Chemical control of protein stability and function in living animals

Supplemental Information

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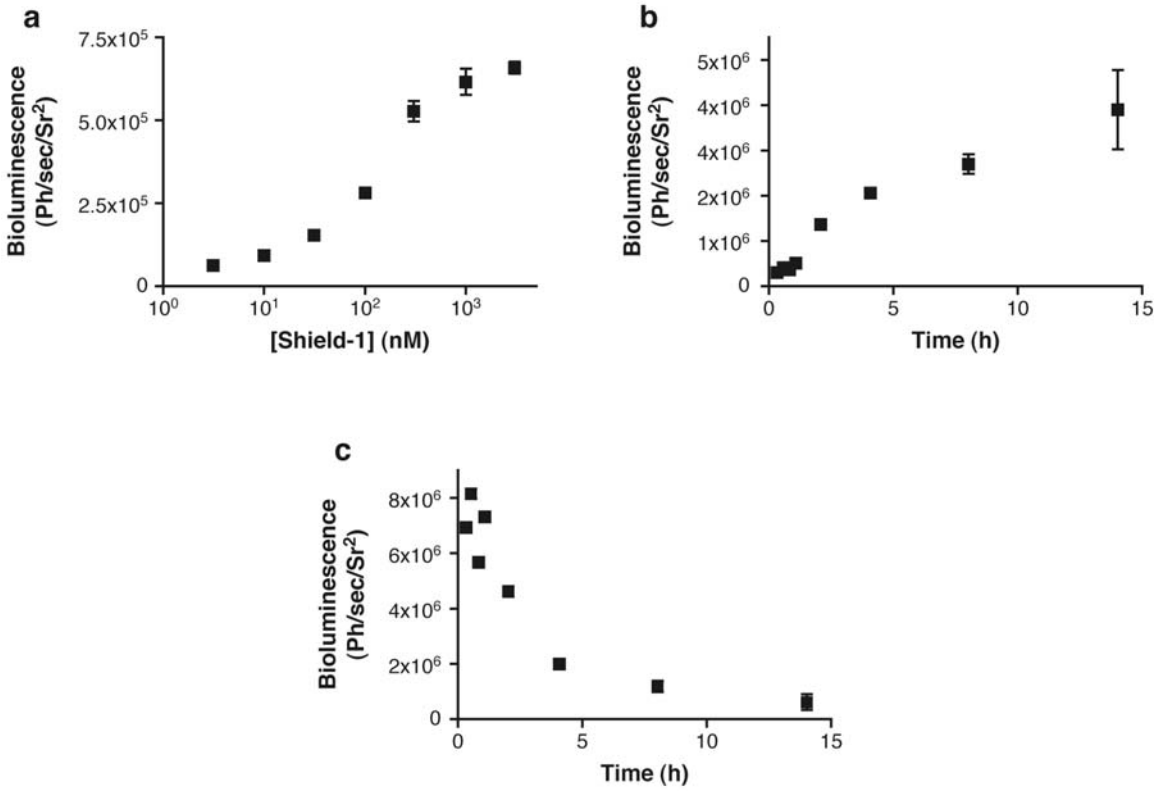

Figure S1. Analysis of L106P-tsLuc in cultured cells. **(a)** HCT116 cells stably expressing L106P-tsLuc were treated with varying concentrations of Shield-1 (3 μM to 3 nM) and monitored for luminescence. Data are represented as the average luminescence ± SEM (n=4). **(b)** HCT116 cells stably expressing L106P-tsLuc were treated with 1 μM Shield-1, and increases in luminescence were monitored over time. **(c)** HCT116 cells stably expressing L106P-tsLuc were treated with 1 μM Shield-1 for 24 hrs at which point cells were washed with media to remove Shield-1, and decreases in luminescence were monitored over time. Data for panels b and c are represented as the average luminescence ± SEM (n=3).

