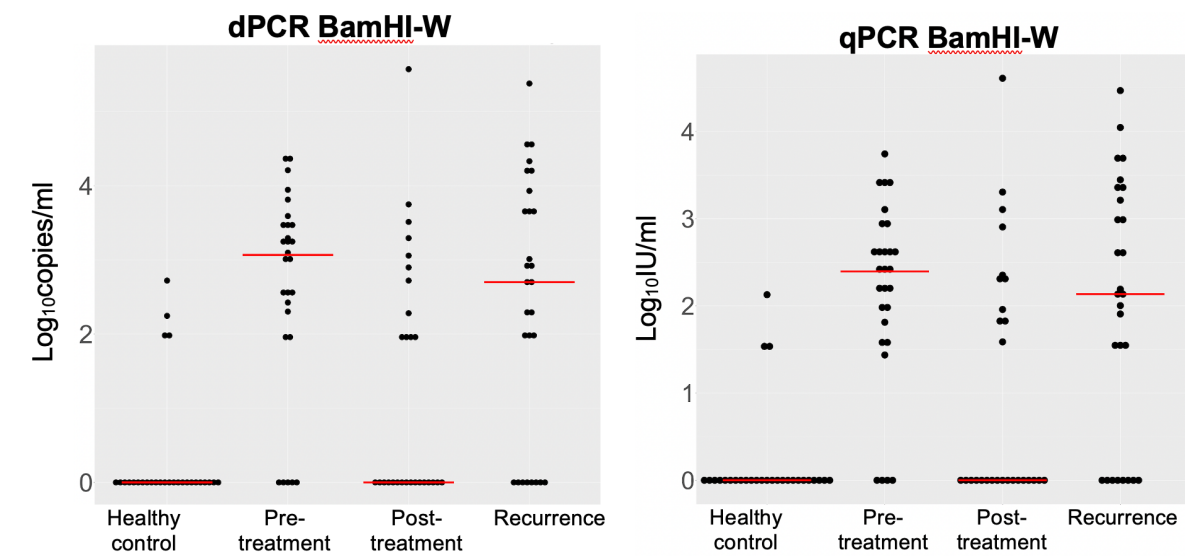
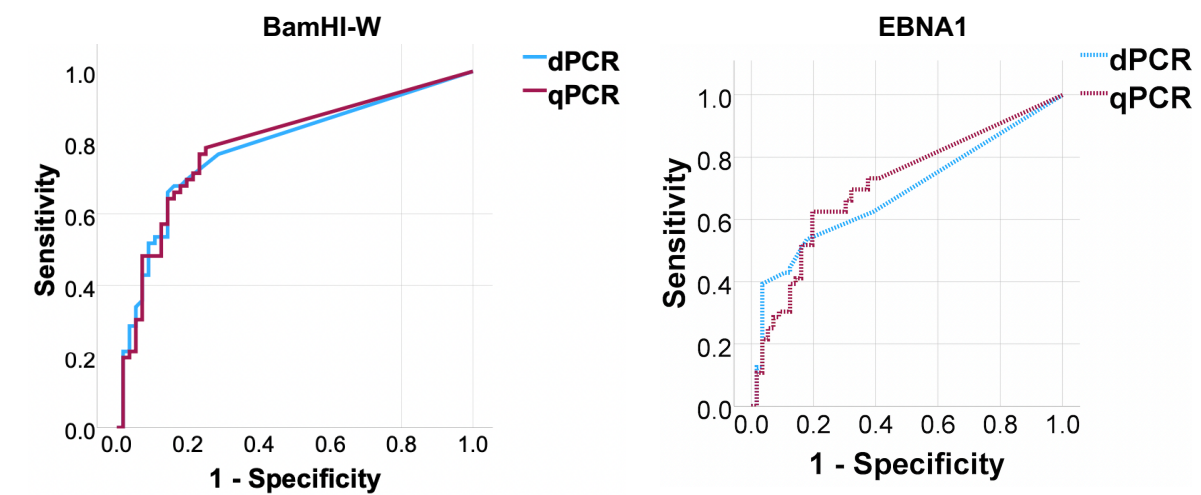


Supplementary Fig. S1. Comparison of serum EBV dPCR and qPCR in NPC.

