



Supplementary Fig. 4

PML-RAR inhibits p53 in PR9-p53ER cells through HDAC recruitment and p53 deacetylation.

(A) PR9-p53ER cells were treated with OHT, Zn and the HDAC inhibitor TSA (50 ng/ml) or RA (10 μ M for 4 hours) or left untreated (n.t.) as indicated. Equal amounts of lysates were analyzed by immunoblotting against p53 (total p53). Anti-RAR immunoblots were performed to detect PML-RAR. (B) Extracts of PR9 -p53ER cells corresponding to equal amounts of p53 were immunoprecipitated with antibodies against p53 ("norm" p53: 1/20th of input), and analyzed by immunoblot using an antibody against acetylated p53. (C) PR9 and PR9-p53ER cells were analyzed with the anti-p53 antibodies DO-1, PAb1801, and FI-393 -recognizing different epitopes of p53 protein- as well as with an anti- ER antibody -recognizing the ER moiety of the p53ER chimera. The DO-1 Antibody recognizes the N-terminal region of p53 and p53ER, and shows only one band in immunoblotting experiments: other antibodies directed versus more C-terminal regions recognize a doublet, suggesting that in U937PR9-p53ER cells alternative usage of 2nd-3rd ATG occurs, as recently described in (Yin, Y., Luciani, M.G. & Fahraeus, R. p53 stability and activity is regulated by Mdm2-mediated induction of alternative p53 translation products. *Nat Cell Biol* **4**, 462-7. (2002)).