Cxcl5 limits macrophage foam cell formation in atherosclerosis

Anthony Rousselle, Fatimunnisa Qadri, Lisa Leukel, Rüstem Yilmaz, Jean-Fred Fontaine, Gabin Sihn, Michael Bader, Amrita Ahluwalia, Johan Duchene.

Online supplement

SUPPLEMENTAL METHODS

Experimental design. 6wks old ApoE$^{-/-}$ and WT mice were placed on a WD (21% fat, 0.2% cholesterol, Harlan Teklad) or remained on CD for the indicated time. ELR^{+}-CXCL chemokines were quantified from aorta tissue mRNA and plasma CXCL5 concentration was assessed by ELISA.

Additionally, ApoE$^{-/-}$ mice fed a WD were treated with a mouse CXCL5 mAb (50 µg/mouse, i.p., every 72h, R&D) or IgG isotype control for 12 wks. Alternatively, ApoE$^{-/-}$-$B_1R^{-/-}$ and ApoE$^{-/-}$-$B_1R^{+/+}$ mice were fed a CD for 60 wks. Brachiocephalic artery and heart were fixed with 4% PFA and embedded in paraffin for histology analysis. Blood was collected for leucocytes, lipids, cytokines and chemokines measurement.

Histology and immunostaining. Serial sections (5µm) of brachiocephalic artery and aortic root were stained with hematoxylin and eosin to determine lesion size. Collagen was detected using Sirius Red coloration. Sections were stained for macrophages (Mac-2 mAb, Acris, 1:200), neutrophils (Ly6G mAb, BioLegend, 1:100), lipid-droplet content (adipophilin mAb, Fitzgerald, 1:1000) and detected with secondary anti-rat Cy3- (1:300) or Alexa 488-(1:500) Ab. Quantification analysis was assessed with ImageJ software.

Chemokine and cytokine measurement. Plasma Cxcl1 (Peprotech), Cxcl2 and Cxcl5 (R&D) and supernatant CXCL6 and IL-6 (R&D) levels were determined by ELISA. Plasma IFNγ, IL-2, IL-4, IL-6, IL-10, IL-12p70 and IL-17 levels were measured using Milliplex technology (Millipore).

Lipids profile. Plasma cholesterol and triglyceride levels were determined with commercially available kits (Abcam and Sigma).

Blood monocyte counting. ApoE$^{-/-}$ mice fed a WD were treated with Cxcl5 mAb or control as above. Blood was collected after 2wks, red blood cell lysed (BD PharmLyse) and cells stained with CD11b (APC), CD115 (PE) and Ly6G (FITC) (all Biolegend). Total monocytes were identified as CD11b^{+}CD115^{+}Ly6G^{-} cells. Data were acquired using BD LSR II flow cytometer and analysed with FlowJo software.

Real-time quantitative PCR. cDNA was synthesized from 1 μg total RNA with M-MLV reverse transcriptase (Promega) using random hexamer nucleotides. Real-time quantitative PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems) on an ABI Prism 7900 sequence detection system with 10 ng of cDNA and 100 nM primers (sequences in Supplemental Table 3). Gene expression was normalized to β–actin (aorta) or HPRT (macrophage) or GAPDH (HUVEC) and expressed as a relative value to control group using the comparative threshold cycle method ($2^{-\Delta\Delta Ct}$).

Microarray analysis. Raw data (CEL files) from 18 mouse aorta samples of an Affymetrix microarray dataset of WT and ApoE$^{-/-}$ mice fed WD for 6, 32 and 78 wks was downloaded from the Gene Expression Omnibus database (identifier: GSE10000), processed and analysed using the R/Bioconductor statistical software. GCRMA normalisation method was applied on the 45101 probes. We filtered out probes with low variation in expression (at least 15% of the samples must have an expression value greater than the 10th percentile of all values in the dataset, and the inter quartile range must be greater than the median), probes having no annotated Entrez Gene identifier, and duplicated probes by keeping the one with the higher variance. There were a total of 19304 probes selected for further analysis. Pearson's correlation coefficients of gene probe expression profiles were computed between the Cxcl5 profile (Affymetrix probe identifier: 1419728_at) and profiles of the selected probes. P-values for significance were assessed using the distribution of all pairwise probe correlation coefficients. 266 probes were significantly correlated to Cxcl5 ($P<=0.01$) and analysed for Biological Functions using Protein ANalysis THrough Evolutionary Relationships (www.pantherdb.org).

***In vivo* model of foam cell formation.** $ApoE^{-/-}$ mice on WD were injected with Cxcl5 (400ng, i.p., every 72h, Peprotech). After 12d, animals were sacrificed for peritoneal cells isolation. By flow cytometry, gated macrophages ($CD11b^{+}CD115^{+)}$ were identified as foam ($FSc^{hi}SSc^{hi}$). In parallel, macrophages were left to adhere (2h) in DMEM and lipids were stained with Oil-Red-O. Cells with over 1/3 of cytoplasm lipids were considered as foam.

Endothelial cell stimulation. Using a cone and plate viscometer, HUVECs were exposed to steady unidirectional high (10dyn/cm^2) or low (2 dyn/cm^2) LSS for 24h. Treatment with oxidized LDL (20μg/ml, BTI) or IL-1β (10ng/ml, Preprotech) was added for the last 4h or an additional 24h. qPCR analysis was carried out from 4h cell lysate. CXCL6 ELISA was performed from 24h supernatant.

Primary macrophage culture. Mouse bone-marrow-derived macrophages (BMDM) flushed out from femur were seeded in 10% FBS DMEM with M-CSF (50ng/mL) for 5-7d. Peritoneal macrophages (PM) isolated 4d after injection of 3% thioglycollate (1mL, i.p.) were left to adhere (2h) in DMEM. Non-adherent cells were PBS washed and primary macrophages were placed in 1% FBS DMEM overnight before further treatment.

