Figure S1. A549 cells from the same batch were pretreated with AMD3100 (10 μM), CCX771 (100 nM) or none for 1 h and then added to the upper well of either a 12-well (10,000 cells/well) or a 48-well (5,000 cells/well) Boyden chamber. Migration index for CXCL12 (100 ng/ml) in the presence or absence of receptor antagonists were determined after 4 h. Cell migration in the absence of CXCL12 was set to 1 and the migration index was calculated as the ratio of cells migrating in the presence and absence of CXCL12. Data are presented as the mean ± SD (n=4-12). CXCL12, C-X-C motif chemokine 12.

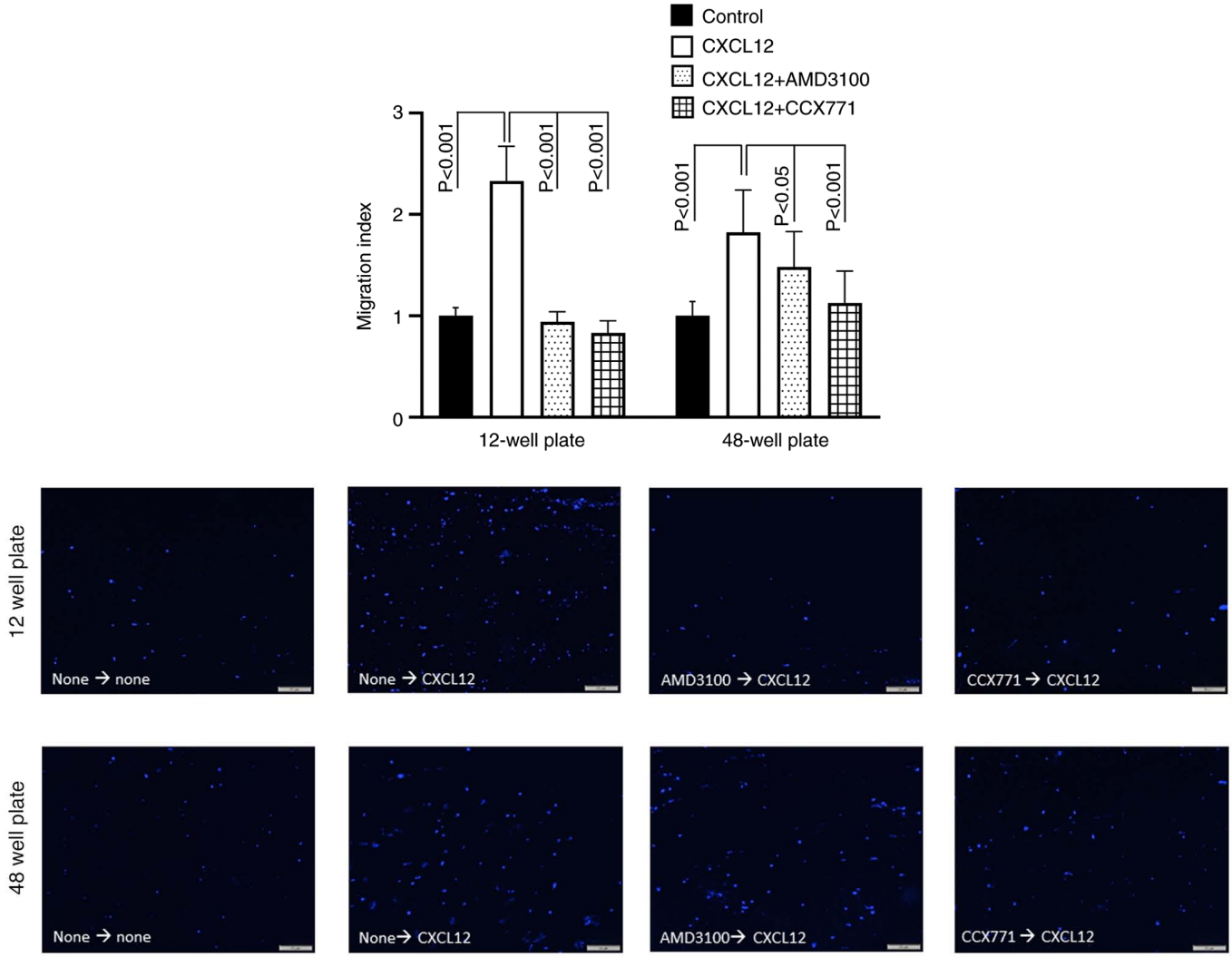

Figure S2. Representative images from chemotactic assay using PTX-treated cells. The various cancer cells (A-E) were treated with the indicated substances or none for 1 h and then tested for chemotactic responses to CXCL12 (100 ng/ml) in a modified Boyden chamber. After 4 h, migrating cells attached to the lower surface of the membrane were stained with DAPI, photographed and counted. Scale bar, 50 μM. CXCL12, C-X-C motif chemokine 12; PTX, pertussis toxin.

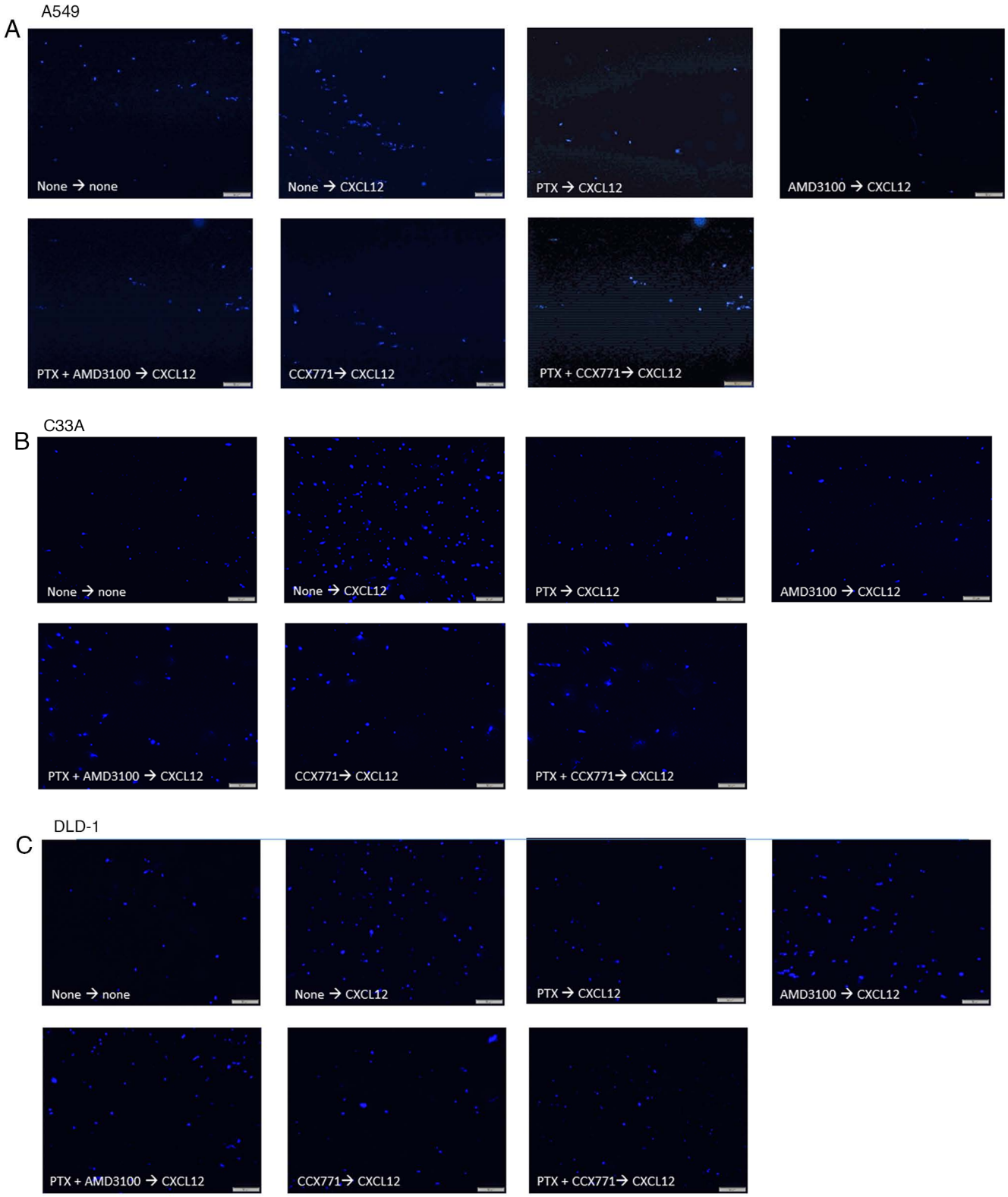

Figure S2. Continued.

