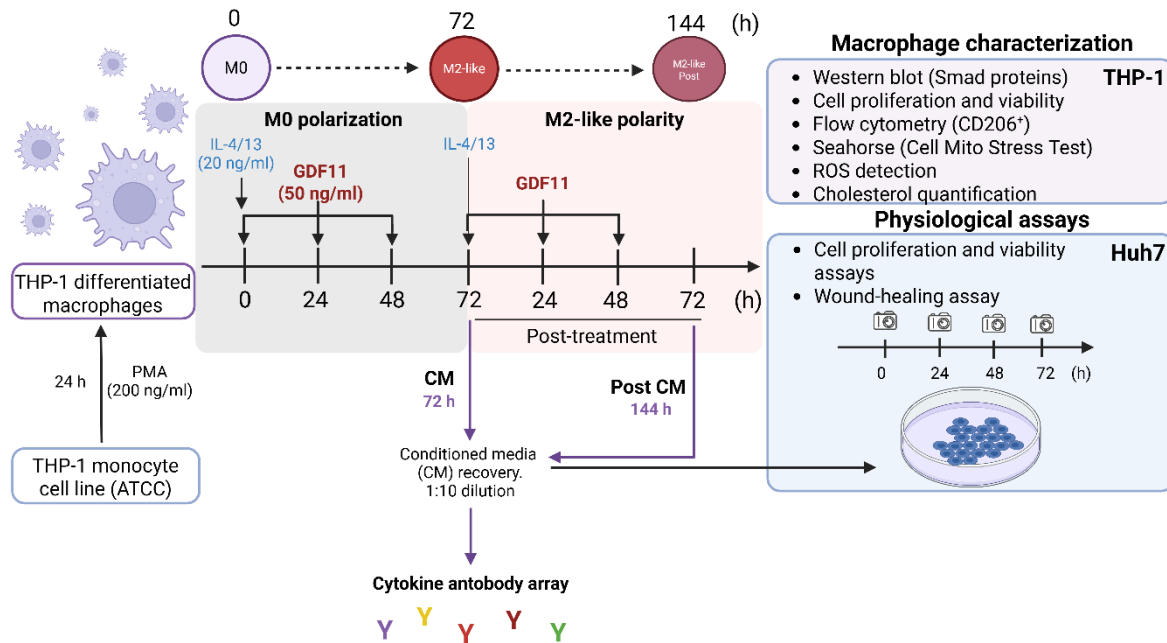
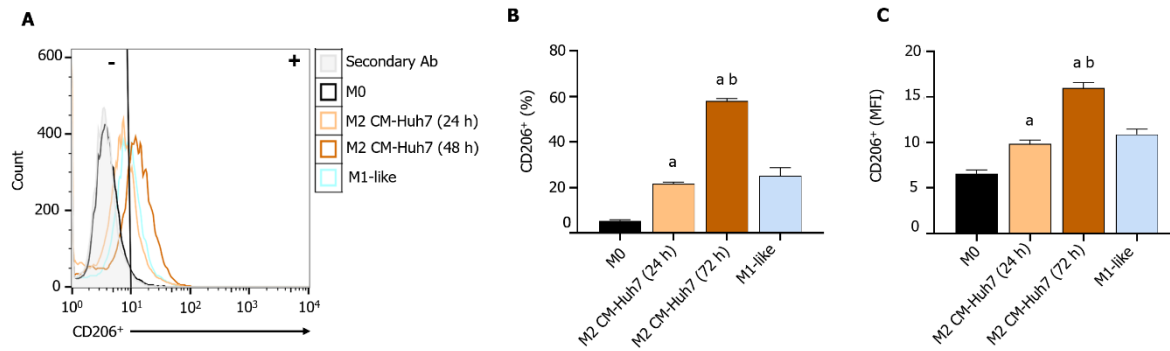
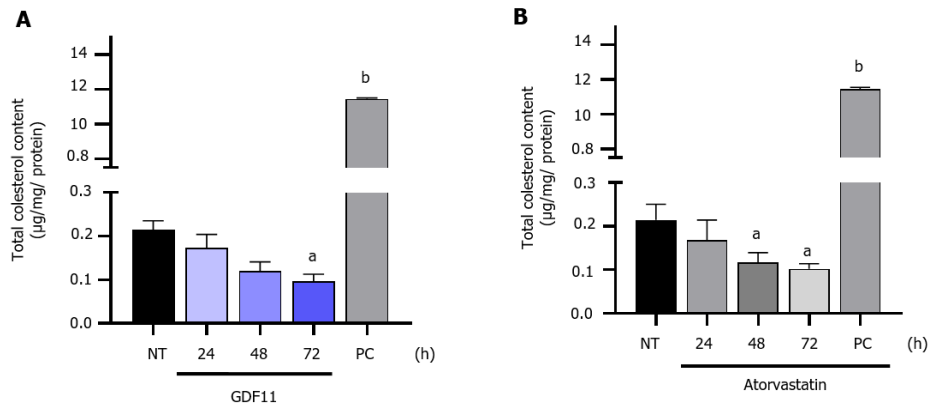




