Supporting Information

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SI Materials and Methods

Cells and Culture Conditions. The human GIST-T1 cell line was established from a gastrointestinal stromal tumor (1). ULTR, a retrovirally transformed human uterine leiomyomatous smooth-muscle cell line was established from tissue obtained from a 49-y-old woman following a hysterectomy for multiple leiomyomas (2). Myometrial samples were obtained from premenopausal women undergoing hysterectomy (3). Tissue samples were minced and digested using 1.5 mg/mL collagenase for 4–6 h at 37 °C. ULTR, primary myometrial cells, and GIST–T1 cells were all maintained in DMEM (Gibco Invitrogen) culture medium, supplemented with 10% (vol/vol) exosome-depleted FBS, and 100 units per mL penicillin–streptomycin (complete medium) at 37 °C with 5% CO_2 in tissue culture flasks. ULTR and GIST–T1 cells were all maintained in DMEM (Gibco Invitrogen) culture medium, supplemented with 10% exosome-depleted FBS and 100 units per mL penicillin–streptomycin (complete medium) at 37 °C with 5% CO_2. FBS-derived exosomes were eliminated by dilution of FBS in DMEM and ultracentrifugation overnight at 100,000 × *g* for 18 h and sterile-filtered using 0.22 μm filter.

Patient Plasma Collection. Blood samples were obtained from seven patients with GIST who had primary or metastatic disease. One patient included in this study had received adjuvant imatinib, whereas the remaining six patients were not exposed to therapy before removal of their primary tumor. All samples were obtained from the University of Kansas Cancer Center's Biospecimen Repository Core Facility after approval from the internal Human Subjects Committee.

Exosome Purification. Exosomes were isolated from the conditioned medium and plasma as previously described (4). Briefly, plasma (1 mL) or CM obtained from cells at ∼80% confluence were centrifuged at 400 × *g* for 10 min to eliminate cells' debris. Supernatant fractions were further spun at 2,000 × *g* for 20 min and 16,500 × *g* for 30 min to remove other types of vesicles, such as microvesicles. The resulting cell-free medium was ultracentrifuged at 100,000 × *g* for 1 h to generate an exosome pellet that was washed once with PBS. The amount of exosomal protein recovered was assessed using detergent-compatible protein assay (Bio-Rad) according to the manufacturer's instructions. On average ∼60 and ∼300 μg of exosomal proteins were recovered from 200 mL of conditioned medium from ULTR and GIST–T1 cell lines, respectively.

Nanoparticle Tracking Analysis. Purified exosomes were resuspended in 100 μL of molecular grade water and analyzed using a NanoSight LM10 instrument (NanoSight). Analysis was performed by applying a monochomatic 404 nm (blue) laser to 300 μL of pure or diluted exosomal preparation and measuring the Brownian movement of each particle. Video files of 30–60 s duration with a frame rate of 25 frame per second were recorded and analyzed using the Nanoparticle Tracking Analysis software version 2.3. The mean, mode, and median vesicle size as well as the concentration of each preparation were obtained after analysis and corrected by the dilution factor when required.

Electron Microscopy. Purified exosome pellets were fixed using 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4 °C overnight. The pellet was rinsed in 0.15 M sodium cacodylate buffer (pH 7.4), followed by a postfixation in 1% osmium tetroxide containing 0.1% potassium ferricynide buffered in 0.1 M cacodylate buffer pellets for 1 h. Pellets were dehydrated through the following series of graded ethanols: 30%, 70%, 80%, 95%, 100%, and 100% at 10 min for each step, and finally in propylene oxide for 10 min. The final resulting pellets were embedded into half propylene oxide/half embed 812 resin (Electron Microscopy Sciences, Inc.) overnight. The next day, the pellets were placed in fresh embed 812 resin and cured in a 60 °C oven overnight. We cut 80 nm sections using a Leica UC7 ultramicrotome. Sections were picked up on Copper thin bar 300-mesh grids and contrasted with 4% uranyl acetate and Sato's lead stain. Samples were examined using a transmission electron microscope JEOL JEM-1400 TEM at 80 KV. Images were captured with a digital camera.

Western Blot. We separated 30 μg of exosomal protein or total cellular lysates on SDS/PAGE gels and transferred them to a nitrocellulose membrane. The membranes were blocked 1 h at room temperature with 5% milk. Primary antibodies were added overnight at 4 °C with gentle shaking. The primary antibodies used included anti–plasminogen activator inhibitor–1 (PAI-1–clone C-9), anti–heat shock protein 60 (HSP60–clone H-1), anti–Asparagine-linked glycosylation homolog-2–interacting protein (Alix-clone 3A9), anti-flotilin (clone C-2), anti-CD9 (clone C4), anti–tumor suppressor gene 1 (TSG101–clone C2), anti-Annexin I (clone EH17a), anti-glucose regulated protein 78 kDa (GRP78–clone G-10), anti–matrix metalloproteinase 2 (MMP2–clone H-76), or anti-β actin (clone-C4), all purchased from Santa Cruz Biotechnology. Antidesmin, antiendoglin, antivimentin, anti–smooth muscle actin (SMA), anti–phospho-(Thr202/Tyr204)-ERK1/2 (clone E10), antitotal ERK1/2 (clone 3A7), anti-phospho(Tyr719)–KIT, and anti–total-KIT antibodies were purchased from Cell Signaling Technology. Finally, anti-CD63 (clone–RFAC4) was purchased from Millipore. Densitometric quantification of the gels or membranes was performed using Unscan-it software (Silk Scientific).

