Supporting Information

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SI Experimental Procedures

DNA Constructs. Full-length human *dlc1* cDNA (GenBank accession number NM_006094) was generated by RT-PCR from total RNA of MCF10A cells and subcloned into a eukaryotic expression vector, pcDNA3.1(+) (Invitrogen) with FLAG tag at the C terminus, via the NheI and XbaI restriction sites. The truncated fragments of deleted in liver cancer 1 (DLC1), DLC1-sterile alpha motif (SAM), DLC1-ΔSAM, DLC1-rhodopsin-GTPase-activating (Rho-GAP)-steroidogenic acute regulatory protein-related lipid transfer (START), DLC1-Rho-GAP, and DLC1-START, were generated by PCR from the full-length pcDNA3.1(+)-DLC1-FLAG. Full-length human *tensin3* (GenBank accession number NM_006094) and *cten* (GenBank accession number AF417489) cDNA were generated by RT-PCR from total RNA of MCF10A cells and subcloned into the eukaryotic expression vector, pEGFPC3 (Clontech), using BglII and SalI sites or BglII and EcoRI sites. The truncated fragments of tensin3 [tensin3 actin-binding domain (ABD) and tensin3ΔABD] were generated by PCR from full-length pEGFPC3-tensin3. The SAM domain of DLC1 was amplified by PCR from full-length pcDNA3.1(+)-DLC1-FLAG and subcloned via the BamHI/XhoI sites into a pGEX6P3 (GE Healthcare) vector. All constructs were verified by DNA sequencing.

Reagents and Antibodies. Unless indicated, chemical reagents were purchased from Sigma-Aldrich. Glutathione Sepharose 4 Fast Flow was purchased from GE Healthcare. Anti-DLC1 rabbit polyclonal antibody, anti-DLC1 goat polyclonal antibody, anti-tensin3 goat polyclonal antibody, anti-paxillin rabbit polyclonal antibody, anti-RhoA rabbit polyclonal antibody, anti-GFP rabbit polyclonal antibody, anti-GST rabbit polyclonal antibody, and anti-β-tubulin rabbit polyclonal antibody were purchased from Santa Cruz Biotech. Anti-Tns3 (tensin3) rabbit polyclonal antibody, anti-COOH-terminal tensin-like protein (cten) mouse monoclonal antibody, and anti-FLAG mouse monoclonal antibody were obtained from Sigma-Aldrich. Antiactive RhoA-GTP mouse monoclonal antibody was from NewEast Biosciences. Anti-phosphotyrosine mouse monoclonal antibody (4G10) was purchased from Upstate Tech. Anti-Akt rabbit polyclonal antibody, anti-Phospho-AKT (Ser473) mouse monoclonal antibody, anti-p44/42 MAPK (ERK1/2) mouse monoclonal antibody, and anti-Phospho-p44/42 MAPK (Thr202/Tyr204) mouse monoclonal antibody were from Cell Signaling Technologies. Anti-epidermal growth factor receptor rabbit polyclonal antibody was from Millipore.

Cell Culture, Transfection, and Inhibitor Treatment. HEK293, A549, and MCF10A cells were obtained from American Type Culture Collection. HEK293 and A549 cells were maintained in DMEM containing antibiotics and 10% FBS (Sigma). MCF10A cells full medium contains DMEM:F12 (1:1) medium supplemented with antibiotics, EGF (10 ng/mL), insulin (10 μg/mL), cholera toxin (1 μg/mL), hydrocortisone (1 μg/mL), and heat-inactivated horse serum (5%) (Invitrogen). MCF10A cells serum-starved medium contains no horse serum and EGF. MCF10A cells EGF treatment medium contains no horse serum. MCF10A cells transfection medium contains no antibiotics. Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere. Transient transfections were carried out with jet polyethylenimine according to the manufacturer's instructions. Rho-associated protein kinase (ROCK)-specific inhibitor Y27632 was obtained from Calbiochem. For treatment, cells were incubated with 10 μM Y27632 for 1 h at 37 °C.

