

“Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors”

## Overview

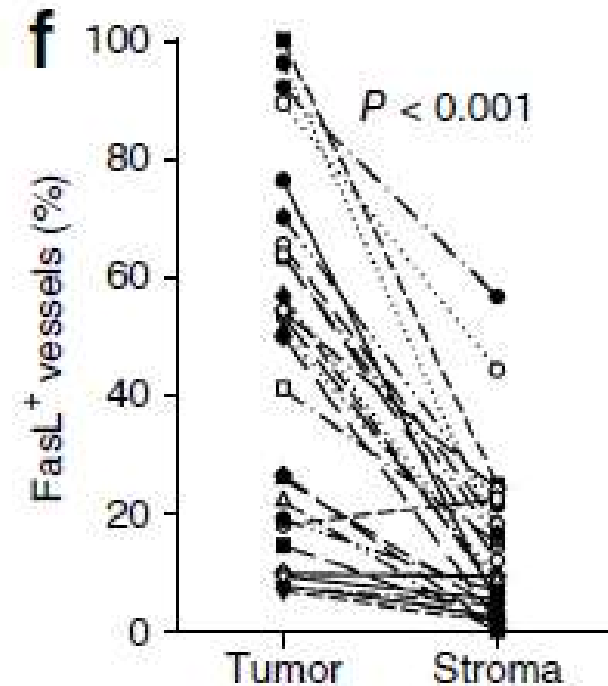
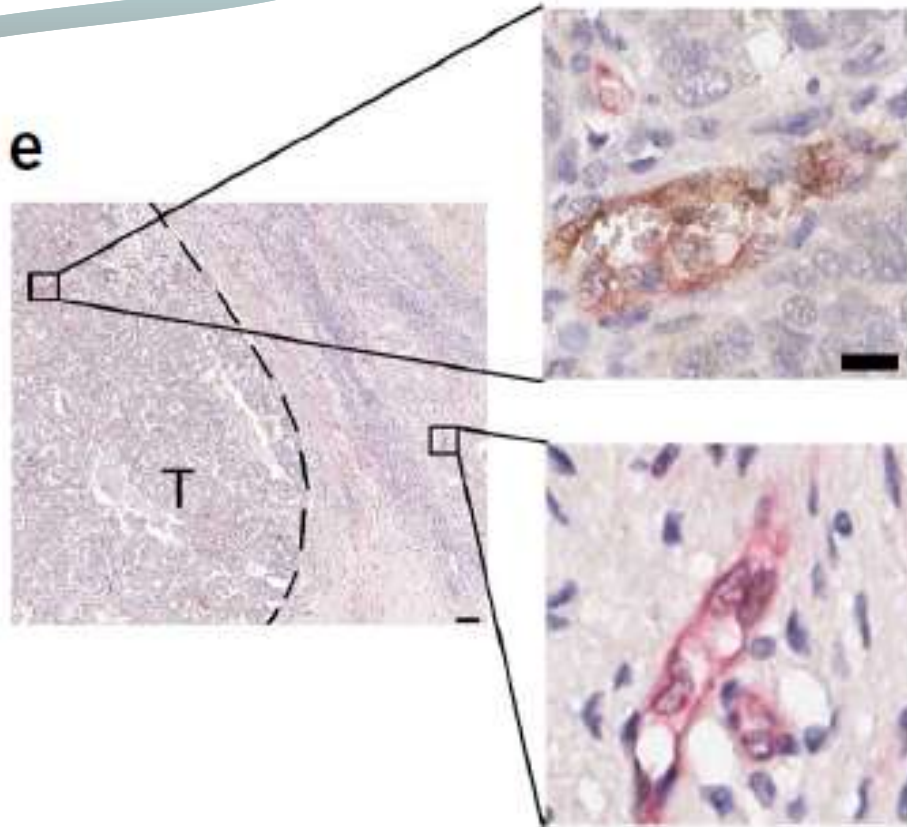
- Tumor needs to evade immune system and sustain vascularization in order to survive
- Vascularization → Tumor exposure to CD8+ T-cell infiltration
- proangiogenetic factors like VEGF-A deregulate VCAM1 and ICAM1 in endothelial cells → attenuated T-cell adhesion
- Endothelium serves as selective immune barrier
- FasL (CD95L) is a T-cell apoptosis inducing factor
- Tumor vascularization microenvironment eludes FasL (tumor itself: principle of Fas-counterattack)

## Results

1.

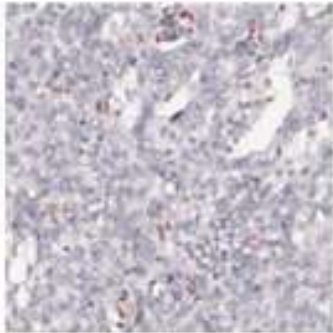
**human tumor endothelium expresses FasL (normal Endothelium CD34)**

→ shown using tissue microarrays

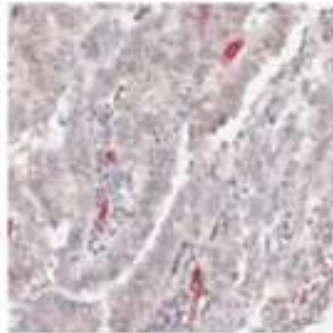


FasL expression on the endothelium within tumor islets compared to the endothelium in the surrounding stroma

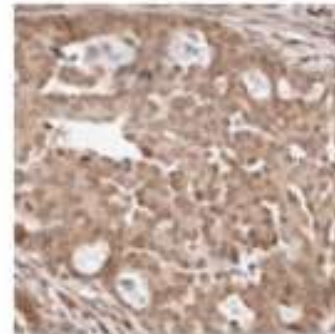
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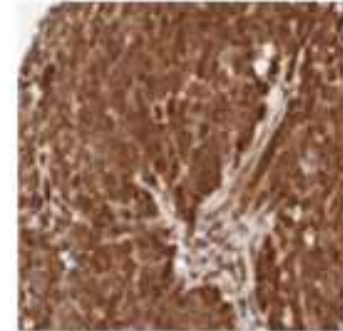
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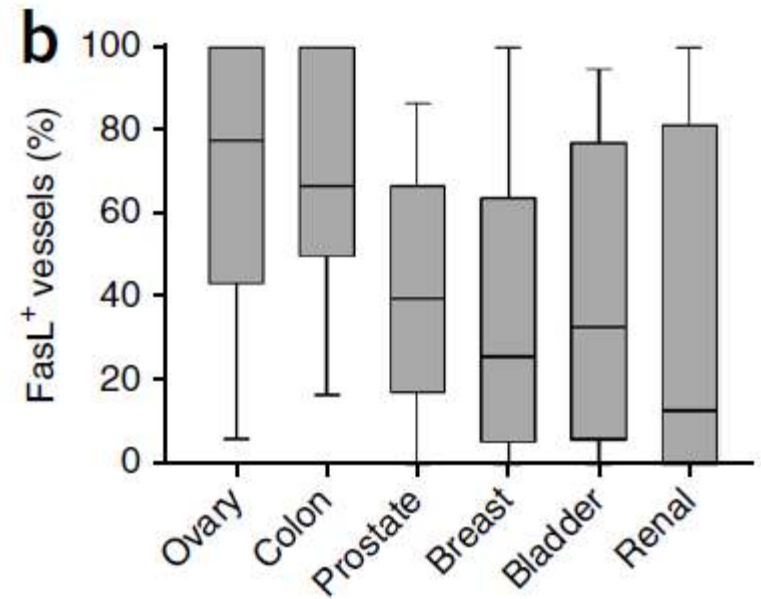
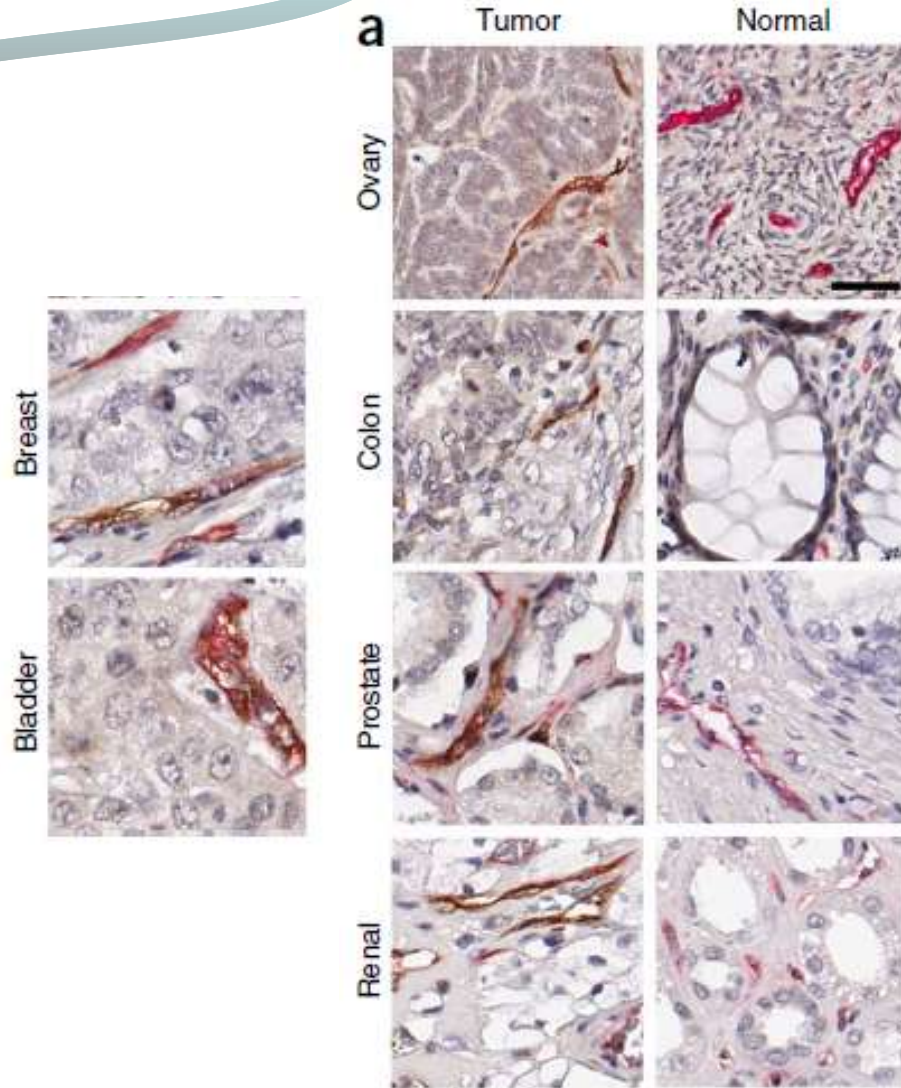
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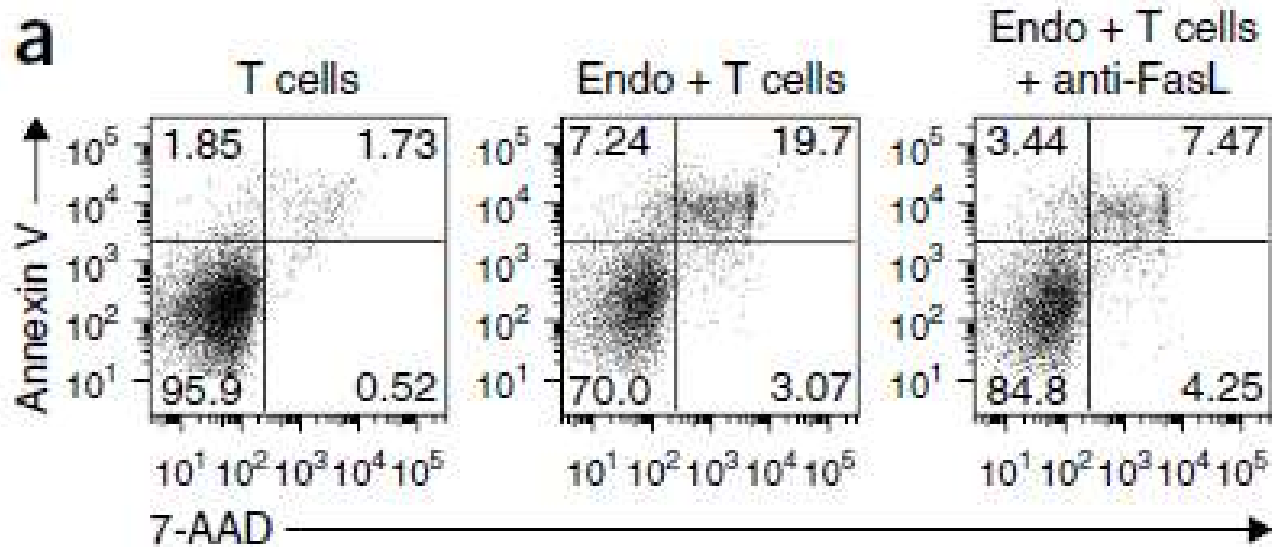
FasL expression depending on tumor staging (breast cancer)



## Results

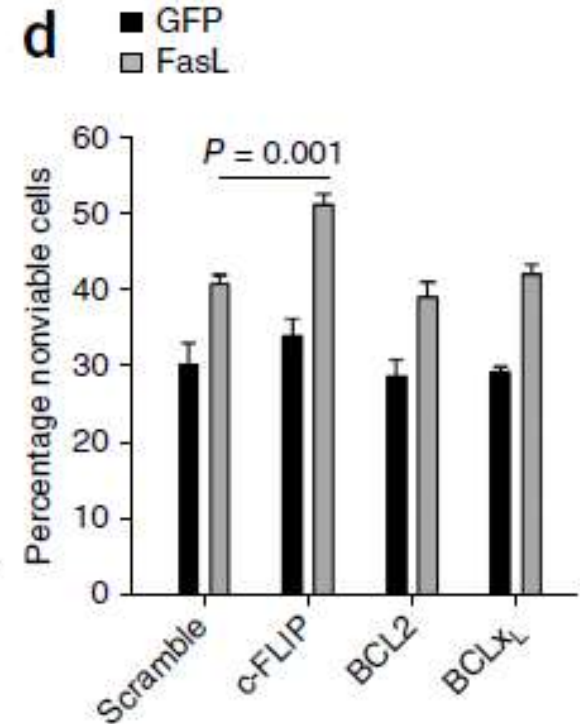
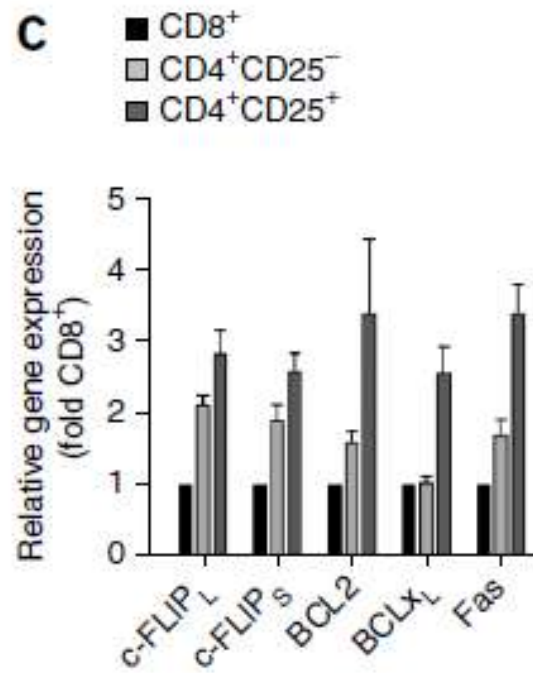
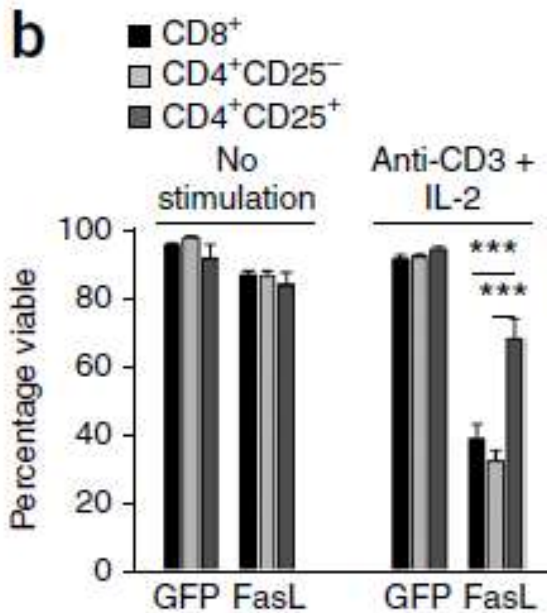
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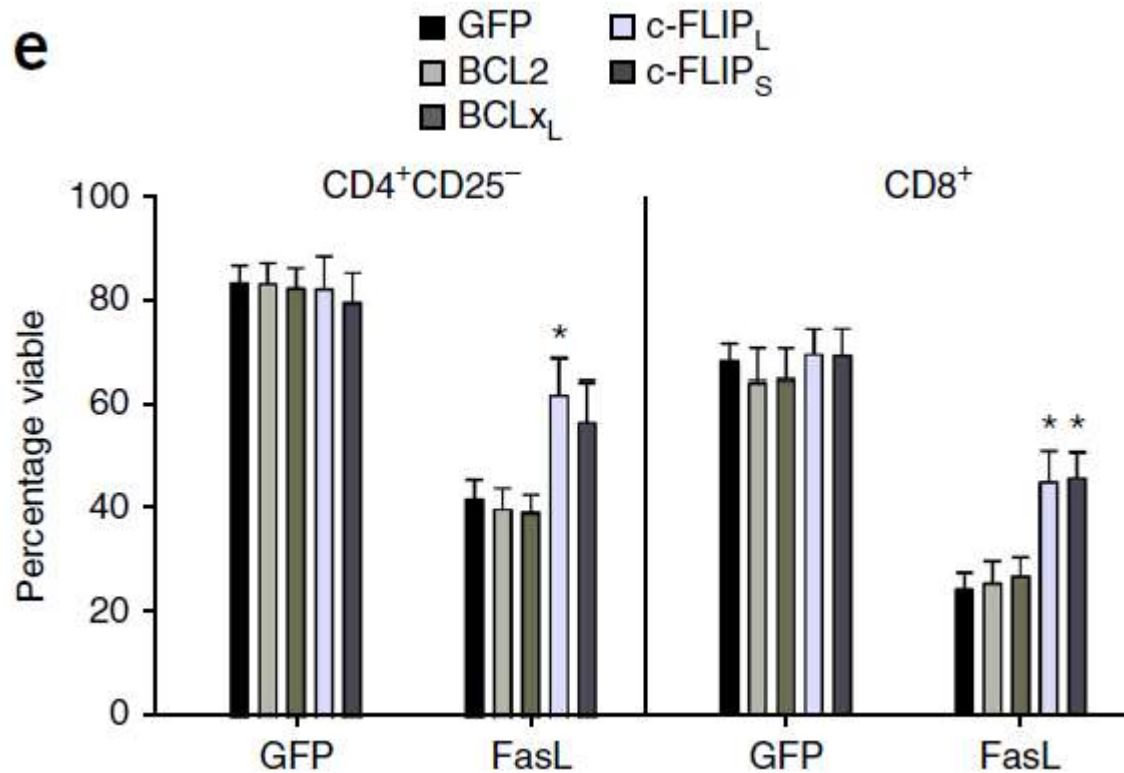
## Human endothelial cells expressing FasL kill effector T cells



