



TSP-1/CD47 SIGNALING WITHIN A TUMOR MICROENVIRONMENT : from molecular modelling to peptide-based drugs preclinical development

Albin JEANNE

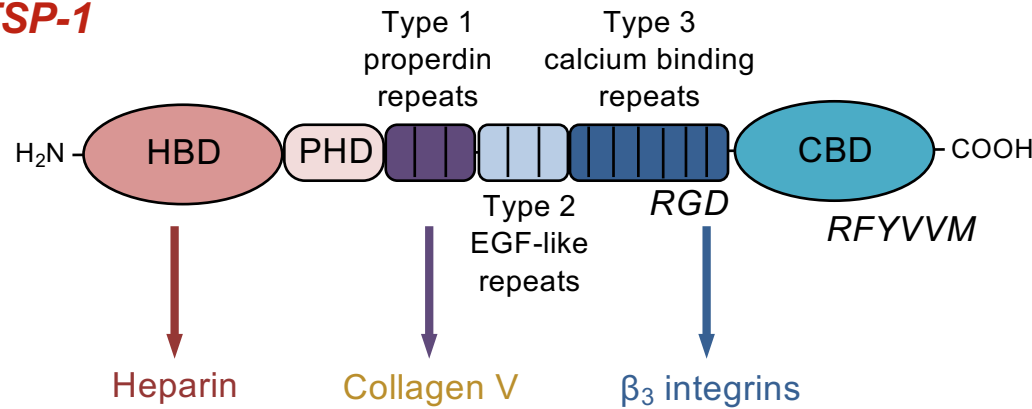
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**10^{ème} Forum du Cancéropôle du Grand-Est
De la recherche fondamentale à la clinique
Nancy - Jeudi 24 novembre 2016**



Thrombospondin-1 (TSP-1): a main actor within a tumor microenvironment (1)

TSP-1

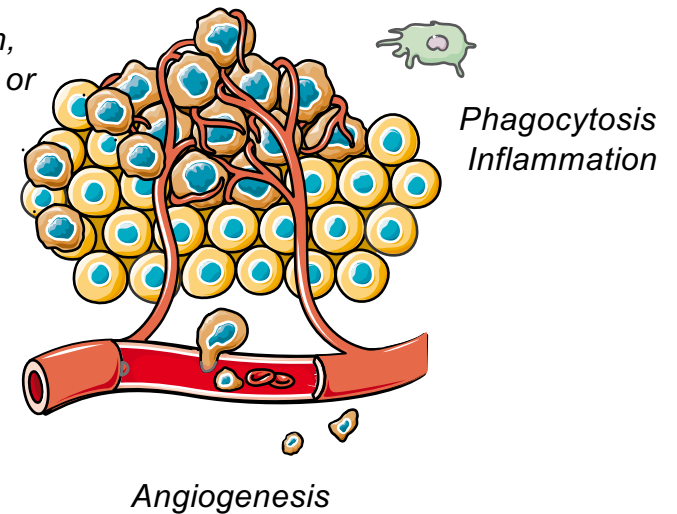


Heparin
 HSPGs
 LRP-1
 $\alpha 3\beta 1$
 $\alpha 4\beta 1$

Collagen V
 Fibronectin
 Laminin
 HSPGs
 CD36

β_3 integrins

Tumor cell adhesion, proliferation, survival or apoptosis



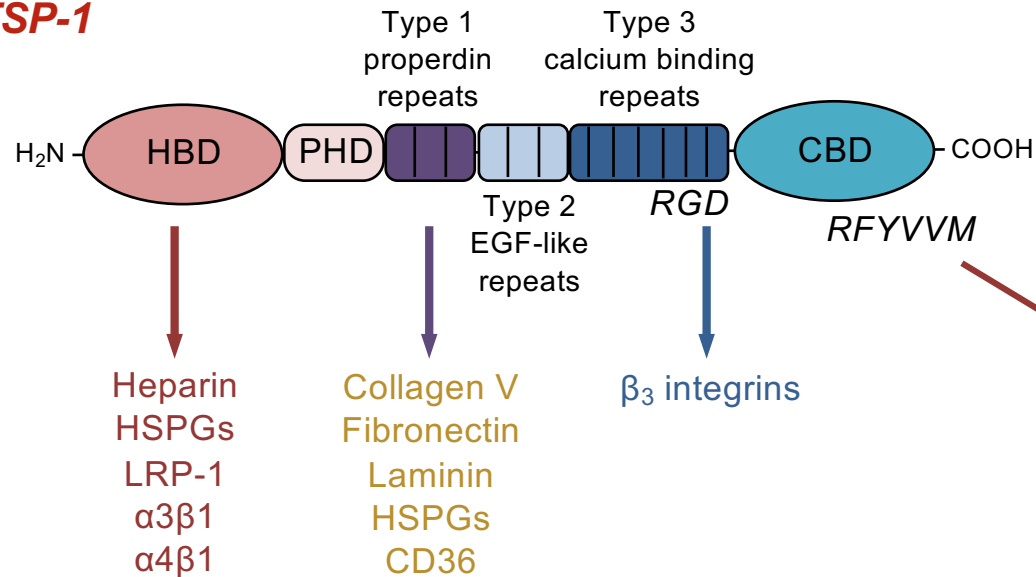
TSP-1: a key actor in tumor progression

- 450 kDa multimodular matricellular protein
- Multifaceted functionalities
- Overexpressed within tumor stroma and in high circulating levels in several cancers

Jeanne et al., Front Pharmacol. 2015

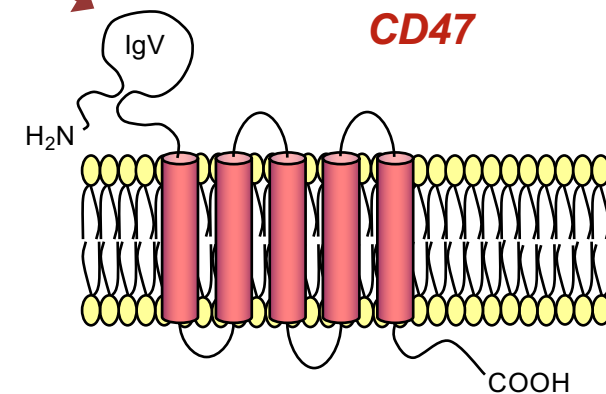
Thrombospondin-1 (TSP-1): a main actor within a tumor microenvironment (2)

TSP-1



TSP-1:CD47 interaction:

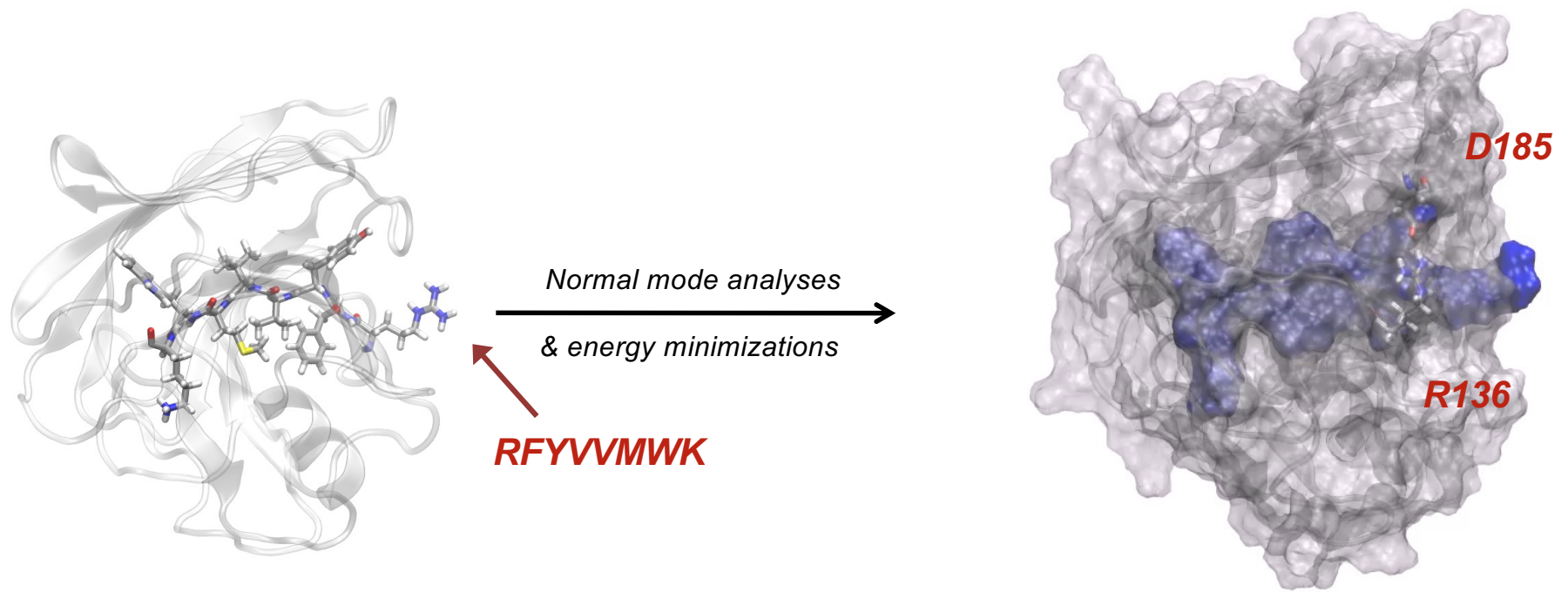
- Promotes tumor invasion and metastatic dissemination
Borsotti et al., Pigment Cell Melanoma Res. 2015
- Inactivates antitumor immunity
Soto-Pantoja et al., Cancer Res. 2014
- Drives drug resistance
Hirata et al., Cancer Cell 2015



