# “Jedi cells patrol the mouse”

Technical Journal Club

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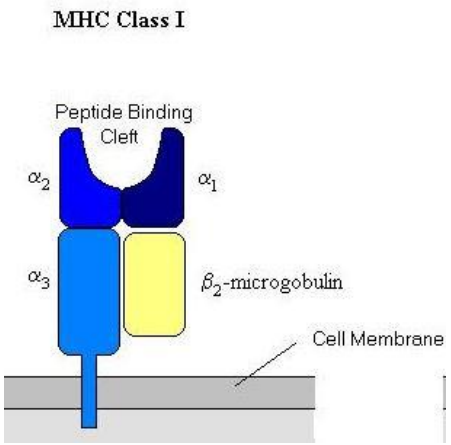
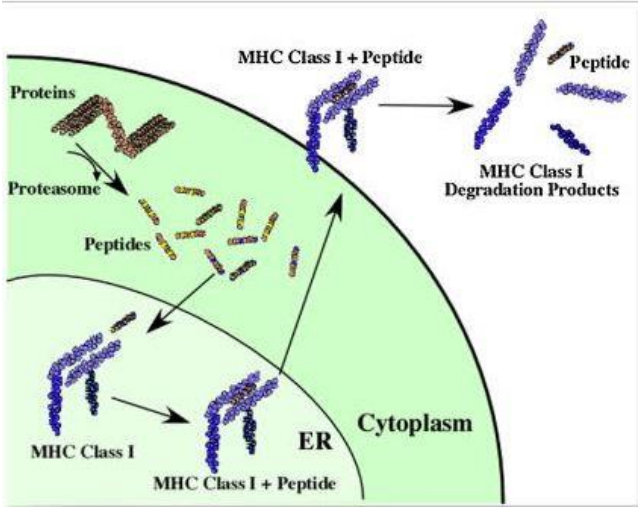
# Introduction

- JEDI= **J**ust **E**GFP **D**eath **I**nducing t- cells
  - Specific CD-8  T cells
- All EGFP expressing cells can be killed
- Enabeling the visualization  of  a t- cell antigen and studying t- cell interaction
- Applicable and useful in disease models (autoimmune disease models, cancer etc.)
  - Characterize the function of rare cell populations

# Introduction

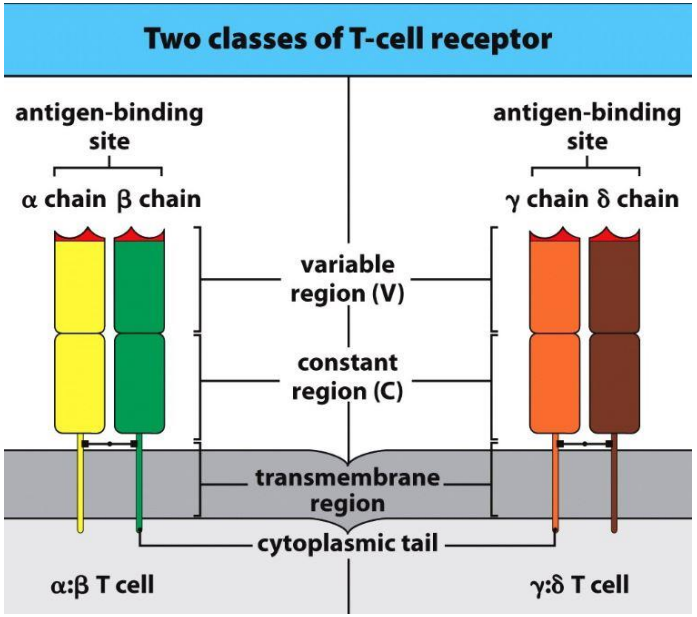
- MHC= major histocompatibility complex
- CD- 8 T- cells =  cytotoxic t- lymphocytes
- CD 8 T- cells scan the cell surface and commit binding with their t-cell receptor only with MHC- 1 complex
- Adaptive immune system
- Function: Elimination of pathogens over specific antigen presenting molecules



# Introduction

- T- cell receptor (TCR) = proteincomplex , which is specific for one single peptide MHC- 1complex
- 2 protein subunits ( family of immunoglobulins)
- Variable and constant domain
- Binding does not only depends on affinity, but also on local environment of the cell
- Activation of TCR determines the development of the cell (cytotoxic or helper cell)



# Introduction

**Obstacles**

•The study of t- cell interaction with their antigen- expressing targets has been limited by…

•Technological difficulties/ restrictions  in tracking and monitoring T- cell interactions

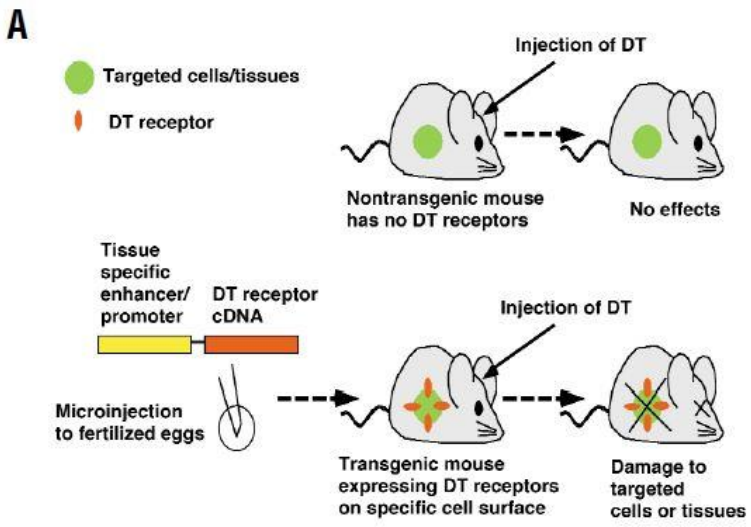
•Lack of animals and reagents that are suitable to study antigen reaction in specific cell types

