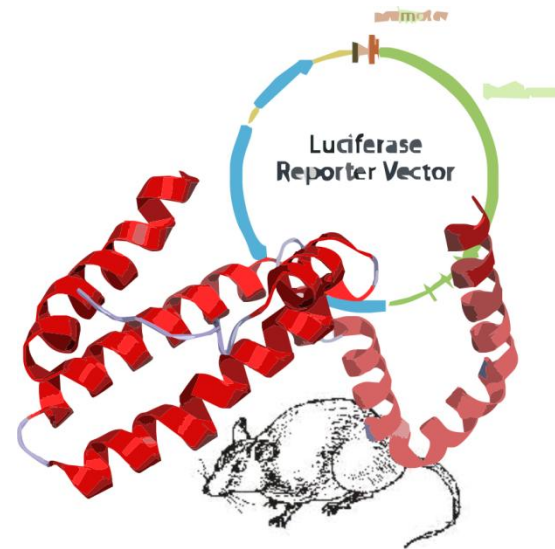
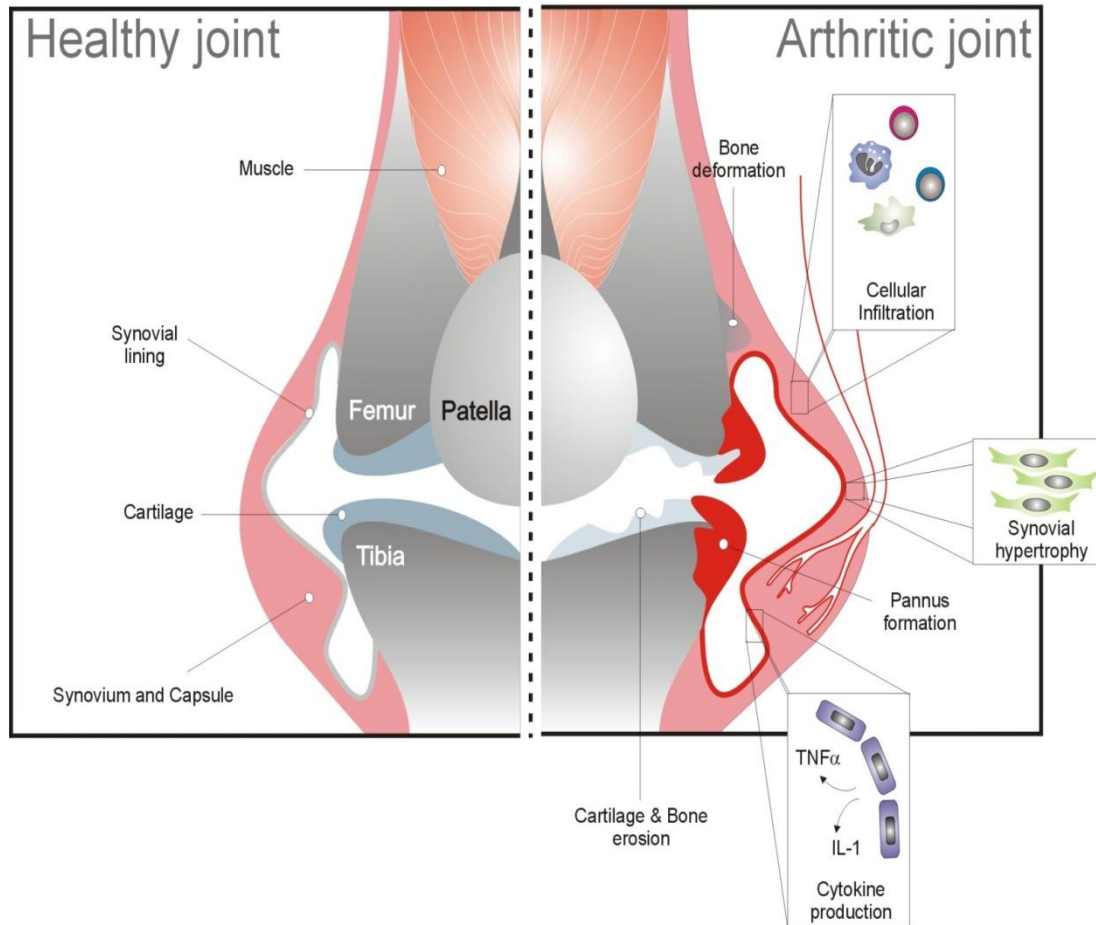


# Intra-articular overexpression of interleukin-10 using disease-inducible promoters diminished synovitis and cartilage proteoglycan depletion in experimental arthritis

