Tumor-extrinsic discoidin domain receptor 1 promotes mammary tumor growth by regulating adipose stromal interleukin 6 production in mice

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Running Title: Host DDR1 promotes tumor growth through IL-6

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ABSTRACT
Discoidin domain receptor 1 (DDR1) is a collagen receptor that mediates cell communications with the extracellular matrix (ECM). Aberrant expression and activity of DDR1 in tumor cells are known to promote tumor growth. Although elevated DDR1 levels in stroma of breast tumors are associated with poor patient outcome, a causal role for tumor-extrinsic DDR1 in cancer promotion remains unclear, however. Here, we report that murine mammary tumor cells transplanted to syngeneic recipient mice in which Ddr1 has been knocked out (KO) grow less robustly than in WT mice. We also found that the tumor-associated stroma in Ddr1-KO mice exhibits reduced collagen deposition compared with the WT controls, supporting a role for stromal DDR1 in ECM remodeling of the tumor microenvironment. Furthermore, the stromal-vascular fraction (SVF) of Ddr1-KO adipose tissue, which contains committed adipose stem/progenitor cells and preadipocytes, was impaired in its ability to stimulate tumor cell migration and invasion. Cytokine array–based screening identified interleukin 6 (IL-6) as a cytokine secreted by the SVF in a DDR1-dependent manner. SVF-produced IL-6 is important for SVF-stimulated tumor cell invasion in vitro, and using antibody-based neutralization, we show that tumor promotion by IL-6 in vivo requires DDR1. In conclusion, our work demonstrates a previously unrecognized function of DDR1 in promoting tumor growth.

INTRODUCTION
Excessive adiposity has been linked to increased breast cancer recurrence and mortality in both pre- and post-menopausal women1–6. The underlying mechanisms of adiposity-associated cancer burden are likely multifactorial, including elevated production of hormones, cytokines, reactive oxygen species, and extracellular matrix (ECM). Obesity is also associated with altered adipose tissue homeostasis and metabolic reprogramming. These changes in adipose tissue could collectively impact tumor progression through both systemic and paracrine mechanisms. Given the abundance of tumor-surrounding adipocytes in breast tissue, communication between tumor and mature adipocytes has naturally been the primary focus of mechanistic studies of obesity-related cancer burden7–9. However, it is increasingly evident that, in addition to mature adipocytes, altered abundance and properties of human adipose stem/stromal cells (ASCs) and elevated fibrosis contribute to tissue remodeling associated with tumor-associated adipose tissue2, 10–12. For example, we and others previously showed that human ASCs are a significant source of local estrogens that stimulate ERα+ breast tumor growth11, 13–15.

Discoidin Domain Receptor 1 (DDR1) is a cell-surface tyrosine kinase receptor that binds to, and is activated by, collagens16–19. DDR1 is predominantly expressed in normal epithelial cells and its aberrant expression is associated with multiple solid cancer types. For these reasons, the current literature on DDR1 function in cancer biology has exclusively been focused on its activity in tumor cells20–22. However, comparative gene expression profiling shows that stromal DDR1 expression in invasive breast cancer is significantly elevated versus normal breast stroma23 (6.4-fold, p= 1x10^-15), suggesting a possible DDR1 function in stromal cells during cancer progression. In support, we previously reported a DDR1-dependent signaling pathway that regulates adipose production of estrogens in human ASC cultured in vitro15. Furthermore, we found that the DDR1 function in human ASCs is not shared by other collagen receptors including integrins or DDR215, indicating a
uniquely important role of DDR1 in regulating endocrine/paracrine ASC functions. Despite these lines of emerging evidence, there lacks any in vivo evidence that definitively establishes a causal relationship between stromal DDR1 and cancer progression.

In the current work, we utilized a Ddr1 knockout (KO) mouse model and syngeneic mouse mammary tumor cells to examine the role of host DDR1 in mammary tumor progression. To complement in vivo tumor studies, we assessed in vitro the tumor cell-promoting ability of the stromal-vascular fraction (SVF) of mouse adipose tissue, which is enriched with multipotent stem/progenitor cells and functionally similar to human ASCs. We conducted comprehensive cytokine profiling to identify the adipose stroma-secreted cytokine IL-6 as an important mediator of stromal DDR1 function in tumor pathogenesis. For the first time, our data provide compelling mechanistic insight into the role of stromal DDR1 in breast tumor growth in vivo.

