Supplemental Figure Legends

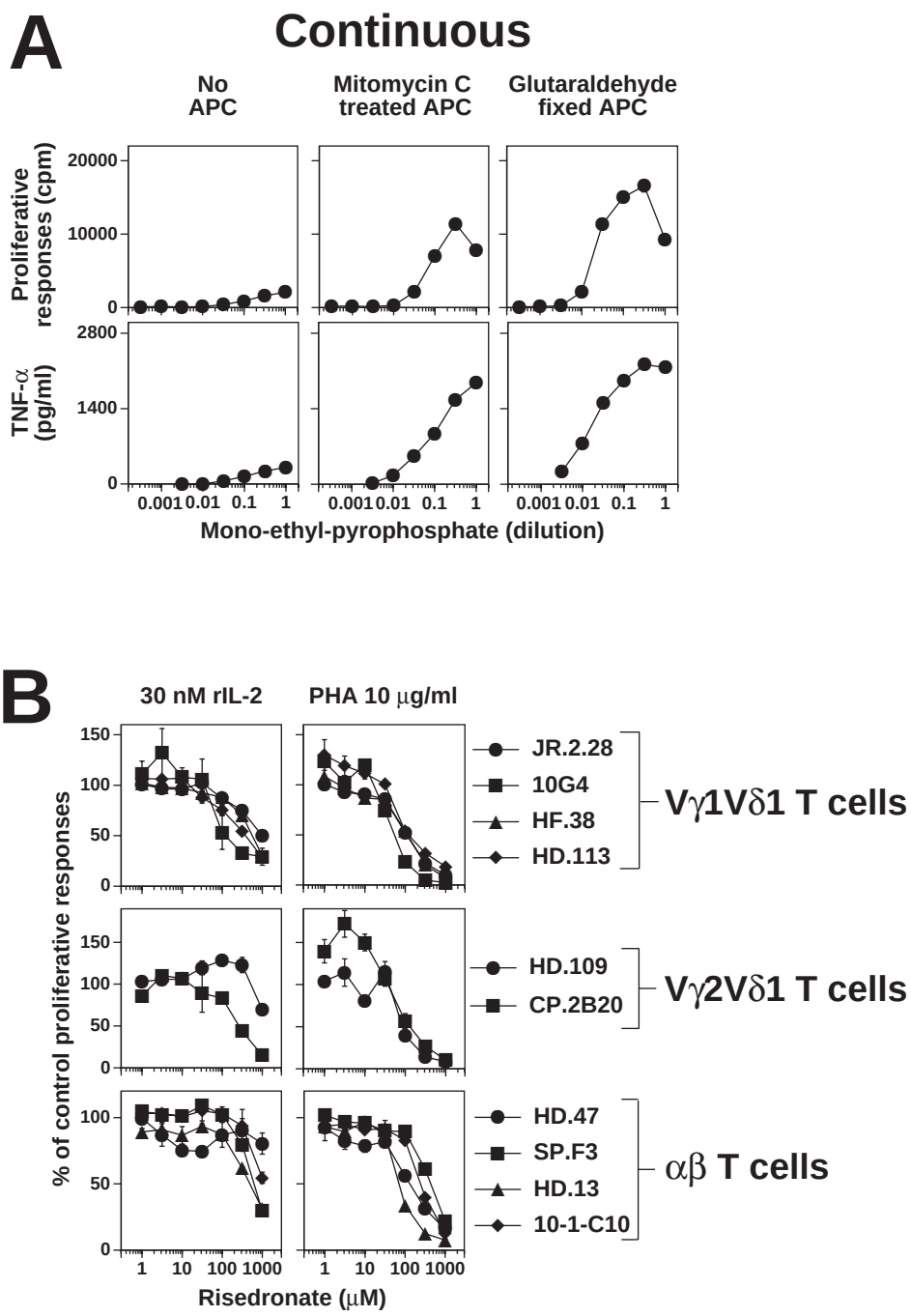
Supplemental Figure 1. Aminobisphosphonate exposure can inhibit T cell proliferation. *A*, Continuous culture of Vγ2Vδ2 T cells with mono-ethyl pyrophosphate stimulates both proliferation and TNF-α release (control experiment performed at the same time as Fig. 2*A*). Mit. C treated or glutaraldehyde fixed CP.EBV cells were cultured continuously with mono-ethyl pyrophosphate and the $CD4^+$ Vγ2Vδ2 T cell clone, JN.23. Supernatants were collected at 16 h for the measurement of TNF-α. The cells were then pulsed with ^{3}H-thymidine and harvested 18 h later. *B*, Risedronate inhibits the proliferation of γδ and αβ T cells to IL-2 or the PHA mitogen. Four Vγ1Vδ1, two Vγ2Vδ1, and four αβ T cell clones were tested for proliferation in response to rIL-2 or PHA in the presence of risedronate. Percentages of maximum control response are plotted.

