

Disease-regulated Local Interleukin-10 Gene Therapy Diminishes Synovitis and Articular Cartilage Damage in Experimental Arthritis

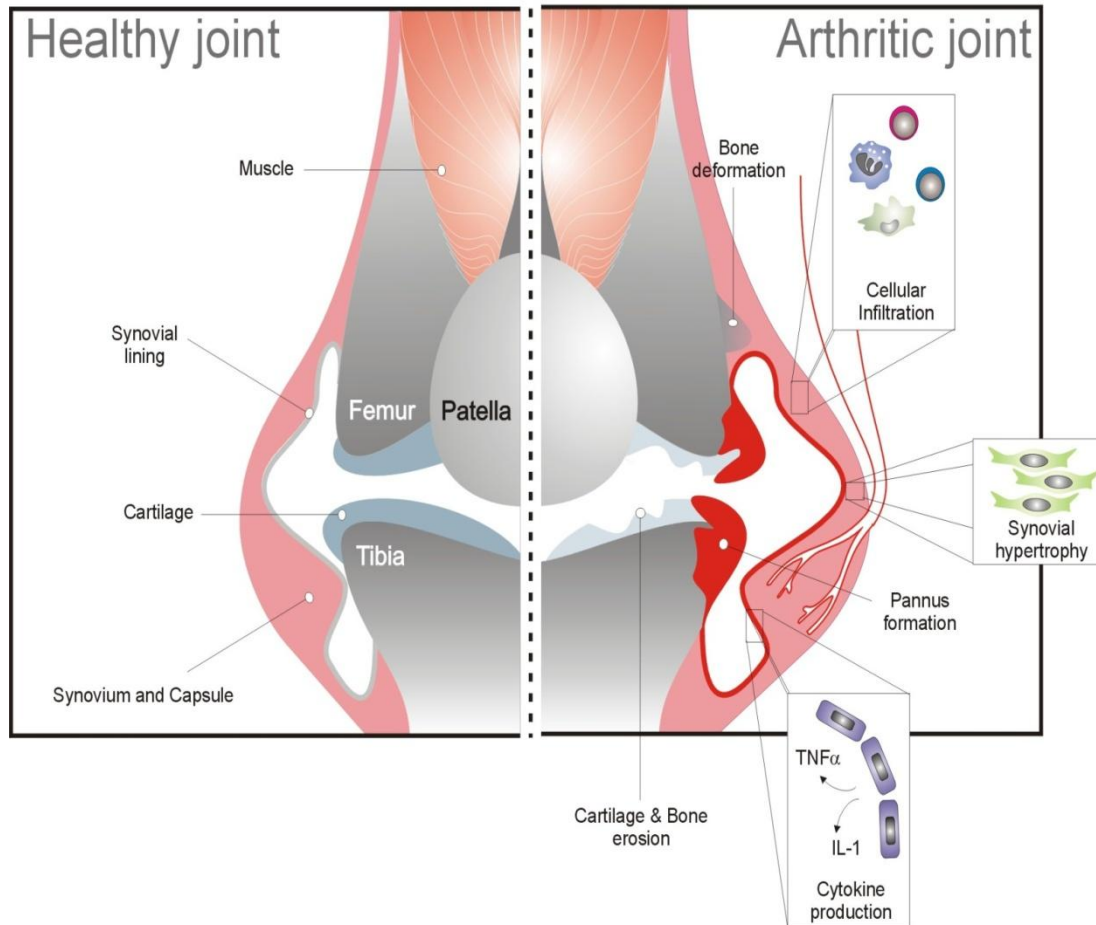
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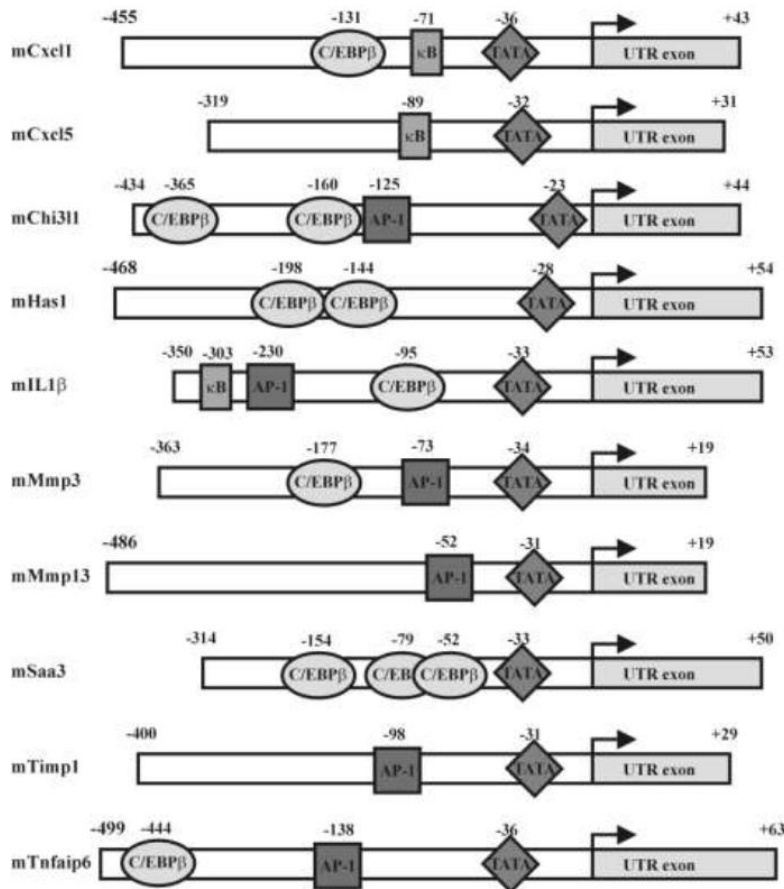
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Rheumatoid Arthritis (RA)



