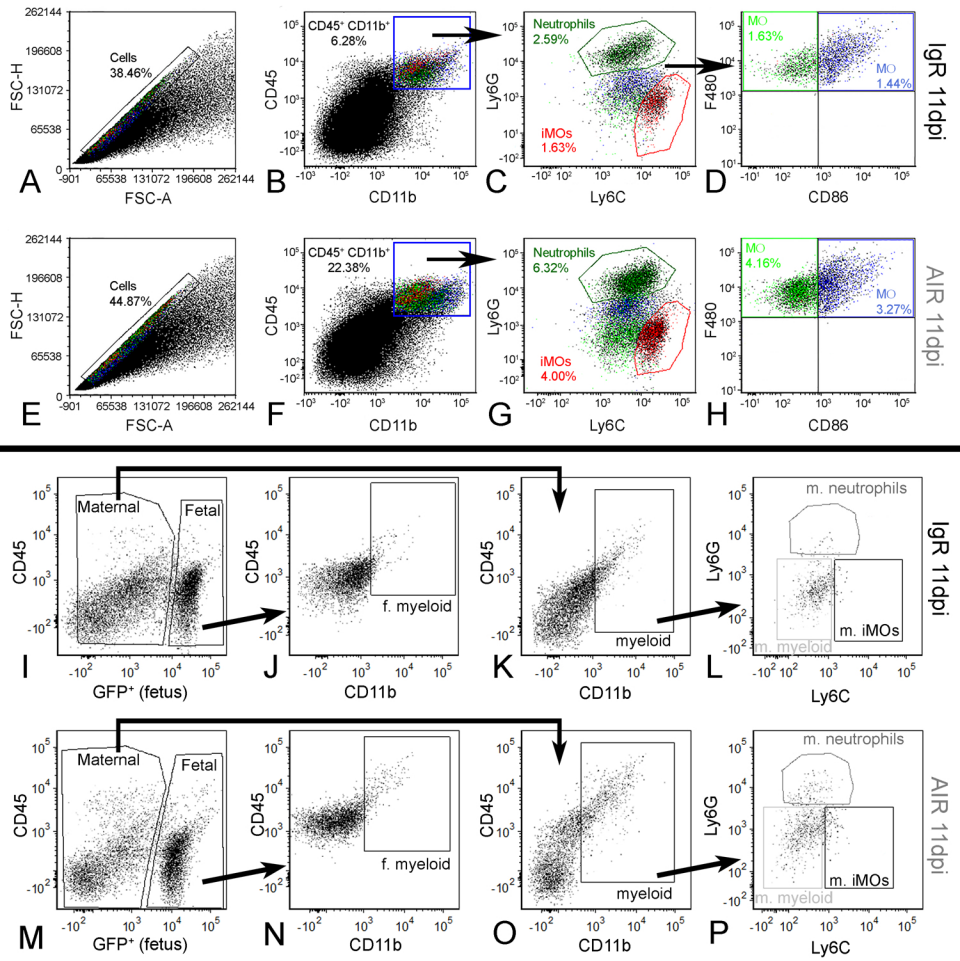
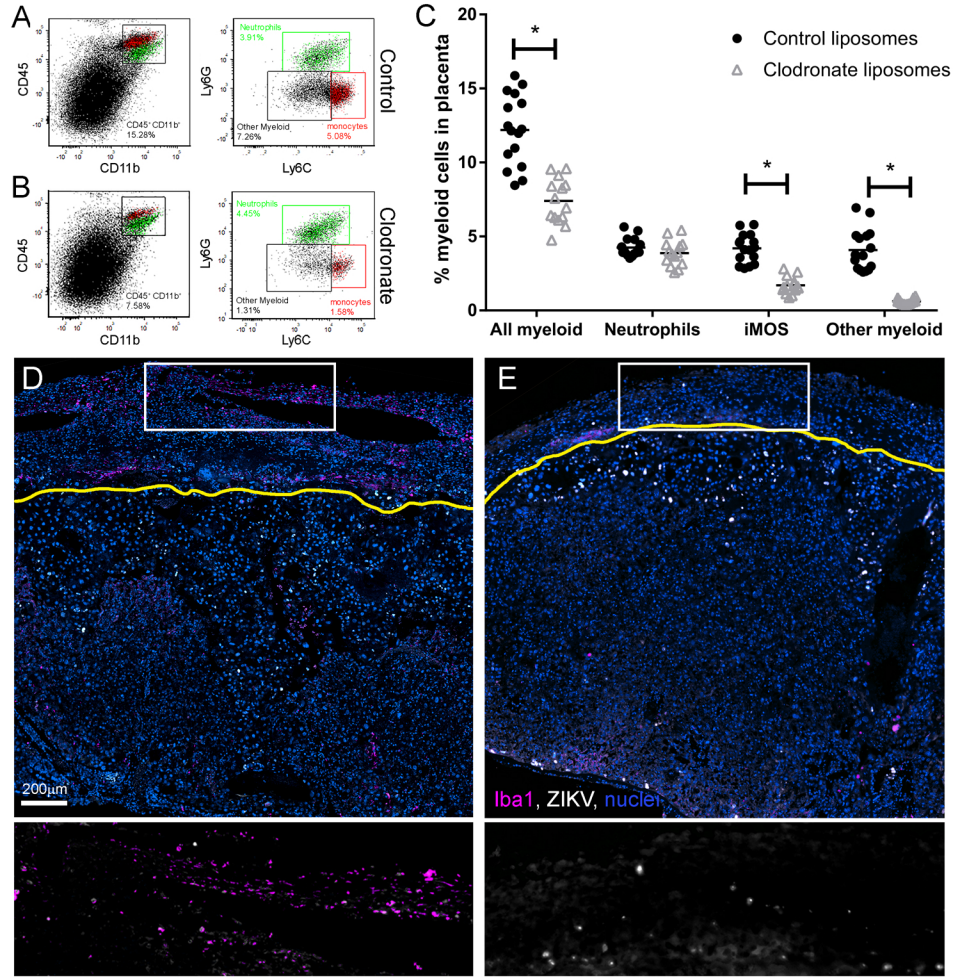



