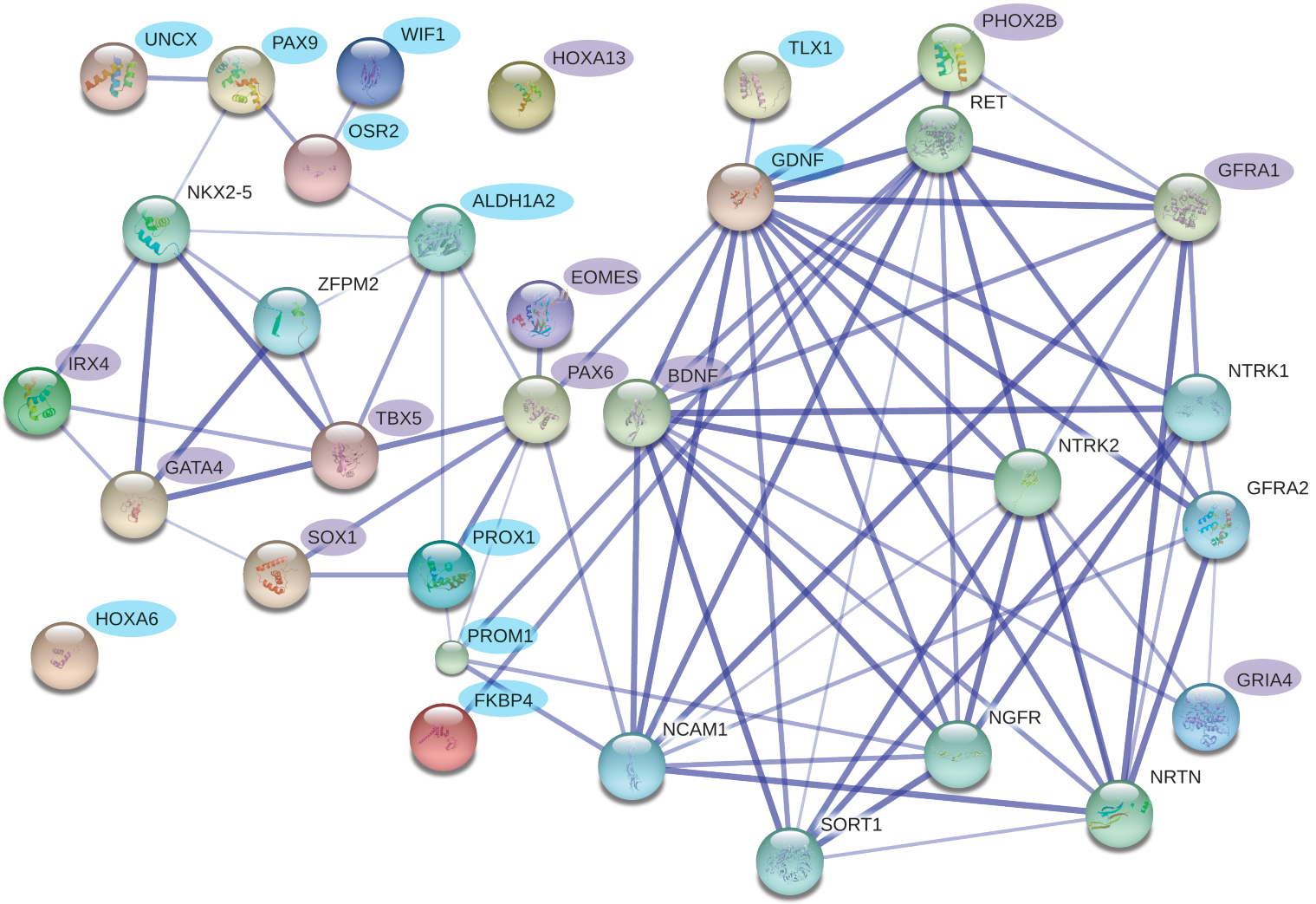
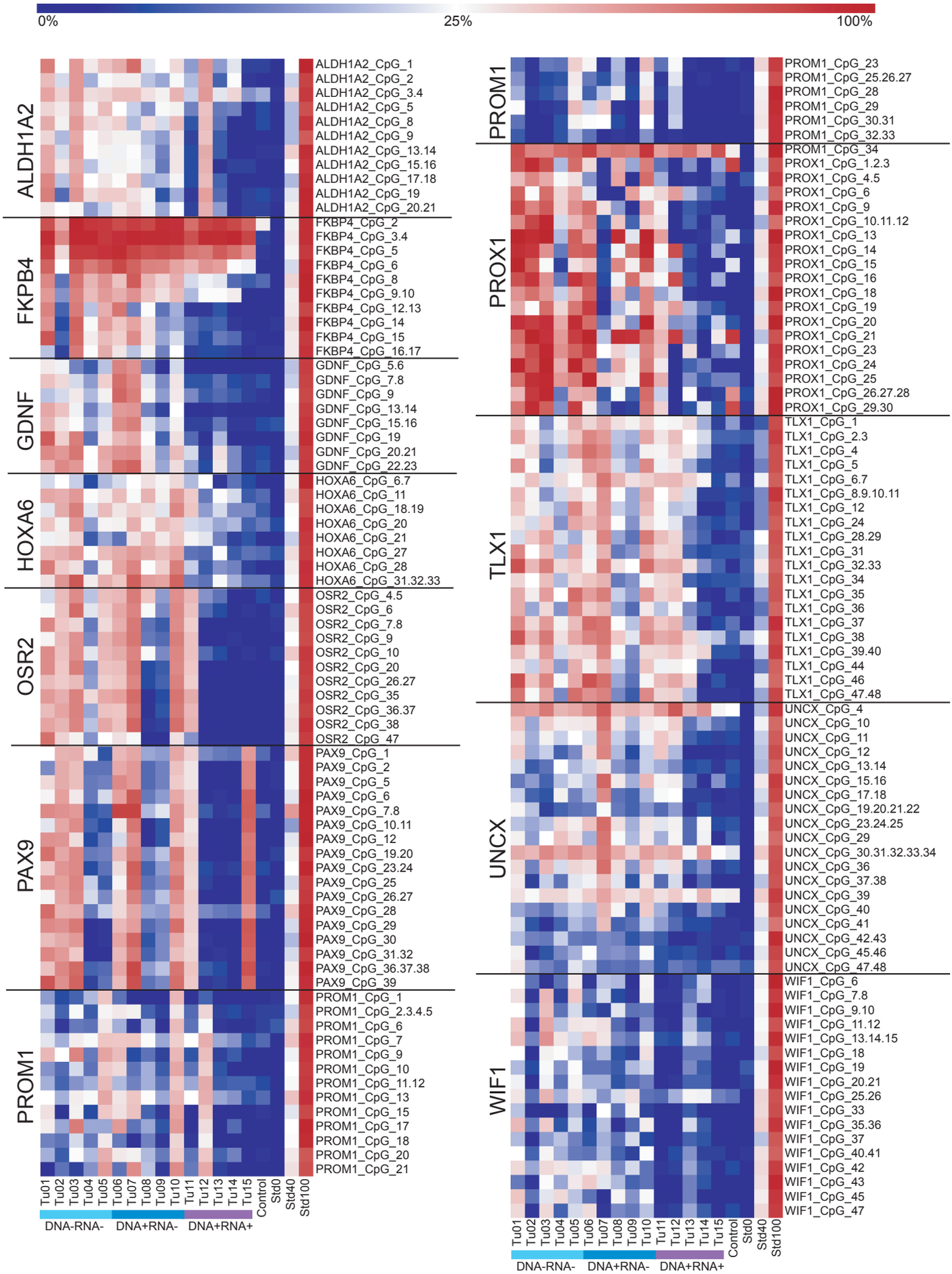
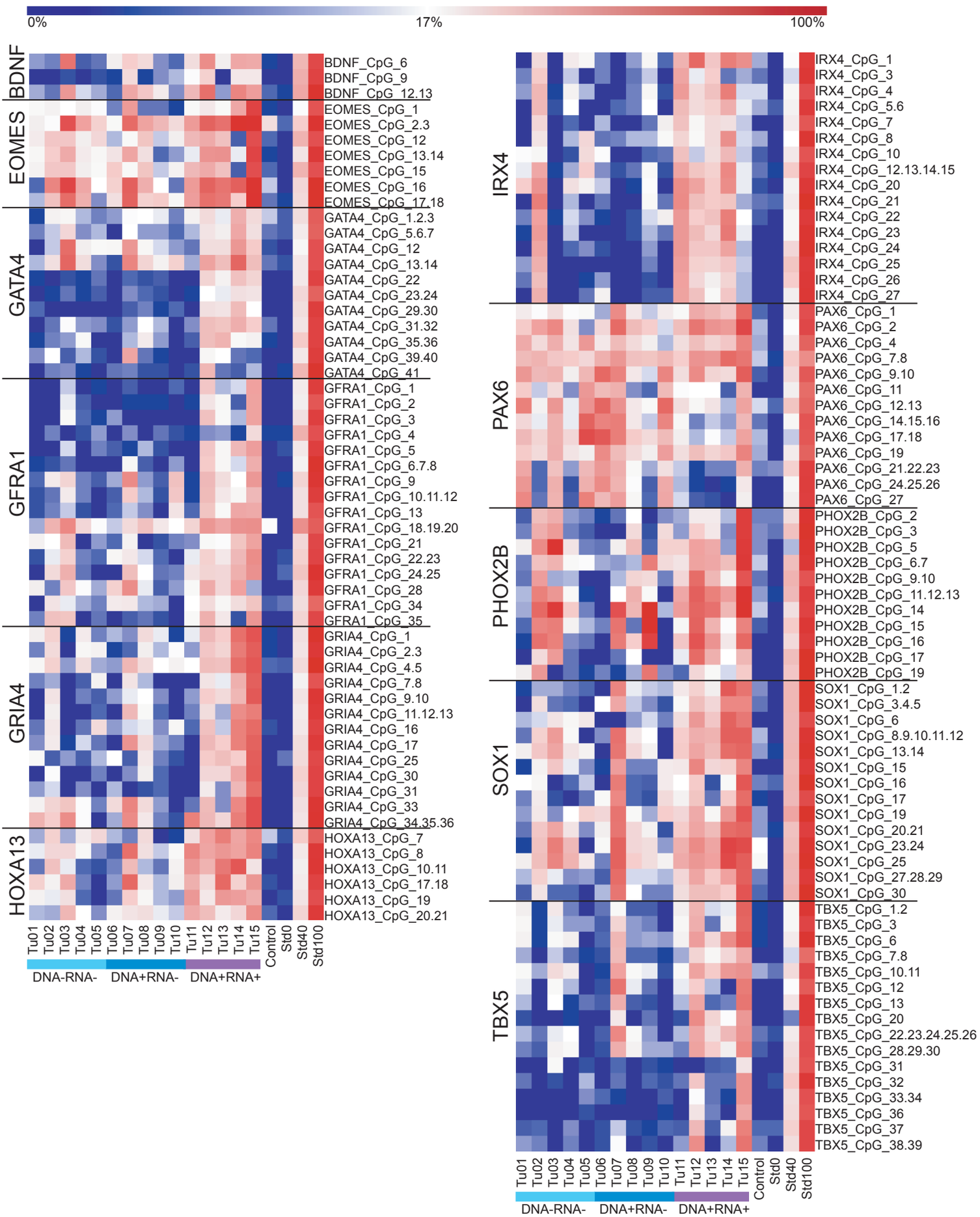


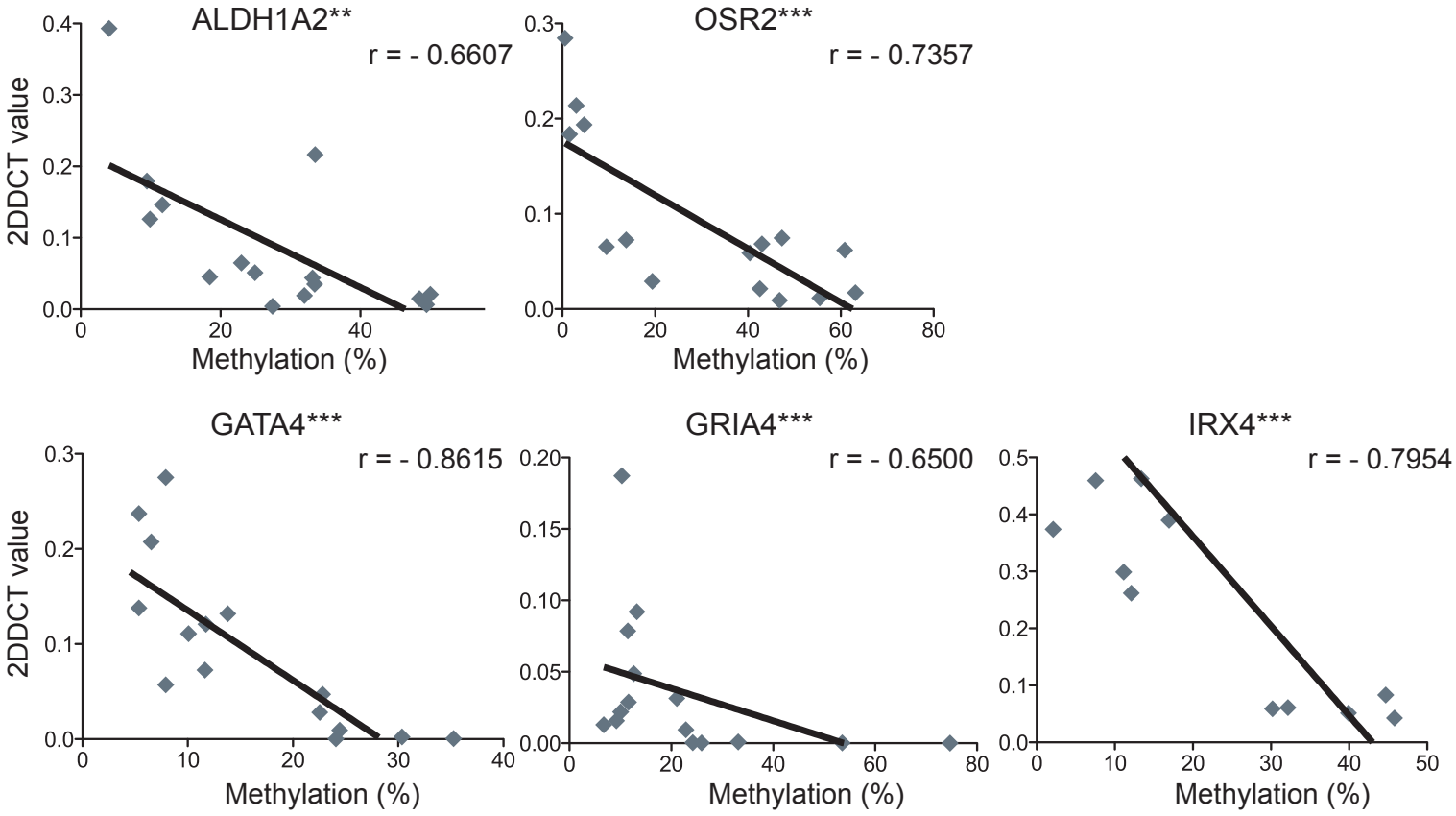
Supplemental Figure 1. Interaction gene analysis network for the 22 selected candidate genes which were identified as being hypermethylated either in the non-HPV-driven (highlighted in blue) or in the HPV-driven tumors (highlighted in purple) (STRING confidence view, enhanced, predicted functional partners scores >0.997) (23).



