

# Inmunobiología linfocitaria

i+12

Instituto de Investigación  
Hospital 12 de Octubre



Reunión de grupos del **área 6** para fomentar la colaboración intra-área e intra-instituto

**Enfermedades inflamatorias y trastornos inmunitarios**

José R. Regueiro, 30 Marzo 2017, 20 min

# Áreas de investigación i+12

1. Cáncer
2. Enfermedades crónicas y patologías sistémicas
3. Enfermedades raras y de base genética
4. Epidemiología y evaluación de las tecnologías y servicios sanitarios
5. Trasplantes, ingeniería de tejidos y medicina regenerativa
6. Enfermedades inflamatorias y trastornos inmunitarios
7. Enfermedades infecciosas y SIDA
8. Neurociencias y salud mental

# ÁREA 6 - ENFERMEDADES INFLAMATORIAS Y TRASTORNOS INMUNITARIOS

Asma y Enfermedades Inmunoalérgicas (H12O)

Bases Moleculares y Celulares en Enfermedades  
Reumáticas (UCM)

Enfermedades Inflamatorias y Autoinmunes  
(H12O)

**Inmunobiología Linfocitaria (UCM)**

Inmunodeficiencias e Inmunología del  
Trasplante (H12O)

# Past

- Established 2000, recognized 2004 by UCM
- External evaluation in 2005, 2007 and 2008 by ANEP with the highest scores/funds among immunology groups at the UCM.
- Last internal evaluation by UCM (2014):
  - First of 8 immunology teams at UCM in funds
  - Fifth of 32 groups at the UCM School of Medicine.
- Joined in 2011 the Hospital 12 de Octubre Health Research Institute

# Present: research personnel as of 2017

- Permanent staff: 7 (3 RyC)
- Postdocs (RyC, JdIC...): 6 (4 RyC)
- PhD students: 13
- **Total:** 26

# Current funding: 1.351 K€

MINECO >> ISCIII, CDTI, FECYT

Agency	Active	Principal Investigator	Reference	Short title	Funds (€)
ISCIII	2017	Martínez-Naves, Eduardo	PI13/00218	Unconventional lymphocytes in intestinal inflammation	102.245
CDTI	2017	Reche, Pedro A	Art 83 4155463	Tolerance to mites and grass pollen with new vaccines	76.600
MINECO	2017	Regueiro/Fdez.-Malavé	SAF2014-54708-R	Intra and extracellular T cell activation physiopathology	332.750
MINECO	2017	Goicoechea de Jorge, Elena	SAF2014-52339-P	Role of microRNAs in complement nephropathy	157.300
MINECO	2017	Reche/Palomares	IPT2012-063909000	Desarrollo de fármacos inmunoestimulantes bacterianos	62.500
MINECO	2017	Reche, Pedro A	BIO2014-54164-R	Vaccine epitope prediction: application to rinovirus	108.900
FECYT	2018	Corell, Alfredo	11509	Inmunomedia 4.0	8.000
MINECO	2018	Gómez del Moral M	RTC-2015-3805-1	New antitumoral vaccine based on Ca10	117.140
MINECO	2019	Cárdenas, Paula P	IJCI-2014-19262	Lymphocyte Immunobiology	6.000
MINECO	2019	Rodríguez de Córdoba S	SAF2016-81876REDT	Complement excellence network	20.000
MINECO	2020	Cubero/Martínez-Naves	SAF2016-78711-R	Role of JNK and ER stress in gut-liver alcoholic inflammation	193.600
MINECO	2020	Goicoechea de Jorge, Elena	RYC-2013-13395	Complement physiopathology	40.000
MINECO	2020	Pedro Roda-Navarro	SAF2016-75656-P	Dual specificity phosphatases in T cells	80.000
MINECO	2020	Tortajada Alonso, Agustín	IJCI-2015-25222	Lymphocyte Immunobiology	6.000
MINECO	2021	Cubero, F Javier	RYC-2014-15242	Compound function of Jnk1 and Jnk2 in liver inflammation	40.000
					<b>1.351.035</b>

# Q1 papers IF>5 > Jan 2011

Authors	Reference > 2011	IF>5
Mohs, A. et al	J Hepatol. 2017 Apr;66(4):743-753.	11
Marin AV, et al.	J Allergy Clin Immunol.2017 Jan;139 (1):347-49	12
Kuttkat N, et al	Gut 2016 (gutjnl-2015-31119)	14
Ramírez-Muñoz	Front Immunol. 2016 Feb 19;7:59	6
Alcover et al.	Front Immunol. 2016 Dec 21;7:632	6
Muñoz-Ruiz M,	Nat Immunol 2016 Jun; 17, 721–727.	20
Recalde S et al	J Am Soc Nephrol. 2016 May;27(5):1305-11	9
Valés-Gómez M	J Allergy Clin Immunol. 2016 Mar;137(3):942-5.	12
Cubero FJ, et al	Gastroenterology 2016 Apr;150(4):968-81	17
Józsi M, et al.	Trends Immunol. 2015 Jun;36(6):374-84	10
Cubero FJ, et al	J Hepatol. 2015 Jan;62(1):140-9.	11
Garcillán B, et al	Front Immunol. 2015 Jan 29;6:20.	6
Valoti E. et al.	J Am Soc Nephrol. 2015 Jan;26(1):209-19	9
Torres JM, et al	J Clin Invest. 2014 Dec 1;124(12):5239-48.	14
Dopfer EP, et al	Cell Rep. 2014 Jun 12;7(5):1704-15.	7
Garcillán B, et al	J Allergy Clin Immunol. 2014 Apr;133:1205-8.	11
Pickering MC,	Kidney Int. 2013 Dec;84(6):1079-89.	9
Adolph TE, et al	Nature. 2013 Nov 14;503(7475):272-6.	42

Authors	Reference > 2011	IF>5
Cubero FJ, et al.	Cell Death Differ. 2013 Nov;20(11):1580-92.	8
Alcorlo M et al.	Proc Natl Acad Sci USA. 2013 Aug 13;110(33):13504-9	10
Medraño-Fernandez I,	Cell Mol Life Sci. 2013 Jul;70(13):2395-410.	6
Iborra S, et al.	J Exp Med. 2013 Jul 1;210(7):1463-79.	14
Tortajada et al.	J Clin Invest. 2013 Jun;123(6):2434-46	13
Abós-Gracia B, et al.	J Allergy Clin Immunol. 2013 May;131(5):1393-9.	11
Goicoechea de Jorge E	Proc Natl Acad Sci USA. 2013 Mar 19;110:4685-90.	10
Gilsanz A, et al.	Cell Mol Life Sci. 2013 Feb;70(3):475-93.	6
Malik TH, et al.	J Am Soc Nephrol. 2012 Jul;23(7):1155-60.	10
Vernon KA, et al.	Am J Kidney Dis. 2012 Jul;60(1):121-5.	6
Johnson S, et al.	PLoS Pathog. 2012;8(10):e1002981.	8
Tortajada et al.	Kidney Int. 2012 Jan;81(1):56-63. doi	9
Marcus N, et al.	J Allergy Clin Immunol. 2011 Nov;128(5):1050-7	11
Gil J, et al.	J Clin Invest. 2011 Oct;121(10):3872-6.	13
Cardenas PP, et al	Nucleic Acids Res. 2011 Nov;39(21):9250-61.	8
Cubero FJ, et al.	Hepatology. 2011 Oct;54(4):1470-2	12
Gutiérrez-López MD,	Cell Mol Life Sci. 2011 Oct;68(19):3275-92.	7
Iborra S, et al.	Blood. 2011 May 12;117(19):5102-11.	10

# Models and techniques

- Human samples: immunodeficiencies, renal disorders
- Animal models: immunodeficiencies, liver inflammation, infection (malaria)
- Biochemistry: fluorescence correlation spectroscopy, DNA repair analysis, complement activation
- Cell biology: confocal microscopy, flow cytometry, gamma irradiator, lymphocyte immortalization
- Molecular biology: vectors, cell transfection, sequencing, CRISPR/Cas9, copy number variation, miRNA
- Bioinformatics: T/B cell epitope prediction



# Research training programs

- Aneca-certified postgraduate programs
  - Master's degree in Immunology since 2010
  - PhD program in Biomedical Research >2013
- Weekly Department meetings

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

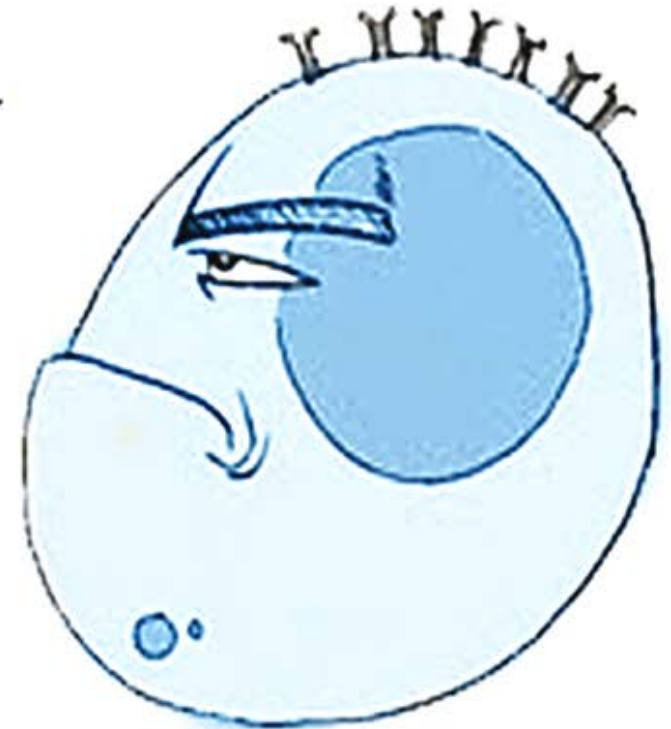


Antigen-presenting cells  
and inflammation



Epitomics

Complement  
physiopathology



T cell physiopathology  
and DNA repair

# i+12 connections

Transl. biophysics  
(F Monroy), Area 1

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling



Gastroenterology  
(P Martínez)

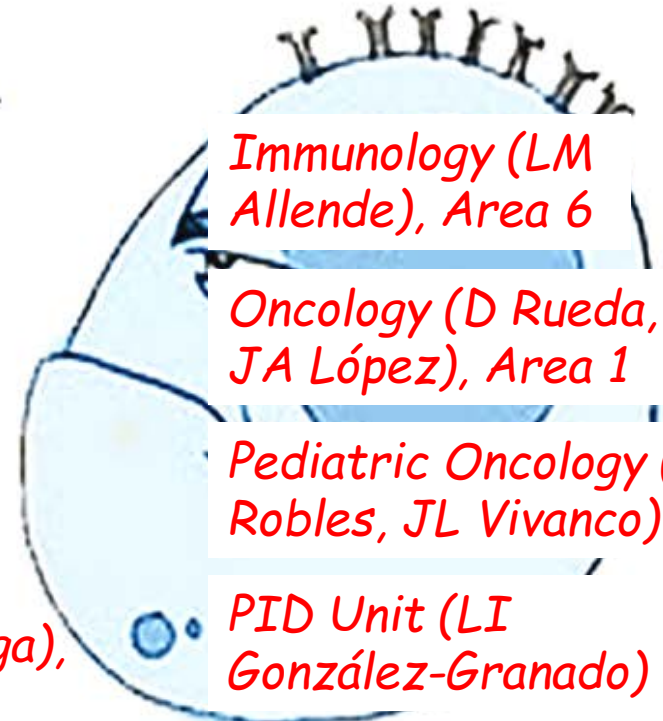
Antigen-presenting cells  
and inflammation



Epitomics

Nephrology (M Praga),  
Area 2

Complement  
physiopathology



Immunology (LM  
Allende), Area 6

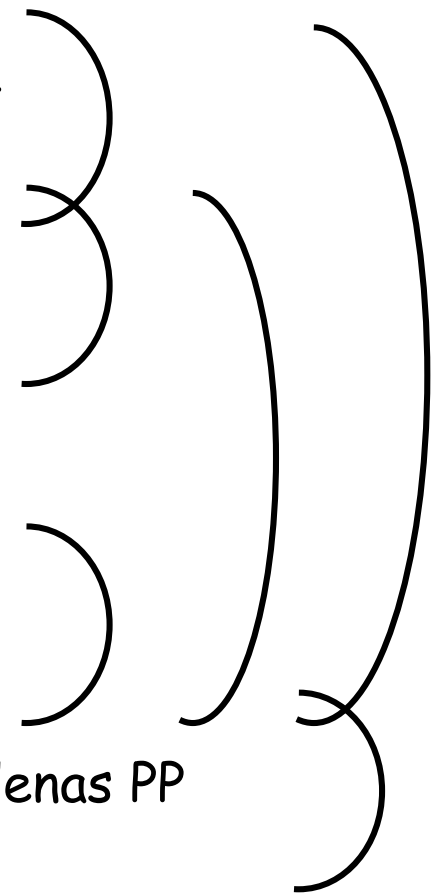
Oncology (D Rueda,  
JA López), Area 1

Pediatric Oncology (L  
Robles, JL Vivanco)

PID Unit (LI  
González-Granado)

T cell physiopathology  
and DNA repair

# Topics and people

- Antigen-presenting cells and inflammation
    - Martinez-Naves E, Gomez del Moral M, Cubero FJ
  - Epitomics
    - Reche PA
  - Cell adhesion and signaling
    - Lafuente EM
  - Immune synapse and lymphocyte signaling
    - Roda-Navarro P
  - T cell physiopathology and DNA repair
    - Regueiro JR, Fernandez-Malave E, Recio MJ, Cardenas PP
  - Complement physiopathology
    - Goicoechea E, Tortajada A
- 

