Figure S1

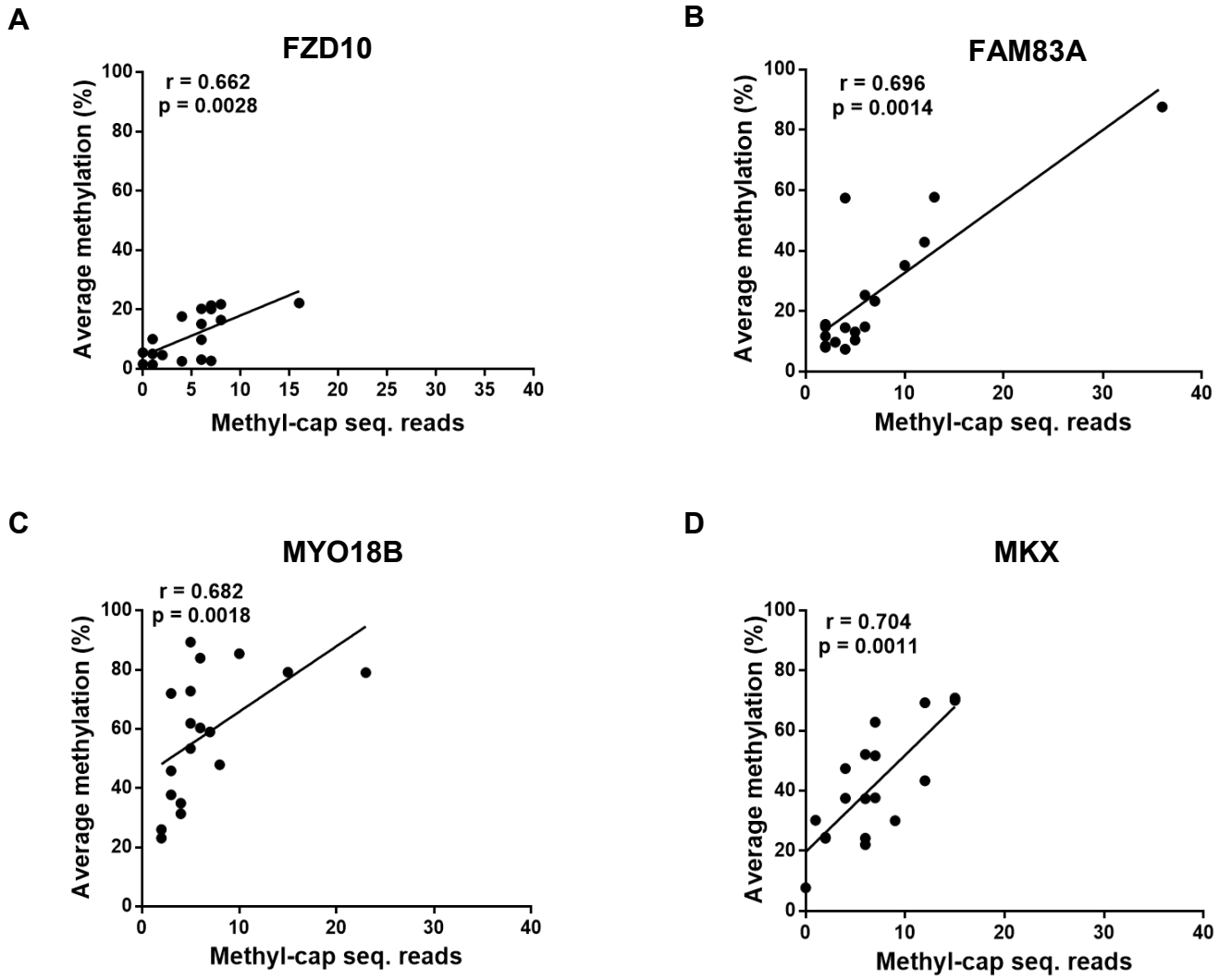

Figure S1. Spearman correlation of MethylCap-seq reads and methylation percentage obtained with bisulfite pyrosequencing for **A)** *FZD10*, **B)** *FAM83A*, **C)** *MYO18B* and **D)** *MKX* showing successful verification of MethylCap-seq data by pyrosequencing for discovery set 1 (n=18).

