

Supplementary Figure 4. Expansion of Scgb1a1+ marked cells during urethane- and MCA/BHTtriggered field cancerization of the lungs. A and B, Data summary of alveolar mG+ cells (A) and single-channel (endogenous mT and mG fluorescence and Hoechst 33258 nuclear stain) images (B) corresponding to Figure 3A, showing non-neoplastic alveolar regions of saline-, EC-, and MCA/BHT-treated mT/mG; Scgb1a1. Cre mice at six months into treatment (n = 8 mice/group). Note the few mG+ cells of saline-treated mice and increased numbers in carcinogen-treated mice. C, Single-channel images corresponding to Figure 3B. Shown are non-neoplastic distal lung regions of EC-treated mT/mG; Scgb1a1. Cre mice at six months into treatment (n = 22), stained for the lung cell markers Clara cell secretory protein (CCSP), acetylated α-tubulin (TUBA1A), and surfactant protein C (SFTPC). Note mG+CCSP-TUBA1A1-SFTPC+ cells in the alveoli. **D** and **E**, Data summary (D) and representative bioluminescence images (merged with photographic images; E) of R26.Luc:Scgb1a1.Cre FVB mice before and seven months after saline (one intraperitoneal injection of 100 μ L; n = 6) or EC (one intraperitoneal injection of 1 g/Kg in 100 μ L saline; n = 5) treatment. Note that in this model light is emitted exclusively by Scgb1a1+ marked cells. Note also that this signal was exclusively detected over the lungs and over the female pelvis during the productive phase of the menstrual cycle, emitted by endometrial cells that also express Scgb1a1 (data not shown). Note the ~30% reduction in the signal of saline-treated mice over the seven months of the experiment that is likely attributable to reduced light penetrance through the thoracic wall of the growing mouse and not to reduction of Scgb1a1+ marked cells. Note the ~30% increase in the signal of EC-treated mice over the same time period that integrally shows expansion of Scgb1a1+ cells in response to EC. Measurements were from five non-overlapping fields/lung. Data are given as mean \pm SD. **, and ***: P < 0.01, and P < 0.001 for comparison of the indicated columns with saline by one-way ANOVA with Bonferroni post-tests (A) and of urethane and saline treatments at the 7-month-time-point by two-way repeated measures ANOVA with Bonferroni post-tests (D). mG, membranous green fluorescent protein fluorophore; mT, membranous tomato fluorophore.