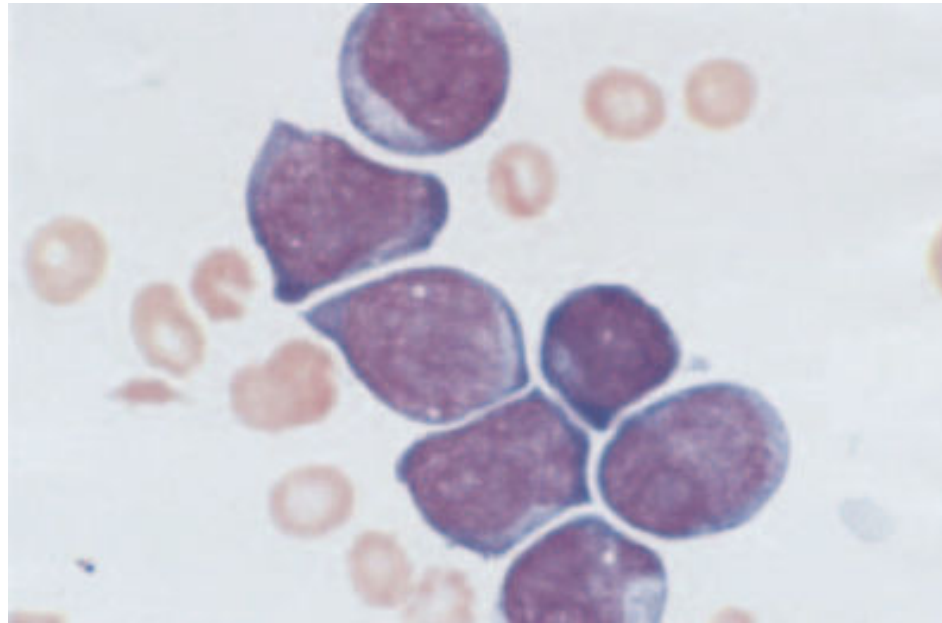


# Role of *ERG* in malignant myeloproliferative disorders associated with Down syndrome



Dr Ashley Ng  
Cancer and Haematology Division  
The Walter and Eliza Hall Institute of Medical Research

# Overview

- Down syndrome
  - Transient Myeloproliferative Disorder
  - Acute Megakaryocytic Leukemia
- Genetic basis for leukemogenesis
  - Evidence for a chromosome 21 gene
- Genetic proof
  - Hsa21 gene in trisomy predisposing to malignant myeloproliferation

# Down Syndrome

Commonest human aneuploidy

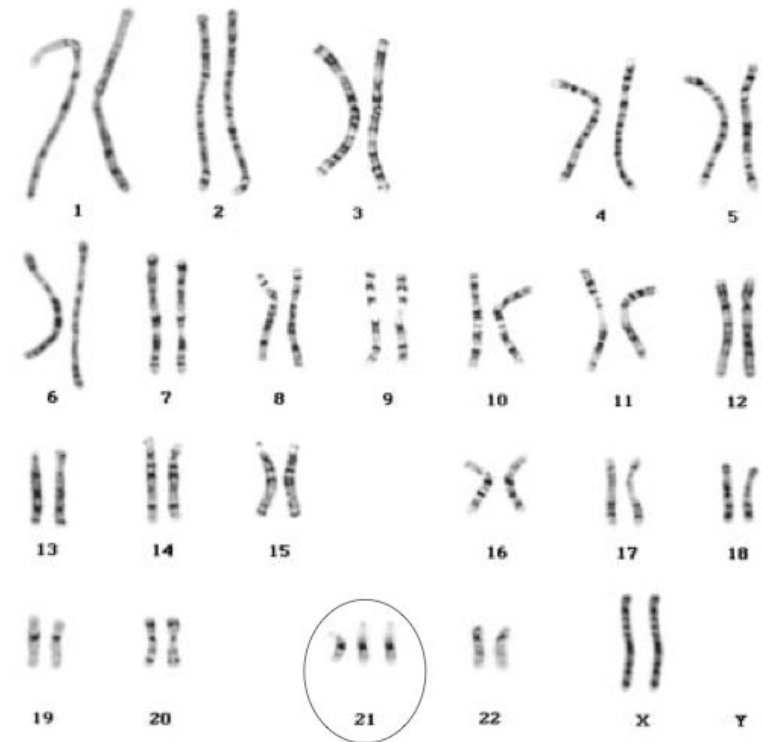
$1/800$  live births

Risk increases with maternal age

- $1/1562$  20-24yo
- $1/214$  35-39yo
- $1/19$  >45yo

Meiotic or mitotic non-dysjunction (+21)

- Trisomy 21\*
- Mosaic Trisomy 21
- Partial Trisomy 21



Antonarakis, Nature Rev Genetics, 2004

\*Lejeune, *Etudes des chromosomatiques de neuf enfants mongoliens*, C.R. Acad. Sci., 1959

# Down Syndrome

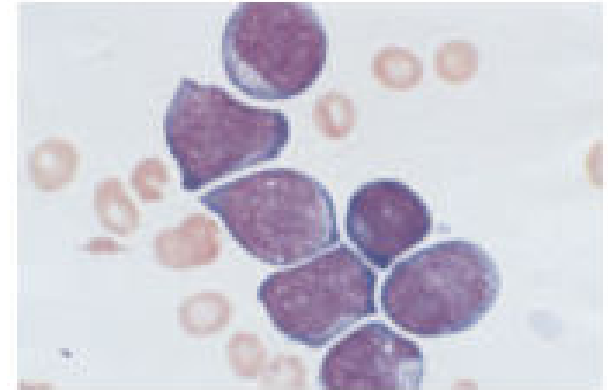
- Acute Megakaryocytic Leukemia  
(DS-AMKL)
- Transient Myeloproliferative Disorder  
(DS-TMD)

# Natural History DS-TMD and DS-AMKL

- Megakaryoblasts in blood film
- Uniquely associated with DS (~incidence 10%)

## DS-TMD (< 3 months)

- ± Asymptomatic
- Megakaryoblasts (>20%) blood film
- Spontaneous resolution (60%)
  - Infiltration (liver fibrosis, failure), ascites, thrombocytopenia, leukocytosis, hydrops
- DS-AMKL (20%)



