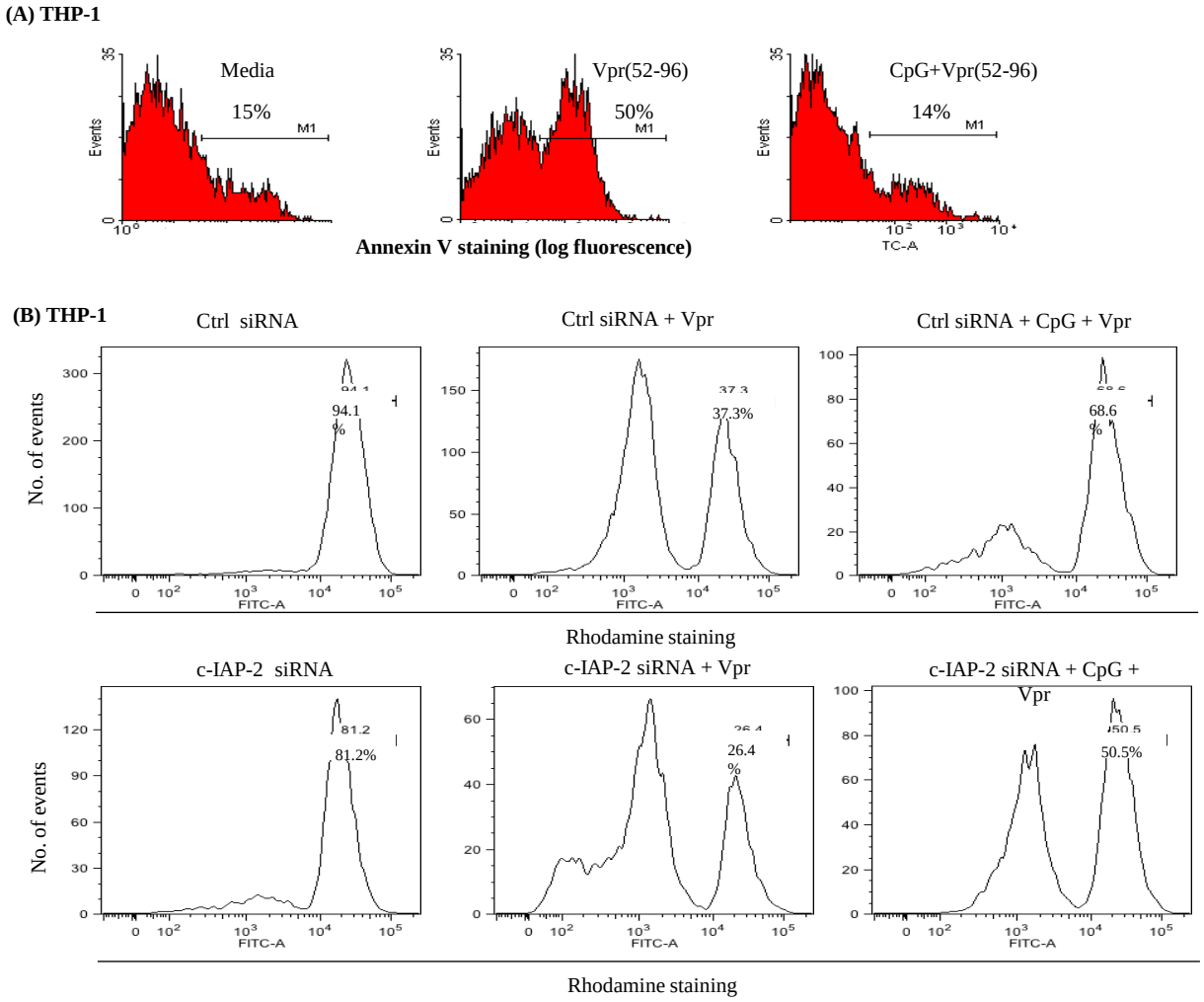
