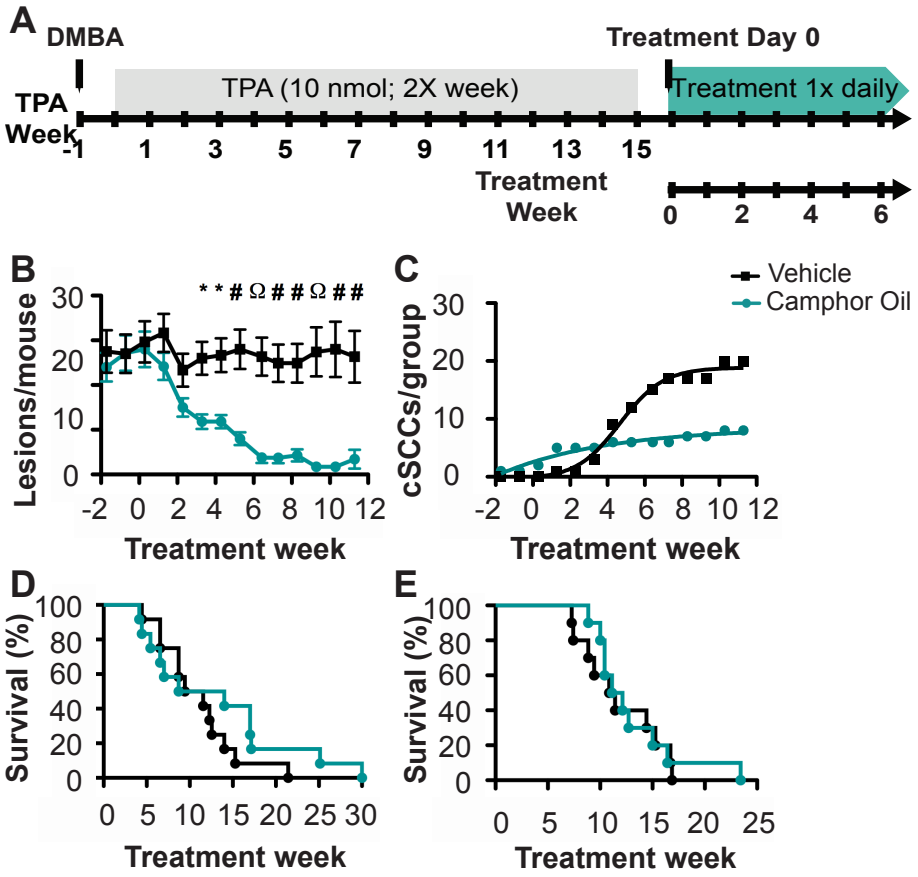
Supplemental figures



Sup. Fig. 1. **CWO reduces tumor burden in an independent cohort.**

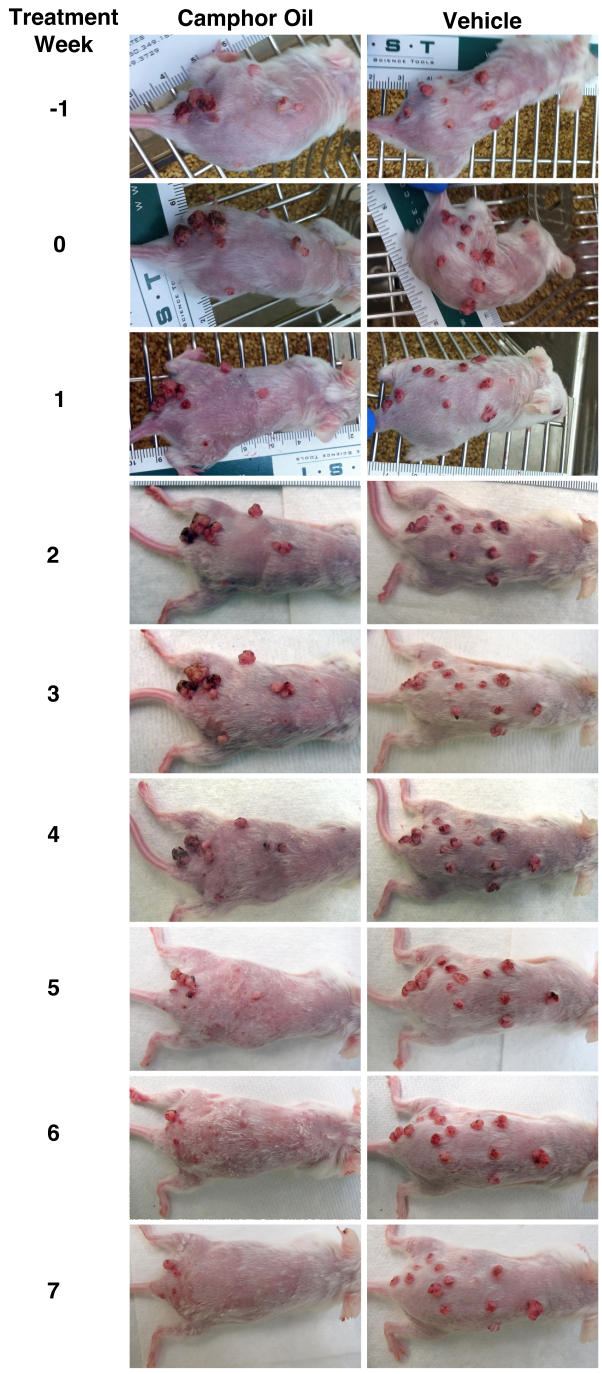
A. Mice were treated once with DMBA to induce mutations followed by twice weekly TPA (10 nM) for 15 weeks. Animals were then split into matched cohorts and treated daily with topical CWO (20%) in vehicle (acetone) or vehicle only.

B. Topical CWO treatment significantly reduces the total lesion burden (CWO=teal, vehicle=black). Two-way ANOVA [*N*=12 mice per group, P<0.0001 F(1,252)=106.11]. **P*<0.05, $^{\#}P$<0.01, $^{\Omega}P$<0.001 Bonferroni post hoc.