## DS-AMKL (< 4 years)

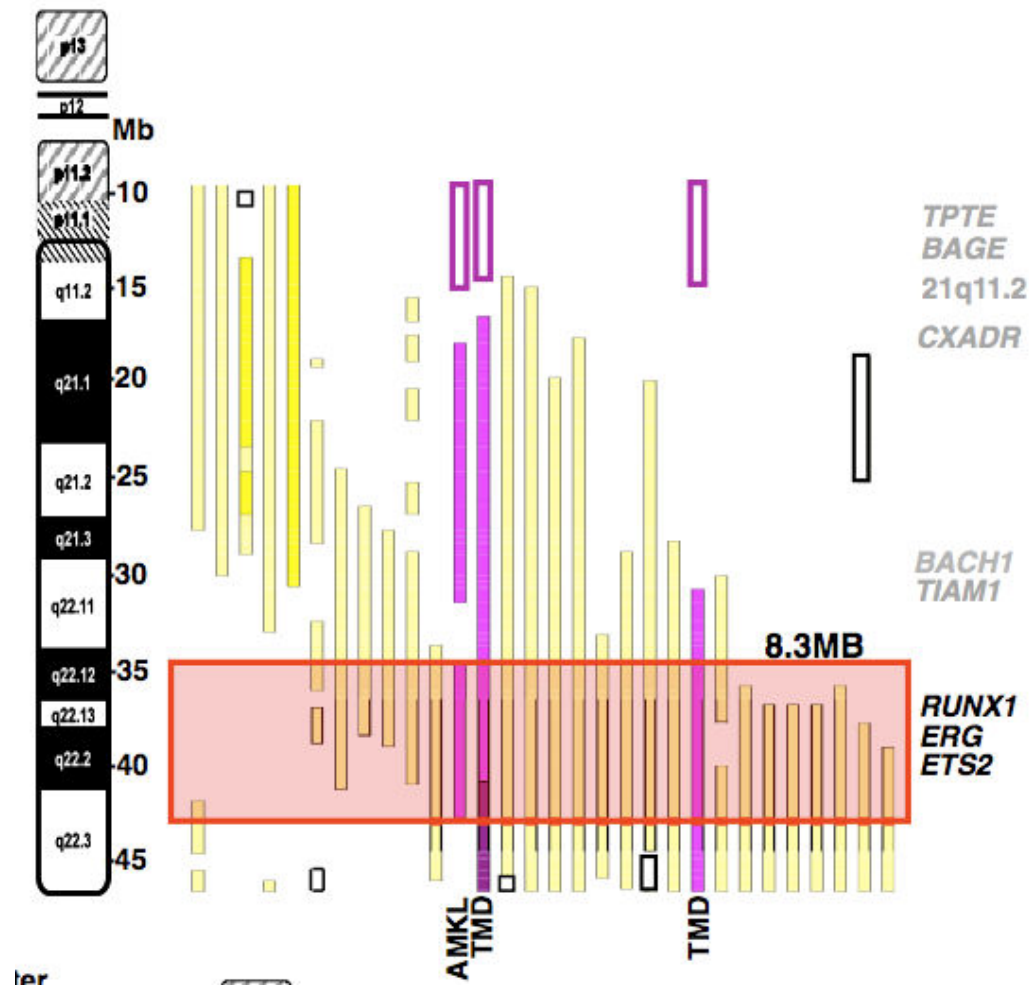
- Megakaryoblasts (>20%) on blood film
- No previous (symptomatic) DS-TMD in majority
- No spontaneous resolution
- Additional cytogenetic abnormalities

*Massey, et al, POG-9481, Blood 2006*

# Cancer is a genetic disease

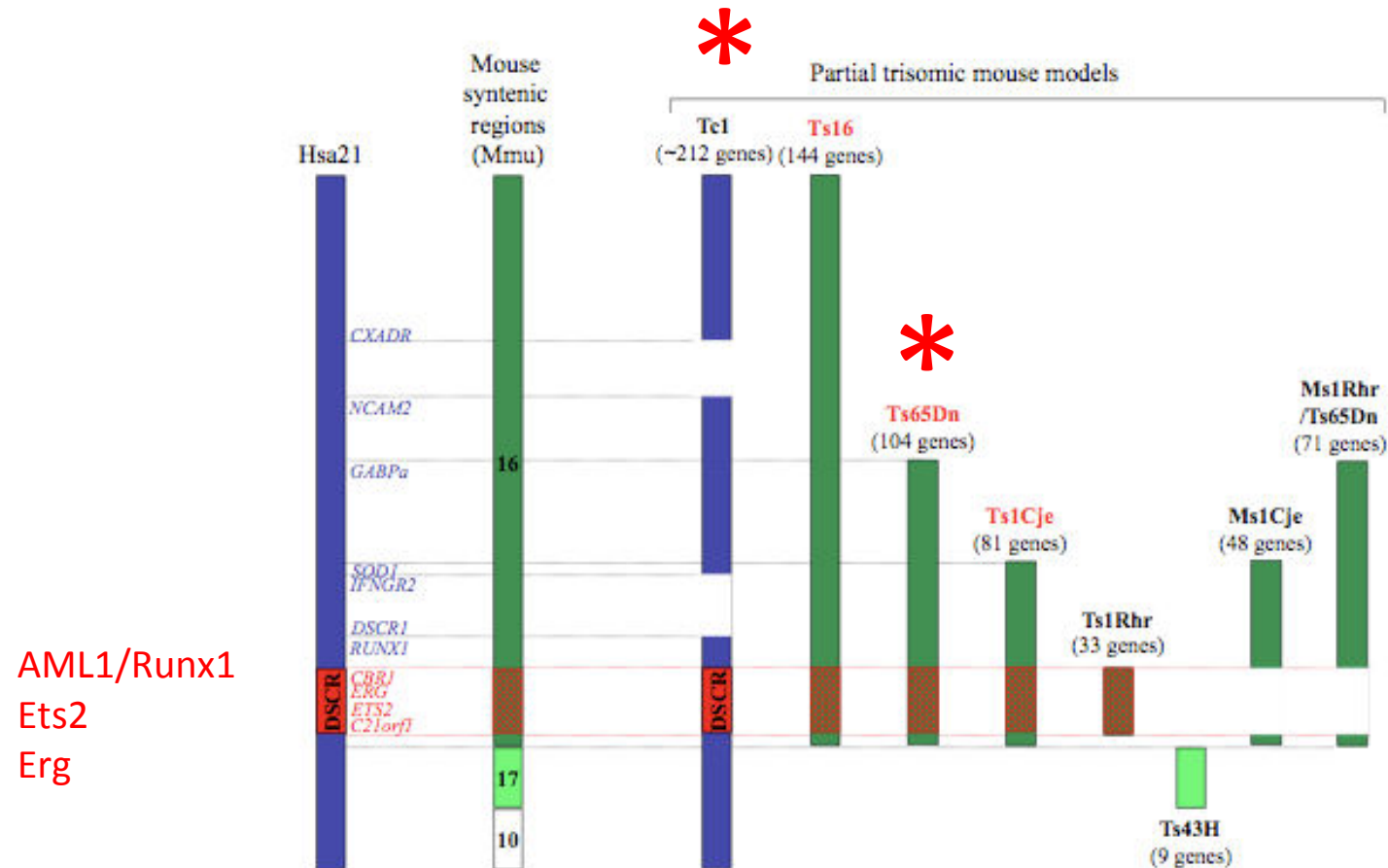
- DS-TMD and DS-AMKL are associated with (at least) two unique genetic lesions
  - GATA1s mutation
    - Weschler, et al., Nature Genetics, 2002
    - Rainis et al., Blood 2003
  - Trisomy of chromosome 21 gene

