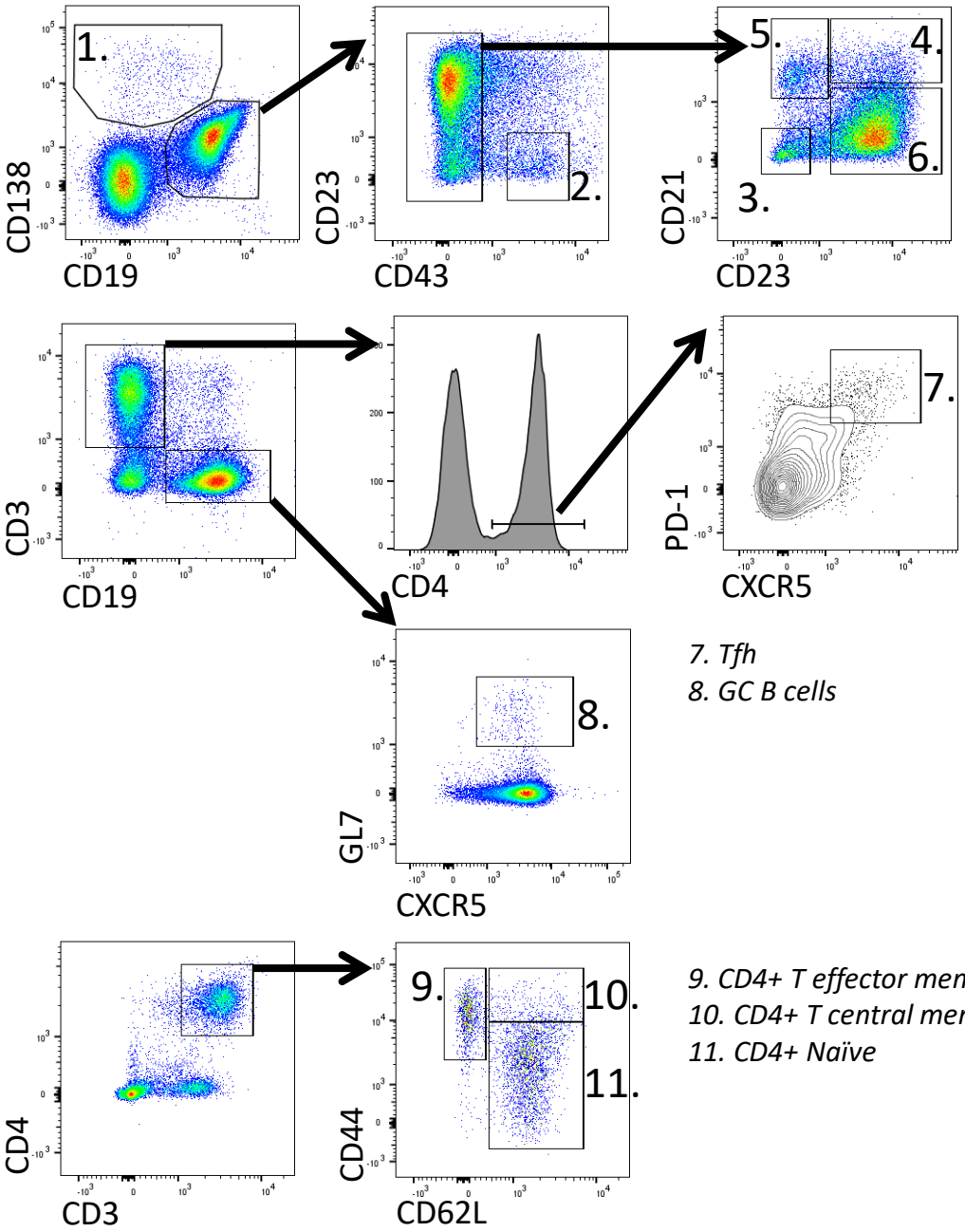
Chakraborty et al
B Cell FcγRIIb and Atherosclerosis
Supplemental Material

