Supplementary Materials: The Interaction Between Laminin-332 and $\alpha 3\beta 1$ Integrin Determines Differentiation and Maintenance of CAFs, and Supports Invasion of Pancreatic Duct Adenocarcinoma Cells

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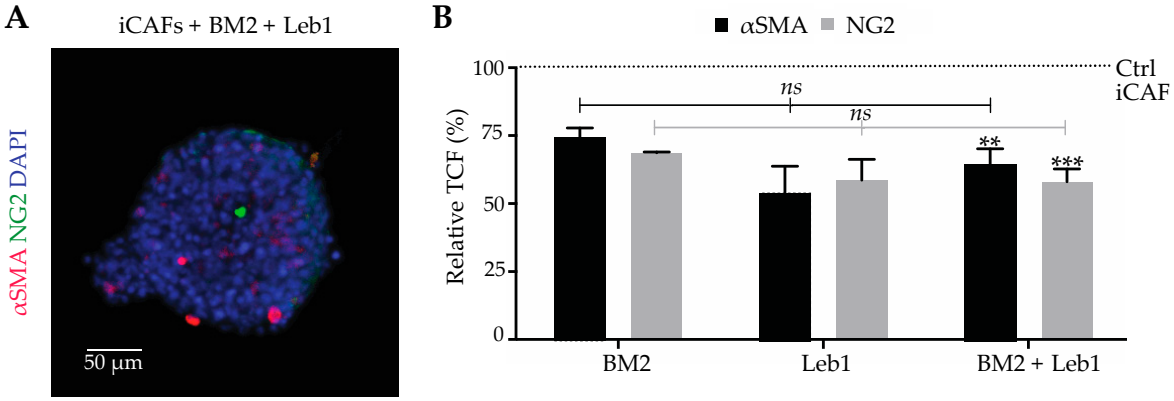

Figure S1. Double treatment of iCAFs with both BM2 and lebein1 did not yield a synergistic effect as compared to single treatment with either BM2 or lebein-1. (**A**) As in Figure 4A, spheroids of iCAFs were treated with both BM2 (20 µg/mL) and lebein1 (10 µg/mL) for 12 h. (**B**) After immunofluorimetric staining, αSMA and NG2 were quantified and normalization to the corresponding signals of non-treated iCAF spheroids. Means ± SEM of three independent experiments are shown and compared with non-treated iCAF spheroids with a *t*-test (**, $p < 0.01$; ***, $p < 0.001$) Both CAF-markers were significantly reduced. However, the reduction of the double treatment with BM2 and lebein-1 did not significantly differ from the signal reductions observed for the individual treatment with either of the inhibitor of integrin-laminin-332 interaction. The values of individual treatment do not differ from the values of double treatment, ruling out that cellular interaction with laminin-332 via receptors other than laminin-binding integrins play a major role.

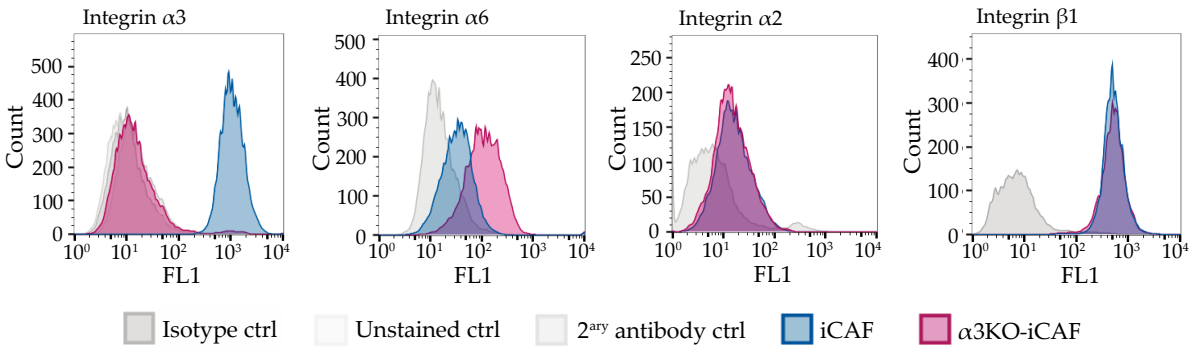

Figure S2. Flow cytometric expression analysis of integrin α3, α6, α2 and β1 subunits to compare iCAFs and integrin α3KO-iCAFs. After fluorescence-assisted cell sorting, a pure population of integrin α3β1 knocked-out iCAFs was obtained. This histogram presents three different controls, to assure that the population is indeed knock-out: isotype control, unstained cells control and secondary antibody control. The α3KO-iCAFs appear to up-regulate α6 integrin slightly. Integrin α2 subunit expression was not altered by the knock-out of the integrin α3 subunit, neither was the β1 expression. These three experiments were performed with the isotype control.

