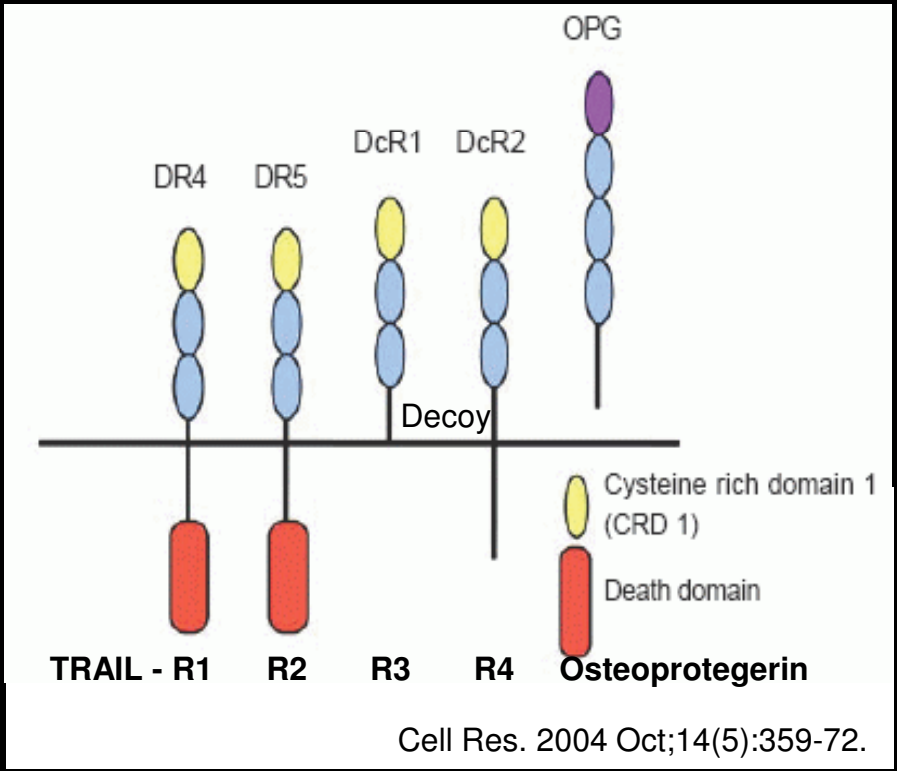
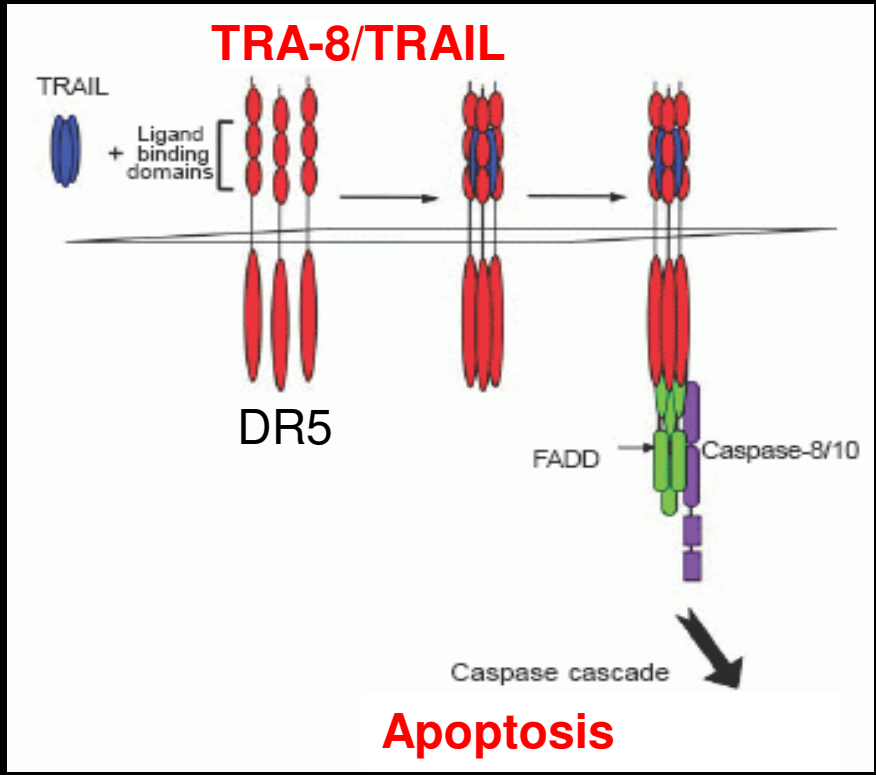
TRAIL, Death Receptors, TRA-8, and Apoptosis

- **DR5** is a pro-apoptotic molecule, which can induce cell death in a variety of cells by engaging the it ligand, TRAIL or anti-DR5 antibody.
- **TRA-8** is a monoclonal anti-human DR5 antibody, which induces apoptosis upon binding to its receptor, DR5.



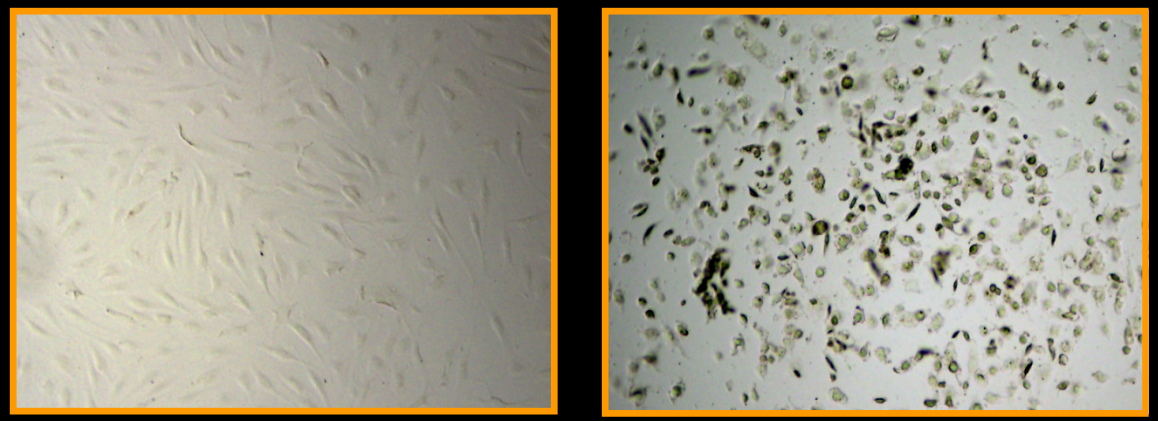
Cell Res. 2004 Oct;14(5):359-72.



Anti-human DR5 Antibody Triggers Apoptosis in RA Synovial Fibroblasts

- TRA-8 can induce apoptosis in human RA synovial fibroblasts.

Rheumatoid Arthritis Synovial Fibroblasts



Isotype control **TRA-8**

- Advantages of TRA-8 compared to TRAIL: Specificity to DR5; Long half-life; No hepatocellular toxicity.

• Can TRA-8 induce apoptosis of pathogenic macrophages and T cells in RA?

Strategies Used to Determine the Arthritis Therapeutic Effects of TRA-8 in Mice

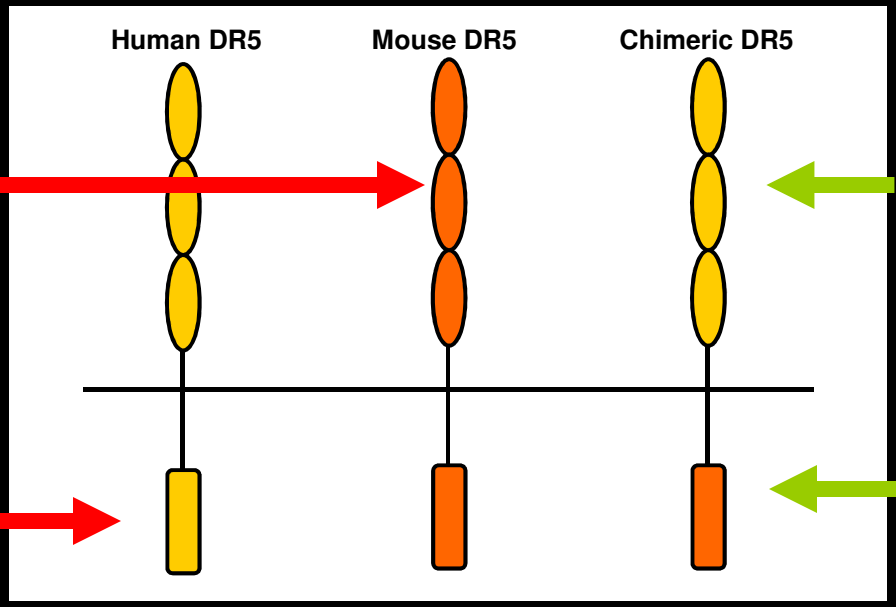
1. Creation of a mouse model with a humanized DR5 reactive with TRA-8.

2. Control of expression through a Cre/loxp system, using both general (Ubiquitous C) and macrophage specific (Lysozyme M) expression Cre mice.

