

Effects of obesity on NK cells in a mouse model of postmenopausal breast cancer

Julia Spielmann^{1*}, Laura Mattheis^{1,2}, Juliane-Susanne Jung¹, Henrik Rauße^{1,3}, Markus Glaß⁴, Ina Bähr¹, Dagmar Quandt^{1,5}, Jana Oswald¹, Heike Kielstein¹

¹Institute of Anatomy and Cell Biology, Medical Faculty of Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

² Department of Internal Medicine I, Medical Faculty of Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany ³Clinic for Psychosomatics and Psychotherapy, Landschaftsverband Westfalen-Lippe Clinic, Lengerich, Germany

⁴Institute of Molecular Medicine, Charles Tanford Protein Center, Medical Faculty of Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

⁵Regenerative Medicine Institute (REMEDI) at CÚRAM Centre for Research in Medical Devices, School of Medicine, College of Medicine, Nursing and Health Sciences, National University of Ireland Galway, Ireland

Corresponding author:

*Julia Spielmann

Institute of Anatomy and Cell Biology

Martin Luther University Halle-Wittenberg, Faculty of Medicine

Grosse Steinstrasse 52

06108 Halle (Saale)

Germany

Email: Julia.Spielmann@medizin.uni-halle.de

Supplementary Table S1: Body and organ weight in short-term experiment

	<i>Short-term experiment</i>						
Time point	20h after 4T1-luc2 injection						
	Co/NaCl	Co/Tumor	DIO/NaCl	DIO/Tumor	Two way ANOVA p-value		
					Interaction	Tumor	Diet
Body weight in g							
Start of experiment	22.4 ± 0.5	23.0 ± 0.6	22.0 ± 0.8	22.6 ± 0.3	0.9694	0.2831	0.4687
End of experiment	25.5 ± 0.3 ^b	25.7 ± 0.3 ^b	30.6 ± 1.1 ^a	30.6 ± 0.5 ^a	0.8015	0.8870	<0.0001
Visceral fat in g	0.5 ± 0.05 ^b	0.57 ± 0.04 ^b	2.02 ± 0.30 ^a	2.05 ± 0.19 ^b	0.9167	0.7965	<0.0001
Spleen weight in g	0.13 ± 0.01 ^{ab}	0.12 ± 0.01 ^b	0.15 ± 0.01 ^{ab}	0.17 ± 0.02 ^a	0.3029	0.6395	0.0041
Liver weight in g	1.15 ± 0.03	1.08 ± 0.01	1.16 ± 0.05	1.16 ± 0.03	0.3443	0.4126	0.1824

Values represent means ± SEM, n=7 mice/group. Bold p-values highlight significant results analyzed by Two-way ANOVA ($p \leq 0.05$). Different superscript letters (a,b) indicate significant differences between individual experimental groups analyzed by Tukey's multiple comparison test ($p \leq 0.05$).

Supplementary Table S2: Body and organ weight in long-term experiment

	<i>Long-term experiment</i>						
Time point	21 days after 4T1-luc2 injection						
	Co/NaCl	Co/Tumor	DIO/NaCl	DIO/Tumor	Two way ANOVA p-value		
					Interaction	Tumor	Diet
Body weight in g							
Start of experiment	22.0 ± 0.5	20.4 ± 0.6	20.9 ± 0.2	21.1 ± 0.5	0.1038	0.2073	0.7561
End of experiment	27.4 ± 0.8 ^c	25.8 ± 0.5 ^c	37.8 ± 1.2 ^a	31.8 ± 1.1 ^b	0.0223	0.0003	<0.0001
Visceral fat in g	0.71 ± 0.05 ^b	0.17 ± 0.04 ^c	2.4 ± 0.20 ^a	0.53 ± 0.10 ^{bc}	<0.0001	<0.0001	<0.0001
Spleen weight in g	0.12 ± 0.01 ^a	1.28 ± 0.08 ^b	0.15 ± 0.02 ^a	1.44 ± 0.09 ^b	0.3965	<0.0001	0.1876
Liver weight in g	1.15 ± 0.04 ^b	1.55 ± 0.06 ^a	1.55 ± 0.07 ^a	1.66 ± 0.07 ^a	0.0416	0.0008	0.0008

