SUPPLEMENTAL MATERIAL

Shao et al., http://www.jem.org/cgi/content/full/jem.20082251/DC1

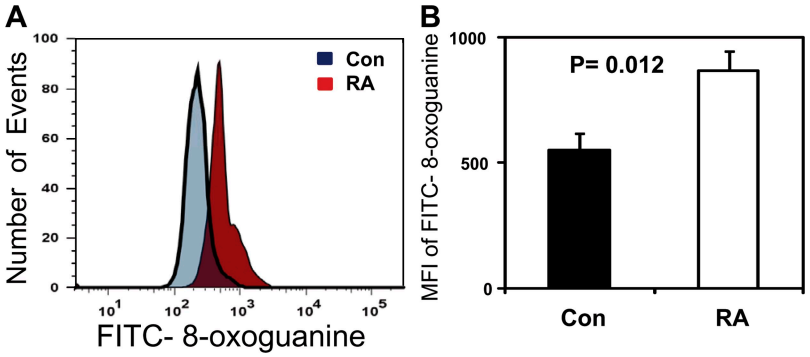


Figure S1. Unprimed CD4 T cells in rheumatoid arthritis accumulate damaged DNA. $CD4^+CD45RO^-$ T cells were isolated from RA patients and controls, and damaged DNA was detected with FITC-conjugated probe binding to the DNA adduct 8-oxoguanine by flow cytometry (OxyDNA Assay kit; EMD). (A) Representative histogram of a flow cytometric analysis of 8-oxoguanine in control and RA T cells. (B) Mean fluorescence intensity (MFI) of FITC–8-oxoguanine was determined. Results are shown as means ± SEM. Mean fluorescence intensity of 10 RA patients and 10 controls was analyzed in four independent experiments.

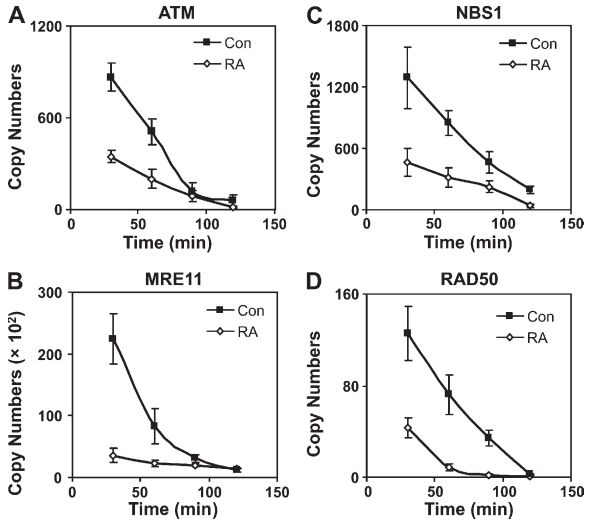
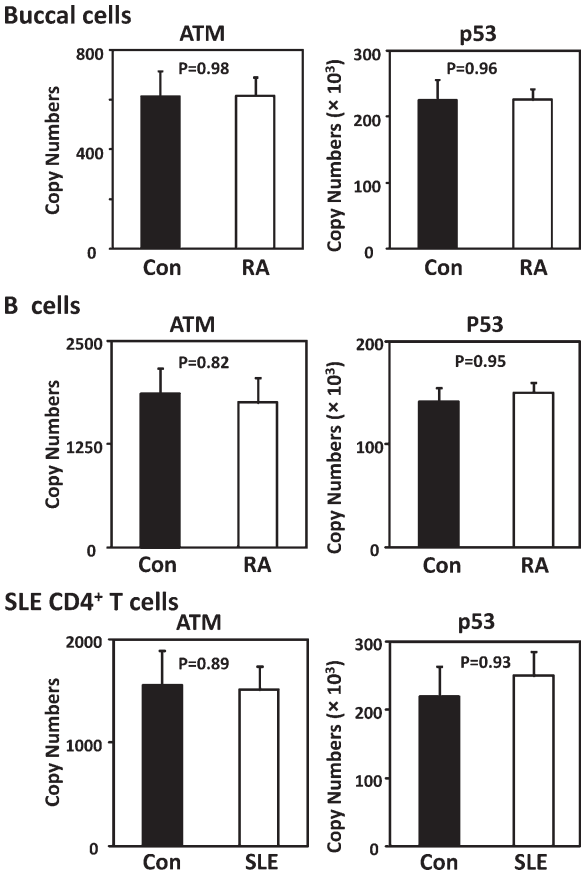