3. Murine models of inflammatory arthritis (Collagen II-induced arthritis).

Generation of human/mouse Chimeric DR5 Transgenic Mice

Human, mouse and chimeric DR5



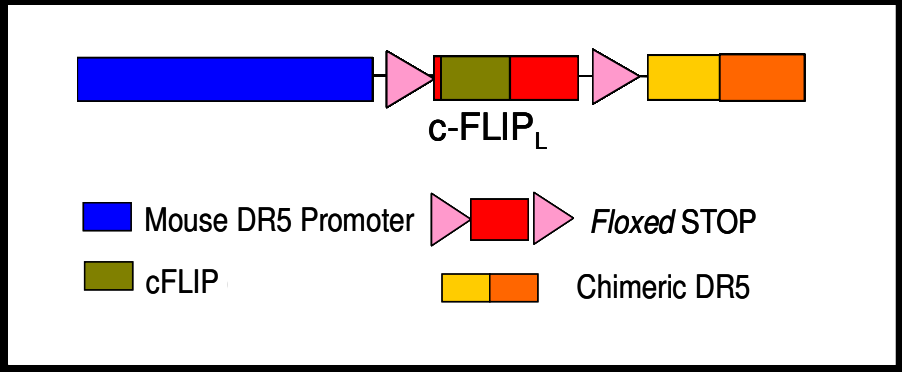
Not recognized by anti-hu DR5

Recognized by anti-hu DR5

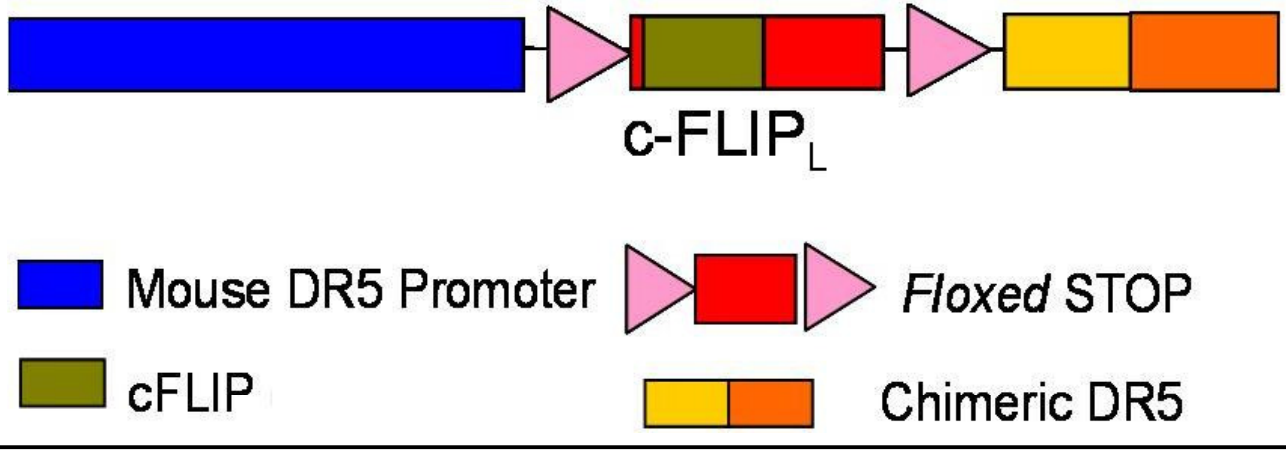
Doesn't trigger apoptosis in mouse cells

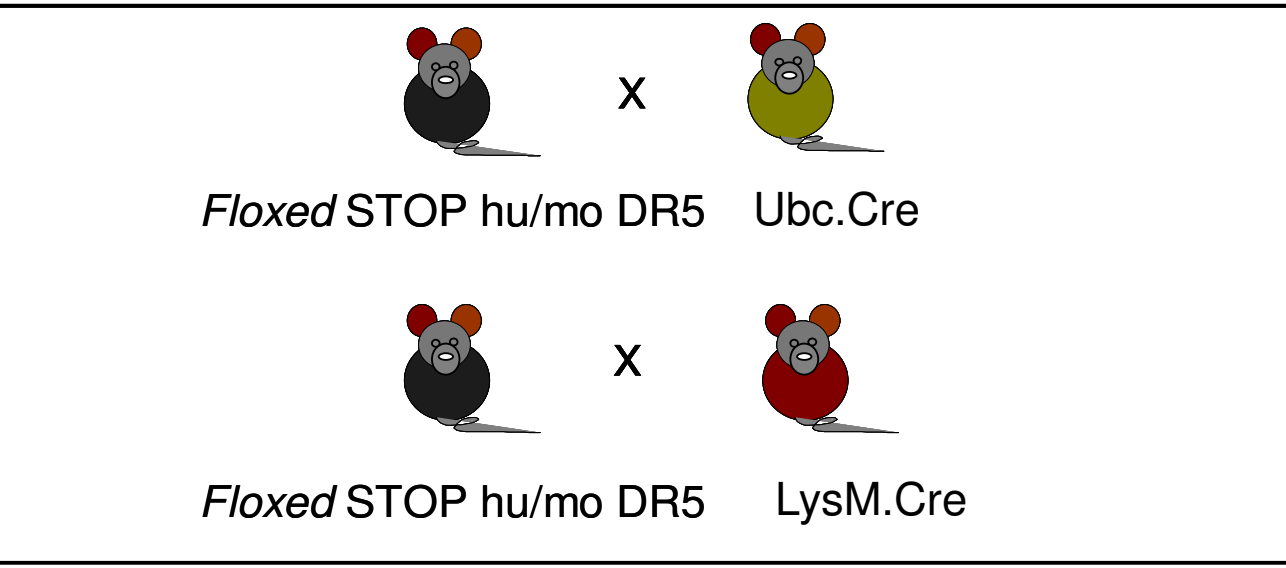
Trigger apoptosis in mouse cells

Generation of hu/mo chimeric DR5 Tg mice

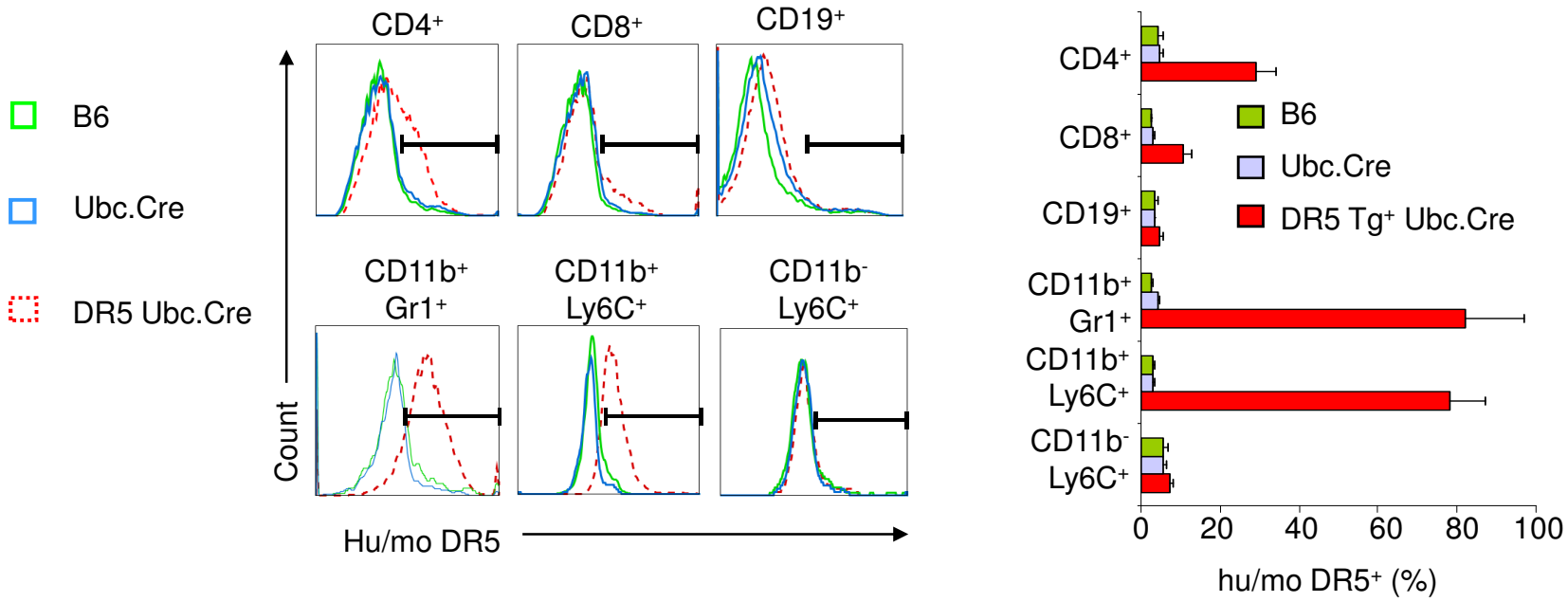


Generation of human/mouse Chimeric DR5 Transgenic Mice





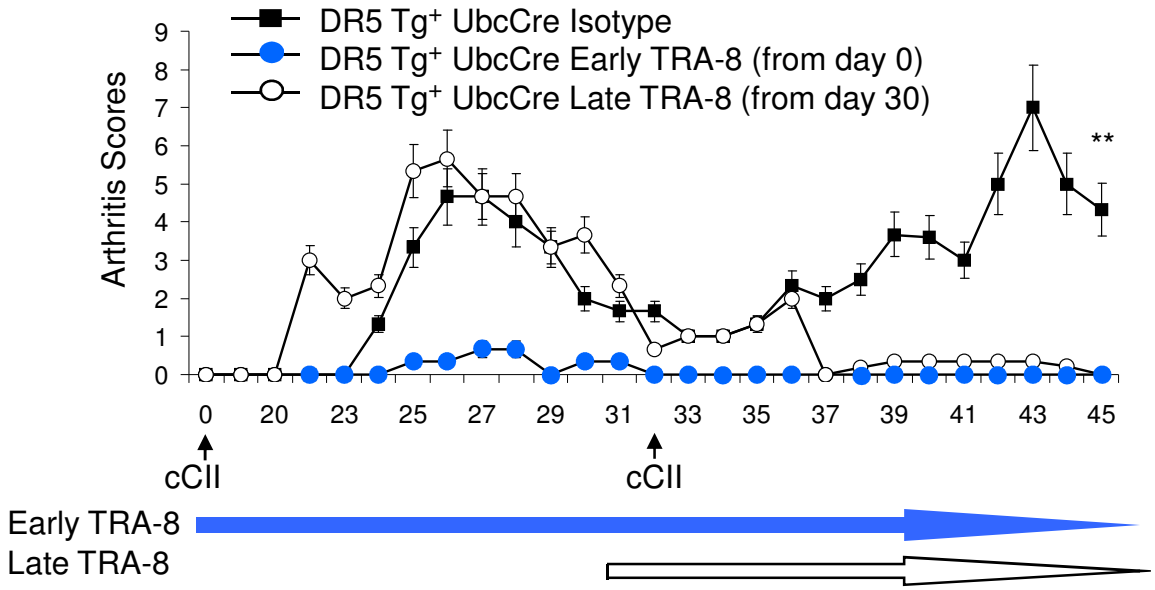
Tg DR5 Expression in Draining LN of Ubc.Cre DR5 Mice (2-mo post CII)

