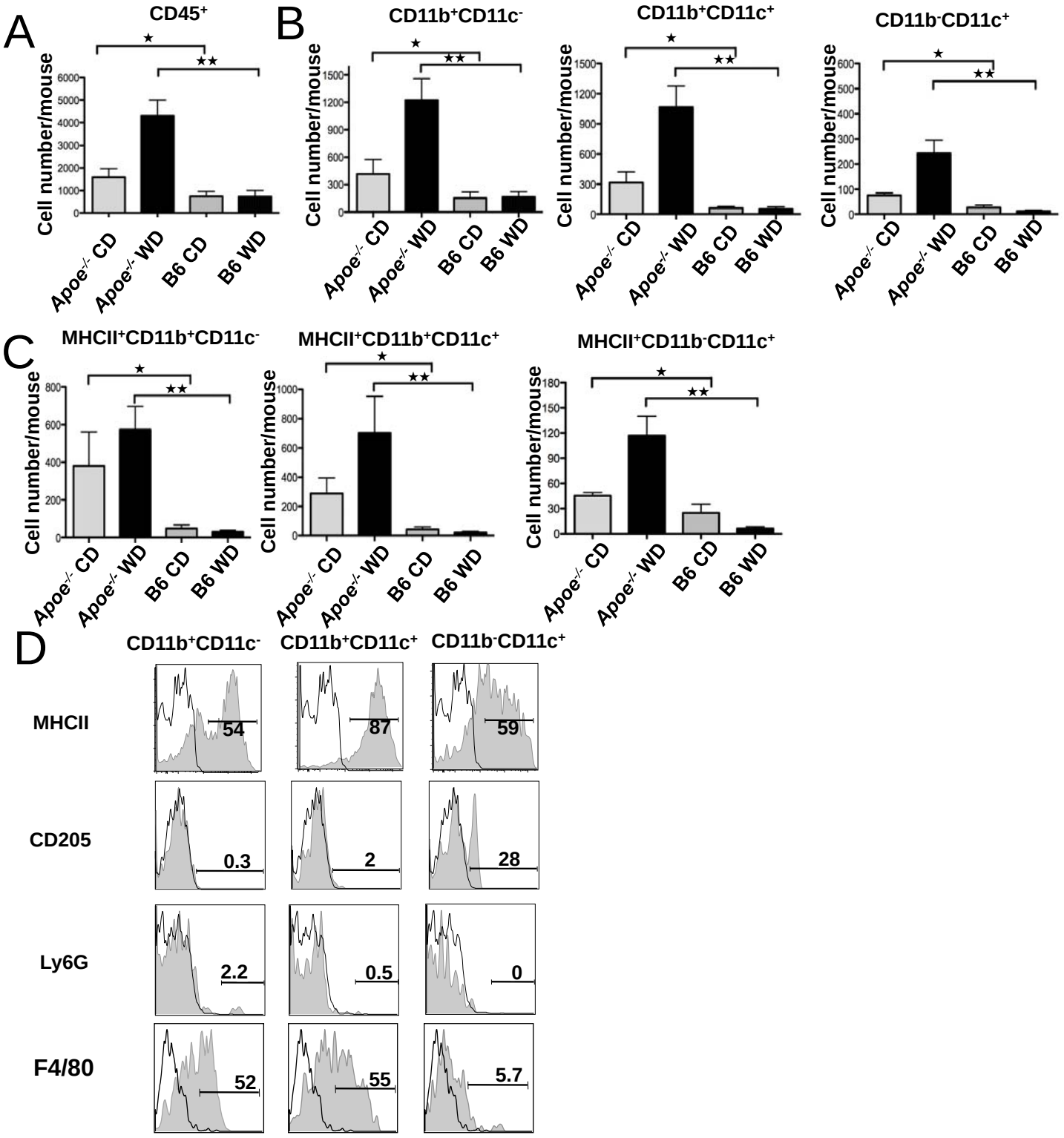
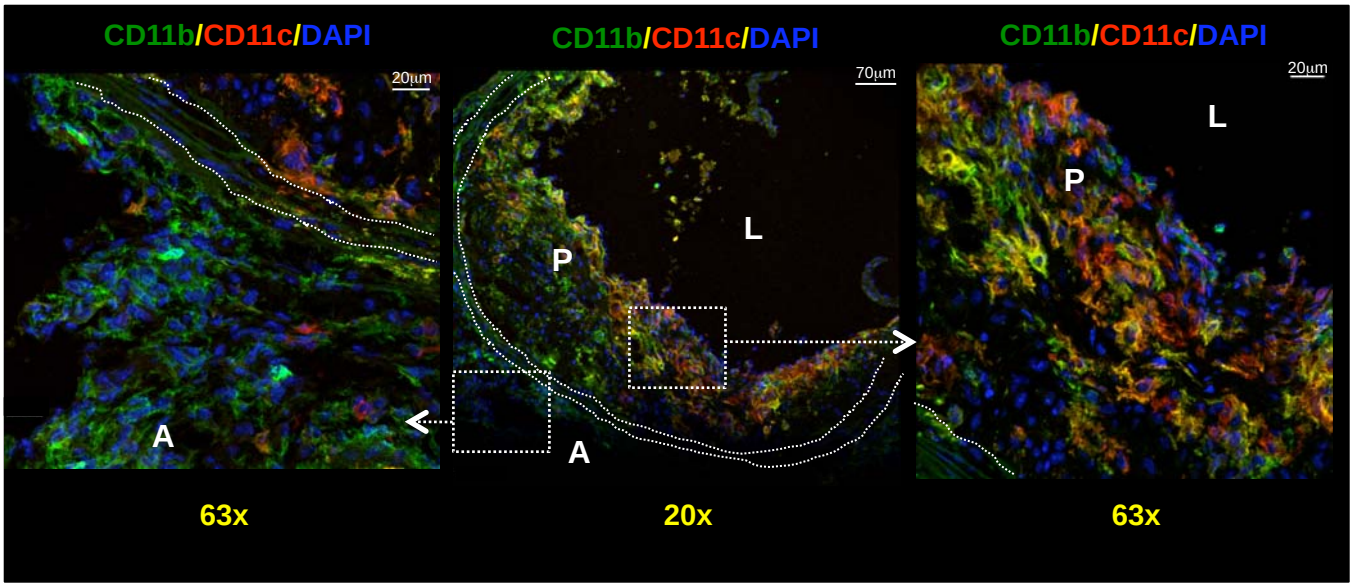
Supplementary Data

