Kimura et al., http://www.jcb.org/cgi/content/full/jcb.201503023/DC1

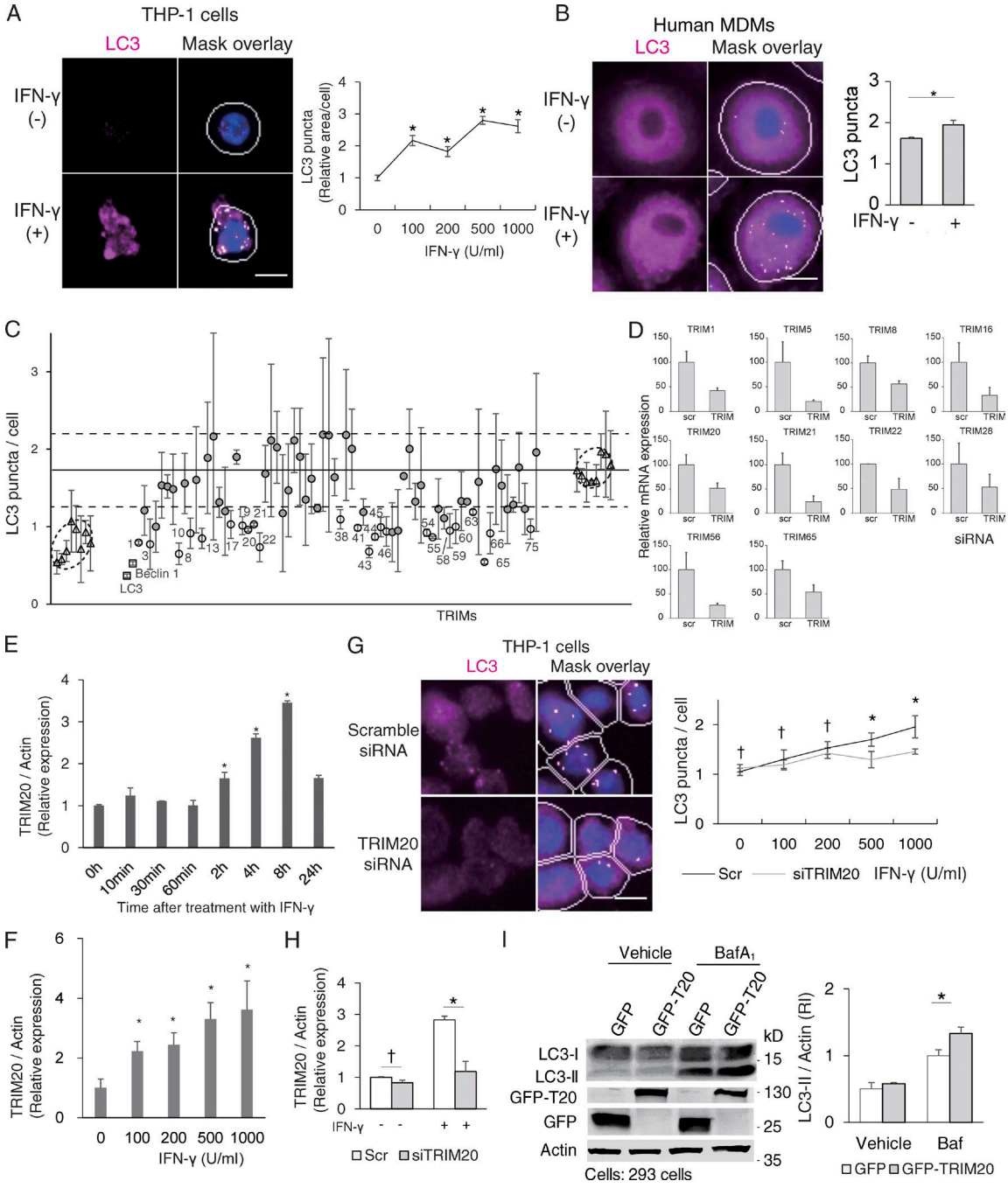

Figure S1. **TRIMs regulate IFN-γ–induced autophagy.** (A and B) HC image analysis of LC3 puncta in THP-1 cells (A) or human MDM cells (B) treated with IFN-γ for 4 h. HC and mask overlays are as in Fig. 1. White outlined profiles are program-assigned masks (large outlines are primary objects/cells; smaller masks are outlines of secondary objects/LC3+ autophagic profiles). (C) Screen data from Fig. 1 B showing average ± range. (D) Knockdown efficacy of TRIMs were determined by RT-PCR. (E and F) THP-1 cells were treated with escalating doses of IFN-γ for 4 h (E) or 1,000 U/ml of IFN-γ for indicated times (F), and TRIM20 mRNA levels were determined by quantitative RT-PCR. Values are standardized to no IFN-γ control (E) or 0 h time point (F). (G) THP-1 cells were subjected to TRIM20 or scrambled siRNA, treated with IFN-γ for 4 h, and HC analysis performed. (H) Knockdown of TRIM20 mRNA levels was examined by quantitative