Regulation of angiogenesis and tissue perfusion by redox signaling

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Outline

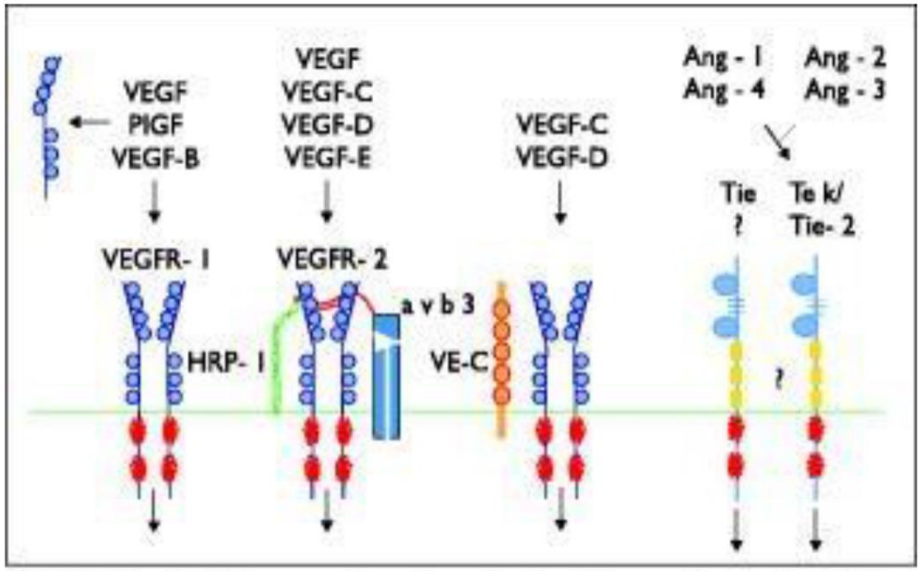
- Angiogenic factors and angiogenesis inhibitors
- Redox regulation of angiogenesis
- Thrombospondin-1, a gatekeeper of nitric oxide signaling
- Therapeutic targeting of the thrombospondin-1 receptor CD47
- Thinking outside the angiogenesis box to understand the effects of endogenous and therapeutic angiogenesis inhibitors in cancer
- Radiation injury stress as a therapeutic target

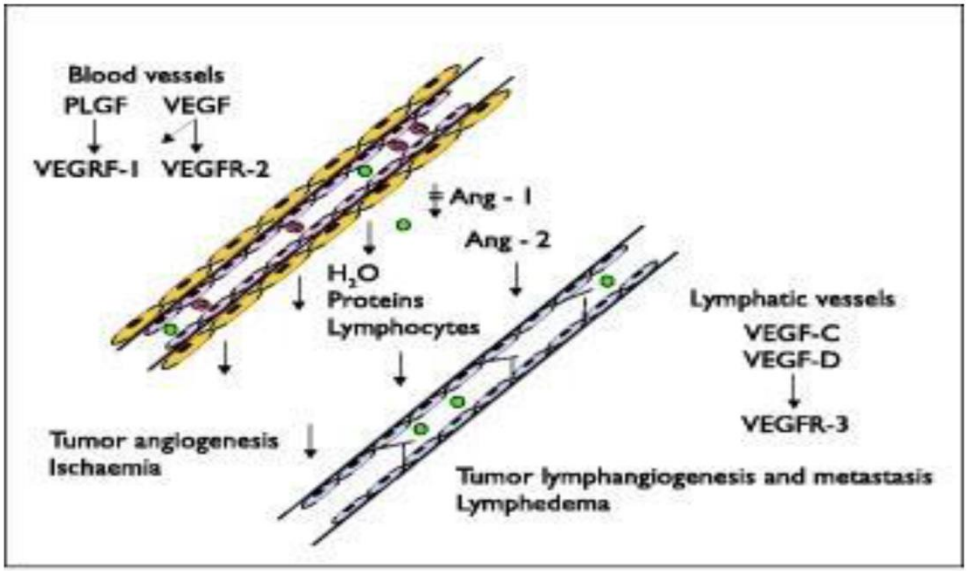
Processes required for developmental and pathological vascularization

- **vasculogenesis**: de novo formation of a vascular plexus
- **arteriogenesis**: defines the direction of blood flow and arborization
- **angiogenesis**: development of a new vessels by branching from an established vascular bed
- **lymphangiogenesis**: establishing lymphatic vessels to drain ultrafiltrate from vascularized tissues

Angiogenic Factors

Vasculogenesis, angiogenesis, and lymphangiogenesis utilize a related family of angiogenic factors





K. Alitalo (2001)

The Angiogenic ‘Balance’

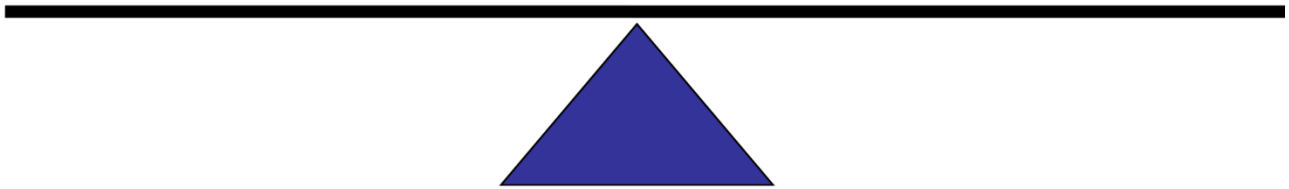
The angiogenic ‘balance’

Angiogenic factors

- VEGFs A-D
- FGF-1,2
- PLGF
- Angiopoietin-1
- IL-8
- Estrogens
- Adrenomedullin
- PDGF

Angiogenesis inhibitors

- Thrombospondins-1,2
- Angiostatin (fragment of plasminogen)
- Endostatin (fragment of coll. XVIII)
- Interferon α
- Vasostatin (fragment of calreticulin)
- TIMP2
- IL-12
- Soluble VEGFR1 (sFlt1)
- Arresten/Canstatin/Tumstatin (Coll. IV)
- Vasoinhibins (prolactin/growth hormone)



Therapeutic angiogenesis inhibitors

- VEGF antagonists: Avastin (bevacizumab) (FDA approved 2004-date for colon Ca, NSCLC, glioblastoma, renal Ca), Ramucirumab(IMC-1121B) (VEGFR2 Ab, phase III)
- VEGF siRNA: Bevasiranib (phase III)
- VEGFR kinase inhibitors: Cediranib/Recentin/AZD2171 (phase III), Vandetanib/AZD6474 (phase III)
- Decoy VEGFR: Aflibercept/AVE0005 (phase III)
- mixed kinase inhibitors: Sunitinib/Sutent (FDA approved 2006), Sorafenib/Nexavar (FDA approved 2005 for renal CC), Votrient (pazopanib HCl, FDA approved Oct 2009), Axitinib (phase III)
- mTOR inhibitor: Torisel (temsirolimus, FDA approved 2007 for renal CC), everolimus (Afinitor)
- integrin antagonists: EMD 121974 (phase II)
- steroids: 2-methoxyestradiol (phase I)
- cytokines: interferon-α (phase II/III)
- thrombospondin-1 mimetic: ABT-510 (phase II)
- fumagillin-based: TNP-470, PPI-2458 (phase I)
- thalidomide analogs: thalidomide (FDA approved 2006, multiple myeloma), CC-5013 (phase I), lenalidomide (FDA approved 2005, multiple myeloma)

http://www.cancer.gov/cancertopics/factsheet/Therapy/angiogenesis-inhibitors
Nature Biotechnology 26, 1055 - 1056 (2008)

Angiogenesis Inhibitors

Hypertensive and prothrombotic activities of angiogenesis inhibitors

Drug	Side-effect (incidence)	Study design and comments	Refs
Bevacizumab	Hypertension (35%)	154 patients: 20% new onset and 80% an exacerbation of pre-existing hypertension	1
Bevacizumab	Hypertension (15%) Thrombosis (6%)	Phase II trial in unresectable hepatocellular carcinoma	180
Bevacizumab	Venous thromboembolism (11.9%)	Meta-analysis of 7,956 patients from 15 randomized controlled trials	181
Bevacizumab Sorafenib Sunitinib	Hypertension (23–34%) Hypertension (11–75%) Hypertension (19%)	Review of clinical data for several cancers	182
Sorafenib	Hypertension (23.4%)	Meta-analysis of 4,599 patients with renal cell carcinoma	2
Sorafenib Sunitinib	Hypertension and proteinuria (7 patients)	Noted to resemble pre-eclampsia	183
Cediranib	Acute hypertension in rats	NO signalling proposed to be responsible	6
Telatinib	Hypertension and proteinuria (18 patients)	Phase I trial noted decreased response to nitroglycerin	184
Various inhibitors	Haemostasis and thrombosis	Review of clinical study and trial data	3
Various inhibitors	Hypertension and proteinuria	Editorial proposes key role for NO depletion in renal vasculature	120

NO, nitric oxide

Isenberg et al , *Nature Rev Cancer* 2009

Cardiac dysfunction

Cardiac dysfunction caused by angiogenesis inhibitors

Table 1 Summary of the characteristics and cardiovascular side effects of the main antiangiogenic drugs

Drug	Area of application	Targets	Adverse effects	Frequency	Mechanism	Reversibility
Bevacizumab	Advanced lung, breast, colon–rectum carcinoma	Circulating VEGF	Contractile dysfunction/HF	Low	Hypertension	Reported
			Hypertension	Moderate, dose dependent	Endothelial dysfunction	Unknown
			Thrombo-embolism	Moderate	Endothelial dysfunction	Variable
Sunitinib	Renal cell cancer, GIST	VEGFR, PDGFR, c-Kit, CSF-1R, FLT3, RET, and >50 others	Contractile dysfunction/HF	Low	Mitochondrial dysfunction	Partial
			Hypertension	Moderate, dose dependent	Endothelial dysfunction	Unknown
			Thrombo-embolism	Moderate	Endothelial dysfunction	Variable
			Arrhythmia/QT prolongation	Rare	HERG K+ blockade	Unknown
Sorafenib	Renal cell cancer, hepatocarcinoma	VEGFR, PDGFR, c-Kit, FLT3, Raf-1/B-Raf and >15 others	Contractile dysfunction/HF	Rare	Cell signalling, survival block	Unknown
			Hypertension	Moderate, dose dependent	Endothelial dysfunction	Unknown
			Thrombo-embolism	Moderate	Endothelial dysfunction	Variable

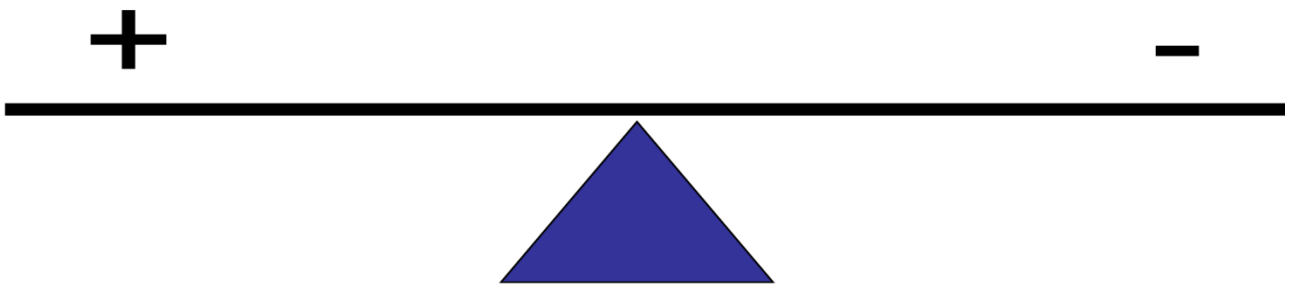
Modified from Cheng and Force[6] and Suter and Ewer.[35]

Tocchetti Eur J Heart Fail. 2013 May;15(5):482-9

Redox Signaling

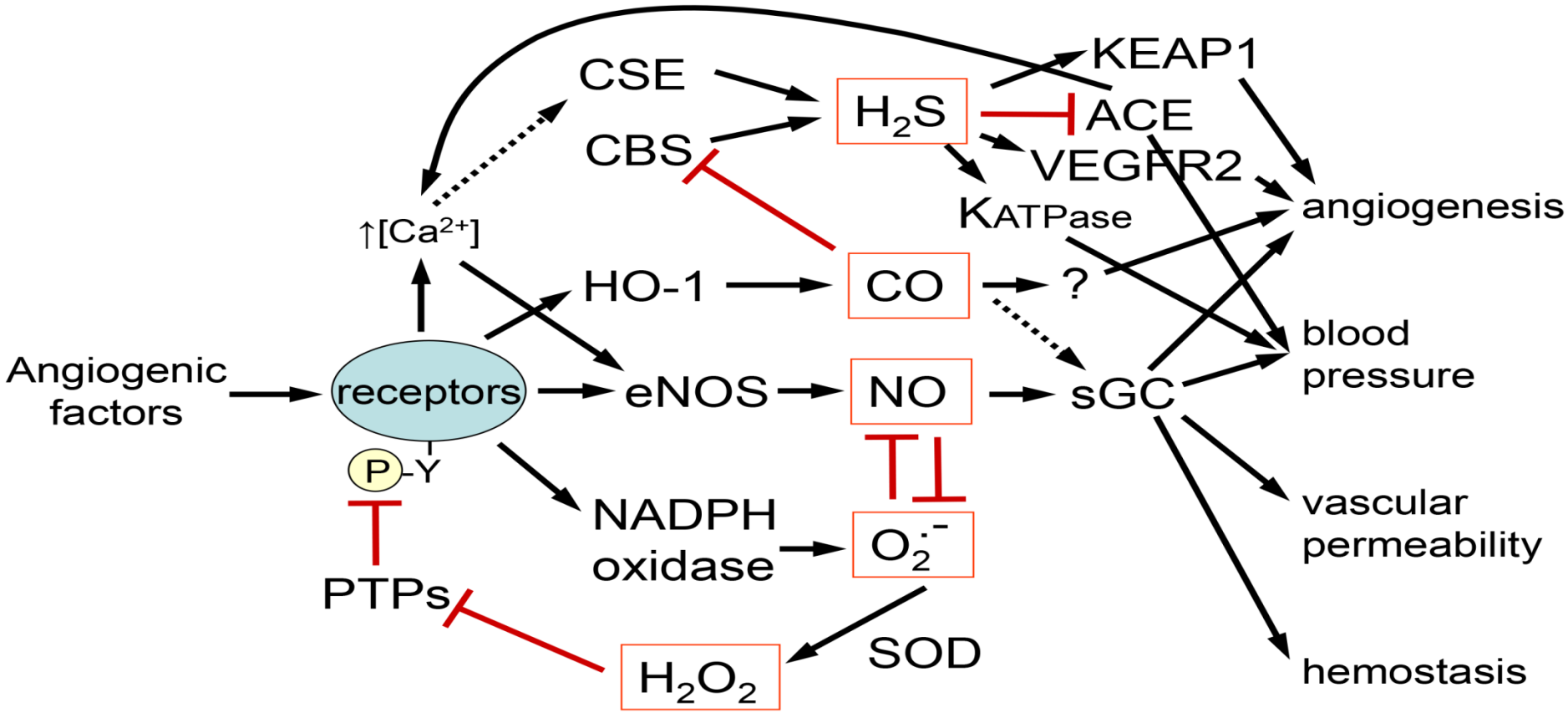
Roles of redox signaling in the angiogenic balance

- Direct effects on angiogenesis
- Interactions with angiogenic factors
- Interactions with angiogenesis inhibitors



Vascular Signaling

Roles of gasotransmitters in vascular signaling

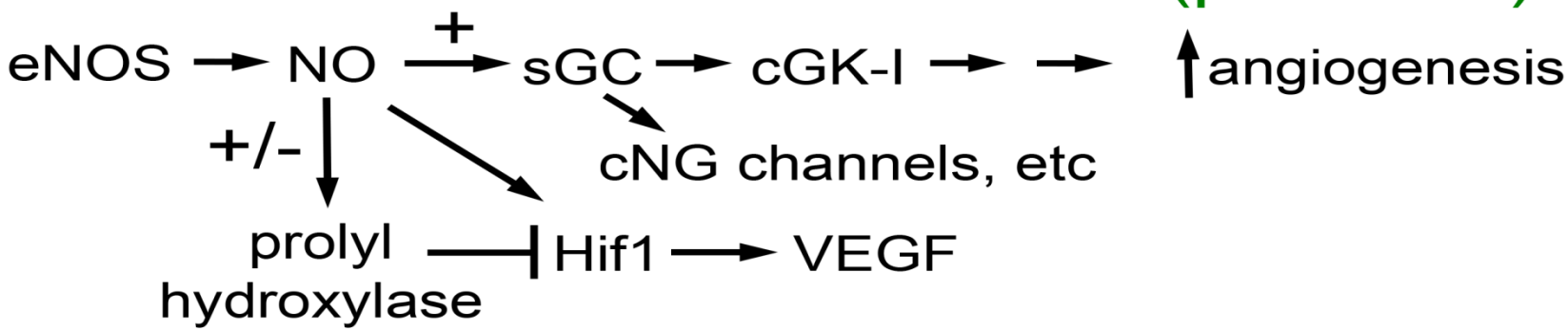


Miller et al *Chem Rev* 2009.

