Landes Bioscience

www.landesbioscience.com

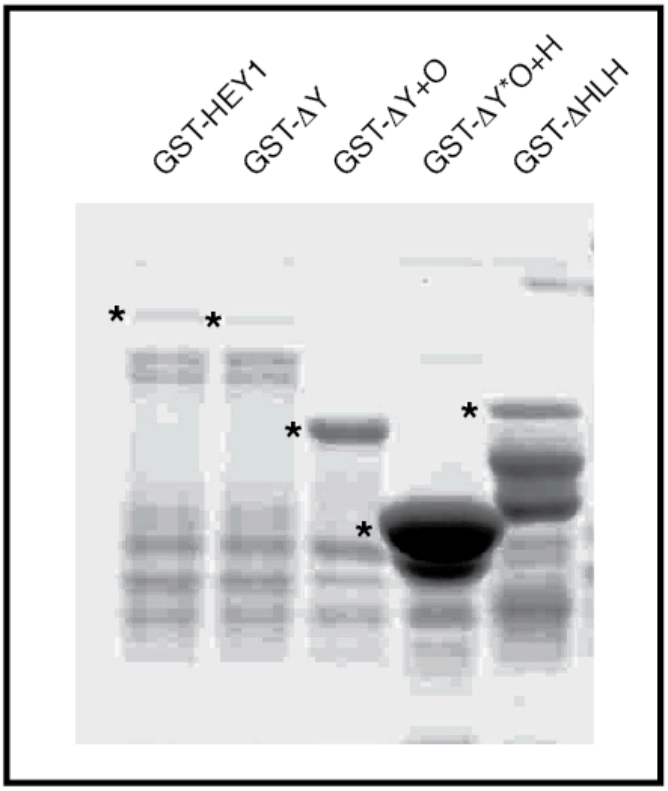
Supplemental Material to:

Irene López-Mateo, Ma. Ángeles Villaronga,
Susana Llanos and Borja Belandia

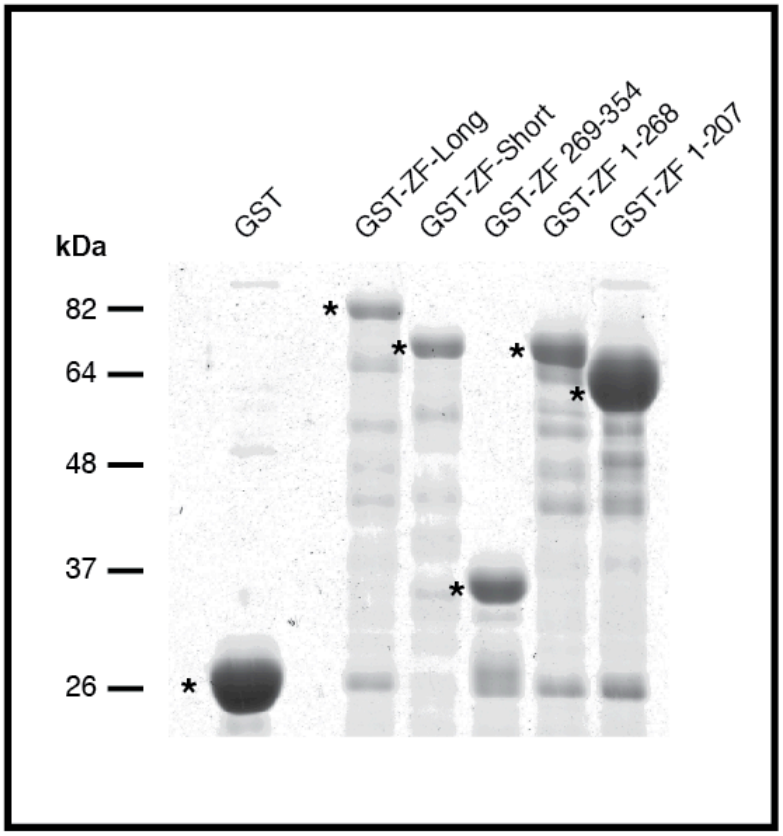
The transcription factor CREBZF is a novel positive regulator of p53

