Differential CpG methylation in the *Interferon Gamma* (*IFNG*) promoter correlates with viral load and transcriptional control in people with HIV

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Introduction

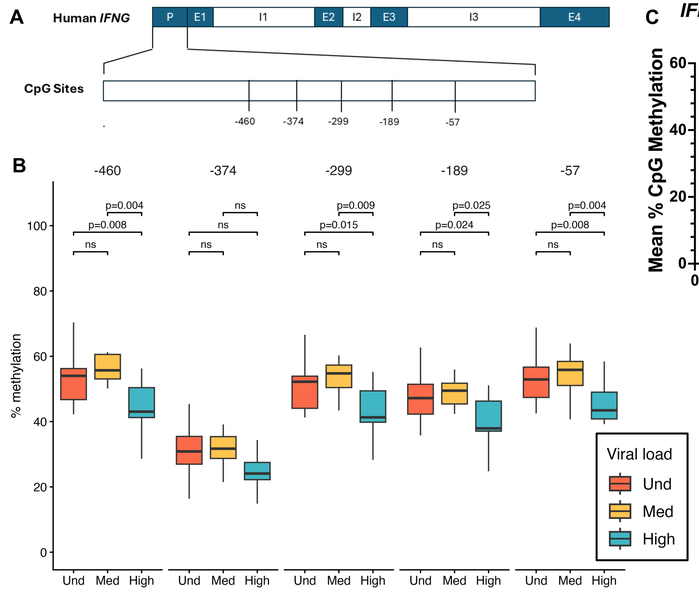
- **HIV persistence** is facilitated by immune evasion strategies that **reshape host immune responses**.
- **Interferon Gamma** (***IFNG***) encodes a key antiviral and immunoregulatory cytokine whose transcription **is governed by promoter CpG methylation**.[1]
- **Clinical and *ex vivo* studies have yielded contrasting results**, as acute HIV infection is marked by elevated IFN-γ plasma expression,[2] yet peripheral blood mononuclear cells (PBMCs) from viremic people with HIV (PWH) produce less IFN-γ compared to PBMCs from people without HIV.[3]
- PBMC subset analysis shows that ***IFNG* promoter methylation** strongly **correlates with variations in** either IFNG **transcription** or IFN-γ **expression**.[4,5,6]
- **HIV** infection is associated with **chromatin modifications** across **genes involved with IFN signaling**.[7]

Hypotheses

- **HIV antigen exposure** will be associated with **epigenetic remodeling** at the ***IFNG* promoter** in PBMCs from PWH.
- **Differential methylation** in the *IFNG* promoter will correlate with variations in **IFNG mRNA expression**.

Results

CpG demethylation of the *INFG* promoter in the PBMCs of PWH represents a **threshold−dependent epigenetic control of IFNG transcription** that emerges **during high HIV viremia**.



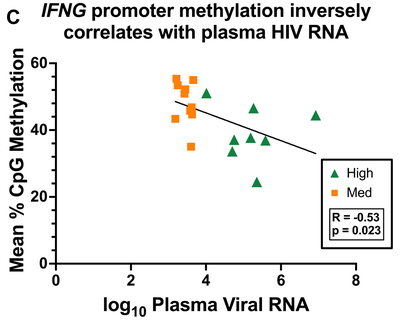


Fig. 1 (**A**) Gene map of ***IFNG* promoter CpG sites**, (**B**) and corresponding **methylation rates** per viral load group. (**C**) **Reduced promoter methylation** is significantly **associated with higher plasma HIV RNA** across participants with detectable HIV expression (Spearman p=0.023).

