Selective inhibitory peptides of the calcineurin phosphatase activity

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RESEARCH TEAM

Principal Investigator

Full Professor at the CNIC and Senior Tenured Scientist at the CSIC (*Profesor de Investigación)*
Group Leader of the "Gene Regulation in Cardiovascular and Inflammation Diseases" laboratory
Head of the "Vascular Biology and Inflammation Department"

The Group: Gene regulation in cardiovascular and inflammatory diseases

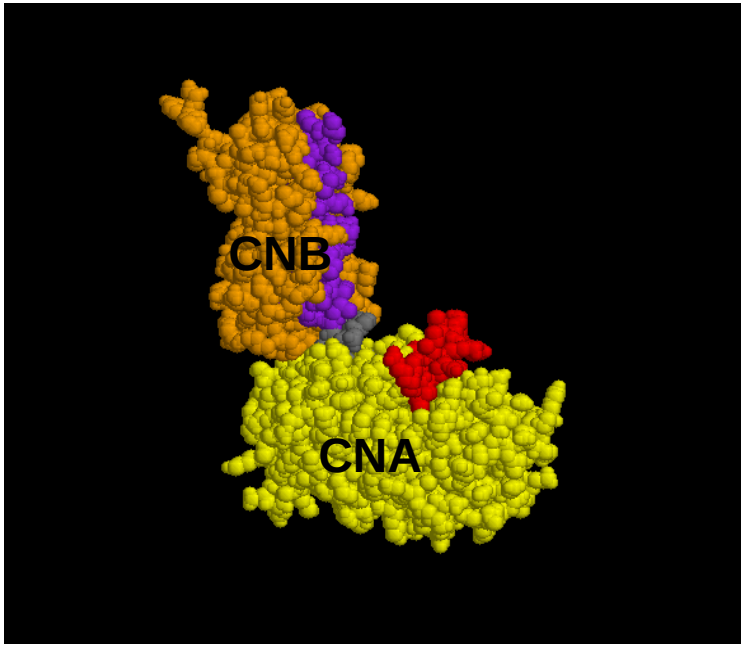
- Group established in 1995.
- Focused in studying regulation and function of the **calcium/CN/NFAT pathway in lymphocyte activation**, angiogenesis, **inflammation** and **cardiovascular remodelling** programs.
- Central research line in recent years: **mechanisms underlying CN interaction with NFAT** (and with other substrates and regulators). Important sequences for these interactions identified.
- **Mechanisms** by which **CN interacts with its substrates** and by which **immunosuppressive drugs inhibit CN**

TARGET

Calcineurin: Quick guide

What is it and what does it do? Calcineurin is a protein **phosphatase**, activated physiologically by Ca2+–calmodulin.

What does it look like?
Calcineurin is **a heterodimer** of an A catalytic subunit and a B subunit.



TARGET

Calcineurin: Quick guide

What are its substrates?

Substrates that contribute to **transcriptional signalling**, in particular nuclear factor of activated T cells (NFAT), **and** calcineurin's substrates in **nontranscriptional pathways** (vast majority of the identified substrates).

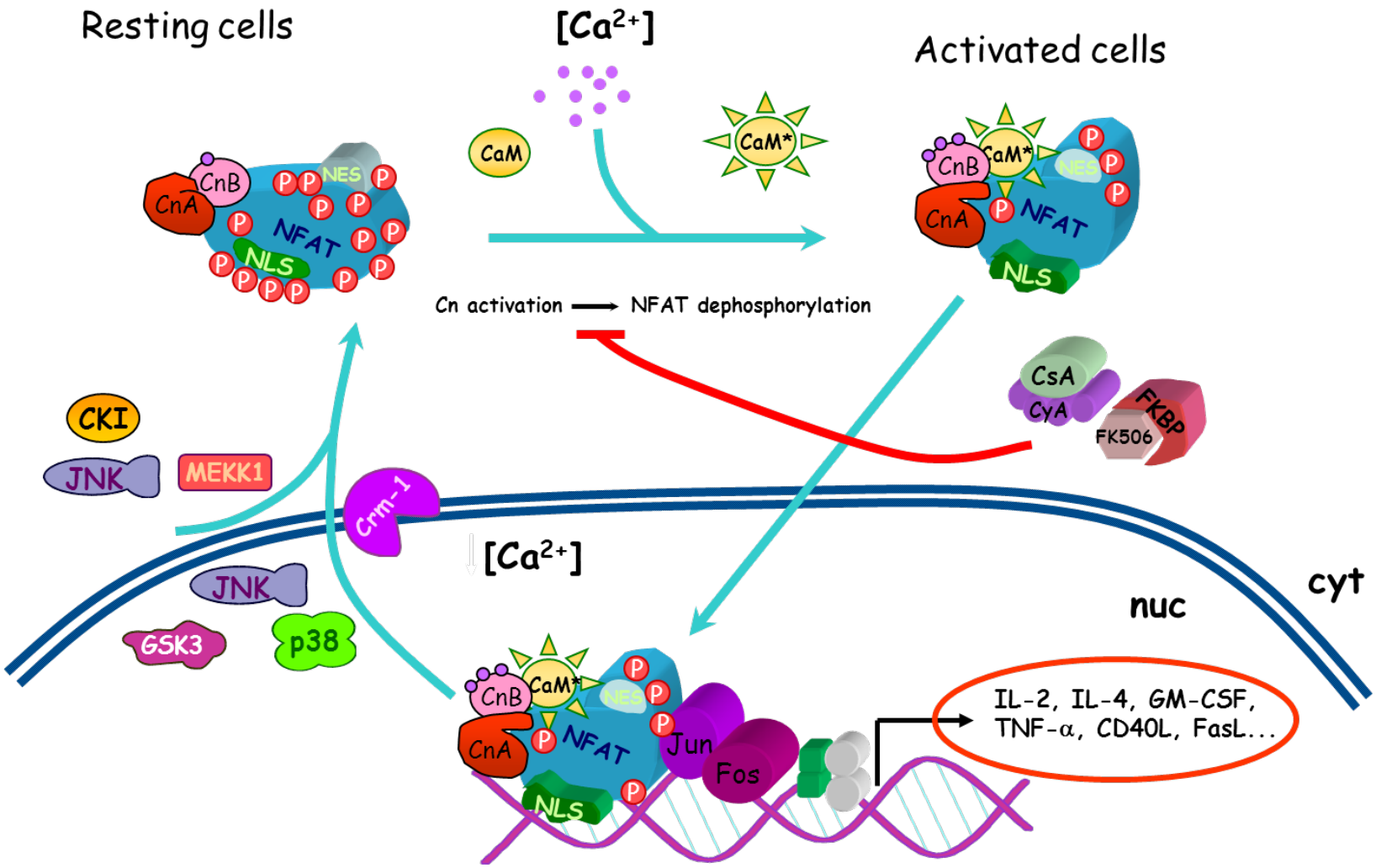
What are its inhibitors?

