# SCIENTIFIC ANNEX ON PROJECT 2, MONOCLONAL ANTIBODY 9F8

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# OLFACTOMEDIN-LIKE 3 (OLFML-3)


- **Extracellular matrix-related protein** produced by tumor stromal cells (endothelial cells, pericytes, cancer associated fibroblasts) binds **BMP4** and **PDGF**
- **Pro-angiogenic properties** in normal and tumor angiogenesis
- Olfml-3 targeting impaired endothelial cell sprouting and pericyte migration
- Olfml-3 targeting reduced tumor growth, vascularization and pericyte coverage (LLC1, 4T1, GC38 tumor models)
- A recombinant  monoclonal antibody (chimeric rat Fv / mouse Fc) has been generated and sequenced

**Anti-Olfml-3 mAb ( 9F8)**

**Olfml-3 knock-out mouse**

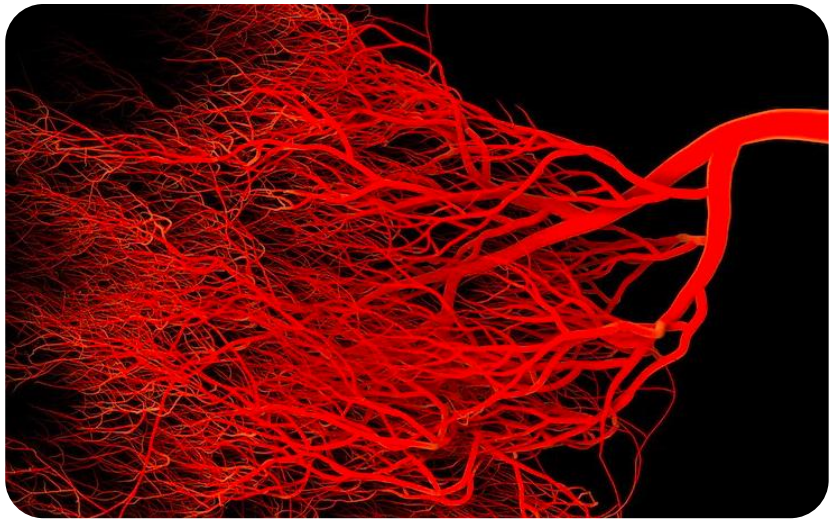
1 Matricellular protein Olfactomedin-like 3 (Olfml-3) creates an angiogenic environment

3 Blocking Olfml-3 with an antibody or deleting the Olfml-3 gene decreases tumour growth

3 Blocking Olfml-3 expression increases efficacy of a checkpoint inhibitor

4 Olfml-3 blocking does not elicit compensatory pro-angiogenic responses

# ANGIOGENESIS, A FUNDAMENTAL ASPECT OF TUMOR GROWTH




- The agressive biology and progression of cancers requires an expanding vascular supply. Such expansion (angiogenesis) is a complex process orchestrated by many molecules. The soluble protein **VEGF** (Vascular Endothelial Growth Factor) plays a central role

- **VEGF** activates the angiogenesis process by binding to the **VEGF receptor (VGEF-R)** in endothelial cells

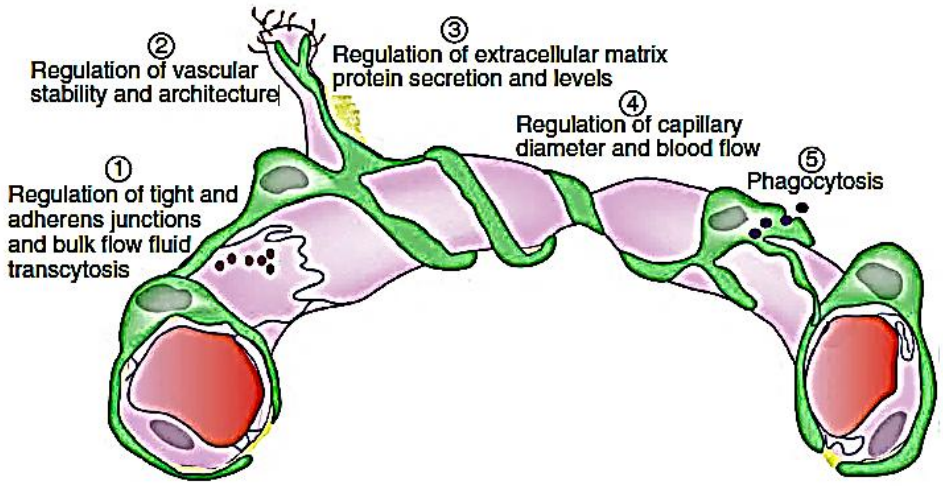
- Anti-**VEGF** products have been approved as part of a combined regimen for the treatment of many solid tumours

- The best known example is **bevacizumab (AVASTIN)** (Roche), a monoclonal antibody that binds to **VEGF** and deactivates it

- Unfortunately a variety of **tumor resistance mechanisms** become rapidly operative following initiation of anti-angiogenic therapy

**Because of this mechanism of tumor resistance, currently available anti-angiogenic strategies have found limited use in the clinical setting**

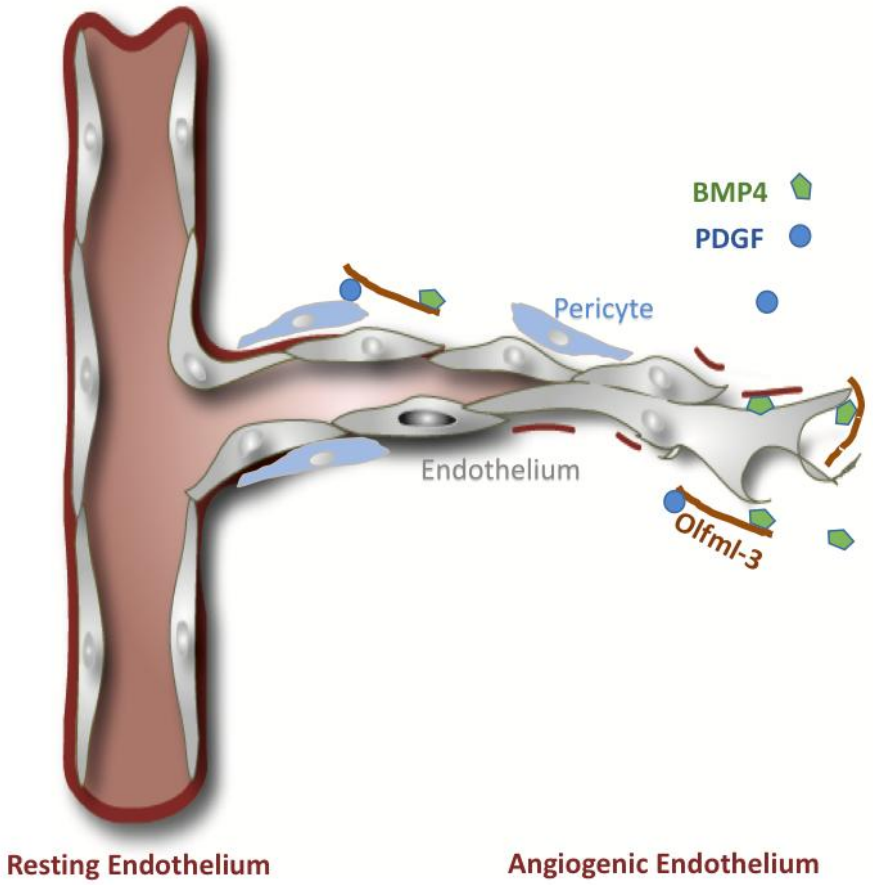
# THE ROLE OF PERICYTES IN ANGIOGENESIS




- Blood vessels are composed of two interacting cell types. Endothelial cells form the inner lining of the vessel wall, and pericytes envelop the surface of the vascular tube. Pericytes are also called mural cells
- Malignant blood vessels are characterized by abnormal pericyte coverage
- Higher levels of PDGF-Rß (a major driver of pericyte recruitment) are consistenly associated with poorer outcomes and survival

**Pericytes stabilize angiogenic blood vessels and lead to tumor growth and bad prognosis in most malignant contexts**

# THE SOLUTION: BLOCK THE ACTIVITY OF A PRO-ANGIOGENIC PROTEIN WITH A DIFFERENT MECHANISM OF ACTION




- Matricellular protein Olfml-3 creates an angiogenic environment in tumors and is associated with vascular growth factors BMP-4 and PDGF
- Tumor-derived Olfml-3 is produced by (and modulates the activity of) both angiogenic vascular **endothelial cells** and accompanying **pericytes**
- Blockage of Olfml-3 is highly effective in reducing angiogenic tumor vascularization, pericyte coverage and tumor growth in murine models of malignancy

# COMPETITIVE ADVANTAGE OF BLOCKING OLFML-3 WITH A MONOCLONAL ANTIBODY

- There is a growing body of evidence indicating that malignant vascular resistance to traditional anti-VEGF anti-angiogenic strategies is substantially mediated via pericyte activity