Viability of T-cells co-incubated with tumor endothelium and FasL-block







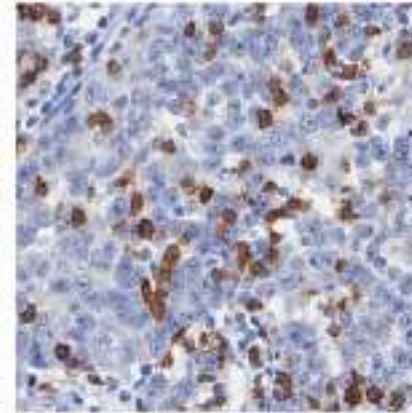
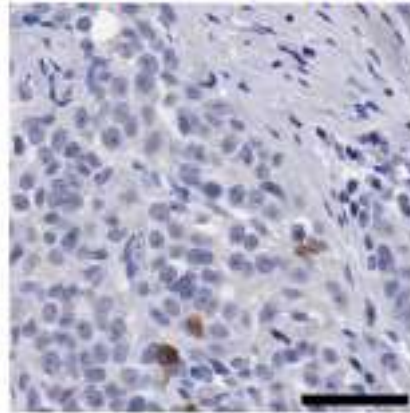
survival of CD8<sup>+</sup> and CD4<sup>+</sup>CD25<sup>-</sup> cells if transduced with anti-apoptotic genes

T

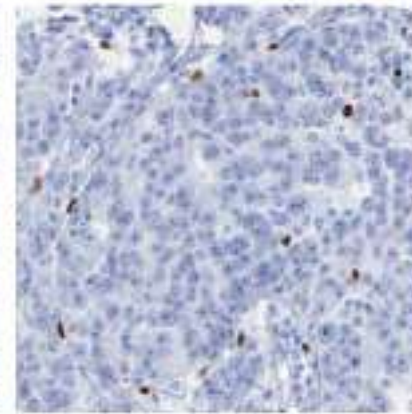
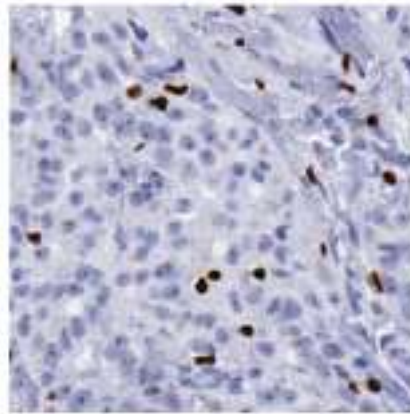
FasL<sup>+</sup> vessels

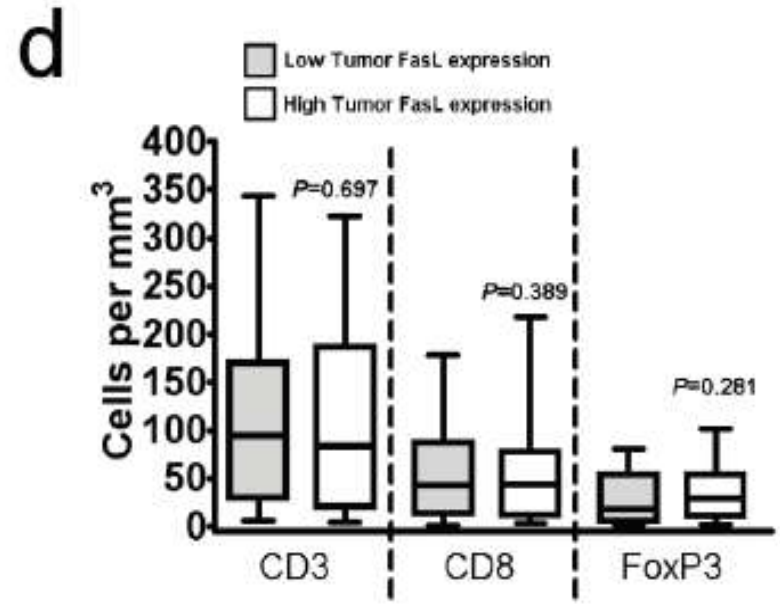
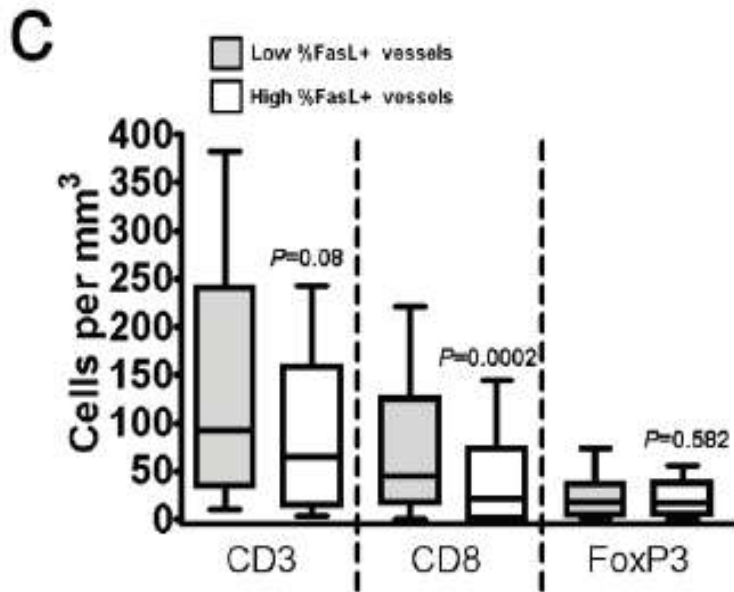
FasL<sup>-</sup> vessels

CD8



FoxP3



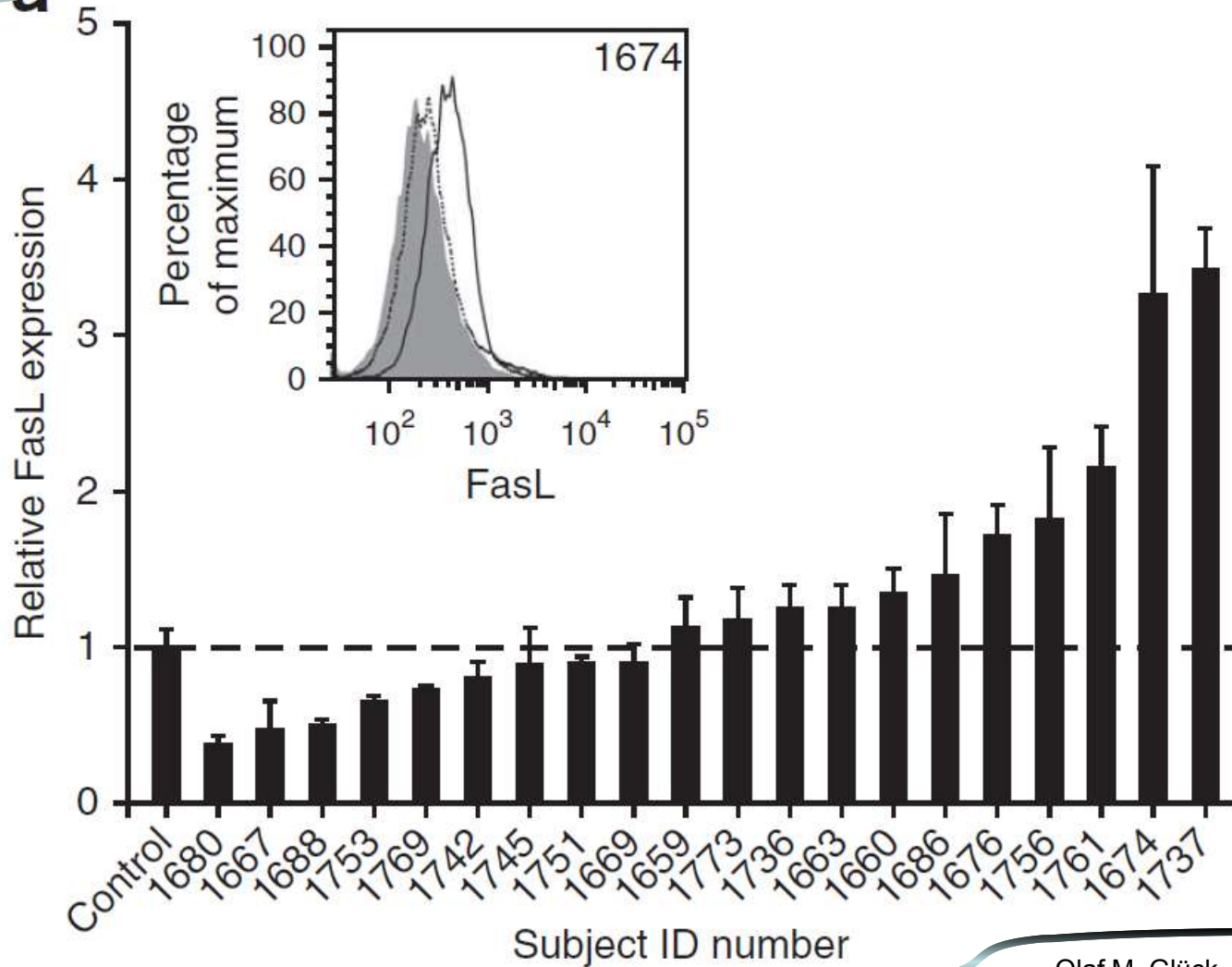


## Results

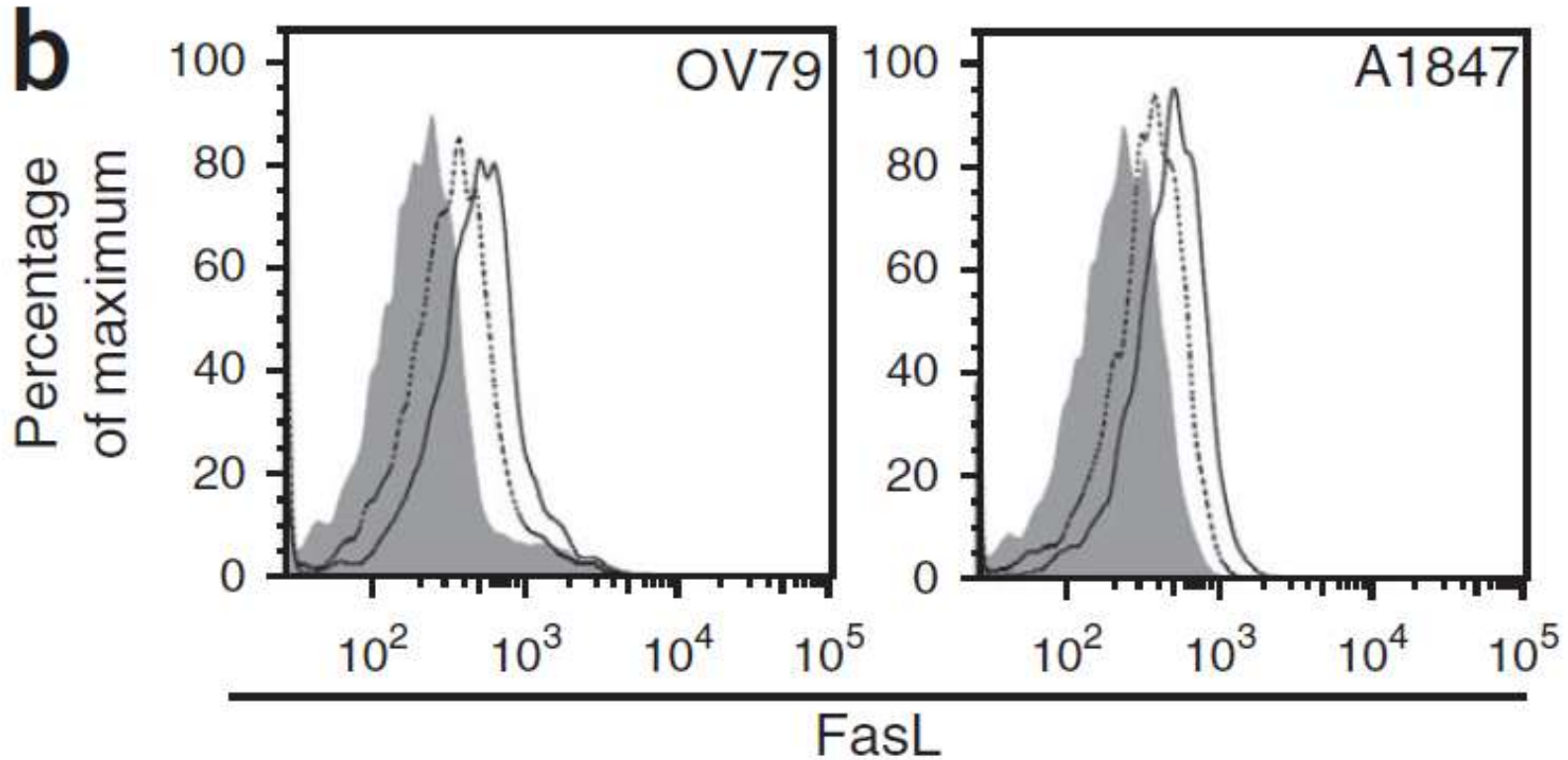
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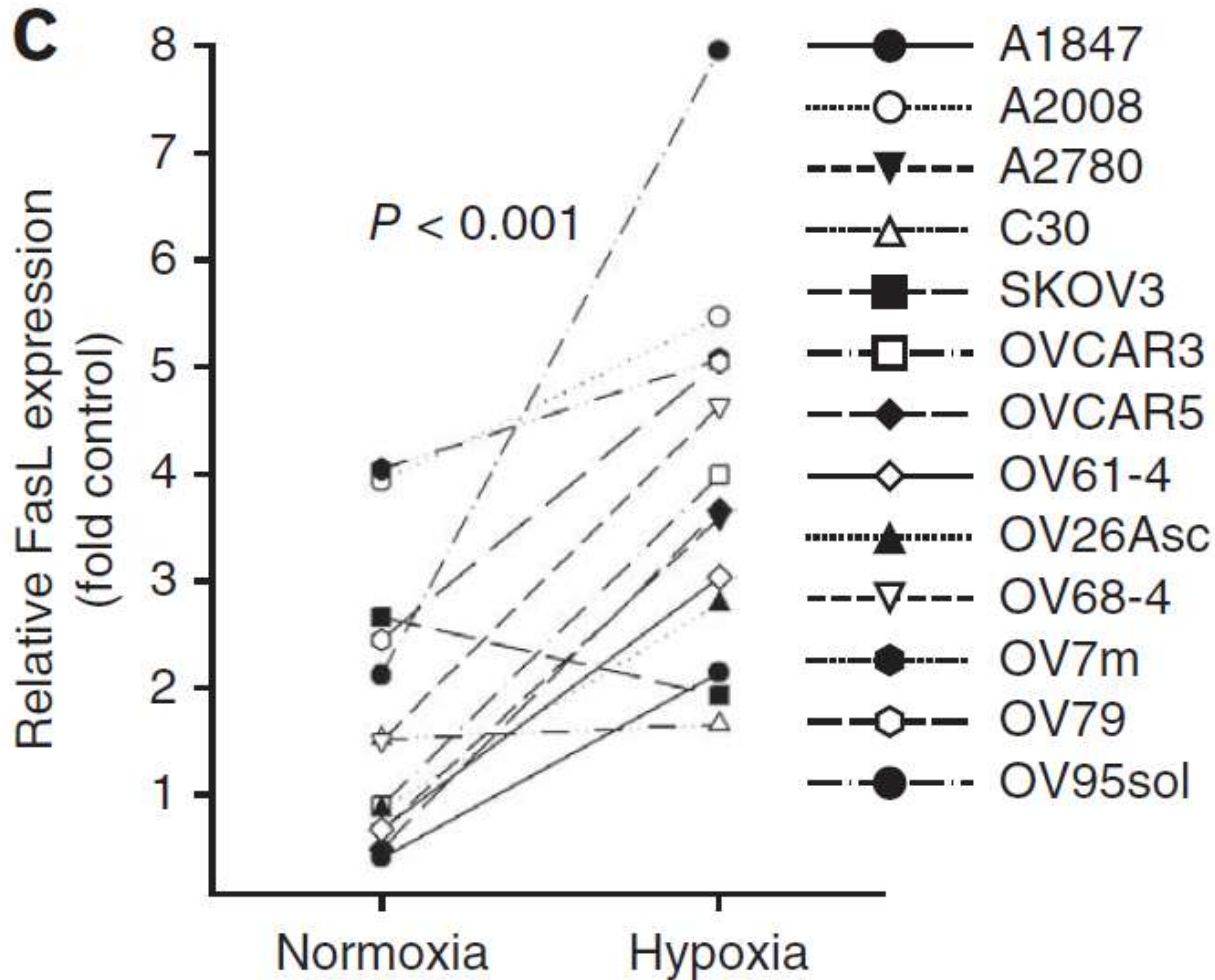
#### **Tumor-derived factors induce endothelial FasL expression**