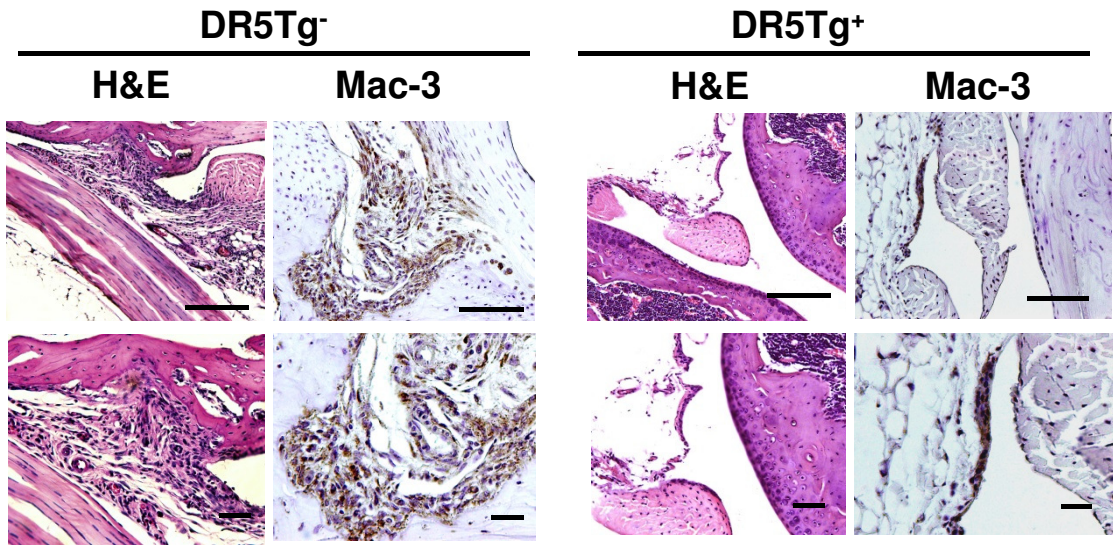


Induction of CIA. CIA was induced using DR5 Tg mice of C57BL/6 background that were 8- to 16-weeks old. Mice were immunized by intradermal administration of chicken Type II collagen in complete CFA, followed by injection of chicken CII in IFA on day 30 after the primary injection. To ensure a higher incidence of CII arthritis, an adenovirus expressing mouse IL-17 ($2x10^9$ pfu/mouse) was administered IV to all mice 2 days prior to the primary immunization with CII.

TRA-8 treatment of CIA mice. TRA-8 (dissolved in PBS, 0.2 mg per mouse) or IgG1 isotype control was administered *i.v.* or *i.p.* twice/week starting on day 0 (early treatment) or on day 30 (late treatment) until mice were sacrificed.

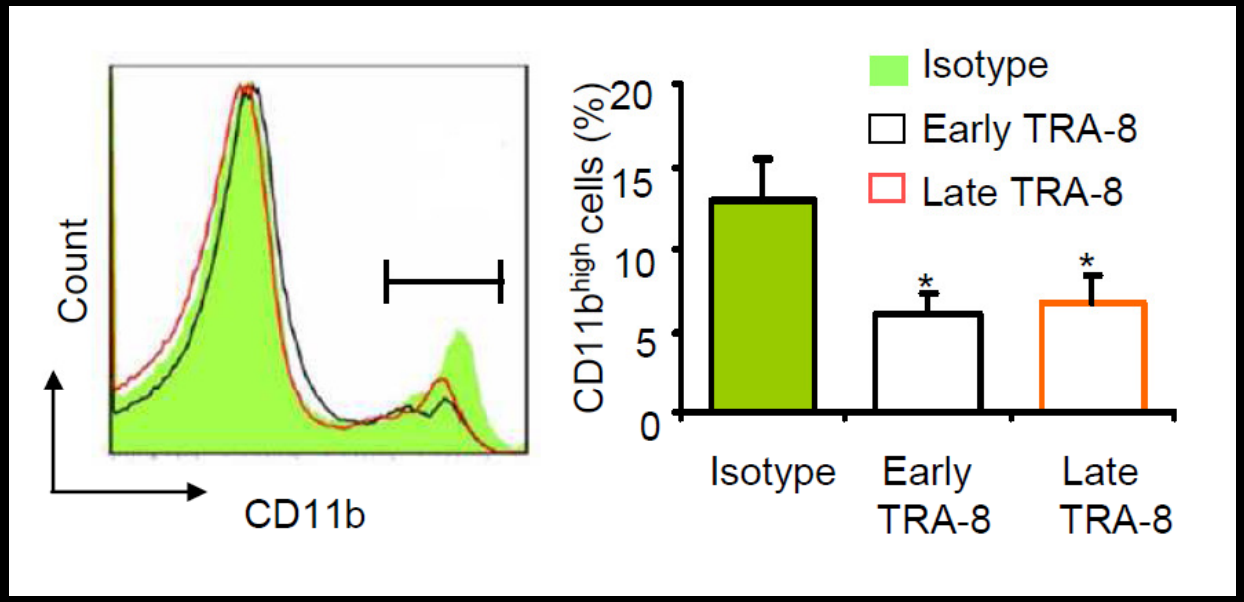
TRA-8 Suppresses the Development of CIA in Ubc.Cre DR5 Mice





Day 60 post 1° cCII

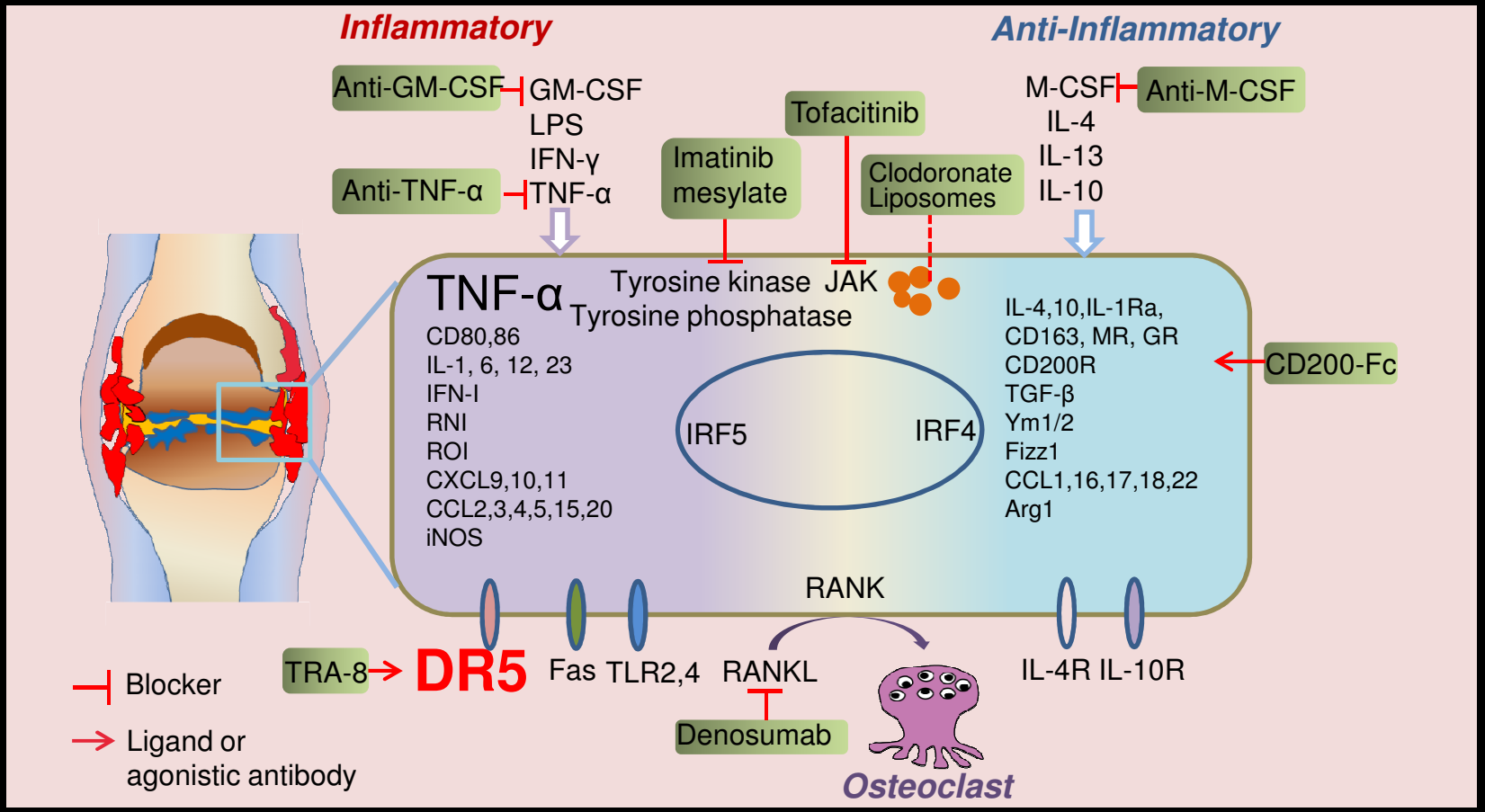
TRA-8 Treatment Depletes Macrophages



Question: What is the TRA-8 effect if TgDR5 expression is restricted in macrophages?