Figure S2

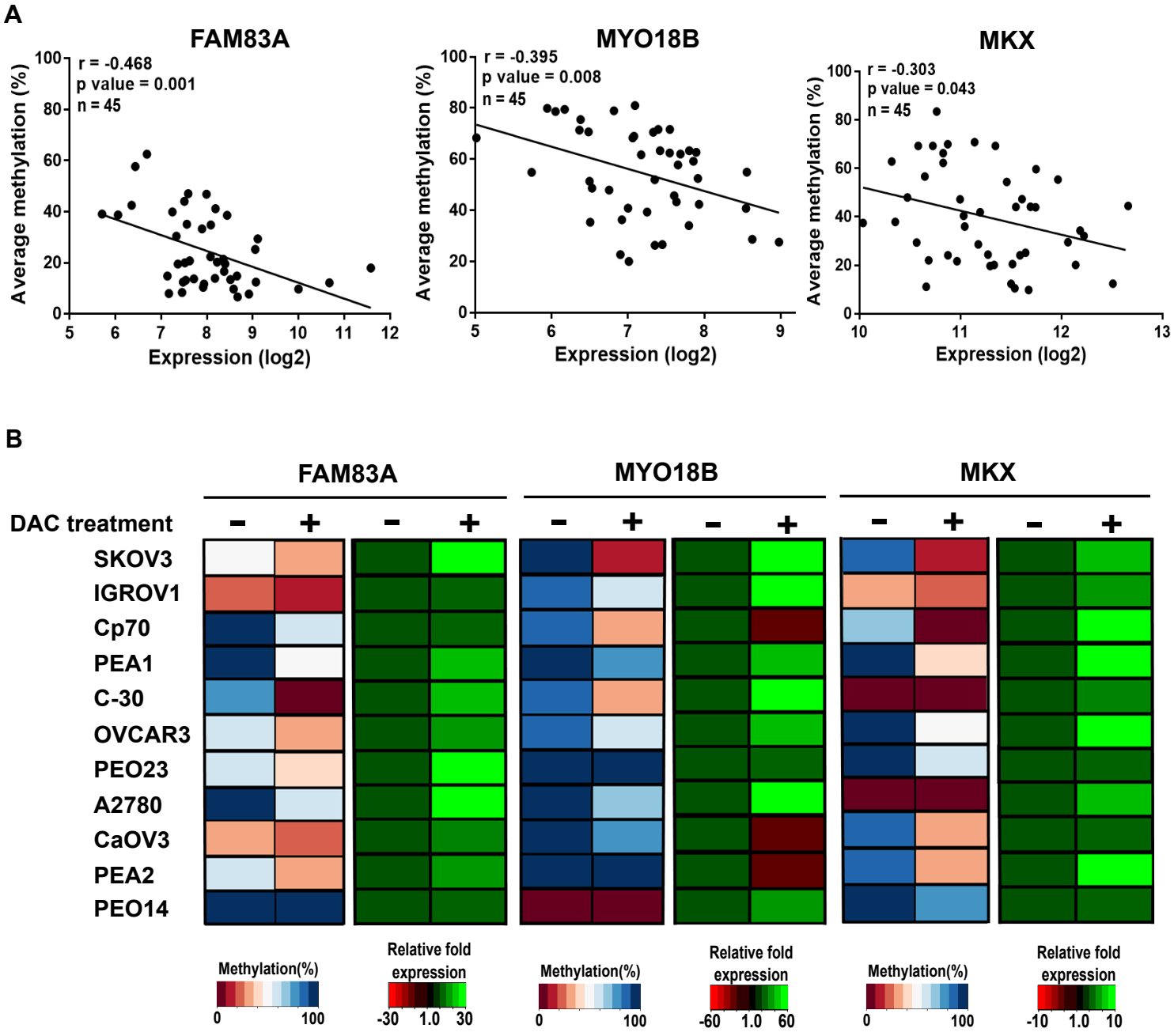

Figure S2. A) Correlation analysis of average methylation as determined by bisulfite pyrosequencing and microarray-based expression levels [18] of *FAM83A*, *MYO18B* and *MKX* in HGSOC patients (n=45) showing a significant inverse correlation between methylation and their correspondent expression using Pearson correlation testing. **B)** Heat maps of average methylation percentage and relative mRNA expression of *FAM83A*, *MYO18B* and *MKX* in various ovarian cancer cell lines (n=11), treated with or without demethylating agent 5-aza-2'-deoxycytidine (DAC) for 72 hrs (DAC + or -) showing demethylation (from blue to dark red) in most of the cell lines with subsequent upregulation of mRNA (from black to green).

