Supporting Information

Britschgi et al. 10.1073/pnas.1217072110

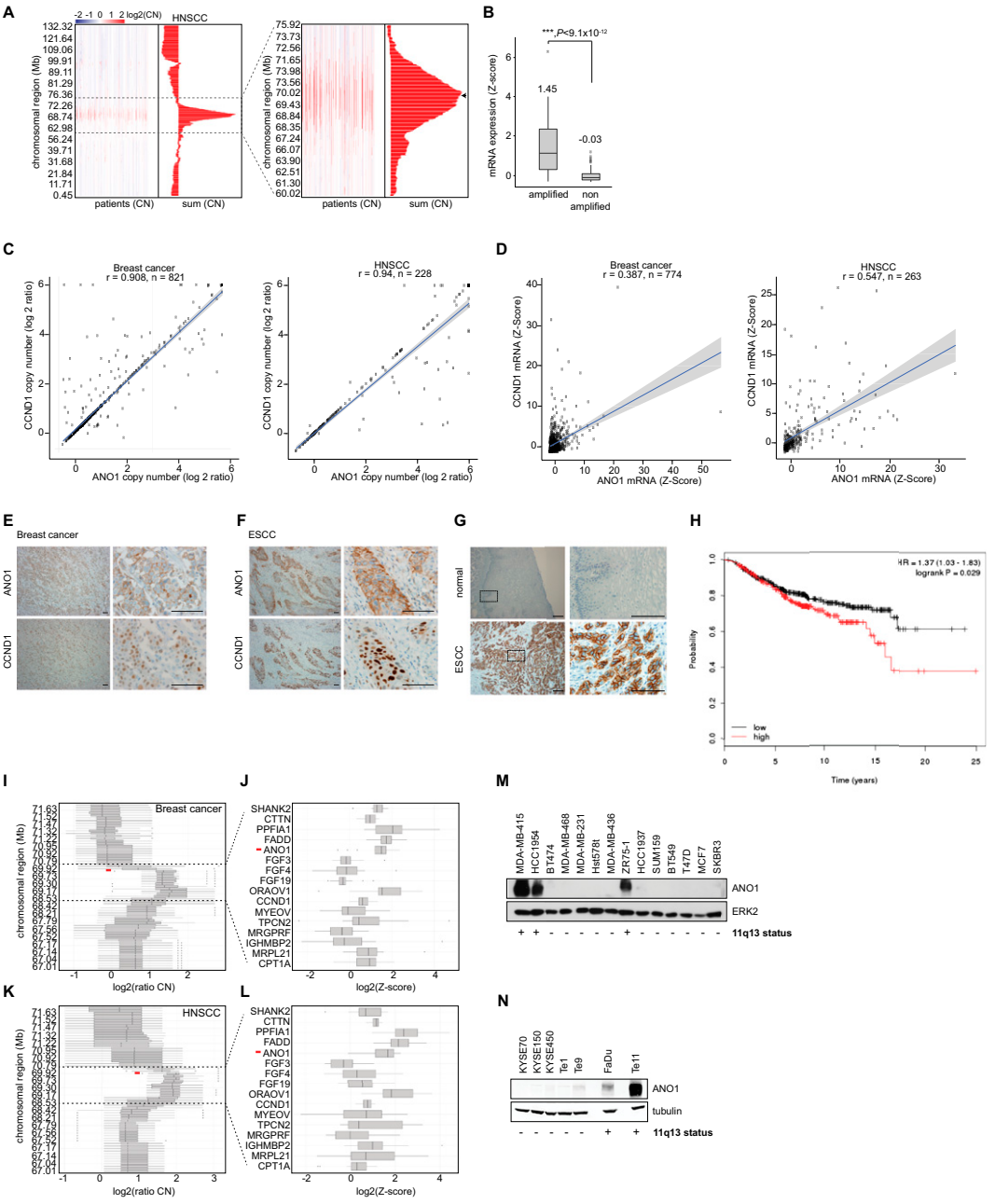

Fig. S1. Anoctamin-1 (ANO1) is amplified and overexpressed in squamous cell carcinoma of the head and neck (HNSCC), esophageal squamous cell carcinoma (ESCC), and breast cancer. (*A*) Copy number (CN) variation in HNSCC as described for Fig. 1*A* ($n = 288$). Data source: *The Cancer Genome Atlas* (https://tcga-data.nci.nih.gov/tcga/). (*B*) Box plots of ANO1 mRNA levels in non–11q13-amplified and 11q13-amplified HNSCC tumor tissue. Normalized gene expression values (*z*-scores) were plotted ($n = 231$). Data source: *The Cancer Gene Atlas*. (*C*) Correlation between copy number variation of *ANO1* and cyclin D1 (*CCND1*) in breast tumors (*Left*) and HNSCC (*Right*). The solid line shows the correlation; the shaded area shows the 95% confidence intervals. Data source: *The Cancer Gene Atlas*. (*D*) Correlation between expression of ANO1 and CCND1 in breast tumors (*Left*) and HNSCC (*Right*). Normalized gene-expression values (*z*-scores) were plotted. The solid line shows the correlation; the shaded area shows the 95% confidence intervals. Data source: *The Cancer Gene Atlas*. (*E* and *F*) Representative images of ANO1 and CCND1 