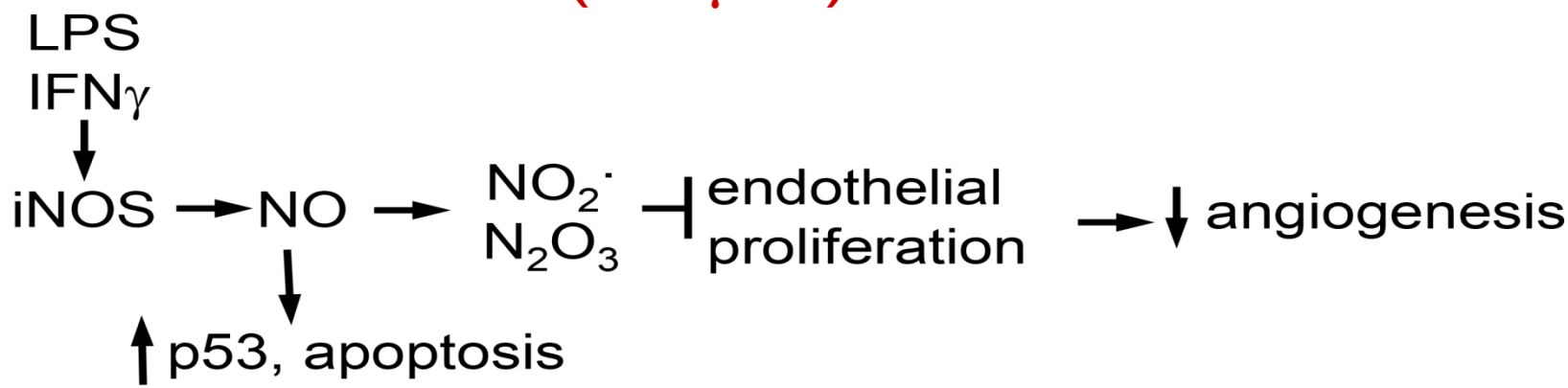
Signaling Thresholds

Signaling thresholds account for the biphasic effects of NO on angiogenesis

Low to moderate concentrations (pM – nM)



High concentrations (~1 μM)



Mitogenic H2O2 Signaling vs Oxidative Stress

Mitogenic H_2O_2 Signaling vs Oxidative Stress

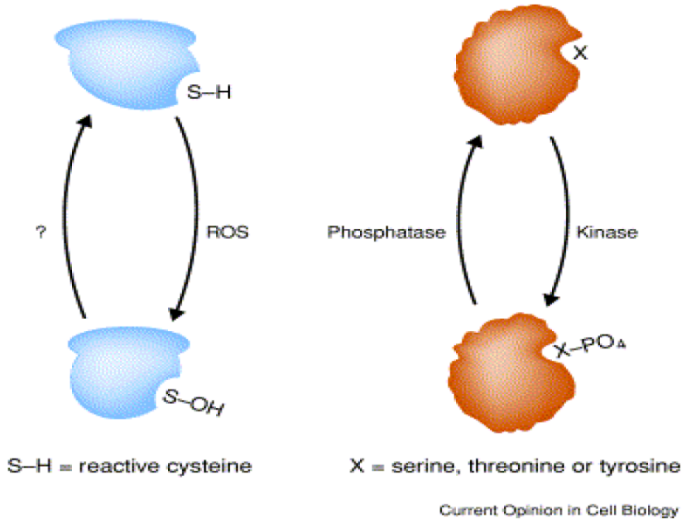
Applied $[H_2O_2]$ (μM)	0.1	1	10	100	1000
Intracellular $[H_2O_2]$ (μM)	0.01	0.1	1	10	100
Biologic Response	[-----Proliferation -----]		[-Growth Arrest / Cell Death-]		
Signaling Pathways Affected	hnRNP-C1/C2		NF-κB AP-1 Tyrosine Kinases Tyrosine Phosphatases MAP Kinases		

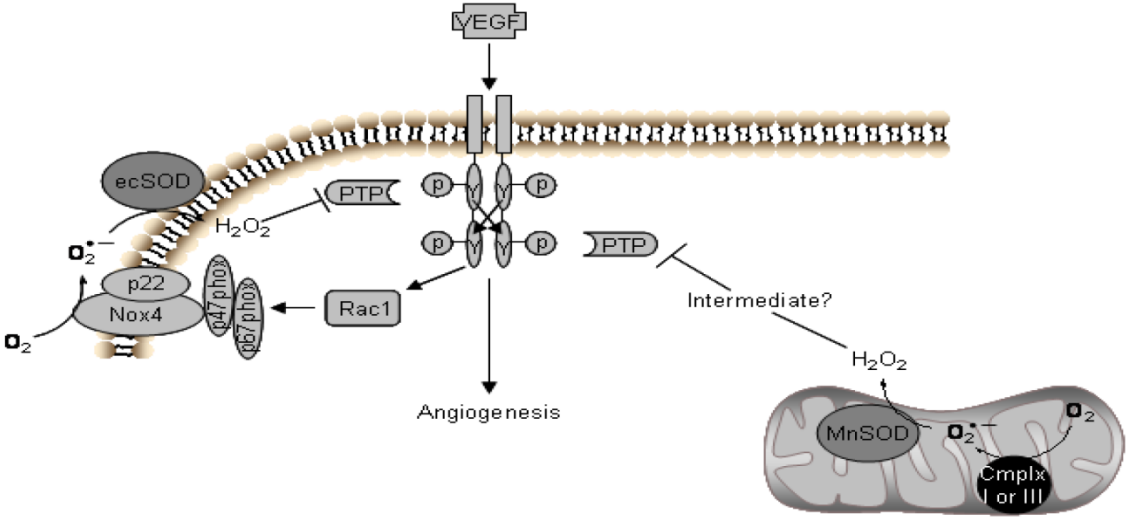
Stone & Collins, Endothelium. 2002 9:231-8

TYR-Kinase Receptors

H_2O_2 as a second messenger for Tyr-kinase receptors

reversible inactivation of protein tyrosine phosphatases

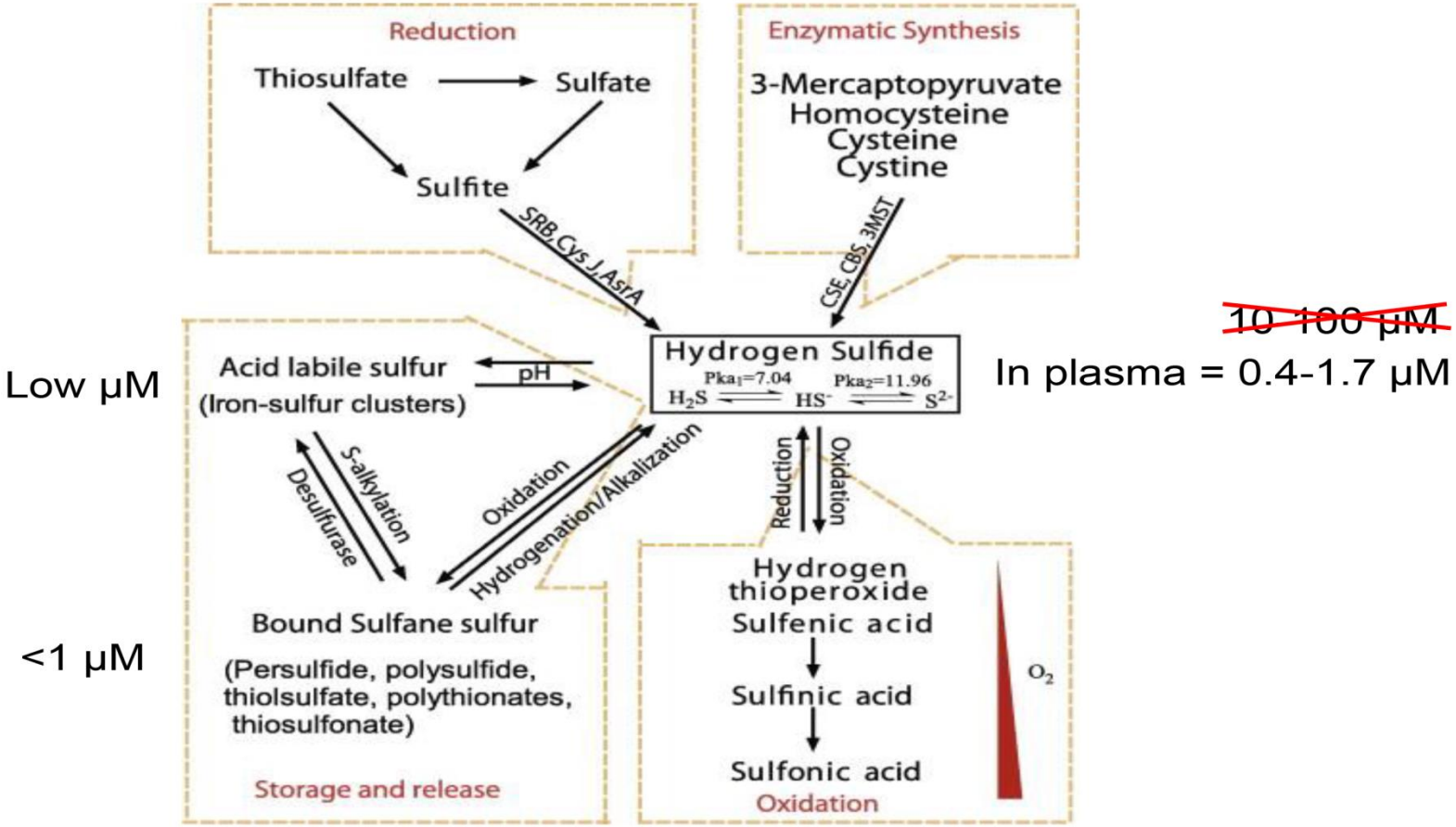




Finkel, *Curr Opin Cell Biol.* 15:247-54, 2003; Dev Cell. 2:251-2, 2002; Miller *Chem Rev* 2009.

Hydrogen Sulfide

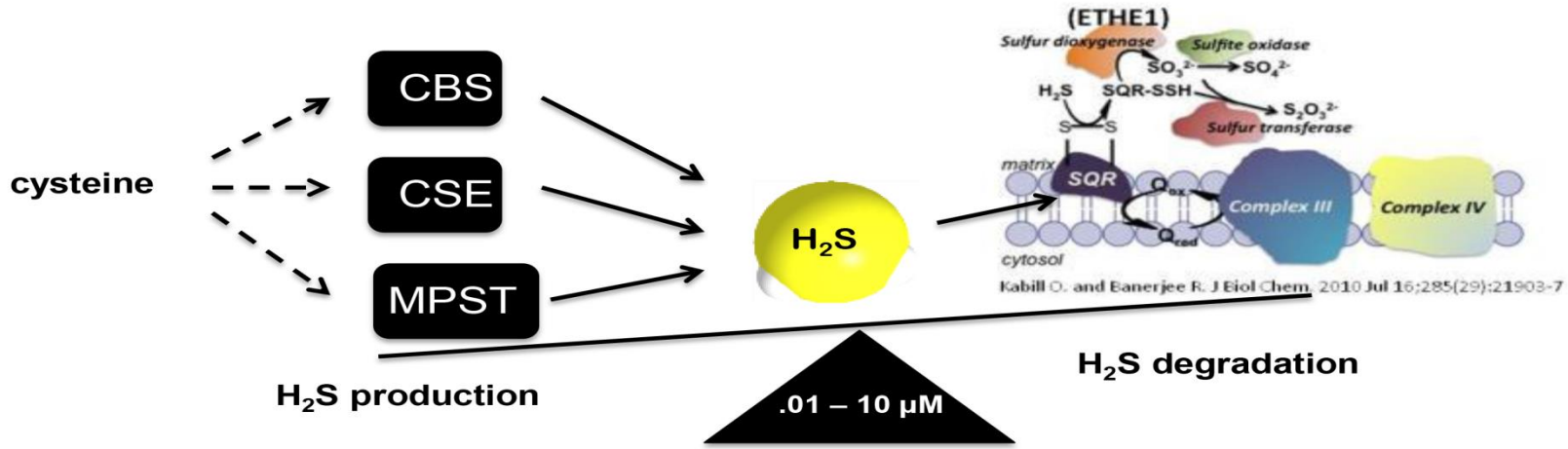
Hydrogen Sulfide (H_2S)



Kolluru, Nitric Oxide. 2013 Nov 30;35:5-20

Hydrogen Sulfide Signaling

H_2S signaling



Biochemical Targets:

- Metal ligation
  - Fe^{3+}, Zn^{2+}
- Thiol modification of glutathione and sulfhydration of protein thiols
  - CysSSH, CysSSSCys

→

Signaling targets:

VEGFR2
KEAP1
PTEN
NFkB p65
Actin
Tubulin
K^+ channels
IK(Ca) K^+ channel
SK(Ca) K^+ channel

→

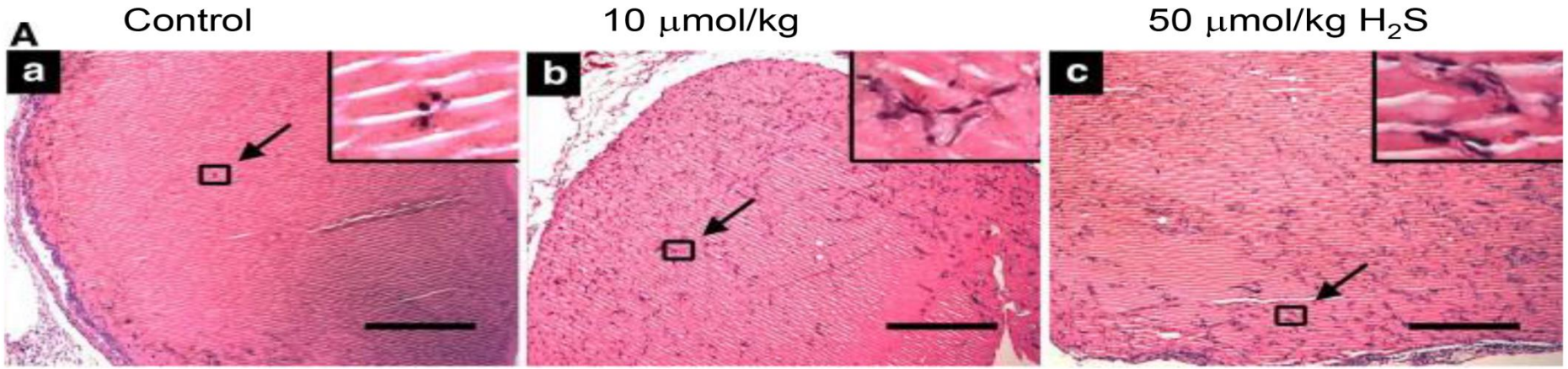
Physiological Functions:

