Supplemental Data

Supplemental Methods

Tissue processing. Samples of chorion comprised both the smooth chorion (SC) and the chorionic plate (CP), which was denuded of chorionic villi (Fig. S1A and B), unless otherwise specified that only the SC was used (Fig.9). Once that the tissues were dissected under a microscope, we processed them following a method previously described (Genbacev et al., 2011) that we modified to improve the recovery of hematopoietic cells (Barcena et al., 2009). Specifically, we added a final enzymatic digestion treatment of the chorion, amnion and chorionic villi preparations with 181 U/ml collagenase I-A, 0.12 mg/ml DNase I, 0.70 mg/ml hyaluronidase type I-S, and 1 mg/ml BSA in PBS at 37°C. Digestion continued for 5-60 min until total cellular dissociation was observed. For tissues 5-6 wks of gestational age, it was not possible to separate the membrane portion from the placental chorionic villi, therefore we prepared cells from whole chorion. In older specimens (≥ 7 wks) the CP was dissected from chorionic villi and those tissues were prepared separately. For 7-12 wks specimens, transplantation and FACS were performed on the dissociated cells without further fractionation. For samples older than 12 wks, the light-density (LD) fraction was obtained (by centrifugation over Nycoprep (1.077 g/ml; Greiner Bio-One, Monroe, NC) for 30 min (25°C) at 600 × *g*) for FACS and transplantation, as previously described (Barcena et al., 2009). For older samples (≥14 wks) in which the amnion has begun to fuse to the chorion, manual separation of these two membranes was performed, as shown macroscopically in Fig S1C and at a histological level in Fig. S1D and E (before and after amnion removal, respectively). This step was followed by enzymatic dissociation and isolation of the LD fraction was then subjected to our standard mature lineage(lin) depletion to enrich in hematopoietic progenitors prior to transplantation. The mAbs used for depletion were all unconjugated and obtained from BioLegend (San Diego, CA), used at 1μg/10^6 target cells, and recognized CD235a (erythrocytes), CD14 (macrophages), CD19 and CD29 (B cells), CD3 (T cells) and CD56 (NK cells). After washing to remove the excess unbound mAbs, the cells were subjected to negative magnetic selection by using BioMag magnetic beads coated with goat anti-mouse IgG antibody (Qiagen Inc., Germantown, MD). The resulting LD lin^- cell suspension was

counted and transplanted into mice. For FACS analyses, no lineage depletions were performed.

Human hematopoietic engraftment of mice. NSG-3GS mice were only used for the transplant experiment associated with the FISH analyses shown in Fig. 9; NSG mice were used in all other experiments. Mice were irradiated with 175 or 200 cGy using an RS2000 X-Ray Biological Irradiator (RAD Source Technologies, Inc., Alpharetta, GA, USA) 1 to 3 hours before the procedure. Female and male mice were transplanted as adults (≥8 wks old) by tail-vein injection using a 28g U100 insulin syringe (BD, Franklin Lakes, NJ, USA). The cells were suspended in 200 µL of PBS (Mediatech, Inc., Manassas, VA, USA). For several days before and for the first month after transplant, the standard chow was replaced with irradiated Global 2018 rodent diet with 4100 ppm Uniprim® (Harlan Laboratories, Inc., Hayward, CA, USA). Most of the transplanted mice were analyzed between 100 and 150 days post-transplant. Mice were sacrificed by carbon dioxide asphyxiation followed by cervical dislocation. BM was harvested by using a syringe with a 27-gauge needle to flush the femurs with approximately 3 ml of culture medium as described (Mahajan et al., 2015). Spleen and liver specimens were held in culture medium on ice until cell isolation. At the conclusion of each experiment, mice were examined for signs of poor health or pathologies such as tumors of the liver, spleen, and/or thymus. Any moribund animals were removed from the study. Mouse BM, spleen and liver were processed as previously described (Varga et al., 2010).

Immunolocalization and fluorescence microscopy. We applied previously reported methods (Prakobphol et al., 2006). The binding of antibodies that were not direct conjugates was detected by using the appropriate species-specific secondary antibody, a rhodamine-conjugated donkey anti-mouse IgG for anti-CD45 (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and AF 633-conjugated goat-anti-rabbit IgG for anti-vimentin (Invitrogen). As controls, serial sections were stained with the secondary antibody alone or mouse-IgG_1-FITC (BD Pharmingen).

FISH. In brief, 2-10 x 10^3 sorted cells were placed in ice cold fresh fixative (v/v 3:1 methanol:acetic acid) for 30 minutes at 4°C. Then the cells were dropped onto a slide and dried under controlled conditions (25°C, 38% relative humidity). We performed FISH using X-and Y-chromosome specific probes following manufacturer's protocol

(Abbott Molecular) and methods we have previously reported (Qi et al., 2015).

Supplemental References

Barcena, A., Muench, M. O., Kapidzic, M. and Fisher, S. J. (2009). A new role for the human placenta as a hematopoietic site throughout gestation. *Reprod Sci* **16**, 178-187.

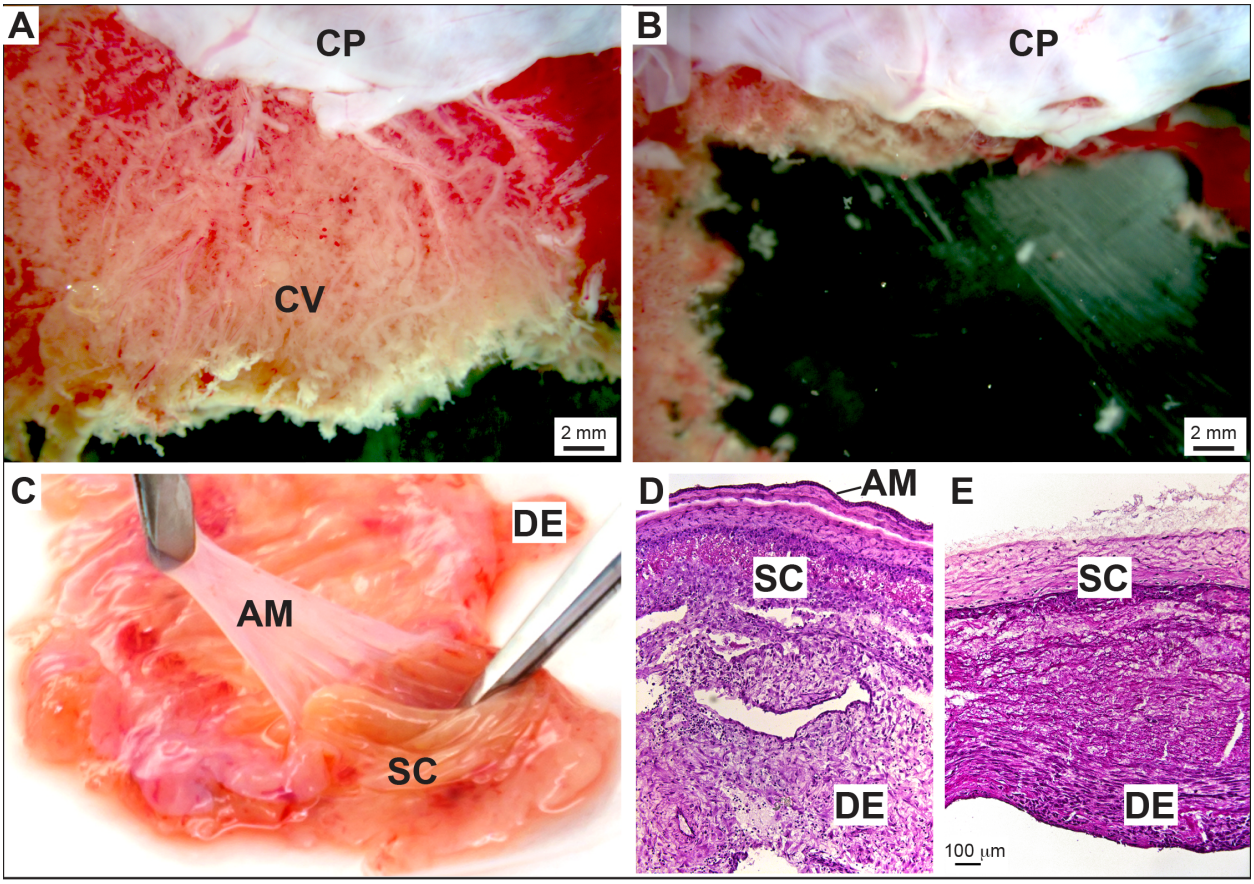
Genbacev, O., Donne, M., Kapidzic, M., Gormley, M., Lamb, J., Gilmore, J., Larocque, N., Goldfien, G., Zdravkovic, T., McMaster, M. T., et al. (2011). Establishment of human trophoblast progenitor cell lines from the chorion. *Stem Cells* **29**, 1427-1436.

