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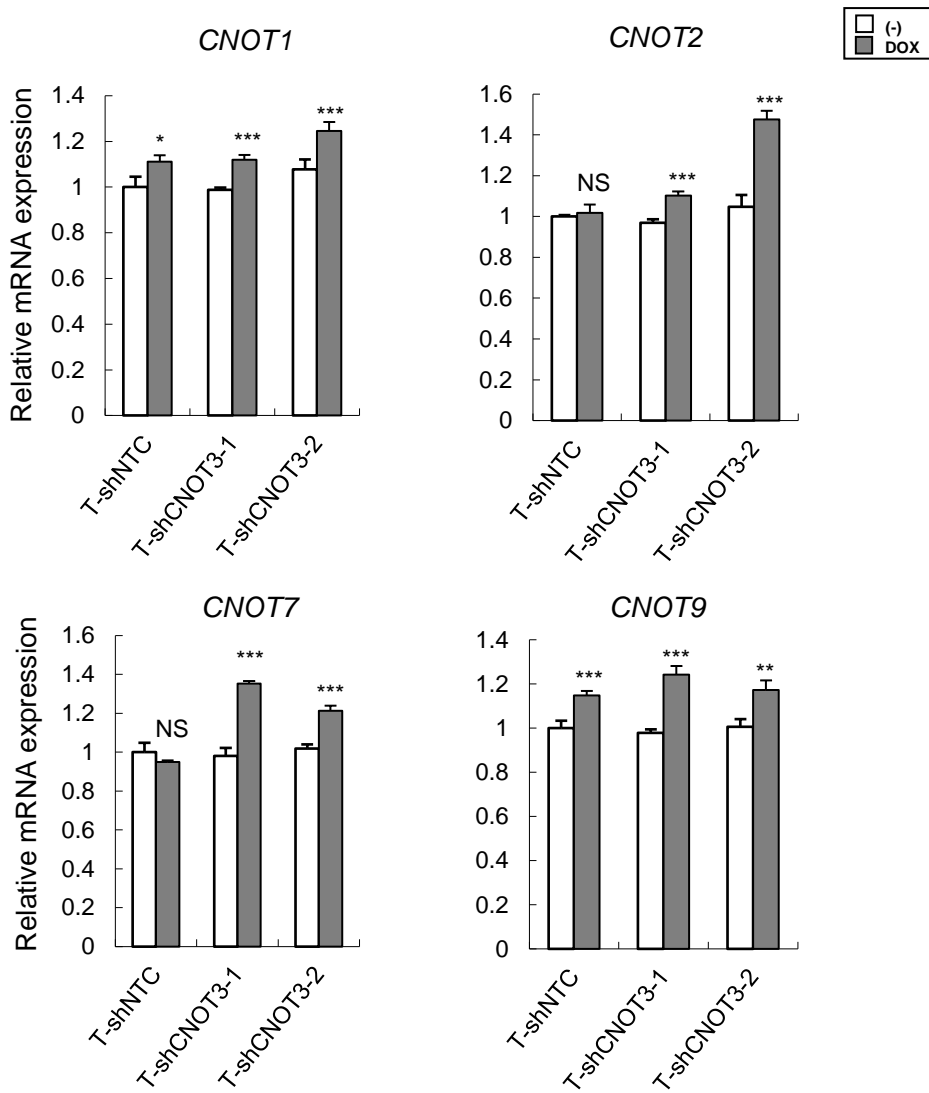
OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY GRADUATE UNIVERSITY  
沖縄科学技術大学院大学

