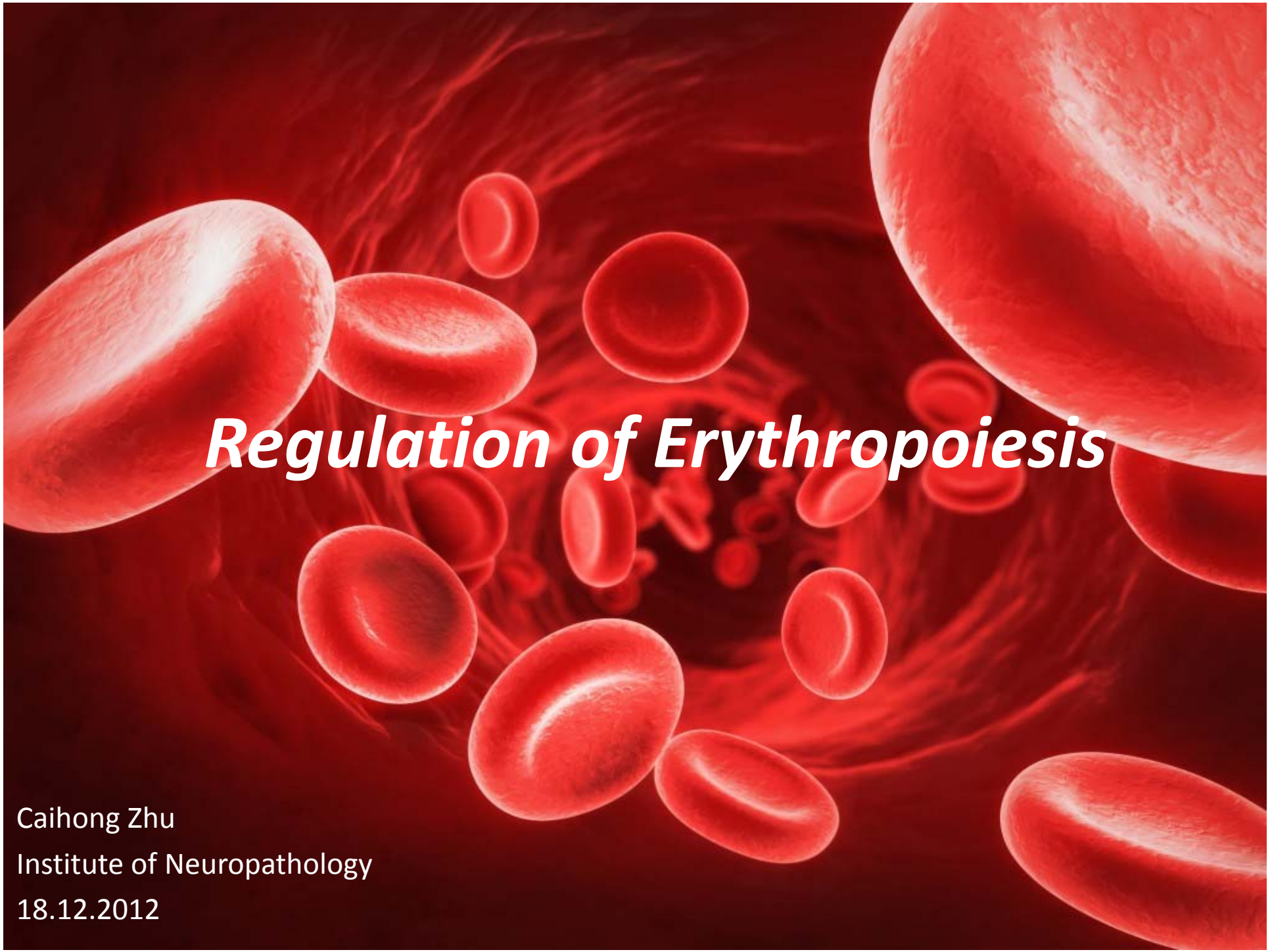


Regulation of Erythropoiesis

Caihong Zhu
Institute of Neuropathology
18.12.2012

-Introduction of the erythropoiesis

-EPO and erythropoiesis

-HIF pathway in osteoblast modulates erythropoiesis

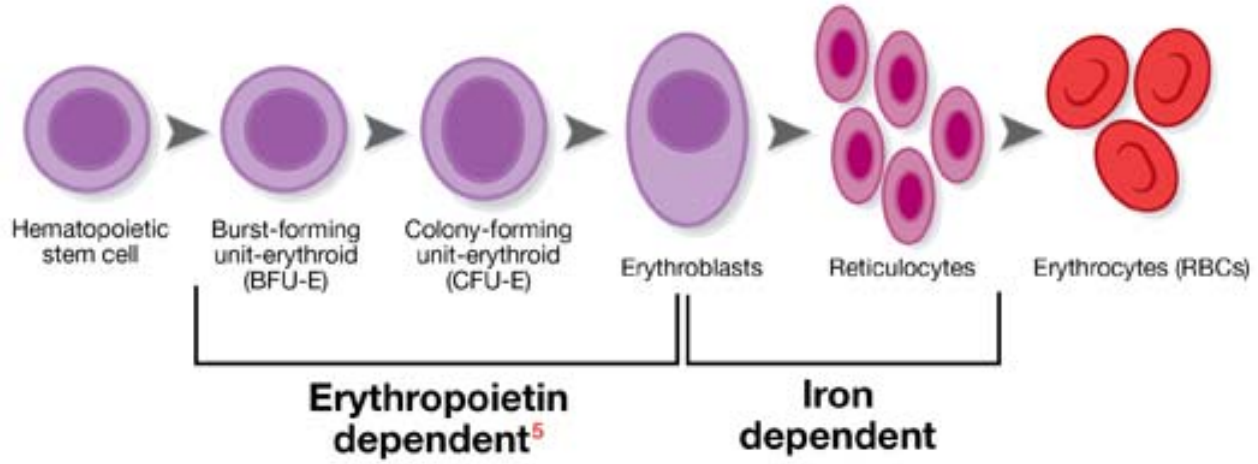
-PDGF-BB modulates erythropoiesis

-Hepcidin and erythropoiesis

Introduction

Erythropoiesis (erythro=red blood cell; poiesis= to make) is the process by which red blood cells (erythrocytes) are produced

yolk sac → spleen and liver → bone marrow

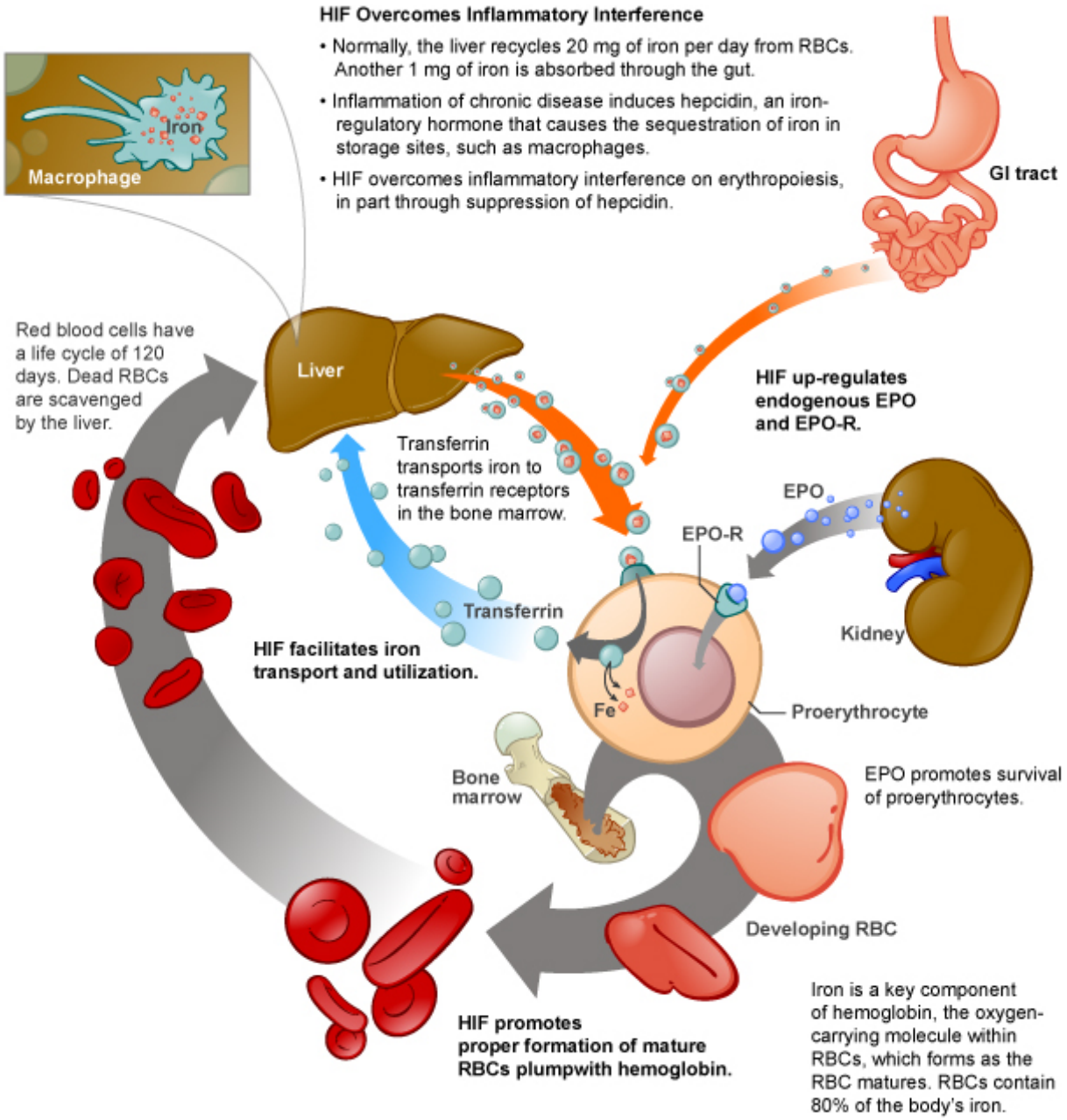


EPO is primarily produced in fetal liver and adult kidney
Iron is absorbed from diet through gut and recycled from splenic and liver macrophages

Introduction

- The hRBC lifetime is about 120 days
- Every day 1% of hRBC (200 billion) get aged and replaced by new-born RBC
- Erythrocytes deliver oxygen from lung to other tissues
- Production = Destruction
  Production > Destruction → polycythemia, thrombosis, stroke
  Production < Destruction → anemia, hypoxia

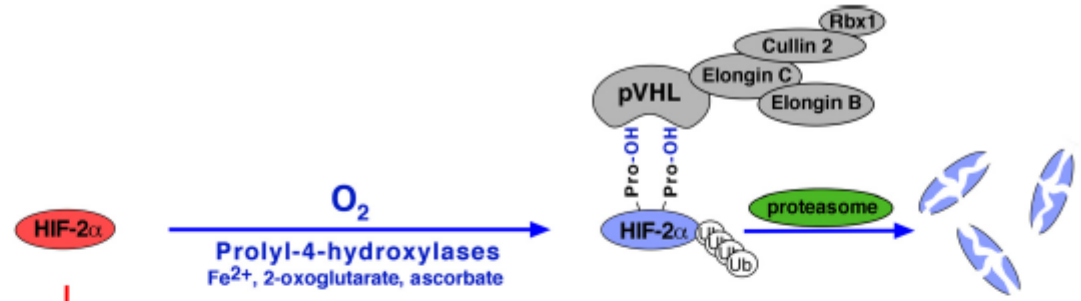
Essential role of HIF in erythropoiesis



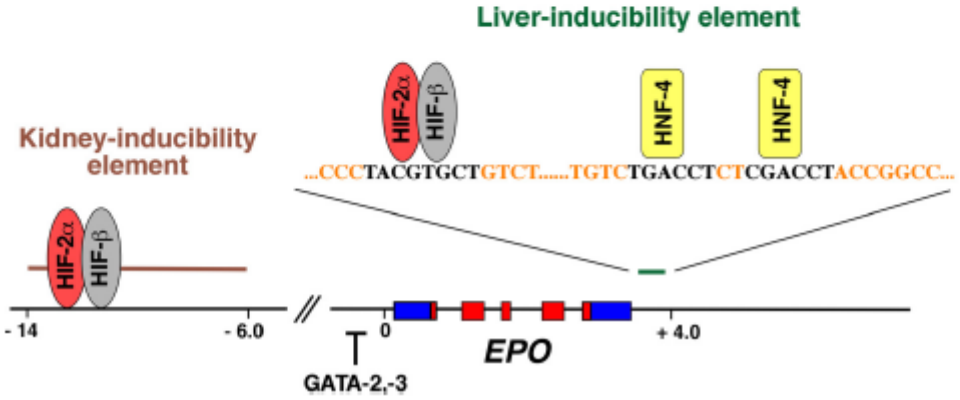
HIF-EPO

HIF-Hepcidin

Stabilization of HIF induces EPO expression in kidney and liver



Prolyl-4-hydroxylase domain:PHD
van Hippel-Lindau: VHL, an E3 ligase uniquitinates HIF for proteasomal degradation



Interstitial fibroblast **hepatocytes**

Cell

The HIF Signaling Pathway in Osteoblasts Directly Modulates Erythropoiesis through the Production of EPO

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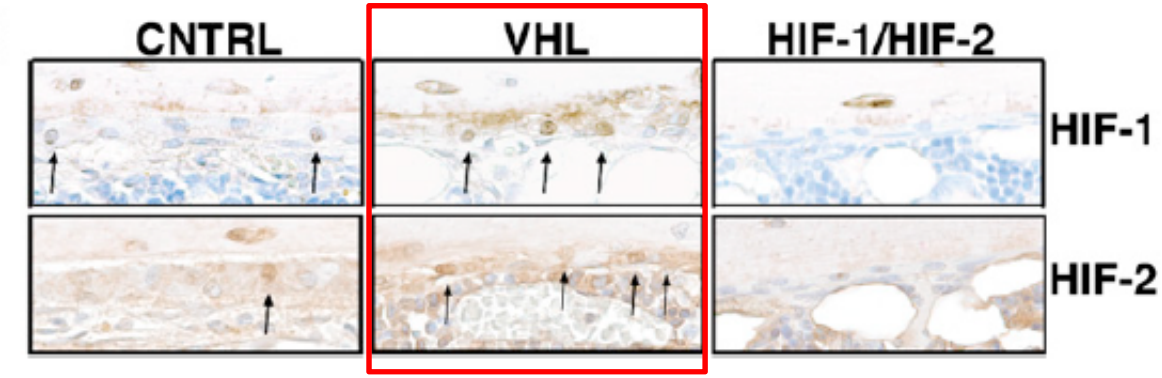
Background

