SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Estimates of the total number of cfDNA template copies per ddPCR reaction (dark gray bars) and DNA purification efficiency (dotted line) (CPP1 control) for each plasma sample for patients with residual disease and/or recurrence. (A-F) cfDNA and purification efficiency estimates for pt. 4, 8, 16, 10 18, and 15 respectively. The cfDNA template copies were calculated as the average of the absolute template copy numbers determined using two reference ddPCR assays targeting reference regions on chromosomes 3 and 7. These cfDNA quantities are used to estimate the minimal ctDNA/cfDNA ratio detectable for each sample. For a sample with 500 cfDNA template copies per reaction a positive detection of ctDNA is unlikely if the actual ctDNA/cfDNA fraction in that sample is less than 1/500 (0.2%). We use the measure to estimate the likelihood of a given plasma sample to be truly negative if our ctDNA specific ddPCR assays do not give a signal. We also use it to evaluate if the sensitivity and specificity of our ctDNA ddPCR assays are sufficient for detection of the ctDNA present in a sample, if any. Light grey bars indicate samples that tested positive using the ddPCR lymphocyte DNA contamination assay. In such samples our QC approach over-estimates the minimal ctDNA/cfDNA ratio detectable. ctDNA positive samples are indicated by an asterisk.

Supplementary Figure 2. Estimates of the total number of cfDNA template copies per ddPCR reaction (dark gray bars) and DNA purification efficiency (CPP1 control) (dotted line) for each plasma sample for patients with no residual disease and/or recurrence. (A-E) cfDNA and purification efficiency estimates for pt. 1, 2, 5, 6, and 19 respectively. The cfDNA template copies were calculated as the average of the absolute template copy numbers determined using two reference ddPCR assays targeting reference regions on chromosomes 3 and 7. These cfDNA quantities are used to estimate the minimal ctDNA/cfDNA ratio detectable for each sample. For a sample with 500 cfDNA template copies per reaction a positive detection of ctDNA is unlikely if the actual

ctDNA/cfDNA fraction in that sample is less than 1/500 (0.2%). We use the measure to estimate the likelihood of a given plasma sample to be truly negative if our ctDNA specific ddPCR assays do not give a signal. We also use it to evaluate if the sensitivity and specificity of our ctDNA ddPCR assays are sufficient for detection of the ctDNA present in a sample, if any. ctDNA positive samples are indicated by an asterisk.

Supplementary Figure 3. Boxplot showing genome equivalents of cell free DNA analyzed per SSV. (A) Boxplot of individual patients without residual disease and/or recurrence. (B) Boxplot of individual patients with residual disease and/or recurrence. (C) Boxplot of patients with or without residual disease and/or recurrence.

Supplementary Figure 4. Clinical application of cfDNA for monitoring colorectal cancer patients in patients with residual disease and/or recurrence. cfDNA was isolated from serial plasma samples collected every third month starting prior to surgery and ending at month 36 post-surgery or in relation to recurrence of disease. Shown are the results of monitoring the level of cfDNA in patients surgically treated with curative intend for: (A) A stage III colon cancer (Pt. 4); (B) A stage I rectum cancer (Pt. 16); (C) A stage III rectum cancer (Pt. 8); (D) A stage IV rectum cancer with focal metastasis in the lung. The initial treatment included resection of the primary tumor and radio frequency ablation to eliminate the lung metastasis (Pt. 10); (E) A stage IV colon cancer with focal metastasis in the liver. The initial treatment included a colon resection to remove the primary tumor and a partial hepatectomy to eliminate the liver metastasis (Pt. 18); (F) A stage II rectum cancer with subsequent local recurrence treated with radiation therapy (Pt. 15). The quantified levels of cfDNA are plotted as cfDNA GEs. Vertical dotted lines indicate surgery or RFA. Grey shaded regions indicate chemotherapy. Arrows indicate radiological and molecular examinations and they were negative unless specified otherwise. Blue shaded regions indicate lead time decided by detection of ctDNA. * QC indicates contamination with lymphocyte DNA.