## CNOT3 targets negative cell cycle regulators in non-small cell lung cancer development

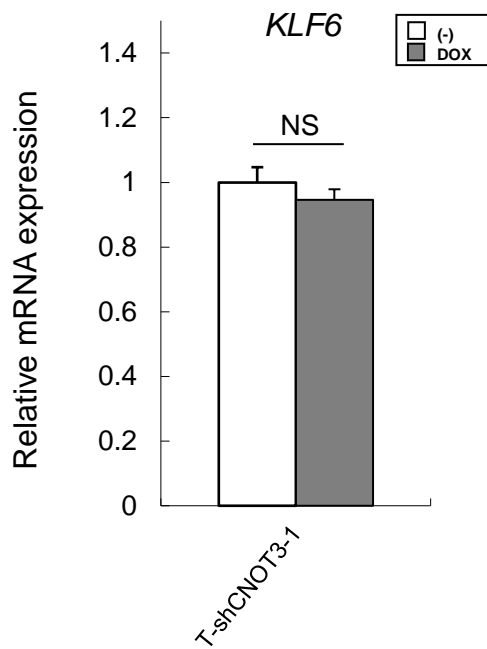
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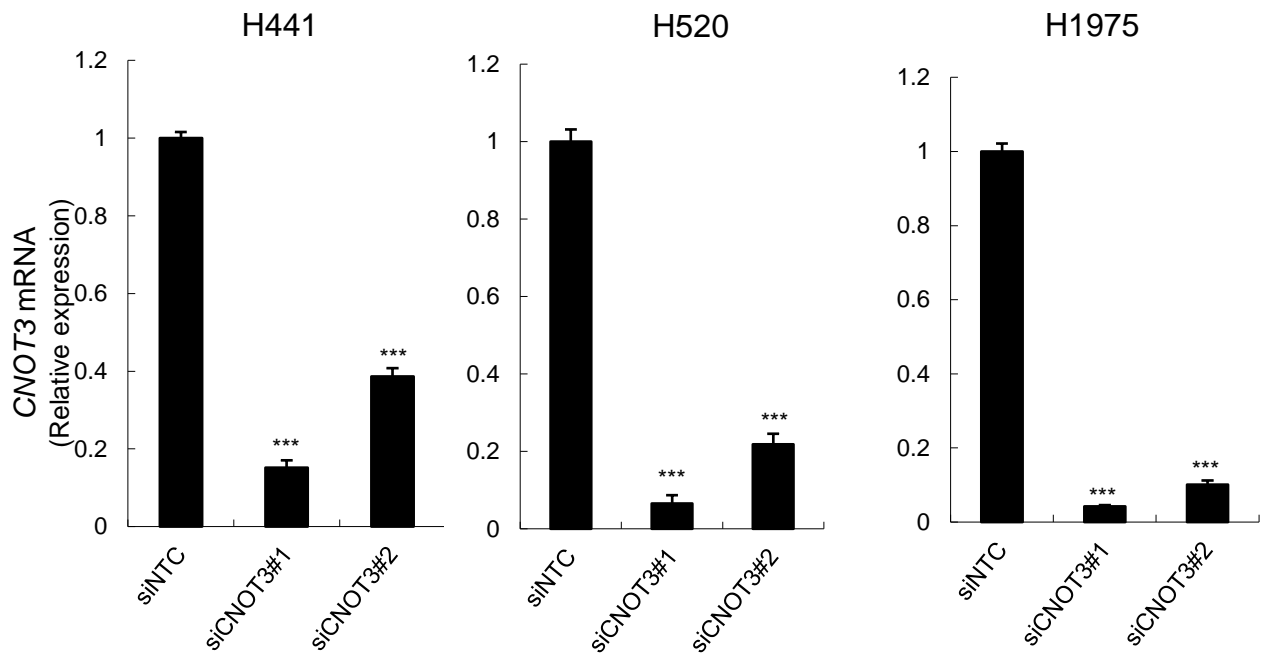




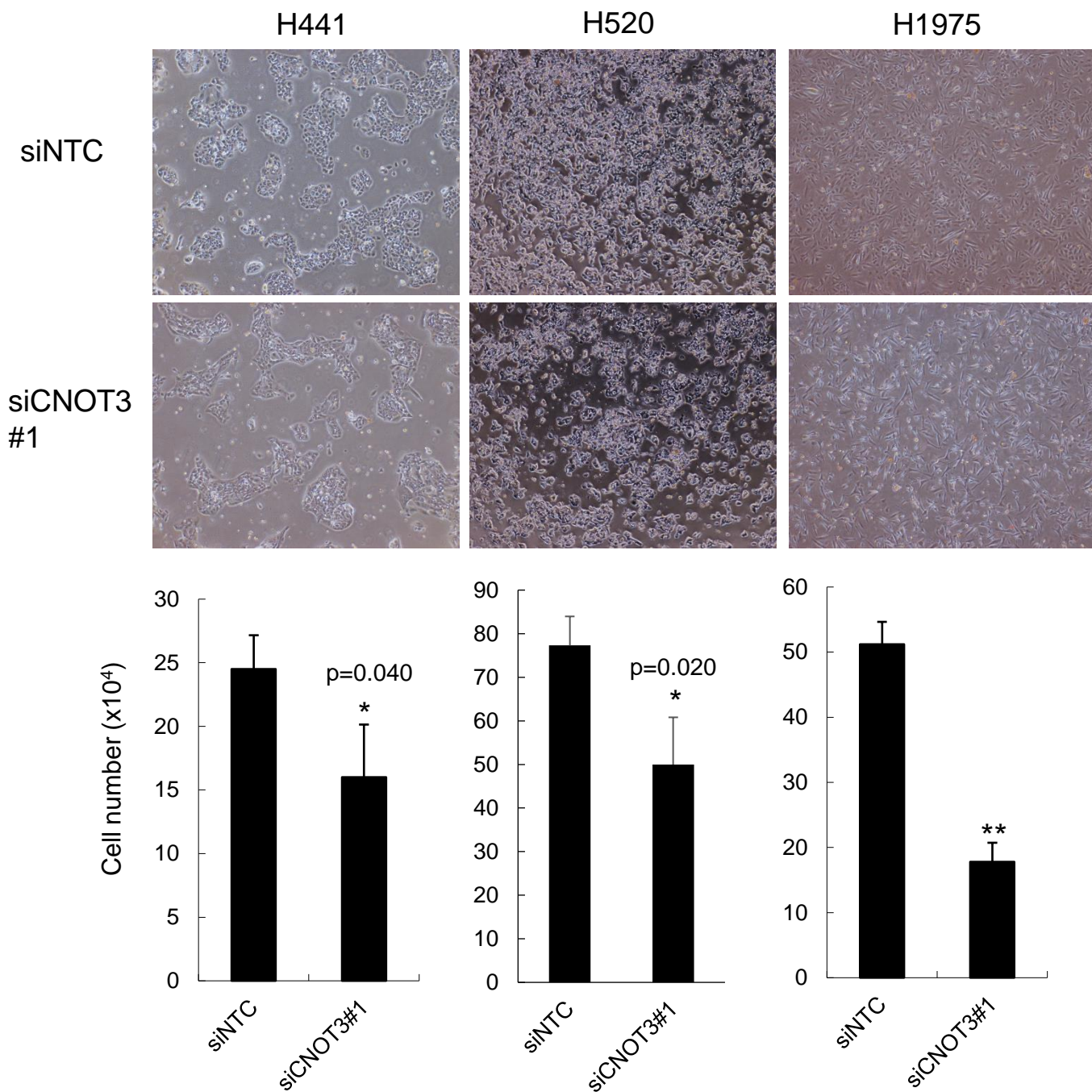
Supplementary Figure S2. qRT-PCR for *CNOT1*, *CNOT2*, *CNOT7*, and *CNOT9* using the cDNA from the same samples with Figure 2a. NS: not significant, \*p<0.05, \*\*p<0.01, or \*\*\*p<0.005 vs each (-) sample by two-sided Student's paired t-test. Data are presented as mean  $\pm$  SD from three technical replicates.