# High Resolution Analysis of Human Segmental Trisomies



# Mouse models of Down Syndrome

Myeloproliferative phenotype \*

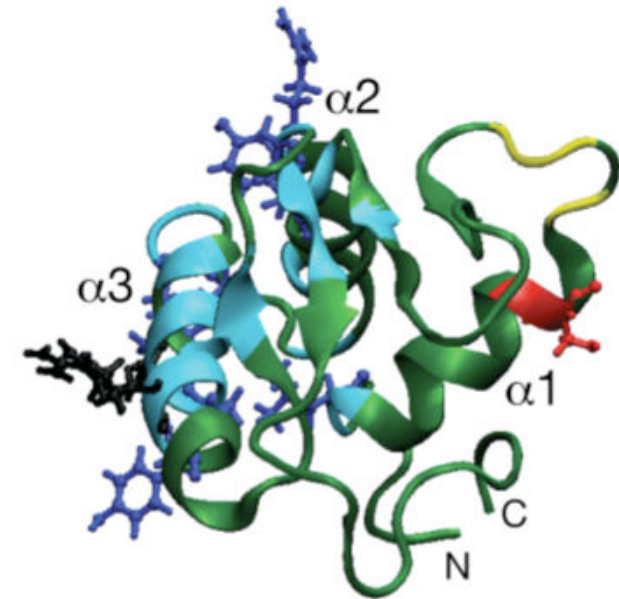
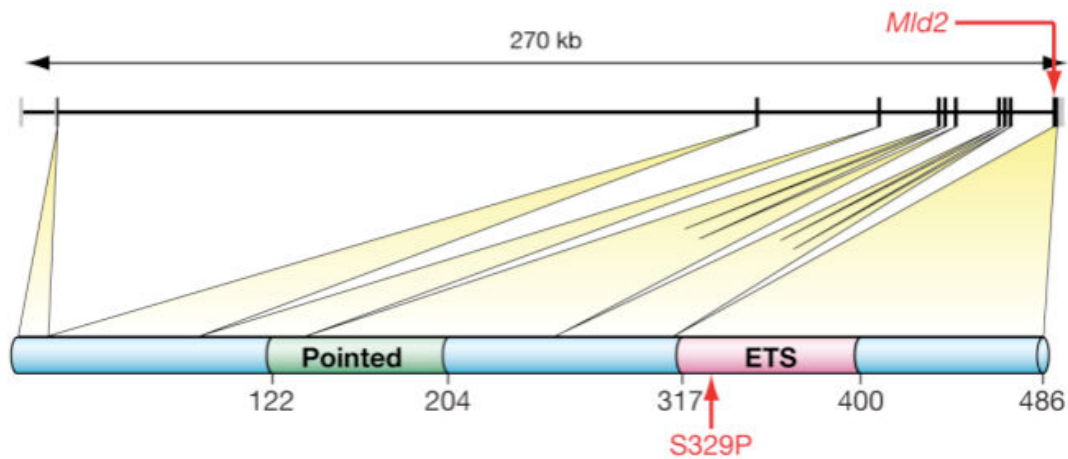


# Ts(17<sup>16</sup>)65Dn DS model

- Myeloproliferative Phenotype
- Absence of GATA1 mutations
- Critical gene in segmental trisomy
  - Drives the myeloproliferative phenotype
  - Syntenic DSCR implicated in DS-TMD and DS-AMKL
    - *RUNX1*
    - *ETS2*
    - *ERG*

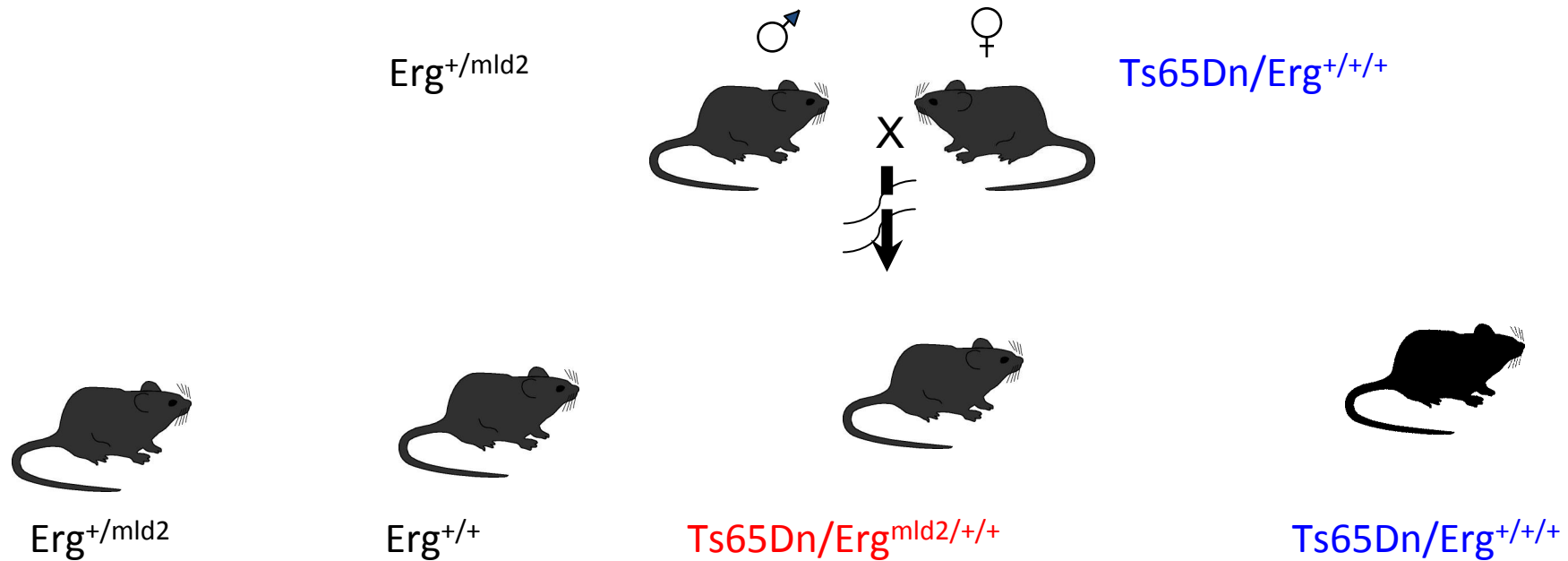
# Mld2 is a non-functioning allele of *Erg*

