Supplementary Information

Breast Cancer Extracellular Matrix Invasion Depends on Local Mechanical Loading of the Collagen Network.

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Supplementary Results

Displacement of Embedded Magnetic Alginate Bead in Collagen Hydrogel

Magnetic alginate beads embedded in collagen hydrogels exhibited displacement when exposed to a static external magnetic field. A localized mechanical loading was induced on the collagen gel by the displacement of the magnetic alginate beads. This displacement was determined by the distance separating the magnetic alginate bead from the magnet, as well as the magnet size. The displacement of embedded magnetic alginate beads was highest near the "big" cubic 10 (L) x 10 (W) x 10 (H) mm neodymium magnets and lower near the "small" rectangular 13 (L) x 6 (W) x 3 (H) mm magnets. While bead displacement caused by the small magnet effectively plateaued by 72h, bead displacement caused by the big magnet did not plateau over 7 days in culture, though it slowed between days 5-7 (**Fig. S1F**).

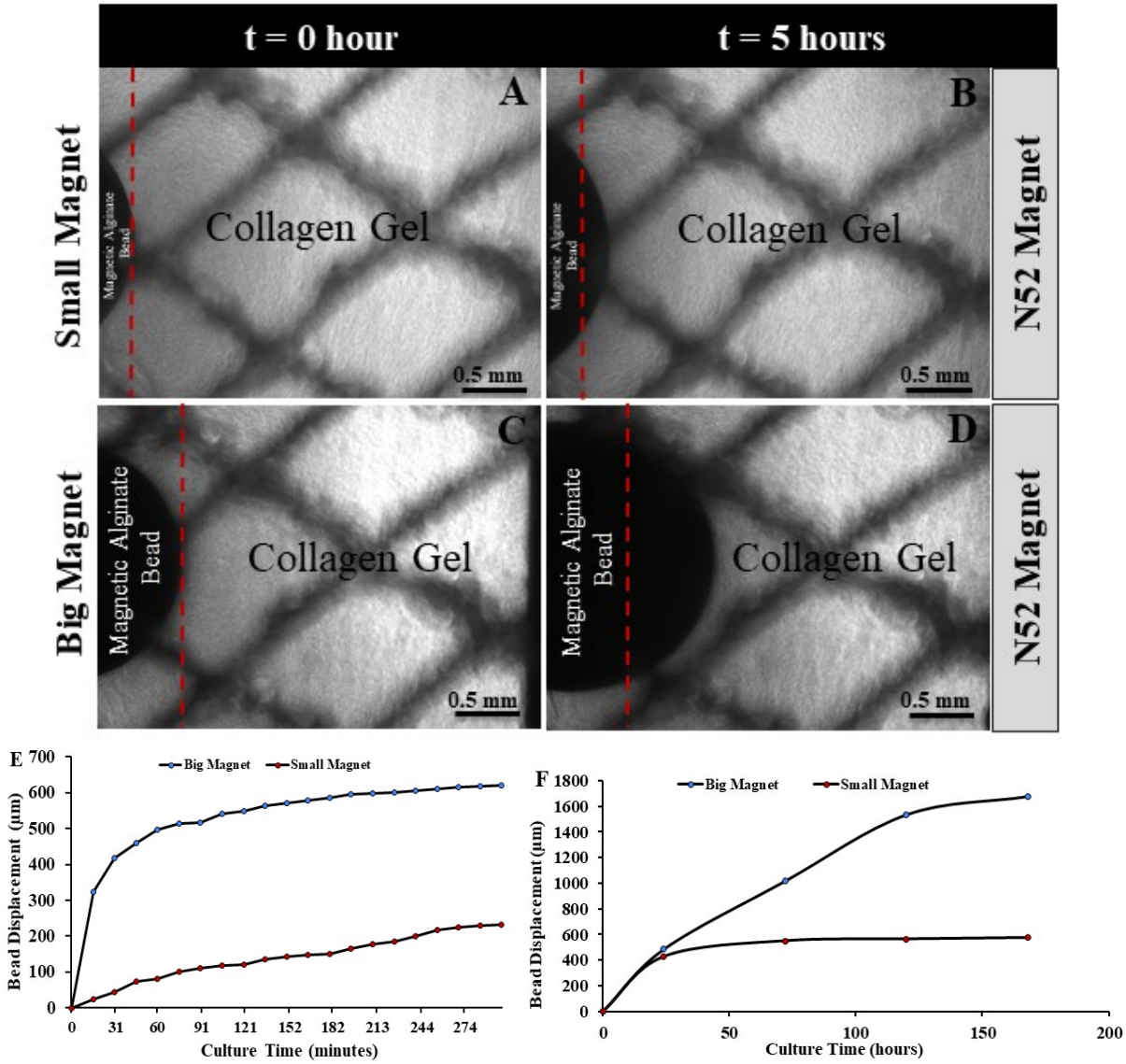

Figure S1. Phase contrast microscope images for embedded magnetic alginate bead in 1.5 mg/mL collagen gel demonstrating bead displacement for 5 hours of culture time **(A)** before exposure to a small static external magnetic field and **(B)** after 5 