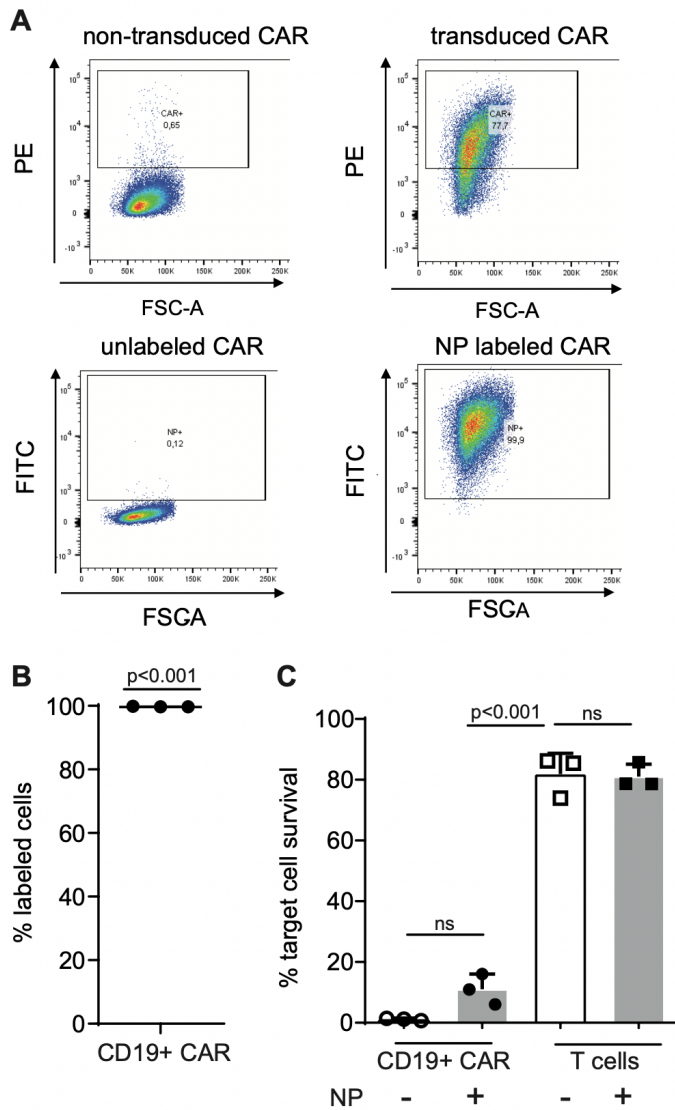


Figure S2

A: NP labeling of human CD19 CAR T cells and flow cytometry plots of labeling efficiency **B:** Quantification of labeling efficiency of human CD19 CAR T cells with iron oxide NP after 24 h as assessed by flow cytometry **C:** killing activity of unlabeled vs NP labeled human CD19 CAR T cells of NALM6 target cells in comparison to human T cells as assessed by flow cytometry.

