

AAV-Mediated Targeting of the Activin A-ACVR1^{R206H} Signaling in Fibrodysplasia Ossificans Progressiva

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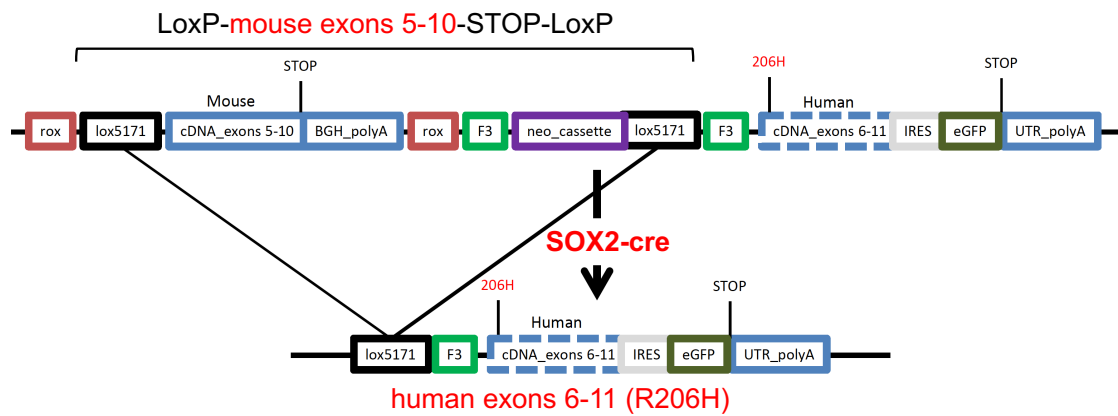
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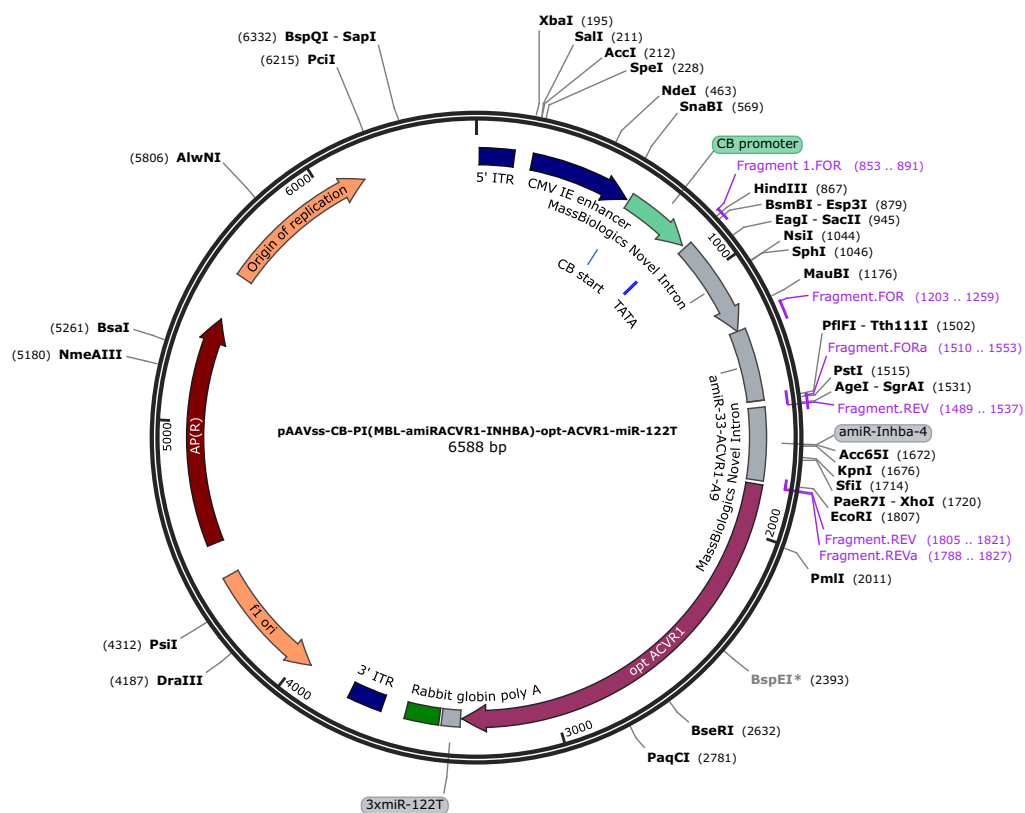
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Supplementary information

- Supplementary Figures S1-S4
- Supplementary Table S1



Supplementary Figure S1. Diagram showing the targeting construct to generate mice harboring a conditional knock-in allele of human *Acvr1*^{R206H} (*Acvr1*^{R206H/+})



Supplementary Figure S2. Construction of AAV vector genome.

