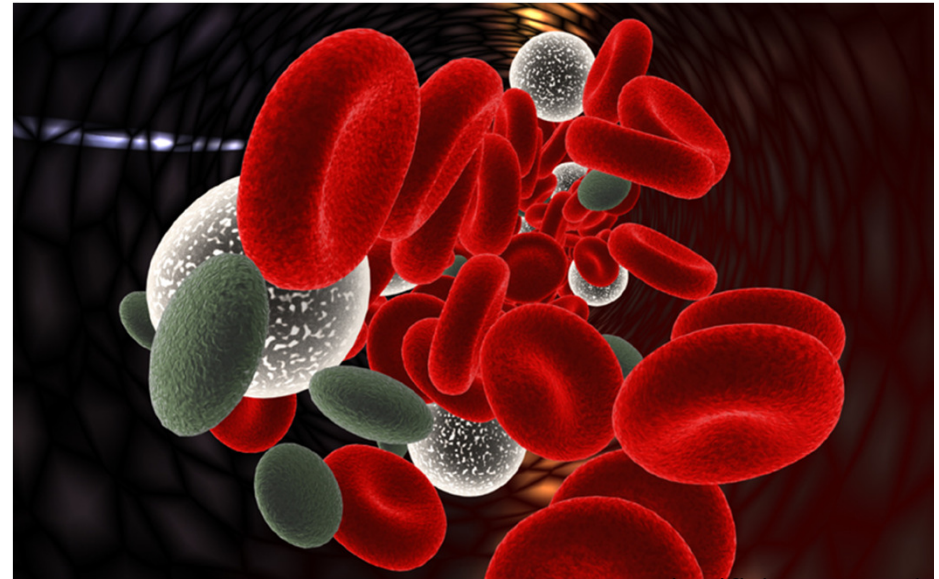


Thioredoxin-interacting (TXNIP) protein regulates the differentiation of erythroid precursors