- Osteoblasts are an essential component of bone and the bone marrow microenvironment for the regulation of skeletal and hematopoietic homeostasis, they are required to maintain hematopoiesis in the bone marrow;

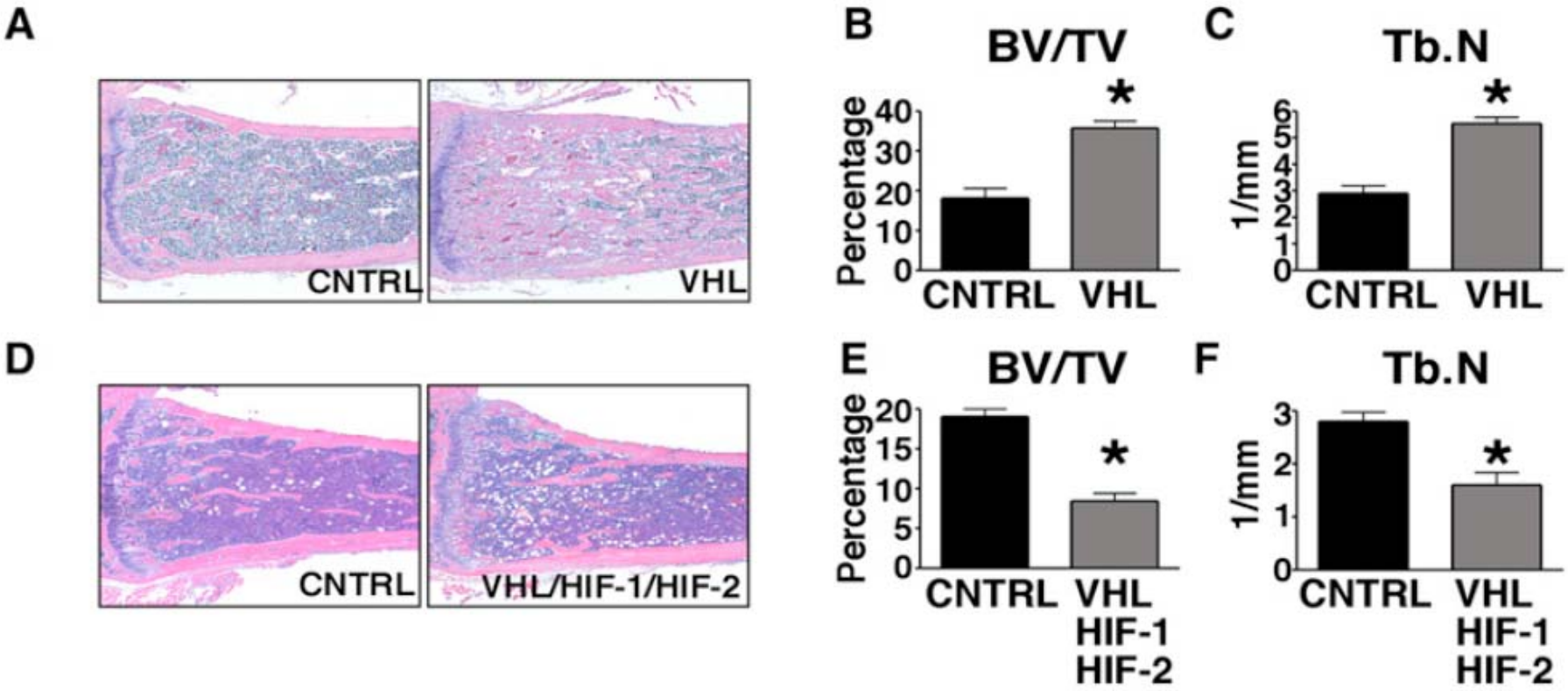
- Role of osteoblats in erythropoiesis remains unknown.

Stabilization of HIFs after VHL depletion



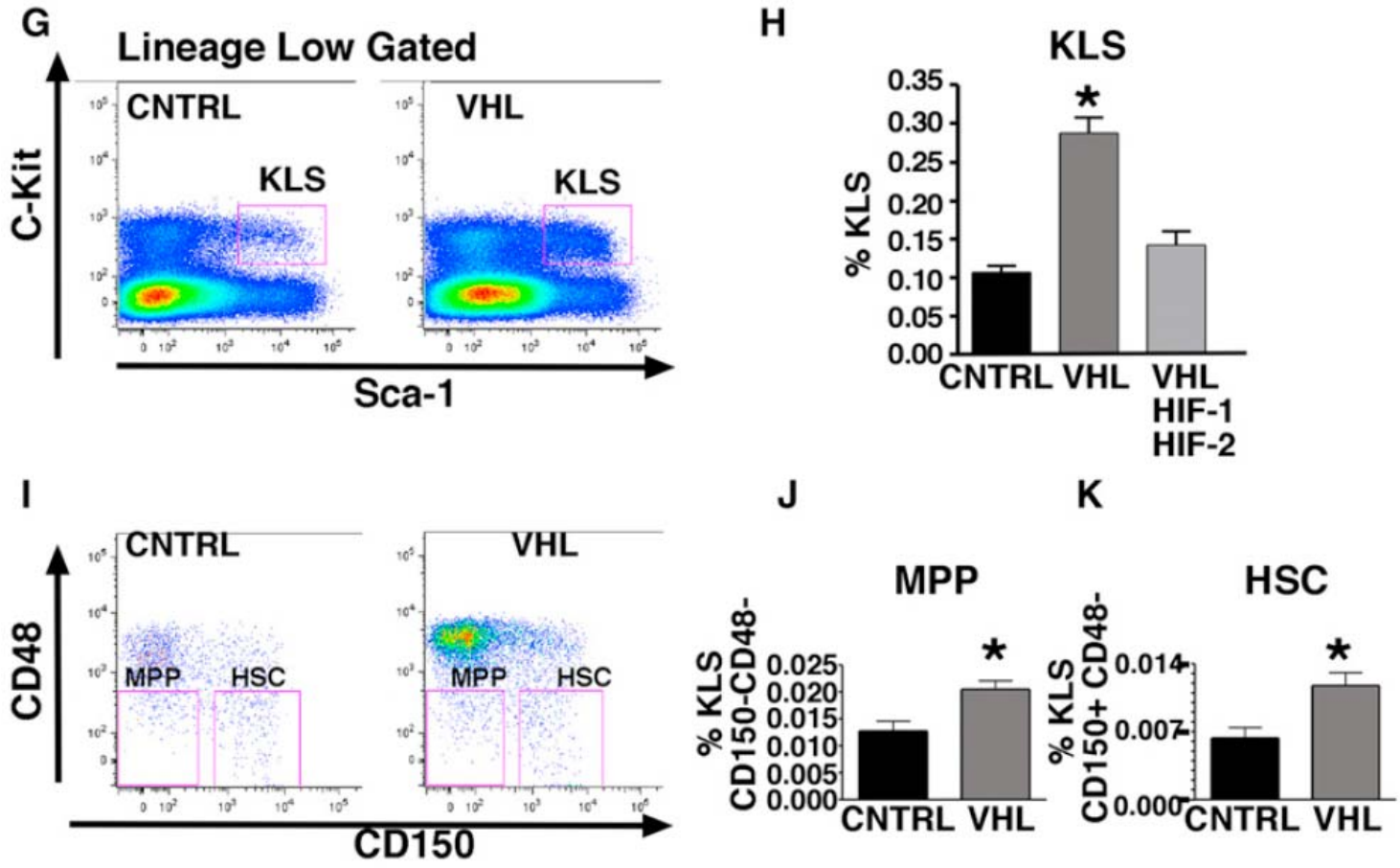
OSX-Cre: osterix promoter, osteoblast specific
VHL: OSX-Cre x floxed VHL (Osteoblasts specific depletion of VHL)
HIF-1/HIF-2: OSX-Cre x floxed HIF-1 x floxed HIF-2 (Osteoblasts specific depletion of HIFs)

Augmented HIF activity in osteoblasts increases trabecular osteoblastic cells and trabeculae



BV/TV: trabecular bone volume
Tb.N: trabecular number

Augmented HIF activity in osteoblasts expands the HSC niche



KLS: cKIT^{high} Lineage^{low} Sca1^{+} progenitors inculde HSC and multipotent progenitors (MPP)

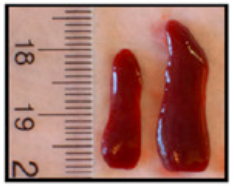
Selective expansion of erythroid lineage in OSX-VHL mice leads to HIF-dependent polycythemia

A

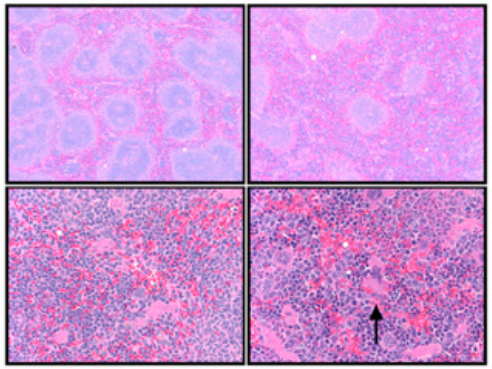
Myeloid	Control	OSX-VHL	OSX-VHL/HIF-1/HIF-2	Normal Range
Neutrophils Absolute	641 +/- 125	1337 +/- 937	733 +/- 281	825- 2604
Monocytes Absolute	118 +/- 35	138 +/- 91	73 +/- 1	0- 279
Platelets (K/uL)	1130 +/- 115	990 +/- 321	825 +/- 198	675- 1338
Eosinophils Absolute	70 +/- 31	44 +/- 27	85 +/- 47	0- 279
Red Blood Cells (M/uL)	10 +/- 0.4	16 +/- 1.4*	10 +/- 0.9	7 - 8.8
Lymphoid				
Lymphocytes Absolute	6819 +/- 2211	1596 +/- 845*	4317 +/- 1463	3685- 7812

B

C



D



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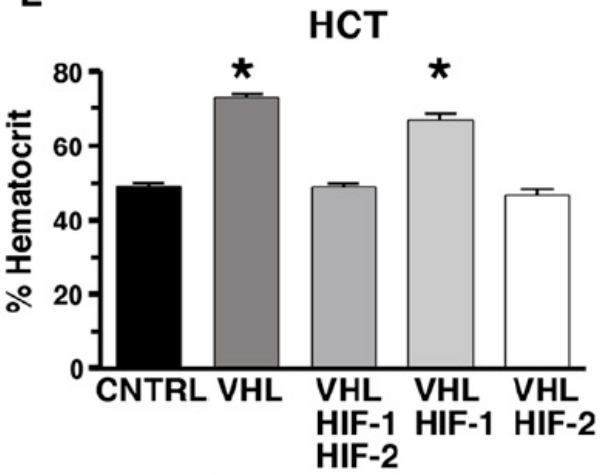
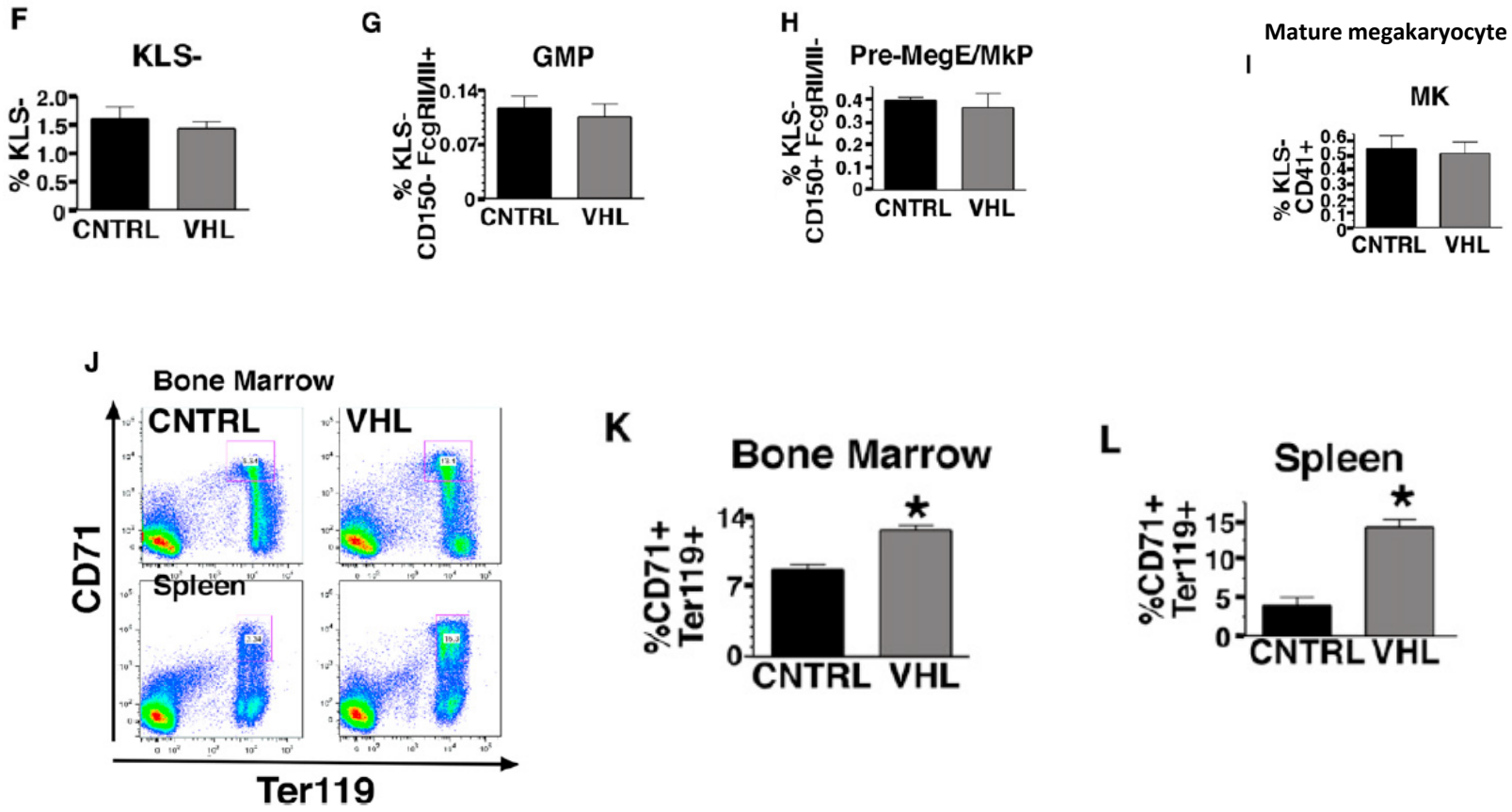
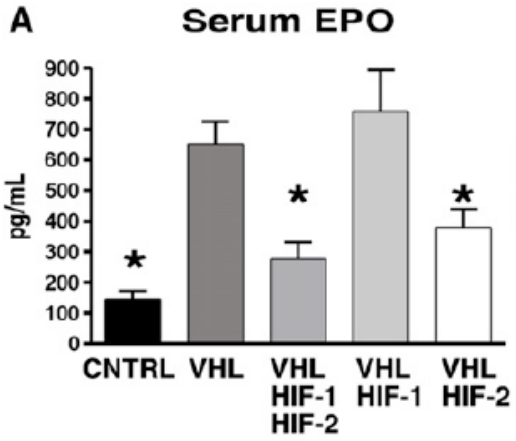
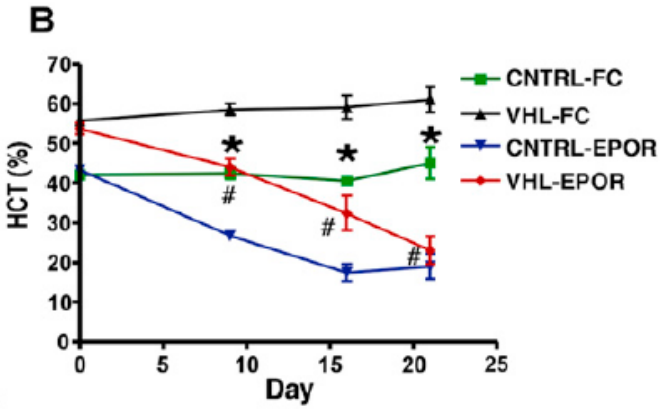