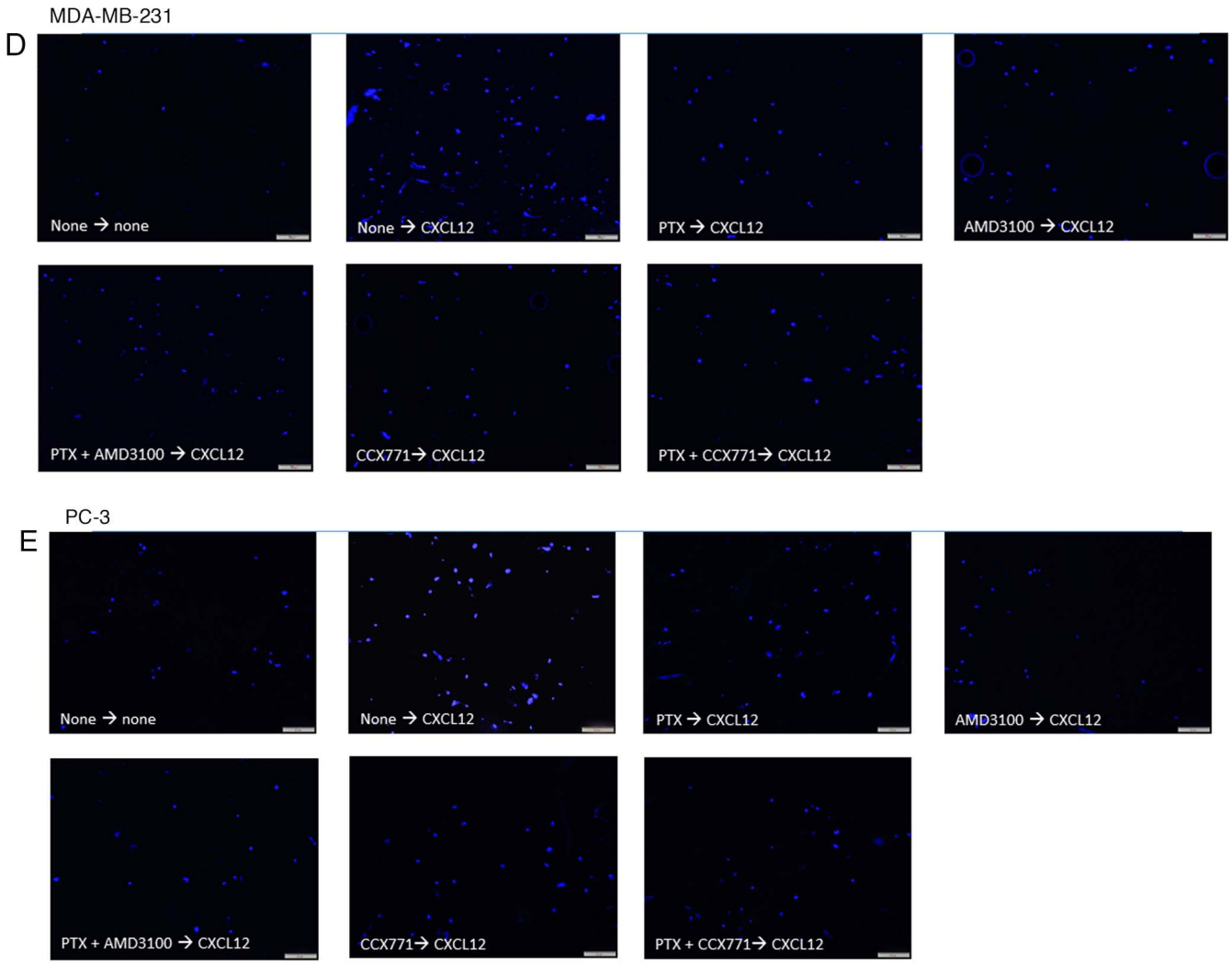

Figure S3. Analysis of the effects of β-arrestin siRNA on β-arrestin protein levels. Cells maintained in serum-free culture medium were transfected with either β-arrestin1 siRNA, β-arrestin2 siRNA or both using siPORT Amine Transfection Agent. After 2 days, the protein expression levels of β-arrestin1 or β-arrestin2 were analyzed by western blotting. To control for protein loading, membranes were re-probed with anti-GAPDH antibodies. Immunoreactive protein bands were normalized to GAPDH levels, and normalized protein levels in untreated controls were set to 1. Data are presented as the mean ± SD (n=1-3). No statistical analysis has been provided for experiments with n<3 (A549 and PC3 cells tested for β-arrestin1 protein levels). Although β-arrestin1 protein levels tended to decrease in C33A cells transfected with β-arrestin1 siRNA, this decrease was not statistically significant due to the small number of replicates. *P<0.05 and ***P<0.001 vs. control. β1/β-arr1, β-arrestin1; β2/β-arr2, β-arrestin2; siRNA, small interfering RNA; ns, not significant.

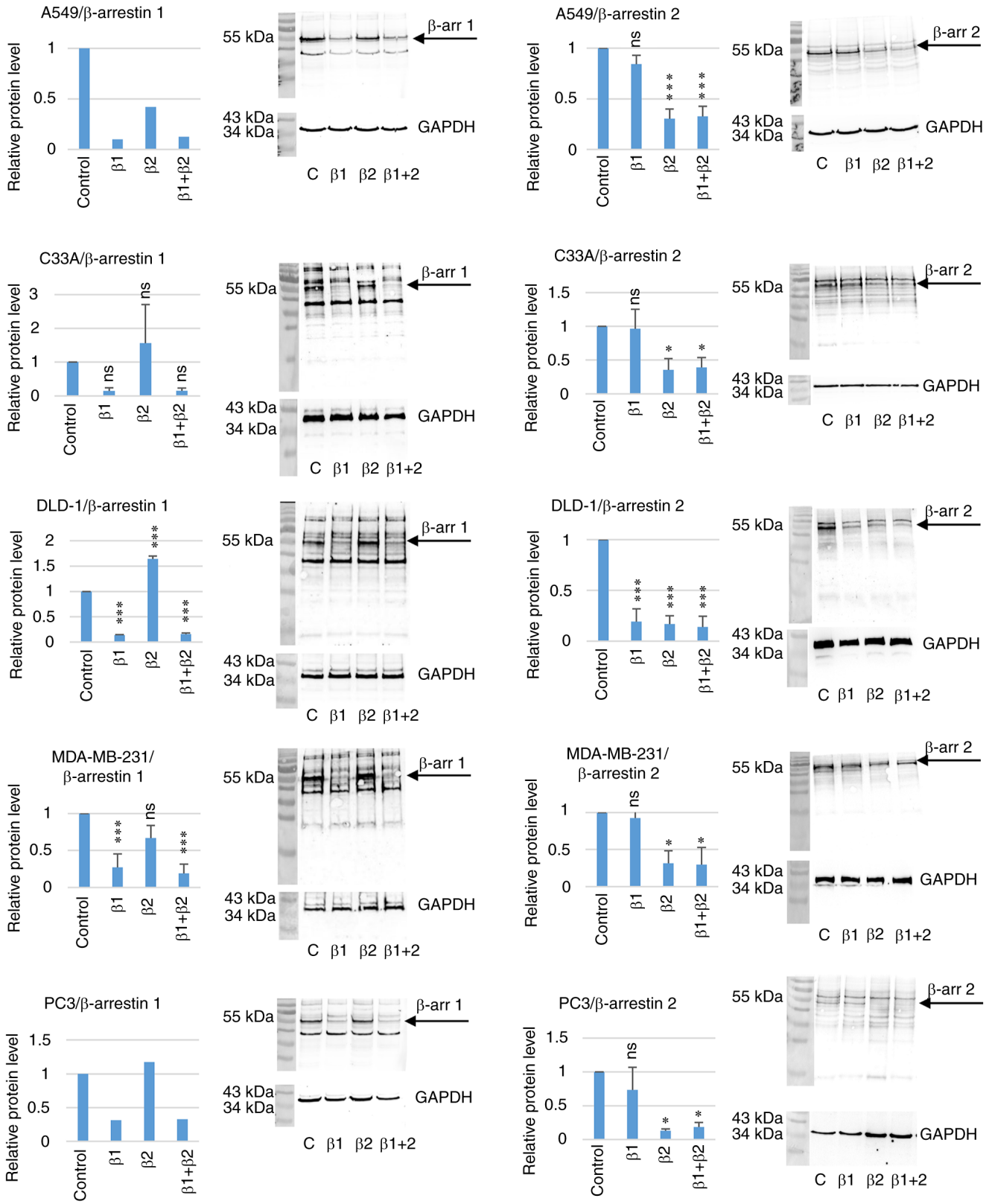

Figure S4. Analysis of the effects of β-arrestin siRNA on β-arrestin mRNA levels. Cells maintained in serum-free culture medium were transfected with either β-arrestin1 siRNA, β-arrestin2 siRNA or both using siPORT Amine Transfection Agent. After 2 days, the mRNA expression levels of β-arrestin1 or β-arrestin2 were analyzed by reverse transcription-quantitative PCR. Gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method and normalized to β-actin. Normalized β-arrestin mRNA levels in untreated controls were set to 1. Data are presented as the mean ± SD (n=4-6). Both A549 cells transfected with β-arrestin 1 siRNA and DLD cells transfected with β-arrestin 2 siRNA showed a notable decrease in their respective mRNA levels. However, this decrease was not statistically significant due to the large variability among groups. *P<0.05, **P<0.01 and ***P<0.001 vs. control. β1, β-arrestin1; β2, β-arrestin2; siRNA, small interfering RNA; ns, not significant.