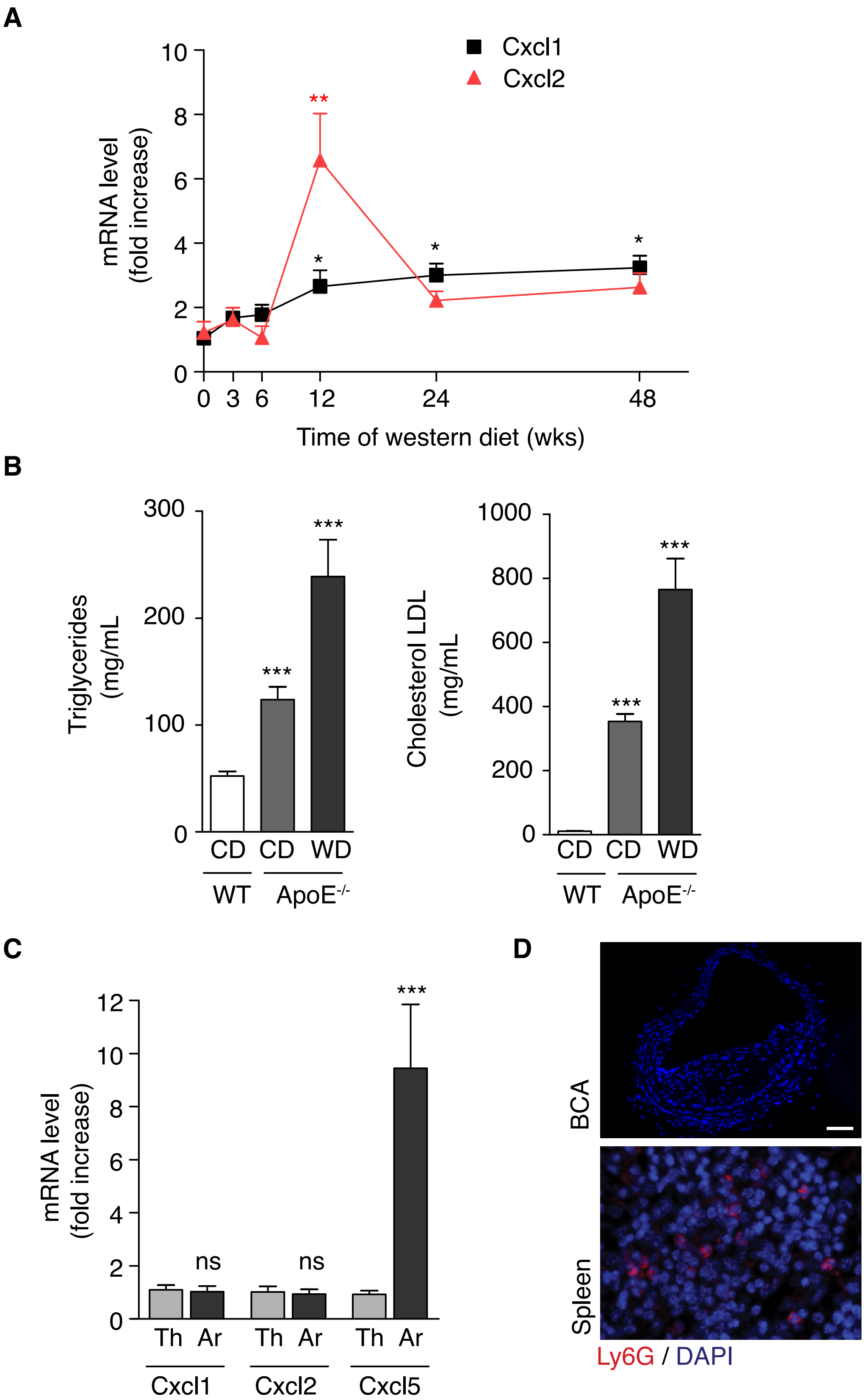
Macrophage stimulation. PMs or BMDMs were stimulated with Cxcl5 (100ng/mL) with (20μg/mL) or without acLDL. In some experiments, cells were also treated with either Cxcl5-, Cxcr2-, IL-6-, or control IgG antibodies (20μg/mL, 20min prior to Cxcl5 stimulation). In addition, some macrophages were polarised into classically-activated macrophages (M1) with IFNϒ (10ng/mL, 4h) or alternatively-activated macrophages (M2) with IL-4 (10ng/mL, 4h) and co-treated with Cxcl5. qPCR analysis was carried out from 4h cell lysate. ABCA1 Western Blotting and IL-6 ELISA were performed from 24h or 48h cell lysate or supernatant respectively.

***In vitro* model of foam cell formation.** acLDL-loaded (20μg/mL) PMs were treated (100ng/mL) or not with Cxcl5 for 24h in 2.5% serum containing medium. 4% PFA fixed macrophages were stained for lipids with 0.5% Oil-Red-O. Cells with over 1/3 of cytoplasm lipids were considered as foam.

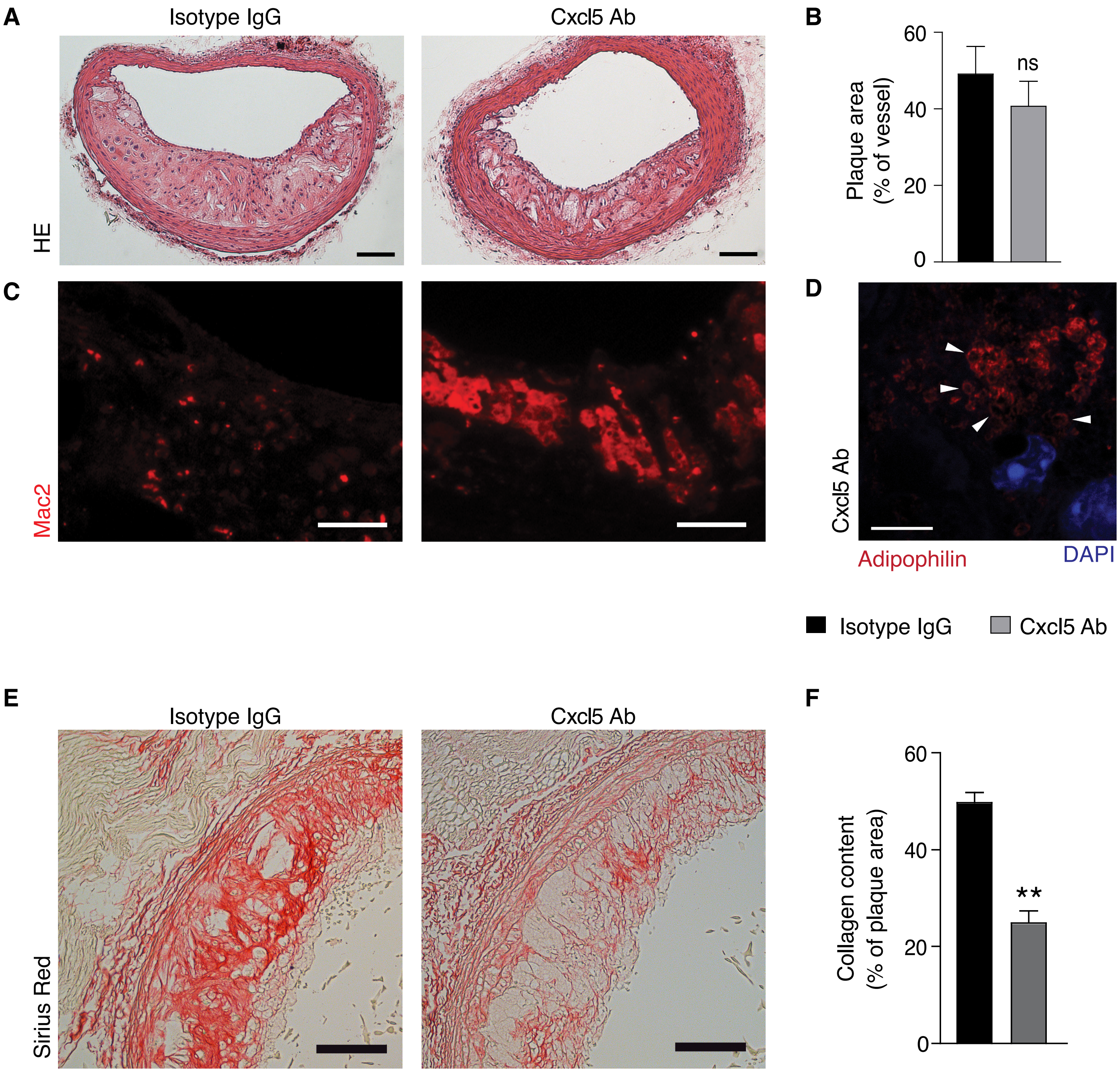
Cholesterol efflux assay. PMs were incubated with acLDL (20μg/mL) with (100ng/mL) or without Cxcl5 for 48h. NDB cholesterol (1μg/mL) was added for 6h. Medium was then replaced by fresh serum-free medium containing ApoAI (10μg/mL, BTI) for 4h. Fluorescence-labelled cholesterol was measured from collected supernatant and cell lysate (0.1N NaOH). Cholesterol efflux was calculated by dividing supernatant fluorescence by the sum of fluorescence in the media and cells.