Confocal Microscopy. Purified GIST–T1–derived exosomes (TEXs) (300 μg) were labeled using the green fluorescent linker dye PKH67 kit (Sigma-Aldrich), according to the manufacturer's instructions. ULTR cells were washed three times with DMEM. The obtained cell pellets (20×10^6 cell per mL) were resuspended in 1 mL of diluent C. Red fluorescent linker dye, PKH26 kit (Sigma-Aldrich), was added at 5×10^6 M to the cell pellets for 5 min at room temperature. The reaction was stopped by addition of 2 mL of human AB serum for 1 min. The labeled cells were then washed twice with DMEM and resuspended in complete medium, and a protein assay was performed before use. After incubation for 24 h at 37 °C, the cells were washed and resuspended in fresh complete medium. PKH67-labeled TEXs were added at 300 μg/mL for 1, 2, 4, 6, or 24 h. The cells were then washed five times with cold PBS and fixed for 15 min with 4% paraformaldehyde (Sigma). After several washes, the cells were resuspended in complete medium and analyzed by confocal microscopy.

Flow Cytometry. GIST–T1 cells were trypsinized, washed, resuspended in 100 μL of PBS–BSA 0.5% (FACS buffer), and incubated at 4 °C for 15 min with human AB serum. Labeling was performed according to standard techniques at 4 °C. Directly conjugated monoclonal antibodies used for the study included anti–CD117–APC and anti–IgG1κ–Allophycocyanin isotype control (BD Pharmingen). Saturating concentrations of primary antibodies were added to the cells without washing and incubated for an additional 30 min at 4 °C. The labeled cells were

then washed twice in cold FACS buffer and fixed with 2% formalin in PBS. The analysis was performed using a FACS Vantage flow cytometer (Beckton Dickinson). At least 30,000 events were acquired from each sample and analyzed using CellQuest software (Beckton Dickinson). For exosome analysis by flow cytometry, 30 μg of TEXs or BSA (negative control) were incubated with 10 μL of 4 μm aldehyde/sulfate latex beads (Invitrogen) for 15 min in a total volume of 50 μL of PBS followed by overnight incubation in 500 μL PBS at 4 °C with agitation. The reaction was stopped by addition of 100 mM glycine and incubation for 30 min at room temperature. Exosomes or BSA-coated beads were washed tree times with PBS–BSA 1%, incubated with either anti–c-KIT (Cell Signaling) or isotype control in PBS–BSA 1% for 30 min at 4 °C, washed, and analyzed on a FACS calibur (Becton Dickinson). At least 10,000 events were acquired from each sample and analyzed using CellQuest software (Beckton Dickinson).

Zymogram. Conditioned medium collected from TEX-treated or -untreated ULTR, GIST-T1 cell lines, and primary myometrial cells cultured in serum-free medium (SFM) were differentially ultracentrifugated to deplete exosomes and other microvesicles. The obtained "vesicle-free" conditioned medium was concentrated (~10-fold) with a centricon concentrator YM10K (Amicon). We mixed 5 or 10 μg of concentrated medium protein with nonreducing 2× sample buffer, and they were run on a 10% SDS/PAGE containing 0.1% gelatin. After electrophoresis, gels were renatured with 2.5% Triton X-100 for 1 h and then incubated overnight in developing buffer (50 mM Tris·HCl, pH 7.6, 5 mM $CaCl_2$, and 1 μM $ZnCl_2$) at 37 °C. The next day, gels were stained and destained in 0.3% Coomassie Blue R250 in 40% methanol and 7.5% acetic acid for 2–3 h. Gelatinolytic activity was detected as clear bands in the background of blue staining.

Quantitative RT-PCR. Total cellular RNA was purified using TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. After quantitation using Tecan Spectrophotometer, 1 μg of total RNA was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen). The cDNA (100 ng) was added to the real-time PCR mixture containing SYBR green (SSoAdvanced SYBR Green Supermix, Biorad) and the appropriate primers. The primers used were as follows: MMP1, forward 5′-TGTCACACCTCTGACATTCACCAA-3′ and reverse 5′-AAATGAGCATCCCCTCCAATACCT-3′; vimentin, forward 5′-TAGGGGCGCTCTTGTCCCCCA-3′ and reverse 5′-CCGCCGAACATCCTGCGGTA-3′; SMA, forward 5′-TGGCTTGGCTTGTCAGGGCTT-3′ and reverse 5′-CCCGGGGGCTGTTAGGACCTT-3′; CD34, forward 5′-AGAAAGGCTGGGCGAAGACCCT-3′ and reverse 5′-AGTGGGGAAGGGTTGGGCGT-3′; Anoctamine 1 (ANO1), 5′-CCAAGTTCCTGACCAGGCGCGG-3′ and reverse 5′-CCGATGTCCTCGATGGCGCAG-3′; MMP2, 5′-TGTCCAGAGGCAATGCAGTGGG-3′ and reverse 5′-ATCTGGGTTGCCGCAGCGTG-3′; endoglin (5), forward 5′-ACACGCGTGAGTACCCCAGG-3′ and reverse 5′-CTTCCCAGCGGGGAGGTGCT-3′; PAI-1, forward 5′-ACCGATTCGACCAGTTATTTGAC-3′ and reverse 5′-TCTTGTTCTCTGCTGCCTTCAG-3′; and β Actin, forward 5′-AGAAAATCTGGCACCACACC-3′ and reverse 5′-CAGAGGCGTACAGGGATAGC-3′, which was used as reference gene. All reactions were run in triplicate using a CFX96 Touch (Biorad), and relative expression was calculated by using the comparative threshold cycle (Ct) method.

