XI Encuentro de Cooperación Farma-Biotech

Development of Novel Anti-Calcineurin Drugs for the Treatment of Inflammatory and Autoimmune Diseases

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2. The Product
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   b) Innovative mechanisms of action
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THE INSTITUTION

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Instituto de Salud Carlos III

Fundaciónprocnic

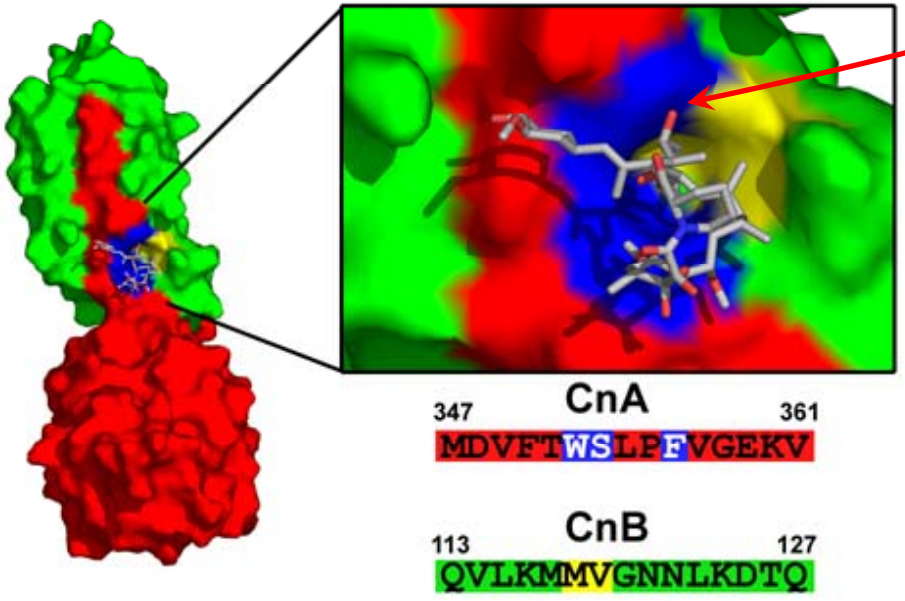
cnïc

THE PRODUCTS

1. The CN inhibitor LxVP peptide

2. Permeable CN Inhibitors Leads:

- CnInh5
- CnInh22
- CnInh14

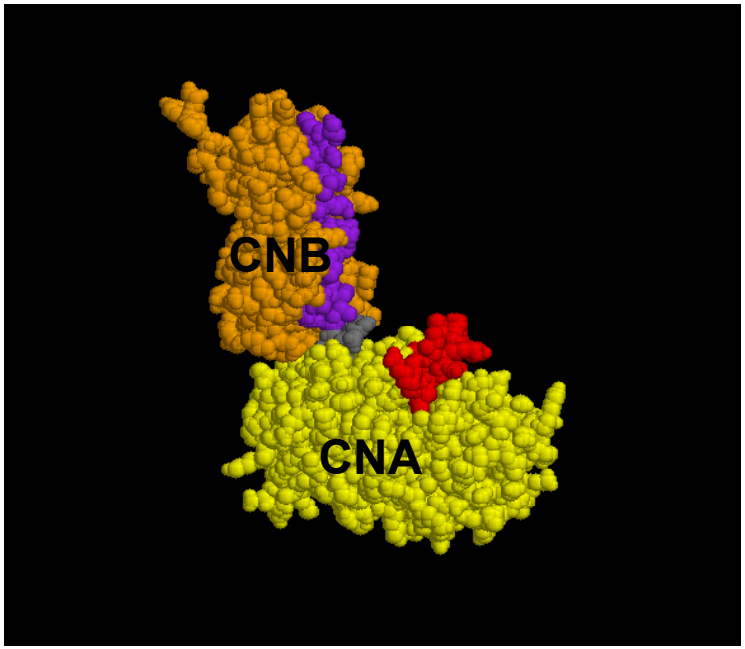


TARGET INDICATIONS

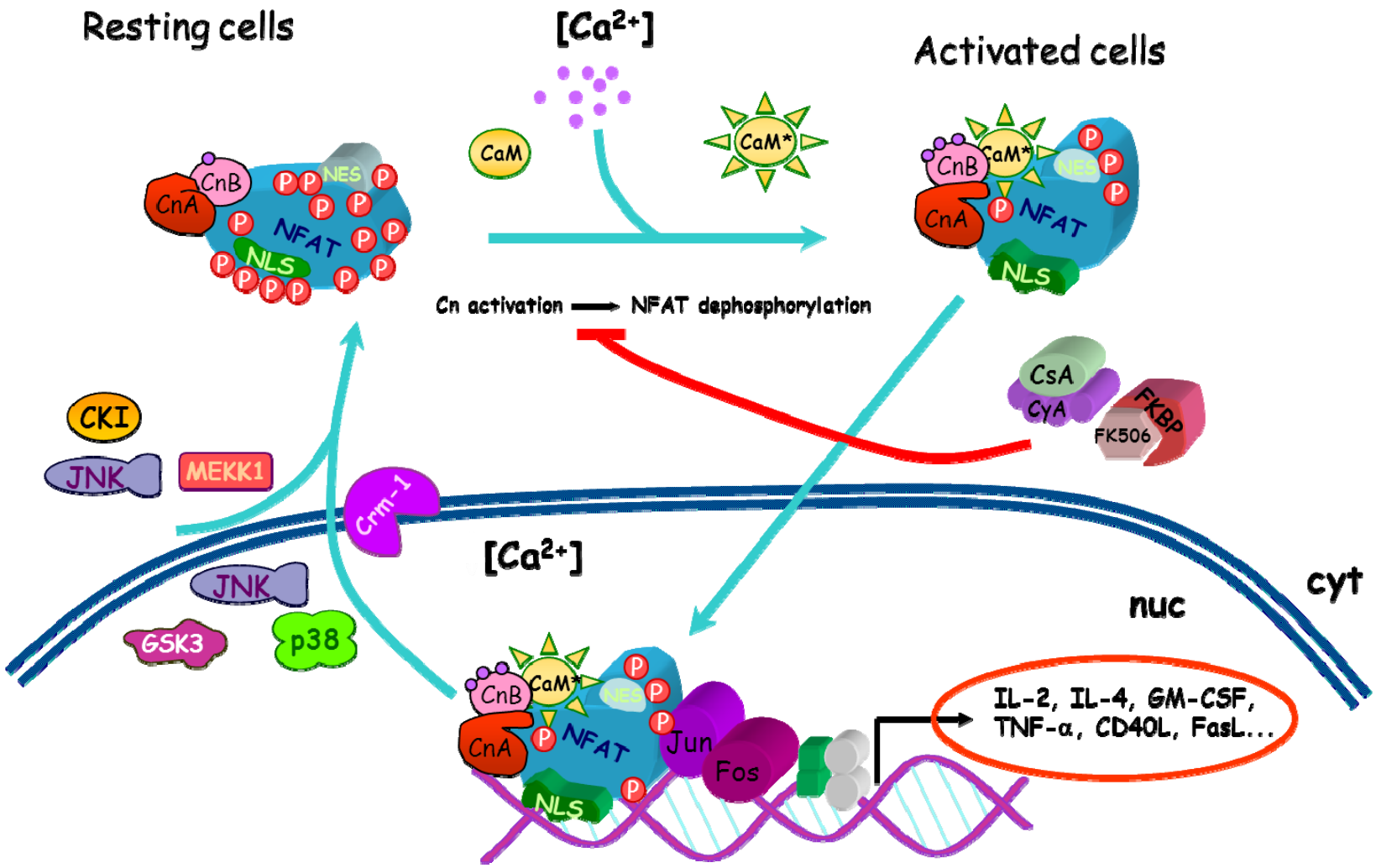
Calcineurin: Quick guide

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

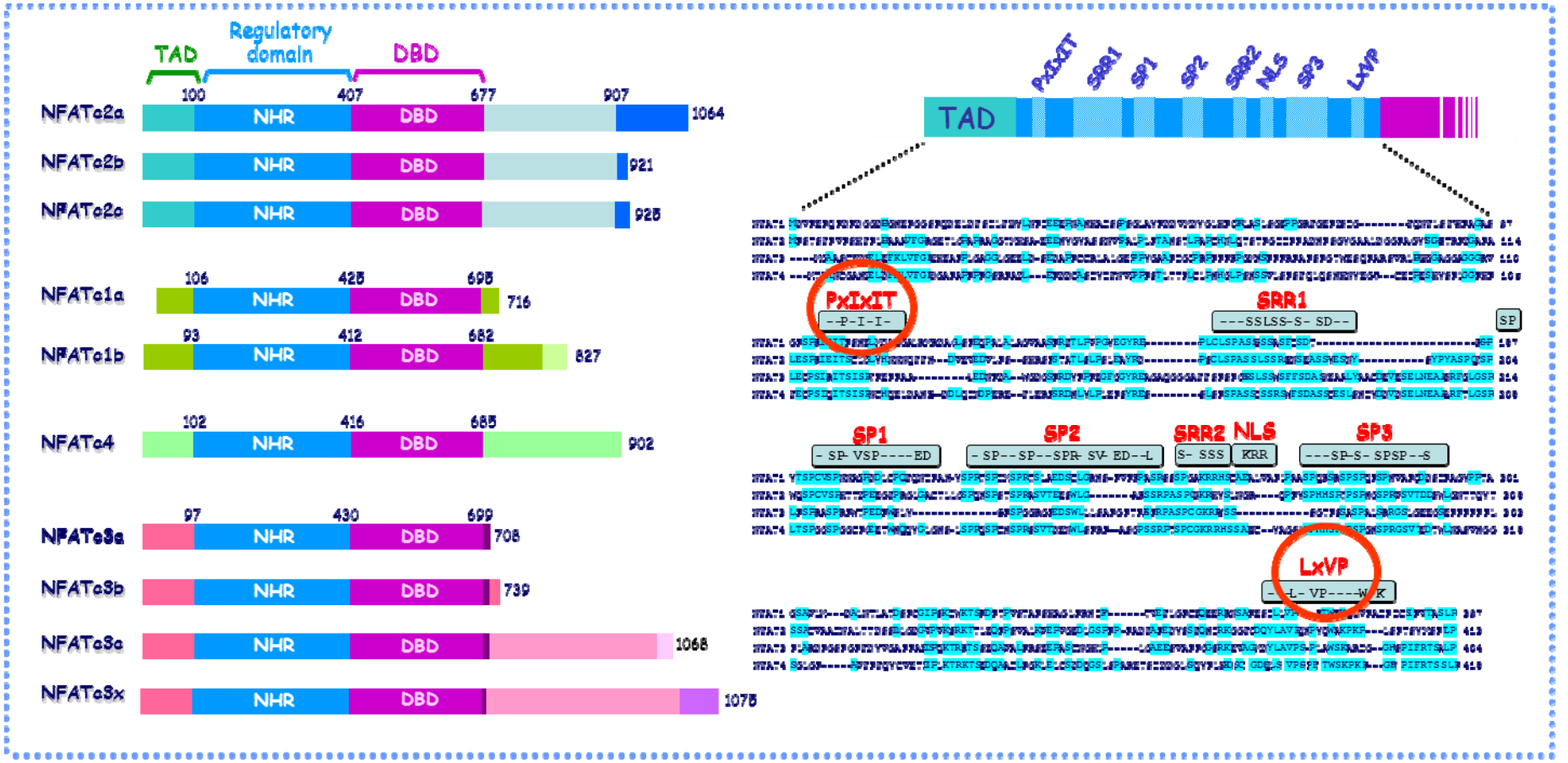
What does it look like?
Calcineurin is **a heterodimer** of an A catalytic subunit and a B subunit.



