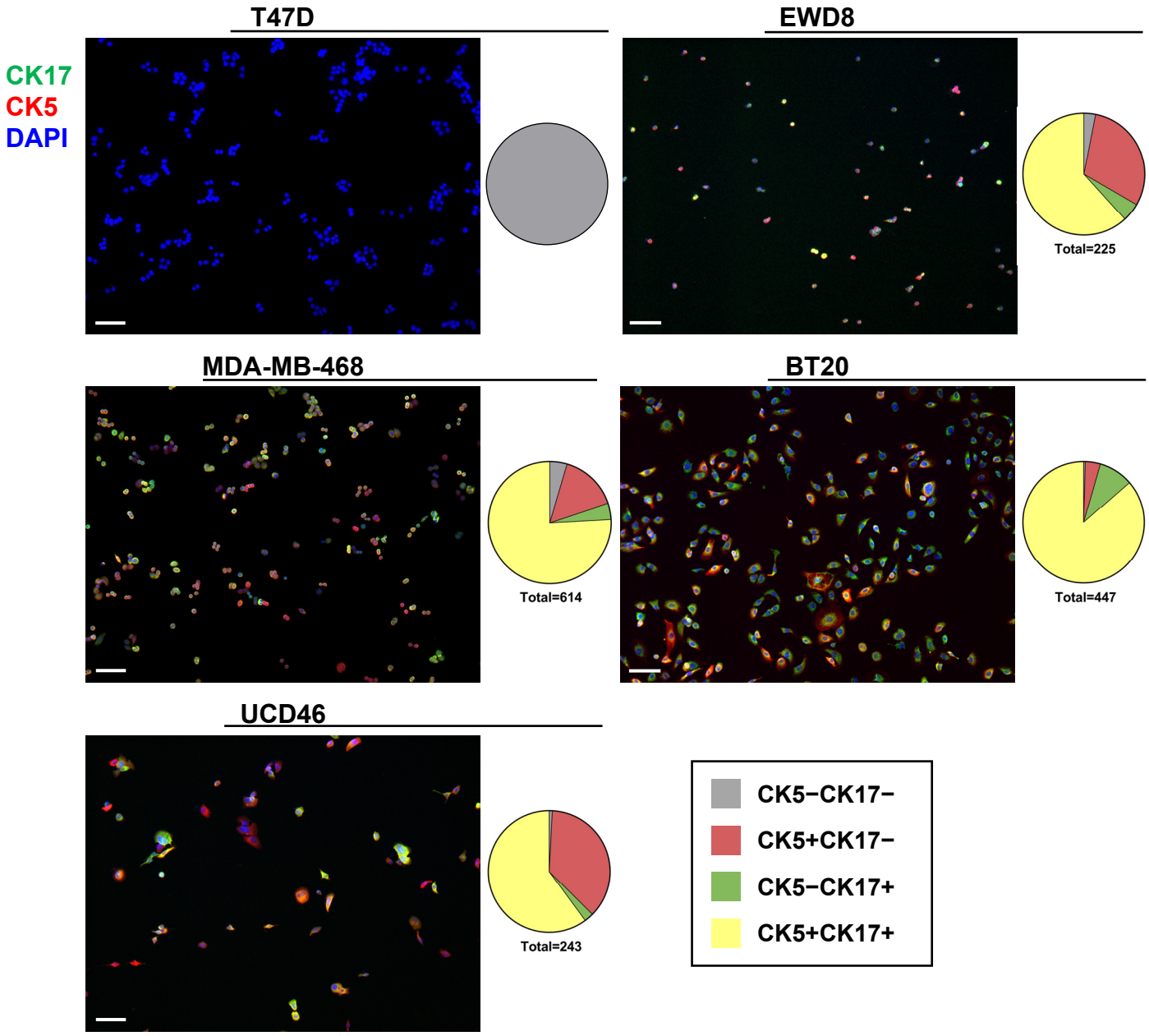


Figure S1. Immunocytochemistry was performed for CK5 (red), CK17 (green), and DAPI (blue) in the indicated cell lines and visualized by fluorescent microscopy. Pie charts indicate the proportion of cells that are CK17+ (green), CK5/17+ (yellow), CK5+ (red), or CK5/17− (grey). No CK5 or CK17 was detected in T47D cells. Scale bars, 100 μm.

