Figure S1. Classical IFN-β signalling pathway in GIC. (A and B) Basal mRNA expression levels of IFNAR1 (light grey) and IFNAR2 (dark grey) were assessed in T-325, ZH-161, ZH-305 or S-24 cells by (A) reverse transcription-quantitative PCR. Protein levels were assessed by (B) immunoblot for IFNAR1 and (C) flow cytometry for IFNAR2. (D and E) Activation of the classical signalling pathway defined by MxA induction in response to IFN-β1a (0, 10, 100 and 1,000 IU/ml) was detected at the (D) mRNA and (E) protein level. ARF-1 or HPRT-1 served as housekeeping genes and β-actin was used as loading control. GIC, glioma-initiating cell; IFN, interferon; IFNAR, IFN-α/β receptor; ARF-1, ADP ribosylation factor 1; HPRT-1, hypoxanthine phosphoribosyltransferase 1.

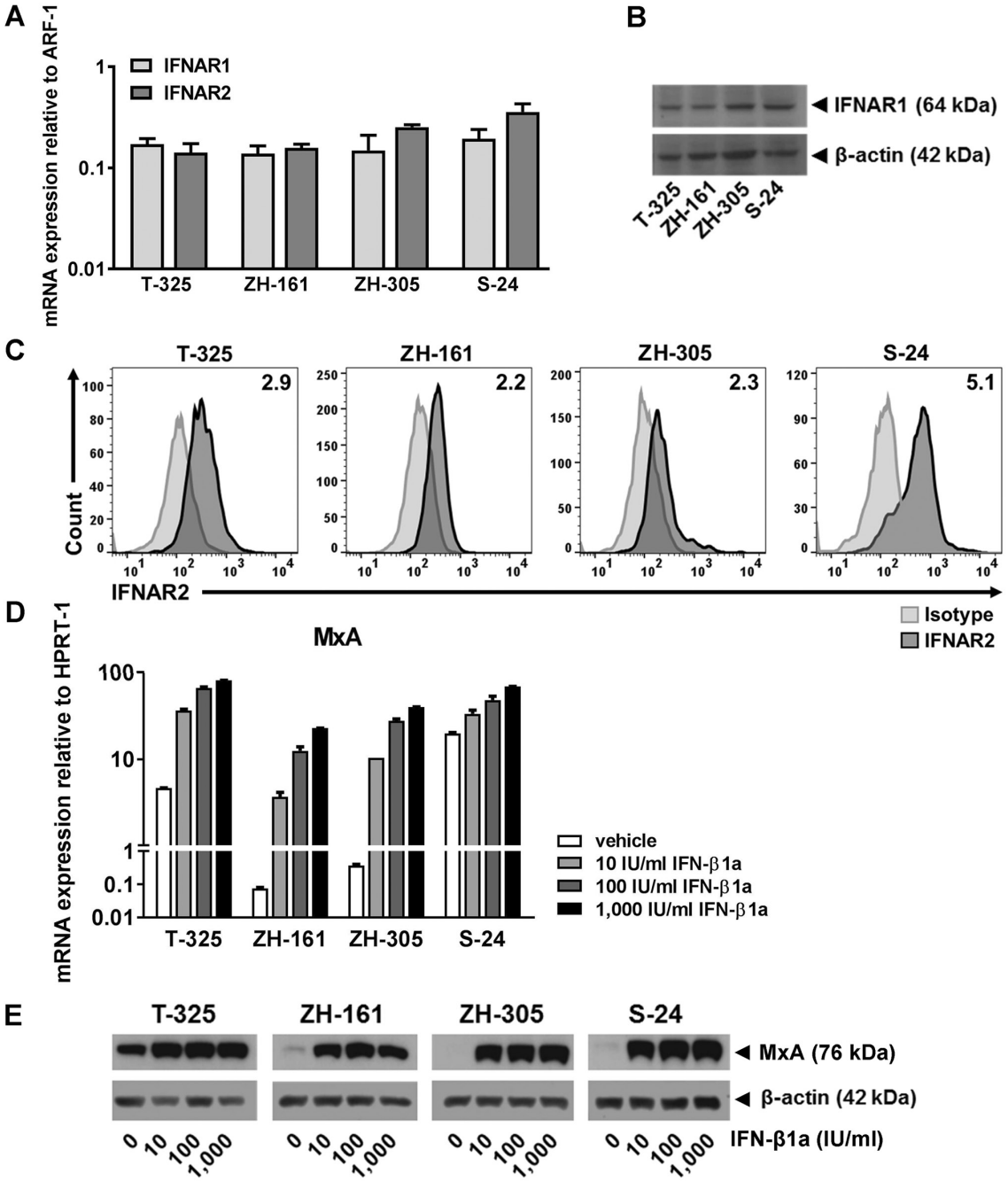

Figure S2. Characterization of XAF-1 as an IFN response gene. (A) Induction of XAF-1 in response to IFN-β1a stimulation at the mRNA and protein level in ZH-161, ZH-305 or S-24 cells. (B) Efficiency of XAF-1 gene silencing was determined at 24, 48 and 72 h. ARF-1 served as housekeeping gene and β-actin as loading control. IFN, interferon; si, small interfering RNA; XAF-1, XIAP associated factor 1; ARF-1, ADP ribosylation factor 1; scr, scrambled control.