Figure S3

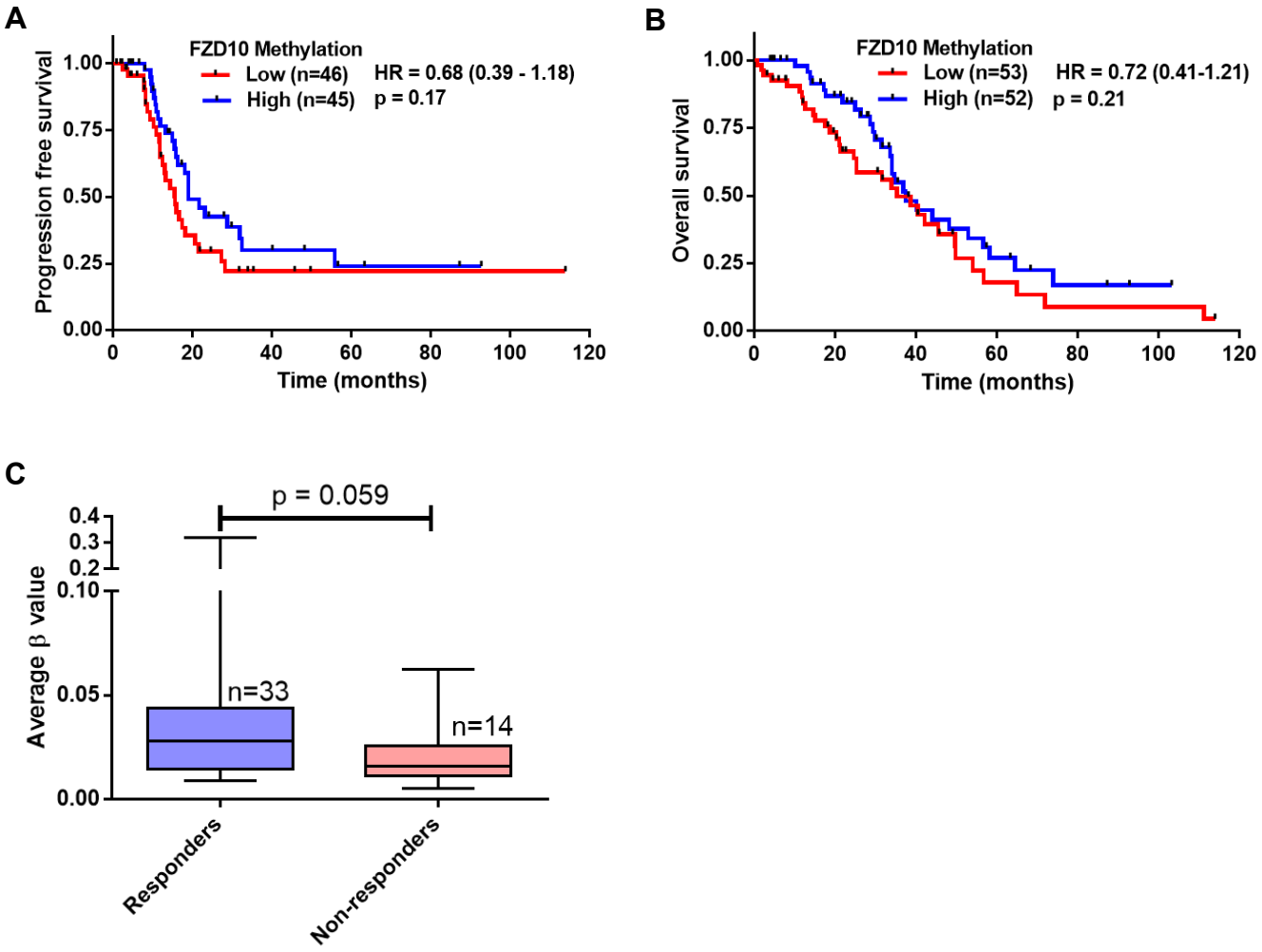

Figure S3. A-B) Kaplan–Meier plots showing PFS (**A**) and OS (**B**) for two user-defined patient groups based on *FZD10* methylation levels using univariate Mantel-Cox log-rank survival analysis on the HGSOC TCGA cohort (Set 5, n=91 and n=105, respectively). Average methylation β-value for 'Low' methylation group patients = 0.01 (0.01-0.02) and for 'High' methylation group patients = 0.06 (0.02-0.61). **C**) Average methylation β-value of *FZD10* in extreme responder (blue bars, n=33) and non-responder groups (red bars, n=14) of the TCGA cohort (Set 5) shows higher *FZD10* methylation in the extreme responder compared to the extreme non-responder group.