Adhesion Assay. We coated 96-well culture plates with collagen-1 (10 μg/mL) and fibronectin (FN) (20 μg/mL) overnight at 4 °C. The next day, wells were washed with 1× PBS. Nonspecific binding sites were blocked with 3% BSA in SFM for 1 h at 37 °C. Untreated ULTR or cells treated with TEXs for 24 h were trypsinized and neutralized with soybean trypsin inhibitor, washed, and resuspended in SFM . Five thousand cells were seeded per well in triplicate in a well precoated with ECM proteins and incubated for 30 min at 37 °C. Bound cells were fixed with 3.7% paraformaldehyde and stained with 0.1% crystal violet. Number of adherent cells per four field per well were counted, and the mean number of adherent cells as well as SEM were calculated.

Viability Assay. Untreated ULTR cells or ULTR cells treated with 2.5, 5, or 10 μg of ULTR-derived exosomes (UEXs) or TEXs (8,000 cells per well) were cultured for 24 h in 96-well plates. Cell viability was assessed after 24 h by addition of the Cell Titer Blue Cell Viability Assay reagent (Promega). The number of viable cells was assessed by the determination of a fluorescent at 560 nm and 590 nm after 2 h using the fluorescent Infinite 200 PRO plate reader (Tecan US).

Measurement of MMP Levels. MMP1, MMP2, MMP7, MMP9, and MMP10 amounts were measured using the bead-based assay, Multiplex MAP Assay (Millipore), run on a Luminex ELISA platform according to the manufacturer's instructions. The SensoLyte Plus 520 MMP1 Assay Kit (Anaspec) was used to specifically detect endogenous activity of MMP1 according to the manufacturer's instructions.

Invasion Assay. The assay was performed using the modification of procedures previously described (6). We added 50 μL of type I collagen solution at 1 mg/mL to the upper compartment of each well in a 24-well transwell plate (8 μm pore size; Costar) and allowed them to gel overnight at 37 °C. GIST–T1 cells (2.5×10^5) in 100 μL of either serum-free DMEM or resuspended in exosome-free conditioned medium were derived from ULTR cells, ULTR cells treated with TEXs, or ULTR cells treated with GIST-patient– or healthy-donor–derived exosomes to the upper chamber. SFM was added to the lower compartments. In indicated experiments, inhibitors of MMP1, FN439 (Sigma), or recombinant MMP1 (Sigma) was added to the upper chamber at final concentrations of 5 μM and 30 nmol/mL, respectively. The invasion proceeded for 48 h at 37 °C. After incubation, the filters were fixed and stained with crystal violet. The cells that reached the underside of the filter were counted in five randomly chosen microscope fields and averaged. Three invasion chambers were used per condition.

siRNA Knockdown of MMP1. Cultured ULTR cells (2×10^5 cells per well) were transfected with 20 pmol human MMP1 siRNA (sc-41552), fluorescein conjugate transfection control siRNA–Glo (sc-36869), or negative control–siRNA (sc-37007), all obtained from Santa Cruz Biotechnology. After 24 h, transfected cells were serum-starved overnight and either left untreated or followed by TEX treatment (100 μg) for 24 h. Protein extracts from cells were then analyzed by Western blotting, and derived conditioned medium was used for invasion assay.

Proliferation Assay. ULTR cells (1×10^4 cells per well) were plated in each well of a 96-well plate and incubated for 24 h in SFM. The cells were then either left untreated or treated with 5 μg of TEXs or UEXs. Cell proliferation was measured using Promega's CellTiter 96 Aqueous One Solution cell proliferation assay according to the manufacturer's protocol (Promega). This assay is based on the cellular conversion of a tetrazolium salt into a soluble formazon product as a measure of proliferation. After 24, 48, and 72 h of incubation with exosomes, the Aqueous One Solution reagent was added and incubated for 2 h. The intensity of the color was measured at 490 nm using a Tecan plate reader.

Immunofluorescent Staining of Tissue Sections. Paraffin-embedded GIST tumors were obtained from University of Kansas Cancer Center's CCSG Biospecimen Repository Core Facility. Tissue

sections were deparaffinised in xylene, rehydrated through a series of ethanol baths. Immunofluorescent staining was performed after antigen retrieval by heating in citrate buffer and blocking steps using anti-MMP1 (Santa Cruz), anti-SMA (Santa Cruz), and anti-KIT (Cell Signaling) antibodies overnight at 4 °C. The slides were washed and incubated with Alexa Fluor 488– or 555–labeled secondary antibodies (Invitrogen, Life Technologies) for 1 h at room temperature. The slides were washed and mounted using Vectashield mounting medium (Vector Labs) and visualized under fluorescent microscope.

