SUPPLEMENTAL FIGURE 1: Expression of ISG54-mGFP promotes cell death. ***A,*** HeLa cells were transfected with plasmids mGFP or ISG54-mGFP and the number of cells expressing GFP fluorescence was quantified with time by flow cytometry. A low percentage of cells expressed ISG54-mGFP at 24 hrs, and that number dramatically decreased with time. ***B,*** Comparison of cell death in cultures transfected with ISG54-mGFP plasmid by PI staining of GFP negative cells, GFP positive cells, and the total population of cells. ***C***, ISG54 promotes cell death in an inducible system. HeLa cells were co-transfected with tetracycline-inducible pRev-ISG54-mGFP and pLib-rtTAm2-iresTRSID-iresPuro coding for TetRepressor/Transactivator. Cells were stimulated with 2μg/ml doxycycline for 24, 48, and 72 hrs and stained with PI. GFP positive and negative cells in the culture were evaluated for cell death by PI staining with flow cytometry.

SUPPLEMENAL FIGURE 2: Physiological levels of ISG54-GFP promotes apoptosis in various cell lines. ***A,*** ISG54 promotes apoptosis in HT1080 cells. HT1080 cells expressing ISG54-mGFP or mGFP were stained with PI at 24, 48 and 72 hrs posttransfection, and analyzed with flow cytometry. The percent of cells positive for both GFP and PI was quantified. ***B***, ISG54 promotes apoptosis in HEC1B cells. HEC1B cells expressing ISG54-mGFP or mGFP, were stained with PI at 24, 48 and 72 hrs posttransfection, and analyzed with flow cytometry. The percent of cells positive for both GFP and PI was quantified. ***C***, Dose response of ISG54 expression to various concentrations of IFNα. HeLa cells were stimulated with increasing amounts of IFNα for 6 hrs as indicated and ISG54 protein was detected by Western blot with anti-ISG54 antibodies. Cross reactive protein with slightly slower migration is visible without IFN. Protein band intensity of ISG54 was analyzed with Image J software. Using 1000 U/ml as the standard of 100%, 500 U/ml corresponded to 116%, and 100 U/ml corresponded to 87%. ***D,*** Levels of ISG54 protein expressed in transfected cultures compared to endogenous ISG54 induced in response to IFNα. HeLa cells were untreated, or treated with 1000 or 5000 U/ml IFNα for 48 hrs to serve as endogenous samples. HeLa cells were transfected with ISG54-mGFP plasmid for 24 or 48 hrs, and cells expression ISG54-GFP were isolated by FACS. Lysates were prepared from the respective cells and the same amount of protein was evaluated by Western blot with polyclonal antibody to human ISG54. A representative experiment is shown, and the protein levels were compared with Image J software. Results indicated that ISG54-mGFP expression was equivalent to 98% of endogenous ISG54 induced by 5000 U/ml IFNα at 24 hrs, and 91% of endogenous ISG54 induced at 48hrs.

SUPPLEMENTAL FIGURE 3: Effectors of ISG54-mediated cell death. ***A***, HeLa cells were transfected with mGFP or ISG54-mGFP. One culture transfected with ISG54-mGFP was treated with the pan-caspase ZVAD-FMK peptide inhibitor (BD Biosciences) at 20μM 4 hrs following transfection. Cells were harvested 24, 48, or 72 hours post transfection and analyzed for cell death by PI staining and flow cytometry. ZVAD-FMK addition resulted in nearly 50% decrease in number of PI positive cells. ***B***, Cells were transfected with mGFP, ISG54-mGFP, or co-transfected with ISG54-mGFP and adenoviral E1B-55K or with ISG54-mGFP and adenoviral E4 orf6. GFP-positive cells were analyzed by flow cytometry for PI staining at 24, 48, or 72 hours post transfection.

