Supporting Information

Sugita et al. 10.1073/pnas.1419699112

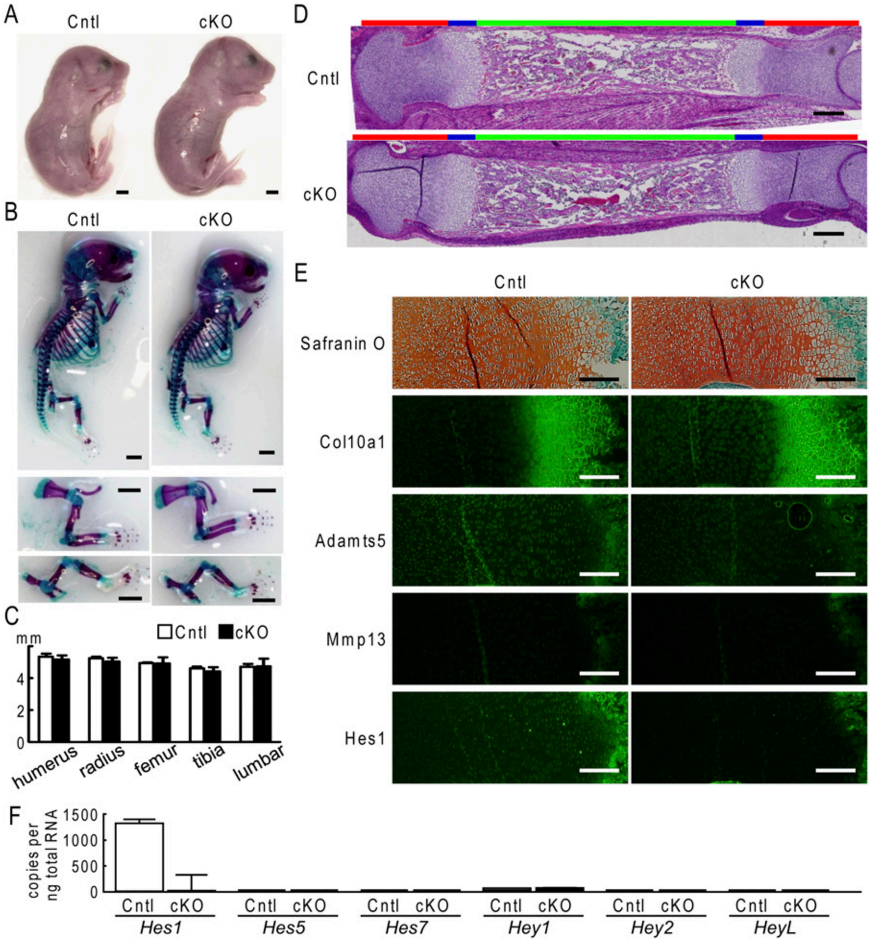

Fig. S1. Normal skeletal formation in *Sox9-Cre;Hes1*$^{fl/fl}$ mouse embryos. (*A*) Gross appearance of *Hes1*$^{fl/fl}$ (Cntl) and *Sox9-Cre;Hes1*$^{fl/fl}$ (cKO) littermate embryos (E18.5). (Scale bars, 1 mm.) (*B*) Double staining with Alizarin red and Alcian blue of the whole skeleton (*Top*), upper extremities (*Middle*), and lower extremities (*Bottom*) of Cntl and cKO littermate embryos (E18.5). (Scale bars, 1 mm.) (*C*) Length of long bones and vertebra (first to fifth lumbar spines) of Cntl and cKO littermate embryos (E18.5). Data are expressed as means ± SD of five mice per group. (*D*) H&E staining of whole tibias of Cntl and cKO littermate embryos (E18.5). (Scale bars, 200 μm.) Upper bars indicate lengths of the proliferative zone (red), hypertrophic zone (blue), and bone area (green). (*E*) Safranin-O staining and immunofluorescence with antibodies to Col10a1, Adamts5, Mmp13, and Hes1 in proximal tibias of Cntl and cKO littermate embryos (E18.5). (Scale bars, 200 μm.) (*F*) mRNA levels of Hes1, Hes5, Hes7, Hey1, Hey2 and HeyL in costal chondrocytes from Cntl and cKO littermate embryos (E18.5). Data are expressed as means ± SD of three mice per group.

