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# Bloom syndrome protein restrains innate immune sensing of micronuclei by cGAS

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**Cellular innate immune sensors of DNA are essential for host defense against invading pathogens. However, the presence of self-DNA inside cells poses a risk of triggering unchecked immune responses. The mechanisms limiting induction of inflammation by self-DNA are poorly understood. BLM RecQ-like helicase is essential for genome integrity and is deficient in Bloom syndrome (BS), a rare genetic disease characterized by genome instability, accumulation of micronuclei, susceptibility to cancer, and immunodeficiency. Here, we show that BLM-deficient fibroblasts show constitutive up-regulation of inflammatory interferon-stimulated gene (ISG) expression, which is mediated by the cGAS–STING–IRF3 cytosolic DNA-sensing pathway. Increased DNA damage or down-regulation of the cytoplasmic exonuclease TREX1 enhances ISG expression in BLM-deficient fibroblasts. cGAS-containing cytoplasmic micronuclei are increased in BS cells. Finally, BS patients demonstrate elevated ISG expression in peripheral blood. These results reveal that BLM limits ISG induction, thus connecting DNA damage to cellular innate immune response, which may contribute to human pathogenesis.**

## Introduction

The innate immune system plays a critical role in maintaining the health of the host. This is mediated, in part, by innate immune sensors of DNA that induce inflammatory signals in response to non-self-DNA from pathogens or damaged self-DNA (Härtlova et al., 2015). These responses are under tight control, and imbalances in these processes can cause a wide range of diseases, including immunodeficiency, autoinflammation, and autoimmunity.

Cyclic GMP-AMP synthase (cGAS) is an innate immune sensor of DNA that recognizes cytosolic DNA, resulting in the activation of the signaling adaptor stimulator of interferon genes (STING), the transcription factor interferon regulatory factor 3 (IRF3) and the expression of type I/III IFN and IFN-stimulated genes (ISGs; Chen et al., 2016). cGAS recognizes cytosolic DNA derived from invading microbes (Gao et al., 2013; Lahaye et al., 2013; Sun et al., 2013) and also self-DNA from engulfed tumor cells (Woo et al., 2014), damaged mitochondria (Rongvaux et al.,

2014; White et al., 2014; West et al., 2015), and nuclear DNA damage (Dou et al., 2017; Glück et al., 2017; Harding et al., 2017; Mackenzie et al., 2017; Yang et al., 2017). After damage, nuclear DNA can accumulate in the cytosol in the form of micronuclei and chromatin fragments. Activation of cGAS by damaged nuclear DNA contributes to auto-tumor immunity, cellular senescence, and autoinflammatory disorders (Li and Chen, 2018). While multiple studies have demonstrated that induction of inflammatory cytokines upon exogenous DNA damage is mediated through the cGAS–STING pathway, the factors that normally keep endogenous DNA damage-mediated inflammation in check remain poorly understood.

Monogenic human diseases that collectively belong to a group referred to as type I interferonopathies have led to the identification of several factors that normally limit pathogenic inflammation (Crow and Manel, 2015). These interferonopathies are characterized by a stable ISG signature in blood samples and

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clinical manifestations of inflammation, with certain features restricted to mutant genotypes (Meyts and Casanova, 2016). These observations raise the possibility that other monogenic diseases may involve aspects of pathology that are, albeit currently unrecognized, related to enhanced type I IFN signaling, thereby potentially revealing the normal function of such genes in keeping inflammation in check.

Several studies have documented the link between deficiencies in genome integrity and induction of an IFN signature. Patients with monogenic mutations in Artemis show an IFN signature in blood, and this was attributed to cell extrinsic effects through NETosis (Gul et al., 2018). Fibroblasts from Werner syndrome, mutated in the RecQ helicase WRN, demonstrate an increased transcriptional level of *IFNB* (Yu et al., 2015). Rats with a missense mutation in *Atm* show an IFN signature in the brain (Quek et al., 2017), and mice knockout for *Atm* show a *Sting*-dependent IFN response (Härtlova et al., 2015). DNase II deficiency can cause an interferonopathy in human patients (Rodero et al., 2017b) and *Dnase2*-deficient murine cells accumulate extranuclear DNA that activates a *Sting*-dependent inflammatory response (Lan et al., 2014). Whether monogenic mutations in genes required for genome integrity can increase micronuclei frequency resulting in an inflammatory response is not known.

Bloom syndrome (BS) is a rare human autosomal recessive disorder characterized by marked genetic instability associated with an increased predisposition to all types of cancers affecting the general population (reviewed in Amor-Gu eret, 2006). BS patients also present a pre- and postnatal growth retardation with normal proportioning, frequently associated with sun-sensitive facial erythema and immunodeficiency manifest by recurrent respiratory tract and gastrointestinal infections (Cunniff et al., 2017). BS is caused by mutations in both copies of the *BLM* gene, encoding the BLM RecQ-like helicase (Ellis et al., 1995). BLM protein displays ATP and Mg<sup>2+</sup>-dependent 3'-5'-DNA helicase activity and plays an essential role in maintaining genome integrity at the crossroads of the response pathways induced by DNA damage and stalled replication forks (Karow et al., 2000; Wu and Hickson, 2003; Amor-Gu eret, 2006; Ralf et al., 2006; Bugreev et al., 2007; Chan et al., 2007; Rao et al., 2007). The BS cellular phenotype includes chromosome breaks, a high rate of sister chromatid exchange (SCE), a slowing of the replication fork speed associated with endogenous activation of the ATM-Chk2-γH2AX pathway, and an increase in the frequency of blocked replication forks and anaphase bridges (Amor-Gu eret, 2006; Rao et al., 2007; Chan and Hickson, 2011). BS cells also accumulate micronuclei (Yankiwski et al., 2000).

Here we identify an inflammatory ISG signature in BLM-deficient cells and determine how BLM connects DNA damage to innate immune-sensing pathways.

## Results

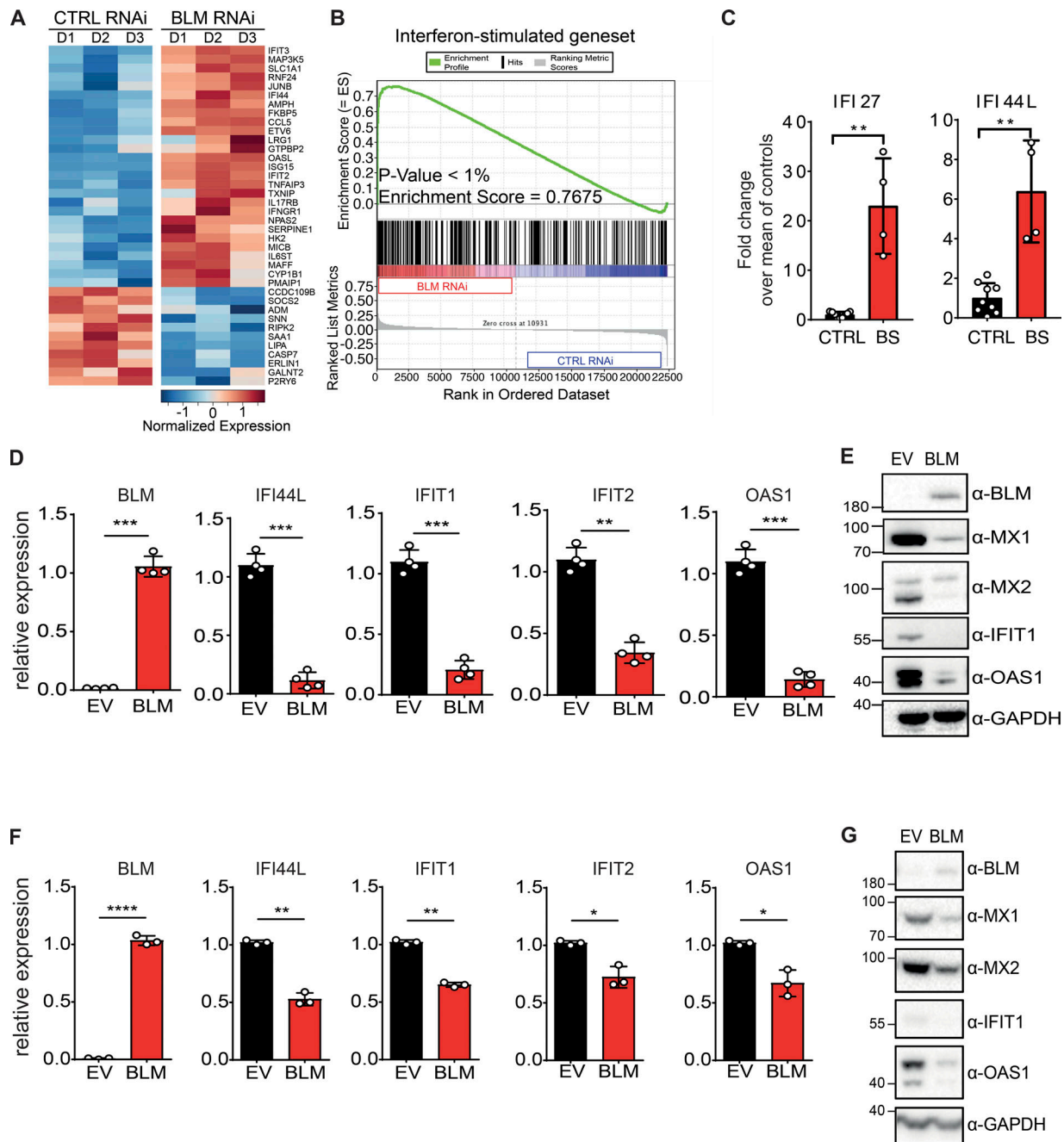
We examined the transcriptional profile of cells following a previously reported down-regulation of BLM (Chabosseau et al., 2011). Expression of ISGs was induced upon down-regulation of BLM (Fig. 1 A and Fig. S1; Schoggins et al., 2011). ISG expression

was significantly enriched in BLM-depleted cells, as shown by a gene set enrichment analysis (Fig. 1 B).

