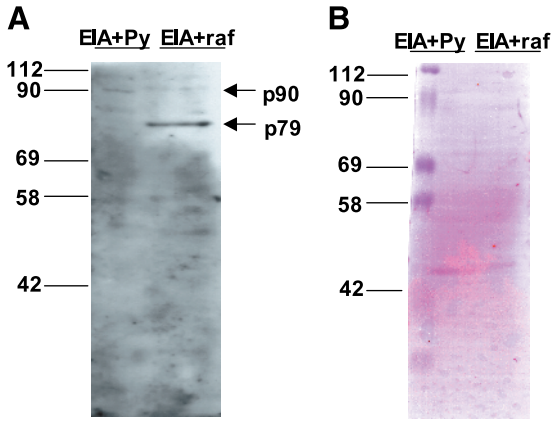

Figure W1. EIA + raf cells express a high level of p79 gag-raf polyprotein. PC EIA + Py and PC EIA + raf cells, grown in normal conditions (5% calf serum), were harvested, and whole-cell lysates were immunoblotted with anti–C-Raf antibodies (A). For control of loading, the filter was stained with Ponceau S (B).

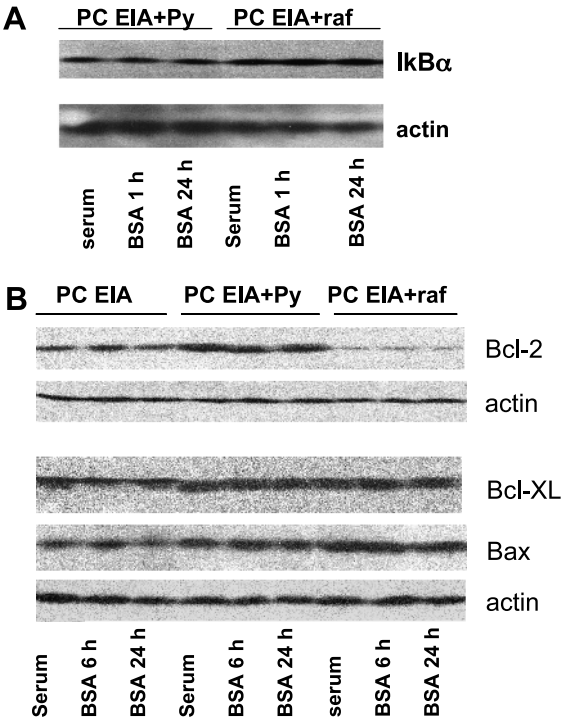

Figure W2. NF-κB is not activated in EIA + raf and EIA + Py cells after growth factor deprivation. EIA + raf cells show lower levels of Bcl2 and equal levels of Bcl-XL and Bax with respect to EIA + Py cells. Cells were kept in the presence of serum or deprived of serum in the presence of 0.2% BSA for 48 hours. At the end of this period in the serum-starved medium, cells were harvested. Whole-cell lysates were prepared and immunoblotted using anti-IκBα, anti-Bcl2, anti–Bcl-XL, and anti-Bax antibodies as outlined in Materials and Methods.

