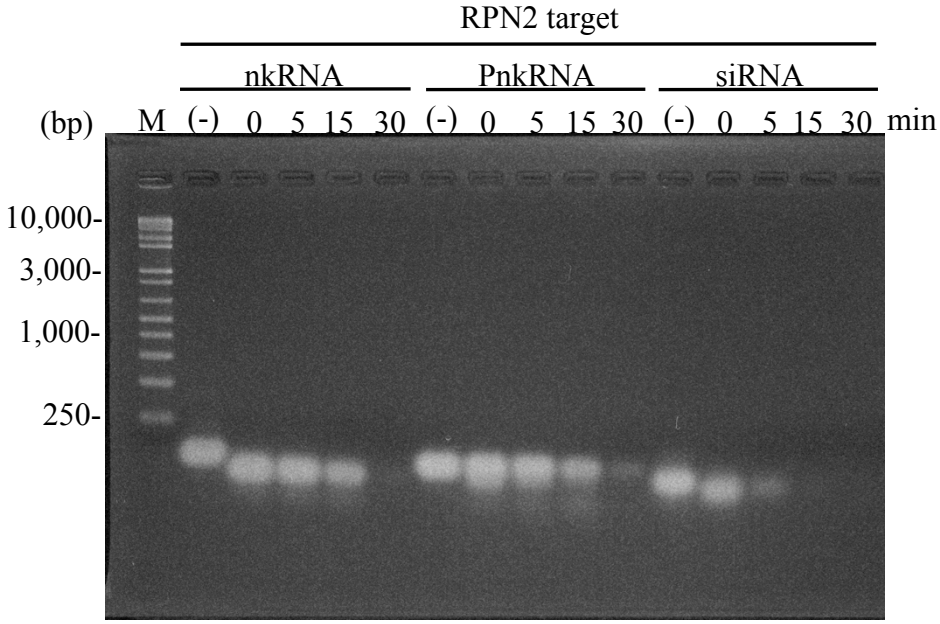
Supplementary Information

A novel platform to enable inhaled naked RNAi medicine for lung cancer

Yu Fujita, Fumitaka Takeshita, Takayuki Mizutani, Tadaaki Ohgi, Kazuyoshi Kuwano, Takahiro Ochiya

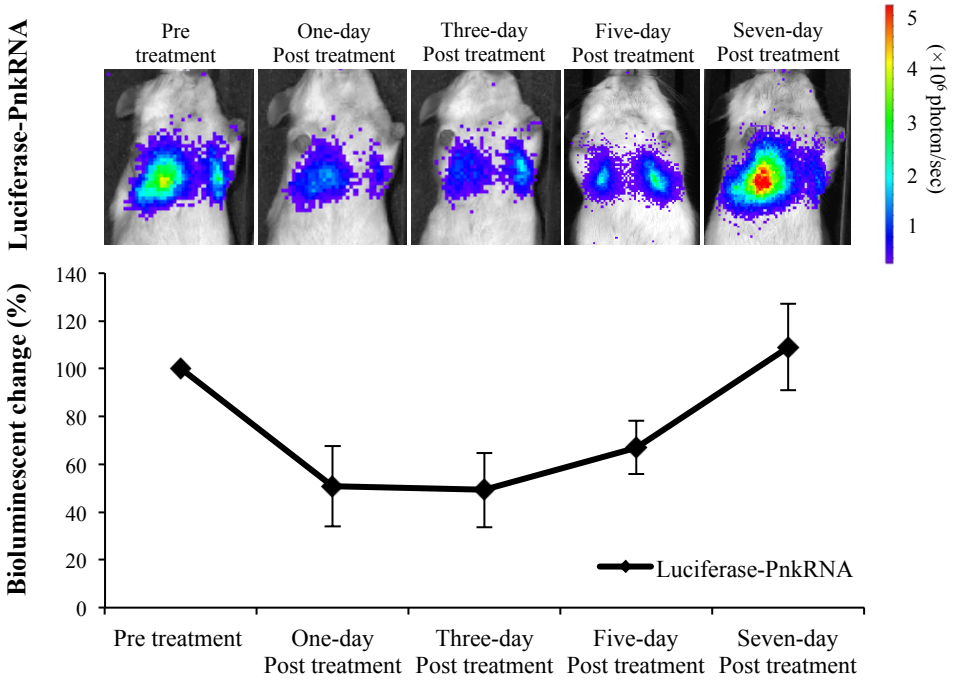
Supplementary Figure 1



Supplementary Figure 1. Stability of siRNA, PnkRNA, and nkRNA directed against human RPN2.

