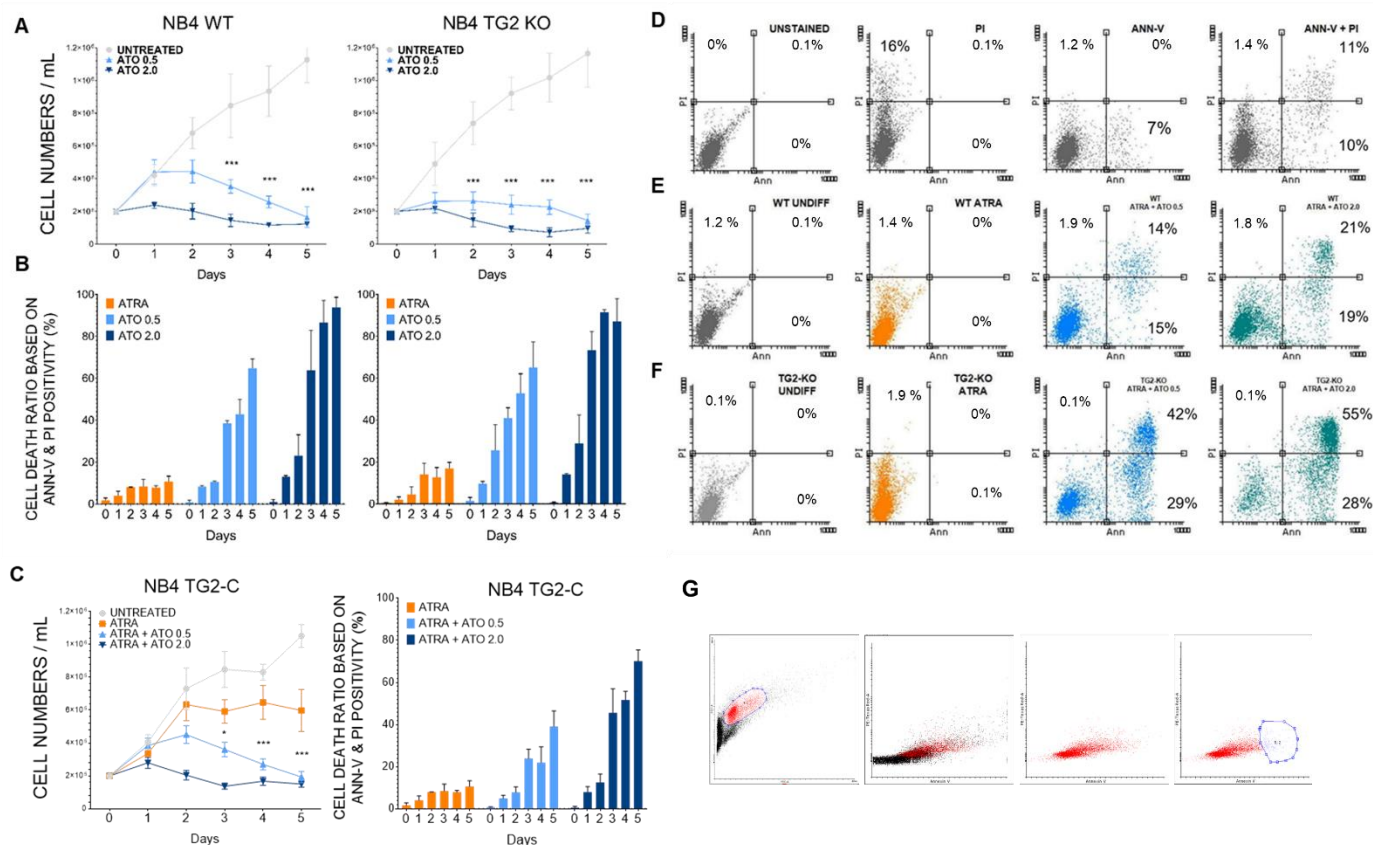
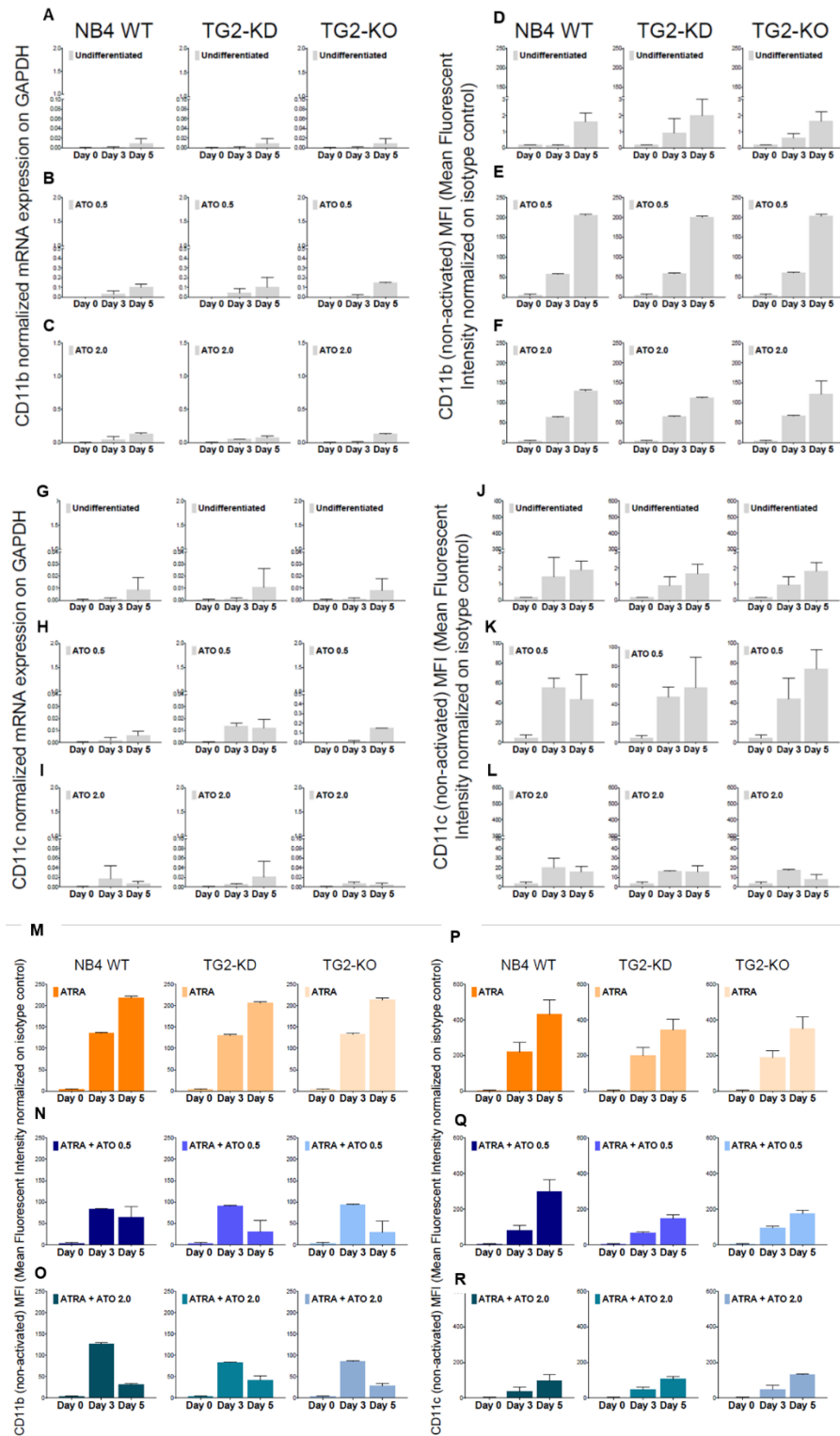


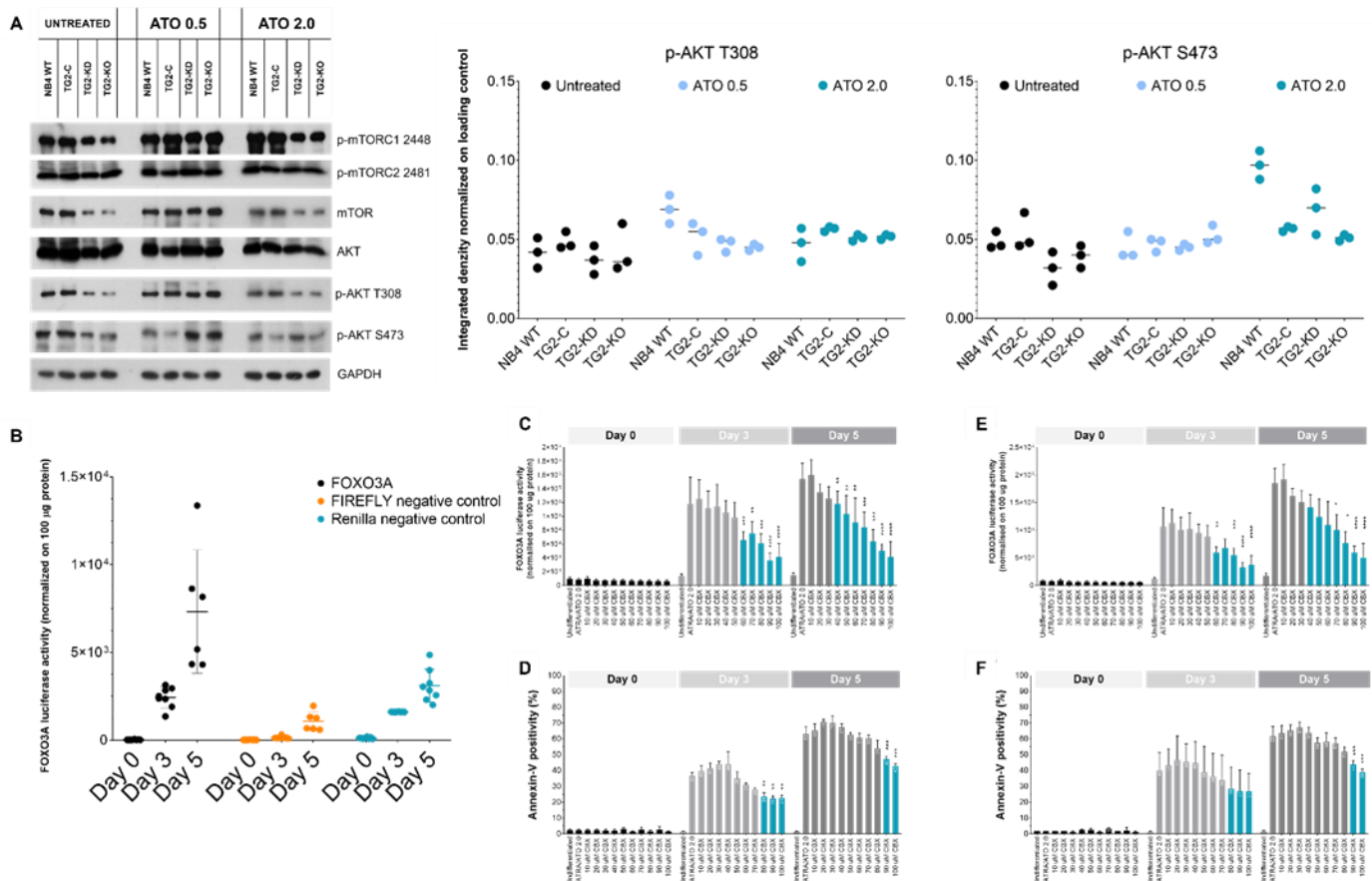
SUPPLEMENTARY FIGURES



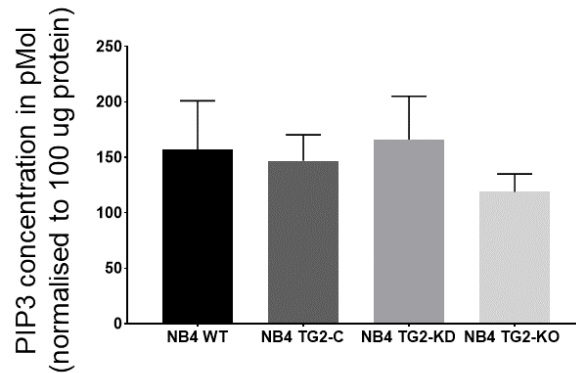
Supplementary Figure 1. ATO-induced cell death. (A) Cell number changes measured in KOVA Glasstic® Slide cell number counting chambers for NB4 WT, NB4 TG2-KD, and NB4 TG2-KO cells treated with 0.5 μ M or 2.0 μ M ATO on the indicated days (n=9). (B) FACS analysis