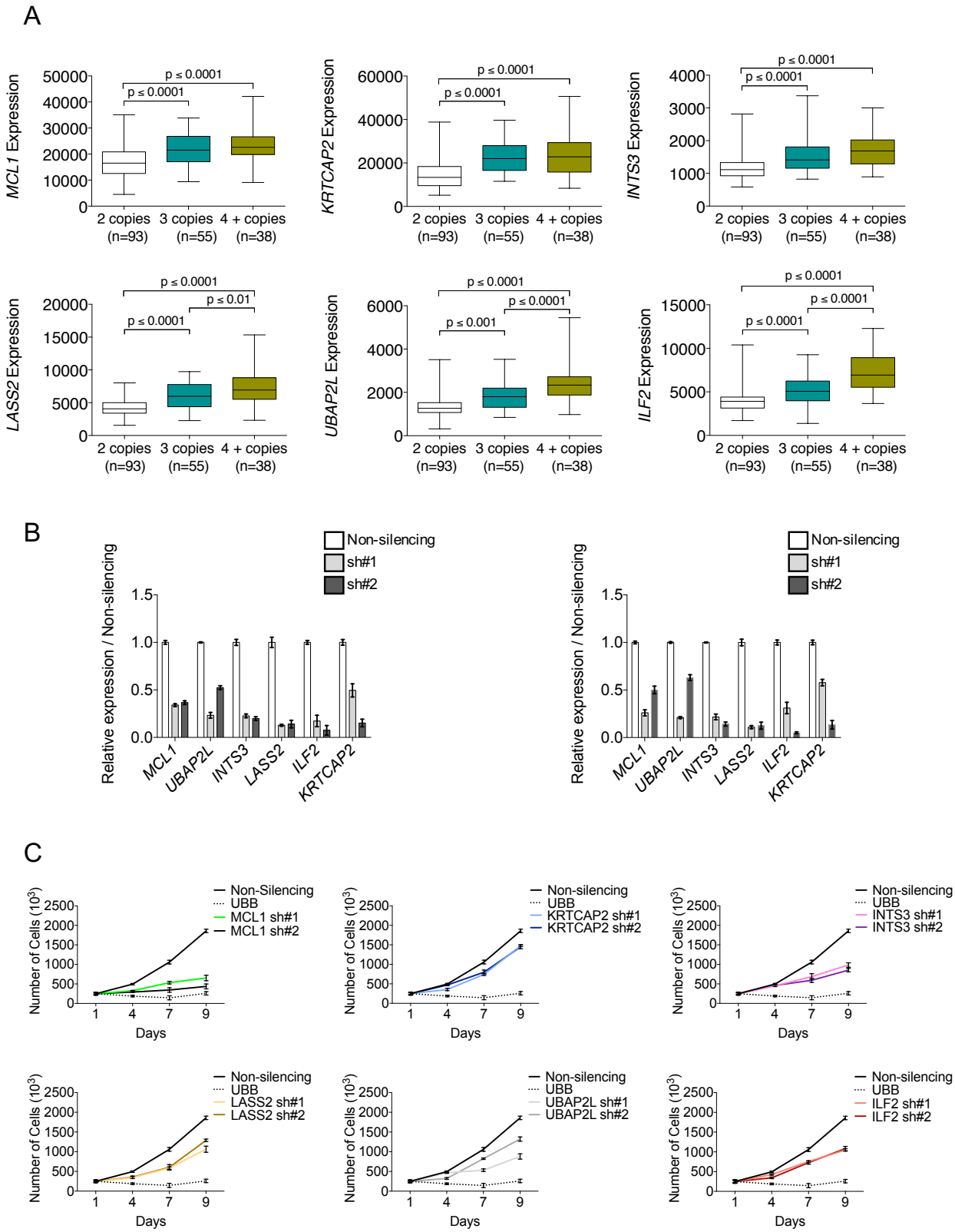
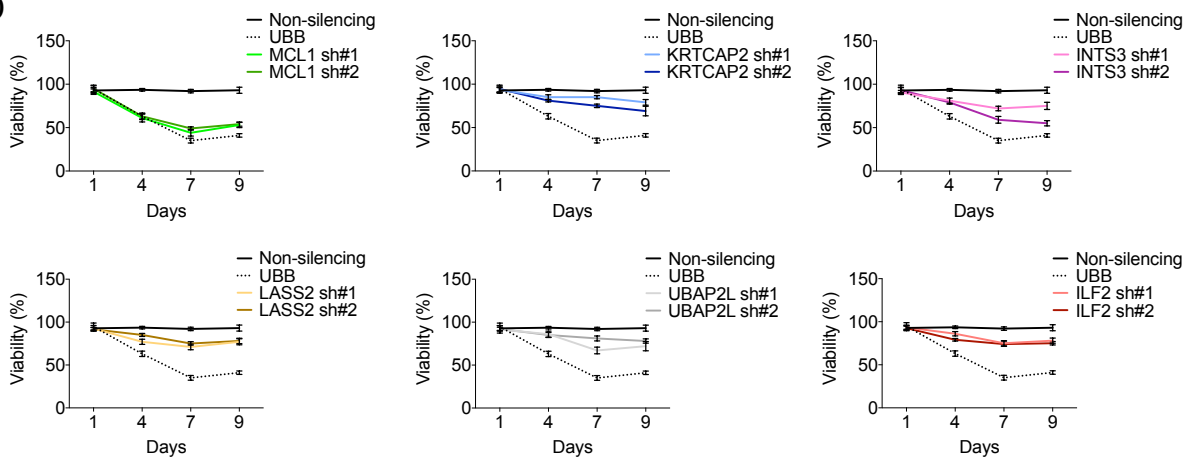
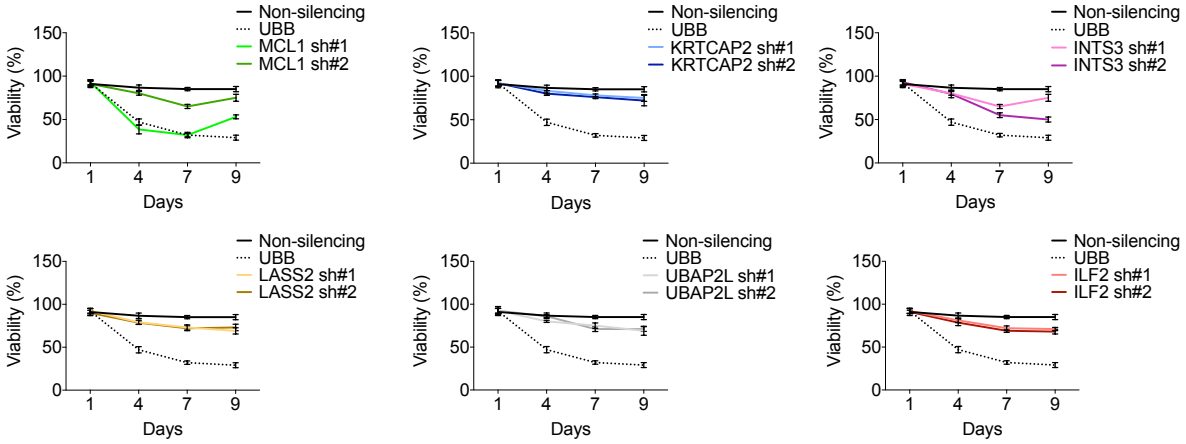

A
MCL1 Expression
KRTCAP2 Expression
INTS3 Expression
LASS2 Expression
UBAP2L Expression
ILF2 Expression
p ≤ 0.0001
p ≤ 0.01
p ≤ 0.001
2 copies (n=93)
3 copies (n=55)
4 + copies (n=38)
B
Non-silencing
sh#1
sh#2
Relative expression / Non-silencing
MCL1
UBAP2L
INTS3
LASS2
ILF2
KRTCAP2
C
Number of Cells (10^3)
Days
Non-Silencing
UBB
MCL1 sh#1
MCL1 sh#2
KRTCAP2 sh#1
KRTCAP2 sh#2
INTS3 sh#1
INTS3 sh#2
LASS2 sh#1
LASS2 sh#2
UBAP2L sh#1
UBAP2L sh#2
ILF2 sh#1
ILF2 sh#2

D




E




F

| shRNA | JJN3 Apoptosis (%) | H929 Apoptosis (%) |
|---|---|---|
| Non-silencing | 8.2 ± 0.9 | 8.6 ± 1.4 |
| UBB | 28.3 ± 0.6 | 42.1 ± 0.6 |
| MCL1 sh#1 | 30.7 ± 2.4 | 58.2 ± 1.3 |
| MCL1 sh#2 | 33 ± 2.9 | 25.3 ± 1.1 |
| INTS3 sh#1 | 16.2 ± 2.1 | 18.1 ± 3.1 |
| INTS3 sh#2 | 22.8 ± 3.2 | 19.4 ± 2.6 |
| UBAP2L sh#1 | 17.7 ± 1.7 | 18.2 ± 3.7 |
| UBAP2L sh#2 | 11.4 ± 1.7 | 11.4 ± 2.2 |
| LASS2 sh#1 | 13.3 ± 2.1 | 15.4 ± 3.2 |
| LASS2 sh#2 | 13.1 ± 2.3 | 16.2 ± 3.1 |
| KRTCAP2 sh#1 | 11.8 ± 1.5 | 12.8 ± 1.5 |
| KRTCAP2 sh#2 | 16.4 ± 0.9 | 17.8 ± 1.1 |
| ILF2 sh#1 | 13 ± 0.4 | 15.3 ± 3.1 |
| ILF2 sh#2 | 14.7 ± 0.8 | 17.8 ± 2.9 |

G

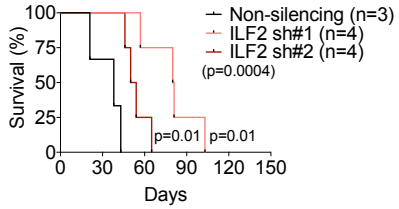


**Figure S1, related to Figure 1. 1q21 shRNA Screen Identifies *ILF2* as a MM-Critical Gene.**

**(A)** Expression of *MCL1*, *KRTCAP2, INTS3*, *LASS2, UBAP2L*, and *ILF2* in MM plasma cells isolated from 186 untreated patients with available 1q21 fluorescence in situ hybridization (FISH) results and enrolled in the Total Therapy 2 trial. Patients were stratified based on the number of 1q21 copies as evaluated by FISH analysis. Expression of the 6 genes was evaluated by microarray analysis. The bottom and top edges of each box indicate the interquartile range of expression; the whiskers indicate the minimum and maximum values of expression; the horizontal line inside each box shows the median value of expression.