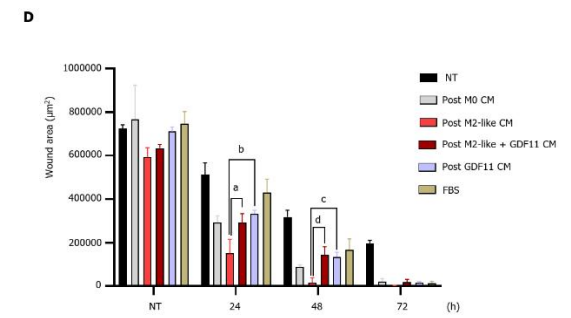
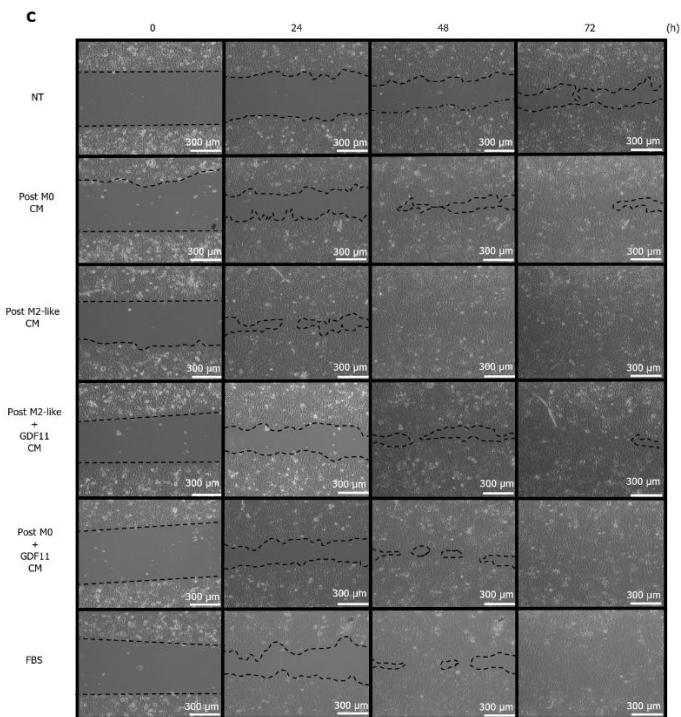
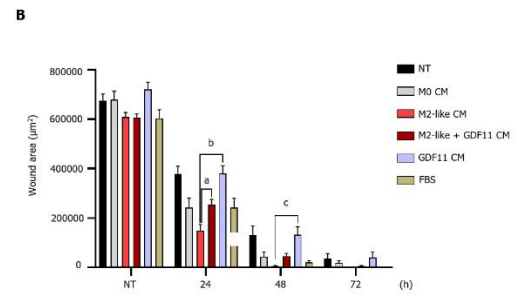
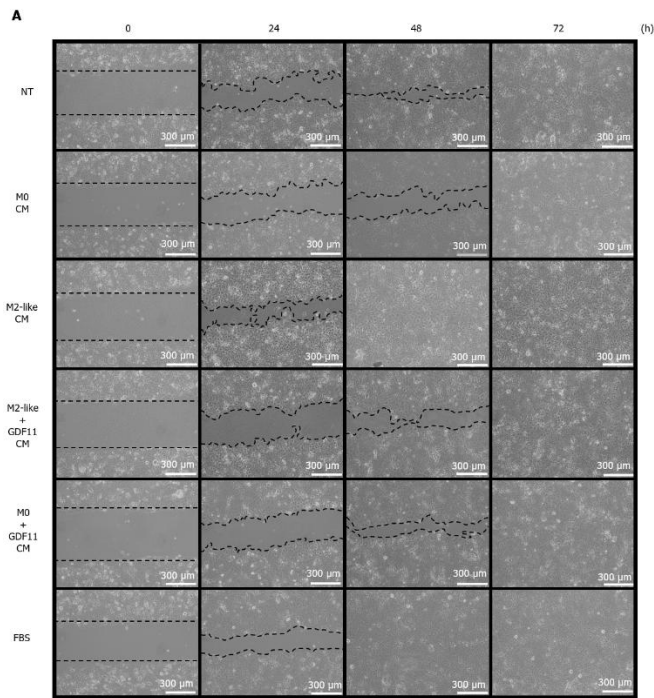
Supplementary Figure 1 Experimental design of the study. Image created using Biorender. PMA: Phorbol myristate acetate; ATCC: American Type Culture Collection; IL: Interleukin; GDF11: Growth differentiation factor 11; CM: Conditioned media; CD206: Cluster of differentiation 206; ROS: Reactive oxygen species.

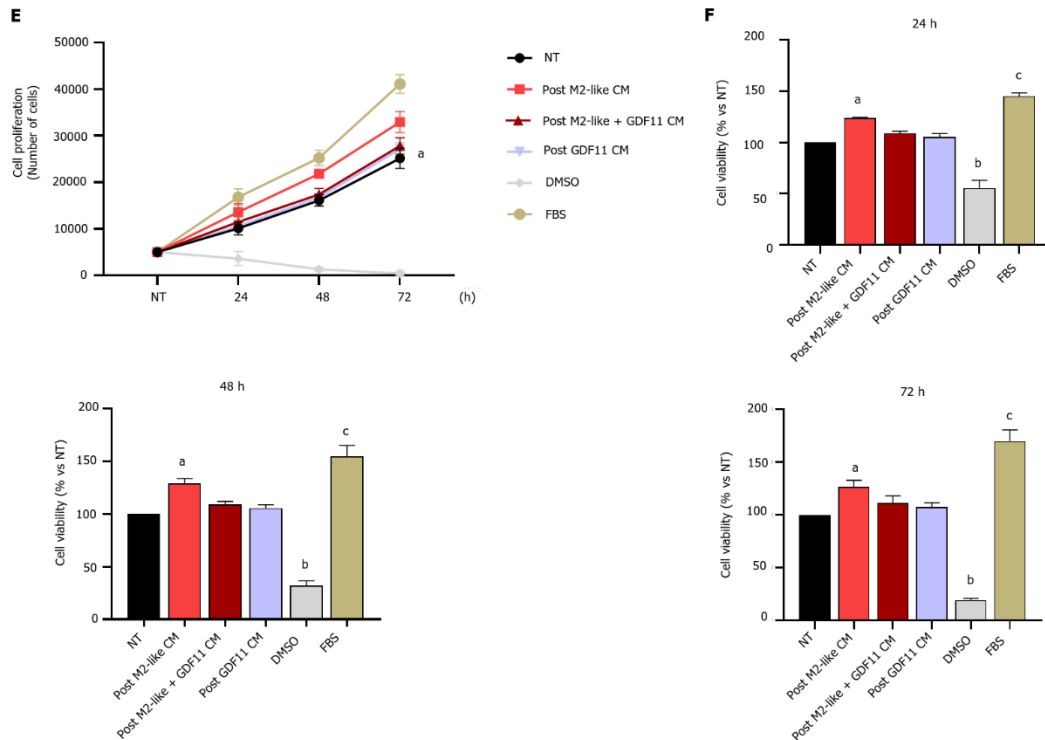


Supplementary Figure 2 Effects of the conditioned media (CM) derived from Huh7 cells on macrophages. Flow cytometry analysis was performed to evaluate cluster of differentiation 206 (CD206⁺) subsets. Macrophages were treated with conditioned media derived from HCC cells, as stated in the Materials and Methods section. A: Fluorescence histogram; B: percentage (%) of CD206⁺ cells; C: Median fluorescence intensity of CD206⁺ subset. Each column represents the mean $\pm$ SEM of at least three independent experiments. ^a $P \leq 0.01$ vs M0; ^b $P \leq 0.05$ vs M2 conditioned media-Huh7 (24 hours). CM: Conditioned media; CD206: Cluster of differentiation 206.