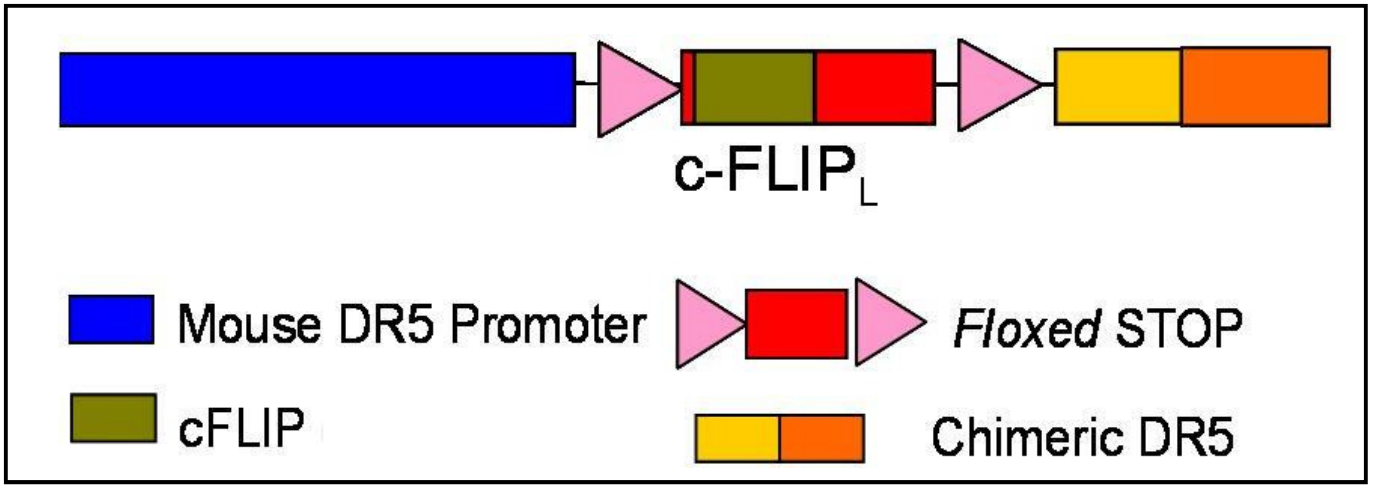
M1/M2 Imbalance in Rheumatoid Arthritis - Can It Be Corrected by TRA-8?

Current therapeutic targets related to macrophages



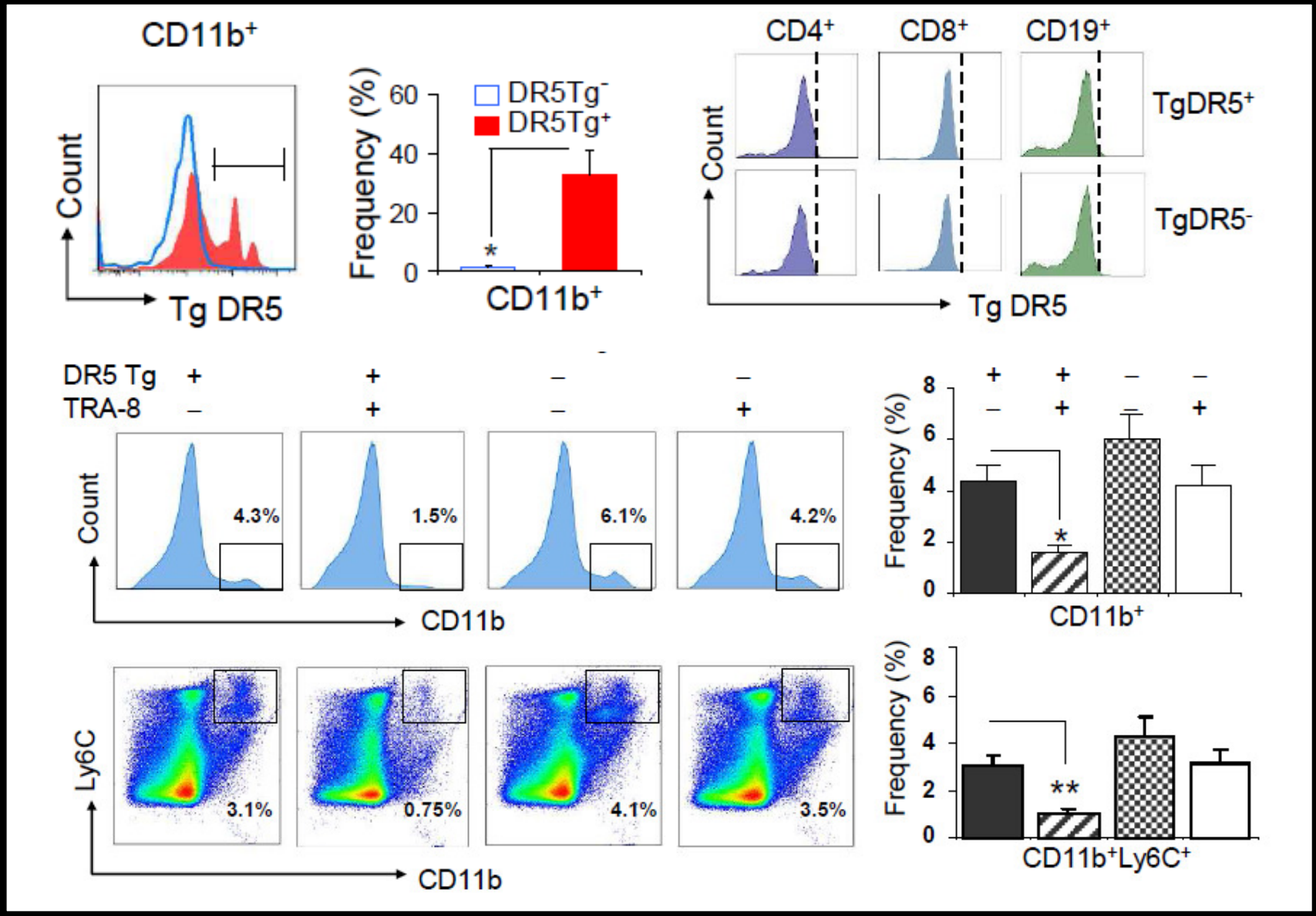
Adapted from J. Li, et al 2012

Generation of Chimeric DR5 Transgenic LysM.Cre Mice

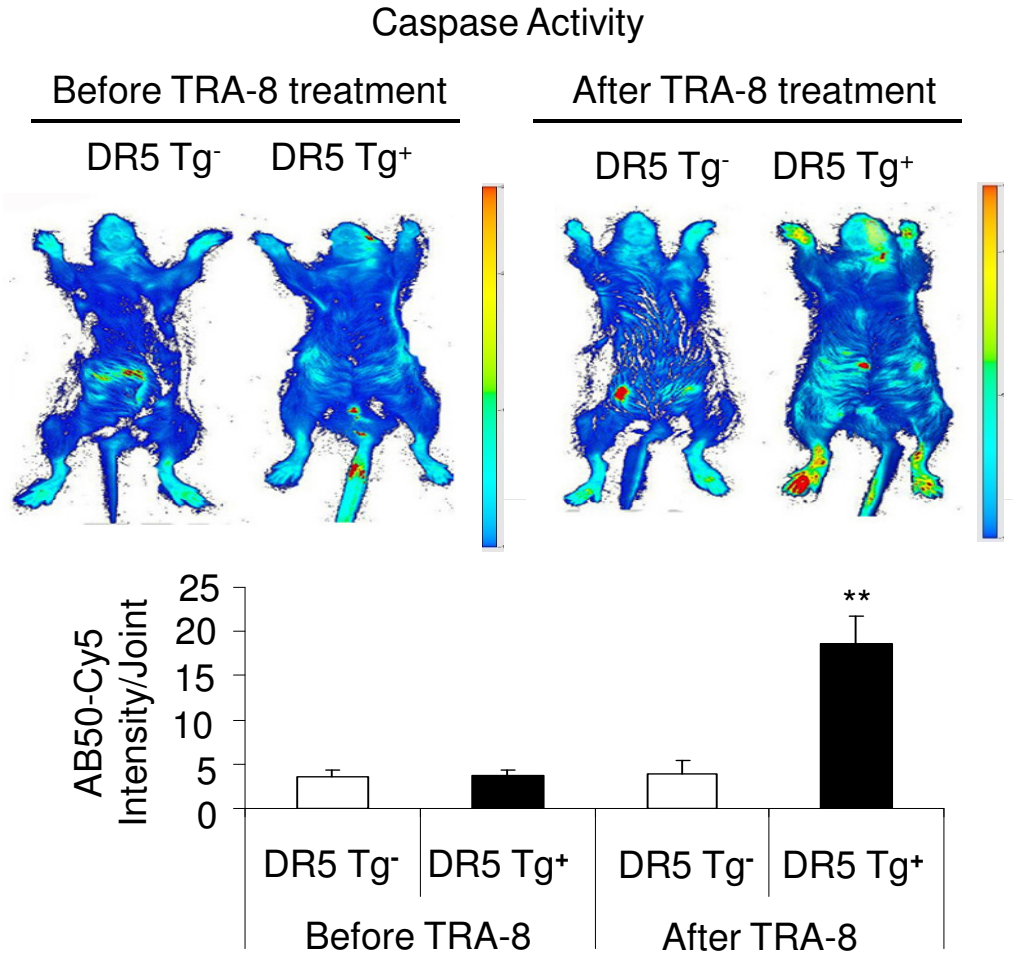


Floxed STOP hu/mo DR5 x LysM.Cre

TRA-8 Treatment Eliminates Inflammatory Macrophages in DR5 Transgenic LysM.Cre CIA Mice

