Table DS1: Oligonucleotides used for the study

Sr. No.	Oligonucleotide name	Sequence (5´-3´)
1	Motif validation_1	CAGTGAGCGAGCTTCCGCTTGACATCCCAATA
2	Motif validation_2	CAGTGAGCGAGCTTCCTCTTGACATCCCAATA
3	Motif validation_3	CAGTGAGCGAGCTTCCTCTCGACATCCCAATA
4	Motif validation_4	CAGTGAGCGAGCTTCCTCCCGACATCCCAATA
5	Motif validation_5	TATTGGGATGTCAAGCGGAAGCTCGCTCACTG
6	Motif validation_6	CGGTAATACAGATCGATTATGCCCCAATAACC
7	MSMEG_3902_binding	TGGGTGAGATCAGCACGCTTCGCGAGATCCTG
9	MSMEG_5103_binding	AGTGCTGCCCGCCAACGCTTCTACGACGCCCT
10	Ms_murA_4934_99bp_F	GGTCCTGGTGTAGATGCGAG
11	Ms_topA_6160_F	TGGCGTAGCGGATTATCGTC
12	Ms_3434_3432_F	CGTAGTCGACTCTGTCACCG
13	32mer_6200-6300Kb	GAAAGTATCCGGCGGGACGCTTGCGCAGCAGT
14	32mer_4900-5100Kb	TCCCGTCGATATGGACTCTTGGGGAAGATCAG
15	32mer_3700-3900Kb	AGTGAGCTATTACGCACTCTTTCAAGGGTGGC

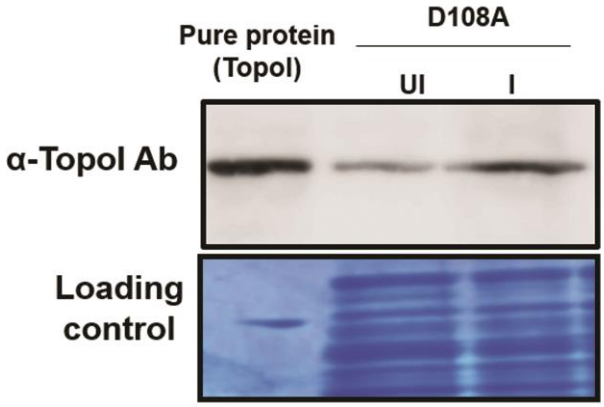

Figure S1.

Immunoblot showing MsTopoI D108A induction. MsTopoI D108A expression was induced by the addition of 25 ng/ml of tetracyclin for 4h. The expression was analysed using anti-TopoI antibodies. Lower panel shows the duplicate gel stained with Coomassies blue. UI= uninduced, I= induced.

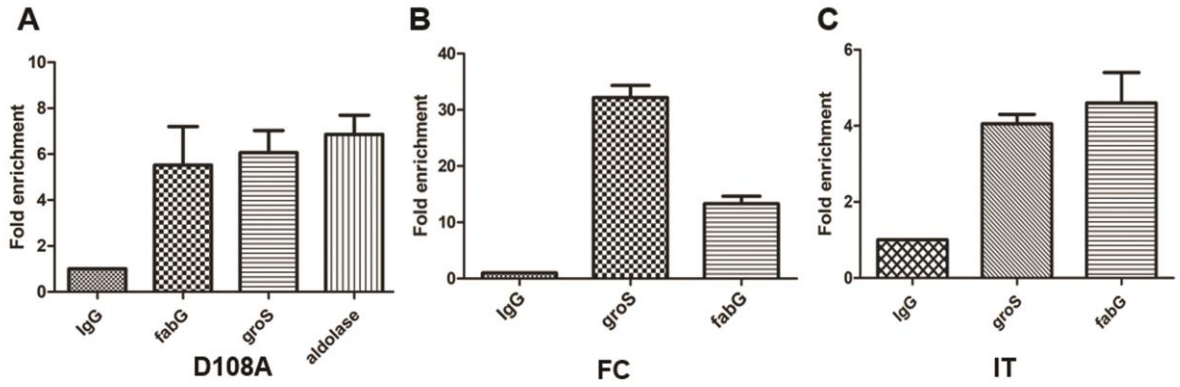

Figure S2.