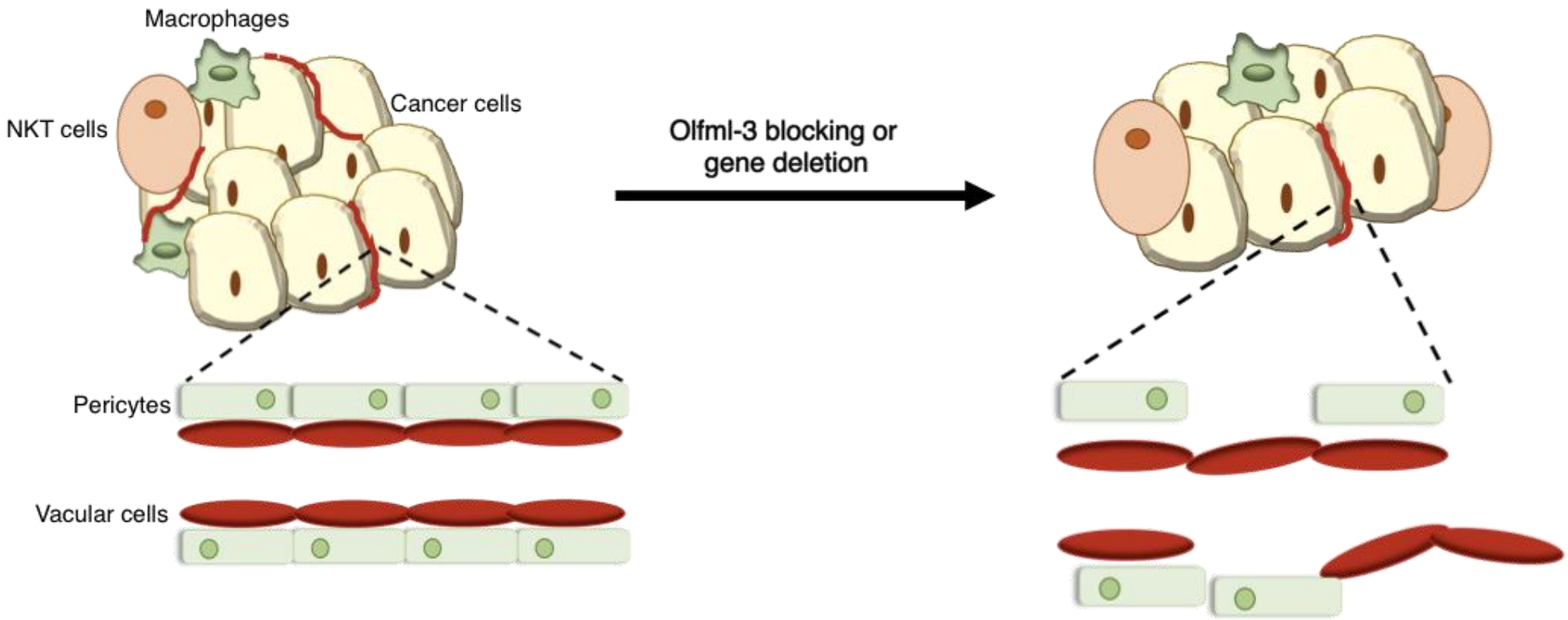
- This suggests two broad clinical spaces for  modulation through anti-Olfml-3 approaches

- This approach is, in many ways, similar to that of bi-specific antibodies

**An alternative anti-angiogenic strategy to current anti-VEGF approaches. Anti-Olfml-3 antibodies exert this function by  affecting the BMP-4 pathway**

**An anti perycte effect by afecting PDGF**

# SUMMARY OF RESULTS




Decreased lung, breast and colon tumor growth
Decreased tumor angiogenesis and pericyte coverage
Decreased tumor-associated macrophage content
Increased Natural-Killer T lymphocyte content
Improved immune checkpoint therapeutic targeting
Decreased mouse breast carcinoma metastases


MOLECULAR CANCER THERAPEUTICS

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# Targeting Olfactomedin-like 3 Inhibits Tumor Growth by Impairing Angiogenesis and Pericyte Coverage

Marijana Miljkovic-Licina, Philippe Hammel, Sarah Garrido-Urbani, Boris P.-L. Lee, Mehdi Meguenani, Chiraz Chaabane, Marie-Luce Bochaton-Piallat, and Beat A. Imhof

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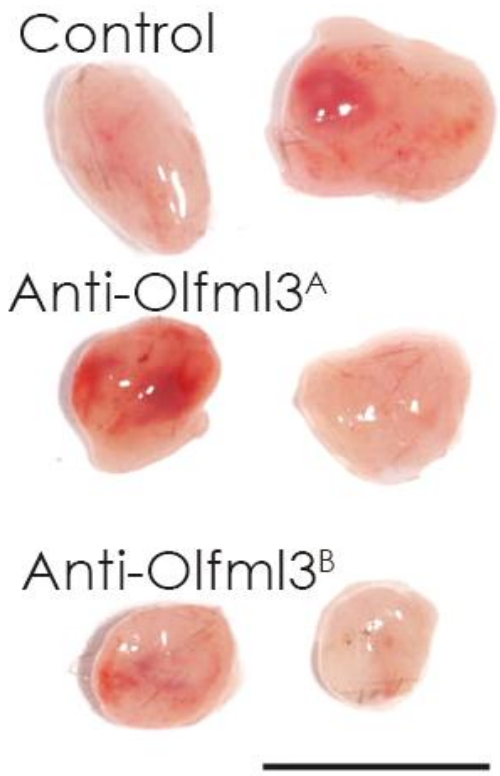
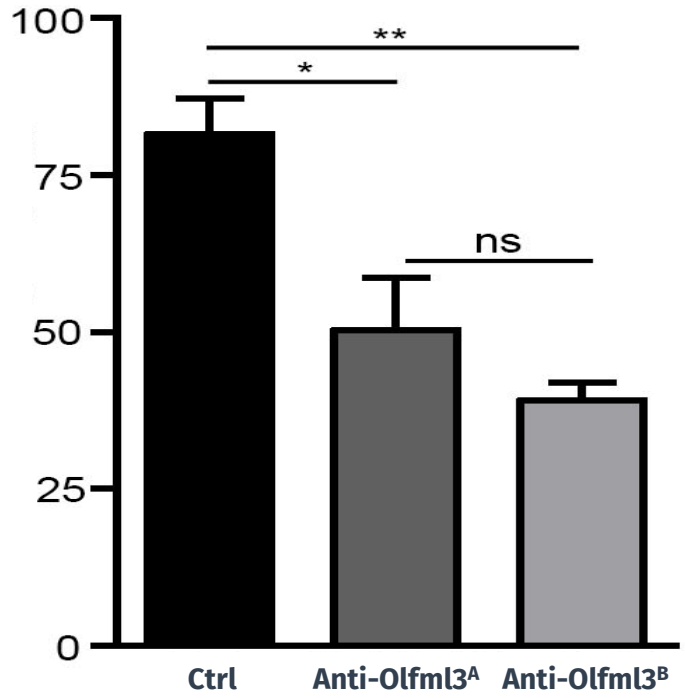
# ANTIBODIES AGAINST MOUSE OLFML-3 REDUCE TUMOR GROWTH