Mahajan, M. M., Cheng, B., Beyer, A. I., Mulvaney, U. S., Wilkinson, M. B., Fomin, M. E. and Muench, M. O. (2015). A quantitative assessment of the content of hematopoietic stem cells in mouse and human endosteal-bone marrow: a simple and rapid method for the isolation of mouse central bone marrow. *BMC Hematol* **15**, 9.

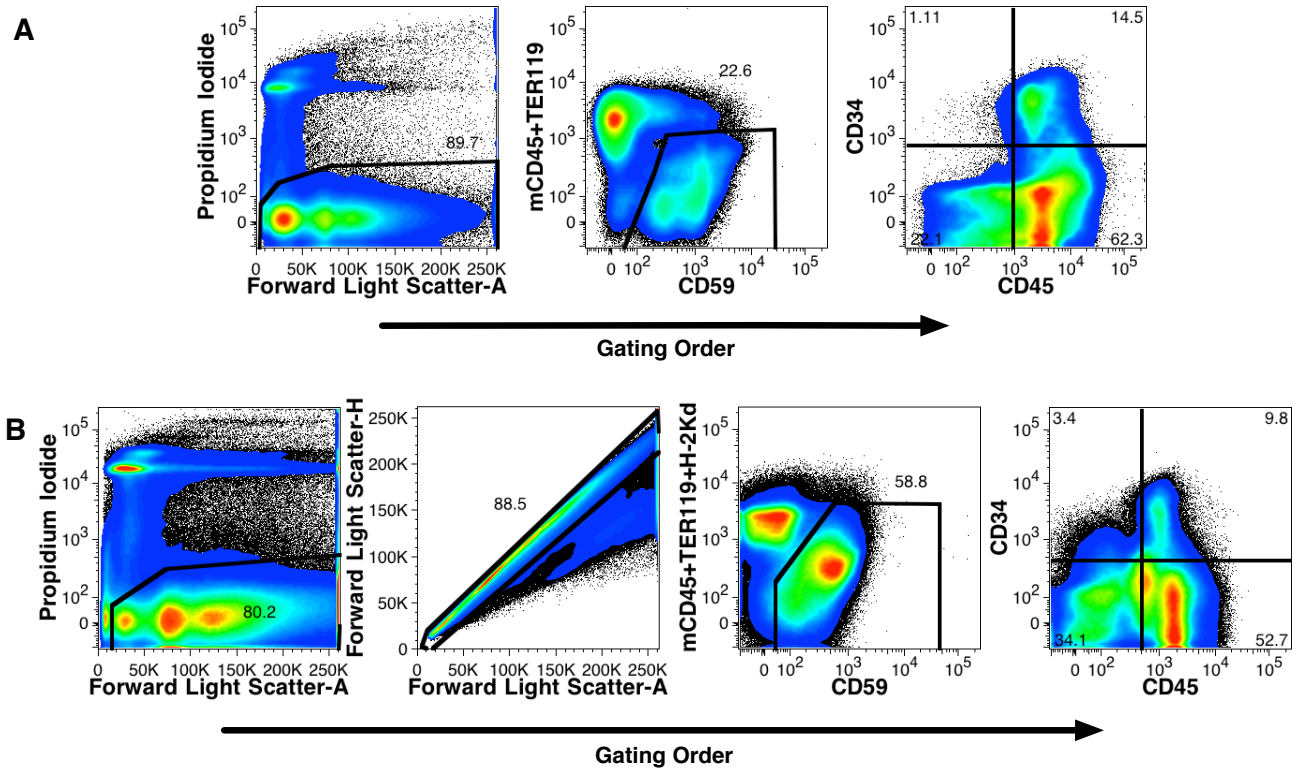
Prakobphol, A., Genbacev, O., Gormley, M., Kapidzic, M. and Fisher, S. J. (2006). A role for the L-selectin adhesion system in mediating cytotrophoblast emigration from the placenta. *Dev Biol* **298**, 107-117.

Qi, Z., Jeng, L. J., Slavotinek, A. and Yu, J. (2015). Haploinsufficiency and triploinsensitivity of the same 6p25.1p24.3 region in a family. *BMC Med Genomics* **8**, 38.

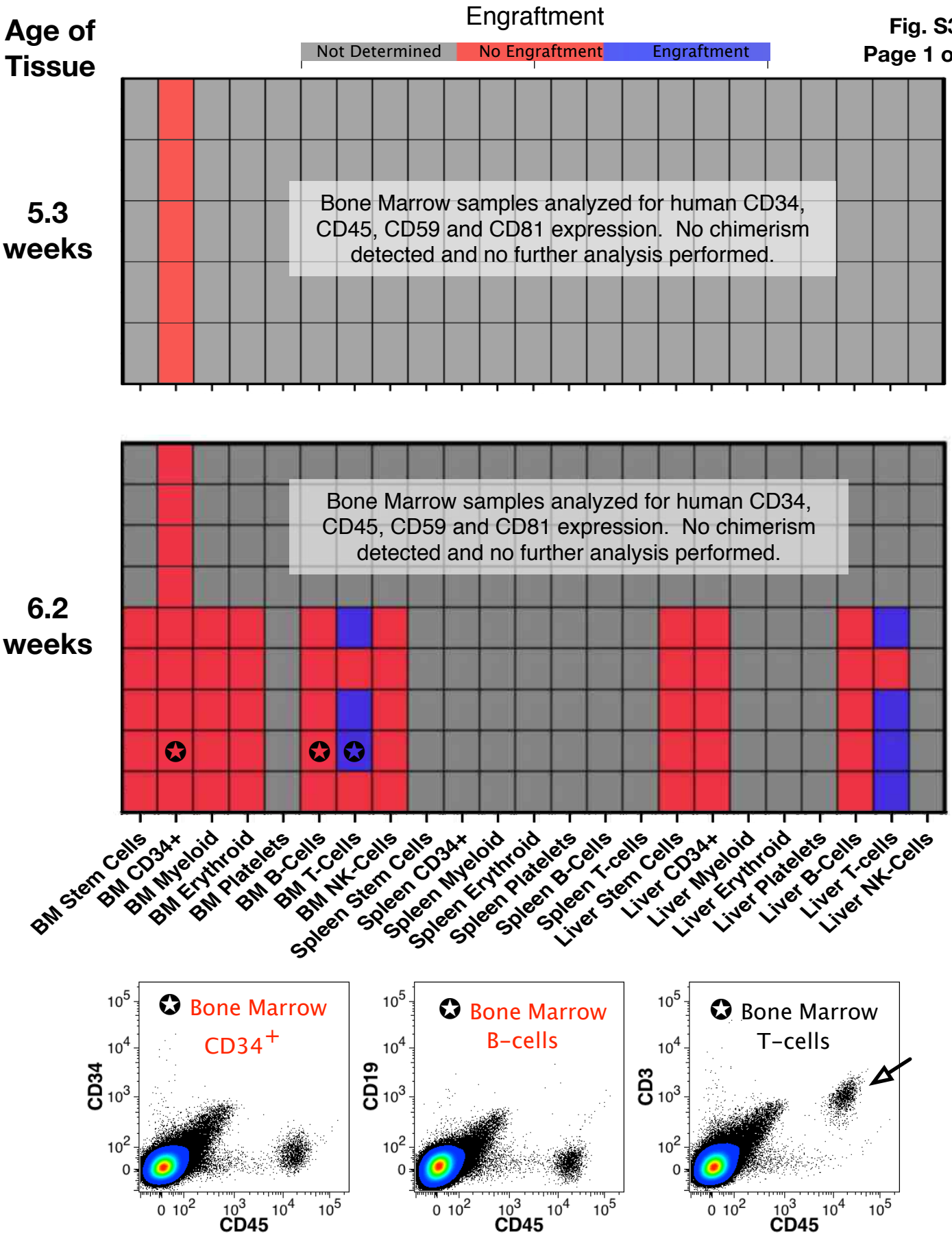
Varga, N. L., Barcena, A., Fomin, M. E. and Muench, M. O. (2010). Detection of human hematopoietic stem cell engraftment in the livers of adult immunodeficient mice by an optimized flow cytometric method. *Stem Cell Stud* **1**.