Eline Vermeij  
NVGCT spring symposium  
15-03-2013



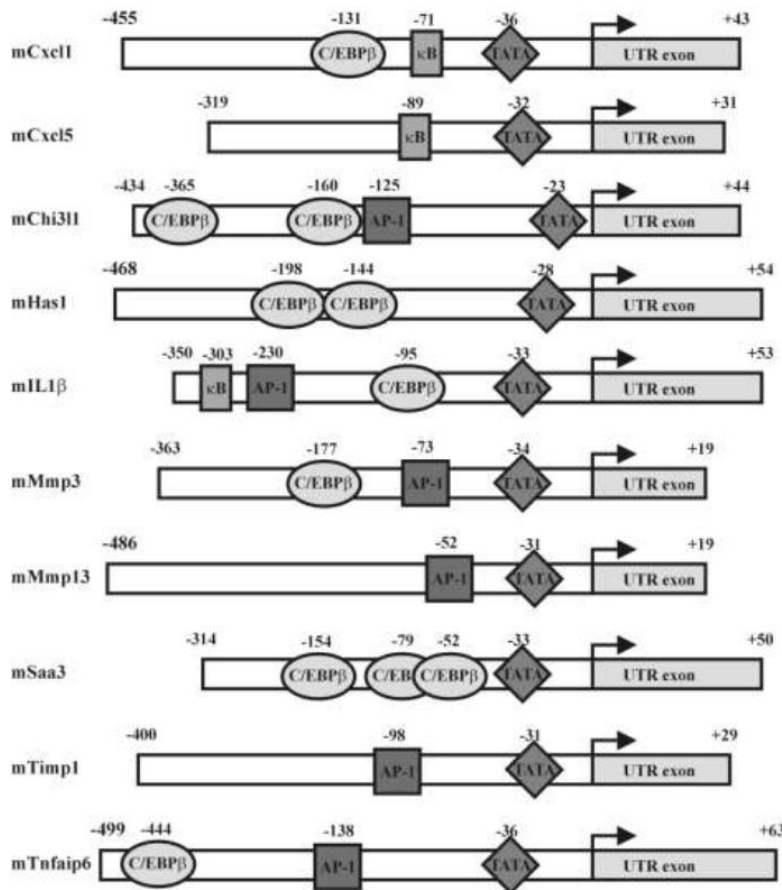
# Rheumatoid Arthritis



- In about 30% of the RA patients, the disease course is characterized by an intermittent pattern of exacerbation and complete remission
- Conventional treatments
  - Repeated administration
  - Side effects
- Gene therapy with disease-inducible promoters

# Disease-inducible promoters

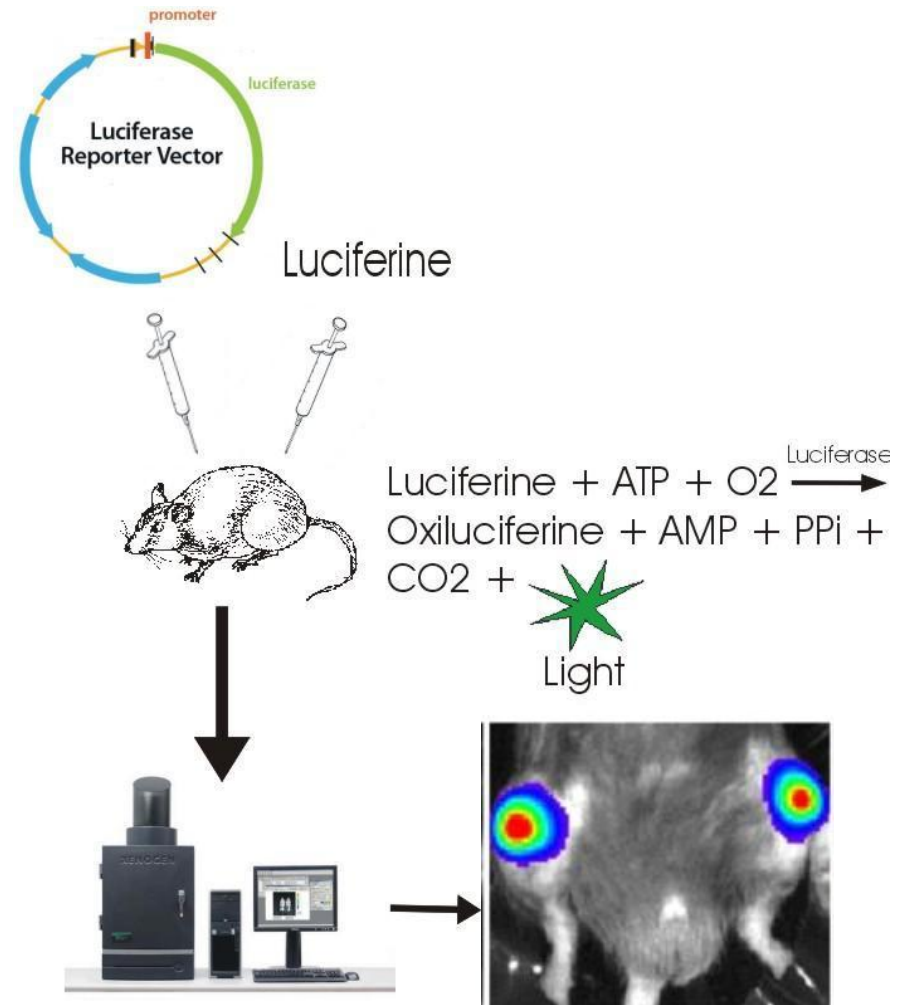
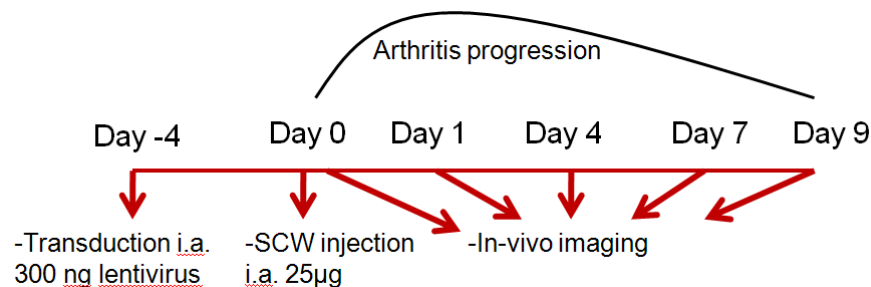
- Selection of promoters from endogenous genes differentially regulated in synovium of collagen induced arthritis mice by computational approach
- Disease-inducible promoters:
  - Only produce a therapeutic gene during flare of disease
    - No production during remission, so less side effects
  - Local treatment