Bacterial Expression of Recombinant Proteins and GST Pull-down. The BL21 strain of *Escherichia coli* was transformed with pGEX6P3-DLC1-SAM or empty vector and induced with 0.1 mM IPTG for 16 h at 18 °C. The bacterial cultures were harvested and pellets were resuspended in PBS containing complete protease inhibitors (Roche). The suspension was sonicated for three times (10 s each) on ice. Triton X-100 was added to a final concentration of 1% and the lysates were centrifuged at 15,700 × *g* for 15 min under 4 °C. Purification of GST-tagged proteins was performed with glutathione resin (GE Healthcare). The resin was washed with PBS for three times. The purity of the proteins was determined by SDS-PAGE followed by Coomassie staining. Pull-down was performed by incubating whole cell lysates with GST-DLC1-SAM or GST beads for 3 h, followed by washing the beads with lysis buffer for three times at 4 °C as described (1). Proteins that bound to GST or GST-SAM were resolved on SDS-PAGE and identified by Western blot analysis.

Lysate Preparation, Immunoprecipitation, and Western Blotting. HEK293, A549, and MCF10A cells were lysed with cold lysis buffer (1% Nonidet P-40, 50 mM Tris pH 7.4, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10% glycerol, and protease inhibitor cocktail diluted at 1:1,000). To prepare MCF10A cell lysates, cell pellets were sonicated in 0.2 mL lysis buffer on ice, and the lysate was spun down at 15,700 × *g* for 15 min at 4 °C (Centrifuge 5415R; Eppendorf). The supernatants were collected and the protein concentrations of the lysates were determined using the Bio-Rad Protein Assay Kit. After clearance of the lysate with appropriate preimmune serum and protein G (Roche), immunoprecipitation was performed using indicated antibodies following established protocols (2). Western blotting was carried out as described previously (2).

RNA Preparation and Real-Time RT-PCR analysis. Total RNA was extracted by QIAGEN RNeasy Mini Kit (74104), and the reverse transcription reaction was performed using QIAGEN QuantiTect Reverse Transcription Kit (205311) following the manufacturer's protocol. After mixing the resulting template with FastStart SYBR Green Master (04673492001; Roche) and indicated primers as below, the real-time RT-PCR reaction was performed with the Stratagene Mx3005P QPCR System (QIAGEN-SABiosciences) using protocols as described previously (3, 4).

DLC1 Forward Primer: 5′-ggatggatgaggagaagctgaa-3′
DLC1 Reverse Primer: 5′-ggtctgcgtggagttggaaa-3′
cten Forward Primer: 5′-cccaccatgaagttcgtgatg-3′
cten Reverse Primer: 5′-cggtatgaagagctgtcccttatg-3′
tensin3 Forward Primer: 5′-gttgaaagggtgctcgaatga-3′
tensin3 Reverse Primer: 5′-gaactttctgctatttcctccaatg-3′
tensin1 Forward Primer: 5′-gtgcaaaggggactgaattcg-3′
tensin1 Reverse Primer: 5′-ggctacaagactctccaagtgg-3
tensin2 Forward Primer: 5′-cctccagccccgagaagatc-3′
tensin2 Reverse Primer: 5′-tgctggttgaagttctcataggag-3′
β-tubullin Forward Primer: 5′-aacacggatgagacctactgcat-3′
β-tubulin Reverse Primer: 5′-gggtgcggaagcagatgt-3′

Knockdown of Protein Expression by siRNA. SiRNA oligonucleotides specific for human *cten* (5) were synthesized by GenePharma. SiCten-I: 5′-UUCUCAUUGACAUGGUGCUCUGGGC-3′; siCten-II: 5′-GGAGGAAUCUGAAGCCUUGGACAUA-3′. SiRNA oligos targetting *dlc1* (sc-43725) and the control siRNA (sc-44236) were obtained from Santa Cruz Biotech. The acces-

sion number of On-TargetPlus SMARTpool siRNA oligonucleotides against *tensin3* (Thermo Fisher Scientific-Dharmacon) was L-009997-00. SiRNA oligos were transfected into subconfluent cells using DharmaFECT 1 Transfection Reagent (T-2001-01) according to the manufacturer's instructions.

