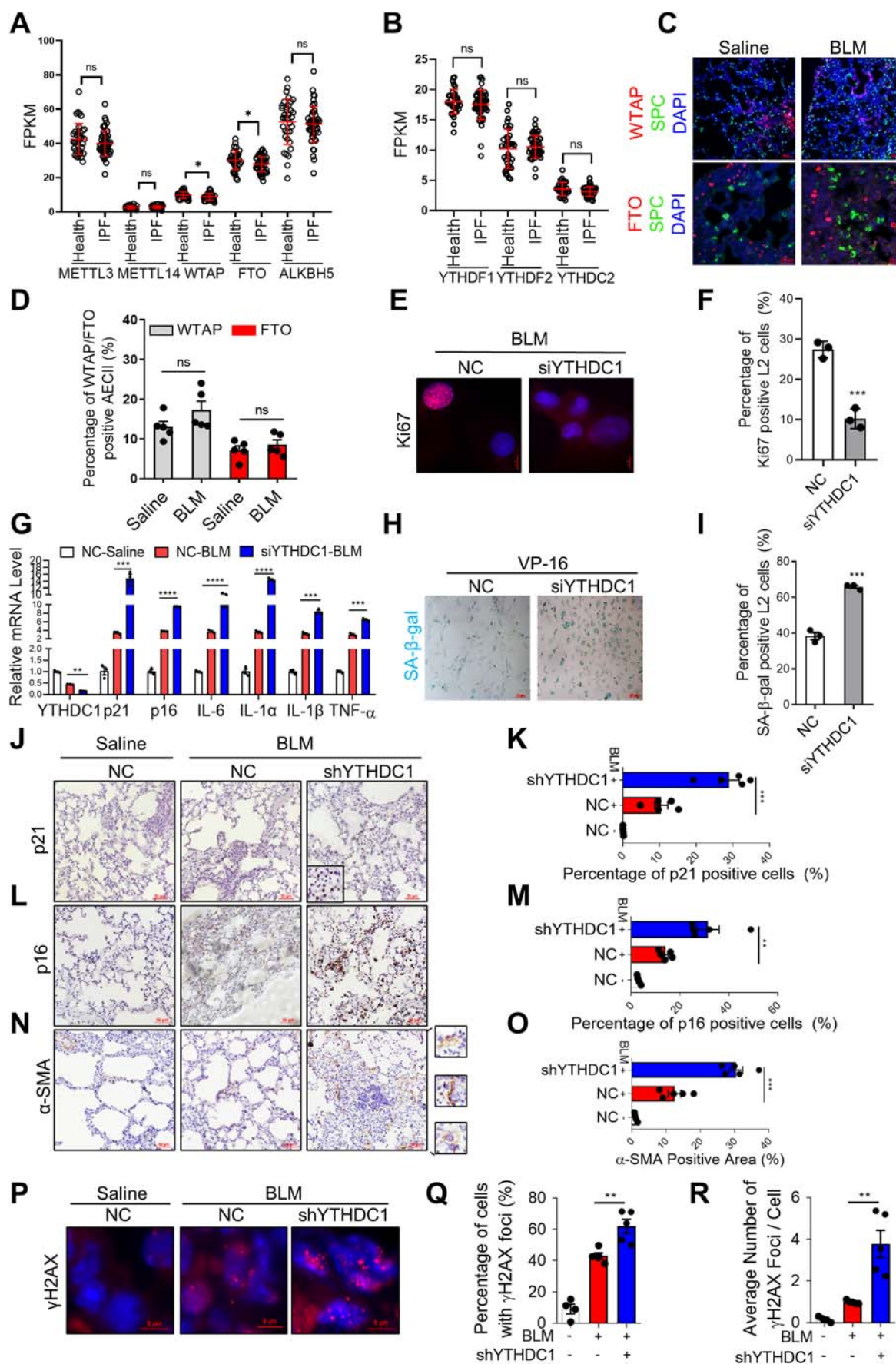
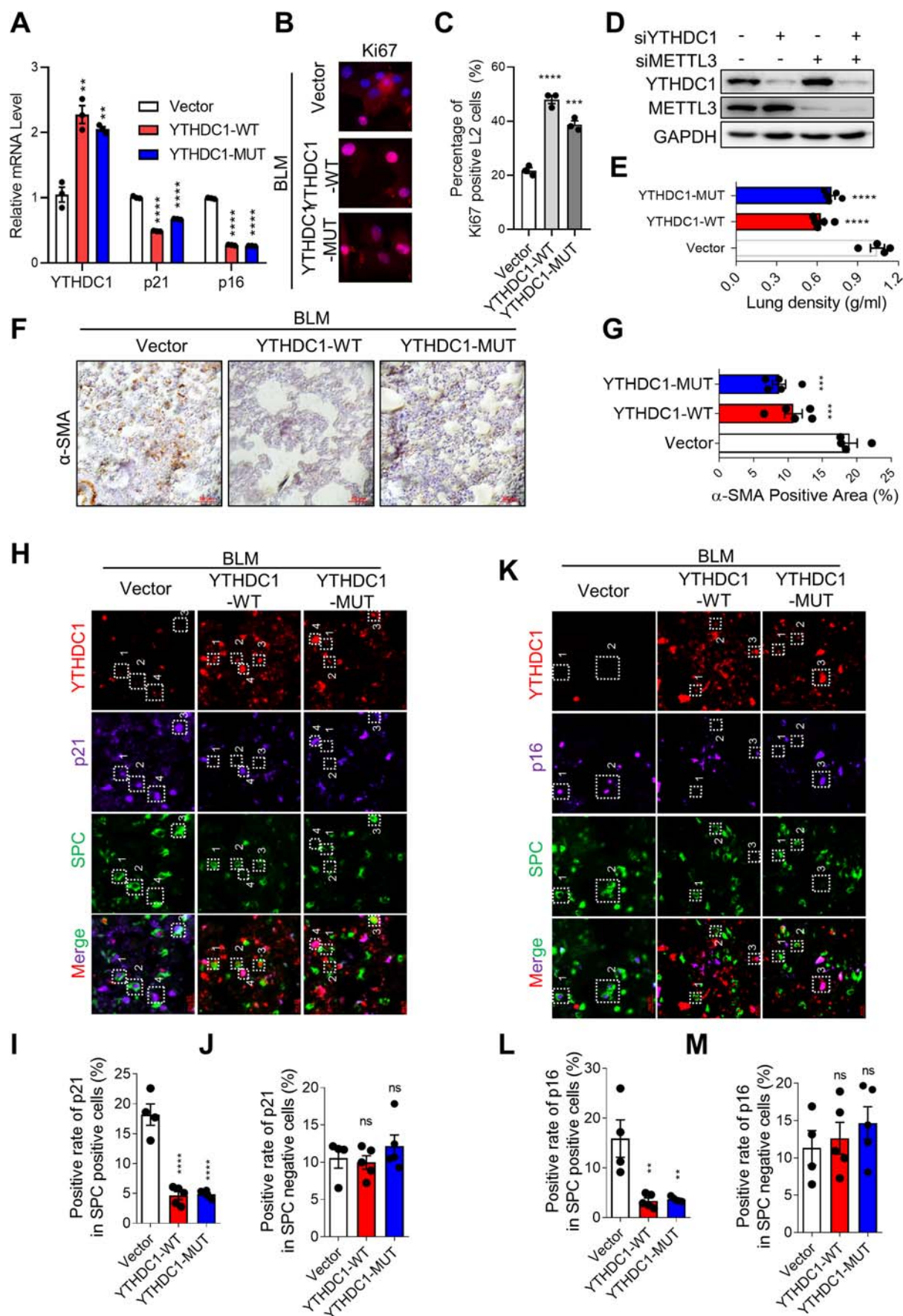


Expanded View Figures

Figure EV1. Knockdown of YTHDC1 accelerates stress-induced pulmonary epithelial cell senescence.