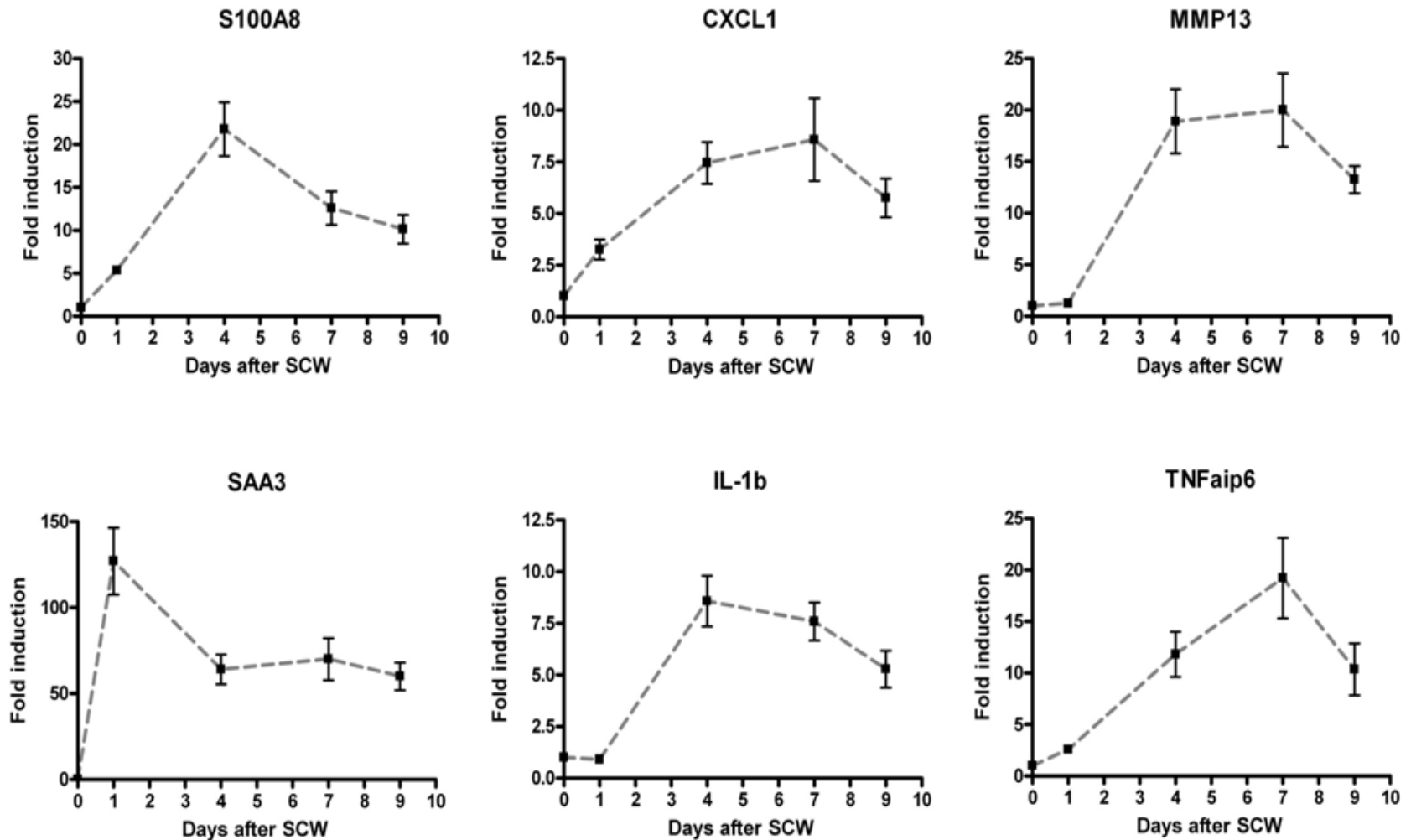
*Question: Which promoter is suitable for gene therapy?*

# In-vivo profiling of inducible promoters

- 300 ng lentivirus intra-articular in knee joint
- Induction SCW arthritis 4 days after transduction
- Imaging at day 0, 1, 4, 7 and 9