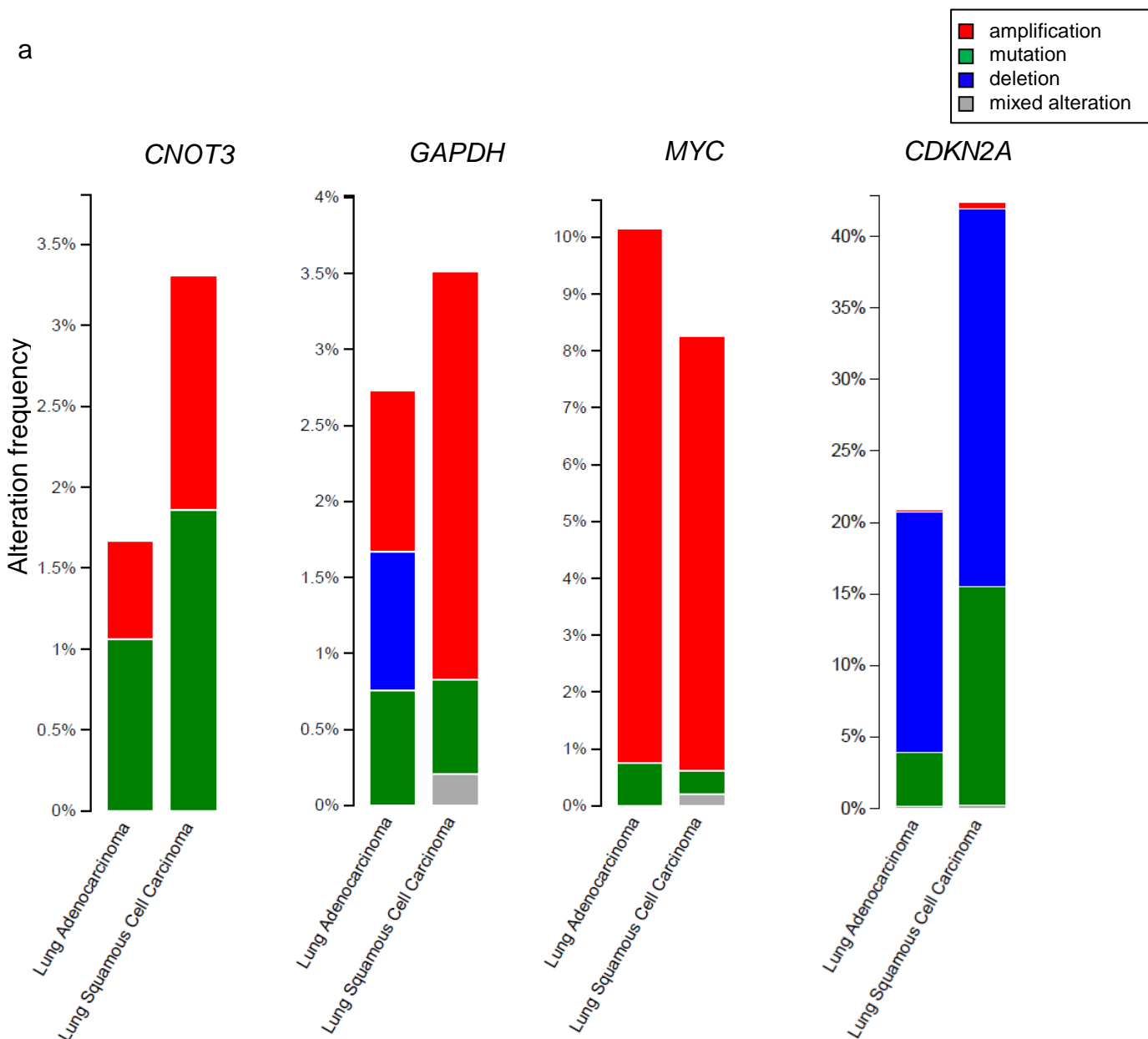
Supplementary Figure S3. qRT-PCR for *KLF6* using the cDNA from the same samples of A549-T-shCNOT3-1 cells with Figure 2a. NS: not significant by two-sided Student's paired t-test. Data are presented as mean  $\pm$  SD from three technical replicates.



