

Precision RNAi for Fibrodysplasia Ossificans Progressiva: a combinatorial, unimolecular, allele selective approach.

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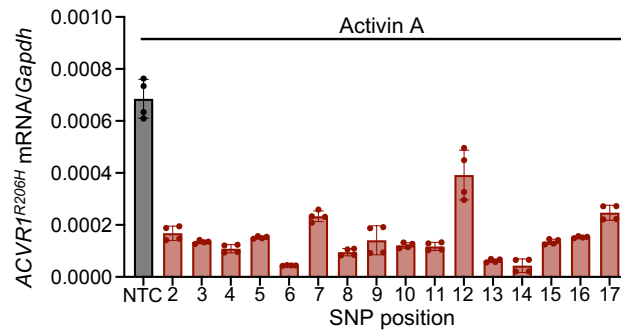
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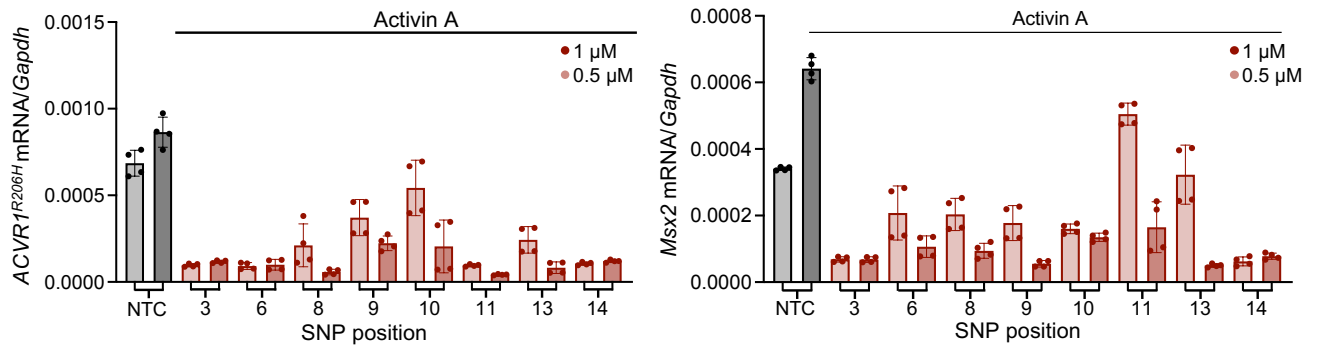
Supplemental information:

- 20 Extended Figures
- Uncropped western blot images
- 6 Extended Tables

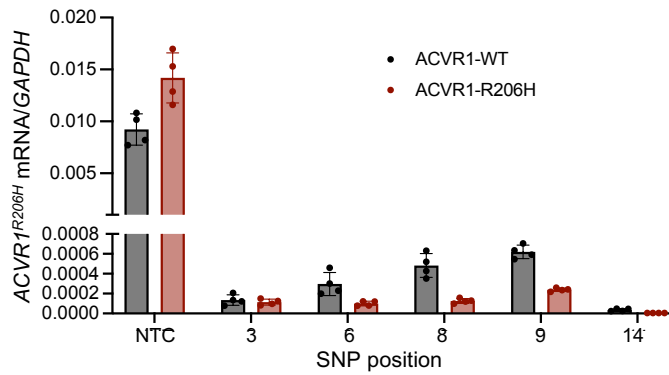
a Silencing Efficacy of *ACVR1^{R206H}* After Activin A Stimulation



b Silencing Efficacy of *ACVR1^{R206H}* and *Msx2* After Activin A Stimulation at Two Different Doses



c Primary Mismatch Screening in Human FOP iPSCs



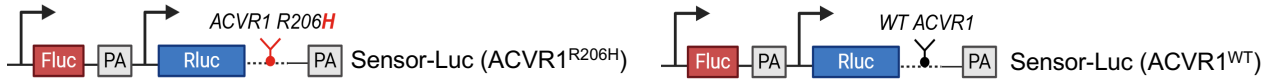
Extended Figure 1. Screening of siRNA candidates targeting human *ACVR1^{R206H}* mRNA (Primary mismatch).

a. Mouse bone marrow-derived stromal cells expressing human *ACVR1^{R206H}* (*ACVR1^{(R206H)Fl};Prrx1-cre* BMSCs) were treated with a panel of 16 siRNAs (1 μ M) and three days later, cells were stimulated with Activin A (50 ng/ml) for twelve hours. mRNA levels of *ACVR1^{R206H}* were measured by RT-PCR and normalized to *Gapdh* (n = 4). NTC: non-targeting control.

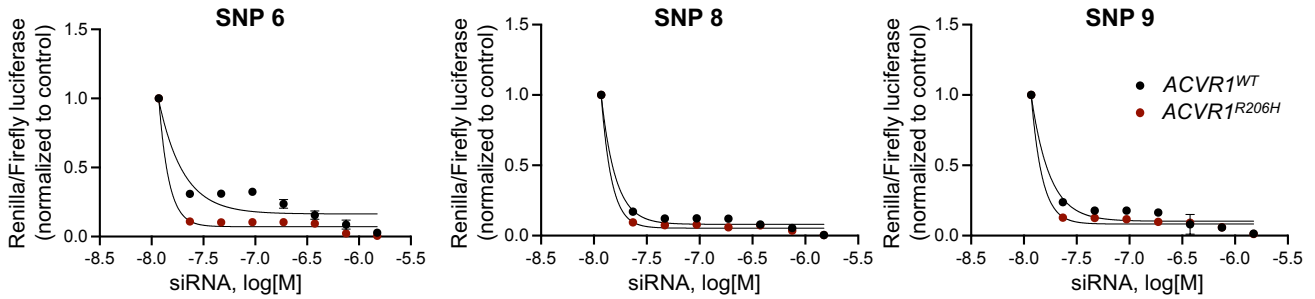
b. *ACVR1^{(R206H)Fl};Prrx1-cre* BMSCs were treated with each siRNA at 1.0 and 0.5 μ M and three days later, cells were stimulated with Activin A (50 ng/ml) for twelve hours. mRNA levels of *ACVR1^{R206H}* and *Msx2* were measured by RT-PCR and normalized to *Gapdh* (n = 4).

c. Human FOP iPSCs were treated with 1 μ M siNTC or siACVR1 and cultured under osteogenic conditions for four days. mRNA levels of *ACVR1^{+/+}* and *ACVR1^{R206H}* were measured by RT-PCR and normalized to *GAPDH* (n = 4). Data are representative of three independent experiments. Values represent mean \pm SD by one-way ANOVA tests.

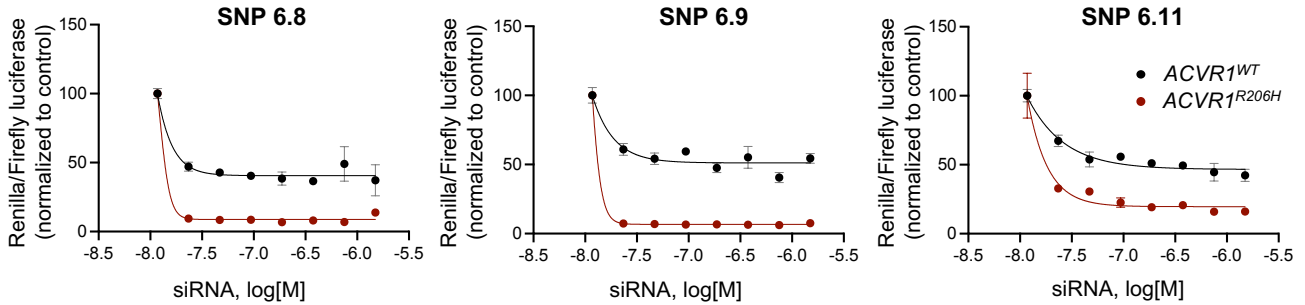
a Construction of *ACVR1^{R206H}* and *ACVR1^{WT}* SNP Reporter Plasmid



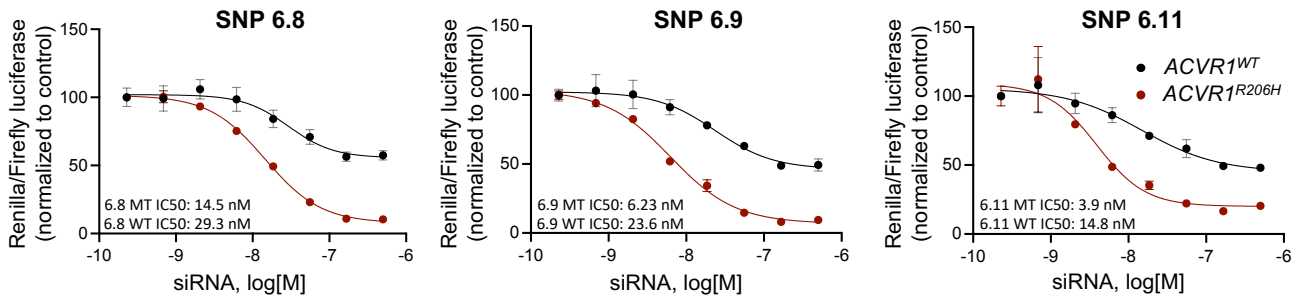
b Primary Mismatch at Positions 6, 8, and 9 Enables Potent *ACVR1^{R206H}* Silencing with Minimal *ACVR1^{WT}* Discrimination



c Secondary Mismatch at Position 9 Reveals Potent Silencing of *ACVR1^{R206H}*, but not *ACVR1^{WT}* (High dose)



d Secondary Mismatch at Position 9 Reveals Potent Silencing of *ACVR1^{R206H}*, but not *ACVR1^{WT}* (Low dose)



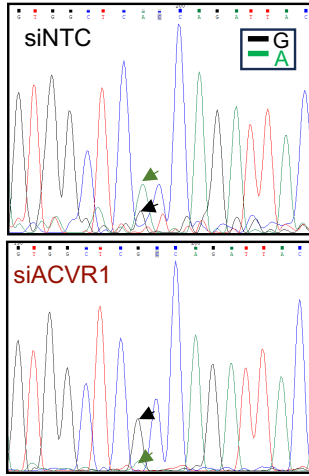
Extended Figure 2. Screening of siRNA candidates targeting human *ACVR1^{R206H}* mRNA in 7-point dose response (Primary and secondary mismatch).

a. Schematic diagram showing the reporter plasmids that contain firefly luciferase (Fluc), *Renilla* luciferase (RLuc), with complementary sequences of human *ACVR1^{R206H}* or *ACVR1^{WT}* incorporated into the 3' UTR of RLuc.

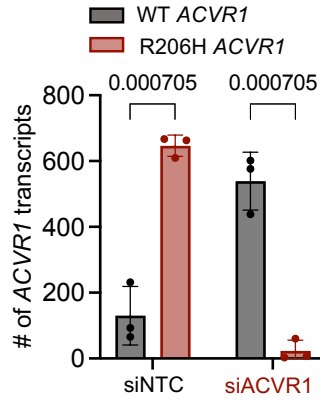
b-d. 1 day after siRNA treatment, HEK293 cells were transiently transfected with the reporter plasmids in 7-point dose response. Two days later, a luciferase assay was performed to measure RLuc and normalized to Fluc. Lower activities indicate higher silencing efficacy of siRNAs (n = 3). Data are representative of three independent experiments. Values represent mean \pm SD by one-way ANOVA test **(b-d)**.

Selective *ACVR1^{R206H}* Silencing in FOP iPSCs

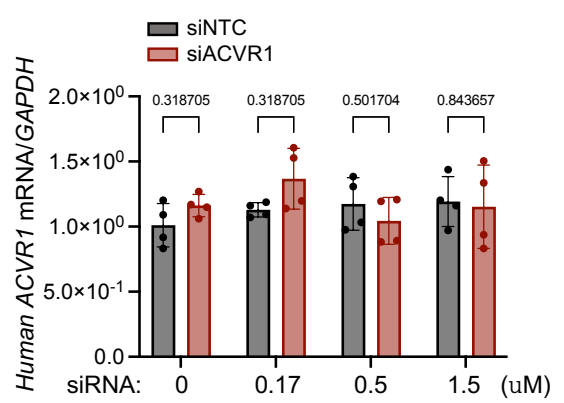
a Sanger sequencing selectivity



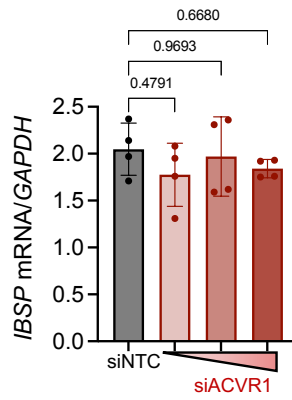
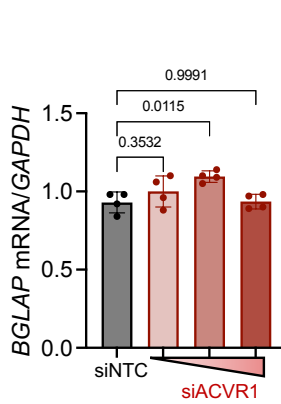
b Transcript number selectivity



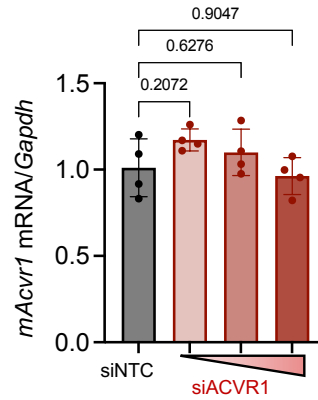
c Human *ACVR1* is unaffected in human WT BMSCs



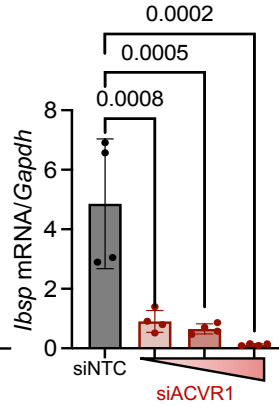
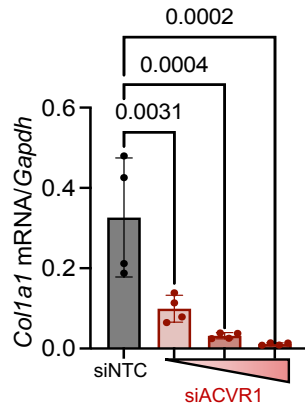
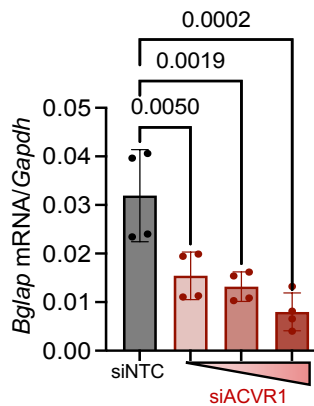
d Osteogenic markers are unaffected in human WT BMSCs



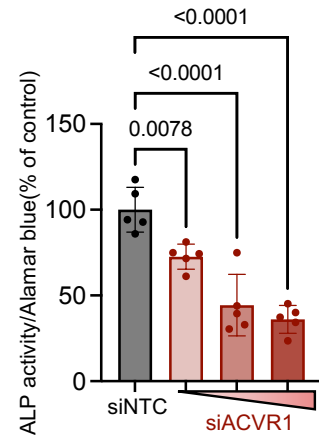
e No effect of siACVR1 on mouse *Acvr1* silencing in FOP BMSCs



f siACVR1 Suppresses Osteogenic Gene Expression in *ACVR1^{R206H}* BMSCs



g Early-Stage Osteogenesis



Extended Figure 3. siACVR1 treatment inhibits osteogenic development in FOP cells, but not in WT cells.

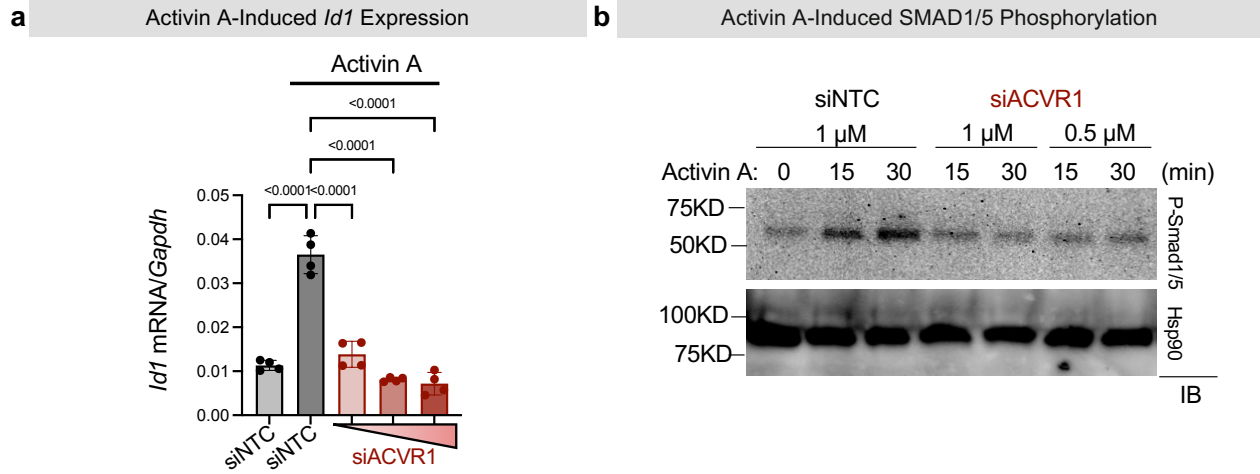
a, b. Human FOP iPSCs were treated with 1 μ M siNTC or siACVR1, cultured under osteogenic conditions for four days, and subjected to Sanger sequencing (n = 4). Black (Guanine, G) and green (Adenine, A) indicate wild type and R206H mutation in *ACVR1* gene, respectively (**a**). Alternatively, extracted RNA was subjected to next-generation sequencing (NGS) to quantify human *ACVR1^{R206H}* and *ACVR1^{WT}* transcripts (**b**).

c, d. Human bone marrow-derived stromal cells (BMSCs) were treated with siNTC or siACVR1 in a 3-point dose response and cultured under osteogenic conditions for four days. mRNA levels of human *ACVR1* (**c**) and osteogenic marker genes (**d**) were measured by RT-PCR and normalized to *GAPDH* (n = 4).

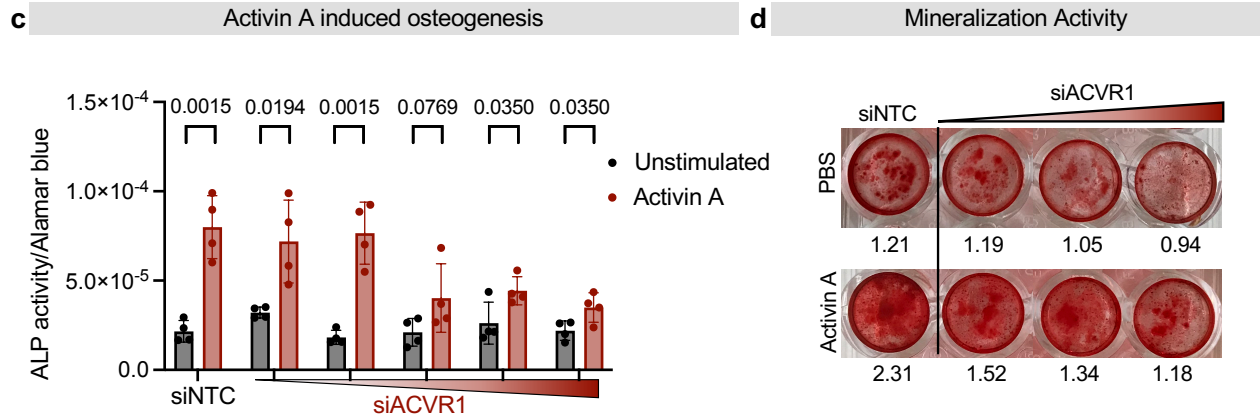
e-g. *ACVR1^{(R206H)Fl};Prrx1-cre* BMSCs were treated with siNTC or siACVR1 in a 3-point dose response and cultured under osteogenic conditions for four days. mRNA levels of mouse *Acvr1* (**e**) and osteogenic marker genes (**f**) were measured by RT-PCR and normalized to *Gapdh* (n = 4).

Alternatively, osteogenic differentiation and cell proliferation were assessed by alkaline phosphatase activity (ALP) and alamar blue staining, respectively (n = 5, **g**). Data are representative of two-three independent experiments. Values represent mean \pm SD by two-sided unpaired t-tests (**b, c**) and one-way ANOVA test (**d, e, f, g**).