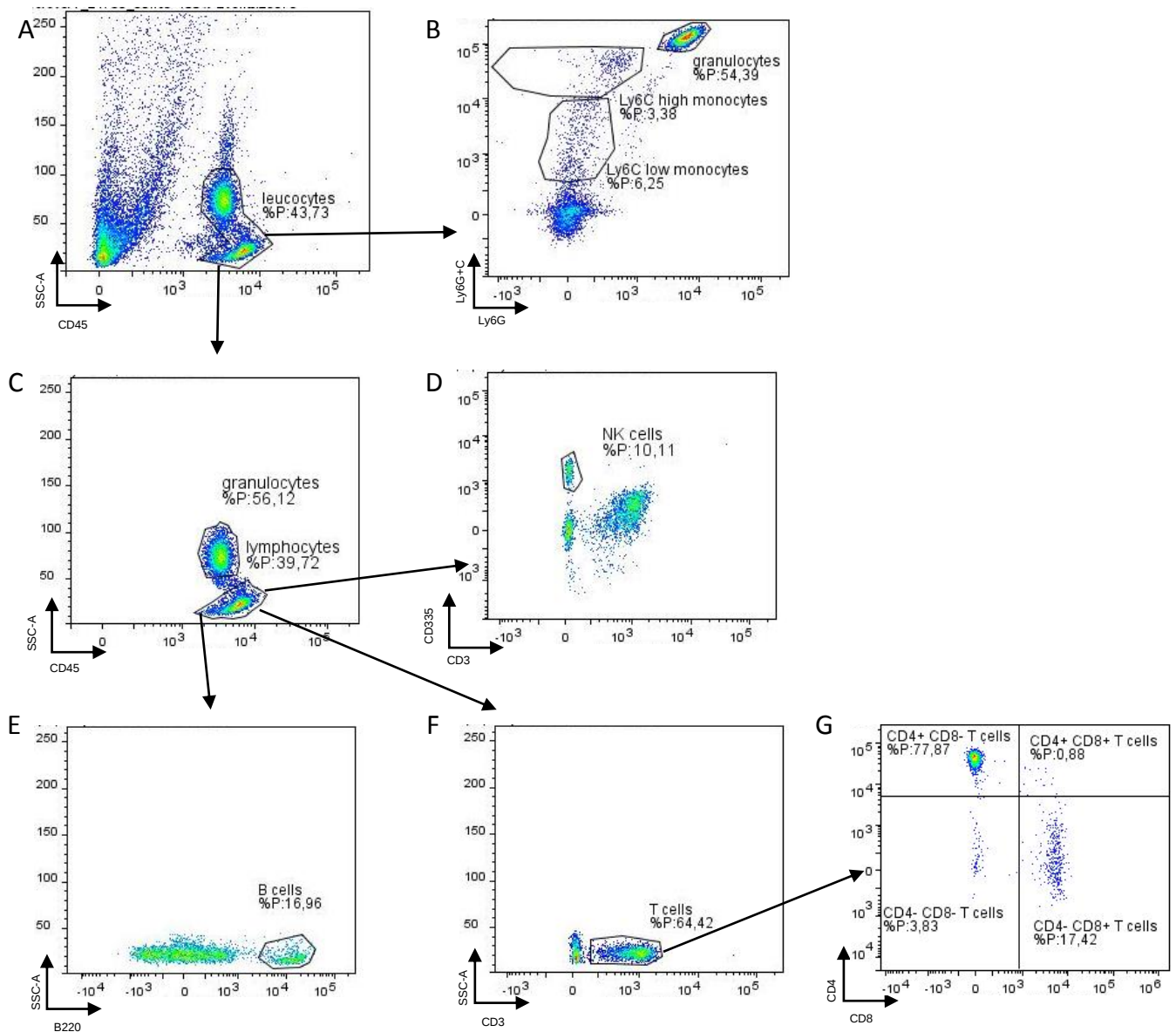
Values represent means ± SEM, n=7 mice/group. Bold p-values highlight significant results analyzed by Two-way ANOVA ($p \leq 0.05$). Different superscript letters (a,b,c) indicate significant differences between individual experimental groups analyzed by Tukey's multiple comparison test ($p \leq 0.05$).