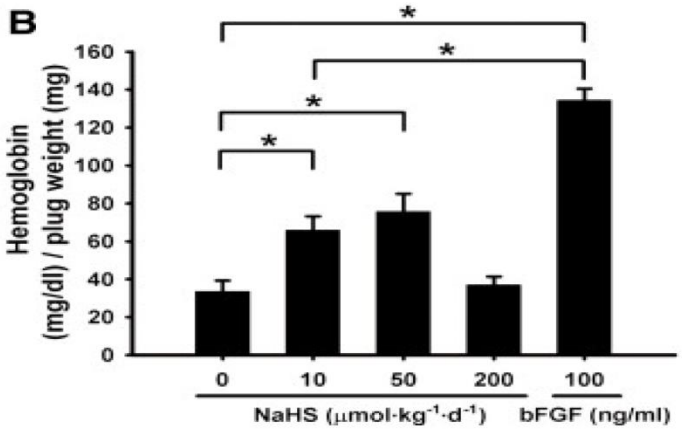
Neuromodulation
Metabolic hibernation
I/R injury protection
Oxygen sensing
Vasodilation
Angiogenesis
Pro/anti-inflammatory
T cell activation

Greiner *Antioxid Redox Signal* 2013, Paul & Snyder *Nat Rev MCB* 2012; Ida *PNAS* 2014

Angiogenesis

H_2S stimulates angiogenesis

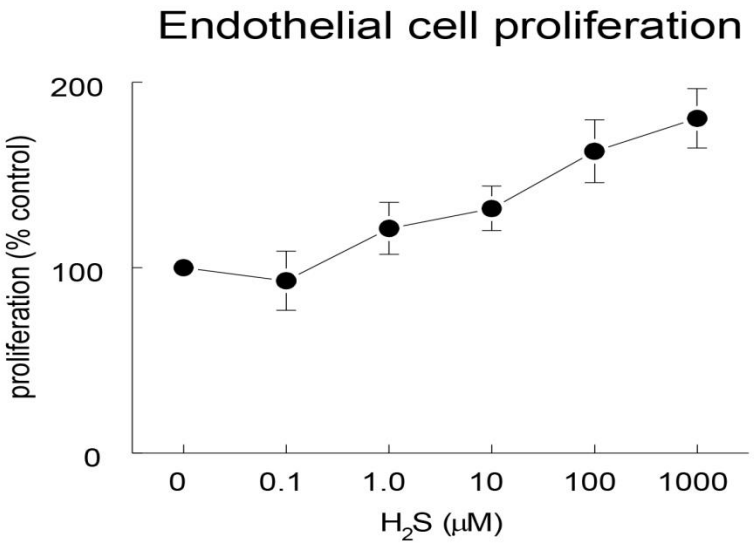


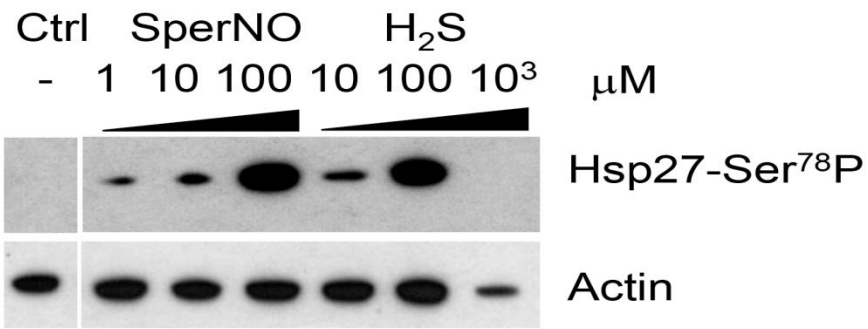


Cai, Cardiovasc Res. 2007 76:29-40

Hydrogen Sulfide Responses

H_2S responses in endothelial cells

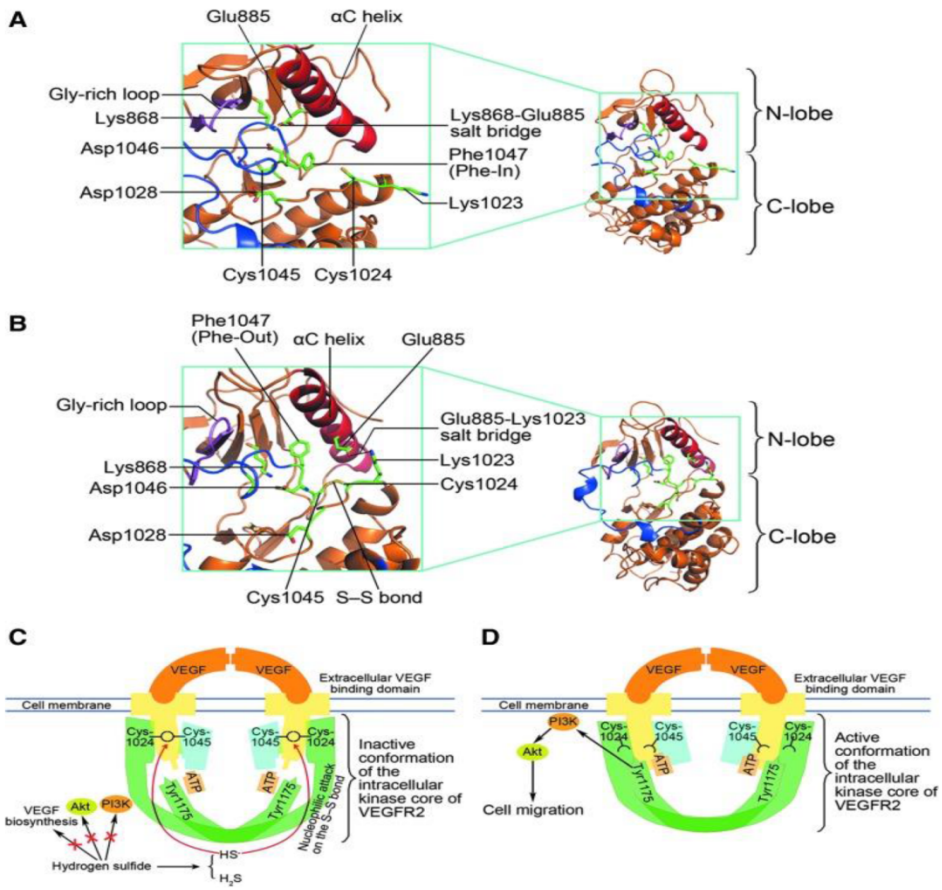




Isenberg *Br J Pharmacol*, 2007

VEGF Receptor-2

VEGF receptor-2 is an H_2S target



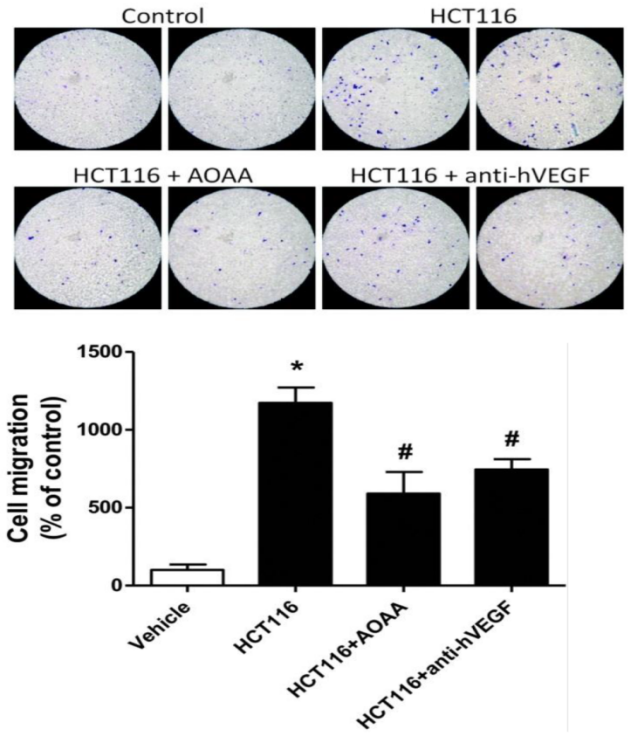
- VEGFR2 knockdown inhibits H_2S-induced endothelial cell migration
- mutant VEGFR2 (C1045A) devoid of the Cys1045-Cys1024 disulfide bond has elevated kinase activity
- siRNA knockdown of cystathionine γ-lyase attenuates VEGF-induced endothelial cell migration

Tao Antioxid Redox Signal. 2013 Aug 10;19(5):448-64

Driving Angiogenesis

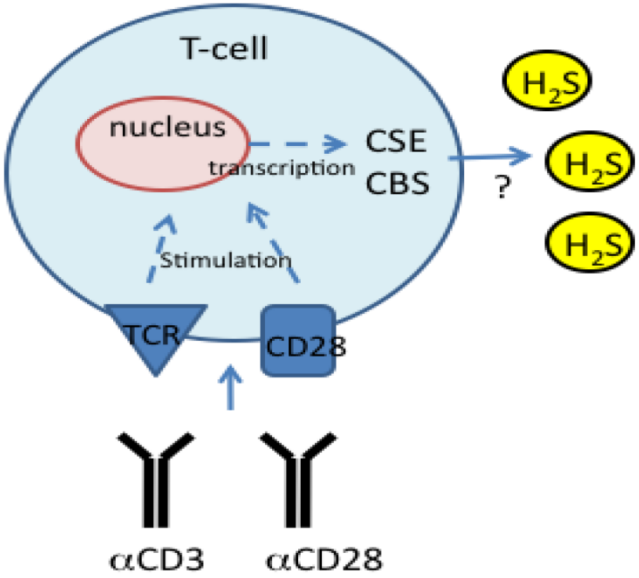
Sources of H_2S to drive angiogenesis

Cancer cells



Hellmich Antioxid Redox Signal. 2014 Jun 20. [Epub]

