Supplementary Materials for

Clathrin-coated structures support 3D directed migration through local force transmission

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Other Supplementary Material for this manuscript includes the following:

Movies S1 to S3
Figure S1
Supplementary Figure 1. **Characterization of ligands association with collagen networks.**

a, Pre-polymerized, 3D collagen network was incubated for 30 min with 100 nM Alexa488-labelled EGF before to be imaged by spinning disk microscopy. Collagen fibers (left panel) were stained using an anti-collagen I antibody. Scale bar: 10 µm.

b, Decellularized mouse liver matrix was incubated for 30 min with 100 nM Alexa488-labelled EGF before to be imaged by spinning disk microscopy. Collagen fibers (left panel) were stained using an anti-collagen I antibody. Scale bar: 25 µm.

c, Alexa488-labelled (left panel), Dil-LDL (right panel)-decorated fibers produced as in Fig. 1a were spotted on a glass coverslip. Scale bar: 5 µm.

d, Images showing Alexa-488-labelled EGF on Alexa548-labelled fibers embedded in a supporting 3D collagen network and imaged by spinning disk microscopy 1h or 24 h after preparing the gel, as indicated. Scale bar: 10 µm.

e, f, FRAP experiments of Alexa488-EGF on collagen fibers produced as in Fig. 1a and spotted on a glass coverslip (e) or embedded in a 3D network (f) with or without adding 100nM Alexa488-EGF (free EGF) in the medium 30 min before the beginning of the experiment, as indicated.
Figure S2
Supplementary Figure 2. **EGF on collagen fibers is functional.** a, MDA-MB-231 cells were serum-starved for 6h before to be trypsinized and incubated in suspension at 37°C with soluble 30nM Alexa488-EGF or with non-decorated (Naked) fibers or with Alexa488-EGF-decorated fibers for 5 or 30 min, as indicated. Cells were then harvested at 4°C and subjected to Western blot analysis using the indicated antibodies. Actin was used as a loading control. Control cells were incubated in suspension at 37°C for 5 min in the absence of fibers or EGF. b, Densitometry analyses of bands depicted in a when using the anti-phospho-Erk antibody. Controls were set to 1. c, MDA-MB-231 cells were serum-starved for 2h before to be trypsinized and incubated at 37°C with Alexa-548-labeled, Alexa488-EGF-decorated fibers produced as in Fig. 1a for the indicated time periods. Cells were then fixed and stained with DAPI before to be imaged by spinning disk microscopy. Scale bar: 5 µm. d, Quantification of the evolution in time of the average EGF-associated fluorescence on fibers (dark circles) and in cells (open circles) as in c, as indicated. Results are expressed as mean ± SD.
Figure S3
Supplementary Figure 3. **Gefitinib treatment does not prevent EGFR recruitment at CCSs.**

