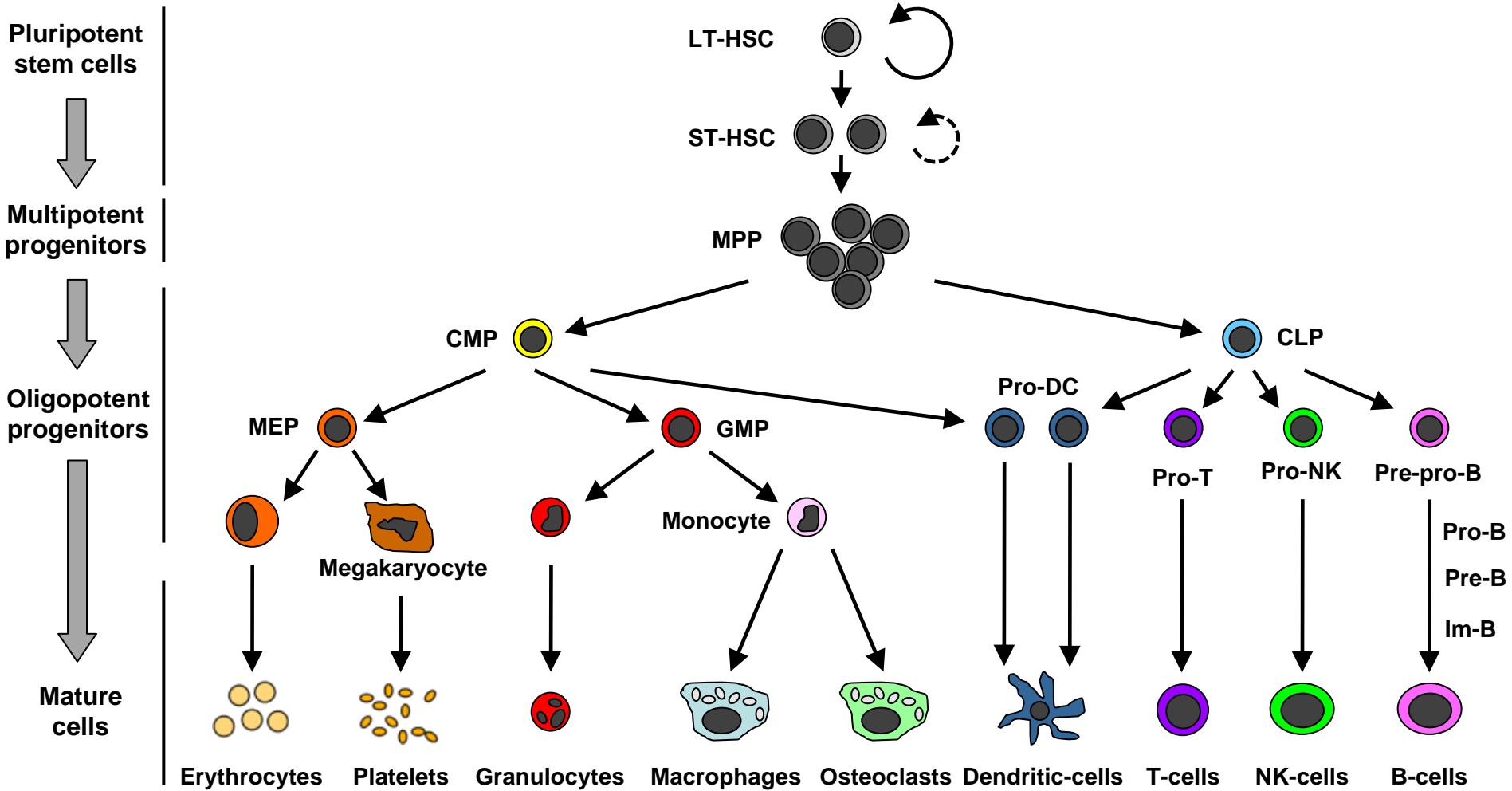


Spatial analysis of hematopoietic stem and progenitor cells in bone marrow

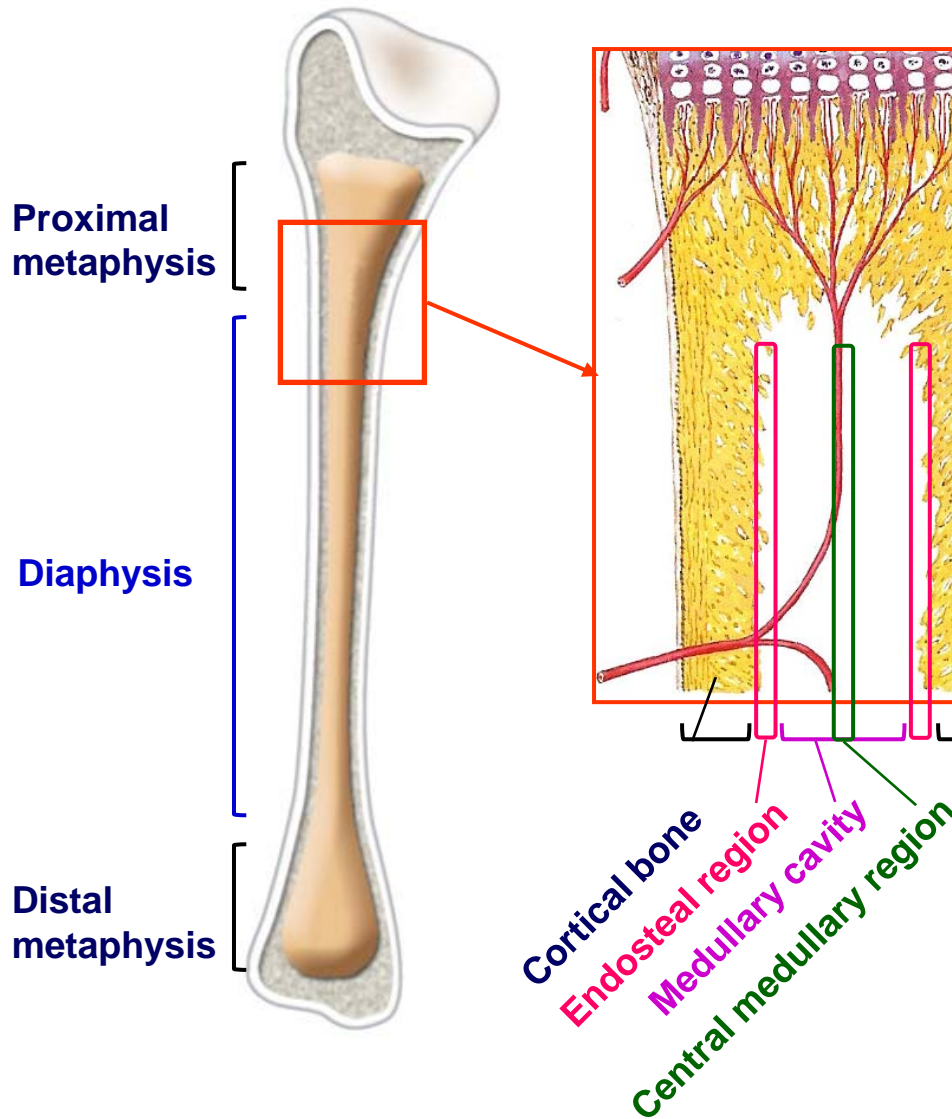
Leslie E. Silberstein, MD

Children's Hospital Boston, Dana-Farber Cancer Institute, Brigham and
Women's Hospital, Immune Disease Institute