•Function of  many cell population is not well characterized

•Limitation of current methods to deplete specific cells

# Introduction

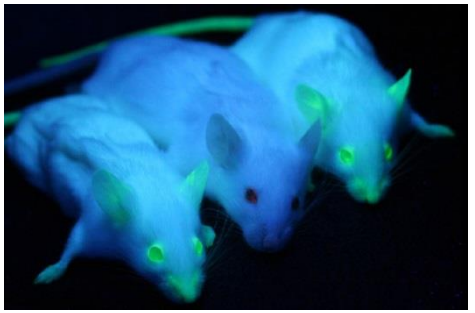
- Depletion of a cell over antibodies (few available and expensive, e.g. Rituximab, Natalizumab)
- Human diphteria toxin receptor (DTR) under cell type specific promoter
- Toxin receptor-mediated conditional cell knockout (TRECK) procedure
- Few depleting antibodies and repeated admin of diphteria toxin can be toxic
- Cell renewal quickly after lack of constant administration



**…How to proceed ?**

# Introduction

**EGFP as a model of antigen ?**



•Enhanced green fluorescent protein can be fusioned gen- specifically with any other protein

•Spatial and temporal distribution of GFP tagged cells can be easily assessed (eg. via flow cytometry and immunohistochemistry)

•100 of EGFP expressing mice are available

•EGFP expressing cancer cell lines, virus, bacteria etc.

| Mouse Strain | Reporter | Gene/Promoter | Gene/Promoter |
|---|---|---|---|
| B6.CBA-Tg(Acrv1-EGFP)2727Redd/J | EGFP | *Acrv1* | acrosomal vesicle protein 1 (mouse) |
| STOCK Tg(CAG-Bgeo/GFP)21Lbe/J | EGFP | *ACTB* | Actin, beta (chicken) |
| B6.129(Cg)-Tg(CAG-Bgeo/GFP)21Lbe/J | EGFP | *ACTB* | Actin, beta (chicken) |
| STOCK Tg(HIST1H2BB/EGFP)1Pa/J | EGFP | *ACTB* | Actin, beta (chicken) |
| B6.Cg-Tg(HIST1H2BB/EGFP)1Pa/J | EGFP | *ACTB* | Actin, beta (chicken) |
| C57BL/6-Tg(CAG-EGFP)1310sb/LeySopJ | EGFP | *ACTB* | Actin, beta (chicken) |
| STOCK Tg(CAG-Bgeo,-NOTCH1,-EGFP)1Lbe/J | EGFP | *ACTB* | Actin, beta (chicken) |
| STOCK Tg(CAG-Bgeo,-TEL/AML1,-EGFP)A6Lbe/J | EGFP | *ACTB* | Actin, beta (chicken) |
| B6.Cg-Tg(CAG-Ub*G76V/GFP)1Dant/J | EGFP | *ACTB* | Actin, beta (chicken) |
| B6.Cg-Tg(CAG-Ub*G76V/GFP)2Dant/J | EGFP | *ACTB* | Actin, beta (chicken) |
| CB6-Tg(CAG-EGFP/CETN2)3-4Jgg/J | EGFP | *ACTB* | Actin, beta (chicken) |
| B6;C3-Tg(CAG-DsRed,-EGFP)5Gae/J | EGFP | *ACTB* | Actin, beta (chicken) |
| B6.FVB-Tg(CAG-EGFP,-ALPP)2.6Ggc/J | EGFP | *ACTB* | Actin, beta (chicken) |
| STOCK Ils2tm1(ACTB-EGFP,-tdTomato)Luo/J | EGFP | *ACTB* | Actin, beta (chicken) |
| STOCK Ils2tm2(ACTB-tdTomato,-EGFP)Luo/J | EGFP | *ACTB* | Actin, beta (chicken) |
| STOCK Ils2tm2(ACTB-tdTomato,-EGFP)LuoTrp53tm1Tyj Nf1tm1Par/J | EGFP | *ACTB* | Actin, beta (chicken) |
| B6.129(FVB)-Alcamtm1Jawe/J | EGFP | *Alcam* | activated leukocyte cell adhesion molecule (mouse) |
| STOCK Ascl1tm1Reed/J | EGFP | *Ascl1* | achaete-scute complex homolog 1 (Drosophila) (mouse) |
| B6.129S-Atoh1tm4.1Hzo/J | EGFP | *Atoh1* | atonal homolog 1 (Drosophila) (mouse) |
| BKa.Cg-Ptprcb Bmi1tm1Ilw Thy1a/J | EGFP | *Bmi1* | Bmi1 polycomb ring finger oncogene (mouse) |
| STOCK Tg(Cp-EGFP)25Gaia/J | EGFP | CBF1 | C promoter (Cp) binding factor 1 (CBF1) |
| STOCK Tg(Cp-EGFP)25Gaia/ReyaJ | EGFP | CBF1 | C promoter (Cp) binding factor 1 (CBF1) |
| B6.129S6-Ccr6tm1(EGFP)Irw/J | EGFP | *Ccr6* | chemokine (C-C motif) receptor 6 (mouse) |
| B6.129S2-Cd207tm2Mal/J | EGFP | *Cd207* | CD207 antigen (mouse) |
| B6.129S2-Cd207tm3Mal/J | EGFP | *Cd207* | CD207 antigen (mouse) |
| Strain Name: NOD/ShiLt-Tg(Cd4-EGFP)1Lt/J | EGFP | *Cd4* | Cd4 molecule (rat) |
| Strain Name: B6.NOD-Tg(Cd4-EGFP)1Lt/J | EGFP | *Cd4* | Cd4 molecule (rat) |
| STOCK Cdx2tm1Yxz/J | EGFP | *Cdx2* | caudal type homeobox 2 (mouse) |

# Paper 1

- Generating mice with a specific TCR over **somatic cell nuclear transfer- SCNT**

*Science*. 2010 April 9; 328(5975): 243–248. doi:10.1126/science.1178590.