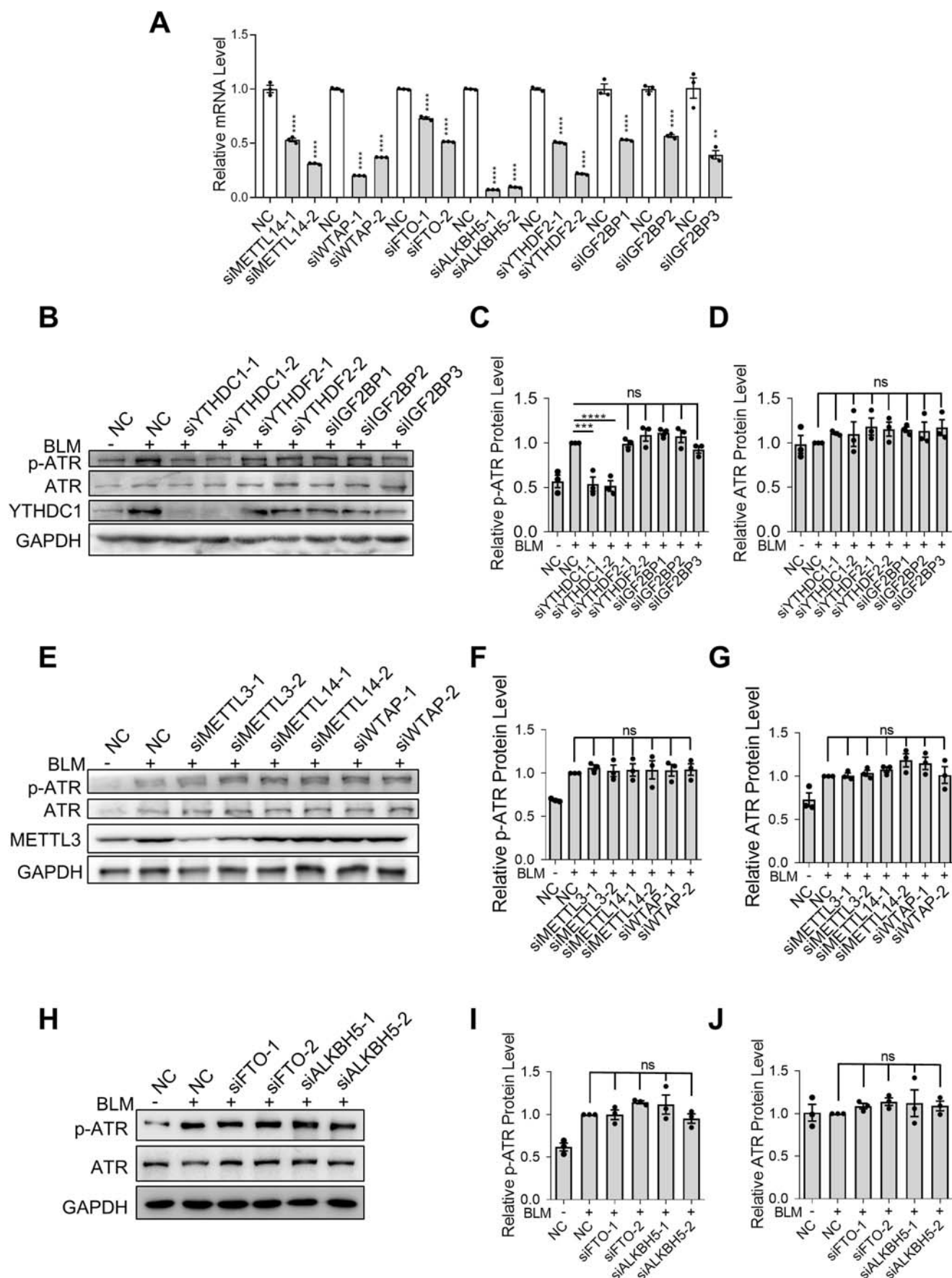
(A) METTL3, METTL14, WTAP, FTO and ALKBH5 expression levels in normal (35 people) and IPF (49 people) human lung tissues were determined using published datasets (Data ref: Ahangari et al, 2019). (B) YTHDF1, YTHDF2 and YTHDC2 expression levels in normal (35 people) and IPF (49 people) human lung tissues were determined using published datasets (Data ref: Ahangari et al, 2019). (C) Immunofluorescence was performed to determine the localization of WTAP/FTO (red) and SPC (green) in mice lungs that treated with saline or BLM for 7 days. Scale bar: 100 μ m. (D) Quantification of panel C. The percentage of double-positive cells in WTAP/FTO-positive cells was calculated ($n = 5$ per group). (E) IF detection of Ki67 in control or YTHDC1-depleted L2 cells. Cells were treated with BLM for 4 days. Scale bar: 10 μ m. (F) Quantification of E. The percentage of Ki67 positive cells was calculated ($n \geq 100$ cells $\times$ three repeats). (G) YTHDC1, p21, p16 and SASP factors were detected by RT-qPCR using L2 cells transfected with NC or siYTHDC1. Forty-eight hours after transfection, cells were treated with BLM or saline for 4 days. (H) SA- β -gal staining of L2 cells transfected with NC or siYTHDC1. Forty-eight hours after transfection, cells were treated with VP-16 for 1 days and released to 7 days and subjected to SA- β -gal staining. Scale bars: 100 μ m. (I) Quantification of H. The percentage of SA- β -gal positive cells was calculated ($n \geq 100$ cells $\times$ three repeats). (J) Representative images of p21 in the mice lungs ($n \geq 4$ per group). C57/BL6 mice transfected with indicated AAV vectors were treated with BLM for 7 days ($n \geq 4$ per group). Inset shows positive signals at high magnification. from the mice lungs. Scale bar: 50 μ m. (K) Quantification of panel J. The percentage of p21 positive cells was calculated. (L, M), as in panels J,K, except using p16 antibody to perform the IHC. ($n \geq 4$ per group). Scale bar: 50 μ m. (N,O) as in panels J,K, except using α -SMA antibody to perform the IHC. ($n \geq 4$ per