Supplemental Figure I

Pre-gated on FSc/SSc
and Live/Dead staining



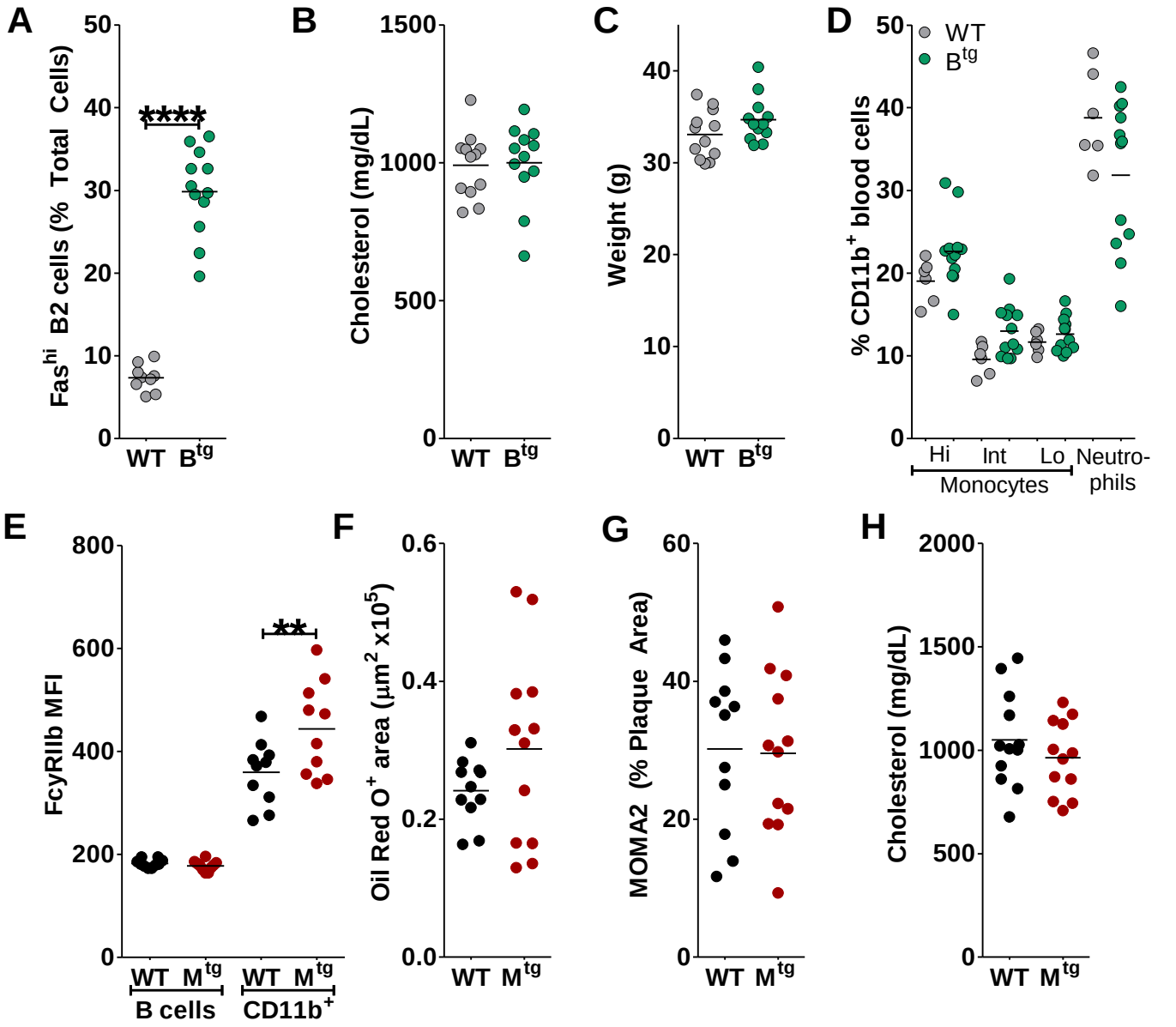
1.Plasma cells
2. B1 Cells
3. T1
4. T2
5. Marginal zone
6. Follicular