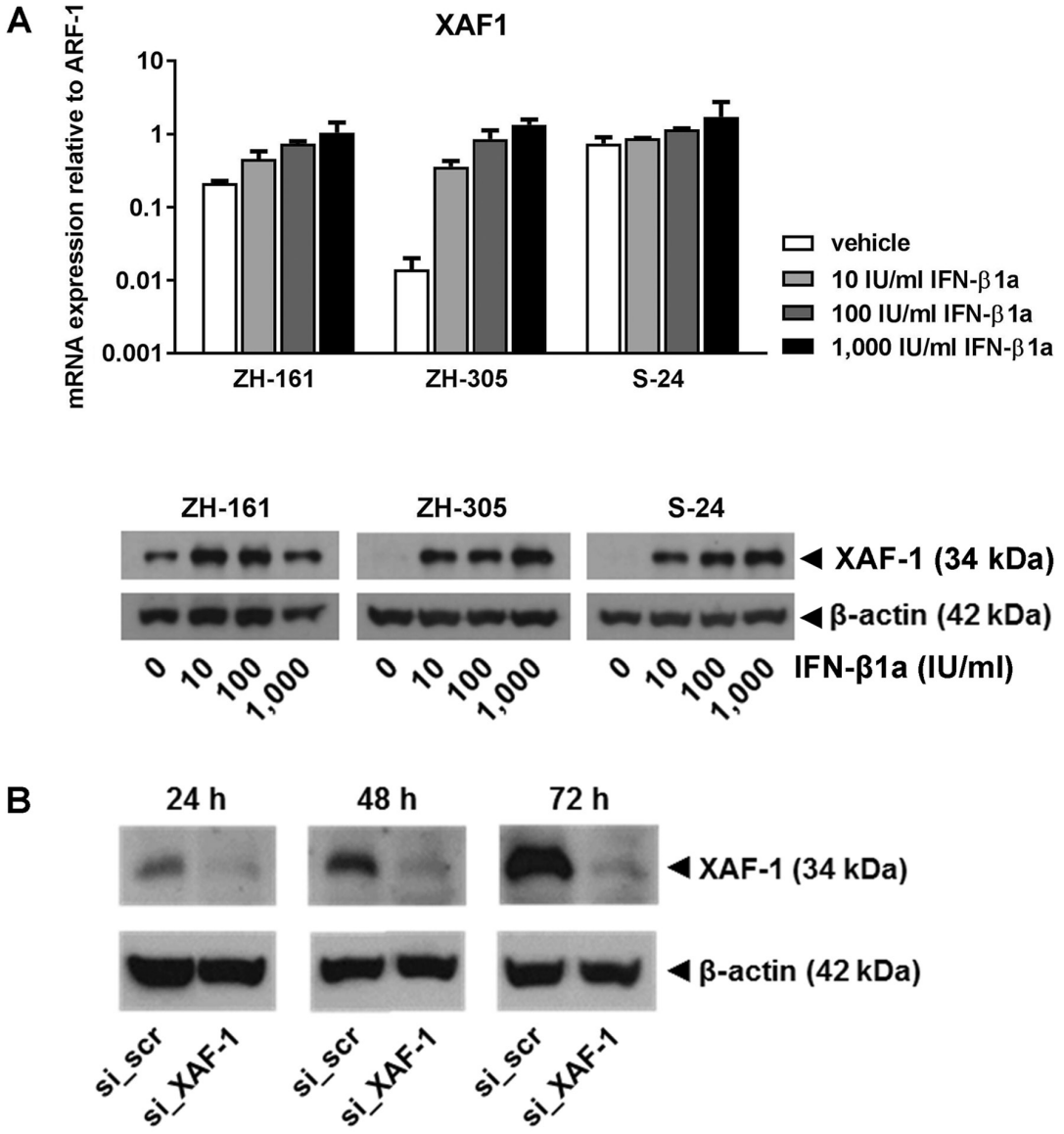

Figure S3. Determination of senescence-associated β-galactosidase activity. Senescence-associated β-galactosidase staining of cytospins of S-24 or ZH-161 cells treated with vehicle, IFN-β1a (1,000 IU/ml), TG02 (100 nM) or both in sequence for 24 h was performed as previously described (12). IR cells (20 Gy; 5 days) served as a positive control. Scale bar, 100 μm. Data are presented as mean and SD. Data were analyzed by one-way ANOVA with Tukey's post hoc test. ***P<0.001 vs. vehicle treatment. IFN, interferon; IR, irradiated; ctr, control.