1. Taguchi T, et al. (2002) Conventional and molecular cytogenetic characterization of a new human cell line, GIST-T1, established from gastrointestinal stromal tumor. *Lab Invest* 82(5):663–665.
2. Zaragoza DB, Wilson RR, Mitchell BF, Olson DM (2006) The interleukin 1beta-induced expression of human prostaglandin F2alpha receptor messenger RNA in human myometrial-derived ULTR cells requires the transcription factor, NFkappaB. *Biol Reprod* 75(5):697–704.
3. Fitzgerald JB, Chennathukuzhi V, Koohestani F, Nowak RA, Christenson LK (2012) Role of microRNA-21 and programmed cell death 4 in the pathogenesis of human uterine leiomyomas. *Fertil Steril* 98(3):726–734 e722.
4. Gupta A, et al. (2010) Autophagy inhibition and antimalarials promote cell death in gastrointestinal stromal tumor (GIST). *Proc Natl Acad Sci USA* 107(32):14333–14338.
5. Matsui N, et al. (2008) Endosonographic detection of dumbbell-shaped jejunal GIST using double balloon enteroscopy. *Endoscopy* 40(Suppl 2):E38–E39.
6. Bracke ME, Boterberg T, Bruyneel EA, Mareel MM (2001) Collagen invasion assay. *Methods Mol Med* 58:81–89.

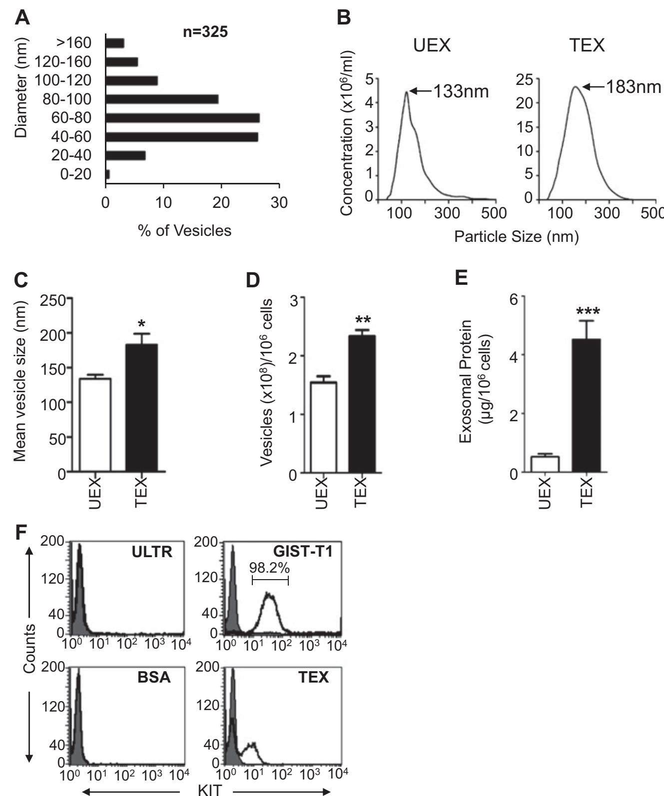

Fig. S1. GIST–T1 cells constitutively secrete exosomes. (*A*) Quantitative size distribution analysis of TEXs from EM pictures shows a size ranging from 50 to 160 nm. Data from 10 independent electron micrograph field quantifications are represented ($n = 325$ individual vesicles counted). (*B*) Nanosight size distribution of purified UEXs and TEXs. (*C*) Quantitative analysis of the mean vesicle size of exosomes secreted by ULTR and GIST–T1 cells. (*D*) Number of total vesicles released by each cell line using Nanosight. (*E*) Total protein content for UEX and TEX isolates ($n = 19$). Data are presented as means ± SEM. *$P = 0.0121$; **$P = 0.0022$; ***$P < 0.0001$(t test). (*F*) Flow cytometry analysis of surface KIT (nonshaded curve) or isotype control (shaded curve) on GIST–T1 cells, ULTR cells, and exosomal surface of TEXs. BSA was used as a negative control.