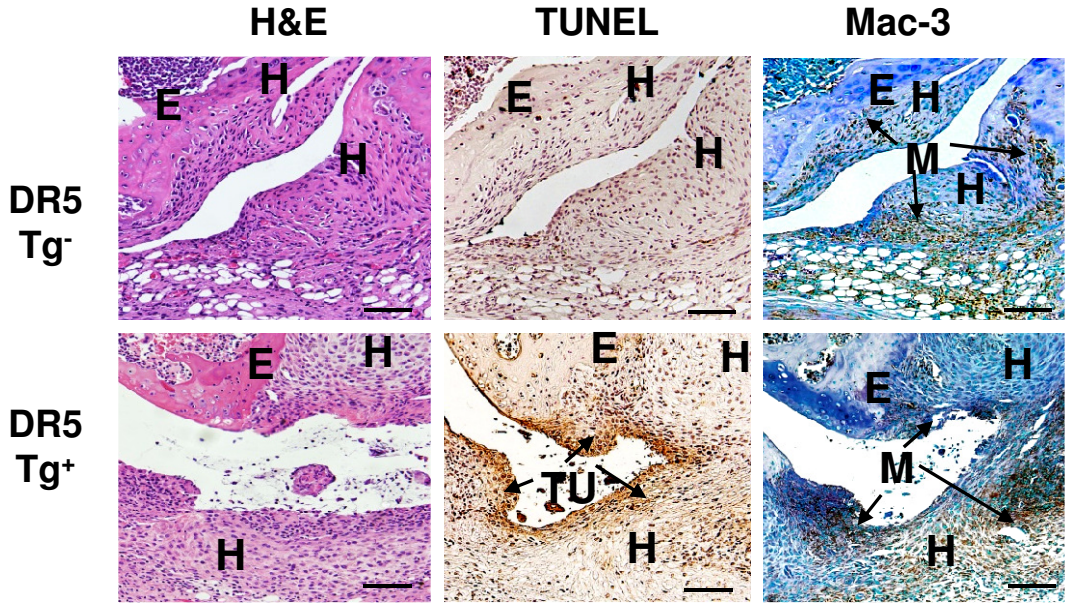


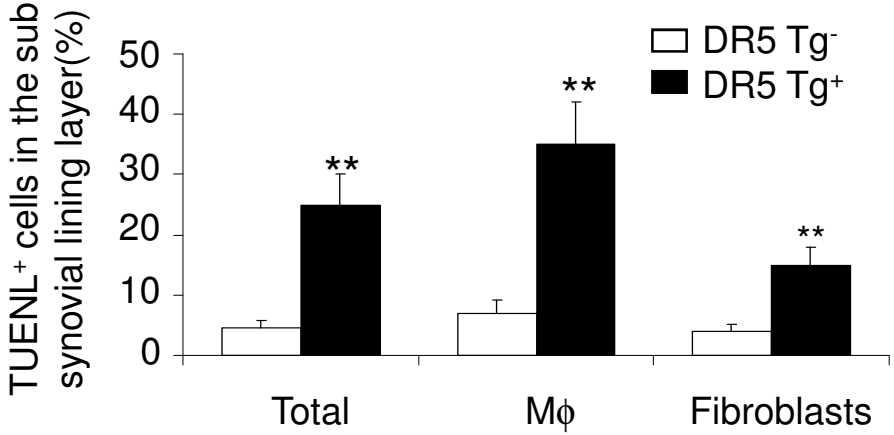
Apoptosis Induced by TRA-8 in Joints of DR5 Transgenic LysM.Cre Mice with CIA



Measurement method: Mice were induced to develop CIA. At 8 week after the primary cCII injection, Mice were then treated with TRA-8 (0.2 mg on day 0 and day 3) and apoptosis imaging using AB50-Cy5 was performed on the same mice on day 6 after initiating TRA-8 treatment.

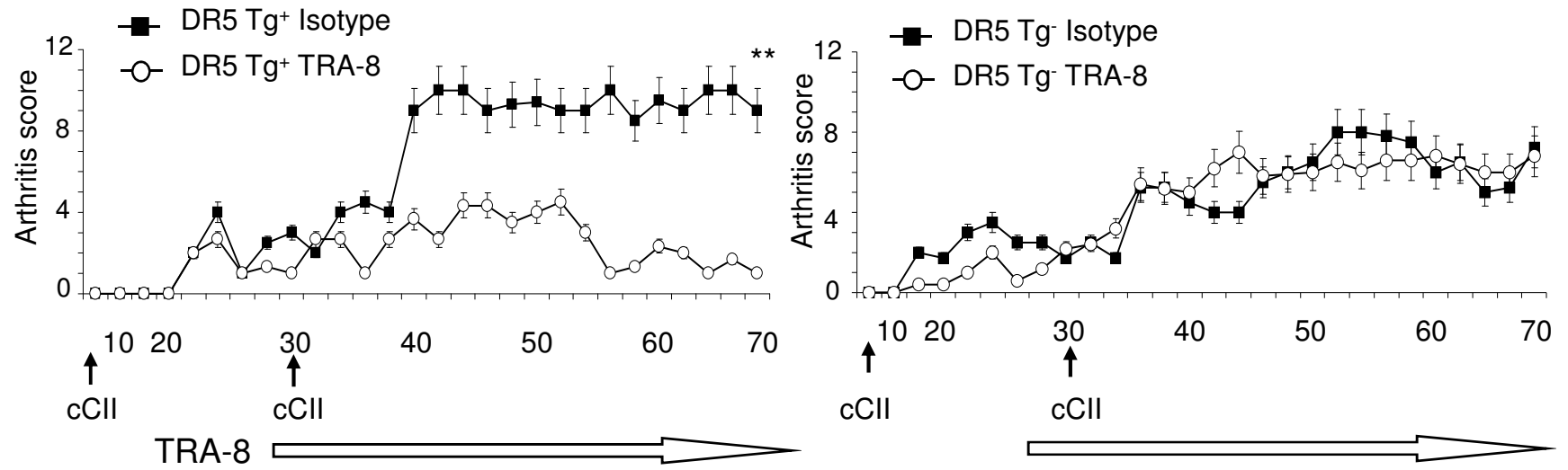
Apoptosis Induced by TRA-8 in Joints of DR5 Transgenic LysM.Cre Mice with CIA

