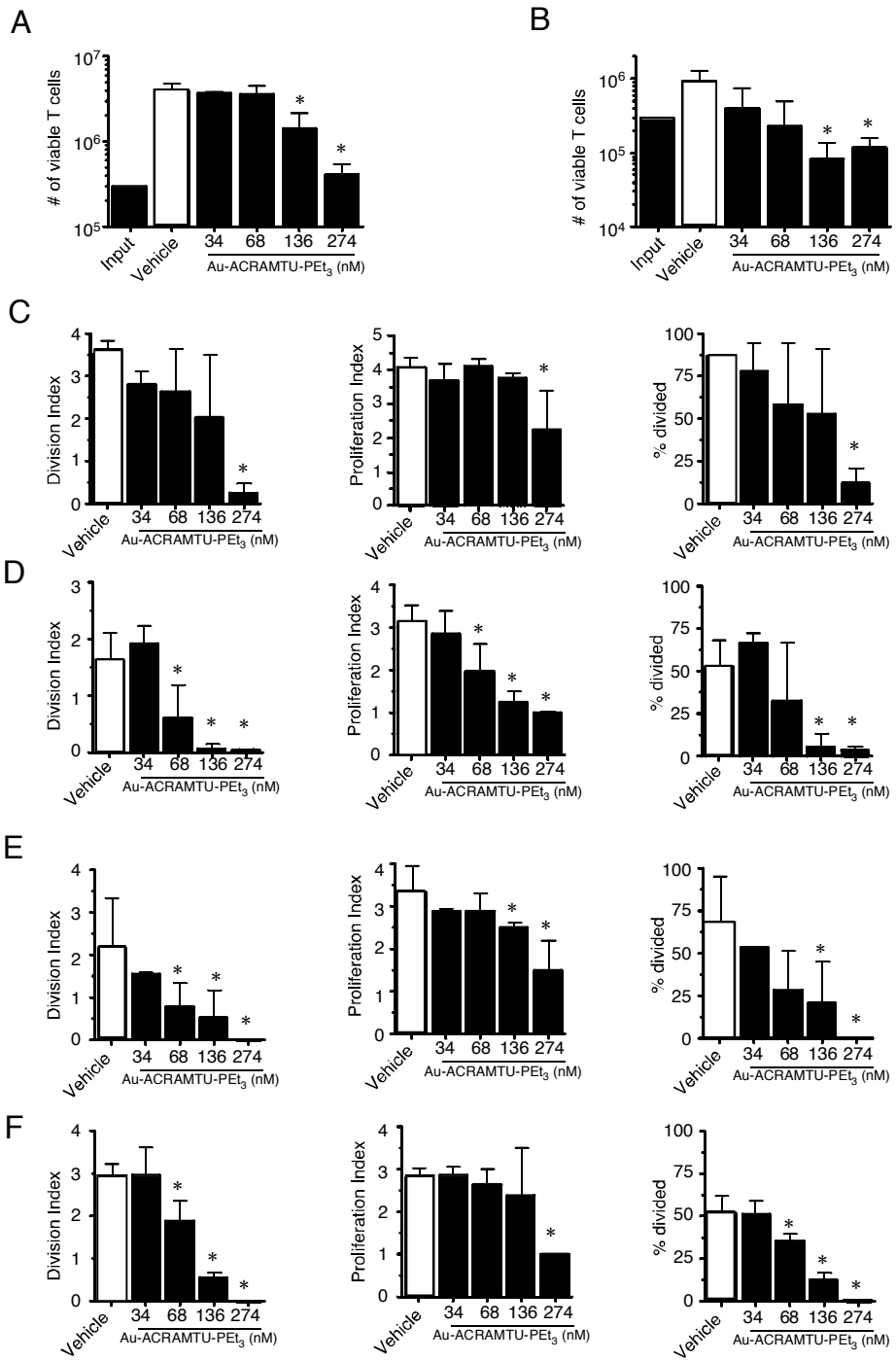

Figure 1. Incubation with Au-ACRAMTU-PEt3 decreases the division and proliferative indexes and the percentage of naïve murine and human T cells that divide. Splenocytes were isolated from naïve C57BL/6 mice, and $CD8^+$ T cells (A) or $CD4^+$ T cells (B) were purified by magnetic microbeads and the number of viable cells was determined on day 3 anti-CD3/CD28 stimulation in the presence of increasing Au-ACRAMTU-PEt_3. The division index, proliferation index, and % divided were determined for murine $CD8^+$ (C) and $CD4^+$ T cells (D). Similar measurements were made for human $CD8^+$ (E) and $CD4^+$ (F) T cells on day 5 anti-CD3/CD28 stimulation in the presence of increasing Au-ACRAMTU-PEt_3. Four subjects were used in 4 independent experiments. The mean and SD are plotted. *, significant difference between vehicle- and Au-ACRAMTU-PEt_3-treated cells , $p<0.05$.