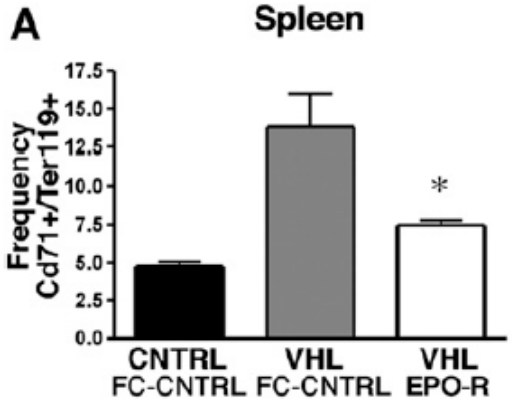
Selective expansion of erythroid lineage in OSX-VHL mice leads to HIF-dependent polycythemia

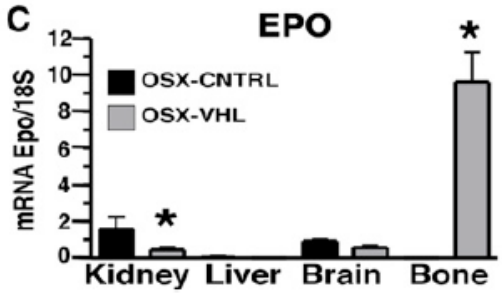


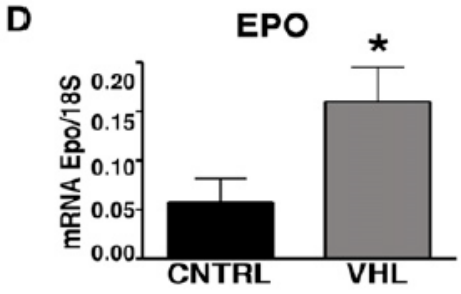
Increased HCT is EPO dependent and associated with increased EPO expression in bone and decreased EPO expression in kidney





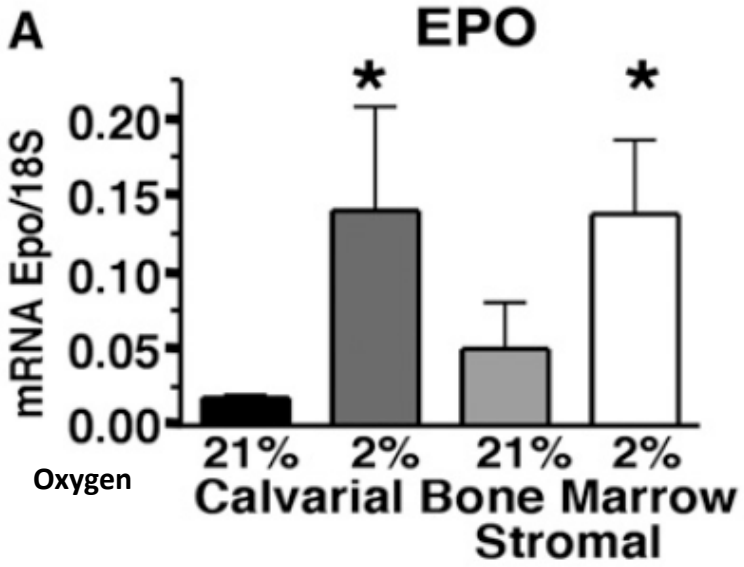




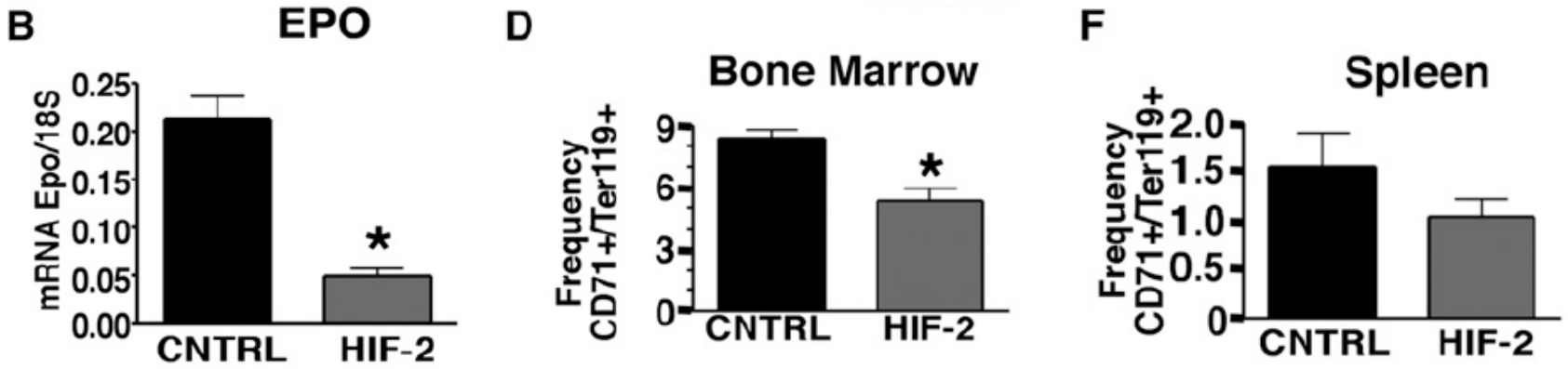


Primary osteoblasts (postnatal 3 days)

Upregulation of EPO in primary osteoblasts under hypoxia

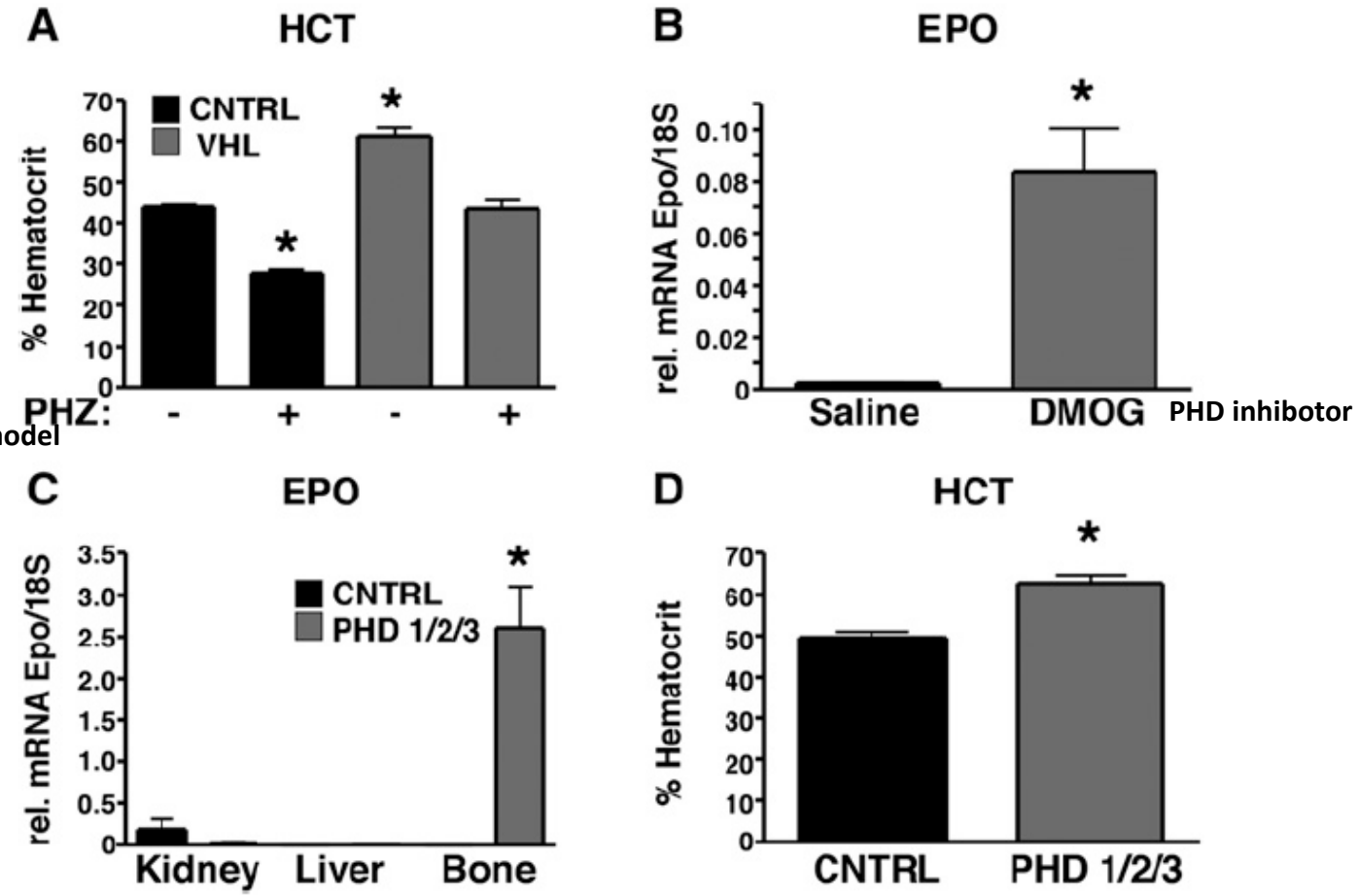


HIF signaling in osteoblasts under physiological condition



HIF signaling in osteoblasts regulates homeostasis of hematopoiesis in bone marrow but not in spleen.

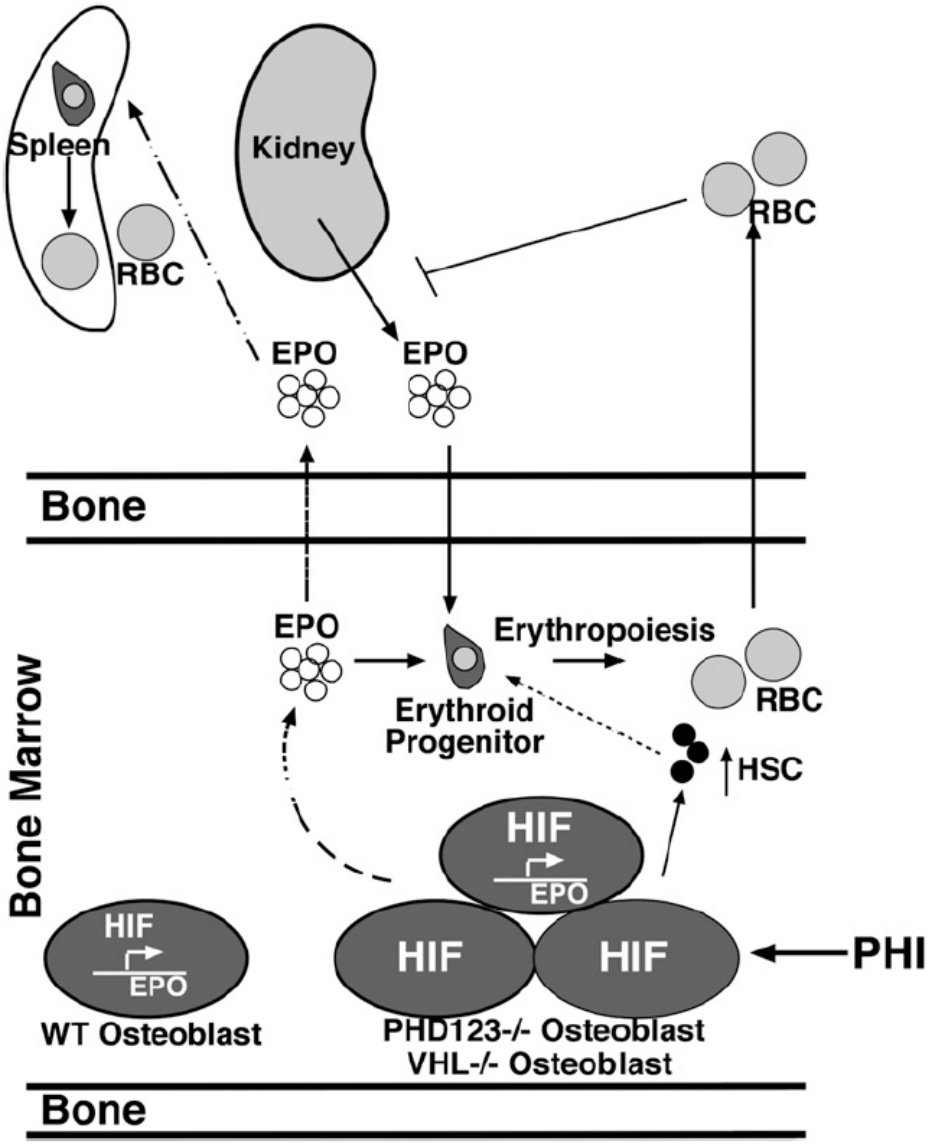
Modulation of PHD/VHL/HIF pathway in osteoblasts is sufficient to induce EPO expression and protect from anemia



phenylhydrazine (PHZ) model of hemolytic anemia

OSX-Cre x floxed PHD1/2/3

The HIF signaling in osteoblasts directly modulates erythropoiesis through production of EPO



Conclusion

- **Osteoblast** is another cell type besides **renal interstitial cells**, **hepatocytes**, **glial cells** (out of 14) that can express EPO and induce erythropoiesis after VHL inactivtion;

- New **crosstalk** between osteoblast and hematopoitic compartment, **local** production of EPO under hypoxia condition by osteoblasts in bone marrow microenvironment is sufficient to drive erythropoiesis;

- **Small molecules** that inhibit PHD could pharmacologically activate HIF pathway and rescue anemia (in trial). In patients with renal failure, in addition to hepatocytes, **osteoblasts** can also produce EPO to increase RBC production after PHD inhibition.

