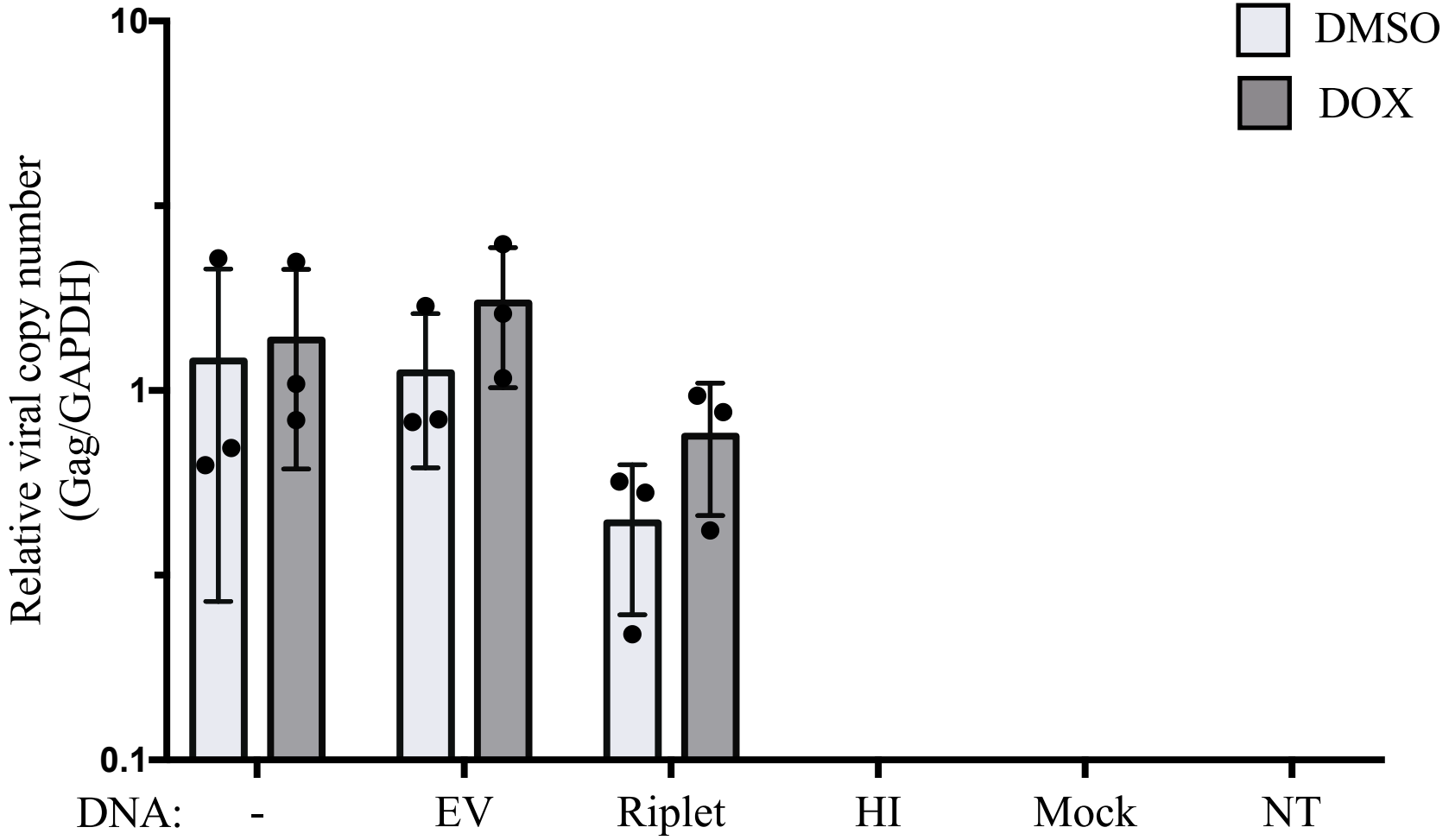