7. Tfh
8. GC B cells

9. CD4+ T effector memory
10. CD4+ T central memory
11. CD4+ Naïve

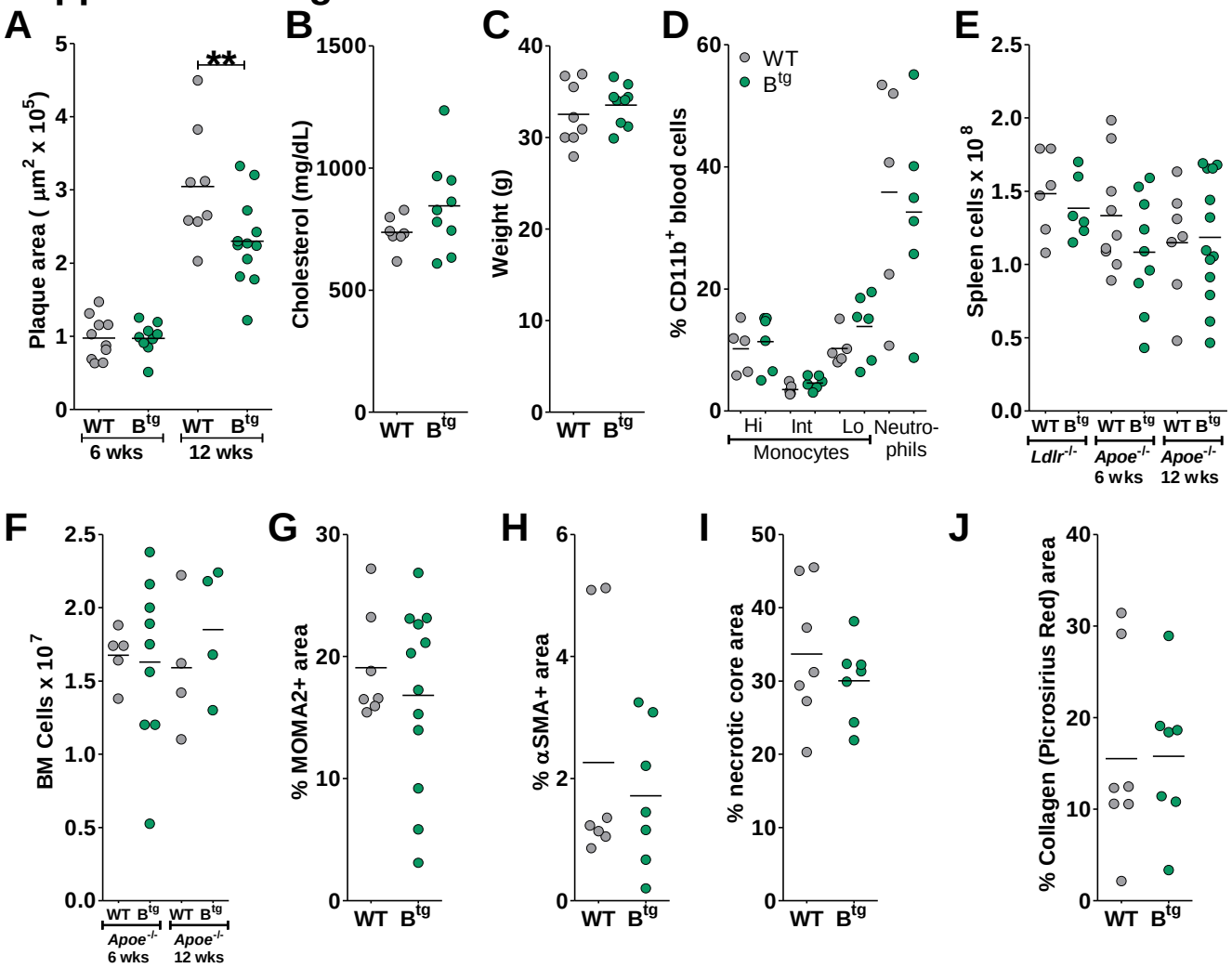
Supplemental Figure I. Gating strategies used in flow cytometry. In some experiments, GC B cells were gated as B220^{+} IgM^{+} CD95^{+} GL7^{+}. B1 cells are additionally CD19^{+} IgM^{+} B220^{lo}.