Hematopoietic system

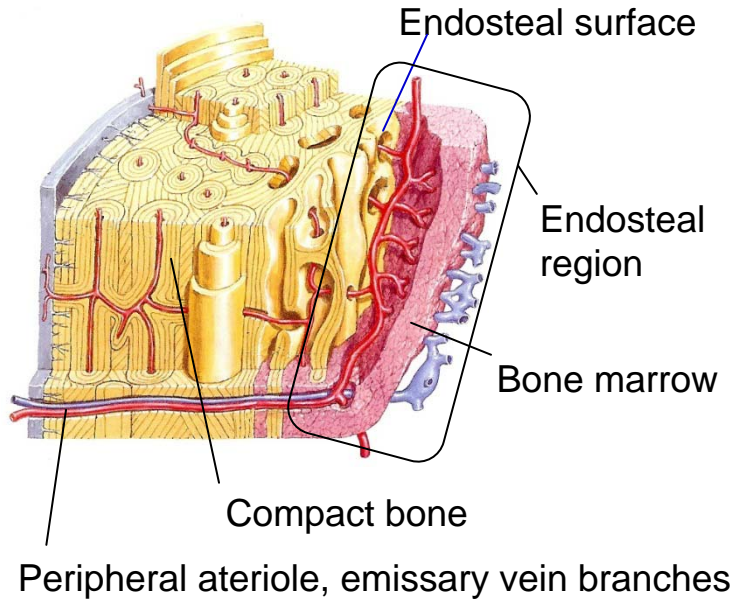


Anatomy of femoral bone marrow cavity



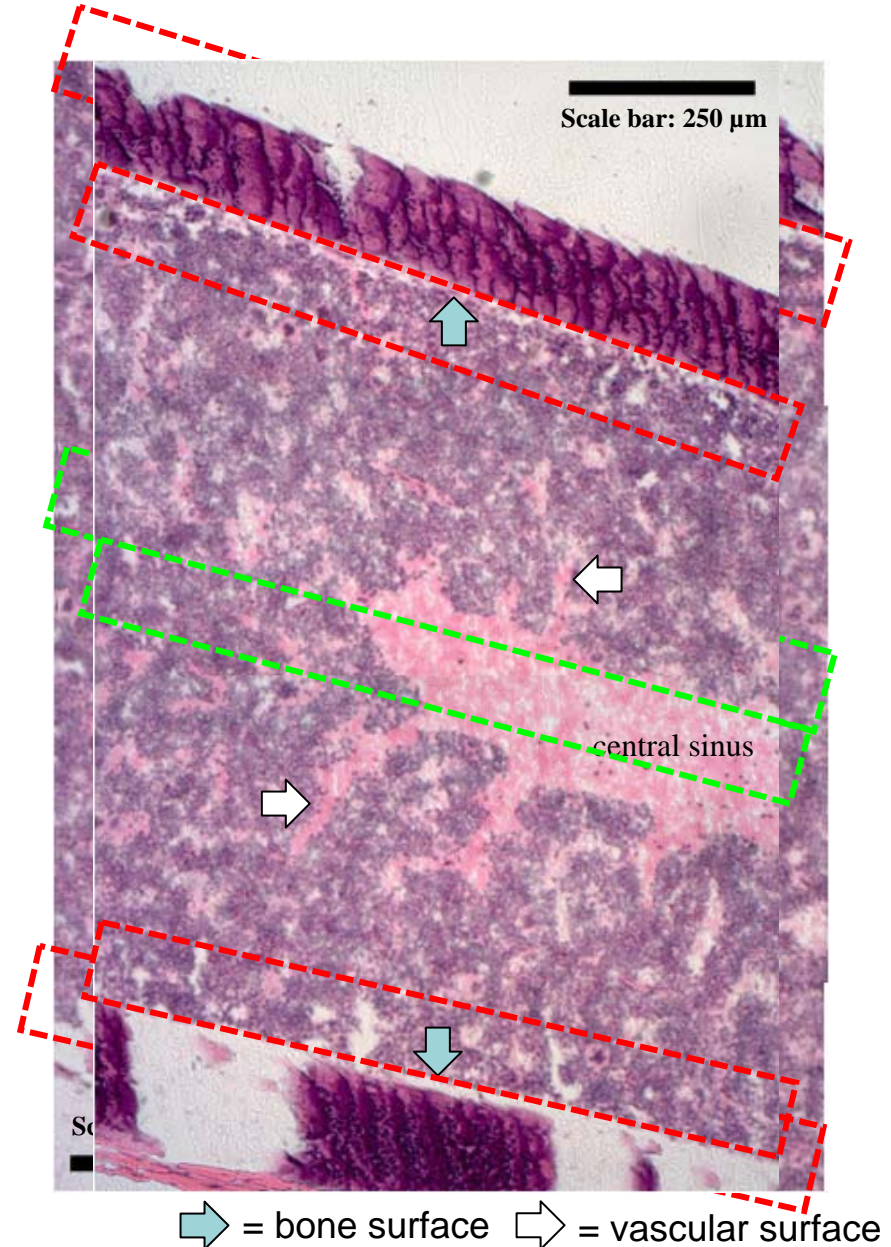
Diaphysis

Diaphysis: Cortical bone, medullary cavity, vasculature, fat, low density of trabecular bone



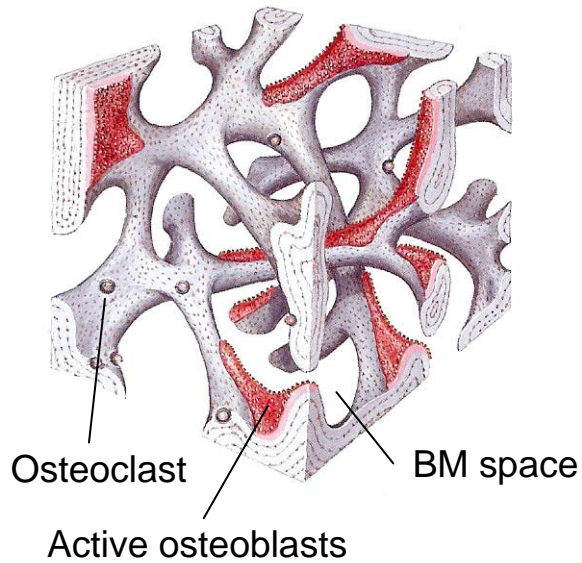
Endosteal Region (ER): Within 20 cell dia. ($\sim 100 \mu\text{m}$) of the endosteal surface. Cells in the ER may be in contact with osteoblasts, stromal, reticular, or other hematopoietic cells, and vascular endothelial cells

Central Medullary Region (CMR): 20 cell wide zone in the center of the BM diaphysis. Cells in the CMR may be in contact with vascular endothelial cells, stromal, reticular cells, B cells, T cells, and other hematopoietic cells, small number of osteoblasts.

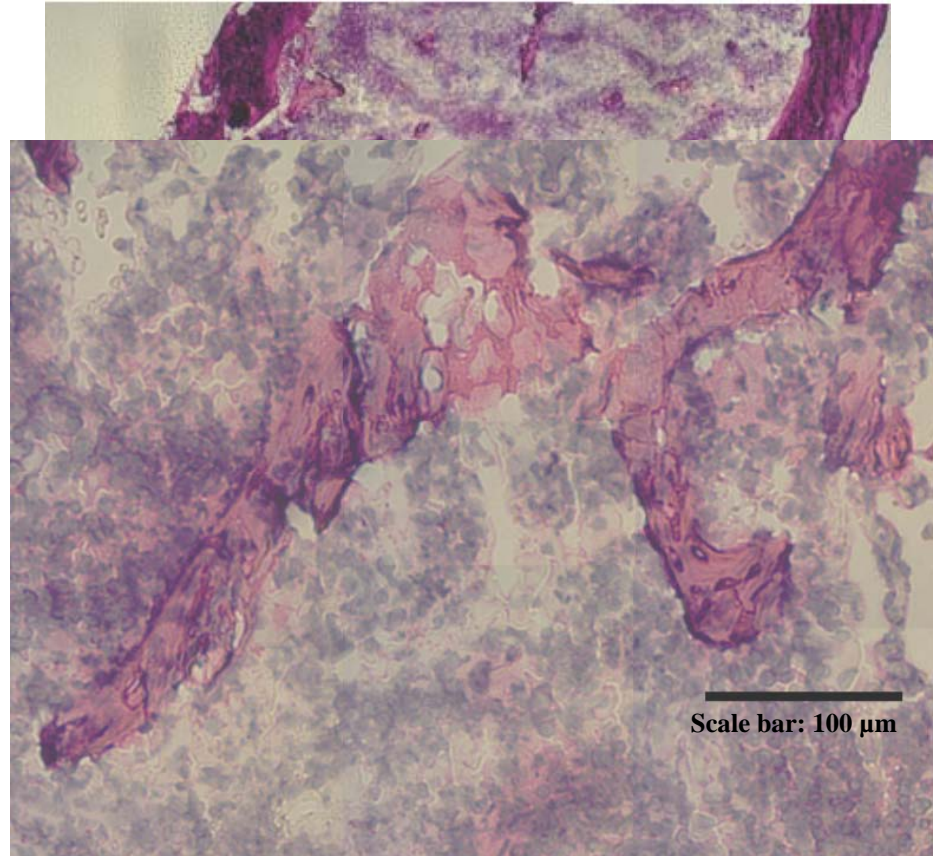


Metaphysis

Metaphysis: Trabecular bone, fat, vasculature

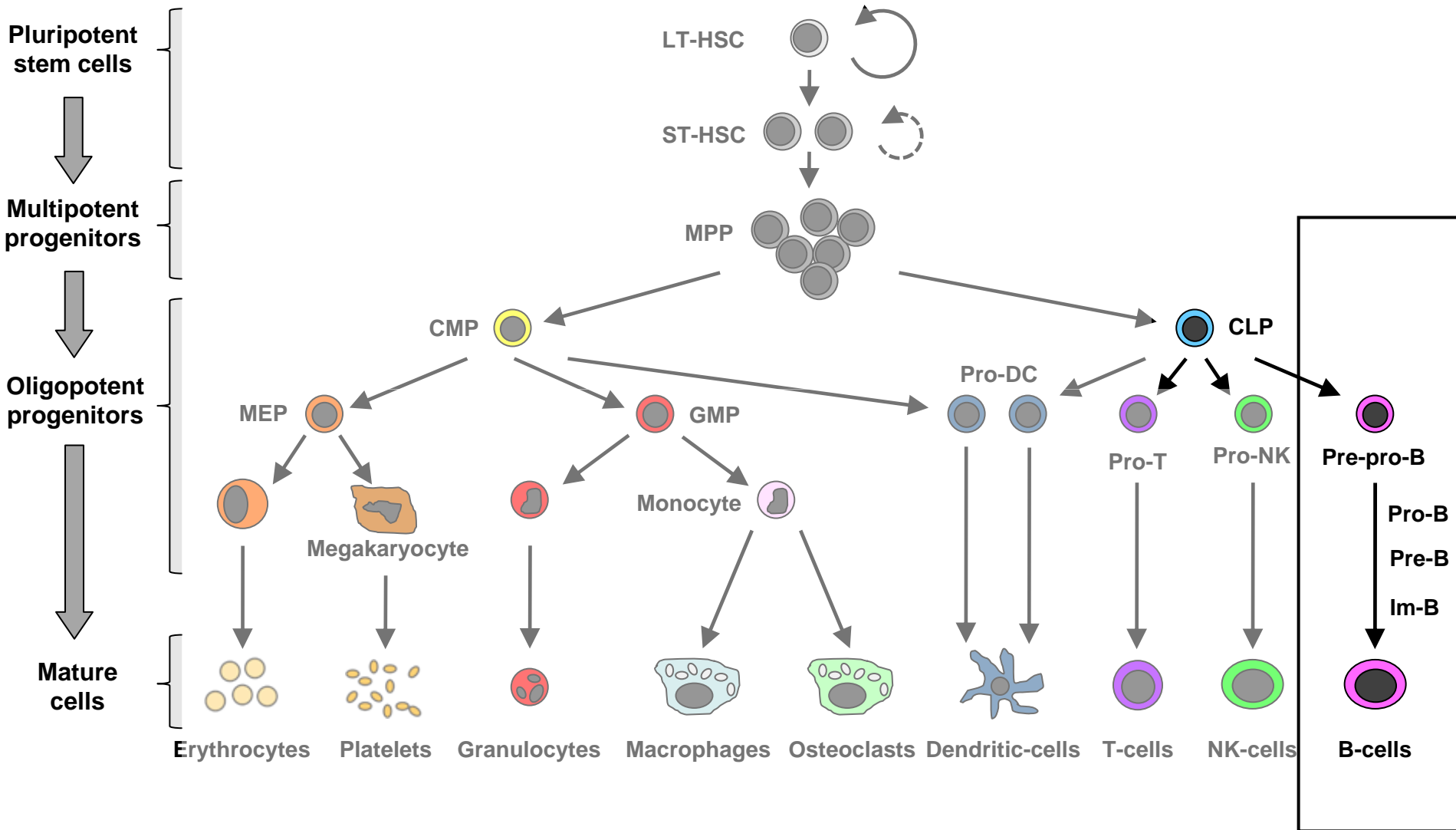


- Higher bone surface area to BM volume ratio compared to the cortical bone of the diaphyseal region.
- Highest density of arteries and veins within the BM.
- Trabecular surface covered with osteoblasts and osteoclasts. Bone marrow spaces are filled with hematopoietic cells, BM stromal and reticular cells, fat, and a vasculature system.