PATHWAY

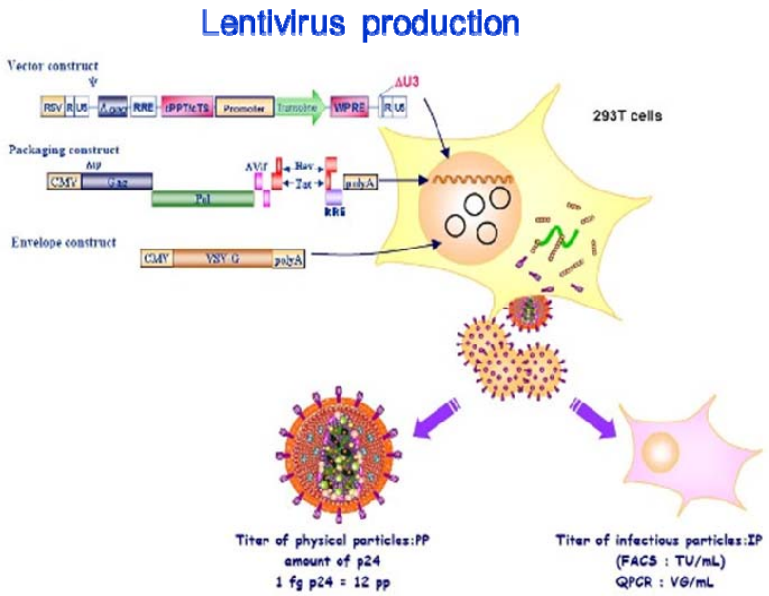

Resting cells
[Ca2+]
Activated cells
CaM
CaM*
CnB
CnA
NES
NFAT
NLS
P
Cn activation
NFAT dephosphorylation
CsA
CyA
FKBP
FK506
CKI
JNK
MEKK1
Crm-1
[Ca2+]
cyt
nuc
JNK
GSK3
p38
Jun
Fos
IL-2, IL-4, GM-CSF,
TNF-α, CD40L, FasL...

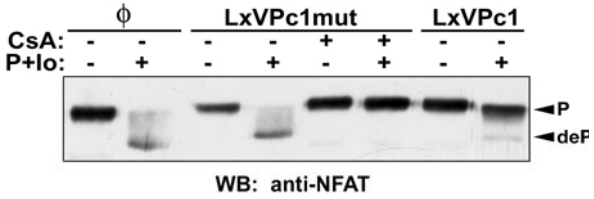
RATIONALE

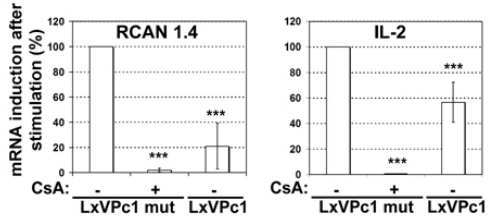


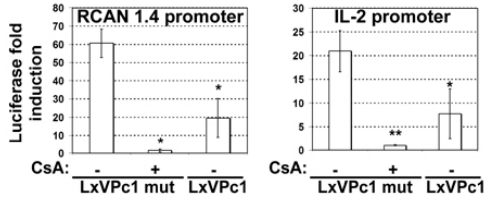
(Rodriguez *et al.* Mol. Cell, 2009)
(Martinez *et al.* PNAS 2009)
(Martinez *et al.* JBC 2006)

MECHANISM OF ACTION







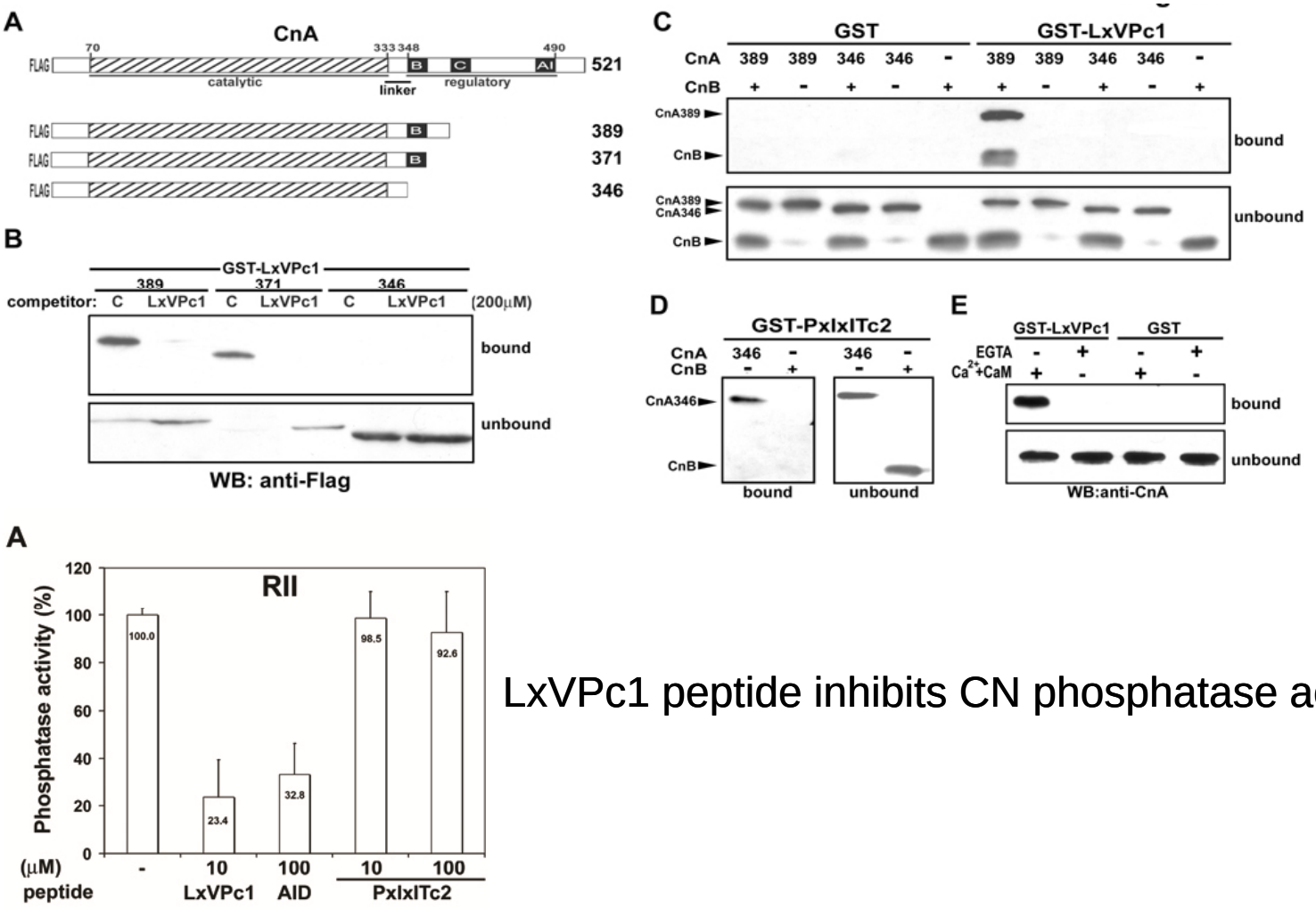


Cytokine network

	LxVPmut		LxVPmut+CsA		LxVPc1	
	Control	PIo	Control	PIo	Control	PIo
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