Figure S4

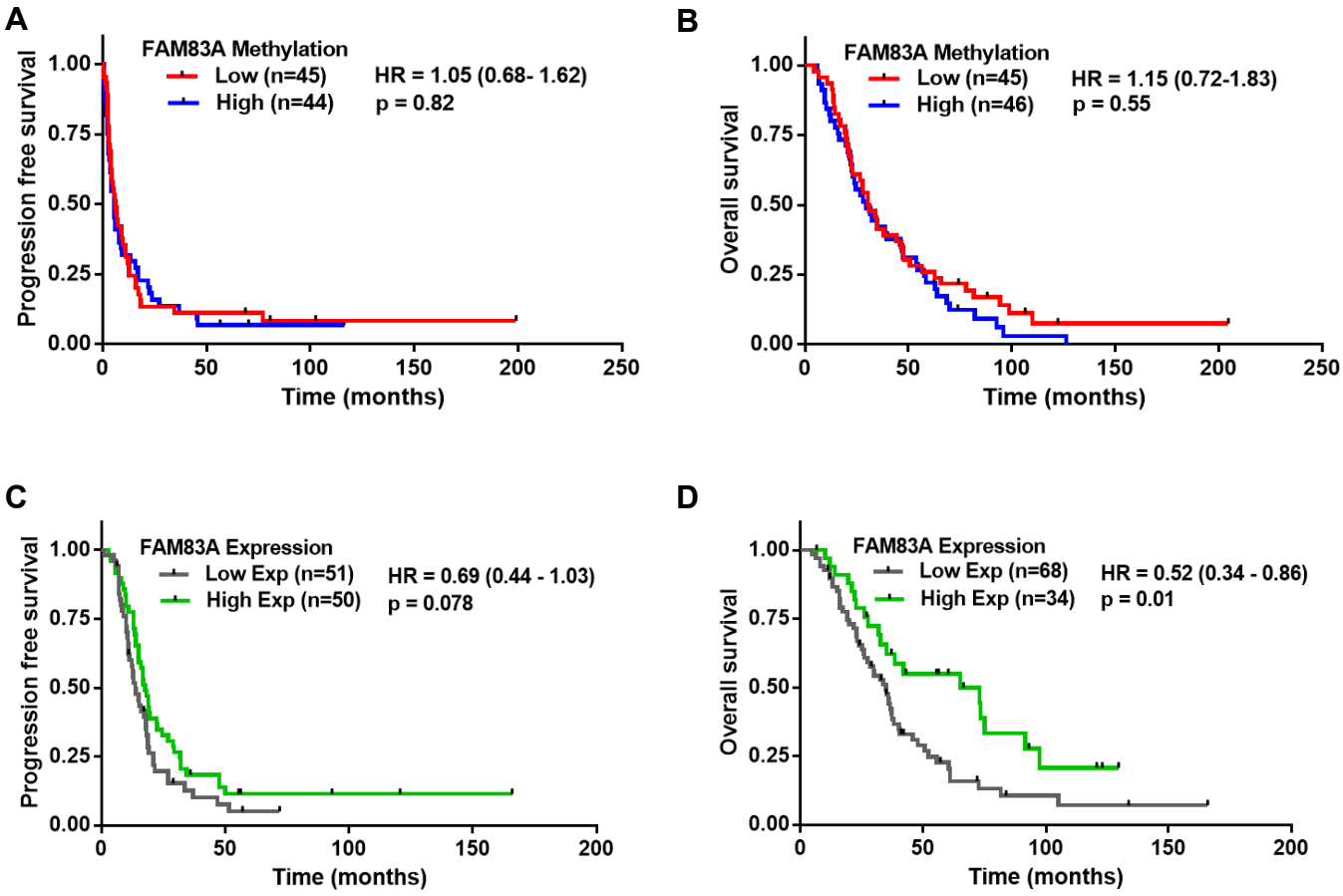

Figure S4. A-B) Kaplan–Meier plots showing PFS (**A**) and OS (**B**) for the two user-defined patient groups based on *FAM83A* methylation using univariate Mantel-Cox log-rank survival analysis in HGSOC cohorts (Set 4, n=89 and n=91, respectively). Average methylation β-value for 'Low' methylation group patients = 0.19 (0.07-0.29) and for 'High' methylation group patients = 0.36 (0.30-0.59). **C-D**) Kaplan–Meier plots showing PFS (**C**) and OS (**D**) for the two patient clusters based on *FAM83A* expression using univariate Mantel-Cox log-rank survival analysis in HGSOC cohorts (Set 6, n=101 and n=102, respectively).