ChIP qPCR to assess the TopoI enrichment on selected genes as described in Materials and Methods. D108A **(A)**, FC **(B)** and IT **(C)**. The data represented is mean±SD from three independent experiments.

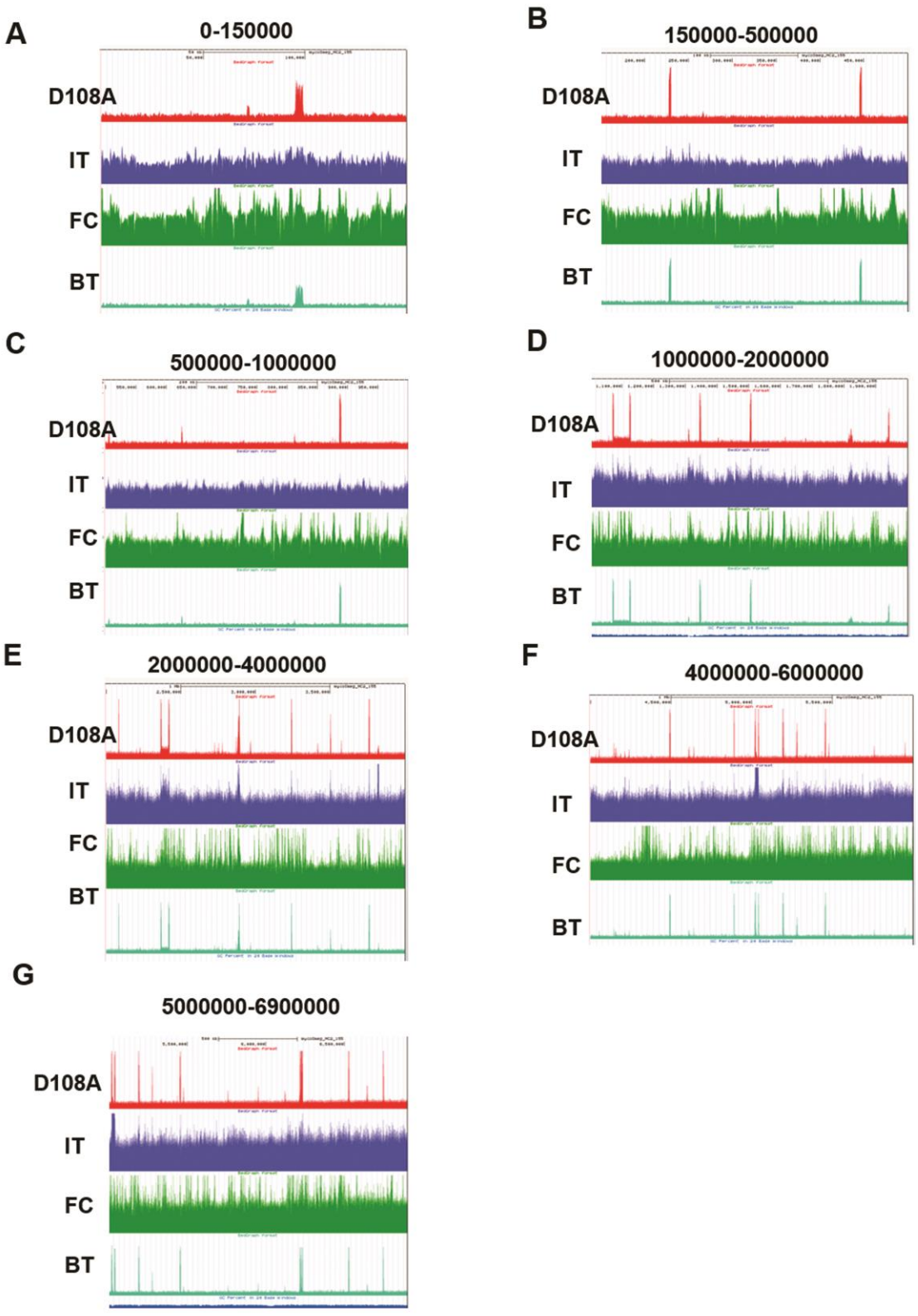

Figure S3.