Supplementary Figures and Figure Legends

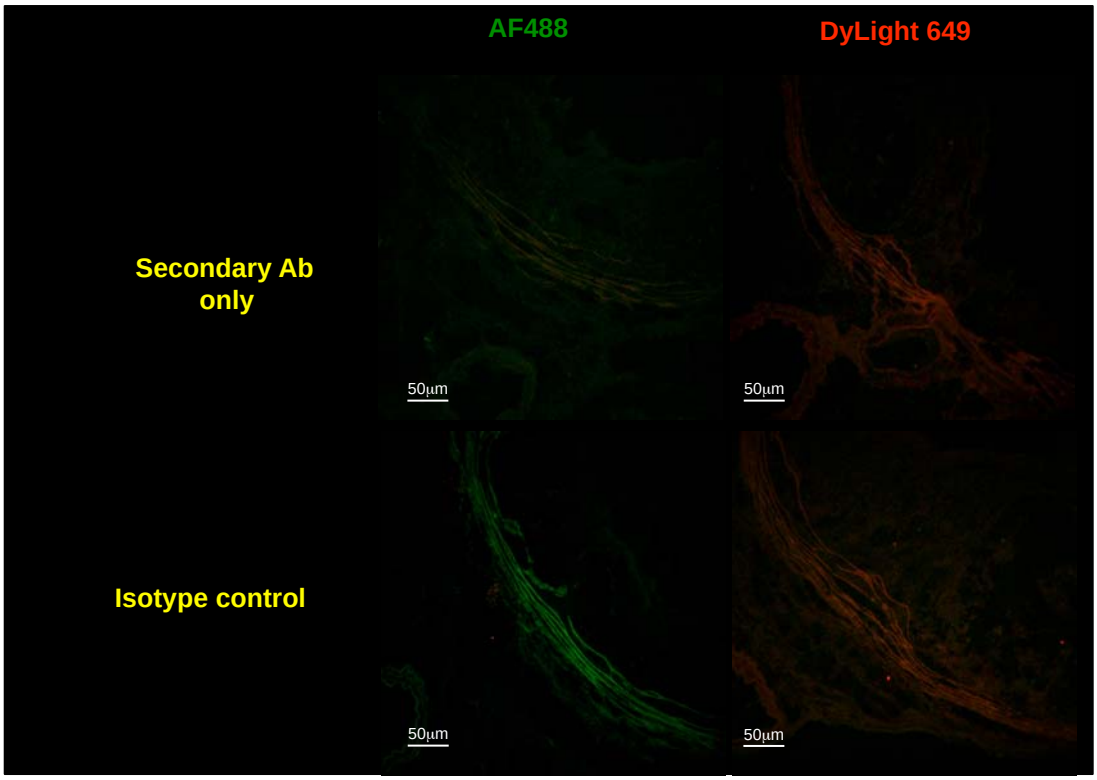


Supplementary figure 1. Myeloid cell populations in mouse aorta analyzed by flow cytometry (A). Total number of live CD45$^+$ leukocytes in aortas of 12 wks chow diet (CD) or western diet (WD)-fed *Apoe*$^{-/-}$ (n=7) and C57BL/6 (B6) (n=6) mice (**B).** Absolute number of live CD45$^+$, CD11b$^+$CD11c$^-$, CD11b$^+$CD11c$^+$ and CD11b$^-$CD11c$^+$ cells in aortas from *Apoe*$^{-/-}$ and C57BL/6 mice on CD and WD, numbers of mice as in (**A). (C)** Total number of MHCII$^+$ CD11b$^+$CD11c$^-$, CD11b$^+$CD11c$^+$ and CD11b$^-$CD11c$^+$ cells in aortas of *Apoe*$^{-/-}$ (n=7) and C57BL/6(B6)(n=6) mice fed for 12 wks with CD or WD as indicated. *p<0.05; ** p<0.01. **(D).** MHCII, CD205, Ly6G and F4/80 expression in CD11b$^+$CD11c$^-$, CD11b$^+$CD11c$^+$ and CD11b$^-$CD11c$^+$ cells in aortas of *Apoe*$^{-/-}$ fed with WD for 12 wks. All gates set by FMO.

