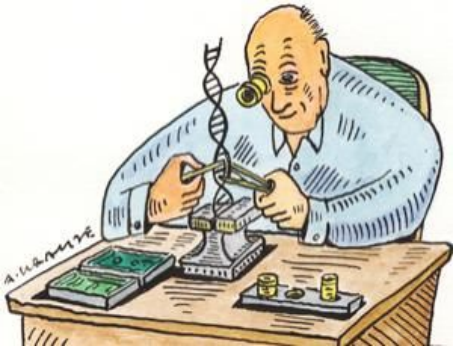
UniversitätsSpital Zürich

Designer Cells Finely Tuned for Therapy



Gianluca Spaltro

MD-PhD student
Prof. Manz group

Technical Journal Club, 12.07.2016

RESEARCH ARTICLE

SYNTHETIC BIOLOGY

Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis

Lina Schukur,[1] Barbara Geering,[1] Ghislaine Charpin-El Hamri,[2] Martin Fussenegger[1,3]*

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16 Dec 2015

New trends in therapies

Targeted therapy

Avoids healthy cells and goes directly to affected cells

Personalized medicine

Drug A

Drug B

Drug C

Medical decisions are taken according to genetic (and economical) background

However...

Drugs administration is still the same:

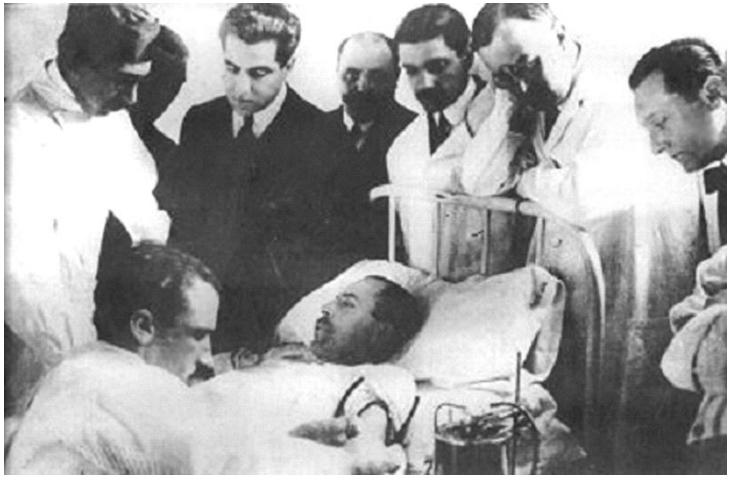
- Once/twice a day
- Full or empty stomach
- Dose depends on body weight
- “Trial and error” approach

While...

Cell intrinsic therapeutic capabilities:

- Ability to sense biological macromolecules
- Autonomously regulate dosage and bioavailability
- Adapt therapeutic effect to external stimuli

History of “cell-based” therapies



Dr. Luis Agote, Buenos Aires, 1914

First blood transfusions in early 1900s and whole organ transplantations

History of “cell-based” therapies

Bone Marrow Transplantation (2008) **42,** S49–S52; doi:10.1038/bmt.2008.121

Hematopoietic cell transplantation for correction of primary immunodeficiencies

A H Filipovich[1]

[1]Immunodeficiency and Histiocytosis Program, Division of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

“ The first hematopoietic cell transplants (HCT) with durable success in humans were reported in 1968 in three patients with primary immunodeficiencies (PIDs) who received grafts from HLA-matched siblings. Significant progress has been made with HCT for PID over the past 40 years, allowing the vast majority of children with PID, worldwide, access to relatively well-matched and safer procedures to correct their otherwise lethal disorders. Important advances (...) ”

History of “cell-based” therapies

1990: Birth of gene therapy

Two patients with PID were treated with their own *ex vivo* genetically corrected lymphocytes.

Later on:

- Ex vivo engineered autologous HSC transplant
- Ex vivo engineered T-cells

Limitations

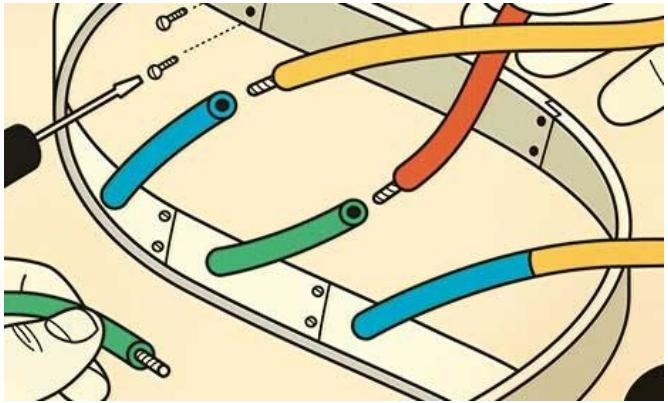
- Inefficiency
- Graft rejection
- GvHD
- Vector-mediated mutagenesis
- Inadequate therapeutic effects

Need for bioengineering tools to create safe and predictable systems that can regulate cellular behaviour

Synthetic biology

“The design and construction of new biological parts, devices, and systems and the redesign of existing, natural biological systems for useful purposes”

MIT/Harvard/Berkeley consortium



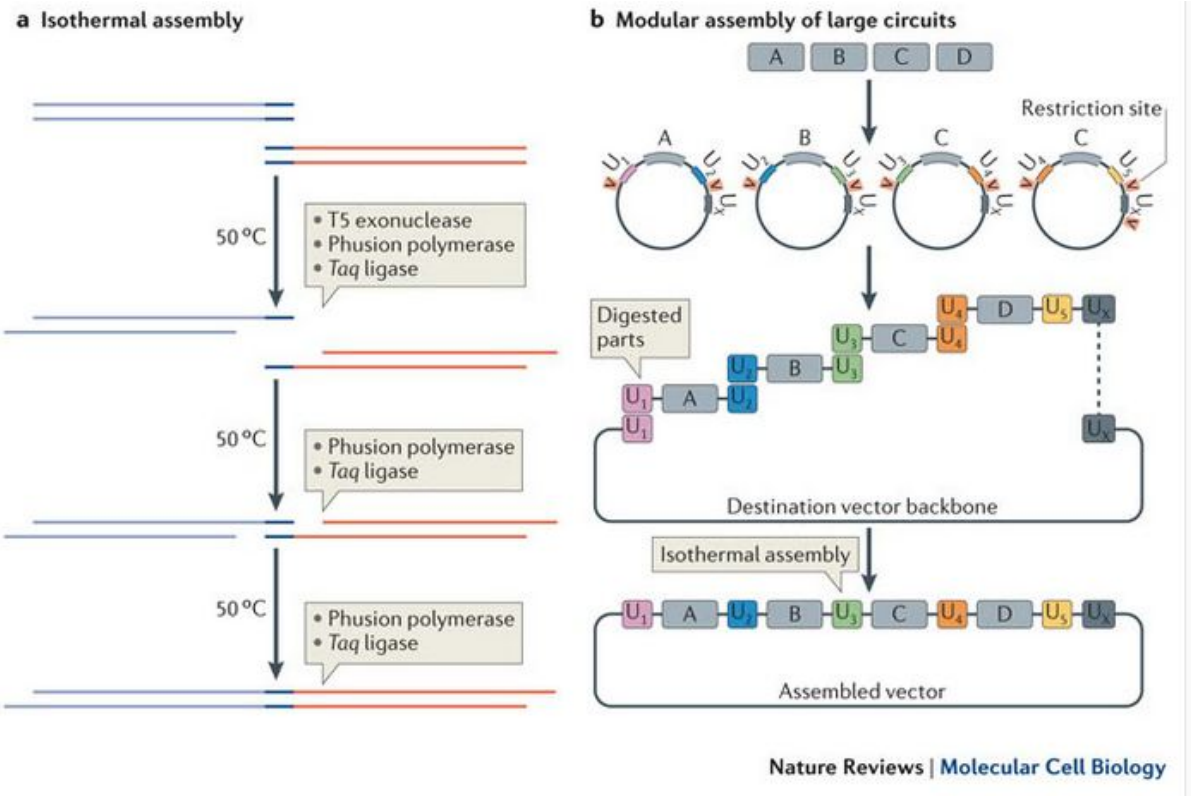
http://syntheticbiology.org

Building biological systems

Biological components from different organisms can be combined into artificial networks that can operate either in parallel or together with natural biological systems.