**a**, Quantification of the average α-adaptin staining fluorescence intensity on HB-EGF-decorated fibers as compared to non-decorated fibers in MDA-MB-231 cells. Data are expressed as the mean percentage ± SD fluorescence normalized to fluorescence associated with non-decorated fibers (*P<0.01, Student’s t-test. N=3). **b**, Alexa488-labelled, EGF-decorated fibers (green) and Alexa548-labelled, non-decorated fibers (Naked, blue) were sequentially spotted on a glass coverslip and MRC5 cells were allowed to spread on this composite network for 15 min before to be fixed and stained for α-adaptin (red). Scale bar: 10 μm. **c**, Quantification of the enrichment of average α-adaptin staining fluorescence intensity on Alexa488EGF-, or DiL-LDL-decorated fibers (as indicated) as compared to non-decorated fibers in MRC5 cells as in b and treated with the indicated siRNA. Data are expressed as the mean percentage ± SD over average fluorescence on non-decorated fibers (*P<0.01, One Way Analysis of Variance – ANOVA. N=3). **d**, Quantification of the average α-adaptin staining fluorescence intensity on Alexa488EGF-, or DiL-LDL-decorated fibers (as indicated) as compared to non-decorated fibers in cancer-associated fibroblasts (CAFs) or osteoblasts, as indicated. Data are expressed as the mean percentage ± SD fluorescence normalized to fluorescence associated with non-decorated fibers (*P<0.01, Student’s t-test. N=3). **e**, Quantification of the AP-2 fluorescence ratio on EGF-decorated versus non-decorated fibers in MDA-MB-231 cells treated or not with soluble EGF as indicated. A ratio=1 indicates no preferential accumulation of AP-2 fluorescence on EGF-decorated fibers (*P<0.01, Student’s t-test. N=3). **f**, MDA-MB-231 cells were serum-starved for 2h before to be stimulated or not with 10nM EGF with or without 10μM Gefitinib for 5 min. Cells were then harvested and subjected to...
Western-blot analyses using the indicated antibodies. Total Erk (t-Erk) antibodies were used as a loading control. g, Genome-edited MDA-MB-231 cells expressing mCherry-tagged μ2-adaptin were serum-starved for 2h before to be stimulated with 30nM Alexa488-EGF for 5min in the presence or not of 10μM Gefitinib, as indicated, and imaged by TIRF microscopy. Scale bar: 2 μm. h, Quantification of the average enrichment of Alexa488-EGF fluorescence intensity in CCSs over background (non-CCSs areas of the plasma membrane; * P<0.01, Student’s t-test. N=3). Values for background were set to 0. All results are expressed as mean ± SD.
Supplementary Figure 4. β1-integrin and vinculin equally distribute between EGF-decorated and non-decorated fibers. a, MDA-MB-231 cells incubated in suspension with Sir-Actin for 30 min were allowed to spread on a composite network composed of Alexa488-EGF-decorated fibers and fluorescently labelled, naked fibers and imaged by spinning disk microscopy. The Sir-Actin signal was used to monitor the speed of extension of the main cell protrusion. Data show the mean velocity of protrusion extension for protrusions associated with either EGF-decorated or naked fibers, as indicated. Data are expressed as mean ± SD speed in μm per min. (* P<0.01, Student’s t-test. N=3). b, Alexa488-labelled, EGF-decorated fibers (green) and Alexa548-labelled, non-decorated fibers (Naked, blue) were sequentially spotted on a glass coverslip and MDA-MB-231 cells were allowed to spread on this composite network for 15 min before to be fixed and stained for β1-integrin (red). Scale bar: 10 μm. c, Quantification of the average β1-integrin staining fluorescence intensity on EGF-decorated and non-decorated (Naked) fibers (ns: non-significant, Student’s t-test. N=3). Values for non-decorated fibers were set to 100%. d, Alexa488-labelled, EGF-decorated fibers (green) and Alexa548-labelled, non-decorated fibers (Naked, blue) were sequentially spotted on a glass coverslip and MDA-MB-231 cells were allowed to spread on this composite network for 15 min before to be fixed and stained for vinculin (red). Scale bar: 10 μm. e, Quantification of the average vinculin staining fluorescence intensity on EGF-decorated and non-decorated (Naked) fibers (ns: non-significant, Student’s t-test. N=3). Values for non-decorated fibers were set to 100%. All results are expressed as mean ± SD. f, Alexa488-labelled, EGF-decorated fibers (green) and Alexa548-labelled, non-decorated fibers (Naked, blue) were sequentially spotted on a glass coverslip and MDA-MB-231 cells were allowed to spread on this composite network for 15 min before to be fixed and stained for phosphorylated myosin-II (p-myosin, red). Scale bar: 10 μm. g, Quantification of the average phosphorylated myosin-II staining.
fluorescence intensity on EGF-decorated and non-decorated (Naked) fibers (ns: non-significant, Student’s t-test. N=3). All results are expressed as mean ± SD. h, MDA-MB-231 cells incubated in suspension with Sir-Actin for 30 min were allowed to spread on a composite network composed of Alexa488-EGF-decorated or HB-EGF-decorated fibers (as indicated) and fluorescently labelled, naked fibers and imaged by spinning disk microscopy. The Sir-Actin signal was used to identify the main cell protrusion. Data show the mean ± SD ratio of the proportion of cells developing their main protrusion on EGF- or HB-EGF-decorated fibers versus cells developing their main protrusion on naked fibers (* P<0.01, One Way Analysis of Variance – ANOVA. N=3). In some experiments, soluble EGF or Gefitinib were added during cell spreading, as indicated. i, Alexa548-labelled, LDL-decorated fibers (green) and Alexa488-labelled, non-decorate fibers (Naked, blue) were sequentially spotted on a glass coverslip and MDA-MB-231 cells were allowed to spread on this composite network for 20 min before to be fixed and stained with phalloidin (red). Scale bar: 10 µm. j, Quantification of the percentage of cells developing their longest protrusion along non-decorated (Naked) or LDL-decorated fibers as in d (* P<0.01, Student’s t-test. N=3).
Figure S5
Supplementary Figure 5. **Characterization of CCS/collagen fibers movements at the cell surface.**

