Figure EV1. **DIS3 loss increases DNA damage and ssDNA in multiple myeloma cells.**

A. Representative pictures of comet assays performed under alkaline conditions in RPMI-8226 cells. Data presented are from the same experiments reported in Fig EV2D. Analysis was performed using Casplab software, error bars indicate s.e.m. (n = 3 independent experiments, 50 cells for each shRNA). ****P < 0.0001, two-way ANOVA, scale bar is 20 μm.

B. Western blot of DIS3, RPA, phospho-RPA levels in RPMI-8226 cells, 72 h after infection with control shRNA (shScr) or shRNA targeting (shDIS3).

Source data are available online for this figure.

Figure EV2. **DNA:RNA hybrids accumulate in cells after DIS3 depletion and induce genome instability.**

A. Immunostaining with S9.6 (green) and DAPI (blue) in U2OS cells, infected with control shRNA (shScr) or shRNA against DIS3 (shDIS3) and then treated (RNase H) or not (UNTR) with RNase H for 1 h. Histograms show S9.6 signal intensity per cell, either in the nucleus or in the cytoplasm. More than 100 cells were counted in each replicate, error bars indicate s.e.m. (n = 3 biological replicates). ****P < 0.0001, ns—not statistically significant one-way ANOVA, scale bar is 10 μm.

B. Immunostaining with S9.6 (green) and DAPI (blue) in RPMI-8226 cells, infected with control shRNA (shScr) or shRNA against DIS3 (shDIS3) and then treated (RNase H) or not (UNTR) with RNase H for 1 h. Histograms show S9.6 signal intensity per cell. More than 100 cells were counted in each replicate, error bars indicate s.e.m. (n = 3 biological replicates). ****P < 0.0001, ns—not statistically significant one-way ANOVA, scale bar is 10 μm.

C. Immunostaining with S9.6 (green), anti-DDX21 (Sordet et al, 2009), and DAPI (blue) in shScr and shDIS3 infected cells, before (-4OHT) and after (+4OHT) damage induction, and treatment (RNase H) or not (UNTR) with RNase H for 1 h. Histograms below show the median of the S9.6 signal intensity per nucleus after nucleolar signal removal, using Cell Profiler software. 150 cells were counted in each replicate, error bars indicate s.e.m. (n = 3). ****P < 0.0001, ns—not statistically significant one-way ANOVA, scale bar is 10 μm.

D. Representative pictures of comet assays performed under alkaline conditions in RPMI-8226 cells, treated (RNase H) or not (UNTR) with RNase H. Relative comet tail moments are plotted for shScr (black circles) and shDIS3 (red circles). Data presented are from the same experiments reported in Fig EV1A. Analysis was performed using Casplab software, error bars indicate s.e.m. (n = 3 independent experiments, 50 cells for each shRNA). ****P < 0.0001, ns—not statistically significant, one-way ANOVA, scale bar is 20 μm.
Figure EV2.
Figure EV3. **DIS3 loss does not affect DDR foci formation.**

A U2OS cells were transfected with siCTRL or with siDIS3. 48 h later, cells were 3 Gy irradiated or left untreated (NO IR), fixed, and probed for MDC1, pS/TQ, pATM, and γH2AX at different time points (hours) after irradiation. Histograms show quantification of the average foci number per cell with Cell Profiler. At least 200 cells were counted for each point, error bars indicate s.e.m. (n = 3 biological replicates). Differences between control and DIS3 silenced cells for each antibody tested were not statistically significant, two-way ANOVA, scale bar is 10 μm.

B RAD51 and BRCA1 protein levels were assayed by western blot. Lamin B was used as a loading control.
Figure EV3.
Figure EV4. Cell cycle progression and NHEJ processes are not impaired in DIS3 silenced cells.

A Immunostaining of DNA-PK (upper panel) and 53BP1 (lower panel) in U2OS cells transfected with siCTRL or with siDIS3, at different time points (0.5, 2, 24 h) after exposure to 3 Gy irradiation or not irradiated (NO IR). Histograms show the average foci number per cell, analyzed with Cell Profiler. At least 200 cells were counted for each point, error bars indicate s.e.m. (n = 3 biological replicates). Differences between control and DIS3 silenced cells for each antibody tested were not statistically significant, two-way ANOVA, scale bar is 10 μm.

B Cell cycle analysis of U2OS control cells compared to DIS3 silenced cells, using propidium iodide (PI) staining, and flow cytometry. FACS analysis was performed 48 h after transfection.

C Cyclin A level assessed by western blot analysis in CTRL and DIS3 depleted U2OS cells. Lamin B was loaded as the loading control.

D Log fold change (log FC) before and after DIS3 ablation (RNA-seq data from 44 of various proteins belonging to R-loop processing and DNA repair mediators).
Figure EV4.
Figure EV5. DIS3 loss hampers DSBs repair.
A Representative pictures of comet assays performed under alkaline conditions in AID-DiVA cells infected with control shRNA (shScr) or shRNA against DIS3 (shDIS3), treated with 4OHT for 4 h to induce DSBs followed by treatment with auxin for 2 h to allow DSBs repair. Plots represent three biological replicates, with 100 cells for each point, error bars indicate s.e.m. The analysis is performed using Casplab software. ****P < 0.0001, *P < 0.05, ns—not statistically significant, two-way ANOVA, scale bar is 20 μm.

B AID-DiVA cells silenced (shDIS3) or not (shScr) for DIS3 were treated with 4OHT for 4 h to induce DSBs. The relative growth (+ 4OHT/− 4OHT) was assessed by trypan blue exclusion at different time points after 4OHT administration. Results are shown as mean ± s.e.m. of three independent experiments. **P < 0.01, *P < 0.05 two-way ANOVA.

Figure EV6. Differentially expressed genes between DIS3 mutant and WT samples.
A Canonical Interferon signaling pathways by IPA. The abundance of transcripts that are increased or decreased in DIS3 mutated samples are colored with various intensities according to log2 fold change, represented in red or green, respectively.

B Differentially expressed IFN-related pathways (N = 14 out of 26 tested) between DIS3 mutant vs DIS3 WT. Enrichment scores of each pathway were compared between DIS3 mutant vs DIS3 WT using a t-test. FDR is calculated using the Benjamini–Hochberg procedure. Red color dots represent the median value in each group (88 DIS3 mutated DIS3, 765 DIS3 WT samples), the line the interquartile range.

C Log2 expression of 72 IFN-related genes belonging to any of the 26 curated IFN signatures and IPA canonical IFN signaling pathways were represented by violin plots (blue, DIS3 WT samples; orange, DIS3 mutant). Red color dots represent the median in each group (88 DIS3 mutated DIS3, 765 DIS3 WT samples), the line the interquartile range.
Figure EV6.