- disease course with flares and remission
- Conventional treatment includes biological drugs:
 - repeated administration
 - systemic
- Goal: Gene therapy using disease-inducible promoters
 - local
 - only active during flare

Disease-inducible promoters

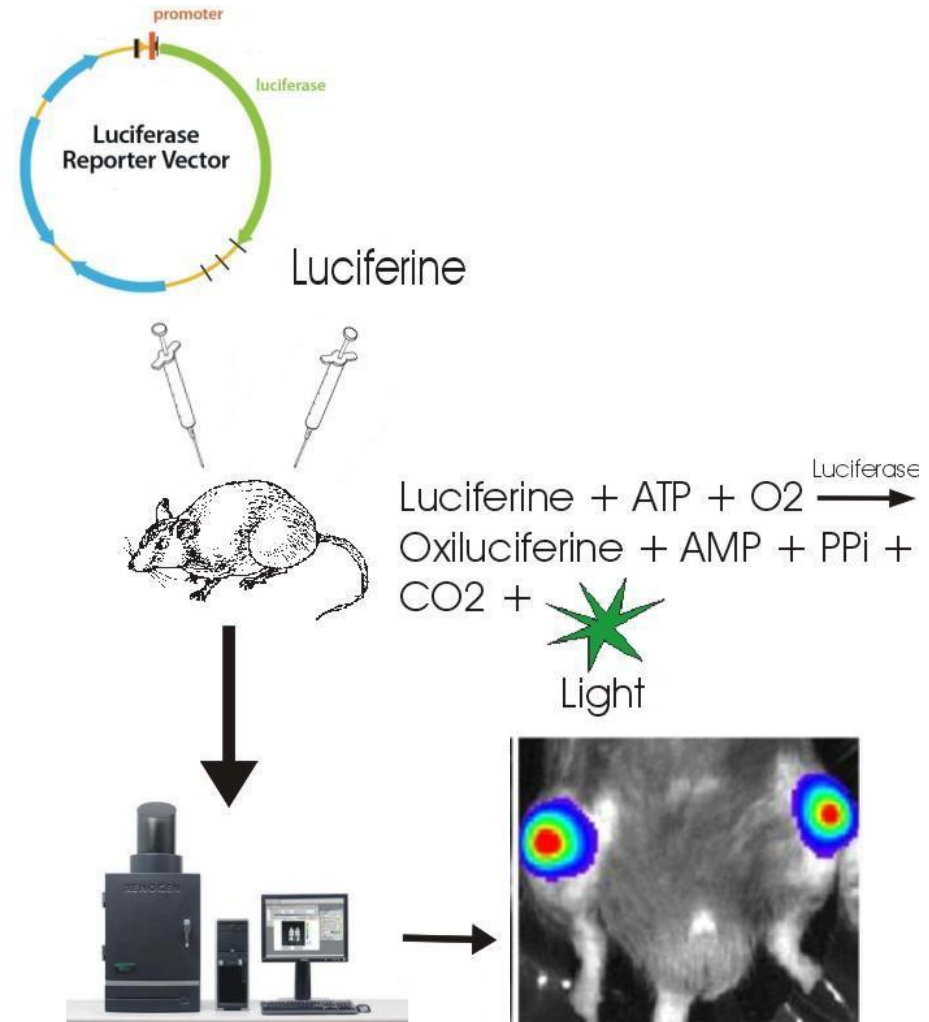
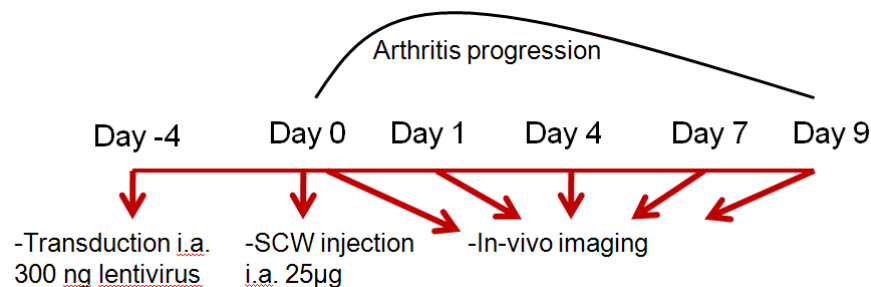