RT-PCR. Values are standardized to control (Scr, scrambled; no IFN-γ). (I) LC3-II conversion in HEK293 cells transfected with GFP-TRIM20 (T20) or GFP. Data, means ± SE; $n \geq 3$ experiments, except in C. Bars, 5 µm. *, $P < 0.05$; †, $P \geq 0.05$ (*t* test in B or ANOVA in other panels).

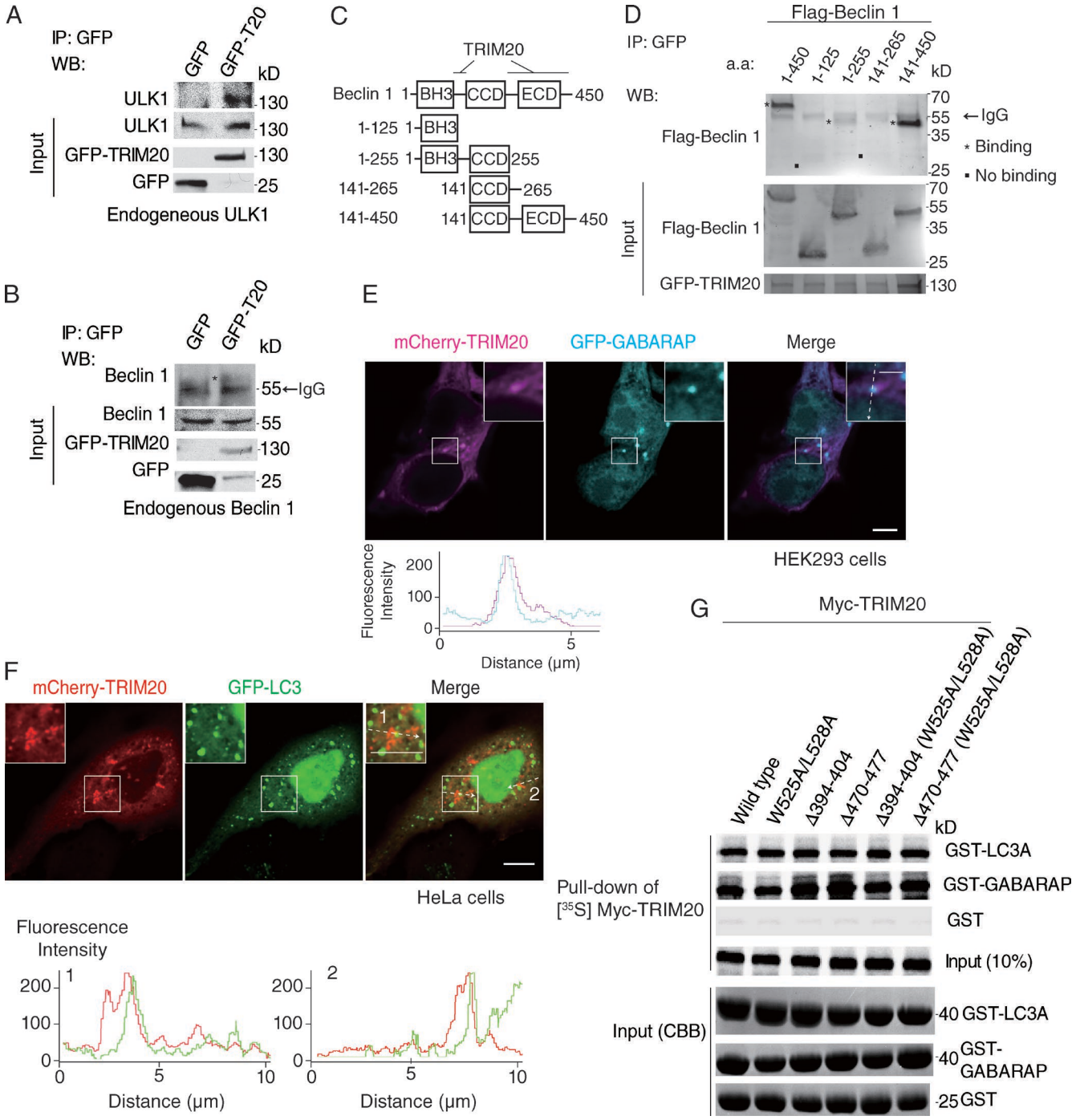

Figure S2. **TRIM20 interacts with ULK1, Beclin 1, and mAtg8s.** (A and B) Coimmunoprecipitation analysis of GFP-TRIM20 with endogenous ULK1 (A) or Beclin 1 (B) in HEK293 cells extracts. (C) Beclin 1 domains and deletion constructs used. (D) Coimmunoprecipitation analysis of interactions between deletion variants of Flag-Beclin 1 (asterisks and squares in the top blot denote presence or absence of Flag-Beclin 1, respectively) and GFP-TRIM20 in HEK293 cells. (E) Confocal microscopy of HEK293 cells coexpressing mCherry-TRIM20 with GFP-GABARAP. Line tracings correspond to dashed line. (F) Confocal microscopy of HeLa cells coexpressing mCherry-TRIM20 with GFP-LC3B in the presence of bafilomycin