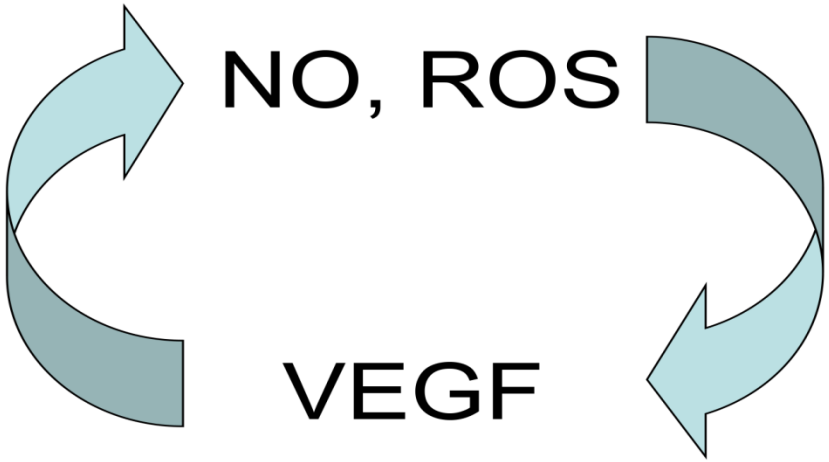
Activated T cells



Miller, J Biol Chem. 2012 Feb 3;287(6):4211-21

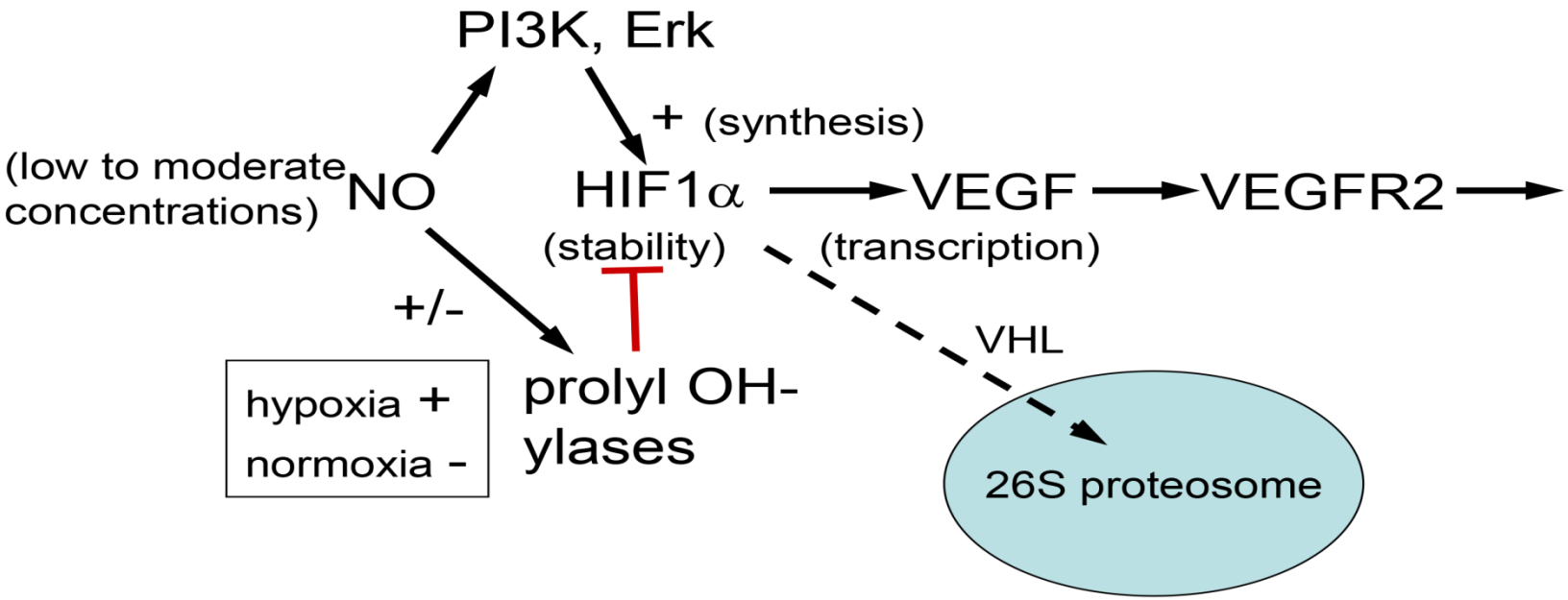
Redox and VEGF

Redox and the angiogenic factor VEGF



Pro-Angiongenic Activities

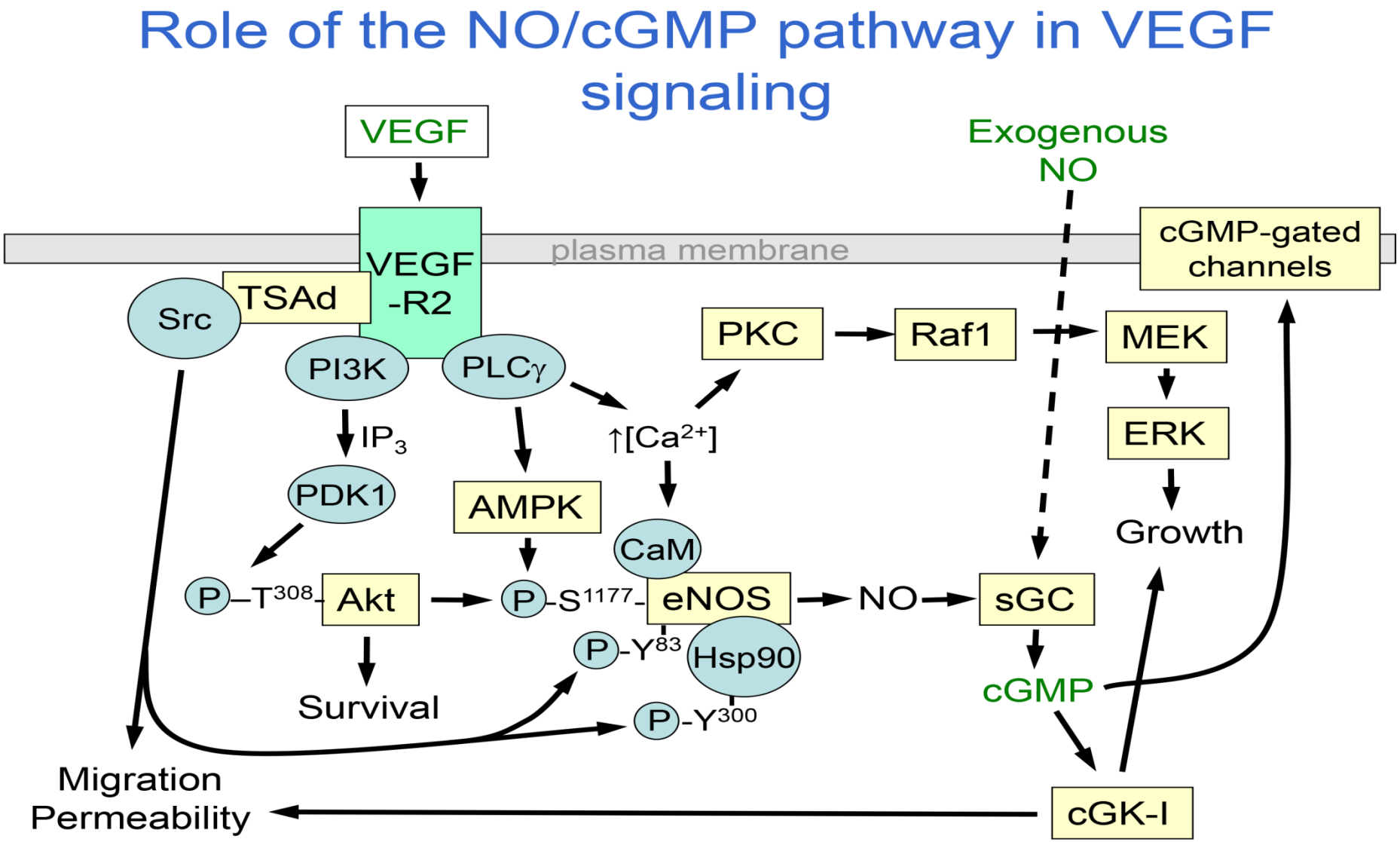
Pro-angiogenic activities of NO through induction of VEGF



Kasuno *J Biol Chem* 279:2550, 2004

Callapina, *Free Radic Biol Med*. 39:925-36 2005.

Role of NO/cGMP



Redox and Angiogenic Balance

Redox and the angiogenic balance

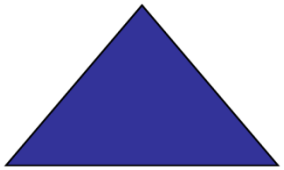
Angiogenic factors

- VEGFs A-D
- VEGFR2,R3
- FGF-1,2
- Angiopoietins-1,2
- Tie2
- IL-8
- Estrogens
- PDGF-B
- Adrenomedullin

Angiogenesis inhibitors

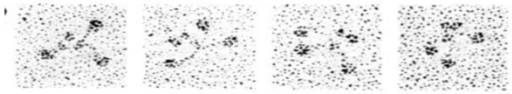
- Thrombospondins-1,2
- Angiostatin (frag. of plasminogen)
- Endostatin (frag. of coll. XVIII)
- Interferon α
- Vasostatin (frag. of calreticulin)
- Fumagillin/TNP470
- IL-12
- NSAIDs

= Redox targets &/or regulators



Thrombospondin-1

The first endogenous angiogenesis inhibitor: thrombospondin-1



TSP1 (nM) 100

10

1

0.1

0.01

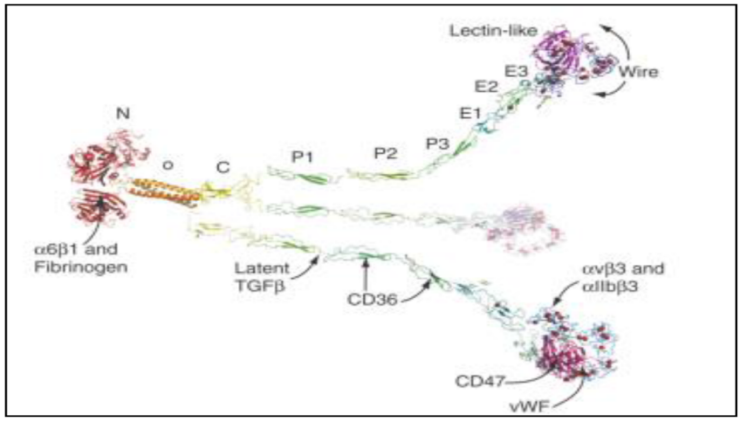
Wounds
Inflammation
Diabetes
Aging

Cancer

Acute & chronic inflammation, acute hemostasis

Normal cells in serum

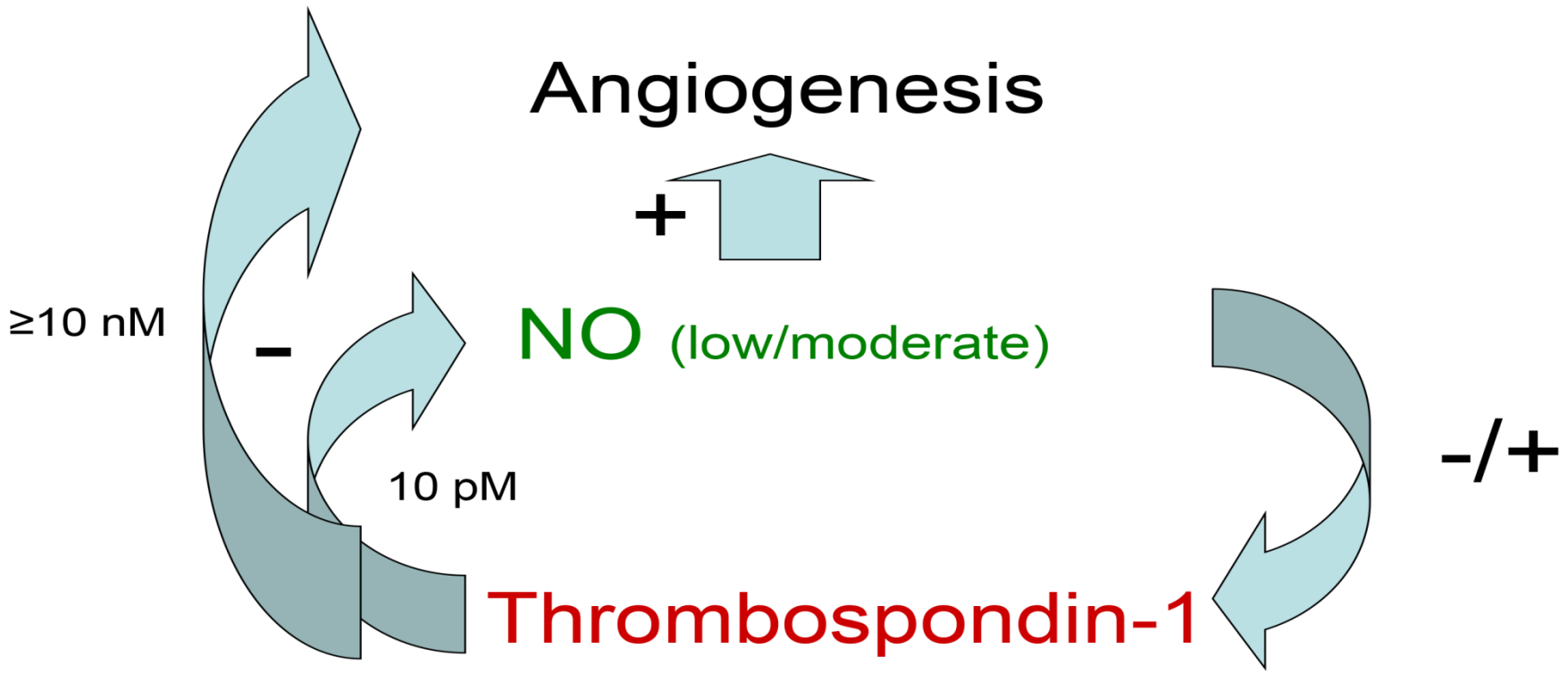
Tumor cells



} Normal plasma TSP1

Thrombospondin-1 Inhibition

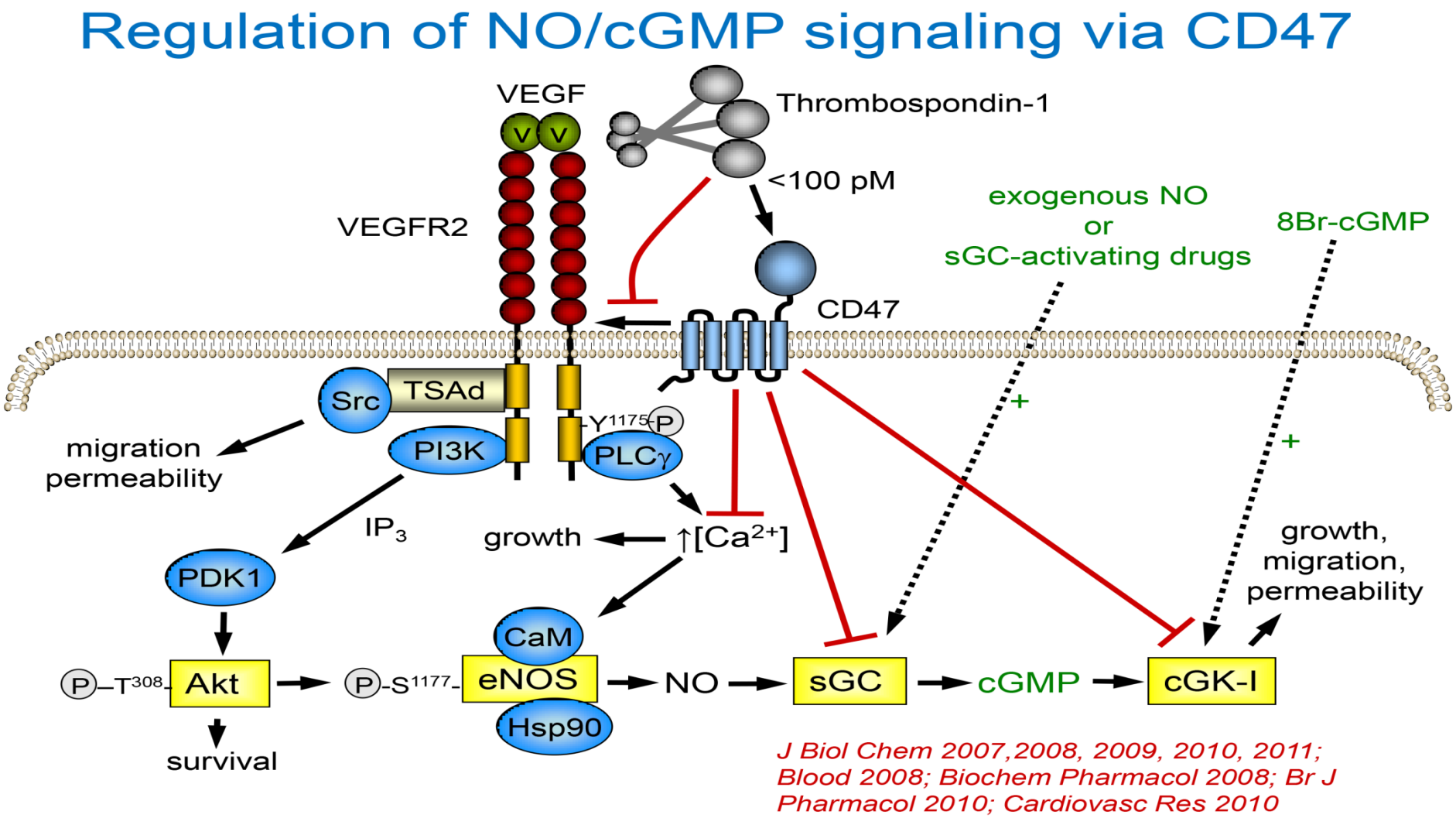
NO sensitizes endothelial cells to thrombospondin-1 inhibition



Isenberg et al, *PNAS* 2005

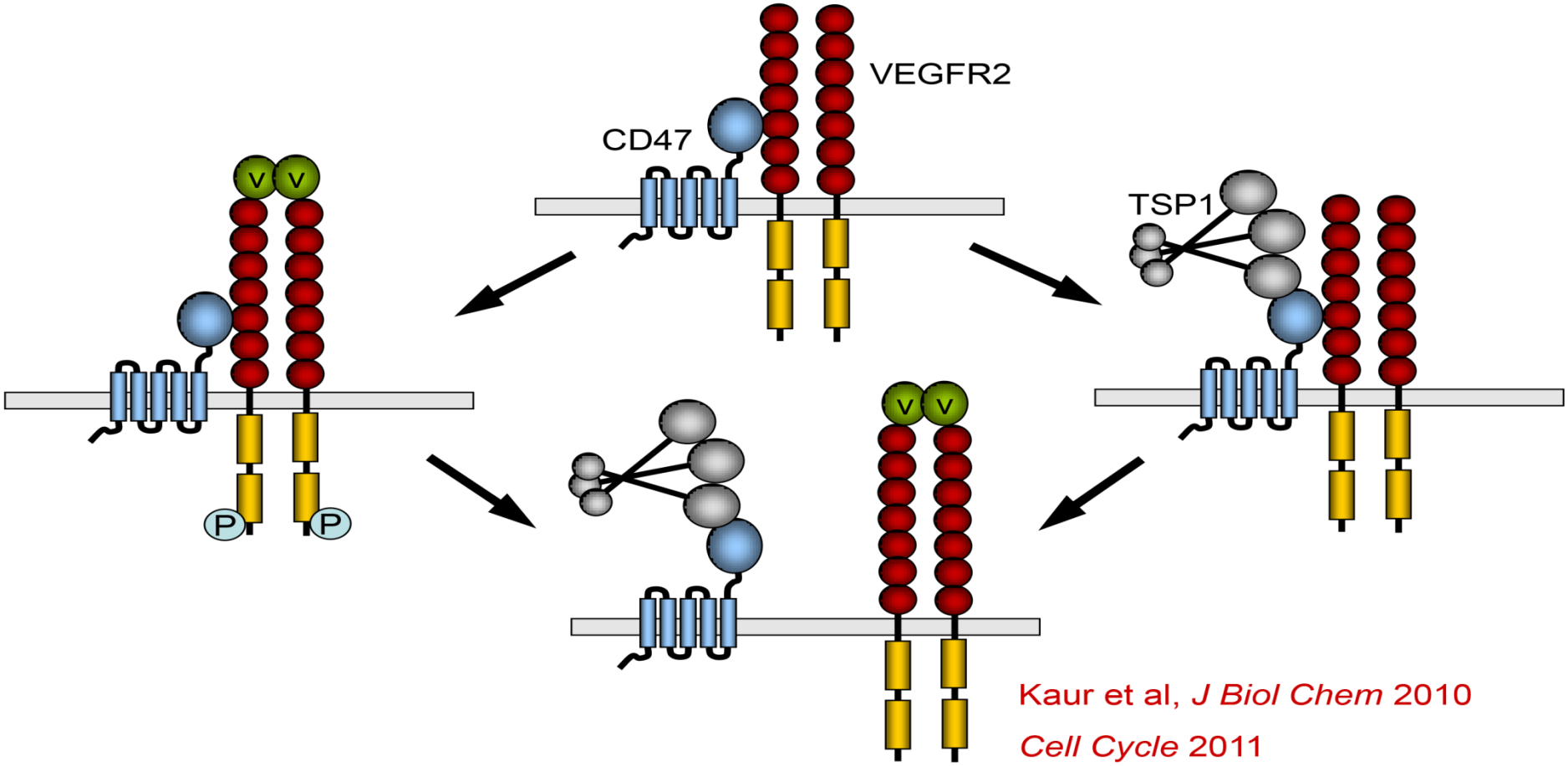
Ridnour et al *PNAS* 2005

Regulation via CD47



TSP1

TSP1 dissociates CD47 from VEGFR2 in the presence of VEGF and inhibits phosphorylation