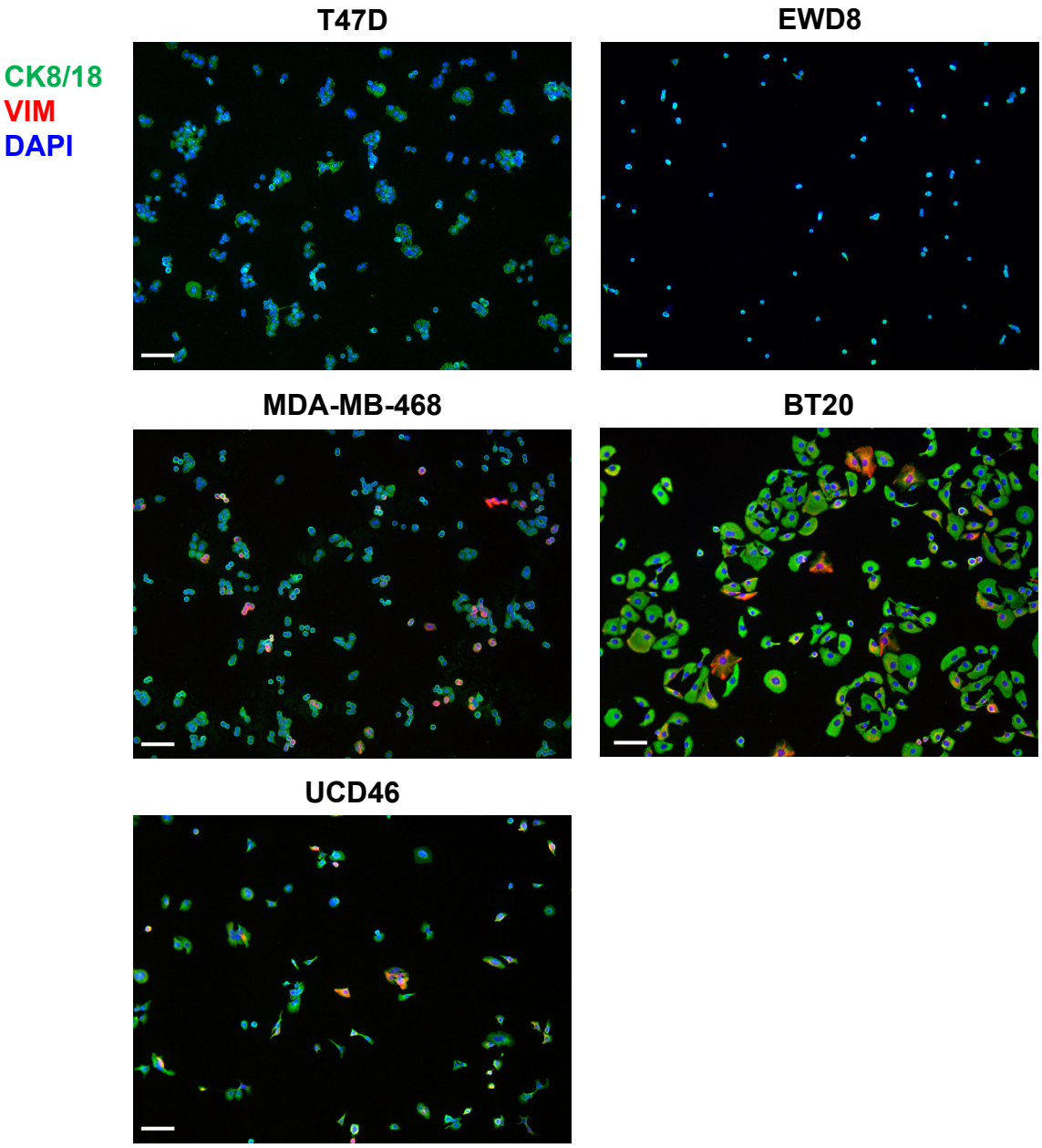
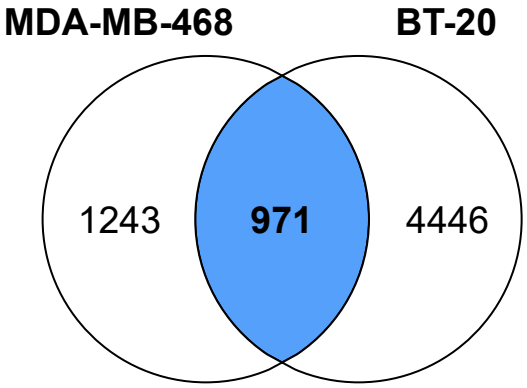


Figure S2. Immunocytochemistry was performed for CK8/18 (green), Vimentin (red), and DAPI (blue) in the indicated cell lines and visualized by fluorescent microscopy. Scale bars, 100 μm.