A1. Line tracings correspond to numbered dashed lines. (G) GST pull-down analysis of interaction between radiolabeled Myc-TRIM20 harboring single or double mutants (corresponding to Fig. 4 D) and GST-GABARAP. Data representative of three independent experiments. IP, immunoprecipitation; WB, Western blot. Bars: (E, main images) 5 μm; (E, insets) 2 μm; (F) 10 μm.

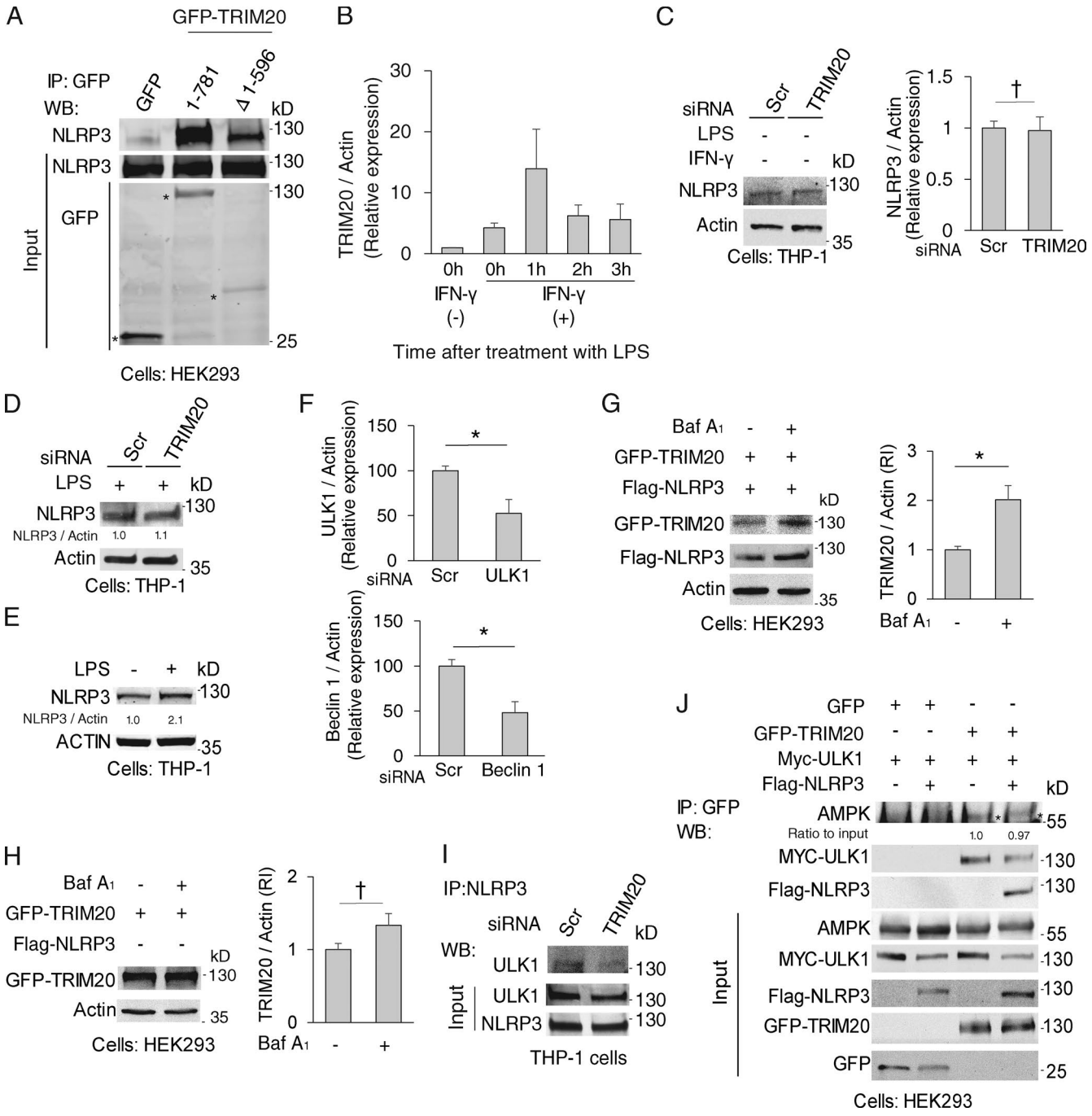

Figure S3. **TRIM20 degrades NLRP3 through autophagy.** (A) Coimmunoprecipitation analysis of deletion variants of TRIM20 (as GFP fusions; asterisks denote fusion products on the bottom blot) with NLRP3 in HEK293 cells. (B) THP-1 cells were treated with IFN-γ for 3 h and additionally treated with 2.5 µg/ml of LPS for indicated periods, and TRIM20 mRNA levels were determined by quantitative PCR. Values are standardized to IFN-γ–untreated control. (C and D) Levels of NLPR3 in lysates from THP-1 cells untreated with IFN-γ and subjected to TRIM 20 or scrambled (Scr) siRNA, untreated or treated with LPS as indicated. (E) THP-1 cells were treated with 1.0 µg/ml of LPS for 3 h, and levels of NLRP3 in lysate were determined by immunoblots. (F) Knockdown efficacies of ULK1 and Beclin 1 by siRNA were examined by quantitative RT-PCR. (G and H) Levels of GFP-TRIM20 were determined in cells coexpressing (G) or not (H) NLRP3 after autophagy induction (EBSS, 3 h) in the presence or absence of bafilomycin A1 (Baf A_1). RI, relative intensity. (I) Coimmunoprecipitation analysis of ULK1 in NLRP3 protein complexes in the presence and absence of TRIM20 knockdown. Lysates from THP-1 cells subjected to each knockdown and treatment of 200 U/ml IFN-γ for 3 h with additional LPS (1.0 µg/ml) treatment for 2 h followed by immunoprecipitation with anti-NLRP3. Immunoblots were probed as indicated. (J) Coimmunoprecipitation analysis of AMPK in GFP-TRIM20 complexes in HEK293T cells expressing Myc-ULK1 and Flag-NLRP3 (or not). Data, means ± SE; $n \geq 3$ experiments; *, $P < 0.05$; †, $P \geq 0.05$ (C, *t* test; D, ANOVA). IP, immunoprecipitation; WB, Western blot.

