Appendix

Yosef et al,

p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling

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Appendix Figure Legends

Appendix Figure S1. Effect of Etoposide on Primary BJ fibroblast. (A) Experimental design and reference time frame scheme. (B) Representative growth curves of growing (G) and Etoposide treated (DNA damage-induced senescent (DIS)) BJ fibroblasts. The time frame corresponds to the scheme presented in plot A. Each curve was performed at least twice, and each time point was determined in triplicate. (C) Pictures of growing and DIS BJ cells stained for SA-β-galactosidase activity at day 7 post etoposide treatment. (D) Quantification of BrdU incorporation (4 hours) in G and DIS cells transduced with sip21 or siCtrl, 4 days post siRNA washout; (E) Western blots of p16, p21 and p53 in G and DIS BJ cells. (F) Survival of DIS BJ cells, transduced with siRNAs targeting p21 or control siRNA three weeks following Etoposide treatment; the indicated time points are following siRNA washout. Data are presented as means ± SEM of 3 repeats, each performed in triplicate.

Appendix Figure S2. p21 knockdown reduces cancer senescent cells survival following DNA damage. (A) Pictures of growing (G) and DNA-damage induced senescence-like (DIS) H1299 lung cancer cells stained for SA-β-galactosidase (pH 6.0) activity at day 7 post etoposide treatment. Pictures are at the same magnification. (B) Immunofluorescence analysis of p-γH2AX in G and DIS BJ cells transduced with sip21 or siCtrl; representative images of DIS cells in at least two independent experiments are shown. (C) Cell cycle analysis of G and DIS H1299 cells; representative histograms of DAPI-Area of at least two independent experiments are shown. (D) Survival of G and DIS H1299 cells infected with small hairpin RNA (shRNA) targeting p21 (shp21) or control
shRNA targeting Luciferase (shLuci) prior to etoposide treatment. Western blots show p21 protein levels in the corresponding samples. Data are presented as mean±S.E.M of three repeats, performed in triplicates. Data was analyzed using Student’s t-test. ***P < 0.0005.

Appendix Figure S3. Cell Cycle pathway from WikiPathways. (A) An overview of the series of events that takes place in a cell leading to its division and duplication. In Blue are the genes that their expression is altered following p21 knockdown only in DIS cells are depicted blue in the pathway map. ([http://www.wikipathways.org/index.php/Pathway:WP179](http://www.wikipathways.org/index.php/Pathway:WP179)).

Appendix Figure S4. DNA damage response pathway from WikiPathways. (A) An overview of gene products, processes and changes in the DNA damage response pathway following p21 knockdown are depicted blue in the pathway map. ([http://www.wikipathways.org/index.php/Pathway:WP707](http://www.wikipathways.org/index.php/Pathway:WP707)).

Appendix Figure S5. TGF-β signaling pathway from WikiPathways. (A) The interactions and intersections between canonical and non-canonical TGF-β signaling that are altered following p21 silencing in DIS cells are depicted blue in the pathway map. ([http://www.wikipathways.org/index.php/Pathway:WP366](http://www.wikipathways.org/index.php/Pathway:WP366)).

Appendix Figure S6. Focal adhesion pathway from WikiPathways. (A) Cell-matrix adhesions and interactions that are altered following p21 silencing in DIS cells are depicted in blue the pathway map. ([http://www.wikipathways.org/index.php/Pathway:WP306](http://www.wikipathways.org/index.php/Pathway:WP306)).
Appendix Figure S7. Hepatic stellate cell activation pathway from Ingenuity. (A) Predicted changes in the activation state of hepatic stellate cells (HSCs) in liver fibrosis following p21 knockdown are depicted in the pathway map. Green: downregulated genes; Red: upregulated genes. (https://targetexplorer.ingenuity.com/pathway/ING/ING:1nilk#!/api/rest/v1/client/searchPathwayNodes?pathwayId=ING:1nilk&rows=0&facetLimit=5000&responseType=default).

Appendix Figure S8. Quantitative analysis of the distribution of γH2AX foci in G and DIS cells transduced with sip21 or siCtrl. ImageStreamX analysis of the distribution of numbers of γH2AX foci was performed on G and DIS cells transduced with sip21 or siCtrl 3 days after siRNA washout. DIS cells stained with the secondary antibodies only and DAPI served as a negative control. Average percent of positive cells in each sample was calculated. Data are presented as mean±S.E.M of three repeats. **P < 0.005.

Appendix Figure S9. Partial rescue of DIS cell death following p21 knockdown by z-VAD-fmk. (A) Percentage survival of DIS BJ cells treated with siRNAs targeting p21 or control siRNA, with or without incubation with the cell-permeable, irreversible pan-caspase inhibitor z-VAD-fmk (z-VAD). Treatment with the inhibitor only partially rescues the cells from sip21-induced decrease in cell viability. The western blots show p21, cleaved PARP and caspase-3 proteins level following siRNA treatment. Data were presented as mean±S.E.M of three biological triplicates. Data was analyzed using Student’s t-test. *P < 0.05.
Appendix Figure S10. Schematic presentation of molecular events that occur in senescent cells following p21 knockdown.
**Appendix Table Legends**

**Appendix Table S1, related to Figure 2: Cell cycle pathway altered genes.**
List of genes significantly changed in cell cycle pathway from WikiPathways following p21 silencing.

**Appendix Table S2, related to Figure 2: DNA damage response pathway altered genes.** List of genes significantly changed in DNA damage response pathway from WikiPathways following p21 silencing.

**Appendix Table S3, related to Figure 2: TGF-β signaling pathway altered genes.** List of genes significantly changed in TGF-β signaling pathway from WikiPathways following p21 silencing.

**Appendix Table S4, related to Figure 2: Focal adhesion pathway altered genes.** List of genes significantly changed in Focal adhesion pathway from WikiPathways following p21 silencing.
Appendix Figure S1
Appendix Figure S2
Appendix Figure S3
Appendix Figure S4
Appendix Figure S5

TGF beta Signaling Pathway

[Diagram of TGF beta Signaling Pathway]

LEGEND

[Legend for TGF beta Signaling Pathway]
Appendix Figure S6
Appendix Figure S7
Appendix Figure S8
Appendix Figure S10
## Appendix tables

### Appendix Table S1.

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