

**Title:** ImmunopET imaging of TIGIT in the glioma microenvironment

Sarah R. Vincze<sup>1#</sup>, Ambika P. Jaswal<sup>1#</sup>, Stephen C. Frederico<sup>1#</sup>, Michal Nisnboym<sup>1,2</sup>, Bo Li<sup>1</sup>, Zujian Xiong<sup>1</sup>, ReidAnn E. Sever<sup>1</sup>, Chaim T. Sneiderman<sup>1</sup>, Mikayla Rodgers<sup>5</sup>, Kathryn E. Day<sup>3</sup>, Joseph D. LaToche<sup>3</sup>, Lesley M Foley<sup>3</sup>, T. Kevin Hitchens<sup>3,7</sup>, Robin Frederick<sup>3</sup>, Ravi B. Patel<sup>4</sup>, Costas G. Hadjipanayis<sup>1</sup>, Itay Raphael<sup>1</sup>, Jessie R. Nedrow<sup>3,4\*</sup>, W. Barry Edwards<sup>5\*</sup>, and Gary Kohanbash<sup>1,6\*</sup>

<sup>1</sup>Department of Neurological Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

<sup>2</sup>Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel-Aviv University, Israel

<sup>3</sup>In Vivo Imaging Facility, University of Pittsburgh Medical Center, UPMC Hillman Cancer Center, Pittsburgh, PA, USA

<sup>4</sup>Department of Radiology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

<sup>5</sup>Department of Biochemistry, University of Missouri, Columbia, MO, USA

<sup>6</sup>Department of Immunology, University of Pittsburgh, Pittsburgh, PA, USA