A



B

Hallmark pathway	MDA-MB-468 *P* value	BT-20 *P* value
ESTROGEN_RESPONSE_LATE	0.001527	0.000677
KRAS_SIGNALING_DN	0.00157	0.000147
INTERFERON_ALPHA_RESPONSE	0.002463	0.000149
IL2_STAT5_SIGNALING	0.002725	0.003883
EPITHELIAL_MESENCHYMAL_TRANSITION	0.00277	0.000342
HYPOXIA	0.002793	0.00037
UV_RESPONSE_DN	0.005362	0.002051
INTERFERON_GAMMA_RESPONSE	0.00551	0.000139

Figure S3. Common differentially expressed genes and pathways in MDA-MB-468 and BT20 cells with CK5 knockdown. A. Venn diagram shows the number of common genes altered in MDA-MB-468 and BT20 shCK5-22 vs shCont cells by RNA-seq analysis (q<0.05). **B.** GSEA analysis identified significantly regulated pathways in in MDA-MB-468 and BT20 shCK5-22 vs shCont. Common altered pathways are indicated with *P* values for each cell line. **C.** MDA-MB-468 shCK5-22 cells were transfected with siCont or siZEB1 then assayed after 48 h by immunoblot for ZEB1, CK5, Ecad, VIM, and β-actin. Fold change to siCont is indicated below the blots.

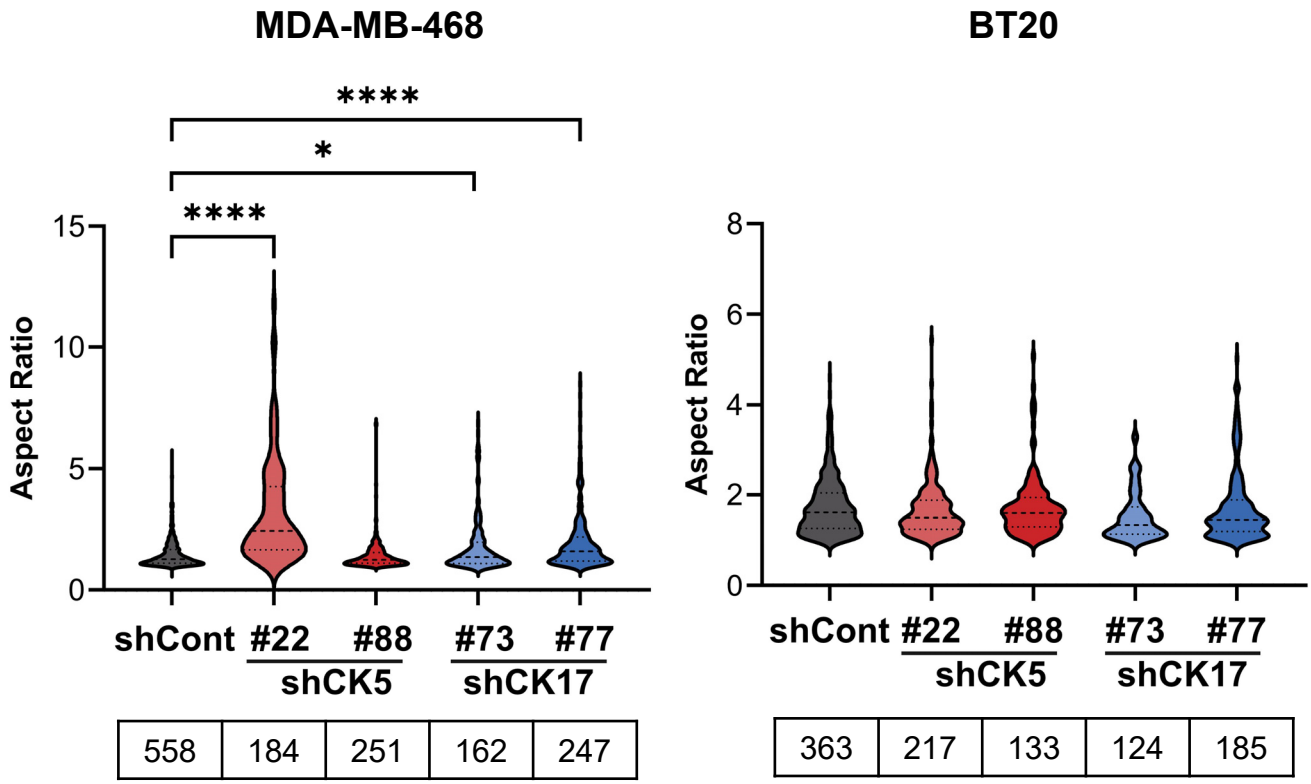


Figure S4. Aspect ratios of BLBC cells with knockdown of CK5 or CK17. Brightfield images were used to measure the length and width of each cell in five separate fields. Aspect ratios were calculated by dividing the longer length by the shorter length and displayed as violin plots. Number of cells measured in each group is indicated below the graph. ANOVA/Kruskal-Wallis tests were used to determine statistical significance. *P<0.05, ****P<0.0001.