- ENU mutagenesis
- Mutation Ser → Pro DNA binding region



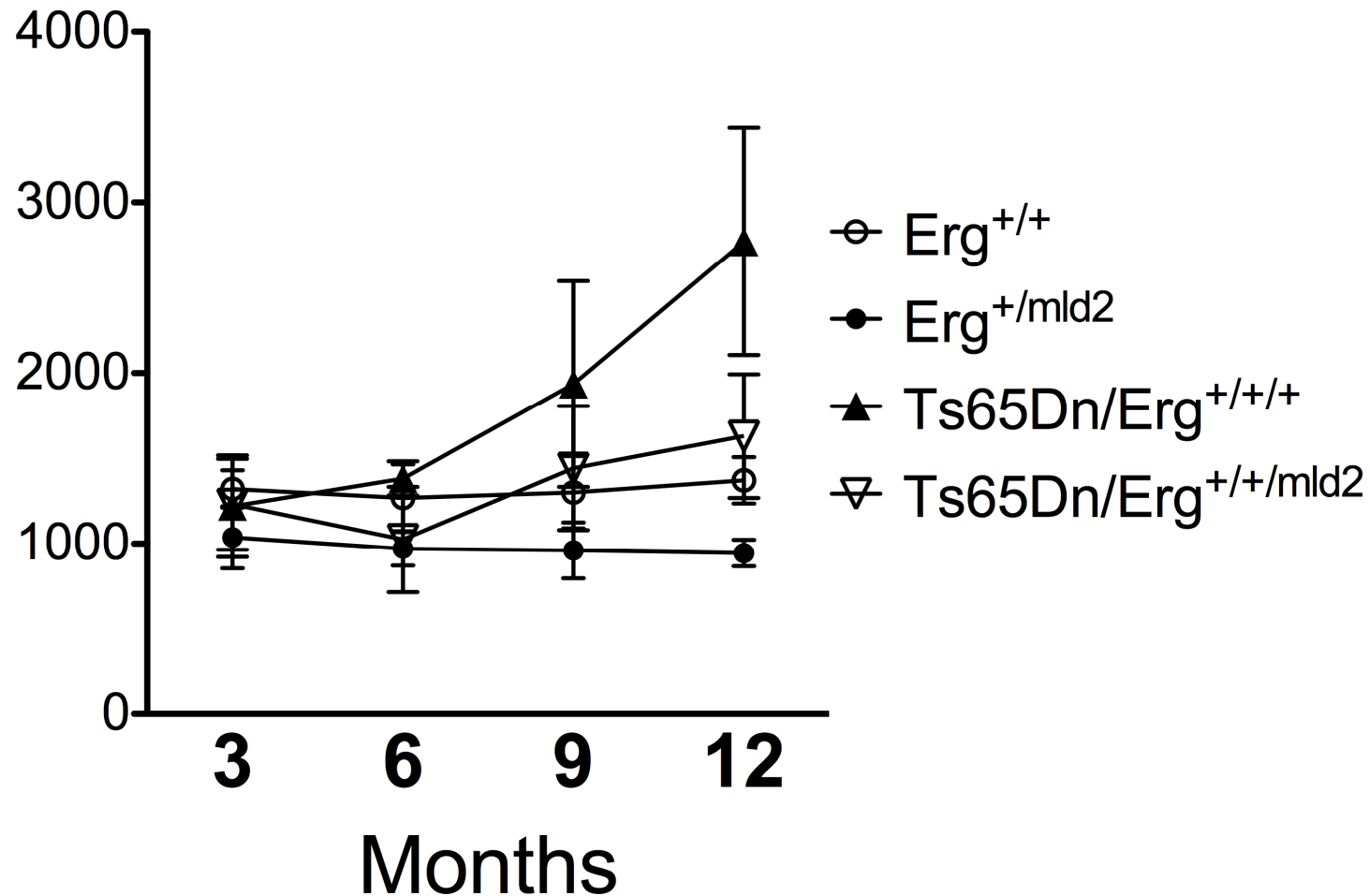
QIQLWQFLLLELLP	DSSNSNCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Mus musculus</i> Erg <sup>Mld2</sup>
QIQLWQFLLLELLS	DSSNSNCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Mus musculus</i> Erg <sup>WT</sup>
QIQLWQFLLLELLS	D <b>S</b> ANAS <b>C</b> ITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Mus musculus</i> Fl-1
QIQLWQFLLLELLS	DSSNS <b>S</b> CITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Homo sapiens</i> Erg
QIQLWQFLLLELLS	DSSNSNCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Rattus norvegicus</i> Erg
QIQLWQFLLLELLS	DSSNSNCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Gallus gallus</i> Erg
QIQLWQFLLLELLS	DSSNSNCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Xenopus laevis</i> Erg
QIQLWQFLLLELLS	D <b>S</b> CNS <b>S</b> CITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDFH	<i>Danio rerio</i> Erg
QIQLWQFLLLELLS	DSSN <b>A</b> NCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKDF <b>A</b>	<i>S. purpuratus</i> Erg

