

Enhanced lymphocyte infiltration in the liver of LDL receptor and Myeloid Differentiation 1 double knockout mice on high fat diet

Mrityunjoy Biswas¹, Susumu Tomono¹, Kenji Kasai², Hidekazu Takagi¹, Masanori Inui¹, Bristy Basak¹, Fumiaki Nagaoka¹, Tatsuya Yamazaki¹, Naoko Morita¹, Akinori Okumura¹, and Sachiko Akashi-Takamura^{1*}

Supplementary Information

Fig.S1. Representative FACS results of monocytes, neutrophils, and B cells in peripheral cells, related to Figure 3a.

Fig.S2. Representative FACS results of MD-1 in peripheral cells, related to Figure 3a.

Fig.S3. Representative FACS results of B cells, B1a cells, and B1b cells in peritoneal cells, related to Figure 3b.

Fig.S4. Representative FACS results of mast cells in peritoneal cells, related to Figure 3b.

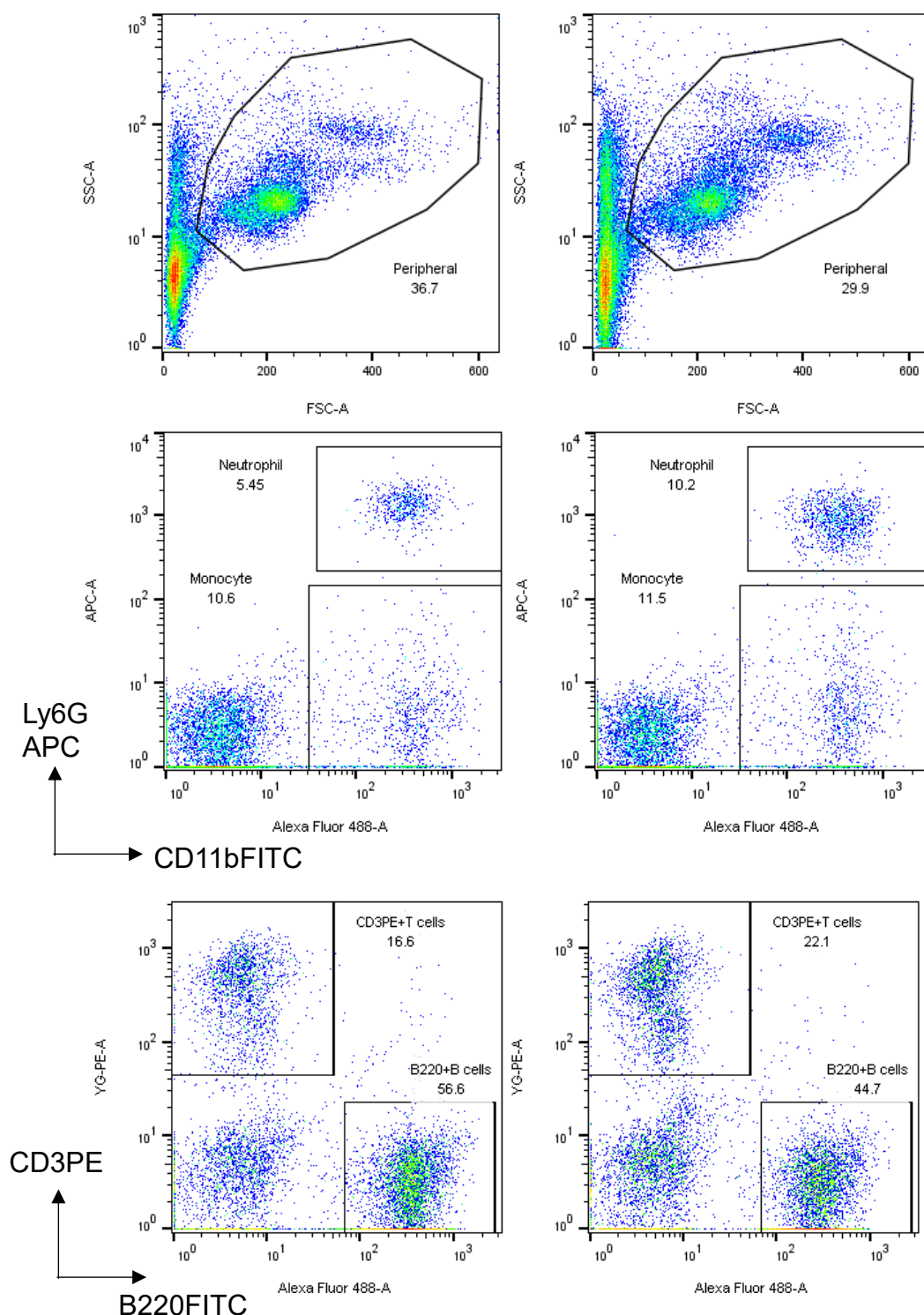
Fig.S5. Representative HE-stained liver sections of LDLr^{-/-}/MD-1^{-/-} mice or LDLr^{-/-}/MD-1^{+/-} mice and the percentage of lymphocyte infiltration area versus total liver area under (a) High Fat Diet for 24 weeks after 7 weeks of age and (b) Normal Chow Diet for 36-37 weeks.