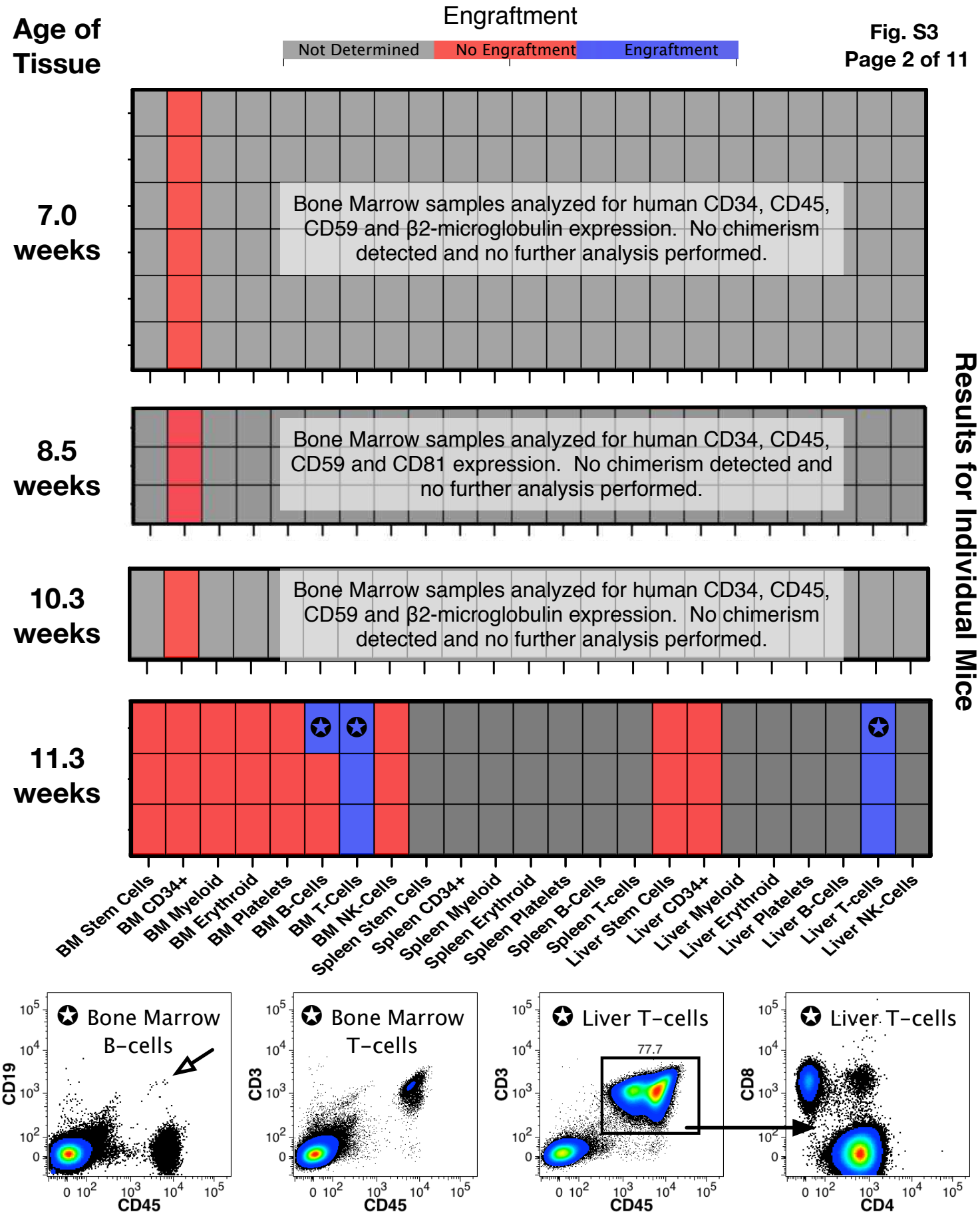
Supplemental Figure S1. Dissection and histology of the fetal human membranes. **A**. A portion of the chorionic plate (CP) of a 19 wk specimen is visualized under a dissection microscope and is shown intact (with the chorionic villi (CV) attached). After dissecting the CV, we obtain the denuded chorion, which is the CP without the CV (**B**). **C.** Manual separation of the amnion (AM) from the smooth chorion (SC) of a 22 wk specimen. A tissue section of the 22 wk amniochorion stained with hematoxylin and eosin is shown prior the removal of the amnion (**D**) and after the removal of the amnion (**E**). On the maternal side of the fetal membrane is the decidua (DE), which is manually removed prior the cell isolation.



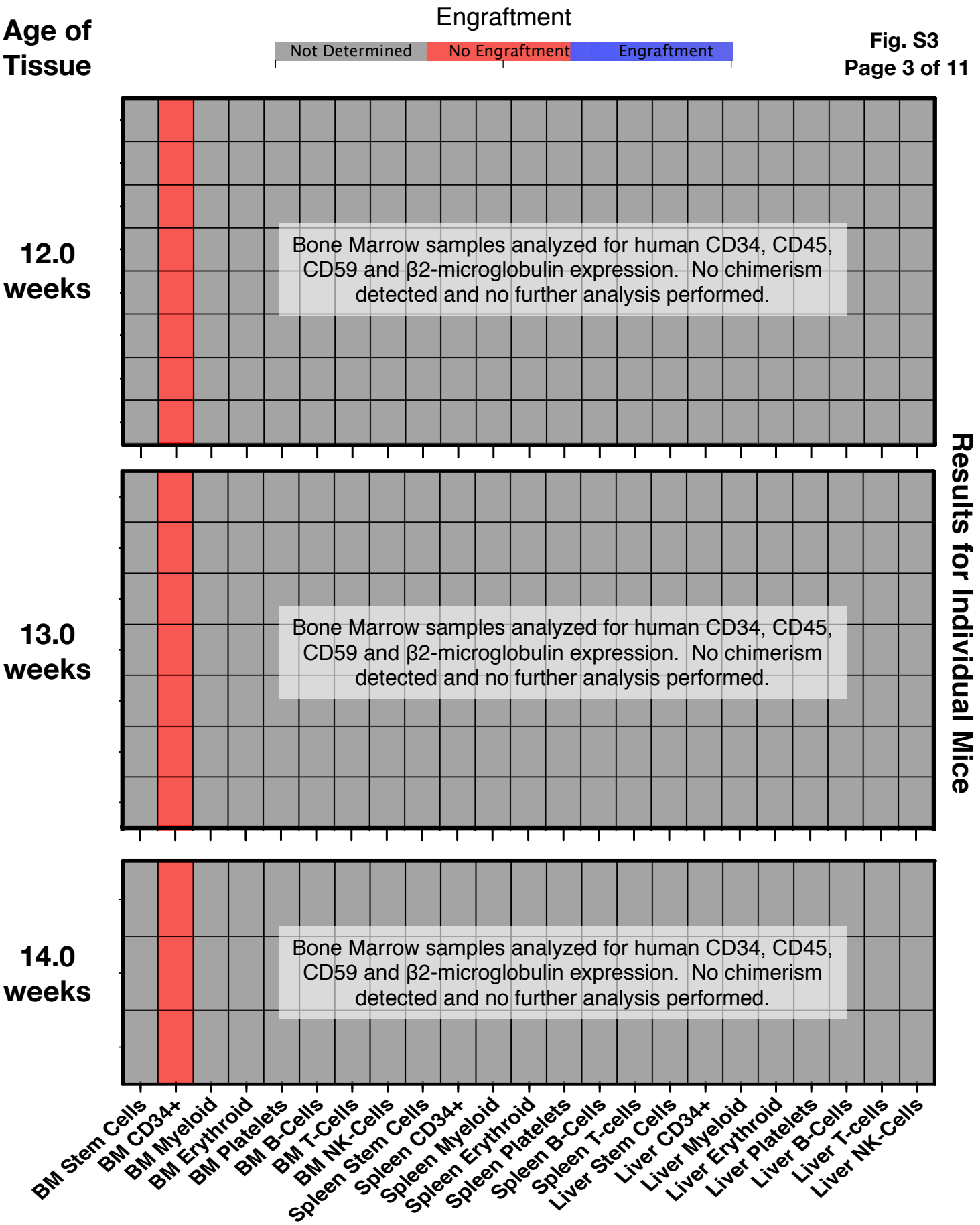
Supplemental Figure S2. Complete gating strategy used to define human cells in mouse BM. A. Live human cells were defined by their expression of CD59 and lack of expression of mouse markers, as well as the lack of propidium iodide staining as indicated in the gates. In some early studies the mouse markers were limited to CD45 and TER119, whereas the class I major histocompatibility antigen H-2k^{d} was stained in later experiments to further stain non-hematopoietic cells of mouse origin (**B**). Additionally, the use of forward-light scatter area (A) and height (H) parameters to exclude doublets was employed in some later analyses. Data shown correspond to engraftment data shown in Figs. 5A (**A**) and 6 (**B**).

