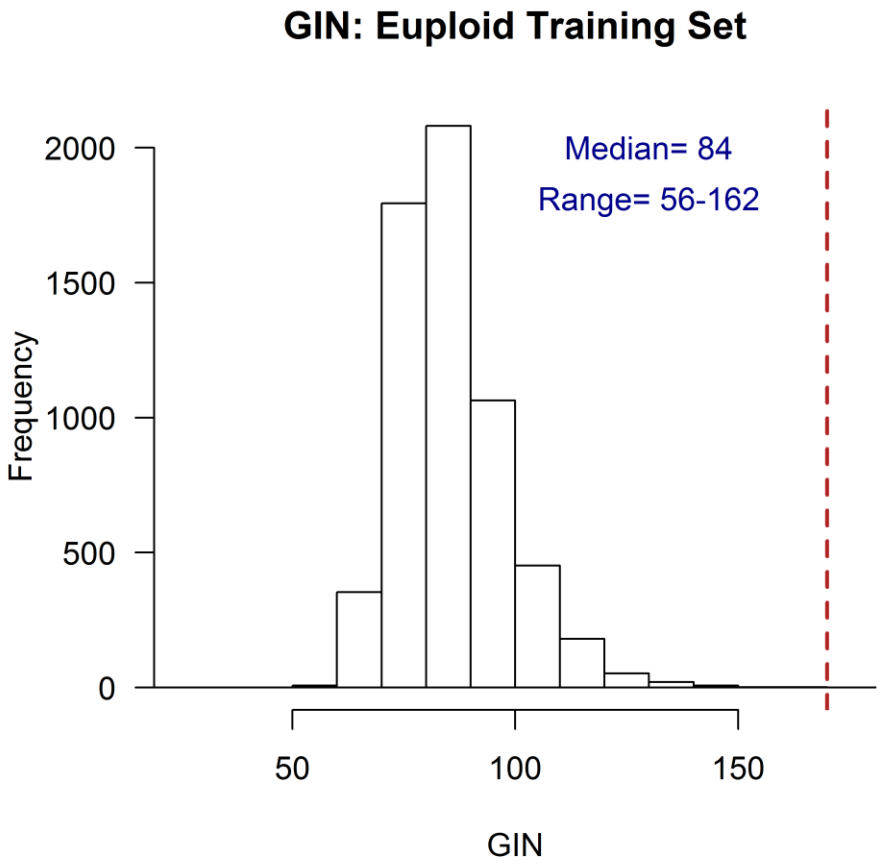
Supplementary Table 1. Patient clinical characteristics (N=44 patients)

Patient number	Diagnosis	Immunotherapy Treatment	PFS (months)*	Best Response	Number Aliquots	Baseline GIN	Baseline total cfDNA (GE)	Comments
1	Basal cell carcinoma	Nivolumab	18.43+	PR	19	167	2052	
19	Hepatocellular carcinoma	Nivolumab	0.8	PD	2	987	84013	
20	NSCLC adenocarcinoma	Nivolumab	1.4	PD	2	122	2255	
24	NSCLC adenocarcinoma	Nivolumab	13.5	SD	17	126	18013	
26	Appendiceal cancer	Pembrolizumab	0.9	PD	1	115	2145	
29	Endometrial stromal sarcoma	Nivolumab + SBRT	1.5	PD	4	145	743	Hyper-progresson
30	Head and neck SCC	Pembrolizumab	5.9	SD	12	221	206	
31	Glioblastoma	Nivolumab + Temozolomide	2.8	PD	11	130	660	
32	NSCLC adenocarcinoma	Nivolumab	2.1	PD	3	111	5995	
34	Melanoma	Pembrolizumab	8.83+	PR	11	128	1980	
35	NSCLC adenocarcinoma	Nivolumab	15.67+	PR	16	122	1815	
40	Cutaneous SCC	Pembrolizumab	13.40+	CR	11	4654	14823	