Suplemental Figure II



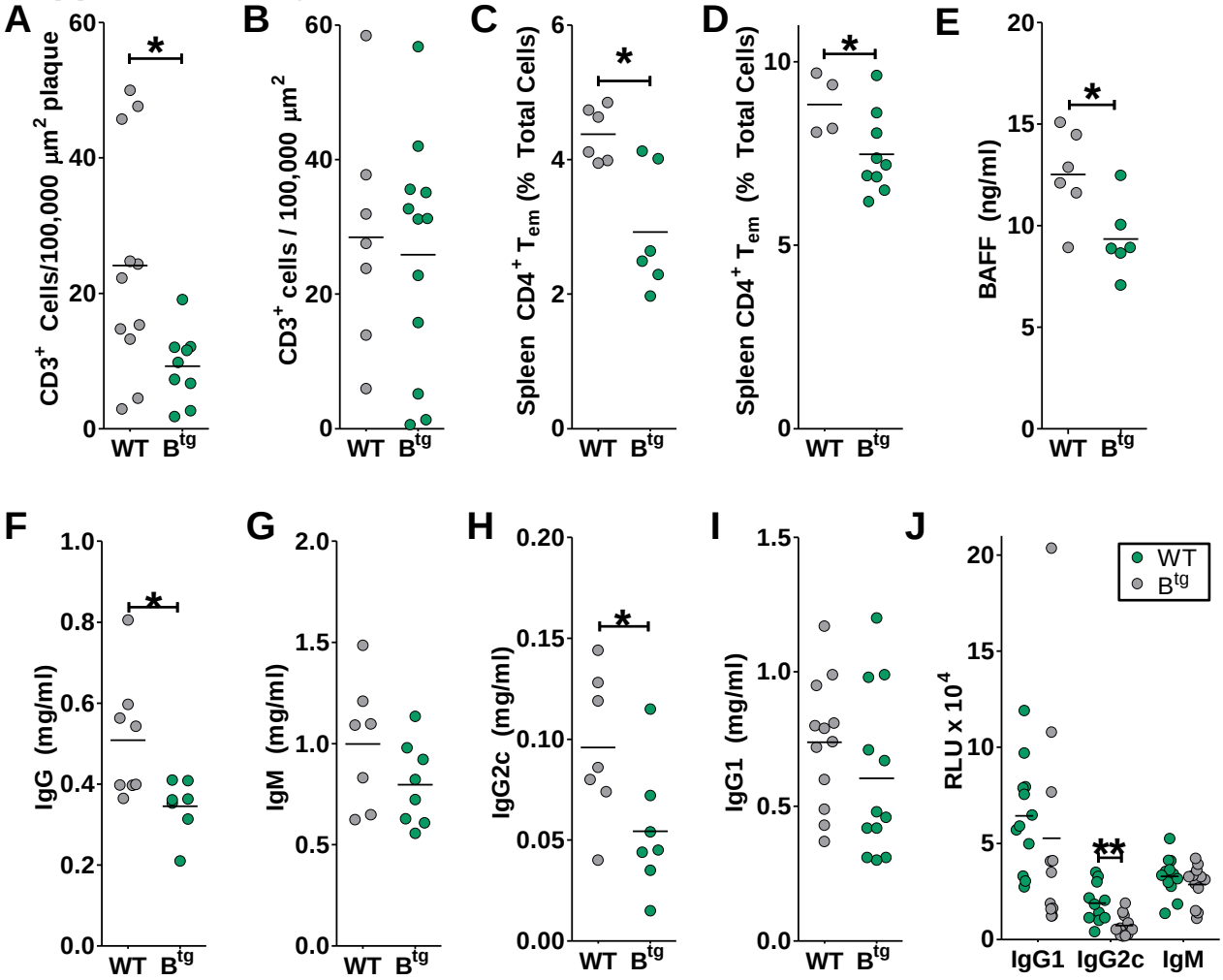
Supplemental Figure II. **A-D.** Analysis of *Ldlr*$^{-/-}$ chimeric mice transplanted with WT control or B^{tg} bone marrow after 6 weeks western diet. **A.** Proportion of B cells expressing high levels of Fas (CD95^{hi} GL7^{-}). **B**. Serum total cholesterol. **C**. Body weight. **D**. Blood monocyte subsets (CD11b^{+} Ly6G^{-} CD115^{+} and Ly6C lo, int or hi) and neutrophils (CD11b^{+} Ly6G^{+}). **E-H**. Analysis of *Ldlr*$^{-/-}$ chimeric mice transplanted with WT control or FcγRIIb-macrophage transgenic (M^{tg}) bone marrow after 6 weeks western diet. **E**. Mean flurorescent intensity for FcγRIIb on B cells and CD11b^{+} cells. **F**. Atherosclerotic plaque area. **G**. Macrophage (MOMA2)-positive area of plaque. **H**. Serum total cholesterol.