hours of magnetic field exposure **(C)** before exposure to a big static external magnetic field and **(D)** after 5 hours of magnetic field exposure **(E-F)** line graph, showing bead displacement in function of magnet size (big, small magnets) during 5 hours and 7 days of culture time (n=2 bead/ magnet size).

Directional Alignment and Morphology of MDA-MB-231 Cells in Magnetically Actuated Tissue Construct

Human breast carcinoma cells responded to *in situ* dynamic force loading by altering alignment and morphology. Alignment of MDA-MB-231 cells was apparent in phase contrast micrographs at 24h and 72h of culture when the beads were exposed to a stronger and weaker magnetic field associated with shorter (5 mm) and longer (13 mm) bead-magnet distances (**Fig. S2**). Elongated cells cultured in a collagen gel

near a magnetic alginate bead (MAB) without an external magnet were oriented in many directions with no clear preferential alignment. Cell aspect ratio (AR) depended on magnetic actuation condition (one-factor ANOVA, $F=13.8$, $p<0.001$, $n=30$ cells/group). The AR for cells in the compressed region adjacent to a bead actuated by a strong magnetic field and a weak magnetic field were 4.0±2.1 and 2.4±0.2, respectively (mean±standard deviation). The AR for cells adjacent to a bead not exposed to a magnetic field was 2.0±0.2 (**Fig. S2D**).

