**Supplementary Figure S1. Experimental design and quality control analysis for Methyl-Seq data.**  (A) FVB mice were bred to become pregnant (parous, P) and underwent a complete pregnancy (Pr), lactation (L), and Involution (I). Animals were then euthanized immediately after involution (Early, E), or 6.5 months post-involution (Late, Lt). Nulliparous (NP) age matched controls were included at each time point. Mammary glands were harvested and genomic DNA was isolated and processed for input into the SureSelectXT Mouse Methy-Seq Enrichment Platform for DNA methylation analysis. (B) Quantitative RT-PCR of cell type specific transcripts. Each data point represents an animal, and bars represent mean ± SEM.

**Supplemental Figure S2.** All samples from both time points were used in a hierarchal clustering analysis. 10 samples at the early time point and 11 samples at the late time point were included. (E=early, L=late, P=parous, NP=nulliparous)

 **Supplemental Figure S3.** Mapping efficiency was above 50 for all samples in both time points. One sample was an outlier in the early time point (P12) and one at the late time point (NP4) and were removed from further analysis. Both Pearson Correlation plots and Principal Component Analysis are displayed.

**Supplemental Figure S4. Full representation of CpG methylation analysis in the confirmation of Methyl-Seq DNA methylation by bisulfite sequencing.** (A)The percent methylation for each CpG site afterbisulfite sequencing on mammary gland genomic DNA is represented. Each of the top differentially methylated genes chosen for validation are depicted. (B) Bisulfite sequencing data of all clones from each animal are shown. Each circle represents a CpG site and each horizontal line represents DNA from a separate clone. Open circles = unmethylated, and closed circles = methylated.

**Supplemental Fig S5. Imprinted genes display approximately 50% DNA methylation.** Gene regions known to be imprinted were chosen to confirm the validity of the Methyl-Seq assay. For each gene a β value was calculated for each window. The frequency of each particular β value is graphically represented for each gene.

**Supplemental Figure S6. Supervised hierarchical clustering and cell type content analysis.** (A) Heatmaps displaying supervised hierarchical clustering of hypermethylated and hypomethylated windows including dendrograms (Red=Methylated, Green=Unmethylated).

**Supplemental Fig S7. Igf1r is central to a top signaling network in Ingenuity pathway analysis.** Ingenuity pathway analysis (IPA) identified this signaling network to be significantly altered by DNA methylation. The hyper and hypomethylated genes identified to be persistently differentially methylated by the meta-analysis were input into IPA (Supplemental Data 1). Red symbols represent hypermethylation, green symbols represent hypomethylation and intensity depicts methylation difference between nulliparous and parous.

**Supplemental Fig S8. Hypermethylation of the Igf1r aligns with sites of histone methylation.** (A) The UCSC Genome Browser was used to align the Igf1r with other epigenetic modifications. The red bar indicates the region of differential DNA methylation identified in the SureSelectXT Methyl-Seq Assay. Black bars represent sites of histone methylation, the intensity corresponds to the degree of methylation. (B)Igf1r bisulfite sequencing data are depicted. Each animal is numbered and each horizontal line represents DNA from a separate clone from that animal. The percent methylation of each animal is listed under each CpG site. Open circles = unmethylated, and closed circles = methylated. The lower panel represents a combined result from all clones in all animals.

**Supplemental Fig S9. Differentially methylated regions of IGF pathway genes align with histone methylation sites.** The UCSC Genome Browser was used to align the Irs1, Igf1, and Igfbp4 with other epigenetic modifications. The red bars indicate the regions of differential DNA methylation identified in the SureSelectXT Methyl-Seq Assay. Black bars represent sites of histone methylation, the intensity corresponds to the degree of methylation.

**Supplemental Data 1.** This spreadsheet contains the results of the differential methylation analysis, the meta-analysis and the pathway analysis. In the meta-analysis sheets, the first 3 columns represent the chromosomal locations, followed by the significance level (stat, p-value, and FDR). Weight.1 and weight.2 represent the weight of early and late data respectively. For the meta-analysis we weighted both as 1. The effect size represents the difference in DNA methylation between NP and P at the early and late time points (effect size.1=early, effect size.2=late), and the p-value for each is next depicted. We then filtered the data by trimmed means as in the Materials and Methods section and the new values are listed in columns N and O. We then annotated the gene name, which genomic feature the loci resides in, and the distance to the nearest promoter (distance to feature). We then annotated the mean coverage and the number of CpG sites in the differentially methylated region. The differential methylation sheets include similar data without the columns pertaining to the meta-analysis. The 3Ingenuity Pathway Analysis (IPA) spreadsheets contain the pathway analysis result. The pathway name, followed by the statistical significance, and molecules represented in our data for each pathway are detailed on each sheet. The analysis was first conducted on the hyper and hypomethylated genes separately, then the combined hyper and hypermethylated genes were analyzed together.