SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: CONSORT diagram depicting study sample and patient numbers and overall strategy for the analysis of these plasma samples by lpWGS.

Supplementary Figure 2: FIRSTANA and PROSELICA trial outlines. These previously reported clinical trials evaluated docetaxel and cabazitaxel in castration-resistant prostate cancer.

Supplementary Figure 3: A-C: Plasma ctDNA lpWGS copy-number profiles from three separate patients are depicted in A-C from three patients (p_182, p_109, p_136), showing 500kb bins tiled across the genome (dark grey points) and assigned segments (orange lines). Chromosomes are represented by alternating background rectangles (light grey and mid grey). Copy-ratio data is plotted on a log2-ratio scale (y-axis). Patient C has the typical features of bi-allelic CDK12 loss; Patient B has a high LST score, and all patients have AR amplification. D: Comparison of CNA frequencies between the cohort herein of baseline lpWGS cfDNA samples, and a previously published cohort of whole-exome mCRPC biopsy samples from the Stand Up To Cancer/PCF Dream Team study (Robinson *et al*, 2015) (23). The frequencies of CNA events are shown on the y-axis: gains (pink), amplifications (red), shallow deletions (light blue) and deep deletions (dark blue). The data indicate that cfDNA lpWGS can provide robust PC genomic copy number data.

Supplementary Figure 4: Technical replicate analyses of cfDNA lpWGS. A. lpWGS CNA profiles are shown for patient p_33 Cycle 1. Initial sample (50% tumour fraction), and samples serially-diluted with germline DNA, are shown as 500kb bins (dark grey points) with assigned segments (orange lines). B. Predicted tumour fraction values correlated with actual dilution target value. Pearson correlation r-value is shown. Blue line indicates linear model fit. C. Ten samples were prepared in duplicate and sequenced utilizing separate runs; comparison of binned CNA values and the Pearson correlation r-value is shown. Blue line indicates linear model fit.

Supplementary Figure 5: Biological replicate analysis of cfDNA lpWGS. A. Comparison of ctDNA lpWGS binned CNA data values between same-patient screening (SCR) and Cycle 1 Day 1 (C1) samples, n=88 patients taken 1-4 weeks apart. The Pearson correlation r-value is shown. The blue line indicates a linear model fit. B. Comparison of the estimated tumour-derived fraction (as a proportion of total cfDNA analysed; ctDNA/cfDNA) between same-patient Screening and Cycle Day 1 samples, n=88 patients taken 2-4 weeks apart. Pearson correlation r-value is shown. Blue line indicates a linear model fit.

Supplementary Figure 6: Association of baseline, pre-treatment, lpWGS tumour fraction and RPFS and PSAPFS. A-D. Univariable analysis of PSA progression-free survival (PSAPFS) and radiographic progression-free survival (RPFS), by median baseline (i.e. average across C1 and SCR samples) ctDNA tumour fraction (high = yellow, low = blue) for both the FIRSTANA (A-B) and PROSELICA (C-D) cohorts. Kaplan-Meier plots with confidence intervals and matching risk tables are shown. Dashed lines indicate time to 50% survival.

Supplementary Figure 7: Association of longitudinal ctDNA detectability and overall survival. A-C. Univariable analyses of overall survival (OS), radiographic progression-free survival (RPFS) and PSA progression-free survival (PSAPFS) for individuals with or without

detectable (>5%) tumour fraction at both baseline and on-treatment timepoints. Patients with both baseline and on-treatment samples available used (n=135). Patients split into four groups: ctDNA detectable at both baseline and on-treatment, detectable at baseline but not on-treatment, not-detectable at baseline but detectable on-treatment, and not-detectable at either timepoint. Kaplan-Meier plots with matching risk tables are shown. Dashed lines indicate median survival time.

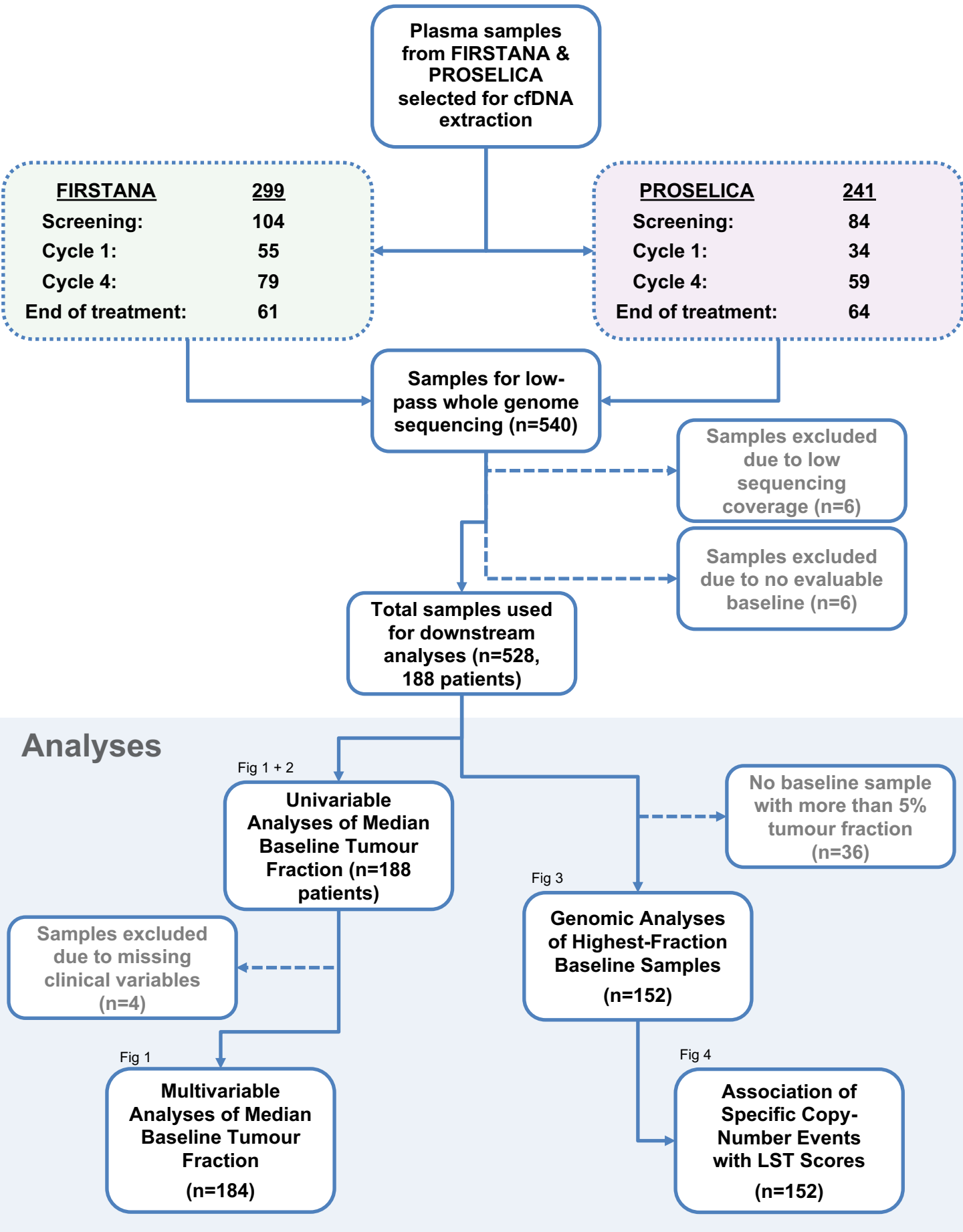
Supplementary Figure 8: A-I. Association of mCRPC clinical variables with median baseline tumour fraction. Categorical variables were compared using Wilcoxon Rank-Sum tests (p-values shown) and continuous variables (median baseline) compared using Pearson Correlation (r-values and p-values shown).