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

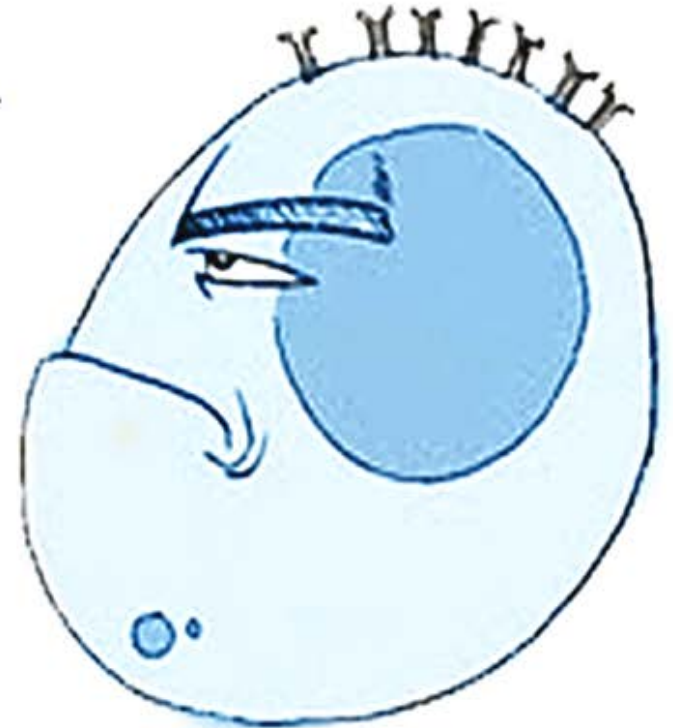


Antigen-presenting cells  
and inflammation



Epitomics

Complement  
physiopathology

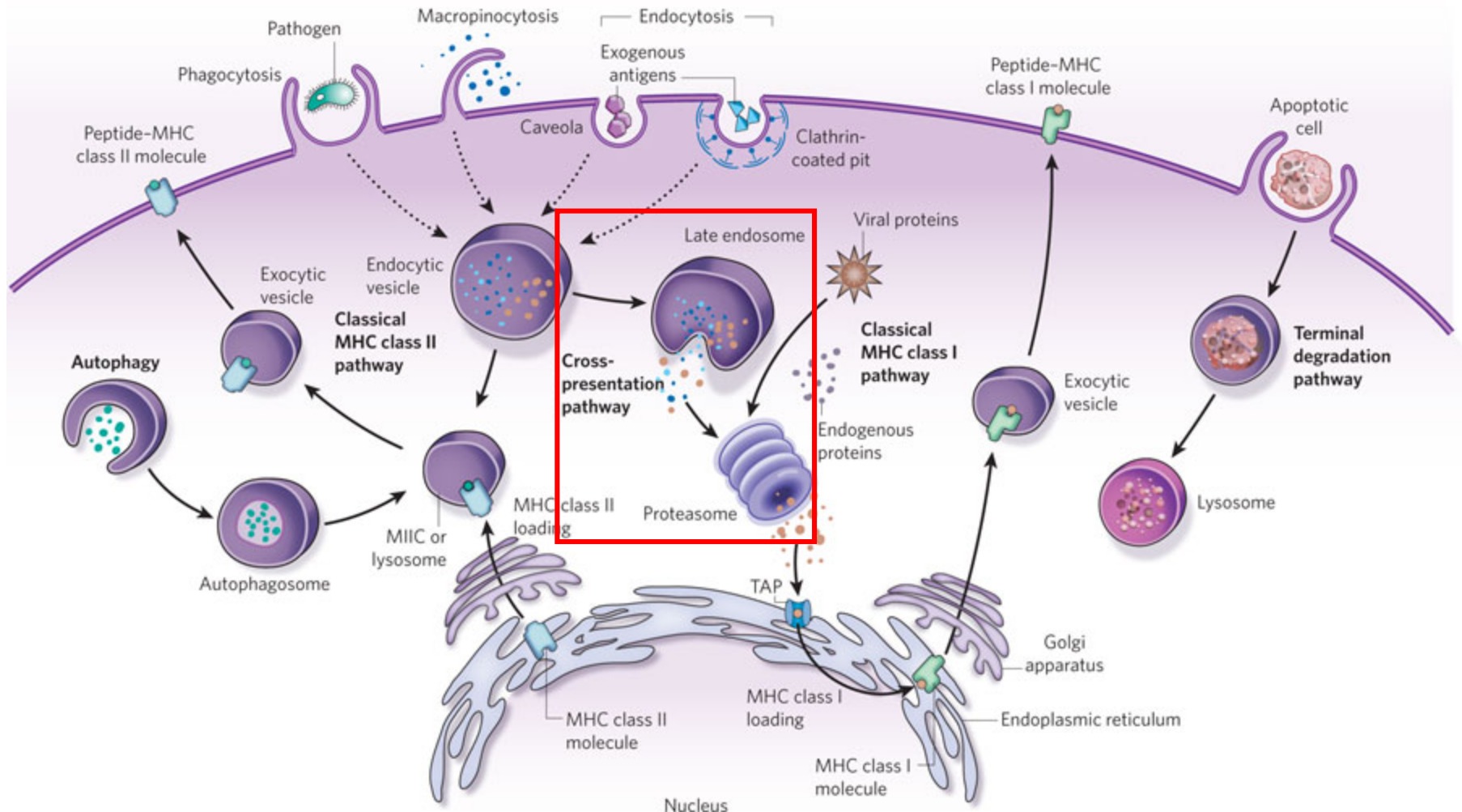


T cell physiopathology  
and DNA repair

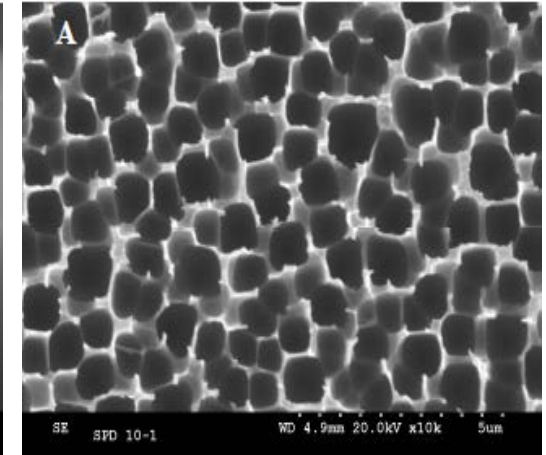
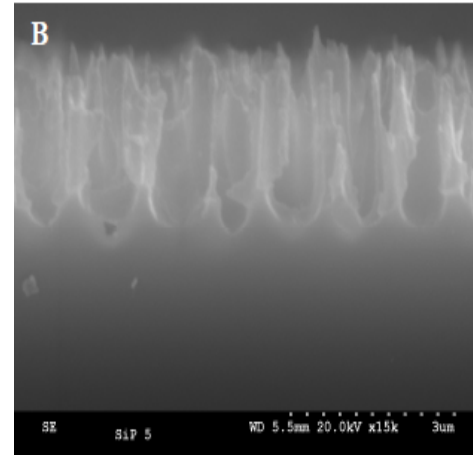
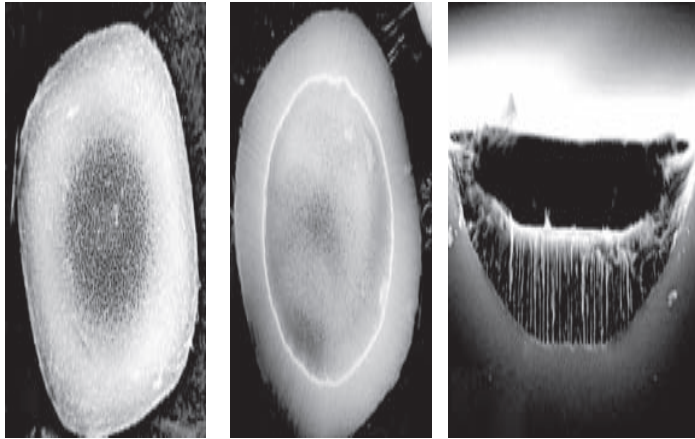
# The APC side of immunity: antigen cross-presentation

Th/B lymphocytes

Tc lymphocytes

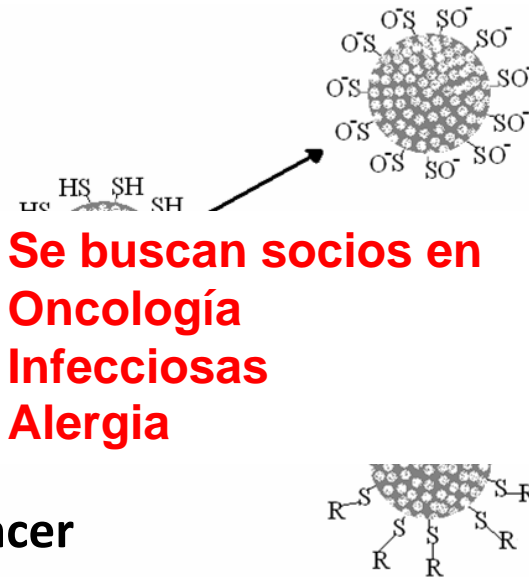


# Micropartículas de silicio mesoporoso que aumentan la respuesta Tc y Th/B

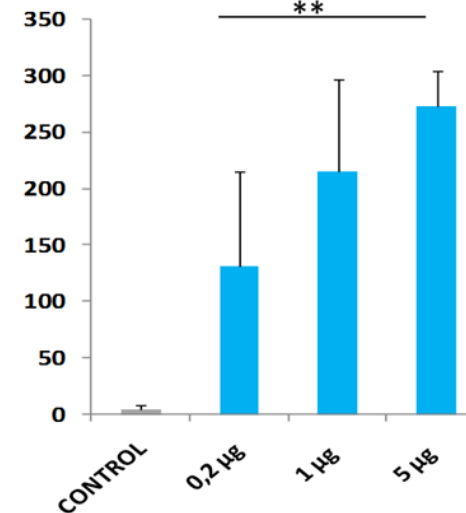


- **Tamaño: 0,1-10µm**
- **Porosidad: 65-71%**
- **Tamaño poro: 5-20 nm**
- **Biocompatible**
- **Estable a bajo pH**
- **Fácil carga**
- **Se puede funcionalizar**
- **Aplicaciones: vacunas, cáncer**

**Se buscan socios en  
Oncología  
Infecciosas  
Alergia**



**IgG1 específica**



Gomez del Moral M

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

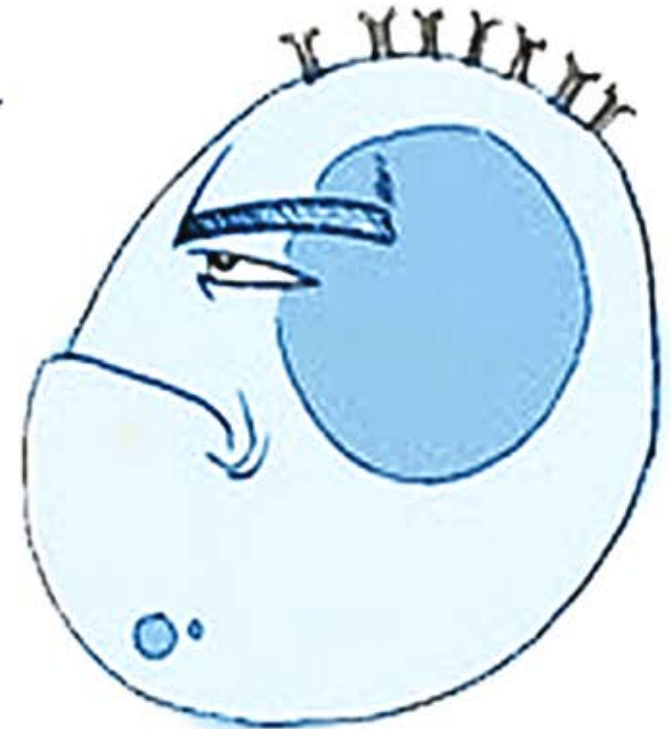


Antigen-presenting cells  
and inflammation



Epitomics

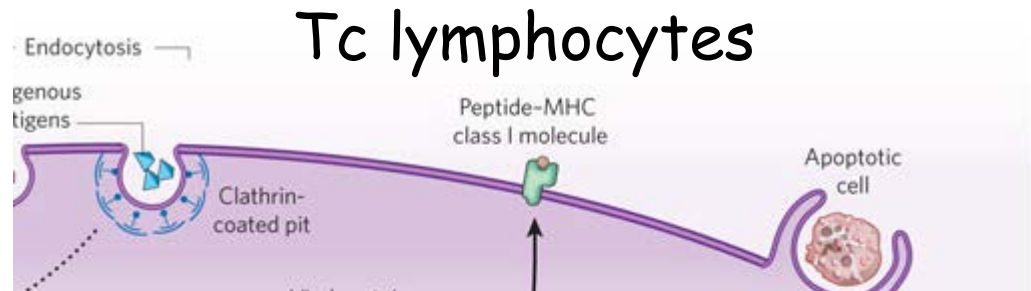
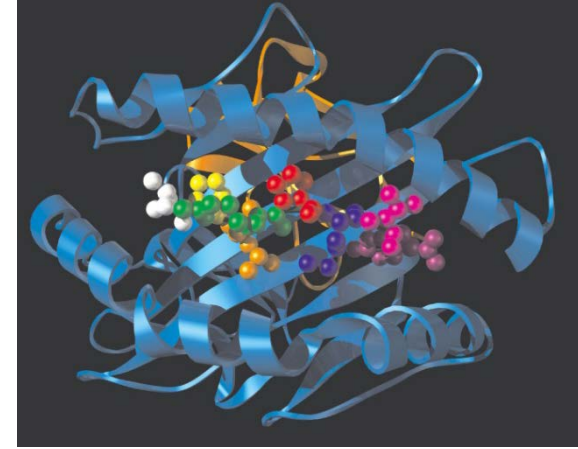
Complement  
physiopathology



T cell physiopathology  
and DNA repair



- # T cell epitope prediction for
- Vaccination (Smallpox, Flu)
  - Allergy immunotherapy
  - Cancer immunotherapy

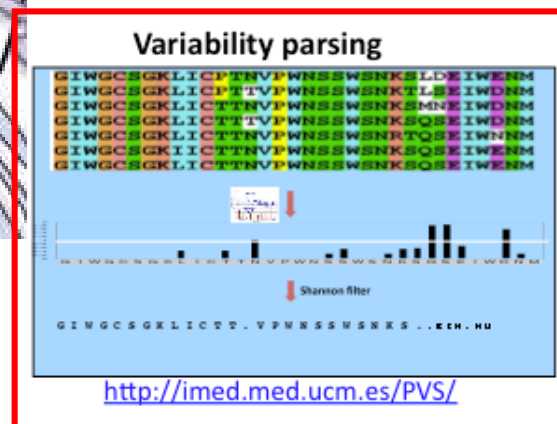
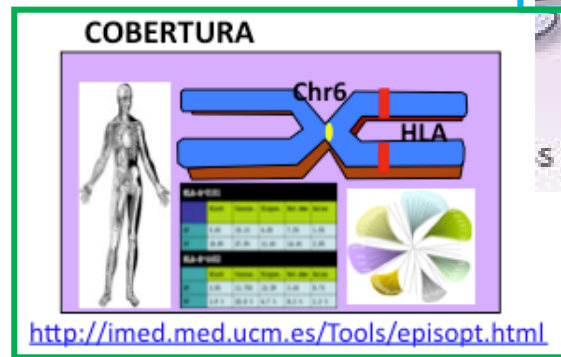
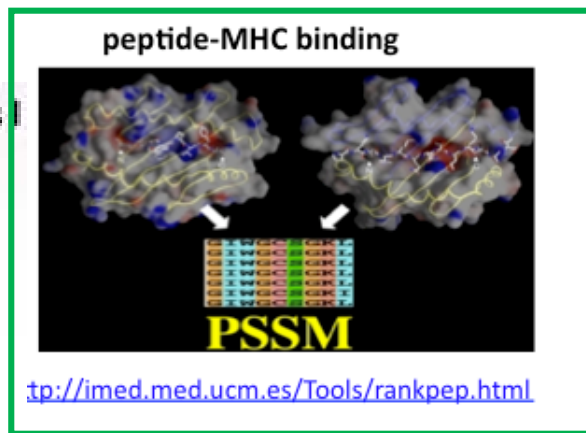
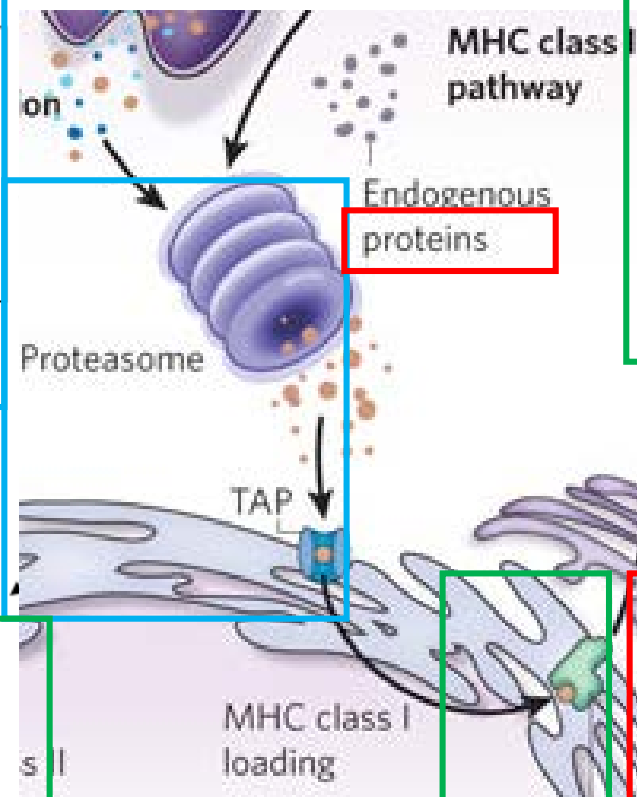
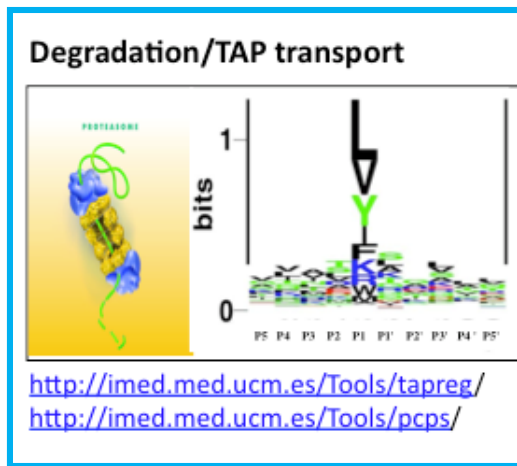