Cyclosporin A (CsA) and **FK506** bind tightly to the abundant intracellular proteins cyclophilin A and FKBP12, respectively, and the resulting ligand–protein complex binds to calcineurin and impedes access of protein substrates to the active site.

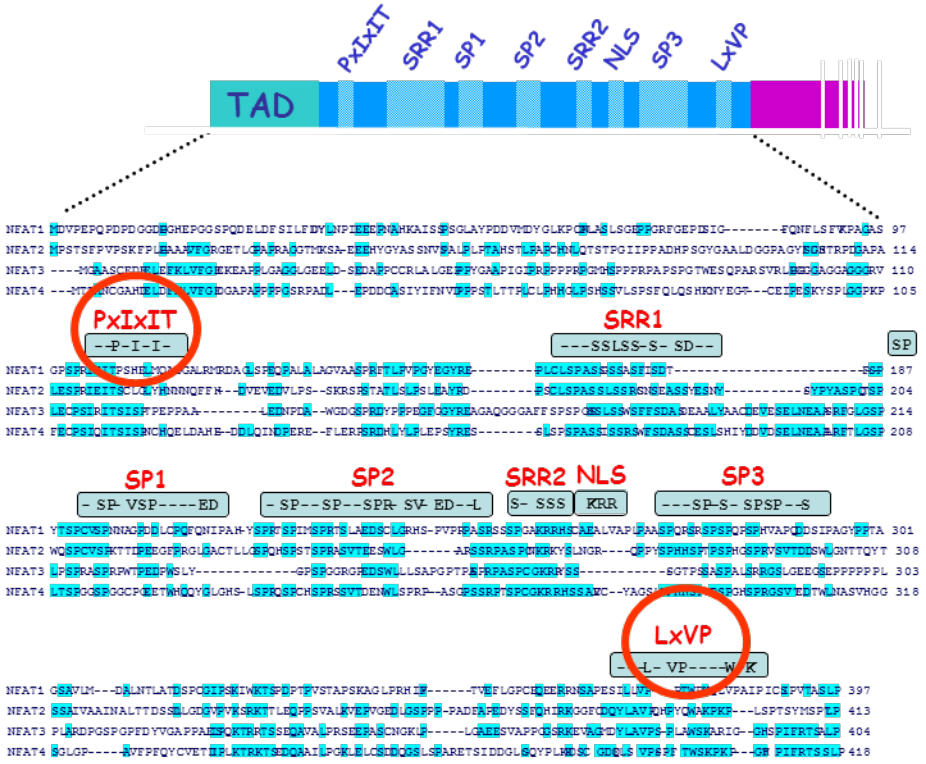
DOES IT HAVE ANY CLINICAL RELEVANCE?

Calcineurin signalling is prominent in **transplant rejection and autoimmune diseases**

PATHWAY



RATIONALE



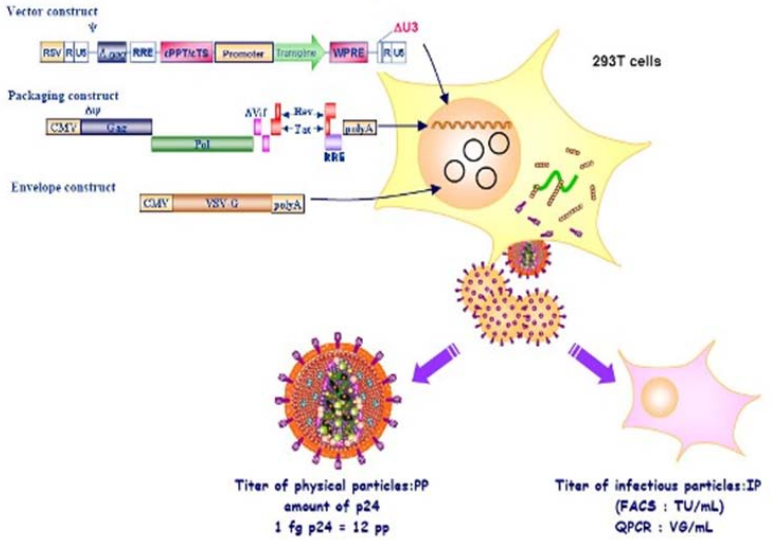
(Rodriguez *et al.* Mol. Cell, 2009)

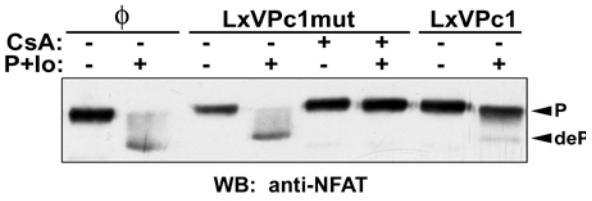
(Martinez *et al.* PNAS 2009)

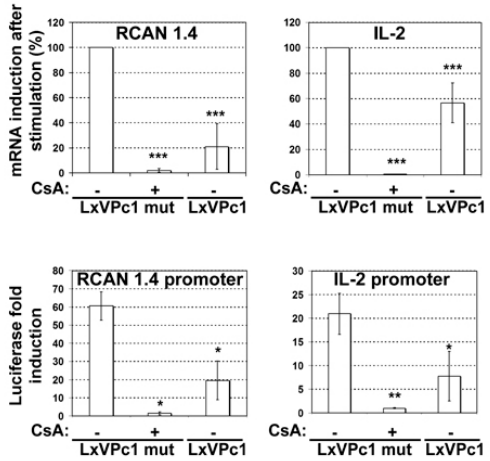
(Martinez *et al.* JBC 2006)