Figure S5

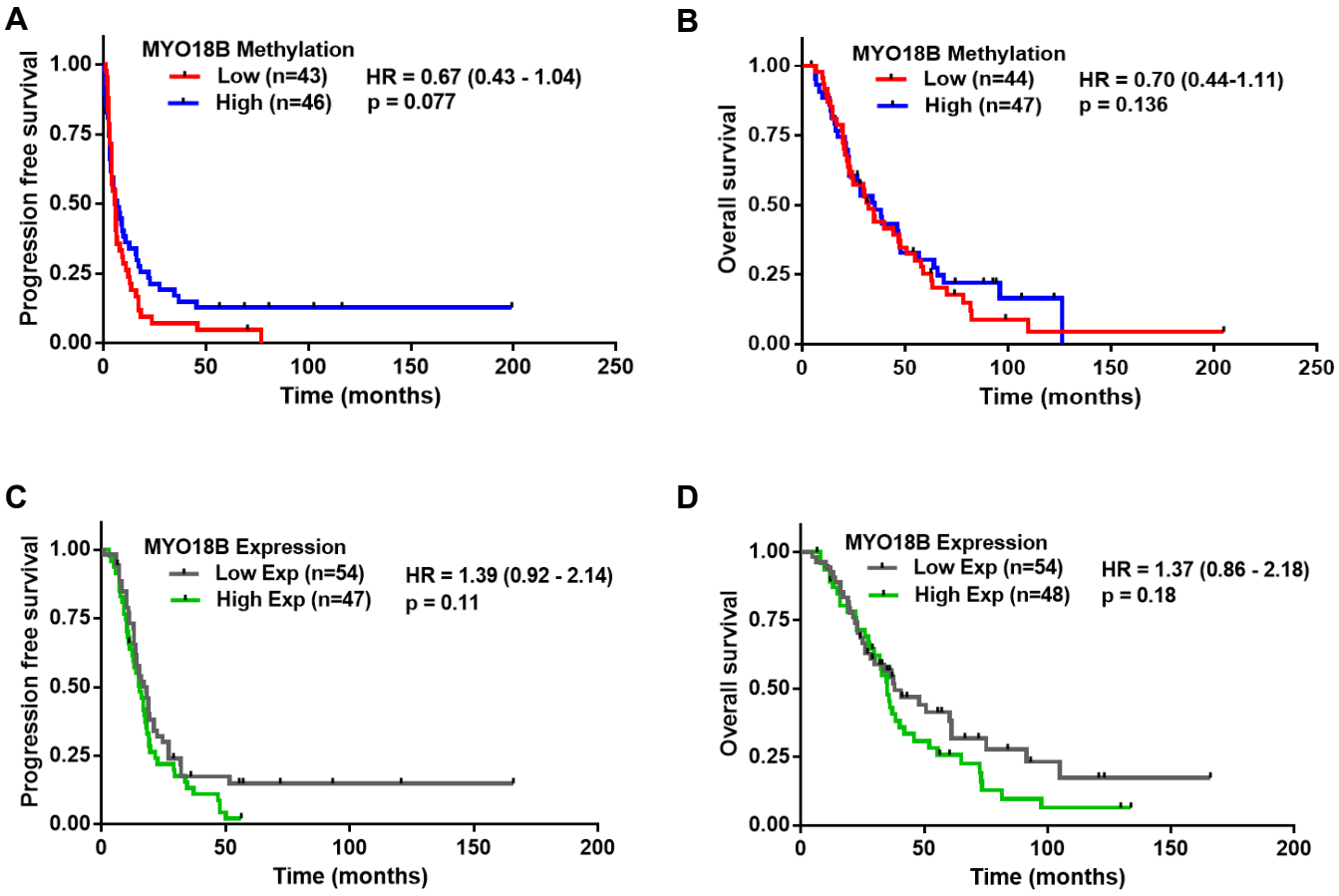

Figure S5. A-B) Kaplan–Meier plots showing PFS (**A**) and OS (**B**) for the two user-defined patient groups based on *MYO18B* methylation using univariate Mantel-Cox log-rank survival analysis in HGSOC cohorts (Set 4, n=89 and n=91, respectively). Average methylation β-value for 'Low' methylation group patients = 0.47 (0.25-0.57) and for 'High' methylation group patients = 0.66 (0.58-0.74). **C-D**) Kaplan–Meier plots showing PFS (**C**) and OS (**D**) for the two patient clusters based on *MYO18B* expression using univariate Mantel-Cox log-rank survival analysis in HGSOC cohorts (Set 6, n=101 and n=102, respectively).

