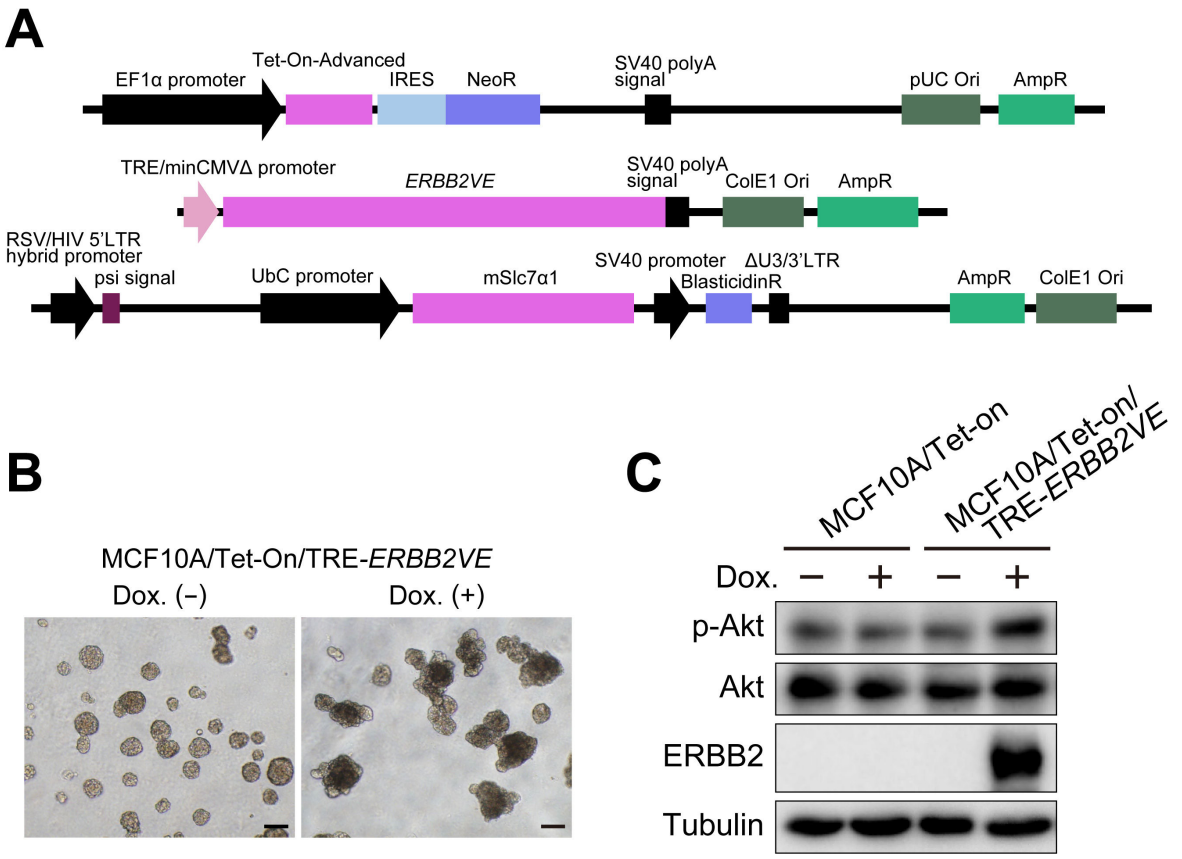
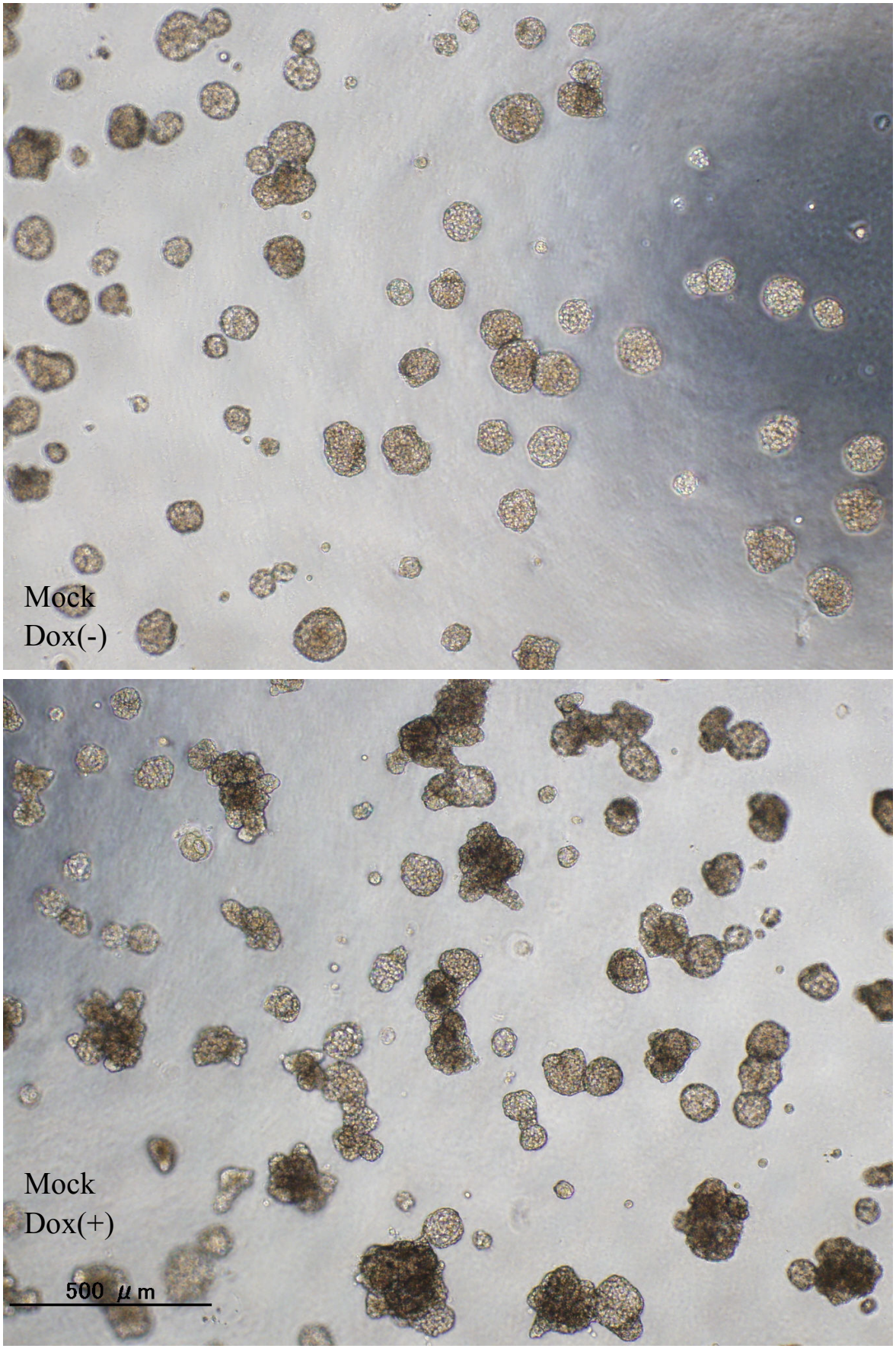
