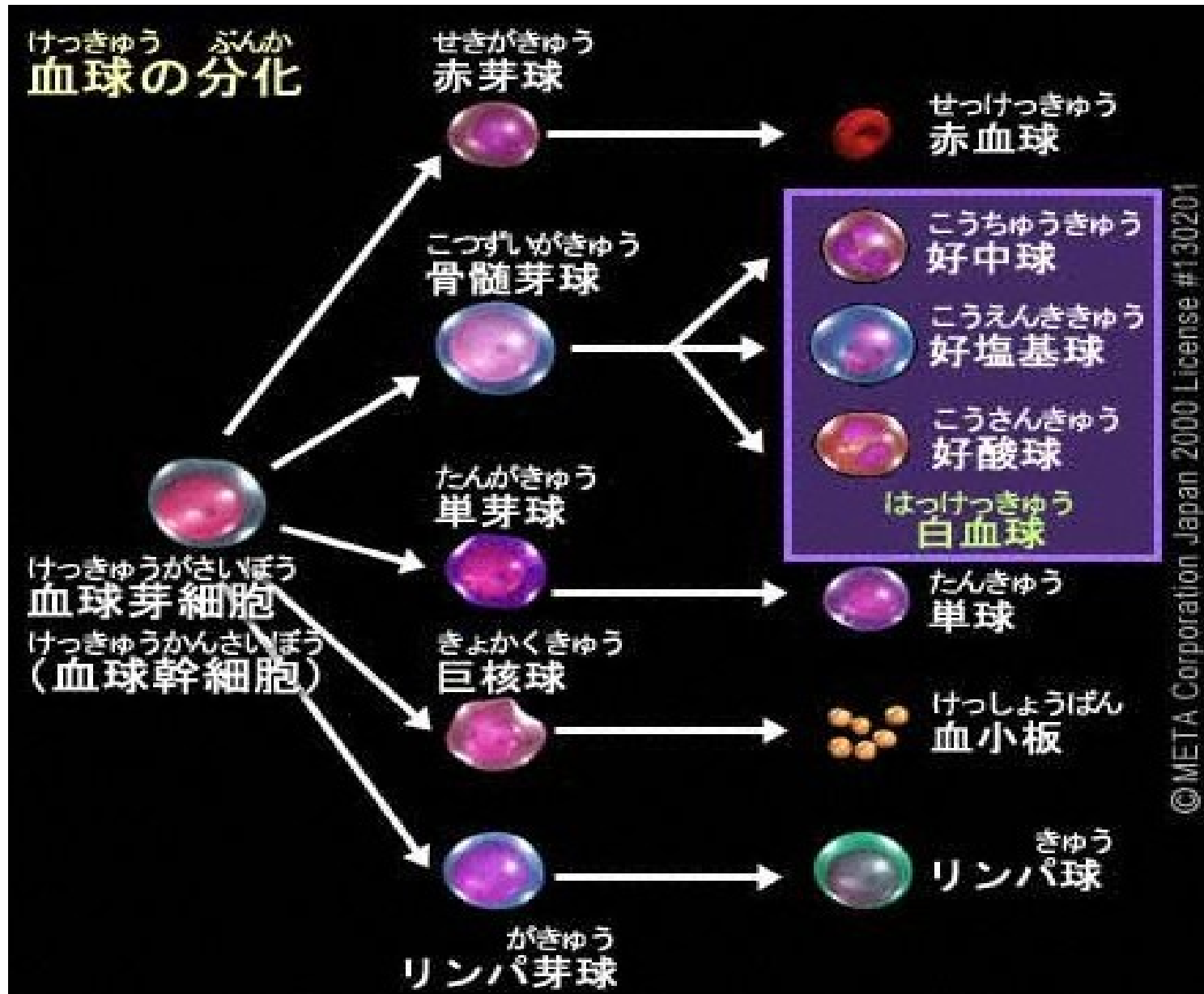
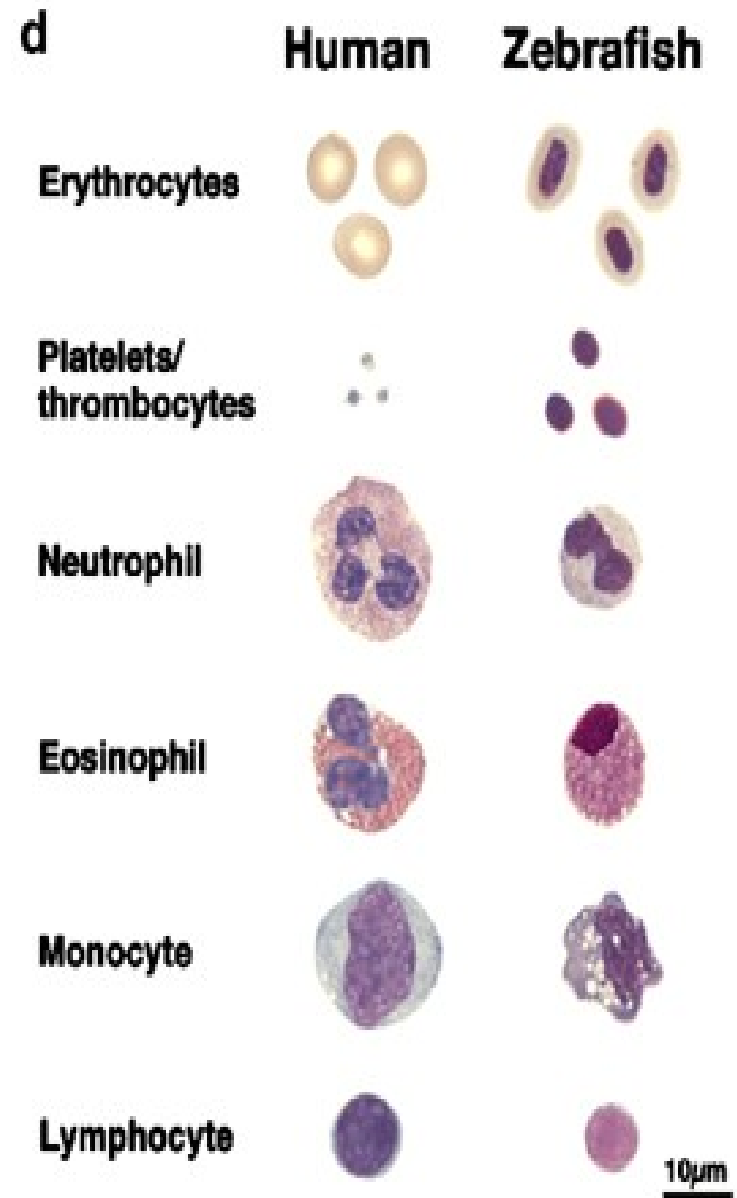
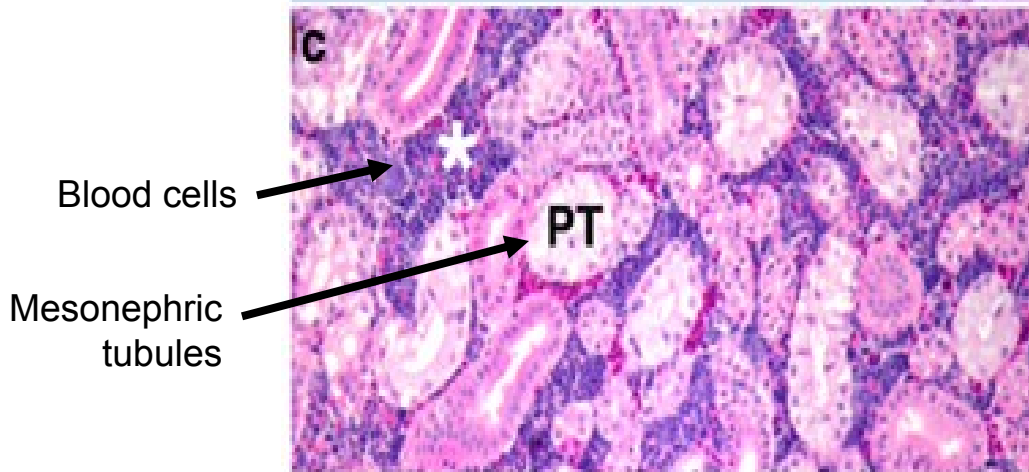
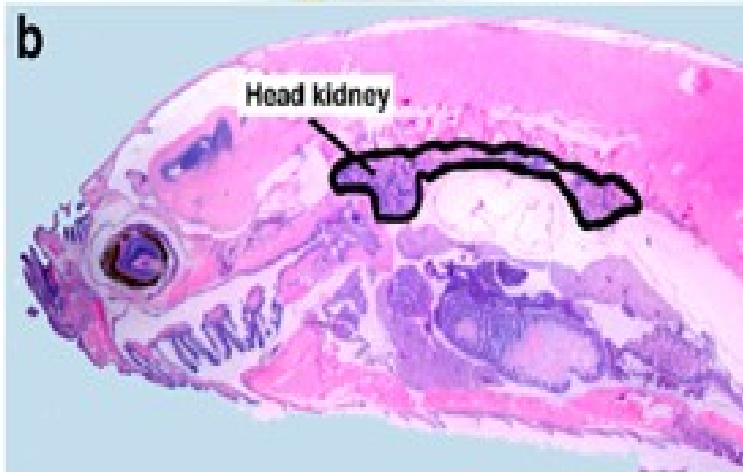
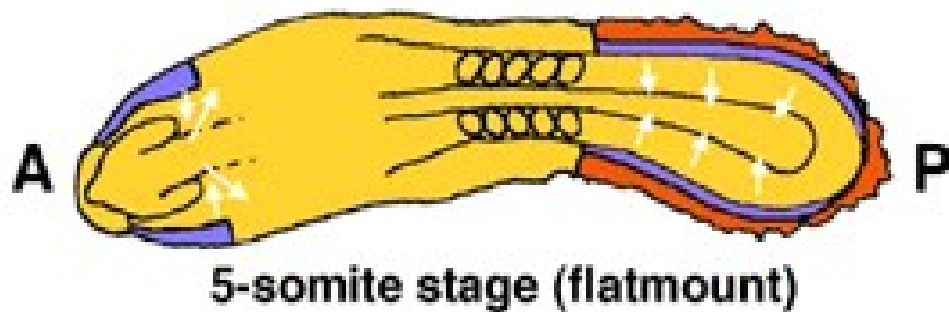
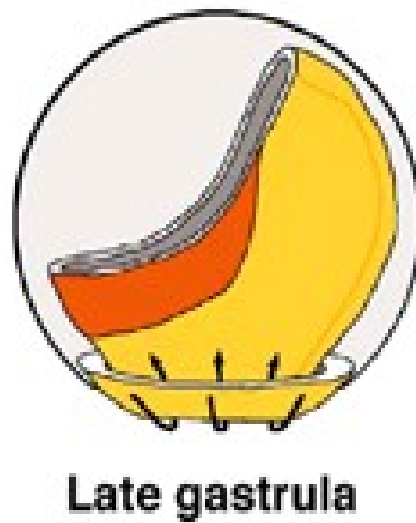
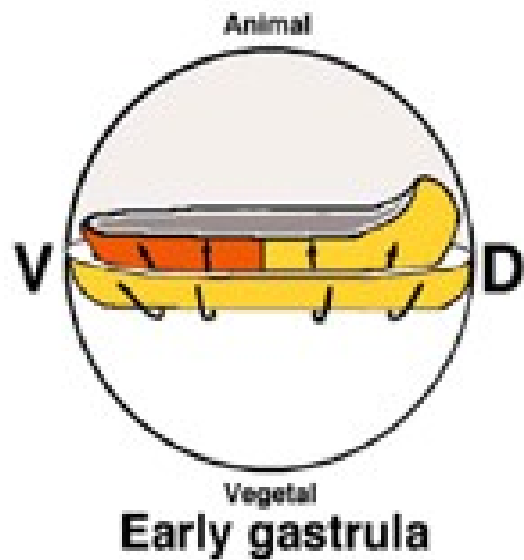


5. DEVELOPMENT OF BLOOD

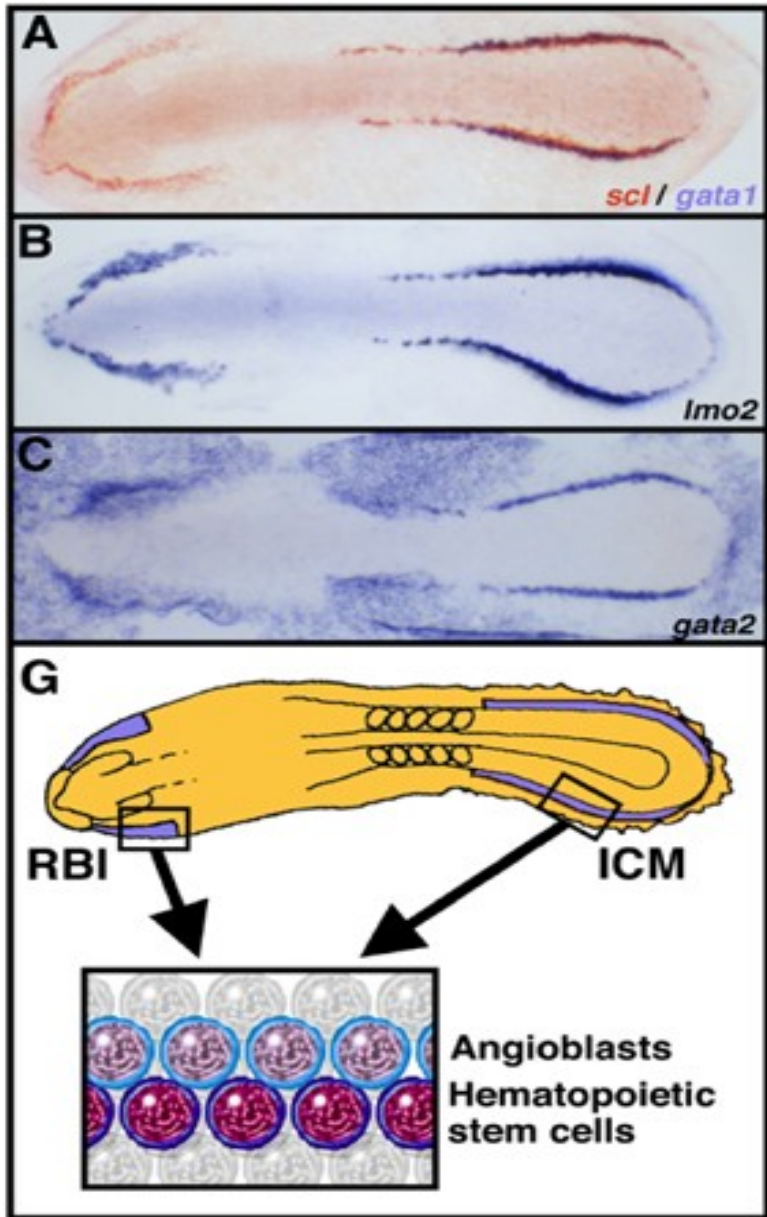






 primitive hematopoiesis
 ventral mesoderm

Fish gastrulation movie

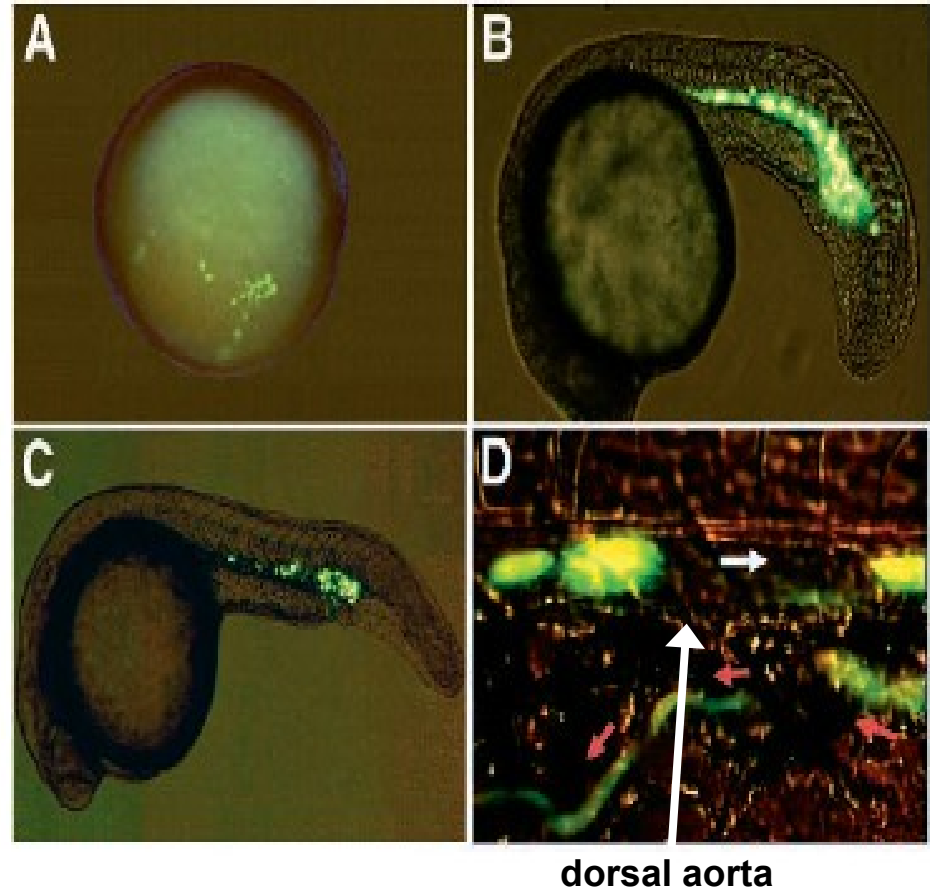


scl, gata1/2, lmo2 - transcriptional factors that specify early hematopoietic tissue (*gata2* expressed also in ectoderm)