Supplemental Figure III



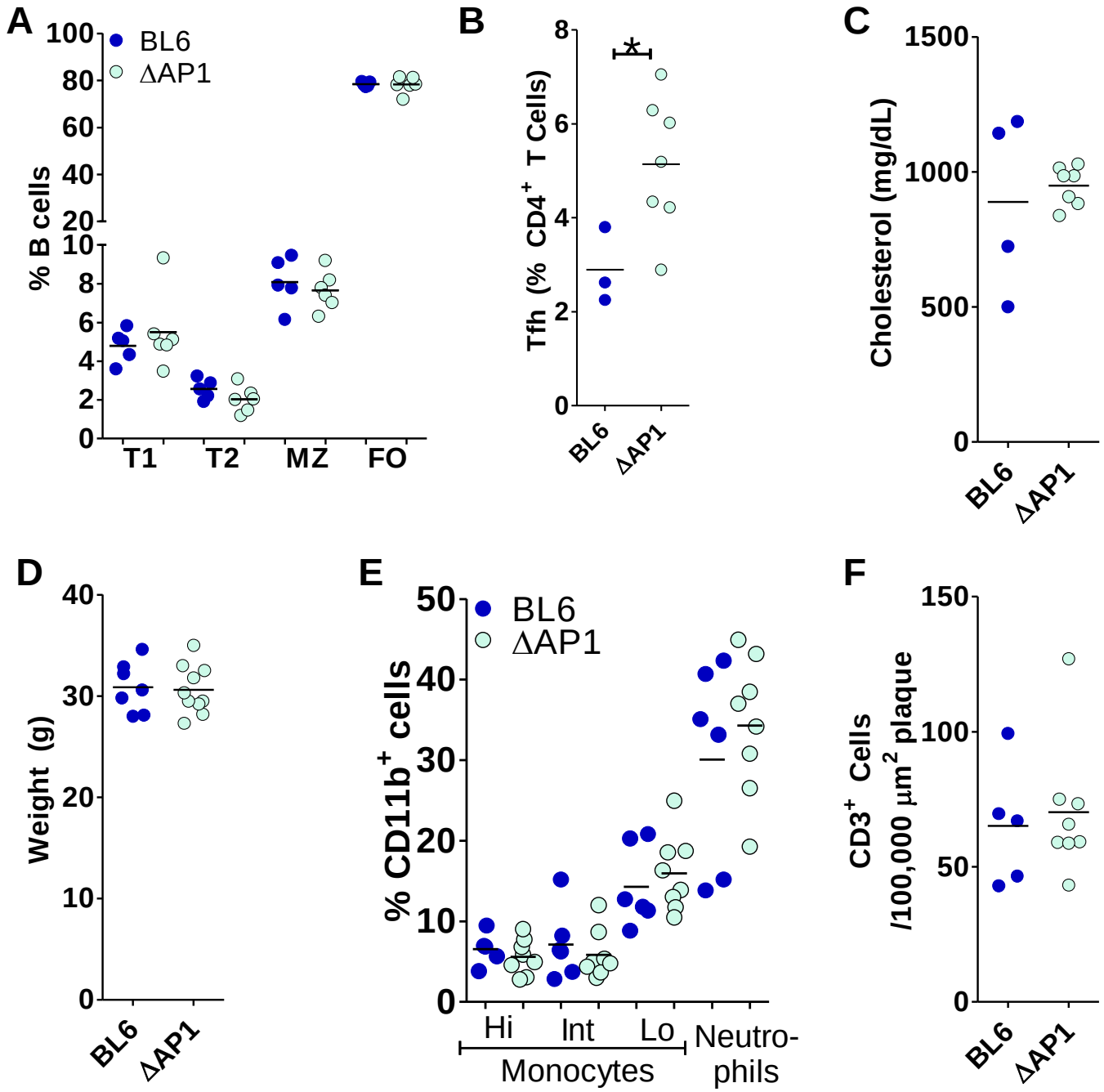
Supplemental Figure III. **A-D**. Analysis of *Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice after 6 or 12 weeks western diet. **A**. Atherosclerosis quantified using Oil Red O-stained aortic root cryosections. **B**. Serum total cholesterol. **C**. Body weight. **D**. Blood monocyte subsets (CD11b^{+} Ly6G^{-} CD115^{+} and Ly6C lo, int or hi) and neutrophils (CD11b^{+} Ly6G^{+}). **E.** Total cell numbers in spleens from each B^{tg} cohort. **F**. Bone marrow cell numbers in *Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice after 6 or 12 weeks western diet. **G-J**. Analysis of atherosclerotic plaques for proportions of positive staining for MOMA2 (G), α-SMA (H), necrotic core (I) or collagen (J) in aortic roots from *Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice after 12 weeks western diet.