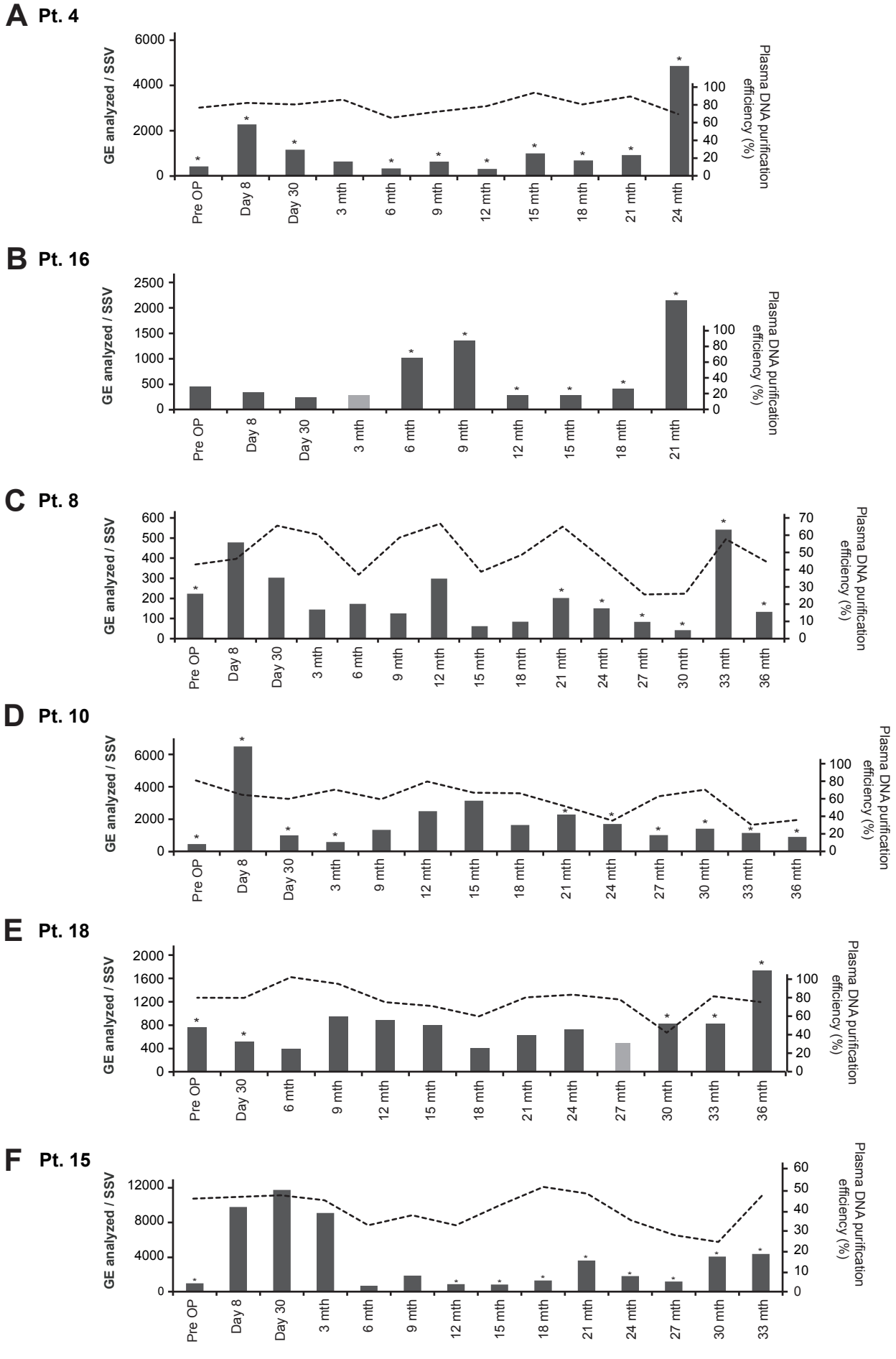
Supplementary Figure 5. Clinical application of cfDNA for monitoring colorectal cancer patients in patients without residual disease and/or recurrence. cfDNA was isolated from serial plasma samples collected every third month starting prior to surgery and ending at month 36 post-surgery or in relation to recurrence of disease. Shown are the results of monitoring the level of cfDNA and ctDNA in patients surgically treated with curative intend for: (A) A stage II colon cancer (Pt. 1); (B) A stage II rectum cancer (Pt. 2); (C) A stage II colon cancer (Pt. 5); (D) A stage II rectum cancer (Pt. 6); (E) A stage IV colon cancer with focal metastasis in the liver. The initial treatment included a colon resection to remove the primary tumor and radiofrequency ablation of liver metastasis (Pt. 19). The quantified levels of cfDNA are plotted as cell-free GEs. Data are only shown for informative assays (See Supplementary Table 4 for a complete list of assays). Vertical dotted lines indicate surgery or RFA. Grey shaded regions indicate chemotherapy. Arrows indicate radiological and molecular examinations and they were negative unless specified otherwise.