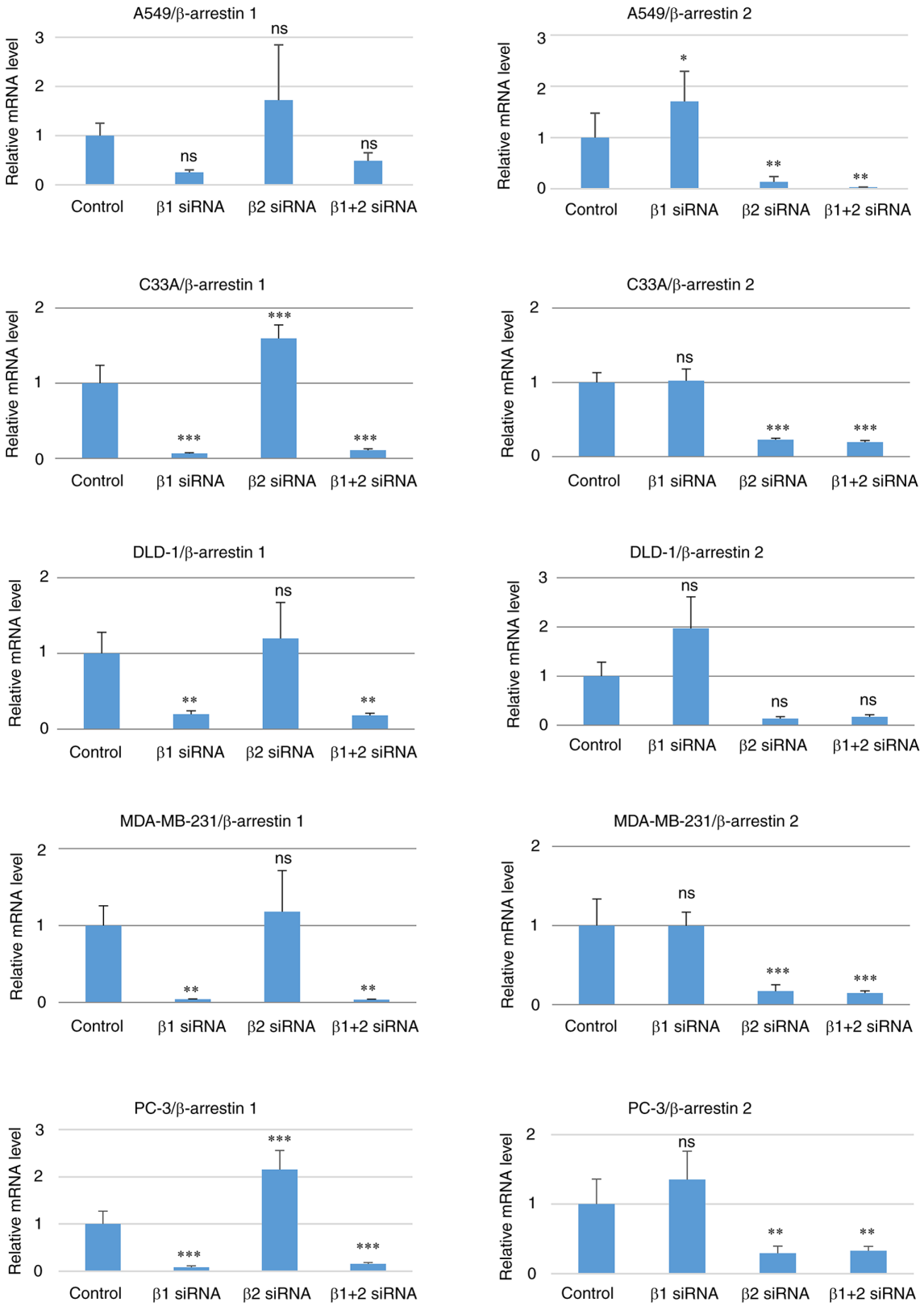

Figure S5. Representative images from chemotactic assay using β-arrestin-depleted cells. The various cancer cells (A-E) were transfected with small interfering RNA against both β-arrestin1 and β-arrestin2 mRNAs. Scale bar, 50 μM. CXCL12, C-X-C motif chemokine 12; PTX, pertussis toxin.

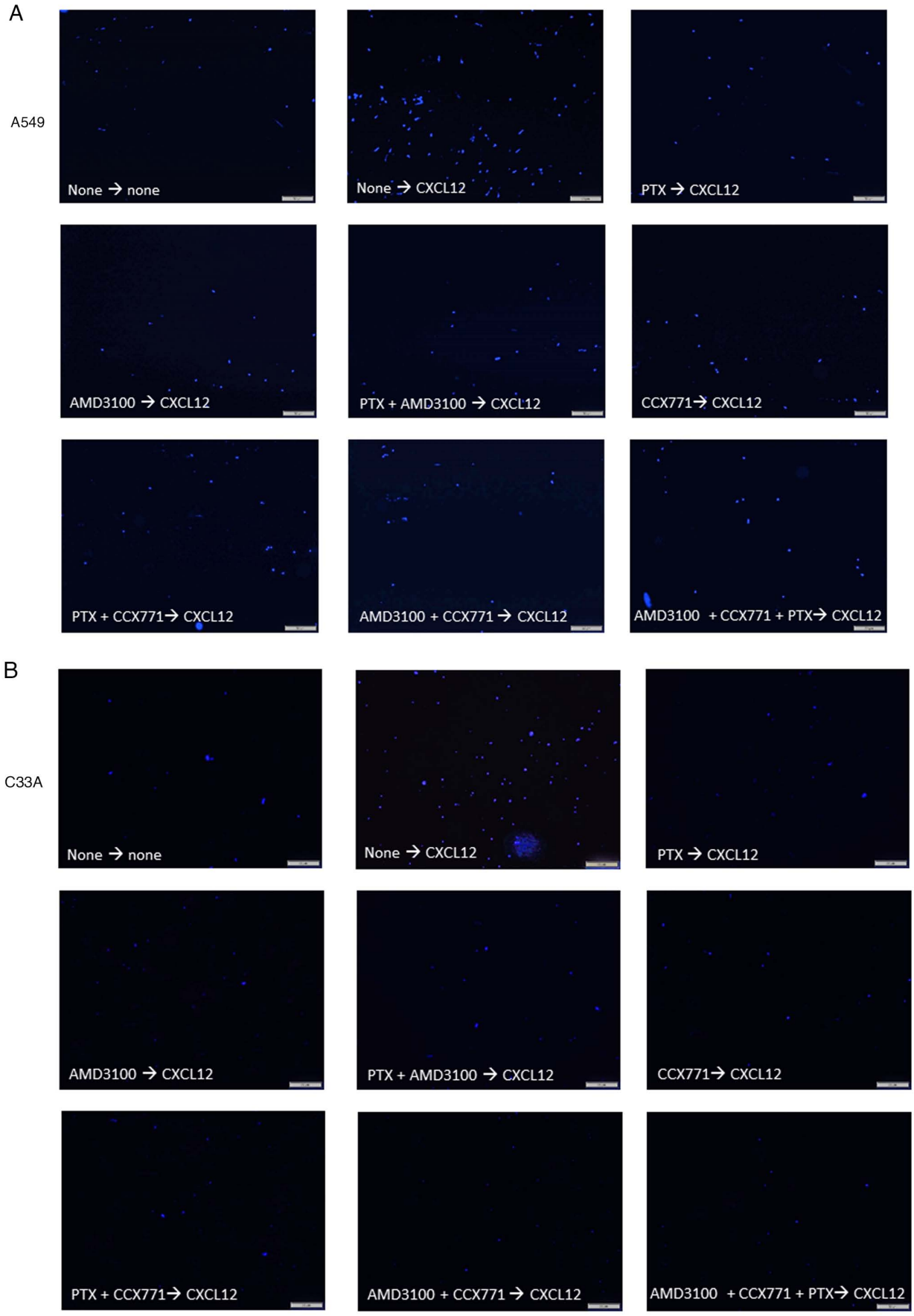

Figure S5. Continued.

