SUPPLEMENTAL DATA

Greta Caprara, Raffaella Zamponi, Marina Melixetian and Kristian Helin

Isolation and characterization of DUSP11, a novel p53 target gene

MATERIALS AND METHODS

Yeast two-hybrid screen (YTH)

Yeast strains, methods and plasmids pPC97 (DNA binding domain) and pPC86 (activation domain) are described by Chevray and Nathans [34]. Full-length *DUSP11* cDNA was cloned into pPC97 and used as bait, and a proquest human fetal brain library, cloned into pPC86, was used as prey. $1.14x10^6$ clones were screened using Two-Step Selections as previously described [35]. From the resulting clones, DNA was prepared and sequenced.

Primer list

Primers for the generation of *DUSP11* phosphatase-inactive mutant (Cys152→Ser152)

Forward primer: 5'-CTTATTGGTGTCCACT**C**TACCCATGGTTTAAACAGG-3'

Reverse primer: 5'-CCTGTTTAAACCATGGGTA**G**AGTGGACACCAATAAG-3'

shRNA oligonucleotides

shDUSP11-a (MSCV-based pLMP retroviral vector):

TGCTGTTGACAGTGAGCGCGAACTTGGACTGATTATTGATTAGTGAAGCCACAGA
TGTAATCAATAATCAGTCCAAGTTCTTGCCTACTGCCTCGGA

shDUSP11-b (MSCV-based pLMP retroviral vector):

TGCTGTTGACAGTGAGCGCTCTGTTATTCCAGTCTTAAATTAGTGAAGCCACAGA
TGTAATTTAAGACTGGAATAACAGATTGCCTACTGCCTCGGA

p21 (pRetroSuper-based retroviral vector):

Forward oligo:

GATCCCCGACCATGTGGACCTGTCACTTCAAGAGAGTGACAGGTCCACATGGTCT
TTTTGGAAA

Reverse oligo:

GGGCACTGTCCAGGTGTACCAGAAGTTCTCTCTGGTACACCTGGACAGTGAAAA
ACCTTTTCGA

Real-time quantitative PCR (qPCR) primers

ACTIN

Forward primer: 5'-TGCAGGTTGGATGGTCAGACAC-3'

Reverse primer: 5'-GCCAAGACCACCAGCACG-3'

p21

Forward primer: 5'-GGCAGACCAGCATGACAGATTTC-3'

Reverse primer: 5'-CGGATTAGGGCTTCCTCTTGG-3'

DUSP11

Forward primer: 5'-CATTGAGAACTTCCCTTGCAAAT-3'

Reverse primer: 5'-AAAGATGGCCTTTGGGTCAATA-3'

Chromatin immunoprecipitation (ChIP) primers

Promoter AchR

Forward primer: 5'-CCTTCATTGGGATCACCACG-3'

Reverse primer: 5'-AGGAGATGAGTACCAGCAGGTTG-3'

Promoter p21

Forward primer: 5'-GTGGCTCTGATTGGCTTTCTG-3'

Reverse primer: 5'-CTGAAAACAGGCAGCCCAAG-3'

Promoter HDM2

Forward primer: 5'-GGTTGACTCAGCTTTTCCTCTTG-3'

Reverse primer: 5'-GGAAAATGCATGGTTTAAATAGCC-3'

Promoter DUSP11 (SITE A)

Forward primer: 5'-TGGGCTTCATCGTTTGTGGT-3'

Reverse primer: 5'-GACATCGCCCCTTTCAGCTAC-3'

Promoter DUSP11 (SITE B)

Forward primer: 5'-TTTAATAAGTGGAGTGGCGAAATG-3'

Reverse primer: 5'-CAAATTGTATGTTAGCCTTGTTTCCTT-3'

FIGURES LEGENDS

Supplementary Figure 1. FACS analysis of RKO cells or RKO cells expressing E6 treated with Actinomycin D or Doxorubicin.
(**A**) Cells were treated with 5 nM Actinomycin D (ActD) for 48h, before fixation. +: treated cells; -: untreated cells. Percentage of apoptotic and G1 arrested cells, are indicated.
(**B**) Cells were treated with 0.3 μg/ml Doxorubicin (Doxo) for 48h, before fixation. +: treated cells; -: untreated cells. Percentage of apoptotic and G1 arrested cells are indicated.