These data suggested the possibility that ISGs could also be induced in BLM-deficient cells from BS patients. We examined ISG expression in primary fibroblasts from a BS patient carrying a homozygous mutation that generates a premature termination codon at position 784 (AGS721; Table S1 and Fig. S2, A-C). As expected, mutant cells demonstrated an increased frequency of SCE (Fig. S2, D and E). BLM protein could not be detected in these slow-dividing cells due to its S-phase restricted expression (Dutertre et al., 2000). Expression of *IFI44L* and *IFI27* was increased in BLM-deficient cells as compared with control cells (Fig. 1 C).

To determine if ISG expression was the result of BLM deficiency, we rescued expression of WT BLM in immortalized BS cells (Fig. 1 D). Of note, the induction of ISGs in BS cells was subject to a clonal effect, and we used polyclonal conditions hereafter (data not shown). Transcriptional levels of *IFI44L*, *IFIT1*, *IFIT2*, and *OAS1* were significantly reduced upon rescue with WT BLM (Fig. 1 D). At the protein level, we found that *MXI*, *MX2*, *IFIT1*, and *OAS1* are expressed in immortalized BS cells (Fig. 1 E). Rescue of WT BLM expression reduced the levels of these proteins, confirming the ISG transcriptional signature of BLM-deficient cells (Fig. 1 E). Similar to immortalized cells, rescuing WT BLM expression in primary BS cells significantly reduced expression of *IFI44L*, *IFIT1*, *IFIT2*, and *OAS1* at the RNA level (Fig. 1 F). Protein levels of *MXI*, *MX2*, *IFIT1*, and *OAS1* were also reduced by WT BLM expression in BS cells (Fig. 1 G). We conclude that BS cells are characterized by an increased expression of ISGs that can be attenuated by reintroducing WT BLM.

We next sought to determine if BLM was required in WT cells to suppress ISG expression. We inhibited BLM expression in primary fibroblasts using siRNA (Fig. 2 A). As a result, RNA levels of *IFI44L*, *IFIT1*, *IFIT2*, and *OAS1* were significantly increased (Fig. 2 A). Consistently, protein levels of *MXI*, *MX2*, and *IFIT1* were also increased upon transient down-regulation of BLM (Fig. 2 B). Of note, a baseline level of *MXI*, *IFIT1*, and *OAS1* proteins was also detected in control cells, which could result from the siRNA transfection. These results were reproduced in an independent source of primary human fibroblasts (Fig. S3). ISGs comprise multiple innate immune sensors and signaling components (Schoggins et al., 2011), and cells with a higher level of ISG expression are expected to show an increased sensitivity to stimulation with double strand RNA (Kaiser et al., 2004; Tissari et al., 2005; Tohyama et al., 2005). To test if ISG induction upon BLM down-regulation results in such functional sensitization, we reduced BLM protein expression with siRNA and treated cells with increasing concentrations of poly(I:C) (polyinosinic-polycytidylic acid). As expected, *CXCL10*, *IFIT2*, and *OAS1* transcripts were induced upon poly(I:C) treatment in a dose-dependent manner in control cells. This induction was significantly increased with concomitant down-regulation of BLM (Fig. 2 C). Accordingly, *IFIT1* protein was induced by poly(I:C) treatment and further increased with down-regulation of BLM (Fig. 2 D). We conclude that BLM is required to limit functional sensitization to an innate immune stimulus in cells, consistent



**Figure 1. BS cells exhibit an ISG signature.** (A) Heatmap representing the Z-score for normalized expression values of 38 ISGs between control (CTRL RNAi) and BLM-depleted (BLM RNAi) HeLa cells. ISGs were selected from another study (Schoggins et al., 2011). (B) Gene set enrichment analysis of ISGs in control (CTRL RNAi) and BLM-depleted (BLM RNAi) HeLa cells (see Materials and methods). (C) mRNA level expression of *IFI44L* and *IFI27* determined by RT-qPCR in primary CTRL and BS fibroblasts. Data are the combination of three independent experiments ( $n_{CTRL} = 2, 3, \text{ and } 4$ , and  $n_{BS} = 1, 2, \text{ and } 1$ , respectively, for each experiment). Values were normalized to the average of controls within each experiment (mean with SD; Mann-Whitney,  $**P < 0.01$ ). (D) mRNA level expression of *BLM*, *IFI44L*, *IFIT1*, *IFIT2*, and *OAS1* determined by RT-qPCR in SV40-immortalized BS cells transduced with BLM or EV (mean with SD of  $n = 4$  independent experiments; paired *t* test,  $**P < 0.01$ ,  $***P < 0.001$ ). (E) Protein levels of *BLM*, *MX1*, *MX2*, *IFIT1*, *OAS1*, and *GAPDH* in cells, as in D (representative of  $n = 4$  independent experiments). (F) Expression of *BLM*, *IFI44L*, *IFIT1*, *IFIT2*, and *OAS1* in primary BS cells transduced with BLM or EV (mean with SD of  $n = 3$  independent experiments; paired *t* test,  $*P < 0.05$ ,  $**P < 0.01$ ,  $****P < 0.0001$ ). (G) Protein levels of *BLM*, *MX2*, *MX1*, *IFIT1*, *OAS1*, and *GAPDH* in cells, as in F (representative of  $n = 3$  independent experiments).

with the up-regulation of ISGs in BLM-deficient cells. To determine if the ISG signature was present at the transcriptome level in BLM-deficient cells, we knocked out *BLM* using clustered regularly interspaced short palindromic repeat (CRISPR) lentivectors in

SV40-immortalized fibroblasts to perform a microarray gene expression analysis (Fig. 2 E). Two significant hallmark gene sets corresponding to ISGs were enriched in the transcriptome of *BLM* knockout cells (Fig. 2, F and G). Among genes that were