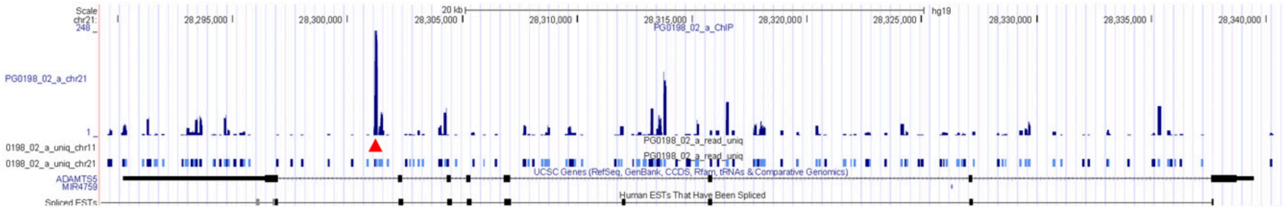

Fig. S2. ChIP-seq data from *ADAMTS5*. Genome Browser display of the *ADAMTS5* locus on chr21 from the human assembly. The University of California Santa Cruz genome browser (genome.ucsc.edu) was used to visualize these findings. The highest peak is evident between +37,134 and +37,623 bp from the transcription start site (red arrowhead).

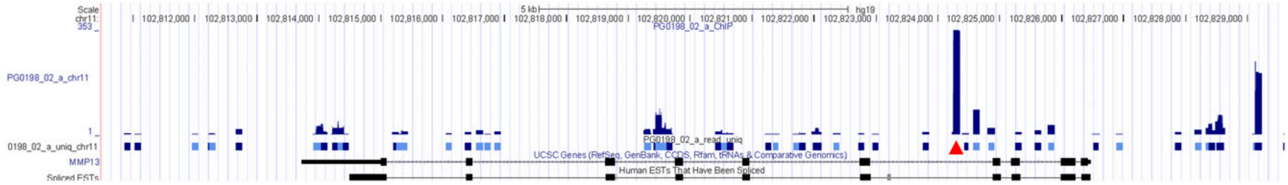

Fig. S3. ChIP-seq data from *MMP13*. Genome Browser display of the *MMP13* locus on chr11 from the human assembly. The highest peak is evident between +2,082 and +2,182 bp from the transcription start site (red arrowhead).