<sup>7</sup>Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA, USA

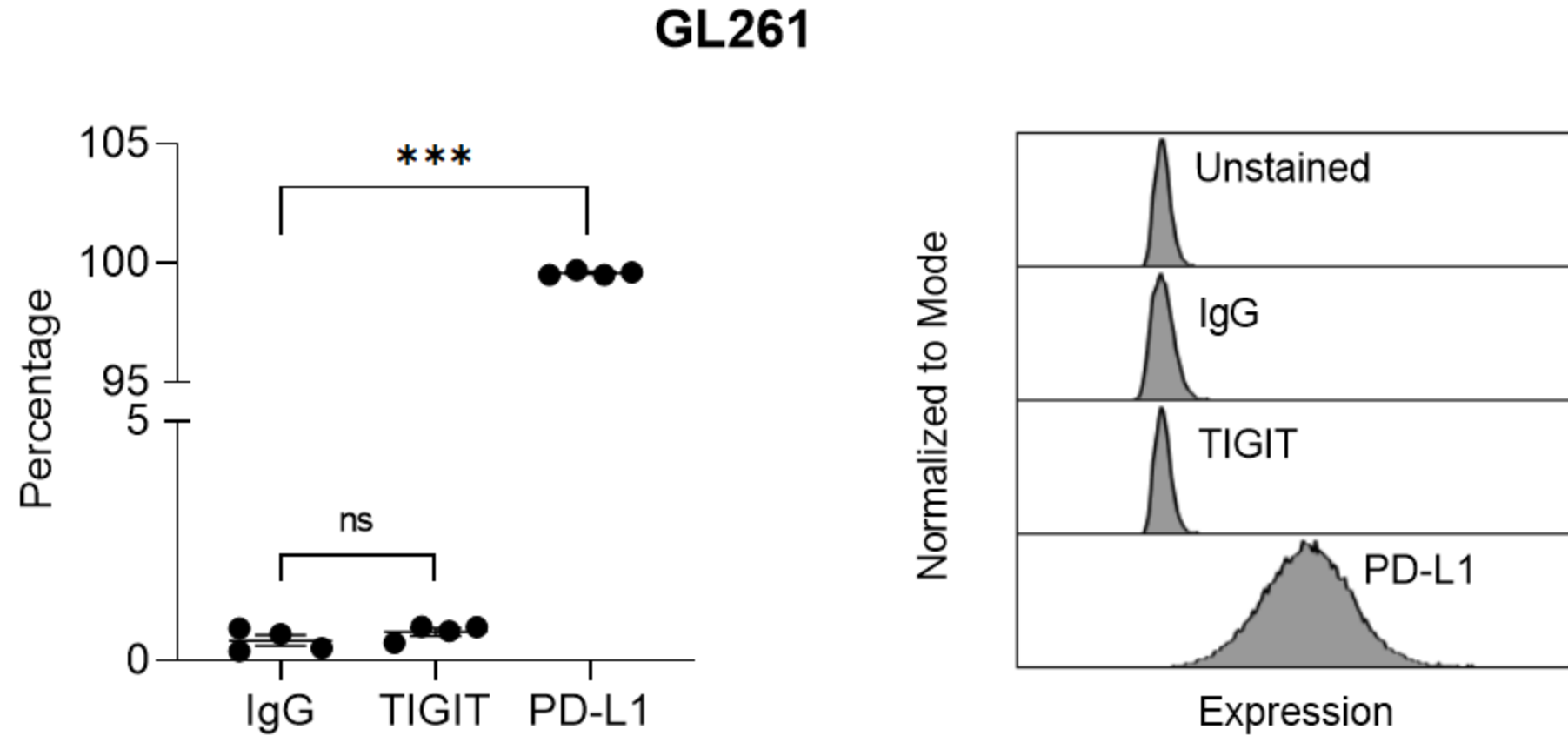
# Co-first authors

**\*Corresponding author:**

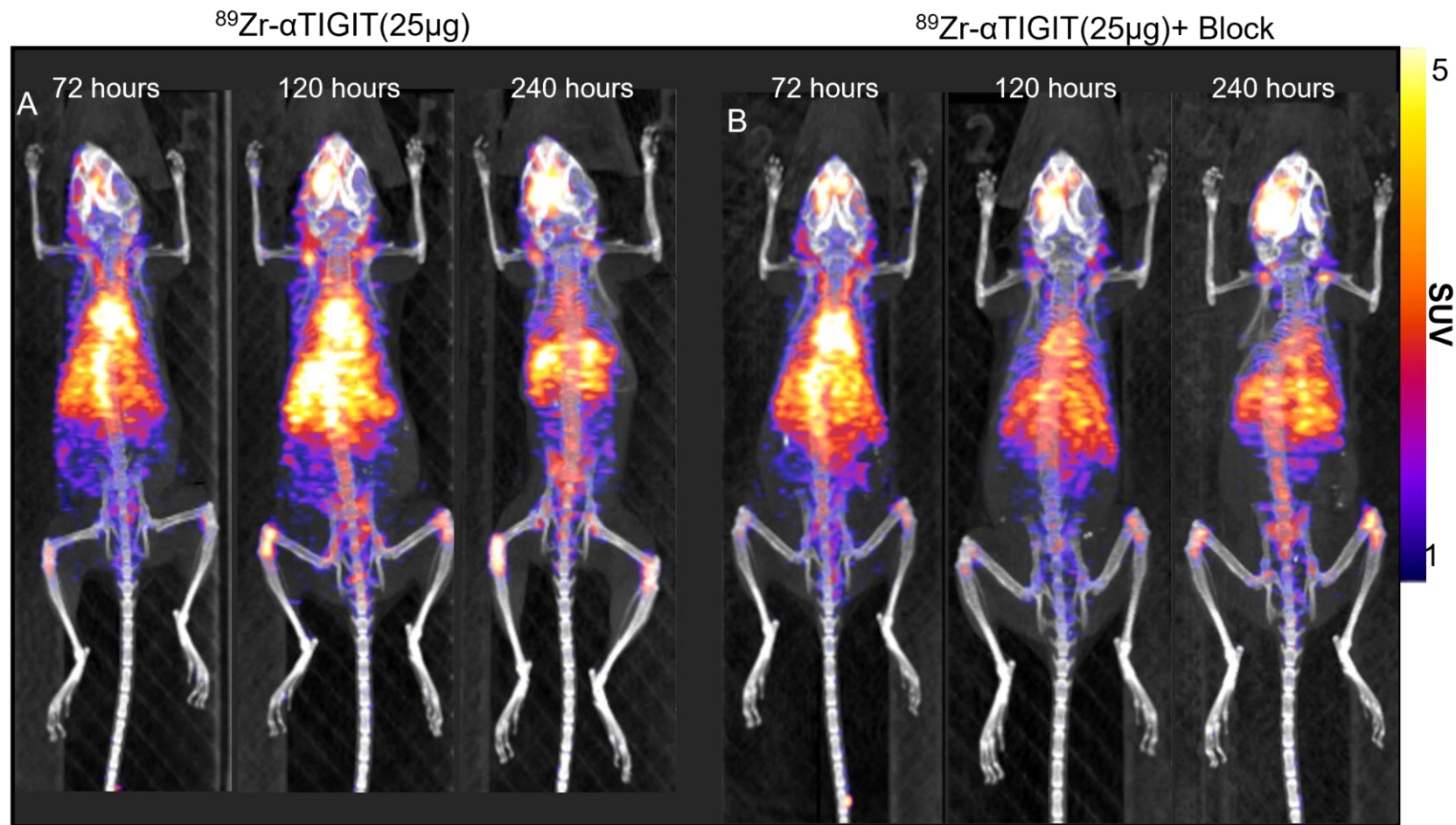
Jessie R. Nedrow, Department of Radiology, University of Pittsburgh.  
5117 Centre Avenue, Suite G. 17b, Pittsburgh, PA 15213, USA  
Phone Office: 412.623.7239; Email: [nedrowj@upmc.edu](mailto:nedrowj@upmc.edu).