- Microarray on joints of mice with experimental arthritis
- Find genes upregulated in arthritic mouse joints
- Isolate promoter from endogenous gene

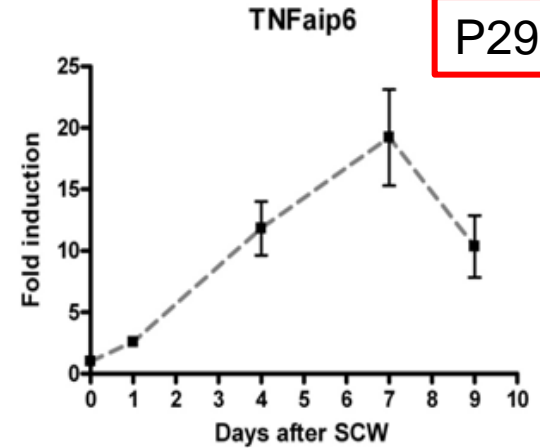
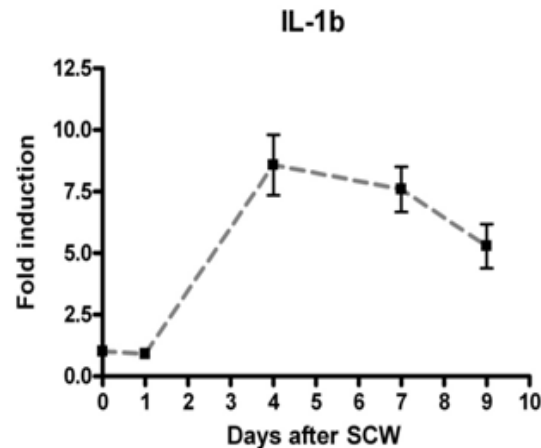
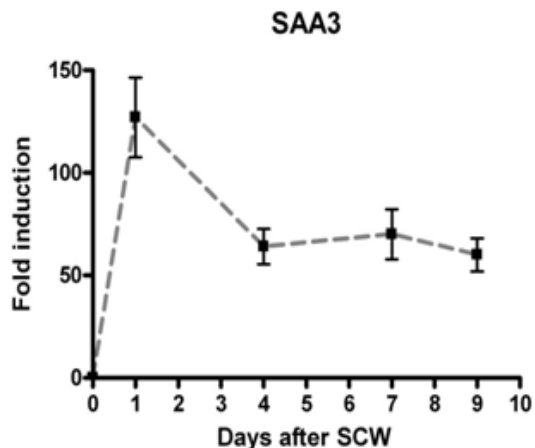
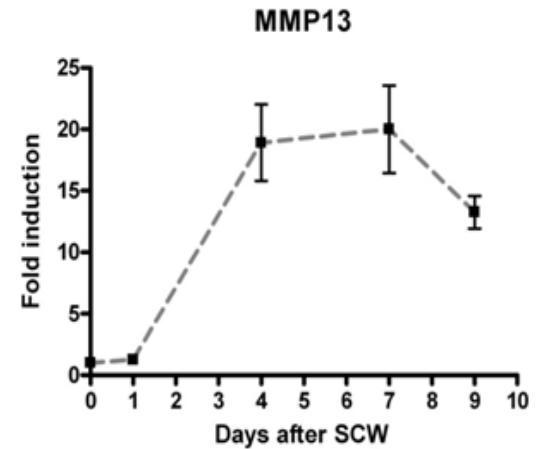
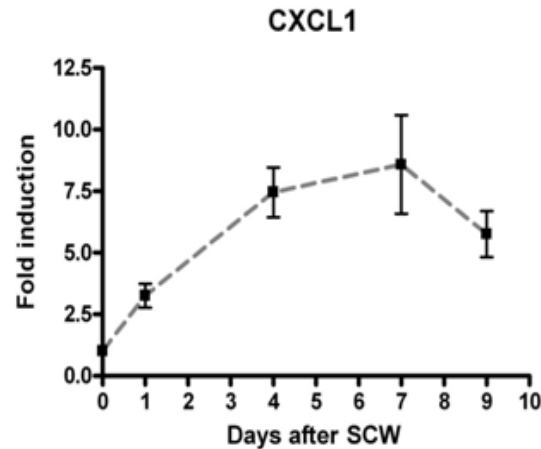
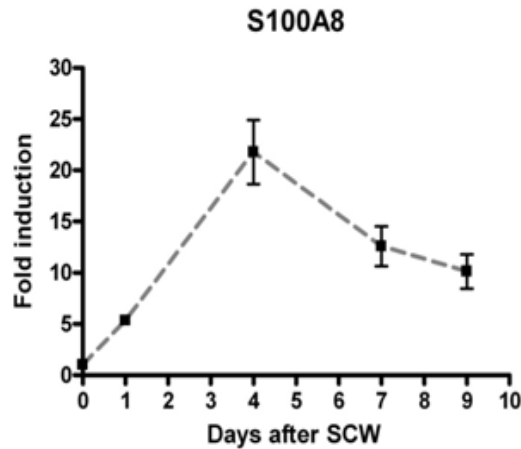
Question: Which promoter is suitable for gene therapy?