Supplementary Figure 9: Three whole-genome CNA profiles are shown for patient p_168, across Screening, Cycle 4 and End of Study timepoints. 500kb bins are shown (dark grey points) with assigned segments (orange lines). This responding patient shows marked differences in CNAs during treatment, with no detectable alterations at Cycle 4, and return of detectable CNAs (similar in pattern to baseline) in relapse sample.

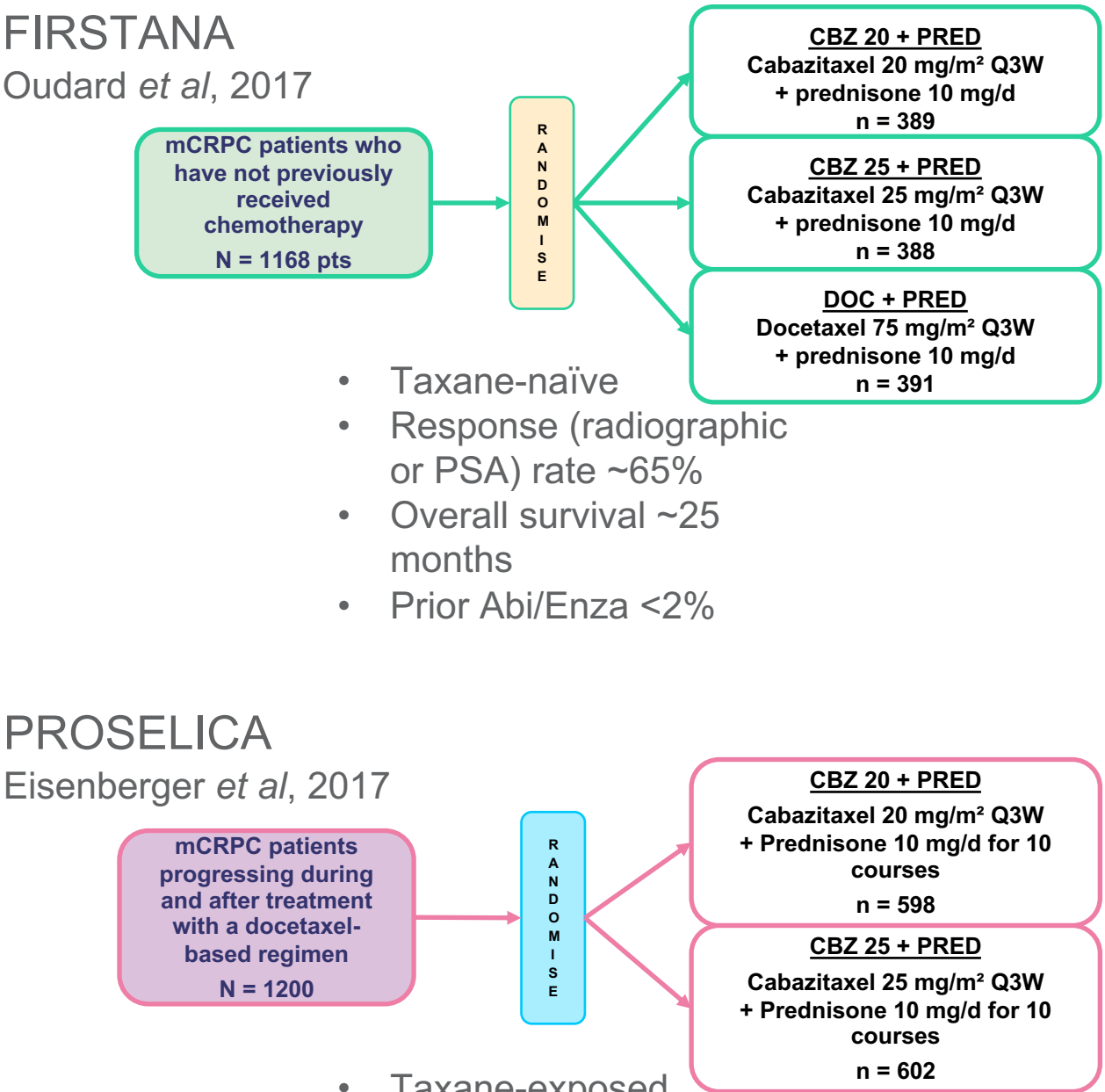
Supplementary Figure 10: A. Correlation of LST score values with Tumour Fraction (Log10); Pearson r-value is shown. B. Comparison of LST score between prior Abi/Enza treatment in PROSELICA only; Wilcoxon Rank-Sum tests p-values shown. C. Whole-genome CNA profiles across three samples from patients p_145, p_133 and p_148, illustrating variable large-scale transition (LST) values across tumour-derived copy number profiles. 500kb bins are shown (grey values) with lines illustrating assigned segment boundaries. Line color dependent on segment width (green segments > 10Mb in size, orange segments < 10Mb).