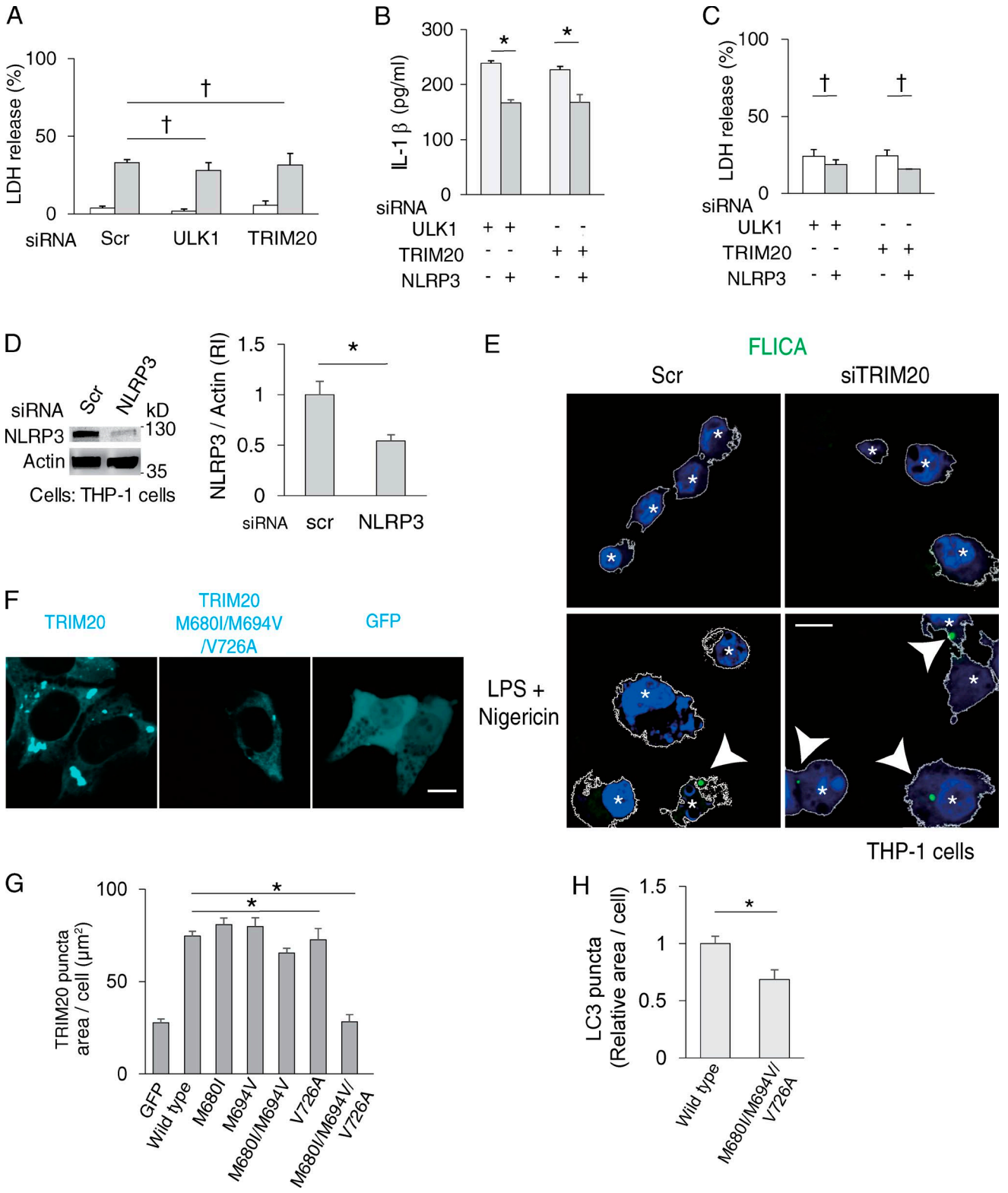

Figure S4. **Effects of TRIM20 on inflammasome activity and FMF-associated variants of TRIM20 decrease number of TRIM20 and LC3 puncta.** (A) LDH release of supernatants analyzed in Fig. 6 D. (B and C) Supernatants were harvested from THP-1 cells that had been subjected to double knockdowns as indicated, treated with IFN-γ and LPS and additionally stimulated with nigericin (20 μM) for 30 min. IL-1β and LDH release was measured. (D) Knockdown efficacy of NLRP3 by siRNA was examined by immunoblotting. RI, relative intensity. (E) Confocal microscopy of THP-1 cells that had been subjected to knockdown of TRIM20, treated with IFN-γ, and then treated with or without LPS (2 h) and nigericin (10 min) and stained for active caspase-1 (FLICA) and nucleus (TO-PRO-3). Arrowheads, FLICA-positive puncta; asterisks, cell nuclei; white outline, cell boundary. (F) Confocal microscopy images of GFP-TRIM20 (wild-type