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Label: Supplementary Figures 1-8
Filename: Supplementary Figures_final.pdf

Supplementary Information

The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and survival of Ly6C^{-} monocytes

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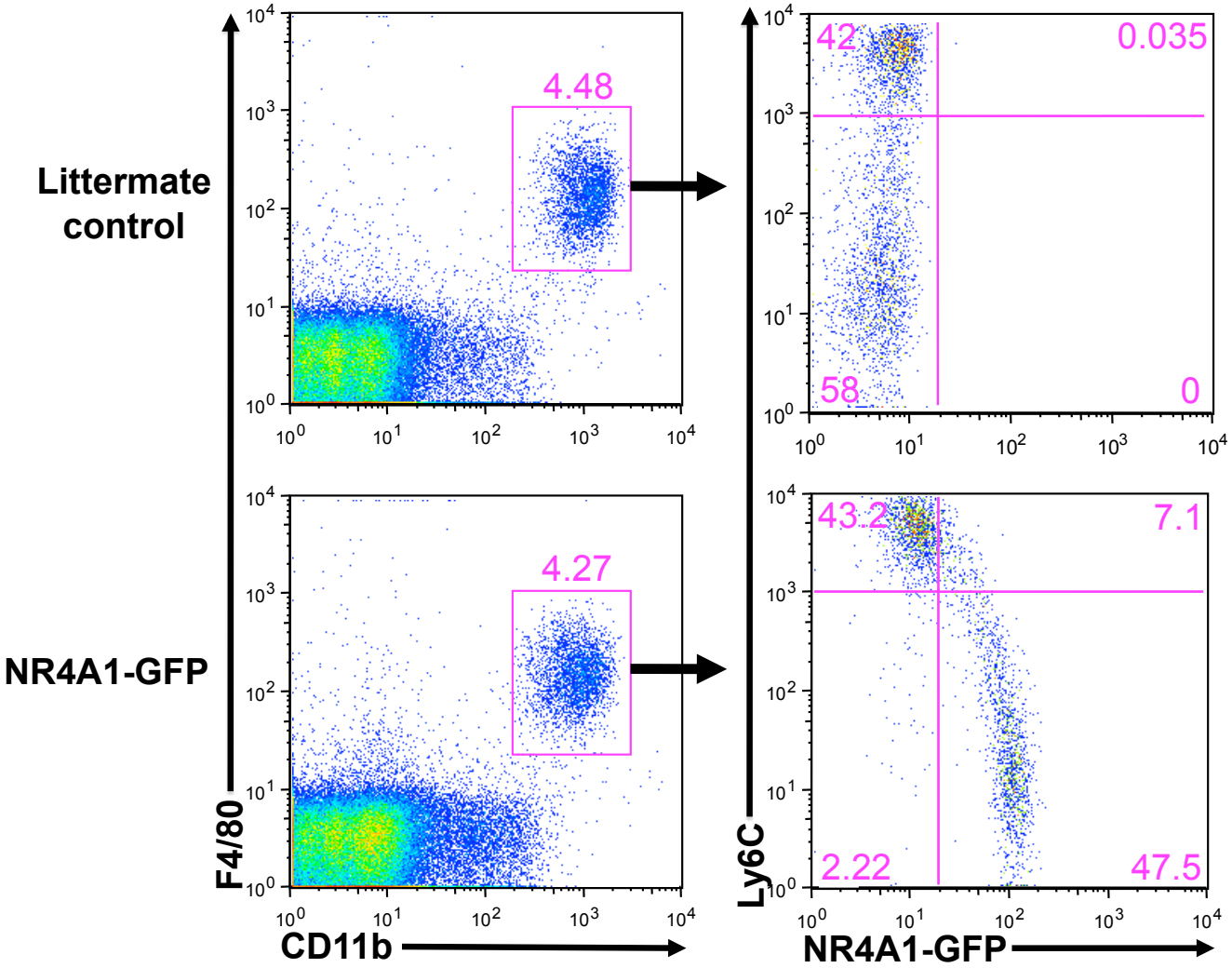
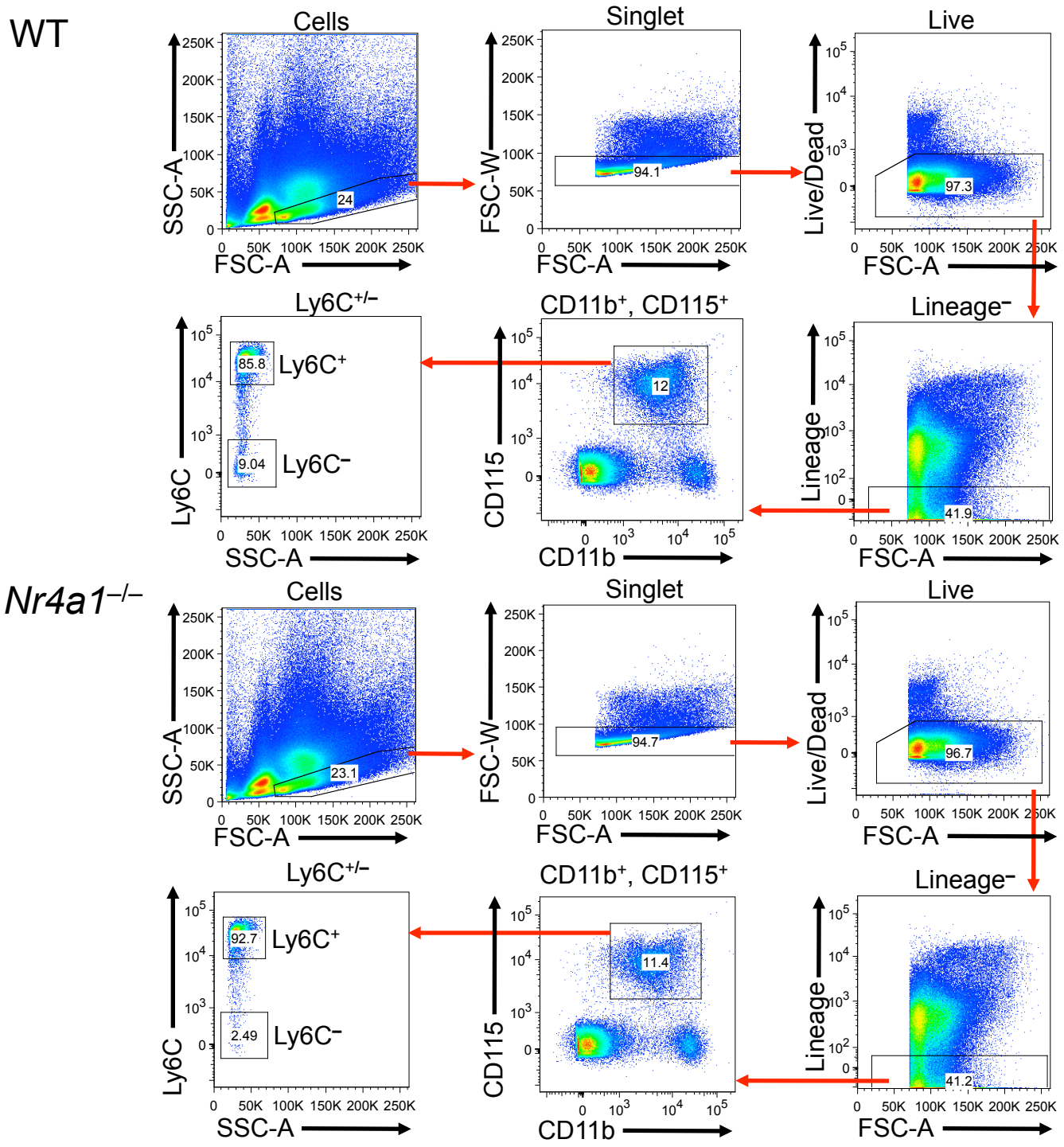
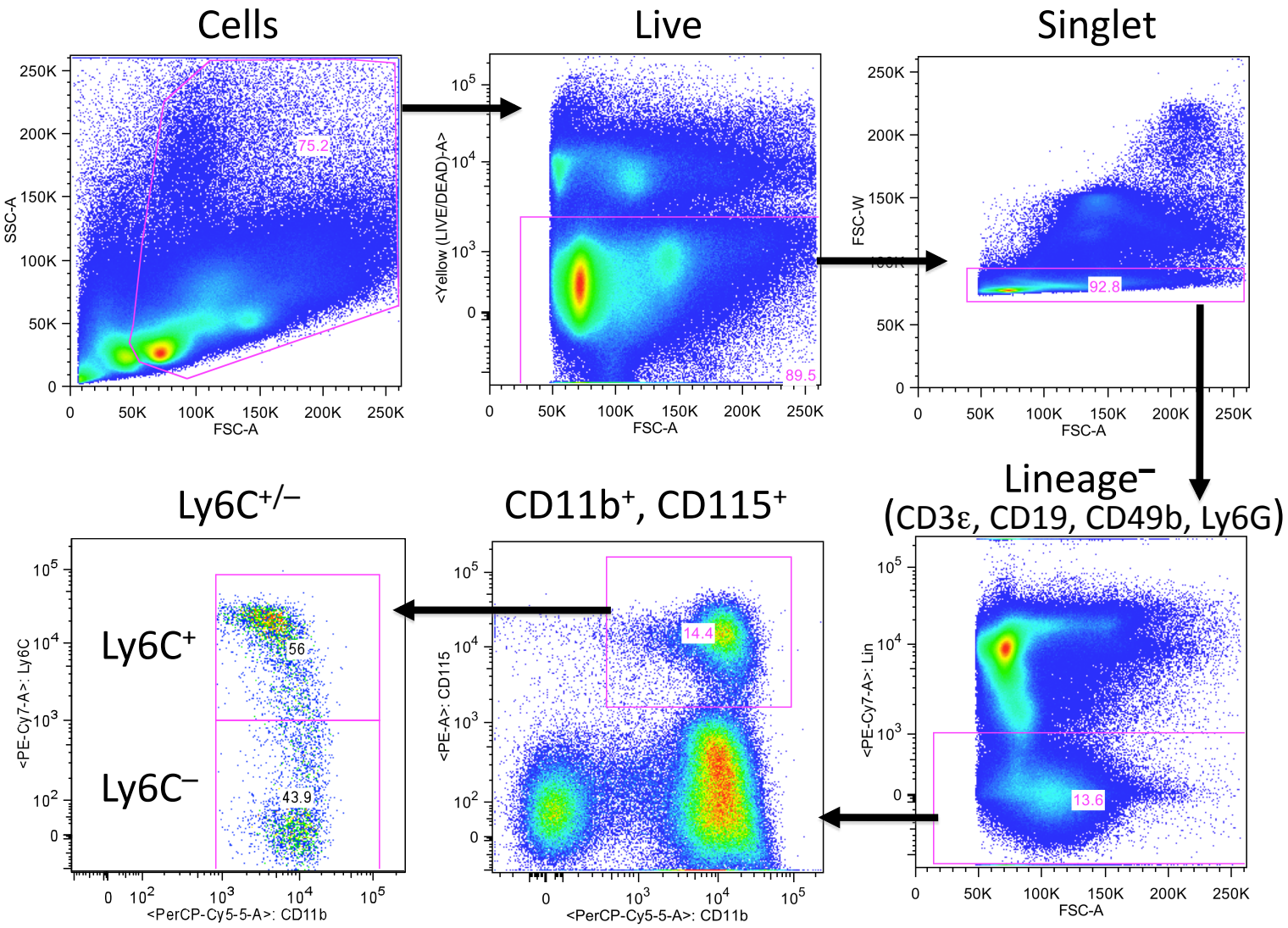
Supplementary Figures 1-8



