Supplemental Materials

IgE contributes to atherosclerosis and obesity by affecting macrophage polarization, macrophage protein network, and foam cell formation

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Major Resource Table. Animal, diet, antibody, and ELISA kit information.

Animals

Species/Strain	Vendor or source	Background strain	Catalog number	Sex
Apoe$^{-/-}$	The Jackson Laboratory, Bar Harbor, ME	C57BL/6J	002052	Male and female
Ige$^{-/-}$	Provided by Dr. Hans Oettgen**,** Boston Children's Hospital, Harvard Medical School, Boston, MA	C57BL/6J	N/A	Male and female
C3$^{-/-}$	The Jackson Laboratory, Bar Harbor, ME	C57BL/6J	029661	Male and female
Fcer1a$^{-/-}$	Provided by Drs. Marie-Helene Jouvin and Jean-Pierre Kinet, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA	C57BL/6J	N/A	Male and female
Nhe1$^{+/-}$	The Jackson Laboratory, Bar Harbor, ME	C57BL/6J	003012	Male and female

Cultured Cells

Name	Vendor or source	Sex (F, M or unknown)
Bone-marrow-derived macrophages	C57BL/6J	Male

Diets

Name	Catalog number	Company name and address
Normal laboratory diet	5053	Lab Supply, Fort Worth, TX
Atherogenic diet	D12108c	Research Diets Inc. New Brunswick, NJ

Antibodies and ELISA kits

Immunostaining and Western blot antibody	Dilution	Concentration	Catalog number	Company name and address
MAC3	1:900	0.56 µg/ml	553322	BD Biosciences, San Jose, CA
CD31	1:500	1 µg/ml	553370	BD Biosciences, San Jose, CA
α-SMA	1:500	unknown	F3777	Sigma-Aldrich, St. Louis, MO
MHC-II	1:250	2 µg/ml	556999	BD Biosciences, San Jose, CA
CD4	1:90	5.56 mg/ml	553043	BD Biosciences, San Jose, CA
CD8	1:100	5 µg/ml	14-0081-85	eBiosciences, San Diego, CA
MYH11	1:2000	0.25 µg/ml	702544	Thermo Fisher Scientific, Waltham, MA

MCP1	1:100	2 μg/ml	AF-479-NA	R&D Systems, Minneapolis, MN
CD49b	1:100	5 μg/ml	14-5971-85	eBiosciences, San Diego, CA
FcεR1	1:50	20 μg/ml	06-727	Millipore, Burlington, MA
Complement C3	1:1000	0.08 μg/ml	PA5-21349	Thermo Fisher Scientific, Waltham, MA
iNOS	1:1000	4 μg/ml	PA1-036	Thermo Fisher Scientific, Waltham, MA
Arg-1	1:1000	0.5 μg/ml	678802	BioLegend, San Diego, CA
MAC2	1:1000	1 μg/ml	CL8942LE	Cadarlane Laboratories, Burlington, NC
pERK	1:1000	unknown	4370	Cell Signaling Technology, Danvers, MA
ERK	1:1000	unknown	9107	Cell Signaling Technology, Danvers, MA
pp38	1:1000	unknown	4631	Cell Signaling Technology, Danvers, MA
p38	1:1000	unknown	9228	Cell Signaling Technology, Danvers, MA
GAPDH	1:2000	unknown	2118	Cell Signaling Technology, Danvers, MA
FACS antibody	**Dilution**	**Concentration**	**Catalog number**	**Company name and address**
CD45-PerCP-Cyanine5.5	1:100	0.2 μg/test	45-0451-82	Invitrogen, Carlsbad, CA
CD45-FITC	1:100	0.5 μg/test	11-0451-82	eBiosciences, San Diego, CA
Ly-6C-FITC	1:250	0.2 μg/test	53-5932-82	Invitrogen, Carlsbad, CA
F4/80-PerCP-Cyanine5.5	1:100	0.2 μg/test	123128	BioLegend, San Diego, CA
CD11b-APC	1:100	0.2 μg/test	17-0112-82	eBiosciences, San Diego, CA
FcεR1-FITC	1:250	0.2 μg/test	11-5898-82	eBiosciences, San Diego, CA
c-kit-APC	1:100	0.2 μg/test	17-1171-82	eBiosciences, San Diego, CA
CD200R3-PE	1:100	0.2 μg/test	142206	BioLegend, San Diego, CA
CD63-PE/Cy7	1:100	0.2 μg/test	143910	BioLegend, San Diego, CA
ELISA kit	**Dilution**	**Concentration**	**Catalog number**	**Company name and address**
Insulin	N/A	N/A	90080	Crystal Chem, Elk Grove Village, IL
IL-1β	N/A	N/A	88-7013-22	Invitrogen, Carlsbad, CA
TNF-α	N/A	N/A	88-7324-22	Invitrogen, Carlsbad, CA
IL-6	N/A	N/A	88-7064-88	Invitrogen, Carlsbad, CA
MCP-1	N/A	N/A	88-7391-88	Invitrogen, Carlsbad, CA
IFN-γ	N/A	N/A	88-7314-88	Invitrogen, Carlsbad, CA
Total triglyceride	N/A	N/A	T7532	Pointe Scientific, Canton, MI