Supplemental Figure IV



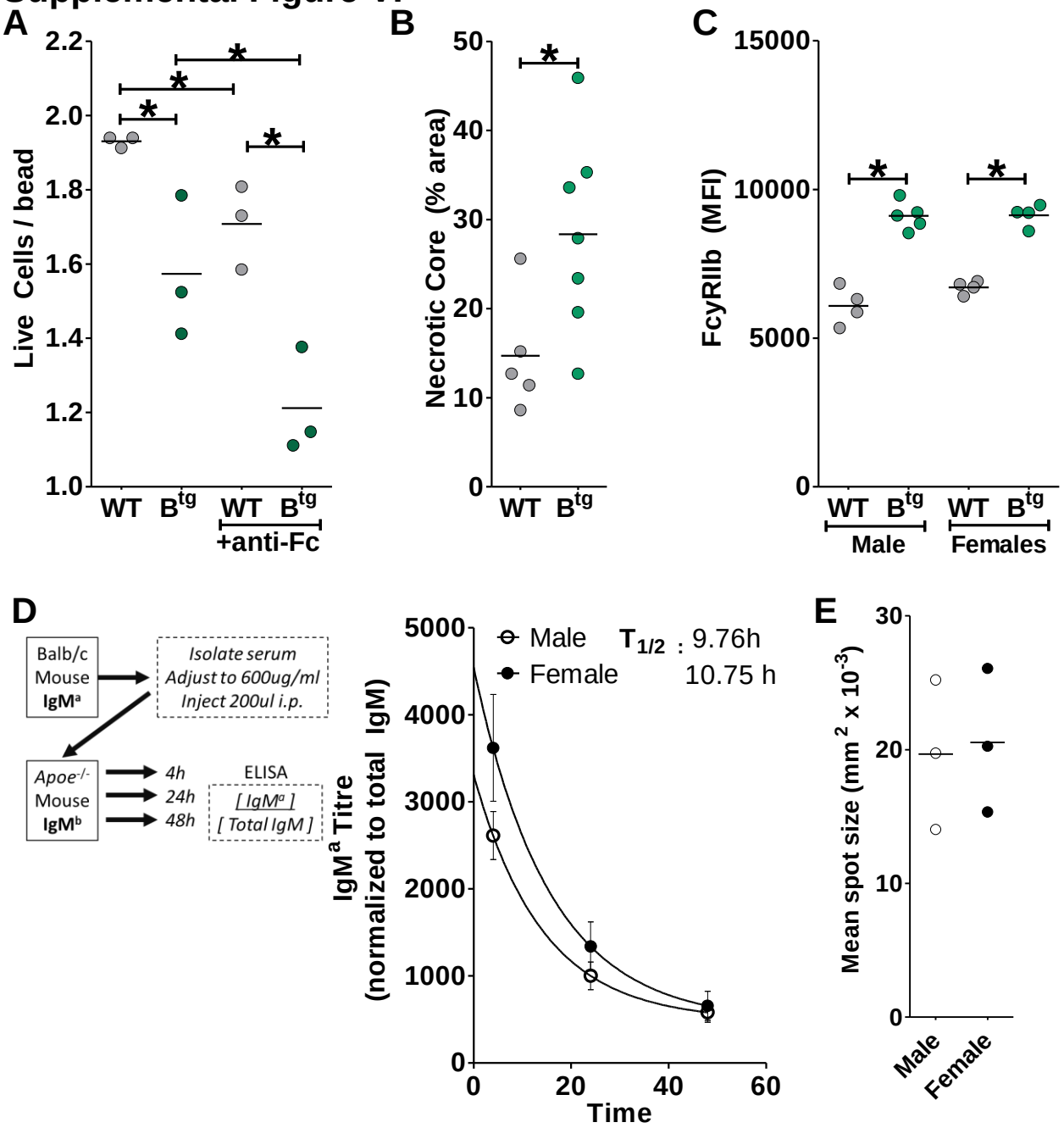
Supplemental Figure IV. **A+B**. Levels of plaque $CD3^+$ T cells in aortic roots from *Ldlr*$^{-/-}$ chimeric mice transplanted with WT control or B^{tg} bone marrow after 6 weeks western diet (A) *or Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice after 12 weeks western diet (B). **C-D**. Proportion of spleen effector memory $CD4^+$ T cells ($CD3^+$ $CD4^+$ $CD62L^-$ $CD44^{hi}$) in *Ldlr*$^{-/-}$ chimeric mice transplanted with WT control or B^{tg} bone marrow after 6 weeks western diet (C) *or Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice after 12 weeks western diet (D). **E**. Serum BAFF levels in *Ldlr*$^{-/-}$ chimeric mice transplanted with WT control or B^{tg} bone marrow after 6 weeks western diet. **F-J**. Serum antibody titres in *Ldlr*$^{-/-}$ chimeric mice transplanted with WT control or B^{tg} bone marrow after 6 weeks western diet.

