Supplementary Materials

Reversing an obesogenic diet to control diet partially rescues pro-inflammatory lipid-immune memory in splenocardiac aging

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Supplementary Table 1. Arachidonic acid (AA) derived lipid mediators in heart and spleen baseline no-MI) and post MI (MI-d1)

Heart -No-MI

Lipid Metabolites	Retention time	Q1	Q3	CON no-MI	OBD no-MI	OBD-R no-MI
AA	17.8	303	259	298895844±758215	8022258±1886985	5920851±1388521
LXB_4	6.6	351	221	112±17	96±11	121±11
PGE_2	6.7	351	175	19±3	135±63	63±13
PGD_2	6.9	351	233	19±1	165±87	65±11
PGF_{2a}	7.2	353	193	65±13	431±201	308±38
8-iso-PGF_{2a}	7.2	353	193	141±42	1532±743	1059±284
5-HETE	14.7	319	115	201±112	158±41	95±14
11-HETE	15.2	319	167	137±55	845±401	499±157
12 HETE	14.2	319	179	852±66	3393±1867	12816±3080
15-HETE	14.8	319	219	186±44	392±162	204±42
20-OH-LTB_4	6.5	351	195	80±24	60±7	84±12
12-HHT	12.4	279	163	272±102	2186±1087	1613±245
5,6 EET	16.1	319	115	619±171	2004±474	635±279
8,9 EET	16	319	155	105±27	133±29	93±9
14,15 EET	16	319	219	44±15	102±39	56±11
TXB_4	7.1	369	169	15±2	53±28	41±4
LTD_4	9.8	497	189	28±6	27±3	28±3

Spleen No-MI

Lipid Metabolites	Retention time	Q1	Q3	CON no-MI	OBD no-MI	OBD-R no-MI
AA	17.8	303	259	16494038±3523980	15866604±2280754	14814635±323049 2
LXB_4	6.6	351	221	402±39	502±42	339±37
PGE_2	6.7	351	175	7917±959	8821±943	5364±395

PGD_2	6.9	351	233	8834±1829	5825±545	4784±1023
PGF_{2a}	7.2	353	193	2285±422	2724±171	18212±158
8-iso-PGF_{2a}	7.2	353	193	2097±370	2549±159	1791±175
5,6 EET	16.1	319	191	460±42	693±338	667±160
8,9 EET	16	319	155	3350±736	4809±1062	4162±306
5-HETE	14.7	319	115	166±46	127±16	83±9
11-HETE	15.2	319	167	5927±494	6212±1029	4127±225
12-HETE	14.2	319	179	186443±13056	246539±49309	130596±13242
15-HETE	14.8	319	219	2023±359	1931±277	1287±164
20-HETE	13.4	319	289	482±33	590±119	321±34
TXB_2	6.3	369	169	7822±441	6737±974	5561±344
12-HHT	11.5	279	163	41623±4586	89886±14232	106418±30246
LTB_4	10.8	335	195	757±64	723±76	501±63
LTC_4	10.8	626	189	294±46	285±26	280±19
LTD_4	9.8.	497	189	45±13	53±10	36±5
LTE_4	11.2	440	189	87±20	91±18	43±5
20-OH-LTB_4	6.5	351	195	10404±227	9444±1183	7075±495

Heart MI-d1

Lipid Metabolites	**Retention time**	**Q1**	**Q3**	**CON MI-d1**	**OBD-MI-d1**	**OBD-R MI-d1**
AA	17.8	303	259	2671439±646430	6624182±2221334	3869293±490976
LXB_4	6.6	351	221	157±27	140±35	161±10
PGE_2	6.7	351	175	135±61	130±50	343±137
PGD_2	6.9	351	233	404±228	198±73	462±186
PGF_{2a}	7.2	353	193	372±156	308±145	669±287
8-iso-PGF_{2a}	7.2	353	193	1428±716	948±559	1978±732
5-HETE	14.7	319	115	672±399	376±52	1637±548
11-HETE	15.2	319	167	1054±562	724±324	1558±511
12 HETE	14.2	319	179	59381±38389	24768±10231	84743±30809

15-HETE	14.8	319	219	419±183	377±112	785±269
20-OH-LTB_4	6.5	351	195	84±25	106±51	172±18
12-HHT	12.4	279	163	3749±2141	2027±862	5353±1688
5,6 EET	16.1	319	115	3858±848	2805±915	2643±625
8,9 EET	16	319	155	1652±1181	638±326	5157±1758
14,15 EET	16	319	219	213±60	134±41	165±36
TXB_4	7.1	369	169	176±75	84±37	278±105
LTD_4	9.8	497	189	32±6	31±5	31±3

Spleen MI-d1

Lipid Metabolites	**Retention time**	**Q1**	**Q3**	**CON MI-d1**	**OBD MI-d1**	**OBD-R MI-d1**
AA	17.8	303	259	19390813±1816500	17219956±4494830	16754003±288635 2
LXB_4	6.6	351	221	462±22	831±69	493±57
PGE_2	6.7	351	175	6253±895	9991±397	7579±1084
PGD_2	6.9	351	233	4883±361	7978±707	8017±832
PGF_{2a}	7.2	353	193	1946±222	3123±221	2236±338
8-iso-PGF_{2a}	7.2	353	193	1750±210	3057±123	2033±362
5,6 EET	16.1	319	191	843±217	477±232	718±305
8,9 EET	16	319	155	5495±557	6474±732	9037±1648
5-HETE	14.7	319	115	176±20	204±33	135±17
11-HETE	15.2	319	167	5204±720	6897±613	6664±1012
12-HETE	14.2	319	179	148319±29717	205192±29155	207003±64955
15-HETE	14.8	319	219	1857±238	3225±422	2154±323
20-HETE	13.4	319	289	368±77	501±62	490±147
TXB_2	6.3	369	169	6989±622	7679±414	7943±2454
12-HHT	11.5	279	163	87092±19439	115497±8152	99207±6926
LTB_4	10.8	335	195	1043±24	881±84	730±47
LTC_4	10.8	626	189	396±31	390±24	265±30

LTD_4	9.8.	497	189	63±6	45±13	28±6
LTE_4	11.2	440	189	132±18	211±86	123±18
20-OH-LTB_4	6.5	351	195	9982±560	11679±713	8841±1265

Supplementary Table 2. Eicosapentaenoic acid (EPA) derived lipid mediators in heart and spleen baseline (d0) and post MI (MI-d1)

Heart no-MI

Lipid Metabolites	Retention time	Q1	Q3	CON no-MI	OBD no-MI	OBD-R no-MI
EPA	17	301	257	26249±3084	1500±306	23049±4579
5-HEPE	13.3	317	115	20±16	1±0	3±1
11-HEPE	12.8	317	167	2±1	0±0	5±3
12-HEPE	13	317	176	214±77	34±16	2168±889

Spleen no-MI

Lipid Metabolites	Retention time	Q1	Q3	CON no-MI	OBD no-MI	OBD-R no-MI
EPA	17	301	257	3686±770	214±38	2824±349
5-HEPE	13.3	317	115	6±1	1±1	1±0
11-HEPE	12.8	317	167	189±31	16±3	86±4
12-HEPE	13	317	179	15415±1234	858±143	9187±359
15-HEPE	12.8	317	219	36±6	1±1	14±2
18-HEPE	12.6	317	259	16±5	2±1	12±2

Heart MI-d1

Lipid Metabolites	Retention time	Q1	Q3	CON MI-d1	OBD MI-d1	OBD-R MI-d1
EPA	17	301	257	28872±5797	964±181	29182±1439
5-HEPE	13.3	317	115	17±8	0±0	37±11

11-HEPE	12.8	317	167	18±10	0±0	22±8
12-HEPE	13	317	179	4996±3197	112±43	6522±2904

Spleen MI-d1

Lipid Metabolites	Retention time	Q1	Q3	CON MI-d1	OBD MI-d1	OBD-R MI-d1
EPA	17	301	257	6757±1239	282±46	4077±849
5-HEPE	13.3	317	115	10±1	2±1	4±1
11-HEPE	12.8	317	167	198±17	18±3	211±15
12-HEPE	13	317	179	20333±1903	850±86	18108±1660
15-HEPE	12.8	317	219	28±2	2±1	25±4
18-HEPE	12.6	317	259	25±2	3±2	35±6

Supplementary Table 3. Docosahexanoic acid (DHA)) derived lipid mediators in heart and spleen baseline (d0) and post MI (MI D1).