Total cholesterol	N/A	N/A	C7510	Pointe Scientific, Canton, MI
HDL cholesterol	N/A	N/A	H7511	Pointe Scientific, Canton, MI
Histamine	N/A	N/A	RE59221	IBL, Hamburg, Germany

Supplemental Table

Supplemental Table I. Primers for quantitative real-time PCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
C3	CCAGCTCCCCATTAGCTCTG	GCACTTGCCTCTTTAGGAAGTC
Lrp1	CGGTGTGACGGTGAAAGAGA	CCCCAGACAACTGTGCTCAT
TFR	GGACGCCATGACTTTGGATG	GCCATGACAGGCACTAGACC
LPL	CAAGACCTTCGTGGTGATCCA	GTACAGGGCGGCCACAAG
iNOS	ACATCGACCCGTCCACAGTAT	CAGAGGGGTAGGCTTGTCTC
TNF-α	CAAAGGGAGAGTGGTCAGGT	GGCAACAAGGTAGAGAGGC
Arg-1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
IL-10	GAAGCATGGCCCAGAAATCA	TGCTCCACTGCCTTGCTCTT
MCP-1	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
IL-6	CCTTCCTACCCCAATTTCCAA	AGATGAATTGGATGGTCTTGGTC
CD68	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA
Mrc1	TGATTACGAGCAGTGGAAGC	GTTCACCGTAAGCCCAATTT
GAPDH	TGTCATACTTGGCAGGTTTCT	CGTGTTCCTACCCCCAATGT