Wilson B. Edwards. Department of Biochemistry. University of Missouri.  
222 Schweitzer Hall, Columbia, MO 65211.  
Phone: 573.882.4845; E-mail: [wbe59z@missouri.edu](mailto:wbe59z@missouri.edu).

Gary Kohanbash, Department of Neurological Surgery, University of Pittsburgh, Children's Hospital of Pittsburgh of UPMC, 7128 Rangos Research Building, 530 45<sup>th</sup> Street, Pittsburgh, Pennsylvania 15201, USA. Phone: 412.692.9456; Email: [gary.kohanbash2@chp.edu](mailto:gary.kohanbash2@chp.edu)

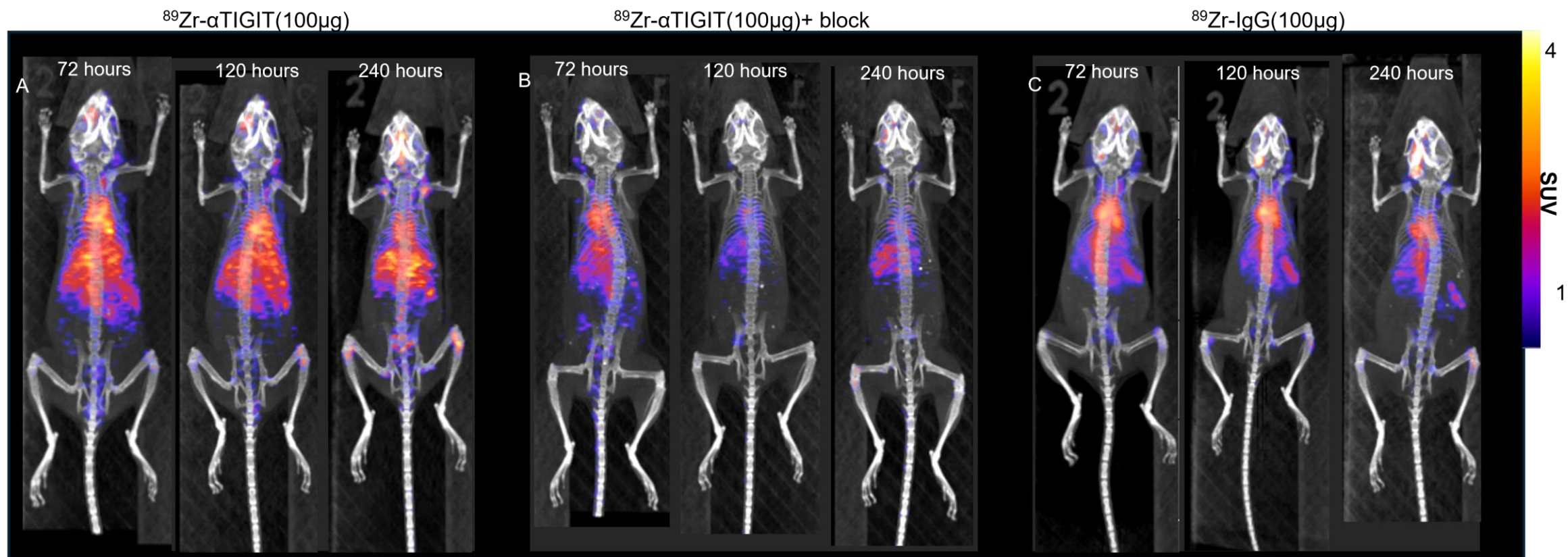


**Supplementary Figure S1.** Mean expression percentages of TIGIT and the IgG isotype control were comparable to the unstained control and in sharp contrast to the positive control, PD-L1, (IgG=0.42%, TIGIT=0.6%, PD-L1=99.6%, indicating that TIGIT is not expressed on GL261 cells.