**(B)** Relative expression of *MCL1*, *UBAP2L, INTS3*, *LASS2, ILF2*, and *KRTCAP2* in shRNA-transduced JJN3 cells (left) or H929 cells (right), as evaluated by real-time PCR. The mean and SD of duplicates from one representative experiment are shown. Data were normalized to GAPDH and expressed as percentages of the non-silencing control.

**(C)** Representative growth curves of shRNA-transduced H929 cells. The mean and SD of duplicates from one representative experiment are shown. Every experiment was performed 3 times and included two different shRNAs per gene (sh#1 and sh#2), a non-silencing shRNA as a negative control, and an UBB-targeting shRNA as a positive control. All growth curves relative to each independent experiment were performed simultaneously.

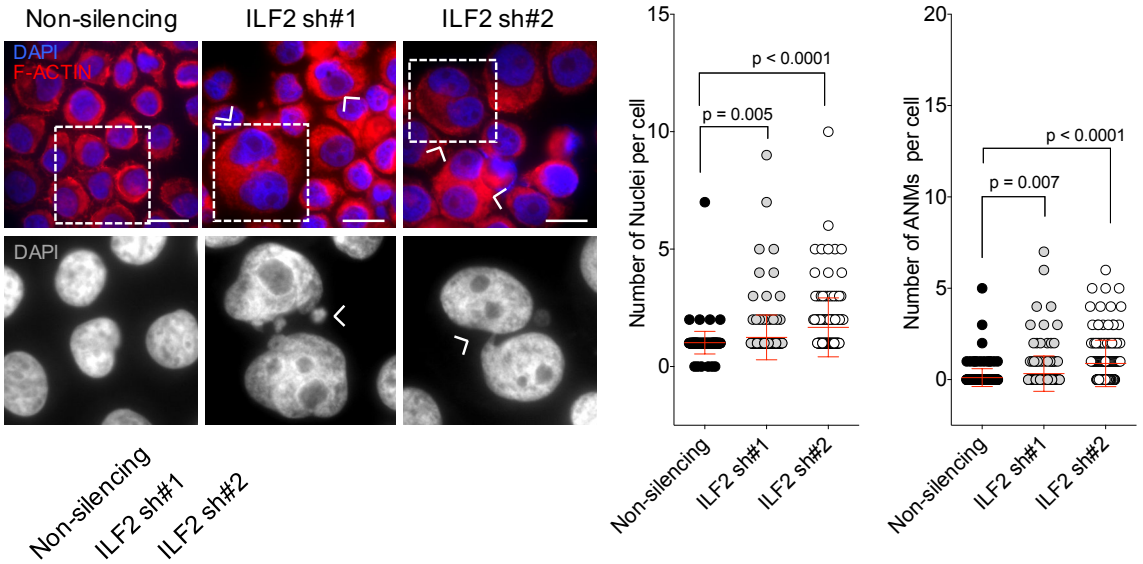
**(D)** Representative viability curves of shRNA-transduced JJN3 cells. The mean and SD of duplicates from one representative experiment are shown. Every experiment was performed 3 times and included two different shRNAs per gene (sh#1 and sh#2), a non-silencing shRNA as a negative control, and an UBB-targeting shRNA as a positive control. All growth curves relative to each independent experiment were performed simultaneously.

**(E)** Representative viability curves of shRNA-transduced H929 cells. The mean and SD of duplicates from one representative experiment are shown. Every experiment was performed three times and included two different shRNAs per gene (sh#1 and sh#2), a non-silencing shRNA as a negative control, and an UBB-targeting shRNA as a positive control. All growth curves relative to each independent experiment were performed simultaneously.

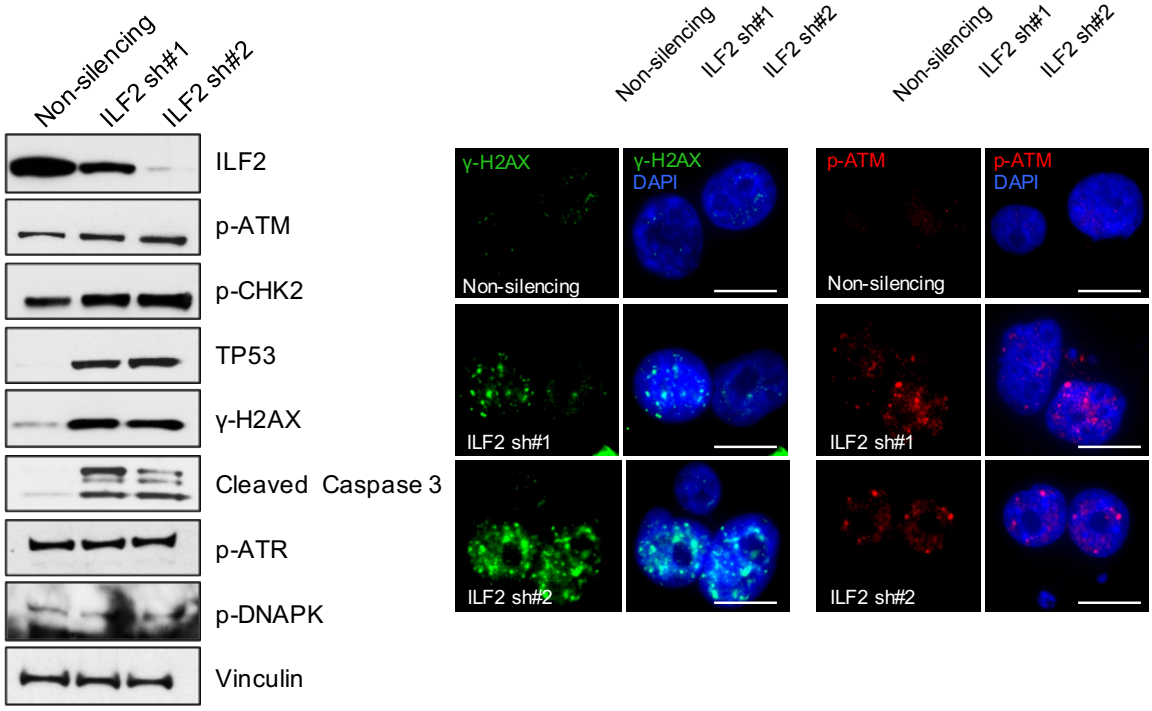
**(F)** Frequency of apoptosis in JJN3 and H929 cells transduced by the indicated shRNAs. The mean and SD of duplicates from one representative experiment are shown. Every experiment included two different shRNAs per gene (sh#1 and sh#2), a non-silencing shRNA as a negative control, and an UBB-targeting shRNA as a positive control. All apoptosis assays relative to each independent experiment were performed simultaneously.

**(G)** Survival curves of NOD/SCID mice injected subcutaneously with $2x10^6$ H929 cells transduced with non-silencing or ILF2 shRNAs (n=3 or 4 mice per group as indicated). Survival curves were analyzed by Mantel–Cox log-rank test.

