CANCER AND INFLAMMATION PROGRAM

Giorgio Trinchieri

Cancer, Inflammation, and Microbiota

The multiple links between inflammation and cancer

COMMENSAL MICROBIOTA

Chronic Inflammation (Infections, aseptic)

Intrinsic / oncogene induced Inflammation

DNA damage, Mutations (ROS, RNS)

Tumor Promotion

Immune Response

Response to therapy

Co-morbidity

Immuno Evasion

Cancer Initiation and Progression

Tumor growth

Cancer-associated Inflammation

Angiogenesis

Infiltration and metastasis
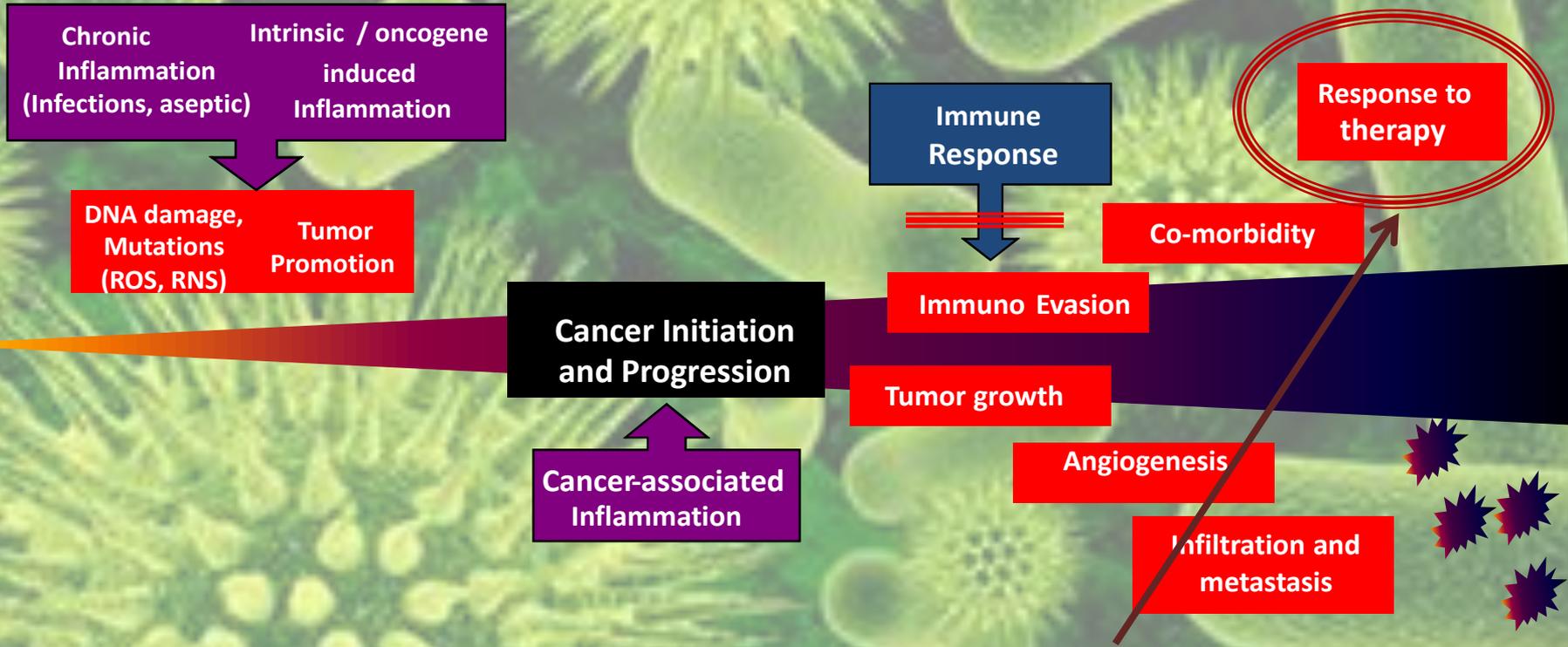
Inflammation and response to immunotherapy and chemotherapy

Humans have co-evolved with microbial partners
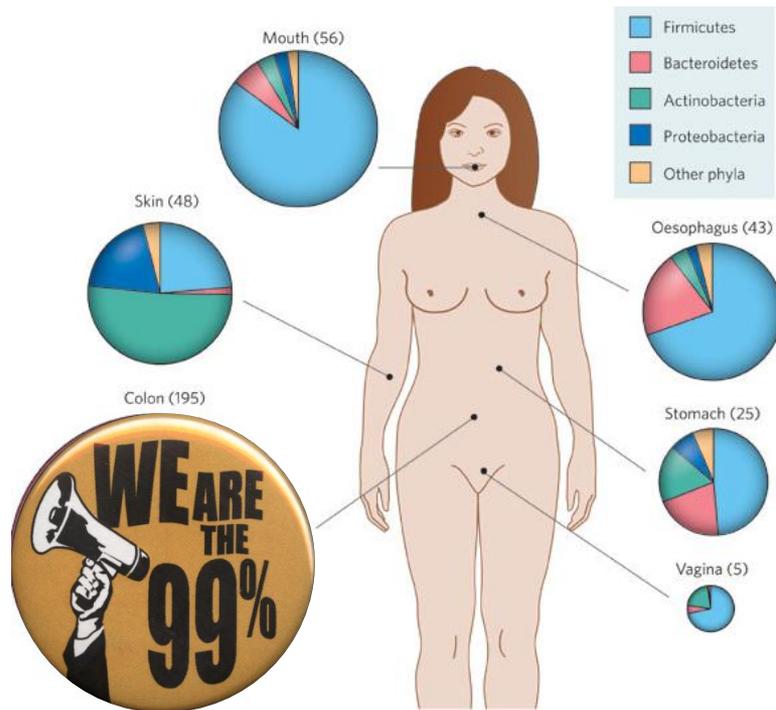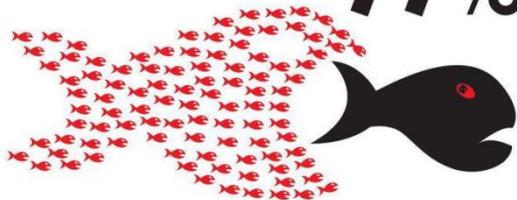
- **We are a composite of species: bacterial, fungi, viruses**

- **In and on our bodies, our microbial cells outnumber the human cells by about 10 fold**

- **In the intestine, the total microbial DNA (the microbiome) may contain 100 times more genes than our 'own' human genome**

- **The microbiome is an integral part of our genetic landscape and regulate metabolic functions**

- **The development of the immune system is dependent on interactions with the commensal microbiota**

Colon rectal carcinoma

Hepatocarcinoma
Mammary carcinoma
Thymic lymphoma

Tumors

Immune response to influenza virus infection

Experimental immune encephalomyelitis

Development of the immune system

Inflammatory bowel disease

Gut microbiota

Mucosal immunity

Bacteria or MAMPs transmucosal translocation

Systemic inflammatory tone, oxidative stress, leukocyte or epithelial cell genotoxicity

NAFLD

Obesity
Metabolic syndrome
Insulin resistance

Autoimmune arthritis

Hepatobiliary-pancreatic autoimmune disease

The price of immunity

How Commensal Bacteria affect
Local and Systemic Inflammation/Immunity?
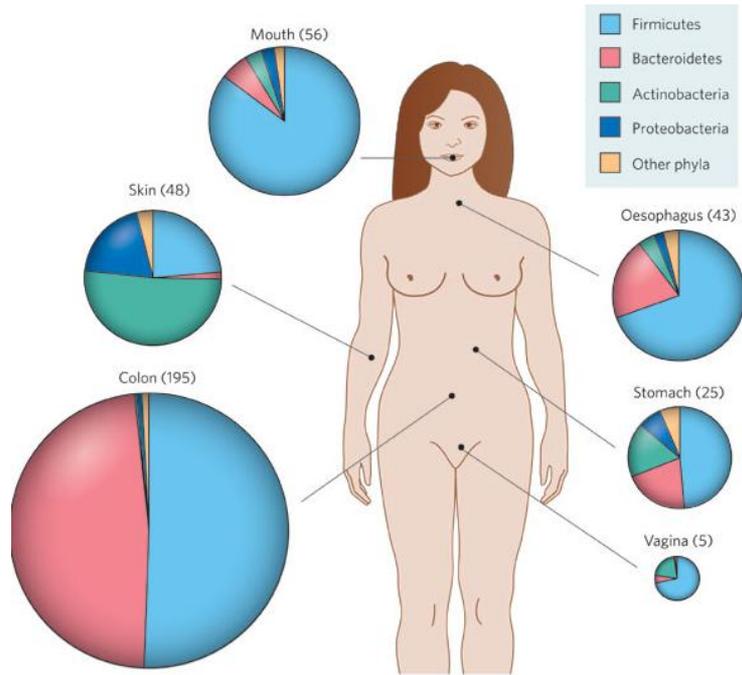
Gut commensals regulate mucosal immunity

Intestinal microbiota

Mucosal Immunity
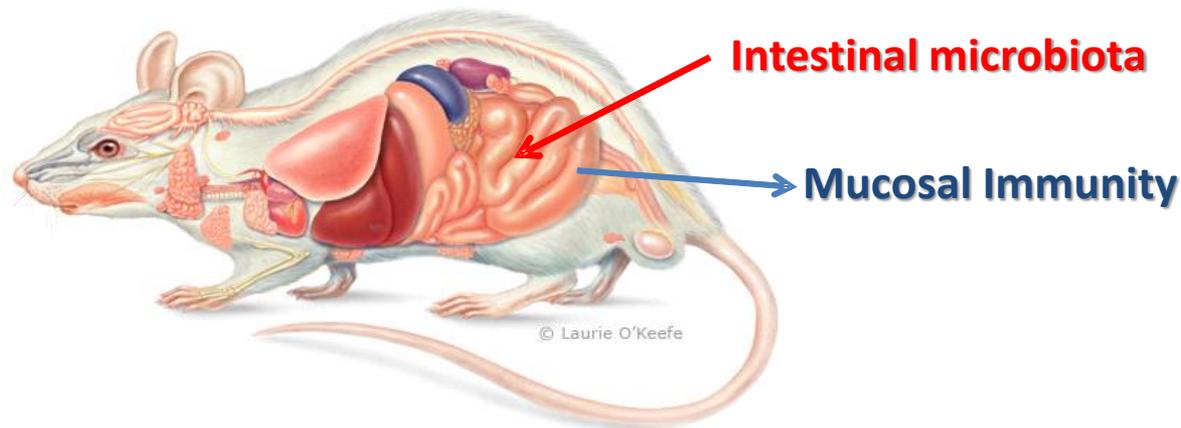
How Commensal Bacteria affect Local and Systemic Inflammation/Immunity?
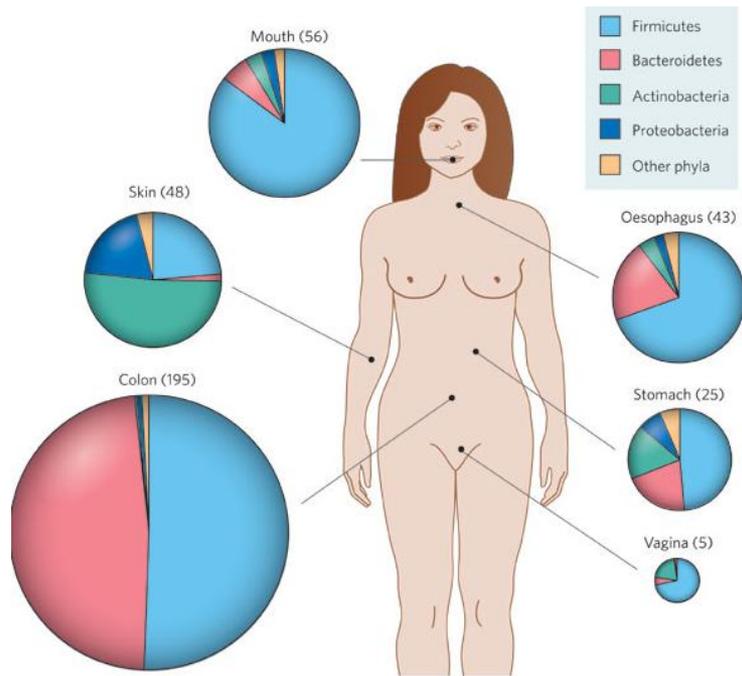
Science **337**, 1115 (2012)

Compartmentalized Control of Skin Immunity by Resident Commensals

Shruti Naik,[1,2] Nicolas Bouladoux,[1] Christoph Wilhelm,[1] Michael J. Molloy,[1] Rosalba Salcedo,[3,4] Wolfgang Kastenmuller,[5] Clayton Deming,[6] Mariam Quinones,[7] Lily Koo,[8] Sean Conlan,[6] Sean Spencer,[1,2] Jason A. Hall,[9] Amiran Dzutsev,[3,4] Heidi Kong,[10] Daniel J. Campbell,[11,12] Giorgio Trinchieri,[3] Julia A. Segre,[6] Yasmine Belkaid[1]*
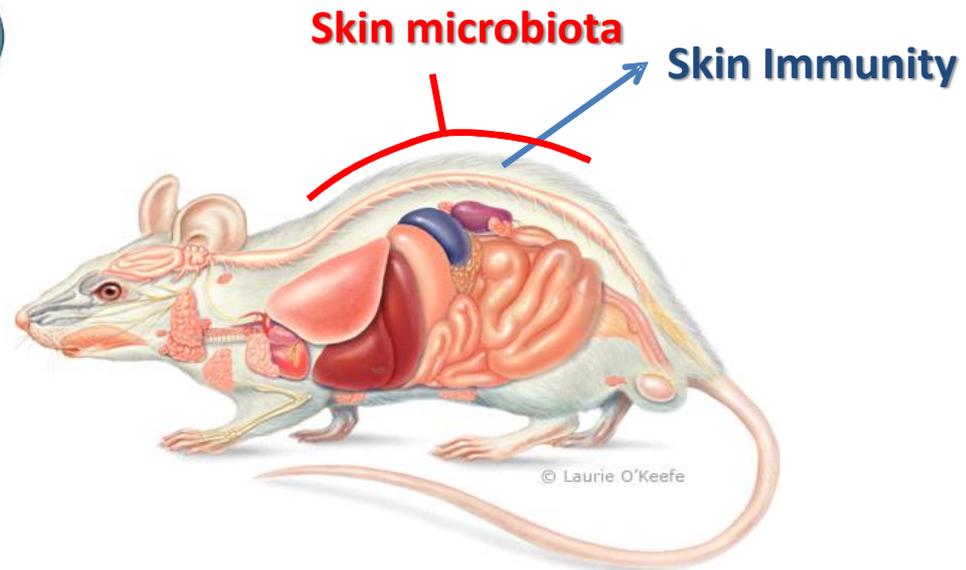
Skin microbiota

Skin Immunity

© Laurie O'Keefe

How Commensal Bacteria affect
Local and Systemic Inflammation/Immunity?
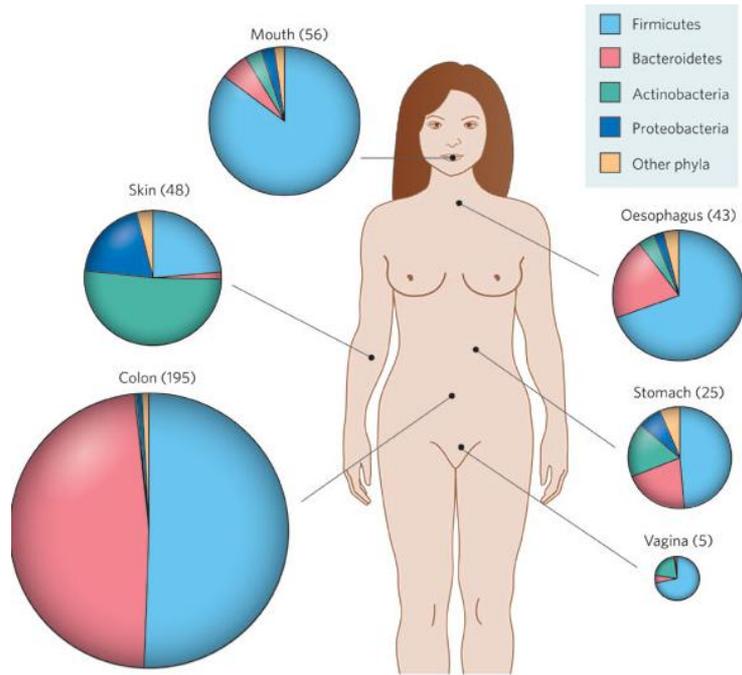
Gut commensals regulate systemic immunity and "Inflammatory Tone"

Intestinal microbiota
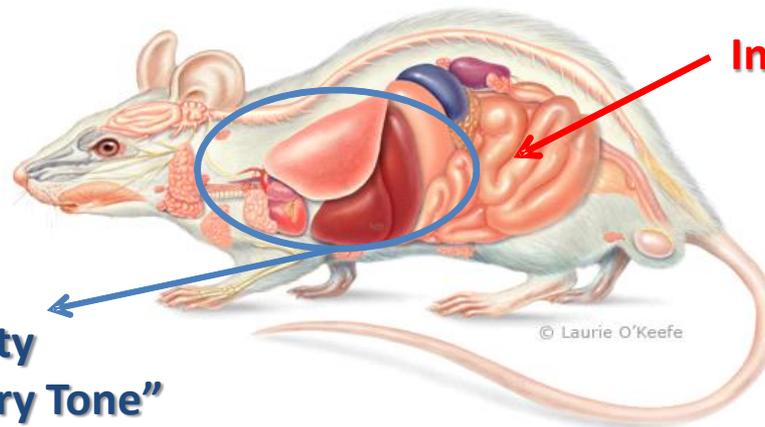
**Systemic Immunity
and "Inflammatory Tone"**

Response to cancer therapy

How Commensal Bacteria affect
Local and Systemic Inflammation/Immunity?