## Científicos españoles avanzan hacia la vacuna universal de la gripe con la ayuda de la bioinformática

- ▶ Han desarrollado dos nuevos prototipos para que no haya que actualizarla cada año
- ▶ Ambas vacunas se desarrollarían a partir de epítomos T
- ▶ Los científicos están contactando con varias farmacéuticas para su desarrollo



# Herramientas bioinformáticas para la predicción de epítomos y diseño de vacunas de epítomos



**Se buscan socios en  
Oncología  
Infecciosas  
Alergia**

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

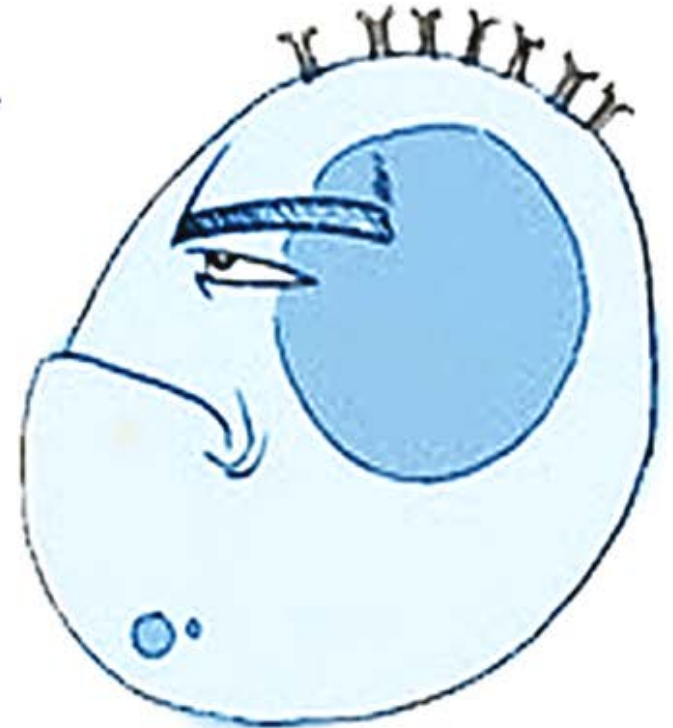


Antigen-presenting cells  
and **inflammation**



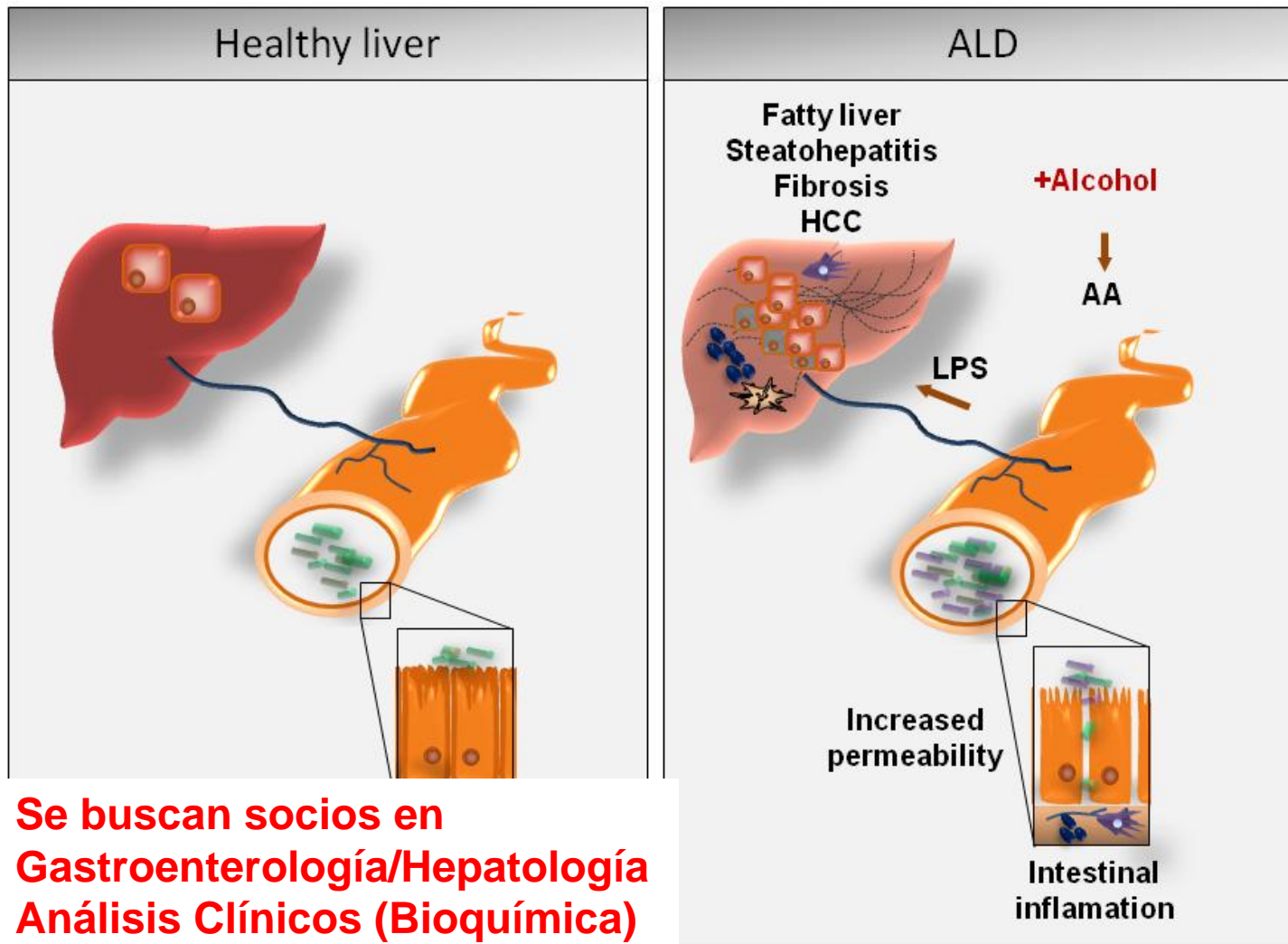
Epitomics

Complement  
physiopathology



T cell physiopathology  
and DNA repair

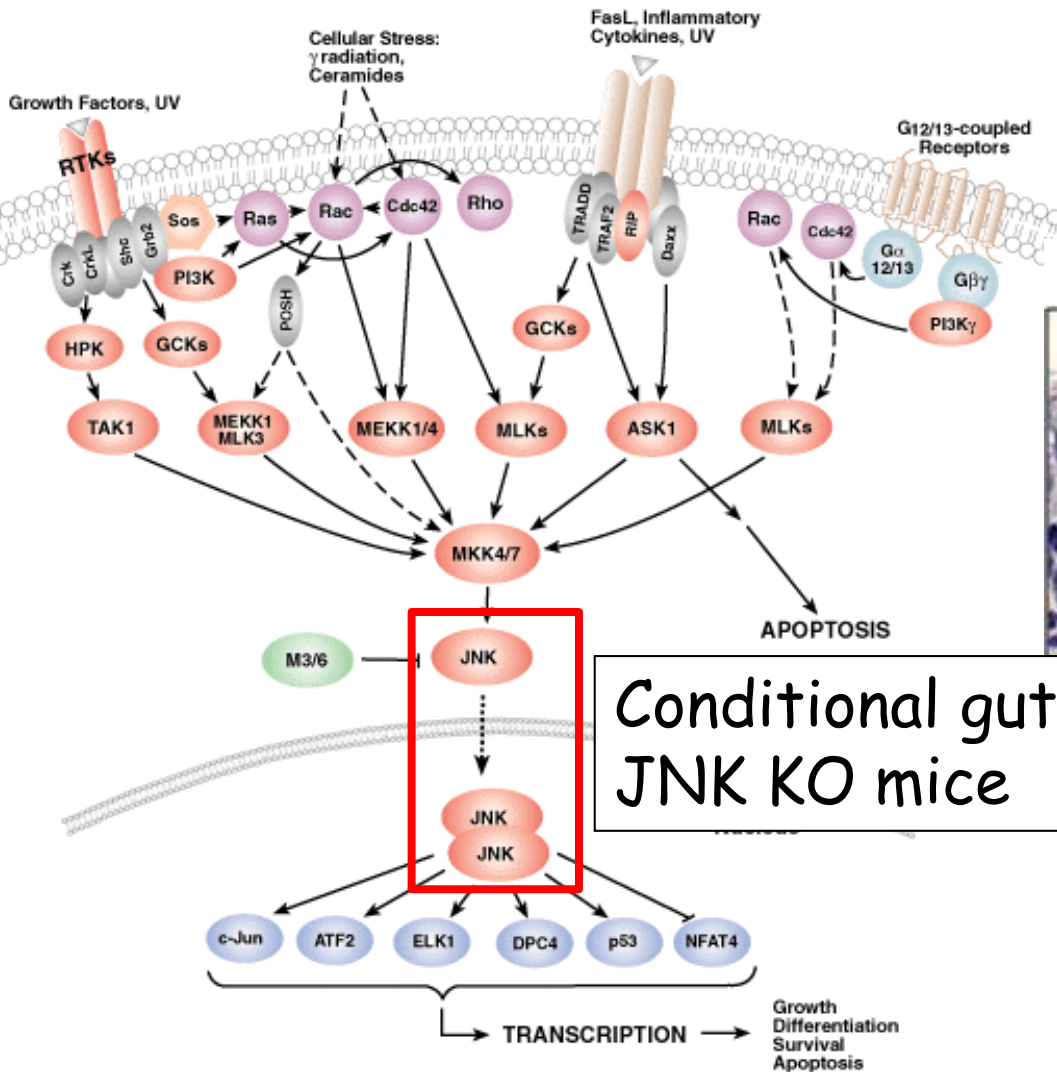
# The gut-liver axis in alcoholic liver disease (ALD)



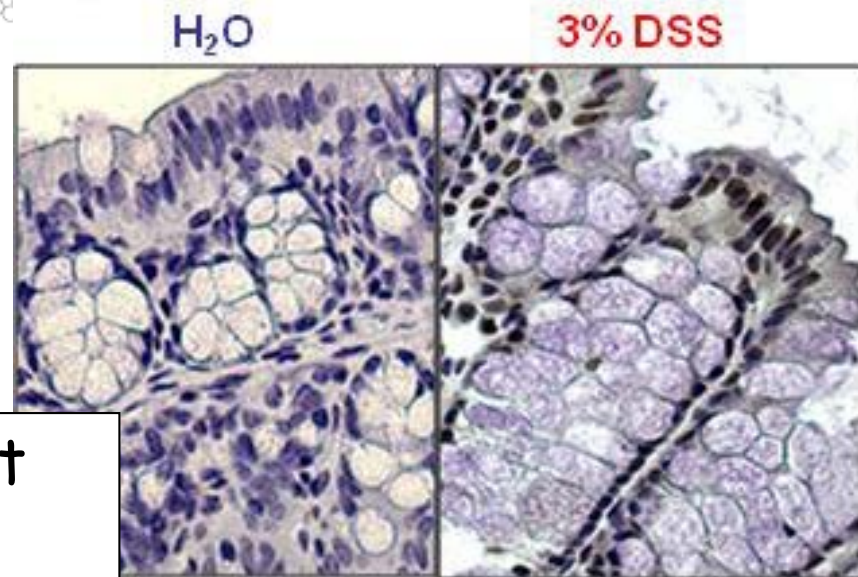
**Se buscan socios en  
Gastroenterología/Hepatología  
Análisis Clínicos (Bioquímica)**

# JNK signaling in gut and liver inflammation and cancer

## SAPK/JNK Signaling Cascades



*Experimental models of alcohol-driven colon (CRC) and liver cancer (HCC)*



**Gut phospho-JNK**

Martinez-Naves E, Cubero FJ

# Research interests

Cell adhesion  
and signaling

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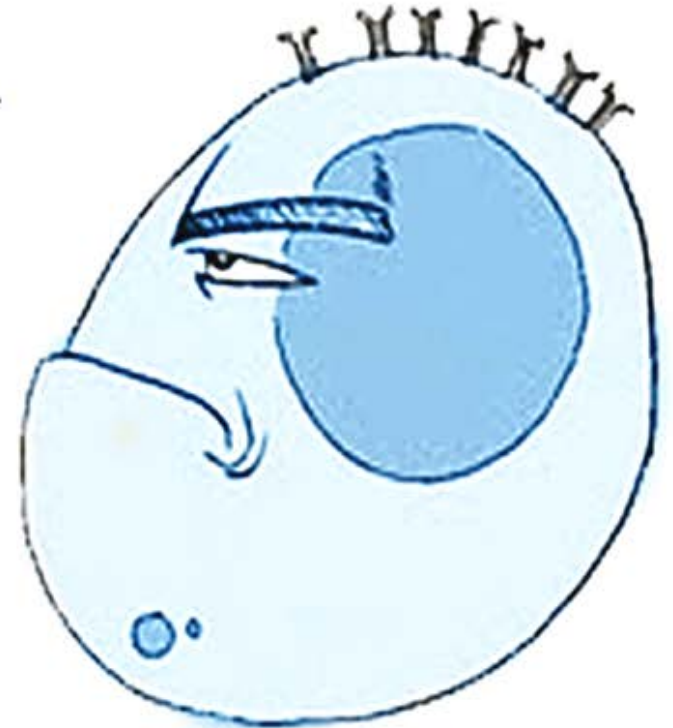


Antigen-presenting cells  
and inflammation



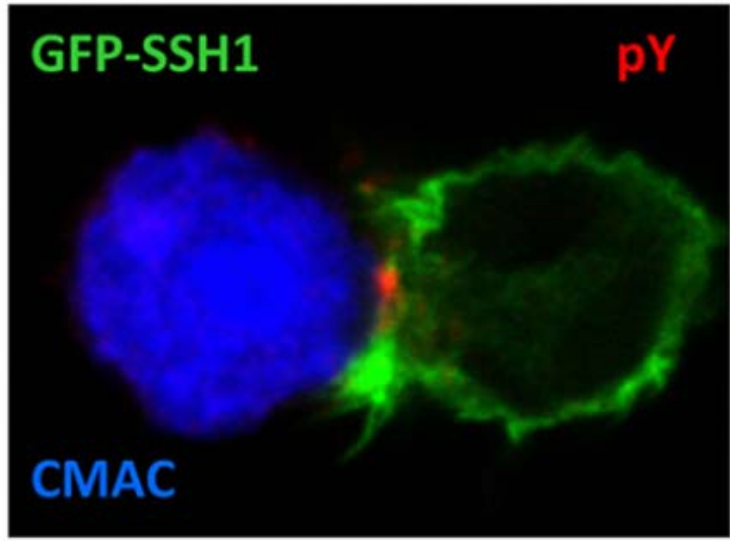
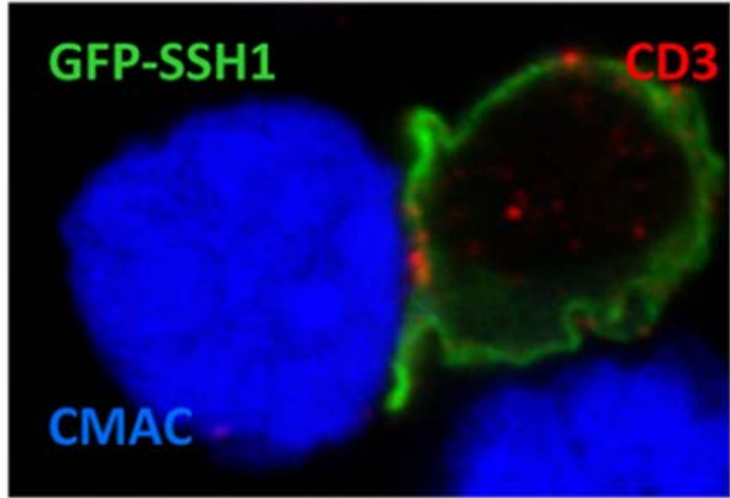
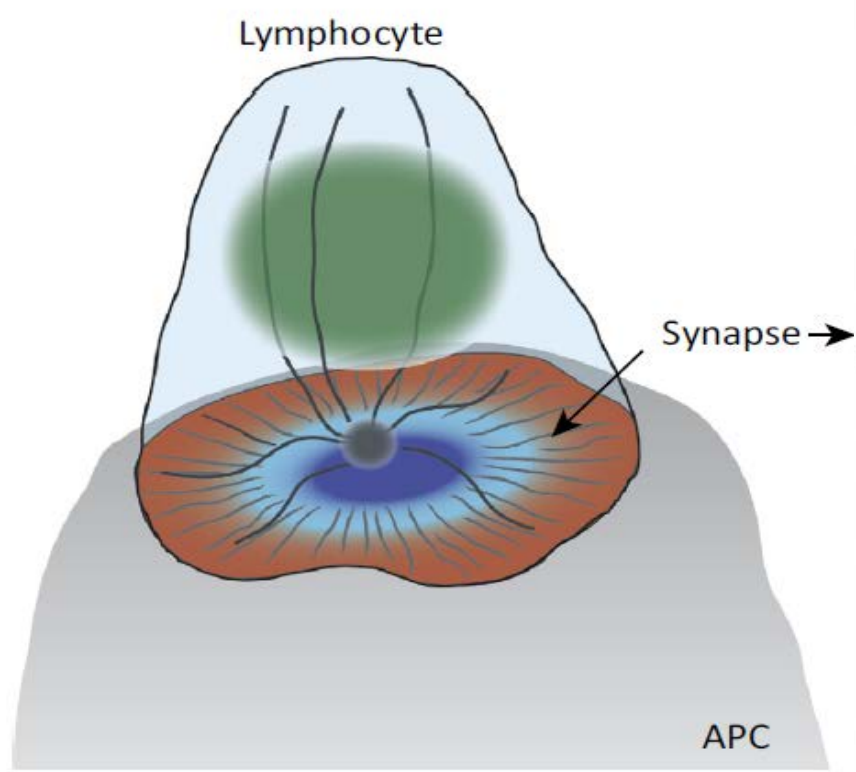
Epitomics

