Supporting Information for the article:

Three-dimensional cell culture conditions affect the proteome of cancer-associated fibroblasts

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Supporting Information

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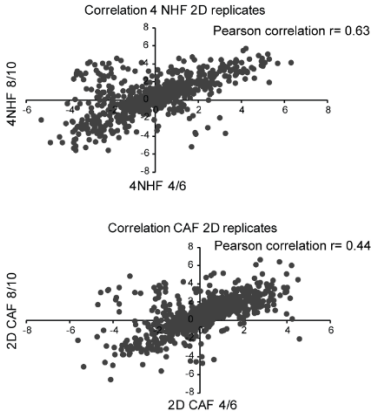
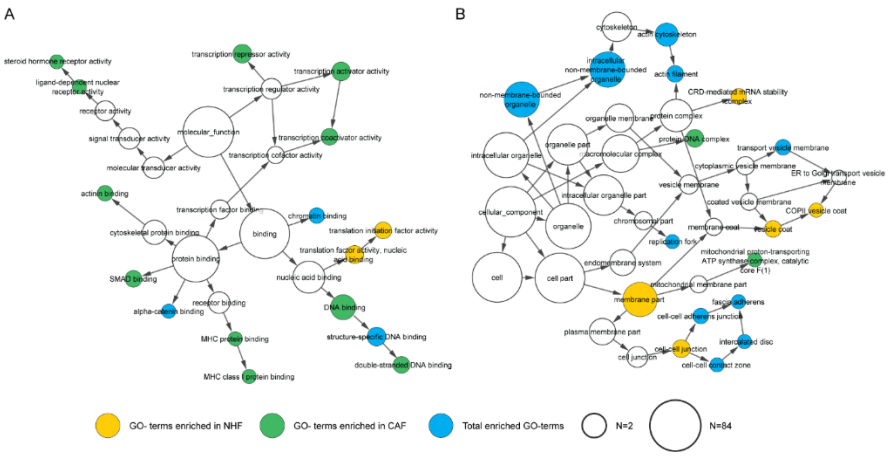
- **Supplementary Table S1:** SILAC ratios of NHF and CAF from 2D culture
- **Supplementary Table S2:** Collagen I (Corning) and Matrigel (growth factor reduced, Corning)
- **Supplementary Table S3:** SILAC-ratios from 2D versus 3D culture conditions
- **Supplementary Table S4:** SILAC ratios of NHF and CAF from 3D culture
- **Supplementary Table S5:** Santi et al. 2017. Comparison of the CAF consensus proteome to 2D vs 3D data
- **Supplementary Table S6:** Significantly regulated proteins in 2D and 3D normalized to the SuperSILAC mix

Supplementary Figure S1. Correlation of biological replicates of NHF and CAF proteomes from cells grown in 2D.



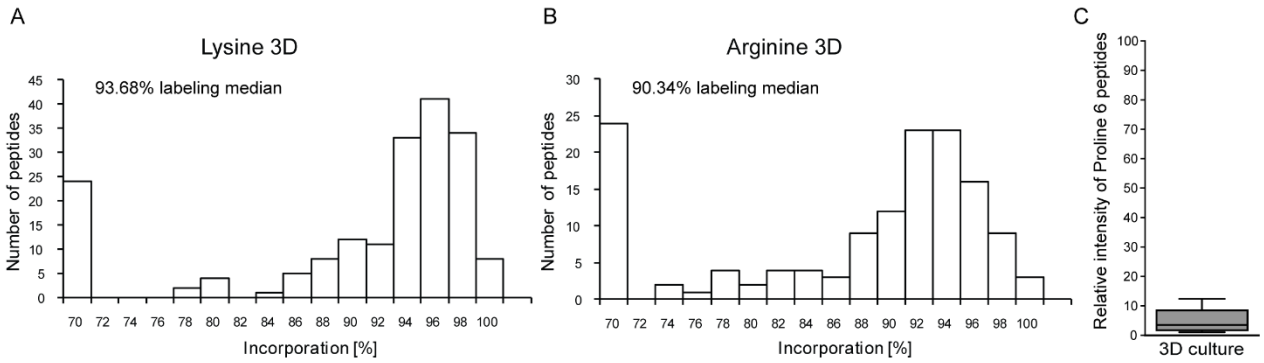
The two biological replicates were analyzed with a SILAC- amino acid swap between experiments.

Supplementary Figure S2. Enriched GO-terms of differentially regulated proteins comparing NHF and CAF grown in 2D.



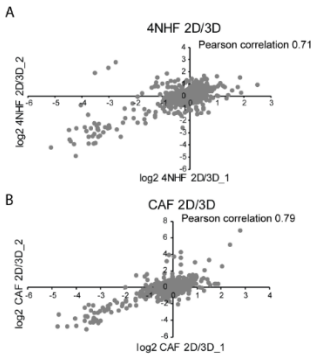
Shown are significantly enriched GO-terms of **(A)** Molecular function and **(B)** Cellular component, of significantly dysregulated proteins between NHF and CAF ($p<0.01$). Colored circles indicate $p<0.03$ for the GO-term enrichment.