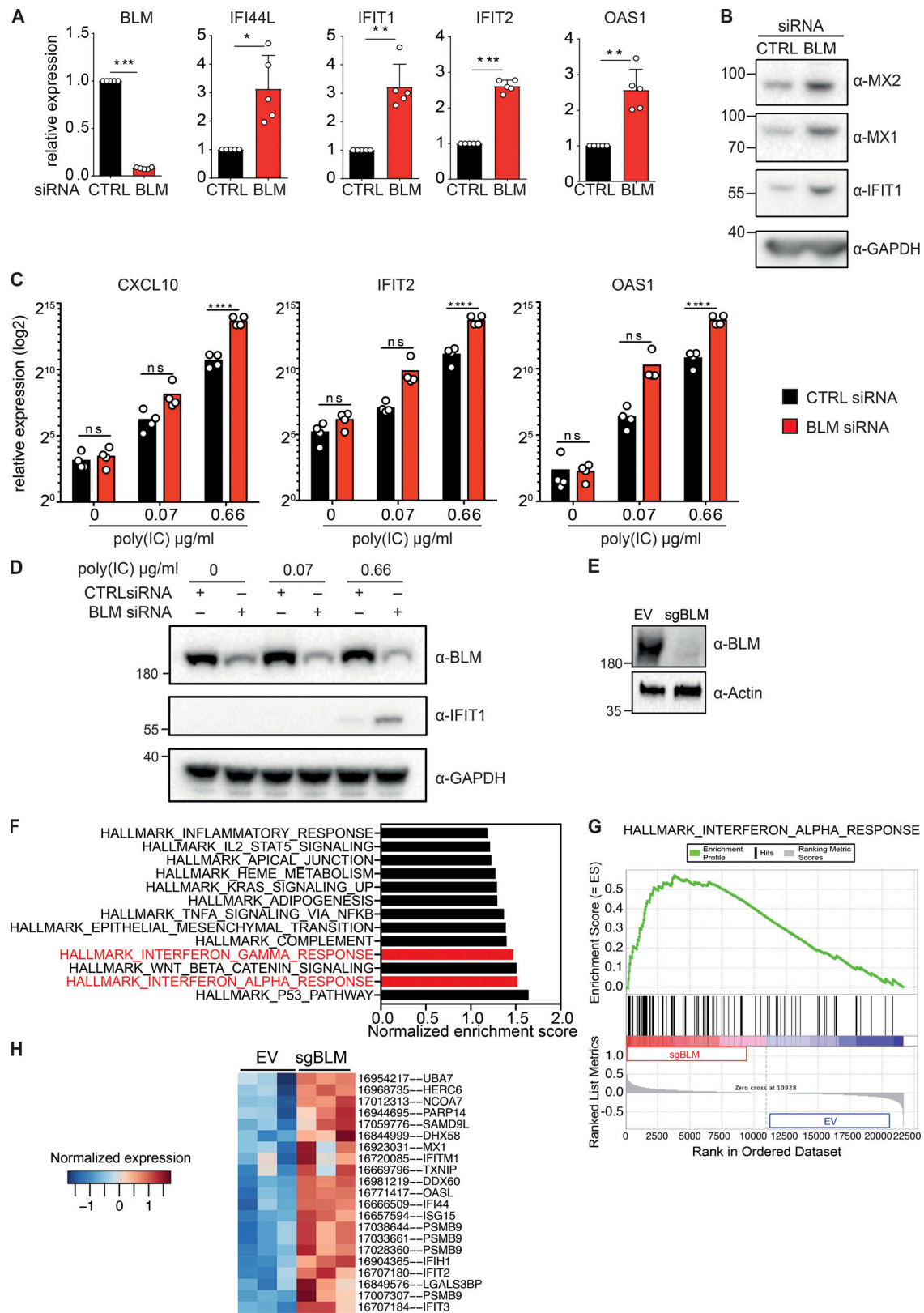


Figure 2. **Down-regulation of BLM induces ISGs.** (A) mRNA level expression of *BLM*, *IFI44L*, *IFIT1*, *IFIT2*, and *OAS1* determined by RT-qPCR in primary human fibroblasts treated with control (CTRL) or BLM siRNA for 72 h (mean with SD of  $n = 5$  independent experiments; one-sample  $t$  test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). (B) Protein levels of *MX2*, *MX1*, *IFI1*, and *GAPDH* in primary human fibroblasts treated with control or BLM siRNA for 72 h (representative of  $n = 5$  independent experiments). (C) mRNA level expression of *CXCL10*, *IFIT2*, and *OAS1* determined by RT-qPCR in primary human fibroblasts treated with control or BLM siRNA for 48 h followed by 24 h of treatment with poly(I:C) (mean with SD of  $n = 4$  independent experiments; one-way ANOVA with Tukey post-test, \*\*\*\* $P < 0.0001$ ). (D) Protein levels of *BLM*, *IFI1*, and *GAPDH* in primary human fibroblasts treated with control or BLM siRNA for 48 h followed by 24 h of



treatment with poly(I:C) (representative of  $n = 4$  independent experiments). **(E)** Protein levels of *BLM* and *Actin* in SV40-immortalized cells after transduction with CRISPR-Cas9 lentivector targeting *BLM* (sgBLM) and the corresponding control lentivectors (EV; representative of three independent replicates). **(F)** Normalized enrichment score of the hallmark gene sets that were significantly enriched in *BLM* knockout cells over control cells, as in E. Microarray-based analysis of three independent replicates. **(G)** Enrichment of the hallmark IFN $\alpha$  response gene set in *BLM* knockout cells. **(H)** Heatmap representing the Z-score for normalized expression values of the hallmark IFN $\alpha$  response genes that were significantly deregulated at the gene level between *BLM* knockout cells and control cells ( $P < 0.05$ ).

significantly deregulated in *BLM*-deficient cells over control cells, all gene set ISGs were up-regulated (Fig. 2 H). We conclude that the ISG signature was significantly enriched in *BLM*-deficient cells at the transcriptome level.

We next aimed to identify the signaling pathway involved in ISG expression upon *BLM* deficiency. Since *BLM* functions in DNA repair, we hypothesized that the cGAS–STING–IRF3 pathway could be activated as a result of *BLM* deficiency. We inhibited the expression of *cGAS*, *STING*, and *IRF3* with shRNA vectors in SV40-immortalized BS and control cells (Fig. 3 A and Fig. S4, A and B). As previously observed, expression of *IFI44L*, *IFIT1*, and *OAS1* was increased in BS cells. Inhibition of *cGAS*, *STING*, or *IRF3* expression significantly inhibited expression of these genes (Fig. 3, B and C). Of note, one of the shRNA targeting *IRF3* also reduced the expression of *STING* in this cell type. To confirm the implication of the cGAS–STING–IRF3 pathway, we knocked out these genes using CRISPR lentivectors in SV40-immortalized BS and control cells (Fig. 3 D and Fig. S4, C and D). Similar to shRNA-mediated down-regulation, expression of *IFI44L*, *IFIT1*, and *OAS1* was significantly reduced in knockout cells (Fig. 3, E and F). We conclude that *BLM* is required to limit ISG expression resulting from engagement of the cGAS–STING–IRF3 pathway.

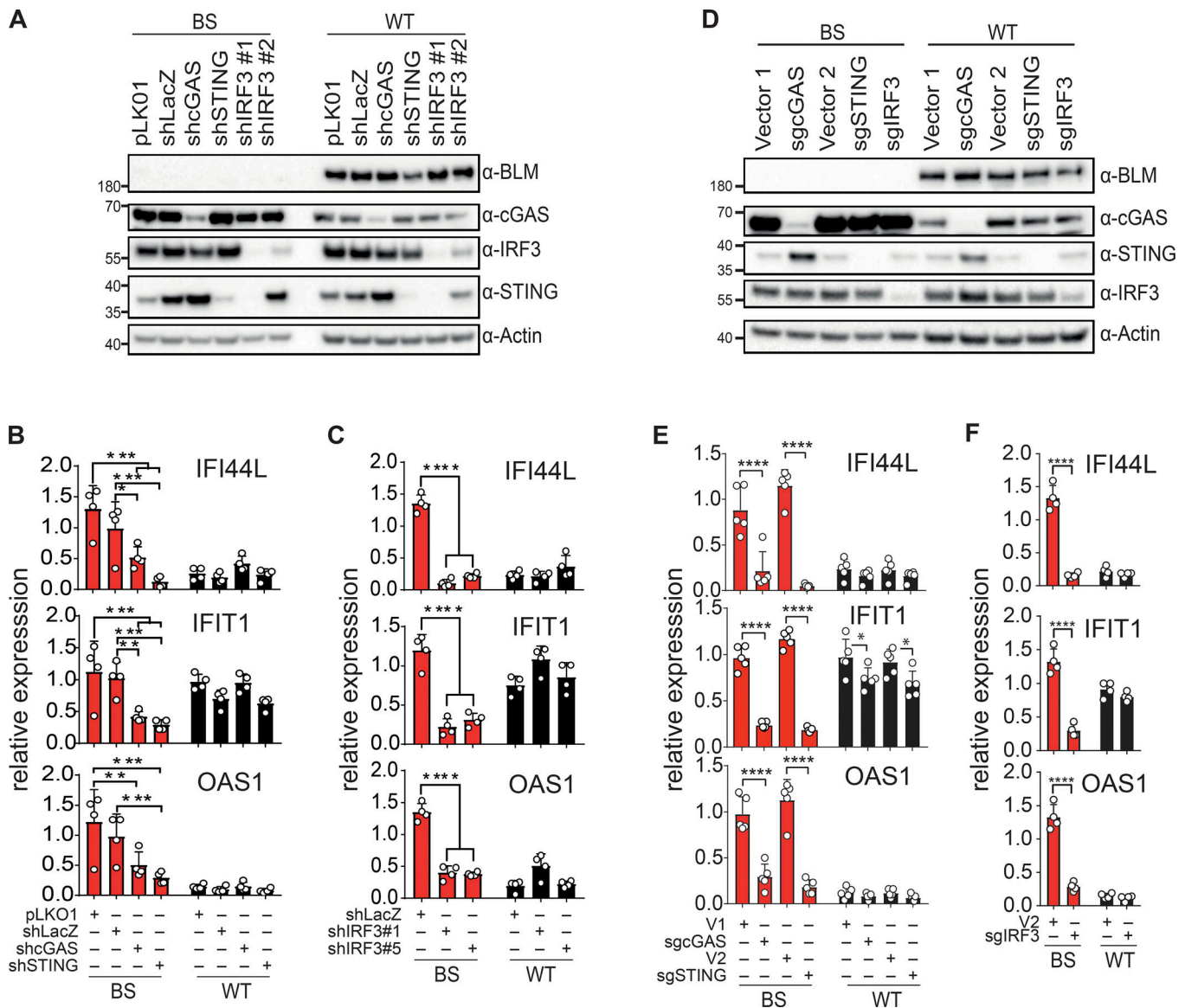
The cGAS–STING–IRF3 pathway is triggered by the accumulation of cytosolic DNA that activates cGAS. Accumulation of cytosolic DNA is counterbalanced by the activity of the DNaseIII TREX1, and TREX1 deficiency leads to increased ISG expression in a cGAS-dependent manner (Ablasser et al., 2014). To determine if cytosolic DNA might be implicated in cGAS activation in *BLM*-deficient cells, we knocked out *TREX1* in control and in *BLM* knockout cells (Fig. 4 A). Inhibiting TREX1 expression in *BLM*-deficient cells increased expression of *IFI44L*, *IFIT1*, *ISG20*, *MXI*, and *OAS1* (Fig. 4 B). At the protein level, *MXI* and *IFIT1* were strongly increased upon TREX1 disruption in *BLM* knockout cells (Fig. 4 A). These results suggested that TREX1-sensitive DNA was responsible for the cGAS-dependent ISG expression in *BLM*-deficient cells.

