Supplementary Information

Blockade of α4 integrins reduces leukocyte–endothelial interactions in cerebral vessels and improves memory in a mouse model of Alzheimer's disease

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Running title: Role of α4 integrins in Alzheimer's disease

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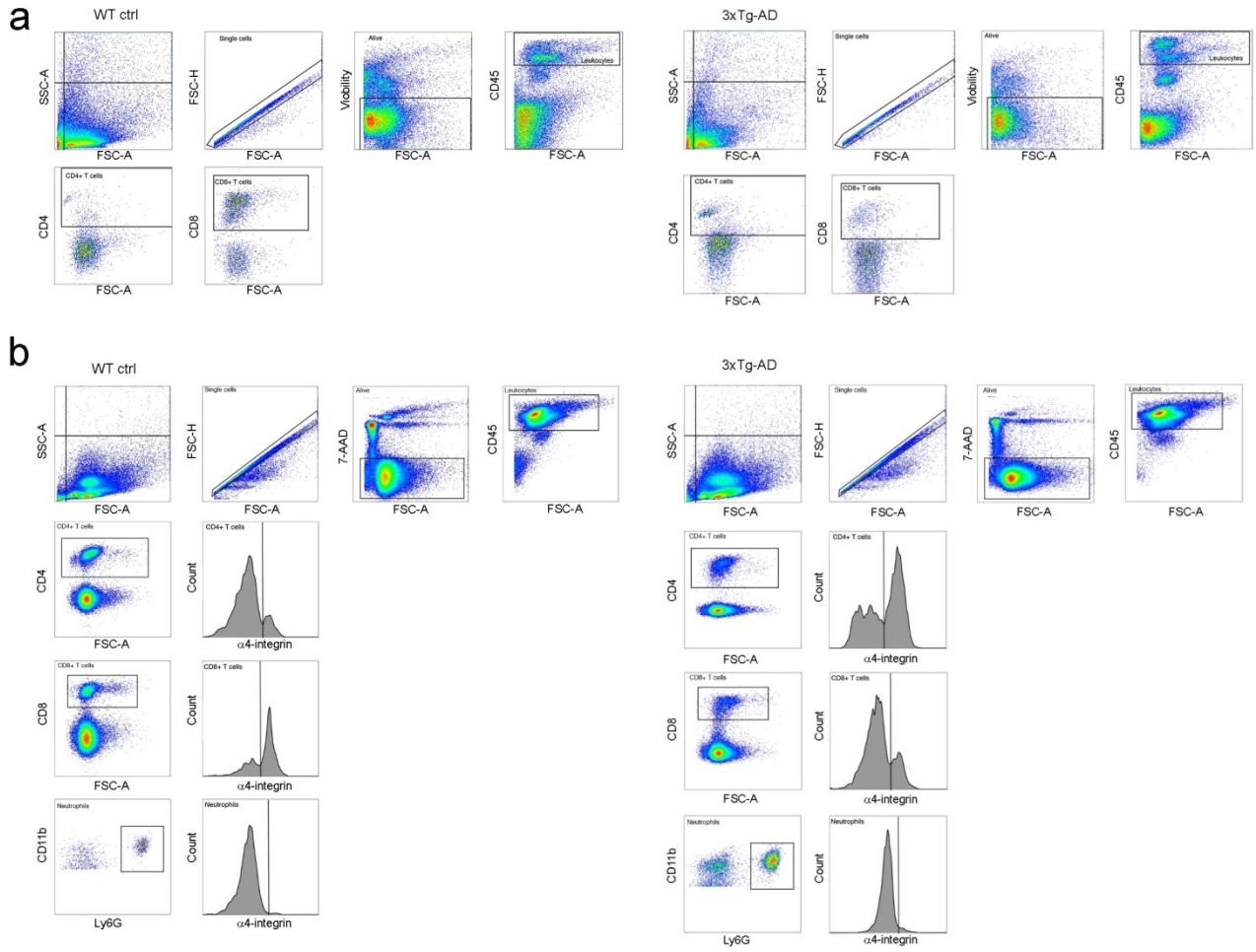

Supplementary Figure 1. Gating strategies for flow cytometry experiments.

Representative gating strategy used during FACS analysis for the identification of (**a**) brain-infiltrating $CD45^{+}$ cells and (**b**) peripheral leukocytes in wild-type control (WT ctrl) and 3xTg-AD mice by simultaneous labeling with antibodies recognizing lymphocyte and neutrophil markers. Doublets were removed from the analysis by the sub-gate identification of singlets using FSC-A and FSC-H. **a**) Dead cells were removed from the analysis using Viobility 405/520 fixable dye. $CD45^{high}$ cells were sub-gated using FSC-A plus a CD4 double gate to define $CD4^{+}$ T cells, and FSC-A plus a CD8 double gate to define $CD8^{+}$ T cells. **b**) Dead cells were removed from the analysis using 7AAD. $CD4^{+}$ and $CD8^{+}$ T cells were identified by gating on $CD45^{+}$ cells, whereas $Ly6G^{+}$ neutrophils were detected by gating CD11b/Ly6G on $CD45^{+}$ leukocytes. The α4 integrin expression in each population ($CD8^{+}$ T cells, $CD4^{+}$ T cells and $Ly6G^{+}$ neutrophils) is shown in the histograms.