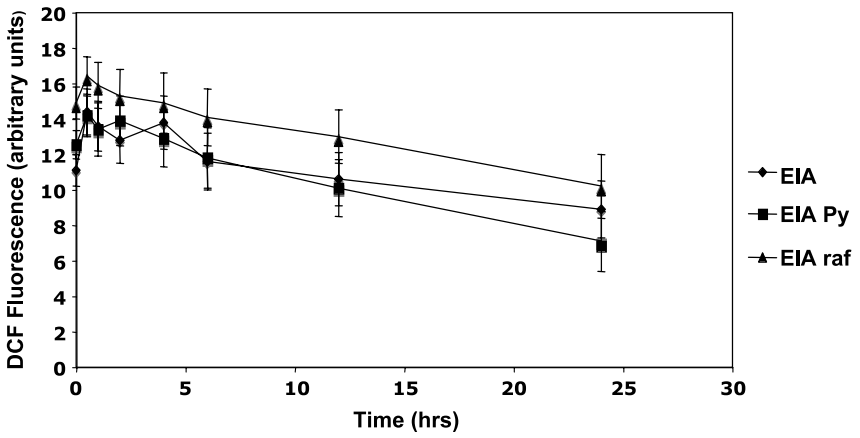

Figure W3. Estimation of intracellular ROS level. Cells were kept in the presence of serum or deprived of serum in the presence of 0.2% BSA for various periods. Floating and adherent cells were collected by mild trypsinization, washed in PBS, and resuspended in PBS, 10 μM 5,6-carboxy-2′,7′-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Inc, Eugene, OR), 5 μg/ml propidium iodide at 37°C, and kept in DCFH-DA thereafter. DCFH-DA is a compound taken up by the cells and trapped in a nonfluorescent deacylated form (DCFH). DCFH is oxidized by ROS to a fluorescent form. After 1 hour of incubation, cells were analyzed by FACScan with excitation at 495-nm and emission at 525-nm wavelengths. Nonintact cells leak DCFH but were stained by propidium iodide and excluded. The results shown are means $\pm$ SD of at least three different experiments.

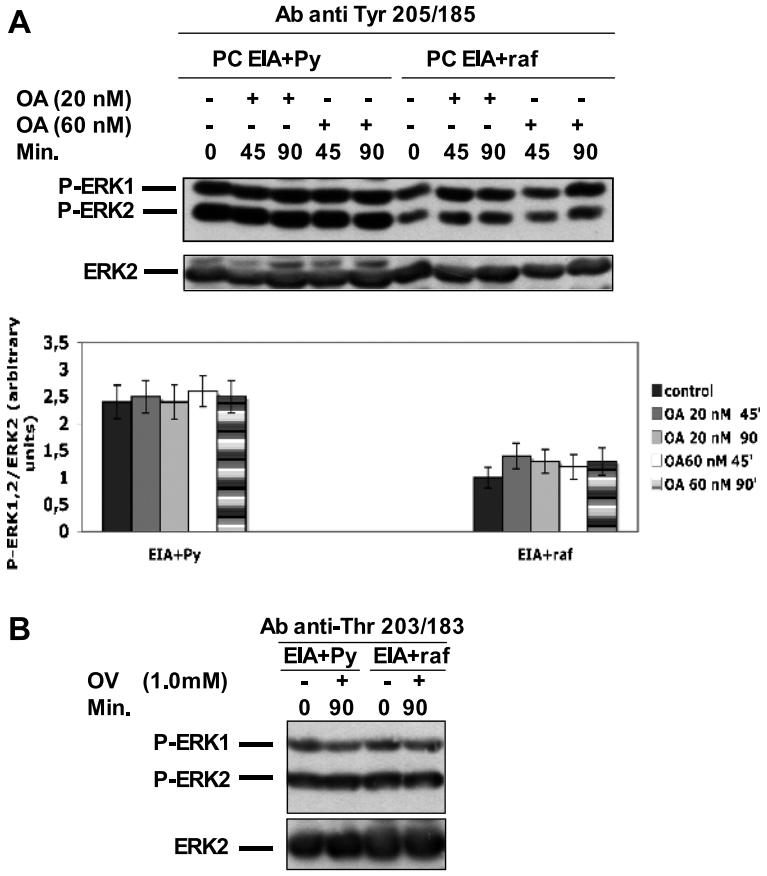

Figure W4. OA treatment does not increase Tyr 205/185 phosphorylation, and OV treatment does not increase Thr 203/183 phosphorylation neither in EIA + raf nor in EIA+Py cells. EIA + raf and EIA+Py cells were treated with OA (A) or OV (B) for the indicated concentrations and times. Whole cell lysates were prepared and immunoblotted using anti–P-ERK1/2 (anti–P-Tyr 205/185 antibodies, able to detect P-Tyr 205/185 irrespective of the state of Thr 203/183) (A) and anti–P-ERK1/2 (anti–Thr 203/183 antibodies, able to detect P-Thr 203/183 irrespective of the state of Tyr 205/185) (B), and anti-ERK2 antibodies, as outlined in Materials and Methods. In (A), the results shown are means ± SD of three independent experiments. In (B), a representative experiment of three independent experiments is shown.

