Supplementary Material

An Investigation of the Inflammatory Landscape in the Brain and Bone Marrow of the APP/PS1 Mouse

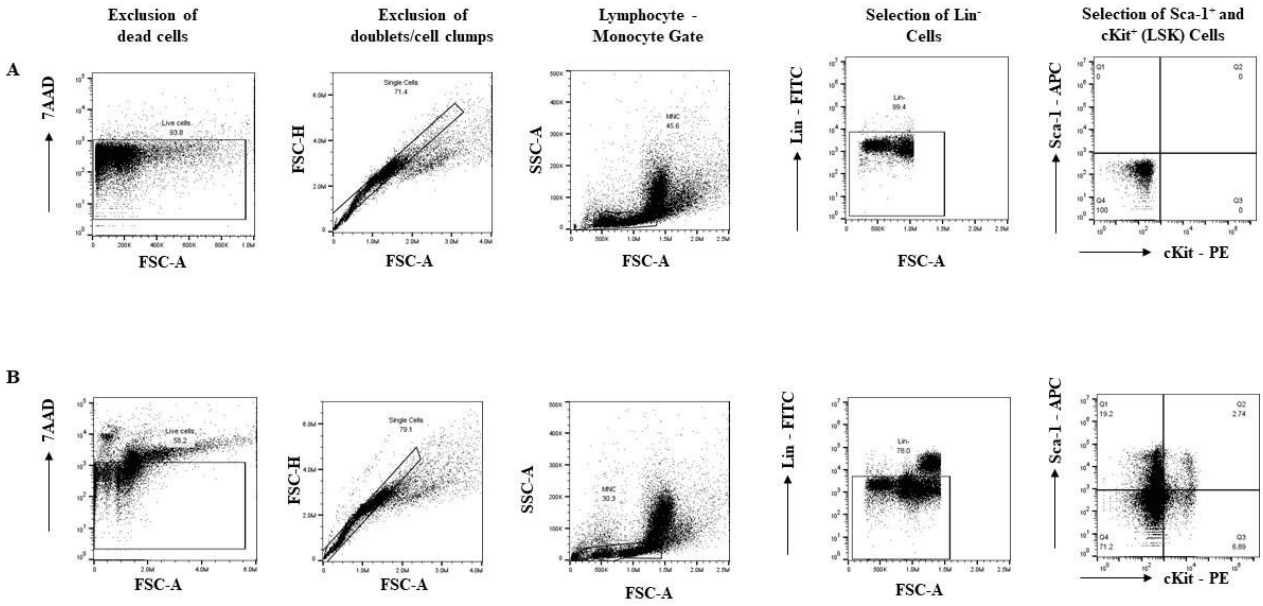
Supplementary Table 1. List of antibodies used in the study.

Antibody	Catalog#	Vendor	Concentration
Lineage cocktail – FITC	133302	Biolegend	1 µL/100 µL
cKit – PE	105808	Biolegend	0.5 µL/100 µL
Sca1 – APC	122512	Biolegend	0.25 µL/100 µL
7AAD	420404	Biolegend	0.3 µL/100 µL
Aqua Blue	L34966A	Invitrogen	0.1 µL/100 µL
CD45 – BV605	103140	Biolegend	0.5 µL/100 µL
Ly6G – PE-CF594	562700	BD Biosciences	0.125 µL/100 µL
CD11b – APC eFluor 780	47-0112-82	Invitrogen	0.06 µL/100 µL
Ly6C – FITC	553104	BD Biosciences	0.1 µL/100 µL
CD115 – APC/Cy 7	135532	Biolegend	0.2 µL/100 µL
F4/80 – PE	123110	Biolegend	0.75 µL/100 µL
CX3CR1 – BV421	149023	Biolegend	0.2 µL/100 µL
CCR2 – BV711	747964	BD Biosciences	0.725 µL/100 µL
CD80 – PerCP/Cy5.5	104722	Biolegend	0.5 µL/100 µL
CD206 – BV785	141729	Biolegend	0.375 µL/100 µL
Flk2 (CD135) – PE-CF594	562537	BD Biosciences	0.725 µL/100 µL
CD48 – PerCP/Cy5.5	103422	Biolegend	0.2 µL/100 µL
CD150 – BV650	115931	Biolegend	0.725 µL/100 µL
CD34 – Alexa Fluor 700	560518	BD Biosciences	0.5 µL/100 µL
FCRγ – BV421	742562	BD Biosciences	0.5 µL/100 µL
IL17rα – PE/Cy7	135014	Biolegend	0.5 µL/100 µL
CD31 (Host - Rb)	ab28364	Abcam	1:50
IB4 - FITC	ALX-650-001F-MC05	Enzo	1:50
F4/80 (Host - Rat)	14-4801-82	Invitrogen	1:50
CD11b (Host - Rb)	ab133357	Abcam	1:4000
DAPI	40043	Biotium	-
633 Goat Anti Rb	20122	Biotium	1:250
488 Goat Anti Mouse	20010	Biotium	1:250
488 Goat Anti Rat	A11006	Invitrogen	1:100
OX 42	MA5-27726	Invitrogen	1:100
IBA1	BS-1014R	Invitrogen	1:100
CD34	Ab81289	Abcam	1:250
CD80	66406-1-IG	Thermofisher	1:100
CX3CR1	14-6093-81	Thermofisher	1:100
S100A8	MAB 3059	R & D Systems	1: 1000
S100A9	MAB 2065	R & D Systems	1: 1000
RAGE	Sc- 365154	Santacruz	1: 1000
HMGb1	3935	CST- cell signaling	1: 1000