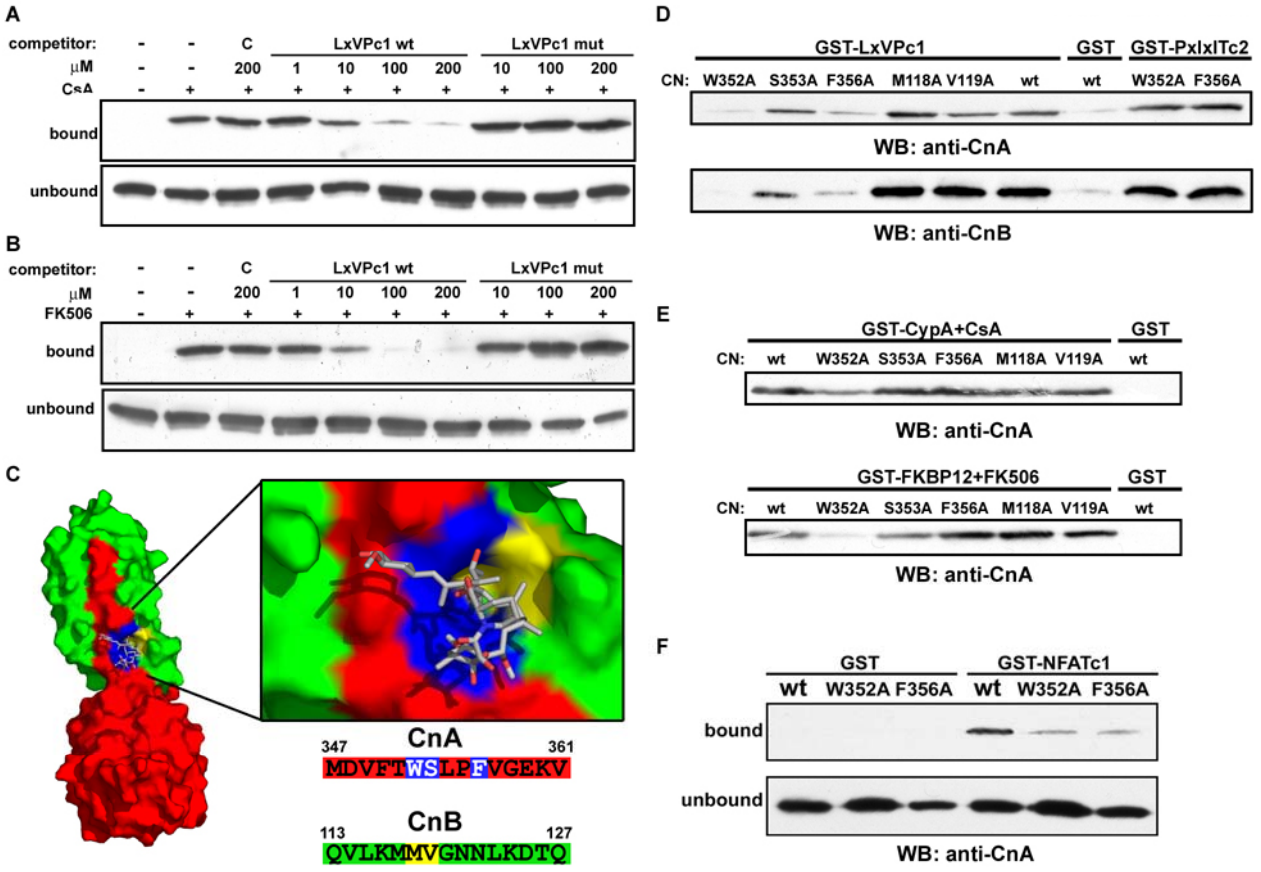
Rq values= mean + stdv (3 endogenous genes)

MECHANISM OF ACTION

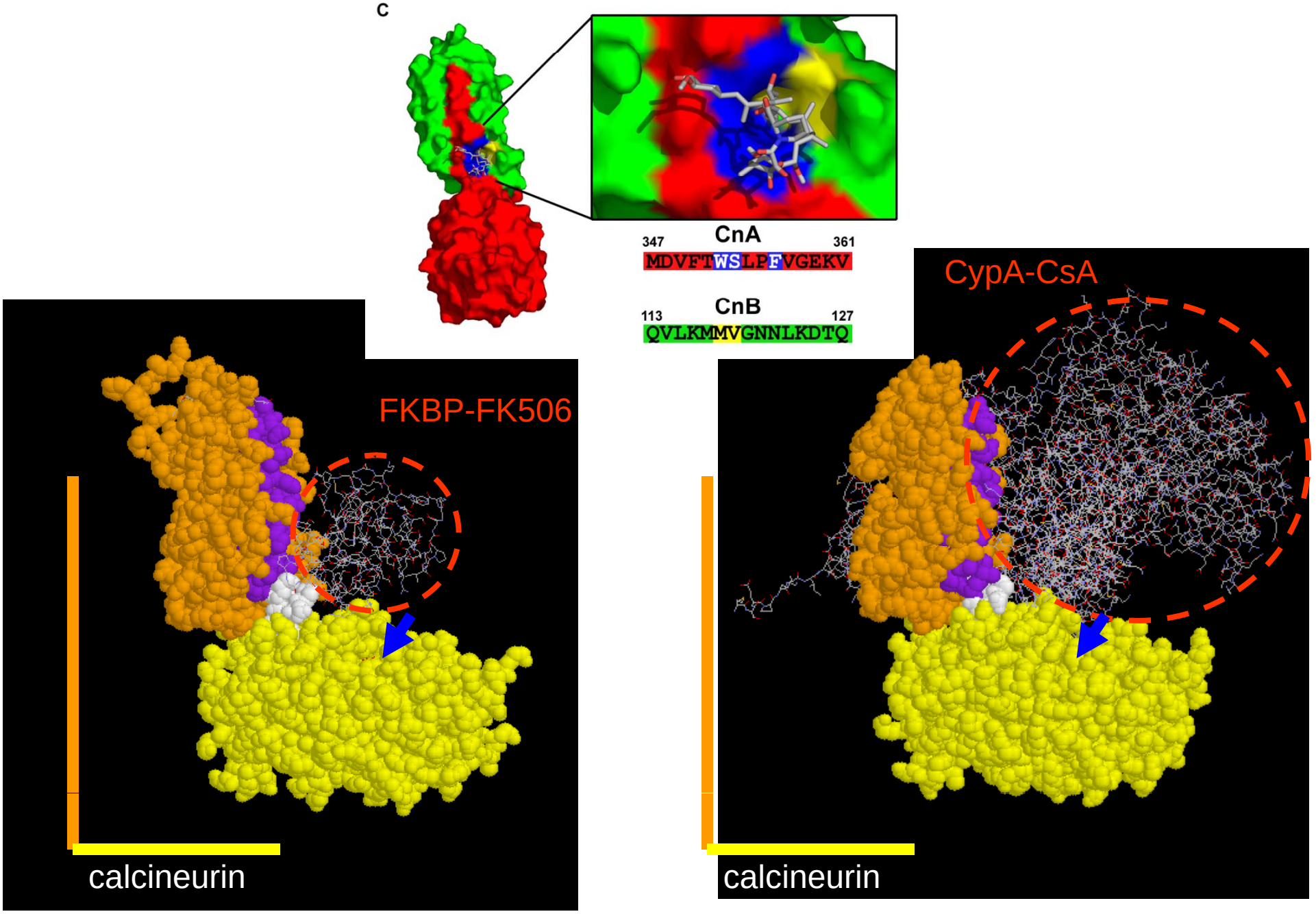


LxVPc1 peptide inhibits CN phosphatase activity

MECHANISM OF ACTION



Immunosuppressant-immunophilin complexes bound to calcineurin



DIFFERENTIAL FEATURES FACING THE MARKET

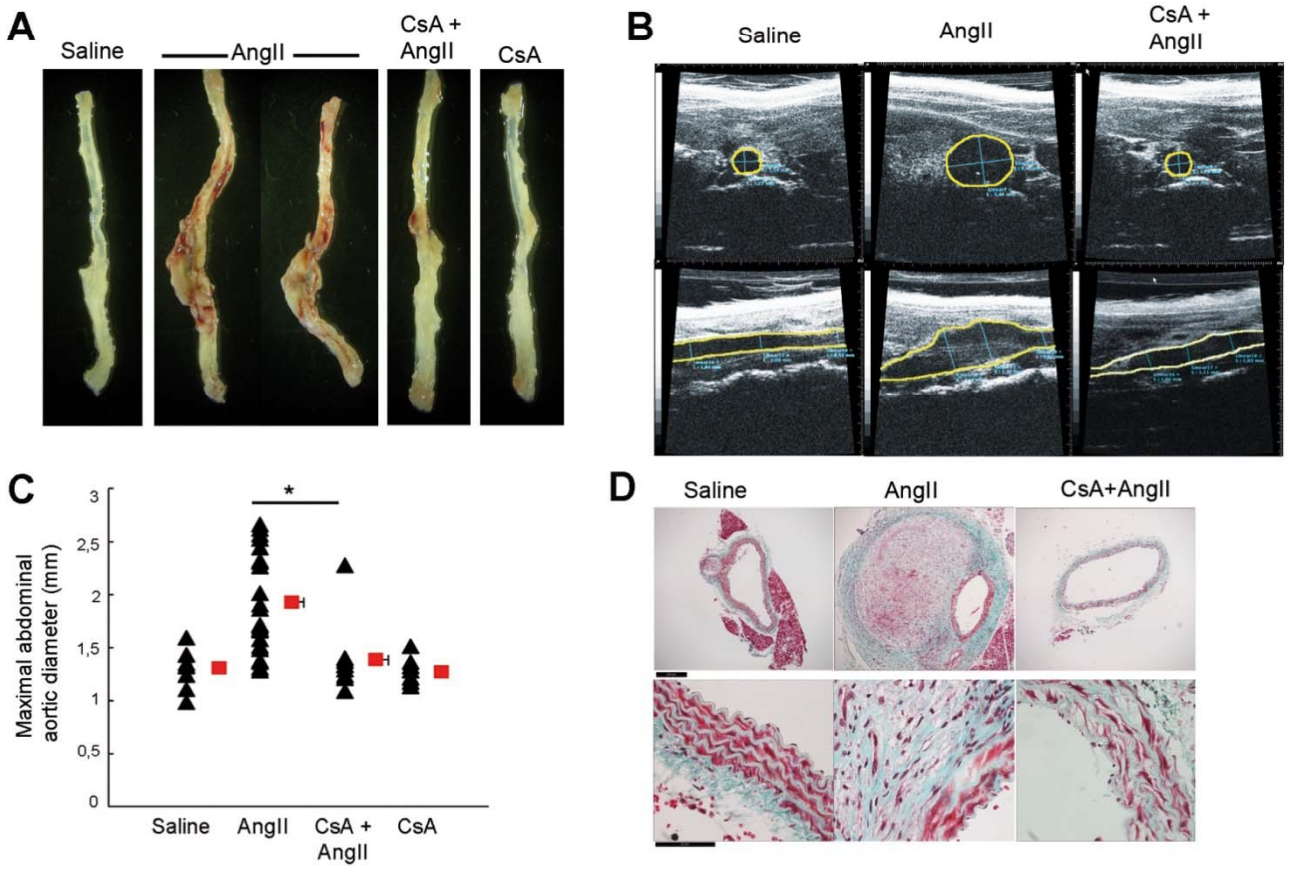
PEPTIDE

- **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**

PERMEABLE CN INHIBITORS

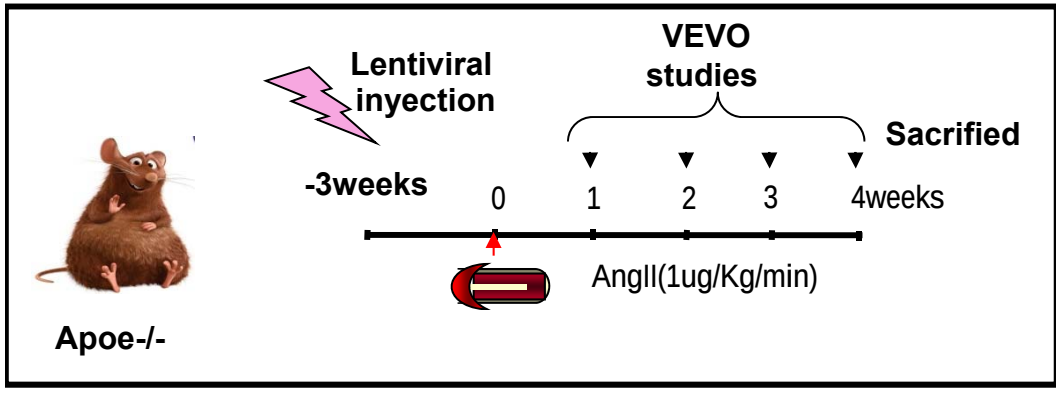
- We have identified a set of hits selected based on the mode of action of the LxVP peptide.
- As the peptide does, **these molecules inhibit the phosphate activity of CN and the NFAT-regulated transcription of reporter constructs**. These molecules are expected to be safer than those inhibitors currently used for transplantation, and with the potential to be efficacious new anti-inflammatory compounds
- Due to this specifity → **reduction of adverse effects**.