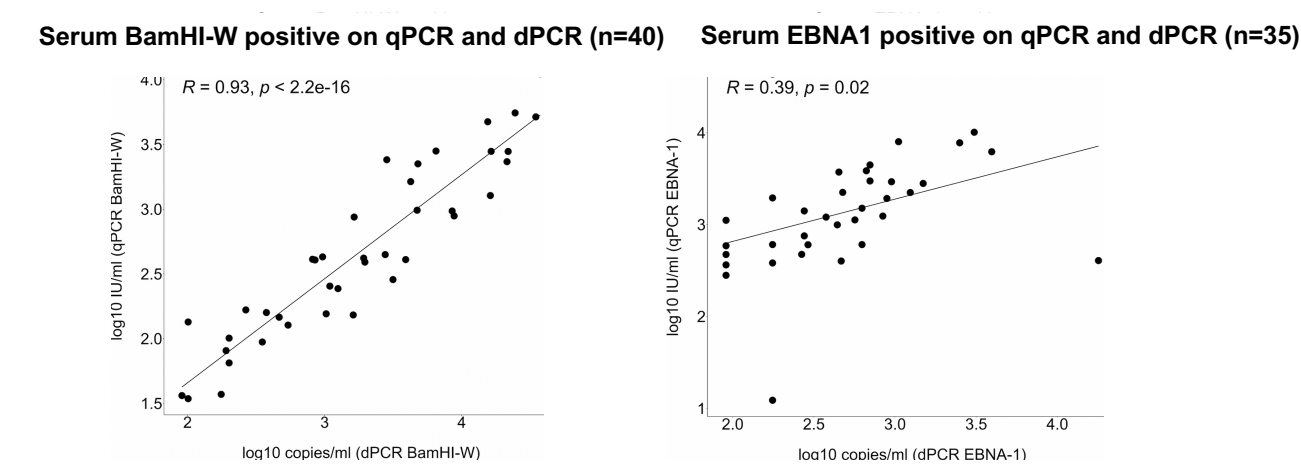
(a) BamHI-W levels across timepoints and patient groups



