

Role for BLT1 in regulating inflammation within adipose tissue immune cells of aged mice

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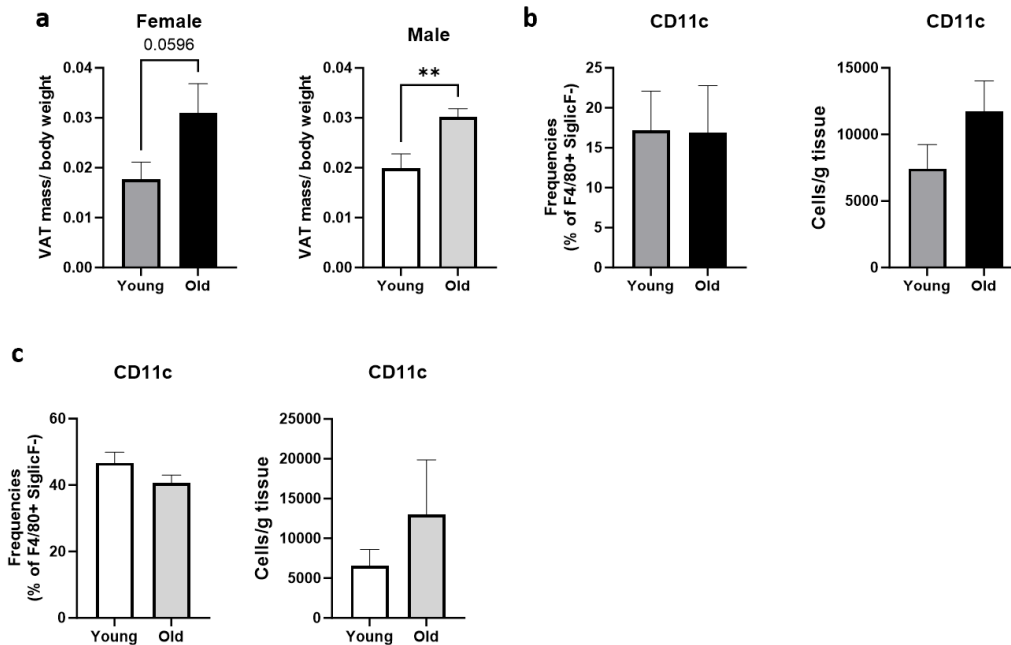
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Supplementary Table 1: Antibodies used for flow cytometry

Experiment	Antibody	Fluorophore	Company	Catalogue	Clone
BLT1 y vs. o (Fig. 1, 2)	CD11c	PE	Biolegend	117308	N418
	SiglicF	BV605	BD Biosciences	740388	E50-2440
	F4/80	BV421	Biolegend	123132	BM8
	CD3e	AF488	Biolegend	100321	145-2C11
	Ly6G	PerCP/Cy5.5	Biolegend	127615	1A8
	CD19	BUV395	BD Biosciences	563557	1D3
	CD11b	BV785	Biolegend	101243	M1/70
	CD45.2	BUV737	BD Horizon	612778	104
	LTB4-R1 (BLT1)	unconjugated	Millipore	MABF2769	7A8
LPS stim BMDM (Fig. 3)	Anti-mouse IgG1	AF647	Biolegend	406618	RMG1-1
	F4/80	PE	invitrogen	2049425	BM8
	CD206	FITC	Biolegend	141704	C068C2
	CD86	BV421	BD Biosciences	564198	GL1
	CD45.2	BUV737	BD Horizon	612778	104
	LTB4-R1 (BLT1)	unconjugated	Millipore	MABF2769	7A8
	Anti-mouse IgG1	AF647	Biolegend	406618	RMG1-1

BMDM polarization (Fig. 4)	F4/80	BV421	Biolegend	123132	BM8
	CD206	FITC	Biolegend	141704	C068C2
	CD11c	PE	Biolegend	117308	N418
	CD45.2	BUV737	BD Horizon	612778	104
	LTB4-R1 (BLT1)	unconjugated	Millipore	MABF2769	7A8
	Anti-mouse IgG1	AF647	Biolegend	406618	RMG1-1
BLT1 antagonist (Fig. 5)	CD206	FITC	Biolegend	141704	C068C2
	CD11c	PE	Biolegend	117308	N418
	SiglicF	BV605	BD Biosciences	740388	E50-2440
	F4/80	BV421	Biolegend	123132	BM8
	CD3e	PE-Cy7	invitrogen	25-0031-82	145-2C11
	Ly6G	PerCP/Cy5.5	Biolegend	127615	1A8
	CD19	BUV395	BD Biosciences	563557	1D3
	CD11b	BV785	Biolegend	101243	M1/70
	CD45.2	BUV737	BD Horizon	612778	104
	CD69	BV711	Biolegend	104537	H1.2F3
	LTB4-R1 (BLT1)	unconjugated	Millipore	MABF2769	7A8
	Anti-mouse IgG1	AF647	Biolegend	406618	RMG1-1