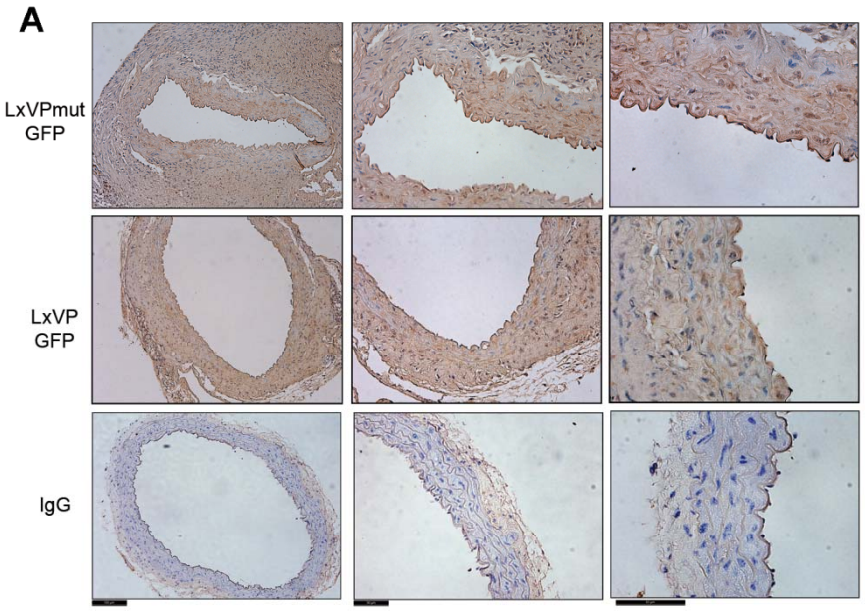
CURRENT STATUS OF DEVELOPMENT

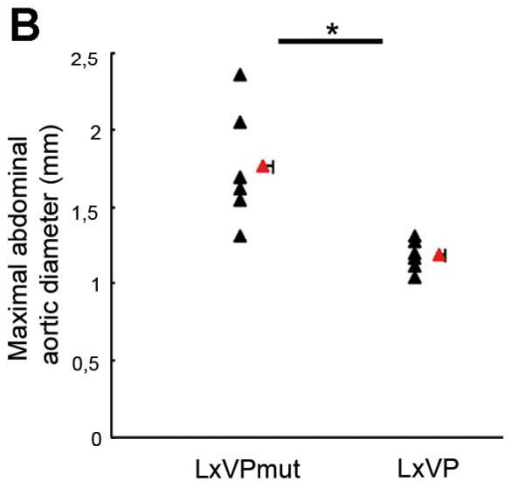


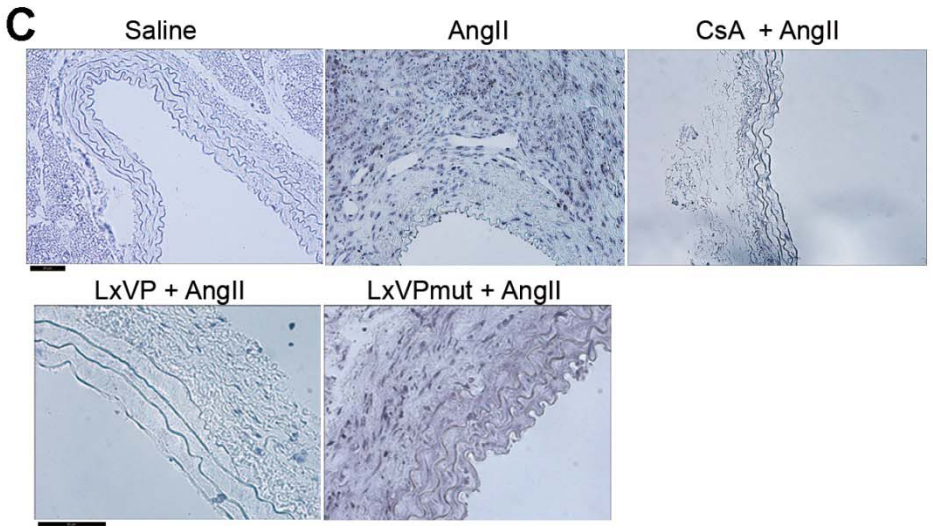
JEM

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



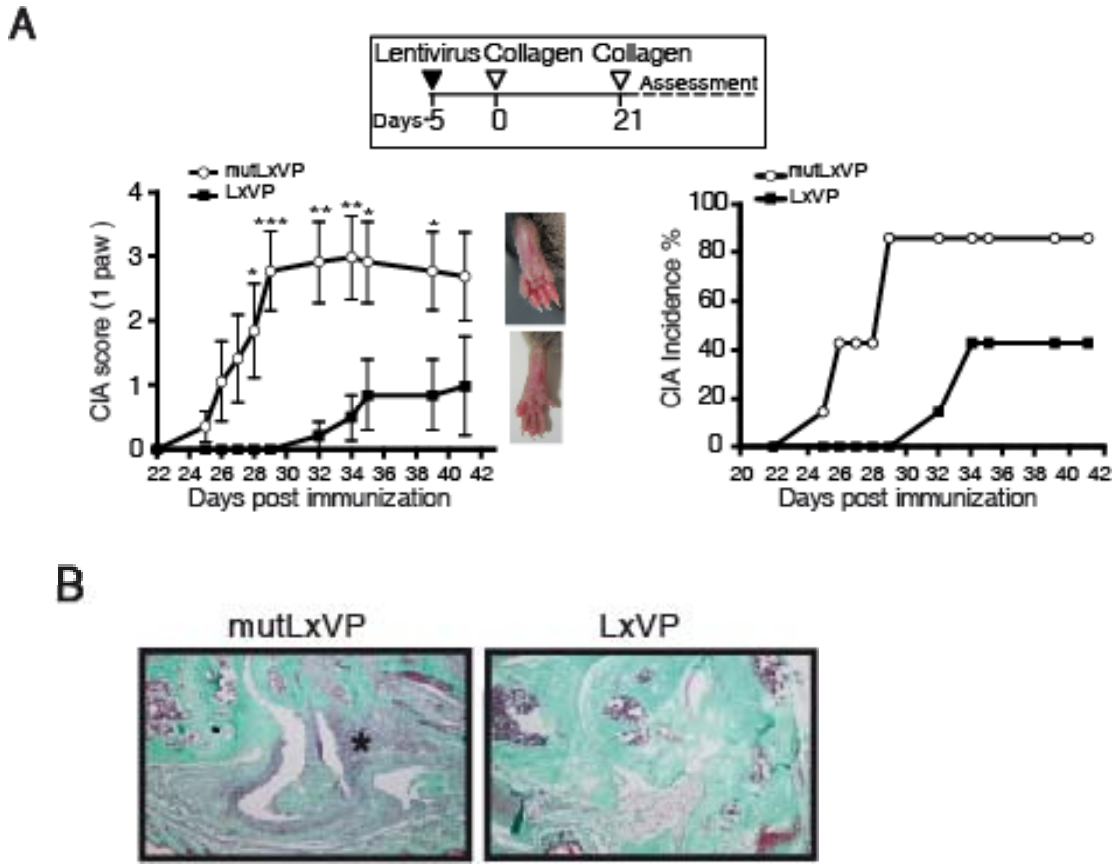






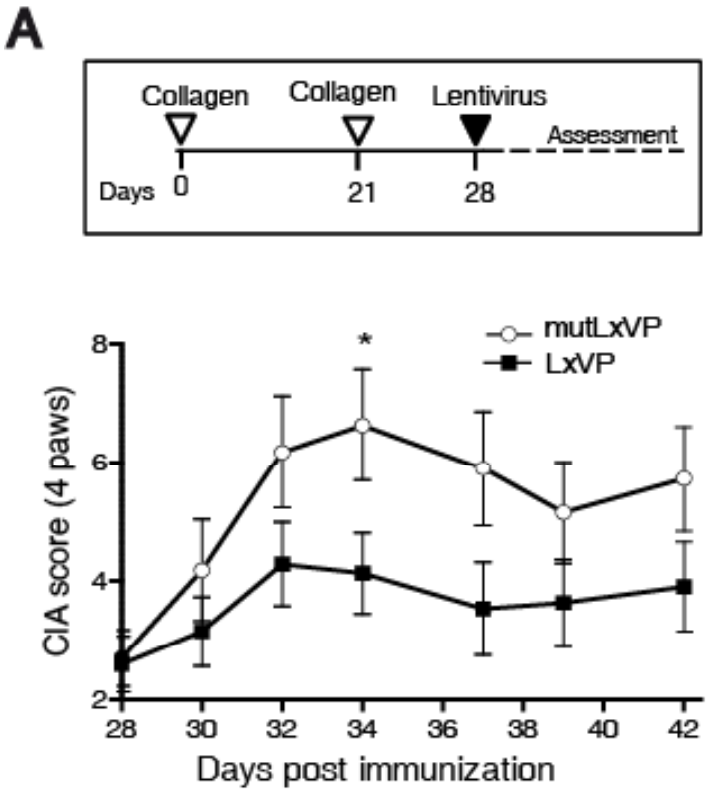
CURRENT STATUS OF DEVELOPMENT

LxVP peptide has prophylactic effects in Collagen-induced arthritis (CIA)