AAV vector genomes containing CBA (CMV enhancer/chicken β -actin) promoter, MBL intron (MassBiologics), amiR-ACVR1^{R206H}, amiR-Inhba #4, ACVR1^{opt}, three tandem miR-122-TS (target sequence), and poly A sequences.

Supplementary Table S1: Sequences of primers and gblocks

1. Codon-optimized ACVR1 (ACVR1^{opt})

atggctgatggagtgatgatcctgctgtcctgattatgattgcctgcccagccccagcatggaagatgaaaaacctaagtaaccctaagctgtatatg
tgcgtgtgtagggcctgagctgcggaaacgaggatcactgcgagggccagcagtgttcagctccctgtccatcaatgacggctccacgtgtaccaga
agggctgtcttcagggtgatgagcagggcaagatgacctgtaagacaccaccttcccaggacagggcagtgagtgctgtcagggcgattgggtgaacc
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acctgacatccctggccaagctgatgaaggagtgttggtatcagaatcctagcgccagggtgaccgacctgcgcatcaagaaaactctgactaaatcg
acaatagcctggataaactgaaaaccgactgctga

2. amiR-ACVR1^{R206H}

agggctctgcgtttgctccaggtagtcgctgctccctgggcctgggcccactgacagccctgggtgccttgccggctgcacacctctggcgggcagc
tgtgtgaatctggtgagccactgtgttctggaatacctgacagtggcagatcagattacacacggaggcctgacctgactgccacgggtccgtggcc
aaaggagatctaagggcaccgctgagggcctacctaaccatcgtggggaataaggacagtgtcaccc

3. amiR-INHBA #1

ggcagccttgagtggttctgccccctgggcacacaaacagagctgaagaccacctgggcacctccttggtggcgcataacctctggcgggca
gctgtgtcttcttcttcttctgccccctgttctggtggtaccagggcaagatcaggaaagaaacacagaggcctgacctggccctcgagagactgcctga
ctgaaggccctatcaggtgggggaggggatcctgatagagggcactgctgccactgttggggcccaag

4. amiR-INHBA #2

ggcagccttgagtggttctgccccctgggcacacaaacagagctgaagaccacctgggcacctccttggtggcgcataacctctggcgggca
gctgtgtttgacctgttctctggtatgttctggtggtaccatccagagatcatagtggcaaacacagaggcctgacctggccctcgagagactgcctga
ctgaaggccctatcaggtgggggaggggatcctgatagagggcactgctgccactgttggggcccaag

5. amiR-INHBA #3

ggcagccttgagtggttctgccccctgggcacacaaacagagctgaagaccacctgggcacctccttggtggcgcataacctctggcgggca
gctgtgttcttcaagtatcttcttctgttctggtggtaccacagagagaatcctgaaggaaacacagaggcctgacctggccctcgagagactgcctga
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6. amiR-INHBA #4

ggcagccttgagtggttctgccccctgggcacacaaacagagctgaagaccacctgggcacctccttggtggcgcataacctctggcgggca
gctgtgtgatctccaggtctgtccatgttctggtggtaccatcgagcagtgcccgagatcacacagaggcctgacctggccctcgagagactgcctg
actgaaggccctatcaggtgggggaggggatcctgatagagggcactgctgccactgttggggcccaag

7. amiR-INHBA #5

ggcagccttgagtggttctgccccctgggcacacaaacagagctgaagaccacctgggcacctccttggtggcgcataacctctggcgggca
gctgtgtttgatgatgtttgacctgttctggtggtaccatgtgtcaattcgtcataaaacacagaggcctgacctggccctcgagagactgcctgact
gaaggccctatcaggtgggggaggggatcctgatagagggcactgctgccactgttggggcccaag