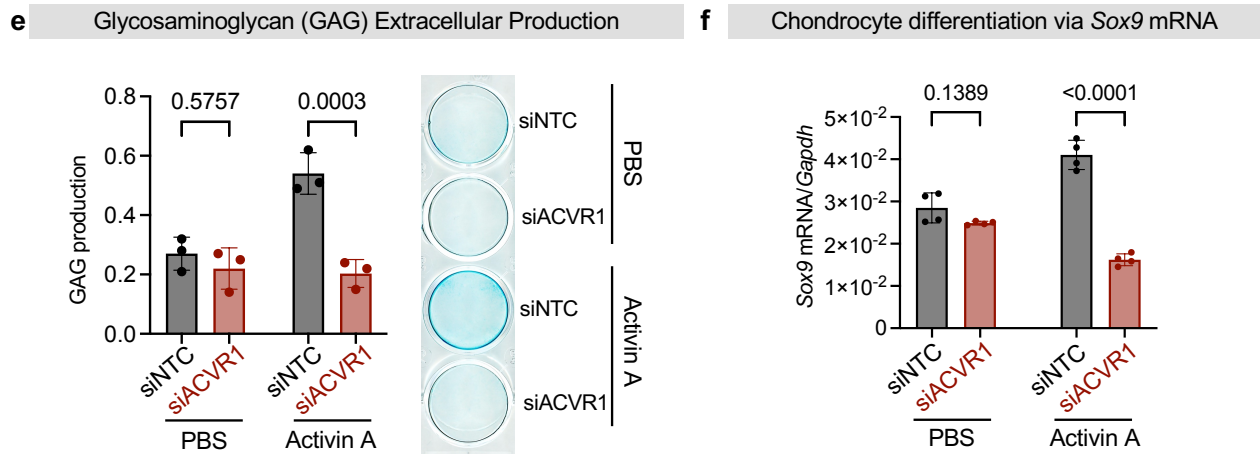
siACVR1 Inhibits Activin A Signaling in Murine ACVR1^{R206H} BMSCs



siACVR1 Attenuates Activin A-induced Osteogenesis in ACVR1^{R206H} BMSCs



siACVR1 Mitigates Activin A-induced Chondrogenesis in ACVR1^{R206H} chondroprogenitors

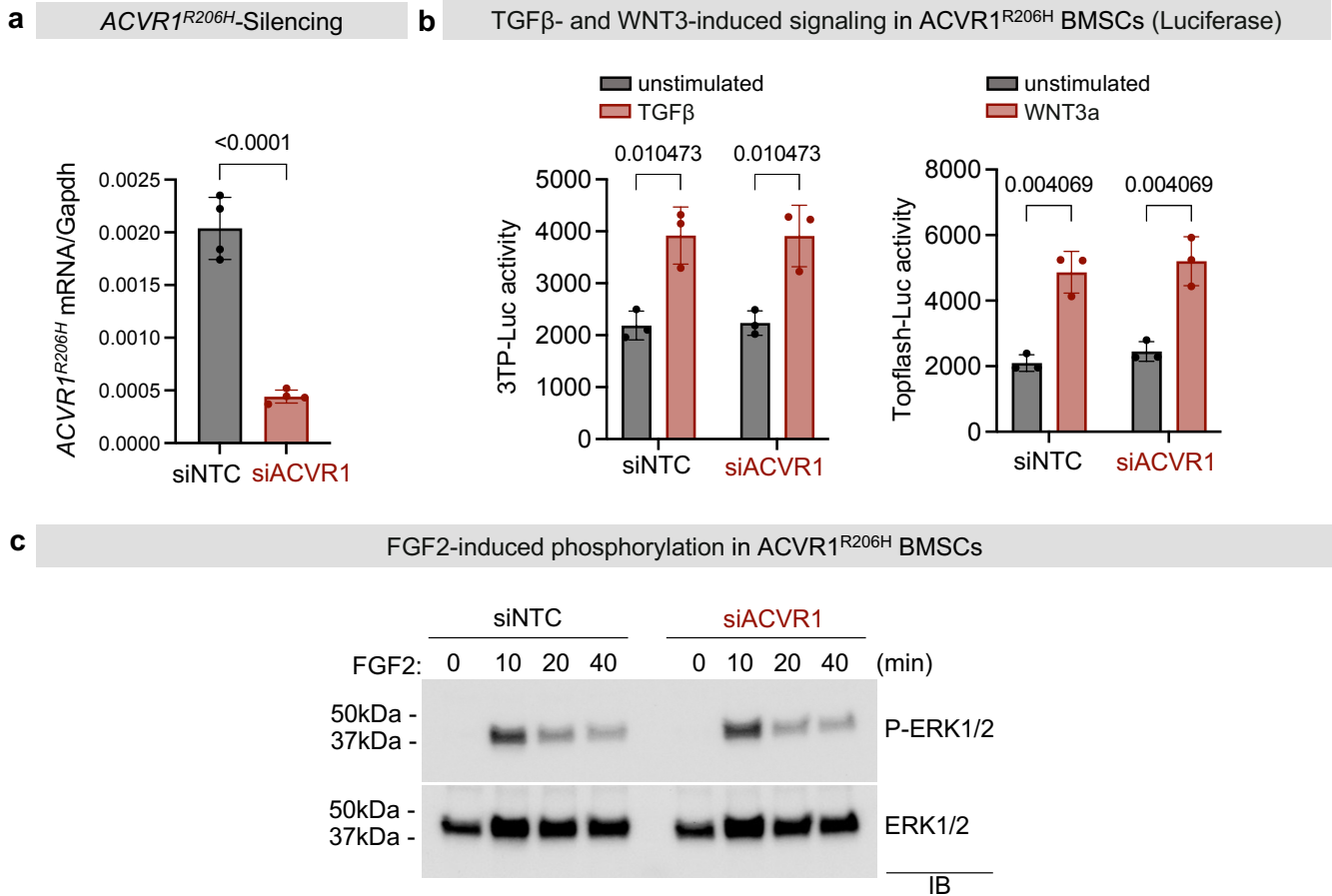


Extended Figure 4. siACVR1 treatment inhibits Activin A-induced signaling, osteogenesis, and chondrogenesis in FOP cells.

a, b. *ACVR1^{(R206H)Fl};Prrx1-cre* BMSCs were treated with siNTC or siACVR1 in a 3-point dose response and three days later, stimulated with Activin A (50 ng/ml). Twelve hours later, *Id1* mRNA expression were evaluated by RT-PCR (n = 4, **a**). Alternatively, siRNA-treated cells were stimulated with Activin A (100 ng/ml) for 15 or 30 min and immunoblotted for phospho-Smad1/5. Hsp90 was used as a loading control (**b**).

c, d. *ACVR1^{(R206H)Fl};Prrx1-cre* BMSCs were treated with siNTC or siACVR1 in a 3- or 5-point dose response and cultured under osteogenic conditions in the presence or absence of Activin A (50 ng/ml). Early and late osteogenic differentiation was assessed by measuring ALP activity at day 4 (**c**) and extracellular matrix mineralization at day 14 (**d**).

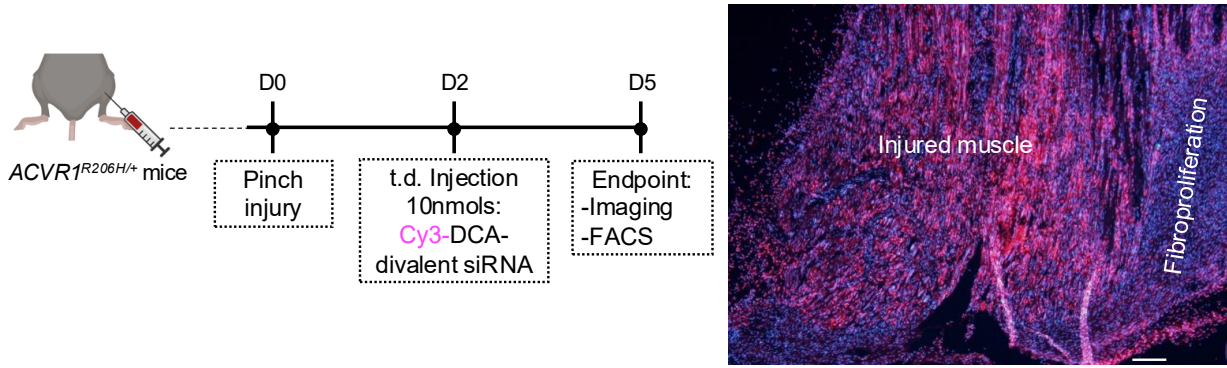
e, f. *ACVR1^{(R206H)Fl};Prrx1-cre* chondroprogenitors were treated with siNTC or siACVR1 and cultured under chondrogenic conditions in the presence or absence of Activin A (50 ng/ml). Chondrocyte differentiation was assessed by alcian blue staining (**e**) and expression of chondrogenic gene *Sox9* (**f**). Representative images of Alcian Blue staining and relative quantification are displayed (**e**, n = 3). Data are representative of two-three independent experiments. Values represent mean \pm SD by two-sided unpaired t-tests (**c**, **f**) and one-way ANOVA test (**a**).



Extended Figure 5. siACVR1 treatment does not affect other osteogenic signaling in FOP cells.

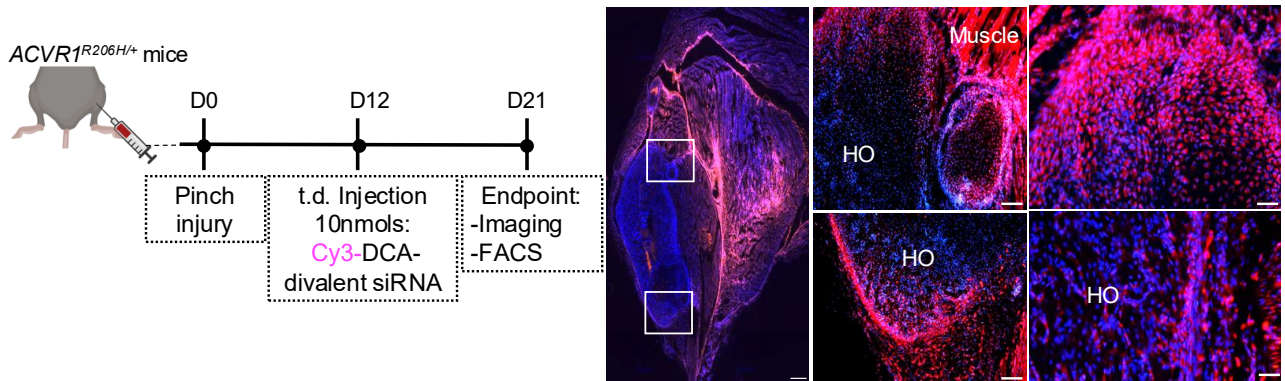
ACVR1^{(R206H)Fl};Prrx1-cre BMSCs were treated with 1 μ M siNTC or siACVR1 and three days later, knockdown efficiency of *ACVR1^{R206H}* was assessed by RT-PCR (n = 4, **a**). siRNA-treated cells were transfected with 3TP-Luc or Topflash-Luc reporter gene and one day later, the cells were stimulated with TGF β (10 ng/ml) and WNT3a (50 ng/ml), respectively. Luciferase activity was measured one day post-stimulation (n = 3, **b**). Alternatively, siRNA-treated cells were stimulated with FGF2 (20 ng/ml) for 10, 20, or 40 min and immunoblotted for phospho-ERK1/2. ERK1/2 was used as a loading control (**c**). Data are representative of two independent experiments. Values represent mean \pm SD by two-sided unpaired t-tests (**a**, **b**).

a DCA-Conjugated siRNA Accumulates in Injured Muscle at the Early Stage of HO



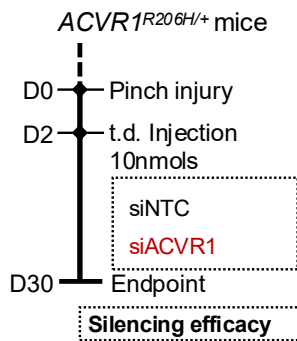
DCA-monovalent siRNA.Cy3/DAPI

b DCA-Conjugated siRNA Accumulates in Regenerating Muscle and Heterotopic Bone at the Late Stage of HO

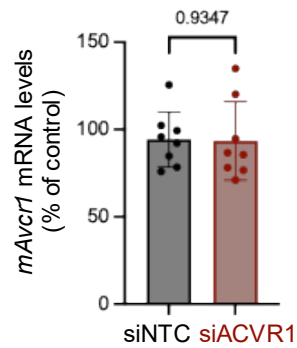


DCA-monovalent siRNA.Cy3/DAPI

c In vivo Study Design



d No Significant Mouse *Acvr1* silencing

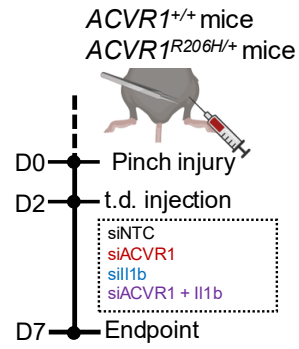
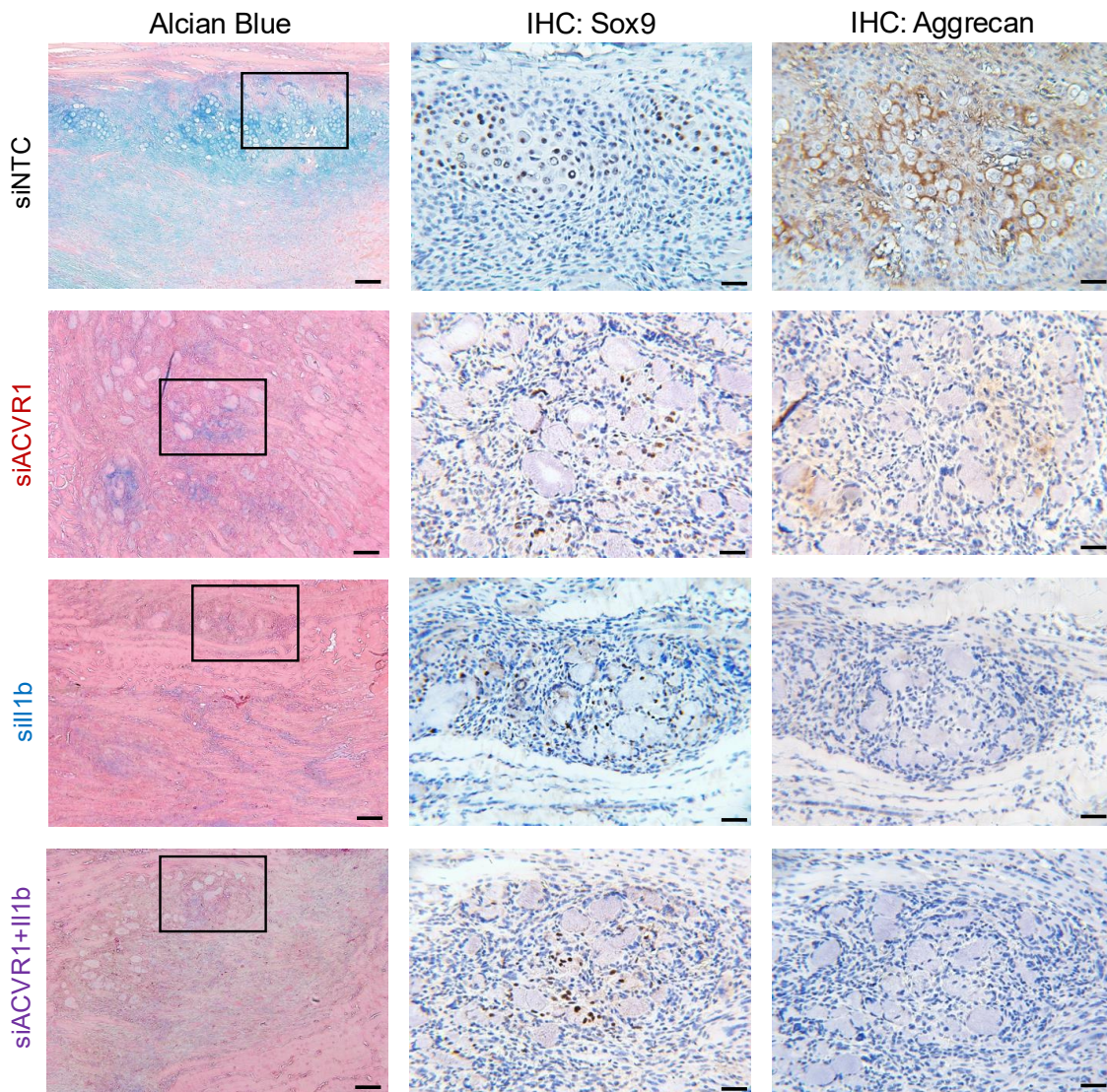


Extended Figure 6. Biodistribution of DCA-conjugated monovalent siRNA in injured muscle of FOP mice.

a. Schematic diagram showing study design for the early stage of HO progression (five day post-injury, **left**). A single dose of Cy3-labeled, DCA-conjugated monovalent siRNA was injected transdermally (t.d.) into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice two day post-injury. Three days later, siRNA-targeted cells in the cryo-sectioned muscle were visualized by fluorescent microscopy (n = 3, **right**). Scale bars: 100 μ m.

b. Schematic diagram showing study design for late stage of HO progression (21 day post-injury, **left**). A single dose of Cy3-labeled, DCA-conjugated monovalent siRNA was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice twelve day post-injury. nine days later, siRNA-targeted cells in the cryo-sectioned muscle were visualized by fluorescent microscopy (n = 3, **right**). HO: heterotopic ossification. Scale bars: 200 μ m (**left**), 100 μ m (**middle**), 50 μ m (**right**).

c, d. Schematic diagram showing study design (**c**). A single dose of siNTC or siACVR1 (10 nmol) was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice two day post-injury. 28 days later, mRNA levels of endogenous mouse *Acvr1* in HO lesions were assessed using Quantigene 2.0 assay (n = 8, **d**).

a**In vivo study design****b****siACVR1 suppresses chondrogenic anlagen formation in FOP mice**

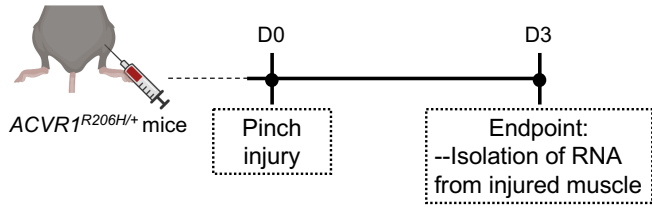
Extended Figure 7. Local delivery of DCA-conjugated siRNA targeting *ACVR1*^{R206H} and/or *Il1b* suppresses chondrogenic analgen formation in injured muscle of FOP mice.

a. Schematic diagram showing study design.

b. 10 nmol DCA monovalent siRNAs were injected t.d. into the tibial muscle of 8-week-old *ACVR1*^{R206H/+} mice two day after pinch injury (n = 3). Five days later, injured muscles were harvested, paraffin-sectioned, and stained with alcian blue for histopathologic analysis (left). Alternatively, immunohistochemistry was performed to examine the expression of early (Sox9, middle) and late (Aggrecan, right) chondrogenic genes in the injured muscle of FOP mice. Scale bars: 100 μ m (left) and 50 μ m (middle, right).

