Supplementary Information

Engineered Self-Regulating Macrophages for Targeted Anti-inflammatory Drug Delivery

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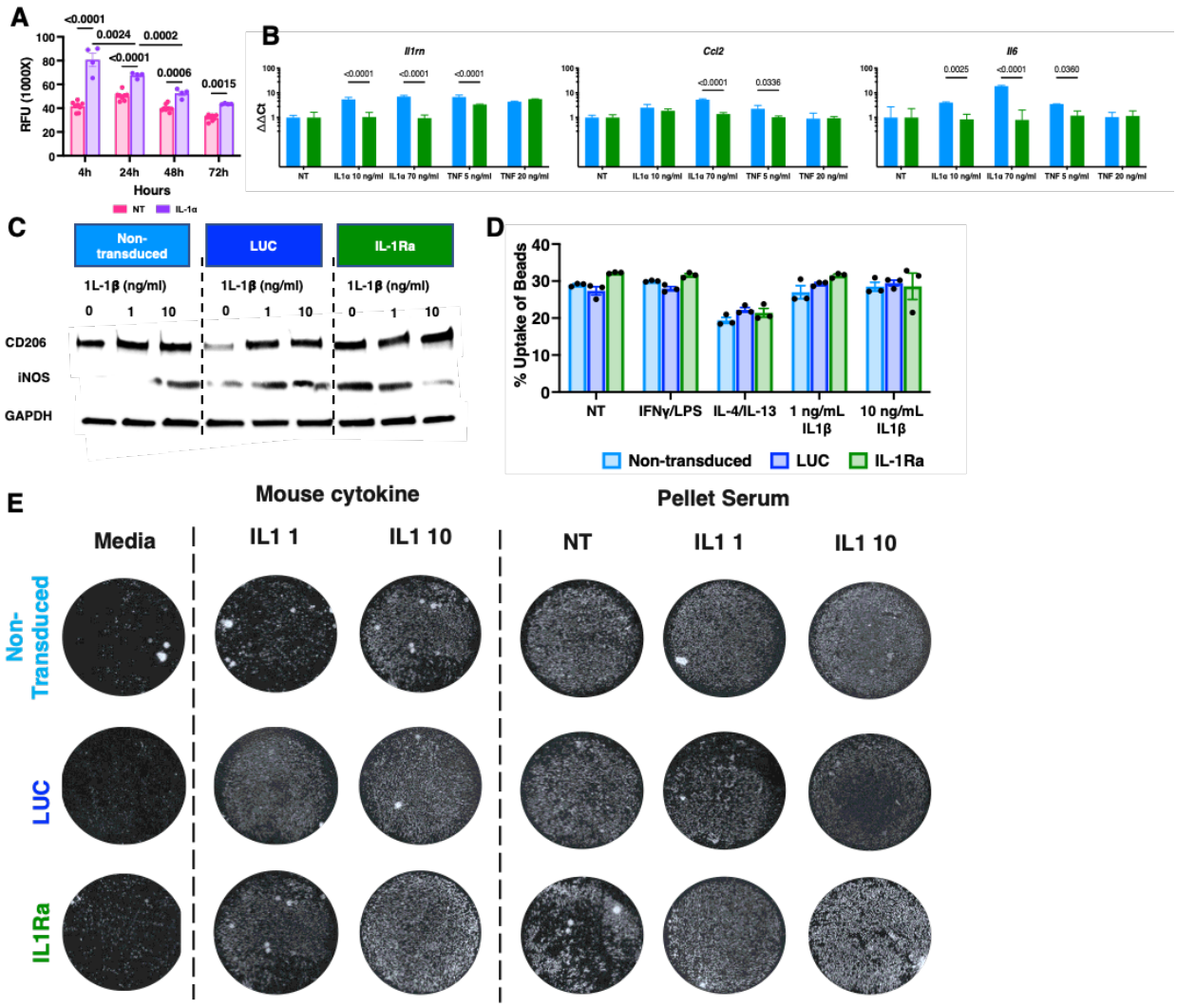
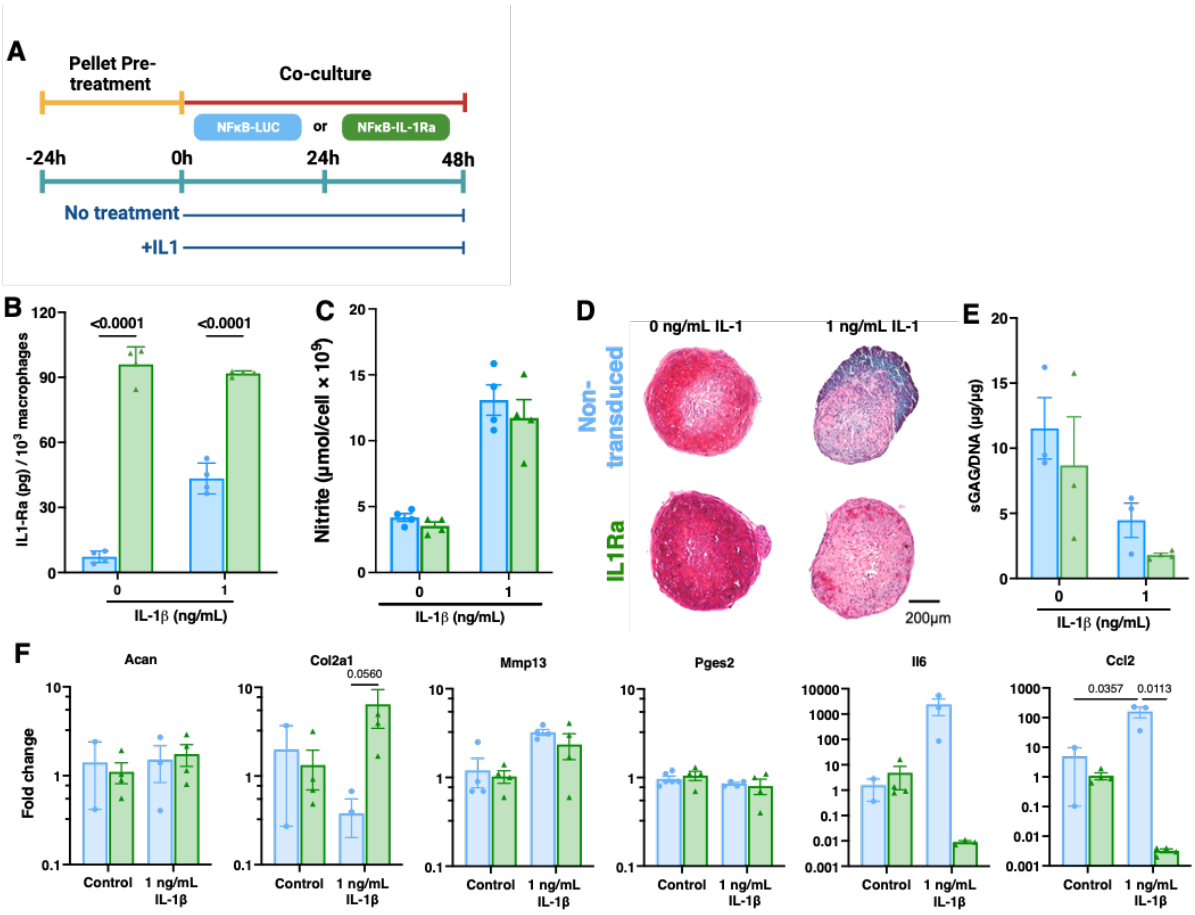
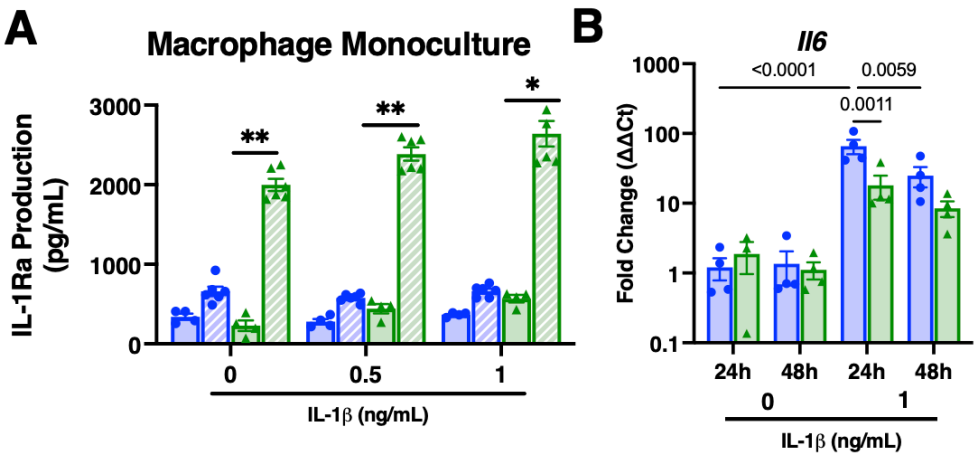