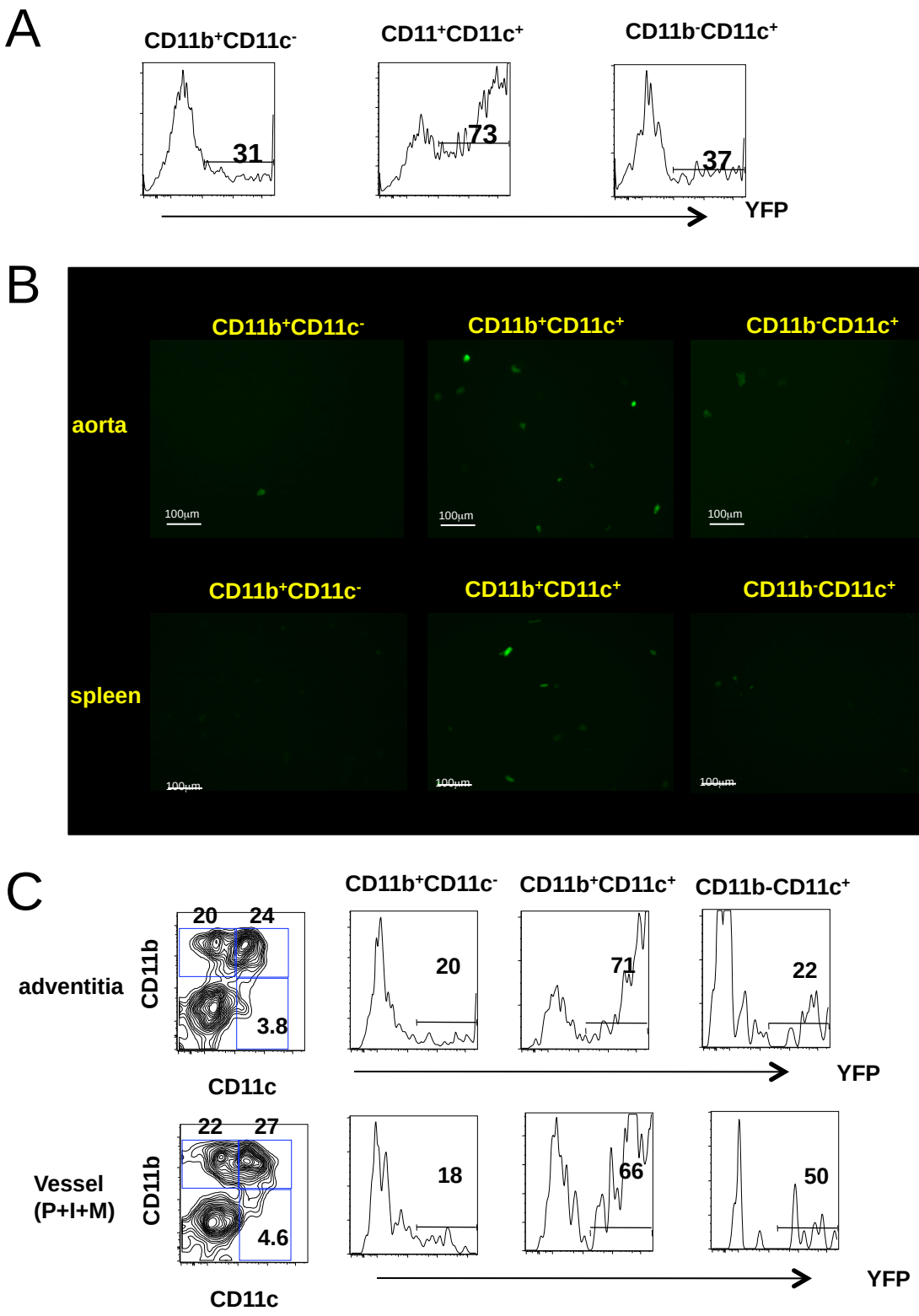
A



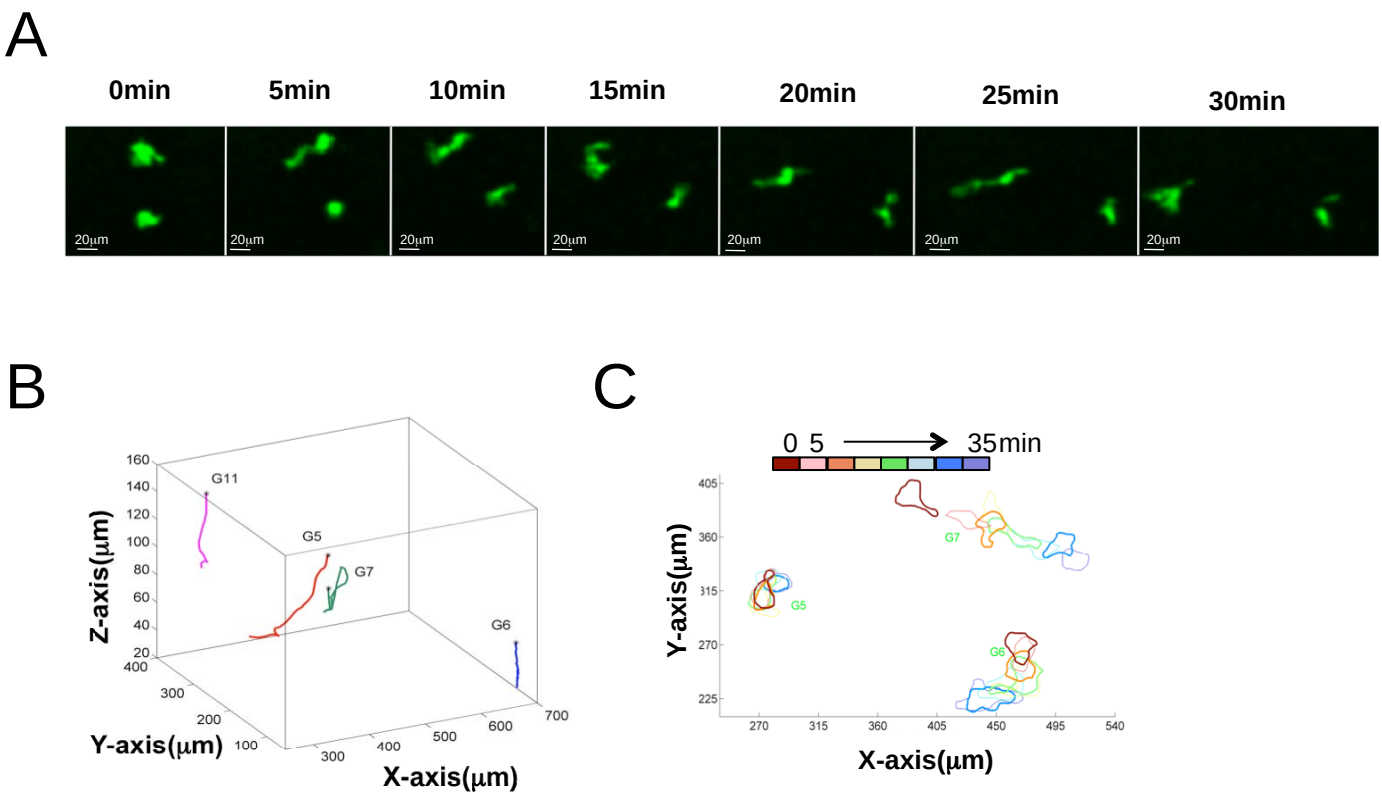
B



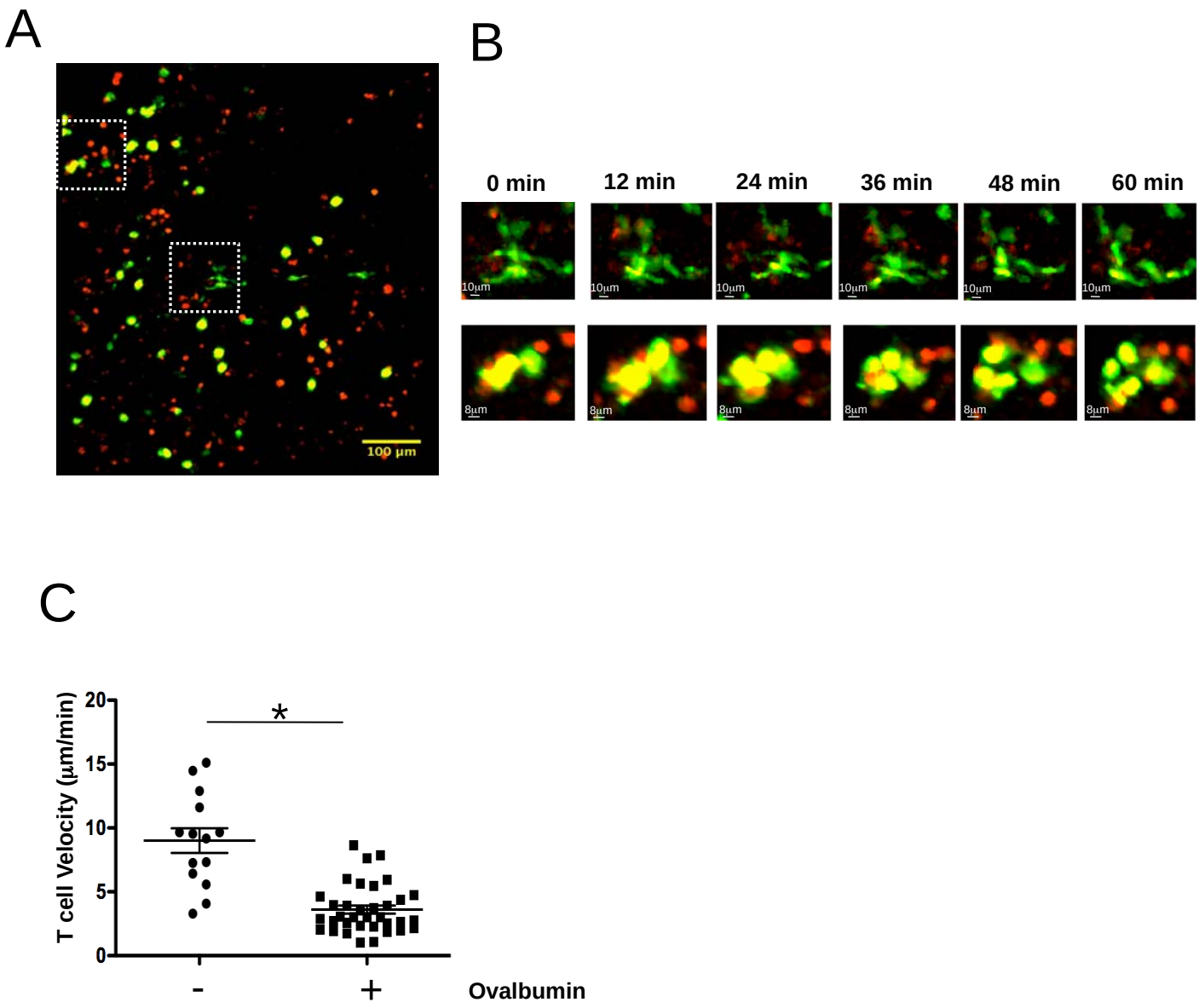
Supplementary figure 2. (A). Localization of CD11b^{+}CD11c^{-} (green), CD11b^{+}CD11c^{+} (yellow), and CD11b^{-}CD11c^{+}(red), DAPI (blue) cells in aortic root of *Apoe*$^{-/-}$ mice fed with WD as seen by immunofluorescence. Dotted white lines indicate border of lamina muscularis; L-lumen; P-plaque; A-adventitia. **(B)** Aortic roots of *Apoe*$^{-/-}$ mice stained with secondary antibody only or isotype controls for CD11b and CD11c staining as seen by immunofluorescence. Autofluorescence of elastic lamina is visible.