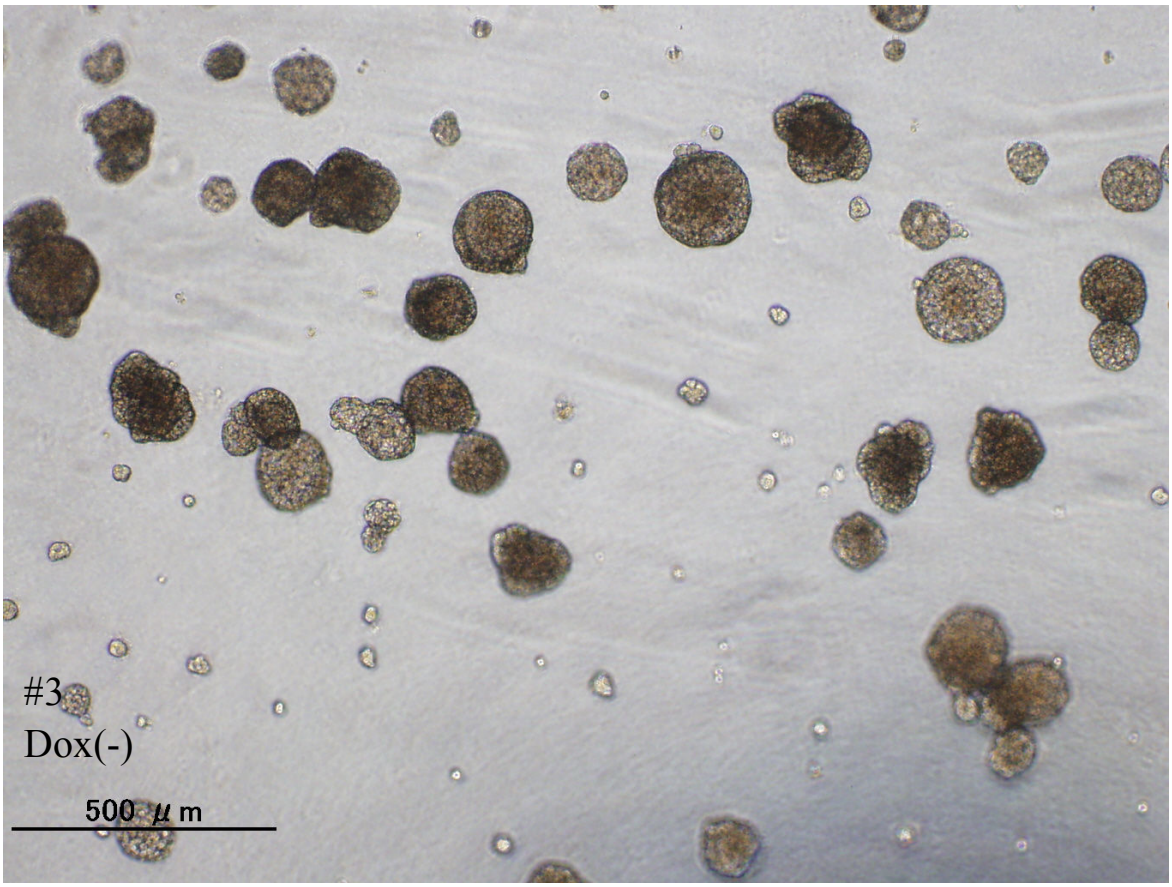