Supplementary Figure 6. Correlation between cfDNA and ctDNA estimates. The correlation between cfDNA and ctDNA levels was analyzed by scatter plot and Spearman correlation for (A) All plasma samples, (B) All ctDNA positive plasma samples, (C) All ctDNA positive samples with less than 63.9 ctDNA GEs/mL plasma, (D) All ctDNA positive samples with more than 63.9 ctDNA GEs/mL plasma.

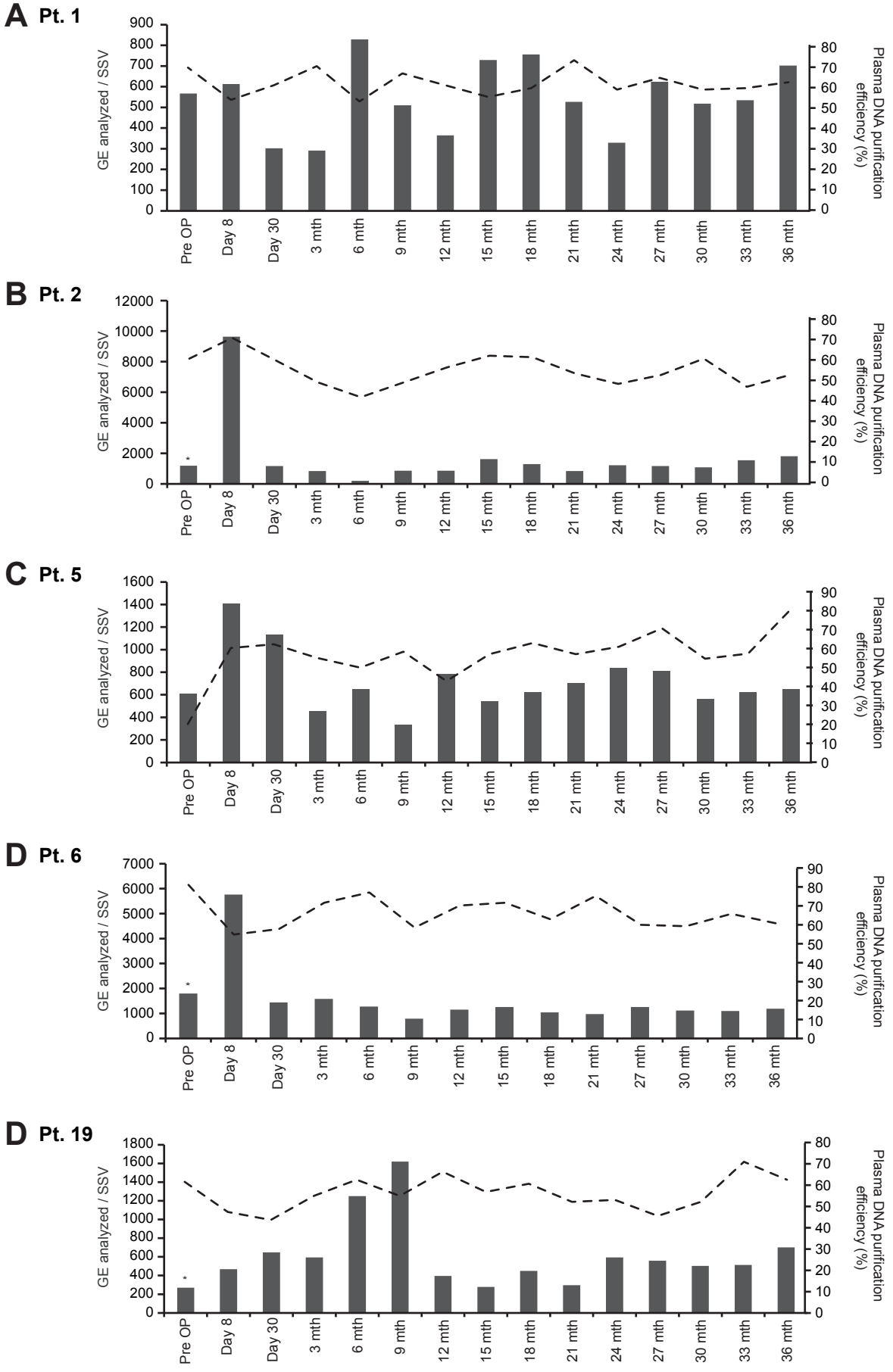
Supplementary Figure 7. cfDNA levels in ctDNA negative plasma samples. cfDNA GE per mL plasma is plotted for all ctDNA negative samples according to time of