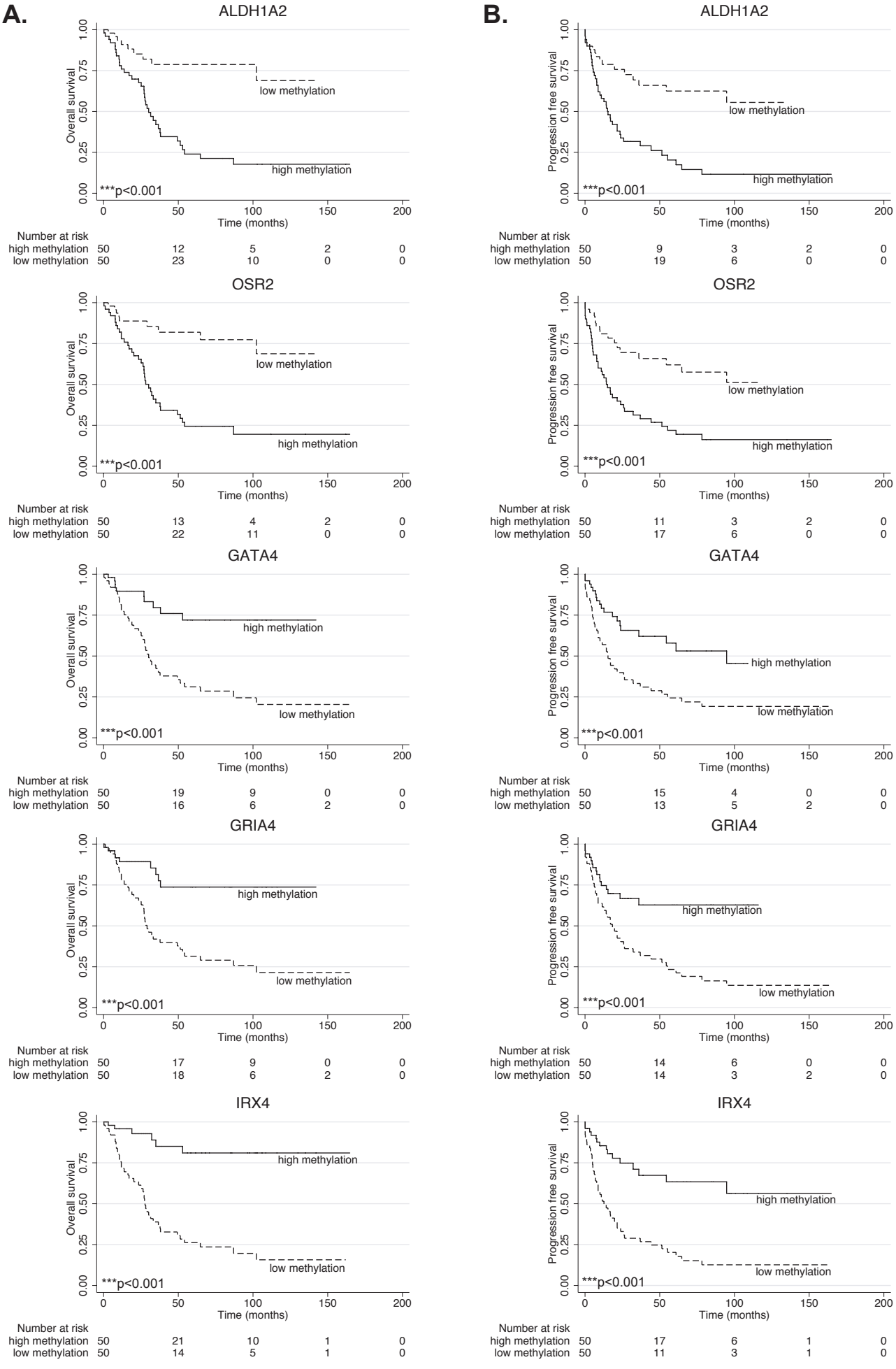
Supplemental Figure 2. Confirmation of DMRs by quantitative methylation analysis. Methylation patterns of gene promoter DMRs, which were identified as hypermethylated in the non-HPV-driven tumors (Tu01-Tu10) by array analysis. A healthy mucosa sample served as control; standards for 0%, 40% and 100% methylation were included.



