Supplementary Data

Immunohistochemistry (IHC)

IHC for phosphorylated-AKT1 (p-AKT1^{Ser473}) (#4060, 1:200 dilution; Cell Signaling Technologies, MA) was done on sections taken from visible BCCs. Antigen retrieval was carried out using DeCloaker Solution (Biocare, CA) for 20 min at 98 °C, and cooled sections were pre-blocked with 5% goat serum/PBS for 1 h, followed by incubation with antibodies to p-AKT1^{Ser473} overnight at 4 °C. Sections were washed, and endogenous biotin, biotin receptors, or avidin binding sites present in tissues were blocked using the Biotin-Avidin System kit (Vector Laboratories, CA). Antigen detection was carried out using the VECTASTAIN® Elite ABC with the peroxidase-based detection system (Vector Laboratories, CA).

Quantitative RT-PCR (qPCR)

We validated the Affymetrix GeneChip array differential expression of a selection of target genes by Taqman RT-PCR. Taqman real-time RT-PCR was carried out using prevalidated gene expression primer-probe assays from Applied Biosystems, CA:

18S rRNA - Hs_99999901_s1, *Fst* - Mm00514982_m1, *Tgm2* - Mm00436980_m1, *Eif4ebp1* - Mm01962435_g1, *Gadd45a* - Mm00432802_ml, *Trib3* - Mm00454876_m1, *Ndrg* – Mm00440447_m1, *Dtx4* - Mm00549843_m1.

We did two technical replicates for each Taqman PCR run and three biological replicates for each gene. The relative expression for each Affymetrix DE gene was obtained using the comparative Ct method, in which the mean expression value of the DE gene of interest was compared to the mean expression of an internal control gene (18S rRNA) for each sample, and the gene expression values for tazarotene-treated samples were compared to that of the DMSO-treated samples. Relative gene expression was calculated by first obtaining the ΔCt value for each DE gene of interest (mean Ct DE gene – mean Ct 18s rRNA) and then calculating the ΔΔCt value (ΔCt DE gene of interest for tazarotene-treated sample - ΔCt DE gene for DMSO-treated sample). The fold change was calculated using the equation 2^n where n=ΔΔCt. Graphs were plotted where the baseline is set at zero.

Western Blotting.

Western blotting was carried out according to manufacturer's instructions or as previously described (8). Primary antibodies used were mouse monoclonal anti-AKT1 (#2967, 1:1000 dilution; Cell Signaling Technologies, MA), rabbit polyclonal anti-β-actin (#ab8227, 1:1000 dilution; abcam, MA), mouse monoclonal anti-β-tubulin (#T4026; 1:5000 dilution, Sigma Aldrich, MO). Secondary antibodies used were HRP-linked

anti-mouse IgG (#7076, 1:5000 dilution; Cell Signaling Technologies, MA) or HRP-linked anti-rabbit IgG (#7074, 1:5000 dilution; Cell Signaling Technologies, MA). Signal detection was done using SuperSignal West Pico Chemiluminescent Substrate (Pierce-Thermo Scientific, IL).

Cell Proliferation Assays

For tazarotene treatment, approximately 5000 ASZ001, myr-HA-AKT1, HA-AKT1 and pLNCX' cells were plated in 96 well plates for 2 days and serum-starved for 2 h prior to drug application. Cells were incubated in 10 μM tazarotene or 0.1% DMSO. After 48 h, cell numbers were assessed(8). For in vitro treatment with PI3K inhibitors, the latter were dissolved in 100% DMSO to give a stock concentration of 30 mM (XL147) and 100 mM (XL765 and LY942002) respectively, which were further diluted with DMSO to generate working concentrations.

FACS analysis

One million cells were trypsinized, washed in PBS and fixed in 2% paraformaldehyde at room temperature for 10 min and then permeabilized with 0.1% Triton-X100 for 10 min. Cells were labeled with mouse monoclonal HA-tag antibodies conjugated with AlexaFluor 488 (#2350, 1:100 dilution; Cell Signal Technology, MA) in 2% FBS for 60 min on ice, and analyzed using the FACSCalibur flow cytometer. FACS data was processed and analyzed with CellQuest Pro software (BD Biosciences, CA).

BCC assessments in vivo. The 5 month skin biopsy sections were scanned digitally, and total microscopic BCC number and cross-sectional size for each mouse biopsy was assessed using image analysis software (Aperio, CA). The data were then standardized to plot the BCC number and size for a 1 cm skin surface length of skin. Mice were monitored from age 5 months to assess the age when the first visible tumor appeared. We measured the number and approximate total volume (the sum of the length x width x height of each tumor) of visible BCCs for each mouse at age 7 months.

Graphs and Statistics. Graphs were plotted using Microsoft Excel and GraphPad Prism^{TM} software, and the latter was used for statistical analysis. For cell proliferation assays, triplicate data were obtained from each condition, and a Student t test was performed on the data from the means of the experimental and control conditions, unless stated. A two-way ANOVA test was used to detect statistical significance between the different doses of drug on the ASZ001 cells. Statistical significance between the drug groups and vehicle control was assessed using the Student t test comparing the vehicle-treated group to the drug-treated group.