SUPPLEMENTAL MATERIAL

Supplementary figure I. Germinal center formation following loss of APOE in mice maintained on a chow diet.

(A) A full depiction of the western blot shown in Fig1. Control and experimental mice (10 to 12 weeks of age) were administered an oral dose of tamoxifen and plasma levels of APOE protein were determined by Western blot. Transferrin (TRF) protein was used as a loading control.

(B) Plasma levels of APOE protein in control and experimental mice and *Apoe*$^{-/-}$ mice (20 weeks of age) maintained on a chow diet as determined by ELISA. N=3-5.

(C) Representative FACS analysis depicting germinal center B cell formation in the spleen of mice 21 days after administration of tamoxifen. Quantification of the indicated B cell subsets is shown on the right. N=16 for controls, n=13-14 for experimental mice.

(D) Confocal microscopy of germinal centers at day 21 following tamoxifen administration. Cryosections of spleens corresponding to the indicated genotypes were stained with eFluor488-labeled GL7 antibody (green) and APC-labelled IgD antibody (purple).

All univariate scatter plots are ± SEM. Significance determined by student t-test.

(E) Flow cytometry analysis (left panel) and absolute cell numbers (right panel) for splenic B cell populations 140 days after tamoxifen administration. B1 cells (CD19+B220lo), B2 cells (CD19+B220+), Marginal Zone (MZ) B cells (CD19+B220+CD21HiCD23lo), Follicular B cells (CD19+B220+CD21loCD23Hi) and germinal center (GC) B cells (B220+CD19+IgD-CD95+GL7+) are gated as indicated. Control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ mice are white bars (n= 12) and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are in blue (n= 11-17 for both genotypes, mixed genders).

All univariate scatter plots are ± SEM. Significance determined by student t-test. *$p < 0.05$, **$p < 0.01$ and ****$p < 0.0001$ compared to control.

Supplementary figure II. No evidence for a general expansion of B cells upon acute loss of APOE.

(A) Flow cytometry analysis of lymph nodes at day 10 following tamoxifen administration in mice of both genders maintained on chow diet. Left top panel shows mesenteric lymph nodes and bottom left panel inguinal. The relative percentages of germinal center (GC) cells are shown. The right panel shows the absolute cell numbers for B2 cells (B220+CD19+) and GC cells (B220+CD19+IgD-CD95+GL7+). Control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ mice are white bars (n= 10) and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are in blue (n= 7).

(B) Analysis of the peritoneal cavity B cells in control and experimental mice at day 10. The panel on the left shows representative FACS plots and the absolute cell numbers are indicted on the right for B1 (CD19+B220lo), B2 (CD19+B220+), B1a (CD19+B220loCD5+) and B1b (CD19+B220loCD5-). Control mice n=4 and experimental mice n=5.

(C) Flow cytometry analysis of bone marrow B-cell differentiation in control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice aged 12 to 14 weeks and administered tamoxifen 10 days earlier. Cells were gated to calculate absolute cell numbers for B1 (CD19+B220lo), B2 (CD19+B220+), immature B (CD19+B220+IgM+IgD-), recirculating (CD19+B220+IgM+IgD+), pro-B (CD19+B220+ IgM−IgD-c-Kit+CD25−) and pre-B (CD19+B220+ IgM−IgD-c-Kit-CD25+) populations as shown in the graphs on the right. Percentages in each gated quadrant are indicated on the left. Control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ mice are white bars (n= 8) and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are in blue (n= 7-9).

All univariate scatter plots are ± SEM. Lack of significance determined by student t-test.

Supplementary figure III. No evidence for a general expansion of T cells upon acute loss of APOE.

(A) Flow cytometry analysis of spleens 10 days after tamoxifen administration in mice of mixed genders maintained on a chow diet. Left top panel shows CD4 and CD8 profile on CD3+ gated cells. Left bottom panel indicates CD44 and CD62L percentages on CD3+CD4+ gated spleen cells. Quantification of total spleen cell numbers is shown in the graphs in the right panel for CD4 (CD3+CD4+) CD8 (CD3+CD8+), CD4 Naïve (CD3+CD4+CD62L+CD44-) and CD4 effector/memory (CD3+CD4+CD62L-CD44+) cells. Control $Apoe^{fl/+}$ $ROSA26^{CreERt2/+}$ mice are white bars (n=7) and experimental $Apoe^{fl/-}$ $ROSA26^{CreERt2/+}$ mice are in blue (n= 9).

(B) Spleen analysis at 21 days following tamoxifen administration. Control mice n=15, experimental mice n=14, mixed genders.

(C) Analysis of T cells populations in the spleen 140 days after tamoxifen administration. Control n=10-12 and experimental n=17.

All univariate scatter plots are ± SEM. Significance determined by student t-test. *$p < 0.05$, **$p < 0.01$ compared to control.

Supplementary figure IV. No activation of spleen myeloid populations during the transition to hyperlipidemia.

(A) Left panel shows FACS analysis of macrophages (MF), inflammatory monocytes (IM) and patrolling monocytes (PM) as determined by F4/80 and Ly6C expression on lineage (lin)-CD11c-Ly6G-CD11b+ gated cells at day10 post-tamoxifen. Numbers indicate percentage of cells in quadrants. Right panel indicates absolute cell numbers for neutrophils (lin-CD11b+Ly6G+), CD11b+CD11c+ cells, eosinophils (Lin-CD11b+CD11c-Ly6G-SSChi), inflammatory monocytes (Lin-CD11b+CD11c-Ly6G- SSClo F4/80-Ly6Chi), macrophages (Lin-CD11b+CD11c-Ly6G- SSClo F4/80+Ly6C+) and patrolling macrophages (Lin-CD11b+CD11c-Ly6G- SSClo F4/80+Ly6C-). Control $Apoe^{fl/+}$ $ROSA26^{CreERt2/+}$ mice are white bars (n= 8) and experimental $Apoe^{fl/-}$ $ROSA26^{CreERt2/+}$ mice are in blue (n= 9).

(B) Nile Red and CD11c (green) staining of aortas (luminal side) at day 10 and 140 after tamoxifen administration in mice maintained on chow diet. Nuclear staining was performed with DAPI (blue).

(C) FACS analysis of digested aortas to determine the presence of CD4+ and CD19+ lymphocytes. Control $Apoe^{fl/+}$ $ROSA26^{CreERt2/+}$ mice are white bars (n= 7) and experimental $Apoe^{fl/-}$ $ROSA26^{CreERt2/+}$ mice are in blue (n= 6).

