Figure Legends to Supplemental Data.

Supplemental Figure 1. Time course of apoptosis following FLLL32 treatment. (A) PARP cleavage and pSTAT3 were evaluated by immunoblot following treatment of A375 cells with FLLL32 for various durations of time. (B) A375 cells were pulse-stimulated for various durations of time (2, 4 or 8 hours) with FLLL32 and incubated for an additional 16 hours. PARP cleavage and pSTAT3 were evaluated by immunoblot analysis. Membranes were probed with β-actin as a loading control and all blots represent data from at least two independent experiments.

Supplemental Figure 2. FLLL32 induced caspase-dependent apoptosis. Flow cytometric analysis of annexin V/PI staining following a 48 hour treatment of Hs294T human melanoma cells with FLLL32 in the presence of the Z-VAD-FMK pan-caspase inhibitor or the Z-FA-FMK control compound. Inhibitors were used at 50µM and the percentage of cells in each quadrant are shown.

Supplemental Figure 3. IFN-γ-induced signal transduction was not adversely affected by FLLL32. Hs294T cells were pre-treated for 16 hours with FLLL32 or other STAT3 pathway inhibitors (WP1066, JSI-124, Stattic) and subsequently treated with IFN-γ (10 ng/mL) for 15 minutes. IFN-γ-induced pSTAT1 and pSTAT3 were evaluated by immunoblot. Total STAT1, STAT3 and β-actin were also measured to control for loading.

Supplemental Figure 4. IC_{50} values of other STAT3 pathway inhibitors. Cells were cultured with various doses of each inhibitor for 48 hours and apoptosis was assessed by annexin V/PI staining. IC_{50} values were determined as described in the Materials and Methods.
Bill et al.
Supplemental Figure 1A-B

A: Continuous Treatment

B: Pulse Treatment

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Hs294T

Pan-caspase Inhibitor vs Negative Control

DMSO

2 μM FLLL32

4 μM FLLL32

APC Annexin V
Supplemental Figure 3

Hs294T

IFN-γ (10 ng/mL): - +
FLLL32 (μM): 0 0 2 4 6 8
pSTAT3
STAT3
pSTAT1
STAT1
β-actin

IFN-γ (10 ng/mL): - +
WP1066 (μM): 0 0 1 2 4 6
pSTAT3
STAT3
pSTAT1
STAT1
β-actin

IFN-γ (10 ng/mL): - +
JSI-124 (μM): 0 0 .1 5 1 2
pSTAT3
STAT3
pSTAT1
STAT1
β-actin

IFN-γ (10 ng/mL): - +
Stattic (μM): 0 0 1 2 4 6
pSTAT3
STAT3
pSTAT1
STAT1
β-actin

Bill et al.
IC50 values (μM) for other STAT3 pathway inhibitors in human melanoma cell lines.

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