C. Fewer malignant SCCs formed with CWO treatment than with vehicle treatment. Boltzmann fits, difference between fits F(4,20)=122.4 P<0.0001; CWO: R^2=0.93, Max cSCC=8.5 weeks; Vehicle: R^2=0.99, Max cSCC=18.89.

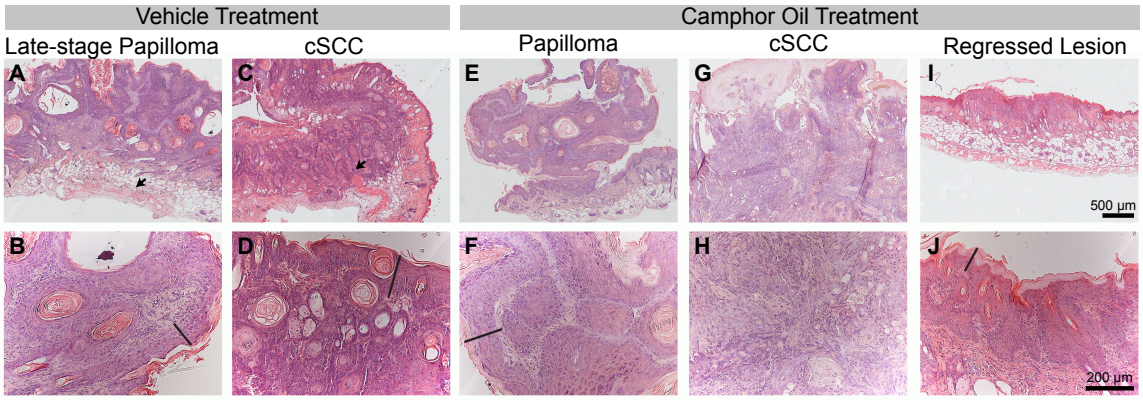
D. Kaplan-Meyer Survival analysis of CWO and vehicle treated groups reveals no significant difference in survival curves (median CWO=11.36, vehicle=10.5, Log-rank P=0.29), but longer overall survival time for a subset of treated mice.