**a**, Representative images of traction force map of MDA-MB-231 cells on 5 kPa polyacrylamide gels spotted with non-decorated (left panel) and EGF-decorated (right panel) collagen fibers. Scale bar, 10 μm. Black arrows show the direction of the traction stress. White lines and dashed lines represent collagen fibers and cell contours, respectively. Color code gives the magnitude of traction stress in picoNewton per square μm.

**b**, Average force (strain energy) exerted by MDA-MB-231 cells seeded on 5 kPa polyacrylamide gels spotted with non-decorated or LDL-decorated collagen fibers. At least 15 cells from 3 independent experiments were analyzed per conditions. Mean ± SD is shown (* P<0.01, Student’s t-test. N=3).

**c**, Genome-edited MDA-MB-231 cells expressing mCherry-tagged μ2-adaptin (red) were seeded onto Alexa488-labelled collagen fibers (green) spotted on a glass coverslip for 1h and imaged by spinning disk microscopy by focusing on the dorsal surface of the cells. Scale bar: 10 μm.

**d**, Kymograph depicting the sequence of events occurring over time in the boxed area shown in a.

**e**, Representative still image (left panel) from a movie of MDA-MB-231 cell overexpressing GFP-tagged α-adaptin subunit of AP-2 migrating on a glass coverslip. The cell was imaged every 15 s for 15 min by spinning disk microscopy. Scale bar: 10 μm. The right panel show a kymograph from corresponding to the dash line on the left panel. Arrow indicate the direction of migration.

**f**, kymograph of regions at the leading edge of control or blebbistatin-treated MDA-MB-231 cells overexpressing GFP-tagged heavy chain of myosin-II and mCherry-tagged μ2-adaptin subunit of AP-2 (CCSs) and imaged every 2 s for 2 min by spinning disk microscopy. Arrows indicate the direction of migration.

**g**, Track plots representing the direction of the flow of individual CCSs imaged as in d. Arrows indicate the direction of cell migration. Red tracks indicate CCSs flowing in the opposite direction of migration. At least 60 CCSs from 3 cells were tracked.
Figure S6
Supplementary Figure 6. **Characterization of collagen fibers remodelling.** a, Alexa647-labelled, EGF-decorated fibers (green) and Alexa548-labelled, non-decorate fibers (Naked, red) were sequentially spotted on a glass coverslip. Scale bar: 100 µm. b, Kymographs depicting collagen fibers remodelling in the boxed area shown in c upon seeding MDA-MB-231 cells on the composite network for 120 min. c, Quantification of the average evolution of EGF-decorated or LDL-decorated (green, as indicated) and non-decorated (Naked, red) collagen fibers circularity upon MRC5, CAFs or osteoblasts seeding on a composite 2D network as in Fig. 3g (* P<0.01, One Way Analysis of Variance – ANOVA. N=3). d, Quantification of the average evolution of EGF-decorated or naked collagen fibers circularity upon cell seeding on either a 2D network composed of only one type of fibers (red) or of both ligand-decorated and naked fibers (approximate ratio 1:1; green; * P<0.01, One Way Analysis of Variance – ANOVA. N=3). e, Quantification of the average evolution of EGF-decorated (green) and non-decorated (Naked, red) collagen fibers circularity upon cell seeding on a composite 2D network as in Fig. 3g and when using cells treated with the indicated siRNAs or with Gefitinib (* P<0.01, One Way Analysis of Variance – ANOVA. N=3). f, Quantification of the ratio of average α-adaptin staining fluorescence intensity on EGF-decorated versus naked fibers in control of siCHC-treated MDA-MB-231 cells as indicated (* P<0.01, Student’s t-test. N=3).
Figure S7
Supplementary Figure 7. Characterization of cell migration in 3D, ligand-decorated networks.

