

Supplementary Figure 2. Changes in pulmonary marked cells during urethane- and MCA/BHTinduced lung adenocarcinoma. A, Top left: schematic of multi-hit urethane administration tailored to yield 90% tumor incidence in C57BL/6 mice: ten weekly intraperitoneal injections of 1 g/Kg urethane (grey arrows) were initiated at six weeks after birth (pink arrow) and lungs were examined six months after the first urethane injection (black arrow). Bottom left: MCA/BHT regimen tailored to yield 90% tumor incidence in C57BL/6 mice. Four weekly intraperitoneal injections of 15 mg/Kg MCA (red arrows) initiated at six weeks after birth (pink arrow) were followed by eight weekly intraperitoneal injections of 200 mg/Kg BHT (blue arrows) and lung examination at six months after first MCA (black arrow). Lung tumor incidence, number and mean diameter are depicted in table and plot, respectively. **B**, Photographs and green epifluorescence images of tumor-bearing lungs (top two rows), as well as merged fluorescent microscopic images of lung sections (of Hoechst 33258 stain and endogenous mT and mG fluorescence) taken from non-affected, hyperplastic/proliferative and frank neoplastic regions (bottom three rows) from experiment from Figure 1C (n = 30, 22, 18,and 20/group, respectively). Top two rows: arrows indicate lung tumors. Note the absence of mG+ fluorescence in mT/mG tumors, the mG+ fluorescence of mT/mG; Scgb1a1. Cre and mT/mG; Sftpc. Cre tumors, and the split mG+ and mG- tumors of mTmG;Lyz2.Cre mice. Bottom three rows: white arrows indicate mG+ cells in apparently nonaffected alveolar areas of mT/mG; Scgb1a1.Cre mice; green arrow indicates rare mG+ cell in nonaffected airway of mT/mG; Lyz2. Cre mouse. Note absence of mG+ fluorescence in mT/mGhyperplasias and tumors (dashed outlines), mG+ fluorescence of mT/mG; Scgb1a1.Cre and mT/mG; Sftpc. Cre lesions, and split mG+ and mG- lesions of mTmG; Lyz2. Cre mice. C and D, Single-channel (endogenous mT and mG fluorescence and Hoechst 33258 nuclear stain) and merged images of lung neoplasias (dashed outlines) of mT/mG and mT/mG;Scgb1a1.Cre mice treated with MCA/BHT as in A (n = 8/group). Note absence of mG+ fluorescence in mT/mG tumors and the mG+ tumors of mT/mG; Scgb1a1.Cre mice. **E**, XY plot of percentage of mG+ tumors/lung versus mG+ tumor cells/tumor averaged per lung from B (n = 30, 22, 18,and 20/group, respectively). Five

non-overlapping fields/lung were examined. Data are given as mean  $\pm$  SD. mG, membranous green fluorescent protein fluorophore; mT, membranous tomato fluorophore.