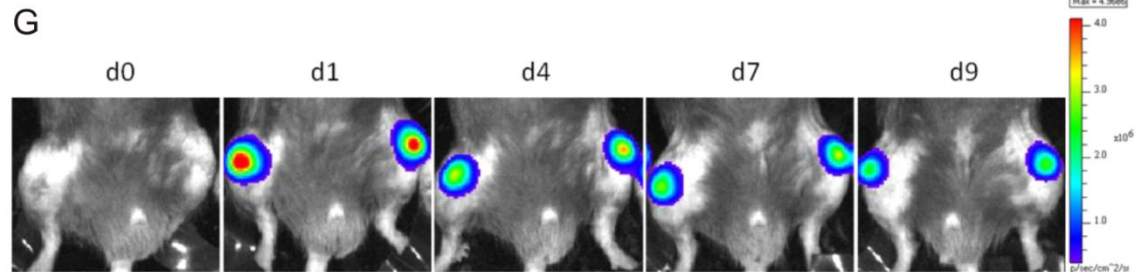
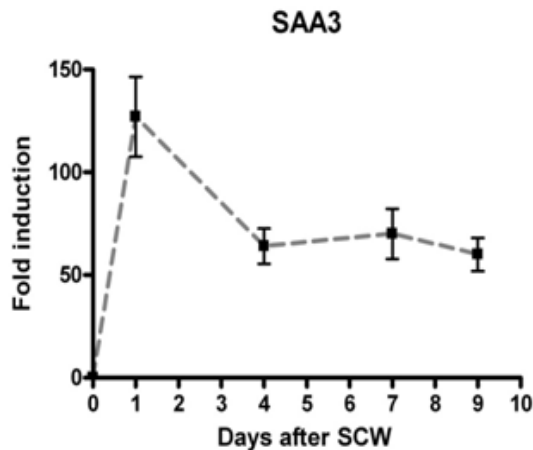


# Kinetics of 6 different inducible promoter reporters



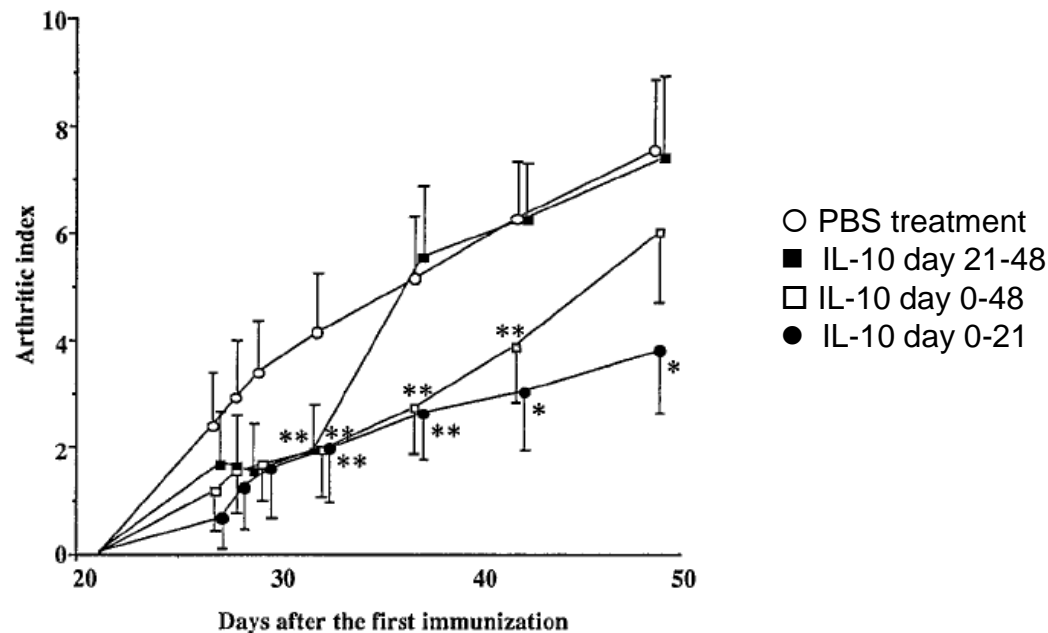
## Kinetics of 6 different inducible promoter reporters

- Saa3 promoter was selected
  - Highest fold induction
  - Early peak at day 1 after arthritis induction
- Transgene → Interleukin-10



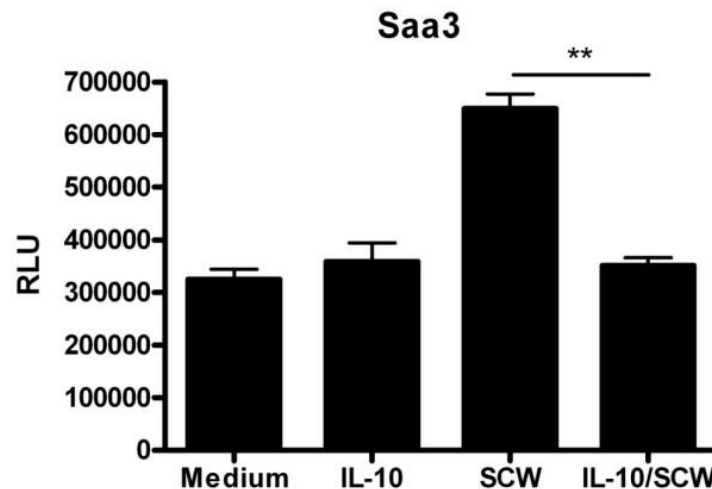
## Transgene → Interleukin-10

- IL-10 is a broad spectrum anti-inflammatory cytokine
  - Produced by Th1 and -2 cells, B-cells, monocytes, macrophages
  - Inhibits production of several pro-inflammatory cytokines
  - Induces production of IL-1 receptor antagonist (IL1RA)
  - Short half-life in serum: between 1.1 – 2.6 hours