Western blotting. Proteins from macrophage lysate were separated on 10% SDS-polyacrylamide gel, transferred to PVDF membranes, blocked (Odyssey) and ABCA1 was detected by incubation with rabbit anti-mouse Ab (1/500, Novus) at 4˚C overnight followed by donkey-anti-rabbit IgG (IRDyeTM 800CW, 1/10000, Odyssey, 30min).

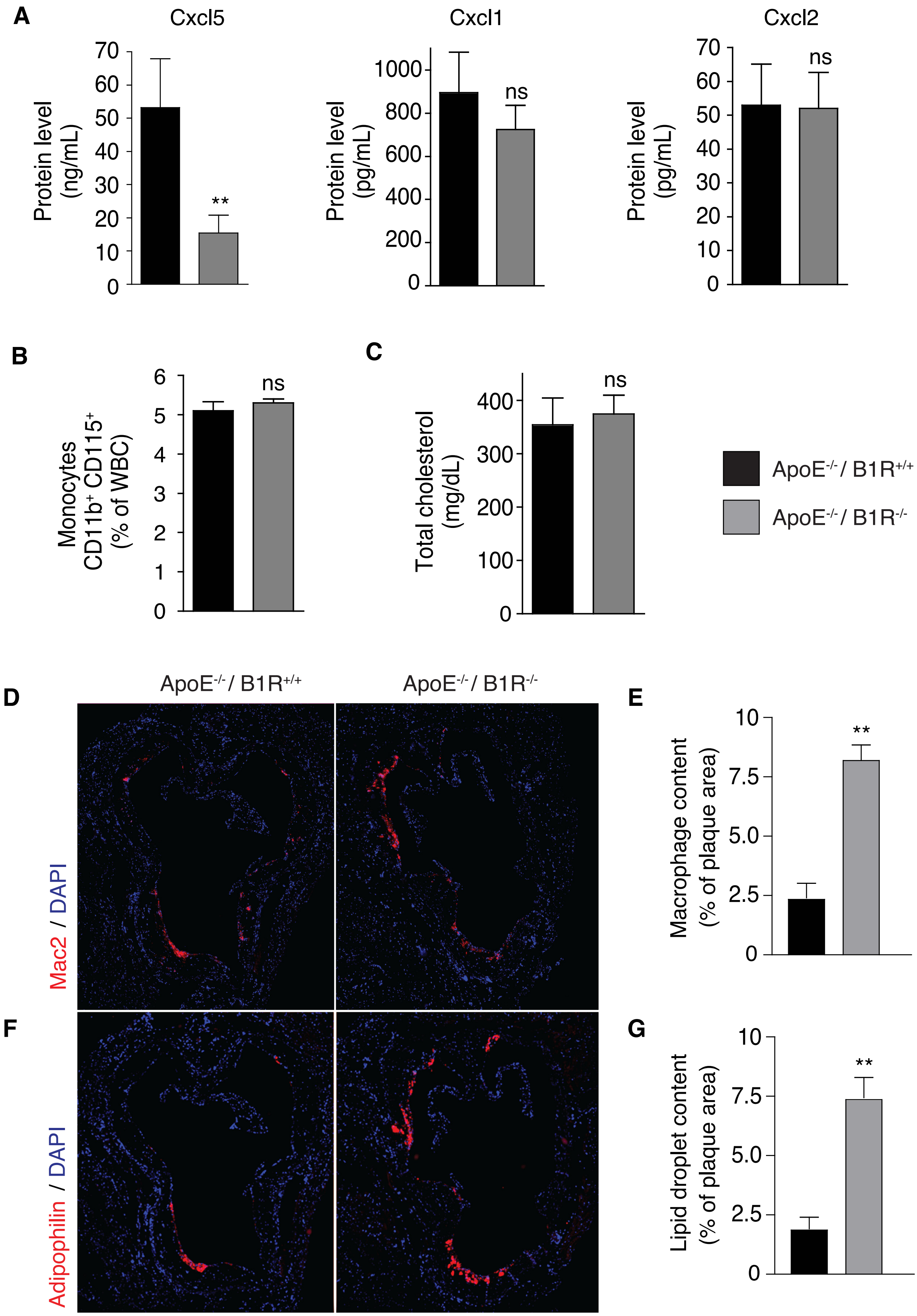
SUPPLEMENTAL FIGURES



Supplemental Figure 1. Expression of ELR$^+$-Cxcl chemokines in atherosclerosis.
(A) Assessment of Cxcl1 (■) and Cxcl2 (▲) mRNA during the progression of atherosclerosis in aorta of ApoE$^{-/-}$ mice (n=6-8 per time point) fed a WD for the indicated time. (B) Triglycerides and LDL cholesterol were measured in plasma of WT and ApoE$^{-/-}$ mice fed either chow (CD) or western (WD) diet for 12wks (n=8 mice per group). (C) Aortic Cxcl1, Cxcl2 and Cxcl5 mRNA expression was measured in ApoE$^{-/-}$ mice under CD for 12wks in thoracic aorta (Th) and aortic arch (Ar) (n=8 mice per group). Data are mean SEM. ***P<0.001, **P<0.01, *P<0.05. ns, not significant. (D) Representative images of brachiocephalic artery (BCA) and spleen from ApoE$^{-/-}$ mice fed a WD for 12wks and stained with anti-Ly6G Ab for neutrophil detection. Spleen was used as a positive control for Ly6G immunostaining. Scale bar= 75μm.