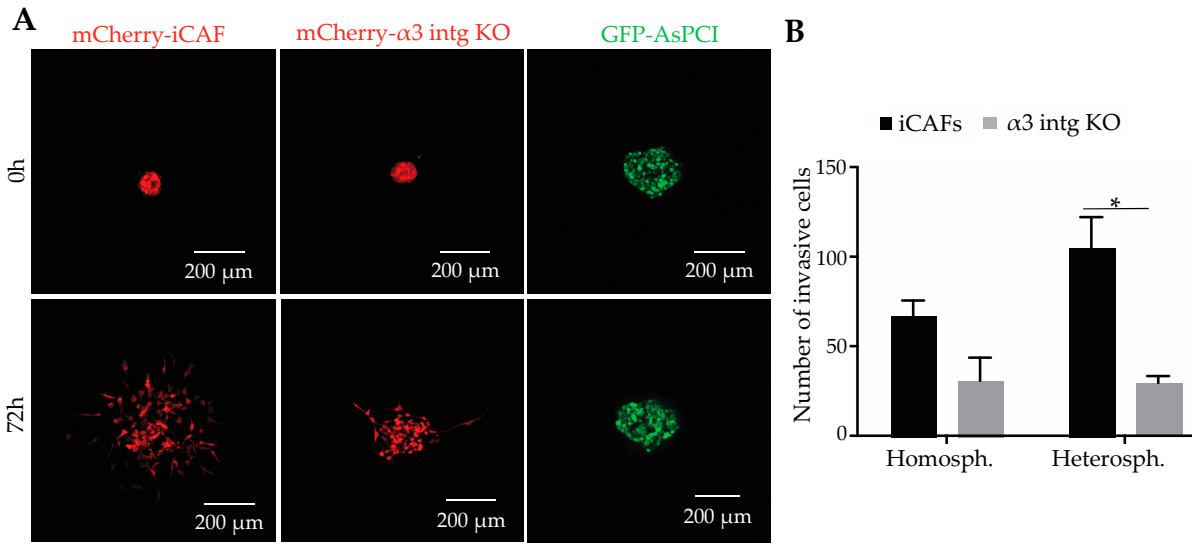

Figure S3. Progression of control homospheroids consisting of only one cell type (mCherry-iCAFs, mCherry-α3KO-iCAFs and GFP-AsPC-I) in the spheroid invasion assay, for comparison to heterospheroids (Figure 6C). (**A**) mCherry-iCAFs (red) were able to invade the surrounding gel. So are mCherry-α3KO-iCAFs (red), although in lower numbers. In contrast, GFP-AsPC-I cells (green) failed to leave the spheroid, unless when co-cultured with mCherry-iCAFs or with mCherry-α3KO-iCAFs (Figure 6C). The latter stimulated less GFP-AsPC-I cells to invade the gel as shown in figure Figure 6E. (**B**) The number of invasive mCherry iCAFs and mCherry-α3KO-iCAFs were compared when in homospheroids or in heterospheroids. The invasive cells were count as shown in Figure 6D. Means ± SEM of three independent experiments are shown and compared by t-test (*, $p < 0.05$).