SUPPLEMENTARY DATA 1 (separate file)
List of genes identified in the elastic-net regression as associated with high LST scores that overlap with published data from CRISPR screens determining genomic alterations that sensitize to PARP inhibitor treatment.

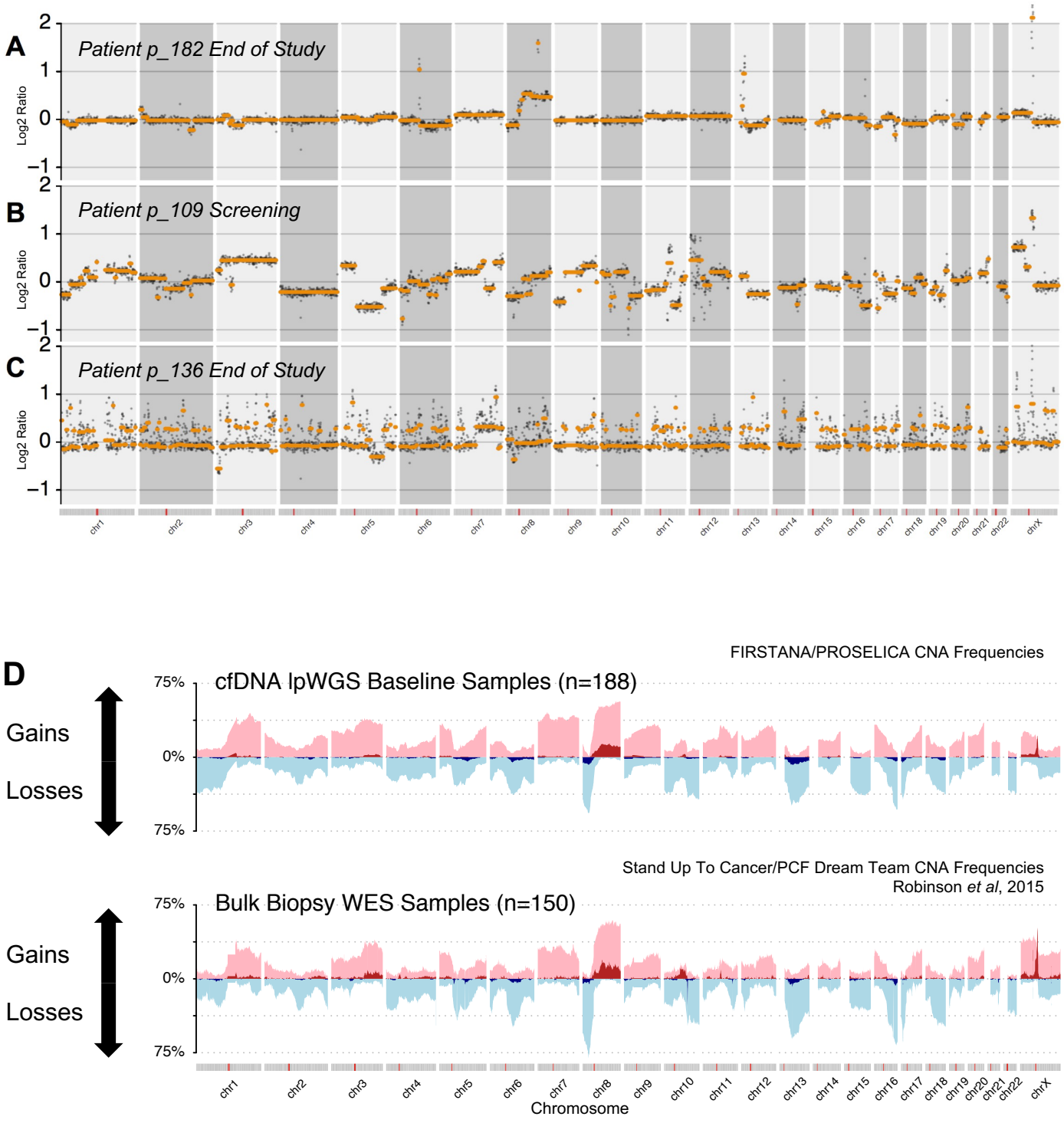
Supplementary Figure 1 – Study outline



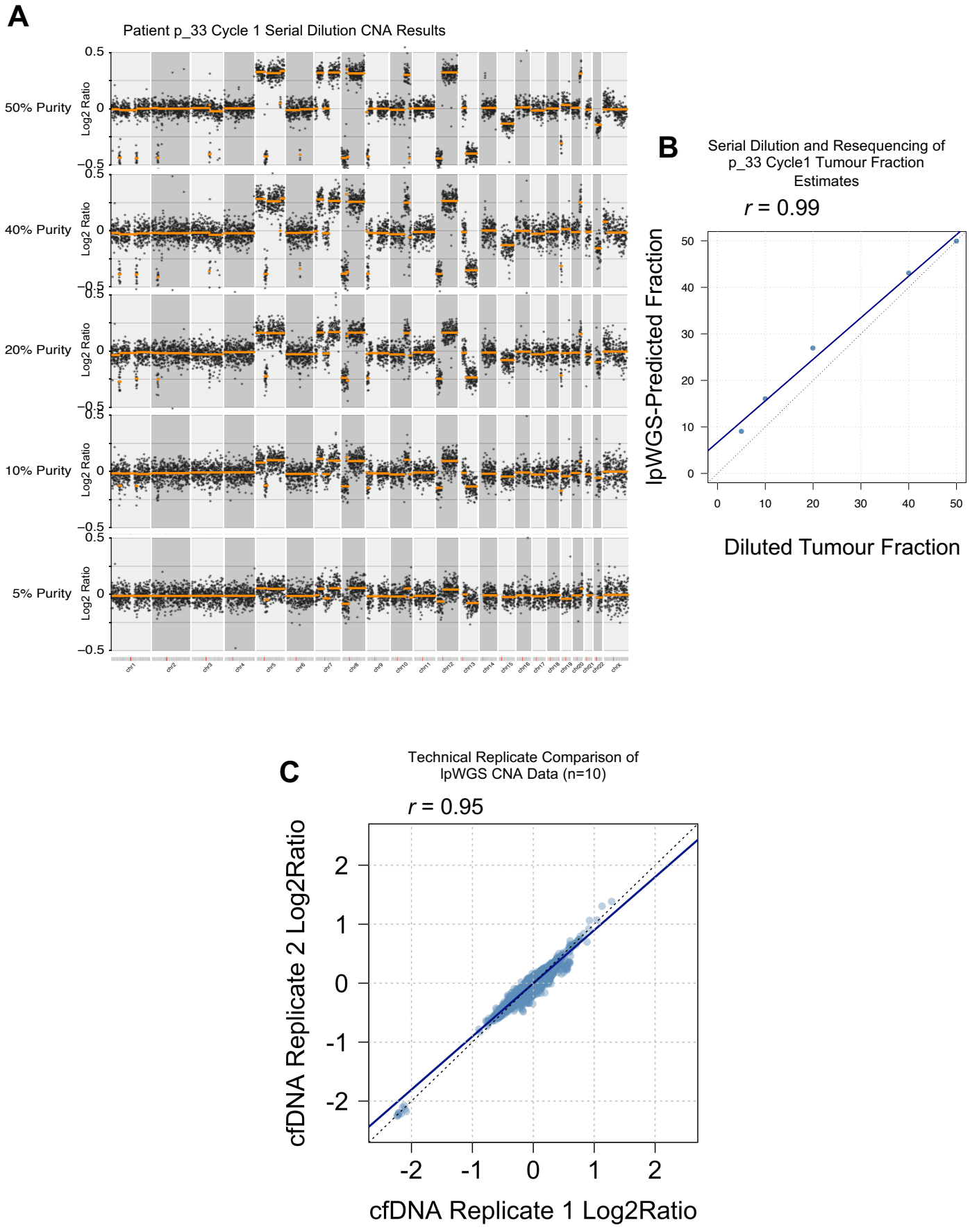
Supplementary Figure 2 – FIRSTANA and PROSELICA trial outlines



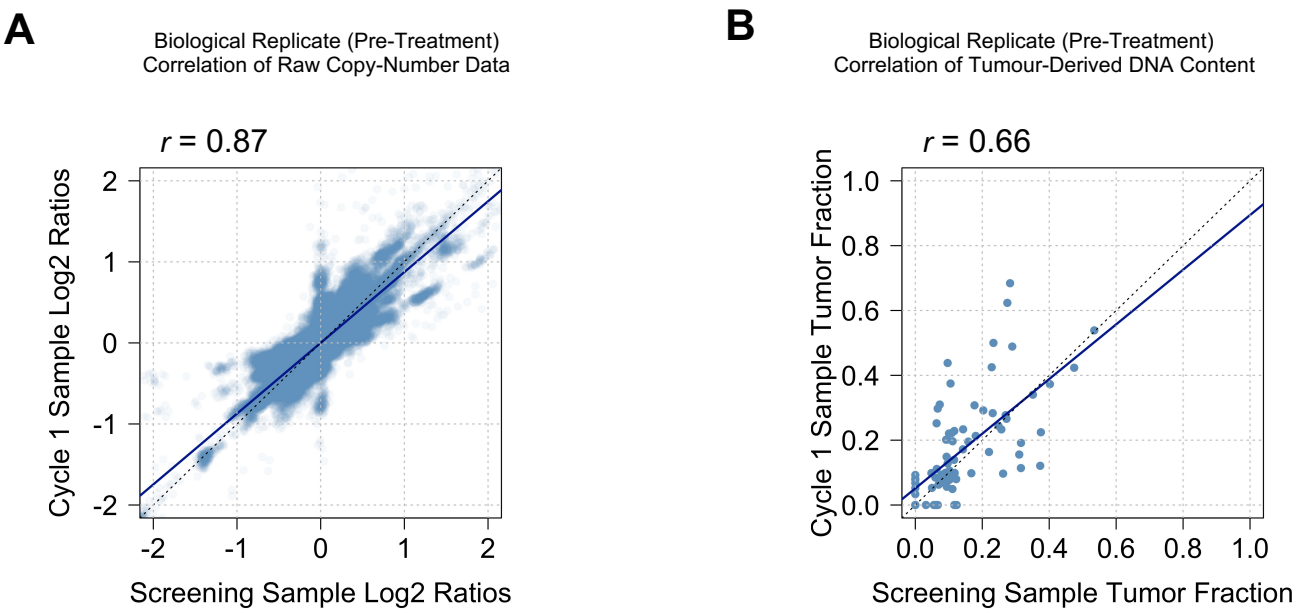
Supplementary Figure 3 – Example lpWGS copy-number profiles



