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## Supplemental information

# *Mds1*$^{CreERT2}$, an inducible Cre allele specific to adult-repopulating hematopoietic stem cells

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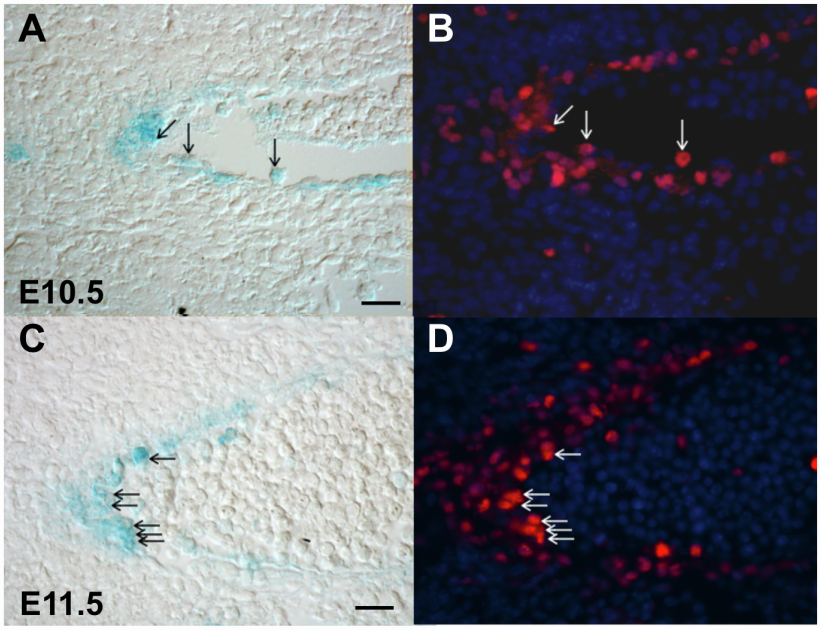
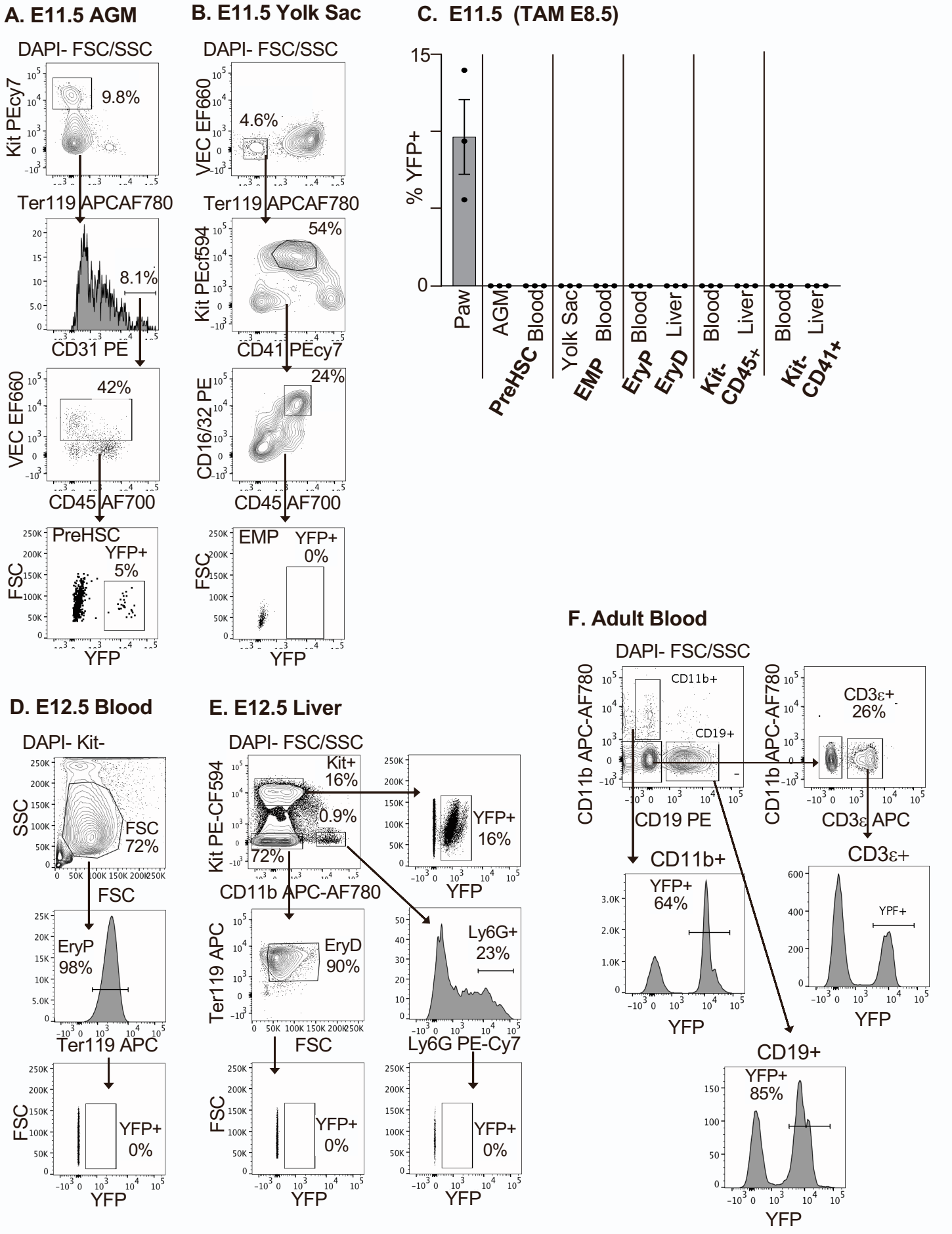
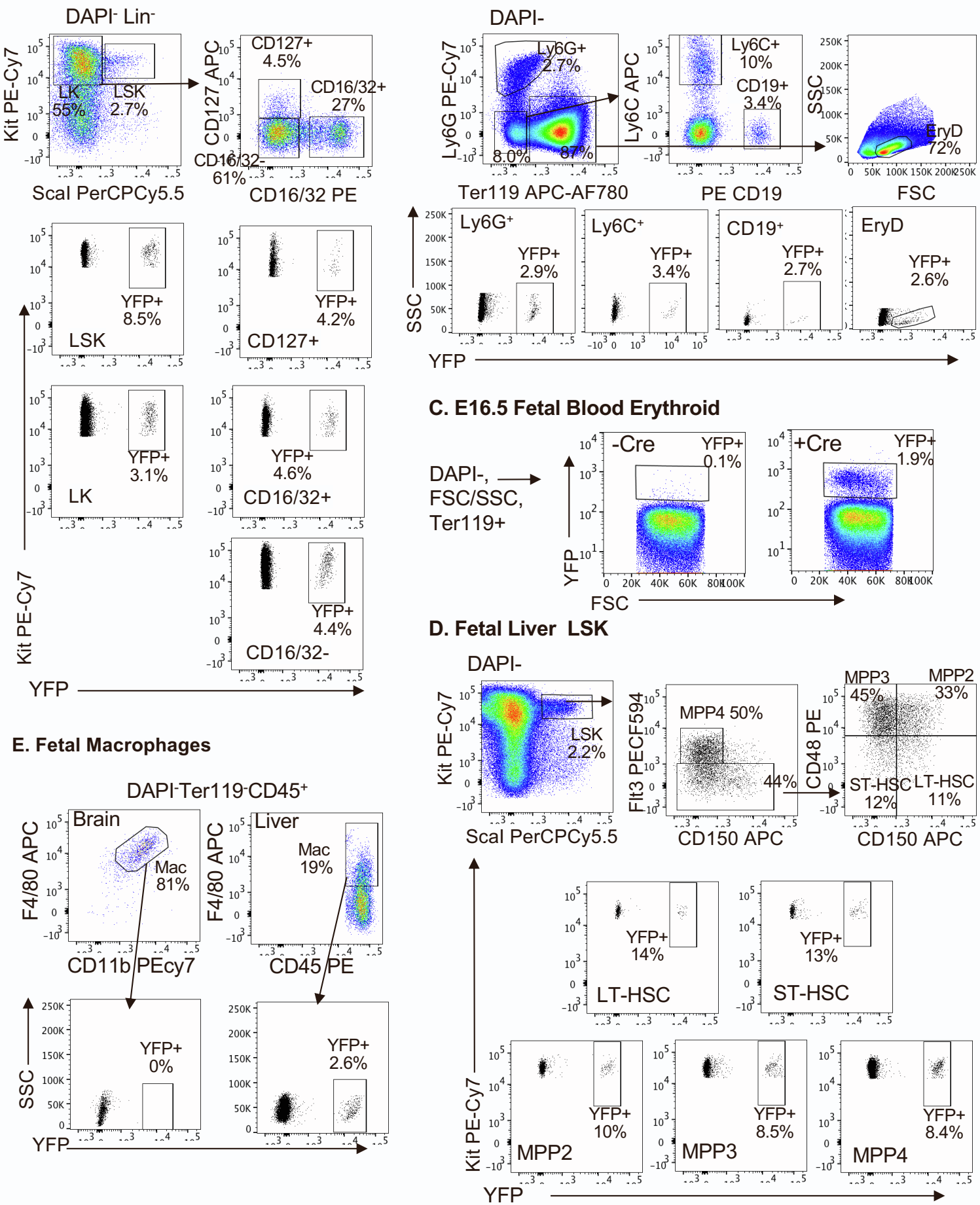


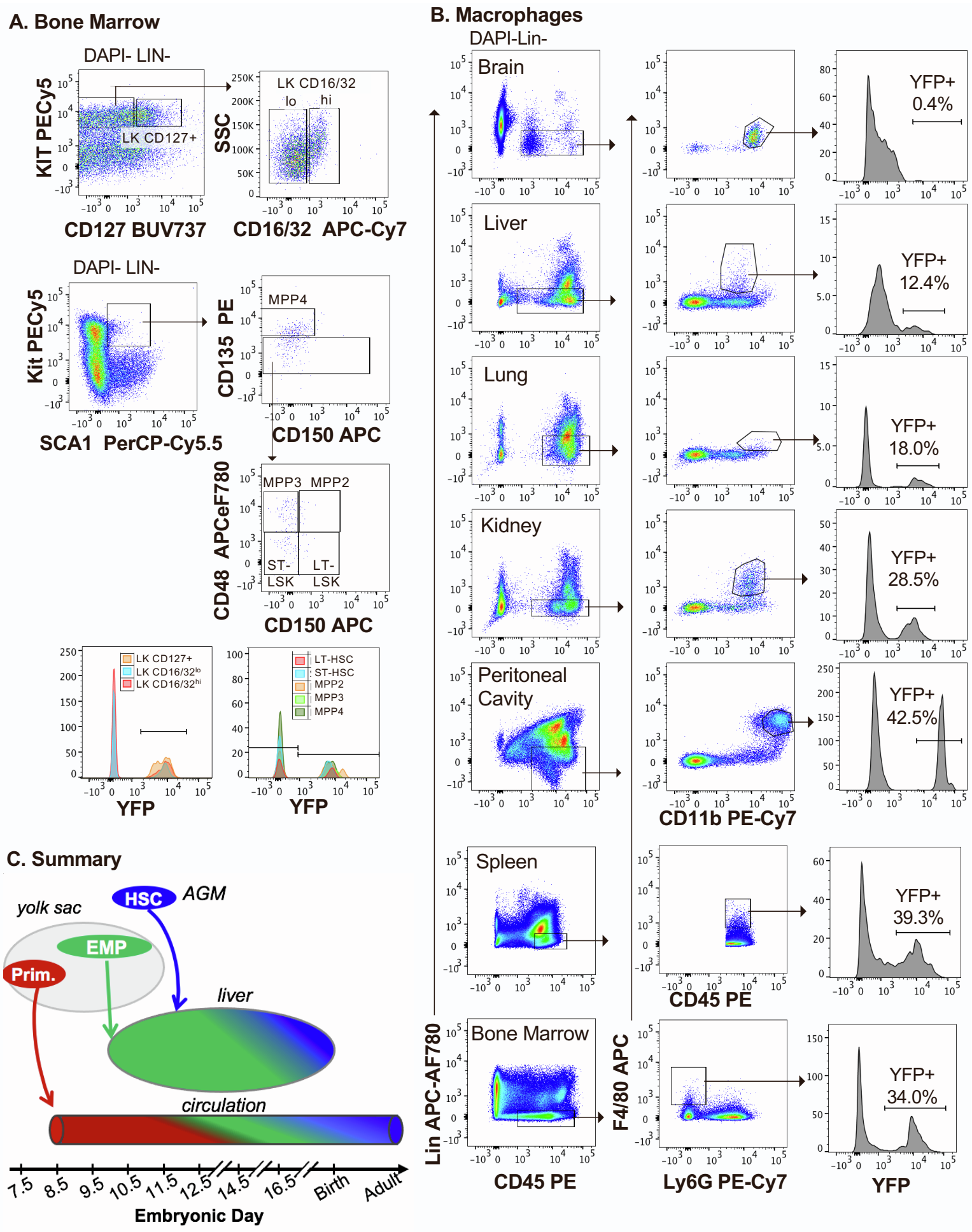

**Supplemental Figure 1. *Mds1*$^{lacz}$ labeling and *Runx1* immunohistochemistry relating to Figure 1H.**
**A-D.** Photomicrograph of E10.5 (A, B) or E11.5 (C, D) aorta, left is ventral. β-galactosidase-positive cells (A, C) that also express Runx1 detected by immunocytochemistry (B, D) are indicated by arrows. Size Bars= 20um


A. E11.5 AGM
DAPI- FSC/SSC
Kit PEcy7
9.8%
Ter119 APCAF780
8.1%
CD31 PE