BIOLOGICAL EFFECTS

Lentivirus production





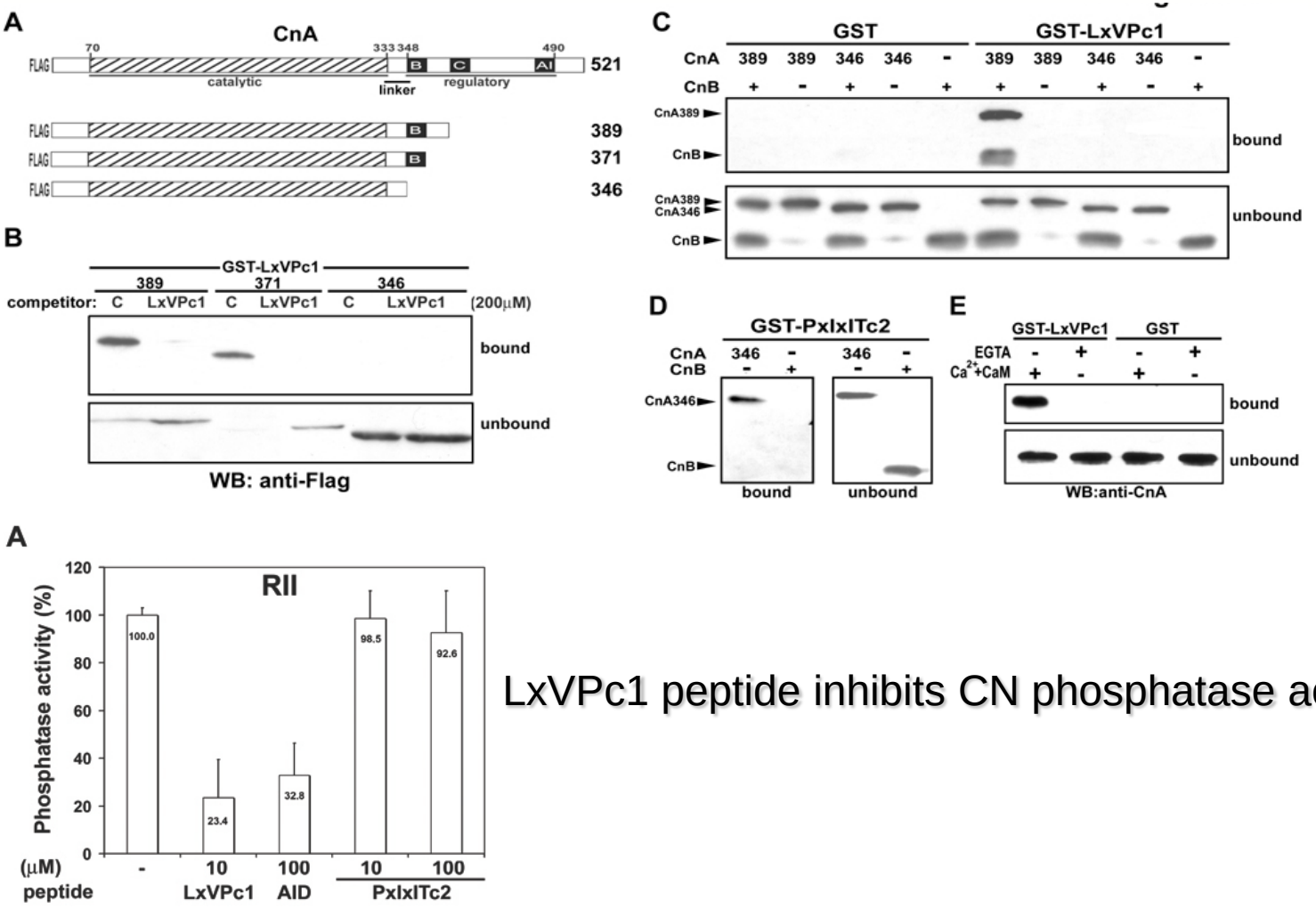


Cytokine network

	LxVPmut		LxVPmut+CsA		LxVPc1	
	Control	Plo	Control	Plo	Control	Plo
IL-2	1.0∓0.0	485.6∓37.1	0.4∓0.0	0.5∓0.0	0.8 0∓0.1	123.1∓30.2
IL-3	1.0∓0.0	580.3∓44.4	0.9∓0.1	0.0∓0.0	3.6∓0.2	89.4∓21.9
IL-4	1.0∓0.0	6.1∓0.4	0.4∓0.0	0.0∓0.0	0.4∓0.0	0.5∓0.1
IL-8	1.0∓0.0	155.5∓11.9	0.3∓0.0	1.9∓0.2	1.1∓0.1	47.9∓11.7
IL-10	1.0∓0.0	5.4∓0.4	0.9∓0.0	0.0∓0.0	0.8∓0.1	1.0∓0.3
IL-12A	1.0∓0.0	3.6∓0.3	2.0∓0.1	0.1∓0.0	0.9∓0.1	2.7∓0.7
IL-12B	1.0∓0.0	0.9∓0.1	0.2∓0.0	0.4∓0.0	0.3∓0.0	0.0∓0.0
IL-13	1.0∓0.0	11.6∓0.8	0.6∓0.0	0.3∓0.0	1.2∓0.1	3.4∓0.8
IL-18	1.0∓0.0	1.1∓0.1	0.6∓0.0	5.1∓0.4	0.8∓0.1	0.7∓0.2
TNFα	1.0∓0.0	12.5∓0.9	1.0+0.0	0.2∓0.0	0.9+0.1	7.9∓1.9

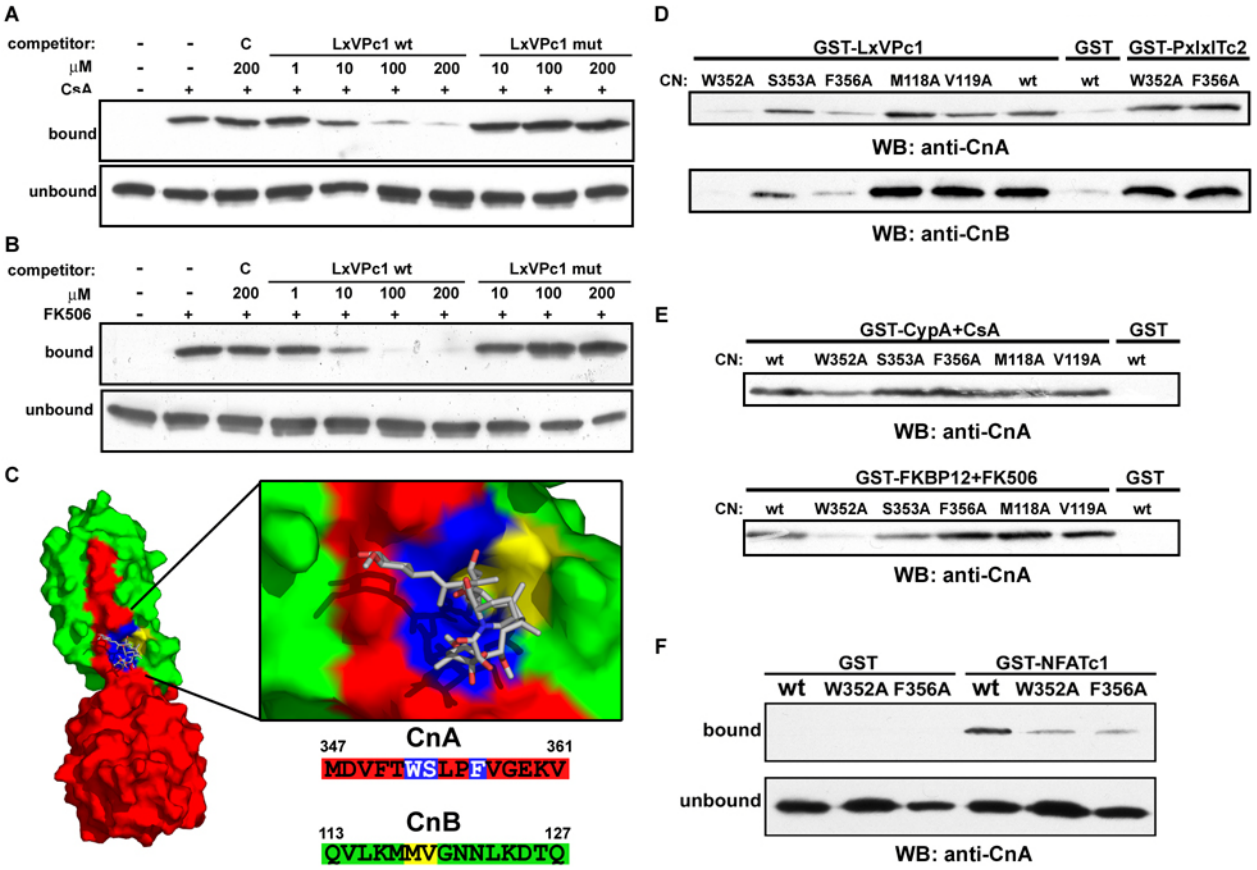
Rq values= mean + stdv (3 endogenous genes)