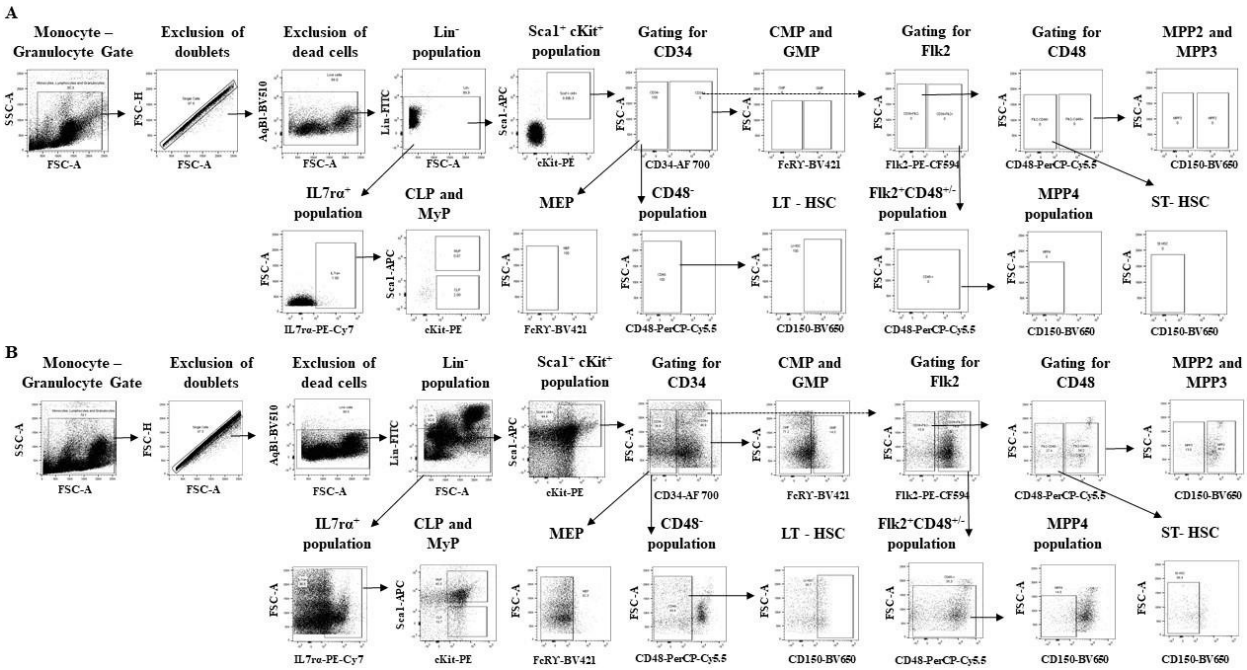
Supplementary Table 2. List of primers used in the study

	Primer Sequences	
Gene	**Forward**	**Reverse**
NLRP1	5'GGAGCCTTGTTCTTCAAAGACACA3'	5'TTGATCAGAAGTGATAGAGGAGACC3'
NLRP3	5'ACCAGCCAGAGTGGAATGAC-3'	5' ATGGAGATGCGGGAGAGATA3'
NLRC4	5'TACACAGCAGGAACGAAGACTCAG3'	5'GGCTTCCACAGATGACCCACA3'
AIM2	5'AAGAGAGCCAGGGAAACTCC3'	5'TGTCTCCTTCCTCGCACTT3'
IL-1β	5'AGTTGACGGACCCCAAAAG3'	5'CTTCTCCACAGCCACAATGA3'
IL-18	5'ACAACTTTGGCCGACTTCAC3'	5'GTCTGGTCTGGGGTTCACTG3'
CMA1	5'CCTGGGTTCCAGCACCAA3'	5'GGCGGGAGTGTGGTATGC3'
PU.1	GGGAGAGCCATAGCGACCAT3'	5'TAGGAGACCTGGTGGCCAAGA3'
CCL2	5'CCCAATGAGTAGGCTGGAGA3'	5'AAAATGGATCCACACCTTGC3'
S100A8	5'AAATCACCATGCCCTCTACAAG3'	5'CCCACTTTTATCACCATCGCAA3'
S100A9	5'ATACTCTAGGAAGGAAGGACACC3'	5'TCCATGATGTCATTTATGAGGGC3'
HMGB	5'GCTCAGAGAGGTGGAAGACCA3'	5'GGTGCATTGGGATCCTTGAA3'
RAGE	5'CTTGCTCTATGGGGAGCTGTA3'	5'CATCGACAATTCCAGTGGCTG3'
TLR4	5'CACTGTTCTTCTCCTGCCTGAC3'	5'TGGTTGAAGAAGGAATGTCATC3'
β-actin	5'CCATCATGAAGTCTGACGTTG3'	5'CAATGATCTTGATCTTCATGGTG3'