Supplementary Figure 1. NR4A1-GFP expression in blood monocytes. Live F4/80$^+$ CD11b$^+$ monocytes from the peripheral blood of a NR4A1-GFP transgenic reporter mouse and NR4A1-GFP negative littermate control were assessed for Ly6C and GFP expression. Note: Nearly all F4/80$^+$ cells in the blood are also CD11b$^+$.

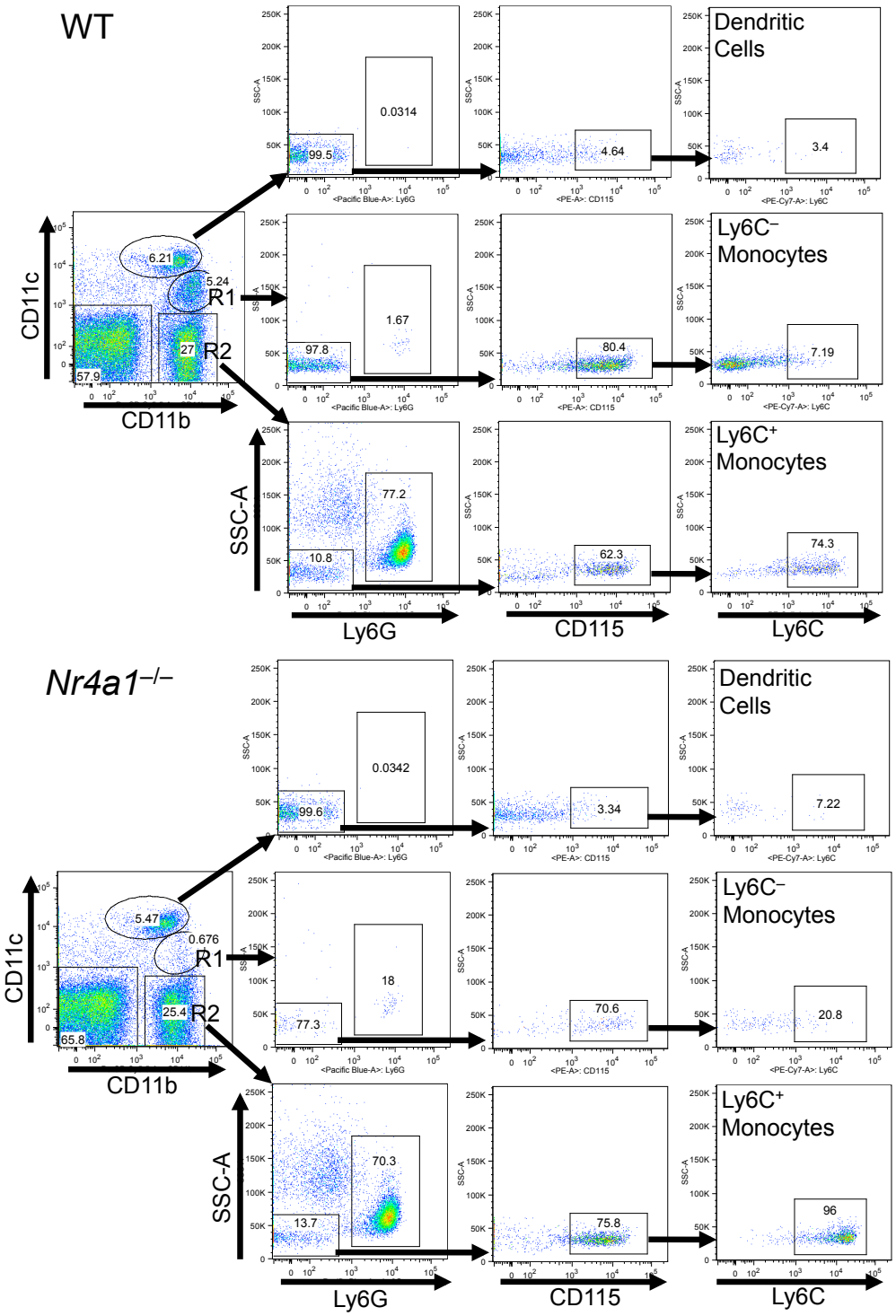


Supplementary Figure 2. Gating strategy for bone marrow monocyte subsets. Gating strategy for identification of monocyte populations by flow cytometry of mouse bone marrow. Live, single, Lin⁻ (CD3ε, CD19, CD49b, Ly6G, CD117) cells with low side scatter (SSC) were plotted for CD115 and CD11b expression. CD115⁺, CD11b⁺ monocytes were then subdivided based on their + or – expression of Ly6C.