of Annexin-V and PI-stained NB4 cell lines upon single ATO treatments. The percentage of cell death is represented as mean $\pm$ SD (n=9). Measurements were conducted in triplicate; values were validated by Flowing software 2.5.1. (C-E) The percentage of cell death is calculated by using the quadrant method. The quadrants are representing the following populations: LL (lower left) -living cells, LR (lower right) - Annexin-V positive cells, UL (upper left) - PI positive cells, UR (upper right) – double positive cells. (C) The first row represents the control measurements by which the quadrant was determined. (D-E) Graphs represent the ATRA + ATO treated NB4 WT and NB4 TG2-KO cells raw data at day 5. Measurements were conducted in triplicates; values were validated by Flowing software 2.5.1. (G) Gating strategy of the Annexin-V-stained cells for cell sorting.



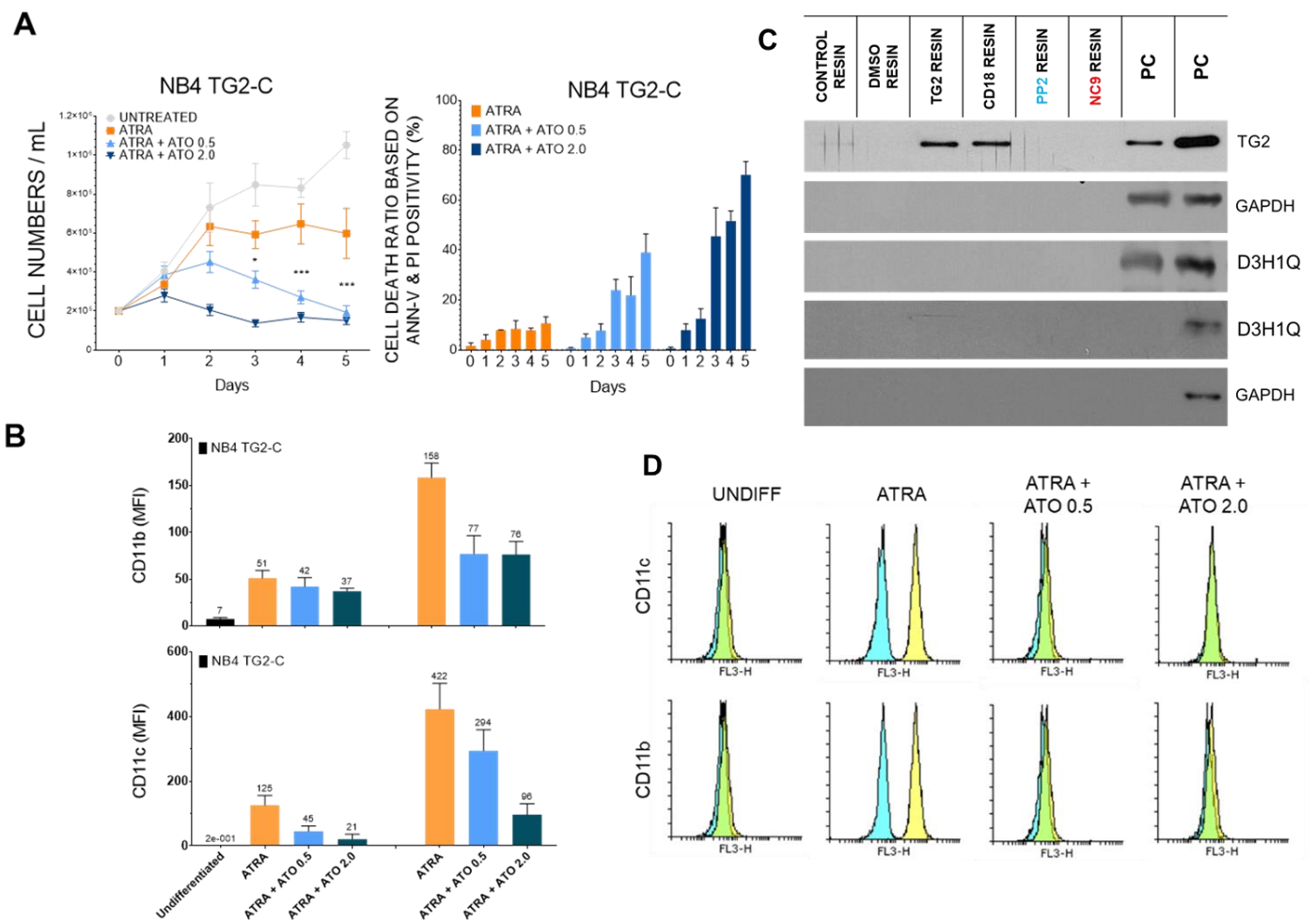
Supplementary Figure 2. Expression of “non-active” leukocyte $\beta 2$ integrin CD11b/CD18 and CD11c/CD18. (A-C) mRNA expression of differentiation markers CD11b in untreated or ATO treated NB4 cells (n=3). (D-F) Flow cytometry analysis of cell surface expression of non-activated differentiation marker CD11b (n=3). (G-I) mRNA expression of differentiation markers CD11c in untreated or ATO treated NB4 cells (n=3). (J-L) Flow cytometry analysis of cell surface expression of non-activated differentiation marker CD11c/CD18 (n=3). (M-O) Flow cytometry analysis of cell surface expression of non-activated differentiation marker CD11b/CD18 upon ATRA + ATO treatment (n=3). (P-R) Flow cytometry analysis of cell surface expression of non-activated differentiation marker CD11c/CD18 upon ATRA + ATO treatment (n=3). Measurements were conducted in triplicate; values were validated by Flowing software 2.5.1.