Statistical Analysis. All statistical analyses were performed using Excel. All data based on statistical analysis were shown as mean ± SD. Statistical significance was analyzed by using paired Student's *t* test. All *p* values were two-tailed and the level of statistical significance was set at $p < 0.05$.

1. Heering J, Erlmann P, Olayioye MA (2009) Simultaneous loss of the DLC1 and PTEN tumor suppressors enhances breast cancer cell migration. *Exp Cell Res* 315:2505–2514.
2. Li C, Iosef C, Jia CY, Han VK, Li SS (2003) Dual functional roles for the X-linked lymphoproliferative syndrome gene product SAP/SH2D1A in signaling through the signaling lymphocyte activation molecule (SLAM) family of immune receptors. *J Biol Chem* 278:3852–3859.
3. Savage J, Conley AJ, Blais A, Skerjanc IS (2009) SOX15 and SOX7 differentially regulate the myogenic program in P19 cells. *Stem Cells* 27:1231–1243.
4. Kennedy KA, et al. (2009) Retinoic acid enhances skeletal muscle progenitor formation and bypasses inhibition by bone morphogenetic protein 4 but not dominant negative beta-catenin. *BMC Biol* 7:67.
5. Katz M, et al. (2007) A reciprocal tensin-3-cten switch mediates EGF-driven mammary cell migration. *Nat Cell Biol* 9:961–969.

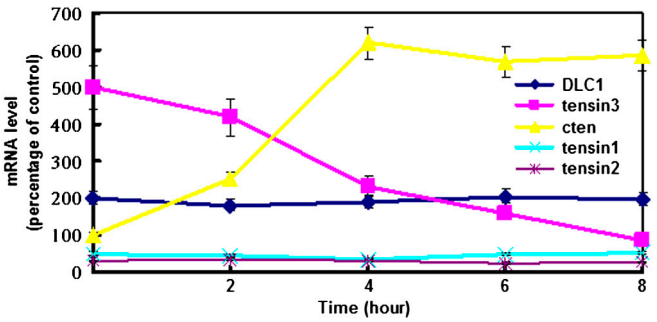

Fig. S1. Effect of EGF on the transcription of tensins and *dlc1* in MCF10A cells. Serum-starved MCF10A cells were stimulated with EGF (10 ng/mL) for the indicated time intervals and mRNA was analyzed using real-time RT-PCR and normalized to that of β-tubulin (control). The mRNA levels of tensin family members and DLC1 at the indicated time points were presented. Data presented are averages of five independent sets of experiments with error bars represent the SD.

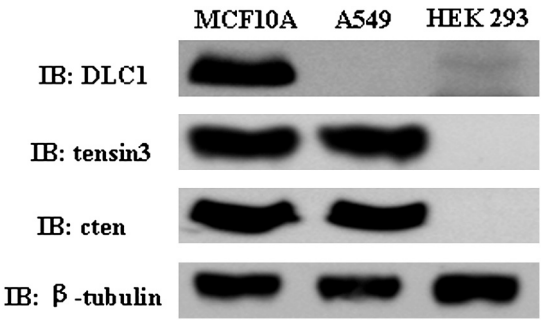

Fig. S2. Endogenous expression levels of the tensin family proteins and DLC1 in different cell lines. Lysate of the MCF10A, A549, or HEK293 cells was subjected to immunoblot (IB) analysis using the indicated antibodies. Anti-β-tubulin was used as the loading control.