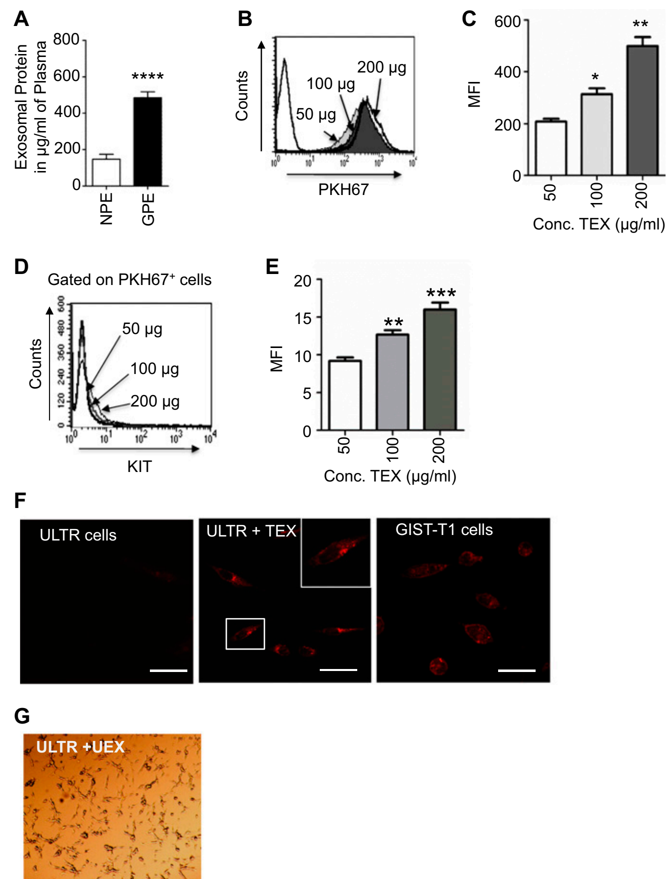

Fig. S2. ULTR cells undergo morphological changes after acquisition of oncogenic KIT via uptake of TEXs. (*A*) Total protein content in exosomes purified from plasma of GIST patients (486.2 ± 32.87 µg, n = 12) and healthy donors (147.1 ± 26.89 µg, n = 10) measured by BCA assay. ****$P < 0.0001$ (student t test). (*B*) Analysis of dose dependency of PKH67–labeled TEX uptake by ULTR cells using flow cytometry (gray shade) compared with control untreated cells (white curve). (*C*) Analysis of the mean fluorescence intensity (MFI) obtained in *B*, indicative of the number of vesicles internalized by ULTR cells (n = 3). Analysis of the percentage of ULTR cells expressing KIT at the cellular surface (*D*) and MFI (*E*) after TEX uptake by flow cytometry. Statistical values were calculated using an unpaired, two-tailed t test. *$P = 0.014$; **$P < 0.01$; ***$P < 0.001$. Error bars indicate the mean ± SEM (*F*) KIT expression in untreated ULTR, TEX-treated ULTR, and GIST–T1 cells detected by immunofluorescence. (Scale bar, 10 µm.) (*G*) Morphology of 24-h UEX-treated ULTR cells, as evaluated by phase contrast microscopy.

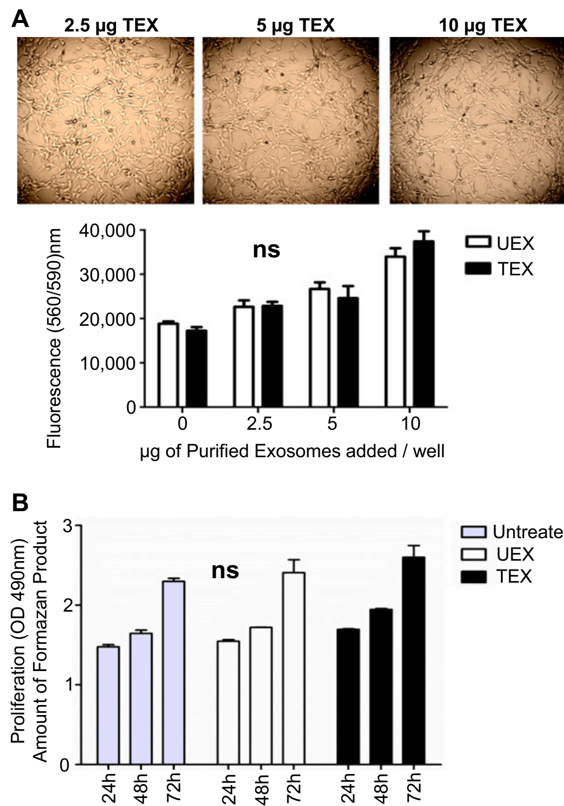

Fig. S3. Exosomes derived from ULTR and GIST–T1 cells have no significant effect on cellular viability or proliferation. (*A*) Effect of various amounts of exosomes purified from ULTR cell (UEX) or GIST–T1 cell (TEX) conditioned medium on ULTR cells' viability. ULTR cells were left untreated or cultured for 24 h with 2.5, 5, or 10 μg of UEXs or TEXs in SFM in 96-well plates. Cellular morphology (*A*, *Upper*) was assessed by phase contrast microscopy, and cell viability (*A*, *Lower*) was estimated by using the Cell Titer Blue reagent. Data quantification represents calculated fluorescence relative to untreated cells obtained for triplicate experiments. No statistically significant changes were observed. (*B*) Kinetic analysis of the impact of UEXs or TEXs on ULTR cell proliferation. ULTR cells were left untreated or cultured for 24 h with 10 μg of UEXs or TEXs in SFM in 96-well plates. Cell proliferation was estimated by using the CellTiter 96 Aqueous One Solution. Data quantification represents calculated optical density measured at 490 nm obtained for triplicate experiments. No statistically significant changes were observed (ns).