Angiogenesis

Isenberg et al., Nat. Rev. Cancer 2009

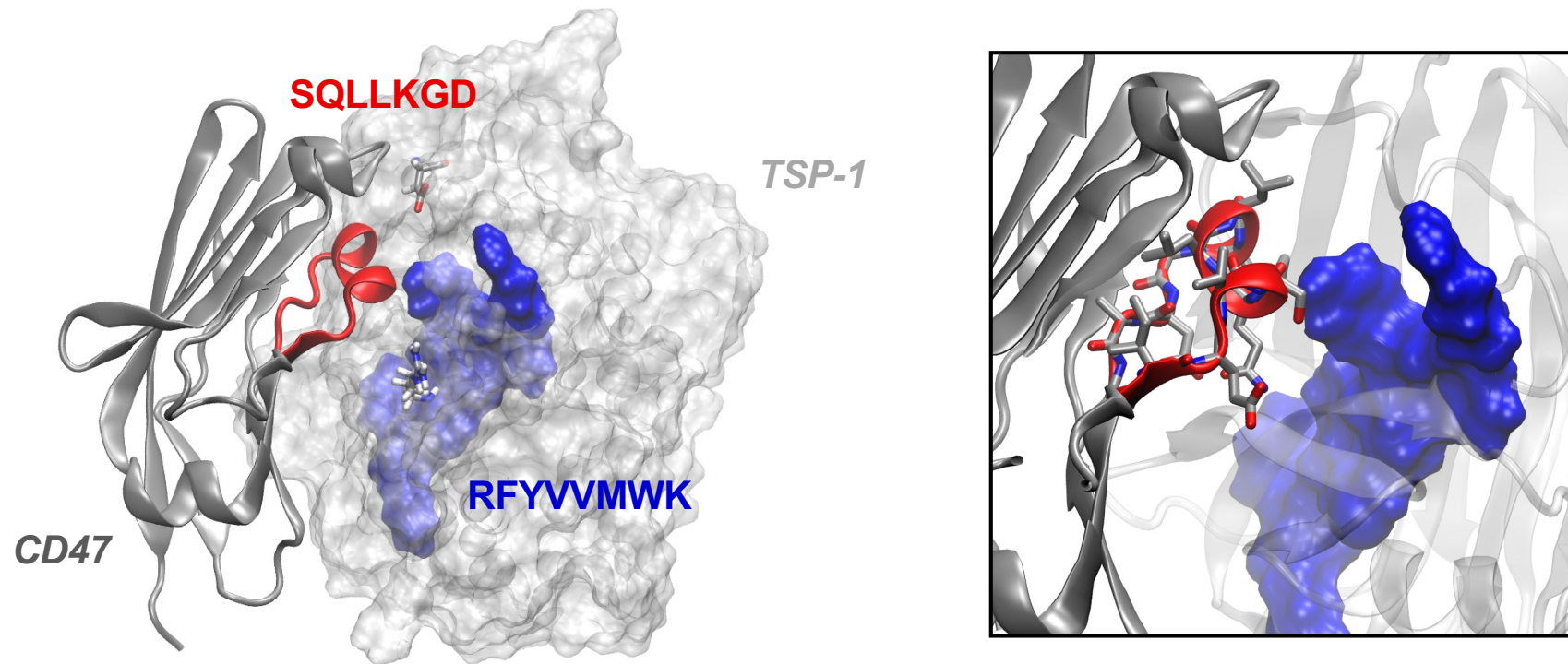
TSP-1:CD47 interaction analysis (1)



*TSP-1 carboxy-terminal domain
(PDB ID code 1UX6)*

TSP-1:CD47 interaction analysis (2)

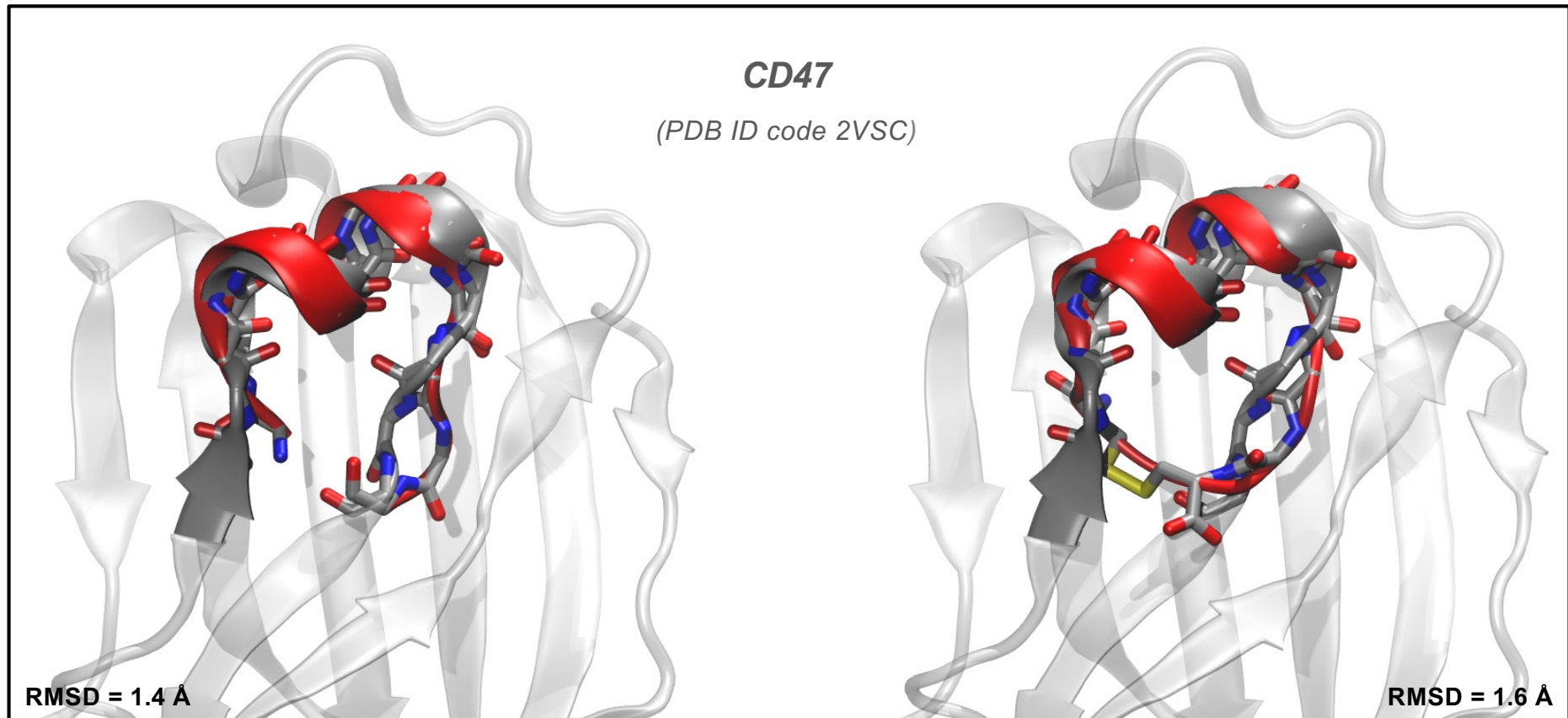
Protein/protein docking (GRAMM-X, RosettaDock)