Supplementary Table: The details of NAS score, related to Figure 5b.

Peripheral Cells

LDLr^{-/-}/MD-1^{-/-}

LDLr^{-/-}/MD-1^{+/-}

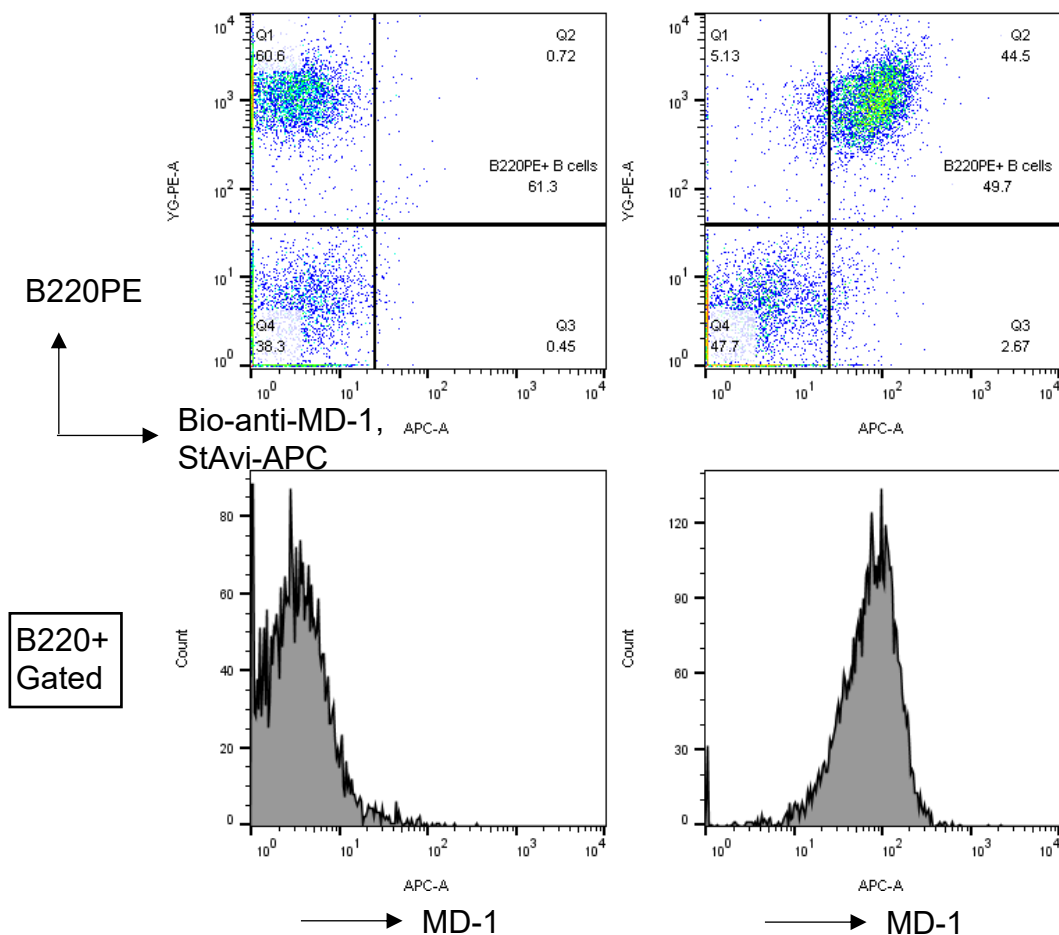


Supplementary Figure 1. Representative FACS results of monocytes, neutrophils, and B cells in peripheral cells, related to Figure 3a. Peripheral blood cells were stained with CD11b-FITC; Ly6G-APC to determine the amounts of