NETosis, platelets and cancer

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Major causes of cancer-related death

- disseminated disease
- thrombosis
- distant organ failure

Major causes of cancer-related death

- disseminated disease
- thrombosis
- distant organ failure
- >50% of patients with solid cancer have renal insufficiency at time of diagnosis

Tumor-induced effects on organs that are not affected by primary tumor growth or metastases?

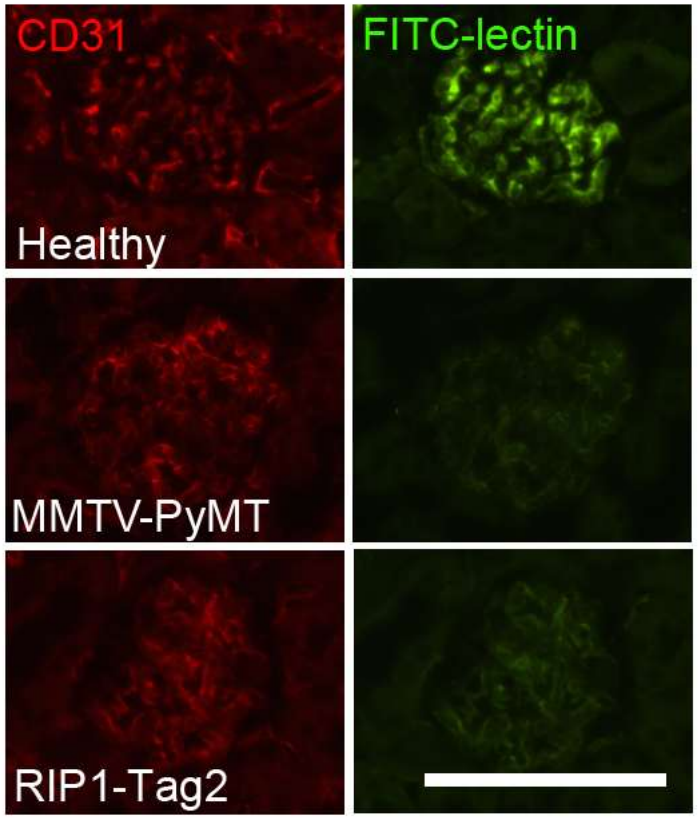
Tumor-induced effects on distant organs in mice with cancer

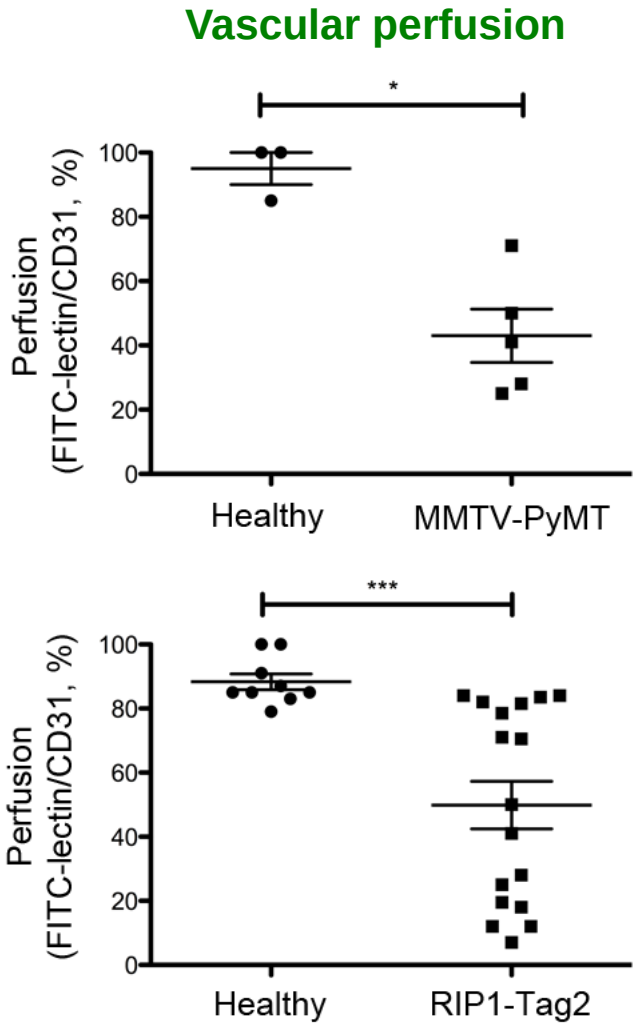
RIP1-Tag2
pancreatic insulinoma,
liver and lung metastases
Strain: C57BL/6

MMTV-PyMT
mammary carcinoma,
lung metastases
Strain: FVB/n

Perfusion and leakage of kidney and heart in RIP1-Tag2 and MMTV-PyMT mice?

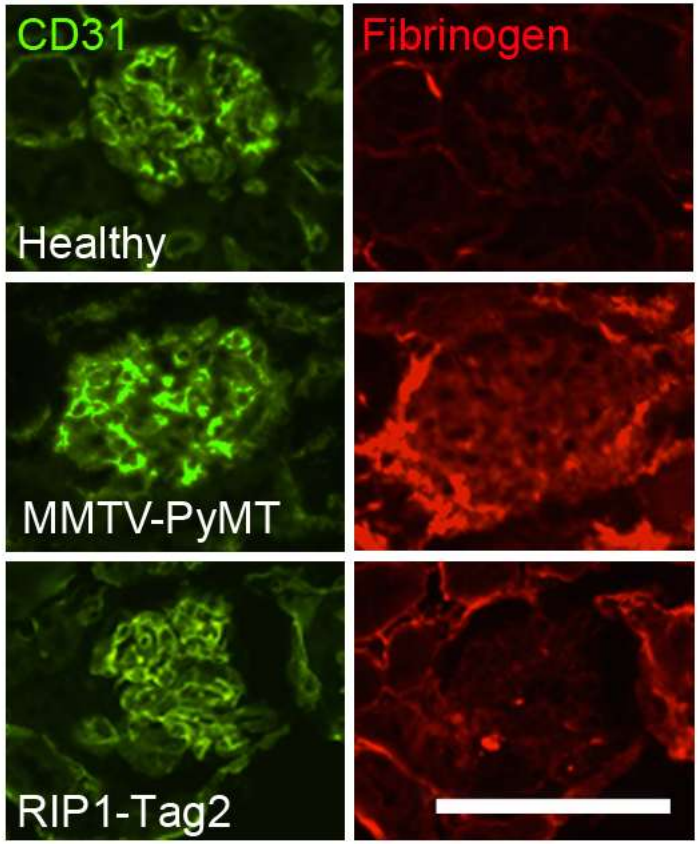
Reduced vascular perfusion in kidney from tumor-bearing mice

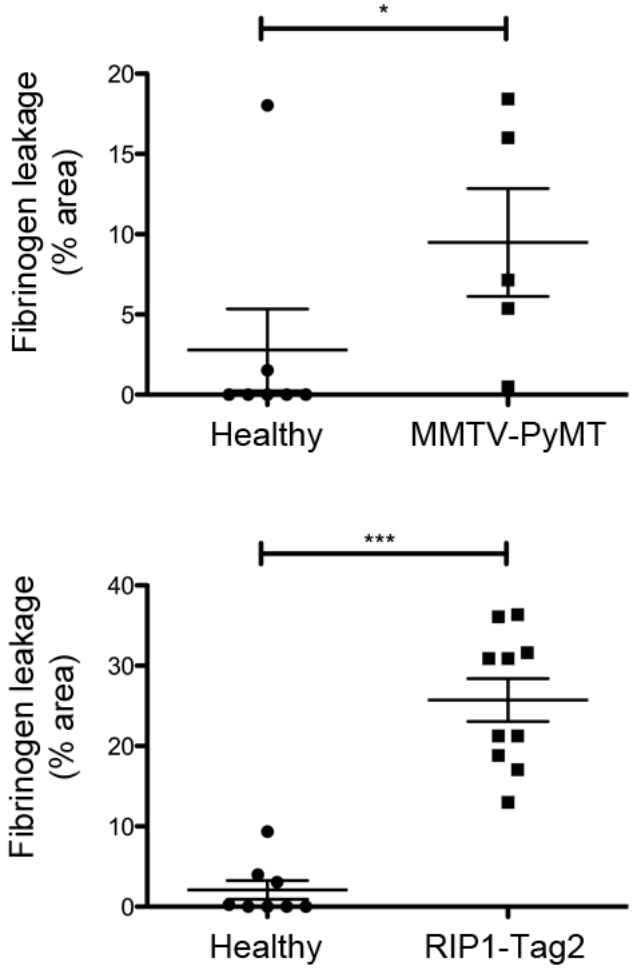




Cedervall et al, Cancer Res, 2015

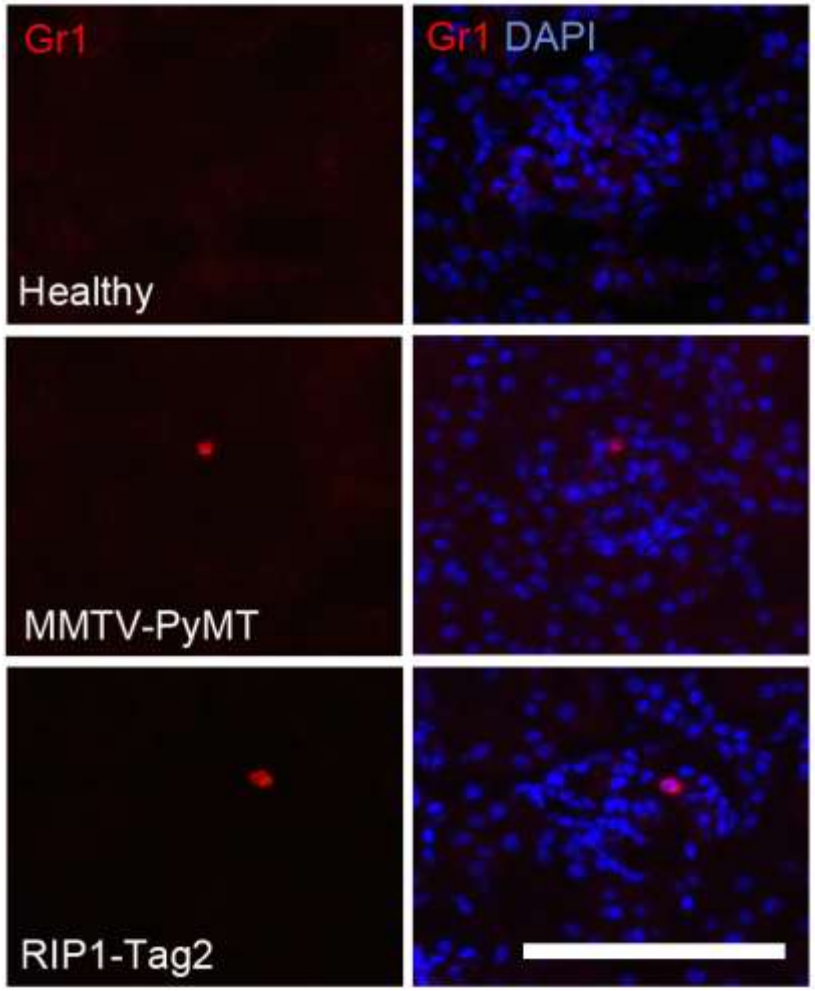
Increased vascular leakage in kidney from tumor-bearing mice





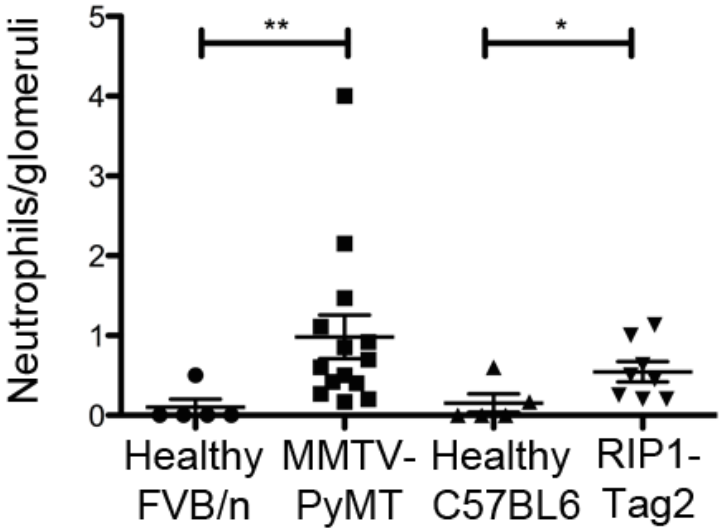
Cedervall et al, Cancer Res, 2015

Increased number of neutrophils in kidney from tumor-bearing mice



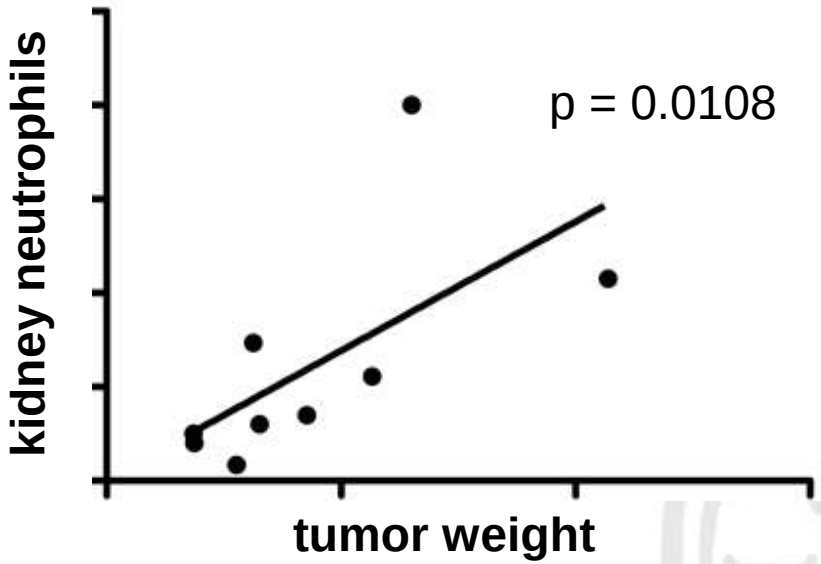
Gr1 = granulocytes (neutrophils)

