Electronic Supplementary Information:

Targeting transdifferentiated hepatic stellate cells and monitoring the hepatic fibrogenic process by means of IGF2R-specific peptides designed *in silico*

Florian Weber,[a,b] Tommaso Casalini,[c,d] Gina Valentino,[a,b] Lorine Brülisauer,[b] Nico Andreas,[e] Andreas Koeberle,[f,g] Thomas Kamradt,[e] Alessandro Contini,[h] and Paola Luciani*[a,b]

[a.] *Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Bern, Switzerland;*
E-Mail: paola.luciani@dcb.unibe.ch
[b.] *Department of Pharmaceutical Technology, Institute of Pharmacy, Friedrich Schiller University Jena, Jena, Germany*
[c.] *Institute of Mechanical Engineering and Material Technology, Department of Innovative Technology, SUPSI, Manno, Switzerland*
[d.] *Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland*
[e.] *Institute of Immunology, Jena University Hospital, Jena, Germany*
[f.] *Michael Popp Institute and Center for Molecular Biosciences (CMBI), University of Innsbruck, Innsbruck, Austria*
[g.] *Chair of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich Schiller University Jena, Jena, Germany*
[h.] *Dipartimento di Scienze Farmaceutiche-Sezione di Chimica Generale e Organica "A. Marchesini", Università degli Studi di Milano, Milano, Italy*
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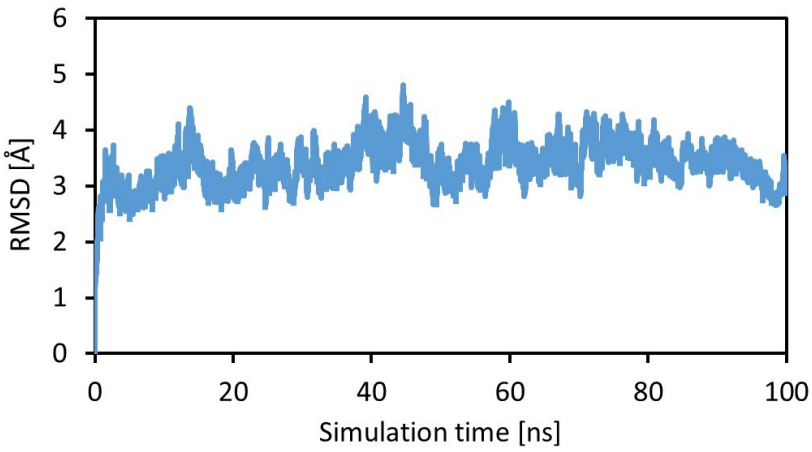

Figure S1. Root mean square displacement (RMSD) as function of simulation time.