Supplemental Figure 2. Continuous bisphosphonate exposure of Vγ2Vδ2 T cells and APC inhibits Vγ2Vδ2 T cell proliferation but not TNF-α release. *A*, Continuous exposure to high concentrations of bisphosphonates inhibits proliferation but not TNF-α release by Vγ2Vδ2 T cells. Various bisphosphonates including risedronate (compound 2) were tested for their stimulation of proliferation and TNF-α release by the JN.24 CD4 Vγ2Vδ2 T cell clone using glutaraldehyde-fixed CP.EBV B cells. *B*, Pulsing of bisphosphonates into fixed APC stimulate both proliferation and TNF-α release by Vγ2Vδ2 T cells without inhibition of proliferation at high concentrations. Various bisphosphonates were pulsed for 1 h in PBS into glutaraldehyde-fixed CP.EBV, washed, and then used to stimulate proliferation and TNF-α release by the JN.24 CD4 Vγ2Vδ2 T cell clone.

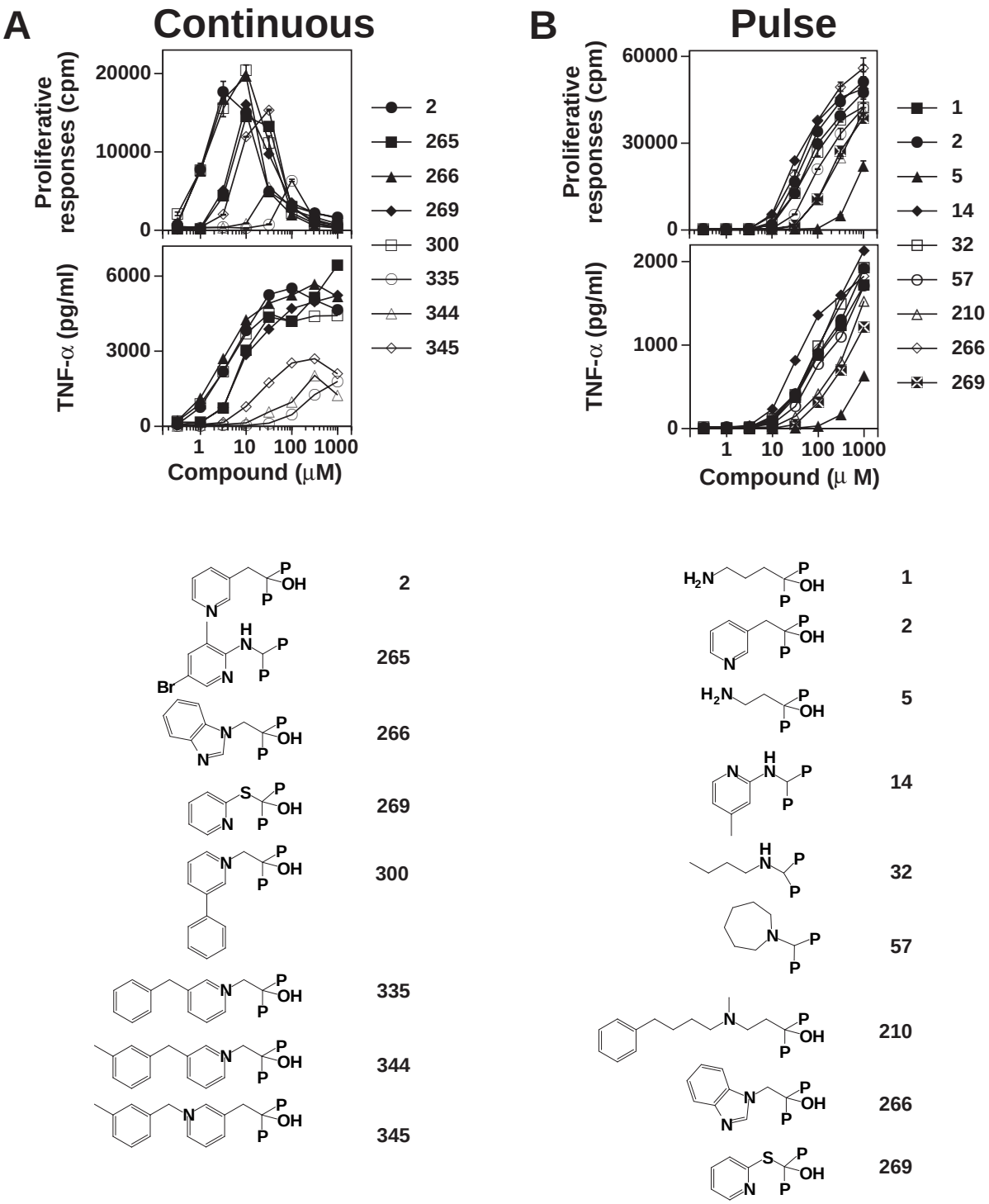
Supplemental Figure 3. Aminobisphosphonates pulse rapidly into APC and their pulsing is not affected by low temperatures or monensin treatment of APC. *A*, Risedronate pulses rapidly into APC to stimulate Vγ2Vδ2 T cells. Mit. C-treated or glutaraldehyde fixed Va-2 APC were