E. Similar survival results were found in the first cohort (Fig 1). Median CWO= 11.64, vehicle=11.15, Log-rank P=0.74).



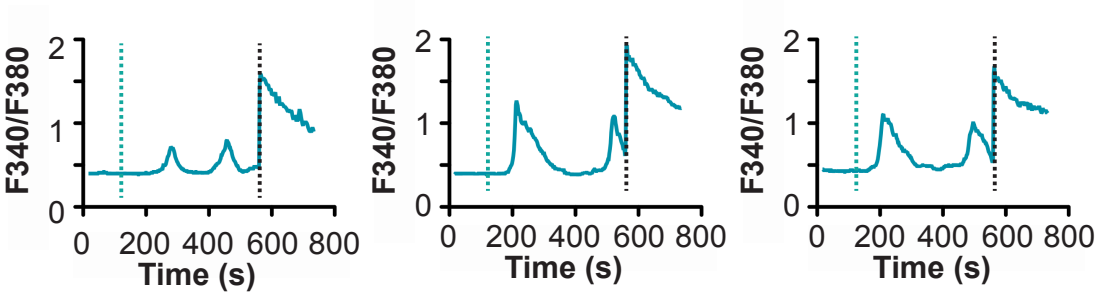
Sup. Fig. 2. **CWO promotes reduction in existing skin lesions through progressive tumor loss**.

Examples images of single CWO (left) and vehicle (right) treated animals are shown over seven weeks of treatment in a second independent cohort. With CWO treatment, tumors progressively regressed (Week 0 = 10 tumors, Week 7 = 0 tumors); whereas in control mice, tumors were stable in number and grew in size and grade (Week 0 = 13 tumors, Week 7 = 13 tumors).

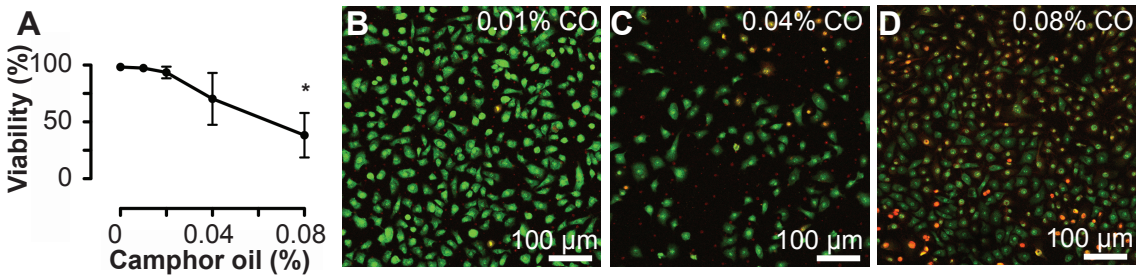


Sup. Fig. 3. CWO and vehicle tumors appear similar by histology.

A, B. A late-stage papilloma from a vehicle treated mouse is shown. Note the largely intact layering of the epidermal-dermal boundary (B, black line), panniculus carnosus (arrow) and dermal fat.
C, D. A fully-converted cSCC from a vehicle-treated animal. Notice the loss of epidermal layering (D, line), and the loss of integrity of the panniculus carnosus (C, arrow).
E, F. A papilloma from a CWO treated mouse is shown with intact layering of the epidermis (F).**G, H**. A cSCC from a CWO treated mouse is shown. Note the lack of organization in H.
I, J. Histology from a regressed lesion in a CWO treated mouse is shown. Here, the epidermal layer is thick (line), a sign of hyperproliferation, but normal skin layering seems to be intact.