Supplemental Figures

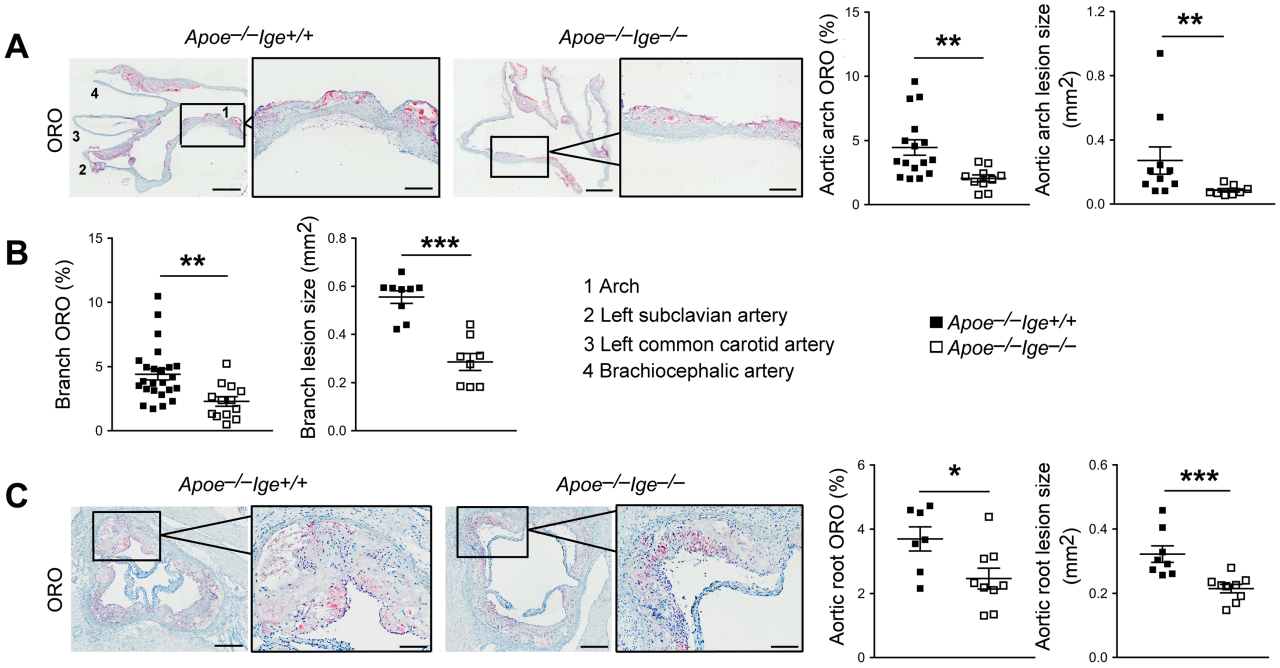

Figure I. IgE deficiency reduces atherosclerotic lesion size and lesion lipid deposition in *Apoe*$^{-/-}$ mice after 12 weeks of an atherogenic diet. ORO staining determined lipid deposition and intima areas in aortic arch (**A**, scale: 1 mm, inset: 300 μm), three branches (brachiocephalic artery, left common carotid artery, and left subclavian artery) (**B**), and aortic root (**C**, scale: 1 mm, inset: 300 μm). Representative images in panels **A** and **C** are shown to the left. n=8~24 mice per group. All data are mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001.