Heart no-MI

Lipid Metabolites	Retention time	Q1	Q3	CON no-MI	OBD no-MI	OBD-R no-MI
	17.7			298895844±941579	57346983±1365056	474535926±1123840
DHA		327	283	31	2	55
4-HDHA	15.7	343	101	508±202	39±8	395±120
7-HDHA	15.2	343	141	117±27	21±2	110±23
13-HDHA	15	343	193	287±58	95±27	734±231
14-HDHA	15.1	343	205	165±17	39±11	1450±346
17-HDHA	14.9	343	245	474±116	166±25	1144±345
RvD_2	8.1	375	175	3±2	57±15	12±4

Spleen no-MI

Lipid Metabolites	Retention time	Q1	Q3	CON no-MI	OBD no-MI	OBD-R no-MI

DHA	17.7	327	283	51226562±7207586	6920141±1414355	36921934±7222149
4-HDHA	15.1	343	101	62±10	7±1	54±9
7-HDHA	14.5	343	141	22±4	3±1	13±3
13-HDHA	14.2	343	193	799±285	92±17	603±102
14-HDHA	14.2	343	205	10705±3645	1511±251	10841±1925
17-HDHA	14.1	343	245	1764±293	242±38	1350±248
$Maresin_1$	10.7	359	221	137±55	25±15	55±7
$Maresin_2$	11.5	359	221	133±25	16±2	67±9
RvD_2	8.1	375	175	17±6	141±66	11±3
RvD_5	11.3	375	199	13±1	2±1	9±1
PD_1	10.7	359	153	107±22	14±1	63±10
PDX	11.3	359	153	116±22	18±2	68±14
$MCTR_1$	10.2	650	191	1090±278	96±15	818±55
$MCTR_3$	11	464	191	159±82	601±347	202±67
$PCTR_1$	10.2	650	231	123±20	13±5	58±13
$PCTR_3$	11	464	231	91±21	7±6	3±3

Heart MI-d1

Lipid Metabolites	**Retention time**	**Q1**	**Q3**	**CON MI-d1**	**OBD MI-d1**	**OBD-R MI-d1**
	17.7			287834661±802879		483016699±6471011
DHA		327	283	91	62667575±3193322	6
4-HDHA	15.7	343	101	1844±692	168±60	4745±1618
7-HDHA	15.2	343	141	459±205	36±10	979±272
13-HDHA	15	343	193	1638±669	139±70	2657±741
14-HDHA	15.1	343	205	4253±2255	317±154	8116±3280
17-HDHA	14.9	343	245	2600±984	460±229	7379±2122
RvD_2	8.1	375	175	5±2	33±10	27±12

Spleen MI-d1

Lipid Metabolites	Retention time	Q1	Q3	CON MI-d1	OBD MI-d1	OBD-R MI-d1
DHA	17.7	327	283	68532316±8159720	8025973±2218398	71711737±8711975
4-HDHA	15.1	343	101	40±5	7±3	38±12
7-HDHA	14.5	343	141	16±2	1±0	9±3
13-HDHA	14.2	343	193	389±89	65±29	345±93
14-HDHA	14.2	343	205	7067±1351	792±296	4948±1321
17-HDHA	14.1	343	245	1301±281	194±73	790±215
$Maresin_1$	10.7	359	221	213±6	6±4	147±17
$Maresin_2$	11.5	359	221	192±22	21±4	158±14
RvD_2	8.1	375	175	9±0	34±12	26±5
RvD_5	11.3	359	199	27±2	4±2	22±6
PD_1	10.7	359	153	190±7	19±3	106±7
PDX	11.3	359	153	204±6	19±2	111±3
$MCTR_1$	10.2	650	191	1645±141	159±24	1145±135
$MCTR_3$	11	464	191	457±77	42±19	214±13
$PCTR_1$	10.2	650	231	141±12	8±4	40±19
$PCTR_3$	11	464	231	129±18	3±3	90±14

Data is represented as Mean±SEM. Values are in pg/50 mg tissue. Q1= Precursor ion mass, Q3= Diagnostic fragment ion mass. n = 5

Methods

Animal care and compliance

Animal usage and monitoring were conducted according to the "Guide for the Care and Use of Laboratory Animals" (8th Edition. 2011), AVMA Guidelines for the Euthanasia of Animals (2020 Edition) were approved by the Institutional Animal Care and Use Committees at the University of South Florida, Tampa, USA. The study is reported following ARRIVE 2.0 guidelines for experiment procedures [1].

Study design and diet intervention

Male mice C57Bl/6J were housed in the USF-MDD Research Center animal facility in a 12-hour light-dark cycle and with ad libitum access to food and water unless otherwise indicated. All animals used in this study were males. The experimental procedures were initiated when mice were 12 weeks (3 months) of age unless otherwise indicated. Male C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). Mice were randomized to two main groups 1) control (fed on standard lab chow, Tekad global # 2918) and 2) OBD (fed on 10% w/w safflower-enriched diet; Research Diets Inc # D111020001) for 6 months (mo). After 6 mo of dietary intervention, OBD group mice were randomized again to the two groups:1) OBD-continued to the 10% safflower diet, 2) OBD-R- Diet was switched to standard lab chow. The third group, CON, continued to the lab chow diet. The diet was continued for the next 4 mo, and then mice were sacrificed with and without myocardial infarction. The study design indicates the timeline and output parameters (Fig 1A).

Coronary artery ligation micro-surgery

The CON, OBD, and OBD-R mice groups were subjected to left anterior descending coronary ligation (MI surgery), specifically at the end of the mice's dark cycle (6:00 p.m. onwards). In brief, mice were anesthetized initially with 3-4% isoflurane, and then a further 1.5-2% was maintained during non-reperfused surgical occlusion. The left anterior descending coronary artery was ligated using nylon 8-0 sutures (ARO Surgical Instruments, Newport Beach, CA); to develop acute heart failure, the mice were monitored after the surgery [2]. Post surgery mice of fractional shortening above 15% were excluded from the study.

Echocardiography

The echocardiography was performed using Vevo 3100 (VisualSonics Inc., Canada) in vivo imaging system equipped with probes up to 40 MHz and a resolution of 30 μm. For the echocardiography, mice were anesthetized using 1-1.5% isoflurane in a 100% oxygen mix. Heart rates (>400 beats/min), respiratory rate, and body temperature (35–37°C) were monitored continuously during the whole process. The echocardiography images were acquired at long-axis B-mode from mice during d0 (pre-surgery) and d1(MI) time frames. They were analyzed using Vevo Lab software in a blinded manner. To validate the equal ischemic intensity and myocardial infarction quality, the mice with fractional shortening of more than 10- 15% were excluded from the post-MI samples [2]

Necropsy pre- and post-MI

For necropsy, samples were collected from d0 (pre-surgery) and d1 (MI) mice. Mice were anesthetized using 2% isoflurane in a 100% oxygen mix. Heparin (4 IU/g) injection was administered intraperitoneally. After 5 minutes of post-heparin administration, the blood was collected from the carotid artery and centrifuged for 5 minutes to separate plasma. The LV, RV, lungs, spleen, and adipose tissue were collected and weighed individually. All samples were immediately snap-frozen and stored separately at -80 ˚C for further biochemical and molecular analysis. [3]

Histology

Each group had LV mid-cavity, spleen, and adipose transverse sections; pre- and post-MI were embedded in paraffin and sectioned for histological measurements. Sections were stained with hematoxylin and eosin. For LV, 40X images were taken at remote, peri-infarct and infarct area. For the spleen, images were taken at 2X and 10X resolutions. Adipose tissue images were taken at 20x. All the images were acquired using the Keyence BZ-X810 fluorescence microscope. At least 3-4 areas were captured in each section with n=4/proup [4]. The adipose size and white pulp area is measured using a Keyence BZ-X800 analyzer with hybrid cell count software.

Isolation of macrophages (MΦs) from spleen, bone marrow, and peritoneal cavity

As previously described, spleen, bone marrow, and peritoneal macrophages were isolated from CON, OBD, and OBD-R mice [5] [4]. Briefly, the peritoneal cavity was lavaged twice with 10 ml of ice-cold RPMI 1640 media with 10% FBS and 1% antibiotics (Thermo Fischer Scientific). The cell pellet was resuspended in 6 ml of RPMI 1640 media, plated in the dishes, further incubated at 37°C overnight to allow the cells to adhere, and subsequently washed with fresh media to remove any unattached cells for RT PCR.

Macrophage functional (phagocytosis) assay using pHrodo-Red labeled Escherichia Coli.

Isolated Mϕs (~1×10^5/ml) from the spleen, bone marrow, and peritoneum of CON, OBD, and OBD-CON mice were incubated in RPMI 1640 with 10% FBS at 37°C with 5% CO2 for 30 min. Before adding pHrodo E. coli bioparticle (Thermo Fisher Scientific, MA), images were taken on KEYENCE BZ-X810 using 20X objective. Cells were then incubated at 37°C with pHrodo E. coli bioparticles per the manufacturer's instructions, and images were captured at 0, 10, 20, and 30 min to visualize bacteria internalization by the Mϕs as red [3].

Immunofluorescence

As previously described, the Immunofluorescence was performed on LV transverse and spleen sections [2]. Briefly, tissues were deparaffinized, and antigen retrieval was done using a 1X antigen retrieval solution. For bone marrow, splenic, and peritoneal macrophages for isolated and seeded on Millicell EZ Slide 8 Well (Millipore # MPPEZGS0816) with and without LPS (100ng/ml) for 4hrs. Cells were fixed with 4% paraformaldehyde for 10 mins at washed with PBS. Cells were permeated with 0.1% Triton X and blocked with 10% goat serum. For tissues, antigen retrieval was done using 1X dako solution, washed with 1X with PBS and blocked with 10% goat serum. Both cells and tissues were then incubated with antibodies against CCL2 and F4/80 (overnight). Further, incubated with secondary antibodies were conjugated with Alexa-488 and -555 for 1h. Alexa Fluor 488 was used for co-staining the myocyte area. Nuclei were stained with Hoechst. Microscopy was performed on a KEYENCE-BZ_810 high-resolution microscope. The images represent the 7-8 section area for 3-4 mice/group.