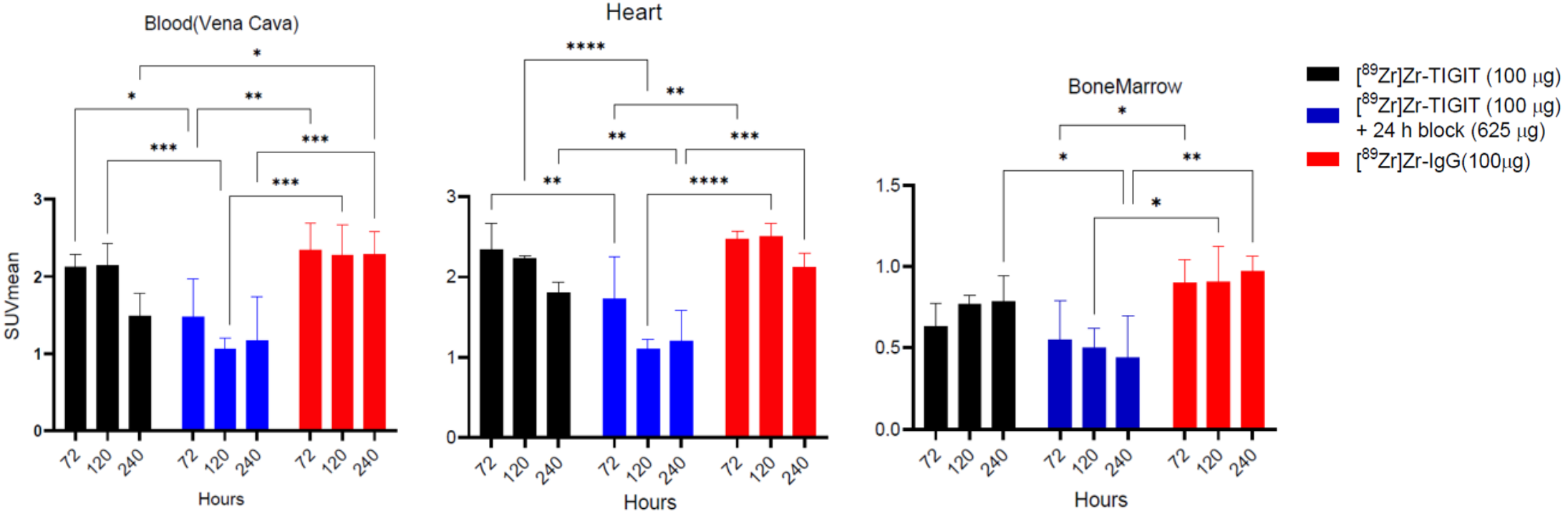
**Supplementary Figure S2.** Whole body PET/CT images at 72,120 and 240-hours post-injection of  $^{89}\text{Zr-}\alpha\text{TIGIT}(2.5\text{-}2.7\text{MBq}/25\mu\text{g})$  alone and  $^{89}\text{Zr-}\alpha\text{TIGIT}(2.5\text{-}2.7\text{MBq}/25\mu\text{g})$  plus unconjugated anti-TIGIT antibody( $250\mu\text{g}$ ) administered 24-hours prior to tracer.

