Supplemental Materials

Molecular Biology of the Cell

Poletto et al.

Nucleophosmin modulates stability, activity and nucleolar accumulation of base excision repair proteins

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SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods

Real-time PCR (Q-PCR)

Q-PCR analyses were performed using a CFX96™ Real-Time PCR Detection System (BioRad). Primers sequences are:

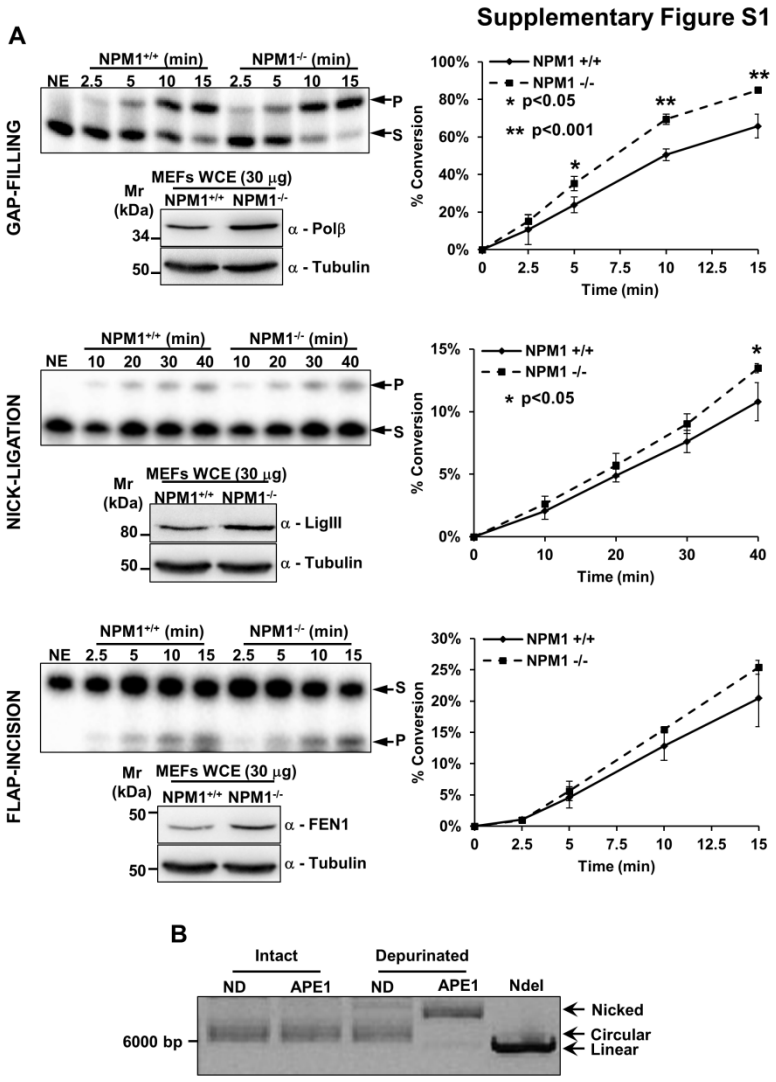
APE1_for: 5'-CGTCACAGCGATGCCAAAGC-3';
APE1_rev: 5'-ATCTGGAGGGTCCTCGTACAGG-3';
FEN1_for: 5'-ATTCGCTCTGCTCCGAACATTCCT-3' (Kleppa et al., 2012);
FEN1_rev: 5'-TCAGCAATTAGTTTGGCAAGGCCG-3' (Kleppa et al., 2012);
Polβ_for: 5'-TGGACTCTGAGTACATTGCTA-3';
Polβ_rev: 5'-TGGCTGTTTGCTGGATTC-3';
Polδcs_for: 5'-CGGCTACAACATTCAGAA-3';
Polδcs_rev: 5'-GATTGGAAGGAGGAGTCA-3';
LigIII_for: 5'-TTGTCTGCTACGAGAGTTC-3';
LigIII_rev: 5'-AATCTGCTTATCATTCAAGTTGT-3';
XRCC1_for: 5'-AATGGCGAGGACCCGTATGC-3' (Saribasak et al., 2011);
XRCC1_rev: 5'-CACGTAGCGGATGAGCCTCC-3' (Saribasak et al., 2011).

Gene expression analysis was carried out using the CFX Manager™ Software (BioRad) and using both *ACTB* and *GAPDH* as reference genes.

