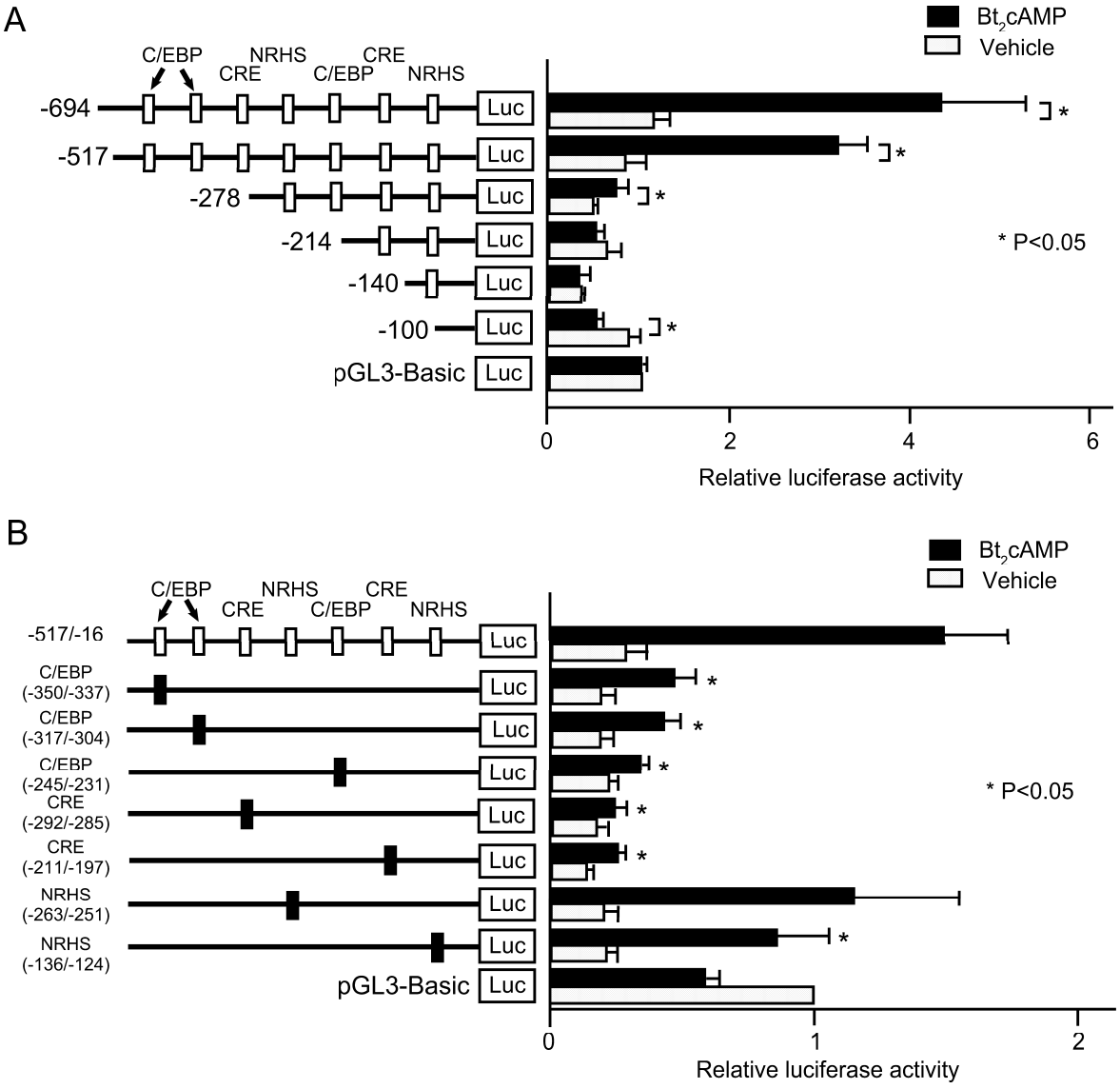
Ishikawa Supplemental Figure 2



Supplemental Figure 2. Identification of regulatory regions responsible for cAMP-dependent aromatase promoter I.3/II activity in MSMCs.

(A) Reporter plasmids containing the 5′-flanking region of human aromatase promoter I.3/II with serial deletions (-694, -517, -278, -214, -140, and -100 to -16 bp) were transfected into MSMCs. Relative positions of the cis-regulatory elements are indicated as white boxes. *P<0.05 (Wilcoxon signed rank test). (B) Site-directed mutagenesis for each cis-regulatory element was performed as described in Materials and Methods, and each reporter plasmid was transfected into MSMCs. White boxes represent positions of each cis-regulatory element within the promoter I.3/II region and black boxes represent the cis-regulatory sequence selectively disrupted by site-directed mutagenesis in each construct. Numbers below the name of each cis-regulatory element indicate the distance from the transcription start site in the promoter I.3/II region. Statistical analysis was performed comparing Bt2cAMP induction of each mutant construct with the -517/-16 bp construct. *P<0.05 (ANOVA). Luciferase activity was normalized to co-transfected renilla luciferase activity and is reported as the average + SEM of data from triplicate experiments. The empty luciferase vector, pGL3-Basic, was arbitrarily assigned a unit of 1. Results are reported as an average from MSMCs from 4 subjects. C/EBP, CCAAT/enhancer binding protein binding site; CRE, cAMP responsive element; NRHS, nuclear receptor half site.