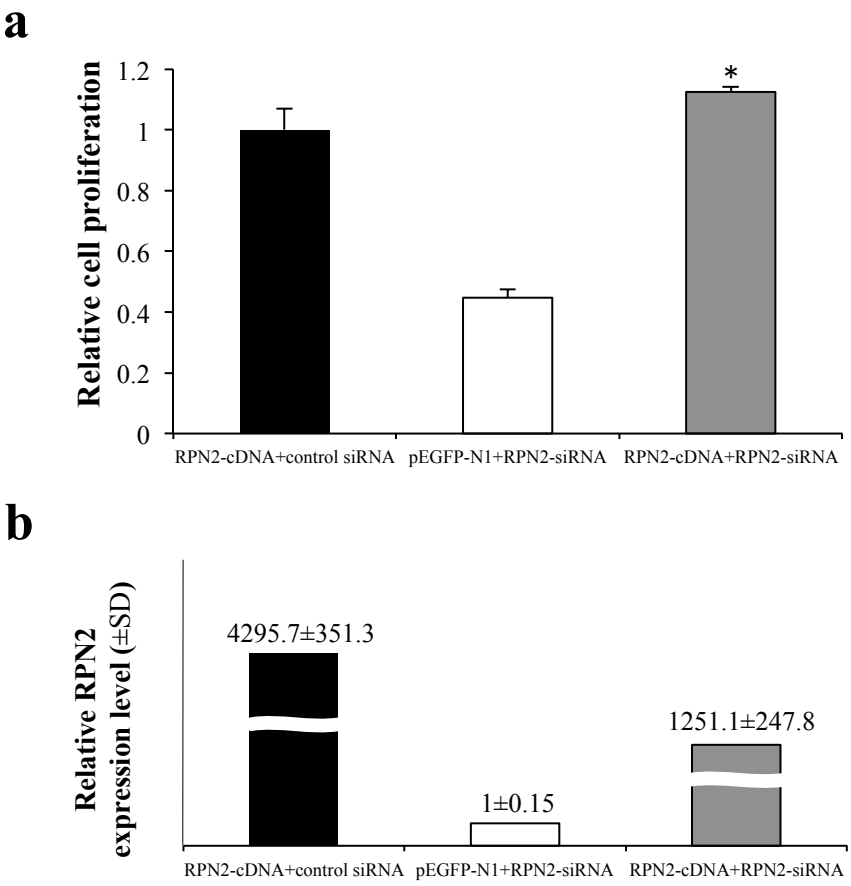
To evaluate *in vitro* stability, each RNA was incubated at 37°C in the presence of RNase and then separated on a 3% agarose gel. Both nkRNA and PnkRNA were more resistant to degradation than siRNA.

