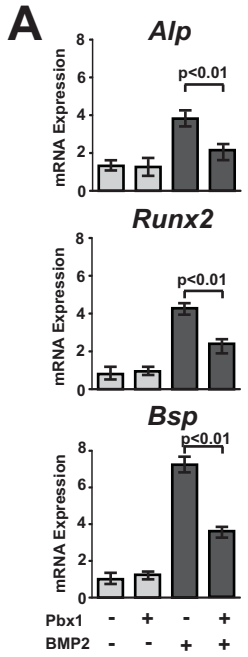

FIG. S1: **Overexpression of Pbx1 results in decreased expression of osteoblast-related genes in mesenchymal progenitor C2C12 cells.** C2C12 cells were infected with 100 p.f.u./cell of recombinant lentivirus encoding for Pbx1 or empty vector. Cells were then treated with BMP2 or vehicle for 5 days and total RNA was isolated from infected cells and relative expression of osteoblast-related genes was determined by quantitative PCR using gene-specific primers. Statistical significance was determined by one-way ANOVA followed by a Bonferroni post-test. Data are presented as the mean of 3 experiments ± S.E.M.

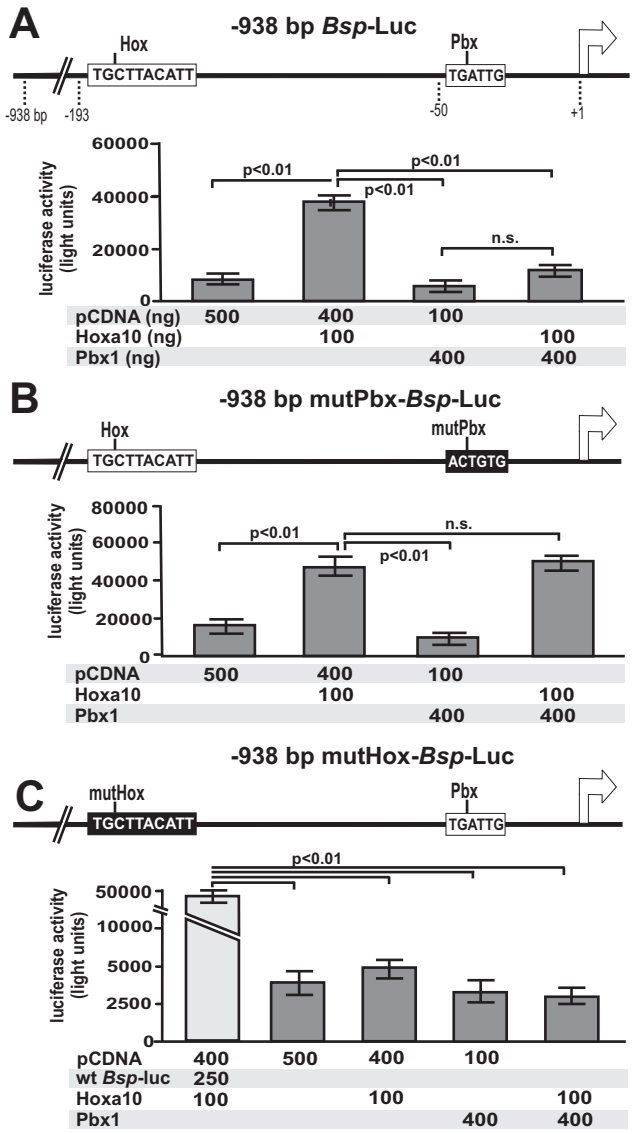

FIG. S2: **Alteration of Pbx1 consensus sequence in the *bone sialoprotein* promoter results in decreased attenuation of Hoxa10 activity.** A) – C) MC3T3E1 preosteoblasts were transfected with 500 ng of indicated luciferase reporter plasmids as well as indicated amounts (ng) of pCDNA3, pCDNA-Hoxa10 and/or pCDNA-Pbx1 and relative luciferase activity was assessed by luminometer. A) Luciferase reporter construct (pGL3-Basic) containing the -938 bp region upstream of the *Bsp* gene transcriptional start site (-938kb *Bsp*-Luc). Hoxa10-mediate luciferase activity was significantly decreased upon co-transfection of Pbx1. The -938 bp *Bsp*-Luc plasmid was modified by site-directed mutagenesis to alter the putative Pbx1 DNA consensus sequence (- 938 bp mutPbx-*Bsp*-Luc) or the Hox DNA consensus sequence (-938 bp mutHox-*Bsp*-Luc). B) Alteration of the Pbx1 DNA consensus sequence resulted in loss of Pbx1 repression of Hoxa10- mediate *Bsp*-luciferase activity. C) Alteration of Hox sequence resulted in a significant decrease in *Bsp*-driven luciferase activity and no significant activation in the presence of Pbx1. Statistical significance was determined by two-way ANOVA followed by a Bonferroni post-test. Data are presented as the mean of 3 experiments ± S.E.M.

