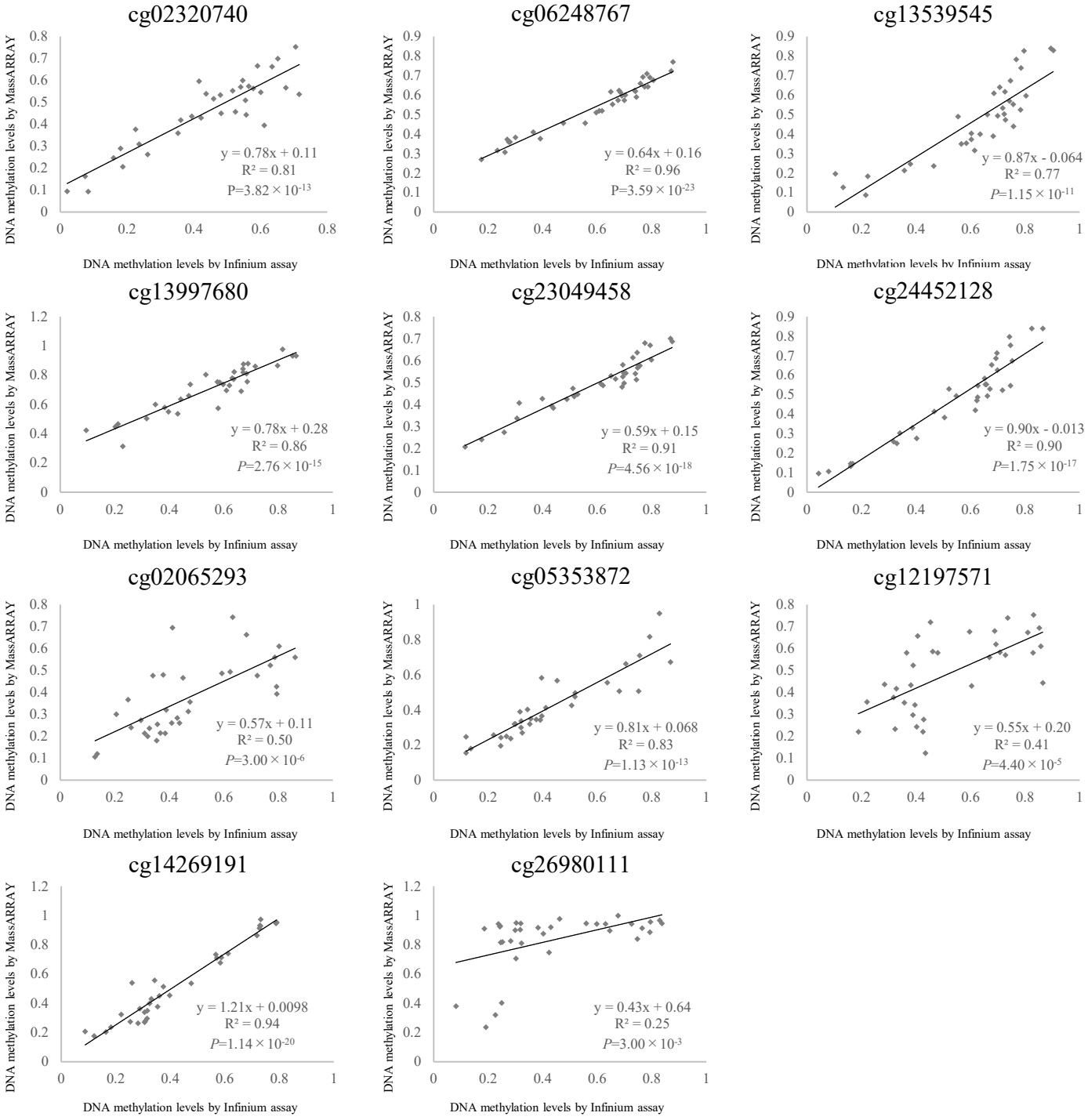

Supplementary Figure 3. Scattergrams showing the significant correlation between DNA methylation levels determined by the Infinium assay and those obtained by MassARRAY in 34 samples of early-onset endometrioid endometrial cancer (patient age <40 yr). For MassARRAY analysis, 500-ng aliquots of DNA were subjected to bisulfite treatment using an EpiTect Bisulfite Kit (QIAGEN GmbH, Hilden, Germany), in accordance with the manufacturer's protocol. Specific PCR primers for bisulfite-converted DNA were designed using the EpiDesigner software package (www.epidesigner.com) (Sequenom, San Diego, CA). To overcome any PCR bias, we optimized the annealing temperature. The PCR products were used as a template for in vitro transcription, and the RNase A-mediated cleavage reaction was subsequently performed using the EpiTYPER Reagent Kit (Sequenom). The fragmented samples were dispensed onto a SpectroCHIP array, and then detected on the MassARRAY analyzer, a compact matrix-assisted laser desorption ionization-time of flight mass spectrometry instrument. The data were visualized using EpiTYPER Analyzer software v1.0 (Sequenom). The DNA methylation level (%) at each CpG site was determined by comparing the signal intensities of methylated and non-methylated templates. Since optimal primers were difficult to design and optimal PCR conditions were difficult to determine for probe CpG sites of cg06248767, cg13997680, cg02065293, cg12197571 and cg26980111 themselves, the nearest CpG sites were analyzed by MassARRAY for each probe. A cluster of consecutive CpG sites, each giving one measured value by the MassARRAY system, is defined as a "CpG unit" in the manufacturer's protocol: for cg02320740, cg13539545, cg23049458 and cg24452128, MassARRAY data for the CpG unit each consisting of probe CpG sites themselves and 2 additional CpG sites in the vicinity are shown.