Quantitative Real-Time PCR

RNA was isolated from BMdms, splenic MΦs, peritoneum MΦ, LV, and spleen tissues using TRIzol. For qPCR, reverse transcription was performed with 2.0 µg of total RNA using the SuperScript® VILO cDNA Synthesis Kit (Invitrogen, CA, USA). Quantitative PCR for *ALOX12, ALOX15, ALOX5, COX-1, COX-2, TNF-α, CCL2, CCR2, IL-6, IL-1b, MRC-1, IFN-g, ARG-1, Il10, and YM-1,* genes was performed using TaqMan probes (Applied Biosystems, CA, USA) on master cycler QuantStudio 6 Pro. Gene levels were normalized to Hprt-1 as the housekeeping control gene. The results were reported as 2-ΔCt (ΔΔCt) values. All the experiments were performed in duplicates with n = 5 / group [6].

Flow cytometry

As per the previously described protocol, single mononuclear cells from LV and splenocytes from CON, OBD, and OBD-R were isolated from the no-MI control day (d0). The cell count for LV mononuclear cells or splenocytes was adjusted to ~1-2 million cells/stain. Isolated cell suspensions were finally suspended in 200µl of 1:500 Fc block and incubated for 10 min on ice. A cocktail of fluorophore-labeled monoclonal antibodies in 2X concentration was added for 30 min on ice as appropriate for each study. We used CD45-PE-cy7, CD11b-BV650, F4/80-Percp, Ly6C-FITC, CX3CR1-PE, CCR2-alexa647 and MHCII BV621 in the cocktail. The live cells were gated on the Invitrogen™ LIVE/DEAD™ Fixable IR Dead Cell Stain Kit. Activated macrophages were defined

as the cell's dual expression of CD11b (Mac-1) and F4/80^{+}surface marker. Data were acquired on BD^{TM} LSRII Flow Cytometer and analyzed with FlowJo software, version 7.6.3.

10x Genomics

Cell Sorting

Single mononuclear cells from LV and splenocytes from CON, OBD, and OBD-R were isolated from the no-MI control day (d0), as per the previously described protocol. The cell counts for LV mononuclear cells or splenocytes were adjusted to ~1-2 million cells/stain. Isolated cell suspensions were finally suspended in 200µl of 1:500 Fc block and incubated for 10 minutes on ice. CD45-PE, fluorophore-labeled monoclonal antibodies in 2X concentration were added for 30 min on ice as appropriate for each study. The live cells were gated on the Invitrogen™ LIVE/DEAD™ Fixable IR Dead Cell Stain Kit. Hematopoietic cells (CD45+, IR-Neg) were sorted on the Aria Fusion (BD Bioscience) under low pressure into DMEM containing 50% FCS for cell visualization and 10X single-cell RNA sequencing or RLT buffer (Qiagen) for RNA extraction.

Single Cell droplet experiments (10x Genomics)

A total of 10000 Hematopoietic cells scRNA-Seq experiments were sequenced to a read depth of ~130,000 reads/cell and run through the 10x Genomics cell ranger platform. Single cell suspensions were prepared as outlined by the 10x genomics Single Cell 3' v2 Reagent Kit user guide. Briefly, samples were washed two times in PBS (Life Technologies) + 0.04% BSA (Sigma). Each wash was performed with a 6-minute centrifugation at 330 x g and resuspension in 1 mL PBS + 0.04% BSA. Sample viability was assessed using Trypan Blue (ThermoFisher) and a hemocytometer, and each sample's appropriate volume was calculated. After droplet generation, samples were transferred onto a pre-chilled 96-well plate (Eppendorf), plates were heat-sealed, and reverse transcription was performed using a thermal cycler (Biorad). After the reverse transcription, cDNA was recovered using Recovery Agent provided by 10x, followed by a Silane Dyna Bead clean-up (ThermoFisher) outlined in the user guide. Purified cDNA was amplified and cleaned using SPRI select beads (Beckman). Samples were diluted 4:1 (elution buffer (Qiagen): cDNA) and run on a Bioanalyzer (Agilent Technologies) to determine cDNA concentration. cDNA libraries were prepared as outlined by the Single Cell 3' Reagent Kits v2 user guide with appropriate modifications to the PCR cycles based on the calculated cDNA concentration (as recommended by 10X Genomics).

Sequencing

The molarity of each library was calculated based on library size as measured by bioanalyzer (Agilent Technologies) and qPCR amplification data (Kappa/ Roche). Samples were pooled and normalized to 10nM, then diluted to 2nM using elution buffer (Qiagen) with 0.1% Tween20 (Sigma). Each 2nM pool was denatured using 0.1N NaOH at equal volumes for 5 min at room temperature. Library pools were further diluted to 20 pM using HT-1 (Illumina) before being diluted to a final loading concentration of 14 pM. 150ul from the 14 pM pool was loaded into each well of an 8-well strip tube and loaded onto a cBot (Illumina) for cluster generation. Samples were sequenced on a HiSeq 2500 with the following run parameters: Read 1 – 26 cycles, read 2 – 98 cycles, index 1 – 8 cycles.[7]

Single-cell RNA-seq Analysis

The sequenced data was processed into expression matrices with 10X Genomics clouds(http://10xgenoics.come/) and data was analyzed Loupe 6.3 (10x genomics)[7]

Lipidomics

To examine the collected lipids, we employed Analyst software 1.7 and a Sciex QTRAP 6500+Triple quadrupole. For the mobile phases A and B, one milliliter of formic acid was dissolved in 1000 milliliters of water and 1000 milliliters of methanol, respectively. The run time and flow rate were 20.5 minutes and 0.5 mL/min, respectively. A 40µL sample was injected into the LC-MS and separated on the Kinetex Polar C-18 column (100 mm x 3mm x 2.6 µm). The column was kept at 50°C and the sample at 5°C. The compounds were separated using progressive elution of the mobile phases, with B initially set at 10% and then increased to 45% between 0.1 and 2 minutes, 80% until 16.5 minutes, 98% at 16.6 minutes, held until 18.5 minutes, and returned to 10%. Data integration was performed using Sciex OS software. Sample extraction was performed using metal bead extraction in methanol [3].

In vitro memory activation assay

BMDMs and splenic macrophages were isolated, starved for 6 hours (hr), and subsequently treated with either the 12-HETE (final concentration 100 nM) for 48 or 96 hr. The medium was then refreshed and changed periodically every 24 hours. Before terminating the experiment, they were treated with LPS (100ng/ml) for 4 hours.

In vitro: ALOX5ap activation by 12-HETE

RAW 264.7 cells were seeded on Millicell EZ Slide 8 Well (Millipore # MPPEZGS0816) with and without 12-HETE (100nM) for 4 hours (hr). Cells were fixed with 4% paraformaldehyde for 10 minutes (min) at washed with PBS. Cells were permeated with 0.1% Triton X and blocked with 10% goat serum. For tissues, antigen retrieval was done using 1X Dako solution, washed with 1X PBS, and blocked with 10% goat serum. Both cells and tissues were then incubated with antibodies against CCL2 and Alox5ap (overnight). Further, incubated with secondary antibodies were conjugated with Alexa-488 and -555 for 1 hr. Nuclei were stained with Hoechst. Microscopy was performed on a KEYENCE-BZ_800 high-resolution microscope. The images represent the 7-8 section area for 3-4 mice/group.

Statistical analysis

Data are expressed as mean ± SEM and bar graphs with individual values. A minimum of 4-5 mice/group is used for all the experiments. Statistical analyses were performed using GraphPad Prism 9. Comparisons between groups using one-way ANOVA with Tukey's multiple-comparisons test *$p < 0.05$ vs CON, #$p < 0.05$ vs OBD. For multiple groups and time point analysis was using two-way ANOVA with Sidak's multiple-comparison test was used. In No-MI vs MI comparisons *$p < 0.05$ vs CON (no-MI), #$p < 0.05$ vs with respective diet group. \$$p<0.05$ vs respective OBD group. For -LPS and +LPS samples. *$p < 0.05$ vs CON(-LPS), #$p < 0.05$ vs with respective,

References

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Major resources table

Antibodies

Target antigen	Source	Catalog#	Working Conc
F4/80-percp cy5.5 (clone BM8)	ebioscience	45-4801-82	1:800
CD11b-BV650 (clone M1/70)	BD Bioscience	563402	1:800
Ly6C-FITC (clone AL21)	BD Bioscience	553104	1:800
CD45-PECY7 (clone 30-FL1)	Biolegend	103114	1:800
CCR2-Alexa-647	Biolegend	150604	1:500
MHCII-BV421	Biolegend	107632	1:500
Goat anti-rabbit Alexa- flour 488	Invitrogen	A11008	1:200
Goat anti-mouse Alexa- flour 555	Invitrogen	A21422	1:200
purified anti-mouse CD16/32	Biolegend	101302	1:500
CCL2/MCP1	Novus biologicals	NBP2-22115	1:100
CCR2-Alexa-647	Invitrogen	PA5-23043	1:100
F4/80	Abcam	ab169111	1:100
ALOX5AP	BIOSS	bs7756R	1:100
CD11b	Abcam	ab8878	1:100

Cultured/Primary Cells

Name	Source	Sex
Peritoneal Macrophages	C57bL/6J	Male
Splenic Macrophages	C57bL/6J	Male