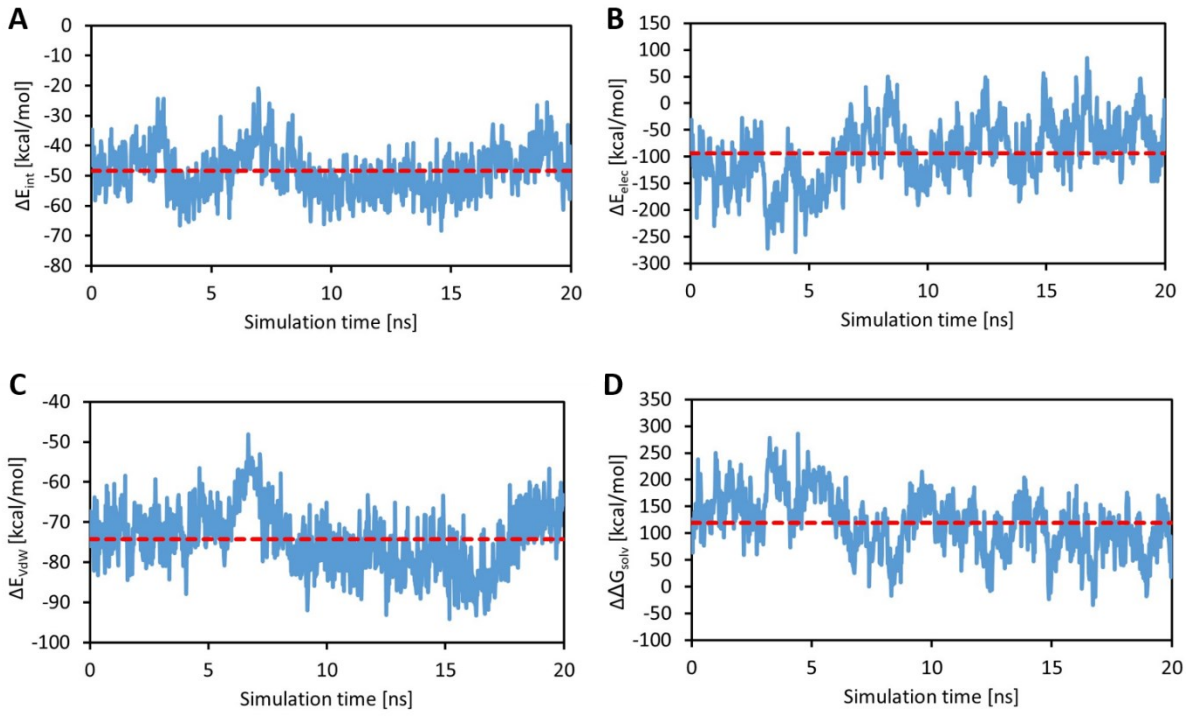

Figure S2. Interaction energy between IGF2R and IGF2 (ΔE_{int}) **(A)** along with specific electrostatic (ΔE_{elec}) **(B)**, van der Waals (ΔE_{VdW}) **(C),** and solvation ($\Delta\Delta G_{solv}$) **(D)** contributions as a function of time

for the last 20 ns of the molecular trajectory, which were employed for post-processing with MMGBSA. The red dotted line represents the average value, reported in the main text in Table 2.

Table S1. Pairwise interaction energy values ΔE_{int} and specific electrostatic (ΔE_{el}), van der Waals (ΔE_{VdW}), and polar solvation ($\Delta\Delta G_{polar}$) contributions resulting from per-residue decomposition; non-polar solvation terms were omitted. Only ΔE_{int} values equal or lower than 2 kcal/mol are reported. Analysis was performed considering the last 20 ns of the molecular trajectory. Results are expressed as mean ± standard deviation.

Receptor	Ligand	ΔE_{VdW} [kcal/mol]	ΔE_{El} [kcal/mol]	$\Delta\Delta G_{polar}$ [kcal/mol]	ΔE_{int} [kcal/mol]
R1571	E12	0.17 ± 0.88	−49.49 ± 4.32	38.55 ± 2.13	−11.65 ± 2.37
R1571	E6	0.52 ± 0.77	−24.87 ± 4.82	15.54 ± 1.58	−9.30 ± 3.34
K1631	E47	−0.66 ± 0.64	−37.83 ± 6.71	33.63 ± 3.51	−5.94 ± 3.23
S1543	D23	−0.31 ± 0.63	−5.99 ± 5.16	2.76 ± 2.27	−4.12 ± 3.07
P1599	L43	−1.18 ± 0.46	−1.94 ± 0.49	0.63 ± 0.10	−3.61 ± 0.59
K1601	D42	−0.35 ± 0.50	−32.66 ± 10.89	30.01 ± 7.92	−3.55 ± 3.28
Q1569	D15	−0.53 ± 0.48	−3.32 ± 4.58	1.50 ± 2.76	−2.95 ± 2.58
K1631	L43	−1.53 ± 0.28	−1.43 ± 0.64	1.50 ± 0.53	−2.76 ± 0.42
H1696	D23	−0.85 ± 0.67	−4.24 ± 3.34	3.04 ± 2.36	−2.72 ± 1.98
Y1542	F19	−1.26 ± 0.29	−0.24 ± 0.23	0.15 ± 0.14	−2.37 ± 0.59
P1599	D42	−1.02 ± 0.24	0.79 ± 0.61	−1.47 ± 0.53	−2.26 ± 0.31