All univariate scatter plots are ± SEM. Lack of significance determined by student t-test.

Supplementary figure V. Formation of lymph node germinal centers following loss of APOE in mice maintained on a western diet.

(A) Representative FACS analysis (left panel) depicting germinal center B cell formation in the spleen of mice 10 days after administration of tamoxifen and maintained on a western diet. Absolute cell numbers are shown on the right. Control $Apoe^{fl/+}$ $ROSA26^{CreERt2/+}$ mice are white bars (n=12-14) and experimental $Apoe^{fl/-}$ $ROSA26^{CreERt2/+}$ mice are in blue (n=10-11).

(B) Flow cytometry analysis of aortic lymph nodes at day 70 following tamoxifen administration in mice maintained on a western diet. The relative percentage of germinal center (GC) cells is shown. The right panel shows the absolute cell numbers for GC cells (B220+CD19+IgD-CD95+GL7+). Control $Apoe^{fl/+}$ n=7 and experimental n=9.

All univariate scatter plots are ± SEM. Significance determined by Mann-Whitney test. *$p < 0.05$.

(C) Flow cytometry analysis of inguinal and mesenteric lymph nodes at day 70 following tamoxifen administration in mice maintained on western diet. The relative percentage of germinal center (GC) cells is shown. The right panel shows the absolute cell numbers for GC cells (B220+CD19+IgD-CD95+GL7+). Control *Apoe*$^{fl/+}$ n=5-7 and experimental n= 8.
All univariate scatter plots are ± SEM. Significance determined by student t-test. *p < 0.05.

Supplementary figure VI. Bone marrow HSC and B cell progenitor activation at day 70 following tamoxifen administration and maintained on a western diet.
(A) Flow cytometry analysis of hematopoietic stem cell populations and multi-potential progenitors in control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice aged 12 to 14 weeks administered tamoxifen 10 weeks earlier. Control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ mice are white bars (n= 7) and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are in blue (n= 6-8).
(B) Flow cytometry analysis of bone marrow B-cell differentiation in control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice aged 12 to 14 weeks administered tamoxifen 10 weeks previously. Cells were gated to calculate absolute cell numbers for B1 (CD19+B220lo), B2 (CD19+B220+), immature B (CD19+B220+IgM+IgD-), recirculating (CD19+B220+IgM+IgD+), pro-B (CD19+B220+ IgM−IgD-c-Kit+CD25−) and pre-B (CD19+B220+ IgM−IgD-c-Kit-CD25+) populations as shown in the graphs on the right. Percentages in each gated quadrant are indicated on the left. Control (n=7-8) are shown as white bars and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are in blue (n= 9).
All univariate scatter plots are ± SEM. Significance determined by student t-test. *p < 0.05, **p < 0.01 compared to control.

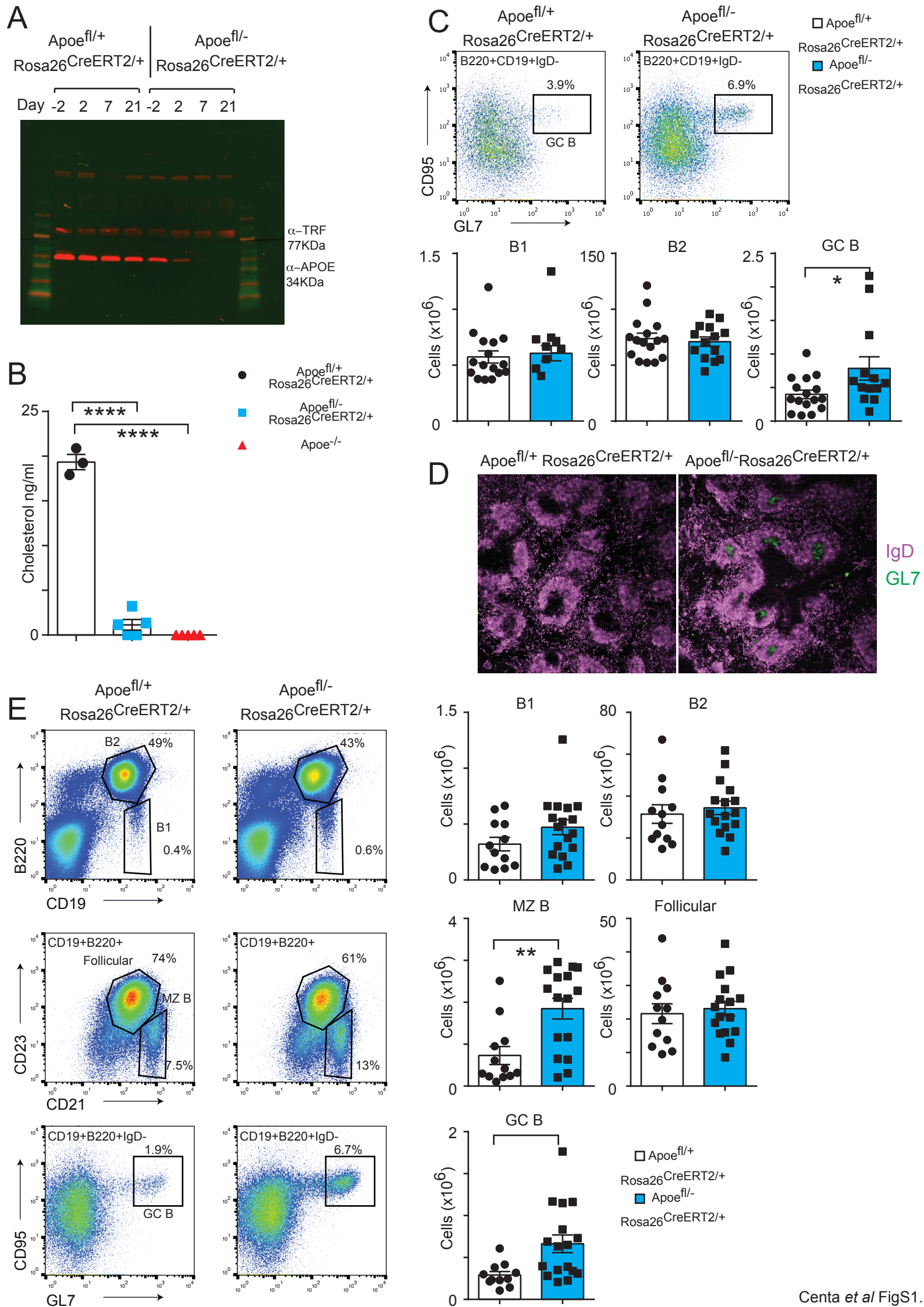
Supplementary figure VII. Evaluation of the acute immune response in the spleen of different models of atherosclerosis.
(A) Splenic germinal centre response determined by flow cytometry. Control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ mice are white bars (n= 9), experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are blue bars (n=17), *Apoe*$^{-/-}$ mice are red bars (n=5) and *Ldlr*$^{-/-}$ mice are in grey bars (n=4). The relative percentage of germinal center (GC) cells is shown in the top panel. The bottom panel shows the absolute cell numbers for GC cells (B220+CD19+IgD-CD95+GL7+), B1 cells (CD19+B220lo) and B2 cells (CD19+B220+). Tamoxifen was administered to *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ and *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ and then all the four strains, 10-12 weeks of ages of mixed genders were maintained on a western diet for a further 14 days.
(B) Splenic T cell response determined by flow cytometry. Control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ mice are white bars (n= 9), experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are blue bars (n=17), *Apoe*$^{-/-}$ mice are red bars (n=5) and *Ldlr*$^{-/-}$ mice are in grey bars (n=4). The top panel indicates CD44 and CD62L percentages on CD3+CD4+ gated spleen cells. Quantification of total spleen cell numbers is shown in the graphs in the bottom panel for CD4 (CD3+CD4+) CD8 (CD3+CD8+), CD4 Naïve (CD3+CD4+CD62L+CD44-) and CD4 effector/memory (CD3+CD4+CD62L-CD44+) cells. Tamoxifen was administered to *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ and *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ and then all the four strains, 10-12 weeks of ages of mixed genders were maintained on a western diet for a further 14 days. All univariate scatter plots are ± SEM. Significance determined by one-way ANOVA. *p < 0.05, **p < 0.01 compared to control.