## Lewis lung carcinoma (LLC1) tumor model

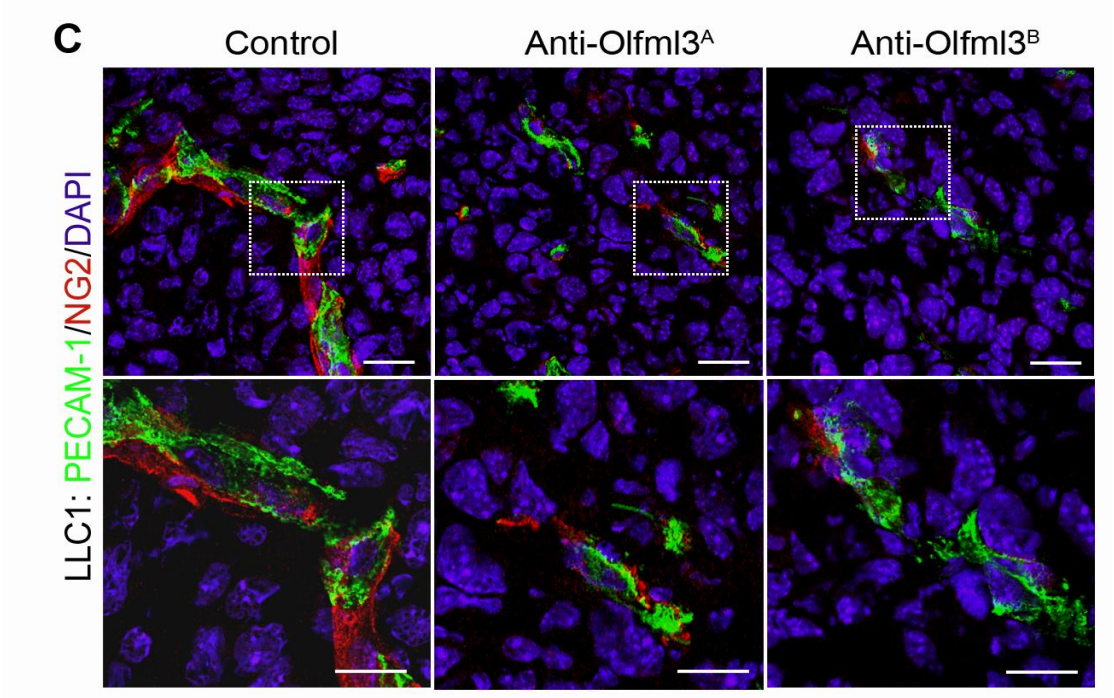


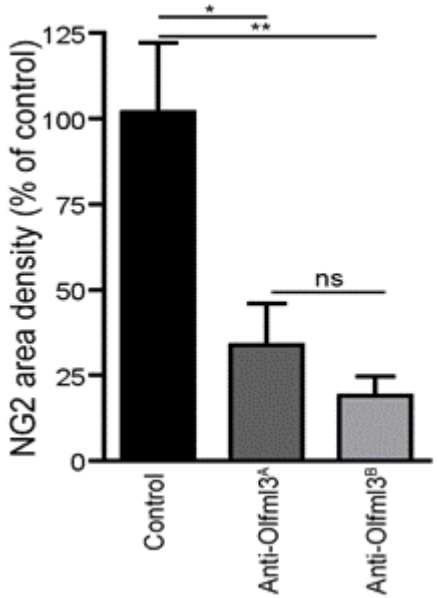

## Tumor weight
(mg)



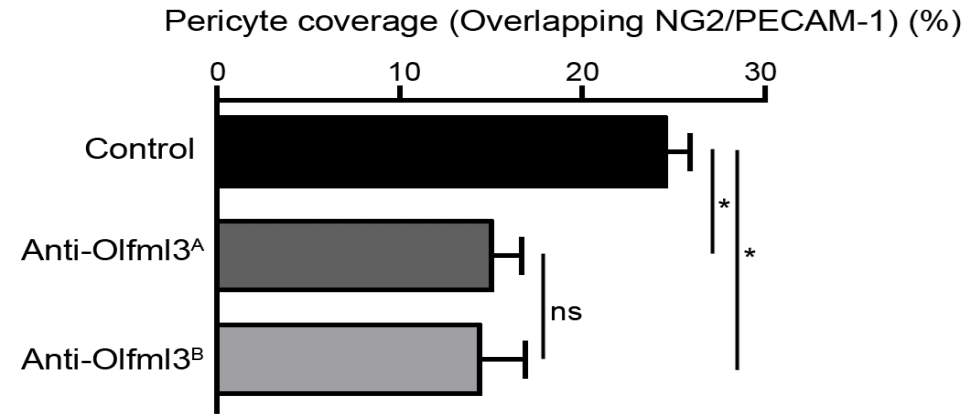

* p<0.05; ** p<0.01

# ANTIBODIES AGAINST MOUSE OLFML-3 REDUCE PERICYTE COVERAGE



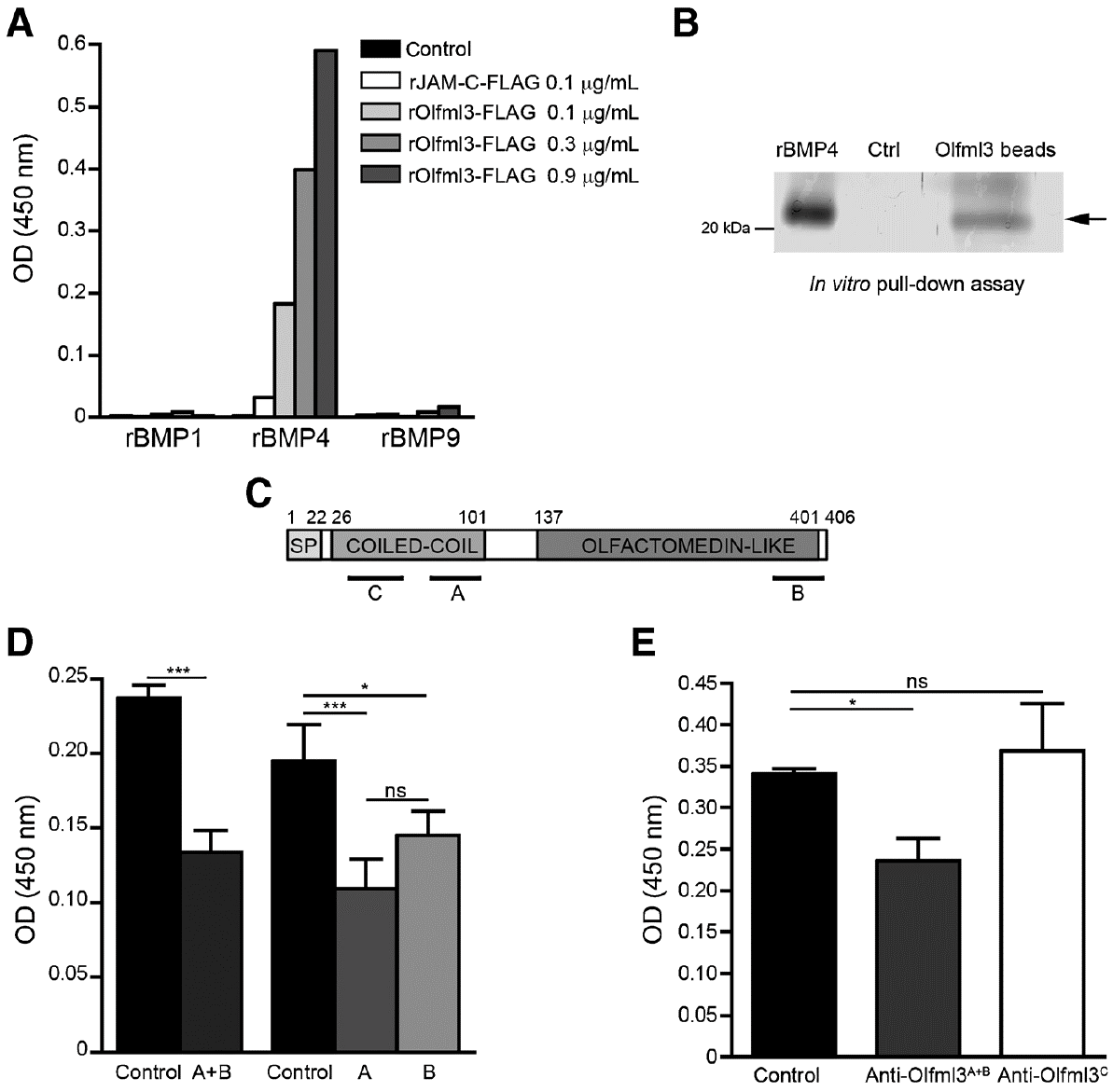




* p<0.05; ** p<0.01

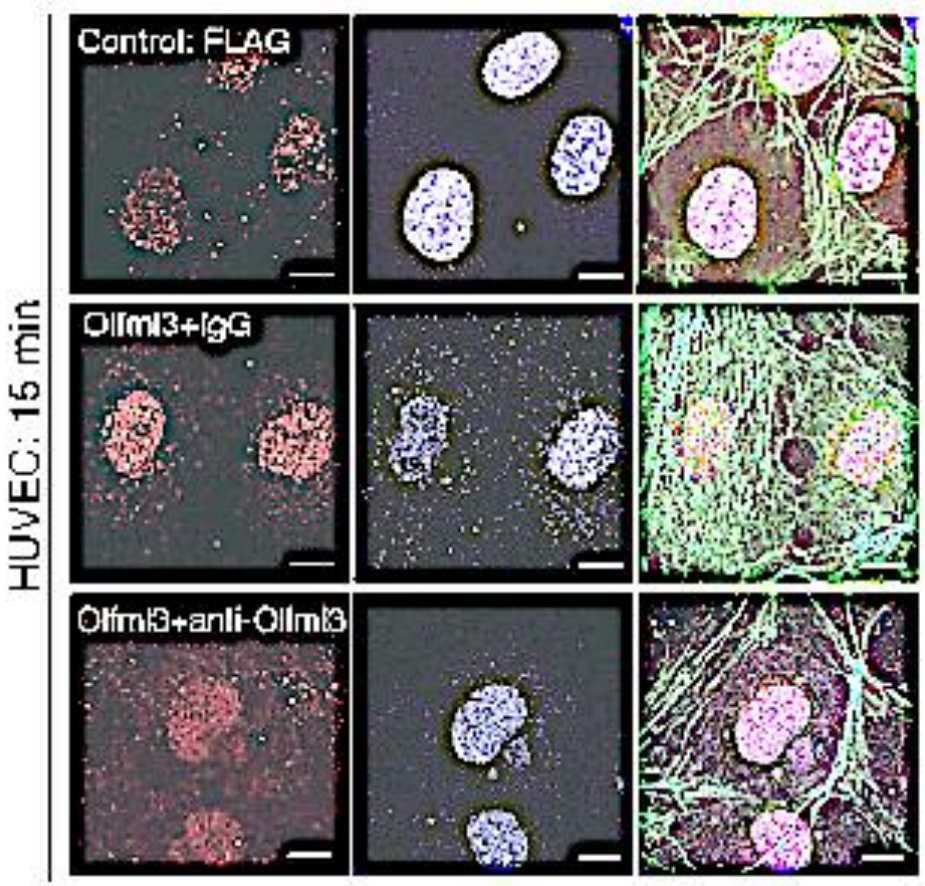


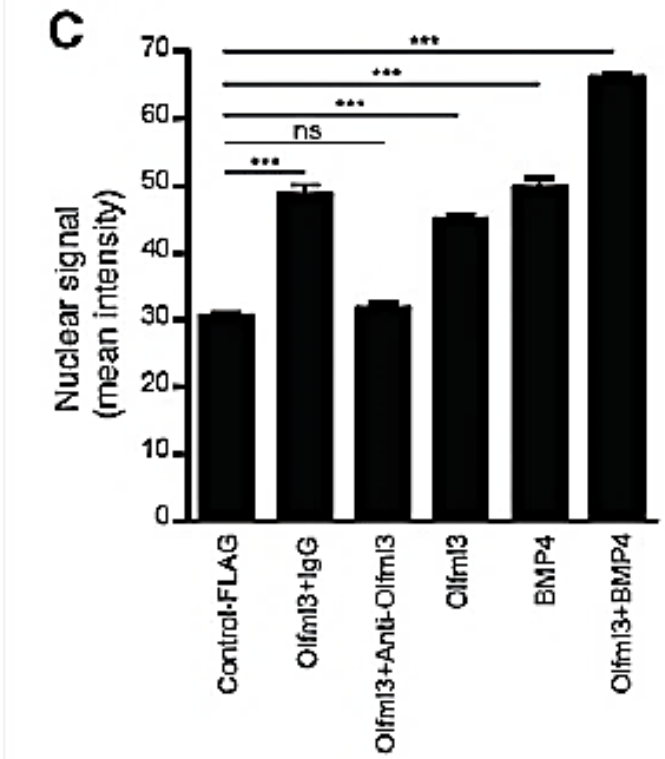

Miljkovic-Licina et al., 2012

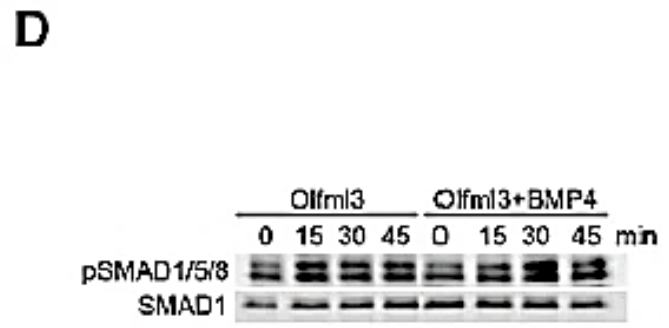
# OLML-3 SPECIFICALLY BINDS TO BMP4. ANTI-OLFML-3 ANTIBODIES BLOCK THIS BINDING ACTIVITY



