biology

MDPI

Supplementary Material to

Endotoxin tolerance acquisition and altered hepatic fatty acid profile in aged mice

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1. Supplementary Tables

Table S1. Sample sizes of sample types, age groups, and LPS treatments. Numbers in brackets represent the number of female mice within the sample size.

	young			old		
	control	non-tolerant	tolerant	control	non-tolerant	tolerant
lung	7 (4)	7 (4)	8 (4)	8 (5)	8 (5)	8 (4)
liver	7 (4)	7 (4)	8 (4)	8 (5)	8 (5)	8 (4)
serum TNF	NA	5 (3)	8 (4)	NA	7 (5)	8 (4)
serum IL-1β	NA	NA	NA	NA	8 (5)	7 (3)
serum IL-6	NA	NA	NA	NA	7 (5)	8 (4)

Table S2. Real-Time PCR primers and conditions.

Primer	**Forward Primer Sequence (5'→3')**	**Reverse Primer Sequence (5'→3')**	**NCBI Accession number**	**µl primer [10 µM/ 20 µl reaction]**	**Annealing Temp-erature [°C]**	**qPCR program**
Cpt1a	CTCAGTGGGAG CGACTCTTCA	GGCCTCTGTGGT ACACGACAA	NM_01349 5.2	0.5	60	• 95°C for 15 min 40 cycles: • 95°C for 15 sec • Annealing [°C] for 20 sec • 72°C for 20 sec • 65°C for 5 sec
Il10	GCCCAGAAATC AAGGAGCAT	GAAATCGATGA CAGCGCCT	NM_01054 8.2	0.5	60	
Il1b	CCAAAAGATGA AGGGCTGCTT	GGAAGGTCCAC GGGAAAGAC	NM_00836 1.3	0.5	60	
Il6	AAGAAATGATG GATGCTACCAA ACTG	GTACTCCAGAA GACCAGAGGAA ATT	NM_03116 8.2	0.4	60	
Ppara	CCTTCCCTGTGA ACTGACG	CCACAGAGCGC TAAGCTGT	NM_00111 3418.1	0.5	60	
Ppia	GCGTCTCCTTCG AGCTGTTT	CACCCTGGCAC ATGAATCCT	NM_00890 7.1	0.5	60	
Tlr2	CACTGCCCGTA GATGAAGTC	TACCTCCGACA GTTCCAAGA	NM_01190 5.3	0.5	60	
Tlr4	TCCCTGCATAGA GGTAGTTCC	TCAAGGGGTTG AAGCTCAGA	NM_02129 7.3	0.5	60	
Tnf	CCATTCCTGAGT TCTGCAAAGG	AGGTAGGAAGG CCTGAGATCTTA TC	NM_01369 3.2	0.5	60	