## Transnuclear mice with pre-defined T Cell Receptor specificities against Toxoplasma gondii obtained via SCNT

**Oktay Kirak**[1,4], **Eva-Maria Frickel**[1], **Gijsbert M. Grotenbreg**[1,3], **Heikyung Suh**[1], **Rudolf Jaenisch**[1,2,4], and **Hidde L. Ploegh**[1,2,4]

[1]Whitehead Institute for Biomedical Research, Cambridge, MA 02142

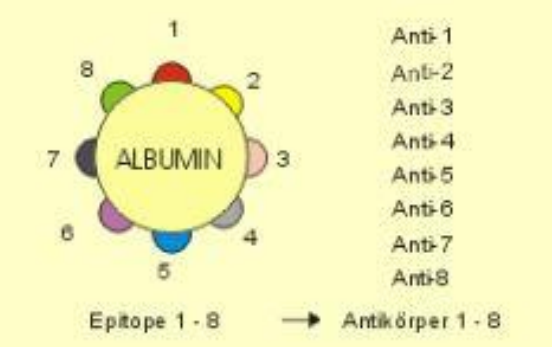
[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142

# Advantage of SCNT approach

- Normally TCR are isolated over a T- cell culture and repeated antigen stimulation
  → time consuming and elaborate
- Random genomic integration of TCR alpha or beta chain and expression from nonendogenous promoters
- Epigenetic reprogramming via SCNT enables TCR expression from endogenous loci without genetic modifications
- Relatively faster and easier than cell cultured TCR`s and no experimentally induced alterations
- Specificity of B and T- cell receptors is determined over the broad combination of V, D, J gene arrangements
- SCNT of lymphocytes with known specificity (V,D,J) will be tranferred to embryonic stem cells

# SCNT approach

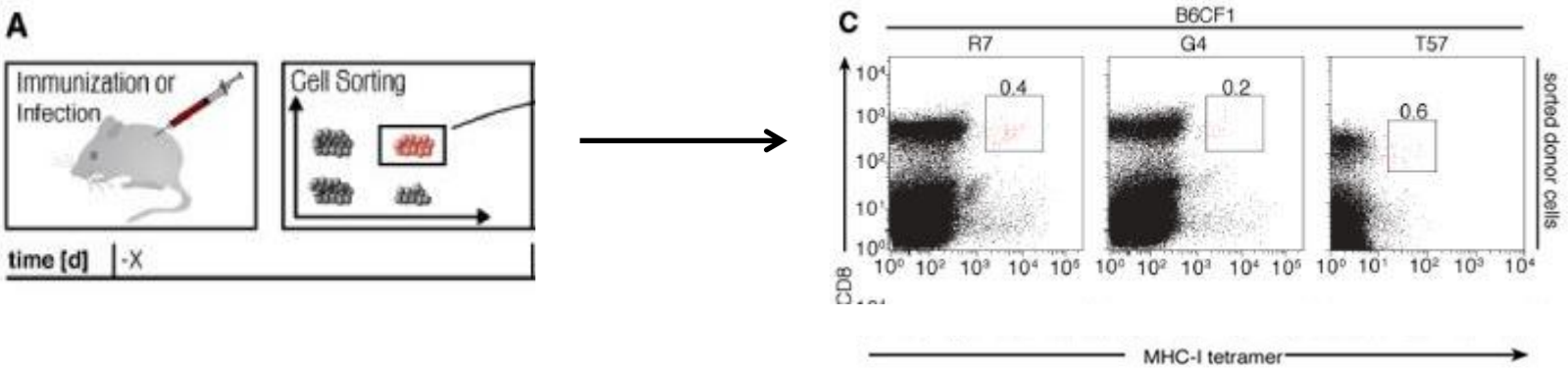
- Specificity was determined via indentification of 3 processed epitopes (R7), (T57) and (G4) restricted by MHC1 recognized by CD- 8 T- cells, specific for T. gondii
- CD8 T- cells specific for R7, G4 and T57 where used for SCNT



# SCNT approach

**Isolation of donor cells**

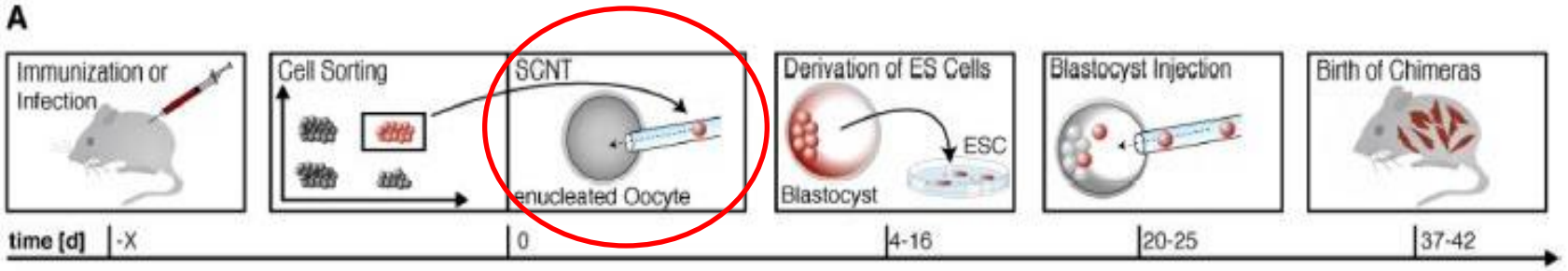
- Infection of BL/6 x Balb/c F1 (B6CF1) mice with Toxoplasma gondii (i.p. injection)
- Isolation and homogenisation of the spleen during peak of  acute infection and placement into a dish containing 6 mL red blood cell (RBC) lysis buffer
- The spleen is minced and incubated in the RBC lysis buffer for 5 min at RT
- After several centrifugation steps and removal of supernatant fluorescently labeled antibodies to detect cells of interest and fluorescently labeled MHC-I tetramer are added to cell suspension
- Cell sorting of CD 8 T- cells via FACS



# SCNT approach

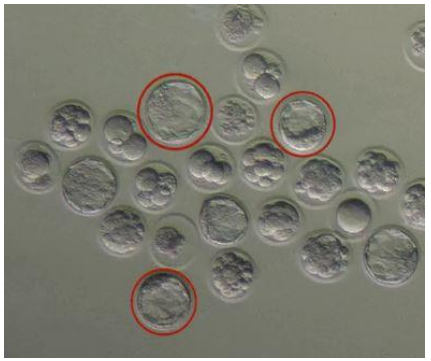
**Somatic Cell Nuclear Transfer**

- Isolation of oocytes from euthanized pseudo- pregnant mice (previous HCG stimulation)
- Oocytes arrested at metaphase-II and where inhibited for further division using Cytochalasin B
- Preparation and enucleation of oocytes
- Nucleus isolation from determined CD-8 cells under an inverted microscope
- Nucleus insertion into enucleated oocytes from Bl6/Balb/c mice can be performed now