40B	Bladder Urothelial Carcinoma	Atezolizumab + SBRT	1.8	PD	3	186	2002	Hyper-progression
43	Melanoma	Ipilimumab/Nivolumab	13.50+	CR	16	108	1513	
47	Melanoma	Pembrolizumab	3.0	SD	5	127	996	
51	Ocular melanoma	Ipilimumab/Nivolumab	12.0	SD	10	5901	13420	
52	Melanoma	Pembrolizumab	6.37+	SD	5	133	2750	
55	Melanoma	Nivolumab/Ipilimumab	10.03+	CR	3	205	1084	
58	Cervical adenocarcinoma	Pembrolizumab	2.8	PD	1	104	1260	
64	Melanoma	Pembrolizumab	1.6	PD	3	86	1683	
65	Colorectal adenocarcinoma	Nivolumab + SBRT	2.3	PD	3	3164	891	
67	NSCLC SCC	Nivolumab + SBRT	1.7	PD	2	610	2673	
69	NSCLC adenocarcinoma	Pembrolizumab	4.1	SD	4	256	3135	
70	Basal Cell Carcinoma	Pembrolizumab	2.5	PD	2	1815	1397	
75	Breast Cancer	Nivolumab	0.1	PD	1	4152	67375	

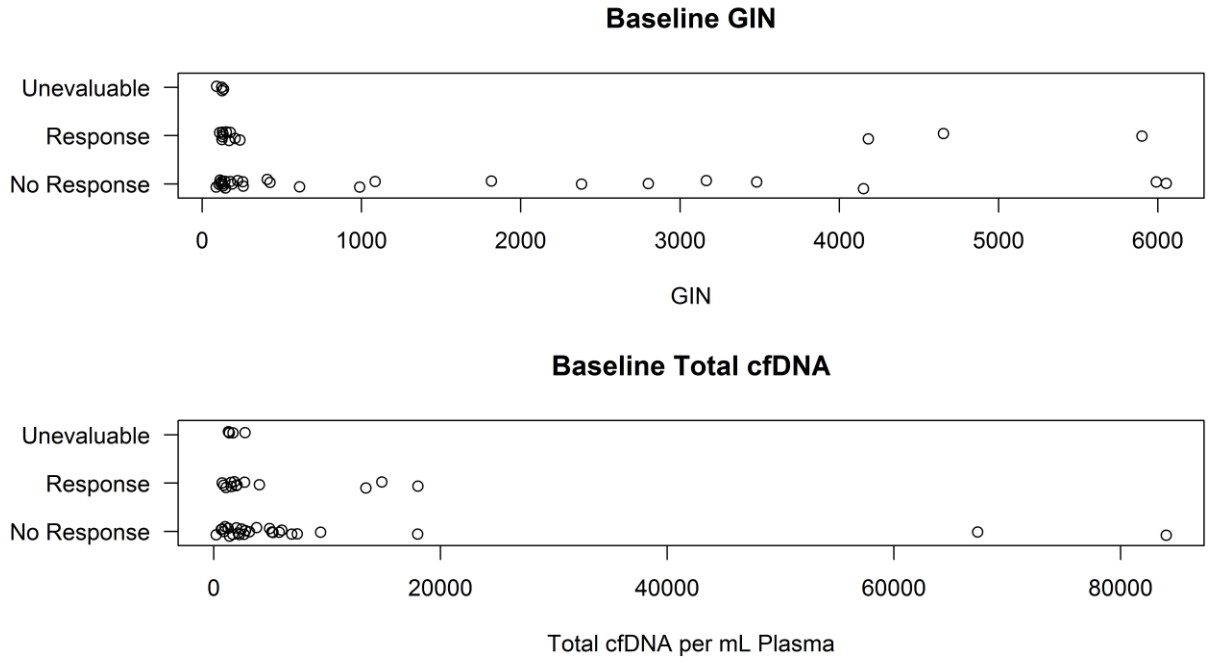
77	Bladder Urothelial Carcinoma	Atezolizumab	1.2	PD	1	2381	5115	
78	Esophageal Cancer SCC	Nivolumab	8.90+	PR	10	130	2723	