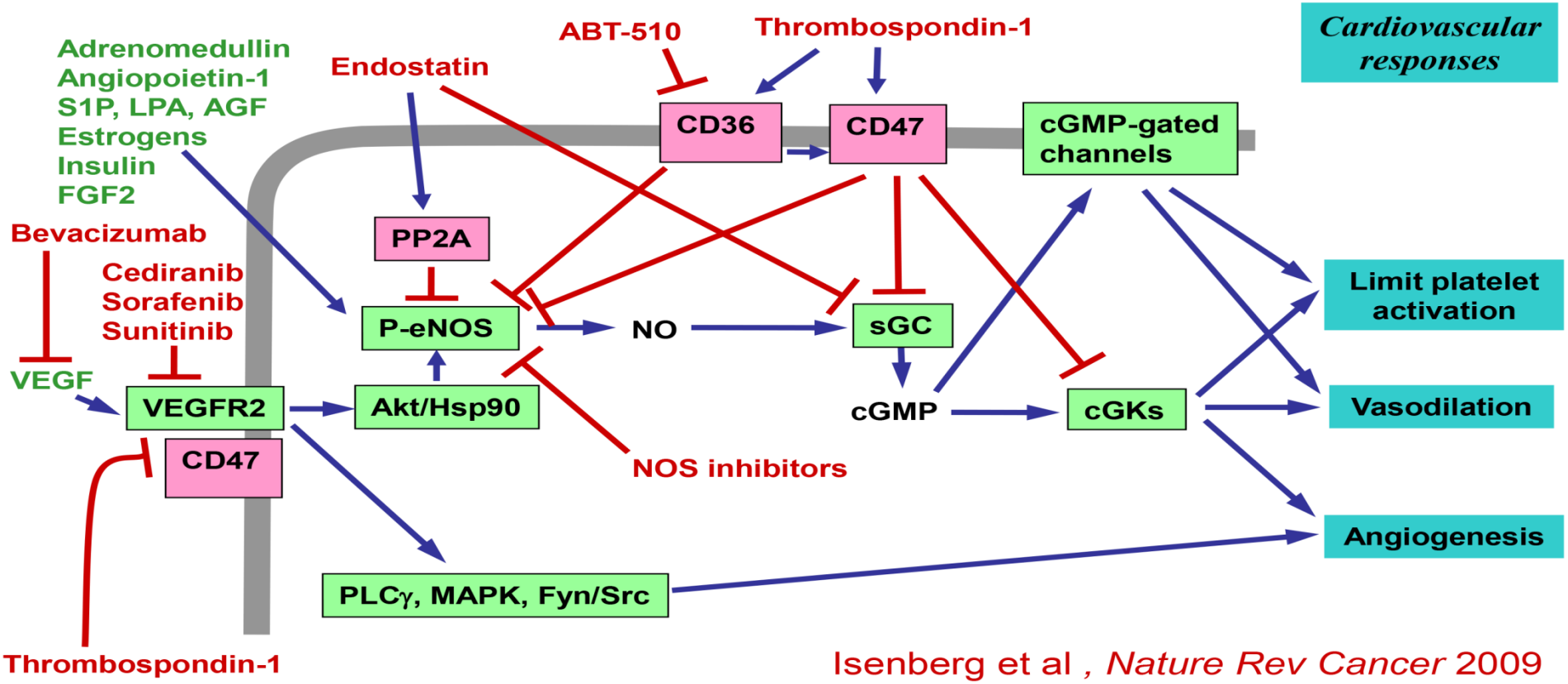


Kaur et al, *J Biol Chem* 2010

Cell Cycle 2011

NO/cGMP signaling

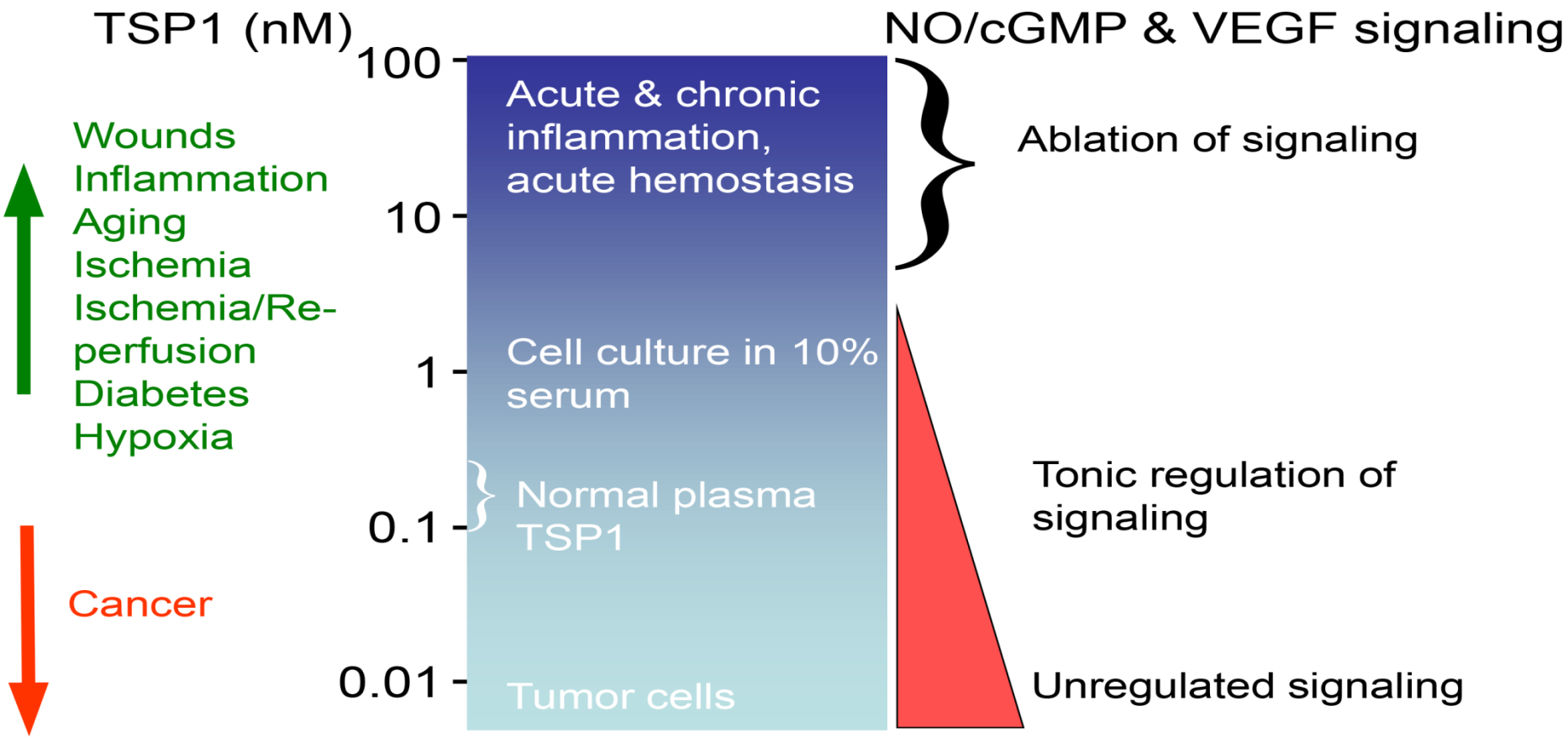
NO/cGMP signaling is a convergence point for angiogenesis inhibitor signaling



Isenberg et al , *Nature Rev Cancer* 2009

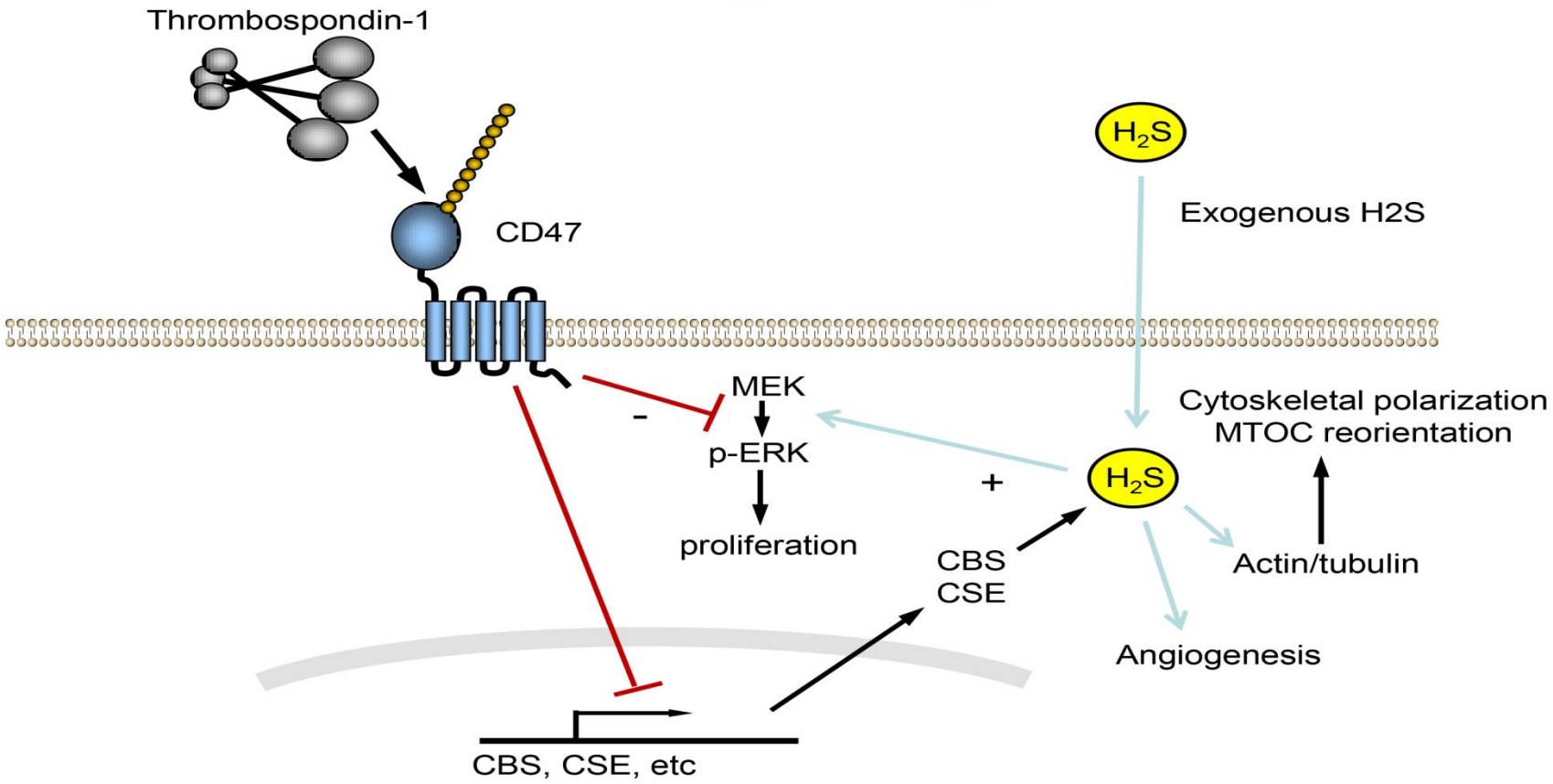
Thrombospondin-1 Levels

Physiological and pathological thrombospondin-1 levels



Thrombospondin-1

Thrombospondin-1 limits H_2S biosynthesis and signaling

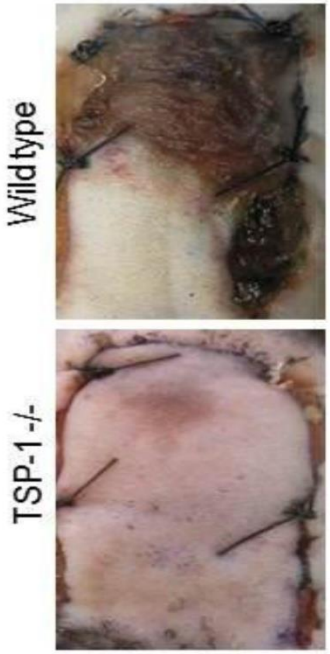


Miller et al. *Matrix Biol* 2013

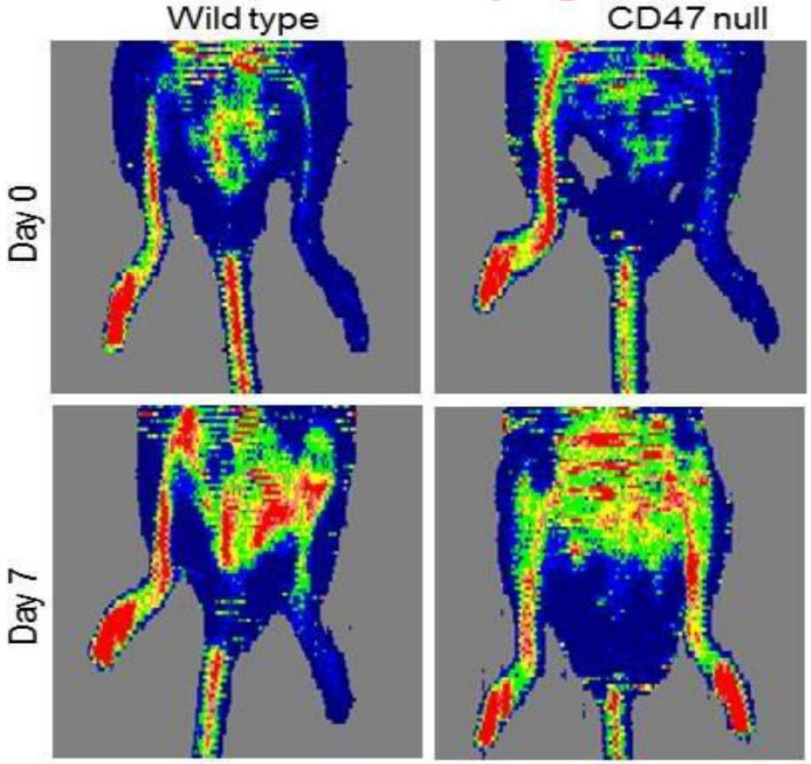
TSP1 Signaling Through CD47

Endogenous TSP1 signaling through CD47 limits recovery from fixed ischemic injuries