www.scientificimages.co.uk

Volker Blank
Lady Davis Institute for Medical Research
McGill University

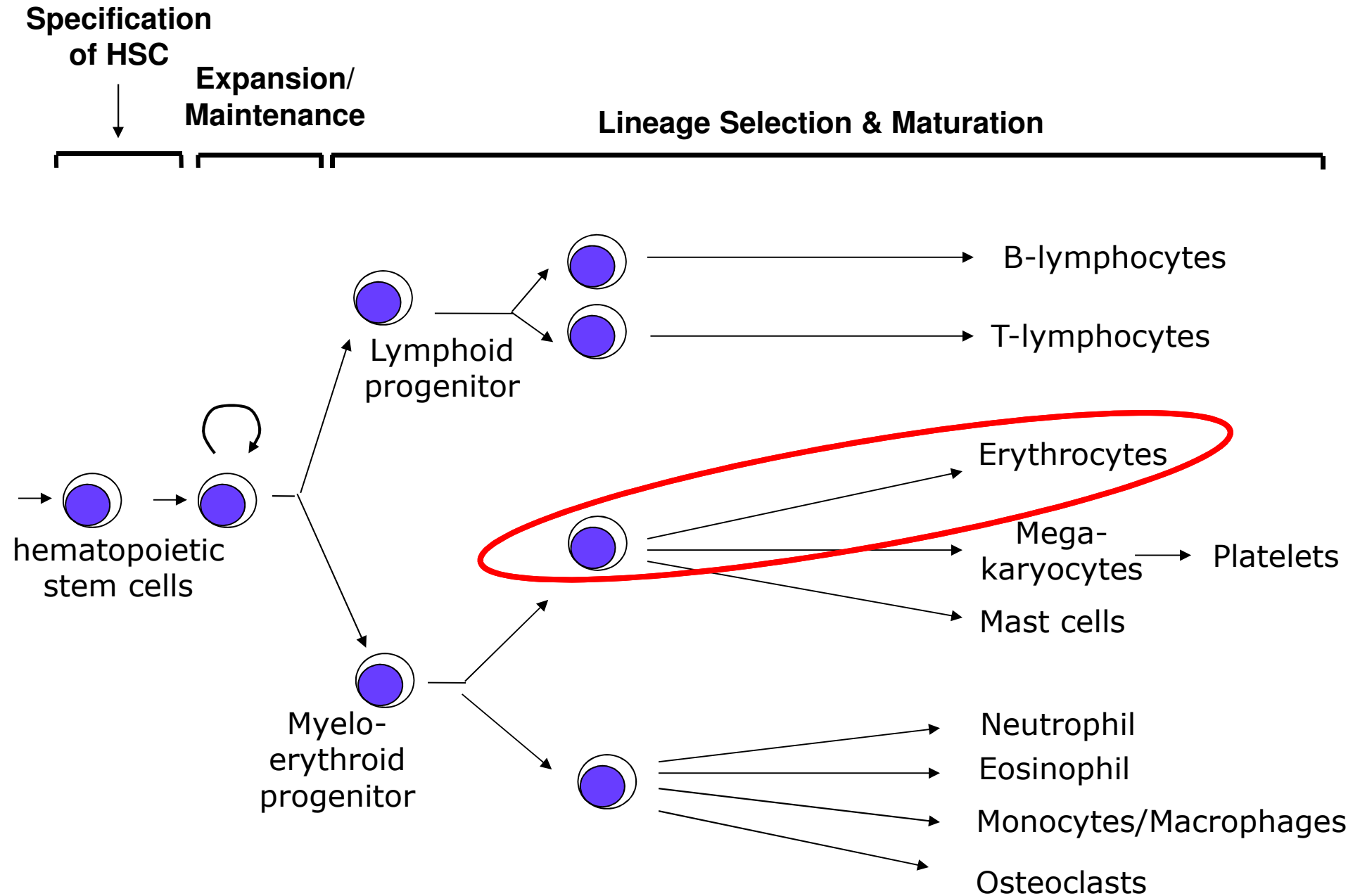


Hôpital général juif
Jewish General Hospital

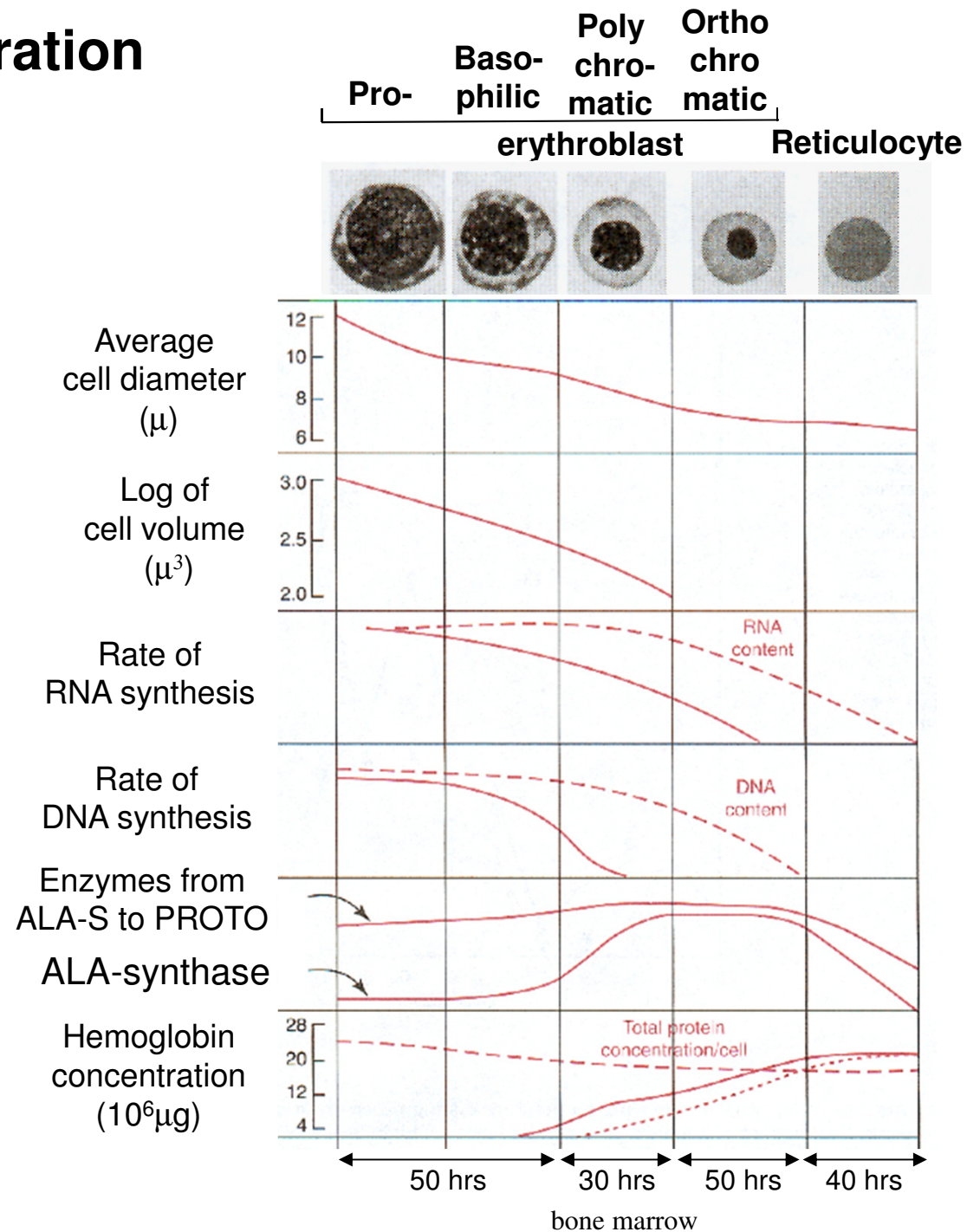
McGill



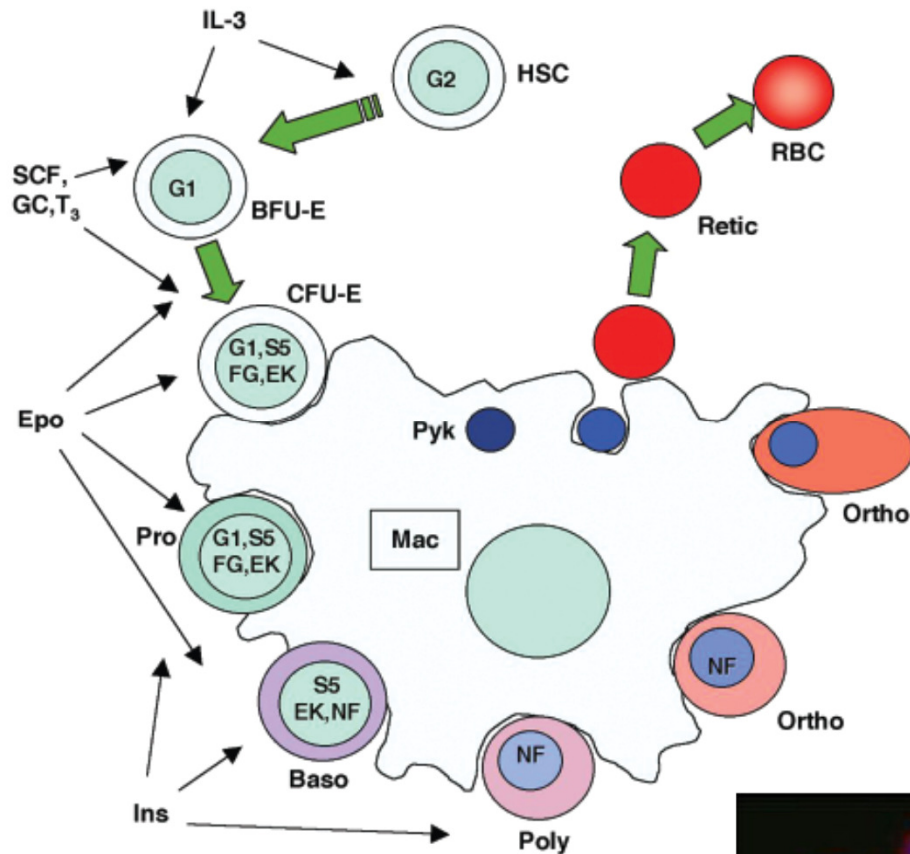
Hematopoiesis



Erythroid maturation - bone marrow



Regulation of erythroid development



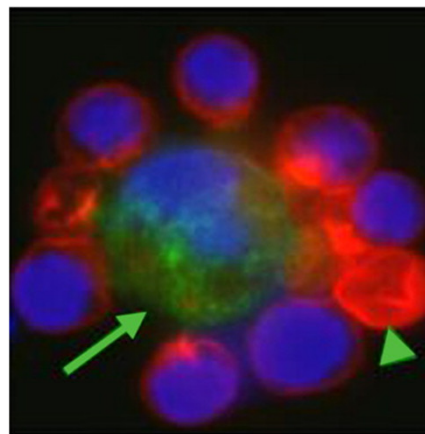
External factors

IL-3
 Stem Cell Factor (SCF)
 Glucocorticoids (GC)
 Thyroid hormone (T3)
 Erythropoietin (Epo)
 Insulin (Ins)

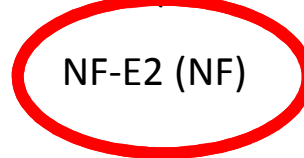
Transcription factors

GATA-2 (G2)
 GATA-1 (G1)
 STAT5 (S5)
 FOG (FG)
 EKLF (EK)
 NF-E2 (NF)