Supplementary figure VIII. The kinetics of humoral immunity to modified LDL epitopes.

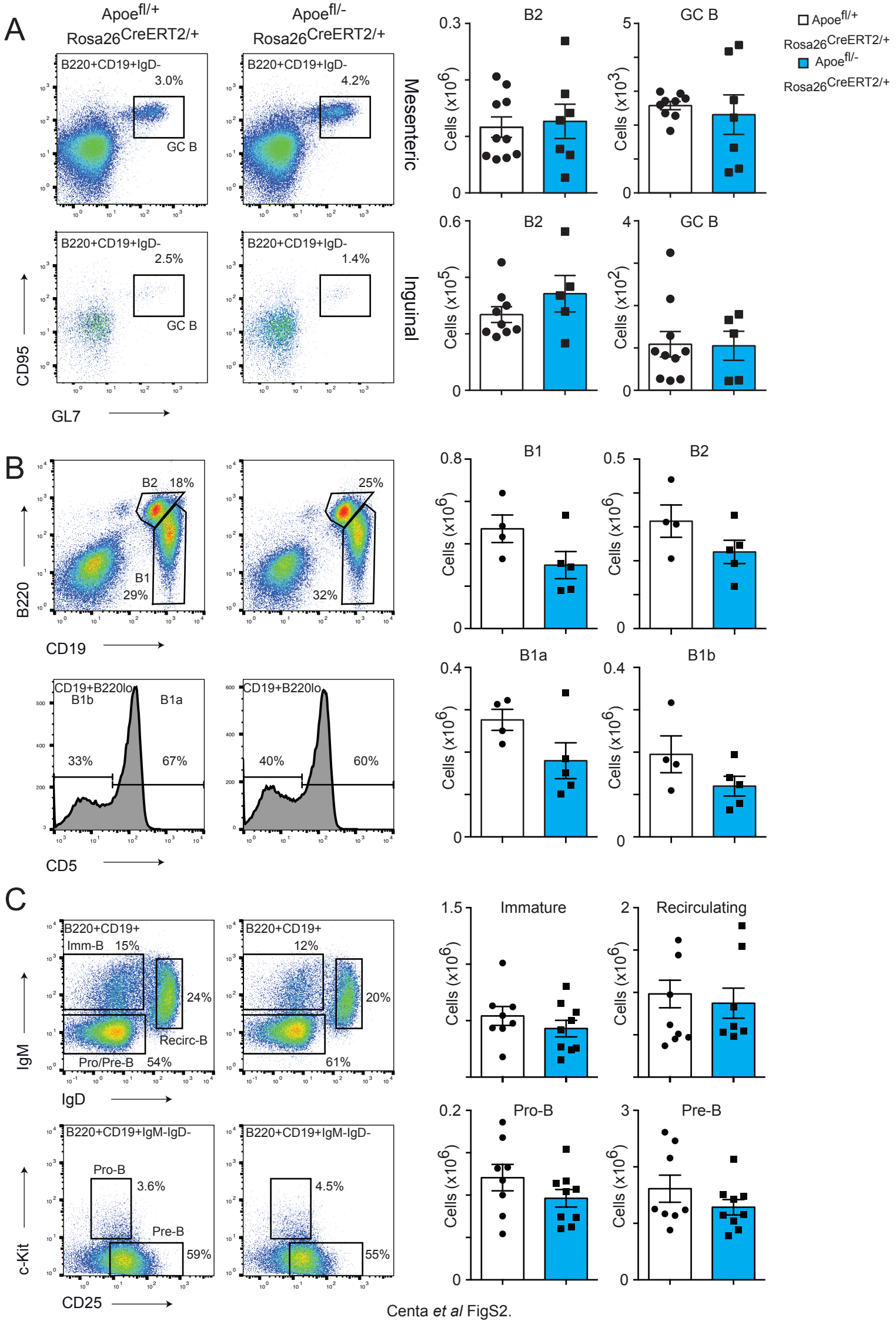
(A) Appearance of T15-id IgM antibodies 10 to 140 days post-induction of APOE deletion, and mice bled prior to tamoxifen administration (pre-bleed) assessed by ELISA on plasma. Control *Apoe*$^{fl/+}$ *ROSA26* $^{CreERt2/+}$ mice are white bars (n= 3-15) and experimental *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ mice are in blue (n= 3-13).
(B) Production of IgM antibodies and (C) appearance of IgG antibodies against copper oxidised (top panel) and MDA modified LDL (bottom panel). *$p < 0.05$ compared to control mice. Values are plotted as relative light units/100ms.
All univariate scatter plots are ± SEM. Significance determined by student t-test. *$p < 0.05$ compared to control.