Figure S2. mRNA stability of ATM, and the MRE11 complex is intact in RA T cells. $CD4^+CD45RO^-$ T cells from RA patients and demographically matched controls were treated with 25 µg/ml actinomycin D for 2 h to block mRNA synthesis. Cells were collected, and RNA was isolated at 30, 60, 90, and 120 min after adding actinomycin D. Real-time PCR amplification was performed to quantify ATM (A), MRE11 (B), NBS1 (C), and RAD50 (D) copy numbers from the same amount of total RNA at different time points. Data are presented as means ± SEM from four independent experiments with one RA patient and one control each.



xFigure S3. ATM and p53 expression in RA non–T cells and CD4 T cells from SLE patients is intact. Transcript levels of ATM and p53 were quantified by qPCR and standardized by 18S ribosomal RNA. (A) Mucosal cells were collected by buccal smear from five RA patients and five age-matched controls. (B) B cells were purified with CD19-specific microbeads from the blood of nine RA patients and nine age-matched controls. (C) $CD4^+$ T cells were isolated from 10 patients with active SLE and 10 age-matched controls. Results are given as means ± SEM of patient cells and control cells.

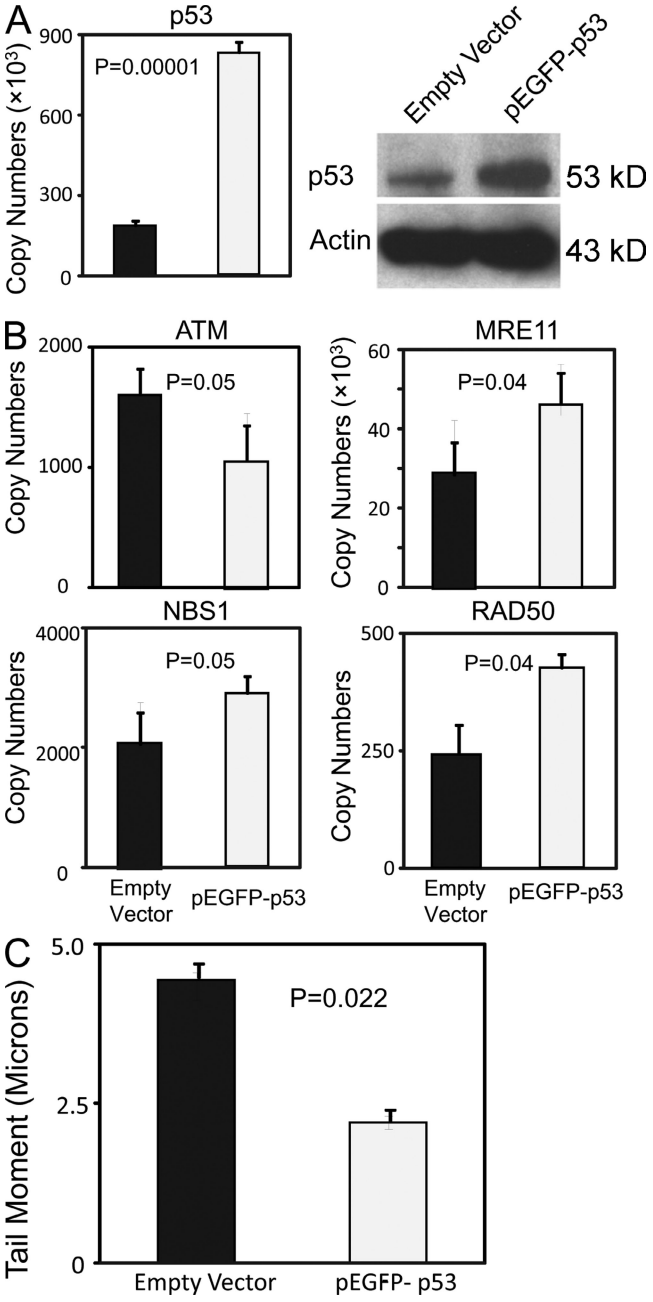


Figure S4. Overexpression of p53 in healthy T cells optimizes DNA repair. $CD4^+CD45RO^-$ T cells from healthy donors were transfected with pEGFP-N1 (empty vector) or pEGFP-p53 by nucleofection. (A) After 24 h, p53 transcript levels were quantified by qPCR. Results are shown as means ± SEM from four experiments with different donors. p53 protein levels were quantified by Western blotting with actin serving as loading control. Results are representative of four independent experiments. (B) Transcript levels of ATM, MRE11, NBS1, and RAD50 were quantified by qPCR. (C) The impact of p53 overexpression on DNA damage repair was determined by comet assay in T cells transfected with empty vector or pEGFP-p53. Mean TMs from >50 cells (yellow fluorescence identifying the coexpression of red fluorescence [ethidium bromide for DNA] and green fluorescence [GFP]) were determined. All results are presented as means ± SEM of four independent experiments with different control individuals.

