Supplementary Figure Legends

Figure S1. Doxorubicin induces hCLCA2 in non-mammary cell types. Data extracted from the GEO database for A673 Ewing's sarcoma cells and HT1080 fibrosarcoma cells.

Figure S2. ChIP analysis with anti-p53 antibody 1C12 (Cell Signaling) showing p53 binding to the consensus elements in the hCLCA2 promoter (h2 p53) and to an established binding site from the p21 promoter (p21) but not to an adjacent region in the hCLCA2 promoter (h2 nc). Cells were infected with hCLCA2-Ad. An anti-CD34 mAb (Pharmingen) served as an additional negative control. Input, 10% of the lysate was subjected to PCR directly. Relative positions of the PCR products from the hCLCA2 promoter are depicted in the schematic.

Figure S3. Knockout of p53, p21, or Bax fails to prevent growth-inhibition by hCLCA2. A, growth curves. Cell lines were infected with Ad-GFP or hCLCA2-Ad in a 24 well plate, and viable cells were counted daily on a ViCell instrument. hCLCA2-Ad-infected cell number was normalized to Ad-GFP-infected cell number and plotted. B, cell death. Cell lines were infected with adenoviruses and cell mortality was measured daily by ViCell counting. Pifithrin (30μM) was added at the time of infection.

Figure S4. Transduction of hCLCA2 causes intracellular acidification. Cells were infected with adenoviruses and analyzed using a fluorescent pHi indicator as described in Supp. Methods. The standard curve used to produce the data in Figure 5E is shown.

Figure S5. hCLCA2 mediates ionomycin-inducible whole-cell current in breast cancer cells. MCF7 cells were infected with Ad-GFP or Ad-hCLCA2. 24 hours later, cells were trypsinized, then allowed to spread 4 hours on coverslips. Using whole cell patch-clamp

techniques, cells were voltage clamped. A ramp protocol, which changed the membrane voltage from -100 to +100 mV in 1s was used for determining the membrane conductance. In control MCF7 cells and cells transduced with hCLCA2, there was a small leak current (A, B). However, when the control cells were exposed to the calcium-ionophore ionomycin there was a significant current (47.7±13.7pA) that could be attributed to endogenous hCLCA2 expressed in these cells (C). When hCLCA2 expressed cells were exposed to ionomycin, a significantly larger outwardly rectifying current (219.7±37.9) could be recorded (D). In the absence of extracellular Ca2+, the current amplitude was much smaller suggesting that the current is Ca2+ dependent. Using a voltage step protocol, we have obtained similar results, in that exposure of hCLCA2-transduced cells to ionomycin induced an outwardly rectifying current. E, averages of 2-4 recordings.