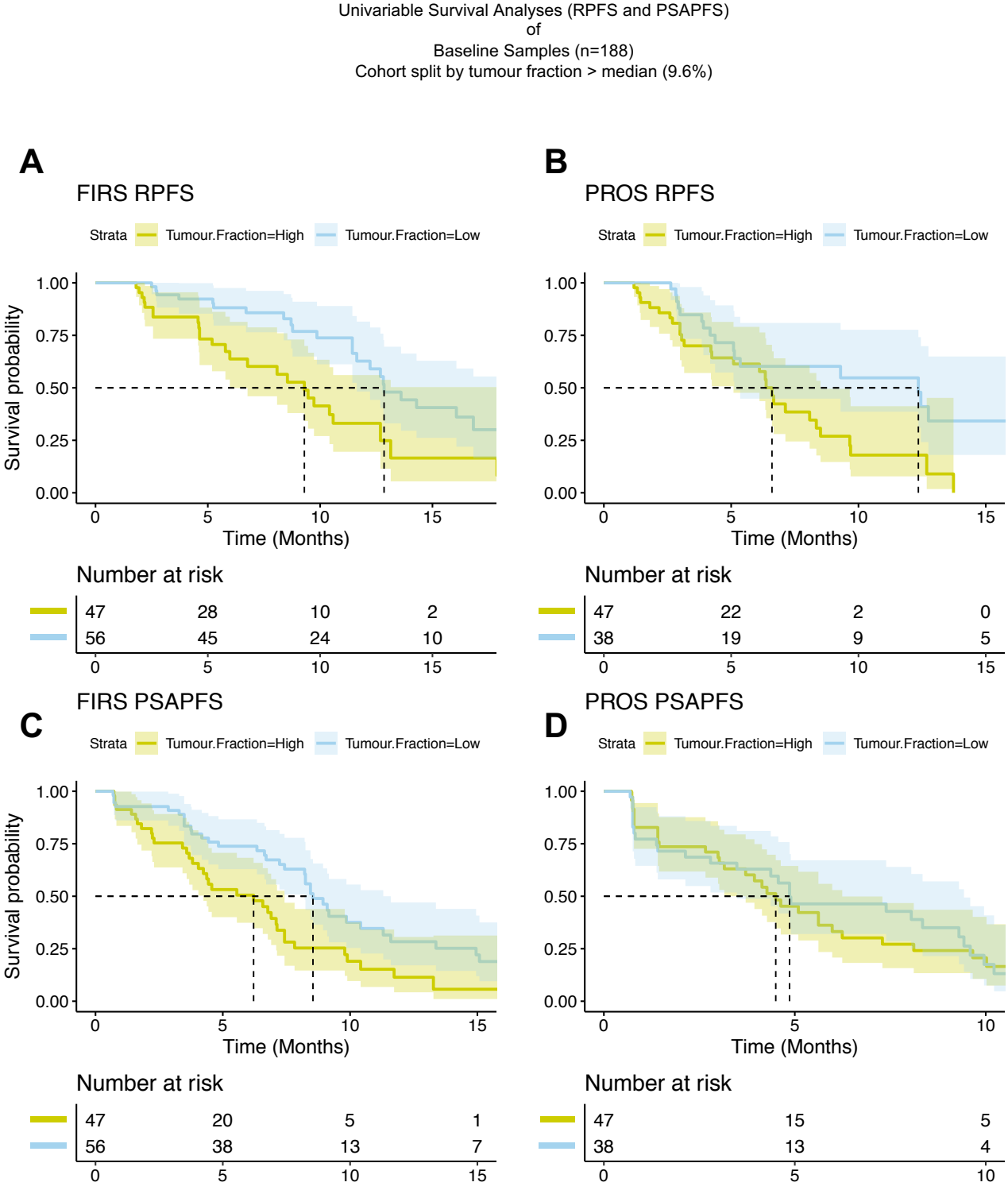
Supplementary Figure 4 – Technical replicate analyses of cfDNA lpWGS



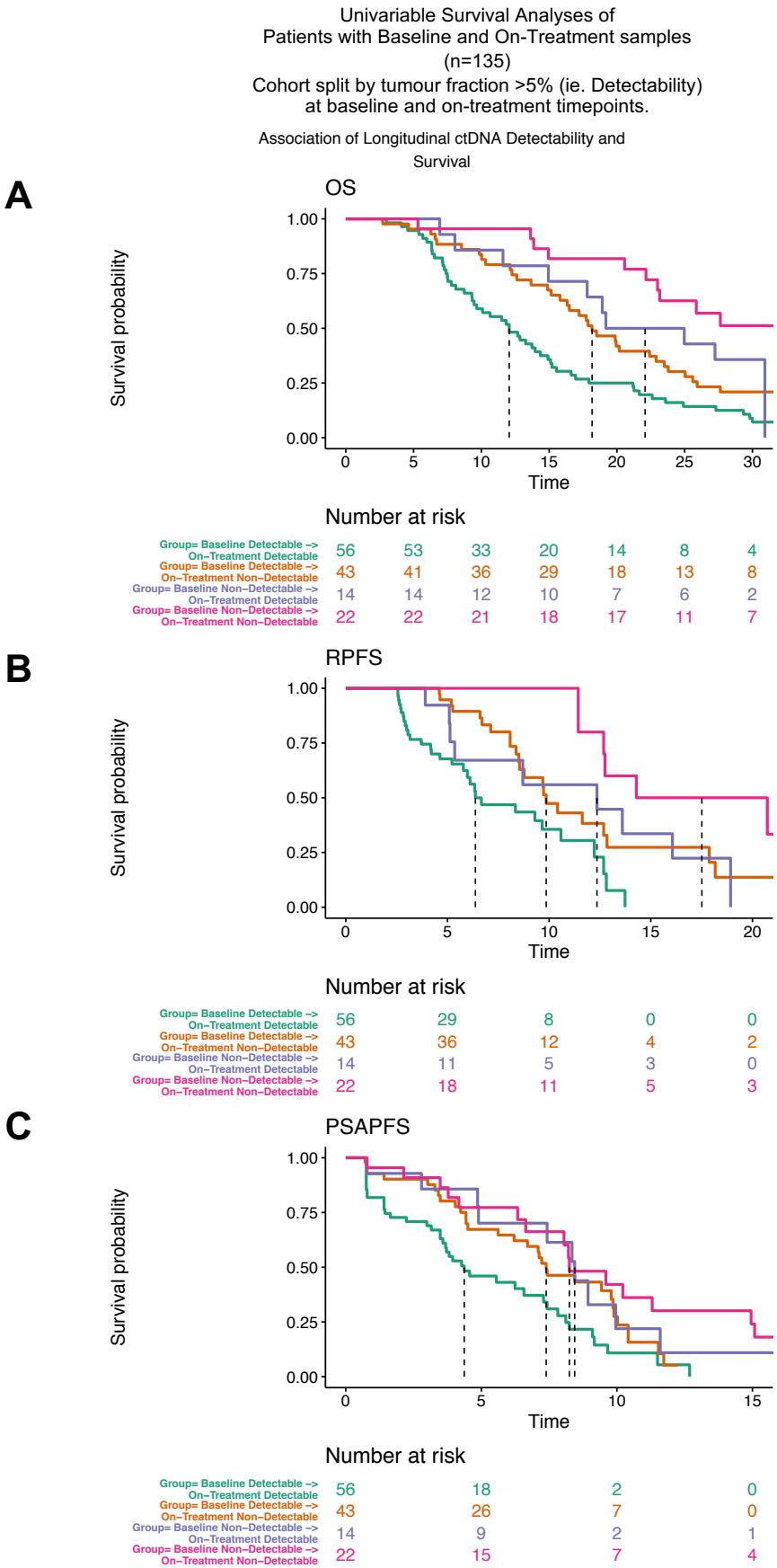
Supplementary Figure 5 – Biological replicate analyses of cfDNAIpWGS