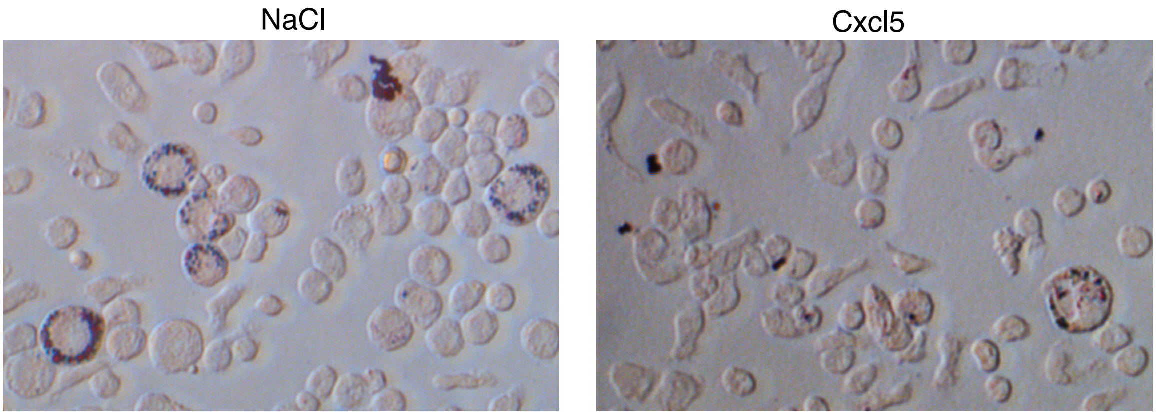
Supplemental Figure 2. Blockade of Cxcl5 augments macrophage foam cell formation.
ApoE$^{-/-}$ mice were fed a WD for 12wks and treated with either IgG isotype control (IgG) or anti-Cxcl5 Antibody (Cxcl5 Ab). Brachiocephalic artery and heart were removed, cut and stained. Representative images from brachiocephalic artery lesions of (A) hematoxylin-eosin staining (HE) and (C) Mac2 immunostaining for macrophage detection. (B) Quantification of plaque size in brachiocephalic artery lesions. (D) Representative image of brachiocephalic artery section from Cxcl5 Ab treated mice immunostained for adipophilin. White arrows indicate lipid-droplet. Representative images from aortic root lesions of (E) picosirius red staining for collagen detection. (F) Quantification of collagen content in aortic root lesions. (B and F) Data represent mean ± SEM. n =7- 8. **P<0.01. ns: not significant. Scale bars 100µm(A and E), 50µm (C), 10µm (D).



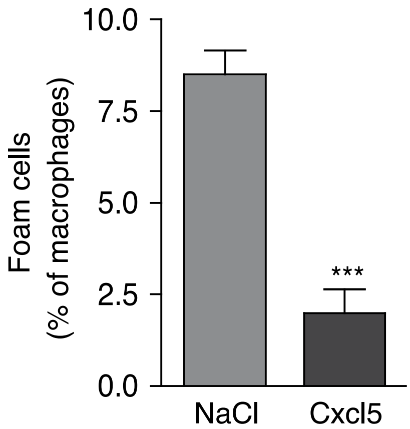
Supplemental Figure 3. Reduction of Cxcl5 in ApoE$^{-/-}$ B1R$^{-/-}$ mice is correlated with an increase of foam macrophage cells in atherosclerotic plaques.

ApoE$^{-/-}$ / B1R$^{+/+}$ and ApoE$^{-/-}$ / B1R$^{-/-}$ mice were fed a CD for 60wks. (A) Cxcl1, Cxcl2 and Cxcl5 levels were measured in plasma by ELISA. (B) Monocytes were measured in blood by flow cytometry analysis. (C) Cholesterol level was determined in plasma. (D-G) Heart was removed and aortic root section were analysed. Representative images of aortic root sections immunostained for Mac2 (D) and Adipophilin (F). Quantification of macrophage (E) and lipid-droplet (G) content in atherosclerotic plaque area within the aortic root. Data in A, B, C, E and G represent mean ± SEM. n = 8.**P<0.01. ns, not significant.