TopoI binding and cleavage sites *M. smegmatis* (mc^2 155) genome. UCSC browser zooms of different genomic coordinates- 0-0.15 Mb **(A)**, 0.15 Mb- 0.5 Mb **(B)**, 0.5 Mb-1 Mb **(C)**, 1-2 Mb **(D)**, 2-4 Mb **(E)**, 4-6 Mb **(F)** and 5-6.9 Mb **(G)**.

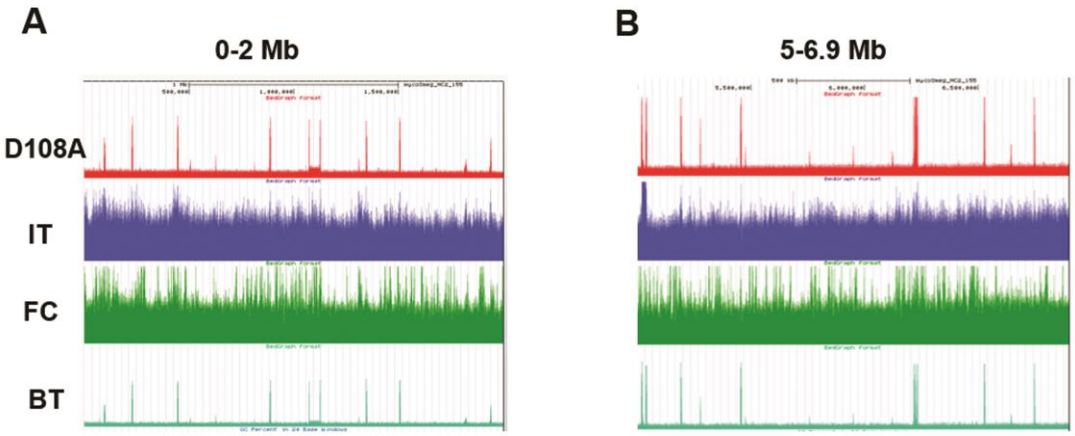

Figure S4

TopoI functional sites near Ori of *M. smegmatis* (mc^2 155) genome. UCSC browser zooms for Ori region depicting the specific binding and cleavage peaks for TopoI in 0-2 Mb genomic coordinates **(A)** and 5Mb-6.9 Mb **(B)**.

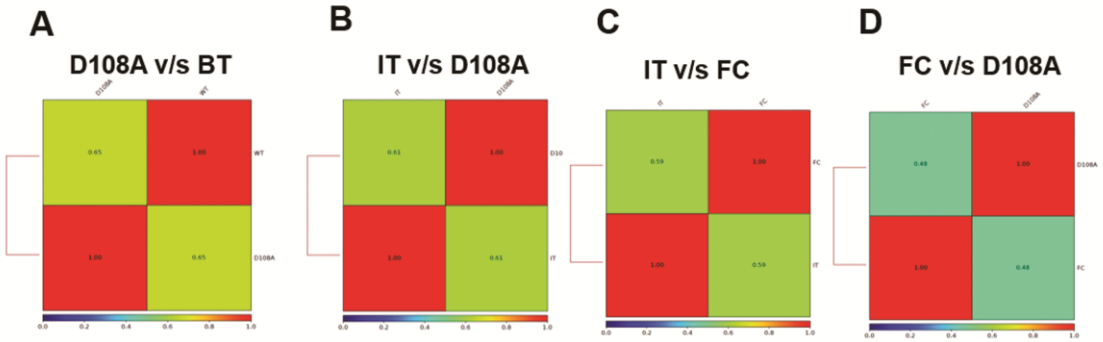

Figure S5.

Calculation of correlation coefficient. Pearson correlation coefficient between D108A and BT **(A)**, IT and D108A **(B)**, IT and FC **(C)**, and FC and D108A **(D)** ChIP-seq data.