Supplementary figure IX. A redundant function for the spleen in protecting against atherosclerosis.
(A) Flow cytometry and quantification of B cell populations in the peritoneal cavity of *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/-}$ mice either sham-operated (blue bars n=11-12) or splenectomised (yellow bars n=8-13) and maintained on western diet for 70 days following tamoxifen administration. B2 cells are defined as CD19+B220+, B1 cells CD19+B220lo, B1a CD19+B220loCD5+ and B1b as CD19+B220loCD5-.
(B) Plasma antibody titres in sham operated (blue bars n=10) and splenectomised (yellow bars n=10) mice.
(C) Plasma cholesterol levels in sham-operated (blue bars n=12) and splenectomised (yellow bars n=12) mice.
(D) Representative figures of Oil Red O stained aortic root sections of male *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/-}$ mice either sham-operated (blue) or splenectomised (yellow). Quantification is shown in the right panel. Significance determined by 2-way ANOVA. Percentage difference between the two genotypes was calculated by area under the curve measurements.
All univariate scatter plots are ± SEM. Significance determined by student t-test unless otherwise stated. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control mice.

Supplementary figure X. Analysis of T cell subsets in germinal center deficient mice
(A) Flow cytometry analysis of the splenic T cell compartment following bone marrow transplant of *Pax5*$^{fl/+}$ *Aicda-Cre Apoe*$^{-/-}$ (blue bars n=10) or *Pax5*$^{fl/-}$ *Aicda-Cre Apoe*$^{-/-}$ (grey bars n=11) donors into *Apoe*$^{fl/-}$ *ROSA26* $^{CreERt2/+}$ hosts. Analysis was performed at day 70 following tamoxifen administration in mice were maintained on a western diet. Absolute cell numbers for the indicated T cell subsets are depicted.



Centa *et al* FigS1.



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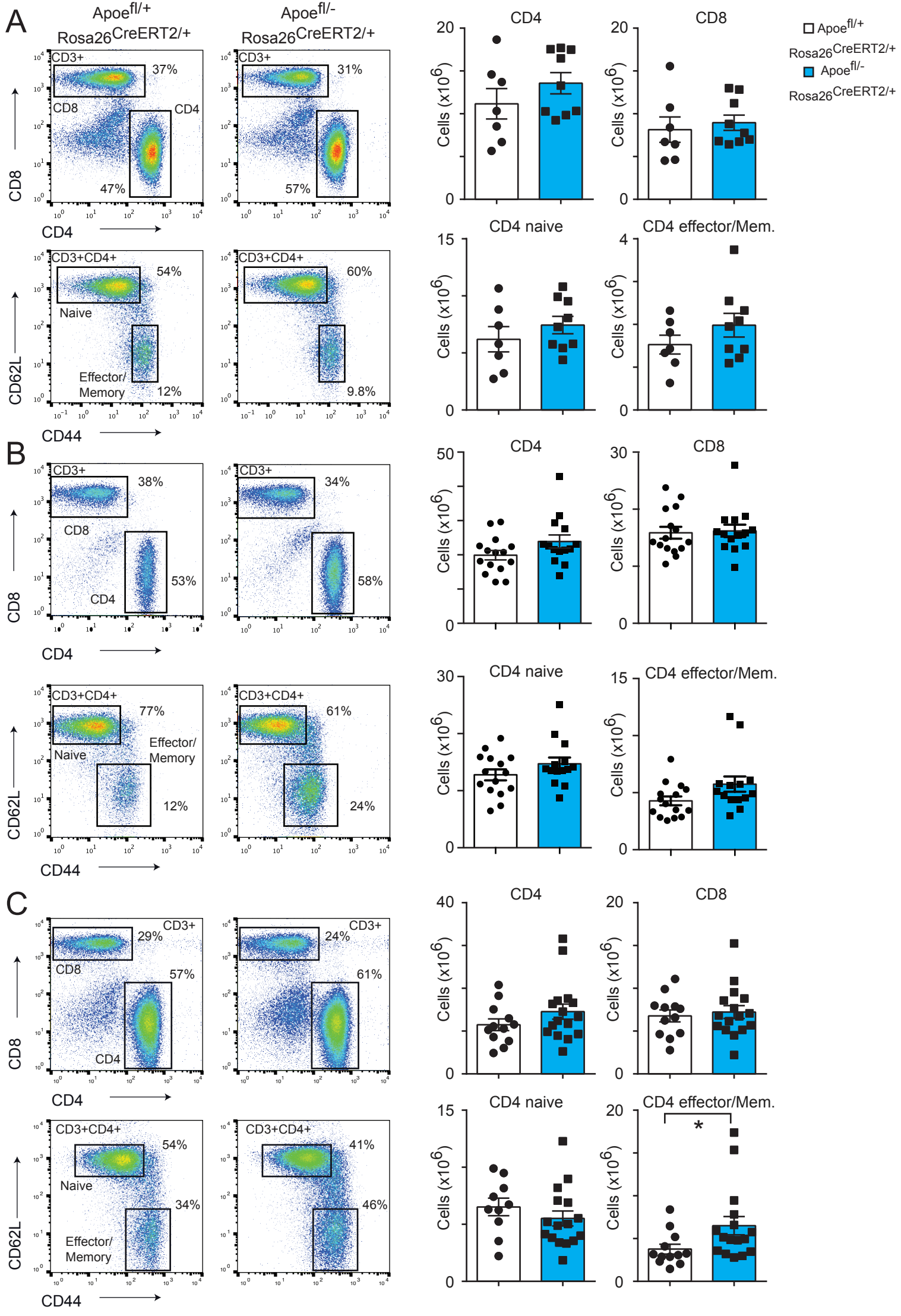