Fig. S4. TEX uptake by smooth muscle cells enhances adhesion to ECM components in an integrin-independent mechanism. (*A* and *B*) Phase contrast microscopy pictures of adhesive abilities of untreated ULTR cells and TEX-treated ULTR cells to (*A*) FN-1– (20 μg/mL) and (*B*) type I collagen– (10 μg/mL) coated wells. In the represented experiments, adhesion was conducted for 30 min ($n = 3$). (*C*) Down-regulation of FN-1 and up-regulation of intercellular adhesion molecule–1 (ICAM-1) protein expression in TEX-treated ULTR cells, as assessed by immunoblotting. (*D*) Immunoblot analysis of various integrin chains before and after TEX treatment in ULTR cells. (*E*) Characterization of cellular and exosomal proteins derived from human primary myometrial cells by immunoblotting of pKIT/KIT and exosomal markers, such as CD9 and flotilin. GIST–T1 cells were used as positive control, and β actin was used as a loading control. (*F*) Immunoblot analysis of pERK1/2$^{Thr202/Tyr204}$, pAKT^{S473}, and pAKT^{T308} levels in primary myometrial cells (Myo1, 2, and 3) treated with 300 μg/mL TEX. β actin was used as a loading control in all cases.

Fig. S5. ULTR cells display a unique transcriptomic profile after TEX uptake. (*A*) BioAnalyzer profiles representing the quality and size distribution of total RNA extracted from GIST–T1 cells (lane 1), ULTR cells (lane 2), ULTR cells treated with TEX for 24 h (lane 3), and purified TEX (lane 4). Note that ribosomal RNA 18S and 28S were only detectable in the cellular preparation, and specific enrichment of small RNA was observed in TEX and TEX-treated ULTR cells. (*B*) Corresponding electropherogram profiles for samples analyzed in *A*. ULTR cells treated with TEX display an enrichment in small RNA (black ovals) as shown by the size distribution compared with untreated GIST–T1 and ULTR cells.

Fig. S6. TEX enhances secretion of active MMPs in primary and ULTR smooth muscle cells. (*A*) Analysis of Interstitial Cell of Cajal (ICC) and tumor-associated markers in primary myometrial cells cocultured with 300 μg/mL of TEX. β actin was used as a loading control. (*B*) Endogenous expression levels of ICC- and tumor-associated transcripts in purified TEX by qRT-PCR; data are expressed as fold change ± SEM of each target transcript relative to β actin as endogenous control (n = 3 separates isolates). (*C*) Enhancement of TEX-induced MMP activity in the culture medium of TEX-treated ULTR cells as measured by gelatin zymography. (*D*) Gelatin–gel zymography analysis of basal and induced MMP activities in the conditioned medium of untreated ULTR cells (ULTR) and CM of ULTR cells after TEX treatment for 24 h (ULTR + TEX). Exosome-associated MMP activities were visualized using purified TEX. (*E*) Comparative basal and induced MMP activity in primary myometrial cell–derived conditioned medium before and after TEX treatment compared with untreated ULTR cells. Levels of MMP1 (*F*), MMP2, and MMP9 (*G*) released in the conditioned medium of untreated primary myometrial cells before and after treatment for 24 h with 300 μg of TEX by Luminex ELISA. Statistical values were calculated using an unpaired, two-tailed t test. *$P = 0.017$; **$P < 0.001$; ****$P < 0.0001$. Error bars indicate the means ± SEM. ND, not detected.

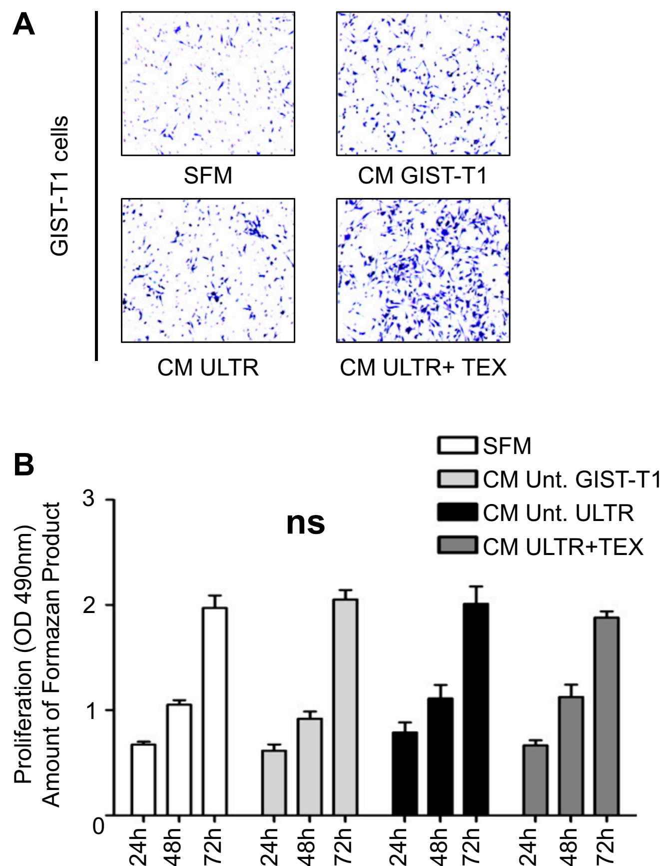

Fig. S7. TEX-challenged ULTR-cell-derived conditioned medium enhances GIST–T1 cell invasion without affecting cellular proliferation. (*A*) Microphotographs of representative collagen type I invasion assay of GIST–T1 cells in the presence of SFM, CM GIST–T1, CM ULTR, or CM ULTR + TEX after 48 h (original magnification, 20×). (*B*) Kinetic analysis of the impact of various conditioned medium on GIST–T1 cell proliferation. GIST–T1 cells were cultured in SFM for 24, 48, and 72 h or with 100 μL of 24 h CM from untreated GIST–T1 cells, ULTR cells, or ULTR + TEX in 96-well plates. Cell proliferation was estimated by using the CellTiter 96 Aqueous One Solution. Data quantification represents calculated optical density measured at 490 nm obtained for triplicate experiments. No statistically significant changes were observed (ns).