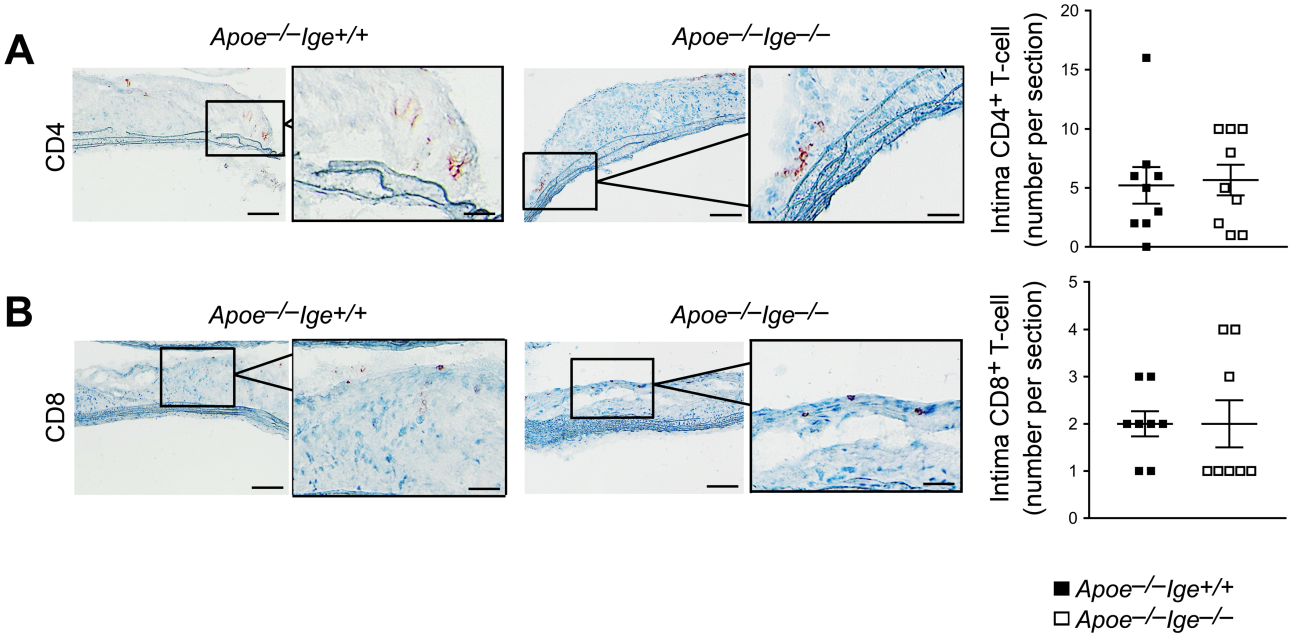

Figure II. IgE deficiency does not affect aortic arch intima $CD4^+$ T-cell and $CD8^+$ T-cell contents in *Apoe*$^{-/-}$ mice after 12 weeks of an atherogenic diet. Intima $CD4^+$ T cells (**A**) $CD8^+$ T cells (**B**) were presented as number per section. Scale: 200 μm, inset scale: 70 μm.