RBI – rostral blood islands

ICM – inner cell mass

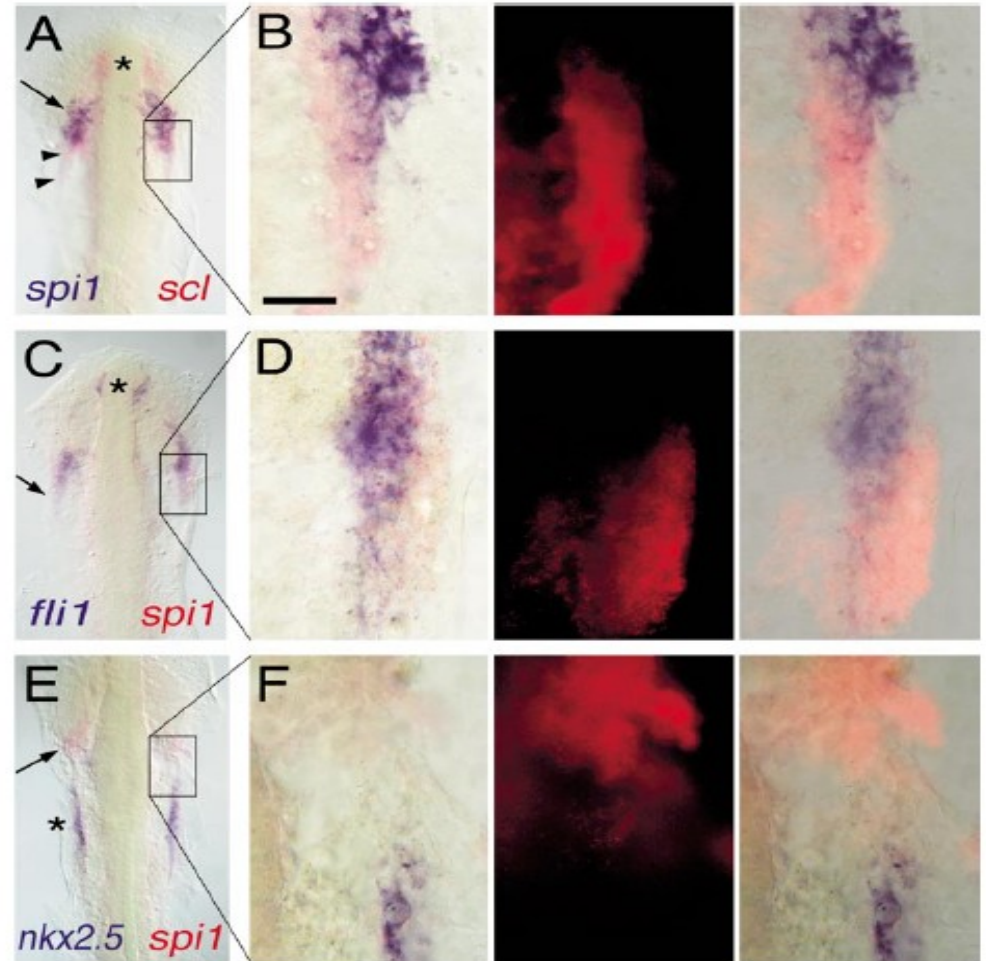
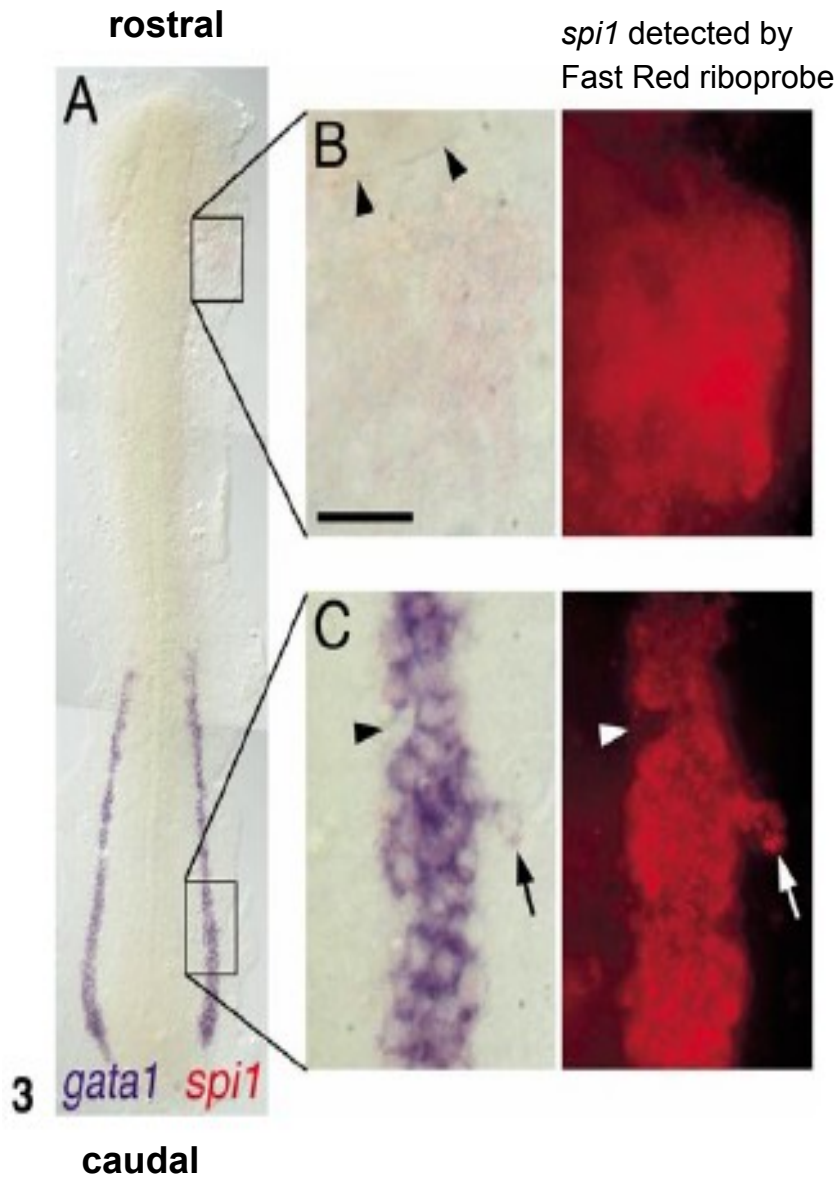
gata1 promoter-driven GFP = early erythroid lineage



5-somite stage embryo – hematopoiesis starts officially

MYELOPOIESIS STARTS IN RBI @ 10-SOMITE STAGE

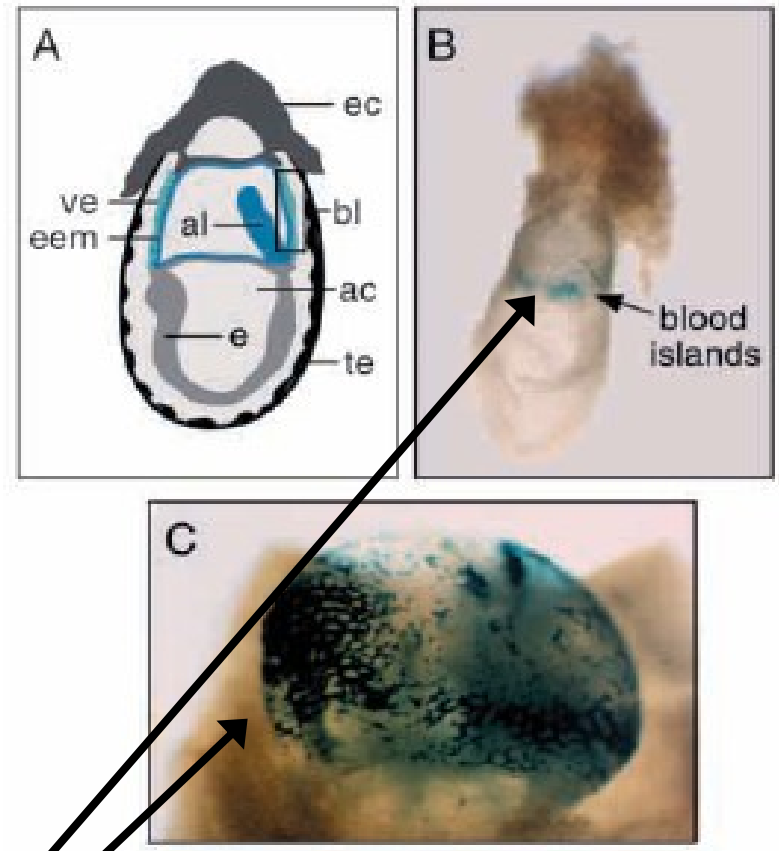
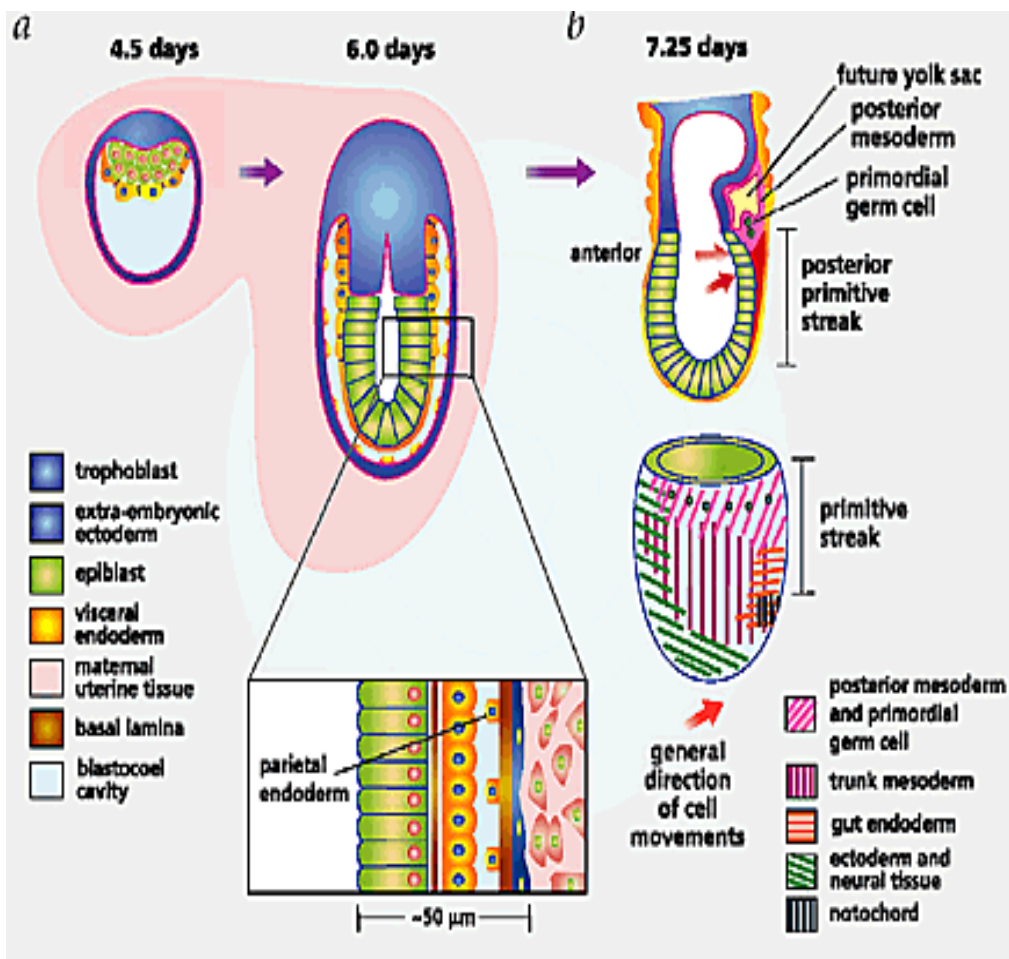
spi1 – marker of myeloid lineage



scl – early hematovascular cell fate

fli1 – early vascular fate

nkx2.5 – heart fate



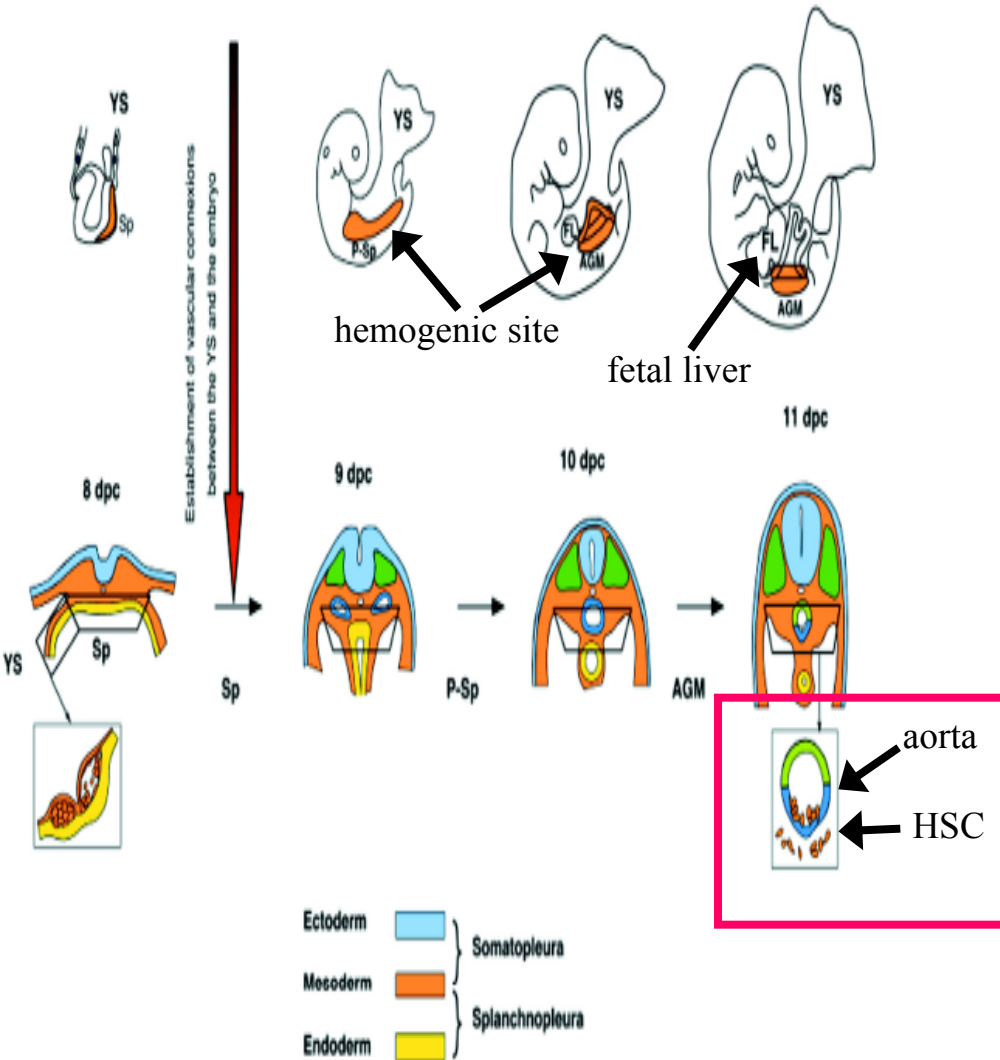
8.5 dpc – yolk sac encloses entire embryo
 blood islands merge to form vascular channels