RESULTS
Genetic ablation of host Ddr1 blunts mammary tumor growth

To interrogate the role of host DDR1 in cancer progression, we used a previously established Ddr1 whole-body knockout (KO) mouse model on the C57BL/6 genetic background. We first confirmed DDR1 protein expression in WT mouse SVF and its depletion in the counterpart from homozygous KO mice (lanes 2 and 3, Figure 1a). The two Ddr1-dependent protein bands in WT mouse stroma are likely DDR1 isoforms due to alternative splicing. As a positive control for DDR1, we used primary ASCs isolated from human breast tissue (lane 1, Figure 1a). For the in vivo tumor study, we orthotopically injected two syngeneic murine mammary tumor cell lines, AT-3 and M-Wnt, into 8-10 weeks old female Ddr1 WT or homozygous KO recipient mice. To avoid potential animal cage-based variation, pairs of WT and KO mice from the same litter were used in tumor and cell culture experiments throughout our study. No significant body weight difference was observed between the WT and KO cohorts at the time of tumor study (data not shown).

The AT-3 cell line was derived from an MMTV-PyMT transgenic mouse mammary tumor, whereas M-Wnt was established from an MMTV-Wnt-1 transgenic mouse mammary tumor. We chose these two tumor cell lines because both are syngeneic with C57BL/6 mice and have been used as models for triple negative breast cancer. Tumor sizes were assessed by caliper measurement over a period of 4-7 weeks, and tumors were weighed upon harvest. In both AT-3 (Figure 1b-d) and M-Wnt (Figure 1e-g) syngeneic tumor models, tumors grew more robustly in WT mice versus Ddr1 KO counterparts. Because DDR1 in tumor cells is also known to promote tumor progression, we examined DDR1 expression in tumors from WT and Ddr1 KO hosts. DDR1 protein levels in KO hosts were not lower than those in WT counterparts (Supplementary Figure 1a), thus further supporting a tumor-extrinsic activity of host DDR1 in promoting tumor growth in vivo. Based on Ki67 and phospho-histone H3 staining, we did not find any significant difference in tumor cell proliferation between the WT and KO cohorts (Supplementary Figure 1b-c). However, tumors from Ddr1 KO hosts displayed elevated apoptosis as measured by TUNEL (Supplementary Figure 2a). The same tumors from KO hosts also expressed less Ctnnb1 (beta-catenin) and Cdh2 (N-cadherin) but more Cdh1 (E-cadherin) versus those tumors in WT hosts (Supplementary Figure 2b), suggesting reduced epithelial-mesenchymal transition (EMT) for tumors in Ddr1 KO hosts.

Host DDR1-dependent ECM remodeling in the tumor microenvironment
Under various physiopathological conditions such as hypertensive nephropathy, collagen-triggered DDR1 activation is known to induce an inflammatory response, which in turn leads to excessive collagen synthesis and exaggerated fibrosis. To determine whether a similar DDR1-dependent positive feedback loop occurred in the mammary tumor microenvironment, we first conducted intratumoral collagen histochemistry with picrosirius red (PSR) on M-Wnt tumors harvested from Ddr1 WT and KO mice. Intratumoral PSR staining intensity was significantly reduced in tumors from Ddr1 KO mice versus their WT counterparts (Figure 2a-b), consistent with reduced collagen in Ddr1 KO. In further support, Col1a1 mRNA levels were markedly dampened in M-Wnt tumors in Ddr1 KO mice (Figure 2c). Expression of Col3a1 and α-smooth muscle actin (α-SMA), another hallmark for accumulation of tumor-associated stromal cells, followed the same trend, albeit not statistically significant (Figure 2c). Taken together, our findings support the notion that host DDR1 contributes to matrix remodeling in the tumor microenvironment.

**Mouse SVF stimulates tumor cell migration and invasion in a stromal DDR1-dependent manner**

Because we previously showed that DDR1 is important for the paracrine action of primary human ASC cultured in vitro, we asked whether host mouse DDR1 played a similar role in the current animal models. Upon isolation of SVF from WT and Ddr1 KO mice, we first verified DDR1 depletion in KO-SVF by immunoblotting (Figure 3a). MTT assay indicated no appreciable difference in cell proliferation between WT- and KO-SVF populations (Figure 3b). Using a Boyden chamber-based co-culture system, we found that medium conditioned by WT-SVF significantly stimulated both migration (Supplementary Figure 3) and invasion (Figure 3c-d) of both M-Wnt and AT-3 murine mammary tumor cells. In stark contrast, medium conditioned with Ddr1 KO-SVF had substantially impaired ability to stimulate tumor cell migration (Supplementary Figure 1) and invasion (Figure 3c-d). Consistent with the in vivo finding of elevated collagen deposition in tumors from WT host versus Ddr1 KO host (Figure 2), medium conditioned with WT-SVF stimulated Col1a1 mRNA expression in tumor cells to a greater extent than that conditioned with KO-SVF (Figure 3e). Thus the in vitro system with SVF-conditioned medium recapitulates the observed DDR1 effect on the tumor microenvironment in the syngeneic mouse tumor models.

