Immunoblotting for total Cx43 protein levels in whole cell extracts from MCF7 cells treated with vehicle, R-pep, or ACT.

#### **Supplementary Figure 2**

(A) Immunoblotting for LC3B processing in MCF7 cells treated with vehicle, R-pep, or ACT, in the presence or absence of chloroquine (100  $\mu$ M). (B) Immunoblotting for pAKT and pERK1/2 in whole cell extracts from MCF7 cells treated with vehicle, R-pep, or ACT.

### Supplementary Figure 3.

(A) MCF10A cells were treated with R-pep (200  $\mu$ M), or ACT1 (200  $\mu$ M) and assessed for Immunofluoresence staining and imaging of Cx43 (green). Wheat germ agglutinin (WGA) in red was used to stain cell membranes. (B) MCF10A cells were treated with R-pep (200  $\mu$ M) or ACT1 (200  $\mu$ M) and assessed for gap-FRAP.

#### Supplementary Figure 4.

Connexin 43 activity and expression after ACT1 treatment in BT474 cells. BT474 cells were treated with vehicle, R-pep (200  $\mu$ M), or ACT1 (200  $\mu$ M) and assessed for (A) gap-FRAP. Dunnett's Multiple Comparison Test was used to determine statistical significance \* = p<0.05 vs Vehicle or R-Pep; ± SEM (B) Immunofluoresence staining and imaging of Cx43 (green) in BT474 cells treated with R-pep or ACT1. Wheat germ agglutinin (WGA) in red was used to stain cell membranes.

p27 expression levels are not altered by ACT1. Whole cell extracts isolated from MCF10A, MCF7, and BT474 cells treated with R-pep (100  $\mu$ M), or ACT1 (100  $\mu$ M) were immunoblotted for p27.

## Supplementary Figure 6.

Differential expression of Cx26 and Cx46 in non-transformed breast epithelial cells and breast cancer cell lines. Whole cell extracts isolated from MCF10A, MCF7, BT474, and MDA MB 231 cells were immunoblotted for Cx26 and Cx46.











