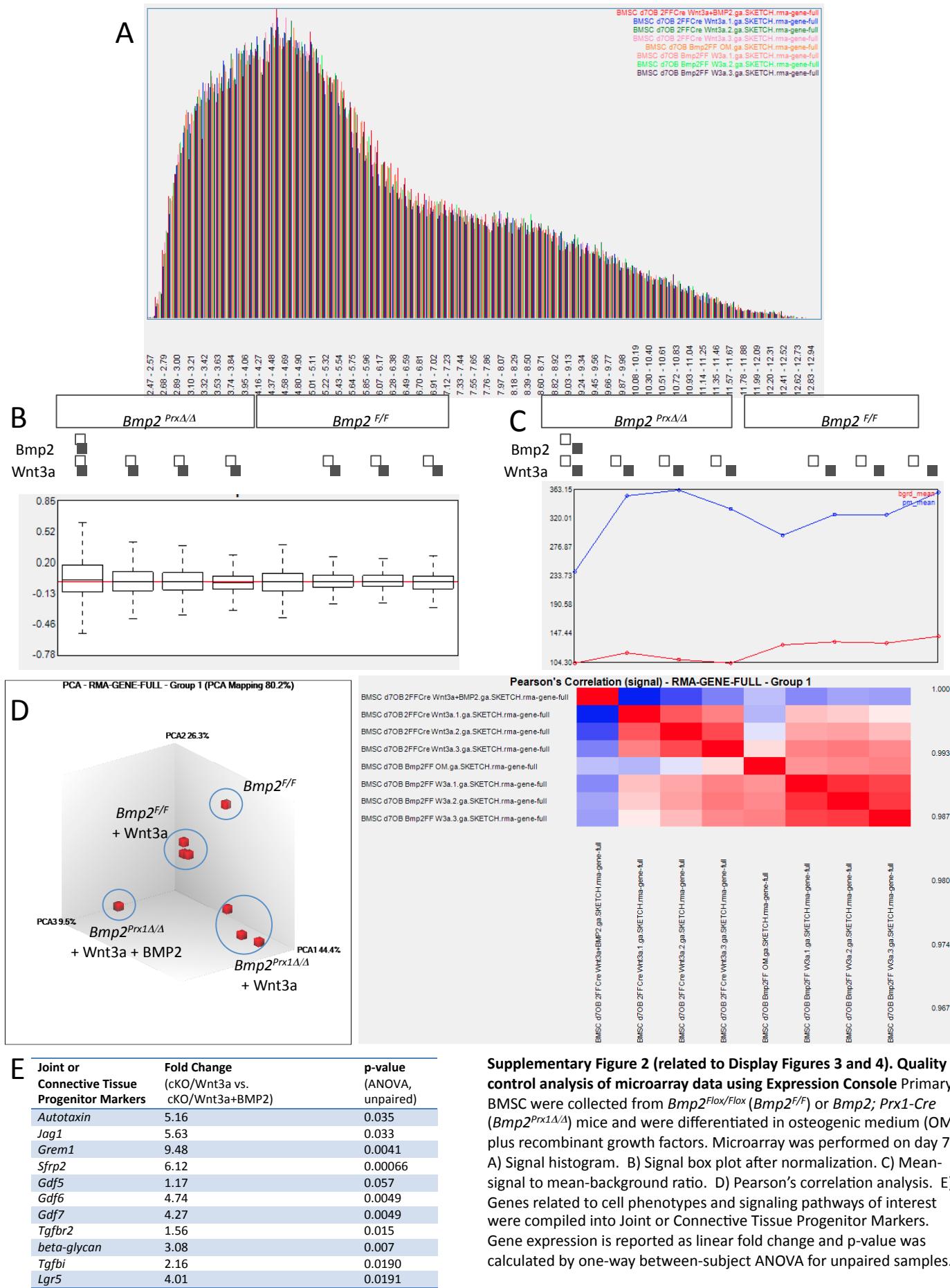
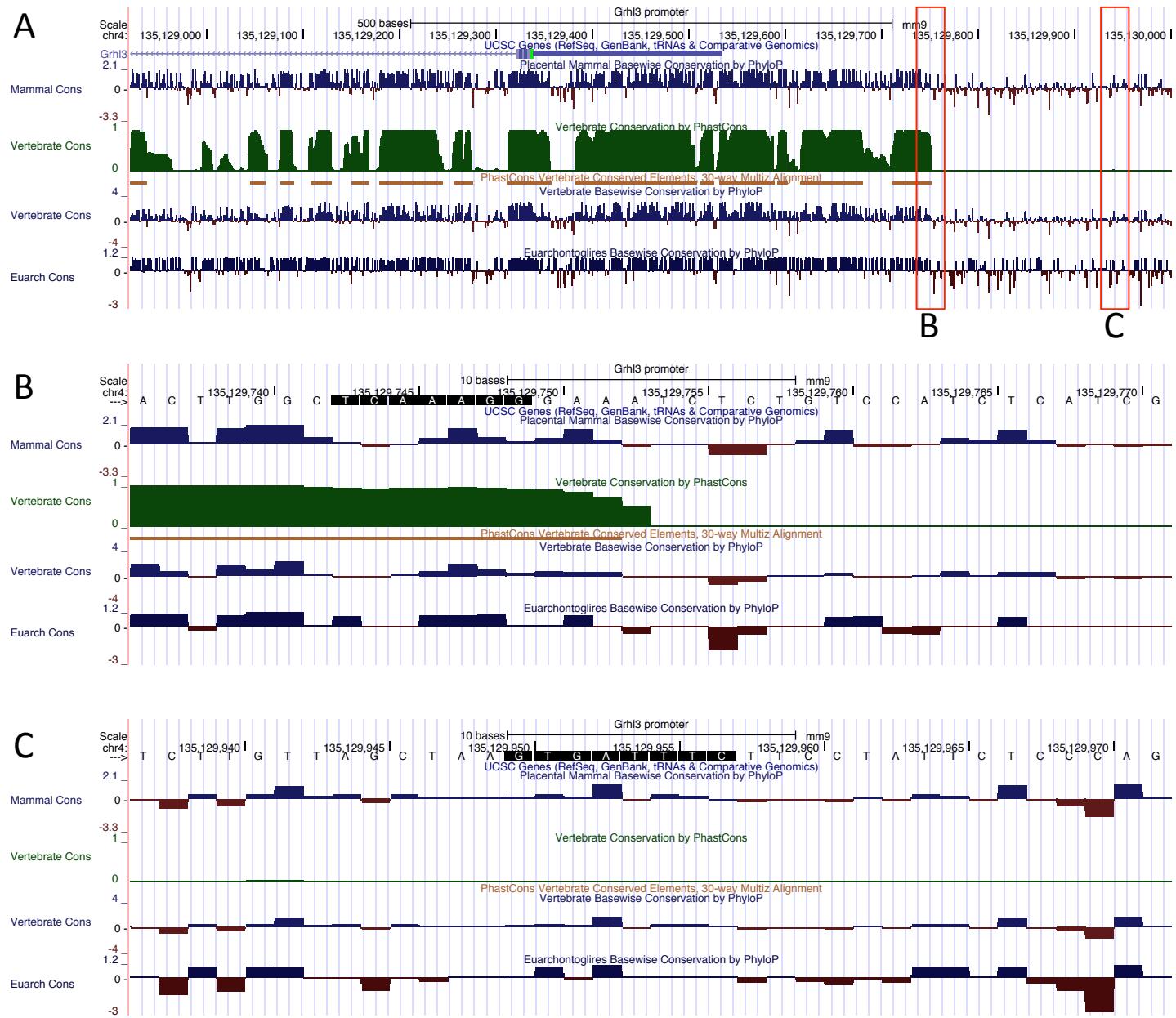