Antibodies for Western Blotting and immuno-fluorescence

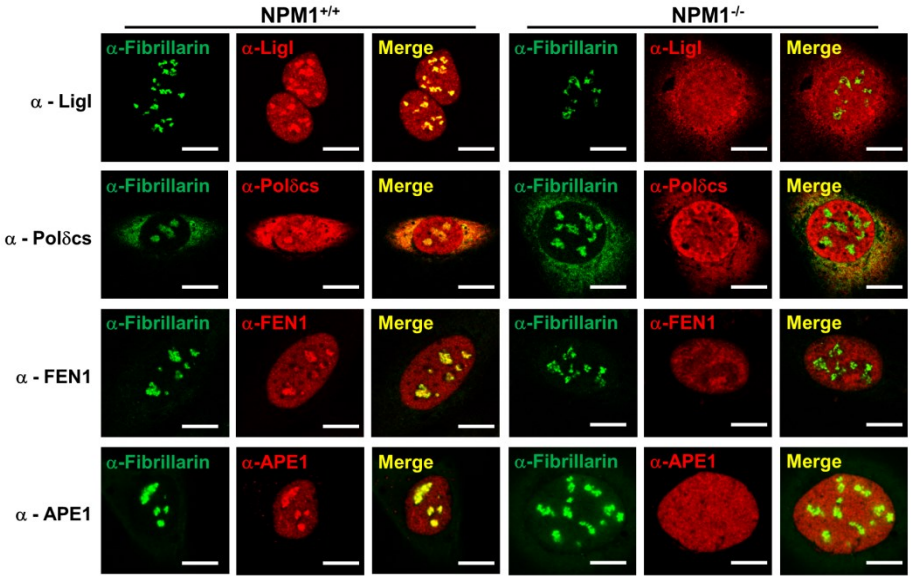
Polyclonal α-APE1 was from Novus (NB 100-101), monoclonal or polyclonal α-NPM1 were from Invitrogen (32-5200) and Abcam (ab15440), respectively, polyclonal α-LigI was a kind gift from Prof Alan Tomkinson (University of New Mexico), monoclonal α-LigIII was from SantaCruz (sc-135883), monoclonal α-XRCC1 was from Thermo Scientific (MS-434-P0), polyclonal α-FEN1 was from Abcam (ab17993), monoclonal α-PCNA was from SantaCruz (sc-56), monoclonal α-Polδcs was from Abcam (ab10362), polyclonal α-Polβ was from Abcam (ab26343).

Supplementary Figures and Legends



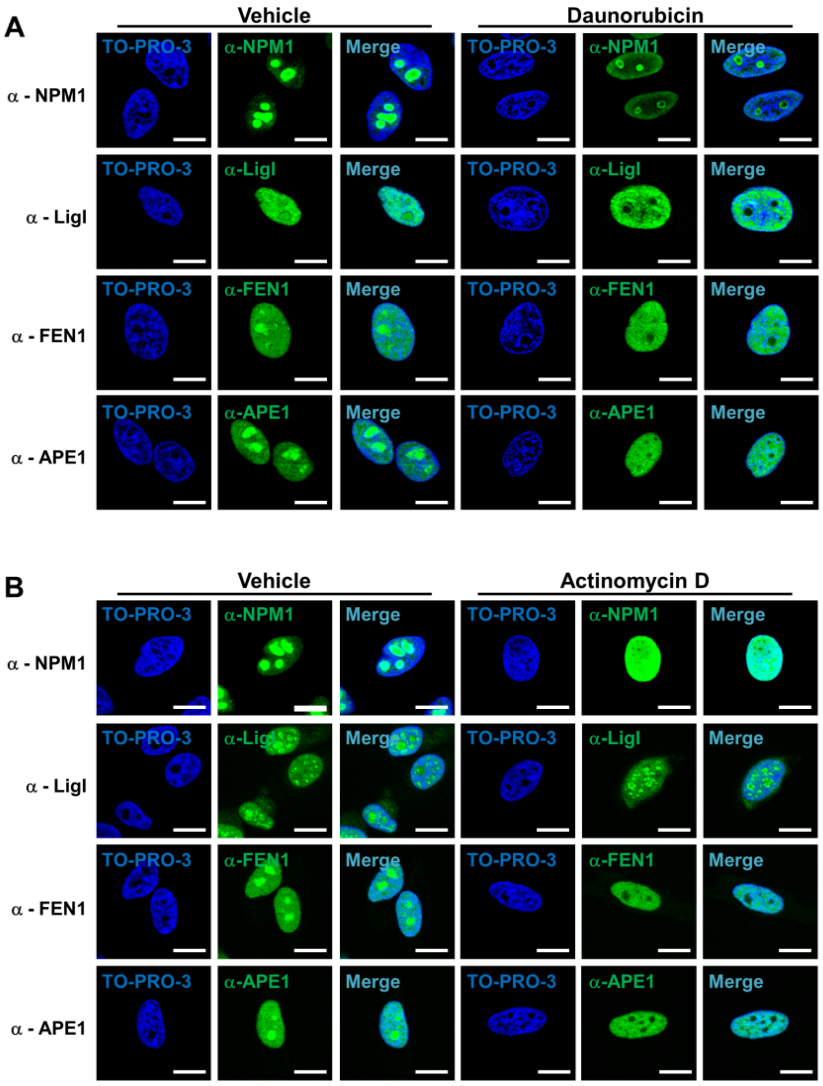
Supplementary Figure S1. Comparison of NPM1$^{+/+}$ and NPM1$^{-/-}$ whole cell extracts at equal whole protein content reveals low differences in the BER capacity. A. *In vitro* BER assays using NPM1$^{+/+}$ and NPM1$^{-/-}$ whole cell extracts at the same protein concentration reveal a slight proficiency in the gap-filling activity for the NPM1$^{+/+}$ cells, but no significant differences in the nick-ligation and flap-incision capacities. For each enzymatic activity we report a representative denaturing gel analysis, along with a Western blotting highlighting the difference in Polβ, LigIII, or FEN1 amounts in the cell extracts used in the assays (*left-hand side*). Graphs (*right-hand side*) describe the percentage of substrate (S) converted to product (P) as a function of time. Values reported are the mean ± SD of at least three independent experimental replicates. B. APE1 incision experiments on the reporter plasmid used in the *in vivo* BER assays. A representative agarose gel shows the generation of spontaneously nicked forms of the plasmid only upon heat-induced depurination. Incision of the substrate with recombinant APE1 results in complete nicking of the depurinated reporter plasmid, therefore indicating the successful introduction of AP-sites. The NdeI-linearized substrate served as migration marker and shows that our protocol does not generate detectable amounts of double-strand breaks on the reporter. "ND": not digested.