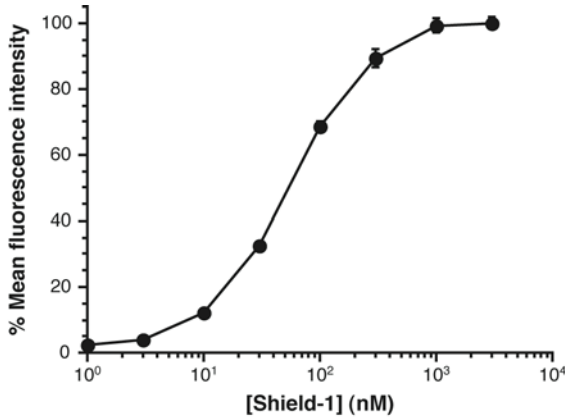

Figure S2. HCT116 cells stably expressing an L106P-tomato fusion were titrated with three-fold dilutions of Shield-1 (3 μM to 1 nM) and monitored by flow cytometry. Data are presented as the average mean fluorescence intensity ± SEM relative to that of the maximum fluorescence intensity observed (n=3).

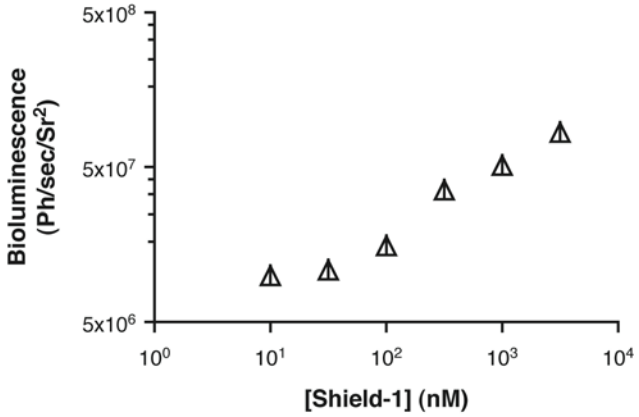

Figure S3. Conditional regulation of a secreted protein. HCT116 cells stably expressing a fusion of the L106P destabilizing domain to *Gaussia* luciferase were treated with various concentrations of Shield-1 (3 μM to 10 nM) and assayed for luminescence in the cell culture media. Data are represented as the average luminescence ± SEM (n=3).

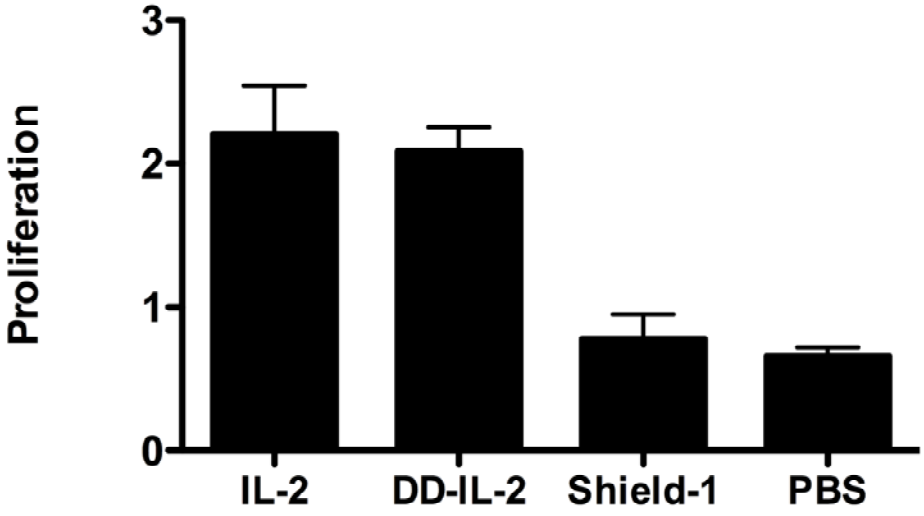

Figure S4. Functional activity of IL-2 fused to L106P. HCT116 cells expressing IL-2 fused to the destabilizing domain L106P (DD-IL-2) were treated with Shield-1 (1μM) for 24 hr, at which point the culture media was collected and filtered. Levels of DD-IL-2 in the media were quantified by ELISA. DD-IL-2 was then added to NK-92 cells, a human NK cell line dependent on IL-2 for growth. Cells were alternatively treated with equivalent levels (as determined by ELISA) of recombinant human IL-2, Shield-1 (1μM), or PBS alone. Cell numbers were determined after 48 hr and proliferation determined relative to the number of cells at the start of the assay (n=3 ± SEM).