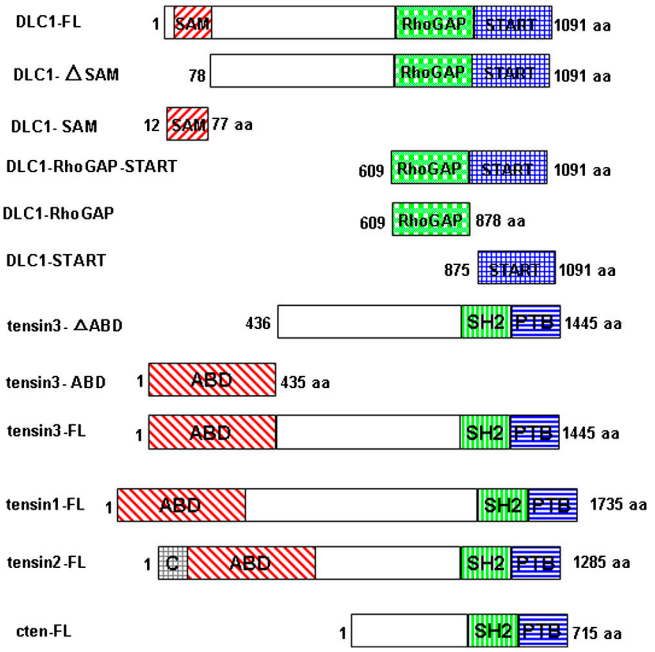

Fig. S3. Schematic diagram of the different constructs of DLC1 and tensin family members in this study. The full-length (FL) and various segments of tensin3 and cten cDNAs were subcloned into the expression vector, pEGFP-C3, resulting in the expression of proteins as fusion to the C terminus of enhanced GFP. The full length and various segments of DLC1 cDNA were subcloned into pcDNA3.1(+) vector. A construct produced a C-terminal FLAG-tag protein when expressed in cells.

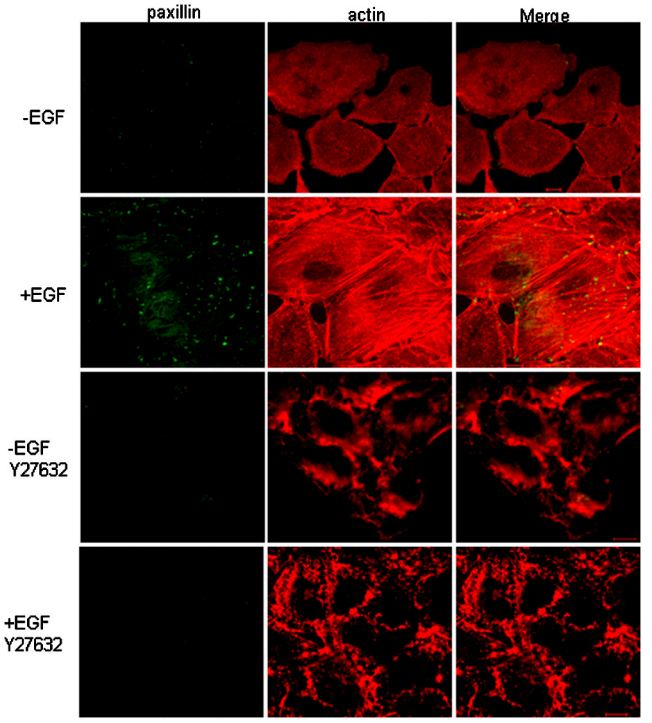

Fig. S4. Effects of EGF and Y27632 treatment on the formation of ROCK-mediated actin stress fibers and focal adhesions. Serum-starved MCF10A cells were incubated without or with EGF (10 ng/mL for 24 h) and Y27632 (10 μM for 1 h) as indicated. Confocal immunofluorescence analysis was performed using anti-paxillin (focal adhesions, green) and rhodamine phalloidin (actin stress fibers, red). All confocal microscopy images are representative of at least three independent experiments. (Scale bars: 10 μm.)