To determine the durability of the SVF effect on tumor cells, M-Wnt and AT-3 tumor cells were retrieved from the exposure to SVF-conditioned medium and assessed alone for their invasive behaviors in the absence of the conditioned medium. As shown in Supplementary Figure 4a-b, tumor cells that had been exposed to medium conditioned with WT-SVF retained the more aggressive invasive behavior versus their counterparts exposed to either medium alone or medium conditioned with KO-SVF. Of note, neither WT nor KO-SVF-conditioned medium had any effect on proliferation of M-Wnt or AT-3 tumor cells (Supplementary Figure 4c-d). Thus, our findings clearly indicate that DDR1 in mouse SVF confers to tumor cells a prolonged invasive phenotype.

**Stromal DDR1 promotes tumor cell invasion by regulating SVF-secreted IL-6**

To identify the DDR1-dependent, SVF-secreted factor(s) that promote tumor cell invasion, we conducted a cytokine screen using a commercial array consisting of 111 cytokines and chemokines. SVF-conditioned medium from two independent pairs of WT and Ddr1 KO mice
was used for probing the cytokine/chemokine array. Levels of several cancer-related, SVF-secreted factors were reduced in conditioned medium from KO-SVF versus WT-SVF, including IL-6, IL-11, CCL17, and VEGF (Figure 4a, data not shown). Because our previously published work showed that human ASC-secreted IL-6 contributes to the tumor-promoting action of ASC, we chose to focus on the functionality of the differential levels of IL-6 between mouse WT- and KO-SVF. We first used RT-qPCR (Figure 4b) and ELISA (Figure 4c) to confirm reduced IL-6 mRNA and protein levels, respectively, in KO-SVF versus WT-SVF in multiple independent pairs of WT and KO samples. Consistent with our published result in human ASCs, mRNA and protein expression of Cofilin, an upstream regulator of IL-6 production in human stromal compartment, was also substantially reduced in mouse KO-SVF as compared to WT-SVF (Figure 4d-f).

To determine whether DDR1-dependent IL-6 secretion contributes to SVF-promoted tumor cell invasion, we used an IL6-neutralizing antibody to reduce IL-6 activity in WT-conditioned medium. Pre-treatment with the anti-IL-6 antibody obliterated the difference in invasion stimulation between WT and KO-SVF (Figure 5a; compare columns 1-2 versus 3-4 in Figure 5b), clearly indicating that IL-6 is an important mediator of the DDR1-dependent effect on tumor cell invasion. In a reciprocal experiment, addition of recombinant IL-6 reduced the difference in stimulation of tumor invasion between WT- and KO-SVF from 1.7 to 1.4 fold (Figure 5c; compare column 1 with 2, and 3 with 4 in Figure 5d). Because exogenous IL-6 did not completely eliminate the difference between WT- and KO-SVF, we infer from this result that additional DDR1-regulated, SVF-secreted factors besides IL-6 likely play roles in mediating host DDR1 signaling.

**IL-6 stimulation of mammary tumor growth in vivo is host DDR1-dependent**

To interrogate the *in vivo* functional relationship between DDR1 and IL-6 in cancer progression, we systemically administered the anti-IL-6 neutralizing antibody in M-Wnt-bearing WT and *Ddr1* KO mice. Tumor sizes were monitored by caliper in a 5-week period following tumor transplantation (Figure 6a) and tumors were weighed upon harvest at the end point (Figure 6b-c). Consistent with the *in vitro* co-culture findings (Figure 5a-b), IL-6 neutralization in WT mice significantly mitigated tumor growth (compare columns 1 and 3 in Figure 6c). Notably, the same antibody treatment in *Ddr1* KO mice did not lead to any further reduction in tumor growth (compare columns 2 and 4 in Figure 6c). Taken together with the findings from the above *in vitro* cytokine experiments, these *in vivo* results strongly suggest that host DDR1 is an important upstream regulator of IL-6 production and its tumor-promoting function.