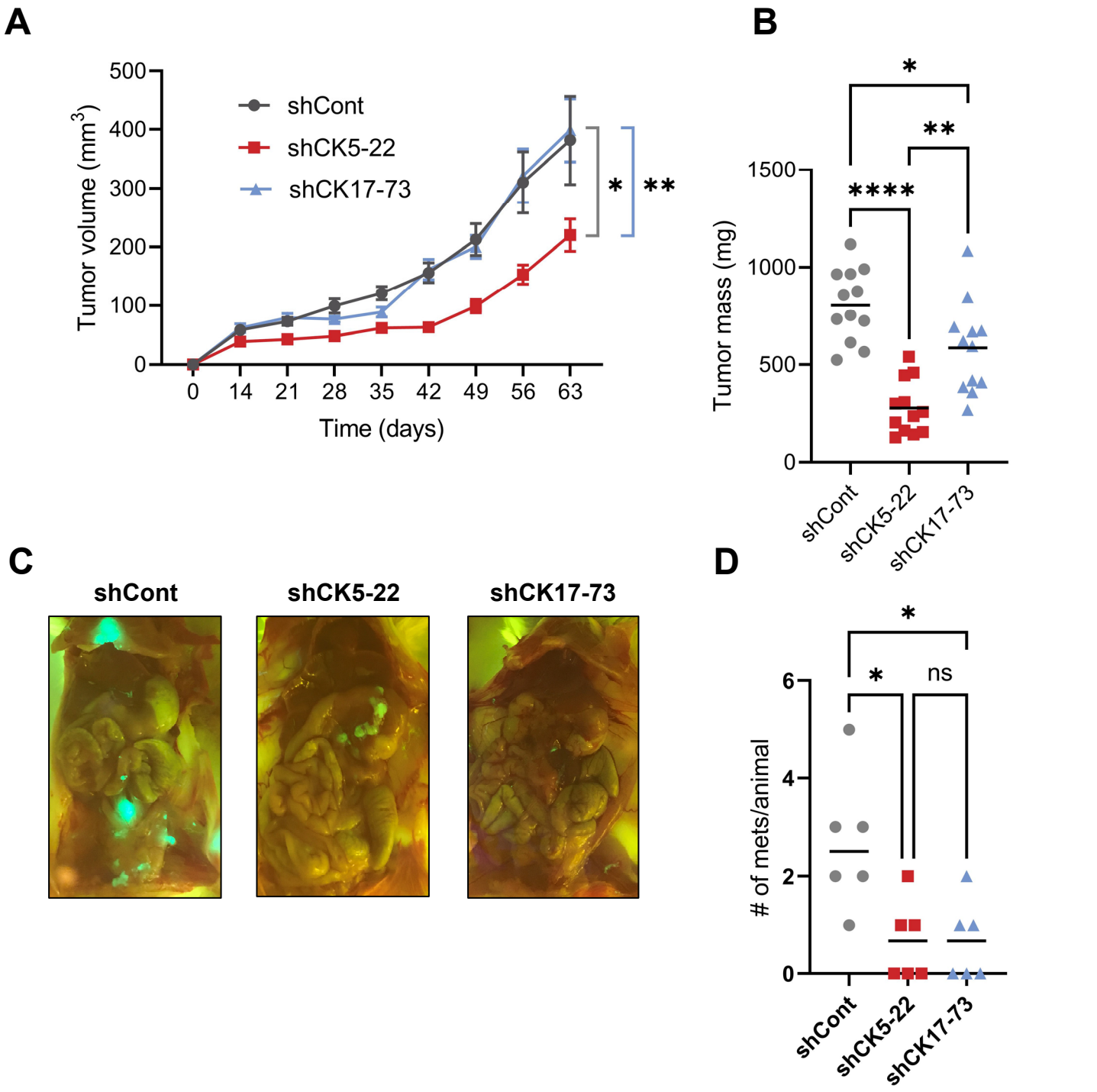


Figure S5. Knockdown of CK5 or CK17 in MDA-MB-468 cells decreases gross metastases. A. 1 x 10^6 GFP+ shCont, shCK5-22, or shCK17-73 cells were injected bilaterally into opposing fourth mammary fat pads of 8-week-old female NSG mice. Six mice were used per group. Tumors were measured weekly and volumes estimated by the formula $l(w^2)/2$, plotted as mean ± s.e.m. Two-way ANOVA with multiple comparisons was used to determine statistical significance, indicated for the last timepoint. **P*<0.05, ***P*<0.01. **B.** Final tumor mass (mg) is plotted with mean ± s.e.m. One-way ANOVA/Tukey tests are indicated. **P*<0.05, ***P*<0.01, *****P*<0.0001. **C.** Representative images of animals from each group at necropsy under an Illumatool. **D**. The number of GFP+ metastases visible at necropsy is plotted per group. ANOVA/Tukey significance is indicated, **P*<0.05, ns=not significant.