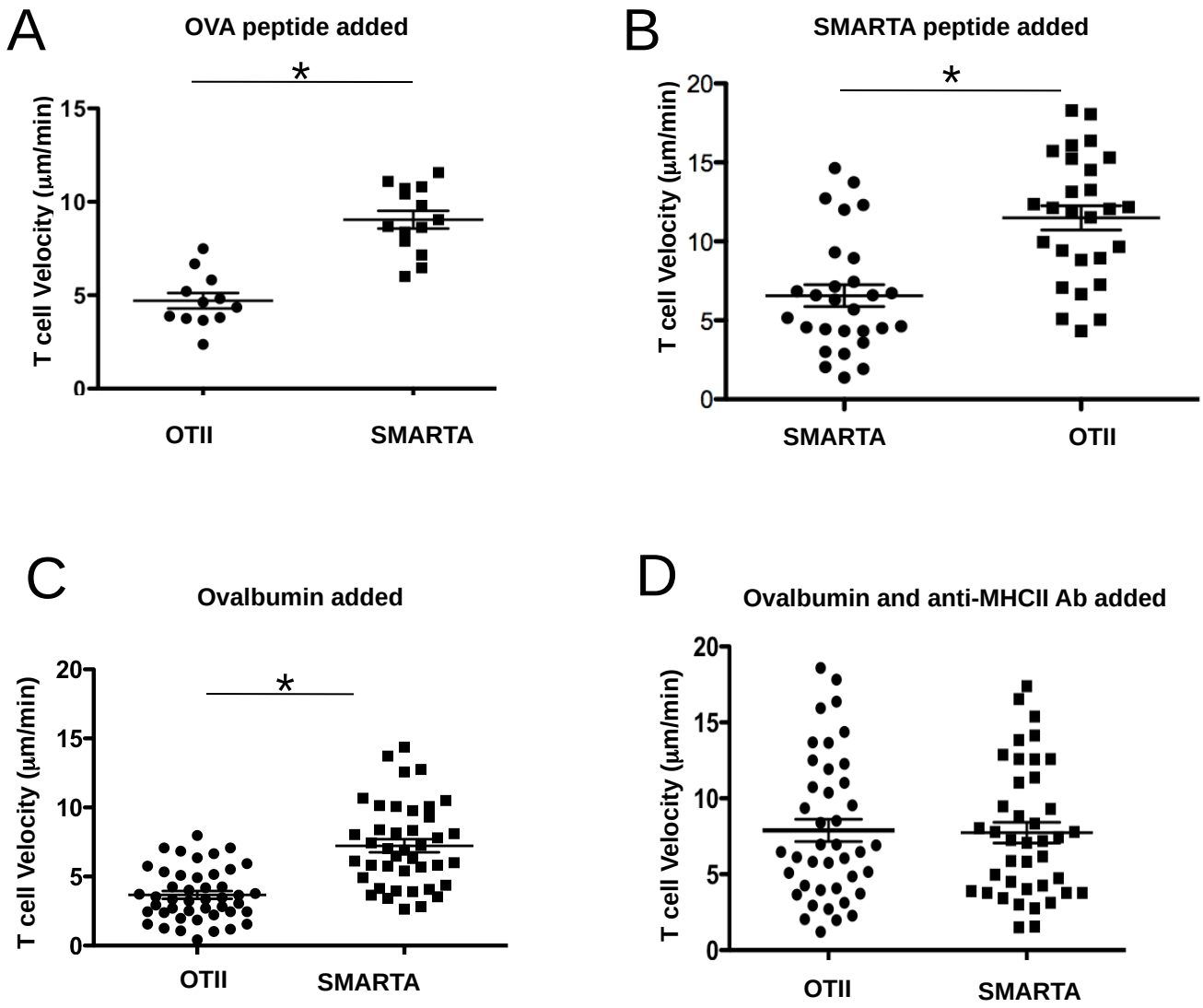
Supplementary figure 3. (A). Expression of YFP among CD11b^{+}CD11c^{-}, CD11b^{+}CD11c^{+} and CD11b^{-}CD11c^{+} populations in aorta of CD11c^{YFP}*Apoe*$^{-/-}$ mice. **(B).** YFP expression in CD11b $^{+}$CD11c^{-}, CD11b^{+}CD11c^{+} and CD11b^{-}CD11c^{+} cells sorted from aortas and spleens as seen by fluorescence microscopy. **(C).** Distribution of CD11c^{YFP+} cells between adventitia and the rest of the vessel (plaque+intima+media(P+M+I)). Representative of 2 independent experiments.