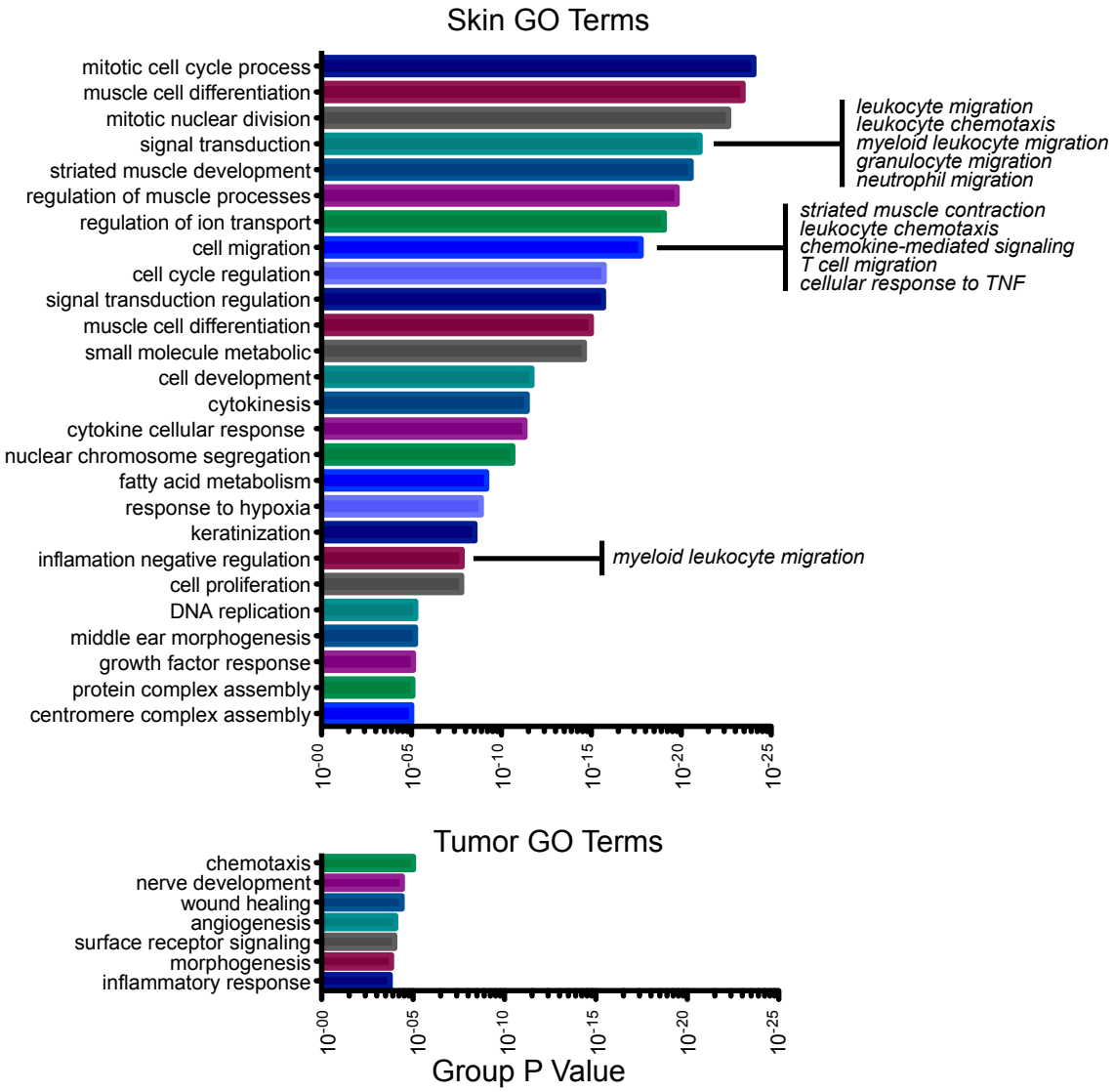


Sup. Fig. 4. CWO induces waves of calcium signaling in human keratinocytes. Representative example traces show recurrent calcium waves after 0.04% CWO treatment. Teal dashed line indicates CWO application. Black dashed line indicates histamine control.