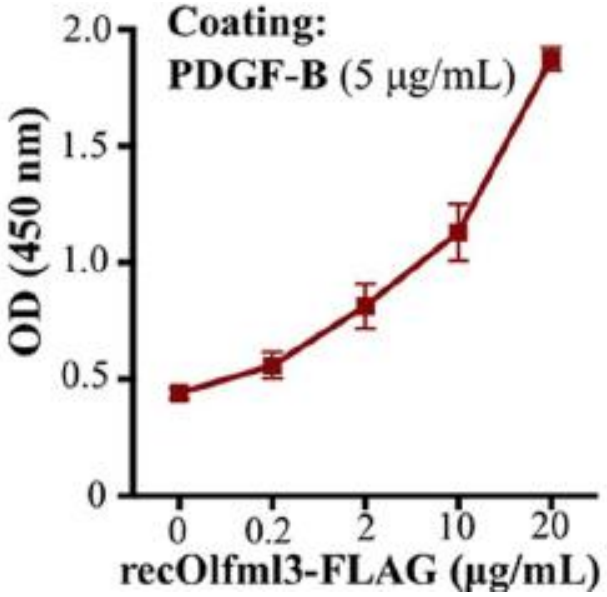
# ANTI-OLFML-3 ANTIBODY REDUCES BMP4 SIGNALING AND NUCLEAR TRANSFER OF PSMAD1/5/8

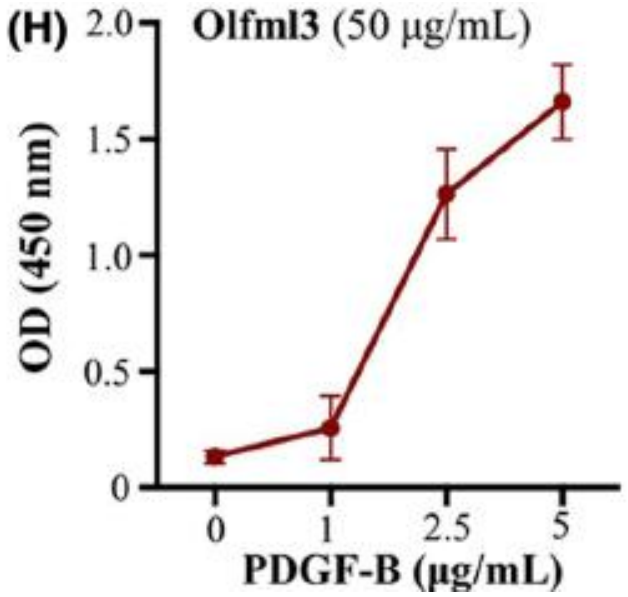


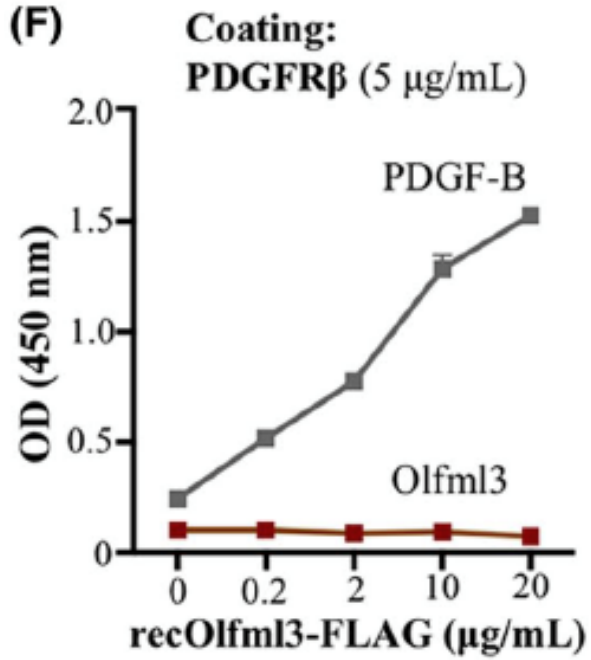



# OLFML-3 BINDS TO PDGF-B BUT NOT TO PDGFRß

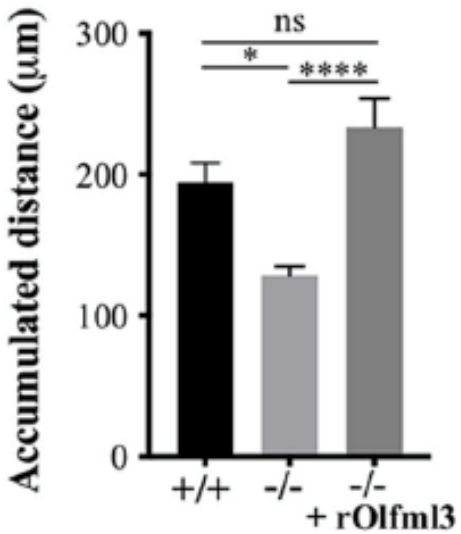


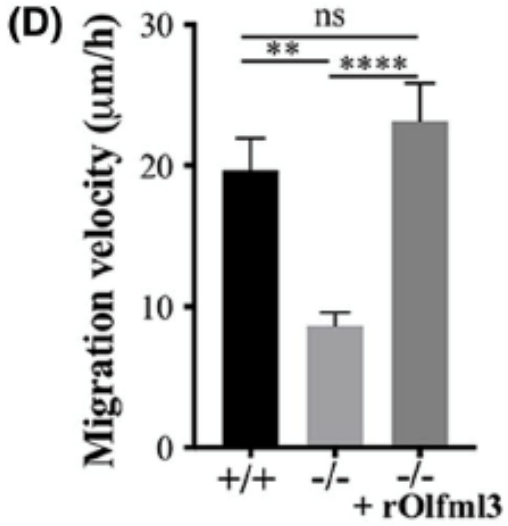



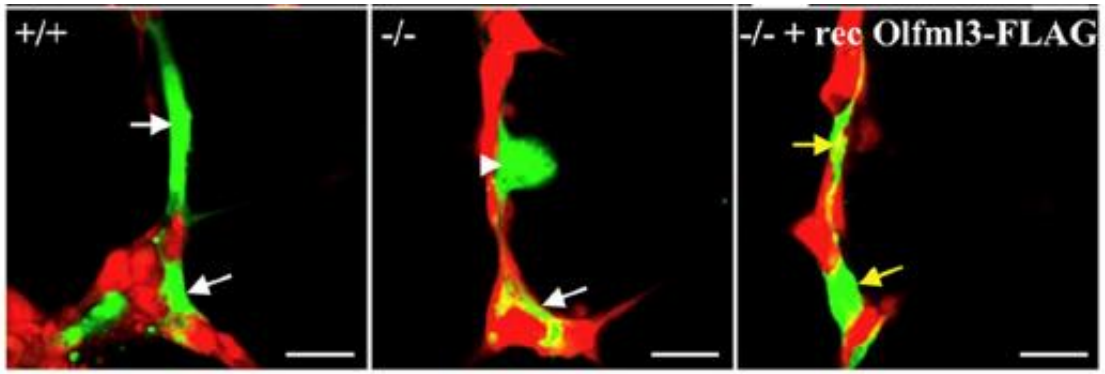



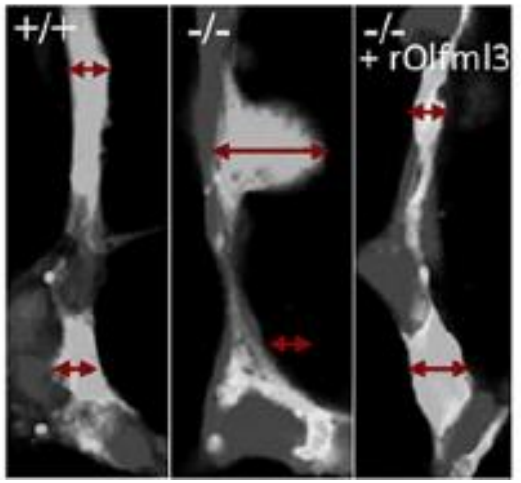
# OLFML-3 PROMOTES MIGRATION OF PERICYTES AND CLOSE ASSOCIATION WITH VASCULAR ENDOTHELIUM

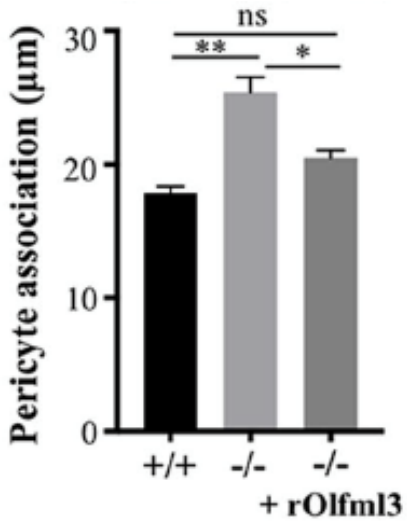
(COCULTURE OF OLFML-3 KO PERICYTES WITH ENDOTHELIAL CELLS)