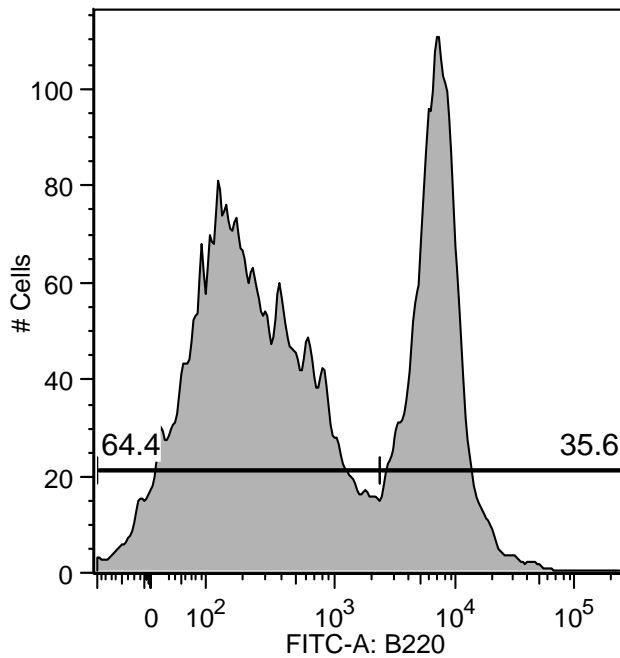
➡ = bone surface ⇨ = vascular surface

B cell developmental pathway

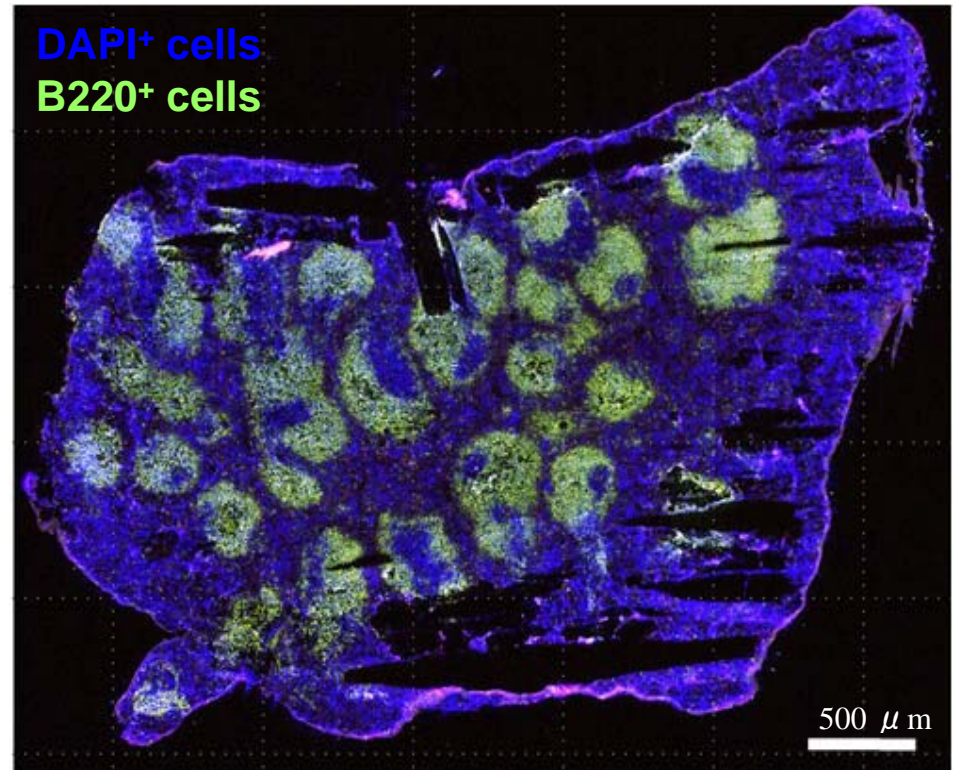


Validation of LSC analysis in murine spleen sections

Flow cytometry (FC):



Laser Scanning Cytometry



% of B220+ cells = 23.9 ± 5.5 % (n = 7)

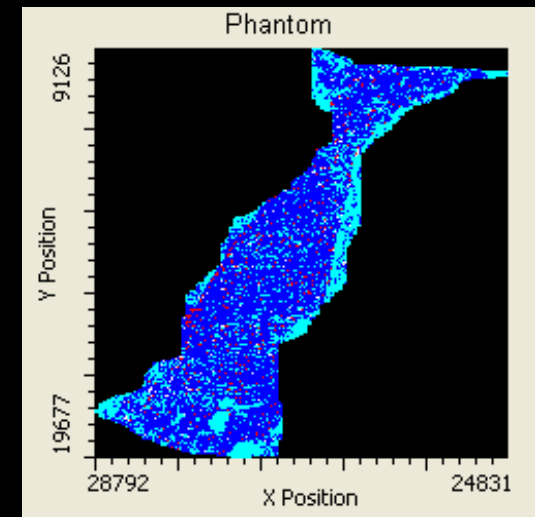
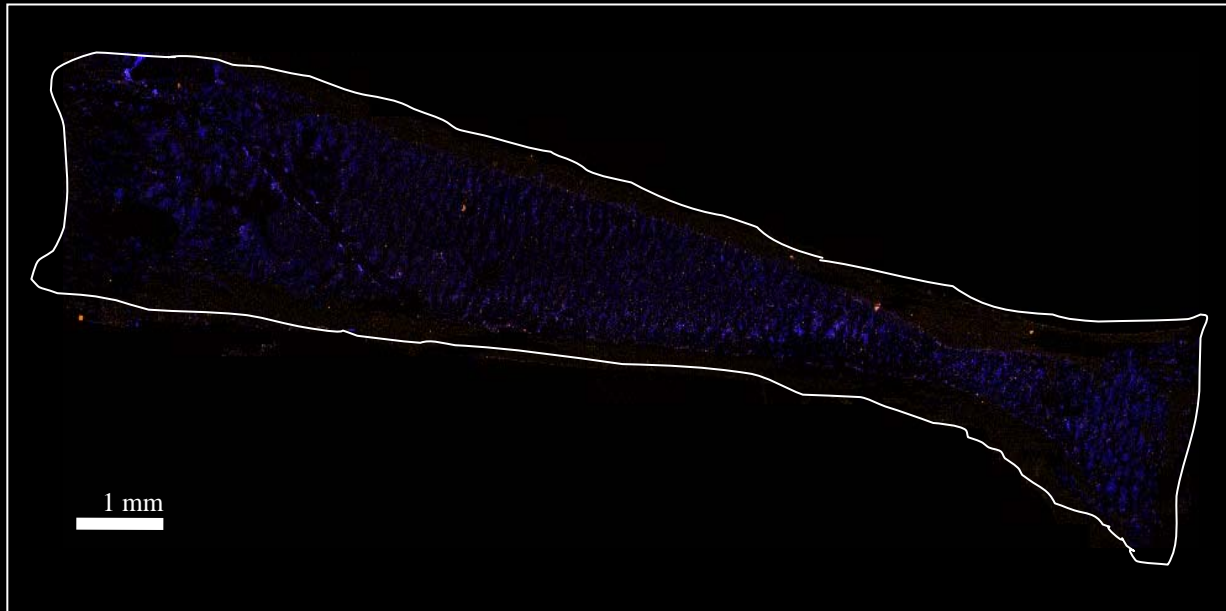
Typical distribution of B220+ cells in B cell follicles