Figure S6

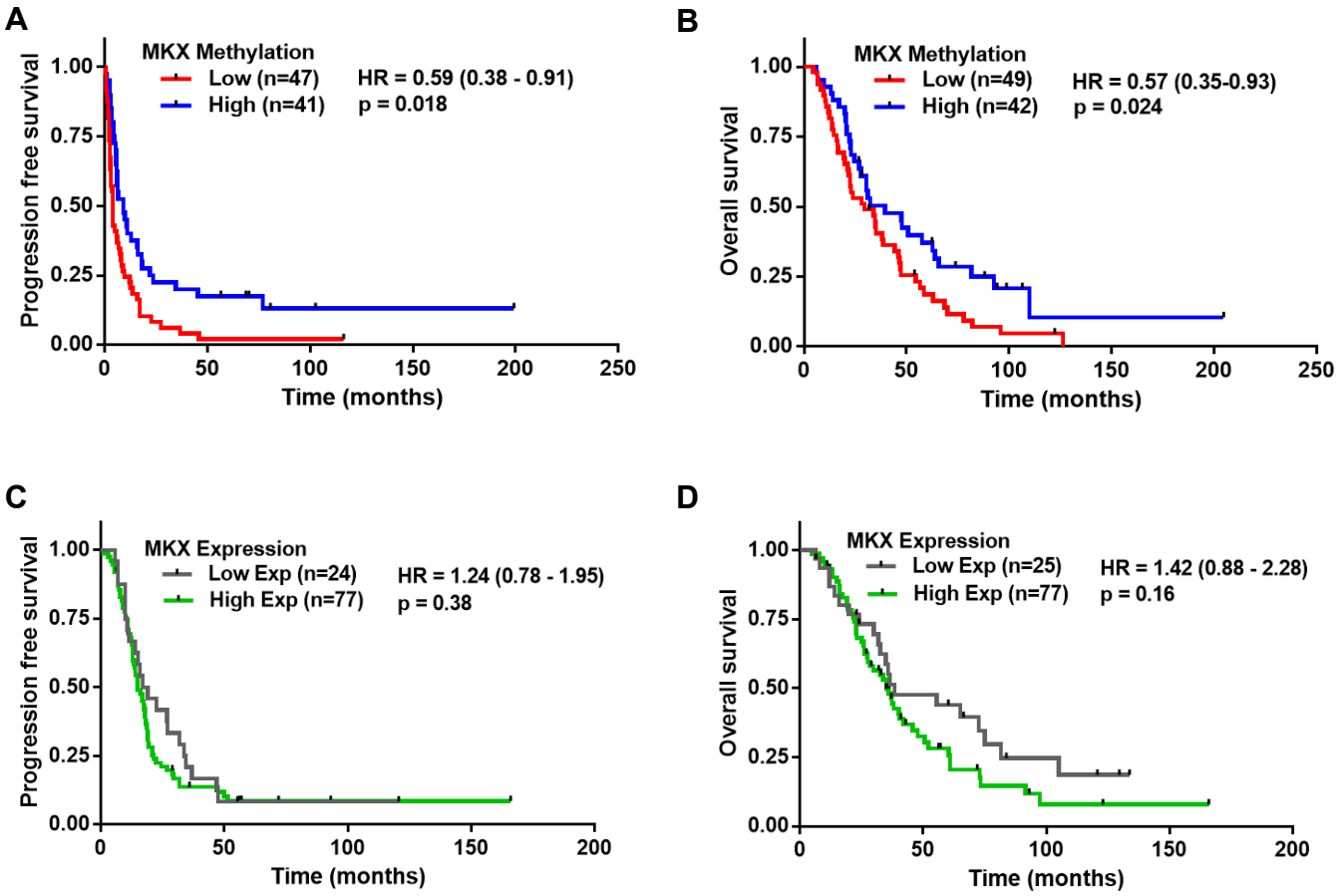

Figure S6. A-B) Kaplan–Meier plots showing PFS (**A**) and OS (**B**) for the two user-defined patient groups based on *MKX* methylation using univariate Mantel-Cox log-rank survival analysis in HGSOC cohorts (Set 4, n=89 and n=91, respectively). Average methylation β-value for 'Low' methylation group patients = 0.50 (0.17-0.65) and for 'High' methylation group patients = 0.74 (0.66-0.85). **C-D**) Kaplan–Meier plots showing PFS (**C**) and OS (**D**) for the two patient clusters based on *MKX* expression using univariate Mantel-Cox log-rank survival analysis in HGSOC cohorts (Set 6, n=101 and n=102, respectively).