**DISCUSSION**

To date, published studies of DDR1 in cancer have been limited to its action in tumor cells. Using genetically engineered mice and syngeneic tumor models, we demonstrate the importance of host DDR1 in tumor growth, thus significantly extending the current understanding of DDR1 tumor-promoting function. We further show that DDR1 in adipose tissue-derived SVF is a previously unappreciated regulator of stromal IL-6 secretion that affects tumor cell migration and invasion. Notably, we provide compelling *in vivo* evidence for a functional link between host DDR1 and the tumor-promoting activity of IL-6. Given the cell surface localization of DDR1 and its innate tyrosine kinase activity, pharmacologic abrogation of host DDR1 function in tumor abrogation could mitigate cancer burden.
Emerging evidence indicates that adipose tissue-derived stem/progenitor cells are a significant source of stroma-secreted factors that have profound impact on tissue regeneration as well as pathogenesis including in cancer\textsuperscript{2, 35, 36}. Obesity is a well-known factor associated with poor prognosis for multiple cancer types including breast cancer. Furthermore, obesity-associated fibrosis is an increasingly recognized hallmark of adipose dysfunction that is tightly associated with other adiposity-related changes such as inflammation\textsuperscript{37}. As a known collagen receptor, DDR1 is a key player in a collagen-initiated positive feedback loop that ultimately results in excessive ECM accumulation in various non-cancer disease models\textsuperscript{19}. Our in vitro and in vivo data suggest that the same positive feedback loop most likely also manifests in the mammary tumor microenvironment. In this regard, DDR1-dependent secretion of various inflammatory factors, including those detected in our cytokine assay, could serve dual functions: they promote tumor growth and at the same time exacerbate obesity-associated ECM remodeling and inflammation. We propose that stromal DDR1 is part of a signaling network that links ECM, stromal cells, and tumor cells in the same tumor microenvironment (model, Figure 6d). While our current work used mice receiving normal (not high fat) diet for studying primary tumor growth, it will be important to investigate the role of host DDR1 in other aspects of cancer biology including obesity-associated tumor progression and metastasis.

Tumor-promoting functions of IL-6 in both tumor and host cells have been well documented as important in inflammation and breast cancer\textsuperscript{33, 34, 38-45}. In further support, high circulating IL-6 levels are associated with poor prognosis in breast cancer patients\textsuperscript{46}. While our in vitro co-culture experiments focused on IL-6 secretion by adipose SVF, multiple cell types including tumor and other stromal cells most likely contribute to IL-6 levels in the tumor microenvironment and in circulation. However, it is also worth noting that genetic ablation of host Ddr1 in our syngeneic mouse models completely eliminates the effect of IL-6 neutralizing antibody on tumor growth. Furthermore, neither AT-3 nor M-Wnt mammary tumor cells express appreciable amount of IL-6 (Supplementary Figure 5). We therefore favor the possibility that stromal IL-6 predominantly contributes to the tumor-promoting activity of IL-6 observed in our study, and that this adipose stromal pool of IL-6 is under the tight control of SVF DDR1. In support, it was reported previously that mouse preadipocytes express significantly higher levels of IL-6 than mature adipocytes\textsuperscript{37}.

Our work does not exclude the possible involvement of other DDR1-dependent, SVF-secreted factors besides IL-6 that could also contribute to tumor promotion. Consistent with this possibility, Ddr1 KO-conditioned medium still exhibited lower invasion-promoting activity than WT-conditioned medium even in the presence of an excessive amount of recombinant IL-6 (Figure 5c-d; 10 ng/ml recombinant IL-6 versus 0.7 and 0.2 ng/ml for endogenous IL-6 in WT- and KO-SVF, respectively). Further, with IL-6 neutralizing antibody, tumor growth in Ddr1 KO mice was still slower versus WT counterparts. While this difference could be due to incomplete IL-6 neutralization in vivo, an alternative explanation is that host DDR1 regulates expression of additional tumor-promoting factors besides IL-6. Potential candidates for future investigation include those cytokines and chemokines that displayed differential levels between WT and Ddr1 KO-derived SVF. While the current whole-body Ddr1 KO animal model allows us to distinguish DDR1 in tumor versus host cells, DDR1 from multiple host cell and
tissue types besides adipose tissue-derived stromal cells could also influence tumor growth. There is currently no suitable Cre-lox system in vivo to create adipose SVF-specific gene KO in mice. More sophisticated tissue-specific mouse models could shed light on this question.

The current study of syngeneic mouse tumor models significantly extends our previously published work using cultured primary human ASCs. While human samples bear obviously more clinical relevance, the use of genetically engineered mouse models circumvents individual-based variation associated with clinical cohorts. Furthermore, compared to xenograft tumor models, syngeneic tumor models used in our current work ensure an immune-competent host environment that more faithfully recapitulates the tumor microenvironment in humans. It is satisfying that both lines of complementary investigation in mouse and human systems clearly point to an unequivocal role of a conserved DDR1-dependent signaling pathway that dictates production of a tumor-promoting secretome in breast cancer.