LacZ driven by β -globin promoter
 (X-gal staining – primitive erythroblasts)

bl - blood islands
 ec - ectoplacental cone
 ac - amniotic cavity
 te - trophectoderm
 al - allantois
 eem - extraembryonic mesoderm (blue)
 ve - visceral endoderm
 e - embryonic ectoderm

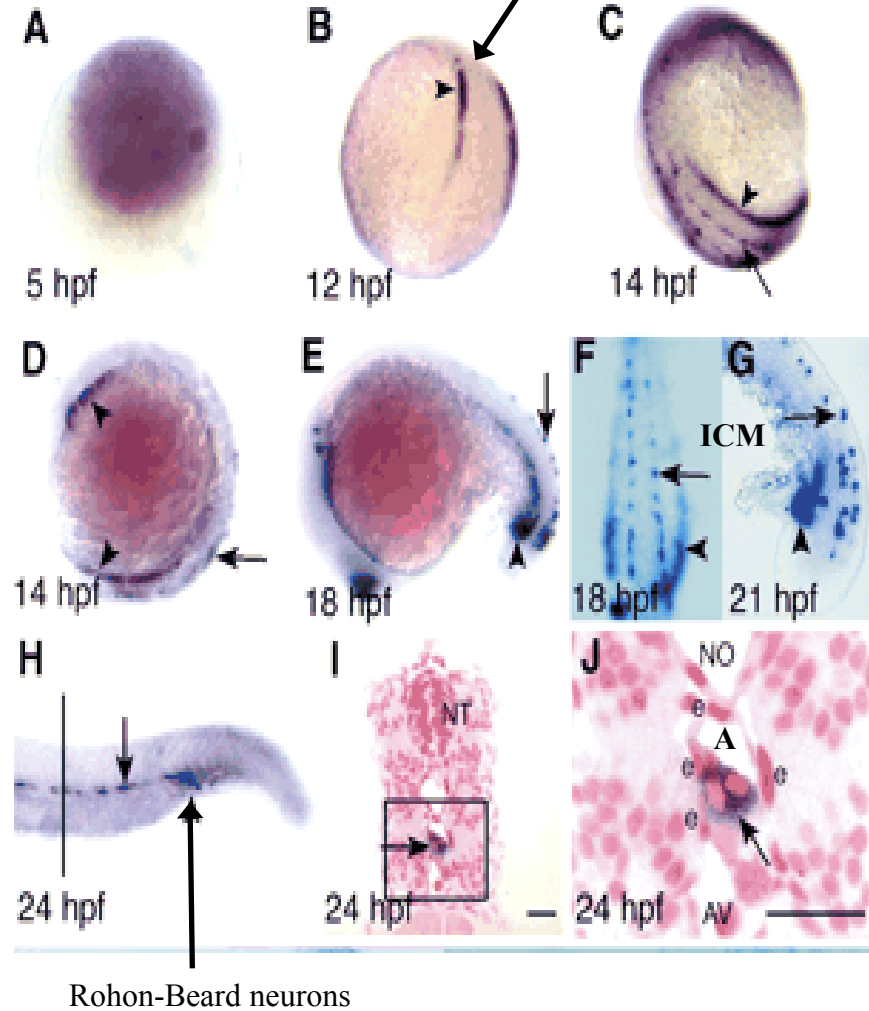
AORTA-GONAD MESONEPHROS (AGM)

Development of intra-embryonic hemogenic site



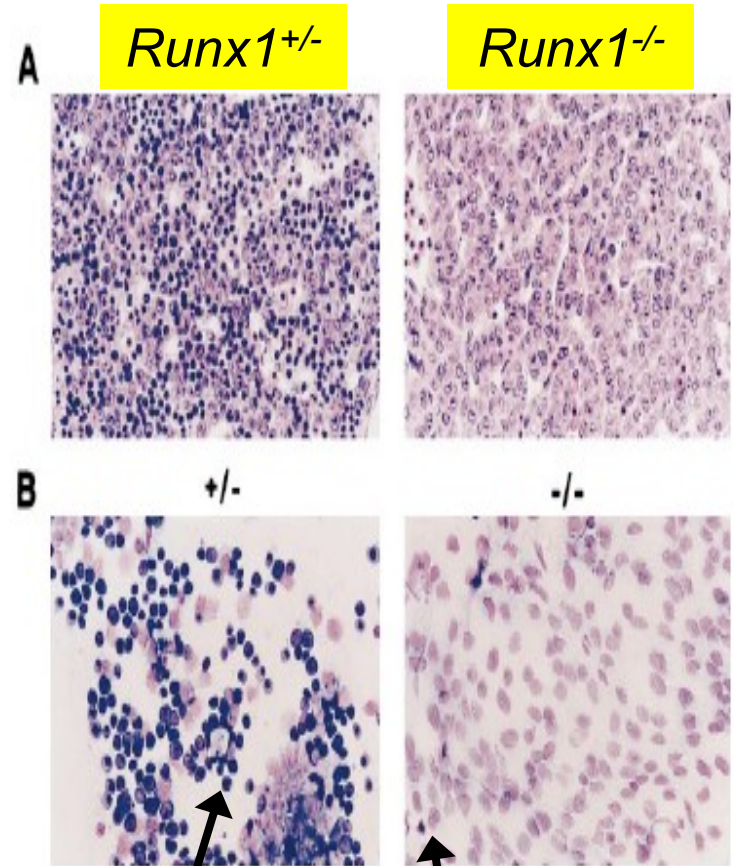
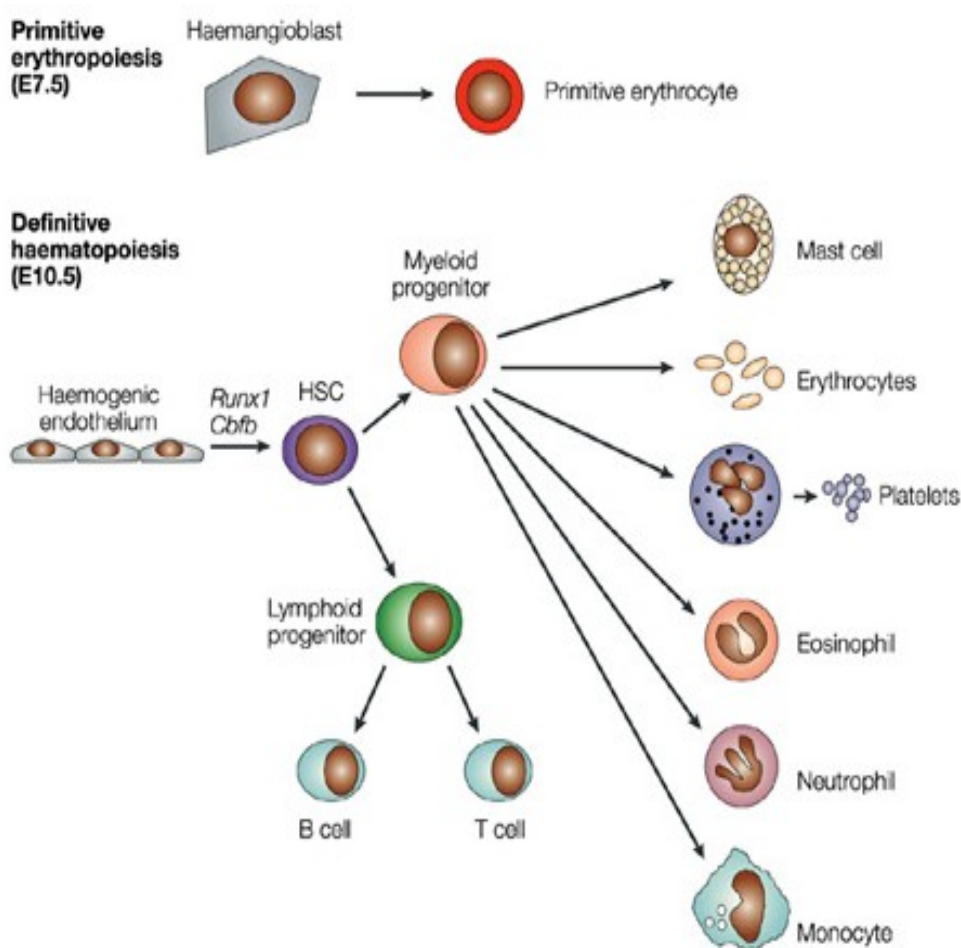
RUNX1 riboprobe

lateral plate mesoderm



A- aorta
 NO- notochord
 AV- axial vein
 e - endothelial cell

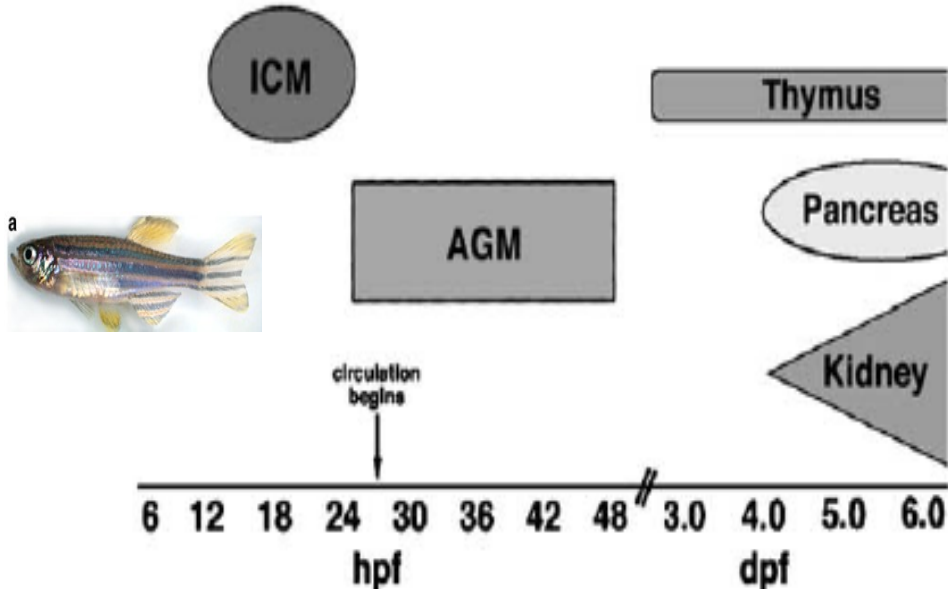
RUNX1 (CBFA2, AML1) is necessary for 'definitive' hematopoiesis to start at AGM



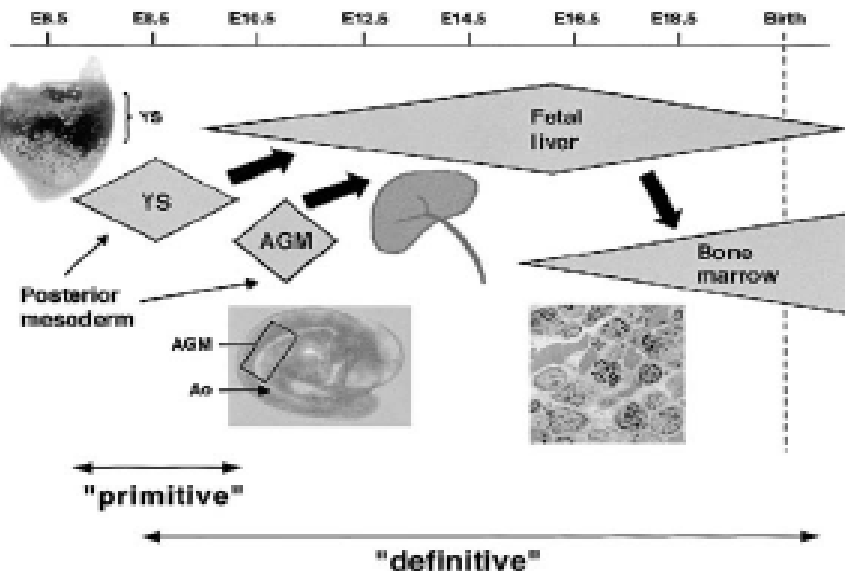
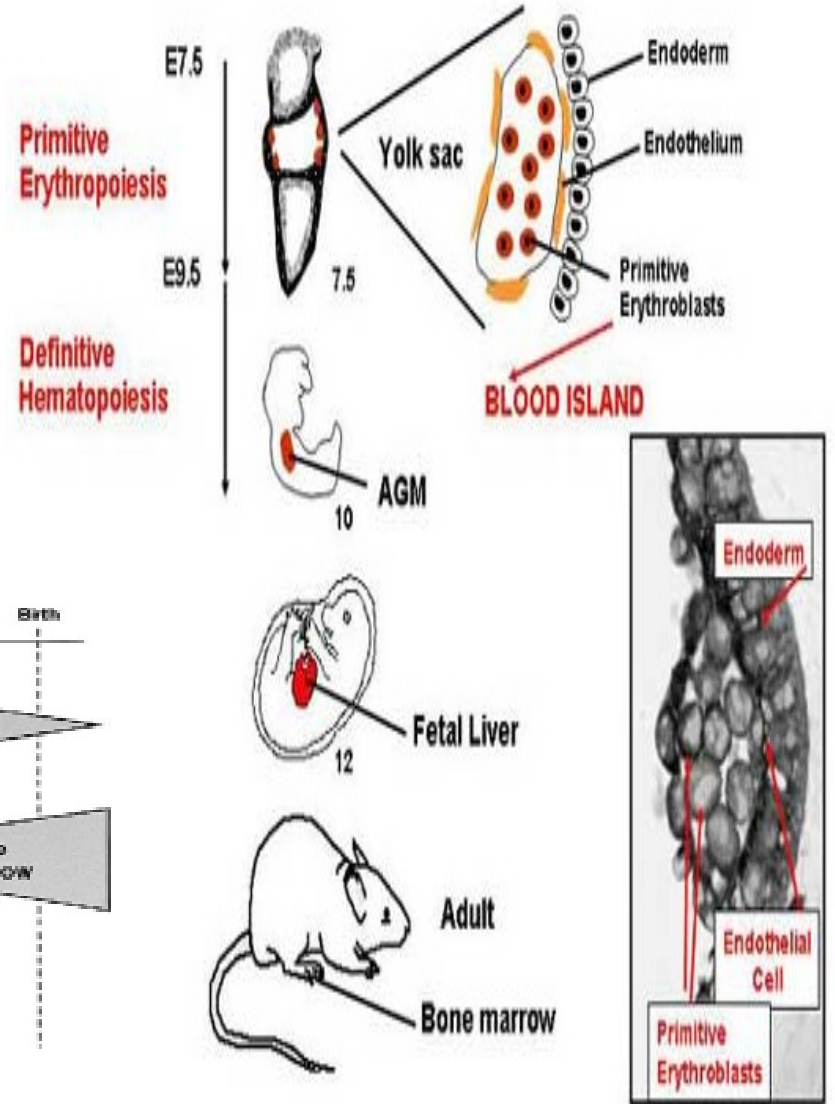
E11.5: **dark-** hematopoietic cells