A




B

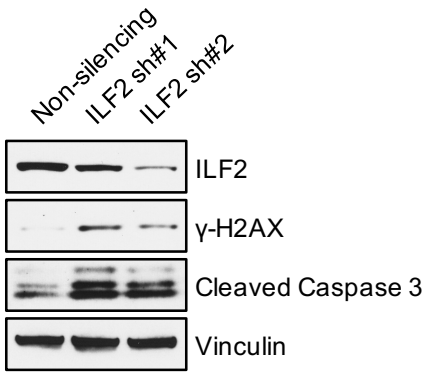


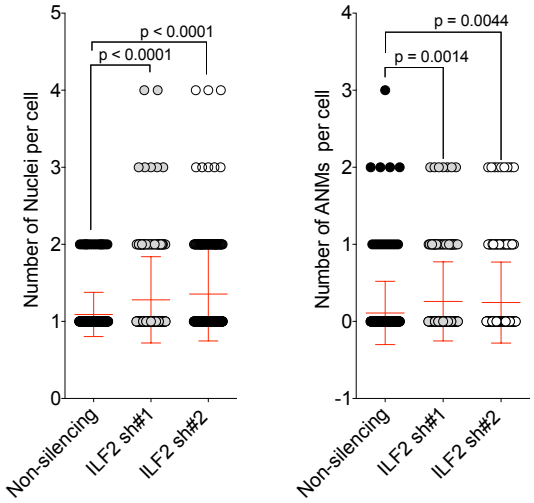

C

| JJN3 | % $G_0/G_1$ | % S-phase | % $G_2/M$ |
|---|---|---|---|
| Non-silencing | 42.55 ± 0.27 | 50.12 ± 0.16 | 7.32 ± 0.35 |
| ILF2 sh#1 | 43.27 ± 0.41 | 40.03 ± 0.42; p=0.0002 | 16.69 ± 0.71; p=0.0005 |
| ILF2 sh#2 | 38.59 ± 0.07; p=0.001 | 40.36 ± 0.57; p=0.0008 | 21.04 ± 0.64; p=0.00009 |

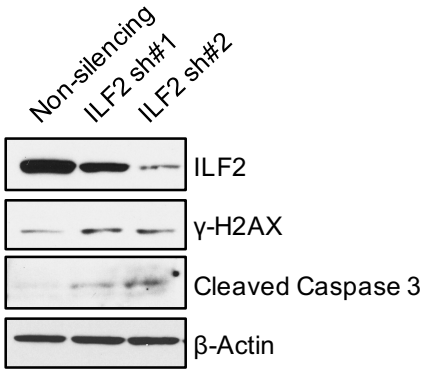
| H929 | % $G_0/G_1$ | % S-phase | % $G_2/M$ |
|---|---|---|---|
| Non-silencing | 57.46 ± 1.17 | 30.16 ± 2.19 | 12.36 ± 1.27 |
| ILF2 sh#1 | 67.01 ± 1.13; p=0.001 | 24.10 ± 2.04; p=0.04 | 8.93 ± 1.86 |
| ILF2 sh#2 | 71.97 ± 1.99; p=0.002 | 19.32 ± 2.8; p=0.01 | 8.55 ± 0.67; p=0.03 |

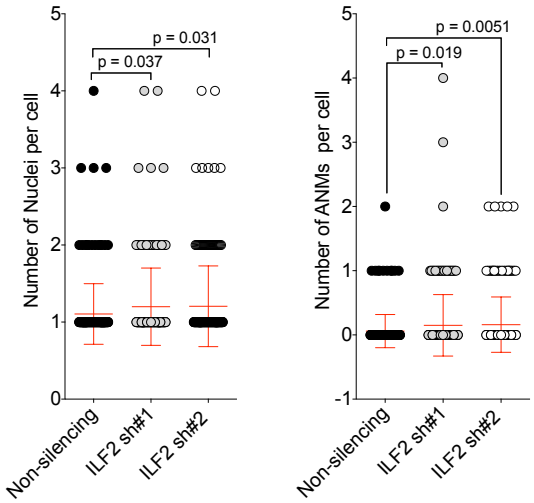
D


Non-silencing
ILF2 sh#1
ILF2 sh#2
ILF2
γ-H2AX
Cleaved Caspase 3
Vinculin

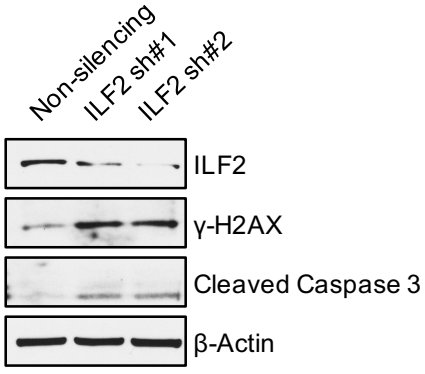

Number of Nuclei per cell
p < 0.0001
p < 0.0001
Number of ANMs per cell
p = 0.0044
p = 0.0014
Non-silencing
ILF2 sh#1
ILF2 sh#2

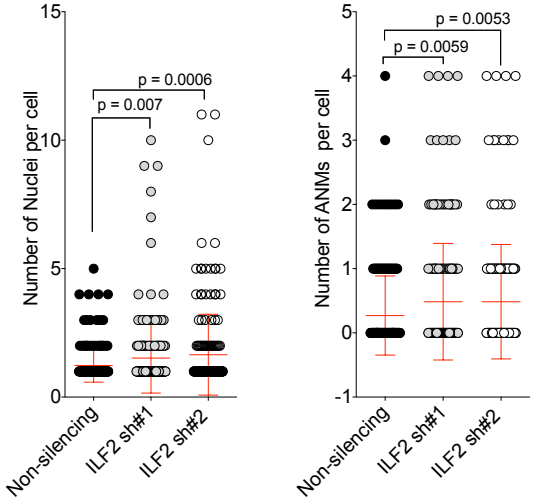
E

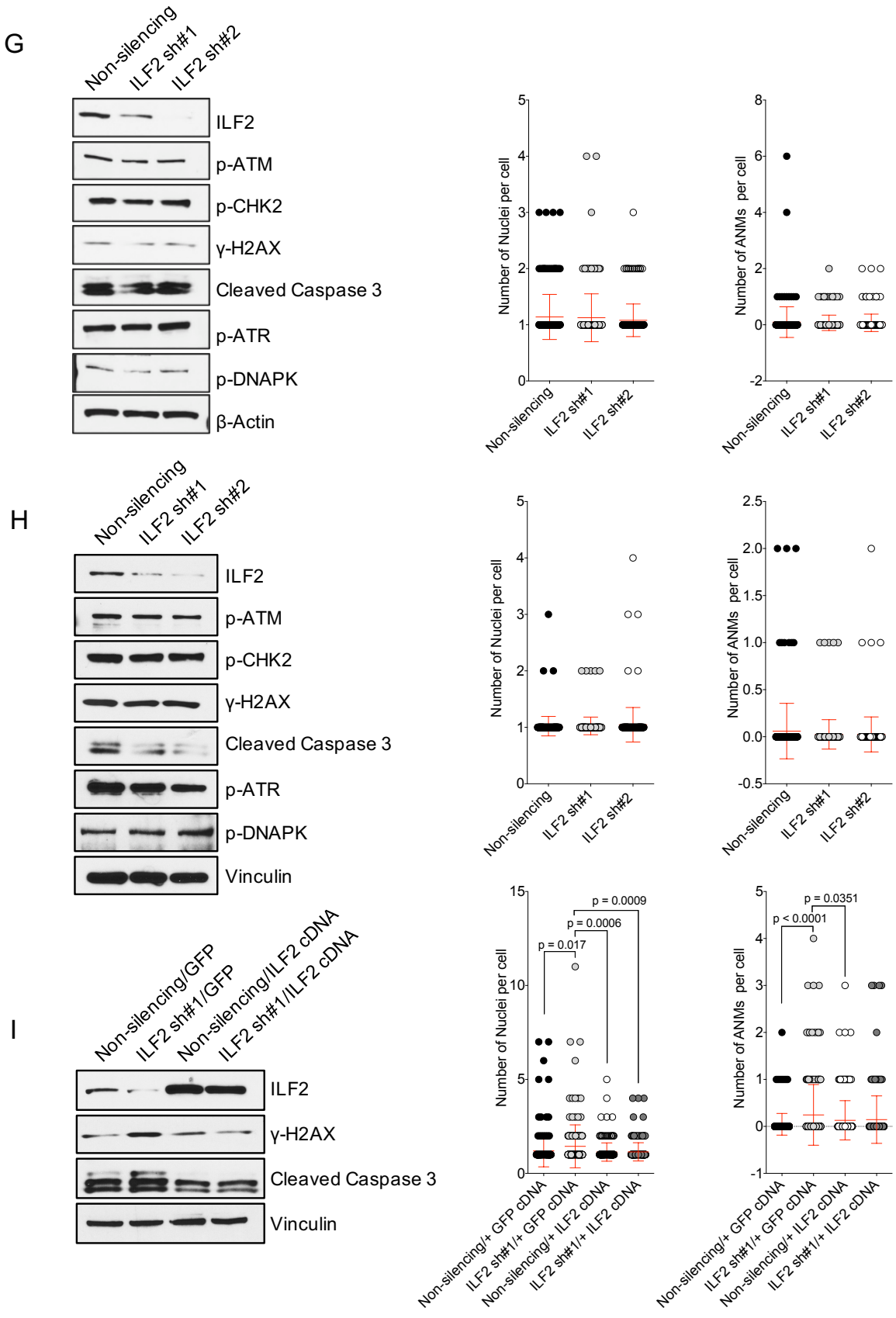

Non-silencing
ILF2 sh#1
ILF2 sh#2
ILF2
γ-H2AX
Cleaved Caspase 3
β-Actin


Number of Nuclei per cell
p = 0.031
p = 0.037
Number of ANMs per cell
p = 0.0051
p = 0.019
Non-silencing
ILF2 sh#1
ILF2 sh#2

F


Non-silencing
ILF2 sh#1
ILF2 sh#2
ILF2
γ-H2AX
Cleaved Caspase 3
β-Actin


Number of Nuclei per cell
p = 0.0006
p = 0.007
Number of ANMs per cell
p = 0.0053
p = 0.0059
Non-silencing
ILF2 sh#1
ILF2 sh#2


G
Non-silencing
ILF2 sh#1
ILF2 sh#2
ILF2
p-ATM
p-CHK2
γ-H2AX
Cleaved Caspase 3
p-ATR
p-DNAPK
β-Actin
Number of Nuclei per cell
Number of ANMs per cell
H
Vinculin
I
Non-silencing/GFP
ILF2 sh#1/GFP
Non-silencing/ILF2 cDNA
ILF2 sh#1/ILF2 cDNA
p = 0.017
p = 0.0006
p = 0.0009
p < 0.0001
p = 0.0351

