

Supplemental Data

Galectin-3 Ablation Enhances Liver Steatosis, but Attenuates Inflammation and IL-33-Dependent Fibrosis in Obesogenic Mouse Model of Nonalcoholic Steatohepatitis

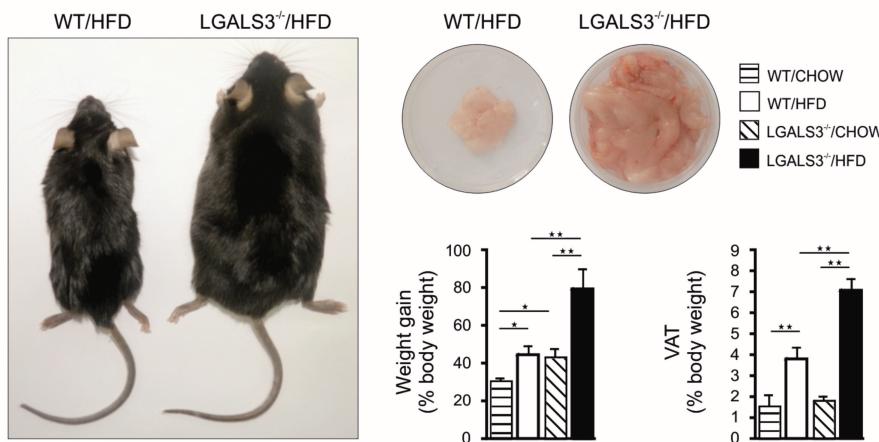
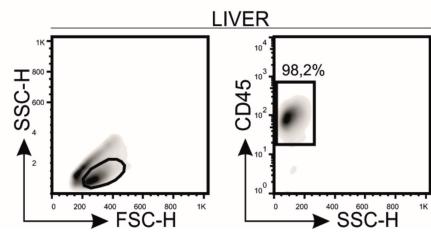
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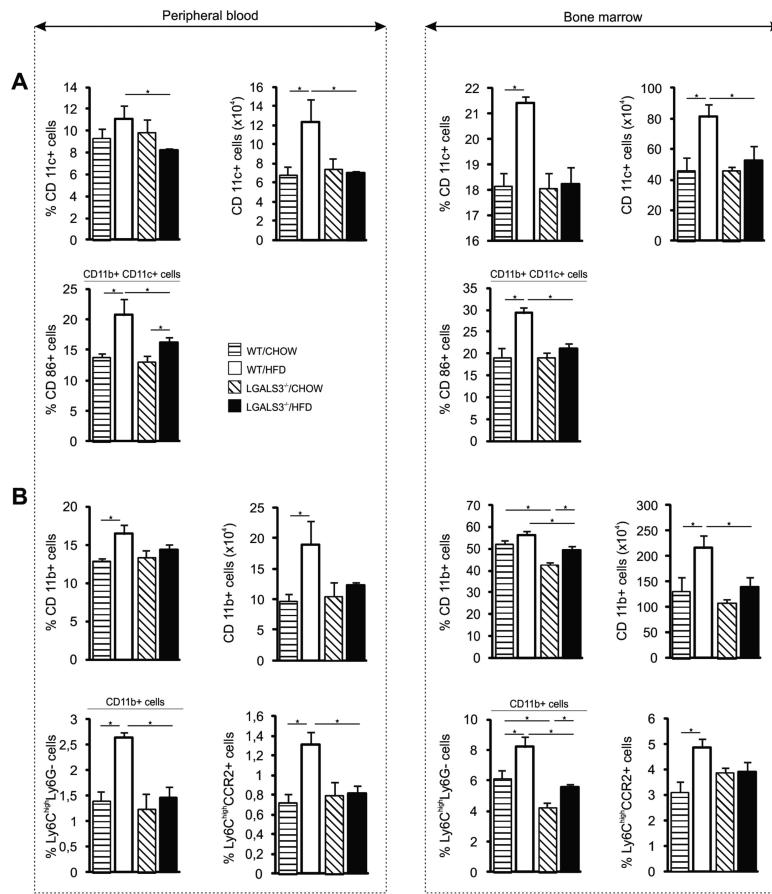
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Supplementary Table S1. Primers used for qRT-PCR analysis.