Molecular interaction modeling
(TSP-1:CD47 docking experiments)

Design of peptides derived from the TSP-1 binding sequence of CD47 (1)

Peptides structures prediction and structural alignments with CD47 (PEP-FOLD, VMD)



IEVSQLLKGDAS
(TAX2-l)

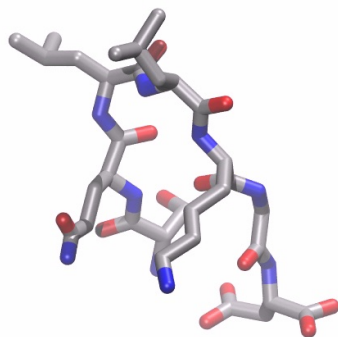
CEVSQLLKGDAC
(TAX2-c)

TAX2 linear and cyclic forms (red) as compared to the natural folding of the peptide into CD47 (silver)

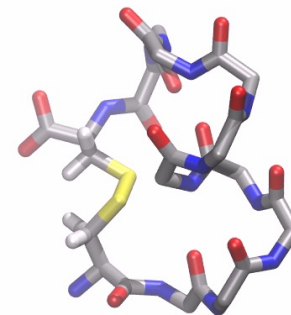
TAX2 peptides were filed for worldwide patent (WO/2013/007933 A1)

Design of peptides derived from the TSP-1 binding sequence of CD47

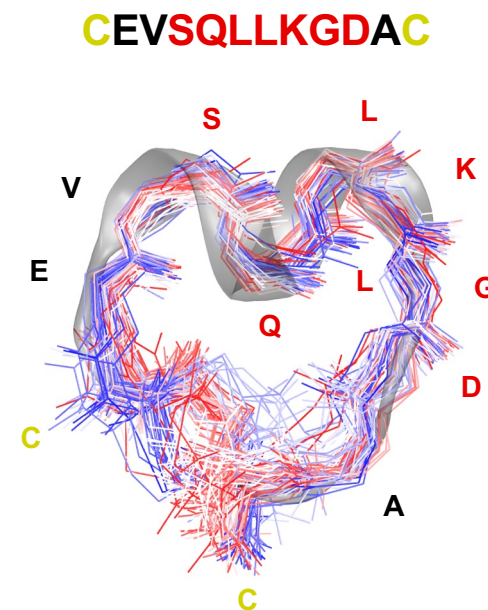
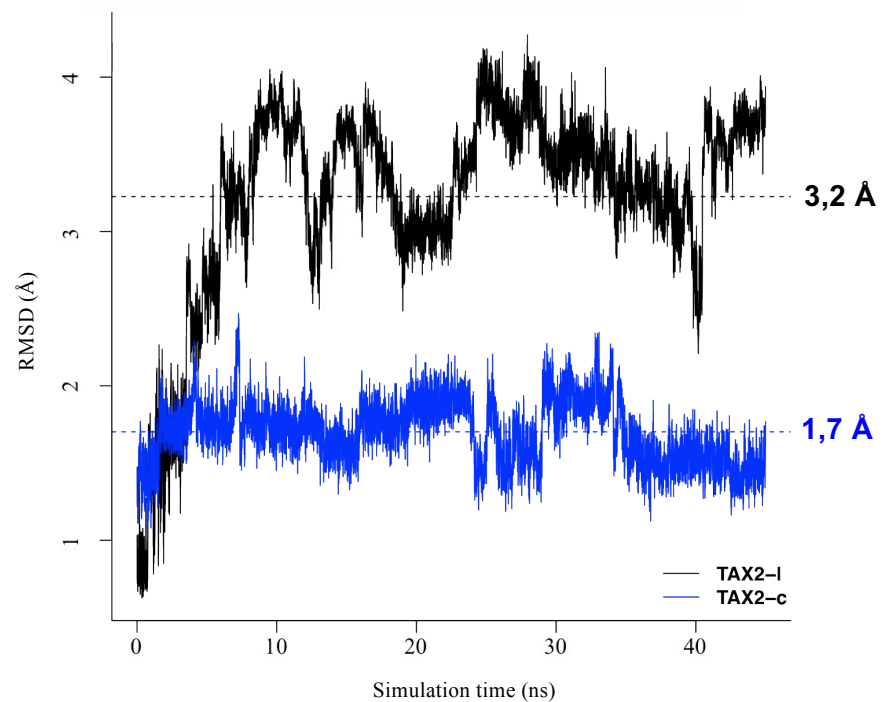
Molecular dynamics trajectories (NAMD)



TAX2-l



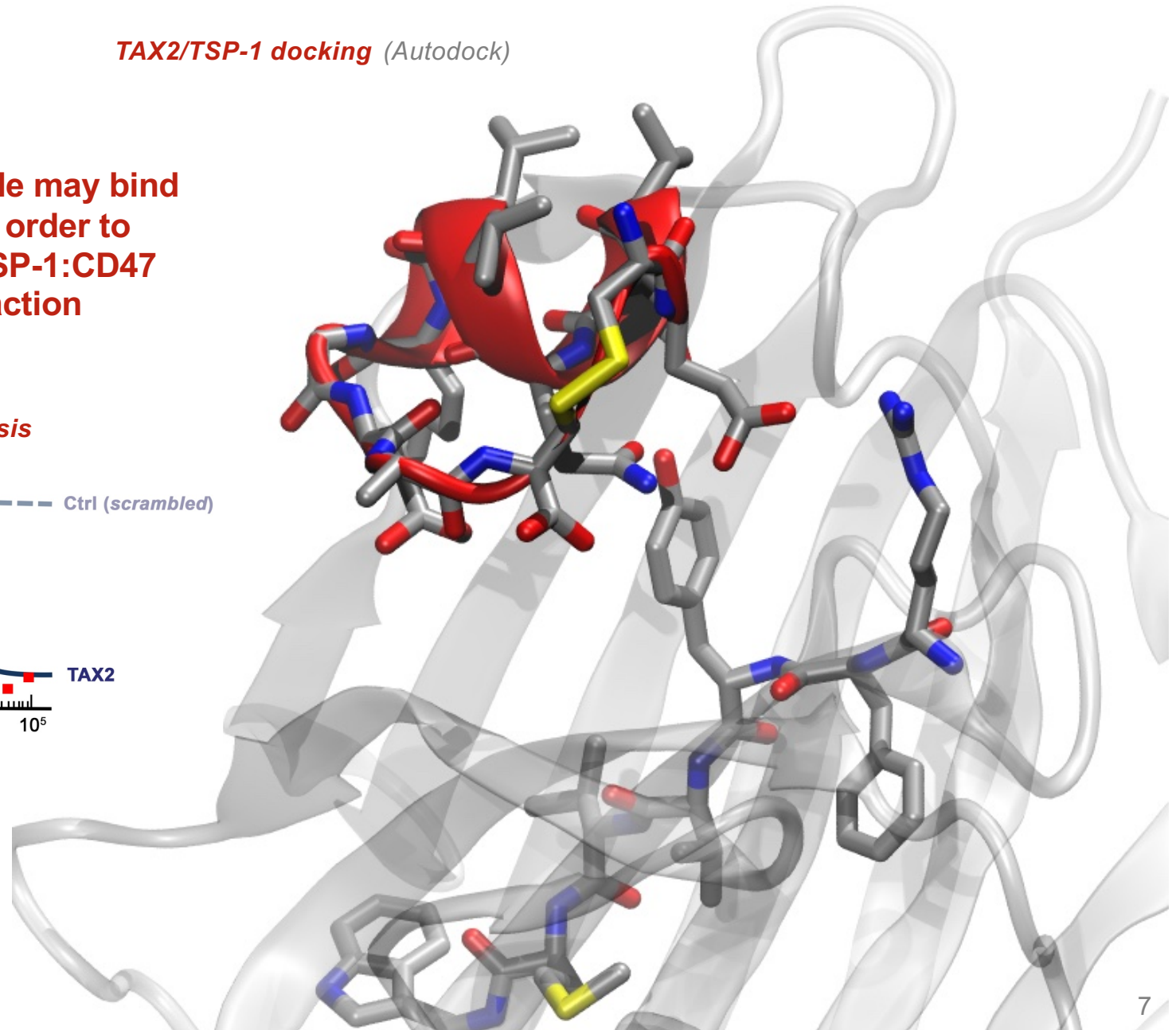
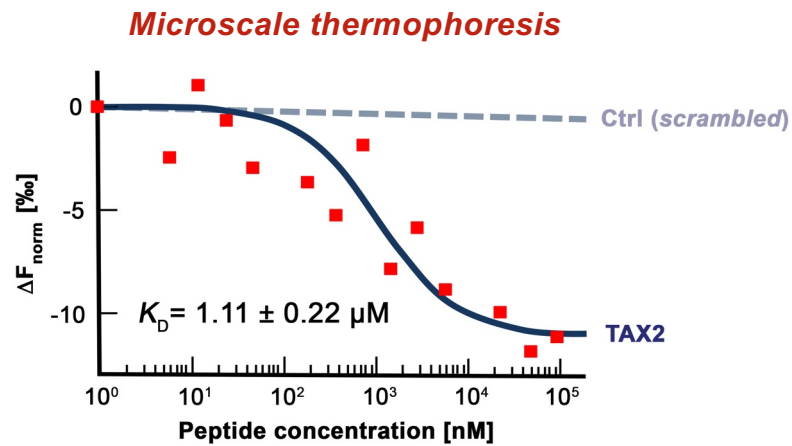
TAX2-c