Supplementary Figure S4. qRT-PCR for *CNOT3* using the cDNA from the same samples with Figure 6a. \*\*\*p < 0.005 vs siNTC by Tukey-Kramer post hoc test. Data are presented as mean  $\pm$  SD from three technical replicates.

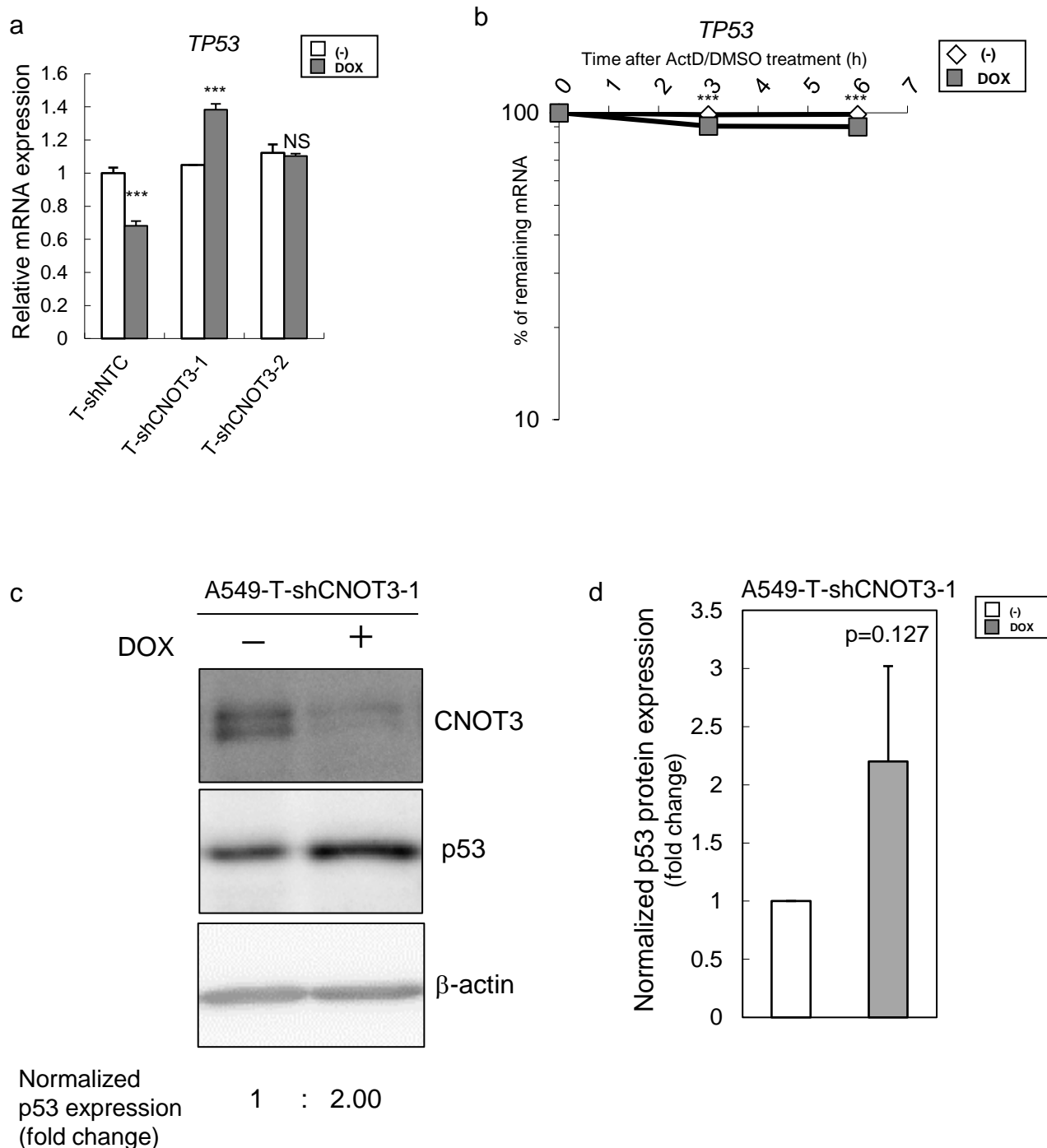


Supplementary Figure S5. Cells ( $10 \times 10^4$  for H441 and H520 or  $25 \times 10^4$  for H1975) were seeded in 12-well (H441 and H520) or 6-well (H1975) plates. Transfection of siNTC or siCNOT3#1 was performed under basically the same condition with Figure 6a, with some modification in proportion to the well area. Representative pictures were taken (above) and cells were counted (below) 3 days after siRNA transfection with duplicates (H1975) or triplicates (H441 and H520). \* $p < 0.05$ , \*\*  $p < 0.01$  vs siNTC by two-sided Student's paired t-test. Data are presented as mean  $\pm$  SD from two or three biological replicates.