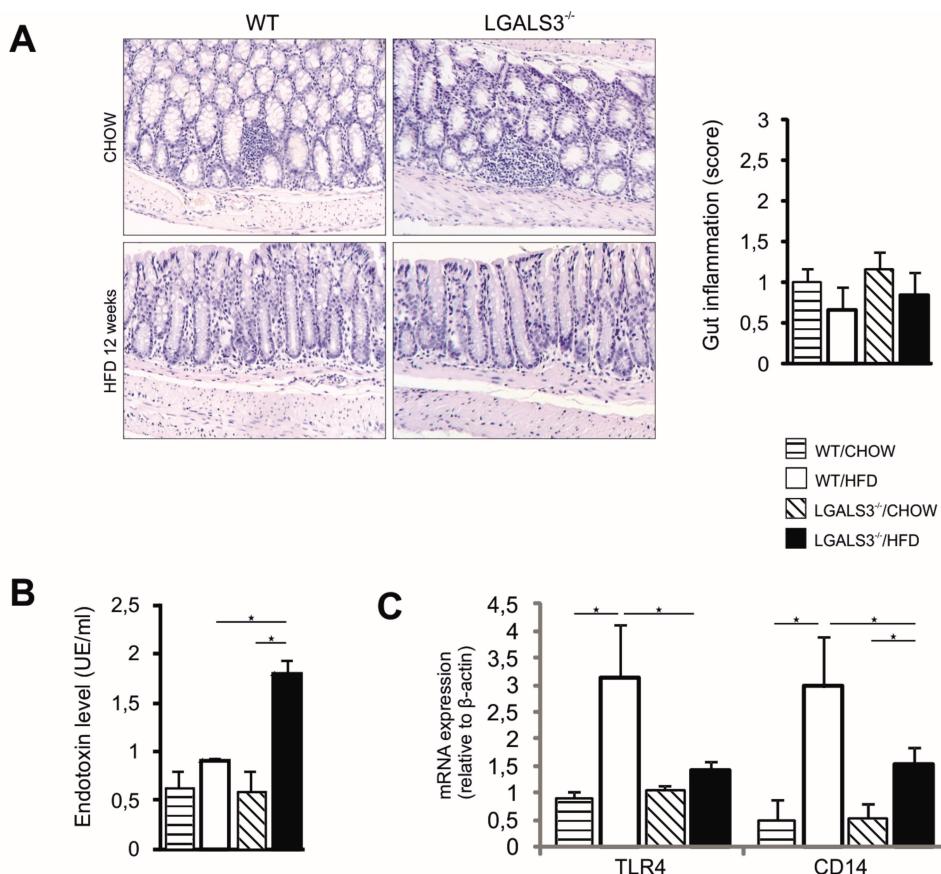
	Sense (5' to 3')	Antisense (5' to 3')
Procol.α1	GCTCCTCTAGGGGCCACT	CCACGTCTCACCATGGGG
α-SMA	ACTGGGACGACATGGAAAAG	CATCTCCAGAGTCCAGCACA
CD11c	ATGGAGCCTCAAGACAGGAC	GGATCTGGGATGCTGAAATC
F4/80	TGCATCTAGCAATGGACAGC	GCCITCTGGATCCATTGAA
CCL2	CAGGTCCCCTGTCATGCTCT	GTCAGCACAGACCTCTCT
NLRP3	AGCCTTCAGGATCCTCTC	CTGGGCAGCAGTTCTTC
Caspase 1	AGATGGCACATTCCAGGAC	GATCCTCCAGCAGCAACTTC
IL-1β	TCTTGAAGTTGACGGACCC	TGAGTGATACTGCCGCTG
IL-6	TCCATCCAGTGCCTCTG	TICCACGATTCCCAGAGAAC
TNF-α	CCACATCCCTCCAGAAAAA	AGGGTCTGGGCCATAGAACT
TLR4	CGTTTACACCTCTGCCCTCACTACAG	ACACTACCACAATAACCTCCGGCTC
CD14	AGGGTACAGCTGCAAGGACT	CTTCAGCCCAGTGAAAGACA
Nox2	CGGTGTGAGTGCATATCATC	GCTCTCCTTCTCAGGGTT
P22phox	CCTGSGCGATAGAGTAGGC	TCATGGGGCAGATCGAGT
P47phox	ATGACCTCAATGGCTCACC	CTATCTGGAGCCCCCTGACA
P67phox	CTATCAGCTGGTCCCACGA	GCAGTGGCCTACTTCCAGAG