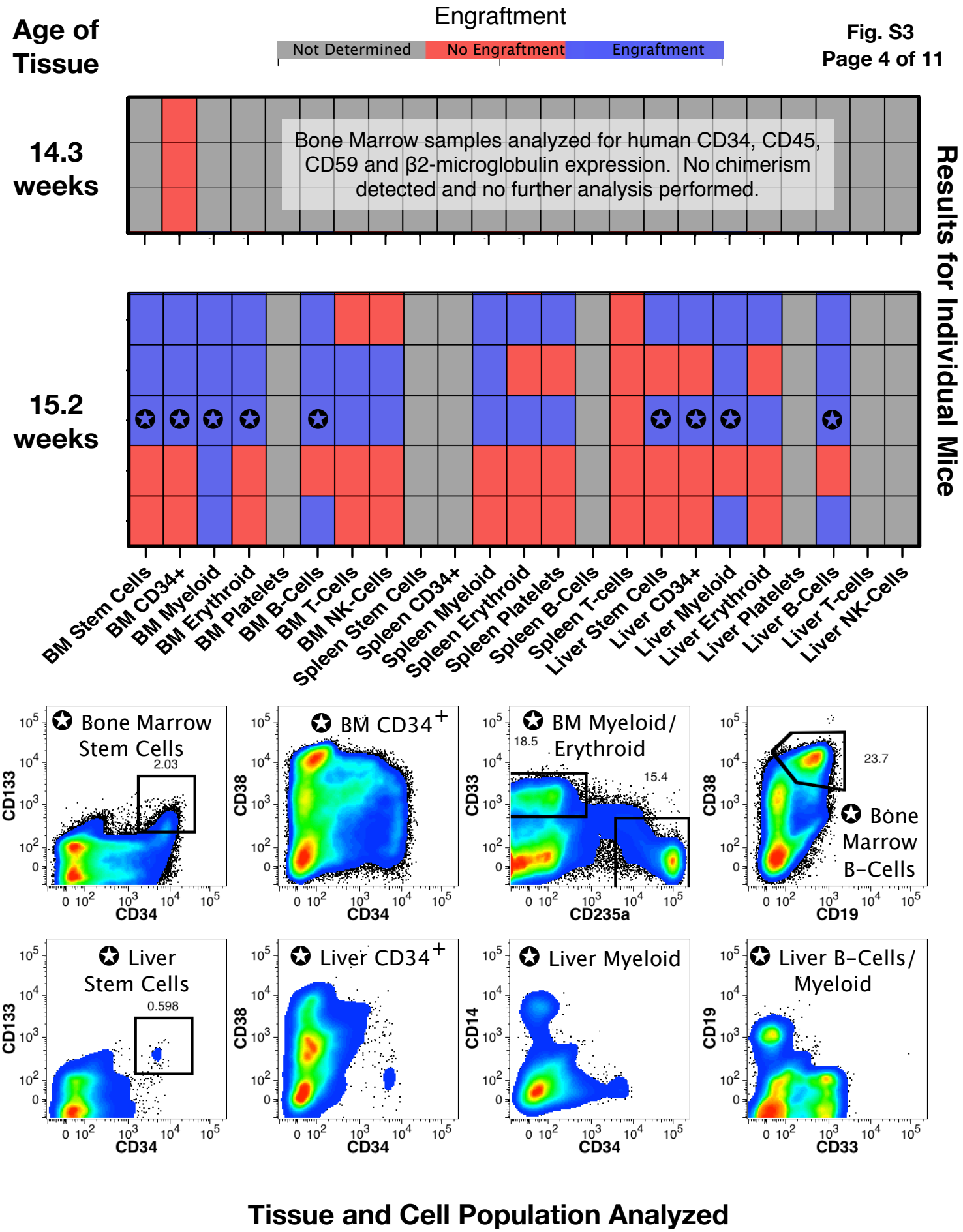


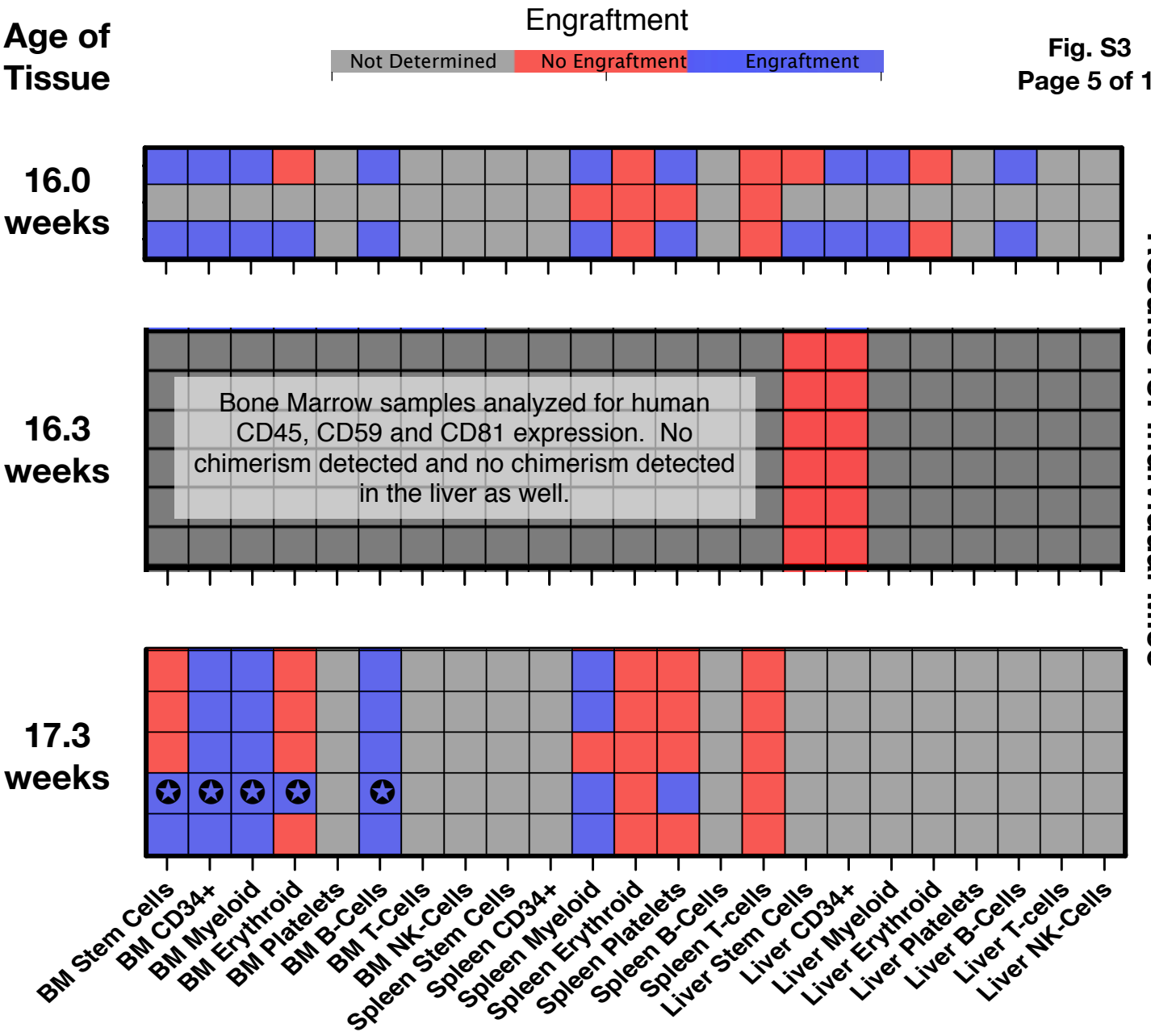
Results for Individual Mice

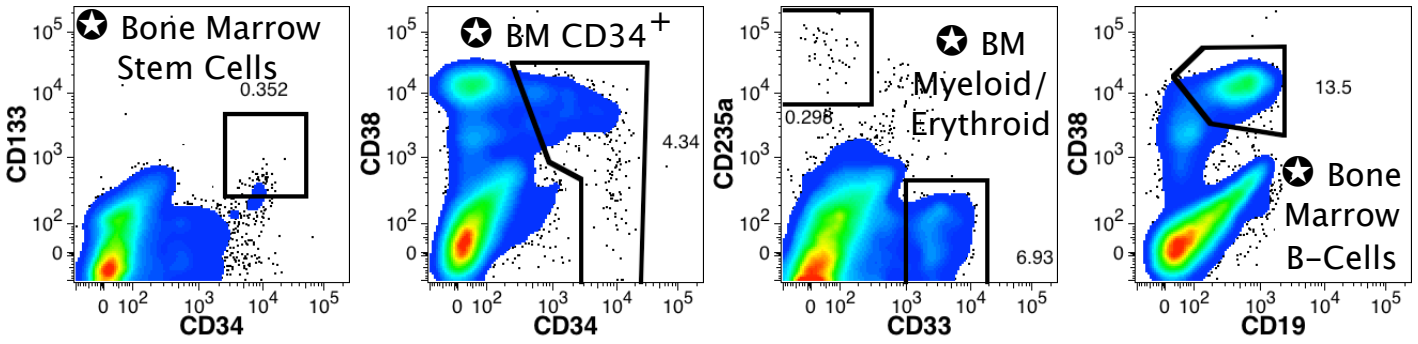


Tissue and Cell Population Analyzed

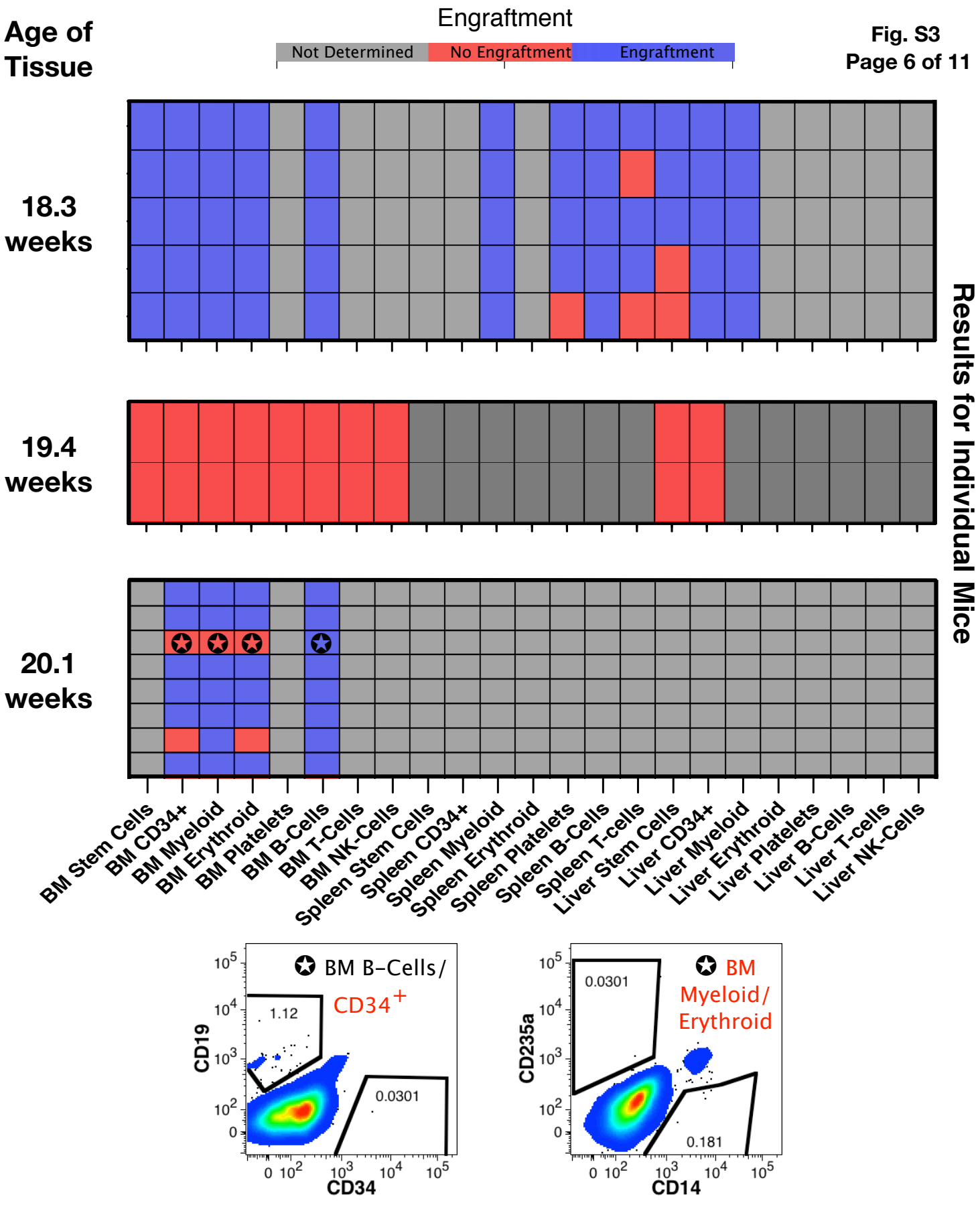


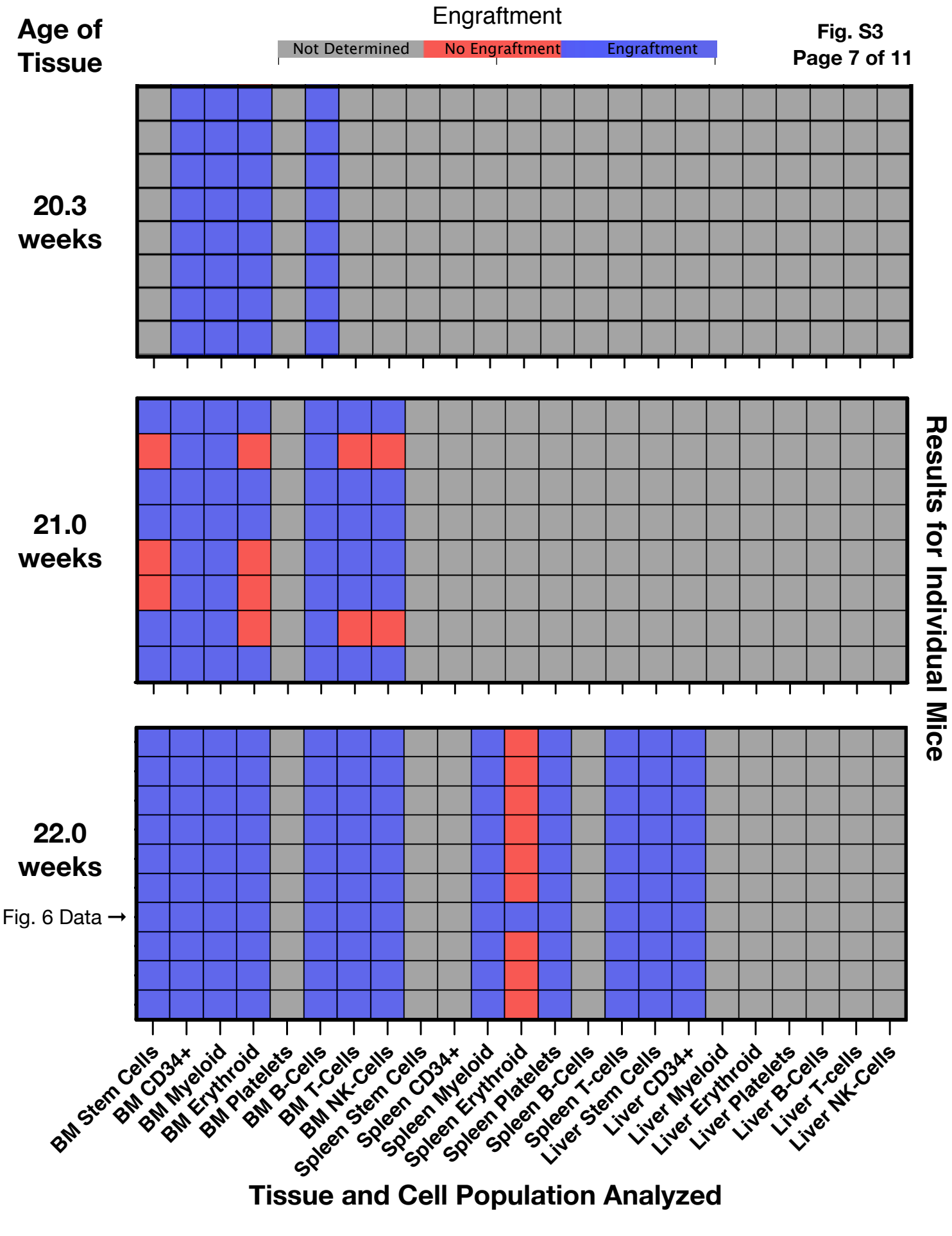


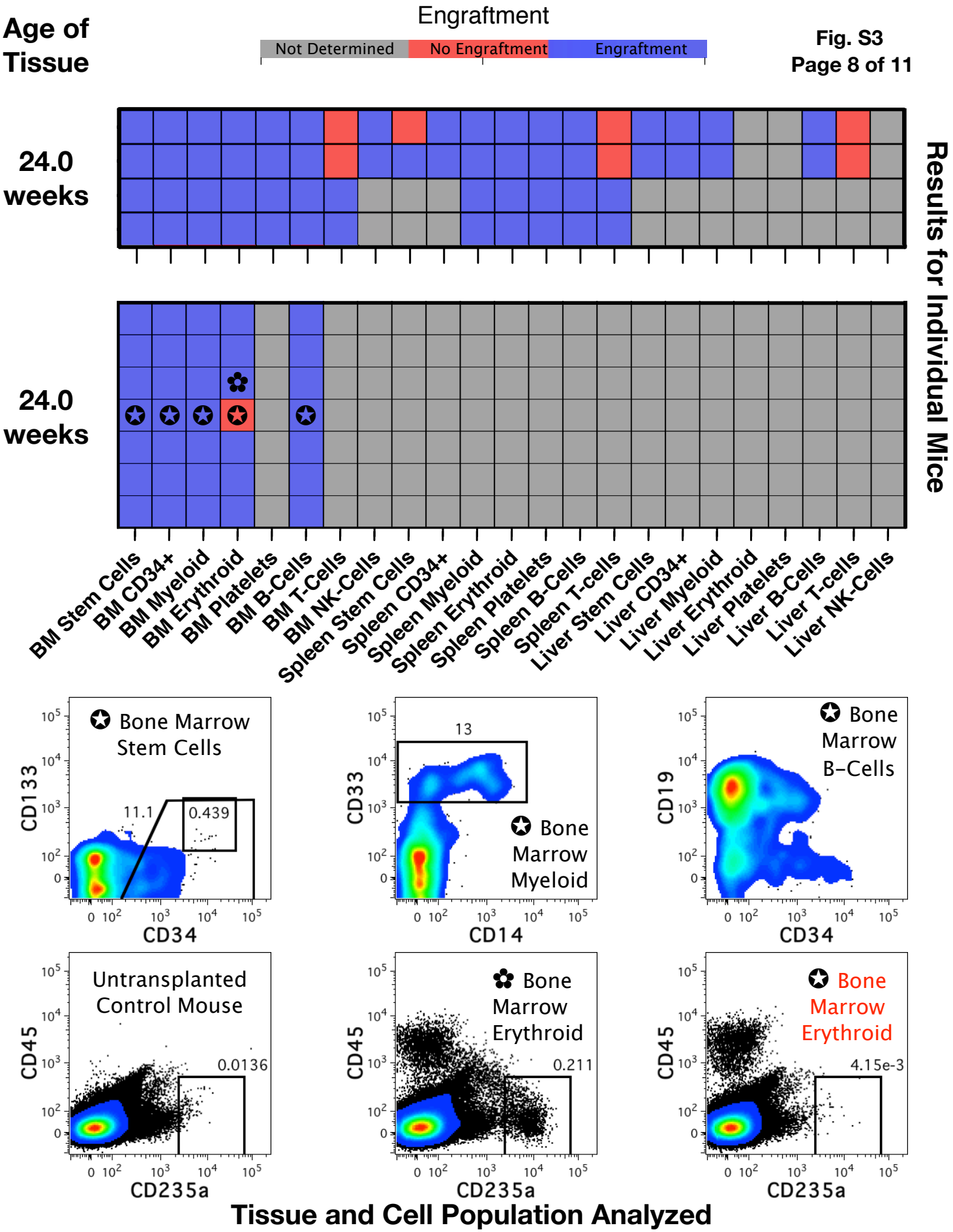


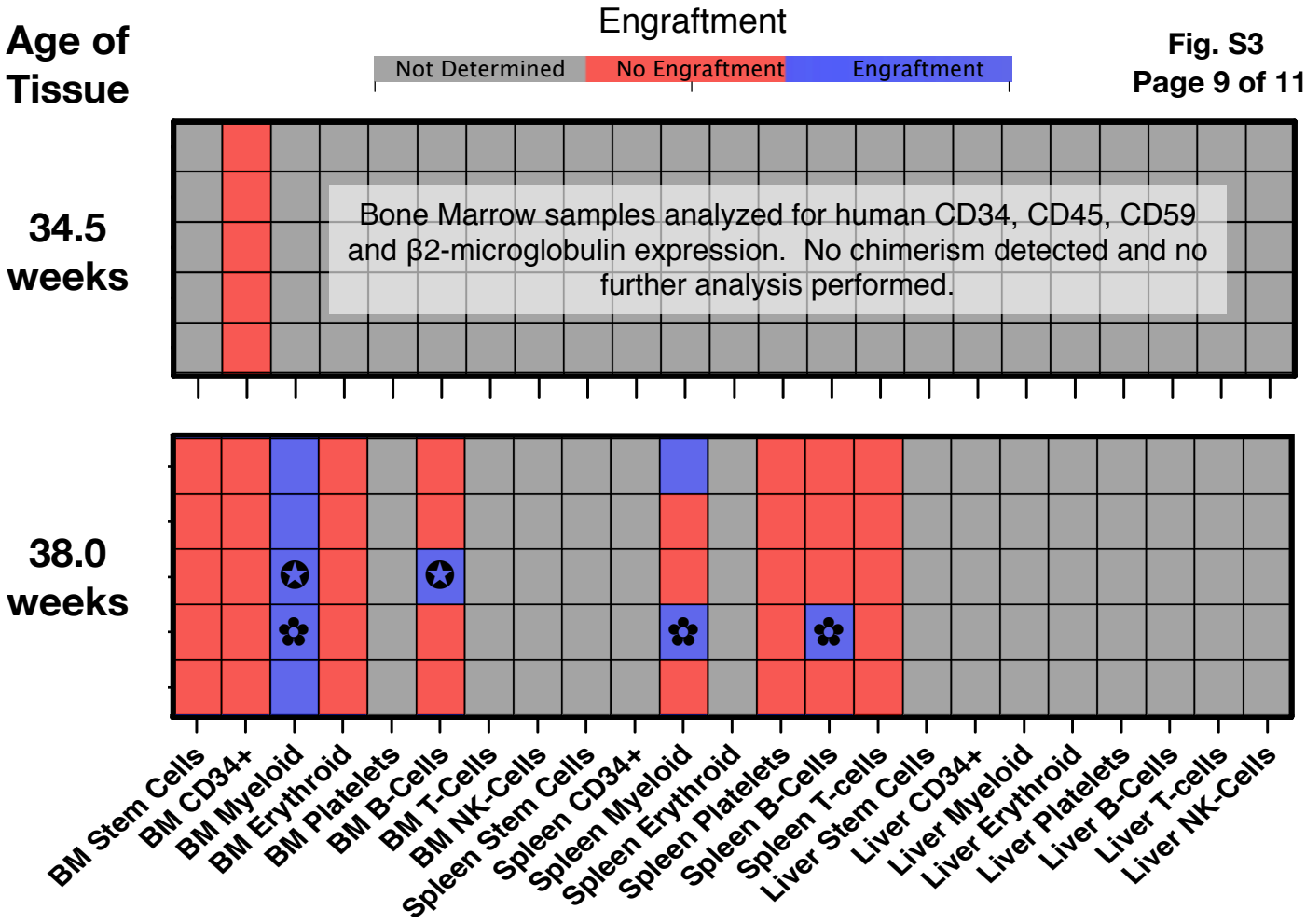