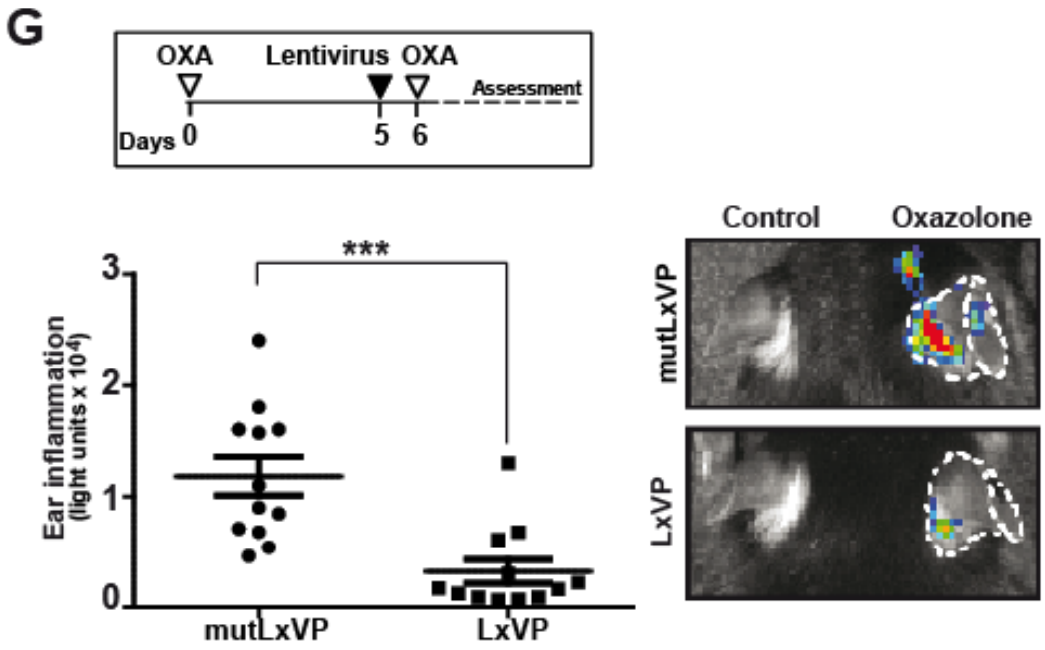
CURRENT STATUS OF DEVELOPMENT

LxVP peptide has therapeutic effects in CIA



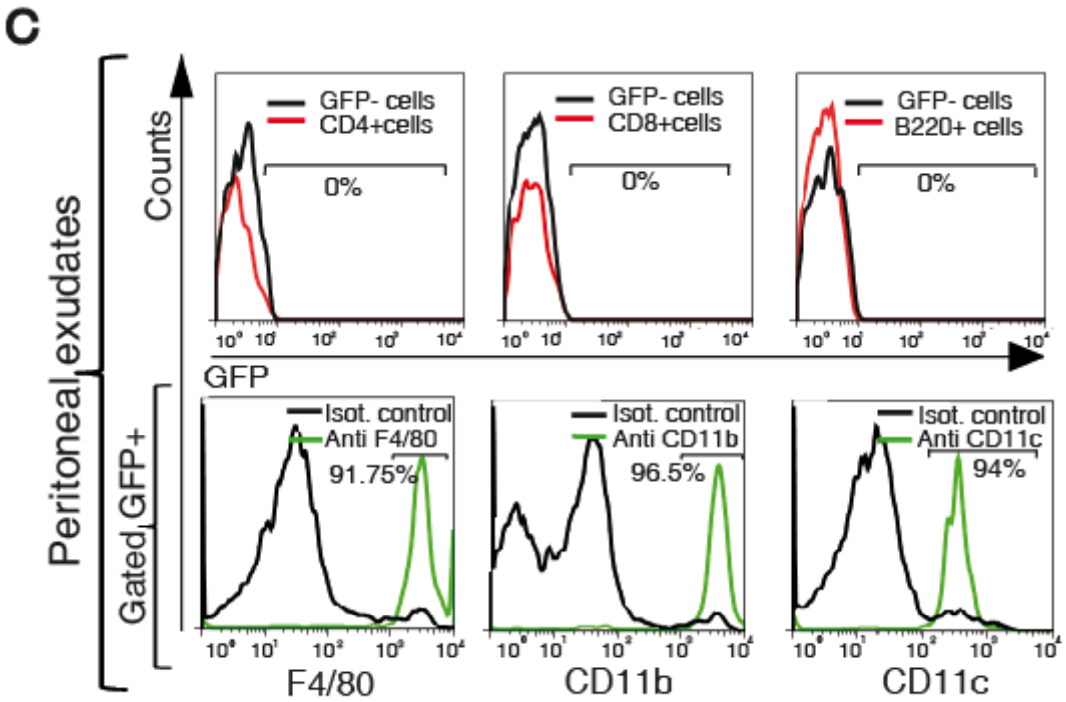
CURRENT STATUS OF DEVELOPMENT

LxVP peptide has beneficial effects in contact hypersensitivity



CURRENT STATUS OF DEVELOPMENT

Lentiviruses deliver the LxVP peptide exclusively to macrophages

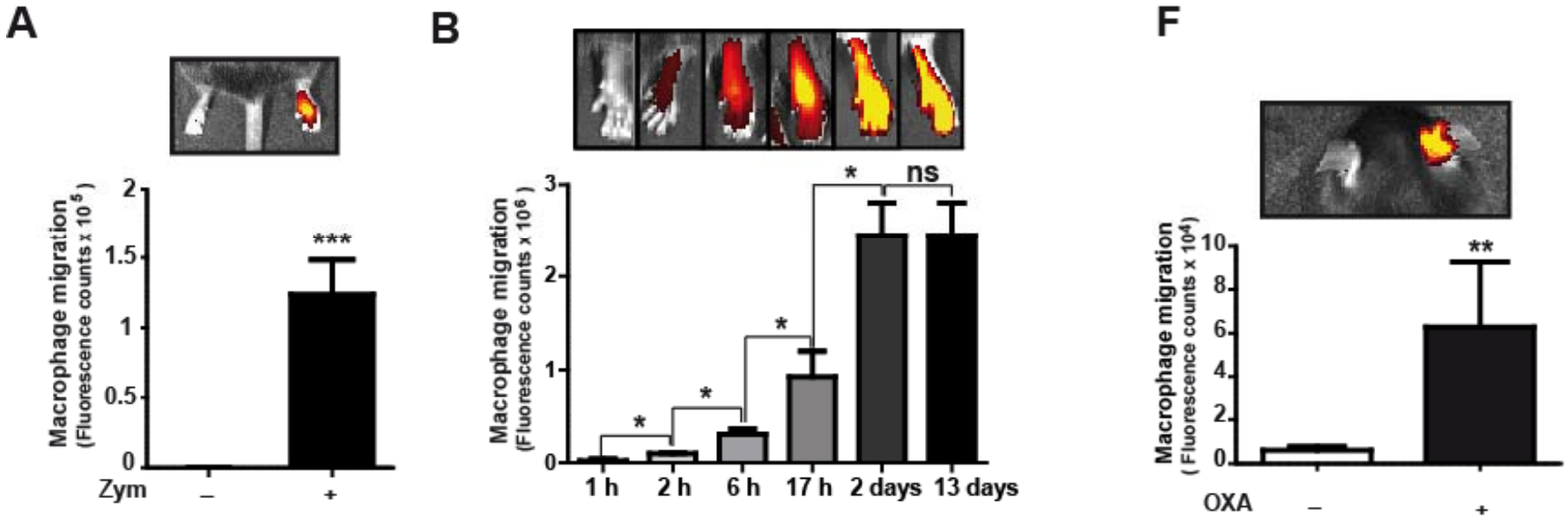


CURRENT STATUS OF DEVELOPMENT

Macrophages transduced with the LxVP peptide migrate specifically to inflammatory foci

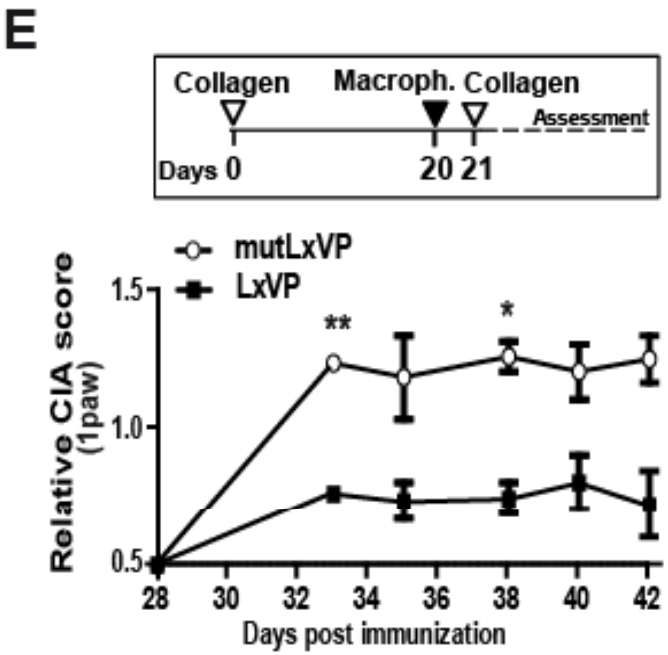
Specific migration to inflammed paws…

and inflammed ears



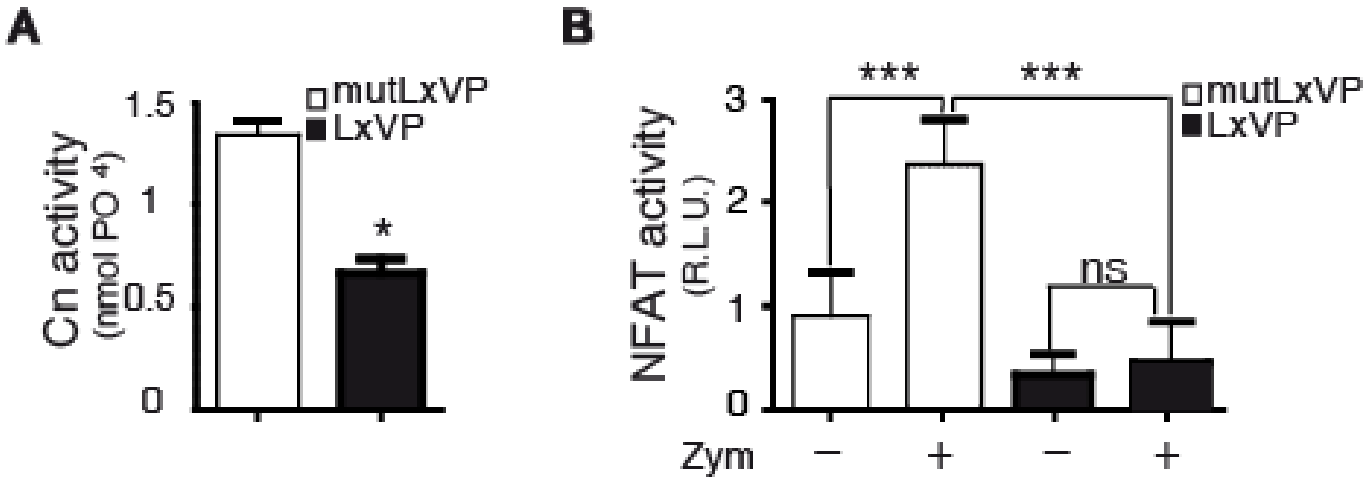
CURRENT STATUS OF DEVELOPMENT

Cell therapy: Macrophage transfer to arthritic paws exerts anti-inflammatory effects