We next sought to determine if activation of a cGAS-dependent ISG expression in *BLM*-deficient cells could be the result of their characteristic DNA replication stress (Rao et al., 2007). We used aphidicolin and hydroxyurea to induce replication-dependent DNA damage in control and BS cells. We first treated *BLM* knockout cells and control cells with aphidicolin (Fig. 4 C). Aphidicolin treatment alone slightly increased ISG expression, which was significant for *IFIT1*, *ISG20*, and *MXI* but not for *IFI44L* and *OAS1* in control cells (Fig. 4 D). In contrast, aphidicolin treatment in *BLM* knockout cells significantly increased *IFIT1*, *ISG20*, *MXI*, *IFI44L*, and *OAS1* expression (Fig. 4 D). *IFIT1* and *MXI* protein levels were also increased by aphidicolin

treatment in *BLM* knockout cells (Fig. 4 C). We next treated *BLM* knockout cells with hydroxyurea (Fig. 4 E). Hydroxyurea significantly increased the expression of *ISG20* and *IFIT1*, but not *MXI* or *OAS1* in control cells, indicative of a slight induction of ISG expression (Fig. 4 F). In *BLM* knockout cells, hydroxyurea treatment further increased expression of *IFIT1*, *ISG20*, *MXI*, and *OAS1* as compared with untreated *BLM* knockout cells (Fig. 4 F). At the protein level, induction of *MXI* and *IFIT1* was also enhanced by hydroxyurea in *BLM* knockout cells (Fig. 4 E). Altogether, these results show that *BLM* is required to limit ISG expression induced by DNA damage generated during replication.

DNA-damaging agents lead to the accumulation of misplaced DNA fragments that activate the cGAS pathway (reviewed in Li and Chen, 2018). Our data suggested that cGAS-dependent ISG expression in *BLM*-deficient cells could be the result of unresolved DNA damage. *BLM*-deficient cells are characterized by the accumulation of micronuclei in the cytosol (Yankiwski et al., 2000), and micronuclei resulting from DNA damage activate the cGAS pathway (Harding et al., 2017; Mackenzie et al., 2017). We quantified the frequency of micronuclei and their association with cGAS in *BLM*-deficient cells using a GFP-cGAS reporter as previously described (Fig. 5 A; Raab et al., 2016; Mackenzie et al., 2017). As expected, the frequency of micronuclei was significantly increased in engineered *BLM*-deficient cells (Fig. 5 B). This increased level was similar to that in BS cells (Fig. S5 A). We found that cells containing cGAS-positive micronuclei were also increased in *BLM*-deficient cells (Fig. 5 C). Within micronuclei, the frequency of GFP-cGAS micronuclei was not different in control and *BLM*-deficient cells (Fig. S5 B), suggesting that *BLM* determines the rate of micronuclei production but not their susceptibility to cGAS association. Similarly, in primary fibroblasts, the frequency of micronuclei, and of cells with cGAS-positive micronuclei, was higher in BS cells (Fig. 5 D and Fig. S5 C). Treatment with aphidicolin, which increased ISG expression in BS cells (Fig. 4, C and D), increased the frequency of micronuclei, and of cells with cGAS-positive micronuclei, in both control and *BLM*-deficient cells (Fig. 5 E and Fig. S5 D). Interestingly, the frequency of cells with cGAS-positive micronuclei was increased in *BLM*-deficient cells over control following aphidicolin treatment (Fig. 5 E). Next, we rescued *BLM* expression in BS cells. In agreement with the inhibition experiment, rescue of *BLM* expression significantly reduced the frequency of micronuclei and of cells with cGAS-positive micronuclei (Fig. 5 F and Fig. S5 E). We conclude that cGAS recognizes micronuclei in *BLM*-deficient cells.

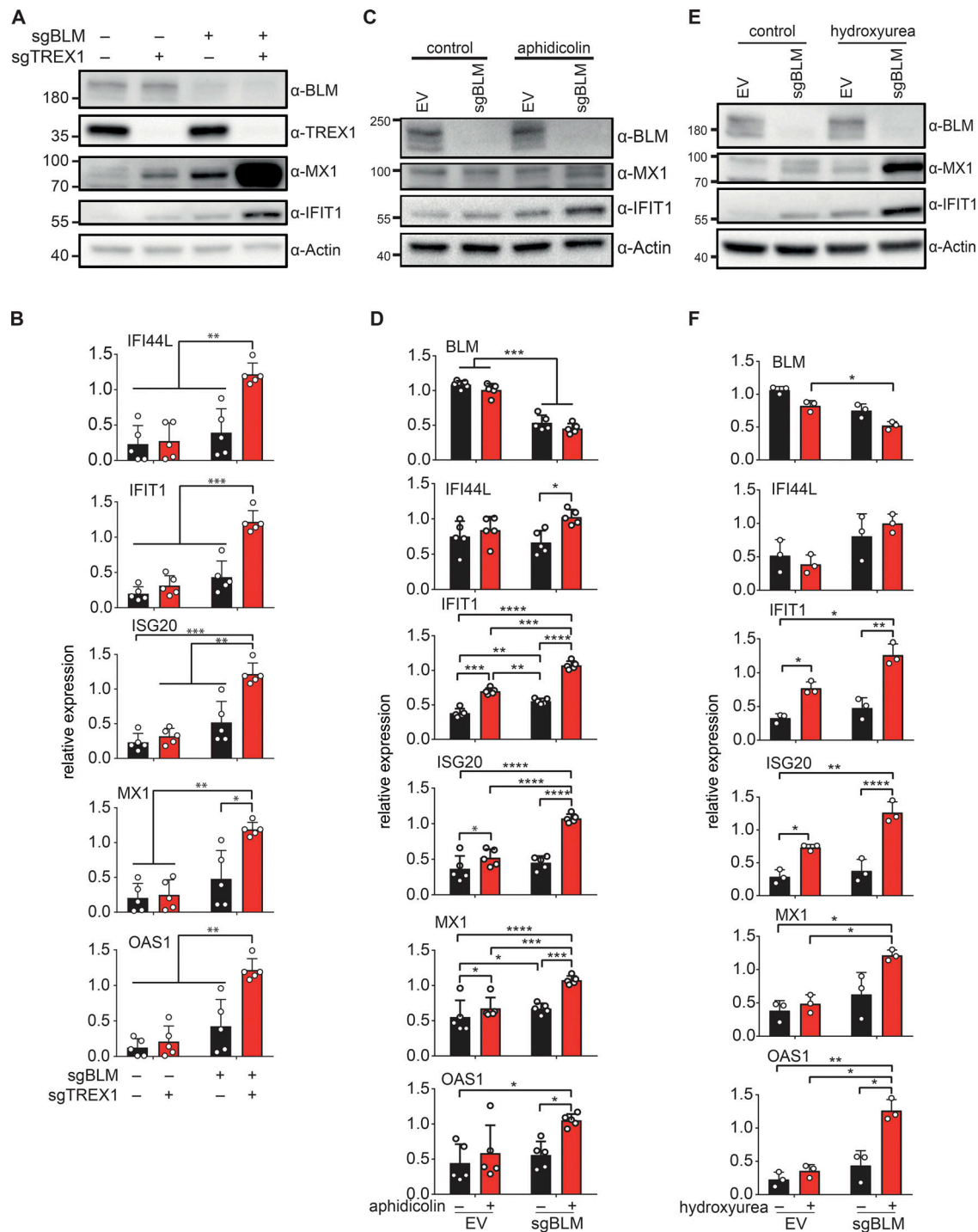
We finally sought to determine whether the expression of ISG in BS cells was physiologically relevant in BS patients. In monogenic interferonopathies, leukocytes are characterized by