Supplementary Figure 2



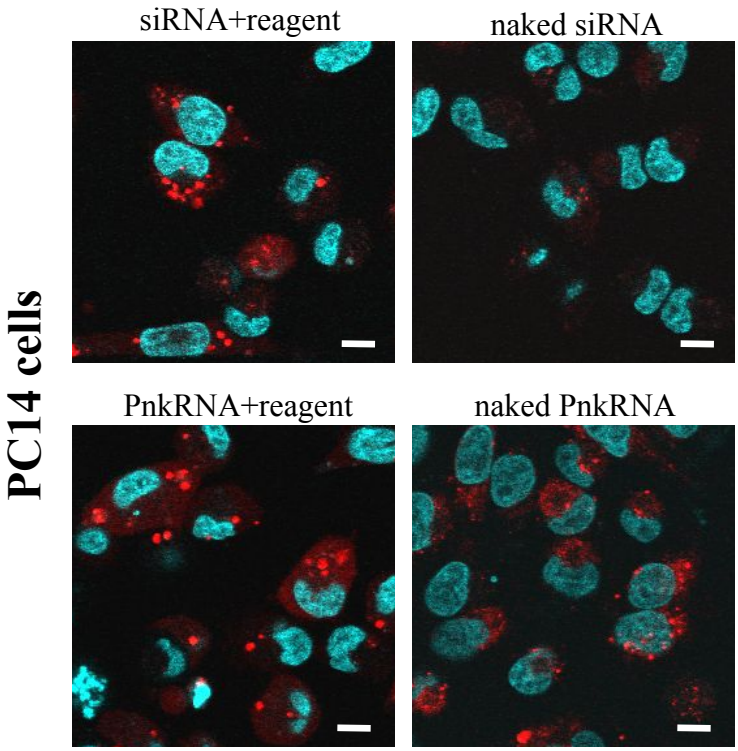
Supplementary Figure 2. The pharmacodynamics of luciferase-PnkRNA *in vivo*. Monitoring luciferase inhibition by PnkRNA *in vivo* with bioluminescent imaging. Representative images from pre-treatment to on seven-day post-treatment.

Supplementary Figure 3



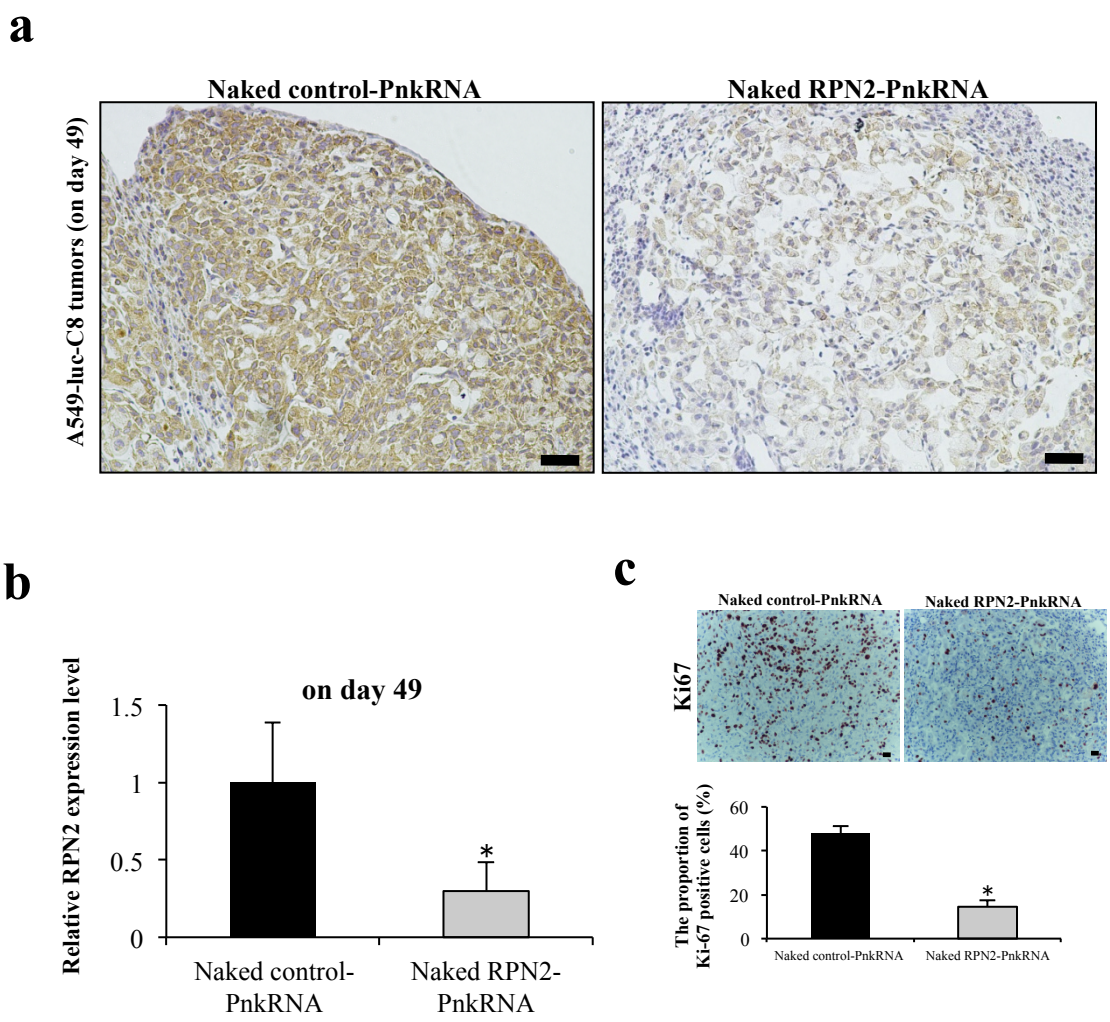
Supplementary Figure 3. Rescue of RPN2-siRNA induces phenotype. (a) Rescue of RPN2-siRNA induces a phenotype. A549-luc-C8 cells were transfected with RPN2-cDNA or control vector combined with siRNA. Cell proliferation was measured 96 h after cotransfection. (b) The expression of RPN2-mRNA after the rescue of RPN2-siRNA. Measurements of human RPN2 expression levels by real-time RT-PCR. The data are represented by the means ± SD (*n*=3). *, P<0.05 versus the pEGFP-N1+RPN2-siRNA group.