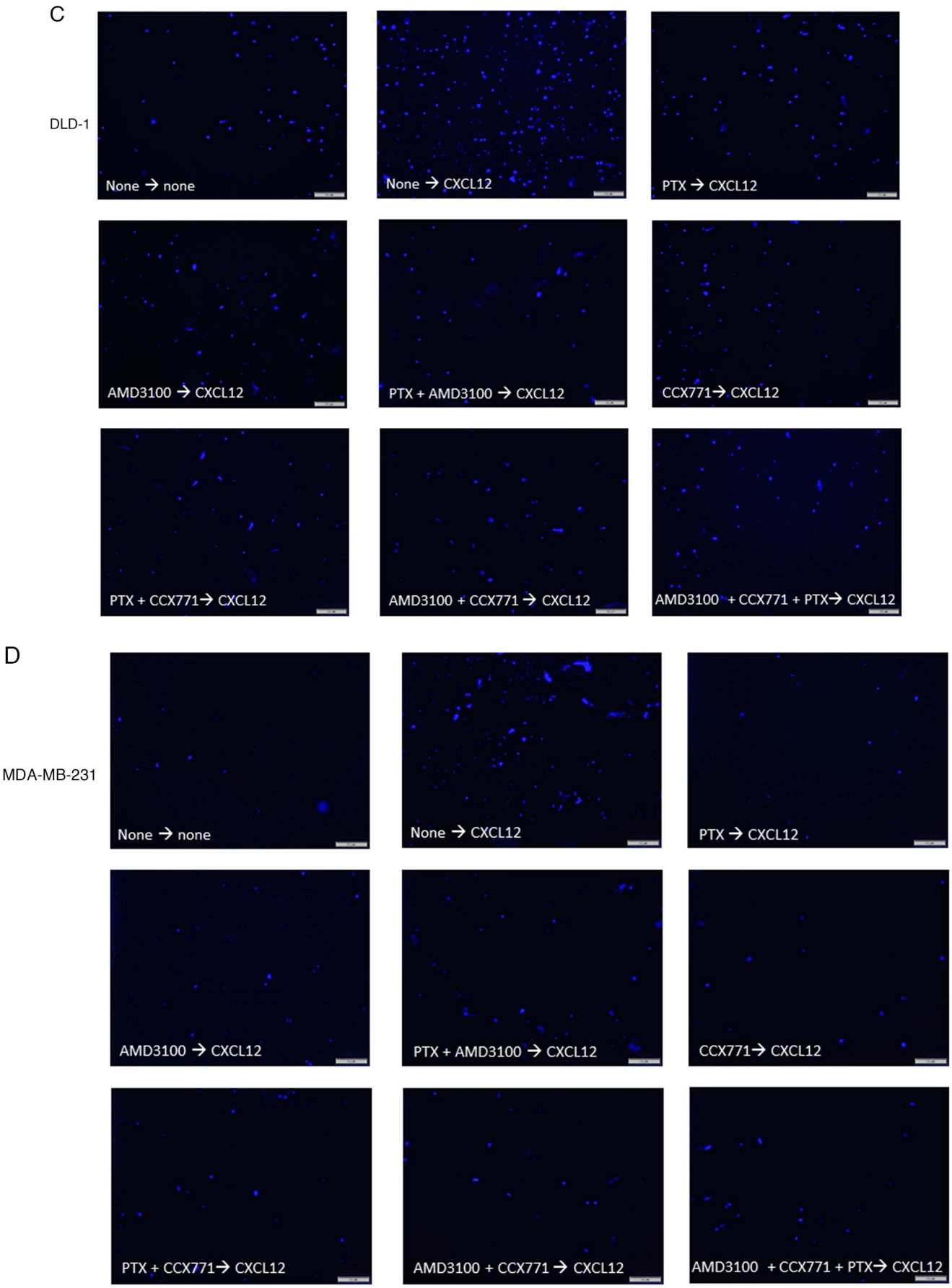

Figure S5. Continued.

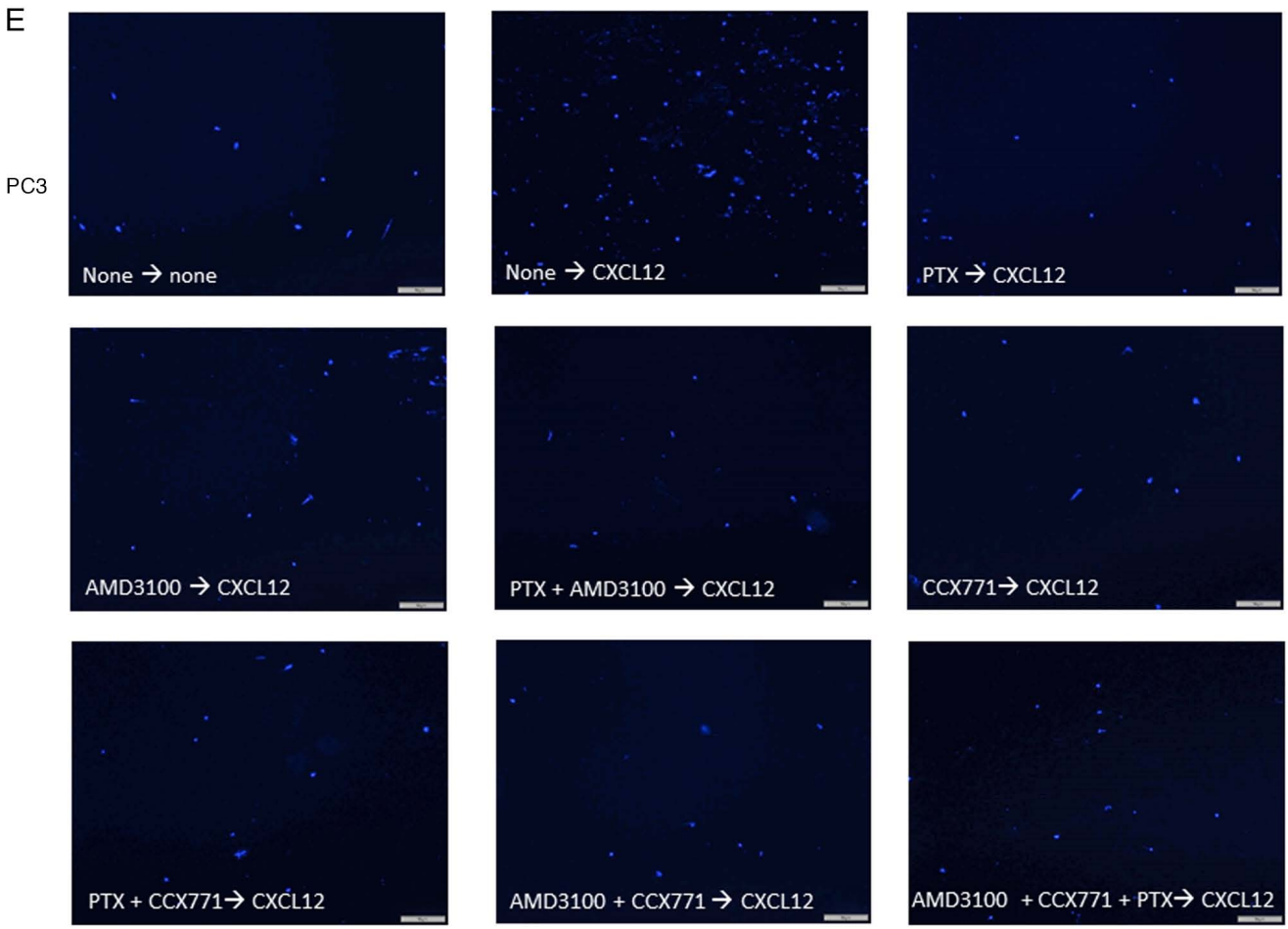

Figure S6. Transfection of cancer cells with control siRNA exhibits no major effect on the molecular mechanisms involved in CXCL12-dependent chemotaxis. Cancer cells were transfected with control siRNA and assayed for CXCL12 (100 ng/ml)-induced chemotaxis after 2 days. In certain experiments, cells were additionally treated with either none, AMD3100 (10 μM, 1 h), CCX771 (100 nM, 1 h), PTX (100 ng/ml, 24 h) or their combinations. Data are presented as the mean migration index ± SD (n=5-12). [a]P<0.001 vs. control; [b]P<0.001 vs. none. CXCL12, C-X-C motif chemokine 12; PTX, pertussis toxin; siRNA, small interfering RNA.