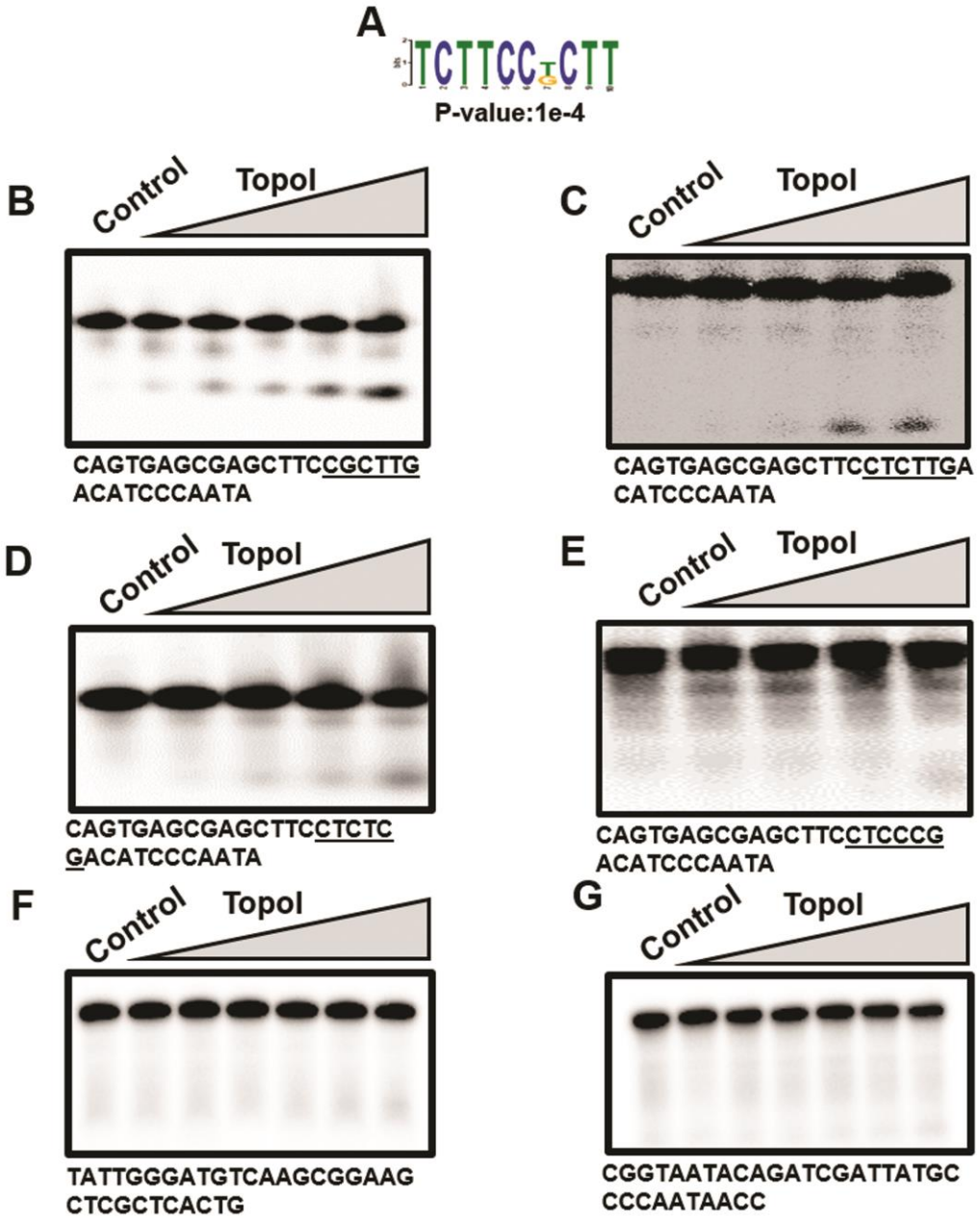

Figure S6.

DNA TopoI cleavage motif and cleavage analysis. **(A)** Motif generated from cleavage sites *in vivo* using MEME suite. **(B)** Cleavage assay was performed with consensus motif containing oligonucleotides with increasing concentration of MsTopoI (0.01-0.16 µM) and the resultant products were analysed on 8M urea 12% polyacrylamide gel. **(C, D and E)** Cleavage assay with oligonucleotides having substitutions in consensus motif. With more substitutions cleavage is diminished. **(F)** Cleavage assay with the oligonucleotides designed from the region lacking both binding and cleavage peaks. **(G)** Cleavage assay with nonspecific oligonucleotides taken from the laboratory stock, which of non-mycobacterial origin.