Mouth (56)

Firmicutes
Bacteroidetes
Actinobacteria
Proteobacteria
Other phyla

Skin (48)

Oesophagus (43)

Colon (195)

Stomach (25)

Vagina (5)

Intestinal microbiota

Sensing of luminal bacteria

© Laurie O'Keefe
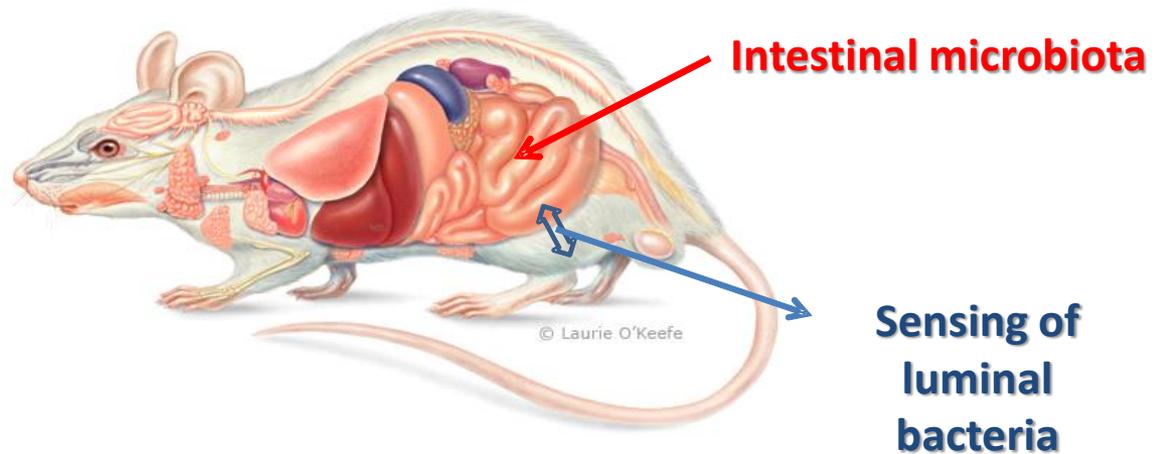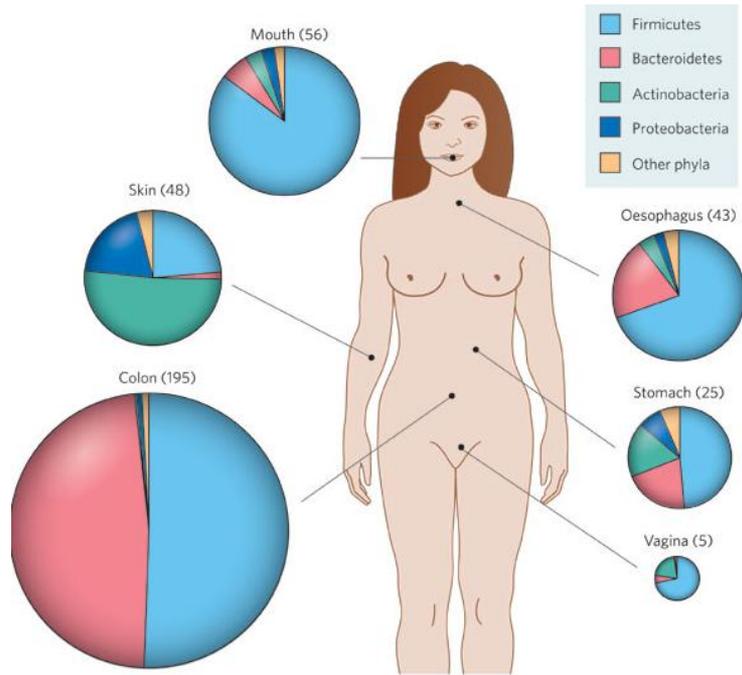
Small intestine

Colon

Gut associated lymphoid tissue

Peripheral immune system

Bacteria
Inert molecules
<600 Da
Cations

Tight junction
Adherens junction
Claudins/occludin
ZO1/2
Actomyosin
JAM-A
Desmosome

Dendritic cell

Paracellular transport pathway

Dietary antigens

Microbes

M cell
Endosome
Lysosome
Golgi
MIIC
Epithelial exosomes

APC

Degradative route 90%
Non-degradative route <10%

Transcellular transport pathways

T cell

M cells

Mucus secreting Goblet cells

Outer mucus layer
Inner mucus layer

sIgA

Plasma cell

Dendritic cell

CD4$^+$ T cell

T$_H$1
T$_H$2
T$_H$17
Treg

Peyer' patch

Colonic patch

Autoimmunity
Allergy
Immunoactivation
Metabolic diseases
Carcinogenesis

Mesenteric lymph nodes

LUMEN

Flagellin, ATP

CCL20

CCL20

Epithelium

Basal membrane

Lamina Propria

CX3CR1+

TSLP, TGF-β, RA

CD103+
CD11b+
CCR6+

LUMEN

Flagellin, ATP

CCL20

CCL20

Epithelium

Basal membrane

Lamina Propria

CX3CR1+

TSLP, TGF-β, RA

CD103+
CD11b+
CCR6+

CP IEC

0.7%

72.0%

8.3%

CP w/o IEC

CD103

21.8%

38.2%

14.1%

CD11b

Dendritic cells residing in the CP-associated epithelium have CD103+CD11b+ phenotype

CD11c
Basal membrane

CP (view from the lumen)

Crypts

IE DCs concentrate around CP-associated crypts
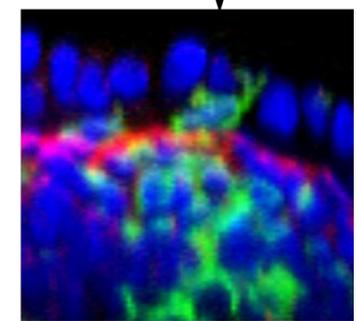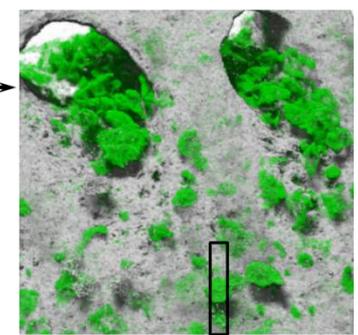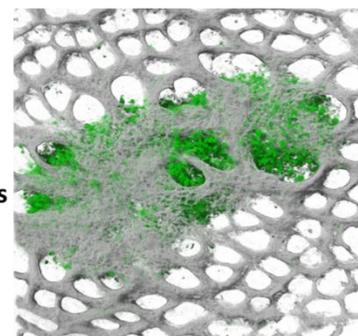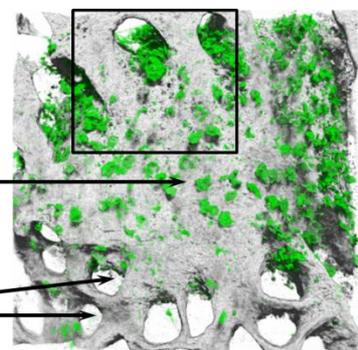
Nuclei, CD11c, Collagen IV
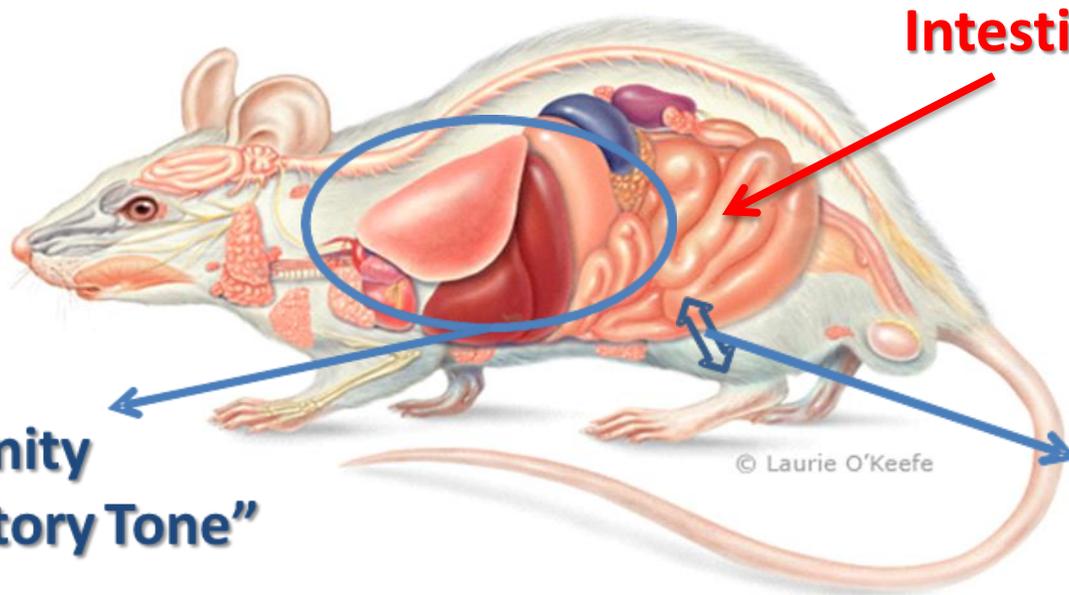
Intestinal microbiota

Systemic Immunity and "Inflammatory Tone"

Sensing of luminal bacteria

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Tumor cell line

Subcutaneous transplanted tumor

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Is the response to cancer therapy regulated by the commensal bacteria?

Systemic anti-IL-10R + Intratumor CpG-OGN immunotherapy
Platinum compound (oxaliplatin, cisplatin) chemotherapy

Intestinal microbiota
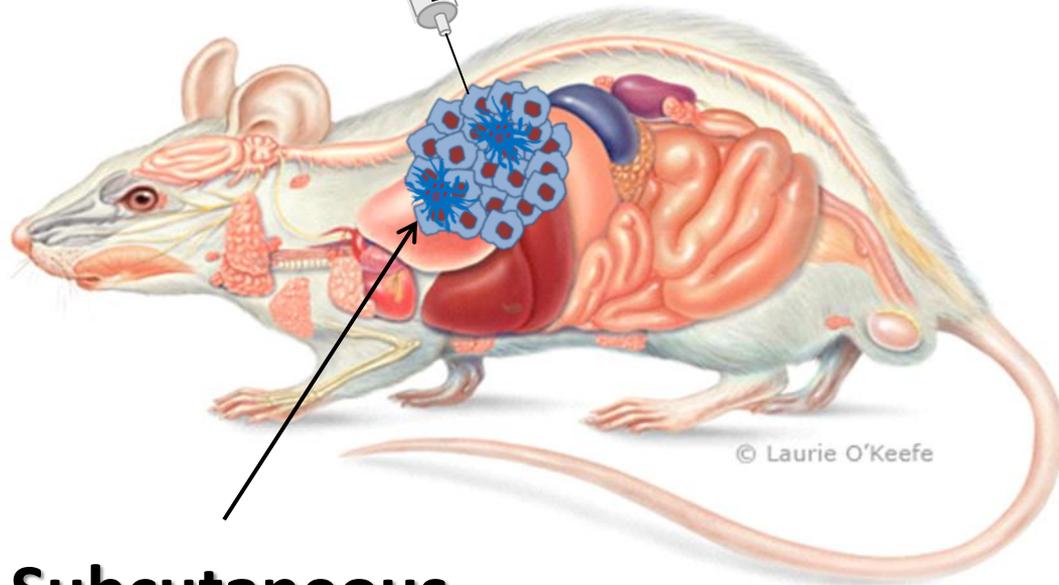
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Subcutaneous transplanted tumor

Is the response to cancer therapy regulated by the commensal bacteria?

Systemic anti-IL-10R + Intratumor CpG-OGN immunotherapy
Platinum compound (oxaliplatin, cisplatin) chemotherapy

ANTIBIOTICS

Neomycin
Vancomycin
Imipenem
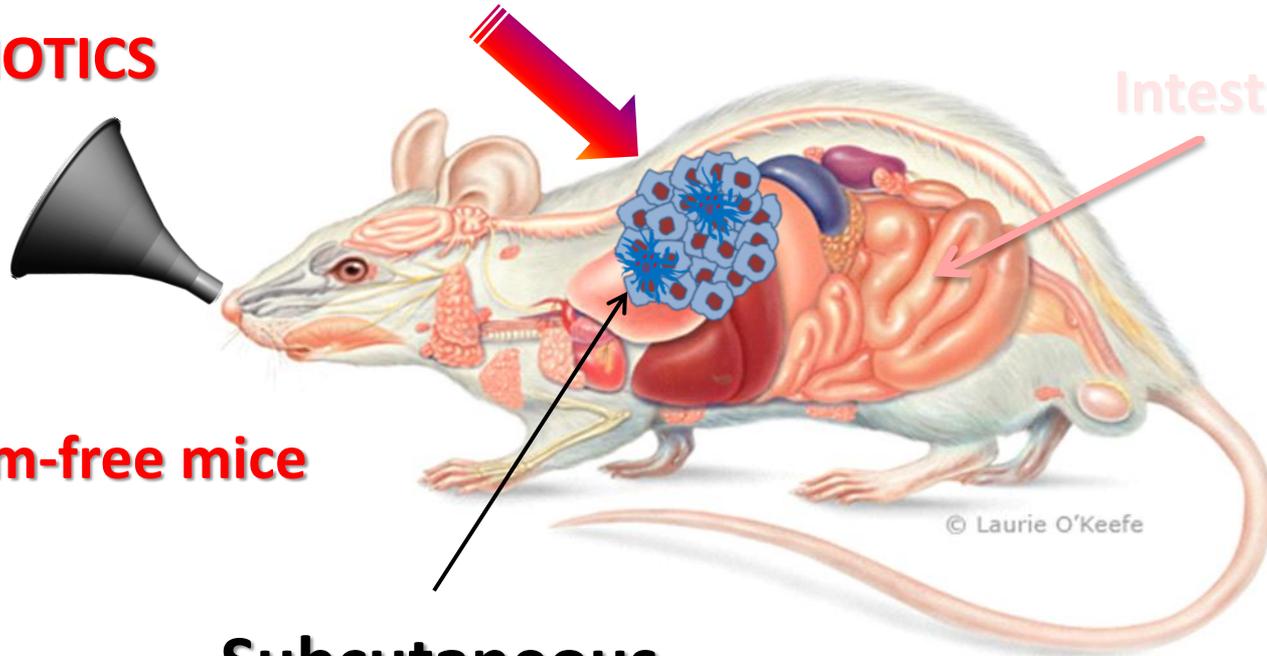
Intestinal microbiota

or Germ-free mice

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Subcutaneous transplanted tumor

