

SUPPLEMENTAL MATERIALS

I. Supplemental Table 1. Gene List for dChip Clustering Analysis

II. Supplemental Table 2. Primers Used for Hey1 Expression Cloning, siRNAs, qPCR and ChIP Analysis.

III. Supplemental Fig. 1.

Suppl. Fig. 1. Hey1 acts synergistically with BMP9 in osteogenic differentiation and the Hey1 knockdown phenotype can be rescued by Runx2. (A) Overexpression of Hey1 potentiates BMP9-induced ALP activity. Subconfluent cells were infected with AdBMP9, AdGFP, and/or AdHey1. ALP activity was determined at the indicated time points. (B) Histologic evidence of the Runx2 rescue effect on the Hey1^{KD} MSCs. Magnification, 100x. (C) MicroCT images (slices) of ectopic bone formation *in vivo*. The MSC implantation experiments were carried out as described in **Fig. 5A** and **Fig. 5C**. (D) Adenovirusmediated Hey1 expression enhanced bone matrix mineralization in MEFs. The *in vivo* experiments and the histologic analysis were carried out as described in **Fig. 5C**.

Supplemental Table 1. Genes whose expression was significantly regulated by BMP9 in mesenchymal stem cells (> 2-fold, p < 0.05)

Gene Description	Accession No.	GFP Control					BMP-9					Fold change	P-value
		C1	C2	C3	Mean	SE	B1	B2	B3	Mean	SE		
growth hormone	BB026315	133.8	119.6	121.7	124.0	12.9	1005.68	672.3	753.9	810.3	99.9	6.53	0.0191
Hey1	NM_010423	447.3	436.0	422.8	435.1	46.3	2421.98	2953.5	1509.4	2290.8	431.3	5.26	0.0485
EST	AK017880	550.3	582.0	354.8	496.4	73.4	3135.6	1893.9	2144.9	2391.5	377.6	4.82	0.0335
PGS2	M88242	289.3	175.9	62.3	175.9	65.6	869.87	620.8	979.9	823.8	105.9	4.68	0.0105
EST	NM_133874	387.5	423.8	430.9	412.2	64.6	2409.22	1814.0	1574.3	1920.5	254.0	4.66	0.0217
EST	BM220945	334.2	378.3	321.5	346.7	22.6	1678.45	1566.1	1434.5	1558.7	72.7	4.5	0.0018
MLLT7	AB032770	320.4	262.0	212.2	261.8	50.9	1173.71	989.2	1135.9	1093.3	67.1	4.18	0.0008
Smad7	BF226166	360.1	559.3	458.0	458.7	73.5	1727	1874.1	1999.1	1876.2	94.5	4.09	0.0004
Serpine1	NM_008871	784.8	1832.3	795.0	1135.4	359.7	3858.56	4516.0	5154.7	4505.4	394.5	3.97	0.0033
fibromodulin	BB235530	366.1	596.2	1237.5	732.8	261.8	3240.93	2921.8	2486.4	2876.1	224.0	3.93	0.0037
Bcat1	X17502	458.0	765.1	736.1	652.2	102.6	2369.32	2133.0	3025.5	2509.7	267.3	3.85	0.0117
calpain 1	BC026138	762.4	667.7	705.9	710.9	131.9	2851.01	2340.6	2578.4	2575.3	180.8	3.62	0.0017
MMP11	NM_008606	308.7	237.8	425.9	321.5	57.3	1228.92	1376.3	794.8	1133.1	176.0	3.52	0.0338
transthyretin	BC024702	358.1	304.1	326.9	327.7	73.8	1274.55	1103.6	891.8	1073.1	136.0	3.27	0.0160
EST	BM231053	1414.0	1692.6	1436.5	1516.8	104.0	5511.26	4761.6	4127.1	4802.7	409.8	3.17	0.0113
Smad6	AF010133	1248.0	1780.8	1269.9	1449.5	204.1	4777.73	4553.6	4328.7	4515.3	197.5	3.12	0.0004
importin 4a	NM_024267	816.1	859.0	969.4	873.0	163.6	3221.42	2772.5	2318.3	2724.0	325.7	3.12	0.0154
Kruppel-like factor 10	NM_013692	292.7	418.5	237.3	316.6	56.2	1036.56	955.3	867.6	949.2	54.7	3	0.0013
C1qtnf3	NM_030888	98.3	82.3	103.6	94.0	11.9	330.27	250.7	265.9	279.6	26.6	2.98	0.0098
Tsta3	NM_031201	962.3	913.2	909.8	930.0	78.7	3045.09	2658.4	2432.7	2705.9	198.7	2.91	0.0060
Kcnk5	BI107337	230.8	212.1	182.1	208.5	21.7	677.14	573.9	531.9	590.2	47.1	2.83	0.0064
EST	BC022622	373.2	340.0	383.3	363.8	47.2	1199.55	989.8	939.6	1027.2	97.3	2.82	0.0097
EST	BF578055	530.8	554.0	632.1	571.6	50.9	1869.8	1602.5	1325.5	1589.7	172.8	2.78	0.0206
Hspa5	AJ002387	1929.3	2286.7	1839.1	2022.5	165.3	6142.37	5185.3	5255.7	5525.4	310.8	2.73	0.0020
Rassf5	NM_018750	116.5	151.7	147.7	141.5	17.8	428.83	392.1	299.4	372.9	39.7	2.64	0.0158
lunatic fringe homolog	AK004642	269.7	332.6	292.9	301.9	31.4	808.19	805.7	738.6	782.4	34.0	2.59	0.0005
EST	AK009669	1036.0	1026.7	899.2	1005.5	69.5	2518.71	2319.6	2746.1	2536.1	127.0	2.52	0.0016
EST	BC024445	348.8	475.3	373.1	400.1	49.8	939.93	964.5	1001.8	973.0	32.9	2.43	0.0013
Limk1	NM_010717	163.1	164.7	170.2	166.2	8.5	404.14	337.4	422.8	387.6	27.1	2.33	0.0094
Tmeff1	BM114154	293.0	317.8	292.0	300.9	13.6	671.85	685.0	671.4	673.9	11.7	2.24	0.0000
EST	AV174022	1902.5	1722.3	1540.0	1743.6	121.7	714.36	621.2	678.2	673.4	30.0	-2.59	0.0094
pleiotrophin	BF178348	17512.1	12745.4	13816.3	14669.7	1456.1	4860.16	5007.2	5766.8	5217.2	472.6	-2.81	0.0156
laminin B1 subunit 1	M15525	231.4	350.6	244.2	276.9	40.2	95.91	89.8	105.2	96.2	6.9	-2.88	0.0426
MME	NM_008604	2093.5	2087.2	1800.9	1997.5	99.8	696.07	660.1	679.1	679.3	17.3	-2.94	0.0047
EST	BB008324	2078.5	1625.2	1507.5	1740.3	176.6	662.22	686.0	416.0	588.6	88.2	-2.96	0.0106
EST	NM_134072	398.6	453.0	545.8	465.5	44.5	154.61	143.8	168.4	156.5	8.5	-2.97	0.0173
Synaptopodin	BB426294	930.7	1168.5	1227.0	1106.5	98.0	389.16	459.1	252.4	366.7	62.2	-3.02	0.0054
GSTM1	NM_010358	4579.9	4361.6	2984.7	3988.1	507.2	1022.37	1188.6	1164.8	1130.8	71.4	-3.53	0.0281
EST	BC028307	867.1	740.7	901.3	834.5	51.5	222.07	193.4	228.7	214.5	20.0	-3.89	0.0029
Plscr1	NM_008880	612.6	741.7	509.9	621.8	69.2	160.44	201.6	114.3	157.4	27.3	-3.95	0.0124