Supplemental Figure 1 (related to Display Figures 1-2). *Bmp2*-deficient cells express essential signaling molecules of the canonical Wnt pathway and are able to activate Tcf/Lef-dependent transcription. A-B) Primary BMSC from *Bmp2^{F/F}* or *Bmp2^{Prx1Δ/Δ}* mice were differentiated in osteogenic medium (OM) plus recombinant growth factors. A) SDS-page electrophoresis and immunoblot analysis was performed on total protein harvested on day 7. B) QPCR analysis was performed on RNA harvested on day 7. C) Immortalized mouse limb bud cells from E13.5 embryos (MLB13 cells) were transfected with plasmid-based RNAi to establish a clonal population of cells with stable knockdown of *Bmp2*. QPCR analysis was performed on RNA from cells treated with 20 ng/mL Wnt3a for 24 h. Data are expressed as fold change relative to control MLB13 cells. p* <0.05 vs. control MLB13 cells grown in osteogenic medium.



Supplementary Figure 2 (related to Display Figures 3 and 4). Quality control analysis of microarray data using Expression Console Primary BMSC were collected from *Bmp2*^{Flux/Flux} (*Bmp2*^{F/F}) or *Bmp2*; *Prx1-Cre* (*Bmp2*^{Prx1A/A}) mice and were differentiated in osteogenic medium (OM) plus recombinant growth factors. Microarray was performed on day 7. A) Signal histogram. B) Signal box plot after normalization. C) Mean-signal to mean-background ratio. D) Pearson's correlation analysis. E) Genes related to cell phenotypes and signaling pathways of interest were compiled into Joint or Connective Tissue Progenitor Markers. Gene expression is reported as linear fold change and p-value was calculated by one-way between-subject ANOVA for unpaired samples.