CpG/anti-IL-10R anti-tumor immune therapy model
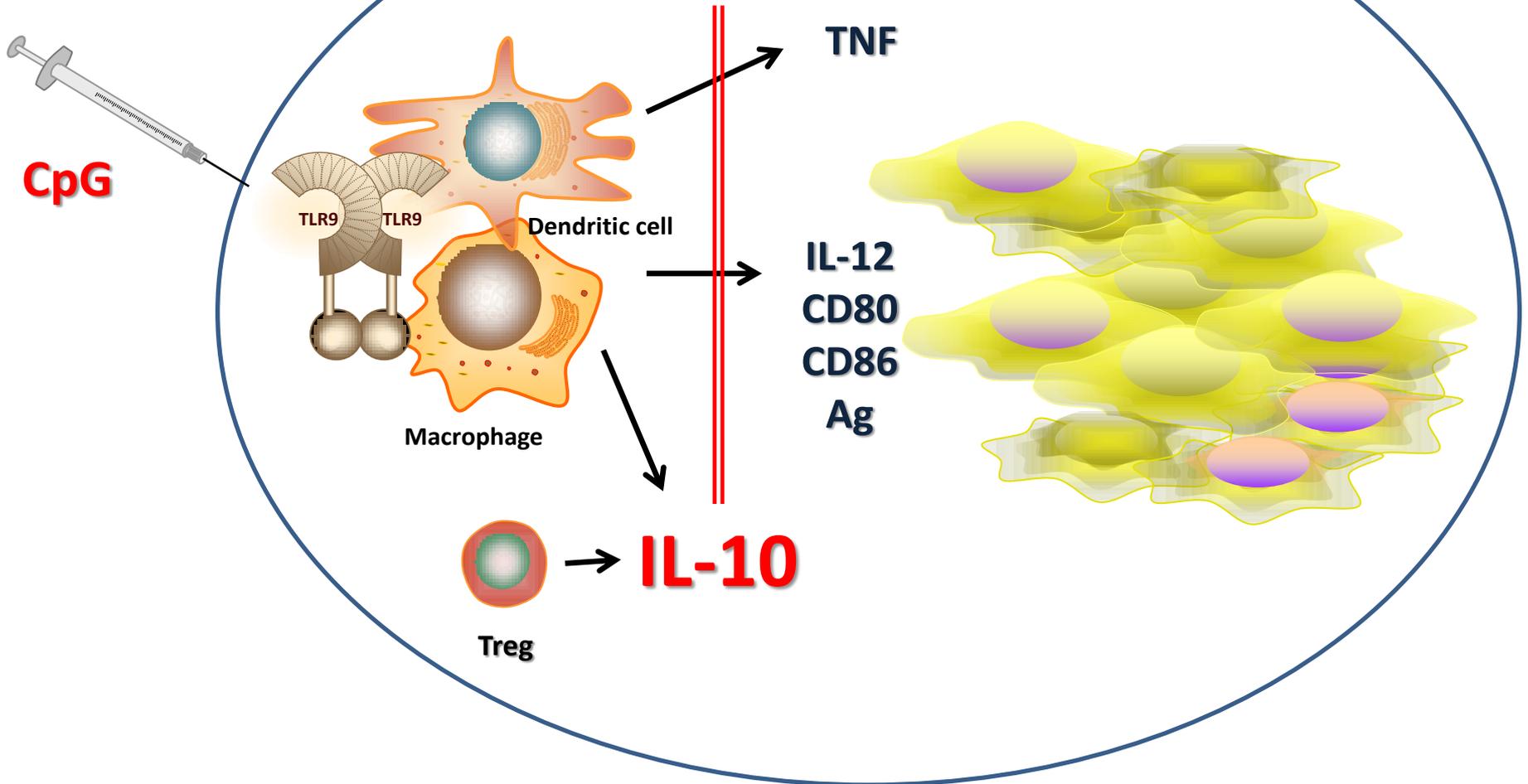
Redirecting In vivo Elicited Tumor Infiltrating Macrophages and Dendritic Cells towards Tumor Rejection
Cristiana Guiducci, Alain P. Vicari, Sabina Sangaletti, Giorgio Trinchieri, and Mario P. Colombo.
Cancer Res 2005; 65(8): 3437-46

CpG/anti-IL-10R anti-tumor immune therapy model

Tumor

CpG

TLR9 TLR9

Dendritic cell

Macrophage

Treg

Anti IL-10R

TNF

IL-12
CD80
CD86
Ag

Hemorragic Necrosis (6-48 h)

Adaptive anti-tumor Immunity >7 days

Th1
Cytotoxic T cells

Antibiotics suppress the anti-tumor effect of anti-IL-10R/CpG therapy
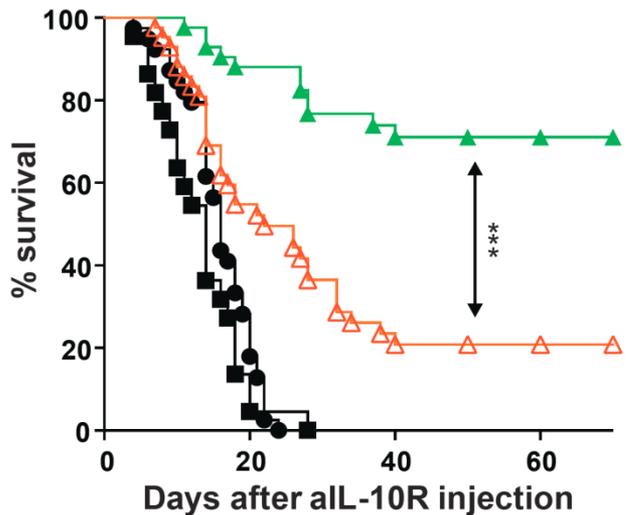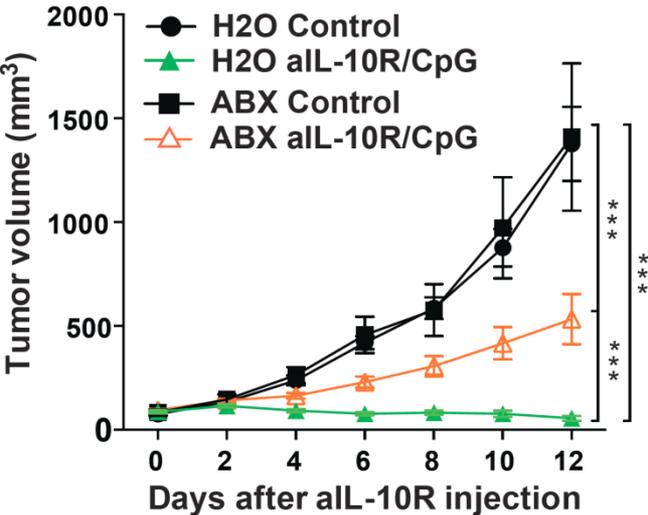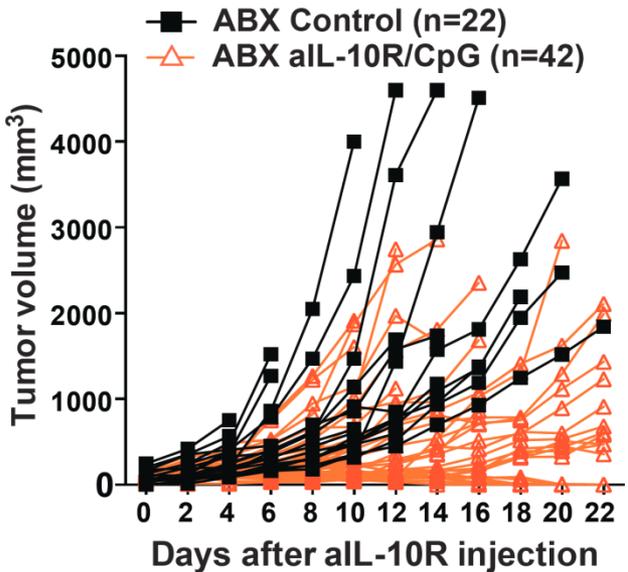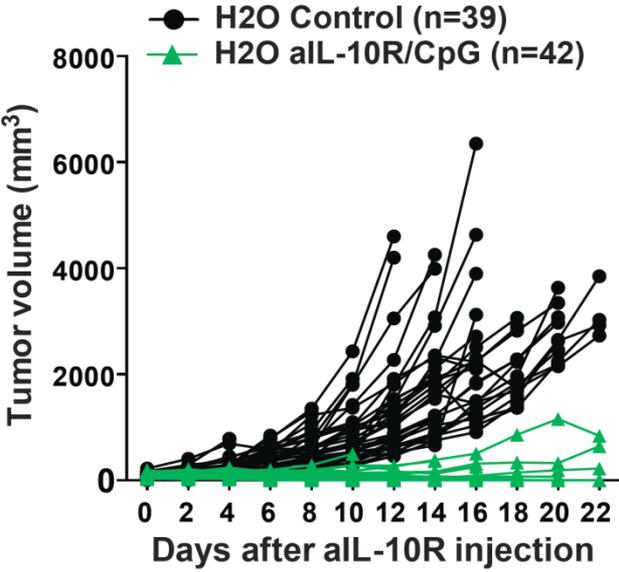
MC38 subcutaneous tumor

ABX suppresses early necrosis of the tumor

Aspect of tumors 72 h after CpG treatment

WT (BL6Ncr)　　　**TNFKO**

% Necrosis after treatment

H2O untreated

H2O aIL-10R CpG

ABX aIL-10R CpG

MCA38

1 cm
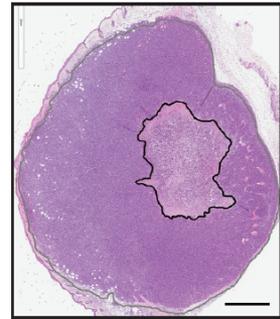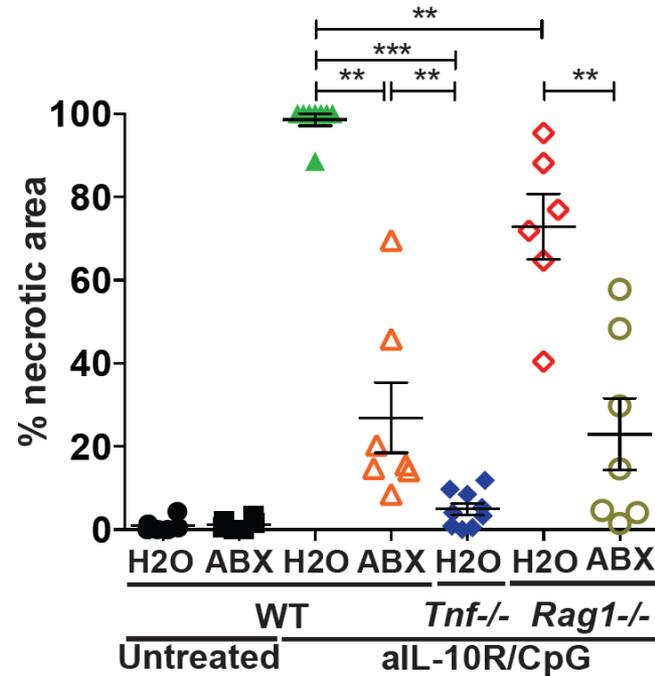
TNF but not T or B cells are required for CpG-induced early tumor necrosis

ABX decreases inflammatory cytokine production in the treated tumors

mRNA expression analyzed by Nanostring
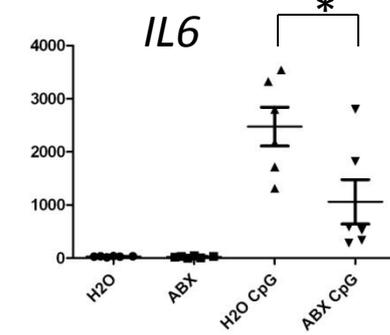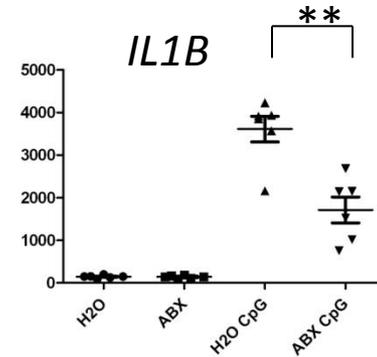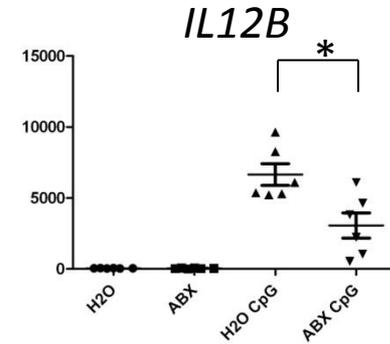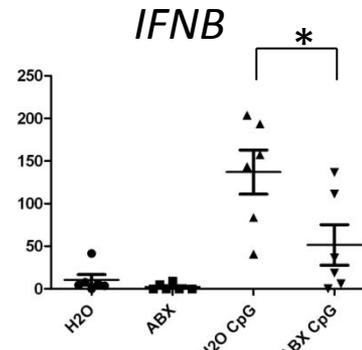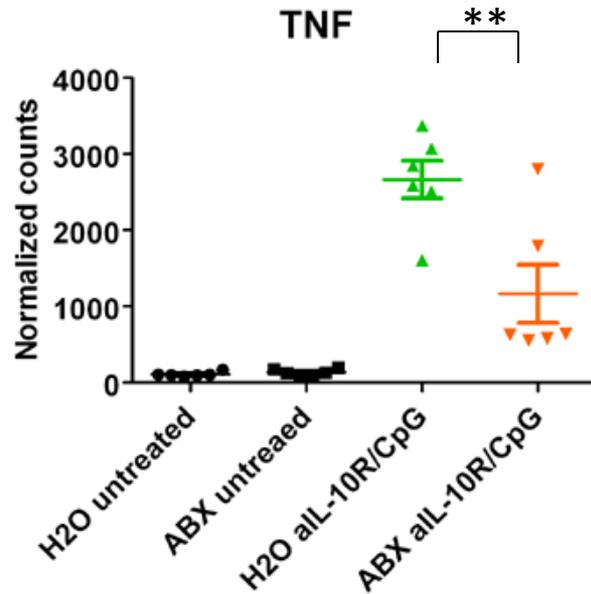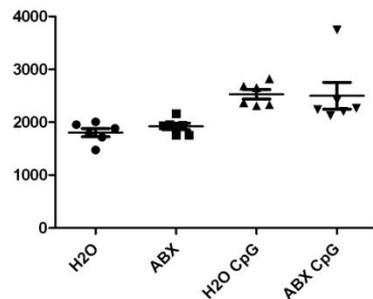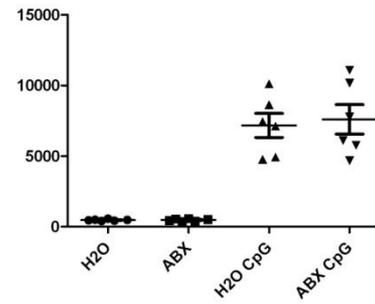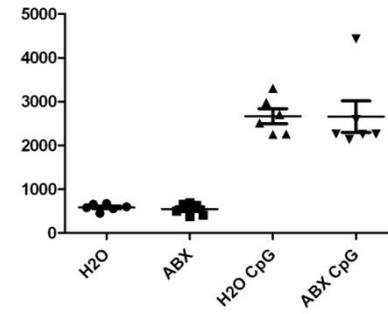
**Tumors were harvested 3 h after anti-IL-10R/CpG.**

ABX decreases inflammatory cytokine production by tumor-infiltrating myeloid cells following aIL-10R/CpG