- hypoxic tumors induce more FasL than normoxic ones
- FasL expression can be decreased by pharmacologically suppressing tumor derived factors

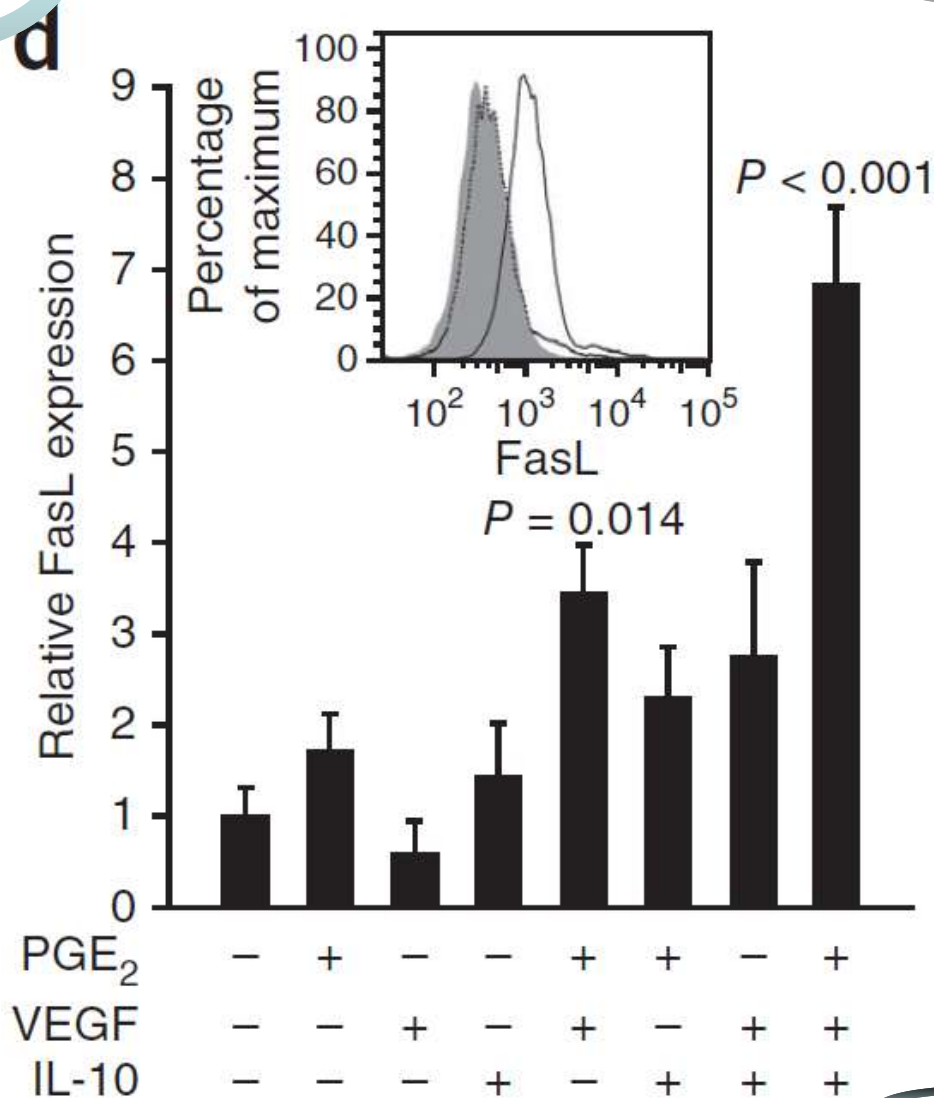


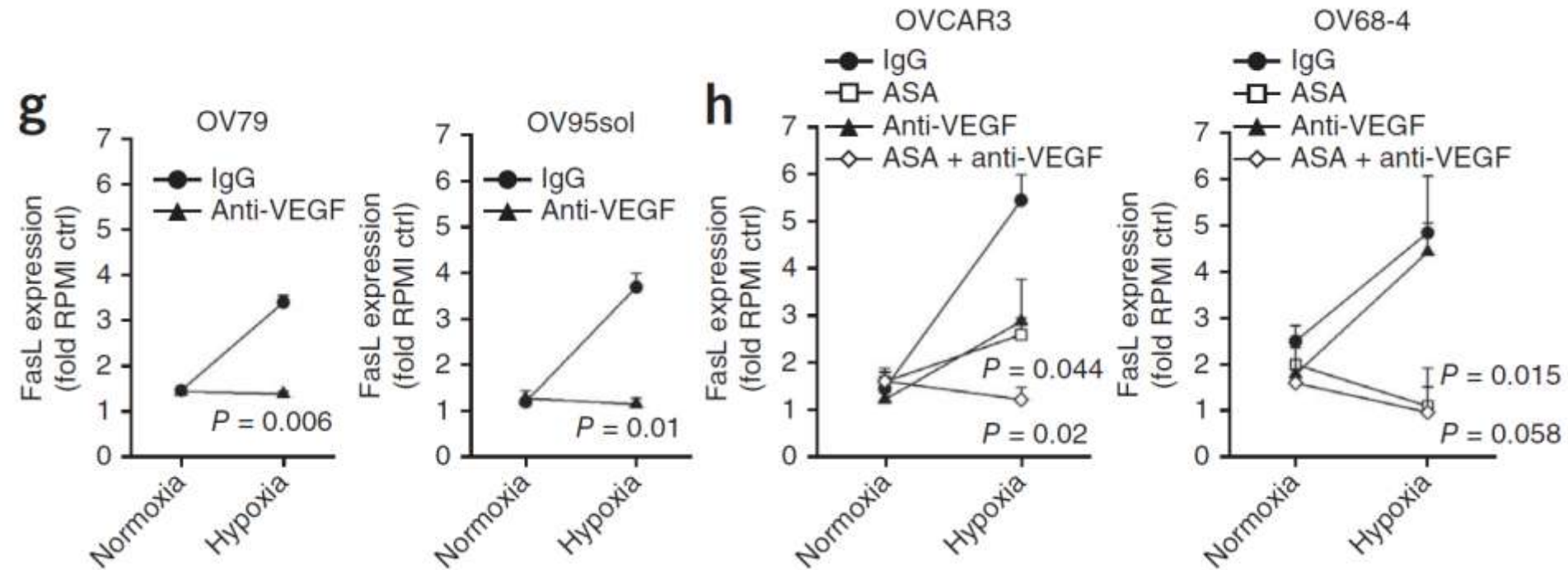
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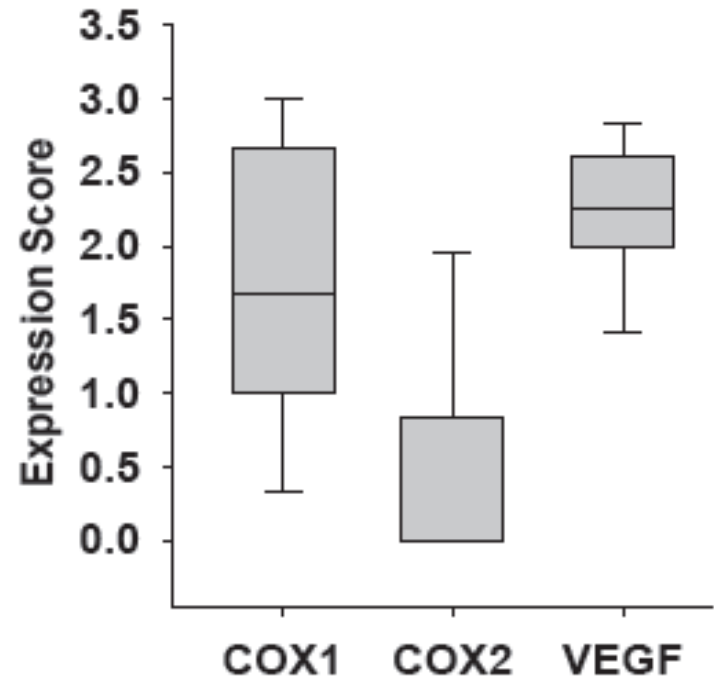
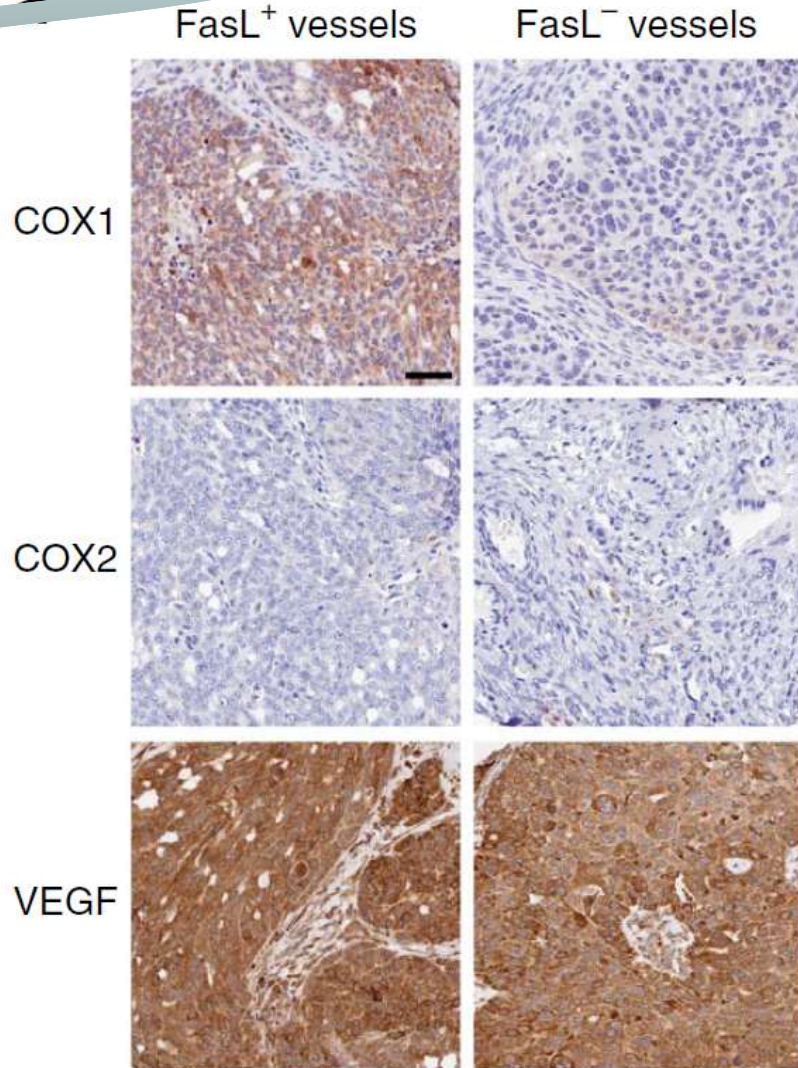
indirect pharmacological suppression of FasL

## Results

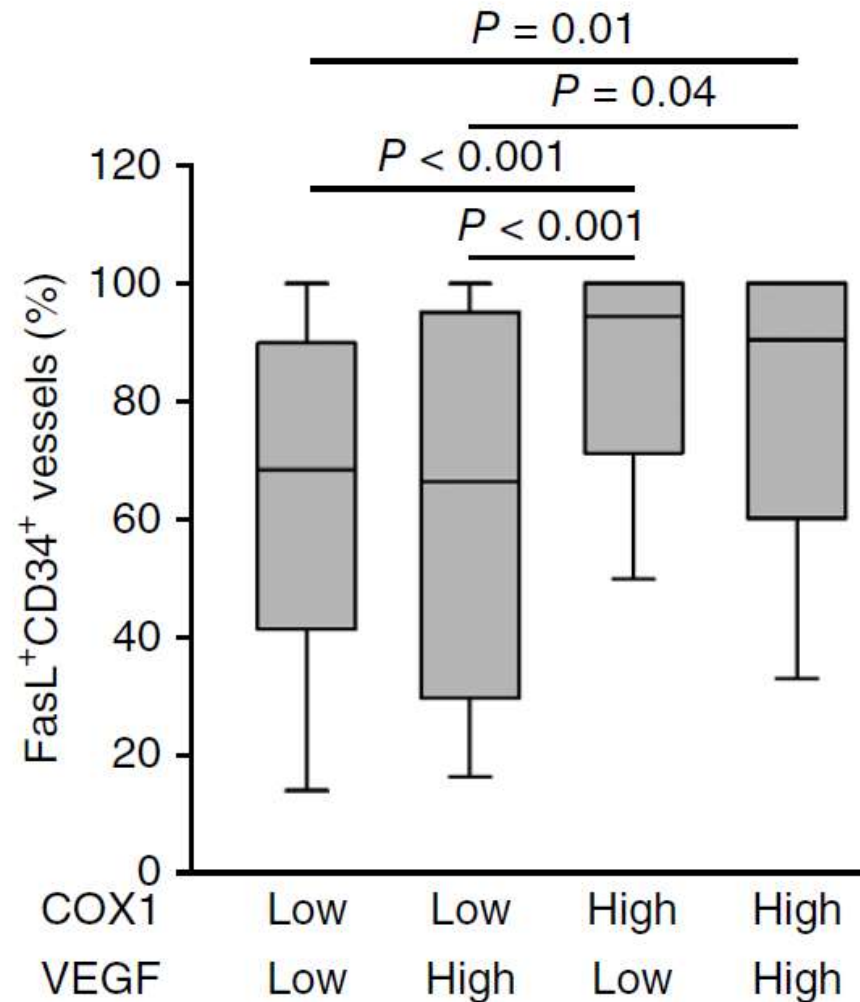
### 4.