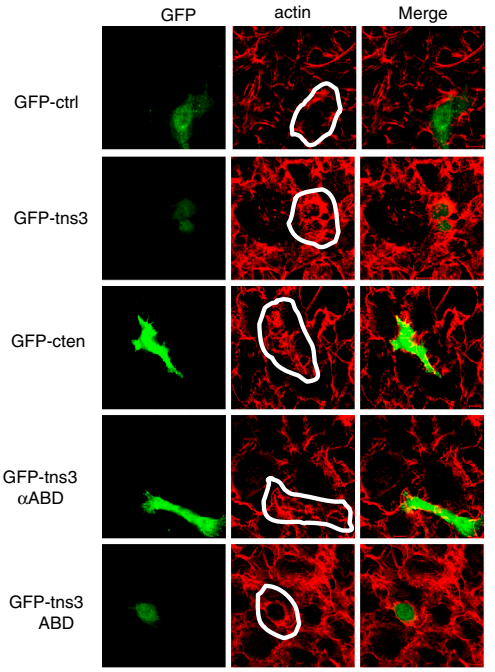

Fig. S5. Distinct effects of overexpressing cten, tensin3, or a truncated version of tensin3 (i.e., tensin3ΔABD or ABD alone) on the actin cytoskeleton in MCF10A cells. All confocal microscopy images are representative of at least three independent experiments. (Scale bars: 10 μm.)

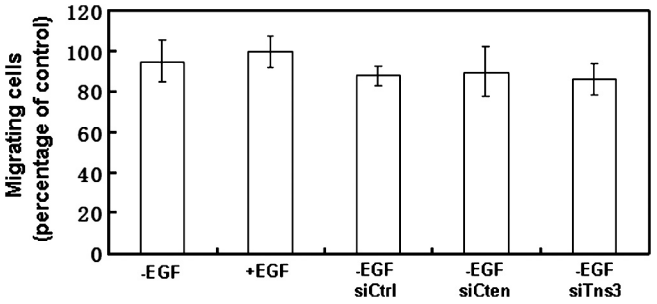

Fig. S6. Effect of EGF treatment or knockdown of cten and tensin3 on A549 cells migration. A549 cells without transfection or transfected with the indicated siRNA oligonucleotides were plated in transwell chambers containing full medium without or with EGF (10 ng/mL). Cell migration was quantified 24 h later as described for Fig. 5*A*. Percentage of migrating cells relative to cells treated with EGF (set at 100%) was presented. Data shown are means of nine independent measurements with standard errors (mean ± SD). Scrambled siRNA control, siCtrl.