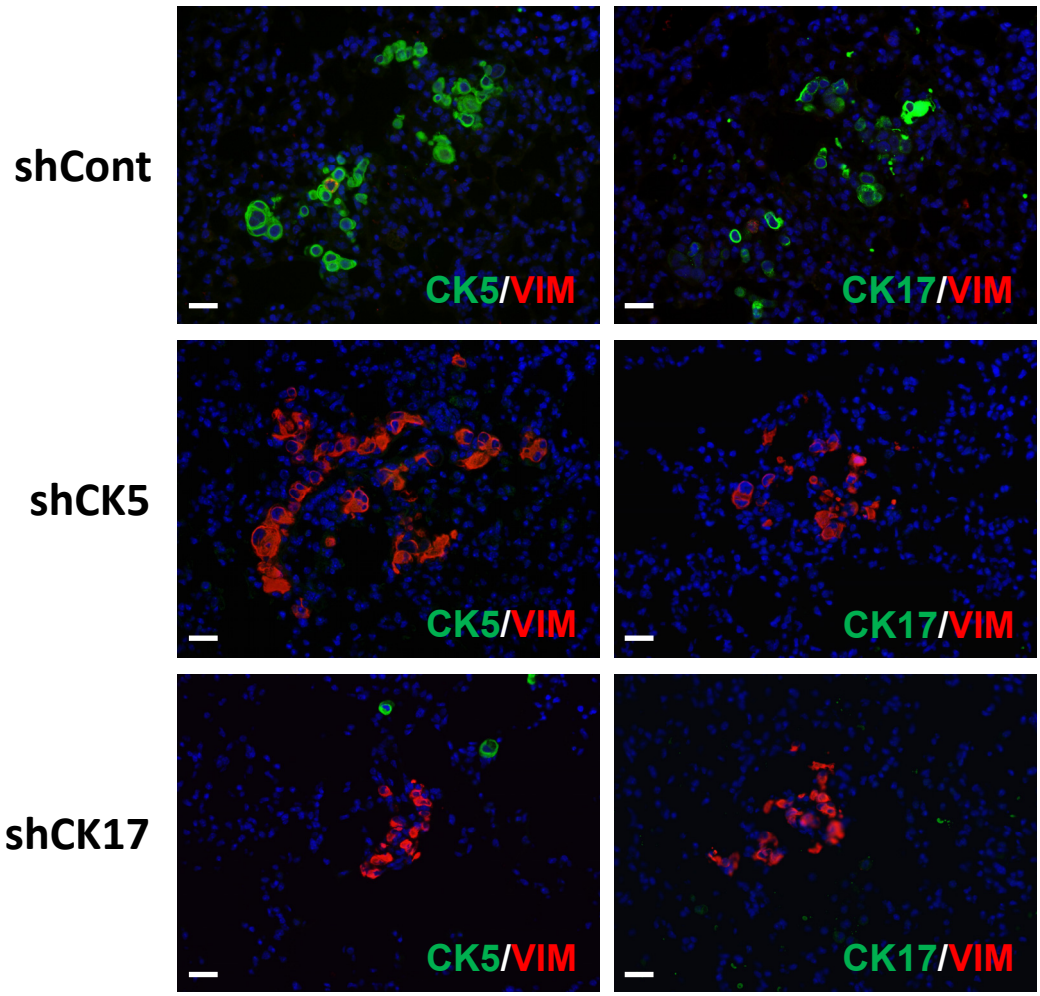
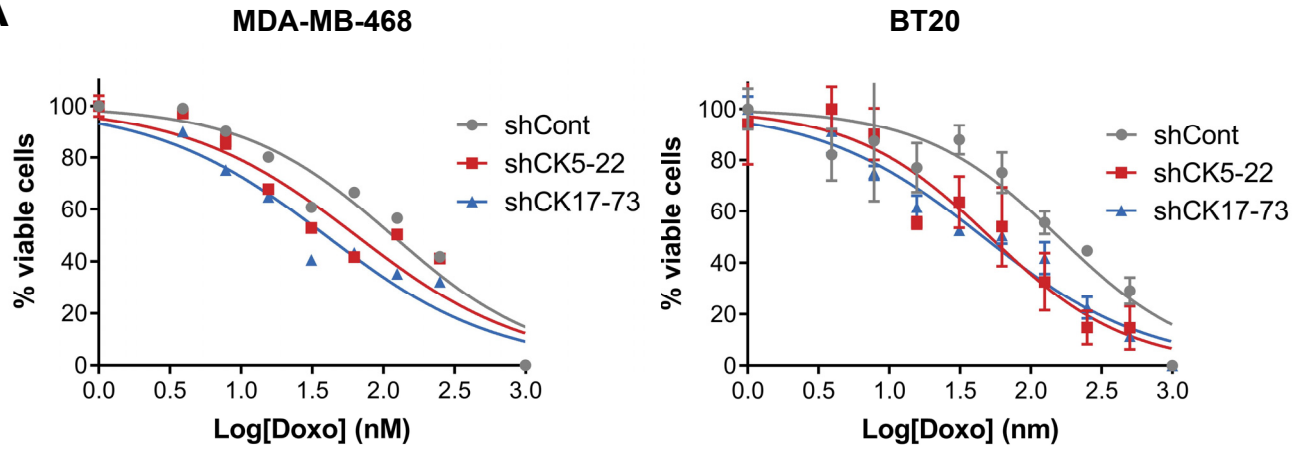
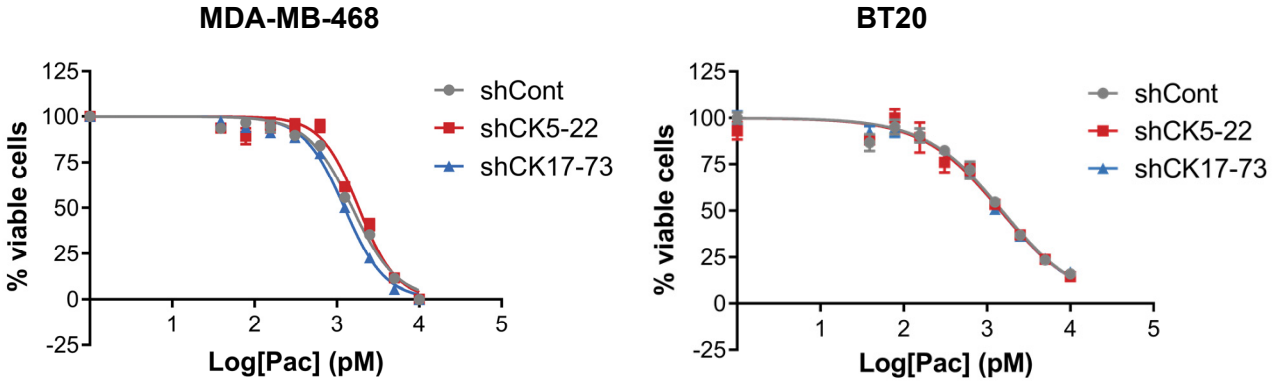