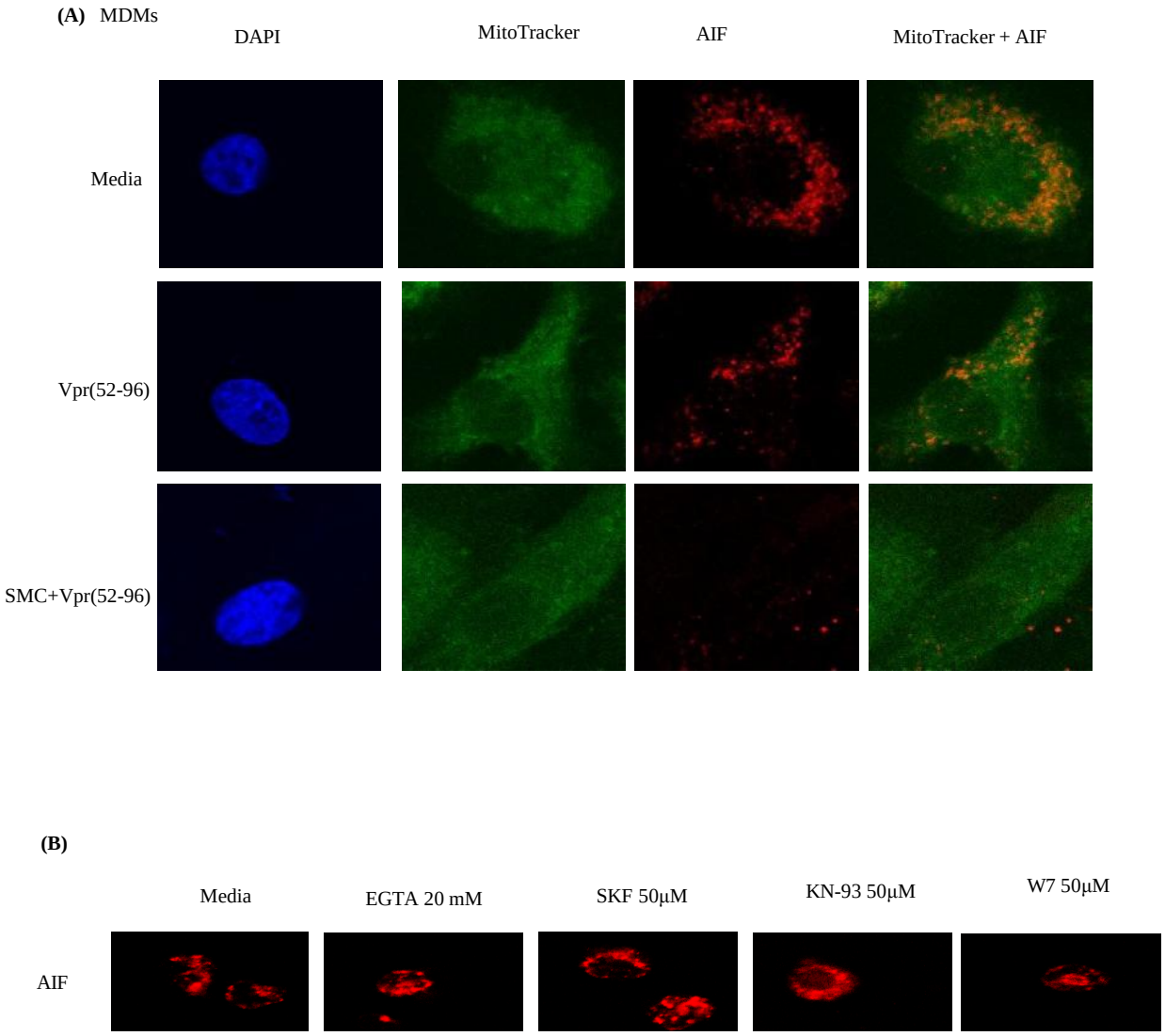
Supplementary-Fig 1



