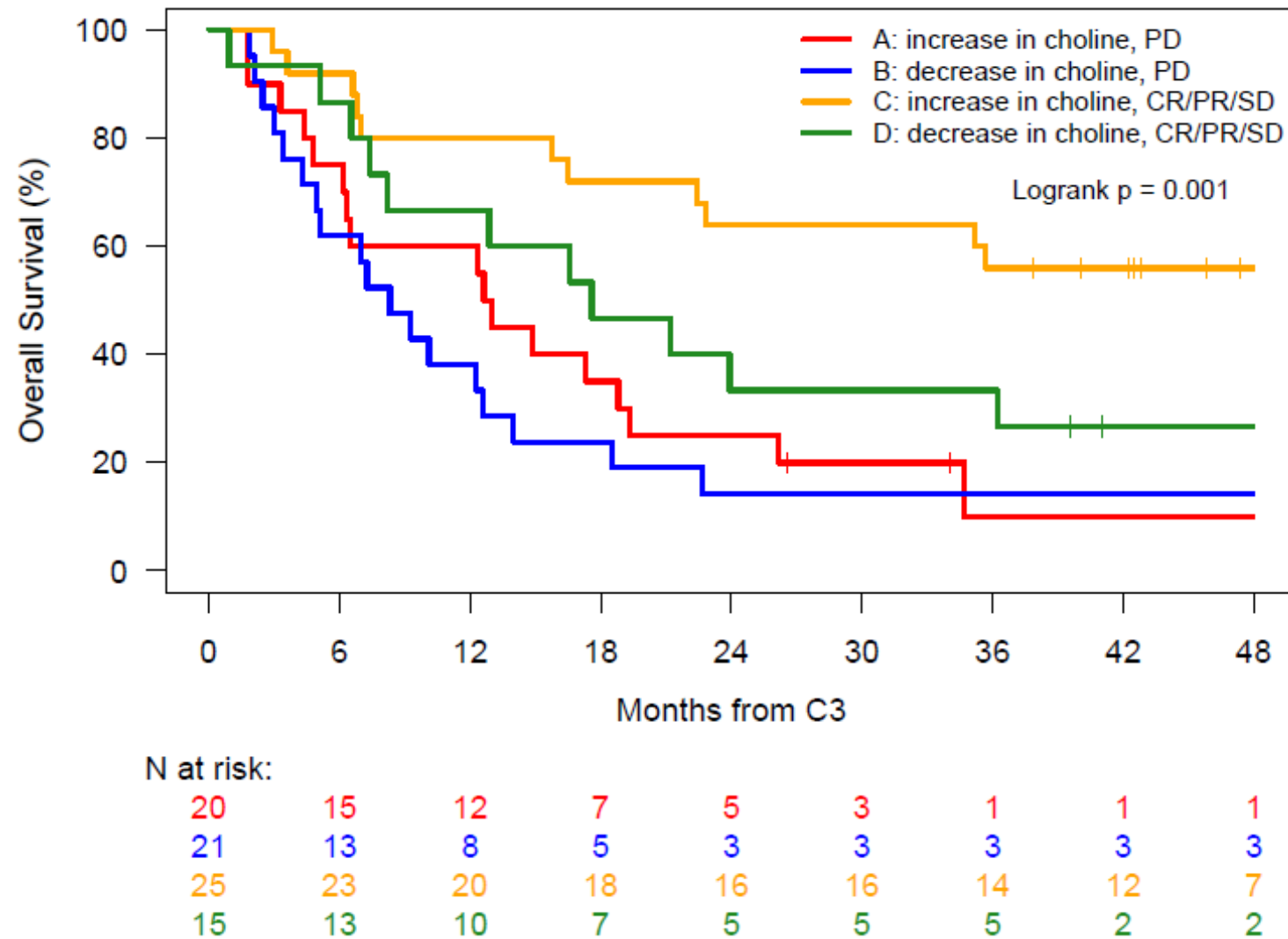