Design of peptides derived from the TSP-1 binding sequence of CD47 (2)

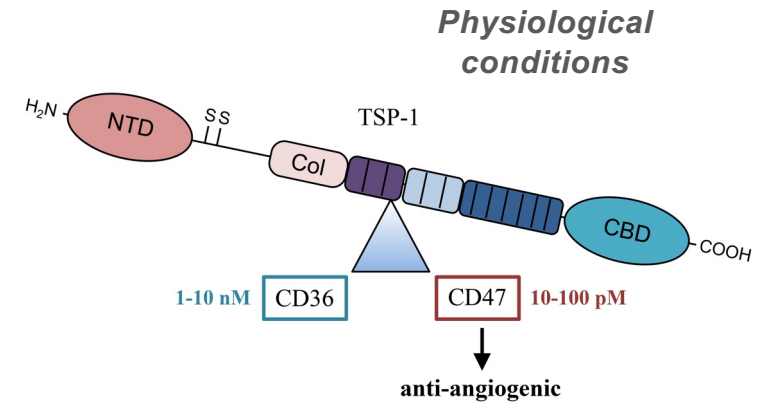
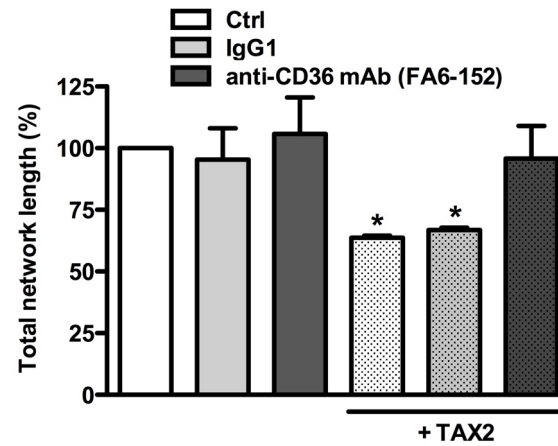
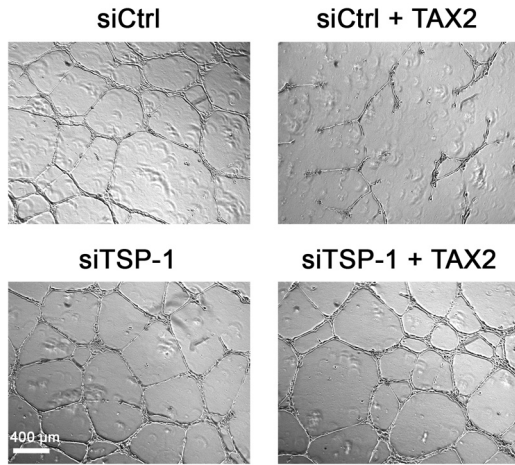
TAX2/TSP-1 docking (Autodock)

➔ TAX2 peptide may bind TSP-1 in order to prevent TSP-1:CD47 interaction

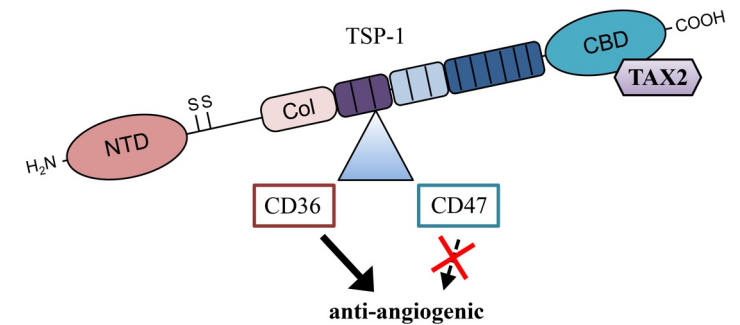


TAX2 mechanism of action

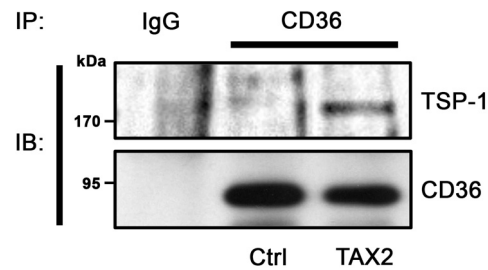
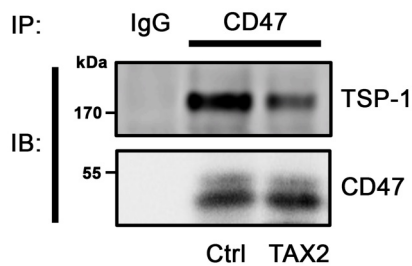
Tube formation assay