Ingley et al. 2004 IUBMB Life 56(4):177-184

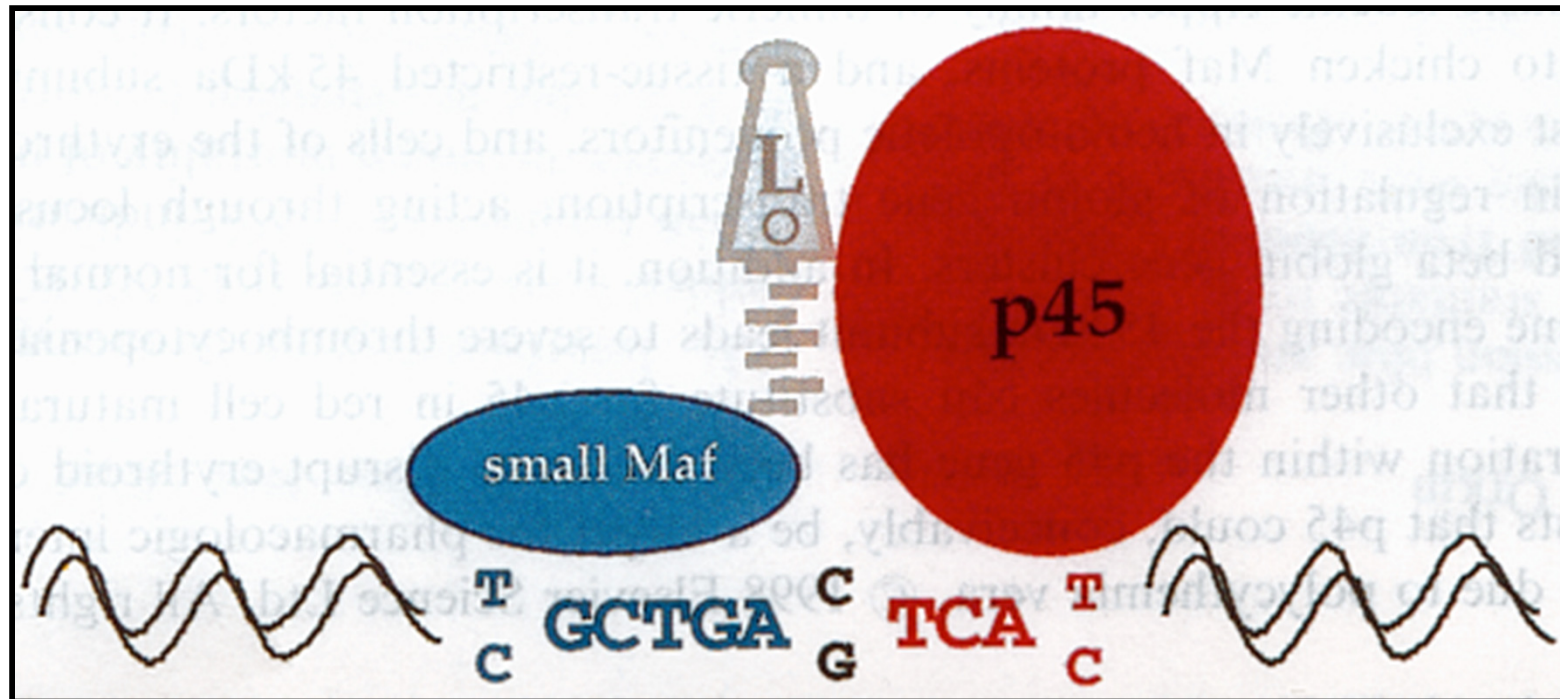


Lee et al. 2006 Blood 108: 2064-2071

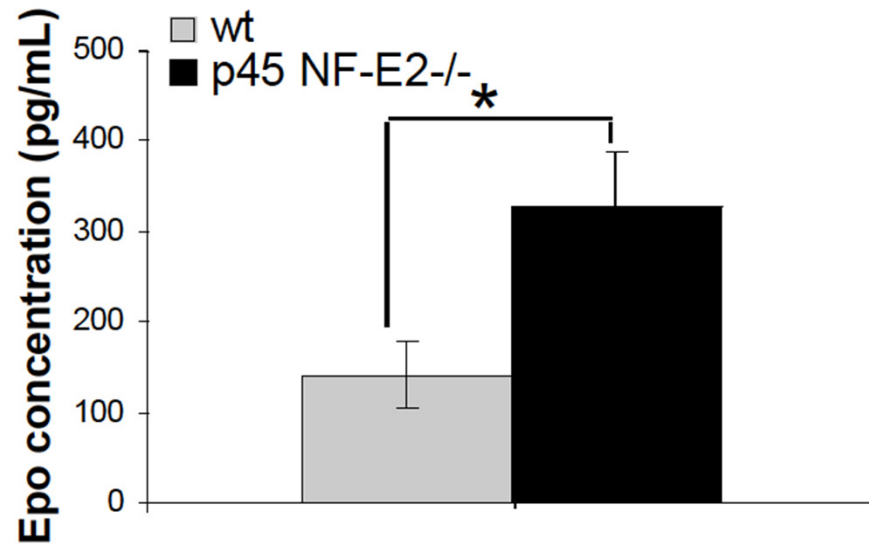


NF-E2 (nuclear factor erythroid 2)

- heterodimer (two subunits: p45 & small Maf)
- basic-leucine zipper
- large subunit specifically expressed in erythroid, megakaryocytic and mast cell lineages

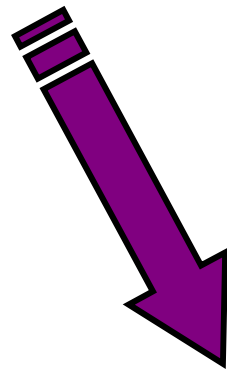
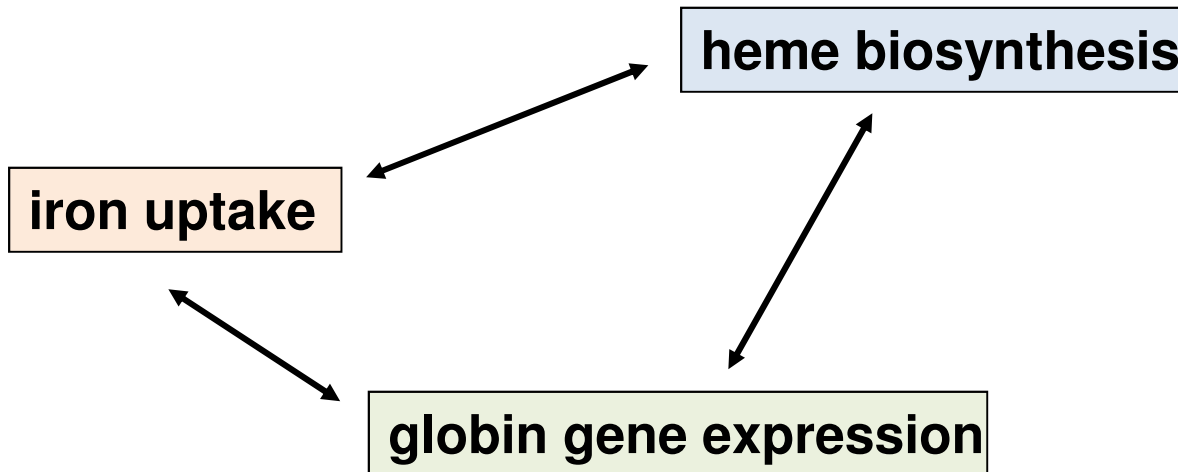


NF-E2^{-/-} mice



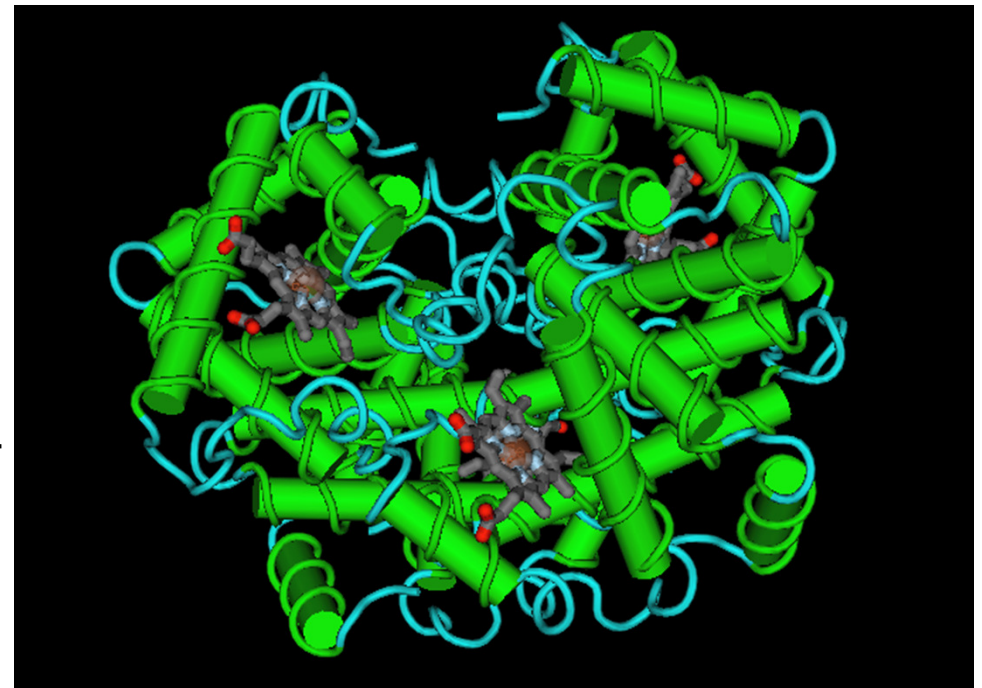
- increase in spleen erythropoiesis
- partial block in erythroid progenitor populations
- increase in GATA-1 levels
- cell cycle: increase of cells in G1