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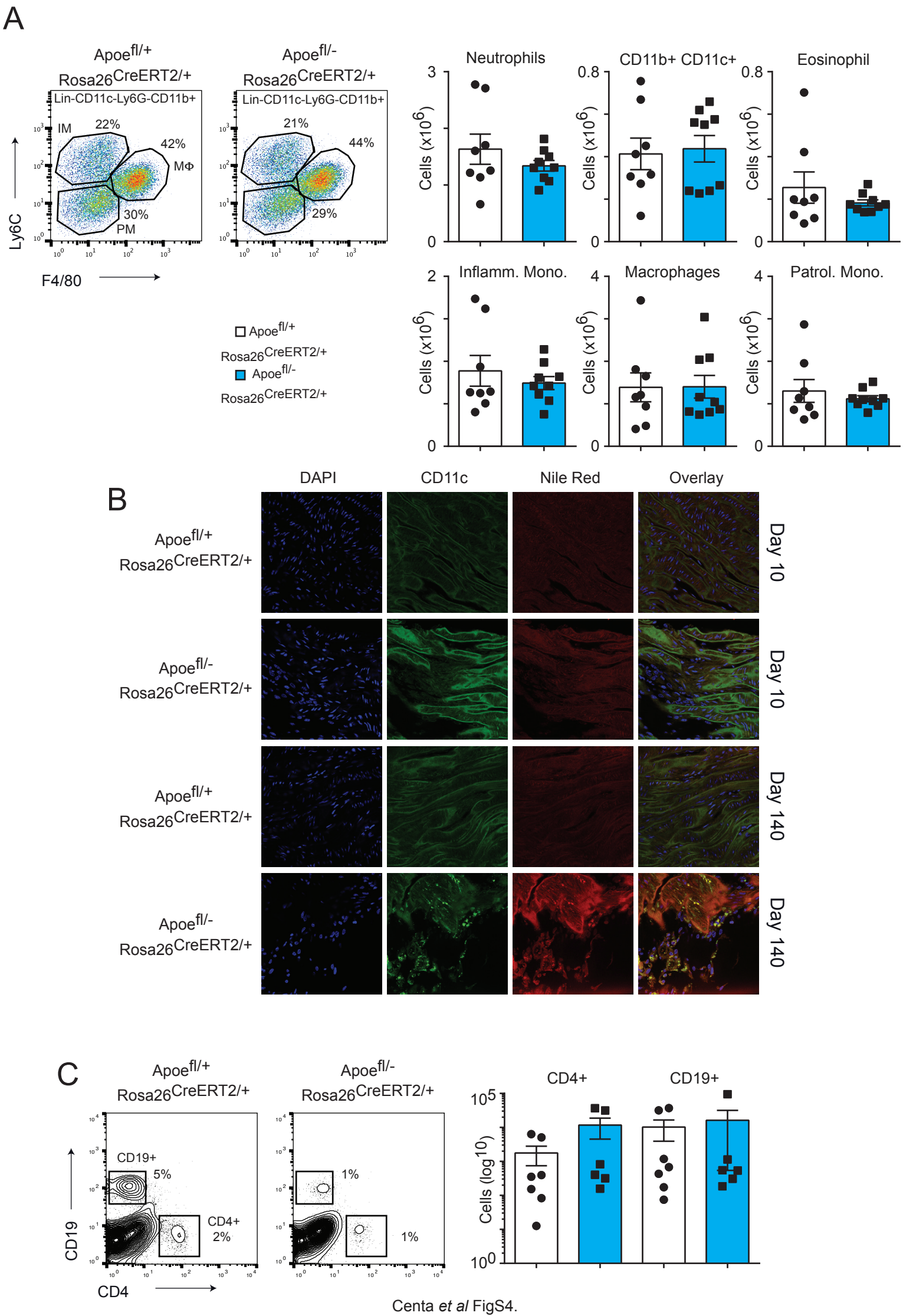

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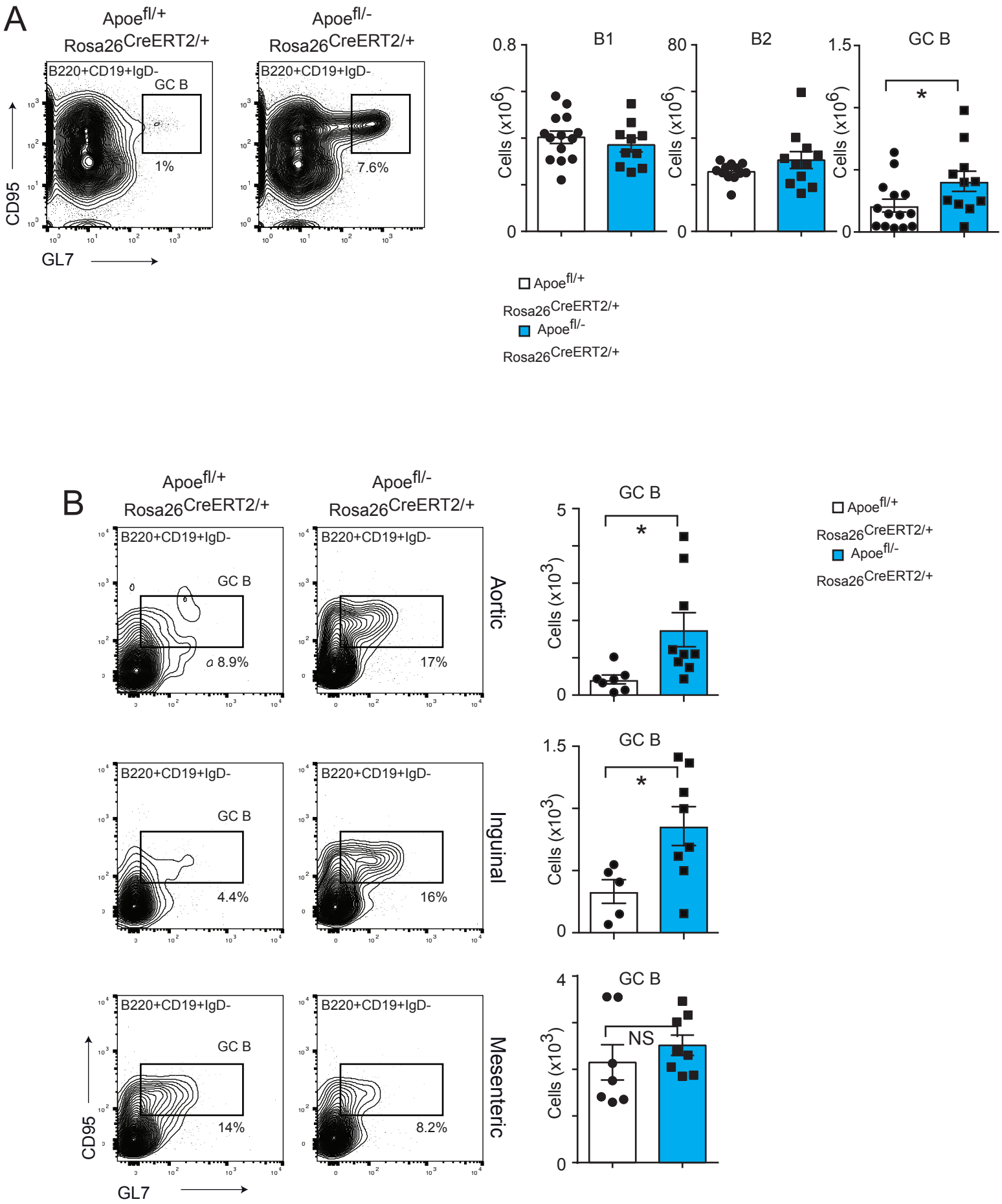