expression in primary human breast tumors (*E*) and ESCC (*F*). (Scale bars: 5 μm.) (*G*) Representative images of ANO1 expression across a tissue microarray of ESCC. (Scale bars: 5 μm.) (*H*) Kaplan–Meier plot showing overall survival of breast cancer patients in correlation to expression of ANO1. Red line, patients with high expression of ANO1; black line, patients with low expression of ANO1. $P = 0.029$, $n = 791$. Data source: K–M plot (http://kmplot.com/analysis/) (1). (*I* and *K*) Copy number status of chromosome 11q13 for 58 breast cancer cell lines (*I*) and 59 HNSCC/ESCC (*K*). The peak amplified region spanning

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chromosome 11q13 is indicated by gray lines. (*J* and *L*) Expression levels of genes contained in the peak amplified region in breast cancer (*J*) or HNSCC (*L*) cell lines. Normalized gene expression values (*z*-scores) were plotted. (*M* and *N*) Immunoblotting of lysates from 14 different breast cancer cell lines (*M*) and seven different HNSCC/ESCC cell lines (*N*). Tubulin and extracellular signal-regulated kinase 2 (ERK2) were used as loading controls throughout the study. Cell lines were defined as 11q13 amplified based on an *ANO1* and *CCND1* copy number >2.

