Supplementary Figure S2

A

Relative Increase in cell death

- Control
- Imipramine
- Promethazine

mSCLC  hSCLC

B

H&E

Saline  Imipramine  Promethazine

DAPI + CC3

C

DAPI + PH3

Saline  Imipramine  Promethazine

PH3+ cells/Tumor Area (arbitrary units)

D

DAPI + SYN  DAPI + CC3

Imipramine treatment for 30 days

E

% survival (MTT)

DMDO  Imipramine  Imipramine + Necro 50 µM  Imipramine + Necro 50 µM
**Supplementary Figure 2: Induction of cell death and decreased proliferation following imipramine and promethazine treatment.**

**A,** Relative increase in cell death based on quantification of Annexin V and PI staining by FACS analysis of mouse (mSCLC, Kp1) and human (hSCLC, H82) cells cultured in 2% serum and treated for 24 hours with 50µM imipramine and 30µM promethazine. Values from three independent experiments are shown as mean ± s.e.m. **B,** Representative H&E staining (top), and CC3 immunostaining (bottom) of tumor sections from NSG mice implanted subcutaneously with mSCLC (Kp1) cells and treated daily with saline, imipramine, and promethazine for 14 days. “N” depicts necrotic areas. **C,** Representative immunostaining of phospho-Histone 3 (PH3) in tumor sections (white dashed areas) from *Rb/p53/p130* mutant mice treated daily with saline, imipramine, and promethazine for 30 days. Right: Quantification of the percentage of PH3 positive cells per tumor area of saline (n=180 tumors from 10 mice), imipramine- (n=160 from 9 mice; \(P=0.0006\)), and promethazine-treated tumors (n=83 from 6 mice; \(P=0.0011\)). **D,** Representative immunostaining of Synaptophysin and CC3 in lung sections from *Rb/p53/p130* mutant mice treated daily with imipramine for 30 days. Normal lung neuroendocrine cells are indicated with white arrows. Note the absence of signal for CC3 in the lungs, similar to Fig. 3b. The pale green staining is the autofluorescence background of the paraffin-embedded tissue. **E,** Effects of the combined treatment of imipramine (50µM) and the necrosis inhibitor necrostatin-1 (abbreviated as Necros) on the survival of mSCLC (Kp1) cells after 24 hours of treatment, as measured by the MTT viability assay. Values from three independent experiments are shown as mean ± s.e.m. The unpaired t-test was used to calculate the p-values of imipramine-treated cells versus control DMSO-treated cells and of imipramine-treated cells versus necrostatin- treated cells combined with imipramine. \(*P<0.05, **P<0.01, ***P<0.001, ns, not significant.\)