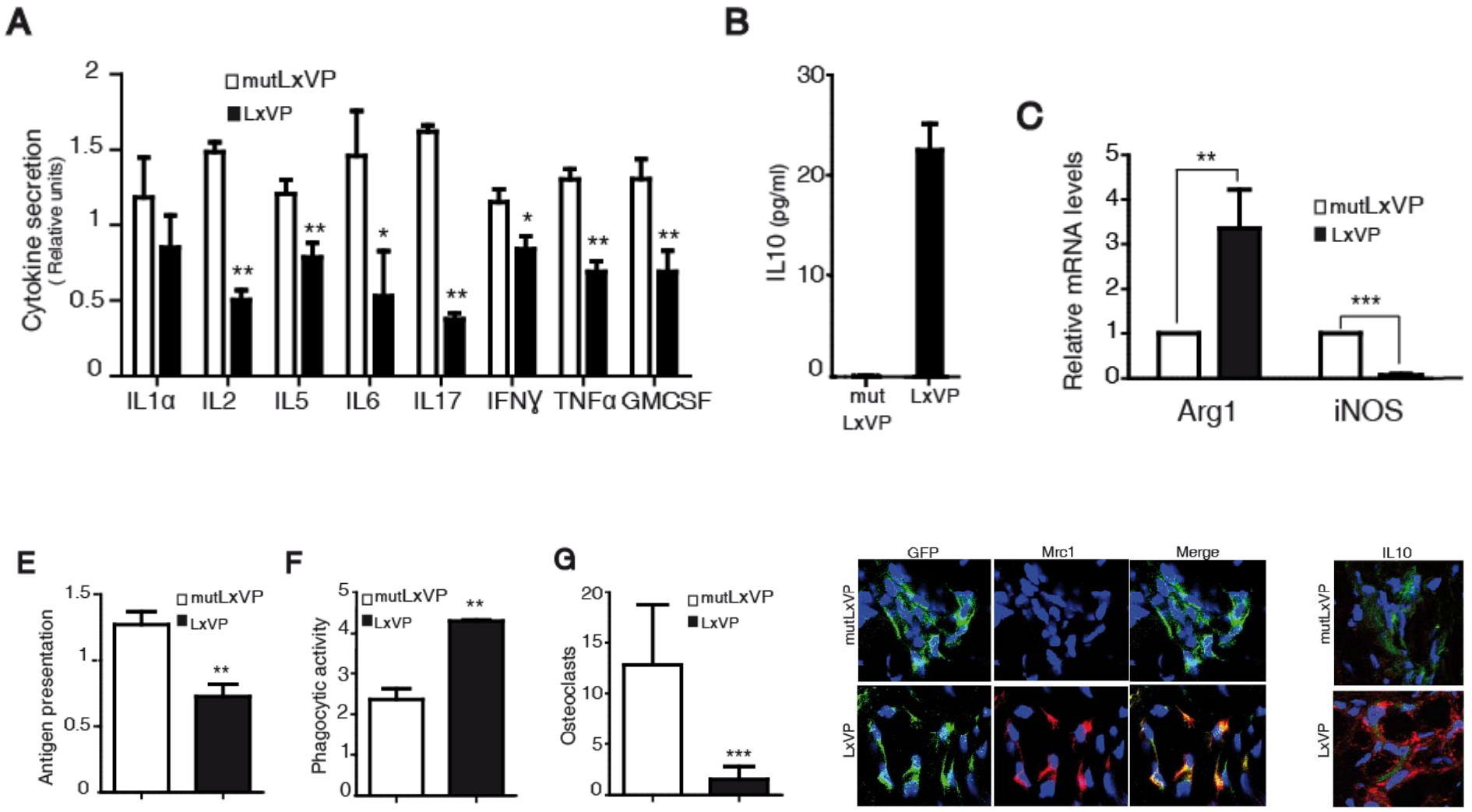
CURRENT STATUS OF DEVELOPMENT

The LxVP peptide abrogates CN/NFAT signalling



CURRENT STATUS OF DEVELOPMENT

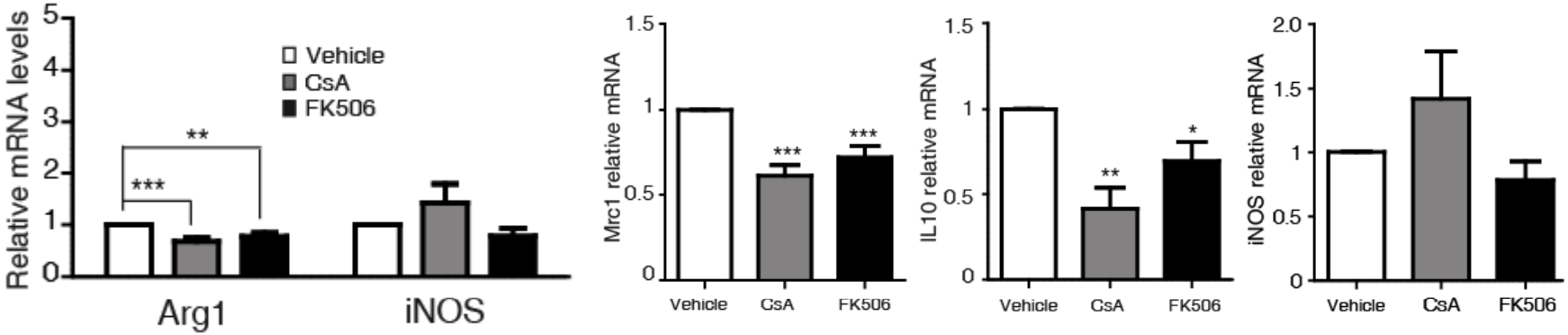
LxVP promotes M2-like macrophage polarization