MECHANISM OF ACTION

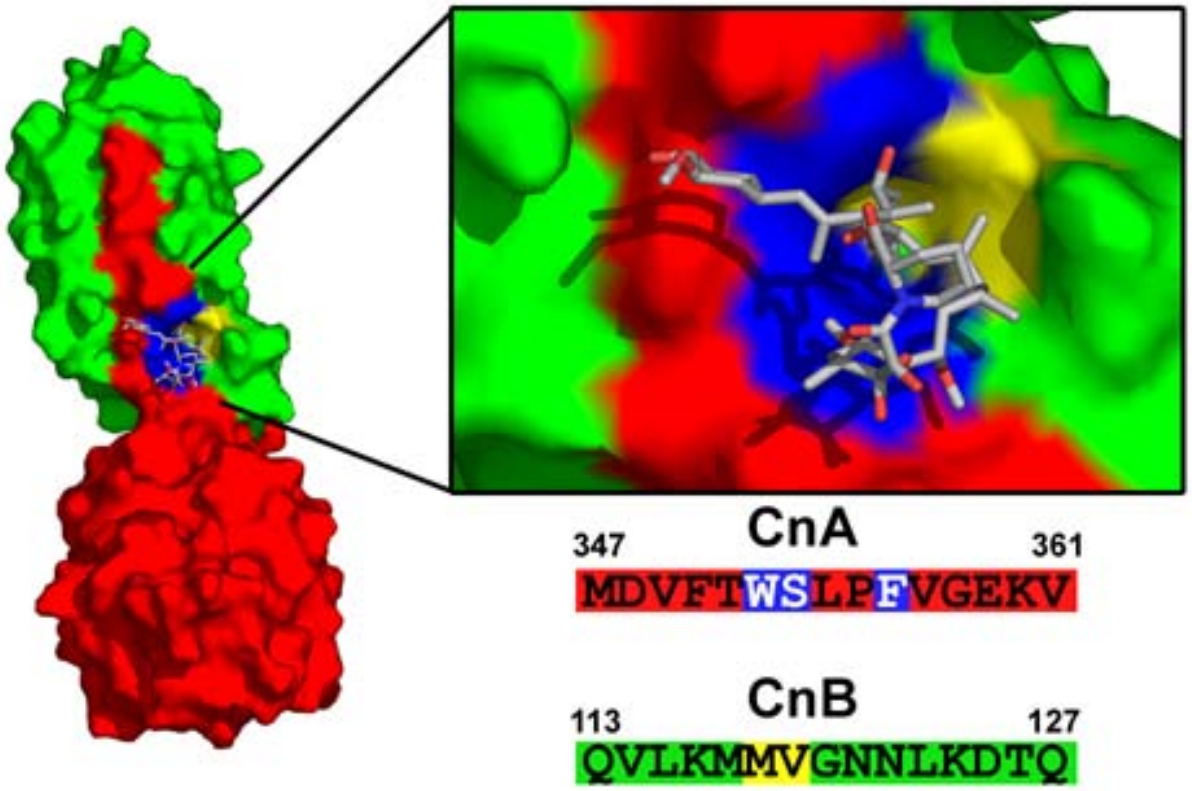


LxVPc1 peptide inhibits CN phosphatase activity

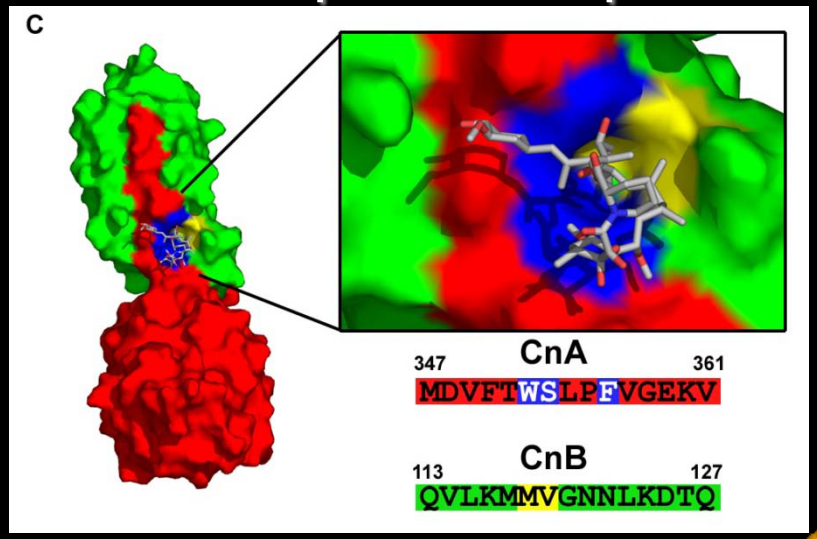
MECHANISM OF ACTION



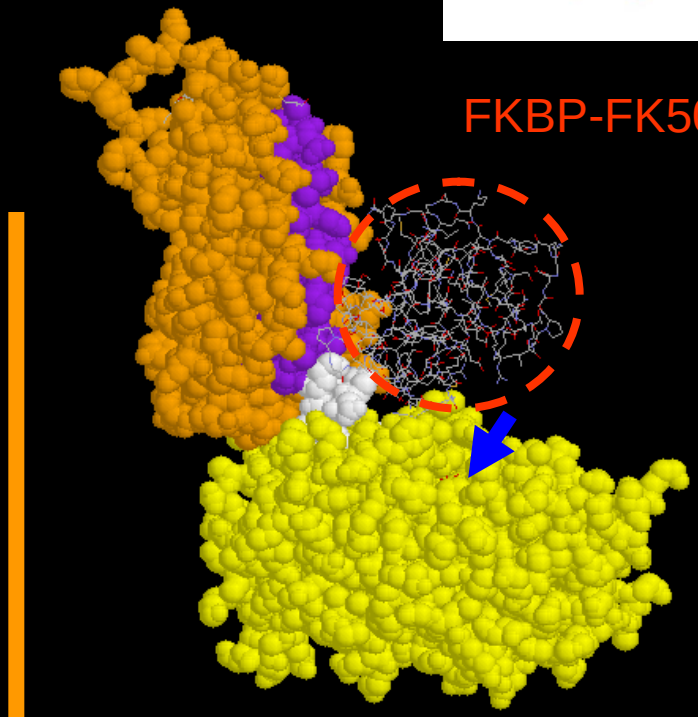
PRODUCT



Immunosuppressant-immunophilin complexes bound to calcineurin

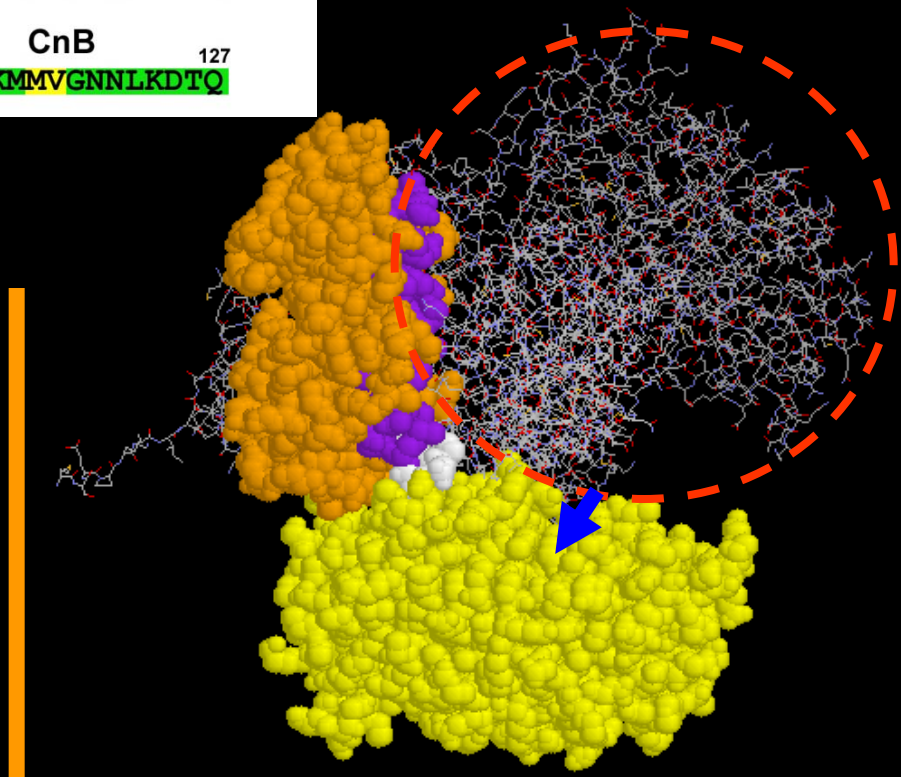


FKBP-FK506



calcineurin

CypA-CsA



calcineurin

DIFFERENTIAL FEATURES FACING THE MARKET

- **NFAT sequence** with a high ability to **inhibit CN phosphatase activity** identified.

- Sequence able to **inhibit CN phosphatase activity by itself** (unlike what has been reported for other immunosuppressive agents like CsA and FK506).

- Due to this specifity → **reduction of adverse effects**.

CURRENT STATUS OF DEVELOPMENT

In vitro:

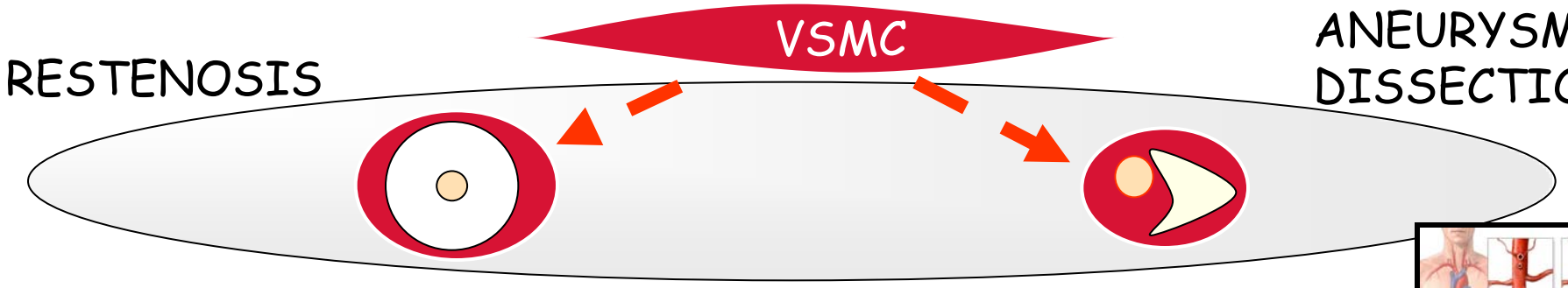
- Peptide able to efficiently block the CN pathway in different cell lines and primary cultures (murine and human), using different delivery methods.
- Strong evidences supporting the specificity of the peptide without affecting other pathways.
- The peptide modifies the cytokine expression profile in immune cells.

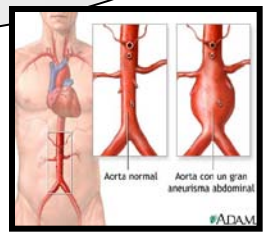
In vivo:

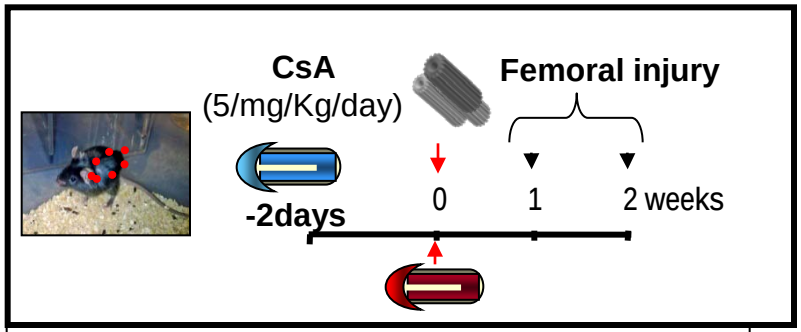
- We are able to efficiently infect immune cells, using lentiviral vectors.
- The peptide has strong therapeutic effects in different animal models where inflammatory processes are involved (subsidiary patent in preparation).
- Evidences supporting the use of the peptide in both systemic and *ex vivo* therapeutic developments.