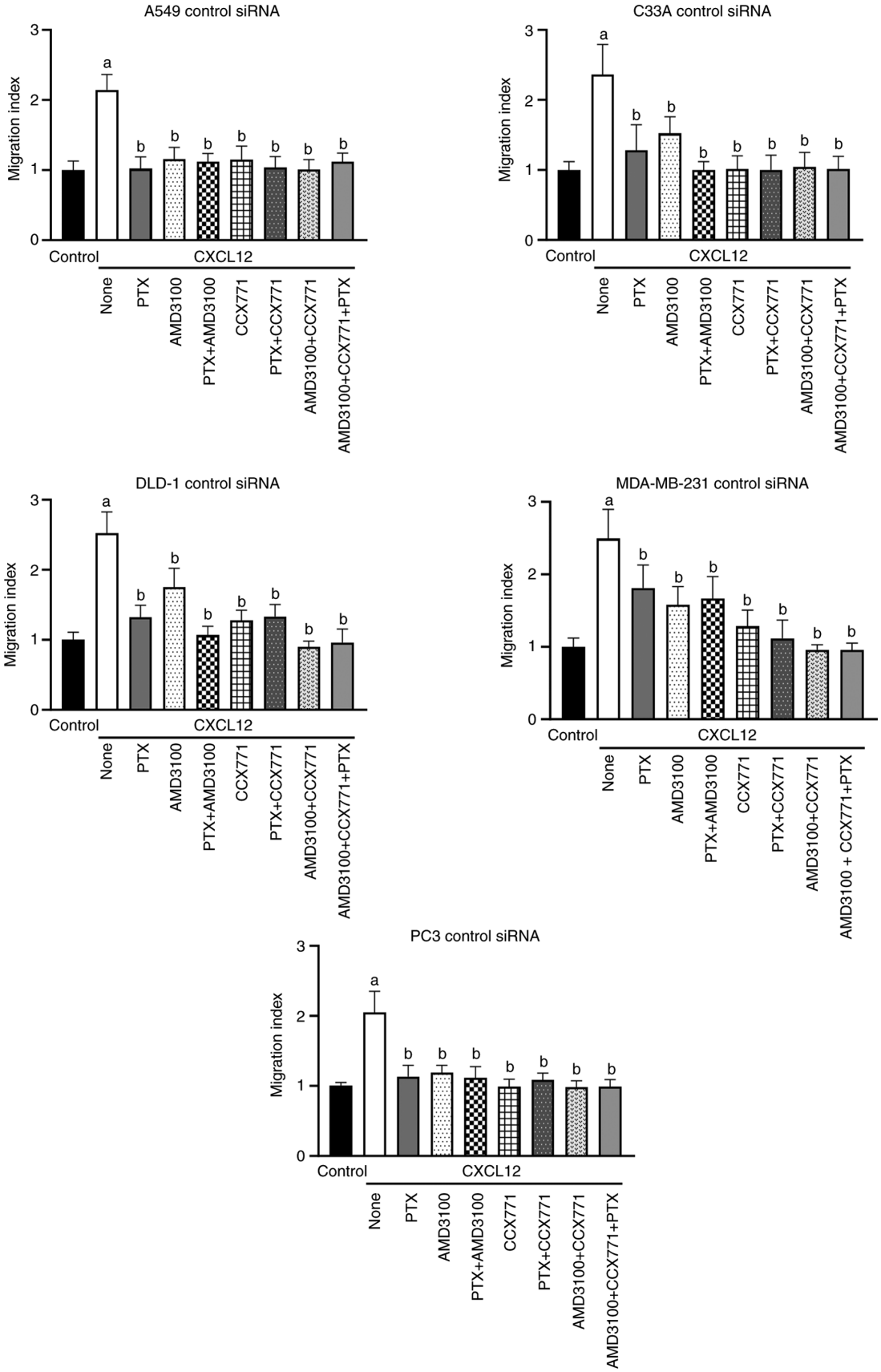

Figure S7. Representative images from chemotactic assay using control small interfering RNA-transfected cells (A-E). Scale bar, 50 μM. CXCL12, C-X-C motif chemokine 12; PTX, pertussis toxin.

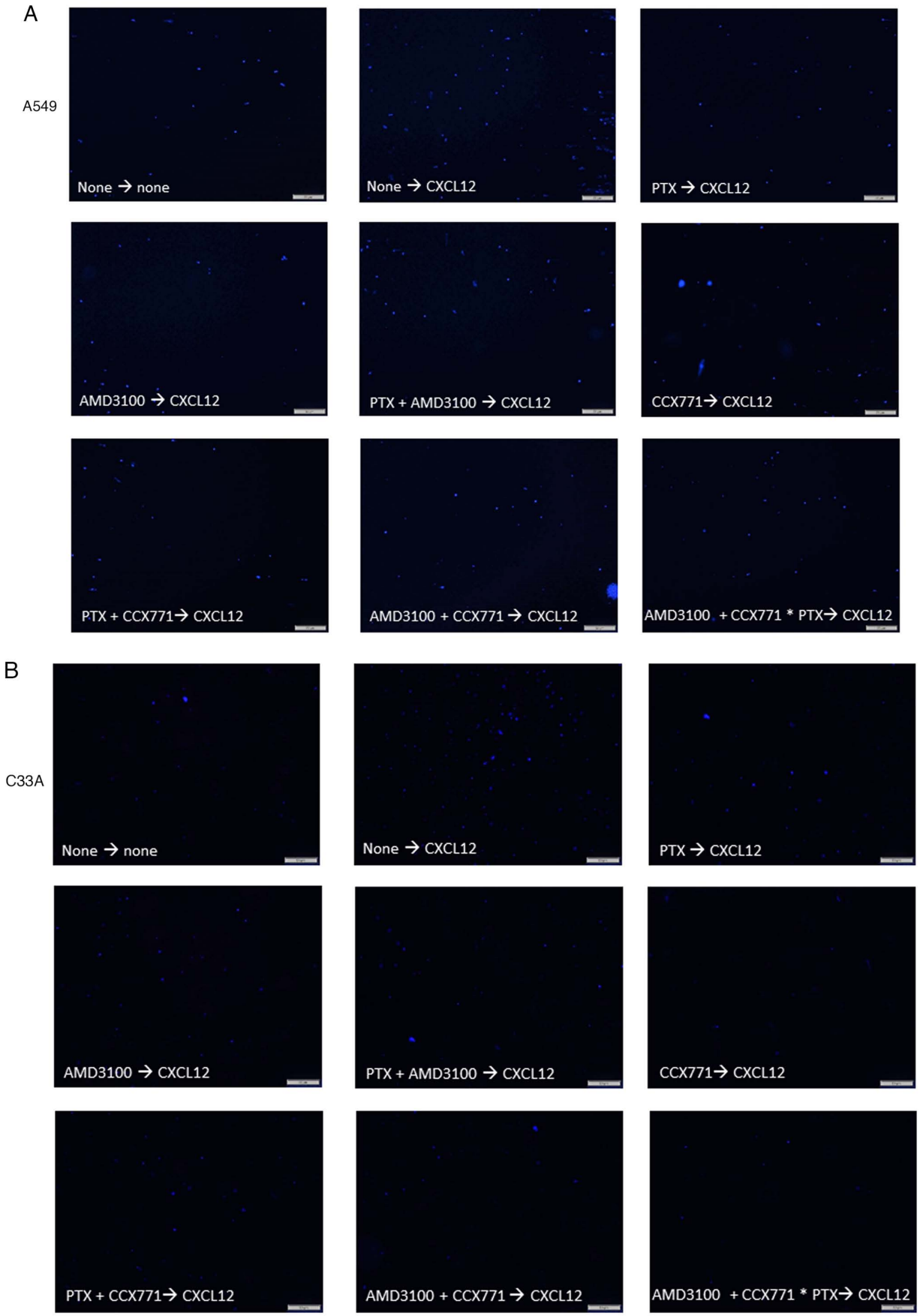

Figure S7. Continued.