Germ-free mice display decreased inflammatory cytokine production by tumor-infiltrating myeloid cells following anti-IL-10R/CpG
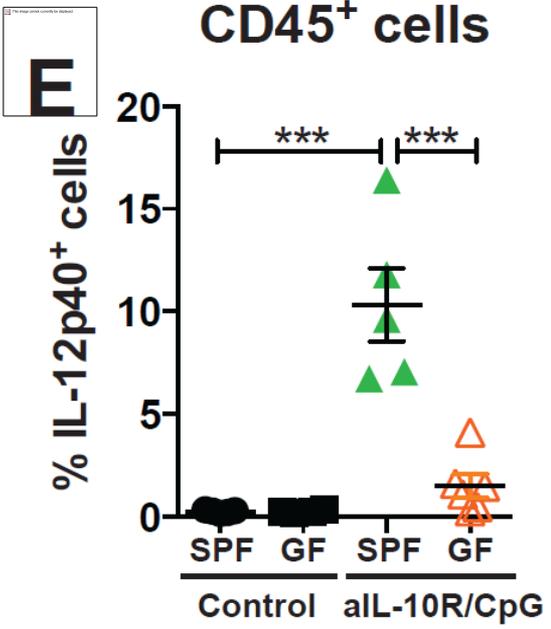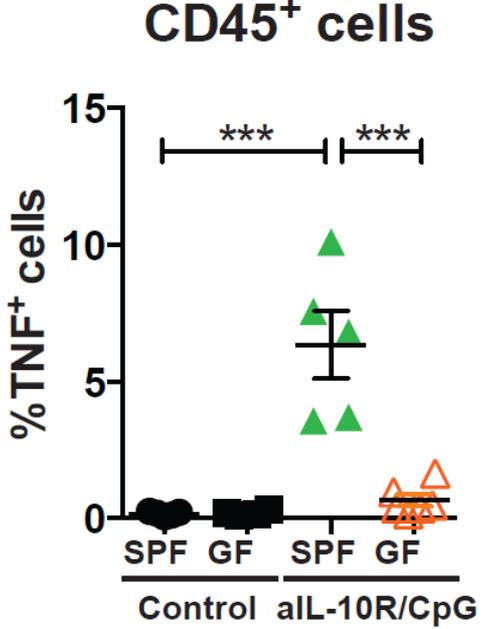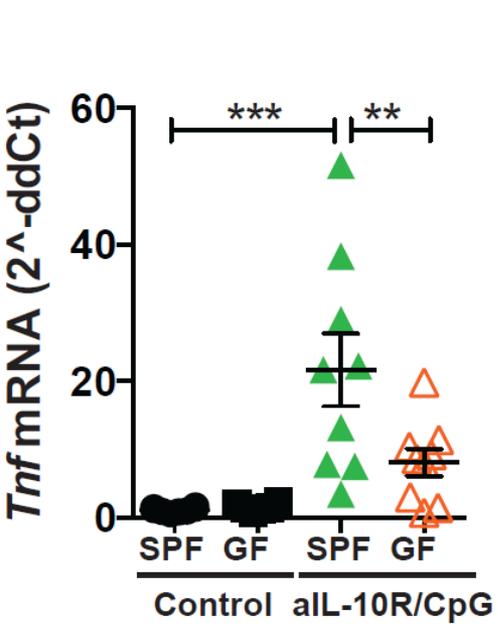
Oral LPS restores the TNF production impaired by ABX

25 mg/kg BW of LPS was orally administered 3 times/week,
2 weeks prior and 1week after MCA38 injection

Real-time PCR

Intracellular cytokine staining (flow)

aIL-10R/CpG

Anti-IL-10R/CpG
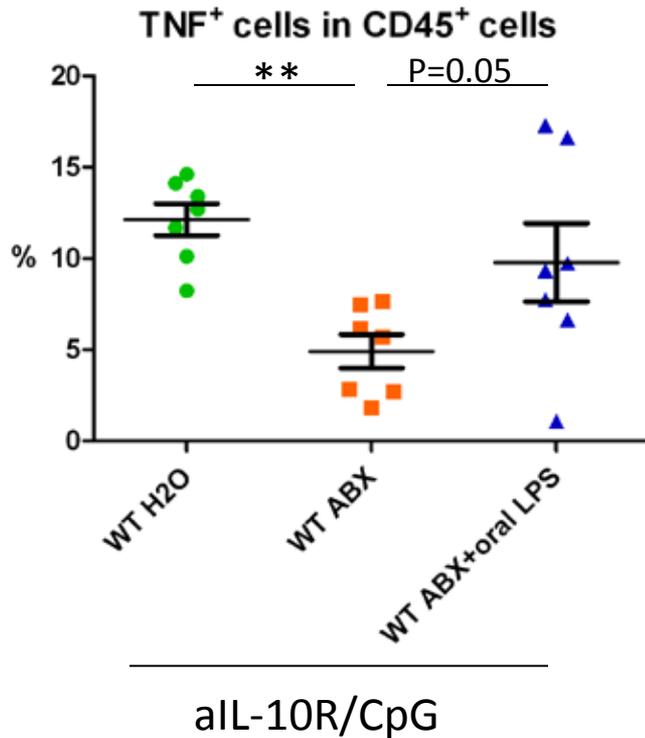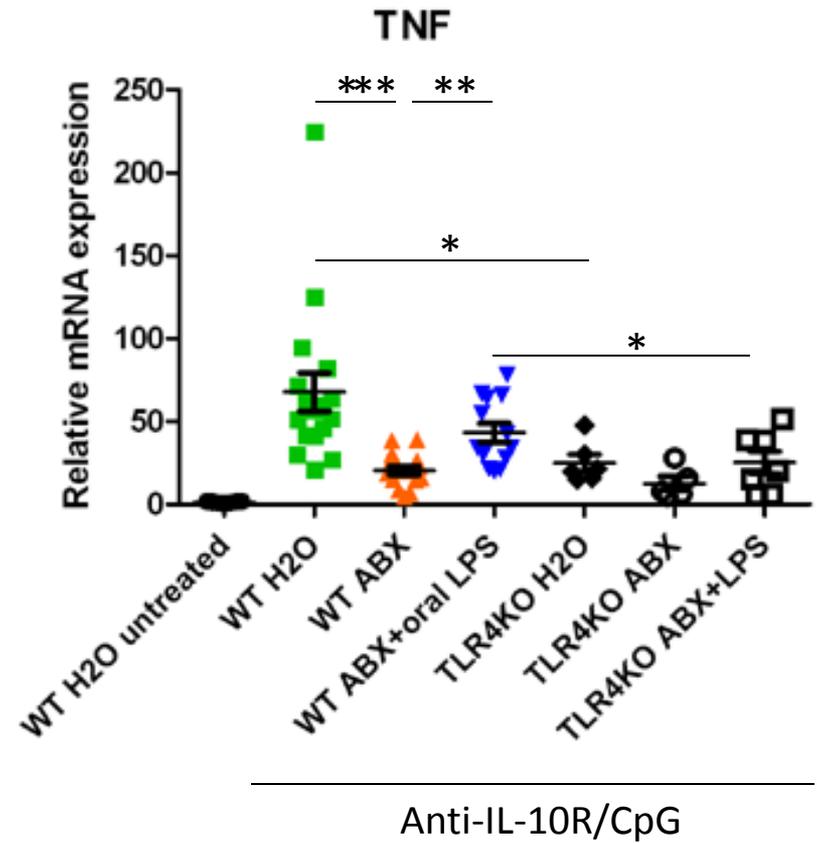
Recovery of gut microbiota and TNF response after ABX withdrawal

Bacteria number
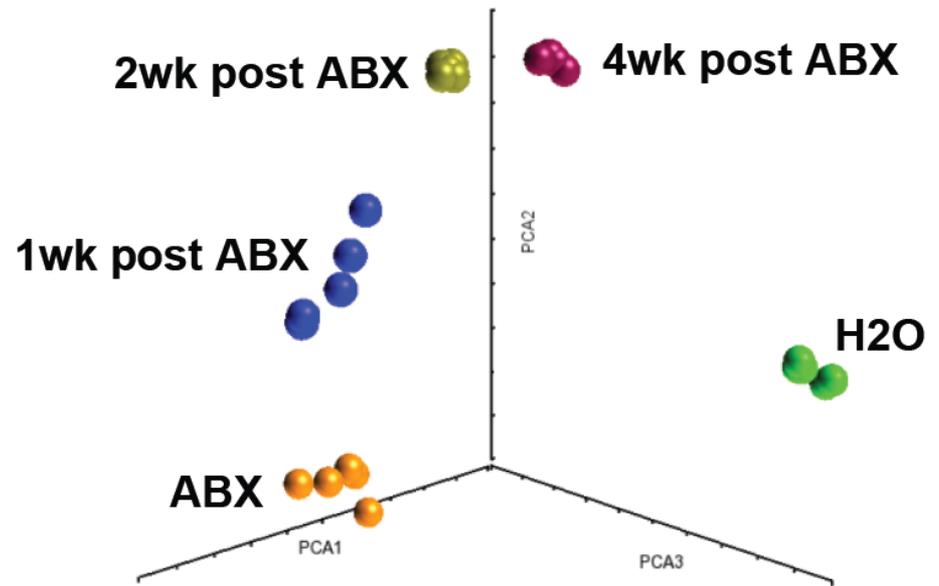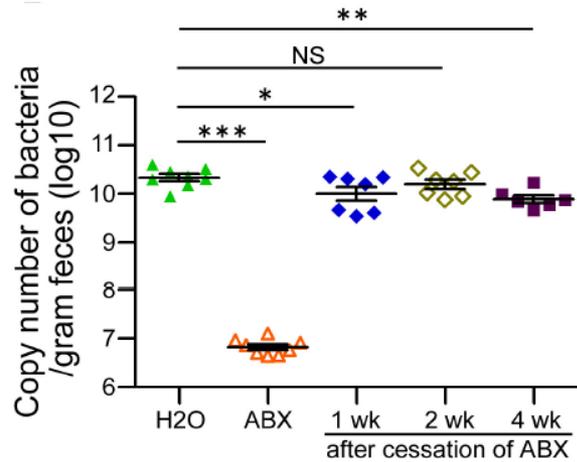
Intratumor TNF response to CpG

2wk post ABX

4wk post ABX

1wk post ABX

H2O

ABX

ABX removal

Recovery of gut microbiota and TNF response after ABX withdrawal

Day: 0 21 28 35 42 49

Bacilli ; Lactobacillales;Leuconostocaceae; Weissella
Bacilli ; Lactobacillales; Streptococcaceae; Lactococcus
Clostridia;Clostrideales; Ruminococcaceae
Clostridia;Clostrideales; Lachnospiraceae

Bacilli; Lactobacillales;Leuconostocaceae ;Lactobacillae

Clostridia;Clostrideales; Ruminococcaceae
Clostridia;Clostrideales; Lachnospiraceae

Clostridia;Clostrideales; Ruminococcaceae
Clostridia;Clostrideales; Lachnospiraceae

ABX
ABX withdrawal

454 sequencing
of 16S from fecal microbiota

Composition of fecal microbiota can be used to segregate mice with high and low intratumoral TNF

Unweighted Unifrac
H$_2$O- drinking mice
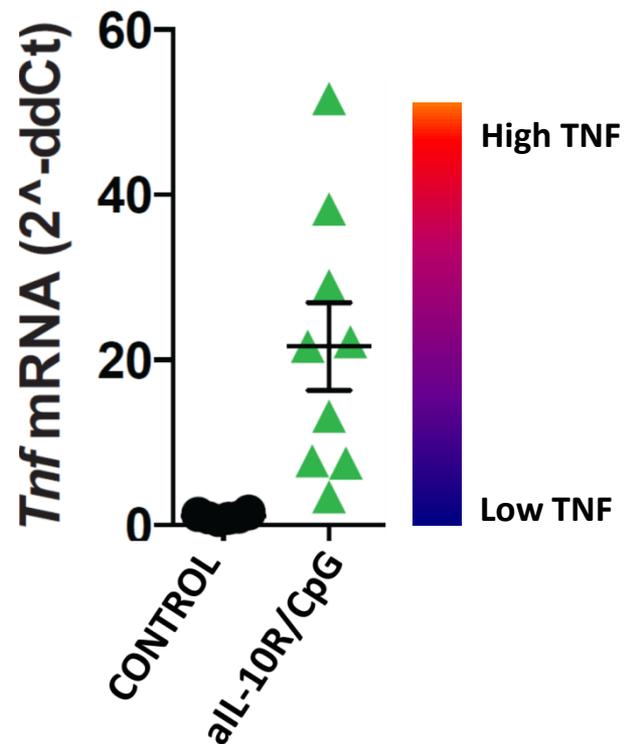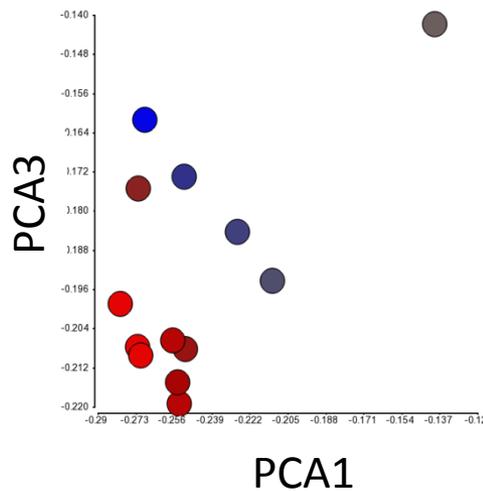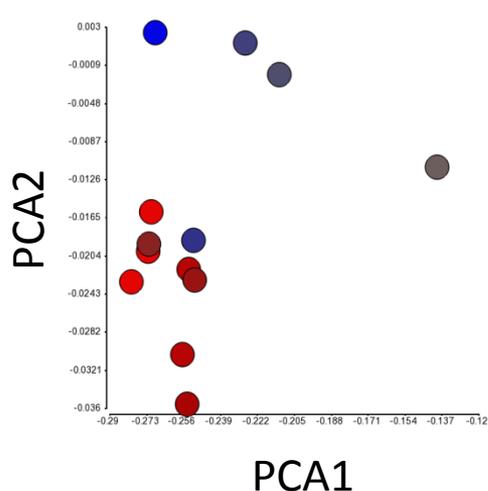
16S rDNA analysis using 454 pyrosequencing

Identification of genera correlating with TNF levels in different microbiota perturbation experiments

Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Lactococcus G+

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Incertae_Sedis G+

ABX Recovery

Single ABX

Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alistipes G−

Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Ruminococcus G+

Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus G+

Bacteroidetes;Bacteroidia;Bacteroidales;unclassified;unclassified G−

Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides G−

H2O

Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;unclassified G−

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Turicibacter G+

Positive correlation with TNF

Negative correlation with TNF

# Correlation of gut microbiota species and TNF response after CpG
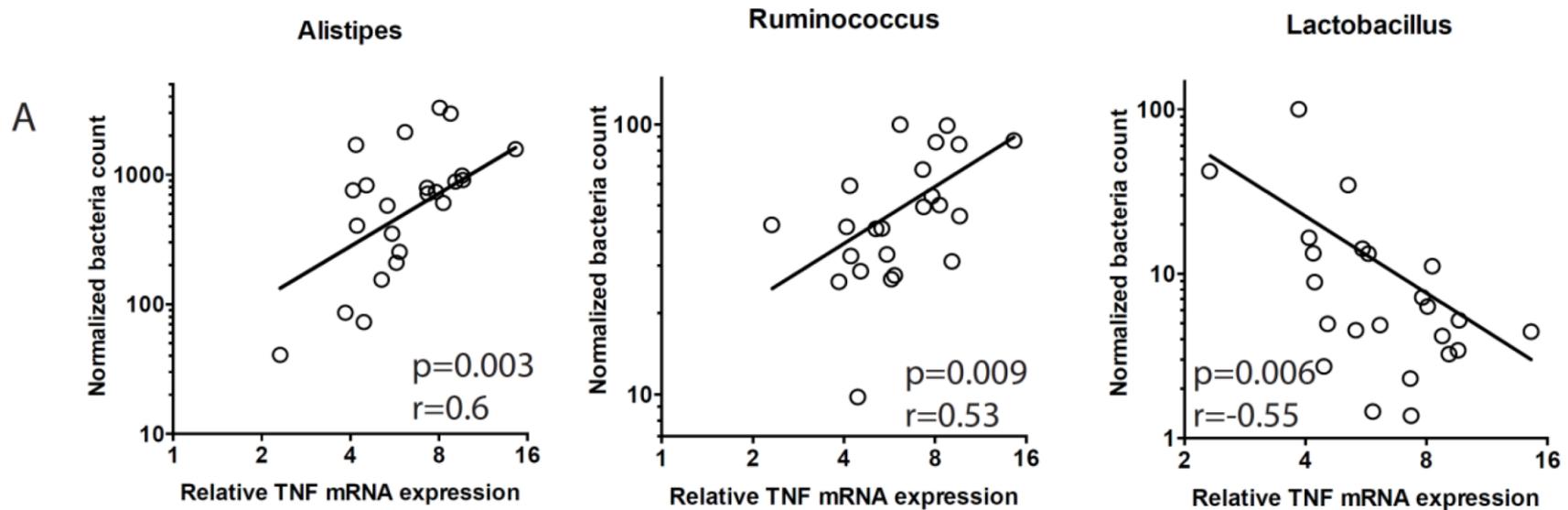
I.G. inoculation of *A. shahii* augments and *L. fermentum* attenuates the TNF response after CpG
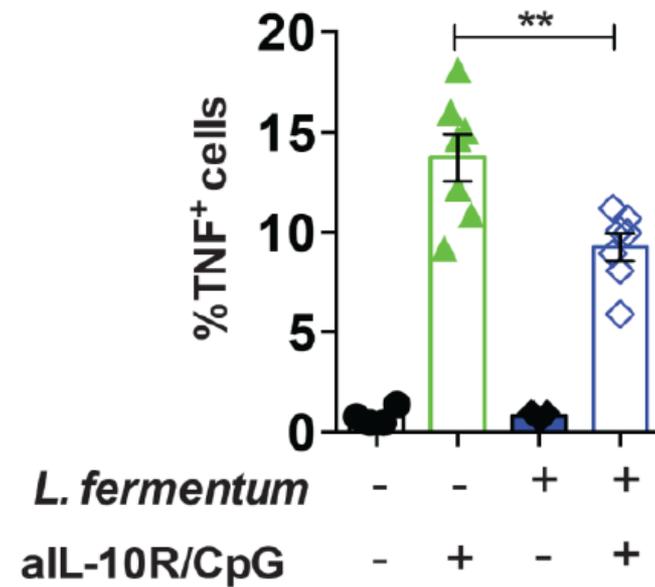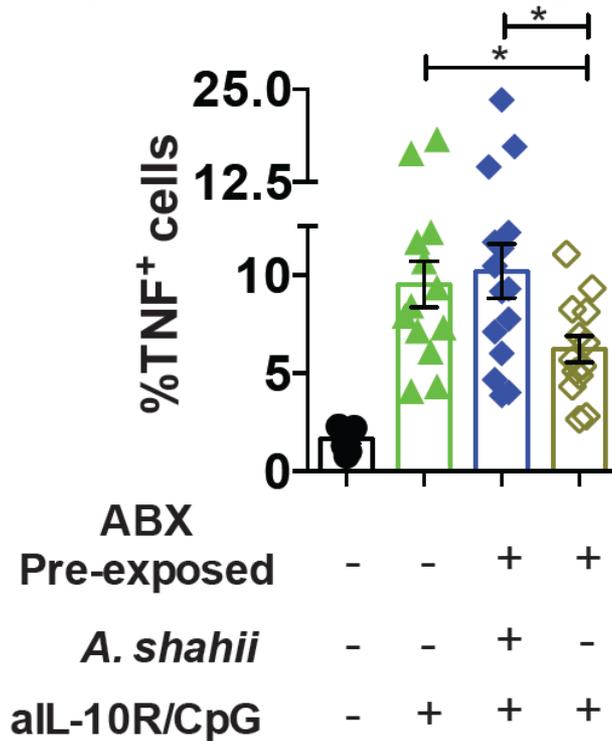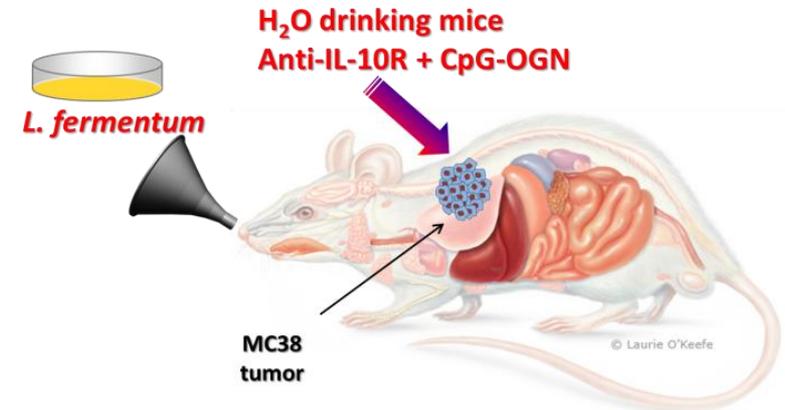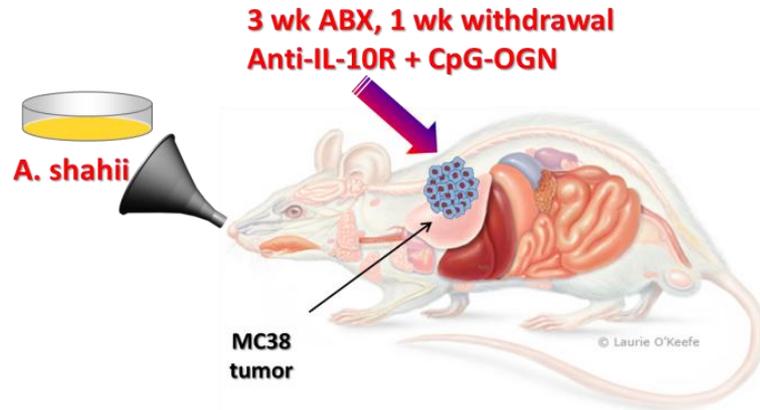
Antibiotics treatment impairs the anti-tumor effect of chemotherapy with platinum compounds
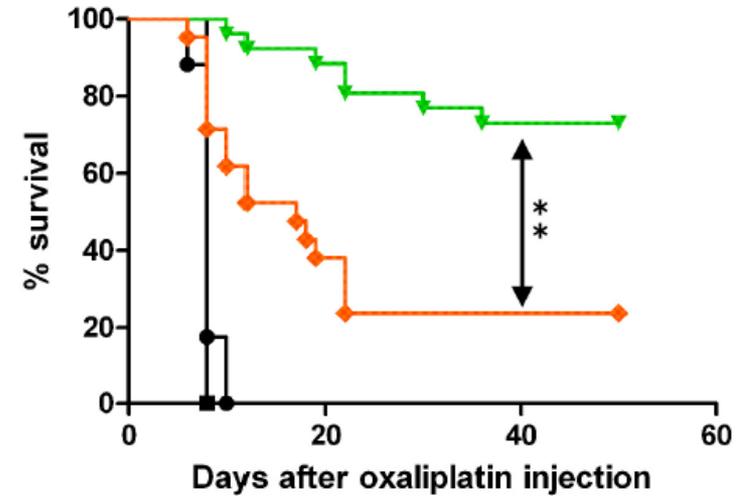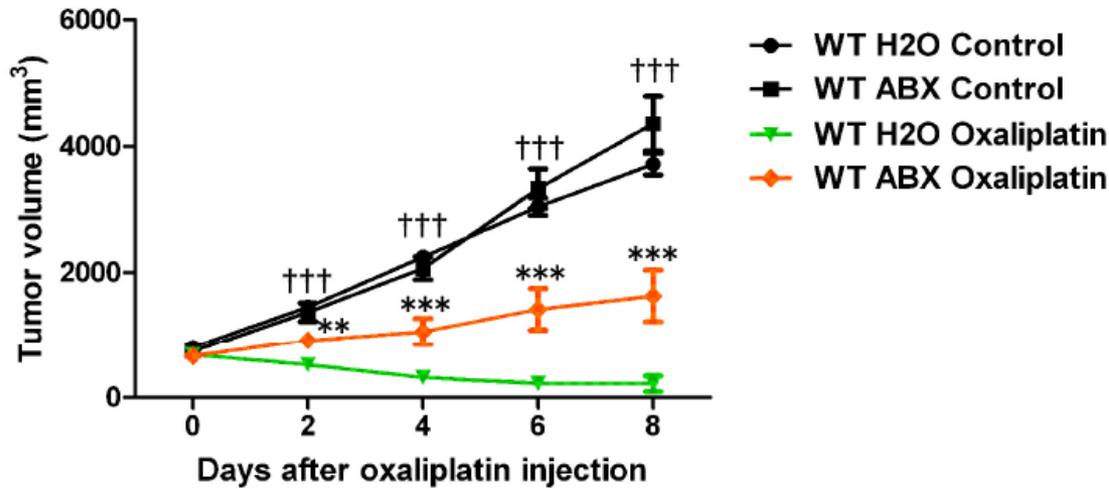

*Oxaliplatin (10mg/kg) was i.p. injected on day 7 after EL4 s.c. tumor inoculation*
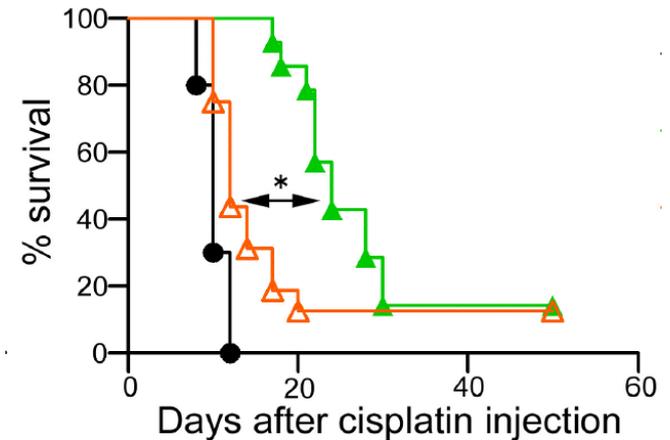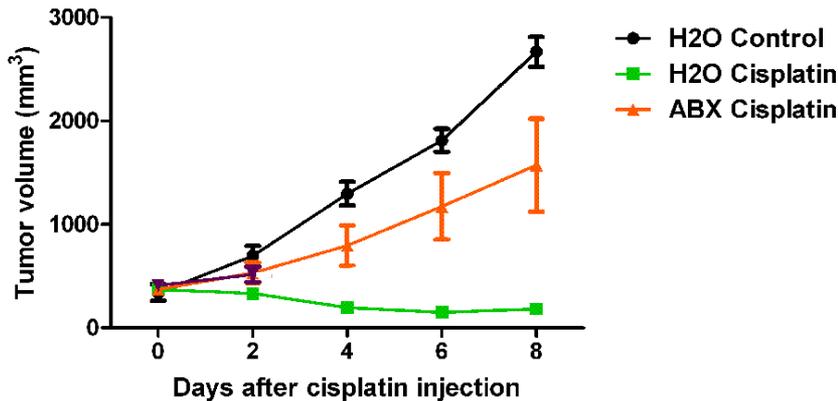
Cisplatin (5mg/kg) was i.p. injected on day 7 after EL4 s.c. tumor inoculation

Oxaliplatin and CpG (+/- anti-IL-10) but not cisplatin induce Immunogenic Cell Death
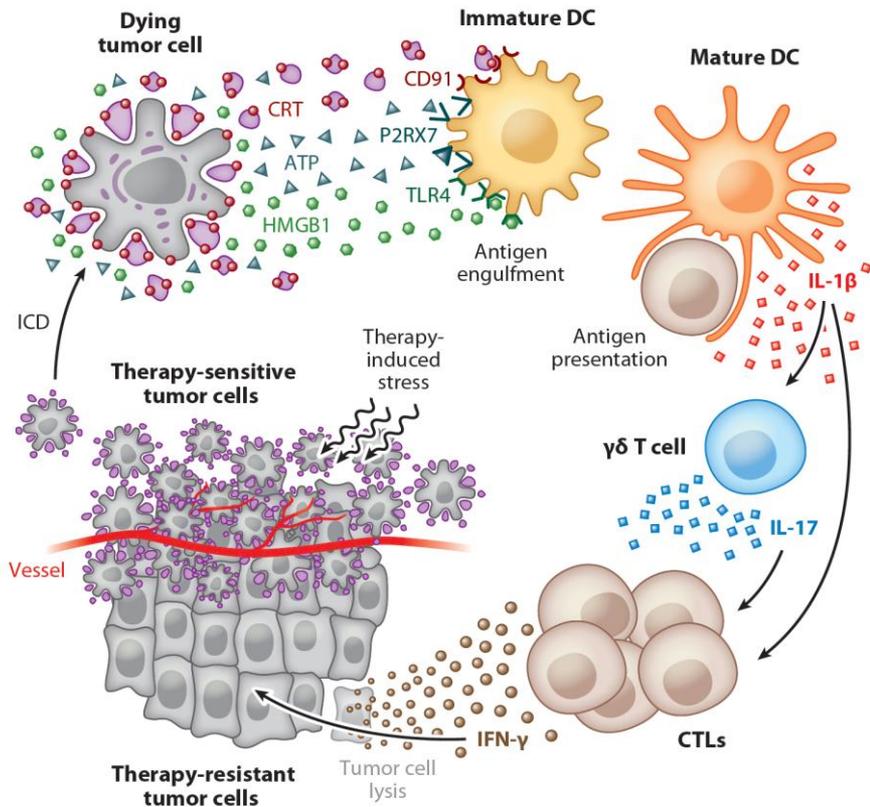
Immunogenic Cell Death in Cancer Therapy

Guido Kroemer,[1,3,6–9,*] Lorenzo Galluzzi,[5,8,*] Oliver Kepp,[1,5,9] and Laurence Zitvogel[2,4,9]
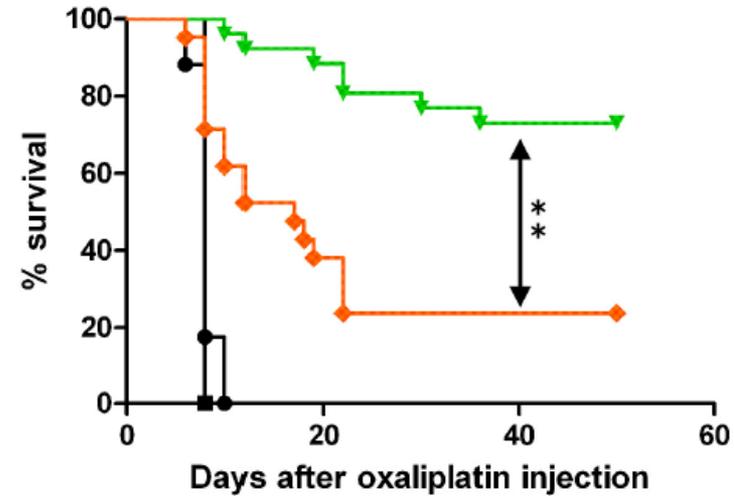
...fter EL4 s.c. tumor inoculation
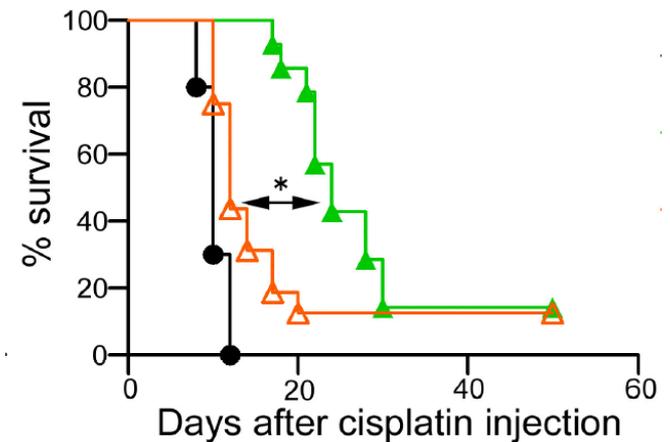
...r EL4 s.c. tumor inoculation