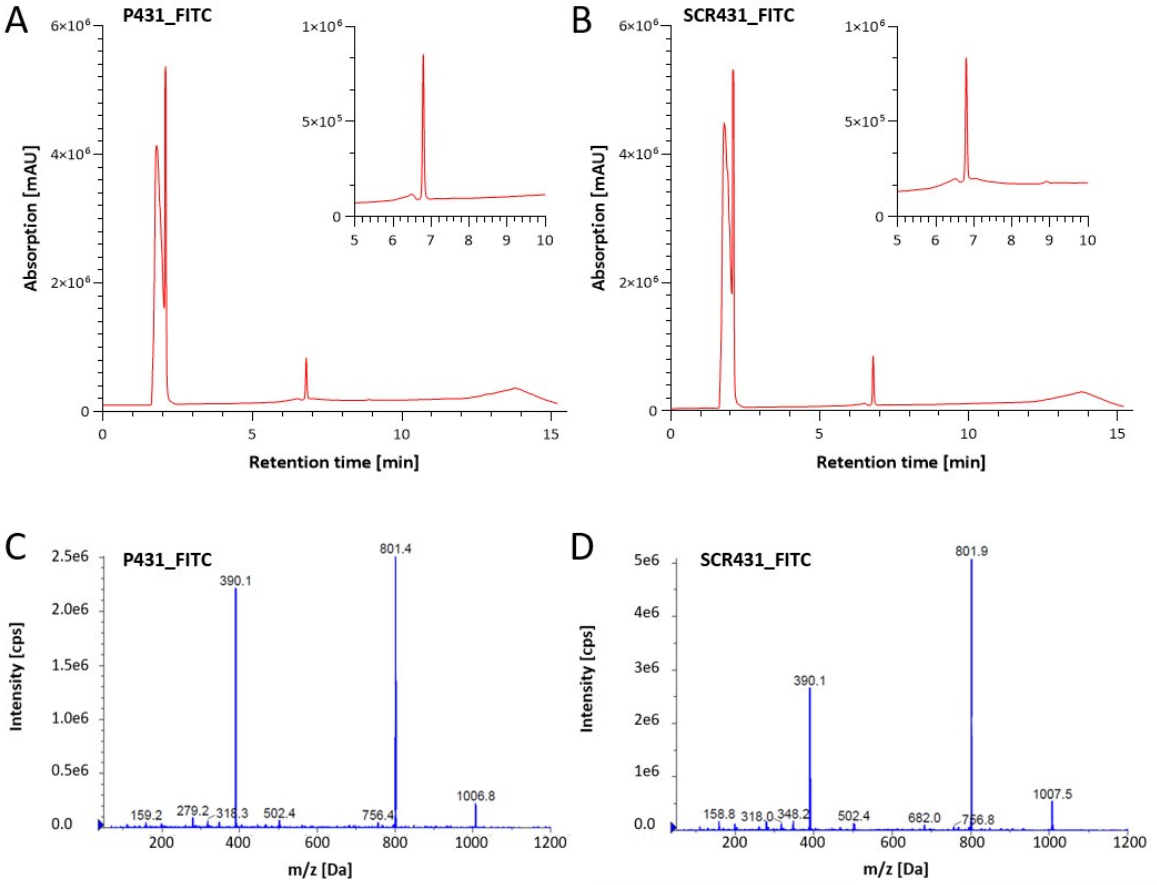

Figure S3. Representative chromatograms (HPLC, UV-detection, λ = 220 nm) of the sequence P431_FITC **(A)** and SCR431_FITC **(B)** after purification and corresponding mass spectra **(C)** and **(D)**, respectively. The peak eluted between 2 and 3 min represents the injection peak and the solvent DMSO.