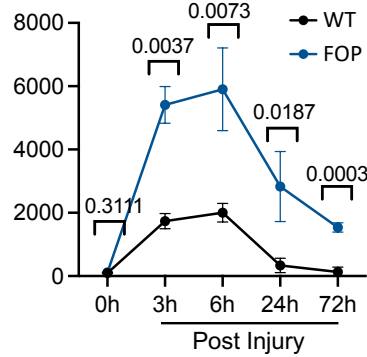
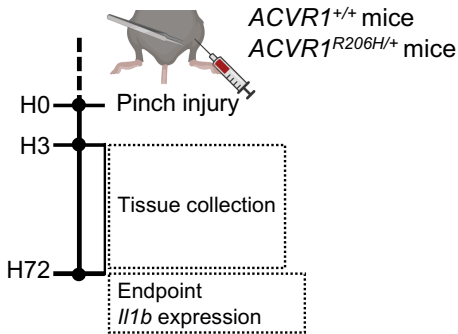
a *Il1b* Expression is Significantly Upregulated in Injured Muscle (FOP vs WT)

ACVR1^{+/+} mice
ACVR1^{R206H/+} mice

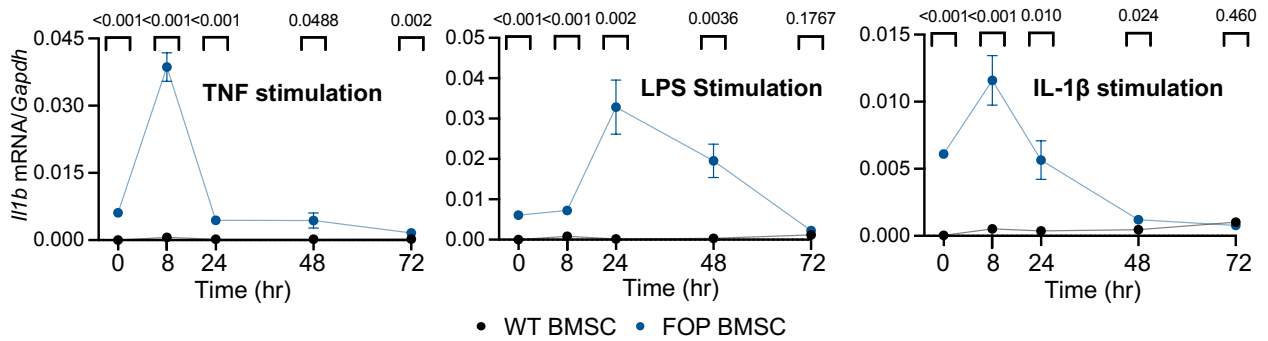


Position	Gene Sym	Fold Regu	p Value
A05	Ccl12	4.54	0.047385
B01	Ccl4	2.78	0.031127
B04	Ccl7	5.51	0.005180
C04	Csf1	3.10	0.022442
C09	Cxcl10	5.13	0.015512
D09	Il10ra	4.22	0.046363
E04	Il17b	2.57	0.038532
E07	Il1b	9.10	0.000446
E08	Il1r1	2.19	0.046101
E09	Il1rn	4.73	0.018493
G03	Pf4	5.10	0.024994

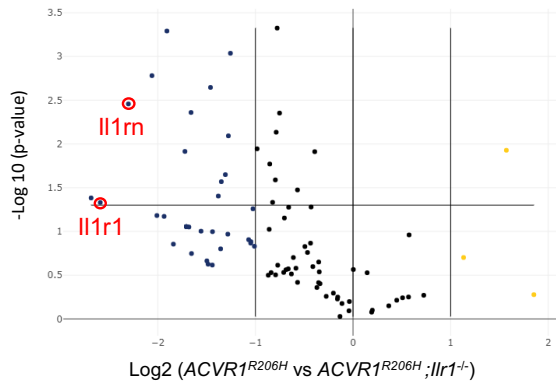
b *Il1b* Expression is Significantly Upregulated in Injured Muscle (FOP vs WT)



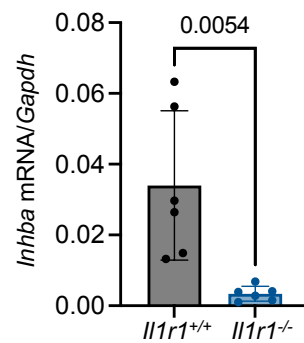
c Elevated and Persistent Expression of *Il1b* in ACVR1^{R206H} BMSCs Following Inflammatory Stimuli



d Reduced IL-1 Signaling in Injured Muscle of ACVR1^{R206H}; *Il1r1*^{-/-} mice

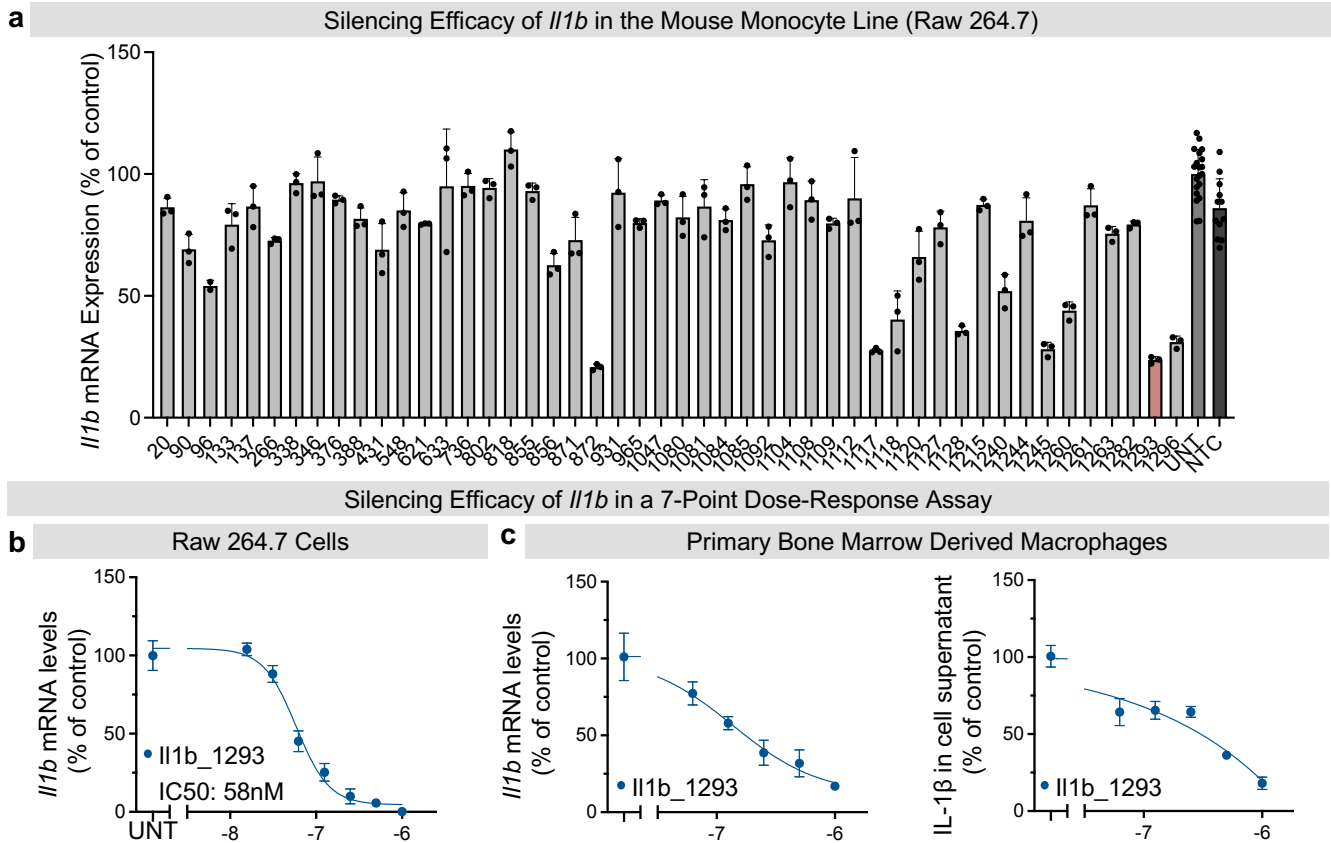


e Reduced Expression of Activin A



Extended Figure 8. IL-1 signaling mediates trauma-induced HO in FOP mice.

- a.** Schematic diagram showing study design (**left**). 8-week-old WT ($ACVR1^{+/+}$) and FOP ($ACVR1^{R206H/+}$) mice underwent pinch injury on the tibial muscle and injured tissues were collected three days post-injury. RT₂ profiler PCR array demonstrates that *Il1b* is the most highly expressed gene in injured muscle of FOP mice among 84 inflammatory genes tested ($n = 3$, **right**).
- b.** Schematic diagram showing study design (**left**). 8-week-old WT and FOP mice underwent pinch injury on the tibial muscle and injured tissues were collected 0, 3, 6, 24, and 72 hr post-injury. mRNA levels of *Il1b* were measured by RT-PCR and normalized to *Gapdh* ($n = 4$, **right**).
- c.** $ACVR1^{(R206H)Fl}$ (black) and $ACVR1^{(R206H)Fl};Prrx1-cre$ (blue) BMSCs were stimulated with TNF (10 ng/ml), LPS (1 μ g/ml), or IL-1 β (20 ng/ml) for 8, 24, 48, and 72 hrs and mRNA levels of *Il1b* were measured by RT-PCR and normalized to *Gapdh* ($n = 4$).
- d, e.** 8-week-old $ACVR1^{R206H/+}$ and $ACVR1^{R206H/+};Il1r1^{-/-}$ mice underwent pinch injury on the tibial muscle and injured tissues were collected three days post-injury. RT₂ profiler PCR array was performed to assess inflammatory gene expression ($n = 3$, **d**). Alternatively, mRNA levels of *Inhba* were measured by RT-PCR and normalized to *Gapdh* ($n = 6$, **e**). Data are representative of three independent experiments. Values represent mean \pm SD by two-sided unpaired t-tests (**e**) and one-way ANOVA tests (**b, c**).

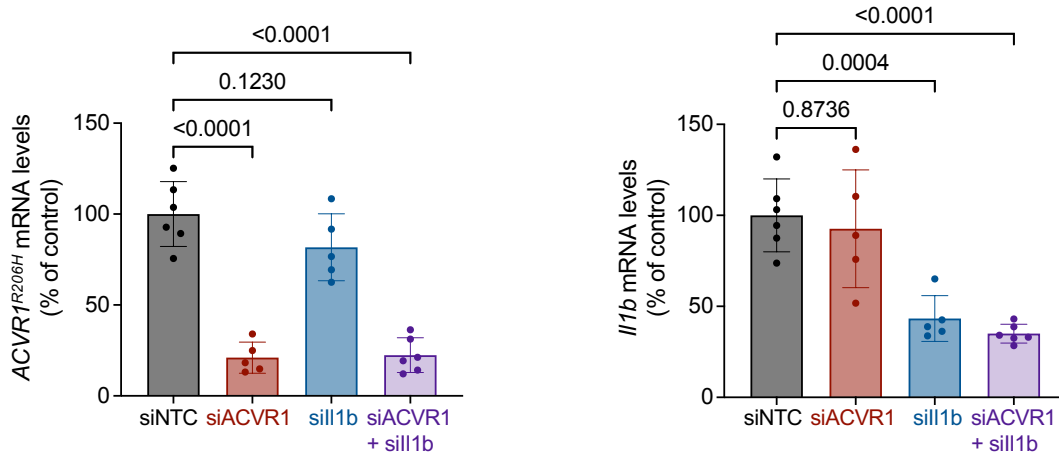


Extended Figure 9. Development of the siRNA targeting mouse *Il1b* mRNA.

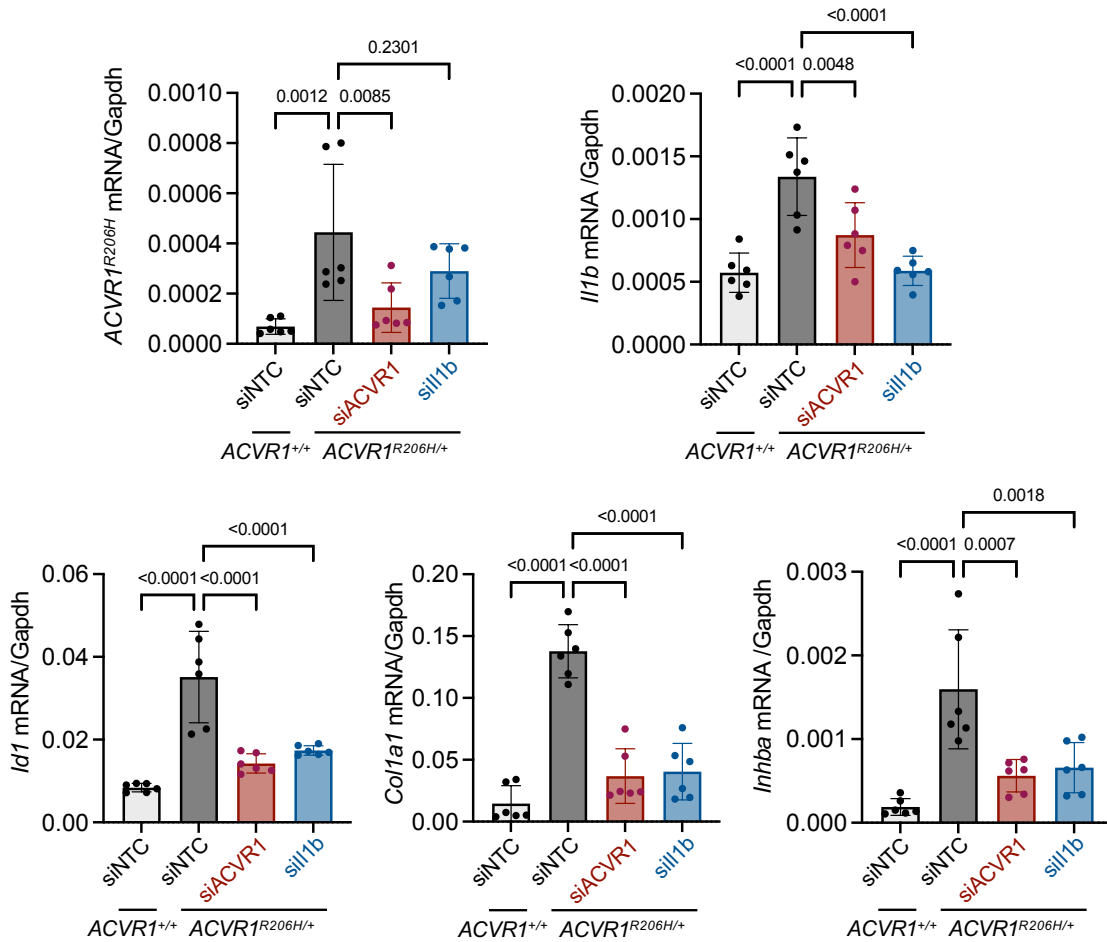
a, b. The mouse monocyte line (Raw264.7) was treated with a panel of 48 siRNAs targeting mouse *Il1b* mRNA, UNT, and siNTC. 3 days later, cells were stimulated with LPS (25 ng/mL) for 8 hrs and mRNA levels of *Il1b* were measured using the Quantigene 2.0 assay ($n = 3$). UNT: untreated control, siNTC: non-targeting control siRNA (**a**). Alternatively, Raw264.7 cells were treated with the lead siRNA_1293 in 7-point dose response, stimulated with LPS, and mRNA levels of *Il1b* were measured ($n = 3$, **b**).

c. Mouse bone marrow-derived monocytes (BMMs) were cultured for six days in the presence of M-CSF (50 ng/ml) to differentiate into macrophages (BMDMs). The cells were treated with the lead siRNA_1293 in 7-point dose response and stimulated with LPS for 8 hrs. mRNA levels of *Il1b* (**left**) and protein levels of IL-1 β in the cell supernatant (**right**) were measured by the Quantigene 2.0 and ELISA assays, respectively ($n = 3$). Data are representative of three independent experiments. Values represent mean \pm SD by one-way ANOVA tests.

a Silencing Efficacy of *ACVR1^{R206H}* and *Il1b* in FOP Muscle After Single or Dual Treatment of siACVR1 and siIl1b



b Single Silencing of *ACVR1^{R206H}* or *Il1b* Suppresses Activin A and Osteo-Chondrogenic Gene Expression



Extended Figure 10. Gene expression in injured muscle tissues of siRNA-treated FOP mice.

a. A single dose of siNTC, siACVR1, sill1b, and siACVR1+sill1b (10 nmol) was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice 2 days post-injury. Four weeks later, mRNA levels of *ACVR1^{R206H}* and *Il1b* were measured by the Quantigene 2.0 assay (n = 5-6). This is the same experiment of **Figure 3c**.

b. A single dose of siNTC, siACVR1, and sill1b (10 nmol) was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice two days post-injury. Three days later, mRNA levels of the indicated genes in injured muscle tissues were measured by RT-PCR (n = 6). Data are representative of two-three independent experiments. Values represent mean ± SD by one-way ANOVA tests.

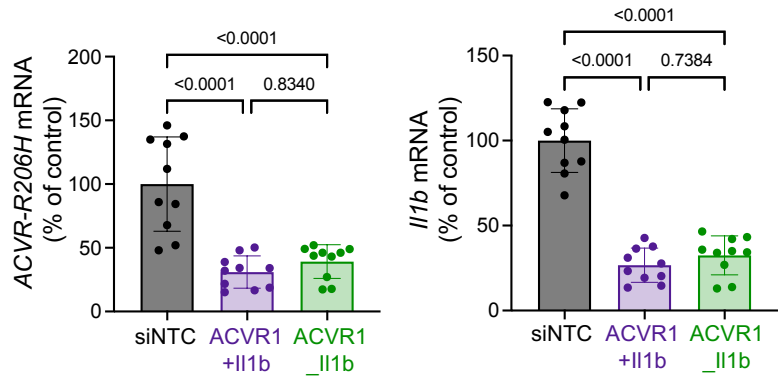
Extended Figure 11. Biodistribution of DCA-conjugated divalent siRNA in FOP mice.

a. A single dose of Cy3-labeled, unconjugated monovalent (Unc) siRNA, DCA-conjugated monovalent (DCA Mono) siRNA, or DCA-conjugated divalent (DCA Div) siRNA (10 nmol) was injected t.d. into the tibial muscle of 8-week-old wildtype mice. One- and three-months later, Cy3 fluorescence in individual tissues was visualized via IVIS-100 optical imaging (n = 5). This is the same experiment of **Figures 4a and b.**

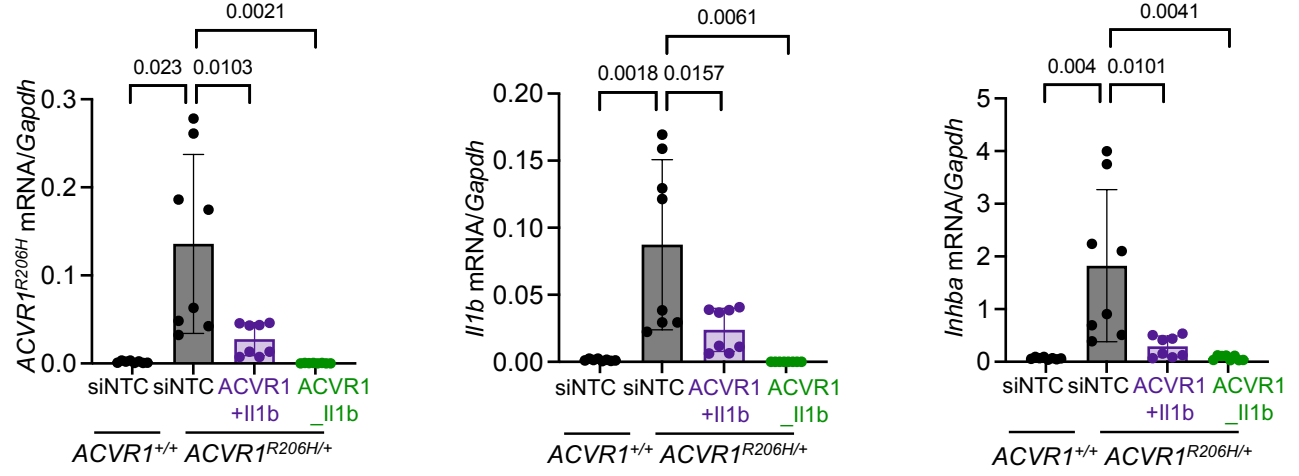
b, c. A single dose of Cy3-labeled DCA-conjugated divalent (DCA Div) siRNA (10 nmol) was injected t.d. into the tibial muscle of *ACVR1^{R206H/+}* mice two days post-injury. Three days later, Cy3-labeled cells in cryo-sectioned muscle was visualized by fluorescence microscopy (n = 3, **b**). Scale bars: 50 μ m. Alternatively, cells were isolated from digested muscle near the site of injury and subjected for FACS analysis to assess the frequency of Cy3-labeled cell populations (n = 3, **c**).

d. Schematic diagram showing study design for late stage of HO progression (**left**). A single dose of Cy3-labeled DCA-conjugated divalent siRNA (10 nmol) was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice twelve days post-injury. 9 days later, Cy3-labeled cells in cryo-sectioned muscle was visualized by fluorescence microscopy (n = 3, **right**). HO: heterotopic ossification, OB: osteoblast, CC: chondrocyte. Scale bars: 100 μ m (**left**), 50 μ m (**right**). Data are representative of two independent experiments. Values represent mean \pm SD by multiple unpaired t-tests (**c**).