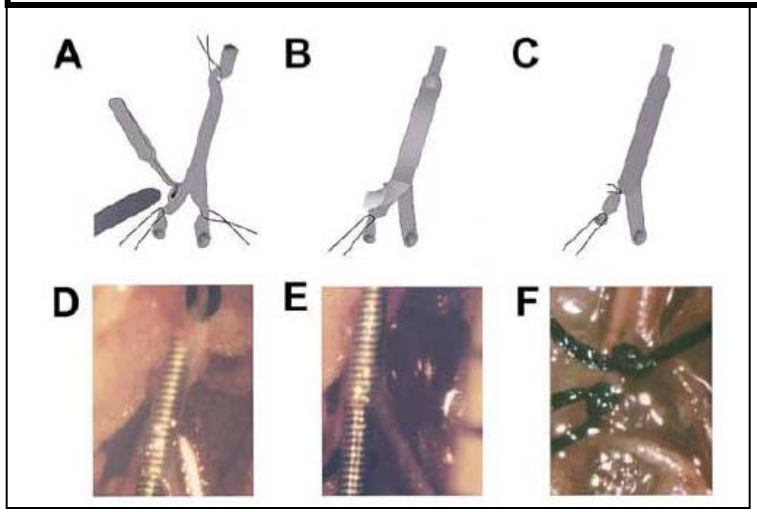
AngII and the CN/NFAT signalling pathway in the Cardiovascular System