VEC EF660
42%
CD45 AF700
PreHSC
YFP+
5%
FSC
YFP
B. E11.5 Yolk Sac
DAPI- FSC/SSC
VEC EF660
4.6%
Ter119 APCAF780
Kit PEcf594
54%
CD41 PEcy7
CD16/32 PE
24%
CD45 AF700
EMP
YFP+
0%
FSC
YFP
C. E11.5 (TAM E8.5)
% YFP+
15
0
Paw
AGM
Blood
PreHSC
Yolk Sac
Blood
EMP
Blood
EryP
Liver
EryD
Blood
Kit-
CD45+
Liver
Blood
Kit-
CD41+
Liver
D. E12.5 Blood
DAPI- Kit-
SSC
FSC
72%
FSC
EryP
98%
Ter119 APC
FSC
YFP+
0%
YFP
E. E12.5 Liver
DAPI- FSC/SSC
Kit PE-CF594
Kit+
16%
0.9%
72%
YFP+
16%
YFP
CD11b APC-AF780
Ter119 APC
EryD
90%
FSC
YFP+
0%
YFP
Ly6G+
23%
Ly6G PE-Cy7
YFP+
0%
YFP
F. Adult Blood
DAPI- FSC/SSC
CD11b APC-AF780
CD11b+
CD19+
CD19 PE
CD3ε+
26%
CD3ε APC
CD11b+
YFP+
64%
YFP
CD3ε+
YPF+
YFP
CD19+
YFP+
85%
YFP