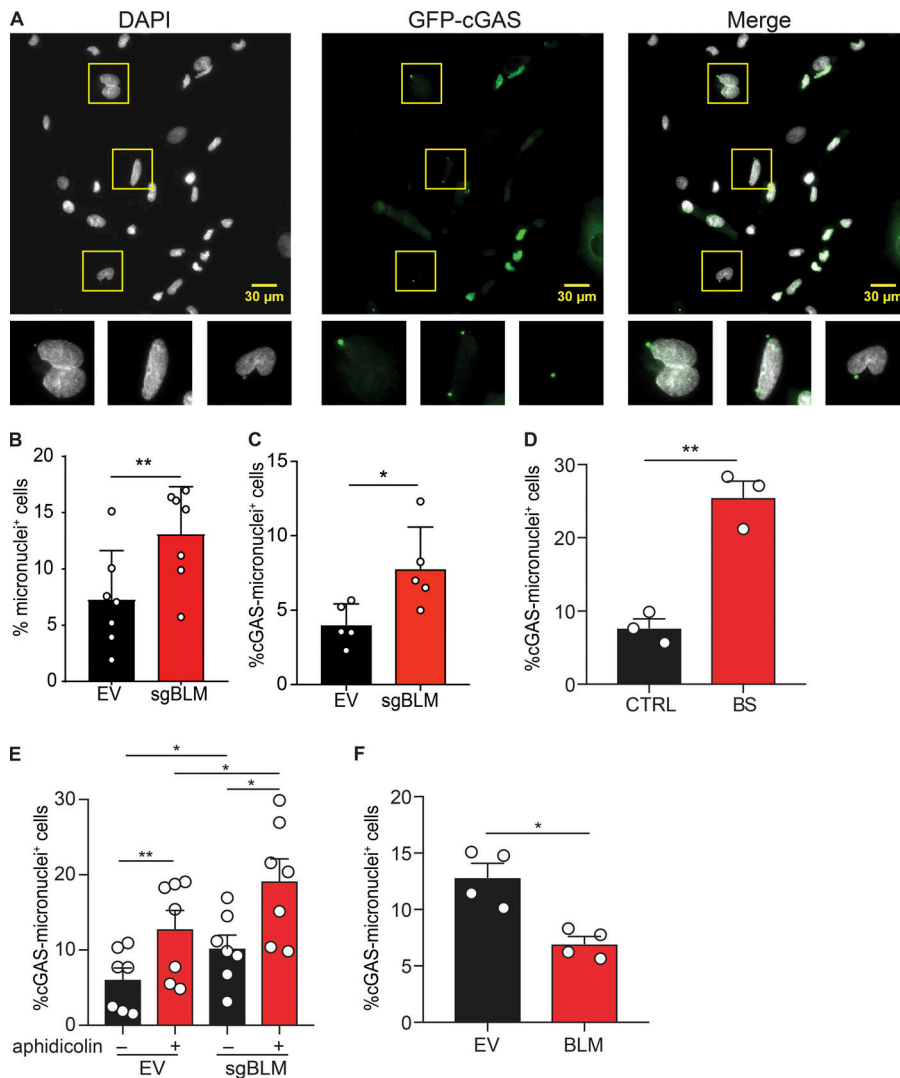
**Figure 3. ISG signature induction in BS cells requires the cGAS-STING-IRF3 pathway.** (A) Protein levels of *BLM*, *cGAS*, *STING*, *IRF3*, and *Actin* in SV40-immortalized control (WT) or BS cells transduced with control shRNA lentivector (pLKO1, shLacZ) or shRNA lentivectors against *cGAS*, *STING*, or *IRF3* (representative of  $n = 4$  independent experiments). (B) mRNA level expression of *IFI44L*, *IFIT1*, and *OAS1* determined by RT-qPCR in cells transduced with control shRNAs or shRNA against *cGAS* or *STING*, as in A (mean with SD of  $n = 4$  independent experiments; two-way ANOVA with Tukey post-test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). (C) mRNA level expression of *IFI44L*, *IFIT1*, and *OAS1* determined by RT-qPCR in cells transduced with control shRNAs or shRNAs against *IRF3*, as in A (mean with SD of  $n = 4$  independent experiments; two-way ANOVA with Tukey post-test, \*\*\*\* $P < 0.0001$ ). (D) Protein levels of *BLM*, *cGAS*, *STING*, *IRF3*, and *Actin* in SV40-immortalized control (WT) or BS cells transduced with CRISPR-Cas9 lentivectors directed against *cGAS*, *STING*, or *IRF3* or control lentivectors (V1 or V2; representative of  $n = 5$  independent experiments). (E) mRNA level expression of *IFI44L*, *IFIT1*, and *OAS1* determined by RT-qPCR in SV40-immortalized control (WT) or BS cells transduced with CRISPR-Cas9 lentivectors directed against *cGAS*, *STING*, or control lentivectors (V1 or V2), as in D (mean with SD of  $n = 5$  independent experiments; two-way ANOVA with Tukey post-test, \* $P < 0.05$ , \*\*\*\* $P < 0.0001$ ). (F) mRNA level expression of *IFI44L*, *IFIT1*, and *OAS1* determined by RT-qPCR in SV40-immortalized control (WT) or BS cells transduced with CRISPR-Cas9 lentivector directed against *IRF3* or control lentivector (V2), as in D (mean with SD of  $n = 4$  independent experiments; two-way ANOVA with Sidak post-test, \*\*\*\* $P < 0.0001$ ).

an increased expression of ISGs that is sustained over time and that can be used to derive a discriminative IFN score (Rice et al., 2013). In addition, elevated IFN $\alpha$  protein can be detected in the serum using a Simoa digital ELISA (Rodero et al., 2017a). We examined these parameters in available samples from two BS patients, AGS721 and AGS1513 (Table S1). AGS1513 carried the homozygous *BLM* mutation p.C1055S that we previously described (Foucault et al., 1997; Fig. S2 F). Increased ISG expression

was consistently detected in three and four independent samples over a period of 1.5 and 2 yr, respectively (Fig. 6 A). The combined IFN score of all samples was significantly higher than that of control samples (Fig. 6 B). We next measured the concentration of IFN $\alpha$  in the plasma from the AGS1513 patient and in the cerebrospinal fluid (CSF) from the AGS721 patient. The plasma IFN $\alpha$  concentration of AGS1513 was increased 97 $\times$  as compared with the average of all healthy donors (Fig. 6 C). The



**Figure 4. DNA damage and down-regulation of TREX1 enhance ISG expression in BLM-deficient cells. (A)** Protein levels of *BLM*, *TREX1*, *MX1*, *IFIT1*, and *Actin* in SV40-immortalized cells after transduction with CRISPR-Cas9 lentivector targeting *BLM* (sgBLM) and/or *TREX1* (sgTREX1) or corresponding control lentivectors (-; representative of  $n = 5$  experiments). **(B)** mRNA level expression of *IFI44L*, *IFIT1*, *ISG20*, *MX1*, and *OAS1* determined by RT-qPCR in cells, as in A (mean with SD of  $n = 5$  independent experiments; two-way ANOVA with Tukey post-test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). **(C)** Protein levels of *BLM*, *MX1*, *IFIT1*, and *Actin* in SV40-immortalized control cells (EV) and BLM-deficient cells (sgBLM) treated with 0.4  $\mu\text{M}$  of aphidicolin for 24 h (representative of  $n = 5$  independent experiments). **(D)** mRNA level expression of *BLM*, *IFI44L*, *IFIT1*, *ISG20*, *MX1*, and *OAS1* determined by RT-qPCR in cells, as in C (mean with SD of  $n = 5$  independent experiments; two-way ANOVA with Tukey post-test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ ). **(E)** Protein levels of *BLM*, *MX1*, *IFIT1*, and *Actin* in SV40-immortalized control cells (EV) and BLM-deficient cells (sgBLM) treated with 0.5  $\mu\text{M}$  of hydroxyurea for 24 h (representative of  $n = 3$  independent experiments). **(F)** mRNA level expression of *BLM*, *IFI44L*, *IFIT1*, *ISG20*, *MX1*, and *OAS1* determined by RT-qPCR in cells, as in E (mean with SD of  $n = 3$  independent experiments; one-way ANOVA with Tukey post-test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\*\* $P < 0.0001$ ).



CSF IFN $\alpha$  concentration of AGS721 was also increased compared with a previously reported set of CSF samples from patients with retinal vasculopathy and cerebral leukodystrophy that is not associated with increased type I IFN signaling (Fig. 6 C; Rodero et al., 2017a). We conclude that BS patients are characterized in vivo by a sustained up-regulation of IFN signaling.