Supplementary Figure S3. **Labeling efficiency of fibroblasts in 3D culture using reduced lysine and arginine concentrations.**



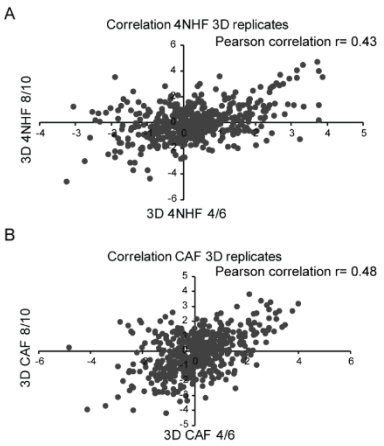
(A) Lysine and **(B)** arginine isotope incorporation. SILAC media was supplemented with 42 mg l^{-1} heavy L-arginine and 73 mg l^{-1} heavy L-lysine. Median label incorporations are indicated. **(C)** Relative intensity of proline 6 containing peptides of heavy labeled NHF and CAF from 3D culture. Cells were cultured using the final concentrations of 84 mg l^{-1} L-arginine, 146 mg l^{-1} L-lysine and 125 mg l^{-1} proline (n=8).

Supplementary Figure S4: Correlation of biological replicates of NHF and CAF proteomes comparing cells grown in 2D and 3D.



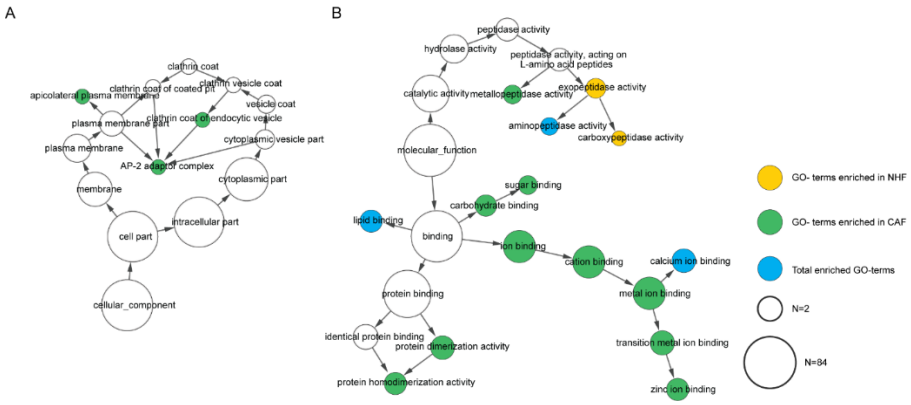
(A) Quantitative proteomics analysis of NHF in 2D and 3D culture and **(B)** CAF in 2D and 3D culture. Biological replicates were performed using swapped SILAC labels.

Supplementary Figure S5: Correlation of biological replicates of NHF and CAF cultured in 3D.



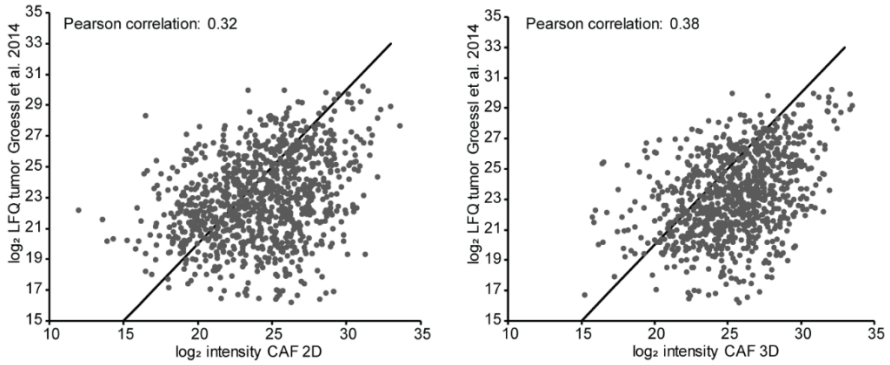
(A) Quantitative proteomics analysis of NHF in 3D culture and **(B)** CAF in 3D culture. Biological replicates were performed using swapped SILAC labels.

Supplementary Figure S6: GO-term enrichment analysis of dysregulated proteins comparing NHF and CAF grown in 3D.



Shown are significantly enriched GO-terms of **(A)** Molecular function and **(B)** cellular compartment. Significant regulated proteins ($p<0.05$) from a one sample t-test of 3D CAF vs 3D NHF were considered for GO-term enrichment. Colored circles indicate $p<0.03$ for the GO-term enrichment.

Supplementary Figure S7. Data from Groessl et al. 2014 in comparison with the 2D and 3D CAF proteomes of this study.



893 proteins of 3D and 983 proteins of 2D CAF cultures, respectively, aligned with the published *ex vivo* breast cancer biopsy analysis [1]. 2D CAF showed a slightly decreased Pearson correlation compared to 3D.

References

1. Groessl, M.; Slany, A.; Bileck, A.; Gloessmann, K.; Kreutz, D.; Jaeger, W.; Pfeiler, G.; Gerner, C., Proteome profiling of breast cancer biopsies reveals a wound healing signature of cancer-associated fibroblasts. *J Proteome Res* **2014,** *13* (11), 4773-82.