Gene espression (microarray analysis) of total EL4 tumors
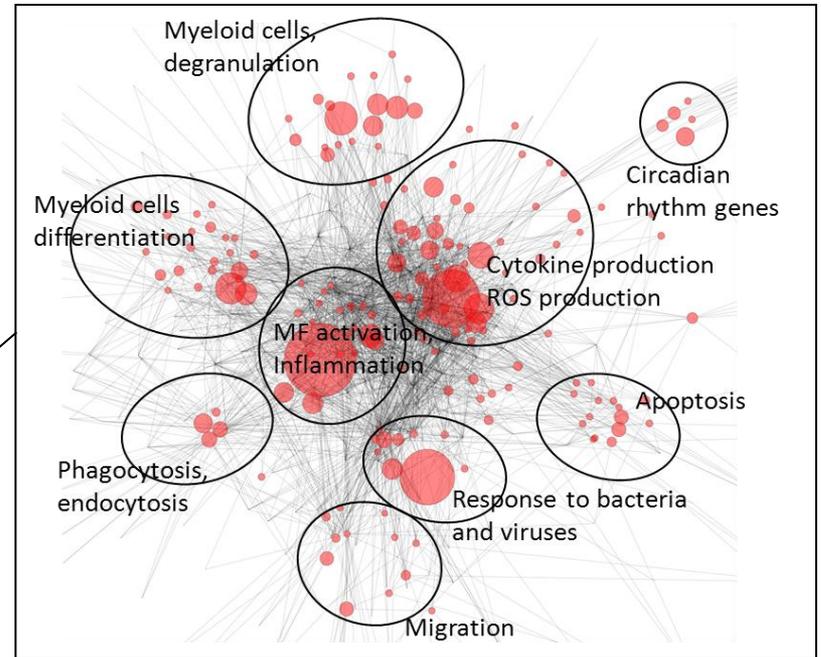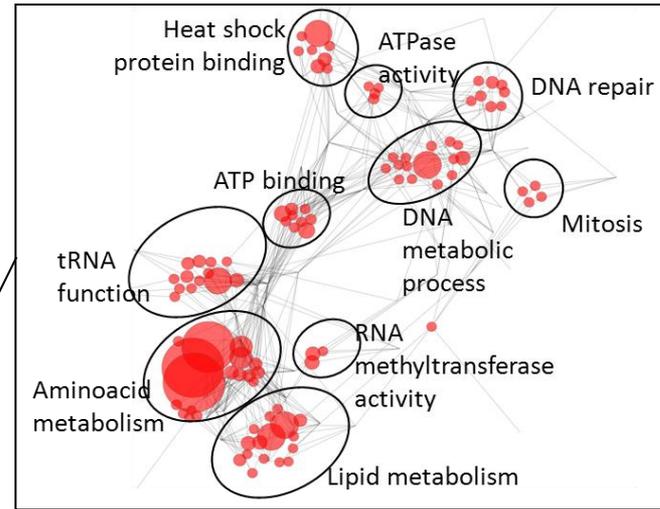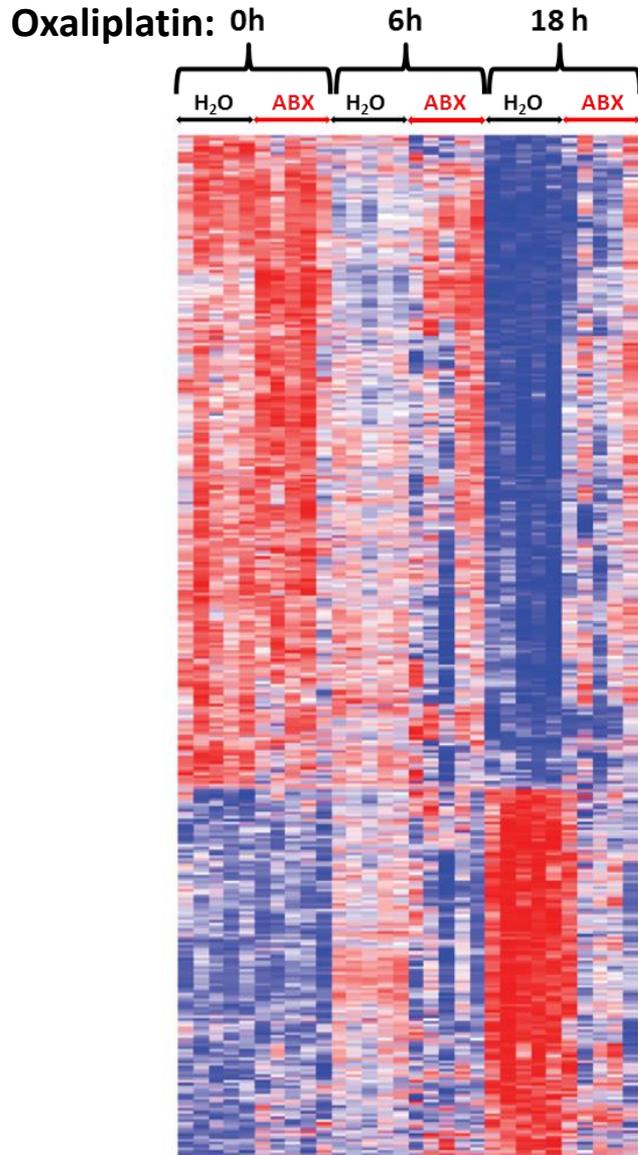
Oxaliplatin tumor treatment requires MyD88 but, unlike CpG, neither TLR4 nor TNF
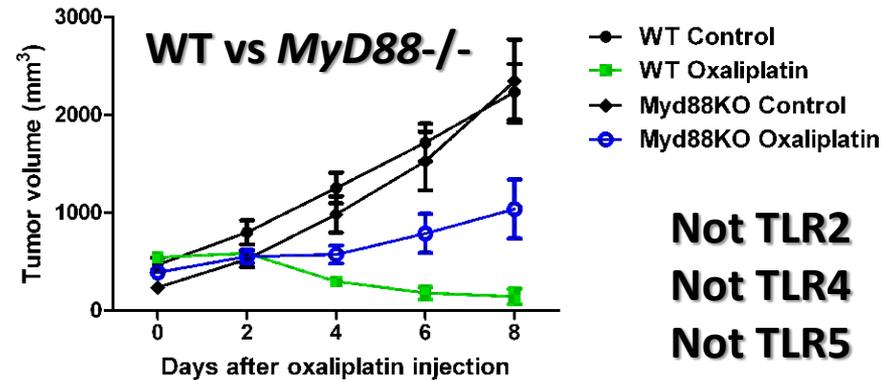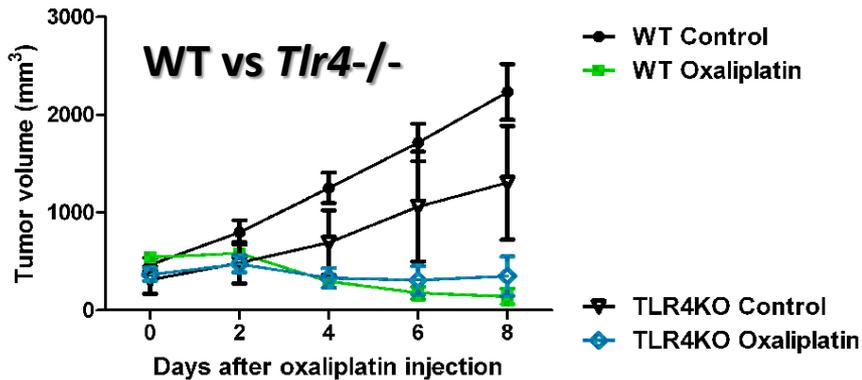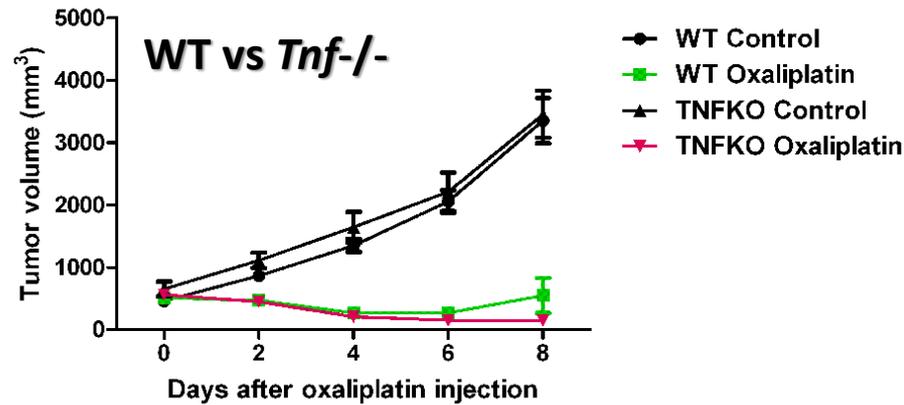
Not TLR2
Not TLR4
Not TLR5
Not TLR9
Not IL-1R
Not IL-18R

Oxaliplatin (10mg/kg) was i.p. injected on day 7 after EL4 s.c. tumor inoculation

Oxaliplatin and CpG (+/- anti-IL-10) but not cisplatin induce Immunogenic Cell Death
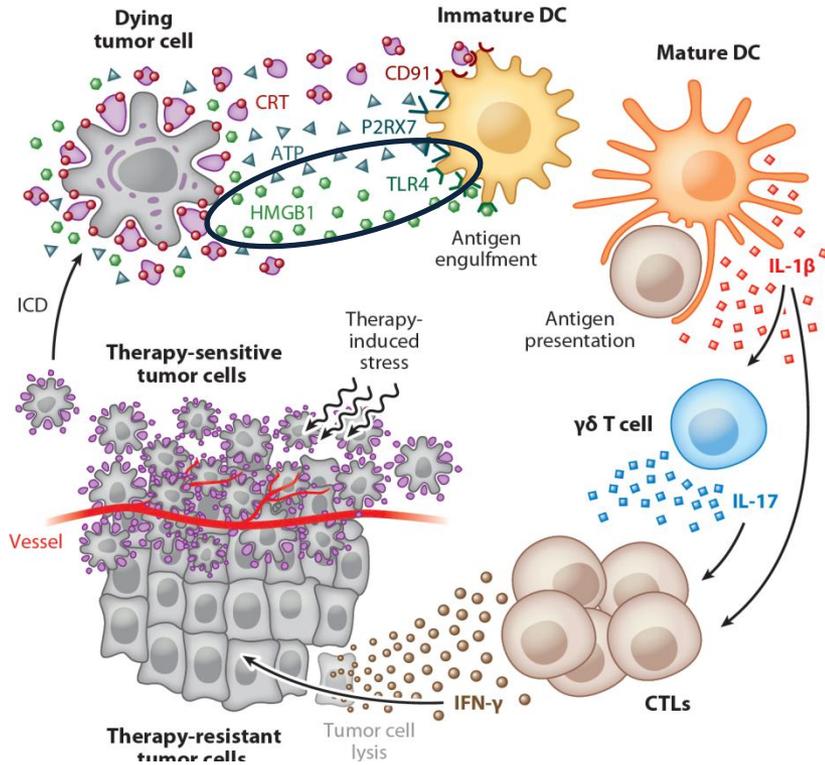
Immunogenic Cell Death in Cancer Therapy

Guido Kroemer,[1,3,6−9,*] Lorenzo Galluzzi,[5,8,*] Oliver Kepp,[1,5,9] and Laurence Zitvogel[2,4,9]
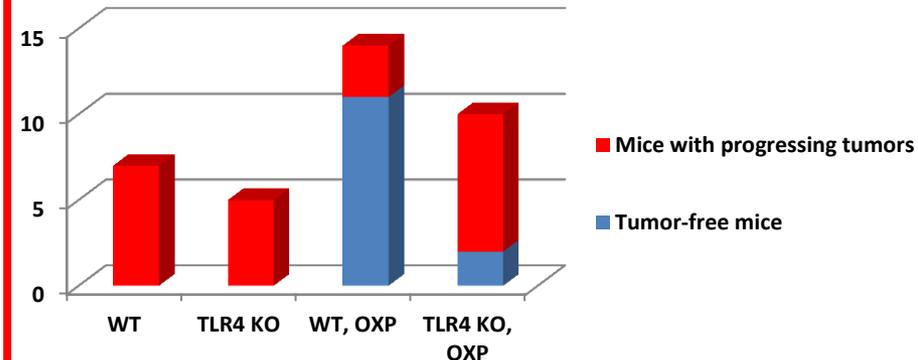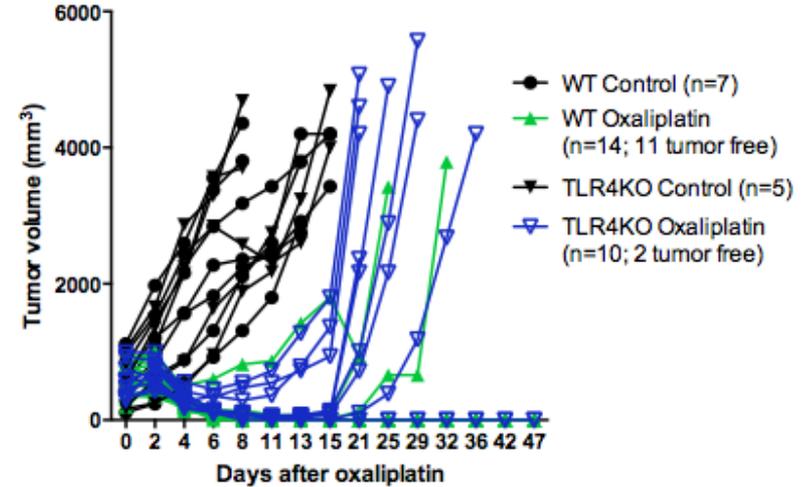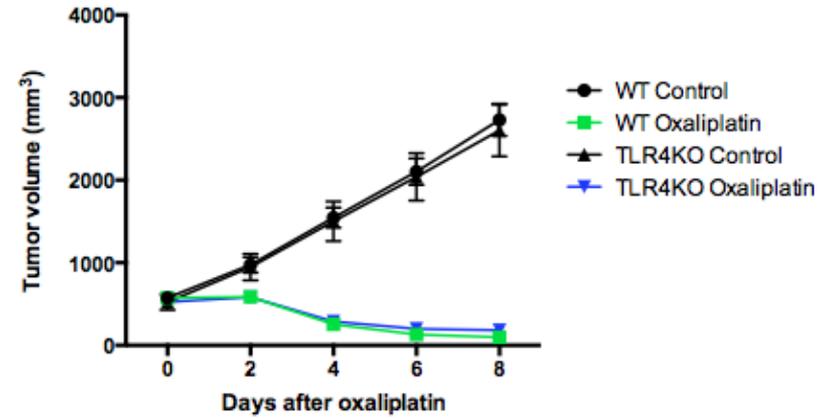
Antibiotics treatment impairs oxaliplatin chemotherapy by preventing ROS production by NOX2 (*Cybb*) expressing myeloid cells

H2O-drinking

ABX-treated

**Oxaliplatin-induced L-012 Bioluminescence (ROS)**
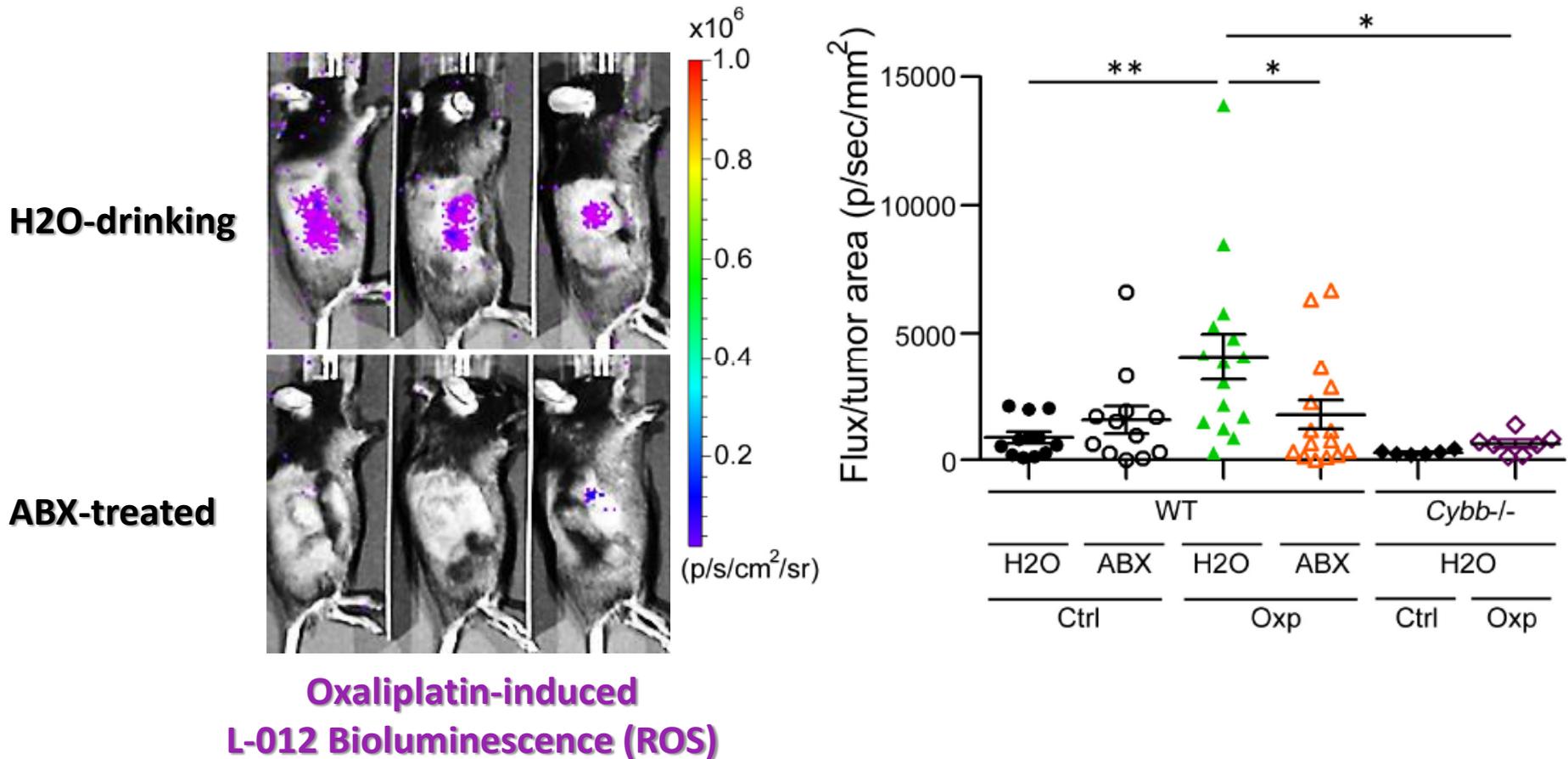
- ➢ **EL4 tumors-bearing B6 mice were treated with 10mg/kg oxaliplatin**
- ➢ **ROS-induced bioluminescence using the L-012 probe was analyzed 24 hours after oxaliplatin injection**