Supplementary Figure 2. Ectopic expression of DUSP11 inhibits cell proliferation.
U2OS cells were stably transfected as described in Fig. 5A and plated at low density. Cells were then fixed and stained with crystal violet.

Supplementary Figure 3. Estimation of transfection efficiency for the cells used to perform the Colony formation assay, described in Figure 5.
Cells were transfected with the indicated plasmids plus one tenth of the pCMV EGFP expression plasmid. The percentage of cells expressing EGFP was analyzed by FACS.

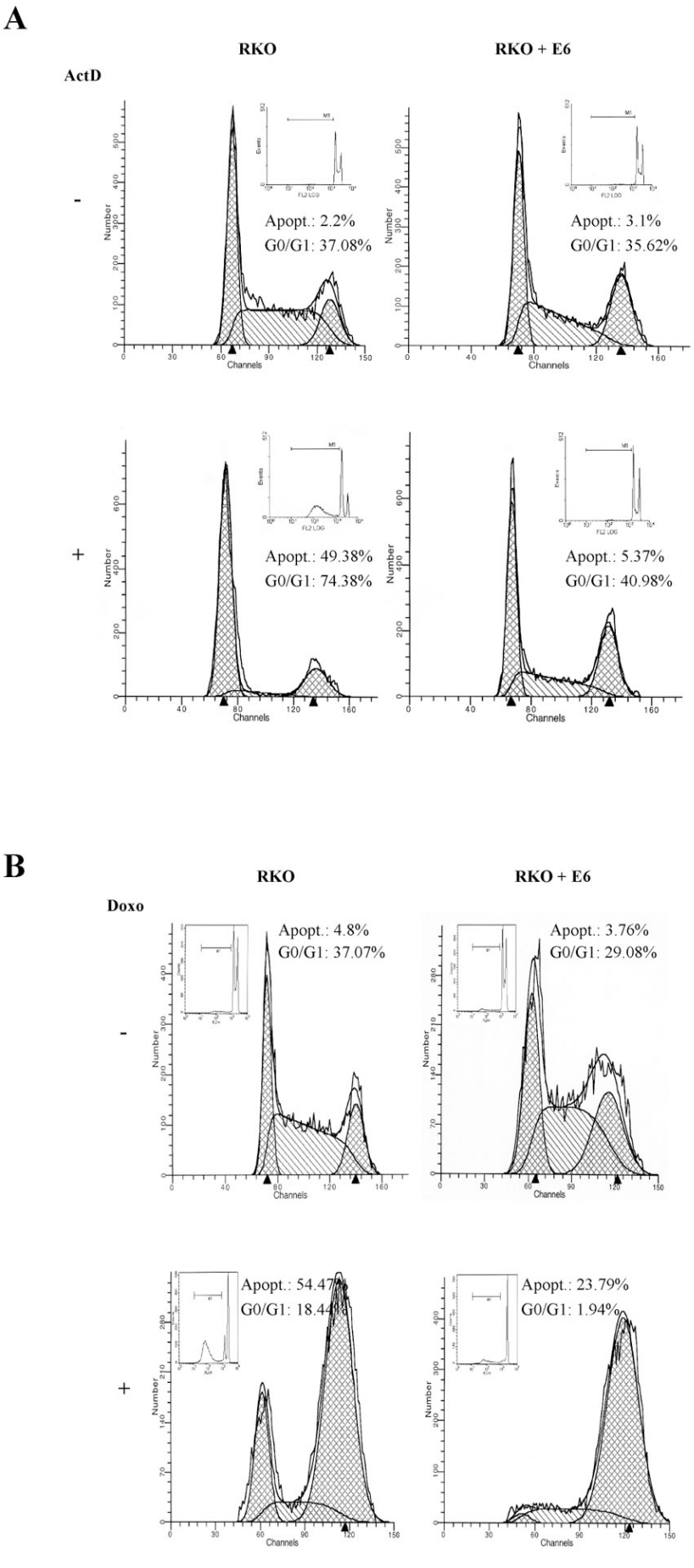
Supplementary Figure 4. Inhibition of *DUSP11* expression by shRNA stimulates cell proliferation and protects from DNA damage.
(**A**) U2OS cells were infected as described in Fig. 6B. Once selected, cells were plated at low density. Cells were then fixed and stained with crystal violet.
(**B**) U2OS cells were infected as described in Fig. 6C. Once selected, cells were treated with UV 15 J/m^2 and then plated at low density. Cells were then fixed and stained with crystal violet.
(**C**) U2OS cells were infected as described in Fig. 6D. Once selected, cells were treated with Doxorubicin 0.3 μg/ml and then plated at low density. Cells were then fixed and stained with crystal violet.