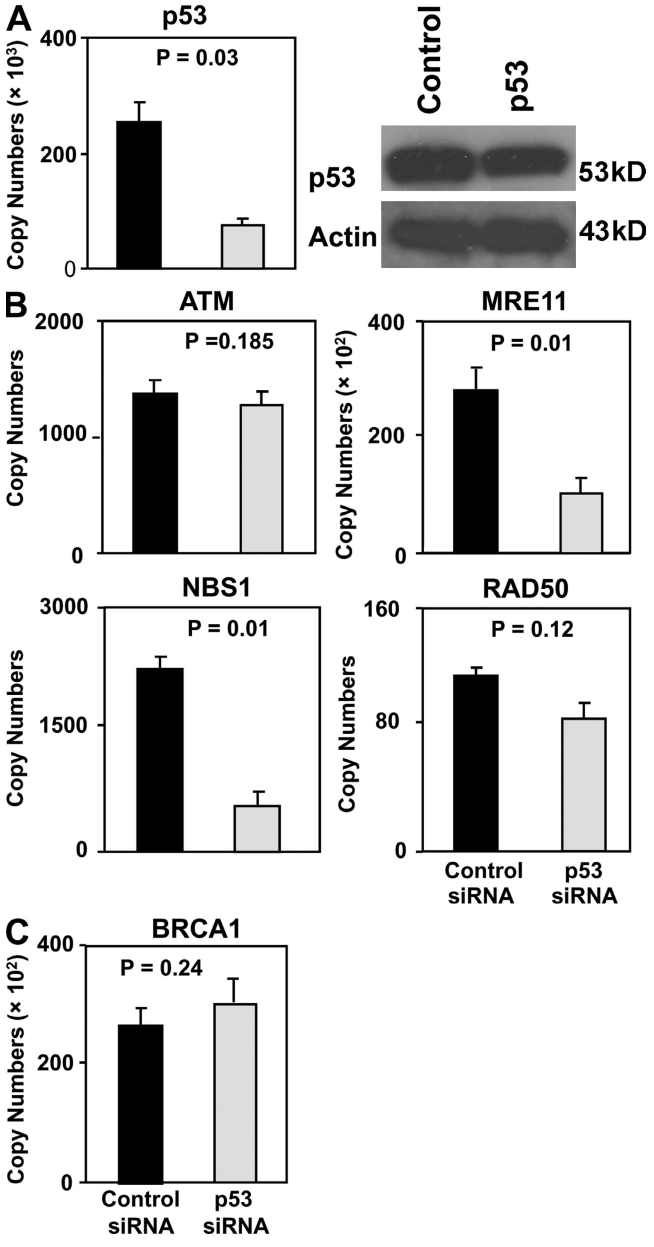


Figure S5. Silencing of p53 in CD4 T cells leads to down-regulation of MRE11, NBS1, and RAD50 but leaves ATM unaffected. CD4 T cells from control donors were transfected with control or siRNA p53 oligonucleotides by nucleofection. (A) After 24 h, p53 transcript levels were quantified by qPCR. p53 protein levels were quantified by Western blotting with actin serving as loading control. (B) Transcript levels of MRE11, NBS1, RAD50, and ATM were quantified by qPCR. (C) BRCA1 transcripts were measured as control. All transcript levels are shown as means ± SEM of three experiments with different healthy donors. The Western blot is representative of three independent experiments.