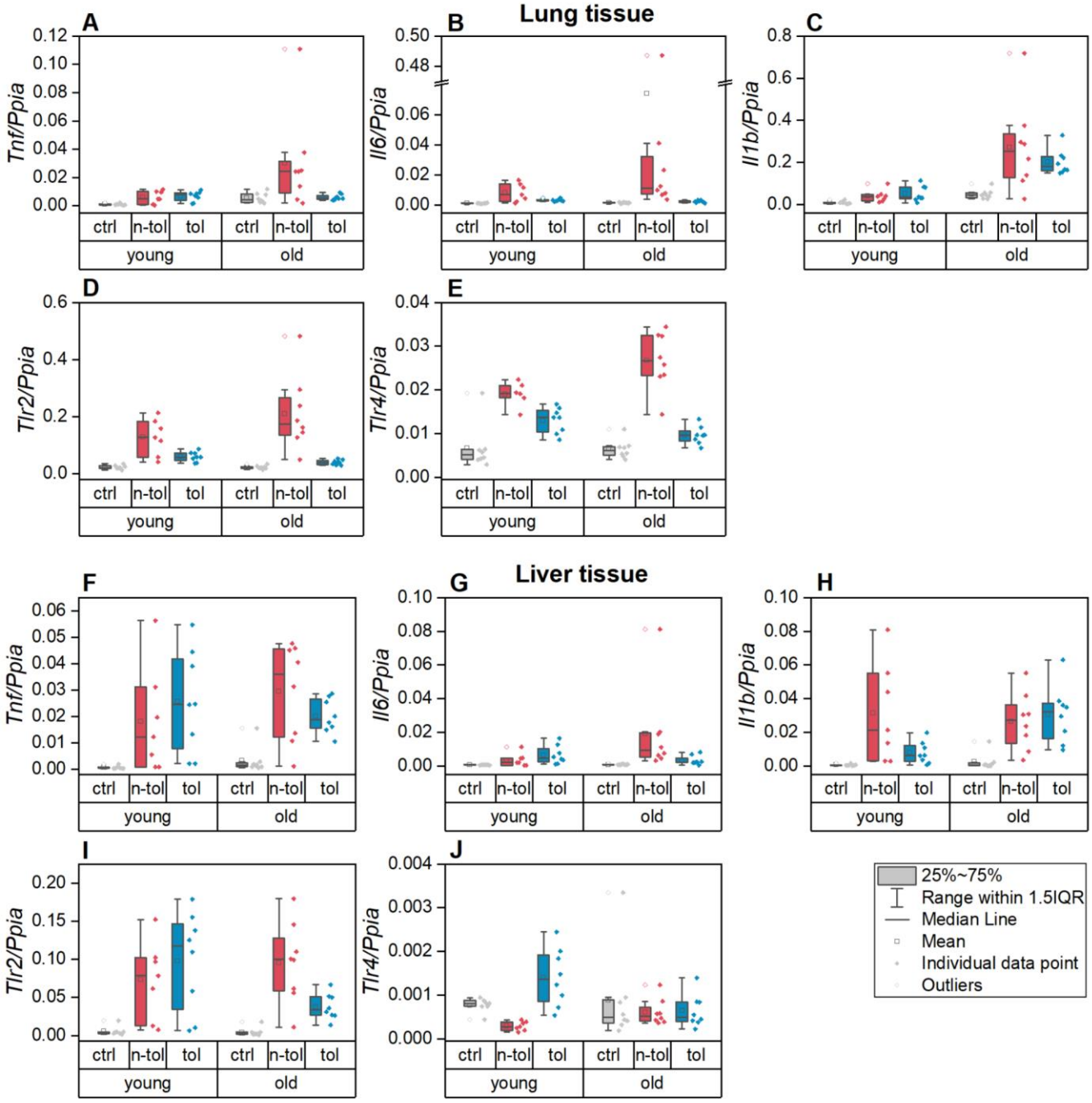

Figure S1: Relative gene expression in lung (A-E) and liver (F-J) tissue. Data are shown for young and old mice as well as control (ctrl), non-tolerant (n-tol), and tolerant (tol) mice in each age group. Note: Results of two-factorial ANOVA in Suppl. Table. S3.

Table S3. Analyses of hepatic fatty acid abundance and gene expression with a two-factorial ANOVA. Significance codes: '***' $p < 0.001$, '**' $p < 0.01$, '*' $p < 0.05$, '.' $p < 0.1$, ' ' $p < 1$. For cases where $0.05 < p < 0.1$, the exact p-value was added in brackets.

Gene expression in lung tissue	Age	Treatment	Age* Treatment
Tnf		**	
Il6			
Il1b	***	. (0.0658)	
Tlr2		***	
Tlr4	***	***	***
Gene expression in liver tissue			
Tnf			
Il6			. (0.067)
Il1b			. (0.051)
Tlr2			*
Tlr4	. (0.091)	**	**
Cpt1a	***	*	. (0.0539)
Ppara	*	***	
Elovl6	*		**
FAs			
Total FA		*	
C18 [% total FA]	***	*	
C16 [% total FA]	***	*	
C18:2 [% total FA]			
C18:1 [% total FA]	***	*	
C18:0 [% total FA]	***	**	
C16:0 [% total FA]	***	*	
C16:1 [% total FA]	***		
Ratio C18:C16	***	*	