McFarlane skin flaps



Femoral artery ligation

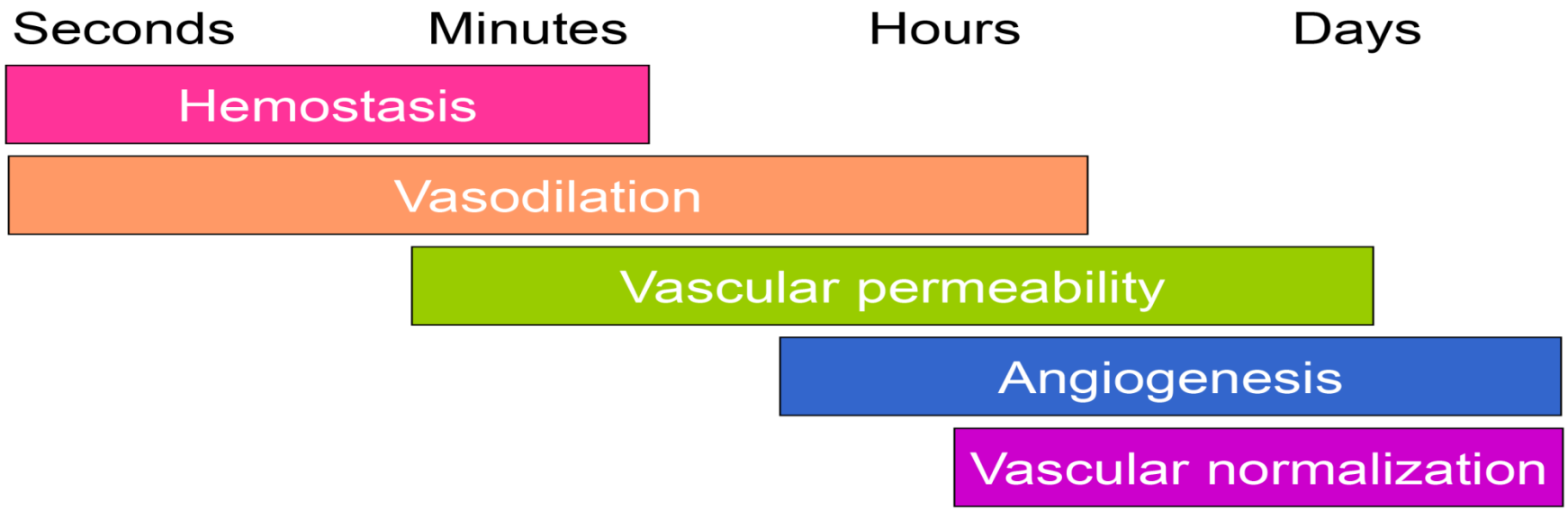


Skin grafts

Blood 2007; *Circ Res* 2007; Ann Surg 2008

Thrombospondin-1 and Nitric Oxide

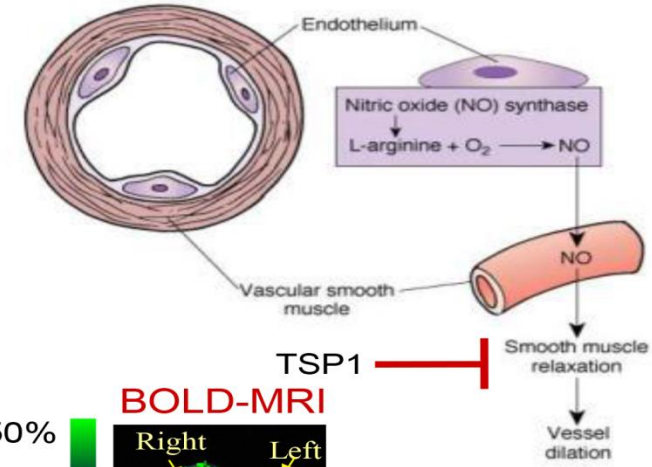
Temporal aspects of thrombospondin-1 and nitric oxide responses in tissues

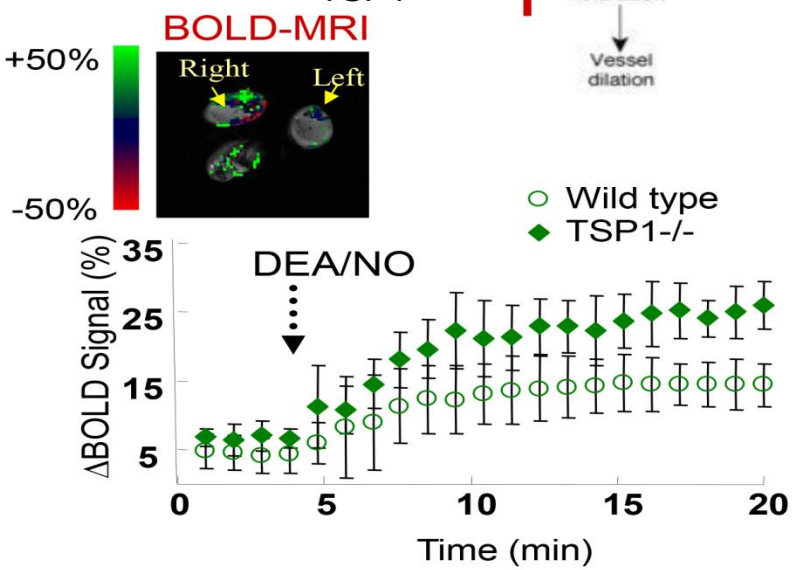


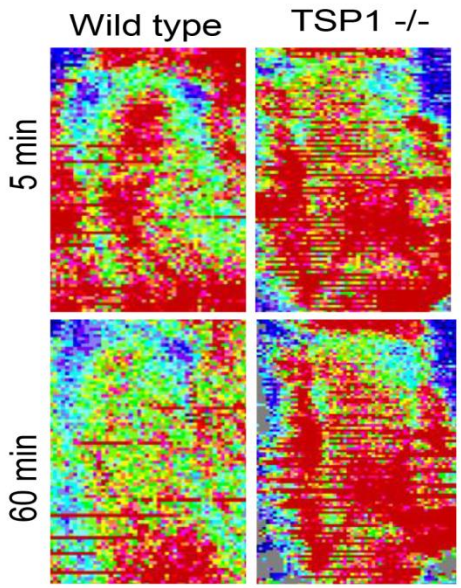
Isenberg et al , *Nature Rev Cancer* 2009

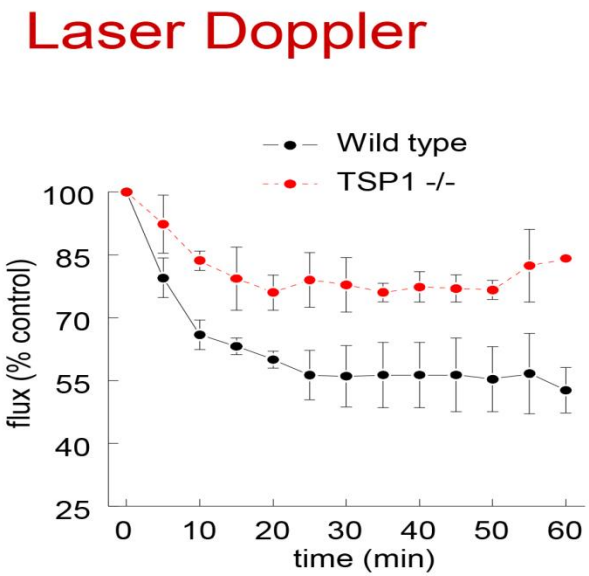
Endogenous TSP1

Endogenous TSP1 acutely limits tissue perfusion in healthy and ischemic tissue







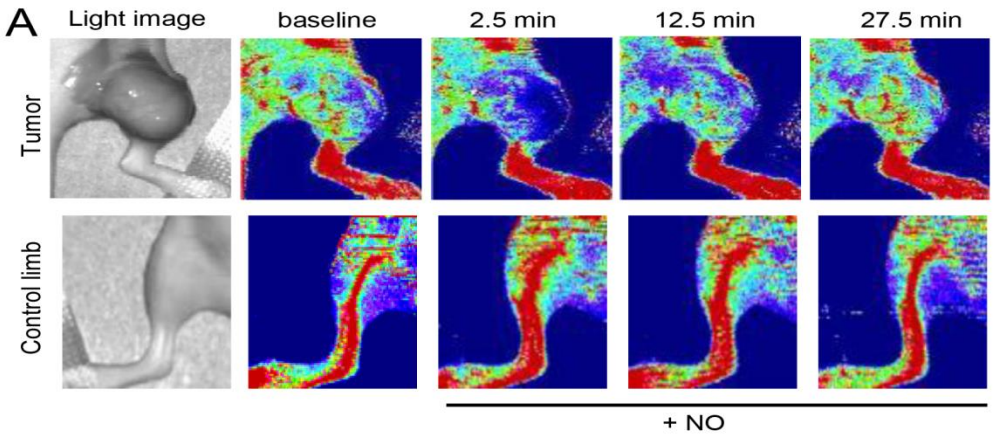


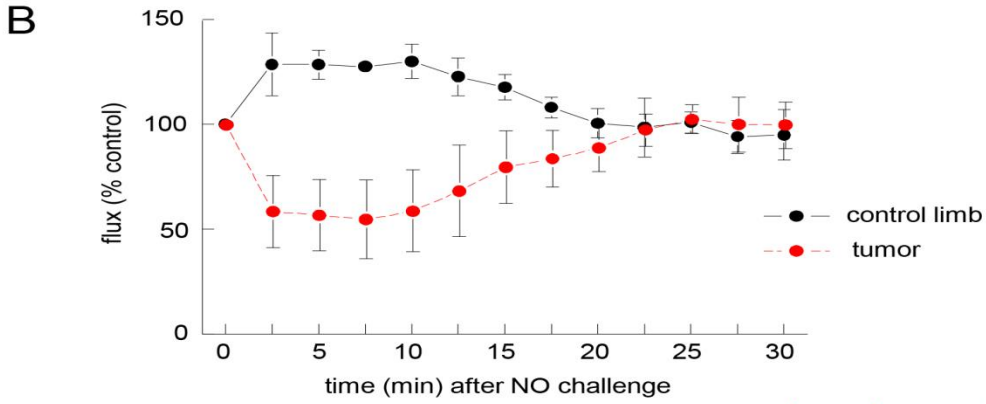
Flap image

Isenberg et al, *Blood* 2007
Isenberg et al, *ATVB 2007*

NO's Effect on Blood Flow

NO increases blood flow in healthy tissue while decreasing flow in the tumor



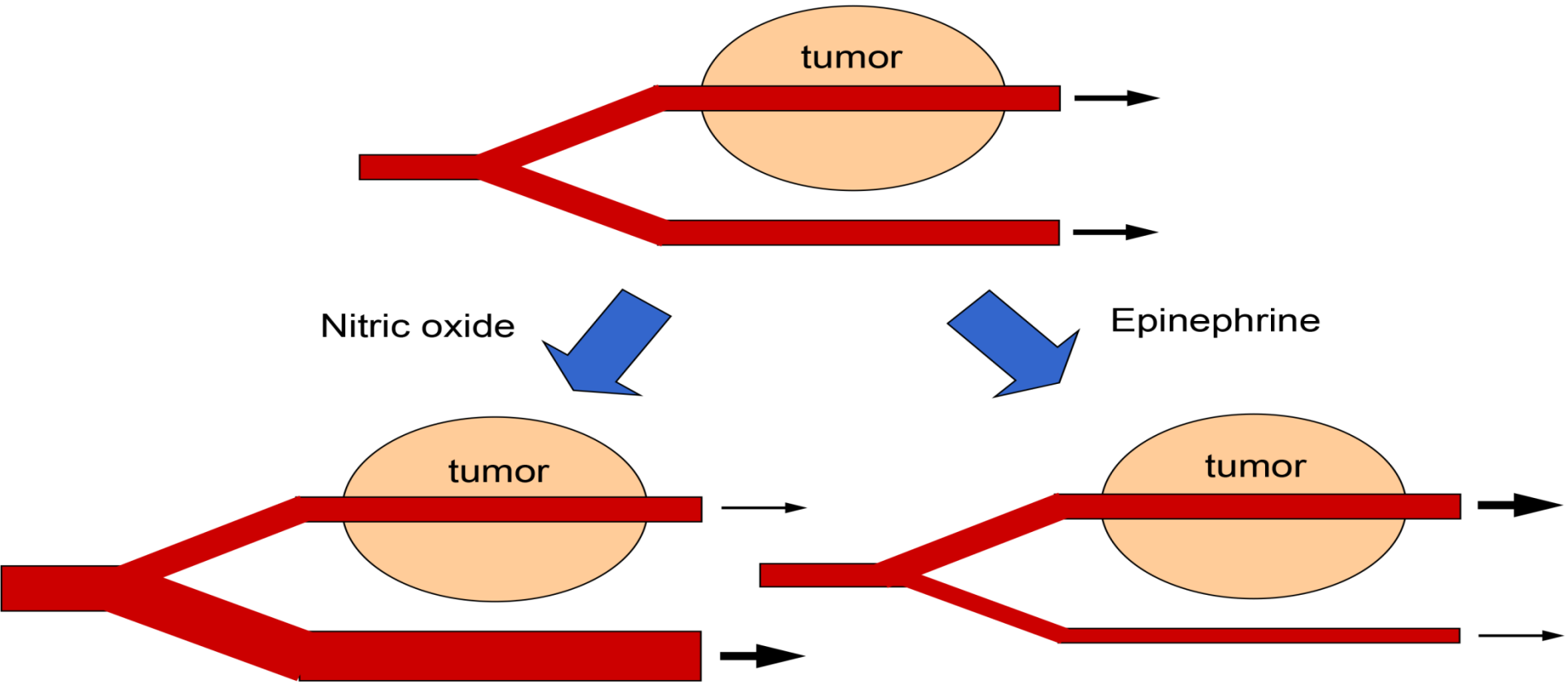


= a "steal effect"

Isenberg et al *Neoplasia*, 2008

Vasoactives and Blood Flow

Vasoactives indirectly modulate tumor blood flow

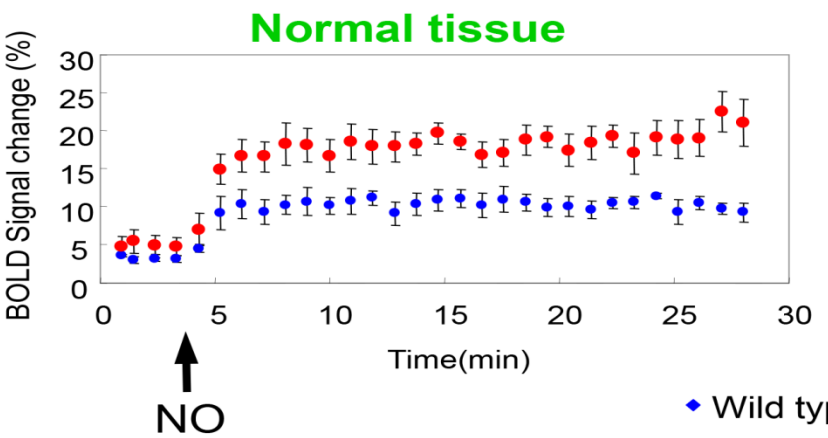


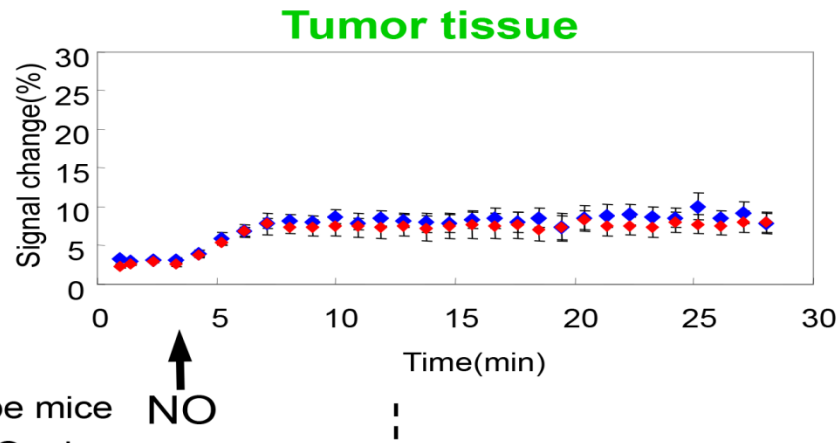
Isenberg et al , *Nature Rev Cancer* 2009

Tumors Neutralize Host TSP1

Tumors neutralize the ability of host TSP1 to limit their vascular perfusion

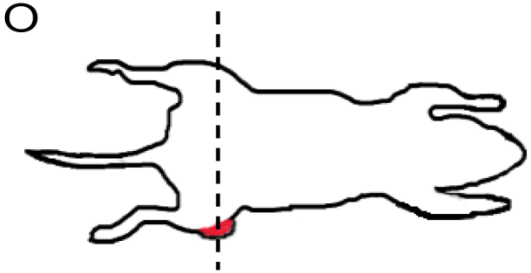
BOLD MRI data of B16 melanoma on wild type and TSP1 null mice.