**Supplemental Figure 2. Gating strategies and support data for Figure 2.** **A.** Gating strategy for E11.5 AGM and circulating pre-HSC. Live (DAPI$^-$), FSC/SSC gated cells were further gated Kit+ Ter119-, then CD31+ (PECAM), and finally gated as VEcadherin+ (VEC) with CD45 used to help visualize the populations. **B.** Gating strategy for EMP in the yolk sac or bloodstream. Live (DAPI$^-$), FSC/SSC gated cells were further gated as Ter119- VEcadherin- (to exclude circulating pre-HSC). Then Kit+ CD41$^{mid}$ cells were further gated as CD16/32+. CD45 was used to help further visualize the EMP population. **C.** Analysis of E11.5 *Mds1*$^{CreERT2}$ *Rosa26*$^{LSL\text{-}YFP}$ fetuses after TAM delivery at E8.5. Analyses were performed as in Figures 2H,I, with YFP+ cells in the paws serving as a positive control for *Mds1*$^{CreERT2}$ expression in these fetuses. Average ±SEM of 3 individuals are plotted. **D.** Gating strategy for cells in fetal blood quantitated in Figure 2I, J and below panel E. Primitive erythroid cells (EryP) are gated out of Kit$^-$ Live (DAPI$^-$) blood as Ter119$^+$. **E.** Gating strategy for fetal liver quantitated in Figure 2I, J and below panel E. Live (DAPI$^-$), FSC/SSC gated cells were further gated as Kit$^+$, CD11b$^+$, Kit-CD11b$^-$. CD11b$^+$ were further gated as GR1+, and Kit-CD11b- that are gated by Ter119+ and FSC to gate maturing definitive erythroblasts (EryD). YFP gating demonstrates positivity found Kit+ cell, but not EryD or GR1+ populations. **F**. After RBC lysis, adult peripheral blood cells were initially gated by live (DAPI-) FSC/SSC then gated as CD11b+ myeloid cells, CD19+ B-cells and from the double negative CD3e+ T-cells. Then each population was gated for YFP+.