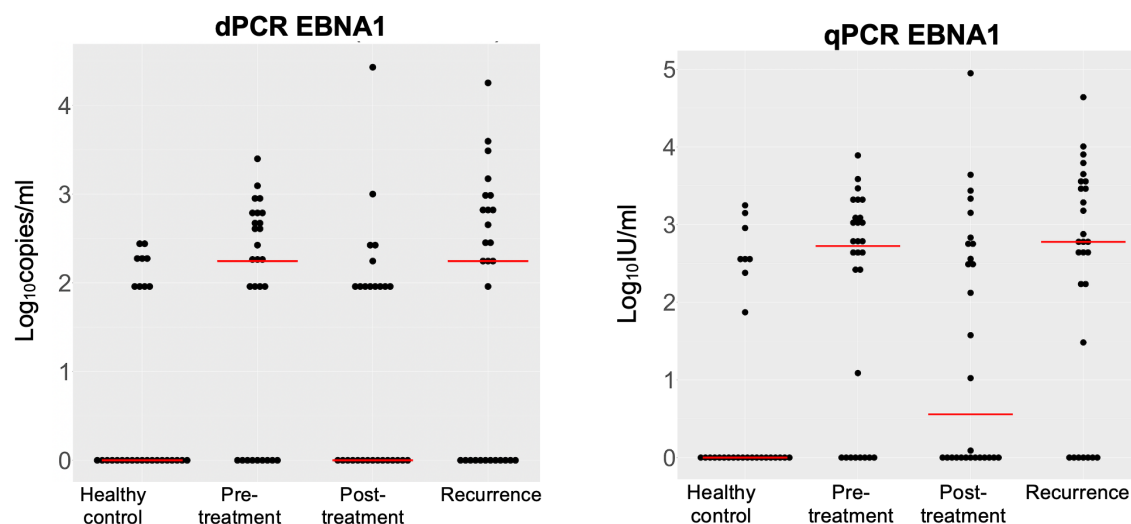
(b) ROC curves for BamHI-W and EBNA1



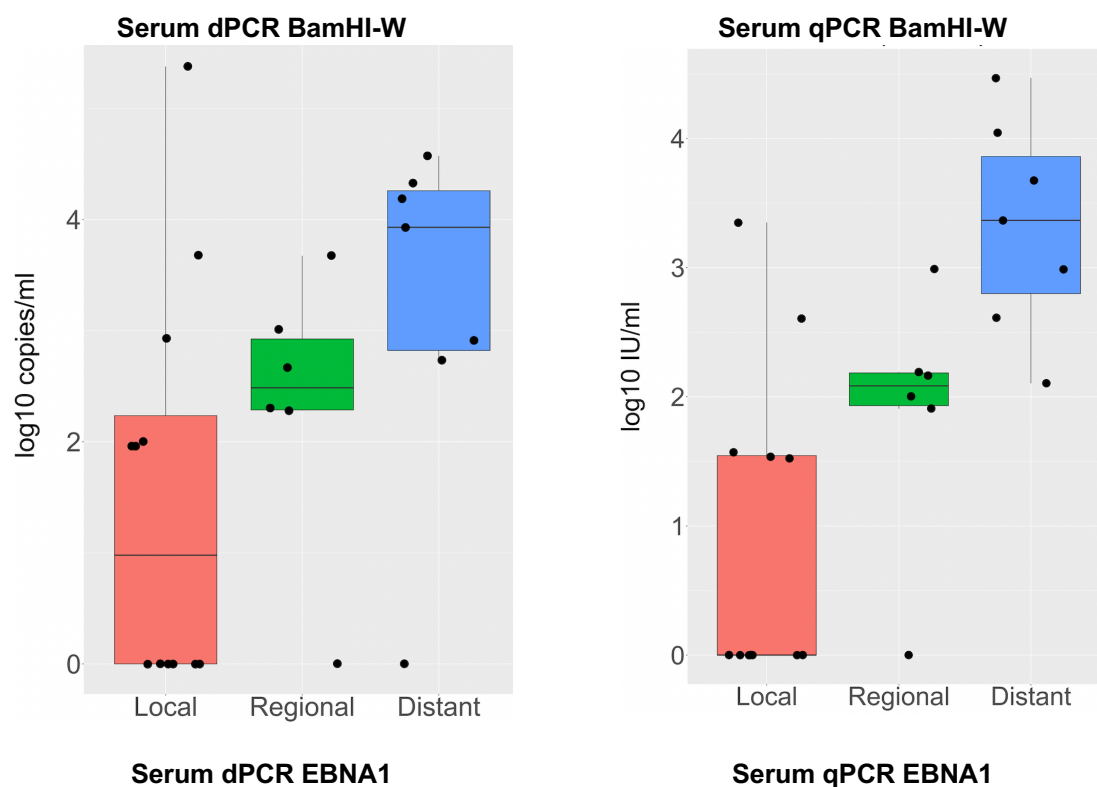
(c) Correlation of dPCR vs qPCR for both targets

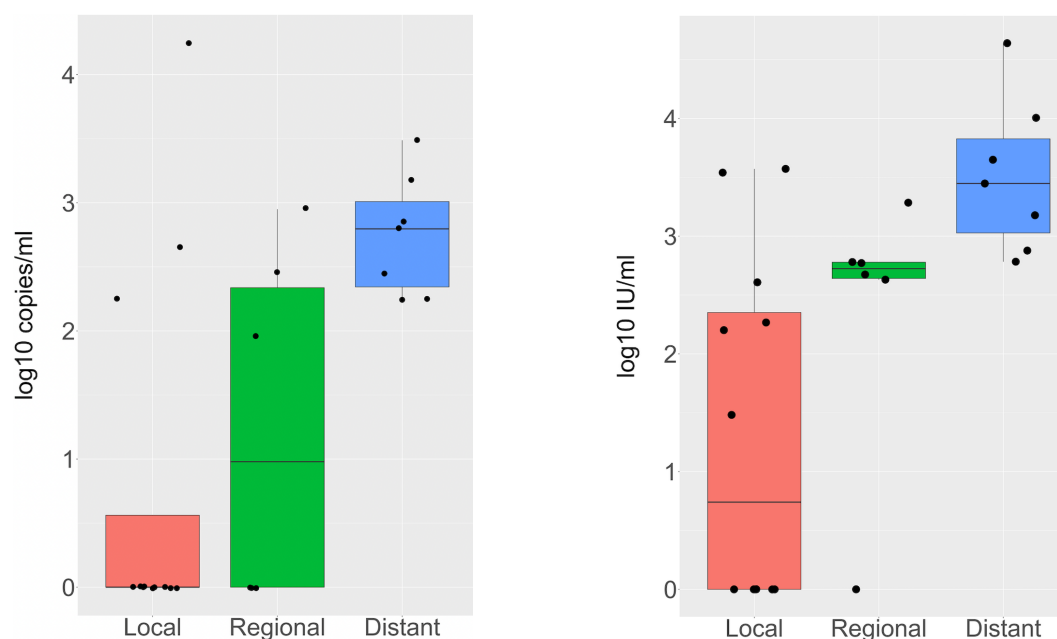


Supplementary Fig. S2. Serum EBV DNA levels (EBNA1) in NPC patients (n=28) at 3 time points (pre-treatment, post-treatment and at recurrence) and healthy controls (n=28). Median values indicated by red lines.



Supplementary Fig. S3. Serum EBV DNA levels for NPC cases with recurrence, based on site of recurrent disease.





Supplementary Table S1. Clinical characteristics.