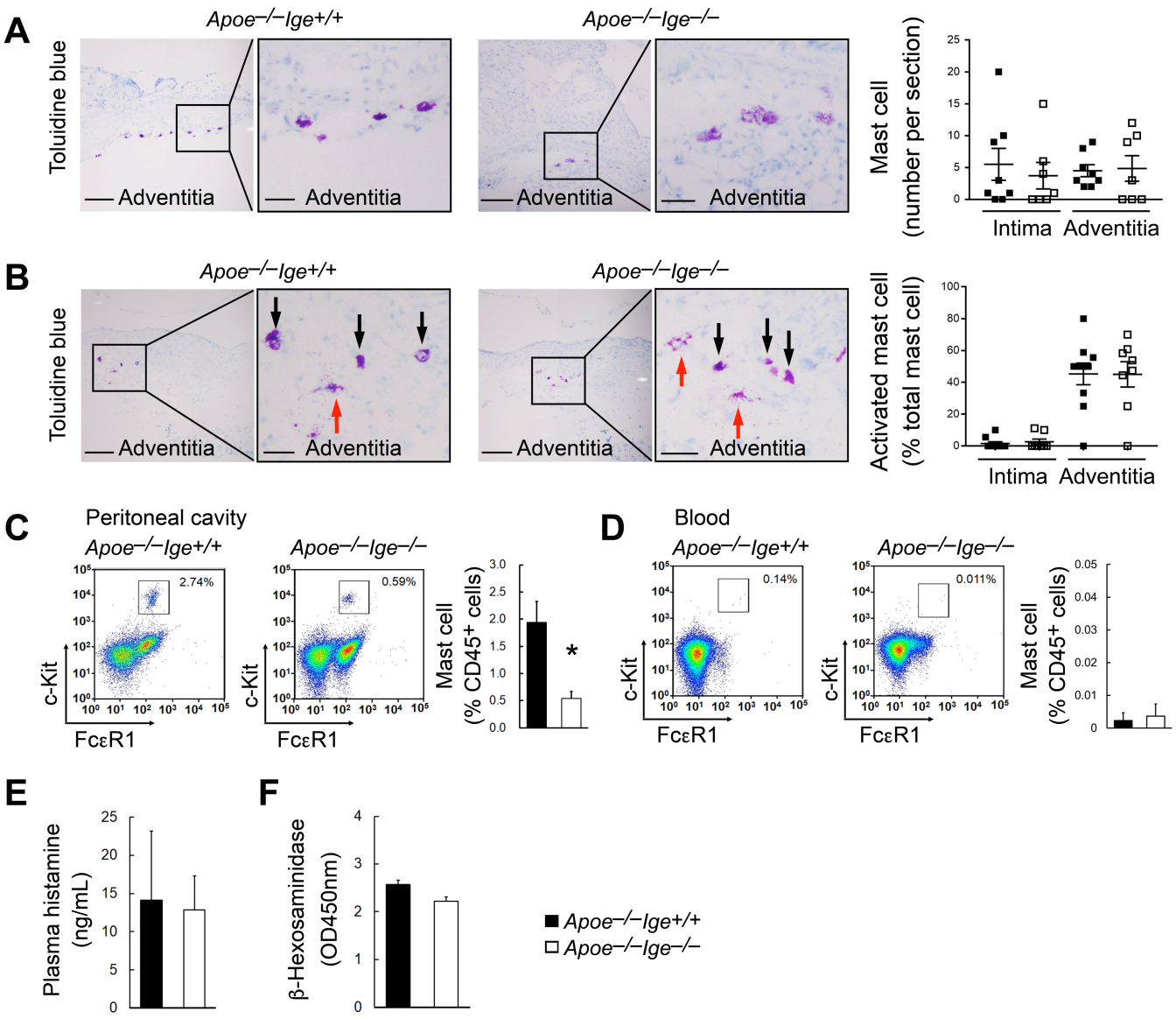

Figure III. IgE deficiency in *Apoe*$^{-/-}$*Ige*$^{-/-}$ mice does not affect atherosclerotic lesion mast cell number and activation. **A.** Quantification of toluidine blue-positive mast cells in aortic arch intima and adventitia from *Apoe*$^{-/-}$*IgE*$^{+/+}$ and *Apoe*$^{-/-}$*Ige*$^{-/-}$ mice. **B.** Percentage of activated (degranulated, red arrows) mast cells over total mast cells (activated red arrows and non-activated black arrows) in both the aortic arch intima and adventitia from *Apoe*$^{-/-}$*IgE*$^{+/+}$ and *Apoe*$^{-/-}$*Ige*$^{-/-}$ mice. Scale: 200 μm, inset: 50 μm. **C-D.** FACS analysis of FcεR1^{+}c-Kit^{+} mast cells in the peritoneal cavity and FcεR1^{+}c-Kit^{+} mast cell progenitors in blood. **E-F.** Plasma histamine level and β-hexosaminidase activity. Representative images of panels **A-D** are shown to the left.