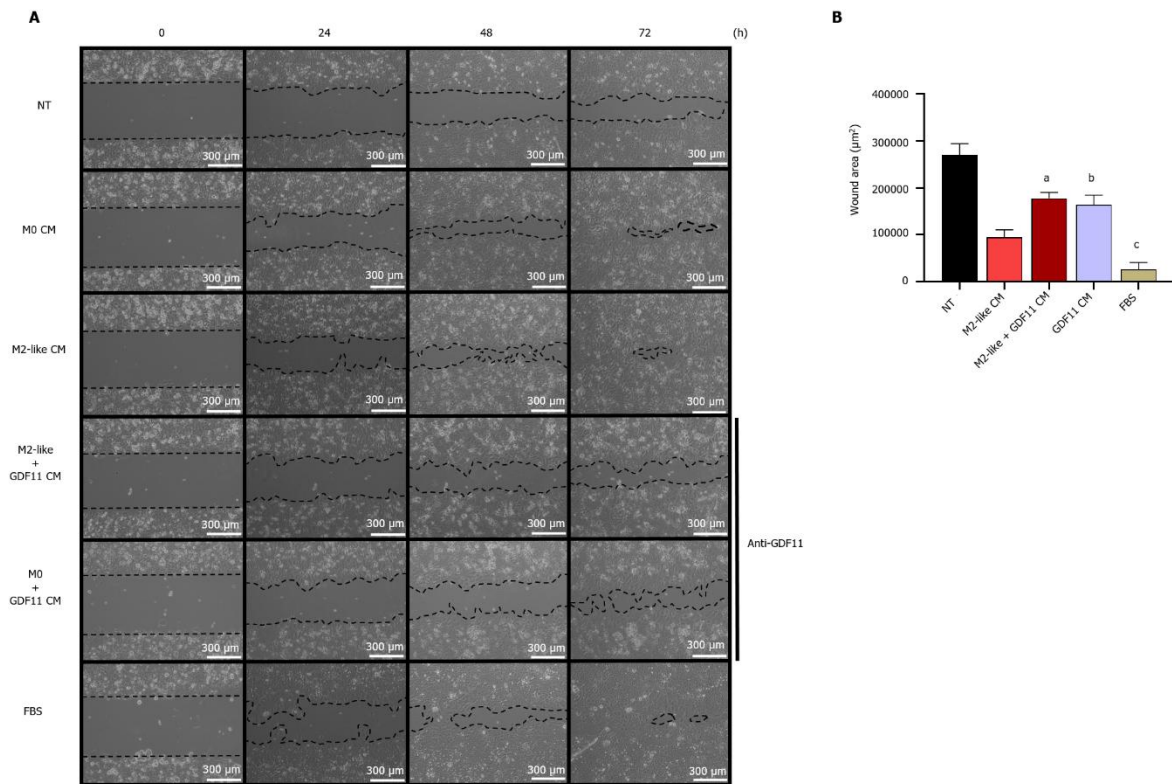


Supplementary Figure 3 Growth differentiation factor 11 decreases cholesterol content in macrophages. Cholesterol was biochemically quantified. A: Cholesterol content from growth differentiation factor 11-treated macrophages; B: Cholesterol from Atorvastatin-treated macrophages. Liver tissue from a mouse fed a high cholesterol diet (2%) for eight months. Each column represents the mean $\pm$ SEM of at least three independent experiments in triplicate. ^a $P \leq 0.05$ *vs* not treated cells; ^b $P \leq 0.0001$ *vs* not treated cells. NT: Not treated cells; GDF11: Growth differentiation factor 11.