incubated at 37°C with risedronate or HMBPP for the indicated time followed by washing three times in PBS (HMBPP results are from Sarikonda et al. (38) and are shown for comparison with permission (copyright 2008, The American Association of Immunologists, Inc.)). APC were resuspended in supplemented RPMI media and incubated with the CD8αα Vγ2Vδ2 T cell clone, 12G12, for 24 h. The cultures were then pulsed with ^{3}H-thymidine and harvested 18 h later. *B*, Low temperatures and monensin have minimal effect on bisphosphonate stimulation of Vγ2Vδ2 T cells. Non-stimulatory CP.EBV (EBV transformed B cells) and stimulatory Daudi (a Burkitt lymphoma cell line) were pulsed with the bisphosphonates indicated for 1 h at 37°C in the presence or absence of monensin (20 μM), or at 4°C, before addition of the CD4^{+} Vγ2Vδ2 T cell clone, JN.24. Culture supernatant was collected 16 h later and TNF-α levels measured. *C*, Minimal effects of low temperatures and monensin on both direct and indirect stimulation of Vγ2Vδ2 T cells. Glutaraldehyde fixed CP.EBV B cells were pulsed with either HMBPP, HMB−OH, or risedronate for 1 h at either 37°C or 4°C in the presence or absence of monensin (20 μM). Proliferative and TNF-α responses were assessed for each condition.