Gr1+ cells/glomeruli



Cedervall et al, Cancer Res, 2015

Number of kidney neutrophils correlates with primary tumor burden

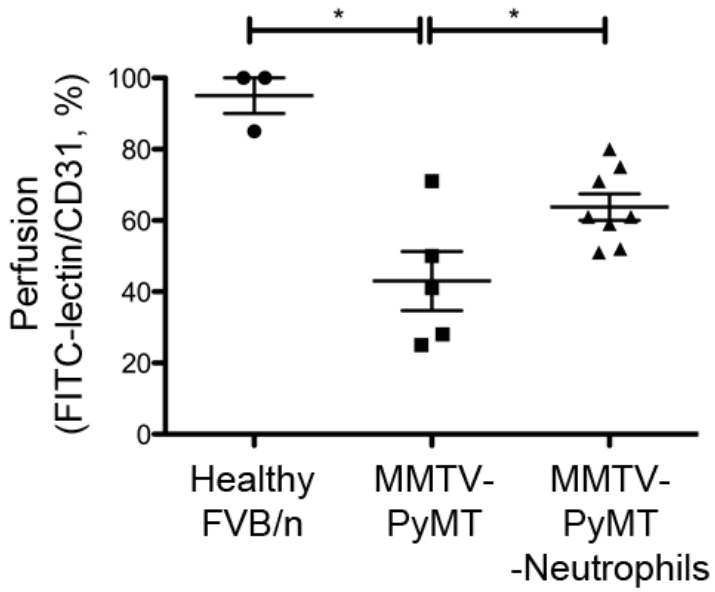


Cedervall et al, Cancer Res, 2015

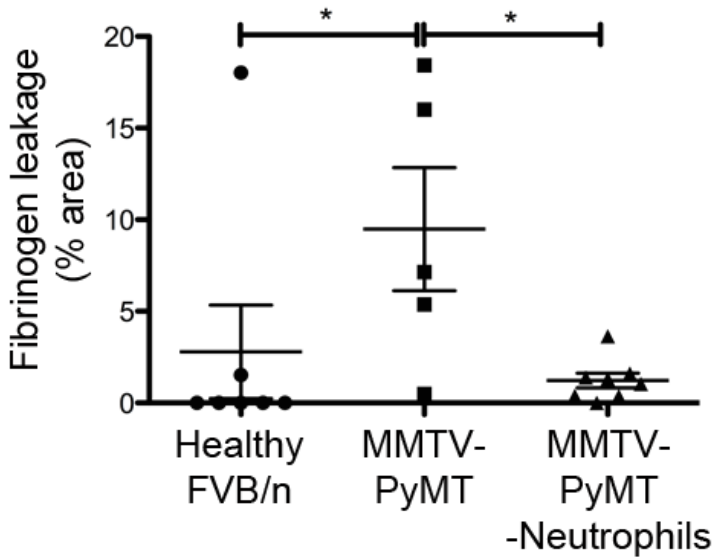
Neutrophil depletion restores kidney vascular perfusion and prevents leakage

Neutrophil depletion by injection of an anti-Gr1 antibody

Vascular perfusion

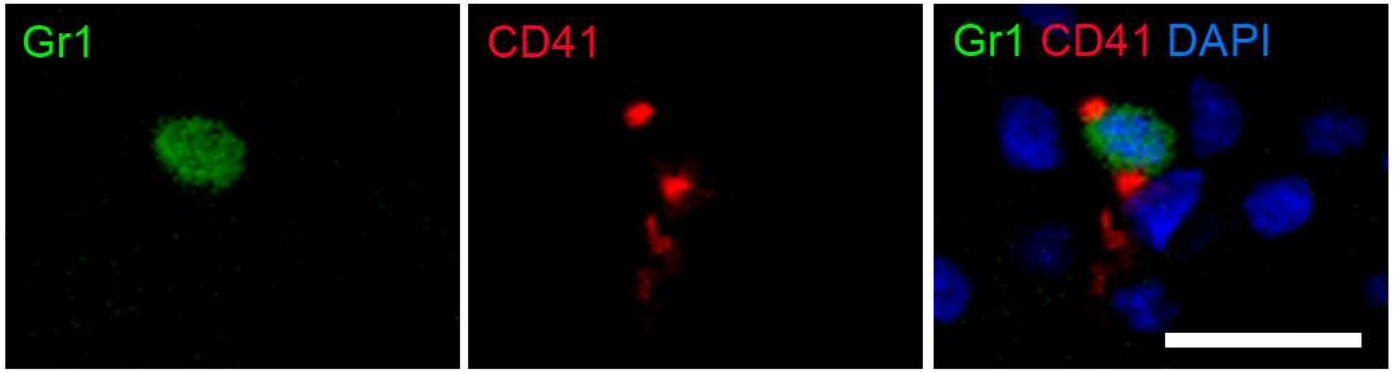


Vascular leakage



Cedervall et al, Cancer Res, 2015

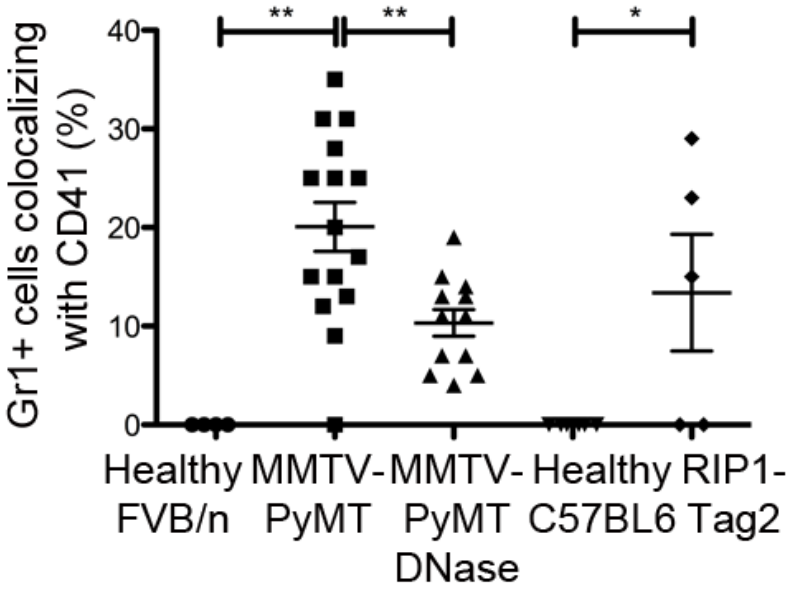
Platelet-neutrophil complexes accumulate in peripheral vasculature in mice with cancer



Gr1=granulocytes (neutrophils)

CD41=platelets

DAPI = nuclei



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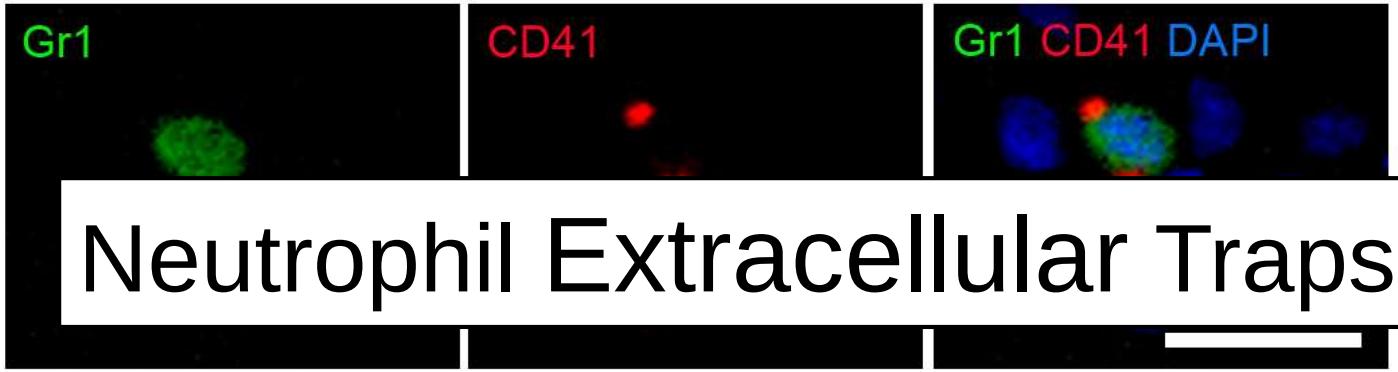

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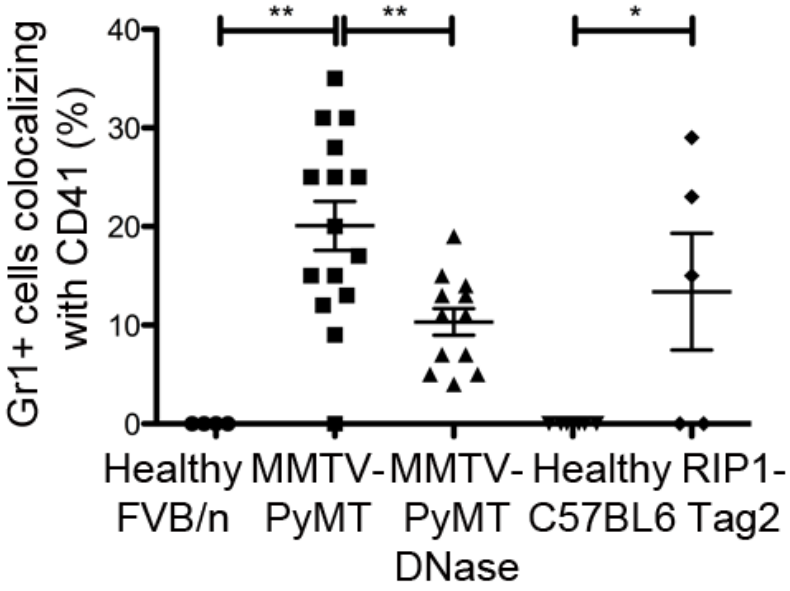
41=platelets

PI = nuclei

Neutrophil Extracellular Traps?



Cedervall et al, Cancer Res, 2015

Neutrophil extracellular traps – NETs

Scanning electron micrograph of Staph aureus bound to NETs (Brinkmann et al 2004)

- 2004: novel defense mechanism to bacterial infections
- Formed when neutrophils externalize their DNA together with histones and anti-bacterial granule proteins like elastase & myeloperoxidase.
- Traps and kills bacteria.
- Cytotoxic towards the endothelium.

NETs in non-infectious disease

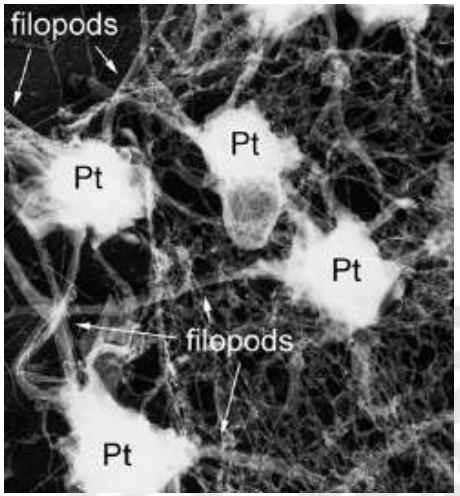
NETosis implicated in various inflammatory diseases:

- **thrombosis**
- **cancer**
- **postsurgical complications**
- **autoimmunity**
- **diabetes**
- **atherosclerosis**
- **arthritis**

Fuchs et al PNAS 2010, Villanueva J Immunol 2011, Demers et al, PNAS 2012, Wong et al Nat Med 2015, Warnatsch et al, Cedervall et al Cancer Res 2015, Clin Exp Immunol 2017, Kubes, Nat Med 2017, Wolach Sci Translational Med 2018

NETs activate platelets and are prothrombotic

- Clark et al Nat Med 2007
- Fuchs et al PNAS 2010
- Von Bruhl et al J Exp Med 2012
- Demers et al PNAS 2012
- Now more than 200 papers!



