Supplementary Materials and Methods

Transplantation into nude mice

Urethane-induced lung tumors of approximately equal sizes (ϕ=1 mm) from both *Nrf2*$^{-/-}$-and *Nrf2*$^{+/+}$-mice were transplanted subcutaneously into the backs of nude mice. The tumor diameters were measured using a digital caliper every month. The tumor volumes were calculated using the following formula: length (mm) × width (mm) × width (mm) ×0.5 (1).

Quantitative real time PCR

Total RNA was extracted from the tissues using ISOGEN (Nippon Gene). First-strand cDNA was synthesized from the total RNA using random hexamers and Superscript III Reverse-Transcriptase (Invitrogen). Real-time reverse-transcription (RT)-PCR was performed using 2X SYBR Green PCR master mix (Invitrogen) and the ABI PRISM 7300 sequence detector system (PE-Applied Biosystems), as described previously (2). The sequences of TaqMan probes and primers are listed in Supplementary Table S1.

Flow cytometry

Analyses of the bone marrow cells were performed using FACS-Caliber (BD Pharmingen). Quantification of ROS level with 2,7-dichlorodihydrofluorescein diacetate (DCFDA), separation of MDSCs, and T cells has been described (2).

Histological analysis and immunohistochemistry

A piece of the right lung was fixed in Mildform (Wako) for 2 days and processed for paraffin embedding for histological analyses. Sections of 2.5-μm thick were stained with hematoxylin-eosin (HE). Five step sections (each 200 μm apart) were stained with HE. For immunohistochemistry, a rabbit polyclonal anti-β-galactosidase antibody (Abcam) was used for the detection of a nuclear Nrf2-β-galactosidase fusion protein. Anti-sera against CD3 (T cell) (AbD Serotec), pancytokeratin (epithelial cell; Santa Cruz Biotechnology), Ki67 (cell proliferation; DAKO) and Cyp2e1 (AbFrontier) were used at 1:1000 dilutions. The sections were examined under a fluorescence microscope BZ-8000 (Keyence). The histological diagnosis of adenoma and adenocarcinoma was performed with the criteria (3).

Supplementary Reference

1. Singh A, Boldin-Adamsky S, Thimmulappa RK, Rath SK, Ashush H, Coulter J, et al. RNAi-mediated silencing of nuclear factor erythroid-2-related factor 2 gene expression in non-small cell lung cancer inhibits tumor growth and increases efficacy of chemotherapy. Cancer Res 2008;68:7975-84.

2. Satoh H, Moriguchi T, Taguchi K, Takai J, Maher JM, Suzuki T, et al. Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung. Carcinogenesis 2010;31:1833-43.

3. Wang Y, Zhang Z, Yao R, Jia D, Wang D, Lubet RA, et al. Prevention of lung cancer progression by bexarotene in mouse models. Oncogene 2006;25:1320-9.

Supplementary Figure Legends

Supplemental Figure 1.

Expression of enzymes involved in the urethane metabolism. **A**, The bioactivation and detoxification pathway of urethane (ethyl carbamate). **B**, mRNA expression level of *Cyp2e1* in the lungs at 1 or 6 hours (h) after urethane injection in both genotype groups. **C**, Immunostaining of Cyp2e1 in *Nrf2*$^{-/-}$- and *Nrf2*$^{+/+}$-mouse lungs at 1 day after urethane administration (right). The mouse groups administered with PBS were used as controls (left). The black boxes indicate higher magnification views in each figure. AW: airway. Arrows indicate the positively stained cells. Black scale bar, 100 μm; red scale bar, 10 μm. **D**, mRNA expression level of *Gstp1/p2* in the lungs at 1 or 6 hours (h) after urethane injection in *Nrf2*$^{-/-}$- and *Nrf2*$^{+/+}$-mouse groups. **E**, mRNA expression level of the microsomal epoxide hydrolase (mEH) in the lungs at 1 or 7 days (d) after urethane injection in both genotype groups.

Supplemental Figure 2.

Urethane administration induces the cellular cluster formation consisting of inflammatory and epithelial cells in the perivascular region of the *Nrf2*$^{-/-}$-mouse lung. **A**, HE staining of lungs from

the *Nrf2*$^{-/-}$- and *Nrf2*$^{+/+}$-mice 24-h after urethane treatment. The arrows indicate each cellular cluster. Representative histological observations in the lungs of two independent mice (upper and lower rows) of either *Nrf2*$^{-/-}$ or *Nrf2*$^{+/+}$. PV: pulmonary vein; AW: airway. Scale bars, 200 μm. **B**, Urethane-induced cell clusters in the *Nrf2*$^{-/-}$-mice contain CD3^{+} T lymphocytes and pan-cytokeratin^{+} epithelial cells. Serial sections of the cell cluster from the urethane-treated *Nrf2*$^{-/-}$-lung (inlet in A) were subjected to immunostaining against CD3 (in brown, left), pan-cytokeratin (in brown, middle) and Ki67 (in brown, right). The black boxes indicate a higher magnification area in each Fig. The arrows in each panel indicate the positively stained cells. Black scale bar, 50 μm; red scale bar; 10 μm.

Supplemental Figure 3.

Experimental design of urethane-induced lung carcinogenesis. **A**, The very short-term observation protocol with a single injection and 4-weeks latency. **B**, The short-term observation protocol with a single injection and 8-weeks latency. **C**, The middle-term observation protocol including a single injection with 16-weeks latency. **D**, The long-term observation protocol including four contiguous weekly injections and 6-months latency.

Supplemental Figure 4.

Pathway analyses using IPA software. **A**, Comparison of molecular and cellular functions

among the deregulated genes in the large-size lung tumors ($\phi > 2.0$ mm) of *Nrf2*$^{+/+}$ mice (n=8) and middle-size lung tumors (1.5 mm > ϕ > 1.0 mm) of *Nrf2*$^{-/-}$ mice (n=8). **B**, Comparison of the canonical pathway among commonly deregulated genes in the *Nrf2*$^{+/+}$ and *Nrf2*$^{-/-}$ lung tumors. The red line indicates the threshold of significant difference compared to the normal lung tissue ($P < 0.05$).

Supplemental Figure 5.

Summary of lung carcinogenesis in *Nrf2*$^{+/+}$-(upper) and *Nrf2*$^{-/-}$-(lower) mice. *Nrf2*$^{-/-}$-mice suffer from more abundant tumor initiation than *Nrf2*$^{+/+}$ -mice do (Left). Nrf2 activates Kras signaling robustly and leads to malignant transformation of tumors. Tumors in *Nrf2*$^{-/-}$-mice fail to activate the signaling due to the lack of Nrf2-mediated downstream gene activation (Middle). The *Nrf2*$^{+/+}$-tumor cells potently induce bone marrow MDSCs-ROS accumulation, which leads to the suppression of anti-tumor immune responses mediated by CD8^{+}-single positive T cells. Therefore, *Nrf2*$^{+/+}$-mice harbor abundantly the lung adenocarcinoma (Right).