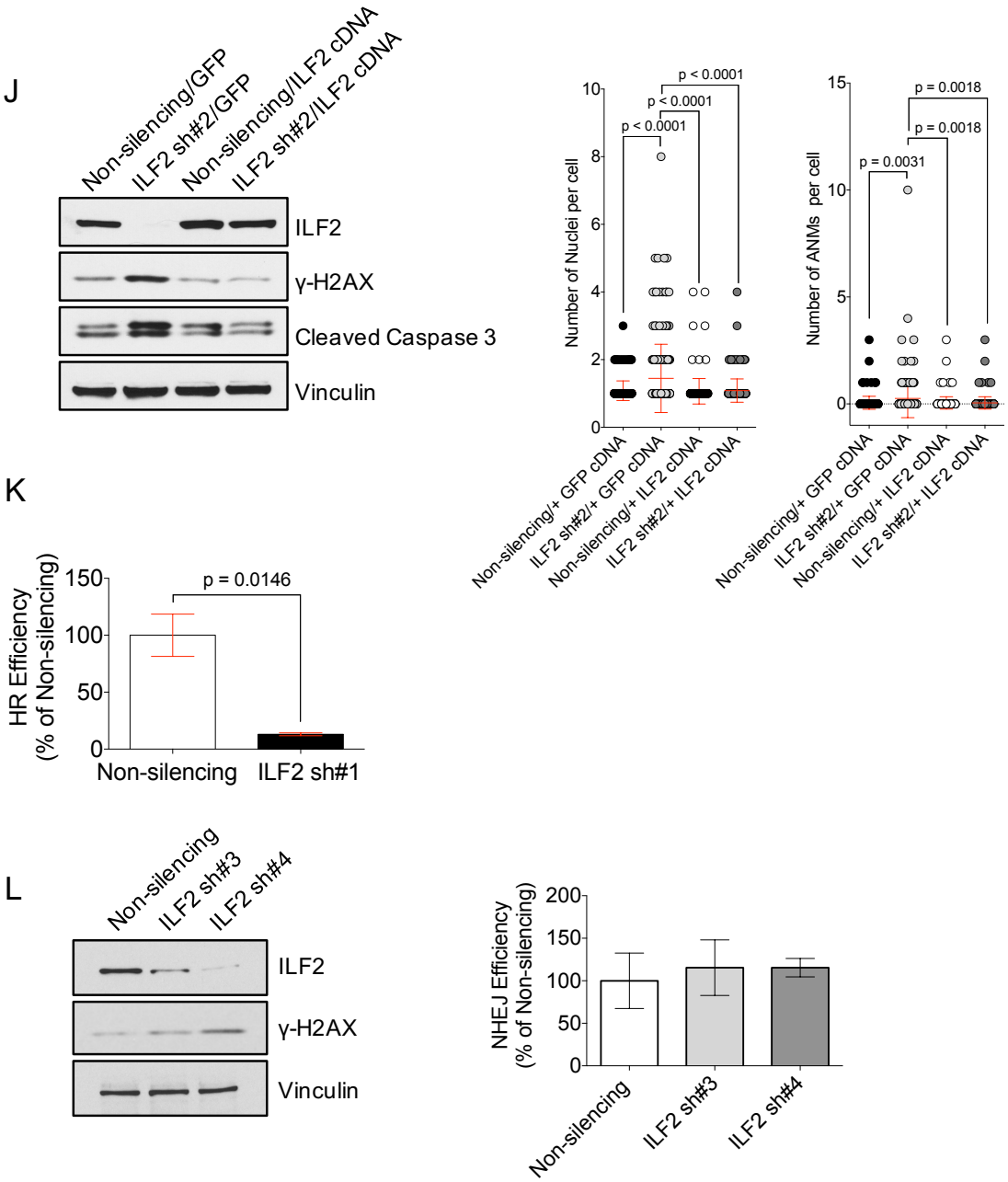

J
Non-silencing/GFP
ILF2 sh#2/GFP
Non-silencing/ILF2 cDNA
ILF2 sh#2/ILF2 cDNA
ILF2
γ-H2AX
Cleaved Caspase 3
Vinculin
Number of Nuclei per cell
p < 0.0001
p < 0.0001
p < 0.0001
Number of ANMs per cell
p = 0.0018
p = 0.0018
p = 0.0031
Non-silencing/+ GFP cDNA
ILF2 sh#2/+ GFP cDNA
Non-silencing/+ ILF2 cDNA
ILF2 sh#2/+ ILF2 cDNA
K
HR Efficiency (% of Non-silencing)
p = 0.0146
Non-silencing
ILF2 sh#1
L
Non-silencing
ILF2 sh#3
ILF2 sh#4
ILF2
γ-H2AX
Vinculin
NHEJ Efficiency (% of Non-silencing)
M
GFP
ILF2 Over-Ex.
ILF2
γ-H2AX
Vinculin

**Figure S2, related to Figure 2. ILF2 Is Involved in the Homologous Recombination of DNA Double-Strand Breaks in MM Cells.**

**(A)** Representative images of multinucleation in H929 cells transduced with a non-silencing shRNA or one of two different ILF2 shRNAs (ILF2 sh#1 and ILF2 sh#2); cell cytoplasm is stained with F-actin (red), and nuclei are stained with DAPI (blue). Arrowheads indicate multinucleated cells (upper panel on the left); scale bars represent 10 μm. Wide-field microscopy magnifications of the cells inside the squares of the upper panel; arrowheads indicate a bud (bottom panel in the middle) or a nucleoplasmic bridge (bottom panel on the right); nuclei are stained with DAPI. Quantitative analysis of the number of nuclei per cell (middle panel) and overall abnormal nuclear morphologies (ANMs) per cell in H929 cells transduced with non-silencing or ILF2 shRNAs; error bars denote SD (panel on the right).

**(B)** Representative Western blot analysis of ILF2, p-ATM, p-CHK2, TP53, γH2AX, cleaved caspase 3, p-ATR, and p-DNAPK in H929 cells transduced with non-silencing or ILF2 shRNAs; a representative vinculin is shown as the loading control for all proteins (left). Representative anti-γH2AX (middle) and anti–p-ATM (right) immunofluorescence in H929 cells transduced with non-silencing or ILF2 shRNAs (γH2AX: green; p-ATM: red; DAPI: blue); scale bars represent 10 μm.

**(C)** Cell cycle analysis of non-silencing and ILF2 shRNA-transduced JJN3 (upper panel) and H929 (bottom panel) cells. The mean and SD of triplicates from one representative experiment are shown.

**(D)** Representative Western blot analysis of ILF2, γH2AX, and cleaved caspase 3 in L363 cells transduced with non-silencing or ILF2 shRNAs; vinculin was the loading control (left). Quantitative analysis of the number of nuclei per cell (middle panel) and overall ANMs per cell

in L363 cells transduced with non-silencing or ILF2 shRNAs (panel on the right); error bars denote SD.

**(E)** Representative Western blot analysis of ILF2, γH2AX, and cleaved caspase 3 in XG-6 cells transduced with non-silencing or ILF2 shRNAs; β-actin was the loading control (left). Quantitative analysis of the number of nuclei per cell (middle panel) and overall ANMs per cell in XG-6 cells transduced with non-silencing or ILF2 shRNAs (panel on the right); error bars denote SD.

**(F)** Representative Western blot analysis of ILF2, γH2AX, and cleaved caspase 3 in OPM2 cells transduced with non-silencing or ILF2 shRNAs; β-actin was the loading control (left). Quantitative analysis of the number of nuclei per cell (middle panel) and overall ANMs per cell in OPM2 cells transduced with non-silencing or ILF2 shRNAs (panel on the right); error bars denote SD.

**(G)** Representative Western blot analysis of ILF2, p-ATM, p-CHK2, γH2AX, cleaved caspase 3, p-ATR, and p-DNAPK in OCI-MY5 cells transduced with non-silencing or ILF2 shRNAs; a representative β-actin is shown as the loading control for all proteins (left). Quantitative analysis of the number of nuclei per cell (middle panel) and overall ANMs per cell in OCI-MY5 cells transduced with non-silencing or ILF2 shRNAs (panel on the right); error bars denote SD.

**(H)** Representative Western blot analysis of ILF2, p-ATM, p-CHK2, γH2AX, cleaved caspase 3, p-ATR, and p-DNAPK in NCU-MM1 cells transduced with non-silencing or ILF2 shRNAs; a representative vinculin is shown as the loading control for all proteins (left). Quantitative analysis of the number of nuclei per cell (middle panel) and overall ANMs per cell in NCU-MM1 cells transduced with non-silencing or ILF2 shRNAs (panel on the right); error bars denote SD.

**(I)** Representative Western blot analysis of ILF2, γH2AX, and cleaved caspase 3 in JJN3 cells transduced with non-silencing or ILF2 shRNAs and infected with a GFP- or ILF2-expressing lentiviral vector; vinculin was the loading control (left); ILF2 shRNA #1 (ILF2 sh#1) was used for this experiment (panel on the left). Quantitative analysis of the number of nuclei per cell (middle panel) and overall ANMs per cell in JJN3 cells transduced with non-silencing shRNA or ILF2 sh#1 and infected with a GFP- or ILF2-expressing lentiviral vector (panel on the right); error bars denote SD.