group). Scale bar: 50 μ m. (P) Immunofluorescence (IF) detection of γ H2AX foci in the mice lung from panel J. ($n \geq 4$ per group). Scale bar: 5 μ m. (Q) Quantification of panel P. The percentage of cells with γ H2AX foci was calculated. (R) Quantification of panel P. The average number of γ H2AX foci per cell. Data information: All values are mean $\pm$ SEM. The unpaired Student's two-tailed t -test was used to determine the statistical significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). $n =$ number of biological replicates. Source data are available online for this figure.





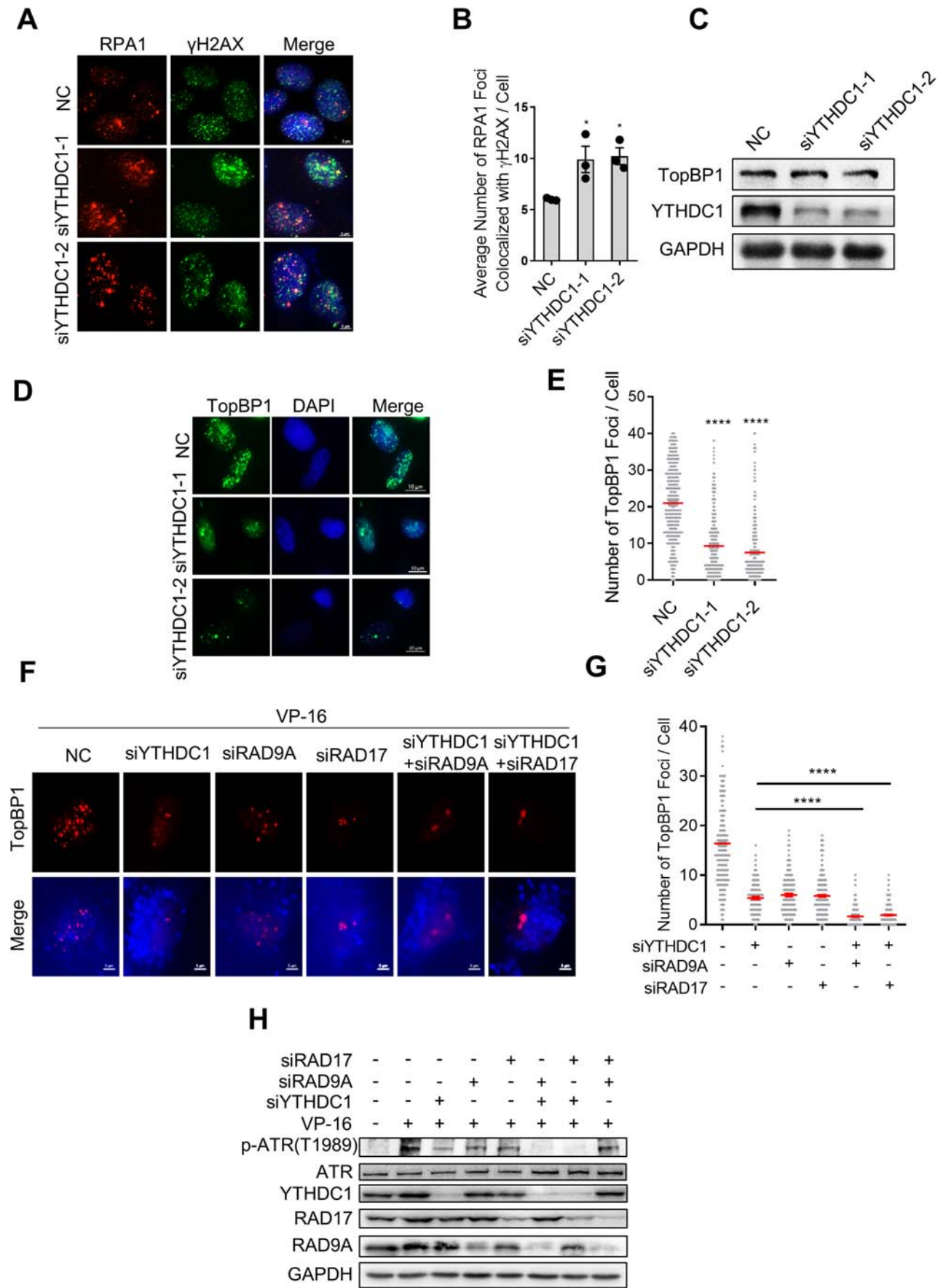
◀ **Figure EV2. YTHDC1 counteracts stress-induced pulmonary epithelial cell senescence independent of its m6A binding activity.**

(A) RT-qPCR analysis of YTHDC1, p21 and p16 mRNA level in the L2 cells overexpressed with Vector, YTHDC1-WT or YTHDC1-MUT. Forty-eight hours after transfection, cells were treated with BLM for 4 days and subjected to RT-qPCR. ($n = 3$). (B) IF detection of Ki67 in L2 cells overexpressed with Vector, YTHDC1-WT or YTHDC1-MUT. Cells were treated with BLM for 4 days. Scale bar: 10 μm . (C) Quantification of B. The percentage of Ki67 positive cells was calculated ($n \geq 100$ cells $\times$ three repeats). (D) Immunoblot analysis of YTHDC1 and METTL3 in L2 cells transfected with indicated siRNAs. Forty-eight hours after transfection, cells were treated with BLM for 4 days prior to analysis. ($n = 3$). (E) Quantification of mice lung density. C57/BL6 mice transfected with indicated AAV vectors were treated with BLM for 7 days ($n \geq 4$ per group). (F) Representative images of α -SMA IHC in the mice lungs from panel E. ($n \geq 4$ per group). Scale bar: 50 μm . (G) Quantification of panel F. The percentage of α -SMA positive cells was calculated. (H) Immunofluorescence was performed to determine the localization of YTHDC1 (red), p21 (purple) and SPC (green) in mice lungs from panel E. ($n \geq 4$ per group). Scale bar: 20 μm . (I) Quantification of panel H. The percentage of p21 positive cells in SPC positive