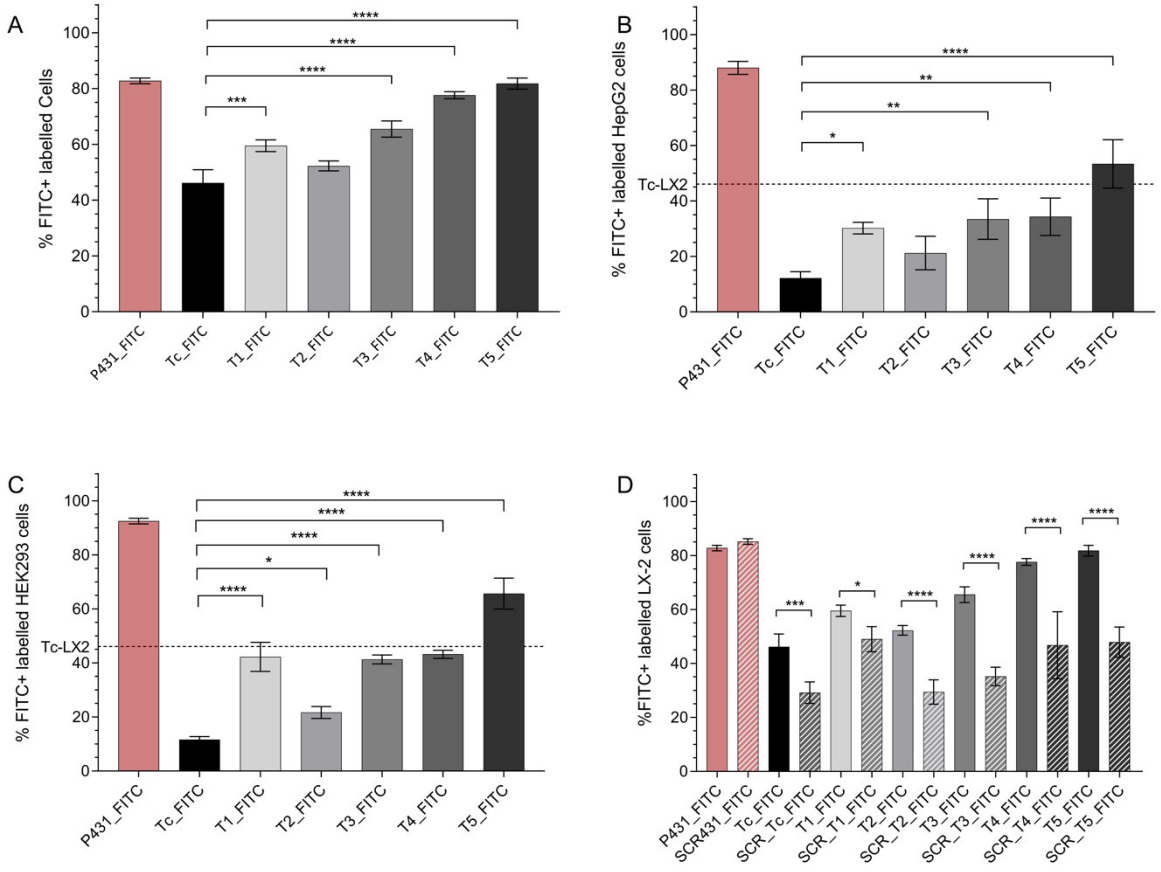

Figure S4. Flow cytometry data reporting %FITC+ of LX-2 cells after the incubation with targeting peptides **(A)**, %FITC+ of HepG2 cells after the incubation with targeting peptides **(B)**, %FITC+ of HEK293 cells after the incubation with targeting peptides **(C)**. (**D**) represents the %FITC+ events of LX-2 cells after the incubation with targeting peptide and their associated scrambled sequences. All cell lines were incubated with 10 μM peptide for 1 h at 37 °C. The dotted line represents the %FITC+ events of LX-2 cells after the incubation with 10 μM **Tc** (lowest %FITC+ on LX-2 cells). All sequences except **P431** and **T5** show a lower binding to HepG2 cells and HEK293 cells in respect to LX-2 cells.

Table S2. Displayed are the results of the multiple comparison test (one-way ANOVA, Holm-Sidak's multiple comparison test) for LX-2 cells after incubation with targeting peptides. The concentration of the peptides was 10 µM (1 h, 37 °C). Results were obtained by flow cytometry.

Multiple comparisons	Mean Diff.	Summary	Adjusted P value
P431_FITC vs. Tc_FITC	36.6	****	<0.0001
P431_FITC vs. T1_FITC	23.27	****	<0.0001
P431_FITC vs. T2_FITC	30.5	****	<0.0001
P431_FITC vs. T3_FITC	17.27	****	<0.0001
P431_FITC vs. T4_FITC	5.17	ns	0.051
P431_FITC vs. T5_FITC	1	ns	0.6144
Tc_FITC vs. T1_FITC	−13.33	****	<0.0001
Tc_FITC vs. T2_FITC	−6.1	*	0.0316
Tc_FITC vs. T3_FITC	−19.33	****	<0.0001
Tc_FITC vs. T4_FITC	−31.43	****	<0.0001
Tc_FITC vs. T5_FITC	−35.6	****	<0.0001
T1_FITC vs. T2_FITC	7.23	*	0.0112
T1_FITC vs. T3_FITC	−6	*	0.0316
T1_FITC vs. T4_FITC	−18.1	****	<0.0001
T1_FITC vs. T5_FITC	−22.27	****	<0.0001
T2_FITC vs. T3_FITC	−13.23	****	<0.0001
T2_FITC vs. T4_FITC	−25.33	****	<0.0001
T2_FITC vs. T5_FITC	−29.5	****	<0.0001
T3_FITC vs. T4_FITC	−12.1	****	<0.0001
T3_FITC vs. T5_FITC	−16.27	****	<0.0001
T4_FITC vs. T5_FITC	−4.167	ns	0.0938