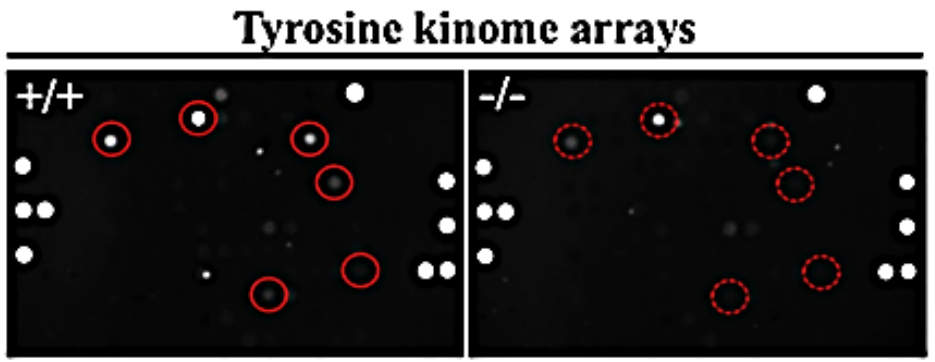



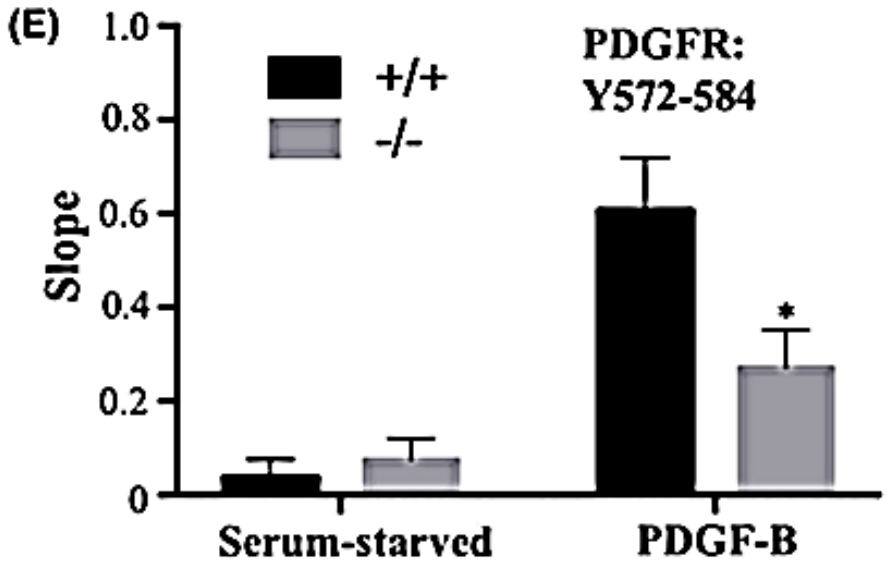



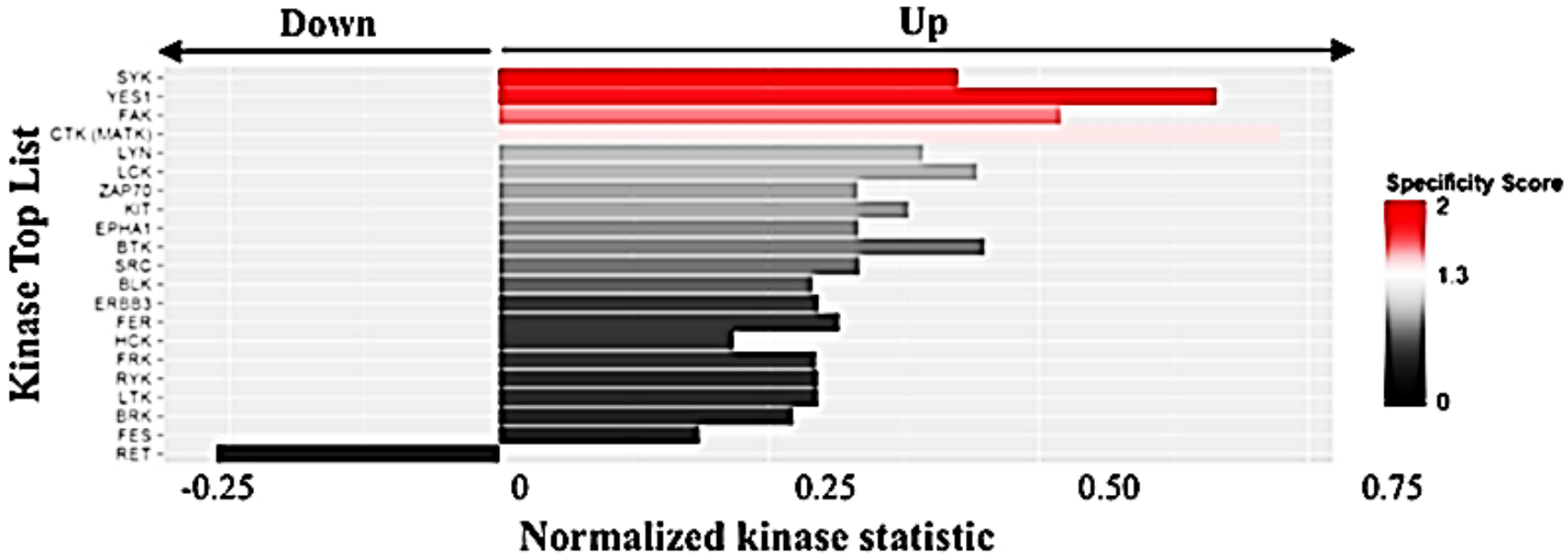



# PFGF-B NEEDS OLFML-3 ASSOCIATION TO INDUCE SIGNALING IN PERICYTES, AND DOES NOT WORK WITH OLFML-3-/- PERICYTES



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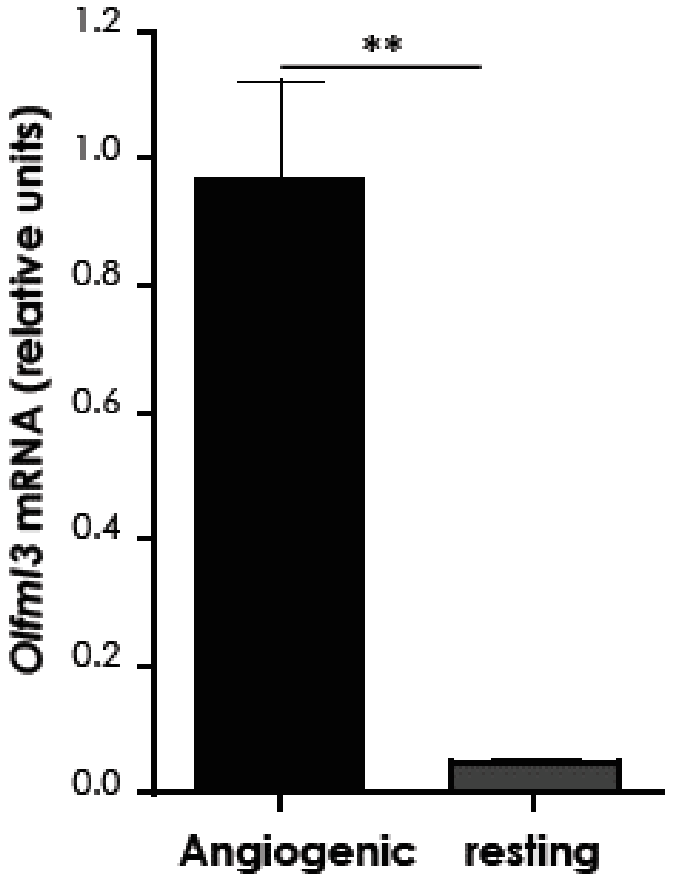
# DIFFERENTIAL GENE EXPRESSION OF OLFML-3 IN ANGIOGENIC VS. RESTING CELLS (ENDOTHELIAL CELL LINE)

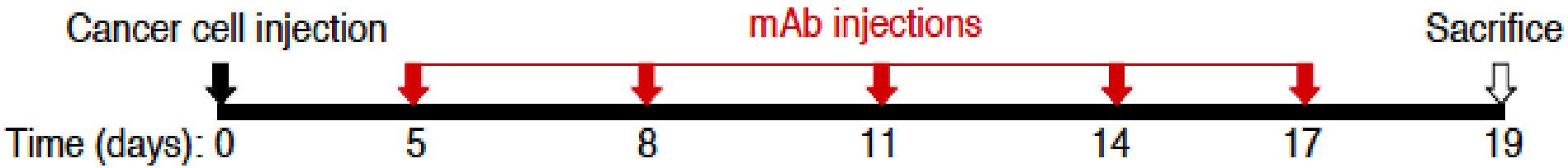
**Olfml-3 mRNA**
(relative units)