METHODS AND MATERIALS

Primary cells isolation and culture

Primary stromal vascular fraction (SVF) was isolated from mouse inguinal fat pad using standard procedures. Briefly, harvested fat tissue was digested for 15-18 hours on rotating shaker at 37 °C, using DMEM/F12 (Stem Cell Technologies, Cat. #36254) supplemented with 10% gentle collagenase/hyaluronidase (Stem Cell Technologies, Cat.#07912) and 1% penicillin/streptomycin (P/S) plus 2% fetal bovine serum (FBS). Debris was removed by a 70 μm cell strainer (BD biosciences, Cat. #352350), followed by centrifugation at 100 g, for 5 min at 4 °C. Red blood cells were removed by lysis using ammonium chloride solution (Stem Cell Technologies, Cat. #07800) at room temperature for 5 min, followed by centrifugation at 600 g for 5 min at 4 °C. Cell pellets were washed and resuspended in SVF culture medium (DMEM/F12 supplemented 10% FBS and 1% P/S).

In vivo tumor study and IL-6 antibody neutralization

Ddr1+/− mice on the pure C57BL/6 background were bred to generate Ddr1+/+ (WT) and Ddr1−/− (KO) litters, which were used in all in vitro and in vivo experiments. Murine mammary tumor cells (M-Wnt and AT-3) were propagated up to 70% confluency, harvested by trypsinization (0.05% trypsin-EDTA), washed and resuspended in PBS. Cells were injected in the mouse mammary gland fat pad, using 1X10⁵ cells/100 μl for M-Wnt and 2x10⁵ cells/100 μl for AT-3 per injection. Tumor volume was measured by caliper at indicated time points, and tumor growth for 4 to 7 weeks. Tumor weight was measured at the time of termination. In vivo IL-6 neutralization was by intraperitoneal injection of IL-6 neutralizing antibody (BD Biosciences, clone MP5-20F3, Cat. #554398) or isotype control anti-IgG (BD Biosciences, clone R3-34, Cat. #554682) at 100 μg per mouse every 3 days starting 2 days before tumor inoculation. All animal experiments were performed after obtaining approval from the University of Texas Health San Antonio (UTHSA) Institutional Animal Care and Use Committee (IACUC). All methods were carried out in accordance with the IACUC-approved guidelines.

Picosirius Red staining

Tumors harvested from mice were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin, and sectioned at 3 μm thickness. The picrosirius red staining was performed according as described by the manufacturer using 0.2 % phosphomolybdic acid (Electron Microscopy Sciences, Cat. #RT-26357-01), Sirius red,
0.1% in saturated picric acid (Electron Microscopy Sciences, Cat. #RT-26357-02) and 0.01 N hydrochloric acid (Electron Microscopy Sciences, Cat. #RT-26357-03). The intensity of positive staining was estimated by measuring the optical density, O.D. = log [I_B / I_O], where I_B is average intensity in the background, and I_O is the average intensity of the stained area. The percent positive staining was calculated by taking the ratio of area of pixels stained to the total area of pixels in the background. A minimum of 10 fields per tumor section were measured and averaged by Image J.

**Cell migration and invasion assay**

Tumor cells grown to 80% confluency were harvested by trypsinization, washed with PBS twice, resuspended in culture medium without FBS, and co-cultured with but physically separated from, either Ddr1 WT-SVF or KO-SVF in a Transwell system. Briefly, 5x10^4 tumor cells were seeded in the top chamber. The bottom chamber was filled with medium with 1% FBS alone or medium with 1% FBS plus 3X10^4 WT-SVF or KO-SVF. After 12 hours of co-culture at 37°C, unmigrated cells on the upper side of the insert were gently removed by a cotton swab. Migrated cells on the undersurface of the inserts were stained by crystal violet staining. 6 fields/insert were counted under an optical microscope.

For cell invasion assay, the inserts were overlaid with ice-cold Matrigel Basement membrane matrix-Growth Factor Reduced (Corning, Cat. #354483) at 5 mg/ml, with 50 μl Matrigel/well. Matrigel was allowed to settle for 30 min at 37°C. 5X10^4 tumor cells were seeded on the top chamber and either medium alone or SVF were seeded in the bottom chamber. After 20 hours, invaded cells were stained by crystal violet and counted by optical microscopy as described above in the migration assay. Invasion assay in Figure 5 was conducted using 10 ng/ml recombinant mouse IL-6 (R&D, Cat. #406-ML-005) supplementation or 1 μg/ml anti-IL6 neutralizing antibody (R&D, Cat. #MAB406) in the bottom chamber with either WT- or KO-SVF. For the experiment shown in Supplementary Figure 2a-b, tumor cells were removed from the top chamber of the inserts (0.4 μm pore; Millipore, Cat. #MCHT12H48) after 3-day incubation with either medium alone or SVF seeded in the bottom chamber. Tumor cells were then examined in the Boyden chamber assay for cell invasion with 10% FBS-containing medium in the bottom chamber, without SVF-conditioned medium.