79	Endometrial sarcoma	Nivolumab + Vismodegib + Trametinib + anastrozole	1.9	PD	3	408	5775	
82	Thyroid Cancer	Pembrolizumab	1.9	PD	3	142	2448	
83	Ovarian Cancer	Nivolumab + SBRT	0.9	PD	2	2800	4923	
84	Melanoma	Nivolumab	0.7	PD	1	6053	6875	
90	Cutaneous SCC	Pembrolizumab	6.80+	SD	4	124	1705	
97	Adenoid cystic carcinoma of base of tongue	Pembrolizumab + SBRT	5.83+	SD	5	90	1375	
98	Spindle Cell Sarcoma	Pembrolizumab	5.80+	SD	3	121	1293	
100	Neuroendocrine carcinoma of unknown primary	Nivolumab + trametinib	2.2	PD	4	5990	2791	
104	Colorectal adenocarcinoma	Pembrolizumab	2.1	PD	2	3481	17978	
108	Breast Cancer	Pembrolizumab	0.9	PD	1	425	7356	
110	Esophageal Cancer	Nivolumab	4.0	SD	4	1085	9419	
111	Breast Cancer	Pembrolizumab	5.5	SD	4	254	3781	
114	NSCLC SCC	Nivolumab	5.1	PR	4	175	729	
121	NSCLC adenocarcinoma	Atezolizumab	3.67+	PR	2	150	873	
125	GE junction adenocarcinoma	Pembrolizumab + SBRT	2.0+	PR	2	4182	4022	Pseudo-progression
