

1 Supplementary information. Pintado-Berninches et al:

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3 Supplementary figure legends

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5 Supplementary Figure 1: **Expression of GSE24.2 and GSE4 in control and AT cells.** a) C-736, C-
6 JY, AT-3189, AT-719, AT-L137IIB lymphoblasts and C-1787, C-3490 and AT-3487 fibroblast cells
7 transduced with LV-CMV-eGFP (GFP) or LV-CMV-GSE24.2-eGFP were obtained. Expression of
8 GSE24.2 was determined by RT-QPCR using specific primers. Expression levels are expressed as
9 relative expression using GAPDH expression as control. Experiments were performed in
10 triplicates. b) C-736, C-JY, AT-3189, AT-719, AT-L137IIB lymphoblasts and C-1787, C-3490 and
11 AT-3487 fibroblast cells transduced with LV-CMV-eGFP (GFP) or LV-CMV-GSE4-eGFP (GSE4)
12 were obtained. Relative expression of GSE4 was determined by RT-QPCR using GAPDH as
13 control. Experiments were performed in triplicates

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15 Supplementary Figure 2: **Immunofluorescence staining of γ -H2AX.** C-1787, C-3490 and AT-3487
16 cells transduced with either GFP, scramble, GSE24.2 or GSE4 CMV virus for 48 hours were, fixed
17 and incubated with antibodies against γ -H2AX and secondary fluorescent antibody. Nuclear DNA
18 was counterstained with DAPI (blue) bar scale 0-25 μ m is indicated. Representative pictures of
19 the experiments are presented.

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21 Supplementary Figure 3: **Immunofluorescence staining of 53BP1.** C-1787, C-3490 and AT-3487
22 cells transduced with either GFP, scramble, GSE24.2 or GSE4 CMV virus for 48 hours were, fixed
23 and incubated with antibodies against 53BP1 and secondary fluorescent antibody. Nuclear DNA
24 was counterstained with DAPI (blue) bar scale 0-25 μ m is indicated. Representative pictures of
25 the experiments are presented.

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28 Supplementary Figure 4. **Role of SOD1 in AT cells expressing GSE24.2 or GSE4.** a) Western blott
29 of SOD1 in cells expressing GSE4. C-736 cells, AT-719 cells expressing GFP, GSE24.2 or GSE4 were
30 used to determine *sod1* protein levels. Protein extracts were obtained and used for western blot
31 analysis of expression of *sod1* by using a specific antibody. As a loading control the membranes
32 were hybridized also with a β -actin antibody. The ratio between expression detected by both
33 antibodies is indicated below each western lane using C-736 cells as control. Experiment was
34 repeated three times with similar results. b) C-736 and AT-3189 cells expressing scramble, GFP
35 or GSE4 were transduced with pGKshSOD1 lentiviral vector. RNA was extracted and *Sod1*
36 expression determined by RT-QPCR and represented in relation to C-736 GFP cells. c) Cells of b)

37 were used to determine the percentage of cells with high intensity with DHE and represented.
38 The experiments were performed in triplicates and repeated three times with similar results.

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40 **Supplementary Figure 5. Long term growth and bleomycin sensitivity of AT cells expressing**
41 **GSE24.2 or GSE4.** a) Growth of AT cells expressing GSE4. AT-L137IIB and AT-719 lymphoblasts
42 were transduced with either LV-CMV-eGFP or LV-CMV-GSE4-eGFP lentiviral vectors. Cells were
43 allowed to grow and every week the number of cells was monitored and expressed as the ration
44 between cells infected with GSE4 to cells expressing GFP. Growth was monitored until 60 days
45 after viral transduction. The experiment was repeated twice with similar results. b) Cell viability
46 to bleomycin. AT-L137IIB and AT-719 lymphoblasts expressing GFP, GSE24.2 or GSE4 were
47 exposed during 72 hours to increasing concentrations of bleomycin. After, the percentage of live
48 cells was determined by MTT assay. C-736 cells were used as controls in every assay. The
49 experiments were repeated three times with similar results and average values and standard
50 deviations of representative experiment showed.

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52 **Supplementary Figure 6. Expression of DKC1 gene and dyskerin protein in control and AT cells.**

53 a) Expression of DKC1. C-736, C-JY, AT-719, AT-L137IIB and AT-3189 cells expressing GFP,
54 GSE24.2 or GSE4 were used to extract RNA and the expression of DKC1 determined by RT-qPCR.
55 The expression level of the control cell line C-736 was considered as 1. b) **Expression of dyskerin.**
56 C-736, C-JY, AT-719, AT-L137IIB and AT-3189 and AT-3487 expressing GFP, GSE24.2 and GSE4
57 were used to obtain protein extracts and used for western blot analysis of expression of dyskerin
58 by using a specific antibody. As a loading control the membranes were hybridized also with a β -
59 actin antibody. The ratio between expression detected by both antibodies is indicated below
60 each western lane using C-736 cells as control. Experiment was repeated three times with
61 similar results.

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63 **Supplementary Figure 7. Expression levels of ribosomal RNA genes in control and AT cells.** C-

64 736, C-JY, AT-719, AT-L137IIB and AT-3189 cells expressing GFP, GSE24.2 or GSE4 were used to
65 extract RNA and the expression of rRNA genes 45S (a), 28S (b), 18S (c) and 5.8S (d) determined
66 by RT-qPCR. The expression level of the control cell line C-736 was considered as 1.

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