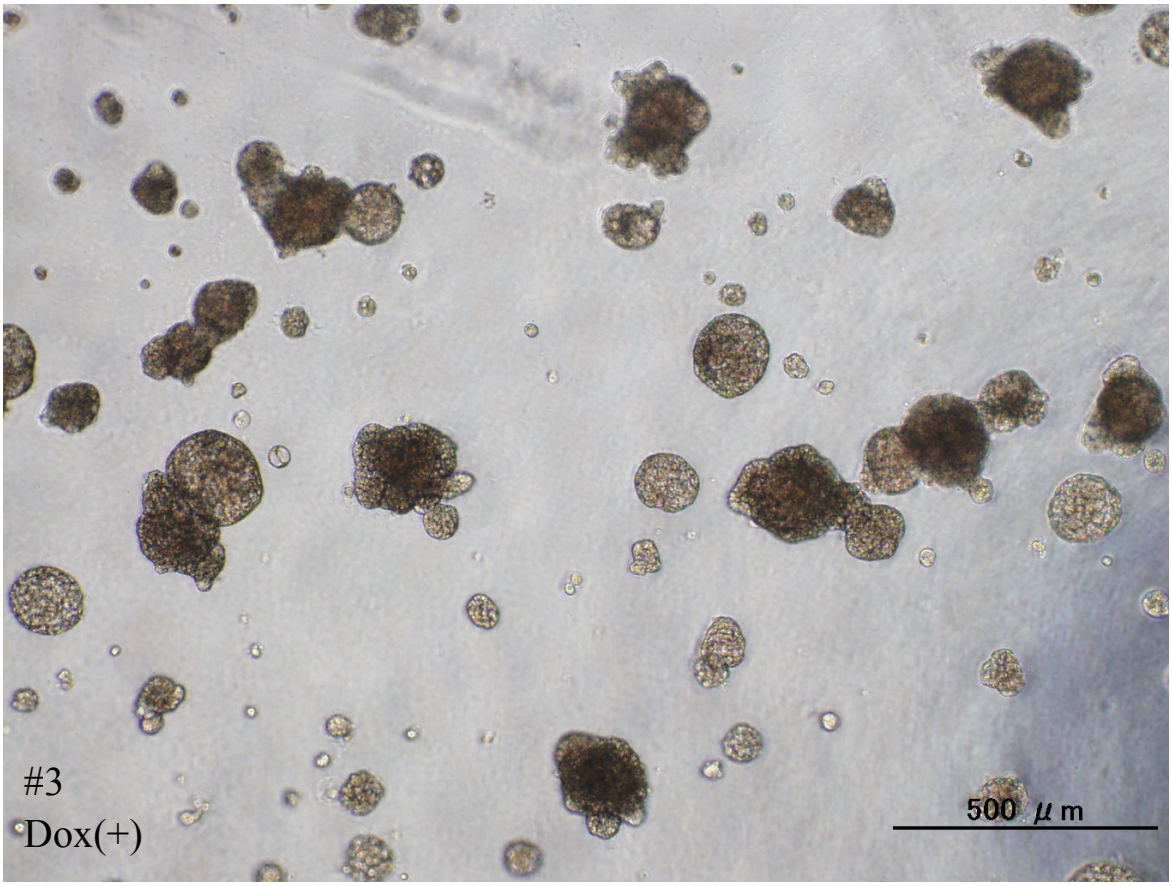
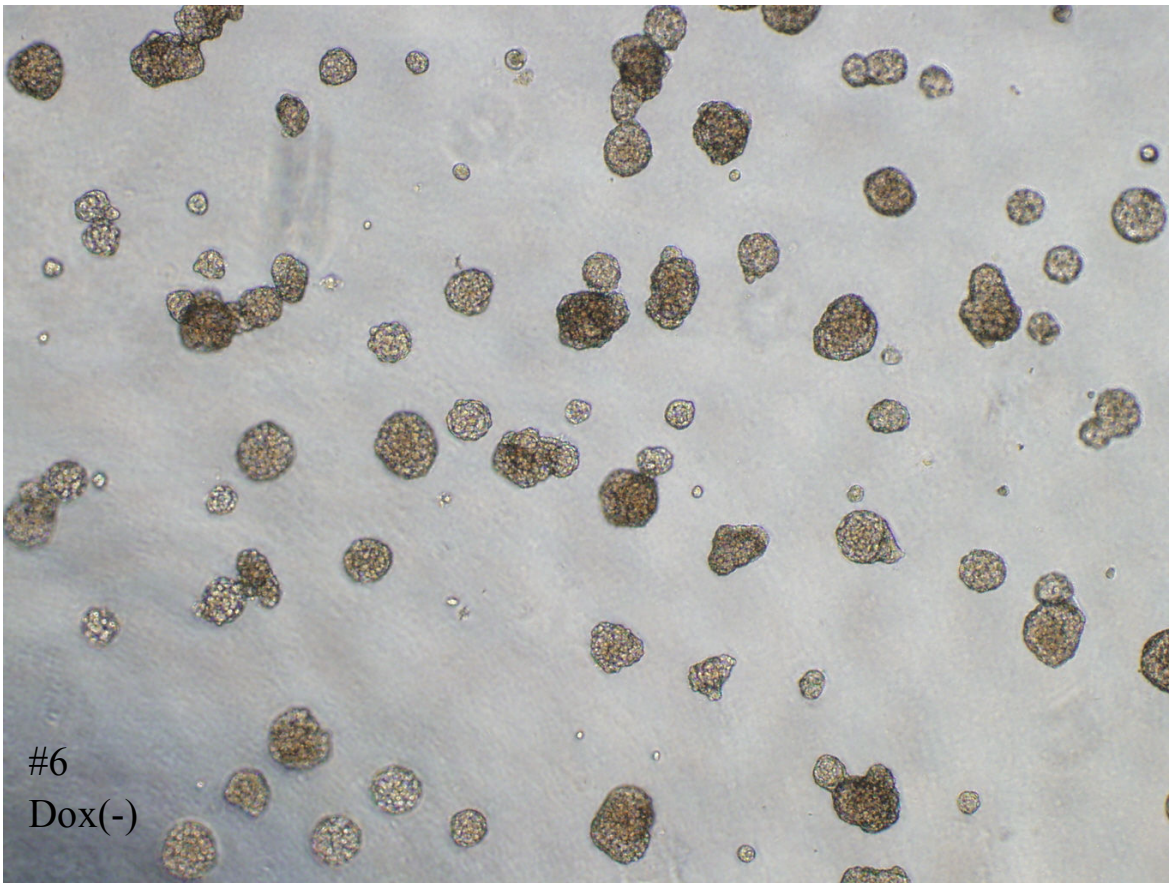