sampling. Black dots indicate plasma samples with less than 5000 GEs cfDNA. Green dots indicate samples with more than 5000 GEs cfDNA, but no obvious course. Orange dots indicate samples with more than 5000 GEs cfDNA in patients with complications related to surgery. Purple dots indicate samples with more than 5000 GEs cfDNA in patients with trauma from the resection at day 8 post OP. Blue dots

indicate samples with more than 5000 GEs cfDNA and from a patient suffering from CLL. Gray dots indicate samples with more than 5000 GEs cfDNA due to chemotherapy.

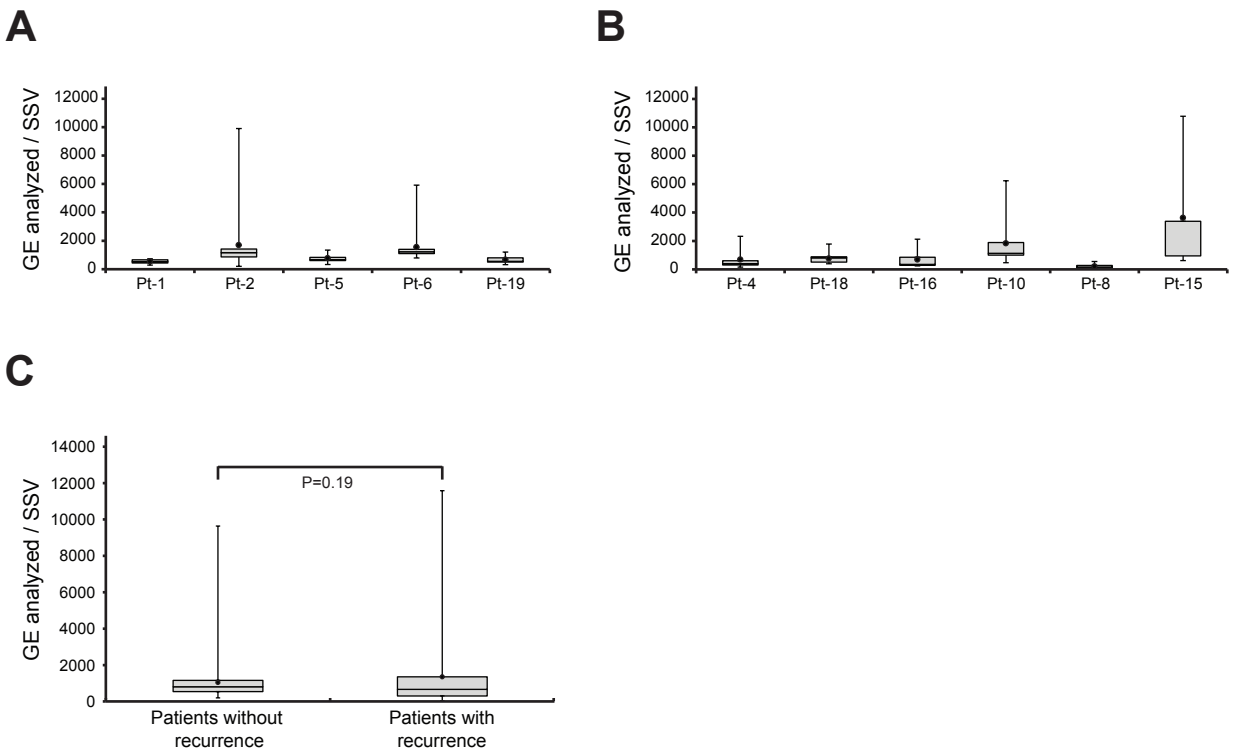