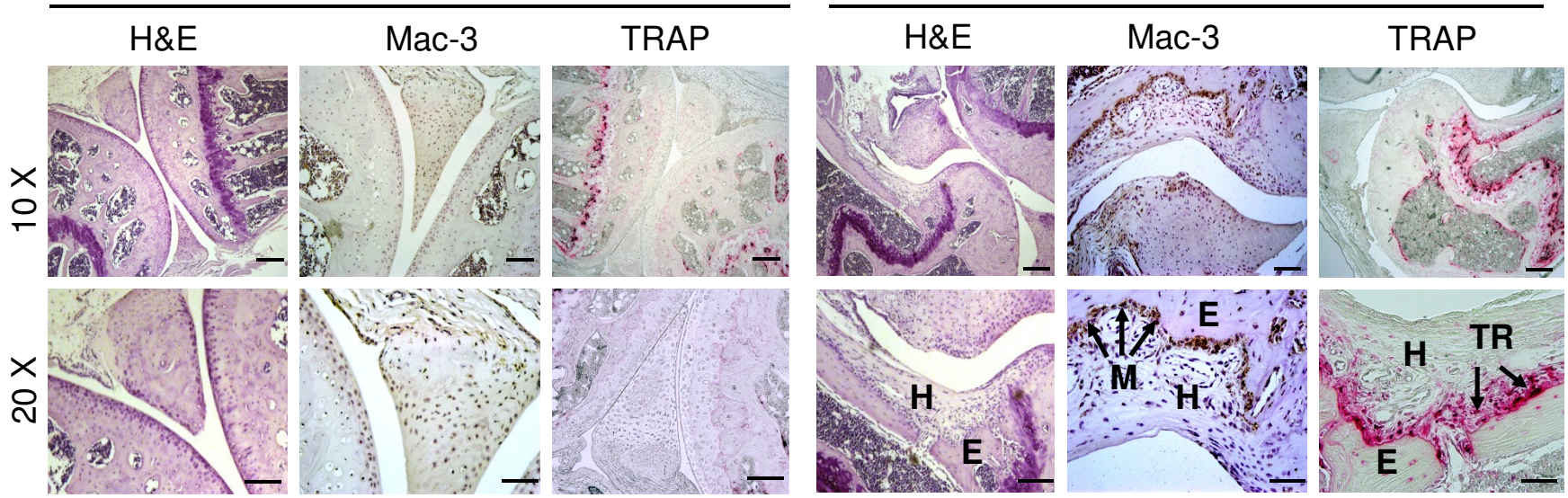




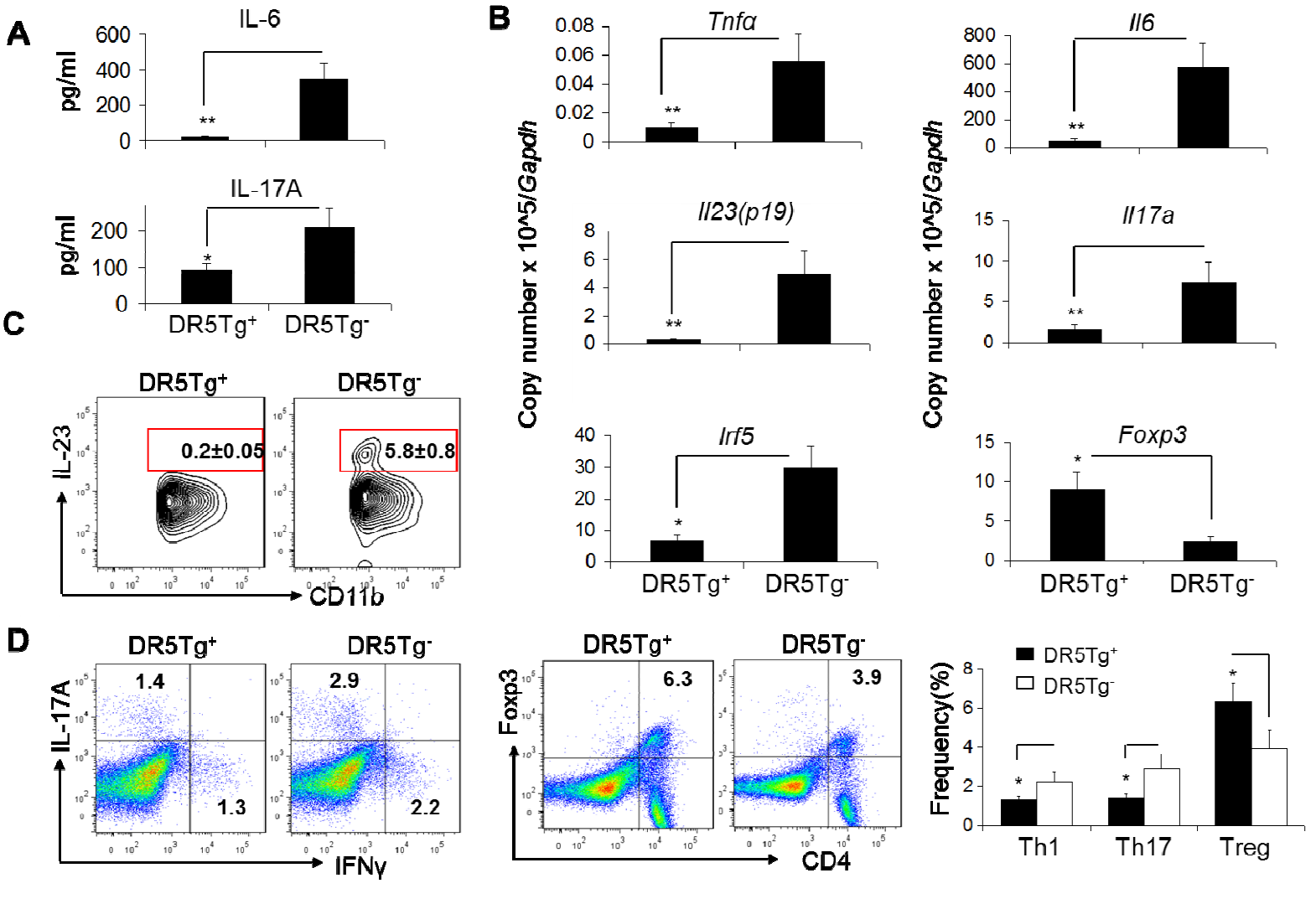
Measurement method: Mice were induced to develop CIA. At 8 week after the primary cCII injection, Mice were then treated with TRA-8 (0.2 mg on day 0 and day 3) and were sacrificed for in situ staining of joint apoptosis (TUNEL) and joint macrophage (Mac-3) infiltration.

TRA-8 Treatment Ameliorates the Severity of CIA in DR5 Transgenic LysM.Cre Mice





Immuno-modulatory Effects of TRA-8 in DR5 Transgenic LysM.Cre Mice with CIA

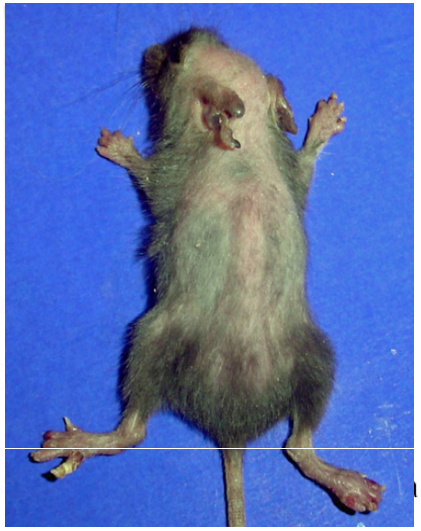


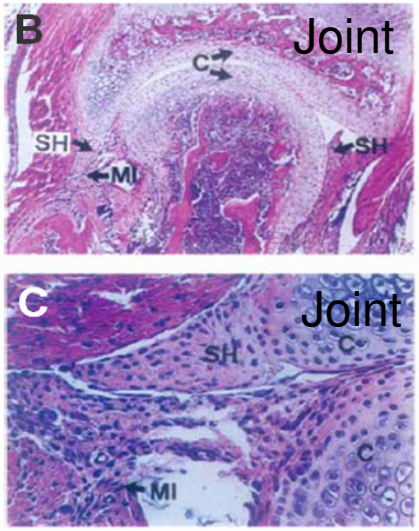
Summary I

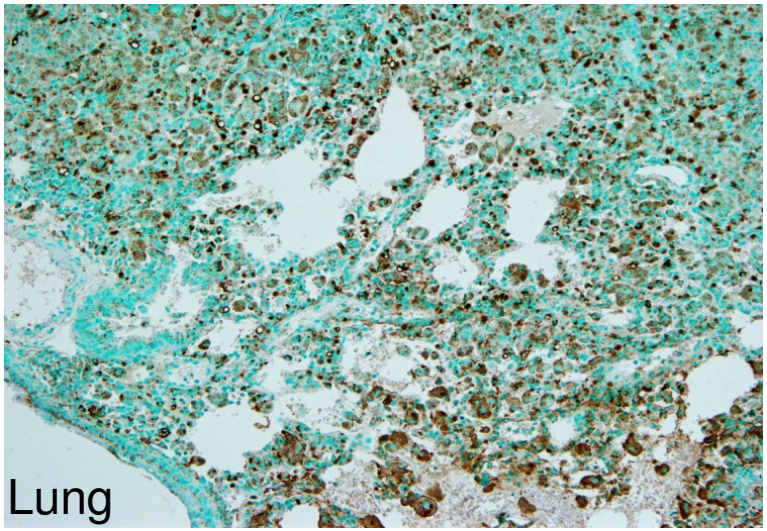
1. In human/mouse DR5 Tg Ubc.Cre mice with CIA, Tg DR5 is expressed on $CD11b^+$ macrophages and $CD4^+$ T cells; In DR5 Tg LysM.Cre mice, Tg DR5 is restrictively expressed on macrophages;
2. TRA-8 (anti-human DR5) treatment results in depletion of pathogenic macrophages and Th17 cells, increased Treg cells in draining LNs of DR5 Tg CIA mice;
3. TRA-8 is potential novel biologic agent for rheumatoid arthritis.

QUESTION: What is the TRA-8 effect in a systemic autoimmune disease model?

Chimeric DR5 Transgenic x Viable Motheaten Mice (*me^v/me^v*)

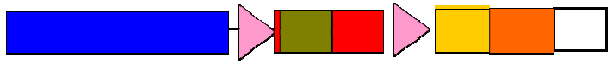






Prominent disorders of SHP-1 (*PTPN6*) deficient mice:

1. Myeloid hyper-proliferation and inappropriate activation.
2. Rapidly progressive patchy dermatitis, limb necrosis, recurrent infections, and premature death consequent to hemorrhagic pneumonitis.