Supplemental Figure V



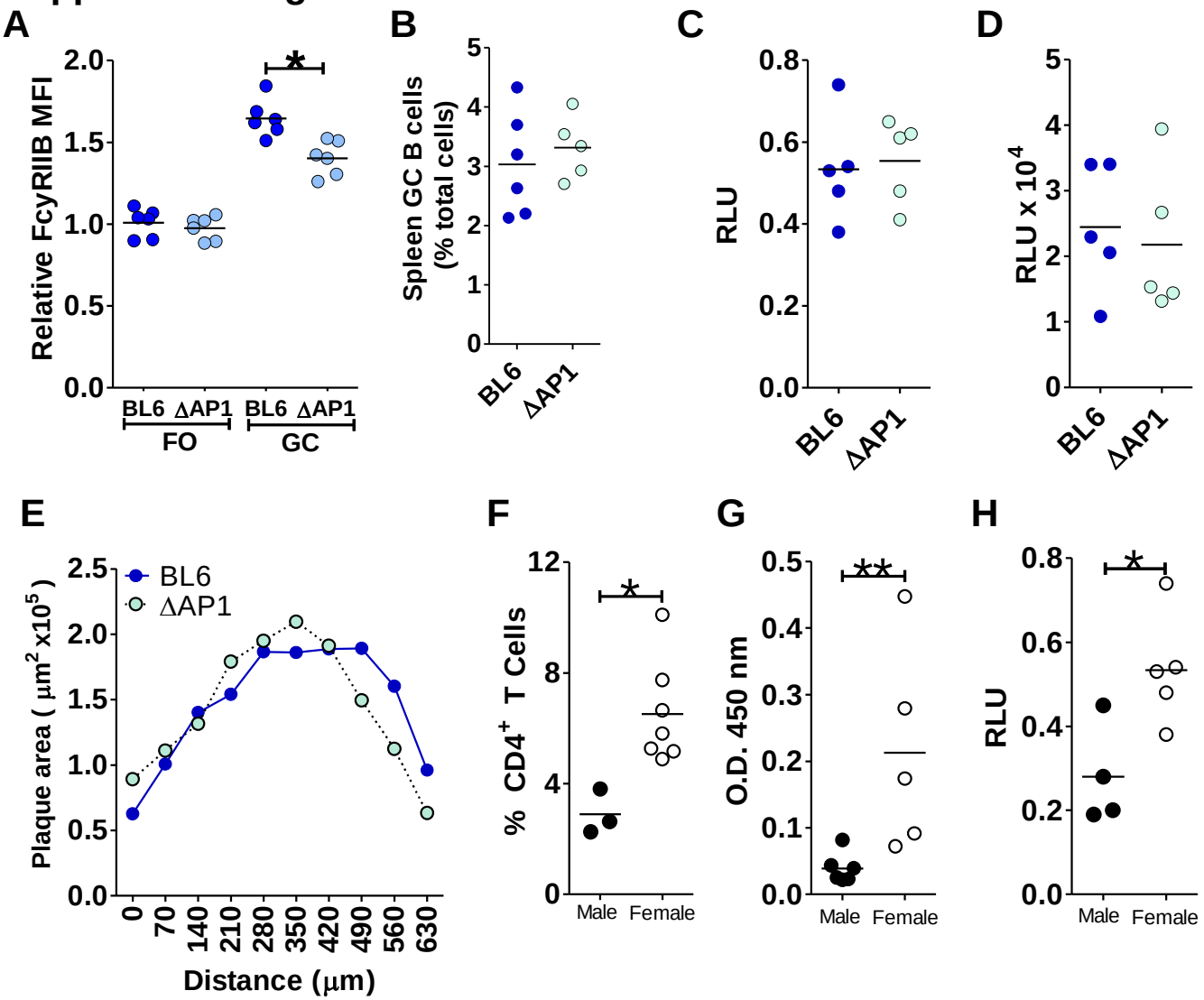
Supplemental Figure V. A-F. Analysis of male *FcγRIIb*^{BL6} *Apoe*$^{-/-}$ (BL6) or *FcγRIIb*$^{\Delta AP-1}$ Apoe$^{-/-}$ (ΔAP-1) mice after 6 weeks western diet. Proportions of spleen B cell subsets (see figure S1). **B**. Follicular helper T cells (CD3^{+} CD4^{+} PD-1^{hi} CXCR5^{hi}). **C**. Body weights. **D**. Serum total cholesterol. **E**. Blood monocytes subsets (CD11b^{+} Ly6G^{-} CD115^{+} and Ly6C lo, int or hi) and neutrophils (CD11b^{+} Ly6G^{+}). **F.** Plaque CD3+ Cells. *p<0.05.

