Cell Reports Medicine, Volume *5*

Supplemental information

Cell-free DNA fragmentomics and second malignant neoplasm risk in patients with *PTEN* hamartoma tumor syndrome

Darren Liu, Lamis Yehia, Andrew Dhawan, Ying Ni, and Charis Eng

Table S1. *PTEN* Variation Spectra in Patients with PHTS, Related to Table 1.						
Characteristic	**N**	**Overall**, N = 99[a]	**No Cancer**, N = 50[a]	**PMN**, N = 23[a]	**SMN**, N = 26[a]	***P*-value**[b]
Variation Classification	99					0.3
P/LP		91 (92%	49 (98%)	19 (83%)	24 (92%)	
VUS		4 (4%)	1 (2%)	2 (9%)	1 (4%)	
Conflicting (VUS, LB)		4 (4%)	1 (2%)	2 (9%)	1 (4%)	
Variation Effect	99					
Missense		29 (29%)	18 (36%)	5 (22%)	6 (23%)	
Nonsense		26 (26%)	11 (22%)	7 (30%)	8 (31%)	
Frameshift truncating		16 (16%)	7 (14%)	4 (17%)	5 (19%)	
Splice site		11 (11%)	8 (16%)	2 (8.7%)	1 (4%)	
Large Deletion		11 (11%)	6 (12%)	3 (13%)	2 (8%)	
Other		6 (6%)	0 (0%)	2 (9%)	4 (16%)	
Variation Site	99					0.2
Exonic		80 (81%)	38 (76%)	18 (78%)	24 (92%)	
Intronic		11 (11%)	8 (16%)	2 (9%)	1 (4%)	
Exonic and Intronic		5 (5%)	4 (8%)	1 (4%)	0 (0%)	
Promoter		3 (3%)	0 (0%)	2 (9%)	1 (4%)	

[a] n (%)
[b] Pearson's Chi-squared test; Fisher's exact test
P/LP, pathogenic/likely pathogenic; VUS, variant of uncertain significance; LB, likely benign

Figure S1. Cumulative cfDNA Fragment Size Frequency Distribution in Patients with PHTS, Related to Figure 1. Each line represents the median cumulative fragment size frequency across the mono-, di-, and tri-nucleosome fraction grouped by (A-C) cancer status, (D-F) SMN status, and (G-I) SMN status and plasma draw time.