neutrophils and monocytes. CD3-PE; B220-FITC; were used to detect T and B cells, respectively.

Peripheral Cells

LDLr^{-/-}/MD-1^{-/-}

LDLr^{-/-}/MD-1^{+/-}

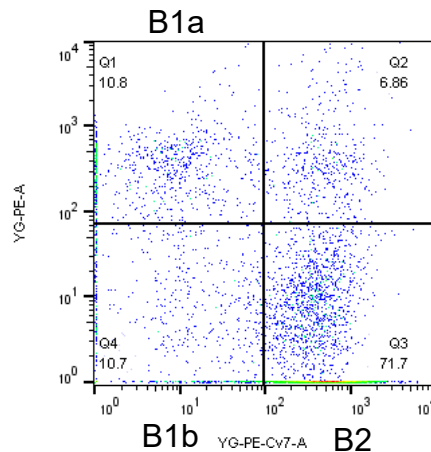
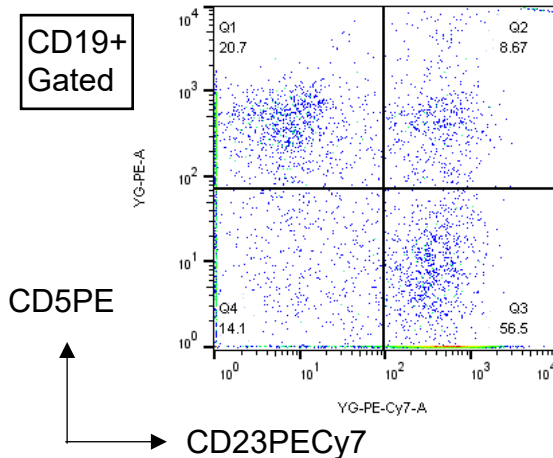
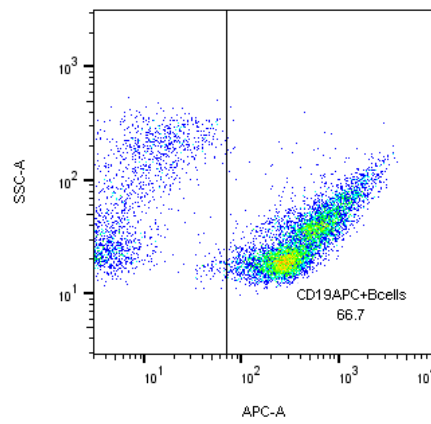
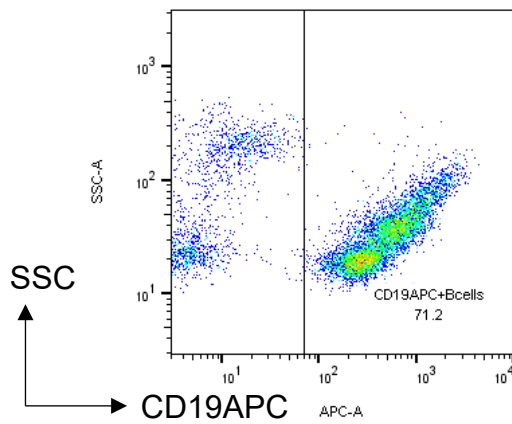
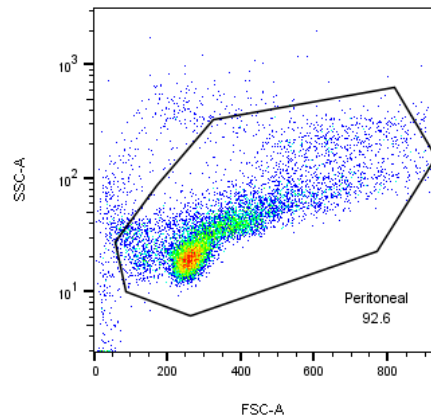
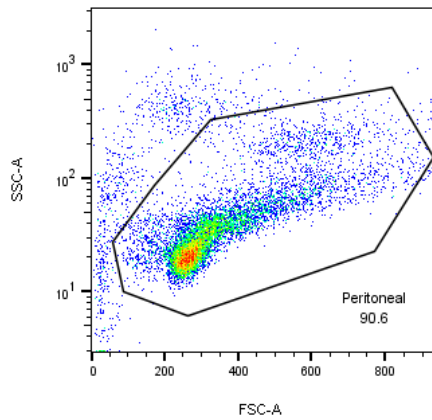