Figure S7

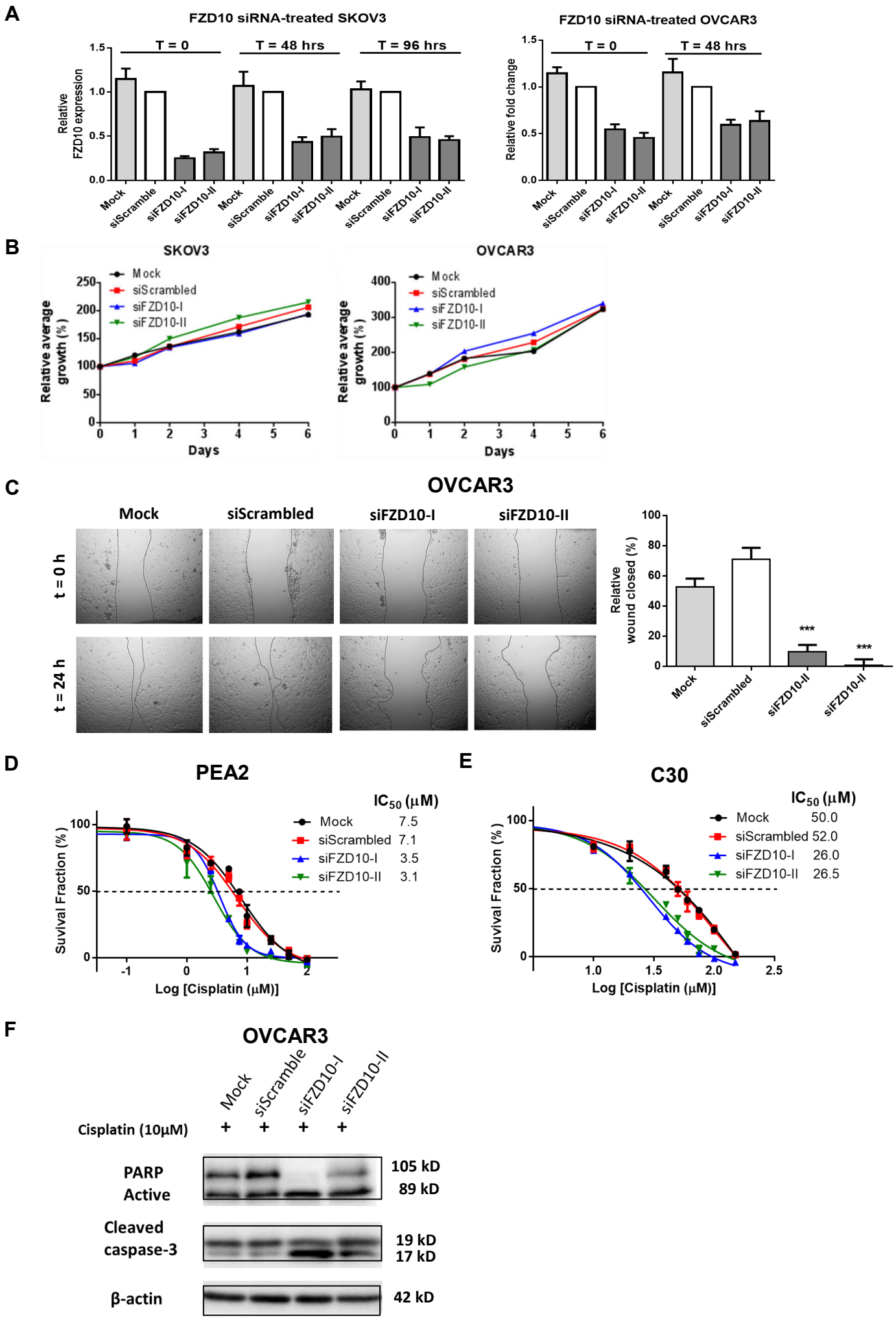
A
FZD10 siRNA-treated SKOV3
T = 0
T = 48 hrs
T = 96 hrs
Relative FZD10 expression
Mock
siScramble
siFZD10-I
siFZD10-II
FZD10 siRNA-treated OVCAR3
Relative fold change
B
SKOV3
OVCAR3
Mock
siScrambled
siFZD10-I
siFZD10-II
Relative average growth (%)
Days
C
OVCAR3
Mock
siScrambled
siFZD10-I
siFZD10-II
t = 0 h
t = 24 h
Relative wound closed (%)
***
D
PEA2
IC50 (μM)
Mock 7.5
siScrambled 7.1
siFZD10-I 3.5
siFZD10-II 3.1
Suvival Fraction (%)
Log [Cisplatin (μM)]
E
C30
IC50 (μM)
Mock 50.0
siScrambled 52.0
siFZD10-I 26.0
siFZD10-II 26.5
F
OVCAR3
Mock
siScramble
siFZD10-I
siFZD10-II
Cisplatin (10μM)
PARP Active
105 kD
89 kD
Cleaved caspase-3
19 kD
17 kD
β-actin
42 kD

Figure S7. A) Relative mRNA expression of *FZD10* in SKOV3 and OVCAR3 cells treated with non-targeted scrambled siRNA (siScrambled) or two FZD10 targeted siRNAs (siFZD10-I and siFZD10-II), after 48 hrs of transfection (T=0), after 48 hrs of replating of siRNAs-treated cells (T=48 hrs) and after 96 hrs of replating (T=96 hrs). **B)** Growth curve of SKOV3 and OVCAR3 cells after treatment with siFZD10 in comparison to siScrambled or mock control over 6 days. **C)** Representative microphotographs (4x magnification) for wound healing assay on FZD10 siRNAs treated OVCAR3 cells for T=0 and T=24 hrs, along with the quantification of relative wound. Each bar represents % of wound closed ± SD from 3 independent experiments. *** $p<0.001$ for FZD10 siRNAs treated cells in comparison to the siScrambled, by student t-test. **D-E)** Short term MTT survival assay on siRNA treated PEA-2 **(D)** and C-30 cells **(E)** in the presence of cisplatin at indicated concentration after 96 hrs. * $p<0.05$; ** $p<0.01$ for siFZD10-I or siFZD10-II relative to scrambled control, Student t test. IC50 were calculated and are shown for each group in the inset. **F)** Protein levels of cleaved PARP and caspase 3 in OVCAR3 cells transiently transfected with either FZD10 siRNAs, along with treatment of cisplatin for 24 hrs for indicated concentrations.