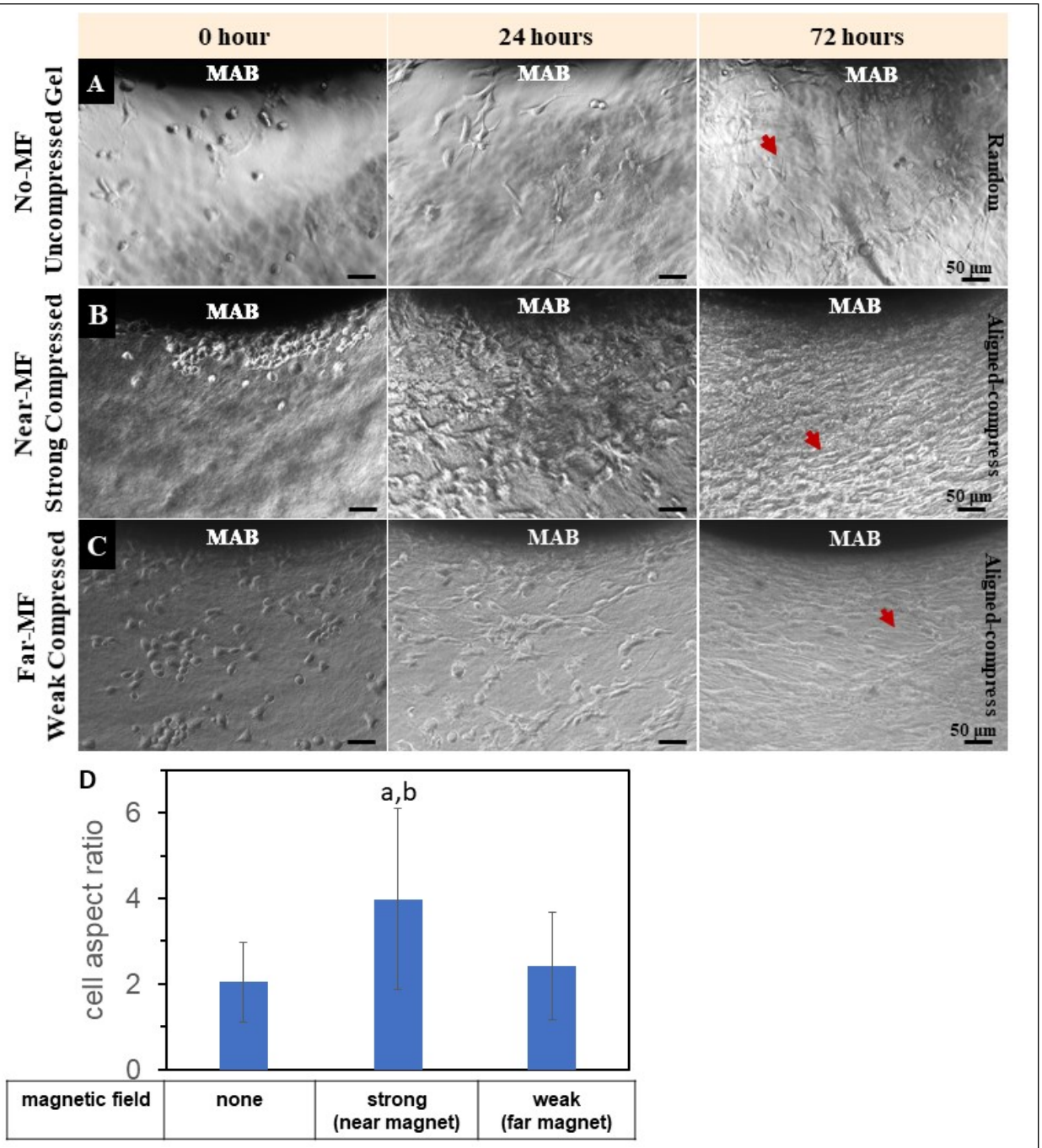

Figure S2. Phase contrast micrographs (20x objective magnification) of embedded MDA-MB-231 cells within a 2 mg/mL collagen hydrogel surrounding an embedded magnetic alginate bead illustrating cancer cell alignment and morphology over 72h of culture **(A)** without applying magnetic field with cells randomly rounded and non-aligned, **(B)** with a stronger magnetic force and more compressed gel induced by a close (~5 mm) magnet with cells elongated and aligned parallel to the bead surface and perpendicular to the direction of the gel compression, and **(C)** with a weaker magnetic force and less compressed gel induced by far (~13 mm) magnet with less pronounced cell elongation and alignment. Red arrows highlight cells equidistant from the bead surface with alignment and morphology characteristic of the condition. Scale bars are indicated. (D) Average cell aspect ratio under the three conditions (n=30 cells/group), quantified from confocal micrographs in Figure 6. a represents difference with control (none), Tukey test, $p<0.001$. b represents difference with weak magnet (far), Tukey test, $p<0.001$.

MDA-MB-231 Cells Proliferation in Response to Magnetic Actuation

Magnetic actuation of breast cancer cells in collagen and photocrosslinked gelatin hydrogels affected proliferation only when the magnetic field was removed and mechanical stress relaxed (**Figs. 7, S3**). **Fig. S3A** shows the horizontal loading condition in collagen constructs, which was followed by the XTT assay. **Fig. S3B** shows the vertical loading condition in photocrosslinked gelatin constructs, which was followed by the Alamar Blue assay. Steady magnetic field application and force loading over 5 days (steady compression) of MDA-MB-231 cells cultured with an embedded magnetic alginate bead did not affect the XTT signal vs. control constructs not exposed to an external magnet (**Fig. S3C**). First-time magnetic field application and force loading after 5 days (first time compression) of MDA-MB-231 cells cultured with an embedded magnetic alginate bead also did not affect the XTT signal vs. control constructs not exposed to an external magnet (**Fig. S3D**).