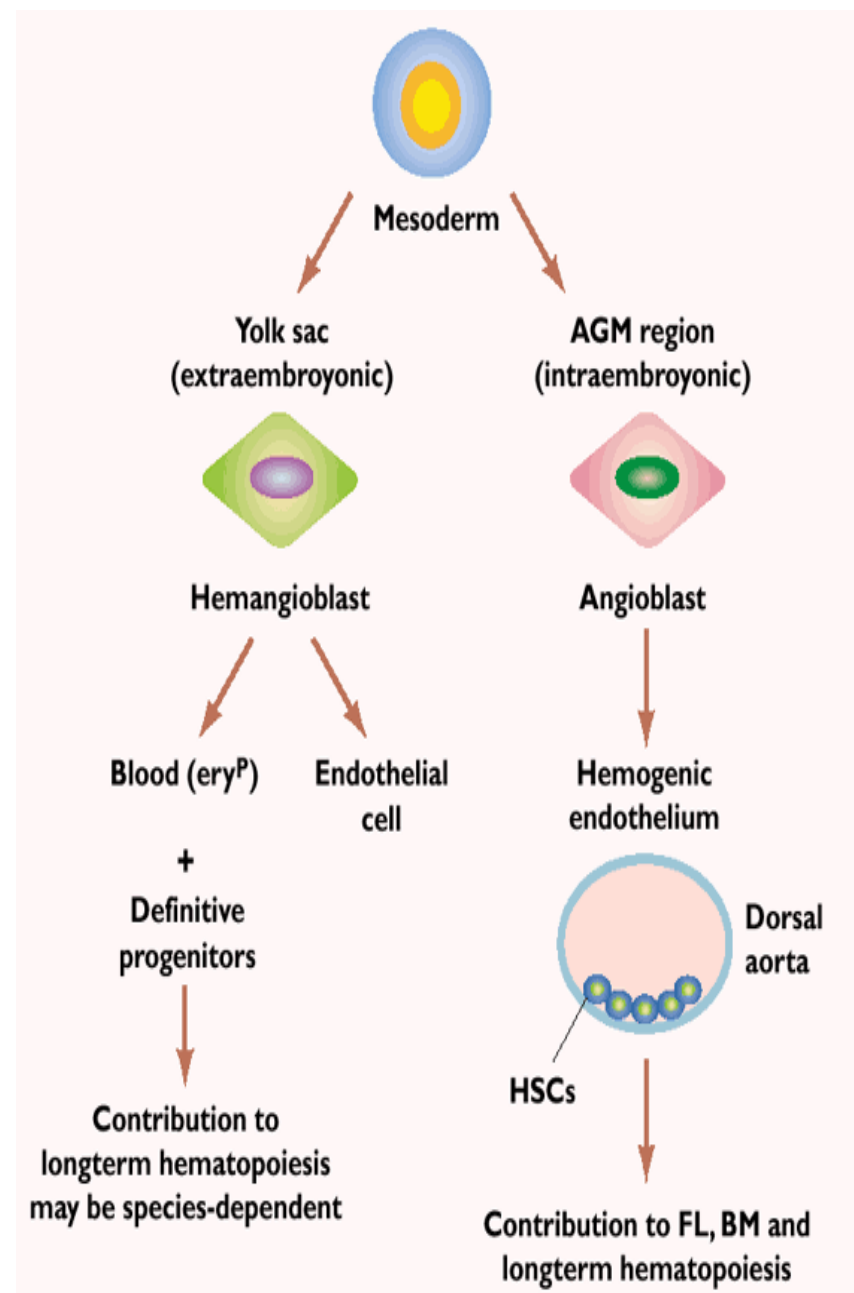
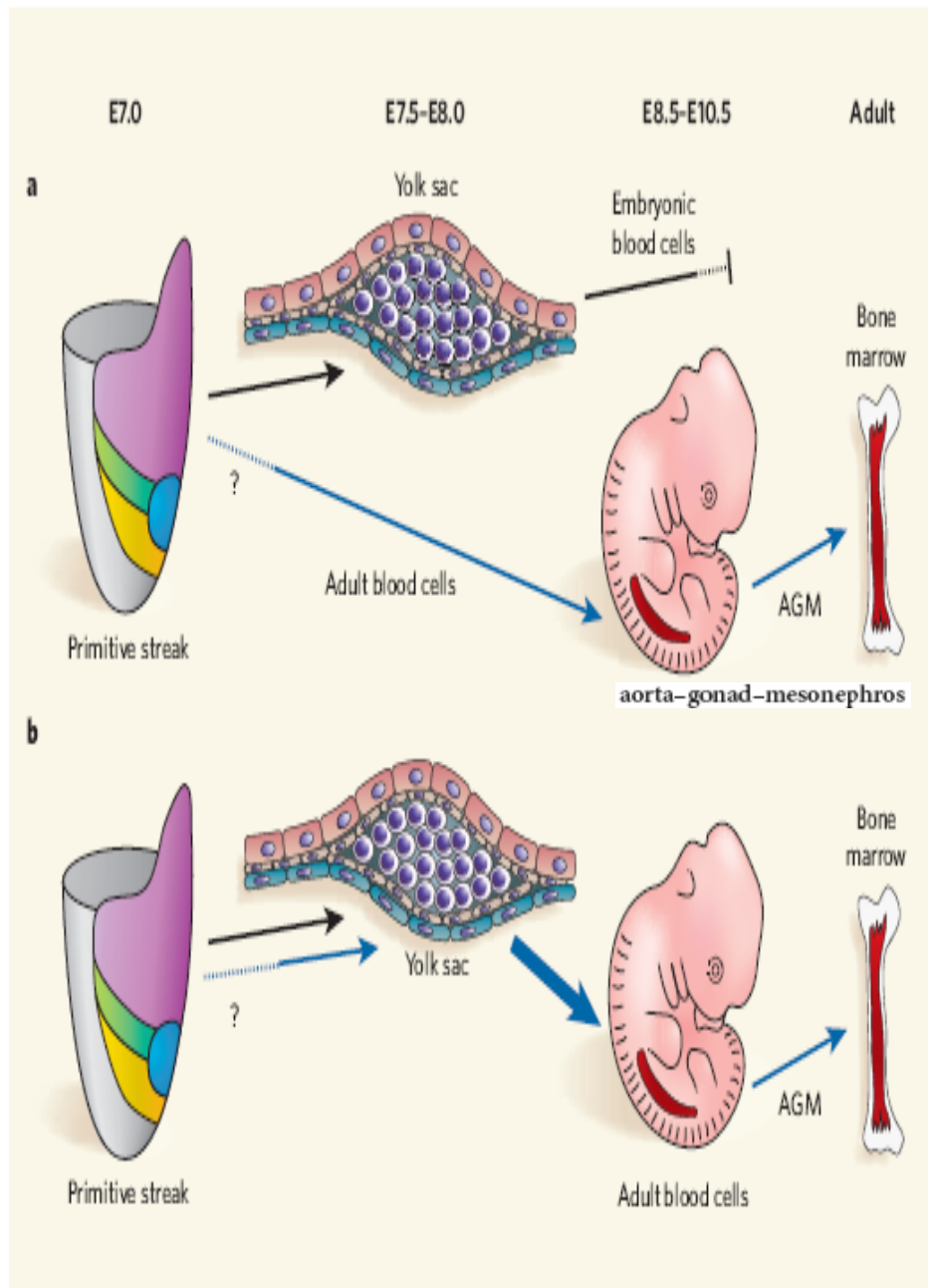
only a few primitive erythrocytes left – likely a carry-over from the 'primitive' hematopoiesis

EXTRAEMBRYONAL vs EMBRYONAL

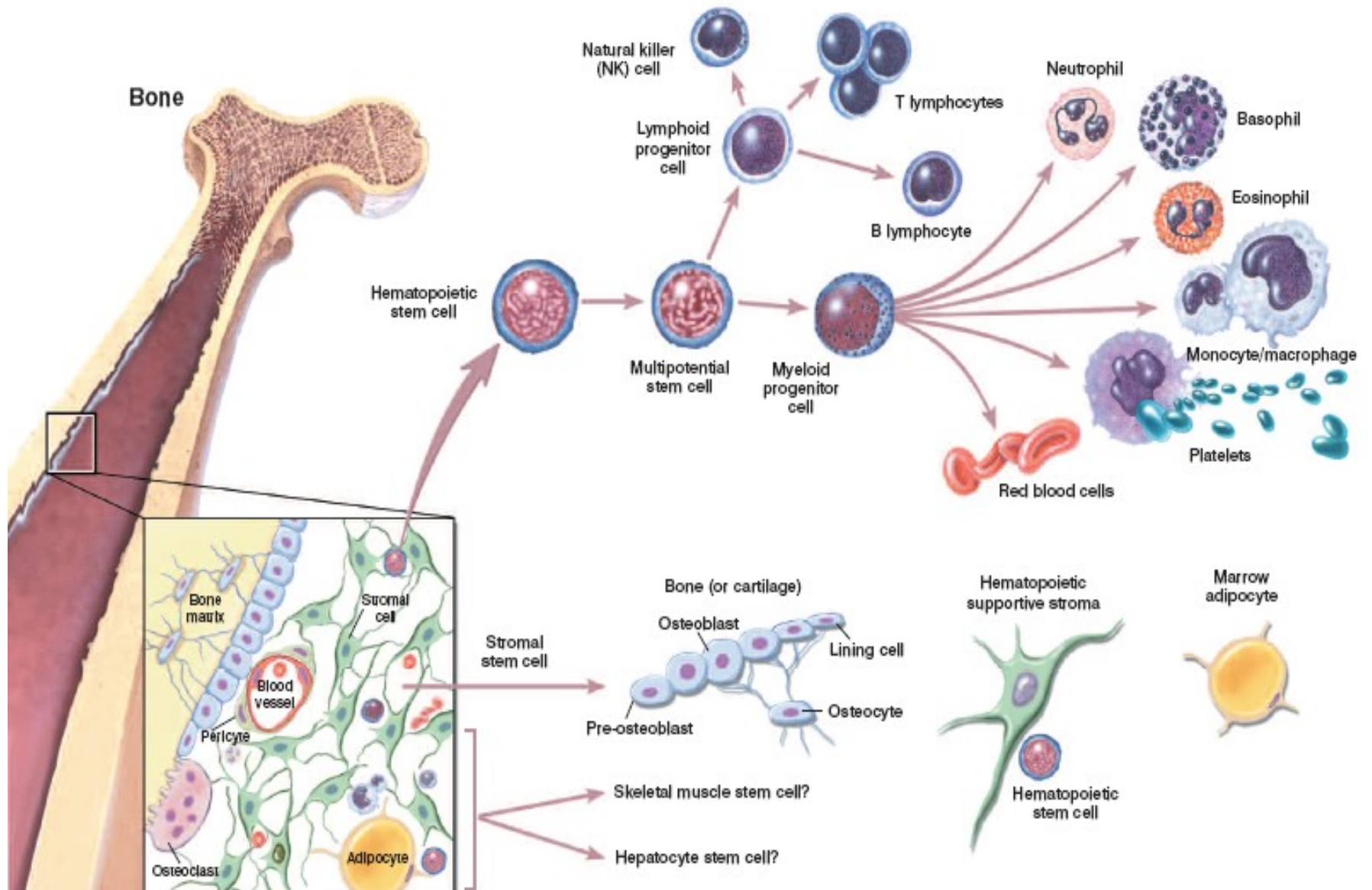


PRIMITIVE vs. DEFINITIVE

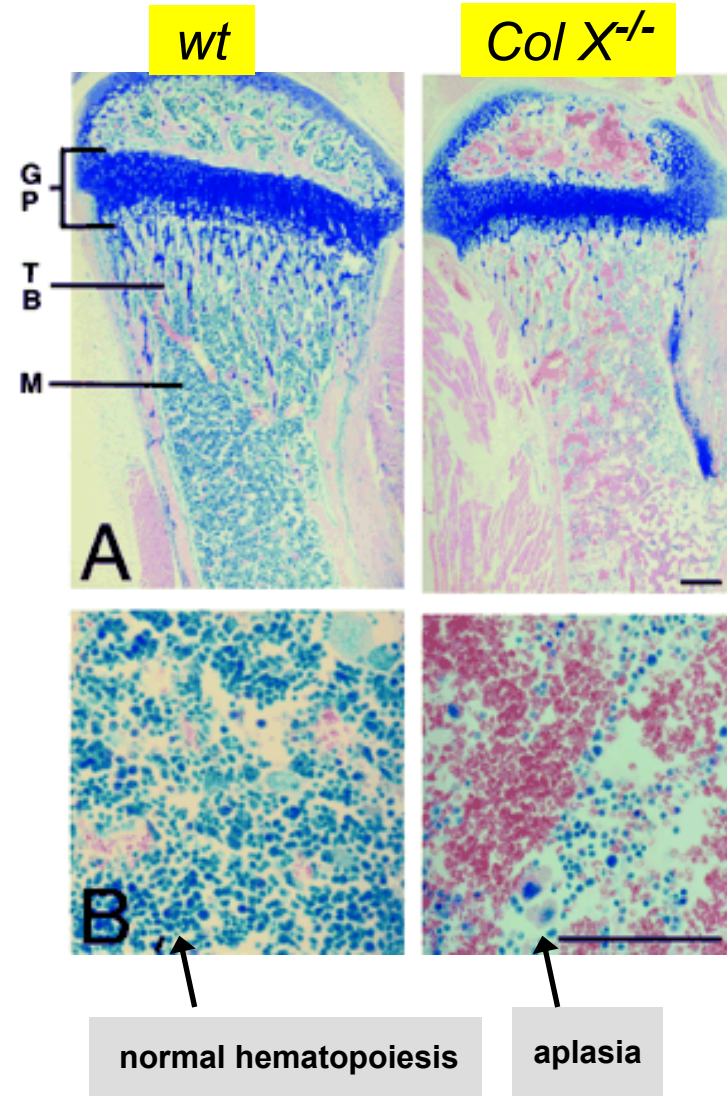
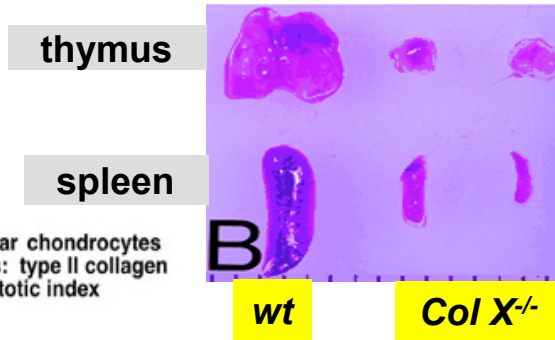
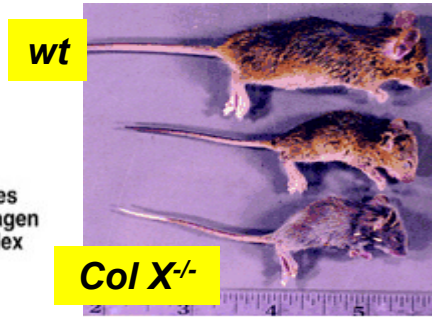
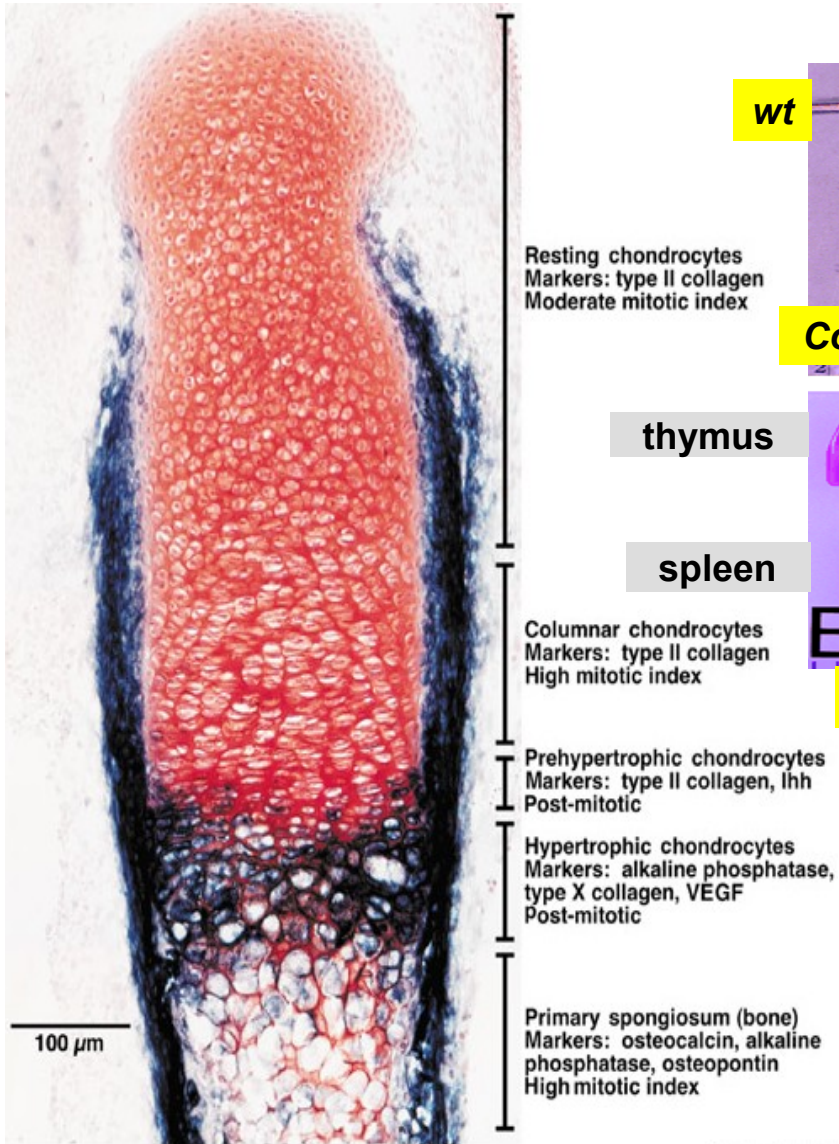