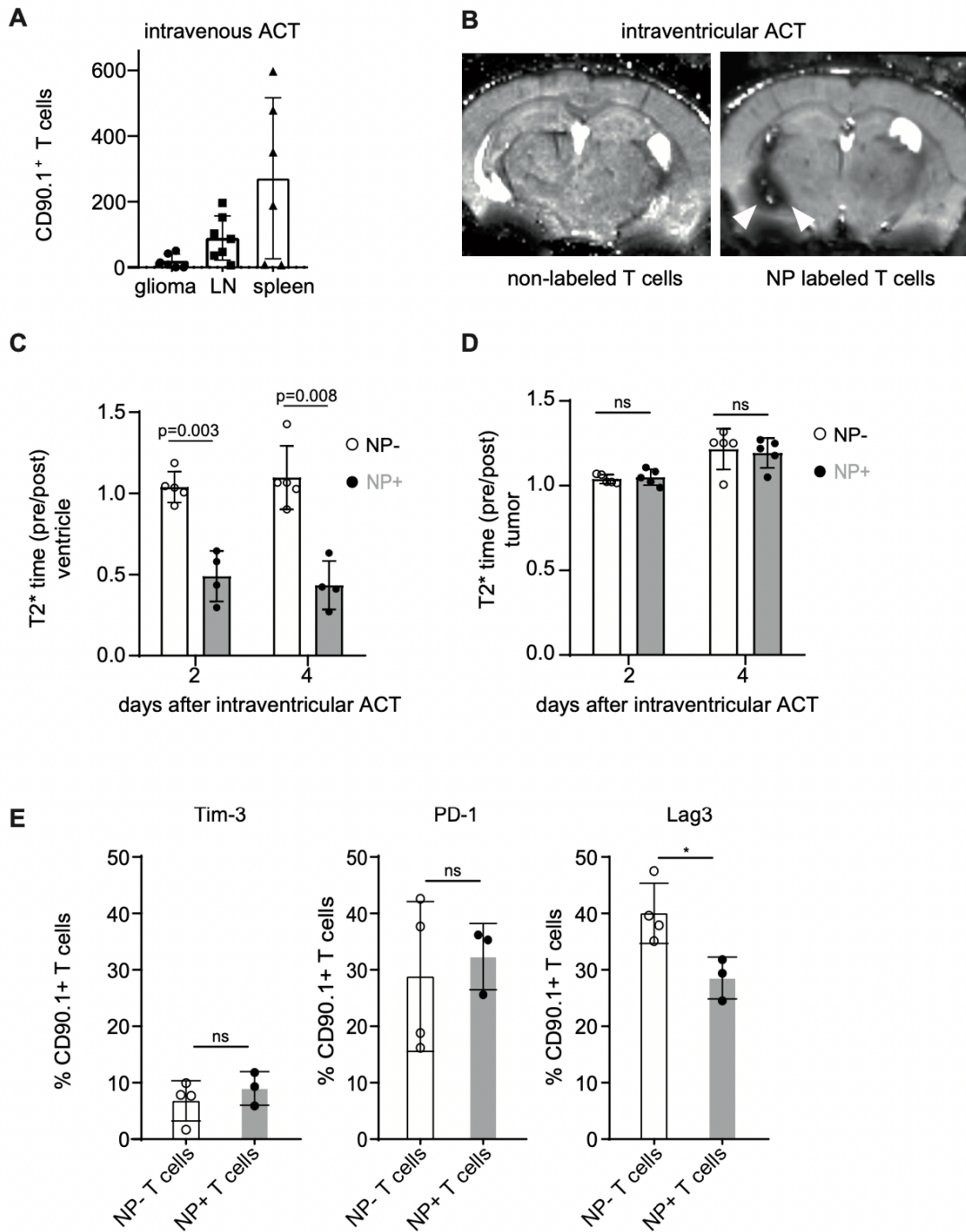


Figure S3

A: PMEL T cell infiltration after i.v. ACT in brain, lymph node and spleen as quantified by flow cytometry **B:** T2* maps of intraventricular ACT comparing unlabeled and iron oxide NP labeled T cell injection **C:** quantification of T2* maps of ventricles after intraventricular ACT after 2 and 4 days comparing unlabeled and iron oxide NP labeled T cells **D:** quantification of T2* maps of tumor area after intraventricular ACT after 2 and 4 d **E:** Tim-3, PD-1 and Lag-3 expression on adoptively transferred, CD90.1+ T cells after intratumoral ACT.

Table S1**Flow cytometry antibodies:**

Antibody	Company	Order number
fixable viability stain eFluor 780	BD Horizon	565388
CD3 murine BV510	BioLegend	100233
CD8 AF700	BioLegend	100730
CD45.1 PE-Cy7	BioLegend	110730
CD45.2 APC	BioLegend	109814
CD90.1 PE-Cy7	BioLegend	202517
CD11b BV711	BioLegend	101241
PD-1 PerCP-eFl710	ThermoFisher	46-9985-82
Lag3 BV785	BioLegend	125219
Tim3 BV421	BioLegend	119723
CD3 human PE	BioLegend	300308
CD19 BV510	BioLegend	363020
R-Phycoerythrin AffiniPure F(ab') ₂ Fragment Goat Anti-Human IgG (H+L)	Jackson ImmunoResearch	109-116-088
anti-CD16/CD32	eBioscience	93 14-0161
Counting beads	Invitrogen	01-1234-42

Antibodies used for clearing:

Antibody	Company	Order number
CD3 (rabbit anti-mouse/human)	Dako	A0452
goat anti-rabbit Alexa 568	Thermofisher	A11011