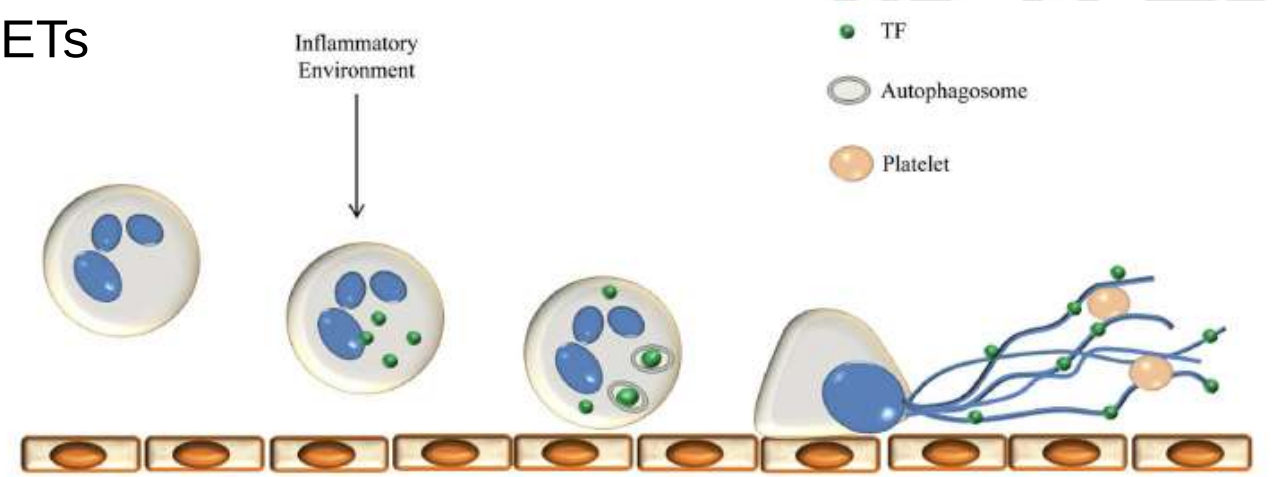
Fuchs et al PNAS 2010

<u>Suggested mechanisms:</u>

Platelet activation by extracellular histones

FXII activation by extracellular DNA

TF bound to NETs



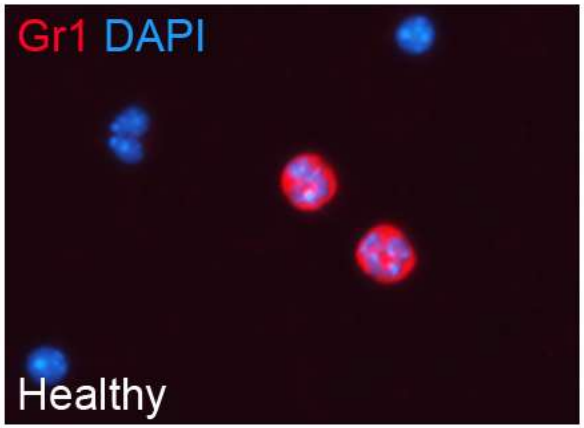
DNase I degrades NETs

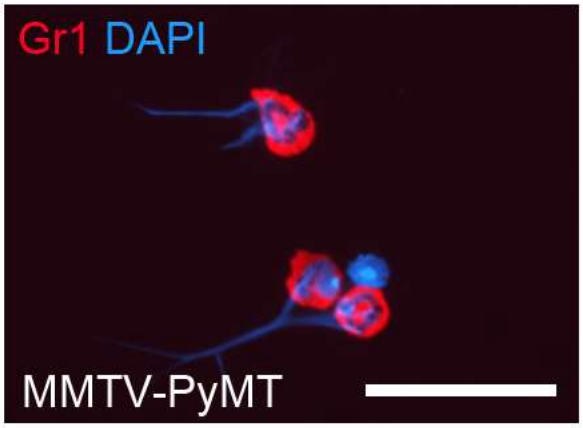
- Several studies have shown that DNase I treatment can degrade NETs *in vivo*

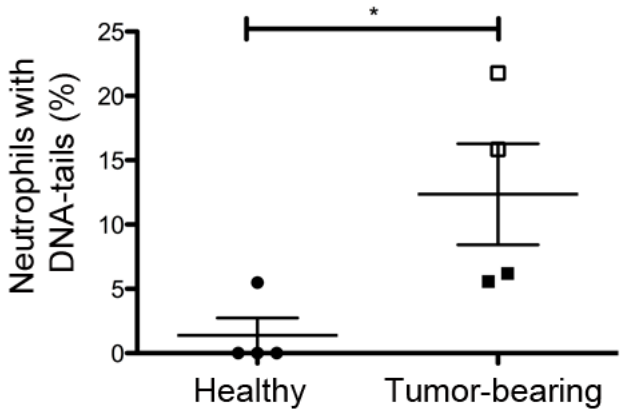
- Host DNases (DNaseI and DNaseI-like3) protects us from NET-induced thrombosis

  Jiménez-Alcázar et al, Science Dec 2017

Neutrophils with extracellular DNA in peripheral vasculature in mice with cancer

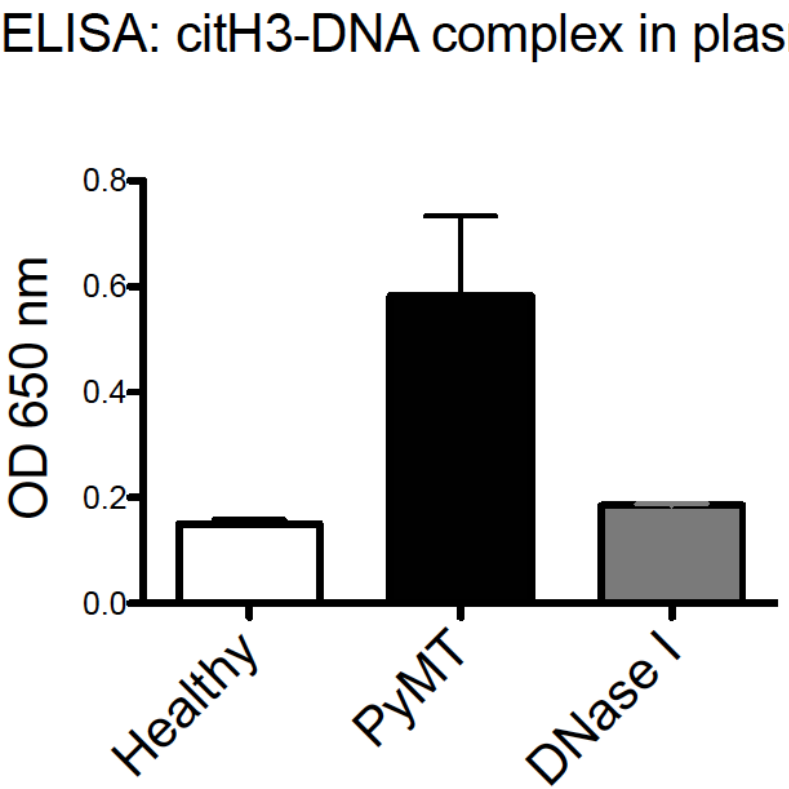




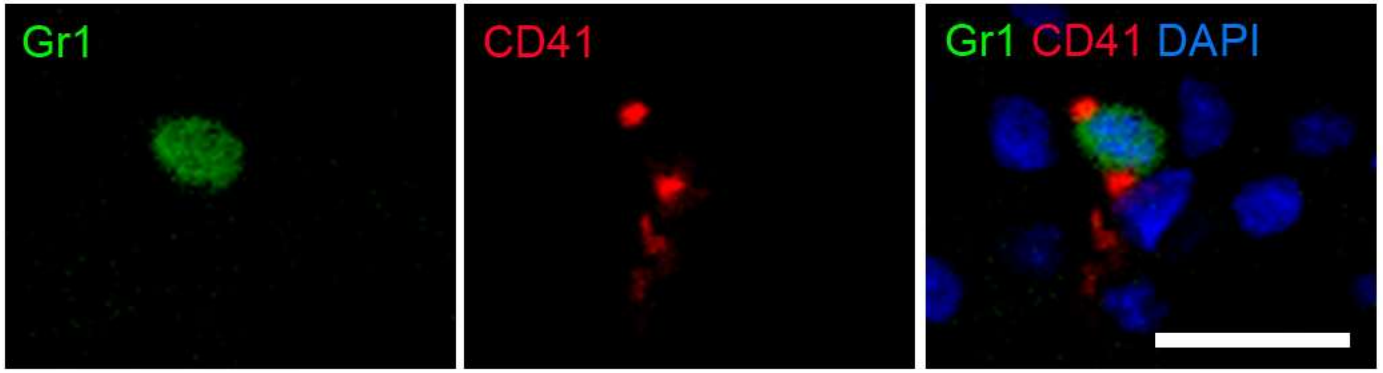


Cedervall et al, Cancer Res, 2015

DNase I treatment removes NETs from the circulation of tumor-bearing mice



DNase I treatment removes kidney platelet-neutrophil complexes in mice with cancer



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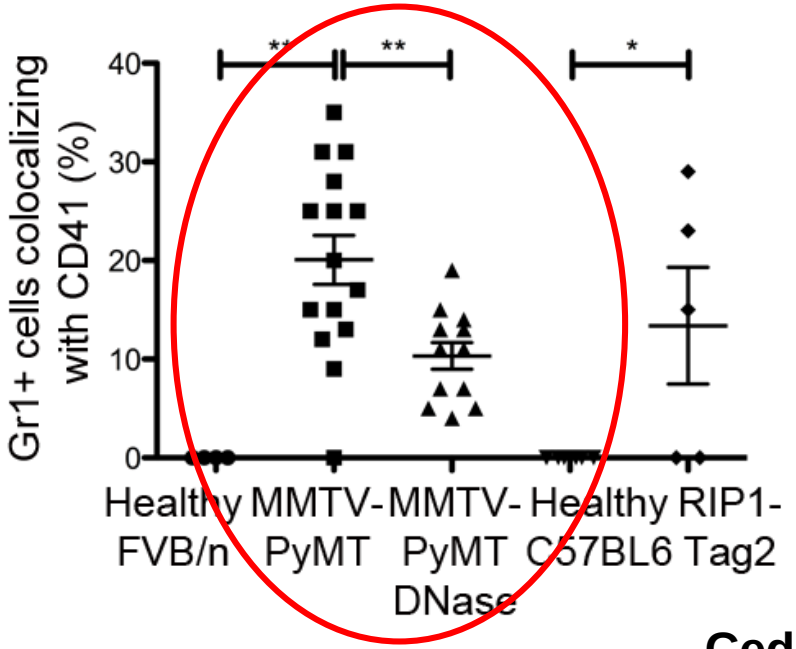
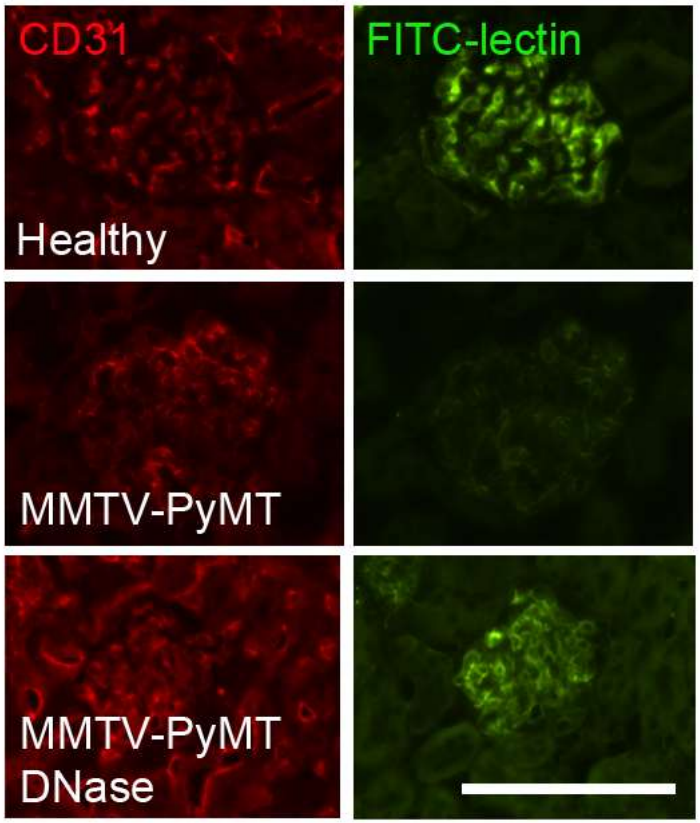
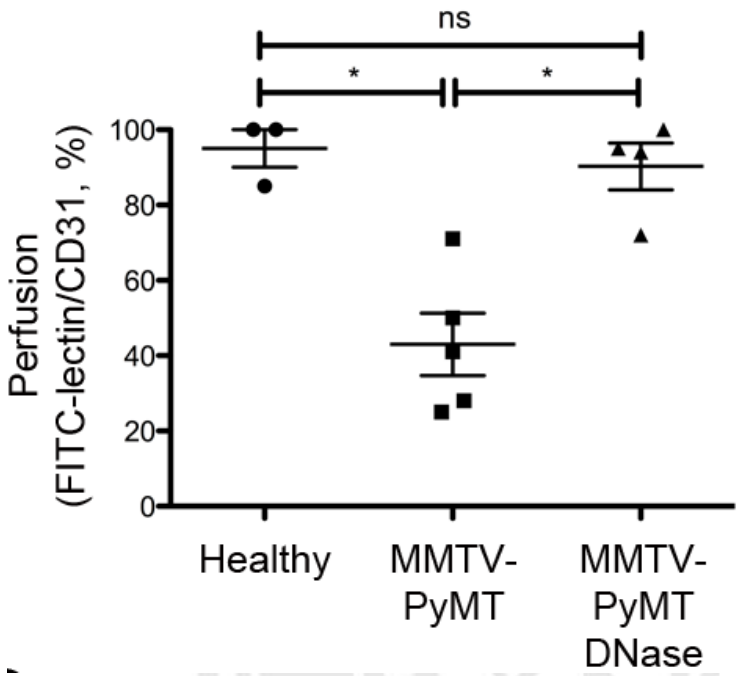


Cedervall et al, Cancer Res, 2015

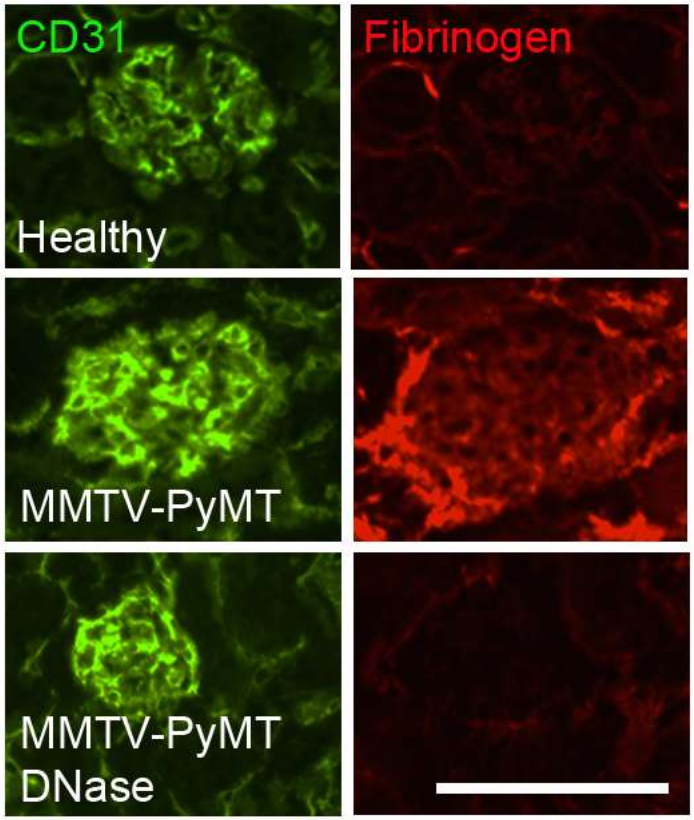
DNase I treatment restores kidney vascular perfusion