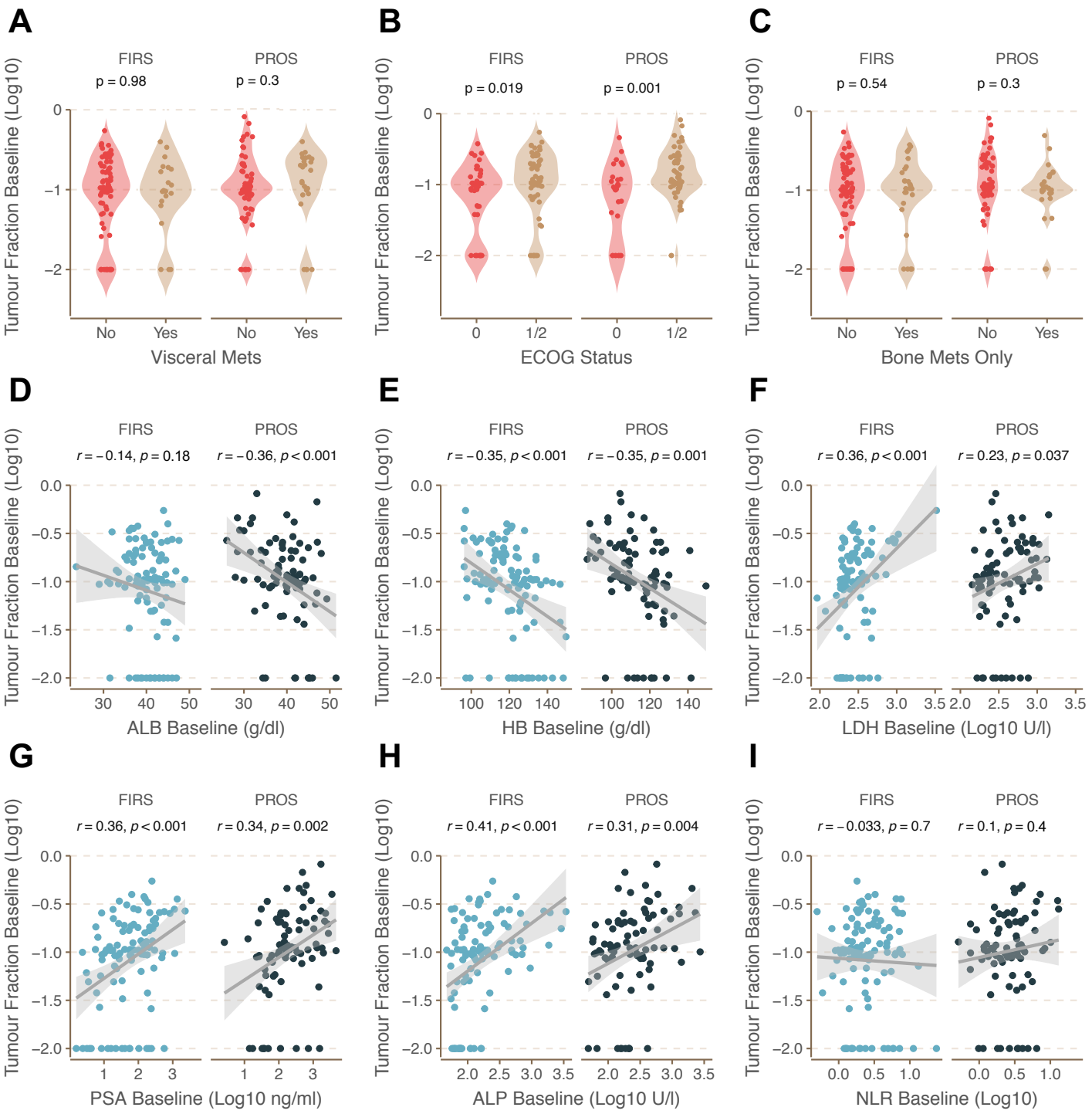
Supplementary Figure 6 – Association of lpWGS tumour fraction and RPFS and PSAPFS