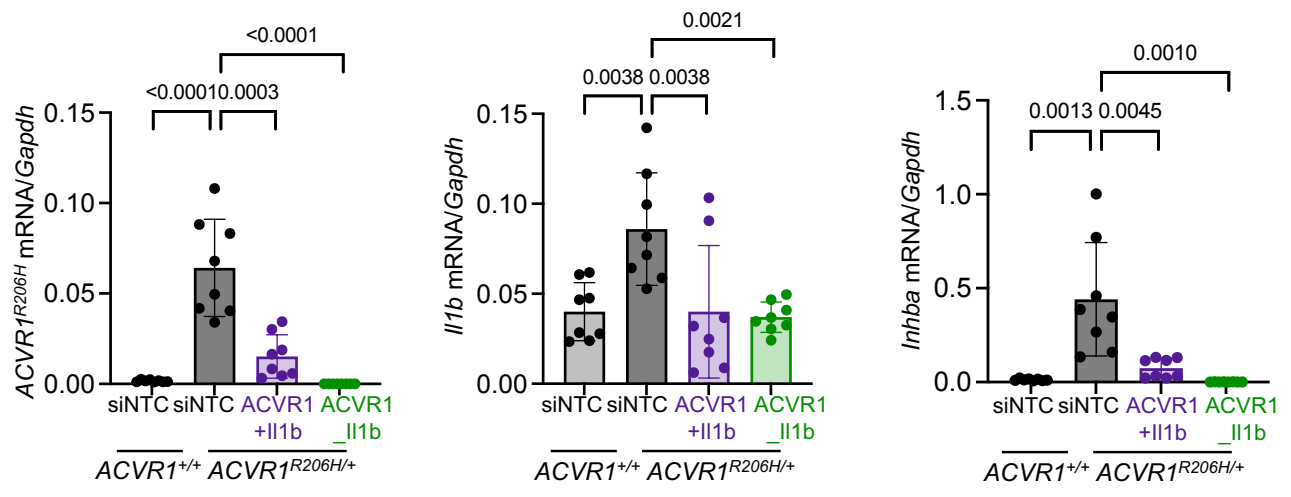
a Silencing Efficacy of *ACVR1^{R206H}* and *Il1b*



b Expression of *ACVR1^{R206H}*, *Il1b*, and *Inhba* in WT, FOP FAPs Treated with siACVR1+siIl1b or siACVR1-Il1b



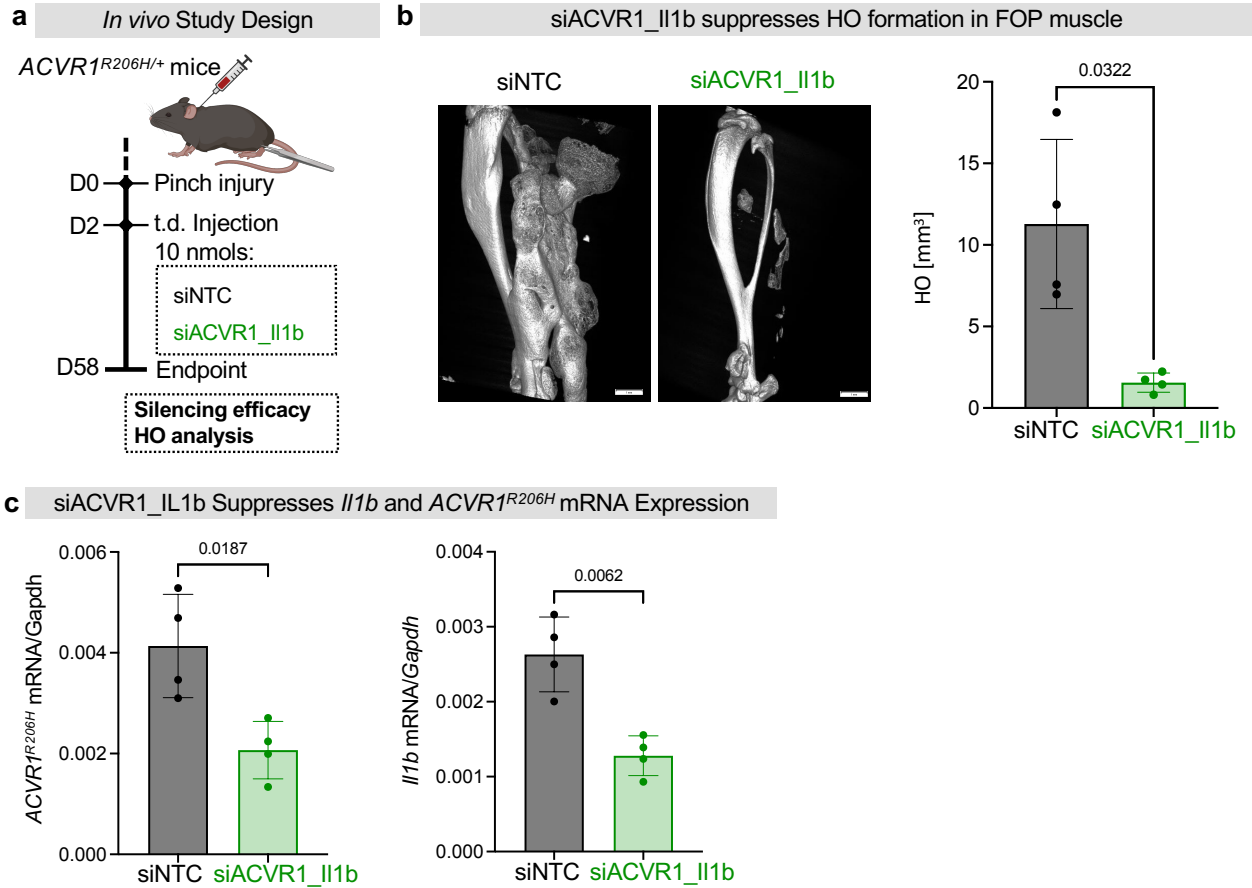
c Expression of *ACVR1^{R206H}*, *Il1b*, and *Inhba* in WT, FOP immune cells Treated with siACVR1+siIl1b or siACVR1-Il1b



Extended Figure 12: Gene expression in injured muscle tissues, FAPs, and immune cells of siRNA-treated FOP mice.

a. A single dose of siNTC, siACVR1+Il1b, or siACVR1_Il1b (10 nmol) was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice 2 days post-injury. 4 weeks later, mRNA levels of *ACVR1^{R206H}* and *Il1b* were measured by the Quantigene 2.0 assay (n = 10, **a**). This is the same experiment of Figure 4c.

b,c. A single dose of siNTC, siACVR1+Il1b, or siACVR1_Il1b (10 nmol) was injected t.d. into the tibial muscle of *ACVR1^{+/+};PDGFR α -GFP* (WT) and *ACVR1^{R206H/+};PDGFR α -GFP* (FOP) mice 2 days post-injury. 3 days later, GFP⁺Sca1⁺CD31⁻CD45⁻ FAPs (**b**) and GFP⁻Sca1⁻CD31⁻CD45⁺ immune cells (**c**) were FACS-sorted from digested muscle near the site of injury. mRNA levels of *ACVR1^{R206H}*, *Il1b*, and *Inhba* were measured by RT-PCR and normalized to *Gapdh* (n = 8). Data are representative of two independent experiments. Values represent mean \pm SD by one-way ANOVA tests (**a**, **b**, **c**).

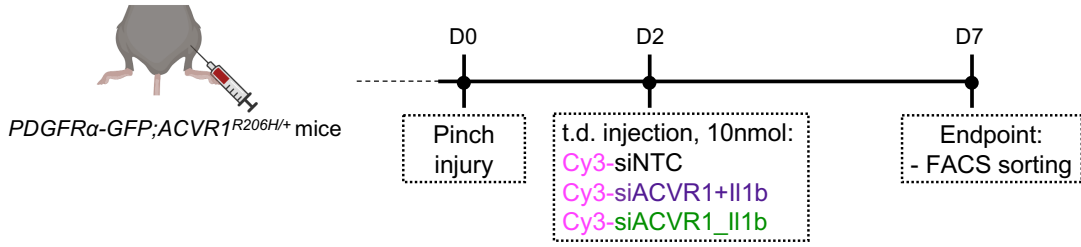


Extended Figure 13. DCA-conjugated siRNA targeting *ACVR1^{R206H}* and *Il1b* suppresses HO in FOP mice for up to 8 weeks.

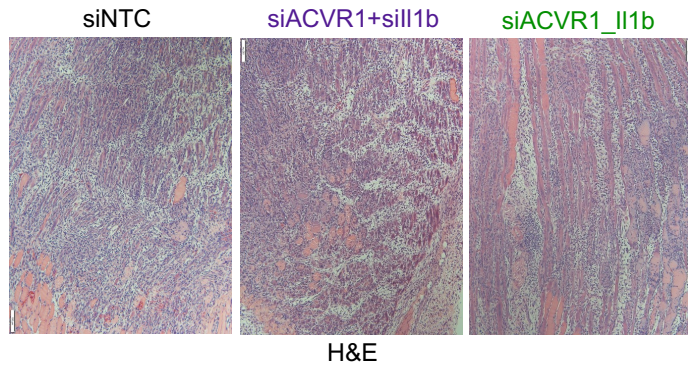
a. Schematic diagram showing study design.

b, c. A single dose of siNTC and siACVR1_II1b (10 nmol) was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+}* mice two days post-injury. Eight weeks later, silencing efficiency of *ACVR1^{R206H}* and *Il1b* in HO lesions and HO formation were assessed by RT-PCR (n = 4, **b**) and microCT imaging and quantification (n = 4, **c**), respectively. Values represent mean ± SD by two-sided unpaired t-tests (**b, c**). Data are representative of two-three independent experiments.

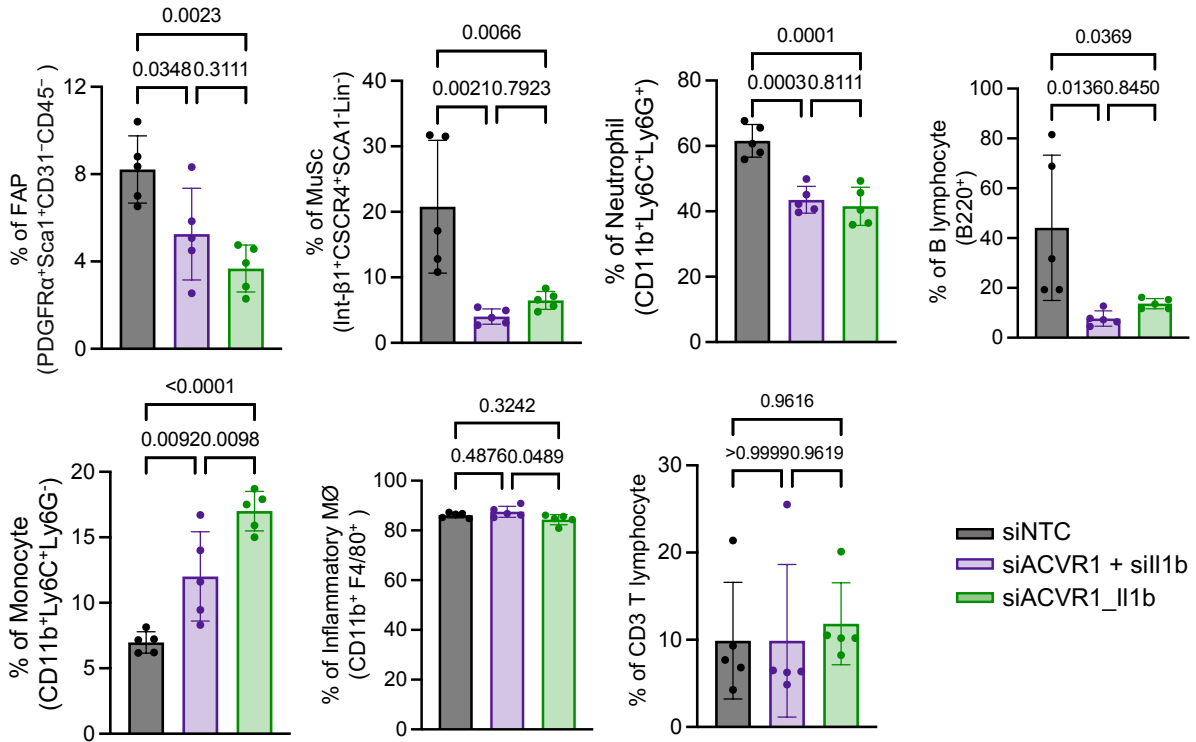
a *In vivo* study design



b FOP muscle tissue 4 days post-injury (t.d. injection)



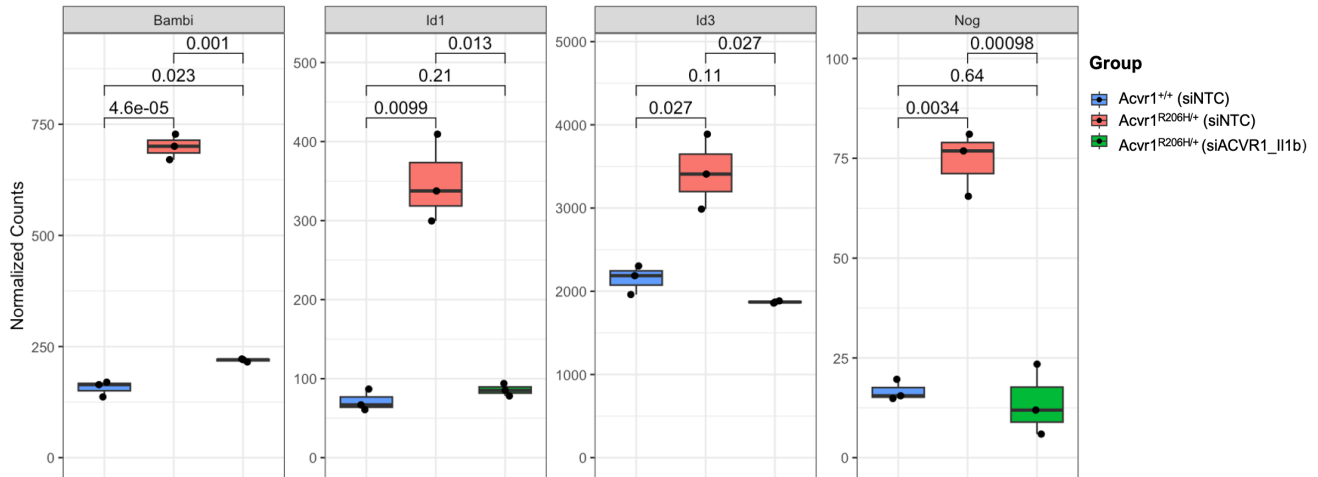
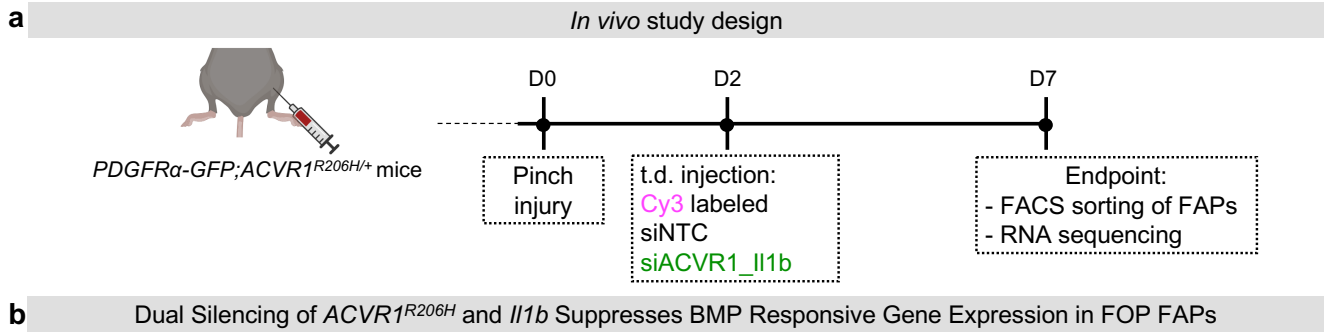
c Alteration of Cell Frequency in Injured FOP Muscle After Treatment of siACVR1+sill1b or siACVR1_II1b



Extended Figure 14. Characterization of cell populations in injured muscle of siRNA-treated FOP mice.

a. Schematic diagram showing study design for the early stage of HO progression.

b, c. A single dose of siNTC, siACVR1+II1b, or siACVR1_II1b (10 nmol) was injected t.d. into the tibial muscle of 8-week-old *ACVR1^{R206H/+};PDGFR α -GFP* mice two days post-injury. Two days later, injured muscles were stained with hematoxylin and eosin (H&E), demonstrating little to no difference in siRNA-treated muscles (n = 3, **b**). Scale bars: 100 μ m. Seven days after injury, cells were isolated from digested muscle near the site of injury and subjected for FACS analysis to assess the frequency of Cy3-labeled cell types (n = 5, **c**). Data are representative of two independent experiments. Values represent mean \pm SD by one-way ANOVA test (**c**).

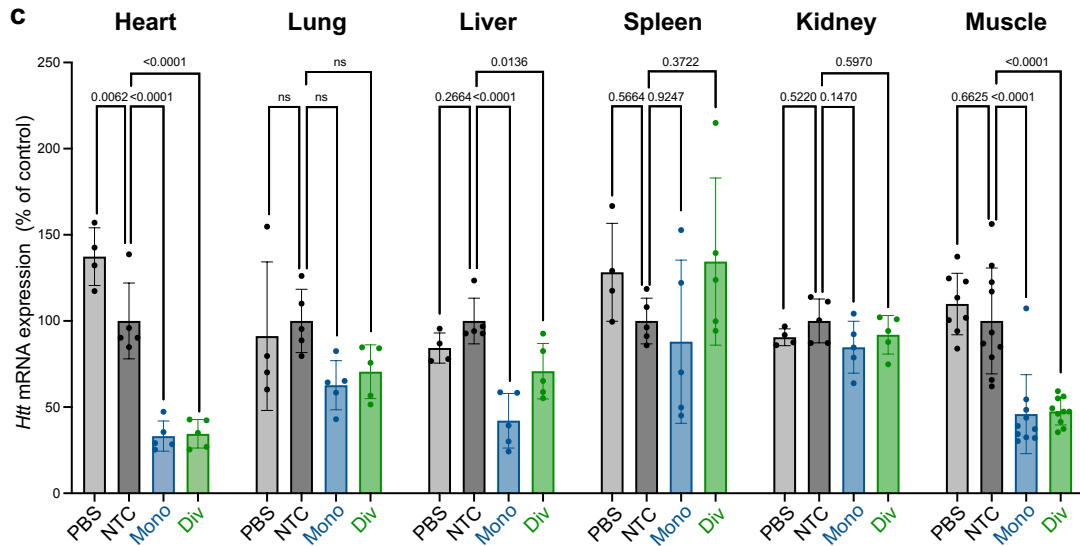
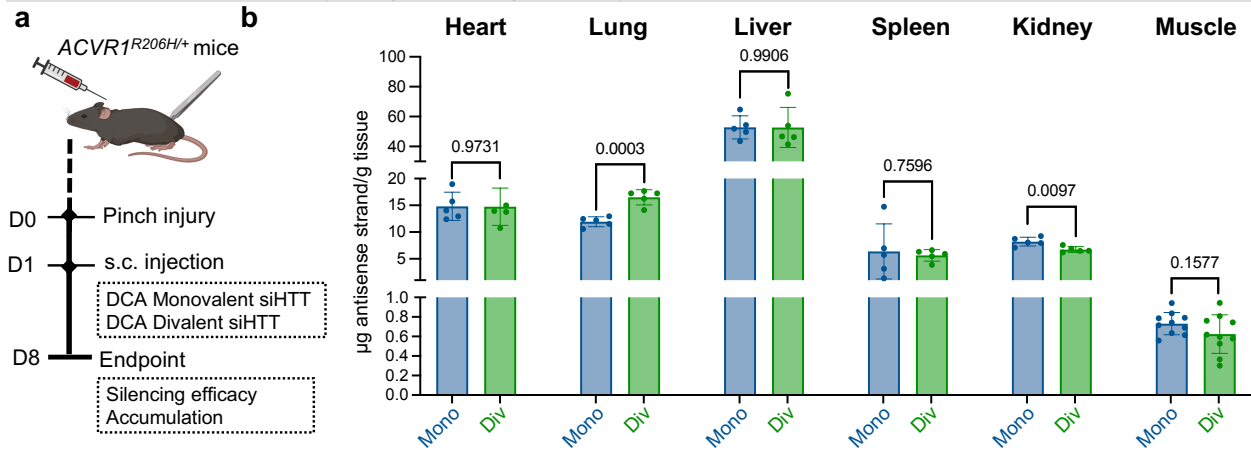


Extended Figure 15. BMP-responsive gene expression in siRNA-treated WT and FOP FAPs.

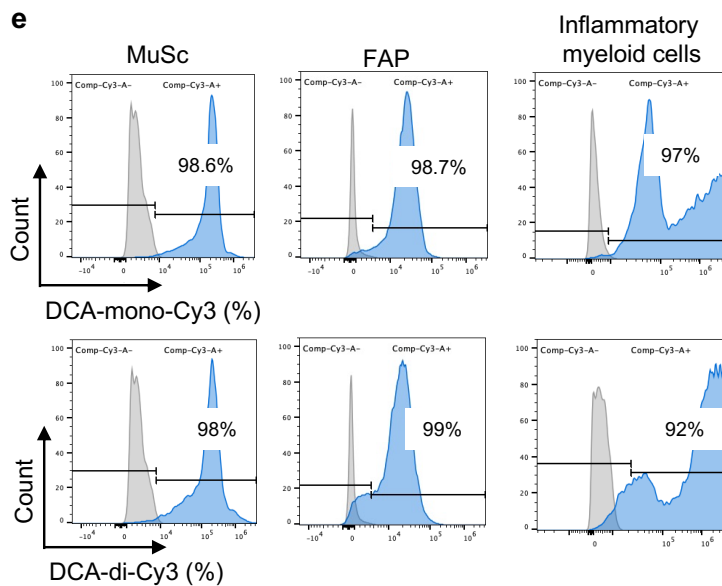
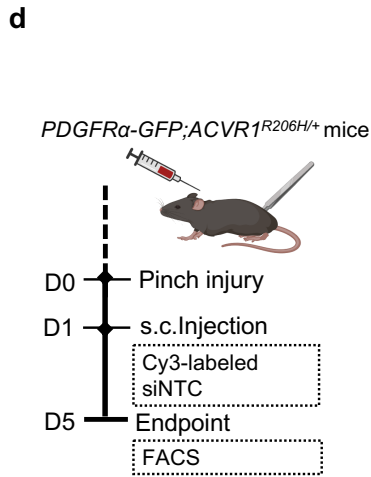
a. Schematic diagram showing study design for the early stage of HO progression.

b. A single dose of siNTC or siACVR1_II1b (10 nmol) was injected t.d. into the tibial muscle of ACVR1^{+/+};PDGFR α -GFP and ACVR1^{R206H/+};PDGFR α -GFP mice two days post-injury. Five days later, GFP⁺Sca1⁺CD31⁻CD45⁻ FAPs were FACS-sorted from digested muscle near the site of injury and then, subjected to RNA sequencing. Normalized transcript numbers of the indicated BMP-responsive genes are displayed (n = 3). This is the same experiment of **Figures 5b-e**. Data are representative of two independent experiments. Values represent mean \pm SD by one-way ANOVA tests (**b**).