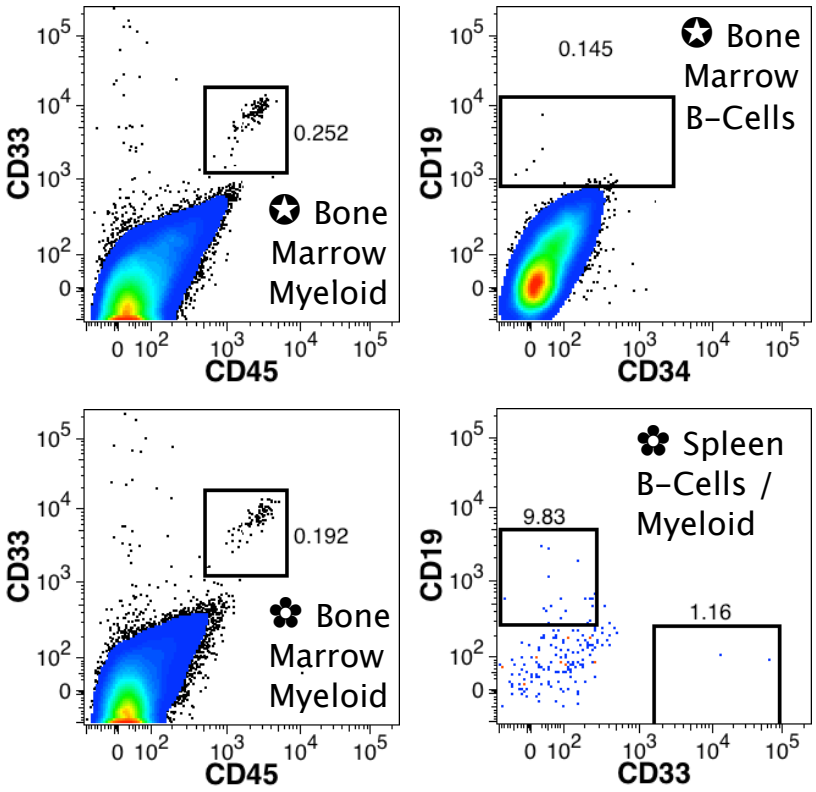
Tissue and Cell Population Analyzed











Tissue and Cell Population Analyzed

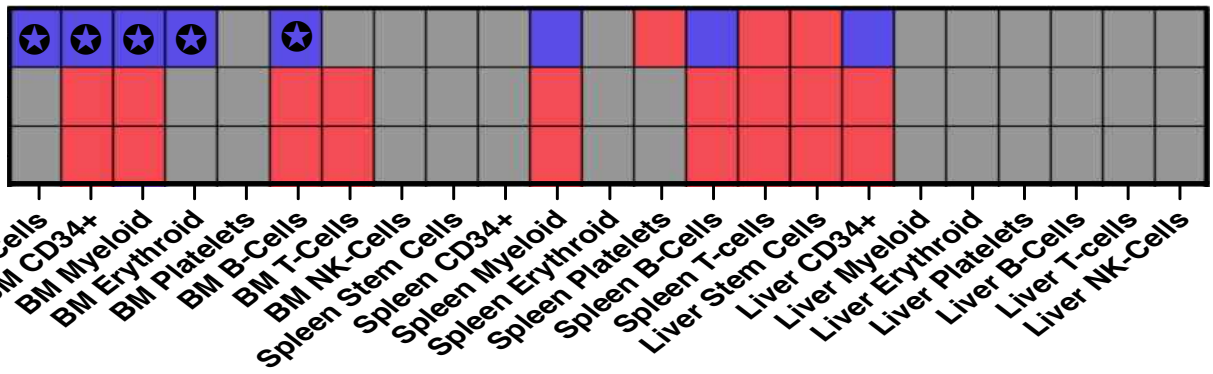
Results for Individual Mice

Age of Tissue

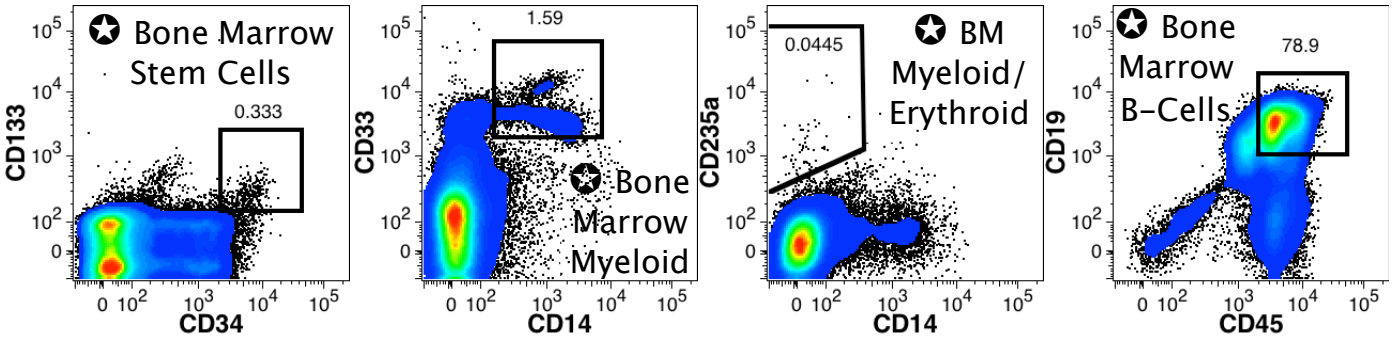
Engraftment

Not Determined | No Engraftment | Engraftment

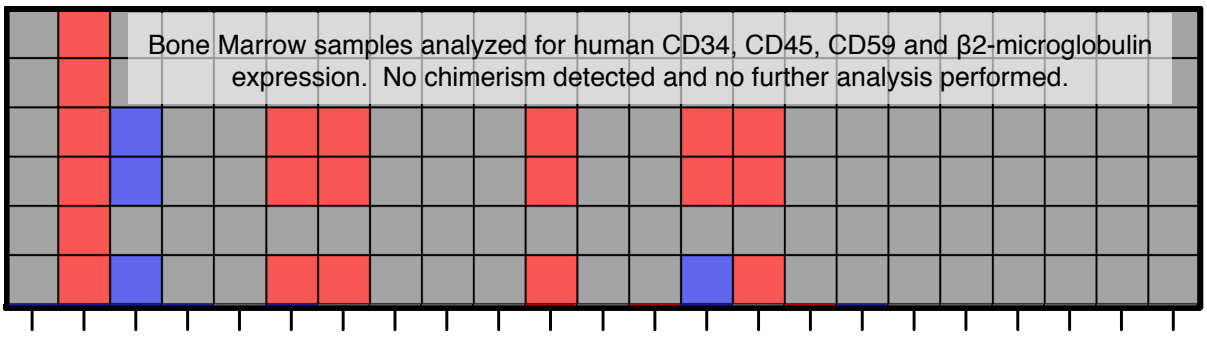