Technical challenges in LSC analysis of Bone Marrow

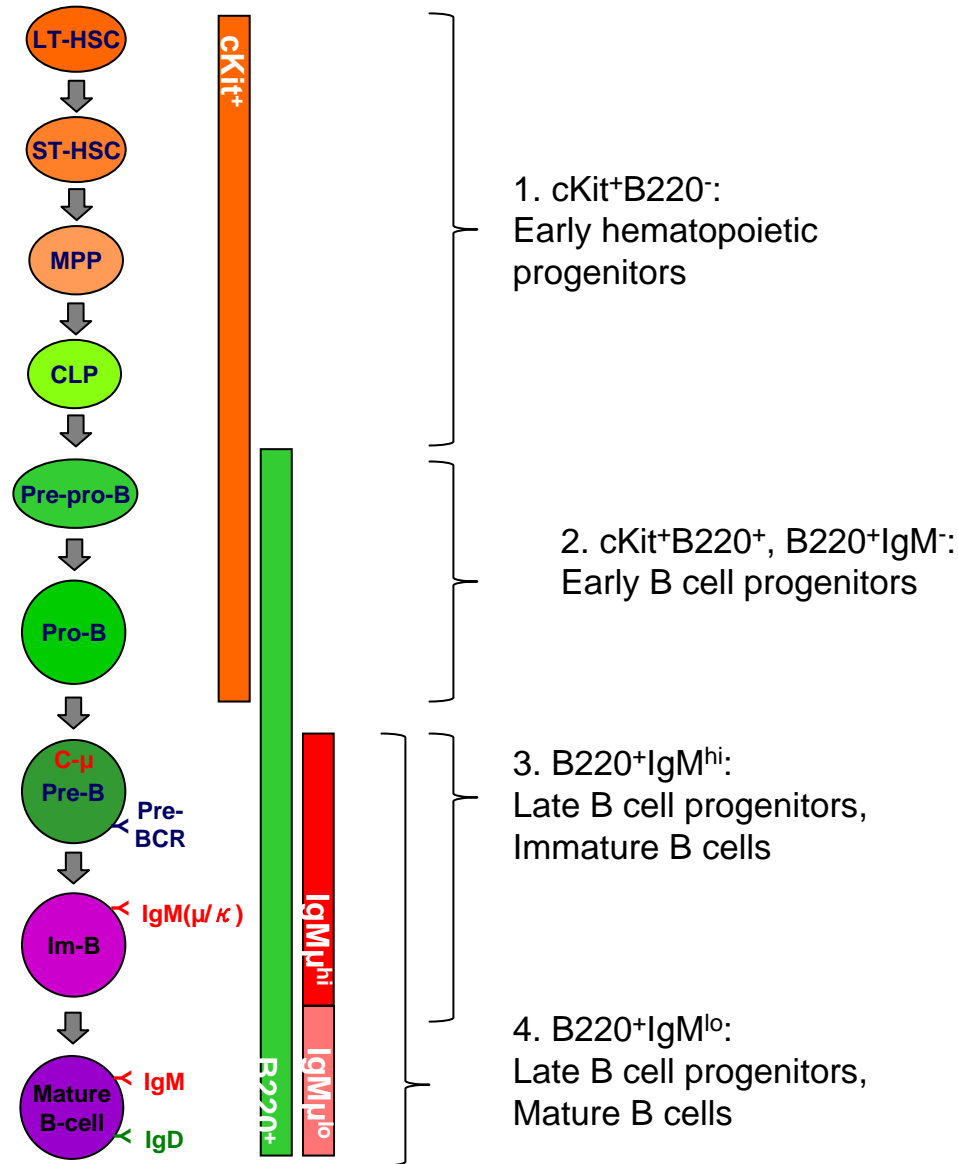
Background: bone thickness prevents generation of uniform complete femoral sections. Prior decalcification results in alteration of anatomic structures

Challenges:

- Cryopreserved
- non-decalcified
- 5 μ m thickness



Spatial distribution and characterization of B-cell Niches

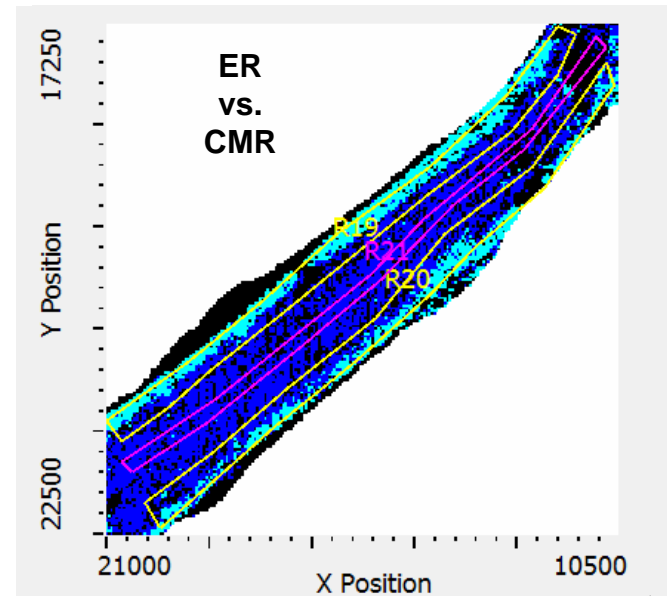
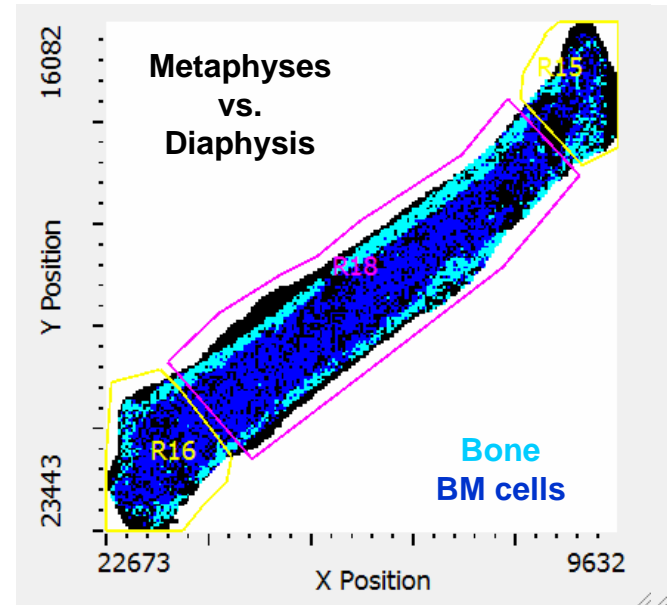


1. cKit⁺B220⁻:
Early hematopoietic progenitors

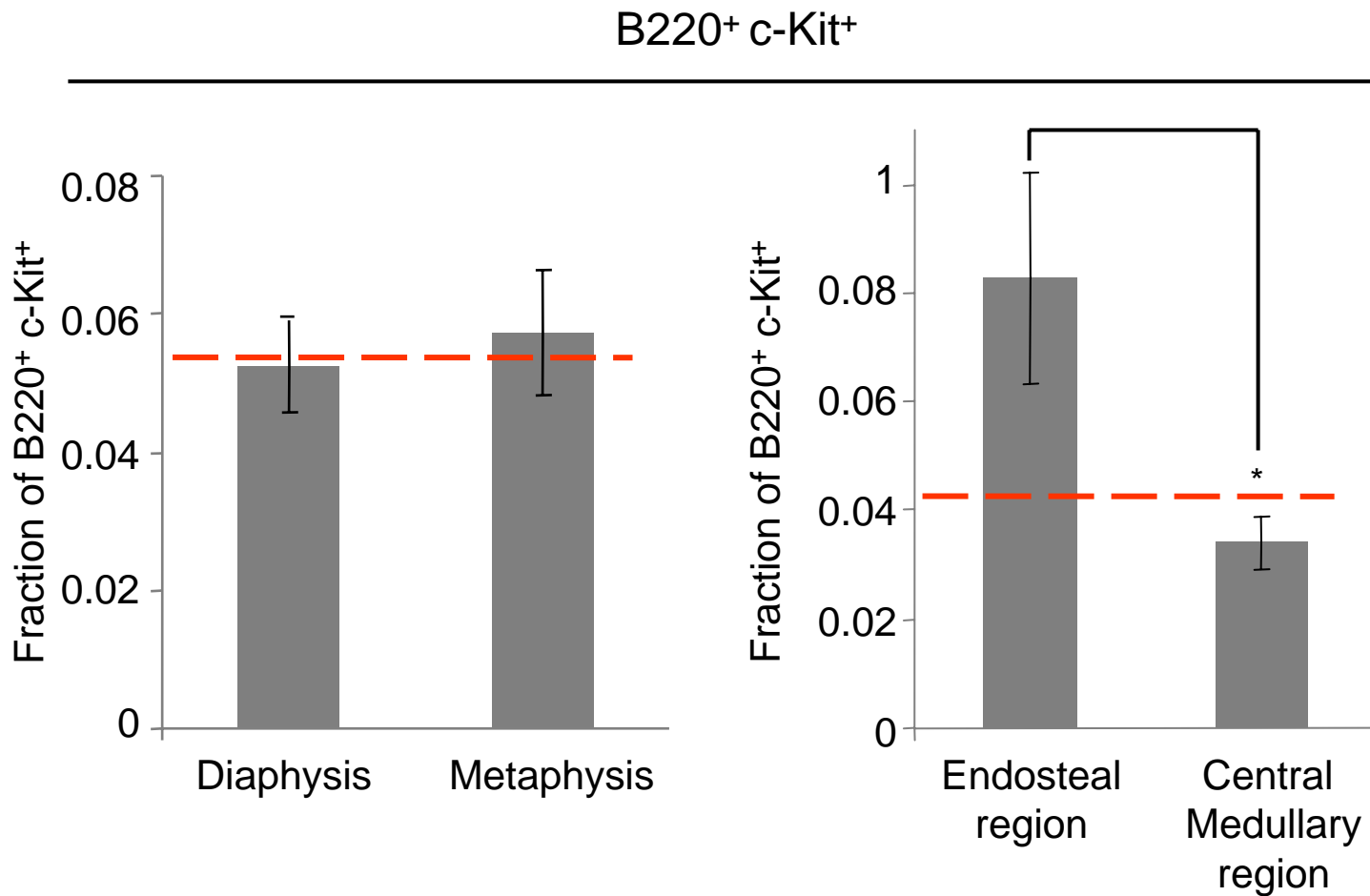
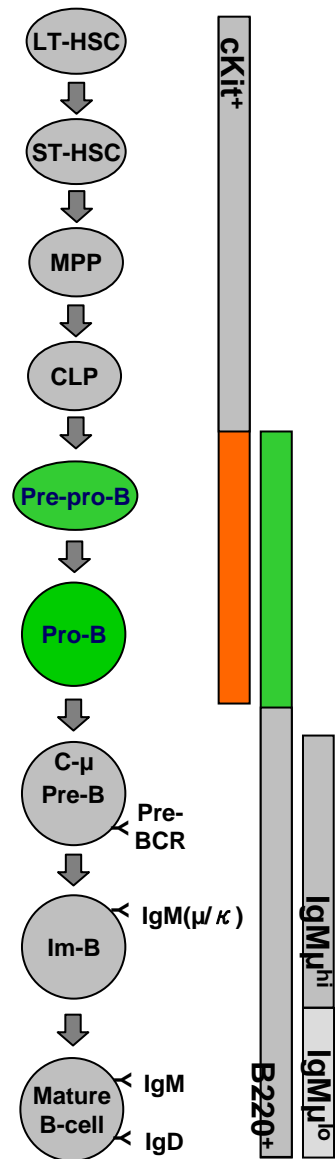
2. cKit⁺B220⁺, B220⁺IgM⁻:
Early B cell progenitors