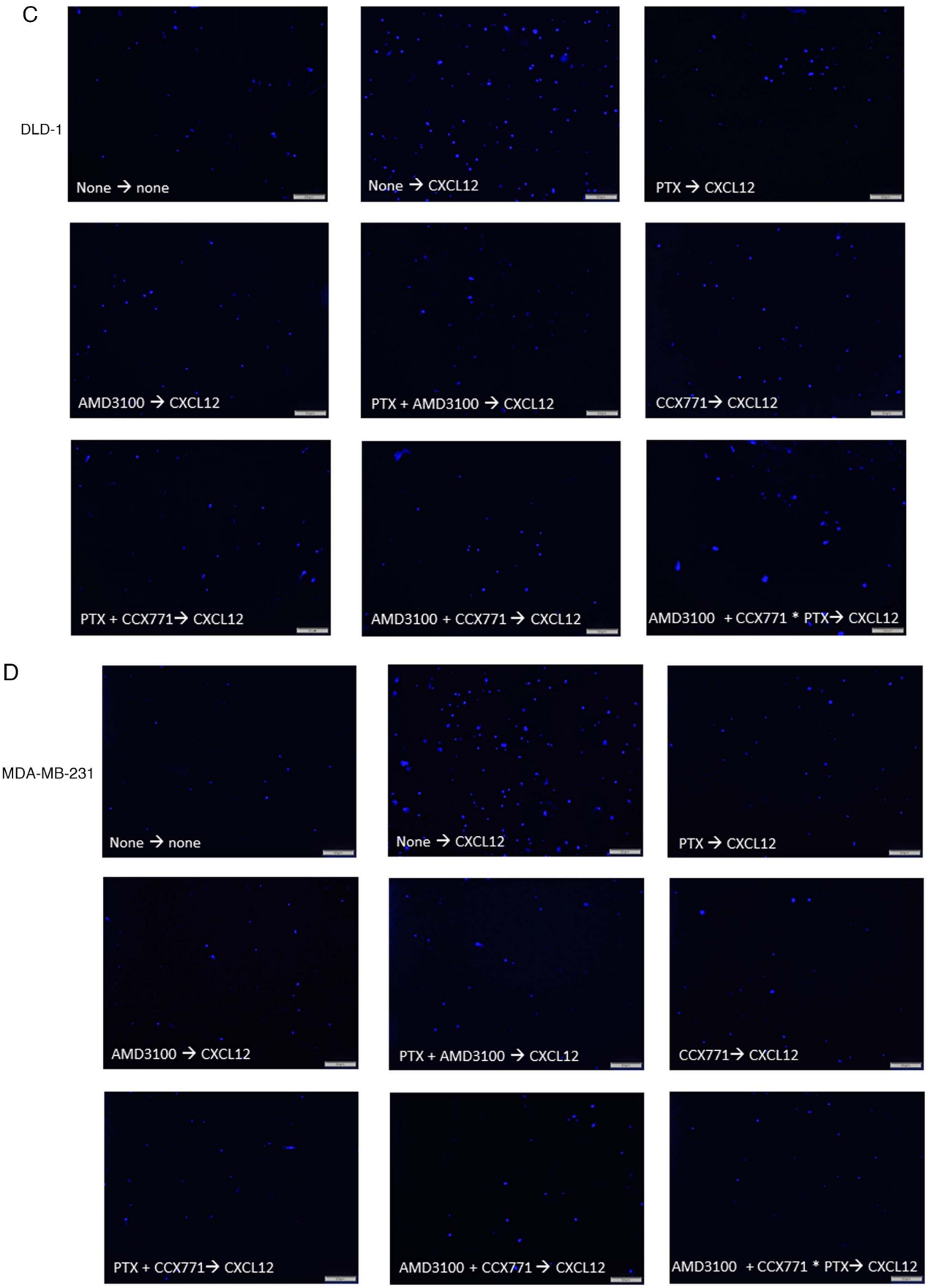

Figure S7. Continued.

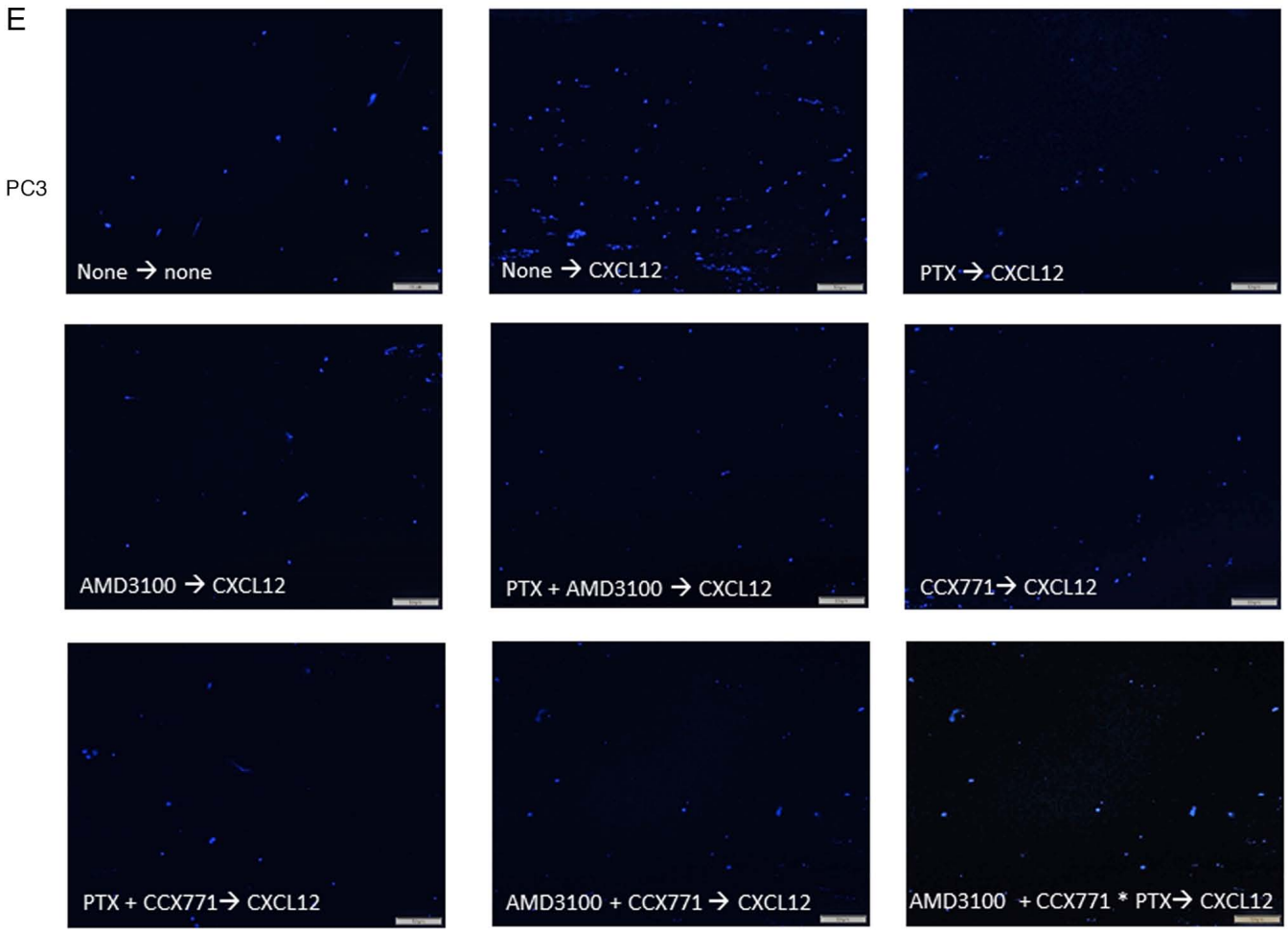

Figure S8. Cancer cell lines express EGFR at different levels that are not affected by inhibitors. Cell lines were treated with PTX (100 ng/ml) for 24 h or AG1478 (2 μM) and Src-I1(1 μM) for 1 h, and subsequently EGFR expression was analyzed by western blotting. To control for protein loading, blots were re-probed with β-actin antibodies. MW, molecular weight; PTX, pertussis toxin; Src-I1, Src inhibitor-1.