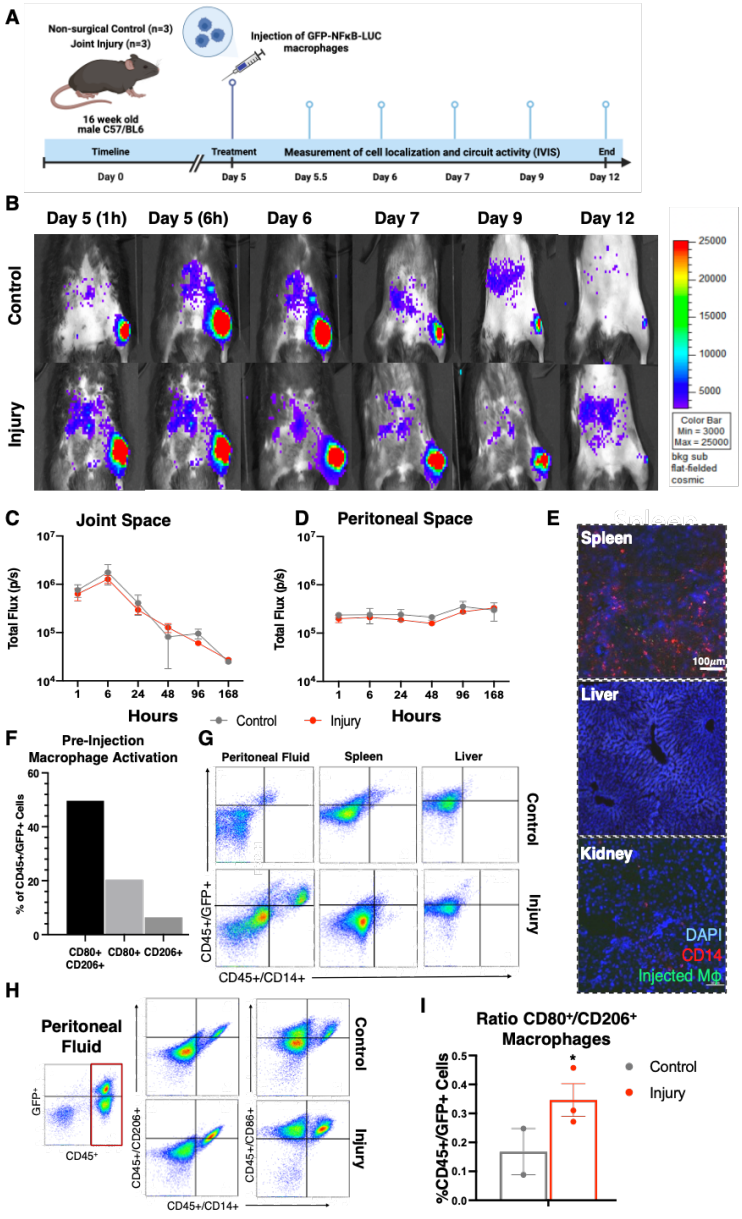
Supplemental Figure 1. Dynamic self-regulation of the NF-κB synthetic promoter in monolayer. (A) LUC macrophages demonstrate circuit activation by 4 hours which is resolved by 72 hours following treatment with IL-1 (n=5). **(B)** Gene expression of IL-1Ra BMDMs in monoculture in reference to non-transduced, non-treated BMDMs when challenged with either no treatment (NT), 10 or 70 ng/mL IL-1α, or 5 or 20 ng/mL of TNF for 48h normalized to GAPDH and R18S (n=4) demonstrated significant reduction in NF-κB responsive genes by IL-1Ra macrophages for both medium and high doses (n=4). **(C)** Western blot analysis revealed that NRE-IL-1Ra macrophages possessed a similar phenotype in comparison to non-transduced and NRE-LUC macrophages in response to IL-1β (n=3). Full replicates are shown in Supplemental Figure 5. **(D)** Flow cytometry of engineered macrophages as compared to non-transduced controls demonstrates little change in phagocytosis of FITC labeled latex beads following a 4-hour incubation (n=3). **(E)** Migration of BMDMs in response to both cytokine and conditioned media stimuli for non-transduced and engineered macrophages over 16 hours (n=3). Data was assessed using a one or two-way ANOVA with Tukey's post hoc test; values represent mean with standard error of the mean (SEM) (n = 3-4); NT, no treatment; RFU, relative luminescence unit; IL-1, Interleukin-1; TNF - tumor necrosis factor.