Supp Fig. 1: Transfection with c-IAP-2 siRNA alone does not affect Vpr-induced mitochondrial depolarization.
(A) THP-1 cells ($1.0x10^6$/ml) were stimulated with 5 μM CpG ODN for 12 h followed by treatment with 1.5 μM Vpr for 24 h and subsequently analyzed by Annexin-V staining for the measurement of apoptosis. Results show a representative of three independent experiments.
(B) THP-1 cells ($0.25x10^6$/0.5ml) were transfected with 1 μg of either c-IAP-2 or non-silencing control siRNA for 48 h either with or without stimulation with CpG ODN (5 μM). Thereafter, cells were treated with 1.5 μM Vpr for 5 h followed by Rhodamine 123 staining and flow cytometry for mitochondrial membrane potential evaluation.

Supplementary-Fig 2

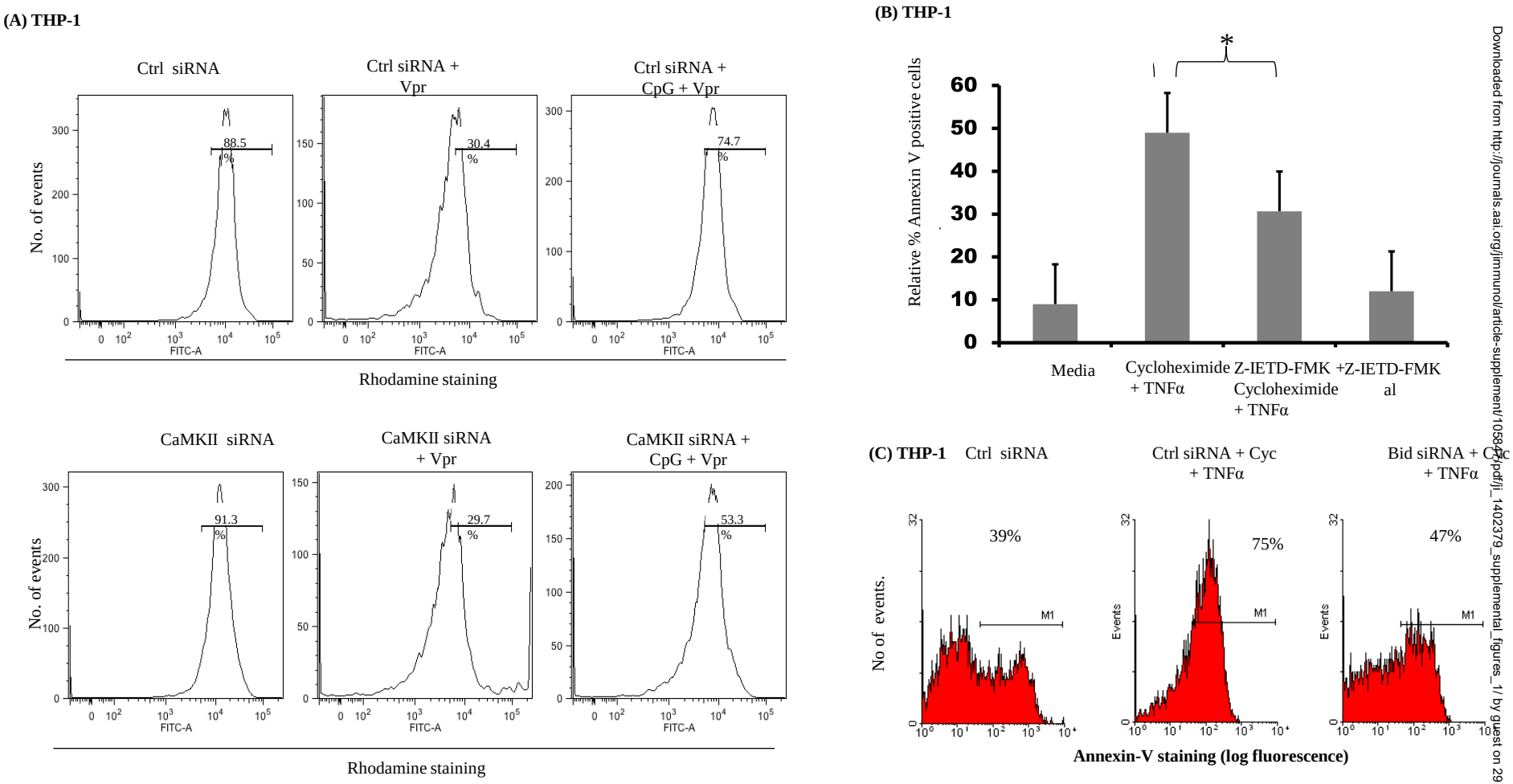


Supp Fig. 2: Pre-treatment with SMC renders MDMs sensitive to Vpr(52-96)-mediated loss of mitochondrial AIF.