1. Györffy B, et al. (2010) An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat* 123(3):725–731.

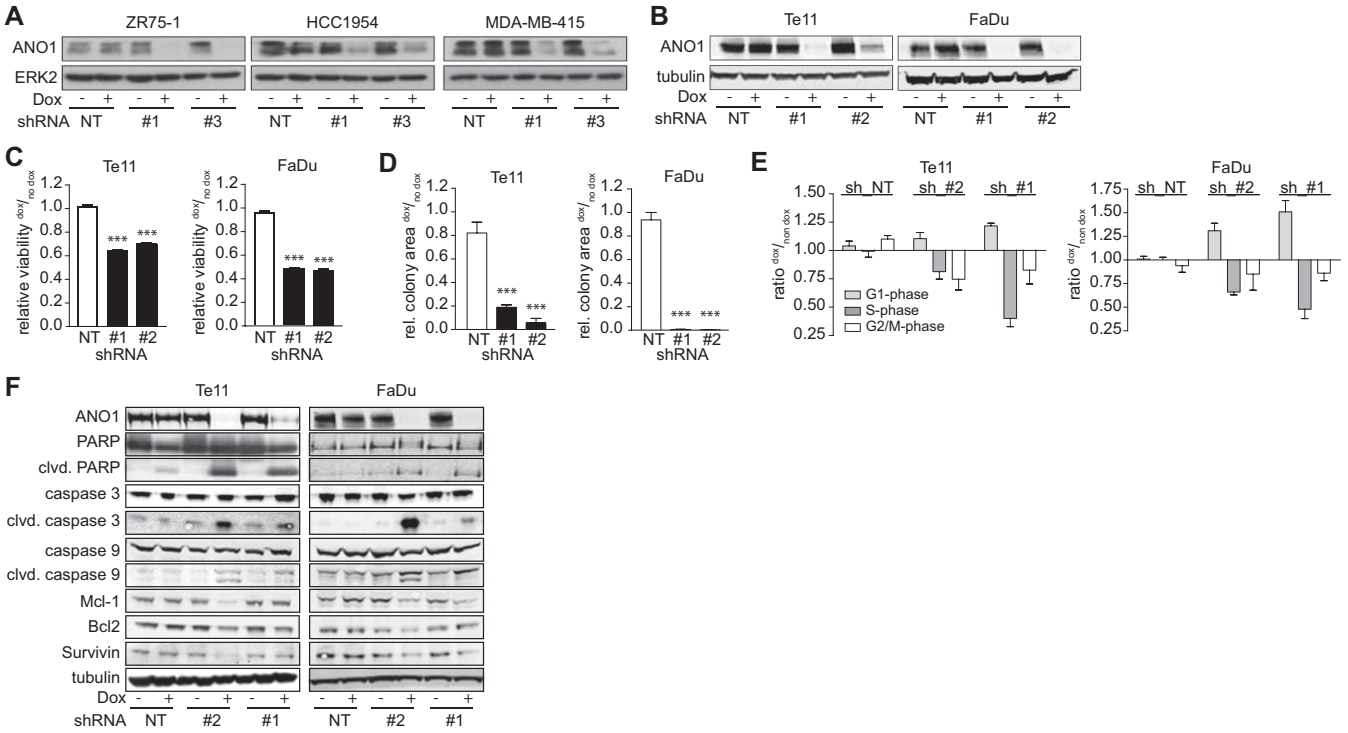

Fig. S2. Knockdown of ANO1 decreases HNSCC, ESCC, and breast cancer cell viability and colony-formation capacity. (*A* and *B*) Immunoblots of breast cell lines ZR75-1, HCC1954, and MDA-MB-415 (*A*) or from Te11 (HNSCC) and FaDu (ESCC) cell lysates (*B*) stably expressing the indicated shRNAs after doxycycline (dox) treatment. NT, nontargeting control shRNA. (*C* and *D*) Bar graphs showing relative viability (*C*) or colony area (*D*) of Te11 and FaDu cells after dox-induced knockdown of ANO1. Data were normalized to the respective non–dox-treated samples. Data are expressed as mean ± SEM; $n = 5$; ***$P < 0.001$. (*E*) Bar graphs showing the relative percentage of cells in the indicated phases of the cell cycle after dox-induced knockdown of ANO1. Data were normalized to the percentage of non–dox-treated cells in the respective cell-cycle phase. Data are expressed as mean ± SEM; $n = 4$. (*F*) Immunoblots of lysates from the indicated cell lines after knockdown of ANO1. PARP, poly(ADP-ribose)polymerase; Mcl-1, myeloid cell leukemia 1; Bcl2, B-cell lymphoma 2.