## Saa3 promoter did not react to IL-10

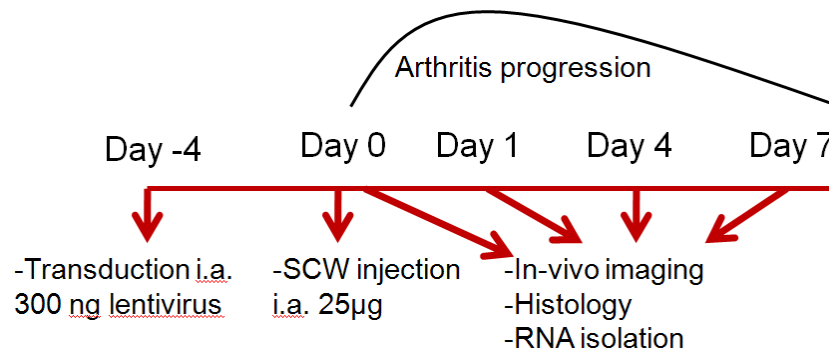
- Stimulation of lentiviral transduced NIH-3T3 fibroblast cells
  - Transduced with Saa3-Luc (50 ng p24<sup>gag</sup> equivalents/well)
  - Stimulated for 6 hours with IL-10 (10 ng/ml), SCW (5 µg/ml) or combination
- IL-10 stimulation did not upregulate the Saa3 promoter
  - Inhibition of SCW stimulation with IL-10





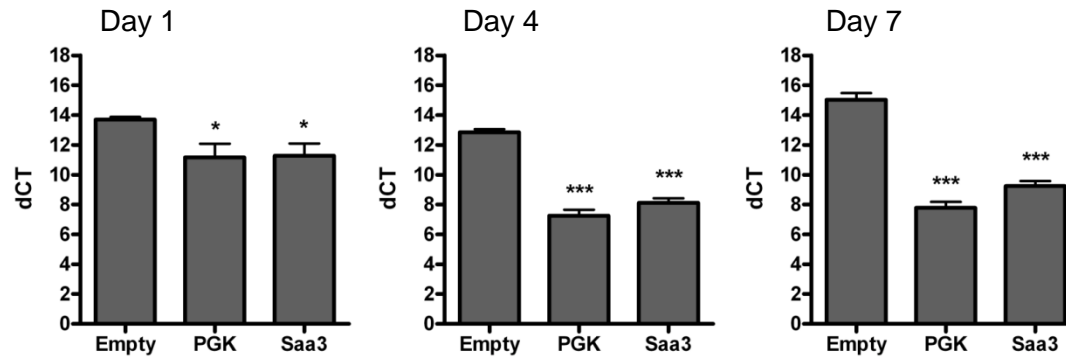
## Experimental setup in-vivo experiment

- Day -4 = i.a. injection lentivirus (300 ng)
  - PGK-Empty (virus control)
  - PGK-IL10 (positive control)
  - Saa3-IL10
- Day 0 = i.a. injection SCW (25 $\mu$ g)
- Day 1,4,7 = isolation knee joint / synovium for histology or RNA isolation + serum for cytokine analysis



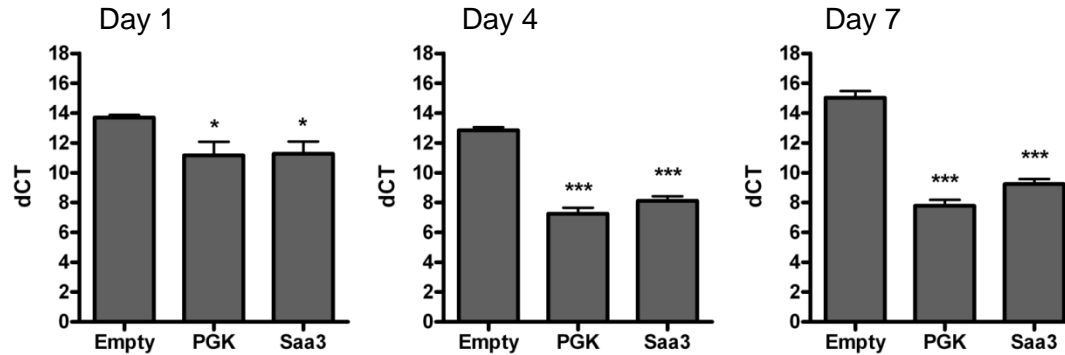
## IL-10 overexpression

- Transgene expression at day 1, 4 and 7 in the arthritic joint
  - IL-10 expression at all days → Saa3 promoter is upregulated

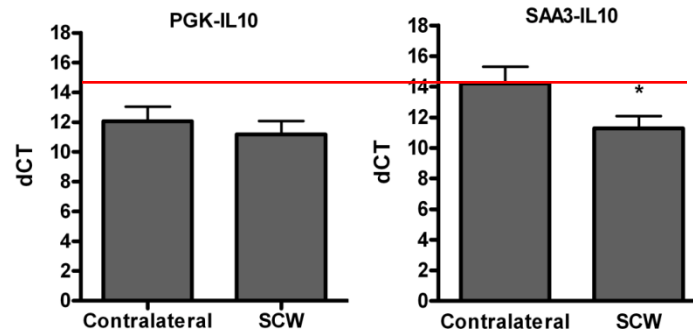


## IL-10 overexpression

- Transgene expression at day 1, 4 and 7 in the arthritic joint
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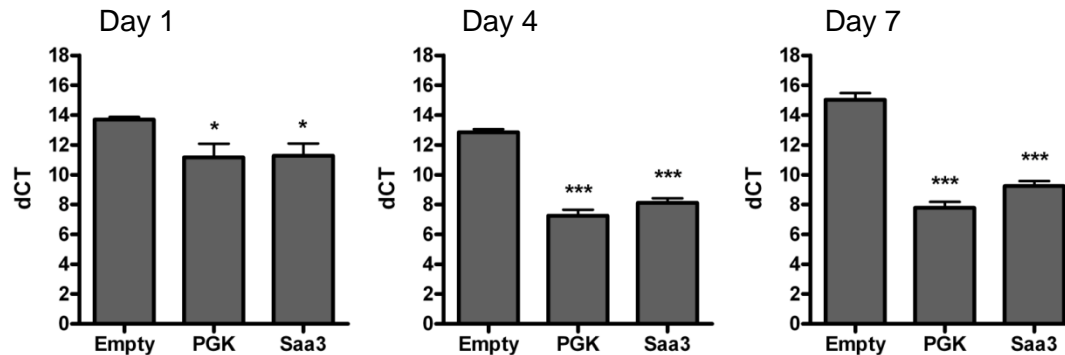