# Oxaliplatin tumor therapy requires ROS production
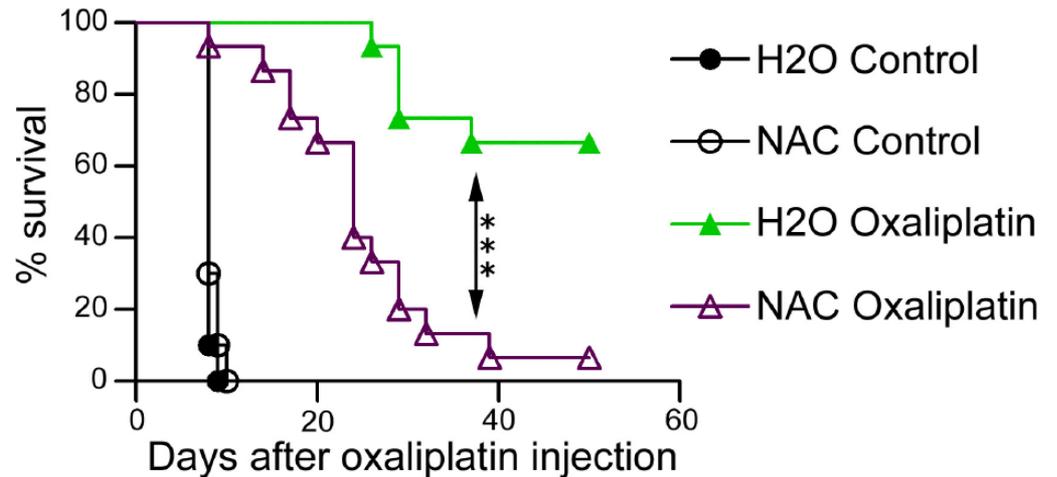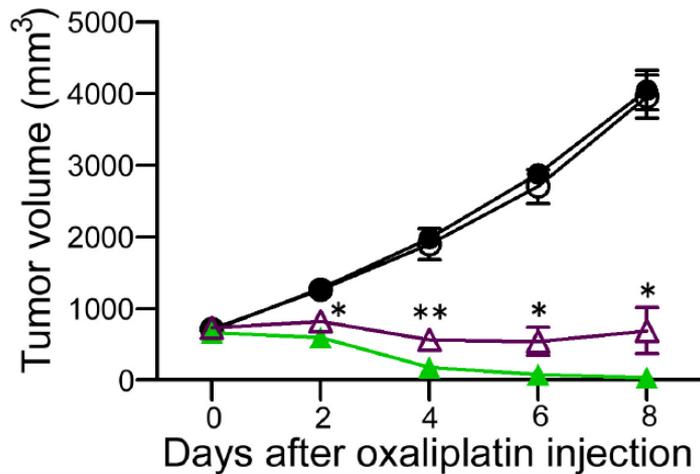
N-acetyl cystein (NAC) decreases oxaliplatin anti-tumor efficacy

Oxaliplatin (10mg/kg) was i.p. injected on day 7 after EL4 s.c. tumor inoculation

Oxaliplatin tumor treatment is ineffective in in NOX2(Cybb)-/- C57Bl6 mice

WT vs *Cybb(Nox2)-/-*
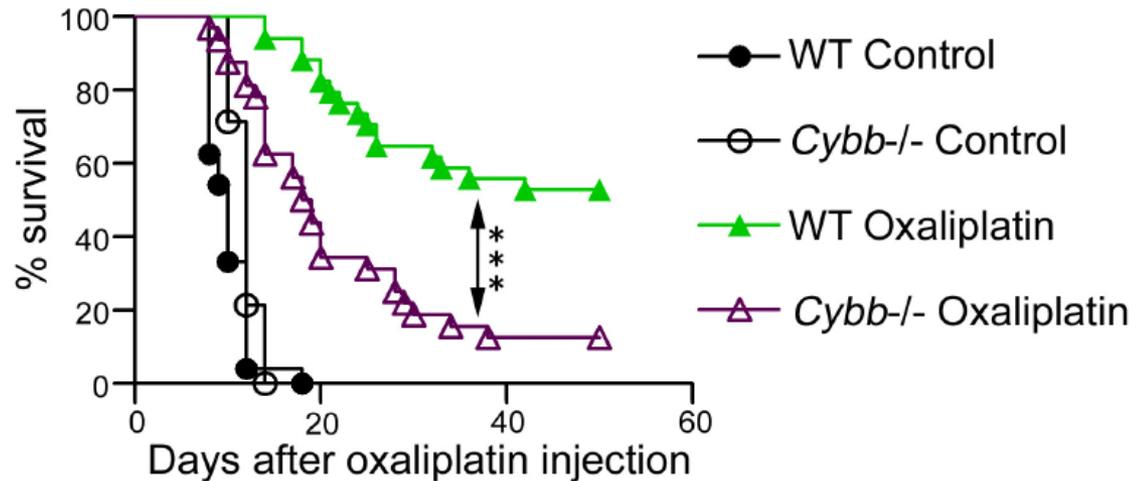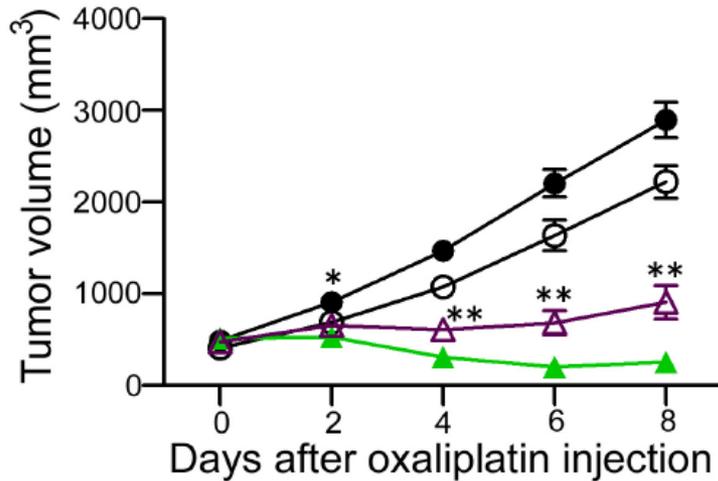
Oxaliplatin (10mg/kg) was i.p. injected on day 7 after EL4 s.c. tumor inoculation

Efficiency of oxaliplatin tumor treatment is reduced by depletion of myeloid cells with anti-GR1 antibody
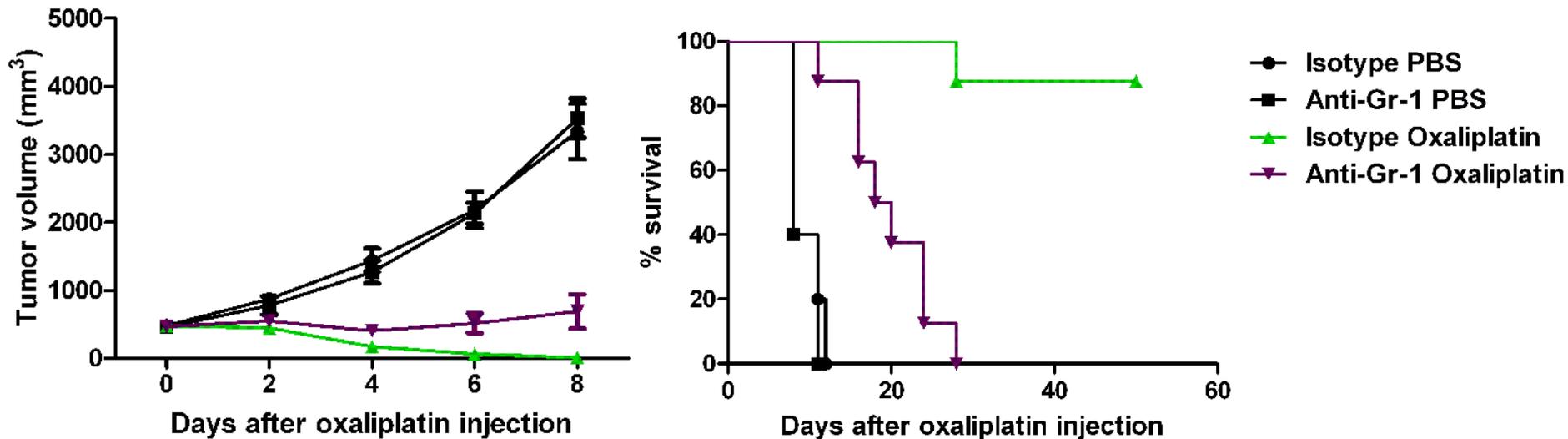
Oxaliplatin (10mg/kg) was i.p. injected on day 7 after EL4 s.c. tumor inoculation

Monocyte/ macrophages **Neutrophils**
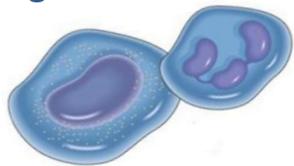
Antibiotics or Germ Free animals
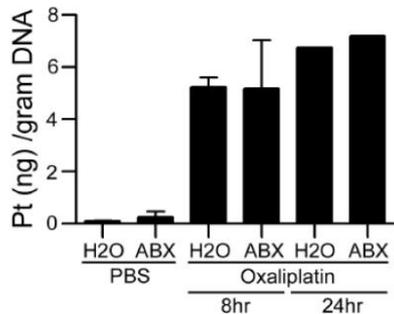
Platinum compounds

ROS

DNA DAMAGE

P53
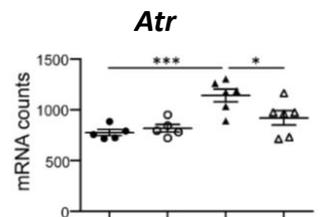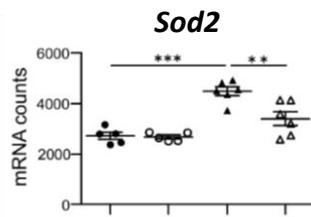
● H2O 0hr (untreated)   ▲ H2O Oxaliplatin 48hr
○ ABX 0hr (untreated)   △ ABX Oxaliplatin 48hr
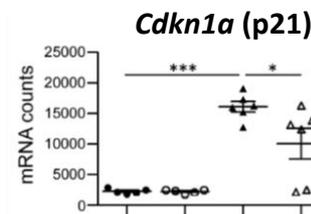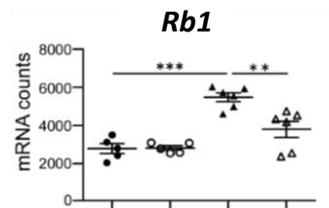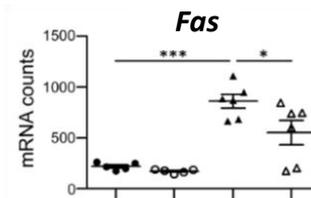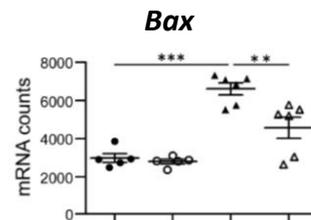
Cell cycle inhibition

Apoptosis

Sod2

Atr

Rb1

Cdkn1a (p21)

Bax

Fas

Platinum DNA Adducts

Pt (ng) /gram DNA

% γH2Ax positive tumor cells

Pan-nuclear
> 4 foci/nucleus

H₂O   ABX
H₂O, oxaliplatin 24h   ABX, oxaliplatin 24h
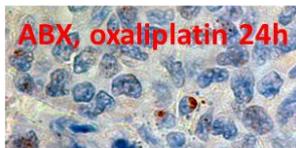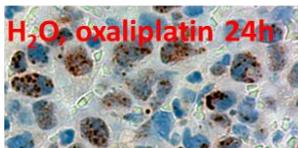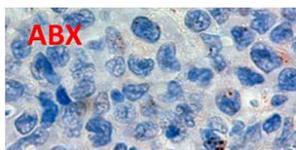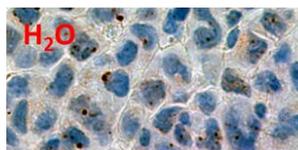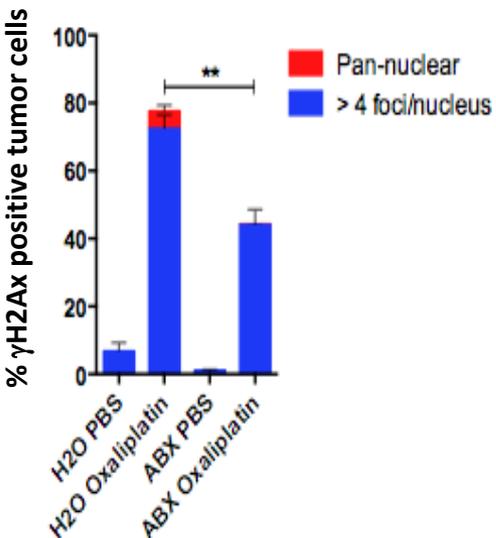
γH2Ax staining

Early response to immunotherapy and chemotherapy

anti-IL-10R/CpG

Oxaliplatin or cisplatin

Tumor

HIGH SYSTEMIC
INFLAMMATORY TONE

GUT MICROBIOTA
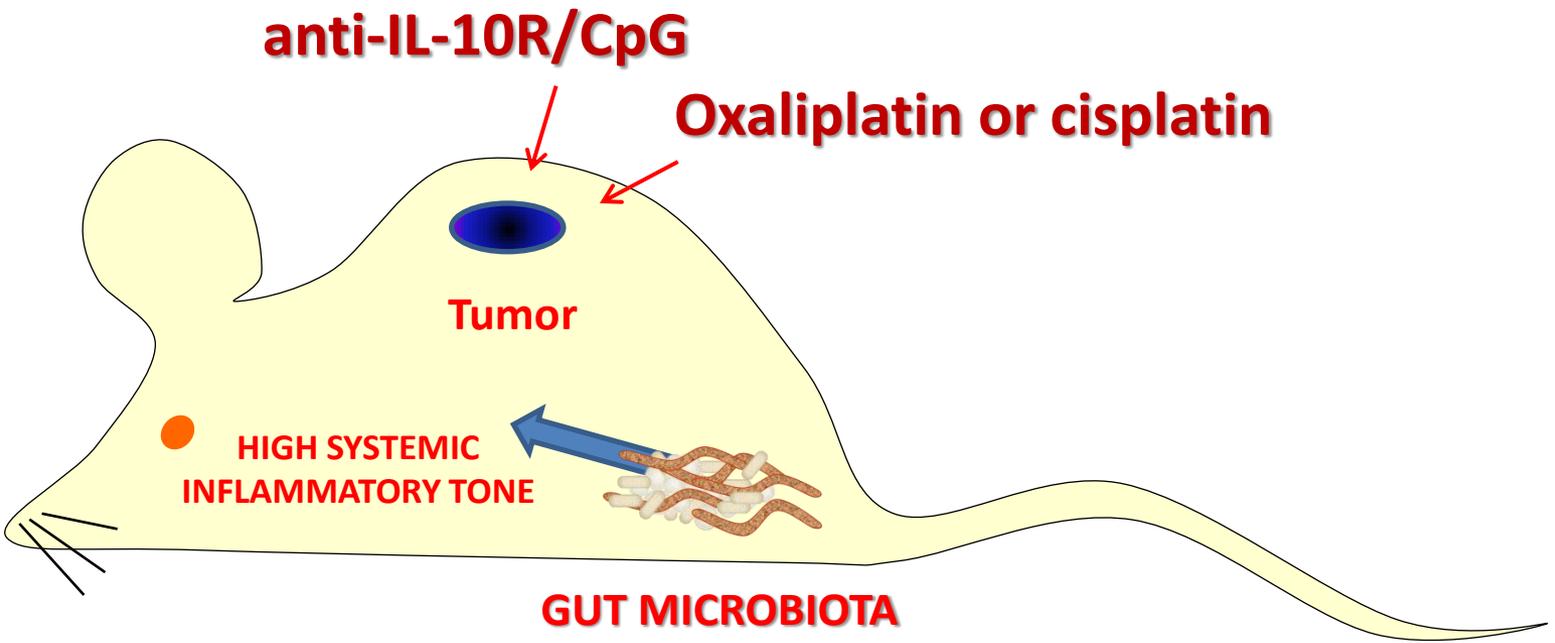
Early response to immunotherapy and chemotherapy