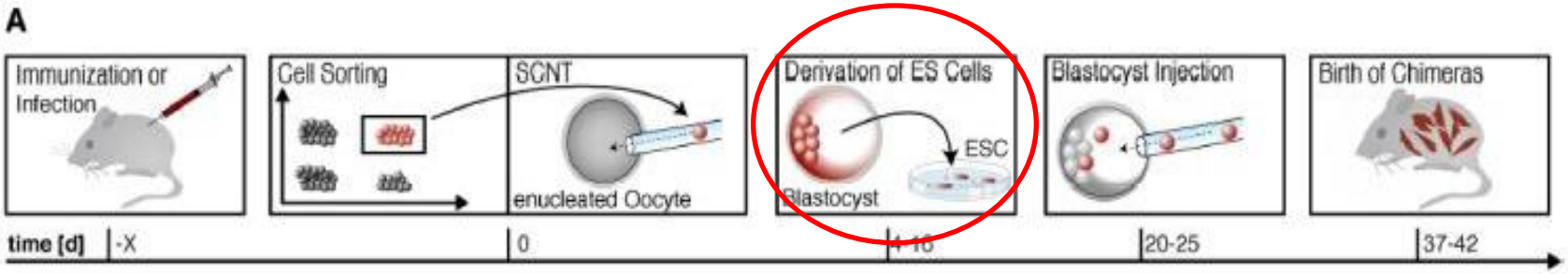


# SCNT approach

**Derivation of embryonic stem cells**



- After medium changes and incubation steps of oocytes, blastocyst state is reached after 3.5 days
- Isolation of blastocytes and further cultured for 5-7 days in an incubator at 37°C, 5% $CO_2$ ES cell medium
- If ESC derivation was successful, ESC colonies should be visible after 7-10 days and isolated into a petri dish



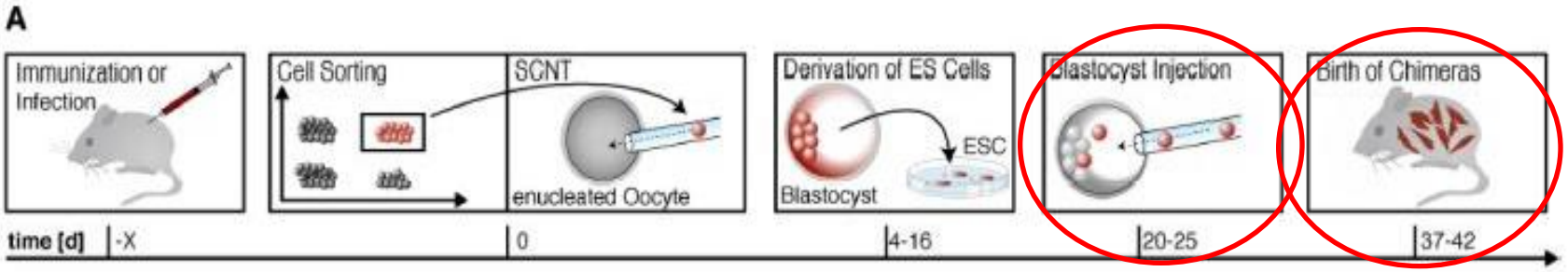
# SCNT approach

**Blastocyst injection and embryo transfer**

- Fertilized embryos are isolated from the oviduct from pregnant mice and cultured for 3 days
- New derived blastocytes gain injection of derived ES cells

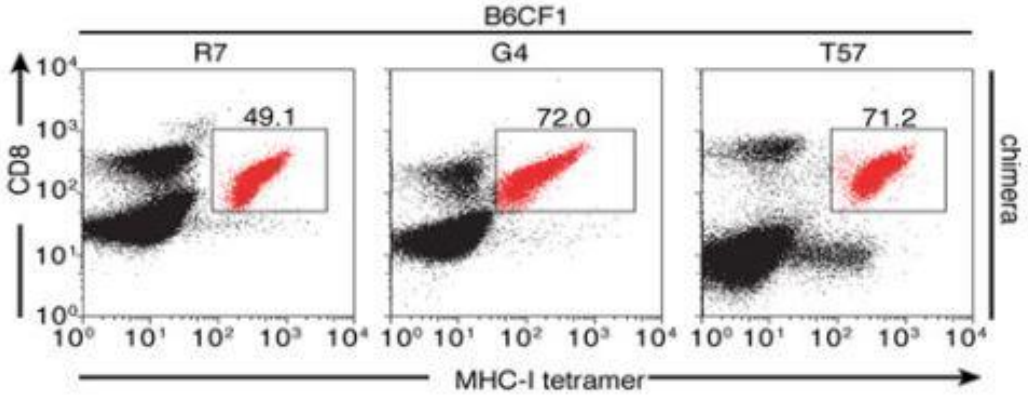
**Embryo Transfer**

- The transfer of embryos into pseudo-pregnant females represents a surgical procedure