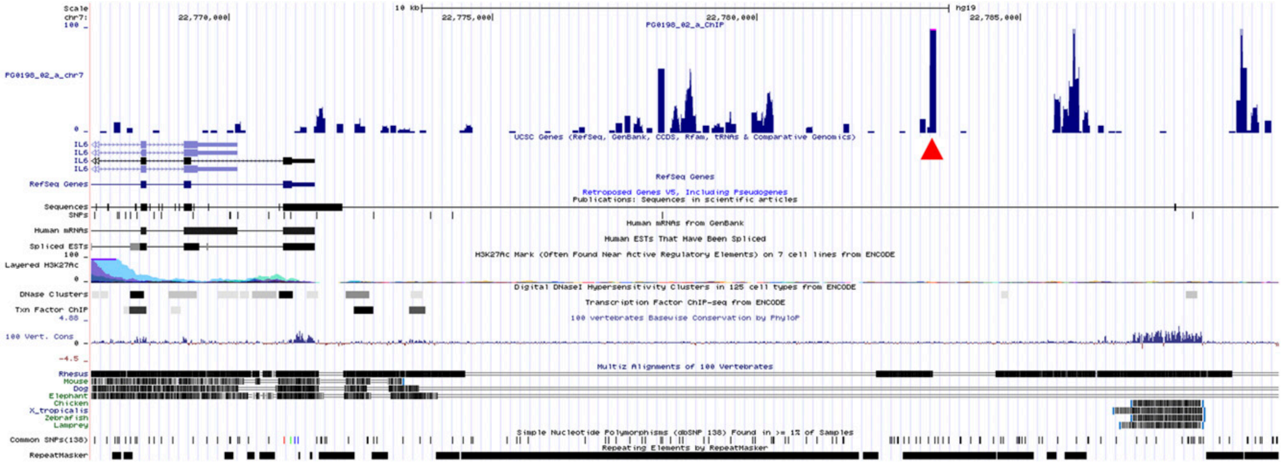

Fig. S4. ChIP-seq data from *IL6*. Genome Browser display of the *IL6* locus on chr7 from the human assembly. The highest peak is evident between +11,591 and +12,144 bp from the transcription start site (red arrowhead).

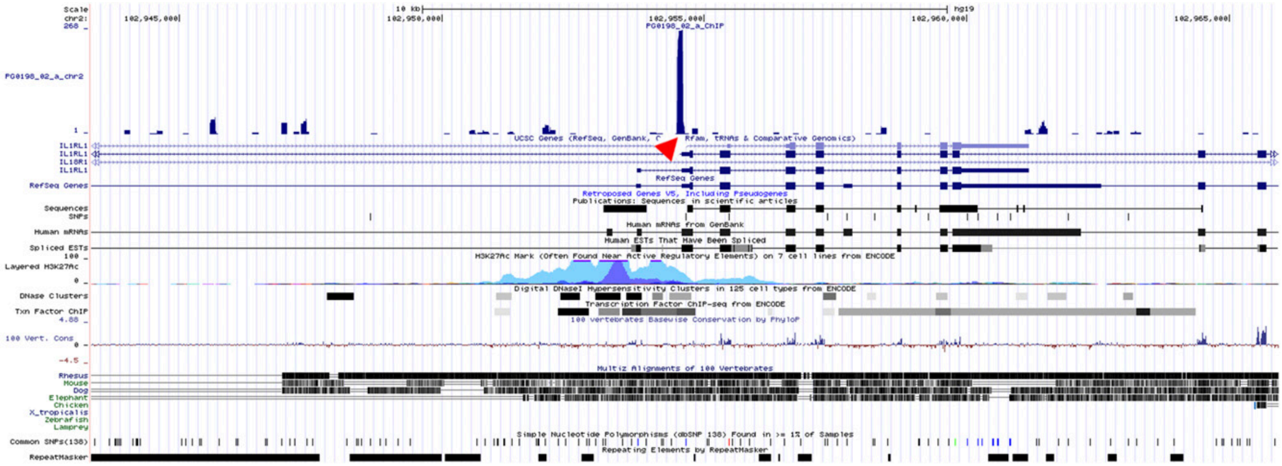

Fig. S5. ChIP-seq data from *IL1RL1*. Genome Browser display of the *IL1RL1* locus on chr2 from the human assembly. The highest peak is evident between +26,346 and +26,980 bp from the transcription start site (red arrowhead).

Table S1. Histomorphometric analyses of subchondral bones in knee joints of *Hes1*$^{fl/fl}$ (Cntl) and *Col2a1-CreERT;Hes1*$^{fl/fl}$ (cKO) littermates (8-wk-old)

Analysis	Cntl	cKO
BV/TV (%)	13.48 ± 10.0	13.48 ± 4.24
Tb.Th (μm)	39.39 ± 13.77	41.52 ± 8.16
Tb.N (mm)	3.02 ± 2.31	3.20 ± 0.50
Tb.Sp (μm)	430.24 ± 374.08	277.06 ± 60.88

Data are expressed as means ± SD of three mice per group. BV/TV, bone volume/tissue volume; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular spacing.