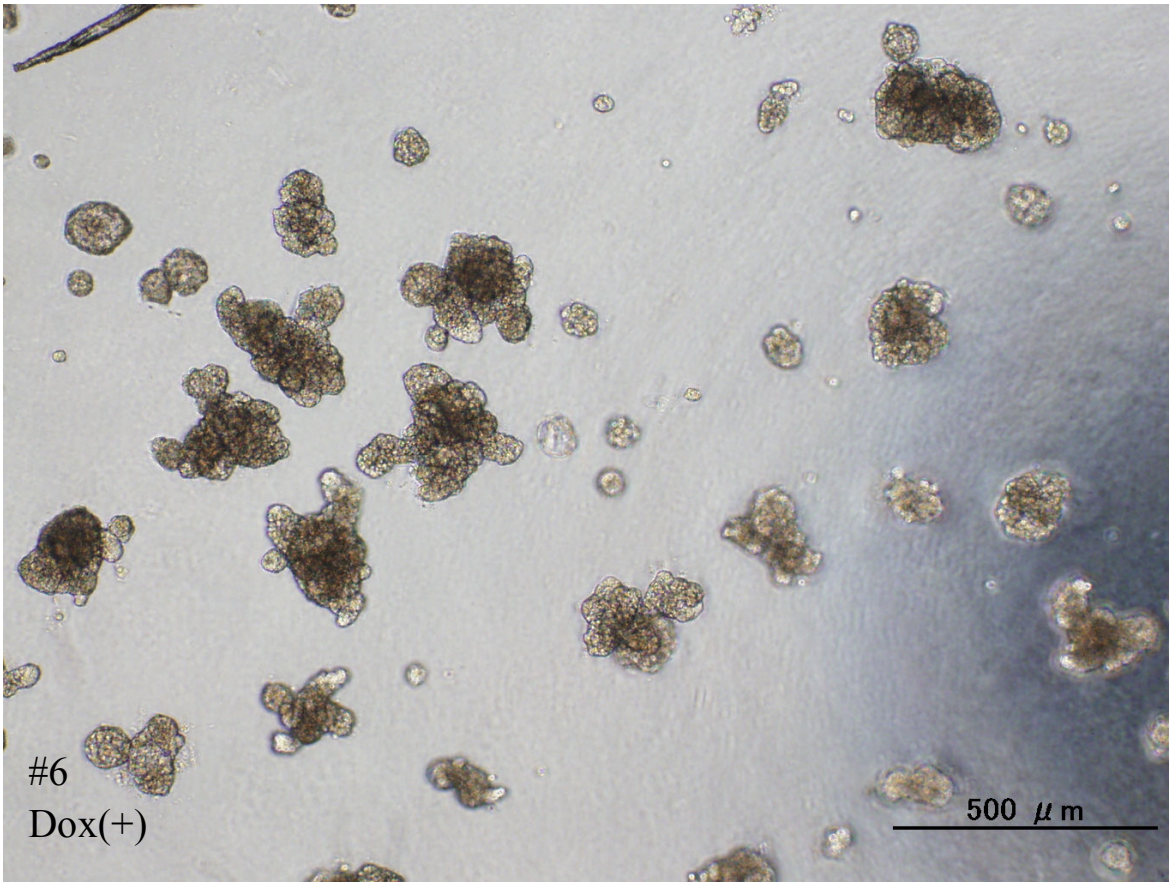
Figure S1. Construction of *ERBB2*-inducible MCF10A strain for screening. (A) Plasmids used for the establishment of the MCF10A clone for screening. *ERBB2VE* gene is under regulation of a tetracycline-responsive DNA element and minimal CMV promoter. *mSLC7a1* gene encodes a mouse ecotropic retroviral receptor. (B) Detection of transforming activities in an isolated MCF10A/Tet-on/*ERBB2VE* clone. MCF10A/Tet-on/*ERBB2VE* cells were cultured on Matrigel for 14 days. In Dox (+) panels, *ERBB2VE* was induced by the addition of Dox at day 7. We selected this parental *ERBB2VE*-inducible MCF10A strain for screening genes for their ability to disrupt lumen formation. Scale bars represent 100 μm. (C) Confirmation of ERBB2 protein induction and its activity. The parental MCF10A/Tet-on cells and their descendant MCF10A/Tet-on/TRE-*ERBB2VE* cells were cultured in the absence (-) or the presence (+) of Dox. Protein levels in the cells were analyzed by immunoblotting using the indicated antibodies. Tubulin was used as a loading control.