or FMF-associated variants) or GFP in HEK293 cells. (G) HC image (epifluorescence) analysis of TRIM20 puncta in HeLa cells expressing GFP-TRIM20 (wild-type or FMF-associated variants) or GFP. (H) HC image analysis of LC3 puncta in HeLa cells expressing GFP-TRIM20 (wild-type or triple mutant TRIM20). Data, means ± SE; $n \geq 3$ experiments; *, $P < 0.05$; †, $P \geq 0.05$ (*t* test or ANOVA). Scr, scrambled. Bars, 5 μm.

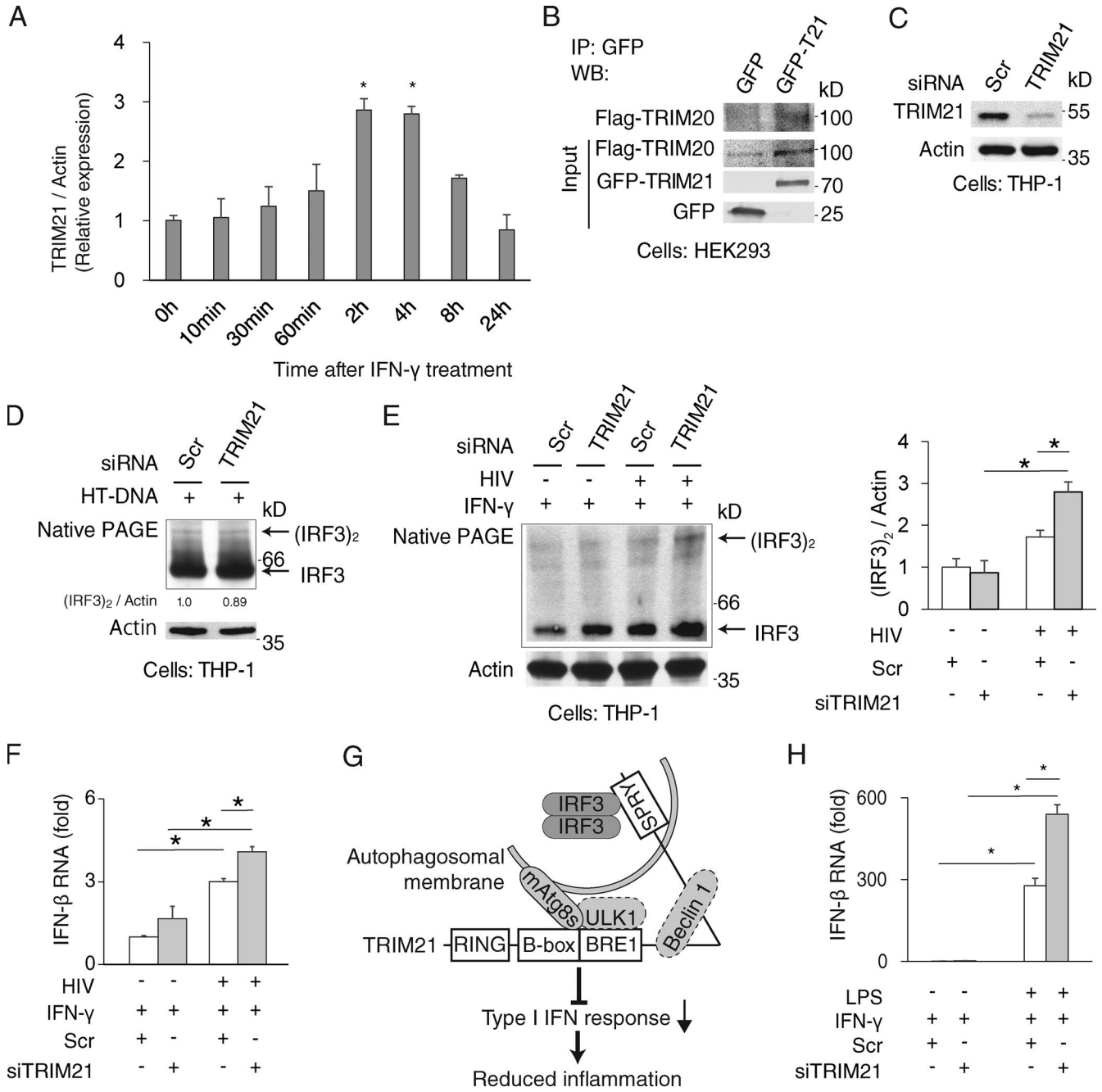

Figure S5. **TRIM21 affects the level of dimerized IRF3 in HIV1 infection.** (A) THP-1 cells were treated with 1,000 U/ml of IFN-γ for indicated times, and TRIM21 mRNA levels were determined by quantitative RT-PCR. (B) Coimmunoprecipitation analysis of GFP-TRIM20 with Flag-TRIM21 in HEK293 cells extracts. (C) Knockdown efficacy of TRIM21 level was examined by immunoblotting. (D) Levels of dimerized IRF3 were assessed by native PAGE from THP-1 cells subjected to TRIM21 or control knockdown, untreated with IFN-γ, and transfected with herring testis DNA (HT-DNA). (E and F) THP-1 cells subjected to TRIM21 or control knockdown were infected with a single-round infection HIV1 virus in the presence of 200 U/ml IFN-γ for 20 h, and the levels of dimerized IRF3 (E) or mRNA levels of IFN-β (F) were determined. (G) Model of TRIM21's dual function in autophagy as a regulator-receptor: TRIM21 assembles autophagy machinery (ULK1, Beclin 1, and mAtg8s) and recognizes substrates (dimerized IRF3) delivering them for autophagic degradation to suppress type I IFN response and inflammation. Dashed outlines (ULK1 and Beclin 1), domain binding location not mapped; solid outline for mAtg8 (GABARAP) reflects mapping data. (H) The effect of TRIM21 knockdown on IFN-β mRNA levels after stimulation of THP-1 cells with 1,000 U/ml IFN-γ for 3 h and then with 2.5 μg/ml LPS for 2 h. Data, means ± SE; $n \geq 3$ experiments; *, $P < 0.05$ (ANOVA).