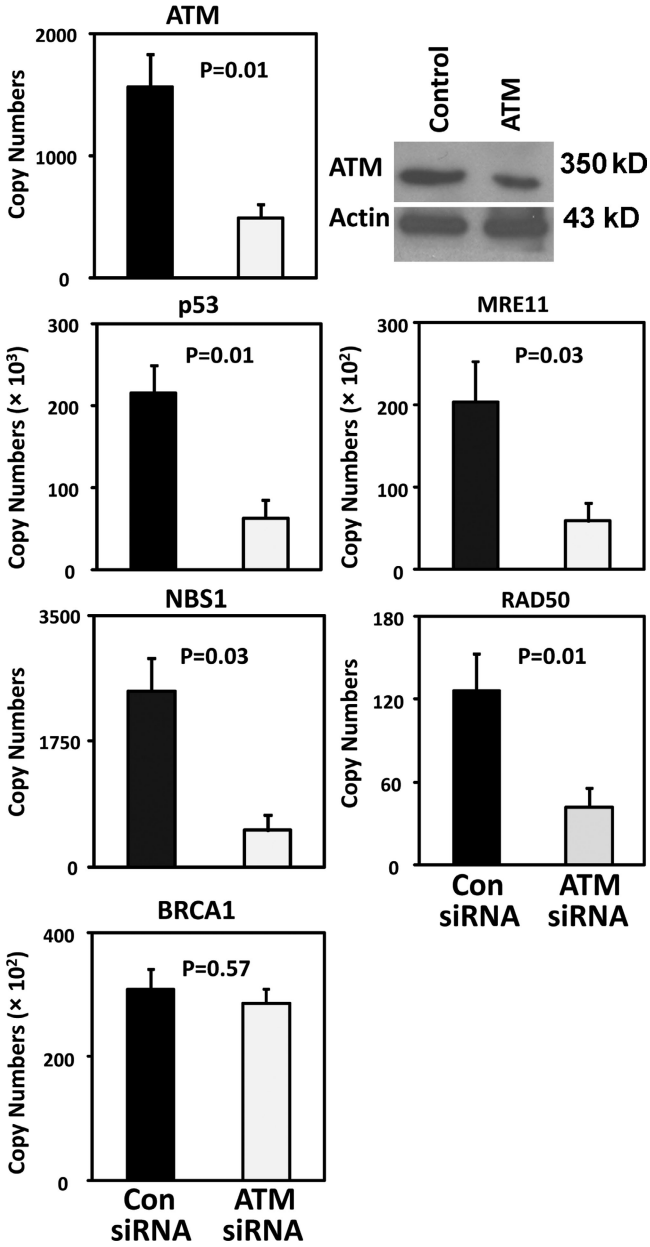


Figure S6. ATM regulates MRE11, NBS1, RAD50, and p53. CD4 T cells from control individuals were transfected with a second set of control or ATM-specific siRNA oligonucleotides (set 2) by nucleofection. ATM transcript levels were quantified by qPCR 24 h after transfection. ATM protein concentrations were measured by Western blotting and representative results from one of three experiments are shown. Transcript levels for MRE11, NBS1, RAD50, and p53 were quantified by qPCR. BRCA1 transcript levels were measured as a control. All transcript levels are shown as means ± SEM of three experiments with different healthy controls.