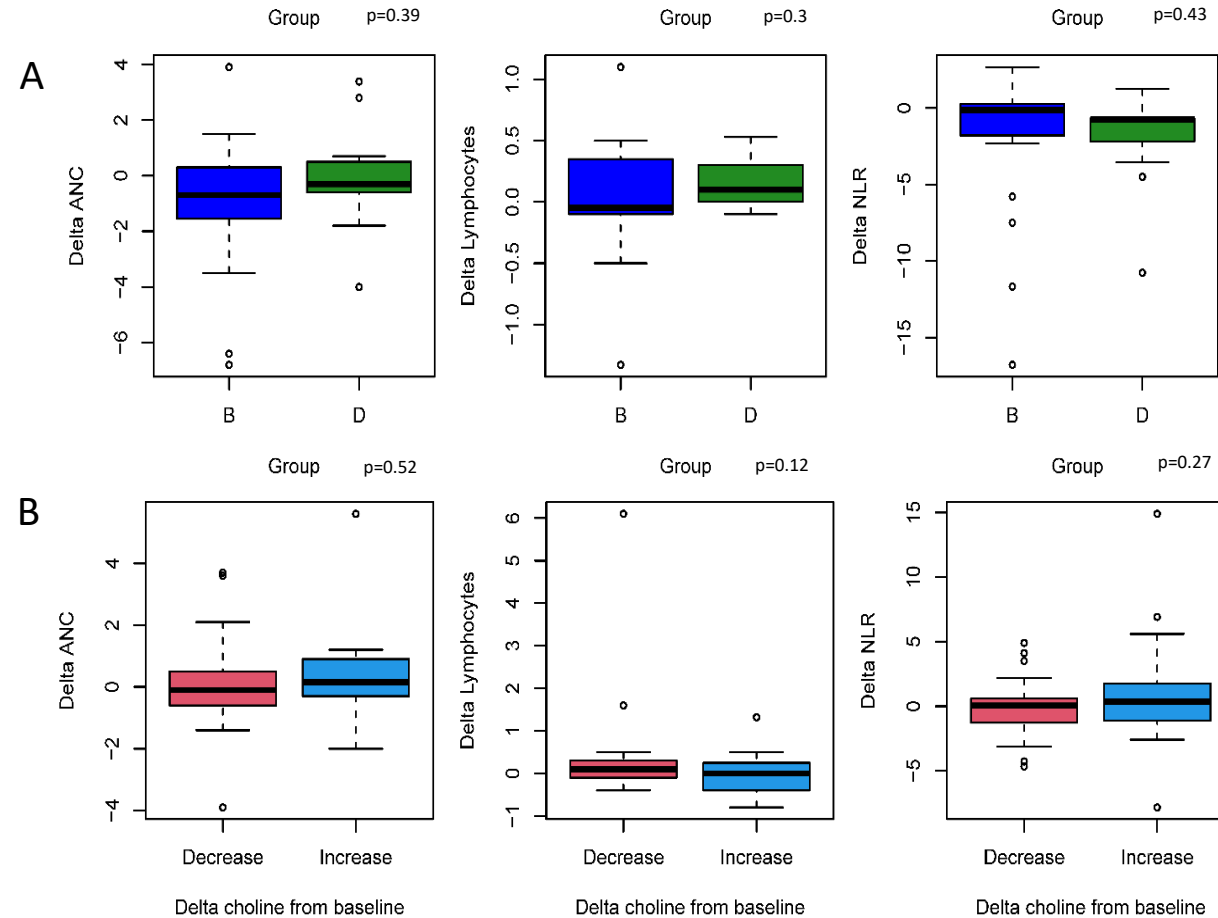