BMDMs	C57bL/6J	Male
RAW 264.7	Unknown	Male

Taqman probes

Probe	Company	Cat #
TaqMan® Gene Expression Assay(il1b)	Applied Biosciences	Mm01336189_m1
TaqMan® Gene Expression Assay(il-6)	Applied Biosciences	Mm00446190_m1
TaqMan® Gene Expression Assay(Arg-1)	Applied Biosciences	Mm00475988_m1
TaqMan® Gene Expression Assay(MRC-1)	Applied Biosciences	Mm00485148_m1
TaqMan® Gene Expression Assay(Ym-1)	Applied Biosciences	Mm00657889_m1
TaqMan® Gene Expression Assay(IFN-g)	Applied Biosciences	Mm01168134_m1
TaqMan® Gene Expression Assay(hprt-1)	Applied Biosciences	Mm01545399_m1
TaqMan® Gene Expression Assay(chemokine (C-C motif) ligand 2)-ccl2	Applied Biosciences	Mm00441242_m1
TaqMan® Gene Expression Assay (Ptgs1, mCG22272)	Applied Biosciences	Mm00477214_m1
TaqMan® Gene Expression Assay (Ptgs2, mCG5001)	Applied Biosciences	Mm00478374_m1
TaqMan® Gene Expression Assay(12-LOX)	Applied Biosciences	Mm00545833_m1
TaqMan® Gene Expression Assay(15-LOX)	Applied Biosciences	Mm00507789_m1
TaqMan® Gene Expression Assay(5-LOX)	Applied Biosciences	Mm01182747_m1

TaqMan® Gene Expression Assay(TNF-a)	Applied Biosciences	Mm00443258_m1
TaqMan® Gene Expression Assay (Gapdh)	Applied Biosciences	Mm99999915_g1
TaqMan® Gene Expression Assay(Ccl5)	Applied Biosciences	Mm01302427_m1
TaqMan® Gene Expression Assay(Il10)	Applied Biosciences	Mm01288386_m1

Chemicals and assay kits

Reagents	**Source**	**Cat #**
Standard lab chow	Tekad global	2918
Obesogenic diet (10% safflower 10%w/w)	Research Diets Inc	D111020001
Nylon 8-0 sutures	ARO Surgical Instruments	T06A08N14-13
RPMI 1640	Gibco	11875-093
DMEM F/12	Gibco	10565-018
FBS	Gibco	A525686-01
100x antimycotic	Gibco	15240-062
Millicell EZ Slide 8 Well	Millipore	MPPEZGS0816
LPS(O55:B5)	Sigma	L2880
Triton X	Sigma	T8787
Goat serum	Vector	S1000
Antigen retreival solution	Dako	S1699
Trizol	Invitrogen	15596018
cDNA Kit: SuperScript™ IV VILO™ Master Mix	Thermo Fisher Scientific	11766050
™ LIVE/DEAD™ Fixable IR Dead Cell Stain Kit	Thermo Fisher Scientific	L34961
12-HETE	Cayman	35550
Hoechst 3342	Thermo Fisher Scientific	H3570

PBS	Thermo Fisher Scientific	10010-023
HBSS	Thermo Fisher Scientific	14025-092
Ultra-pure water	Thermo Fisher Scientific	10977-015
Taqman Fast Advance Master Mix	Applied Biosciences	4444557
WGA-488	Thermo Fisher Scientific	W11261
WGA-647	Thermo Fisher Scientific	W32466

Supplementary legends

S. Figure 1

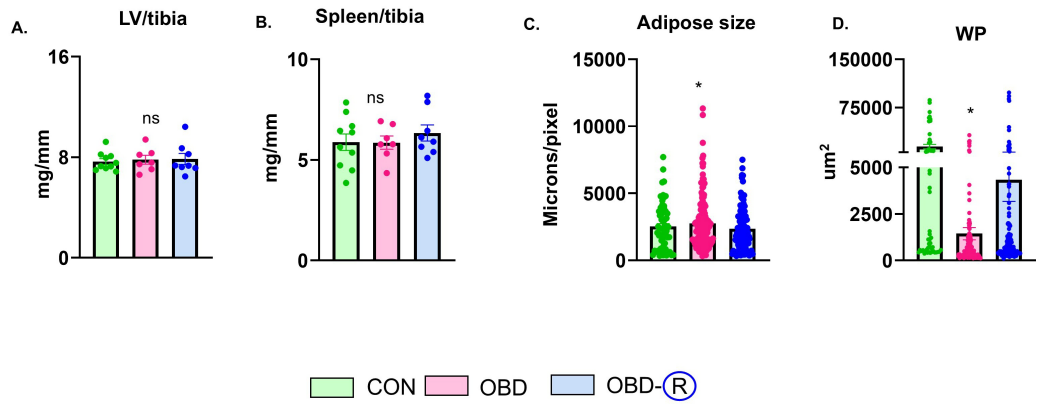

Fig. 1. Obesogenic diet did not impact heart and spleen weight but increased adipose size. The graph representing (A) LV (Left Ventricle)/Tibia (B) Spleen/Tibia (C) adipose size (D) White pulp area (WP)-spleen CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet). Comparisons between groups using one-way ANOVA with Tukey's multiple-comparisons test *p < 0.05 vs CON, #p < 0.05 vs OBD; ns=non-significant. Error bars represent mean ± SEM; n=10-13/group.

S. Figure 2

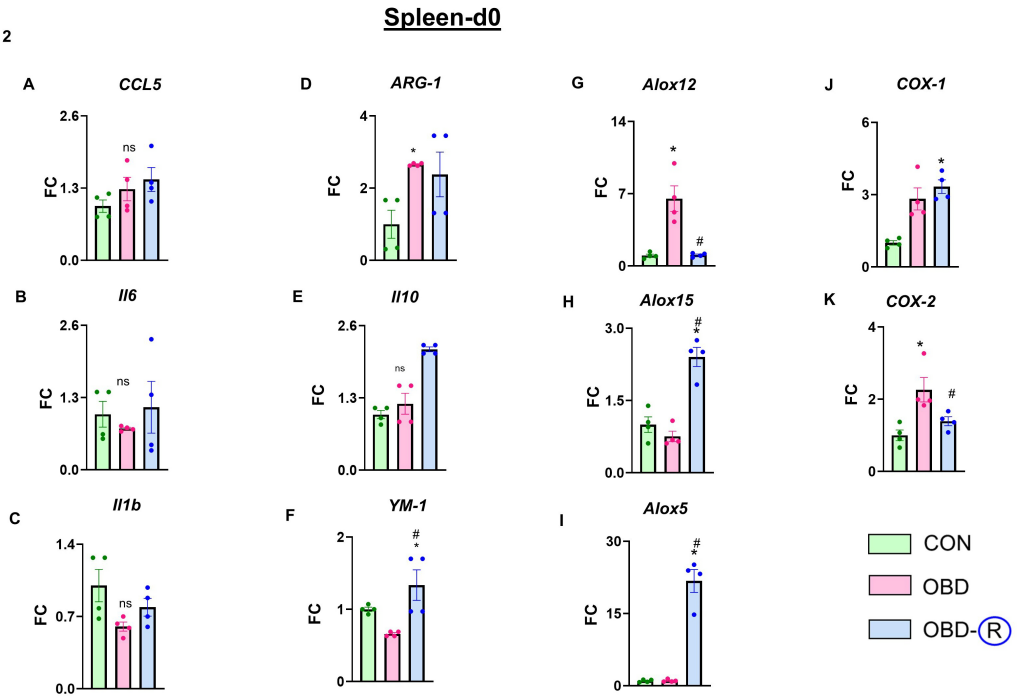

Fig. 2. Prior chronic OBD supplementation retains partial proinflammatory gene expression in the immune hub, the spleen. mRNA expression of proinflammatory cytokines (A) *CCL5* (B) *Il6* (C) *Il1b* in the spleen of CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months of OBD diet + 4 months CON diet) mice. mRNA expression of proresolving cytokines (D) *ARG-1* (E) *IL10* (F) *YM-1* in the spleen of CON, OBD, and OBD-R mice. mRNA expression of (G) *Alox12* (H) *Alox15* (I) *Alox5* (J) *COX-1* (K) *COX-2* in the spleen of CON, OBD, and OBD-R mice. Data is presented in fold change (FC), the CON group is considered as base line with FC 1, and gene expression was normalized to Hprt-1Comparisons between groups were analyzed using one-way ANOVA with Tukey's multiple-comparisons test. *p < 0.05 vs CON, #p < 0.05 vs OBD. Error bars represent mean ± SEM; n=4/group; ns: non-significant.