**b**

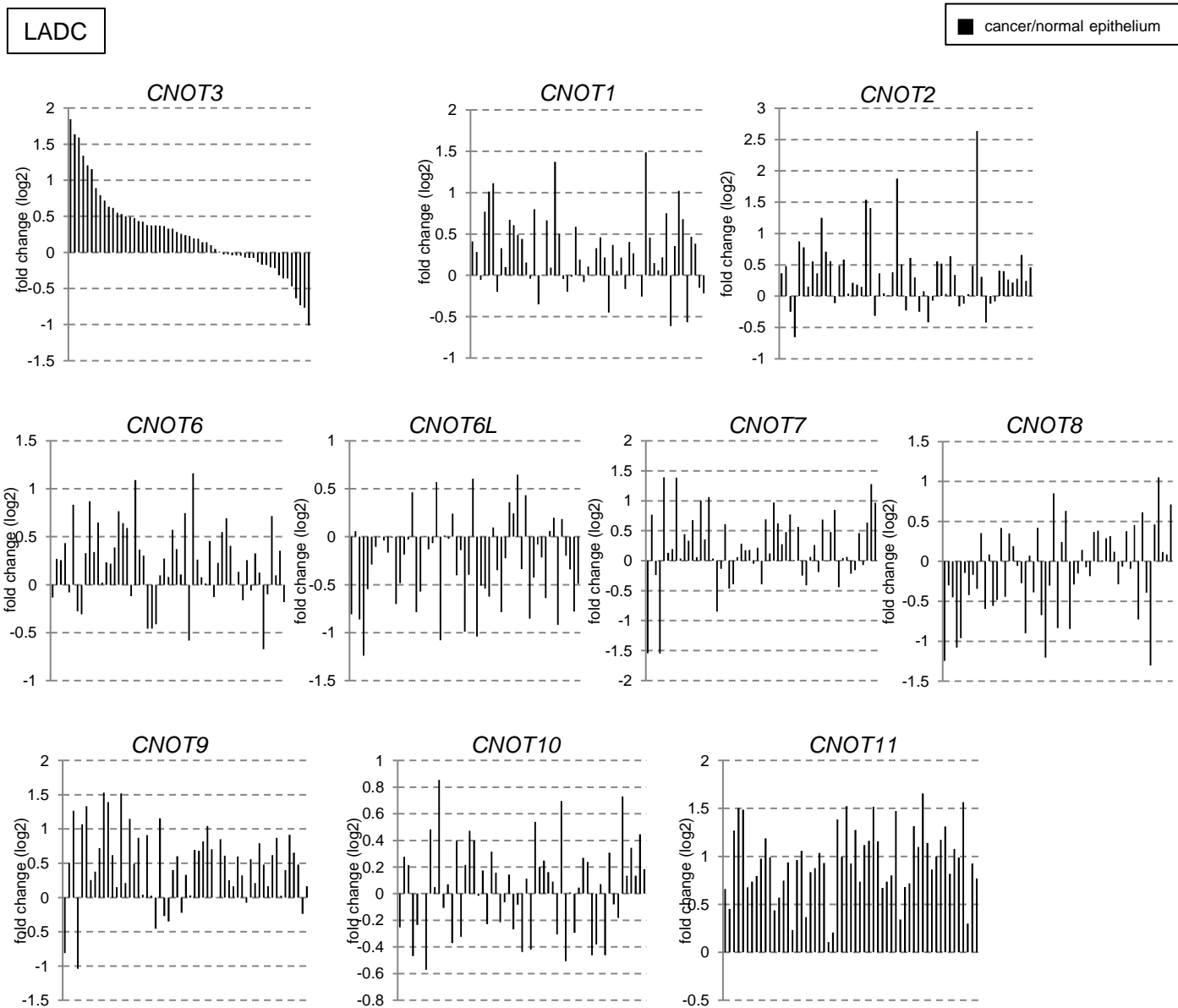
	Gene amplification frequency in total NSCLC	Gene deletion frequency in total NSCLC
<i>CNOT3</i>	0.96% (11/1144)	0% (0/1144)
<i>GAPDH</i>	1.84% (21/1144)	0.52% (6/1144)
<i>MYC</i>	8.74% (100/1144)	0% (0/1144)
<i>CDKN2A</i>	0.26% (3/1144)	21.07% (241/1144)

Supplementary Figure S6. Genomic alteration frequency of each gene in NSCLC using the dataset from Campbell et al. is shown. Mixed alteration (grey) indicates amplification with inframe mutation for *GAPDH* (1 case), amplification with missense mutation for *MYC* (1 case), and deletion with missense mutation for *CKDN2A* (2 cases). The graphs were generated by the cBio Cancer Genomic Portal.