2012; 11(20)
http://dx.doi.org/10.4161/cc.22133

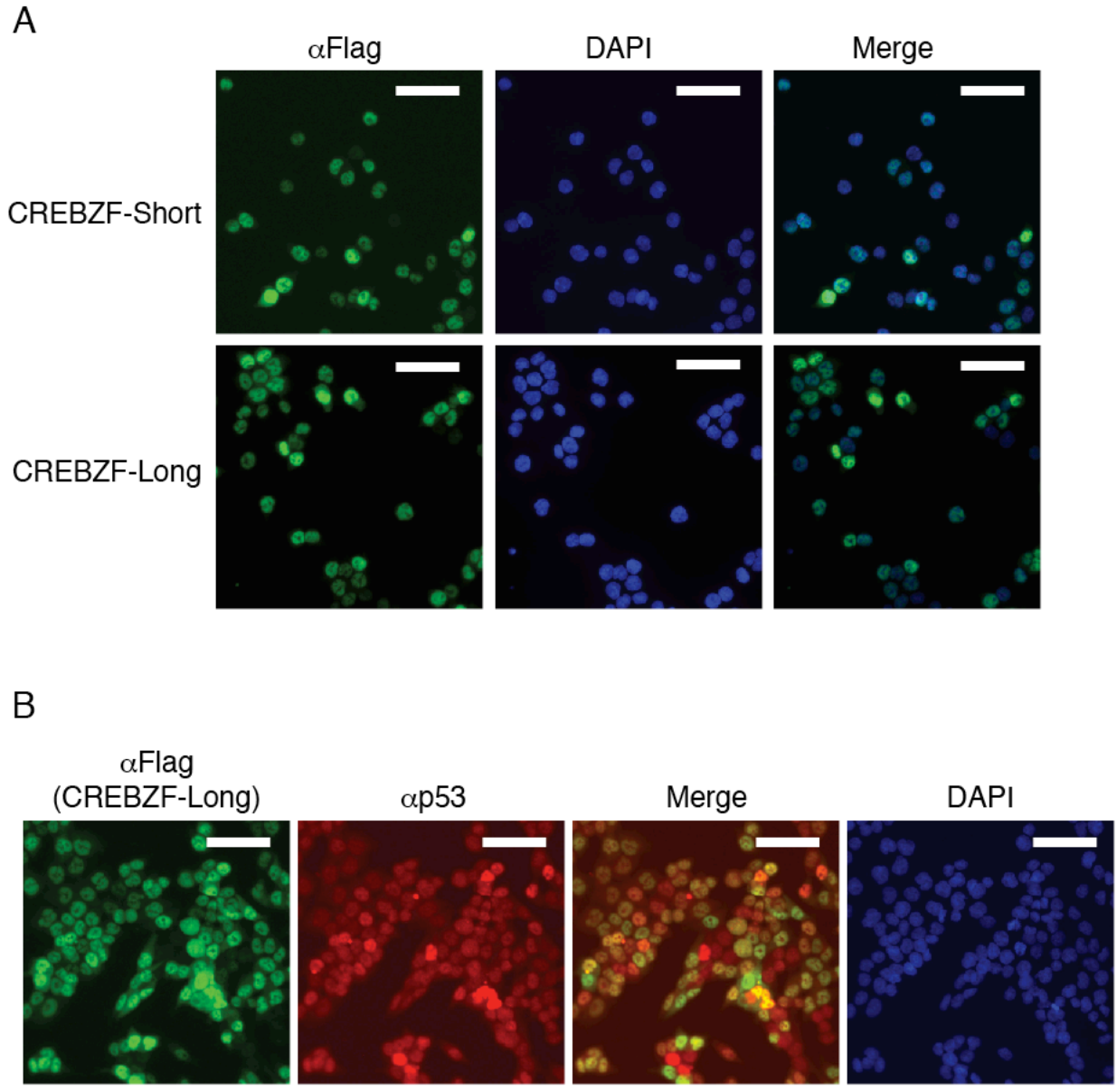
http://www.landesbioscience.com/journals/cc/article/22133