# Erg<sup>+/mld2</sup> x Ts65Dn



? ↓ Erg gene dose prevent/modify phenotype

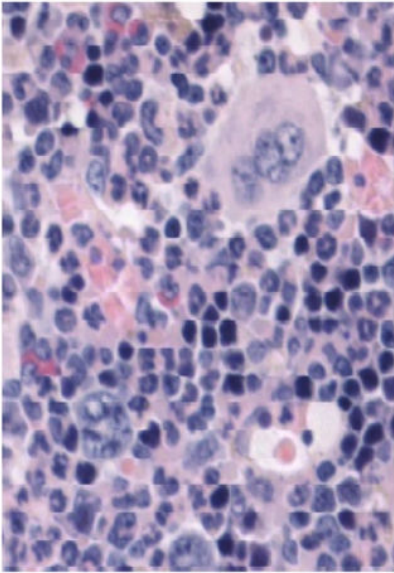
# Thrombocytosis



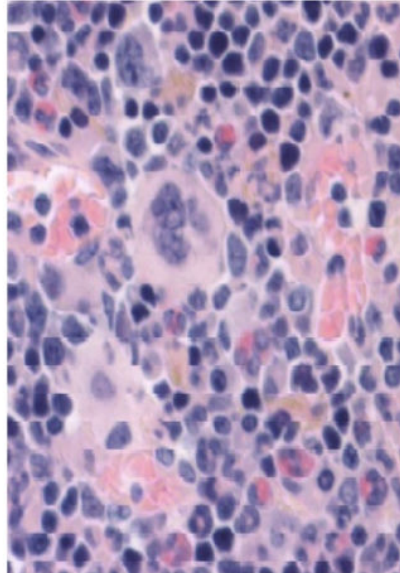
\* $p=0.008$  for Ts65Dn/Erg<sup>+/+/+</sup> vs Ts65Dn/Erg<sup>+/+/mld2</sup> (means with S.D.) shown