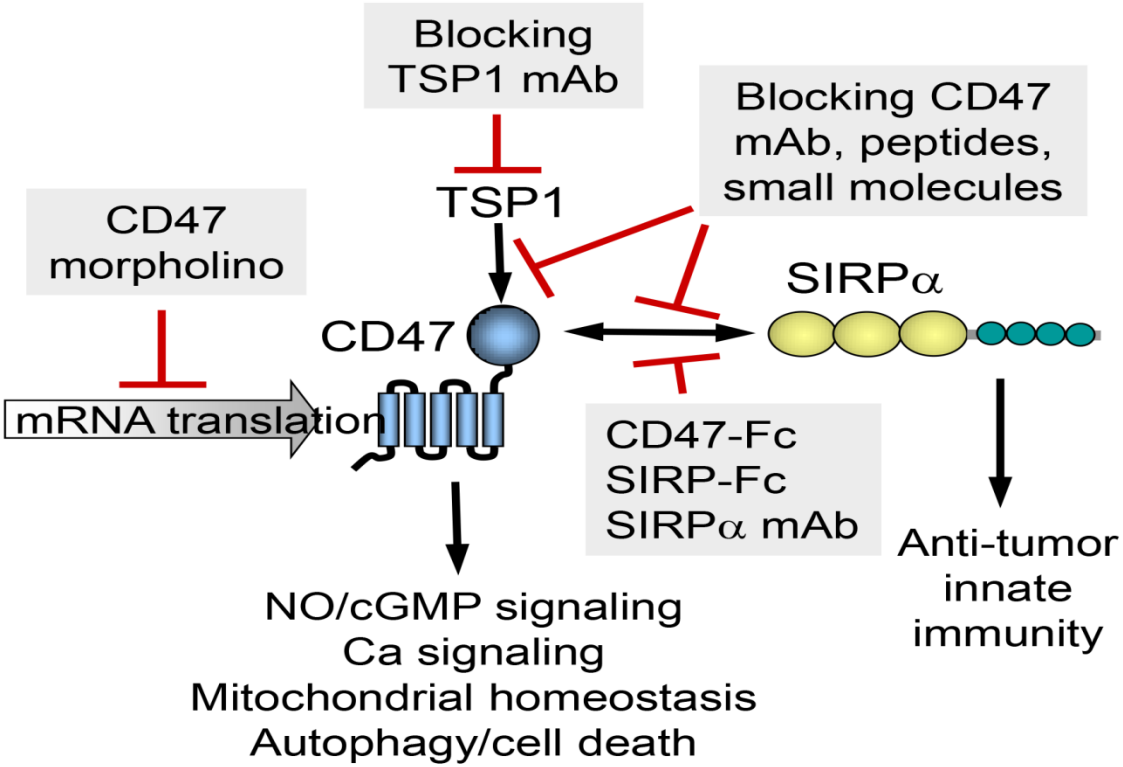
Wild type mice
TSP1 KO mice

Wild type N = 5
TSP1 KO N = 6
Error shown as SE



Therapeutic Strategies

Therapeutic strategies



Soto-Pantoja, et al *Expert Opin Ther Targets* 2012

Significance for human disease

- **Decrease TSP/antagonize CD47 receptor(increase NO signaling**
- **Improve tissue survival following reconstructive surgery**
- **Maintain flow and stimulate vascular remodeling following ischemic injury**
- **Limit ischemic injury associated with peripheral vascular disease and aging**
- **IncreaseTSP/agonize CD47 (decrease NO signaling)**
- **Inhibit tumor angiogenesis (and perfusion?)**
- **Limit bleeding by accelerating platelet aggregation**

CD47 therapeutics

CD47 therapeutics

Clinical Trials

- Humanized anti-CD47 antibody (Hu5F9-G4A, Stanford & Forty Seven Inc.): Phase 1 trials to test the safety of in adult patients with solid tumors (NCT02216409) and to treat AML (NCT02678338).
- Humanized CD47 antibody (CC-90002, Celgene Corporation): Phase 1 trials to treat subjects with advanced solid, breast, and hematological cancers (NCT02367196, NCT02488811).
- SIRPα-Fc fusion protein (TTI-621, Trillium Therapeutics): Phase 1 trial for patients with hematologic malignancies (NCT02663518).

Preclinical Development

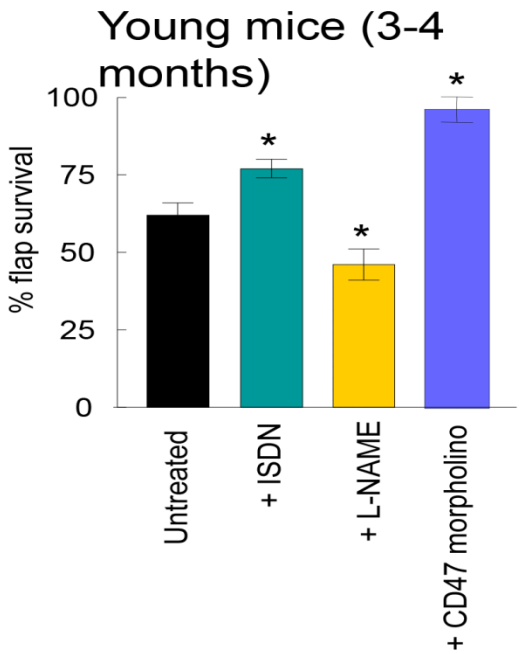
- Bispecific CD47 antibody (NI-1701, Novimmune SA),
- Humanized CD47 antibody that exhibits direct cytotoxic activity for cancer cells (Vasculox, Inc.)
- CD47 antibody (C47B222, Janssen Research & Development , div. Johnson & Johnson)
- CD47 morpholino (RCTI1938, Radiation Control Technologies)
- Small molecule modulators of CD47 (Paradigm Shift Therapeutics)

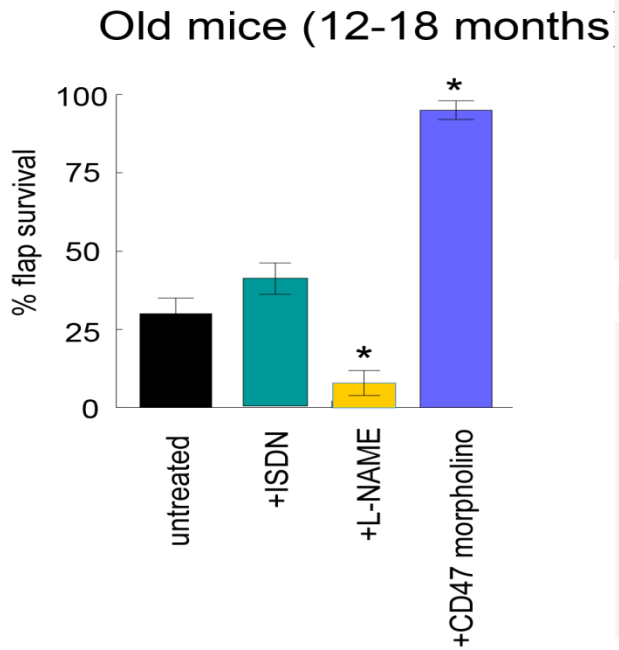
Translational development of CD47 antagonists for vascular pathology

- Protection from fixed ischemic injuries in mice and pigs (skin flaps, hindlimb, full thickness skin grafts)
- Protection from loss of resistance to ischemic injuries with aging or cardiovascular disease in mice
- Protection from ischemia/reperfusion injuries in mice and rats (liver, island skin flap)
- Kidney transplantation in mice and rats

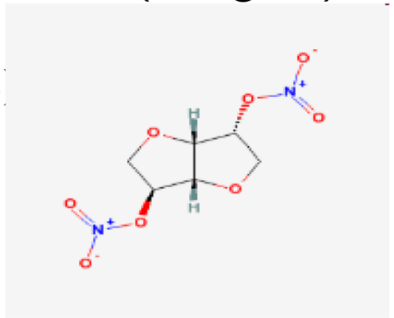
Endogenous Thrombospondin-1

Endogenous thrombospondin-1 limits the ability of NO-donor drugs to enhance ischemic injury survival

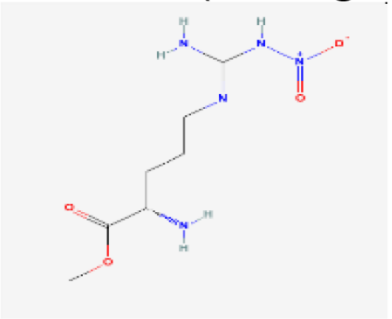




ISDN (1 mg/ml)



L-NAME (0.5 mg/ml)

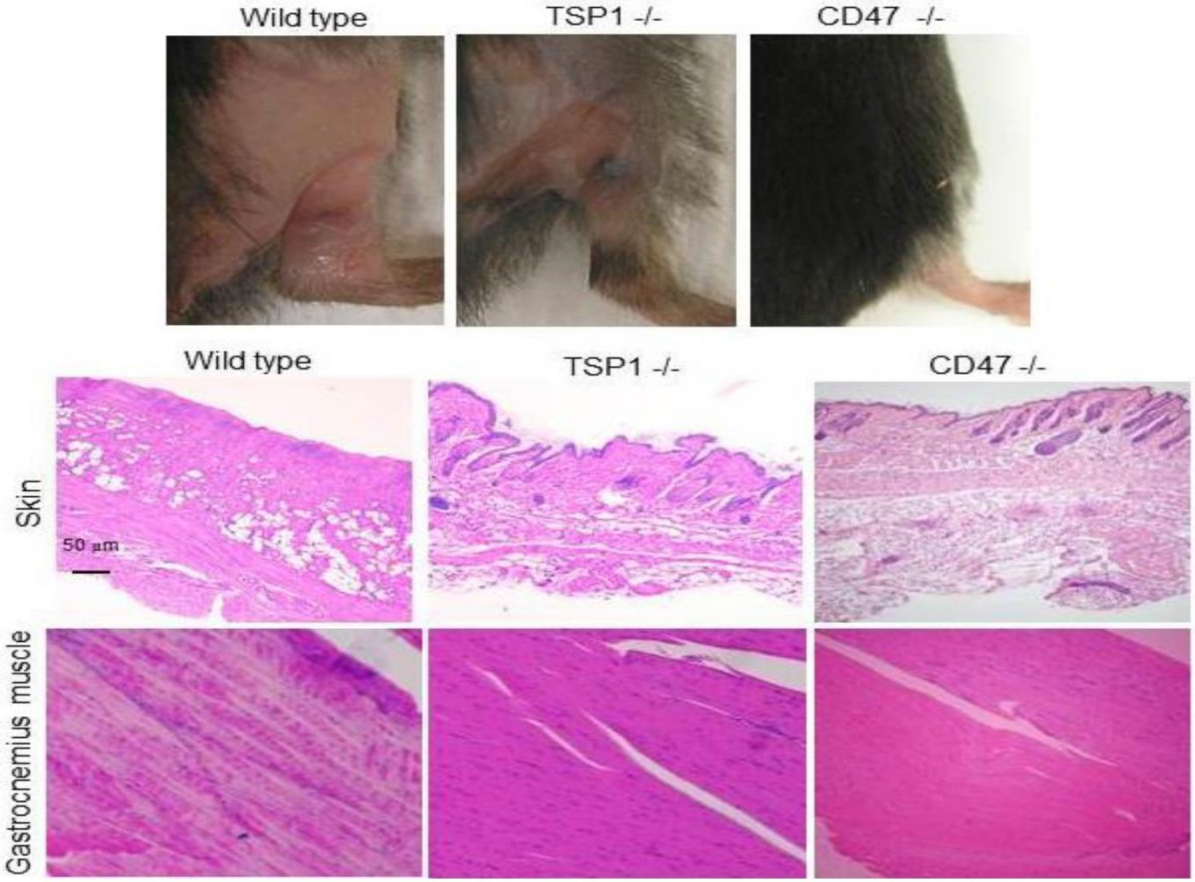


Isenberg et al, *Blood*, 2007; Circe Res 2007; *Arterioscler Thromb Vasc Biol 2007*

TSP1/CD47 Limit Tissue Survival After Irradiation

TSP1/CD47 limit tissue survival after irradiation

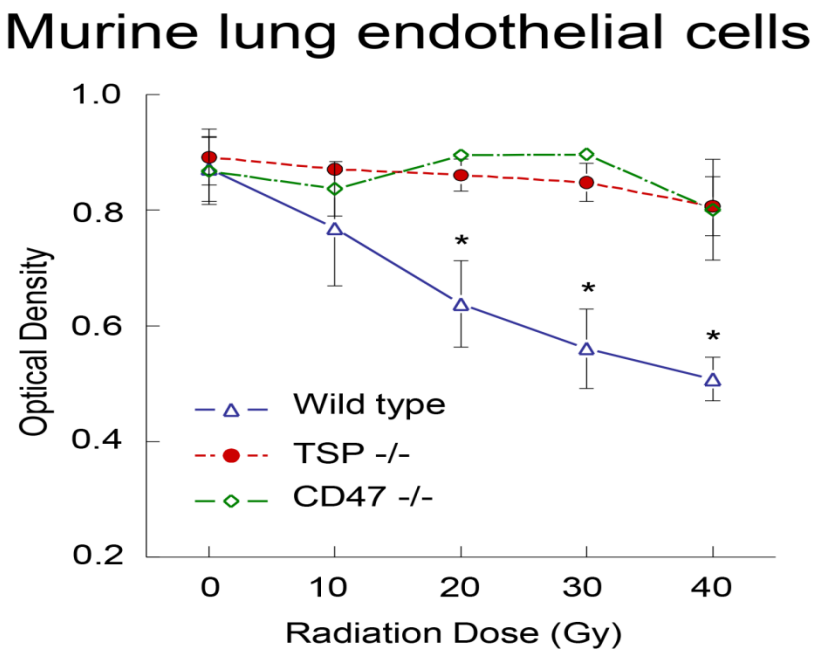
WT, CD47-/- and TSP1-/- mice 60 days post 25 Gy to hindlimb