In vivo study design, silencing efficiency, and siRNA accumulation in individual tissues



DCA-siRNA Provides Efficient Delivery to FOP Relevant Cell Types (FACS)

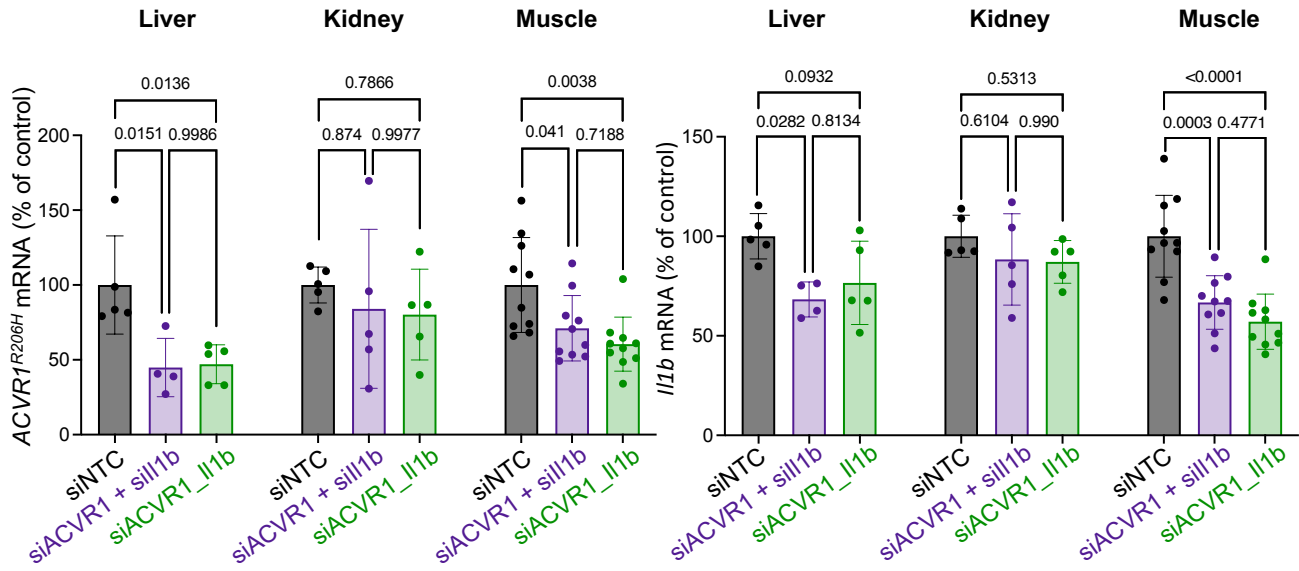


Extended Figure 16. Biodistribution and silencing efficiency in individual tissues following systemic delivery of siRNAs.

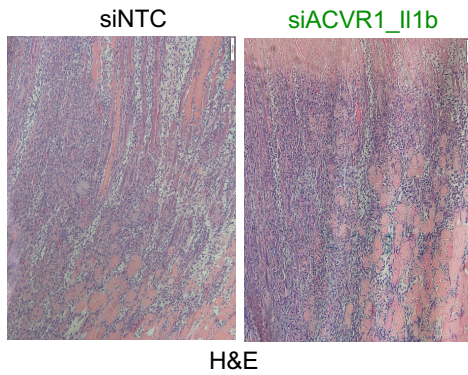
a-c. Schematic diagram showing study design **(a)**. A single dose of DCA-conjugated monovalent (Mono) or DCA-conjugated divalent (Div) siHTT (40 mg/kg) was injected subcutaneously (s.c.) into 8-week-old *ACVR1^{R206H/+}* mice one day post-injury. Seven days later, antisense accumulation **(b)** and knockdown efficiency **(c)** in heart, lung, liver, spleen, kidney, and muscle were quantified by the Quantigene 2.0 assay (n = 4).

d, e. Schematic diagram showing study design **(d)**. A single dose of Cy3-labeled, DCA-conjugated monovalent or divalent siNTC (40 mg/kg) was injected s.c. into 8-week-old *ACVR1^{R206H/+};PDGFR α -GFP* mice one day post-injury. Four days later, cells were isolated from digested muscle near the site of injury and subjected for FACS analysis to assess the frequency of the indicated cell types (n = 6, **f**). FAP (fibroadipogenic progenitor): GFP⁺Sca1⁺CD31⁻CD45⁻, MuSc (muscle satellite cells): Sca1⁻Ter119⁻Cxcr4⁻Int β 1⁺, Inflammatory myeloid cells: F4/80⁺CD45⁺CD11b⁺CD11c⁻. Data are representative of two independent experiments. Values represent mean \pm SD by one-way ANOVA tests.

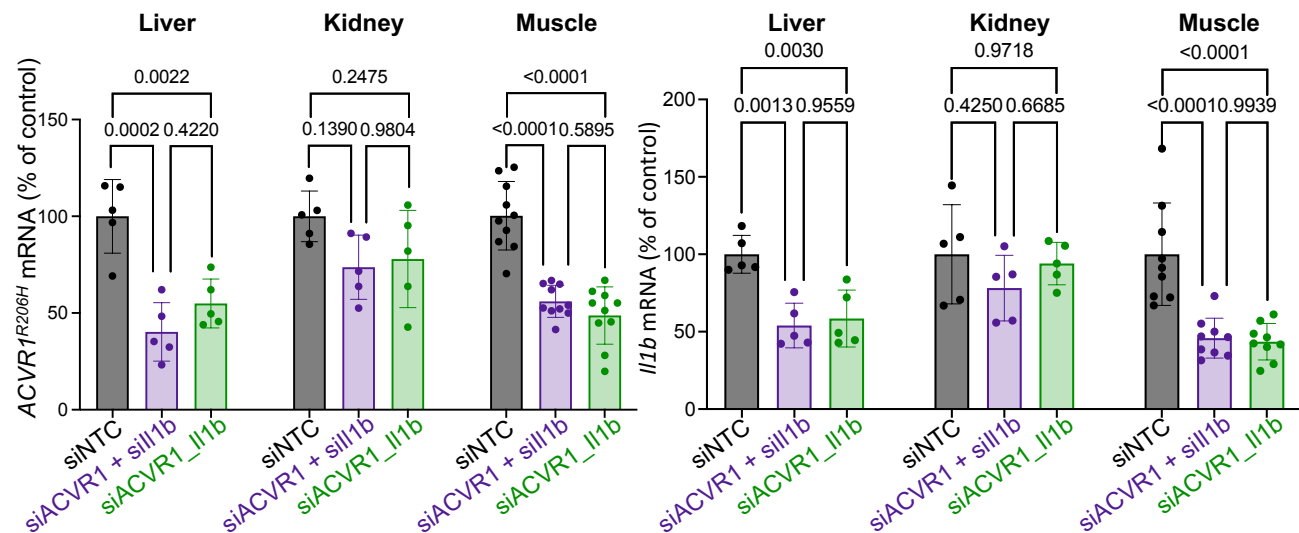
a Silencing Efficacy of *ACVR1^{R206H}* and *Il1b* in FOP Tissues Post-Treated with siACVR1+siIl1b or siACVR1_II1b



b FOP muscle tissue 4 days post-injury (s.c. injection)



c Silencing Efficacy of *ACVR1^{R206H}* and *Il1b* in FOP Tissues Pre-Treated with siACVR1+siIl1b or siACVR1_II1b



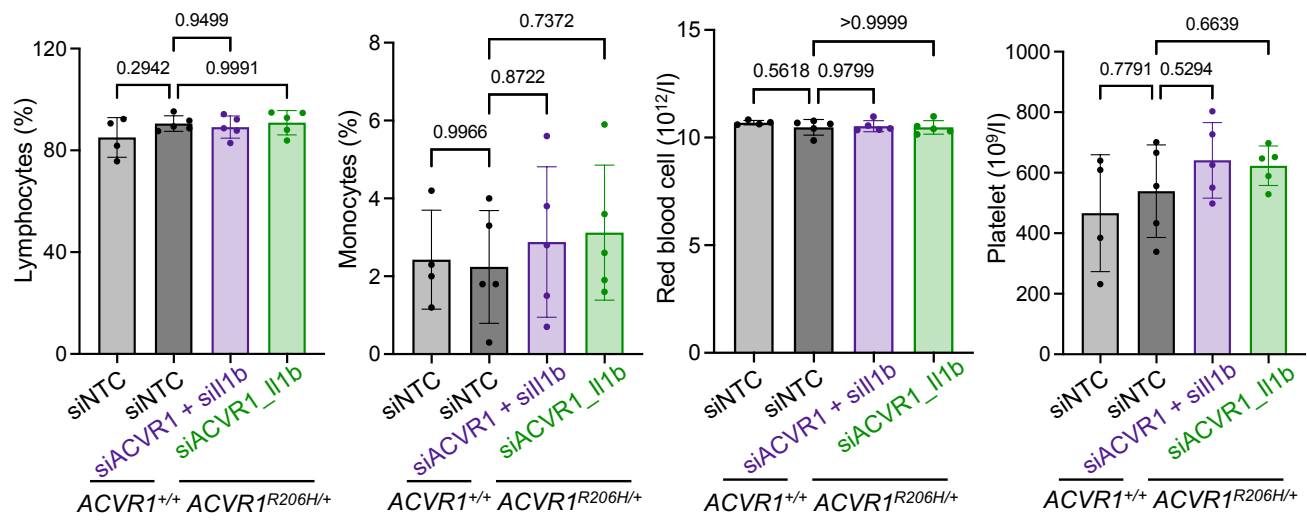
Extended Figure 17. Silencing efficiency of *ACVR1*^{R206H} and *Il1b* in various tissues after systemic delivery of siRNAs.

a. A single dose of siNTC, siACVR1+siIl1b, or siACVR1_Il1b (40 mg/kg) was injected subcutaneously (s.c.) into 8-week-old *ACVR1*^{R206H/+} mice one day post-injury. Four weeks later, mRNA levels of *ACVR1*^{R206H} and *Il1b* in liver, kidney, and muscle were quantified by the Quantigene 2.0 assay (n = 10).

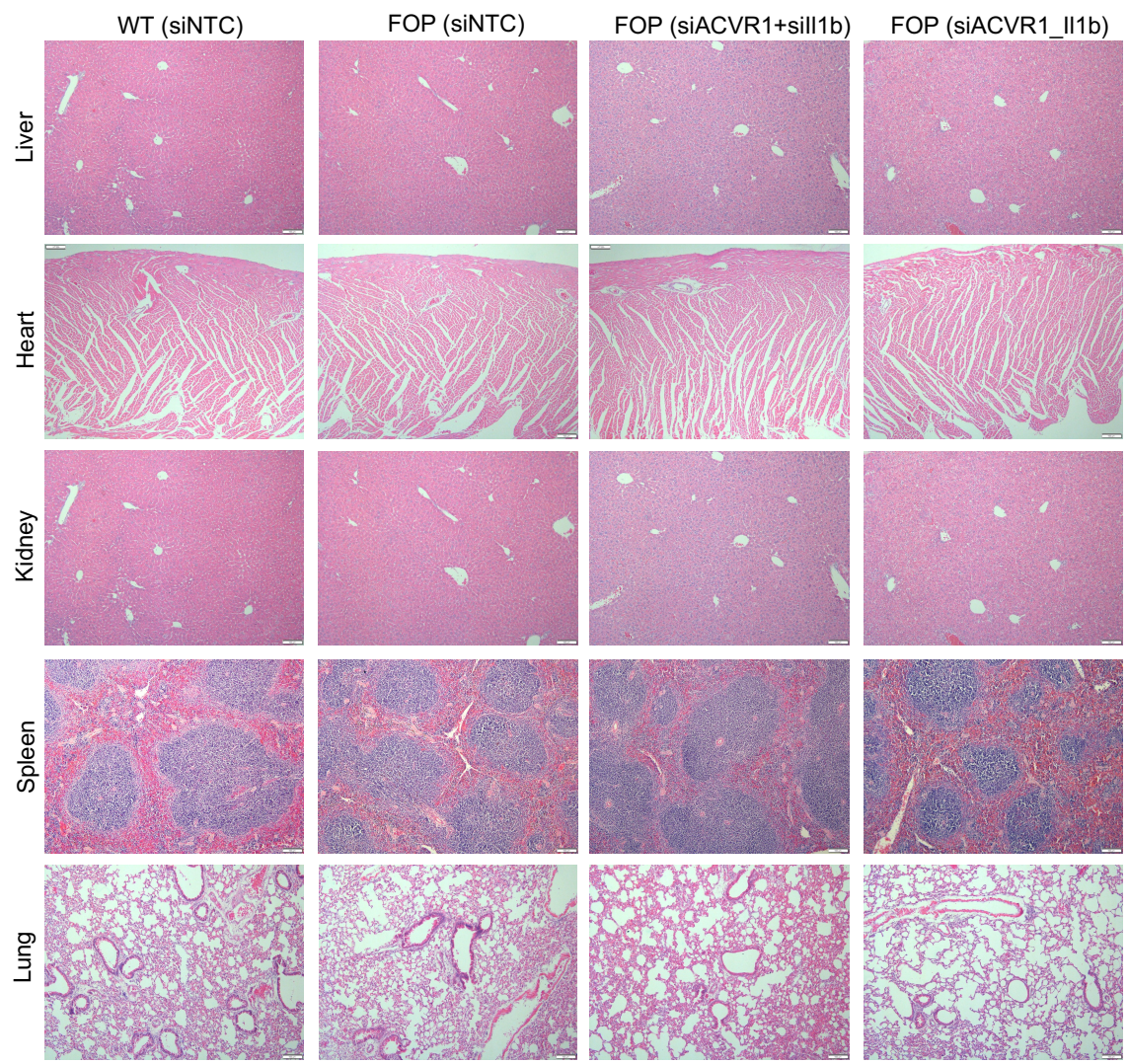
This is the same experiment of **Figure 6a**.

b, c. A single dose of siNTC, siACVR1+siIl1b, or siACVR1_Il1b (40 mg/kg) was injected s.c. into 8-week-old *ACVR1*^{R206H/+} mice 10 days prior to pinch injury. Injured muscles were stained with H&E four days post-injury, demonstrating little to no difference in siRNA-treated muscles (n = 3, **b**). Scale bars: 100 μm. Four weeks later, mRNA levels of *ACVR1*^{R206H} and *Il1b* in liver, kidney, and muscle were quantified by the Quantigene 2.0 assay (n = 5, **c**). This is the same experiment of **Figures 6b and c**. Data are representative of two independent experiments. Values represent mean ± SD by one-way ANOVA tests.

a No Effects of siRNA Treatments on Circulating Lymphocytes, Monocytes, or Red Blood Cells: CBC Test



b Little to No Histological Changes in siRNA-Treated Tissues: H&E Staining

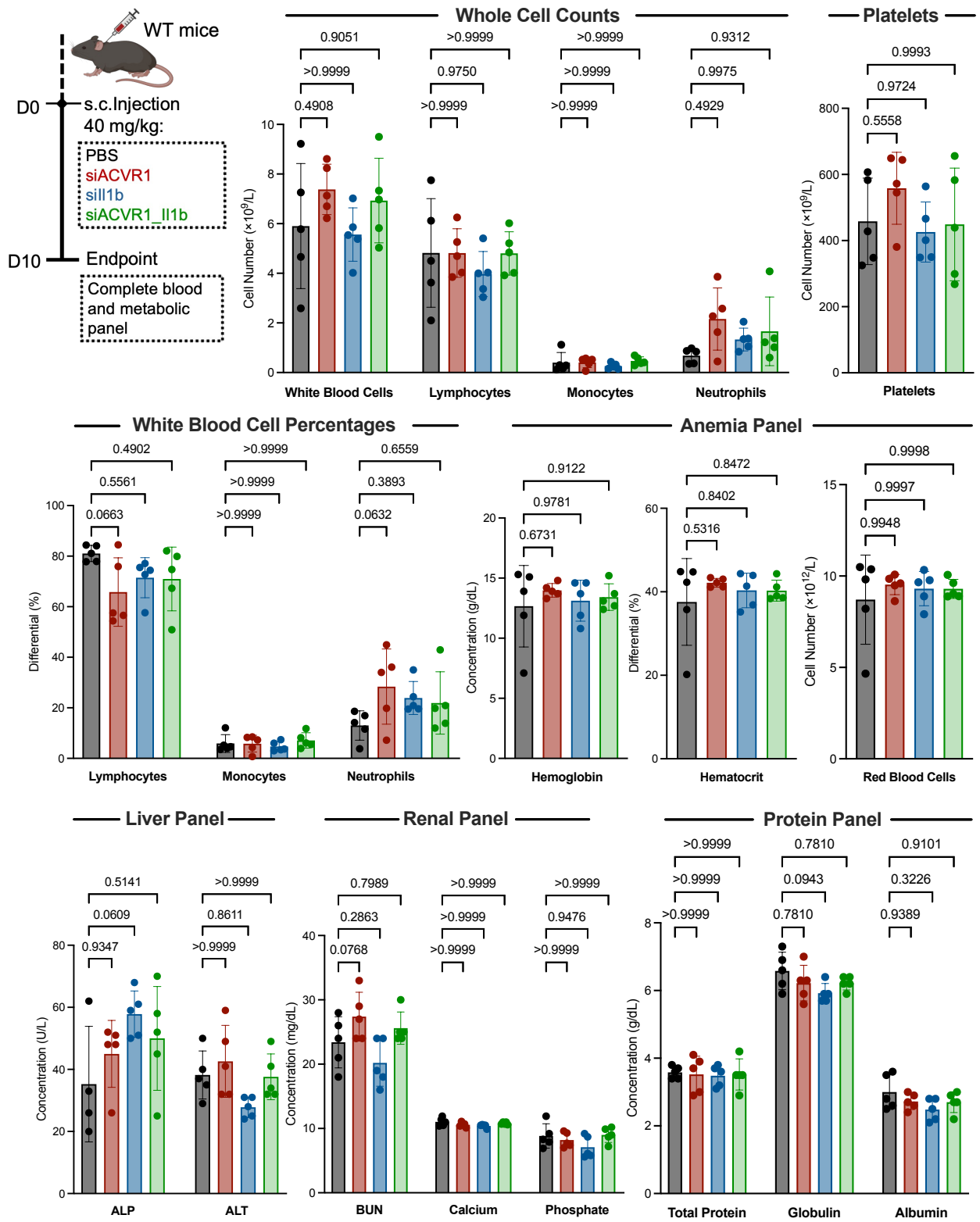


Extended Figure 18. No adverse effects of systemically delivered siRNAs in FOP mice.

a. A single dose of siNTC, siACVR1+sill1b, or siACVR1_II1b (40 mg/kg) was injected s.c. into 8-week-old WT (*ACVR1^{+/+}*) and FOP (*ACVR1^{R206H/+}*) mice 1 day post-injury. Four weeks later, peripheral blood was collected via cheek bleeding and then, subjected to complete blood count (CBC) test (n = 4-5).

b. Alternatively, multiple tissues, including liver, heart, kidney, spleen, and lung, were harvested, paraffin-sectioned, and stained with hematoxylin and eosin (H&E) for histopathologic analysis (n = 5,

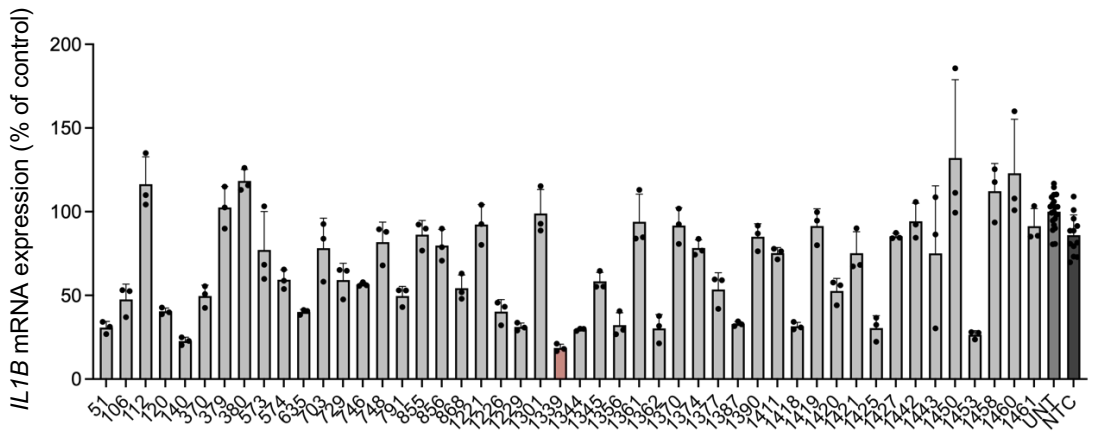
b). Scale bars: 100 μ m. This is the same experiment of **Figure 6a**. Data are representative of two independent experiments. Values represent mean \pm SD by one-way ANOVA tests (**a**).



Extended Figure 19. Toxicology study shows no adverse effects of systemically delivered siRNAs in WT mice.

A single dose of PBS, siACVR1, siIl1b, or siACVR1_II1b (40 mg/kg) was injected s.c. into 8-week-old WT mice (n = 4-5). One week later, peripheral blood was collected via cheek bleeding and then, subjected to complete blood count (CBC) test to assess circulating immune cells, red blood cells, and levels of platelets, hemoglobin, and hematocrit. Alternatively, blood levels of globulin and albumin proteins, alkaline phosphatase (ALP) and alanine aminotransferase (ALT), and Bun, Calcium, and Phosphate were measured to assess blood, liver, and renal toxicities, respectively. Values represent mean \pm SD by one-way ANOVA tests.