cnic

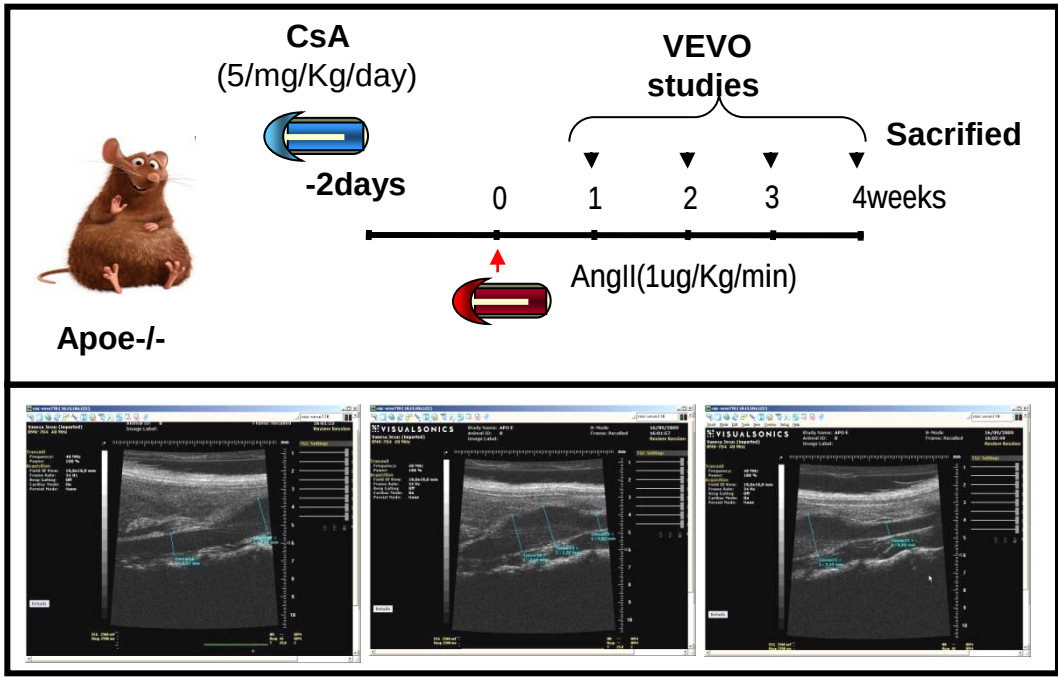






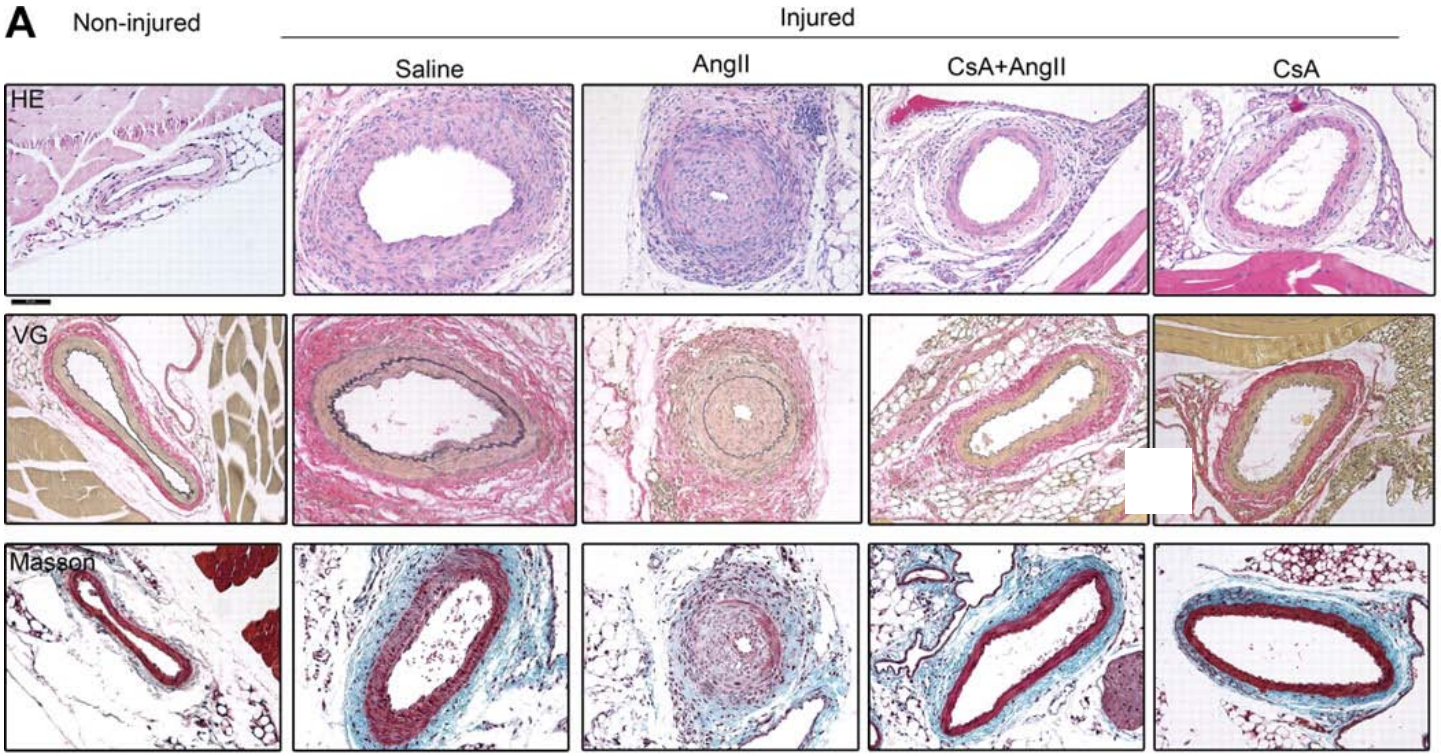


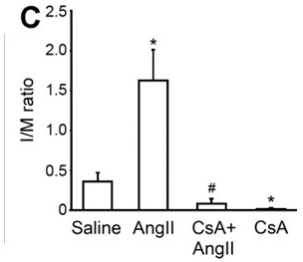
Femoral injury



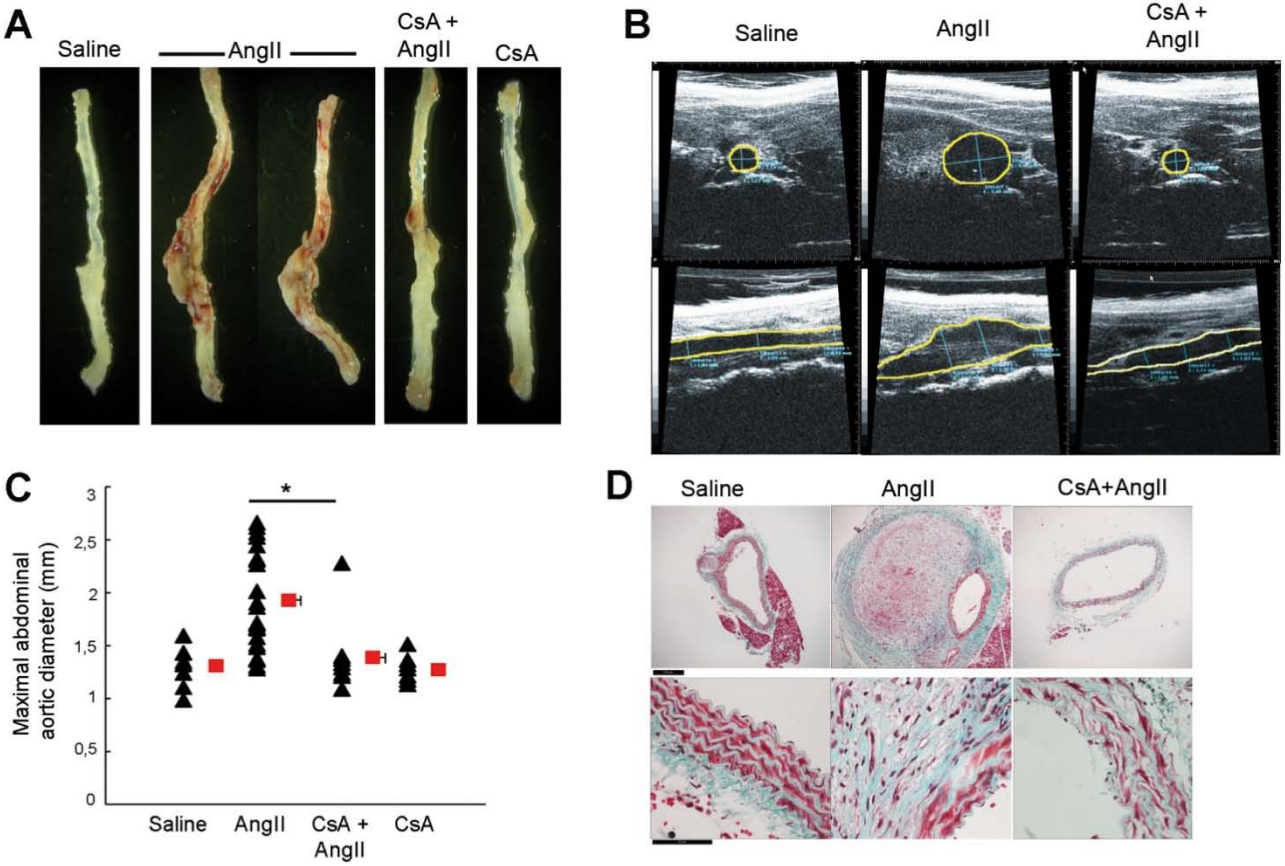
High-frequency US imaging system (VEVO770)