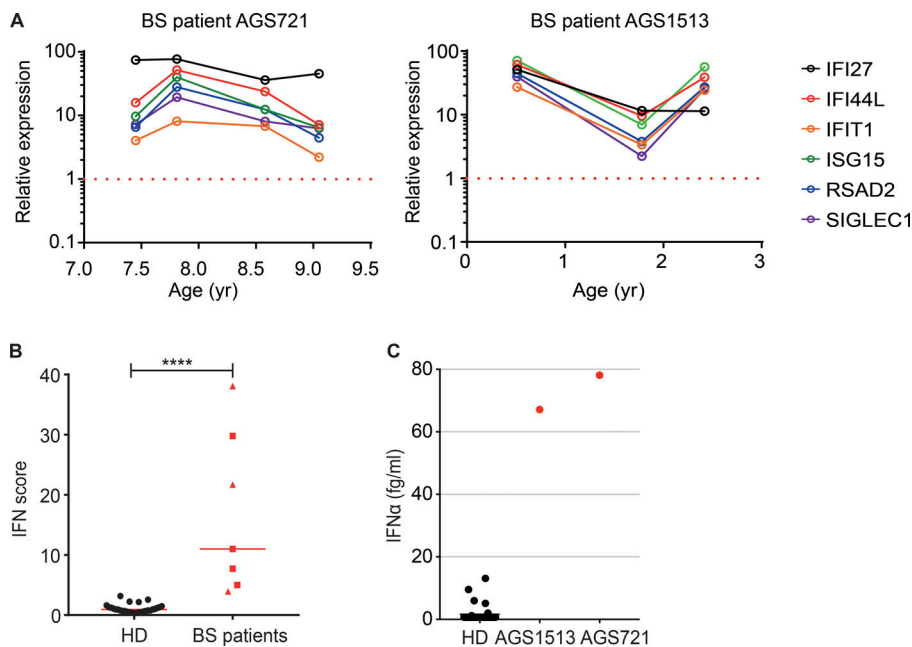
## Discussion

Here, we show that *BLM*-deficient cells present an increased expression of inflammatory genes through the cGAS-STING-IRF3 pathway as a result of DNA damage and micronuclei generation and provide evidence that BS patients exhibit an IFN-gene signature. Altogether, these results establish that *BLM* is essential to prevent the induction of unchecked inflammatory gene responses.

We observed induction of ISGs at the transcriptional and protein levels in peripheral blood cells and plasma from BS patients, in primary BS cells, in SV40-immortalized BS cells and upon CRISPR- and RNA interference (RNAi)-mediated inhibition of *BLM* expression in wild-type cells. Consistent with this, a

previous report noted the up-regulation of an immune gene signature in a panel of BS cells (Nguyen et al., 2014). BS includes an immune deficiency that often leads to life-threatening infections. Patients usually present with low concentrations of one or more plasma immunoglobulins and fail to develop delayed-type hypersensitivity (Hütteroth et al., 1975; Van Kerckhove et al., 1988; Kondo et al., 1992). BS patients are also at risk of developing diabetes mellitus. In mice, the full deficiency of *Blm* is embryonic lethal (Chester et al., 1998; Luo et al., 2000; Goss et al., 2002). Tissue-specific deletion of *Blm* has revealed that *BLM* plays an essential role during B and T lymphocyte development (Babbe et al., 2007, 2009). In agreement with our results, it was previously noted that the levels of *Ifnb* transcripts are increased in Werner-Bloom-Terc triple-deficient mice (Yu et al., 2015). Chronic IFN production was also detected in ataxia telangiectasia and ATM deficiency (Quek et al., 2017; Gul et al., 2018). However, the cellular basis of the immune deficiency in BS is poorly understood, and the function of the *BLM* protein in the immune system is not defined. Our work enables investigation of how innate immune sensing of damaged DNA in BS could contribute to immune-related disorders in patients.





**Figure 6. Increased ISG expression in BS patients.** (A) IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1 expression was measured in the whole blood of two BS patients (AGS721 and AGS1513). Measurements were made at different time points and were normalized to 29 control samples (set to 1; red dotted line). (B) IFN score of the two BS patients (red squares: AGS721; red triangles: AGS1513) and control individuals (bar = median; Mann-Whitney test, \*\*\*\*P < 0.0001). (C) IFN $\alpha$  protein concentration in 36 control sera, in serum from the AGS1513 BS patient, and in CSF from the AGS712 BS patient.

We find that cGAS-positive micronuclei are increased in BLM-deficient cells. For recombinant BLM, preferred substrates are structures mimicking replication forks or homologous recombination intermediates, such as D-loops and X-junctions, and G-quadruplex DNA, which could all contribute to micronuclei generation (Amor-Gu eret, 2006). Consistent with a role of DNA damage in micronuclei generation in BLM-deficient cells, we find that DNA-damaging agents aphidicolin or hydroxyurea treatment potentiated ISG induction. The increased frequency of cGAS-positive micronuclei in BLM-deficient cells over control, following aphidicolin treatment, suggests the interesting possibility that aphidicolin promotes association of cGAS with micronuclei when BLM is not present. In addition to micronuclei, DNA damage can also lead to release of immunostimulatory single-stranded DNA (ssDNA) in the cytosol (Li and Chen, 2018). Release of ssDNA in the cytosol was recently reported in cells simultaneously depleted for SAMHD1 and treated with hydroxyurea (Coquel et al., 2018). Co-inhibition of BLM expression did not modify the quantity of cytosolic ssDNA in this study, contrasting with its effect on micronuclei. We also find that down-regulation of TREX1, a protein that degrades cytosolic DNA (Stetson et al., 2008), enhances ISG induction in BS cells. Altogether, this suggests that BLM normally contributes to resolving nucleic acid structures that would otherwise lead to the release of TREX1-sensitive, immunostimulatory micronuclei. We found that ISG expression in BS cells required cGAS, STING, and IRF3. A low level of ISG expression was also detectable in wild-type fibroblasts, but that did not require cGAS, STING, or IRF3, and was not enhanced by TREX1 down-regulation. This is consistent with ISG expression resulting from tonic IFN signaling (Gough et al., 2012), which is unlikely to result from accumulation of cytosolic DNA in wild-type fibroblasts in this case.

The IFN $\alpha$  concentrations that we measured in two BS patients are in the range of what has been reported in polygenic diseases such as systemic lupus erythematosus or juvenile-onset

dermatomyositis and are lower than in monogenic interferonopathies (Rodero et al., 2017a). How do these data inform our clinical understanding? In monogenic interferonopathies, systemic lupus erythematosus, and juvenile-onset dermatomyositis, there is no strict correlation between the IFN signature and disease (Rodero et al., 2017a), and there is clear distinction in the phenotype of BS. Previously, two constant features (growth delay and susceptibility to all kinds of cancer) and nine non-constant features have been described in BS (German, 1995). The contribution of the IFN signature as a driver of the BS phenotype requires further interrogation, and understanding the clinical relevance of the DNA damage response genes in controlling innate inflammation and pathological processes will possibly require identification of the anatomical and cellular sites where IFN is produced at the highest levels.

ISGs can be induced by radiotherapy and chemotherapy, and the expression of a subset of ISGs was reported to correlate with resistance to treatment and cancer cell survival in another study (Erdal et al., 2017). In this context, contrasting with our results on BLM expression in primary and immortalized fibroblasts, in an MCF7 cancer cell model, BLM was instead required for the accumulation of cytoplasmic DNA foci, phosphorylation of STAT1, and induction of IFI44, IFI6, ISG15, and MX1 in response to irradiation (Erdal et al., 2017). This could suggest that in specific cancer cell types, where ISG expression might contribute to resistance to treatment, BLM is “repurposed” to enhance the production of inflammatory cytoplasmic DNA species. In contrast, we find that in primary fibroblasts, BLM is required to limit these processes. The mechanisms that enable such putative repurposing of BLM in cancer cells are not known. Taken together, our results highlight the multifaceted role of BLM in health and cancer, in the context of cytoplasmic DNA sensing and inflammation.

Altogether, our results indicate that BLM keeps inflammatory gene induction resulting from DNA damage in check. This

extends our basic understanding of how DNA damage is prevented from triggering damaging innate immune activation in sterile conditions and provides new insights into the pathogenesis of rare monogenic human diseases.

## Materials and methods

### Patients

The study was approved by the Comité de Protection des Personnes (ID-RCB/EUDRACT: 2014-A01017-40). Patients are described in Table S1.