Figure S8

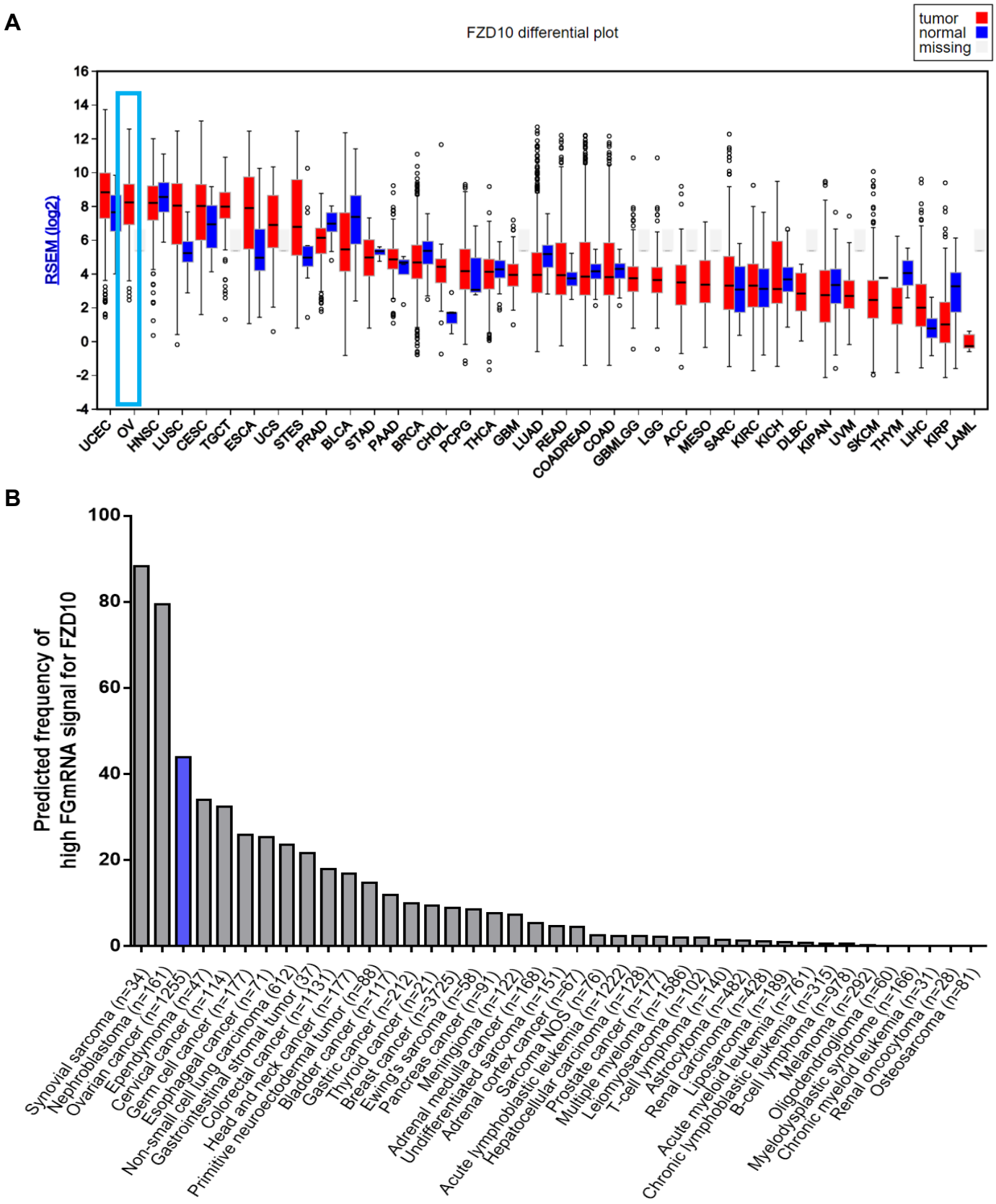

Figure S8. Global relative expression of *FZD10* **(A)** in different types of cancer based on the TCGA data*. **B)** *FZD10* predicted high expression level calculated with FGmRNA profiling [33] (see Material & Methods for details). The x-axis represents the percentage of samples per tumor type that show an overexpression of *FZD10*.

*Marx V. Drilling into big cancer-genome data. Nat Methods 2013;10:293–7