A. Fetal Liver Progenitors
DAPI- Lin-
Kit PE-Cy7
LK- 55%
LSK 2.7%
Sca1 PerCPCy5.5
CD127 APC
CD127+ 4.5%
CD16/32+ 27%
CD16/32- 61%
CD16/32 PE
YFP+ 8.5%
LSK
YFP+ 4.2%
CD127+
YFP+ 3.1%
LK
YFP+ 4.6%
CD16/32+
YFP+ 4.4%
CD16/32-
Kit PE-Cy7
YFP
B. Fetal Liver Lineage
DAPI-
Ly6G PE-Cy7
Ly6G+ 2.7%
8.0%
87%
Ly6C APC
Ly6C+ 10%
CD19+ 3.4%
SSC
EryD 72%
Ter119 APC-AF780
PE CD19
FSC
Ly6G+
YFP+ 2.9%
Ly6C+
YFP+ 3.4%
CD19+
YFP+ 2.7%
EryD
YFP+ 2.6%
SSC
YFP
C. E16.5 Fetal Blood Erythroid
DAPI-, FSC/SSC, Ter119+
-Cre
YFP+ 0.1%
+Cre
YFP+ 1.9%
YFP
FSC
D. Fetal Liver LSK
DAPI-
Kit PE-Cy7
LSK 2.2%
Sca1 PerCPCy5.5
Flt3 PECF594
MPP4 50%
44%
CD150 APC
CD48 PE
MPP3 45%
MPP2 33%
ST-HSC 12%
LT-HSC 11%
CD150 APC
YFP+ 14%
LT-HSC
YFP+ 13%
ST-HSC
Kit PE-Cy7
YFP+ 10%
MPP2
YFP+ 8.5%
MPP3
YFP+ 8.4%
MPP4
YFP
E. Fetal Macrophages
DAPI-Ter119-CD45+
Brain
F4/80 APC
Mac 81%
CD11b PEcy7
Liver
Mac 19%
CD45 PE
SSC
YFP+ 0%
YFP+ 2.6%
YFP

**Supplemental Figure 3. Gating strategies utilized in Figure 3.** **A.** Example of gating for Figure 3A, B and C. Fetal liver cells were gated to assay YFP positivity of progenitors starting with FSC/SSC, lineage (Ter119, GR-1, CD19, CD3e) negative, live ($DAPI^-$) cells that were then gated as $Kit^+$ $Sca1^-$ (LK) or $Kit^+$ $Sca^+$ (LSK). LK were further gated as CD127+ (CD127+), and then Kit+ CD127- cells were further gated into CD16/32+ and - populations. **B.** Maturing lineages within the fetal liver or blood were gated from a population of FSC/SSC live (DAPI-) Kit- cells (middle graph) as Ly6G+ or Ter119+ or double negative cells. In the liver, Ter119+ cells were further refined for high SSC/FSC (right) to gate erythroblast versus circulating erythrocytes (See C for gating of blood erythrocytes). The double negative cells were future discriminated (left) into CD19+ or Ly6C+ cells. Note that this gating strategy removes the lower Ly6C+ granulocytes (Ly6G+) allowing gating of the Ly6C+ monocytes. **C.** Gating of circulating erythrocytes. Due to the decreasing Rosa expression in erythroid cells as they mature (note the already lower value of liver erythroblasts versus other precursors) the gating of low YFP positivity in erythrocytes above Cre-negative littermates is shown. **D.** Example of subgating of E16.5 LSK quantitated in Figure 3C. LSK were further refined into MPP4- CD135 (Flt3+, CD150-) or LT-HSC, ST-HSC, MPP2 and MPP3 out of the LSK Flt3- cells. **E.** Macrophages were identified by their F4/80 positivity in different tissues from FSC, SSC, live (DAPI-), Ter119- cells. In brain and liver additional CD11b positivity was used but not in fetal liver, which is highly erythropoietic as the macrophages associated with erythropoiesis do not express CD11b (Seu et al., 2017).