TAX2 TREATMENT



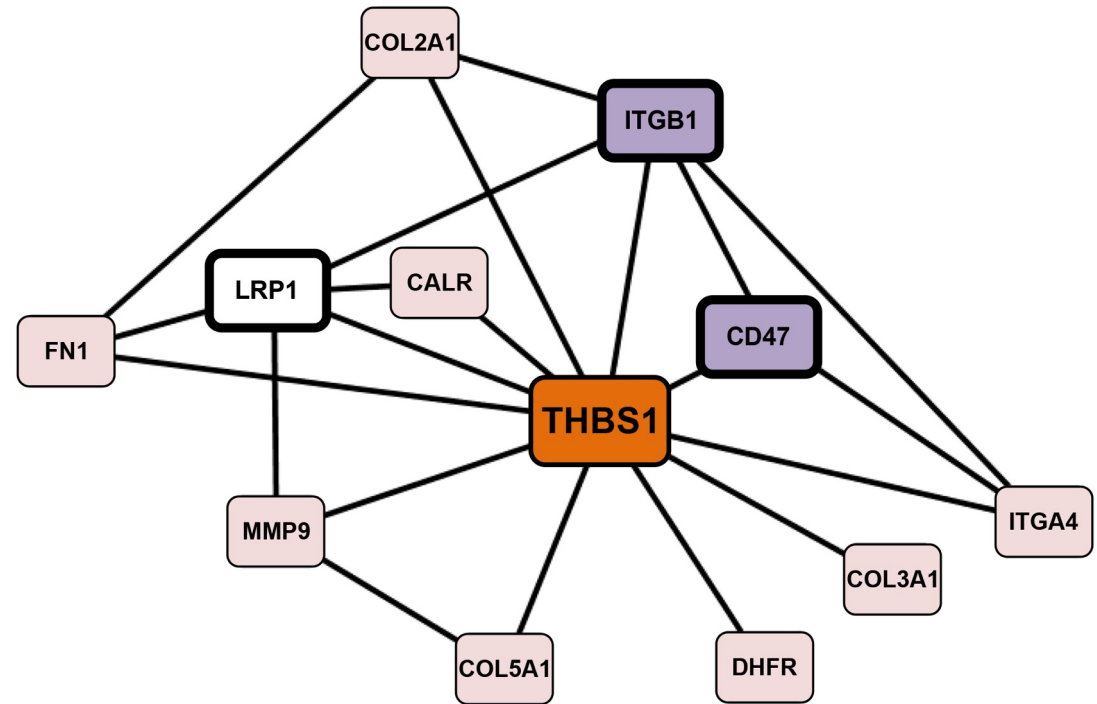
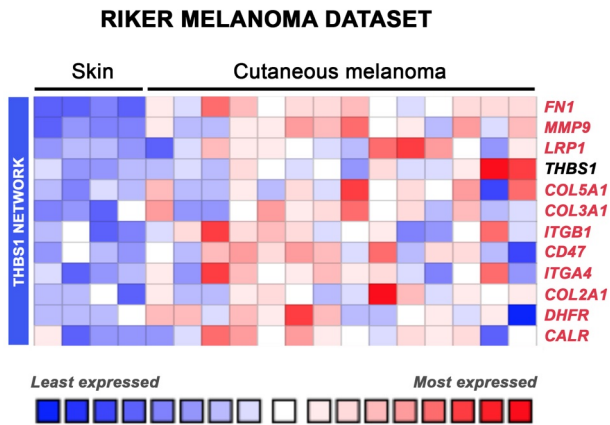
Co-Immunoprecipitation assays



TAX2 induces a switch in TSP-1 binding from CD47 to CD36, leading to subsequent anti-angiogenic responses

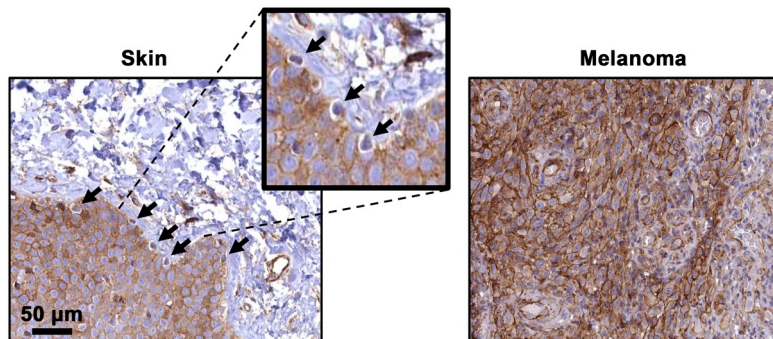
TAX2 potential for impeding tumor progression?

Genomic databases mining



Human tissue microarrays

TSP-1 IHC

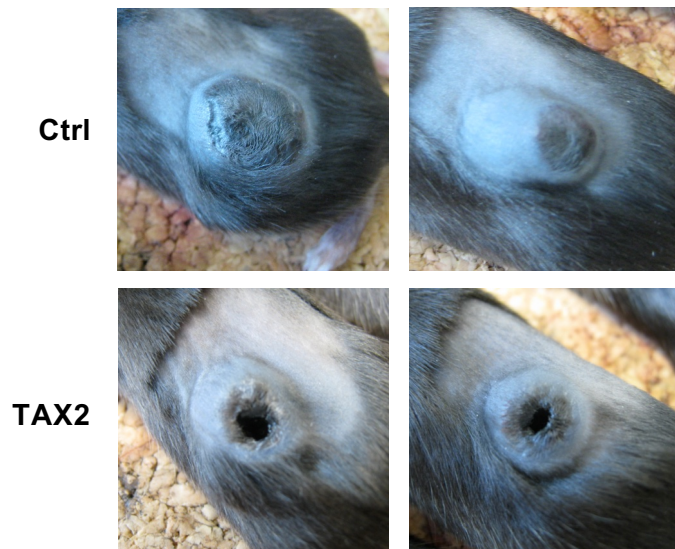


A THBS1-centered network is overexpressed in metastatic melanoma

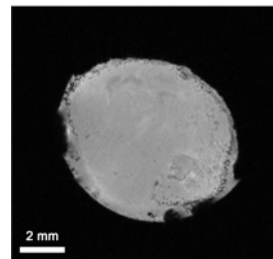
B16F1 melanoma allograft model (1)

Subcutaneous injection of **B16F1** melanoma cells in 9-weeks-old syngeneic inbred **C57BL/6J** mice;
intraperitoneal injection of cyclic peptide (10 mg/kg mouse weight) at days 3, 5 and 7

Tumor photographs at day 20

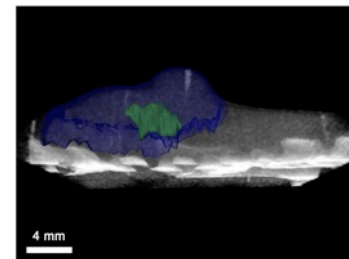


MRI

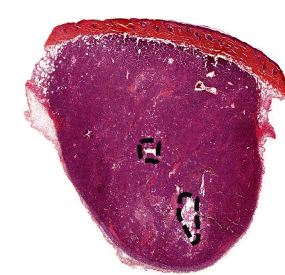


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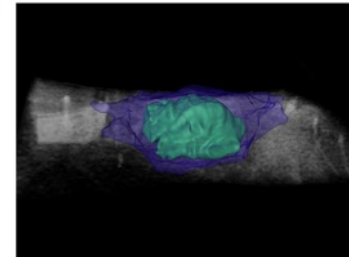
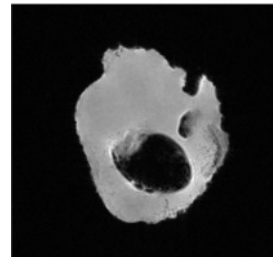
μ CT



HPS



TAX2

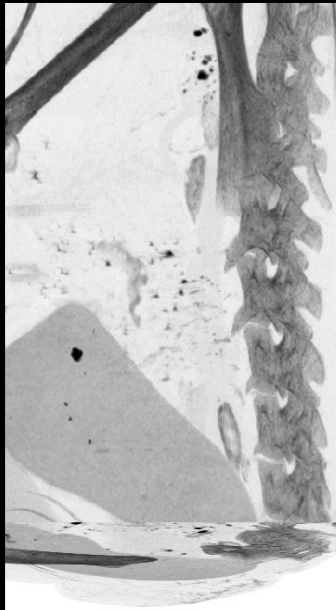


TAX2 treatment induces extensive tumor necrosis

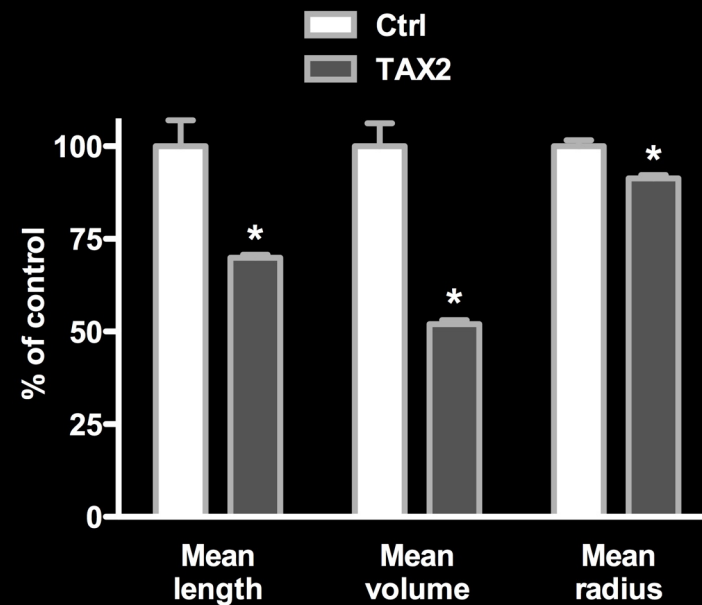
B16F1 melanoma allograft model (2)