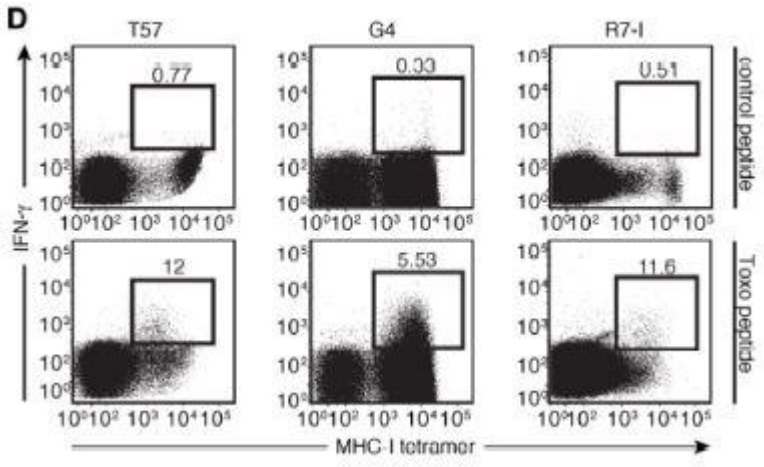
# SCNT approach

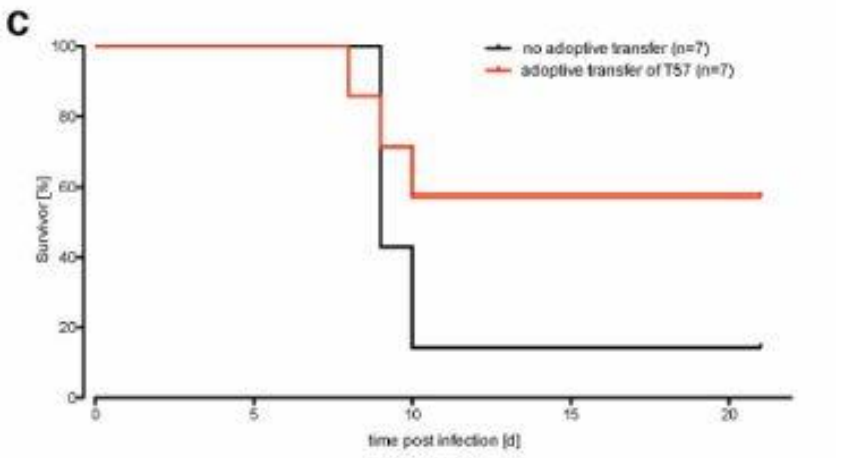
- Litters where analyzed via flow cytometry for successful carry of the corresponding TCR with correct specificity



# SCNT approach

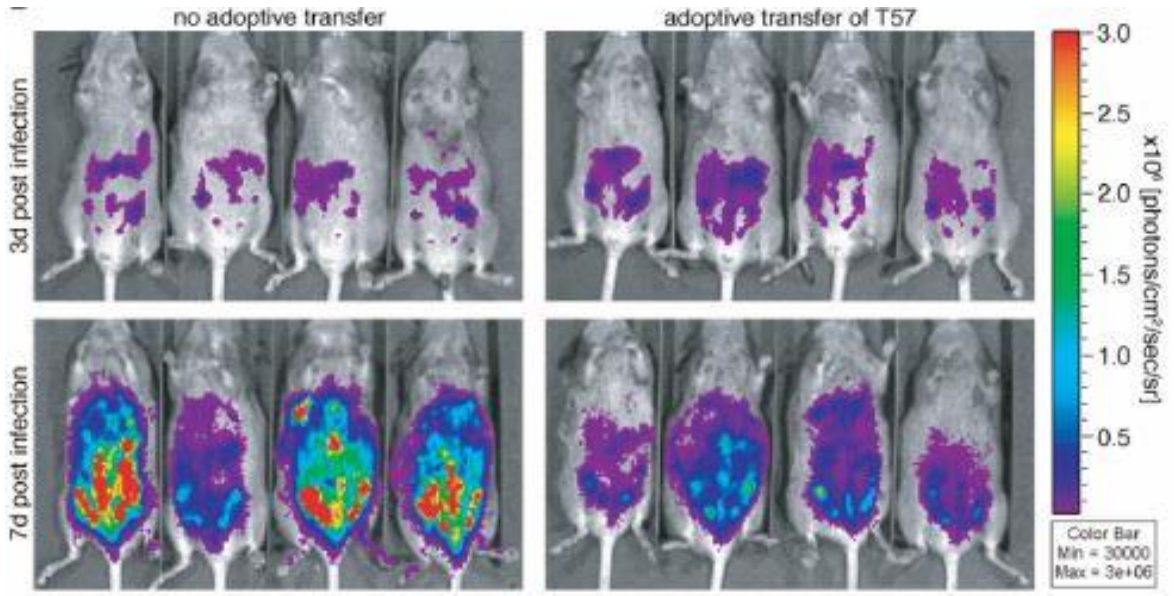
- Stimulation of antigen presenting cells to check ability of CD 8 T- cells to produce Interferon gamma (in vitro)
- In vivo: Testing immuno response towards  infection with T. gondii on chimeric mice and wt Bl6 mice as controls



# SCNT approach

- Live imaging with luciferase expressing t. gondii shows reduced parasite load in the group, which received CD 8 t- cells from chimeric mice



# Conclusion- paper 1

- Successful transgenic mouse model which shows specific CD-8 TCR transfer
- Transnuclear mouse model  which expresses  the TCR from the endogenous locus under endogenous promoter
- No cultured t- cell clones, which is time consuming, elaborate and sensitive to genetic modification
- Applicable also for CD4- T cells and B- cells
- New hallmark to investigate various immunological diseases

# Paper 2

**What`s new ? JEDI cells??**

nature biotechnology

## GFP-specific CD8 T cells enable targeted cell depletion and visualization of T-cell interactions

Judith Agudo[1], Albert Ruzo[1], Eun Sook Park[1], Robert Sweeney[1], Veronika Kana[2], Meng Wu[1], Yong Zhao[1], Dieter Egli[3], Miriam Merad[2,4,5] & Brian D Brown[1,4–6]

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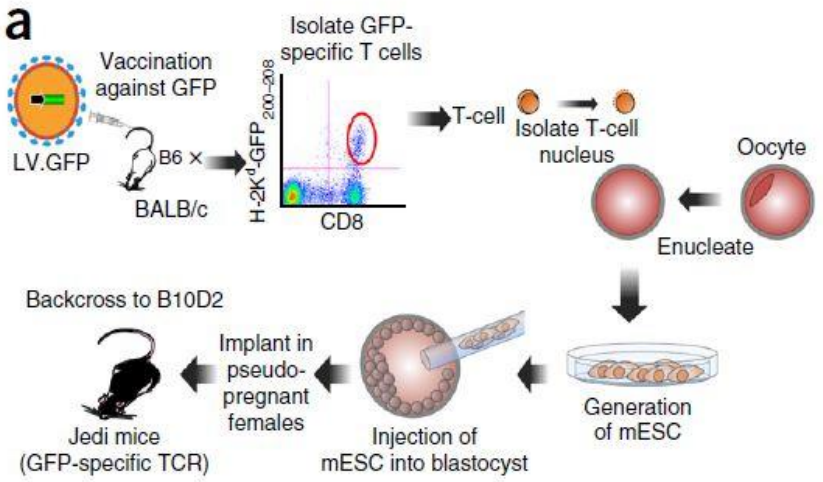
# How to generate the Jedi mice?