Questions

Are other cell types able to produce EPO and induce erythropoiesis?

Is HIF signaling really indispensible for the EPO induction and erythroipoiesis?

nature medicine

PDGF-BB modulates hematopoiesis and tumor angiogenesis by inducing erythropoietin production in stromal cells

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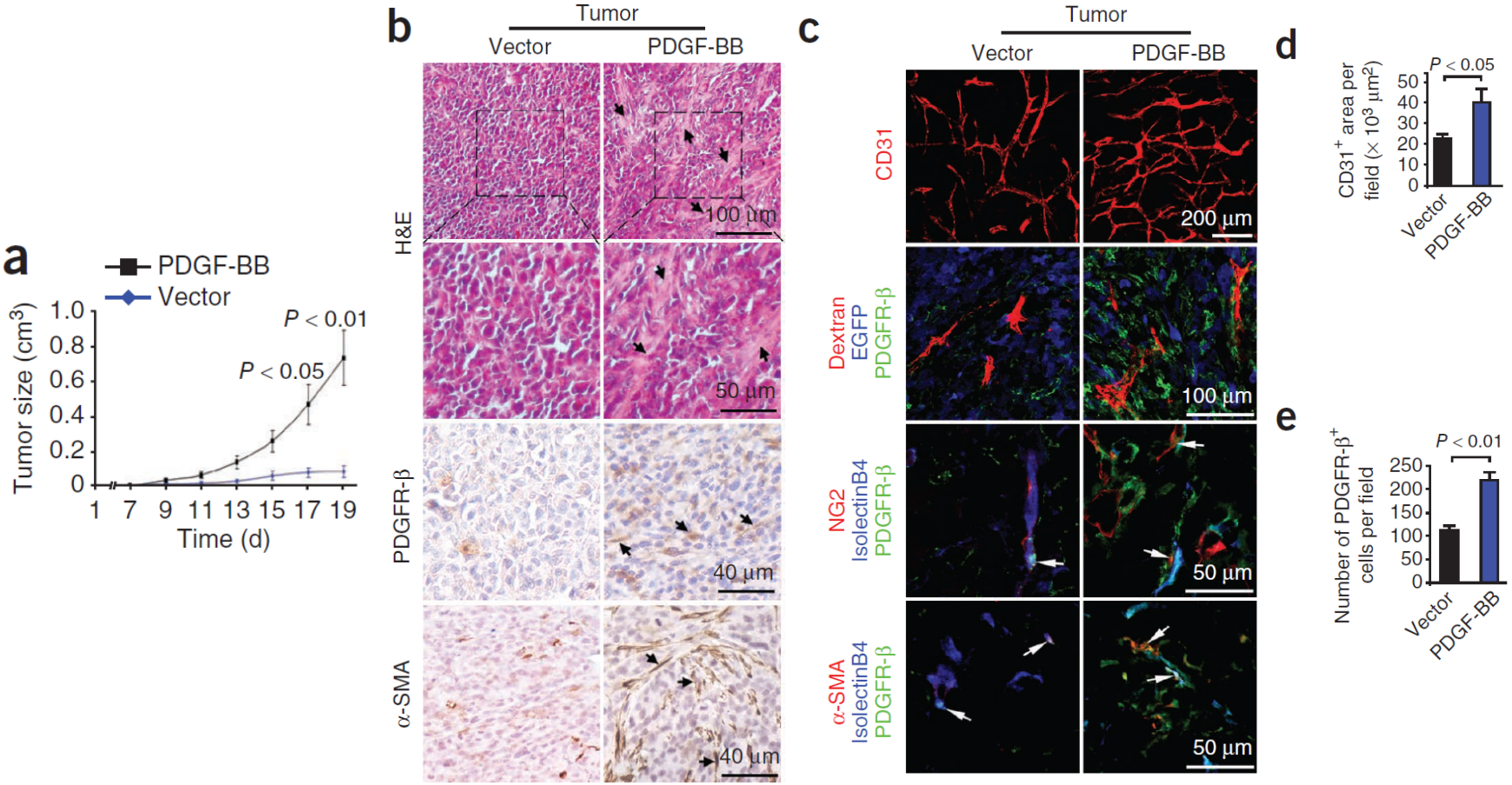
Background

- PDGF-BB: a dimer of the platelet-derived growth factor (PDGF)-B chain, a multifunctional member of PDGF family, signals through receptors PDGFR-α or PDGFR-β

- PDGF-BB stimulates tumor angiogenesis and vascular remodeling

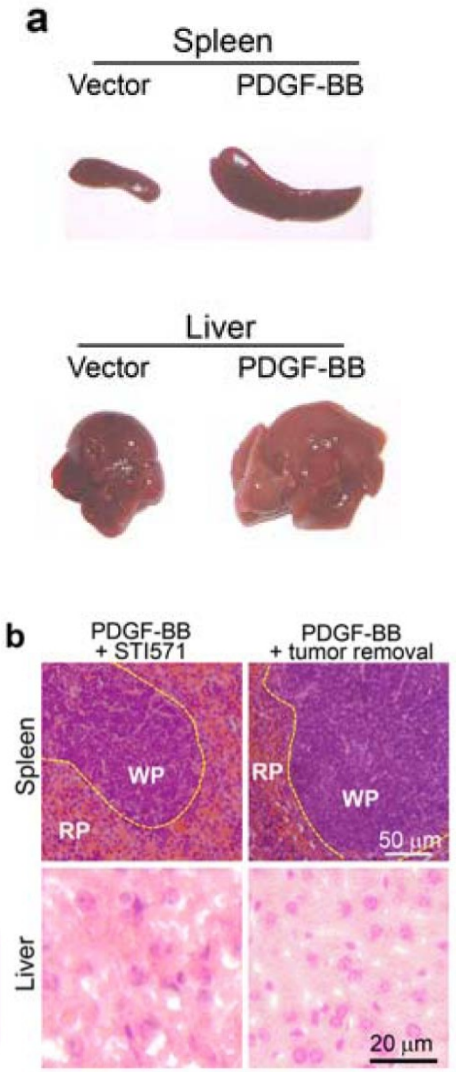
- Is PDGF-BB involved in erythropoieis?

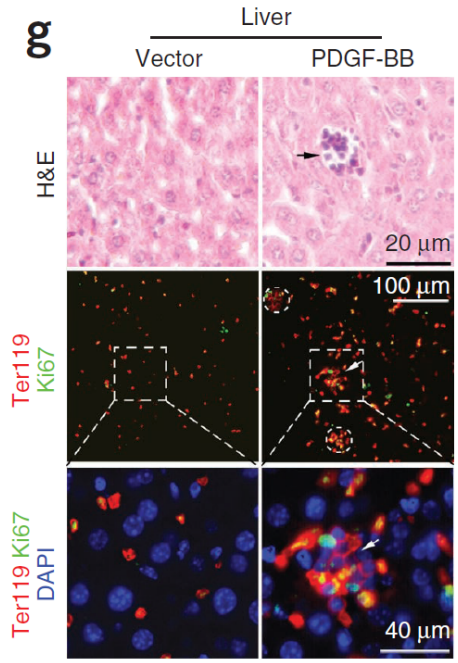
PDGF-BB promotes tumor growth, angiogenesis and stromal expansion

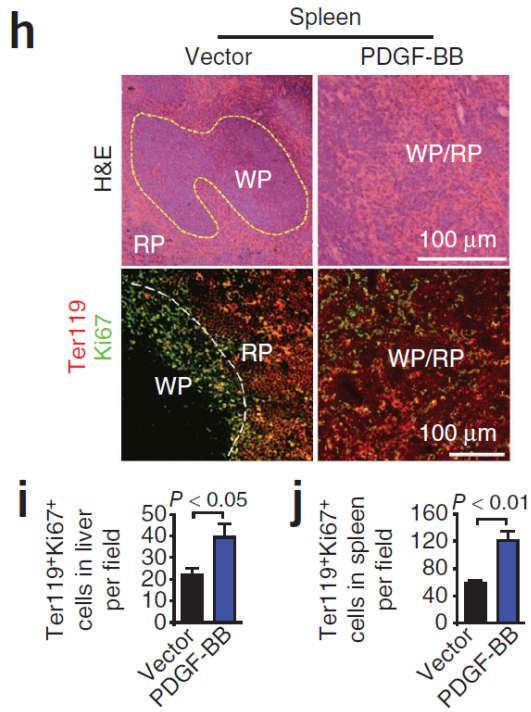


Mouse fibrosarcoma T241 cells are transfected with PDGF-BB and EGFP (PDGF-BB) or only EGFP control (vector) and subcutaneously implanted into the dorsal back of the mice.

PDGF-BB tumor leads to splenomegaly and hepatomegaly, and induces extramedullary hematopoiesis

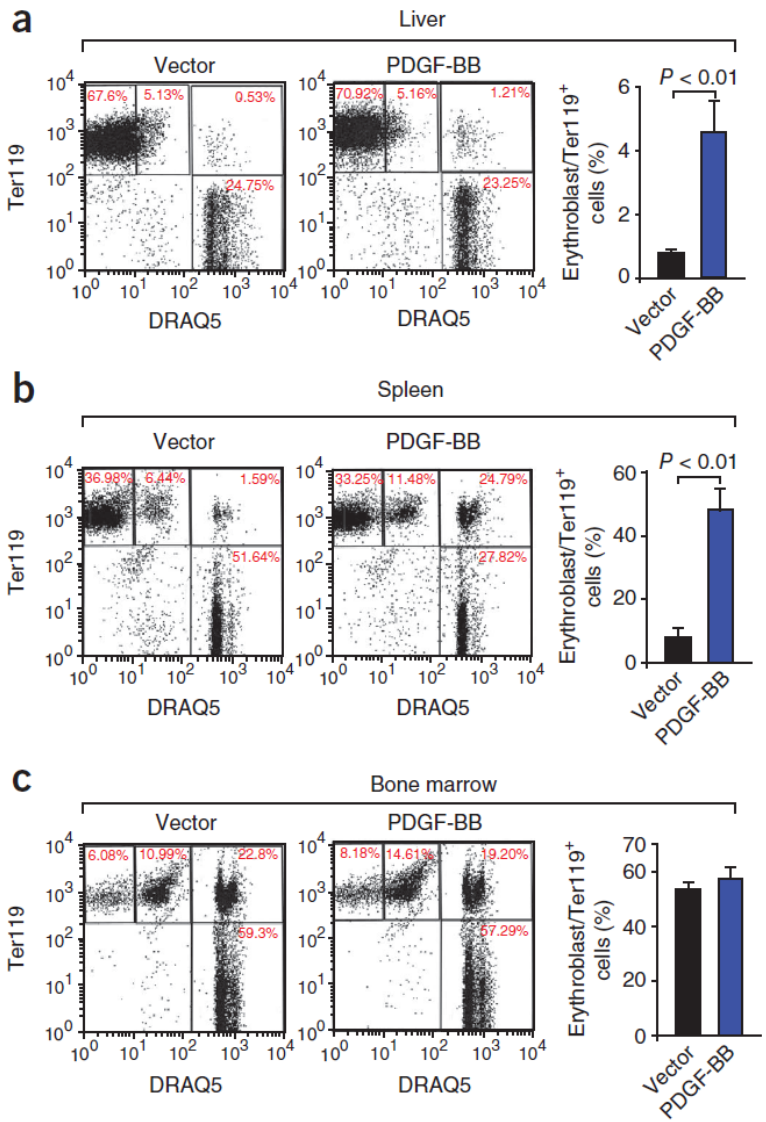






Treatment of imatinib (STI571, a tyrosine kinase inhibitor of PDGFR-β) or removal of tumor reversed the hematopoietic phenotype induced by PDGF-BB tumors

PDGF-BB tumor induces extramedullary hematopoiesis



Ter119^{high} DRAQ5^{+}: erythroblasts; Ter119^{high} DRAQ5^{-}: erythrocytes; Ter119^{low} DRAQ5^{+}: reticulocytes.