38.4 weeks



BM Stem Cells
BM CD34+
BM Myeloid
BM Erythroid
BM Platelets
BM B-Cells
BM T-Cells
BM NK-Cells
Spleen Stem Cells
Spleen CD34+
Spleen Myeloid
Spleen Erythroid
Spleen Platelets
Spleen B-Cells
Spleen T-cells
Liver Stem Cells
Liver CD34+
Liver Myeloid
Liver Erythroid
Liver Platelets
Liver B-Cells
Liver T-cells
Liver NK-Cells

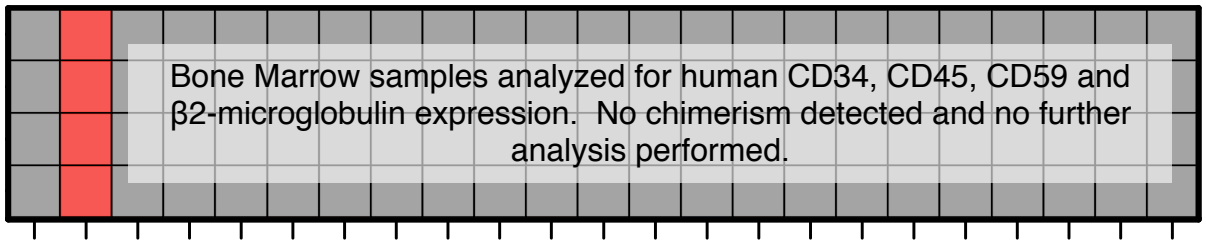


39.0 weeks



Bone Marrow samples analyzed for human CD34, CD45, CD59 and β2-microglobulin expression. No chimerism detected and no further analysis performed.

39.0 weeks



Bone Marrow samples analyzed for human CD34, CD45, CD59 and β2-microglobulin expression. No chimerism detected and no further analysis performed.