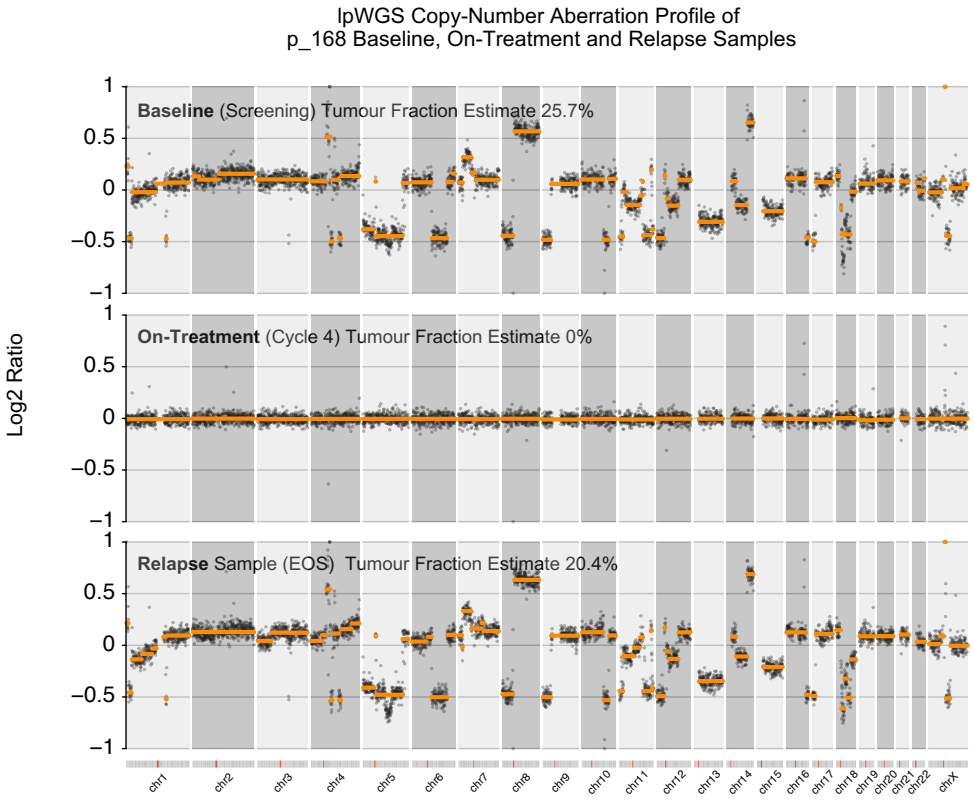
Supplementary Figure 7 – Association of longitudinal ctDNA detectability and Survival



Supplementary Figure 8 – lpWGS tumour fraction and mCRPC clinical variables

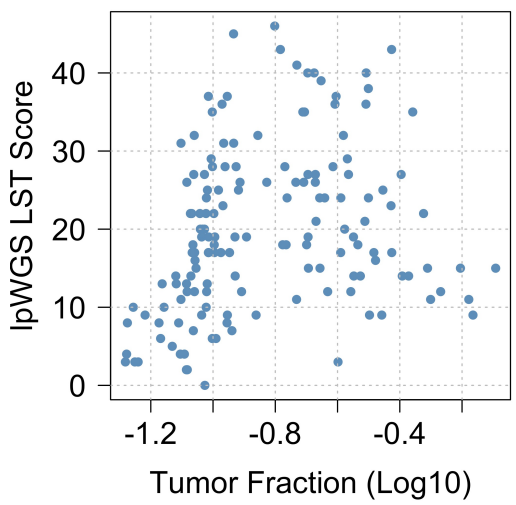


Supplementary Figure 9 – Examples of changes in CN detectability due to tumour fraction changes.



Supplementary Figure 10 – Additional LST Comparisons

A



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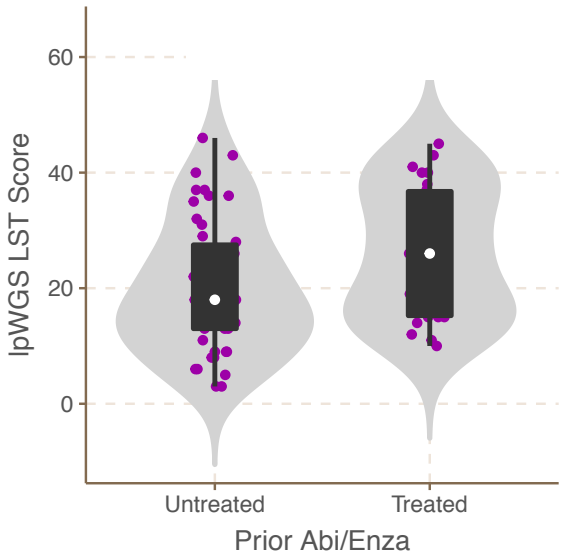
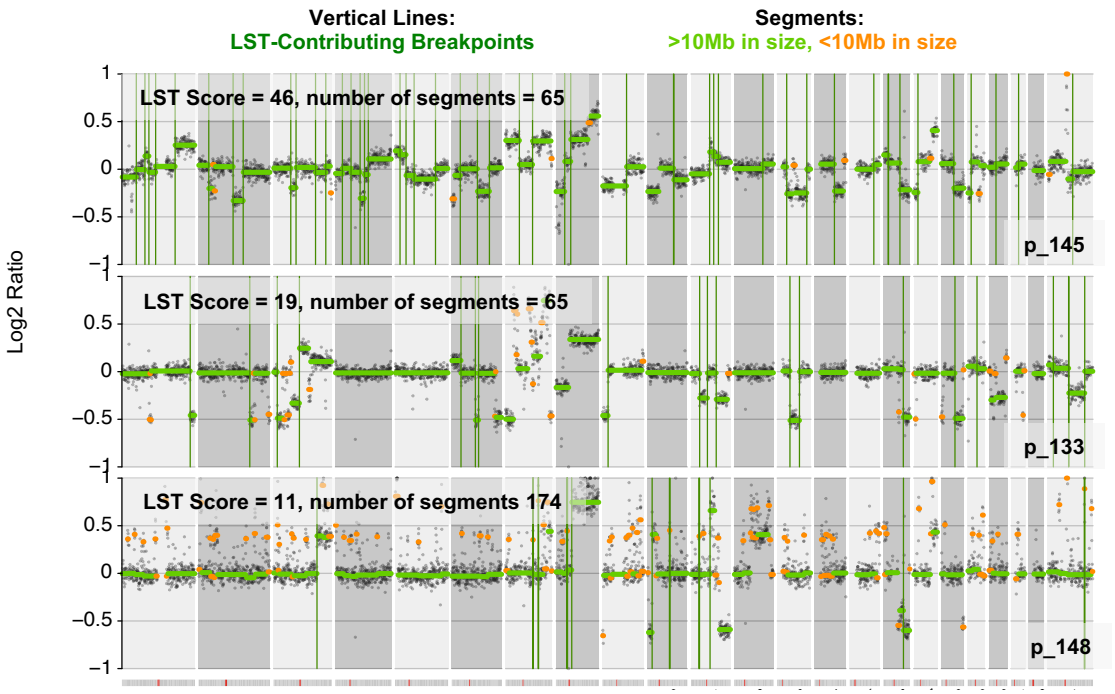


C



Supplementary Table 1 - Comparison of clinical characteristics between the substudy cohorts analysed here and the overall complete FIRSTANA/PROSELICA trial populations.