Complement  
physiopathology



T cell physiopathology  
and DNA repair

# Immunological synapse dynamics: tuning T cell responses by tyrosine phosphatases

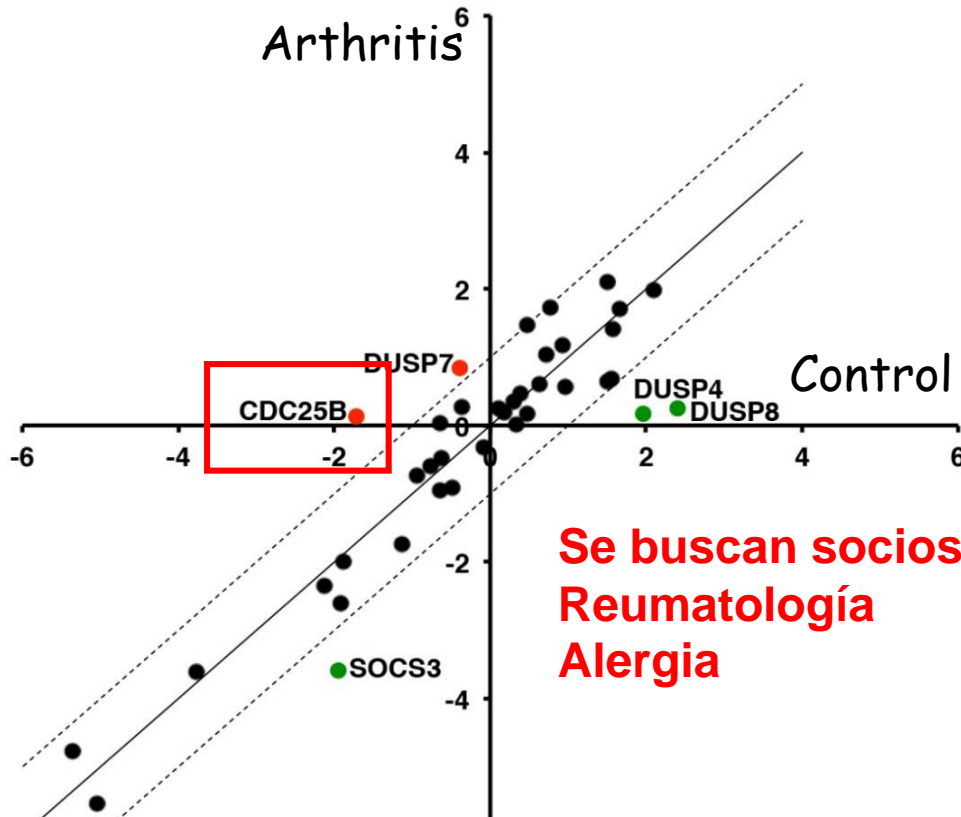


SSH1=SlingSHot 1

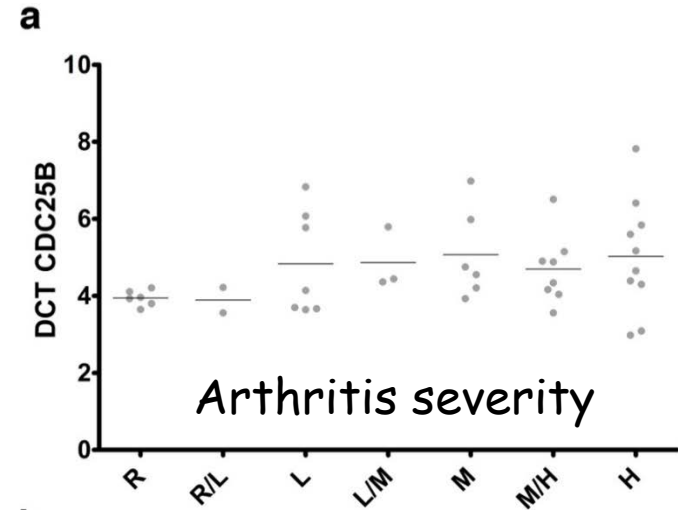
Roda-Navarro P

# Low mRNA of DUSP7 and CDC25B phosphatases in CD4 T cells of early arthritis patients

Pair	Age (C vs RA)	Gender	Smoking
C1 vs RA1	50 vs 46	Male	Ever vs Current
C2 vs RA2	40 vs 49	Female	Never
C3 vs RA3	63 vs 77	Female	Ever vs Never



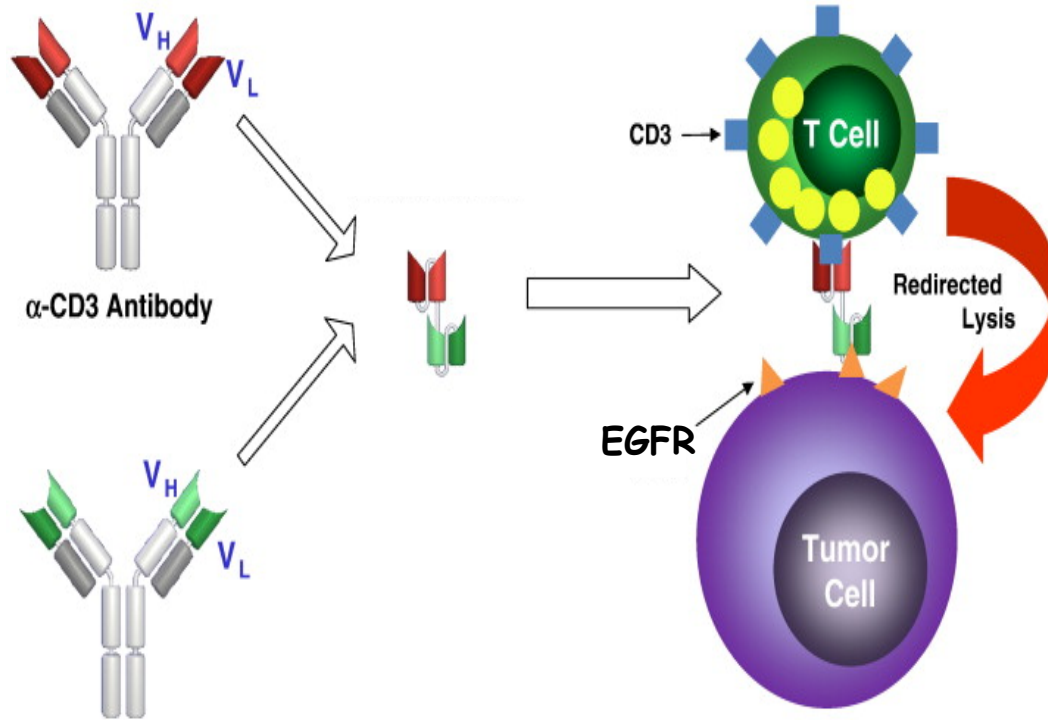
**Se buscan socios en  
Reumatología  
Alergia**





# Fatal attraction: redirecting T cells to target cancer cells

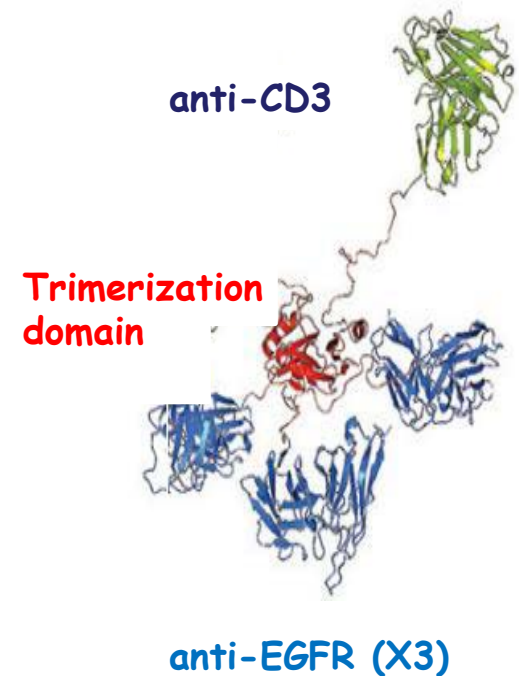
Standard tandem



Anti-EGFR

**Se buscan socios en Oncología**

Asymmetrical tandem trimerbody



# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

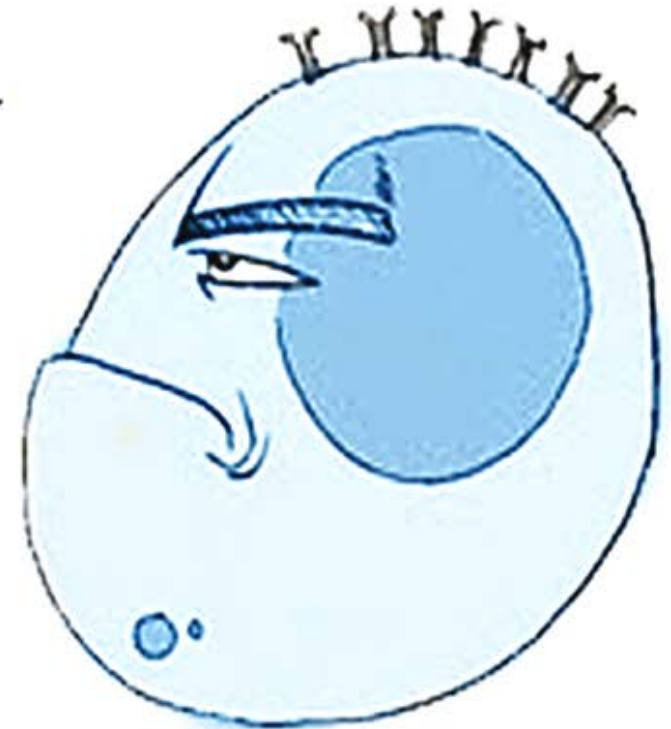


Antigen-presenting cells  
and inflammation



Epitomics

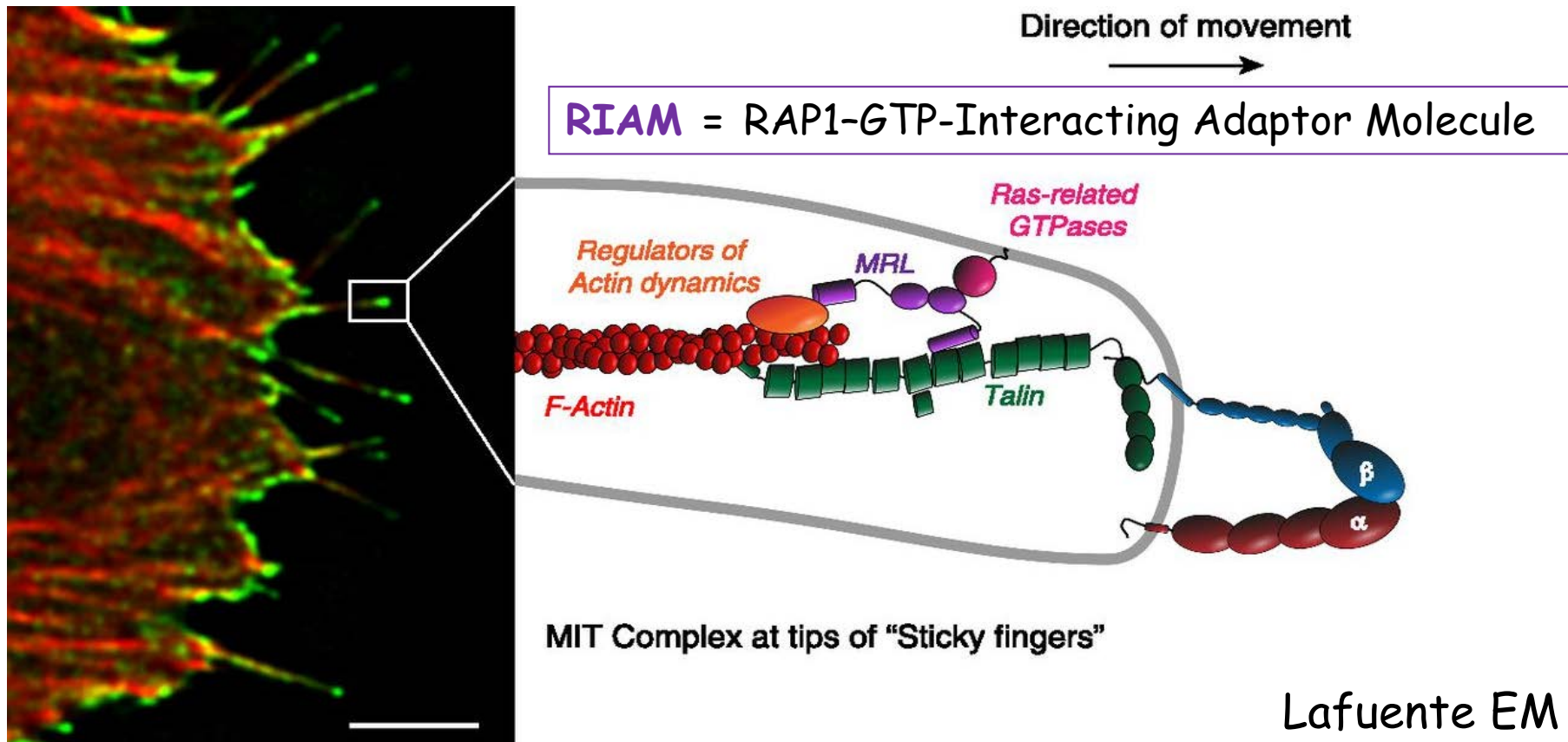
Complement  
physiopathology



T cell physiopathology  
and DNA repair

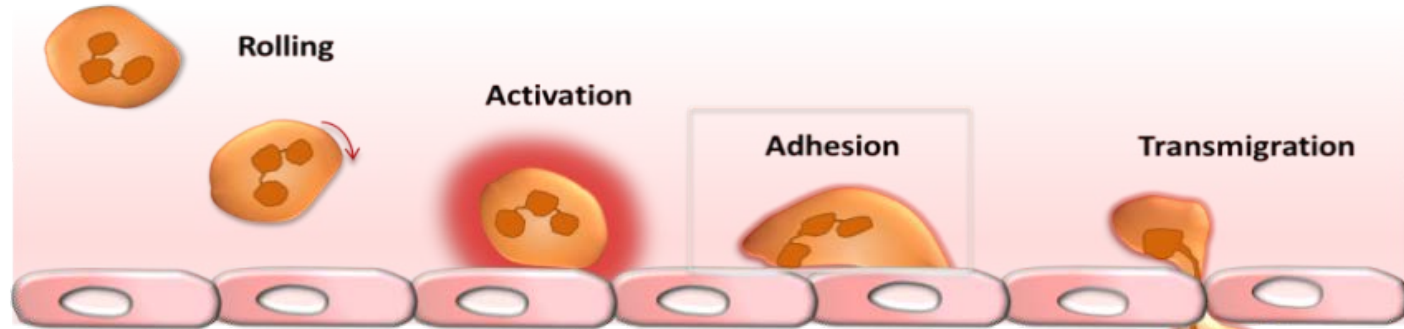
# Integrin-mediated cell adhesion and signaling