cells was calculated ($n \geq 4$ per group). (J) Quantification of panel H. The percentage of p21 positive cells in SPC negative cells was calculated ($n \geq 4$ per group). (K-M) as in panels H-J except using p16 antibody to perform the IF. ($n \geq 4$ per group). Scale bar: 20 μm . Data information: All values are mean $\pm$ SEM. The One-way ANOVA was used to determine the statistical significance (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). n = number of biological replicates except. Source data are available online for this figure.



◀ **Figure EV3. YTHDC1 regulates the activation of ATR.**

(A) RT-qPCR analysis of METTL14, WTAP, FTO, ALKBH5, YTHDF2, IGF2BP1, IGF2BP2 and IGF2BP3 mRNA level in A549 cells transfected with indicated siRNAs. ($n = 3$). (B) Immunoblot analysis of activated ATR, total ATR and YTHDC1 in A549 cells transfected with indicated siRNAs. A549 cells were treated with BLM or saline for 4 h prior to analysis. (C,D), Quantification of panel B. The relative protein p-ATR or ATR levels were determined by normalizing the intensities of p-ATR or ATR to the intensity of GAPDH. ($n = 3$). (E) Immunoblot analysis of activated ATR, total ATR and METTL3 in A549 cells transfected with indicated siRNAs. A549 cells were treated with BLM or saline for 4 h prior to analysis. (F,G) Quantification of panel E. The relative protein p-ATR or ATR levels were determined by normalizing the intensities of p-ATR or ATR to the intensity of GAPDH. ($n = 3$). (H) Immunoblot analysis of activated ATR and total ATR in A549 cells transfected with indicated siRNAs. A549 cells were treated with BLM or saline for 4 h prior to analysis. (I,J), Quantification of panel H. The relative protein p-ATR or ATR levels were determined by normalizing the intensity of p-ATR or ATR to the intensities of GAPDH. ($n = 3$). Data information: All values are mean $\pm$ SEM. The One-way ANOVA was used to determine the statistical significance (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). n = number of biological replicates. Source data are available online for this figure.