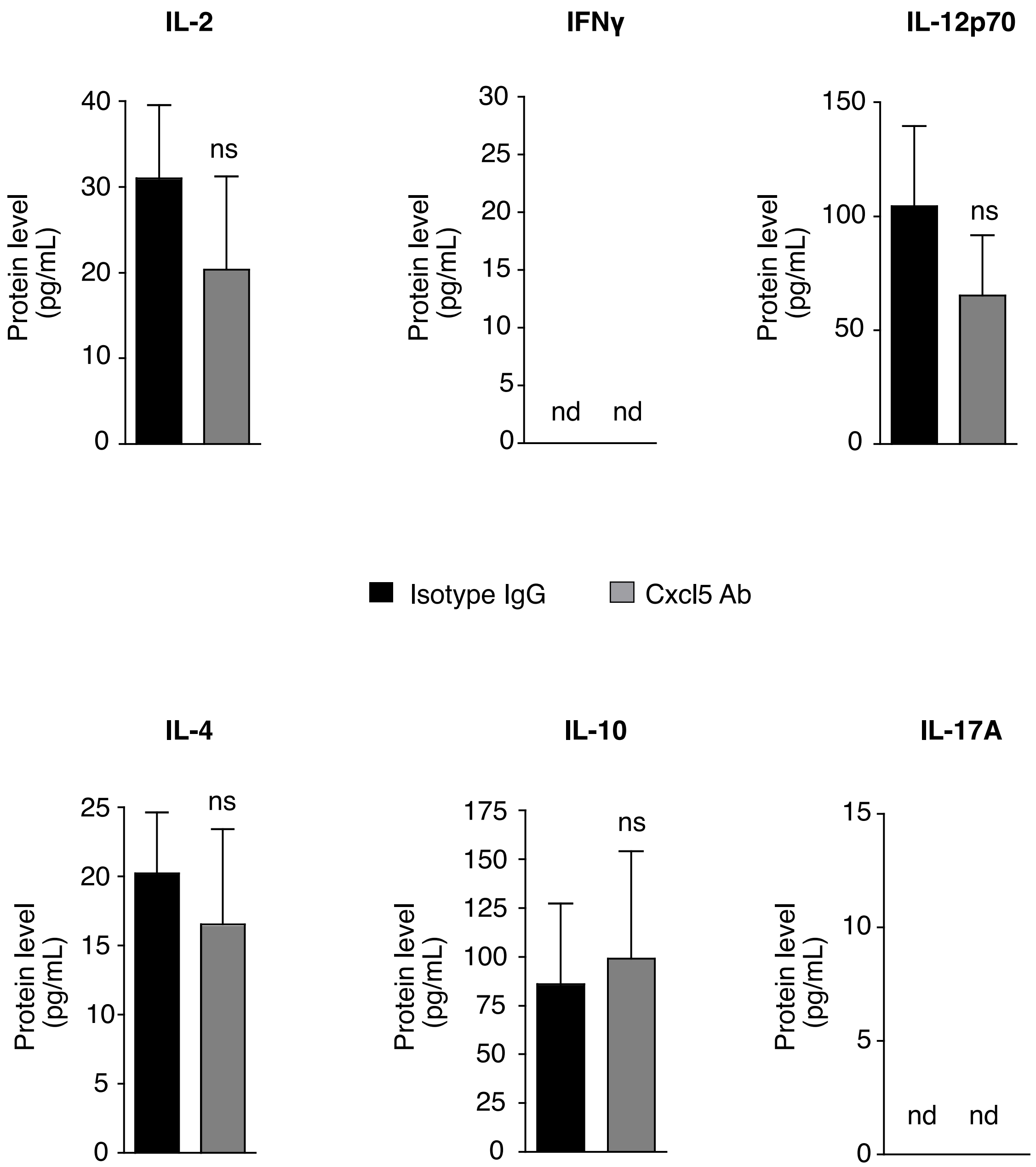
A



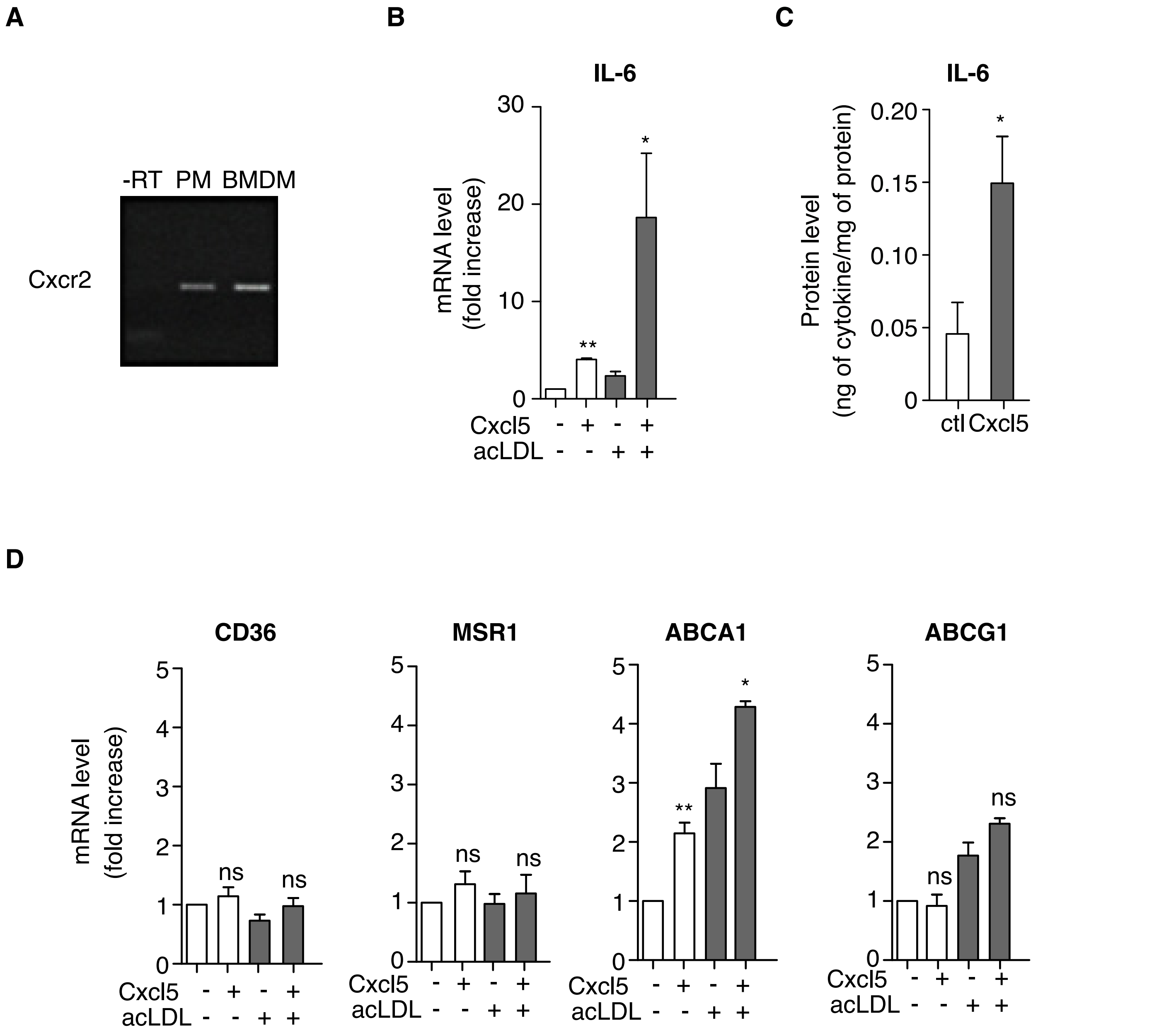
B



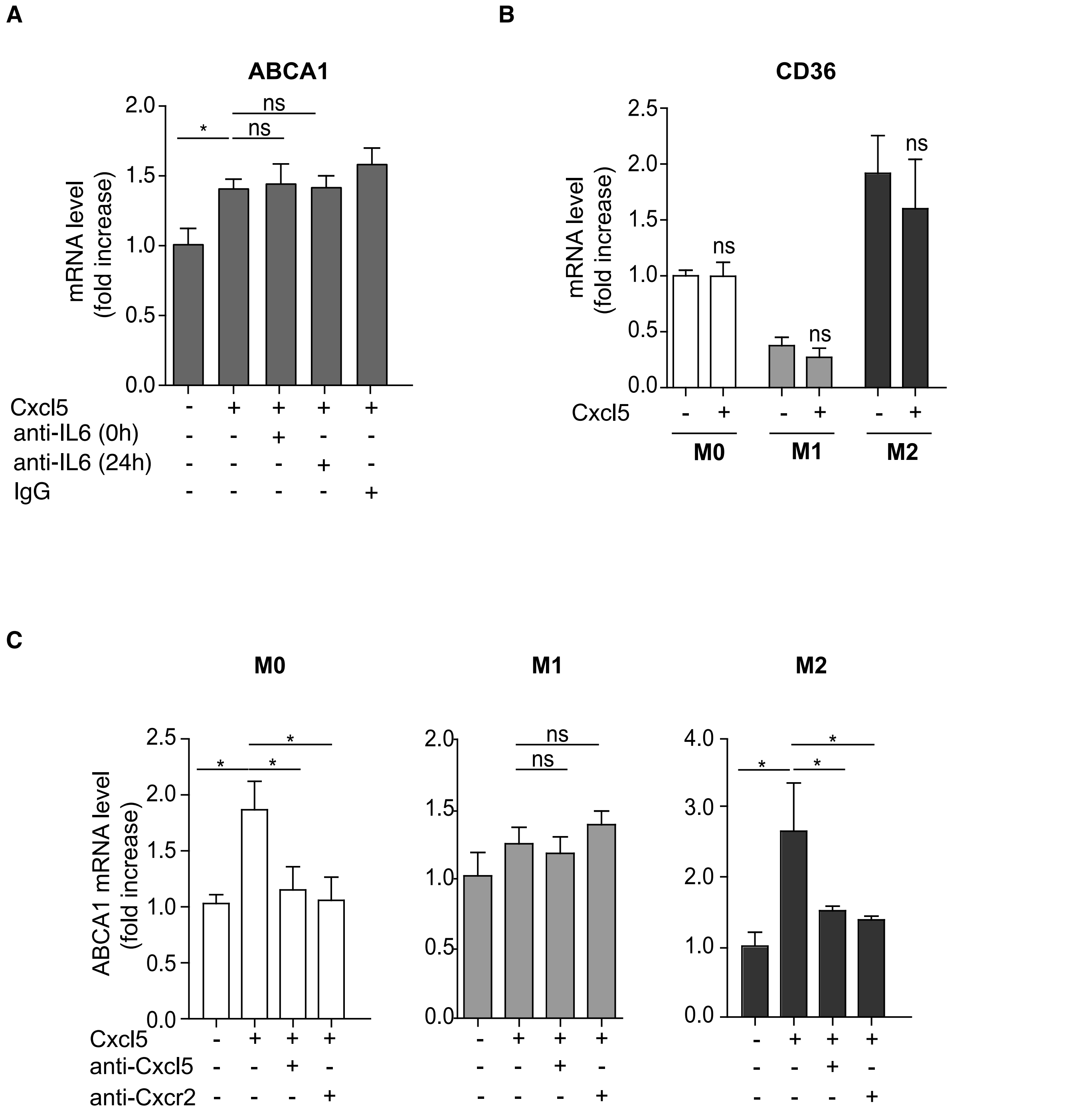
Supplemental Figure 4. Cxcl5 reduces macrophage foam cell formation in vivo.
ApoE$^{-/-}$ mice were fed a WD and treated with either saline or Cxcl5 (i.p. 400ng, every 72h) for 12days. Peritoneal macrophages were isolated and stained with Oil-Red-O. (A) Representative images of peritoneal macrophages stained with Oil-Red-O. (B) Quantification of peritoneal foamy macrophages (Oil-Red-O positive). Data represent mean ± SEM. n = 3. ***P<0.001.