BONE MARROW NICHE IS CRITICAL FOR HEMATOPOIESIS



COLLAGEN TYPE-X DELETION

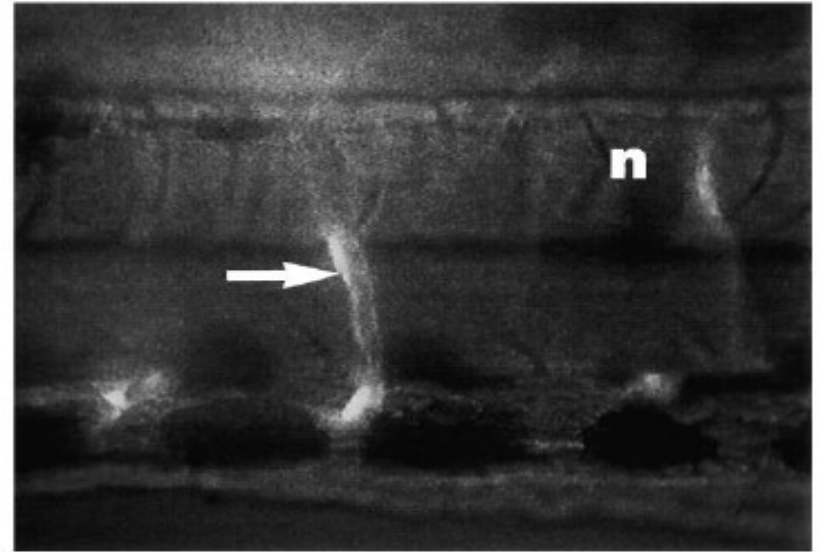
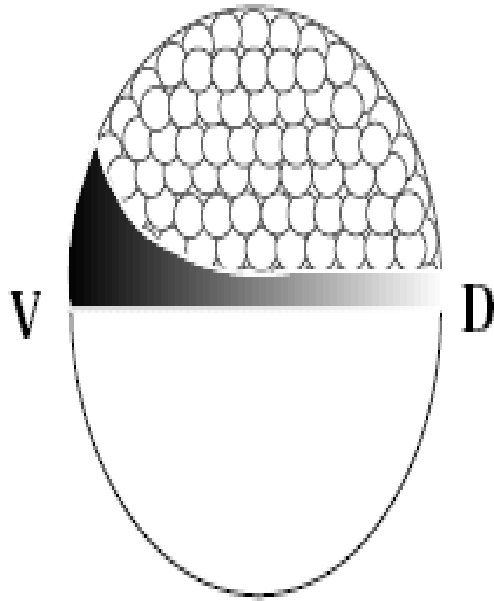


THE HUNT FOR HEMANGIOBLAST AND ITS HEM- ANGIO-DIFFERENTIATION



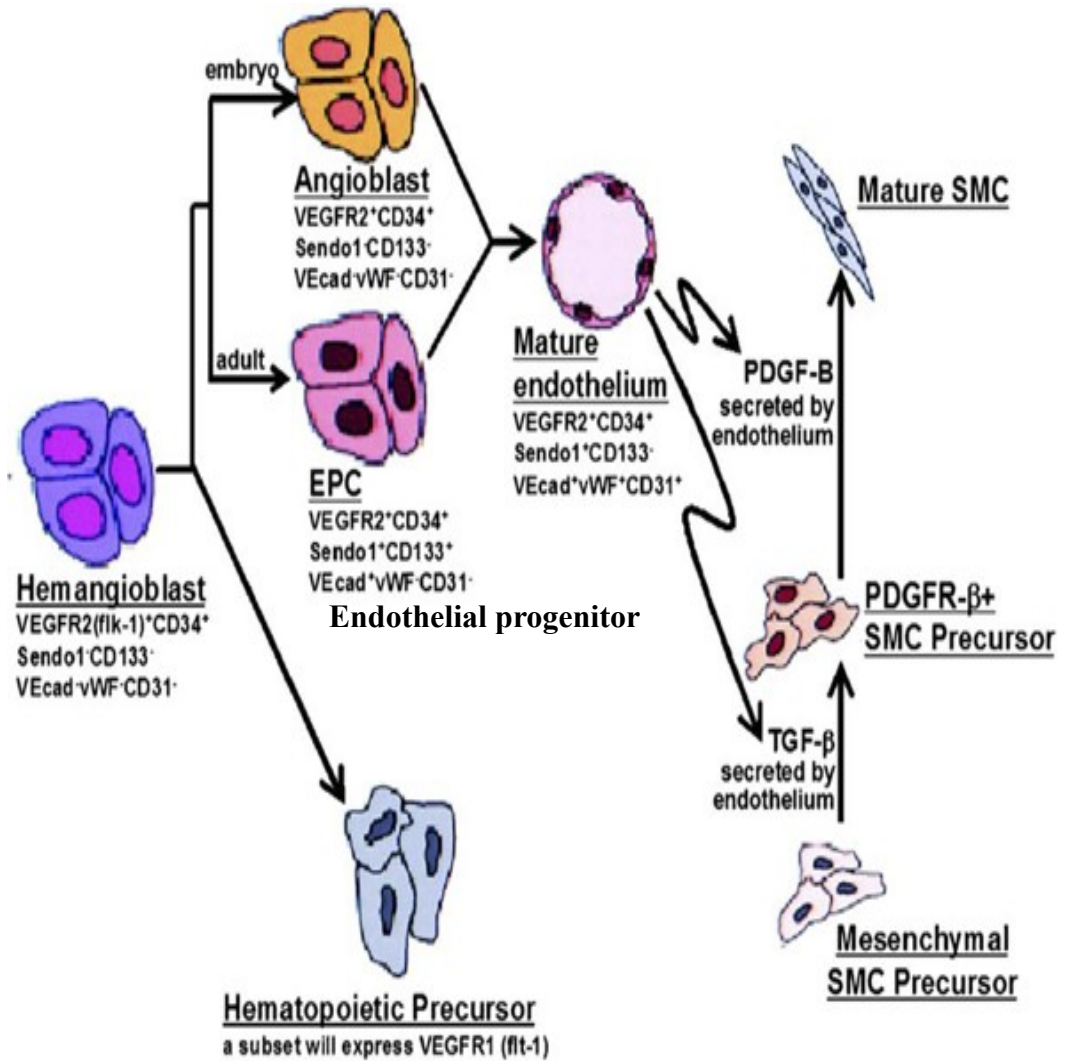
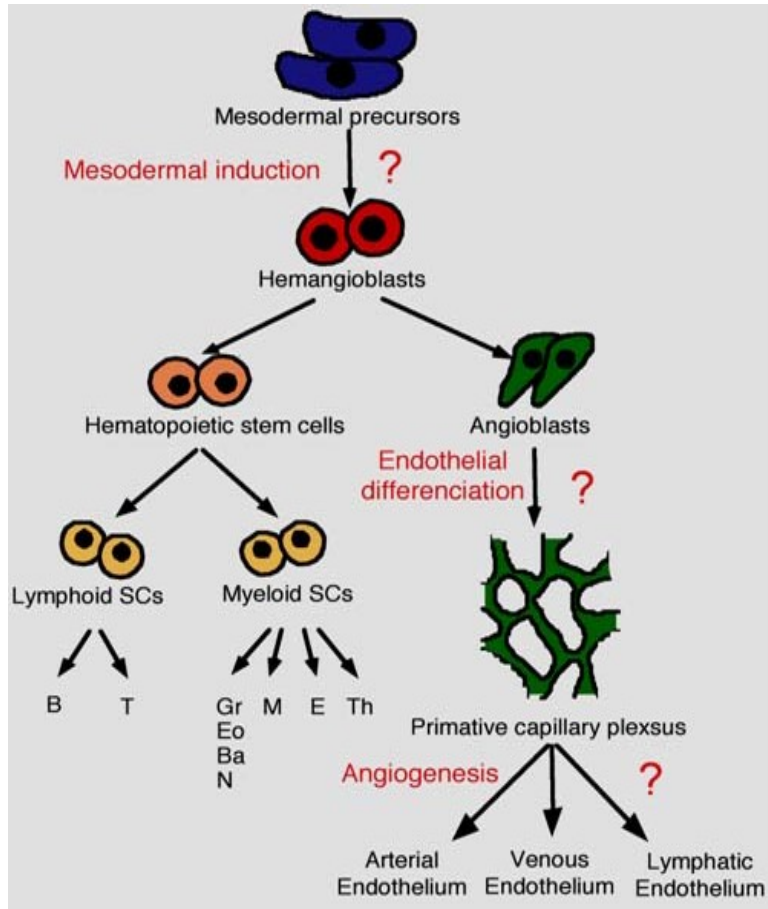
HEMANGIOBLAST

scattered among endothelial and HSC in heart field of the gastrula



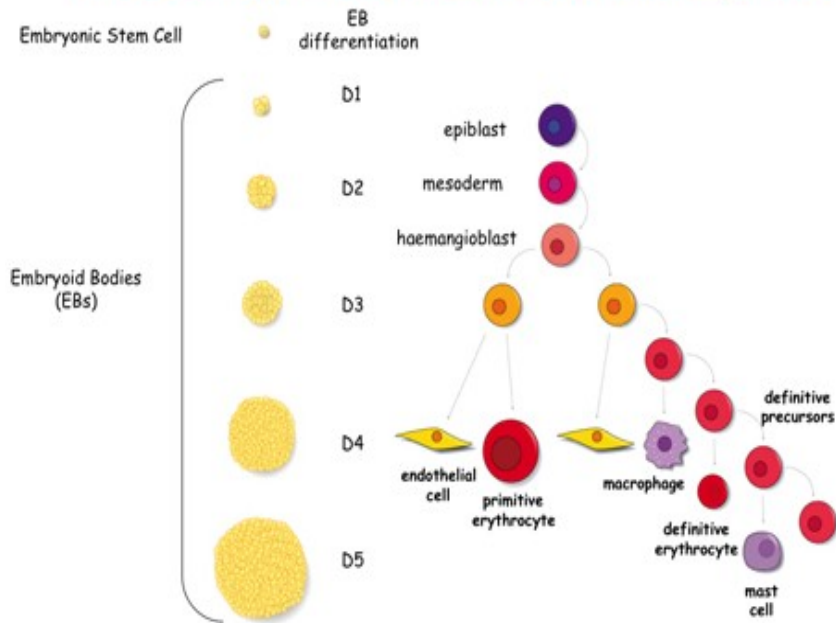
ventral marginal cells of the heart field, n- notochord

Fig. 5. The heart field. Diagrammatic representation of the heart field in the early blastula. The intensity of grey represents the propensity to form heart.

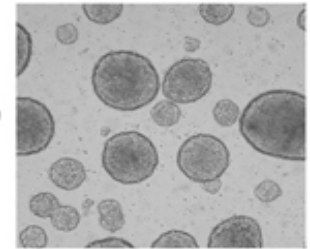
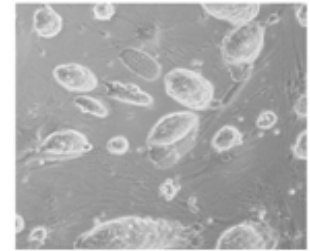
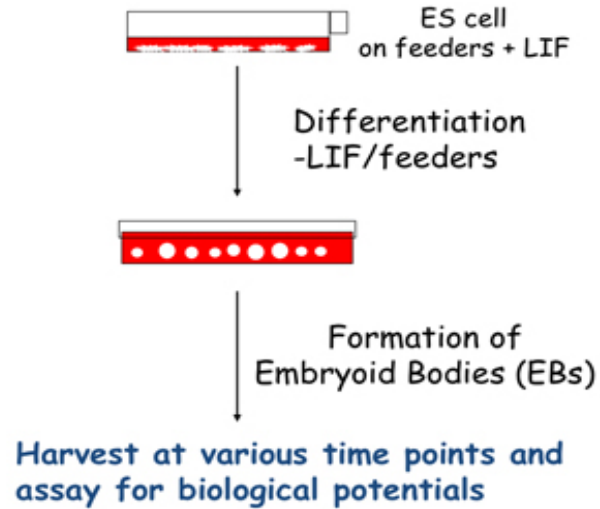


HEMANGIOBLAST or HAEMOGENIC EPITHELIA, or BOTH?

ES/EB as a Model of Yolk Sac Haematopoiesis

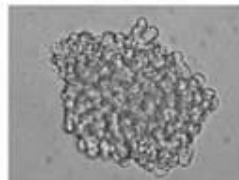
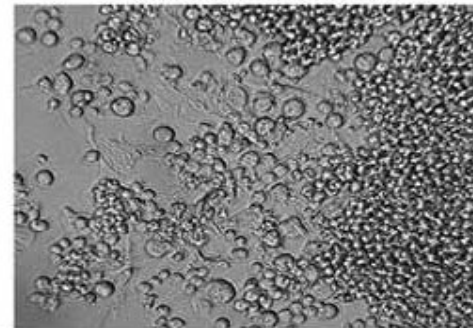
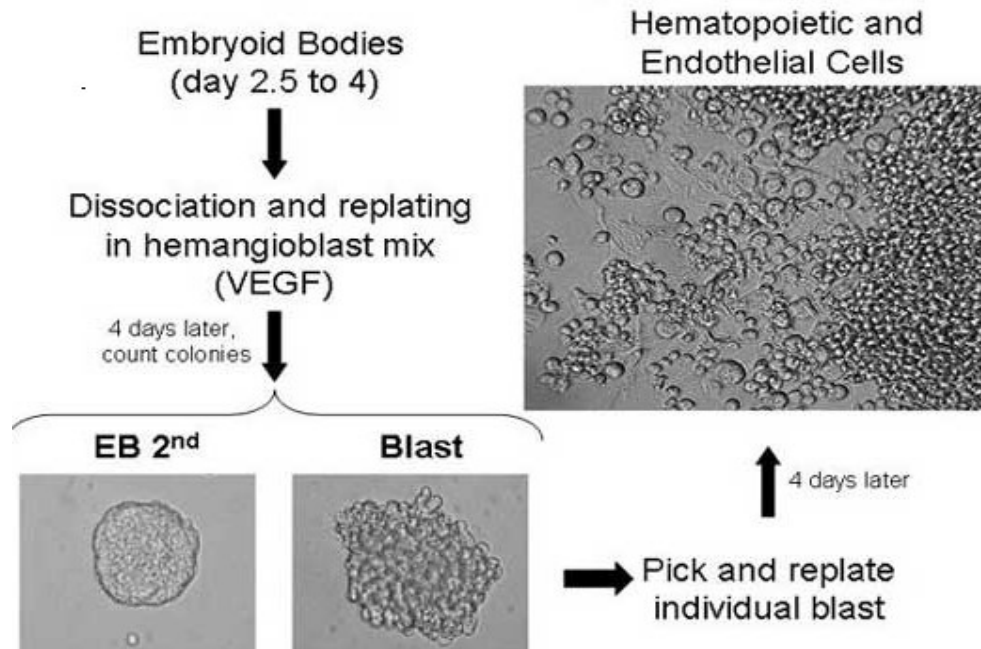


In vitro differentiation of ES cells



blast colony-forming cell

Hemangioblast FLK-1⁺ (BL-CFC) Assay



The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage

NATURE | Vol 457 | 12 February 2009

Christophe Lancrin¹, Patrycja Sroczynska¹, Catherine Stephenson¹, Terry Allen², Valerie Kouskoff³ & Georges Lacaud¹