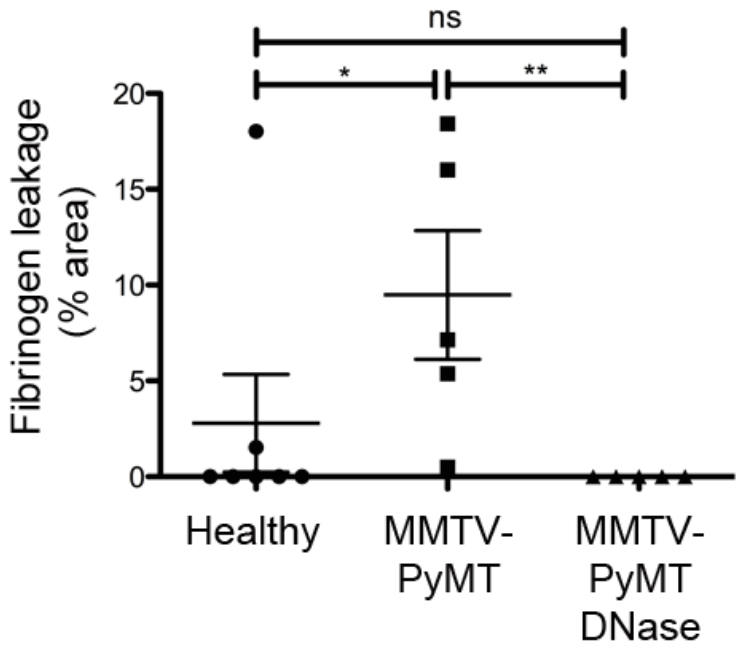


Cedervall et al, Cancer Res, 2015

DNase I treatment prevents kidney vascular leakage

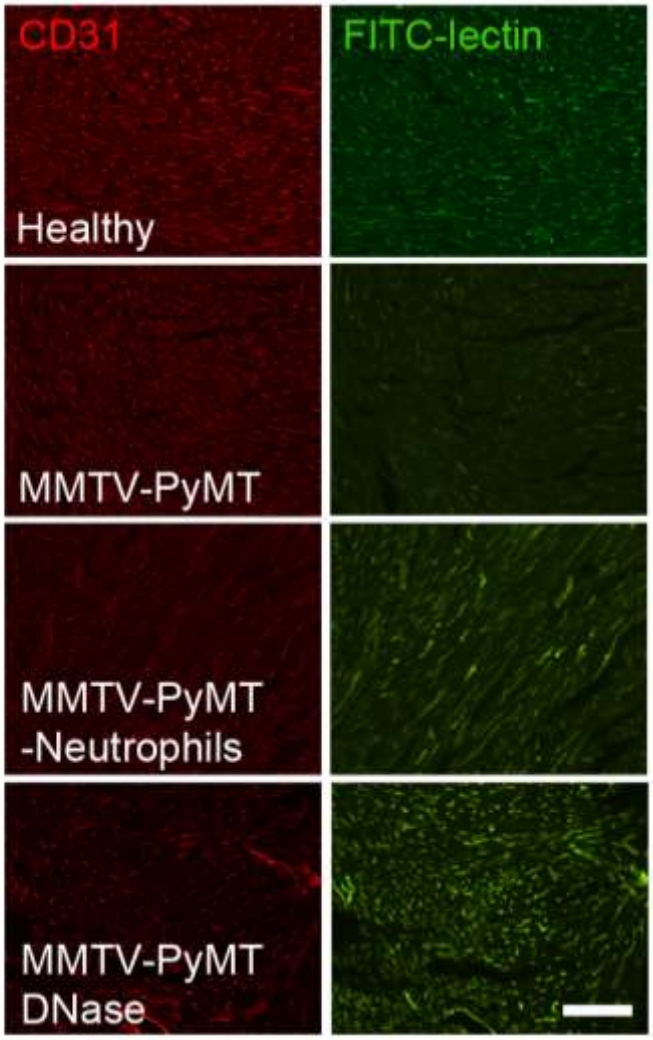


Vascular leakage

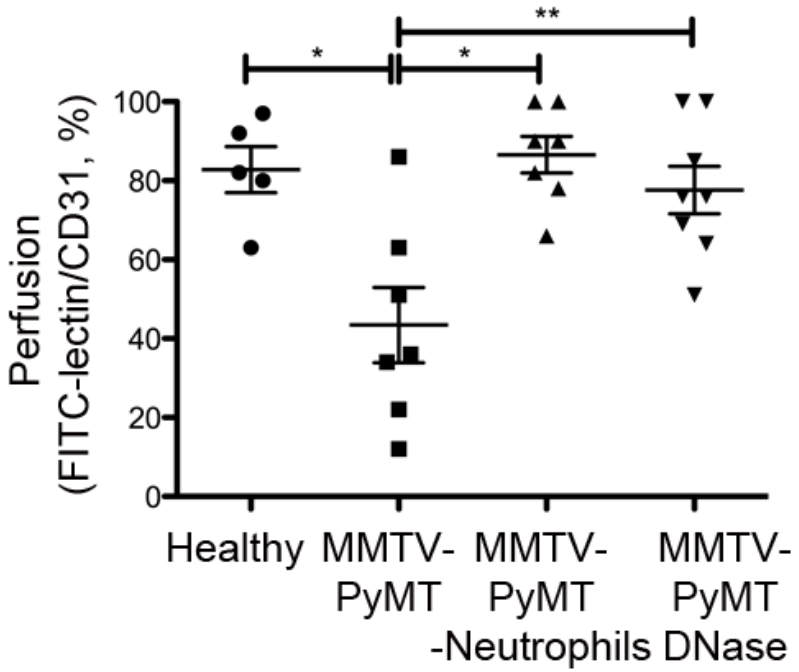


Cedervall et al, Cancer Res, 2015

DNase I treatment restores vascular perfusion in the heart



Vascular perfusion



Cedervall et al, Cancer Res, 2015

Upregulation of inflammatory molecules in kidneys from tumor-bearing mice

Gene	Forward (5'-3')	Reverse (5'-3')
ICAM	CCGCTACCATCACCGTGTA	CACAGGTCTCACCTCCACACT
VCAM	TGGGAACCTGGAACCAAGTA	CTCTGGATCCTTGGGGAAAA
E-selectin	GCGCTTTCTCTCTGCTCTTG	ATGAGCTCACTGGAGGCATT
G-CSF	CCTGGAGCAAGTGAGGAAGA	CTCGGGGTCACACAGCTT
IL1a	TCATTGGCGCTTGAGTCCGCA	TCAGAGAGAGATGGTCAATGGCACA
IL1b	TGAAATGCCACCTTTTGACA	GGGTCCGTCAACTTCAAAGA
IL6	GATGGATGCTACCAAACTGGA	GGTACTCCAGAAGACCAGAGGA
IL10	TGAATTCCCTGGGTGAGAAG	GCTCCACTGCCTTGCTCTTA
IL17	TCCAGAAGGCCCTCAGACTA	AGCTTCCCAGATCACAGAGG
IL18	TGTCTACCCTCTCCTGTAAGAAC	TGTATATCATCAATATTTTCAGGTGG
CCL20	CTTGCTTTGGCATGGGTACT	TGTACGAGAGGCAACAGTCG
CXCL12	CCAAACTGTGCCCTTCAGAT	TAATTTCGGGTCAATGCACA
CX3CL1	CGCGTTCTTCCATTTGTGTA	TTCGTGAGGTCATCTTGTCG
CCL3	ACCATGACACTCTGCAACCA	TAGGAGAAGCAGCAGGCACT
CCL5	GTGCCCACGTCAAGGAGTAT	CACTTCTTCTCTGGGTTGGC
CXCL1	GTCGCGAGCCTTGCCTTCAC	AAGCCAGCGTTCACCAGACAGG
TNFα	GTCTACTGAACTTCGGGGTGA	AGGGTCTGGGCCATAGAACT
IFNγ	CACGGCACAGTCATTGAAAG	TTCCACATCTATGCCACTTGA

ICAM-1

VCAM-1

E-selectin

IL-1β

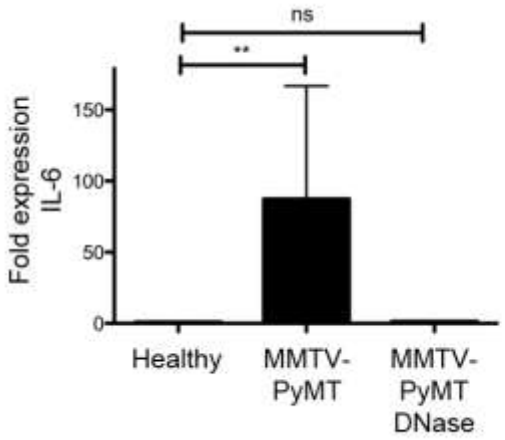
IL-6

CXCL1

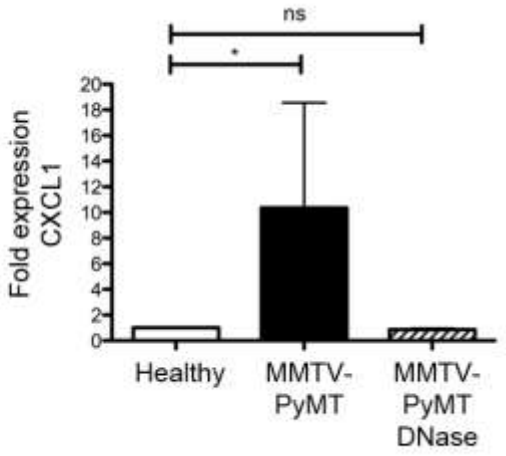
Cedervall et al, Cancer Res, 2015

DNase I treatment suppressed inflammation in the kidney

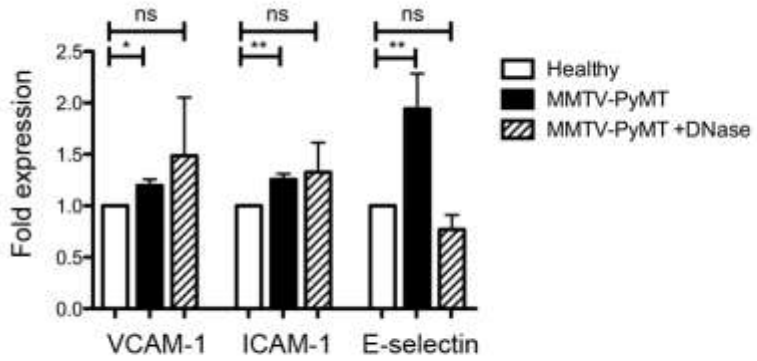
IL-6



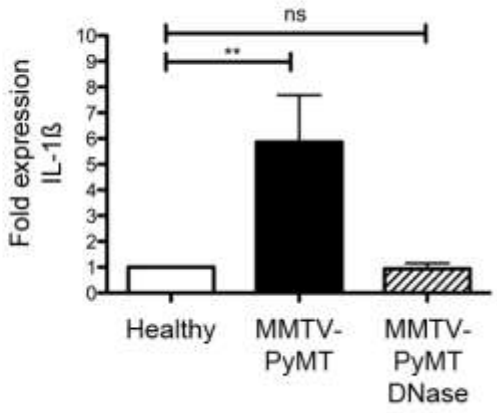
CXCL1



VCAM-1 ICAM-1 E-selectin

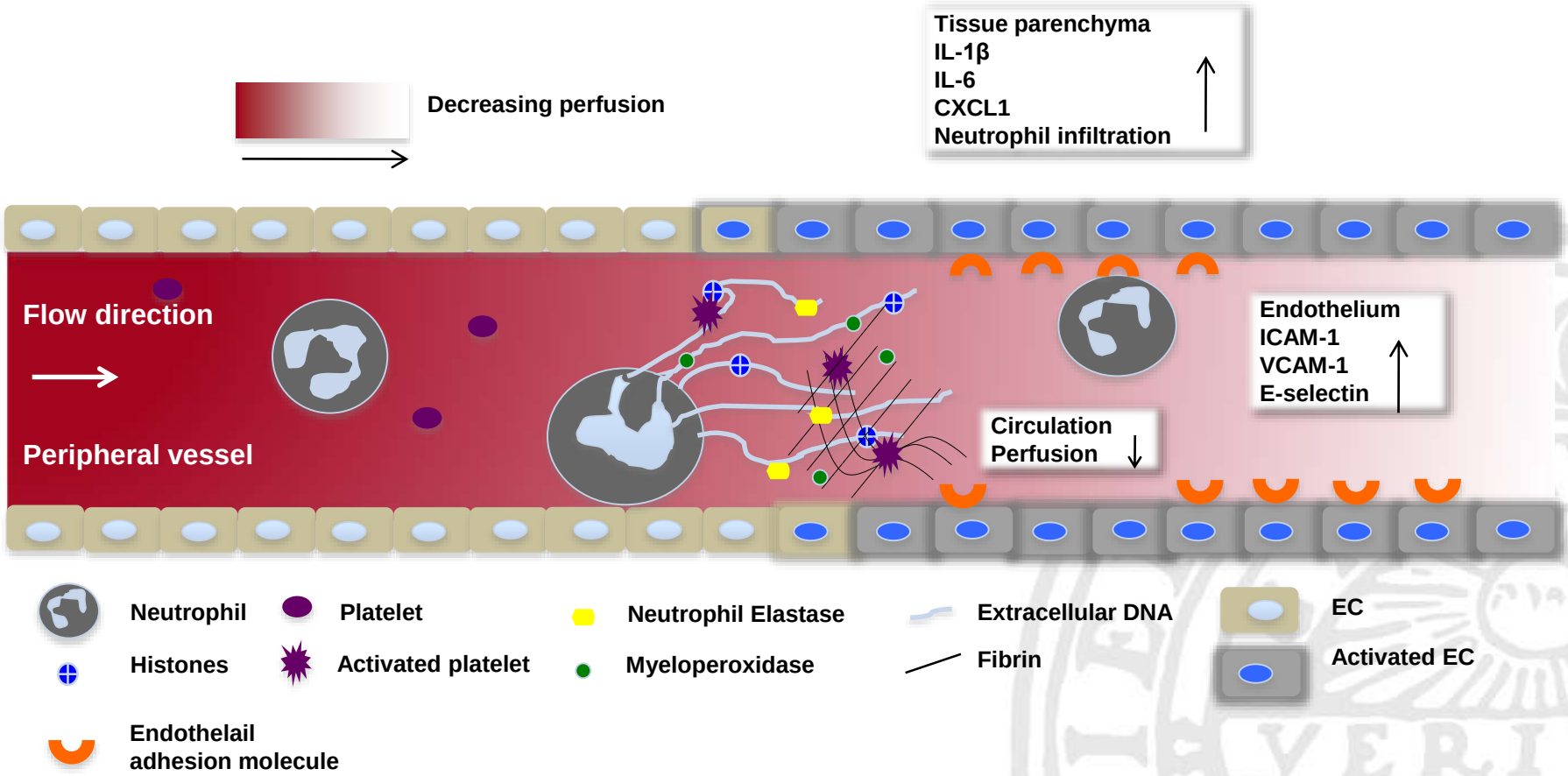


IL-1β



Cedervall et al, Cancer Res, 2015

Tumor-induced intravascular NETs obstructs perfusion and induces systemic inflammation



Cedervall et al, Oncoimmunology, 2015

Can tumor-induced formation of NETs impair distant organ function?