- Transcriptional control
- Translational control
- Protein turnover regulation
- Therapeutic designer cells

Constructing large DNA circuits



I) Pieces of DNA that share terminal sequence overlaps are assembled at 50° using three different enzymes.

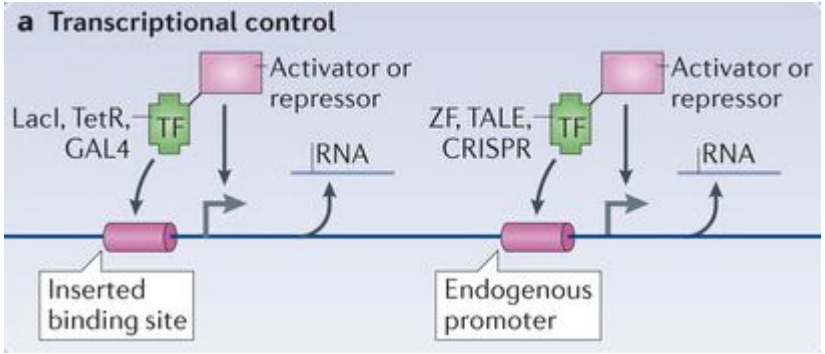
II) T5 exonuclease generates single strand overhangs

III) Phusion DNA polymerase fills the gap

IV) Taq ligase seals the nicks

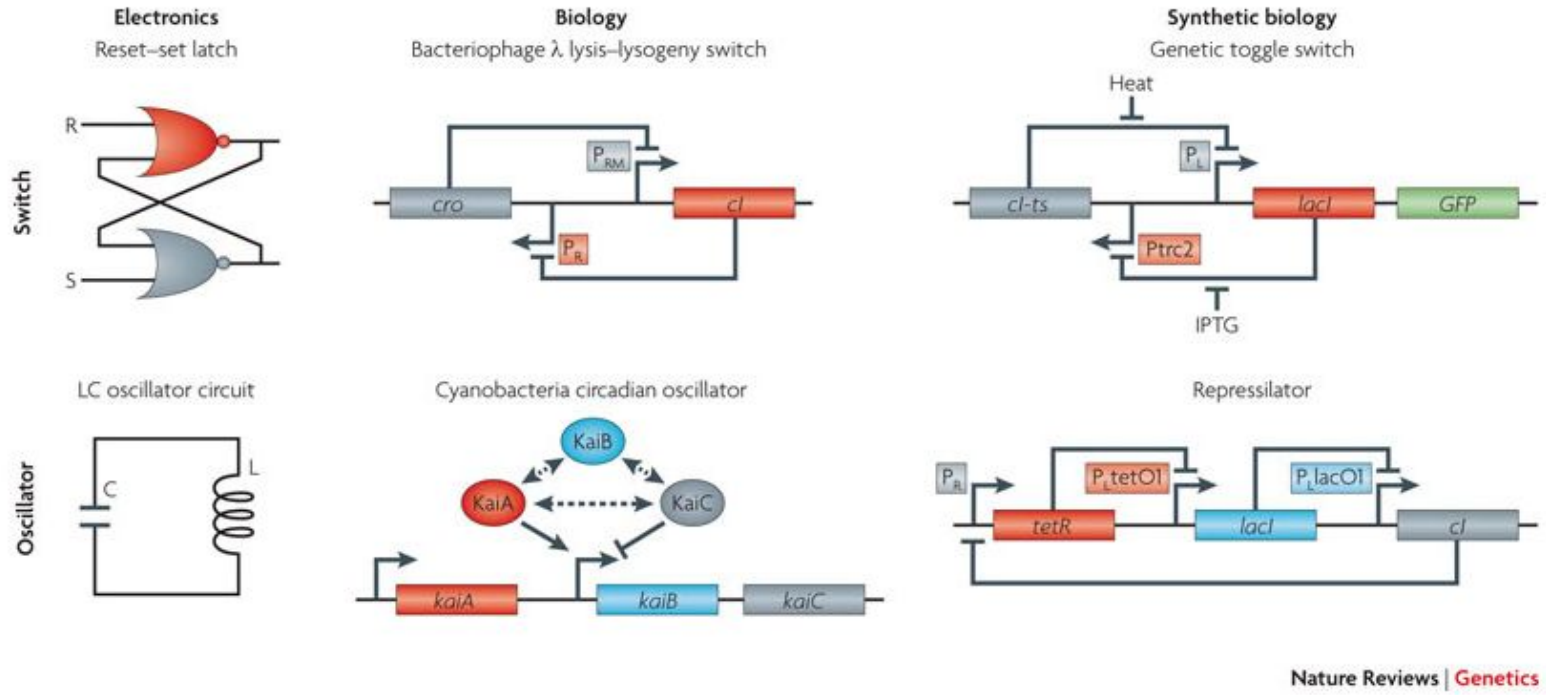
Transcriptional control

- Largest number of mammalian synthetic circuits to date
- Transcription factors: DNA binding domain and transcriptional activation/repression domain
- First generation: existing TF & synthetic promoters
- New generation: programmable TF & endogenous promoter



Nature Reviews | Molecular Cell Biology

Early synthetic biology designs



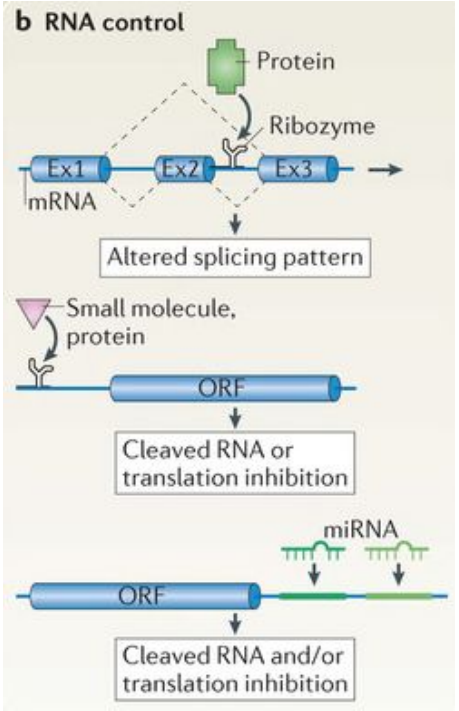
Translational control

- Increasingly important
- Fast-acting (no translation required)
- Fine-tune translational control of synthetic genes