- Transgene expression at day 1 in the arthritic and contralateral non-arthritic joint
  - Saa3 promoter shows inducible production of IL-10

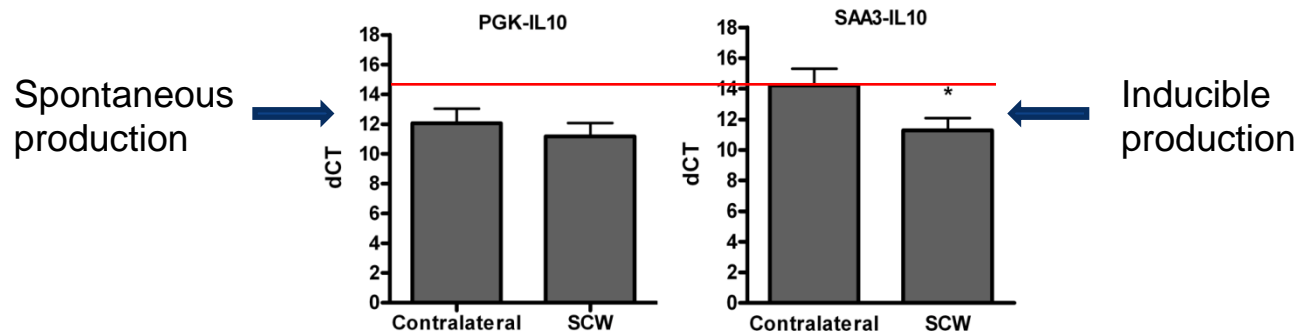


## IL-10 overexpression

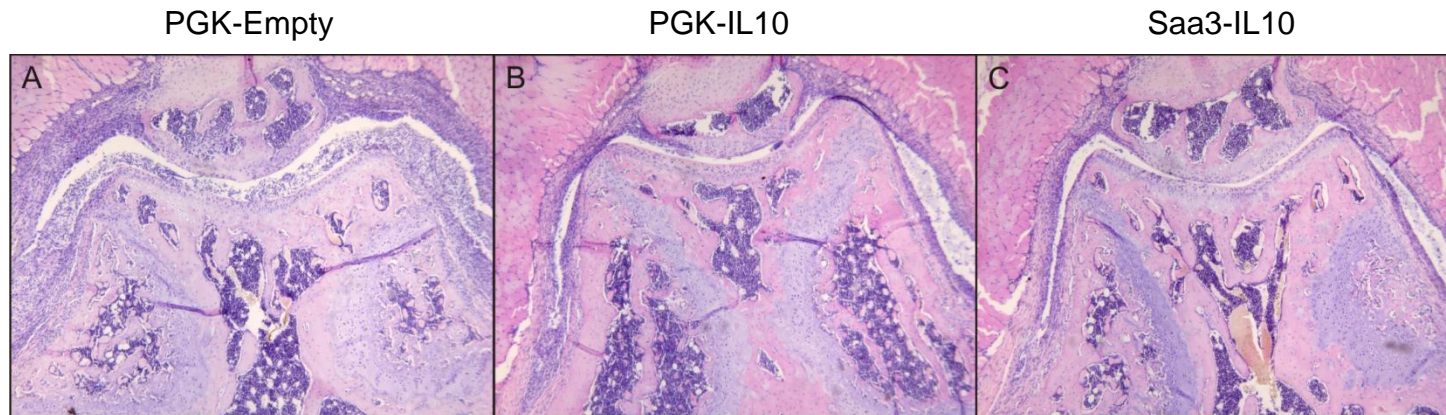
- Transgene expression at day 1, 4 and 7 in the arthritic joint
  - IL-10 expression at all days → Saa3 promoter is upregulated



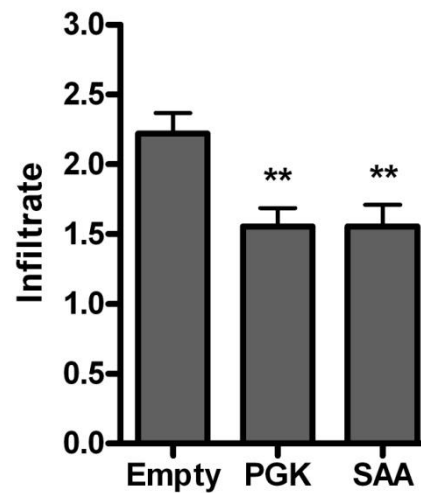
- Transgene expression at day 1 in the arthritic and contralateral non-arthritic joint
  - Saa3 promoter shows inducible production of IL-10