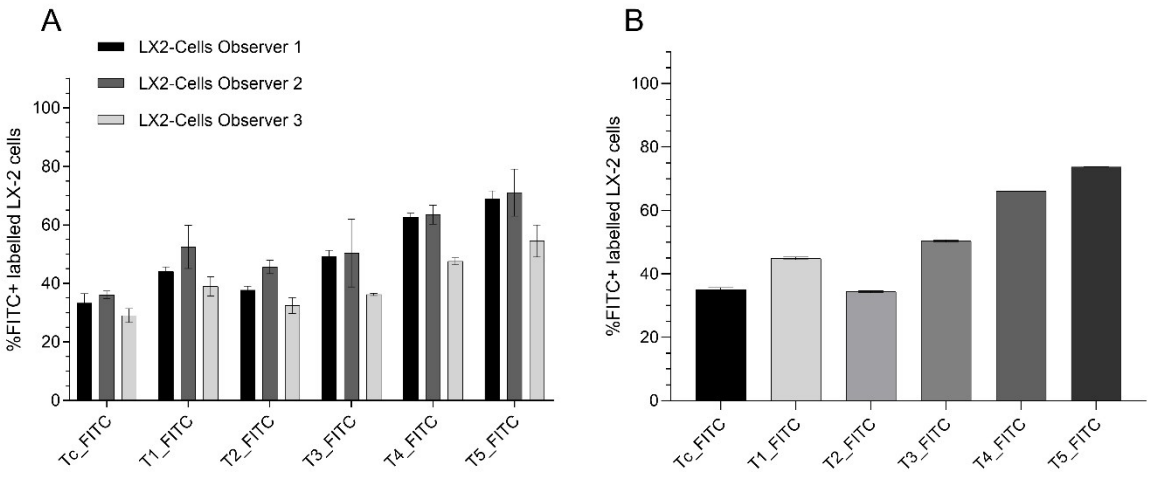

Figure S5. Flow cytometry data reporting %FITC+ of LX-2 cells after the incubation with targeting peptides by different observers **(A)** and in a different lab **(B).** All cell lines were incubated with 10 μM peptide for 1 h at 37 °C.

Table S3. Displayed are the results of the multiple comparison test (one-way ANOVA. Holm-Sidak's multiple comparison test) for the ratios (R_{Active}) of %FITC+ $_{TGF\text{-}\beta1}$/%FITC+ $_{Rol+PA}$ after the incubation with FITC-labelled peptide at a concentration of 10 µM for 1 h at 37 °C. Prior to the incubation. cells were transdifferentiated into the perpetuated state with 10 ng/mL TGF-β_1 or into the quiescent-like state with Rol (10 µM) and PA (300 µM) for 24 h. R_{Active} > 1 represent a specific selectivity for the perpetuated phenotype of the LX-2 cell line. Results were obtained by flow cytometry.

Multiple comparisons	Mean Diff.	Summary	Adjusted P Value
P431_FITC vs. Tc_FITC	−1.22	****	<0.0001
P431_FITC vs. T1_FITC	−0.59	****	<0.0001
P431_FITC vs. T2_FITC	−0.54	****	<0.0001
P431_FITC vs. T3_FITC	−0.18	*	0.0215
P431_FITC vs. T4_FITC	−0.26	**	0.002
P431_FITC vs. T5_FITC	0.04	ns	0.6626
Tc_FITC vs. T1_FITC	0.63	****	<0.0001
Tc_FITC vs. T2_FITC	0.68	****	<0.0001
Tc_FITC vs. T3_FITC	1.04	****	<0.0001
Tc_FITC vs. T4_FITC	0.96	****	<0.0001
Tc_FITC vs. T5_FITC	1.26	****	<0.0001
T1_FITC vs. T2_FITC	0.05	ns	0.6626
T1_FITC vs. T3_FITC	0.41	****	<0.0001
T1_FITC vs. T4_FITC	0.38	***	0.0003
T1_FITC vs. T5_FITC	0.63	****	<0.0001
T2_FITC vs. T3_FITC	0.36	***	0.0001
T2_FITC vs. T4_FITC	0.28	**	0.0011
T2_FITC vs. T5_FITC	0.59	****	<0.0001
T3_FITC vs. T4_FITC	−0.08	ns	0.4433
T3_FITC vs. T5_FITC	0.23	**	0.0054
T4_FITC vs. T5_FITC	0.30	***	0.0006