Supplemental Figure 2. IL-1Ra macrophages offer moderate chondroprotection against inflammatory mediators and inflammatory driven matrix degradation in response to IL-1β. **(A)** Timeline of co-culture experimental conditions. **(B)** LUC/IL-1Ra macrophage production of IL-1Ra in co-culture over 48 hours, as measured by ELISA, following stimulation with 0.5 or 1 ng/ml of IL-1β. **(C)** Concentration of nitric oxide (NO2-) secreted into the media normalized to cell number following treatment with 0.5 or 1 ng/ml of IL-1β. **(D)** Safranin-O (red)/fast green(blue)/hematoxylin-stained tissue sections of miPSC derived cartilage co-cultured with LUC or IL-1Ra macrophages. **(E)** Amount of sGAG per pellet normalized to DNA content and secreted into the media during culture. **(F)** Gene expression of miPSC derived cartilage pellets in co-culture in reference to non-treated (NT) control when challenged with 1 ng/mL IL-1β for 48h normalized to GAPDH and R18S (n=3-4) demonstrated significant reduction in NF-κB responsive genes and some chondroprotective effects when cultured with IL-1Ra macrophages. Data was assessed using a two-way ANOVA with Tukey's post hoc test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; values represent mean with standard error of the mean (SEM) (n = 3-4).