Reduced production of pro-inflammatory cytokines by tumor-associated myeloid cells (TNF and TLR4 required, NOX2 independent)

anti-IL-10R/CpG

Oxaliplatin or cisplatin

ANTIBIOTICS

Reduced ROS production by tumor-associated myeloid cells (NOX2-dependent, TNF and TLR4 independent)

Tumor

LOW SYSTEMIC INFLAMMATORY TONE
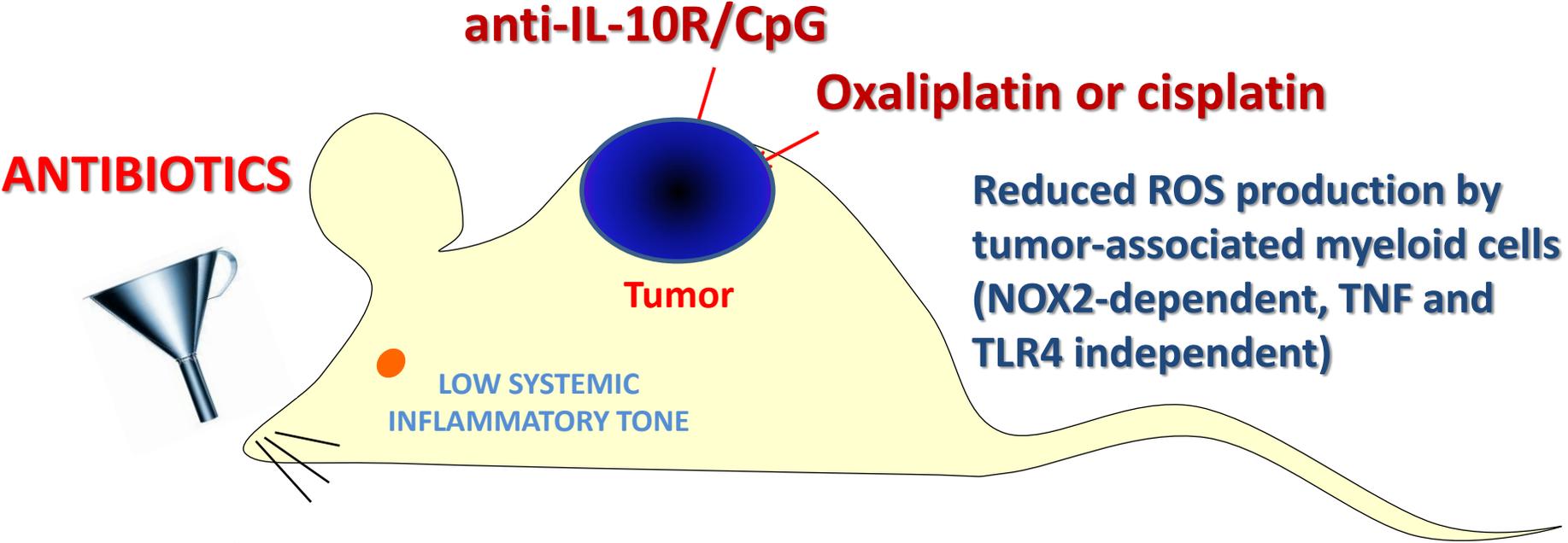
(or Germ-free mice)

Cyclophosphamide (CTX) induces mucosal bacterial translocation that is required for anti-tumor Th17 response
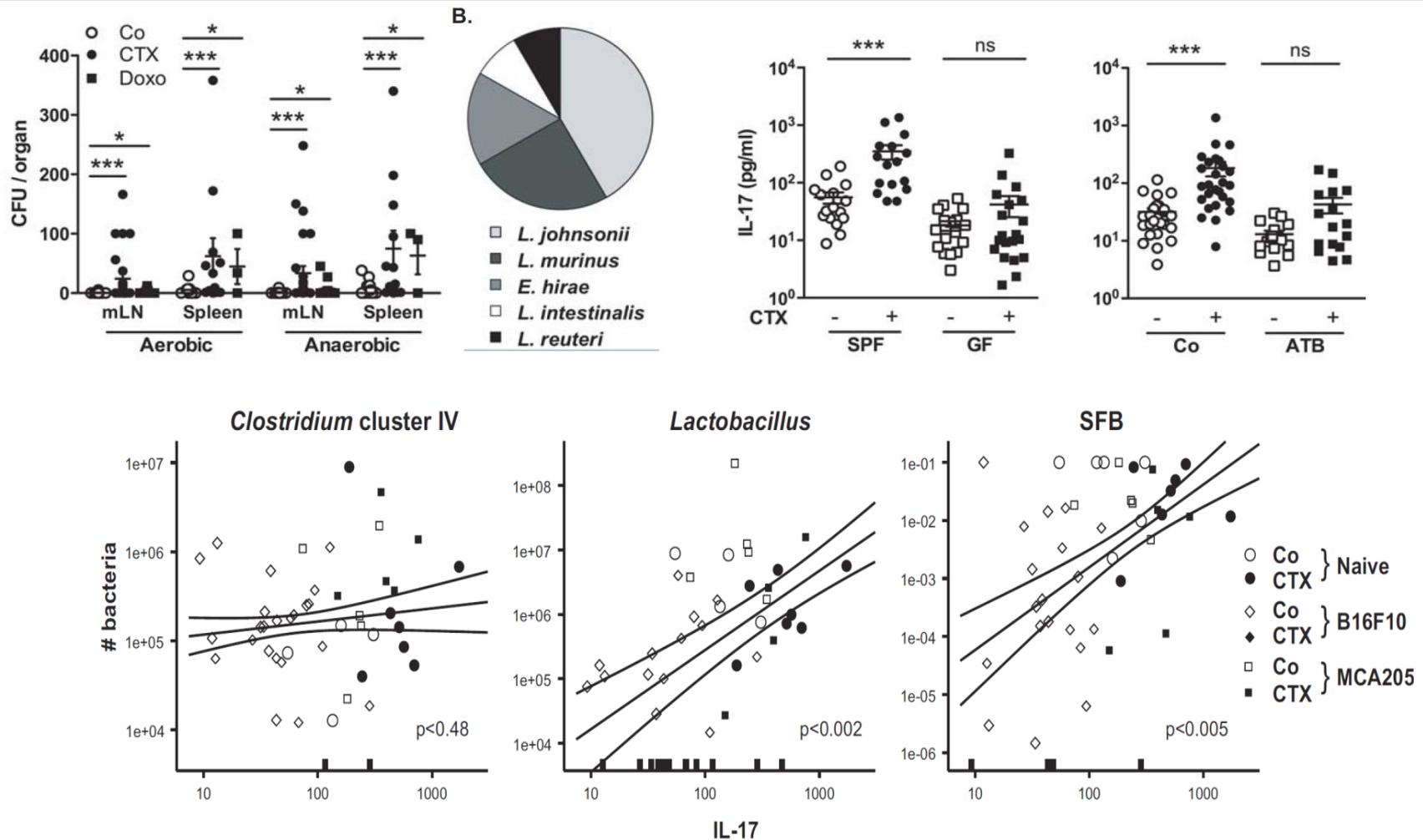
Sophie Viaud, ...and Laurence Zitvogel
**The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide**
Science, 2013, in press

Direct toxicity (Innate) (0-48h)

Adaptive (>7 days)

Antibiotics TX

Dysbiosis

Mucositis

Cyclophosphamide (CTX)

Bacterial translocation

Intestine

IL-17

Dying tumor cell

Immature DC

Mature DC

CRT

CD91

P2RX7

ATP

TLR4

HMGB1

Antigen engulfment

IL-1β

ICD

Therapy-induced stress

Therapy-sensitive tumor cells

Antigen presentation

γδ T cell

IL-17

Vessel

CTLs **IFN-γ**

IFN-γ

Therapy-resistant tumor cells

Tumor cell lysis
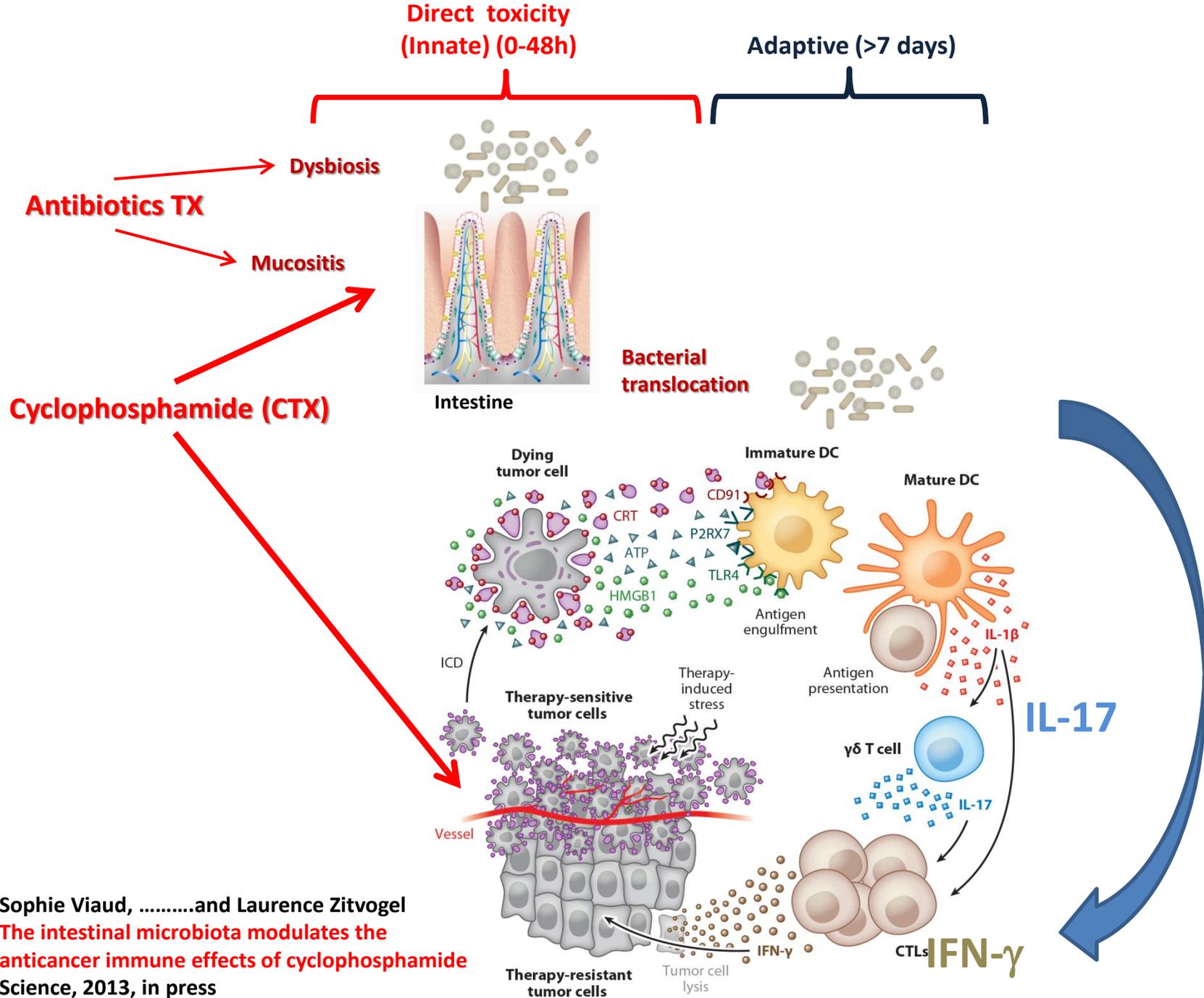
Sophie Viaud, ……….and Laurence Zitvogel
The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide Science, 2013, in press

# Role of intestinal microbiota in regulating systemic inflammation and immunity

Innate:

- **Transcriptional poising of type I IFN-responsive genes**
- **Lowering the threshold of pro-inflammatory cytokine production**
- **Lowering the threshold of induction of ROS production**

Adaptive:

- **Regulation of Th1 / Th17 / CD8 / Treg responses**

Different bacteria types and different TLRs and other innate receptors are likely involved
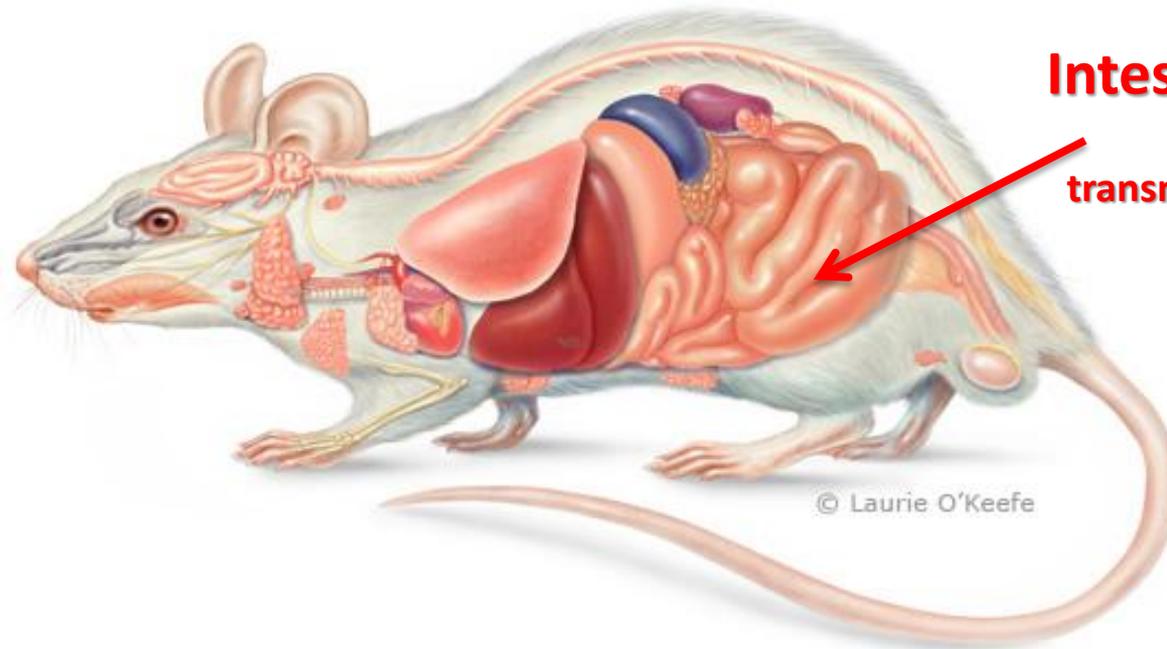
Intestinal microbiota
(dysbiosis?
transmucosal translocation?)

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We are the 99% and We demand a very different budget.

Wellcome Images

Our friendly gut bacteria

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