Supplementary Figure 2. Representative FACS results of MD-1 in peripheral cells, related to Figure 3a. MD-1 expression on B cells (B220⁺) were analyzed by using biotinylated anti-mouse MD-1 mAb (previously we established (clone 7G1) (39)), followed by stained with streptavidin-APC (BioLegend, CA, USA) in peripheral blood cells.

Peritoneal Cells

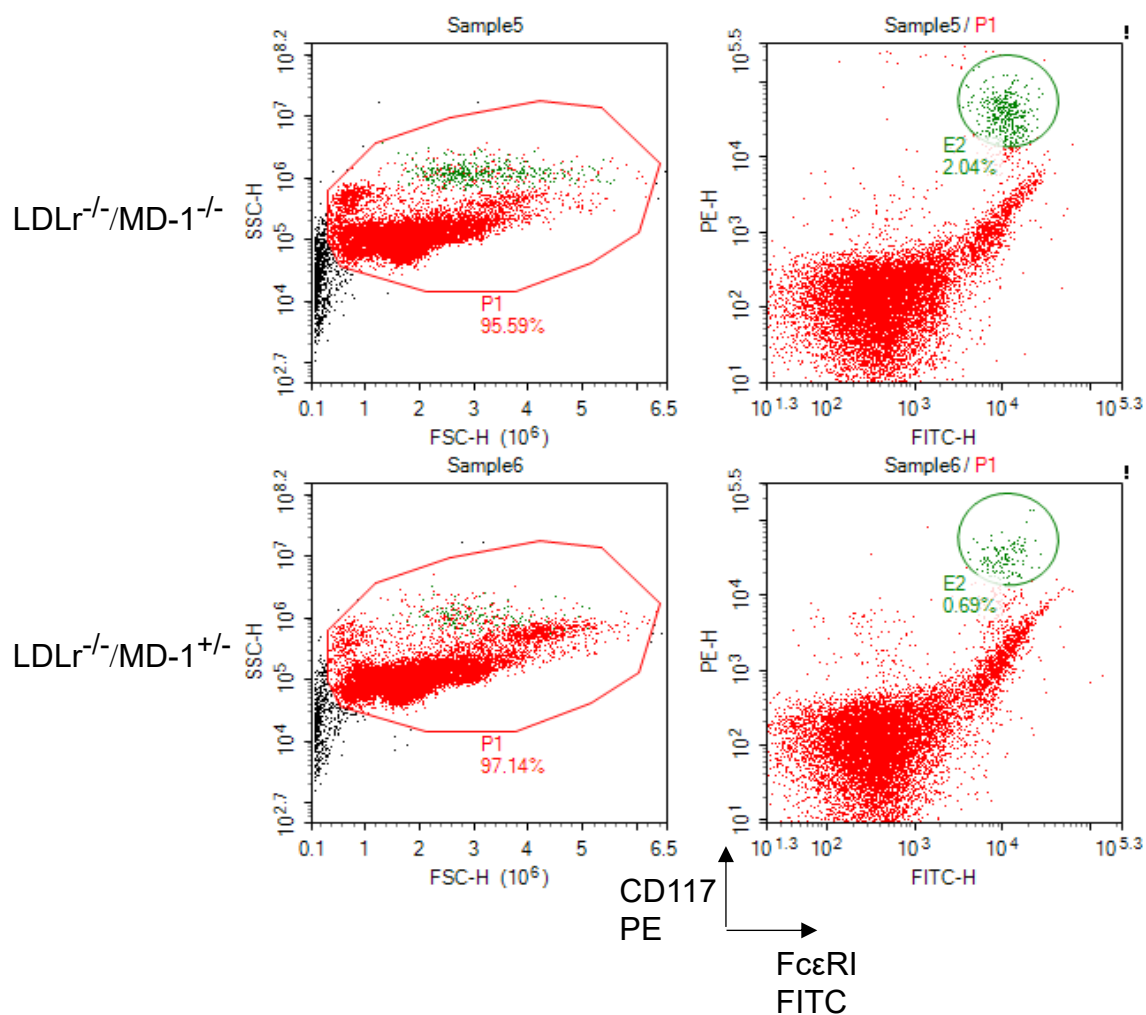
LDLr^{-/-}/MD-1^{-/-}

LDLr^{-/-}/MD-1^{+/-}



Supplementary Figure 3. Representative FACS results of B cells, B1a cells, and B1b cells in peritoneal cells, related to Figure 3b. Representative flow cytometry plots of peritoneal cells. Peritoneal cells were stained to analyze B cells (CD19⁺), B cell subsets (B1a cells: CD5⁺; CD23⁻; B1b cells: CD5⁻; CD23⁻; and B2 cells: CD5⁻; CD23⁺; all calculated as a percentage of CD19⁺ cells.

Peritoneal Mast Cells



Supplementary Figure 4. Representative FACS results of mast cells in peritoneal cells, related to Figure 3b. Peritoneal cells were stained to analyze mast cell populations (E2 = $CD117^{+}; FcεRI^{+}$).

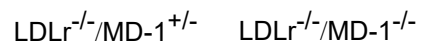
High Fat Diet (24w after 7w of age)

Infiltration area /Total Liver area (%)



Normal Chow Diet (36-37w)

Infiltration area /Total Liver area (%)