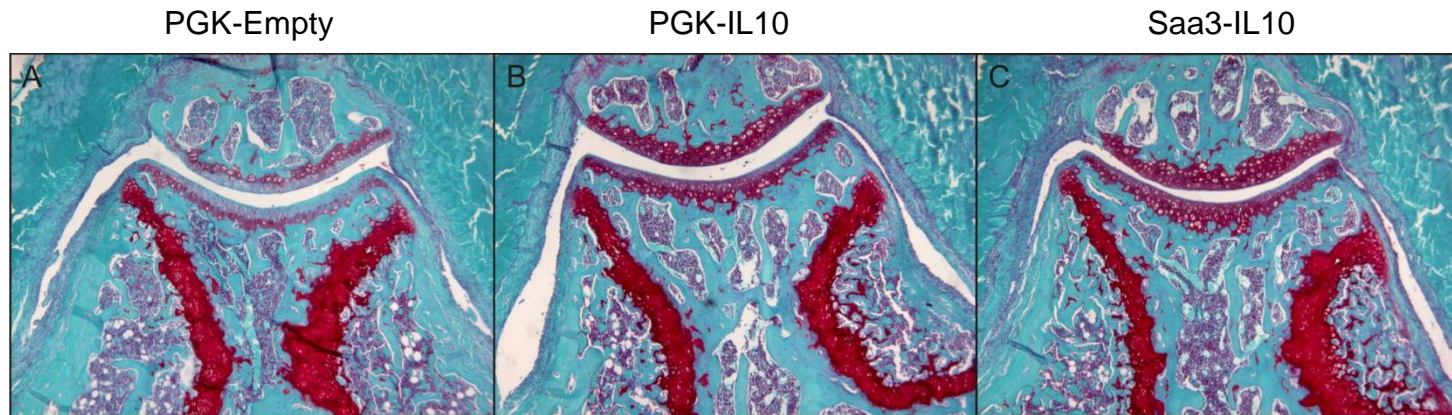
## Less synovitis can be seen at day 4



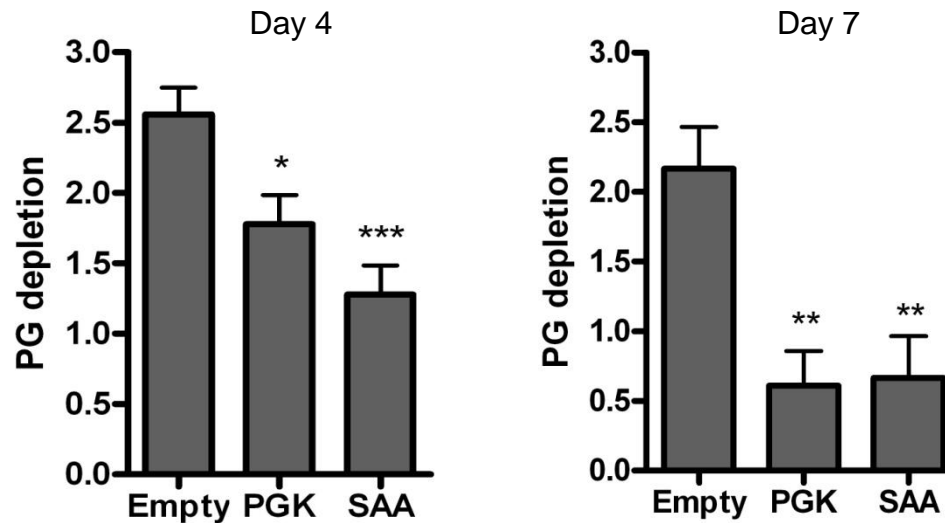
Day 4 after SCW



## Cartilage damage is diminished at day 4 and 7

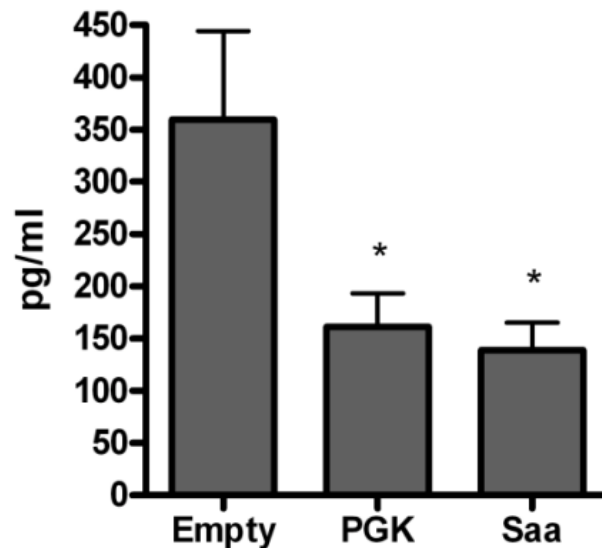


Day 7 after SCW



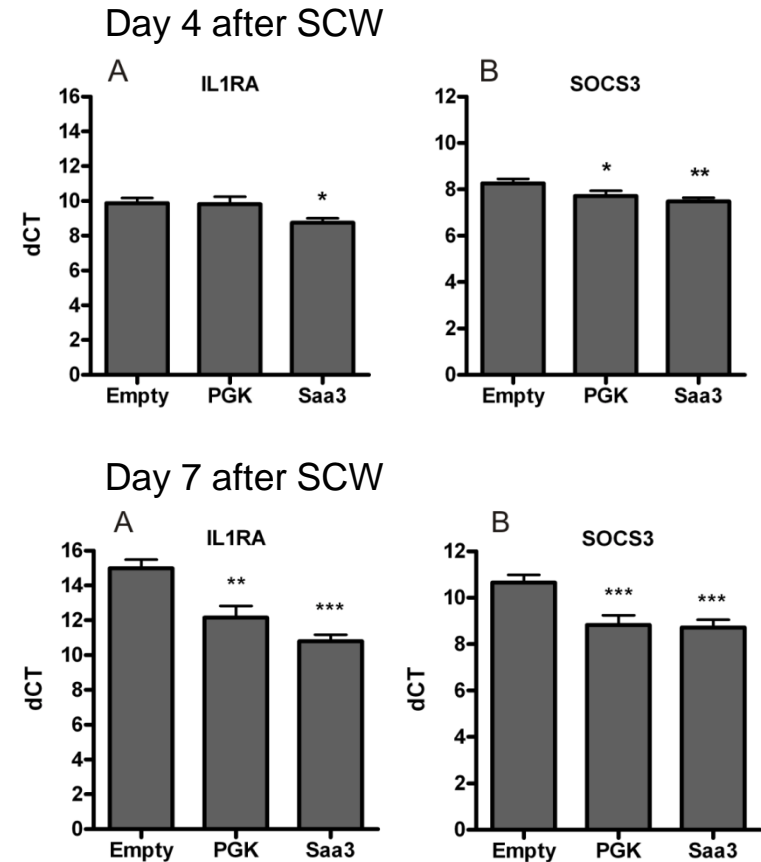
## Effects of IL-10 overexpression on synovial cytokine production and gene expression

- IL-8 protein was significantly downregulated at day 1 after SCW injection
  - Chemokine that plays an important role in the pathogenesis of arthritis



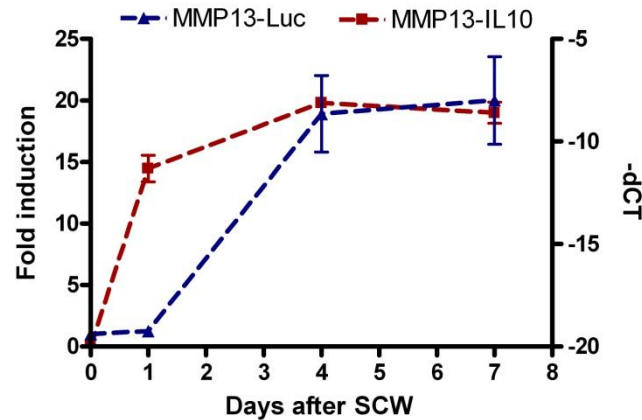
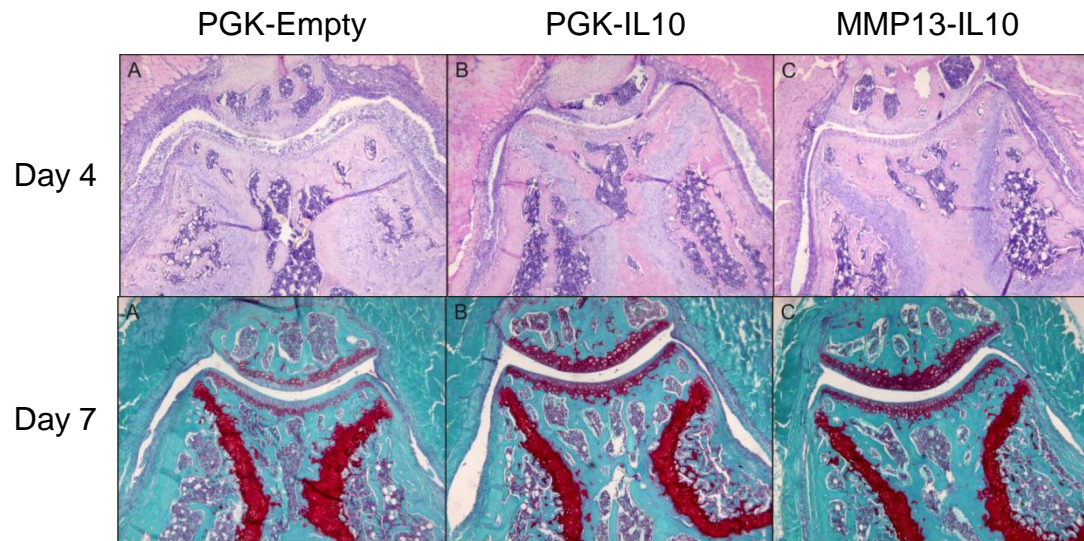
# Effects of IL-10 overexpression on synovial cytokine production and gene expression

- Both IL1RA and SOCS3 are upregulated
  - SOCS 3 upregulation
    - inhibits JAK/STAT pathway and subsequent inflammation  
→ less synovitis (Henningsson *et al.*, 2012)
  - IL1RA upregulation
    - Counteracts detrimental effects of IL-1 on cartilage damage → less PG depletion (Kuiper *et al.*, 1998)





# MMP-13 promoter



- MMP13 promoter is also upregulated, but not according to profile
  - Influence of IL-10 on MMP13 expression profile

## Implications for gene therapy in RA

- Inducible promoters Saa3 and MMP13 both showed effects on synovitis and PG depletion
  - Choice of inducible promoter also depends on the transgene
    - Important to know whether promoter is regulated by transgene
- Saa3 promoter is a good candidate for gene therapy using IL-10

# Acknowledgements

## Department of Rheumatology

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NanoDiaRA