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



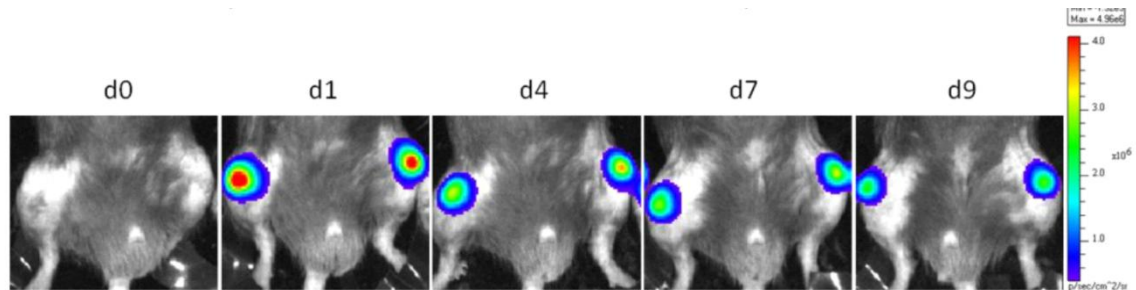
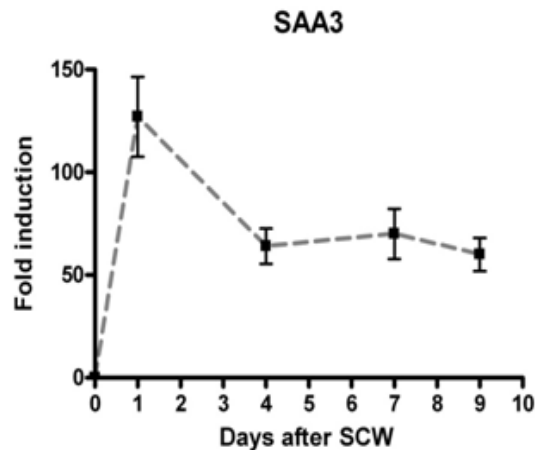
Kinetics of inducible promoter reporters



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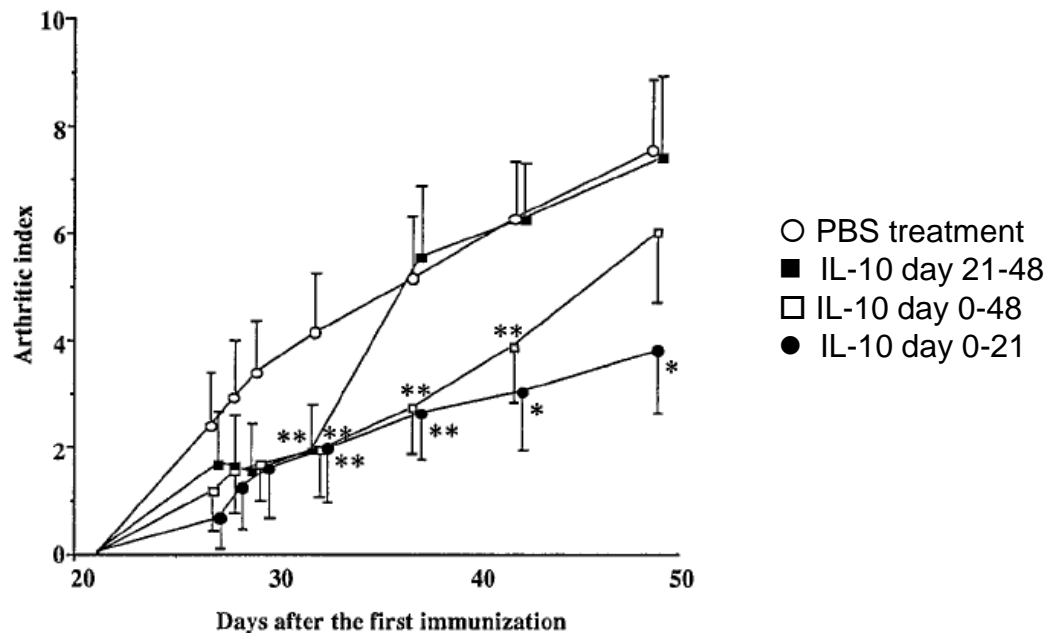
Kinetics of inducible promoter reporters