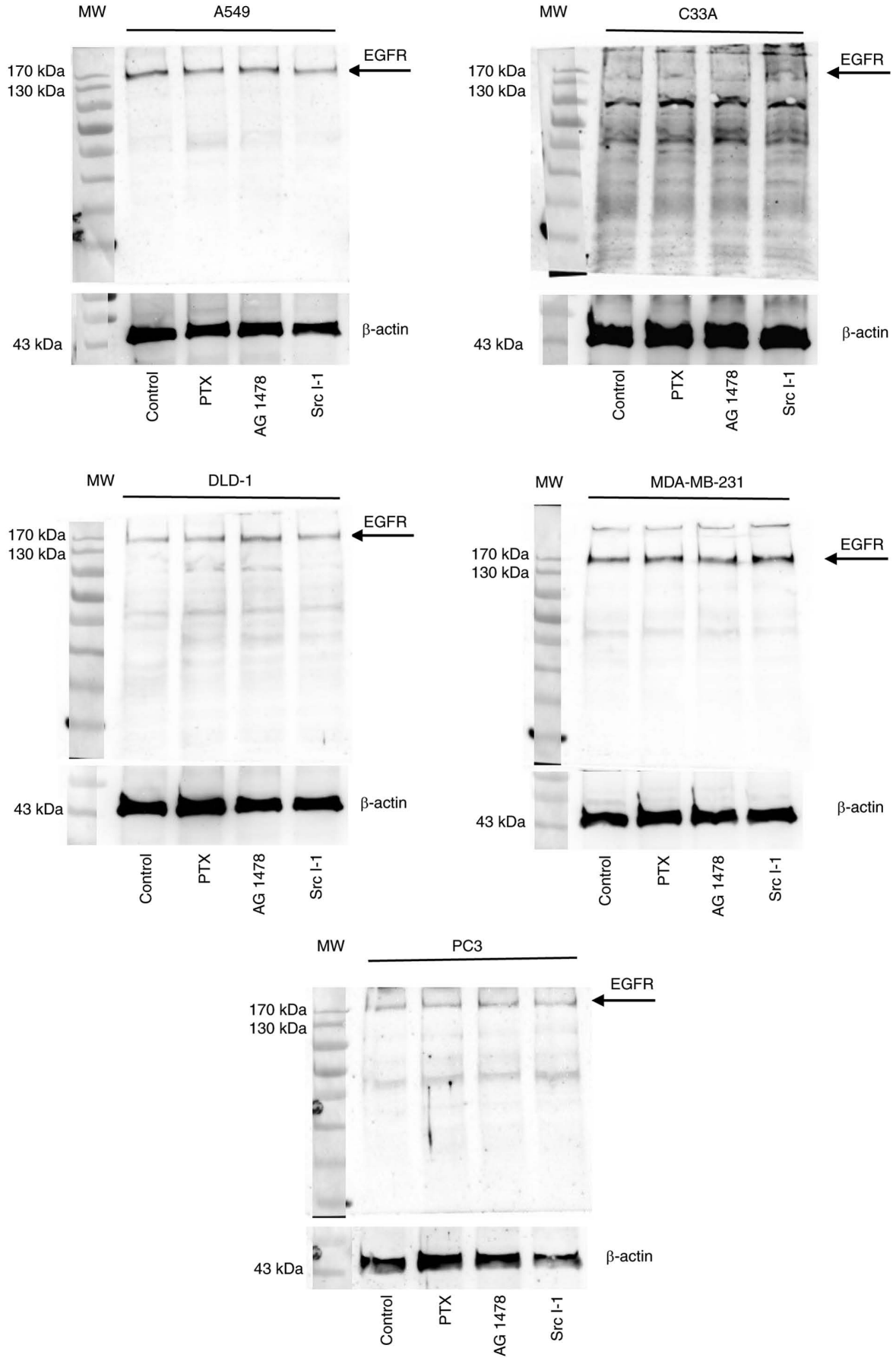

Figure S9. Representative images from chemotactic assay using the various AG1478-treated cells (A-E). Scale bar, 50 μM. CXCL12, C-X-C motif chemokine 12; PTX, pertussis toxin.

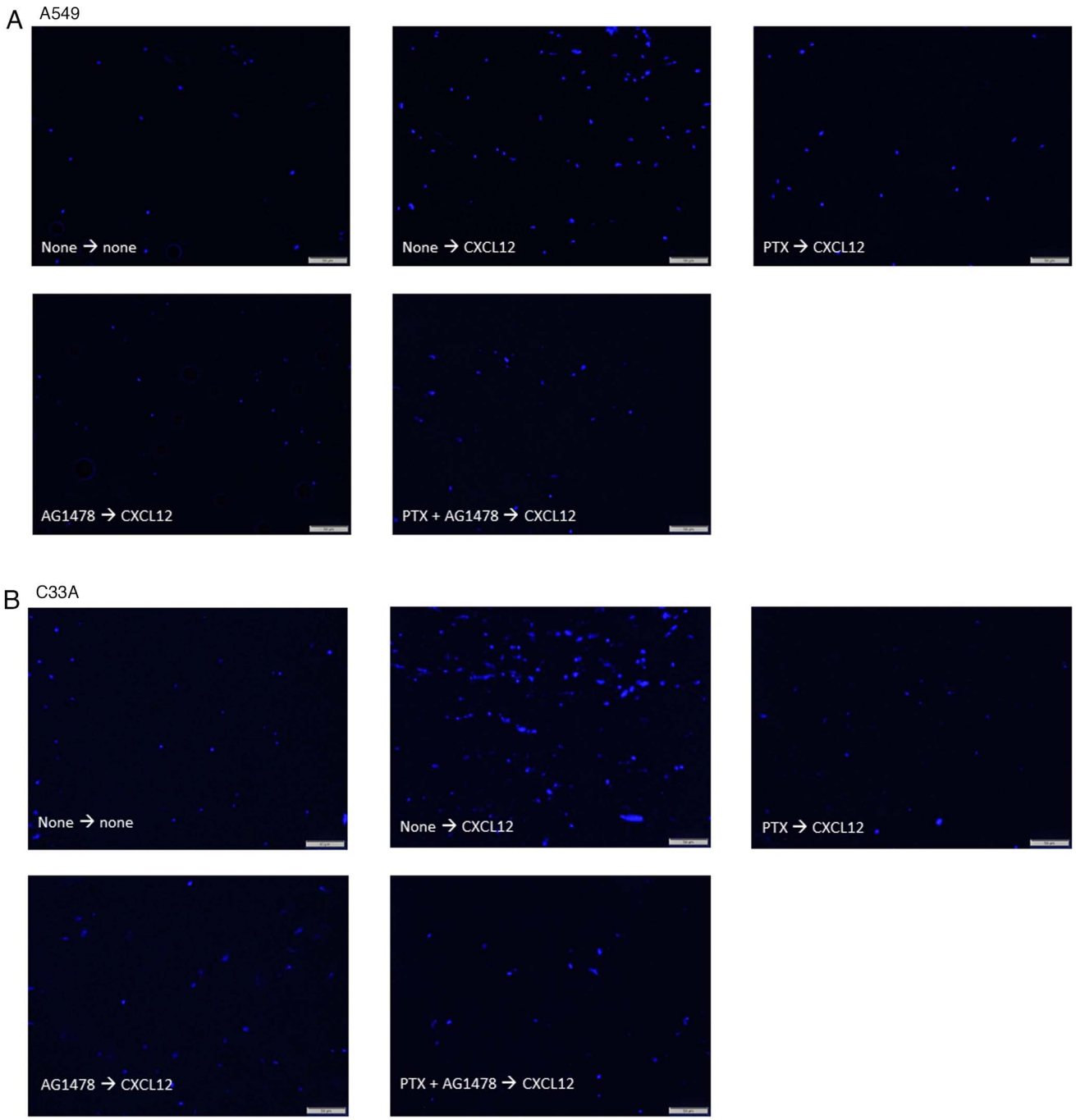

Figure S9. Continued.