126	Breast Cancer	Nivolumab	2.40+	CR	2	236	1550	
132	GE junction adenocarcinoma	Pembrolizumab	1.5	PD	1	176	5225	

***PFS values with a (+) indicate response is ongoing at time of data censoring**

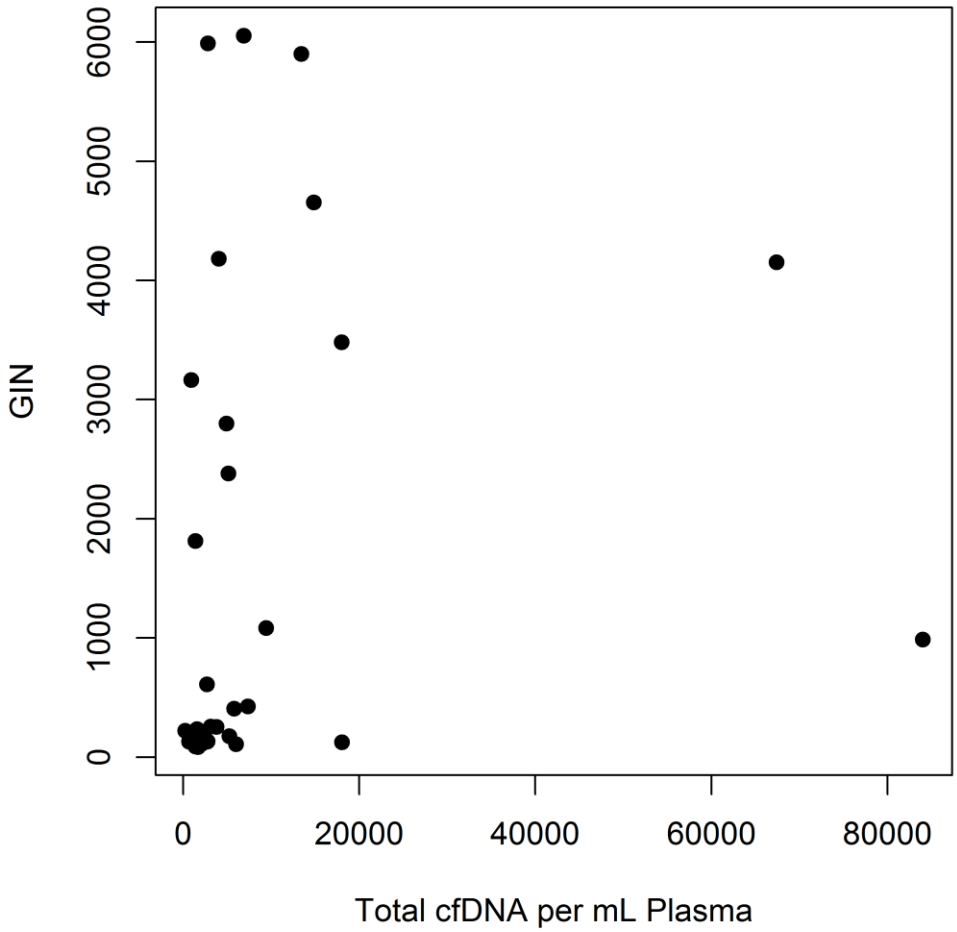
Abbreviations: cfDNA = cell-free DNA; CR = complete response; GE = gene equivalents per mL; GE junction = gastroesophageal junction; GIN = genome instability number; NSCLC = non-small cell lung cancer; PFS = Progression-free survival; PR = partial response; SBRT = stereotactic body radiation therapy; SCC = squamous cell cancer; SD = stable disease



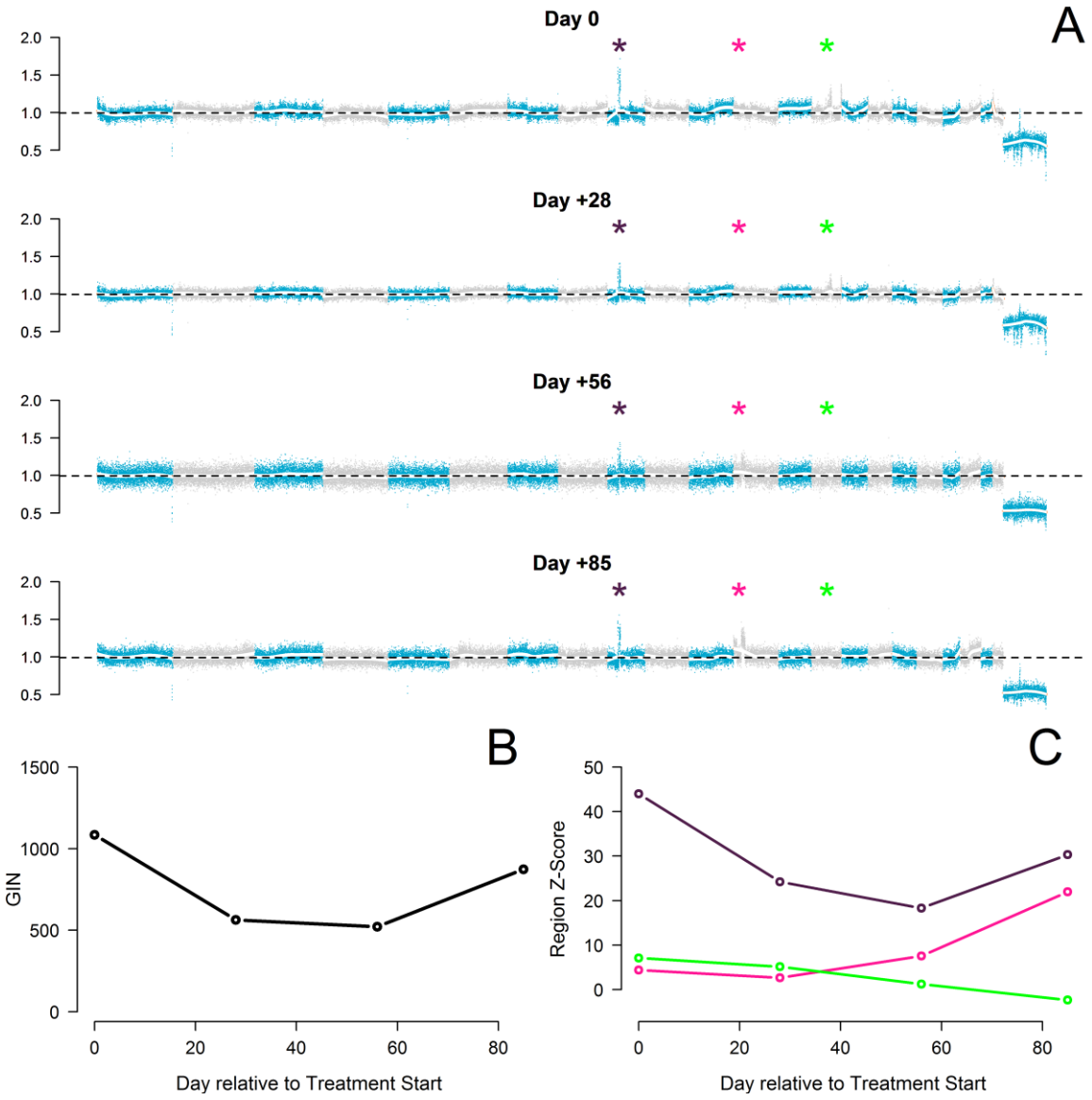
Supplementary Figure 1. GIN values for 6014 samples submitted for noninvasive prenatal testing for which no CNAs were detected using NIPT algorithms. Red dashed line represents the established threshold of a GIN value of 170 based on empirical distribution resulting in no GIN values higher than 170 in this training cohort.