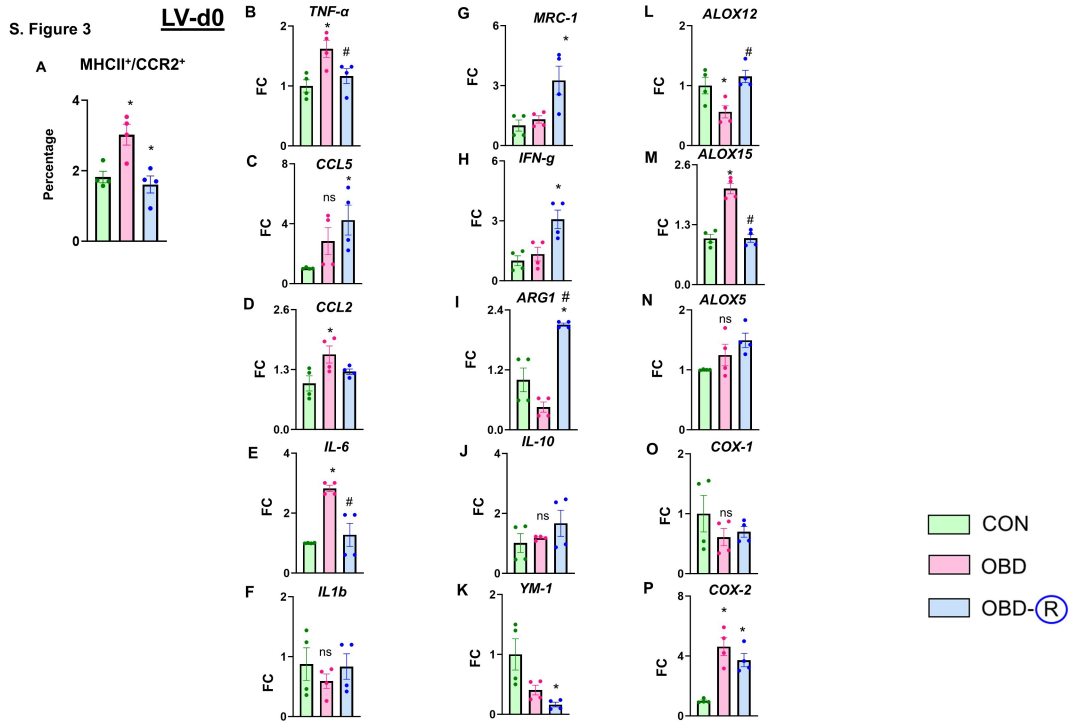

Fig. 3. The heart retains residual inflammation after diet reversal, reflected by a sustained proinflammatory profile despite partial recovery. (A) Flow quantification of MHCHII^{+}CCR2^{+} in the LV of CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months of OBD diet + 4 months CON diet). mRNA expression of proinflammatory cytokines (B)*TNF-α* (C) *CCL5* (D) *CCL2* (E) *Il6* (F) *Il1b* in the spleen of CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months of OBD diet + 4 months CON diet) mice. mRNA expression of proresolving cytokines (G) *MRC-1* (H) *IFN-g* (I) *ARG-1* (J) *IL-10* (K) *YM-1* in the spleen of CON, OBD, and OBD-R mice. mRNA expression of (L) *Alox12* (M) *Alox15* (N) *Alox5* (O) *COX-1* (P) *COX-2* in the spleen of CON, OBD, and OBD-R mice. Data is presented in fold change (FC), the CON group is considered as base line with FC 1, and gene expression was normalized to Hprt-1Comparisons between groups were analyzed using one-way ANOVA with Tukey's multiple-comparisons test. *p < 0.05 vs CON, #p < 0.05 vs OBD. Error bars represent mean ± SEM; n=4/group; ns: non-significant.

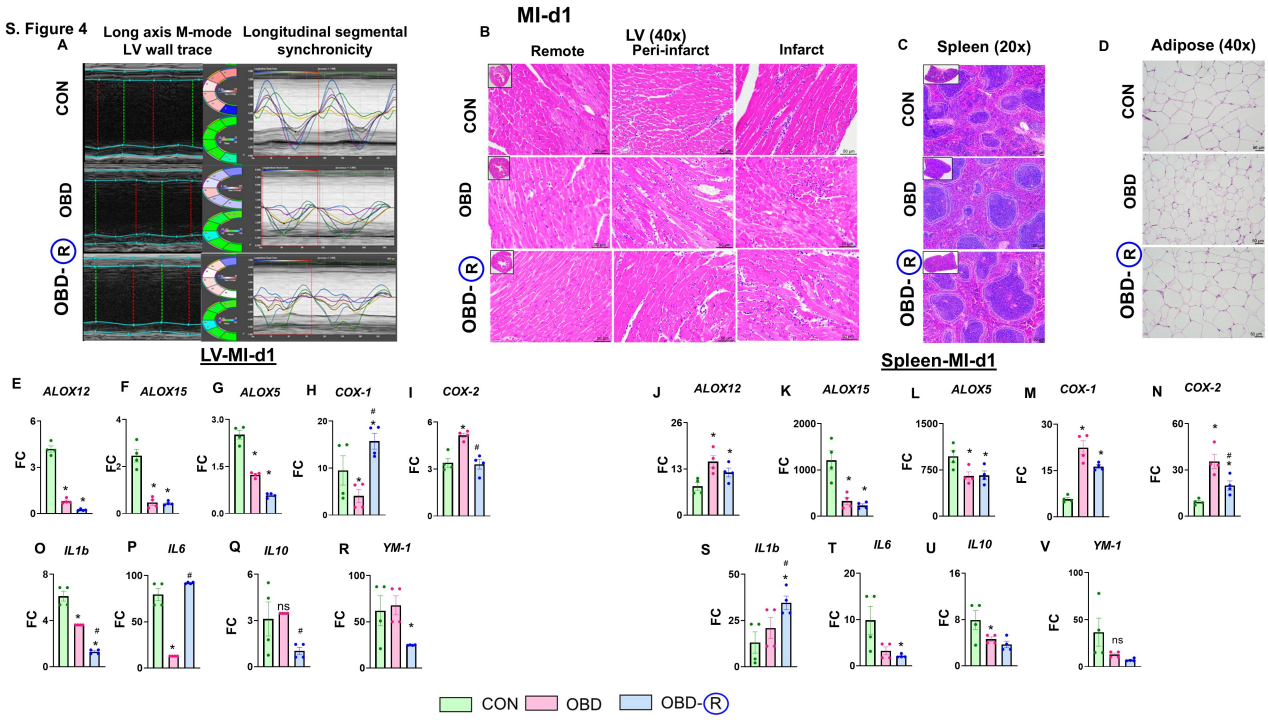

Fig. 4. OBD intake fails to initiate physiological acute inflammation-resolution signaling in the spleen and infarcted LV post-MI. (A) The representative images depicting long-axis M-mode LV wall trace, longitudinal segmental synchronicity changes in left ventricular (LV) in CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) mice at post-MI-d1. Representative Hematoxylin and eosin (H&E) staining of (B) LV (40x)-remote, peri-infarct and infarct, (C) spleen (20x) and (D) adipose (40x) in CON, OBD, and OBD-mice at post-MI-d1 Magnification; 40x:LV 10x:spleen and 40x:adipocytes. Scale bar, 50 µm. mRNA expression of lipoxygenease genes (E) *ALOX-12* (F) *ALOX15* (G) *ALOX5* (H) *COX-1* and (I) COX-2 in LV and (J) *ALOX-12* (K) *ALOX15* (L) *ALOX5* (M) *COX-1* and (N) COX-2 of CON, OBD and OBD-R mice post-MI-d1. mRNA expression of cytokines (O) *IL1β* (P) *Il6* (Q) *IL10* (R) *YM-1* in LV (O) *IL1β* (P) *Il6* (Q) *IL10* (R) *YM-1*in spleen of CON, OBD and OBD-R mice post-MI-d1. is presented in fold change (FC), CON-d0 (No-MI) group is considered as base line with FC 1 and gene expression was normalized to Hprt-1. Comparisons between groups were analyzed using one-way ANOVA with Tukey's multiple-comparisons test. *p < 0.05 vs CON, #p < 0.05 vs OBD. Error bars represent mean ± SEM; n=4/group.