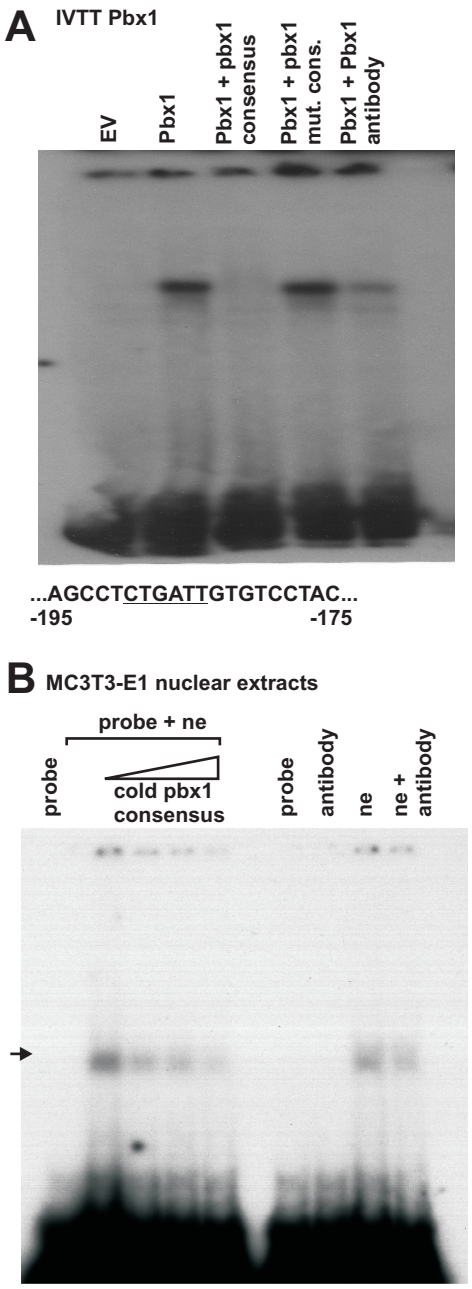

FIG. S3: Specificity of the Pbx1 site in the osteocalcin promoter. Oligonucleotide sequences are shown for the punitive Pbx1 site in the osteocalcin promoter (underline). A) DNA binding activity of IVTT-generated Pbx1 is demonstrated by EMSA. Ten femtomoles of labeled double- stranded oligonucleotides derived from the WT *osteocalcin* promoter or mutant (CTACT) probe as indicated was incubated with 2 µg of IVTT Pbx1 with or without Pbx1 antibody (Santa Cruz) and resolved on a 6.5% non-denaturing acrylamide gel. B) 5 µg of MC3T3 nuclear extract (NE) was incubated with labeled WT *osteocalcin* probe (as above) and resolved on a 4.5% non-denaturing acrylamide gel. Competition with Pbx1 binding-site consensus sequence (TTGATGAGAT) and Pbx1 antibody block-shift controls are shown.

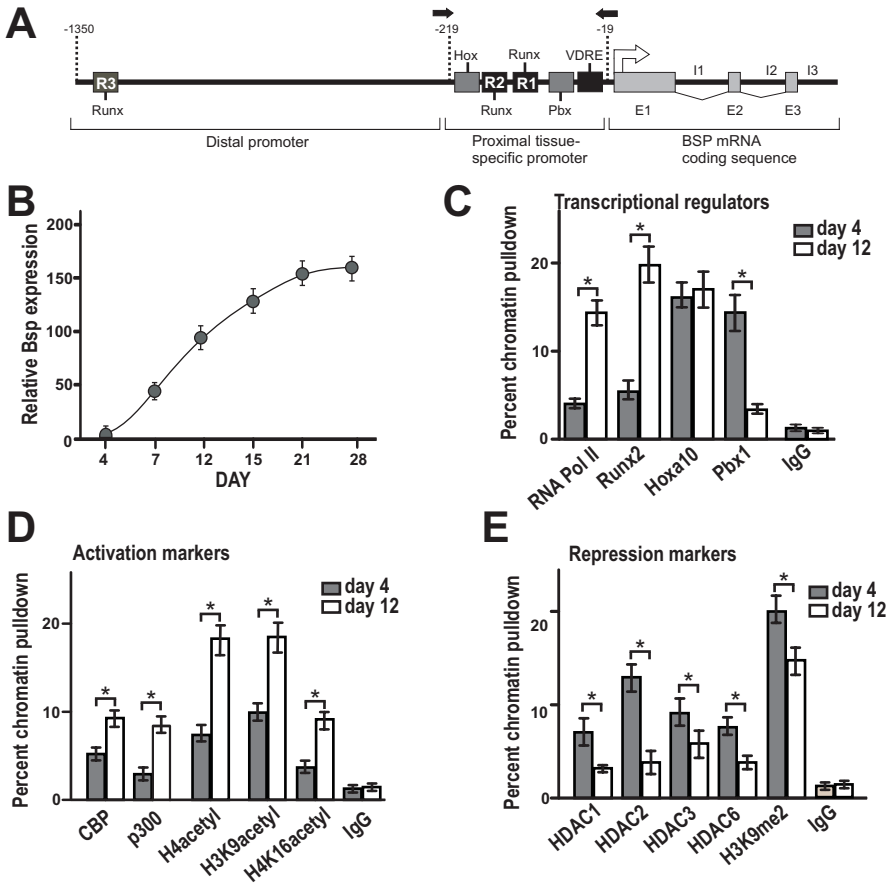

FIG. S4: **Pbx**1 **displays a pattern of gene-repressive functionality on the *bone sialoprotein* promoter**. A) Diagram of rat *bone sialoprotein* promoter displaying relative binding sites and primer sites used for chromatin immunoprecipitation analysis. Rat calvarial osteoblasts were isolated from E18.5 rat pups and cells were collected 48 h after infection after just reaching confluence (day 6). B) Calvarial osteoblasts were analyzed by qPCR to determine at indicated time points to determine levels of *Bsp* gene expression. B) – E) ChIP analysis was performed on cleared lysates from primary osteoblasts using ~5 μg of indicated antibody. Recovered DNA was then quantified by qPCR using primers specific for the proximal promoter region of the bone sialoprotein gene to determine relative occupancy of: C) transcriptional activators, D) activation markers or E) repressive markers. ChIP experiment was repeated 2 times with similar results and one representative experiment is presented ± S.D.