Supplementary Fig.5. Representative HE-stained liver sections of LDLr^{-/-}/MD-1^{+/-} mice or LDLr^{-/-}/MD-1^{-/-} mice and the percentage of lymphocyte infiltration area versus total liver area under (a) High Fat Diet for 24weeks after 7 weeks of age and (b) Normal Chow Diet for 36-37 weeks. (a) (Left): Image of the entire tissue section of Fig.6 mice No.237 and 297 (Scale bar = 5 mm). (Right): The percentage of lymphocyte infiltration area versus total liver area. Fig.6 samples are indicated as red mark with mice number. (*n* = 11). Data are presented as mean. n.s., not significant (Mann-Whitney's U test). (b) (Left): Image of the entire tissue section of Normal Chow Diet (Scale bar = 500 μm). (Right): The percentage of lymphocyte infiltration area versus total liver area. (LDLr^{-/-}/MD-1^{+/-} mice: *n* = 3, LDLr^{-/-} / MD-1^{-/-} mice: *n* = 4). Data are presented as mean. n.s., not significant (Student's *t*-test).

Supplementary Table. The details of NAS score, related to Figure 5b.

Score	Fatty deposits in hepatocytes	Balloon-like degeneration of hepatocytes	Inflammation within the hepatic lobules
0	< 5%	None	None
1	5% ~ 33%	Few	< 2 locations
2	33% ~ 66%	Many	2~4 locations
3	> 66%		> 4 locations
Code	Steatosis	Balloning	Inflammation