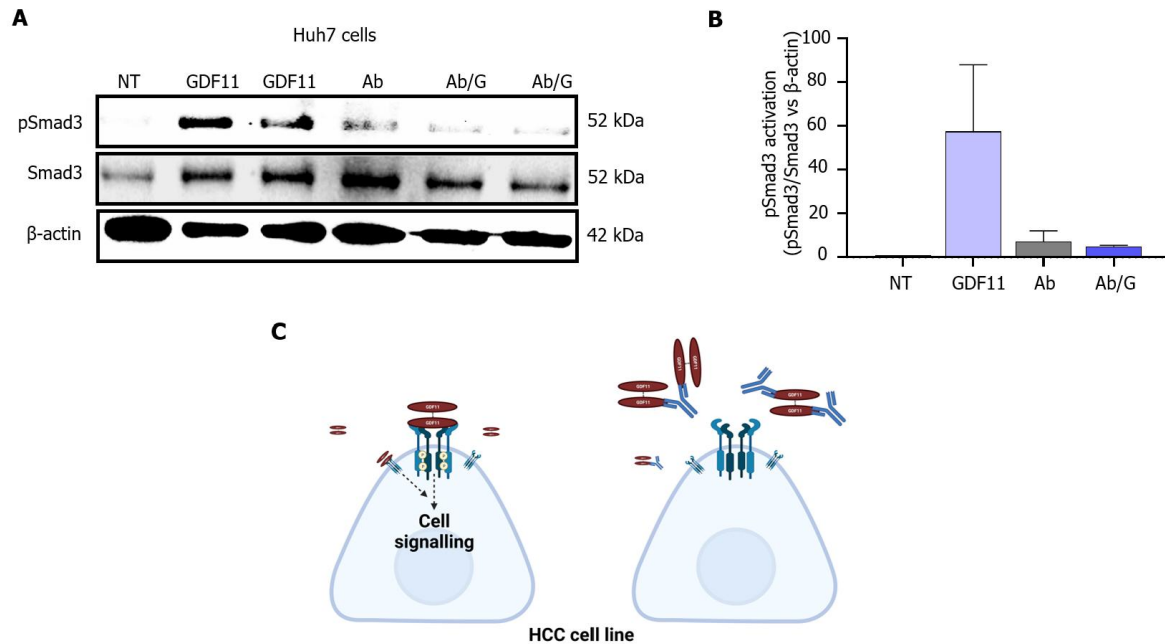




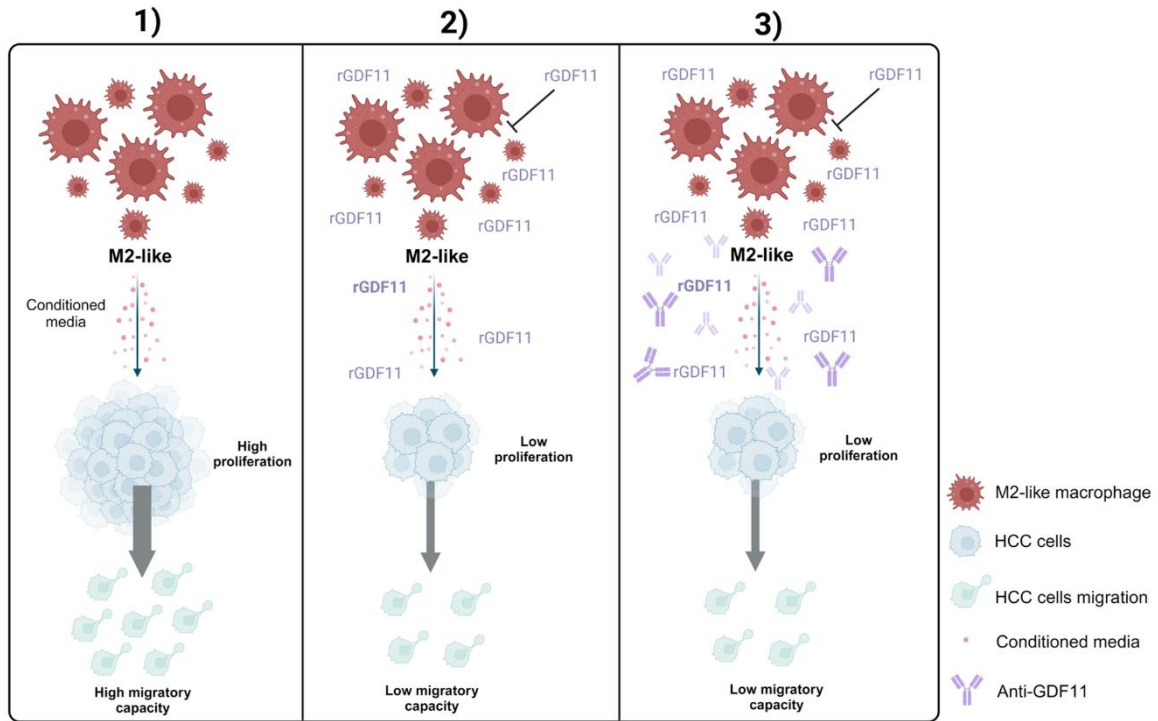
Supplementary Figure 4 Effects of the conditioned media derived from M2-like macrophages on hepatocellular carcinoma cells. Includes complete controls. A: Cell migration determined by a wound healing assay, representative images of at least three independent experiments. Scale bars: 300 μm (100 $\times$, original magnification); B: Wound area (μm^2) quantification determined by Image J software; C: Cell migration determined by a wound healing assay. Scale bars: 300 μm (100 $\times$, original magnification); D: Wound area (μm^2) quantification determined by Image J software. Each column represents the mean $\pm$ SEM of at least three independent experiments in triplicate; E: Cell proliferation; F: Cell viability was assayed by the Cell Counting Kit-8. Fetal bovine serum was used as a control. Dimethyl sulfoxide was used as a negative control. Each column or point represents the mean $\pm$ SEM of at least three independent experiments carried out in triplicate. ^a $P \leq 0.05$ vs not treated cells; ^b $P \leq 0.05$ vs not treated cells; ^c $P \leq 0.01$ vs not treated cells. NT: Not treated cells; CM: Conditioned media; FBS: Fetal bovine serum; GDF11: Growth differentiation factor 11; DMSO: Dimethyl sulfoxide.