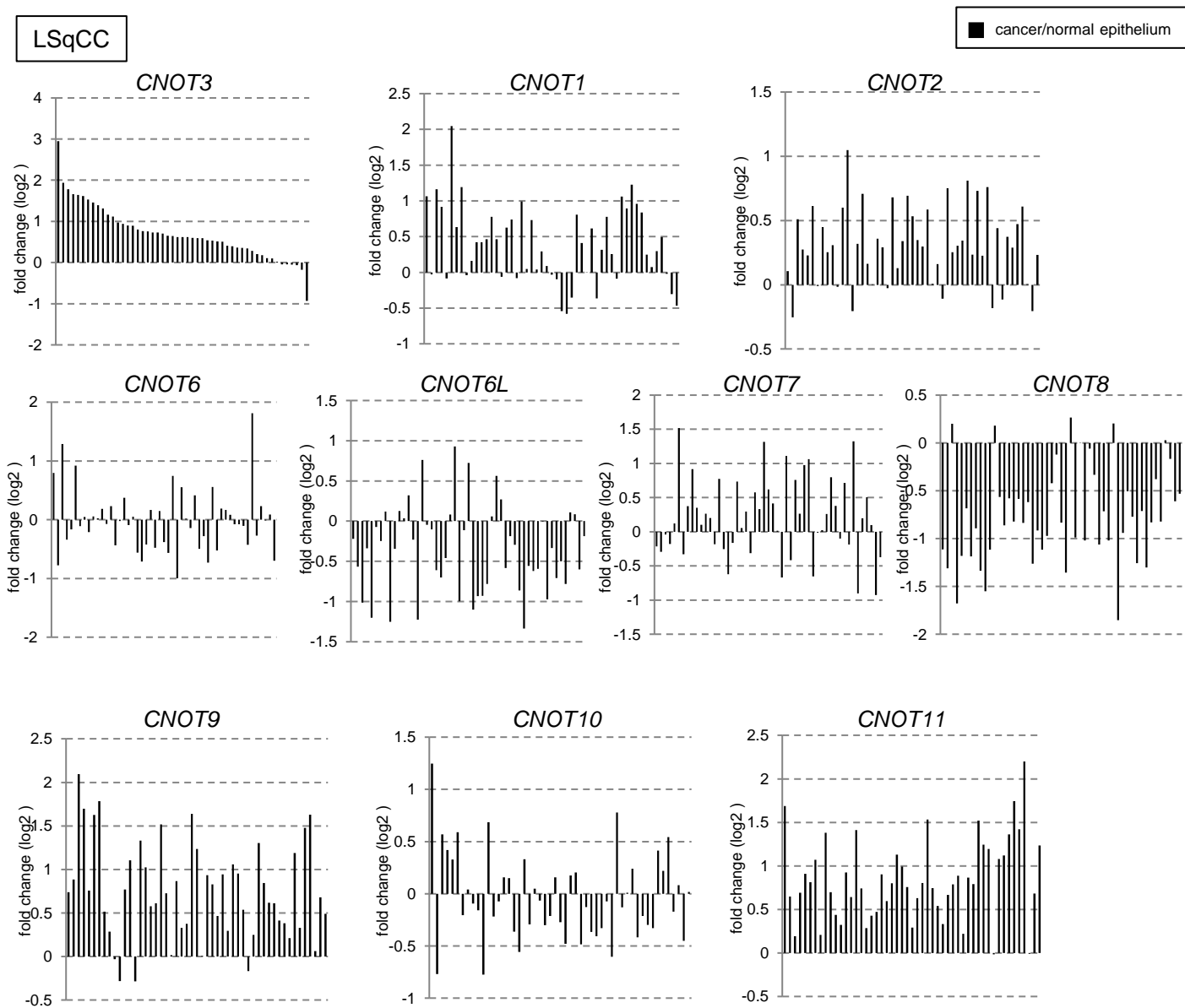


Supplementary Figure S7. (a) qRT-PCR for *TP53* using the cDNA from the same samples with Figure 2a. (b) qRT-PCR for *TP53* using the same samples with Figure 3d. (c) Cell lysate of A549-T-shCNOT3-1 with or without 3 days of DOX treatment was subjected to immunostaining with antibodies, as indicated under *Materials and Methods*. (d) The average value of normalized p53 protein expression using three individual sets of cell lysate of A549-T-shCNOT3-1 with or without 3 days of DOX treatment. NS: not significant or \*\*\* $p < 0.005$  vs each (-) sample by two-sided Student's paired t-test or Welch's t-test. Data are presented as mean  $\pm$  SD from three technical replicates or individual samples.