#### **COX and VEGF regulate endothelial FasL expression *in vivo***

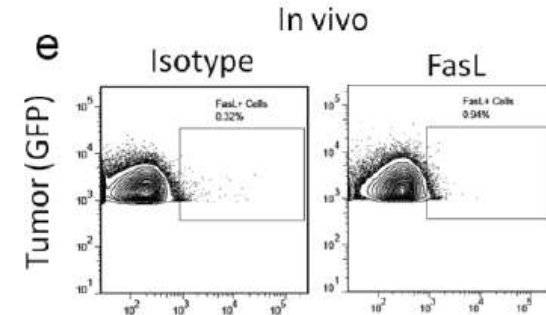
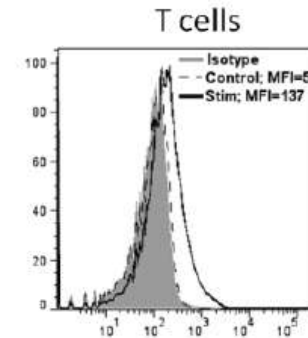
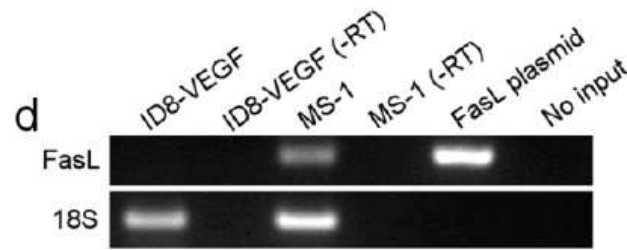
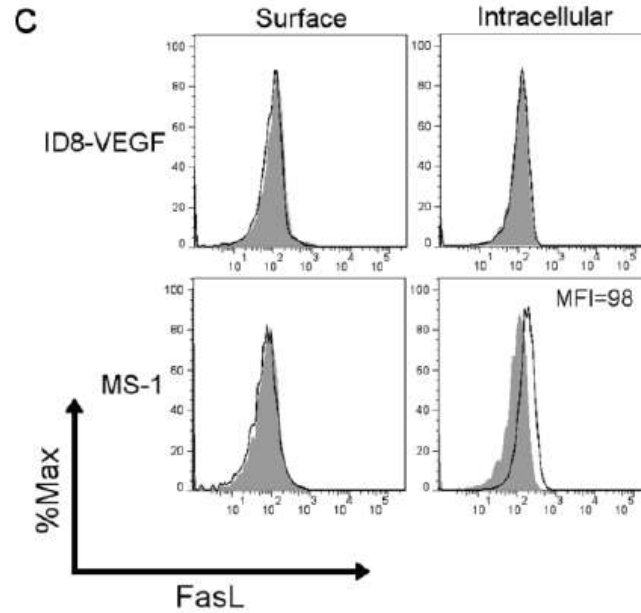
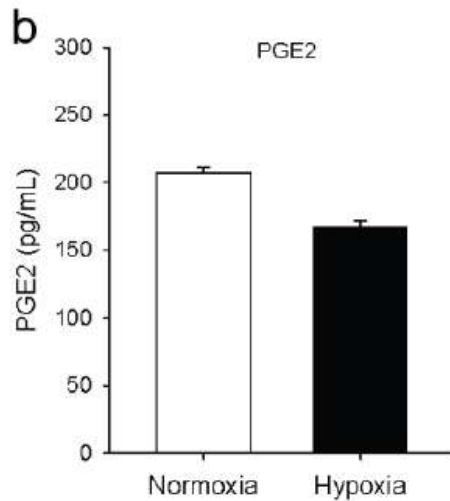
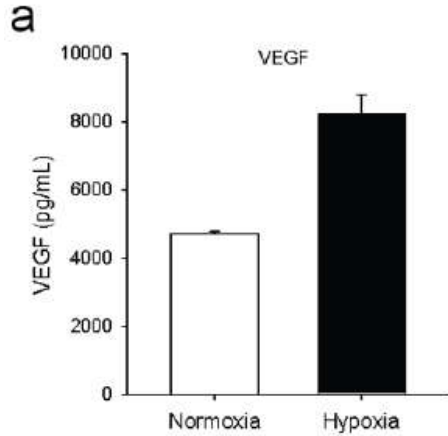
- in vitro, coexpression can induce FasL
- tumor volume correlates with mediators and FasL expression



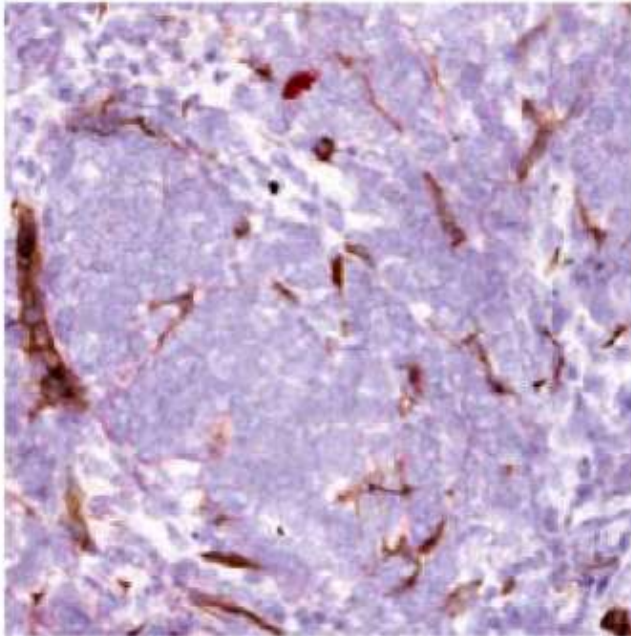
*in vitro*; TMA and Quantification



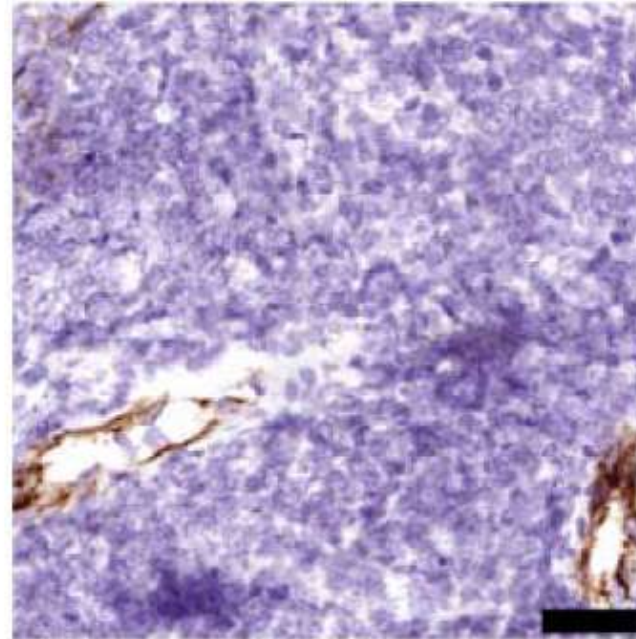
no FasL expression in ID8-VEGF ovarian cancer



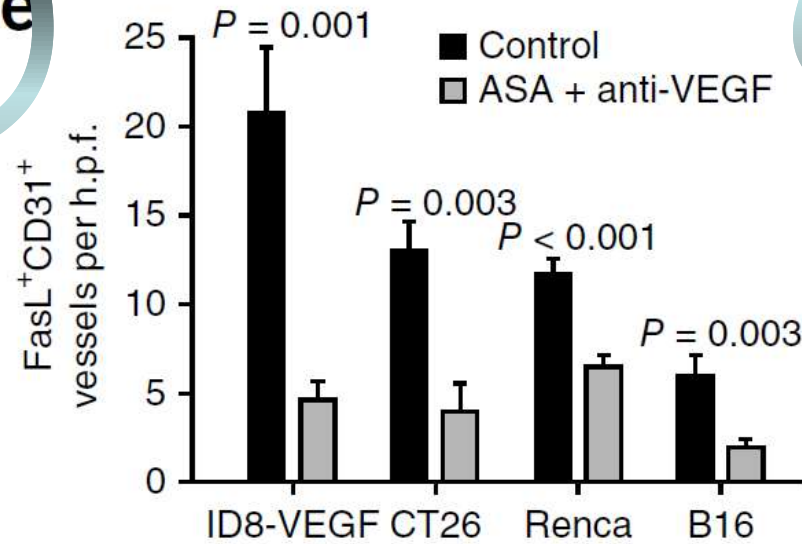
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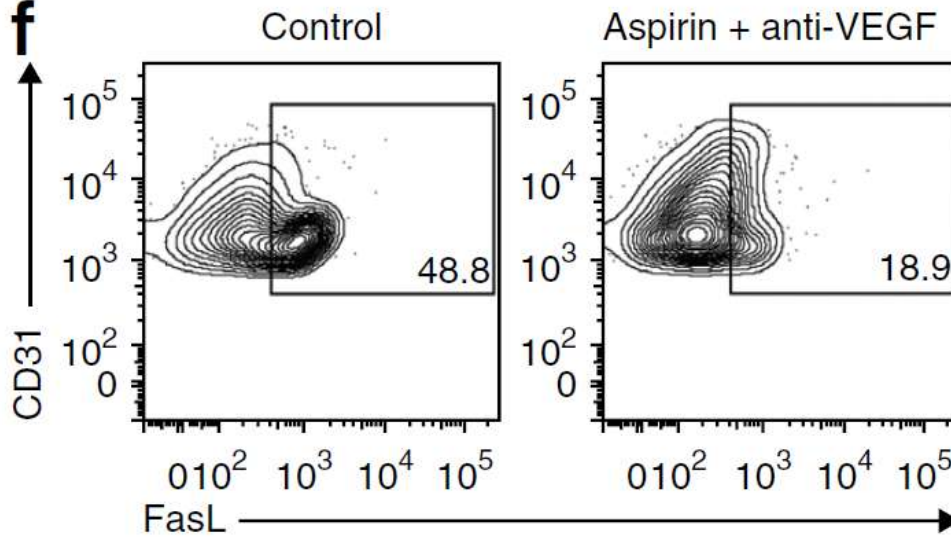
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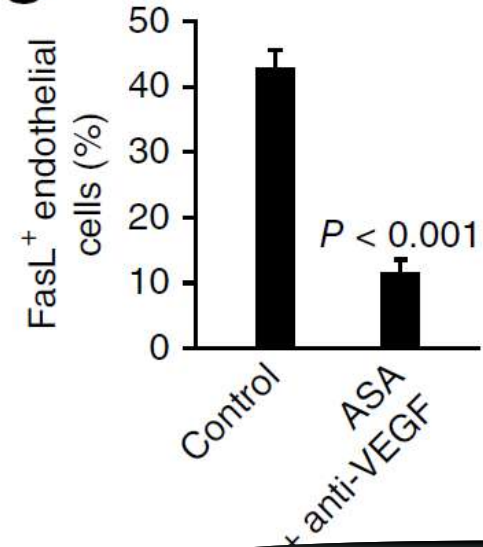
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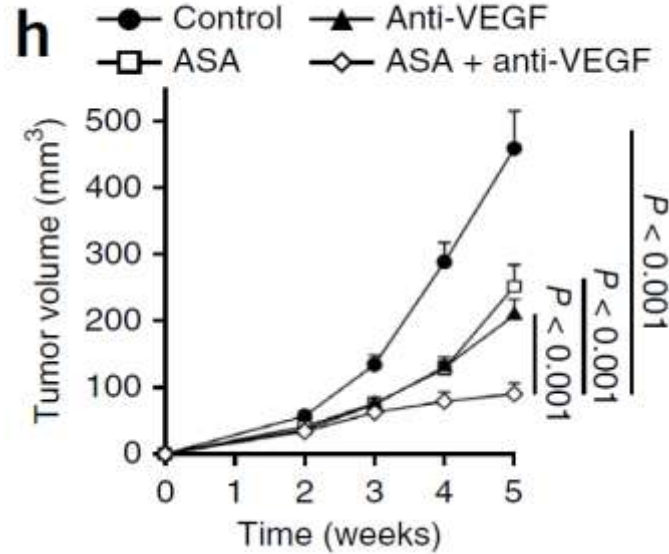
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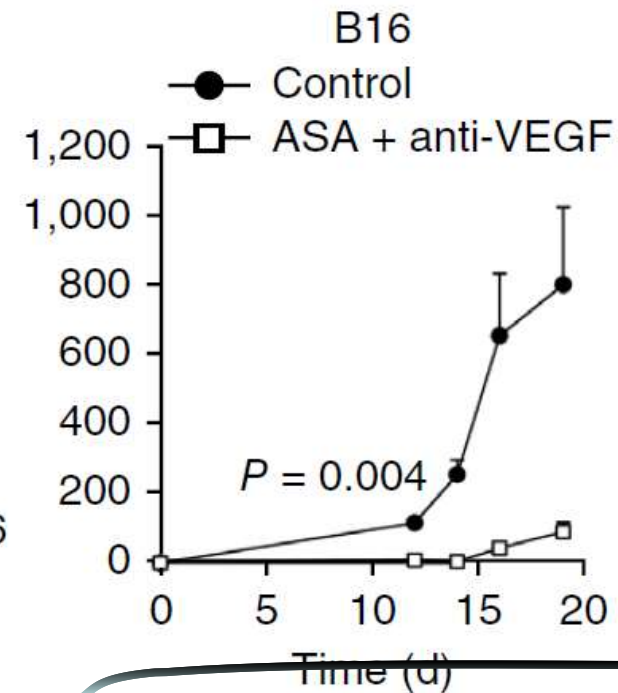
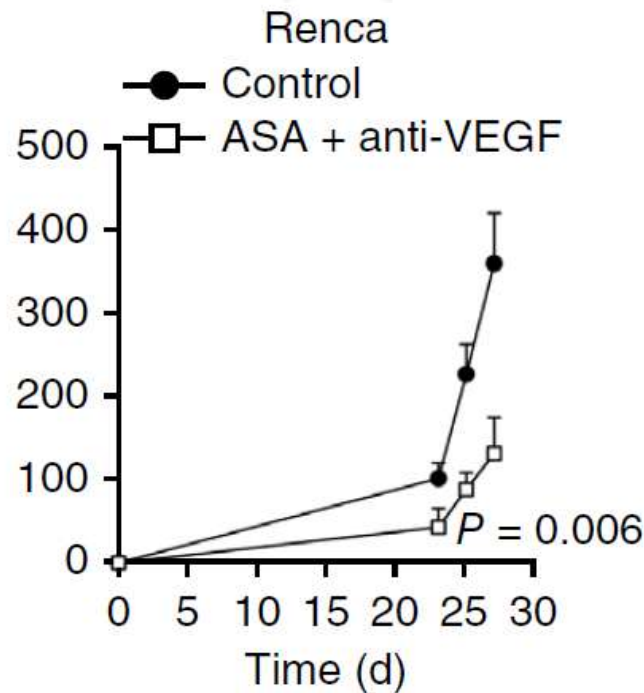
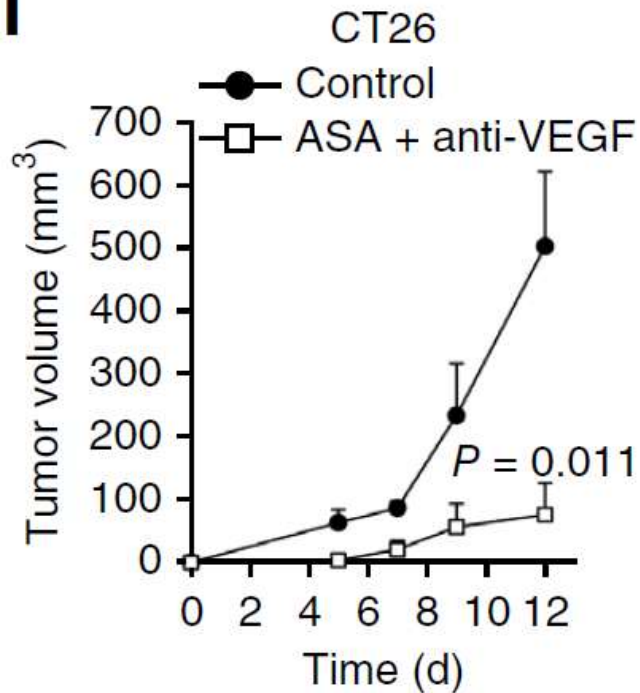
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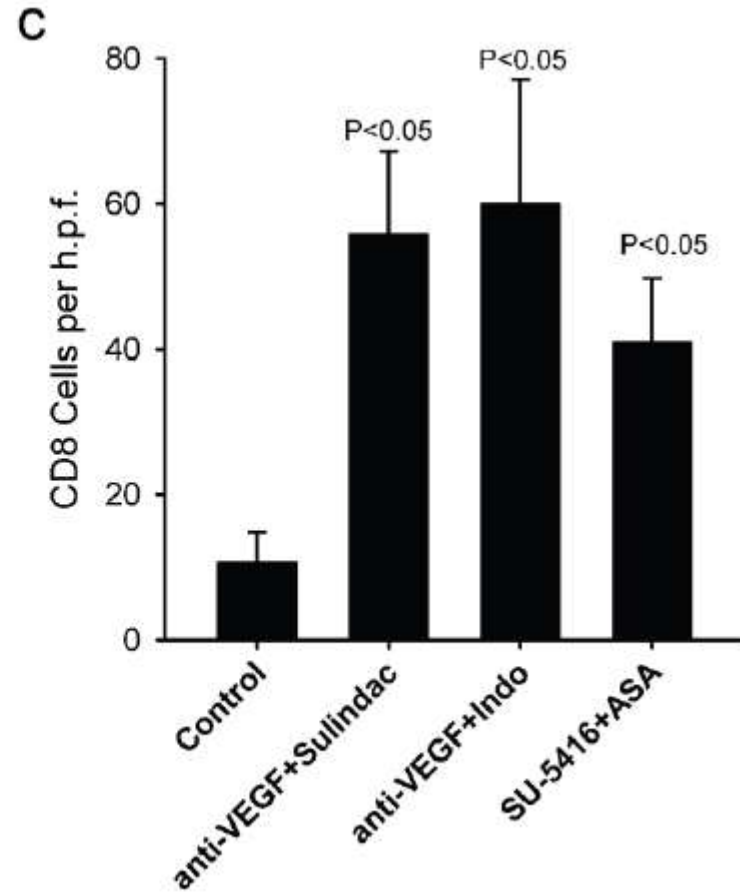
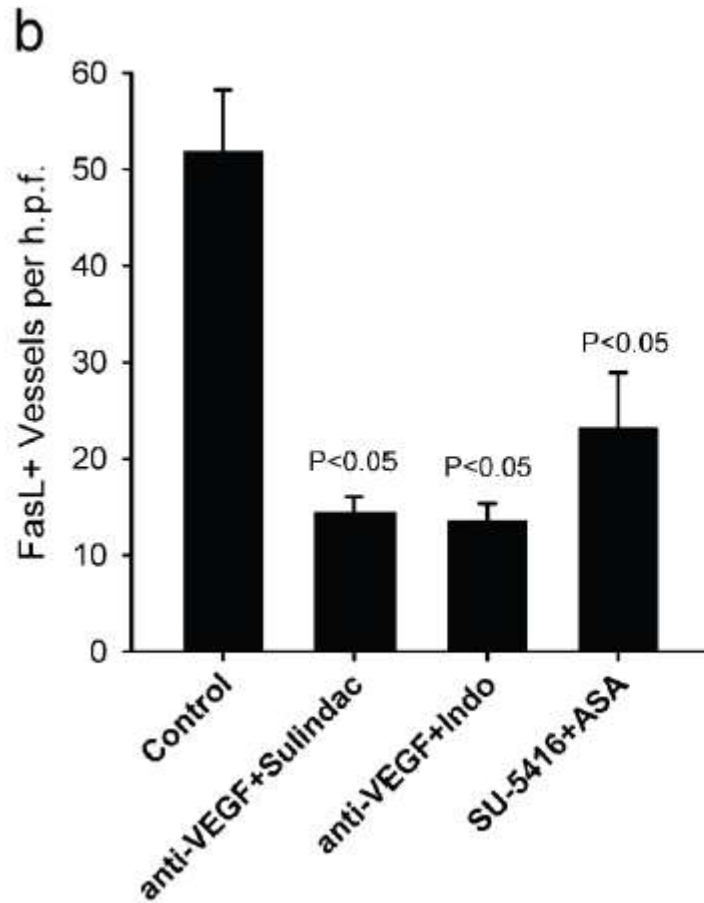






