## Validation data for Necrostatin-1

https://www.invivogen.com/necrostatin-1

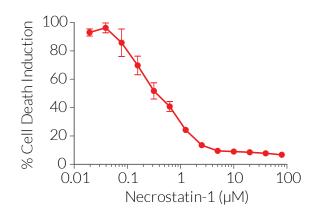
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Version 21G30-MM

Necrostatin-1 is a potent and specific small-molecule inhibitor of the receptor-interacting serine/threonine-protein kinase 1 (RIPK1, aka RIP1). RIPK1, through its kinase and scaffolding functions, is a key regulator of apoptosis, necroptosis, and inflammatory pathways.

The ability of Necrostatin-1 to inhibit RIPK1-RIPK3-MLKL necrosome-mediated necroptosis upon TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ) signaling and Caspase-8 inhibition was validated using InvivoGen's THP1-HMGB1-Lucia<sup>TM</sup> cellular assay (Figure 1).

## Dose-dependent inhibition of RIPK1 activity



## Figure 1: Necrostatin-1 inhibits the $TNF-\alpha$ -induced necroptosis in a dose-dependent manner.

THP1-HMGB1-Lucia<sup>TM</sup> cells were incubated with recombinant human TNF- $\alpha$  (100 ng/ml), Z-VAD-FMK (pan-caspase inhibitor, 25  $\mu$ M), BV6 (cIAP inhibitor, 5  $\mu$ M), and increasing concentrations of Necrostatin-1 (RIPK1 inhibitor). After overnight incubation, the level of HMGB1::Lucia released in the supernatant was assessed by measuring the light signal produced using the QUANTI-Luc<sup>TM</sup> detection reagent. Data are shown as a percentage (%) cell death induction in absence of RIPK1 inhibitor.