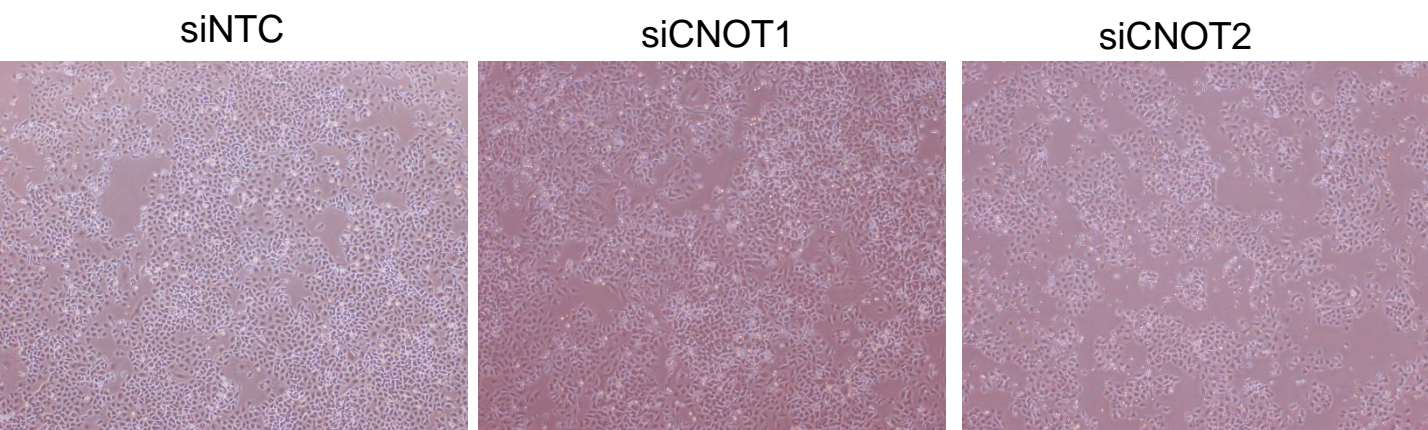


Supplementary Figure S8. Fold change (log<sub>2</sub>) of the expressions of each CCR4-NOT components in LADC in comparison with the paired normal lung epithelium from the same patients using the same TCGA RNA-seq data with Figure 1a is shown. The samples are put into order based on the fold change of CNOT3 expression from the top to the bottom.

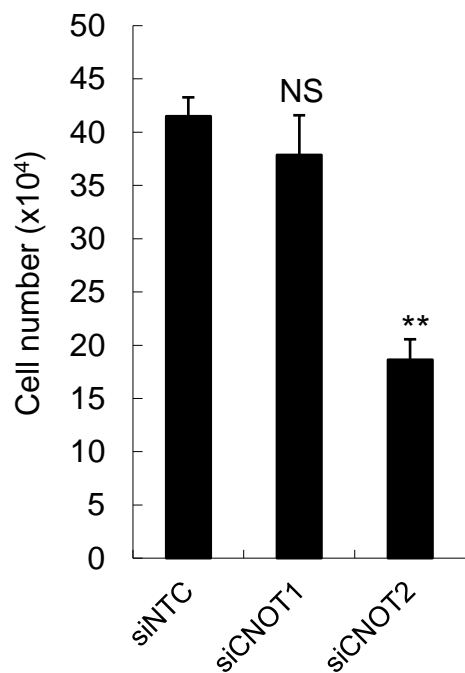


Supplementary Figure S9. Fold change ( $\log_2$ ) of the expressions of each CCR4-NOT components in LSqCC in comparison with the paired normal lung epithelium from the same patients using the same TCGA RNA-seq data with Figure 1b is shown. The samples are put into order based on the fold change of CNOT3 expression from the top to the bottom.