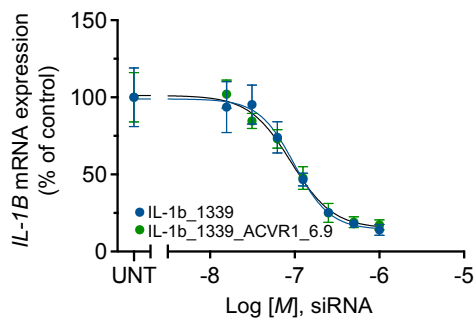
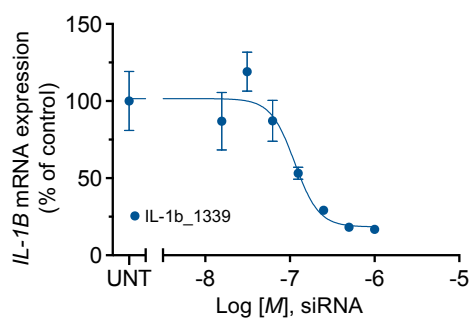
a *IL-1B* is Robustly Silenced by *IL-1B* Targeting siRNAs in HEL92.1.7 Cells



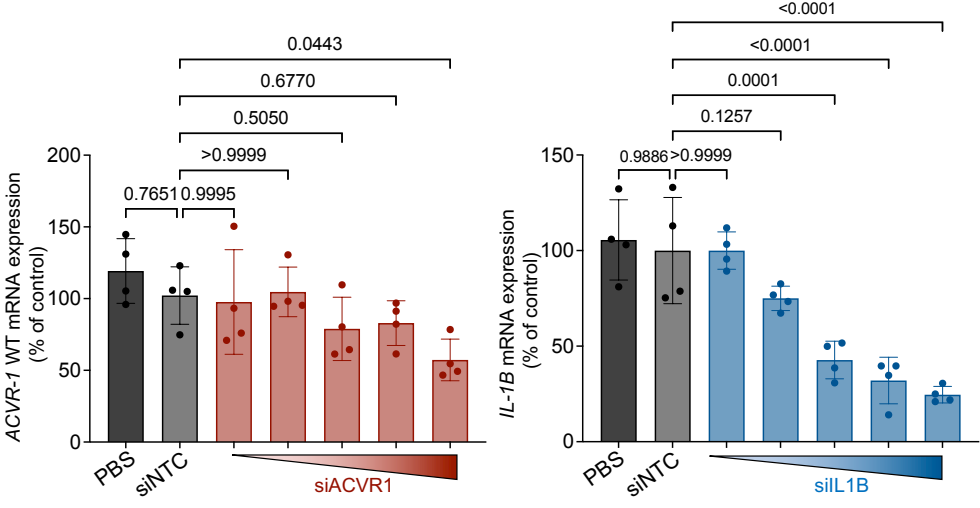
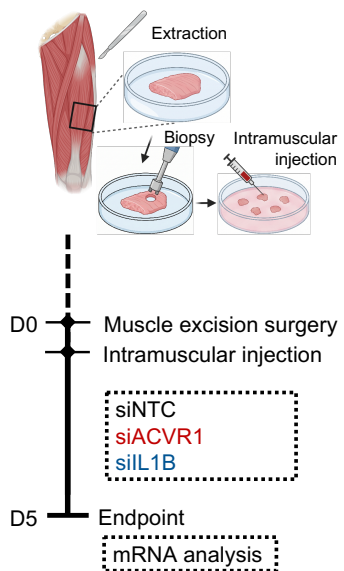
Silencing Efficacy of *IL-1B* in a 7-Point Dose-Response Assay

b Human lymphoblast cell line

c Human peripheral blood mononuclear cells



d Silencing efficacy of siIL1B and siACVR1 in ex vivo Human Muscle Tissue



Extended Figure 20. Development of the siRNA targeting human *IL1B* mRNA.

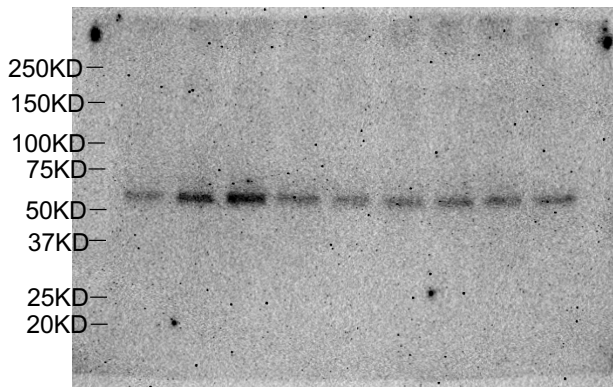
a-c. The human lymphoblastic cell line HEL92.1.7 was treated with a panel of 48 siRNAs targeting human *IL-1B* mRNA and siNTC. Three days later, mRNA levels of *IL-1B* were measured using the Quantigene 2.0 assay (n = 3, **a**). UNT: untreated control, siNTC: non-targeting control siRNA.

HEL92.1.7 cells were treated with the lead siRNA_1339 in a 7-point dose response, and mRNA levels of *IL-1B* were measured (n = 3, **b**). Alternatively, a unimolecular structure comprising of both lead compound IL-1B_1339 and ACVR1_6.9 were tested in human peripheral blood mononuclear cells (PBMCs) in a 7-point dose response, and mRNA levels of *IL-1B* were measured (n = 3, **c**).

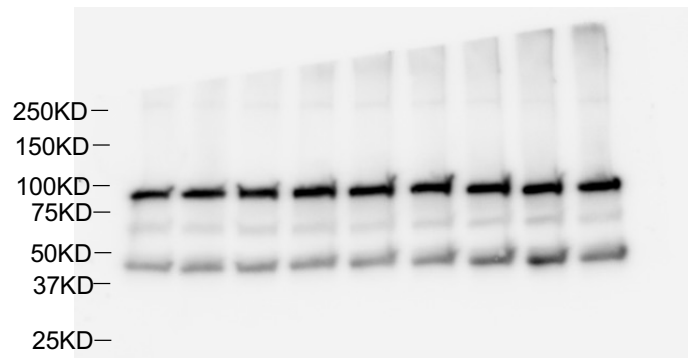
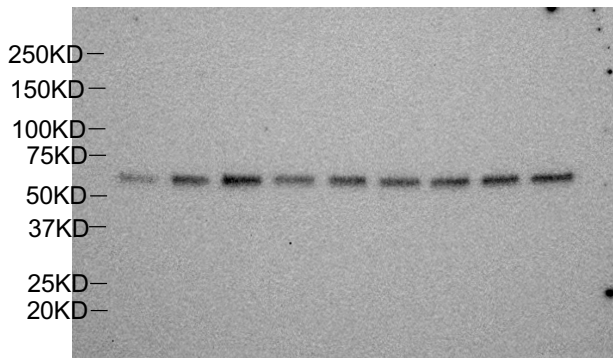
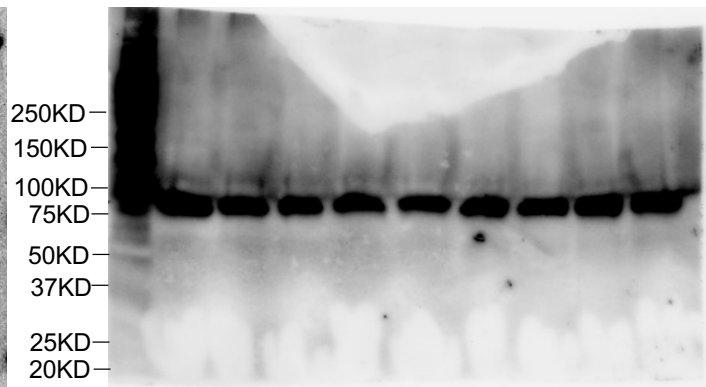
d. Schematic of administering siRNAs into human muscle biopsies (8 mm) in a 5-point dose response following intramuscular injection of siRNAs (50 uL of 200 uM siRNA/biopsy, 10 nmol top dose/biopsy). Both WT *ACVR1* and *IL-1B* mRNA silencing four days post intramuscular injection (n = 4). Values represent mean \pm SD by one-way ANOVA tests (**d**).

Uncropped Western Blot images

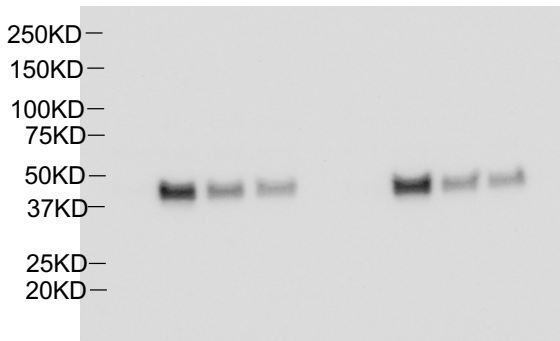
IB: Phospho-Smad1/5



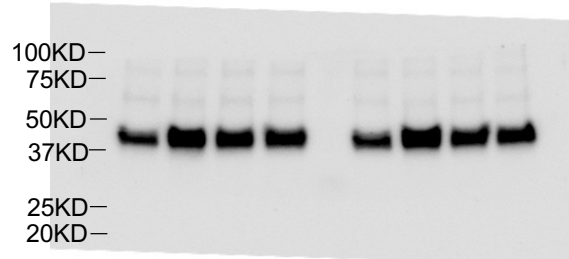
IB: Hsp90



IB: Phospho-ERK1/2



IB: ERK1/2



Extended Table 1. Sequences and chemical modification patterns of human *ACVR1*^{R206H} siRNAs (Primary mismatch).

Oligo Name	Sequence
ACVR1_2	P(mU)#(fU)#(mG)(fA)(fG)(fC)(mC)(fA)(mC)(fU)(mG)(fU)(mU)(fC)#(mU)#(fU)#(mU)#(mG)#(mU)#(mA)#(fA)
	(mA)#(mA)#(mG)(fA)(mA)(fC)(mA)(fG)(mU)(fG)(mG)(mC)(mU)(fC)#(mA)#(mA)-TegChol
ACVR1_3	P(mU)#(fG)#(mU)(fG)(fA)(fG)(mC)(fC)(mA)(fC)(mU)(fG)(mU)(fU)#(mC)#(fU)#(mU)#(mU)#(mG)#(mU)#(fA)
	(mA)#(mG)#(mA)(fA)(mC)(fA)(mG)(fU)(mG)(fG)(mC)(mU)(mC)(fA)#(mC)#(mA)-TegChol
ACVR1_4	P(mU)#(fG)#(mG)(fU)(fG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)(mG)(fU)#(mU)#(fC)#(mU)#(mU)#(mU)#(mG)#(fA)
	(mG)#(mA)#(mA)(fC)(mA)(fG)(mU)(fG)(mG)(fC)(mU)(mC)(mA)(fC)#(mC)#(mA)-TegChol
ACVR1_5	P(mU)#(fU)#(mG)(fG)(fU)(fG)(mA)(fG)(mC)(fC)(mA)(fC)(mU)(fG)#(mU)#(fU)#(mC)#(mU)#(mU)#(mU)#(fA)
	(mA)#(mA)#(mC)(fA)(mG)(fU)(mG)(fG)(mC)(fU)(mC)(mA)(mC)(fC)#(mA)#(mA)-TegChol
ACVR1_6	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_7	P(mU)#(fU)#(mC)(fU)(fG)(fG)(mU)(fG)(mA)(fG)(mC)(fC)(mA)(fC)#(mU)#(fG)#(mU)#(mU)#(mC)#(mU)#(fA)
	(mC)#(mA)#(mG)(fU)(mG)(fG)(mC)(fU)(mC)(fA)(mC)(mC)(mA)(fG)#(mA)#(mA)-TegChol
ACVR1_8	P(mU)#(fA)#(mU)(fC)(fU)(fG)(mG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)#(mC)#(fU)#(mG)#(mU)#(mU)#(mC)#(fA)
	(mA)#(mG)#(mU)(fG)(mG)(fC)(mU)(fC)(mA)(fC)(mC)(mA)(mG)(fA)#(mU)#(mA)-TegChol
ACVR1_9	P(mU)#(fA)#(mA)(fU)(fC)(fU)(mG)(fG)(mU)(fG)(mA)(fG)(mC)(fC)#(mA)#(fC)#(mU)#(mG)#(mU)#(mU)#(fA)
	(mG)#(mU)#(mG)(fG)(mC)(fU)(mC)(fA)(mC)(fC)(mA)(mG)(mA)(fU)#(mU)#(mA)-TegChol
ACVR1_10	P(mU)#(fU)#(mA)(fA)(fU)(fC)(mU)(fG)(mG)(fU)(mG)(fA)(mG)(fC)#(mC)#(fA)#(mC)#(mU)#(mG)#(mU)#(fA)
	(mU)#(mG)#(mG)(fC)(mU)(fC)(mA)(fC)(mC)(fA)(mG)(mA)(mU)(fU)#(mA)#(mA)-TegChol
ACVR1_11	P(mU)#(fG)#(mU)(fA)(fA)(fU)(mC)(fU)(mG)(fG)(mU)(fG)(mA)(fG)#(mC)#(fC)#(mA)#(mC)#(mU)#(mG)#(fA)
	(mG)#(mG)#(mC)(fU)(mC)(fA)(mC)(fC)(mA)(fG)(mA)(mU)(mU)(fA)#(mC)#(mA)-TegChol
ACVR1_12	P(mU)#(fU)#(mG)(fU)(fA)(fA)(mU)(fC)(mU)(fG)(mG)(fU)(mG)(fA)#(mG)#(fC)#(mC)#(mA)#(mC)#(mU)#(fA)
	(mG)#(mC)#(mU)(fC)(mA)(fC)(mC)(fA)(mG)(fA)(mU)(mU)(mA)(fC)#(mA)#(mA)-TegChol
ACVR1_13	P(mU)#(fG)#(mU)(fG)(fU)(fA)(mA)(fU)(mC)(fU)(mG)(fG)(mU)(fG)#(mA)#(fG)#(mC)#(mC)#(mA)#(mC)#(fA)
	(mC)#(mU)#(mC)(fA)(mC)(fC)(mA)(fG)(mA)(fU)(mU)(mA)(mC)(fA)#(mC)#(mA)-TegChol
ACVR1_14	P(mU)#(fA)#(mG)(fU)(fG)(fU)(mA)(fA)(mU)(fC)(mU)(fG)(mG)(fU)#(mG)#(fA)#(mG)#(mC)#(mC)#(mA)#(fA)
	(mU)#(mC)#(mA)(fC)(mC)(fA)(mG)(fA)(mU)(fU)(mA)(mC)(mA)(fC)#(mU)#(mA)-TegChol
ACVR1_15	P(mU)#(fC)#(mA)(fG)(fU)(fG)(mU)(fA)(mA)(fU)(mC)(fU)(mG)(fG)#(mU)#(fG)#(mA)#(mG)#(mC)#(mC)#(fA)

	(mC)#(mA)#(mC)(fC)(mA)(fG)(mA)(fU)(mU)(fA)(mC)(mA)(mC)(fU)#(mG)#(mA)-TegChol
ACVR1_16	P(mU)#(fA)#(mC)(fA)(fG)(fU)(mG)(fU)(mA)(fA)(mU)(fC)(mU)(fG)#(mG)#(fU)#(mG)#(mA)#(mG)#(mC)#(fA)
	(mA)#(mC)#(mC)(fA)(mG)(fA)(mU)(fU)(mA)(fC)(mA)(mC)(mU)(fG)#(mU)#(mA)-TegChol
ACVR1_17	P(mU)#(fA)#(mA)(fC)(fA)(fG)(mU)(fG)(mU)(fA)(mA)(fU)(mC)(fU)#(mG)#(fG)#(mU)#(mG)#(mA)#(mG)#(fA)
	(mC)#(mC)#(mA)(fG)(mA)(fU)(mU)(fA)(mC)(fA)(mC)(mU)(mG)(fU)#(mU)#(mA)-TegChol

Extended Table 2. Sequences and chemical modification patterns of human *ACVR1*^{R206H} siRNAs (Secondary mismatch).

Oligo Name	Sequence
ACVR1_6_2	P(mU)#(fU)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mA)#(mA)-TegChol
ACVR1_6_3	P(mU)#(fC)#(mA)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fU)#(mG)#(mA)-TegChol
ACVR1_6_4	P(mU)#(fC)#(mU)(fU)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mA)(fA)#(mG)#(mA)-TegChol
ACVR1_6_5	P(mU)#(fC)#(mU)(fG)(fU)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mA)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_7	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mU)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fA)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_8	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fU)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mA)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_9	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mU)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fA)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_10	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fA)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mU)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_11	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mA)(fA)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fU)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_12	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fU)(mC)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mA)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_13	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mA)(fU)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fU)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_14	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fA)#(mG)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mU)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_15	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mU)#(fU)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mA)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_16	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fA)#(mU)#(mC)#(mU)#(mU)#(fA)
	(mU)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
ACVR1_6_17	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fA)(mG)(fC)(mC)(fA)(mC)(fU)#(mG)#(fU)#(mA)#(mC)#(mU)#(mU)#(fA)
	(mA)#(mC)#(mA)(fG)(mU)(fG)(mG)(fC)(mU)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol

Extended Table 3. Sequences and chemical modification patterns of mouse *Il1b* siRNAs.

Oligo Name	Sequence
Il1b_NM_008361_1293	P(mU)#(fA)#(mA)(fA)(fG)(fC)(mA)(fA)(mU)(fG)(mU)(fG)(mC)(fU)#(mG)#(fG)#(mU)#(mG)#(mC)#(mU)#(fU) (mC)#(mC)#(mA)(fG)(mC)(fA)(mC)(fA)(mU)(fU)(mG)(mC)(mU)(fU)#(mU)#(mA)-TegChol
Il1b_NM_008361_1263	P(mU)#(fA)#(mA)(fG)(fU)(fC)(mU)(fC)(mA)(fU)(mC)(fC)(mA)(fU)#(mA)#(fU)#(mU)#(mA)#(mU)#(mU)#(fU) (mA)#(mU)#(mA)(fU)(mG)(fG)(mA)(fU)(mG)(fA)(mG)(mA)(mC)(fU)#(mU)#(mA)-TegChol
Il1b_NM_008361_856	P(mU)#(fU)#(mG)(fA)(fA)(fG)(mU)(fC)(mA)(fA)(mU)(fU)(mA)(fU)#(mG)#(fU)#(mC)#(mC)#(mU)#(mU)#(fU) (mA)#(mC)#(mA)(fU)(mA)(fA)(mU)(fU)(mG)(fA)(mC)(mU)(mU)(fC)#(mA)#(mA)-TegChol
Il1b_NM_008361_1245	P(mU)#(fA)#(mG)(fG)(fA)(fA)(mC)(fC)(mU)(fA)(mU)(fU)(mU)(fA)#(mU)#(fU)#(mC)#(mU)#(mC)#(mU)#(fU) (mA)#(mA)#(mU)(fA)(mA)(fA)(mU)(fA)(mG)(fG)(mU)(mU)(mC)(fC)#(mU)#(mA)-TegChol
Il1b_NM_008361_1092	P(mU)#(fU)#(mA)(fA)(fA)(fU)(mA)(fA)(mA)(fU)(mA)(fG)(mG)(fU)#(mA)#(fA)#(mG)#(mU)#(mG)#(mU)#(fU) (mU)#(mU)#(mA)(fC)(mC)(fU)(mA)(fU)(mU)(fU)(mA)(mU)(mU)(fU)#(mA)#(mA)-TegChol
Il1b_NM_008361_1117	P(mU)#(fA)#(mU)(fA)(fG)(fA)(mU)(fC)(mA)(fA)(mC)(fC)(mA)(fA)#(mU)#(fC)#(mA)#(mA)#(mU)#(mU)#(fU) (mG)#(mA)#(mU)(fU)(mG)(fG)(mU)(fU)(mG)(fA)(mU)(mC)(mU)(fA)#(mU)#(mA)-TegChol
Il1b_NM_008361_1108	P(mU)#(fC)#(mC)(fA)(fA)(fU)(mC)(fA)(mA)(fU)(mA)(fA)(mA)(fU)#(mA)#(fC)#(mA)#(mU)#(mA)#(mU)#(fU) (mG)#(mU)#(mA)(fU)(mU)(fU)(mA)(fU)(mU)(fG)(mA)(mU)(mU)(fG)#(mG)#(mA)-TegChol
Il1b_NM_008361_1118	P(mU)#(fA)#(mA)(fU)(fA)(fG)(mA)(fU)(mC)(fA)(mA)(fC)(mC)(fA)#(mA)#(fU)#(mC)#(mA)#(mA)#(mU)#(fU) (mA)#(mU)#(mU)(fG)(mG)(fU)(mU)(fG)(mA)(fU)(mC)(mU)(mA)(fU)#(mU)#(mA)-TegChol
Il1b_NM_008361_1244	P(mU)#(fG)#(mG)(fA)(fA)(fC)(mC)(fU)(mA)(fU)(mU)(fU)(mA)(fU)#(mU)#(fC)#(mU)#(mC)#(mA)#(mU)#(fU) (mG)#(mA)#(mA)(fU)(mA)(fA)(mA)(fU)(mA)(fG)(mG)(mU)(mU)(fC)#(mC)#(mA)-TegChol
Il1b_NM_008361_1080	P(mU)#(fU)#(mA)(fA)(fG)(fU)(mG)(fG)(mU)(fU)(mG)(fC)(mC)(fC)#(mA)#(fU)#(mC)#(mA)#(mG)#(mU)#(fU) (mA)#(mU)#(mG)(fG)(mG)(fC)(mA)(fA)(mC)(fC)(mA)(mC)(mU)(fU)#(mA)#(mA)-TegChol
Il1b_NM_008361_1127	P(mU)#(fA)#(mU)(fC)(fA)(fA)(mC)(fU)(mU)(fA)(mA)(fA)(mU)(fA)#(mG)#(fA)#(mU)#(mC)#(mA)#(mU)#(fU) (mU)#(mC)#(mU)(fA)(mU)(fU)(mU)(fA)(mA)(fG)(mU)(mU)(mG)(fA)#(mU)#(mA)-TegChol
Il1b_NM_008361_1081	P(mU)#(fG)#(mU)(fA)(fA)(fG)(mU)(fG)(mG)(fU)(mU)(fG)(mC)(fC)#(mC)#(fA)#(mU)#(mC)#(mA)#(mU)#(fU) (mU)#(mG)#(mG)(fG)(mC)(fA)(mA)(fC)(mC)(fA)(mC)(mU)(mU)(fA)#(mC)#(mA)-TegChol
Il1b_NM_008361_266	P(mU)#(fA)#(mG)(fU)(fG)(fA)(mU)(fA)(mC)(fU)(mG)(fC)(mC)(fU)#(mG)#(fC)#(mC)#(mU)#(mG)#(mU)#(fU) (mG)#(mC)#(mA)(fG)(mG)(fC)(mA)(fG)(mU)(fA)(mU)(mC)(mA)(fC)#(mU)#(mA)-TegChol
Il1b_NM_008361_1282	P(mU)#(fC)#(mU)(fG)(fG)(fU)(mG)(fC)(mU)(fU)(mC)(fA)(mU)(fU)#(mC)#(fA)#(mU)#(mA)#(mA)#(mU)#(fU) (mU)#(mG)#(mA)(fA)(mU)(fG)(mA)(fA)(mG)(fC)(mA)(mC)(mC)(fA)#(mG)#(mA)-TegChol
Il1b_NM_008361_1296	P(mU)#(fA)#(mU)(fC)(fA)(fA)(mA)(fG)(mC)(fA)(mA)(fU)(mG)(fU)#(mG)#(fC)#(mU)#(mG)#(mG)#(mU)#(fU) (mG)#(mC)#(mA)(fC)(mA)(fU)(mU)(fG)(mC)(fU)(mU)(mU)(mG)(fA)#(mU)#(mA)-TegChol
Il1b_NM_008361_1085	P(mU)#(fA)#(mU)(fA)(fG)(fG)(mU)(fA)(mA)(fG)(mU)(fG)(mG)(fU)#(mU)#(fG)#(mC)#(mC)#(mC)#(mU)#(fU) (mC)#(mA)#(mA)(fC)(mC)(fA)(mC)(fU)(mU)(fA)(mC)(mC)(mU)(fA)#(mU)#(mA)-TegChol
Il1b_NM_008361_133	P(mU)#(fA)#(mC)(fA)(fG)(fG)(mU)(fC)(mA)(fU)(mU)(fC)(mU)(fC)#(mA)#(fU)#(mC)#(mA)#(mC)#(mU)#(fU) (mA)#(mU)#(mG)(fA)(mG)(fA)(mA)(fU)(mG)(fA)(mC)(mC)(mU)(fG)#(mU)#(mA)-TegChol
Il1b_NM_008361_137	P(mU)#(fA)#(mA)(fG)(fA)(fA)(mC)(fA)(mG)(fG)(mU)(fC)(mA)(fU)#(mU)#(fC)#(mU)#(mC)#(mA)#(mU)#(fU)