Hemoglobin synthesis

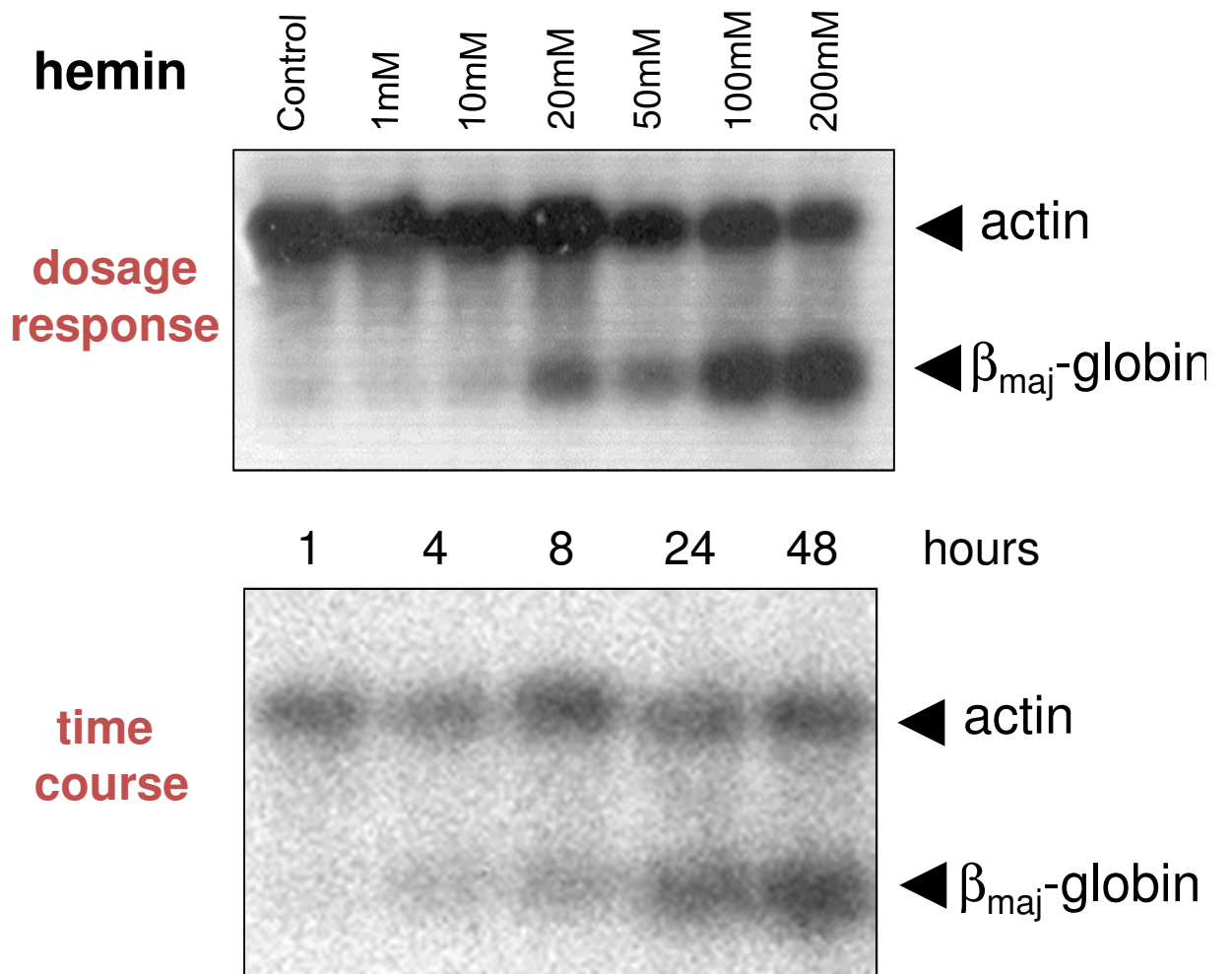
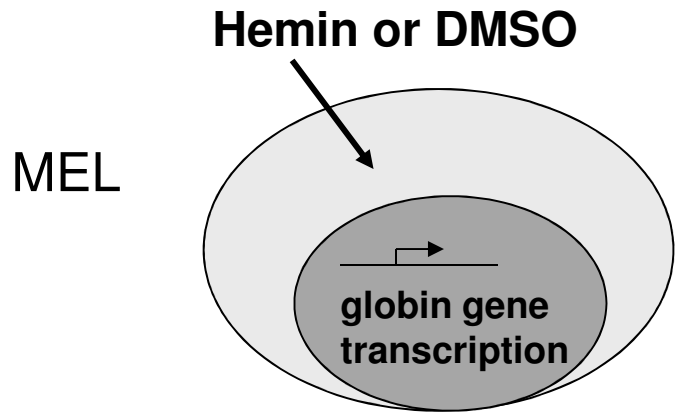


hemoglobin

$\alpha_2\beta_2$ tetramer
 α -chain 141aa
 β -chain 146aa

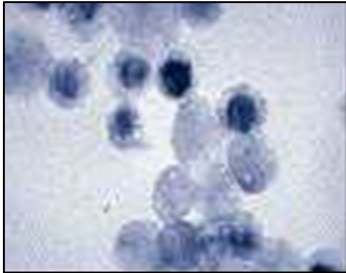


Erythroleukemia cells



Models

MEL & G1-ER cells



untreated

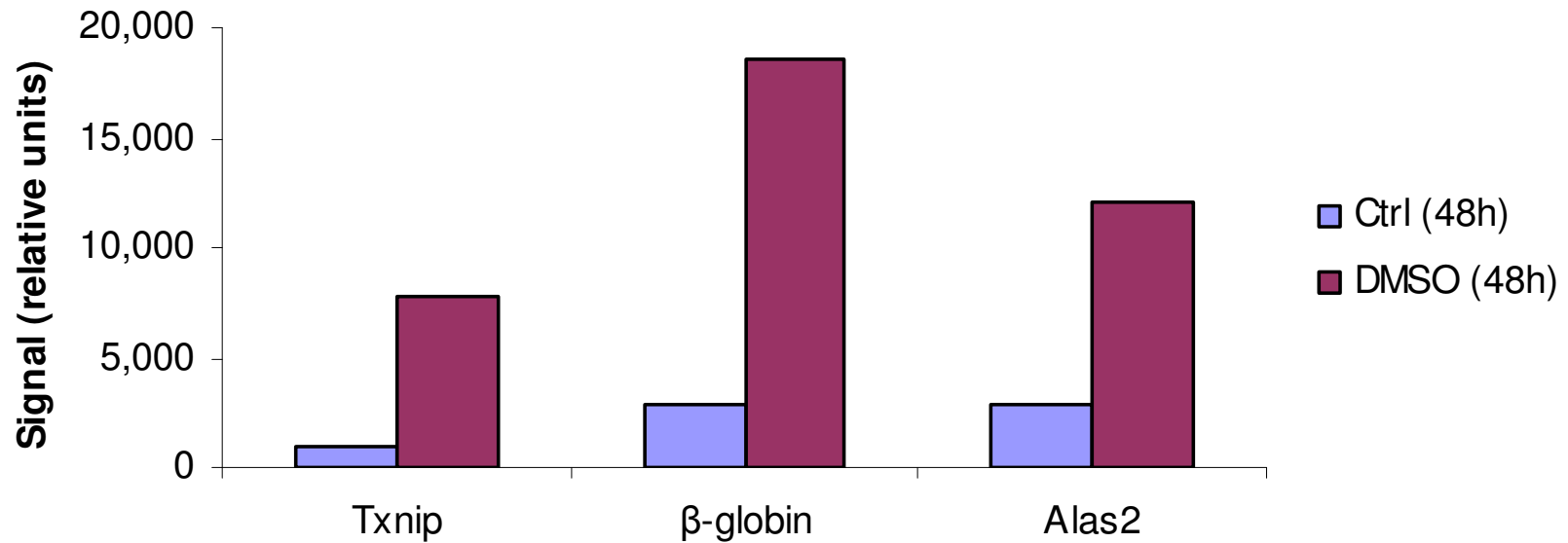
treated
(HMBA 96h)