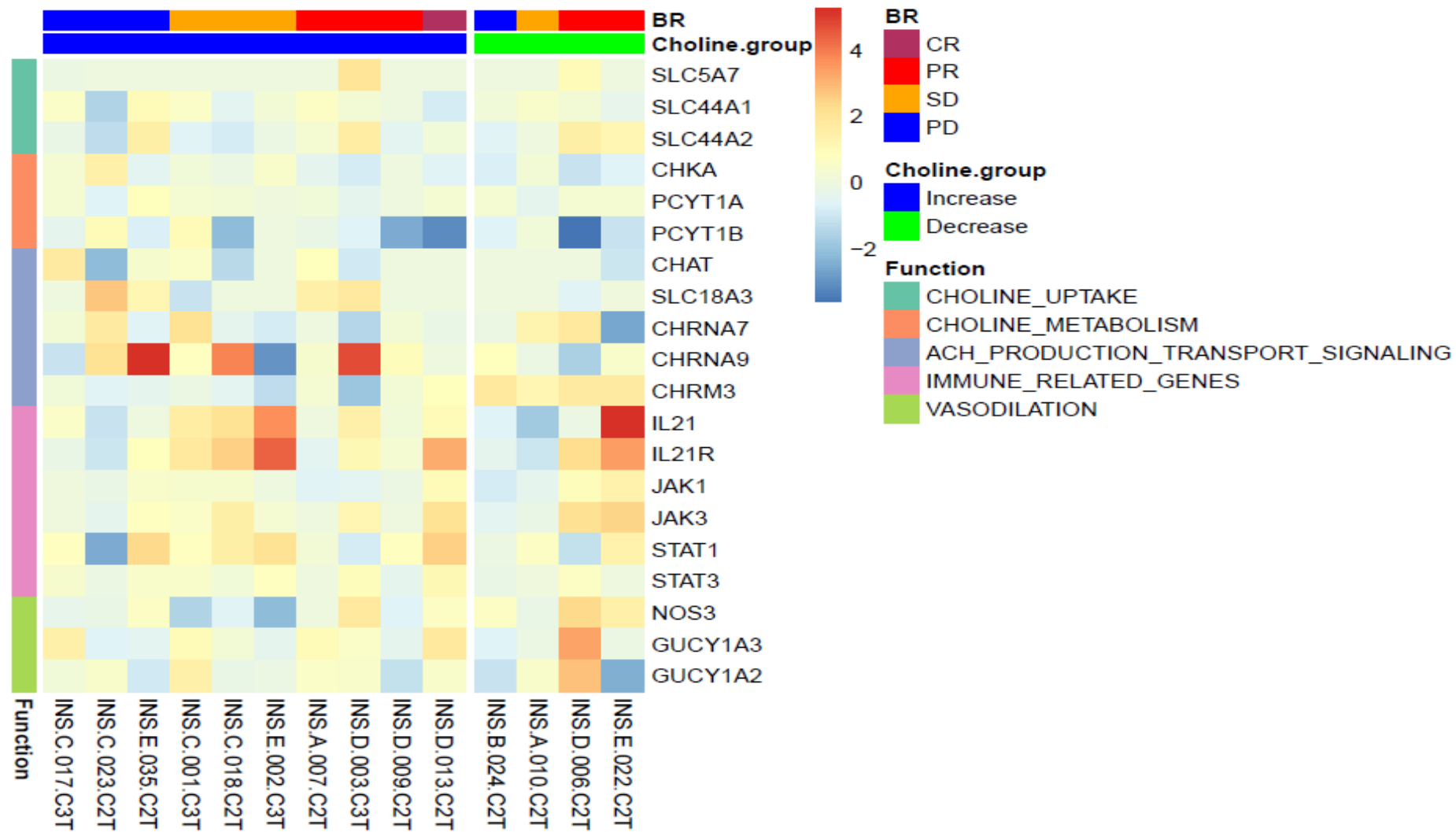
**Supplementary Figure 1.** Two chromatograms with choline at A) 100 and B) 1000 ng/ml.



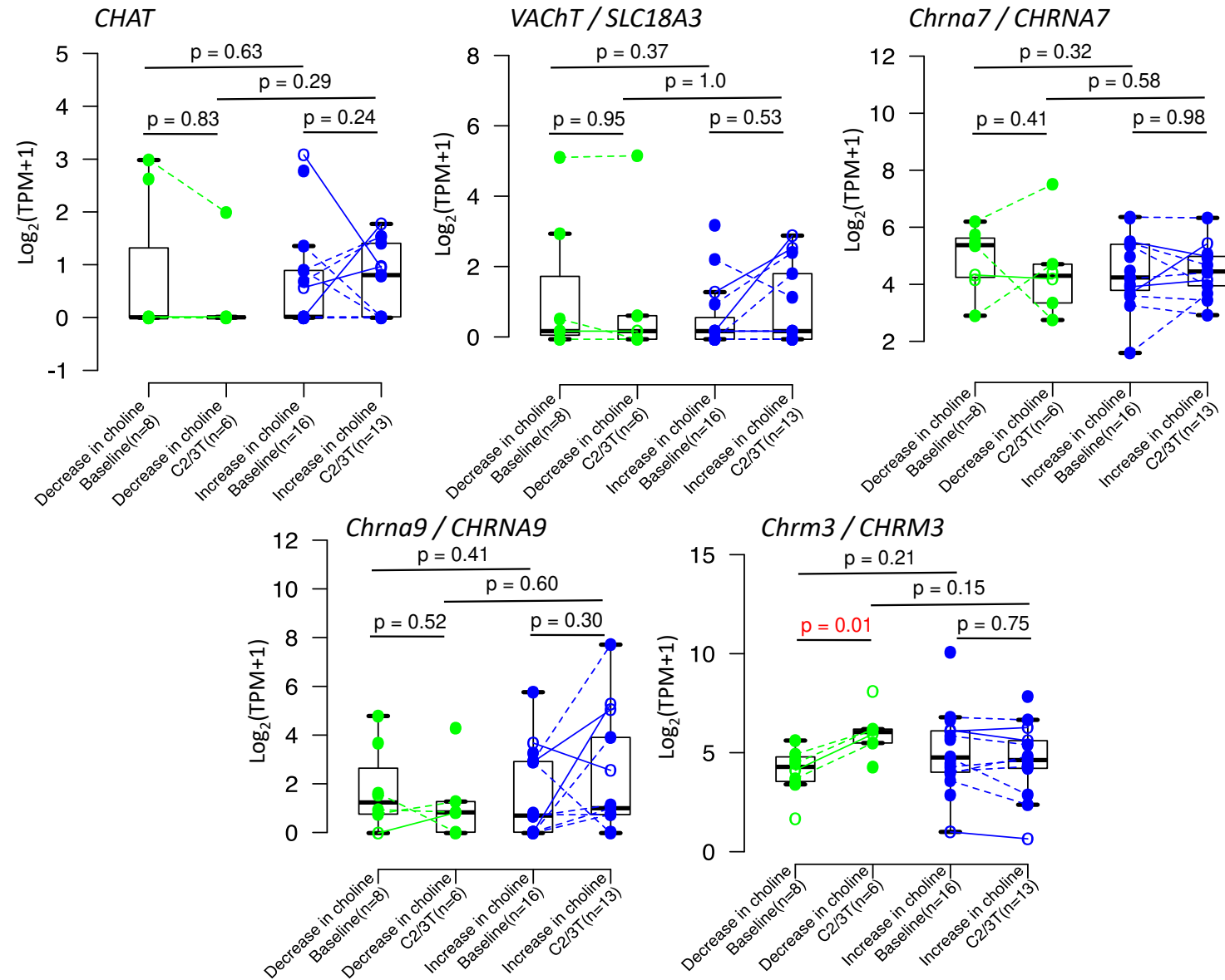
**Supplementary Figure 2.** Kaplan-Meier curve of OS in patients included in  $\Delta$ choline<sub>C3</sub> analysis (n=81) stratified according to cycle 3 RECIST response.