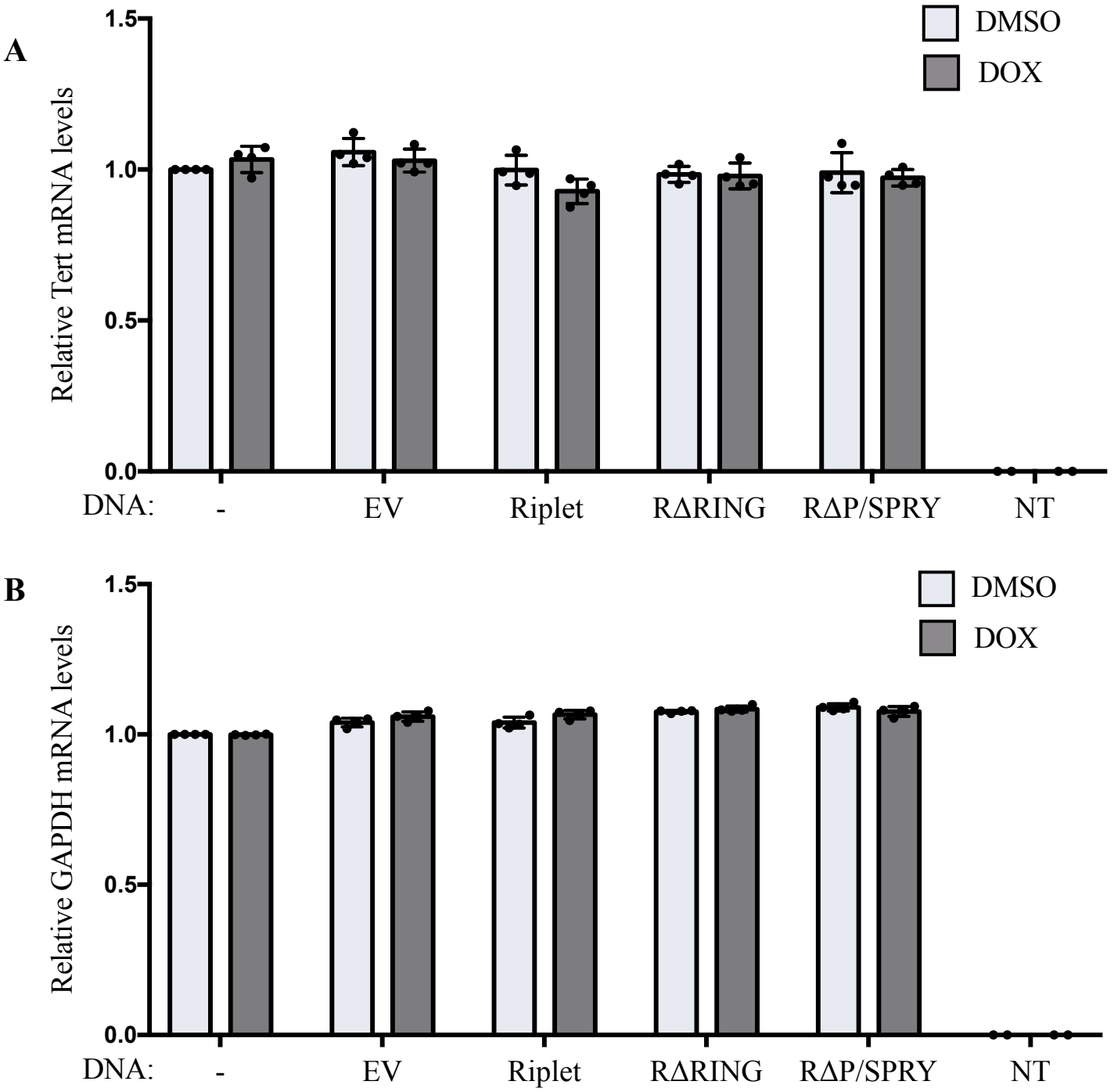
Supplementary Fig 1