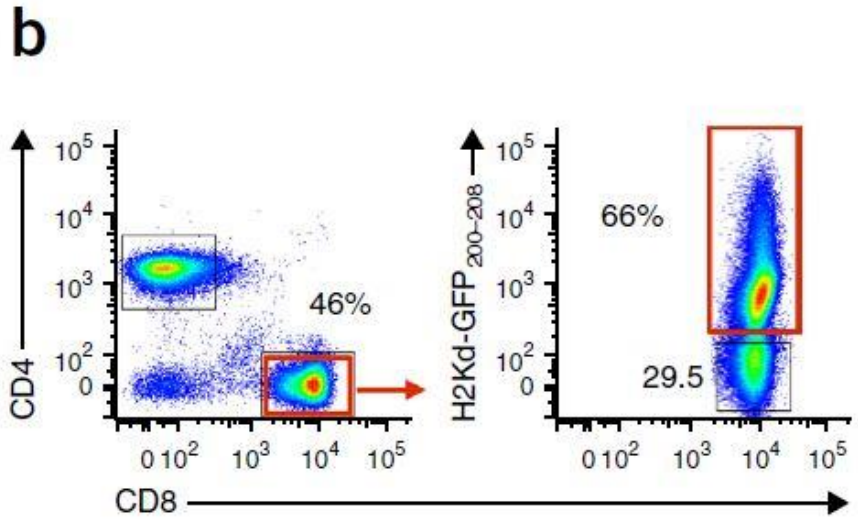
- Jedi mice- express EGFP specific TCR by using the SCNT approach
- Crossing between BALB/c and C57BL/6 and immunized F1 progeny mice with a lentivirus vector encoding EGFP
- After 2 weeks: Tetramer isolation of CD8 T-cells, which express the specific epitope of EGFP on the TCR`s presented on H-2Kd (H2-Kd is a mouse MHC class I protein which contains 1 Ig-like C1-type (immunoglobulin-like) domain)
- Backgross to 8 generations with expression of immunodominant epitope (H-2KD allele)



# Frequency analysis of GFP isotope

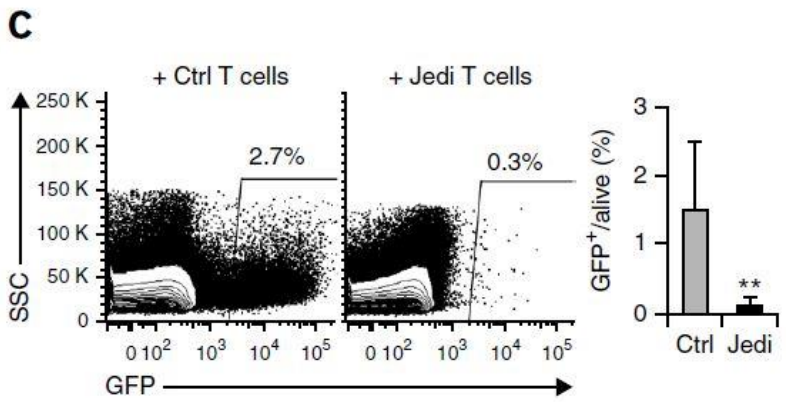
**Flow cytometry of splenocytes**

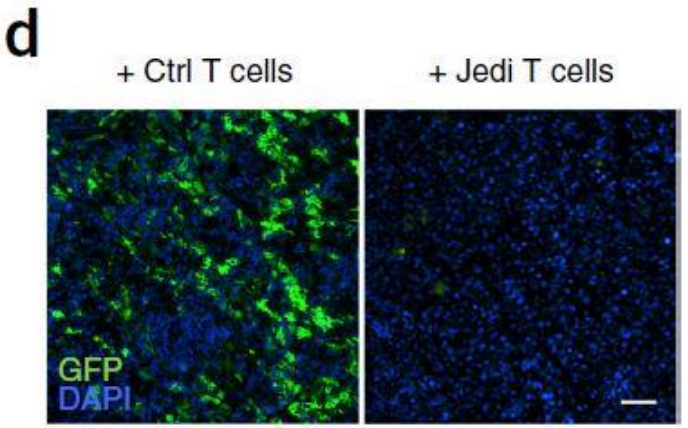
- More than 50% of CD8 T- cells in all jedi mice where specific for GFP H-2Kd using CD3, CD4 and CD8 staining and H-2Kd GFP isotope



# Removal of infected cells detectable?

- Capability of killing EGFP expressing cells through Jedi cells
- Injection of  LVEGFP (lentivirus EGFP fluorescent protein) in mice which has over 95% homology to EGFP
- Injection results into infected splenocytes
- Injection of CD8 T- cells  from Jedi mice and control mice
- Cytotoxic capacity of Jedi cells regarding elimination of EGFP + splenocytes