3. B220⁺IgM^{hi}:
Late B cell progenitors,
Immature B cells

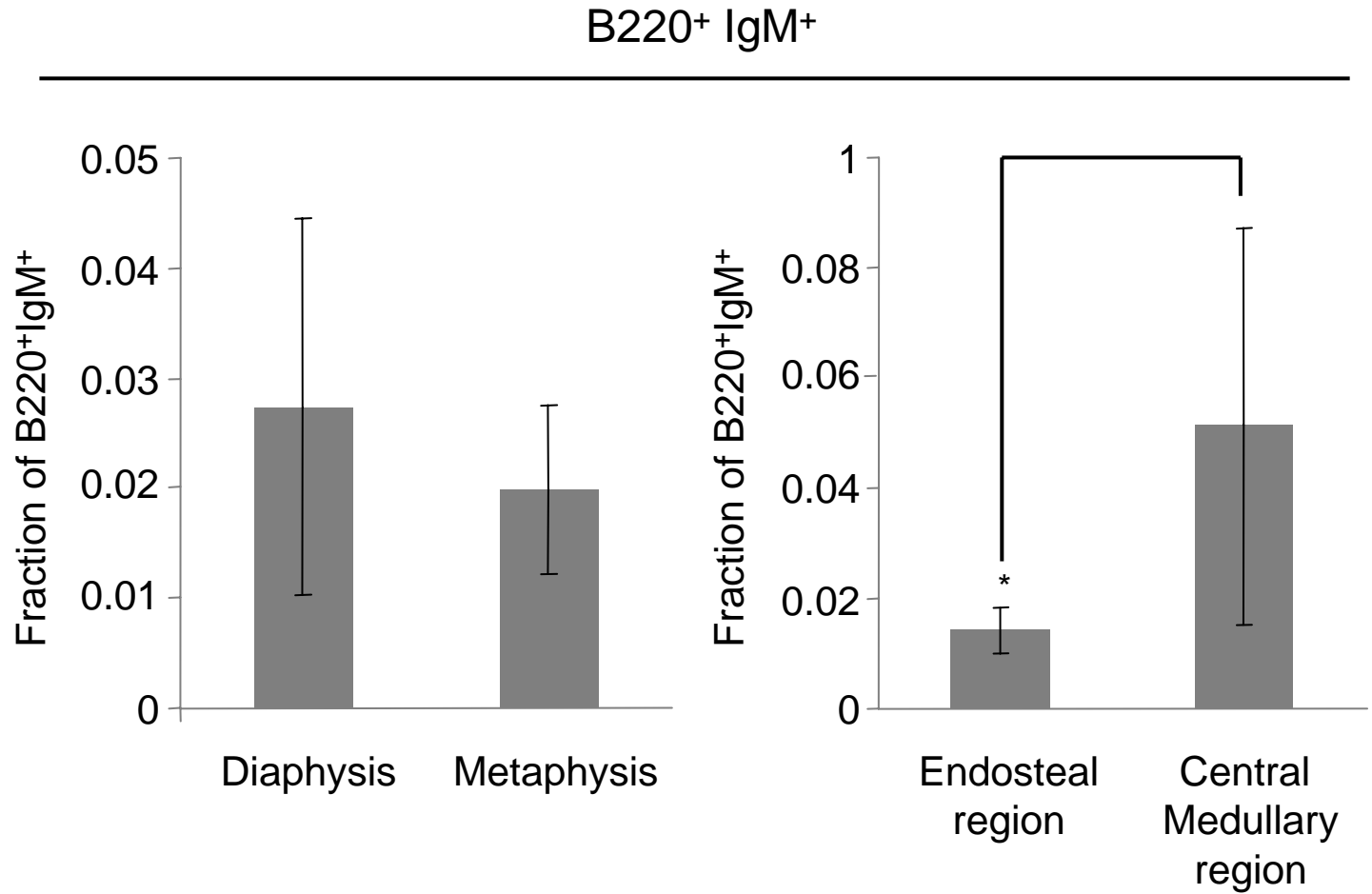
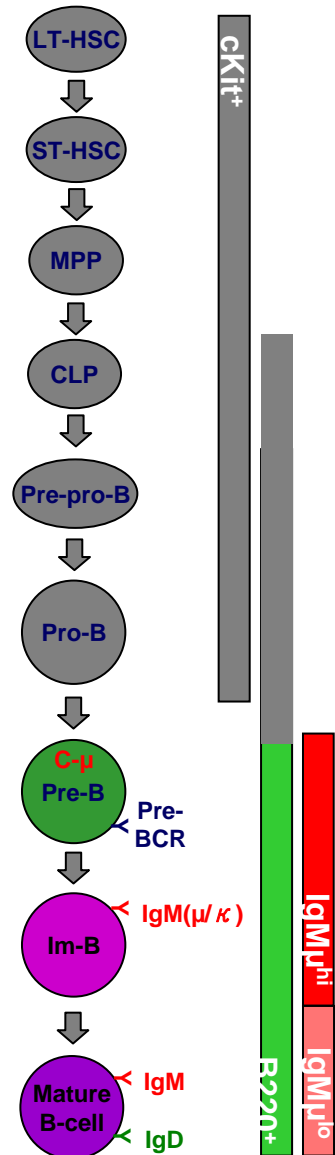
4. B220⁺IgM^{lo}:
Late B cell progenitors,
Mature B cells



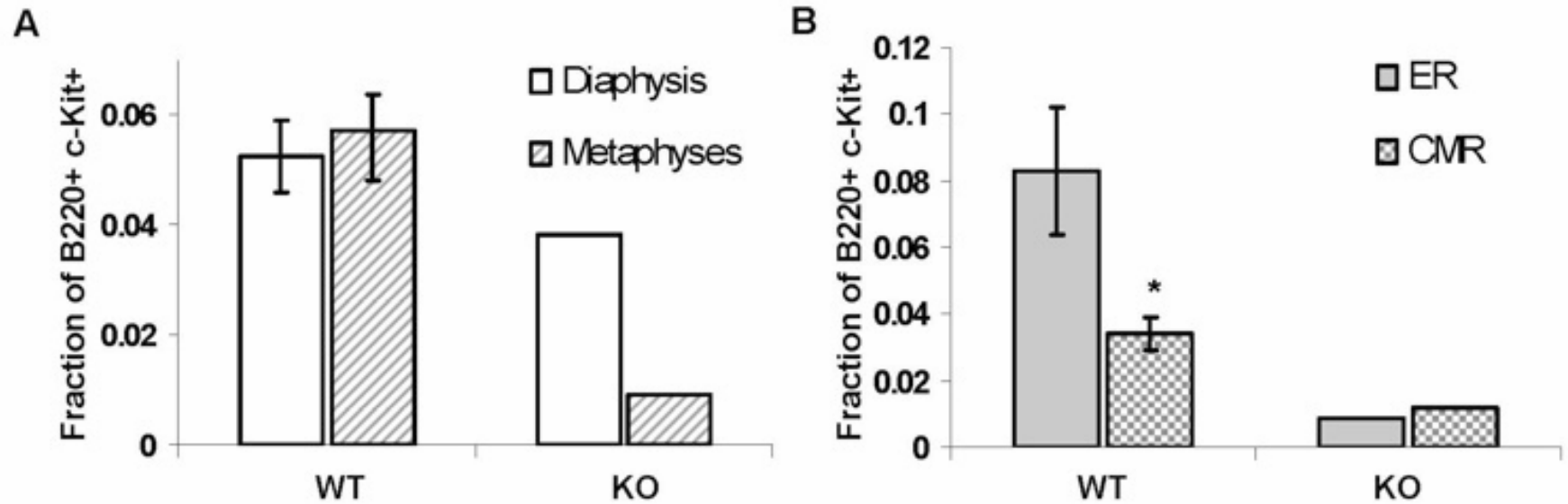
Preferential distribution of progenitor B cells in ER