### Plasmid and reagents

Plasmids used in the study are listed in Table S2. pTRIP-CMV-puro-2A and pTRIP-CMV-EGFP-FLAG-cGAS E225A-D227A were described elsewhere (Gentili et al., 2015). *BLM* open reading frame was amplified from pGFP-*BLM* (Eladad et al., 2005) and inserted in pTRIP-CMV-puro-2A. pCMV-VSVG, psPAX2, pLKO.1-puro, and pLCRISPR-CMV were described elsewhere (Manel et al., 2010; Gentili et al., 2015), and pLentiCRISPRv2 was described in Sanjana et al. (2014). pLentiCRISPRv2 Neo was created by replacing puromycin-resistant gene by neomycin in pLentiCRISPRv2. shRNAs against *LacZ* and *cGAS* were previously described (Lahaye et al., 2013). shRNA against *STING* was purchased from Sigma (TRCN0000163296). shRNAs against *IRF3* were transferred from pLKO1gfp to pLKO.1-puro (Manel et al., 2010). The lentivector with a guide RNA (gRNA) against *cGAS* was previously described (Gentili et al., 2015). gRNA against *TREX1* was inserted in pLentiCRISPRv2 Neo. gRNA against *STING* was previously described (Cerboni et al., 2017). gRNA against *BLM* and *IRF3* was inserted in pLentiCRISPRv2. All plasmids' DNA was purified using Nucleobond Xtra Midi Plus (Macherey-Nagel) and were sequenced over the full *BLM* open reading frame or on the shRNA/single guide RNA inserted before use.

Poly(I:C) was purchased from Invivogen (ref tlr-pic). Aphidicolin (#A0781) and hydroxyurea (#H8627) were purchased from Sigma-Aldrich (#A0781).

### Cell culture, transfection, and lentiviral transduction

Primary human BS fibroblasts were skin fibroblasts from patient AGS721. SV40-immortalized BS fibroblasts (GM08505B) were obtained from CORIELL Institute. Primary human control fibroblasts were described elsewhere (Jenkinson et al., 2016). SV40-transformed and human telomerase reverse transcriptase (hTERT) immortalized human control fibroblasts were described elsewhere (Nicolas et al., 1998; Poinson et al., 2004). All cells were cultured in DMEM with 10% serum (Euromedex; lot S67113-0217), glutamine, and penicillin/streptomycin. Puromycin (1 µg/ml) or neomycin (500 µg/ml) was added according to lentiviral transduction.

For lentivirus production, packaging vectors pCMV-VSVG (1 µg) and psPAX2 (0.4 µg) were cotransfected in 6-well plates with lentiviral vector (1.6 µg) in HEK293T cells using Jetprime transfection reagent (Ozyme) according to the manufacturer's protocol. HEK293T cells were washed 24 h after transfection, and the viruses were collected 48 h after transfection. Viruses

were filtered (0.45 µm) and directly used for transductions. 8 µg/ml of protamine was added in each virus solution prior to each transduction. Cells were transduced for 24 h and then subjected to antibiotics selection (puromycin or neomycin).

For *BLM* complementation, human SV40-immortalized BS fibroblasts (GM08505B) and human primary BS fibroblasts (AGS721) were transduced with pTRIP-CMV-puro-2A or pTRIP-CMV-puro-2A-*BLM*. For shRNAs or CRISPR-Cas9 depletion, human SV40-immortalized BS fibroblasts (GM08505B) and human SV40-immortalized control fibroblasts were transduced with pLKO1-puro, pLCRISPR-CMV, or pLentiCRISPRv2 vectors. Cells were selected for at least 1 wk and harvested for RNA and protein extraction. To visualize *cGAS* in micronuclei, human SV40-immortalized BS fibroblasts (GM08505B) and human SV40-immortalized control fibroblasts were transduced with pTRIP-CMV-EGFP-FLAG-cGAS E225A-D227A.

For siRNA transfection, human primary control fibroblasts (in-house or from the American Type Culture Collection) or HEK293T cells were reverse transfected by siCtrl or siBLM (ON-targetplus SMART-pool; Dharmacon) at a final concentration of 50 nM by using Dharmafect 4 (Dharmacon). siRNAs are listed in Table S3. Cells were harvested at 72 h after transfection for RNA and protein extraction.

For poly(I:C) treatment, HEK293T cells depleted for *BLM* by siRNA were transfected with 0.66 or 0.07 µg/ml of poly(I:C) 48 h after siRNA transfection. Cells were harvested 72 h after siRNA transfection for RNA and protein extractions.

For aphidicolin or hydroxyurea treatments, human SV40-immortalized control fibroblasts depleted for *BLM* by CRISPR-Cas9 were treated by 0.4 µM of aphidicolin or 0.5 µM of hydroxyurea. Cells were washed at 24 h and harvested at 48 h after treatment for RNA and protein extraction.

### Affymetrix data

For RNAi-treated HeLa cells, the microarray data were described previously (Chabosseau et al., 2011). For SV40-immortalized fibroblasts, 48 h after seeding of single guide RNA against *BLM* (sgBLM) and empty vector (EV) cells, total RNA was extracted with a Nucleospin kit and eluted in 40 µl of RNase/DNase free water. Quality of total RNA was assessed using RNA 6000 NanoChips with the Agilent 2100 Bioanalyzer (Agilent). Using 100 ng of total RNA, double-stranded cDNA was synthesized with random hexamers tagged with a T7 promoter sequence. The double-stranded cDNA was subsequently used as a template and amplified by T7 RNA polymerase producing many copies of antisense complementary RNA. In the second cycle of cDNA synthesis, random hexamers are used to prime reverse transcription of the complementary RNA from the first cycle to produce ssDNA in the sense orientation. cDNA was synthesized using the GeneChip WT (Whole Transcript) Sense Target Labeling and Control Reagents kit as described by the manufacturer (Affymetrix). The sense cDNA was then fragmented by uracil DNA glycosylase and apurinic/apyrimidic endonuclease-1 and biotin labeled with terminal deoxynucleotidyl transferase using the GeneChip WT Terminal labeling kit (Affymetrix). Hybridization was performed using 5 µg of biotinylated target, which was incubated with the GeneChip Human gene 2.0 array

(Affymetrix) at 45°C for 16–20 h. After hybridization, non-specifically bound material was removed by washing and specifically bound target was detected using the GeneChip Hybridization, Wash and Stain kit, and the GeneChip Fluidics Station 450 (Affymetrix). The arrays were scanned using the GeneChip Scanner 3000 7G (Affymetrix), and raw data were extracted from the scanned images and analyzed with the Affymetrix Power Tools software package. Microarray data analyses were performed with R (version 3.0.0) using packages from Bioconductor. The quality control was performed using the ArrayQualityMetrics package (Kauffmann et al., 2009) without detecting any outlier among the experiment. Data were normalized using the Robust Multi-array Average algorithm from the Oligo package (Carvalho and Irizarry, 2010). Different expression analysis was performed with the Limma package (Ritchie et al., 2015). For RNAi-treated cells, the threshold was an adjusted P value <5% and a  $-1 > \log_2$  fold-change  $> 1$  ( $n = 560$  probes). ISGs were selected based on a previous study (Schoggins et al., 2011;  $n = 330$  genes). For SV40-immortalized fibroblasts, the threshold was a P value <5%. Gene-set enrichment analyses were performed with javaGSEA software (Subramanian et al., 2005). For SV40-immortalized fibroblasts, hallmark gene sets from MSigDB ( $n = 50$ ) were tested for enrichment. The data have been deposited into the Gene Expression Omnibus database (accession no. GSE123447).

#### Quantitative PCR of gene expression (RT-qPCR)

Total RNA was extracted from cells using Nucleospin RNA (Macherey-Nagel) according to the manufacturer's instructions. cDNA was synthesized from 1  $\mu$ g of total RNA using iScript Advanced cDNA Synthesis Kit (Bio-Rad). Quantitative PCR (qPCR) was done using iTaq Universal SYBR Green (Bio-Rad) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). *ACTB* and *HMBS* were used as internal control genes, except for experiments with aphidicolin or hydroxyurea treatments, where *B2M* and *HPRT* were used as internal control genes. A list of the primers used in the study is provided Table S4. Gene expression was analyzed through CFX Manager software (Bio-Rad) according to the  $2^{\Delta\Delta Ct}$  method.

For comparison of gene expression between BS patient cells and control cultured primary fibroblasts, *IFI27* and *IFI44L* were quantified by RT-qPCR using TaqMan probes (*IFI27*: Hs01086370\_m1; and *IFI44L*: Hs00199115\_m1), the relative abundance of each target transcript normalized to the expression level of *HPRT1* (Hs03929096\_g1), and assessed with the Applied Biosystems StepOne Software v2.1. Values were normalized to the average of controls within each experiment.