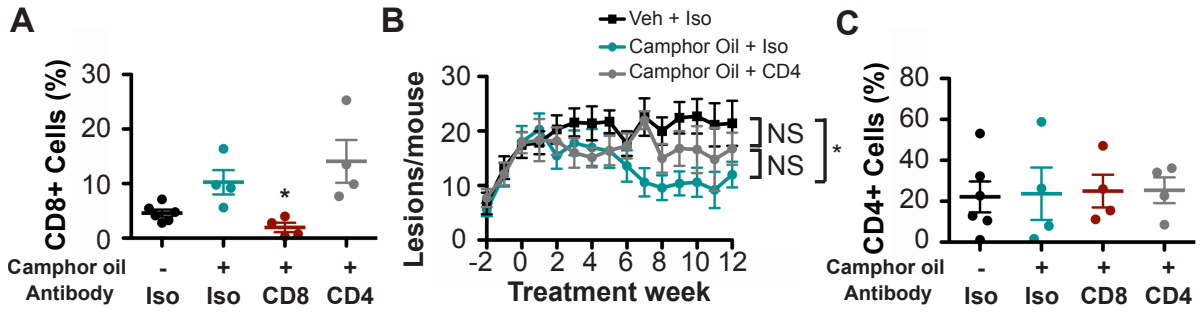
Sup. Fig. 5. Cultured keratinocytes are viable with CWO treatment.

A. Percent of normal human epidermal keratinocytes that are viable following 30-minute incubation with 0-0.08% CWO in keratinocyte media (N=3 experiments with >3800 cells per replicate; One-way ANOVA $p<0.05$, Dunnet's Multiple Comparison Test against 0% *$P<0.05$). **B-C.** Representative images from viability assay showing calcein-AM (green) and ethidium homodimer-1 (red) of normal human epidermal keratinocytes treated with 0.01% CWO (**B**) 0.04% CWO (**C**) and 0.08% CWO. All cells are labeled by calcein, but only dead cells by ethidium homodimer-1.



Sup. Fig. 6. Extended RNA-Seq analysis of tumors and skin treated with CWO.

GO analysis of genes differentially expressed in epidermis and tumors treated with CWO compared to vehicle. GO term fusion was used, and selected GO terms within a fused node related to inflammation are shown.



Sup. Fig. 7. Antibody depletion.