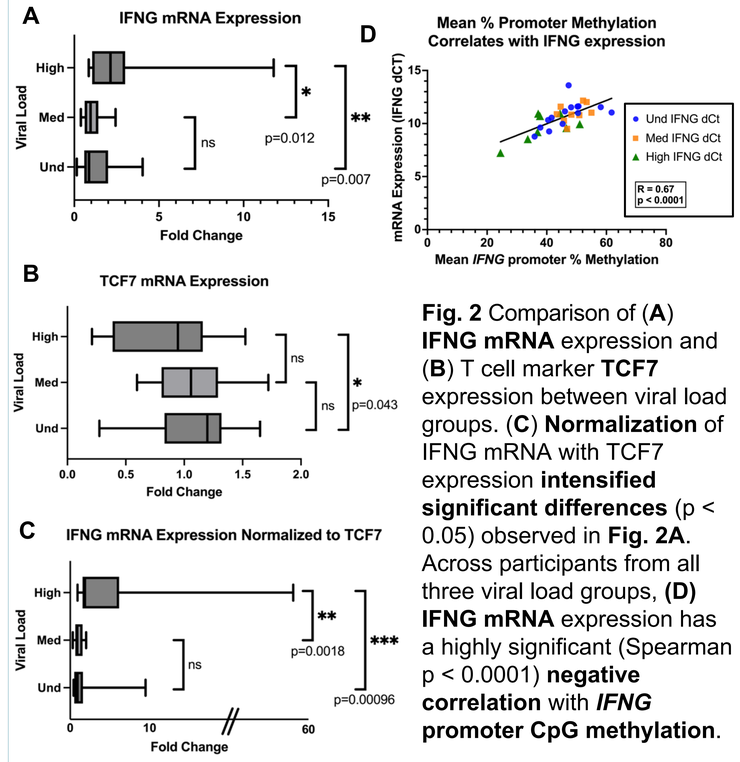


Fig. 2 Comparison of (**A**) **IFNG mRNA** expression and (**B**) T cell marker **TCF7** expression between viral load groups. (**C**) **Normalization** of IFNG mRNA with TCF7 expression **intensified significant differences** ($p < 0.05$) observed in **Fig. 2A**. Across participants from all three viral load groups, **(D) IFNG mRNA** expression has a highly significant (Spearman $p < 0.0001$) **negative correlation** with ***IFNG* promoter CpG methylation**.

Materials, Methods, and Participants

1. **Study participants** enrolled in the San Diego Primary Infection Resource Consortium (PIRC) were selected and **categorized** based on **high** (> 5000 copies/mL), **medium** (500 to 4999 copies/mL) and **undetectable** (< 50 copies/mL) **HIV plasma RNA**.
2. Forward and reverse primers were designed to target the **bisulfite converted *IFNG* promoter** 557 bp upstream from the transcriptional start site. **Genomic DNA extracted** from PBMCs was subject to **bisulfite PCR and sequencing**.
3. FASTQ files were preprocessed using **Trim Galore!** for adapter trimming and **bwa-meth** for alignment to the *IFNG* promoter. Percent methylation at each CpG site was calculated using **Methyldackel** as the proportion of methylated reads among total reads. A **beta-binomial regression** framework was used to account for overdispersion in methylation proportions across samples. **Statistical significance** ($p < 0.05$) was assessed using model-based contrasts.
4. Forward and reverse primers were designed to target **IFNG mRNA expression** and the **T cell marker TCF7**. Total **RNA** was extracted from PBMCs and subject to **RT-qPCR**. Gene transcripts were quantified using **ΔΔCT normalization to RPP30**. Kruskal-Wallis and Dun's **post-hoc tests** were used to determine statistical significance ($p < 0.05$).

	High VL	Medium VL	Undetectable VL
Age (at draw)	30 (27, 38)	35 (30, 39)	41 (29, 46)
Sex			
Male	16	17	16
Female	0	1	2
Race			
White	11	16	15
Black	2	0	1
Other	3	2	2
Ethnicity			
Hispanic	2	2	6
Non-hispanic	14	16	12
Risk Factor			
MSM	14	17	16
Heterosexual/Other	2	1	2
Injection Drug User	2	1	0
ART Status			
On ART at Draw	2	5	18
Not on ART at Draw	14	13	0
Viral Load	145500 (49247, 262163)	2497 (1551, 3979)	< 50
CD4 Count	484 (387, 715)	555 (497, 726)	776 (497, 1054)

Table 1. Participant demographics and viral load categorization at time of sample draw. Covariate analysis using linear models was used to test for differences in age, and ART was not included as a covariate in primary models due to collinearity with viral load group.

Conclusions and Future Directions

- **HIV plasma RNA** levels correlate with **hypomethylation** of the ***IFNG* promoter** and **elevated IFNG transcription** in PBMCs from PWH, reinforcing evidence that **HIV replication influences epigenetic regulation of interferon pathways**.[7]
- Plasma **HIV viral load may drive epigenetic remodeling of host immune genes** in circulating immune cells and amplify INFG responses during active infection. Promoter *IFNG* hypomethylation and transcriptional control occur as a **threshold-dependent epigenetic response during high HIV plasma expression**.
- **Further investigation** of the longitudinal progression of chromatin accessibility at these promoter CpG sites will help **elucidate the epigenetic machinery** affecting interferon pathways, potentially enabling immune evasion and viral persistence.

Acknowledgements and References

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