Supplemental Figure 1.

Overexpression of Riplet does not impact proviral DNA formation. 293TrexhZAP cells (-) or 293TrexhZAP stable cell lines expressing an empty vector (EV) or Riplet were infected with VSV-G pseudotyped HIV-1 Luciferase reporter virus or a mock virus (Mock) for 6 h at 37°C followed by ZAP induction with doxycycline or DMSO as a control. Infected cells were then propagated for 10 days, after which genomic DNA was extracted and real-time quantitative PCR was carried out using primers specific for HIV-1 gag or GAPDH. The extent of viral integration (relative viral copy number) was quantified by qPCR using the $2^{-\Delta\Delta CT}$ method by normalizing the levels of viral DNA detected with Gag-specific PCR primers to cellular DNA detected with GAPDH-specific primers. To control for potential plasmid DNA carryover in the viral preparation, heat-inactivated (HI) virus was used in parallel. An additional sample lacking a template for PCR amplification was included (NT) to control for DNA contamination. Results shown are means ± SD from three independent experiments performed in triplicate.

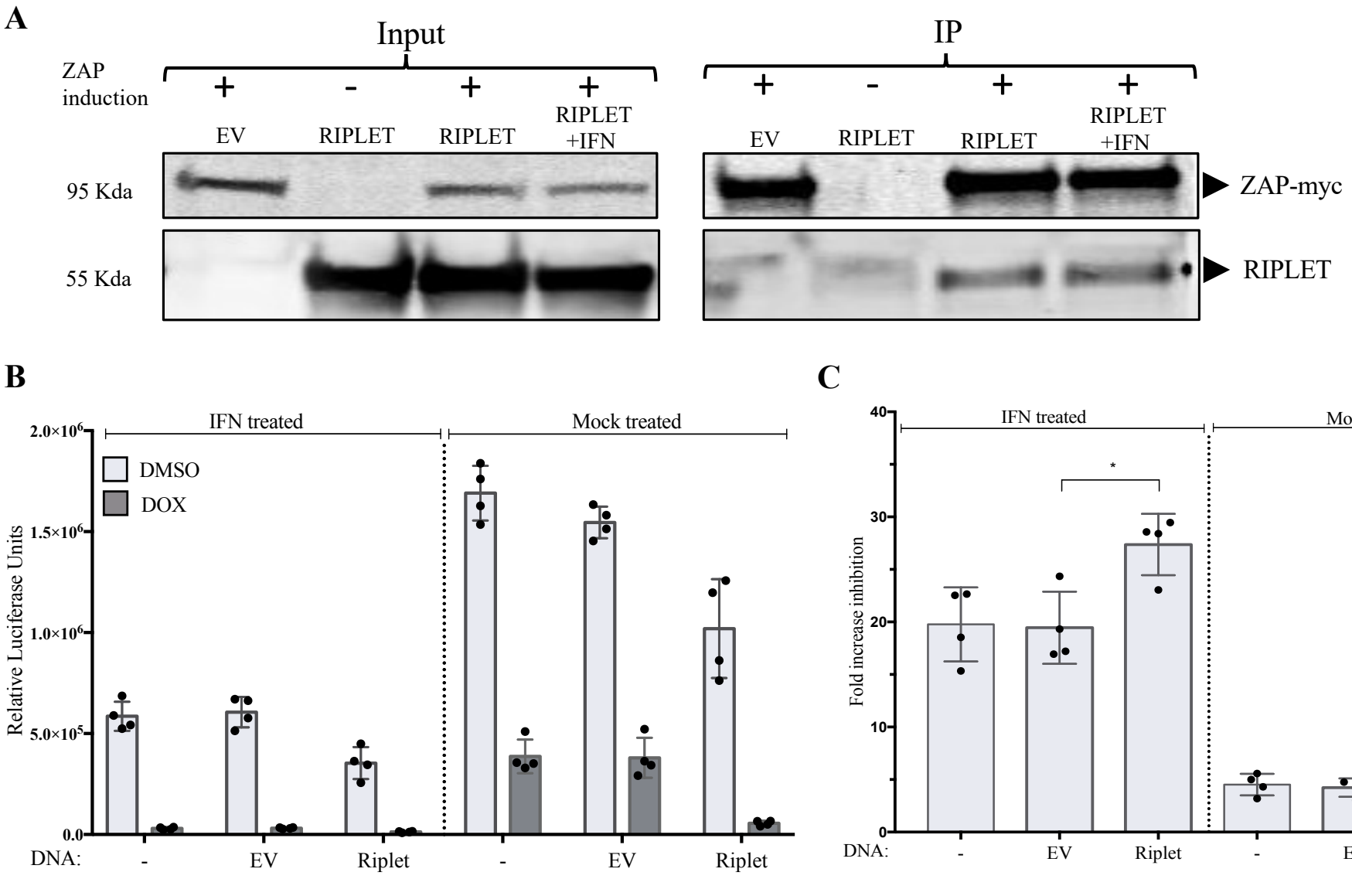
Supplementary Fig 2



Supplemental Figure 2.

Housekeeping mRNA levels are not affected by Riplet overexpression. Cellular transcription was assessed by measuring relative mRNA levels for the cellular Tert **(A)** and GAPDH genes **(B)**. Cell lines untreated (-) or transfected to express an empty vector (EV) or the indicated Riplet constructs were infected with VSV-G pseudotyped HIV-1 Luciferase reporter virus for 6 h at 37°C followed by ZAP induction with doxycycline or DMSO as a control. Two days postinfection, RNA of 8 $x10^6$ cells per sample was isolated and 100 ng of purified cellular RNA was used for qRT-PCR analysis performed with specific primers against the human Tert and GAPDH genes. The obtained values were then compared to the values obtained for 293TrexhZAP cells without ZAP induction (DMSO) which was set to 1. To control for nucleic acid contamination, sample containing no template for PCR amplification (NT) was used in parallel. Data presented are means ± SEMs from four independent experiment done in triplicate.