Supplementary Table I. Effect size statistics

FACS analysis (WT ctrl vs 3xTg-AD)	Cohen's *d*	Magnitude
Brain		
% of $CD4^+$ cells on $CD45^{high}$ (6 months)	-0.3924413	Small
% of $CD4^+$ cells on $CD45^{high}$ (9 months)	-1.938403	Large
% of $CD8^+$ cells on $CD45^{high}$ (6 months)	-0.05060928	Negligible
% of $CD8^+$ cells on $CD45^{high}$ (9 months)	2.790802	Large
Blood		
% α4 integrin$^+$ $CD4^+$ cells (6 months)	-2.75399	Large
% α4 integrin$^+$ $CD4^+$ cells (9 months)	-2.195376	Large
MFI α4 integrin$^+$ expression on $CD4^+$ cells (6 months)	0.2582844	Small
MFI α4 integrin$^+$ expression on $CD4^+$ cells (9 months)	-6.414628	Large
% α4 integrin$^+$ $CD8^+$ cells (6 months)	1.356608	Large
% α4 integrin$^+$ $CD8^+$ cells (9 months)	1.175441	Large
MFI α4 integrin$^+$ expression on $CD8^+$ cells (6 months)	0.6933269	Medium
MFI α4 integrin$^+$ expression on $CD8^+$ cells (9 months)	0.3108755	Small
% α4 integrin$^+$ $Ly6G^+$ cells (6 months)	0.1284168	Negligible
% α4 integrin$^+$ $Ly6G^+$ cells (9 months)	-0.1840141	Negligible
MFI α4 integrin$^+$ expression on $Ly6G^+$ cells (6 months)	0.9542835	Large
MFI α4 integrin$^+$ expression on $Ly6G^+$ cells (9 months)	1.210988	Large

IVM studies	Cohen's *d*	Magnitude
Digital		
Number of interacting cells ≤ Vcrit (WT ctrl vs 3xTg-AD)	-2.835166	Large
% of interacting cells ≤ Vcrit (Untreated vs Anti-α4)	1.582386	Large
% of interacting cells ≤ Vcrit (Untreated vs Isotype)	-0.2805429	Small
Analog		
% of leukocyte rolling (Untreated vs Anti-α4)	1.283774	Large
% of leukocyte rolling (Untreated vs Isotype)	0.5024471	Medium

Behavioral tests	Cohen's d	Magnitude
Y maze		
% Alternation (WT ctrl vs 3xTg-AD	1.507574	Large

Isotype)		
% Alternation (3xTg-AD Isotype vs 3xTg-AD Anti-α4)	-1.209133	Large
CFC		
% Freezing (WT ctrl vs 3xTg-AD Isotype)	1.343395	Large
% Freezing (3xTg-AD Isotype vs 3xTg-AD Anti-α4)	-1.058881	Large
MWM		
Escape latency (WT ctrl vs 3xTg-AD Isotype)	0.7383913	Medium
Escape latency (3xTg-AD Isotype vs 3xTg-AD Anti-α4)	1.307888	Large
Platform location crossing (WT ctrl vs 3xTg-AD Isotype)	1.487006	Large
Platform location crossing (3xTg-AD Isotype vs 3xTg-AD Anti-α4)	-1.553772	Large
Latency to cross platform location (WT ctrl vs 3xTg-AD Isotype)	-2.243153	Large
Latency to cross platform location (3xTg-AD Isotype vs 3xTg-AD Anti-α4)	1.247004	Large

Neuropathology (Isotype vs Anti-α4)	**Cohen's *d***	**Magnitude**
Treatment at 6 months		
Area Cortex Iba-1	0.1447865	Negligible
Area CA1 Iba-1	0.2103128	Small
Area CA1 6E10	0.9596423	Large
Area CA1 HT7	0.2634473	Small
Area CA1 AT180	1.321709	Large
Treatment at 9 months		
Area Cortex Iba-1	0.647467	Medium
Area Cortex 6E10	0.9800084	Large
Area CA1 HT7	-0.2208119	Small
Area CA1 AT180	1.408082	Large

The magnitude was assessed using the thresholds provided by Cohen (1992)[1], i.e. $d < 0.2$ negligible, $d < 0.5$ small, $d < 0.8$ medium, otherwise large

Supplementary Methods

Isolation of brain leukocytes and flow-cytometry analysis

Mice were anesthetized and perfused through the left cardiac ventricle by injecting cold PBS. The brain was digested with 20 U/ml of DNaseI and 1 mg/ml collagenase at 37°C for 45 min. Cells were isolated by passing the digested tissue through a 70-μm cell strainer. They were then resuspended in 30% Percoll and loaded onto 70% Percoll. The tubes were centrifuged at 1300 × g for 20 min at 4°C. Cells were removed from the interphase, washed and labeled with the following anti-mouse antibodies: anti-CD45-PE, anti-CD11b-PE-Vio770, anti-Ly6G-APC-Vio770, anti-CD3 PerCP-Vio700, anti-CD8a VioBright-FITC, and anti-CD4 VioBlue. Cells were acquired by flow cytometry with MACSQuant Analyzer (Miltenyi Biotec). Data were analyzed using FlowJo software.

Supplementary Reference

1. Cohen, J. A power primer. *Psychol Bull*. **112,** 155-159 (1992).