SUPPLEMENTAL FIGURE 4: ISG54 and the ER. ***A,*** ISG54 localizes within the cytoplasm

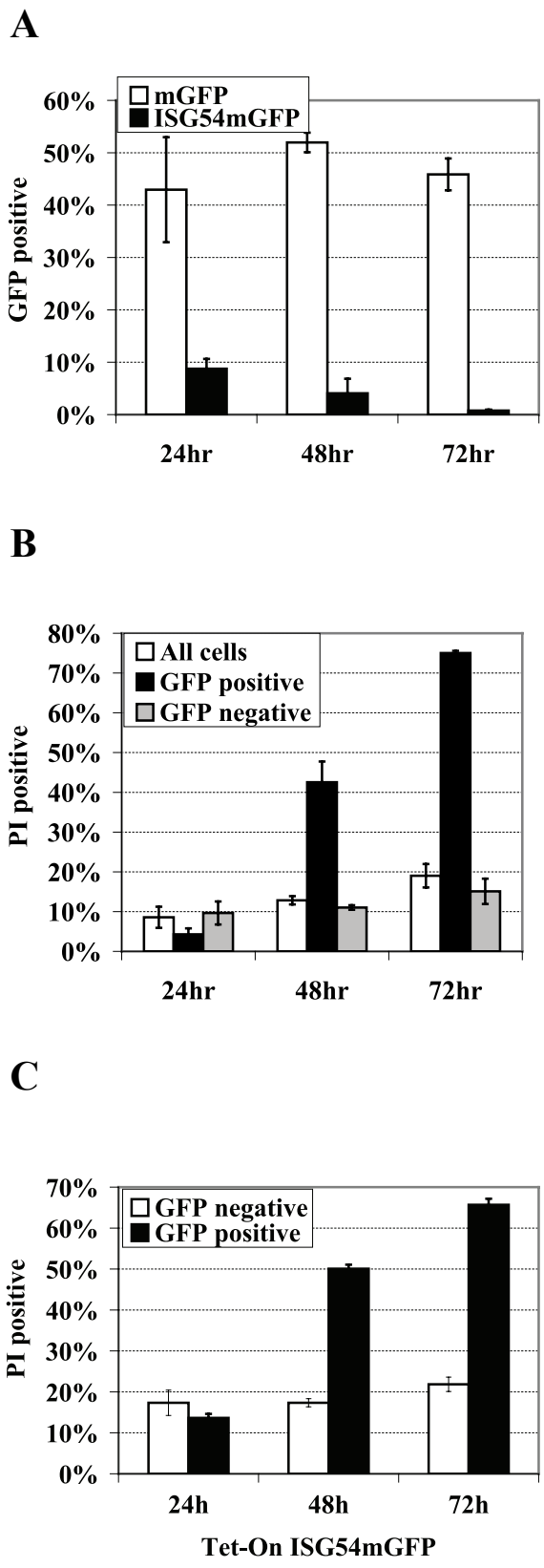
and the ER. HeLa cells expressing ISG54mGFP were stained by immunofluorescence with antibodies to calnexin, an ER marker, and secondary TRITC-conjugated anti-mouse antibodies. Colocalization of ISG54mGFP (green) and calnexin (red) was calculated with ImageJ software. Pearson's coefficient values of colocalization were calculated between 0.05-0.12 indicating only a weak positive correlation. ***B,*** Intensity of ISG54-mGFP signal does not increase in the ER. Image of the cell from (A) (arrow in merged image) was analyzed with LSM5 Pascal software to evaluate the correlation between red and green fluorescence. Graph presents the profile of fluorescence along the white arrow. Red fluorescence peaks crossing into the ER region, but fluorescence of ISG54mGFP remains the same. ***C,*** ER stress inhibitor does not decrease ISG54 induced cell death. HeLa cells were transfected with mGFP or ISG54mGFP and 6 hrs after transfection cells were treated with 15μM or 30μM salubrinal, an ER stress inhibitor. Cells were harvested 24, 48, or 72 hours post transfection and analyzed for cell death with PI staining and flow cytometry. There was no noted effect of salubrinal on ISG54 induced cell death.

SUPPLEMENTAL FIGURE 5: Controls for protein expression. ***A,*** Expression of ISG proteins used in experiments presented in Figure 4B. 40μg of total protein lysate was analyzed by Western blot ISGs with anti-V5 antibodies. ***B ,C, D,*** Expression of ISG54 truncations used in IP experiments presented on Figures 5B, 5C, 5D respectively. In each case 40μg of total protein lysate was analyzed by Western blot for ISG54 truncations with anti-V5 antibodies.