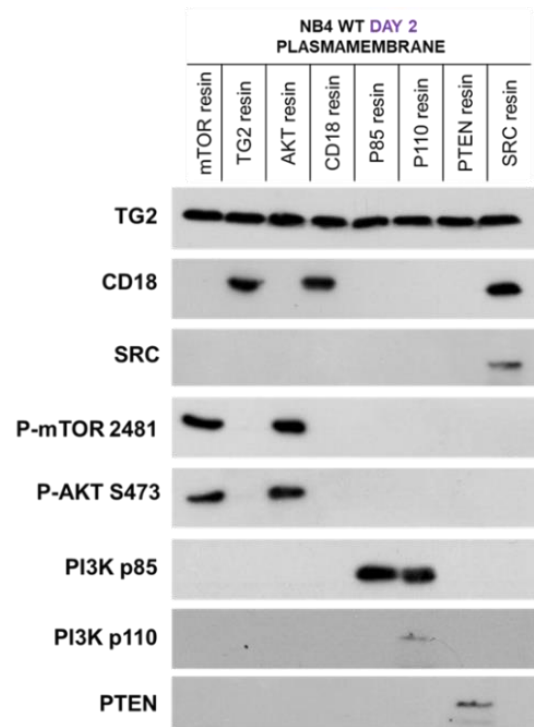
Supplementary Figure 3. CBX inhibits the FOXO3 pathway. (A) Representative western blot showing p-mTORC1-2 (2481, 2448) p-AKT (S473, T308), mTOR, AKT and GAPDH protein expression levels in NB4 cell lines without treatments or with ATO treatment alone (n=3). NB4 WT cells containing a FOXO3A luciferase reporter element were treated with ATRA and ATO and measured using a luminescence-based method in triplicate, with values reported as relative light units (RLU) (n=3). Values were normalized to 100 µg cell lysate protein. (B) FOXO3A luciferase reporter element was measured by a luminescence-based method in triplicate and reported as relative light units in NB4 WT cells using control luciferase construct: Renilla and Firefly. (RLU) (n=3). Values are normalized to 100 µg cell lysate protein. (C-D) NB4 WT cells (left part) and TG2-C (right part) containing a FOXO3A luciferase reporter element were treated with ATRA+ATO 2.0 with or without the FOXO3A inhibitor, CBX at different concentrations. Graph represents the percentage of cell death measured by Annexin-V and PI staining upon FOXO3A inhibition by CBX. The cyan blue color representing the statistically significant differences compared to the treatments without inhibitor. Statistical analysis was conducted by two-way ANOVA (Bonferroni post hoc test; *P<0.05, **P<0.01 and ***P<0.001, ****P<0.0001). Asterisk show the significant differences in ATRA+ATO treated vs. ATRA+ATO+CBX treated cells.



Supplement Figure 4. PIP₃ concentration in undifferentiated NB4 cells. PIP₃ levels in membranes of untreated NB4 cell lines were quantified by ELISA (Echelon Inc.). The mean values $\pm$ SD are shown for PIP₃ levels from three independent experiments. Measurements were conducted in triplicate, and the values were normalized against the protein concentration of the samples.



Supplementary Figure 5. ATO-induced cell death in NB4 TG2-C and control resin IP results from NB4 TG2 WT cells (A) Cell number changes measured in KOVA Glasstic® Slide cell number counting chambers for NB4 TG2-C cells without treatment or with ATRA, ATRA + ATO treatment on the indicated days (n=9). The percentage of cell death is represented as mean $\pm$ SD (n=9). The right panel represents FACS analysis of Annexin-V and PI-stained NB4 cell lines following ATO treatments. The percentage of cell death is represented as mean $\pm$ SD (n=9). Measurements were conducted in triplicate; values were validated by Flowing software 2.5.1. (B) Flow cytometry analysis of cell surface expression of non-activated differentiation marker CD11c/CD18 (n=3) upon ATRA + ATO treatment (n=3). Measurements were conducted in triplicate; values were validated by Flowing software 2.5.1. (C) Representative control IP-western blot showing the resin controls for DMSO, PP2, NC9 treatments. Positive control (PC) was also used as 5 days differentiated cell lysates (n=3). As a control measurements we used the expression of D3H1Q Anti-Sodium Potassium ATPase expression (D) Representative CD11c and CD11b histograms of ATRA, ATRA+ATO treated NB4 WT cells after day 5.



Supplementary Figure 6. Representative co-immunoprecipitation Western-blots showing plasma membrane fractions containing TG2, CD18, SRC, p-mTOR (2481), p-AKT (S473), p85, p110 and PTEN protein levels in ATRA differentiated NB4 WT cells at day 2 (n=3).