Supplemental Figure 3 (related to Display Figure 5). The proximal 5' genomic fragment of the murine Grainyhead-like 3 (*Grhl3*) promoter encodes two previously established TCF/Lef binding motifs. The UCSC Genome Brower at <http://genome.ucsc.edu> was used to examine the murine *Grhl3* locus. A) Panoramic genomic view of the 5' region surrounding the murine locus of *Grhl3*. B) TCF/Lef binding site 1 at mouse chromosome 4: 135,129,743-135,129,749. C) TCF/Lef binding site 2 at mouse chromosome 4: 135,129,950-135,129,957.

TTEST group 1	TTEST group 2	Grlh2	Grlh3	Runx2	Osx1	Dlx3	Dlx5	Col1A1	Alp1	Phospho1	Car3	Axin2	Prx1	Car12
Control/OM	Control/BMP2	0.0071	0.9099	0.0003	0.0001	0.0000	0.0002	0.0002	0.0001	0.9559	0.0878	0.0031	0.0000	
Control/OM	Control/Wnt3a	0.0188	0.0152	0.1656	0.0085	0.7611	0.2787	0.0093	0.1353	0.8926	0.0001	0.0673	0.0009	
Control/OM	Control/BMP2/Wnt3a	0.0358	0.0004	0.0001	0.0000	0.0000	0.0001	0.0006	0.0030	0.0001	0.0005	0.0001	0.0002	0.0027
Control/OM	G2/OM	0.1560	0.0206	0.0459	0.0963	0.4471	0.8421	0.0181	0.0480	0.1383	0.5047	0.1907	0.1143	0.3889
Control/BMP2	G2/BMP2	0.0051	0.0005	0.5519	0.0019	0.4153	0.6651	0.5156	0.0031	0.8480	0.0723	0.1013	0.0663	0.9542
Control/Wnt3a	G2/Wnt3a	0.0004	0.0015	0.8368	0.2461	0.8846	0.2957	0.0554	0.0036	0.5822	0.0000	0.0173	0.1501	0.9886
Control/BMP2/Wnt3a	G2/BMP2/Wnt3a	0.0130	0.0001	0.0002	0.0001	0.0003	0.0027	0.0026	0.0067	0.0005	0.0369	0.0461	0.0002	0.0018
Control/OM	G3/OM	0.1428	0.0079	0.2160	0.0031	0.0771	0.2032	0.0000	0.0091	0.0021	0.5723	0.0529	0.1030	0.0000
Control/BMP2	G3/BMP2	0.1276	0.0002	0.0206	0.0036	0.0007	0.0121	0.0097	0.0033	0.0026	0.2371	0.0236	0.0098	0.0001
Control/Wnt3a	G3/Wnt3a	0.0024	0.0046	0.1551	0.7696	0.0659	0.7975	0.3140	0.0250	0.0410	0.0000	0.0049	0.2394	0.0010
Control/BMP2/Wnt3a	G3/BMP2/Wnt3a	0.4011	0.0000	0.0101	0.0010	0.0009	0.0303	0.0719	0.0241	0.0029	0.1856	0.0020	0.0040	0.0001
Control/OM	G2G3/OM	0.0817	0.0382	0.0686	0.8110	0.0232	0.0081	0.0546	0.4607	0.1249	0.0768	0.0110	0.0029	0.2537
Control/BMP2	G2G3/BMP2	0.0076	0.0002	0.0290	0.0014	0.2511	0.7729	0.0367	0.0054	0.7614	0.6158	0.1129	0.0106	0.0029
Control/Wnt3a	G2G3/Wnt3a	0.0016	0.0008	0.1164	0.1871	0.0825	0.4804	0.0186	0.0510	0.0521	0.0014	0.1461	0.3436	0.4794
Control/BMP2/Wnt3a	G2G3/BMP2/Wnt3a	0.0188	0.0000	0.0004	0.0000	0.0004	0.0034	0.0017	0.0081	0.0027	0.7102	0.0021	0.0002	0.0005

Table S1 (related to Display Figure 6). v rainyheadk like 3 (Grlh3) is a novel transcription factor that acts upstream of Osx1 during osteoblast differentiation. Ae Statistical analysis of QPCR analysis on MLB13^{WT} cells, transfected with siRNA targeting Grlh2, Grlh3, or Grlh2 and Grlh3.