**(J)** Representative Western blot analysis of ILF2, γH2AX, and cleaved caspase 3 in JJN3 cells transduced with non-silencing or ILF2 shRNAs and infected with a GFP- or ILF2-overexpressing lentiviral vector; vinculin was the loading control (left); ILF2 shRNA #2 (ILF2 sh#2) was used for this experiment (panel on the left). Quantitative analysis of the number of nuclei per cell (middle panel) and overall ANMs per cell in JJN3 cells transduced with non-silencing shRNA or ILF2 sh#2 and infected with a GFP- or ILF2-overexpressing lentiviral vector (panel on the right); error bars denote SD.

**(K)** Quantitative analysis of homologous recombination efficiency in JJN3 cells transduced with non-silencing or ILF2 shRNAs. The mean and SD of three independent experiments are shown. ILF2 sh#1 was used for this experiment; data are expressed as percentages of the non-silencing control.

**(L)** Representative Western blot analysis of ILF2 and γH2AX in JJN3 cells transduced with non-silencing or ILF2 shRNAs; vinculin was used as the loading control. Two different shRNAs (sh#3 and sh#4) that do not express GFP were used for this experiment (left). Quantitative analysis of non-homologous end joining efficiency in JJN3 cells transduced with non-silencing

or ILF2 shRNAs. The mean and SD of two independent experiments are shown; data are expressed as percentages of the non-silencing control (panel on the right).

**(M)** Representative Western blot analysis of ILF2 and γH2AX in GFP- and ILF2-overexpressing JJN3 (panel on the left) and H929 cells (panel on the right). Vinculin was used as the loading control.

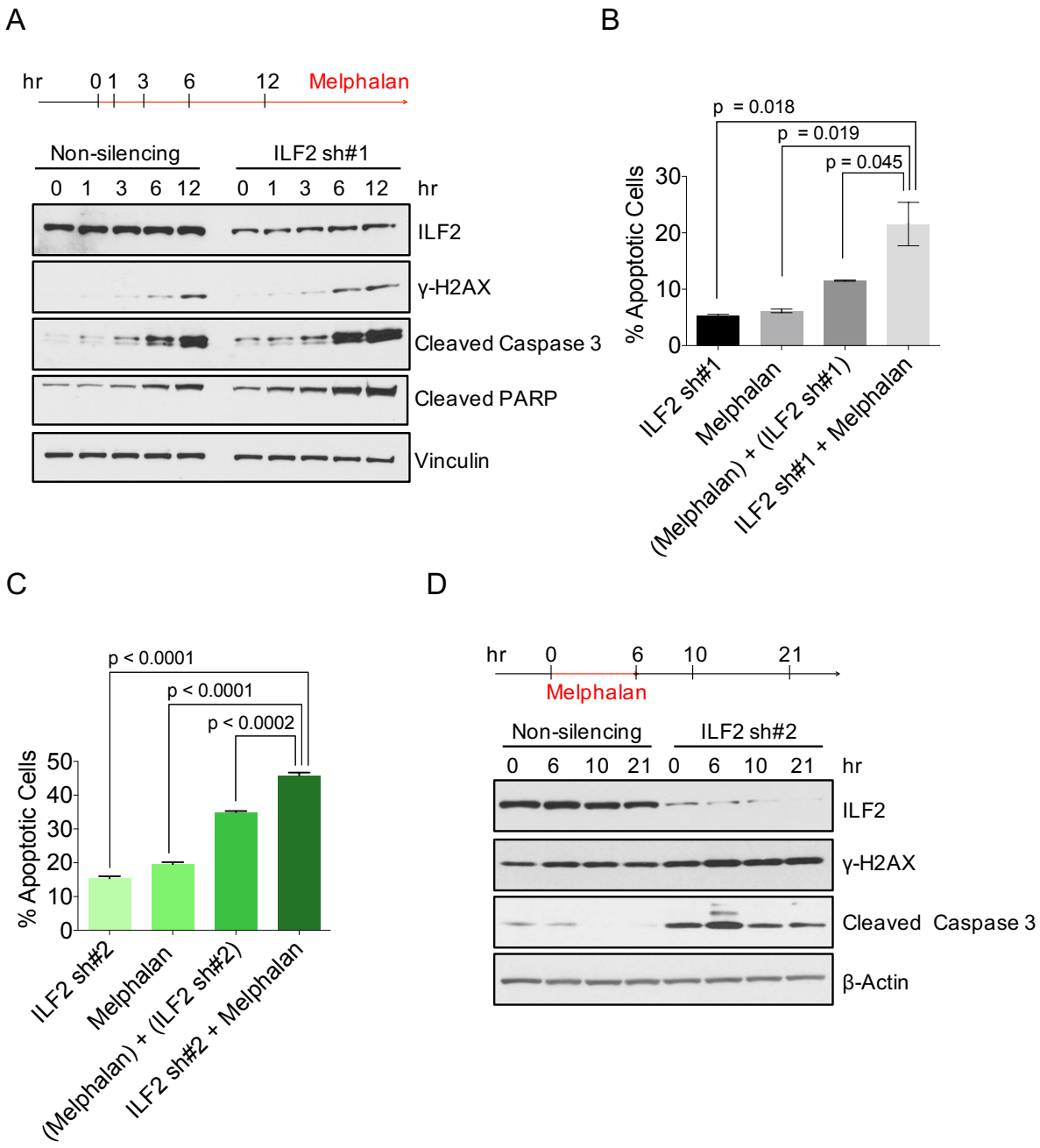


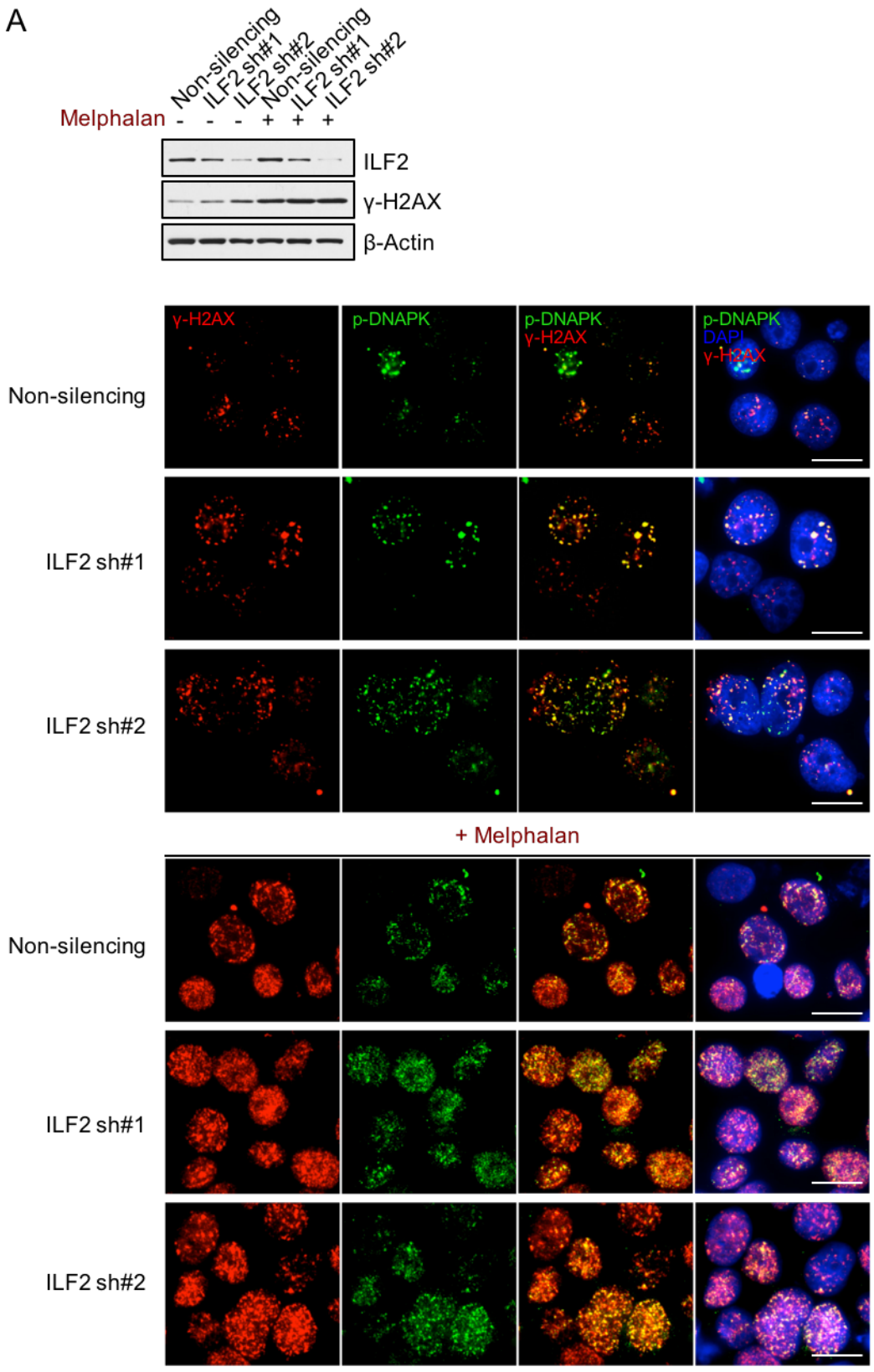

**Figure S3, related to Figure 3. ILF2 Levels Modulate MM Cell Sensitivity to DNA-Damaging Agents.**

**(A)** Representative Western blot analysis of ILF2, γH2AX, cleaved caspase 3, and cleaved PARP proteins in JJN3 cells transduced with non-silencing shRNA or ILF2 shRNA #1 and treated with 25 μM melphalan for the indicated times. Vinculin was used as the loading control.