# Megakaryocytosis Bone marrow

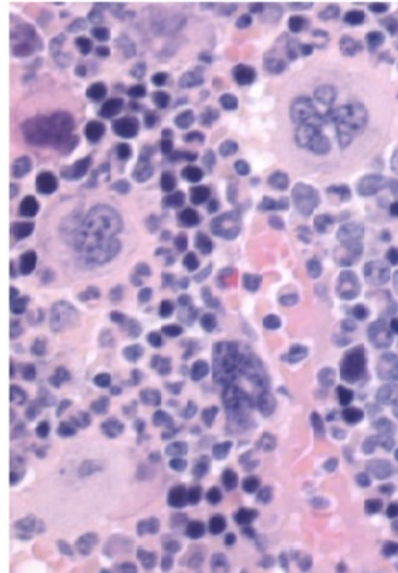
**Erg<sup>+/+</sup>**



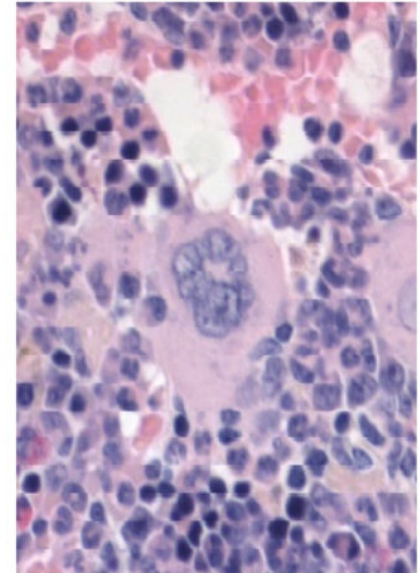
**Erg<sup>+/mld2</sup>**



**Ts65Dn/Erg<sup>+/+/+</sup>**

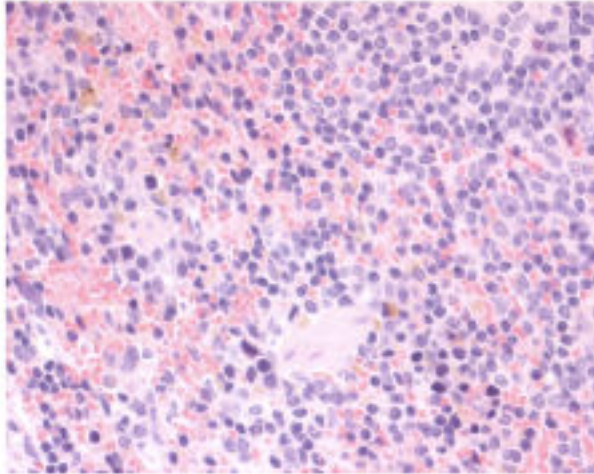


**Ts65Dn/Erg<sup>+/+/mld2</sup>**

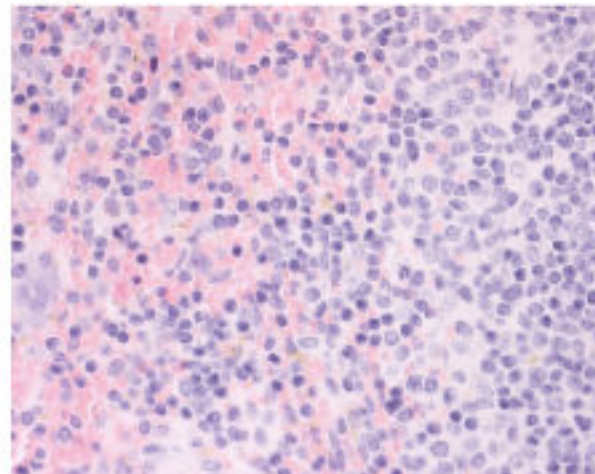


# Megakaryocytosis Spleen

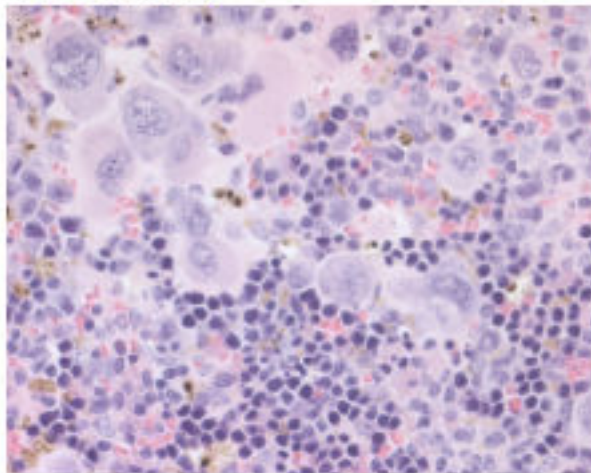