◀ **Figure EV4. YTHDC1 regulates the recruitment of TopBP1 to activate ATR independent of 9-1-1 complex.**

(A) IF detection of RPA1 and γ H2AX foci in control or YTHDC1-depleted A549 cells. Cells were treated with VP-16 for 24 h. Scale bar: 5 μ m. (B) Quantification of A. The average number of RPA1 foci colocalized with γ H2AX foci per cell ($n \geq 100$ cells $\times$ three repeats). (C) Immunoblot analysis of TopBP1 and YTHDC1 in A549 cells transfected with NC, siYTHDC1-1 or siYTHDC1-2. A549 cells were treated with VP-16 for 24 h prior to analysis ($n = 3$). (D) Immunofluorescence (IF) detection of TopBP1 foci in control or YTHDC1-depleted A549 cells. Cells were treated with BLM for 4 h before detection. Scale bar: 5 μ m. (E) Quantification of D. The number of TopBP1 foci per cell ($n \geq 100$ cells $\times$ three repeats). (F) IF detection of TopBP1 foci in A549 cells transfected with indicated siRNAs. Cells were treated with VP-16 for 24 h. Scale bar: 5 μ m. (G) Quantification of F. The number of TopBP1 foci per cell ($n \geq 100$ cells $\times$ three repeats). (H) Immunoblot analysis of activated ATR, total ATR, YTHDC1, RAD17, and RAD9A in A549 cells transfected with indicated siRNAs. Cells were treated with VP-16 or DMSO for 24 h prior to analysis. ($n = 3$). Data information: All values are mean $\pm$ SEM. The One-way ANOVA was used to determine the statistical significance (* $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$). n = number of biological replicates. Source data are available online for this figure.

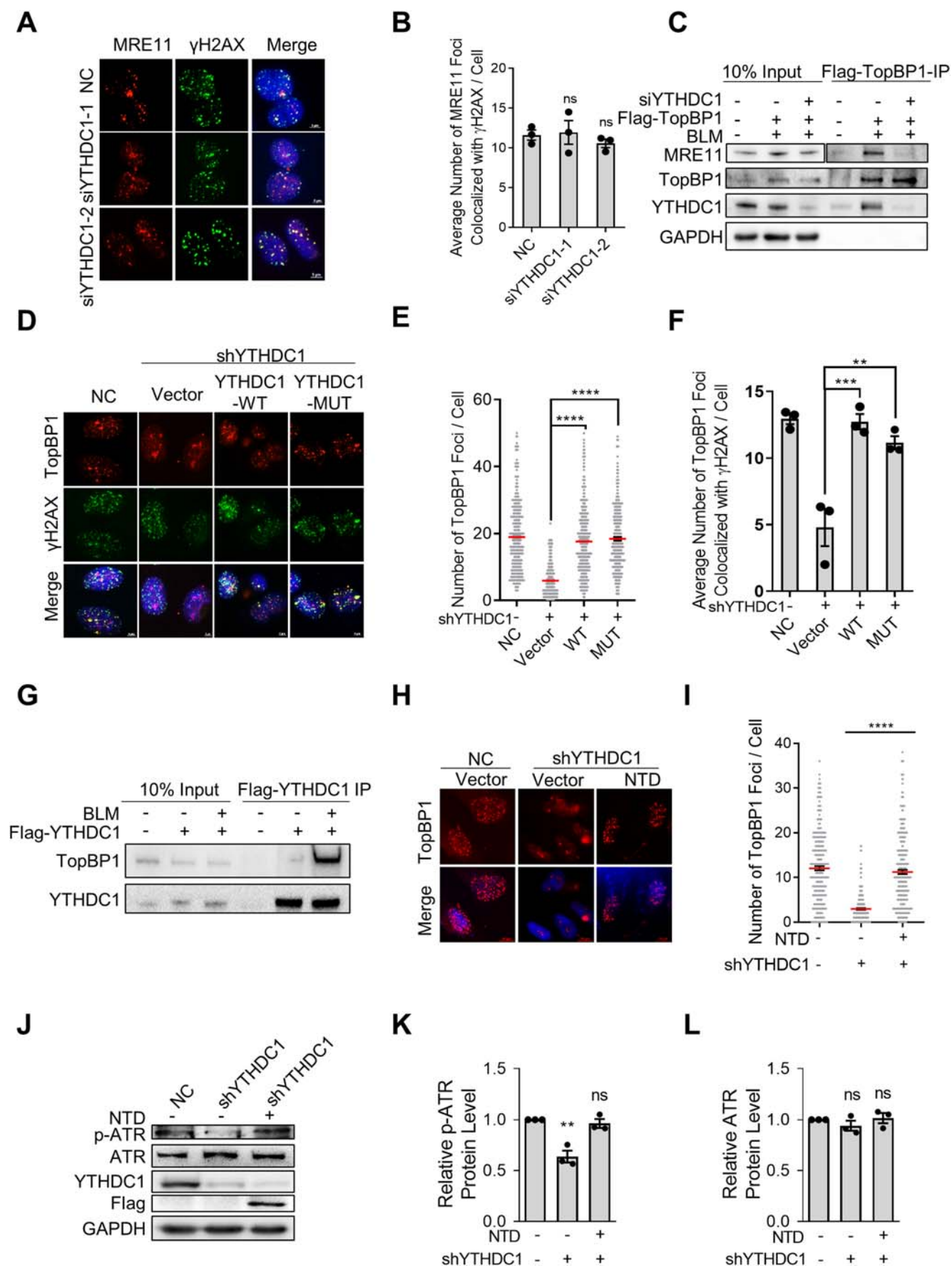


Figure EV5. YTHDC1 regulates the recruitment of TopBP1 independent of its m6A binding activity.

(A) IF detection of MRE11 and γ H2AX foci in control or YTHDC1-depleted A549 cells. Cells were treated with VP-16 for 24 h. Scale bar: 5 μ m. (B) Quantification of A. The average number of MRE11 foci colocalized with γ H2AX foci per cell ($n \geq 100$ cells $\times$ three repeats). (C) Co-IP assay to determine the interaction