Figure S6. Expression of CK5/CK17/vimentin in MDA-MB-468 spontaneous lung metastases. Lungs were collected from mice bearing mammary fat pad tumors derived from MDA-MB-468 shCont, shCK5-22, shCK17-73 cells from the experiment shown in Figure 4 (equal tumor size harvest). Paraffin section of lungs were stained by IHC for CK5 or CK17 (green) and vimentin (VIM, red) and counterstained with DAPI. Scale bars, 20 μm.

A



B



C

	MDA-MB-468		
	shCont	shCK5-22	shCK17-73
Doxorubicin (nM)	**115.70** (97.0-138.5)	**64.02** (50.58-81.72)	**39.17** (33.11-46.46)
Paclitaxel (nM)	**1.54** (1.44-1.65)	**1.24** (1.17-1.31)	**1.85** (1.67-2.04)

	BT20		
	shCont	shCK5-22	shCK17-73
Doxorubicin (nM)	**157.2** (100.1-244.1)	**51.46** (33.08-79.74)	**46.19** (35.48-60.01)
Paclitaxel (nM)	**1.52** (1.33-1.74)	**1.46** (1.22-1.75)	**1.45** (1.28-1.64)

Figure S7. IC50 of doxorubicin (replicates) and paclitaxel in shCont, shCK5-22, and shCK17-73 BLBC cells. The indicated cell lines were treated with varying concentrations of doxorubicin (**A**) or paclitaxel (**B**) in sextuplicate and growth monitored using the IncuCyte. Normalized transformed graphs are plotted for percent cell viability vs Log(conc) drug at 72 h. Error bars represent mean ± SEM. **C**. Table shows IC50 values for doxorubicin and paclitaxel in the indicated cell lines. 95% confidence interval is shown in parentheses.