a, MDA-MB-231 cells were embedded in 3D networks containing either chopped, non-decorated or EGFdecorated fibers or in classical 3D collagen networks polymerized “en masse” and incubated or not with EGF as indicated. Cells were tracked manually using Image J. Data represent cell velocity measured in at least 30 cells per experiments and per conditions in three independent experiments. Results are expressed as mean ± SD (* P<0.01, One Way Analysis of Variance – ANOVA. N=3).

b, MDA-MB-231 cells were embedded in 3D networks containing either chopped, non-decorated or EGFdecorated fibers or in classical 3D collagen networks polymerized “en masse” and incubated or not with EGF as indicated. Cells were tracked manually using Image J. Data represent the percentage of cells dividing over a 24h period and measured in at least 30 cells per experiments and per conditions in three independent experiments. Results are expressed as mean ± SD (n.s= non-significant, One Way Analysis of Variance – ANOVA. N=3).

c, A 3D composite network composed of a EGF-decorated fibers area (Alexa488-EGF) and a non-decorated fibers area (Naked fibers) was generated as described in the material and methods section and imaged by spinning disk microscopy 1h or 24h after assembling the setup, as indicated. Scale bar: 10 µm.

d, A 3D composite network composed of two areas both containing only non-decorated fibers was generated as described in the material and methods section and imaged by spinning disk microscopy. Cells located at the border between the two areas were manually tracked and ranked based on whether they migrated towards the inner area (usually corresponding to EGF-decorated fibers) or towards the external area (usually corresponding to the non-decorated fibers area) of the gel. Data are expressed as the average percentage of cells migrating towards the indicated area. At least 60 cells were tracked per experiments in 3 independent experiments. The
dash line represent the 50% threshold indicating no preferential migration in a given direction. e, Box plots representing the average ratio of MRC5 cells initially located at the border between the non-decorated and EGF-decorated areas and migrating towards the EGF-fibers-containing area versus the non-decorated fibers area SD (* P<0.001, One Way Analysis of Variance – ANOVA). A ratio of 1 indicates no preferential migration towards one or the other area. f, A 3D composite network composed of LDL-decorated fibers area and non-decorated fibers area was generated as described in the material and methods section and imaged by spinning disk microscopy. Scale bar: 10 µm.
Supplementary movies legends:

Supplementary Movie 1. **Dynamics of TCALs on EGF-decorated and non-decorated collagen fibers.** MDA-MB-231 expressing µ2-adaptin-mCherry (red) were allowed to spread on a glass coverslip spotted with EGF-decorated fibers (green) and non-decorated fibers (blue) for 15 min. Time is indicated in seconds. Scale bar = 10µm.

Supplementary Movie 2. **Dynamics of EGF-decorated and non-decorated collagen fibers remodelling.** MDA-MB-231 were seeded on coverslips spotted with EGF-decorated fibers (green) and non-decorated fibers (red) for the indicated time. Wide-field time-lapse depicting cells evolving on the composite 2D substrate is shown on the right panel. Time is indicated in hours:minutes. Scale bar = 10µm.

Supplementary Movie 3. **Cell migration in the composite 3D network.** MDA-MB-231 were embedded in a composite 3D network composed of a EGF-decorated fibers-containing area (top left and green) and of a non-decorated fibers-containing area (top right panel and red) and imaged for the indicated time. Wide-field time-lapse used to track cells is shown in the bottom left panel and merge in the bottom right panel. Time is indicated in minutes. Scale bar = 50 µm.