**RT-qPCR**

RNA samples were reverse-transcribed using the ImProm-II Reverse Transcription System (Promega, Cat. #A3800). Real time PCR was set up using the Luminaris Color High Green High ROX qPCR Master Mix (Thermo Fisher Scientific, Cat. #K0364), and run in an Applied Biosystems 7900HT workstation installed with SDS 2.4 software. All primers used for the RT-PCR were designed using Primer-3 software (Sigma Aldrich). The primer sequences are as follows.

Col1a1-F: GCTCCTCTTAGGGGCCACT; Col1a1-R: ATGGGGGACCCTTAGGCCAT; Col3a1-F: CTGTAACATGGAACTGGGGAAA; Col3a1-R: CCATAGCTGACTGAAAACCACC; α-SMA-F: CCCAGACATCAGGGAGTAATGG; α-SMA-R: TCTATCGGATACTTCAGCGTCA; IL6-F: TCTATACCACTTCACAAGTGC; IL6-R: GAATTGCCATTGCACAACTCTTT; Cofilin-F: ATGACATGAAGGTTCGCAAGT; Cofilin-R: GAATTGCCATTGCACAACTCTTT; Ctnnb1-F: ATGGAGCCGGACAGAAAAGC; Ctnnb1-R: TGGGAGGTGTCAACATCTTTT;
Cdh2-F: AGGCTTCTGGTGAAATTGCAT; Cdh2-R: GTCCACCTTGAAATCTGCTGG; Cdh1-F: CAGTTCCGAGGTCTACACCTT; Cdh1-R: TGAATCGGGAGTCTTCCGAAAA.

**Western Blotting**

Protein lysates were prepared in Laemmli sample buffer and protein amount was estimated using the Pierce BCA Protein Assay Kit (Pierce, Cat. #23225). For DDR-1 Western blot, samples were run on SDS-PAGE and transferred to an H-bond nitrocellulose membrane using standard procedures. Membrane was blocked using 5% BSA and immunoblotted with anti-DDR-1 (D1G6) XP rabbit mAb (Cell signaling, Cat. #5583), GAPDH (14C10) Rabbit mAb (Cell signaling, Cat. #2118), Cofilin mouse mAb (Santa Cruz, Cat. #SC-53934), and α-Tubulin mouse mAb (Calbiochem, Cat. #CP06). Protein detection was done using Pierce™ ECL Plus Western Blotting Substrate (Pierce, Cat. #32132).

**Cytokine array/ELISA assay**

A membrane-based antibody array was used to detect the secretome of Ddr1 WT- and KO-SVF. To reduce the baseline levels of growth factors and cytokines in FBS, SVF was cultured in 1% FBS (heat inactivated) containing medium for 2 days. Cell-free supernatant was collected and used in the Proteome Profiler Mouse XL Cytokine Array Kit (R&D System, Cat. #ARY028) following the manufacturer’s instructions. For ELISA, SVF was incubated in 1% FBS (heat inactivated) medium for 2 days. Concentrations of IL-6 in the WT- and KO-SVF cell supernatant or AT-3/M-Wnt tumor cell supernatant were detected utilizing mouse IL-6 ELISA kit (Invitrogen, Cat. #KMC0061) according to the kit instructions.

**MTT proliferation assay**

1X10^3 cells were seeded into a 96-well plate and incubated for 1-4 days. 50 µl MTT solution (3 mg/ml) were added to the culture medium at the indicated time point and incubated at 37°C for 1 hour until purple precipitate became visible. Medium was removed without disturbing the purple precipitate. 100 µl DMSO were added into each well and mixed homogeneously and the absorbance were recorded at 570 nm. For the experiment shown in Supplementary Figure 2c-d, tumor cells were trypsinized from the top chamber of the inserts (0.4 µm pore; Millipore, Cat. #MCHT12H48) after 3 days of co-culture with either medium alone or SVF seeded in the bottom chamber. Tumor cells were seeded into a 96-well plate and incubated for 2 days to monitor the proliferation rate as described above.