Supplementary Figure 4



Supplementary Figure 4. Endocytosis assay for naked RNAi therapeutic agents on PC14 cells. Naked PnkRNAs are incorporated into the PC14 cell cytoplasm by the mechanism of endocytosis without delivery reagents. The scale bars indicate 10 μm.

Supplementary Figure 5



Supplementary Figure 5. The effect of repeat administration study with naked RPN2-PnkRNA. (a) Immunohistochemical staining for RPN2 proteins in representative tumours of lung cancer models on day 49. The scale bars indicate 100 μm. (b) The expression of RPN2-mRNA after the repeat administration. Measurements of human RPN2 expression levels by real-time RT-PCR. (c) Immunohistochemical analysis of Ki67 in representative tumors of lung cancer models on day 49. The scale bars indicate 100 μm. The data are represented as the means ± SD (n=12). *, P<0.05 versus the naked control-PnkRNA group.

Supplementary Tables

Table 1. Sequence of siRNA, nkRNA, and PnkRNA directed against luciferase mRNA

RNA class	Sequence	Mass
Target siRNA	: 5’- CCUACGCCGAGUACUUCGATT-3’ （sense） / 5’-UCGAAGUACUCGGCGUAGGTT-3’ (antisense)	6605.1/ 6725.2
Target nkRNA	: 5’- ACCUACGCCGAGUACUUCGAUUCCCCACACCGGAAUCGAAGUACUCGGCGUAGGUUCUUCGG-3’	19785.7
Target PnkRNA	: 5’- ACCUACGCCGAGUACUUCGAUUCC-P-GGAAUCGAAGUACUCGGCGUAGGUUC-P-G-3’	17063.4

Table 2. Sequence of siRNA, nkRNA, and PnkRNA directed against human RPN2 mRNA

RNA class	Sequence	Mass
Target siRNA	: 5’- GGCCACUGUUAAACUAGAACA-3’ （sense） / 5’-UUCUAGUUUAACAGUGGCCUG-3’ (antisense)	6703.1/ 6651.0
Target nkRNA	: 5’- AGGCCACUGUUAAACUAGAACACCCCACACCGGUGUUCUAGUUUAACAGUGGCCUGCUUCGG-3’	19794.75
Target PnkRNA	: 5’- AGGCCACUGUUAAACUAGAACACC-P-GGUGUUCUAGUUUAACAGUGGCCUGC-P-G-3’	17072.42
Control PnkRNA	: 5’- AUACUAUUCGACACGCGAAGUUCC-P-GGAACUUCGCGUGUCGAAUAGUAUUC-P-G-3’	17033.4