SUPPLEMENTAL REFERENCES

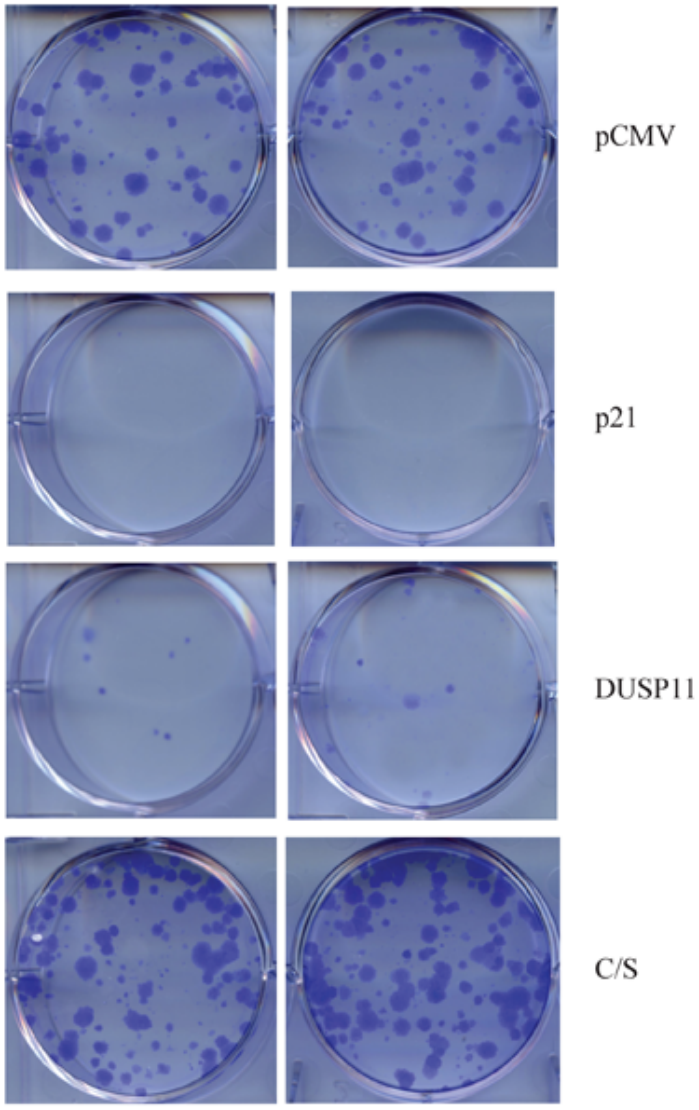
34. **Chevray PM, Nathans D.** Protein interaction cloning in yeast: identification of mammalian proteins that react with the leucine zipper of Jun. Proc Natl Acad Sci U S A. 1992 Jul 1;89(13):5789-93.

35. **Walhout AJ, Vidal M.** High-throughput yeast two-hybrid assays for large-scale protein interaction mapping. Methods. 2001 Jul;24(3):297-306.

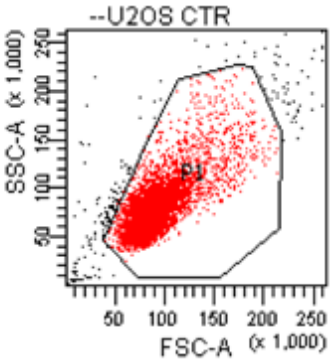
Supplementary Figure 1.

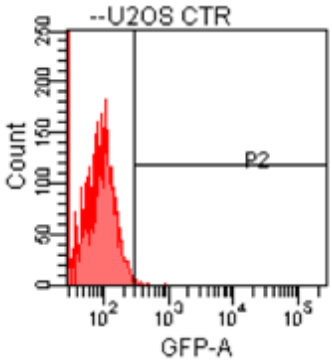


Supplementary Figure 2.



