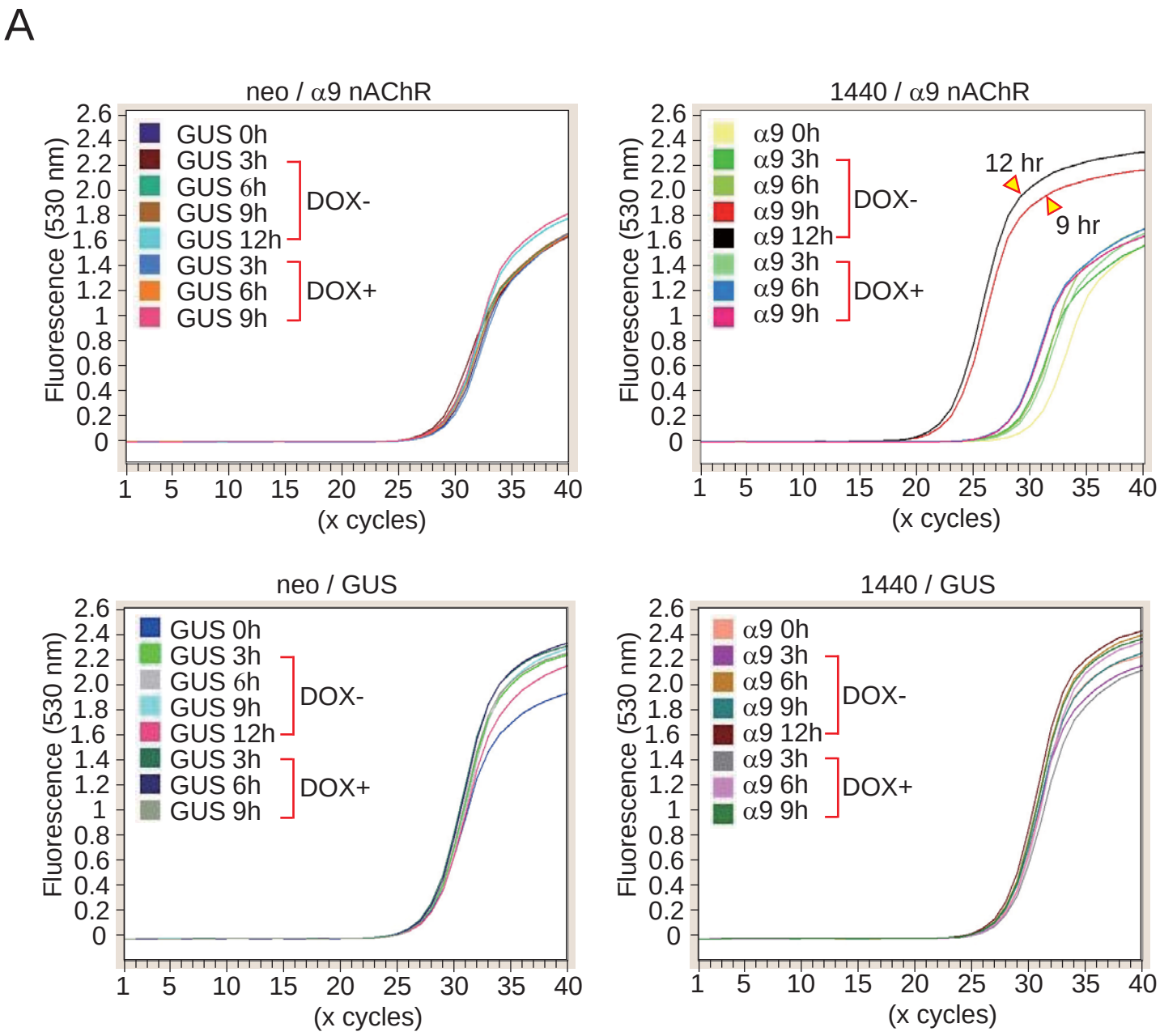
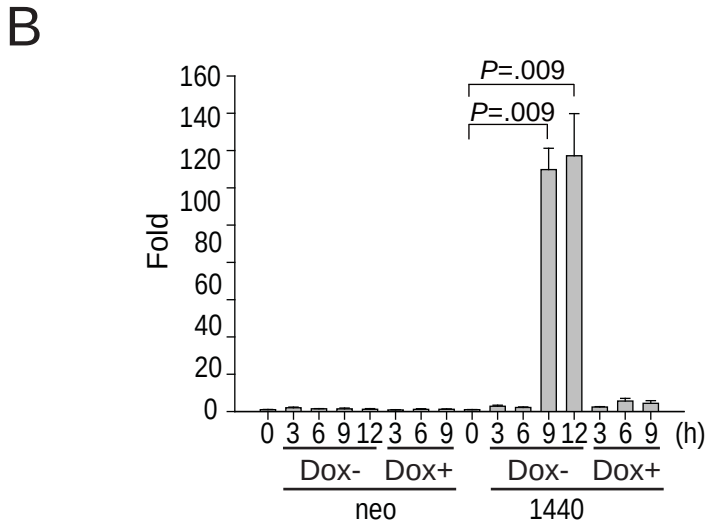
Figure S3