Sup. Fig. 1. *Flow cytometry and FACs gating strategy for myeloid cells in the placenta and fetus*

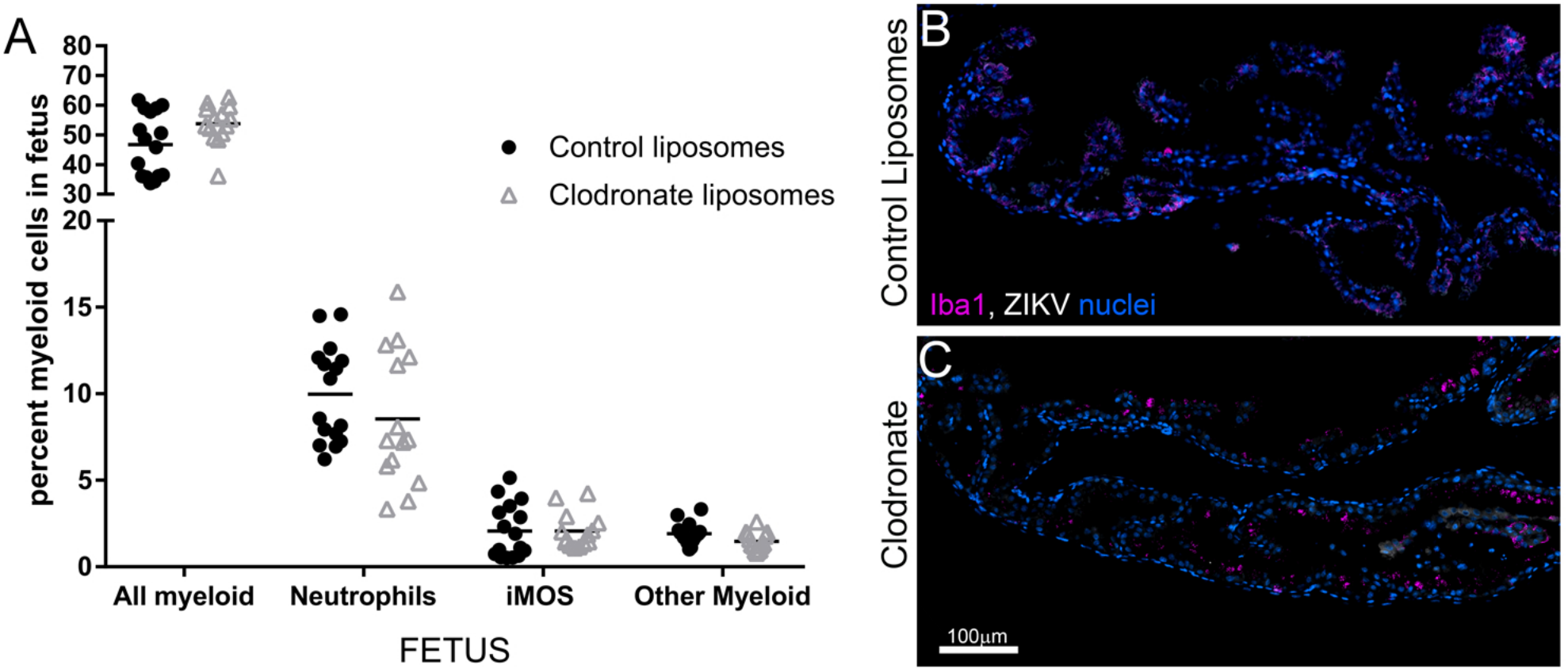
Placental and fetal tissue was isolated and process according to the materials and methods. Flow cytometry gating strategy examples are shown for an IgR (A-D) and AIR (E-H) placenta samples at 11dpi. The same gating strategy was at all time points and also used when analyzing fetal tissue. Flow data were gated on forward scatter height and area to exclude doublets and noise. Plots (C, D and G, H) are derived from the CD45+/CD11b+ gated populations (blue gates in B and F) to clearly shown only myeloid cell populations. Proportions calculated and shown in Fig 1 and 5 are based on the entire placental population.

