

Research Article

Title: Stroma-derived IL-6, G-CSF and Activin-A mediated dedifferentiation of lung carcinoma cells into cancer stem cells

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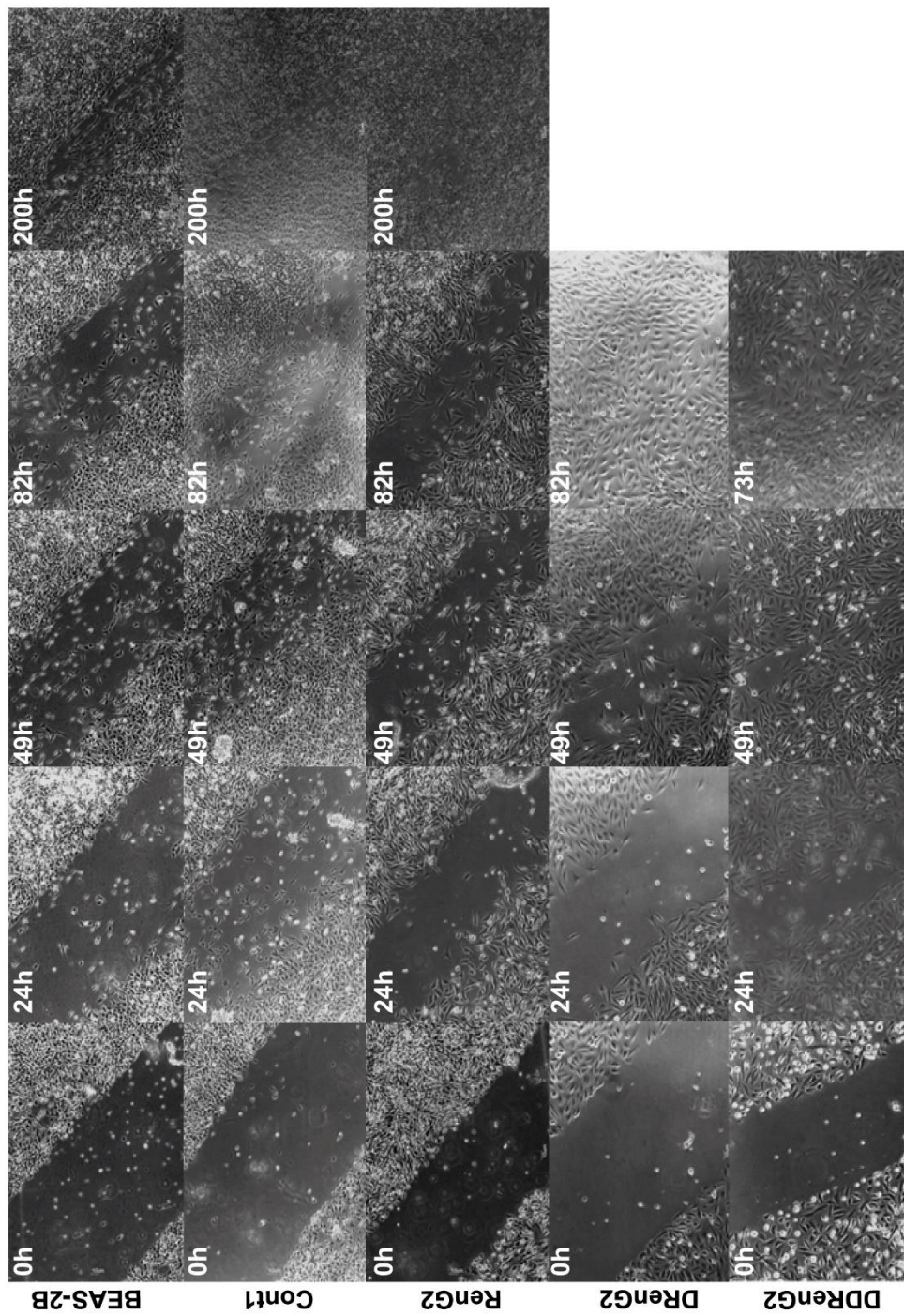
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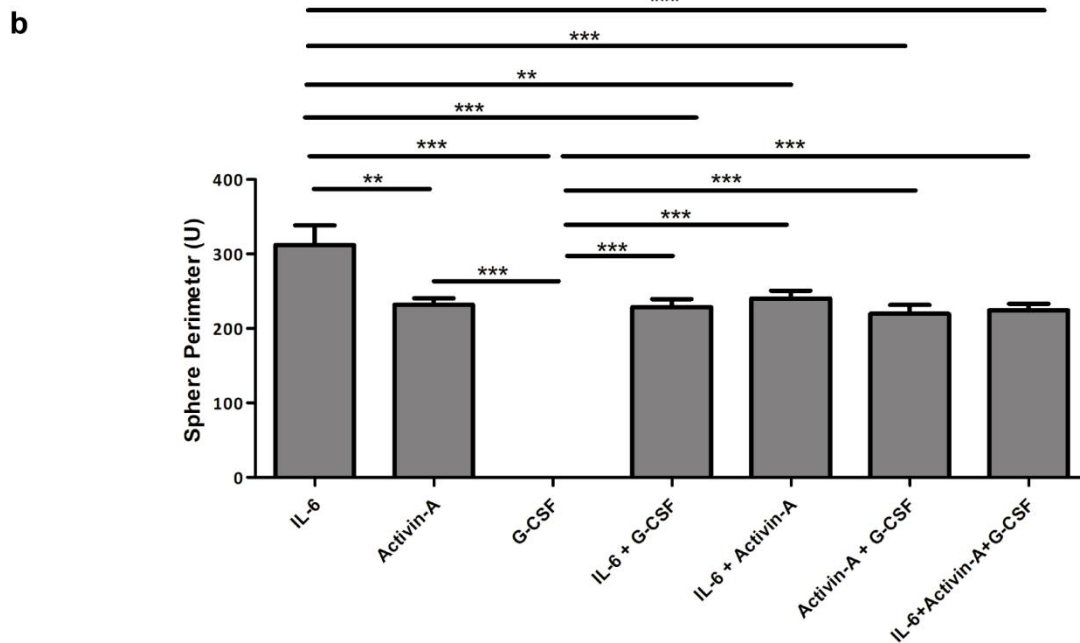
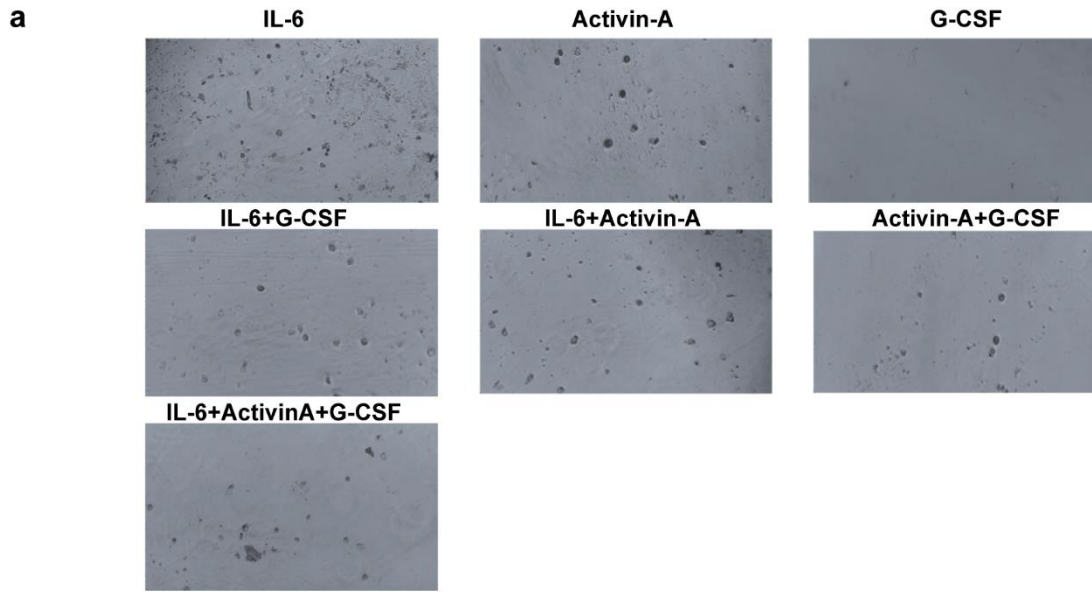
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Supplementary Information