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



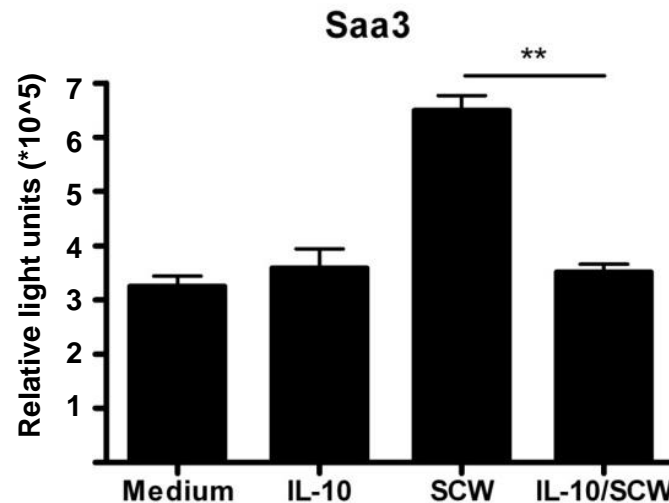
Transgene → Interleukin-10 (IL-10)

- IL-10 is a broad spectrum anti-inflammatory cytokine
 - Produced by many inflammatory cells
 - Inhibits production of several pro-inflammatory cytokines
 - Induces production of anti-inflammatory cytokines
 - Short half-life in serum: between 1.1 – 2.6 hours



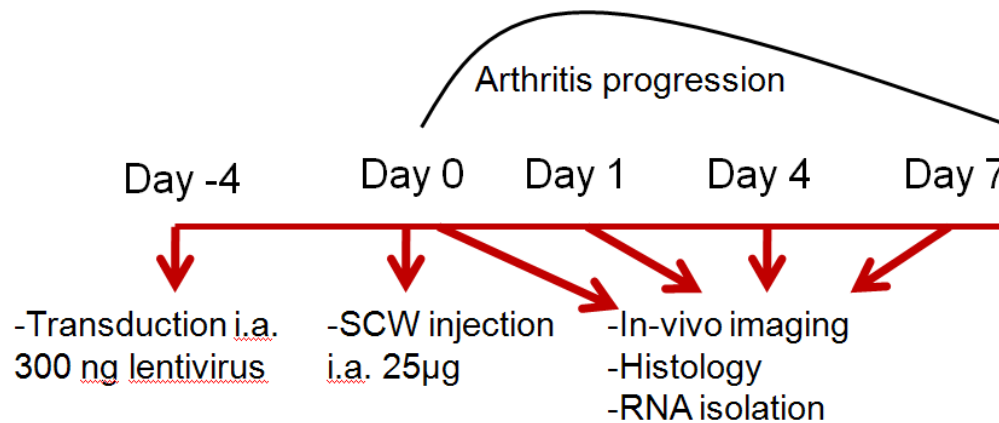
Saa3 promoter response to IL-10

- Stimulation of lentiviral transduced NIH-3T3 fibroblast cells
 - Transduced with Saa3-Luc (50 ng p24^{gag} 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



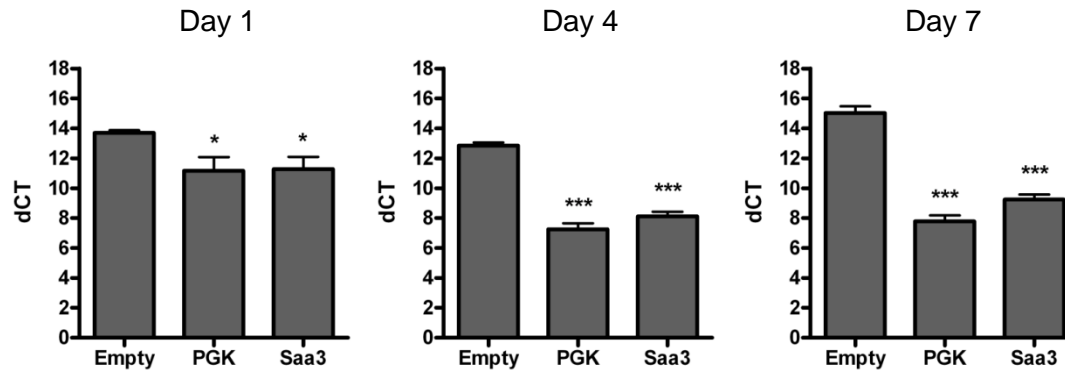
Experimental setup in-vivo experiment

- 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 µg)
- Day 1,4,7 = isolation knee joint / synovium for histology or RNA isolation + serum for cytokine analysis