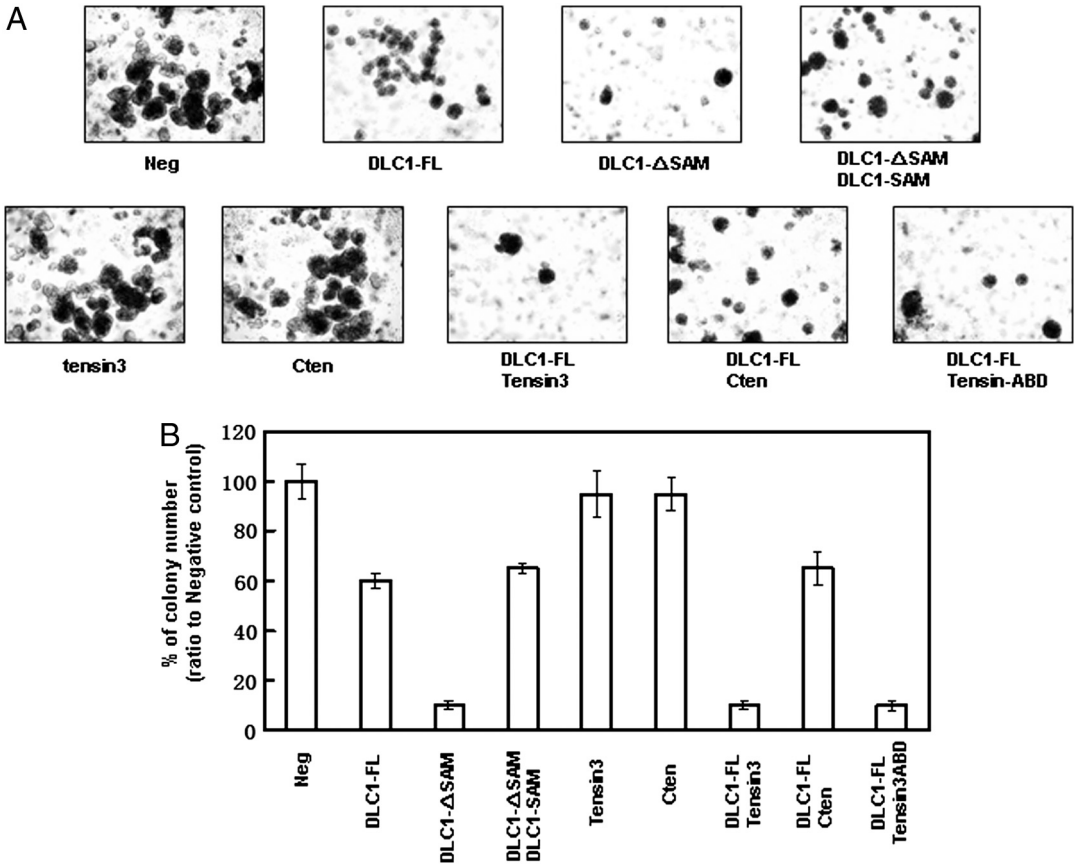

Fig. S7. Effect of tensin3, cten, DLC1, and their segments on colony formation in soft agar. (*A*) Phase contrast images of colony formation in soft agar by HEK293 cells transfected with the indicated constructs after 21 d incubation. (*B*) Colonies formed by cells expressing the various proteins as indicated, relative to that by the control HEK293 cells (set at 100%). Data presented are mean ± SD, $n = 3$.

Table S1. Changes in gene expression for DLC1, tensin family member and the ErbB family receptors between 32 pair-matched colorectal adenomas and normal tissue samples

Gene	Normal	SD	Tumor	SD	Fold change	*p* value
DLC1	86.97	50.97	76.71	49.75	−0.18	0.2216
cten	142.65	96.48	689.57	302.14	2.27	3.67E-11
tensin3	3,781.16	600.64	2,296.84	644.79	−0.72	3.40E-12
tensin1	717.68	203.89	255.91	92.74	−1.49	1.89E-13
ErbB2	289.75	91.42	276.05	135.78	−0.07	0.3132
EGFR	167.45	102.08	118.42	83.77	−0.50	0.0069
ErbB4	49.38	42.96	46.54	44.66	−0.09	0.3935
ErbB3	90.38	49.04	69.62	44.41	−0.38	0.0353

Analysis was done using the microarray data of Sabates-Bellver et al. (1). Specifically, the gene expression data of 32 colorectal adenomas with matched normal mucosa samples were obtained from the National Center for Biotechnology Information Gene Expression Omnibus database under the accession number GSE8671. Comparisons of mean values and standard deviations between the normal and adenomas groups were analyzed using Excel. Statistical significance of the differences was analyzed using paired Student's *t* test. EGFR, epidermal growth factor receptor.

1. Sabates-Bellver J, et al. (2007) Transcriptome profile of human colorectal adenomas. *Mol Cancer Res* 5:1263–1275.

Table S2. Changes in gene expression for DLC1, tensin family members, and the ErbB family receptors between 111 breast cancer samples and 12 normal breast samples

Gene	Normal	SD	Tumor	SD	Fold change	*p* value
DLC1	−0.80	0.81	0.33	0.42	1.13	1.36E-12
cten	−0.22	2.08	1.30	0.91	1.52	7.57E-06
tensin3	0.22	1.44	−0.84	0.60	−1.06	3.56E-06
tensin2	0.34	1.34	−0.74	0.46	−1.08	2.76E-08
tensin1	0.18	0.38	−0.08	0.34	−0.26	0.01
ErbB2	0.08	2.60	2.84	1.22	2.76	1.91E-09
ErbB4	0.24	0.79	1.17	0.76	0.93	0.00

Analysis was done using the microarray data of Finak et al. (1). Specifically, the gene expression data of 111 breast stroma collected from breast cancer patients and 12 normal breast stroma samples, were obtained from the National Center for Biotechnology Information Gene Expression Omnibus database under the accession number GSE9014. Comparisons of mean values and standard deviations between the normal and breast stroma groups were analyzed using Excel. Statistical significance of the differences was analyzed using unpaired Student's *t* test.

1. Finak, et al. (2008) Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med* 14:518–527.