**(B)** Quantitative analysis of the frequency of apoptosis of non-silencing and ILF2 shRNA–transduced JJN3 cells that were treated with 4 μM melphalan for 48 hr. ILF2 shRNA #1 (ILF2 sh#1) was used for this experiment. ILF2 sh#1 indicates the frequency of apoptosis induced by ILF2 depletion; melphalan indicates the frequency of apoptosis induced by melphalan; (melphalan) + (ILF2 sh#1) indicates the sum frequency of apoptosis induced by ILF2 depletion and melphalan; and ILF2 sh#1 + melphalan indicates the frequency of apoptosis of melphalan-treated, ILF2 shRNA–transduced cells. The mean and SD of triplicates from one representative experiment are shown.

**(C)** Quantitative analysis of the frequency of apoptosis in H929 cells transduced with non-silencing or ILF2 shRNAs and treated with 4 μM melphalan for 48 hr. ILF2 sh#2 indicates the frequency of apoptosis induced by ILF2 depletion; melphalan indicates the frequency of apoptosis induced by melphalan; (melphalan) + (ILF2 sh#2) indicates the sum frequency of apoptosis induced by ILF2 depletion and melphalan; and ILF2 sh#2 + melphalan indicates the frequency of apoptosis of melphalan-treated, ILF2 shRNA–transduced cells. The mean and SD of triplicates from one representative experiment are shown.

**(D)** Representative Western blot analysis of ILF2, γH2AX and cleaved caspase 3 in JJN3 cells transduced with non-silencing or ILF2 shRNAs and treated with 25 μM melphalan for 6 h; after 6 hr, the medium was changed, and the cells were allowed to recover. β-actin was used as the loading control.


A
Non-silencing
ILF2 sh#1
ILF2 sh#2
Non-silencing
ILF2 sh#1
ILF2 sh#2
Melphalan - - - + + +
ILF2
γ-H2AX
β-Actin
γ-H2AX
p-DNAPK
p-DNAPK
γ-H2AX
p-DNAPK
DAPI
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+ Melphalan
Non-silencing
ILF2 sh#1
ILF2 sh#2

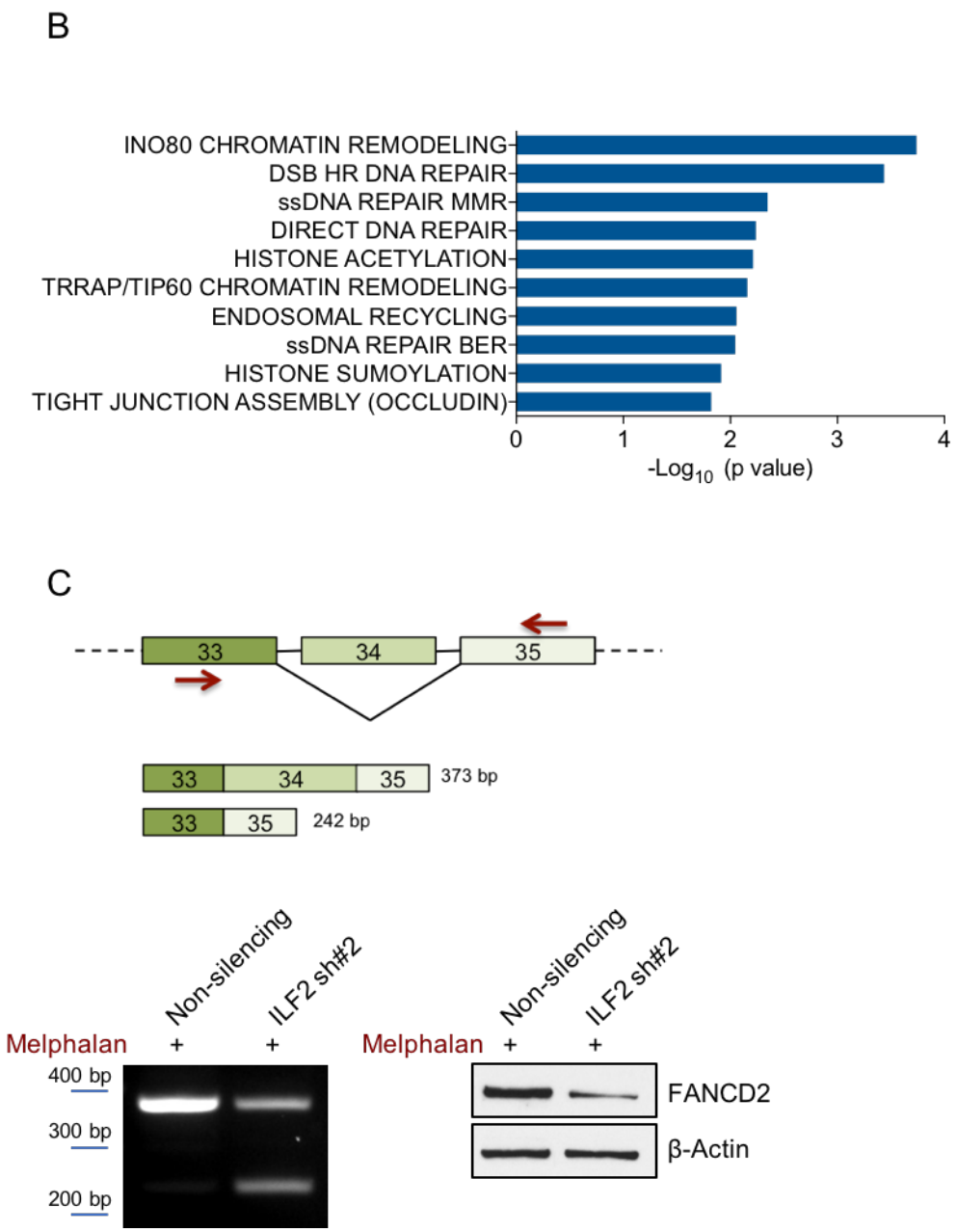



**Figure S4, related to Figure 4. ILF2 Is Required for the RNA Splicing of Genes Involved in DNA Repair.**

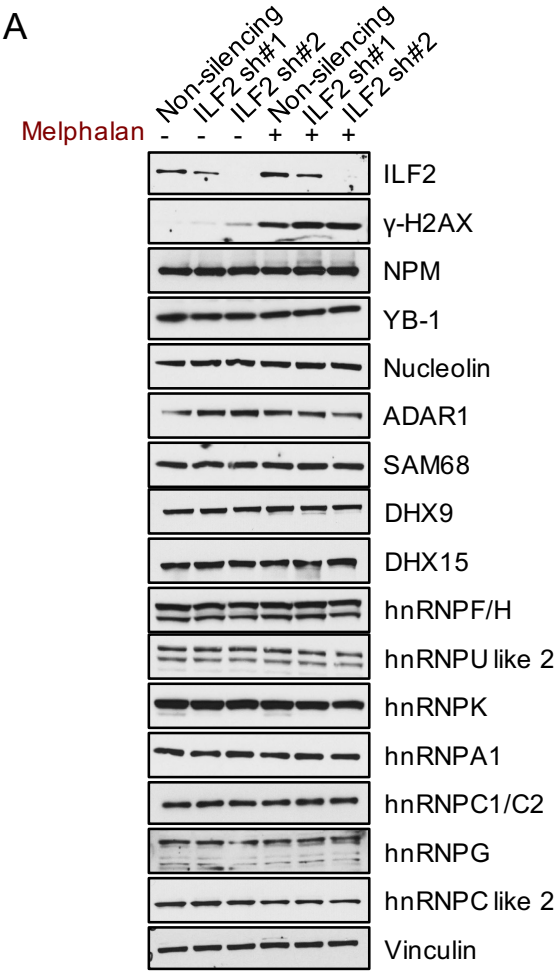
**(A)** Representative Western blot analysis of ILF2 and γH2AX in untreated and melphalan-treated JJN3 cells transduced with non-silencing or ILF2 shRNAs; β-actin was the loading control (upper panel). Representative anti-γH2AX and anti–p-DNAPK immunofluorescence in untreated

(top) and melphalan-treated (bottom) JJN3 cells transduced with non-silencing or ILF2 shRNAs (red: γH2AX; green: p-DNAPK; blue: DAPI); scale bars represent 10 μm.