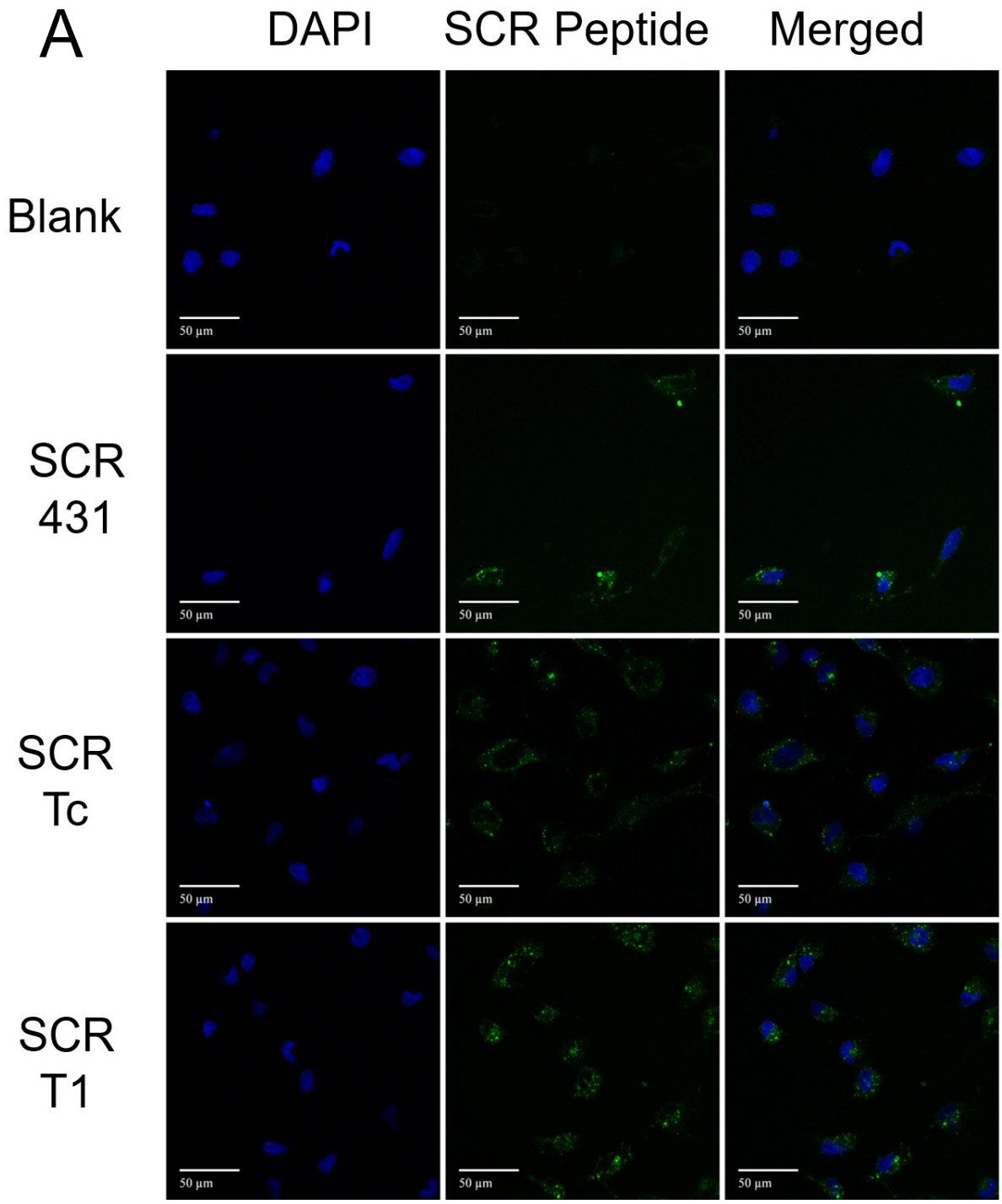

Figure S6A. Representative confocal microscopy images of naive LX-2 cells after a 1 h treatment (37 °C) with the scrambled sequence of **P431**, **Tc**, or **T1** at a concentration of 10 µM. Displayed are the summary z-stacks (> 20 single images per z-stack) for all tested sequences. Left panel: cell nucleus stained with DAPI (blue); middle panel: FITC-labelled peptide (green); right panel: merged images. Scale bar = 50 µm.