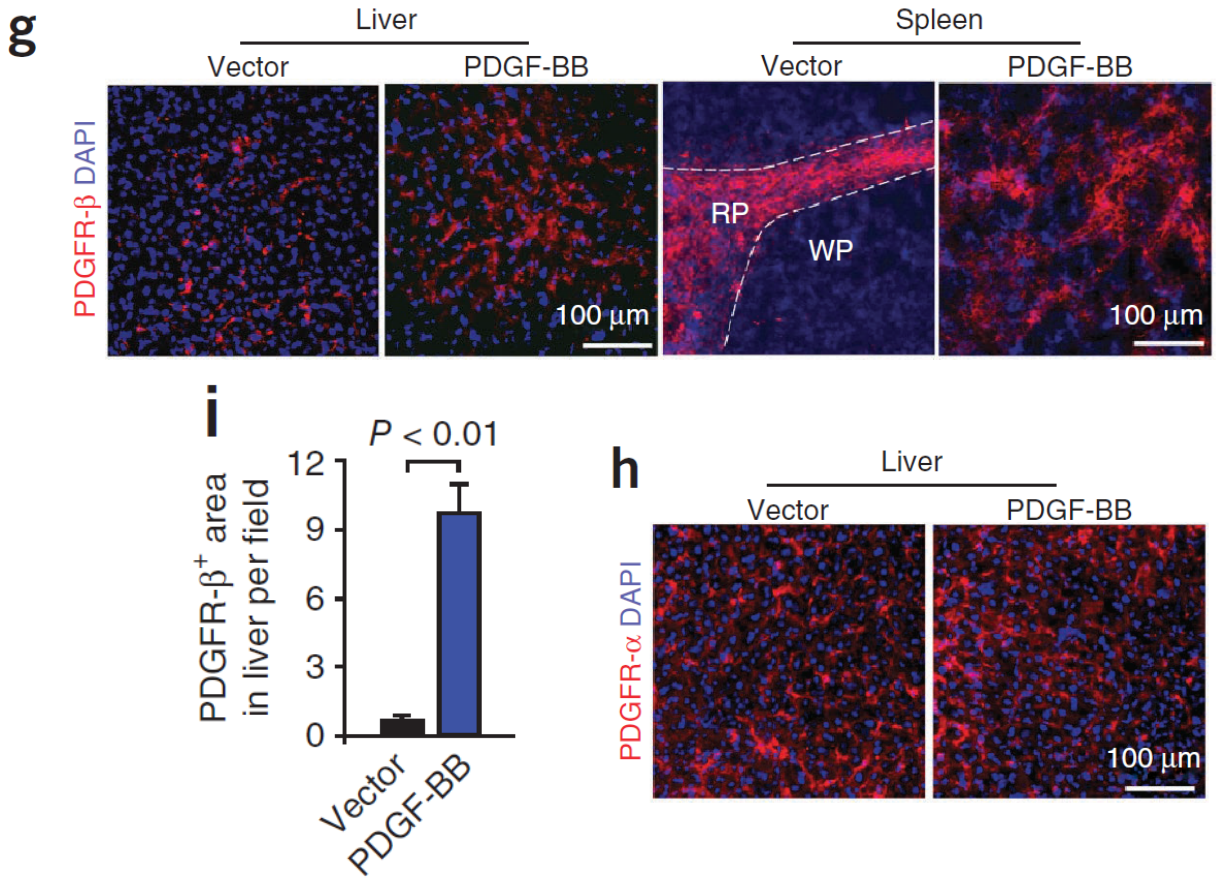
PDGF-BB protects against tumor-induced anemia

Peripheral counts of RBC, hematocrit, haemoglobin, platelet and WBC

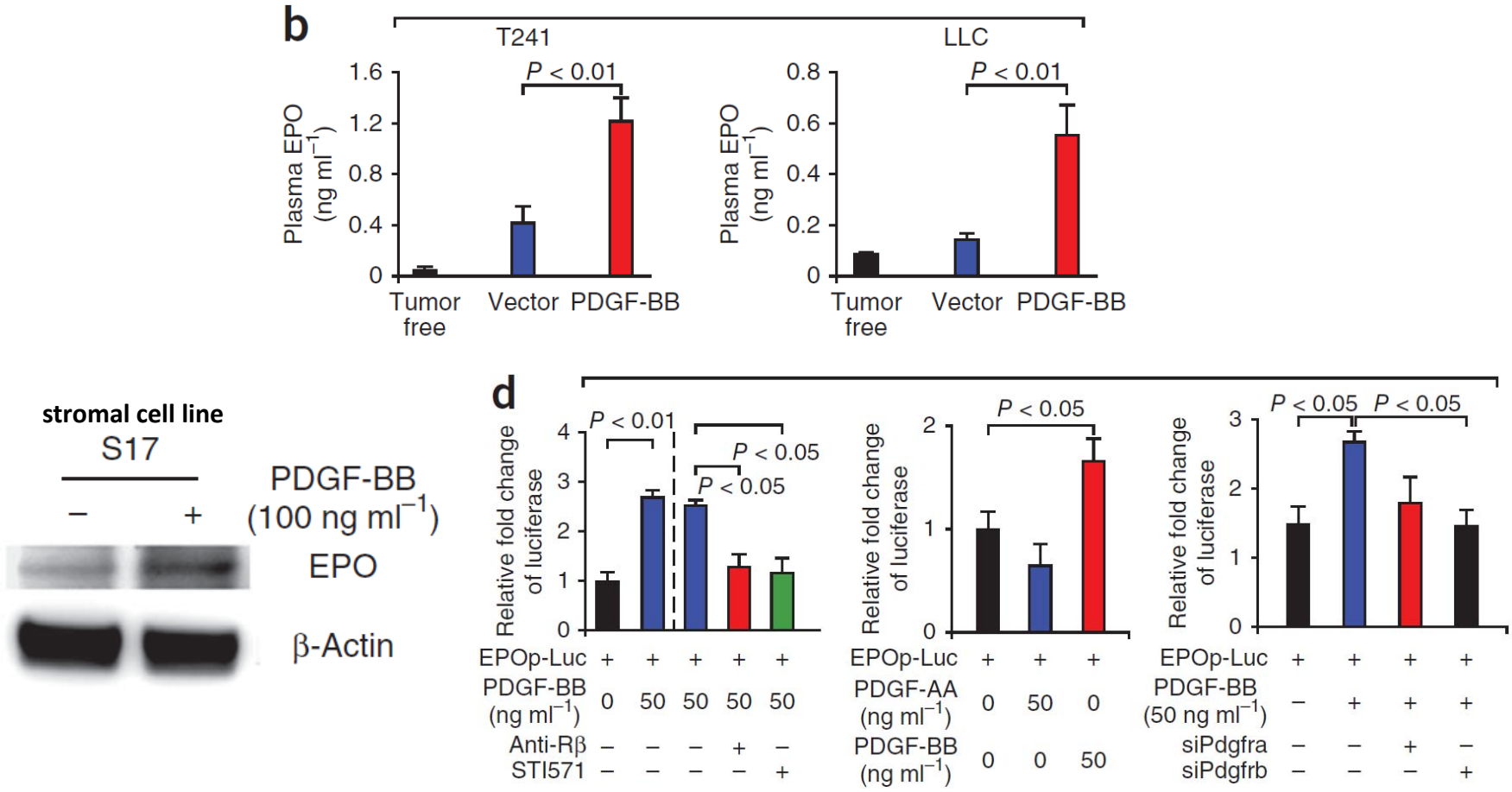
Group	Hemoglobin (g/L)		RBC (1×10^{12}/L)		Hematocrit (%)		Platelet (1×10^{9}/L)	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
Wild-type	119.60	±8.29	8.09	±0.59	38.24	±2.78	239.20	±80.48
	$P<0.05$		$P<0.05$		$P<0.05$			
Vector-T241	102.33	±9.91	6.83	±0.82	32.42	±2.52	272.80	±25.28
	$P<0.05$		$P<0.05$		$P<0.05$			
PDGF-BB-T241	114.20	±7.29	7.64	±0.74	36.74	±2.43	280.14	±71.31

Group	Total WBC (1×10^{9}/L)		Monocyte (1×10^{8}/L)		Granulocyte (1×10^{9}/L)		Lymphocyte (1×10^{9}/L)	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
Wild-type	8.78	±1.79	3.66	±0.42	2.30	±0.62	6.18	±1.13
Vector-T241	7.48	±2.97	4.24	±0.46	2.45	±1.44	5.55	±2.27
	$P<0.05$							
PDGF-BB-T241	12.92	±4.36	5.41	±0.91	3.84	±1.48	8.58	±2.88

PDGF-BB induces PDGFR-β$^+$ cells expansion in liver and spleen, but not PDGFR-α$^+$ cells

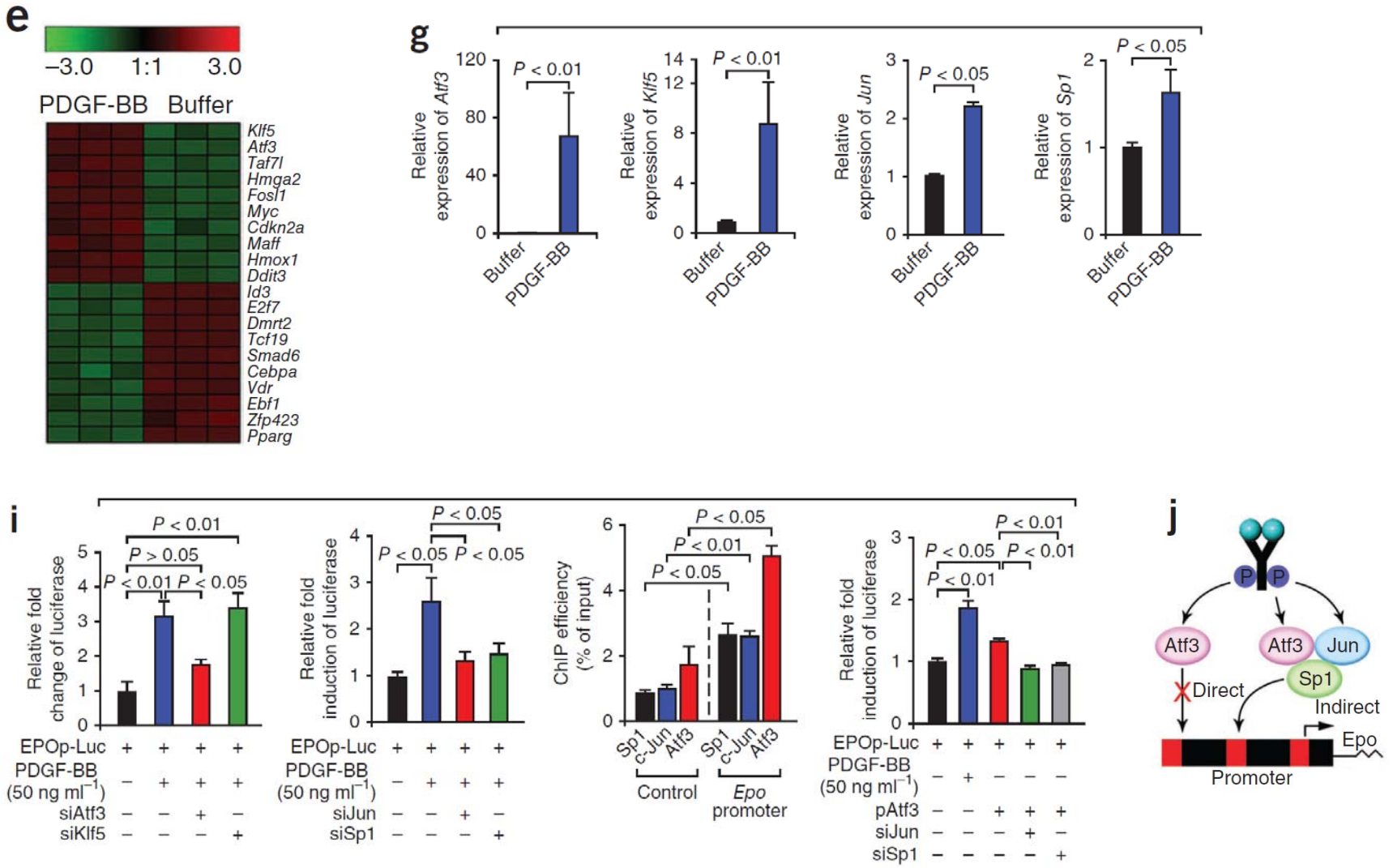


PDGFR-β dependent EPO promoter activity in stromal cells

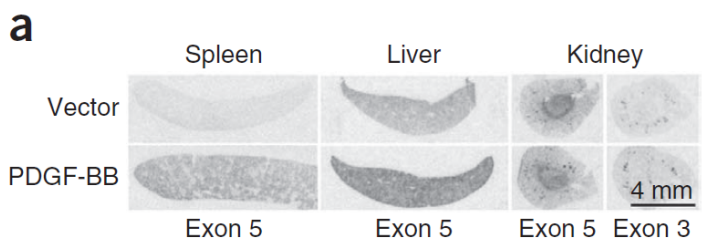


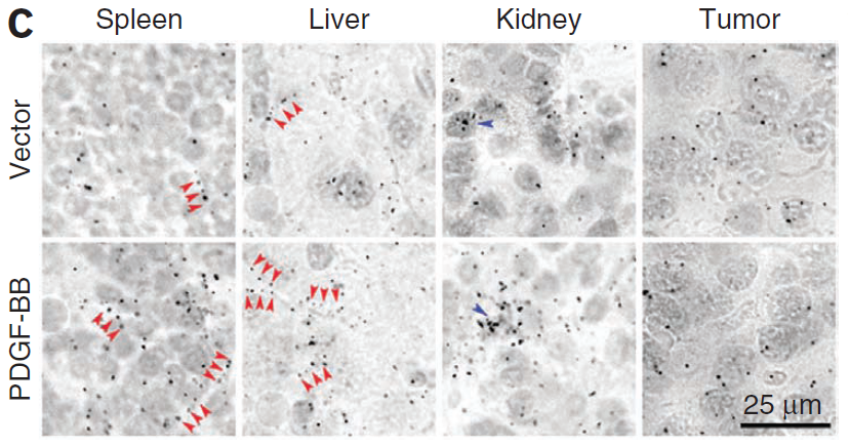
PDGFR-BB transcriptionally induces EPO promoter activity through PDGFR-β but not PDGFR-α

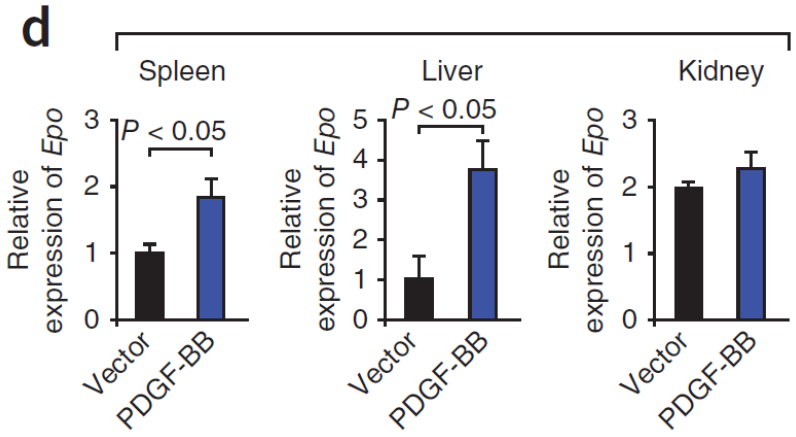
Atf3 mediates PDGF-BB-induced EPO expression

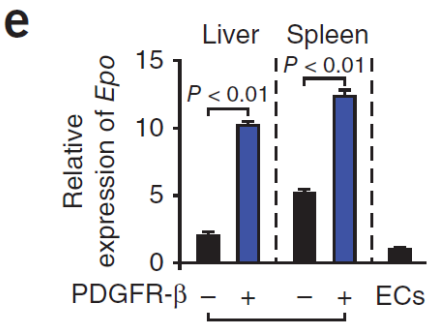


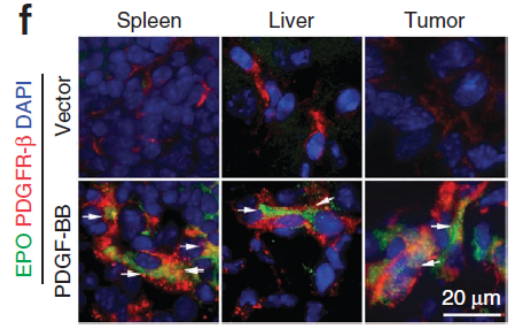
PDGF-BB induces stromal EPO expression *in vivo*



