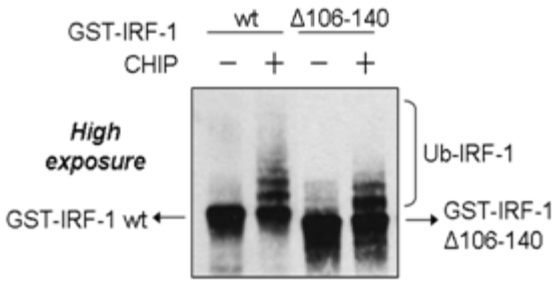

Figure S1. IRF-1 Δ106-140 is deficient in CHIP-dependent ubiquitination relative to IRF-1 wt

In vitro ubiquitination assays were set up as described in the methods using GST-IRF-1 wt or Δ106-140 (expressed and purified using the PURExpress In Vitro Protein Synthesis Kit) as substrate and CHIP as E3. The reaction products analysed by SDS-PAGE/immunoblot using anti-IRF-1 mAb.

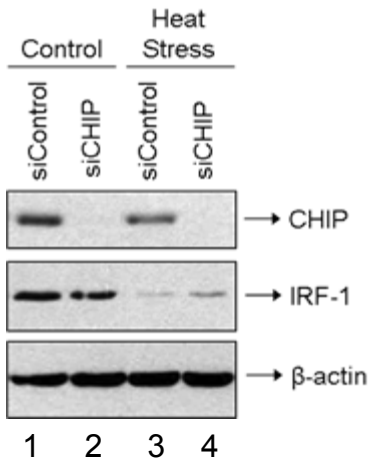

Figure S2. Partial recovery of IRF-1 protein in heat-stressed cells transfected with CHIP siRNA

H1299 cells were transiently transfected with 50 nmol control siRNA (siControl) or 50 nmol siRNA to CHIP (siCHIP) and were harvested 72 hours post transfection. Just prior to harvesting, the cells were heat shocked at 43°C for 30 min (Heat Stress) or were left at 37°C (Control). Harvested cells were lysed in Triton lysis buffer and analysed by SDS-PAGE/immunoblot using anti-CHIP, anti-IRF-1 and anti-β-actin mAbs.

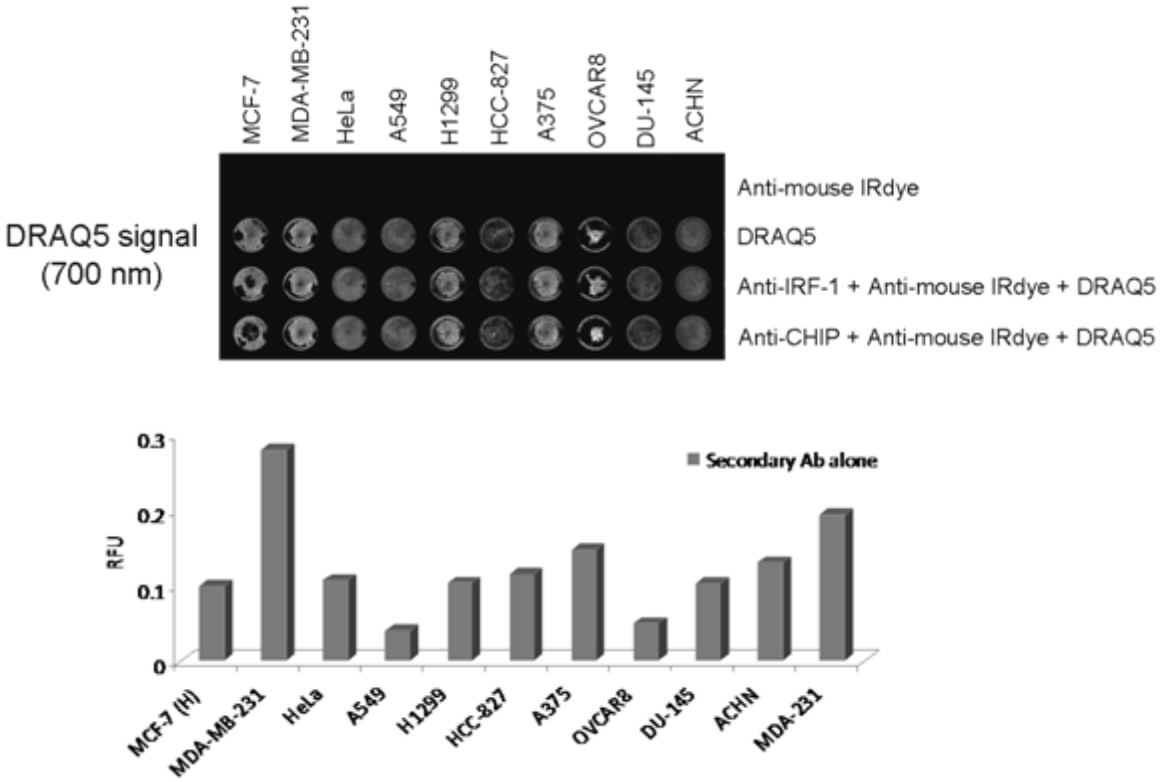

Figure S3. In-cell western (Figure 7D; raw data)
The indicated cells were seeded in a 96-well plate, and in-cell westerns performed with anti-IRF-1 and anti-CHIP mAbs to measure the relative CHIP and IRF-1 levels *in situ* using a Licor Odyssey SA scanner. Shown here is the raw data obtained by taking readings at 700 nm (DRAQ5 DNA stain; used to normalise for cell number across samples) and a graph of the secondary antibody signal alone measured at 800 nm, normalised to the DRAQ5 signal. This secondary antibody background signal was subtracted from the IRF-1 and CHIP data shown in Fig. 7D.

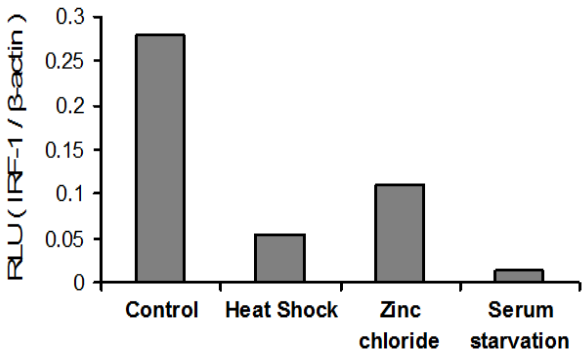

Figure S4. Quantification of IRF-1 loss in A375 cells upon treatment with various stresses
The intensity (in relative light units or RLU) of the IRF-1 signal from the immunoblots shown in Fig. 8A (Input) was quantified using the Scion Imaging software and normalised to the β-actin signal to show the relative loss of IRF-1 protein under the indicated stress conditions. Results are representative of at least 3 independent experiments.