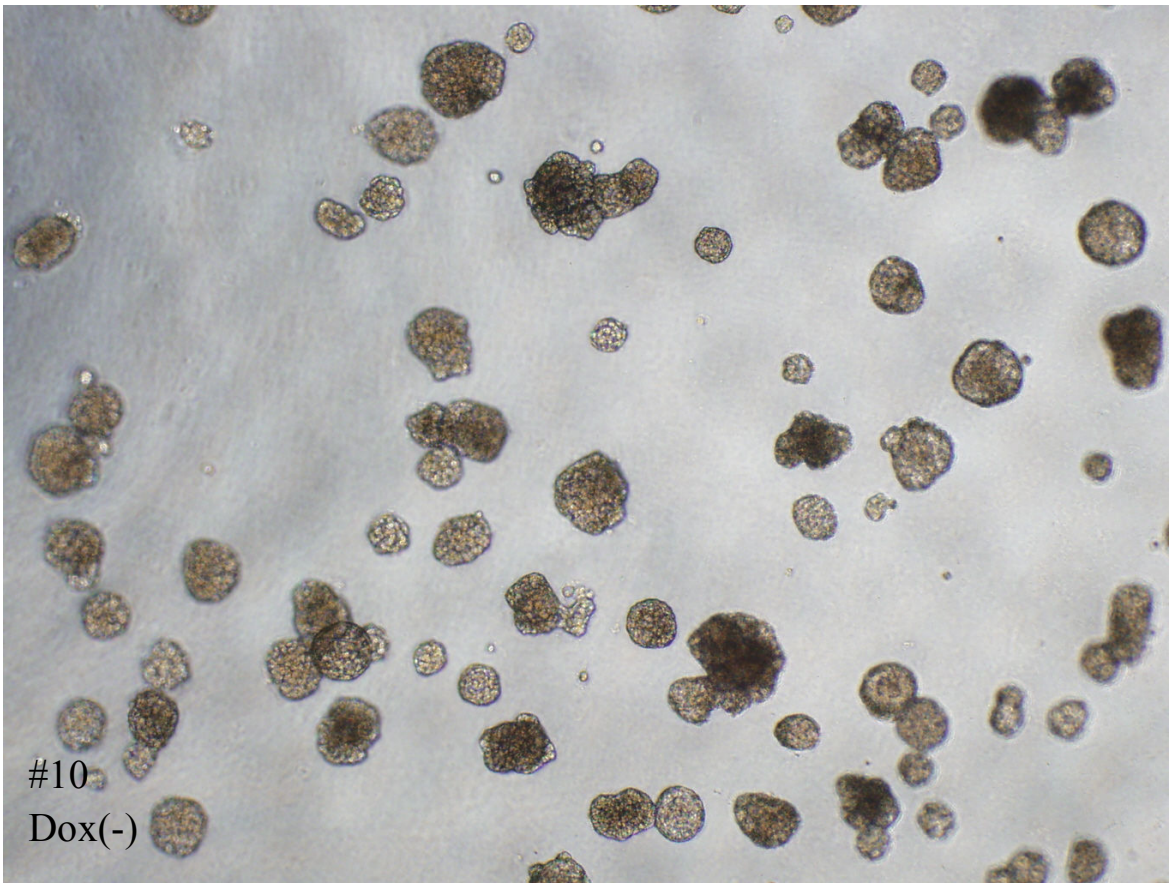

Mock
Dox(-)
Mock
Dox(+)
500 μm


#3
Dox(-)
500 μm


#3
Dox(+)
500 μm


#6
Dox(-)


#6
Dox(+)
500 μm


#10
Dox(-)