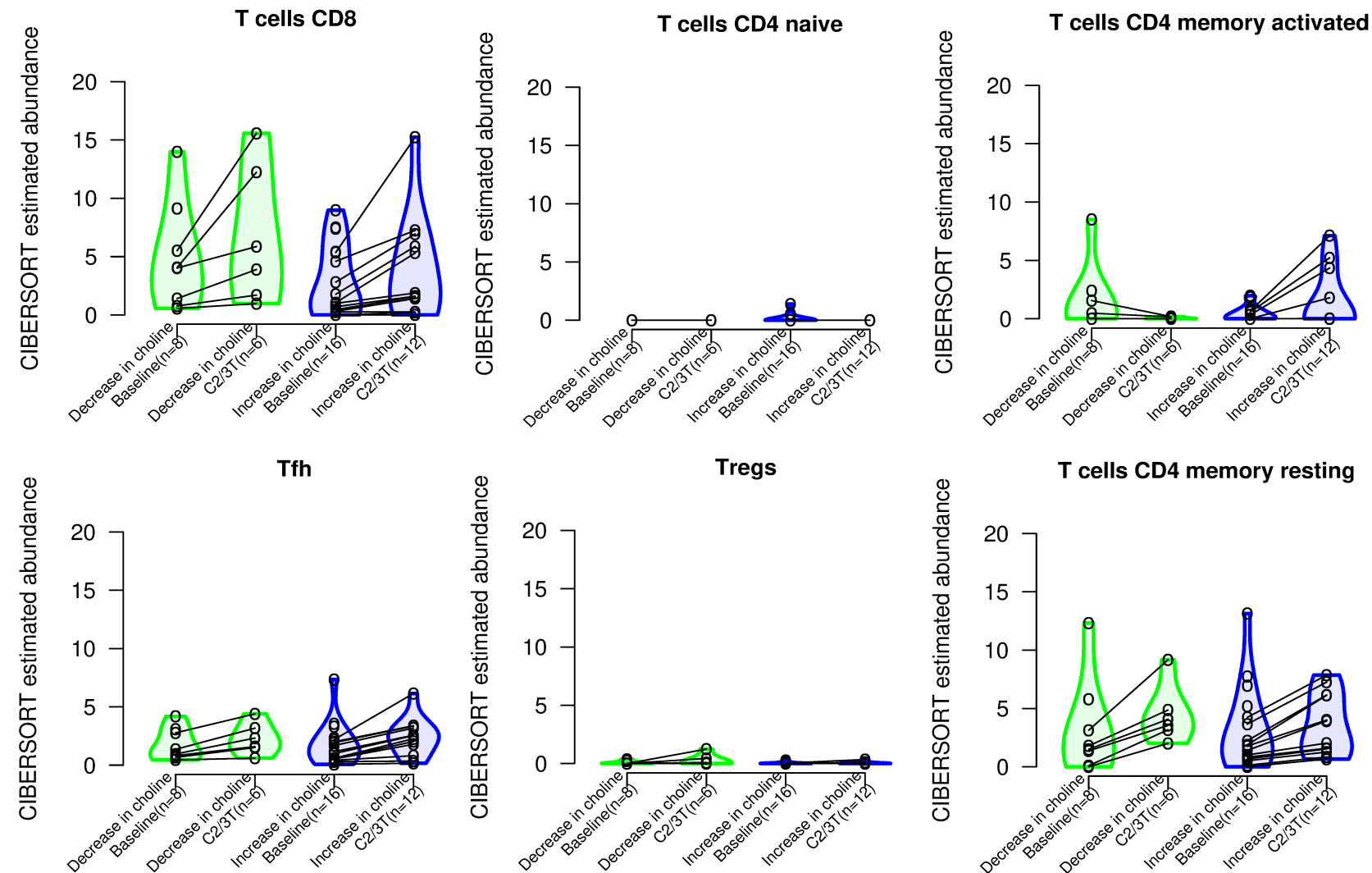
**Supplementary Figure 3. A.** Comparison of delta ANC, delta lymphocytes and delta NLR between blue (patients with a decrease in ctDNA and an increase in serum choline) and green (patients with a decrease in ctDNA and a decrease in choline) sample groups and statistical significance in INSPIRE. **B.** Comparison of delta ANC, delta lymphocytes and delta NLR between patients with decrease in delta choline (red) and increase in delta choline (blue) in the independent validation cohort.



