Isolation of CAF

CAF were isolated from skin or LN clinical specimens. Briefly, the sample was cut into small pieces and digested with collagenase and dispase. Following filtration of large debris, the solution was serial centrifuged, and the final pellet was re-suspended in DMEM supplemented with 10% FBS and seeded in a tissue culture dish. After 30 min, CAF have adhered to the dish whilst other cellular types in suspension were discarded. The identity of CAF was validated by the expression of classical markers by qRT-PCR and flow cytometry.

Treatment of Conditioned media (CM)

In some experiments, EVs and EV-free CM were prepared by differential ultracentrifugation as described (35) or CM was treated with 100 µg/ml Proteinase K (ThermoFisher) overnight and then with 1 mM PMSF (Sigma) to inactivate Proteinase K.

Microarray analysis

Microarray analyses were performed using R (RRID: SCR_001905). We used the Bioconductor (RRID: SCR_006442) package arrayQualityMetrics (RRID: SCR_001335) and custom R scripts for quality control. Additional analyses were done using Bioconductor package Limma (RRID: SCR_010943). Data were normalized using the quantile method. A linear modeling approach was used to calculate log ratios, moderated *t*-statistics, and *P*-values for each comparison of interest. *P*-values were adjusted for multiple testing using the Benjamini–Hochberg method that controls the false discovery rate. After raw data processing, the 25,000 most expressed genes in LN-F (average mean expression ≥ 6) were analyzed. To identify pathways down-regulated by 1205Lu CM treatment, we selected the genes down-regulated with LogFC ≤ -0.5 in 1205Lu-reprogrammed LN-F and submitted these genes to Gene Set Enrichment Analysis (GSEA, RRID: SCR_003199). To analyze genes involved in the regulation of the STAT3 pathway, we identified genes common to the "GO negative regulation of receptor signaling pathway via stat" pathway, and differentially expressed with an absolute LogFC ≥ 0.4. Results were presented in heatmaps generated with Graphpad Prism or Phantasus (https://genome.ifmo.ru/phantasus) softwares.

Hydrogels

Hydrogels were prepared on 20 mm diameter glass coverslips. Briefly, 3-aminopropyl trimethoxysilane (Sigma) was applied on a coverslip and another coverslip was treated with Rain-X glass water repellent. The coverslips were then washed thoroughly with sterilized water and dried. Acrylamide/bis-acrylamide solutions (Fisher Scientific) were mixed to obtain 0.2 kPa (3% acrylamide; 0.06% bis-acrylamide) and 2.8 kPa (7.5% acrylamide; 0.3% bis-acrylamide) hydrogels. Ammonium persulfate (0.05% w/v) and N,N,N0,N0-tetramethylethylenediamine (0.14% v/v) were added to the bis-acrylamide mixture and 50 μl of this mixture was deposited on top of the silanized glass and covered with the X-Rain-treated coverslip. After 10 min polymerization, the upper coverslip was removed and the polyacrylamide surface was photo-activated by exposing the sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino)hexanoate crosslinker (Sigma) to UV light. The stiffness of hydrogels was validated using the Piuma Nanoindenter (Optics11 Life).

Atomic force microscopy (AFM)

The AFM experiments were performed using a Borosilicate Glass spherical tip (5 μm of diameter) attached to a cantilever with a nominal spring constant of 0.06 N/m (Novascan Technologies, Ames, IA USA) mounted on a Bioscope Catalyst (Bruker Nano Surfaces, Santa Barbara, CA, USA) equipped with a Nanoscope V controller and coupled with an optical microscope (Leica DMI6000B, Leica Microsystems Ltd., UK). Before starting the experiment, the periphery of the collagen gel sample was glued (Twinsil®) to the bottom of a 50 mm dish (Willco Glass Bottom Dish), leaving the central part free from glue for performing the AFM experiment. The sample was washed and covered with 4 ml of Leibovitz's medium supplemented with 0.5 % FBS. After the thermal stabilization of the system, the deflection sensitivity of the system in the medium using a clean glass dish area and the spring constant of the cantilever by the thermal tune method were obtained. For each sample, at least 3 different areas were analyzed using the “Point and Shoot” method, collecting a minimum of 100 force-distance curves at just as many discrete points. Force-distance curves were collected on the sample using a velocity of 5 μm/s, in relative trigger mode and by setting the trigger threshold to 2 nN. The apparent Young's (elastic) modulus was calculated using the NanoScope Analysis 1.80 software (Bruker Nano Surfaces, Santa Barbara, CA,

USA), fitting the force curves to the Hertz spherical indentation model and using a Poisson's ratio of 0.5. To avoid large indentation, a minimum and a maximum Force Fit Boundary of 5% and 25% respectively of the whole force curve was considered for the fit. After the base correction step in the analysis, only the force curves having their maximum value at 2 nN were considered for performing the fit. The data analysis was performed using GraphPad Prism. The Kruskal-Wallis test for non-parametric distributions, followed by a Dunns multiple comparison test was applied to the dataset samples.

Cancer cell real-time proliferation and migration

1205Lu Red cell proliferation and migration were monitored by real-time microscopy (37°C, 5% CO_2). Briefly, $2x10^4$ LN-F/well were plated in triplicate in 48 well plates and were treated with control medium, melanoma CM, IL-1β or Y-27632 for 5 days. Then, LN-F were washed twice and 10^4 1205Lu Red cells/well were plated on LN-F confluent monolayers in DMEM. To monitor proliferation, 4 images per well were taken every 2h for 3 days (IncuCyte®, Sartorius, RRID: SCR_0198874) and the count of fluorescent nuclei was normalized over time to the first time-point of each condition. To follow migration, 4 images per well were taken every 10 min for 24 h (video-microscope TIRF-AM, Leica Microsystems). 1205Lu Red cell displacement was quantified with the ImageJ software using the TrackMate plugin. Only cells with tracks followed for the full 24 h were included in the analysis.