**Immunohistochemistry/TUNEL assay**

Tumors were harvested from specified host mice and fixed with 10% neutral-buffered formalin (Fisher Scientific, Cat. #23245685) overnight at 4°C. Paraffin-embedded tumors were cut into 3 µm sections for staining. Slides were deparaffinized and rehydrated by 100% xylene and graded ethanol (100%, 95%, 70%, 50%). The slides were then boiled with antigen-unmasking solution, and washed with PBS. After pretreated with 3% hydrogen peroxide for 10 min, the slides were subjected to immunostaining with anti-Ki67 (Thermo Fisher Scientific, Cat. #MA5-14520; 1:100) and anti-phospho-Histone H3 (Cell Signaling Technology, Cat. 9701; 1:200). After 2 hours of incubation with primary antibody, the ABC Peroxidase Detection System (Vector Lab, Cat. #PK-6105) were used with 3, 3’-diaminobenzidine as substrate (Vector Lab, Cat. #SK-4105) to detect the primary antibody. Enumeration of Ki67 and p-H3 positive cells were done using an image analysis software (ImmunoRatio, http://153.1.200.58:8080/immunoratio/; Institute of Biomedical Technology, University of Tampere, Tampere, Finland)
For TUNEL assay, slides were deparaffinized by graded ethanol (100%, 90%, 70%, 60%) and PBS wash, and subsequently permeabilized by 0.2% Triton X-100 wash for 5 mins. Nick-end DNA fragmentations were labeled with the DeadEnd Fluorometric TUNEL System Kit (Promega; Cat. #G3250) according to manufacturer’s instructions. Slides were washed with PBS and mounted by Vectashield mounting medium with DAPI (Vector Labs, Cat. #H-1200). TUNEL-positive cells were visualized by fluorescence microscopy and quantified by Image J software.

**Statistical methods**
Mean comparison of two groups was assessed by two-tailed Student's *t*-test. Mean differences of multiple groups were examined by one-way ANOVA followed by multiple comparison tests. Two-Way ANOVA analysis was used in tumor growth curve analysis. In all assays, *p*<0.05 was considered statistically significant.

**AUTHOR CONTRIBUTIONS**
RL and YH conceived and supervised the project. RL, XS and KG designed the experiments. XS, KG, BW, DZ, BY, XZ, HC and CZ performed the experiments. XS, KG, MB, SH, TC and BW analyzed the data. RL, YH, TC, XS, KG and BW wrote the manuscript.

**CONFLICT OF INTEREST STATEMENT**
The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Figure 1. Host DDR1 promotes mammary tumor growth in syngeneic mouse models. (a) Immunoblot assessing DDR1 protein expression in primary human ASCs, WT mouse ASCs, and littermate KO ASCs. (b-d) Tumor volume (b), size (c) and weight (d) of AT-3 mammary tumor cells in Ddr1 WT (n=7) and KO (n=8) mice. (e-g) Tumor volume (e), size (f) and weight (g) of M-Wnt mammary tumor cells in Ddr1 WT (n=7) and KO (n=5) mice. Scale bar, 1cm. Data are represented as mean ± SD, *p<0.05; **p<0.01; ***p<0.001; ns: not significant.

Figure 2. Host DDR1 affects ECM remodeling in the tumor microenvironment. (a) PSR staining of tumor (M-Wnt) from Ddr1 WT and KO mice. (b) Quantification of PSR staining. (c) RT-PCR of ECM markers in tumor (M-Wnt) from Ddr1 WT and KO mice. Scale bar, 50 µm. Data are represented as mean ± SD, *p<0.05; **p<0.01; ***p<0.001. The same numbers of mice as shown in Figure 1 were used here.

Figure 3. Stromal DDR1 promotes tumor cell migration and invasion. (a) DDR1 protein expression in Ddr1 WT- and KO-SVF, GAPDH is the loading control. (b) MTT assay assessing proliferation of primary SVF from Ddr1 KO and littermate WT controls. (c) Representative images of invasion of M-Wnt (upper panel) and AT-3 (lower panel) after 20 hours of co-culture with medium alone or WT/KO-SVF. (d) Quantification of invaded cells by cell counting. (e) RT-qPCR is used to assess mRNA levels of Collal in M-Wnt or AT-3 cells following co-culture with medium alone or WT/KO SVF. Scale bar, 50 µm. Data represent mean ± SD of four littermate WT/KO pairs. *p<0.05; **p<0.01; ***p<0.001.