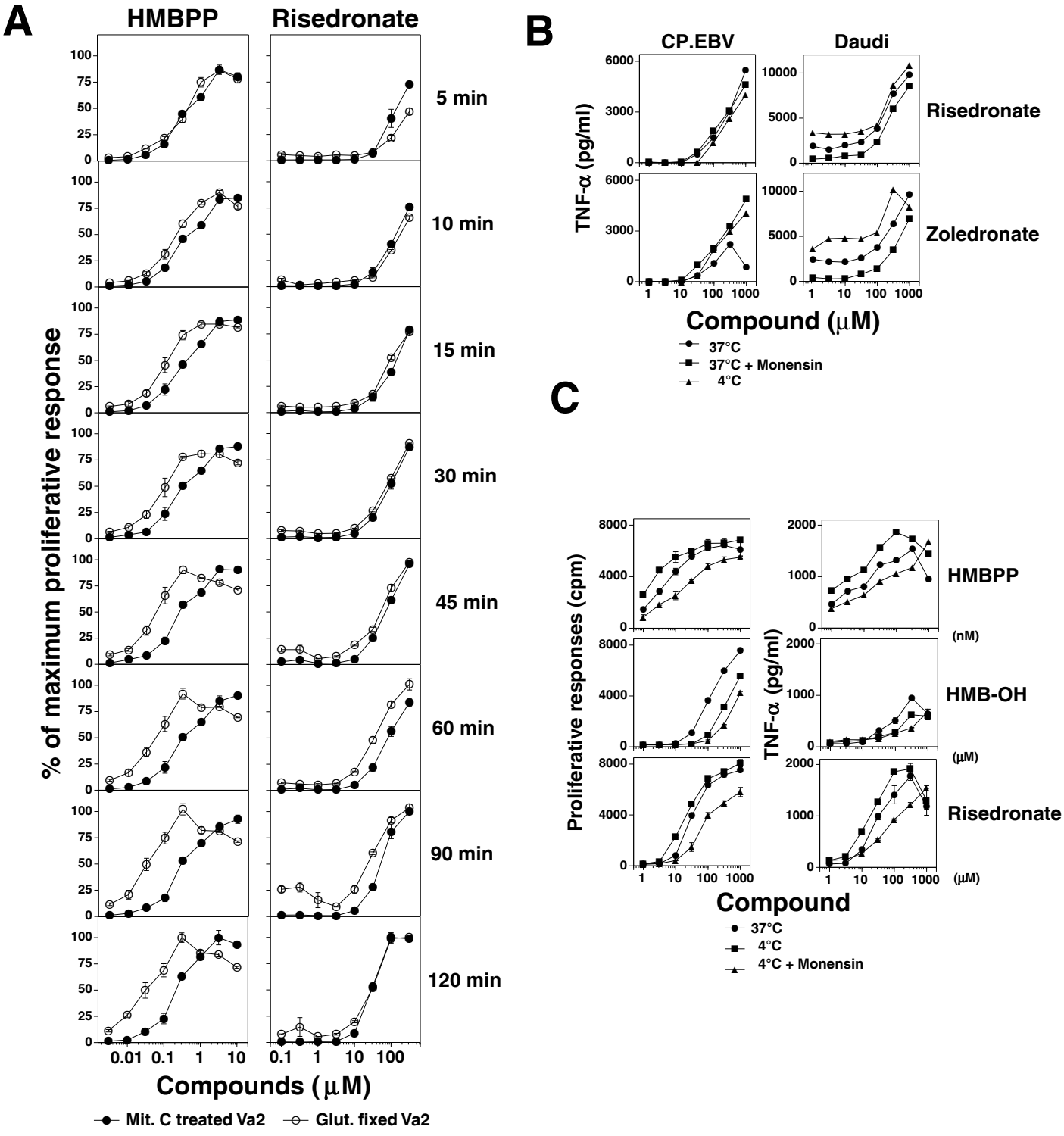
Supplemental Figure 4. Statin concentrations required for the inhibition of Vγ2Vδ2 T cell responses to risedronate increase with the strength of the response but are still lower than those required to inhibit PHA and IL-2 responses. *A*, Mevastatin IC_{50} plotted versus percentage of maximal TNF-α release. *B*, Mevastatin IC_{50} plotted versus risedronate dose. *C*, Mevastatin inhibition of TNF-α release by the JN.24 Vγ2Vδ2 T cell clone stimulated by varying doses of risedronate in the absence (*left panel*) or presence (*right panel*) of 1 mM mevalonate. *D*, Mevastatin inhibition of IL-2-driven proliferation of T cell clones. T cell clones were incubated with 30 nM IL-2 in the presence of mevastatin. The cultures were pulsed with ^{3}H-thymidine at 24 h and harvested 18 h later. *E*, Mevastatin inhibition of Vγ2Vδ2 T cell responses to the mitogen, PHA. The CD4^{+} Vγ2Vδ2 T cell clone, JN.24, was stimulated with PHA (10 μg/ml) in the absence of APC. Responses were determined as in Figure 2*A*.