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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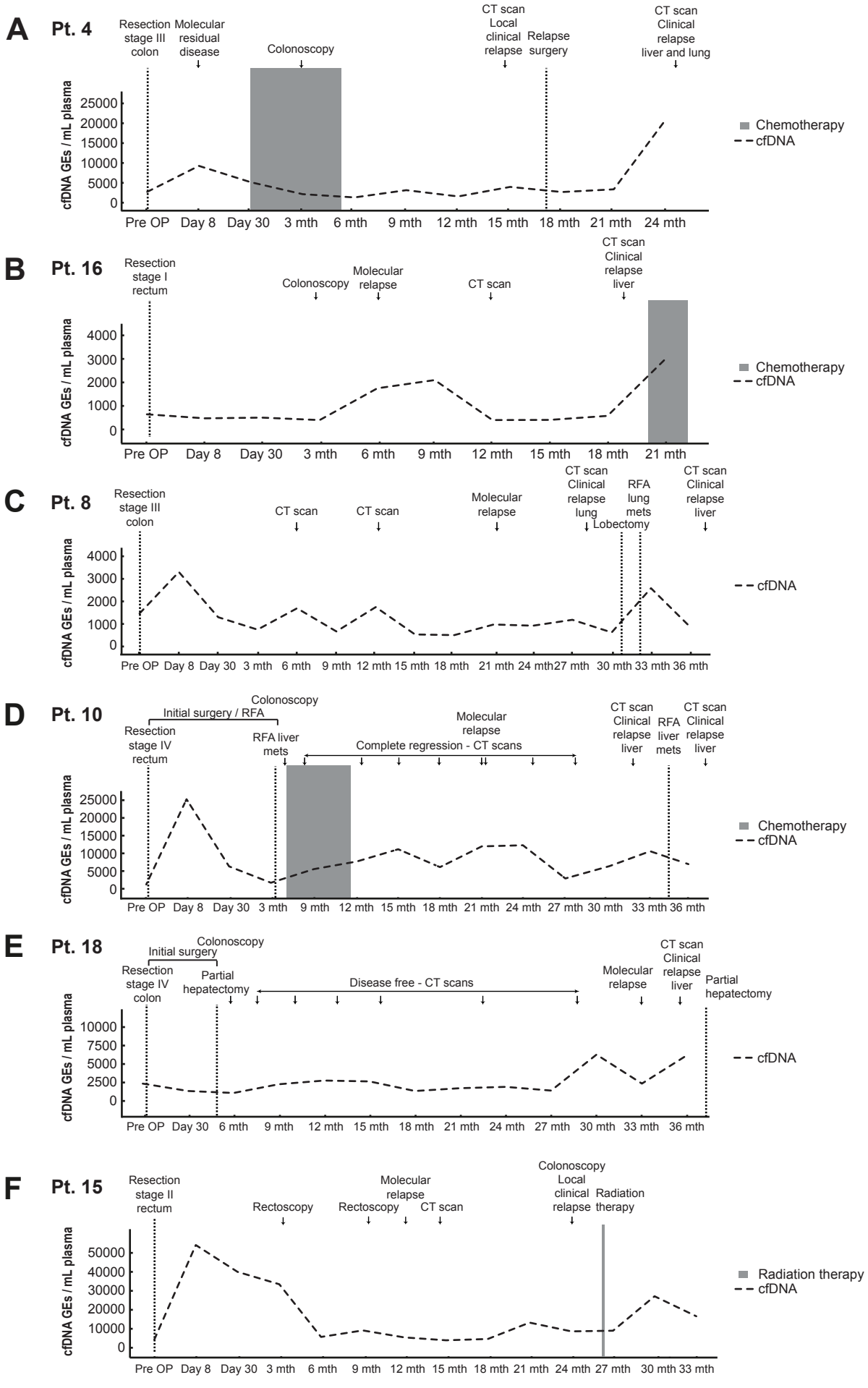
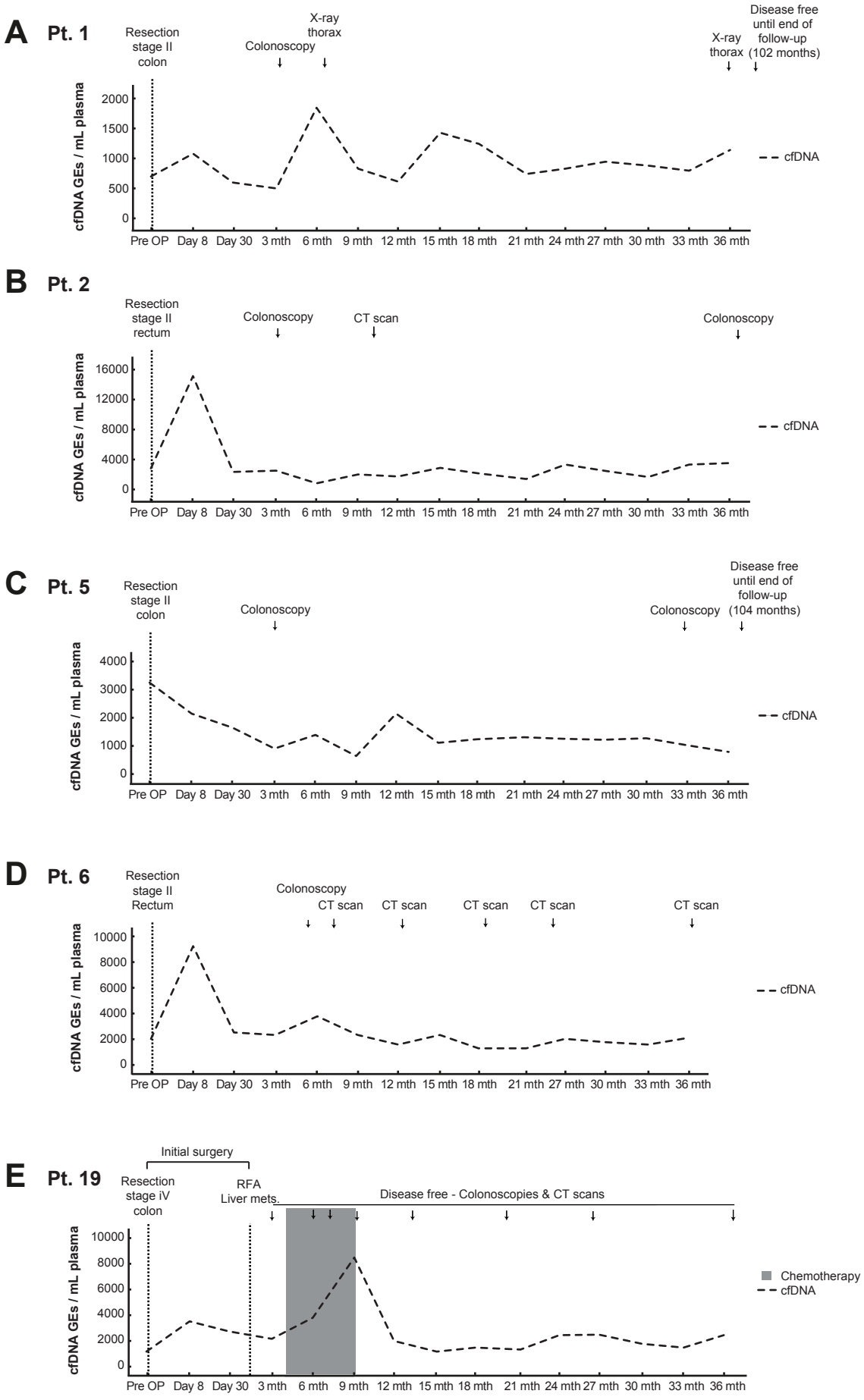
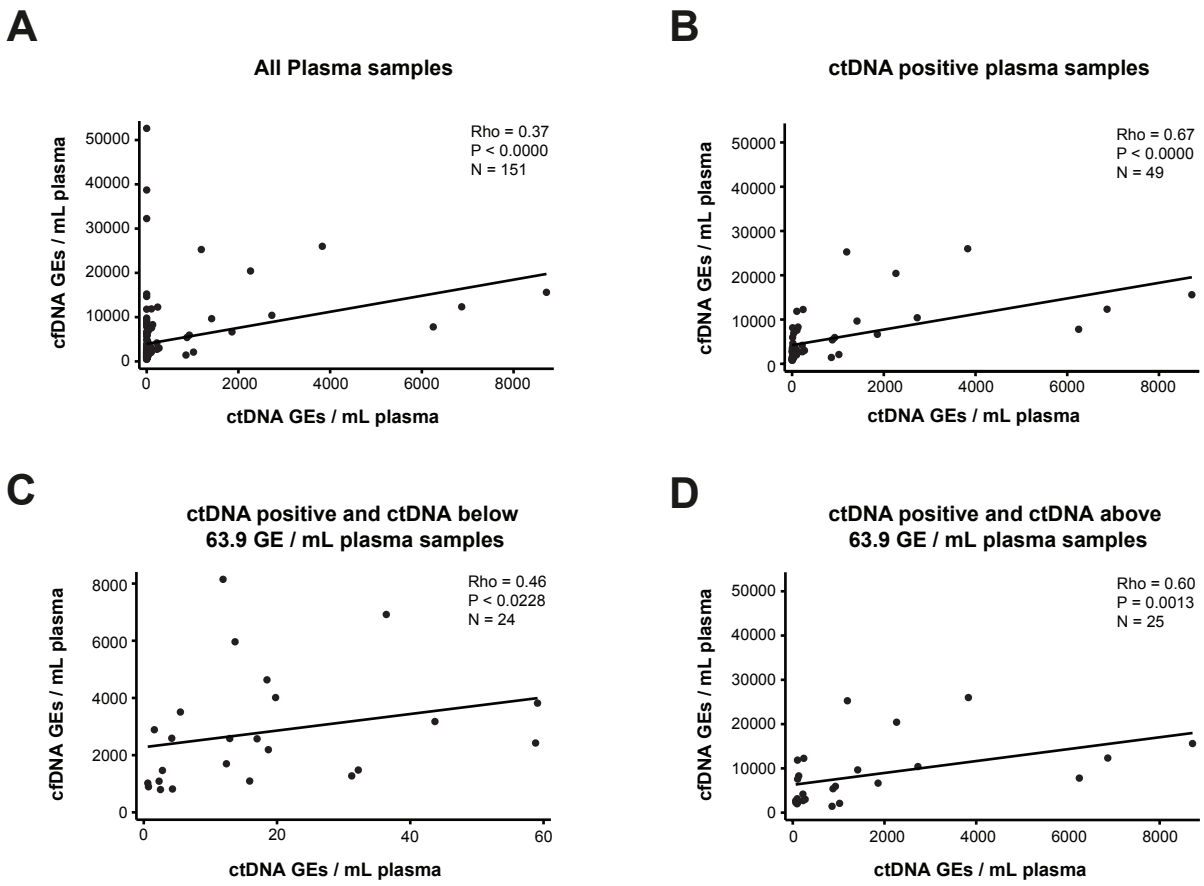


Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7