Supplementary figure 4. (A). Time lapse images of CD11c^{YFP+}APC (green), displayed as maximum intensity projections along z axis (top view) through image stacks encompassing a volume of 760x760x160 mm (x, y, z) in the explanted aorta. (**B)**. 3D projection of tracks (x, y and z) of four APC in the arterial wall. (**C).** Shapes of 3 APC tracked over 35 min (time color coded). See also supplementary movie 1. Representative images from at least four independent experiments.

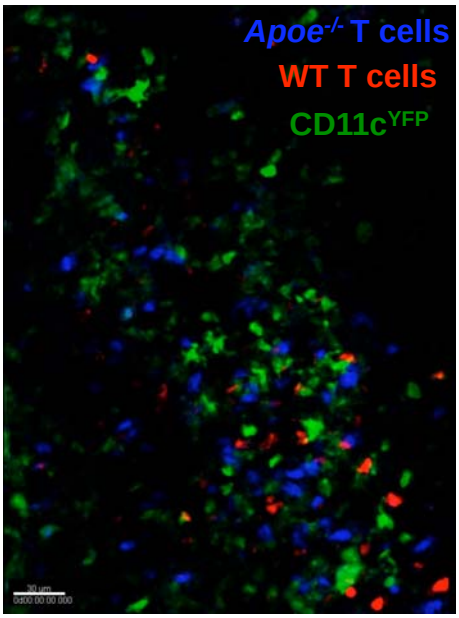


Supplementary figure 5. (**A).** Maximum intensity projection along z axis (top view) through image stacks (760x760x210 µm (x, y, z)). View from the adventitial side of explanted mouse aorta. $CD11c^{YFP}$ (green), SNARF-labeled OTII T cells (red). Interactions of APC and T cells in the adventitiain the presence of Ovalbumin **(B).** Time lapse images of interacting APC (green) and OTII T (red) cells in the presence of ovalbumin, displayed as maximum intensity projections along z axis (top view)**.** Magnified from boxes. **(C)** Mean velocities of individual OTII T cells in the absence or presence of ovalbumin. Average ± SEM is indicated by horizontal line. Representative data of 2 independent experiments. *p<0.05