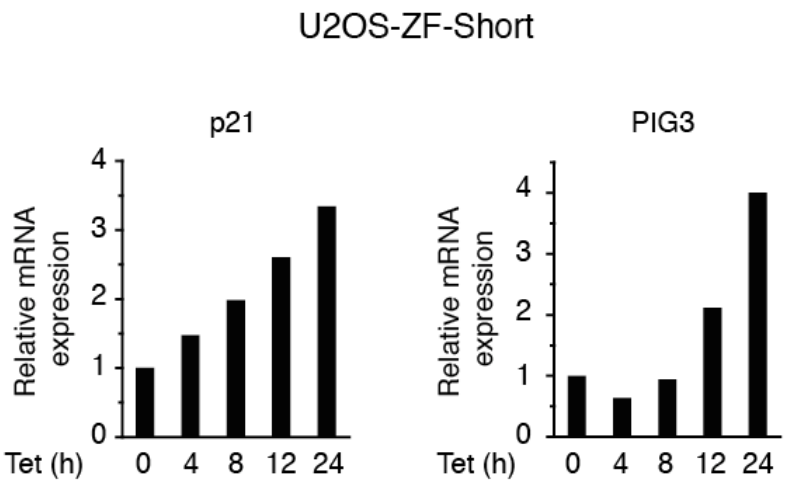
Supplementary figure 1: Coomassie-stained gel shows the levels of the bacterially expressed GST fusion proteins used in the pull-down assays marked with an asterisk



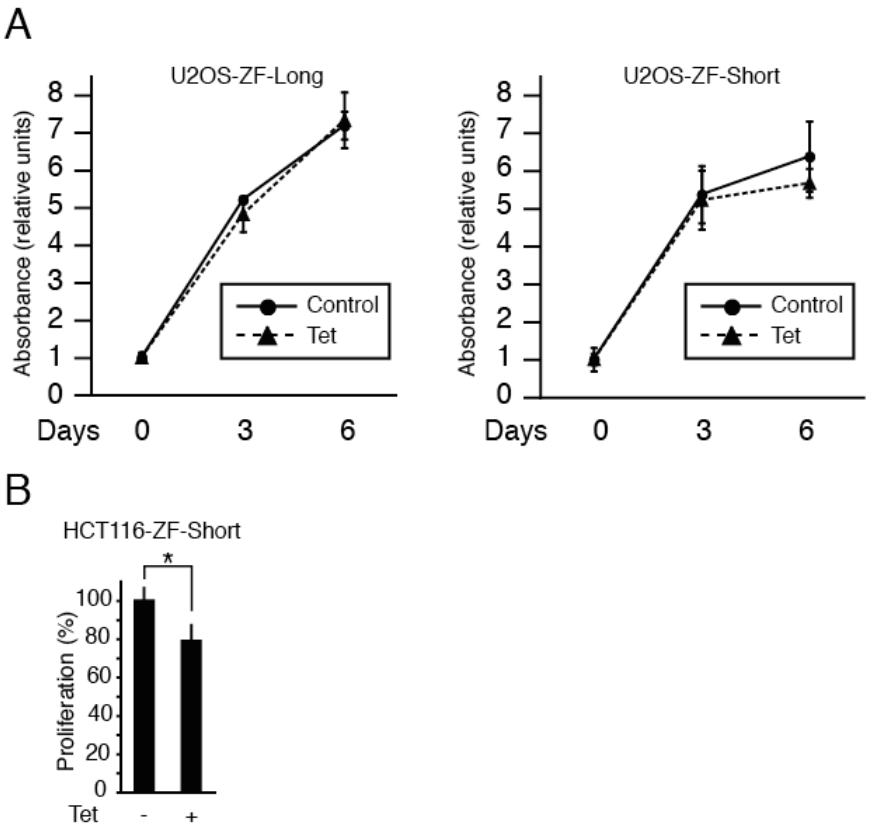
Supplementary figure 2: Coomassie-stained gel shows the levels of the bacterially expressed GST fusion proteins used in the pull-down assays marked with an asterisk



