Supplementary Materials and Methods:

Preclinical chemoprevention experimental design

Mice were weighed and each mammary tumor was manually palpated and measured with digital calipers once a week from 4 weeks of age until sacrifice. Tumor size was assessed by external measurement of the length (L) and width (W) of the tumors in two dimensions using a Vernier caliper as soon as tumors reached measurable size (~pea-sized, 4-5mm in diameter). Tumor volume (TV, expressed in mm^3) was calculated by using the following equation: $TV = (L \times W^2)/2$ as in (1). Animals were closely monitored for signs of morbidity, including declining body condition score. At experimental end point, all mice were anesthetized, weighed to obtain final body weight, and subjected to necropsy. All mammary tumors per mouse were surgically resected, followed by inflation of the lungs with formalin by syringe injection into the trachea, and death was confirmed by cervical dislocation. After tumor resection, all tumors were measured again ex vivo with calipers to calculate tumor volume, all tumors per mouse were weighed to obtain final wet tumor weight per mouse and to determine tumor burden per mouse (total mammary tumor weight/final body weight of mouse prior to tumor resection).

Mammary tumors were divided into two halves, and two slices of tumor fragments (~2 mm thickness) were processed for fixation in 10% neutral-buffered formalin (NBF) for 6-8h at RT or slices were flash frozen in liquid nitrogen to harvest total RNA. Slices prepared for flash freezing for downstream DNA/RNA/protein analyses were purposefully selected from areas that were not visibly necrotic. Tumors that were fluid filled or highly necrotic were utilized for histology only.

Histopathology

Mammary tumor and lung samples were paraffin embedded and sectioned (4-6 μm) at the UTHSC Department of Pathology Tissue Services Core. Tumor and lung sections were stained with hematoxylin and eosin (H&E) (Supplementary Figure 1). Lung metastases were quantified as previously described (1). Immunohistochemical staining and analyses of the percentages of mean proliferating, apoptotic (Supplementary Figure 2) or ERα positive cells (Supplementary Figure 3) using end-stage vehicle (n=4) or DPM-treated (n=5) tumor sections was performed as described in (1).

Gene expression analyses using real-time quantitative PCR (qPCR)

Genes with the highest differential expression (≥ 2.0 fold) changes) were selected for downstream PCR-validation by quantitative real-time PCR (qPCR) using an established primer design algorithm (primerdepot.nci.nih.gov). Two μg of total RNA from each sample was reverse transcribed into cDNA using SuperScript II Reverse Transcriptase (Invitrogen) with an oligo $(dT)_{24}$ primer. All PCR primers were synthesized by IDT and each sample was analyzed for each specific gene primers with Sybr Green I Master mix on the LightCycler 480 System, according to manufacturer protocols (Roche-Applied-Science). The relative level of amplified mRNA was normalized to the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The average C_t value of the endogenous control (GAPDH) for every sample was subtracted from the C_t value for each target gene, resulting in the ΔC_t value. Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method (2), where the comparative cycle threshold ($\Delta\Delta C_t$) was defined

as the difference between $\Delta C_{t,DPM}$ (DPM-treated) minus $\Delta C_{t,Vehicle}$ (vehicle-treated control). To compare expression of PyMT transgene cDNA levels between tumors of DPM-and vehicle treated mice (n=3 tumors/cohort), the Roche Universal Probe Library RT-PCR algorithm was utilized to design primers to two different regions of the PyMT cDNA, and the PCR products were detected using either UPL probe 11 or 149. The mean crossing point (Cp) value was calculated using absolute maximum, second derivative method after confirming equivalent input among samples by expression of the *Ints3* mRNA as in (1)

miRNA-specific real time PCR (miR-RT-qPCR)

DNase I-treated total RNA was poly(A) tailed using poly(A) polymerase (NEB) at 37°C for 1 hour, then terminated by heating at 65℃ for 20 min. Poly(A)-tailed RNA (1.2 μg) was then reverse-transcribed into first-strand cDNA using Superscript II transcriptase with miRNA specific stem-looped RT primer (5′-GCGAGCACAGAATTAATACGACTCA-CTATAGGTTTTTTTTTTTTVN-3′)(3). The forward primers were specific to miRNA mature sequences and the common reverse primer primed the adapter sequence (5′-GCGAGCACAGAATTAATACGACTCAC-3′). Each value of individual miRNA expression is represented as relative to the expression of the U6-snRNA internal control, and fold change was calculated using the $2^{-\Delta\Delta Ct}$ method. (44)

Supplementary Figure Legends:

Supplementary Figure 1: Structure of DPM and H&E-stained end stage tumor sections. (**A**) Structure and source of DPM. (**B-C**) No gross changes in vehicle (**B**) or DPM-treated (**C**) tumor histopathology were observed following H&E-staining of tumor sections, 100x magnification, scale bar = 50 μm.

Supplementary Figure 2: No gross changes in cellular proliferation, apoptosis or microvessel density are observed in end stage tumors in response to DPM therapy. (**A**) Immunostaining for Ki67, activated caspase-3 and CD34 was performed as described in Schwab et al. 2012. Scale bar = =10 μm, 400x magnification, Ki67 and caspase-3, or 50 μm, 100x magnification, for CD34. (**B**) Bar graph of the percentage of tumor cells positive for either Ki67 or caspase-3 was quantitated as described in the materials and methods, however, no statistically significant differences for either marker were found by student's *t*-test.

Supplementary Figure 3: **DPM treatment does not maintain expression of ERα in late stage carcinomas.** Representative images of ERα immunostaining detected in a normal, immature virgin mammary gland harvested from a 6 week old FVB/Nj female mouse (**A**, internal positive control for immunostaining), a vehicle-treated tumor (**B**) or a DPM-treated tumor (**C**), 400x magnification, scale bar =10 μm.

Supplementary Figure 4: **DPM treatment does not reduce the expression of the PyMT transgene in tumors**. qPCR was conducted to compare expression of the PyMT transgene in cDNA prepared from independent end-stage tumors (n=3 tumors/treatment) using two independent primer/probe sets (UPL probe 11, forward primer: 5' AACCCGAGTTCTCCAACAGA 3'; reverse primer: 5' TCAGCAACACAAGGATTCG 3', and UPL probe 149, forward primer: 5' AAAGTGGGACCCCAGCTAC 3'; reverse primer: 5' AATAGGTCGGGTTGCTCAGA 3') and the results analyzed as described in the materials and methods. The mean Cp values (± S.E.M.) did not vary by more than 10% for either assay and were not significantly different by student's *t*-test.

References

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4. Lin EY, Jones JG, Li P, Zhu UY, Whitney KD, Muller WJ, et al. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. American Journal of Pathology. 2003;163:2113-26.