Mice



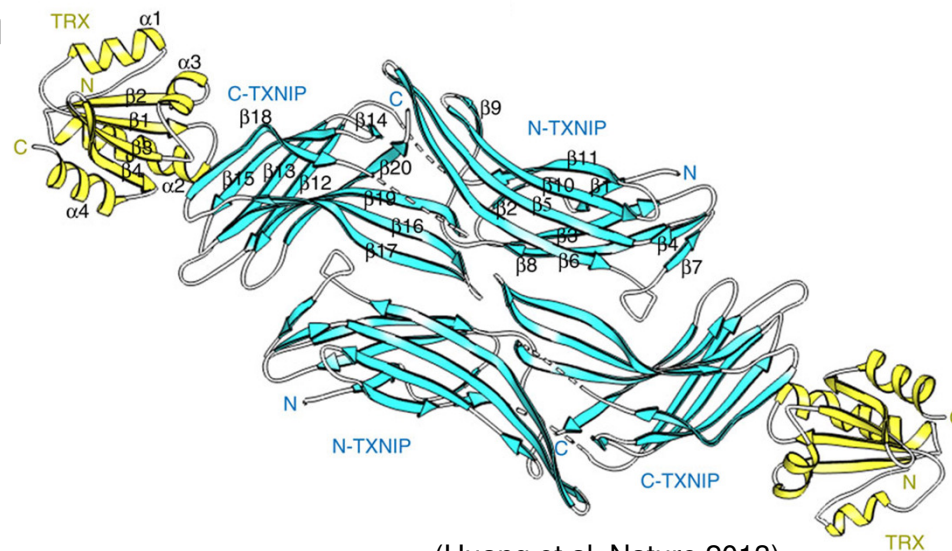
Genechip expression array (Affymetrix 430A)

- MEL cells
- differentially expressed TXNIP



TXNIP (Thioredoxin interacting protein)

- ubiquitously expressed, ~50 kDa
- cytoplasm, nucleus, mitochondria, cell membrane
- tumor suppressor (expression reduced in many tumors)
- overexpression induces cell cycle arrest
- role in oxidative stress by inhibiting activity or bioavailability of thioredoxin



(Huang et al, Nature 2013)

TXNIP ^{-/-} mice

Metabolism

In fasting conditions:

hypoglycemia

↑ insulin

↑ triglycerides

↑ fatty acids in plasma

(Hui et al. 2004, Donnelly et al. 2004, Oka et al. 2006)

Carcinogenesis

hepatocellular carcinoma (40% of mice) (Sheth et al. 2006)

↑ hepatocellular and bladder tumors (carcinogen-induced)

(Kwon et al. 2010, Nishizawa et al. 2011)

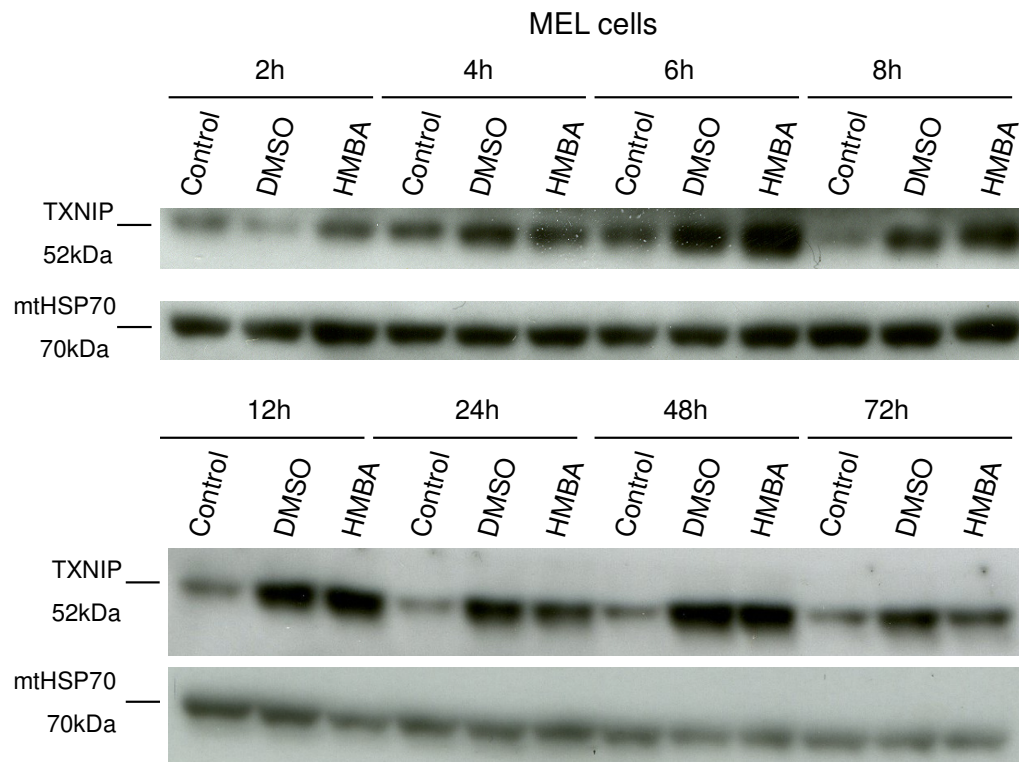
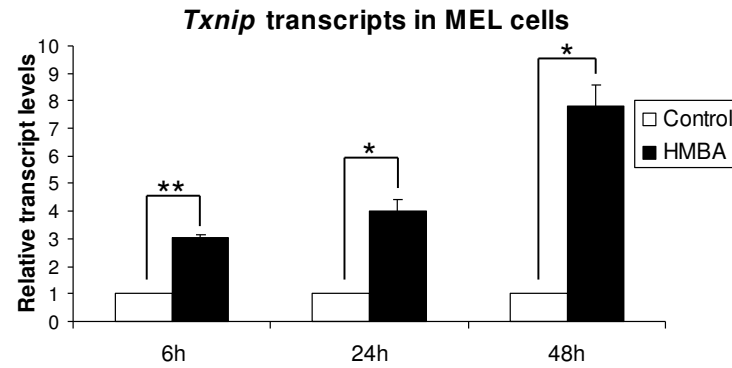
Hematopoiesis

impaired maturation of NK cells (Lee et al. 2005)

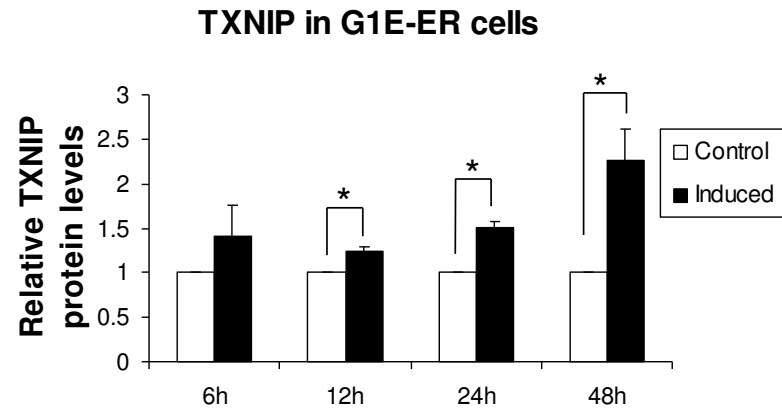
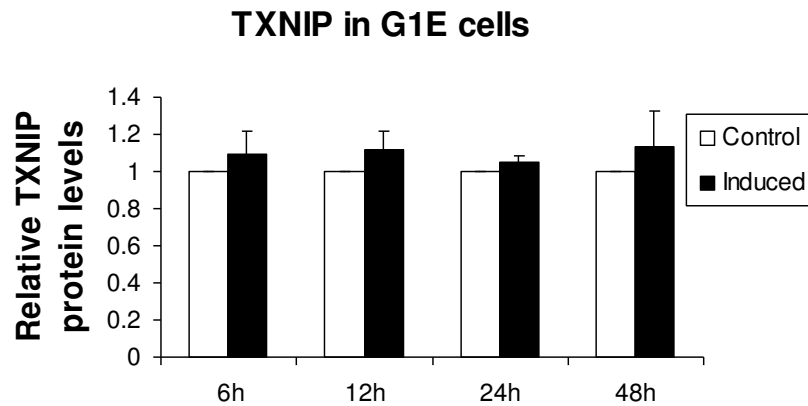
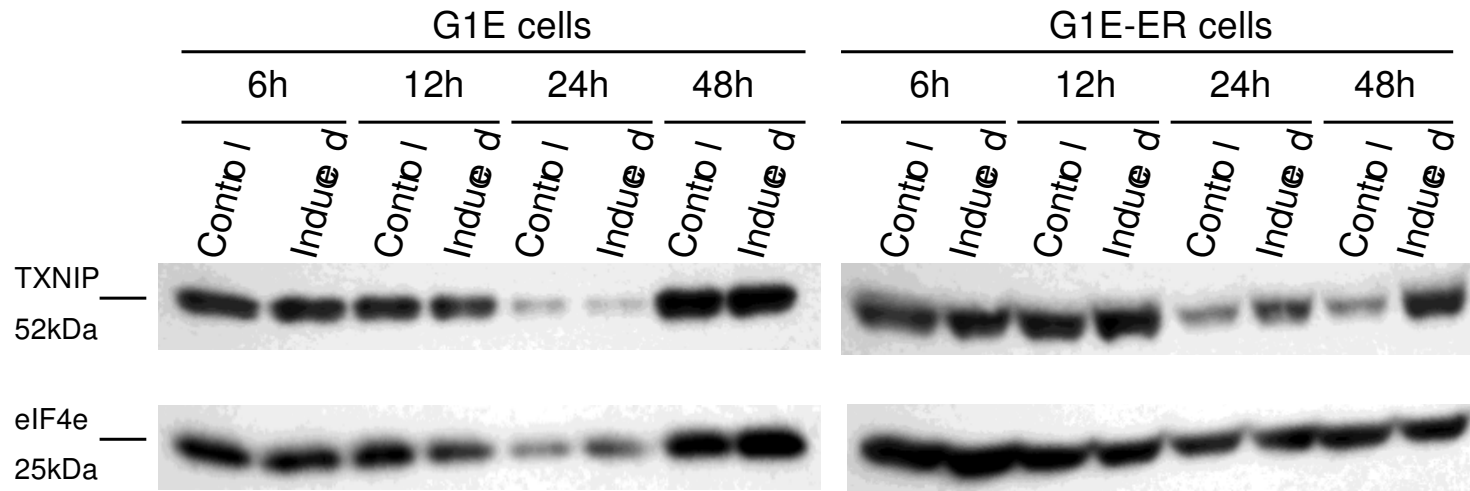
impaired function of dendritic cells (Son et al. 2008)