Supplementary Fig. 1

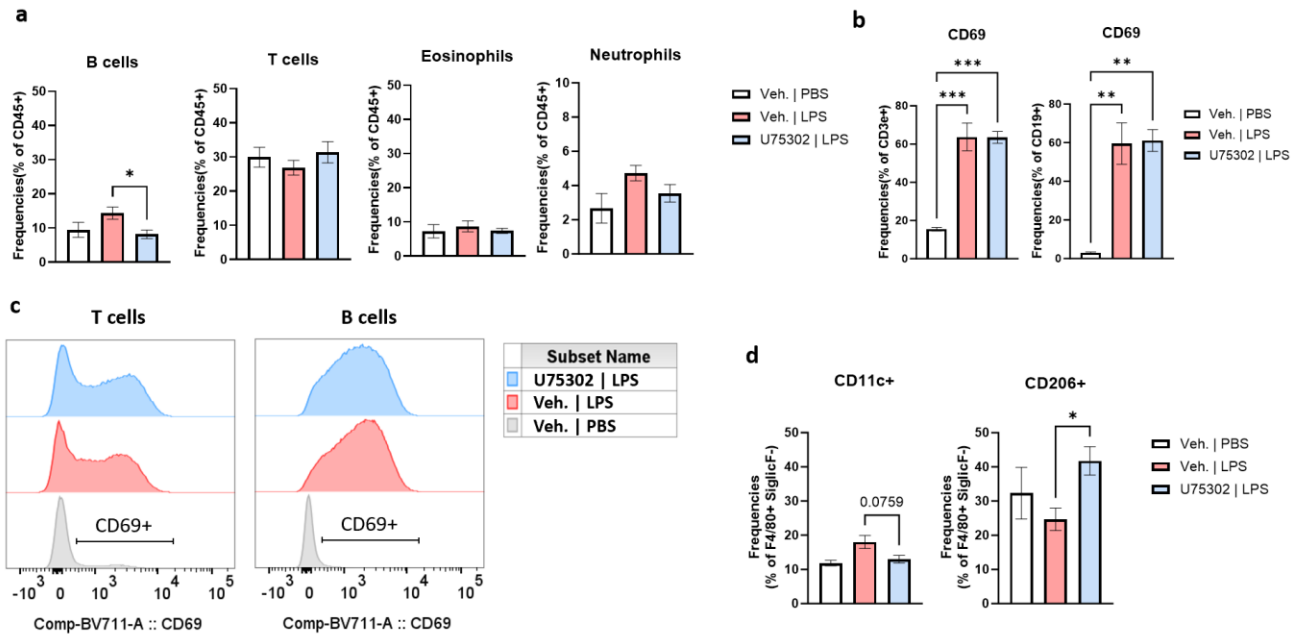


Supplementary Fig. 1: Comparison of CD11c frequencies and cellularity of young (3-8 months) and aged (20-24 months) mice.

a Ratio of VAT mass and body weight of female and male, young and aged mice. **b** Frequency of CD11c⁺ F4/80⁺ SiglecF⁻ macrophages and cells/g tissue in VAT from female mice. **c** Frequency of CD11c⁺ F4/80⁺ SiglecF⁻ macrophages and cells/g tissue of male mice. (n=4-5/group). Error bars represent mean ± SEM. Statistical analysis was conducted using non-parametric T-tests.

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

Supplementary Fig. 2

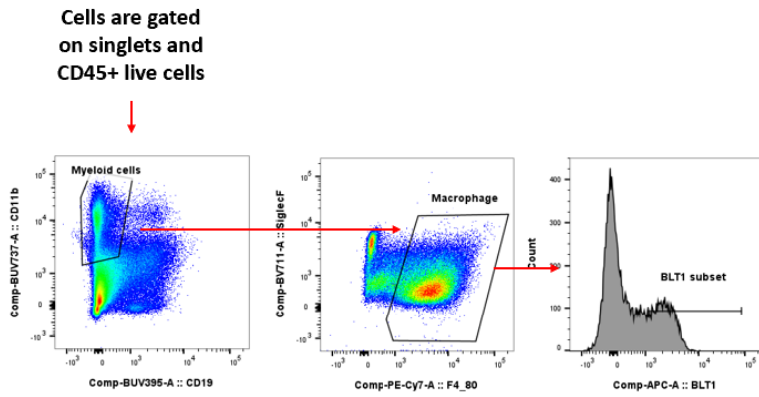


Supplementary Fig. 2: CD11c and CD206 frequencies in BLT1 antagonist (U75302) treated aged (24 months) mice.

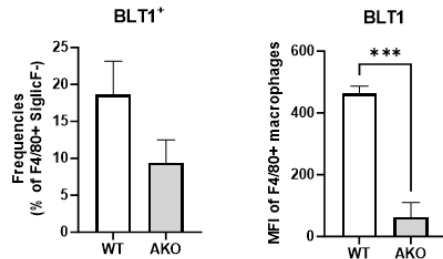
a Immune cell frequency of CD45⁺ live cells in VAT. **b** CD69 population in CD3e⁺ T cells and CD19⁺ B cells of spleen. **c** Histogram of CD69 population in CD3e⁺ T cells and CD19⁺ B cells of spleen. **d** Frequency of CD11c⁺ F4/80⁺ SiglecF⁻ macrophages and CD206⁺ F4/80⁺ SiglecF⁻ macrophages. Male mice were used in this experiment (n=4 in vehicle, n=8 in LPS, n=7 in LPS with BLT1 antagonist). Error bars represent mean ± SEM. Statistical analysis was conducted using one-way ANOVA with a post-hoc test of Tucky. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

Supplementary Fig. 3

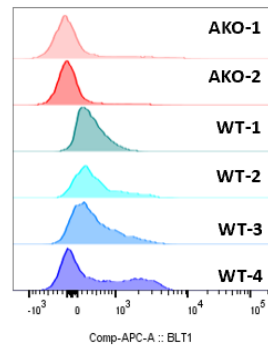
a



b



c



Supplementary Fig. 3: BLT1 expression on VAT macrophages from aged WT and AKO male mice.

a Flow cytometry gating strategy of BLT1⁺ subsets. **b** BLT1 frequency and MFI of F4/80⁺ SiglecF⁻ macrophages in VAT from male mice. **c** Histogram of BLT1 population in F4/80⁺ SiglecF⁻ VAT macrophages of aged male mice. Male mice were used in this experiment (n=4 in WT, n=2 in AKO). Error bars represent mean $\pm$ SEM. Statistical analysis was conducted using non-parametric T-tests. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.