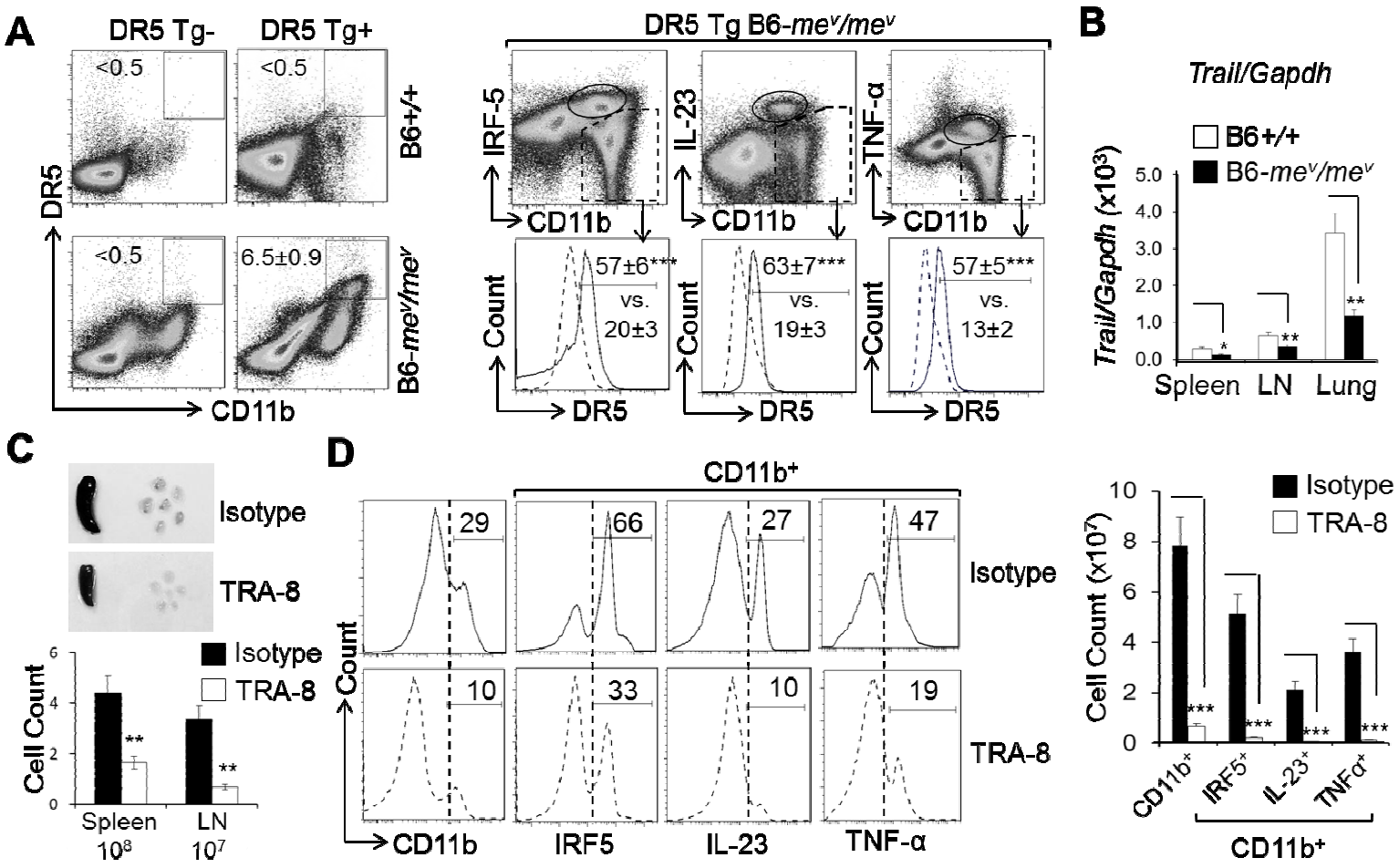
3kb mo DR5 promoter/FloxedSTOP/Chimeric DR5

X

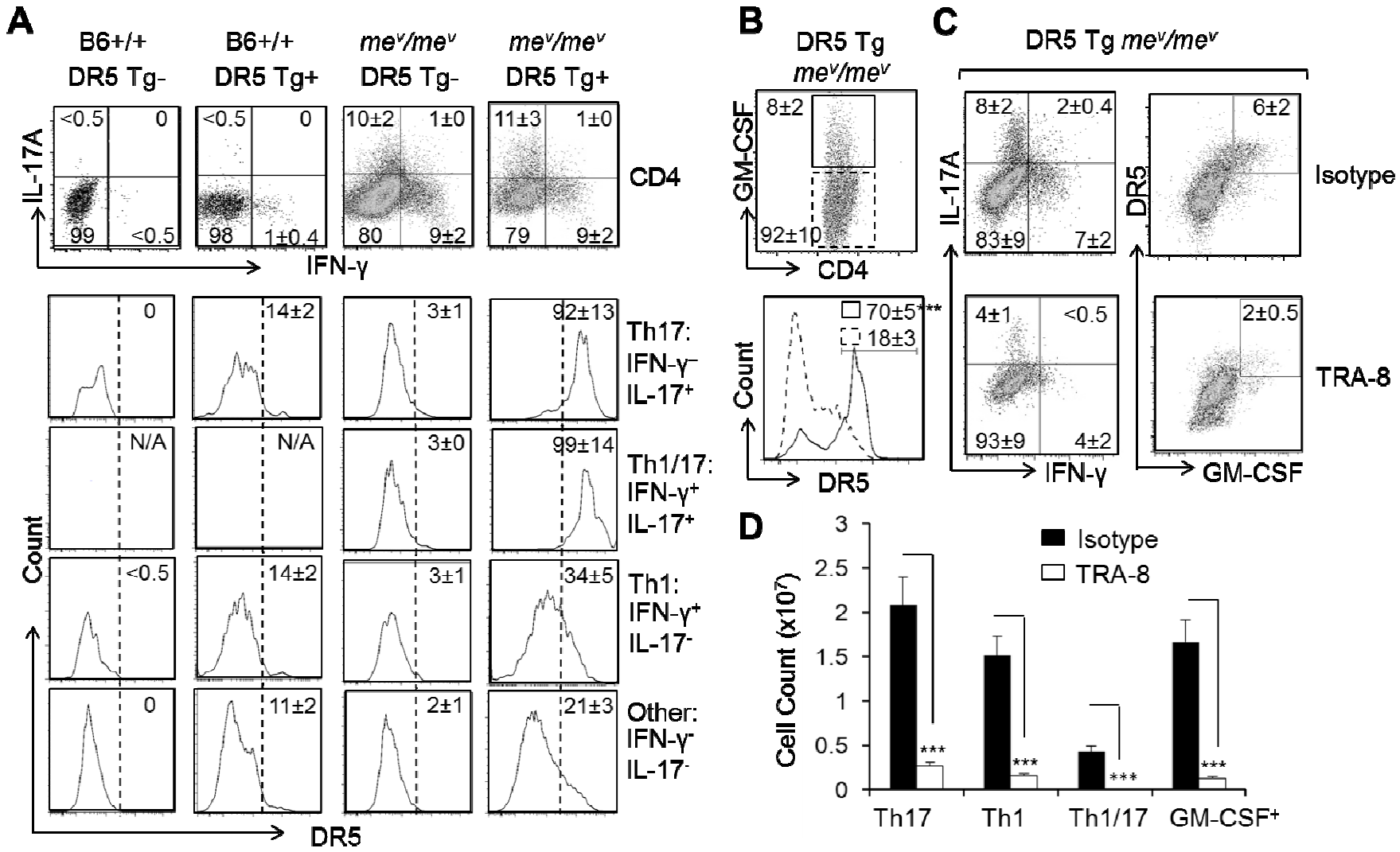
Ubc.Cre
Lyz.Cre

X *me^v/me^v*

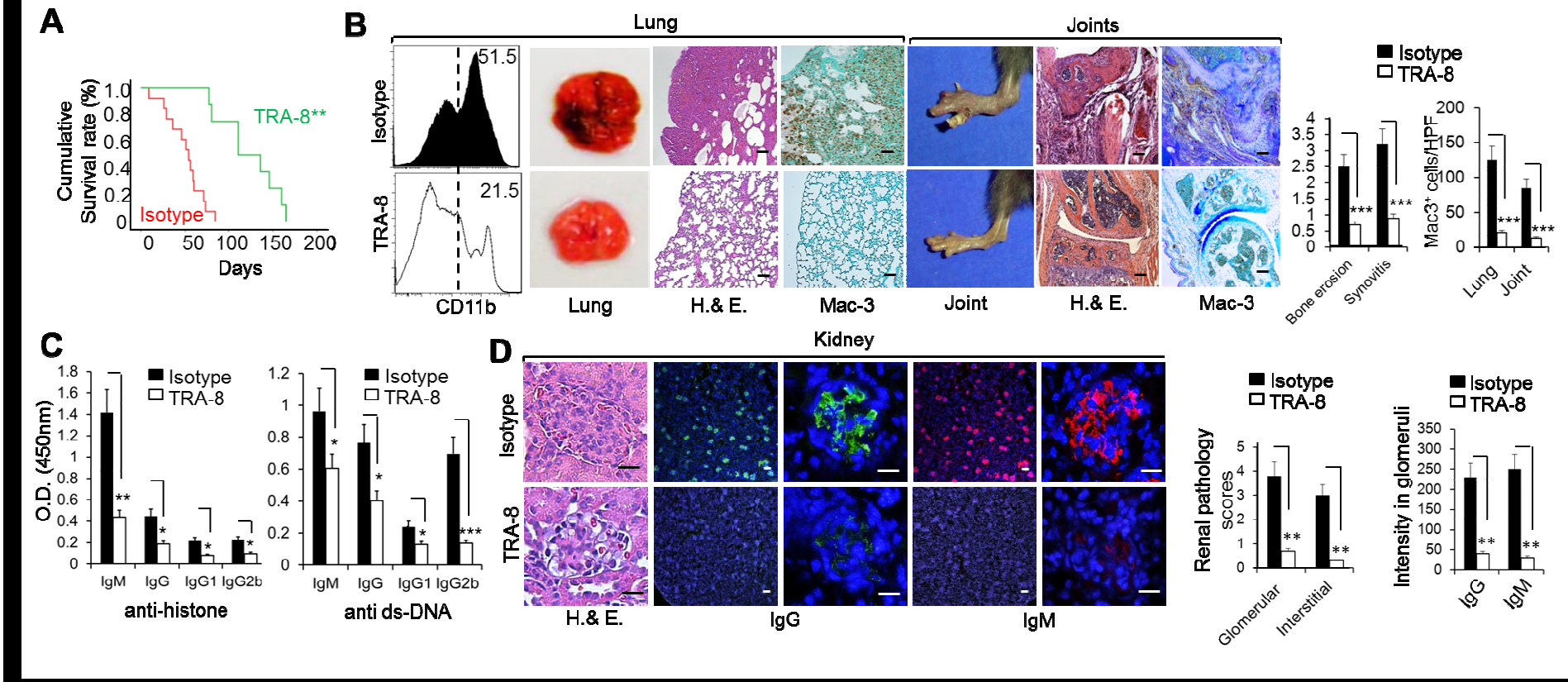
DR5 expression and TRA-8 Induced Depletion of Inflammatory Macrophages in DR5 Tg *me^v/me^v* Mice