Supplementary Figure S2

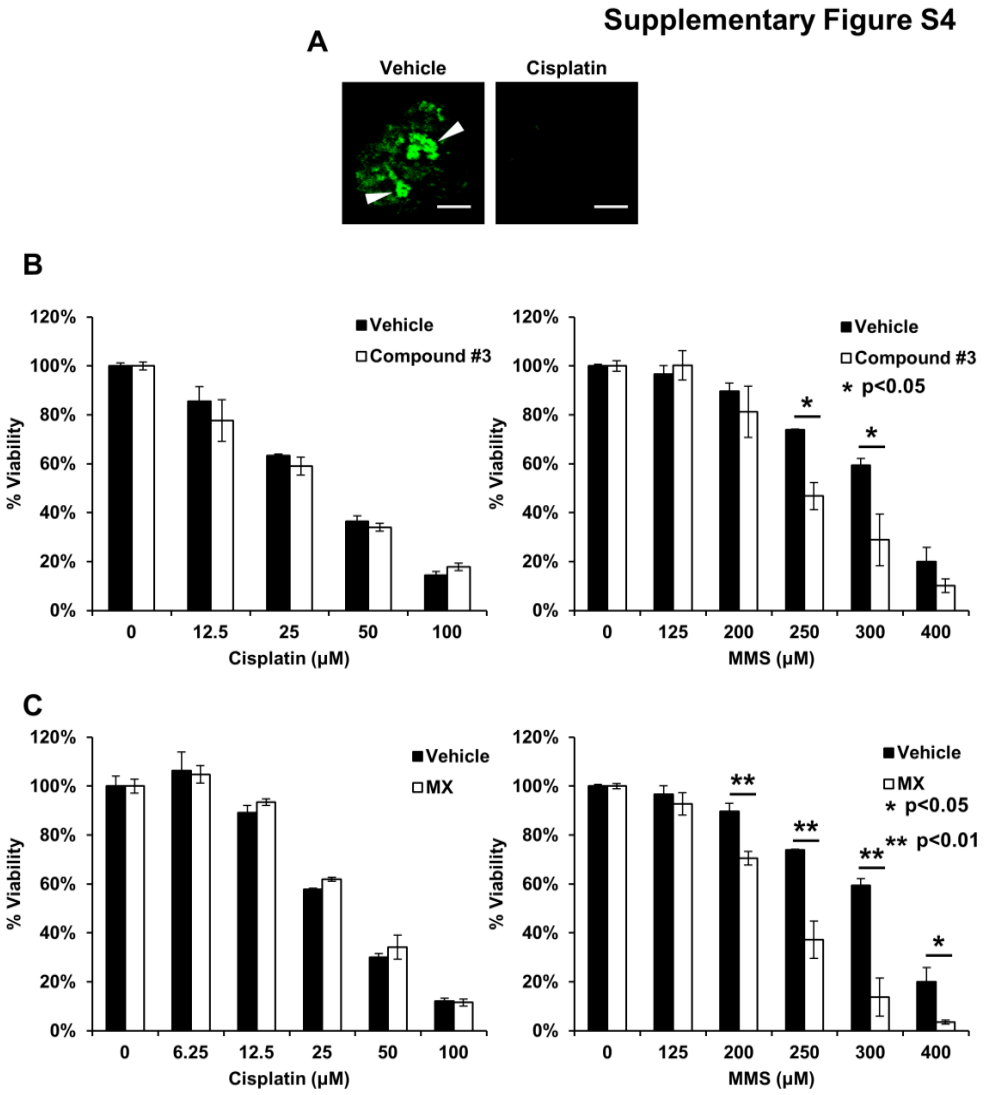


Supplementary Figure S2. Nucleolar structures are intact in NPM1$^{-/-}$ MEFs. Representative immunofluorescence analysis on NPM1$^{+/+}$ and NPM1$^{-/-}$ cells highlights the presence of intact nucleolar structures in both cell lines, as shown by fibrillarin staining (green). BER proteins analyzed (i.e. LigI, Polδcs, FEN1 and APE1) are shown in red and co-localize with fibrillarin only in NPM1$^{+/+}$ cells. Bars correspond to 16 μm.

Supplementary Figure S3



Supplementary Figure S3. Daunorubicin and actinomycin D induce relocalization of NPM1 and BER proteins. A. Representative immuno-fluorescence analysis on the indicated proteins highlight the redistribution upon nucleolar stress induced by daunorubicin treatment (1 μM for 2 hours) in HeLa cells. BER proteins (i.e. APE1, FEN1, LigI) relocalize from nucleoli to the nucleoplasm. B. Immuno-fluorescence analysis in HeLa cells on the indicated proteins upon actinomycin-D (1 μg/ml for 90 minutes). The redistribution from nucleoli to the nucleoplasm is visible for BER proteins, with evident nucleolar emptying for APE1 and FEN1 and peri-nucleolar relocalization for LigI. Nuclei are counter-stained with TO-PRO-3; bars correspond to 16 μm.



Supplementary Figure S4. Inhibition of the APE1 endonuclease activity does not affect cellular sensitivity to cisplatin in APE1^{K4pleA}-expressing cells. A. Fluorouridine (FUrd) incorporation assay on HeLa cells highlights the active transcription in nucleoli of untreated cells (*white arrowheads*); upon cisplatin treatment (100 μM, 6 hours) the nucleolar transcription is completely absent. Bars 4 μm. B. Representative