**i**



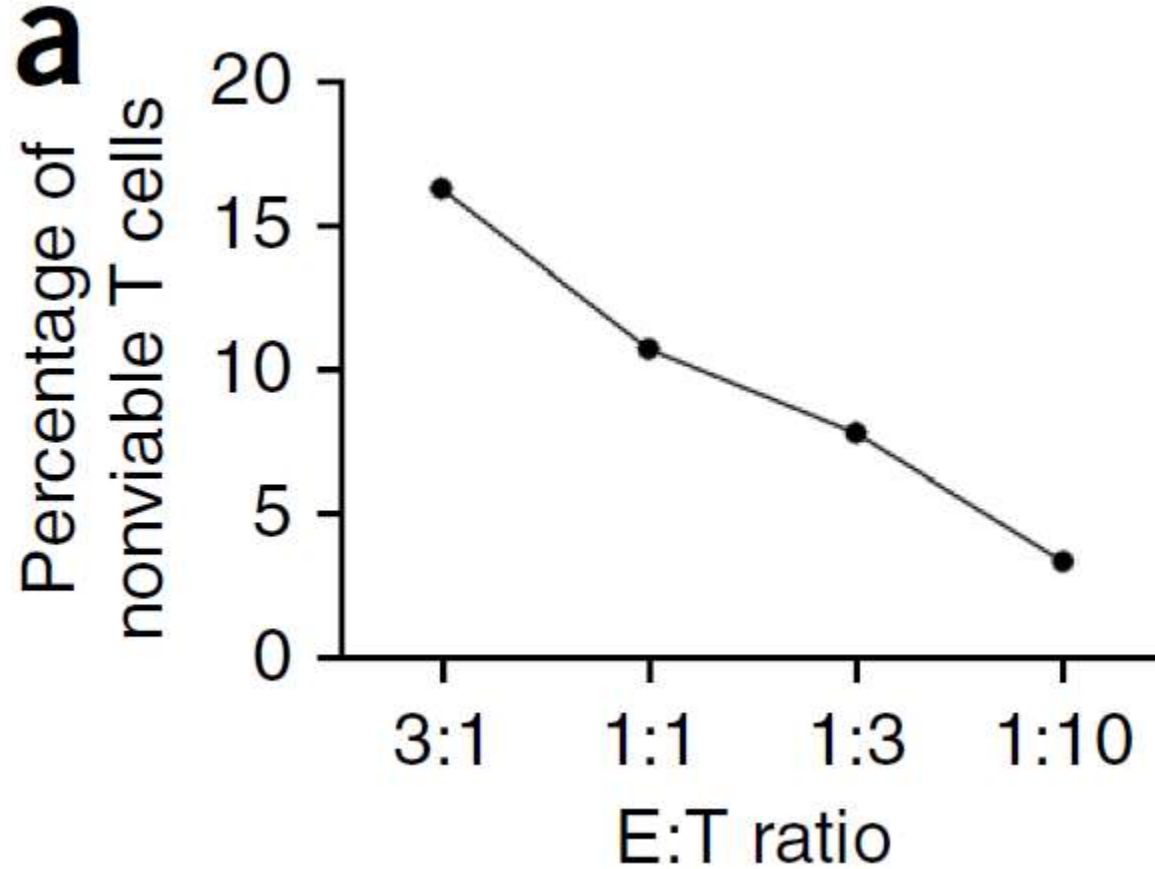


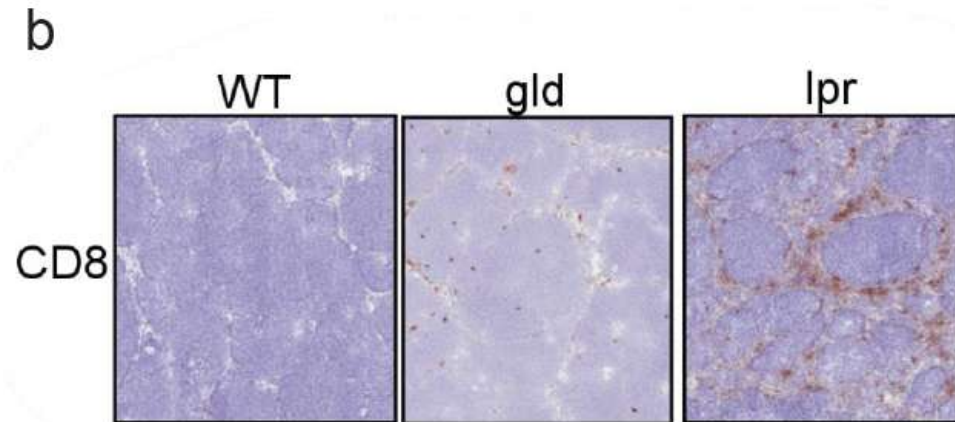
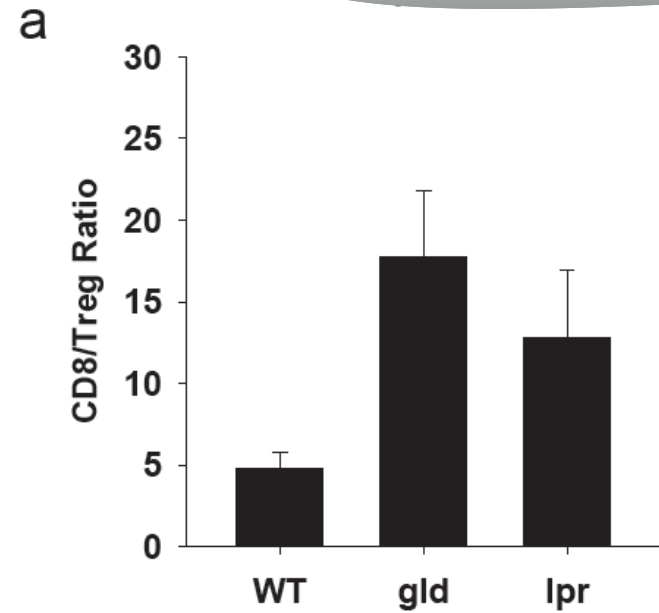
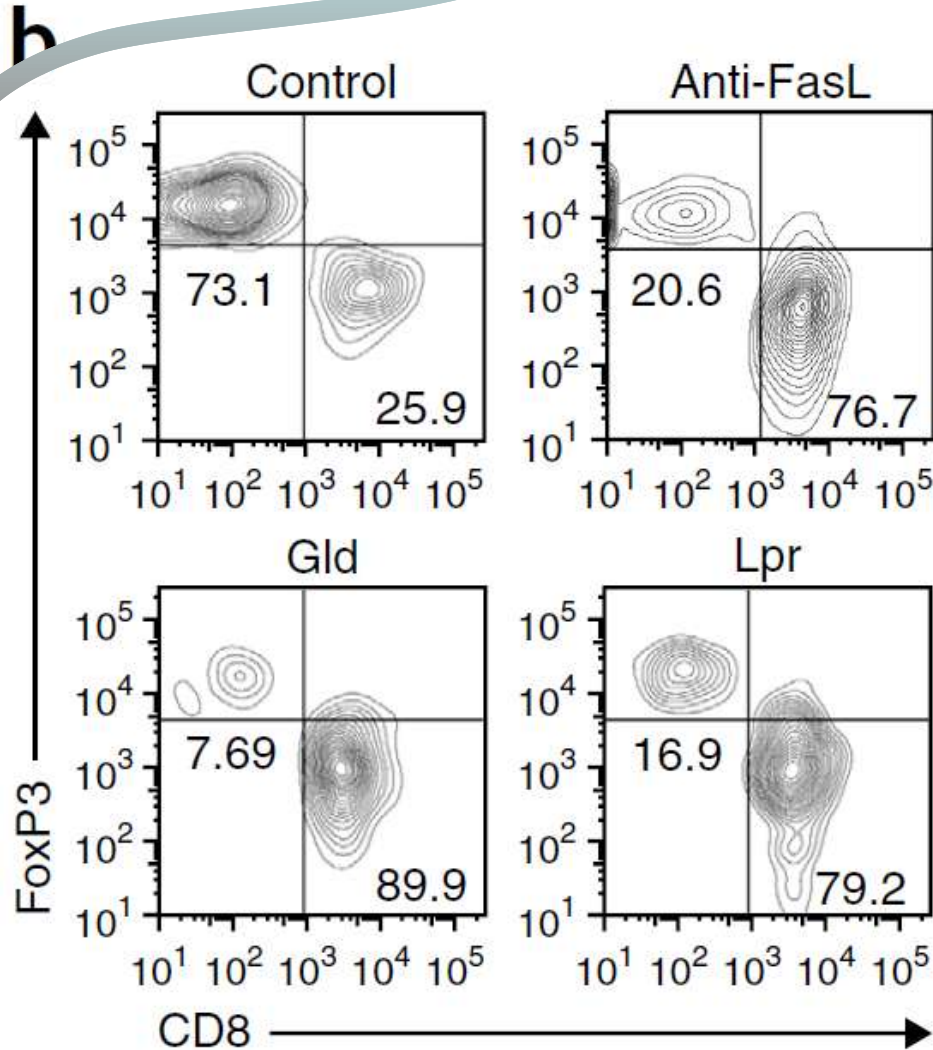
## Results

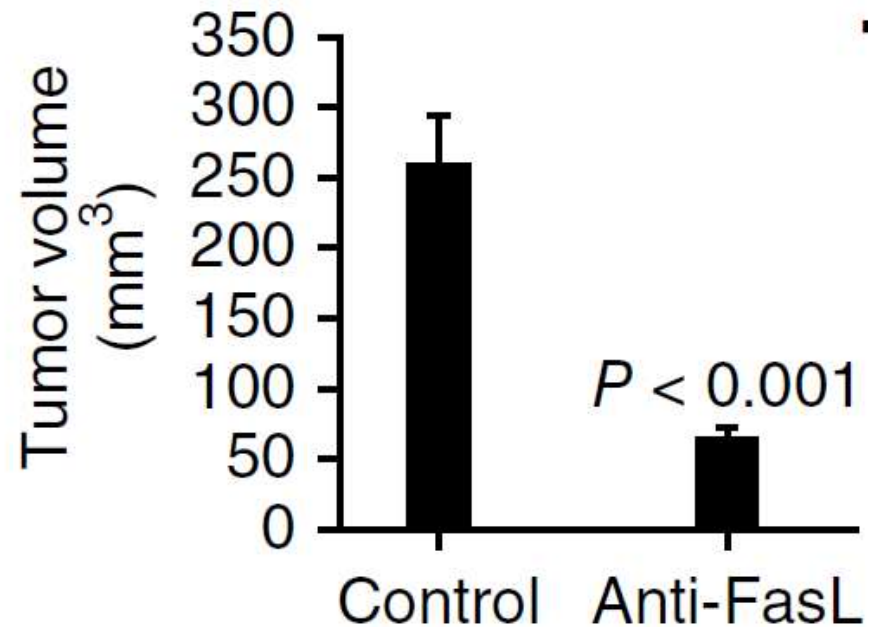
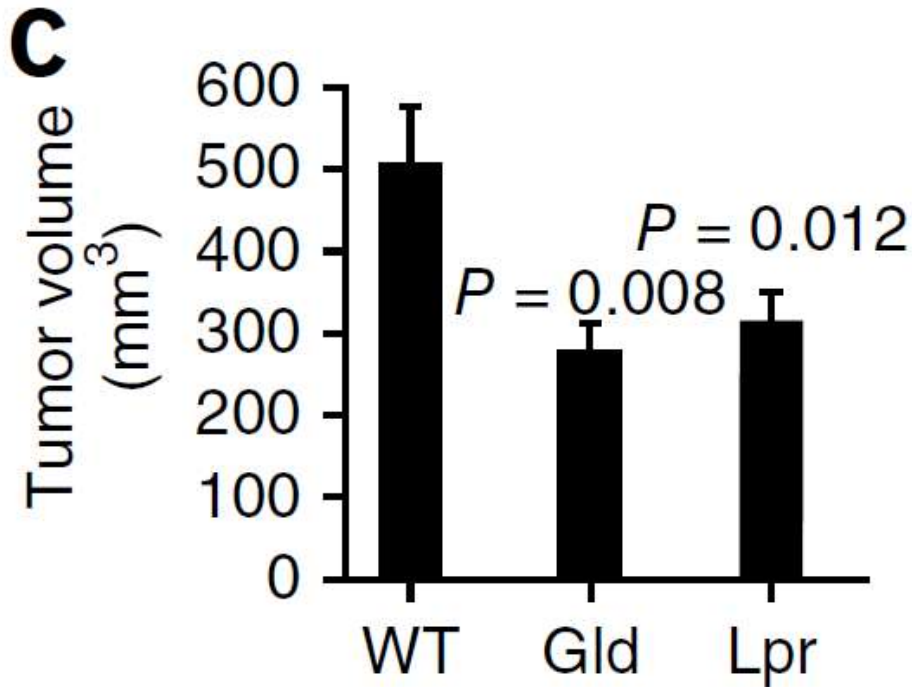
### 5.