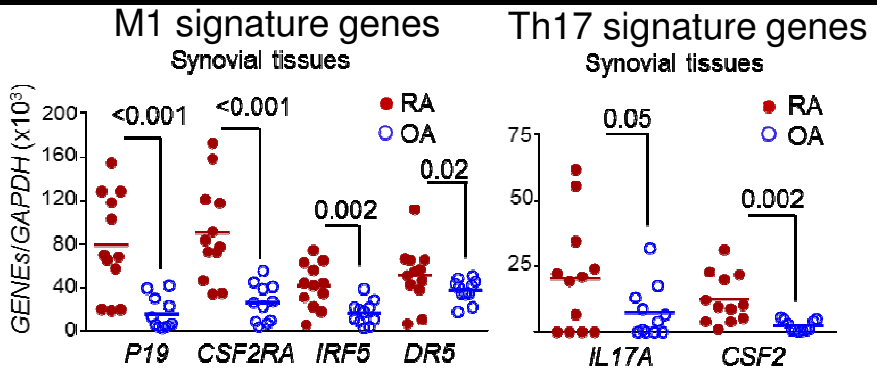
DR5 expression and TRA-8 Induced Depletion of Pathogenic CD4 T Cells in DR5 Tg *me^v/me^v* Mice

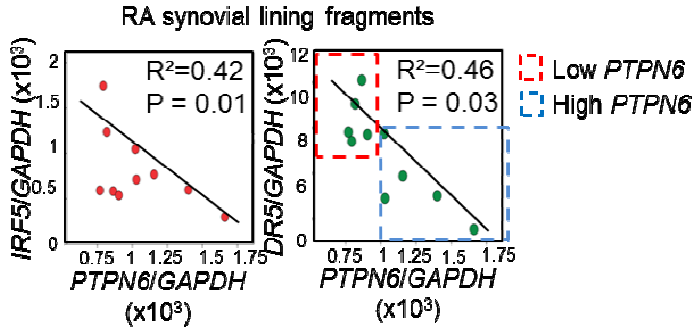


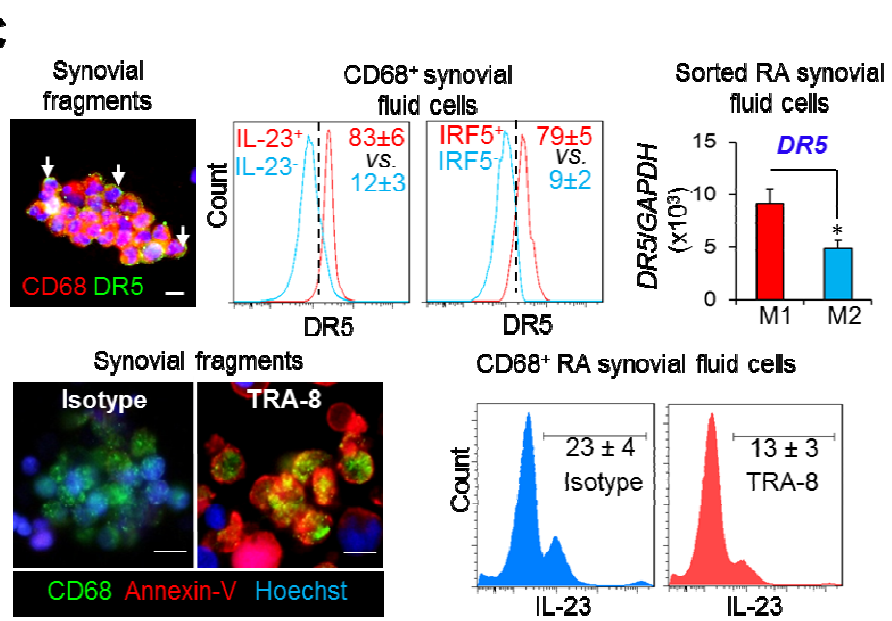
TRA-8 Treatment Reduces Tissue Inflammation and Increases Lifespan of DR5 Tg *me^v/me^v* Mice

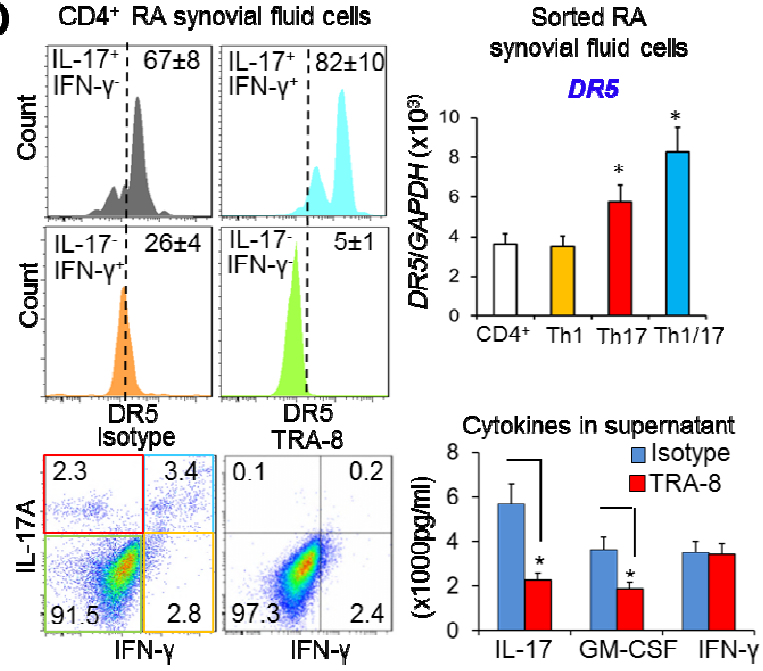


TRA-8 Eliminates Inflammatory M1 Macrophages and CD4 T Cells in Human RA Patients with Low *PTPN6*



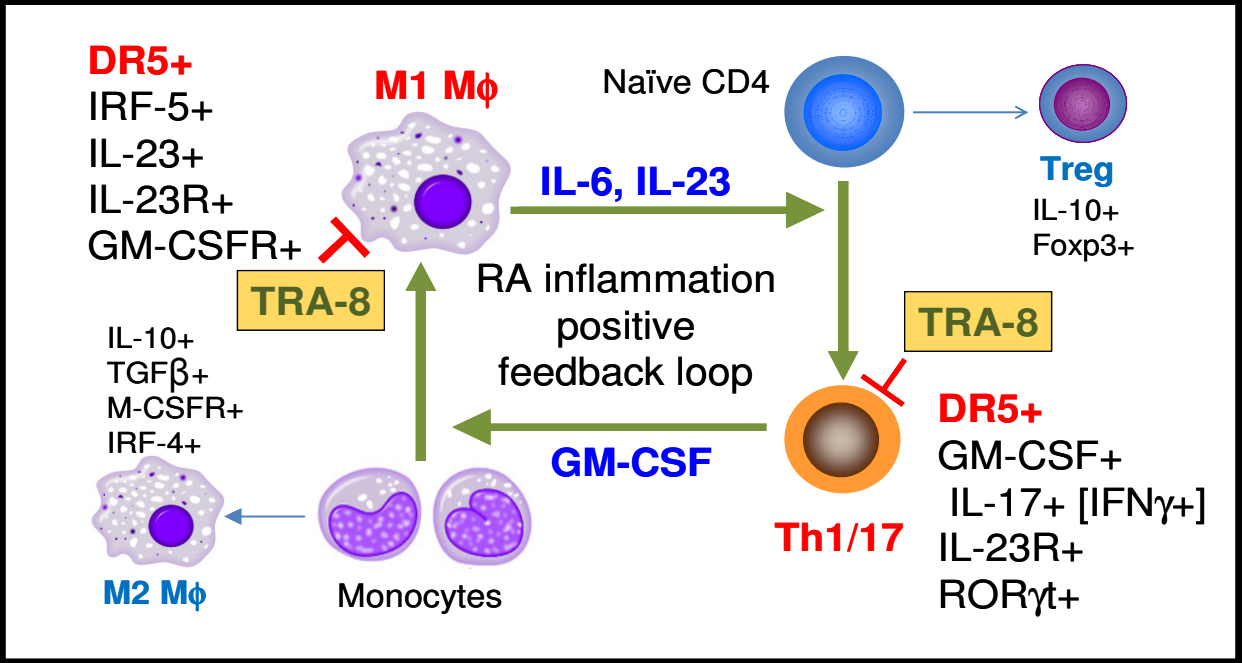






Summary II

1. DR5 expression is upregulated in inflammatory macrophages and pathogenic CD4 T cells in DR5 Tg *me^{v}/me^{v}* Mice;
2. TRA-8 selectively eliminates IRF5^{+} IL-23^{+} pathogenic macrophages and IL-17^{+} GM-CSF^{+} CD4 T cells in both human and mouse autoimmune conditions, resulting in immune homeostasis and resolution of inflammation.
3. Anti-human DR5 (TRA-8) can deplete inflammatory cell populations that result from a hyperactive GM-CSF/IRF5 AXIS, and it is a novel biologic agent for RA and other autoimmune diseases.



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Dr. David M. Spalding
Dr. W. Winn Chatham
Dr. Laura Hughes

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