Supplementary Fig 3



Supplemental Figure 3.

Coimmunoprecipitation of Riplet and ZAP is not affected by treatment with IFNs. (A) 293TrexhZAP cells were transfected with an empty vector control (EV) or DNAs expressing the indicated proteins, and treated with doxycycline to induce ZAP expression (+) or with DMSO control (-) as indicated. Cells were then treated with "universal" type I alpha interferon at 1000 U/ml (+IFN) where indicated. Lysates were prepared at 24 hours post transfection. Left panels (Input): Whole cell lysates were analyzed by gel electrophoresis, blotted, and probed for myc-tagged ZAP with a mouse α-myc antibody (upper panel) or Riplet specific antibodies (lower panel). Right panel (IP): Myc-tagged ZAP was recovered by immunoprecipitation with anti-myc antibodies followed by gel electrophoresis analysis, blotted and probed for myc-tagged ZAP (upper panel) or Riplet (lower panel). Approximate molecular weights of major proteins estimated from size markers are indicated on left. **(B)** 293TrexhZAP cells (-) or 293TrexhZAP stable cell lines expressing the indicated DNAs were infected with VSV-G pseudotyped HIV-luc reporter virus. Fresh media containing doxycycline to induce ZAP expression (100 ng/ml) or DMSO as a control was added at 6 hours post infection. Cells were then treated with universal type I interferons at 1000 U/ml or DMSO as a control for 4 hours. Firefly luciferase reporter activity was measured at 24 hours post infection and normalized to total protein content measured by a Bradford assay for each sample. Data points presented are the mean RLU/mg ±SD values of four independent experiments done in triplicate. **(C)** The fold increase inhibition was calculated for data shown in panel B as the ratio of luciferase expression levels in DMSO treated cells to that in doxycycline treated cells for after normalization to total protein content shown by Bradford assay for each sample. Data points represent the mean ±SD values of four independent experiments. Student's t test was used for statistical analysis. *, $P < 0.05$, **** $P < 0.0001$.