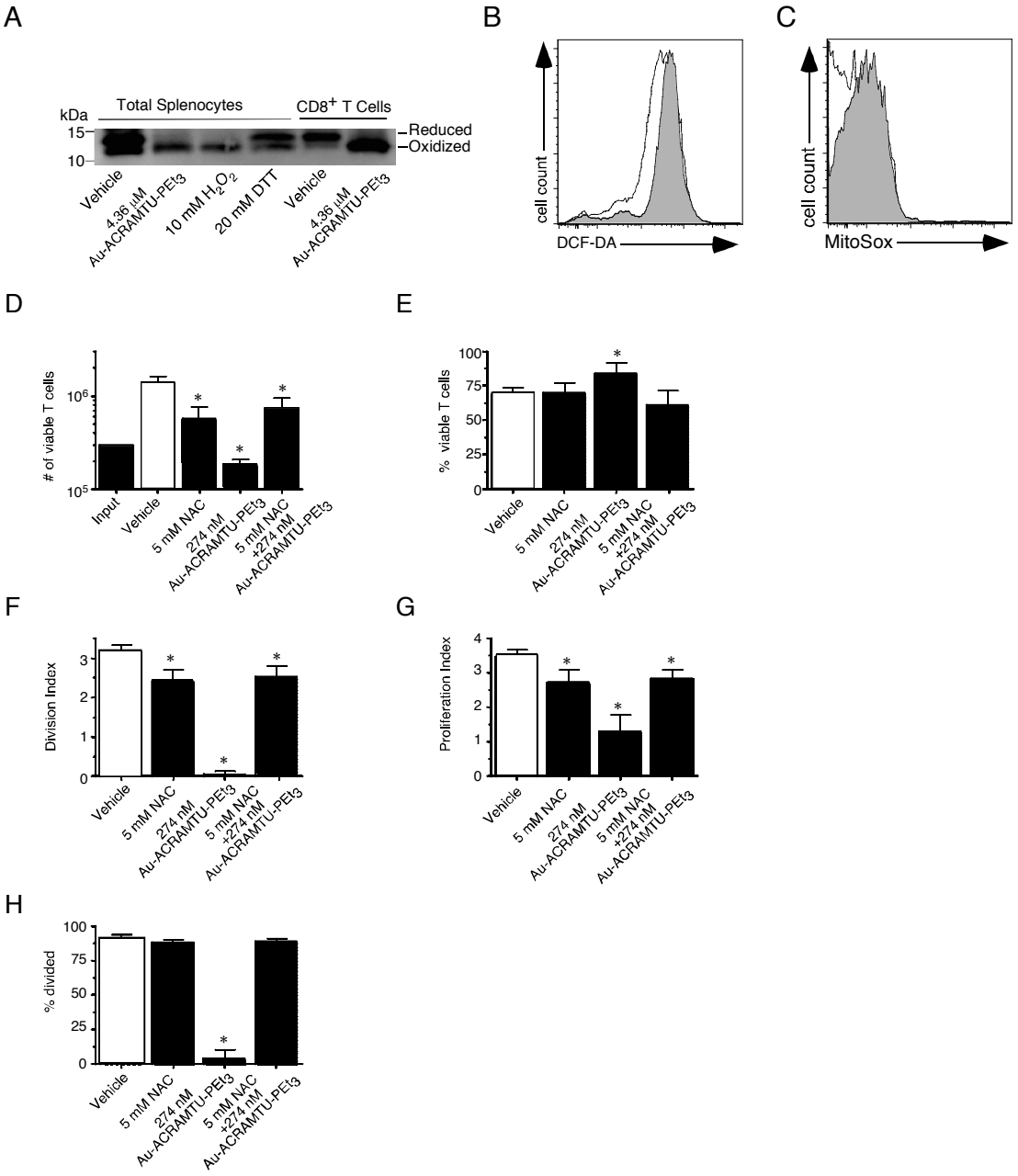

Figure 2. Au-ACRAMTU-PEt$_3$ modulates the redox status of cells. (A) Splenocytes and purified CD8$^+$ T cells (5x10^6) from naive C57BL/6 mice were treated for 30 min in 10 mM H_2O_2 or 20 mM dithiothreitol (DTT) or for 60 min in 4.36 μM Au-ACRAMTU-PEt$_3$ or vehicle. Proteins were then precipitated and alkylated with AMS prior to separation on a 15% polyacrylamide separating gel. This blot is representative of Trx2 oxidation state in the presence of H_2O_2, DTT, Au-ACRAMTU-PEt$_3$, or vehicle in 2 independent experiments. CD8$^+$ T cells were purified from naive C57BL/6 mice and then were preincubated with vehicle or 274 nM Au-ACRAMTU-PEt$_3$ for 60 minutes. Cells were then incubated with either (B) DCF-DA or (C) MitoSox for 30 minutes, washed and then acquired immediately. The open histogram represents vehicle-treated cells, while filled histograms are from Au-ACRAMTU-PEt$_3$ pretreated cells. Staining is representative of 3 mice in two independent experiments. Magnetic microbead purified CD8$^+$ T cells were incubated with 0.1% DMF control or 5mM NAC for 60 minutes at 37^{o}C. Afterwards cells were incubated with 0.1% DMF or 274 nM Au-ACRAMTU-PEt$_3$ for 60 minutes. Cells were then placed on αCD3/αCD28 coated plates and the number of viable cells (D), percentage of viable cells (E), division index (F), proliferation index (G), and % divided (H) were determined on day 3. Four mice were analyzed in 3 independent experiments. The mean and standard deviation are plotted.*, significant difference between vehicle- and Au-ACRAMTU-PEt$_3$-treated cells, $p<0.05$, student's t test..