**Erg<sup>+/+</sup>**



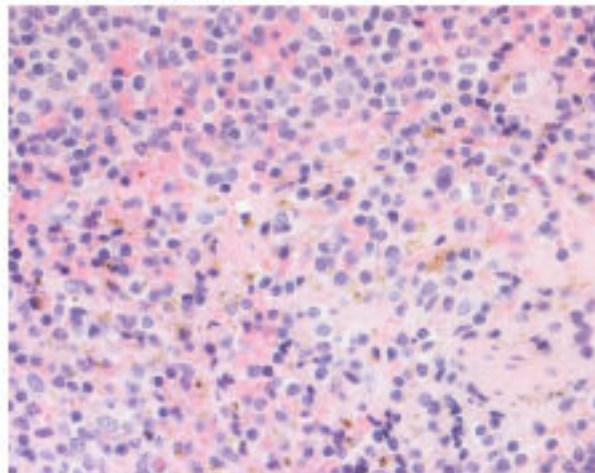
**Erg<sup>+/mld2</sup>**



**Ts65Dn/Erg<sup>+/+/+</sup>**

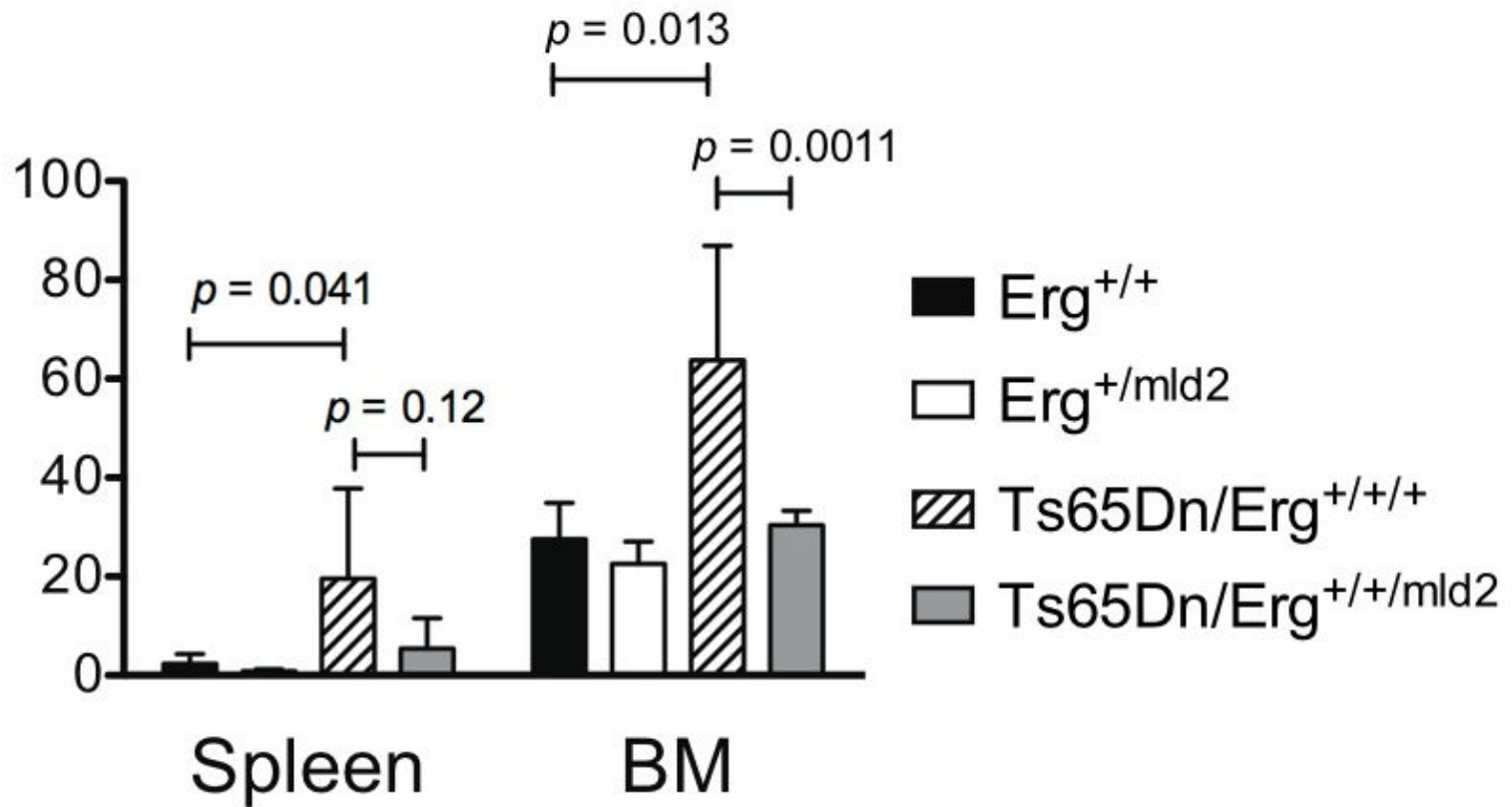


**Ts65Dn/Erg<sup>+/+/mld2</sup>**

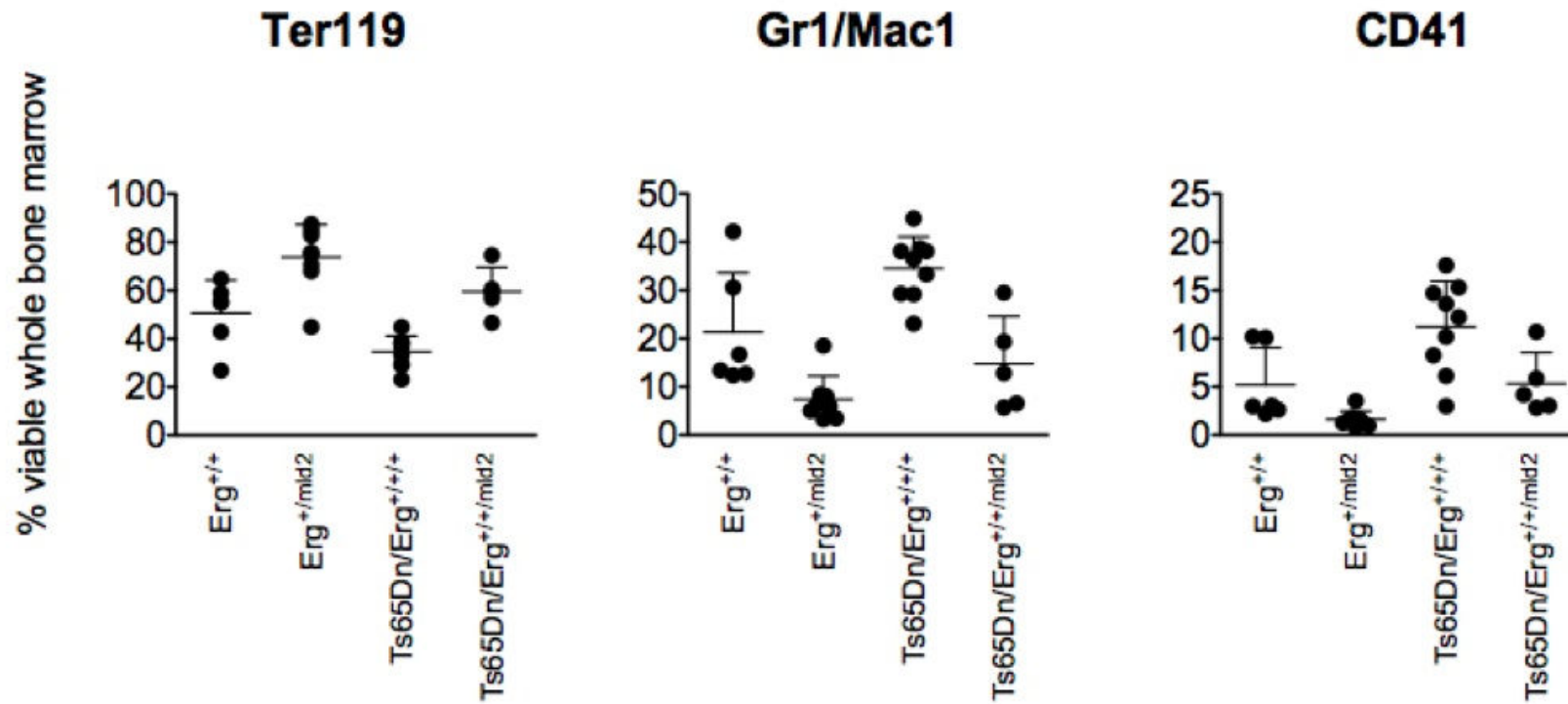


# Megakaryocytosis

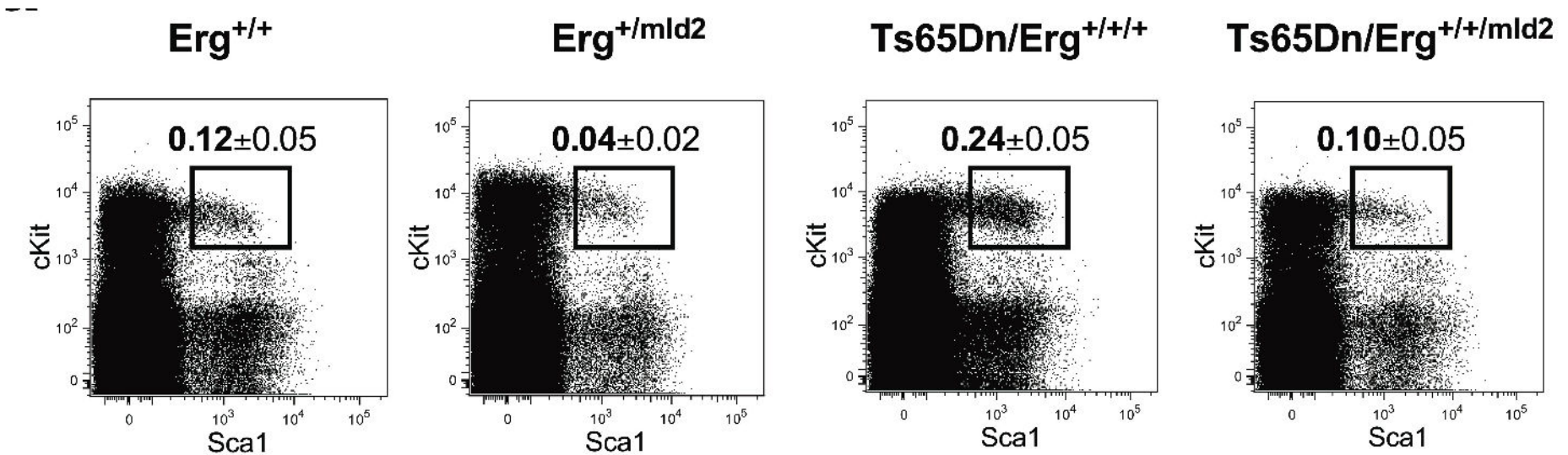
Megakaryocytes/hpf (x200)