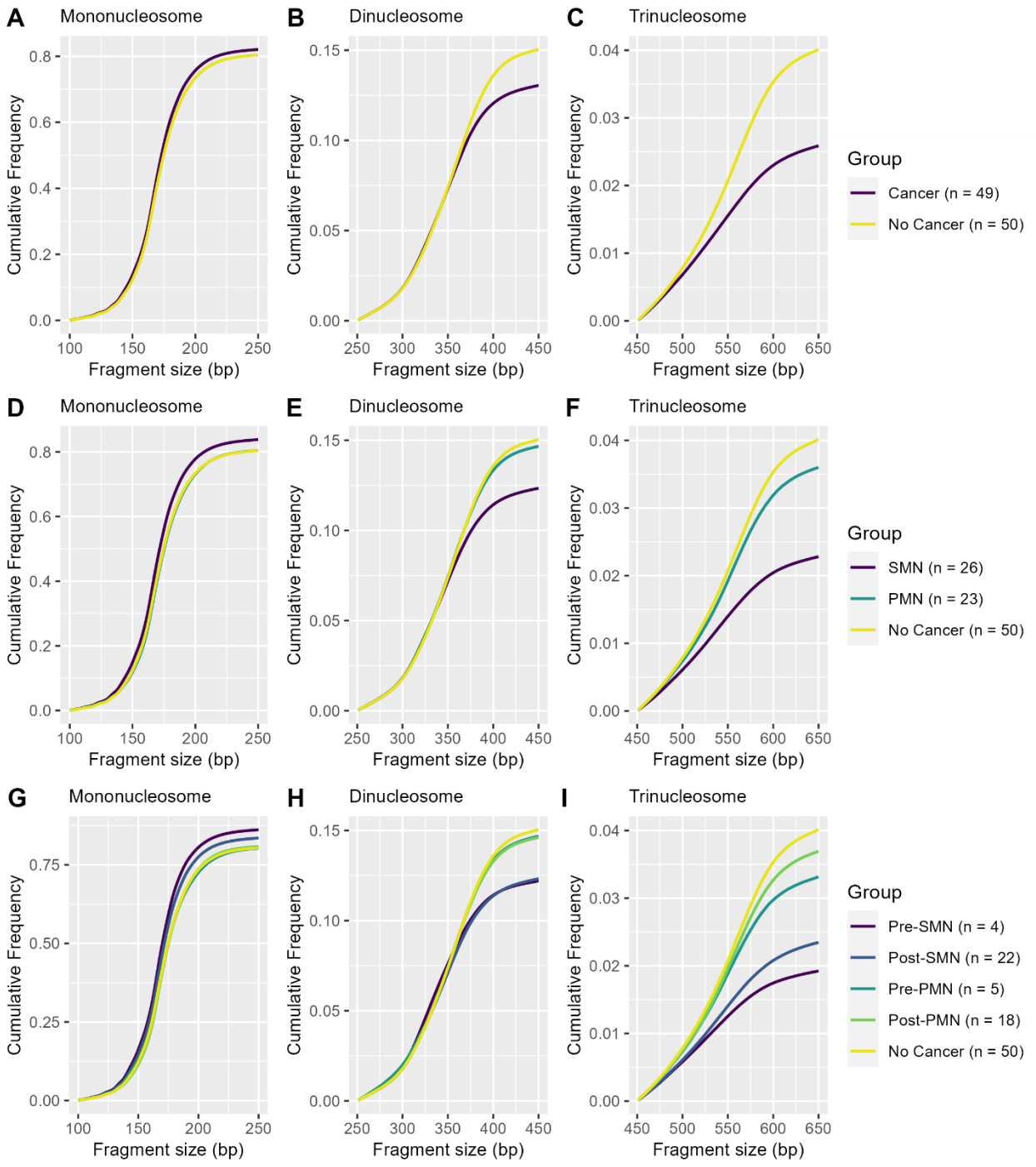

Figure S2. Difference cfDNA Fragment Size Frequency Patients with PHTS, Related to Figure 1. Each line represents the difference in the median fragment size frequency, denoted as ΔF, of each cancer subgroup relative to patients with PHTS and no cancer across each nucleosome fraction grouped by (A-C) cancer status, (D-F) SMN status, and (G-I) SMN status and plasma draw time.