# Supplementary Figure S3



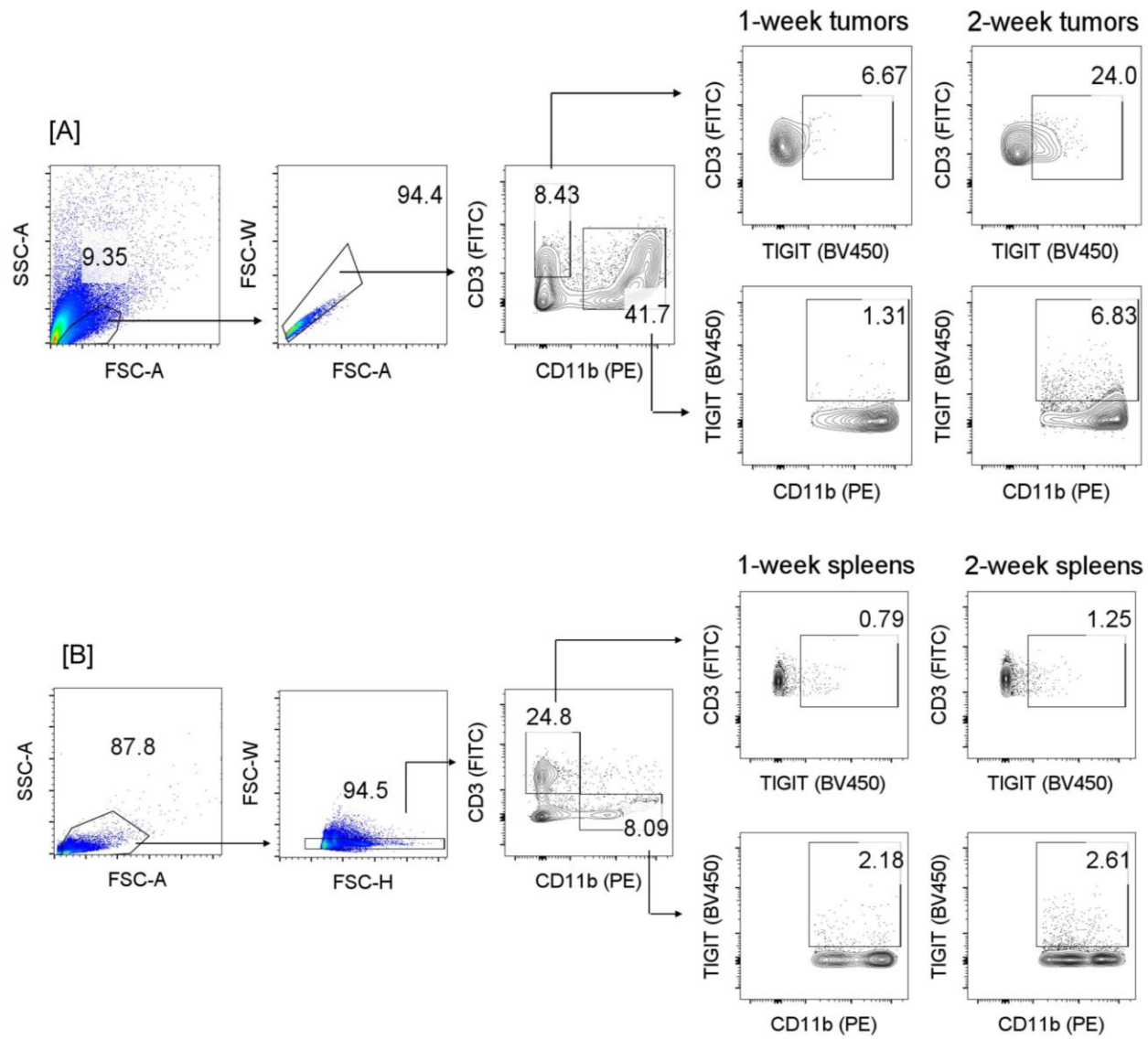
**Supplementary Figure S3:** Whole body PET/CT images of  $^{89}\text{Zr}$ -TIGIT at 72, 120 and 240-hours post injection of (A) 2.9-3.3MBq  $^{89}\text{Zr}$ - $\alpha$ TIGIT with 100 $\mu\text{g}$  (B) 2.9-3.3MBq  $^{89}\text{Zr}$ - $\alpha$ TIGIT with 625 $\mu\text{g}$  unlabeled anti-TIGIT antibody injected 24 hours prior radiotracer injection (C) 3.1-3.5MBq  $^{89}\text{Zr}$ -IgG.