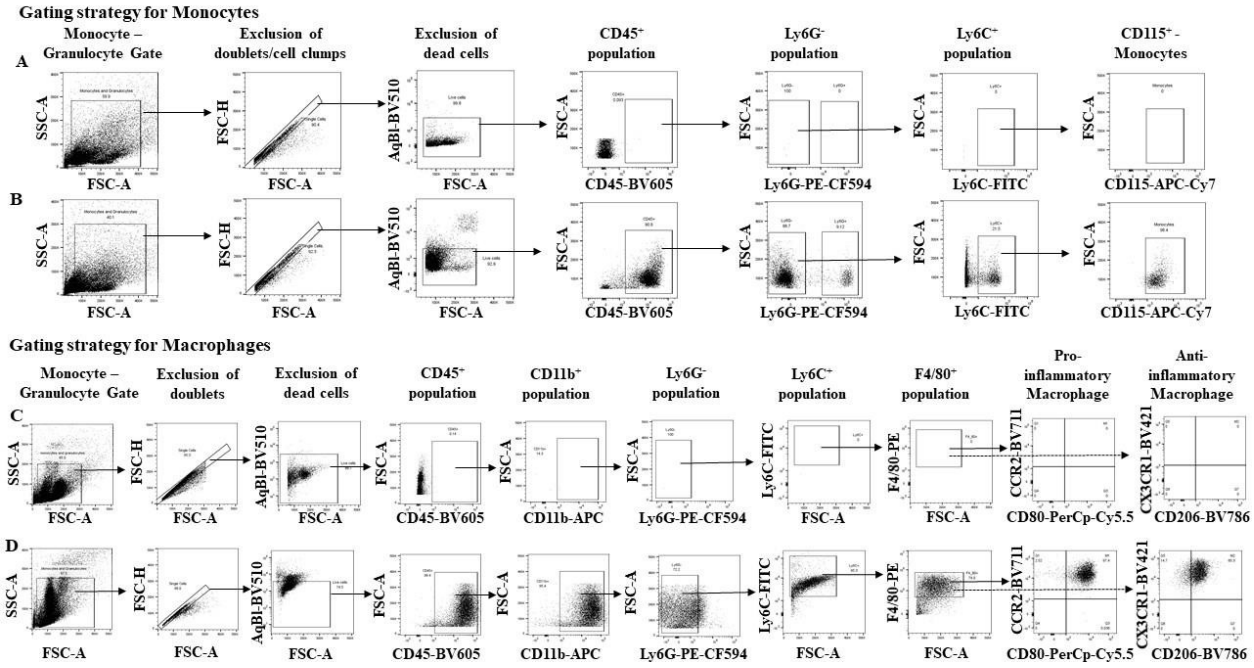
Supplementary Figure 1. Flow cytometry gating strategy for the characterization of lin$^-$ Sca1$^+$cKit$^+$ (LSK) cells. Representative dot plots for sample with isotype control (A) and with fluorescent conjugated antibodies (B). Sequential gating strategy includes exclusion of dead cells and doublets, selection of lymphocyte – monocyte population, selection of lineage-depleted cell population and then selection of cells dual positive for Sca-1 and cKit.



Supplementary Figure 2. Flowcytometry gating strategy for hematopoietic progenitor cells. Representative dot plots for sample with isotype control (A) and with fluorescent conjugated antibodies (B). Sequential gating strategy includes selection of lymphocyte – monocyte population, exclusion of doublets and dead cells, selection of lineage negative cells and then further selection based on the immunophenotypic markers mentioned in the Table 1 for short-term and long-term hematopoietic stem cells (HSCs) and Multipotent progenitor cells, MPP2, MPP3 and MPP4.



Supplementary Figure 3. Flowcytometry gating strategy for monocytes. Representative dot plots for sample with isotype control (A) and with fluorescent conjugated antibodies (B). Sequential gating strategy includes selection of lymphocyte – monocyte population, exclusion of doublets and dead cells, selection of CD45 positive cells, selection of Ly6G negative cells, selection of Ly6C positive cells and finally selection of monocytes specific CD115 positive cells. Flowcytometry gating strategy for macrophages. Representative dot plots for a sample with isotype control (C) and with fluorescent conjugated antibodies (D). Sequential gating strategy includes selection of lymphocyte – monocyte population, exclusion of doublets and dead cells, selection of CD45 positive cells, selection of CD11b positive cells, selection of Ly6G negative cells, selection of Ly6C positive cells, selection of macrophage specific F4/80 positive cells, then pro-inflammatory macrophages were gated by using dual positivity for CD80 and CCR2 and similarly F4/80 positive cells were gated for anti- inflammatory macrophage specific markers of CX3CR1 and CD206.



Supplementary Figure 4. Shown are complete western blots, without cropping, for S100A8, S100A9 and HMGb1 in the bone marrow supernatants of control and APP/PS1 mice.