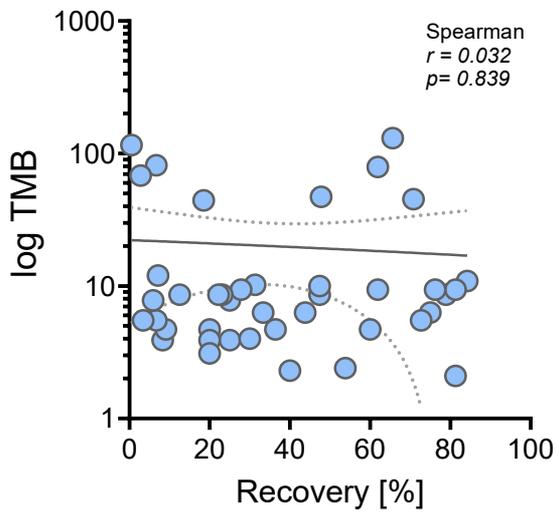


Supplementary Figure 1. Comparison of sequencing quality metrics of FFPE and Fresh Frozen (FF) CRC tissue samples using the TSO500 assay.

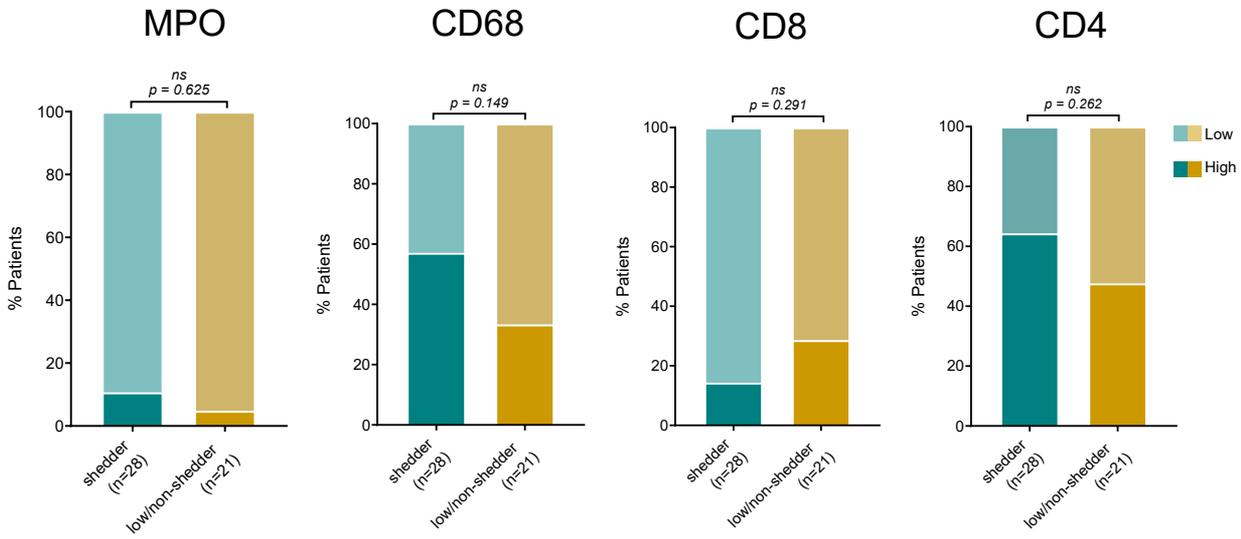
TSO500 pipeline (v1.1) calculated quality metrics of 6 matched FFPE and FF samples are shown. % Aligned Reads: Percentage of all identified reads aligned to HG19 reference; % Read Enrichment: proportion of reads aligning to TSO500 panel region; Median insert size: median DNA fragment length of sequenced inserts in bp; % Target 100x and 250x: percentage of targeted bases with coverage higher 100x or 250x respectively; % Unstable MSI sites: proportion of microsatellite loci classified as unstable; Tumor Mutational Burden: number of somatic mutations per megabase.



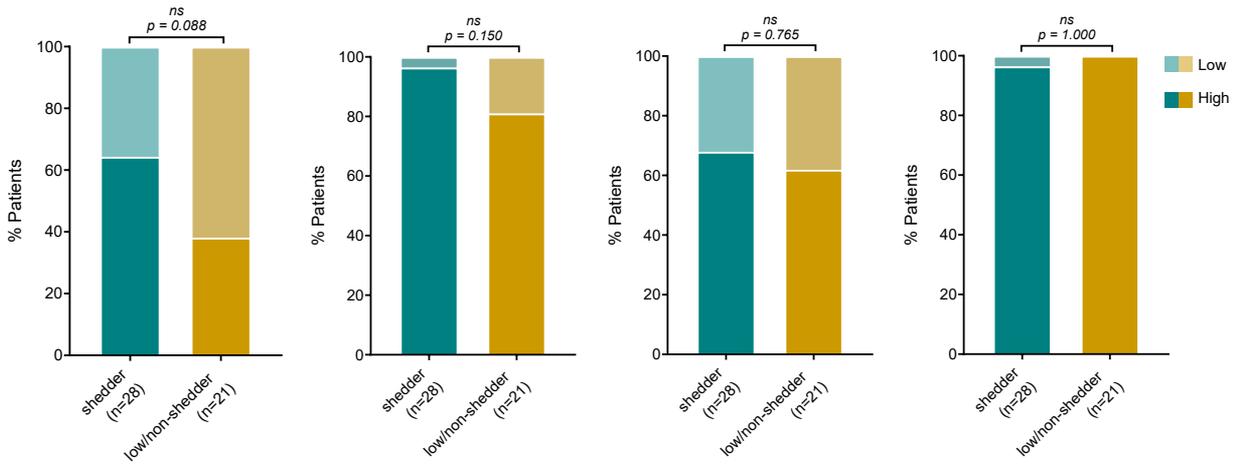
Supplementary Figure 2. Tumor mutational burden (TMB) does not correlate with variant recovery in plasma. Association of TMB with variant recovery in plasma is shown. R- and p-values were calculated using Spearman rank-correlation, a linear regression line with 95% confidence interval is shown for visual reference