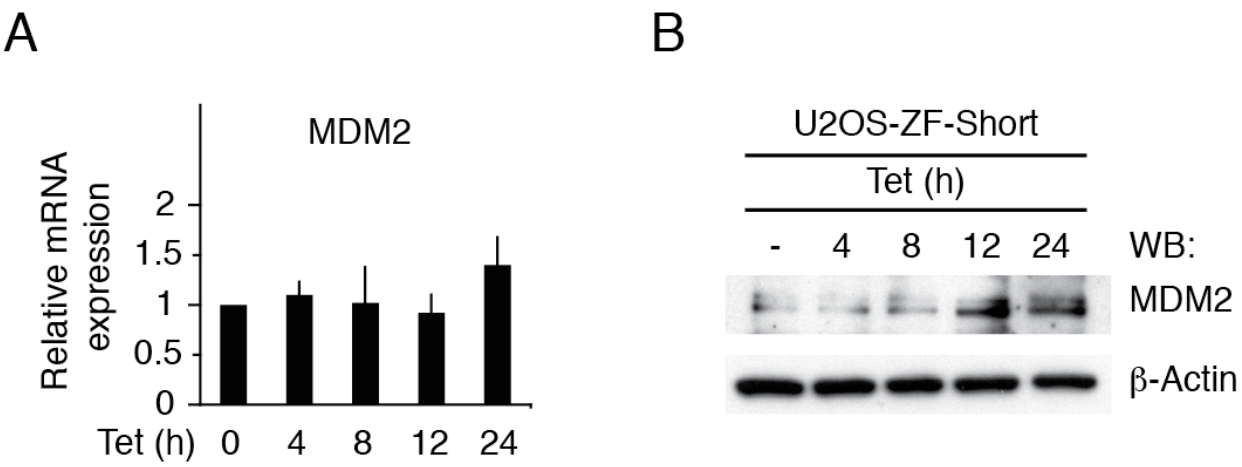
Supplementary figure 3: CREBZF shows nuclear expression in HCT116 cells. (A) HCT116-ZF-Short or Long cells growing in the presence of 1μg/ml tetracycline to induce the expression of flag-tagged CREBZF were assayed by indirect immunofluorescence with anti-Flag antibody. The first column shows the indirect immunofluorescence with anti-Flag antibody (green), the second column shows DAPI staining of DNA (blue) and the third column shows the merge image indicating the degree of colocalization of green and blue fluorescence. (B) HCT116-ZF-Long cells growing in the presence of 1μg/ml tetracycline were assayed by indirect immunofluorescence with anti-Flag antibody and anti-p53 antibody. The first column shows the indirect immunofluorescence with anti-Flag antibody (green), the second column shows the indirect immunofluorescence with anti-p53 antibody (red), the third column shows the merge image indicating the degree of colocalization of green and red fluorescence and the fourth column shows DAPI staining of DNA (blue). Bars, 50 μm.



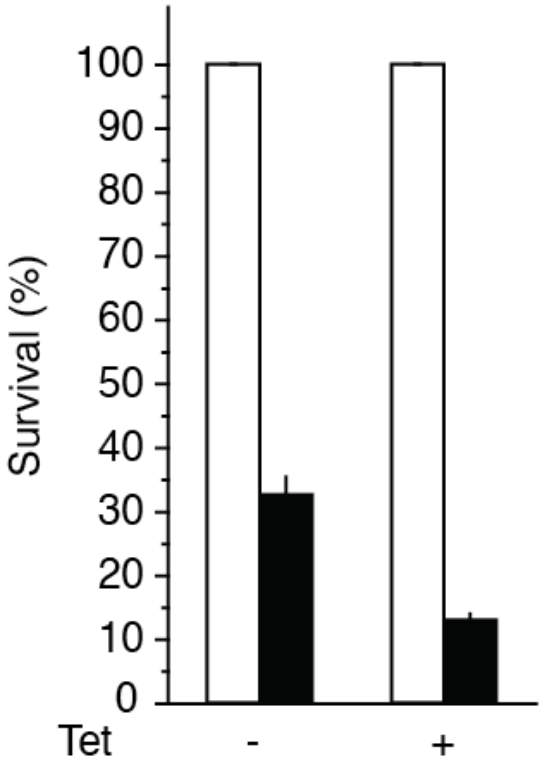
Supplementary figure 4: CREBZF expression in U2OS cells induces the expression of p53-target genes. Quantitative RT-PCR analysis for the expression of p21 and PIG3 transcripts in U2OS-ZF-Short cells treated with 1 μg/ml tetracycline (Tet) for the indicated times. Results from a representative experiment are shown.