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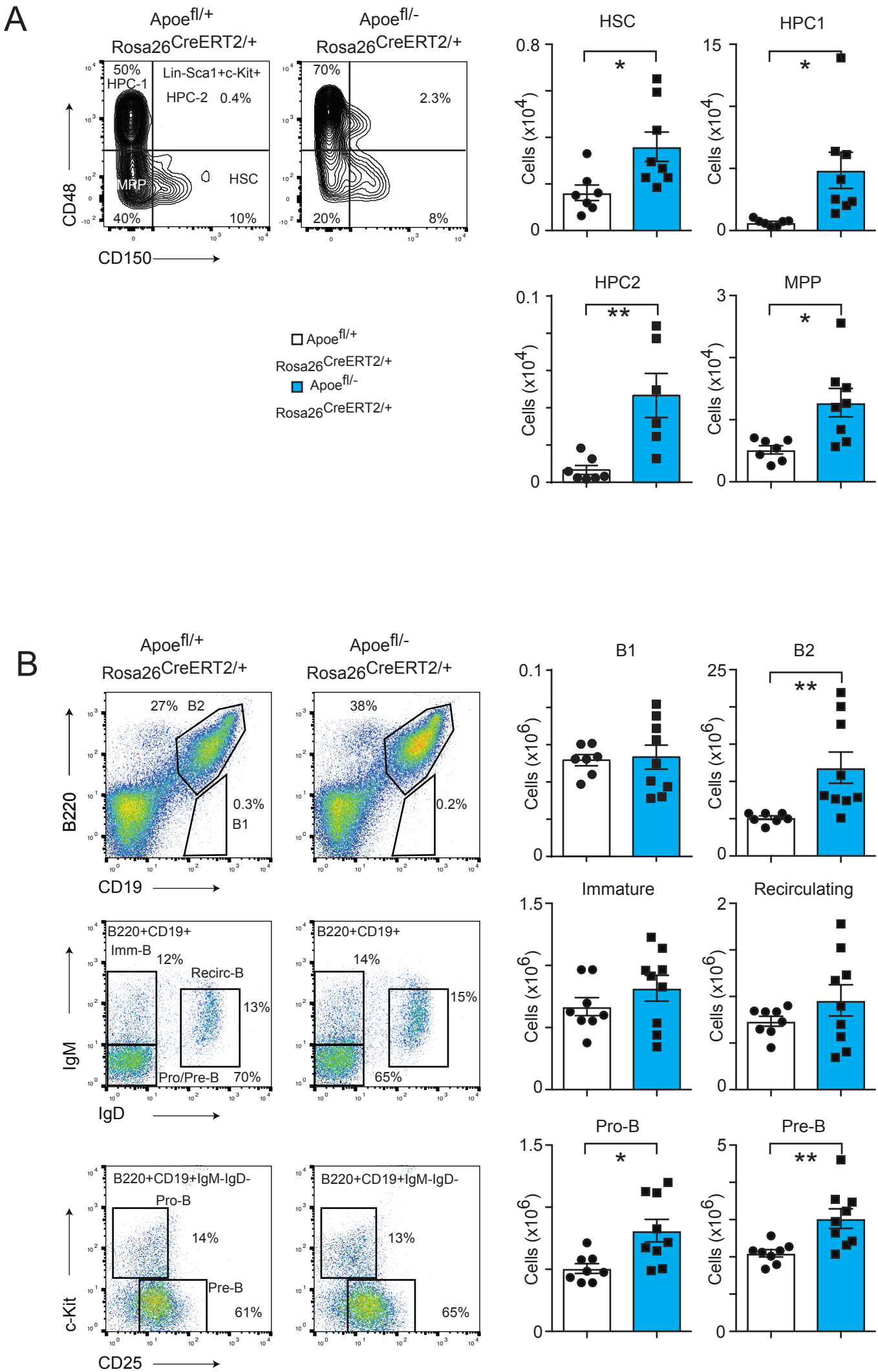