Table S2. List of the top 30 up-regulated genes following Hes1 overexpression

			Signal intensity		
Gene symbol	Definition	Accession no.	GFP	HES1	Fold-increase
Oas2	2′-5′ oligoadenylate synthetase 2	NM_145227	16.5	79805.3	4846.250044
Mx1	Myxovirus (influenza virus) resistance 1	NM_010846	12.6	50459.2	4003.665635
Oas1a	2′-5′ oligoadenylate synthetase 1A	NM_145211	118.0	232615.2	1971.11468
Zbp1	Z-DNA binding protein 1	NM_021394	10.4	19954.0	1927.793365
Vgf	VGF nerve growth factor inducible	NM_001039385	14.5	25513.1	1762.647099
Csprs	Component of Sp100-rs	NM_033616	12.6	18594.3	1480.388952
Trim30a	Tripartite motif-containing 30A	NM_009099	9.2	13041.5	1418.507871
Ifi44l	IFN-induced protein 44 like	NM_031367	9.5	10132.8	1067.758234
Gvin1	GTPase, very large IFN inducible 1	NM_029000	9.6	9953.5	1039.052
Oas1f	2′-5′ oligoadenylate synthetase 1F	NM_145153	124.4	95565.8	768.4231532
Trim30d	Tripartite motif-containing 30D	NM_199146	10.1	7059.6	696.8601153
Gbp6	Guanylate binding protein 6	NM_194336	16.1	9892.7	613.1444228
Mnda	Myeloid cell nuclear differentiation antigen	NM_001033450	22.9	13997.1	611.3529946
Iigp1	IFN inducible GTPase 1	NM_001146275	15.2	8867.6	583.0995978
Mx2	Myxovirus (influenza virus) resistance 2	NM_013606	231.4	118732.2	513.206726
Phf11d	PHD finger protein 11D	NM_199015	72.7	36834.3	506.6135702
Rsad2	Radical S-adenosyl methionine domain containing 2	NM_021384	57.0	28424.6	498.6956843
Phf11a	PHD finger protein 11A	NM_172603	38.1	18836.5	495.0330322
Irf7	IFN regulatory factor 7	NM_016850	52.8	25160.6	476.8225082
Oasl1	2′-5′ oligoadenylate synthetase-like 1	NM_145209	169.4	61313.4	361.9340508
Tgtp2	T-cell specific GTPase 2	NM_001145164	509.2	154260.6	302.9477354
Cmpk2	Cytidine monophosphate (UMP-CMP) kinase 2	NM_020557	18.6	5357.3	288.6413947
Oasl2	2′-5′ oligoadenylate synthetase-like 2	NM_011854	553.0	146729.1	265.3257035
Cxcl10	Chemokine (C-X-C motif) ligand 10	NM_021274	1035.1	262780.8	253.8728529
Il6	Interleukin 6	NM_031168	88.2	21147.6	239.7370111
Usp18	Ubiquitin specific peptidase 18	NM_011909	493.4	118274.2	239.7165883
Npy1r	Neuropeptide Y receptor Y1	NM_010934	30.2	7122.4	235.5499841
Vmn1r65	Vomeronasal 1 receptor 65	NM_030738	8.0	1814.6	226.1623612
Gbp5	Guanylate binding protein 5	NM_153564	41.1	9245.1	225.058535
Phf11b	PHD finger protein 11B	NM_001164327	78.3	17561.8	224.247971