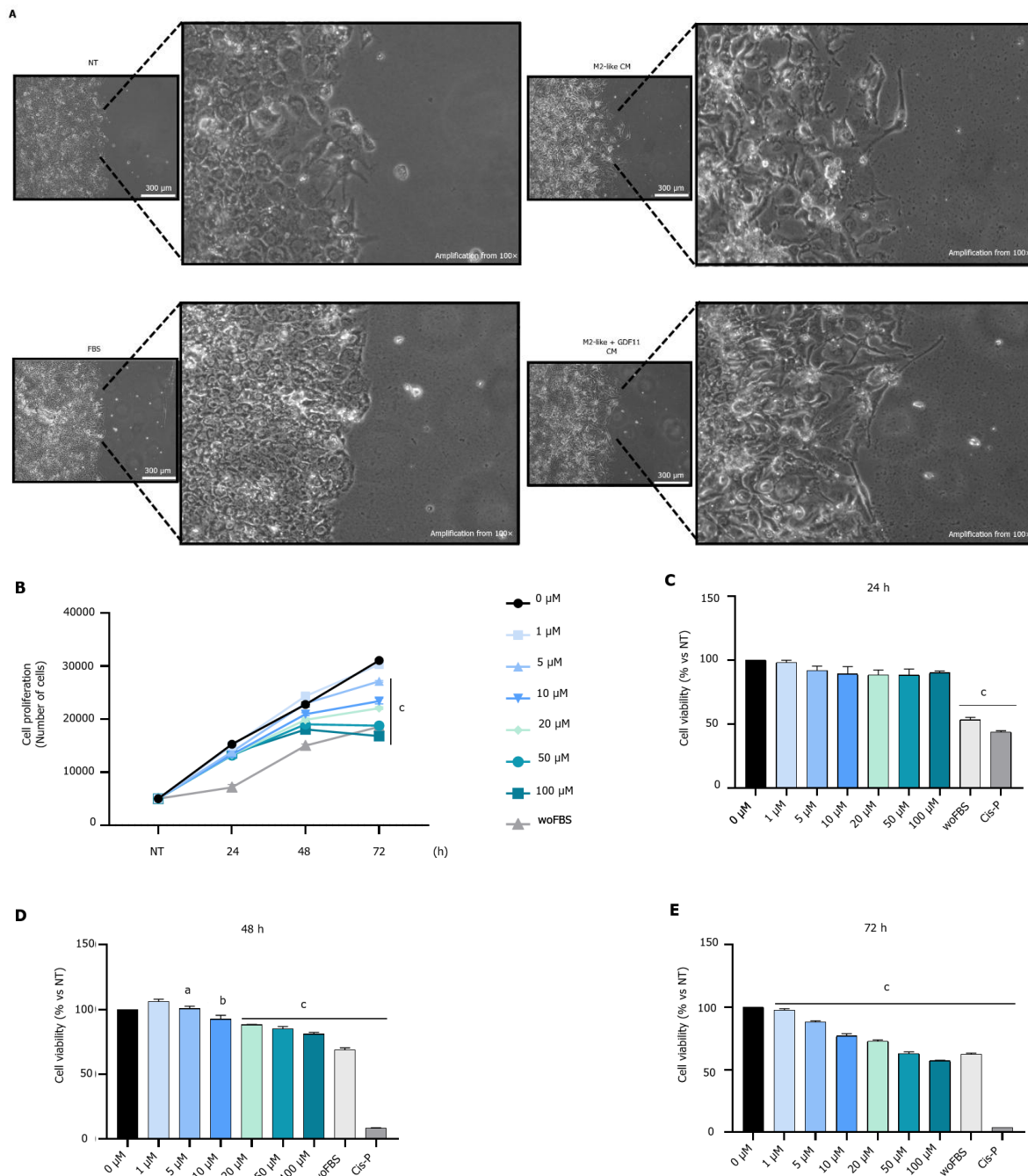
Supplementary Figure 5 Conditioned media from growth differentiation factor 11-treated macrophages decrease the migration capacity of Huh7 cells, even in the presence of anti-growth differentiation factor 11. Includes complete controls. A: Cell migration assay determined by a wound healing assay, representative images of at least three independent experiments. Scale bars: 300 μ m (100 $\times$, original magnification). **B:** Wound area (μ m²) quantification determined by Image J software at 72 hours. Each column represents the mean $\pm$ SEM of at least three independent experiments in triplicate. ^a $P \leq 0.05$ vs M2-like conditioned media; ^b $P \leq 0.05$ vs M2-like conditioned media; and ^c $P \leq 0.01$ vs not treated cells. Anti-growth differentiation factor 11 was used to inhibit the remnants of the recombinant growth differentiation factor 11. Fetal bovine serum was used as a control. NT: Not treated cells; CM: Conditioned media; FBS: Fetal bovine serum; GDF11: Growth differentiation factor 11.



Supplementary Figure 6. Anti-growth differentiation factor 11 repressed the phosphorylation of Smad3 protein. A: Representative images of western blot; B: Densitometries from A. C: Representation of the model. Image created using Biorender. β -actin was used as a loading control. Ab: anti-growth differentiation factor 11. NT: Not treated cells; GDF11: Growth differentiation factor 11; HCC: Hepatocellular carcinoma.