Supplemental Figure 3. IL-1Ra macrophages offer moderate chondroprotection against inflammatory mediators and inflammatory driven matrix degradation in response to IL-1β. **(A)** LUC/IL-1Ra macrophage production of IL-1Ra in co-culture over 48 hours, as measured by ELISA, following stimulation with 0.5 or 1 ng/ml of IL-1β. **(B)** Gene expression of engineered macrophages in monoculture in reference to non-treated control when challenged with 0 or 1 ng/mL IL-1β for 48h normalized to GAPDH and R18S (n=3-4). Data was assessed using a two-way ANOVA with Tukey's post hoc test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; values represent mean with standard error of the mean (SEM) (n = 3-4).



Supplemental Figure 4. Engineered macrophages can maintain their presence in the joint for at least 7 days. (A) Timeline of experimental conditions **(B-D)** NF-κB-LUC macrophages were given by interarticular injection into mice after receiving a joint injury or into non-surgical controls. Localization and activation of the engineered macrophages was monitored through IVIS imaging and quantified for the joint space and peritoneal fluid over time starting 1 hour following injection and continuing for 7 days **(E)** Macrophage activation was examined for inflammatory marker CD80 and anti-inflammatory marker CD206. At 7 days following injection, mouse tissues were examined for injected macrophages through **(F)** immunofluorescence as well as **(G)** flow cytometry on cells isolated from dissociated tissues (n=3). **(H)** Peritoneal fluid possessed the only notable population of engineered macrophages and was examined for macrophage activation post-injection based on the same markers, CD80 and CD206, which are **(I)** quantified.

Supplemental Table 1. qPCR primers

Gene	Forward	Reverse
Ccl2	GGC TCA GCC AGA TGC AGT TAA	CCT ACT CAT TGG GAT CAT CTT GCT
IL1RN	GTC CAG GAT GGT TCC TCT GC	TCT TCC GGT GTG TTG GTG AG
IL6	GAG GAT ACC ACT CCC AAC AGA CC	AAG TGC ATC ATC GTT GTT CAT ACA
R18s	CGG CTA CCA CAT CCA AGG AA	GGG CCT CGA AAG AGT CCT GT
Acan	GCA TGA GAG AGG CGA ATG GA	CTG ATC TCG TAG CGA TCT TTC TTC T
Col2a1	TCC AGA TGA CTT TCC TCC GTC TA	AGG TAG GCG ATG CTG TTC TTA CA
GAPDH	CAT GGC CTT CCG TGT TCC TA	TGT CAT CAT ACT TGG CAG GTT TCT
TNFa	GGT GCC TAT GTC TCA GCC TCT T	GCC ATA GAA CTG ATG AGA GGG AG
PGES2	AAG ACA TGT CCC TTC TGC	CCA AGA TGG GCA CTT TCC
MMP13	TCA AGG CTA TGC ACA CTG GT	CAC TAT GGT CTT TTC AAT GCC TAA
ADAMPTS4	CAG TCA CCT CTA AGC CAA AGA AA	CTT CCG GCG TAG GAT GTG AG

Supplemental Table 2. Flow cytometry antibodies

Conjugate	Specificity	Manufacturer	Catalog Number
Sytox Blue	Live/Dead	Thermo Scientific	S34857
PerCP-Cy5.5	anti-mouse F4/80	Biolegend	123127
PE	anti-mouse CD163	Biolegend	156703
PE	anti-mouse CD14	Biolegend	123309
PE	anti-mouse CD206	BioLegend	141705
PE	anti-mouse CD163	Biolegend	156703
PE	anti-mouse CD80	Biolegend	104707
PE	anti-mouse CD45	Biolegend	103105
PE	anti-mouse CD301	Biolegend	145703
PE/Cy7	anti-mouse CD206	BioLegend	141719
PE/Cy7	anti-mouse CD11b	Biolegend	101215
PE/Cy7	anti-mouse CD80	Biolegend	104733
PE/Cy7	anti-mouse Cx3CR1	Biolegend	149015
APC	anti-mouse CD206	BioLegend	141707
APC	anti-mouse CD11b	Biolegend	101211
APC	anti-mouse CD45	Biolegend	103111