altered HSCs (Jeong et al. 2009, Jung et al. 2013)

Induction of TXNIP in differentiating MEL cells

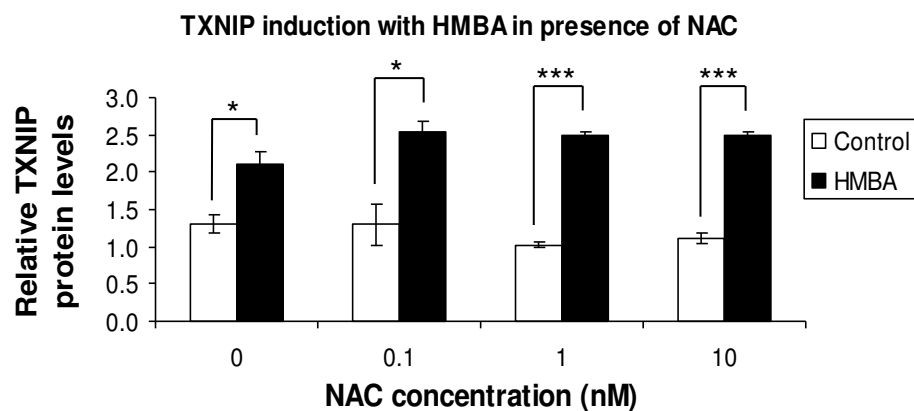
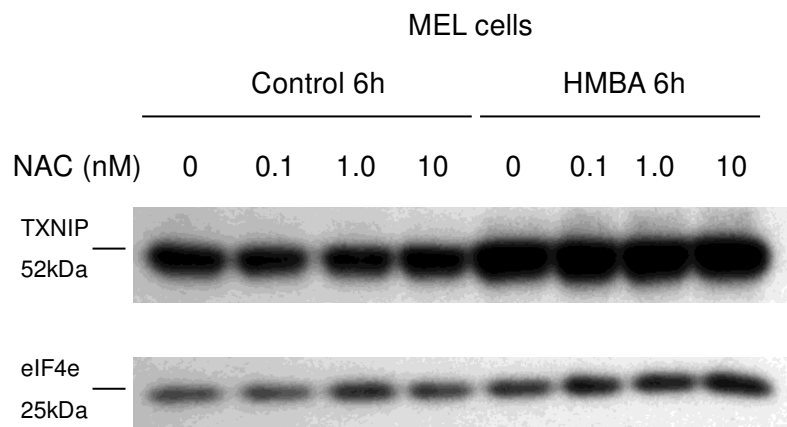