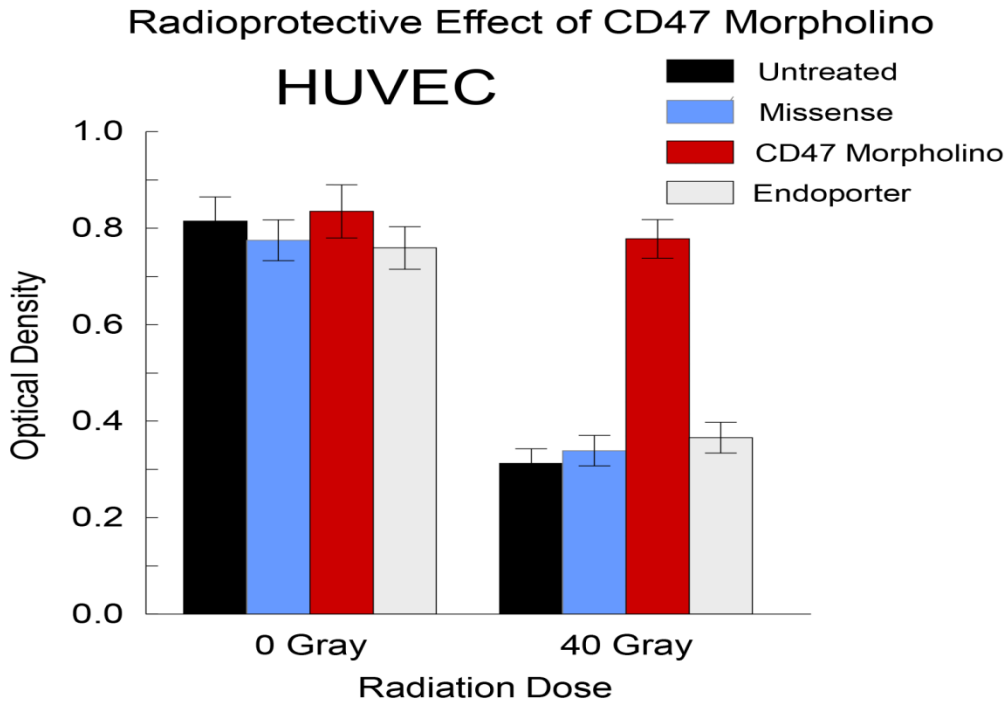


Isenberg et al, *Am J Pathol 2008*

Blocking Thrombospondin-1/CD47 Signaling

The radioprotective effect of blocking thrombospondin-1/CD47 signaling in endothelial cells is cell autonomous

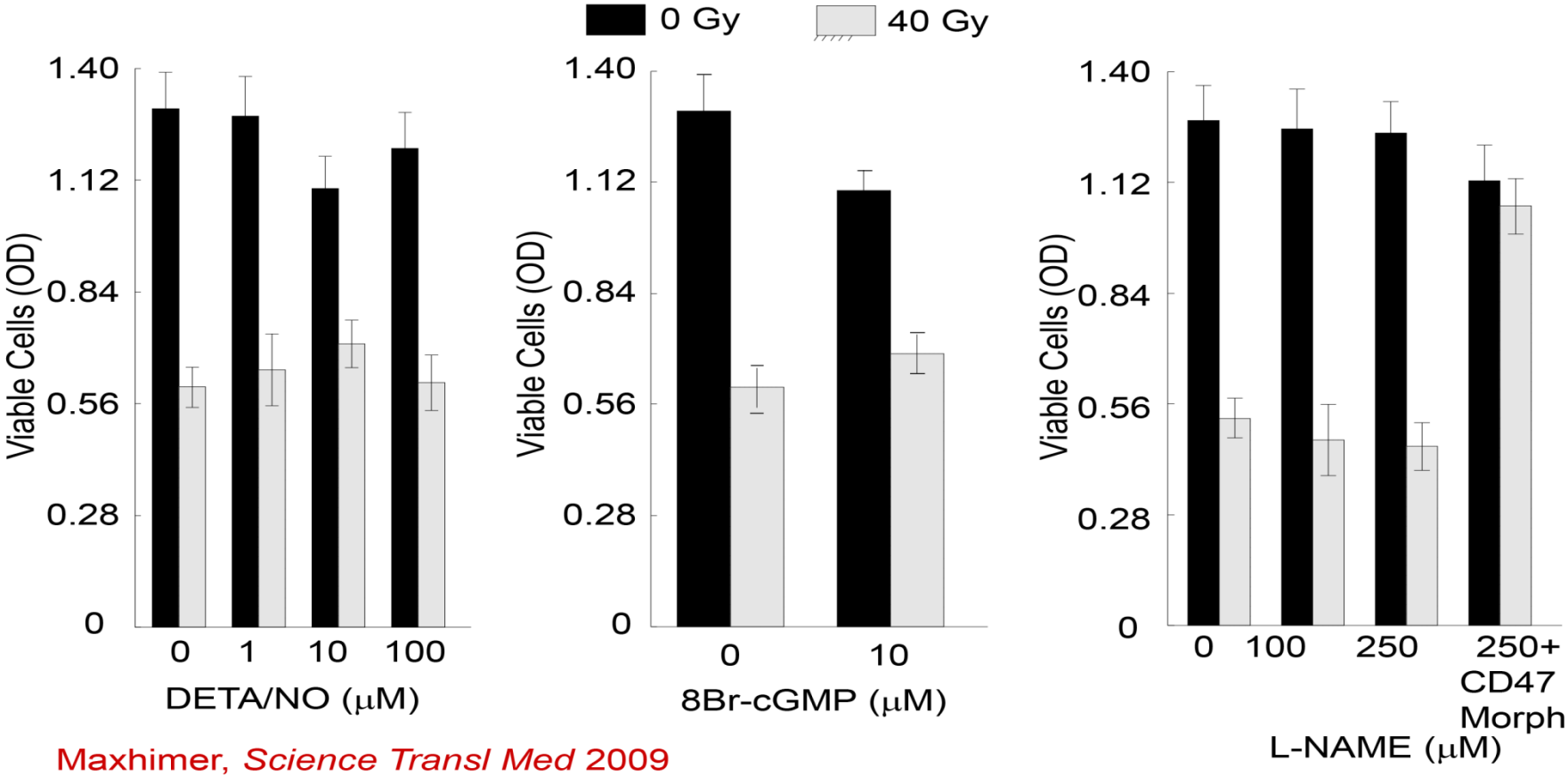




Isenberg, *Am J Pathol 2008;* Maxhimer, *Science Transl Med* 2009

Cell-autonomous Radioprotection

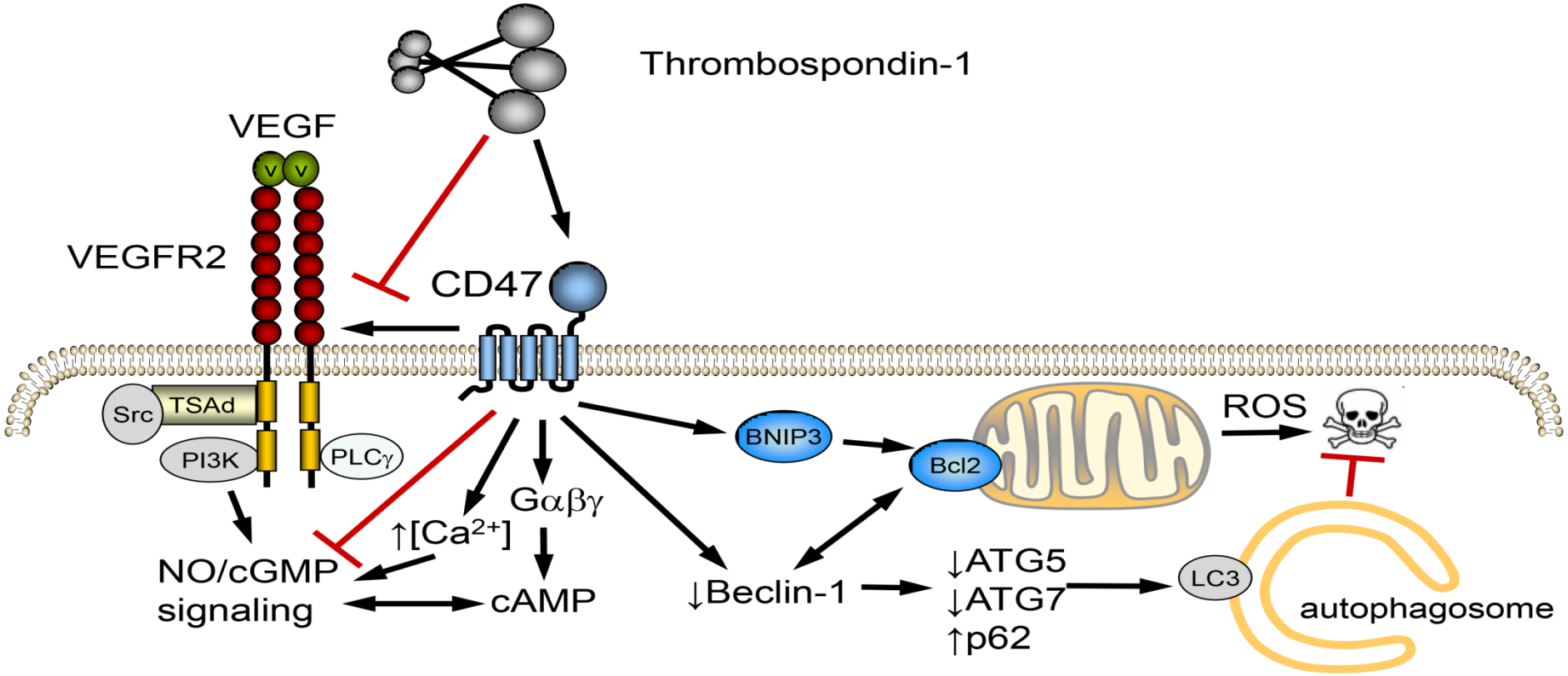
Cell-autonomous radioprotection is independent of NO/cGMP signaling



Maxhimer, *Science Transl Med* 2009

CD47 Cell Survival Control

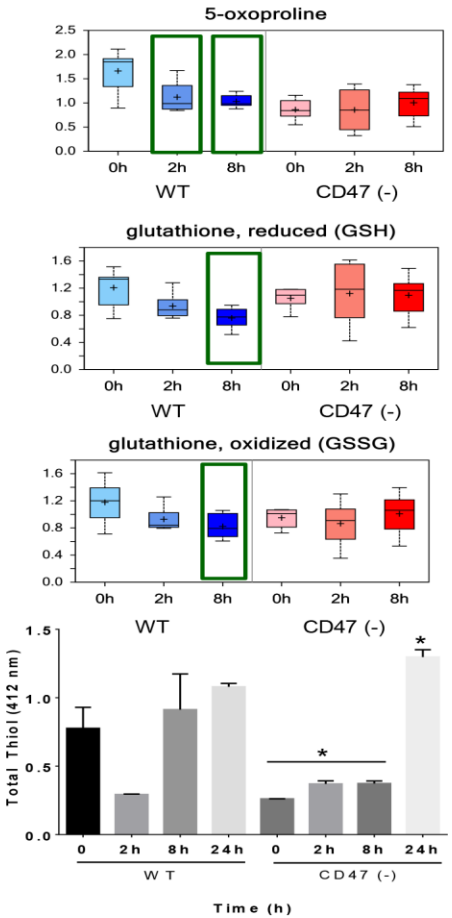
CD47 controls cell survival through regulating autophagy and mitochondrial ROS production

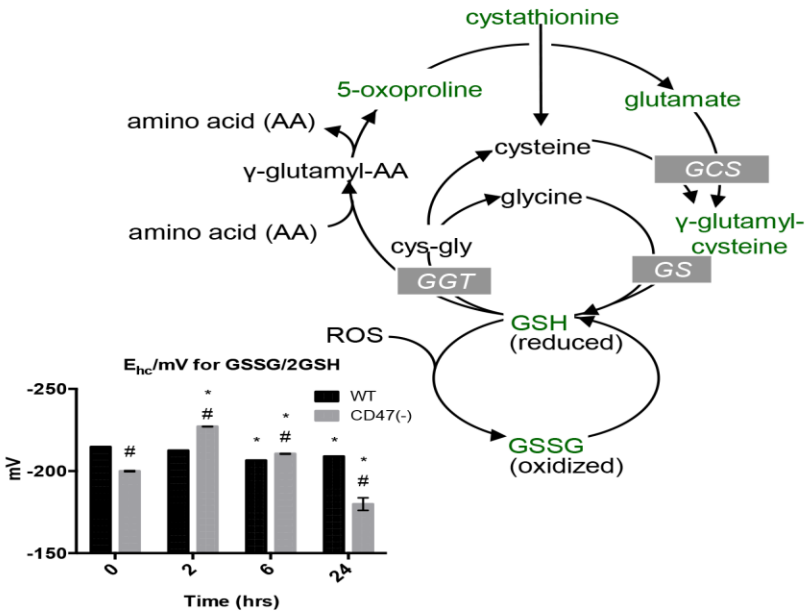


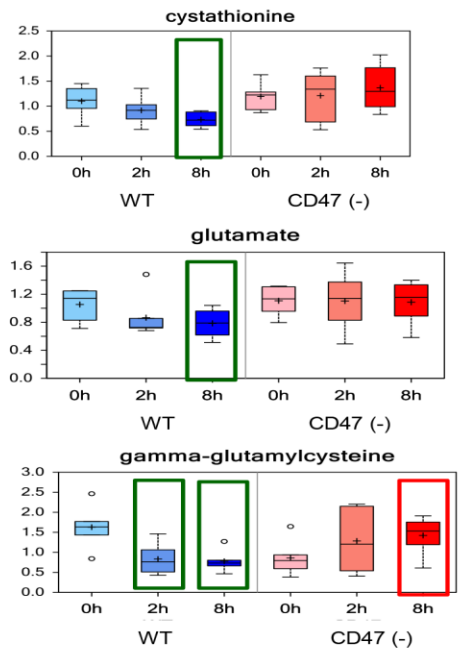
Soto-Pantoja, et al *Expert Opin Ther Targets* 2012

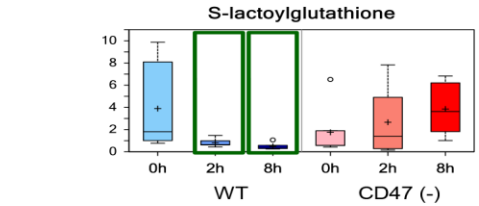
CD47 and stress

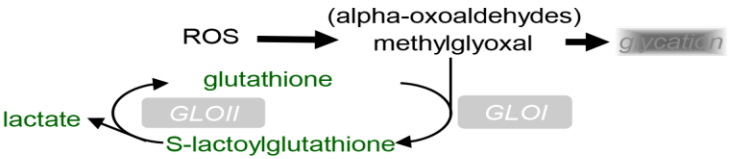
CD47 broadly regulates cellular redox stress responses











Miller et al, *J. Biol. Chem.* 2015

Conclusions

- Thrombospondin-1 is an important pathophysiological inhibitor of vascular nitric oxide signaling via cGMP that limits angiogenesis, hemostasis, and cardiovascular dynamics
- CD47 is a direct binding partner of VEGFR2, and thrombospondin-1 inhibits VEGFR2 signaling by disrupting this complex.
- Thrombospondin-1 signaling through its receptor CD47 limits tissue survival of ischemia, ischemia/reperfusion, and radiation injuries
- Drugs targeting TSP1/CD47 improve survival of ischemia and ischemia/reperfusion injuries in mice, rats, and pigs and have potent radioprotective activities