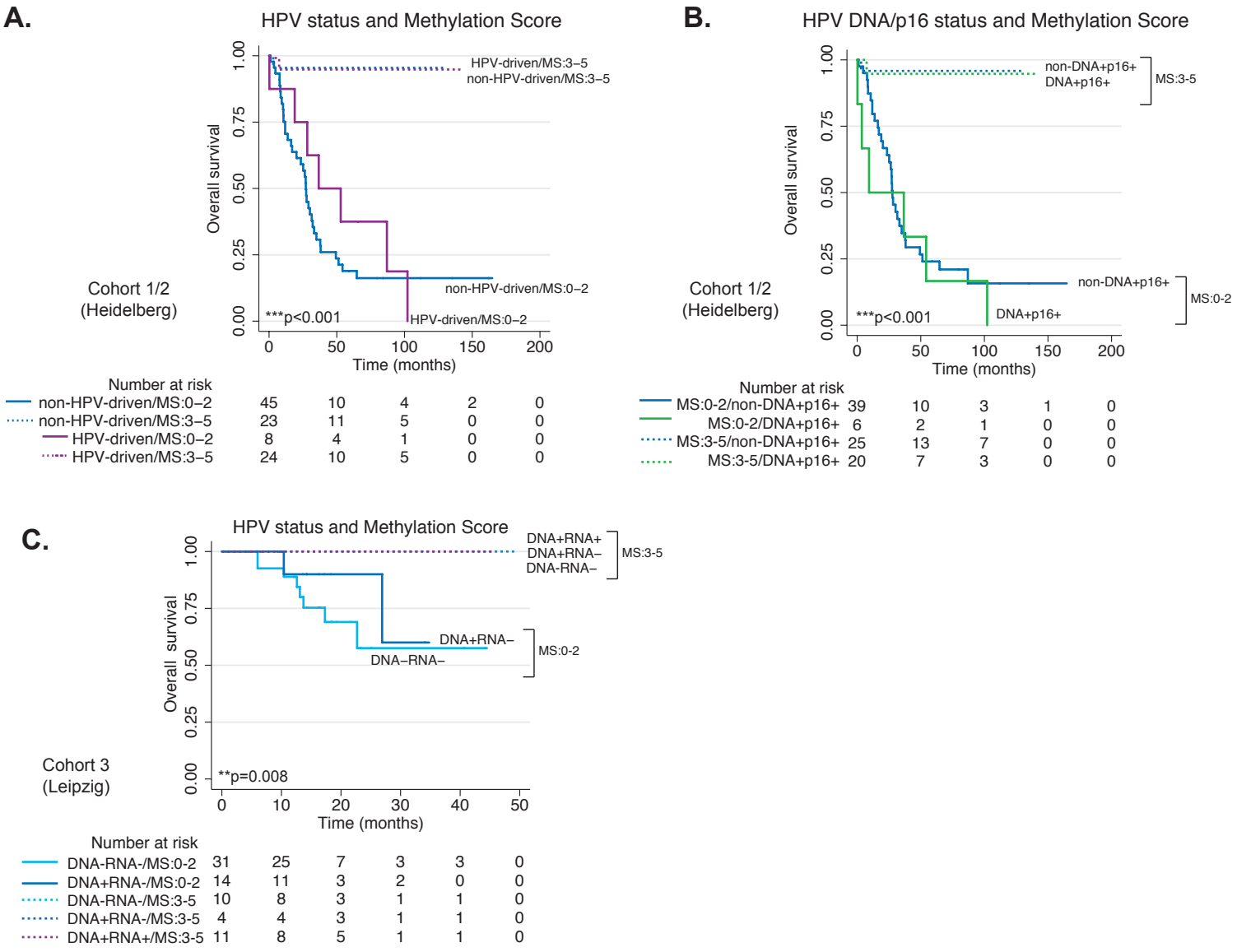
Supplemental Figure 3. Confirmation of DMRs by quantitative methylation analysis. Methylation patterns of gene promoter DMRs, which were identified as hypemethylated in the HPV-driven tumors (Tu11-Tu15) by array analysis. A healthy mucosa sample served as control; standards for 0%, 40% and 100% methylation were included.