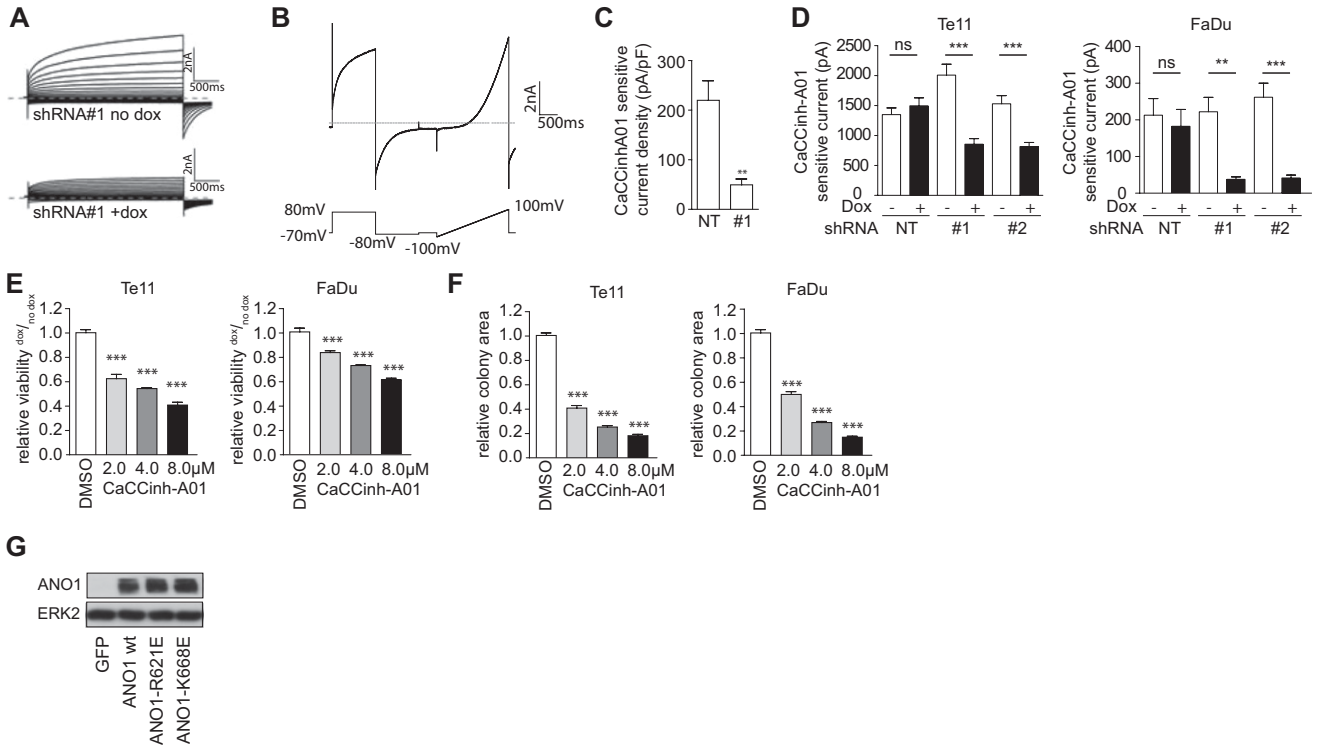

Fig. S3. Inhibition of ANO1 diminishes chloride conduction and decreases HNSCC, ESCC, and breast cancer cell viability and colony-formation capacity. (*A*) Calcium-dependent chloride conductance in Te11 cells before (no dox) and after (+ dox) knockdown of ANO1 as assessed using conventional patch clamp. Representative whole-cell current traces were elicited at potentials ranging from −80 to +100 mV. (*B*) ANO1-like currents in Te11 cells as assessed using conventional patch clamp. Typical ANO1-like current traces were recorded in Te11_shRNA#1 cells without dox in the presence of 1 μM intracellular free Ca^{2+}. (*C*) Quantification of calcium-activated chloride channel inhibitor A01 (CaCCinh-A01)–sensitive current densities in Te11_shRNA#1 cells as assessed using conventional patch clamp and assay conditions as described in *A*. Data are expressed as mean ± SEM; $n = 9–11$. (*D*) CaCCinh-A01–sensitive currents are reduced significantly after 72 h incubation with dox in cells expressing ANO1-targeted shRNA but not in cells expressing nontargeting shRNA as assessed using the QPatch platform. Data are expressed as mean ± SEM; $n = 31–61$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant. (*E* and *F*) Bar graphs showing relative viability (*E*) and colony area (*F*) of the indicated cell lines after inhibition of ANO1 using CaCCinh-A01. Data were normalized to the respective DMSO-treated samples. Data are expressed as mean ± SEM; $n = 4$; $^{***}P < 0.001$. (*G*) Immunoblotting of lysates from MCF10A cells stably transfected with wild-type ANO1 or the pore-mutants ANO1-R621E or ANO1-K668E.