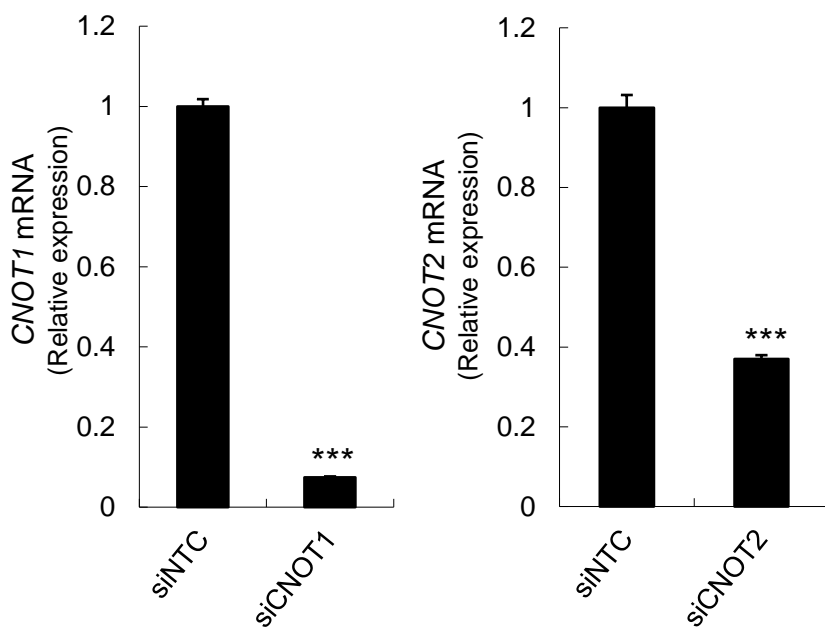
A



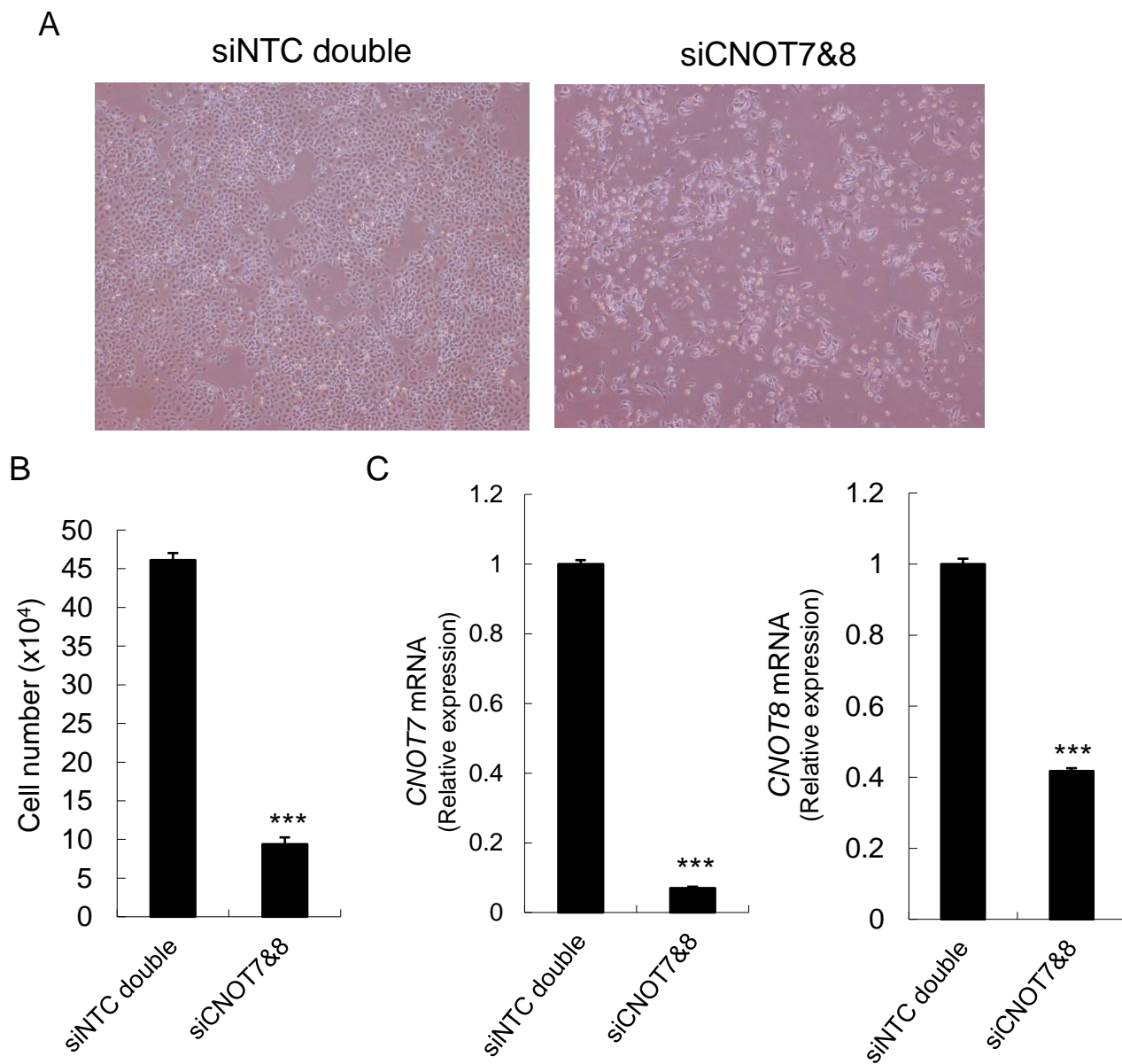
B



C



Supplementary Figure S10. A549 cells ( $5 \times 10^4$ ) were seeded to 12-well plates in duplicates. Representative pictures were taken (A) and cell number was counted (B) 3 days after siRNA transfection. mRNA expression of the cells 3 days after siRNA transfection was also examined (C). NS: not significant, \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs siNTC by Tukey-Kramer post hoc test (B) or two-sided Student's paired t-test (C). Data are presented as mean  $\pm$  SD from two biological replicates or three technical triplicates.



Supplementary Figure S11. A549 cells ( $5 \times 10^4$ ) were seeded to 12-well plates in duplicates. Representative pictures were taken (A) and cell number was counted (B) 3 days after siRNA transfection. mRNA expression of the cells 3 days after siRNA transfection was also examined (C). \*\*\* $p < 0.001$  vs siNTC double by two-sided Student's paired t-test. Data are presented as mean  $\pm$  SD from two biological replicates or three technical replicates.