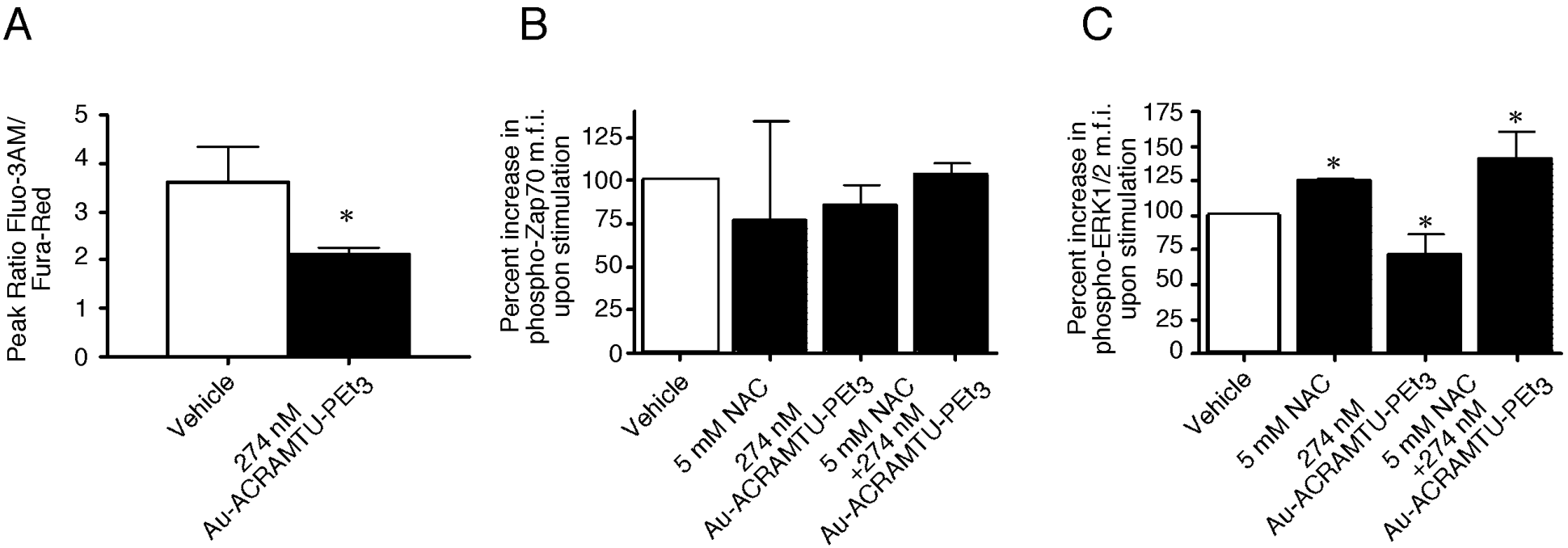

Figure 3. Quantification of T cell signal transduction. (A) Magnetic microbead purified $CD8^+$ T cells were incubated with with 0.1% DMF or 274 nM Au-ACRAMTU-PEt_3 for 60 minutes. Cells were then coated with αCD3/αCD8 antibodies followed by streptavidin crosslinking. The peak ratio of Fluo-3/Fura-Red was determined for 3 mice in 2 independent experiments. The average and standard deviation are plotted. Alternatively,magnetic microbead purified $CD8^+$ T cells were incubated with 0.1% DMF control or 5mM NAC for 60 minutes at 37°C. Afterwards cells were incubated with 0.1% DMF or 274 nM Au-ACRAMTU-PEt_3 for 60 minutes. Cells were then coated with αCD3/αCD8 antibodies followed by streptavidin crosslinking. Intracellular staining for (B) phospho-Zap-70 or (C) phospho-ERK1/2 was performed. The percent increase in m.f.i. relative to the vehicle-treated