**RIAM** (MRL proteins) recruits talin and actin regulators to integrin cytoplasmic tails inducing its activation. MRL= MIG-10, RIAM and Lamellipodin



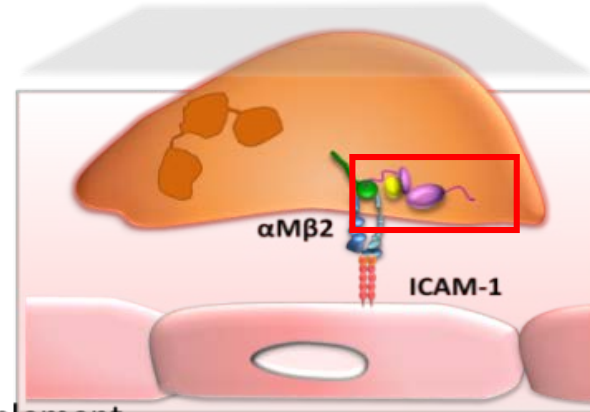
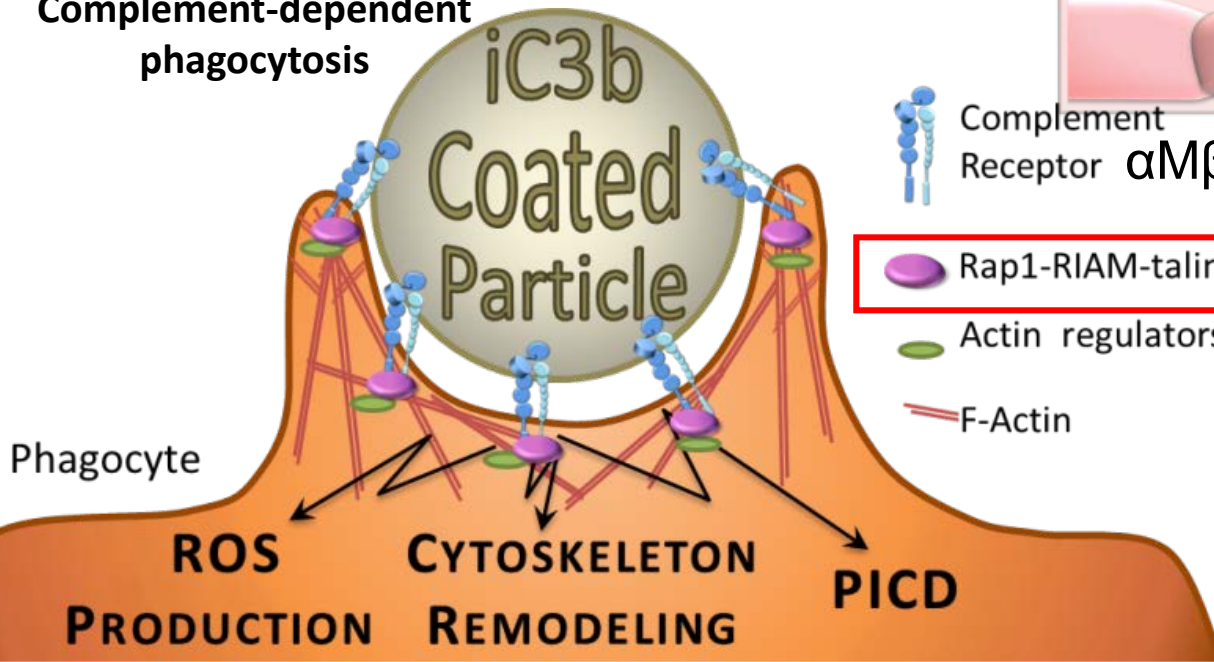
# RIAM function in innate immune responses

Adhesion  
Extravasation/Motility



Se buscan socios en  
Pediatria  
Infecciosas

Complement-dependent  
phagocytosis



Complement  
Receptor  $\alpha M\beta 2$  (CR3/Mac-1)

Rap1-RIAM-talin

Actin regulators

F-Actin

Cell Mol Life Sci. 2013

Lafuente EM

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

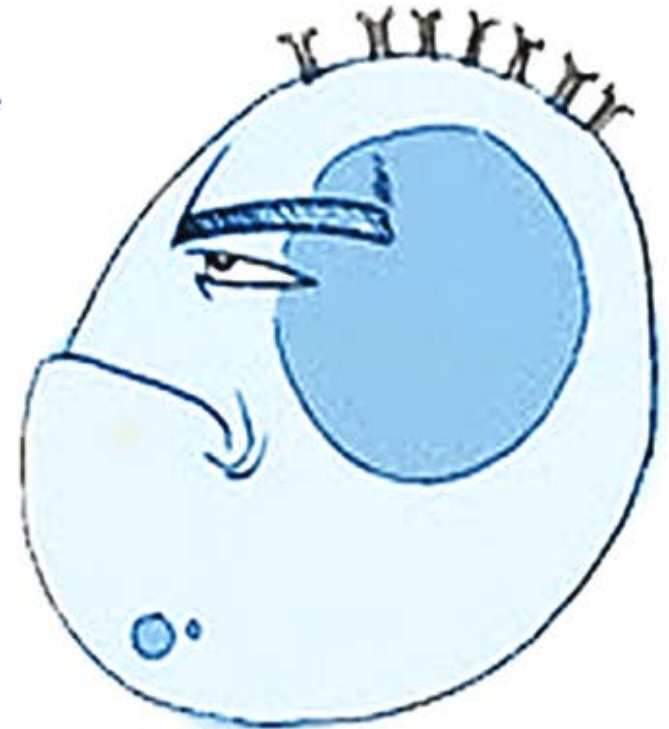


Antigen-presenting cells  
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Epitomics

Complement  
physiopathology

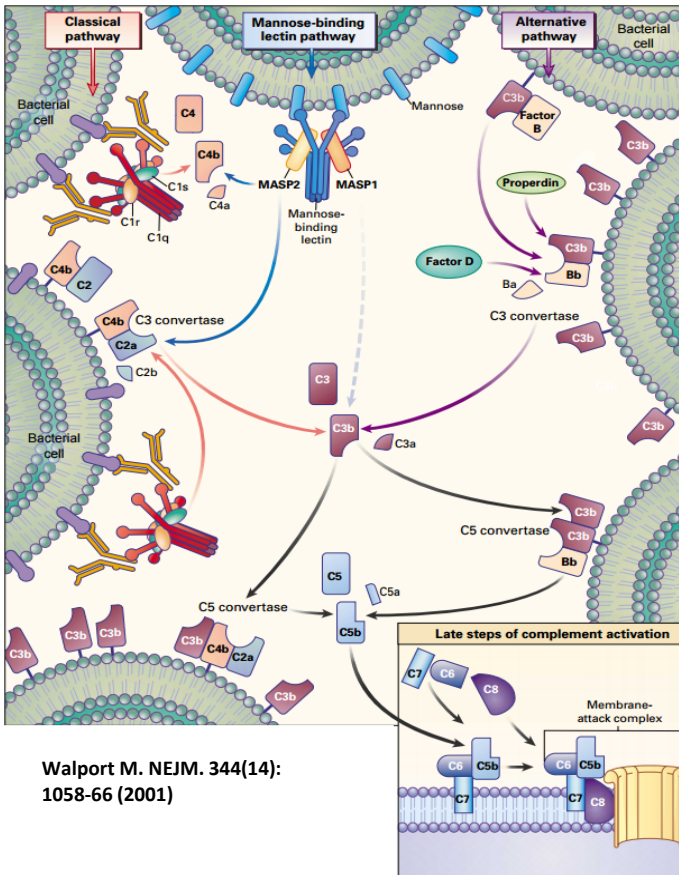


T cell physiopathology  
and DNA repair

# Complement physiology

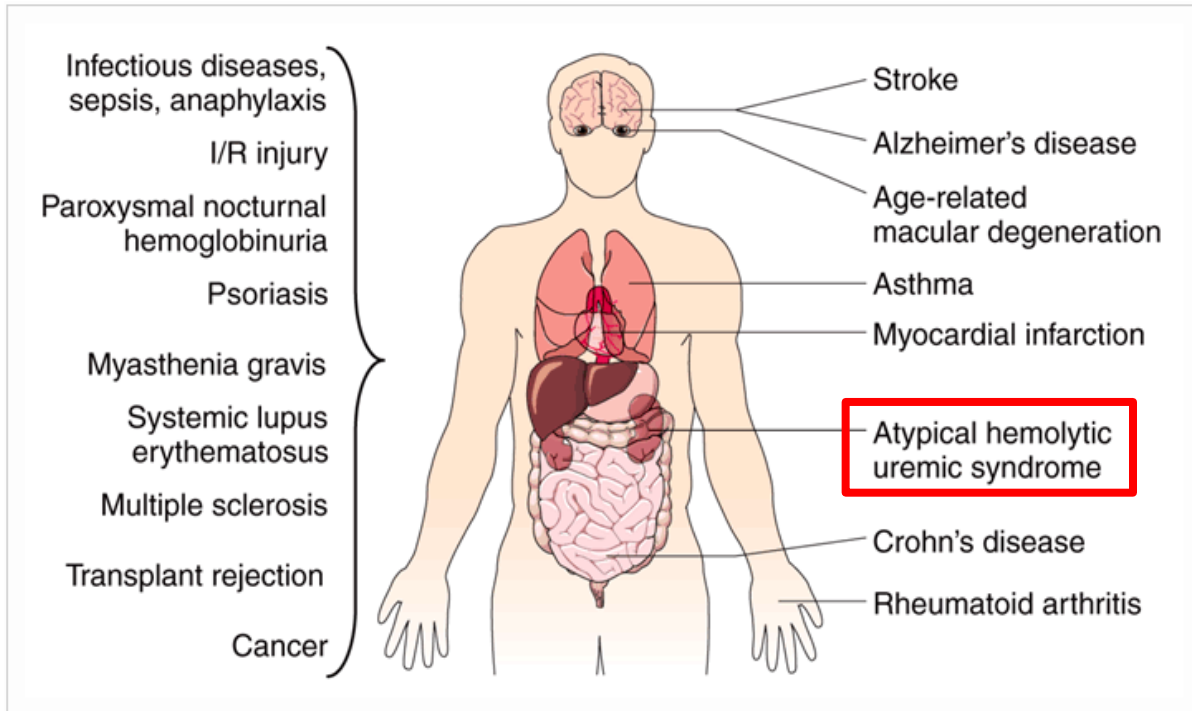
- Humoral innate immunity against infection, injured self tissue and immune complexes; regulates adaptive immunity (B and T cells)
- Hypo- or hyper-complement activation cause or exacerbate several human diseases

## Complement activation pathways



Walport M. NEJM. 344(14): 1058-66 (2001)

## Complement associated human diseases



Daniel Ricklin & John D Lambris  
*Nature Biotechnology* 25, 1265 - 1275 (2007)

Goicoechea E, Tortajada A

# Our main interest is...

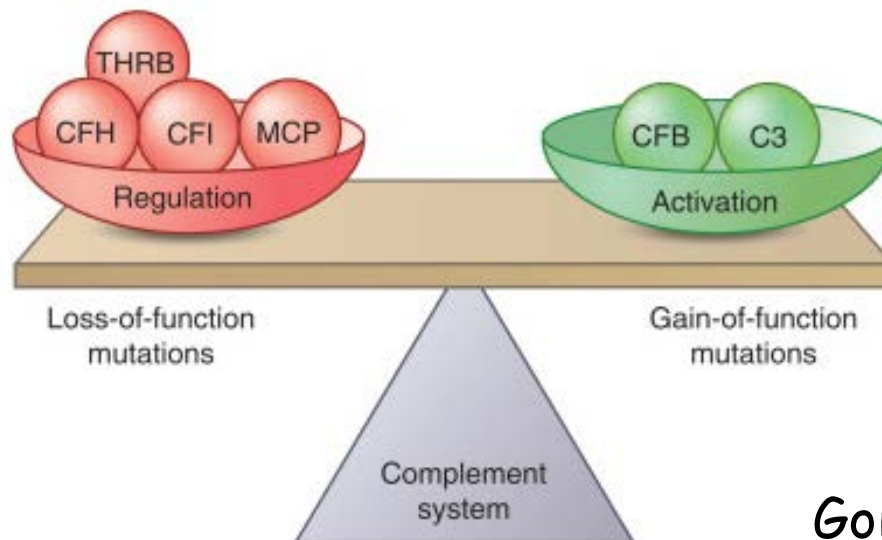
To elucidate the pathogenic mechanisms of complement-mediated renal diseases such as atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathies (C3G).

## Specific aims:

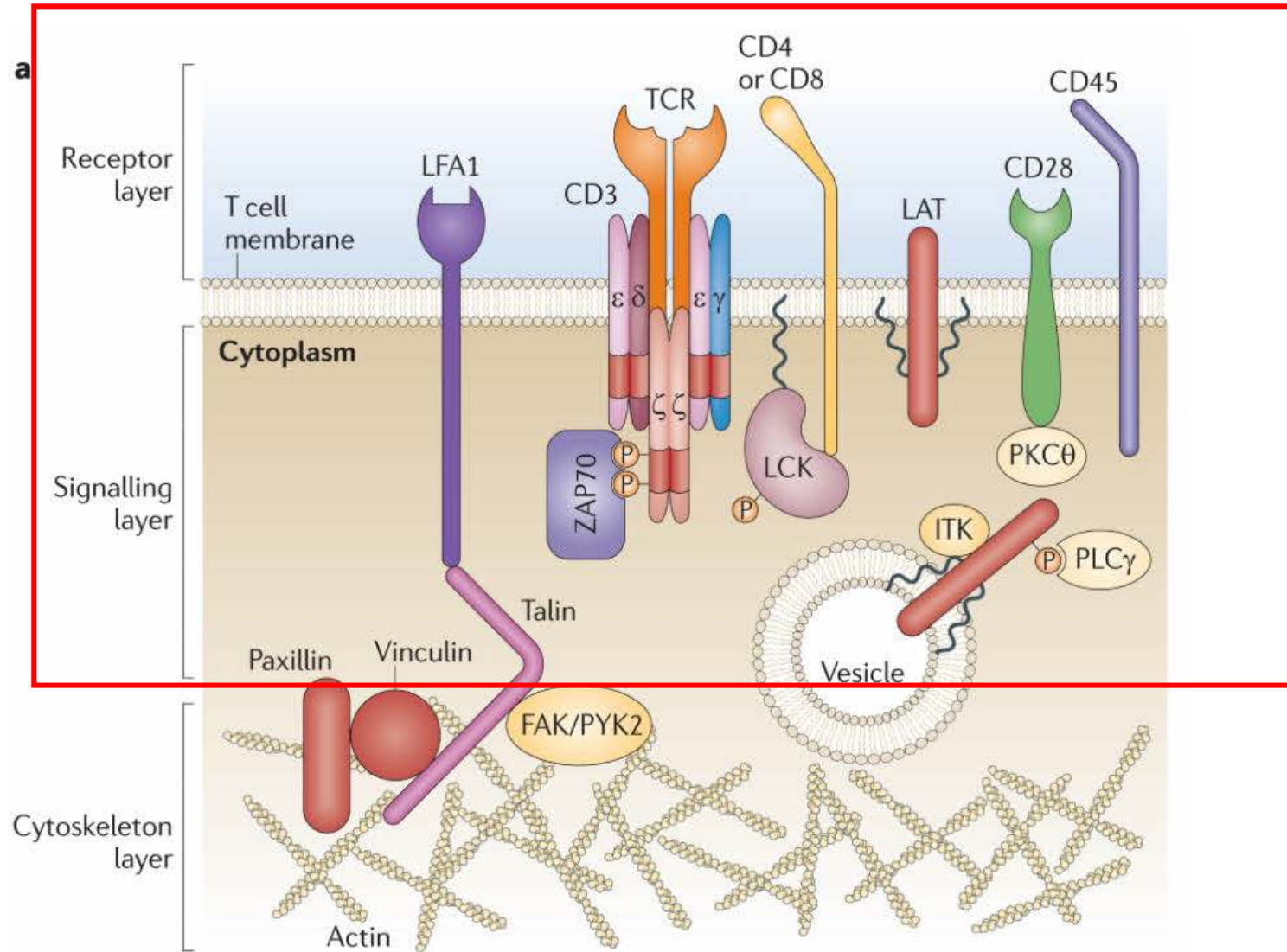
Identification of complement gene variants associated with **aHUS and C3G**