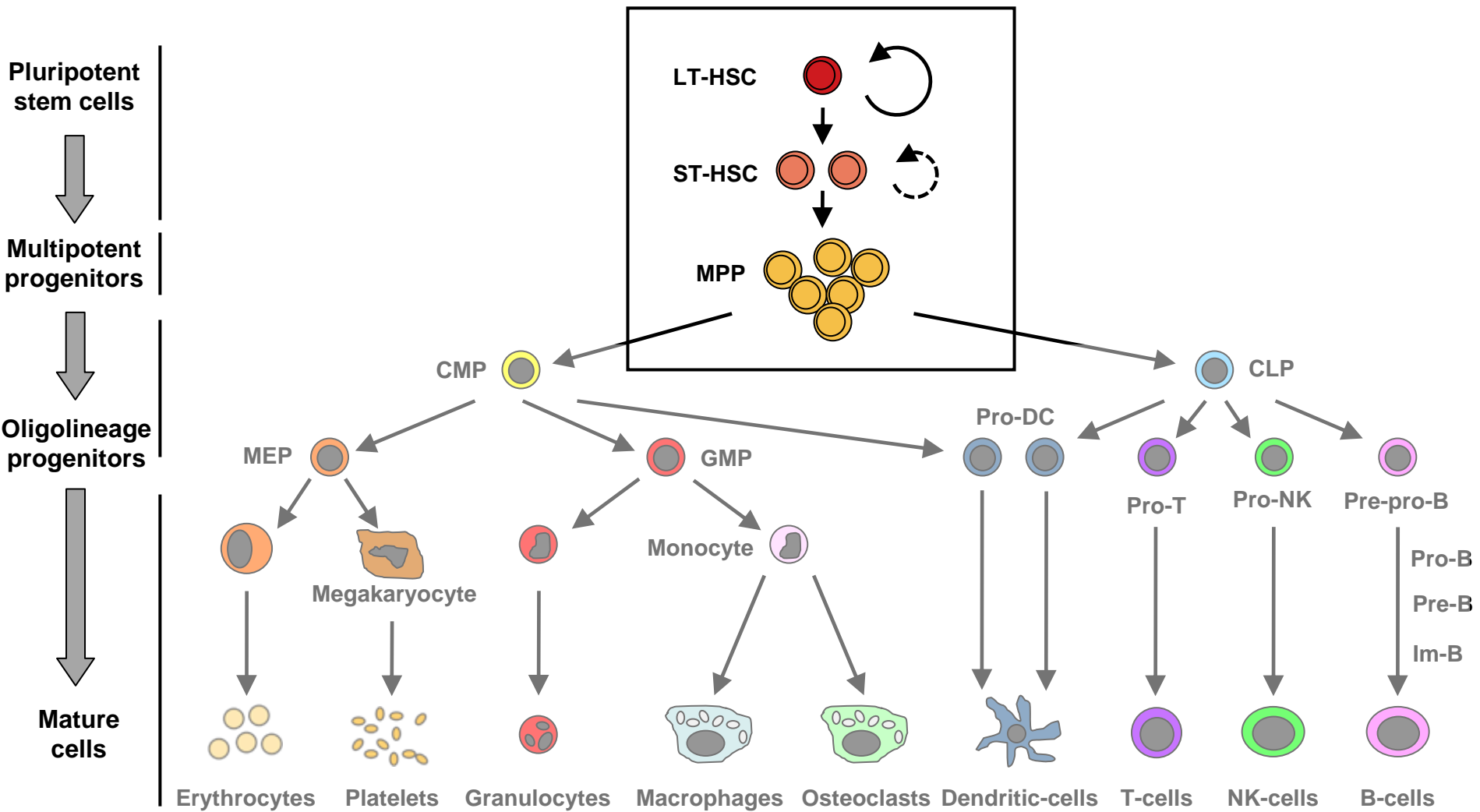
Preferential distribution of mature IgM⁺ B cells in CMR



Focal Adhesion Kinase deficiency alters early B cell progenitor number and distribution in BM regions

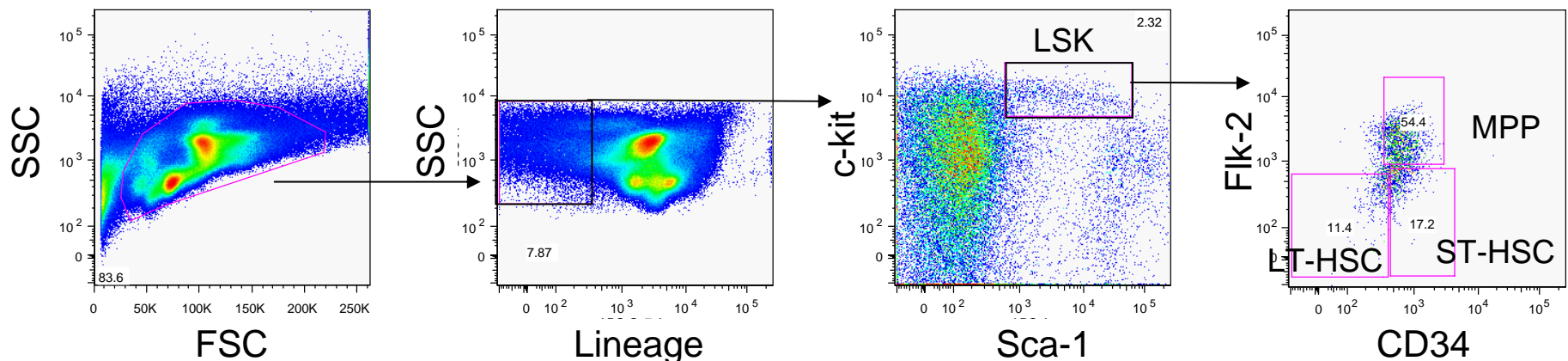


LSC analysis of spatial distribution of early hematopoietic progenitors



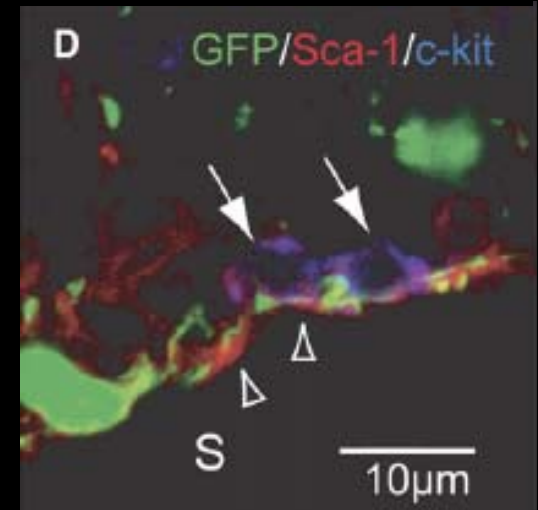
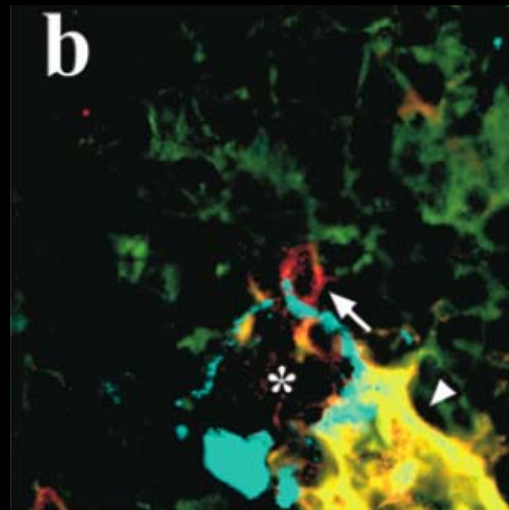
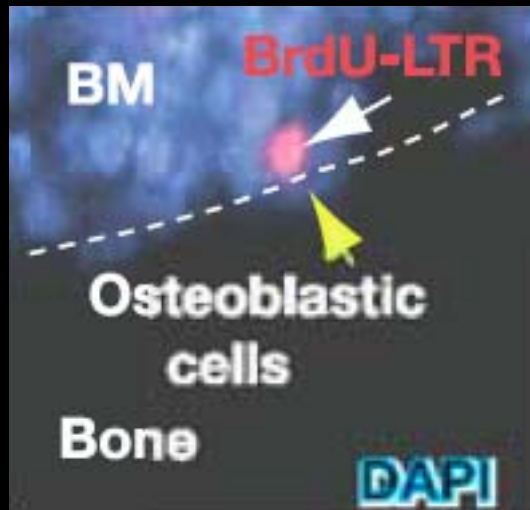
Challenges in imaging of HSPC

- Strict definition of HSC is based on ability to reconstitute the hematopoietic system in marrow-ablated recipient mice
- Phenotypic identification requires complex combinations of markers which yield heterogeneous cell populations enriched in HSC content to different degrees (HSPC)

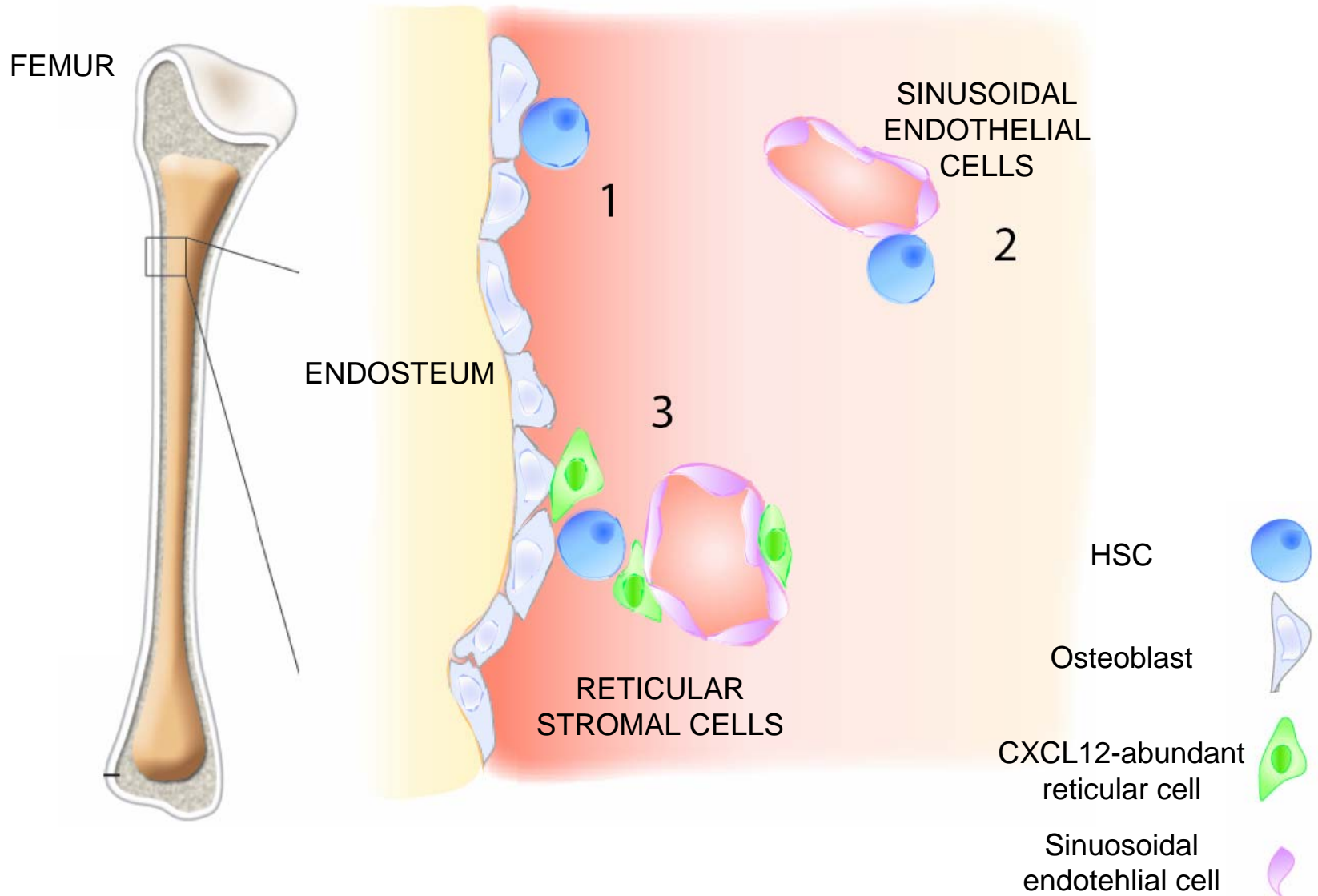


- HSPC populations are highly rare, requires imaging technologies with high quantitative capabilities (0.01-0.02 %)