FACs gating strategy examples are shown for placental cells from IgR (I-L) and AIR (M-P) pregnancies at 11dpi but the same gating was at both time points and also for fetal tissue with the exception that maternal myeloid cells were collected as a bulk population rather fetal cells as is shown here for placenta (see discussion in materials and methods). Cells were first gated for GFP expression to distinguish between maternal and fetal cells (I, M). Fetal cells were gated using CD45+/CD11b+ expression to obtain a bulk fetal myeloid population (J, N). Maternal cells were gated using CD45+/CD11b+ to identify myeloid cells (K, O) and those cells were further analyzed for expression of Ly6C+ to identify inflammatory monocytes (m. iMOs) and Ly6+ to identify neutrophils (m. neutrophils, L, P). The remaining myeloid population was sorted as maternal myeloid cells (m. myeloid).



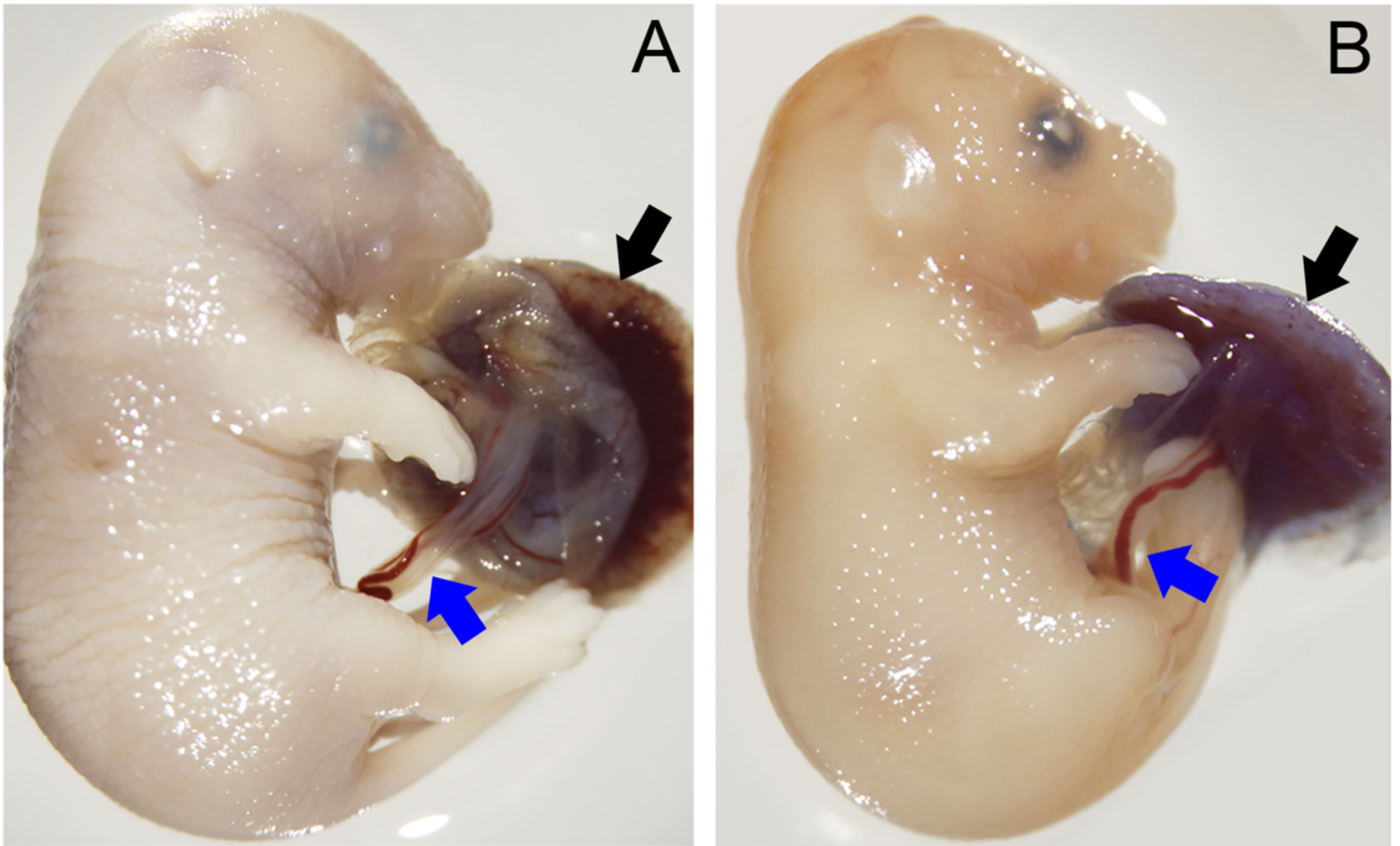

Sup. Fig. 2. *Myeloid cell depletion with clodronate in the placenta of ZIKV infected AIR mice*

Placental tissue collected from 12dpi infected AIR mice treated with either clodronate (A) or control liposomes (B) was analyzed via flow cytometry for markers of myeloid cell populations. An abbreviated gating strategy (described in material and methods) is shown. The relative proportions of all myeloid cells, neutrophils, monocytes (iMOs) and other myeloid cells is from control (black symbols) or clodronate (gray symbols) are shown as a percentage of total placenta cells. A two-way ANOVA with a Sidak's multiple comparisons test was used to determine statistical significance. *=$p<0.05$. Representative section of placenta from control (D) or clodronate (E) treated fetuses are shown. The area above the yellow line indicates the maternal decidua. Sections are labeled immunohistochemically with Iba1 (magenta) and ZIKV NS5 (white). Blue label indicates Hoechst staining of cell nuclei. The white boxes in (D) and (E) indicate the position of the insets shown below each image. In the insets, only the Iba1 and ZIKV labeling is shown for clarity. Scale bar in (D) is also relevant to (E).




Sup. Fig. 3. *Clodronate does not cross the placental barrier during ZIKV infection as it does not deplete myeloid cells in the fetus*

Using the same litters of pregnant, infected AIR mice treated with control of clodronate liposomes shown in Sup. Fig. 3A-C, myeloid populations isolated from whole fetuses were analyzed via flow cytometry as previously described (A). All myeloid populations are plotted as percent cells within the whole fetus. Representative sections of the umbilical cord from 12dpi control (B) or clodronate (C) liposome treated fetuses described in Table I are shown. Sections are labeled immunohistochemically with Iba1 (magenta). and ZIKV NS5 (white). Blue label indicates Hoechst staining of cell nuclei. The scale bar in (C) is also relevant to (A).




Sup. Fig. 4. *Myeloid cell depletion does not cause large scale breakdown of the placental barrier*

Gross histological analysis of Evans Blue dye leakage across the placenta into the umbilical cord or fetus from 11dpi litters treated with control (A) or clodronate (B) liposomes. Black arrows indicate the maternal decidua of the placenta where dye is evident. Blue arrows indicate the umbilical cord where no dye was visually present.