Based on **Aptamers**: oligonucleic acids that bind to a specific target molecules (small molecules, protein or nucleic acid):

→ self-cleaving effector

Or **miRNA** on 3'UTR

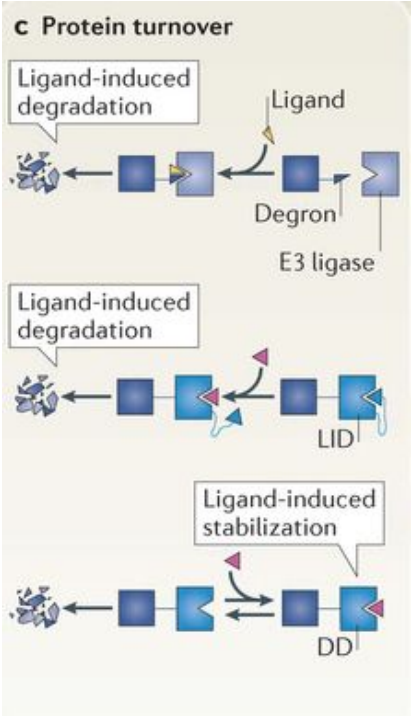


Protein turnover regulation

- Rapid, post-translational control of protein activity

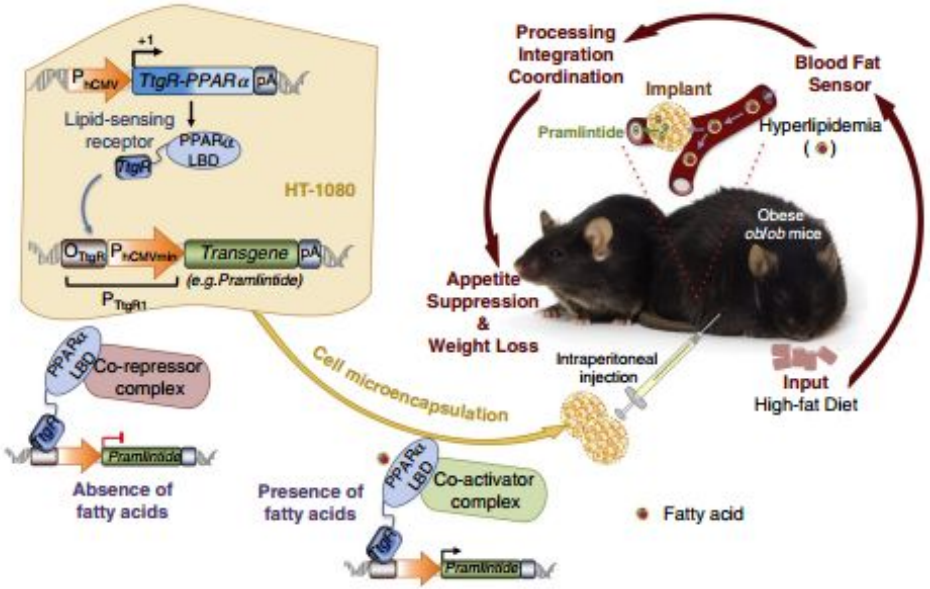
Ligand-induced protein degradation: fusing degradation recognition site (degron) to the target protein. When the ligand is present, the degron is bound by E3 ubiquitin ligase.

Ligand-induced degradation (LID) domain: when fused to a protein confers stability in the absence of a ligand and causes degradation in the presence of the ligand (or viceversa).



Therapeutic designer cells

- Implantation of genetically engineered encapsulated cells to deliver gene control circuits
- Biocompatible and semi-permeable material
- Possibility to treat complex diseases
- Construction of logic gates

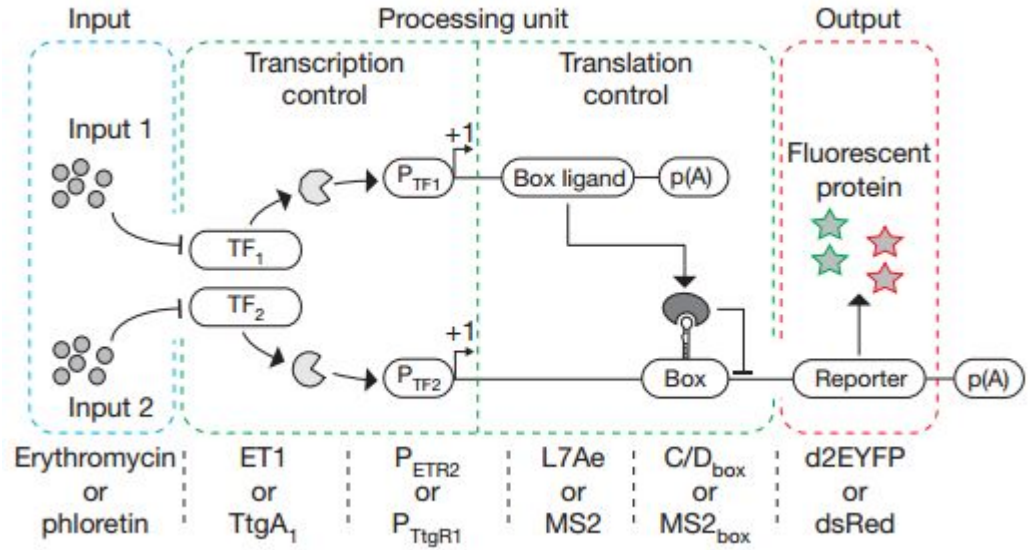


Rossger K. et Al., Nat Commun 2013

Logic gates in mammalian cells

Digital logic gate = device that implements Boolean logic on one or more input to create a single output

Boolean algebra = logical calculus of truth values at the basis of all devices that process information (binary)