Figure 4. Identification of DDR1-dependent cytokines in SVF. (a) Representative images of membrane-based antibody array using cell-free supernatant from WT- and KO-SVF. The boxed dots are a reference protein (blue) and IL-6 (red). (b) mRNA levels of IL-6 in WT/KO-SVF by RT-qPCR. (c) Measurement of secreted IL-6 in cell-free supernatant from WT/KO-SVF by ELISA. (d) mRNA levels of Cofilin in WT/KO-SVF by RT-qPCR. (e) Protein levels of DDR1 and Cofilin in three pairs of WT/KO SVF. GAPDH is a loading control. (f) Quantification of Cofilin protein level normalized by GAPDH. All mRNA analyses and ELISA were done with four pairs of WT and KO. Data represent mean ± SD. *p<0.05; **p<0.01; ***p<0.001.

Figure 5. DDR1-dependent stromal IL-6 secretion is important for SVF to promote tumor cell invasion. (a) Representative images of M-Wnt cell invasion after 20 hours of co-culture with WT/KO-SVF, in the presence of anti-IL6 or anti-IgG (1 µg/ml). (b) Quantification of the cell invasion result in (a). (c) Representative images of M-Wnt cell invasion in the co-culture system in the presence or absence of recombinant IL-6 (10 ng/ml). (d) Quantification of the cell invasion result shown in (c). Data represent mean ± SD of four littermate WT/KO pairs. **p<0.01; ***p<0.001.

Figure 6. IL-6 stimulation of mammary tumor growth in vivo is host DDR1-dependent. (a) Growth curves of M-Wnt tumors in WT/KO host treated with either anti-IL6 or anti-IgG antibodies, each at 100 µg per mouse every 3 days starting 2 days before tumor inoculation. (b) Tumor images upon harvest. Scale bar, 1cm. (c) Tumor weight at the final time point. Data represent mean ± SD of 7 tumors from each group. (d) A model diagram showing the crosstalk between ECM, ASC and tumor cells. Data are represented as mean ± SD.*p<0.05; **p<0.01; ***p<0.001; ns: not significant.
Figure 1. Host DDR1 promotes mammary tumor growth in syngeneic mouse models

(a) Western blot analysis of DDR1 and α-Tubulin in AT-3 and M-Wnt cell lines.

(b) Growth curves showing tumor volume progression for AT-3 cells in WT and KO mice.

(c) Images of AT-3 tumors in WT and KO mice.

(d) Tumor weight comparison between WT and KO mice for AT-3 tumors.

(e) Growth curves showing tumor volume progression for M-Wnt cells in WT and KO mice.

(f) Images of M-Wnt tumors in WT and KO mice.

(g) Tumor weight comparison between WT and KO mice for M-Wnt tumors.
Figure 2. Host DDR1 affects ECM remodeling in tumor microenvironment

(a) WT and KO images showing ECM remodeling.

(b) % Positive PSR Staining

(c) mRNA Level

- Col1a1
- Col3a1
- α-SMA

WT vs. KO comparison with statistical significance indicated.
Figure 3. Stromal DDR1 promotes tumor cell invasion

a) Western blot showing DDR1 and GAPDH expression in WT and KO SVF.

b) Graph showing relative cell proliferation over days with DDR1 WT and DDR1 KO.

M-Wnt

Invaded Cells per Field

Medium alone
WT-SVF
KO-SVF

Col1a1 mRNA Level

Relative Cell Proliferation

Day

DDR1 WT
DDR1 KO

Invaded Cells per Field

SVF:
- WT KO

Col1a1 mRNA Level

SVF:
- WT KO

Col1a1 mRNA Level

SVF:
- WT KO

AT-3

Medium alone
WT-SVF
KO-SVF

Col1a1 mRNA Level

AT-3

Medium alone
WT-SVF
KO-SVF

Col1a1 mRNA Level

AT-3

Medium alone
WT-SVF
KO-SVF

Col1a1 mRNA Level

AT-3

Medium alone
WT-SVF
KO-SVF
Figure 4. Identification of DDR1-dependent cytokines in SVF

**a**

WT-SVF

KO-SVF

**b**

IL-6 mRNA Level

WT    KO

**c**

IL-6 Protein Level (pg/ml)

WT    KO

**d**

Cofilin mRNA Level

WT    KO

**e**

WT    KO

DDR1

Cofilin

GAPDH

**f**

Cofilin Protein Level

WT    KO

* * *
Figure 5. DDR1-dependent stromal IL-6 secretion is important for SVF to promote tumor cell invasion
Figure 6. IL-6 stimulation of mammary tumor growth in vivo is host DDR1-dependent.
Tumor-extrinsic discoidin domain receptor 1 promotes mammary tumor growth by regulating adipose stromal interleukin 6 production in mice

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