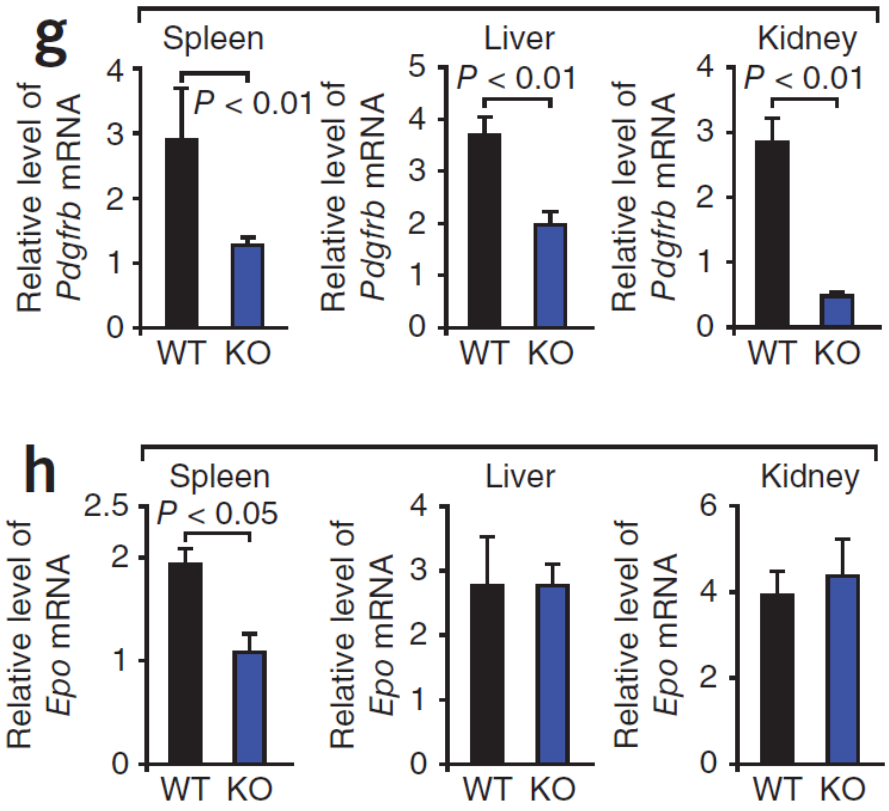








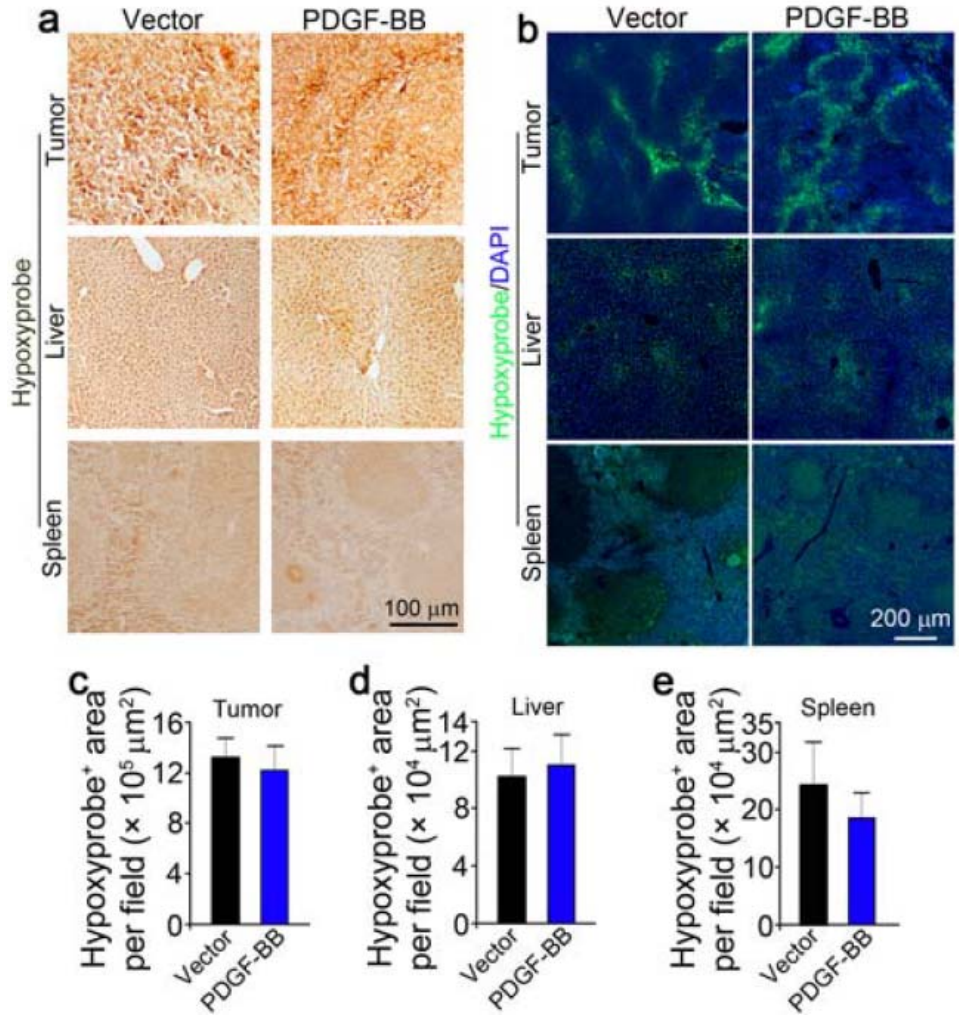
PDGFR-β in physiological EPO maintenance



CAGG-CreER x PDGFR-β $^{flox/flox}$
Global deletion of PDGFR-β by tamoxifen administration

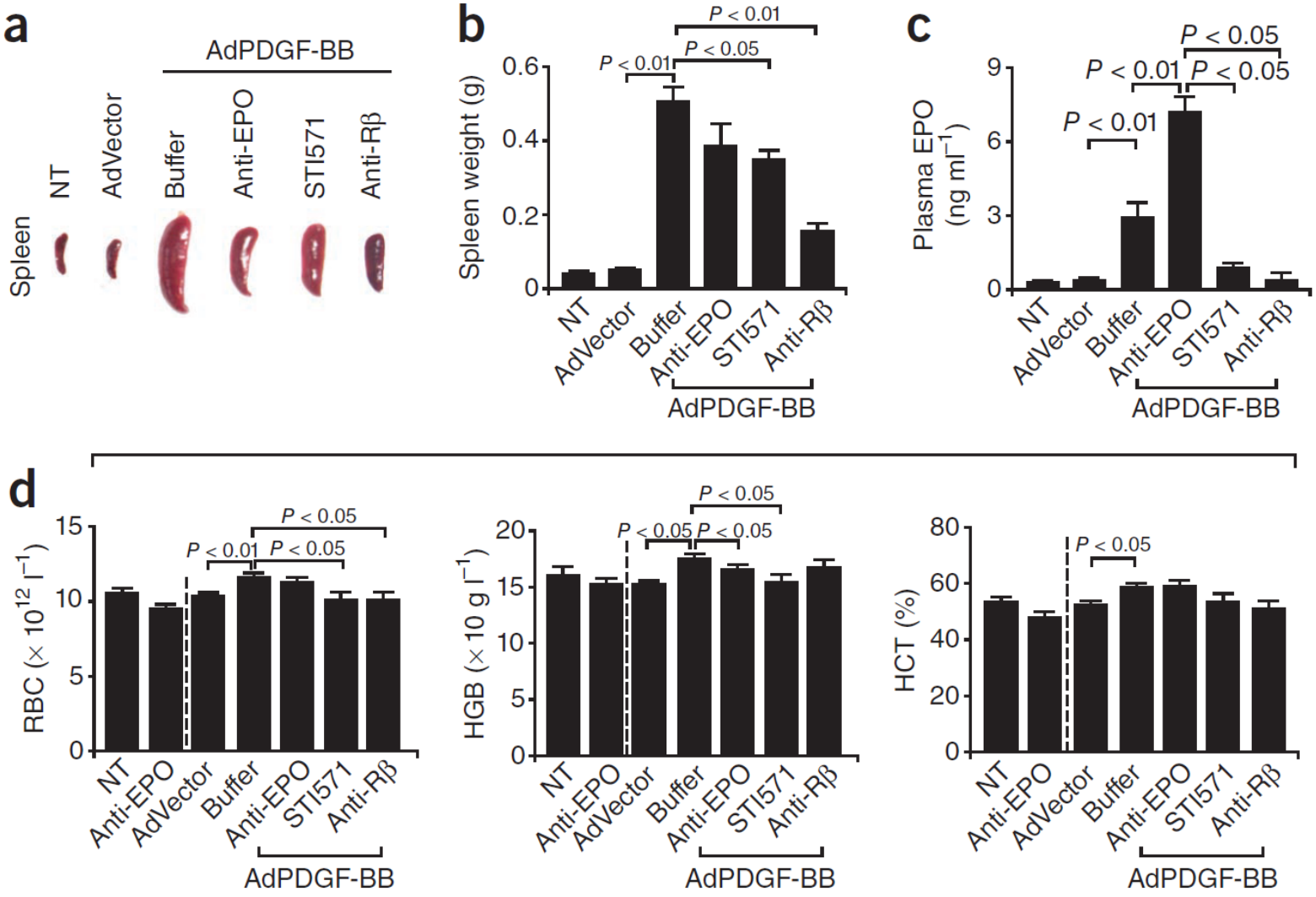
PDGFR-BB signaling through PDGFR-β is required for the physiological maintenance of EPO expression in the spleen, but not in liver or kidney