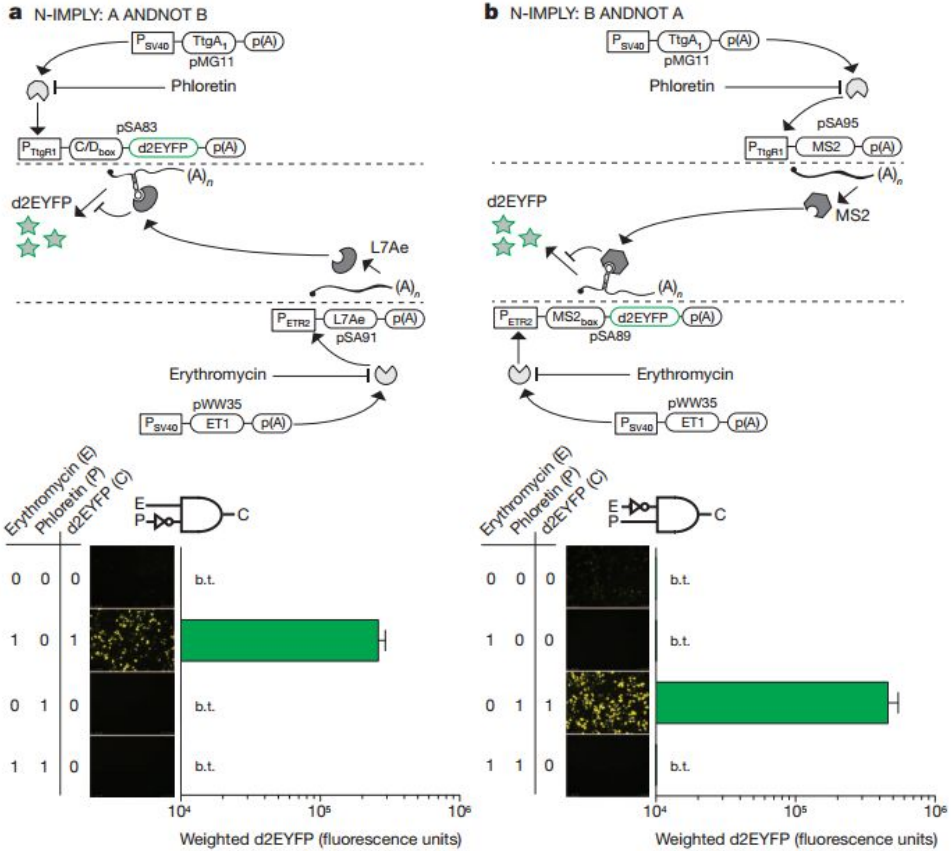


Auslaender S., et Al., Nature, 2012

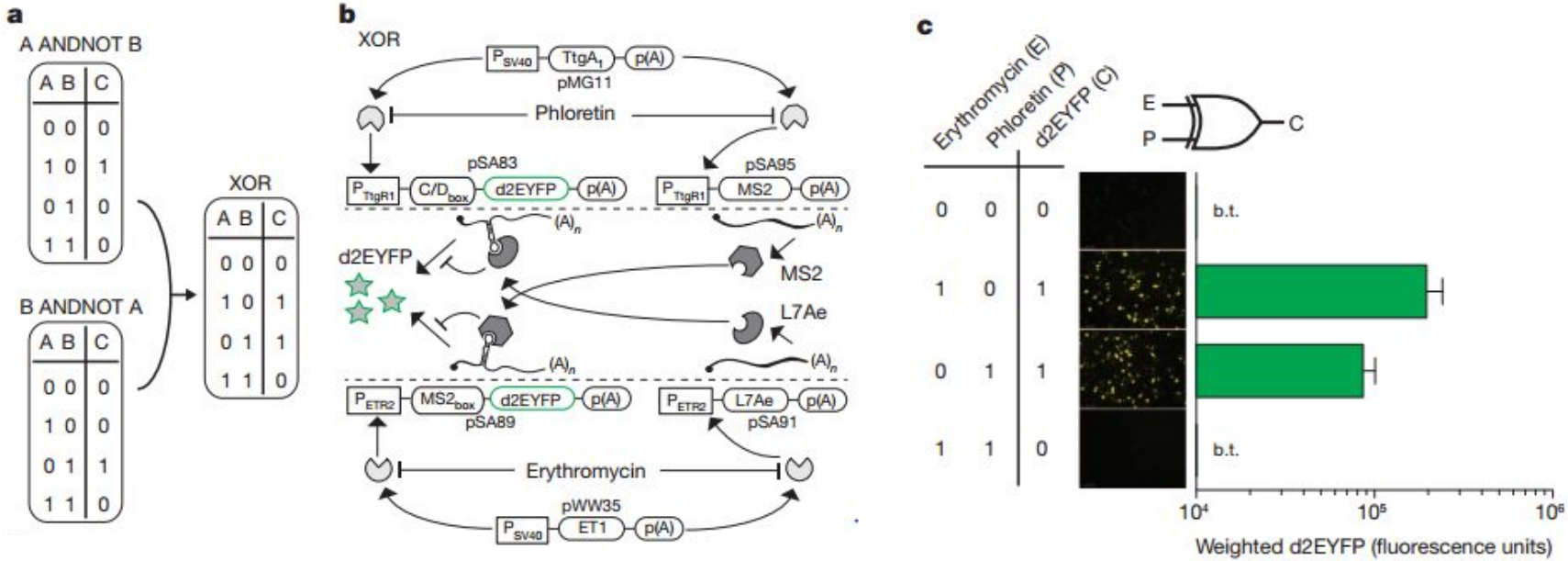
Logic gates in mammalian cells

N-IMPLY

transfected HEK-293 cells are programmed to produce d2EYFP exclusively in the presence of erythromycin and not phloretin (or viceversa)