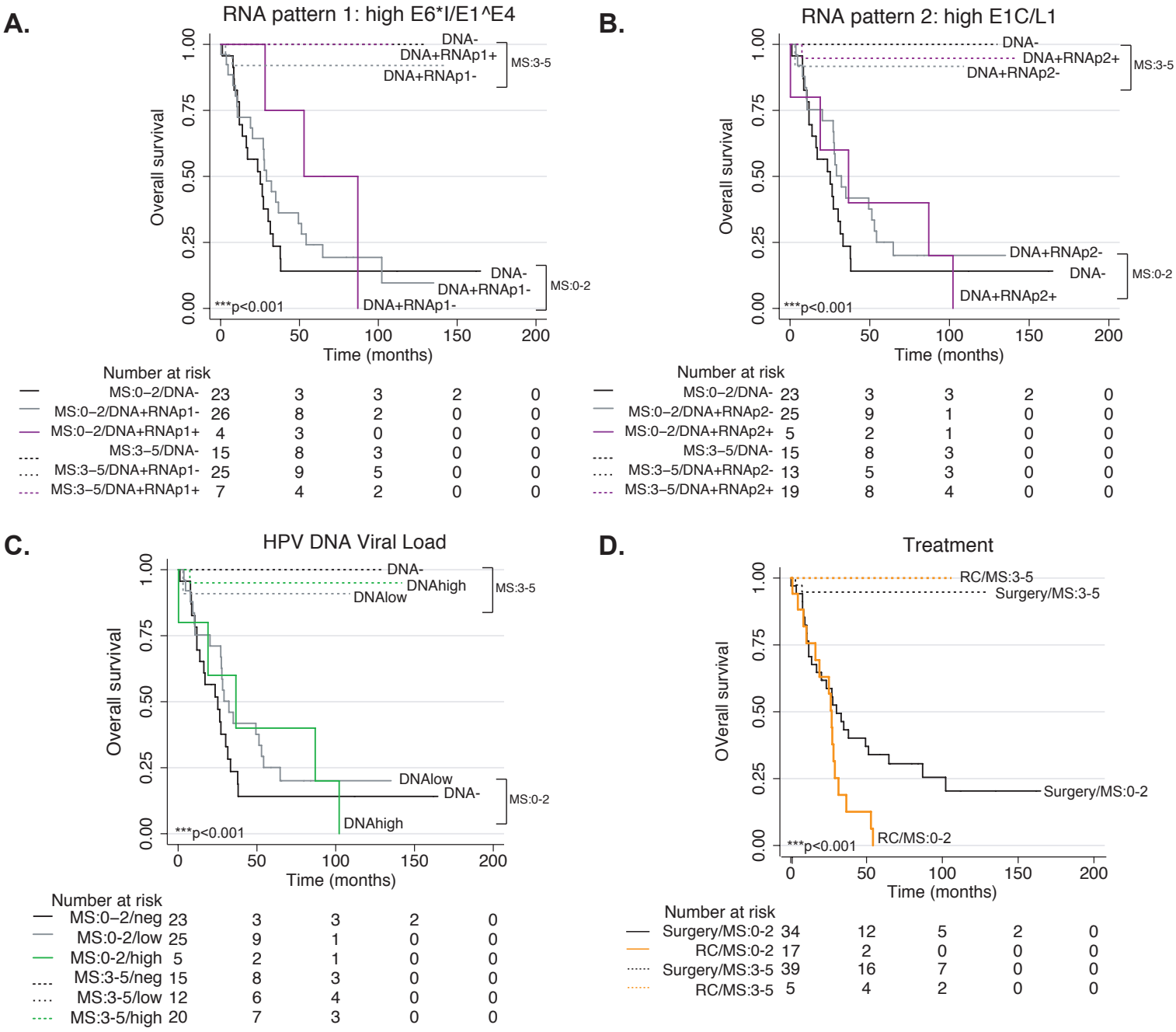
Supplemental Figure 4. Correlation between gene promoter methylation and transcript levels (2DDCT value) in Tu01-Tu15 samples. Statistical non-parametric comparison for correlation between methylation and expression values was performed by Spearman's Rho method (Spearman's rank correlation coefficient r value is depicted in each graph, significance is documented by stars and was derived by Spearman's rho p-value/two-tailed test, **=p<0.01, ***=p<0.001).