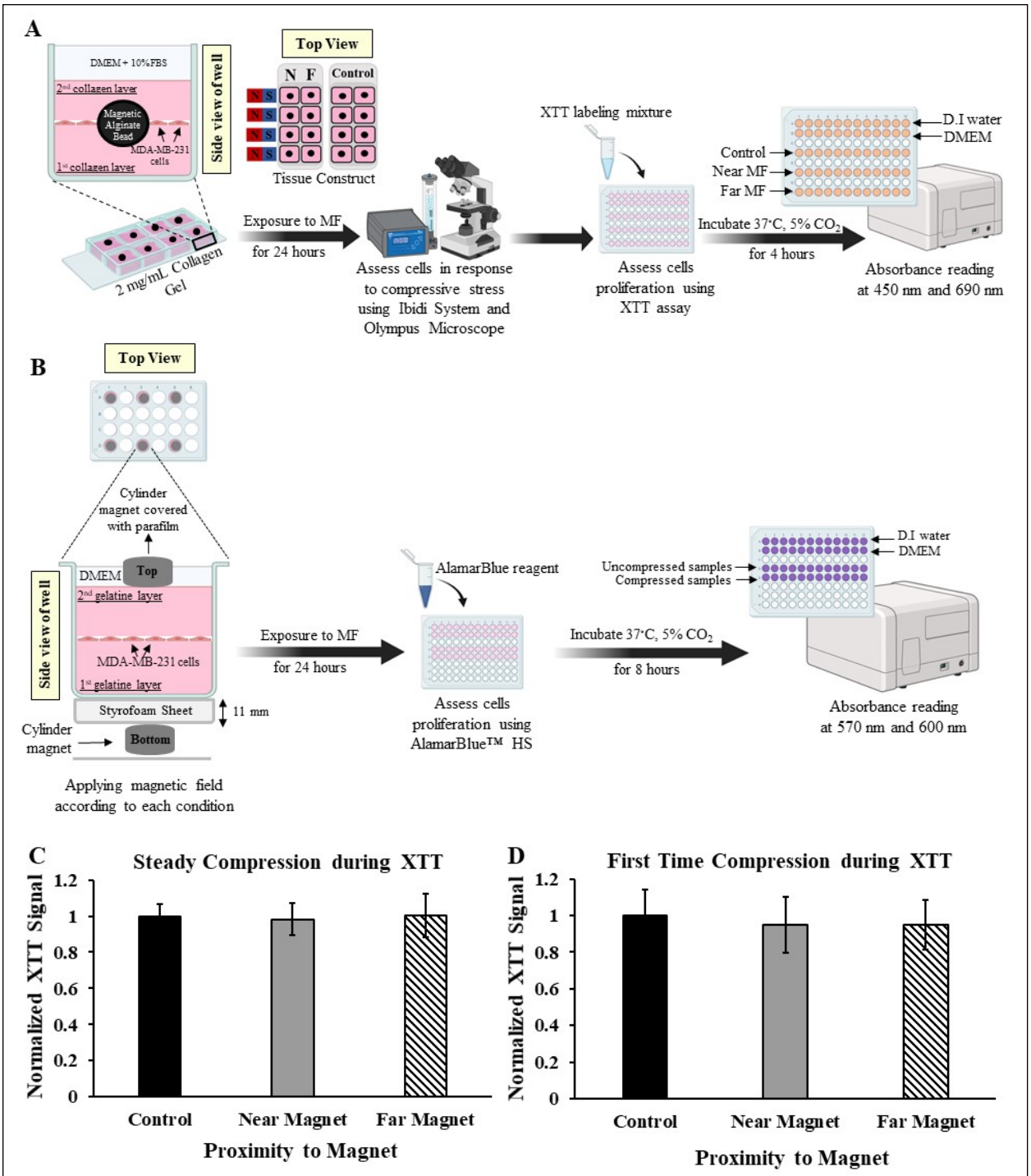

Figure S3. (A-B) Schematic representation of force-loaded constructs and proliferation assays. XTT and Almar Blue measurements were performed on collagen and photocrosslinked gelatin constructs, respectively, with a layer of MDA-MB-231 cells and embedded magnetic alginate beads. **(C,D)** Normalized XTT signal is not significantly impacted by continuous, steady force loading (steady compression) or by first time, rapid force loaing (first time compression) of the constructs due to placement of an external magnet (n=16 constructs/condition).