A
neo / α9 nAChR
Fluorescence (530 nm)
(x cycles)
GUS 0h
GUS 3h
GUS 6h
GUS 9h
GUS 12h
DOX-
DOX+
1440 / α9 nAChR
α9 0h
α9 3h
α9 6h
α9 9h
α9 12h
12 hr
9 hr
neo / GUS
1440 / GUS


B
Fold
P=.009
P=.009
0 3 6 9 12 3 6 9 0 3 6 9 12 3 6 9 (h)
Dox- Dox+ Dox- Dox+
neo
1440

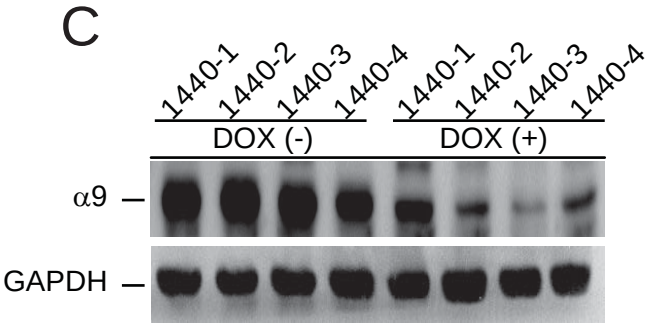

C
1440-1
1440-2
1440-3
1440-4
DOX (-)
DOX (+)
α9
GAPDH

Figure S3. Establishment of transformed human breast epithelial MCF-10A (DOX) cells that overexpressed the α9-nicotinic acetylcholine receptor (α9-nAChR) via a tetracycline-based (Tet-off)-regulatory system. **A, B)** Inducible α9-nAChR expression. Human MCF-10A (DOX) cells were cultured in fresh growth medium containing 10% serum and 1 μg/mL of the tetracycline analog, doxycycline (DOX+). α9-nAChR mRNA expression was induced in MCF-10A (DOX) cells (designated as 1440) in a time-dependent manner after removal of DOX. Cells infected with adenovirus vector alone (designated as neo) were used as a control. Data points represent the means; error bars indicate 95% confidence intervals. Inducible α9-nAChR expression was compared in MCF-10A (DOX-) cells at 0 vs 9 and 12 hours (P = .009). β-glucuronidase (GUS) gene expression from each sample was analyzed for internal control. Data were analyzed using nonparametric tests (Kruskal Wallis and Mann-Whitney test); all P-values are two-sided. **C)** The level of α9-nAChR protein expression in MCF-10A (DOX-) cells increased substantially 24 hours after removal from DOX (lanes 1–4) in comparison to control MCF-10A (DOX+) cells (lanes 5–8).