IL-33	TCCITGCTGGCAGTATCCA	TGCTCAATGTGCAACAGACG
ST2	GCAATTGACACTTCCCAG	ACGATTACTGCCCTCCGTA
IL-13	CCTGGCTCTGCTGCCTT	GGTCTTGTGATGTTGCTCA
TGF-β	ATACAGGGCTTCGATTCA	GTCCAGGCTCAAATATAGG
ChREBP	CTGGGGACCTAACAGGAGC	GAAGCCACCCCTATAGCTCCC
LXR-α	ATCGCCTGCTGAAGACCTCG	GATGGGGTTGATGAACCTCCACC
LXR-β	AAGCAGGTGCCAGGGTCT	TGCATTCTGTCGTGGTTGT
CREBP-1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAAGTCACTGT
PPAR-γ	CCCAATGGTGTGATTACAAA	GAGGGTGTAGAAGGTTCTCATGA
Abca-1	CGCAGTGACCAGAAAACAATGT	TATCAATGAGGCAAGGGTGTGG
Cd36	TCCAGCCAATGCCCTTG	TGGAGATTACTTTTCAGTGCAGAA
FAS	AGAGATCCCGAGACGCTTCT	GCCTGGTAGGCATTCTGTAGT
CPT1	AACCCAGTGCCTAACGATG	GAACCTGGTGGCCAATGAGAT
DAGT1	ACCGCGAGTTCTACAGAGATTGGT	ACAGCTGCATGCCATAGTCCCT
DAGT2	AGCTGGTGAAGACACACAACC	TGATGATAGCATGCCACTCC
β-actin	AGCTCGTTTACACCCCTT	AAGCCATGCCAATGTTGTCT