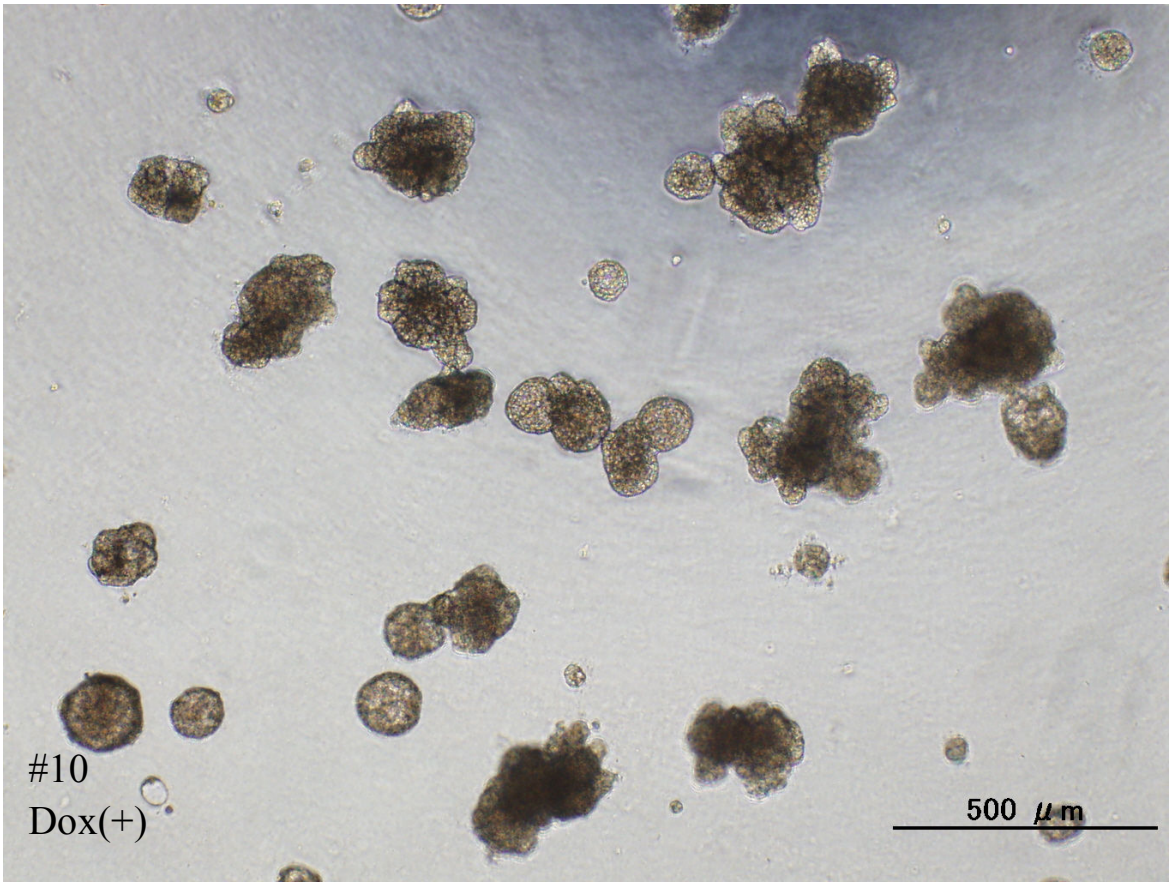

#10
Dox(+)
500 μm

Figure S2. High resolution images of 3D-morphological changes observed after subgroup gene introduction. These are high-resolution pictures of phenotypic changes shown in Figure 1B. Scale bars represent 500 μm.

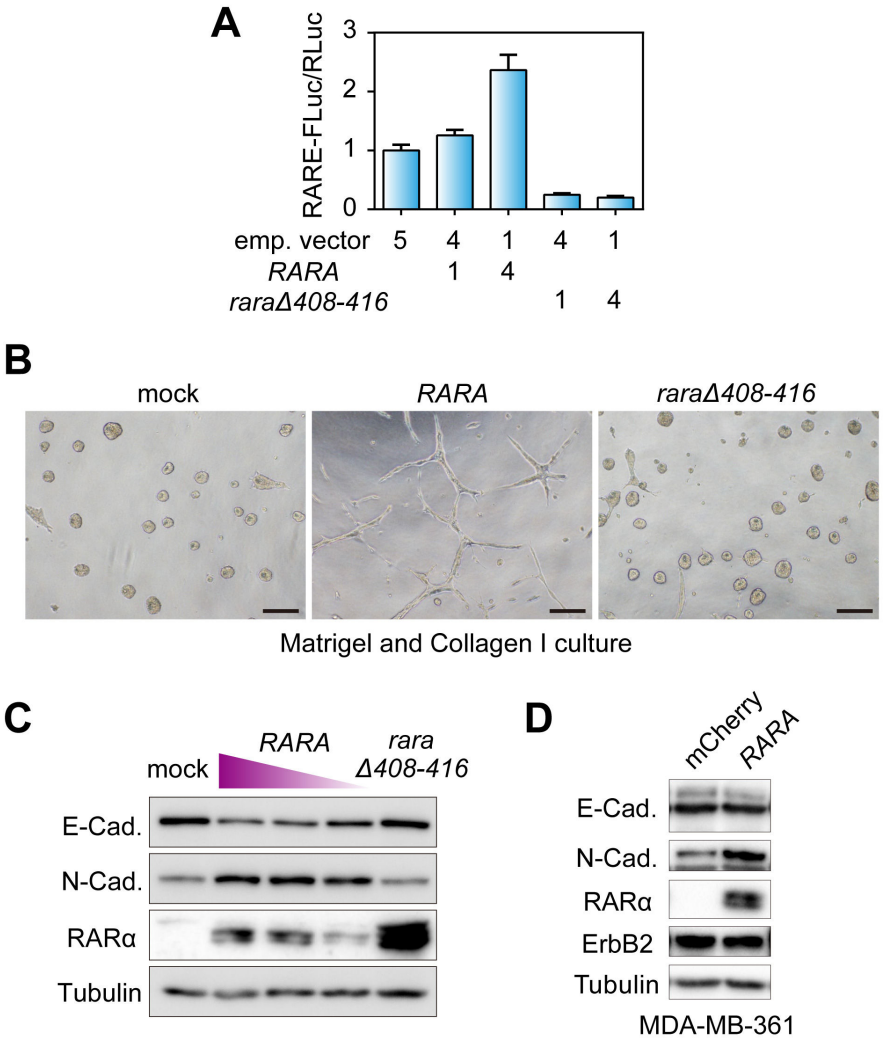


Figure S3. Induction of EMT by RARα in a manner required for its transcriptional activity. (A) Enhanced transcriptional activity by overexpression of *RARA* and dominant negative activity of its mutant lacking the transcriptional activation domain. MCF10A/Tet-on/Eco cells were cotransfected with