Lack of information on spatial distribution and localization of HSC

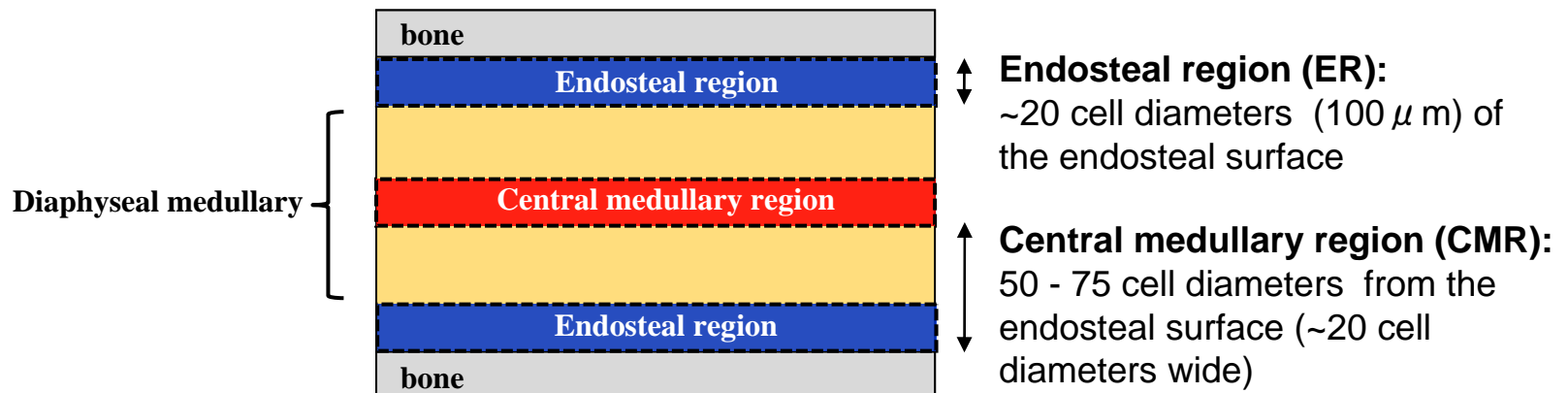
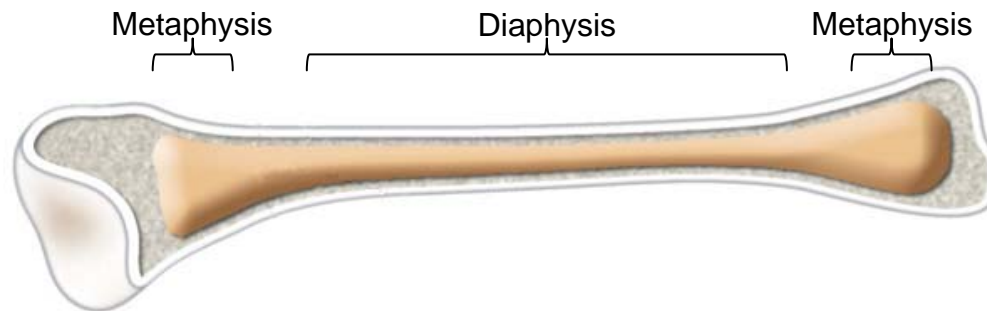


Uncertainty in the identity of HSC niches



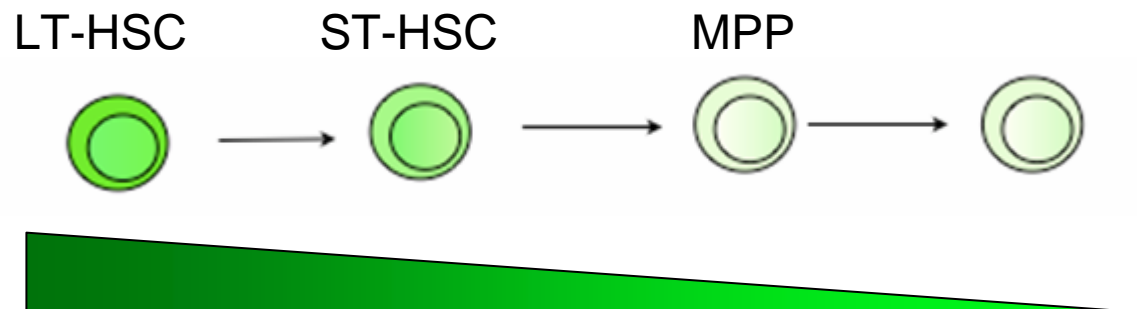
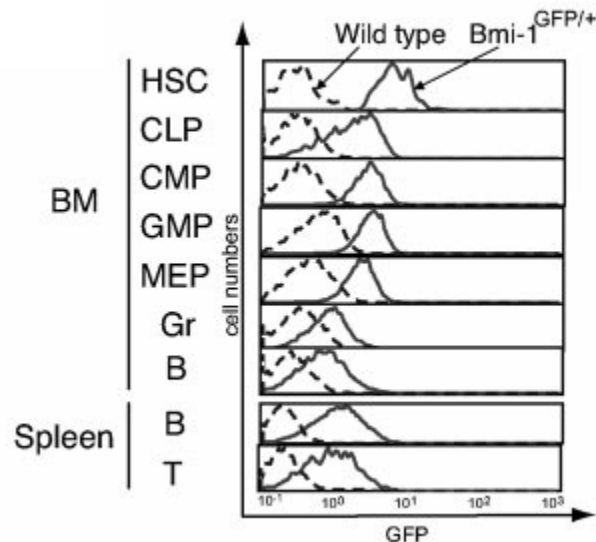
Spatial analysis of HSPCs in the bone marrow using LSC

- Where do HSPCs lodge?
 - **Region** identification
- What are the key components of HSC niche(s)?
 - **Niche** identification



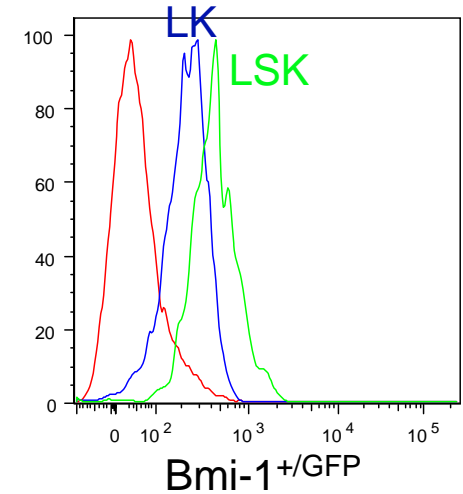
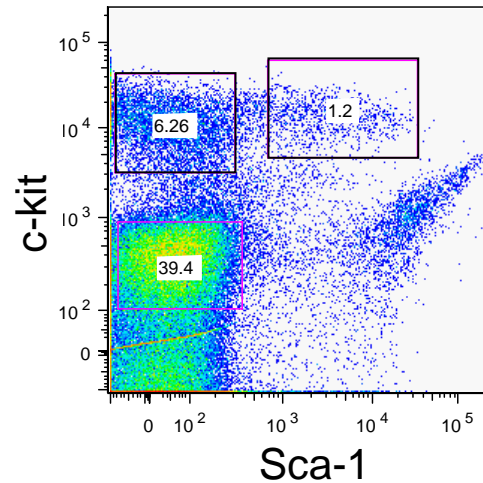
Identifying HSPCs: $bmi-1^{+/GFP}$ Mouse Model

Bmi-1 is a transcription factor involved in regulation of HSC self renewal. $Bmi-1^{+/GFP}$ knock-in transgene is expressed at highest levels in HSCs *Hosen et al. (Stem Cells 2007;25;1635-1644)*

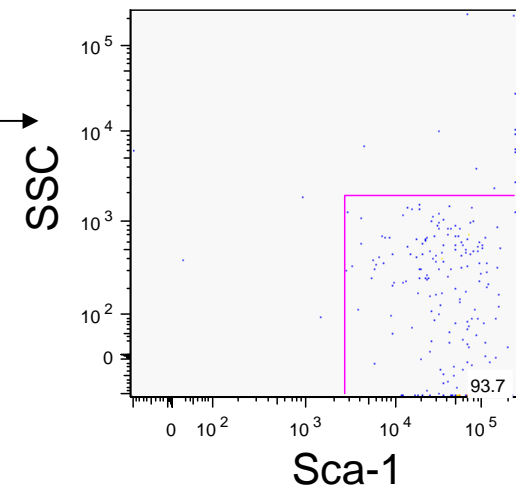
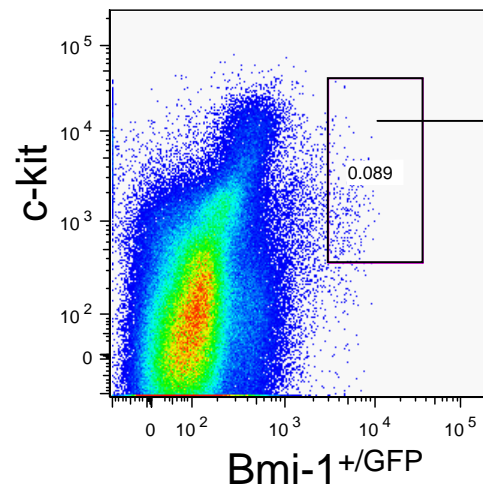


