Fig. E-1
Fracture callus gene expression is altered by cyclooxygenase-2 (COX-2) or 5-lipoxygenase (5-LO) inhibition. Using reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR), mRNA levels for aggrecan (panel A), *Col2a1* (panel B), *Col10a1* (panel C), *Col1a2* (panel D), osteocalcin (panel E), cathepsin K (panel F), COX-2 (panel G), 5-LO (panel H), and osteopontin (panel I) were measured in fracture callus total RNA samples obtained from vehicle-treated (filled circle), celecoxib-treated (inverted triangle), and AA-861-treated (open circle) rats. The RT-qPCR data were normalized to GAPDH mRNA levels. Shown are the means (and standard error of the mean) for the normalized values at four, seven, ten, fourteen, seventeen, and twenty-one days after fracture. The normalized data were compared by two-way analysis of variance with use of time after fracture and drug treatment as the independent variables followed by Holm-Sidak tests when appropriate. Significant differences between treatment groups within each time point are indicated as A for a significant difference between AA-861-treated and vehicle-treated samples, B for a significant difference between AA-861-treated and celecoxib-treated samples, and C for a significant difference between celecoxib-treated and vehicle-treated samples.

TABLE E-1 Reverse Transcriptase-Quantitative Polymerase Chain Reaction (RT-qPCR) Primer Sets Used in This Study

Common Name	Gene	Forward Primer	Reverse Primer	Amplicon Size (bp)	Annealing Temp. (°C)
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	CCT GTG ACT TCA ACA GCA ACT CC	CCA CCA CCC TGT TGC TGT AGC C	106	52
Elongation factor-1α	Eef1a1	TGG TAA GAA GCT GGA AGA TGG	CAA GTG GAG GGT AGT CAG AGA AG	116	52-64
Aggrecan	Acan	CTT GGG CAG AAG AAA GAT CG	GGC TGA CAC CGG ATG GTG GG	101	52-64
Type II collagen	Col2a1	GGA GAA GAA GCA CAT CTG GTT TGG	TGG ACG TTA GCG GTG TTG GG	96	57
Type X collagen	Col10a1	GCG CGA TCA TGG AGC TCA CGG	TTC ATA TGG GAG CCA CTA GG	129	52-61
Type I collagen	Col1a2	GCC ATC TCG CCT GCC ATT CC	AAC AGA CAG GGC CAA CCT CC	89	52-64
Osteocalcin	Bglap	CGG CGC TAC CTC AAC AAT GG	CGC TAG CTC GTC ACA ATT GG	105	52
Cathepsin K	Ctsk	GGG CTA TGG CAC CCA GAA GG	CCA CAG GCA TTG TTC TTA TTC C	114	52-64
Cyclooxygenase-2	Ptgs2	GGA TCA TCA ACA CTG CCT CAA TTC	TGG TAG CCT GCG GGT CTT GC	96	52
5-Lipoxygenase	Alox5	CAT GAT TCG ATT CCG CAA GAA CC	GAC GGC AAA GCC TTA GAT GG	135	52-57
Osteopontin	Spp1	CAG TCA GGC GCG TTC CAA AGC	GAA ATG CGG AAT TCA GA TAC C	123	52-55

TABLE E-2A Effects of Drug Treatment and Time After Fracture on Callus Cell Proliferation Rate

	Time Point (Days After Fracture)			
	2 Days	4 Days	7 Days	10 Days
Group size				
Vehicle	6	6	6	6
Celecoxib	6	6	6	6
AA-861	4	5	4	5
Proliferation rate*				
Vehicle	2.076 ± 0.778	4.221 ± 0.954	1.051 ± 0.248	0.425 ± 0.308
Celecoxib	1.328 ± 0.611	1.841 ± 0.388	1.088 ± 0.737	0.824 ± 0.220
AA-861	3.677 ± 0.863	2.187 ± 0.296	2.020 ± 0.127	1.031 ± 0.331

*The values are given as the mean percentage of BrdU-positive cells and the standard deviation.

TABLE E-2B Two-Way Analysis of Variance: Effect of Time on Proliferation Rates within Treatment Groups*

	Holm-Sidak Test P Values		
	4 Days	7 Days	10 Days
All drug treatments			
Compared with 2 days	0.054	<0.001	<0.001
Compared with 4 days	—	<0.001	<0.001
Compared with 7 days		—	0.003
Vehicle			
Compared with 2 days	<0.001	0.003	<0.001
Compared with 4 days	—	<0.001	<0.001
Compared with 7 days		—	0.059
Celecoxib			
Compared with 2 days	0.120	0.462	0.126
Compared with 4 days	—	0.024†	0.003
Compared with 7 days		—	0.419
AA-861			
Compared with 2 days	<0.001	<0.001	<0.001
Compared with 4 days	—	0.660	0.002
Compared with 7 days		—	0.011

*$P < 0.001$ with power of 1.000 at $\alpha = 0.05$. †P value was below 0.05, but was not significant.

TABLE E-2C Two-Way Analysis of Variance: Effect of Drug Treatments on Proliferation Rates within Time Points*

	Holm-Sidak Test P Values		
	Comparison of Vehicle and Celecoxib	Comparison of Vehicle and AA-861	Comparison of Celecoxib and AA-861
All time points	<0.001	0.109	<0.001
At 2 days	0.025	<0.001	<0.001
At 4 days	<0.001	<0.001	0.313
At 7 days	0.909	0.010	0.013
At 10 days	0.223	0.080	0.545

*$P < 0.001$ with power of 1.000 at $\alpha = 0.05$.

TABLE E-3 Statistical Analysis of Fracture Callus mRNA Levels

mRNA	Two-Way Analysis of Variance P Values			Comparison of Treatment Groups Across All Time Points (Holm-Sidak Test P Values)		
	Effect of Time After Fracture	Effect of Drug Treatment	Interaction Between Time After Fracture and Drug Treatment	AA-861 and Vehicle	AA-861 and Celecoxib	Celecoxib and Vehicle
Type I Collagen	<0.001	<0.001	<0.001	0.439	<0.001	<0.001
Osteocalcin	<0.001	<0.001	<0.001	<0.001	<0.001	0.171
Cathepsin K	0.859	0.432	0.026	—	—	—
Aggrecan	0.005	0.015	0.066	0.374	0.035	0.005
Type II collagen	<0.001	<0.001	<0.001	<0.001	0.048	0.005
Type X collagen	<0.001	<0.001	<0.001	<0.001	<0.001	0.539
COX-2	0.010	0.002	0.002	<0.001	0.025	0.145
5-LO	0.002	<0.001	0.001	<0.001	<0.001	0.005
Osteopontin	0.295	0.106	<0.001	—	—	—