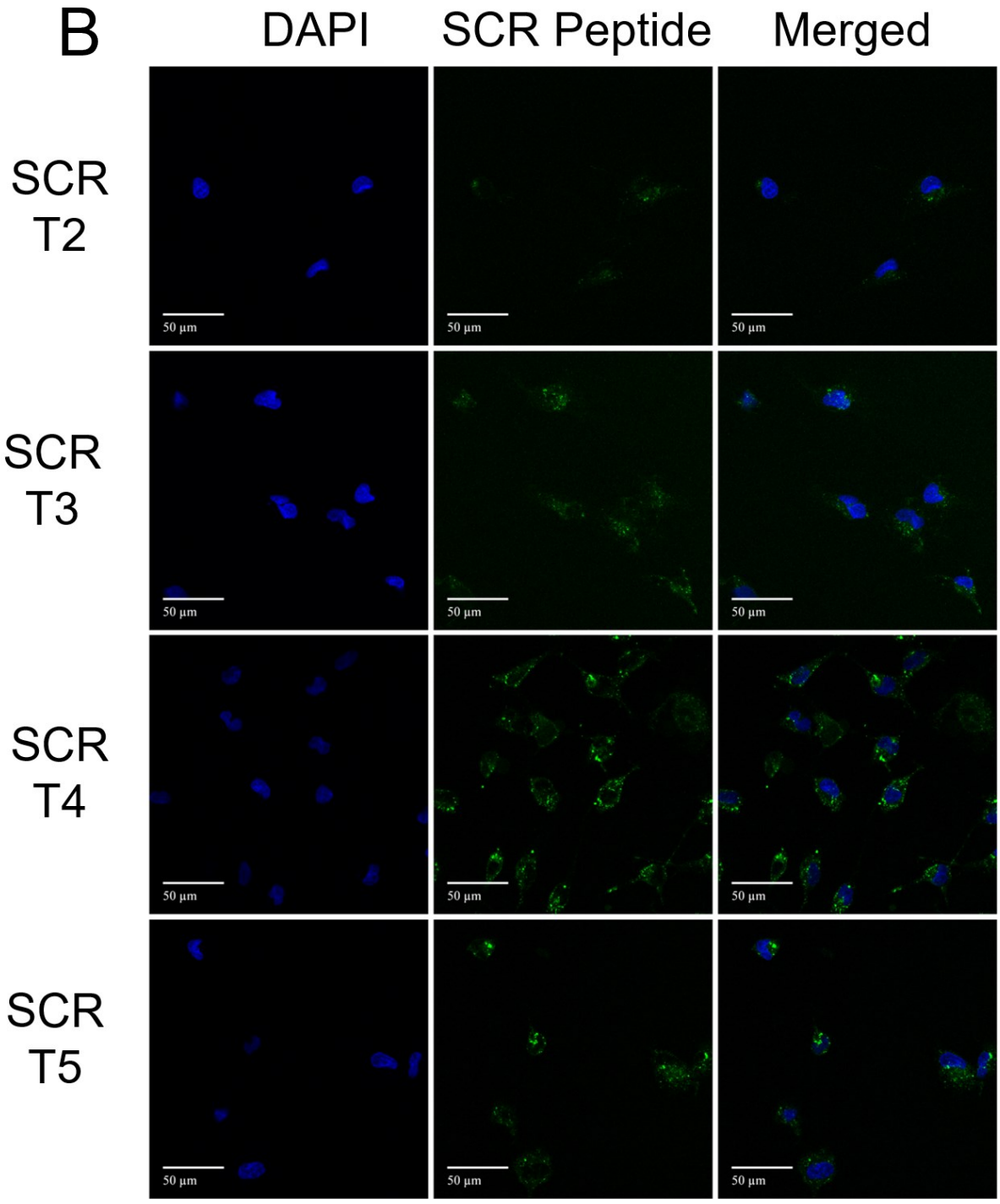

Figure S6B. Representative confocal microscopy images of naive LX-2 cells after a 1-h treatment (37 °C) with the scrambled sequence of **T2**, **T3**, **T4**, or **T5** at a concentration of 10 µM. Displayed are the summary z-stacks (> 20 single images per z-stack) for all tested sequences. Left panel: cell nucleus stained with DAPI (blue); middle panel: FITC-labelled peptide (green); right panel: merged images. Scale bar = 50 µm.