Logic gates in mammalian cells

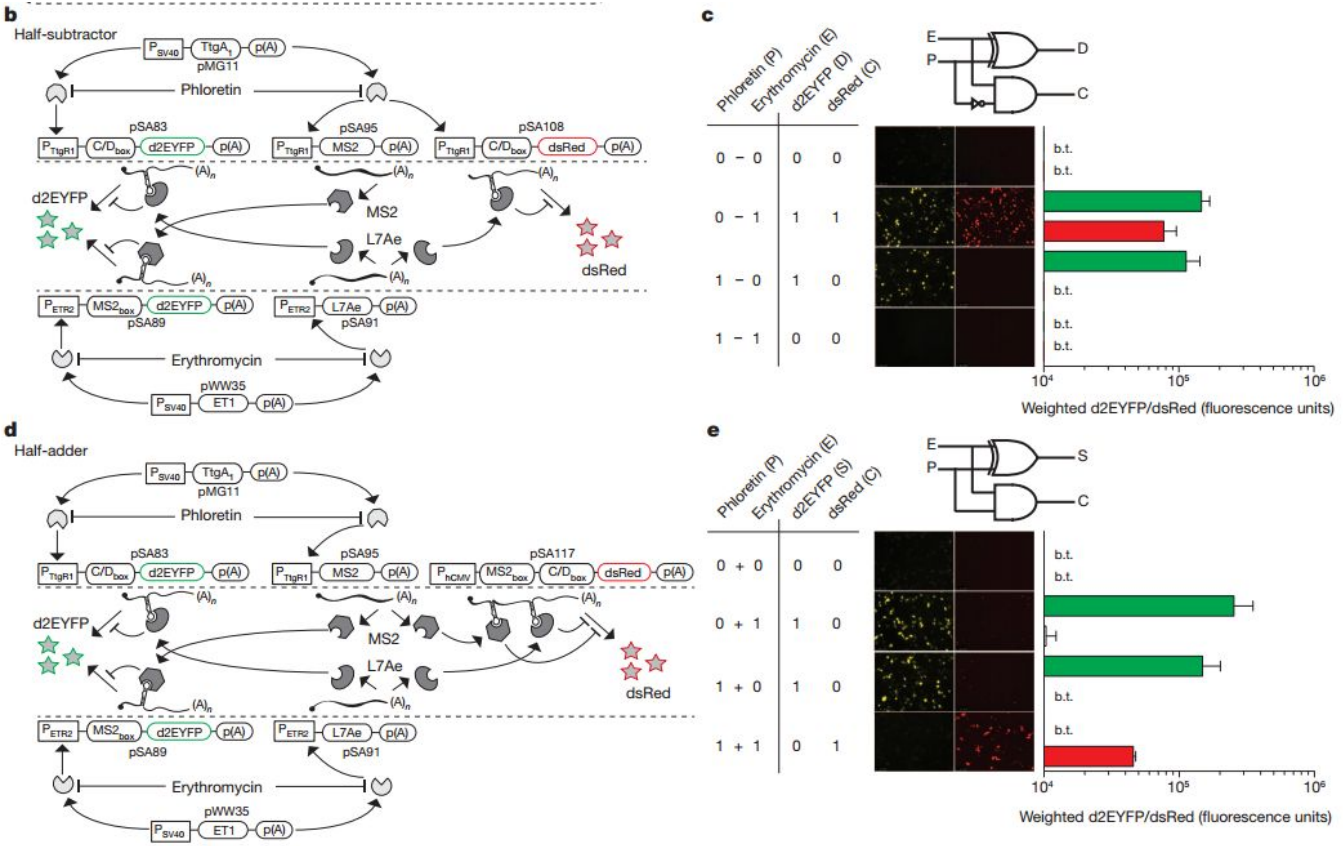


XOR circuit = output is ON when exactly one of the input signals is present

Logic gates in mammalian cells

XOR - N-IMPLY

XOR - AND



RESEARCH ARTICLE

SYNTHETIC BIOLOGY

Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis

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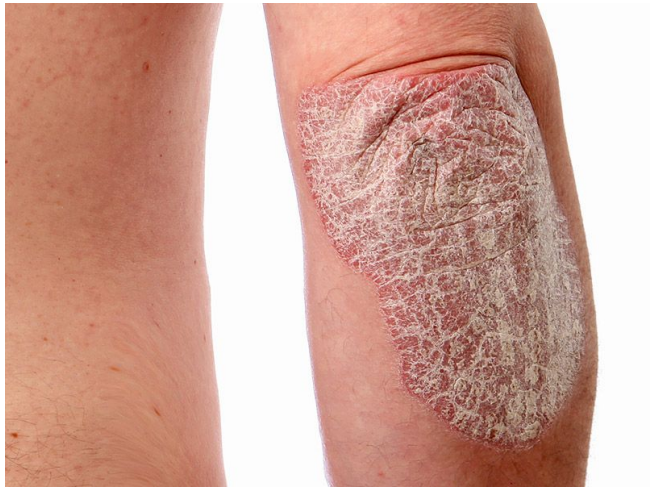
*Corresponding author. E-mail: fussenegger@bsse.ethz.ch

Science Translational Medicine
16 Dec 2015

Psoriasis

- Common chronic relapsing-remitting inflammatory skin disease
- Characterized by itchy red scaly skin lesions
- Associated with increased risk of immune-mediated diseases (Chron's, Ulcerative colitis), cardiovascular diseases, metabolic disorders (diabetes, obesity)
- Causes are largely unknown
- Genetic component and environmental triggers (infections, lifestyle, medications)



Pathophysiology

Erroneous crosstalk between keratinocytes and dendritic cells

Recruitment of immune cells (adaptive and innate) to the epidermis

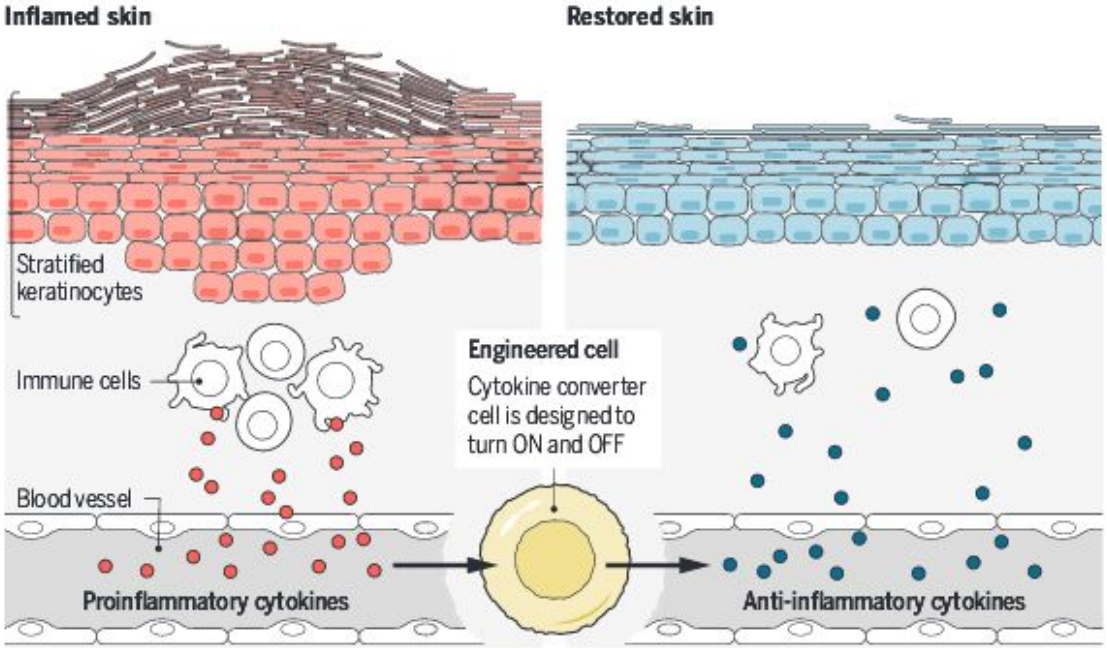
Release of TNF and IL22

Keratinocytes proliferation

Therapy

- Currently no cure (just symptomatic treatment)
- Immunosuppressive drugs
- Phase 2 trial using immunomodulatory cytokines IL4 and IL10
  (however their short half-life requires continuous daily administration)

Therapy


Inflamed skin
Restored skin
Stratified keratinocytes
Immune cells
Engineered cell
Cytokine converter cell is designed to turn ON and OFF
Blood vessel
Proinflammatory cytokines
Anti-inflammatory cytokines