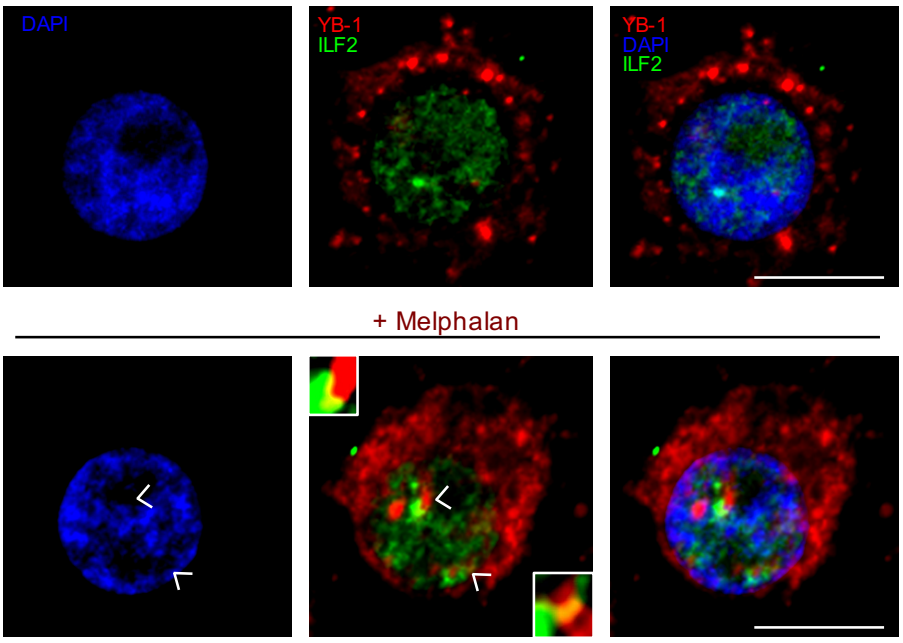
**(B)** Significantly enriched pathways relative to the 1100 events of aberrant splicing in ILF2 shRNA–transduced H929 cells as compared to non-silencing shRNA–transduced H929 cells. ILF2 shRNA #2 was used for this experiment, which was performed in duplicate.

**(C)** Schematic representation of the splicing events involving *FANCD2* mRNA in melphalan-treated, ILF2 shRNA–transduced JJN3 cells, as determined by RNA-Seq analysis (top). RT-PCR analysis with primers localized in exons 33 and 35 of *FANCD2* shows the skipping of exon 34 (bottom left), which results in decreased FANCD2 expression due to the premature termination of protein translation (bottom right).

A


Non-silencing
ILF2 sh#1
ILF2 sh#2
Non-silencing
ILF2 sh#1
ILF2 sh#2
Melphalan - - - + + +
ILF2
γ-H2AX
NPM
YB-1
Nucleolin
ADAR1
SAM68
DHX9
DHX15
hnRNPF/H
hnRNPU like 2
hnRNPK
hnRNPA1
hnRNPC1/C2
hnRNPG
hnRNPC like 2
Vinculin

B


DAPI
YB-1
ILF2
YB-1
DAPI
ILF2
+ Melphalan

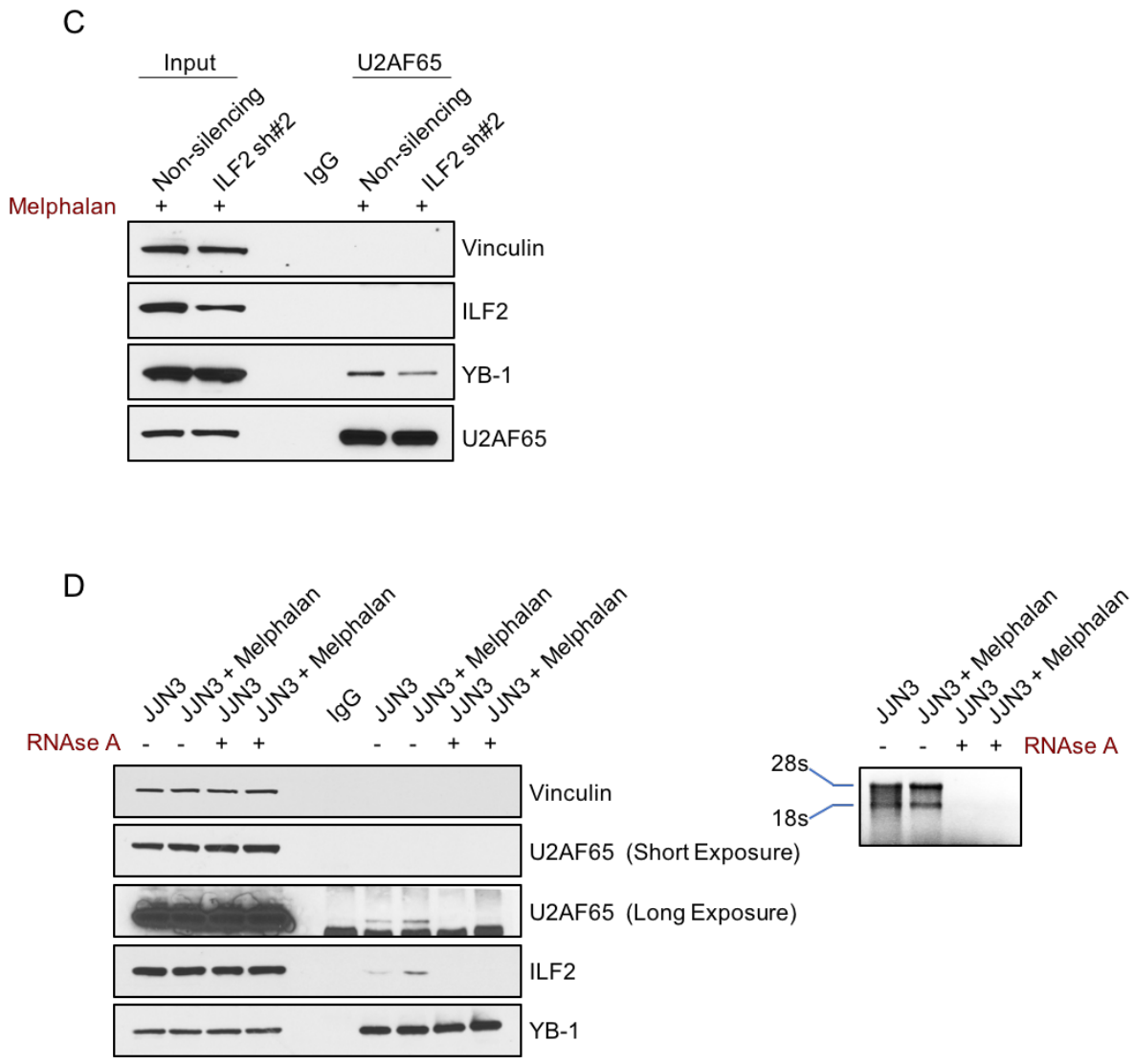



**Figure S5, related to Figure 5. ILF2 Modulates DNA Damage–Induced YB-1 Nuclear Translocation and Splicing Regulation.**

**(A)** Representative Western blot analysis of ILF2, γH2AX, and ILF2 interactors in untreated and melphalan-treated JJN3 cells transduced with non-silencing or ILF2 shRNAs. A representative vinculin is shown as the loading control for all proteins.

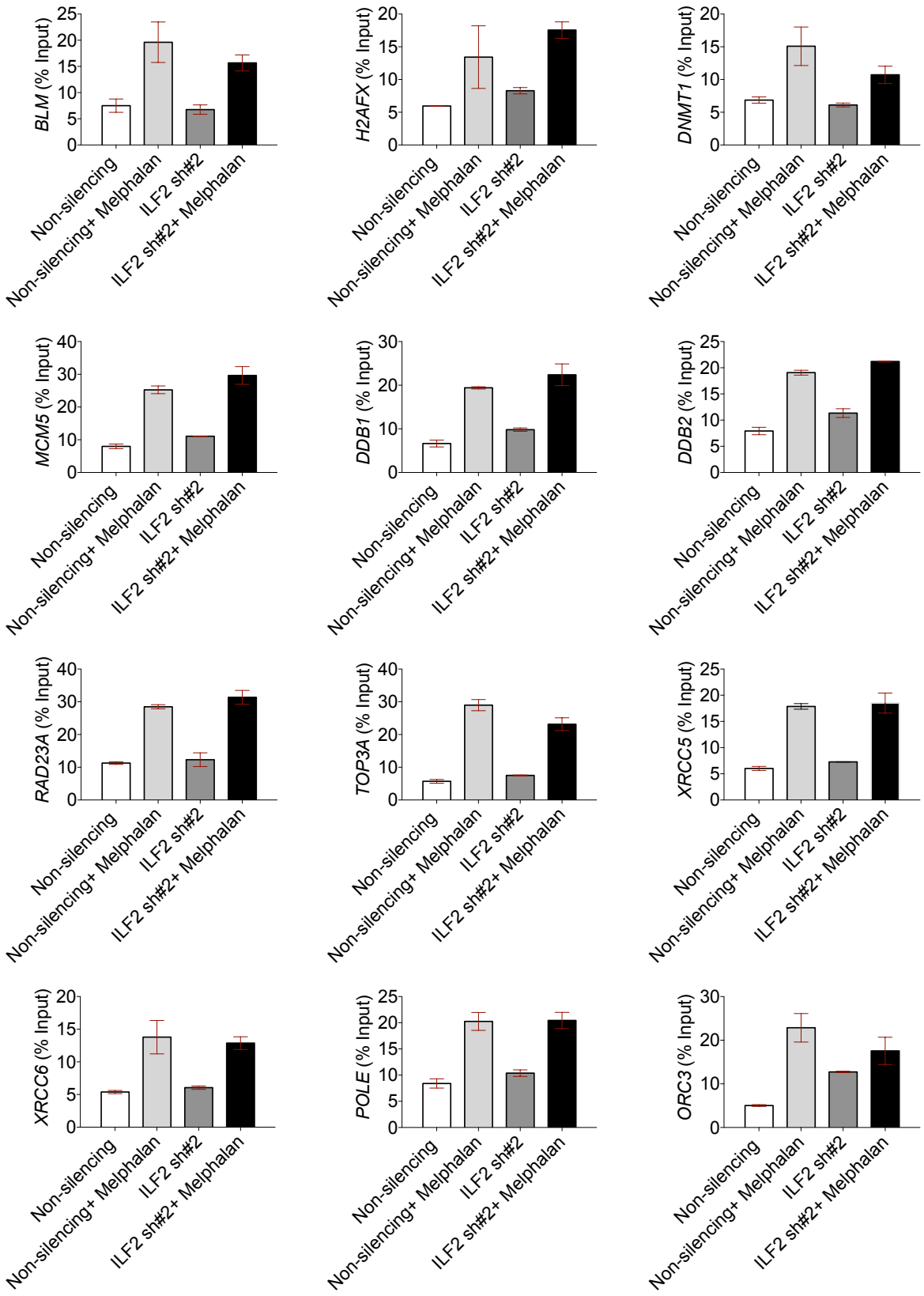
**(B)** Representative confocal microscopy images of anti–YB-1 and anti-ILF2 immunofluorescence in untreated (top) and melphalan-treated (bottom) JJN3 cells (YB-1: red;

ILF2: green; DAPI: blue). Arrowheads indicate areas of eurochromatin inside the nucleus in the panel on the left and YB-1/ILF2 colocalization (yellow) in the middle panel; scale bars represent 10 μm.