(A) MDMs (1.0x106/ml) were treated with 200 nM AEG-730 SMC for 12 h followed by treatment with 1.5 μM Vpr(52-96) for 6 h. Thereafter cells were co-stained for the nuclear stain DAPI (blue), Mitotracker (green) and AIF (red); and visualized using confocal microscope with a 40X lens at 4x magnification.Yellow/Orange represents colocalization of AIF with the mitochondria. The results are representative of three independent experiments.

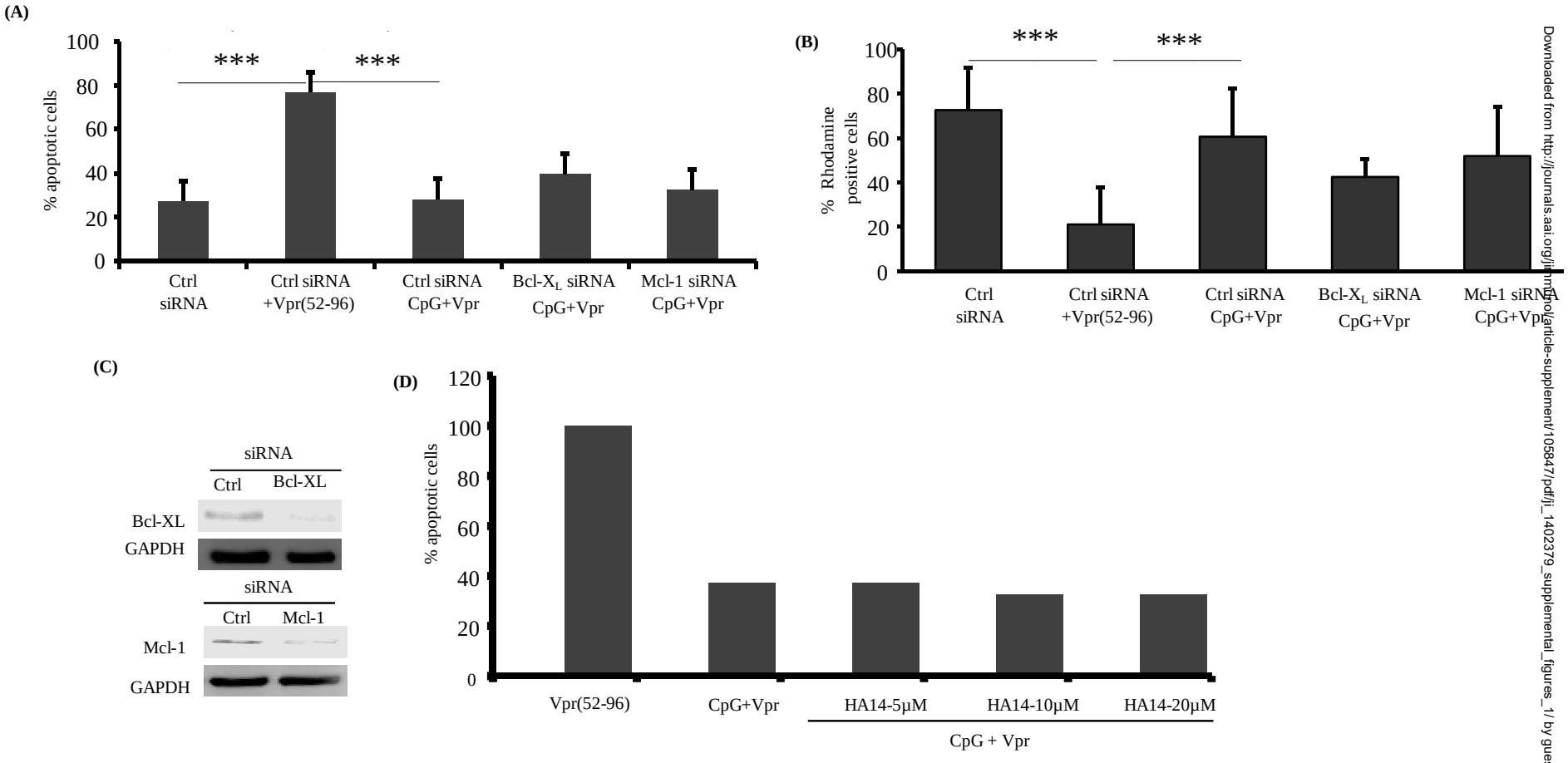
(B) THP-1 cells (1.0x106/ml) were treated with 10 mM EGTA, 20 μM each of W-7, SKF or KN-93 for 14 h and prepared for confocal microscopy as described in Materials and Methods. AIF (red) was visualized using confocal microscope with a 63X lens at 4x magnification. The results are representative of three independent experiments.