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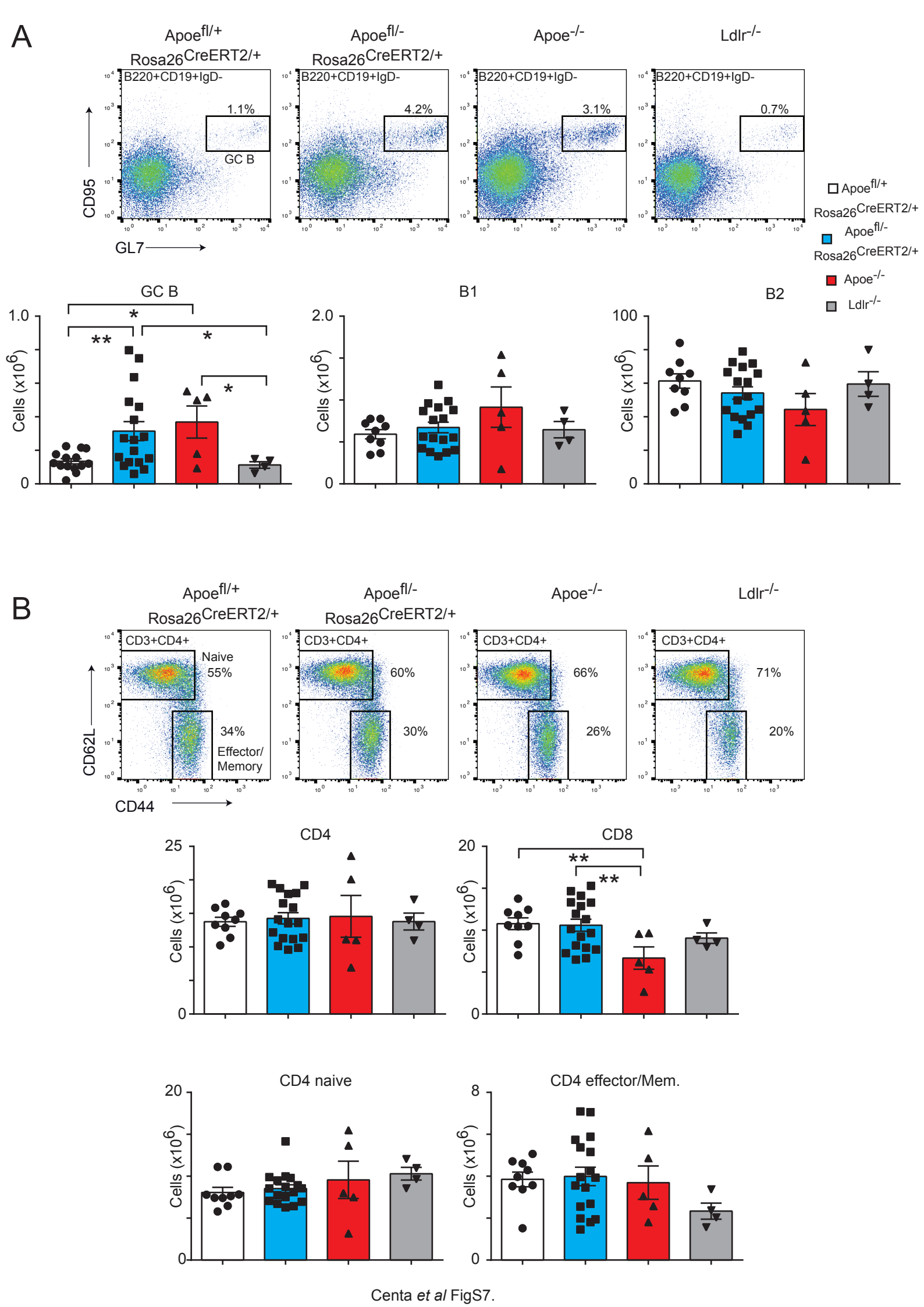
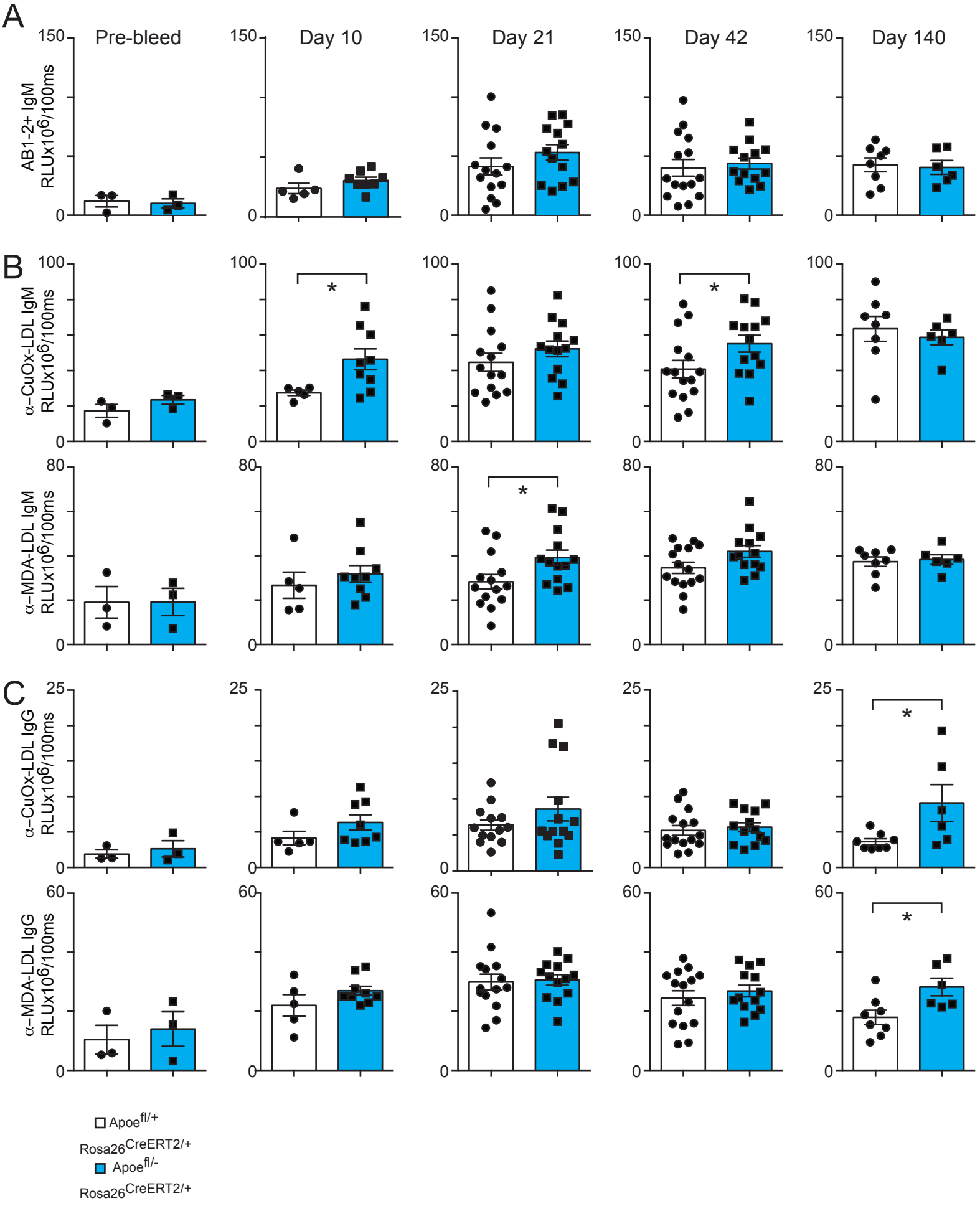


Centa *et al* FigS7.