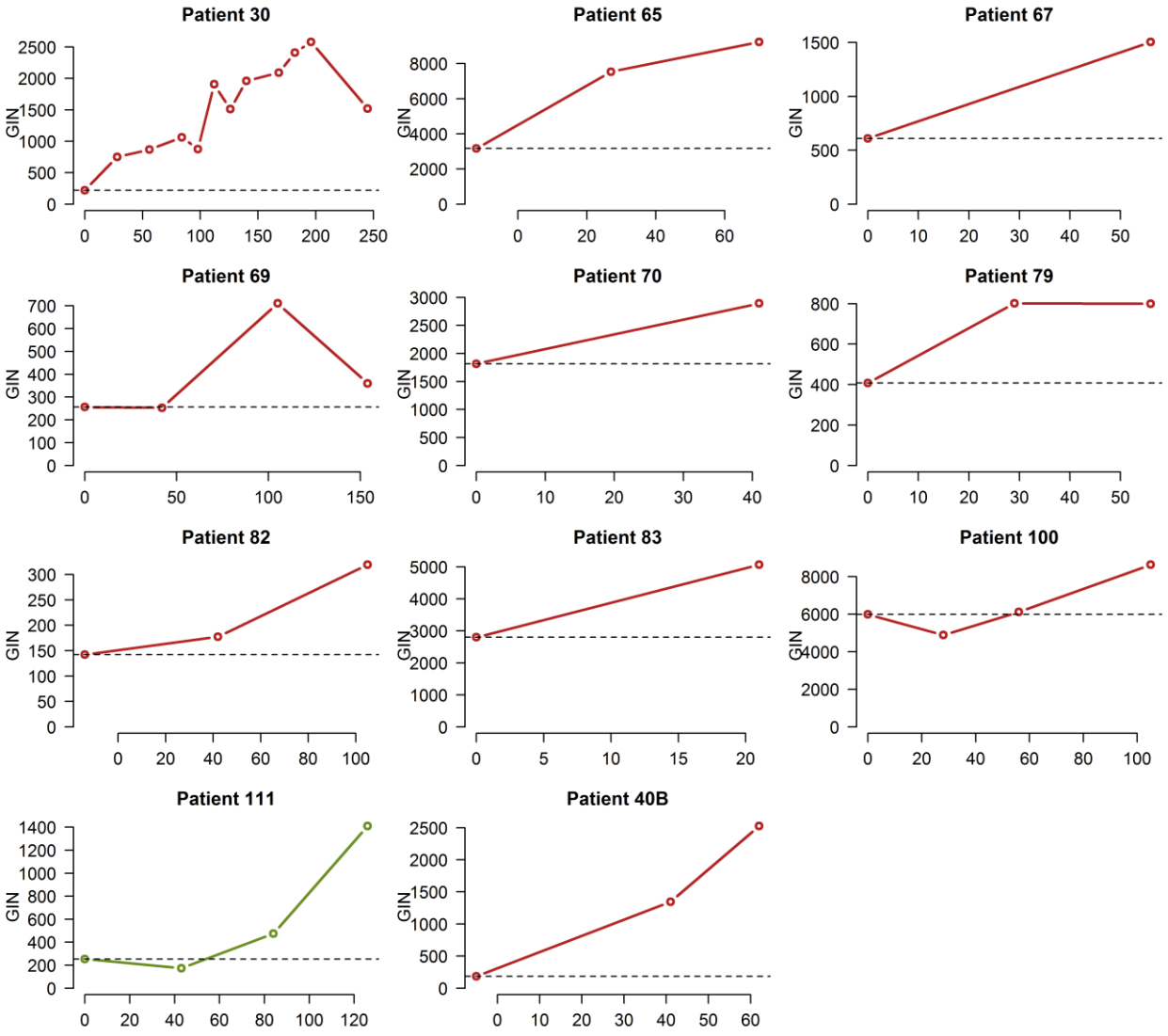
Supplementary Figure 2. Values for all baseline samples. Patients are divided based upon clinical response to immunotherapy treatment (response = stable disease for > 9 months or complete or partial response; No response (progressor) = progressive or stable disease ≤ 9 months; Unevaluable = ongoing stable disease not yet reaching 6 months.
Top: GIN values for the baseline sample from each of 44 patients.
Bottom: Total cfDNA per mL of plasma collected from each patient. Values represent total genome equivalents (GE) based on a single locus droplet digital PCR assay. There was no difference in GIN (p = 0.0.48) or in GE (p =0.49) between responders (N = 13 patients) and non-responders (N = 27 patients) (**Supplementary Table 1**).
Abbreviations: cfDNA = cell-free DNA; GE = gene equivalents per mL; GIN = genome instability number; PCR = polymerase chain reaction.