SUPPLEMENTAL FIGURE 6: ISG54 shRNA reduces apoptosis. ***A***, A linear diagram of the nucleotide positions targeted for shRNA in ISG54 mRNA. Numbers in italics correspond to the first codon nucleotide and the last codon nucleotide. Numbers with a bar correspond to 5' nucleotide position of the shRNA. ***B***, Cells expressing stable knockdown of ISG54 with 1075 shRNA, or cells expressing 136 shRNA that did not reduce ISG54 protein levels, were compared for their apoptotic response to IFNα by annexin V staining and flow cytometry.

SUPPLEMENTAL FIGURE 7: IFN and ISG60 positively influence survival of cells expressing ISG54-mGFP. ***A***, HeLa cells were co-transfected with ISG54-mGFP and pEF-1-V5 empty vector, or ISG54-mGFP and V5-ISG60. The number of cells in the transfected cultures expressing GFP fluorescence was quantified with time by flow cytometry. ***B,*** HeLa cells were transfected with ISG54mGFP and pEF-1-V5 empty vector, or with ISG54mGFP and V5-ISG56 or V5-ISG60. At 24h and 48h cells were harvested, lysed, and 75μg of protein lysate was analyzed for by Western-blot with anti-ISG54 antibodies. ISG54 protein band intensity was analyzed with Image J. In the presence of ISG60, levels of ISG54 increased at 24h and 48hs by 90% and 200% respectively. ***C,*** Cells were transfected with ISG54-mGFP or 2-9TPR-ISG54-mGFP and after 12 hours the cells were untreated or treated with 2000 U/ml IFNα. The number of cells in the transfected cultures expressing GFP fluorescence was quantified with time by flow cytometry.

Supplementary figure 1



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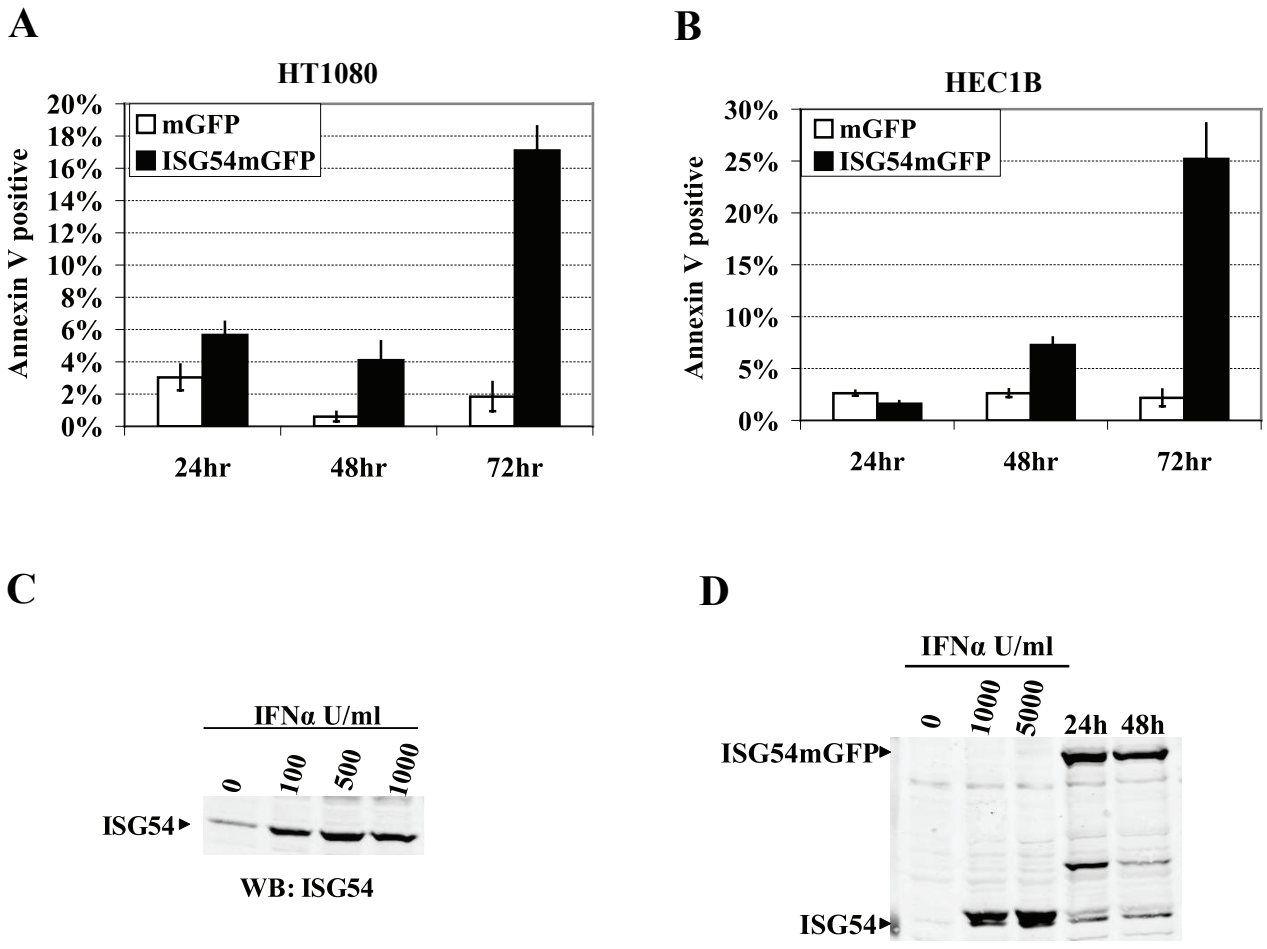