μCT analysis of tumor angiography

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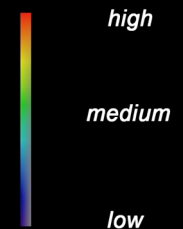


TAX2



TAX2 peptide targeting TSP-1:CD47 highly disturbs tumor-associated vascularization

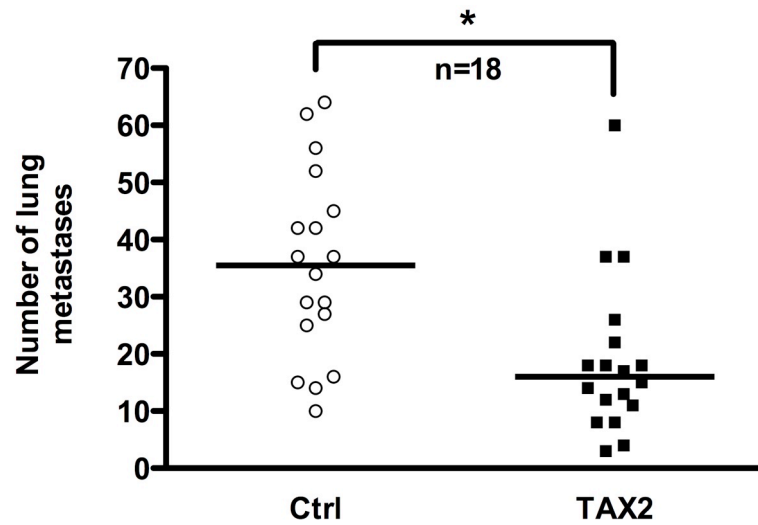
2 mm



B16F10 experimental metastatic model (1)

Intravenous injection of **B16F10** melanoma cells in 9-weeks-old syngeneic inbred **C57BL/6J** mice;
Intraperitoneal injection of cyclic peptide (10 mg/kg mouse weight) at days 0, 3, 5, 7, 10, 12, 14 and 17

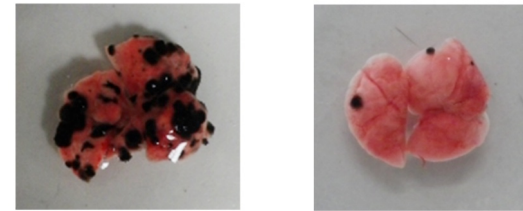
Lung metastases at day 21



Lung photographs at day 21

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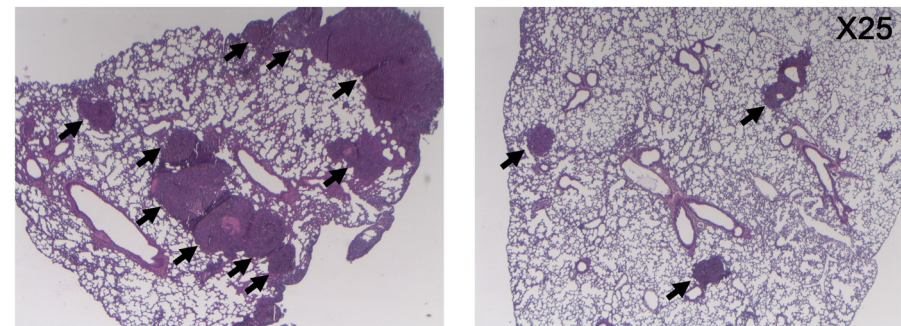
TAX2



HPS staining

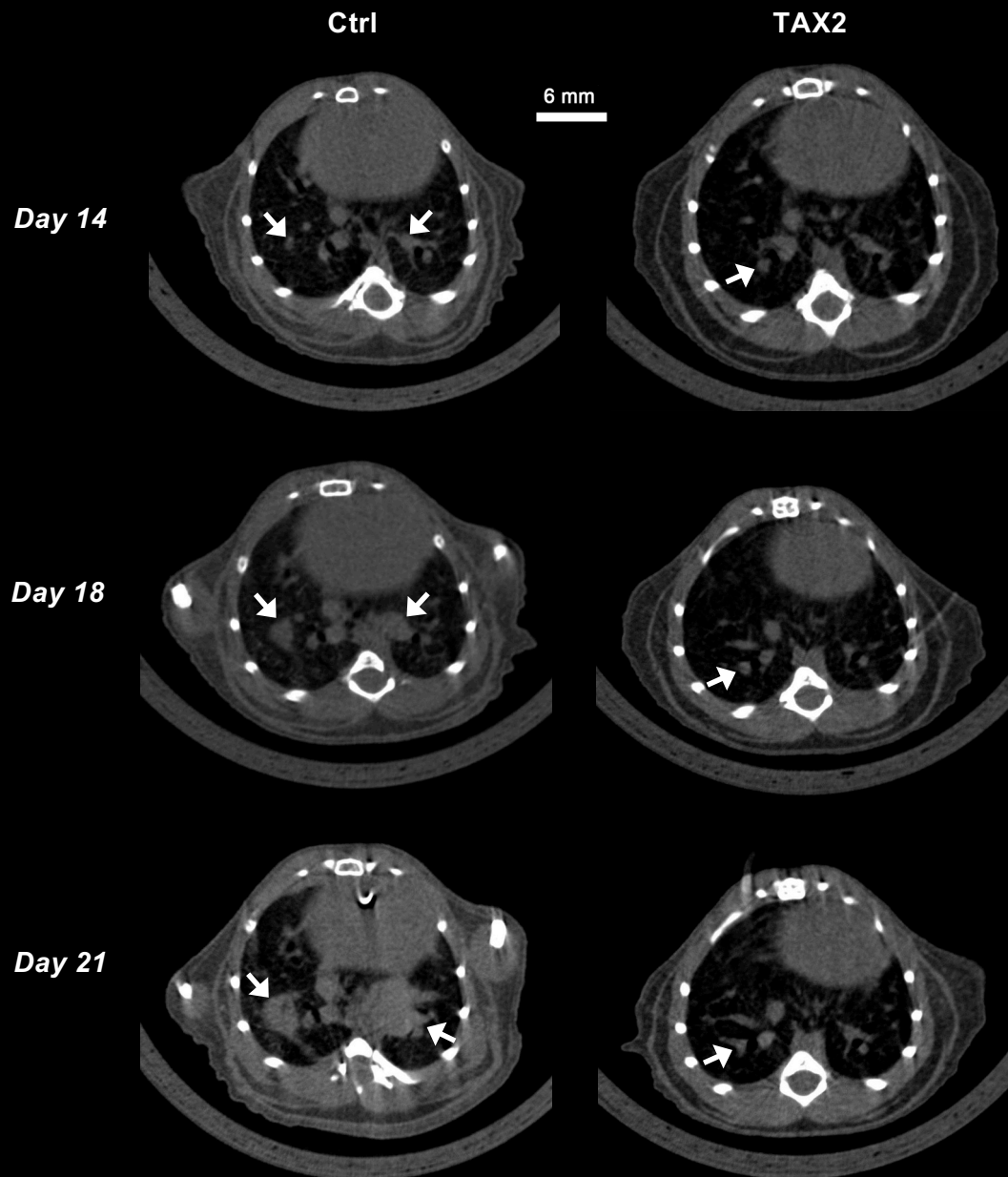
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TAX2



TAX2 peptide reduces the number of experimental lung metastases

B16F10 experimental metastatic model (2)