Renilla-luciferase vector (internal control), RARE-Firefly-luciferase vector, and expression vectors harboring empty, *RARA,* or *raraΔ408-416* genes by calcium phosphate transfection. Their extracts were subjected to dual luciferase assay, and the relative Firefly-luciferase activity was calculated. Numbers below the graphs represent the relative DNA amounts of the expression vectors. See also materials and methods. (B) An essential role of amino acids 408–416 of RARα in the invasive transformation. MCF10A/Tet-on/TRE-*ERBB2VE*/Eco cells overexpressing the indicated genes were cultured in a 1:1 mixture of Matrigel and collagen I for 5 days in the absence of Dox. Scale bars represent 200 μm. (C) An essential role of amino acids 408–416 of RARα in EMT induction. MCF10A/Tet-on/TRE-*ERBB2VE*/Eco cells were infected with the indicated retroviral vectors at higher MOI of *raraΔ408–416* than that of *RARA*. Protein levels were analyzed by immunoblotting using the indicated antibodies. Tubulin was used as a loading control. (D) N-cadherin increase by RARα in MDA-MB-361 breast cancer cells. Cells were infected with retrovirus packaged with pMXs-mCherry-IRES-puro or pMXs-*RARA*-IRES-puro vector together with pVSV-G plasmid and selected with puromycin. Protein levels in the cells were analyzed by immunoblotting with the indicated antibodies.

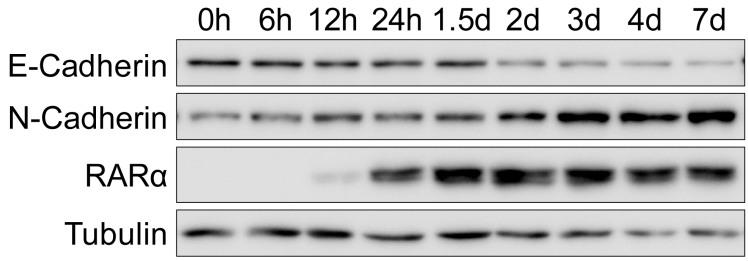


Figure S4. Chronological changes in E-cadherin and N-cadherin expression levels induced by *RARA* expression. MCF10A/Tet-on/TRE-*ERBB2VE*/Eco cells were infected with retroviruses for *RARA* and lysed at the indicated time points. The expression levels of the indicated proteins were analyzed by immunoblotting. Tubulin was used as a loading control.