8. amiR-INHBA #6

Ggcagccttgagtggttctgccccctcgggcacacaaacagagctgaagaccacctgggcacctccttggtggccgcatacctcctggcgggca
gctgtgtcttcttcttcttctgccccctgttctggtggtacccagggcaagatcaggaagaaagacacagaggcctgctggccctcgagagactgcctga
ctgaaggccctatcaggtggggaggggatcctgatagaggcactgctgccactgttggggcccaag

9. miR-122 target sequences

acaaacaccattgtcacactccaacaaacaccattgtcacactccaacaaacaccattgtcacactcca

10. Sequences of qPCR primers

Gene	Forward	Reverse
<i>ACVR1^{opt}</i>	GACTACAAGCCACCCTTCTATG	ACCAGCGATTAGGGATGTTG
<i>ACVR1^{R206H}</i>	TGGTACAAAGAACAGTGGCTTA	CCATACCTGCCTTTCCCGA
<i>Egfp</i>	AGCAAAGACCCCAACGAGAA	GGCGGCGGTCACGAA
Mouse <i>Inhba</i>	GGAGATAGAGGACGACATTGGC	ACGCTCCACTACTGACAGGTCA
Mouse <i>Sox9</i>	CACACGTCAAGCGACCCATGAA	TCTTCTCGCTCTCGTTCAGCAG
Mouse <i>Id1</i>	ACATGAACGGCTGCTACTCAC	GACTTCAGACTCCGAGTTCAGC
Mouse <i>Msx2</i>	GGAAGTGGAAAAGCTGAAAATG	CACAGGTCTATGGAAGGGGTAG
Mouse <i>Tnalp</i>	CACAATATCAAGGATATCGACGTGA	ACATCAGTTCTGTTCTTCGGGTACA
Mouse <i>Osx</i>	ATGGCGTCCTCTCTGCTTGA	GAAGGGTGGGTAGTCATTTG
Mouse <i>Bglap</i>	GCAGCACAGGTCCTAAATAG	GGGCAATAAGGTAGTGAACAG
Mouse <i>Ibsp</i>	CAGGGAGGCAGTGACTCTTC	AGTGTGGAAAGTGTGGCGTT
Mouse <i>Col1α1</i>	ACTGTCCCAACCCCCAAAG	ACGTATTCTTCCGGGCAGAA
Mouse <i>Acht</i>	AGGGAAATCGTGCGTGACAT	GGGCAATAAGGTAGTGAACAG
Mouse <i>Gapdh</i>	ACAGTCCATGCCATCACTGCC	GCCTGCTTCACCACCTTCTTG
Human <i>INHBA</i>	GGATGACATTGGAAGGAGGGCA	ACTGACAGGTCACTGCCTTCCT