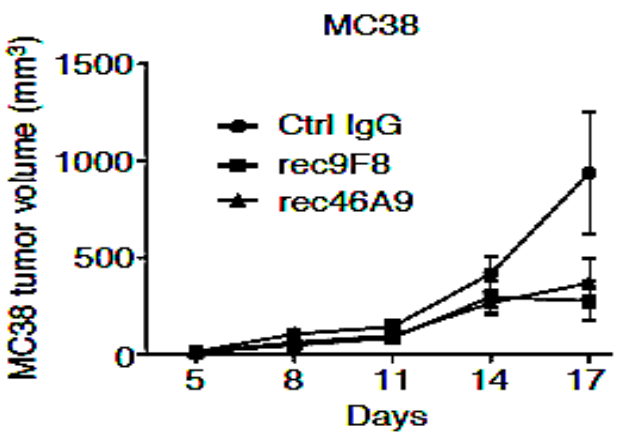


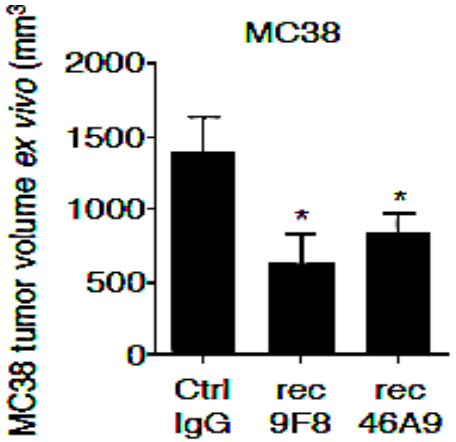
# EXPRESSION OF VASCULAR OLFML-3 IN HUMAN CARCINOMA

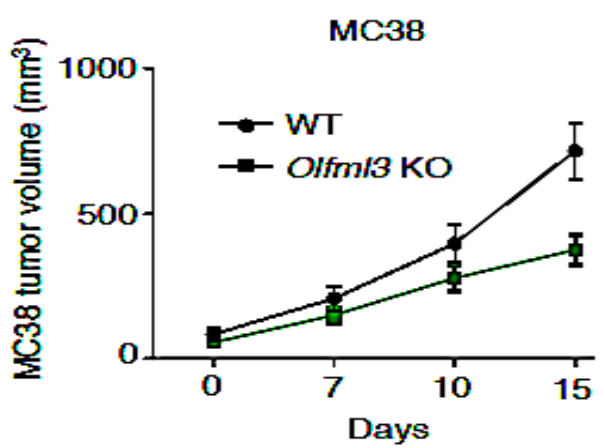
| | Tissue | Vascular expression intensity |
|---|---|---|
| 1 | Normal breast | + |
| | Breast carcinoma | +++ |
| | Ductal breast carcinoma | +++ |
| | Breast carcinoma metastases | +++ |
| | Triple negative | + |
| 2 | Normal colon | + |
| | Colon adenocarcinoma | +++ |
| 3 | Normal esophagus | - |
| | Esophagus adenocarcinoma | - |
| 4 | Normal kidney | +/- |
| | Papillary cell carcinoma | +/- |
| 5 | Normal lung | + |
| | Lung adenocarcinoma | ++ |
| 6 | Normal prostate | + |
| | Prostate adenocarcinoma | ++ |
| 7 | Normal testis | - |
| | Classical seminoma | - |
| 8 | Normal uterus | +/- |
| | Uterus carcinoma | +++ |

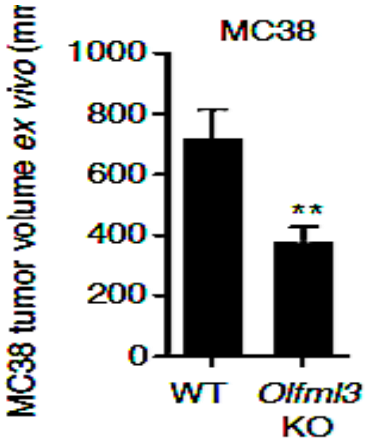
# INHIBITORY EFFECT OF ANTI OLFML-3 ANTIBODY AND OLFML-3 GENE DELETION ON MC38 MOUSE COLORECTAL CARCINOMA MODEL



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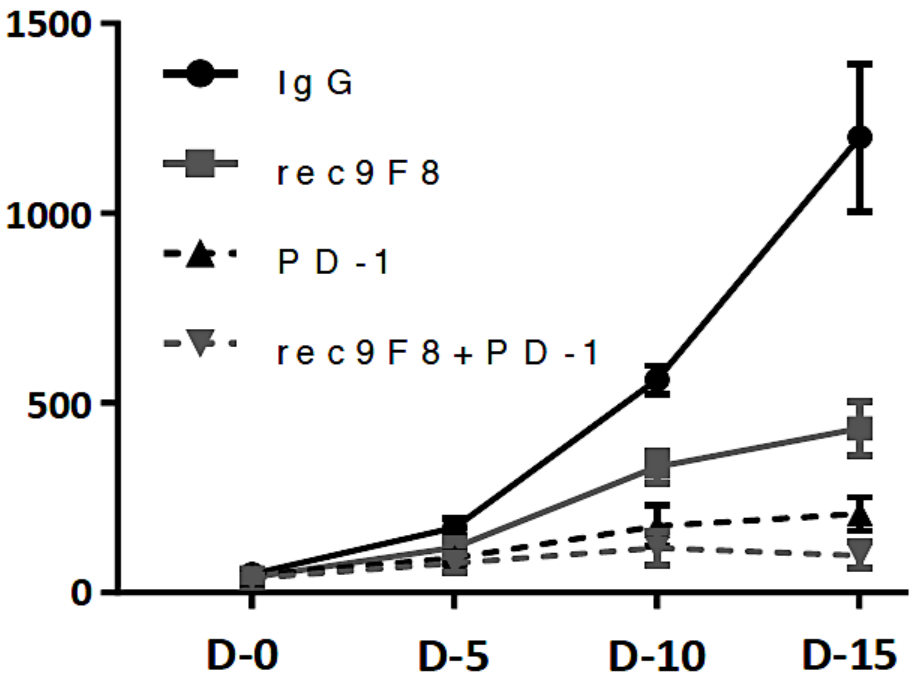
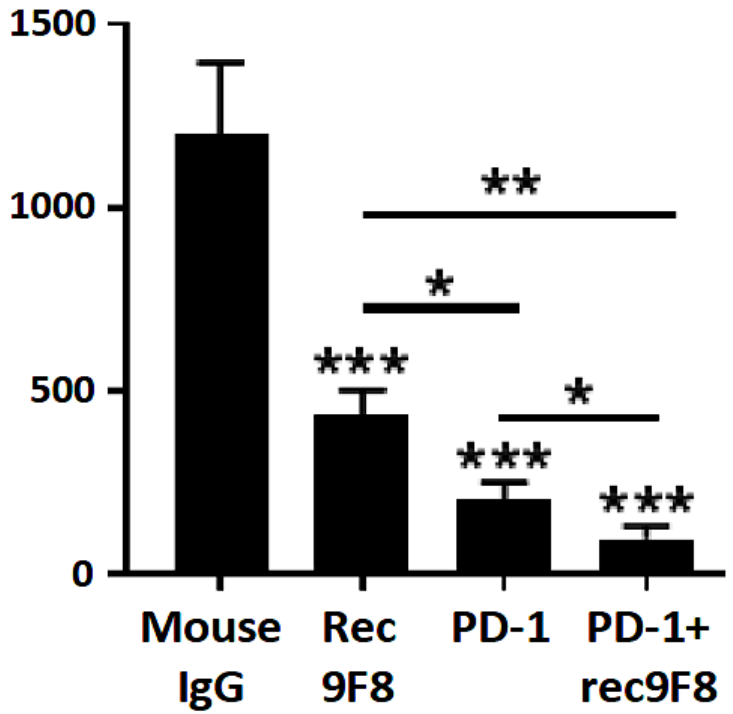
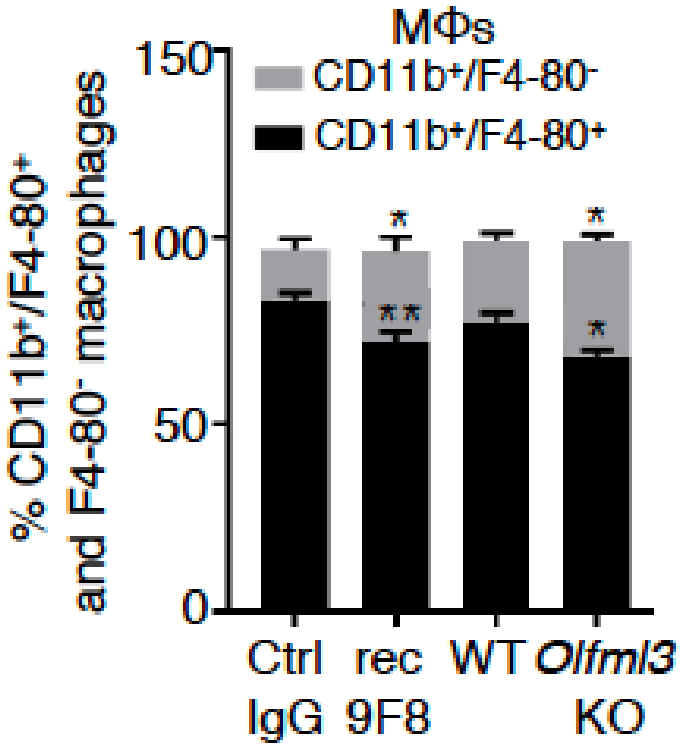
## MC38 tumor volume
(mm3)