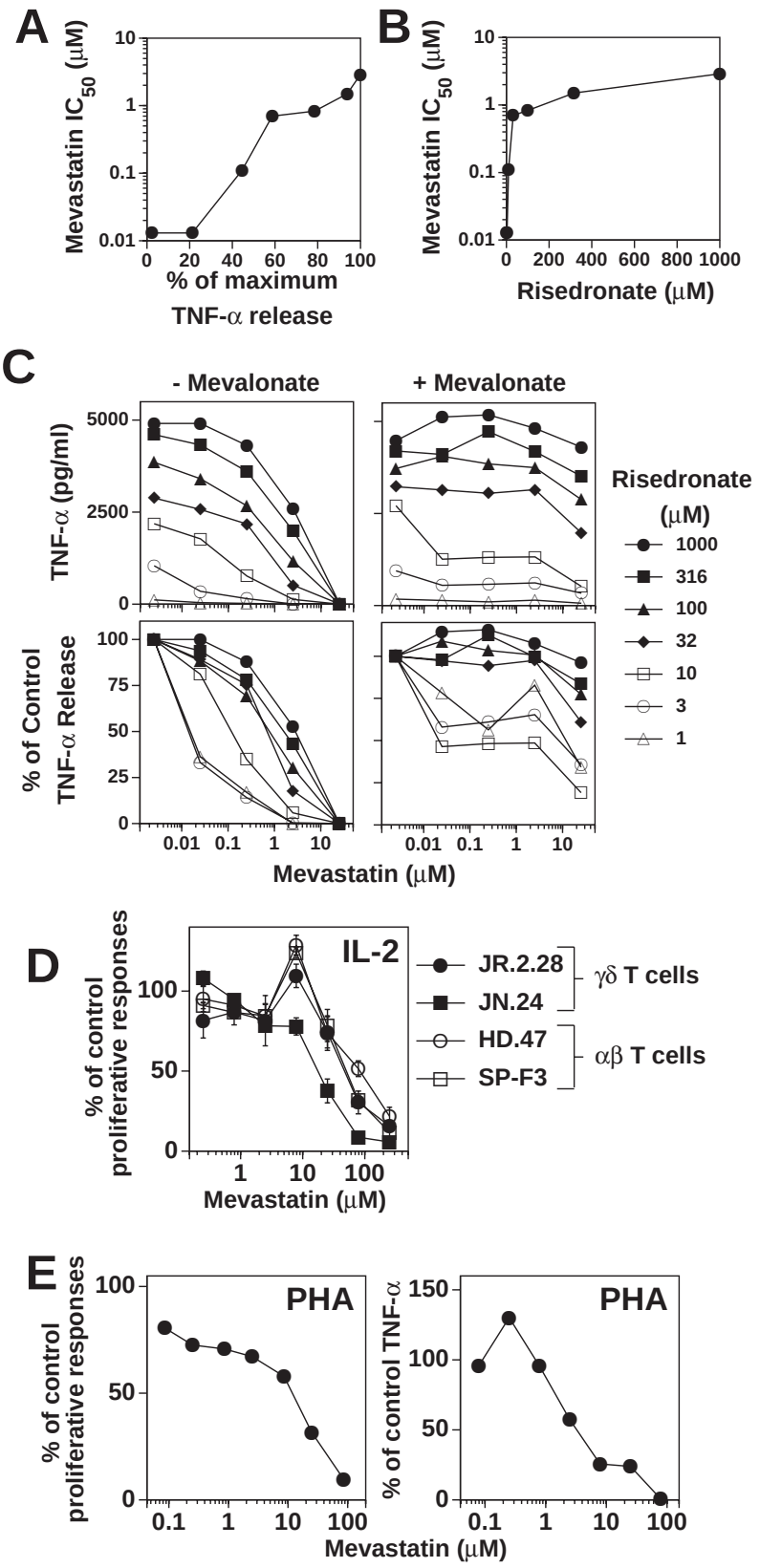
Supplemental Figure 1. Wang et al.



Supplemental Figure 2. Wang et al.



Supplemental Figure 3. Wang et al.



Supplemental Figure 4. Wang et al.