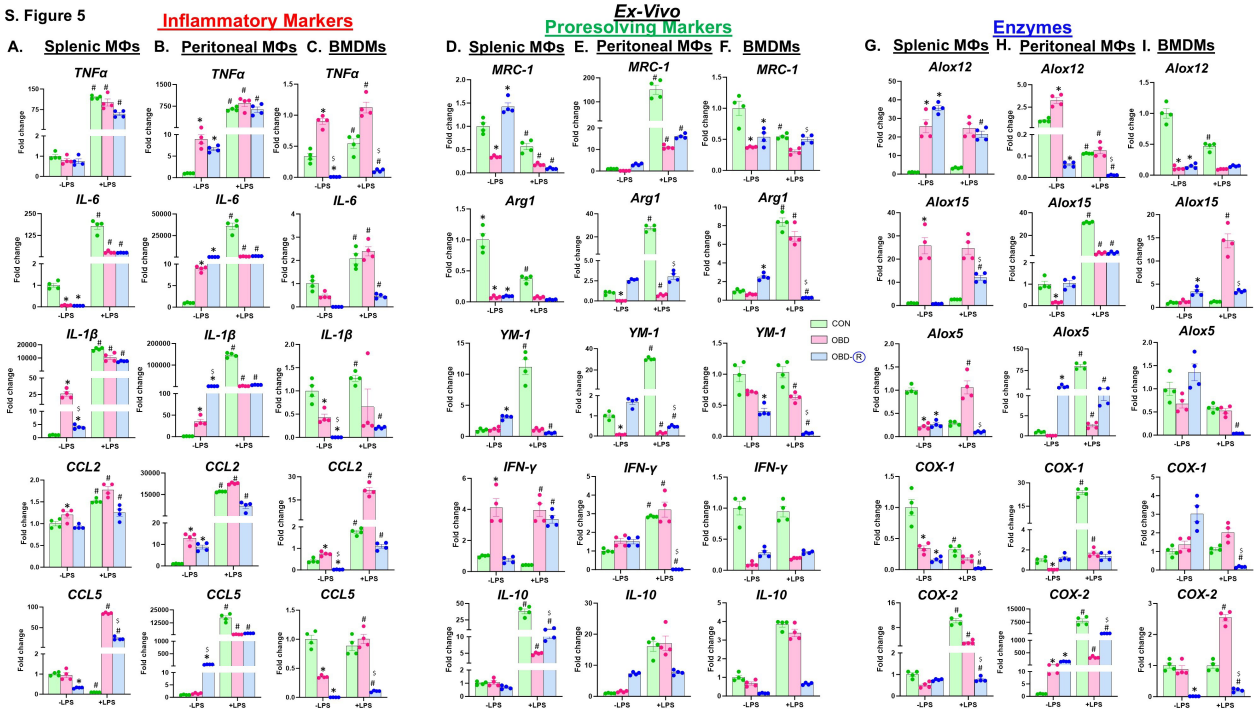

Fig. 5. Expression of immune and bioactive lipid memory markers in splenic Mϕs, peritoneal Mϕs and BMDMs. Expression of proinflammatory markers: *TNF-α, IL6, IL1β, CCL2*, and *CCL5* in (A) splenic Mϕs, (B) peritoneal Mϕs (C) BMDMs isolated from CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) mice in the presence and absence of LPS (100ng/ml) for 4h examining memory phase of Mϕs. Expression of proresolving markers: *MRC-1, Arg1, YM-1, IFN-γ*, and *IL-10* in (D) splenic Mϕs (E) peritoneal Mϕs (F) BMDMs isolated from CON, OBD, and OBD-R mice in the presence and absence of LPS (100ng/ml) for 4h examining memory phase of Mϕs. Expression of LMs inducing markers: *Alox12, Alox15, Alox5, Cox-1*, and *Cox-2* (G) splenic Mϕs, (H) peritoneal Mϕ (I) BMDMs isolated from CON, OBD, and OBD-R mice in the presence and absence of LPS (100 ng/ml) for 4h examining memory phase of Mϕs. Data is presented in the fold change (FC), and gene expression was normalized to Hprt-1. Comparisons between groups were analyzed using two-way ANOVA with Sidak's multiple-comparisons test *$p < 0.05$ vs CON (-LPS), #$p < 0.05$ vs with respective. Error bars represent mean ± SEM; n=4/group

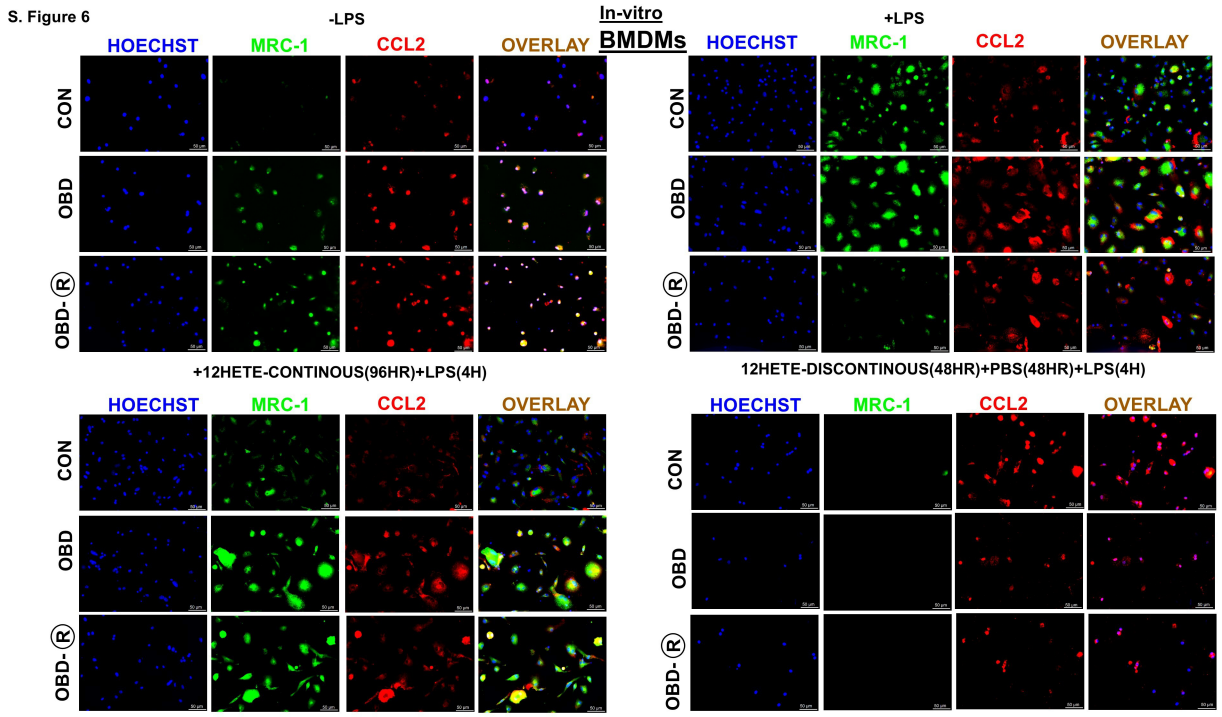

Fig. 6. 12-HETE induced immune memory in BMDMs. Expanded representative immunofluorescence images showing expression MRC-1(green), CCL2 (red), and nuclei (Hoechst-blue) depicting 12-HETE induced immune memory in BMDMs isolated from CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) mice. Magnification; 40x. Scale bar, 50 µm.

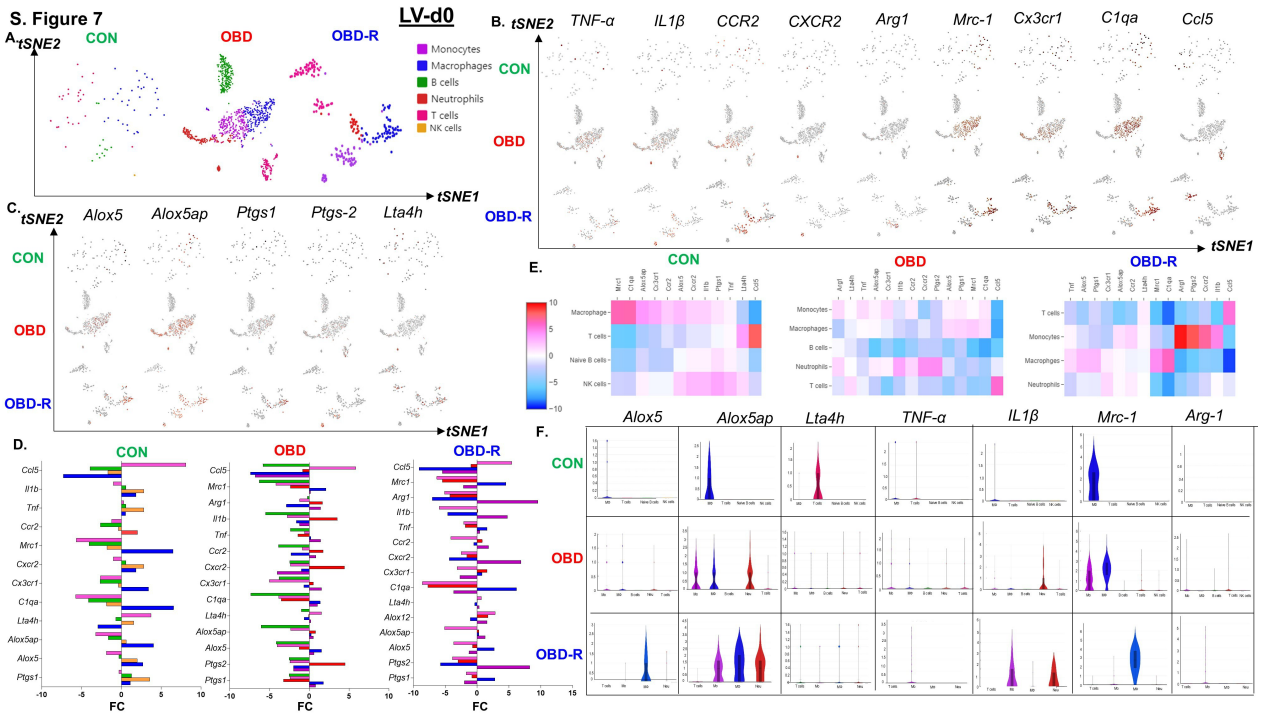

Fig. 7. Impact of diet switch on CD45^{+} immune cells identified by scRNA-seq in the heart and spleen under homeostatic (no-MI) and infarcted setting post-MI. (A) The tSNE plot visualizing immune cells in LV of CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) group in LV at homeostatic conditions (no-MI). Cell lineages are denoted according to the expression of marker genes and colored accordingly. (B) The tSNE plot visualizing expression of TNF-α, IL1β, CCR2, CXCR2, Arg-1, Mrc-1, Cx3cr1, C1qa, and Ccl5 in LV of CON, OBD, and OBD-R group at homeostatic conditions (no-MI). (C) The tSNE plot visualizing the expression of Alox5, Alox5ap, ptgs-1, ptgs-2, and Lta4h in LV of CON, OBD, and OBD-R group in LV at homeostatic conditions (no-MI). (D) Fold change graphs showing GOI (gene of interest) in different cell lineages in No-MI (d0) LV samples. (E) Heat map showing GOI (gene of interest) in different cell lineages in no-MI-d0 LV samples. Blue color represents downregulation, and red represents upregulation. (F) Violin plots of cluster-defining genes in CON, OBD, and OBD-R group in LV at homeostatic conditions (no-MI).