μ CT longitudinal follow-up of lung metastases development

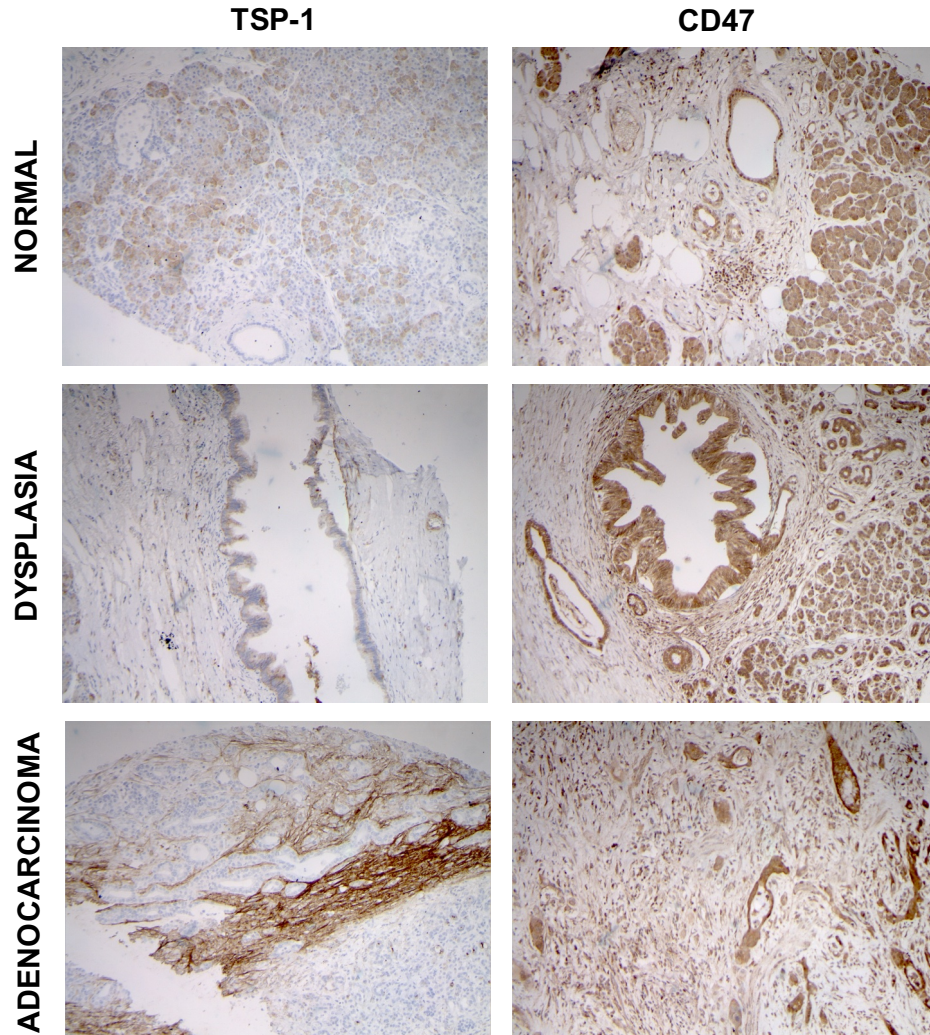


TAX2 peptide inhibits experimental lung metastases development and growth

May TAX2 inhibit growth of human tumor xenografts?

Human tissue microarrays

PANCREATIC CARCINOMA

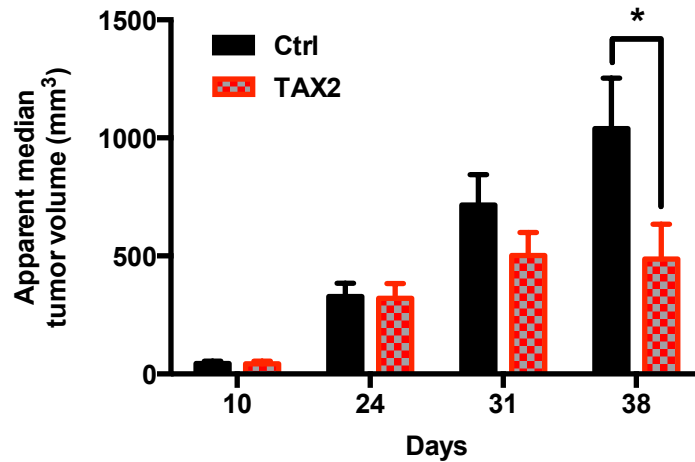


➔ TAX2 molecular targets are (over)expressed within human pancreatic tumors

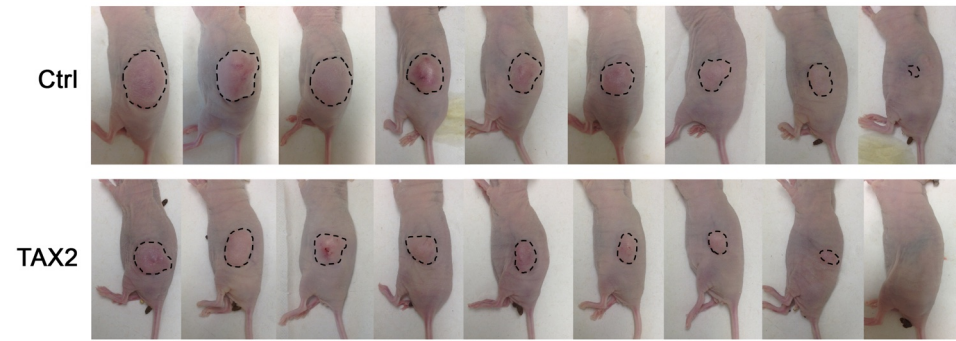
MIA PaCa-2 pancreatic carcinoma xenograft model

3×10^6 MIA PaCa-2 cells/mouse were implanted subcutaneously in inbred BALB/C nu/nu mice ; TAX2 i.p. treatment (10 mg/kg mouse weight) were performed 3 times a week during 4 weeks starting at day 10 after tumor cells s.c. inoculation