MDA-MB-231 Cells Invasion in Response to Magnetic Actuation

The pattern and direction of collagen gel invasion by MDA-MB-231 cells from the surface of magnetic alginate beads depended on the presence and timing of magnetic force loading and unloading. Specifically, occasional leader cells of invasive fronts into the collagen gel retracted and rounded up upon first loading of mechanical stress by addition of an external magnet at day 5 after invasion had begun (**Fig. S4A**, see movie **S7**). With an external magnet in place and force loading continuously during culture, MDA-MB-231 cells invaded on either side of the compressed region, with multiple leader cells aligned obliquely and outward from the bead surface (**Fig. S4B**, see movie **S8**). Sometimes long streams of cells extended from the bead surface from the magnet side of the bead but not in the area of maximum compression (**Fig. S5**). Upon removal of the magnet and force unloading after 5 days of continuous culture with a nearby magnet, invading cells remained elongated (**Fig. S4C**, see movie **S9**). Without any external magnetic force loading, invading MDA-MB-231 cells appeared rounder and invaded in dispersed groups (**Fig. S4D**, see movie **S6**).

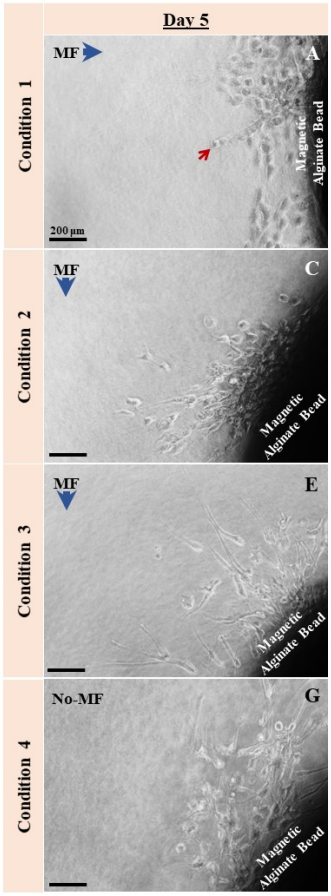

Figure S4. Phase contrast microscope images at 10x magnification, demonstrating MDA-MB-231 breast cancer cells invasion from magnetic alginate bead into surrounding of 2 mg/mL collagen hydrogel matrix under varied magnetic field exposure conditions using a static external magnet **(A)** condition 1, where a static external magnetic field was applied at day five of culture and continued until day six **(B)** condition 2, a static external magnetic field was applied at the first day of culture **(C)** condition 3, where a static external magnetic field was applied at the first day of culture, and then removed at day five, and **(D)** condition 4, without applying magnetic fields to the tissue construct. Red arrow points to a leader cell. Scale bars are indicated.