	FIRSTANA			PROSELICA		
Characteristic	**Biomarker subset**	**Non-biomarker subset**	**p-value[a]**	**Biomarker subset**	**Non-biomarker subset**	**p-value[a]**
	n = 103	**n = 1065**		**n = 85**	**n = 1115**	
	N (%)	**N (%)**		**N (%)**	**N (%)**	
ECOG PS[b] >= 2	3 (2.9)	45 (4.2)	0.5	10 (12)	1110 (10)	0.5
RECIST measurable[b]	54 (52)	561 (53)	0.9	44 (52)	544 (49)	0.6
Visceral disease	20 (19)	230 (22)	0.6	25 (29)	310 (28)	0.7
Pain at baseline[c]	69 (78)	626 (64)	0.019	59 (74)	734 (72)	0.7
Prior Abi/Enza	2 (1.9)	26 (2.4)	0.7	29 (34)	279 (25)	0.085
	Median (IQR)	**Median (IQR)**	**p-value[d]**	**Median (IQR)**	**Median (IQR)**	**p-value[d]**
Age (yr)	68.0 (62.5 – 72.0)	68.0 (63.0 – 74.0)	0.14	67.0 (64.0 - 71.0)	69.0 (63.0 – 74.0)	0.016
LDH (U/L)	263 (205 - 372)	239 (190 – 374)	0.12	350 (222 - 588)	325 (220 - 498)	0.3
ALP (U/L)	128 (79.5 - 244)	125 (79.0 - 264)	0.3	209 (118 - 415)	163 (92.0 - 346)	0.026
Haemoglobin (g/dl)	123 (114 - 132)	128 (117 - 137)	0.005	117 (108 - 124)	120 (108 - 130)	0.043
Albumin (g/dl)	40.1 (37.8 – 43.0)	41.0 (38.0 – 44.0)	0.06	40.0 (36.0 – 43.0)	40.0 (36.7 – 43.0)	0.4
PSA (ng/ml)	75.0 (22.2 - 237)	76.0 (30.0 - 196)	0.4	247 (93.7 - 741)	158 (53.2 - 413)	0.001
PSA doubling time (days)	62 (36 - 100)	62 (41 - 103)	0.11	52 (36 - 86)	58 (37 - 94)	0.3
NLR	3.17 (2.32 – 4.33)	2.87 (2.05 - 4.16)	0.086	3.38 (2.25 - 5.45)	3.38 (2.23 - 5.45)	0.5
Outcome	**N (%)**	**N (%)**	**p-value[a]**	**N (%)**	**N (%)**	**p-value[a]**
>50% PSA response at 12 weeks	55 (53)	580 (54)	0.8	23 (27)	351 (31)	0.4
>50% PSA response at any time	68 (66)	755 (71)	0.3	33 (39)	456 (41)	0.7
Survival	**Median (95% CI)**	**Median (95% CI)**	**p-value[e]**	**Median (95% CI)**	**Median (95% CI)**	**p-value[e]**
OS (months)	21.3 (17.2 – 25.9)	25.1 (23.6 – 26.5)	0.12	13.3 (11.5 – 15.8)	14.1 (13.3 – 15.0)	0.058
RPFS (months)	11.6 (9.86 – 13.6)	13.3 (11.9 – 13.9)	0.13	7.13 (6.11 - 12.4)	8.31 (7.92 – 8.77)	0.8
PSAPFS (months)	7.43 (6.64 – 8.94)	8.74 (8.18 – 9.33)	0.078	4.86 (4.14 – 7.29)	5.55 (4.96 – 5.91)	0.5

ALP = alkaline phosphatase; cfDNA = cell-free DNA; ECOG PS = Eastern Cooperative Oncology Group performance status; IQR = interquartile range; LDH = lactate dehydrogenase; mo = months; PSA = prostate-specific antigen; NLR = Neutrophil-Lymphocyte Ratio; RECIST = Response Evaluation Criteria in Solid Tumours; U = unit, yr = years, OS = overall survival, RPFS = radiographic progression-free survival, PSAPFS = PSA progression-free survival, CI = confidence interval.

a = χ^2 test.

b = Stratification parameters

c = For FIRSTANA 99 assessments were missing (15 in the sub-study and 84 in the main study), For PROSELICA 97 assessments were missing (5 in the sub-study and 92 in the main study).

d = Wilcoxon rank sum test

e = Log-rank test

Supplementary Table 2 – Multivariable model performance. Comparison of multivariable Cox regression model containing all variables of interest with reduced model omitting tumour fraction. Harrell's C index and standard error (SE) values shown. Log-likelihood test comparison p-value shown for overall survival, radiographic progression-free survival and PSA progression-free survival models.

	Concordance Index		
	Full Model (Including Tumour Fraction)	Reduced Model (Excluding Tumour Fraction)	Likelihood Ratio Test
	C (SE)	C (SE)	p-value
Overall Survival (OS)	0.722 (0.021)	0.709 (0.021)	0.021
Radiographic Progression-Free Survival (RPFS)	0.751 (0.028)	0.722 (0.030)	<0.001
PSA Progression-Free Survival (PSAPFS)	0.615 (0.029)	0.591 (0.031)	0.056

Supplementary Table 3 – Prior treatments of matched biopsy cohort.

Matched Cohort Data Prior Treatment Information	Whole Cohort (n=52 patients)		Study Cohort (n=44 patients)	
	Treatment **Any Time**		Treatment **Before mCRPC Biopsy**	
	Total Number	Percentage	Total Number	Percentage
Second Line Hormone Therapy (Abiraterone)	30	57%	28	63%
Second Line Hormone Therapy (Enzalutamide)	35	67%	31	72%
Either Abiraterone OR Enzalutamide	49	94%	44	100%
Chemotherapy (Cabazitaxel)	24	46%	23	53%
Chemotherapy (Docetaxel)	48	92%	42	95%