Supplementary figure 3

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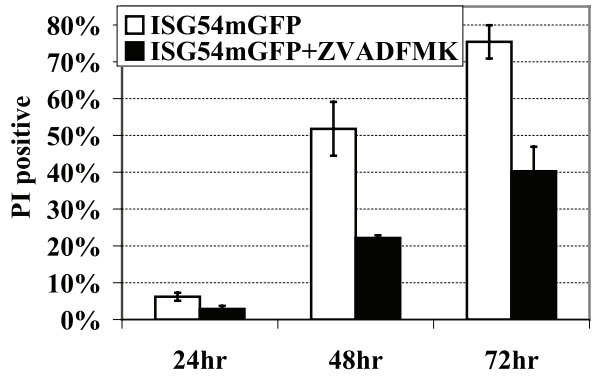

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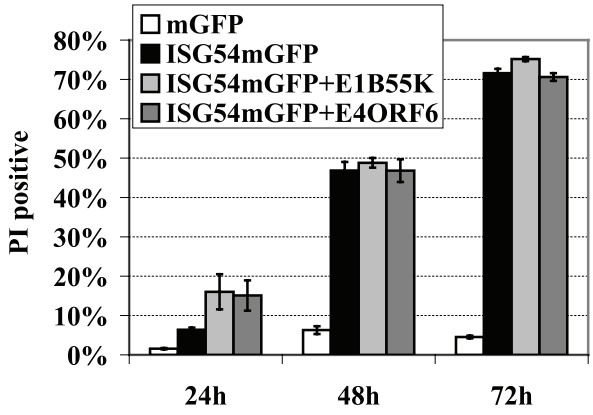

Supplementary figure 4

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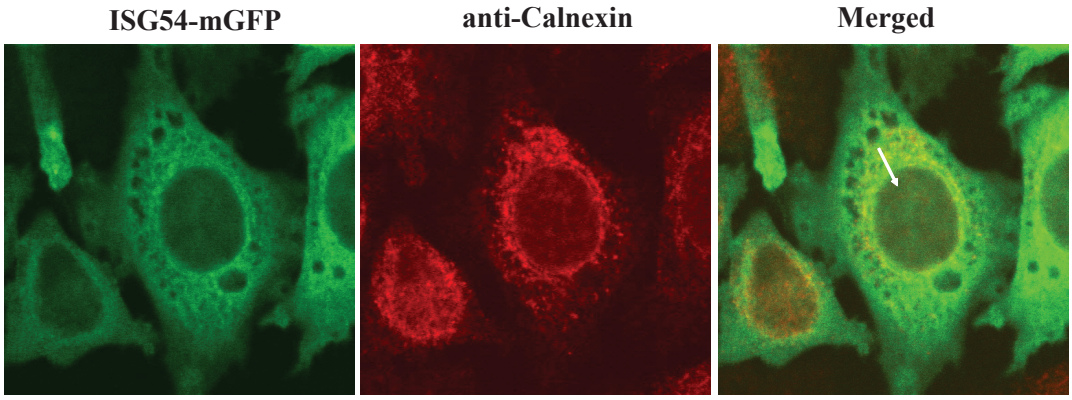