Supplemental Figure 5. Blockade of Cxcl5 does not affect plasma cytokine levels.
ApoE$^{-/-}$ mice were fed a WD for 12wks and treated with either IgG isotype control (IgG) or anti-Cxcl5 Ab (Cxcl5 Ab). Selected plasma cytokines were analysed using Multiplex technology. Data represent mean ± SEM. n=7-8. ns: not significant; nd: not determined (below the limit of detection).



Supplemental Figure 6. Cxcl5 induces IL-6 and ABCA1 expression in peritoneal macrophages in vitro. (A) Cxcr2 mRNA expression was determined by RT-PCR in BMDM and PM. Minus RT (-RT) serves as a negative control. (B-D) PM were cholesterol-loaded or not with acLDL and treated with or without Cxcl5. Macrophage activation was determined by IL-6 expression (B) at mRNA level using qPCR and (C) protein release in the medium using ELISA. (D) Expression of genes involved in cholesterol trafficking was measured by qPCR in PM. Data are mean ± SEM. n=5-8. **P<0.01, *P<0.05. ns, not significant.



Supplemental Figure 7. Stimulation of Cxcl5-Cxcr2 axis induces expression of ABCA1 but not CD36 in M0 and M2 macrophages and is independent of IL-6 pathway.

(A) Cholesterol-loaded BMDM with acLDL were treated with anti-IL-6 Ab 20min prior to (0h) or 24h after Cxcl5 stimulation. ABCA1 mRNA expression was measured by qPCR at t=48h. (B-C) BMDM were treated with Cxcl5 (M0) or in combination with INFϒ (M1) or IL-4 (M2) for 4h. Antibodies against Cxcl5 and Cxcr2 were added 20min prior to Cxcl5, INFϒ and IL-4 treatments. CD36 (B) and ABCA1 (C) mRNA expression in naive (M0), classically-activated (M1) and alternatively-activated (M2) macrophage was determined by qPCR. Data are mean ± SEM. n=6. *P<0.05. ns, not significant.

Supplemental Table 1. List of genes significantly correlated to Cxcl5

Rank	Probeset	EntrezGeneID	Symbol	Correlation (R value)
1	1419728_at	20311	Cxcl5	1.0000
2	1440173_x_at	20344	Selp	0.9135
3	1422412_x_at	53876	Ear3	0.8930
4	1424415_s_at	233744	Spon1	0.8897
5	1421977_at	58223	Mmp19	0.8787
6	1418508_a_at	14784	Grb2	0.8732
7	1451537_at	12654	Chi3l1	0.8572
8	1438676_at	100702	Gbp6	0.8520
9	1418675_at	18414	Osmr	0.8507
10	1417721_s_at	16792	Laptm5	0.8500
11	1423878_at	71683	Gypc	0.8472
12	1444242_at	24059	Slco2a1	0.8453
13	1427747_a_at	16819	Lcn2	0.8408
14	1439015_at	14585	Gfra1	0.8407
15	1450826_a_at	20210	Saa3	0.8395
16	1426594_at	232288	Frmd4b	0.8392
17	1419209_at	14825	Cxcl1	0.8386
18	1450430_at	17533	Mrc1	0.8375
19	1420249_s_at	20305	Ccl6	0.8339
20	1442599_at	83704	Slc12a9	0.8336
21	1415804_at	20312	Cx3cl1	0.8300
22	1420498_a_at	13132	Dab2	0.8285
23	1426246_at	19128	Pros1	0.8282
24	1457539_at	52701	D10Ertd709e	0.8280
25	1450822_at	17110	Lyz1	0.8266
26	1448566_at	53945	Slc40a1	0.8259
27	1436987_at	319909	Ism1	0.8197
28	1423632_at	80290	Gpr146	0.8197
29	1417495_x_at	12870	Cp	0.8196
30	1423140_at	16889	Lipa	0.8190
31	1417019_a_at	23834	Cdc6	0.8187
32	1437046_x_at	75007	Fam63a	0.8176
33	1419417_at	22341	Vegfc	0.8159
34	1456620_at	432486	Gnptab	0.8149
35	1450020_at	13051	Cx3cr1	0.8087
36	1439518_at	105450	Mmrn2	0.8083
37	1424125_at	217826	Kcnk13	0.8083
38	1451680_at	76650	Srxn1	0.8074
39	1417523_at	56193	Plek	0.8066
40	1430124_x_at	58810	Akr1a4	0.8046
41	1436963_x_at	67706	Tmem179b	0.8008
42	1440759_at	100038499	Gm10672	0.8001
43	1443698_at	327959	Xaf1	0.8001
44	1449193_at	11801	Cd5l	0.7993
45	1426604_at	24014	Rnasel	0.7977
46	1435135_at	320024	Nceh1	0.7973
47	1451359_at	210992	Lpcat1	0.7948
48	1435143_at	13713	Elk3	0.7941
49	1436003_at	22329	Vcam1	0.7936
50	1449153_at	17381	Mmp12	0.7936
51	1418580_at	67775	Rtp4	0.7934