Table S3. List of top 30 down-regulated genes following Hes1 overexpression

Gene symbol	Definition	Accession no.	Signal intensity		Fold-increase
			GFP	HES1	
Pde3a	Phosphodiesterase 3A, cGMP inhibited	NM_018779	4710.7	23.8	0.005051014
Zdhhc24	Zinc finger, DHHC domain containing 24	NM_027476	27404.3	726.6	0.026515558
Klra8	Killer cell lectin-like receptor, subfamily A, member 8	NM_010650	306.6	8.7	0.028504689
Olfr677	Olfactory receptor 677	NM_146358	274.3	8.8	0.032230603
Madcam1	Mucosal vascular addressin cell adhesion molecule 1	NM_013591	1500.2	58.3	0.038869904
Plaa	Phospholipase A2, activating protein	NM_172695	249.4	10.1	0.040485766
Naa11	N(α)-acetyltransferase 11, NatA catalytic subunit	NM_001033191	321.3	15.1	0.047056364
Ahi1	Abelson helper integration site 1	NM_026203	6171.1	297.5	0.048214357
Shh	Sonic hedgehog	NM_009170	171.0	9.4	0.054837432
Lrrc6	Leucine rich repeat containing 6	NM_019457	473.5	27.1	0.057170221
Nmur1	Neuromedin U receptor 1	NM_010341	220.1	12.9	0.058800034
Olfr1240	Olfactory receptor 1240	NM_146808	173.8	10.6	0.060909518
Olfr374	Olfactory receptor 374	NM_146338	141.3	9.4	0.066199534
Aspm	Asp (abnormal spindle)-like, microcephaly associated	NM_009791	24063.5	1635.3	0.067959686
Scn3a	Sodium channel, voltage-gated, type III, alpha	NM_018732	397.4	27.4	0.069046182
Mageb4	Melanoma antigen, family B, 4	NM_001033492	136.1	9.9	0.072406481
Ikzf1	IKAROS family zinc finger 1 (Ikzf1)	NM_001025597	136.7	10.3	0.075601771
Olfr761	Olfactory receptor 761	NM_001011829	110.8	8.5	0.076600134
Kmo	Kynurenine 3-monooxygenase	NM_133809	416.3	32.5	0.077960937
Olfr1255	Olfactory receptor 1255	NM_146977	128.6	10.2	0.07956938
Prkcz	Protein kinase C, ζ	NM_008860	113.5	9.9	0.087353479
Lcn12	Lipocalin 12	NM_029958	271.1	23.8	0.087707076
Ace3	Angiotensin I converting enzyme	NM_001101453	119.1	10.7	0.089583886
Trim55	Tripartite motif-containing 55	NM_001081281	304.9	27.9	0.091456105
Fam71b	Family with sequence similarity 71, member B	NM_001013783	120.7	11.2	0.093117221
Rac2	RAS-related C3 botulinum substrate 2	NM_009008	236.1	22.3	0.094641156
Olfr930	Olfactory receptor 930	NM_146272	102.1	10.1	0.098727764
H60a	Histocompatibility 60a	NM_010400	6911.4	693.2	0.100298079
Palm3	Paralemmin 3	NM_028877	136.1	14.5	0.106207869
Flg2	Filaggrin family member 2	NM_001013804	83.7	9.1	0.108836368

Table S4. List of mechanical stress-related genes up-regulated by Hes1 overexpression

Gene symbol	Definition	Accession no.	Signal intensity		Fold-increase
			GFP	HES1	
P2rx7	Purinergic receptor P2X, ligand-gated ion channel, 7	NM_001038887	208.4750897	4600.075202	22.06534703
Stat1	Signal transducer and activator of transcription 1	NM_009283	35.83590909	608.0865616	16.9686378
Lck	Lymphocyte protein tyrosine kinase	NM_010693	674.5735014	10477.8277	15.53252193
Pde2a	Phosphodiesterase 2A, cGMP-stimulated	NM_001143848	228.6839863	1734.16365	7.583231683
Ankrd1	Ankyrin repeat domain 1	NM_013468	1385.022269	8879.072285	6.410779441