	(mG)#(mA)#(mA)(fU)(mG)(fA)(mC)(fC)(mU)(fG)(mU)(mU)(mC)(fU)#(mU)#(mA)-TegChol
Il1b_NM_008361_431	P(mU)#(fC)#(mU)(fA)(fA)(fU)(mG)(fG)(mG)(fA)(mA)(fC)(mG)(fU)#(mC)#(fA)#(mC)#(mA)#(mC)#(mU)#(fU)
	(mU)#(mG)#(mA)(fC)(mG)(fU)(mU)(fC)(mC)(fC)(mA)(mU)(mU)(fA)#(mG)#(mA)-TegChol
Il1b_NM_008361_1128	P(mU)#(fA)#(mA)(fU)(fC)(fA)(mA)(fC)(mU)(fU)(mA)(fA)(mA)(fU)#(mA)#(fG)#(mA)#(mU)#(mC)#(mU)#(fU)
	(mC)#(mU)#(mA)(fU)(mU)(fU)(mA)(fA)(mG)(fU)(mU)(mG)(mA)(fU)#(mU)#(mA)-TegChol
Il1b_NM_008361_1215	P(mU)#(fA)#(mG)(fA)(fC)(fC)(mU)(fC)(mA)(fG)(mU)(fG)(mC)(fG)#(mG)#(fG)#(mC)#(mU)#(mA)#(mU)#(fU)
	(mC)#(mC)#(mC)(fG)(mC)(fA)(mC)(fU)(mG)(fA)(mG)(mG)(mU)(fC)#(mU)#(mA)-TegChol
Il1b_NM_008361_802	P(mU)#(fC)#(mU)(fG)(fC)(fU)(mU)(fG)(mU)(fG)(mA)(fG)(mG)(fU)#(mG)#(fC)#(mU)#(mG)#(mA)#(mU)#(fU)
	(mG)#(mC)#(mA)(fC)(mC)(fU)(mC)(fA)(mC)(fA)(mA)(mG)(mC)(fA)#(mG)#(mA)-TegChol
Il1b_NM_008361_871	P(mU)#(fA)#(mC)(fA)(fC)(fG)(mG)(fA)(mU)(fU)(mC)(fC)(mA)(fU)#(mG)#(fG)#(mU)#(mG)#(mA)#(mU)#(fU)
	(mC)#(mC)#(mA)(fU)(mG)(fG)(mA)(fA)(mU)(fC)(mC)(mG)(mU)(fG)#(mU)#(mA)-TegChol
Il1b_NM_008361_90	P(mU)#(fG)#(mA)(fG)(fU)(fU)(mC)(fA)(mG)(fG)(mA)(fA)(mC)(fA)#(mG)#(fU)#(mU)#(mG)#(mC)#(mU)#(fU)
	(mA)#(mC)#(mU)(fG)(mU)(fU)(mC)(fC)(mU)(fG)(mA)(mA)(mC)(fU)#(mC)#(mA)-TegChol
Il1b_NM_008361_388	P(mU)#(fA)#(mU)(fG)(fA)(fG)(mU)(fC)(mA)(fC)(mA)(fG)(mA)(fG)#(mG)#(fA)#(mU)#(mG)#(mG)#(mU)#(fU)
	(mU)#(mC)#(mC)(fU)(mC)(fU)(mG)(fU)(mG)(fA)(mC)(mU)(mC)(fA)#(mU)#(mA)-TegChol
Il1b_NM_008361_376	P(mU)#(fG)#(mG)(fA)(fU)(fG)(mG)(fG)(mC)(fU)(mC)(fU)(mU)(fC)#(mU)#(fU)#(mC)#(mA)#(mA)#(mU)#(fU)
	(mA)#(mA)#(mG)(fA)(mA)(fG)(mA)(fG)(mC)(fC)(mC)(mA)(mU)(fC)#(mC)#(mA)-TegChol
Il1b_NM_008361_548	P(mU)#(fG)#(mA)(fG)(fA)(fA)(mU)(fA)(mU)(fC)(mA)(fC)(mU)(fU)#(mG)#(fU)#(mU)#(mG)#(mG)#(mU)#(fU)
	(mA)#(mC)#(mA)(fA)(mG)(fU)(mG)(fA)(mU)(fA)(mU)(mU)(mC)(fU)#(mC)#(mA)-TegChol
Il1b_NM_008361_96	P(mU)#(fA)#(mC)(fA)(fG)(fU)(mU)(fG)(mA)(fG)(mU)(fU)(mC)(fA)#(mG)#(fG)#(mA)#(mA)#(mC)#(mU)#(fU)
	(mC)#(mC)#(mU)(fG)(mA)(fA)(mC)(fU)(mC)(fA)(mA)(mC)(mU)(fG)#(mU)#(mA)-TegChol
Il1b_NM_008361_346	P(mU)#(fA)#(mG)(fA)(fA)(fG)(mG)(fU)(mG)(fC)(mU)(fC)(mA)(fU)#(mG)#(fU)#(mC)#(mC)#(mU)#(mU)#(fU)
	(mA)#(mC)#(mA)(fU)(mG)(fA)(mG)(fC)(mA)(fC)(mC)(mU)(mU)(fC)#(mU)#(mA)-TegChol
Il1b_NM_008361_1261	P(mU)#(fG)#(mU)(fC)(fU)(fC)(mA)(fU)(mC)(fC)(mA)(fU)(mA)(fU)#(mU)#(fA)#(mU)#(mA)#(mG)#(mU)#(fU)
	(mU)#(mA)#(mA)(fU)(mA)(fU)(mG)(fG)(mA)(fU)(mG)(mA)(mG)(fA)#(mC)#(mA)-TegChol
Il1b_NM_008361_1104	P(mU)#(fU)#(mC)(fA)(fA)(fU)(mA)(fA)(mA)(fU)(mA)(fC)(mA)(fU)#(mA)#(fA)#(mA)#(mU)#(mA)#(mU)#(fU)
	(mU)#(mU)#(mA)(fU)(mG)(fU)(mA)(fU)(mU)(fU)(mA)(mU)(mU)(fG)#(mA)#(mA)-TegChol
Il1b_NM_008361_338	P(mU)#(fC)#(mU)(fC)(fA)(fU)(mG)(fU)(mC)(fC)(mU)(fC)(mA)(fU)#(mC)#(fC)#(mU)#(mG)#(mG)#(mU)#(fU)
	(mG)#(mG)#(mA)(fU)(mG)(fA)(mG)(fG)(mA)(fC)(mA)(mU)(mG)(fA)#(mG)#(mA)-TegChol
Il1b_NM_008361_1112	P(mU)#(fU)#(mC)(fA)(fA)(fC)(mC)(fA)(mA)(fU)(mC)(fA)(mA)(fU)#(mA)#(fA)#(mA)#(mU)#(mA)#(mU)#(fU)
	(mU)#(mU)#(mA)(fU)(mU)(fG)(mA)(fU)(mU)(fG)(mG)(mU)(mU)(fG)#(mA)#(mA)-TegChol
Il1b_NM_008361_855	P(mU)#(fG)#(mA)(fA)(fG)(fU)(mC)(fA)(mA)(fU)(mU)(fA)(mU)(fG)#(mU)#(fC)#(mC)#(mU)#(mG)#(mU)#(fU)
	(mG)#(mA)#(mC)(fA)(mU)(fA)(mA)(fU)(mU)(fG)(mA)(mC)(mU)(fU)#(mC)#(mA)-TegChol
Il1b_NM_008361_621	P(mU)#(fU)#(mA)(fG)(fA)(fU)(mU)(fC)(mU)(fU)(mU)(fC)(mC)(fU)#(mU)#(fU)#(mG)#(mA)#(mG)#(mU)#(fU)
	(mA)#(mA)#(mA)(fG)(mG)(fA)(mA)(fA)(mG)(fA)(mA)(mU)(mC)(fU)#(mA)#(mA)-TegChol
Il1b_NM_008361_736	P(mU)#(fC)#(mU)(fU)(fC)(fU)(mA)(fU)(mC)(fU)(mU)(fG)(mU)(fU)#(mG)#(fA)#(mA)#(mG)#(mA)#(mU)#(fU)
	(mU)#(mC)#(mA)(fA)(mC)(fA)(mA)(fG)(mA)(fU)(mA)(mG)(mA)(fA)#(mG)#(mA)-TegChol
Il1b_NM_008361_965	P(mU)#(fU)#(mG)(fG)(fA)(fG)(mA)(fU)(mU)(fG)(mA)(fG)(mC)(fU)#(mG)#(fU)#(mC)#(mU)#(mG)#(mU)#(fU)

	(mA)#(mC)#(mA)(fG)(mC)(fU)(mC)(fA)(mA)(fU)(mC)(mU)(mC)(fC)#(mA)#(mA)-TegChol
Il1b_NM_008361_1109	(mU)#(mA)#(mU)(fU)(mU)(fA)(mU)(fU)(mG)(fA)(mU)(mU)(mG)(fG)#(mU)#(mA)-TegChol
	P(mU)#(fA)#(mC)(fC)(fA)(fA)(mU)(fC)(mA)(fA)(mU)(fA)(mA)(fA)#(mU)#(fA)#(mC)#(mA)#(mU)#(mU)#(fU)
Il1b_NM_008361_20	(mG)#(mC)#(mC)(fU)(mA)(fA)(mU)(fA)(mG)(fG)(mC)(mU)(mC)(fA)#(mU)#(mA)-TegChol
	P(mU)#(fA)#(mU)(fG)(fA)(fG)(mC)(fC)(mU)(fA)(mU)(fU)(mA)(fG)#(mG)#(fC)#(mC)#(mU)#(mC)#(mU)#(fU)
Il1b_NM_008361_1047	(mC)#(mA)#(mA)(fA)(mG)(fA)(mG)(fA)(mG)(fC)(mC)(mU)(mG)(fU)#(mG)#(mA)-TegChol
	P(mU)#(fC)#(mA)(fC)(fA)(fG)(mG)(fC)(mU)(fC)(mU)(fC)(mU)(fU)#(mU)#(fG)#(mA)#(mA)#(mC)#(mU)#(fU)
Il1b_NM_008361_1120	(mU)#(mG)#(mG)(fU)(mU)(fG)(mA)(fU)(mC)(fU)(mA)(mU)(mU)(fU)#(mA)#(mA)-TegChol
	P(mU)#(fU)#(mA)(fA)(fA)(fU)(mA)(fG)(mA)(fU)(mC)(fA)(mA)(fC)#(mC)#(fA)#(mA)#(mU)#(mC)#(mU)#(fU)
Il1b_NM_008361_1260	(mA)#(mU)#(mA)(fA)(mU)(fA)(mU)(fG)(mG)(fA)(mU)(mG)(mA)(fG)#(mA)#(mA)-TegChol
	P(mU)#(fU)#(mC)(fU)(fC)(fA)(mU)(fC)(mC)(fA)(mU)(fA)(mU)(fU)#(mA)#(fU)#(mA)#(mG)#(mG)#(mU)#(fU)
Il1b_NM_008361_931	(mC)#(mA)#(mU)(fG)(mU)(fU)(mA)(fA)(mG)(fG)(mA)(mG)(mC)(fU)#(mC)#(mA)-TegChol
	P(mU)#(fG)#(mA)(fG)(fC)(fU)(mC)(fC)(mU)(fU)(mA)(fA)(mC)(fA)#(mU)#(fG)#(mC)#(mC)#(mC)#(mU)#(fU)
Il1b_NM_008361_872	(mC)#(mA)#(mU)(fG)(mG)(fA)(mA)(fU)(mC)(fC)(mG)(mU)(mG)(fU)#(mC)#(mA)-TegChol
	P(mU)#(fG)#(mA)(fC)(fA)(fC)(mG)(fG)(mA)(fU)(mU)(fC)(mC)(fA)#(mU)#(fG)#(mG)#(mU)#(mG)#(mU)#(fU)
Il1b_NM_008361_633	(mC)#(mU)#(mA)(fU)(mA)(fC)(mC)(fU)(mG)(fU)(mC)(mC)(mU)(fG)#(mU)#(mA)-TegChol
	P(mU)#(fA)#(mC)(fA)(fG)(fG)(mA)(fC)(mA)(fG)(mG)(fU)(mA)(fU)#(mA)#(fG)#(mA)#(mU)#(mU)#(mU)#(fU)
Il1b_NM_008361_1084	(mG)#(mC)#(mA)(fA)(mC)(fC)(mA)(fC)(mU)(fU)(mA)(mC)(mC)(fU)#(mA)#(mA)-TegChol
	P(mU)#(fU)#(mA)(fG)(fG)(fU)(mA)(fA)(mG)(fU)(mG)(fG)(mU)(fU)#(mG)#(fC)#(mC)#(mC)#(mA)#(mU)#(fU)
Il1b_NM_008361_1240	(mC)#(mU)#(mG)(fA)(mG)(fA)(mA)(fU)(mA)(fA)(mA)(mU)(mA)(fG)#(mG)#(mA)-TegChol
	P(mU)#(fC)#(mC)(fU)(fA)(fU)(mU)(fU)(mA)(fU)(mU)(fC)(mU)(fC)#(mA)#(fG)#(mC)#(mU)#(mU)#(mU)#(fU)
Il1b_NM_008361_818	(mG)#(mC)#(mA)(fC)(mA)(fA)(mG)(fC)(mC)(fU)(mG)(mU)(mC)(fU)#(mU)#(mA)-TegChol
	P(mU)#(fA)#(mA)(fG)(fA)(fC)(mA)(fG)(mG)(fC)(mU)(fU)(mG)(fU)#(mG)#(fC)#(mU)#(mC)#(mU)#(mU)#(fU)

Extended Table 4. Sequences and chemical modification patterns of human *IL1B* siRNAs.