Combined expression of $Bmi-1^{+}/GFP^{hi}$ and $ckit^{+}$ cells defines a cell population highly enriched in HSPC

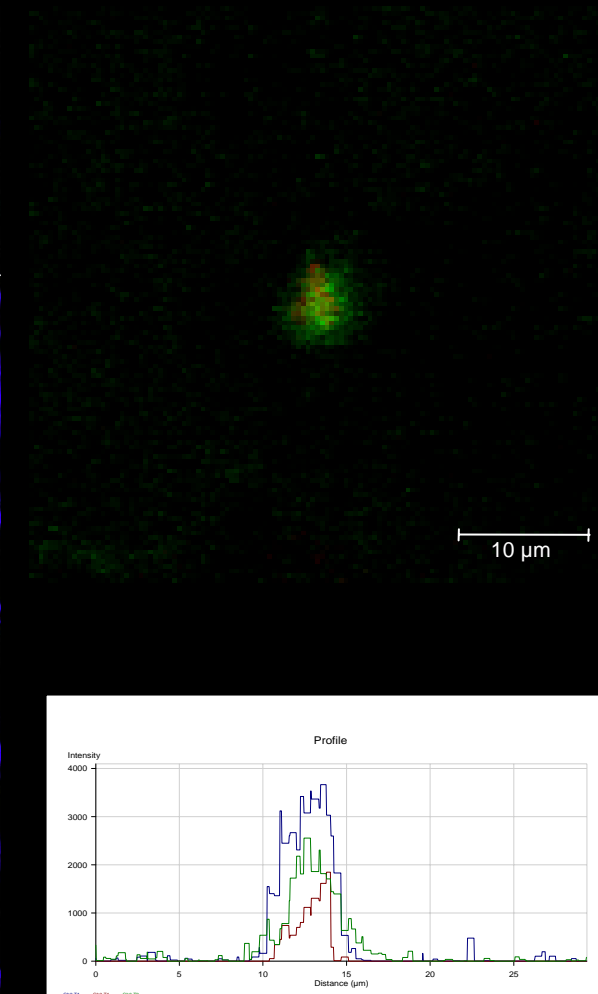
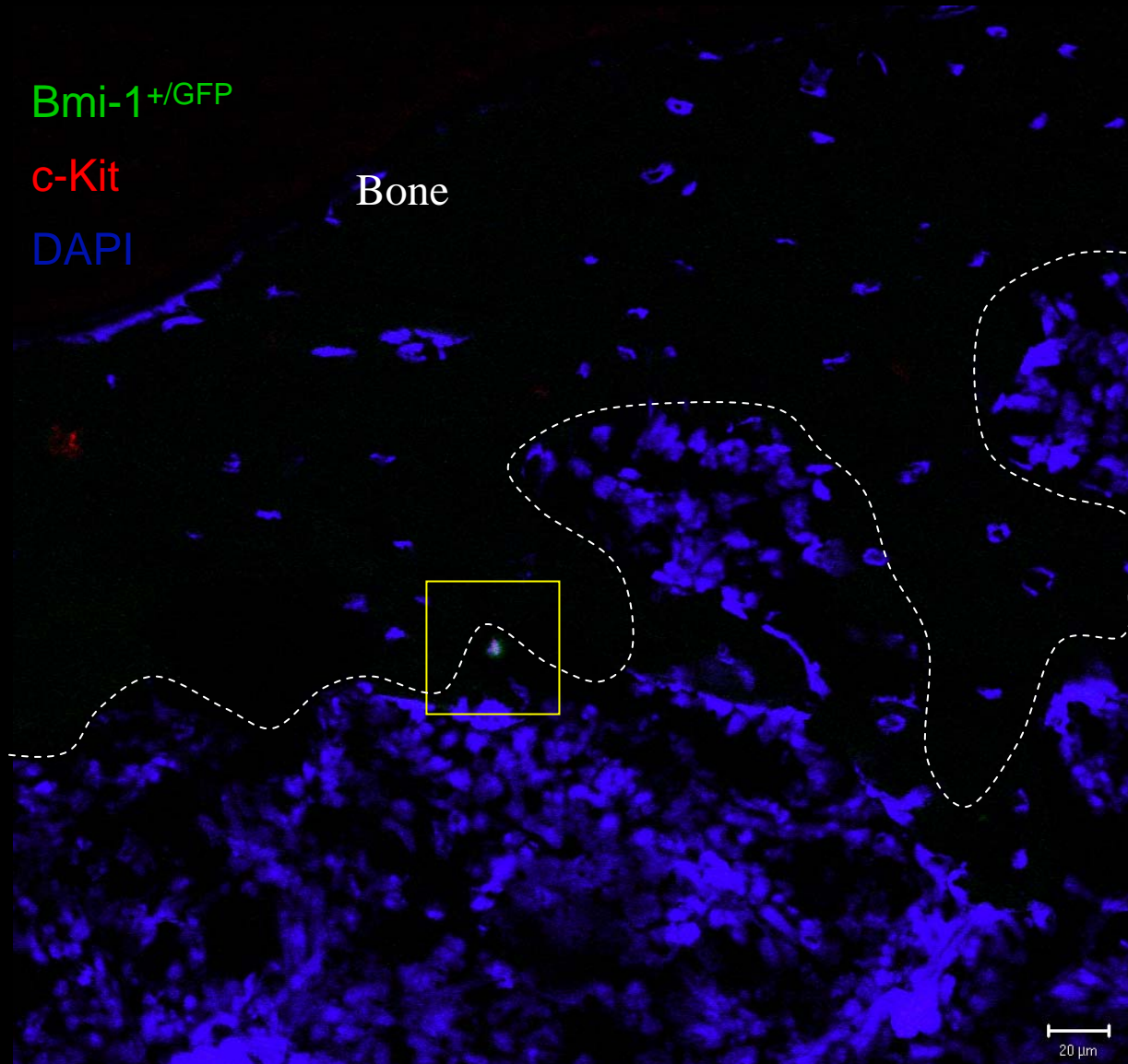
LSK cells express highest levels of $Bmi-1^{+}/GFP$ transgene



c-kit expressing $Bmi-1^{+}/GFP^{hi}$ cells
Co-express Sca-1



Imaging of Bmi-1^{+/GFP} cKit⁺ cells by confocal microscopy

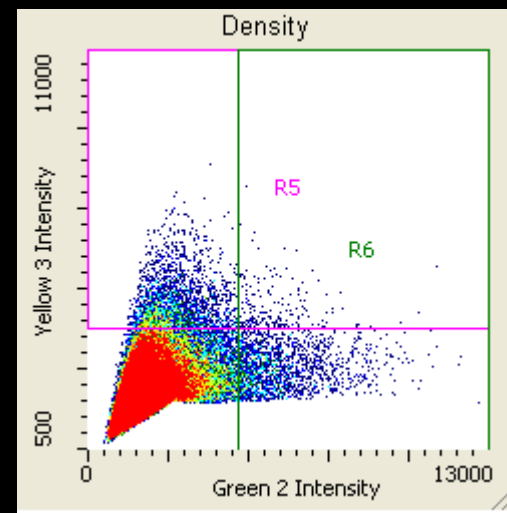
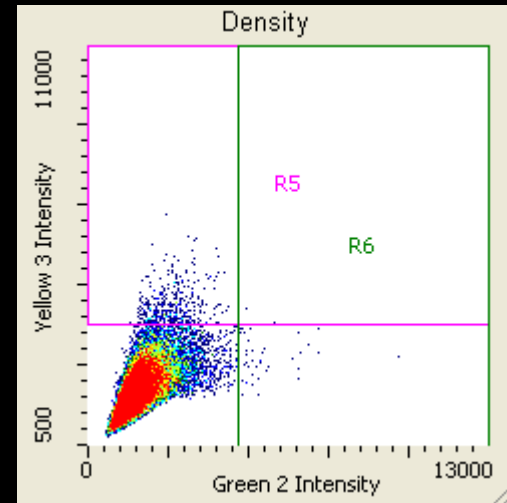


LSC analysis of spatial distribution of HSPC

Negative control

Bmi-1^{+/GFP}

c-kit



FITC antiGFP

LSC analysis of spatial distribution of HSPC

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LSC analysis of spatial distribution of HSPC

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LSC allows single event analysis

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LSC allows single event analysis

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Summary and Conclusions

1. B lymphopoiesis in BM is not compartmentalized as in secondary lymphoid organs.
2. Early progenitor B cells localize preferentially in the endosteum.
3. Mature B cells exhibit an opposite gradient in distribution with preferential localization in the central medullary region
4. HSC localize preferentially in trabecular metaphysis.
5. Quantitative image analyses suggest the importance of specific microenvironments, e.g. niches regulating different stages of hematopoietic development.

Future and on-going studies

- Characterization of niche cells
- Optimization of Bmi-1/GFP model with additional HSPC markers (LSKCD34⁻, LSKCD150⁺)
- Lodgement of transplanted HSC
- Quiescent HSC niche: analysis of BrdU retaining cells and Histone2B/GFP mice
- Cancer stem cell niche: leukemia models (leukemia initiating cells)
- Effects of irradiation and mobilization on HSC niches
- Regulation of HSC niches and distribution by HSC intrinsic and extrinsic pathways

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