Supplementary Figure 3. Gating strategy for blood and spleen monocyte subsets. Gating strategy for identification of monocyte populations by flow cytometry of mouse spleen and blood. Live, single, Lin⁻ (CD3ε, CD19, CD49b, Ly6G) cells were plotted for CD115 and CD11b expression. CD115⁺, CD11b⁺ monocytes were then subdivided based on their + or – expression of Ly6C.

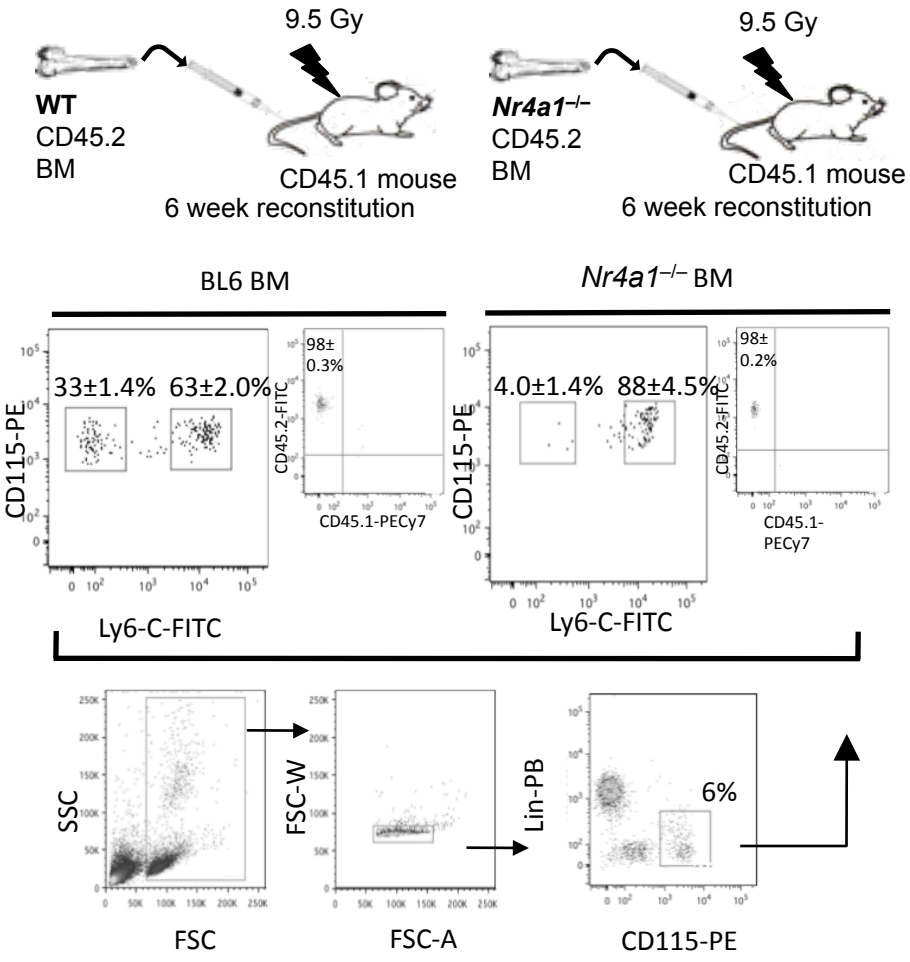
a



b

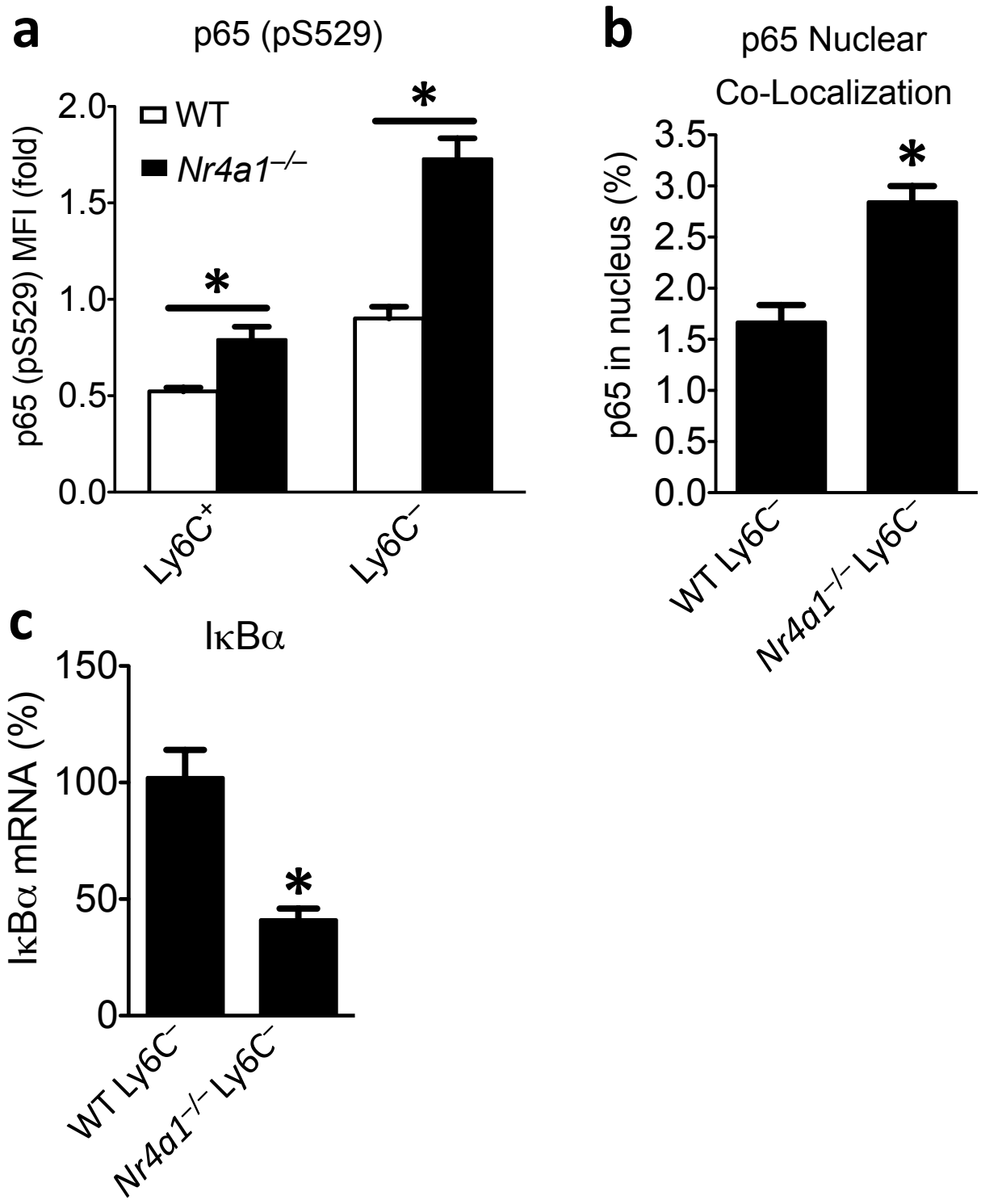
Marker	Ly6C- (R1)	Ly6C+ (R2)
CD11b	+	+
CD115	+	+
Ly6C	-	+
CD62L	**Low (<20%+)**	+
CD11c	**Mid**	-
CCR2	-	+
CX3CR1	**High**	**Mid**
CD43	+	+
F480	**Low (<30%+)**	**Low (<30%+)**
CD103	**Low (<20%+)**	**Low (<20%+)**
CD11a	+	+
Ly6G	-	-
CD135	-	-
B220	-	-
PDCA	-	-
CD8	-	-
CD3ε	-	-
DX5	-	-
CD19	-	-
CD45	+	+

Supplementary Figure 4. Analysis of surface markers on Ly6C⁺ and Ly6C⁻ subsets. (a) The two monocyte populations (Ly6C + and –) in spleen were further examined for expression of a variety of surface markers by flow cytometry in *Nr4a1*$^{-/-}$ or wild-type (WT) control mice. **(b)** The two monocyte populations in wild-type mice varied in their expression of Ly6C, CD62L, CD11c, CCR2 and CX3CR1 (red box).