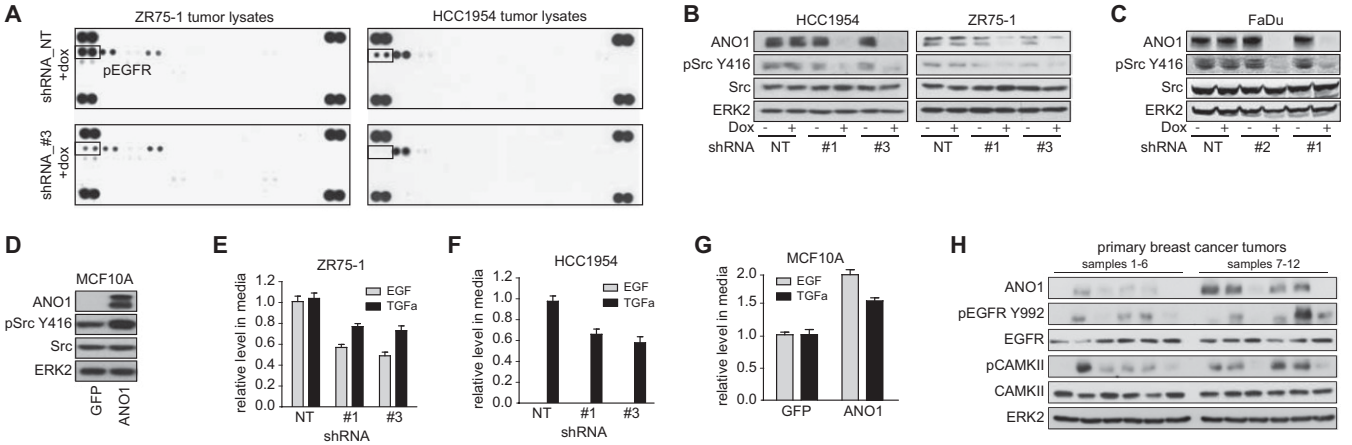

Fig. S4. Knockdown of ANO1 reduces the phosphorylation of EGF receptor (EGFR) and SRC and decreases secretion of EGF and TGF-α. (*A*) Antibody array on ZR75-1 and HCC1954 breast tumor lysates after ANO1 knockdown. The box indicates signals (duplicate) for phosphorylated EGFR (pEGFR). The strong signals on the edges of the array represent positive controls. (*B* and *C*) Immunoblotting of lysates from breast cell lines ZR75-1 and HCC1954 (*B*) or the ESCC line FaDu (*C*) stably expressing the indicated shRNAs after dox treatment. NT, nontargeting control shRNA; pSrc (phosphorylated Src). (*D*) Immunoblotting of lysates from MCF10A cells stably transfected with wild-type ANO1. (*E* and *F*) Relative levels of secreted EGF or TGF-α in the supernatant of ZR75-1 (*E*) or HCC1954 (*F*) cells after knockdown of ANO1. Levels of EGF and TGF-α were measured using ELISA and normalized to the respective non–dox-treated cells. Data are expressed as mean ± SEM; $n = 3$. (*G*) Relative levels of secreted EGF or TGF-α in the supernatant of MCF10A cells stably transfected with wild-type ANO1. Levels of EGF and T TGF-α were measured using ELISA and normalized to MCFA10A cells overexpressing GFP as a control. Data are expressed as mean ± SEM; $n = 3$. (*H*) Immunoblots of lysates from primary human breast tumors as indicated ($n = 12$).