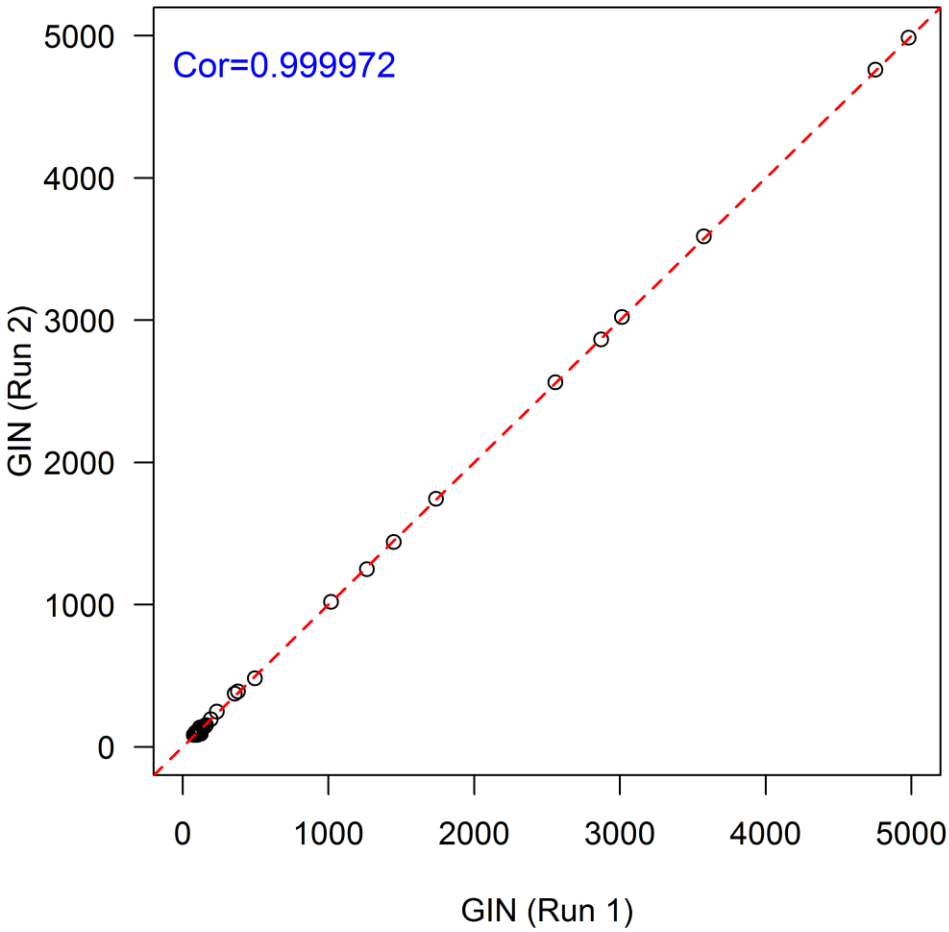
Supplementary Figure 3. Relationship between the total amount of cfDNA and the GIN in 44 baseline plasma samples. Spearman correlation coefficient = 0.49.



Supplementary Figure 4. Overview of Patient 110 where the GIN prediction was discordant with RECIST criteria (stable disease for 4 months (hence labelled as progressor)). A) Genome-wide cfDNA profiles from each of the time points described above each plot. Sequencing reads were assigned to 50 kilobase (kb) non-overlapping segments of the human reference genome. The normalized read counts from each segment of the genome are shown with alternating colors delineating chromosomes. A LOESS regression was performed for each chromosome (white lines). Deviations above and below the median value (dashed black line) indicate amplifications and deletions, respectively. B) GIN values for each collected time point relative to treatment initiation. C) Relative change in CNA-specific z-scores over time, consisent with clonal evolution. Each line corresponds with the genomic locus described by like colored "*" in panel A. Note that the region on Chromosome 9 denoted with the purple * appears in all samples and reflects the GIN; however, the region on Chromosome 14 (green *) decreases over time while the region on Chromosome 12 (pink *) increases in the same samples. Collectively, these data are consistent with clonal selection in the tumor measured in the cfDNA.



Supplementary Figure 5. Examples of GIN profiles of eleven patients predicted to be non-responders by GIN (responder defined as achieving SD>9 months, PR or CR). X axis = GIN value; Y axis = day since start of treatment. The data from patient 111 is colored green due to showing neither an increased or decreased GIN upon initial evaluation. Open circles represent measured samples with time relative to the treatment initiation (day 0; x-axis). Black dashed line represents the baseline GIN for each patient.



Supplementary Figure 6. An independent cohort of 43 samples was processed through two independent sequencing runs and the GIN calculated for each. Cor=pearson correlation coefficient. Red dashed line represents the linear regression of these data.