Oligo Name	Sequence
IL1B_NM_000576_856	P(mU)#(fU)#(mG)(fA)(fA)(fG)(mU)(fC)(mA)(fG)(mU)(fU)(mA)(fU)#(mA)#(fU)#(mC)#(mC)#(mU)#(mU)#(fU)
	(mA)#(mU)#(mA)(fU)(mA)(fA)(mC)(fU)(mG)(fA)(mC)(mU)(mU)(fC)#(mA)#(mA)-TegChol
IL1B_NM_000576_1370	P(mU)#(fU)#(mU)(fG)(fA)(fU)(mU)(fU)(mA)(fA)(mA)(fG)(mA)(fG)#(mA)#(fG)#(mC)#(mA)#(mC)#(mU)#(fU)
	(mC)#(mU)#(mC)(fU)(mC)(fU)(mU)(fU)(mA)(fA)(mA)(mU)(mC)(fA)#(mA)#(mA)-TegChol
IL1B_NM_000576_1427	P(mU)#(fU)#(mA)(fA)(fA)(fU)(mA)(fU)(mU)(fC)(mC)(fC)(mA)(fU)#(mU)#(fU)#(mA)#(mA)#(mA)#(mU)#(fU)
	(mA)#(mA)#(mA)(fU)(mG)(fG)(mG)(fA)(mA)(fU)(mA)(mU)(mU)(fU)#(mA)#(mA)-TegChol
IL1B_NM_000576_1411	P(mU)#(fA)#(mA)(fU)(fA)(fA)(mU)(fC)(mU)(fG)(mA)(fG)(mC)(fU)#(mU)#(fA)#(mU)#(mA)#(mU)#(mU)#(fU)
	(mU)#(mA)#(mA)(fG)(mC)(fU)(mC)(fA)(mG)(fA)(mU)(mU)(mA)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_1390	P(mU)#(fU)#(mU)(fC)(fA)(fG)(mU)(fC)(mU)(fU)(mA)(fA)(mU)(fU)#(mA)#(fA)#(mA)#(mG)#(mG)#(mU)#(fU)
	(mU)#(mU)#(mA)(fA)(mU)(fU)(mA)(fA)(mG)(fA)(mC)(mU)(mG)(fA)#(mA)#(mA)-TegChol
IL1B_NM_000576_112	P(mU)#(fA)#(mA)(fU)(fA)(fA)(mG)(fC)(mC)(fA)(mU)(fC)(mA)(fU)#(mU)#(fU)#(mC)#(mA)#(mC)#(mU)#(fU)
	(mA)#(mA)#(mA)(fU)(mG)(fA)(mU)(fG)(mG)(fC)(mU)(mU)(mA)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_1374	P(mU)#(fG)#(mG)(fA)(fC)(fU)(mU)(fG)(mA)(fU)(mU)(fU)(mA)(fA)#(mA)#(fG)#(mA)#(mG)#(mA)#(mU)#(fU)
	(mC)#(mU)#(mU)(fU)(mA)(fA)(mA)(fU)(mC)(fA)(mA)(mG)(mU)(fC)#(mC)#(mA)-TegChol
IL1B_NM_000576_1443	P(mU)#(fG)#(mA)(fU)(fA)(fU)(mU)(fU)(mG)(fC)(mU)(fC)(mA)(fU)#(mU)#(fU)#(mA)#(mU)#(mA)#(mU)#(fU)
	(mA)#(mA)#(mA)(fU)(mG)(fA)(mG)(fC)(mA)(fA)(mA)(mU)(mA)(fU)#(mC)#(mA)-TegChol
IL1B_NM_000576_1460	P(mU)#(fG)#(mA)(fA)(fC)(fC)(mA)(fU)(mU)(fG)(mA)(fA)(mC)(fA)#(mG)#(fU)#(mA)#(mU)#(mG)#(mU)#(fU)
	(mA)#(mC)#(mU)(fG)(mU)(fU)(mC)(fA)(mA)(fU)(mG)(mG)(mU)(fU)#(mC)#(mA)-TegChol
IL1B_NM_000576_1442	P(mU)#(fA)#(mU)(fA)(fU)(fU)(mU)(fG)(mC)(fU)(mC)(fA)(mU)(fU)#(mU)#(fA)#(mU)#(mA)#(mA)#(mU)#(fU)
	(mU)#(mA)#(mA)(fA)(mU)(fG)(mA)(fG)(mC)(fA)(mA)(mA)(mU)(fA)#(mU)#(mA)-TegChol
IL1B_NM_000576_703	P(mU)#(fC)#(mC)(fA)(fU)(fC)(mU)(fU)(mC)(fU)(mU)(fC)(mU)(fU)#(mU)#(fG)#(mG)#(mG)#(mU)#(mU)#(fU)
	(mC)#(mA)#(mA)(fA)(mG)(fA)(mA)(fG)(mA)(fA)(mG)(mA)(mU)(fG)#(mG)#(mA)-TegChol
IL1B_NM_000576_1421	P(mU)#(fU)#(mU)(fC)(fC)(fC)(mA)(fU)(mU)(fU)(mA)(fA)(mA)(fU)#(mA)#(fA)#(mU)#(mC)#(mU)#(mU)#(fU)
	(mU)#(mU)#(mA)(fU)(mU)(fU)(mA)(fA)(mA)(fU)(mG)(mG)(mG)(fA)#(mA)#(mA)-TegChol
IL1B_NM_000576_855	P(mU)#(fG)#(mA)(fA)(fG)(fU)(mC)(fA)(mG)(fU)(mU)(fA)(mU)(fA)#(mU)#(fC)#(mC)#(mU)#(mG)#(mU)#(fU)
	(mG)#(mA)#(mU)(fA)(mU)(fA)(mA)(fC)(mU)(fG)(mA)(mC)(mU)(fU)#(mC)#(mA)-TegChol
IL1B_NM_000576_380	P(mU)#(fA)#(mA)(fG)(fA)(fU)(mA)(fG)(mG)(fU)(mU)(fC)(mU)(fU)#(mC)#(fU)#(mU)#(mC)#(mA)#(mU)#(fU)
	(mA)#(mG)#(mA)(fA)(mG)(fA)(mA)(fC)(mC)(fU)(mA)(mU)(mC)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_1361	P(mU)#(fA)#(mG)(fA)(fG)(fA)(mG)(fC)(mA)(fC)(mA)(fC)(mC)(fA)#(mG)#(fU)#(mC)#(mC)#(mA)#(mU)#(fU)
	(mA)#(mC)#(mU)(fG)(mG)(fU)(mG)(fU)(mG)(fC)(mU)(mC)(mU)(fC)#(mU)#(mA)-TegChol
IL1B_NM_000576_748	P(mU)#(fC)#(mC)(fA)(fG)(fC)(mU)(fU)(mG)(fU)(mU)(fA)(mU)(fU)#(mG)#(fA)#(mU)#(mU)#(mU)#(mU)#(fU)
	(mU)#(mC)#(mA)(fA)(mU)(fA)(mA)(fC)(mA)(fA)(mG)(mC)(mU)(fG)#(mG)#(mA)-TegChol
IL1B_NM_000576_1450	P(mU)#(fA)#(mC)(fA)(fG)(fU)(mA)(fU)(mG)(fA)(mU)(fA)(mU)(fU)#(mU)#(fG)#(mC)#(mU)#(mC)#(mU)#(fU)
	(mC)#(mA)#(mA)(fA)(mU)(fA)(mU)(fC)(mA)(fU)(mA)(mC)(mU)(fG)#(mU)#(mA)-TegChol
IL1B_NM_000576_1301	P(mU)#(fC)#(mA)(fG)(fA)(fC)(mA)(fC)(mU)(fG)(mC)(fU)(mA)(fC)#(mU)#(fU)#(mC)#(mU)#(mU)#(mU)#(fU)

	(mA)#(mA)#(mG)(fU)(mA)(fG)(mC)(fA)(mG)(fU)(mG)(mU)(mC)(fU)#(mG)#(mA)-TegChol
IL1B_NM_000576_1221	P(mU)#(fG)#(mA)(fA)(fG)(fC)(mG)(fG)(mU)(fU)(mG)(fC)(mU)(fC)#(mA)#(fU)#(mC)#(mA)#(mG)#(mU)#(fU)
	(mA)#(mU)#(mG)(fA)(mG)(fC)(mA)(fA)(mC)(fC)(mG)(mC)(mU)(fU)#(mC)#(mA)-TegChol
IL1B_NM_000576_379	P(mU)#(fA)#(mG)(fA)(fU)(fA)(mG)(fG)(mU)(fU)(mC)(fU)(mU)(fC)#(mU)#(fU)#(mC)#(mA)#(mA)#(mU)#(fU)
	(mA)#(mA)#(mG)(fA)(mA)(fG)(mA)(fA)(mC)(fC)(mU)(mA)(mU)(fC)#(mU)#(mA)-TegChol
IL1B_NM_000576_1461	P(mU)#(fA)#(mG)(fA)(fA)(fC)(mC)(fA)(mU)(fU)(mG)(fA)(mA)(fC)#(mA)#(fG)#(mU)#(mA)#(mU)#(mU)#(fU)
	(mC)#(mU)#(mG)(fU)(mU)(fC)(mA)(fA)(mU)(fG)(mG)(mU)(mU)(fC)#(mU)#(mA)-TegChol
IL1B_NM_000576_573	P(mU)#(fA)#(mC)(fU)(fU)(fU)(mC)(fU)(mU)(fC)(mU)(fC)(mC)(fU)#(mU)#(fG)#(mU)#(mA)#(mC)#(mU)#(fU)
	(mC)#(mA)#(mA)(fG)(mG)(fA)(mG)(fA)(mA)(fG)(mA)(mA)(mA)(fG)#(mU)#(mA)-TegChol
IL1B_NM_000576_1419	P(mU)#(fC)#(mC)(fC)(fA)(fU)(mU)(fU)(mA)(fA)(mA)(fU)(mA)(fA)#(mU)#(fC)#(mU)#(mG)#(mA)#(mU)#(fU)
	(mG)#(mA)#(mU)(fU)(mA)(fU)(mU)(fU)(mA)(fA)(mA)(mU)(mG)(fG)#(mG)#(mA)-TegChol
IL1B_NM_000576_1458	P(mU)#(fA)#(mC)(fC)(fA)(fU)(mU)(fG)(mA)(fA)(mC)(fA)(mG)(fU)#(mA)#(fU)#(mG)#(mA)#(mU)#(mU)#(fU)
	(mA)#(mU)#(mA)(fC)(mU)(fG)(mU)(fU)(mC)(fA)(mA)(mU)(mG)(fG)#(mU)#(mA)-TegChol
IL1B_NM_000576_120	P(mU)#(fG)#(mC)(fC)(fA)(fC)(mU)(fG)(mU)(fA)(mA)(fU)(mA)(fA)#(mG)#(fC)#(mC)#(mA)#(mU)#(mU)#(fU)
	(mG)#(mC)#(mU)(fU)(mA)(fU)(mU)(fA)(mC)(fA)(mG)(mU)(mG)(fG)#(mC)#(mA)-TegChol
IL1B_NM_000576_1387	P(mU)#(fA)#(mG)(fU)(fC)(fU)(mU)(fA)(mA)(fU)(mU)(fA)(mA)(fA)#(mG)#(fG)#(mA)#(mC)#(mU)#(mU)#(fU)
	(mC)#(mC)#(mU)(fU)(mU)(fA)(mA)(fU)(mU)(fA)(mA)(mG)(mA)(fC)#(mU)#(mA)-TegChol
IL1B_NM_000576_140	P(mU)#(fA)#(mA)(fG)(fA)(fA)(mC)(fA)(mA)(fG)(mU)(fC)(mA)(fU)#(mC)#(fC)#(mU)#(mC)#(mA)#(mU)#(fU)
	(mG)#(mG)#(mA)(fU)(mG)(fA)(mC)(fU)(mU)(fG)(mU)(mU)(mC)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_635	P(mU)#(fA)#(mA)(fC)(fA)(fC)(mG)(fC)(mA)(fG)(mG)(fA)(mC)(fA)#(mG)#(fG)#(mU)#(mA)#(mC)#(mU)#(fU)
	(mC)#(mC)#(mU)(fG)(mU)(fC)(mC)(fU)(mG)(fC)(mG)(mU)(mG)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_1418	P(mU)#(fC)#(mC)(fA)(fU)(fU)(mU)(fA)(mA)(fA)(mU)(fA)(mA)(fU)#(mC)#(fU)#(mG)#(mA)#(mG)#(mU)#(fU)
	(mA)#(mG)#(mA)(fU)(mU)(fA)(mU)(fU)(mU)(fA)(mA)(mA)(mU)(fG)#(mG)#(mA)-TegChol
IL1B_NM_000576_51	P(mU)#(fA)#(mG)(fC)(fA)(fA)(mU)(fG)(mA)(fA)(mG)(fA)(mU)(fU)#(mG)#(fG)#(mC)#(mU)#(mG)#(mU)#(fU)
	(mC)#(mC)#(mA)(fA)(mU)(fC)(mU)(fU)(mC)(fA)(mU)(mU)(mG)(fC)#(mU)#(mA)-TegChol
IL1B_NM_000576_1362	P(mU)#(fA)#(mA)(fG)(fA)(fG)(mA)(fG)(mC)(fA)(mC)(fA)(mC)(fC)#(mA)#(fG)#(mU)#(mC)#(mC)#(mU)#(fU)
	(mC)#(mU)#(mG)(fG)(mU)(fG)(mU)(fG)(mC)(fU)(mC)(mU)(mC)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_1425	P(mU)#(fA)#(mA)(fU)(fA)(fU)(mU)(fC)(mC)(fC)(mA)(fU)(mU)(fU)#(mA)#(fA)#(mA)#(mU)#(mA)#(mU)#(fU)
	(mU)#(mU)#(mA)(fA)(mA)(fU)(mG)(fG)(mG)(fA)(mA)(mU)(mA)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_1339	P(mU)#(fG)#(mA)(fA)(fU)(fU)(mG)(fA)(mU)(fU)(mC)(fC)(mA)(fU)#(mA)#(fG)#(mC)#(mU)#(mA)#(mU)#(fU)
	(mC)#(mU)#(mA)(fU)(mG)(fG)(mA)(fA)(mU)(fC)(mA)(mA)(mU)(fU)#(mC)#(mA)-TegChol
IL1B_NM_000576_106	P(mU)#(fC)#(mC)(fA)(fU)(fC)(mA)(fU)(mU)(fU)(mC)(fA)(mC)(fU)#(mG)#(fG)#(mC)#(mG)#(mA)#(mU)#(fU)
	(mC)#(mC)#(mA)(fG)(mU)(fG)(mA)(fA)(mA)(fU)(mG)(mA)(mU)(fG)#(mG)#(mA)-TegChol
IL1B_NM_000576_1377	P(mU)#(fA)#(mA)(fA)(fG)(fG)(mA)(fC)(mU)(fU)(mG)(fA)(mU)(fU)#(mU)#(fA)#(mA)#(mA)#(mG)#(mU)#(fU)
	(mU)#(mA)#(mA)(fA)(mU)(fC)(mA)(fA)(mG)(fU)(mC)(mC)(mU)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_1420	P(mU)#(fU)#(mC)(fC)(fC)(fA)(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fA)#(mA)#(fU)#(mC)#(mU)#(mG)#(mU)#(fU)
	(mA)#(mU)#(mU)(fA)(mU)(fU)(mU)(fA)(mA)(fA)(mU)(mG)(mG)(fG)#(mA)#(mA)-TegChol
IL1B_NM_000576_1229	P(mU)#(fU)#(mA)(fA)(fA)(fU)(mA)(fG)(mG)(fG)(mA)(fA)(mG)(fC)#(mG)#(fG)#(mU)#(mU)#(mG)#(mU)#(fU)

	(mC)#(mC)#(mG)(fC)(mU)(fU)(mC)(fC)(mC)(fU)(mA)(mU)(mU)(fU)#(mA)#(mA)-TegChol
IL1B_NM_000576_1453	P(mU)#(fU)#(mG)(fA)(fA)(fC)(mA)(fG)(mU)(fA)(mU)(fG)(mA)(fU)#(mA)#(fU)#(mU)#(mU)#(mG)#(mU)#(fU)
	(mA)#(mU)#(mA)(fU)(mC)(fA)(mU)(fA)(mC)(fU)(mG)(mU)(mU)(fC)#(mA)#(mA)-TegChol
IL1B_NM_000576_1344	P(mU)#(fA)#(mA)(fA)(fU)(fU)(mG)(fA)(mA)(fU)(mU)(fG)(mA)(fU)#(mU)#(fC)#(mC)#(mA)#(mU)#(mU)#(fU)
	(mG)#(mA)#(mA)(fU)(mC)(fA)(mA)(fU)(mU)(fC)(mA)(mA)(mU)(fU)#(mU)#(mA)-TegChol
IL1B_NM_000576_791	P(mU)#(fG)#(mA)(fG)(fG)(fU)(mG)(fC)(mU)(fG)(mA)(fU)(mG)(fU)#(mA)#(fC)#(mC)#(mA)#(mG)#(mU)#(fU)
	(mG)#(mU)#(mA)(fC)(mA)(fU)(mC)(fA)(mG)(fC)(mA)(mC)(mC)(fU)#(mC)#(mA)-TegChol
IL1B_NM_000576_1356	P(mU)#(fG)#(mC)(fA)(fC)(fA)(mC)(fC)(mA)(fG)(mU)(fC)(mC)(fA)#(mA)#(fA)#(mU)#(mU)#(mG)#(mU)#(fU)
	(mU)#(mU)#(mU)(fG)(mG)(fA)(mC)(fU)(mG)(fG)(mU)(mG)(mU)(fG)#(mC)#(mA)-TegChol
IL1B_NM_000576_1226	P(mU)#(fA)#(mU)(fA)(fG)(fG)(mG)(fA)(mA)(fG)(mC)(fG)(mG)(fU)#(mU)#(fG)#(mC)#(mU)#(mC)#(mU)#(fU)
	(mC)#(mA)#(mA)(fC)(mC)(fG)(mC)(fU)(mU)(fC)(mC)(mC)(mU)(fA)#(mU)#(mA)-TegChol
IL1B_NM_000576_1345	P(mU)#(fC)#(mA)(fA)(fA)(fU)(mU)(fG)(mA)(fA)(mU)(fU)(mG)(fA)#(mU)#(fU)#(mC)#(mC)#(mA)#(mU)#(fU)
	(mA)#(mA)#(mU)(fC)(mA)(fA)(mU)(fU)(mC)(fA)(mA)(mU)(mU)(fU)#(mG)#(mA)-TegChol
IL1B_NM_000576_729	P(mU)#(fU)#(mA)(fU)(fC)(fU)(mU)(fG)(mU)(fU)(mG)(fA)(mA)(fG)#(mA)#(fC)#(mA)#(mA)#(mA)#(mU)#(fU)
	(mG)#(mU)#(mC)(fU)(mU)(fC)(mA)(fA)(mC)(fA)(mA)(mG)(mA)(fU)#(mA)#(mA)-TegChol
IL1B_NM_000576_370	P(mU)#(fC)#(mU)(fU)(fC)(fU)(mU)(fC)(mA)(fA)(mA)(fG)(mA)(fU)#(mG)#(fA)#(mA)#(mG)#(mG)#(mU)#(fU)
	(mU)#(mC)#(mA)(fU)(mC)(fU)(mU)(fU)(mG)(fA)(mA)(mG)(mA)(fA)#(mG)#(mA)-TegChol
IL1B_NM_000576_868	P(mU)#(fC)#(mA)(fA)(fA)(fU)(mU)(fG)(mC)(fA)(mU)(fG)(mG)(fU)#(mG)#(fA)#(mA)#(mG)#(mU)#(mU)#(fU)
	(mU)#(mC)#(mA)(fC)(mC)(fA)(mU)(fG)(mC)(fA)(mA)(mU)(mU)(fU)#(mG)#(mA)-TegChol
IL1B_NM_000576_574	P(mU)#(fU)#(mA)(fC)(fU)(fU)(mU)(fC)(mU)(fU)(mC)(fU)(mC)(fC)#(mU)#(fU)#(mG)#(mU)#(mA)#(mU)#(fU)
	(mA)#(mA)#(mG)(fG)(mA)(fG)(mA)(fA)(mG)(fA)(mA)(mA)(mG)(fU)#(mA)#(mA)-TegChol
IL1B_NM_000576_746	P(mU)#(fA)#(mG)(fC)(fU)(fU)(mG)(fU)(mU)(fA)(mU)(fU)(mG)(fA)#(mU)#(fU)#(mU)#(mC)#(mU)#(mU)#(fU)
	(mA)#(mA)#(mU)(fC)(mA)(fA)(mU)(fA)(mA)(fC)(mA)(mA)(mG)(fC)#(mU)#(mA)-TegChol

Extended Table 5. Sequences of RT-PCR Primers

Mouse Genes	Primer (5'-3')
<i>Id1</i> (Inhibitor of DNA binding 1)	F: ACATGAACGGCTGCTACTCAC R: GACTTCAGACTCCGAGTTCAGC
<i>Tnalp</i> (Tissue-nonspecific alkaline phosphatase)	F: CACAATATCAAGGATATCGACGTGA R: ACATCAGTTCTGTTCTTCGGGTACA
<i>Ibsp</i> (Integrin binding sialoprotein)	F: CAGGGAGGCAGTGACTCTTC R: AGTGTGGAAAGTGTGGCGTT
<i>Osx</i> (Osterix: SP7)	F: ATGGCGTCCTCTCTGCTTGA R: GAAGGGTGGGTAGTCATTTG
<i>Bglap/Osteocalcin</i> (Bone γ -carboxyglutamate protein)	F: GCAGCACAGGTCCTAAATAG R: GGGCAATAAGGTAGTGAACAG
<i>Hprt</i> (Hypoxanthine-guanine phosphoribosyl transferase)	F: CTGGTGAAAAGGACCTCTCGAAG R: CCAGTTTCACTAATGACACAAACG
<i>Acbt</i> (Actin, Beta)	F: AGGGAAATCGTGCGTGACAT R: GGGCAATAAGGTAGTGAACAG
<i>Gapdh</i> (Glyceraldehyde-3-phosphate dehydrogenase)	F: ACAGTCCATGCCATCACTGCC R: GCCTGCTTCACCACCTTCTTG
<i>Msx2</i> (Msh homeobox2)	F: GGAAGTGGAAAAGCTGAAAATG R: CACAGGTCTATGGAAGGGGTAG
<i>Sox9</i> (SRY-Box Transcription Factor9)	F: CACACGTCAAGCGACCCATGAA R: TCTTCTCGCTCTCGTTCAGCAG
<i>Inhba</i> (Inhibin subunit beta A)	F: GGAGATAGAGGACGACATTGGC R: ACGCTCCACTACTGACAGGTCA
<i>Acvr1</i> (activin A receptor, type 1)	F: CCATTGAAGGGCTCATCACAC R: CCGTTCTCTGTACCAGGAAAGG
Human Gene	Primer(5'-3')
<i>ACVR1^{R206H}</i>	F: TGGTACAAAGAACAGTGGCTTA R: CCATACCTGCCTTTCCCGA
<i>ACVR1^{WT}</i>	F: GACGTGGAGTATGGCACTATCG R: CACTCCAACAGTGTAATCTGGCG
<i>INHBA</i>	F: GGATGACATTGGAAGGAGGGCA R: ACTGACAGGTCACTGCCTTCT
<i>ID1</i>	F: GTTGGAGCTGAACTCGGAATCC R: ACACAAGATGCGATCGTCCGCA
<i>GAPDH</i>	F: GGTGTGATCTTAACTGCCG R: CAGATCAGCAGACTCTGGGT

Extended Table 6. A list of inflammatory cytokines and receptors (RT² profiler PCR array)

Chemokines & Chemokine receptors	<i>Ccl1, Ccl11, Ccl12, Ccl17, Ccl19, Ccl2, Ccl20, Ccl22, Ccl24, Ccl3, Ccl4, Ccl5, Ccl6, Ccl7, Ccl8, Ccl9, Cx3cl1, Cxcl1, Cxcl10, Cxcl11, Cxcl12, Cxcl13, Cxcl15, Cxcl5, Cxcl9, Ccr1, Ccr10, Ccr2, Ccr3, Ccr4, Ccr5, Ccr6, Ccr8, Cxcr2, Cxcr3, Cxcr5</i>
Interleukins	<i>Il11, Il13, Il15, Il16, Il17a, Il17b, Il17f, Il1a, Il1b, Il1rn, Il21, Il27, Il3, Il33, Il4, Il5, Il7, Il10ra, Il10rb, Il1r1, Il2rb, Il2rg, Il5ra, Il6ra, Il6st</i>
Others	<i>Aimp1, Bmp2, Cd40lg, Csf1, Csf2, Csf3, Fasl, Ifng, Lta, Ltb, Mif, Nampt, Osm, Pf4, Spp1, Tnf, Tnfsf10, Tnfsf11, Tnfsf13, Tnfsf13b, Tnfsf4, Vegfa, Tnfrsf11b</i>