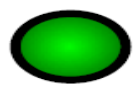
Embryonic Stem (ES) cell

Haemangioblast Fik1⁺

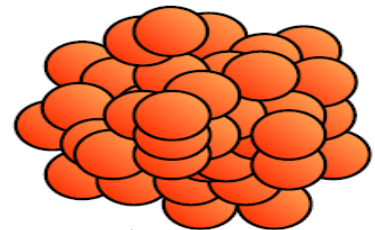
Blast Colony



3 to 3.5 Days
ES differentiation

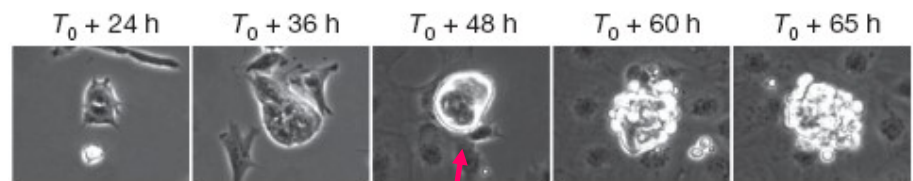
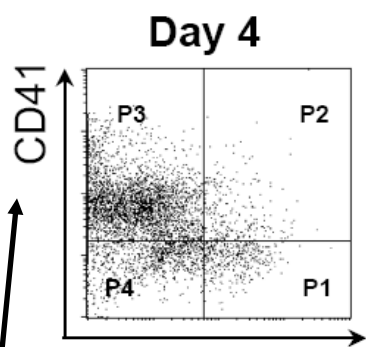


3-4 Days



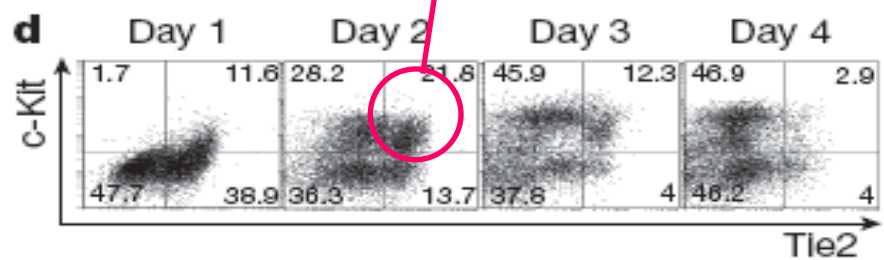
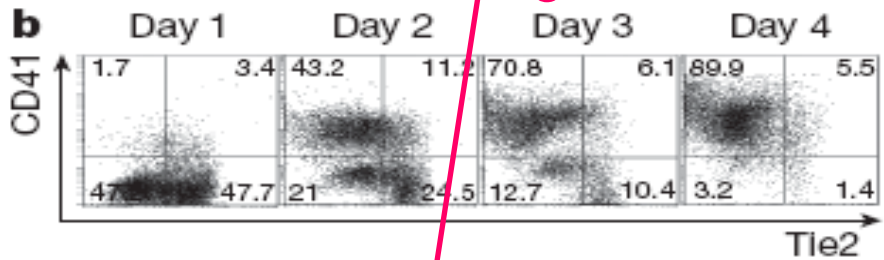
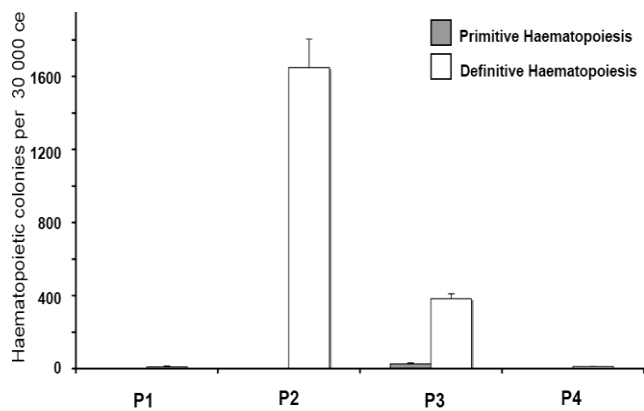
Sort of Fik1⁺ cells

Hemangioblast culture



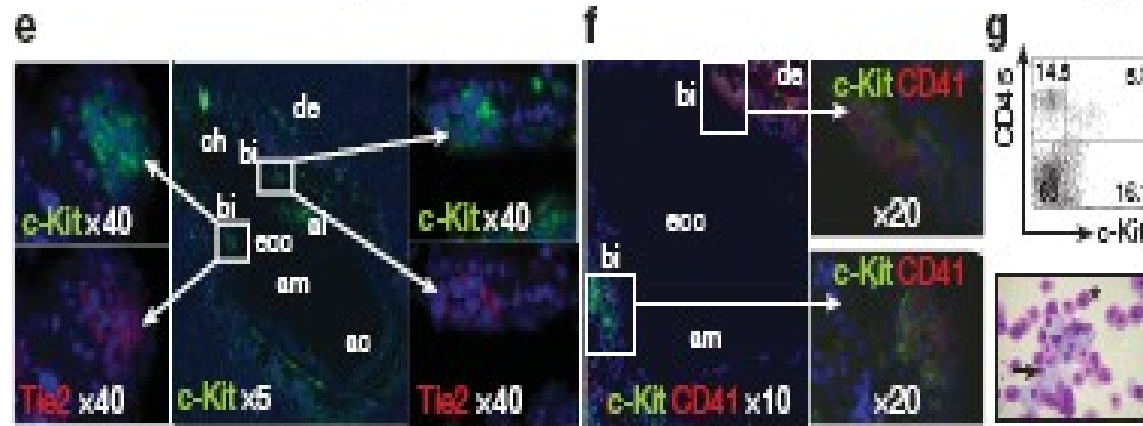
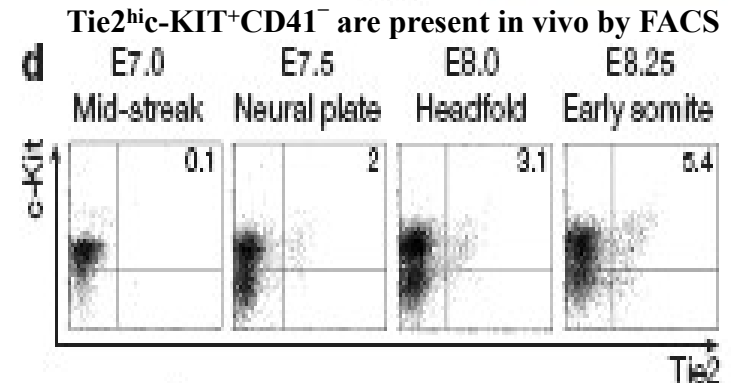
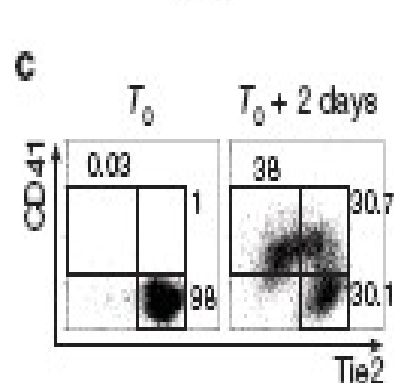
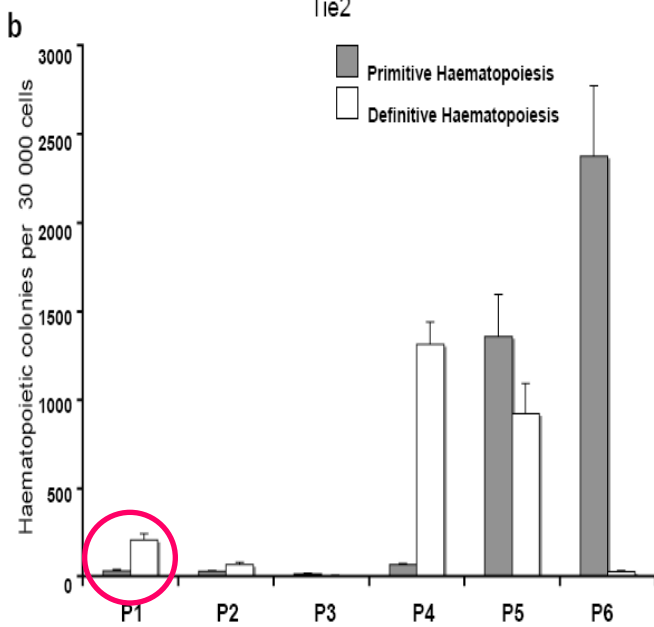
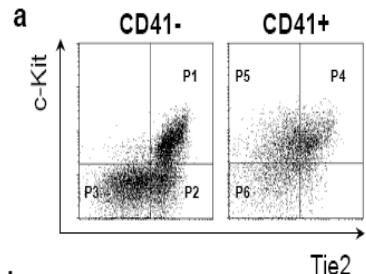
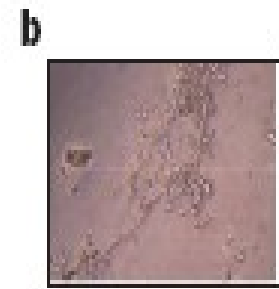
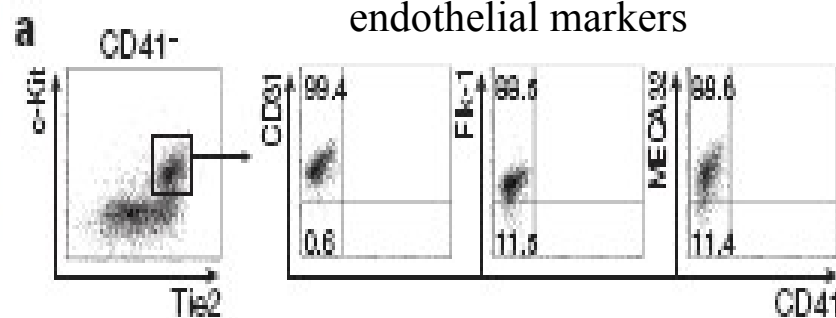
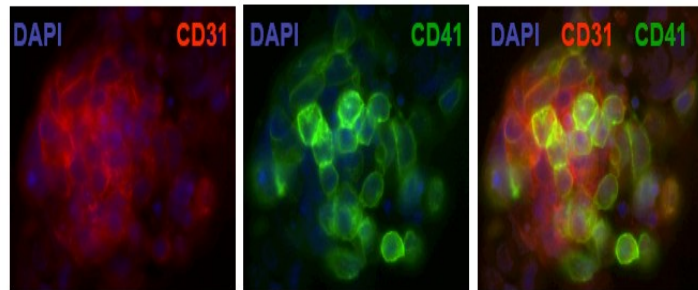
tight adherent structure

Integrin alpha 2B Tie2 Angiopoietin 1 rec.