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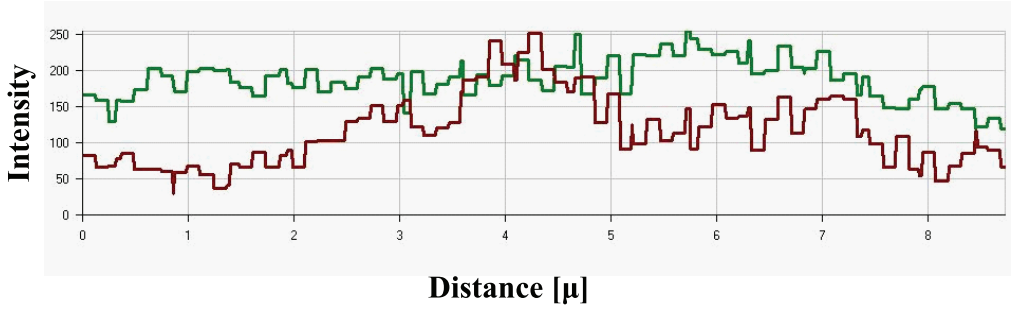

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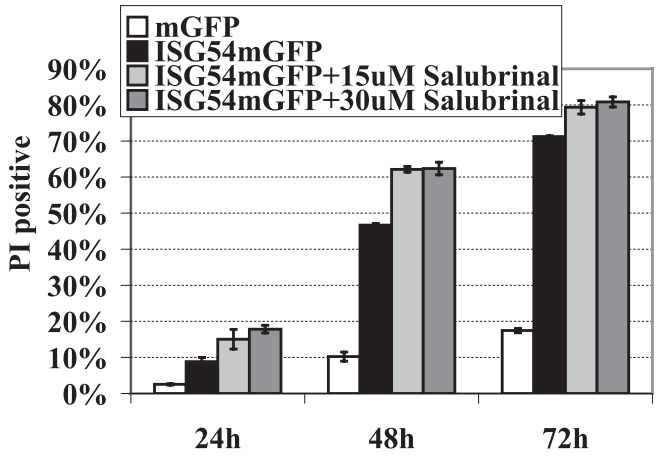