A. Bone Marrow
DAPI- LIN-
KIT PECy5
LK CD127+
CD127 BUV737
SSC
LK CD16/32
lo
hi
CD16/32 APC-Cy7
DAPI- LIN-
Kit PECy5
SCA1 PerCP-Cy5.5
CD135 PE
MPP4
CD150 APC
CD48 APCeF780
MPP3
MPP2
ST-
LSK
LT-
LSK
CD150 APC
LK CD127+
LK CD16/32lo
LK CD16/32hi
YFP
LT-HSC
ST-HSC
MPP2
MPP3
MPP4
YFP
B. Macrophages
DAPI-Lin-
Brain
YFP+
0.4%
Liver
YFP+
12.4%
Lung
YFP+
18.0%
Kidney
YFP+
28.5%
Peritoneal
Cavity
YFP+
42.5%
CD11b PE-Cy7
Spleen
YFP+
39.3%
CD45 PE
Bone Marrow
YFP+
34.0%
Lin APC-AF780
CD45 PE
F4/80 APC
Ly6G PE-Cy7
YFP
C. Summary
yolk sac
HSC
AGM
EMP
Prim.
liver
circulation
7.5
8.5
9.5
10.5
11.5
12.5
14.5
16.5
Birth
Adult
Embryonic Day

**Supplemental Figure 4 Gating strategies utilized in Figure 4 and Summary.** **A.** YFP positivity of more specified hematopoietic progenitors (top) in adult bone marrow was determine by first gating FSC/SSC, lineage (Ter119, GR-1, CD19, CD3e) negative, live (DAPI-) cells that were then gated as kit+ CD127+ (LK CD127+) and then kit+ CD127- further gated into CD16/32 hi and negative. More multipotent progenitors were gated first (middle) out of the Kit+ Sca+ population (LKS) and further refined into MPP4- CD135 (Flt3+, CD150-) or LT-HSC, ST-HSC, MPP2 and MPP3 out of the LSK Flt3- cells. YFP gating of all populations are shown below. **B.** Macrophages were identified by their F4/80 positivity in different tissues from FSC, SSC, live (DAPI-), lineage (Ter119, CD19, CD19, CD3e) negative cells. For most tissues, additional CD11b positivity was used but not in spleen and bone marrow because of evidence that CD11b- macrophages are involved in erythropoiesis in those tissues (Seu et al., 2017). **C.** Overlapping temporal waves of primitive hematopoietic progenitors and erythro-myeloid progenitors (EMPs) emerge in the yolk sac, followed by hematopoietic stem cells (HSCs), which emerge in the aorta-gonad-mesonephros region (AGM). Differentiated progeny of the primitive hematopoietic lineages enter the newly forming bloodstream beginning at E8.25. EMPs, and then the HSCs, colonize the fetal liver, where they differentiate to produce hematopoietic cells that also enter the circulation. Lineage tracing with Mds1Cre$^{ERT2}$ induced at E9.5 with TAM labels HSC (blue), but not primitive hematopoiesis (Prim. red) or EMP (green) and demonstrates that HSC-derived hematopoiesis increases over time in the fetal liver and in the circulation.