Functional characterization of **risk variants**

Identification of disease **biomarkers**



# The T cell side of immunity





# Research interests

Cell adhesion  
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lymphocyte signalling

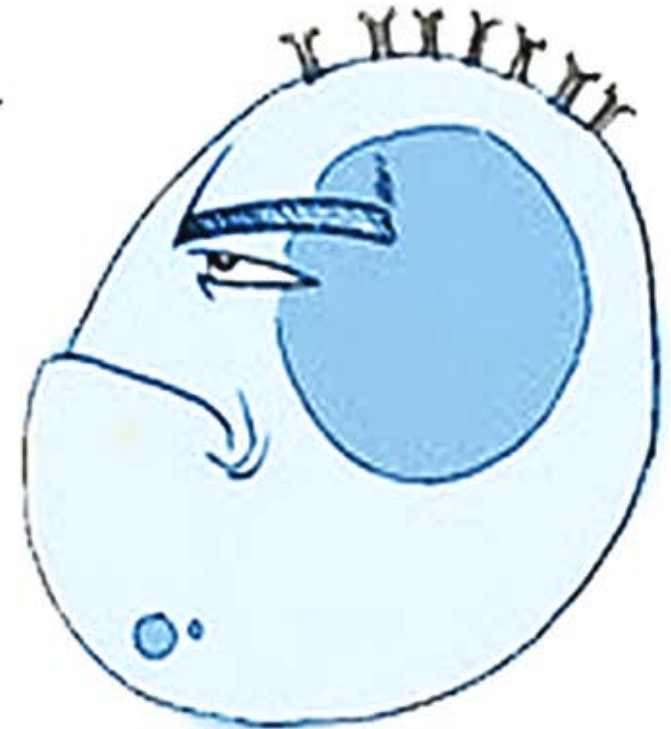


Antigen-presenting cells  
and inflammation



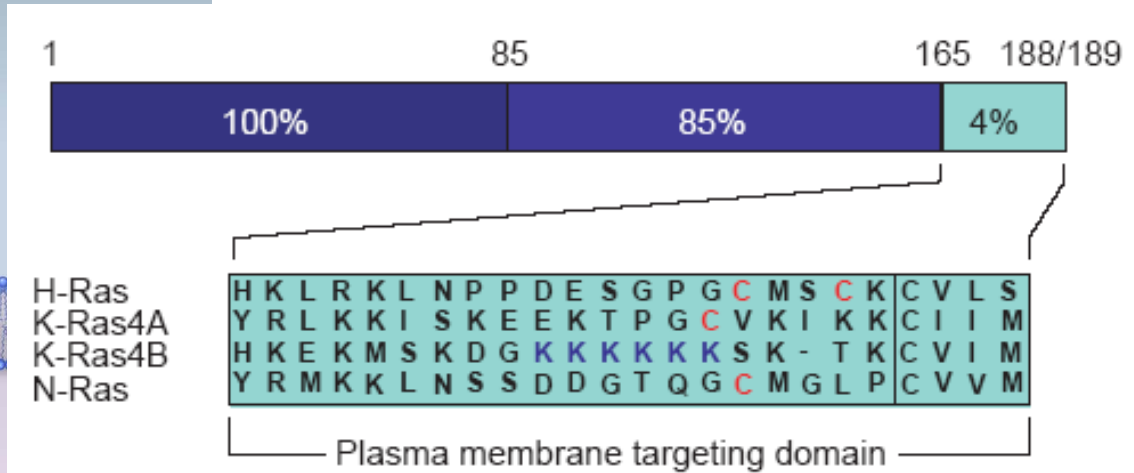
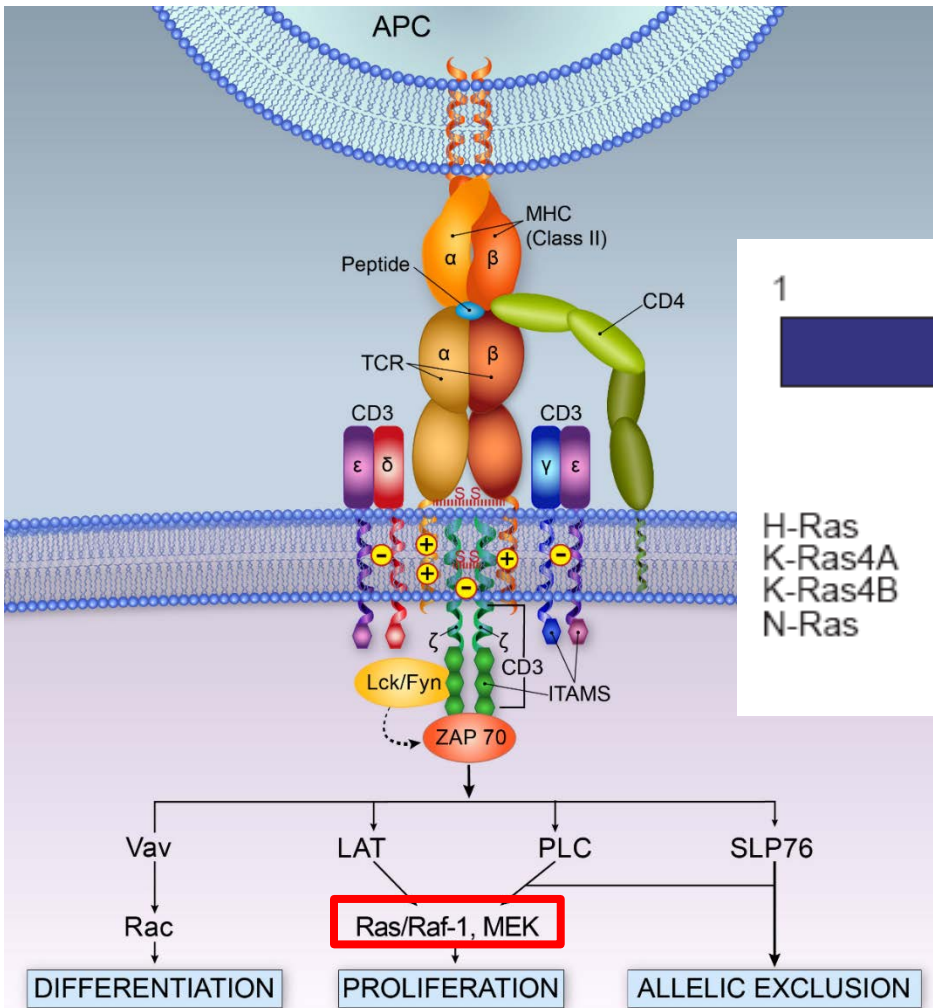
Epitomics