	Plasma		Serum	
	NPC cases (n=38)	Healthy controls (n=12)	NPC cases (n=28)	Healthy controls (n=28)
Age (years)	56.5 ± 3.0	51.3 ± 15.3	49.4 ± 11.7	49.0 ± 11.4
Gender				
Male	29 (76.3%)	6 (50.0%)	17 (60.7%)	18 (64.3%)
Female	9 (23.7%)	6 (50.0%)	11 (39.3%)	10 (35.7%)
Race				
Chinese	30	9	26	22
Malay	5	0	1	2
Indian	0	0	1	0
Others	3	3	0	4
Stage				
I	4 (10.5%)		1 (3.6%)	
II	6 (15.8%)		10 (35.7%)	
III	10 (26.3%)		8 (28.6%)	
IV	18 (47.4%)		9 (32.1%)	
Disease status				
Pre-treatment	21 (55.2%)			
Post treatment (Healthy)	14 (36.8%)			
Post treatment (Recurrence)	3 (7.89%)			
Site of recurrence				
Local	1 (33.3%)		12 (42.9%)	
Regional	2 (66.6%)		6 (21.4%)	
Distant			7 (25.0%)	
Multiple			3 (10.7%)	

Supplementary Table S2. Performance of plasma EBV dPCR and qPCR

(a) Sensitivity and specificity at optimal cut-off levels, and AUC values from ROC analysis.

Target	Method	Cut-off	Sensitivity	Specificity	AUC
BamHI-W	dPCR	31.5 copies/ml	0.958	0.731	0.921
	qPCR	13.9 IU/ml	0.75	0.962	0.865
EBNA1	dPCR	72 copies/ml	0.958	0.769	0.936
	qPCR	204.1 IU/ml	0.583	1	0.792

(b) Sensitivity and specificity values at selected cut-off points. The optimal cut-off was selected by maximising the Youden J index.

Target	Method	Cut-off	Sensitivity	Specificity	Youden J index
BamHI-W	dPCR	10.5 copies/ml	1	0.577	0.577
		31.5 copies/ml	0.958	0.731	0.689
		43.5 copies/ml	0.792	0.769	0.561
		52.5 copies/ml	0.75	0.769	0.519
	qPCR	13.9 IU/ml	0.75	0.962	0.712
		28.2 IU/ml	0.708	0.962	0.67
		29.2 IU/ml	0.667	0.962	0.628
		36.7 IU/ml	0.625	0.962	0.587
EBNA1	dPCR	64.5 copies/ml	0.958	0.692	0.651
		72 copies/ml	0.958	0.769	0.728
		81 copies/ml	0.917	0.769	0.686
		90 copies/ml	0.917	0.808	0.724
	qPCR	204.1 IU/ml	0.583	1	0.583
		454.5 IU/ml	0.542	1	0.542
		528.7 IU/ml	0.5	1	0.5
		586.4 IU/ml	0.458	1	0.458

Supplementary Table S3. Performance of serum EBV dPCR and qPCR.

(a) Sensitivity and specificity at optimal cut-off levels, and AUC values from ROC analysis.

Target	Method	Cut-off	Pre-treatment		
			Sensitivity	Specificity	AUC
BamHI-W	dPCR	390 copies/ml	0.661	0.857	0.784
	qPCR	63.1 IU/ml	0.768	0.768	0.793
EBNA1	dPCR	265 copies/ml	0.536	0.821	0.683
	qPCR	780 IU/ml	0.625	0.804	0.712

(b) Sensitivity and specificity values at selected cut-off points. The optimal cut-off was selected by maximising the Youden J index.

Target	Method	Cut-off	Sensitivity	Specificity	Youden J index
BamHI-W	dPCR	275 copies/ml	0.679	0.821	0.500
		365 copies/ml	0.679	0.839	0.518
		390 copies/ml	0.661	0.857	0.518
		465 copies/ml	0.625	0.857	0.482
	qPCR	26.4 IU/ml	0.786	0.750	0.536
		57.1 IU/ml	0.768	0.750	0.518
		63.1 IU/ml	0.768	0.768	0.536
		65.7 IU/ml	0.750	0.768	0.518
EBNA1	dPCR	90.0 copies/ml	0.625	0.607	0.232
		265 copies/ml	0.536	0.821	0.357
		365 copies/ml	0.446	0.875	0.321
		390 copies/ml	0.429	0.875	0.304
	qPCR	744 IU/ml	0.625	0.786	0.411
		780 IU/ml	0.625	0.804	0.429
		804 IU/ml	0.607	0.804	0.411
		830 IU/ml	0.589	0.804	0.393