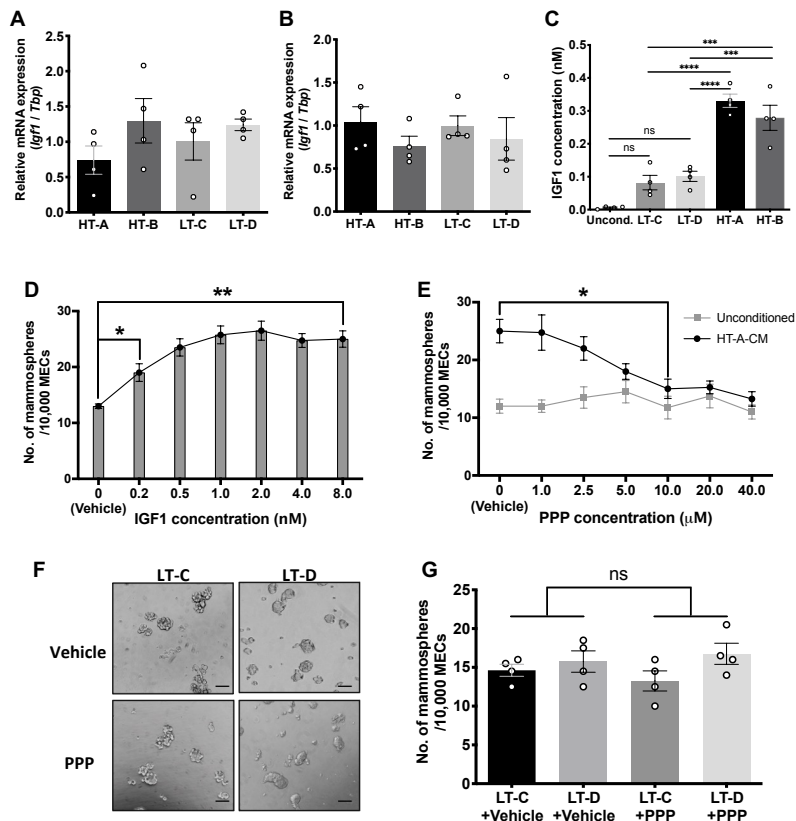
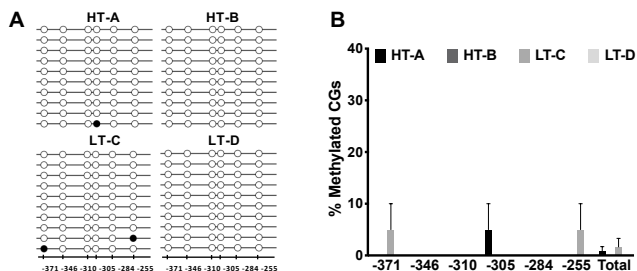


Supplemental Figure S1. The total number of MaSCs was increased in HT compared to LT animals. (A) Gating strategy for cell sorting. MaSC-enriched, basal (Ba.), luminal (Lu.) and stromal (Str.) cells were gated into Lin⁻CD24^{mod}CD29^{hi}, Lin⁻CD24⁺CD29^{hi}, Lin⁻CD24^{hi}CD29^{low} and Lin⁻CD24^{low}CD29^{low} subpopulations, respectively. (B) Total numbers of rudimentary MECs harvested from abdominal/inguinal mammary glands from 5-week-old animals (n=7/group). (C) Estimates of the total number of cells with MaSC markers in 5-week-old mice (n=7/group). The frequency of the MaSC-enriched population was multiplied by the number of MECs isolated from each corresponding sample. (D) Total numbers of rudimentary MECs harvested from abdominal/inguinal mammary glands from 10-week-old animals (n=5/HT-A; n=7/HT-B; n=4/LT-C; n=4/LT-D). (E) Estimates of the total number of cells with MaSC markers in 10-week-old mice (n=5/HT-A; n=7/HT-B; n=4/LT-C; n=4/LT-D). Mean \pm SEM are shown. One-way ANOVA was used for statistical analysis, and pairwise comparisons were performed using Tukey's posttest, $a \neq b \neq c$, $P < 0.05$.



Supplemental Figure S2. (A) *Igf1* mRNA levels in FACS-sorted MaSC-enriched population and (B) luminal epithelial cells from mammary tissues of 5-week-old animals ($n=4$ /group). Relative *Igf1* mRNA levels are expressed as fold change over group LT-C. (C) IGF1 protein concentrations in CM from HT and LT groups. Unconditioned DMEM/Ham's F-12 medium was used as a negative control. Four independent experiments ($n=4$) were performed. (D) Dose-response analysis to determine the effect of recombinant IGF1 on mammosphere numbers in LT-C-CM. (E) Dose-response analysis to determine the effect of picropodophyllin (PPP) on mammosphere numbers in unconditioned medium or in HT-A-CM. (F) Representative images and (G) bar graph showing numbers of mammospheres formed in LT-C-CM or LT-D-CM, supplemented with DMSO vehicle or 10 μM PPP. Scale bar = 100 μm. Four independent experiments ($n=4$) were performed, and data are displayed as mean \pm SEM. One-way ANOVA was used for statistical analysis and pairwise comparisons were performed using Tukey's posttest. $a \neq b$, $P < 0.05$.



Supplemental Figure S3. *Igf1* Pr2 was not differentially methylated in mammary stromal cells from HT and LT groups. (A) Representative Pr2 DNA methylation patterns from each group. Each circle represents a CG site, closed circles are methylated. Each vertical line represents a single clone. (B) Quantification of DNA methylation percentages for each CG site of the Pr2 promoter. Two independent experiments (n=2/group) were performed.