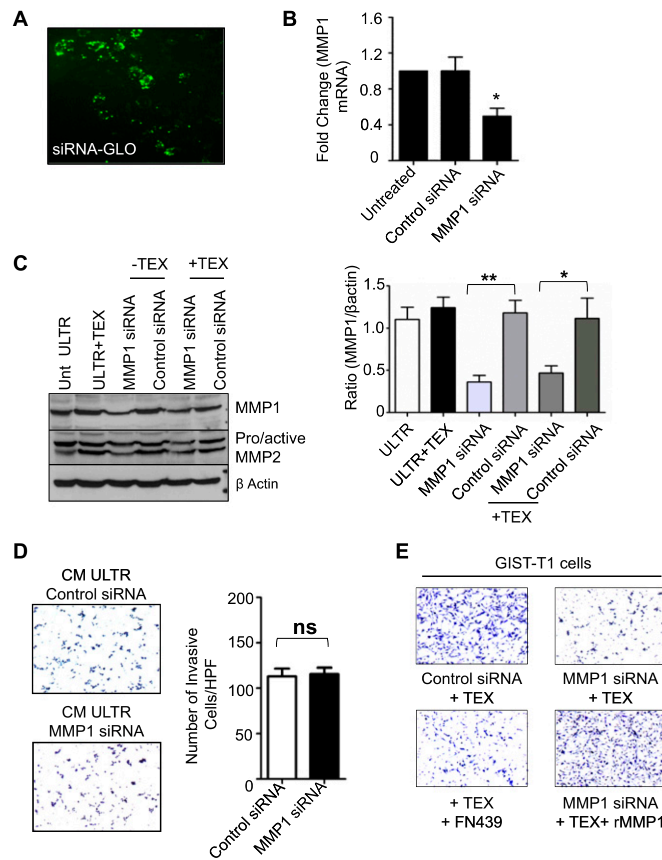

Fig. S8. Efficient knockdown of MMP1 in ULTR cells alters active MMP2 levels. (*A*) Uptake of FITC fluorescent-labeled siRNA (siRNA–Glo) was assayed at 48 h posttransfection in ULTR cells (20× objective). (Scale bar, 10 μm.) (*B*) Quantitative RT-PCR analysis of MMP1 transcript levels in ULTR cells 48 h posttransfection with transfection reagent only (untreated), nontargeting siRNA (control siRNA), or MMP1-targeting siRNA (MMP1 siRNA). The results are expressed as fold changes ± SEM of MMP1 transcript relative to untreated cells. Unpaired, two-tailed *t* test. *$P = 0.047$. Error bars indicate the mean ± SEM. (*C*) MMP1 and MMP2 protein levels are decreased after MMP1 siRNA treatment of ULTR cells. Left panel represents immunoblot analysis of MMP1 and MMP2 proteins in untreated ULTR cells, ULTR cells treated with TEX for 24 h, ULTR cells treated only with control or MMP1 siRNA, and ULTR cells treated with control or MMP1-targeting siRNA followed by TEX challenge. Equal loading of each lane was determined using β actin expression. Right panel represents quantification by densitometric analysis of the ratio MMP1/β actin in untreated ULTR cells, ULTR cells treated for 24 h with 100 μg TEX (ULTR + TEX), ULTR cells transfected with MMP1 siRNA followed by TEX treatment (MMP1 siRNA + TEX), and ULTR cells transfected with nontargeting control siRNA followed by TEX treatment (control siRNA + TEX). Unpaired, two-tailed *t* test. *$P = 0.043$; **$P = 0.0079$. Error bars indicate the means ± SEM. (*D*, *Left*) Representative photomicrographs of invading GIST–T1 cells after 48 h in the presence of cell-free conditioned medium derived from ULTR cells transfected with 20 pmol of control siRNA or MMP1 siRNA placed in the upper compartment of an invasion chamber with a membrane coated with type I collagen. (*D*, *Right*) Quantification of the *Left* panel, represented as mean number of invasive cells per field; the data represent experiments done in triplicate. NS, no significant difference was observed between the two treatment groups (student *t* test). (*E*) siRNA silencing or chemical inhibition of MMP1 in ULTR cells before TEX challenge inhibits invasion of GIST–T1 cells in a collagen I invasion system. Control siRNA–transfected ULTR cells treated with TEX (control siRNA + TEX), MMP1 siRNA–transfected ULTR cells treated with TEX (MMP1 siRNA + TEX), ULTR cells treated with TEX followed by the addition of MMP1 inhibitor FN439 (5 μM) (CM ULTR + TEX + FN439), or MMP1 siRNA–transfected ULTR cells treated with TEX, followed by 30 ng of recombinant MMP1 (MMP1 siRNA+ TEX + rMMP1).