of TopBP1 with MRE11 and YTHDC1 in HEK293T cells. YTHDC1 depleted cells were transfected with Flag-TopBP1 or Vector and treated with BLM for 4 h. Cell lysates were used for immunoprecipitation with Flag-beads. Immunoprecipitates were immunoblotted with MRE11, TopBP1, YTHDC1 and GAPDH antibody, respectively. ($n = 3$). (D) IF detection of TopBP1 and γ H2AX foci in YTHDC1-depleted A549 cells overexpressed with Vector, YTHDC1-WT or YTHDC1-MUT. Cells were treated with VP-16 for 24 h. Scale bar: 5 μ m. (E) Quantification of D. The number of TopBP1 foci per cell ($n \geq 100$ cells $\times$ three repeats). (F), Quantification of D. The average number of TopBP1 foci colocalized with γ H2AX foci per cell ($n \geq 100$ cells $\times$ three repeats). (G) Co-IP assay to determine the interaction between YTHDC1 and TopBP1. HEK293T cells transfected with Flag-YTHDC1 were treated with BLM for 4 h. Cell lysates were used for immunoprecipitation with Flag-beads. Immunoprecipitates were immunoblotted with TopBP1 and YTHDC1 antibody, respectively. ($n = 3$). (H) IF detection of TopBP1 foci in YTHDC1 defect A549 cells overexpressed Vector or NTD of YTHDC1. Forty-eight hours after transfection, cells were treated with VP-16 for 24 h. Scale bar: 5 μ m. (I) Quantification of H. The number of TopBP1 foci per cell ($n \geq 100$ cells $\times$ three repeats). (J) Immunoblot analysis of activated ATR, total ATR, YTHDC1 and Flag from panel H. (K,L) Quantification of panel J. The relative protein p-ATR or ATR levels were determined by normalizing the intensities of p-ATR or ATR to the intensity of GAPDH. ($n = 3$). Data information: All values are mean $\pm$ SEM. The unpaired Student's two-tailed *t*-test was used to determine the statistical significance between two groups. The One-way ANOVA was used to determine the statistical significance for more than two groups (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). n = number of biological replicates. Source data are available online for this figure.