Supplementary Figure 3.





Tube Name: U2OS CTR
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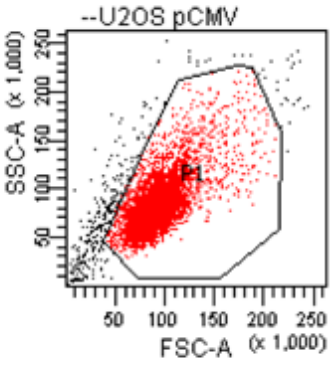
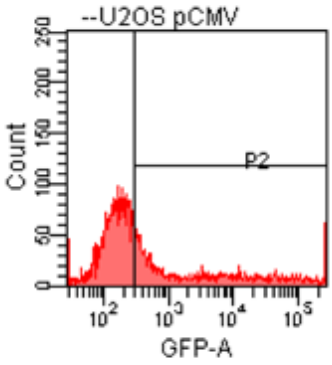
Population	#Events	%Parent	GFP-A Mean
P1	4,975	95.99	88
P2	27	0.54	359





Tube Name: U2OS pCMV
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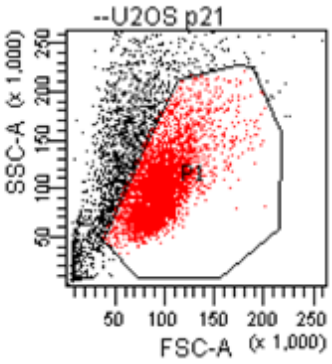
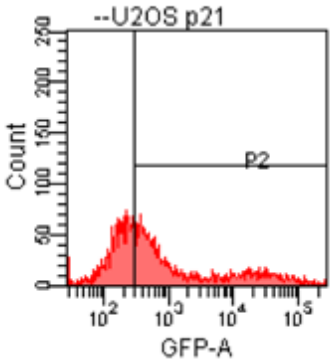
Population	#Events	%Parent	GFP-A Mean
P1	4,950	93.57	15,726
P2	2,185	44.14	35,434





Tube Name: U2OS p21
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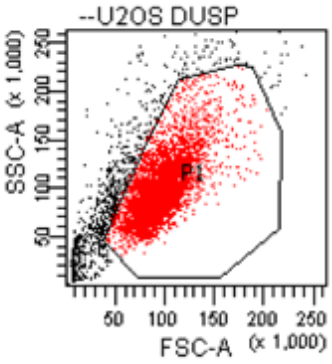
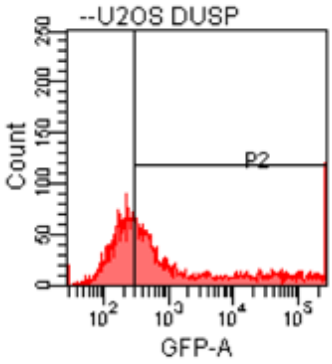
P1	4,390	69.52	9,035
P2	2,663	60.66	14,786





Tube Name: U2OS DUSP
Record Date: Sep 18, 2008 11:18:13 AM

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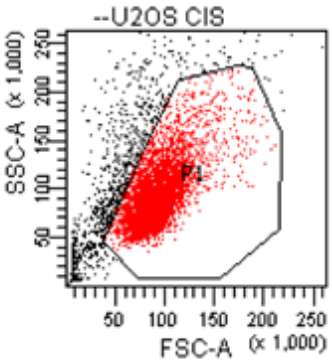
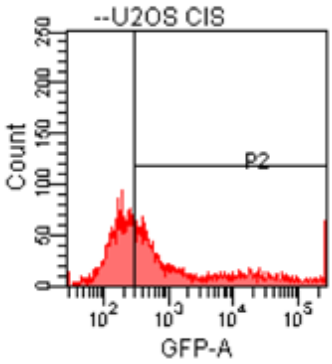
P1	4,759	84.23	26,329
P2	2,987	62.77	41,846





Tube Name: U2OS CIS
Record Date: Sep 18, 2008 11:18:44 AM

Population	#Events	%Parent	GFP-A Mean
P1	4,715	83.25	17,442
P2	2,770	58.75	29,573

Supplementary Figure 4.