#### Western blot

Dry cell pellets were resuspended in 10 mM Tris HCl pH 7.4, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 100 mM NaF, 4 mM  $\text{Na}_4\text{P}_2\text{O}_7$ , 2 mM  $\text{Na}_3\text{VO}_4$ , 1% Triton X100, 0.5% NP-40 IGEPAL, and protease inhibitors. Cells lysis was performed for 5 min on ice and the lysate was cleared by centrifugation. An equivalent amount of proteins was loaded in polyacrylamide gel either according to the number of harvested cells or by dosing protein concentration with Qubit Protein Assay Kit (Thermo Fisher

Scientific). Lithium dodecyl sulfate (LDS) sample buffer was added to lysates (Nupage LDS Sample Buffer 4X; NP0007; Thermo Fisher Scientific) and protein migration was performed in 25 mM Tris pH 8.3, 192 mM glycine, and 0.1% SDS with MINIPROTEAN SFX, 4–20% gels (Bio-Rad) at 50 V for 3 h in a MiniPROTEAN Tetra Vertical Electrophoresis Cell. Protein transfer was performed at 35 V overnight on a polyvinylidene fluoride membrane in 25 mM Tris pH 8.3 and 192 mM glycine. Transfers on membrane were verified by using a Ponceau staining. Membranes were incubated in Tris-buffered saline (TBS) 0.1% Tween-20 and 5% milk for 1 h, and then with the primary antibodies. Membranes were washed 3 times in TBS 0.1% Tween-20 after primary and secondary antibodies. All the antibodies were diluted in TBS 0.1% Tween and 5% milk at the concentration indicated in Table S5. Protein revelation was performed using Chemidoc XRS+ (Bio-Rad) after incubation with Clarity Western ECL (Bio-Rad).

#### IFN score (IS)

Blood was collected into PAXgene tubes (PreAnalytix) and, after being kept at room temperature for between 1 and 72 h, frozen at  $-20^\circ\text{C}$  until extraction. Total RNA was extracted from whole blood using a PAXgene (PreAnalytix) RNA isolation kit. RNA concentration was assessed using a spectrophotometer (FLUOstar Omega; Labtech). RT-qPCR analysis was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems) and cDNA derived from 40 ng total RNA. Using TaqMan probes for *IFI27* (Hs01086370\_m1), *IFI44L* (Hs00199115\_m1), *IFIT1* (Hs00356631\_g1), *ISG15* (Hs00192713\_m1), *RSAD2* (Hs01057264\_m1), and *SIGLEC1* (Hs00988063\_m1), the relative abundance of each target transcript was normalized to the expression level of *HPRT1* (Hs03929096\_g1) and *I8S* (Hs99999001\_sl) and assessed with the Applied Biosystems StepOne Software v2.1 and DataAssist Software v.3.01. For each of the six probes, individual (patient and control) data were expressed relative to a single calibrator (control C25). The median fold-change of the six ISGs, when compared with the median of previously collected 29 healthy controls, was used to create an IS for each individual. Relative quantification is equal to  $2^{-\Delta\Delta Ct}$  (i.e., the normalized fold-change relative to the control data). In this way, we define an abnormal IS as being greater than +2 SDs above the mean of this control group (i.e., 2.466).

#### Single molecule array (Simoa)

The Simoa IFN $\alpha$  assay was developed using a Quanterix Homebrew Simoa assay according to the manufacturer's instructions and using two autoantibodies specific for IFN $\alpha$  isolated and cloned from two APS1/APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy) patients (Rodero et al., 2017a). The 12H5 antibody clone was used as a capture antibody after coating on paramagnetic beads (0.3 mg/ml), and the 8H1 was biotinylated (biotin-to-antibody ratio = 30:1) and used as the detector.

#### Fluorescent microscopy and micronuclei counting

Cells were seeded on coverslips and allowed to grow. Cells were then fixed with 4% paraformaldehyde for 20 min at room temperature, then washed three times with PBS and mounted on



slides with Prolong Gold DAPI (Thermo Fisher Scientific, Molecular Probes) or Fluoromount-G with DAPI (Invitrogen). For cGAS-positive micronuclei visualization, cells were first transduced 24 h with GFP-cGAS lentivector before seeding on coverslips and were allowed to grow for 4 d.

For Fig. 5, B and C, and Fig. S5, A and B, cells were visualized with an upright widefield Leica microscope using a 63× oil objective mounted with a sCMOS Hamamatsu Orca Flash 4.0 camera (pixel size 6.5 μm). Stacks of images were acquired with Metamorph for DAPI or GFP fluorescence. Nuclei and micronuclei per nuclei were manually counted for each image. To identify cGAS-positive micronuclei, and exclude micronuclei located in a cGAS-positive area of the cytoplasm, we defined cGAS-positive micronuclei as  $cGAS_{i_{max}}/cGAS_{i_{min}} > 2$ , where  $cGAS_{i_{max}}$  is maximal cGAS intensity,  $cGAS_{i_{min}}$  is minimal cGAS intensity, and measures are performed by automated quantification in a mask comprising each micronucleus and its surrounding cytoplasmic corona.

In Fig. 5, D-F, and Fig. S5, C-E, cells were visualized with a Leica SP8 scanning head on a DMI8 inverted confocal microscope. Images were acquired using a 20× dry objective (numerical aperture = 0.75) with excitation at 405 (for DAPI) and 488 (for GFP) lasers, and fluorescence signals were collected on hybrid detectors. Stacks of images were acquired with Leica LAS X software. Nuclei, micronuclei, and cGAS-positive micronuclei were detected and quantified for each image with an automated in-house macro in Fiji. In details, micronuclei detection was manually corrected to remove false-positive micronuclei. The frequency of cells with at least one micronucleus was calculated for each experiment. GFP-cGAS-positive micronuclei were identified using a global threshold of intensity above cytoplasmic levels of GFP-cGAS. To account for the different rate of GFP-cGAS transduction between samples within an experiment, the frequency of cells with GFP-cGAS micronuclei was determined either by gating in GFP-cGAS-positive cells (Fig. 5, D and F) or by normalizing to the percentage of transduced cells (Fig. 5 E), based on a threshold of GFP-cGAS intensity in the nucleus.

The number of nuclei analyzed for each experiment is reported in Table S6. All images were analyzed in Fiji.

### Whole-exome sequencing

DNA was extracted from whole-blood samples using an in-house salting-out method. Agilent SureSelect libraries were prepared from 3 μg of genomic DNA extracted from leukocytes and sheared with a Covaris S2 Ultrasonicator as recommended by the manufacturer. We used a 51-Mb SureSelect Human All Exon kit V5 (Agilent Technologies) for exome capture, with multiplexed molecular barcodes for sample traceability. Pooled bar-coded exome libraries were then sequenced on a HiSeq2500 (Illumina) machine, generating 76- + 76-bp paired-end reads, followed by processing using the Genome Analysis Toolkit (GATK), SAMtools, and Picard. Calls were made with the Polyweb application (<https://www.polyweb.fr>).

### Sanger sequencing

Sanger sequencing was performed to confirm the Bloom variants found by exome sequencing.

For P1, Sanger confirmation of the mutation was performed on RNA extracted from whole blood. Whole blood was collected into PAXgene tubes. We used a PreAnalytix RNA isolation kit and RNA concentration, assessed with a spectrophotometer (FLUOstar Omega; Labtech), to extract total RNA. cDNA was generated using Qiagen OneStep RT-PCR kit. The region of the gene was amplified by PCR using specific primers (eurofinsgenomics) designed with Primer3plus (Blm cDNA 2F, 5'-CTC CTGGGGTCACTGTTGTC-3'; Blm cDNA 2R, 5'-AGCCATCACCG AACAGAAG-3').

### SCEs

The assay was performed according to the method described (Gemble et al., 2015). SCE frequency in HeLa cells was previously described (Lahkim Bennani-Belhaj et al., 2010).

### Statistics

Unless indicated, data are represented by the mean with SD bars. Statistical tests are indicated in the legends of each figure.

### Online supplemental material

Fig. S1 shows the ISG signature in HeLa cells treated with BLM siRNA. Fig. S2 shows the characterization of the BLM mutations of patients AGS721 and AGS1513. Fig. S3 shows that depletion of BLM induces ISGs in an independent source of primary human fibroblasts. Fig. S4 shows the expression of additional genes related to Fig. 3. Fig. S5 shows additional quantifications related to Fig. 5. Table S1 shows the mutations and clinical features of patients in the study. Tables S2, S3, S4, and S5 describe the plasmids, siRNAs, primers, and antibodies used in the study, respectively. Table S6 shows the number of primary nuclei analyzed in imaging experiments.

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Author contributions: M. Gratia performed most experiments. C. Conrad and E. Bou Samra performed initial experiments. Y.J. Crow, M. Amor-Gueret, and N. Manel designed and piloted the study. M.P. Rodero contributed to experiments with BS cells. M. Maurin designed image analysis methods and wrote scripts. P. Revy characterized the BLM mutation from one patient. D. Duffy performed Simoa IFN measurements. G.I. Rice performed measured ISG expression in patient samples. R.C. Dale and F. Petit provided patient samples.

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