Figure 2A

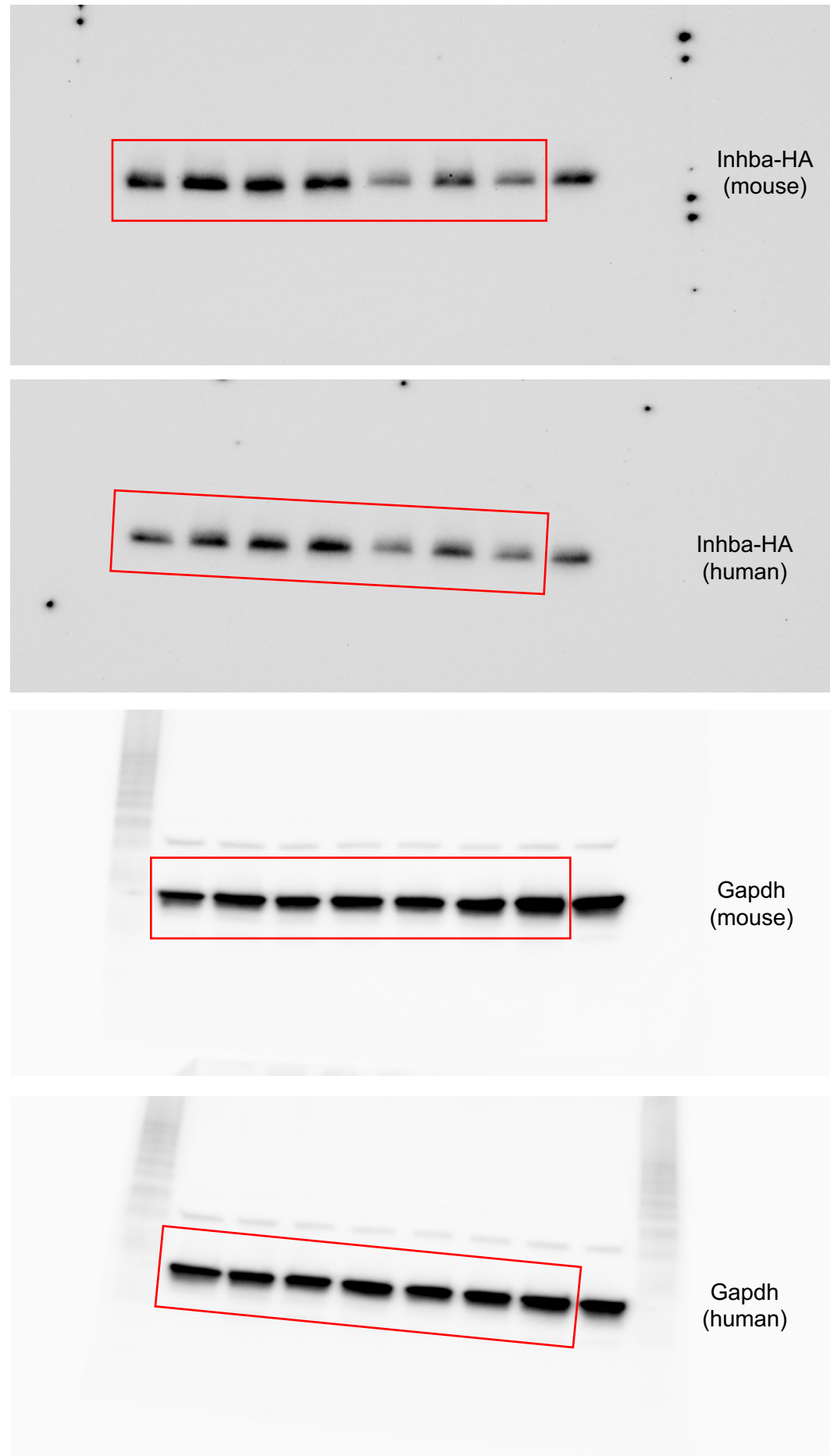


Figure S3: Original images of Figure 2A.

Figure 4G

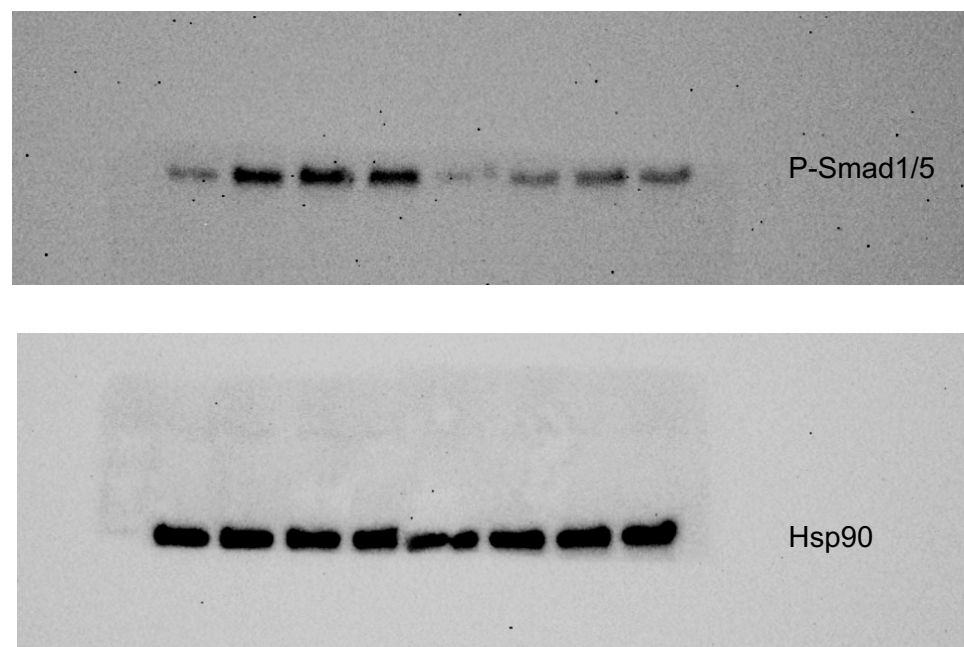


Figure S4: Original images of Figure 4G.