Design

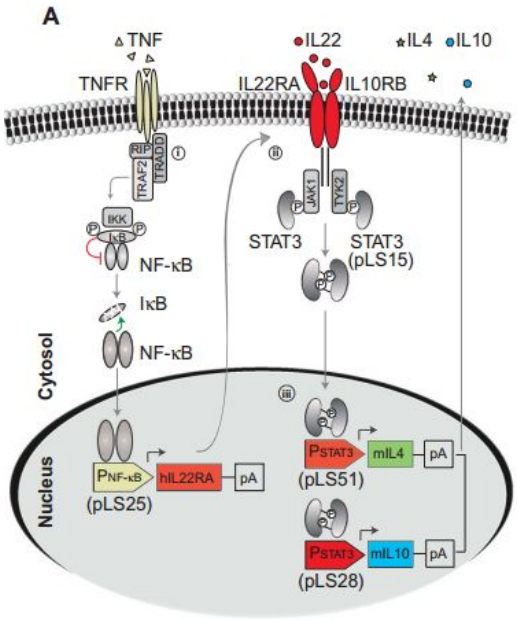
I) TNF activates TNFR1A

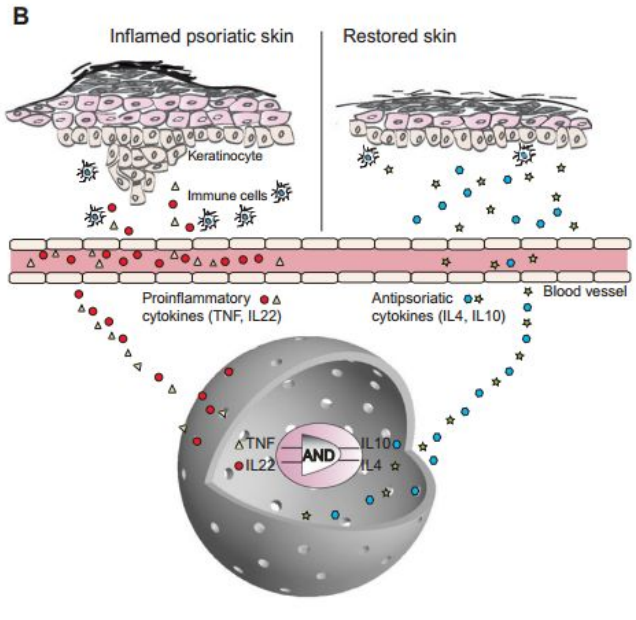
II) NFkB-triggered expression of pLS25

III) In the presence of IL22 ILRA heterodimerizes with endogenous IL10RB

IV) Ectopically expressed STAT3 translocates to the nucleus

V) Activation of Pstat3 promoters driving expression of IL4 and IL10



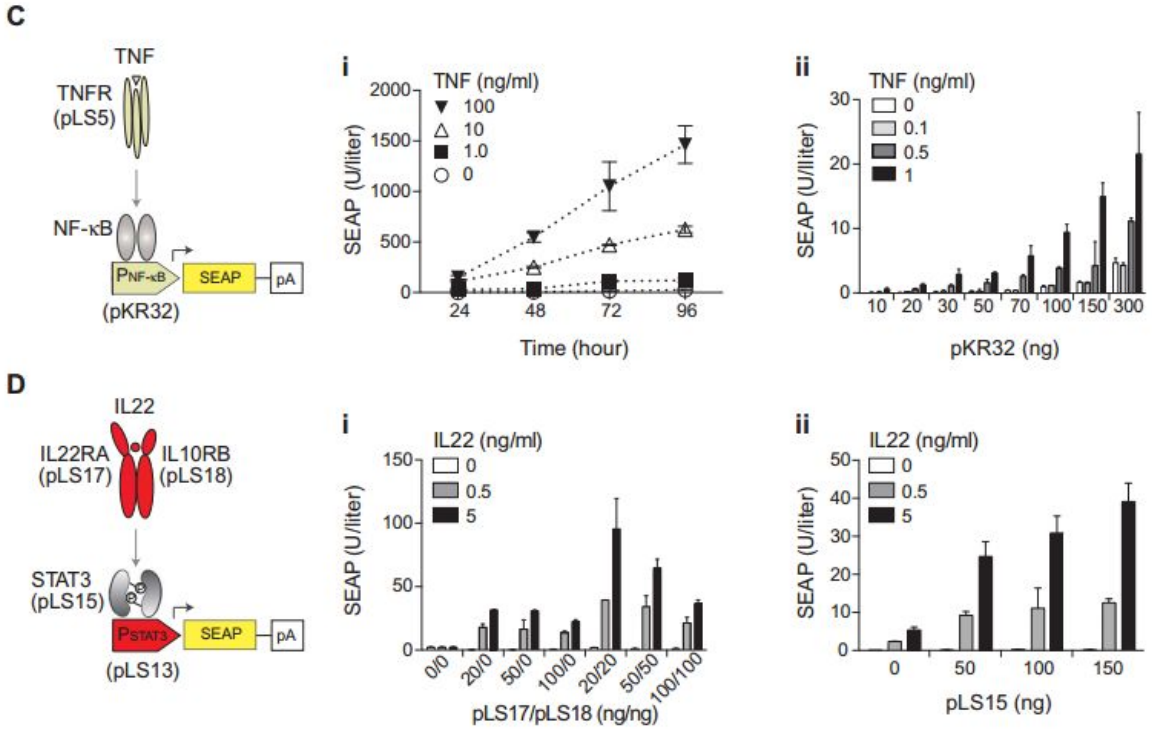


Results 1

In vitro validation:

Synthetic TNF-sensor: TNF-dose responsive SEAP level; TNF and pKR32 can be fine tuned.

Synthetic IL22 receptor: HEK293 co-transfected with different combinations of plasmids and exposed to IL22

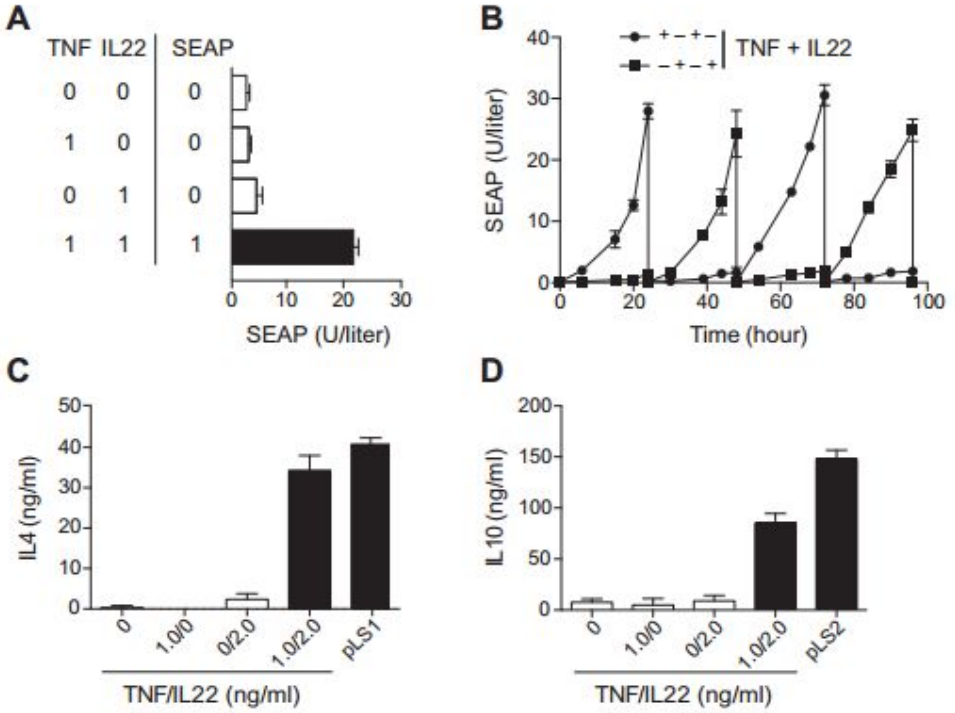


Results 2

Validation of AND gate logic in vitro:

IL22RA under the control of NFkB

- Target gene expression only in the presence of both TNF and IL22
- AND-gate logic is fully reversible (can be switched off and on)
- Anti-inflammatory cytokines are produced when HEK293 are exposed to TNF and IL22