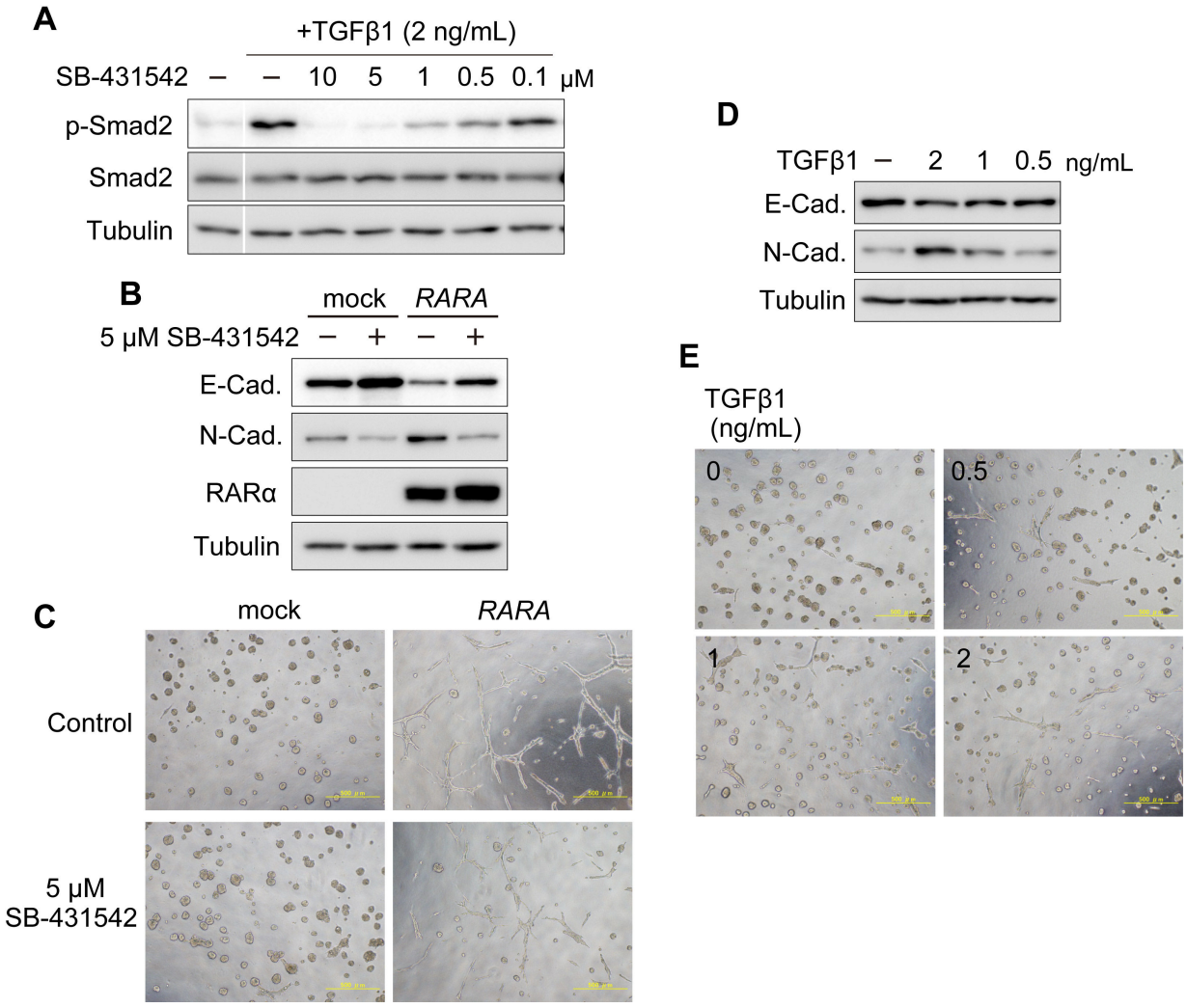


Figure S5. Partial involvement of the TGF-β/SMAD signaling pathway in RARα function. (A) Inhibiton of the phosphorylation of SMAD2 by SB-431542 treatment. MCF10A/Tet-on/TRE-*ERBB2VE*/Eco cells were treated with indicated concentrations of SB-431542, an inhibitor of TGF-β receptor type I, for 30 min, followed by stimulation with recombinant human TGF-β1 at 2 ng/mL for 24 h. Protein levels in the cells were analyzed by immunoblotting using the indicated antibodies. Tubulin was used as a loading control. White line, trimmed margin of unrelated sample lanes. (B) Partial suppression of RARα-induced EMT by SB-431542 treatment. MCF10A/Tet-on/TRE-*ERBB2VE*/Eco cells were infected with retroviruses for *Venus* or *RARA*, and simultaneously treated with 5 μM SB-431542. The concentration of SB-431542 was determined on the

basis of the phosphorylation level of SMAD2 under TGF-β stimulation shown in (A). Protein levels in the cells were analyzed by immunoblotting using the indicated antibodies. Tubulin was used as a loading control. (C) Partial suppression of the RARα-induced invasive phenotype by SB-431542 treatment. Cells indicated in (B) were cultured in a 1:1 mixture of Matrigel and collagen I for 5 days in the absence or presence of SB-431542. Scale bars represent 500 μm. (D) Effects of TGF-β1 on changes in the expression of EMT markers. MCF10A/Tet-on/TRE-*ERBB2VE*/Eco cells were treated with TGF-β1 at the indicated concentrations for 3 days. Protein levels in the cells were analyzed by immunoblotting using the indicated antibodies. Tubulin was used as a loading control. (E) Effects of TGF-β1 on morphology in Matrigel-collagen I 3D cultures. Three days after TGF-β1 treatment, MCF10A/Tet-on/TRE-*ERBB2VE*/Eco cells were embedded in a 1:1 mixture of Matrigel and collagen I. Cells were cultured for 5 days in assay medium supplemented with TGF-β1 at the indicated concentrations. Scale bars represent 500 μm.

Table S1. Oligonucleotide pairs for knockdown vector construction

shZEB1#1	sense	5'-GATCCGGACTAGCAATGTTGATTTGAGCTTCCTGTCACTCAGATTAACATTGTTAGTCCTTTTTG-3'
	antisense	5'-AATTCAAAAAGGACTAACAATGTTAATCTGAGTGACAGGAAGCTCAAATCAACATTGCTAGTCCG-3'
shZEB1#2	sense	5'-GATCCGCCAAATTGGGGTTAGCTTCTGCTTCCTGTCACAGAAGTTAATCCTAATTTGGCTTTTTG-3'
	antisense	5'-AATTCAAAAAGCCAAATTAGGATTAACTTCTGTGACAGGAAGCAGAAGCTAACCCCAATTTGGCG-3'
shZEB2	sense	5'-GATCCGCCATTATCCGGTTAAGGAACGCTTCCTGTCACGTTTCTTAACTGGGTAATGGCTTTTTG-3'
	antisense	5'-AATTCAAAAAGCCATTACCCAGTTAAGAAACGTGACAGGAAGCGTTCCTTAACCGGATAATGGCG-3'

*Anealed oligos were inserted into *Bam*HI and *Hin*dIII restriction sites of knockdown vectors.

Table S2. Gene lists in subgroups

Subgroup	Gene Symbol					
1	*ERBB2*	*C17orf37*	*GRB7*	*GSDMA*	*CSF3*	*CCR7*
2	*PSMB3*	*PSMD3*	*RAPGEFL1*	*LOC100131821*	*KRT25*	
3	*HNF1B*	*RPL23*	*MED1*	*GSDMB*	*RARA*	*IGFBP4*
4	*ORMDL3*	*CDC6*	*TNS4*	*SMARCE1*	*TMEM99*	
5	*C17orf78*	*SOCS7*	*PIP4K2B*	*LASP1*	*KRT28*	
6	*CWC25*	*TCAP*	*PGAP3*	*THRA*	*NR1D1*	
7	*SYNRG*	*DDX52*	*FBXL20*	*PNMT*	*MSL1*	
8	*MRPL45*	*SRCIN1*	*PLXDC1*	*RPL19*	*WIPF2*	
9	*TADA2A*	*DUSP14*	*PCGF2*	*IKZF3*	*MED24*	
10	*MLLT6*	*CACNB1*	*PPP1R1B*	*STARD3*	*KRT24*	