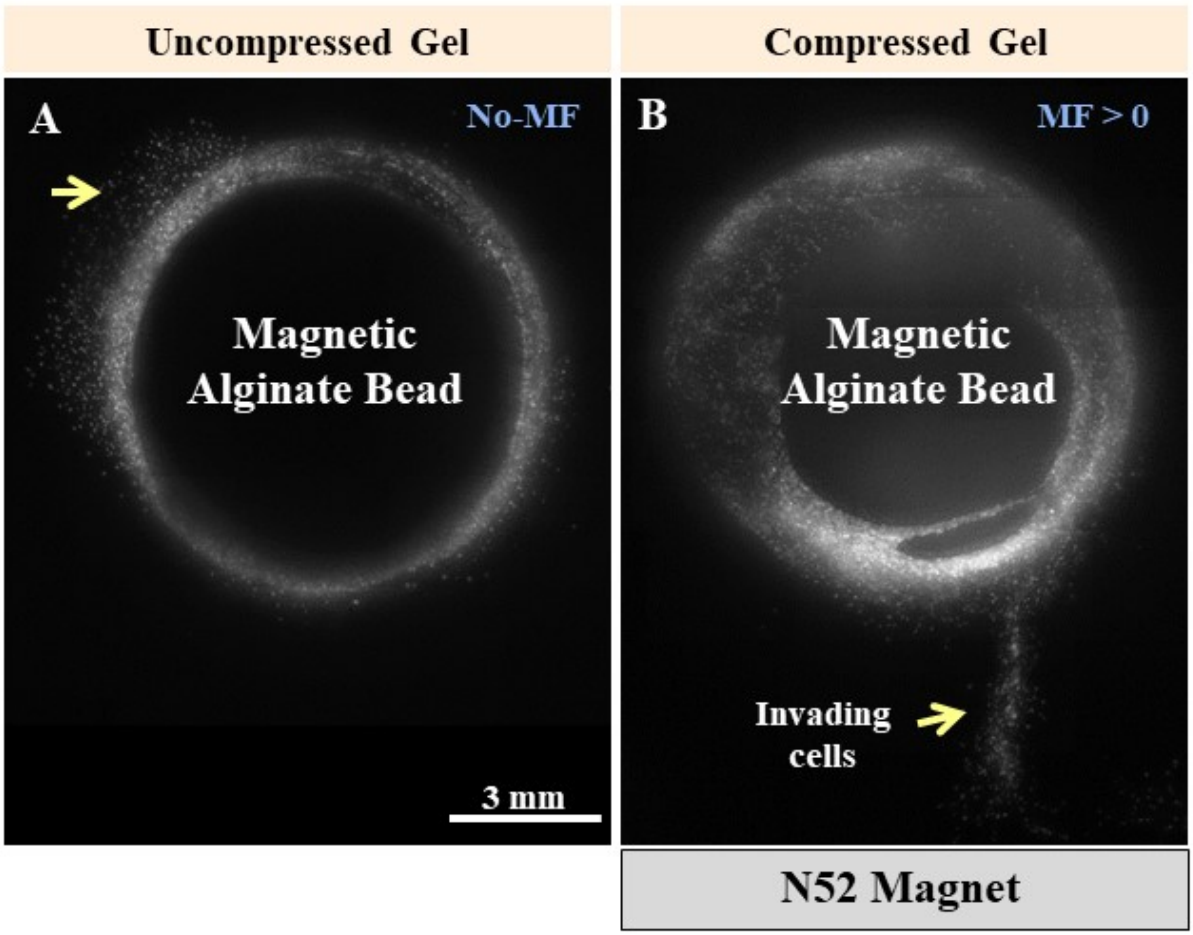

Figure S5. Stitched epifluorescence micrographs (4x objective magnification) of fluorescence signal from stained DNA in MDA-MB-231 cells nuclei, illustrating cancer cells invading into 2 mg/mL collagen hydrogels from a magnetic alginate bead after 7 days of culture **(A)** without exposure to an external magnetic field (control), and **(B)** with continuous exposure to a static external magnetic field (n=12 cell coated magnetic alginate beads/ group, representatives of each group are depicted). Yellow arrows point to the cells invading in the predominant direction. Scale bars are indicated.

Hydrogel Response to Magnetic Bead Actuation

Collagen hydrogels compressed in response to magnetic bead actuation in the gel by an external magnet (**Movies S10, S11**). A large external magnet (strong magnetic field) caused more bead displacement, and a 45% lower graylevel than baseline, of signal in the compressed hydrogel area (**Movie S11, Fig. S6**). In comparison, a smaller external magnet (weaker magnetic field) caused less bead displacement and a 12% lower graylevel than baseline, of signal in the compressed hydrogel area (**Movie S10, Fig. S6**). Videos were recorded over 5 hours.

Figure S6. Normalized graylevel of collagen hydrogels in the compressed region in front of magnetic alginate beads displaced by a stronger (big magnet, blue line), and a weaker (small magnet, orange line) external magnetic field. Graylevel was recorded from the same region of interest in co-registered timelapse phase contrast micrographs over 5 hours, and normalized the the graylevel of the first image in each dataset.

Movie S6: Time-lapse phase contrast videomicroscopy of MDA-MB-231 cells invading a collagen gel from the surface of a magnetic alginate bead, with no external magnetic force loading.

Movie S7: Time-lapse phase contrast videomicroscopy of MDA-MB-231 cells invading a collagen gel from the surface of a magnetic alginate bead, immediately after placement of an external magnet and initial force loading.

Movie S8: Time-lapse phase contrast videomicroscopy of MDA-MB-231 cells invading a collagen gel from the surface of a magnetic alginate bead, with continuous magnetic force loading from a magnet placed outside the culture chamber over 5 days of culture.

Movie S9: Time-lapse phase contrast videomicroscopy of MDA-MB-231 cells invading a collagen gel from the surface of a magnetic alginate bead, immediately after removal of an external magnet and initial force unloading.

Movie S10: Magnetic alginate bead displacement over 5 hours in response to placement of a small (234 mm^3) magnet external to the culture chamber. Frames collected in 5-minute intervals.

Movie S11: Magnetic alginate bead displacement over 5 hours in response to placement of a big (1000 mm^3) magnet external to the culture chamber. Frames collected in 1-minute intervals.