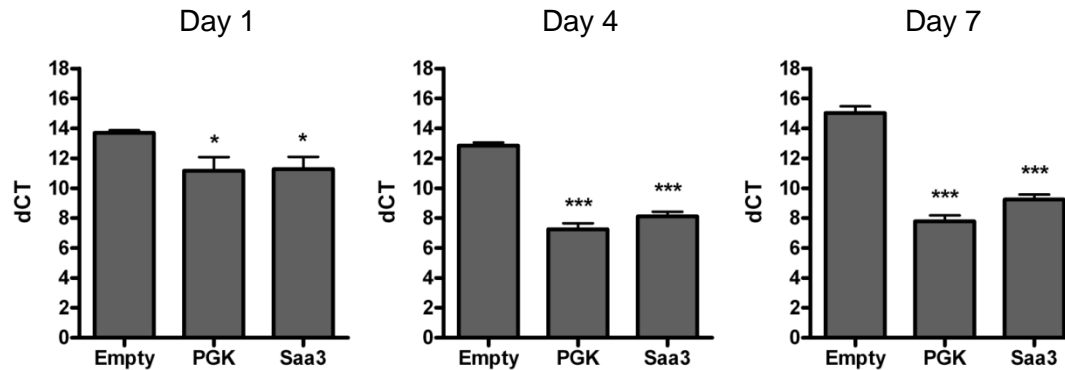
IL-10 overexpression with inducible Saa3 promoter

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

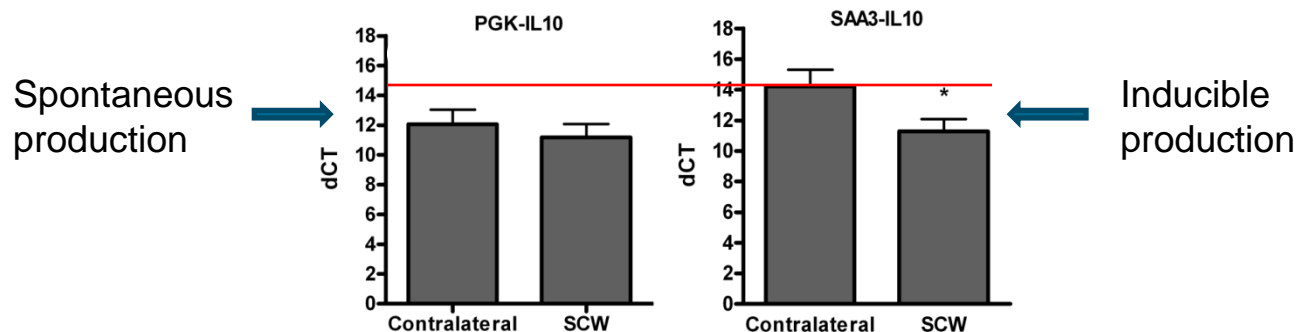


IL-10 overexpression with inducible Saa3 promoter

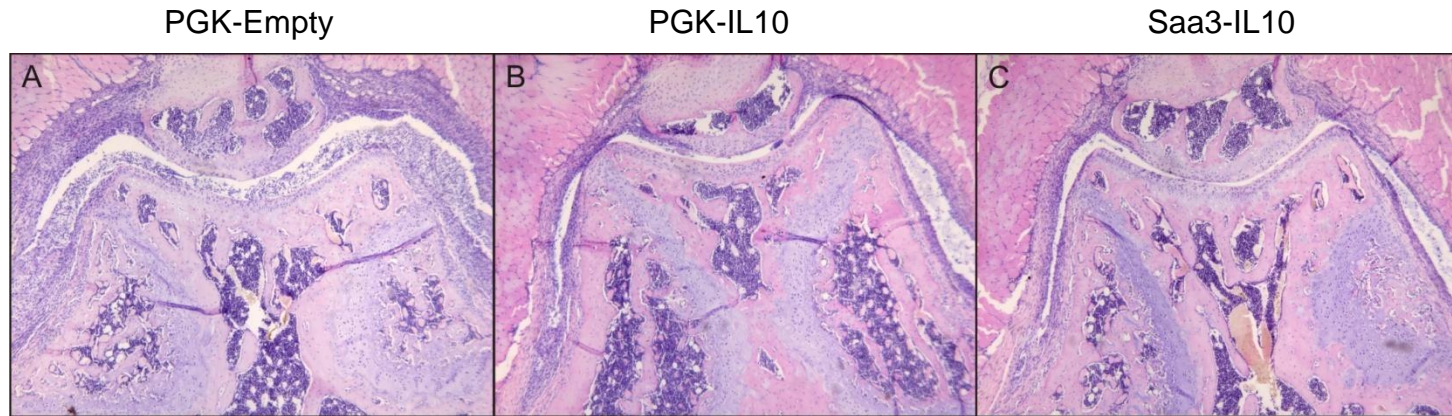
- Transgene expression at day 1, 4 and 7 in the arthritic joint
 - IL-10 expression at all days → Saa3 promoter is upregulated