Hypoxia does not substantially contribute to PDGF-BB-mediated EPO upregulation

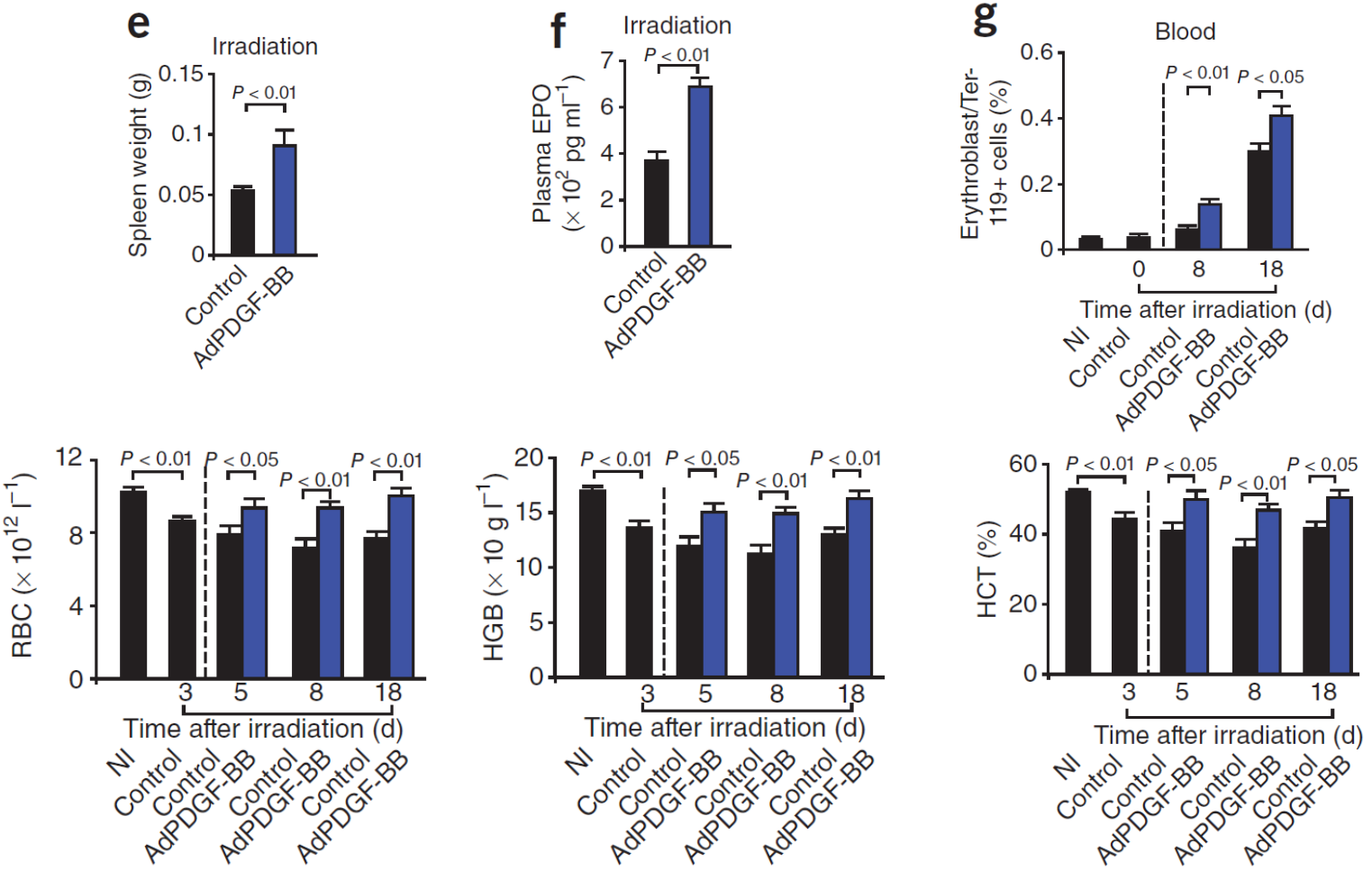


Pimonidazole bind to thiol- containing proteins specifically in hypoxic cells

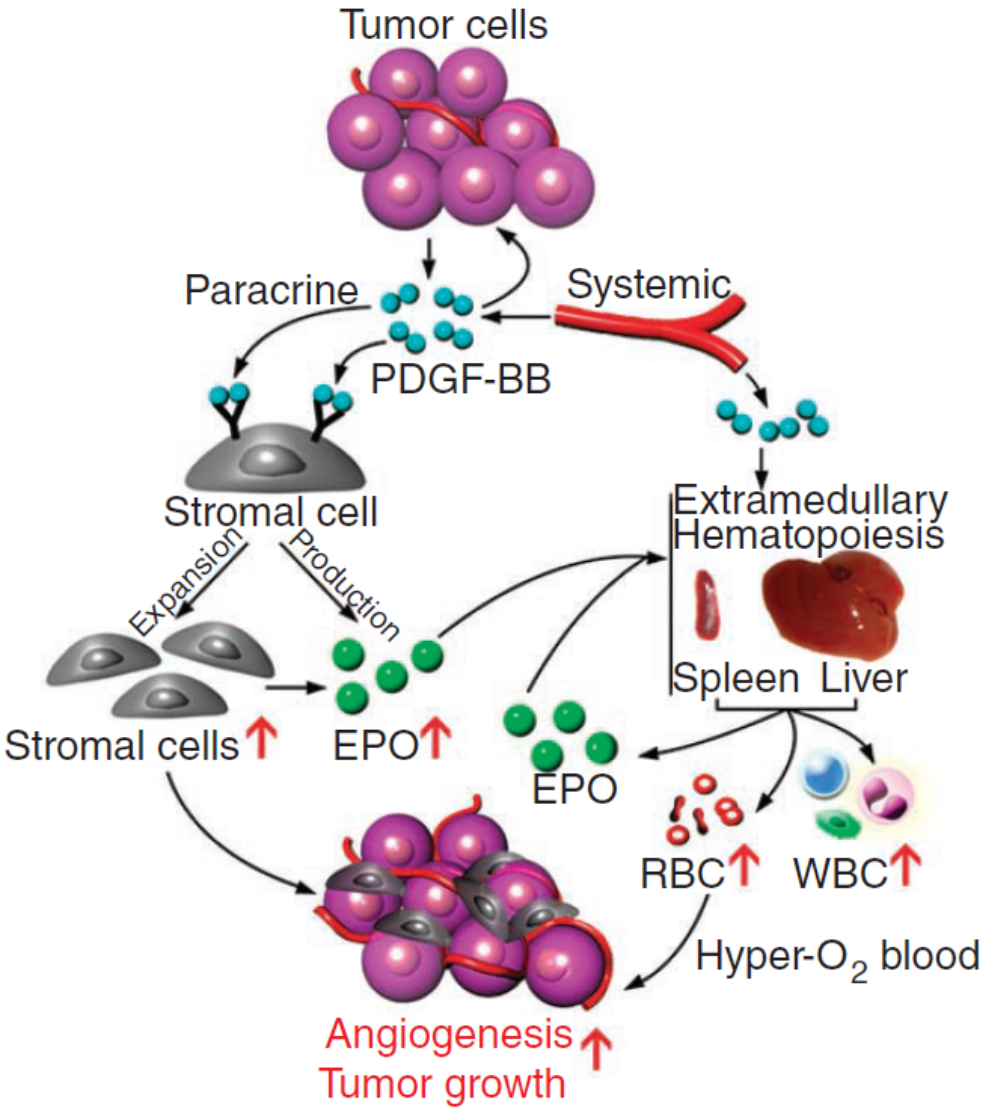
Adenoviral PDGF-BB increased EPO levels and hematopoiesis



Adenoviral PDGF-BB protects against irradiation-induced hematopoietic suppression



Mechanisms of tumor-derived PDGF-BB-induced hematopoiesis, tumor growth and angiogenesis



Conclusion

- **PDGF-BB** acts on stromal cells, pericytes or VSMCs that express PDGFR-β to expand the stromal compartment, leading to enhanced tumor angiogenesis;

- **PDGF-BB** acts on stromal cells, pericytes or VSMCs that express PDGFR-β to activate EPO expression, leading to extramedullary hematopoiesis;

- Combination of PDGF-specific and EPO-specific neutralizing agents for cancer therapeutics.

Research article

Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis

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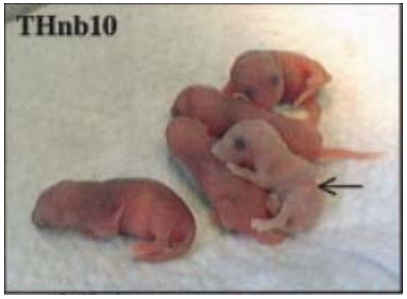
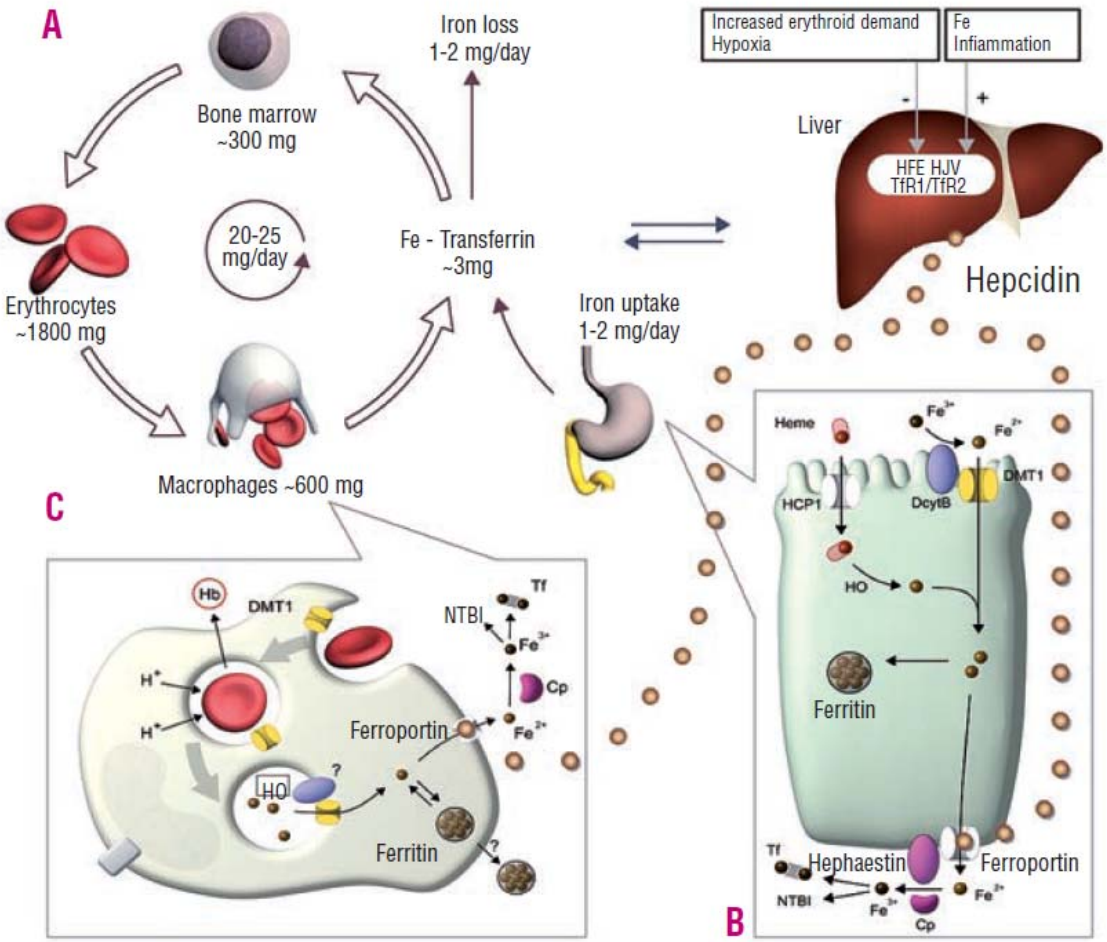
Background

- Iron demand increases when erythropoiesis is stimulated by hypoxia;
- Hepcidin is a **hypoxia**- or **inflammation**- regulated small peptides (25 aa) produced by **hepatocytes**;
- Hepcidin suppresses intestinal iron uptake and release from internal stores by facilitating the internalization and degradation of the only known iron exporter, **ferroportin**, which is expressed in the surface of enterocytes and macrophages;
- Mice transgenically overexpress hepcidin show severe iron-deficiency anemia.

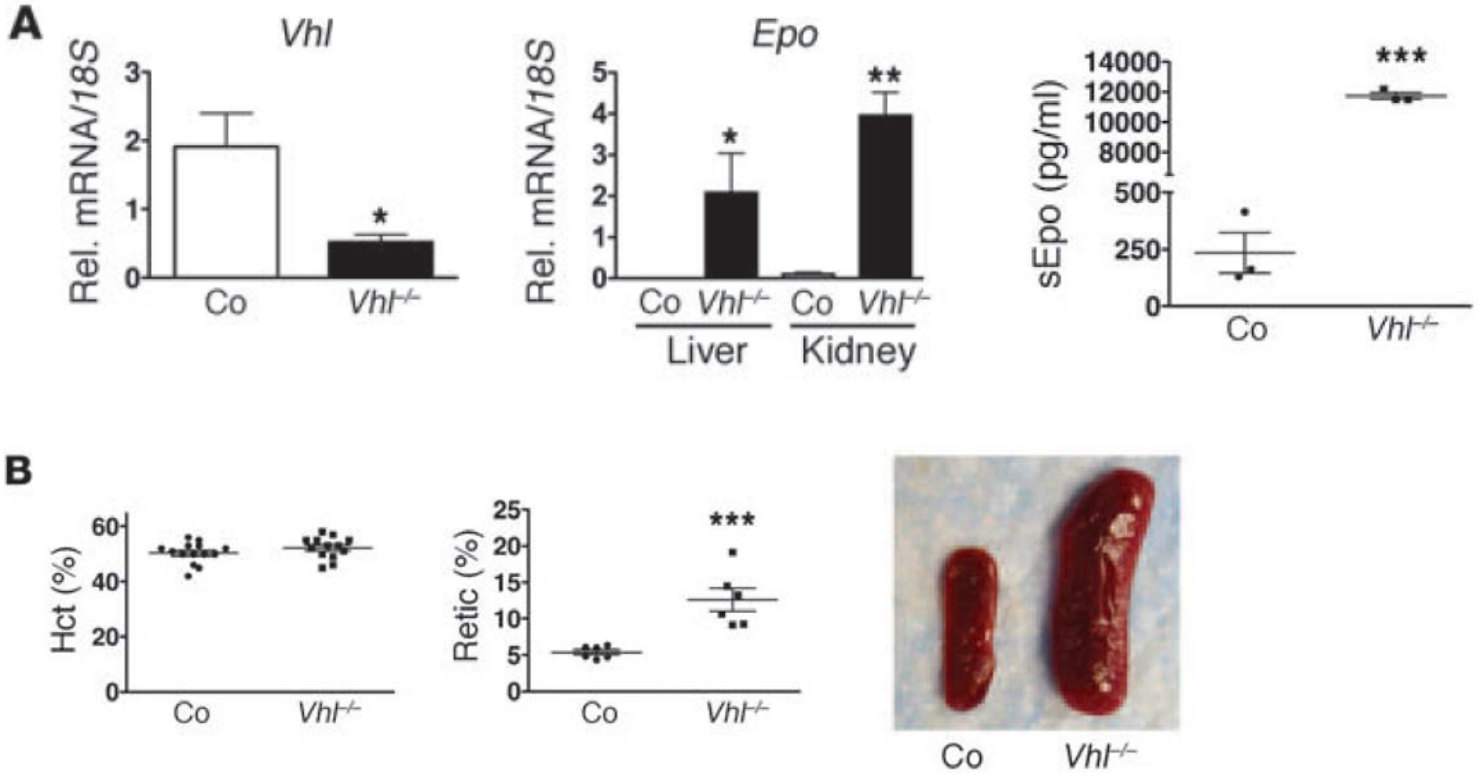


Negative regulation of iron access by hepcidin

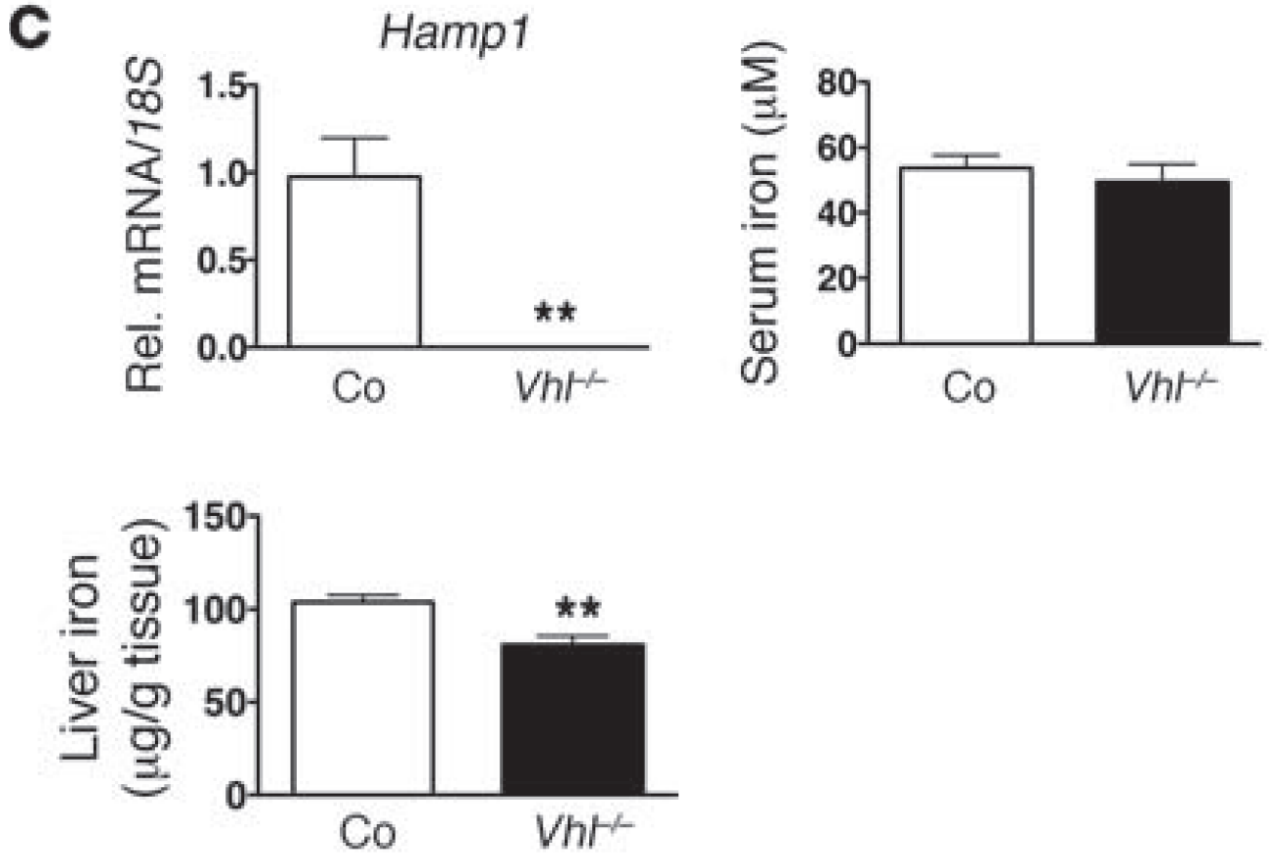


Global inactivation of VHL results in EPO upregulation (HIF target gene) and increased erythropoietic activity