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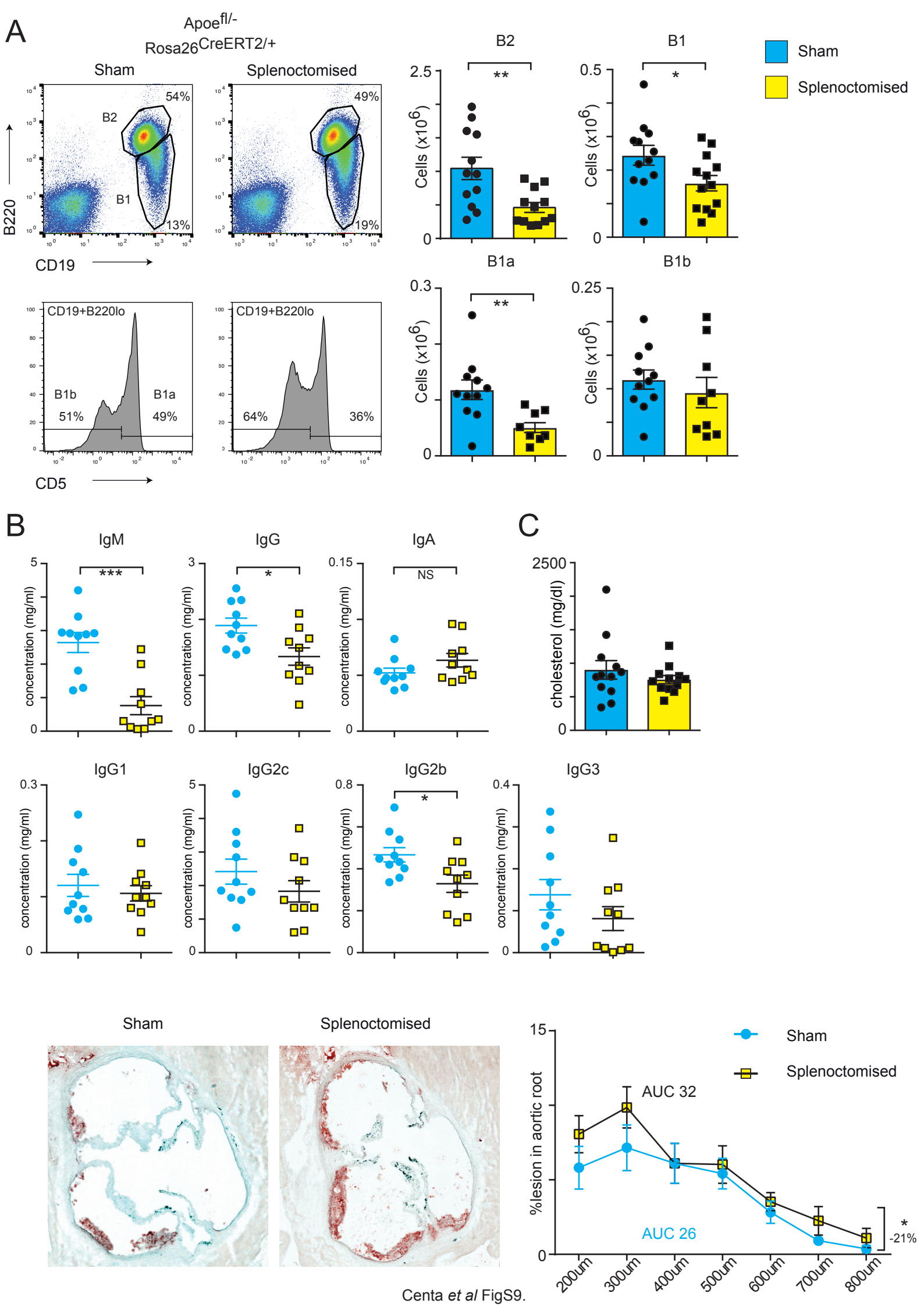

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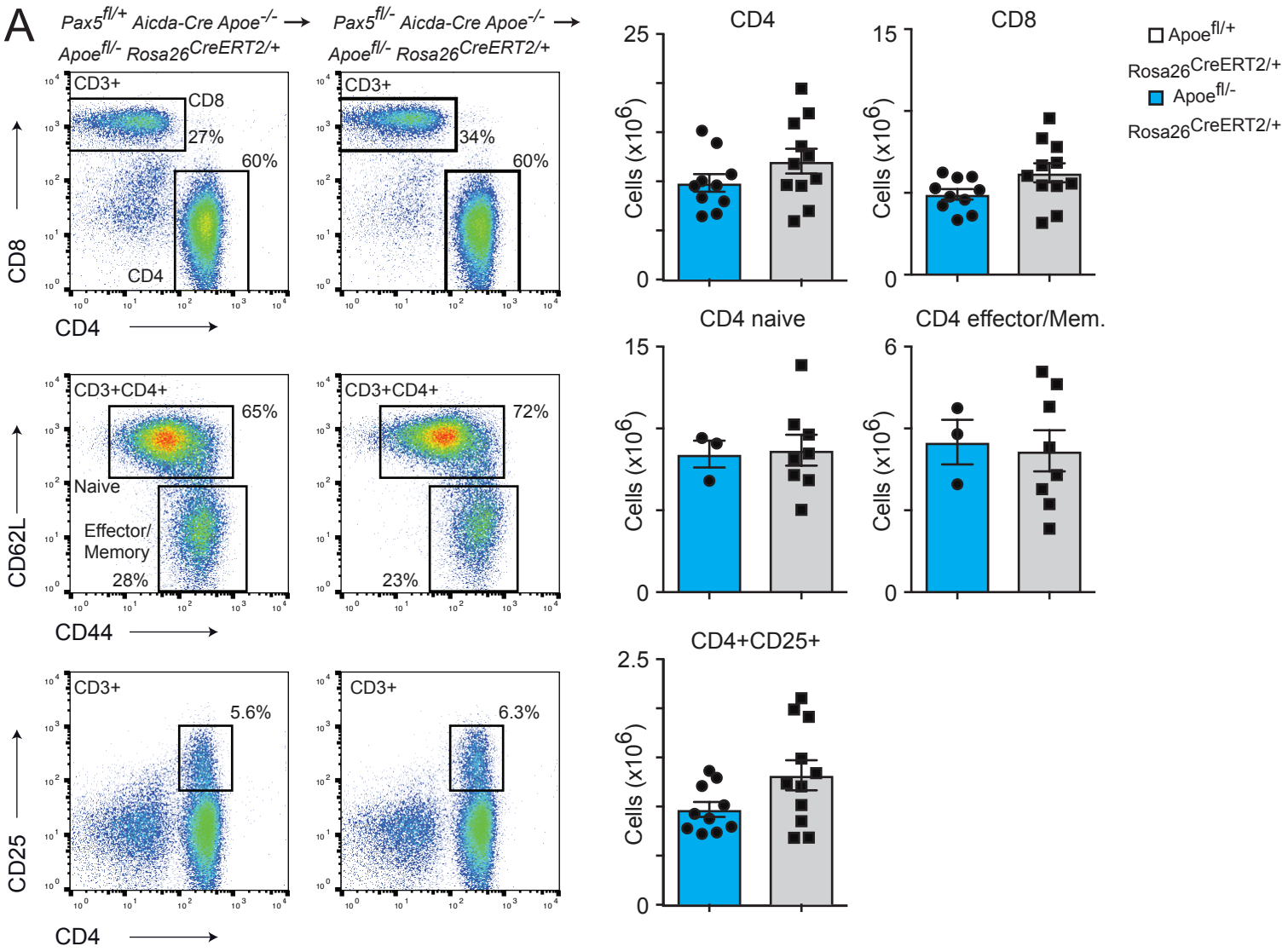

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