Supplementary figure 6. Mean migration velocities of individual OT II T cells or SMARTA T cells interacting with $CD11c^{YFP+}$ APC in the presence of OVA peptide **(A)**, SMARTA peptide **(B),** Ovalbumin **(C)** or Ovalbumin with anti-MHCII antibody **(D).** Aortas, explanted from *Apoe*$^{-/-}$ mice fed with WD for 12 weeks. Average ± SEM is indicated by horizontal lines. Representative data of 3 independent experiments. *p<0.05

A



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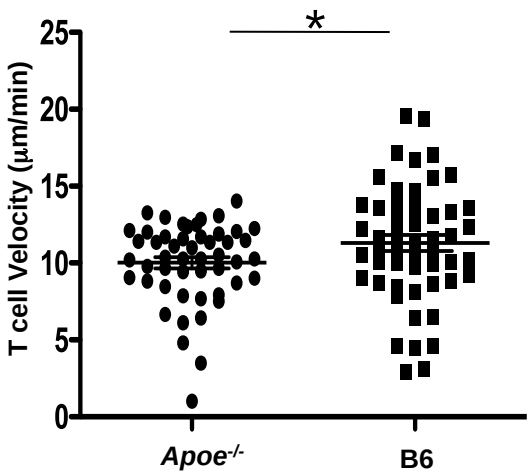

Supplementary figure 7 . Two-photon imaging of antigen-specific T cell-APC interaction in paraaortic lymph node. (A) CD11c^{YFP} APC (green), *Apoe*$^{-/-}$ T cells (blue), B6 T cells (red) in the paraaortic lymph node of atherosclerotic *Apoe*$^{-/-}$ mice. **(B)** Mean migration velocities of individual *Apoe*$^{-/-}$ T cells or B6 T cells. No exogenous antigen added. Lymph nodes, explanted from *Apoe*$^{-/-}$ mice fed with WD for 12 weeks. Average ± SEM is indicated by horizontal lines. Representative data of 3 independent experiments. *p<0.05

Supplementary materials:

Supplementary Movie 1. Z stack from top to bottom showing the localization of $CD11c^{YFP}$DC in different compartments of the atherosclerotic arterial wall (top, adventitia; bottom, plaque). YFP^{+}DC (green); second harmonic (blue); media autofluorescence (brown).

Supplementary Movie 2. Migration of $CD11c^{YFP}$DC in the wall of explanted aorta. Displayed as maximum intensity projection along z axis. Adventitial localization of $YFP^{+}CD11c^{+}$DC. Representative movie of at least 10 independent experiments.

Supplementary Movie 3. Migration and interaction of $CD11c^{+}$DC (green) with OT II T cells (red) in explanted aorta from CD11cYFP mouse in the presence of OVA peptide, maximum intensity projection along z axis through image stacks encompassing a volume of 760x760x210 μm (x, y, z). Adventitial localization of $YFP^{+}CD11c^{+}$DC and CD4 T cells Representative movie of at least 5 independent experiments.

Supplementary Movie 4. Migration and interaction of YFP^{+}DC (green) with OT II T cells (red) in explanted aorta from $CD11c^{YFP}$ mouse in the presence of ovalbumin, maximum intensity projection along z axis through image stacks encompassing a volume of 760x760x210 μm (x, y, z). Adventitial localization of $YFP^{+}CD11c^{+}$DC and CD4 T cells Representative of 2 independent experiments.

Supplementary Movies 5 and 6. Migration and interaction of YFP^{+}DC (green) with OT II T cells (red) or SMARTA speific T cells (blue) in the presence of OVA peptide **(movie S5)** or SMARTA peptide **(movie S6).** Explanted aortas from $CD11c^{YFP}$/*Apoe*$^{-/-}$ mice fed with WD for 12 weeks. Maximum intensity projection along z axis. Adventitial localization of $YFP^{+}CD11c^{+}$DC and CD4 T cells Representative of 3 independent experiments.

Supplementary Movie 7. Migration and interaction of $YFP^{+}CD11c^{+}$DC (green) with untreated *Apoe*$^{-/-}$ CD4 T cells, labeled with SNARF (red) isolated from $CD11c^{YFP}$ /*Apoe*$^{-/-}$ fed 20 weeks with WD, but not with pre-stimulated OTII, labeled with CMRA (blue). No antigen added. Maximum intensity projection along z axis. Adventitial localization of $YFP^{+}CD11c^{+}$DC and CD4 T cells. Representative movie of 2 independent experiments.