**(C)** Western blot analysis of vinculin, ILF2, YB-1, and U2AF65 in the lysates isolated from melphalan-treated non-silencing and ILF2 shRNA–transduced JJN3 cells (input) and immunoprecipitated with anti-IgG or anti– U2AF65 antibodies.

**(D)** Western blot analysis of vinculin, U2AF65 (short or long exposure), ILF2, and YB-1 in lysates isolated from untreated and melphalan-treated JJN3 cells (input) and immunoprecipitated with anti-IgG or anti–YB-1 antibodies. Immunoprecipitates were pre-treated with RNAse A as indicated (left). RNA integrity was checked on a denaturing agarose gel (right).

A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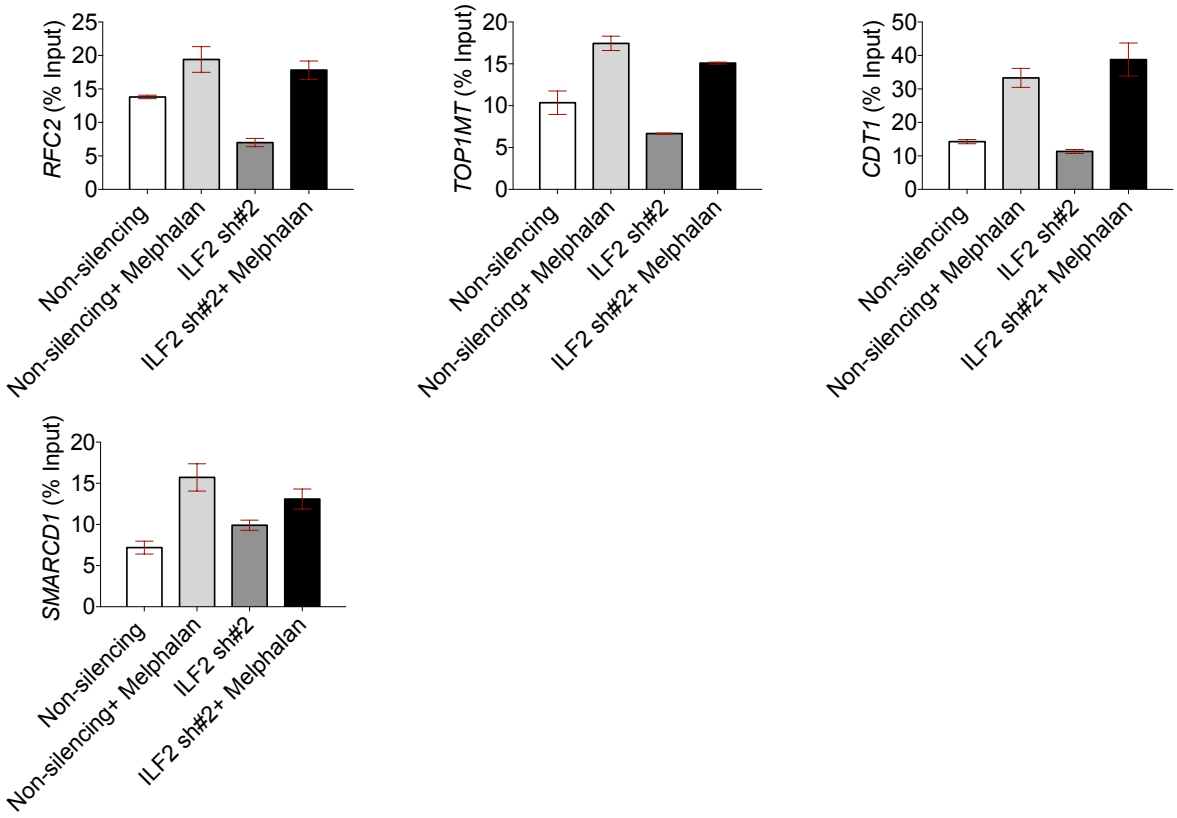
H2AFX (% Input)
DNMT1 (% Input)
MCM5 (% Input)
DDB1 (% Input)
DDB2 (% Input)
RAD23A (% Input)
TOP3A (% Input)
XRCC5 (% Input)
XRCC6 (% Input)
POLE (% Input)
ORC3 (% Input)
Non-silencing
Non-silencing+ Melphalan
ILF2 sh#2
ILF2 sh#2+ Melphalan


RFC2 (% Input)
25
20
15
10
5
0
Non-silencing
Non-silencing+ Melphalan
ILF2 sh#2
ILF2 sh#2+ Melphalan
TOP1MT (% Input)
20
15
10
5
0
Non-silencing
Non-silencing+ Melphalan
ILF2 sh#2
ILF2 sh#2+ Melphalan
CDT1 (% Input)
50
40
30
20
10
0
Non-silencing
Non-silencing+ Melphalan
ILF2 sh#2
ILF2 sh#2+ Melphalan
SMARCD1 (% Input)
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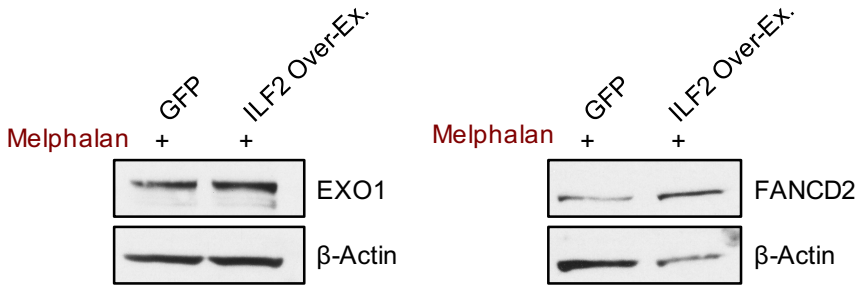
15
10
5
0
Non-silencing
Non-silencing+ Melphalan
ILF2 sh#2
ILF2 sh#2+ Melphalan

B


GFP
ILF2 Over-Ex.
Melphalan + +
EXO1
β-Actin
GFP
ILF2 Over-Ex.
Melphalan + +
FANCD2
β-Actin

C

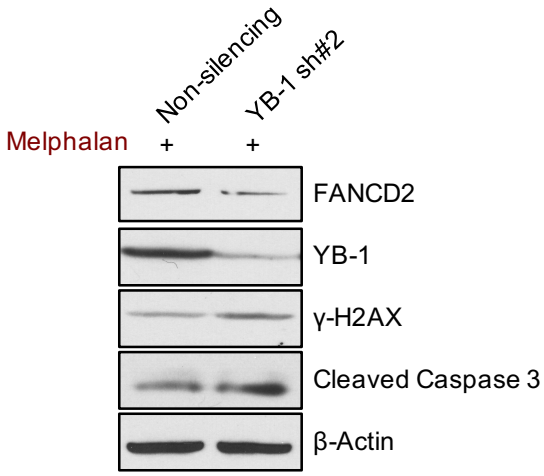

Non-silencing
YB-1 sh#2
Melphalan + +
FANCD2
YB-1
γ-H2AX
Cleaved Caspase 3
β-Actin

**Figure S6, related to Figure 6. ILF2-Mediated YB-1 Nuclear Translocation Regulates the Cotranscriptional Splicing of DNA Repair Transcripts in Response to DNA Damage.**

**(A)** RNA immunoprecipitation–qPCR quantification of YB-1–bound *BLM*, *H2AFX*, *DNMT1*, *MCM5*, *DDB1*, *DDB2*, *RAD23A*, *TOP3A*, *XRCC5*, *XRCC6*, *POLE*, *ORC3*, *RFC2*, *TOP1MT*, *CDT1*, and *SMARCD1* transcripts in untreated and melphalan-treated JJN3 cells transduced with non-silencing or ILF2 shRNA#2. The mean and SD of two independent experiments are shown; data are expressed as percentages of the corresponding input.

**(B)** Representative Western blot analysis of EXO1 (left) and FANCD2 (right) in melphalan-treated GFP- and ILF2-overexpressing JJN3 cells. β-actin was used as the loading control.

**(C)** Representative Western blot analysis of FANCD2, YB-1, γH2AX, and cleaved caspase 3 proteins in melphalan-treated JJN3 cells transduced with non-silencing shRNA or YB-1 shRNA #2. β-actin was used as the loading control.