Supplementary Figure S4



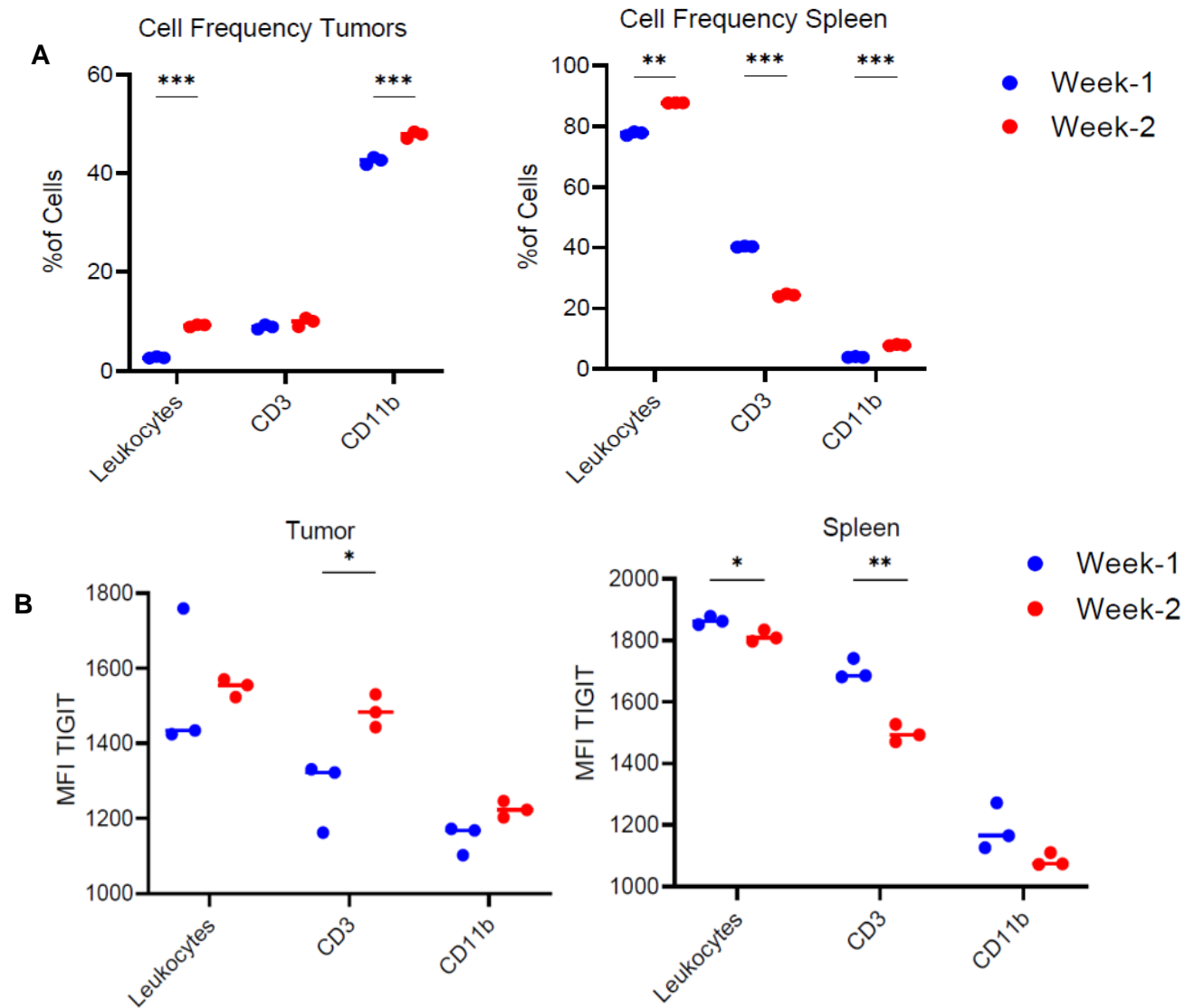
**Supplementary Figure S4.** Bar graph demonstrating variation of SUV mean for Blood, heart, Bone M arrow for  $^{89}\text{Zr-}\alpha\text{TIGIT(100}\mu\text{g, black)}$ ,  $^{89}\text{Zr-}\alpha\text{TIGIT(100}\mu\text{g with 625}\mu\text{g block, blue)}$  and  $^{89}\text{Zr-}\alpha\text{TIGIT (100}\mu\text{g)}$  72, 120, 240 hours post radiotracer injection in GL261 glioma models one week post tumor inoculation. Data represented as  $\pm\text{SD}$

# Supplementary Figure S5



**Supplementary Figure S5.** Gating strategies used for flow cytometry data analysis of tumor (A) and spleen (B) samples.

Supplementary Figure S6



**Supplementary Figure S6. MFI and % values for TIGIT in GL261 gliomas.** (A) Percentage of total Leukocytes, CD3 and CD11b positive cells in week-1 and week-2 tumor and spleen samples by Flow cytometry and (B) Flow cytometry analysis of MFI in week-1 and week-2 tumor and spleen samples