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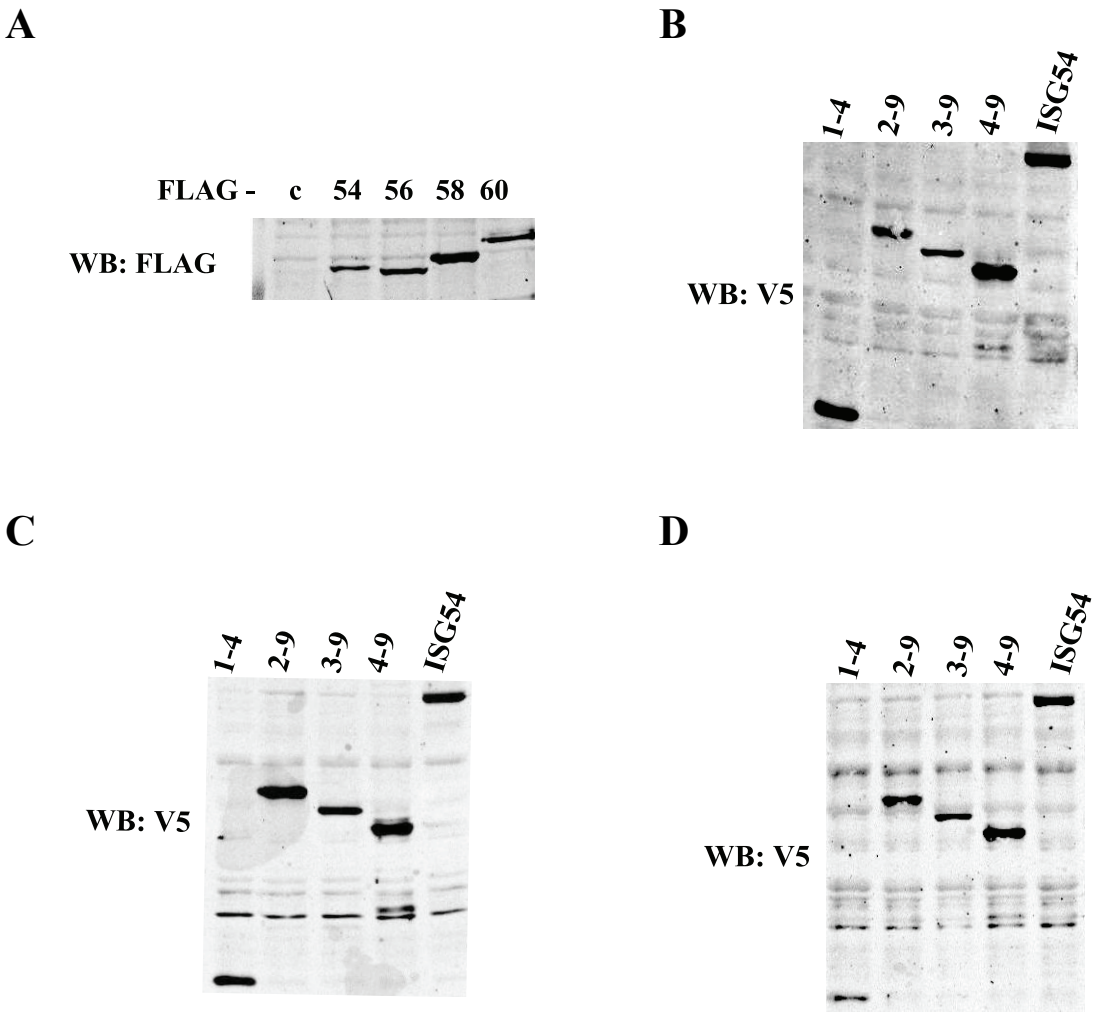

Supplementary figure 6

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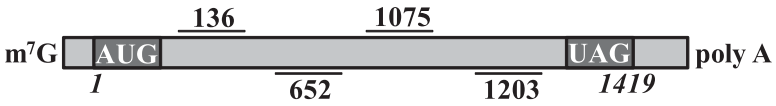

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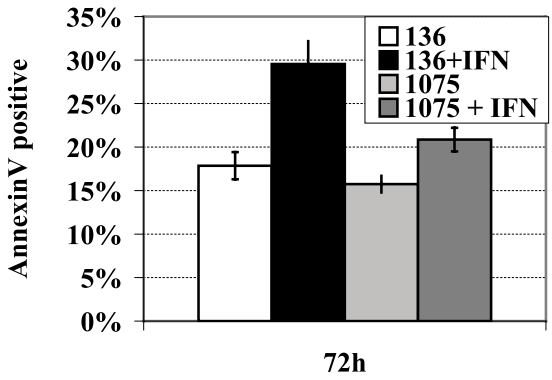

Supplementary figure 7

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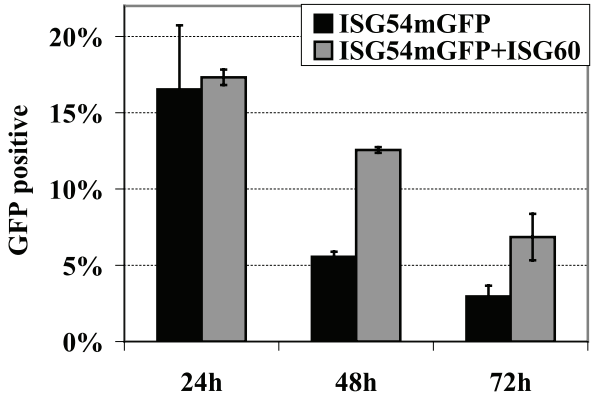

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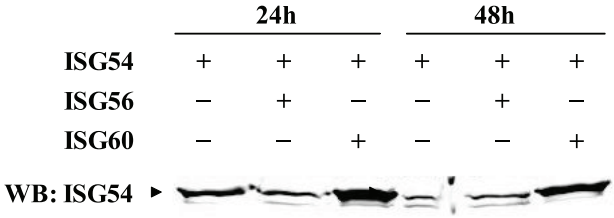

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