A**B**

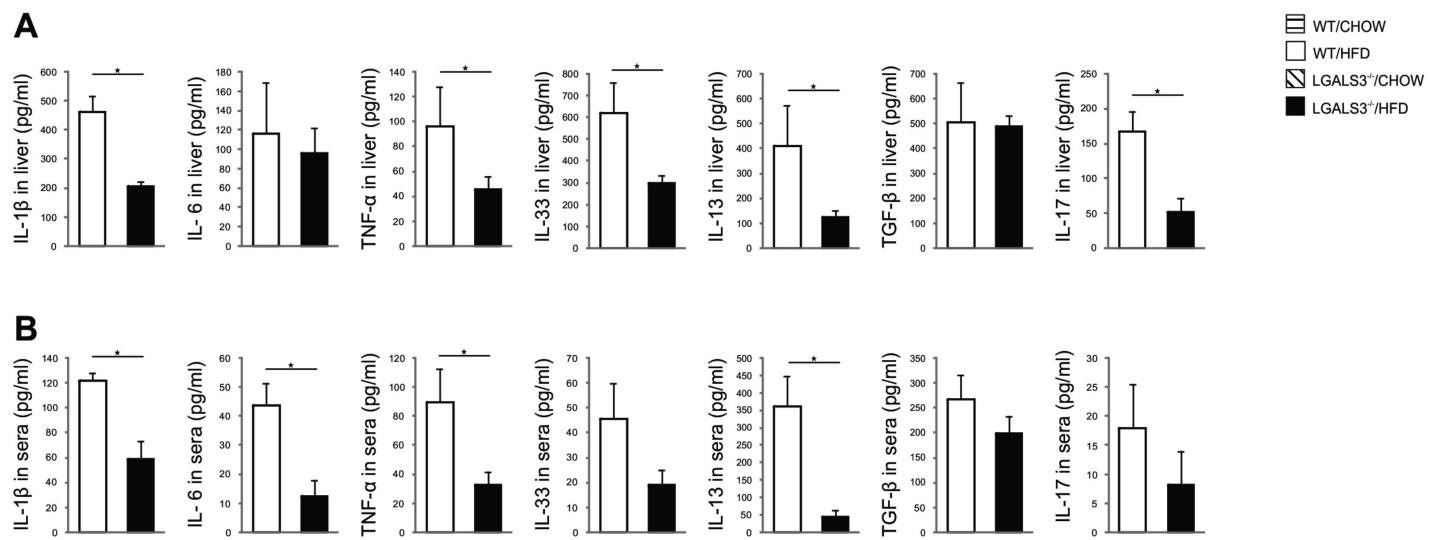
Supplementary Figure S1. Galectin-3 ablation accelerated HFD-induced obesity. (A) Representative images of WT and LGALS3^{-/-} mice after 24 wks on HFD. Representative image of the larger body size of LGALS3^{-/-} vs. WT mice. Representative image of the amount of VAT in WT and LGALS3^{-/-} mice. Weight gain (% body weight) and VAT (% body weight). The results are shown as the means \pm SEM ($n=5-6$ mice/group/experiment), * $P < 0.05$, ** $P < 0.01$. The results are representative of two experiments. (B) Gating region in flow cytometry analysis. Representative flow cytometric plots of gating region used to analyze liver CD45⁺ mononuclear cells.



Supplementary Figure S2. Decreased number of mature dendritic cells and proinflammatory monocytes in peripheral blood and bone marrow in Gal-3 deficient mice on HFD. Peripheral blood and bone marrow cells were analyzed in WT and LGALS3^{-/-} mice on HFD or standard diet for 12 wks. (A) Lower proportions and number of CD11c⁺ DCs and mature myeloid DCs in peripheral blood and bone marrow of LGALS3^{-/-} mice on HFD. (B) Lower proportions of proinflammatory monocytes in peripheral blood and bone marrow of LGALS3^{-/-} mice on HFD. The results are shown as the means \pm SEM ($n=5-6$ mice/group/experiment) * $P < 0.05$, ** $P < 0.01$.



Supplementary Figure S3. Gut inflammation and systemic endotoxin levels. Gut inflammation and endotoxin levels were analyzed in WT and LGALS3^{-/-} mice on HFD or standard diet for 12 wks. (A) H&E staining ($\times 40$). Grading of gut inflammation. (B) Plasma endotoxin levels (UE/ml) (C) Hepatic mRNA expression of TLR-4 and CD14 in WT and LGALS3^{-/-} mice on HFD or standard diet for 24 wks. The results are shown as the means \pm SEM ($n=5-6$ mice/group/experiment). * $P < 0.05$.



Supplementary Figure S4. Proinflammatory and profibrogenic cytokines. Cytokines were analyzed in WT and LGALS3^{-/-} mice on HFD or standard diet for 24 wks. (A) Cytokine levels in the liver homogenates. (B) Cytokine levels in sera. The results are shown as the means \pm SEM ($n=5-6$ mice/group/experiment), * $P < 0.05$, ** $P < 0.01$. The results are representative of two experiments.