LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1: Conditioned media of cultures of prostate-derived cell lines increase cloning efficiency of LNCaP* cells in DMEM-FCS. A) Increase in cloning efficiency provided by conditioned media. DMEM-FCS culture medium was harvested from subconfluent cultures of PNT1a, LNCaP* and Du145 cells (control is naive DMEM-FCS), filtered through 200 nm pore-sized filters and applied to low cell density culture of LNCaP* cells (4 ml of conditioned media mixed to 4 ml of native DMEM-FCS for 10^4 LNCaP* cells plated in 9-cm diameter dish). B) A low molecular weight fraction increases cloning efficiency. Naive (control) or conditioned DMEM-FCS medium harvested from Du145 cell cultures was ultrafiltrated as indicated with 10, 5, 2 kDa pore-sized filters (Vivaspin 15R_Hydrosart membranes; Vivasciences, Sartorius Stedim Biotech S.A., Aubagne, France), and assayed as in A). Note that dialysis of Du145 conditioned medium against DMEM with a 100-500 Daltons porosity Spectra/por Biotech CE membrane (Spectrum® Laboratories ,Broadwick St, Rancho Dominguez, USA) preserves biological activity (data not shown).

Figure S2: Characterization of cell populations transduced with retroviral expression vectors. A) Decreased p53 activity in p53-R175H-transduced cell populations. About 10^6 cells transduced with control or p53-R175H expressing vectors were cotransfected with the indicated p53 firely luciferase reporter (pGL13LUC (1) or p21-luc (2); 75 to 220 ng) and pRL-Tk (a Renilla luciferase expression vector from Promega, France; 75 to 105 ng) using lipofectamine 2000 (Gibco Invitrogen Corporation, Grand Island, NY, USA) according to manufacturer's instructions (3 µl of lipofectamine per µg of DNA). Cells were lysed 2 days later to measure p53 luciferase reporter activity normalized to pRL-Tk activity with a dual-luciferase assay kit (Promega, France). Ratio of normalized p53 luciferase activity between control and p53-R175H transduced cell populations are shown. Data are the average of two independant experiments, in duplicate or triplicate. B) Western Blot analysis of smad7 expression in control and smad7 transduced cell populations. The indicated cell extracts were analyzed with monoclonal antibodies against smad7 (clone 293039; R&D Systems, England), and α-tubulin (clone B-7, Santa Cruz Bioechnology, Heidelberg, Germany).

BIBLIOGRAPHY

1. Pearson, M., Carbone, R., Sebastiani, C., Cioce, M., Fagioli, M., Saito, S., Higashimoto, Y., Appella, E., Minucci, S., Pandolfi, P. P., and Pelicci, P. G. (2000) *Nature* **406**(6792), 207-210
2. el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) *Cell* **75**(4), 817-825

FIGURE S1

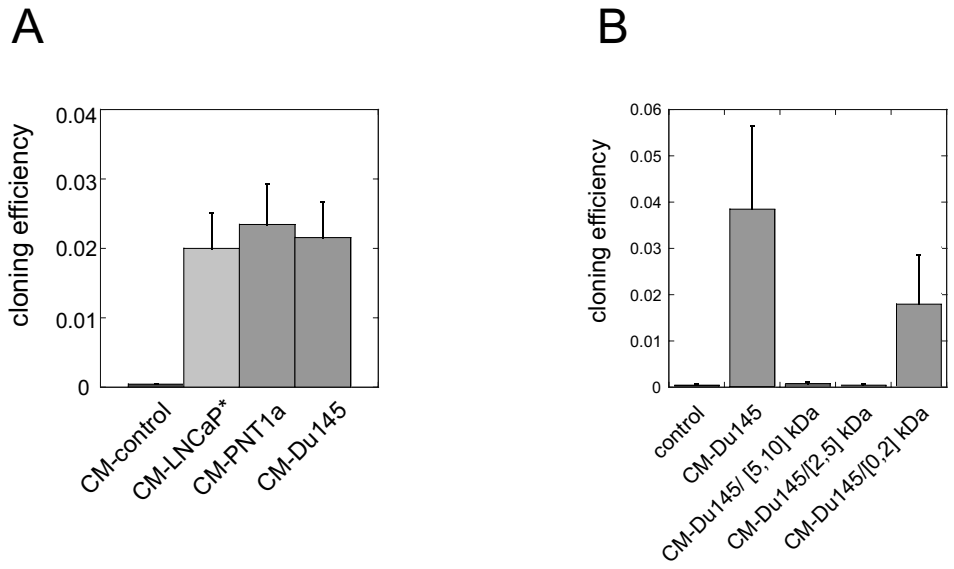
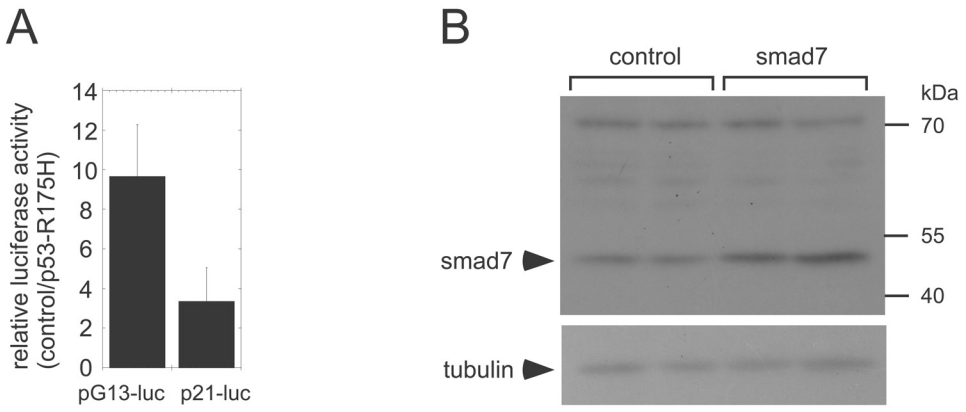

A
B
cloning efficiency
0.04
0.03
0.02
0.01
0
CM-control
CM-LNCaP*
CM-PNT1a
CM-Du145
cloning efficiency
0.06
0.05
0.04
0.03
0.02
0.01
0
control
CM-Du145
CM-Du145/ [5,10] kDa
CM-Du145/[2,5] kDa
CM-Du145/[0,2] kDa

FIGURE S2


A
relative luciferase activity
(control/p53-R175H)
14
12
10
8
6
4
2
0
pG13-luc
p21-luc
B
control
smad7
kDa
70
55
40
smad7
tubulin