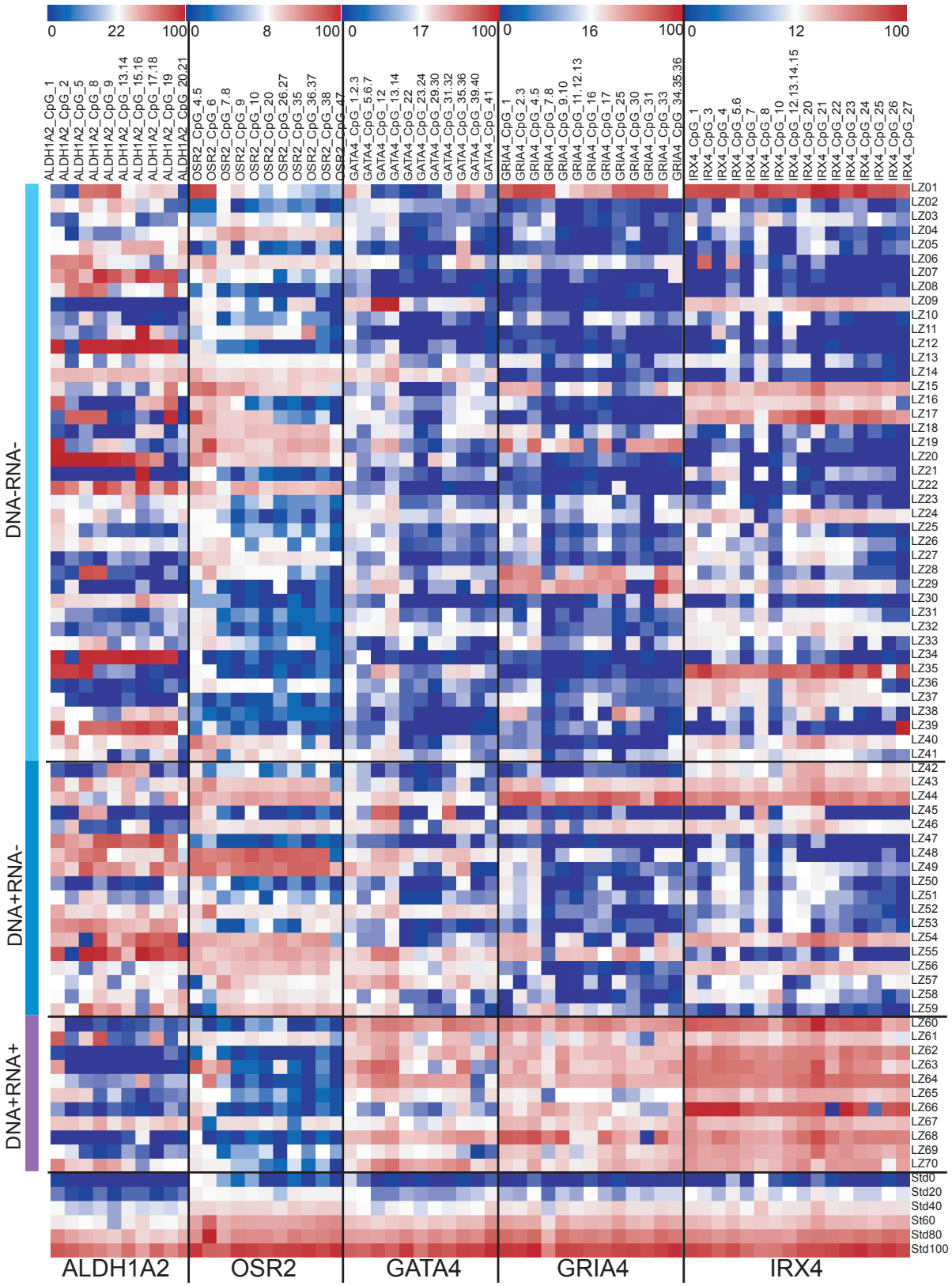
Supplemental Figure 5. Association between the methylation status of five gene promoter DMRs (*ALDH1A2, OSR2, GATA4, GRIA4* and *IRX4*) and clinical outcome of OPSCC patients (cohort 1 and cohort 2, n=100, Heidelberg). Kaplan-Meier plots for overall survival **(A)** and progression-free survival **(B)**. The mean methylation value (mean of CpG units per amplicon) in all 100 tumors was computed and categorized in high (above the median of all samples) and low (below the median of all samples) methylation. P values derived by Log Rank/Mantel-Cox test.



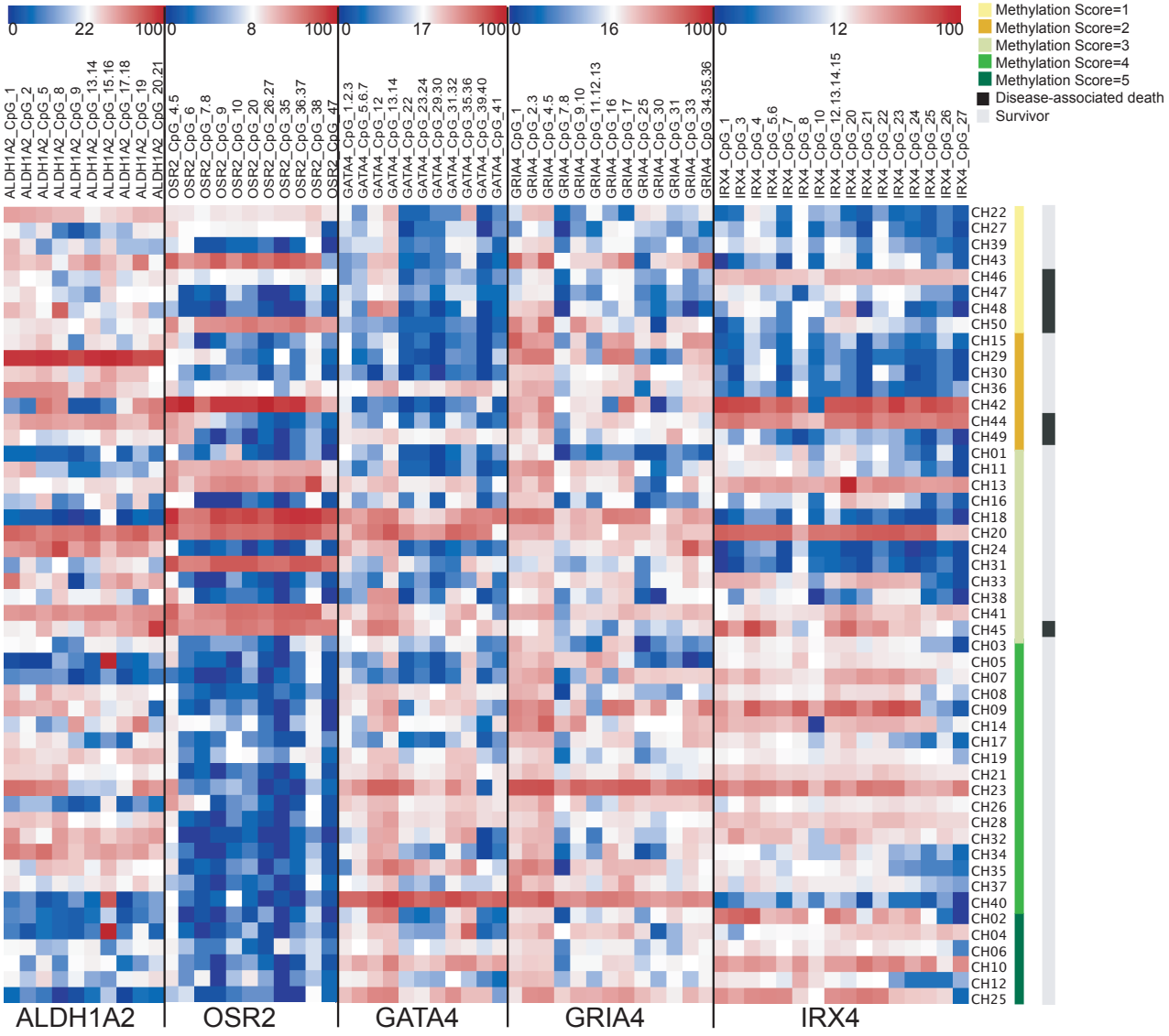
Supplemental Figure 6. Methylation signature score predicts clinical outcome of OPSCC patients independent of various HPV and p16 status **(A)** Kaplan-Meier plot for overall survival with regard to methylation signature score (MS) and stratified for HPV status as defined by both HPV16 DNA positivity and HPV16 RNA positivity (12) (Cohort 1/2, Heidelberg University Hospital). HPV-driven: DNA+RNA+, non-HPV-driven: DNA-RNA and DNA+RNA-. **(B)** Kaplan-Meier plots for overall survival with regard to methylation signature score (MS) and stratified for HPV/p16 status (91/100 patients from cohort 1/2, Heidelberg). The non-DNA+p16+ cases correspond to DNA-p16- (n=28), DNA+p16- (n=6) and DNA+p16- (n=30) (see also Supplemental Table 25). **(C)** Kaplan-Meier plot for overall survival with regard to the methylation signature score (MS) and stratified for HPV status as defined by both HPV16 DNA positivity and HPV16 RNA positivity for cohort 3 (Leipzig). P values derived by Log Rank/Mantel-Cox test.