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

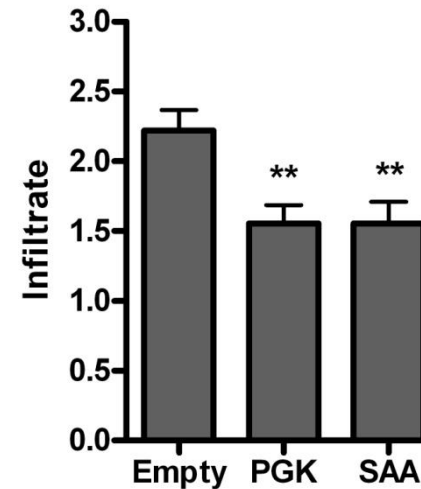


IL-10 expression reduces synovitis

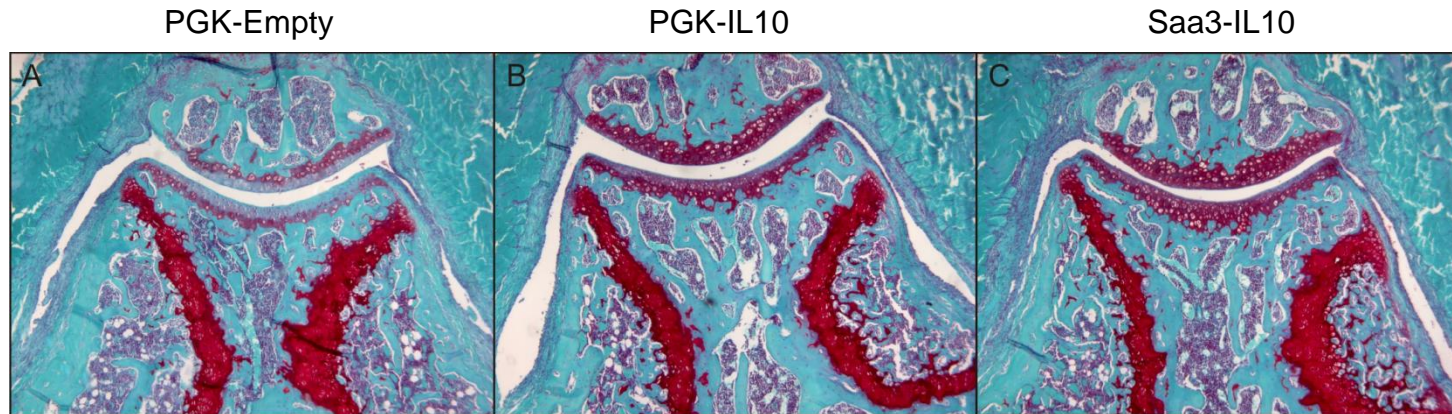


Day 4 after SCW

- Synovitis decreased at day 4

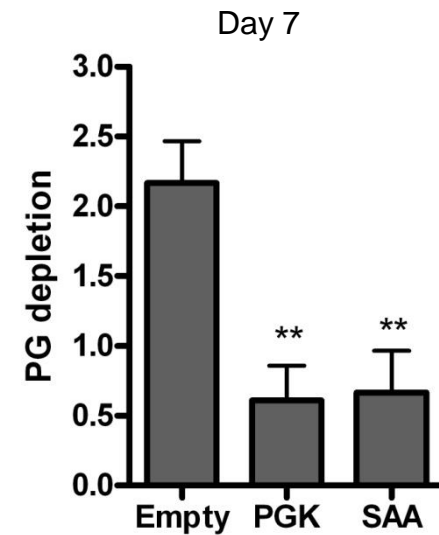
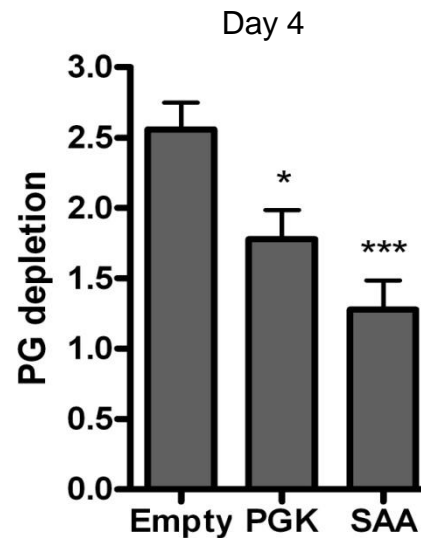