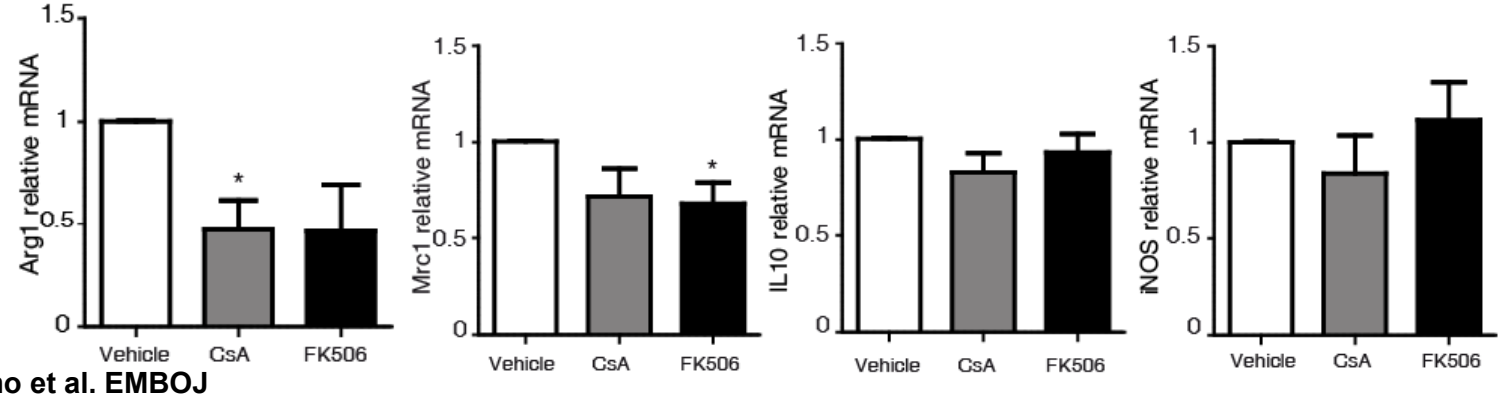
CURRENT STATUS OF DEVELOPMENT

Common immunosuppressants CsA and FK506 do not induce M2 polarization

Peritoneal macrophages

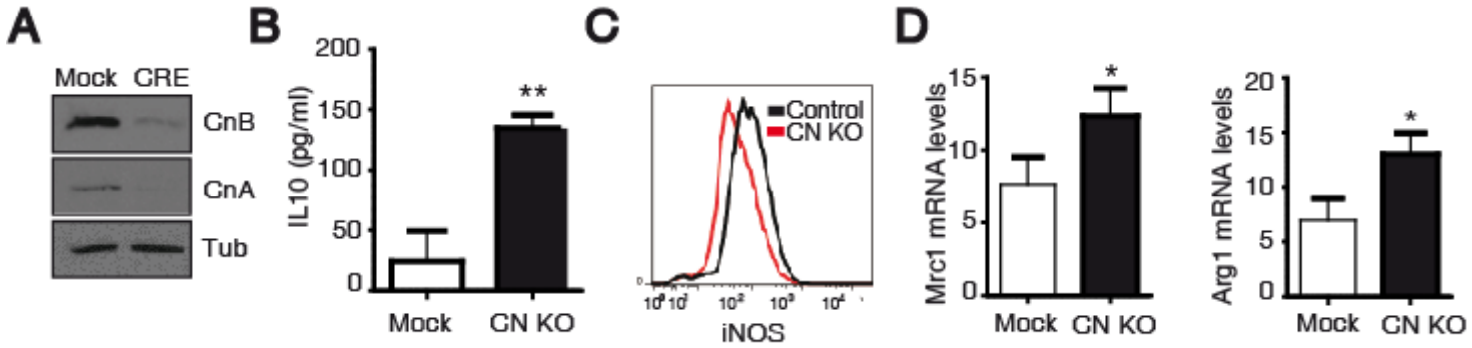


Bone marrow derived macrophages



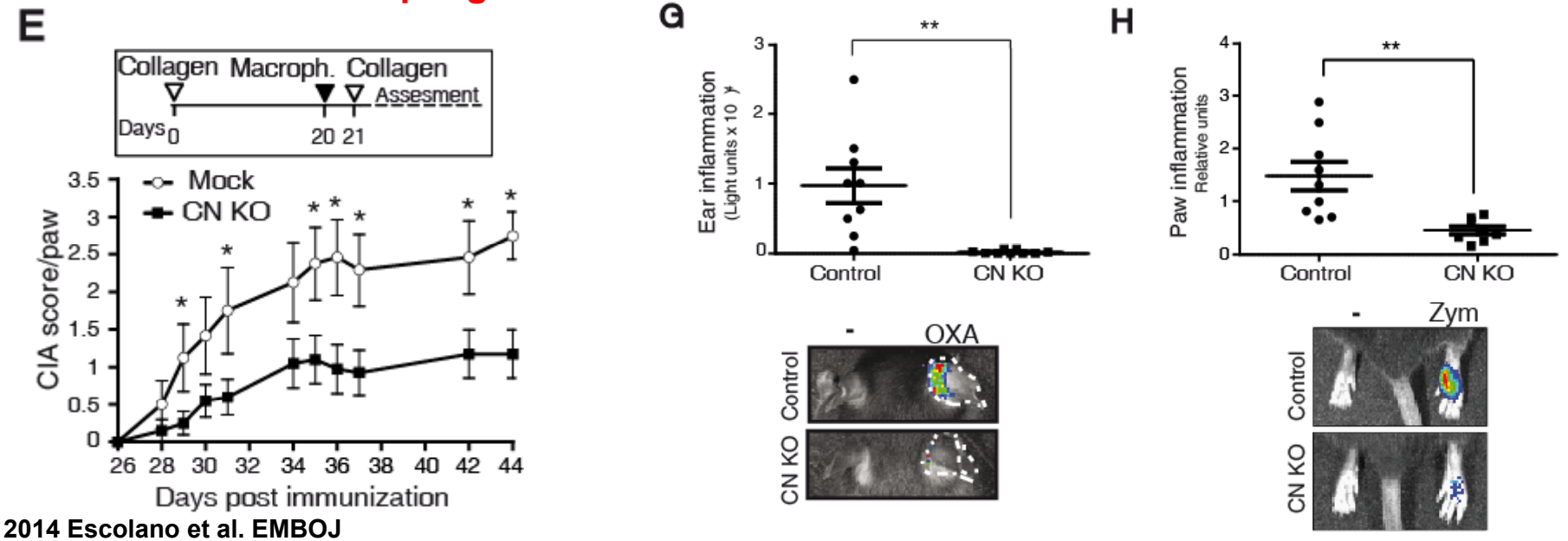
CURRENT STATUS OF DEVELOPMENT

Calcineurin deficiency in macropahes promotes M2-like polarization and confers resistance to inflammation



Cell therapy with CN deficient macrophages

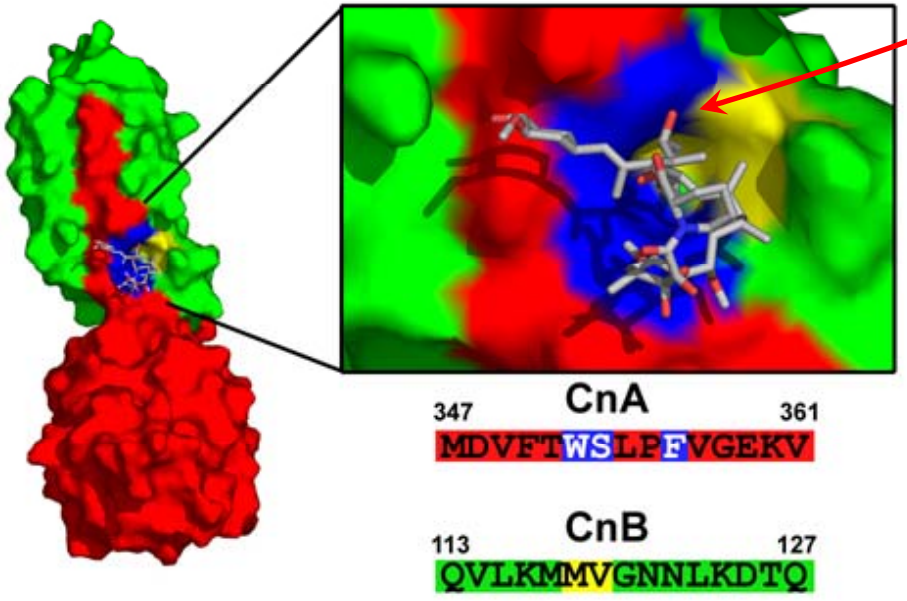
Mice deficient for CN in Mφ are resistant to inflammation



THE PRODUCTS

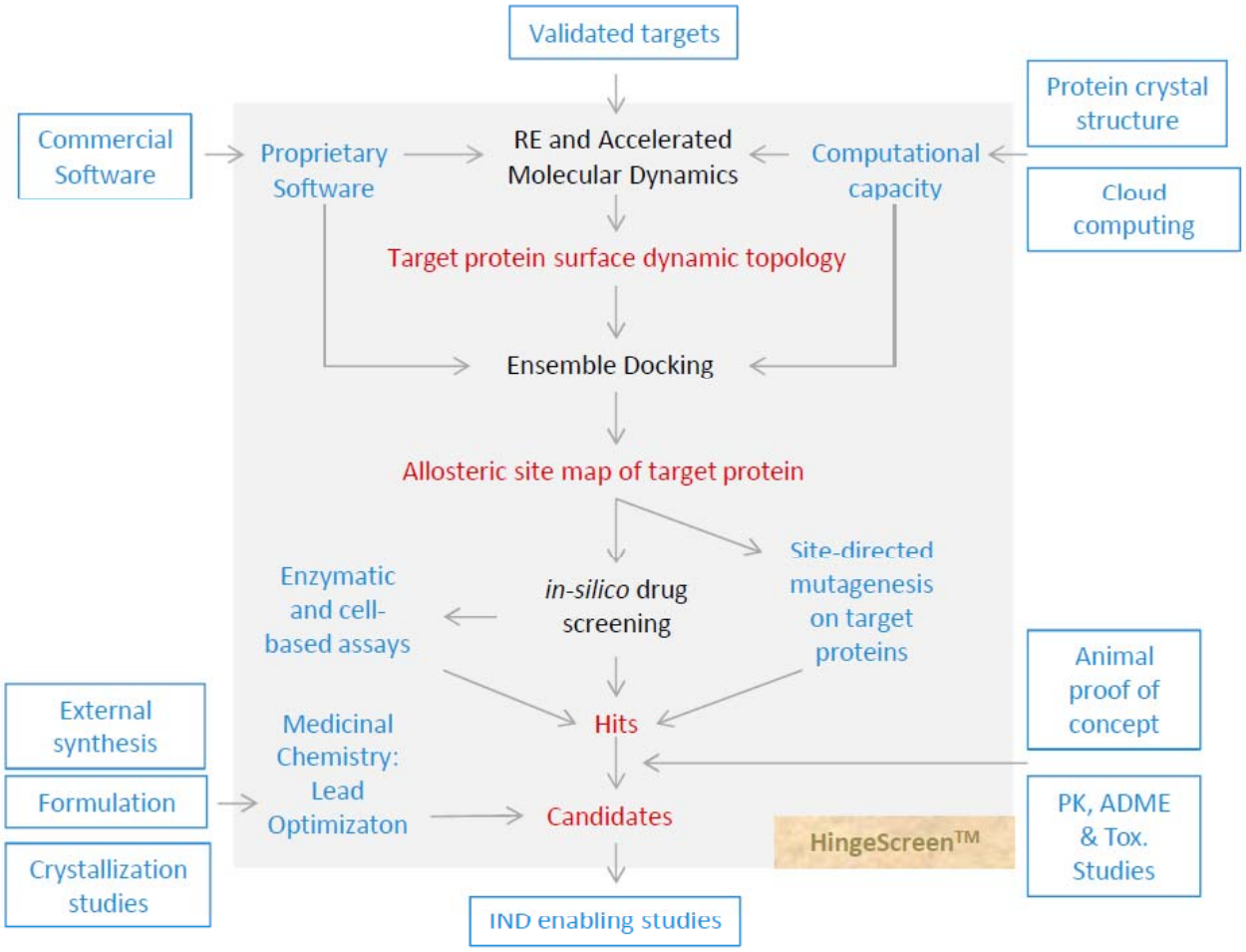
Permeable CN Inhibitors Leads:

- CnInh5
- CnInh22
- CnInh14

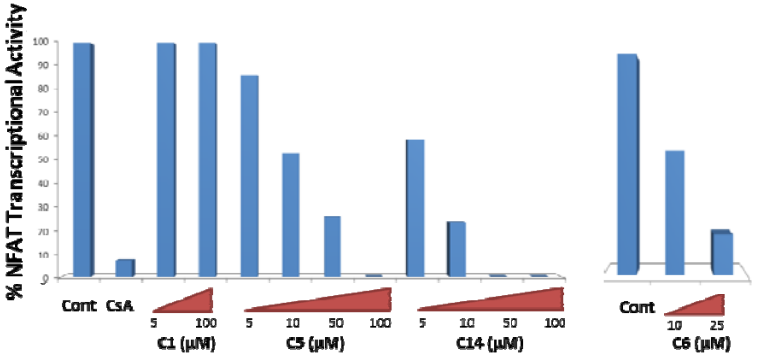


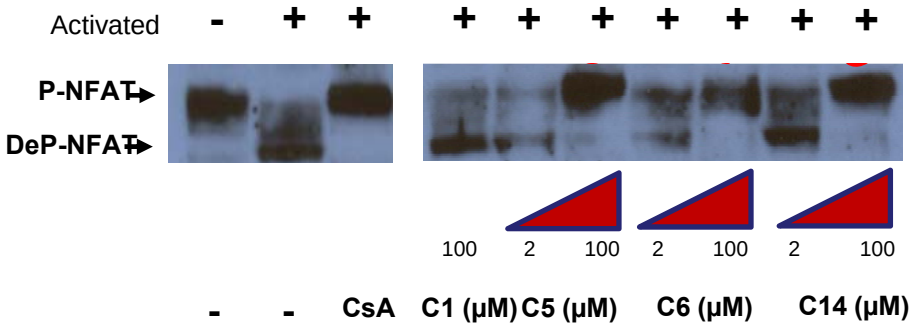
PARTNERING OPPORTUNITIES

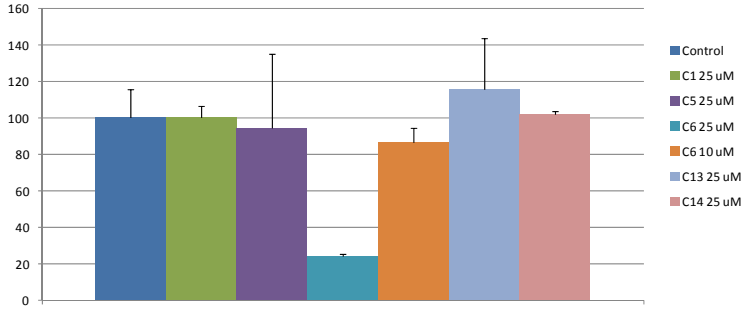
Allinky's technological platform: HINGESCREEN™