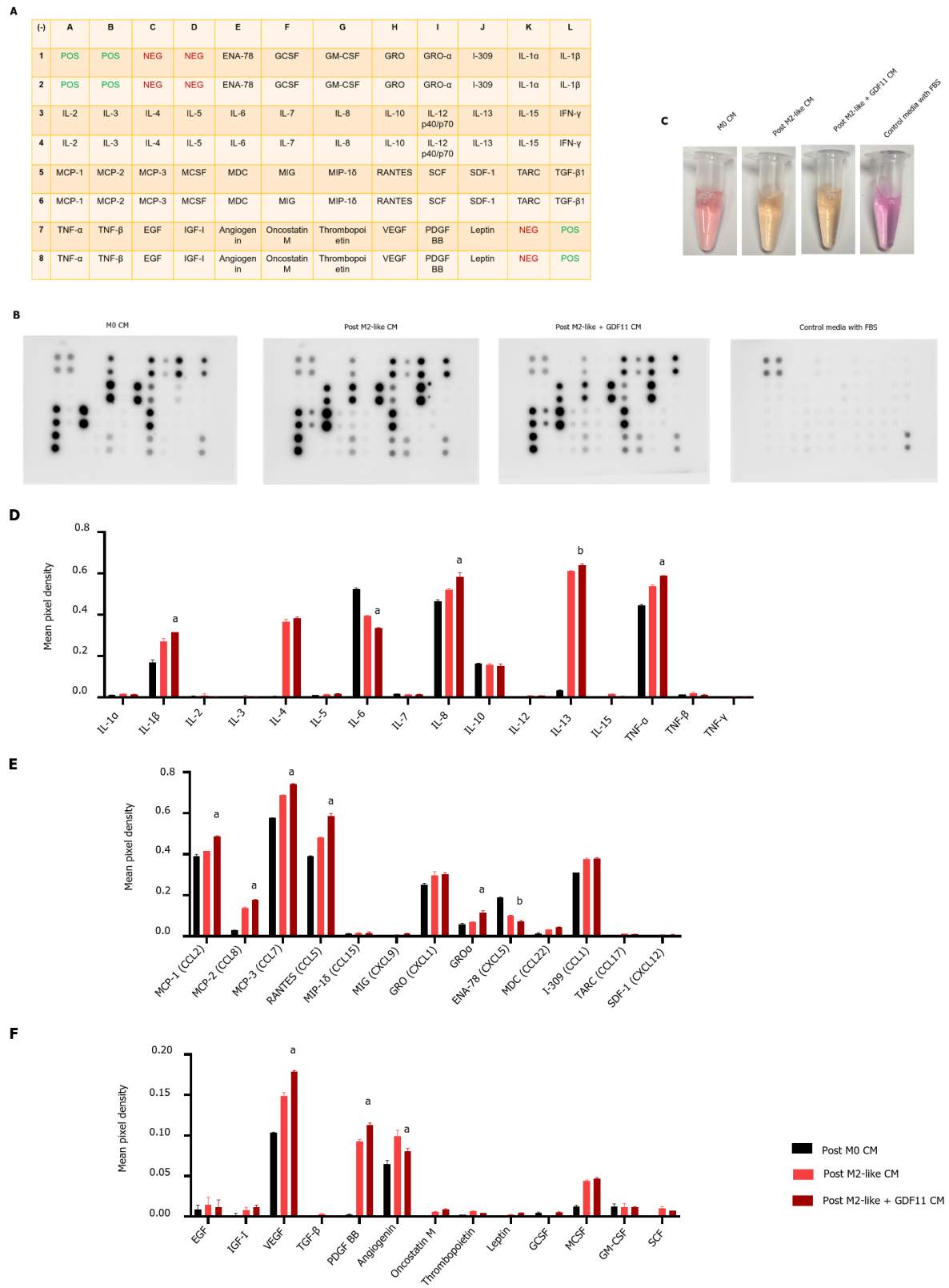


Supplementary Figure 7 The three scenarios of the model. M2-like macrophages exacerbate aggressively on hepatocellular carcinoma cells due to secretion factors (1); growth differentiation factor 11 alters and mitigates M2-like polarization, reducing aggressiveness (2), and using anti-growth differentiation factor 11 in conditioned media did not affect the previous results; hepatocellular carcinoma aggressiveness was reduced (3). Image created using Biorender. GDF11: Growth differentiation factor 11; HCC: Hepatocellular carcinoma.



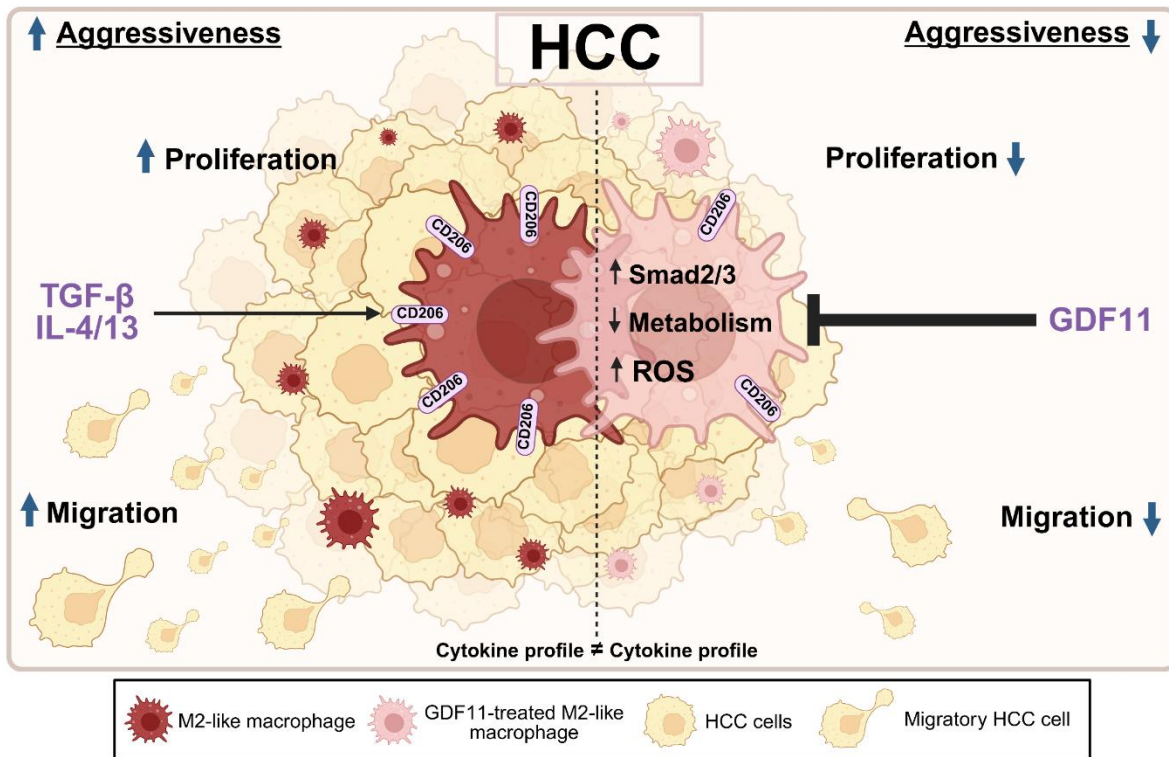
Supplementary Figure 8 Evidence of the migratory morphologies of the cells. A: At the border of Huh7 wound healing culture, representative images of at least three independent experiments. Scale bars: 300 μm (100 $\times$, original magnification); **B:** Proliferation assay using cytarabine at different concentrations; **C-E:** Cell viability using cytarabine at different concentrations, absence of fetal bovine serum and cisplatin were used as negative controls, respectively. Each column represents the

mean $\pm$ SEM of at least three independent experiments in triplicate. ^a $P \leq 0.05$ *vs* not treated cells, ^b $P \leq 0.001$ *vs* not treated cells, and ^c $P \leq 0.0001$ *vs* not treated cells. NT: Not treated cells; CM: Conditioned media; FBS: Fetal bovine serum.



Supplementary Figure 9 Cytokine profile changes induced by growth differentiation factor 11 on post M2-like macrophages. A: Map of interleukin's

location in the cytokine array membranes; B: Representative membranes or dot blots; C: Conditioned media quality derived from M0, post M2-like, and post M2-like + growth differentiation factor 11 macrophages; D: Interleukin and interferon family alteration by growth differentiation factor 11 on post M2-like macrophages, $^aP < 0.0001$ *vs* post M2-like conditioned media, $^bP = 0.0012$ *vs* post M2-like conditioned media; E: Chemokine family alteration by growth differentiation factor 11 on post M2-like macrophages, $^aP < 0.0001$ *vs* post M2-like conditioned media, $^bP = 0.0050$ *vs* post M2-like conditioned media; F: Growth factors alteration by growth differentiation factor 11 on post M2-like macrophages, $^aP \leq 0.0001$ *vs* post M2-like conditioned media. The mean pixel density in each column represents the mean $\pm$ SEM of at least three independent experiments. CM: Conditioned media; GDF11: Growth differentiation factor 11; FBS: Fetal bovine serum.