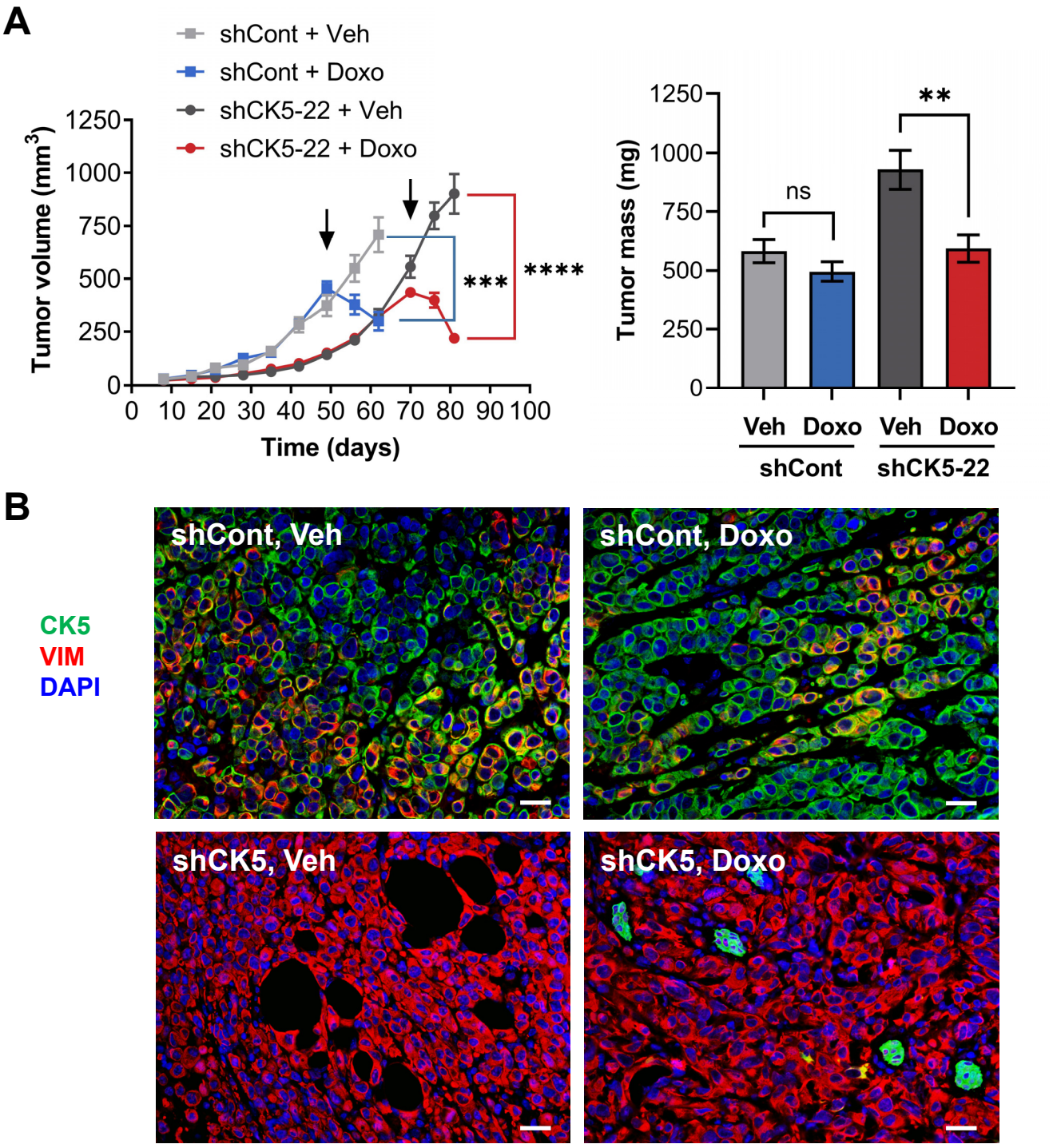


Figure S8. Expression of CK5/vimentin in shCont and shCK5 MDA-MB-468 tumors treated with vehicle or doxorubicin. **A**. MDA-MB-468 shCont and shCK5 tumors were grown to an average of ~450 mm^3 then treated once week with vehicle (Veh) or doxorubicin (Doxo) for two weeks. T-tests at final time point indicated. Mean final tumor mass is down on the right. T-tests are indicated. **B**. Tumors were collected from mice bearing mammary fat pad tumors derived from MDA-MB-468 shCont and shCK5-22 cells following two weeks of Veh or Doxo (5 mg/kg) treatment. Paraffin sections of tumors were stained by IHC for CK5 (green) and vimentin (VIM, red) and counterstained with DAPI. Scale bars, 20 μm.