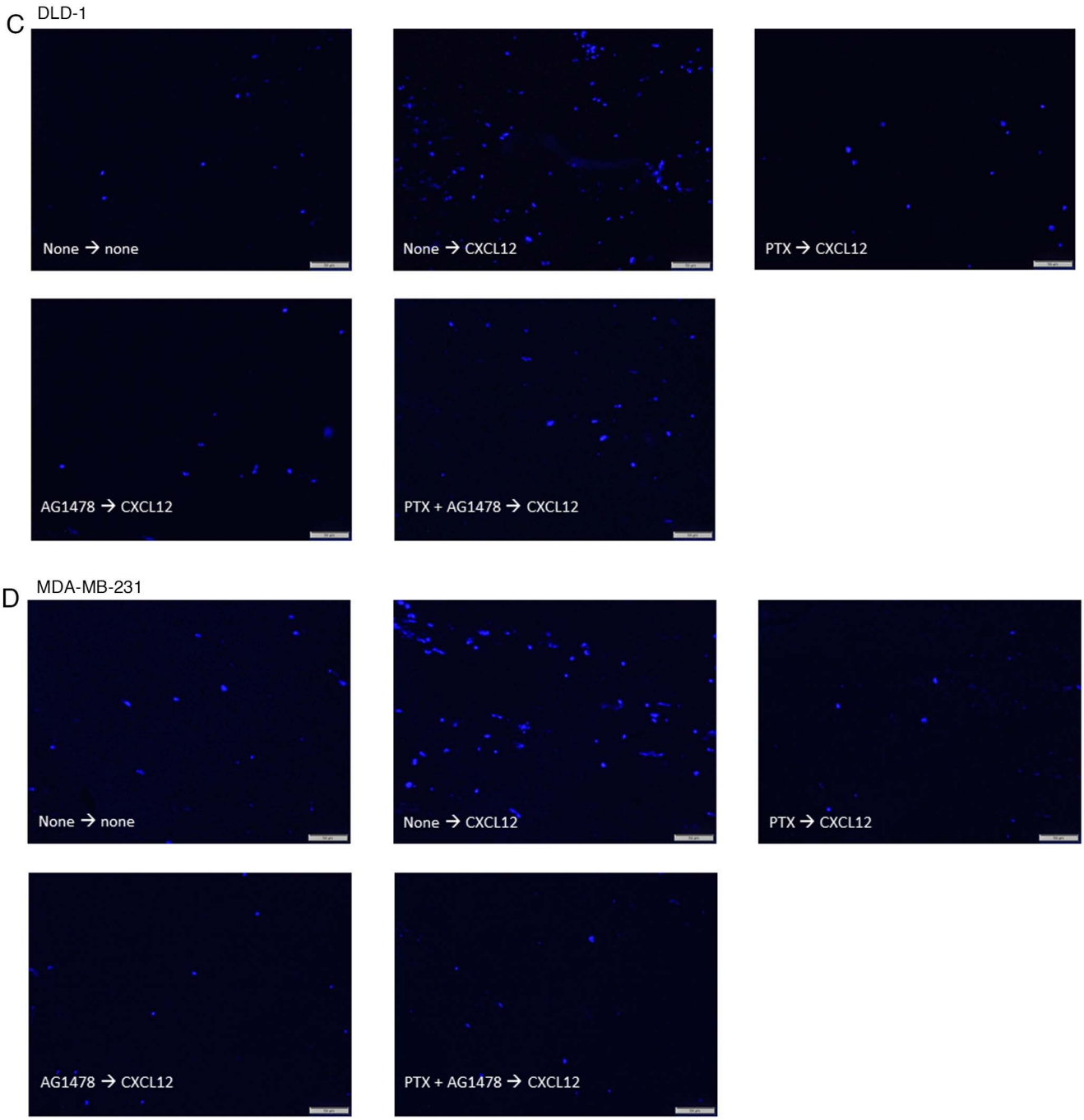

Figure S9. Continued.

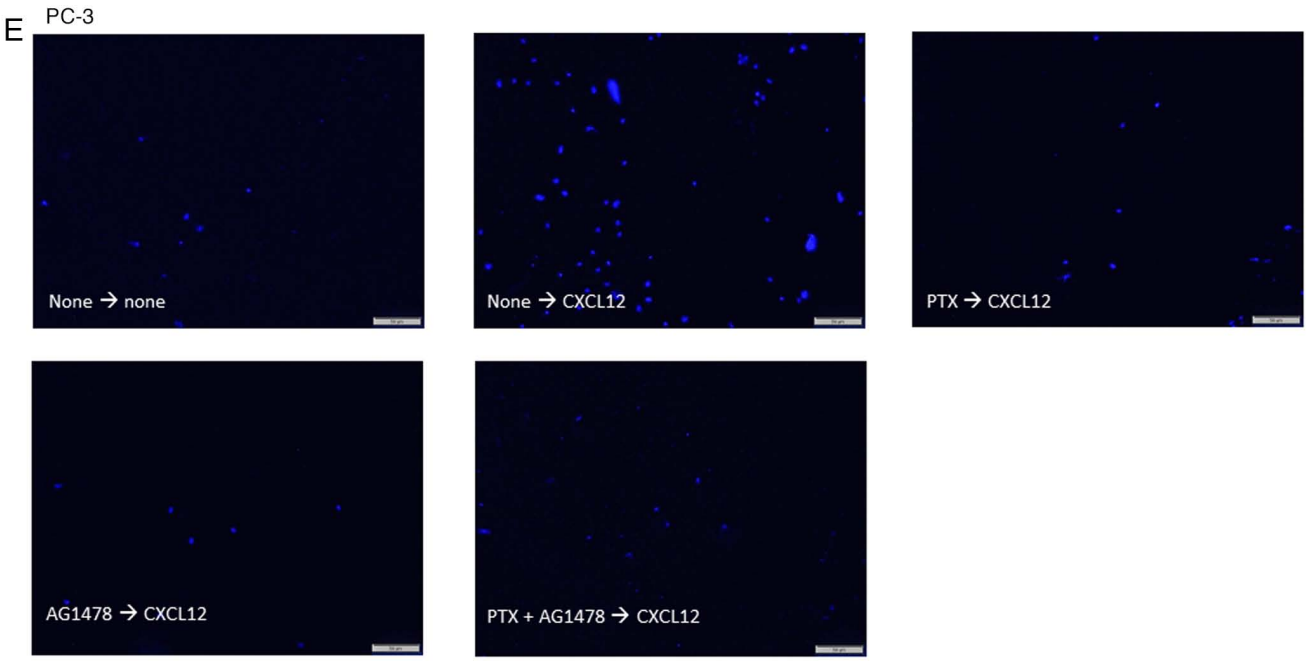

Figure S10. Representative images from chemotactic assay using the various Src-I1-treated cells (A-E). Scale bar, 50 μM. CXCL12, C-X-C motif chemokine 12; PTX, pertussis toxin; Src-I1, Src inhibitor-1.

Figure S10. Continued.

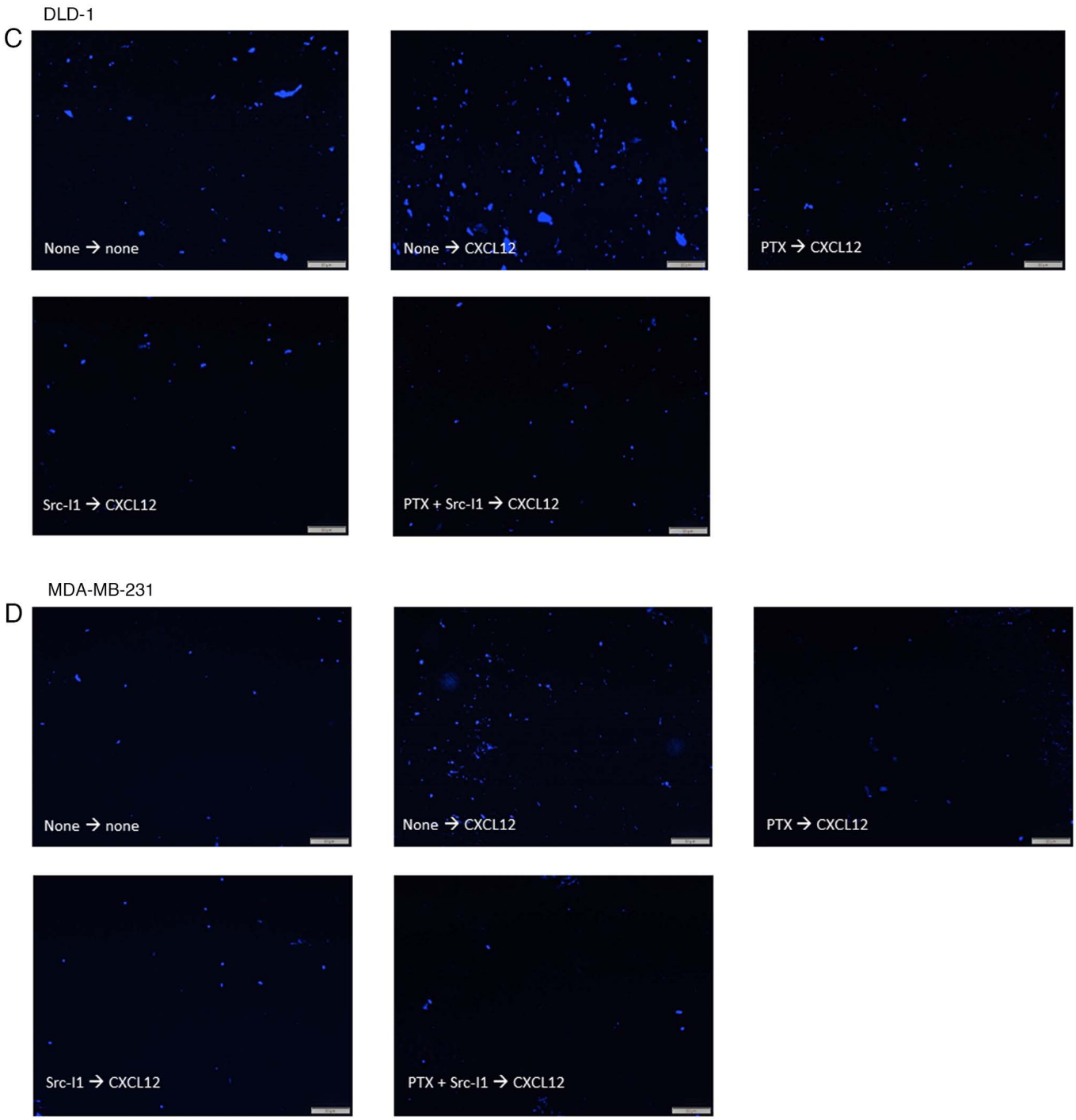

Figure S10. Continued.