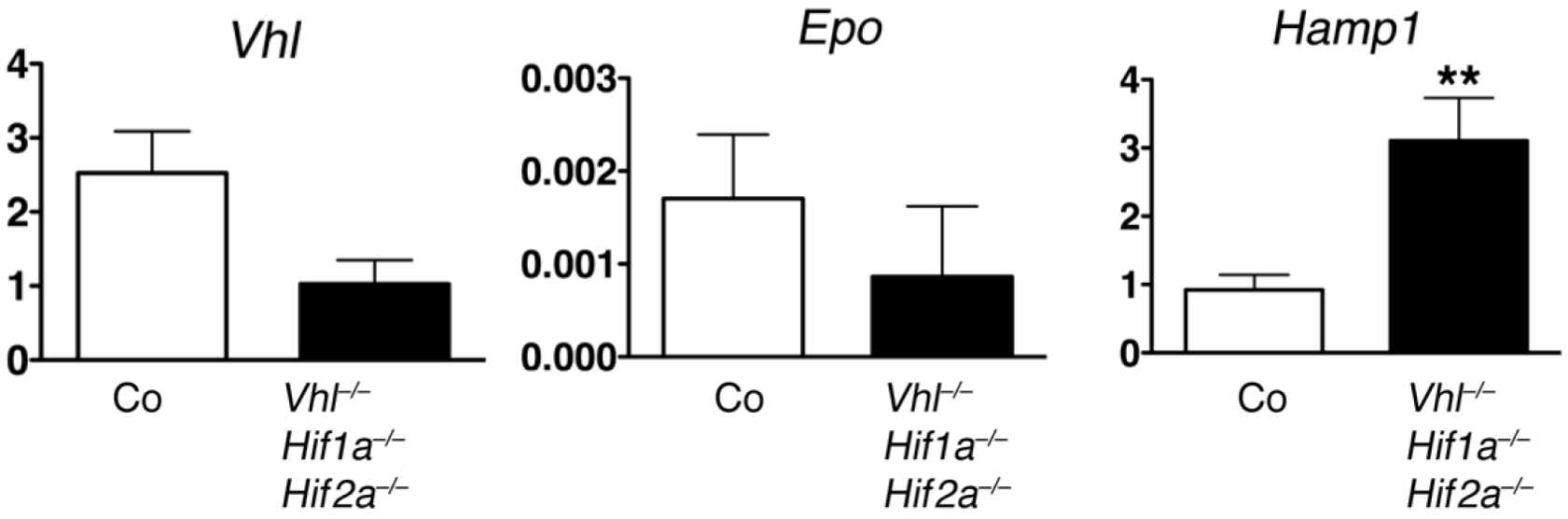
Ubiquitin c promoter - CreERT2



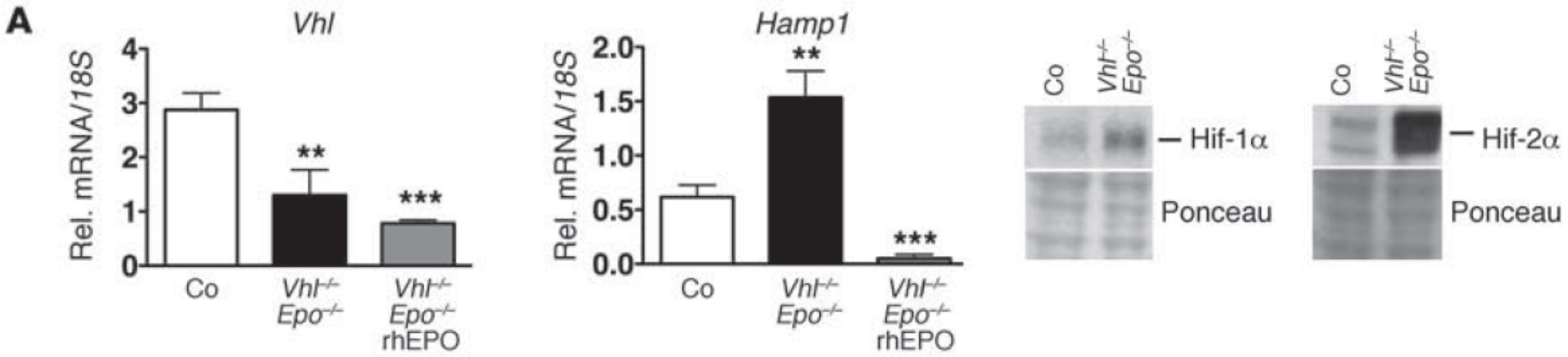
Global inactivation of VHL results in hepcidin suppression and decreased liver iron stores



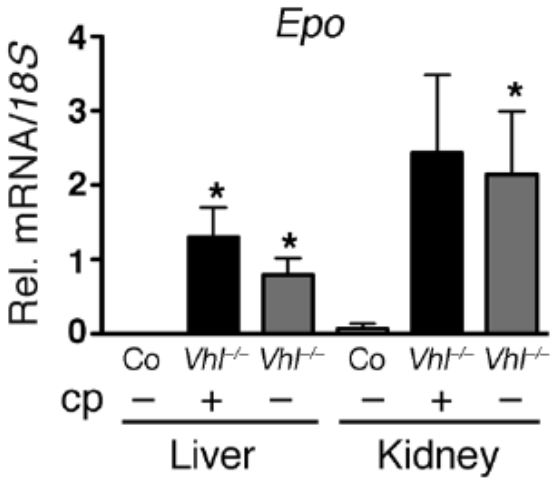
Regulation of EPO and hepcidin by VHL is HIF dependent

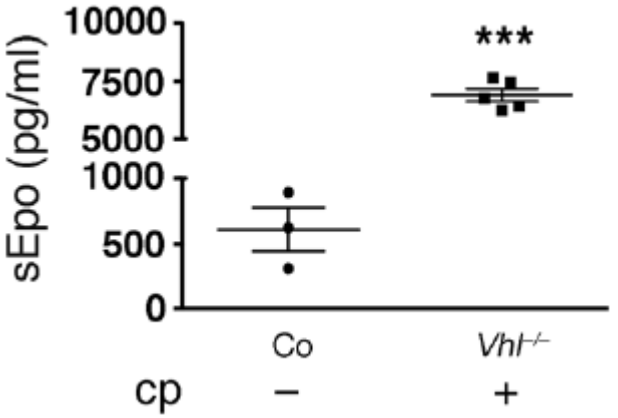


Regulation of hepcidin by VHL is EPO dependent

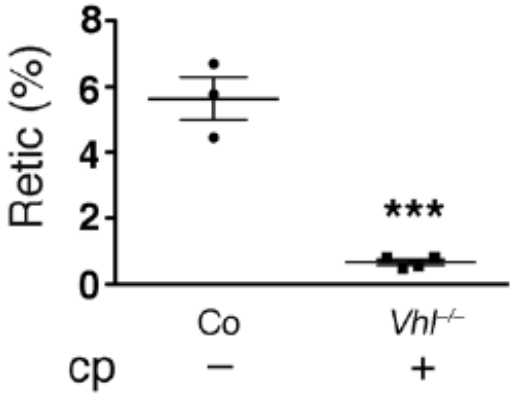


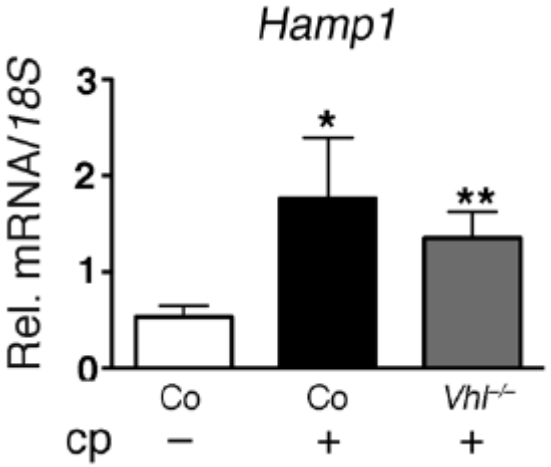
Regulation of hepcidin by VHL requires erythropoiesis



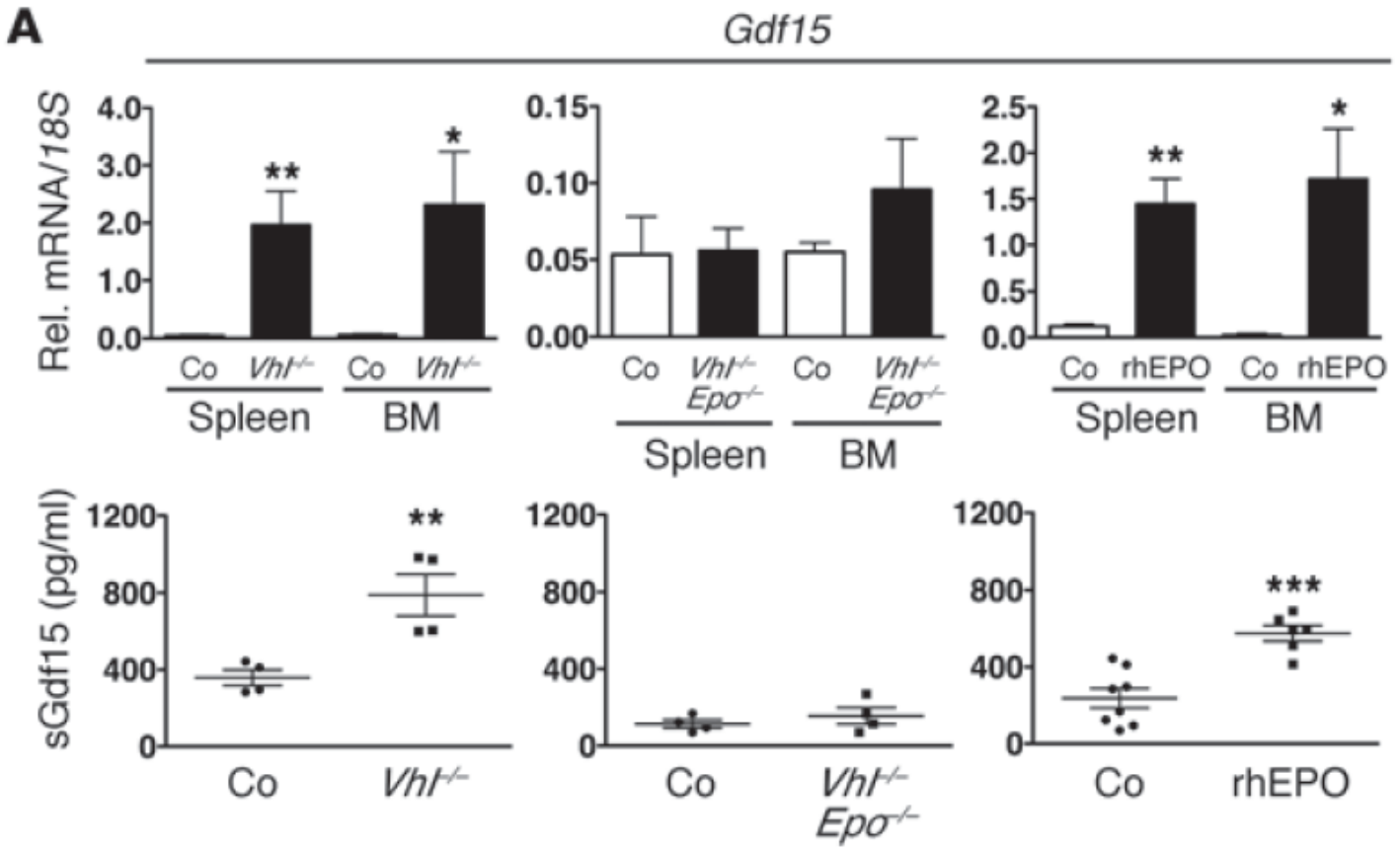


Cp: carboplatin, BM suppression

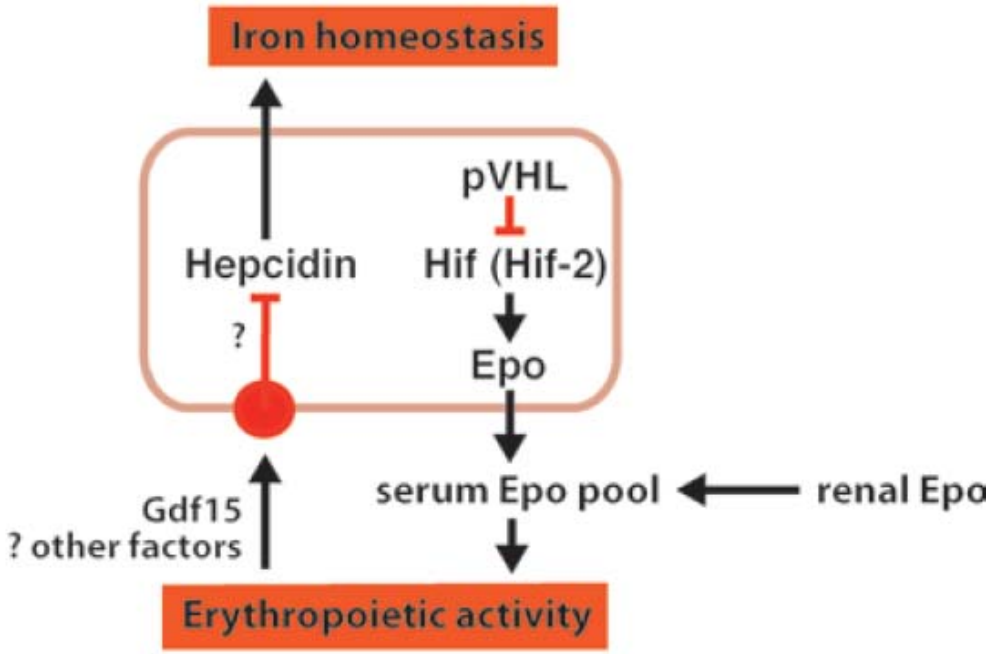




Gdf15 (growth differentiation factor 15) might be a factor that regulates hepcidin expression



Regulation of hepcidin expression by erythropoietin-induced erythropoiesis



Conclusion

- Suppression of hepcidin is not directly regulated by HIF
- Suppression of hepcidin denpends on HIF-induced EPO expression
- Suppression of hepcidin requires EPO-induced erythropoiesis
- Gdf15 may participate in the suppression of hepcidin


Thank you!