sample was calculated. The mean and standard deviation are plotted. *, significant difference between vehicle- and Au-ACRAMTU-PEt_3-treated cells, $p<0.05$, student's t test.

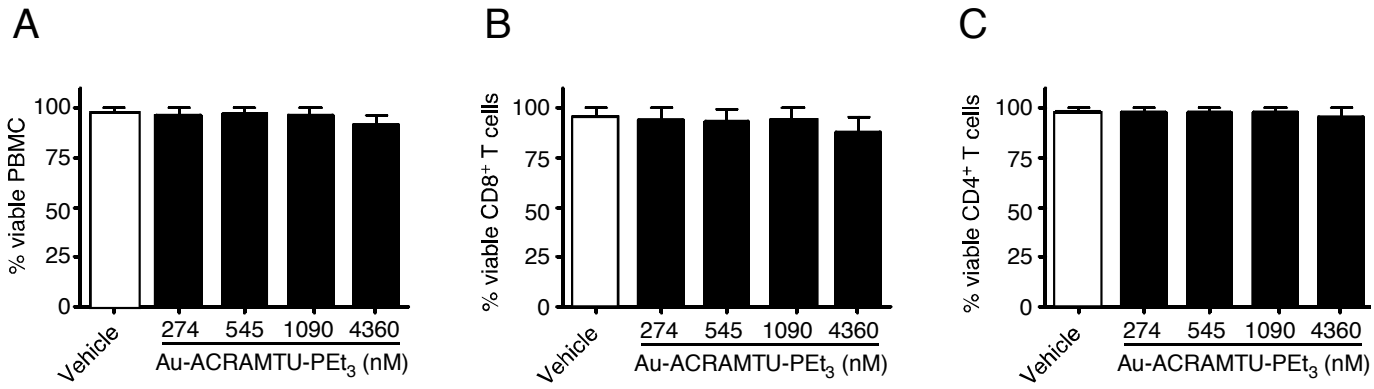

Figure 4. Quantification of cell viability following incubation with Au-ACRAMTU-PEt. (A) PBMCs were incubated with with 0.1% DMF or the indicated concentration of Au-ACRAMTU-PEt_3 for 60 minutes. Cells were then stimulated with PMA/ION in the presence of GolgiPlug and GolgiStop for 5 hours. Following surface staining cells were incubated with 7-AAD, washed and then intracellular staining for cytokines was performed. The percent of cells that were $7AAD^-$ was determined for (A) PBMCs, (B) $CD8^+$ or (C) $CD4^+$ T cells. The mean and standard deviation are plotted. *, significant difference between vehicle- and Au-ACRAMTU-PEt_3-treated cells, $p<0.05$, student's t test.