Supplementary Table S3. Characteristics of the specific primers used for real-time RT-PCR analysis

Gene	Forward and reversed primers	bp	Annealing temperature	NCBI gene bank
Ppia	for: 5' CACCGTGTTCTTCGACATCA 3' rev: 5' TGTCTGCAAACAGCTCGAAG 3'	71	64°C	NM_008907.1
Klrb1c/CD161	for: 5' AACTGAGATTCCTACTGGAC 3' rev: 5' TTGTGCCATTTATCCACTTC 3'	105	60°C	NM_001159904
Klrk1/NKG2D	for: 5' AGTATTGTGCAACAAGGAAG 3' rev: 5' TTTTGAGACAACCAGGAAGC 3'	153	57°C	NM_033078.4
CD335/NCR1/NKp46	for: 5' TAGTAACTGGTCTGTATGACAC 3' rev: 5' CTTGAGCAGAAAGAATTTGC 3'	125	58°C	NM_010746
PDCD1/PD-1	for: 5' ACTAGGGCAATAAAGGGAAC 3' rev: 5' GAATGAGGAGATTCTAACACC 3'	176	57°C	NM_008798
ESR1	for: 5' CAAGGTAAATGTGTGGAAGG 3' rev: 5' GTGTACACTCCGGAATTAAG 3'	140	58°C	NM_007956.5
ESR2	for: 5' ATGCTTCTTTCTCATGTCAG 3' rev: 5' TCTTTGCTCTTACTGTCCTC 3'	188	58°C	NM_010157.3
GPHER1	for: 5' GATCTAGGGAGAAAGCCATC 3' rev: 5' CCTGTTAGTCTCAGAAAACC 5'	181	60°C	NM_029771

RT-PCR, Reverse transcription polymerase chain reaction; Bp, base pair; NCBI, National Center for Biotechnology Information; Ppia, peptidylprolyl isomerase A; Klrb1c/CD161, Killer cell lectin-like receptor subfamily B member 1c; Klrk1/NKG2D, Killer cell lectin-like receptor subfamily k member/ Natural Killer group 2 member D; CD335/NCR1/NKp46, natural cytotoxicity triggering receptor 1; PDCD1/PD-1, programmed cell death protein 1; ESR, estrogen receptor; GPHER1, G protein-coupled estrogen receptor 1.

Supplementary Figure 1: Gating strategy for FACS analysis



Representative plots of the hierarchical gating strategy to identify immune cell populations and NK cells in BALB/c mice. Leucocytes were identified by their CD45 expression and granularity using SSC (A), followed by gating of CD45⁺ SSC^{low} lymphocytes and CD45⁺ SSC^{high} granulocytes (C). Leucocyte gating combined with single cell gating of Ly6G+C and Ly6C expression leads to the identification of Ly6C^{high} monocytes and Ly6C^{low} monocytes (B). Based on the lymphocyte gate, CD3⁻ and CD335⁺ lymphocytes were identified as NK cells (D), B220⁺ SSC^{low} lymphocytes were identified as B cells (E) and CD3⁺ SSC^{low} lymphocytes were identified as T cells (F). Based on the T cell gate, CD4⁺/CD8⁻ and CD4⁻/CD8⁺ cells were gated and identified as T cell subsets (G).