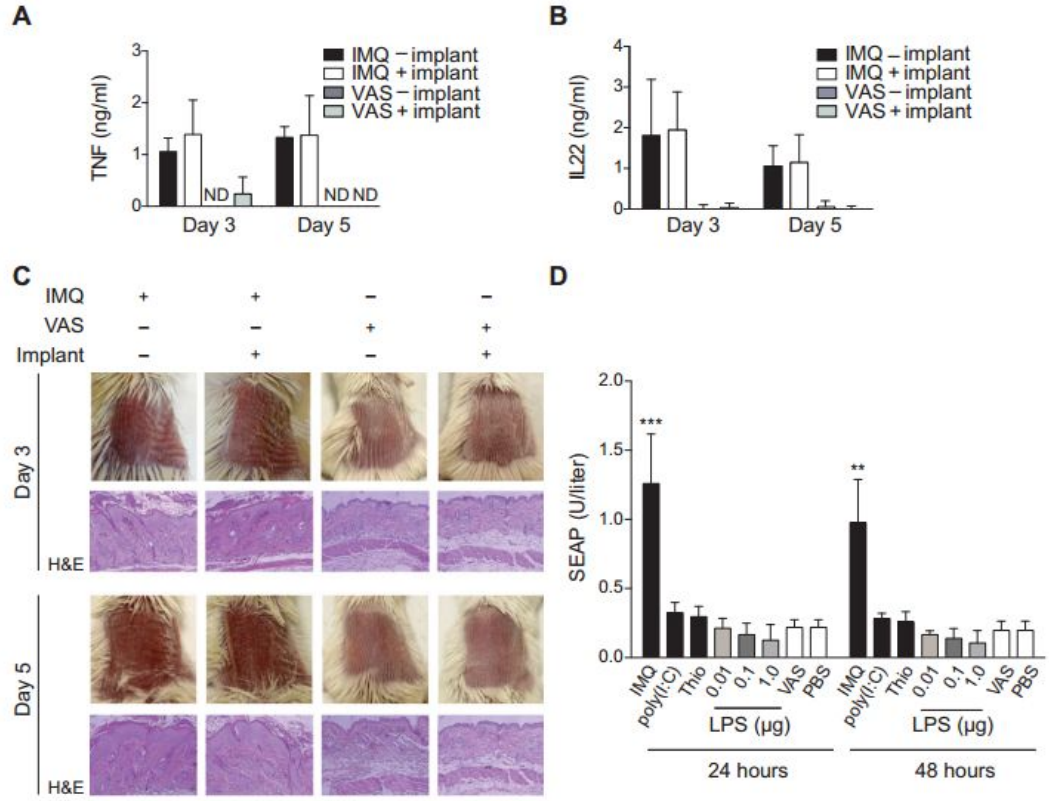


Results 3

Validation in vivo:

Mouse model of Imiquimod-induced psoriasis-like lesions

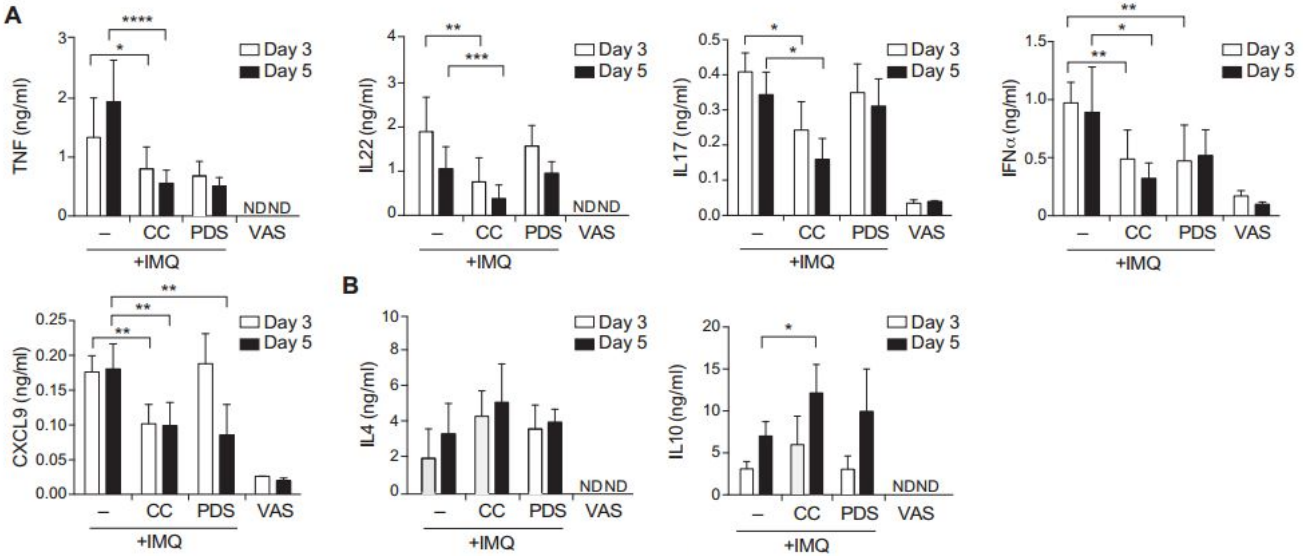
- No significant differences in inflammation levels after implant
- SEAP produced by the implanted designer cells in response to TNF and IL22 was higher in animals with psoriasis (AND-circuit is specifically activated in psoriasis like inflammation)