#### **Endothelial FasL prevents CD8+ cell infiltration in mice**

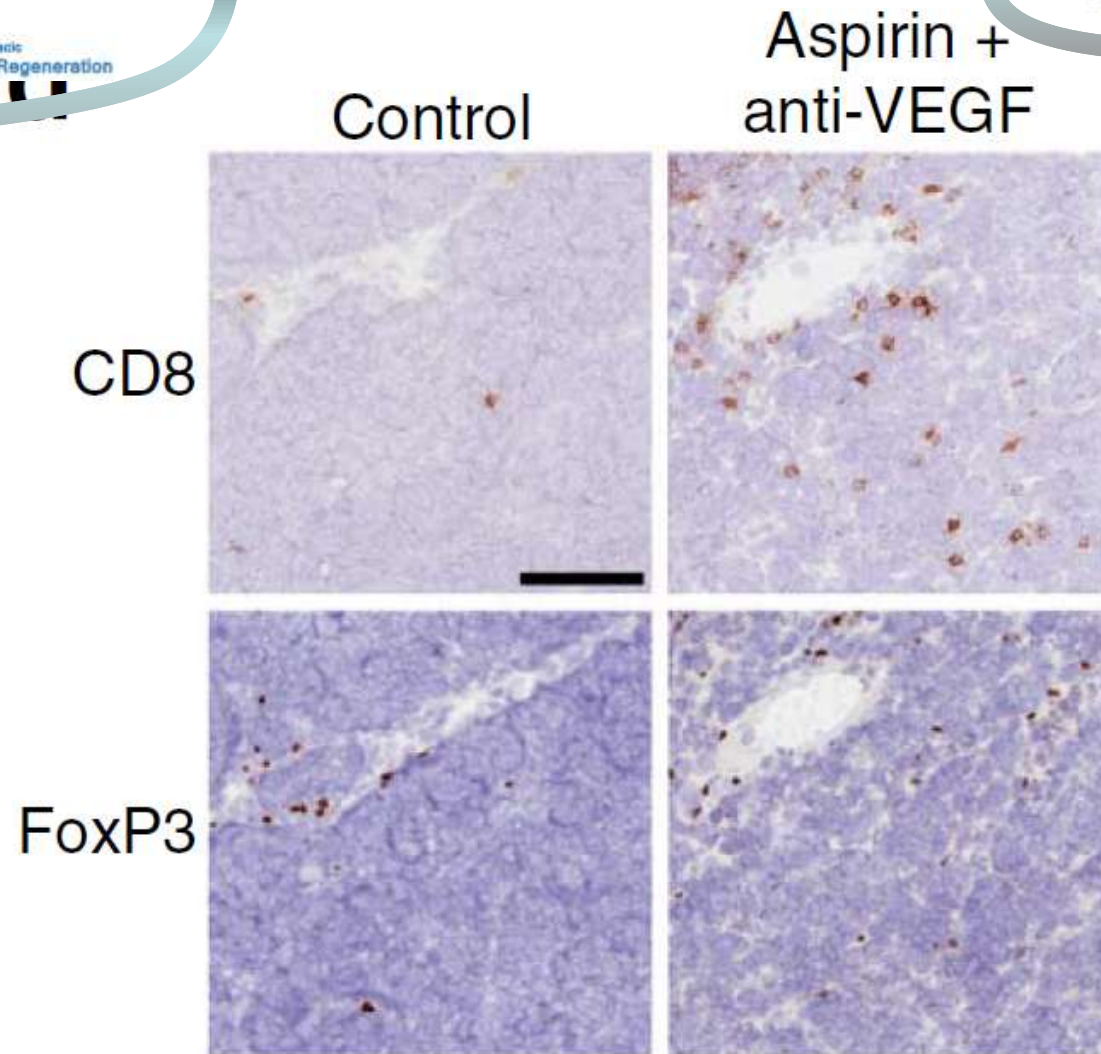
- comparison *in vivo* – *in vitro*
- disruption of Fas-FasL signaling enhances T-cell infiltration  
→ direct effect on tumor growth





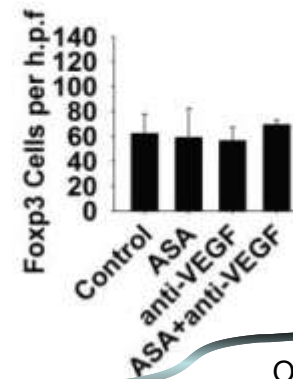
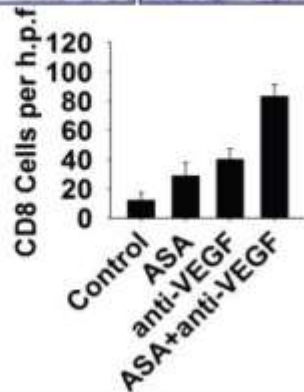
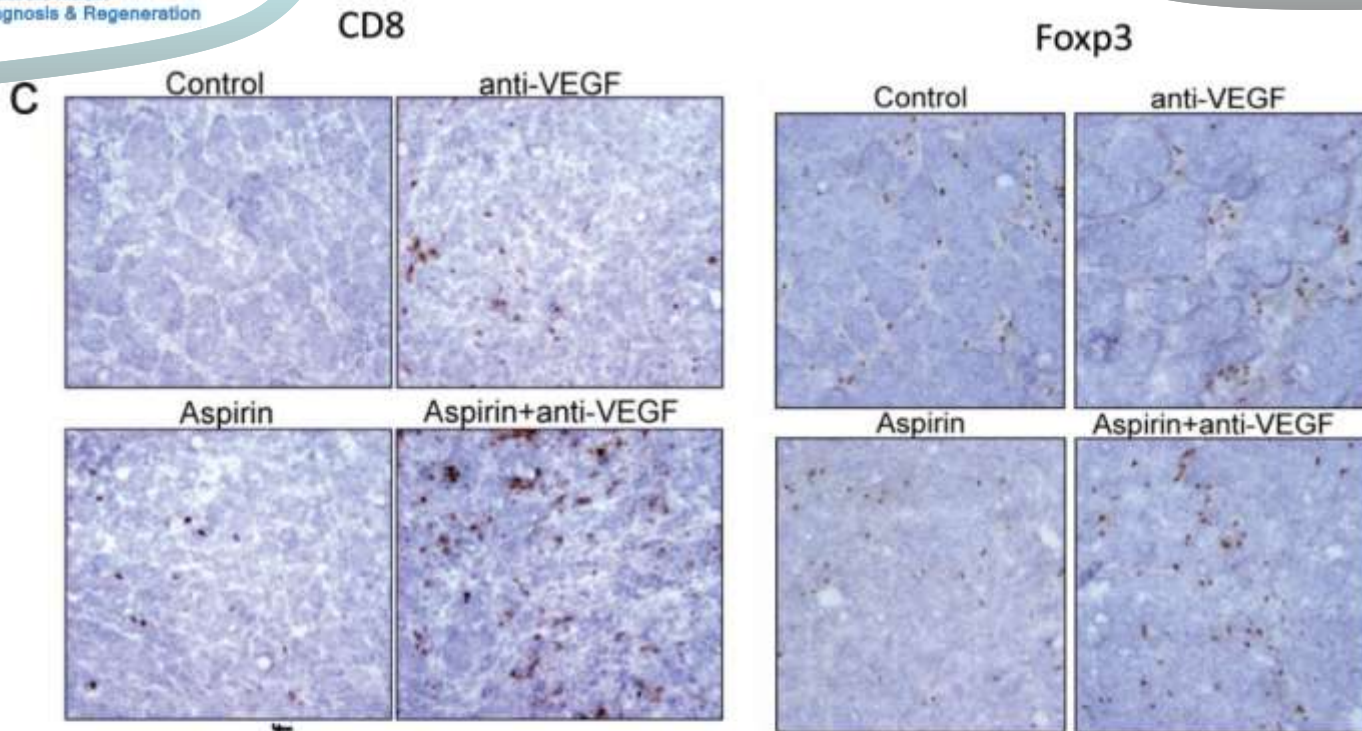


Tumor growth rates in ID8-VEGF tumors



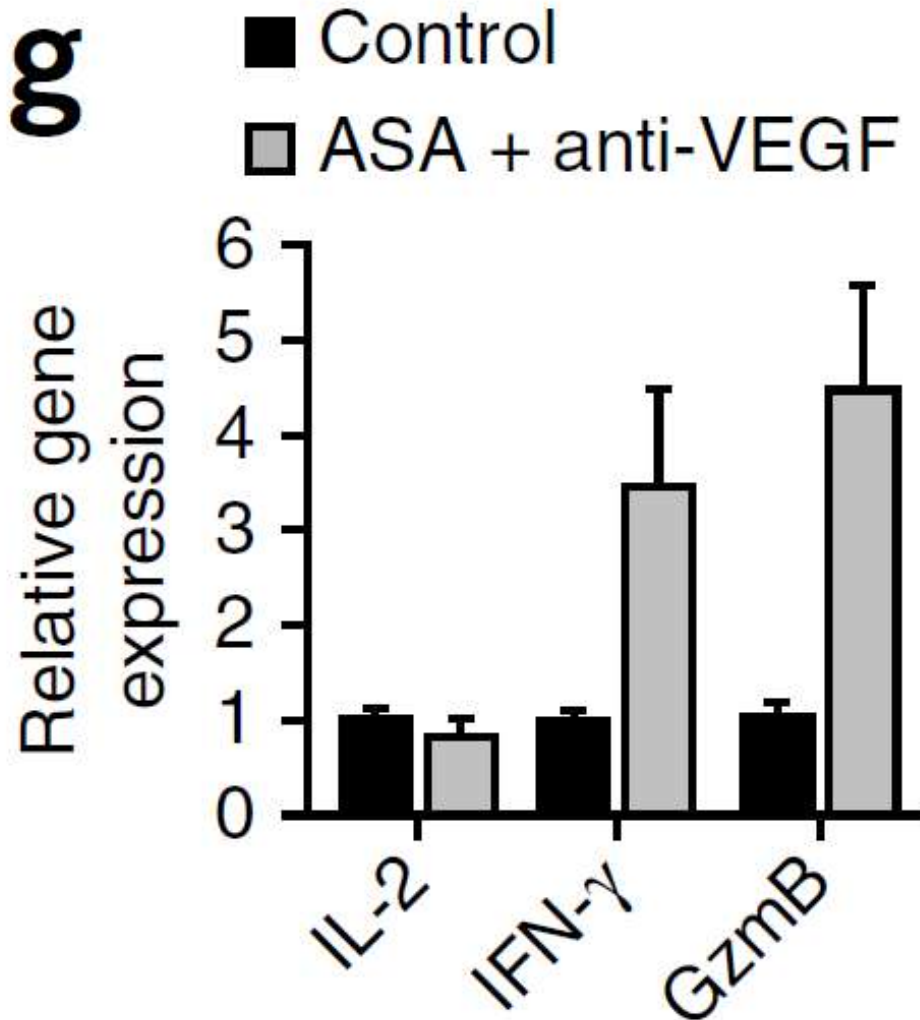
■ CD8    □ FoxP3

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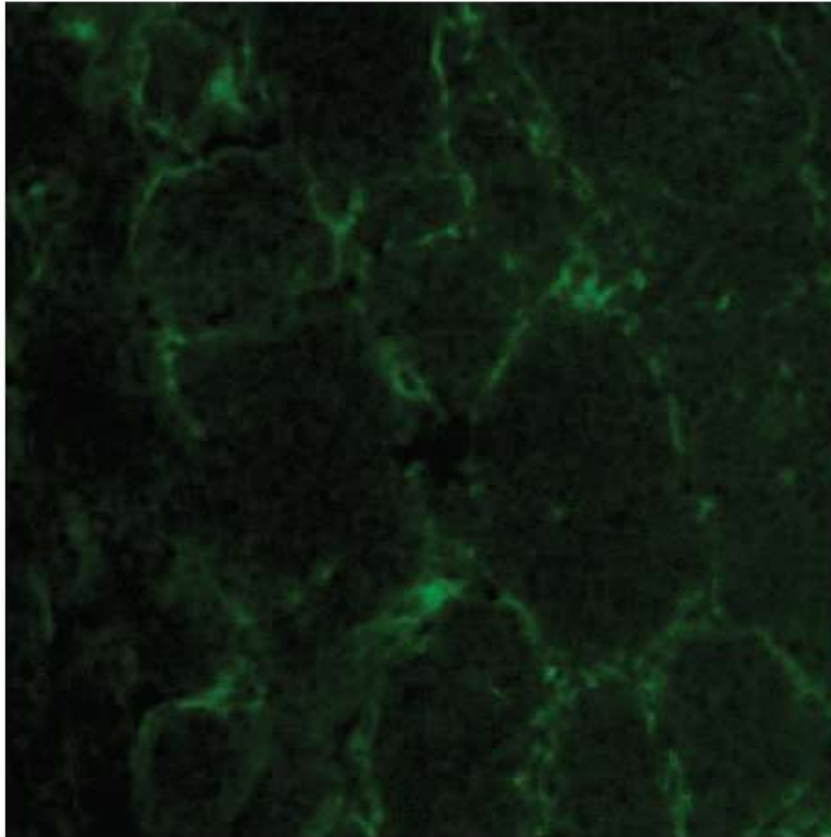