Supplementary Figure 10 Graphical abstract. Image created using Biorender. HCC: Hepatocellular carcinoma; TGF: Transforming growth factor; IL: Interleukin; ROS: Reactive oxygen species; CD206: Cluster of differentiation 206; GDF11: Growth differentiation factor 11.

Supplementary Table 1 Antibodies used in the study

Antibody	Dilution	Secondary	Catalog Number
pSmad3 (S423/425)	1:1000 WB	Rabbit mAb	Cell Signaling C25A9
Smad3	1:1000 WB	Rabbit mAb	Cell Signaling C67H9
pSmad2 (S465/467)	1:1000 WB	Rabbit mAb	Cell Signaling 138D4
Smad2	1:1000 WB	Rabbit mAb	Cell Signaling D43B4 XP(R)
Smurf1	1:200 WB	Mouse mAb	Santa Cruz Biotechnology sc-100616
CD206 (15-2)	0.6 µg FCM	Mouse mAb	Santa Cruz Biotechnology sc-58986
GDF11	30 ng WH	Mouse mAb	Santa Cruz Biotechnology sc-81952
β-actin	1:10000 WB	Rabbit mAb	Millipore-Sigma A3854
Alexa Fluor 488	1:500 FCM	Mouse mAb	Thermo Fisher A32723

CD206: Cluster of differentiation 206; GDF11: Growth differentiation factor 11; WB: Western blot; FCM: Flow cytometry.