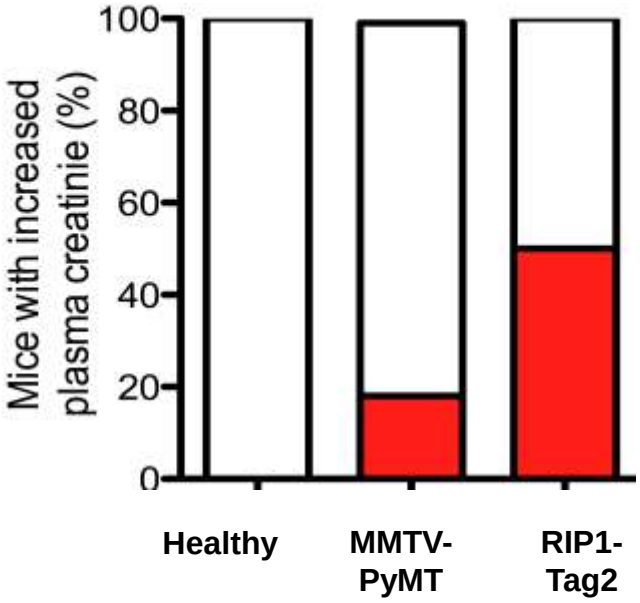
Biomarkers for kidney function

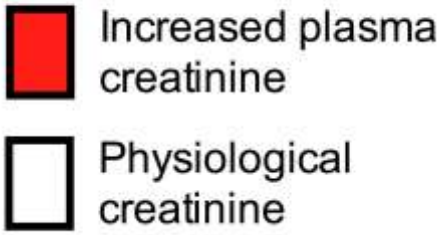
- plasma and urine creatinine
- total protein in urine
- Lipocalin-2/ NGAL in urine and kidney tubuli

  NGAL is an early marker for hypoperfusion (hypoxia) and kidney stress.

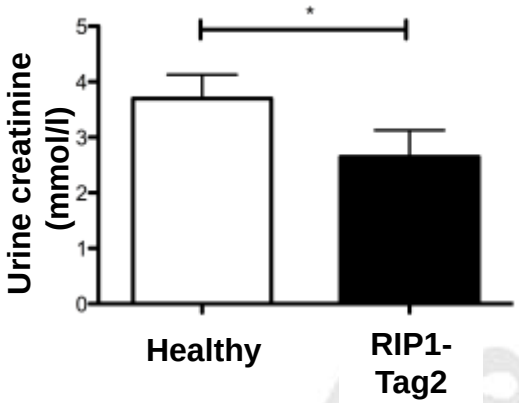
Tumor-bearing mice show clinical signs of renal insufficiency

Plasma creatinine





Urine creatinine



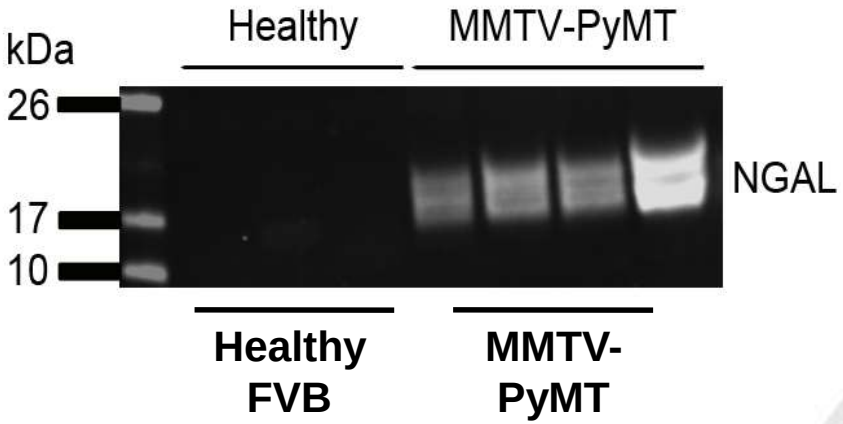
"RIFLE-criteria":

- risk – 1.5-fold increase
- injury – 2-fold increase
- failure – 3-fold increase

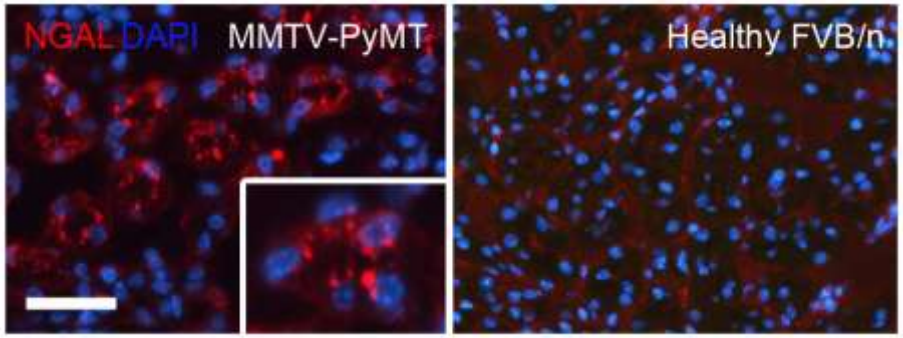
Cedervall et al, Oncoimmunology 2017

Elevated NGAL expression in kidneys from tumor-bearing mice

NGAL Western blot urine



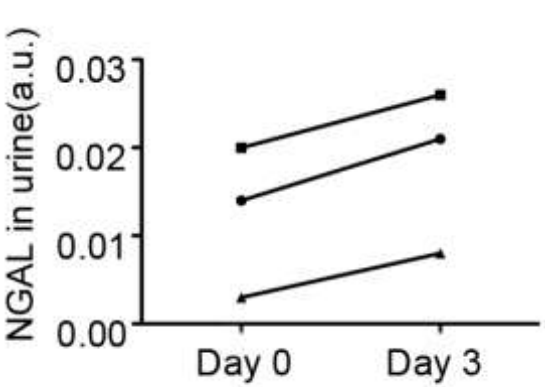
NGAL expression in kidney tubuli cells



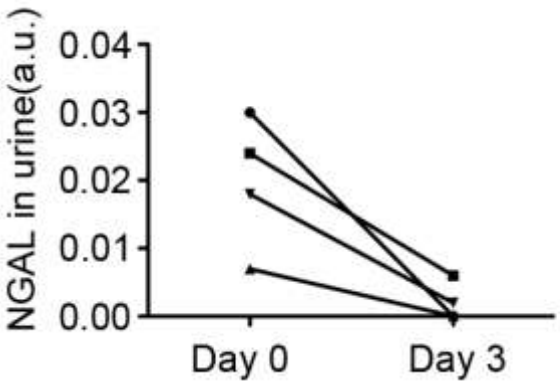
Cedervall et al, Oncoimmunology 2017

NGAL levels in urine decreased after DNase I treatment

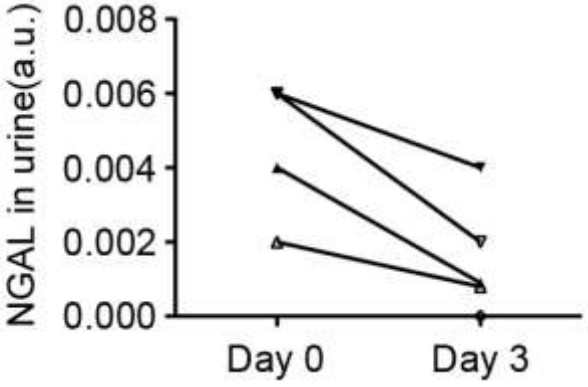
MMTV-PyMT untreated

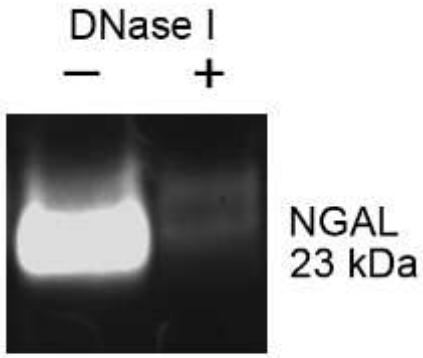


MMTV-PyMT + DNase I



RIP1-Tag2 + DNase I





Cedervall et al, Oncoimmunology 2017

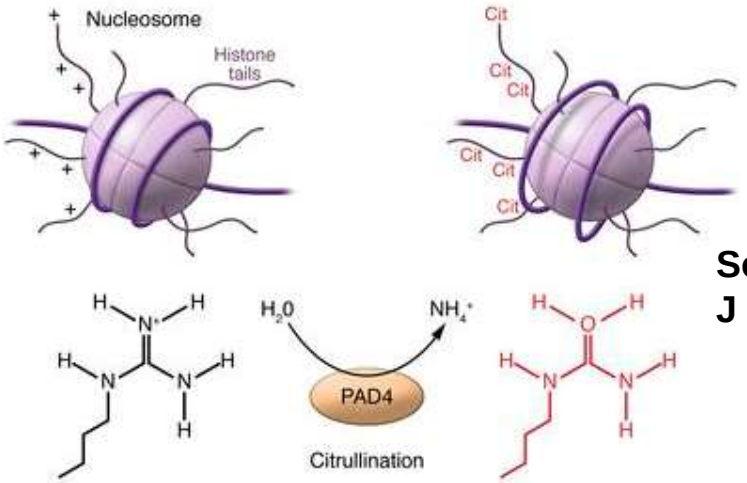
Is DNase I a therapeutic option?

- Established treatment for patients with Cystic Fibrosis (Pulmozyne®, aerosol spray).

- Injection of DNase I showed no safety concerns in patients with the autoimmune disease systemic lupus nephritis (SLE).

Targeting of peptidylarginine deiminase 4 (PAD4) to prevent NETosis

- PAD4 responsible for histone citrullination, essential for NETosis



Sorensen & Borregaard, J Clin Inv 2016

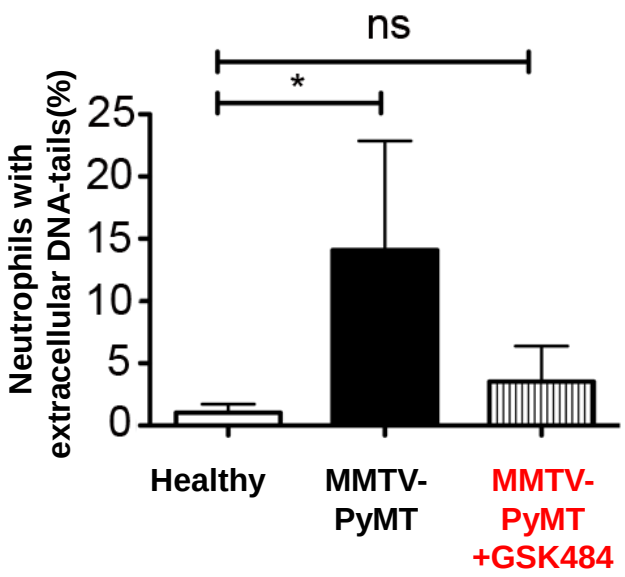
- PAD4-/- mice do not form NETs in response to bacterial infections.

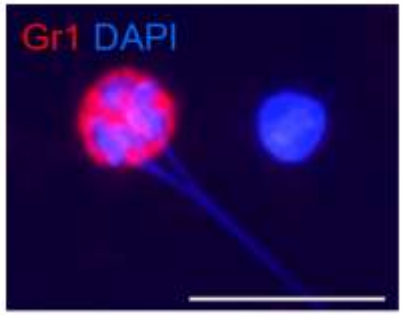
- Inhibitors of PAD4 enzymatic activity recently described and commercially available (GSK484).