viability assays on HeLa cells stably expressing the APE1^{K4pleA} mutant. Cells were treated for 24 hours with the indicated amount of cisplatin in presence of the APE1 endonuclease inhibitor (N-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide) (compound #3 (Rai et al., 2012)). Inhibition of the APE1 AP-site incision activity does not affect cell sensitivity to cisplatin (*left panel*). Treatment with MMS (24 hours) was used as positive control to verify the sensitizing activity of compound #3 at this dosage (*right panel*). C. Representative viability assays on the APE1^{K4pleA}-expressing HeLa clone. Cells were treated with increasing amounts of cisplatin in presence of 10 mM methoxyamine (MX) and cell viability was measured 24 hours later. Inhibition of the APE1 endonuclease function does not affect cell sensitivity to cisplatin (*left panel*). Treatment with MMS (24 hours) was used as positive control to verify the sensitizing activity of MX at the dosage tested (*right panel*).

Supplementary references

Kleppa et al. (2012). Kinetics of endogenous mouse FEN1 in base excision repair. Nucleic Acids Res. *40*, 9044-9059.

Rai, G., Vyjayanti, V. N., Dorjsuren, D., Simeonov, A., Jadhav, A., Wilson 3rd, D. M., and Maloney, D. J. (2012). Synthesis, biological evaluation, and structure-activity relationships of a novel class of apurinic/apyrimidinic endonuclease 1 inhibitors. J Med Chem. *55*, 3101-3112.

Saribasak, H., Maul, R. W., Cao, Z., McClure, R. L., Yang, W., McNeill, D. R., Wilson 3rd, D. M., and Gearhart, P. J. (2011). XRCC1 suppresses somatic hypermutation and promotes alternative nonhomologous end joining in Igh genes. J Exp Med. *208*, 2209-2216.