Supplementary-Fig 3



Supp Fig. 3: Transfection with CaMK-II siRNA alone does not affect Vpr-induced mitochondrial depolarization. (A) THP-1 cells ($0.25x10^6$/0.5ml) were transfected with 1 µg of either CaMK-II siRNA or non-silencing siRNA for 24 h followed by stimulation with 5 µM CpG ODN for 12 h. Thereafter, cells were treated with 1.5 µM Vpr for 5 h followed by Rhodamine 123 staining and flow cytometry for mitochondrial membrane potential evaluation. **(B)** THP-1 cells ($1x10^6$/ml) were treated with 20 mM of Z-IETD-FMK for 2 h before treatment with 20 ng/ml of TNF-α and 25 ng/ml of cycloheximide for 24 h followed by measurement of apoptosis by Annexin-V staining. The results shown are expressed as a mean ± SD of four independent experiments. *p <0.001, is calculated using the Mann-Whitney Test. **(C)** THP-1 cells ($0.25x10^6$/0.5ml) were transfected with 40 nM Bid specific siRNA or non-silencing control siRNA for 48 h. Following transfection, cells were treated with 20 ng TNF-α and 25 µg/ml cyloheximide for 24 h followed by measurement of apoptosis by Annexin-V staining.

Supplementary-Fig 4



Supp Fig. 4: Mcl-1, Bcl-XL and Bcl2 not contribute to CpG ODN- induced protection against Vpr-mediated apoptosis and mitochondrial injury. THP-1 cells (0.25x10^6/0.5ml) were transfected with 40 nM Bcl-XL or Mcl-1 specific siRNAs or non-silencing control siRNA for 48 h. Thereafter the cells were stimulated with 5 uM CpG ODN for 12 h before treatment with 1.5 μM Vpr(52- 96) for **(A)** 24 h followed by measurement of apoptosis by Annexin-V staining or **(B)** for 5 h followed by staining with Rhodamine 123 for mitochondrial membrane potential evaluation by flow cytometry. (**C**). Cell lysates were analyzed for Bcl-XL and Mcl-1 expression by immunoblotting. Results in A and B are expressed as a mean ± SD of four and six independent experiments. The results in C are representative of three independent experiments. *** p <0.001, is calculated using the Mann-Whitney Test.
(D) THP-1 cells (1.0x106/ml) were treated with 5-20 μM HA14-1 prior to stimulation with 5 μM CpG for 12 h followed by treatment with 1.5 μM Vpr(52-96) for 24 h. Subsequently cells were stained with AnnexinV for measurement of apoptosis.