Table S5. List of inflammation-related genes up-regulated by Hes1 overexpression

Gene symbol	Definition	Accession no.	Signal intensity		Fold-increase
			GFP	HES1	
Cxcl10	Chemokine (C-X-C motif) ligand 10	NM_021274	1035.088088	262780.766	253.8728529
Il6	Interleukin 6	NM_031168	88.21168017	21147.60455	239.7370111
Cxcl9	Chemokine (C-X-C motif) ligand 9	NM_008599	9.064536627	1486.693037	164.0120282
Apod	Apolipoprotein D	NM_007470	190.966717	24524.48761	128.4228372
Tlr3	Toll-like receptor 3	NM_126166	79.9117321	4130.837039	51.69249784
Ccl2	Chemokine (C-C motif) ligand 2	NM_011333	713.3851706	32362.18847	45.36425735
Ccl7	Chemokine (C-C motif) ligand 7	NM_013654	335.6995956	11257.86982	33.53554775
P2rx7	Purinergic receptor P2X, ligand-gated ion channel, 7	NM_001038887	208.4750897	4600.075202	22.06534703
Ccrl2	Chemokine (C-C motif) receptor-like 2	NM_017466	68.4913974	1301.218906	18.99828235
Ifng	IFN-γ	NM_008337	11.19467225	187.3166384	16.73265944
Naip6	NLR family, apoptosis inhibitory protein 6	NM_010871	8.477237109	139.4267096	16.44718766
Cd40	CD40 antigen	NM_011611	10.03434907	151.6106737	15.10916878
Zfp36	Zinc finger protein 36	NM_011756	13.95101956	206.4052363	14.79499297
Vnn1	Vanin 1	NM_011704	15.8489333	203.577347	12.84486112
Ccl8	Chemokine (C-C motif) ligand 8	NM_021443	58.99512447	693.6893927	11.758419
Trp73	Transformation related protein 73	NM_011642	24.96404864	258.3631958	10.34941085
S100a8	S100 calcium binding protein A8	NM_013650	16.5642753	169.4070307	10.22725279
Serpinb9	Serine (or cysteine) peptidase inhibitor, clade B, member 9	NM_009256	244.1270736	2282.207769	9.348441923
Ahsg	α-2-HS-glycoprotein	NM_013465	10.07827856	91.8978879	9.118411182
Hmox1	Heme oxygenase (decycling) 1	NM_010442	3628.910479	30898.0466	8.514414111
Naip2	NLR family, apoptosis inhibitory protein 2	NM_010872	22.8900897	192.0525437	8.390204939
Ccl5	Chemokine (C-C motif) ligand 5	NM_013653	10502.19276	84944.65226	8.088277773
Il1rl1	Interleukin 1 receptor-like 1	NM_010743	686.0549997	5483.020086	7.992099887
Pde2a	Phosphodiesterase 2A, cGMP-stimulated	NM_001143848	228.6839863	1734.16365	7.583231683
Ccl12	Chemokine (C-C motif) ligand 12	NM_011331	10.47869892	58.89732639	5.62067169
Ccl3	Chemokine (C-C motif) ligand 3	NM_011337	9.652403763	53.54774776	5.547607527
Tnf	Tumor necrosis factor	NM_013693	8.627355831	47.02896662	5.451144886
Klkb1	Kallikrein B, plasma 1	NM_008455	24.65734731	132.0032586	5.353506072
Ccl4	Chemokine (C-C motif) ligand 4	NM_013652	10.64395506	56.56105382	5.313913249
Tnfaip3	Tumor necrosis factor, alpha-induced protein 3	NM_009397	1400.149691	7259.254592	5.184627498
Cxcl13	Chemokine (C-X-C motif) ligand 13	NM_018866	21.09724589	104.6028322	4.958127367
Nr1h3	Nuclear receptor subfamily 1, group H, member 3	NM_013839	125.2198918	593.848623	4.742446384
Nupr1	Nuclear protein transcription regulator 1	NM_019738	11321.89806	52647.51153	4.65006055
Itgb6	Integrin beta 6 (Itgb6), transcript variant 2	NM_021359	9.408618128	42.78180918	4.547087425
Hyal1	Hyaluronoglucosaminidase 1	NM_008317	36.95654689	164.2328202	4.443943876
Pla2g7	Phospholipase A2, group VII	NM_013737	247.9056351	1087.677442	4.387465583
Il17f	Interleukin 17F	NM_145856	25.30738442	108.8969345	4.302970734
Kl	Klotho	NM_013823	9.212208226	38.68663948	4.199496855
Adora2b	Adenosine A2b receptor	NM_007413	20.32735765	84.9084311	4.177052057
Il33	Interleukin 33	NM_001164724	39.96758039	161.5887215	4.042994845