52	1419043_a_at	60440	Iigp1	0.7931
53	1421138_a_at	18768	Pkib	0.7929
54	1424616_s_at	233575	Pgap2	0.7929
55	1452948_at	69769	Tnfaip8l2	0.7927
56	1426385_x_at	22627	Ywhae	0.7927
57	1422243_at	14178	Fgf7	0.7925
58	1430704_at	73914	Irak3	0.7922
59	1453196_a_at	23962	Oasl2	0.7918
60	1444176_at	242341	Atp6v0d2	0.7915
61	1437726_x_at	12260	C1qb	0.7907
62	1434783_at	380967	Tmem106c	0.7905
63	1448668_a_at	16179	Irak1	0.7905
64	1449516_a_at	50780	Rgs3	0.7902
65	1427348_at	230738	Zc3h12a	0.7891
66	1427994_at	246746	Cd300lf	0.7886
67	1417793_at	54396	Irgm2	0.7882
68	1456111_at	244853	Fam55d	0.7874
69	1449025_at	15959	Ifit3	0.7858
70	1419100_at	20716	Serpina3n	0.7850
71	1417009_at	50909	C1ra	0.7834
72	1418090_at	84094	Plvap	0.7834
73	1449256_a_at	53869	Rab11a	0.7832
74	1421424_a_at	16790	Anpep	0.7831
75	1434380_at	229900	Gbp7	0.7820
76	1421034_a_at	16190	Il4ra	0.7805
77	1419463_at	80797	Clca2	0.7803
78	1433588_at	28006	D6Wsu116e	0.7796
79	**1421839_at**	**11303**	**Abca1**	**0.7795**
80	1425133_s_at	74760	Rab3il1	0.7793
81	1420699_at	56644	Clec7a	0.7790
82	1418626_a_at	12759	Clu	0.7789
83	1419932_s_at	83924	Gpr137b	0.7788
84	1433488_x_at	75612	Gns	0.7788
85	1418021_at	12268	C4b	0.7785
86	1448424_at	20378	Frzb	0.7783
87	1448303_at	93695	Gpnmb	0.7782
88	1448929_at	74145	F13a1	0.7782
89	1425823_at	545366	Cfhr2	0.7780
90	1424603_at	58911	Sumf1	0.7776
91	1449254_at	20750	Spp1	0.7772
92	1434784_s_at	380967	Tmem106c	0.7772
93	1435732_x_at	11984	Atp6v0c	0.7769
94	1451715_at	16658	Mafb	0.7762
95	1429527_a_at	22038	Plscr1	0.7754
96	1428777_at	114715	Spred1	0.7752
97	1435627_x_at	17357	Marcksl1	0.7750
98	1426808_at	16854	Lgals3	0.7748
99	1435133_at	22234	Ugcg	0.7746
100	1423547_at	17105	Lyz2	0.7737
101	1455204_at	71795	Pitpnc1	0.7736
102	1456424_s_at	18830	Pltp	0.7733
103	1451784_x_at	14964	H2-D1	0.7732
104	1429321_at	67702	Rnf149	0.7725
105	1425639_at	216991	Adap2	0.7723
106	1439454_x_at	69742	Tm2d2	0.7720
107	1417040_a_at	51800	Bok	0.7720
108	1420562_at	57277	Slurp1	0.7718
109	1452647_a_at	67728	Dph2	0.7717

110	1448380_at	19039	Lgals3bp	0.7717
111	1451651_at	278180	Vsig4	0.7714
112	1415983_at	18826	Lcp1	0.7714
113	1419449_a_at	14678	Gnai2	0.7712
114	1418133_at	12051	Bcl3	0.7711
115	1451931_x_at	14980	H2-L	0.7684
116	1417492_at	13030	Ctsb	0.7682
117	1426774_at	243771	Parp12	0.7681
118	1448591_at	13040	Ctss	0.7680
119	1436333_a_at	104015	Synj1	0.7680
120	1441216_at	20442	St3gal1	0.7677
121	1454783_at	16164	Il13ra1	0.7676
122	1422062_at	20288	Msr1	0.7672
123	1418318_at	66889	Rnf128	0.7669
124	1427076_at	17476	Mpeg1	0.7667
125	1418736_at	26879	B3galnt1	0.7664
126	1451243_at	215615	Rnpep	0.7661
127	1437760_at	230145	Galnt12	0.7660
128	1422645_at	15216	Hfe	0.7657
129	1451866_a_at	15234	Hgf	0.7656
130	1421172_at	11489	Adam12	0.7656
131	1454086_a_at	16909	Lmo2	0.7650
132	1423593_a_at	12978	Csf1r	0.7649
133	1422978_at	13058	Cybb	0.7648
134	1456212_x_at	12702	Socs3	0.7648
135	1422868_s_at	14544	Gda	0.7645
136	1436589_x_at	101540	Prkd2	0.7632
137	1427103_at	102595	Plekho2	0.7630
138	1433500_at	209773	Dennd2a	0.7619
139	1455357_x_at	67952	Tomm20	0.7618
140	1444052_at	14257	Flt4	0.7609
141	1427327_at	231805	Pilra	0.7607
142	1450764_at	27052	Aoah	0.7601
143	1448163_at	26384	Gnpda1	0.7598
144	1451191_at	12904	Crabp2	0.7595
145	1420196_s_at	100855	Tbc1d14	0.7594
146	1427682_a_at	13654	Egr2	0.7591
147	**1450297_at**	**16193**	**Il6**	**0.7590**
148	1422572_at	56212	Rhog	0.7589
149	1424523_at	140580	Elmo1	0.7583
150	1460619_at	211798	Mfsd9	0.7583
151	1416022_at	16592	Fabp5	0.7582
152	1420697_at	65221	Slc15a3	0.7581
153	1442082_at	12267	C3ar1	0.7579
154	1418539_a_at	19267	Ptpre	0.7572
155	1450678_at	16414	Itgb2	0.7570
156	1438633_x_at	16796	Lasp1	0.7567
157	1455221_at	11307	Abcg1	0.7565
158	1455476_a_at	382034	Gse1	0.7562
159	1449370_at	20677	Sox4	0.7561
160	1436838_x_at	72042	Cotl1	0.7560
161	1430700_a_at	27226	Pla2g7	0.7559
162	1419194_s_at	63986	Gmfg	0.7558
163	1452117_a_at	23880	Fyb	0.7553
164	1424727_at	12774	Ccr5	0.7548
165	1426528_at	18187	Nrp2	0.7544
166	1455660_at	12983	Csf2rb	0.7541
167	1420703_at	12982	Csf2ra	0.7538