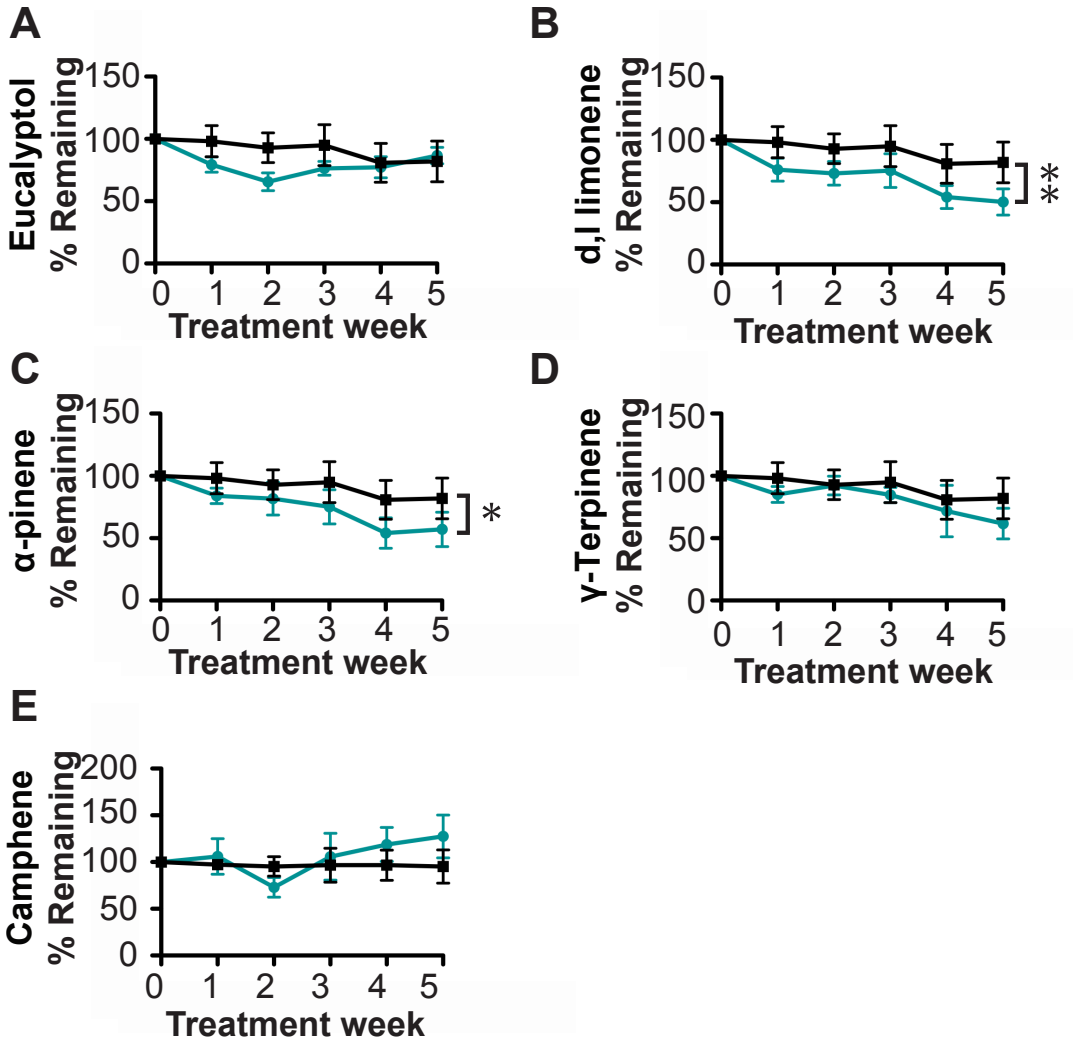
Blood from animals in T cell blocking experiments were analyzed for antibody efficiency at sacrifice. Mice were sacrificed between 12-13 weeks after the start of treatment, and 5-6 days after the final antibody injection.

A. A significant effect of treatment was found on circulating CD8+ T cells ($P<0.01$) Circulating CD8+ cells were significantly reduced with CD8 antibody treatments. Interestingly, circulating CD8+ cells were increased with CWO treatment.

B. Co-treatment with CD4 blocking antibodies and topical CWO resulted in an intermediate effect on tumor reduction. CD4/CWO treatment was not significantly (NS) different from either Isotype/CWO or Isotype/veh groups by two-way ANOVA. CWO treatment had similar effects on tumor burden to previous cohorts, $*P<0.05$

C. No effect of treatment was found on circulating CD4+ T cells, indicating that CD4+ T cells were not sufficiently blocked between injections.

N=4-6 mice per group.



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75 **Sup. Fig. 8. CWO constituents reduce tumor burden.**

76 Pilot studies were performed to identify CWO constituents that reduce tumor burden. Tumors
77 were induced as before and mice were split into groups with equal total tumor burdens. Mice were
78 treated with 20% Eucalyptol (**A**), d,l-Limonene (**B**), α-pinene (**C**), γ-terpinene (**D**), and camphene
79 (**E**). Data are baseline corrected to tumor counts at time zero for each mouse. Two-way ANOVAs
80 reveal significant treatment effects of d,l-limonene ($P<0.01$) and α-pinene ($P<0.05$).

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