# Application in disease models

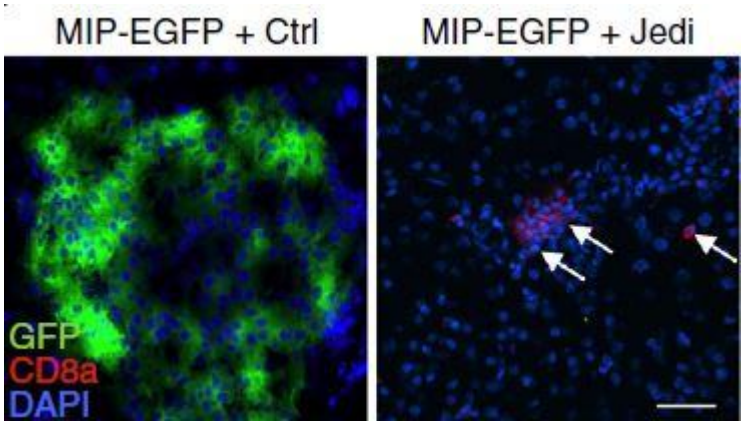
**Advantage :**

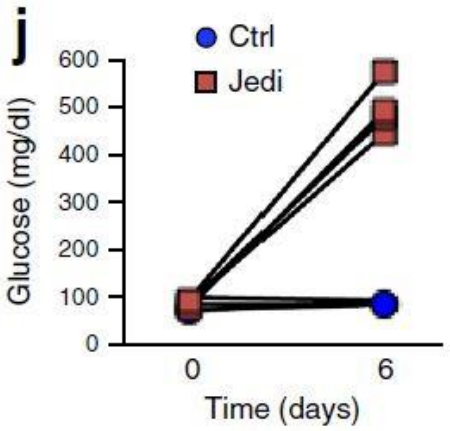
•Qualitative and quantitative assessment at cellular level, in order to test, if all target cells can be killed

•Visualizable disease model: diabetes type 1- Insulin promoter

•Injection of jedi cells in mice which express EGFP under the control of the Insulin promoter (MIP EGFP)

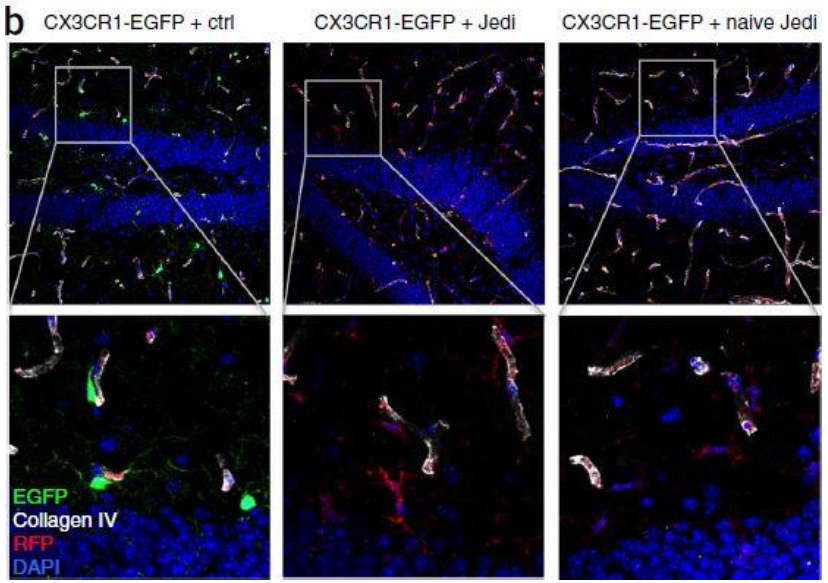
•After 6 days no EGFP expressing beta cells in pancreas where detected

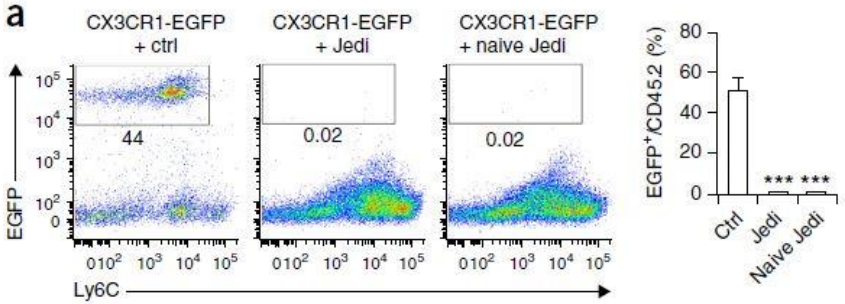


# Application in antigen- dependent T- cell interaction

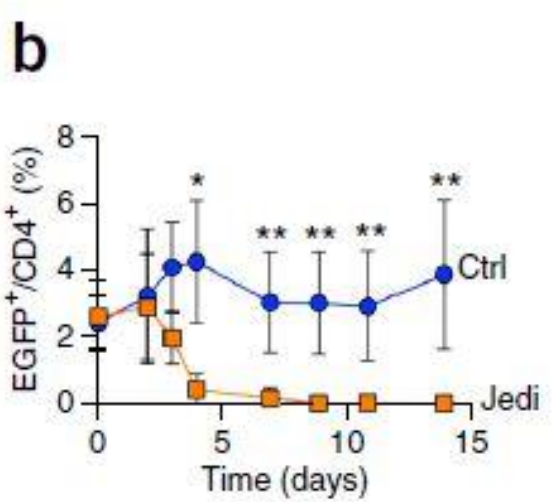
- Antigen- presenting capacity of microglia using CX3CR1- EGFP mice
- Chimeric mouse model to avoid influence of EGFP + monocytes and myeloid cells
- Dentate gyrus 3 weeks after injection of Jedi cells shows no EGFP+ cells
- Conclusion: naive T- cells can enter the brain when microglia present an antigen recognized by the t- cells
- New applicable model to deplete microglia specifically over a long time