Lewis et al, Nat Chem Biol 2015

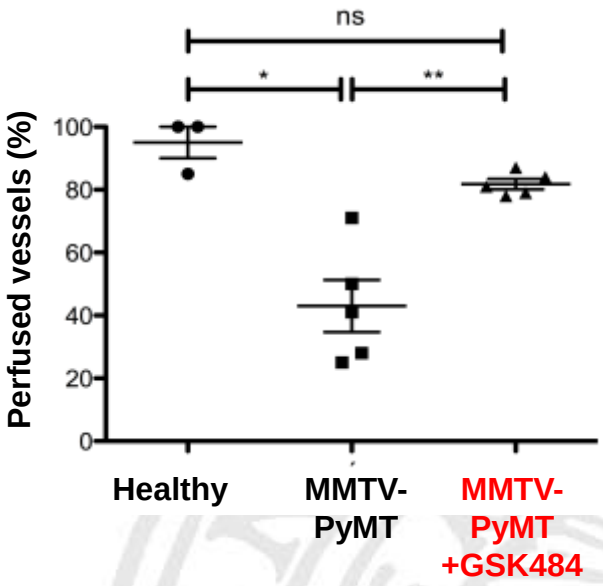
GSK484 suppresses NET formation and restores kidney vascular perfusion

NETs in peripheral blood





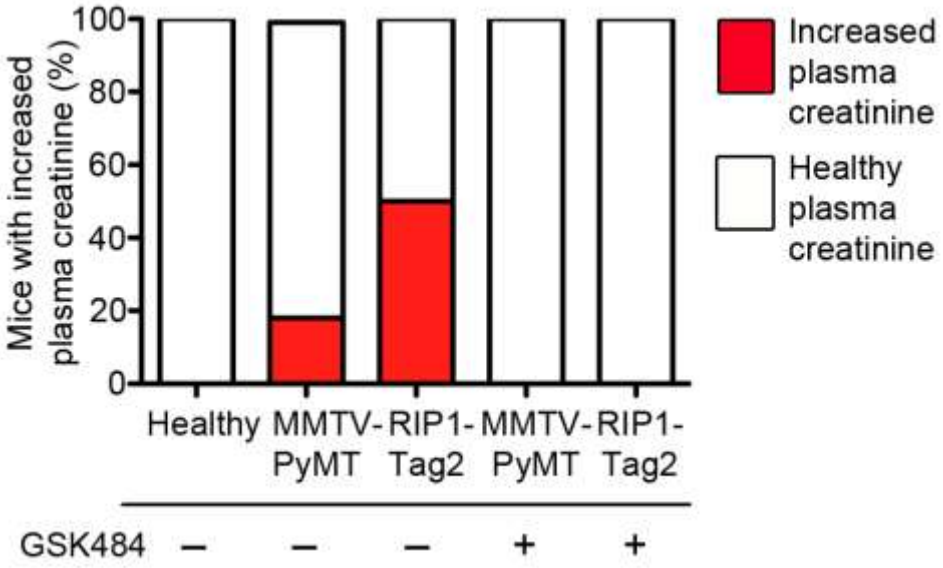
Kidney vascular perfusion



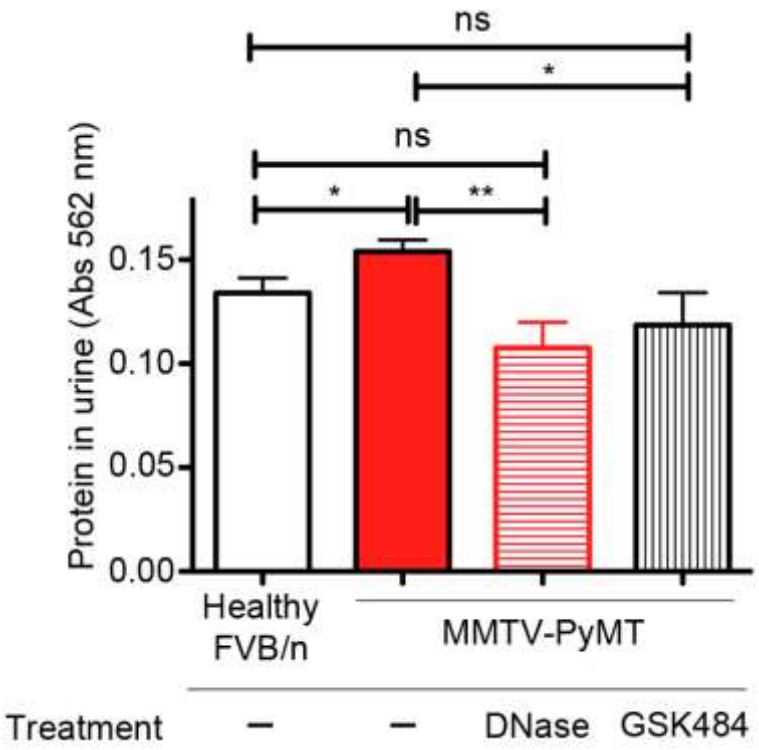
Cedervall et al, Oncoimmunology 2017

Treatment with the PAD4-inhibitor GSK484 improves renal function

Plasma creatinine



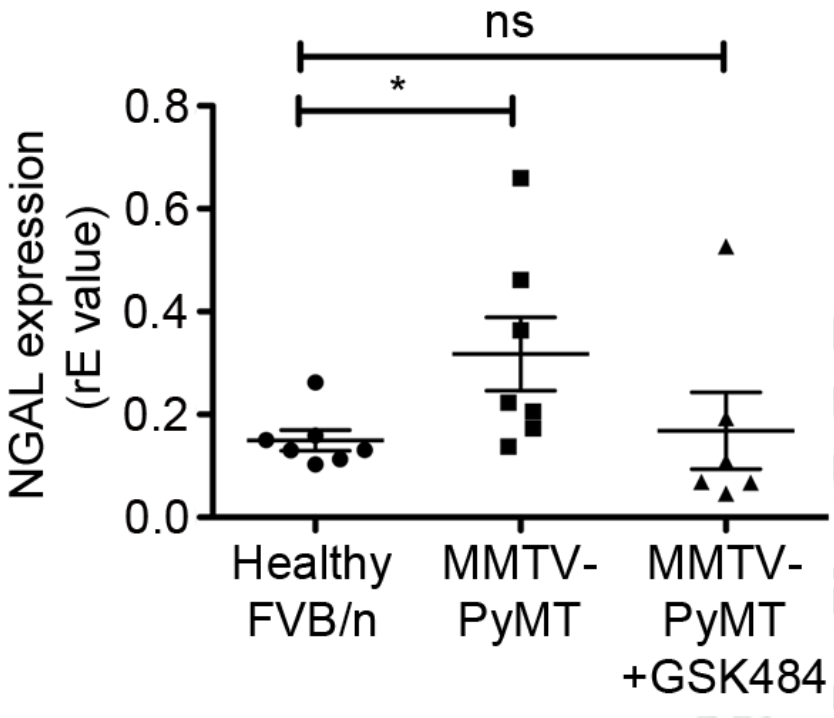
Urine protein



Cedervall et al, Oncoimmunology 2017

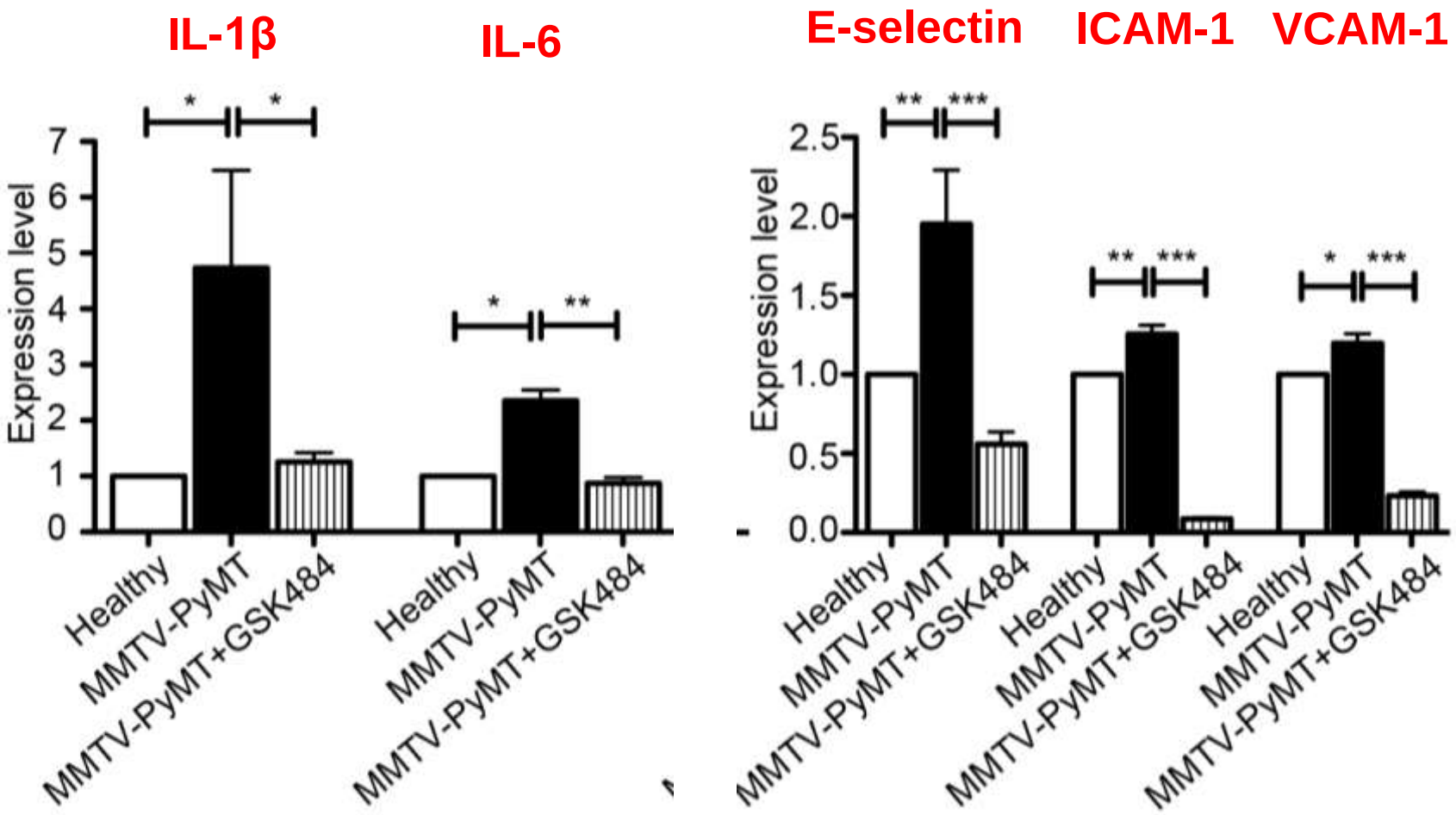
Treatment with the PAD4-inhibitor GSK484 improves renal function

NGAL mRNA expression



Cedervall et al, Oncoimmunology 2017

Suppressed inflammation in the kidney after PAD4-inhibition



Cedervall et al, Oncoimmunology 2017

Conclusions

- Tumor-induced NET formation impairs vascular funcion in distant organs and promotes systemic inflammation.

- Tumor-induced NET formation may be a contributing factor to kidney failure commonly seen in cancer patients.

- NET formation could represent a therapeutic target to prevent cancer-associated kidney injury.

Future – patient samples

- Correlation NETs and biomarkers kidney function?
- Correlation thrombotic events and blood DNase I levels?
- Initiating collaboration on AAV-mediated DNase I delivery in pancreatic cancer patients.