Supplemental Figure VI



Supplemental Figure VI. A. B1 cells purified from peritoneum of *Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice were cultured for 16h with or without anti-FcγRIIB (2.4G2 antibody) then stained with annexin V and propidium iodide. Absolute numbers of remaining live cells (AnnV^{-} PI^{-}) was quantified by flow cytometry using beads for normalization. **B**. Necrotic core area in aortic root plaques from female *Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice after 6 weeks western diet. **C.** FcγRIIb levels on Spleen B1 cells in male and female *Apoe*$^{-/-}$ or B^{tg} *Apoe*$^{-/-}$ mice. **D**. IgM clearance rates determined after transfer of IgM^{a} serum (from Balb/c mice) into male or female *Apoe*$^{-/-}$ mice (each gender received gender matched serum). **E.** Mean spot size of IgM-secreting spleen cells from male and female *Apoe*$^{-/-}$ mice analysed by Elispot. Representative experiment of more than 5.

Supplemental Figure VII



Supplemental Figure VII. A-E. Analysis of *Apoe*$^{-/-}$ or *FcγRIIb*$^{\Delta AP\text{-}1}$ *Apoe*$^{-/-}$ female mice after 6 weeks western diet. **A**. Mean fluorescence intensity (MFI) of anti-FcγRIIb staining on follicular (FO) or germinal center (GC) B cells from *Apoe*$^{-/-}$ or *FcγRIIb*$^{\Delta AP\text{-}1}$ *Apoe*$^{-/-}$ female mice. **B**. Spleen GC B cell levels. **C.** Total serum IgG2c. **D**. Total IgM levels. **E**. Atherosclerotic plaque area. N=6/group. *p<0.05. F-H. Comparison of male and female *Apoe*$^{-/-}$ mice. **F**. Follicular helper T cells. **G**. Anti-dsDNA IgG antiboides. **H**. Total IgG2c.

Supplemental Table I

Target	Clone	Company
Ly6C	7/4	AbD Serotec
Ly-6G	1A8	BD
CD95 (Fas)	Jo2	BD
CD11b	M1/70	Biolegend
CD11c	N418	Biolegend
CD16.2	9E9	Biolegend
CD43	Ly48 gp115	Biolegend
CD44	1M7	Biolegend
CD62L	MEL-14	Biolegend
CD138	281-2	Biolegend
CD3	145-2C11	Biolegend
CD19	6D5	Biolegend
CXCR5	L138D7	Biolegend
PD-1	4B12	Biolegend
CD23	B3B4	Biolegend
CD16/32	93	Biolegend
MHCII	M5/114.15.2	Biolegend
B220	RA3-6B2	Life Tech.
CD4	RM4-5	Life Tech.
IgM	II/41	Life Tech.
GL7	GL7	Life Tech.
CD21	4E3	Life Tech.
CD115	AFS98	Life Tech.
Ki67	SolA15	Life Tech.
CD64	REA286	Miltenyi

Supplemental Table II - Summary of effects of B cell FcγRIIB models on major parameters

FcγRIIb mutation	**FcγRIIB^{Btg}** *(increased FcγRIIB on B cells)*			**FcγRIIB$^{\Delta AP\text{-}1}$** *(decreased FcγRIIb on GC B Cells)*	
Model	***Ldlr$^{-/-}$***	***Apoe$^{-/-}$***		***Apoe$^{-/-}$***	
Gender	**Male**	**Male**	**Female**	**Male**	**Female**
Parameter:					
Plasma Cells	n.d.	↓	↓*	↔	↔*
GC B Cells	n.d.	↓	↓*	↑	↔
B1 Cells	↔*	↔	↓	↔	↔*
IgG2c	↓	↓	↓*	↑	↔
IgG1	↓	↓	↓*	↔	↔*
IgM	↔	↔	↓	↑	↔*
Teff	↓	↓	↓*	↔	↔*
Atherosclerosis	↓	↓	↑	↑	↔
**Data not shown.*					