168	1428758_at	67893	Tmem86a	0.7535
169	1425546_a_at	22041	Trf	0.7534
170	1423776_s_at	223754	Tbc1d22a	0.7533
171	1415686_at	68365	Rab14	0.7531
172	1434308_at	215113	Slc43a2	0.7527
173	1452068_at	67111	Naaa	0.7523
174	1453055_at	214968	Sema6d	0.7519
175	1460273_a_at	17948	Naip2	0.7518
176	1449824_at	96875	Prg4	0.7514
177	1439256_x_at	664862	Gpr137b-ps	0.7509
178	1449360_at	12984	Csf2rb2	0.7507
179	1416094_at	11502	Adam9	0.7507
180	1424470_a_at	223864	Rapgef3	0.7506
181	1424126_at	11655	Alas1	0.7502
182	1460437_at	72318	Cyth4	0.7500
183	1417648_s_at	69178	Snx5	0.7498
184	1426623_a_at	70497	Arhgap17	0.7498
185	1424265_at	74091	Npl	0.7494
186	1434376_at	12505	Cd44	0.7494
187	1455741_a_at	230857	Ece1	0.7494
188	1425797_a_at	20963	Sykb	0.7489
189	1419128_at	16411	Itgax	0.7489
190	1451821_a_at	20684	Sp100	0.7489
191	1456581_x_at	14569	Gdi2	0.7487
192	1449164_at	12514	Cd68	0.7486
193	1421322_a_at	16391	Irf9	0.7484
194	1423722_at	75909	Vmp1	0.7483
195	1425789_s_at	11752	Anxa8	0.7481
196	1436530_at	100034251	Gm11428	0.7478
197	1435449_at	12125	Bcl2l11	0.7474
198	1419609_at	12768	Ccr1	0.7474
199	1449429_at	14226	Fkbp1b	0.7474
200	1422631_at	11622	Ahr	0.7469
201	1419883_s_at	11966	Atp6v1b2	0.7469
202	1428187_at	16423	Cd47	0.7468
203	1448118_a_at	13033	Ctsd	0.7465
204	1452279_at	18636	Cfp	0.7464
205	1419534_at	108078	Olr1	0.7461
206	1442798_x_at	212032	Hk3	0.7460
207	1427691_a_at	15976	Ifnar2	0.7460
208	1457035_at	226691	AI607873	0.7456
209	1438561_x_at	75146	Tmem180	0.7453
210	1436223_at	320910	Itgb8	0.7452
211	1451363_a_at	72121	Dennd2d	0.7449
212	1448550_at	16803	Lbp	0.7442
213	1449156_at	17085	Ly9	0.7437
214	1422648_at	11988	Slc7a2	0.7436
215	1419605_at	17312	Clec10a	0.7434
216	1423571_at	13609	S1pr1	0.7434
217	1449280_at	71690	Esm1	0.7432
218	1421187_at	12772	Ccr2	0.7430
219	1417381_at	12259	C1qa	0.7426
220	1419598_at	68774	Ms4a6d	0.7426
221	1450686_at	330260	Pon2	0.7424
222	1435416_x_at	14755	Pigq	0.7422
223	1419589_at	17064	Cd93	0.7418
224	1417073_a_at	19317	Qk	0.7417
225	1448620_at	14131	Fcgr3	0.7416

226	1460430_at	72065	Rap2c	0.7414
227	1424595_at	16456	F11r	0.7413
228	1443783_x_at	14960	H2-Aa	0.7412
229	1448595_a_at	19716	Bex1	0.7409
230	1442849_at	16971	Lrp1	0.7405
231	1415947_at	433375	Creg1	0.7398
232	1449846_at	13587	Ear2	0.7397
233	1439069_a_at	66776	Pisd-ps3	0.7395
234	1434731_x_at	18477	Prdx1	0.7388
235	1416046_a_at	66848	Fuca2	0.7384
236	1449454_at	12182	Bst1	0.7383
237	1449519_at	13197	Gadd45a	0.7383
238	1459740_s_at	22228	Ucp2	0.7380
239	1424302_at	18733	Lilrb3	0.7380
240	1450424_a_at	16068	Il18bp	0.7378
241	1416968_a_at	101502	Hsd3b7	0.7376
242	1439440_x_at	23999	Twf2	0.7376
243	1416013_at	18807	Pld3	0.7376
244	1437347_at	13618	Ednrb	0.7375
245	1460235_at	12492	Scarb2	0.7374
246	1448313_at	12751	Tpp1	0.7373
247	1415687_a_at	19156	Psap	0.7373
248	1441281_s_at	18081	Ninj1	0.7368
249	1418738_at	20266	Scn1b	0.7368
250	1450241_a_at	14017	Evi2a	0.7365
251	1433741_at	12494	Cd38	0.7364
252	1427020_at	219151	Scara3	0.7360
253	1449033_at	18383	Tnfrsf11b	0.7360
254	1418446_at	20502	Slc16a2	0.7355
255	1438138_a_at	224824	Pex6	0.7354
256	1419042_at	60440	Iigp1	0.7352
257	1446951_at	320452	P4ha3	0.7349
258	1437454_a_at	66958	Tmx2	0.7348
259	1426441_at	18174	Slc11a2	0.7347
260	1448576_at	16197	Il7r	0.7346
261	1448919_at	66205	Cd302	0.7344
262	1424067_at	15894	Icam1	0.7333
263	1451310_a_at	13039	Ctsl	0.7331
264	1418340_at	14127	Fcer1g	0.7329
265	1425733_a_at	13860	Eps8	0.7328
266	1448617_at	12508	Cd53	0.7328
267	1457753_at	279572	Tlr13	0.7325

Supplemental Table 2. List of the Biological Functions of genes coregulated with Cxcl5 generated by PANTHER Classification System.

Biological Process	amount	expected	P value
immune system process	82	17.54	2.55E-33
response to stimulus	55	14.49	9.00E-18
immune response	29	5.19	1.29E-13
cytokine-mediated signaling pathway	19	2.61	3.25E-11
signal transduction	78	37.09	4.17E-11
cell communication	80	38.76	5.27E-11
cellular process	96	56.16	8.41E-09
macrophage activation	**12**	**1.65**	**1.47E-07**
cell surface receptor linked signal transduction	43	18.96	3.97E-07

Supplemental Table 3. List of primers

Gene	Species	Forward	Reverse
Cxcl1	mouse	TGAGCTGCGCTGTCAGTGCCT	AGAAGCCAGCGTTCACCAGA
Cxcl2	mouse	GAGCTTGAGTGTGACGCCCCCAGG	GTTAGCCTTGCCTTTGTTCAGTATC
Cxcl5	mouse	GCATTTCTGTTGCTGTTCACGCTG	CCTCCTTCTGGTTTTTCAGTTTAGC
Cxcr2	mouse	ATGCCCTCTATTCTGCCAGAT	GTGCTCCGGTTGTATAAGATGAC
IL-6	mouse	CTGCAAGAGACTTCCATCCAGTT	GAAGTAGGGAAGGCCGTGG
CD36	mouse	TTTCCTCTGACATTTGCAGGTCTA	AAAGGCATTGGCTGGAAGAA
MSR1	mouse	TGAACGAGAGGATGCTGACTG	TGTCATTGAACGTGCGTCAAA
ABCA1	mouse	CCCAGAGCAAAAAGCGACTC	GGTCATCATCACTTTGGTCCTTG
ABCG1	mouse	ATCTGAGGGATCTGGGTCTGA	CCTGATGCCACTTCCATGA
HPRT	mouse	GTAATGATCAGTCAACGGGGGAC	CCAGCAAGCTTGCAACCTTAACCA
β-actin	mouse	GAAATCGTGCGTGACATCAAAG	TGTAGTTTCATGGATGCCACAG
CXCL8	human	GAATGGGTTTGCTAGAATGTGATA	CAGACTAGGGTTGCCAGATTTAAC
CXCL6	human	GGTCCTGTCTCTGCTGTGC	GGGAGGCTACCACTTCCA
GAPDH	human	CATGTTCGTCATGGGTGTGAA	ATGGACTGTGGTCATGAGTCCTT