a

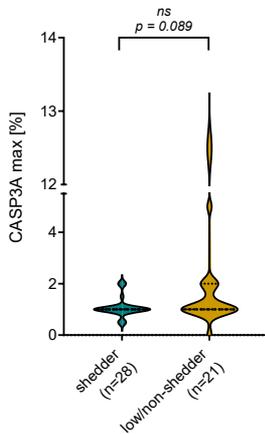
Center



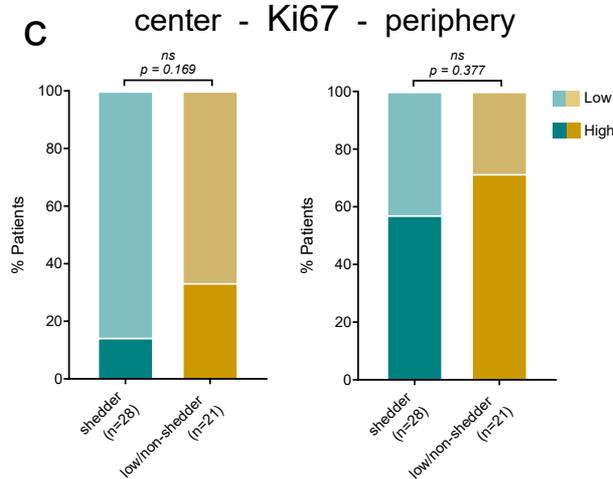
Invasive Front



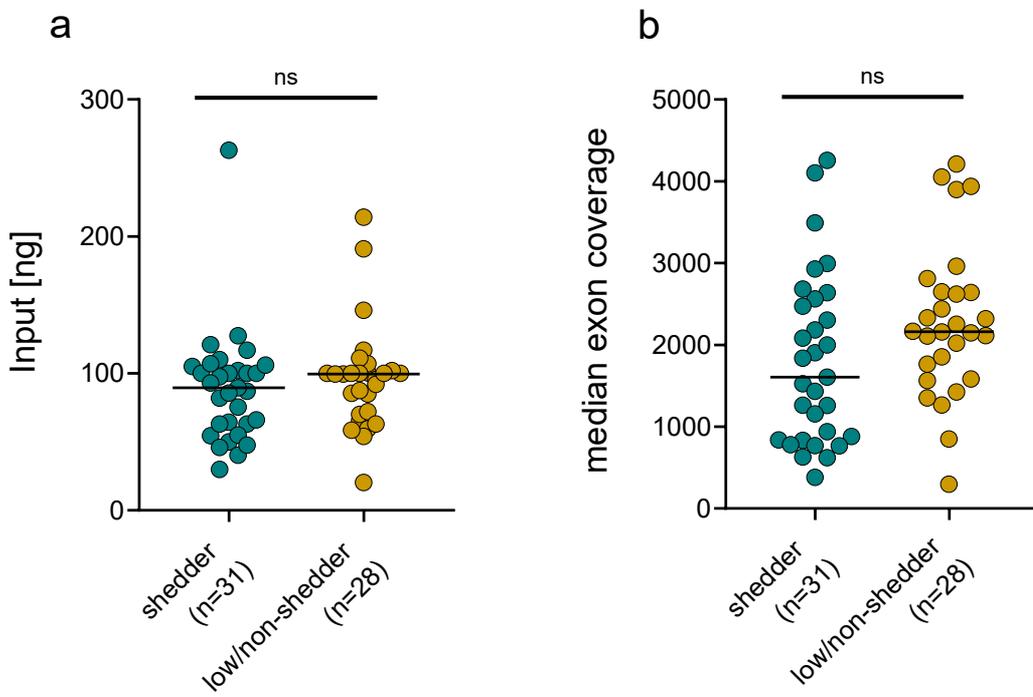
b



c



Supplementary Figure 3. Immune cell distribution and proliferative capacity at tumor center and invasive front of ctDNA shedders vs. low/non-shedders. a) Immunohistochemistry (IHC) assessed proportions of immune cells (MPO: neutrophils, CD68: macrophages, CD8: cytotoxic T-cells, CD4: helper T-cells) at tumor center and invasive front. Bars show relative distribution per shedding group. P-values were calculated from raw patient counts (not shown) using Fisher's exact test. b) Tumor-wide assessment of apoptosis using cleaved Caspase 3 (CASP3A) staining is shown, values indicate percentage of positive cells relative to the area with maximal expression. P-values were calculated using unpaired two-tailed Mann-Whitney U test. c) Assessment of proliferation using Ki67-IHC in tumor center and peripheral regions. Bars show relative distribution per shedding group. P-values were calculated from raw patient counts (not shown) using Fisher's exact test



Supplementary Figure 4. TSO500 library input and median exon coverage for cfDNA libraries. a) cfDNA input amount used for TSO500 library preparation in ctDNA shedder (n=31) and low/non-shedder (n=28) b) Median exon coverage obtained from sequenced plasma cfDNA libraries calculated by the TSO500 pipeline. Horizontal lines indicate mean values. Statistical comparisons were performed using two-sided Mann-Whitney U test