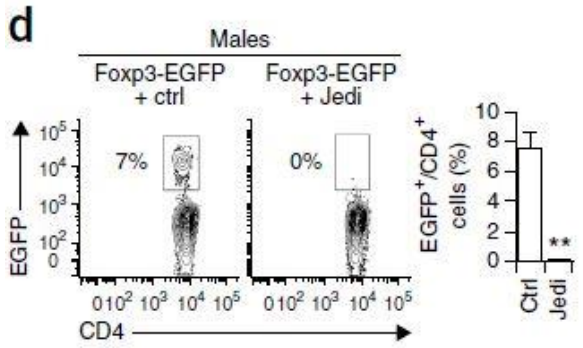


# Jedi cells in a cell depletion model

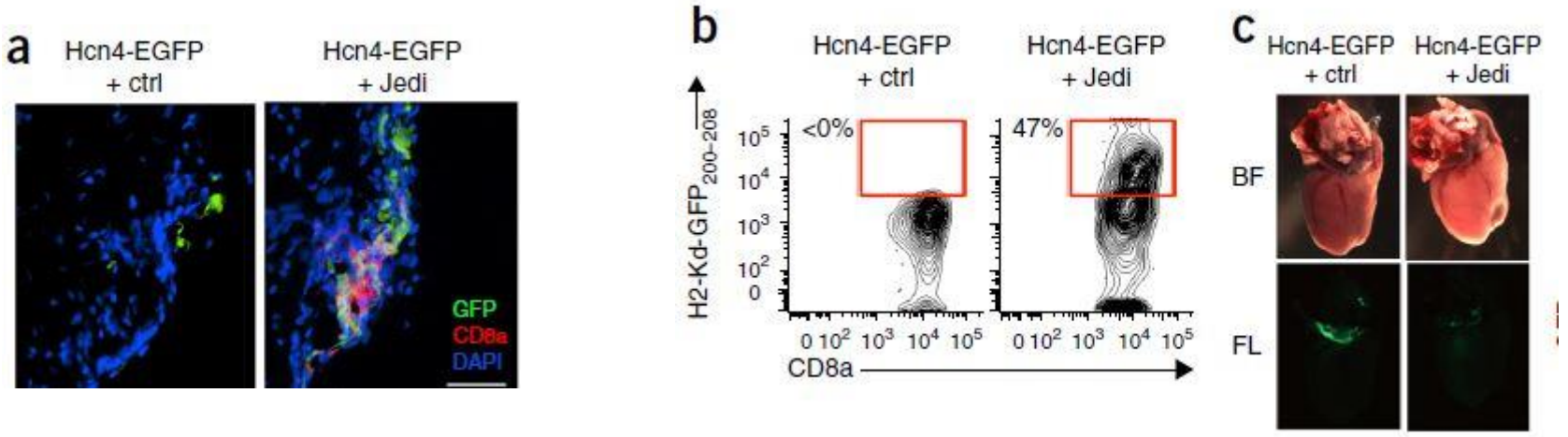
- Foxp3+ regulatory T cells = mice with a Foxp3 deficiency lack regulatory T- cells and develop fatal autoimmune pathology
- All Fox3 cells are EGFP positive
- Injection of Jedi cells and control CD8 cells
- Assessment after 6 days: Complete elimination and mice develop severe immune disregulation
- Depletion lasts for more than 14 days, even though Foxp3 cells are known to be renewing



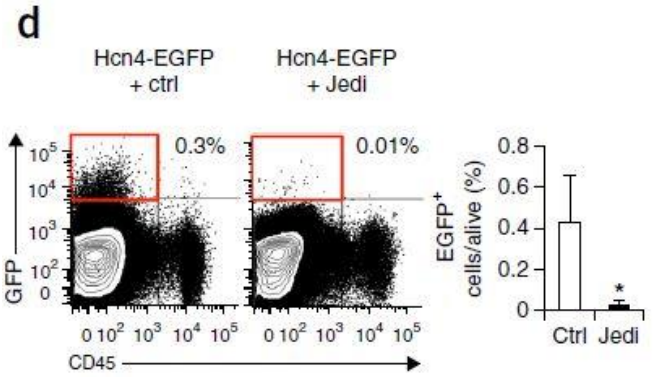
# Jedi cells and rare cell types

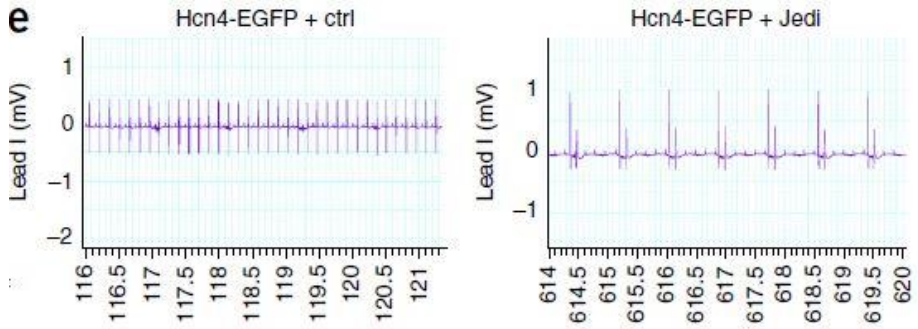
- Hcn4+ cells = heart cells and rare regarding total cell population  < 10 000 per animal
- Function: Pacemaking, but general importance and necessity is strongly discussed
- Hcn4 cells are EGFP positive under the control of the Hcn4 promoter

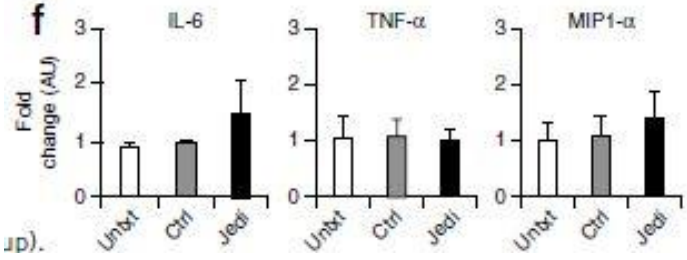


# Jedi cells and rare cell types?

- Flow cytometry of EGFP + cells in the heart 10 days after the t- cell transfer with complete depletion of Hcn4 cells
- CD- 45 staining marks hematopoetic stem cells
- Outcome of depletion: ECG shows bradicardia and av-block in all mice which received jedi cells and had lack of Hcn4 cells
- Lack of inflammation (f) supports the result



# Conclusion- paper 2

- Valuable tool to asses visualization of an antigen
- Monitor T cell trafficking in health and disease
- Testing cell vaccine and discovery targets
- Preclinical testing of immune modulatory drugs
- Broad availability of different EGFP mice
- Many cell populations and their function is not well characterized and can be studied
- New method of cell depletion, which reflects the nature it`s most

**Thank you for your attention!**