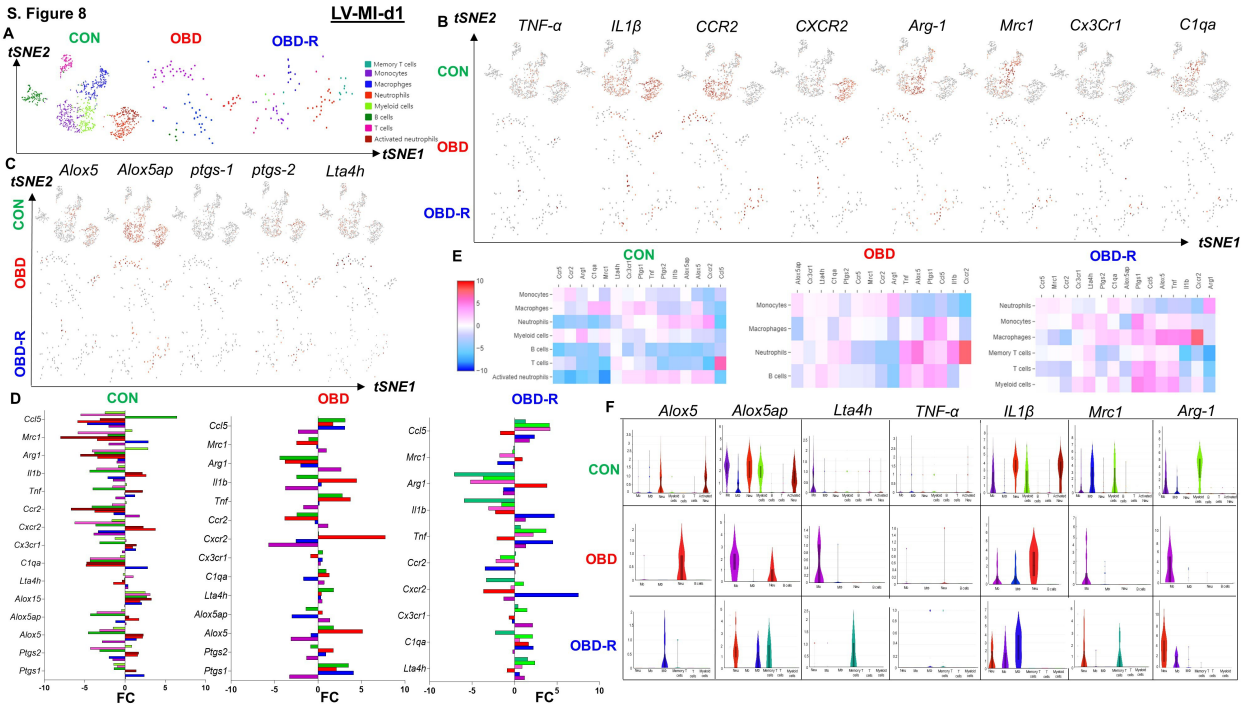

Fig. 8. Impact of diet switch of ScRNA-seq identifying immune and lipid markers in LV post-injury (post-MI d1-infracted). (A) The tSNE plot visualizing immune cells in LV of CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) group in LV at infracted conditions (MI-d1). Cell lineages are denoted according to the expression of marker genes and colored accordingly. (B) The tSNE plot visualizing expression of TNF-α, IL1β, CCR2, CXCR2, Arg-1, Mrc-1, Cx3cr1, and C1qa in LV of CON, OBD, and OBD-R group in LV V at infracted conditions (MI-d1). (C) The tSNE plot visualizing the expression of Alox5, Alox5ap, ptgs-1, ptgs-2, and Lta4h in LV of CON, OBD, and OBD-R group in LV at infracted conditions (MI-d1). (D) Fold change graphs showing GOI (gene of interest) in different cell lineages at post-MI d1 LV samples. (E) Heat map showing GOI (gene of interest) in different cell lineages in LV samples at post-MI-d1. Blue color represents downregulation, and red represents upregulation. (F) Violin plots of cluster-defining genes of the CON, OBD, and OBD-R group in LV at post-MI d1.

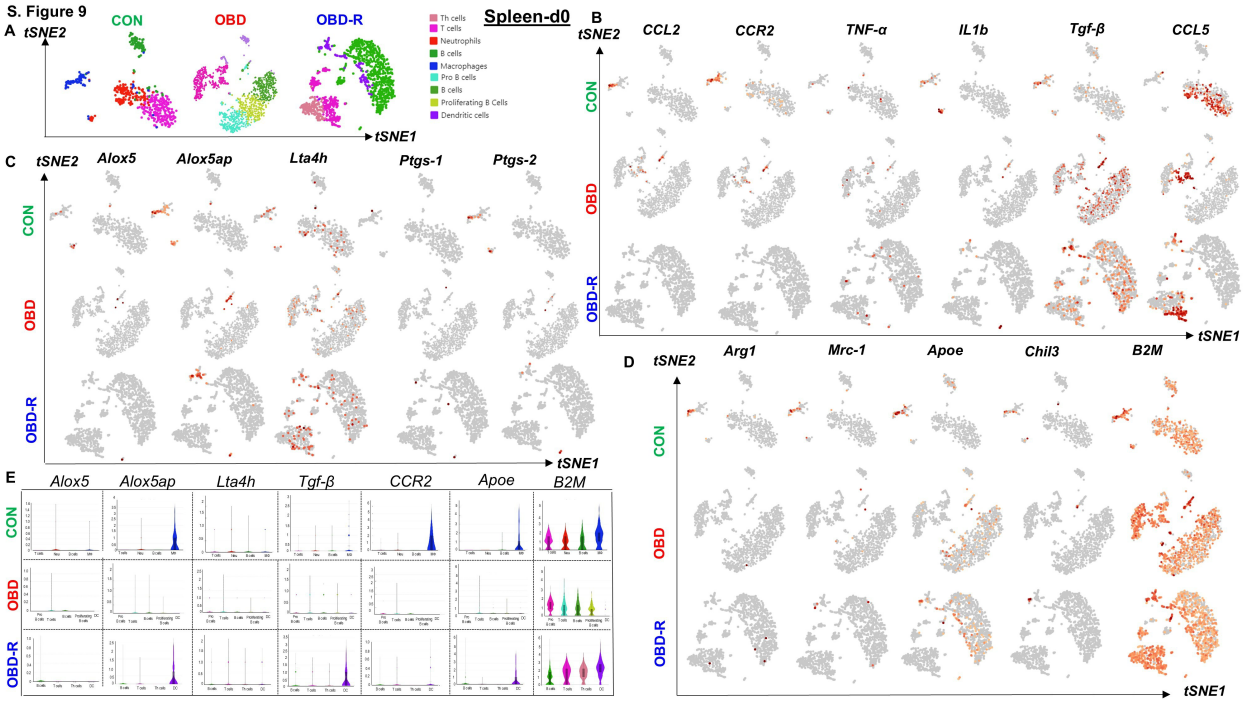

Fig. 9. Impact of diet switch of ScRNA-seq identifying immune and lipid markers in spleen at homeostatic conditions (d0). (A) The tSNE plot visualizing immune cells in LV of CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) group in the spleen at homeostatic conditions (d0). Cell lineages are denoted according to the expression of marker genes and colored accordingly. (B) The tSNE plot visualizing expression of proinflammatory markers: CCR2, CXCR2, TNF-α, IL1β, TGF-β and CCL5 in spleen of CON, OBD, and OBD-R group at d0 (C) The tSNE plot visualizing the expression of Alox5, Alox5ap, Lta4h, Ptgs-1 and Ptgs-2 in LV of CON, OBD, and OBD-R group at d0. (D) The tSNE plot visualizing the expression of proresolving markers: Arg1, Mrc-1, Apoe, Chil3, and B2M in LV of CON, OBD, and OBD-R group at d0. (E) Violin plots of cluster-defining genes in CON, OBD, and OBD-R groups in different cell lineages in the spleen at d0.