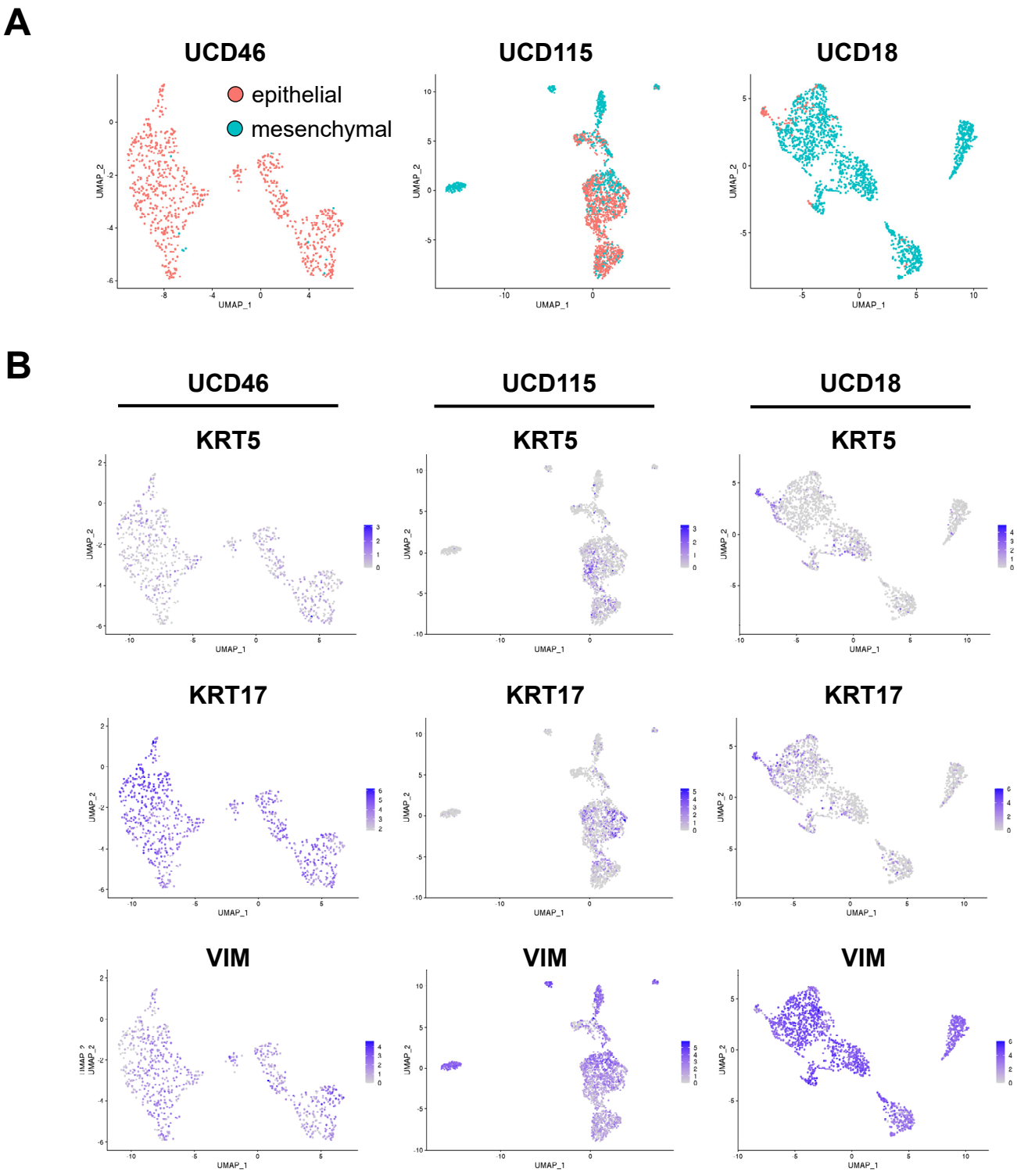


Figure S9. Single cell expression of CK5, CK17, and vimentin in BLBC PDX. A. A published gene signature for generating epithelial-mesenchymal scores was used {Taube, 2010 #2644}. This signature contains 246 genes that are down- or upregulated during EMT, which are designated as “epithelial” and “mesenchymal” respectively. The average expression for epithelial and mesenchymal genes was calculated for each cell and the log2 of the epithelial/mesenchymal ratio calcutated. Cells with positive scores were designated as epithelial (blue) and negative scores as mesenchymal (red). These are indicated for three PDX on the UMAPs. **B**. UMAP plots for CK5 (KRT5), CK17 (KRT17), and vimentin (VIM) in each of the three BLBC PDX.