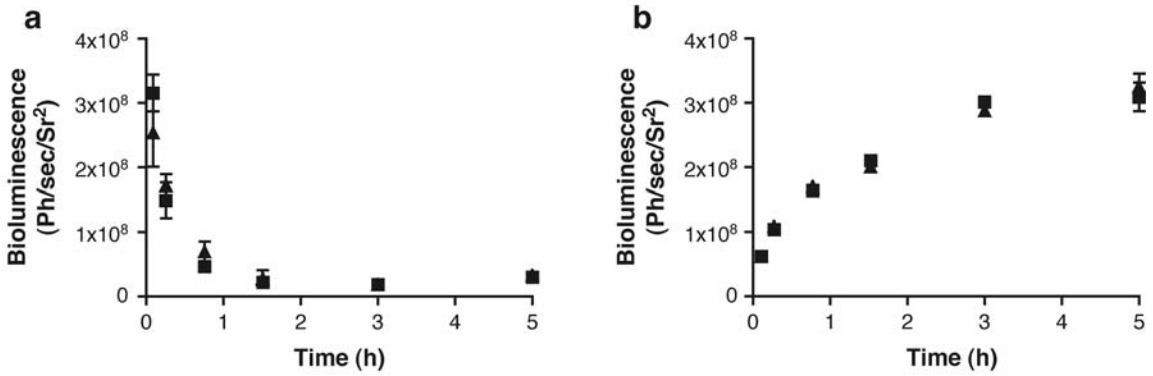

Figure S5. Viral infection does not interfere with L106P-mediated degradation. **(a)** HepG2 cells stably expressing L106P fused to the N-terminus of luciferase (L106P-Luc) were either untreated (triangles) or infected (squares) with a wild-type vaccinia virus for 1 hr at 37 ˚C. Cells were washed to remove excess virus and then treated with 1 μM Shield-1. Cell were monitored for luminescence over time. **(b)** HepG2 cells stably expressing L106P-luc were infected with an empty vvDD vaccinia virus as above. Cells were then treated with 1 μM Shield-1 for 24 hr, at which point cells were washed with media to remove Shield-1, and luminescence was monitored over time. Data for both panels are represented as the average luminescence ± SEM (n=3).

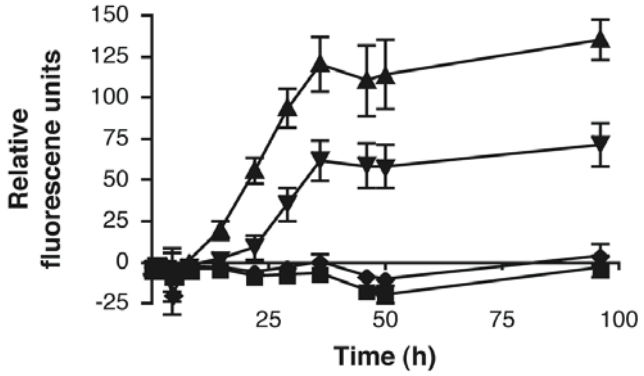

Figure S6. Conditional stability of a virally delivered reporter protein in cultured cells. HCT116 cells were infected with the vvDD L106P-tomato vaccinia strain. Cells were then mock treated (squares) or treated with Shield-1 at 1 μM (triangles), 100 nM (inverted triangles), or 10 nM (diamonds). Cells were monitored for fluorescence over time, and background-subtracted data are represented as the average fluorescence ± SEM (n=3).

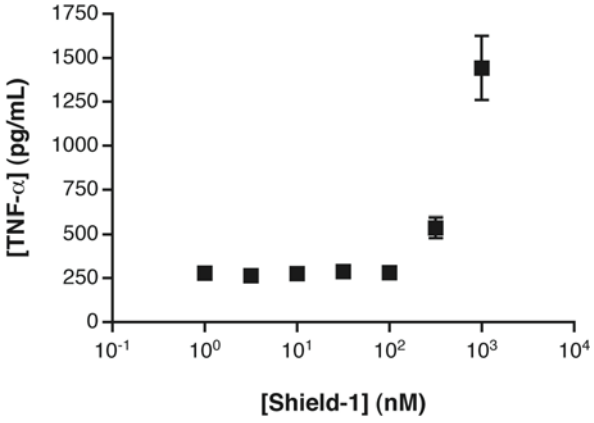

Figure S7. Conditional regulation of TNF-α secretion in cultured cells. HCT116 cells were infected with vaccinia virus strain vvDD expressing L-L106P-TNF-α and then treated with various concentrations of Shield-1 (1 μM to 1 nM). The concentration of TNF-α in the cell culture media was determined by ELISA. Data are represented as the average TNF-α concentration ± SEM (n=3).