Complement  
physiopathology



T cell physiopathology  
and DNA repair

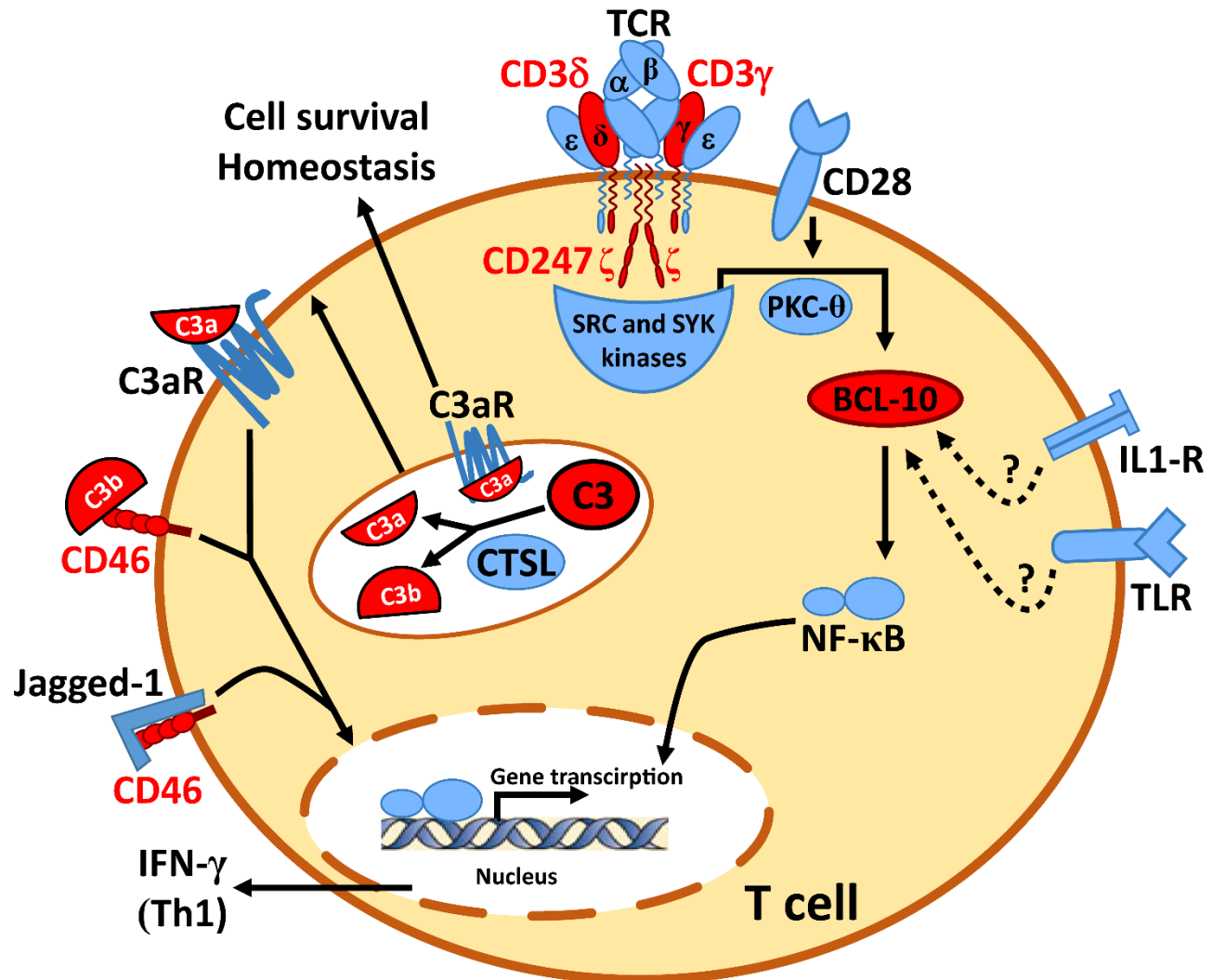
# Ras-mediated TCR signaling



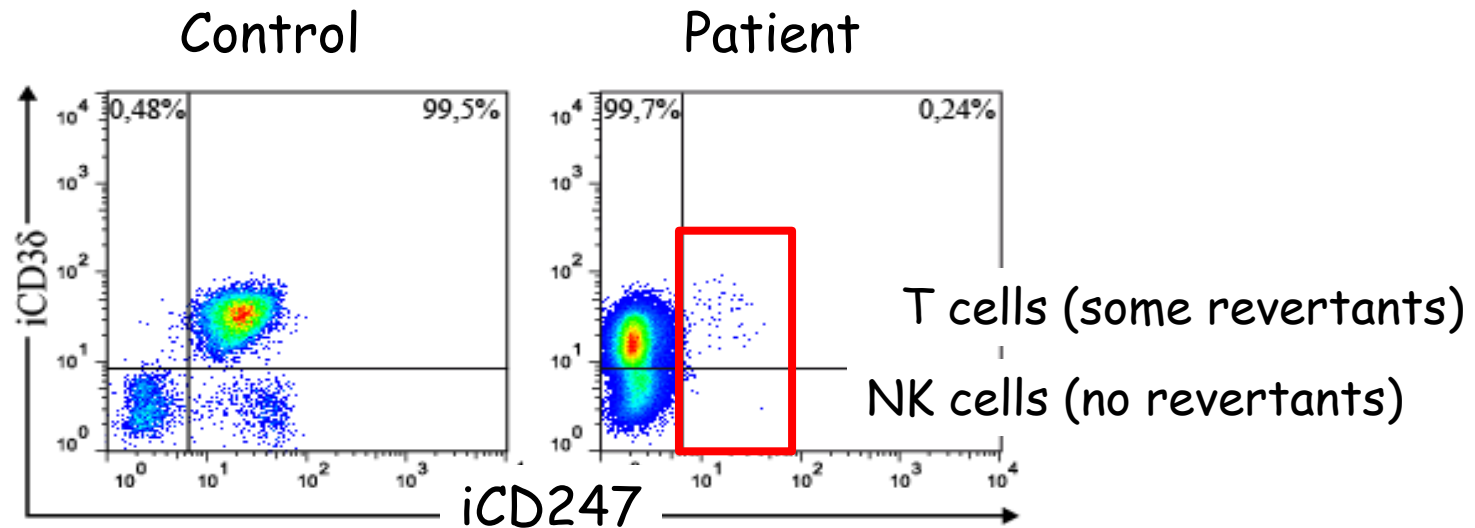
H-ras  
N-ras  
K-ras

H-ras vs N-ras  
role in KO mice

# Human congenital T cell deficiencies

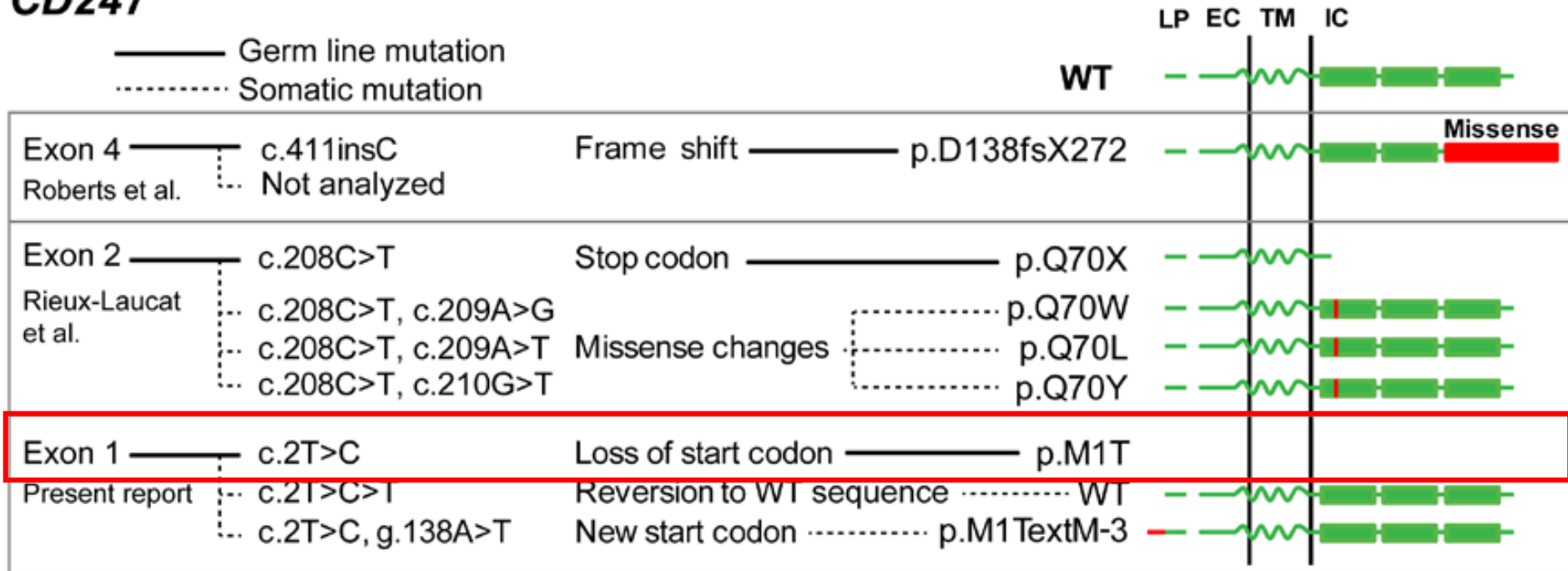


# TCR expression revertants = gene therapy in vivo



# CD247 reversions help to understand T cell selection

## CD247



Líneas celulares  
Vectores de expresión

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

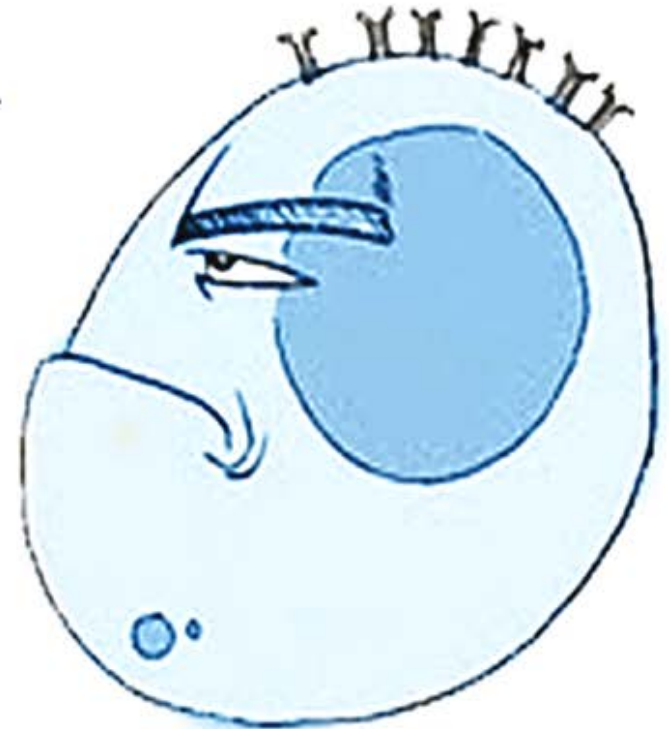


Antigen-presenting cells  
and inflammation



Epitomics

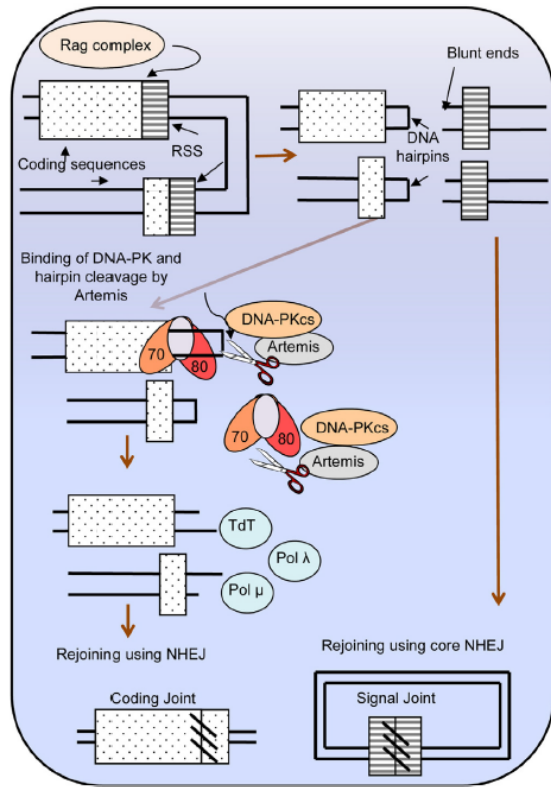
Complement  
physiopathology



T cell physiopathology  
and DNA repair

# Combined T-cell and B-cell Immunodeficiencies in RadioSensitive-SCID

## Development of T and B cell receptor repertoires



T and B Immunodeficiency

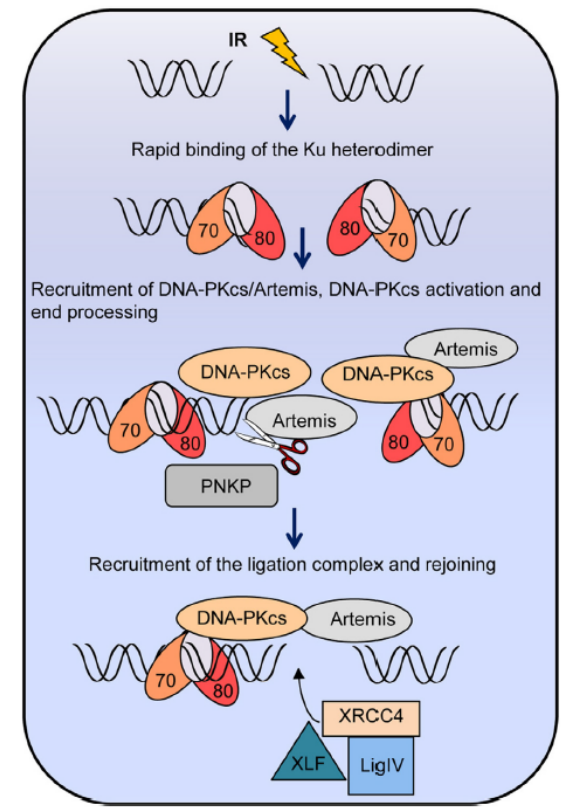
Lymphopenia or T cell lymphopenia

Yes=SCID

```

    graph TD
      A[Lymphopenia or T cell lymphopenia] --> B[Yes=SCID]
      B --> C[CD19 ↓ : SCID T-B-]
      C --> D[SCID T-B-NK-]
      C --> E[SCID T-B-NK+]
      D --> F[Granulocytopenia, Thrombocytopenia, deafness]
      F --> G[Reticular dysgenesis (AK2)]
      G --> H[Chondrosteral dysplasia, deafness]
      H --> I[ADA def (ADA)]
      E --> J[Distinctive clinical features?]
      J -- Yes --> K[Microcephaly + facial dysmorphism]
      K --> L[DNA ligase IV def (LIG4)]
      L --> M[Microcephaly + in utero growth retardation]
      M --> N[Cernunnos* (NHEJ1)]
      J -- No --> O[RAG 1/2 def (RAG1 or RAG2)]
      O --> P[Artemis def (DCLRE1C)]
      O --> Q[DNA PKcs def* (PRKDC)]
  
```

## Non Homologous End Joining repair pathway



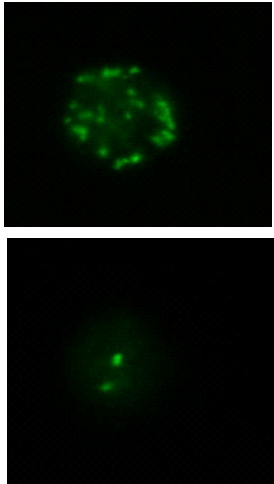
Radiosensitivity  
DNA repair defects

# Técnicas diagnósticas para pacientes con sospecha de radiosensibilidad y defectos de reparación de DNA

## Reparación de roturas de DNA de doble cadena (DSBs)

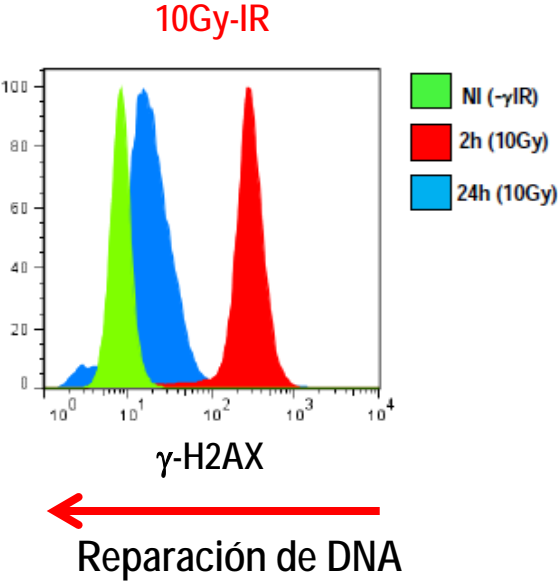
## Radiosensibilidad

### Inmunofluorescencia

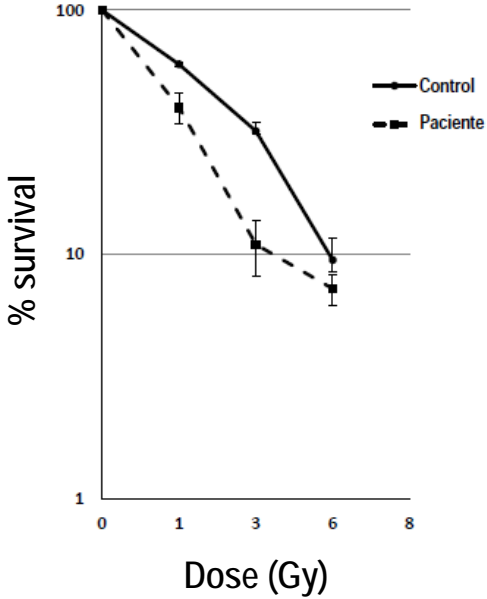


3Gy-IR  
 Formación de foci  
 30 minutos  
 Reparación de DNA  
 24 horas  
 Desaparición foci

### Citometría de flujo



### Supervivencia celular



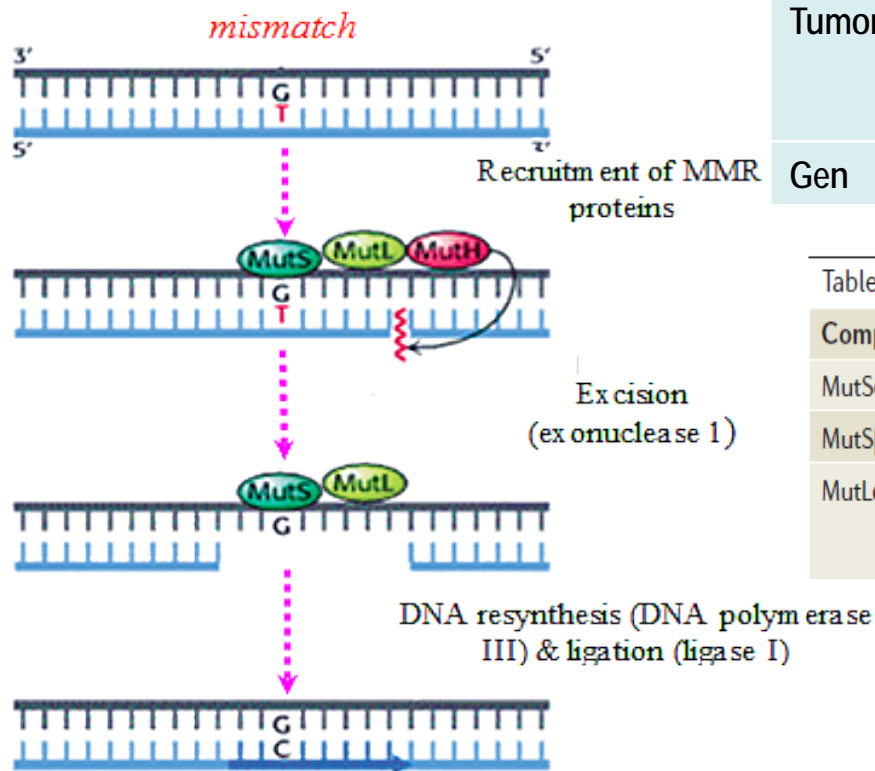
Más rápido  
 Menos invasivo

Recio MJ



# Consortio europeo "Care for Constitutional MissMatch Repair Disease"

CMMRD: autosómica dominante, riesgo de tumores infantiles (hematológicos y del SNC)



Pacientes	P1	P2	P3
Tumores	Linfoma linfoblástico (7 meses)	Linfoma no Hodgking B (2 años); linfomas linfoblásticos pre-T (3 y 8 años)	Glioblastoma
Gen	MLH1	MSH6	PMS2

Table 1 | Human MutS and MutL homologue complexes that are involved in mismatch repair

Complex	Components	Function
MutS $\alpha$	MSH2, MSH6	Recognition of base-base mismatches and small IDLs
MutS $\beta$	MSH2, MSH3	Recognition of IDLs
MutL $\alpha$	MLH1, PMS2	Forms a ternary complex with mismatch DNA and MutS $\alpha$ ; increases discrimination between heteroduplexes and homoduplexes; also functions in meiotic recombination