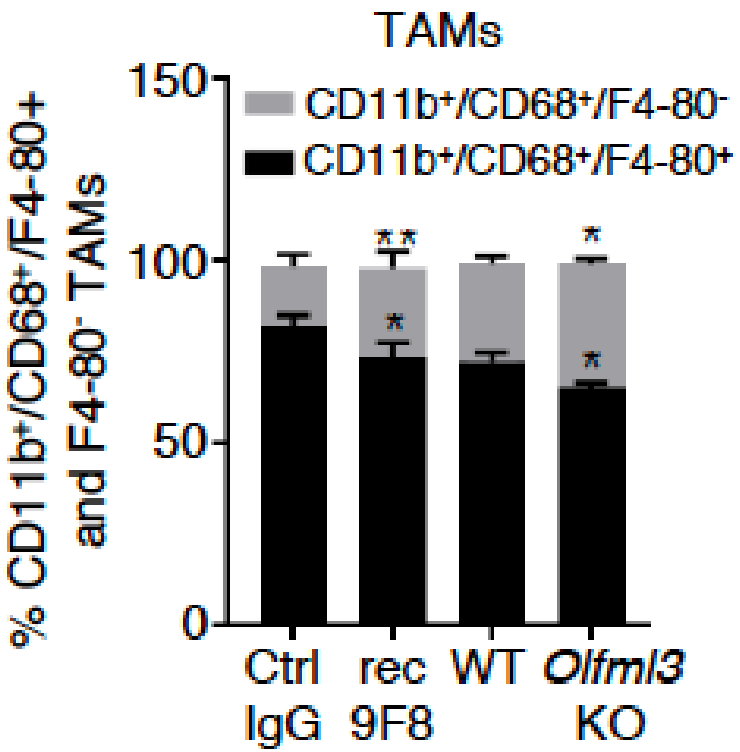


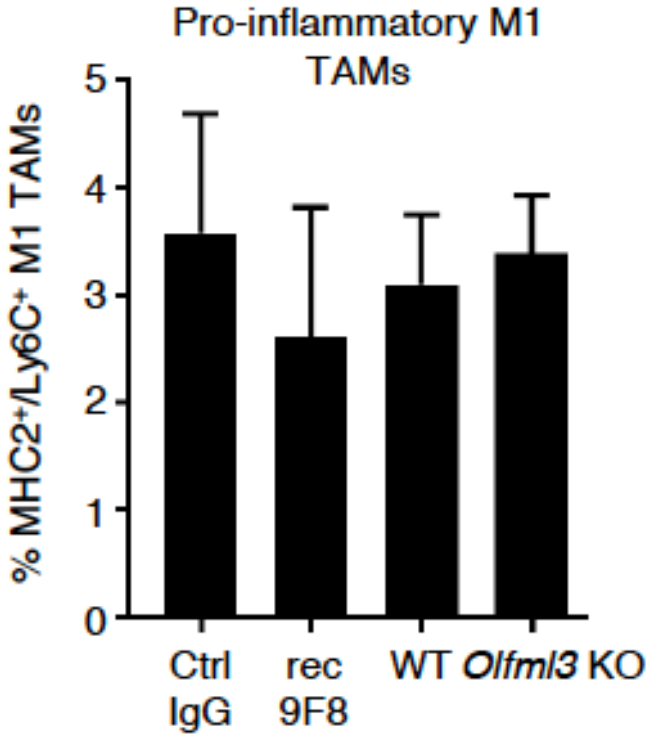

## MC38 tumor volume ex vivo
(mm3)



# ANTI OLFML-3 ANTIBODIES DECREASE THE TUMOUR-ASSOCIATED MACROPHAGE CONTENT

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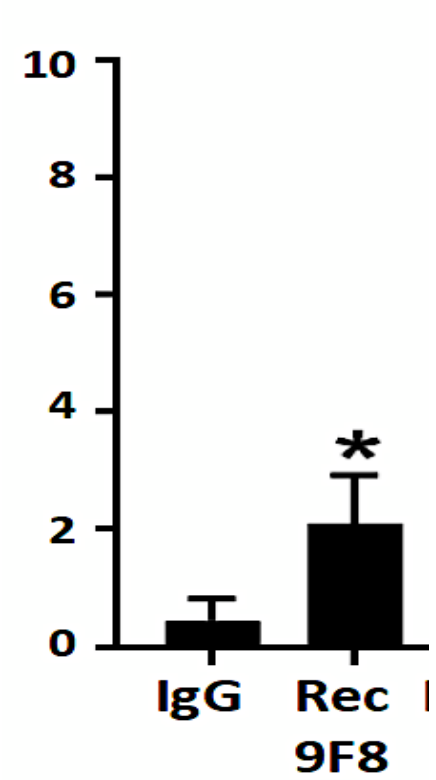
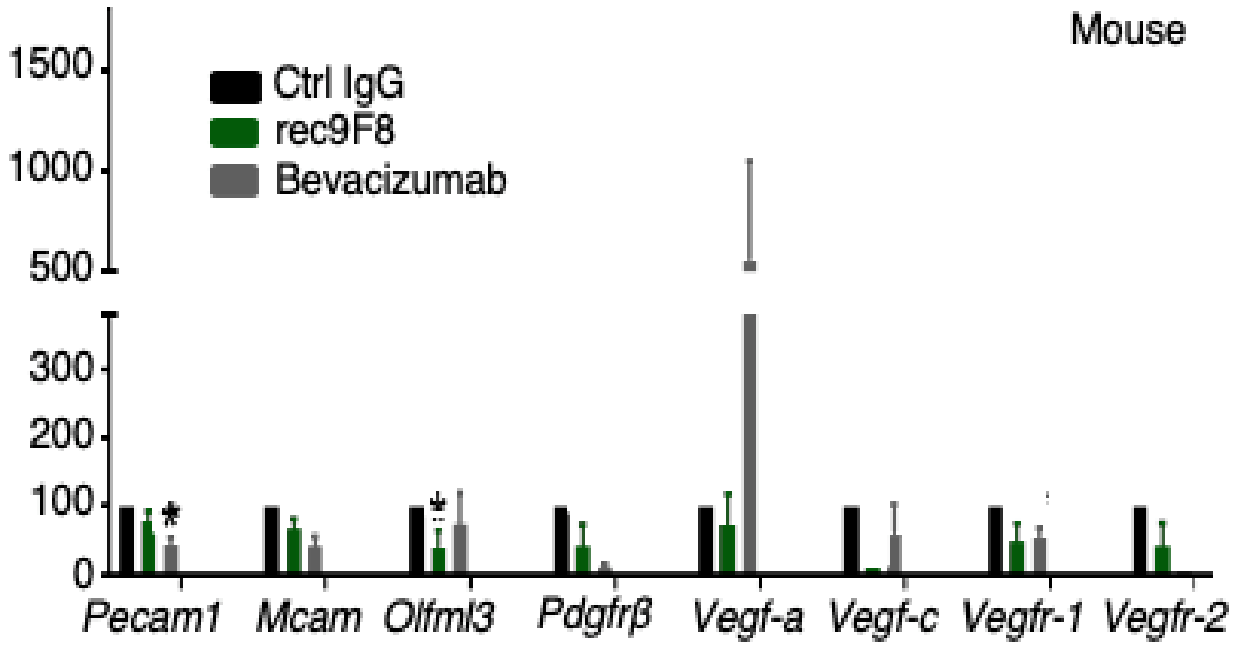
# ANTI OLFML-3 ANTIBODIES INCREASE THE NATURAL-KILLER (NK) T LYMPHOCYTE CONTENT

**% of CD3+/NK1.1+NK cells**

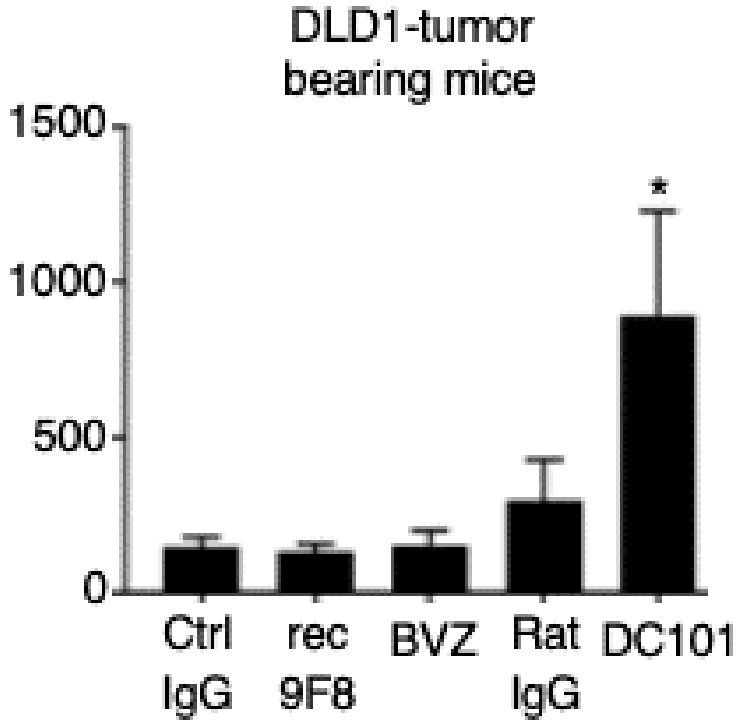


# OLFML-3 ANTIBODY-BASED TUMOUR TREATMENT DOES NOT ELICIT COMPENSATORY PRO-ANGIOGENIC RESPONSES WHILE ANTI VEGF-R2 DOES

**mRNA expression**
(% of control)




**Plasma Vegf-a**
Pg/mL)



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- Selection strategies for potential indications could be **hypothesis based**, focusing on tumor types expressing high levels of Olfml-3

**OR**

- **Empirically directed**, via selection of tumor types where clinical activity with existing anti-angiogenic agents is limited, suggesting *a priori* that a strategy of combining a novel anti-angiogenic approach (Olfml-3) with an existing anti-angiogenic agent would result in a dramatic improvement of activity

**A limited (but by no means exhaustive) list of potential indications for early clinical evaluation of an anti-Olfml-3 strategy is shown in the following pages**

- **Regorafenib** is a multi-kinase inhibitor with marked anti-angiogenic activity. This product was approved on the basis of absolutely marginal improvements (third line setting)