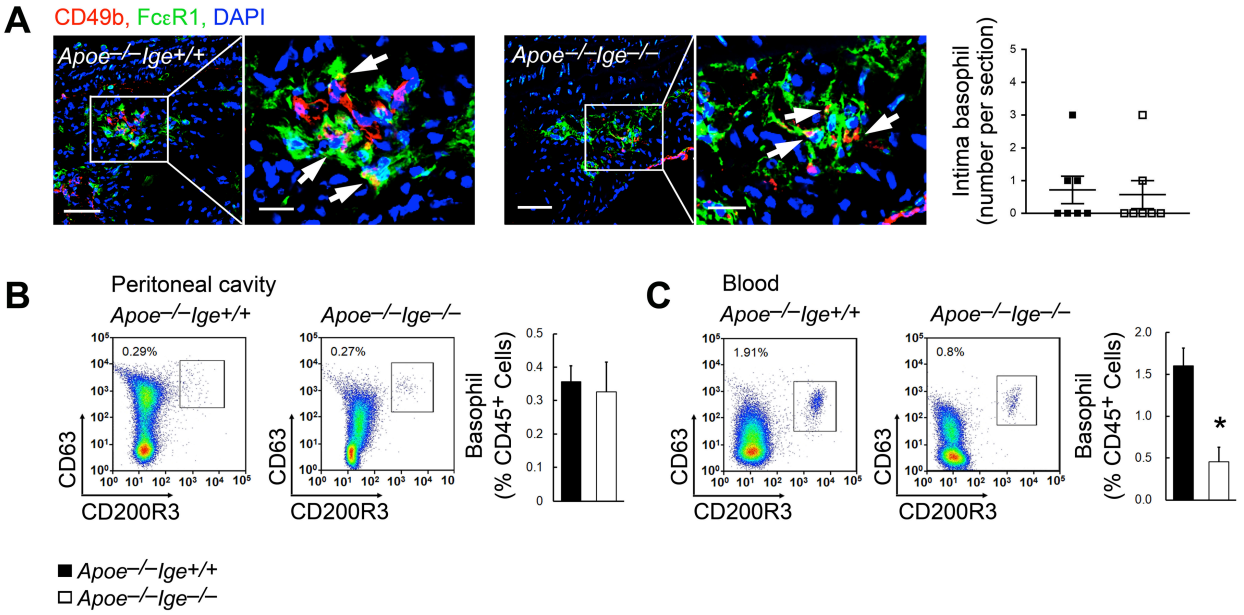

Figure IV. IgE deficiency in *Apoe*$^{-/-}$*Ige*$^{-/-}$ mice does not affect atherosclerotic lesion intima basophils after mice consumed an atherogenic diet for 12 weeks. **A.** Immunofluorescent double staining detected FcεR1^{+}CD49b^{+} basophils in aortic arch intima. Scale: 100 μm, inset: 30 μm. **B-C**. FACS analysis of activated basophils (CD63^{+}CD200R3^{+}) in peritoneal cavity and blood. Representative images of panels **A-C** are shown to the left.

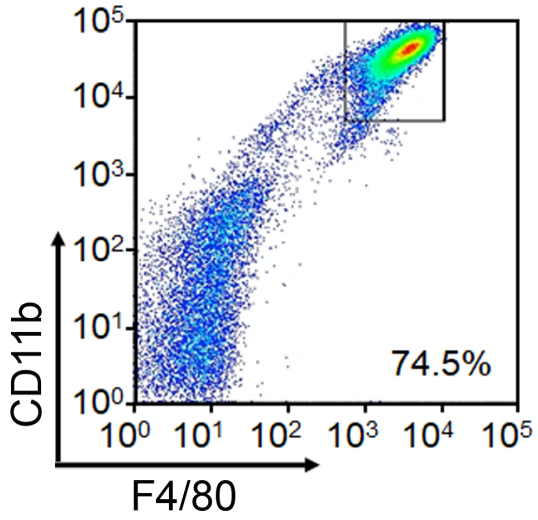

Figure V. FACS analysis assessed the purity of macrophages (F4/80^{+}CD11b^{+}) isolated from the peritoneum.

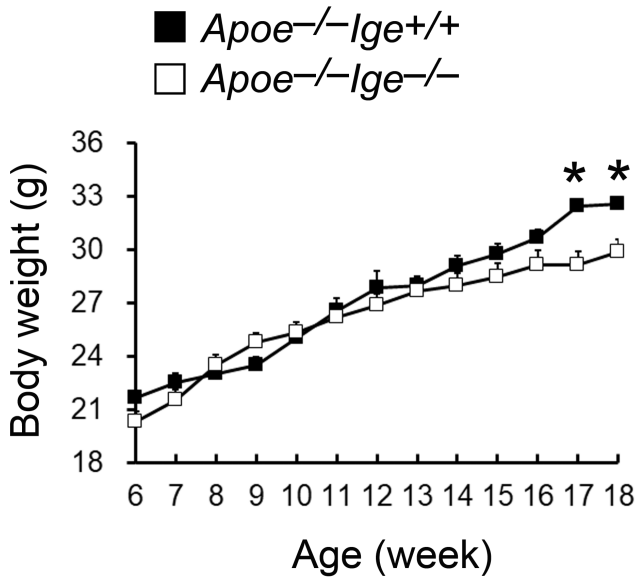

Figure VI. Body weight gain of *Apoe*$^{-/-}$*Ige*$^{-/-}$ and *Apoe*$^{-/-}$*Ige*$^{+/+}$ mice on a normal laboratory diet started with comparable body weight. n=14 mice per group. Data are mean±SEM. *P<0.05.