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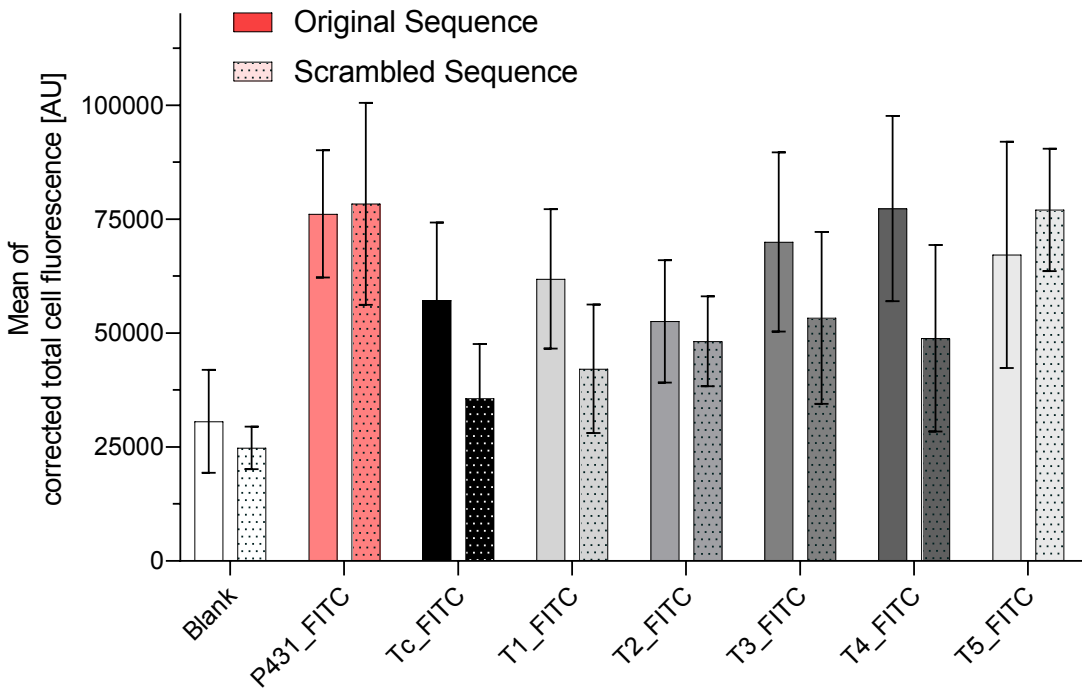

Figure S7. Quantification of the confocal images of LX-2 cells treated with 10 µM FITC-labelled peptides and their corresponding scrambled sequences in PBS for 1 h at 37 °C. Mean of corrected total cell fluorescence (CTCF) of the FITC-signal for all sequences and scrambled sequences were acquired using ImageJ (> 20 images per z-stack). Although no statistically significant differences between the various peptides and their associated scrambled sequences could be obtained for the CTCF analysis (ordinary one-way ANOVA with Tukey multiple comparison test), the obtained pattern of the fluorescence intensity for all peptides is comparable with the flow cytometry data (Figure 5A).

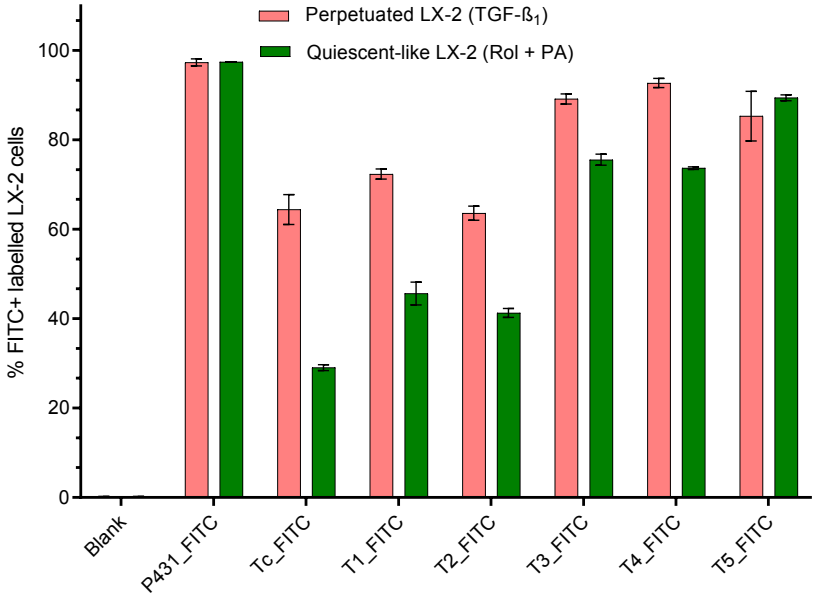

Figure S8. Flow cytometry data expressing %FITC+ perpetuated or quiescent-like LX-2 cells after incubation with targeting peptides. Naïve LX-2 cells were transdifferentiated into the perpetuated state with 10 ng/mL TGF-β_1 or into the quiescent-like state with RoI (10 μM) and PA (300 μM) for 24 h at 37 °C prior to the incubation with peptides. All transdifferentiated cells were then incubated with 10 μM peptide for 1 h at 37 °C.