- Once activity has been confirmed in the third line setting it would the be easy to migrate to earlier CRC treatment stages

- Next logical step would be a combination regimen with **bevacizumab** in the first and second line setting

**Small scale initial clinical trial of an anti-Olfml-3 agent plus regorafenib in a similar third line CRC patient population**

- The combination of an anti-olfml-3 antibody plus and anti-VEGFR2 antibody (DC101) has shown encouraging activity in murine models of TNBC

- ABOLOGIX has shown synergistic activity between an anti-Olfml-3 antibody and a checkpoint inhibitor in a murine model of CRC. This needs to be replicated in the TNBC context

- The recent finding that the checkpoint inhibitor **atezolizumab** combined with **paclitaxel** did not confirm a PFS advantage in the same front-line metastatic TNBC setting emphasizes the scope for improving checkpoint inhibition through combination with mechanistically novel agents

**An anti-Olfml-3 antibody in combination with either i) bevacizumab, ii) atezolizumab or iii) bevacizumab and atezolizumab would represent a highly novel therapeutic strategy in both the front and second line TNBC settings**

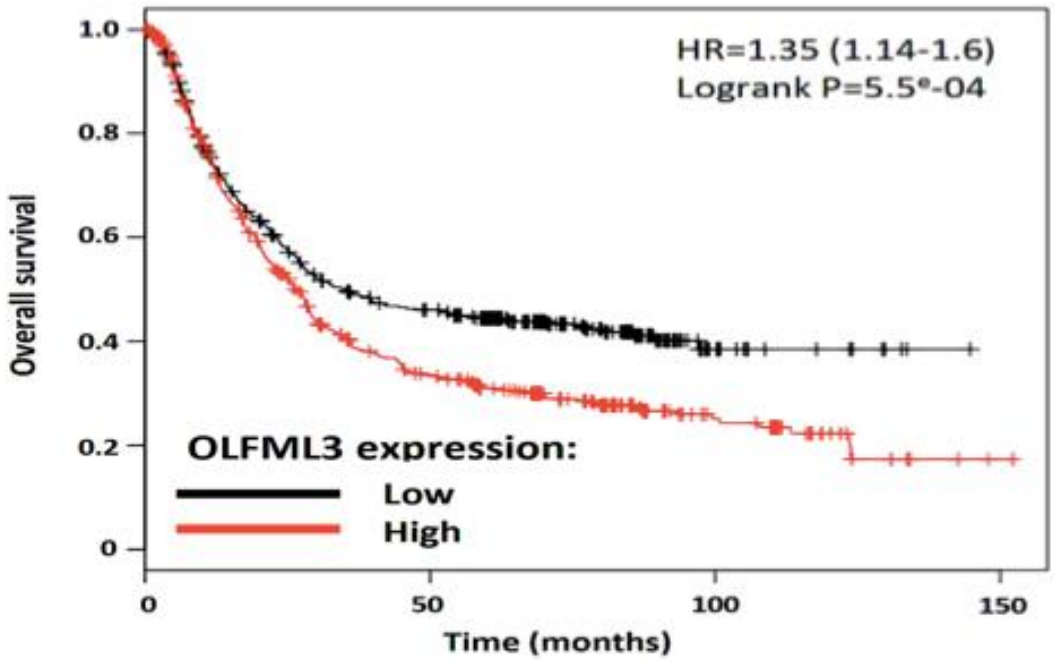
- **AVASTIN (bevacizumab)** currently approved in the US in the second-line treatment of GBM which has progressed following initial therapy
- **GBM** a suitable potential indication for evaluation of an anti-Olfml-3 agent (in combination with **bevacizumab**)

**ABSTRACT**

Glioblastoma (GBM) is a lethal brain tumor containing a subpopulation of glioma stem cells (GSC). Pan-cancer analyses have revealed that stemness of cancer cells correlates positively with immunosuppressive pathways in many solid tumors, including GBM, prompting us to conduct a gain-of-function screen of epigenetic regulators that may influence GSC self-renewal and tumor immunity. The circadian regulator *CLOCK* emerged as a top hit in enhancing stem-cell self-renewal, which was amplified in about 5% of human GBM cases. CLOCK and its heterodimeric partner BMAL1 enhanced GSC self-renewal and triggered protumor immunity via transcriptional upregulation of OLFML3, a novel chemokine recruiting immune-suppressive microglia into the tumor microenvironment. In GBM models, *CLOCK* or *OLFML3* depletion reduced intratumoral microglia density and extended overall survival. We conclude that the CLOCK–BMAL1 complex contributes to key GBM hallmarks of GSC maintenance and immunosuppression and, together with its downstream target OLFML3, represents new therapeutic targets for this disease.

**A single-arm study (n=75-100) in late stage recurrent GBM would be sufficient for an accelerated approval**

- There is a correlation between Olfml-3 expression and survival in gastric cancer patients
- **Ramucirumab** is a VEGFR2 antagonist indicated for the treatment of advanced gastric cancer




**Olfml-3 expression level correlates with survival of gastric cancer patients -** Reference: GSE39582 and The Cancer Genome Atlas

**An initial small clinical trial in the second-line gastric setting with an anti-Olfml-3 agents plus ramucirumab could be envisaged**

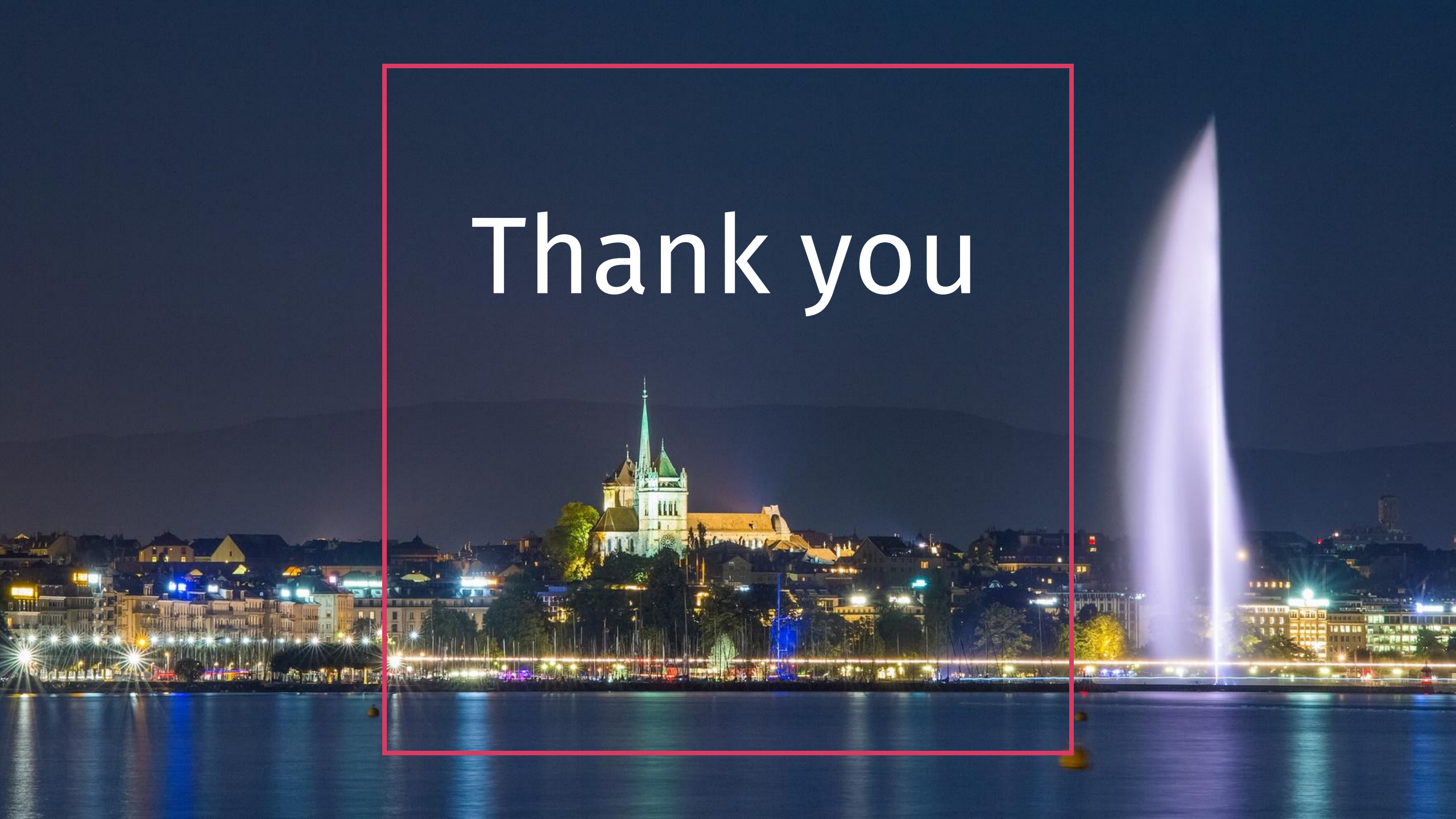
# FRONT-LINE HEPATOCELLULAR CARCINOMA

- **Bevacizumab** is approved in combination with the checkpoint inhibitor **atezolizumab** in certain subgroups of patients with metastatic hepatocellular carcinoma (HCC)

- The preclinical data from ABOLOGIX suggests that blockade of Olfml-3 expression may increase the activity of checkpoint inhibitors

- HCC is a very vascular tumor, and therefore an indication that is highly responsive to anti-angiogenic strategies, suggesting a high probability of clinical activity with an anti-Olfml-3 agent

**Following initial confirmation of clinical activity with an anti-Olfml-3 agent, extension to larger HCC studies in combination with atezolizumab and / or bevacizumab would be possible**


Thank you