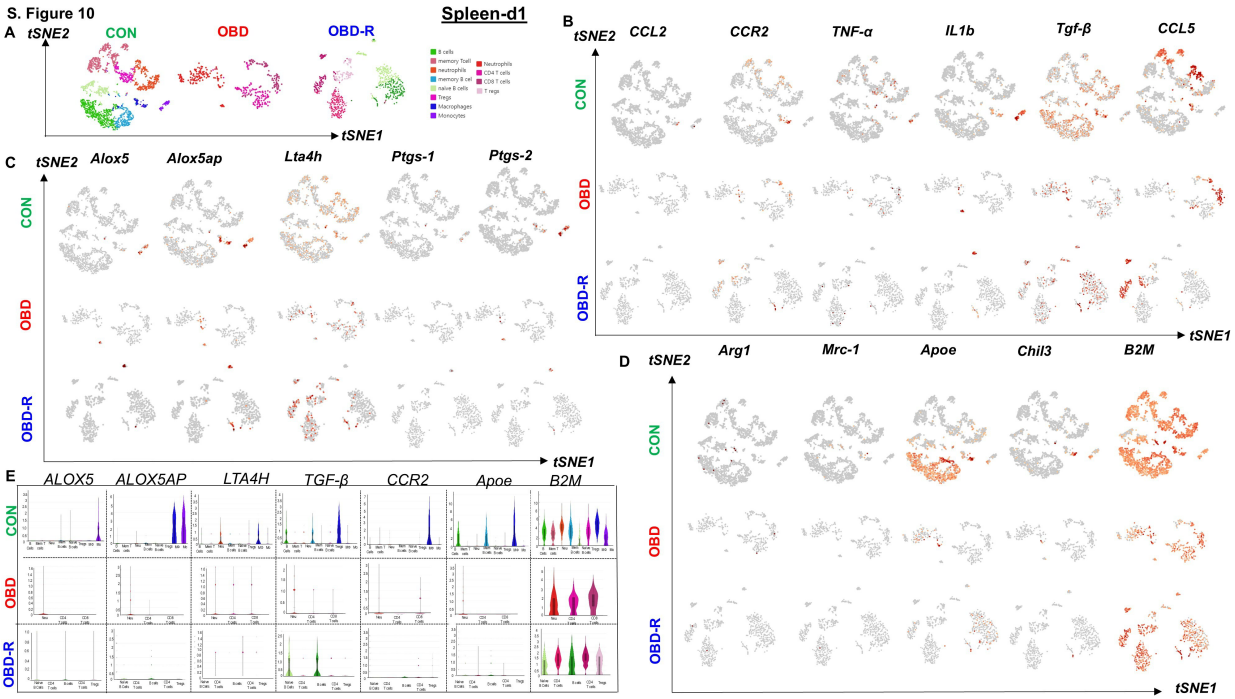

Fig. 10. Impact of diet switch of ScRNA-seq identifying immune and lipid markers in spleen at post-MI d1. (A) The tSNE plot visualizing immune cells in LV of CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) group in the spleen at post-MI-d1. Cell lineages are denoted according to the expression of marker genes and colored accordingly. (B) The tSNE plot visualizing expression of proinflammatory markers: CCR2, CXCR2, TNF-α, IL1β, TGF-β and CCL5 in spleen of CON, OBD, and OBD-R group at post-MI-d1 (C) The tSNE plot visualizing the expression of Alox5, Alox5ap, Lta4h, Ptgs-1 and Ptgs-2 in LV of CON, OBD, and OBD-R group at post-MId1. (D) The tSNE plot visualizing the expression of proresolving markers: Arg1, Mrc-1, Apoe, Chil3, and B2M in LV of CON, OBD, and OBD-R group at post-MId1. (E) Violin plots of cluster-defining genes in CON, OBD, and OBD-R groups in different cell lineages in the spleen at post-MI-d1.

S. Figure 11

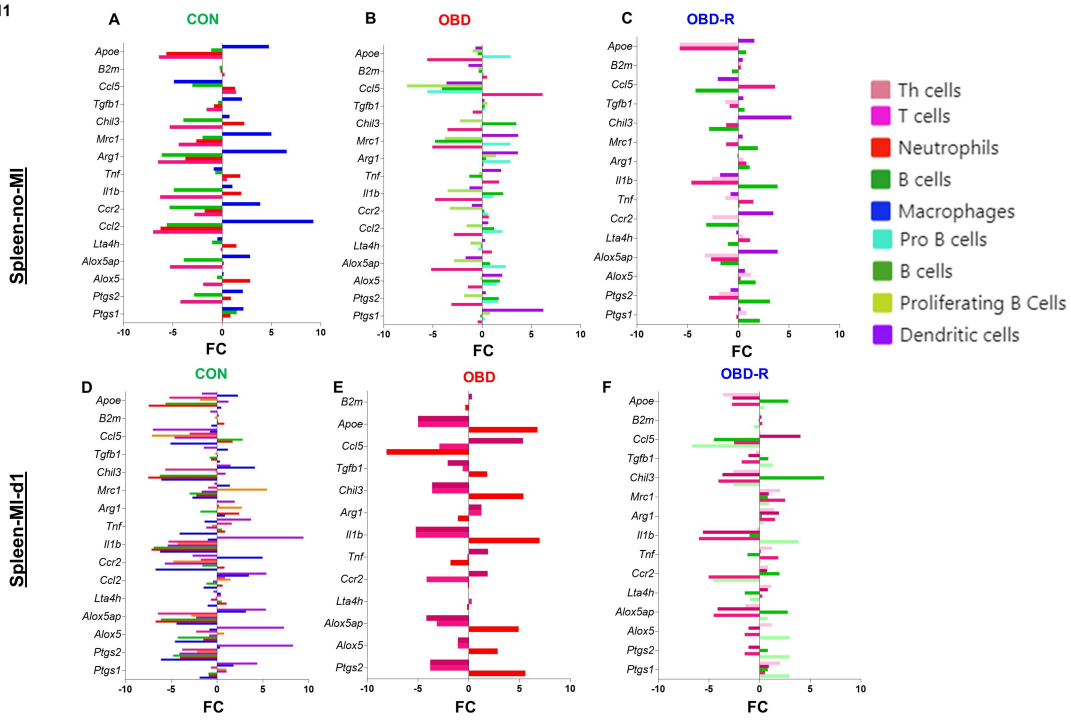

Fig. 11. Fold change graphs showing GOI (gene of interest) in different cell lineage in the spleen at d0 and post-MI-d1 in CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) groups. Cell lineages are denoted according to the expression of marker genes and colored accordingly.

S. Figure 12

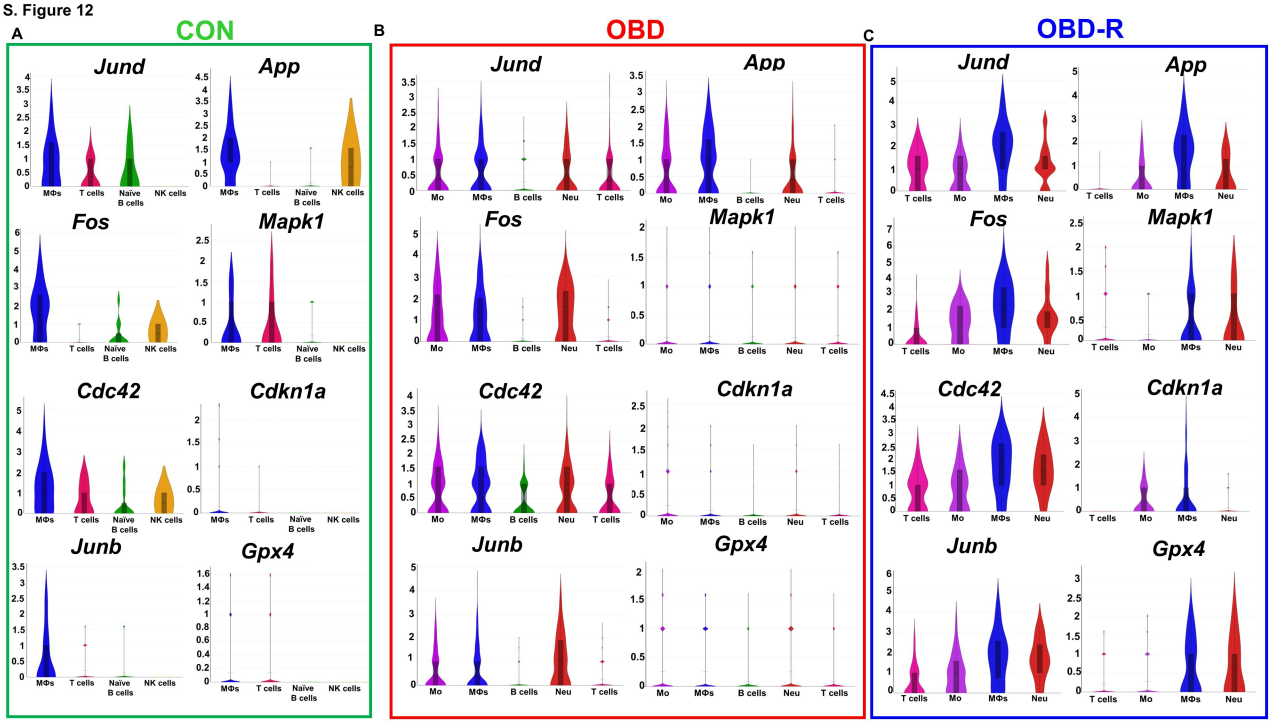

Fig. 12. Violin plots of cluster-defining age-related genes CON (10 months of lab chow), OBD(10 months of omega-6 enriched diet), and OBD-R (10 months OBD diet + 4 months CON diet) groups in different cell lineages in the LV at d0. Cell lineages are denoted according to the expression of marker genes and colored accordingly.

S. Figure 13

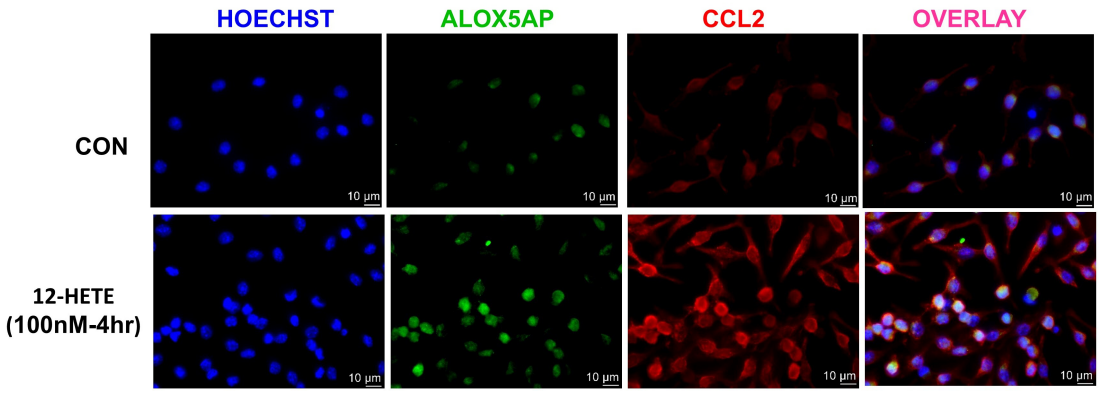

Fig. 13. Expanded representative immunofluorescence images showing ALOX5AP (green) and CCL2 (red) expression in RAW 264.7 cells untreated and treated with 12-HETE (100nM) for 4 hours. Nuclei are labeled with Hoechst (blue). Magnification; 100x. Scale bar, 10 µm; n=3/group.