Results 4

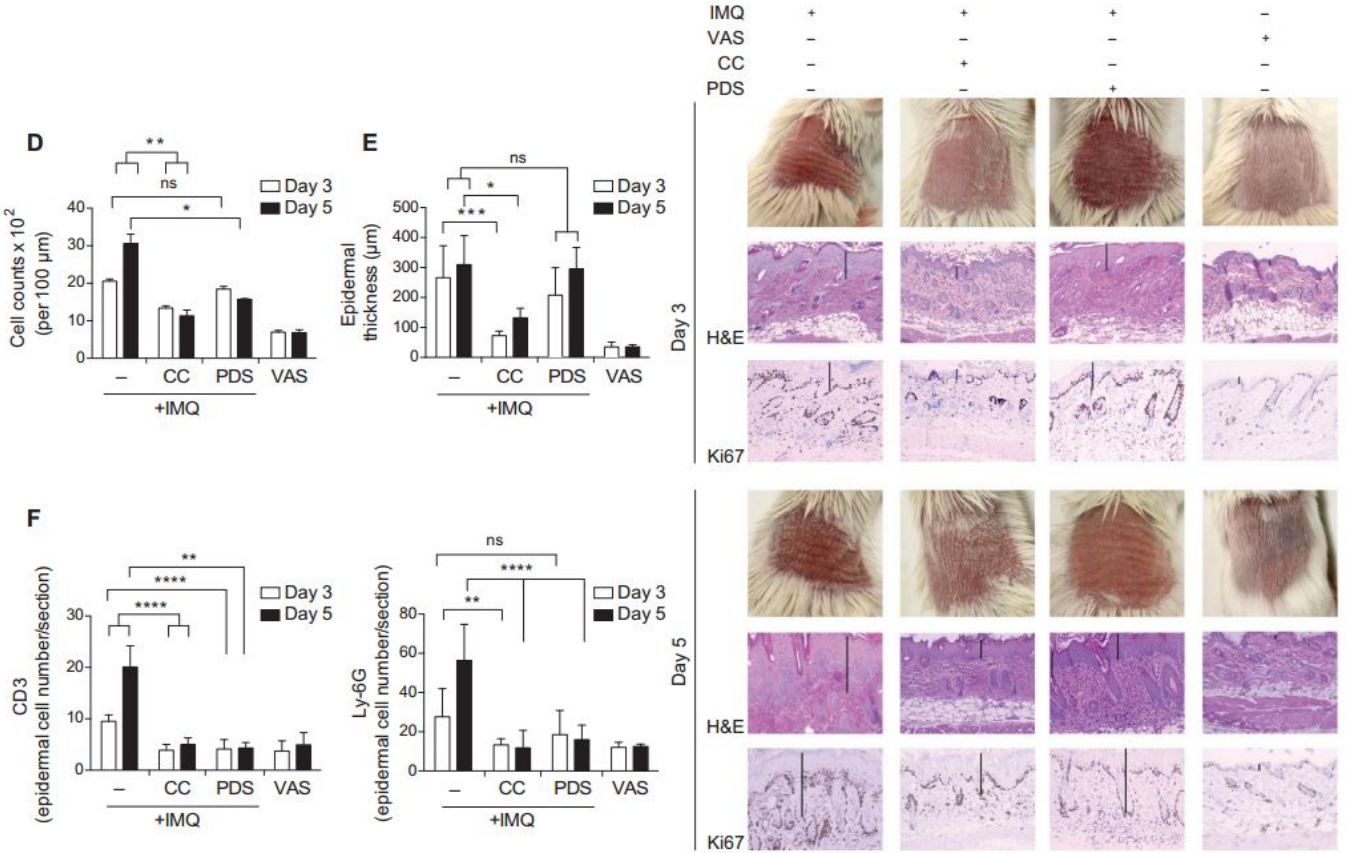
Cytokine converter prevents psoriasis

- First implantation, then +IMQ
- TNF and IL22 decreased in CC
- Low level of IL4 and IL10 due to low level of inflammation



Results 4

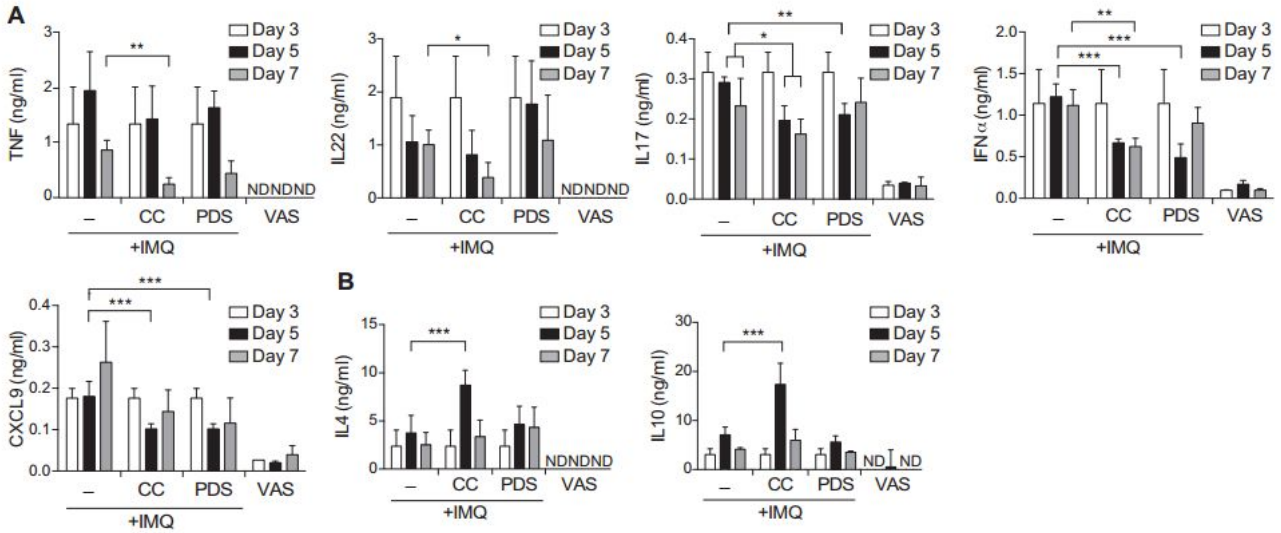
- Despite low IL4 and IL10, CC mice showed normal skin morphology
- Decrease in cell number and epidermal thickness in CC treated
- Reduced immune infiltration and proliferation



Results 5

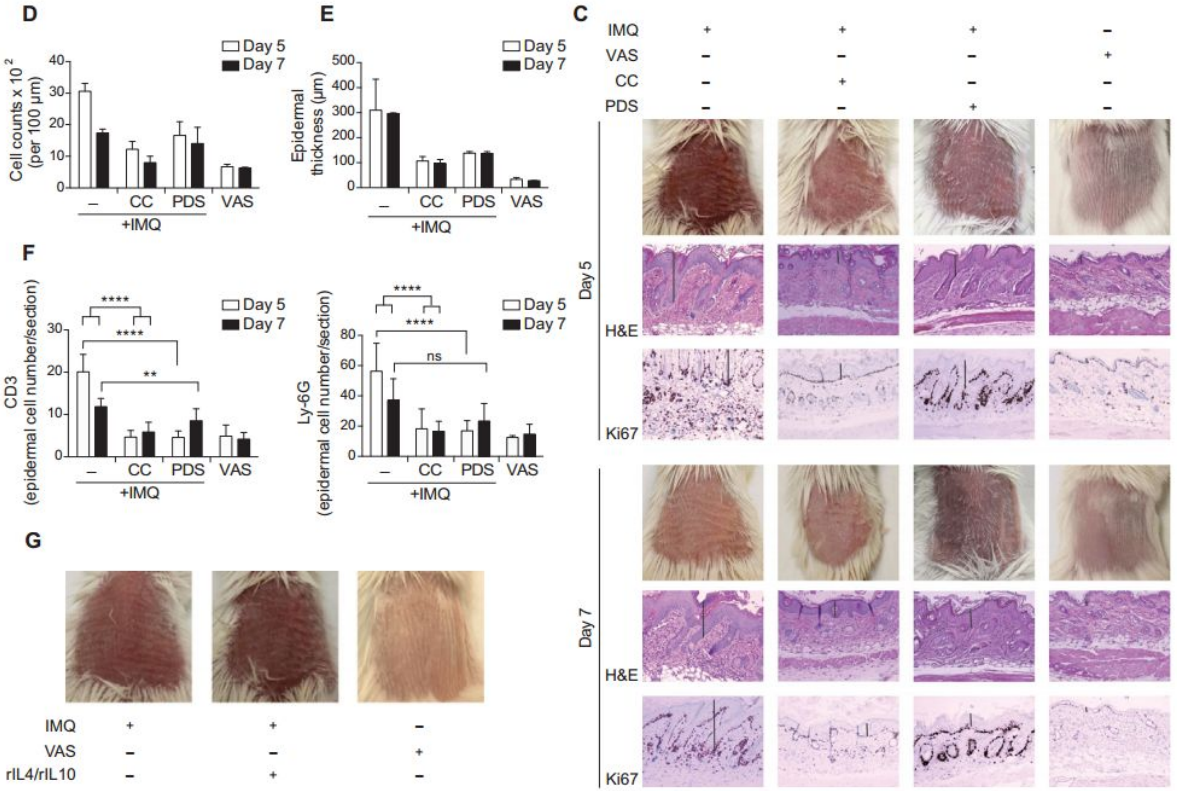
Cytokine converter attenuates established psoriasis

- Reduced proinflammatory cytokines
- Increased of anti-inflammatory cytokines



Results 5

- Reduction of psoriasis symptoms in the skin only in CC treated
- Reduction of 50% in the epidermal cell number and thickness
- Decreased immune cell infiltration



Challenges

- Use of autologous cell
- Scaling the system to provide therapeutic levels in humans
- Development of implant devices
- Recapitulate findings in other autoimmune disease