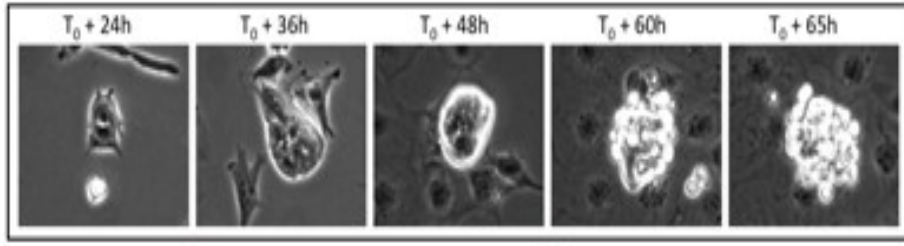


Tie2^{hi}c-KIT⁺CD41⁻ can generate hematopoietic precursors

endothelia in matrigel

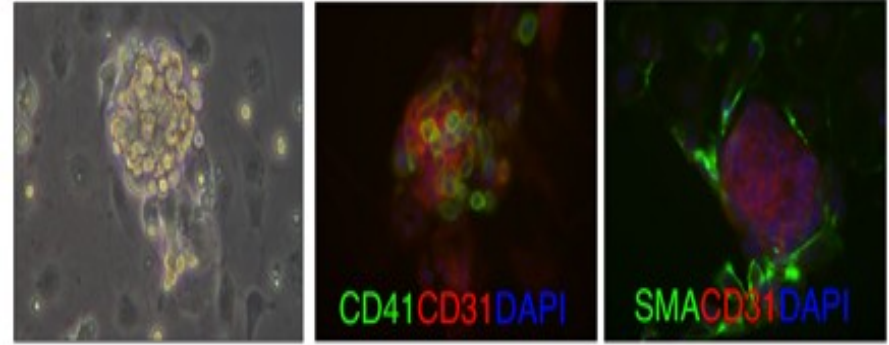


Tie2^{hi}c-KIT⁺CD41⁻ are present in vivo by IHC



Generation of structure of tightly associated cells

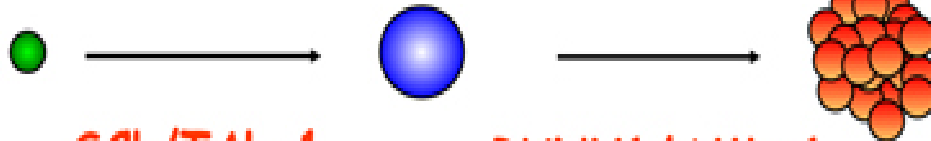
Generation of round cells



Haemangioblast

Haemogenic endothelium

Definitive Haematopoiet progenitors



SCL/TAL-1

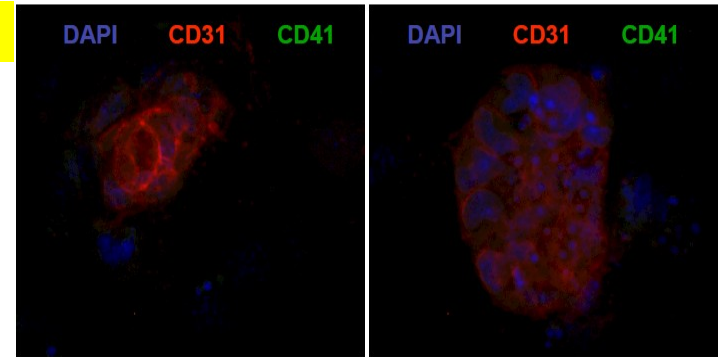
RUNX1/AML-1

Flk1⁺
Bry-GFP⁺

Tie2^{Hi}
c-Kit⁺

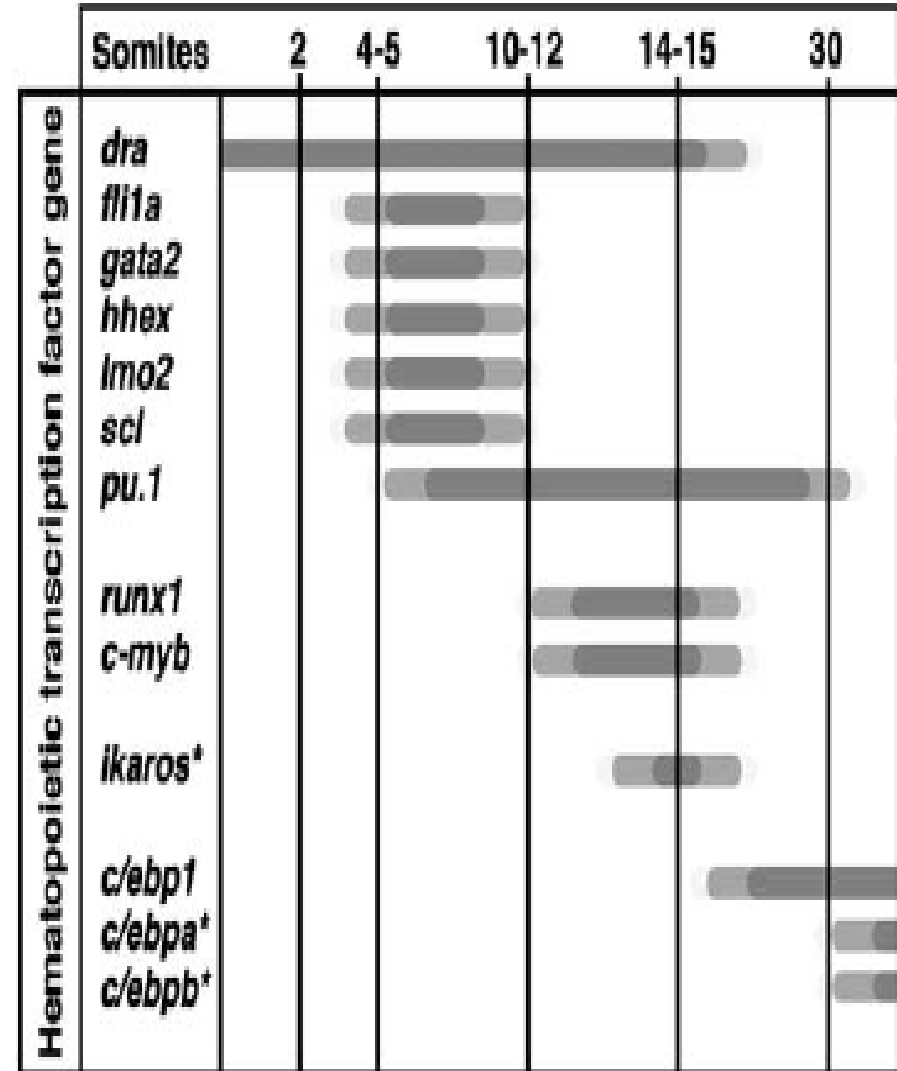
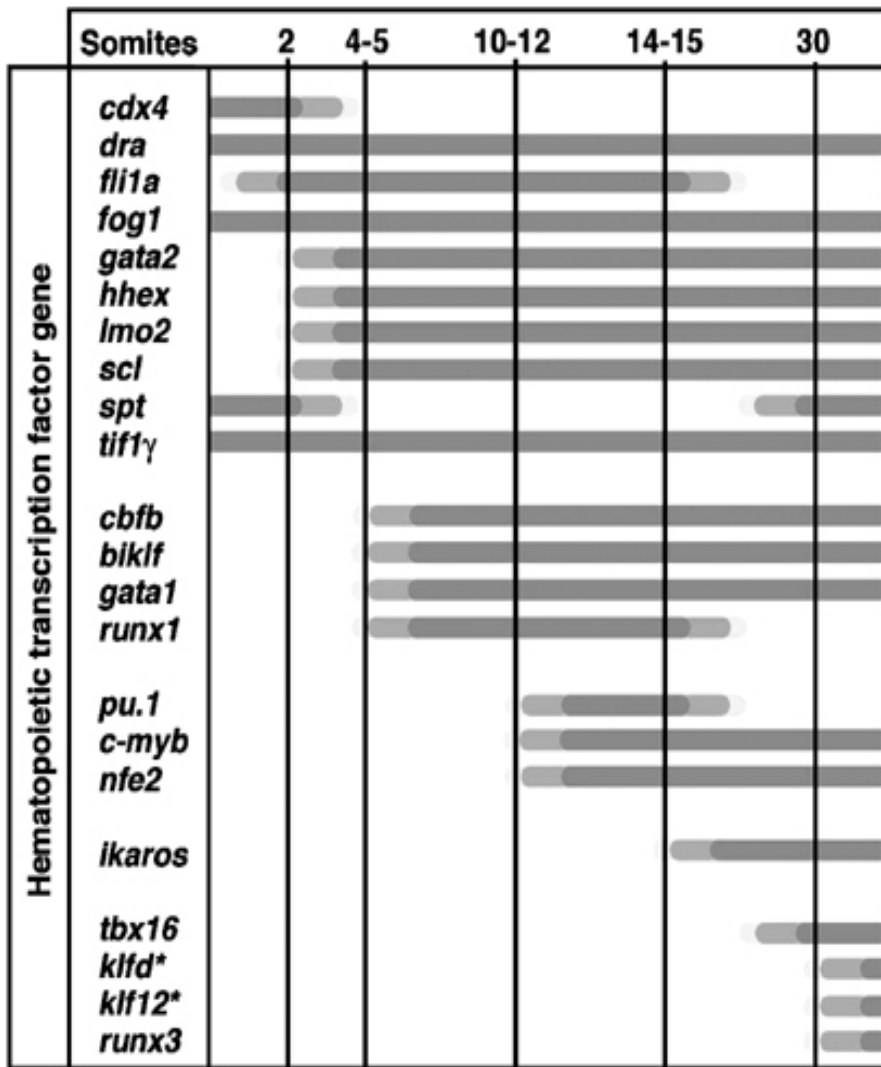
CD41⁺
CD45⁺

Runx1^{-/-}

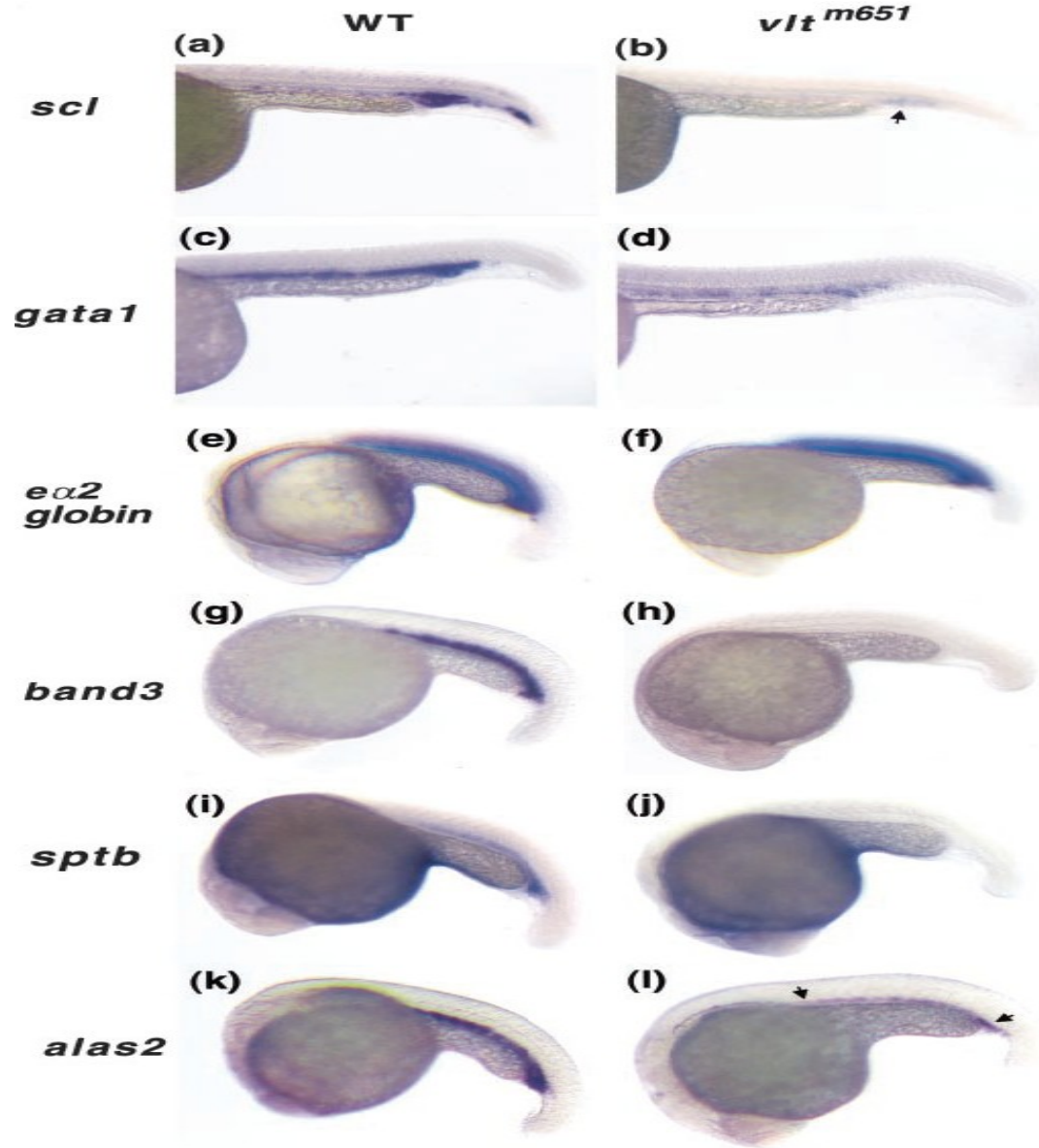


Blast movies 3, 4
Runx^{-/-} movie

TRANSCRIPTIONAL FACTORS IN BLOOD DEVELOPMENT



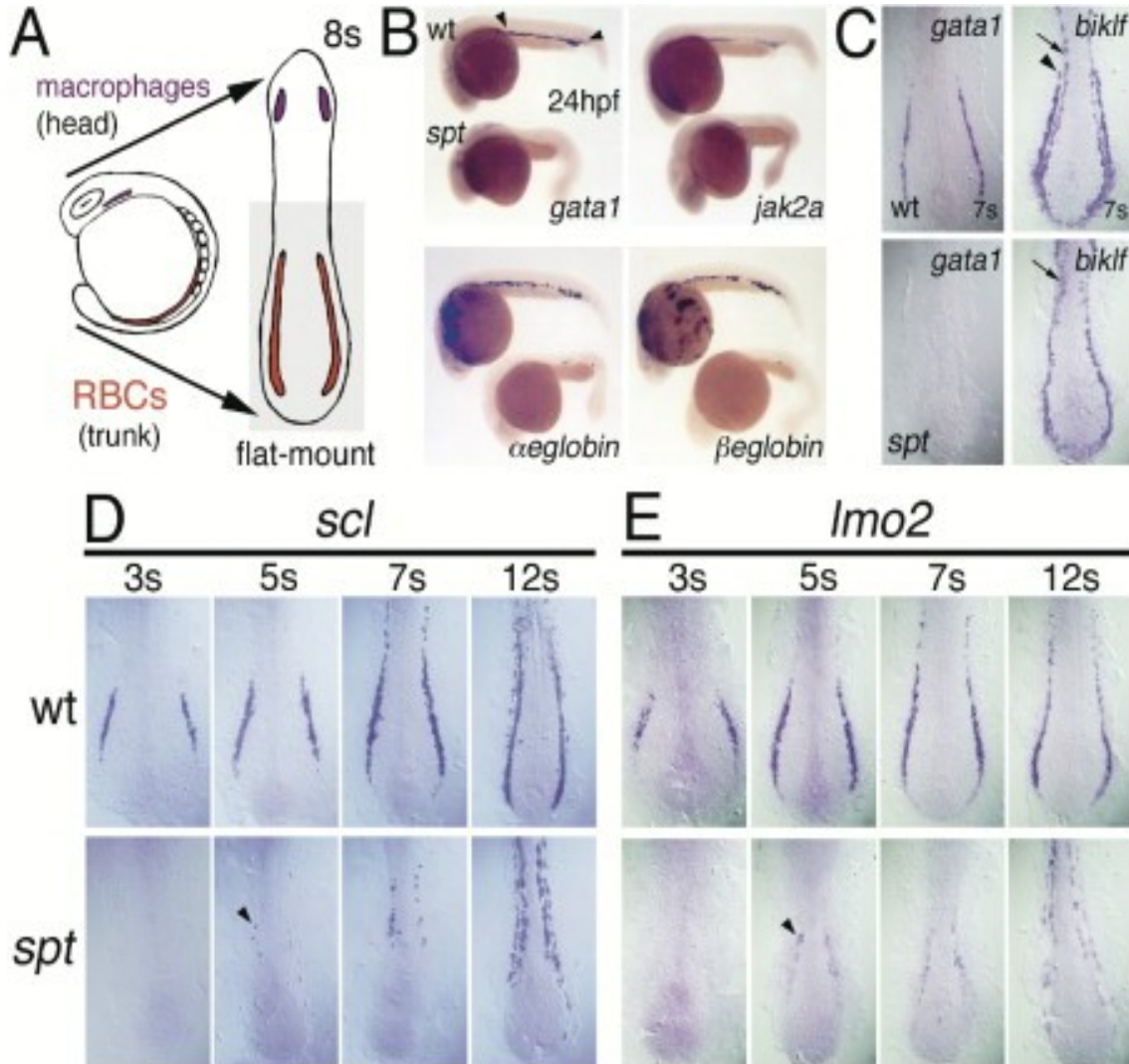
GATA-1 - binds DNA via a zinc finger motif



Dracula fish – loss-of-function mutation in GATA1 – impaired erythroid differentiation

Spadetail/TBX16

DNA binding domain derived from the prototype gene called transcription factor T



impaired erythroid but not myelopoietic differentiation

Moonshine

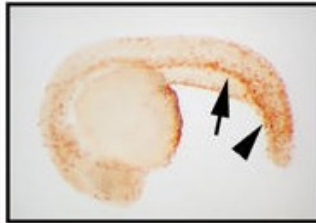
TIM-family of transcriptional factors

B

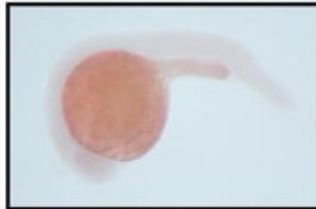
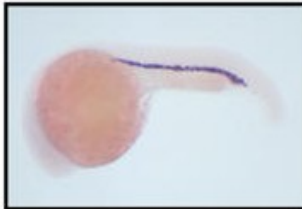
wild-type

montg234^{-/-}

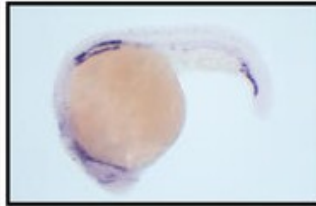
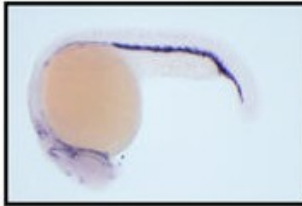
TUNEL



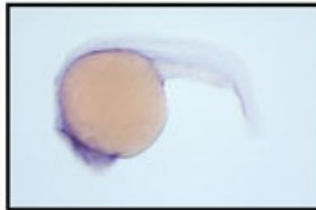
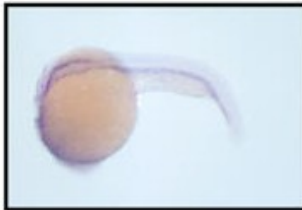
gata1



scl

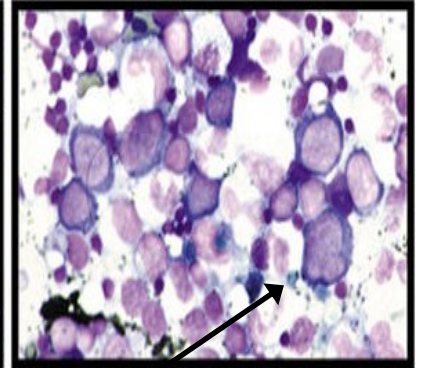
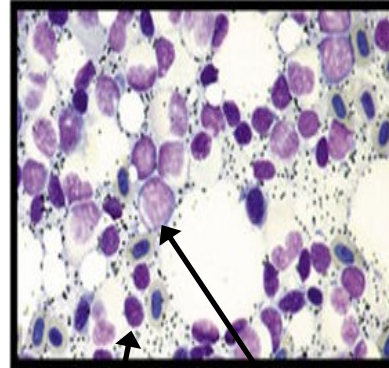