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Cancerfonden

UPPSALA UNIVERSITET

Vetenskapsrådet

NET-inducing signals

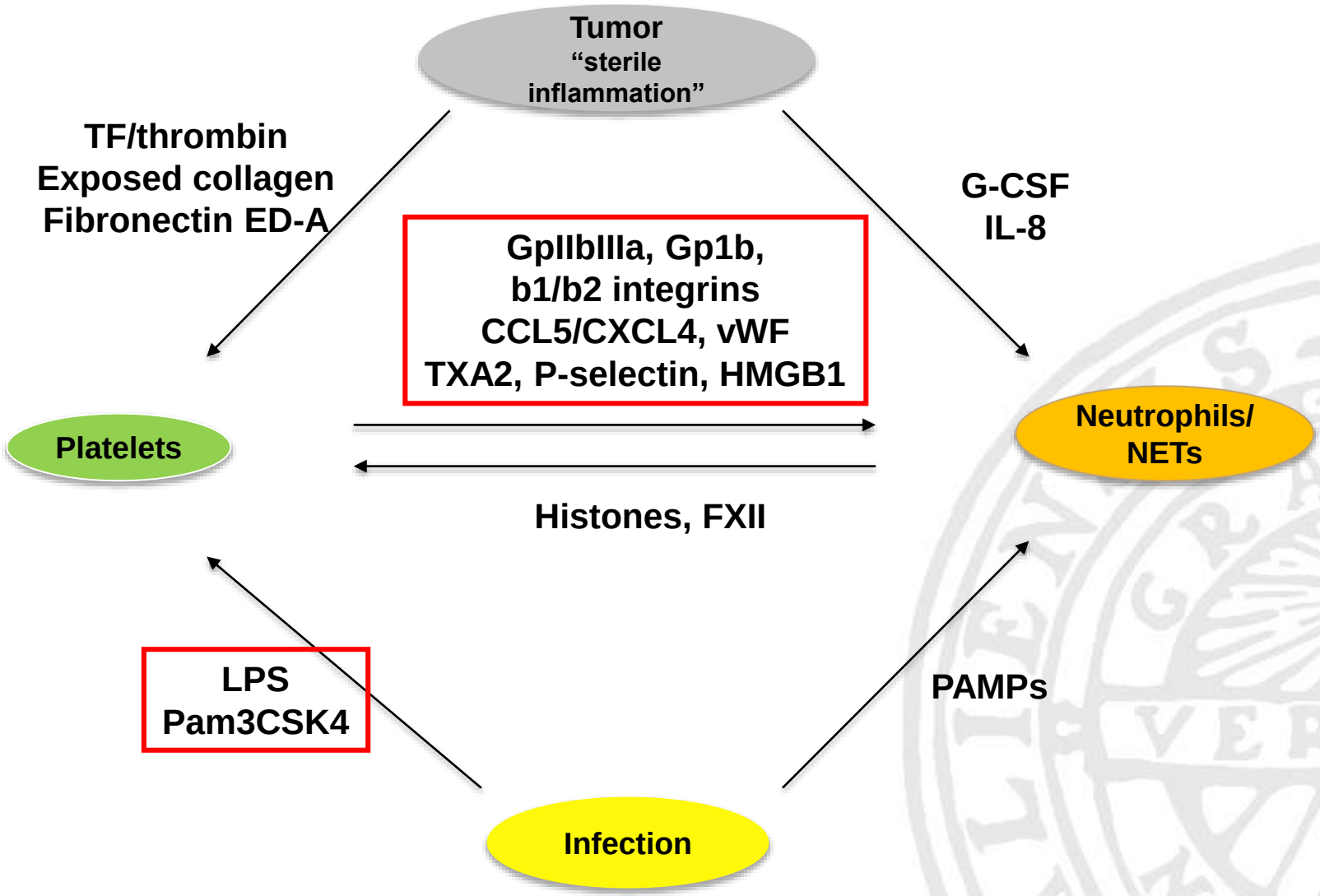
• Infectious agents

- bacterial infections (LPS, PamC3K)
- Virus

• Endogenous factors

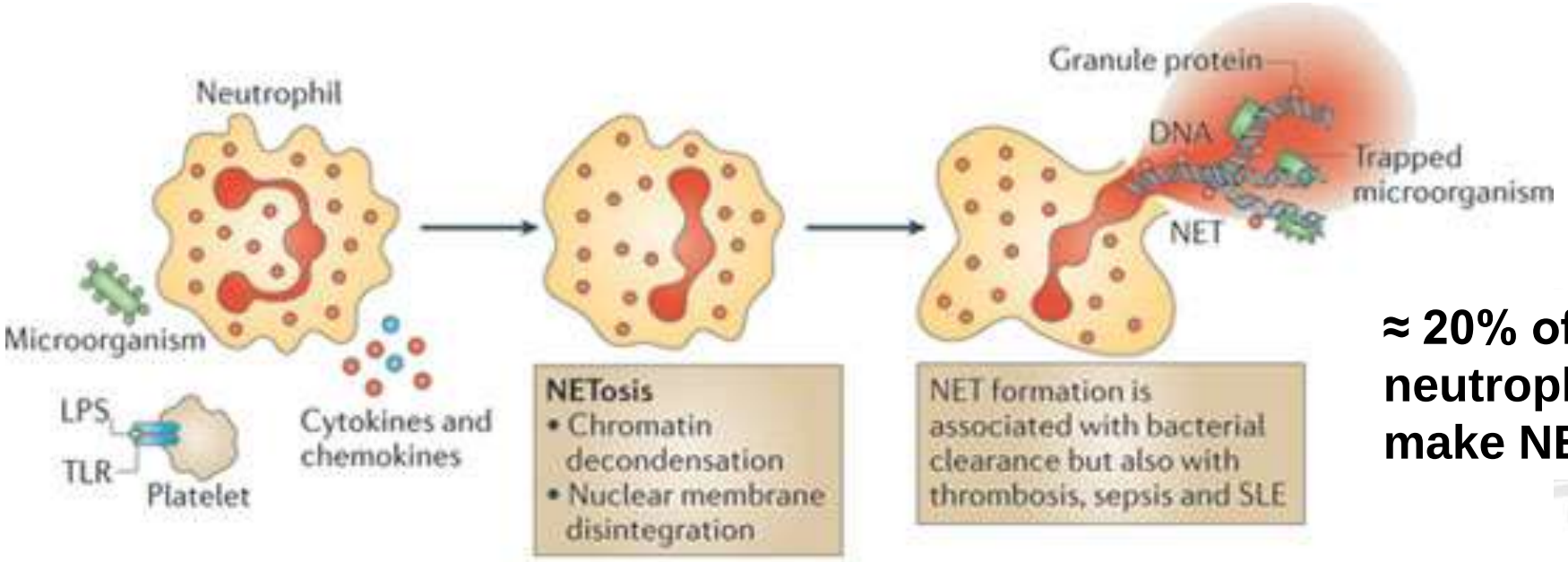
- inflammation
- G/GM-CSF, IL-8, HMGB1
- platelet-mediated signals (integrins, CCL5/CXCL4, vWF, TXA2, P-selectin, HMGB1)
- ischemia – reperfusion
- cholesterol
- high blood glucose

Platelets promote NET formation



Cedervall, Hamidi & Olsson, Thromb Res 2018

Neutrophil extracellular traps – NETs

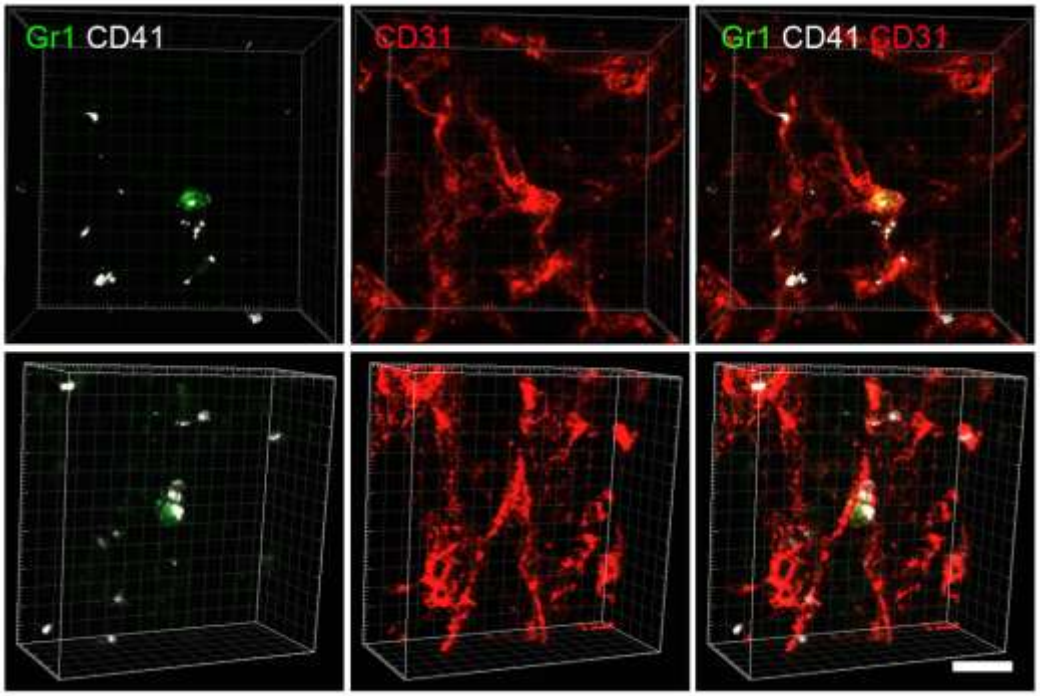


≈ 20% of neutrophils make NETs

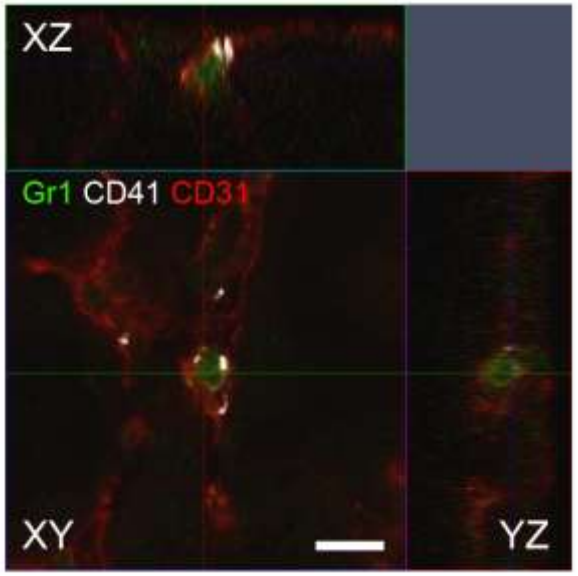
- Activating signal: LPS, IL-8, G-CSF, phorbol ester
- Platelets can induce NETosis; TLR4, P-selectin/PSGL-1

Platelet-neutrophil complexes are located inside the kidney vessel lumen

Kidney tissue from MMTV-PyMT mice



3D confocal reconstruction



Optical sections
three different angles (XZ, XY, YZ)

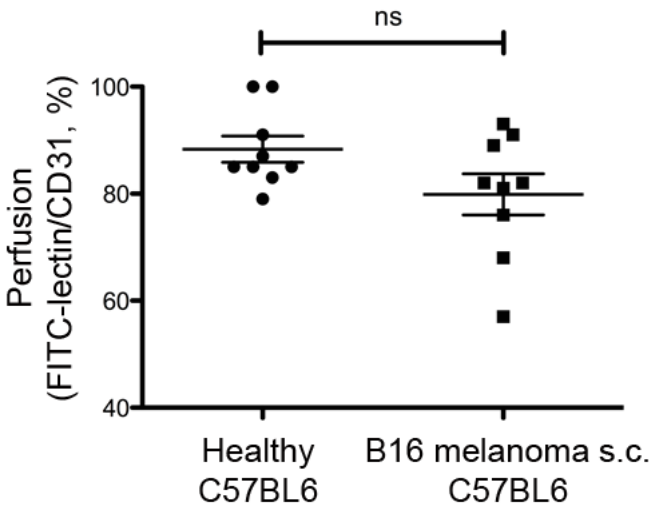
Cedervall et al, Cancer Res, 2015

Which tumor-derived factors are involved in formation of NETs?

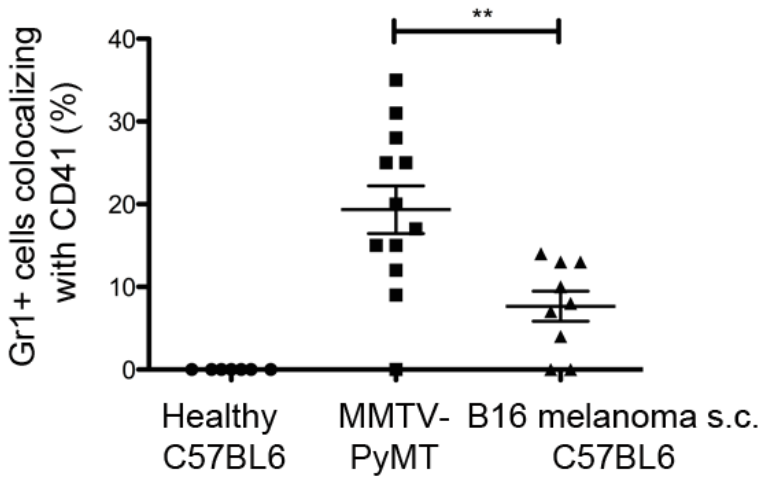
Cedervall et al, Cancer Res, 2015

B16 melanomas do not exhibit impaired function of the peripheral vasculature

Vascular perfusion



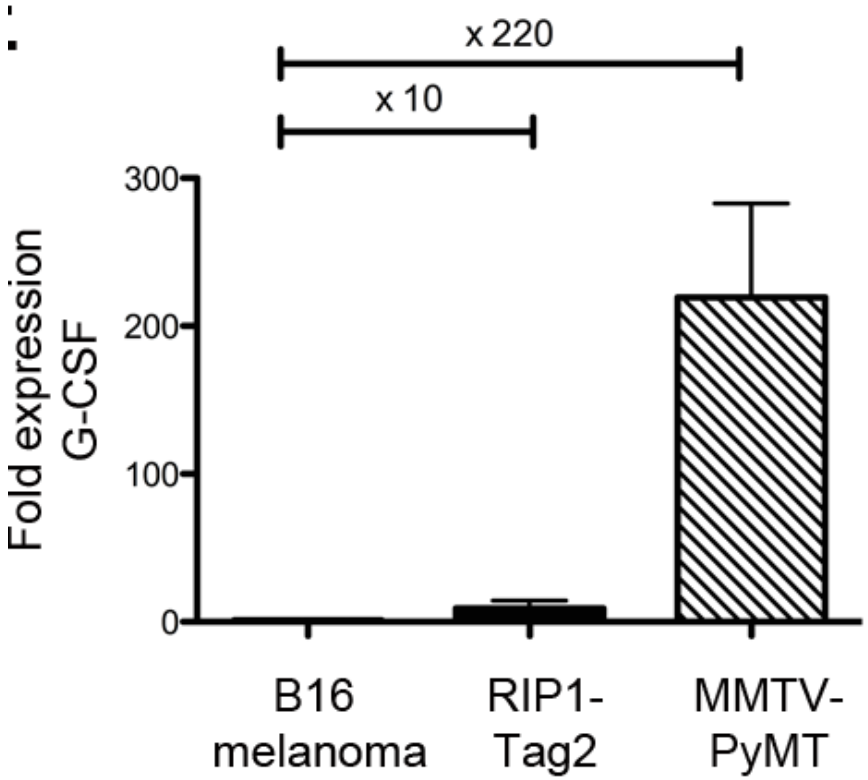
Platelet-neutrophil complexes in the kidney