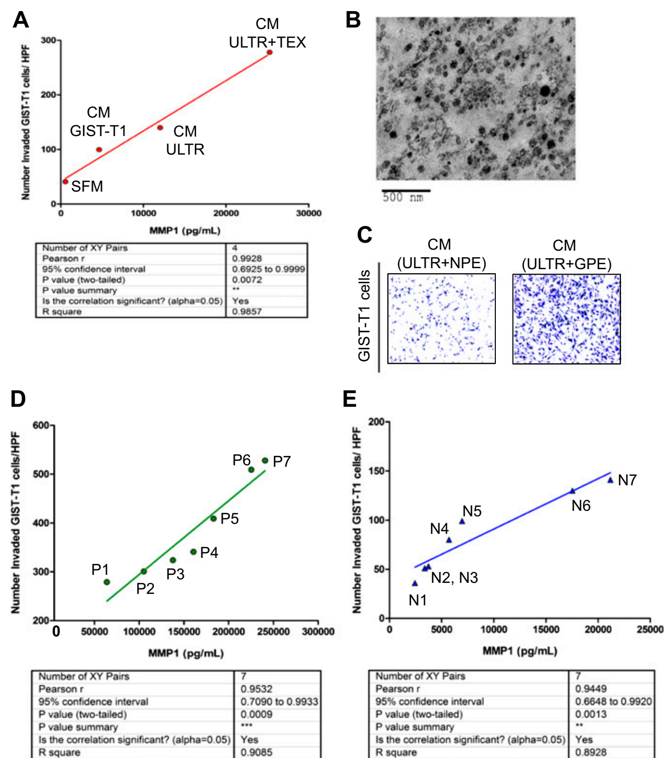

Fig. S9. Linear correlation between MMP1 levels and tumor cell invasion suggests possible direct and indirect effects of exosome-induced MMP1 in invasion. (*A*) Positive correlation between the levels of active MMP1 expression in the SFM as well as conditioned medium of GIST–T1 cells (CM GIST–T1), ULTR cells (CM ULTR), ULTR cells treated with TEX for 24 h (CM ULTR + TEX), and GIST–T1 cell invasiveness ($r = 0.9857$, $P = 0.0072$). (*B*) Representative electron microphotograph of GIST plasma-derived exosomes. (*C*) CM from ULTR cells cultured with GPE exosomes ($n = 7$ each) enhances invasion on type I collagen of GIST–T1 cells. Pearson's correlation values obtained for MMP1 expression and GIST–T1 cell invasiveness after incubation with conditioned medium collected from ULTR cells treated for 24 h with (*D*) GIST-patient–derived exosomes [$r = 0.9085$, $P = 0.0009$, $n = 7$ (P1–7)] or (*E*) healthy-patient–derived exosomes [$r = 0.8928$, $P = 0.0013$, $n = 7$ (N1–7)].

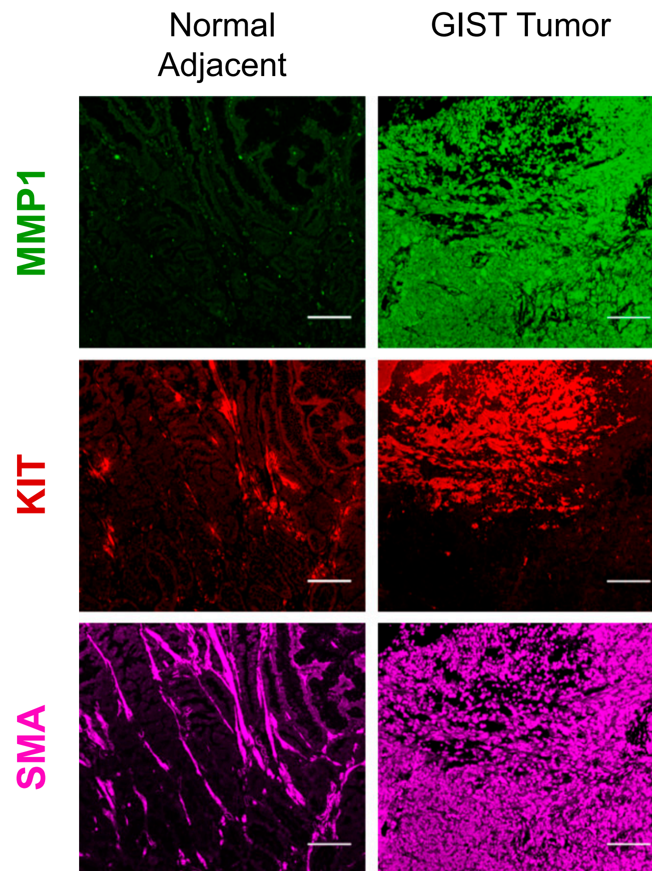

Fig. S10. Expression of MMP1 (green), CD117 (KIT, red), and SMA (magenta) in adjacent normal gastric transverse cross-sections (n = 4) and GIST-patient–derived tumors (n = 5) by immunofluorescence. Normal adjacent gastric tissue from GIST patients lacks detectable MMP1 expression. Control experiments used no primary antibodies. (Scale bar, 100 μm.) GIST-tumor–associated stroma produce enhanced levels of MMP1. The tumors cells were visualized by KIT staining. (Scale bar, 100 μm.)