Supplementary Figure S1 – Migration ability of the different cellular systems. Both BEAS-2B and Cont-1 cell lines failed to close the scratch, even after 200 h. RenG2 cells grossly closed it, but DRenG2 and DDRenG2 did it faster and better in progressively less time. A magnification of 100x was used in all panels.



Supplementary Figure S2 – Proof-of-concept experiment illustrating cytokines-mediated dedifferentiation. (A) Sphere-forming assay performed in different conditions of cytokines' abrogation, showing that spheres were observed whenever either IL-6 or Activin-A was present. G-CSF was unable to induce sphere formation. (B) Perimeter analysis. Corroborating the previous results, bigger spheres were attained when only IL-6 was present. A magnification of 100x was used in all panels. 10 spheres were measured in each condition. Data represent means \pm SEM. Differences between the means were evaluated by one-way ANOVA followed by a Bonferroni post test.

Supplementary Table S1. Statistical significance of drug-resistance assays in Figure 1F and 2F. MTX, methotrexate; Cis, cisplatin; Gem, Gemcitabine. Differences between the means were evaluated by a repeated measures ANOVA followed by a Bonferroni post test in relation to control. n.s., no significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Cell line	MTX (μM)			Cis (μM)			Gem (μM)		
	0.1	10	50	0.1	10	50	0.1	10	50
BEAS-2B	**	***	***	***	***	***	***	***	***
Cont1	***	***	***	***	***	***	***	***	***
RenG2	*	**	***	*	*	**	*	**	**
DRenG2	***	***	***	n.s	n.s	*	n.s	n.s	n.s
DDRenG2	**	***	***	n.s	n.s	*	n.s	n.s	n.s
SC-DRenG2	n.s	n.s	*	–	–	–	–	–	–
SC-DDRenG2	n.s	n.s	*	–	–	–	–	–	–