Supplemental Figure 7. Methylation signature score predicts clinical outcome of OPSCC patients independent of HPV RNA patterns, DNA viral load and treatment. **(A)** Kaplan-Meier plot for overall survival with regard to the methylation signature score (MS) and stratified for HPV16 RNA pattern 1 (high E6*I/E1^E4) (12). **(B)** Kaplan-Meier plot for overall survival with regard to the methylation signature score (MS) and stratified for HPV16 RNA pattern 2 (high E1C/L1) (Hozlinger et al. 2012). **(C)** Kaplan-Meier plot for overall survival with regard to the methylation signature score (MS) and stratified for DNA viral load. **(D)** Kaplan-Meier plot for overall survival with regard to the methylation signature score (MS) and stratified for first-line treatment (surgery versus radiotherapy/chemotherapy (RC); see also Supplemental Tables 25-26).



Supplemental Figure 8. Methylation heatmap of promoter DMRs associated with genes *ALDH1A2, OSR2, GATA4, GRIA4,* and *IRX4* in cohort 3 (Leipzig) with n=70 OPSCC patients (DNA-RNA-: n=41; DNA+RNA-: n=18; DNA+RNA+: n=11). Standards (Std) for different percentages of methylation are also decicted.



Supplemental Figure 9. The five-gene methylation signature is associated with clinical outcome of OPSCC in the Chicago validation cohort (cohort 4). DNA methylation patterns of gene promoter DMRs for *ALDH1A2, OSR2, GATA4, GRIA4* and *IRX4* in cohort 4 (Chicago, n=50). Analysis was performed by MassARRAY as described for cohorts 1/2 (Heidelberg) and cohort 3 (Leipzig). Color bar indicate methylation score (MS) of each patient starting from low to high (none of the patients showed MS=0). Gray/Black bars indicate survival status (OS: disease-specific death or not).