Table S1. Immunohistochemical analysis of ANO1 expression in HNSCC, ESCC, and breast cancer tumor tissue: number of cases, staining intensities, and percentage of positive specimens

ANO1 expression	No. of cases	Tumor positivity (%)
HNSCC	**17**	**100**
Intensity = 0	0	
Intensity = 1+	11	
Intensity = 2+	4	
Intensity = 3+	2	
ESCC	**40**	**90**
Intensity = 0	4	
Intensity = 1+	17	
Intensity = 2+	11	
Intensity = 3+	8	
Breast cancer	**49**	**78**
Intensity = 0	11	
Intensity = 1+	8	
Intensity = 2+	28	
Intensity = 3+	2	

Table S2. Copy numbers of *ANO1* and *CCND1* in human HNSCC, ESCC, and breast cancer cell lines

	Copy number*	
Cell line	*ANO1*	*CCND1*
BT474	n.a.	n.a.
BT549	n.a.	n.a.
HCC1937	n.a.	n.a.
HCC1954	3	3
Hs578t	n.a.	n.a.
MCF7	n.a.	n.a.
MDA-MB-231	n.a.	n.a.
MDA-MB-415	7	7
MDA-MB-436	n.a.	n.a.
MDA-MB-468	n.a.	n.a.
SkBr3	n.a.	n.a.
SUM159	n.a.	n.a.
T47D	n.a.	n.a.
ZR75-1	3	3
FaDu	14	9
KYSE150	n.a.	n.a.
KYSE450	n.a.	n.a.
KYSE70	n.a.	n.a.
Te1	n.a.	n.a.
Te11	9	5
Te9	n.a.	n.a.

Data represent the mean of at least three independent experiments. n.a., not amplified.

*Copy numbers of ANO1 and CCND1 in breast cancer and HNSCC cell lines.

Table S3. IC_{50} values for the inhibition of cell viability in HNSCC and ESCC cell lines by SRC-inhibitors in correlation with *ANO1* amplification status

Cell line	*ANO1* amplification	IC_{50}* (μM)		
		Dasatinib	Saracatinib	Bosutinib
BT474	No	5.3	5.0	6.3
BT549	No	18.8	11.3	14.1
HCC1937	No	1.0	2.1	7.9
HCC1954	Yes	0.1	1.1	0.9
MCF7	No	28.3	14.4	10.1
MDA-MB-415	Yes	0.1	1.7	3.0
MDA-MB-436	No	23.7	15.6	4.3
MDA-MB-468	No	9.5	11.1	7.0
SkBr3	No	3.3	11.3	7.0
SUM159	No	15.6	25.5	17.3
ZR75-1	Yes	2.7	2.6	2.6
FaDu	Yes	0.4	1.6	1.0
KYSE150	No	4.6	9.4	3.0
Te1	No	0.8	5.0	1.5
Te11	Yes	0.1	0.5	0.5
MCF10A-GFP		0.4	1.2	2.6
MCF10A-ANO1		0.2	0.4	0.5

Data represent the mean of three independent experiments.
*IC_{50} values for inhibition of cell viability by Src inhibitors in correlation with *ANO1* amplification status.

Table S4. IC_{50} values for the inhibition of cell viability in HNSCC, ESCC, and breast cancer cell lines by EGFR inhibitors in correlation with *ANO1* amplification status

Cell line	*ANO1* amplification	IC_{50} (μM)				
		Gefitinib	Erlotinib	BIBX1382	AEE788	Lapatinib
KYSE70	No	10.1	10.6	13.9	5.0	6.9
KYSE150	No	15.3	4.7	15.6	1.2	6.6
KYSE450	No	15.5	7.2	10.8	0.5	4.0
Te11	Yes	2.4	0.5	3.2	0.2	1.4
FaDu	Yes	5.5	2.3	9.8	0.4	1.9

Data represent the mean of three independent experiments.