Subcutaneous B16 melanoma

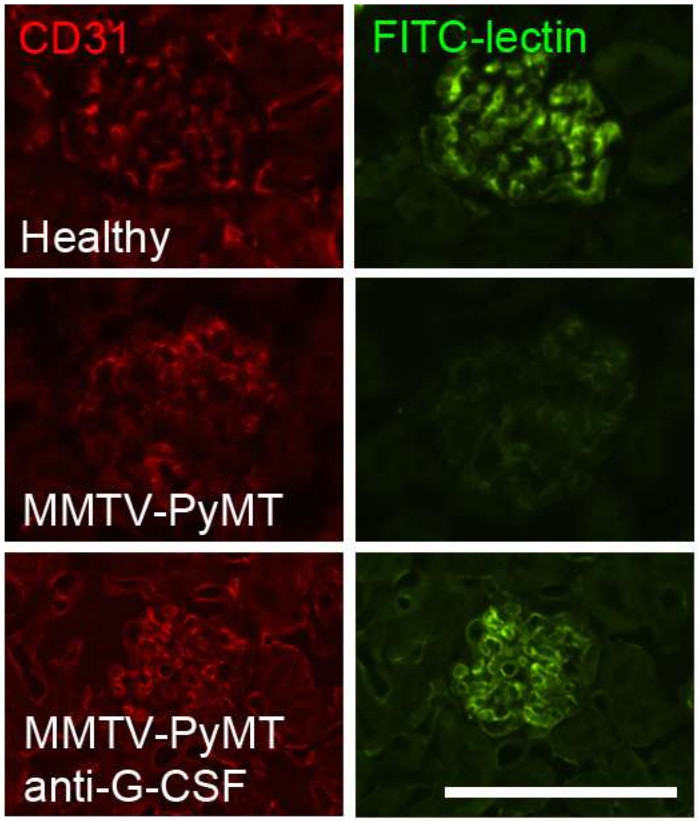
Cedervall et al, Cancer Res, 2015

RIP1-Tag2 and MMTV-PyMT tumors express higher levels of G-CSF than B16 tumors

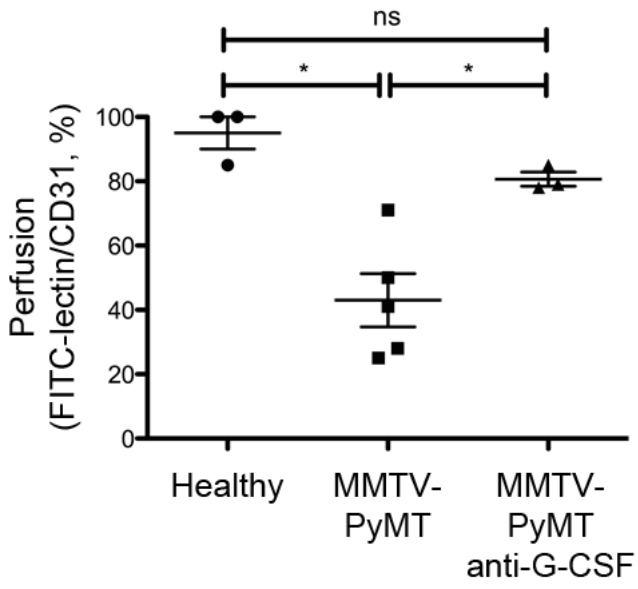


Cedervall et al, Cancer Res, 2015

Anti-G-CSF treatment restores kidney vascular perfusion in tumor bearing mice



Vascular perfusion

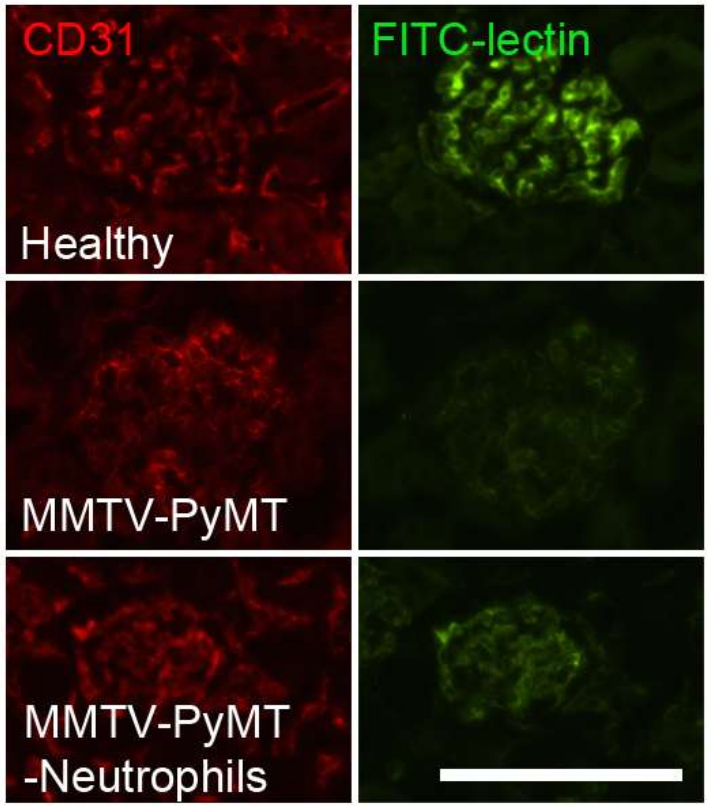


CD31 = blood vessels

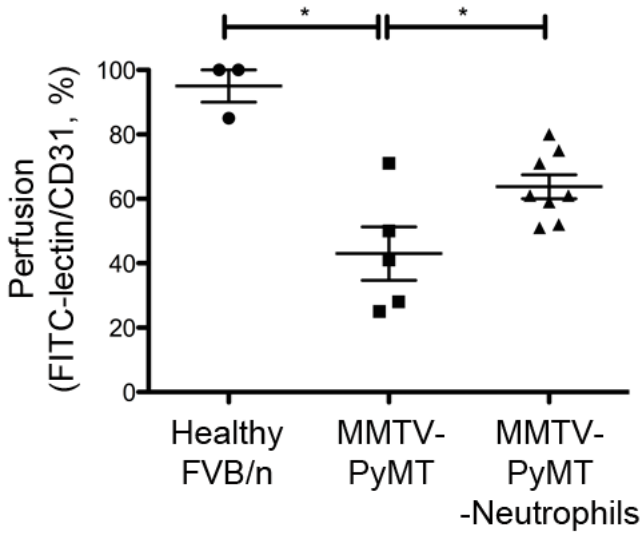
Cedervall et al, Cancer Res, 2015

Neutrophil depletion restores kidney vascular perfusion

Neutrophil depletion by injection of an anti-Gr1 antibody



Vascular perfusion

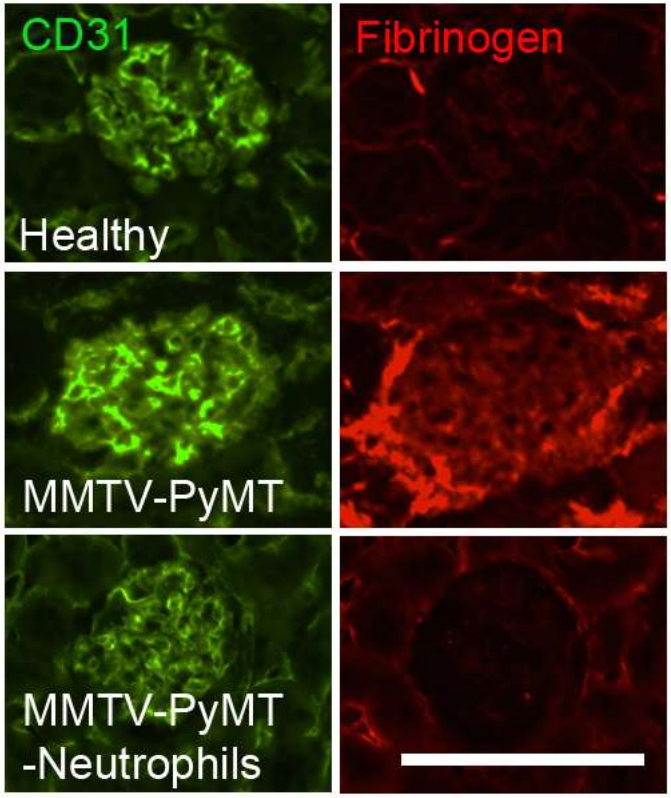


CD31 = blood vessels

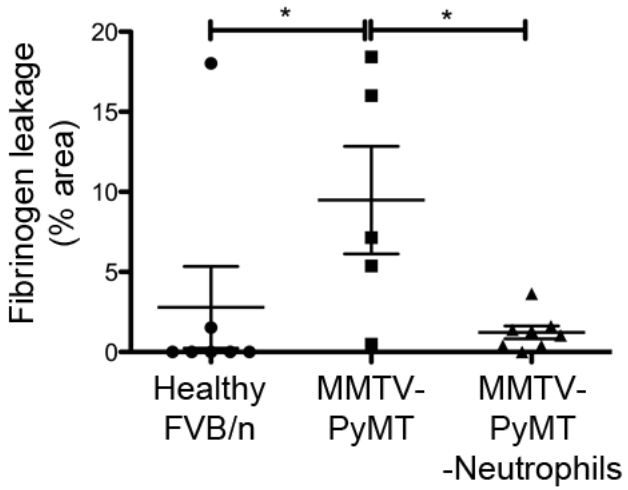
Cedervall et al, Cancer Res, 2015

Neutrophil depletion reduces kidney vascular leakage

Neutrophil depletion by injection of an anti-Gr1 antibody



Vascular leakage



CD31 = blood vessels

Cedervall et al, Cancer Res, 2015

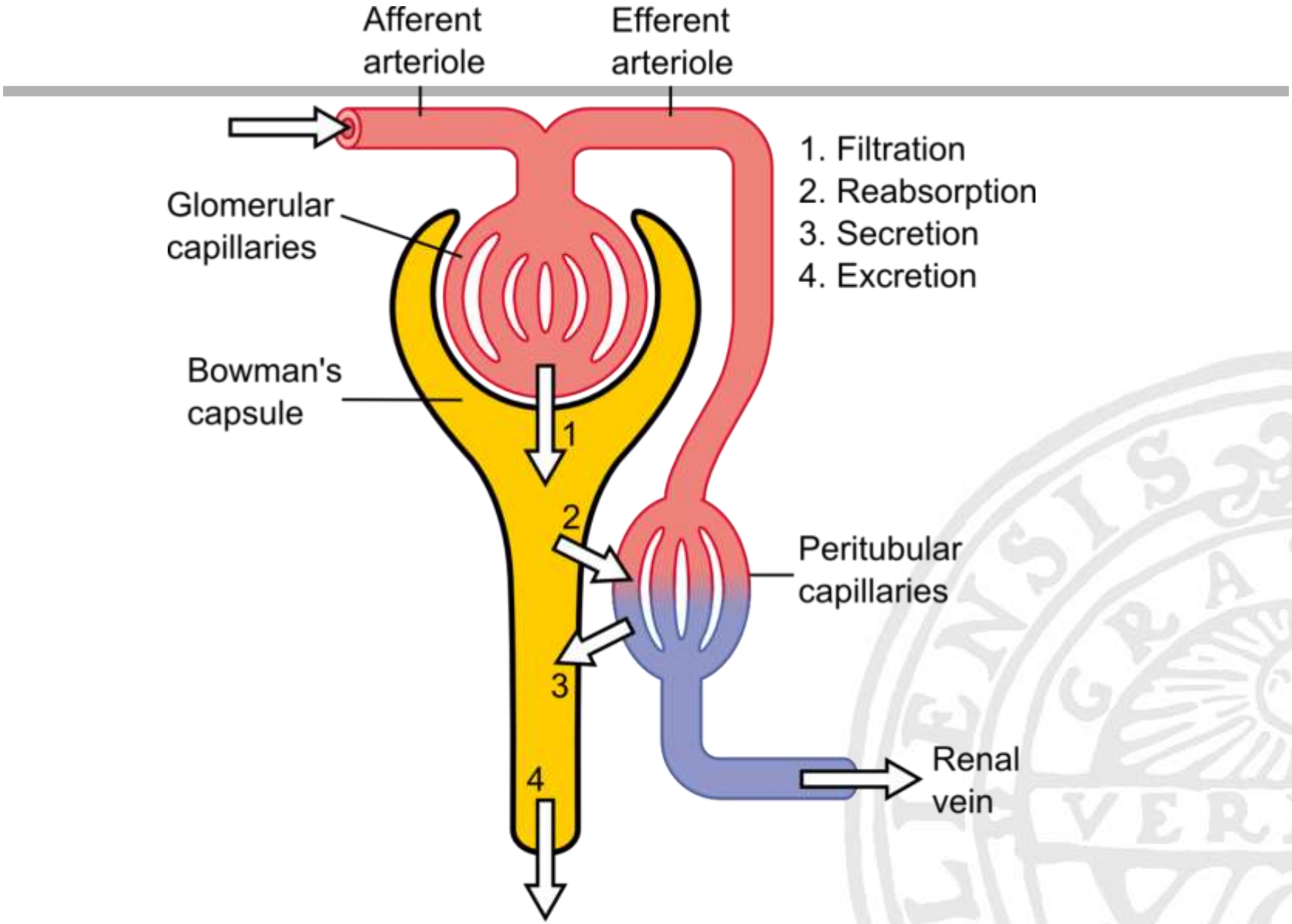
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms

Ofir Wolach,[1,2,3]* Rob S. Sellar,[1,4,5]* Kimberly Martinod,[6] Deya Cherpokova,[6] Marie McConkey,[1] Ryan J. Chappell,[1] Alexander J. Silver,[1] Dylan Adams,[1] Cecilia A. Castellano,[1] Rebekka K. Schneider,[1,7] Robert F. Padera,[8] Daniel J. DeAngelo,[9] Martha Wadleigh,[9] David P. Steensma,[9] Ilene Galinsky,[9] Richard M. Stone,[9] Giulio Genovese,[5,10] Steven A. McCarroll,[5,10] Bozenna Iliadou,[11] Christina Hultman,[11] Donna Neuberg,[9] Ann Mullally,[1,5,9] Denisa D. Wagner,[6] Benjamin L. Ebert[1,5,9†]

April 11, 2018



Excretion = Filtration – Reabsorption + Secretion

Major causes of cancer-related death

- locally disturbed organ function
- systemic organ failure, for instance kidney failure
- thrombosis
- infections
- disturbed balance in body salts and chemicals
- calcium overdose (bone tumors)
- severe weight loss (cachexia) or dehydration

Cancer-associated NET formation – consequences for peripheral vessel and organ function

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IMBIM, Uppsala University