Lin- = NK1.1, CD19, CD3ε

Supplementary Figure 5. Analysis of reconstitution in chimeric studies. Blood monocyte subsets from wild-type (WT) or *Nr4a1*$^{-/-}$ bone marrow chimera analyzed by flow cytometry. Lineage negative (NK1.1, CD19, CD3ε) CD115^{+} cells were analyzed for expression of Ly6C, CD45.2 (donor), and CD45.1 (recipient). Chimerism was 98% for both wild-type and *Nr4a1*$^{-/-}$ bone marrow chimera. Ly6C^{low} Lin^{-} CD115^{+} cells represented 33±1.4% of monocytes in wild-type bone marrow chimera, but 4±1.4% in *Nr4a1*$^{-/-}$ BM recipients.



Supplementary Figure 6. Analysis of NFκB activity in bone marrow monocytes. (a) Relative activity of p65 (pS529) activity in bone marrow monocytes from *Nr4a1*$^{-/-}$ or wild-type (WT) control mice as measured by intracellular flow cytometric analysis using an anti-p65 phosphoserine 529 antibody. (*p<0.05 n=4) **(b)** Nuclear co-localization of p65 in bone marrow Ly6C^{-} monocyte populations from *Nr4a1*$^{-/-}$ and wild-type control mice measured by immunofluorescence microscopy. Monocytes were sorted by FACS, fixed on slides and then stained with p65 antibody and DAPI staining of the nucleus. The percent of p65 staining co-localized in the nucleus was quantified using Imaris software. (*p<0.05 n=6) **(c)** Relative expression of IκBα transcripts in Ly6C^{-} monocytes isolated by FACS from bone marrow of *Nr4a1*$^{-/-}$ or wild-type mice measured by qRT-PCR. (*p<0.05 n=6, expressed as a percentage of wild-type transcript).

Antibody	Company	Clone	Secondary (if not directly conjugated)
CD49b	eBioscience	DX5	
CD3e	BD Pharmingen	145-2C11	
NK1.1	eBioscience	PK136	
CD19	eBioscience	1D3	
B220	eBioscience	RA3-6B2	
Ly6G	Biolegend	1A8	
CD11c	eBioscience	N418	
CD115	Biolegend	AFS98	
CD11b	BD Pharmingen	M1/70	
LY6C	BD Biosciences	AL-21	
F4/80	Biolegend	BM8	
CD62L	BD Biosciences	MEL14	
CD45.1	Biolegend	A20	
CD45.2	Biolegend	104	
CX3CR1	Santa Cruz	T-20	anti-goat IgG-FITC (SC-2777) SC
CCR2	(Mack et al., 2001)	MC21	biotin anti-rat IgG (RG7/11.1) BD, streptavidin-APC (554067) BD
Lineage Cocktail	BD Pharmingen	CD3e (145-2C11), CD11b (M1/70), B220 (RA3-6B2), Ly-76 (TER-119), Ly6C/G (RB6-8C5)	
CD117	eBioscience	2B8	
SCA-1 (Ly6A/E)	Biolegend	E13-161.7	
CD34	BD Biosciences	RAM34	
Nur77	BD Biosciences	12.14	eBiosciences (11-4011-85)
CD11a (LFA-1)	BD Biosciences	M17/4	
H2A.X (pS139)	Biolegend	2F3	
Active Caspase-3	BD Biosciences	C92-605	
p65 (pS529)	BD Biosciences	K10-895.12.50	

Supplementary Figure 7. List of antibodies used for flow cytometry.

Taqman Primers	
Assay ID	**Gene Symbol**
4352932-0912031	Gapdh
Mm01300401_m1	Nr4a1 (Nur77)
Mm00443060_m1	Nr4a2 (Nurr1)
Mm00450074_m1	Nr4a3 (NOR-1)
Mm00477798_m1	Nfkbia (IκBα)
Mm00772472_m1	Cdc2a (Cdk1)
Mm00438064_m1	Ccna2 (Cyclin A2)
Mm00438070_m1	Ccnd2 (Cyclin D2)
Mm00624964_m1	E2F2
Mm00438354_m1	cx3cr1
Mm00492781_s1	Junb
Mm00843434_s1	Cebpb
Mm00488140_m1	Sfpil (Pu.1)

Supplementary Figure 8. List of TaqMan primers used for qRT-PCR.