Induction of TXNIP in differentiating G1E-ER cells

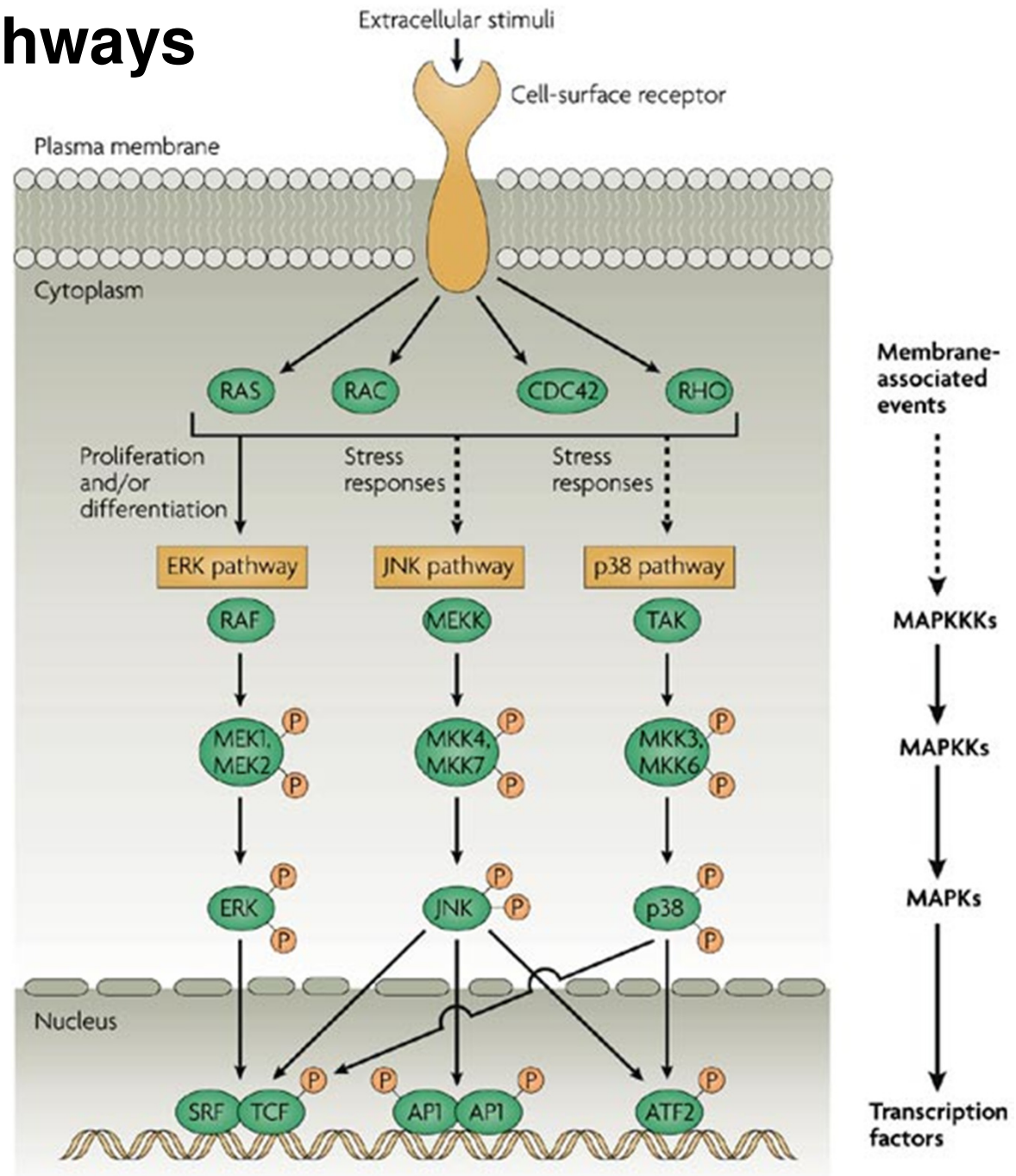


TXNIP & ROS scavenger NAC

- TXNIP upregulation with HMBA unchanged

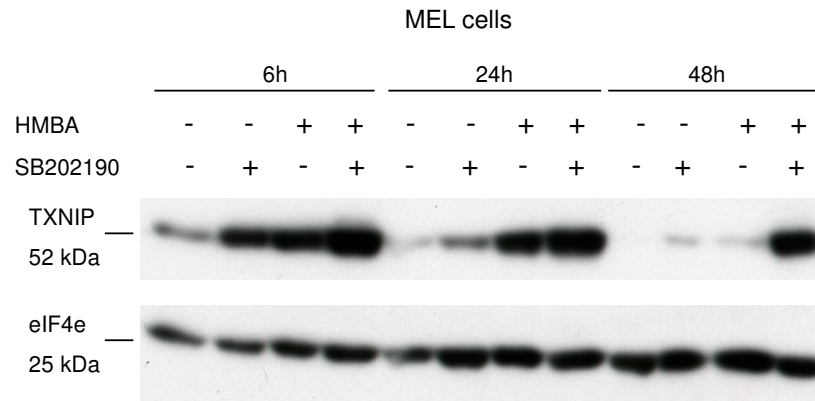


MAP kinase pathways

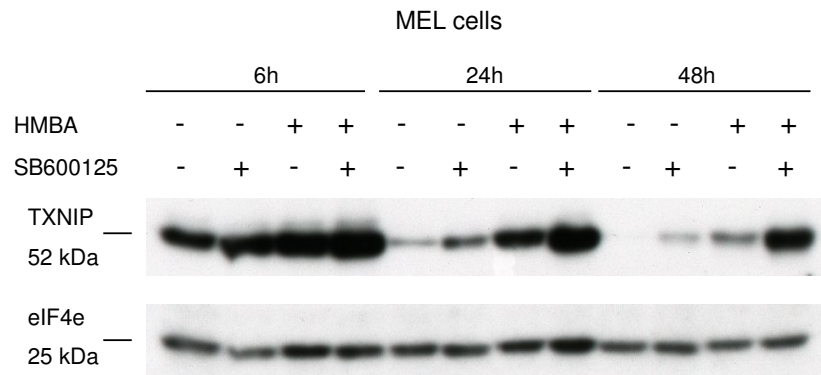


Liu et al. *Nature Reviews Immunology* 7, 202–212 (March 2007)

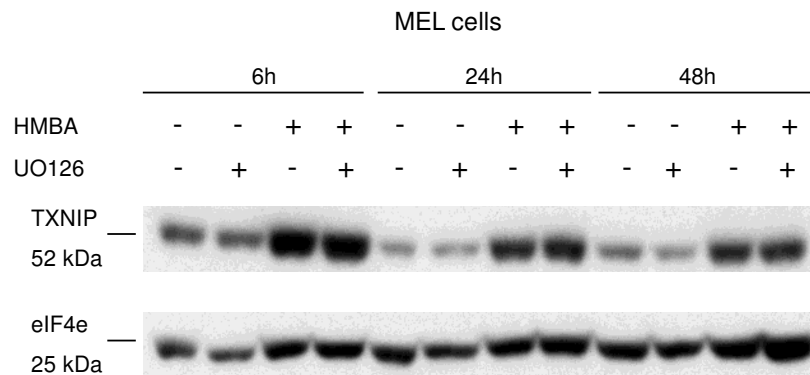
TXNIP – Regulation of MAP kinases



p38 inhibitor



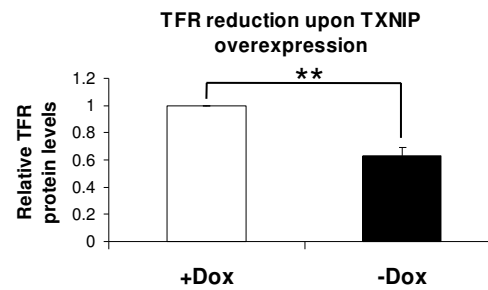
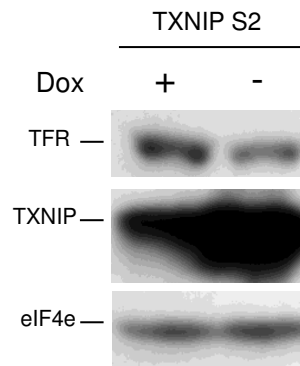
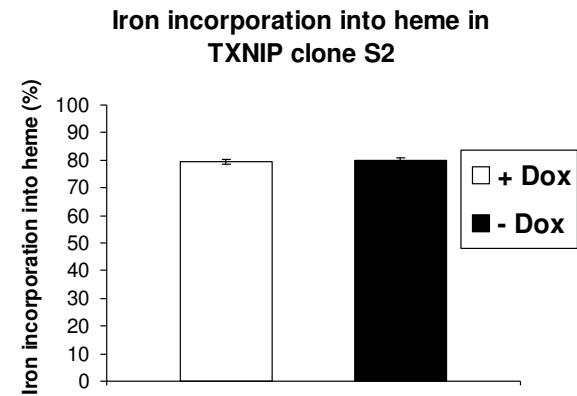
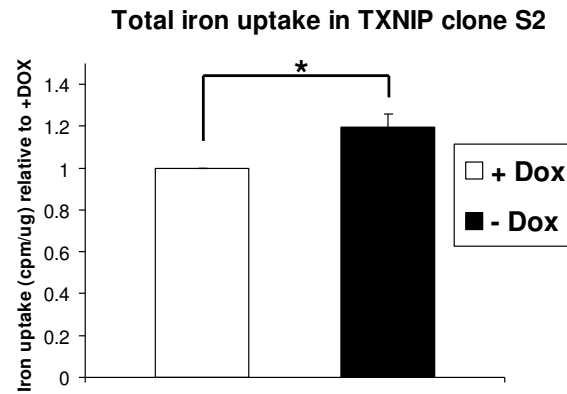
JNK inhibitor