# Bone marrow



# Stem Cells – LSK compartment

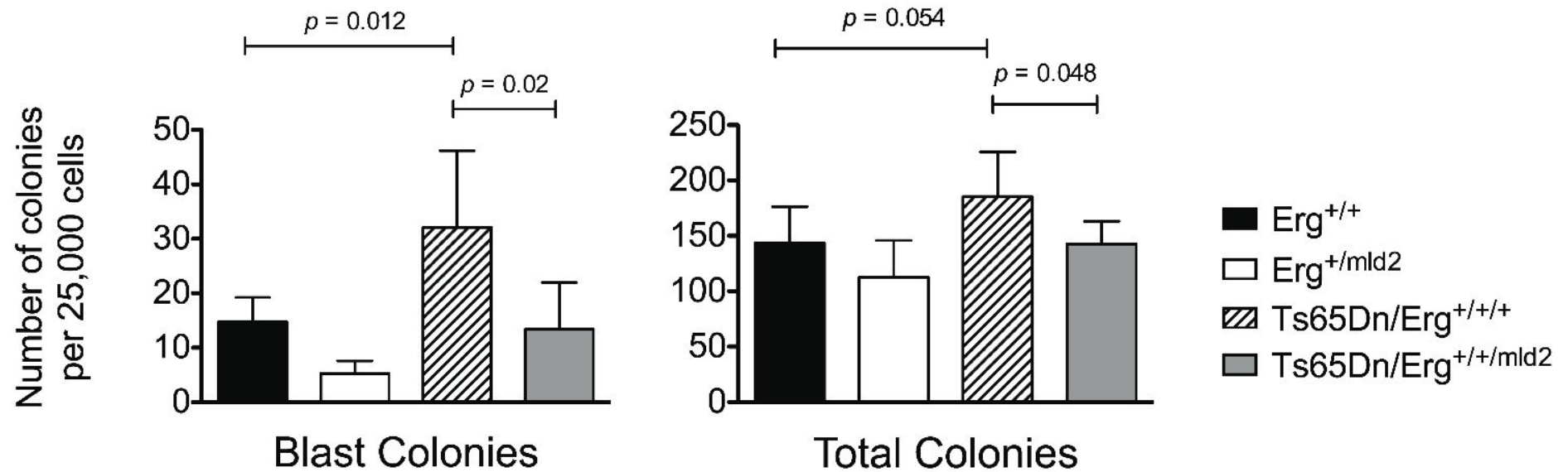


*p=0.038 Ts65Dn/Erg<sup>+/+/+</sup> and Ts65Dn/Erg<sup>+/+/mld2</sup>*

*p=0.048 Ts65Dn/Erg<sup>+/+/+</sup> and Erg<sup>+/+</sup>*

*p=0.57 Ts65Dn/Erg<sup>+/+/mld2</sup> and Erg<sup>+/+</sup>*

# Clonogenic colony assay



# Ts65DnErg<sup>+/+/Mld2</sup>

Disomy of *Erg* and trisomy of 103 other genes orthologous to Hsa21

## Amelioration of Myeloproliferative Disease

- normalisation of thrombocytosis
- normalisation of bone marrow megakaryocytosis
- absent bone marrow reticulin fibrosis
- amelioration of splenic extramedullary haemopoiesis
- normalisation of stem cells numbers
- normalisation of clonogenic progenitors from bone marrow

# Genetic proof

Specifically reducing the *Erg* gene dose to disomy mitigates the MPD phenotype in Ts65Dn (whilst *Runx1* and *Ets2* remain trisomic)

Identified *ERG* as a critical trisomic gene which may predispose to DS-AMKL and TMD in humans

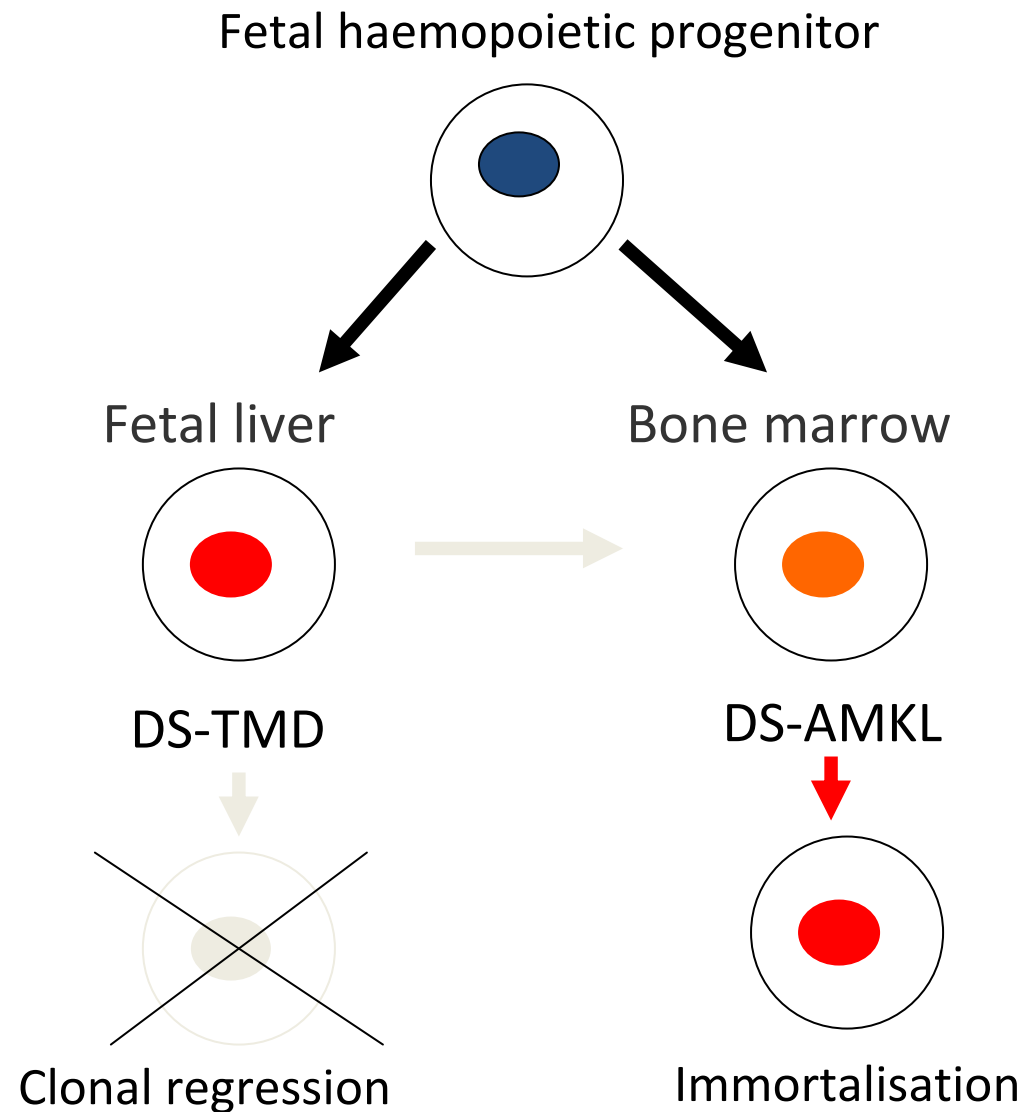
# Model for development DS-TMD & DS-AMKL

## Predisposition

- Trisomy *ERG*

## In Utero Acquisition

- GATA1 mutation(s)
- ± other mutations



# Acknowledgements

## Cancer and Haematology

- *Warren Alexander*
- Craig Hyland
- Jason Corbin
- Stephen Loughran
- Jessie Kiu
  
- Don Metcalf
- Ladina DiRago
- Sandra Mifsud

## Bundoora Mouse Facility

- Louise Inglis
- Tracey Kemp
- Eric Dressler

## Advanced Technologies

- Meagan Blake
- Kim Burchall

## Molecular Medicine

- *Ben Kile*
- Doug Hilton
- Catherine Carmichael

## Histology

- Ellen Tsui
- Steven Mihajlovic
- Yuyin Hoang
- Vera Babo
- Kevin Weston

## FACS laboratory

# Declarations

- NHMRC Research Training Scholarship
- AMGEN/HSANZ New Investigator Scholarship
- Email: [ang@wehi.edu.au](mailto:ang@wehi.edu.au)