gata2



wild type

mon^{tb222}^{-/-}



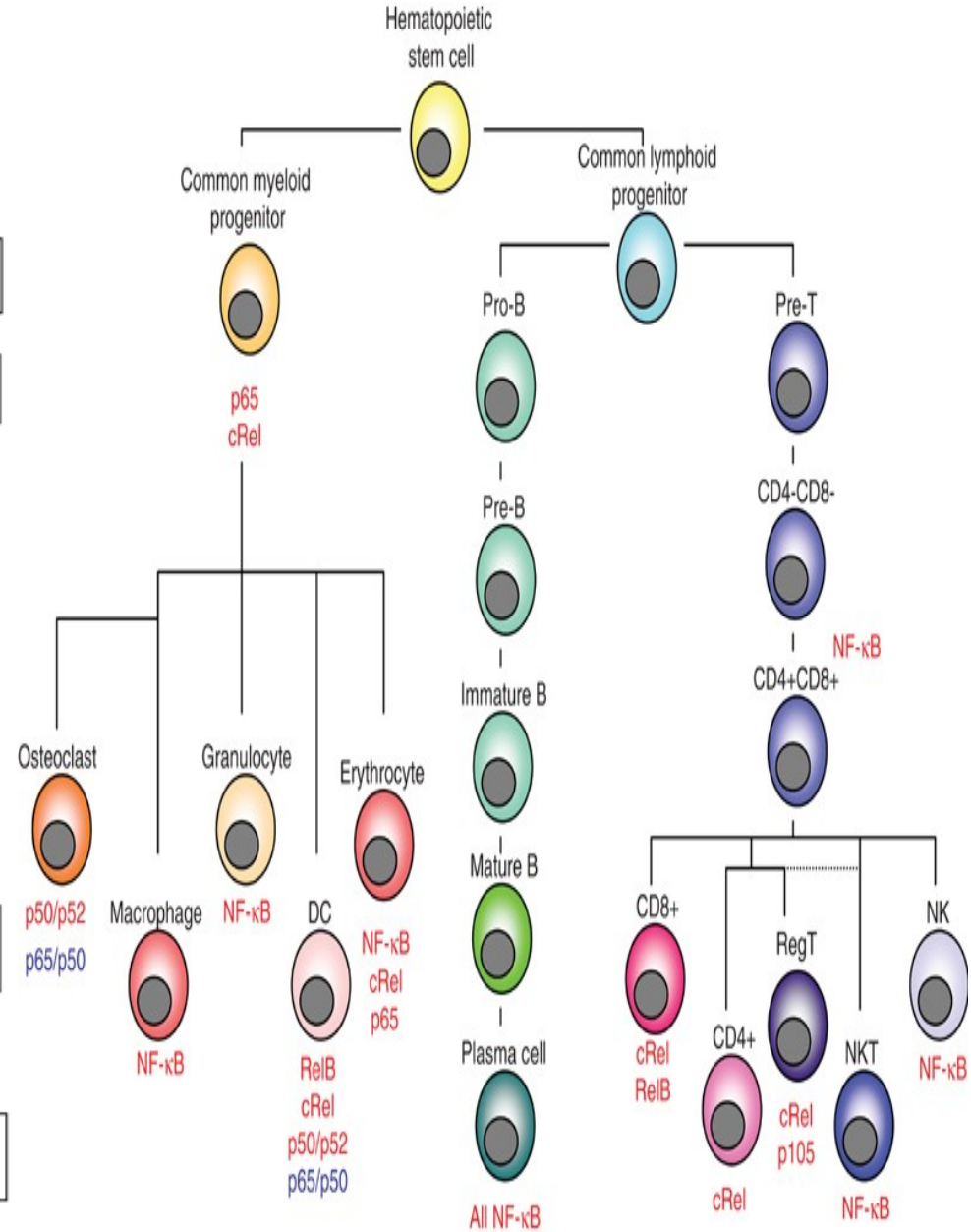
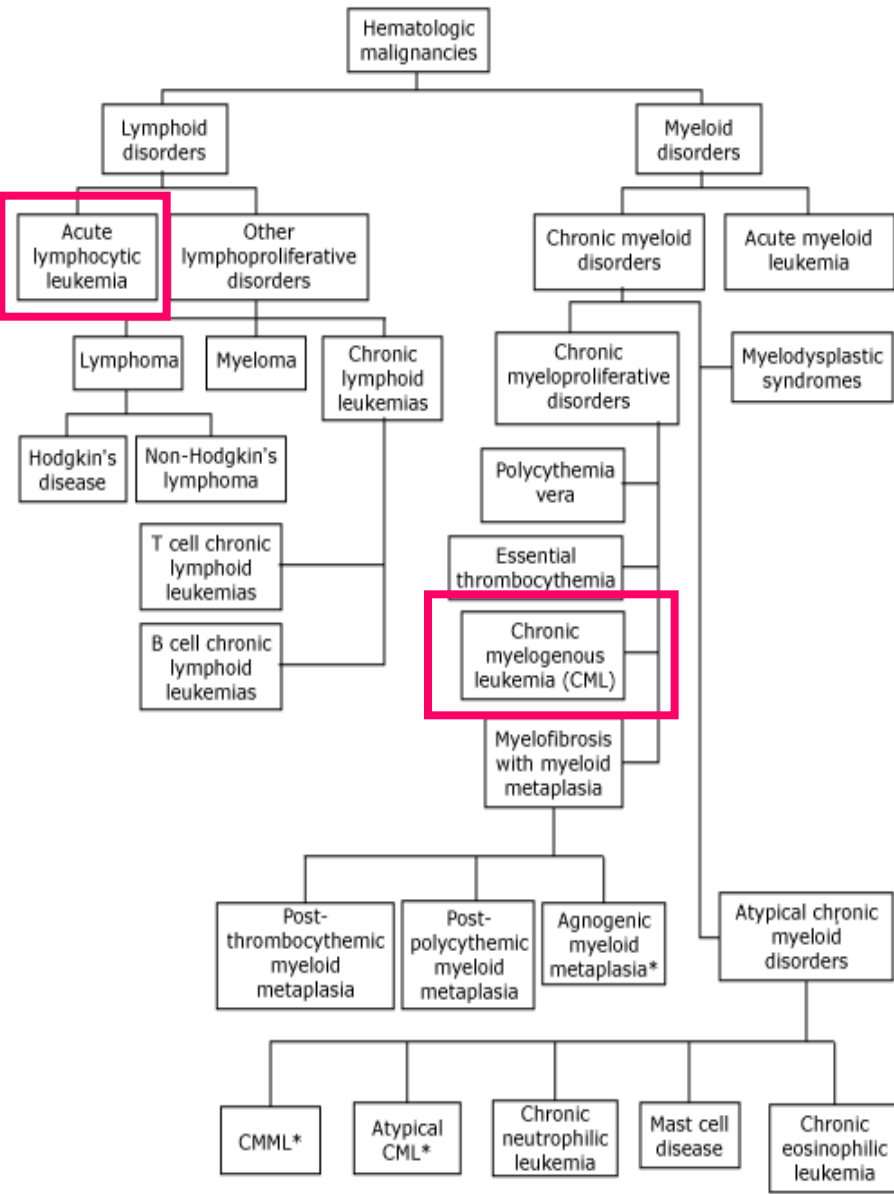
erythrocyte

proerythroblast

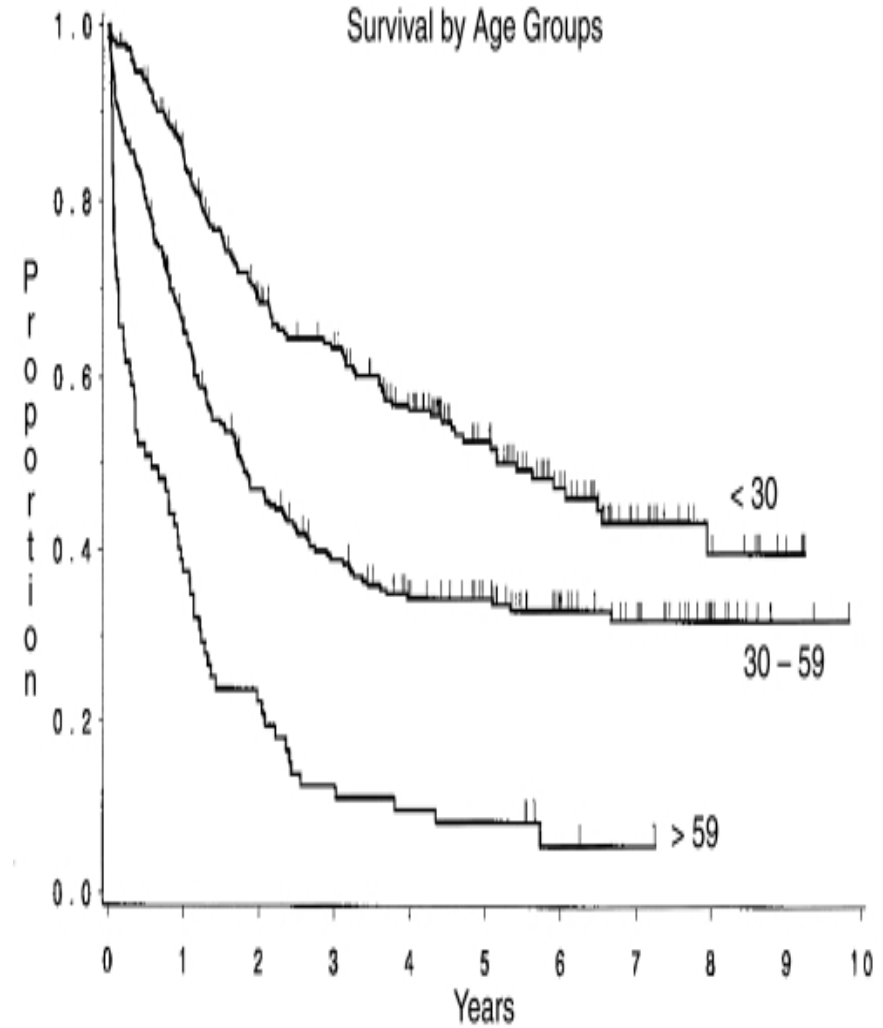
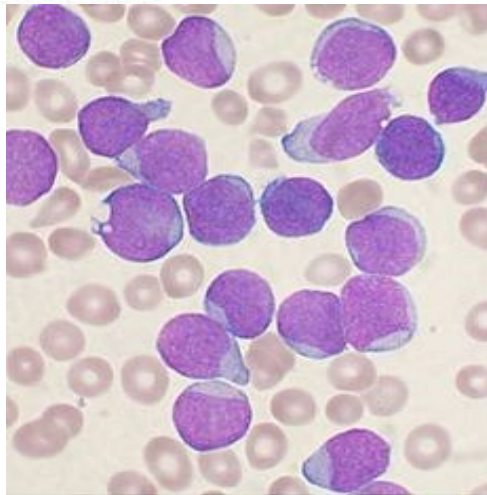
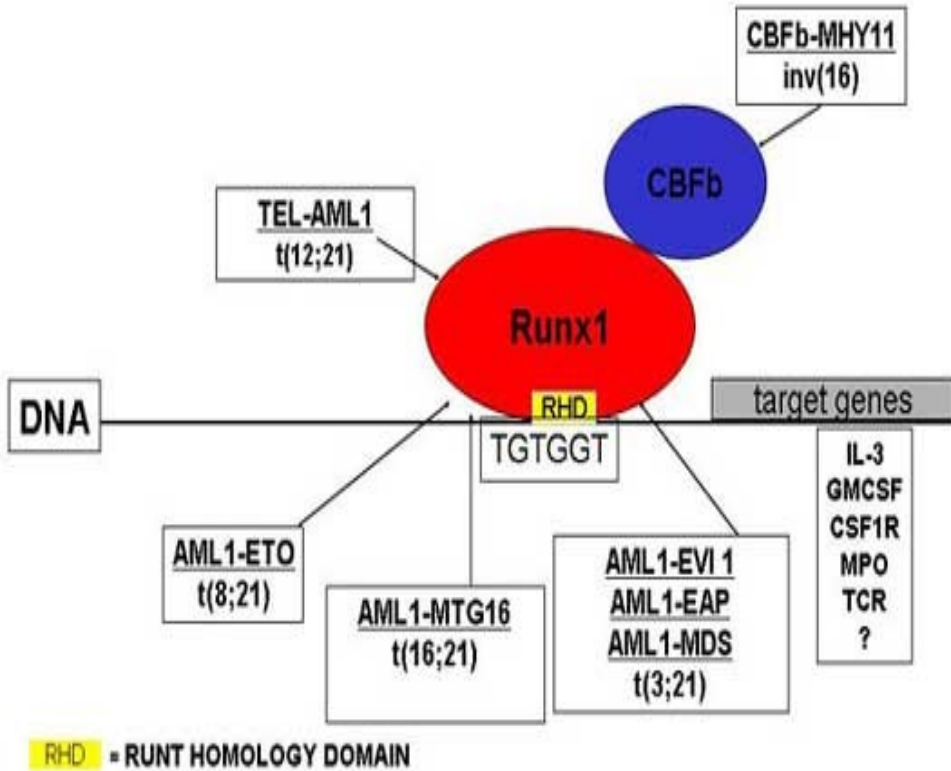
'primitive' – survival of HSC

'definitive' – cardiomegaly and impaired red cell differentiation

WHEN SOMETHING GOES WRONG WITH BLOOD

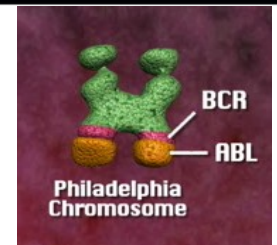
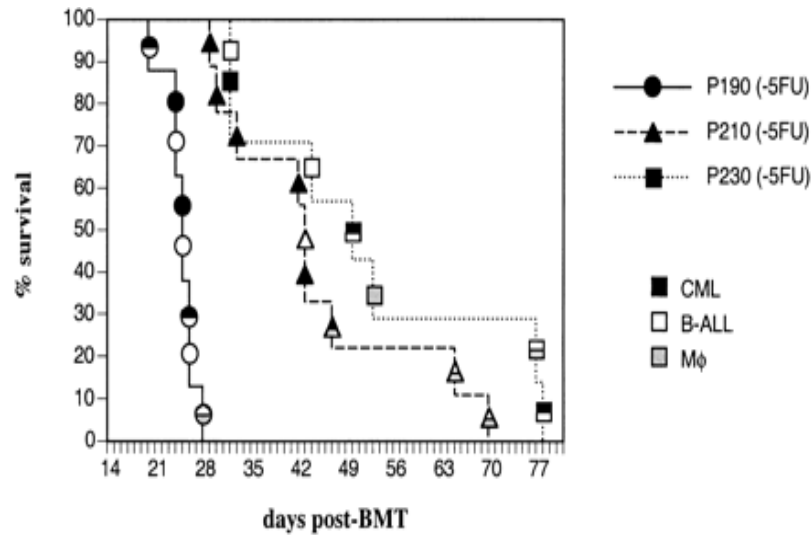
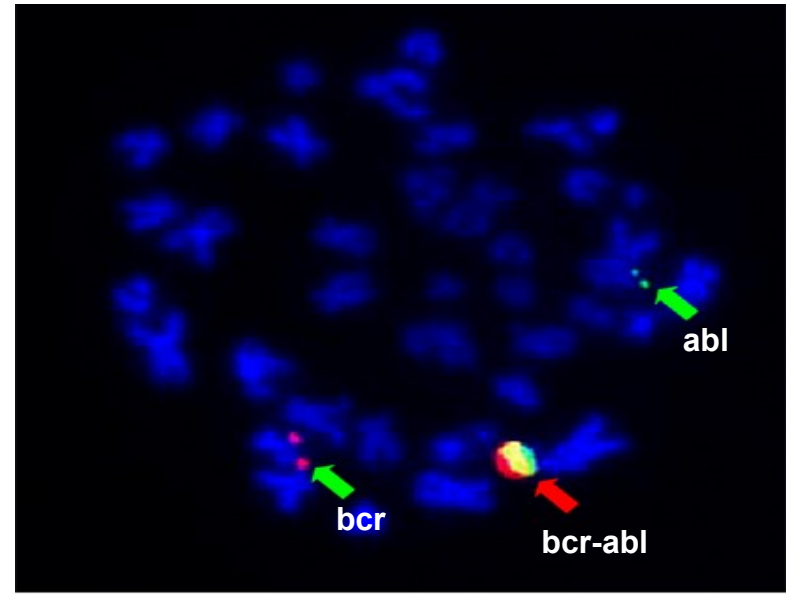
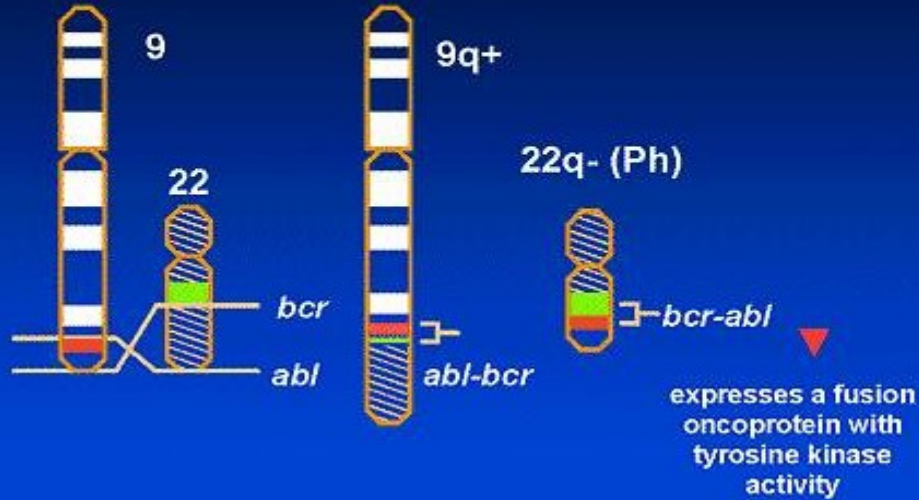


ACUTE LYMPHOID LEUKEMIA



CHRONIC MYELOID LEUKEMIA

The t(9;22) translocation produces the Philadelphia (Ph) chromosome



mice transplanted with patient bone marrow

