Figure S4

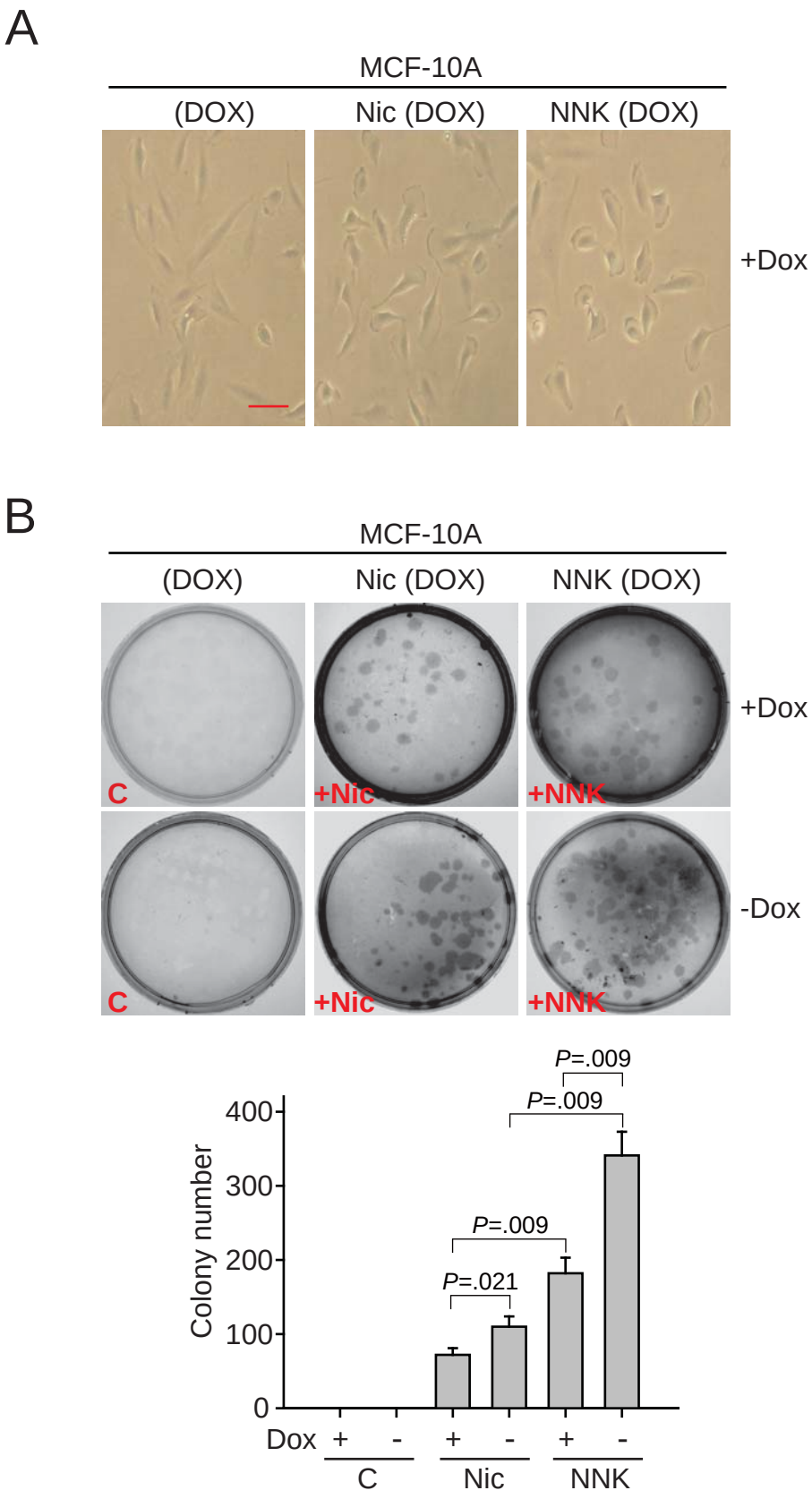
A
MCF-10A
(DOX)
Nic (DOX)
NNK (DOX)
+Dox
B
MCF-10A
(DOX)
Nic (DOX)
NNK (DOX)
+Dox
-Dox
C
+Nic
+NNK
C
+Nic
+NNK
P=.009
P=.009
P=.009
P=.021
Colony number
400
300
200
100
0
Dox + - + - + -
C
Nic
NNK

Figure S4. Establishment of α9-nicotinic acetylcholine receptor (α9-nAChR)-overexpressing transformed human breast epithelial cells: MCF-10A-Nic (DOX) and MCF-10A-NNK (DOX). MCF-10A (DOX) cells were treated with the nicotine metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; 1 μM) or nicotine (Nic; 10 μM) and the medium was replaced every 2 days for 60 days according to previously described methods [9]. **A)** Morphologies of MCF-10A-Nic (DOX) and MCF-10A-NNK (DOX) cells. Both cell lines exhibited subtle changes in morphology, including a slight increase in roundedness compared with the parental MCF-10A (DOX) cells. Scale bar = 25 μm. **B)** Number of colonies scored for the soft-agar plates. To investigate the role of acquired α9-nAChR overexpression in colony formation, MCF-10A-Nic (DOX) and MCF-10A-NNK (DOX) transformed cells were seeded in soft agar with or without DOX. The treatment conditions were the same as those described in Figure 4, B. Parental MCF-10A (DOX) cells that were transiently infected with an α9-nAChR adenovirus did not transform into colonies in soft agar (bars 1–2). These results demonstrate that overexpression of α9-nAChR in MCF-10A (DOX) cells without long-term exposure to NNK or nicotine could not induce malignant cell transformation. The colonies present in a whole area were counted for each plate. C, vehicle control. Data represent the mean of nine samples in each group; error bars are 95% confidence intervals. Comparisons were performed for DOX+ Nic+ vs DOX- Nic+ (P = .021), DOX+ NNK+ vs DOX- NNK+ (P = .009), DOX+ Nic+ vs DOX+ NNK+ (P = .009), and DOX- Nic+ vs DOX- NNK+ (P = .009). Data were analyzed with nonparametric tests (Kruskal Wallis and Mann-Whitney test); all P-values are two-sided.