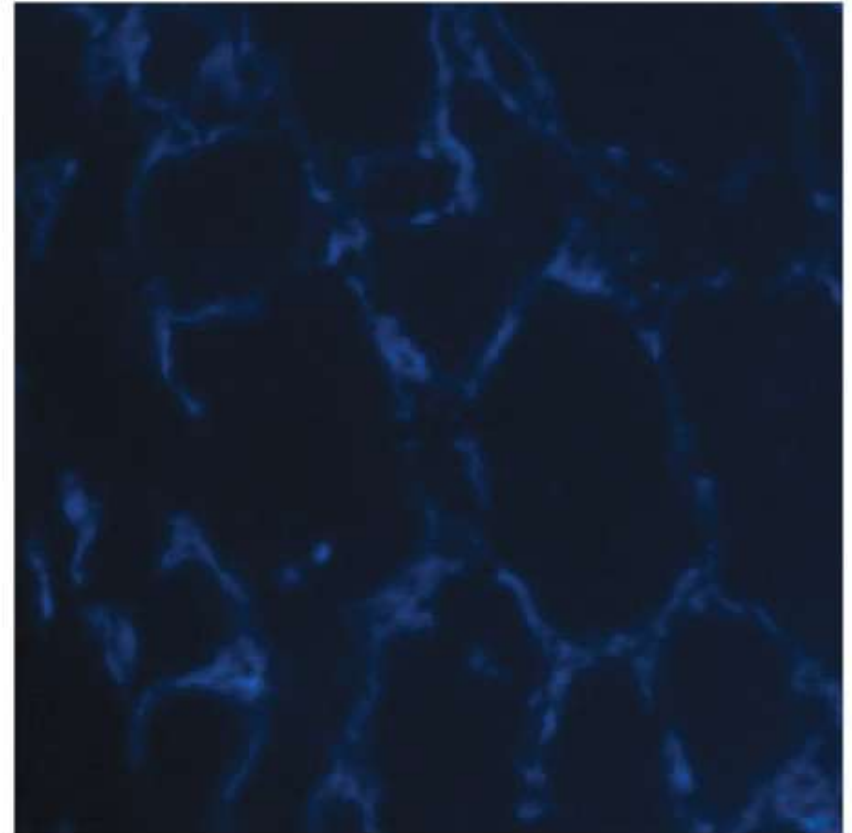
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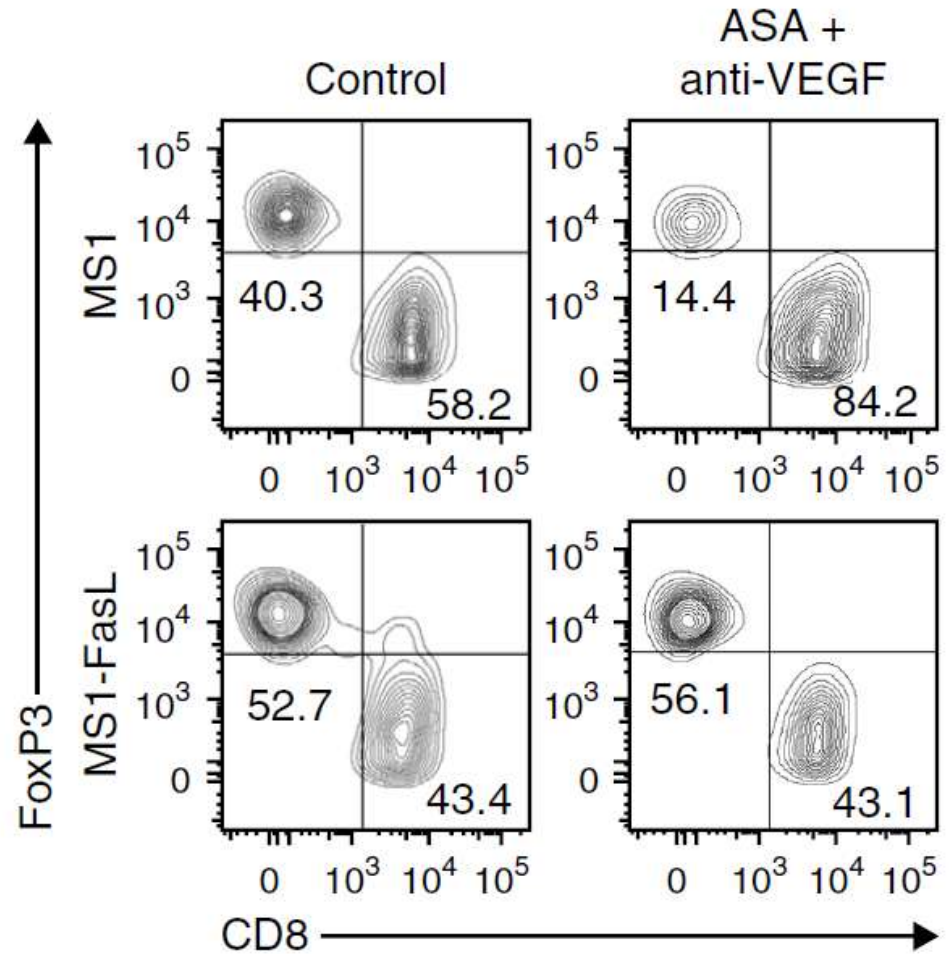
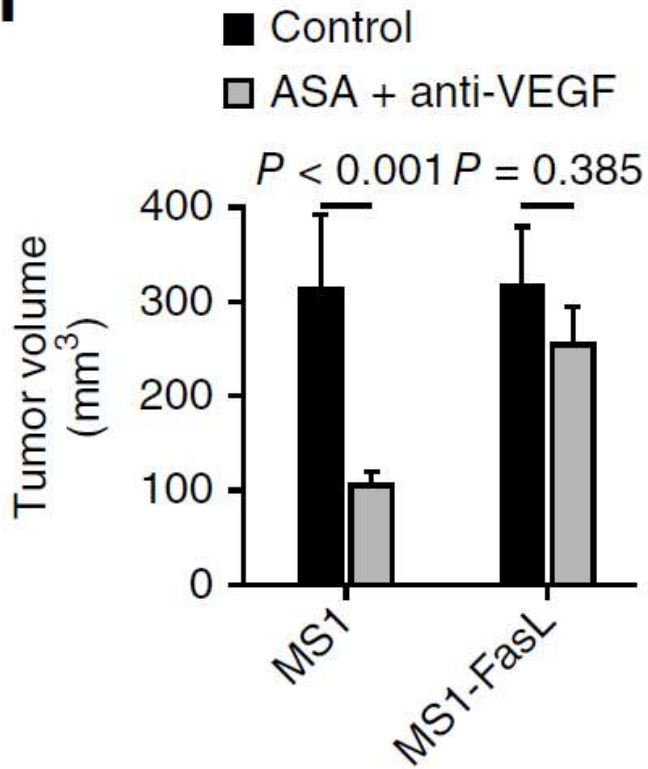
## mouse CD31



## human PSMA



h

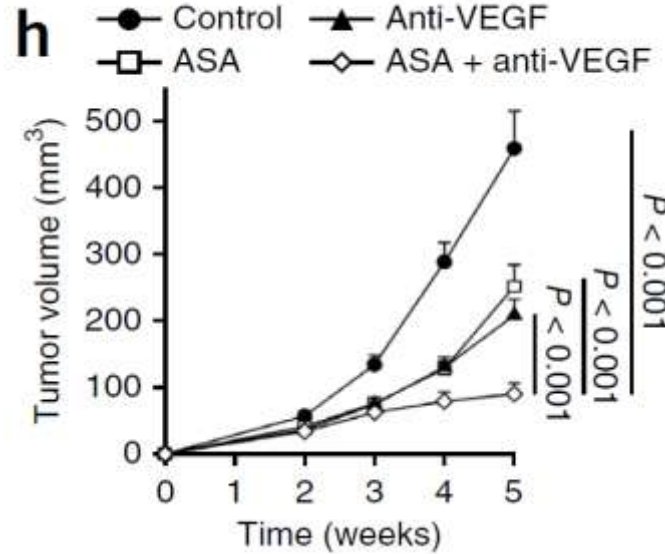


## Results

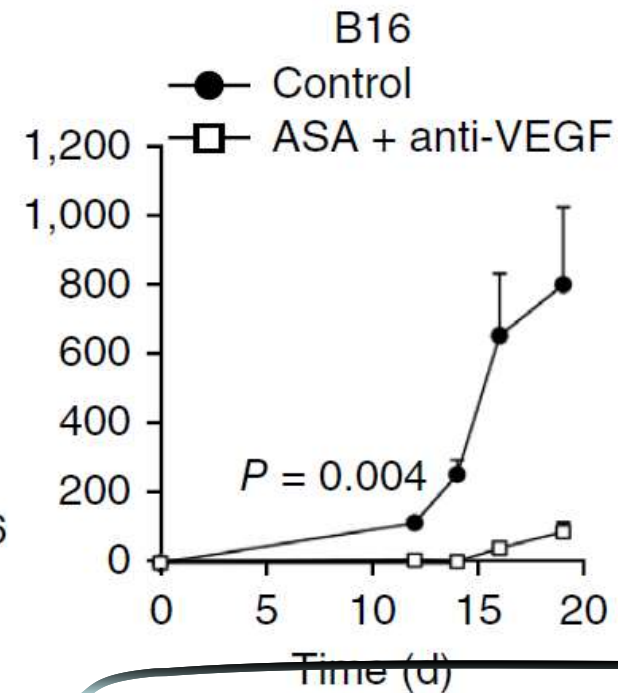
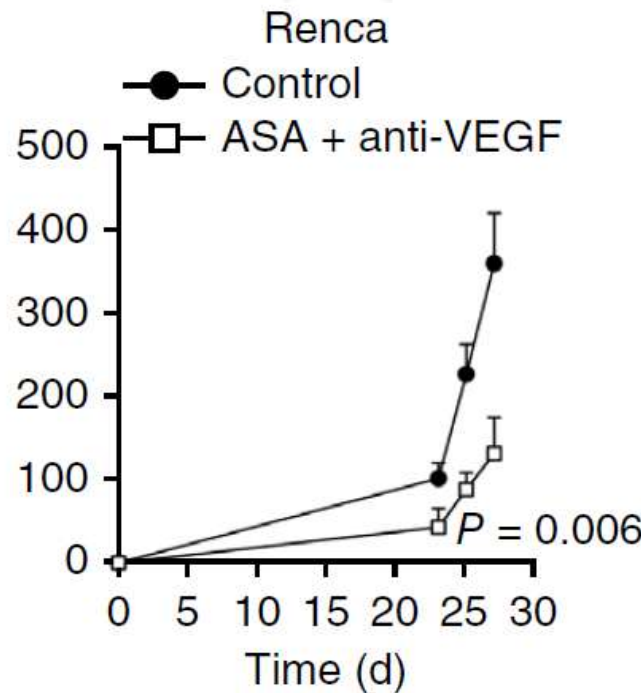
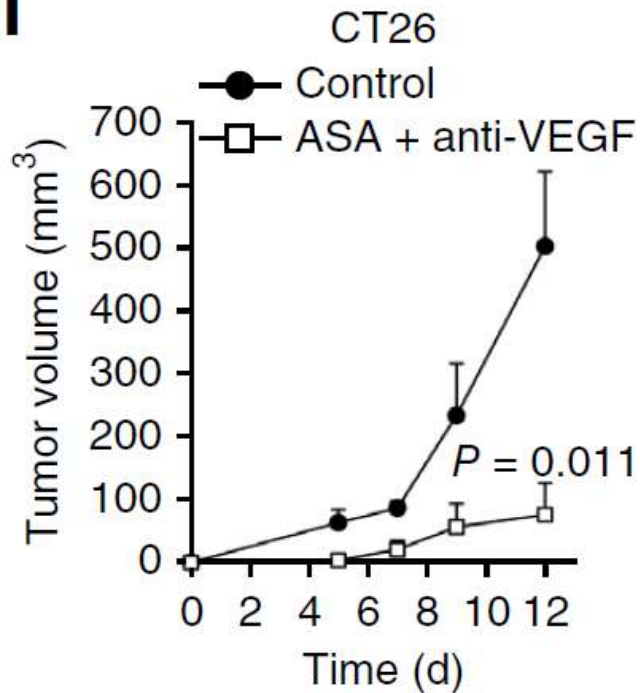
### 6.

#### **Cox and Vegf blockade promotes tumor-suppressing immunity**

- suppression of tumor growth depends on CD8+/Treg ratio
- pharmacological suppression depends on CD8+ cell density

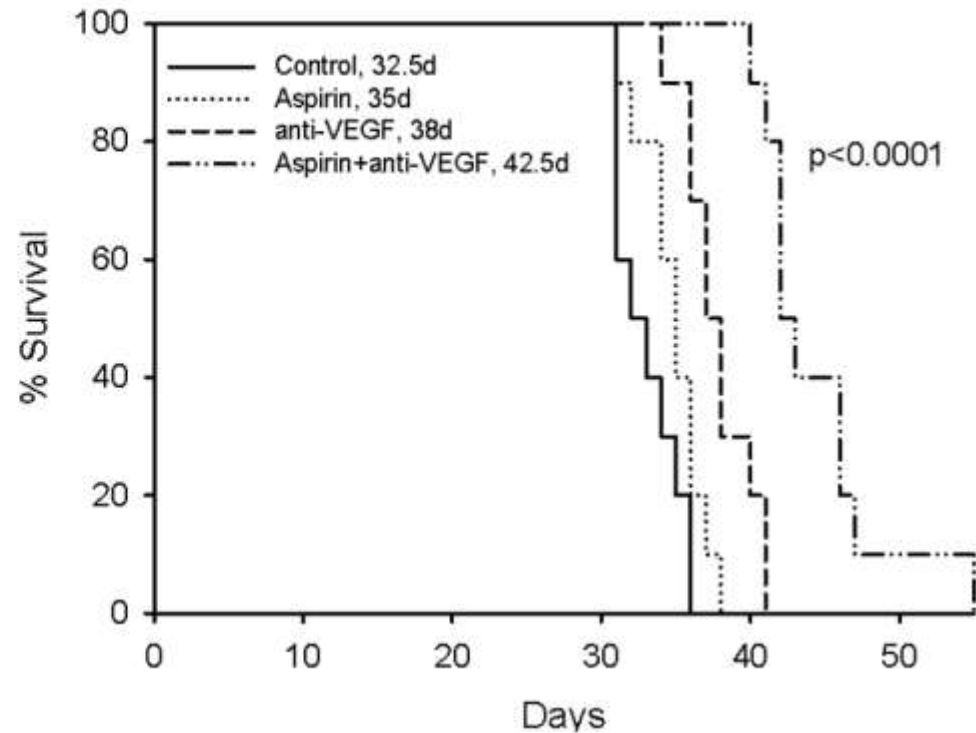
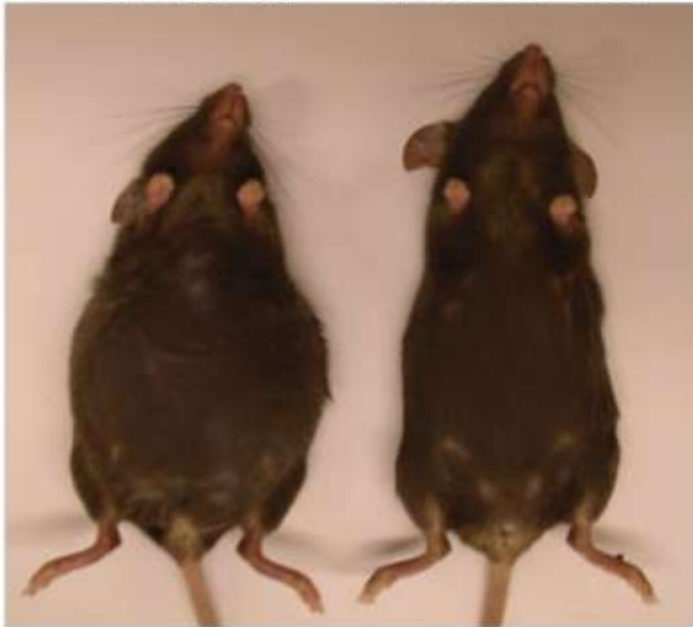