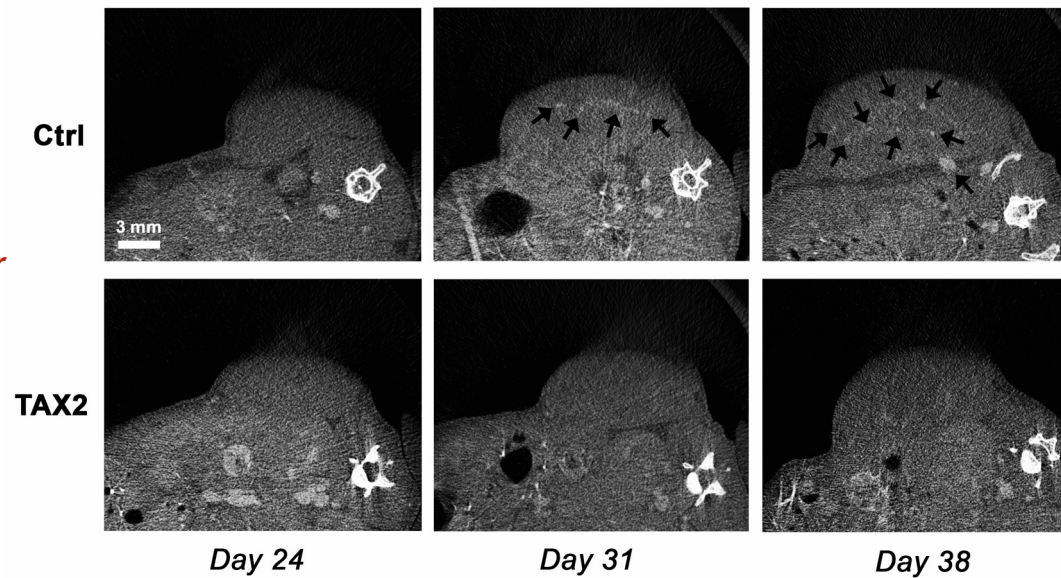
Tumor volume evolution



Tumor photographs at day 38

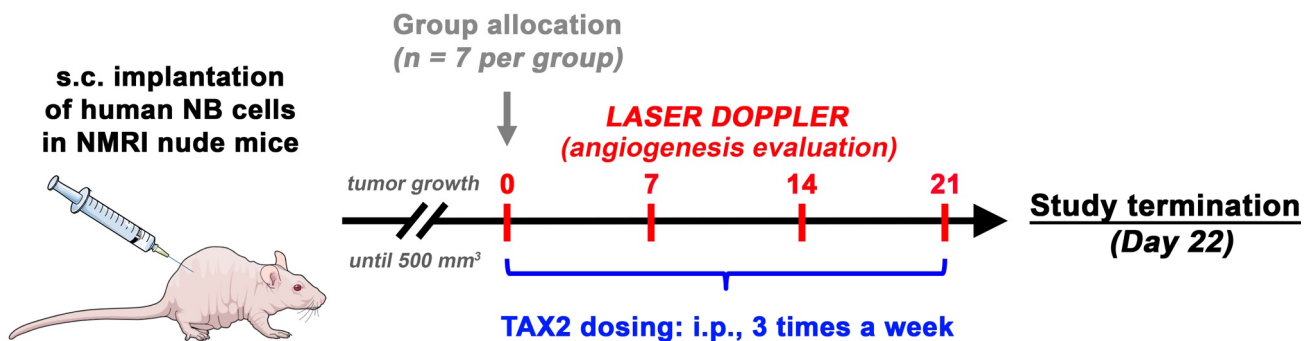


μ CT analysis of tumor angiography

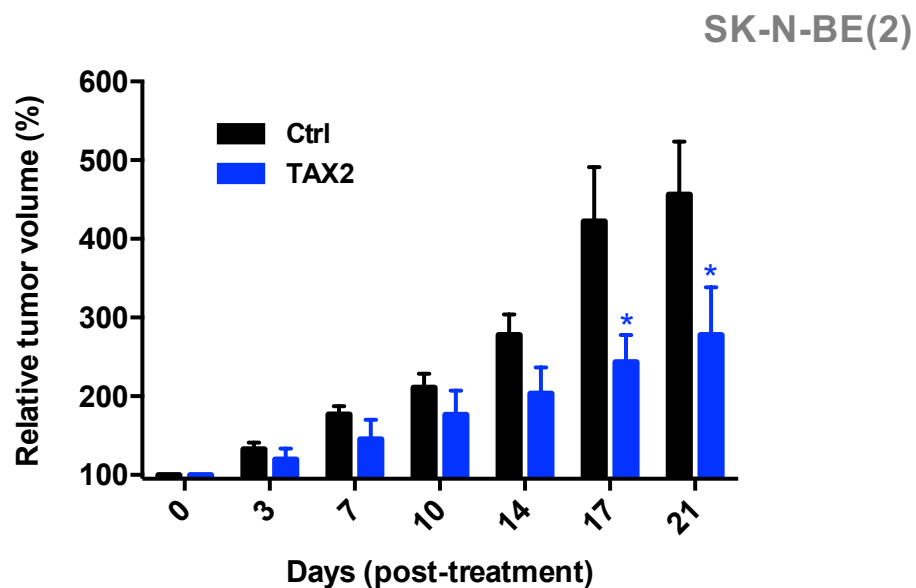


Childhood neuroblastoma xenograft models (1)

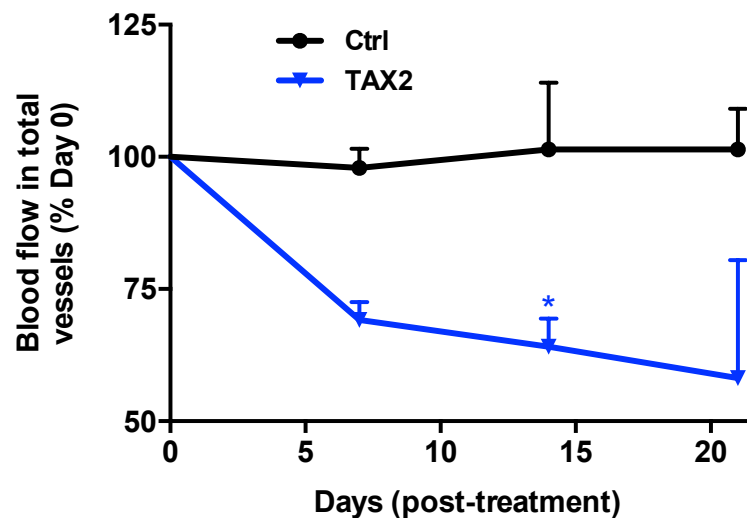
Pre-established neuroblastoma s.c. tumors (0.5 cm³)



Tumor volume evolution



LASER Doppler scanning

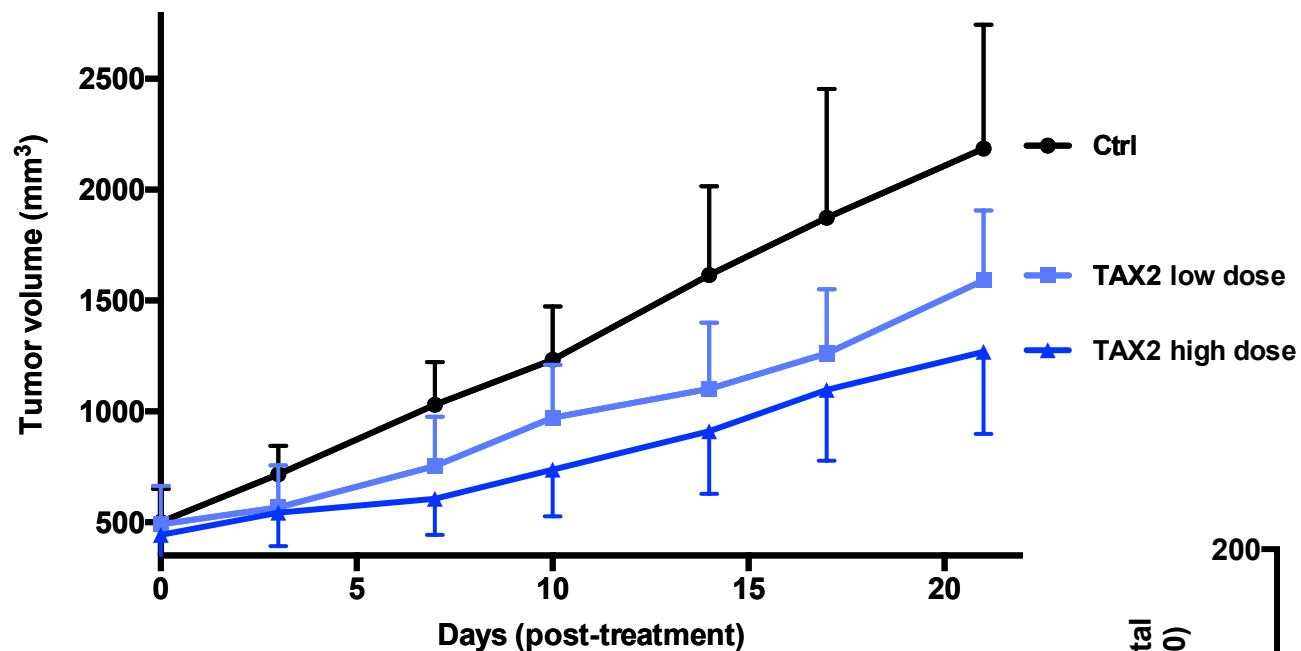


TAX2 treatment inhibits tumor growth and impedes intra-tumoral blood flow in the SK-N-BE(2) neuroblastoma xenografts model

Childhood neuroblastoma xenograft models (2)

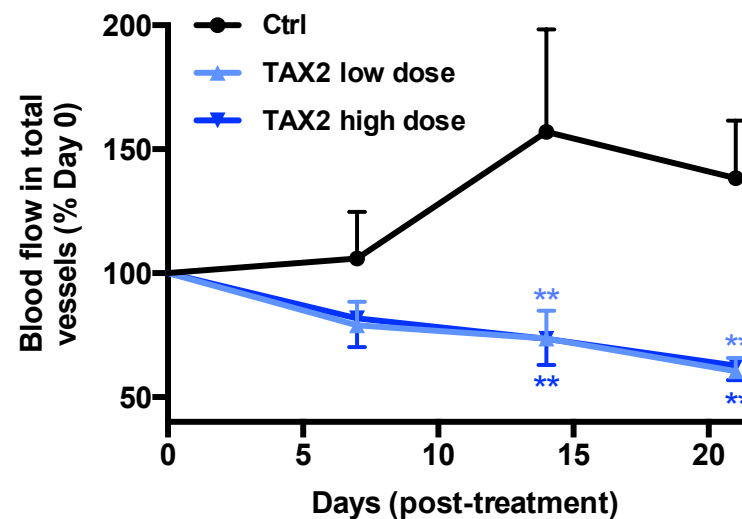
SK-N-SH

Tumor volume evolution



➔ **TAX2-induced anti-tumor effects are likely to be multifaceted**

LASER Doppler scanning



Remerciements

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Team leader: Stéphane DEDIEU

MERCI DE VOTRE ATTENTION