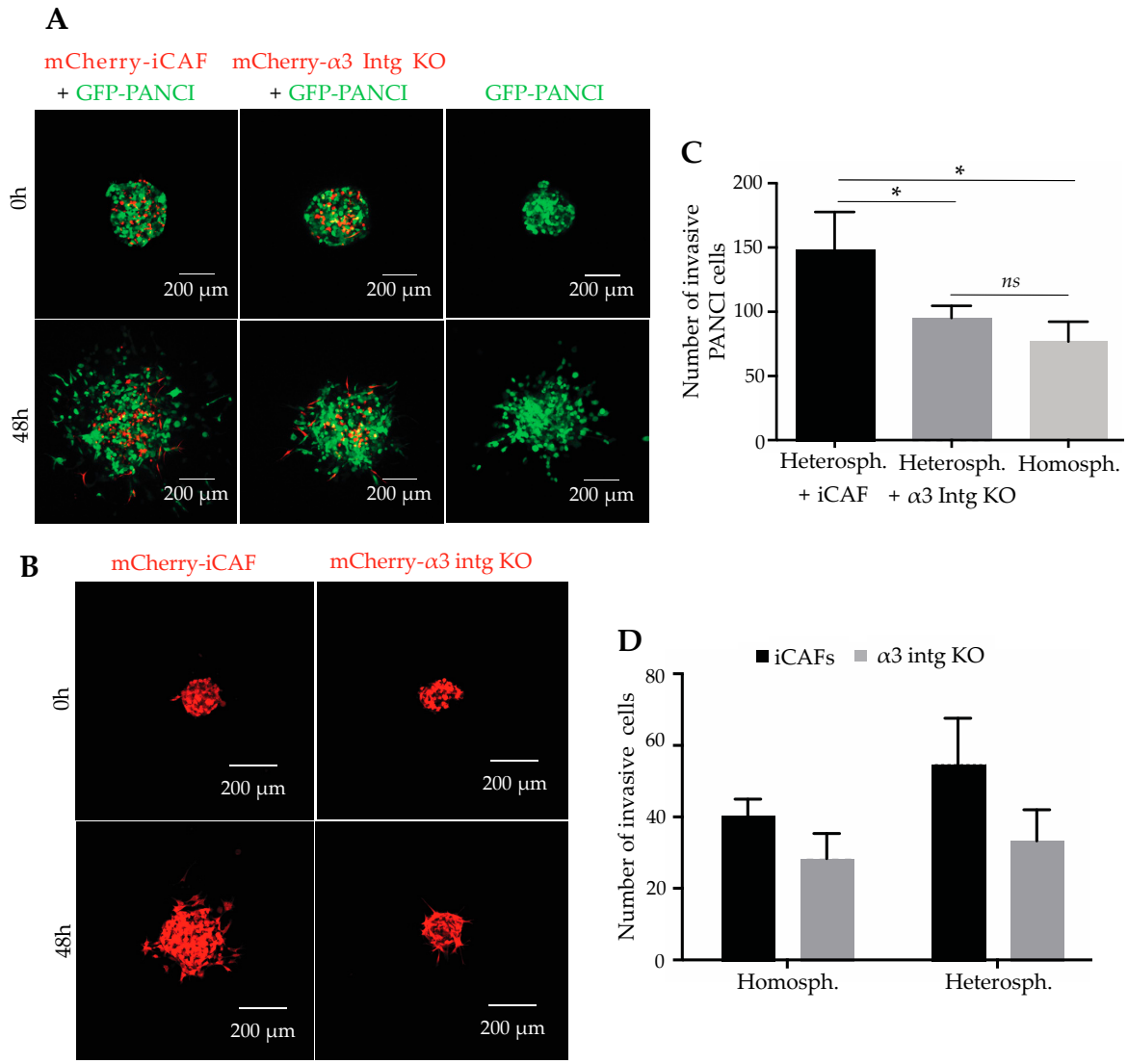

Figure S4. The invasion of GFP-labelled PANC-I also depended on neighbouring mCherry-labelled iCAFs in an α3β1 integrin-dependent manner in the spheroid invasion assay, similarly to the invasion of AsPC-I cells. (**A**) Although the GFP-PANC-I cells invade the surrounding gel from homospheroids, this invasion was further increased when the cancer cells were co-cultures in heterospheroids with the mCherry-iCAF. In contrast, mCherry-α3KO-iCAFs failed to support PANC-I cells invasion significantly. Invasion was measured after 48h due to the higher invasiveness of PANC-I cells. (**B**) The invasion of control homospheroids made of mCherry-iCAFs and mCherry-α3KO-iCAFs, alone. (**C**) Biometric evaluation of invading PANC-I cancer cells. As compared to the homospheroids of PANC-I, the number of invaded cancer cells differed significantly, only if they were cultured in heterospheroids with wild- type iCAFs, but not with integrin α3β1-deficient iCAFs. (**D**) The numbers of invasive mCherry iCAFs and mCherry-α3KO-iCAFs were compared from homospheroids and heterospheroids. Although remarkably more iCAFs, especially the integrin α3β1-bearing iCAFs, invaded from heterospheroids than from homospheroids, suggesting also a promigratory influence of cancer cells on iCAFs, these differences did not reach significance level. The invasive cells in images similar to the ones in (**A**) and (**B**) were count as shown in Figure 6D. Means ± SEM of three independent experiments are shown in (**C**) and (**D**), and compared by t-test (*, $p < 0.05$).