MECHANISM OF ACTION







Novel Drugs that interfere the CN-NFAT signaling pathway

The novel compounds C5, C6 and C14 (but not C1 control compound) inhibit both the transcriptional activity of a NFAT-luciferase reporter (upper panel) and the early dephosphorylation of NFAT (upper right panel) in Jurkat cells activated with Phorbol Ester + Calcium Ionophore. Except for the C6 compound, the drugs employed fail to affect the viability of these cells (bottom-right panel)

Selected CN Inhibitors

	Inhibition of NFAT dephosphorylation			Inhibition of NFAT transcriptional reporter activity				
	25 µM	50 µM	100µM	5 µM	10 µM	25 µM	50 µM	100 µM
C_001	nd	nd	-	-	nd	nd	nd	-
C_005	nd	nd	++++	+	++++	nd	++++	++++
C_015	-	+++	++++	-	nd	++	nd	+++
C_016	+++	++++	++++	nd	nd	-	nd	++++
C_017	nd	nd	+++	-	nd	+	nd	++
C_022	-	+++	++++	-	nd	++	nd	++++
C_023	nd	nd	+++	-	nd	++	nd	++++
C_024	nd	nd	+++	-	nd	++	nd	++
C_014	nd	nd	++++	+	++++	++++	++++	++++

Selected CN Inhibitors

	Inhibition of NFAT dephosphorylation			Inhibition of NFAT transcriptional reporter activity				
	25 µM	50 µM	100µM	5 µM	10 µM	25 µM	50 µM	100 µM
C_001	nd	nd	-	85%	nd	nd	nd	86%
C_005	nd	nd	++++	69%	15%	nd	6%	11%
C_015	-	+++	++++	97%	nd	56%	nd	27%
C_016	+++	++++	++++	nd	nd	91%	nd	0,4%
C_017	nd	nd	+++	79%	nd	68%	nd	51%
C_022	-	+++	++++	104%	nd	56%	nd	1,6%
C_023	nd	nd	+++	104%	nd	56%	nd	0,3%
C_024	nd	nd	+++	110%	nd	58%	nd	1,3%
C_014	nd	nd	++++	67%	3%	2%	0,3%	7%

DIFFERENTIAL FEATURES FACING THE MARKET

PEPTIDE

- **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**

PERMEABLE CN INHIBITORS

- We have identified a set of hits selected based on the mode of action of the LxVP peptide.
- As the peptide does, **these molecules inhibit the phosphate activity of CN and the NFAT-regulated transcription of reporter constructs**. These molecules are expected to be safer than those inhibitors currently used for transplantation, and with the potential to be efficacious new anti-inflammatory compounds
- Due to this specifity → **reduction of adverse effects**.
- Biochemical and transcriptional characterization of the selected hits has allowed the identification of **three lead compounds**.

CURRENT STATUS OF DEVELOPMENT

The proof of concept of these products has been developed based on the activity of the peptide.

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.
- The **permeable compounds** inhibit the CN activity and CN-dependent luciferase reporter expression in living cells and are expected to display similar anti-inflammatory properties that those exerted by the expressed LxVP in vivo (Escolano et al., EMBO J. 2014 May 16;33(10):1117-33)

In vivo:

- Cell and gene therapy approaches using the lentiviral-mediated expression of the LxVP peptide results in prophylactic and therapeutic inhibition of a number of inflammatory diseases, such rheumatoid arthritis and delayed type hypersensitivity.
- Strong evidences supporting the specificity of the peptide without affecting other pathways.
- The peptide modifies the cytokine expression profile in immune cells.

IPR PROTECTION

- “Selective peptides that inhibit the biological activity of Calcineurin”. EPO and USPTO applications pending.

- “LxVP-mediated calcineurin inhibition in macrophages”. PCT application (PCT application date: 18/10/2013).

PITFALLS & RISKS

- **Bioavailability of the compounds when delivered**
- **Possible off-target effects of the new drugs**
- **Those inherent to medical chemistry of lead optimization**

MARKET POTENTIAL

• Respiratory diseases and arthritis continue to be among the most common diseases and they are among the leading causes behind major chronic disability in the working population. In terms of expenditure the **market for inflammatory** diseases can be divided into thirds: one third of inflammatory healthcare spending is **on rheumatoid arthritis**; another third is on **asthma/COPD**; and the final third is on conditions such as **psoriasis, multiple sclerosis, inflammatory bowel disease, ankylosing spondylitis and many others**.

• The **global market for inflammatory therapeutics** was estimated to be **$57.8 billion** in 2010, representing a cumulative **annual growth rate of 7.6%** between 2002 and 2010. Experts forecast that the market will grow with a Compound Annual Growth Rate (CAGR) of 5.8% between 2010 and 2017, to record a sales value of $85.9 billion. While 73.1% of revenue was from the branded market, 26.9% was achieved from generic drugs in 2010.

• The patent expiry of some major drugs by 2017 is expected to make way for the entry of generic drugs, although this impact will be buffered by a number of strong pipeline molecules. Due to the presence of a strong pipeline portfolio for anti-inflammatory agents, the **branded share is forecast to increase to 78.6% in 2017**, while the generics market will decrease to 21.4%.

• **The R&D pipeline for anti-inflammatory therapeutics is strong**. Many of the major pharmaceutical companies such as Abbvie, Amgen Inc., Johnson & Johnson, GlaxoSmithKline, AstraZeneca, Merck, Pfizer, Eli Lilly, Boehringer Ingelheim and Sanofi are either entering or expanding into the market. They are collaborating with or acquiring small and medium-sized enterprise (SME) pharmaceutical companies that have promising anti-inflammatory drugs in their pipeline. Currently, **more than 1,000 molecules are in R&D, with 12% of them in Phase III, 36% in Phase II, 15% in Phase I, 29% in preclinical stage and 7% in the discovery phase**. This indicates that anti-inflammatory R&D will be very active for at least the next 7 to 8 years. The main factor that is driving the big pharmaceutical companies towards the inflammatory therapeutics market is the large patient base.

MARKET POTENTIAL

Indication	PoC	Market	Market Competition
Rheumatoid arthritis	✓	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	In progress	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

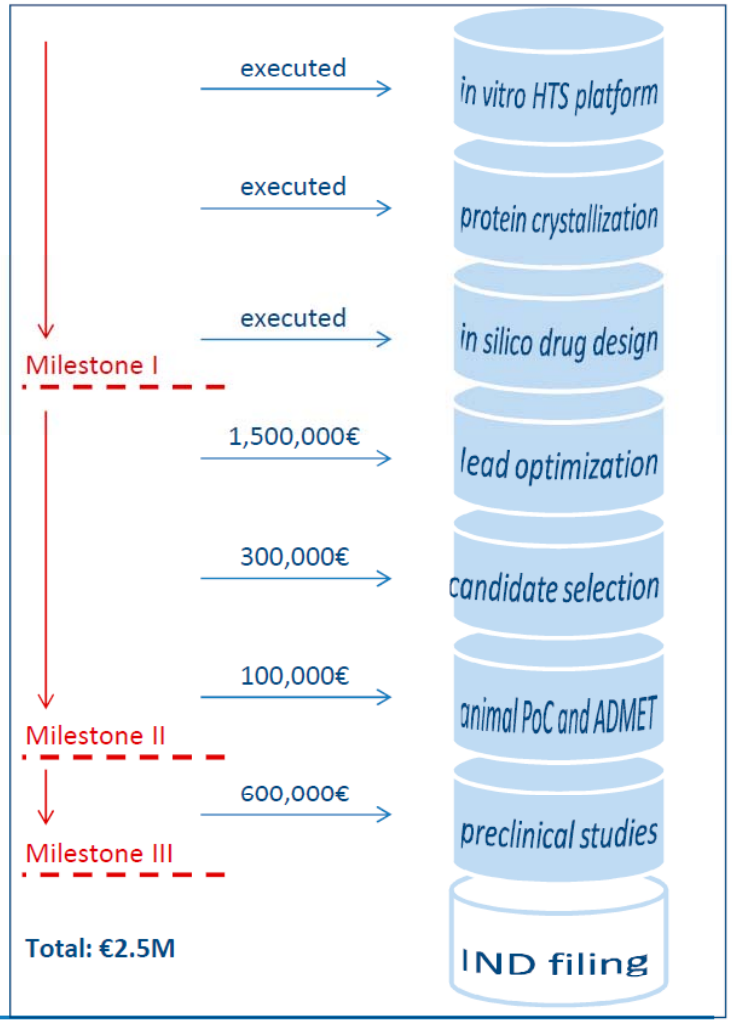
* (DTH and other diseases under currently under analisys)

PARTNERING OPPORTUNITIES

Finance

- Project funding needed up to IND filing is €2.5M
- Milestone-based project financing
- €0.5M in cash committed by Allinky if a pharma partner gets involved
- €2M additional funding is needed
- In kind funding might be eligible
- Know-how from CNIC valued €1.5M
- Know-how from Allinky valued €0.5M

The CNIC and Allinky are actively searching for a pharmaceutical partner to co-develop and/or invest in the discovery and development of new drugs for inflammatory and auto-immune diseases.



cnic Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III

Allinky BIOPHARMA