ERK inhibitor

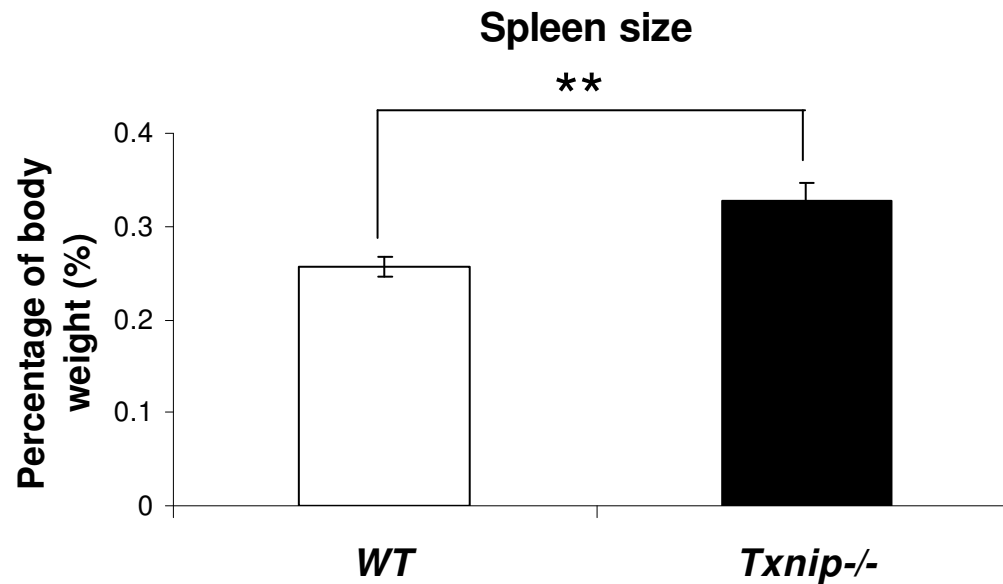
TXNIP overexpression

- increases iron uptake
- decreases TFR expression



Analysis of *Txnip*^{-/-} mice

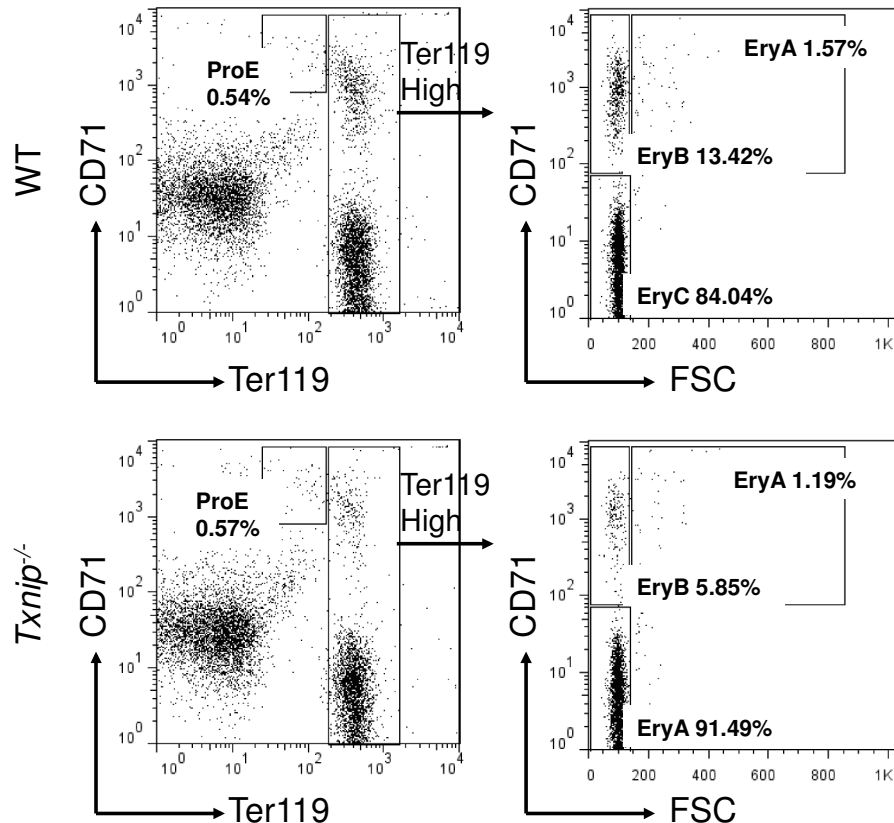
- spleen size
- blood parameters



	<i>WT</i> (n=5)	<i>Txnip</i> ^{-/-} (n=5)	p-value
RBC count, x10 ⁶ /μL	11.08 ± 0.29	11.05 ± 0.41	0.963
HGB level, g/dL	16.86 ± 0.50	17.78 ± 0.43	0.203
HCT, %	51.42 ± 1.37	53.30 ± 1.57	0.393

Erythroid precursor populations in *Txnip*^{-/-} mice

- spleen

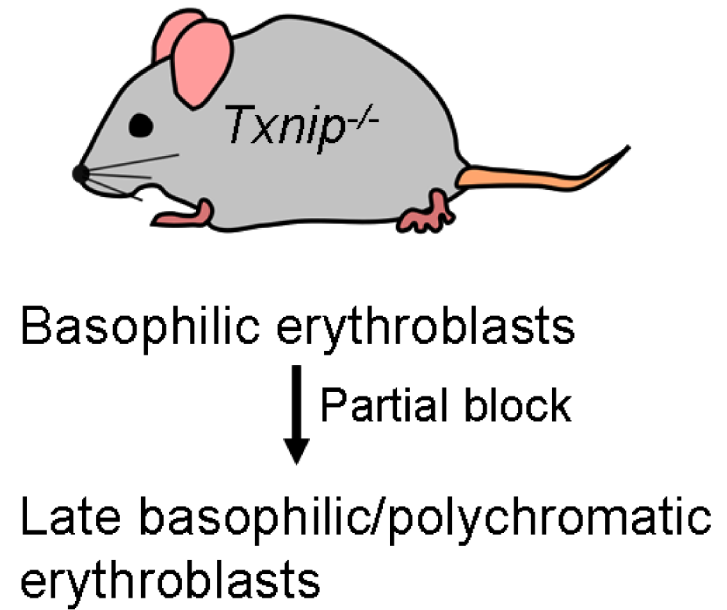
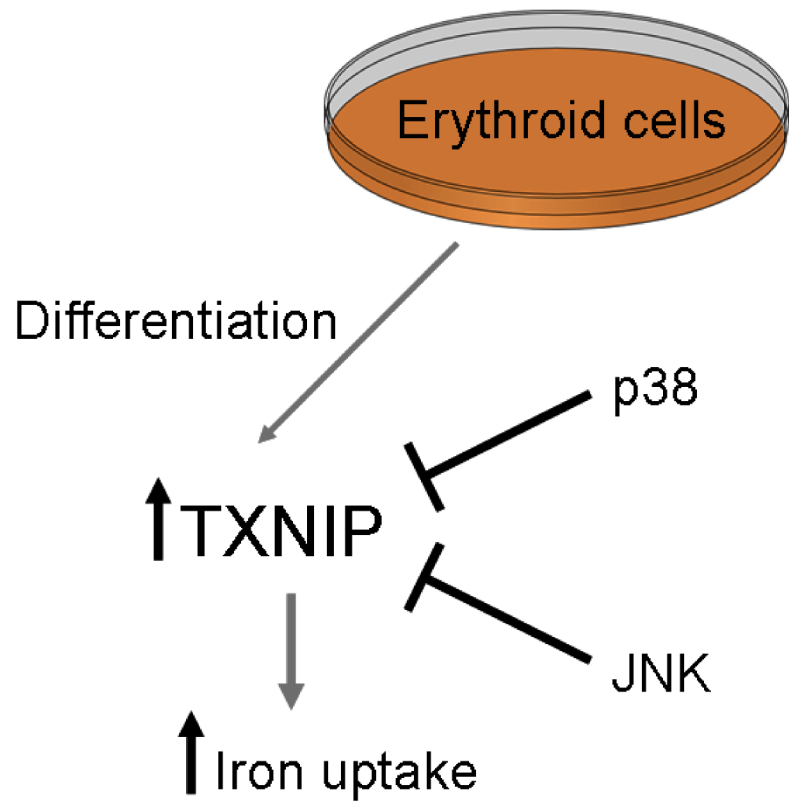


	WT (n=13)	<i>Txnip</i> ^{-/-} (n=14)	p-value
Spleen			
ProE	1.78 ± 0.35	2.37 ± 0.37	0.2580
EryA	2.99 ± 0.63	2.53 ± 0.48	0.5640
EryB	11.08 ± 1.24	6.72 ± 0.64	*0.0057
EryC	84.16 ± 1.61	88.38 ± 1.36	0.0563
Ter119+ cells	47.57 ± 1.87	45.17 ± 2.19	0.4130
Bone marrow			
ProE	6.40 ± 0.91	7.57 ± 0.69	0.315
EryA	14.90 ± 0.82	14.18 ± 0.82	0.546
EryB	25.85 ± 1.48	21.83 ± 1.44	0.063
EryC	52.85 ± 2.39	56.68 ± 2.20	0.251
Ter119+ cells	44.59 ± 2.60	42.97 ± 2.01	0.628

ProE proerythroblasts
EryA basophilic erythroblasts
EryB late basophilic and polychromatic erythroblasts
EryC orthochromatic erythroblasts and erythrocytes

Model

- Role of Txnip in erythroid differentiation



Acknowledgements



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