Cartilage damage is reduced at day 4 and 7



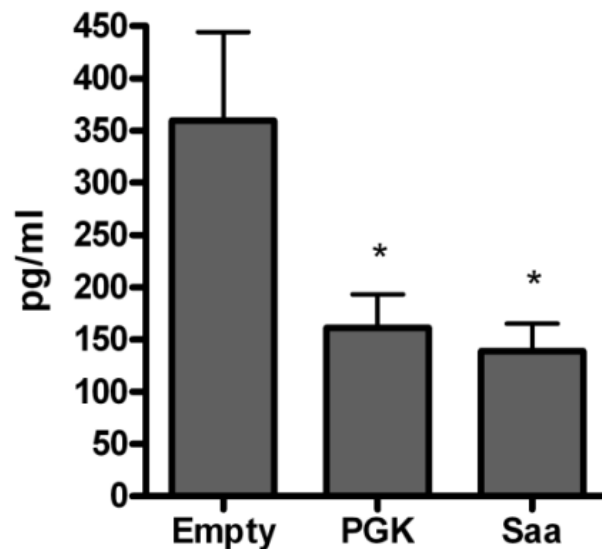
Day 7 after SCW

- Proteoglycan depletion decreased at day 4 and 7



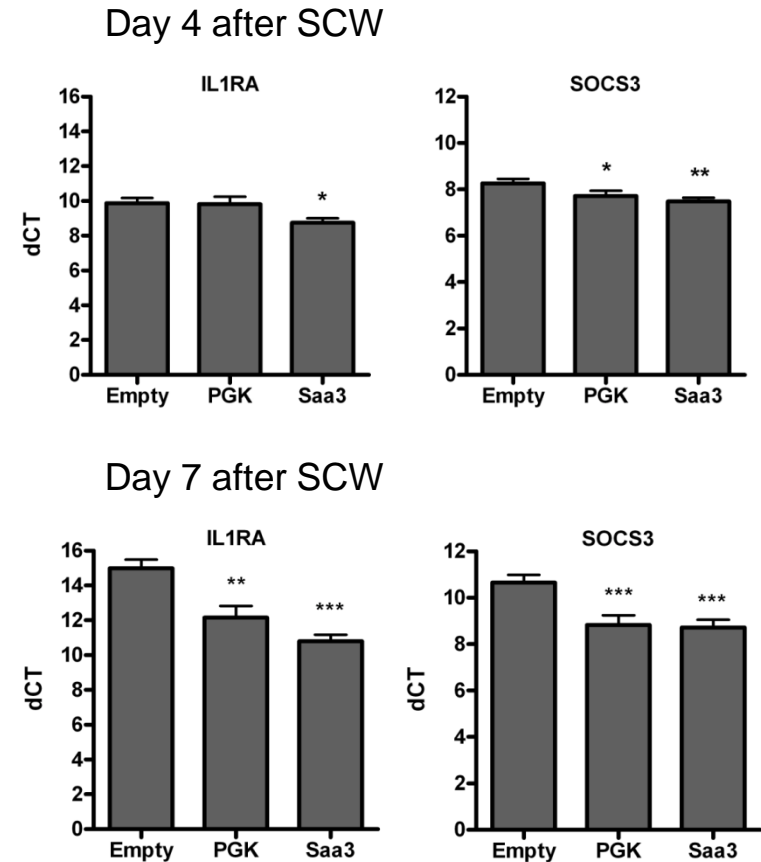
Effects of IL-10 overexpression on synovial chemokine production

- KC protein downregulated at day 1 after SCW injection
 - Important chemokine in the pathogenesis of arthritis



Effects of IL-10 overexpression on synovial gene expression

- Both IL1RA and SOCS3 are upregulated
 - IL1RA upregulation
 - Counteracts detrimental effects of IL-1 on cartilage damage → less proteoglycan depletion¹
 - SOCS 3 upregulation
 - inhibits JAK/STAT pathway and subsequent inflammation → less synovitis²



1) Kuiper et al., 1998
2) Henningsson et al., 2012

Implications for gene therapy in RA

- The Saa3 promoter is inducible in experimental arthritis
- Saa3p-IL10 gene therapy can diminish synovitis and proteoglycan depletion
- Saa3p-IL10 gene therapy can upregulate important anti-inflammatory cytokines and genes
- **Saa3 promoter is a good candidate for gene therapy using IL-10**



Acknowledgements

Department of Rheumatology

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