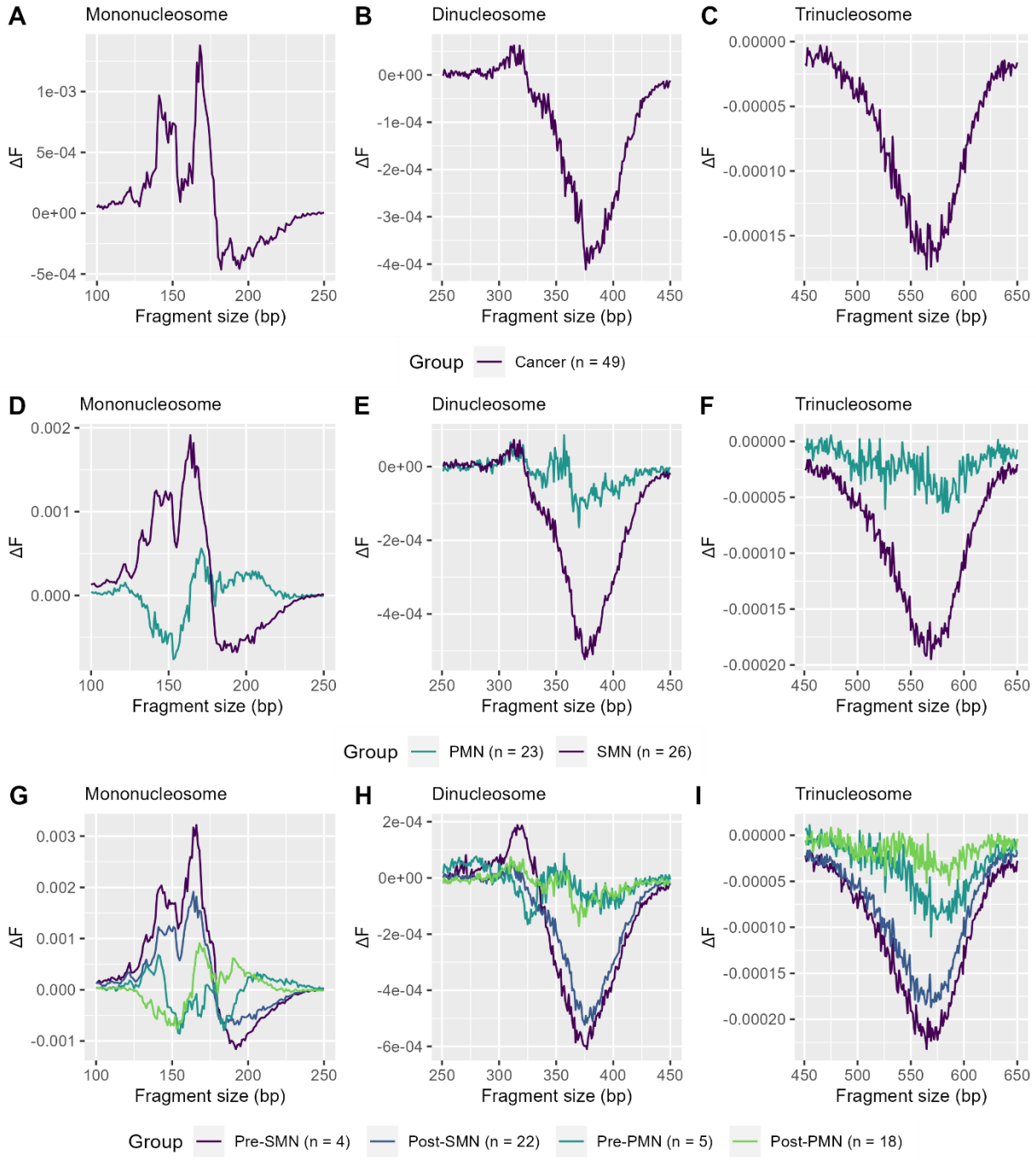

Figure S3. Difference in Cumulative cfDNA Fragment Size Frequency Distribution in Patients with PHTS, Related to Figure 1. Each line represents the difference in the cumulative median fragment size frequency, denoted as ΔCF, of each cancer subgroup relative to patients with PHTS and no cancer across each nucleosome fraction grouped by (A-C) cancer status, (D-F) SMN status, and (G-I) SMN status and plasma draw time.

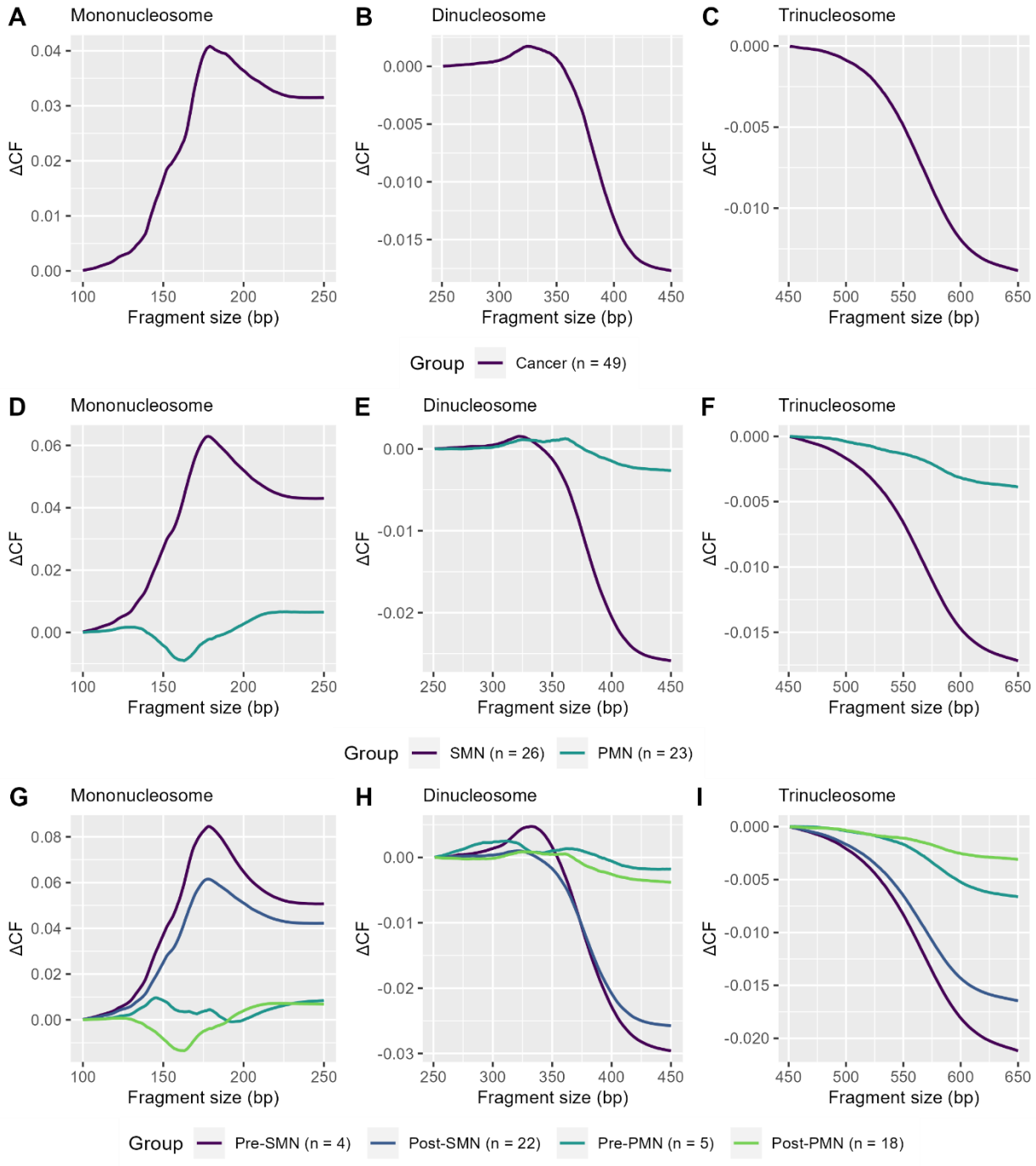

Figure S4. Receiver Operating Characteristic (ROC) curve for Prediction of SMN, Related to Table 2. Performance of models containing age of plasma draw, CC score (i.e., phenotypic burden), and fragment ratios from each nucleosome assessed utilizing Leave-One-Out Cross-Validation (LOOCV).