CURRENT STATUS OF DEVELOPMENT





CURRENT STATUS OF DEVELOPMENT



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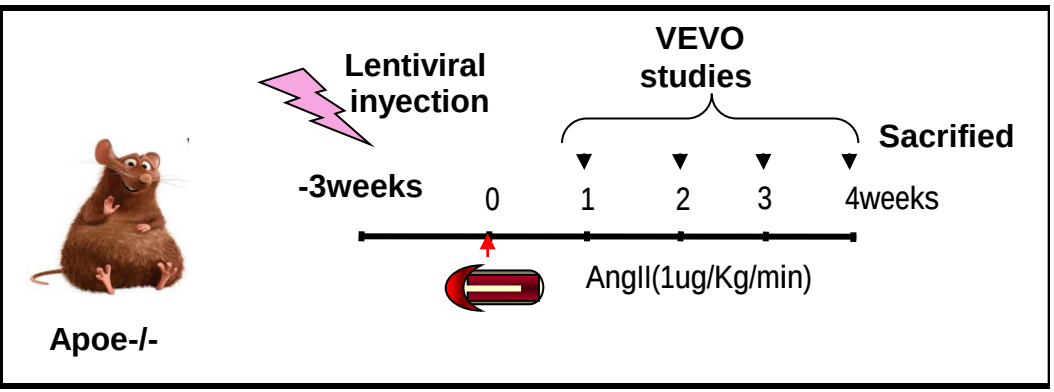
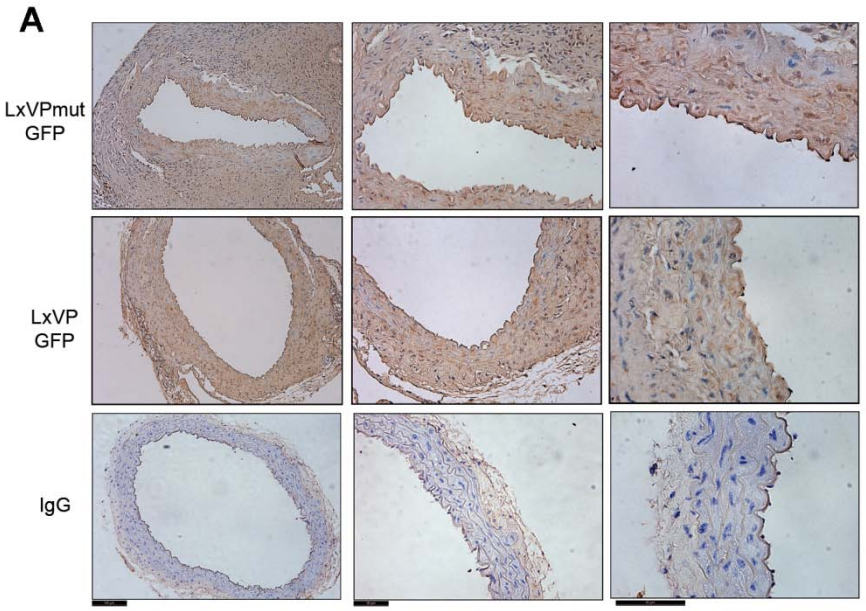
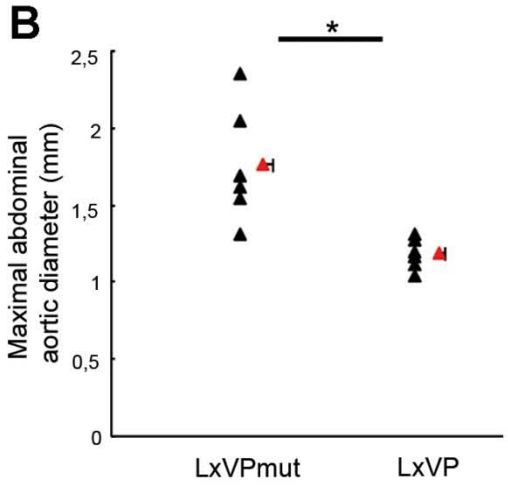
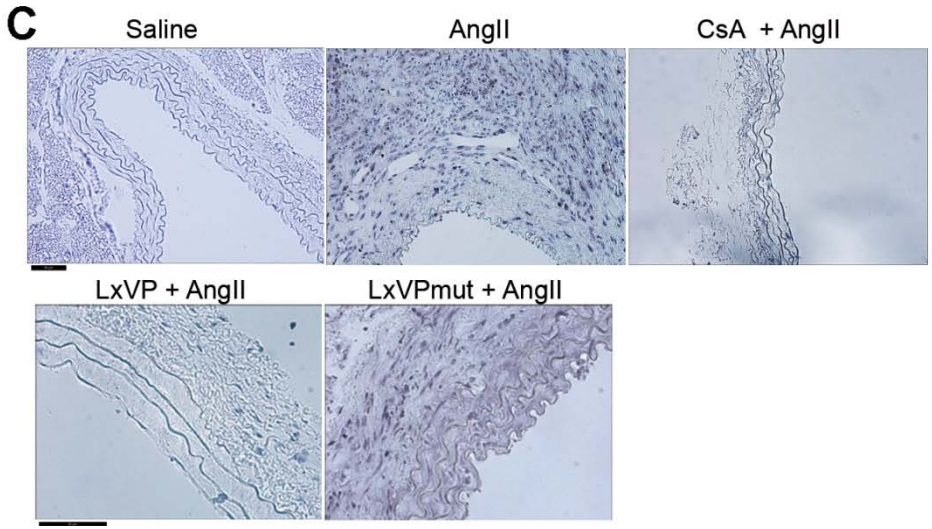
Systemic delivery of LxVP lentivirus inhibits development of AngII-induced AAA.









CURRENT STATUS OF DEVELOPMENT

We have also found that the peptide is therapeutically active in a mouse model of <u>Rheumatoid Arthritis</u> as well as three additional models of <u>inflammatory and cardiovascular diseases</u>… (subsidiary patent application in preparation).

We are glad to provide further details under a confidential agreement!!!

MARKET POTENTIAL

Indication	PoC	Market	Market Competition
Rheumatoid arthritis	✓	**No known cure**- DMARDs, surgery, other therapies 3 cases/ 10,000/annum Rituximab (Roche) 6.100 M$ 2010 (4. 612M€)	⇧⇧⇧
Aneurysm	✓	**Just Medical Devices** 2-4% Incidence US AAA (2.9– 4.9 cm Ø) •1.3% of men aged 45–54 years •12.5% of men aged 75–84 years	⇩
Transplant rejection	Pending	Immunosupressant drugs CAGR: 6,7% 2004-2010 Market value $5.6 billion in 2018	⇧⇧
Other autoimmune diseases	?	?	?

References: Center for Disease Control and Prevention – USA http://www.cdc.gov/dhdsp/data_statistics/fact_sheets/fs_aortic_aneurysm.htm
Thomson Pharma
Solid Organ Transplant Immunosuppressant Market to 2018 http://finance.yahoo.com/news/solid-organ-transplant-immunosuppressant-market-104400902.html

IPR PROTECTION

- Patent granted in Spain: **ES 2 327 694 B1**
- USPTO: **Nº 13/302,917**
- EPO: **EP06841769.0**
- **50% CSIC/ 50% CNIC**
- **Proyecto de la cartera tecnológica de GE.**

PITFALLS & RISKS

- **Delivery:** The delivery may be the main pitfall associated with the product, and we have evaluated different alternatives:

  ✓ The use of gene therapy approaches (*i.e* lentiviral vectors) has different caveats:

  - Uncontrolled DNA integration ⟶ risk of mutations in host genome
  - Difficulty in the approval (ethical issues)
  - Uncontrolled mutations in the vector

  ✓ The use of nanoparticles and Permeable Peptides implies also some drawbacks:

  - Stability in blood of the nanoparticle/product
  - Selectivity and efficiency (half life) of the delivery
  - Biological availability of the peptide inside the target cell
  - Intracellular concentrations achieved

PARTNERING OPPORTUNITIES

- **Co-development**
- **Licensing**

We are open to discuss any other ways of collaboration!!!

THANK YOU!!

Prof. Juan Miguel Redondo
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