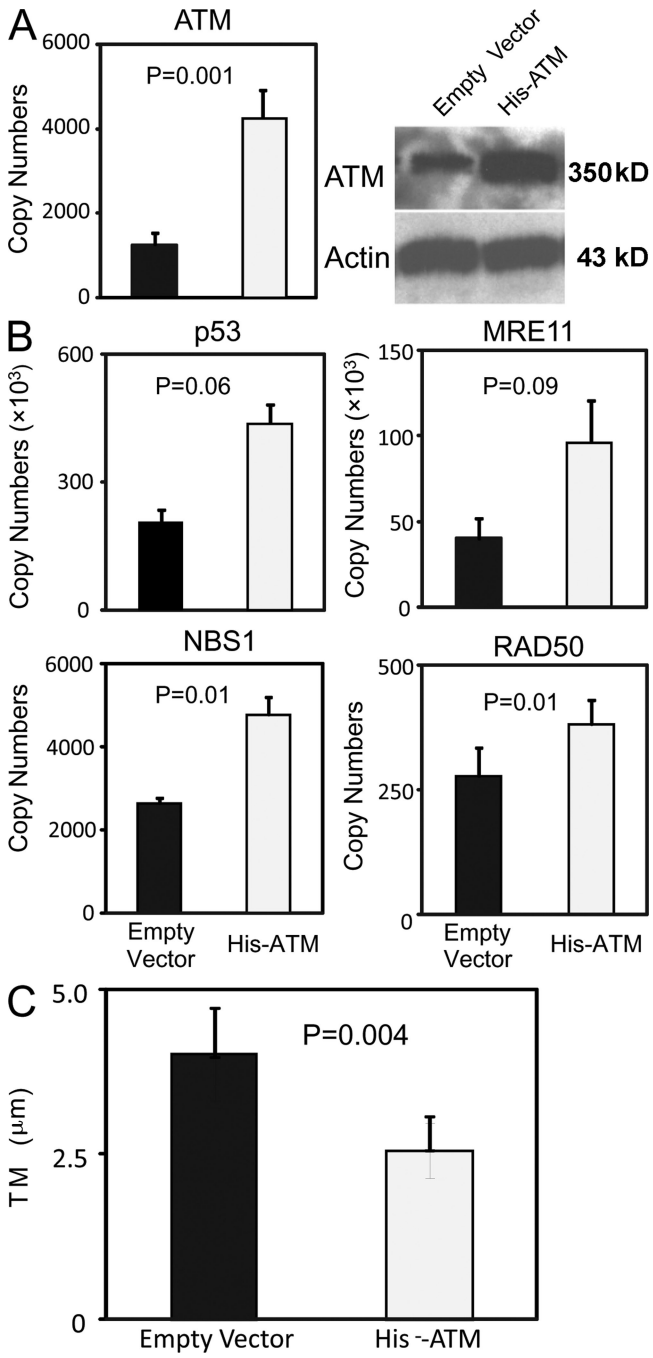


Figure S7. Overexpression of ATM in human CD4 T cells improves DNA damage repair. $CD4^+CD45RO^-$ T cells from healthy control donors were transfected with pcDNA3-His (empty vector) or pcDNA3-FLAG-His-ATM by nucleofection. (A) After 24 h, ATM transcript levels were measured by qPCR. ATM protein levels were quantified by Western blotting with actin serving as loading control, and representative results from one of four experiments are shown. (B) Transcript levels of p53, MRE11, NBS1, and RAD50 were quantified by qPCR. All transcript levels are shown as means ± SEM from four independent experiments with different healthy controls. (C) The effect of ATM overexpression on DNA damage repair was determined by measuring TMs in T cells expressing ectopic ATM compared with control transfected T cells. Successfully transfected cells (yellow cells) were identified by the coexpression of FITC (green fluorescence) and the DNA dye ethidium bromide (red fluorescence). Mean TMs of >50 individual cells were determined. Data are shown as means ± SEM of four experiments comparing T cells from four normal donors transfected with control vectors or His-ATM.