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

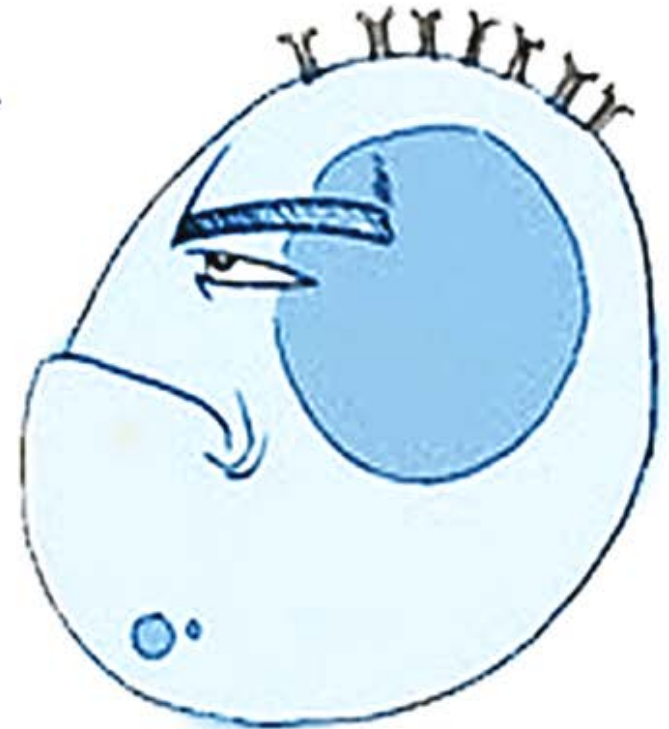


Antigen-presenting cells  
and inflammation



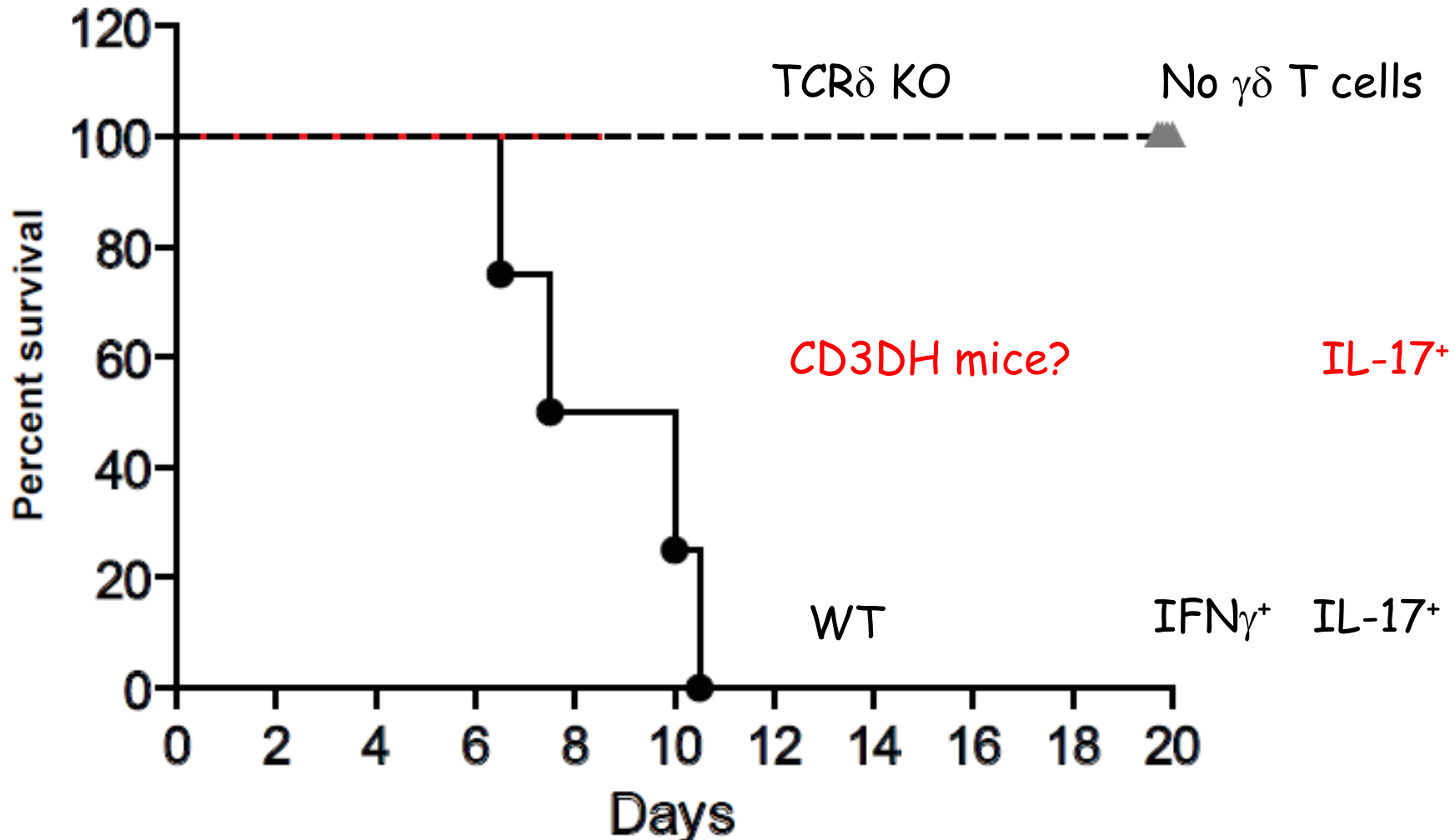
Epitomics

Complement  
physiopathology

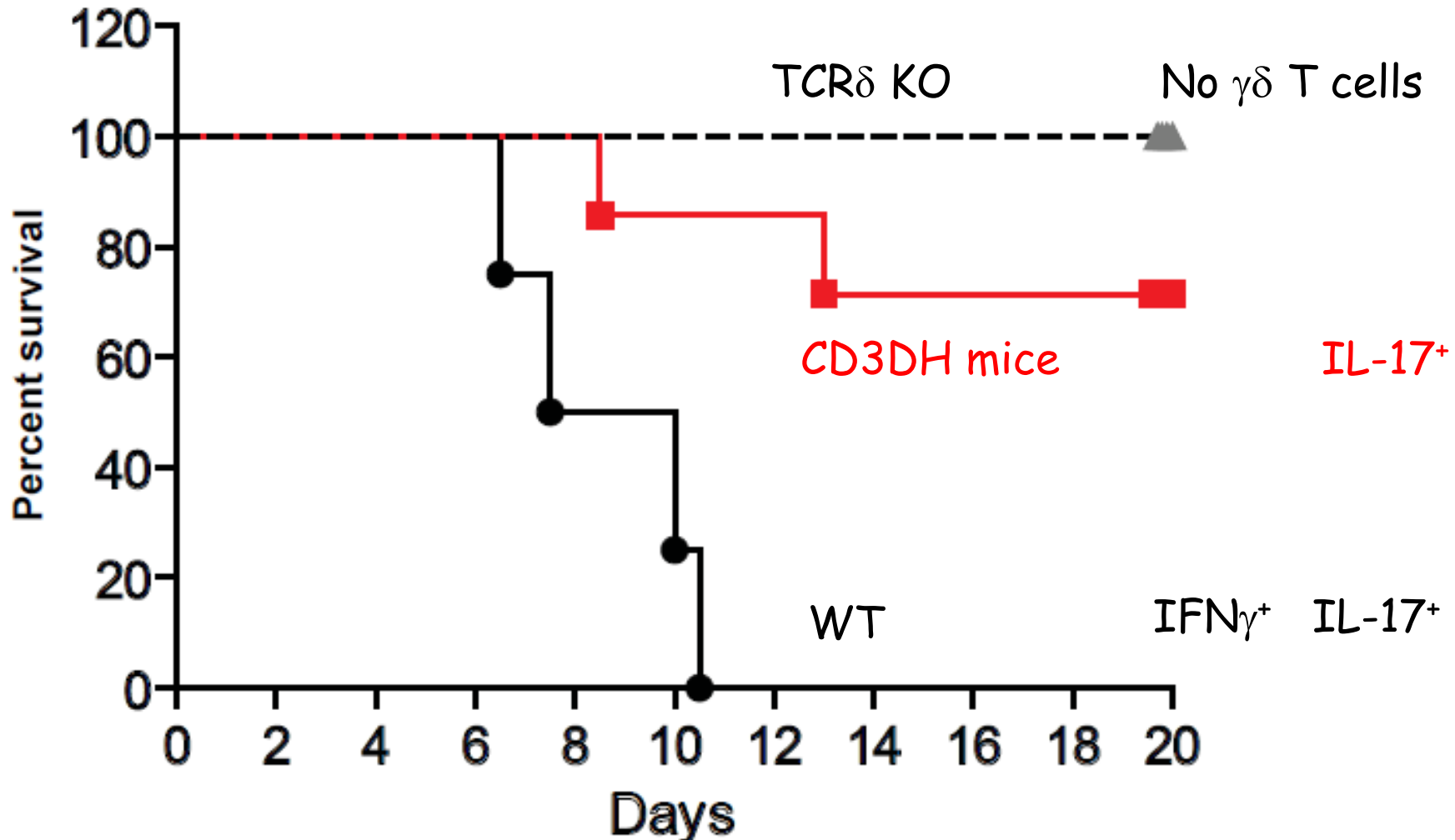


T cell physiopathology  
and DNA repair

# Deadly inflammation after cerebral malaria due to $\gamma\delta$ T cells

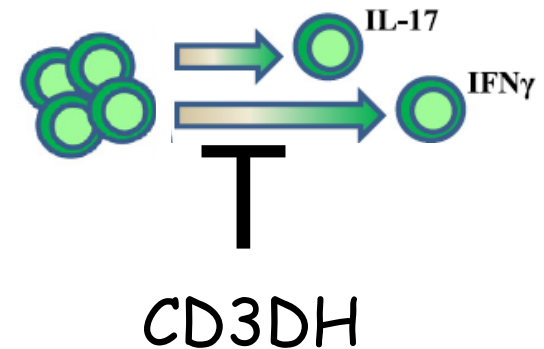
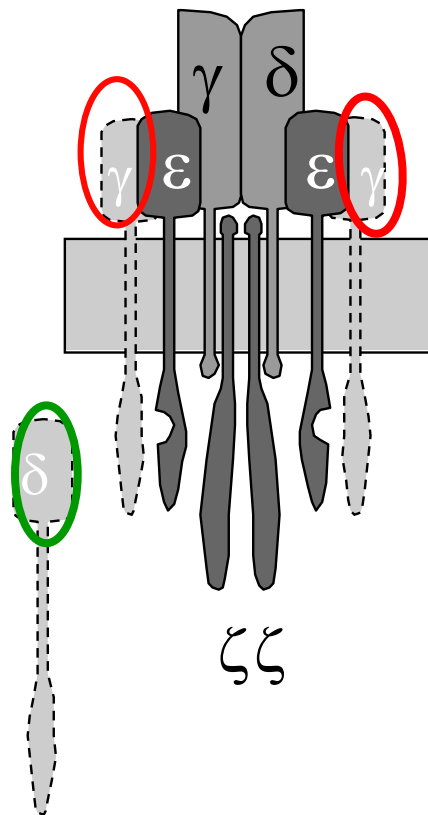


# CD3DH mice: less inflammation, resistant to cerebral malaria



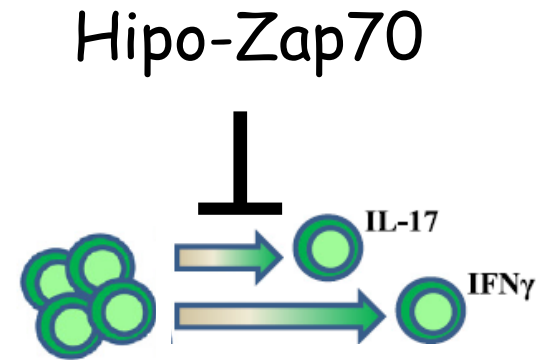
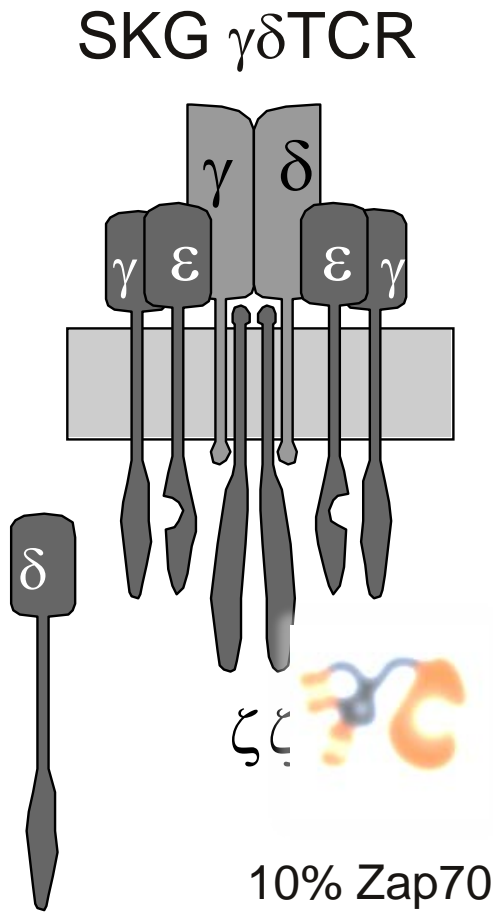
# CD3DH: no IFN- $\gamma$ <sup>+</sup> $\gamma\delta$ T cells

CD3DH  $\gamma\delta$ TCR



Muñoz, M. *et al. Nat Immunol* 17, 721–727 (2016).

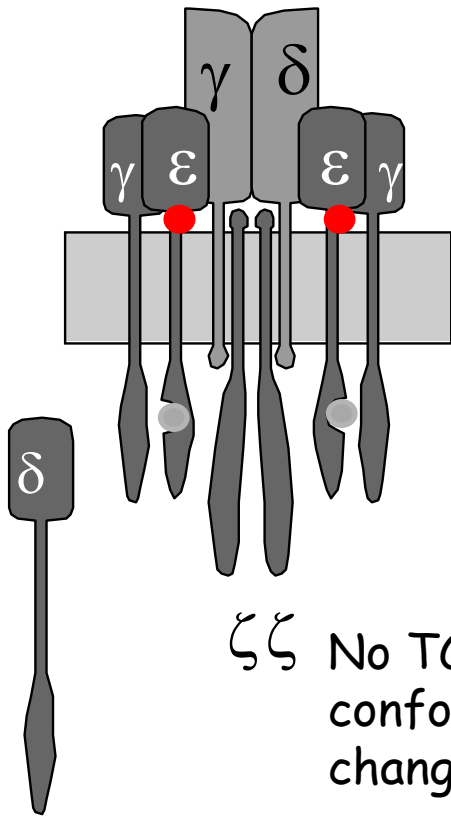
# Hipo-Zap: no $IL-17^+$ $\gamma\delta$ T cells



Wencker, M. *et al. Nat Immunol* 15, 80-87 (2014).

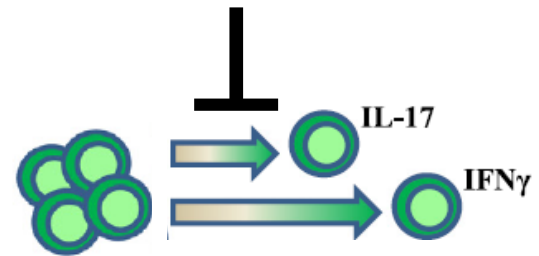
# CD3 $\epsilon$ C80G: no IL-17<sup>+</sup> $\gamma\delta$ T cells

CD3 $\epsilon$  C80G  $\gamma\delta$ TCR



$\zeta\zeta$  No TCR  
conformational  
change (CC)

CD3 $\epsilon$ C80G



Blanco, R. *et al. Sci Signal*  
7, 115 (2014).

# Model for $\gamma\delta$ T cell pathology

- Pivotal role of cerebral interleukin-17-producing  $\gamma\delta$ T cells in the delayed phase of **ischemic brain injury**. Shichita, T. et al. Nat Med 15, 946-950 (2009).
- IL-23-independent induction of IL-17 from  $\gamma\delta$ T cells and innate lymphoid cells promotes experimental **intraocular neovascularization**. Hasegawa E et al. J Immunol. 2013 Feb 15;190(4):1778-87.

**Se buscan socios en**  
**Ictus**  
**Oftalmología**  
**Tgdpatología**



# Take home message

- Ciudad al i+12 (no cuenta, si no)
- Buscad socios en el i+12 > otros sitios
- 2º proyecto en el i+12 (necesario para convocatorias en las que ponen pasta)
- Infraestructura mejor que la UCM (los de la Princesa secuencian allí)

# Citad al i+12 (no cuenta, si no)

- Dpt. de Microbiología I (Inmunología), Facultad de Medicina, Universidad Complutense de Madrid; Instituto de Investigación Sanitaria Hospital 12 de Octubre (**imas12**), Madrid, España.
- Servicio de Inmunología, Hospital Universitario 12 de Octubre; Departamento de Microbiología I (Inmunología), Facultad de Medicina, Universidad Complutense de Madrid; Instituto de Investigación Sanitaria Hospital 12 de Octubre (**imas12**), Madrid, España.

# Áreas de investigación i+12

1. Cáncer
2. Enfermedades crónicas y patologías sistémicas
3. Enfermedades raras y de base genética
4. Epidemiología y evaluación de las tecnologías y servicios sanitarios
5. Trasplantes, ingeniería de tejidos y medicina regenerativa
6. Enfermedades inflamatorias y trastornos inmunitarios
7. Enfermedades infecciosas y SIDA
8. Neurociencias y salud mental

# ÁREA 6 - ENFERMEDADES INFLAMATORIAS Y TRASTORNOS INMUNITARIOS

Asma y Enfermedades Inmunoalérgicas (H12O)

Bases Moleculares y Celulares en Enfermedades  
Reumáticas (UCM)

Enfermedades Inflamatorias y Autoinmunes  
(H12O)

**Inmunobiología Linfocitaria (UCM)**

Inmunodeficiencias e Inmunología del  
Trasplante (H12O)

# Research interests

Cell adhesion  
and signaling

Immune synapse and  
lymphocyte signalling

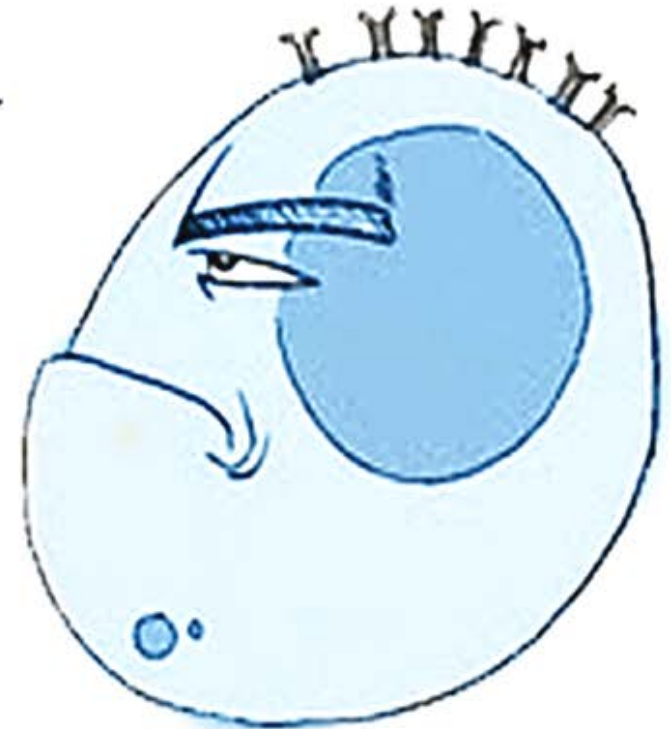


Antigen-presenting cells  
and inflammation



Epitomics

Complement  
physiopathology



T cell physiopathology  
and DNA repair