Supplemental Table 2. Oligonucleotide Primer Sequences

Use	Sequence
simHey1 site-1 forward	5'-aCGACGAGACCGAATCAATAtttt-3'
simHey1 site-1 reverse	5'-aTATTGATTCCGGTCTCGTCGtttt-3'
simHey1 site-2 forward	5'-aGGACGAGAATGGAAACTTGtttt-3'
simHey1 site-2 reverse	5'-aCAAGTTTCCATTCTCGTCctttt-3'
simHey1 site-3 forward	5'-aCCATCTCAACAACCTACGCAtttt-3'
simHey1 site-3 reverse	5'-aTGCGTAGTTGTTGAGATGGtttt-3'
mouse Hey1 CDS qPCR forward	5'-CGCCCTGGCTATGGACTA-3'
mouse Hey1 CDS qPCR reverse	5'-GAGACCAGGCGAACGAGA-3'
mouse Hey1 qPCR forward	5'-GGCCTGCTTGGCTTTTCT-3'
mouse Hey1 qPCR reverse	5'-CCAAGTGCAGGCAAGGTC-3'
Adenoviral Hey1 CDS forward	5'-cgcgtcgaccatgggcATGAAGAGAGCTCACCCAGACTAC-3'
Adenoviral Hey1 CDS reverse	5'-TGCTCTAGAttAGAAAGCTCCGATCTCTGTCCCCCAAG-3'
Retroviral Hey1 CDS forward	5'-CCCAAGCTTGCATGAAGAGAGCTCACCCAGACTAC-3'
Retroviral Hey1 CDS reverse	5'-ccgacgcgtGAAAGCTCCGATCTCTGTCCCCCAAG-3'
ChIP PP-1 forward	5'-GCCTTGCAGGATTAGCTTTAGA-3'
ChIP PP-1 reverse	5'-GGTGTGAACCTGTAATCGGAGT-3'
ChIP PP-2 forward	5'-ACCTTTTCAGAGGAGCTGTGAG-3'
ChIP PP-2 reverse	5'-TCCCGTCTATGAAGCAAACTT-3'
ChIP PP-3 forward	5'-CATGCAGAAGACACTTTGGAAG-3'
ChIP PP-3 reverse	5'-GAGGCAAAATTTTCATGAGTTCC-3'

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