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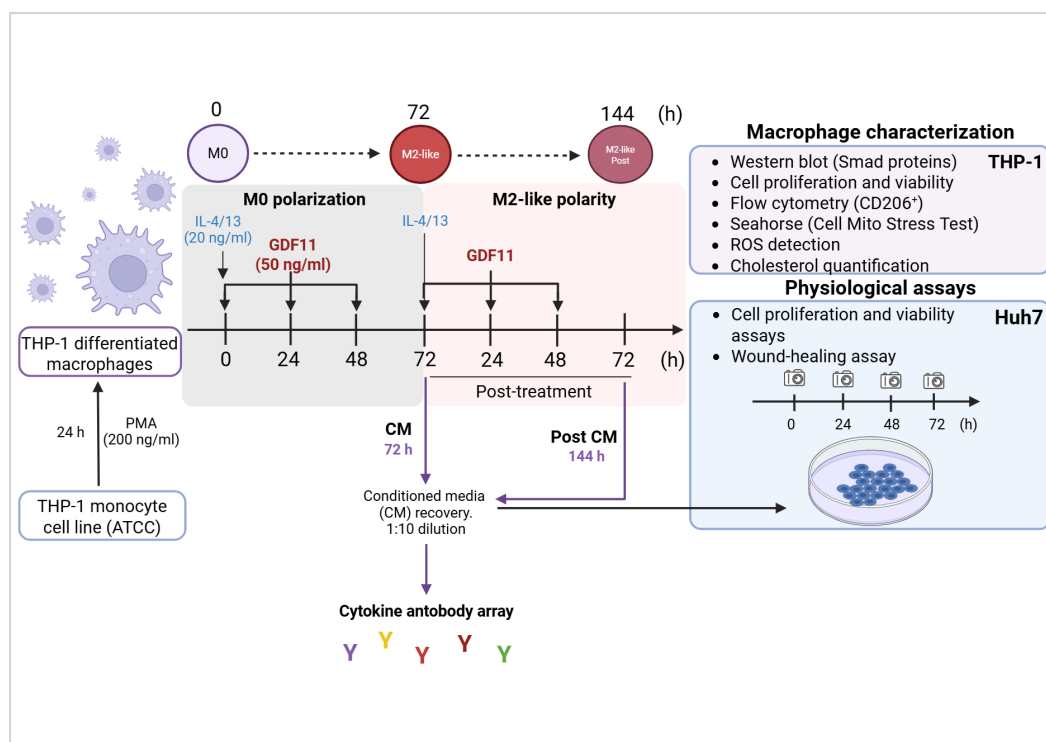
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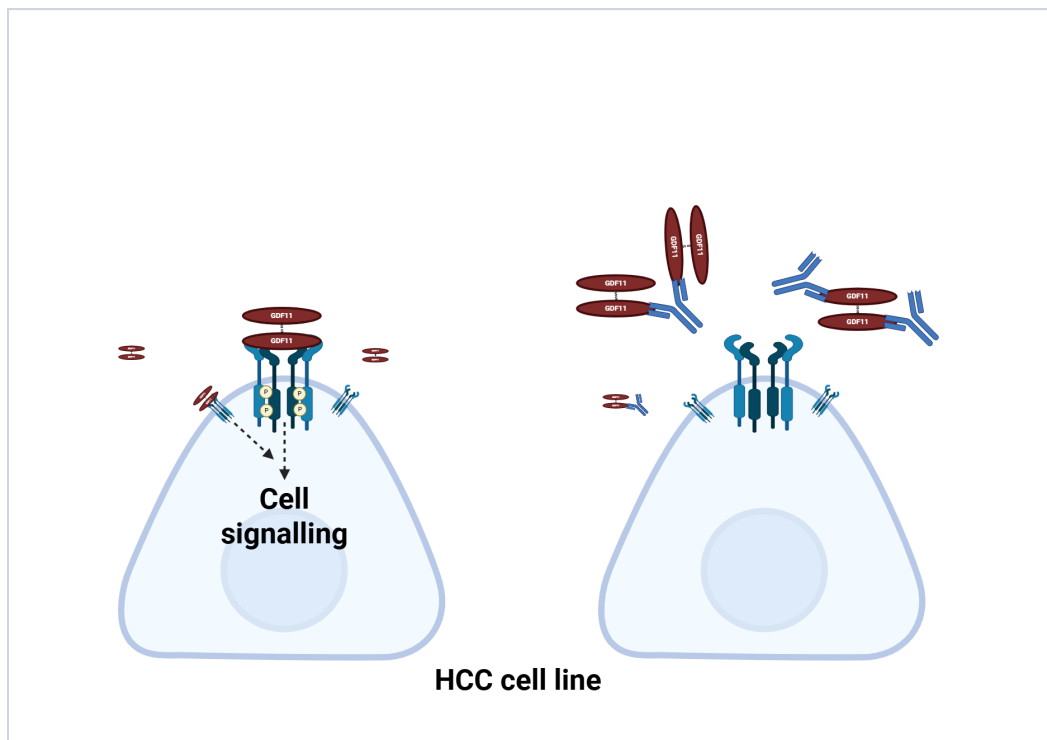
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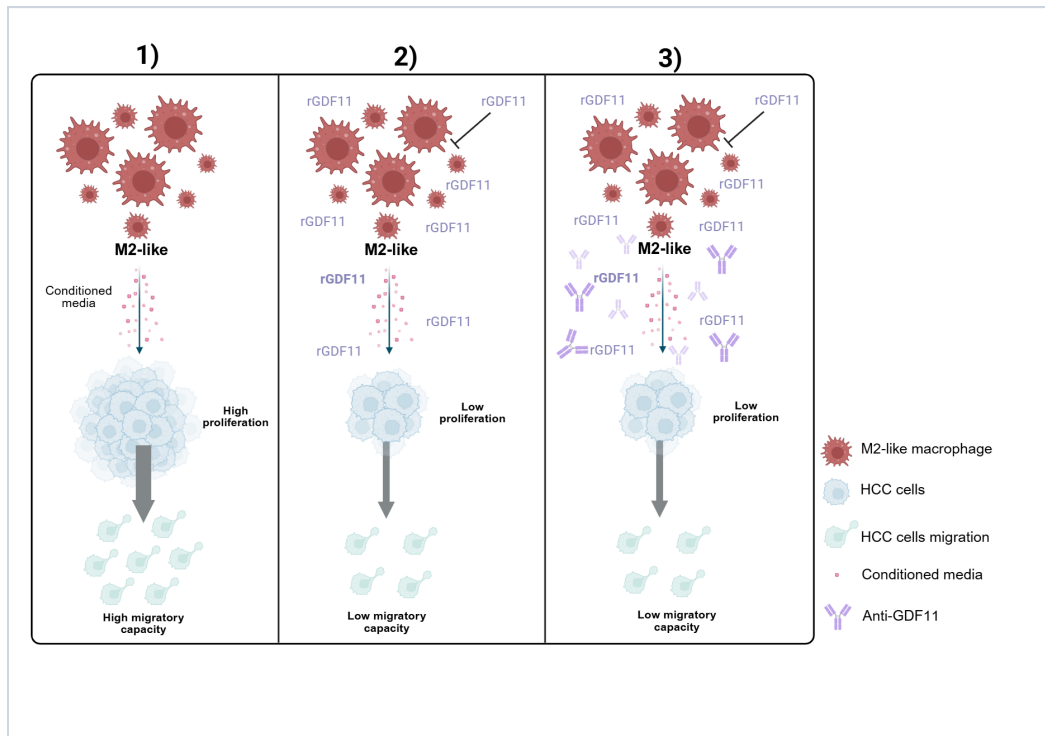
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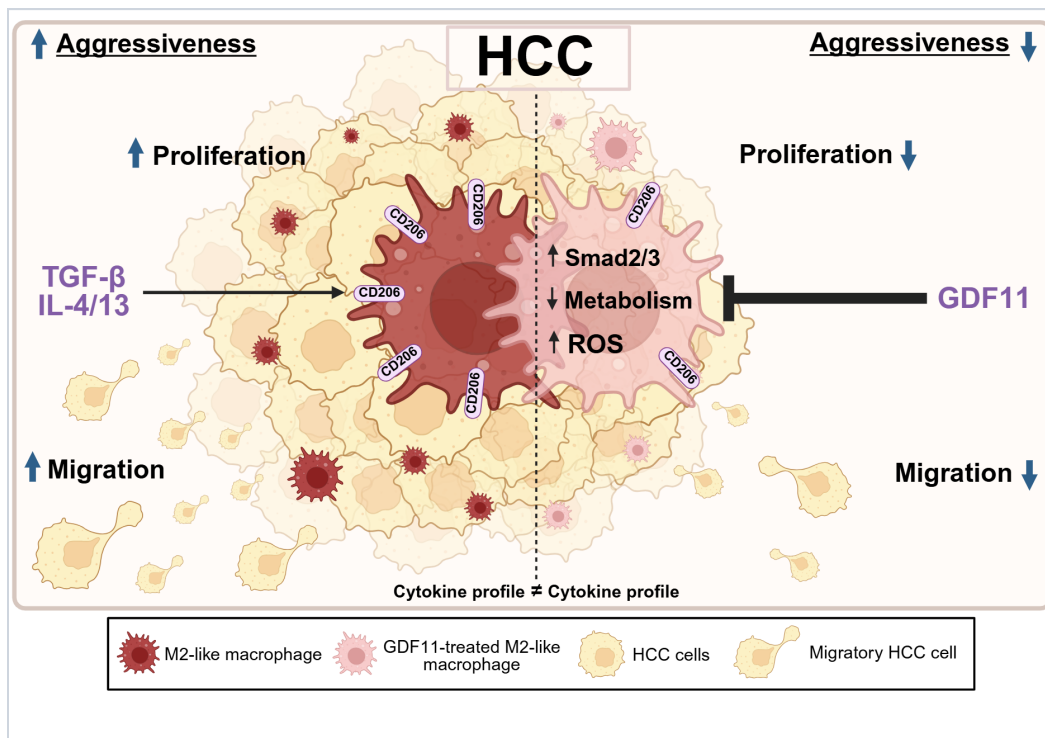
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