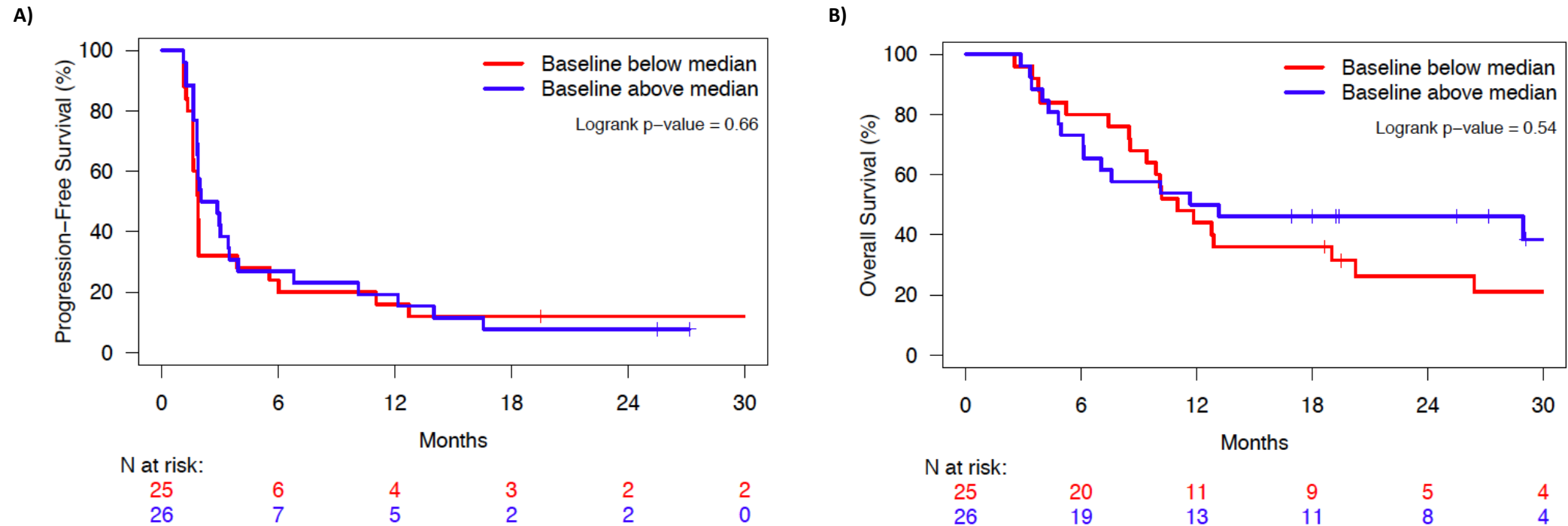
**Supplementary Figure 4.** Heatmap of Choline Gene Expression. Grouped according to according to metabolic function, RECIST response rates and blue (patients with a decrease in ctDNA and an increase in serum choline) and green groups (patients with a decrease in ctDNA and a decrease in choline).



**Supplementary Figure 5.** Comparison of gene expression between blue (patients with a decrease in ctDNA and an increase in serum choline) and green (patients with a decrease in ctDNA and a decrease in choline) sample groups and statistical significance.



**Supplementary Figure 6.** T cell inference comparisons from tumor biopsies in patients from blue (patients with a decrease in ctDNA and an increase in serum choline) and green (patients with a decrease in ctDNA and a decrease in choline) sample groups.



**Supplementary Figure 7.** Kaplan-Meier curves of PFS (A) and OS (B) among patients treated with at least one anti-PD-1 agent within early phase trials stratified at the median choline value at baseline.