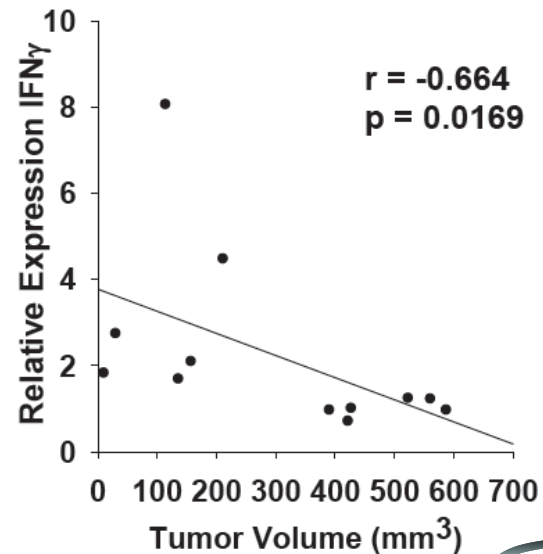
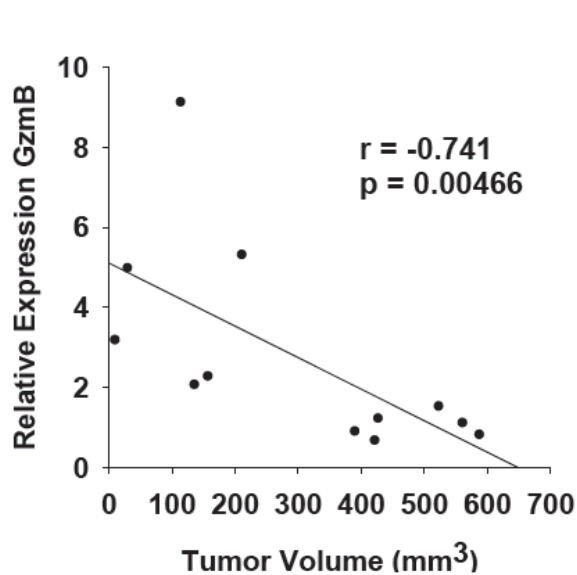
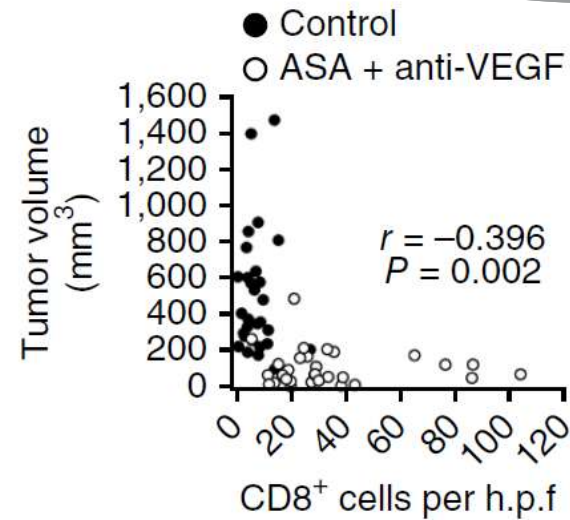
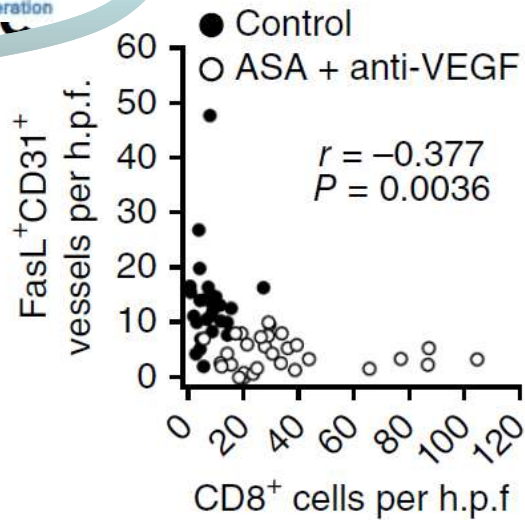
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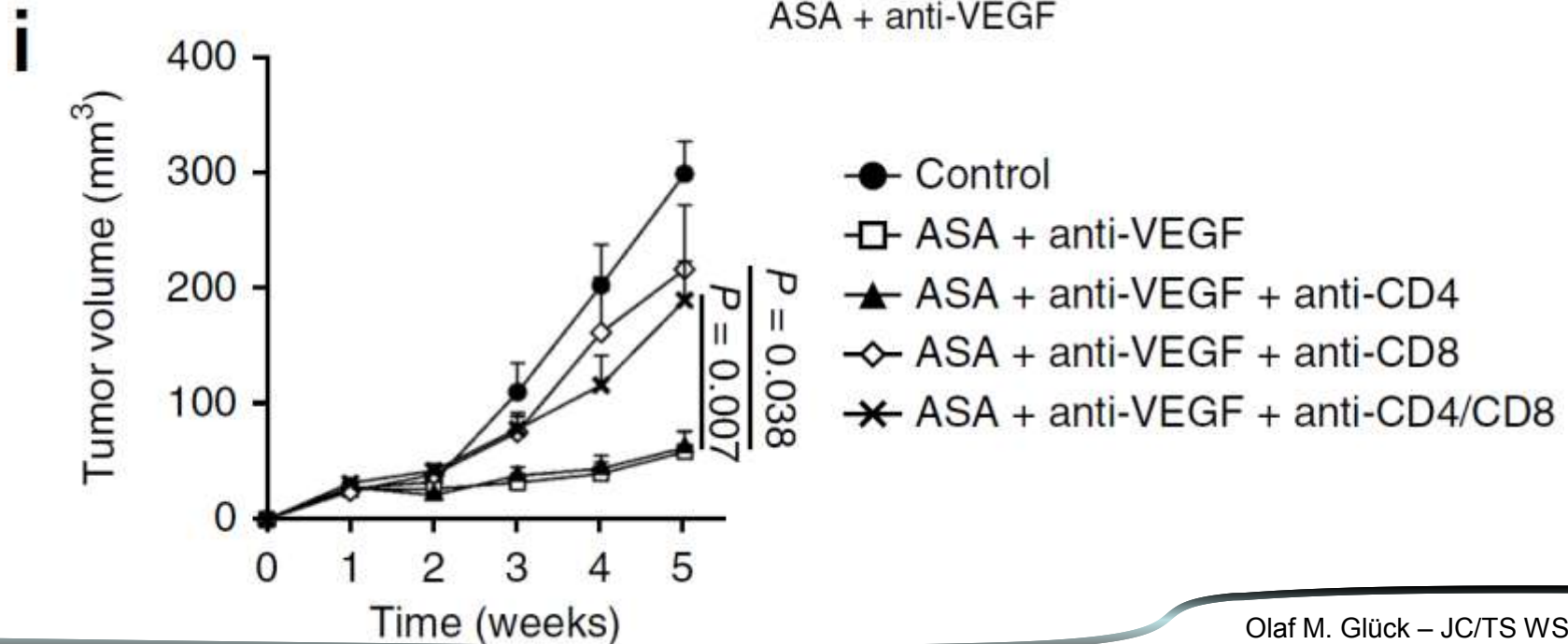


a

Control      Aspirin+  
anti-VEGF









## Results

### 7.

#### **FasL blockade synergizes with adoptive T cell transfer**

- primed CD8+ Cells infiltrate tumor mass
- pretreatment increases CD8+ infiltration

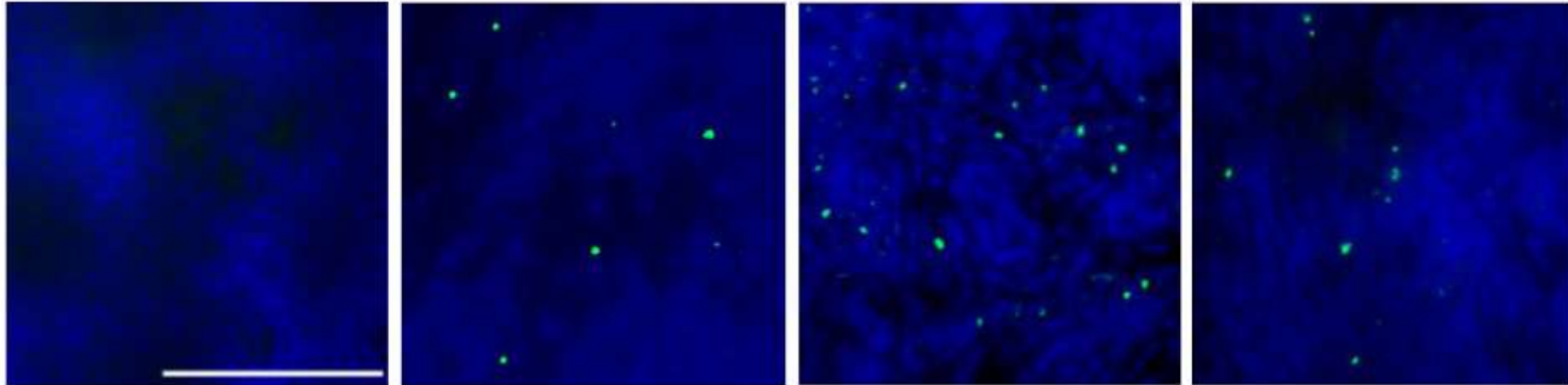
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Control

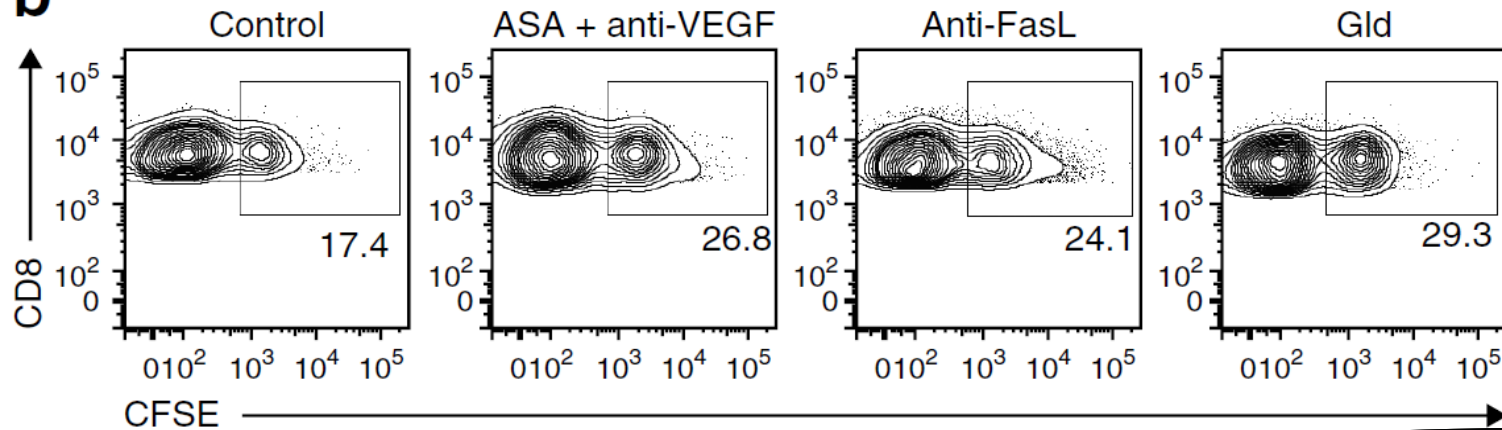
ASA + anti-VEGF

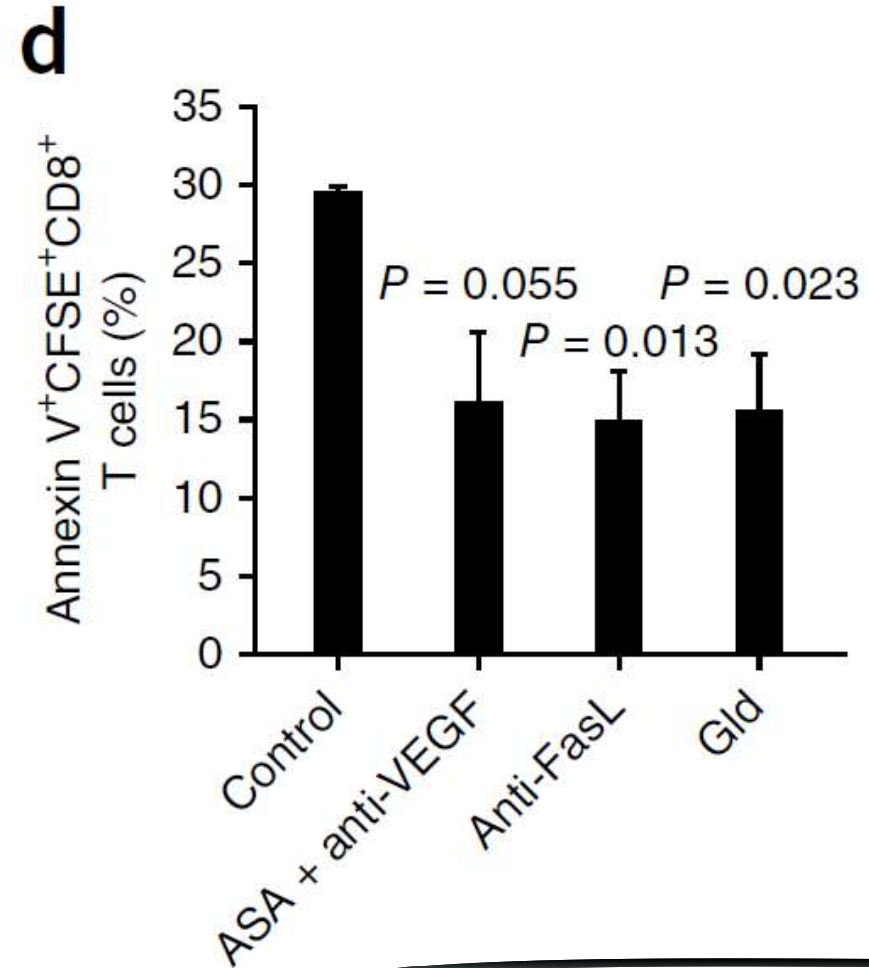
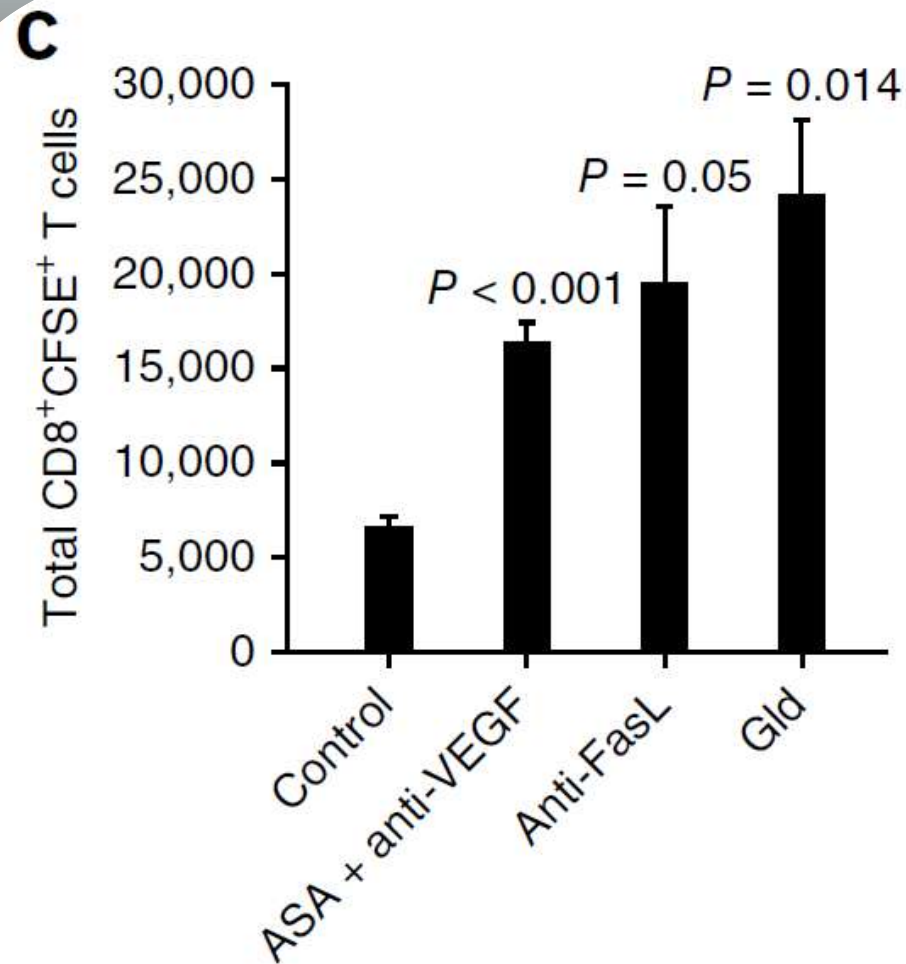
Anti-FasL

Gld

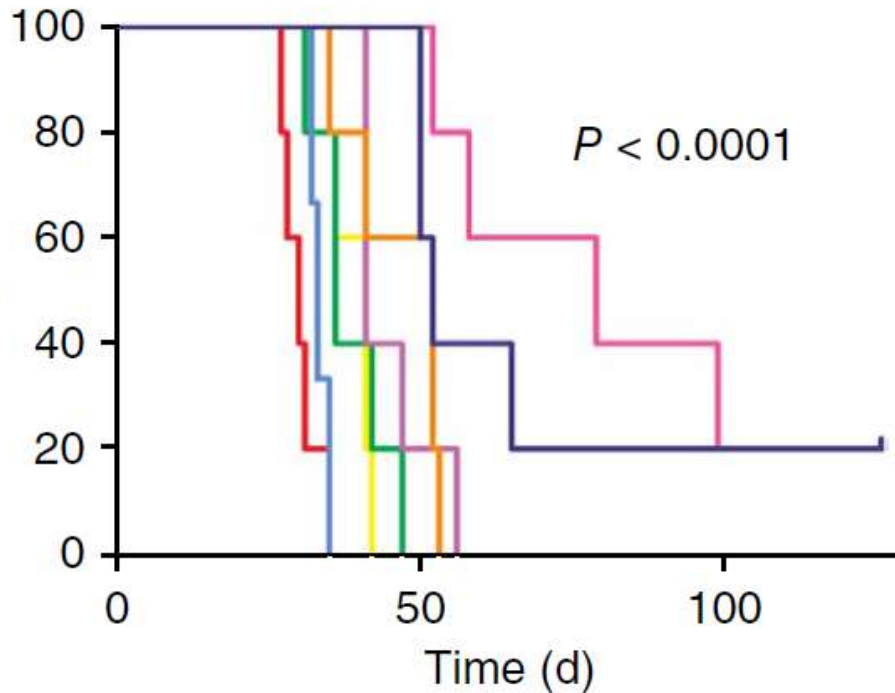


**b**





©



- Control, 30 d
- ASA + anti-VEGF, 41 d
- Anti-FasL, 36 d
- Gld, 33 d
- Control + OT1 T cells, 41 d
- ASA + anti-VEGF + OT1 T cells, 79 d
- Anti-FasL + OT1 T cells, 52 d
- Gld + OT1 T cells, 52 d

## Discussion

- tumors co-opt existing immune-regulatory mechanisms
- thus sustaining tumor growth and promoting immunological tolerance
- tumor-endothelium is not only a physical barrier but also an effective immune regulator
- angiogenic growth factors induce immunosuppression and tolerogenic tumor microenvironment through FasL expression

## Discussion

- Strong inverse correlation between FasL+vessels and CD8+TILs, but no relationship across multiple tumor types
- pharmacological FasL-block, knockout or deficient Fas-FasL signaling increases intratumoral CD8+ T cells and decreases tumor volume
- pharmacological inhibition of angiogenetic factors VEGF-A and PGE<sub>2</sub> can increase CD8+ TILs, but have no effect if FasL is ectopically expressed on tumor-endothelium

# Thank you for your attention