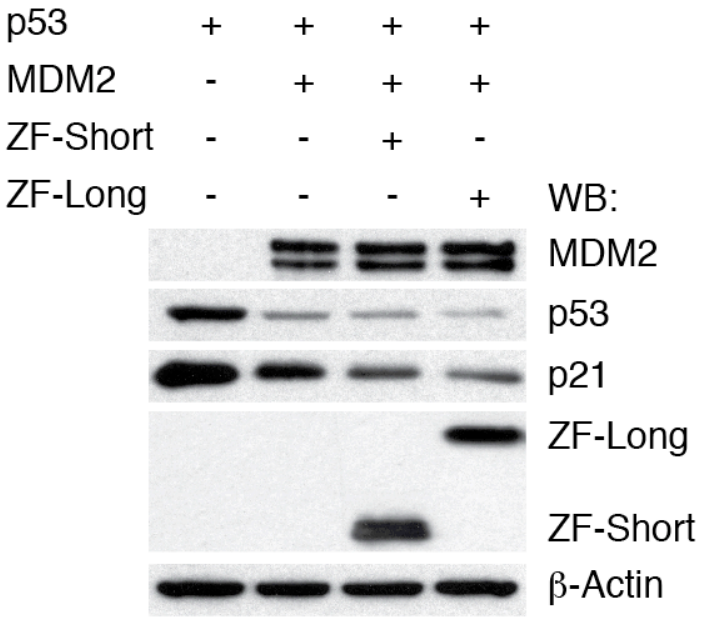
Supplementary figure 5: Effects of CREBZF expression on cellular proliferation. (A) CREBZF expression does not affect U2OS proliferation rate. U2OS cells expressing tetracycline-inducible ZF-Short or ZF-Long were plated into 96-well plates (2500 cells per well). After 24 h 1 μg/ml of tetracycline was added and cell proliferation was measured at different time points. Medium and tetracycline were replaced every 3 days. Cell growth was determined in quadruplicates by using MTS assay. The results shown represent the averages of results of two independent experiments assayed in quadruplicate ± s.d. (B) CREBZF expression attenuates moderately HCT116 proliferation rate. HCT116-ZF-Short cells were untreated or treated with 1 μg/ml tetracycline (Tet) to induce ZF-Short expression. 24 hours after tetracycline treatment cells were seeded at low density (120.000 cells per 60 mm plate). The cells were cultured for another 4 days in the presence or absence of tetracycline and cell proliferation was determined counting the number of control cells (-) or tetracycline treated cells (+). The results shown represent the averages of results of three independent experiments assayed in quadruplicate + s.d. *P<0.05 relative to control (Student´s t-test).



Supplementary figure 6: Effects of CREBZF expression on MDM2 mRNA and protein levels. (A) Quantitative RT-PCR analysis for the expression of MDM2 transcripts in stable U2OS-ZF-Short cells treated with 1 μg/ml tetracycline (Tet) for the indicated times. The results shown represent the averages of results of three independent experiments + s.d. (B) Immunoblot expression of MDM2 and ß-actin in stable U2OS-ZF-Short cells treated with 1 μg/ml tetracycline (Tet) for the indicated times.



Supplementary figure 7: The expression of ZF-short leads to increased sensitivity to the chemotherapy agent 5-fluorouracil. HCT116-ZF-Short cells were untreated or treated with 1 μg/ml tetracycline (Tet) to induce ZF-Short expression. 24 hours after tetracycline treatment cells were seeded at low density (120.000 cells per 60 mm plate). The cells were cultured for another 4 days in the presence (+) or absence (-) of tetracycline and in the absence (white bars) or in the presence (black bars) of 3 μM 5-fluorouracil. Cell survival was determined counting the number of cells. Normalized values for each group are expressed relative to the drug-free controls. The results shown represent the averages of results of two independent experiments assayed in quadruplicate + s.d.



Supplementary figure 8: CREBZF does not prevent MDM2-mediated p53 degradation in p53-null human lung adenocarcinoma H1299 cells. H1229 cells were plated into 60 mm plates and transfected, where indicated, with 0,01 μg p53, 1 μg MDM2, 2 μg ZF-Long or ZF-Short expression plasmids. 24 hours after transfection cells were harvested for immunoblot analysis with the specified antibodies.