BM Stem Cells
BM CD34+
BM Myeloid
BM Erythroid
BM Platelets
BM B-Cells
BM T-Cells
BM NK-Cells
Spleen Stem Cells
Spleen CD34+
Spleen Myeloid
Spleen Erythroid
Spleen Platelets
Spleen B-Cells
Spleen T-cells
Liver Stem Cells
Liver CD34+
Liver Myeloid
Liver Erythroid
Liver Platelets
Liver B-Cells
Liver T-cells
Liver NK-Cells

Tissue and Cell Population Analyzed

Results for Individual Mice

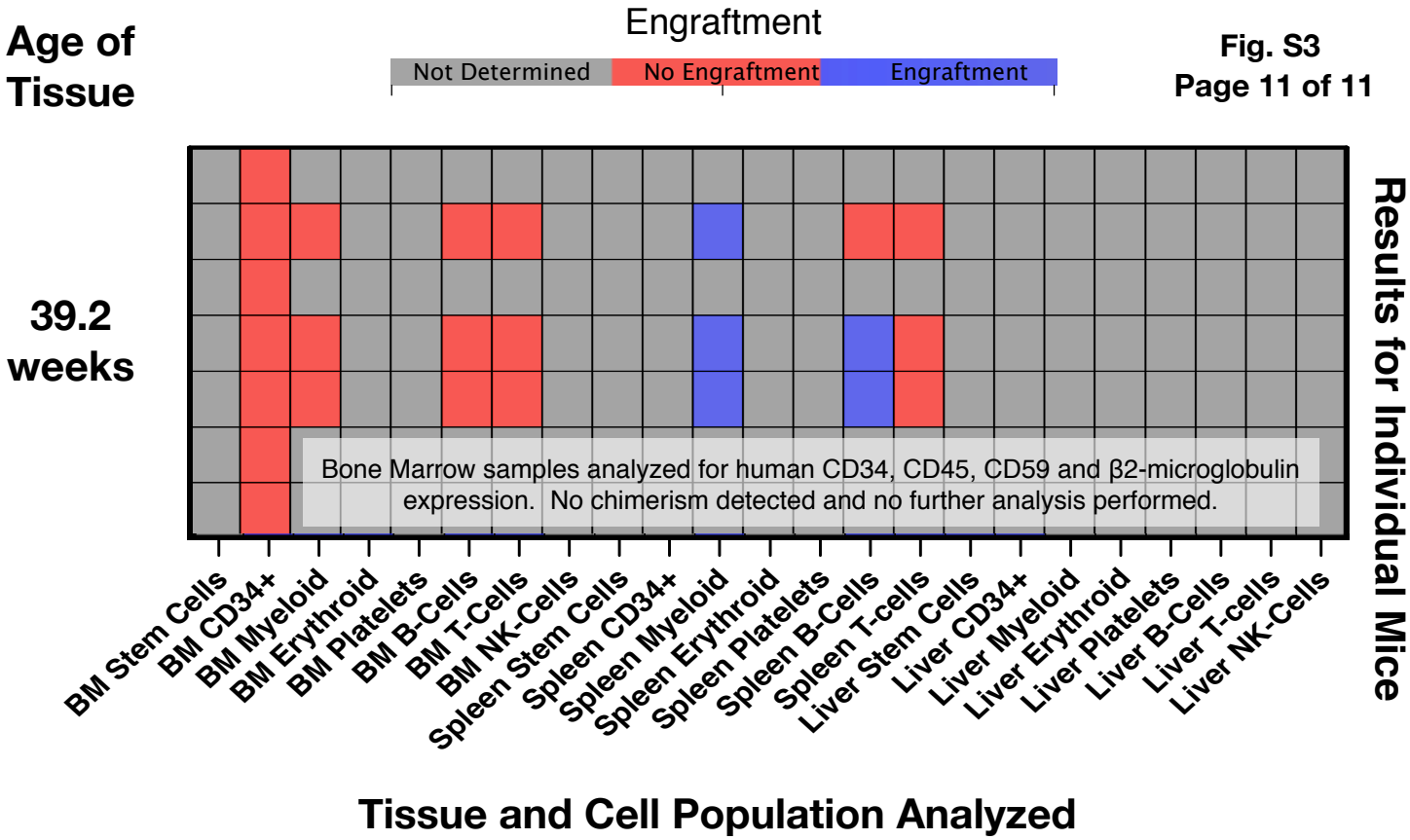
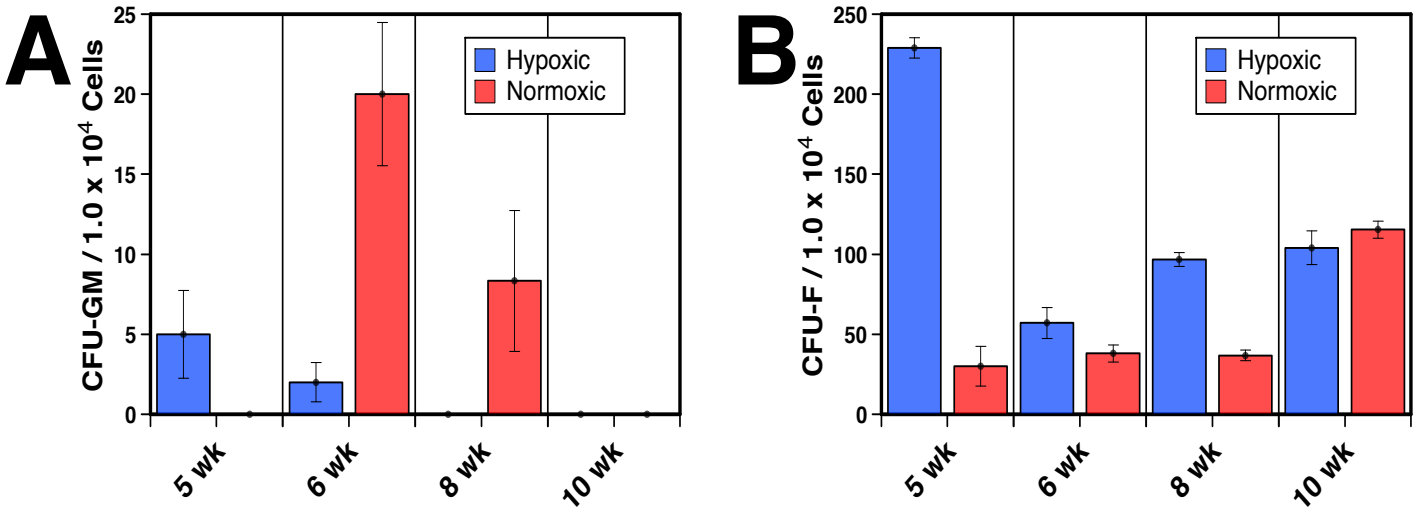


Fig. S3. Overview of hematopoietic engraftment and selected flow cytometric analyses of chorion transplants in immunodeficient mice. Heat maps indicate the lineage analyses performed for the three hematopoietic tissues examined and whether engraftment was observed for each of the 28 transplanted cell isolates. Results are presented according to gestation age of the donor tissue (youngest at the top), which parallels the presentation of the summary findings presented in Fig. 5B of the manuscript. Each row of a heat map represents a single transplanted mouse. At a minimum, all mice were analyzed with a single panel of antibodies staining widely expressed human antigens (CD34, CD45, CD59 and CD81 or β2-microglobulin). If no human chimerism was detected, then no further analysis was performed. Otherwise, mice were analyzed for the presence of human CD34+, myeloid, erythroid and B-lymphoid bone marrow cells in addition to any other indicated tissue/lineage analyses. Fig. 6 in the manuscript presents a broad analysis of hematopoietic engraftment in one mouse. Additional examples of flow cytometry results showing full and partial engraftment are presented herein: 1) First trimester tissues generally did not engraft mice with the exception of T- and B-lymphocyte engraftment observed in some mice (6.2 and 11.3 weeks' gestation grafts), 2) the youngest tissue yielding full engraftment was 15.2 weeks' gestation, 3) another examples of full engraftment is shown for the 17.3 weeks' gestation graft, 4) an example of partial (B-cell) engraftment is shown for the 20.1 weeks' gestation sample, 5) anther example of partial engraftment in which erythroid engraftment could not be documented is shown for one of the 24.0 weeks' gestation samples, 6) partial myeloid engraftment was observed in some transplants of third trimester tissues - e.g, 38.0 weeks' gestation- with very low numbers of CD19+ cells being observed as well, in some cases, and 7) a single case of full engraftment was observed in a mouse transplanted with third trimester cells (38.4 weeks' gestation). The symbols ✪ and ✿ are used to indicate the mouse, tissue and cell lineage represented by the flow cytometry data shown associated with the individual heat maps. Analyses labeled with red text represent negative data and black text represents positive engraftment data.



Supplemental Figure S4. Oxygen levels do not significantly affect the in vitro hematopoietic potential of 1st trimester chorionic hematopoietic progenitors. Prior to 7 wks the chorion and the chorionic villi could not be separated and were analyzed together. Afterwards the chorion was processed separately from the villi. Cells were cultured for 3 wks in either physiological hypoxia (1% O_2, blue) or standard conditions (20% O_2, red). (A) CFU-GM (granulocyte-macrophage) colonies were enumerated at the gestational ages indicated. (B) CFU-F (fibroblast) colonies in the same cultures were also enumerated. Data in A and B are mean ± SEM of 3-5 plates/ sample.

Supplementary Table S1. This table contains a full list of the monoclonal antibodies against human and mouse antigens utilized in flow cytometry analyses, including the fluorochrome label, isotype, clone name and source.

Click here to Download Table S1