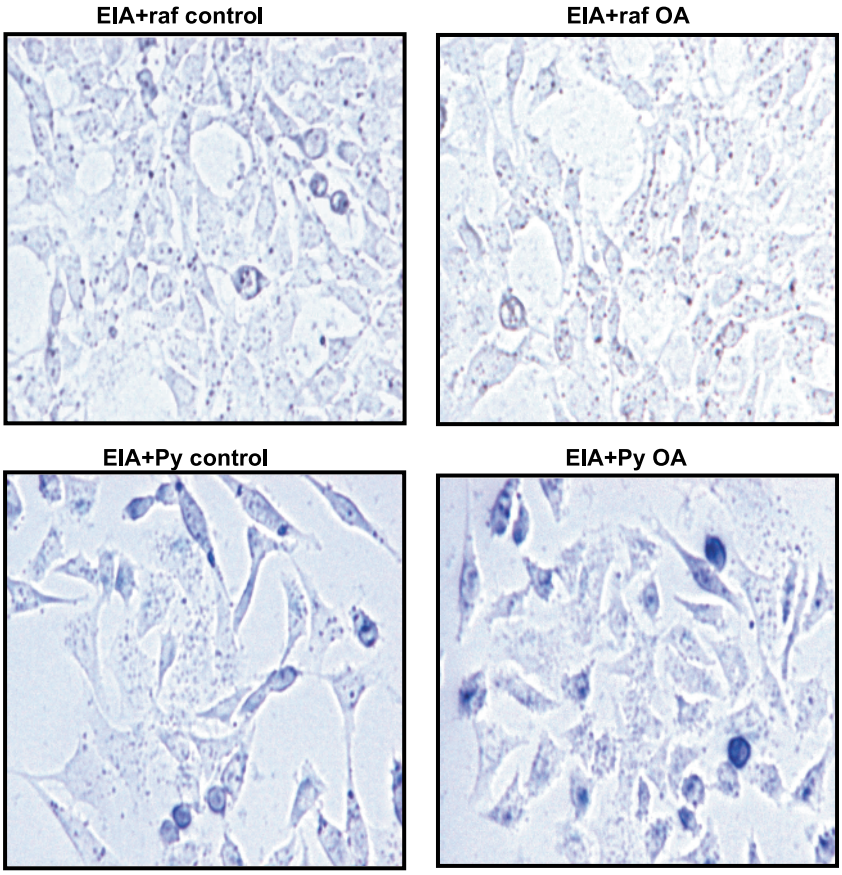

Figure W5. OA treatment does not cause the appearance of the hallmarks of senescence in both EIA + raf and EIA + Py cells. Transformed PC EIA + raf and PC EIA+Py cells were mock-treated or treated with 60 nM OA for 90 minutes and then incubated with regular medium (without OA) for 48 hours. Control and OA-treated cells were subjected to β-galactosidase staining at the end of the 48-hour period in the regular medium without OA.

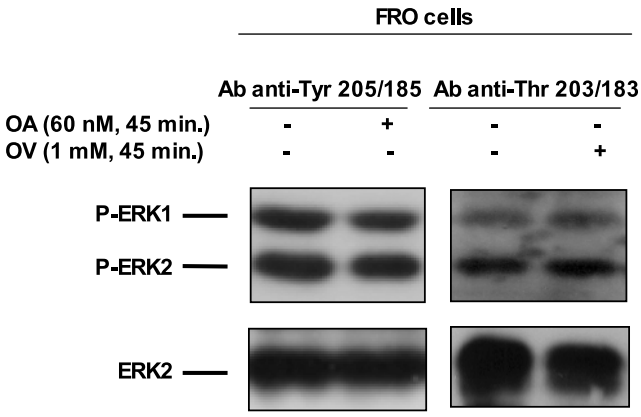

Figure W6. OA treatment does not increase Tyr 205/185 phosphorylation, and OV treatment does not increase Thr 203/183 phosphorylation in FRO anaplastic thyroid cancer cells. FRO cells were treated with OA or OV for the indicated concentrations and times. Whole-cell lysates were prepared and immunoblotted using anti–P-ERK1/2 (anti–P-Tyr 205/185 antibodies, able to detect P-Tyr 205/185 irrespective of the state of Thr 203/183) and anti–P-ERK1/2 (anti–Thr 203/183 antibodies, able to detect P-Thr 203/183 irrespective of the state of Tyr 205/185) and anti-ERK2 antibodies, as outlined in Materials and Methods. A representative experiment of three independent experiments is shown.