

Single domain antibodies as carriers for intracellular drug delivery: a proof of principle study

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Supplementary Figures and Tables

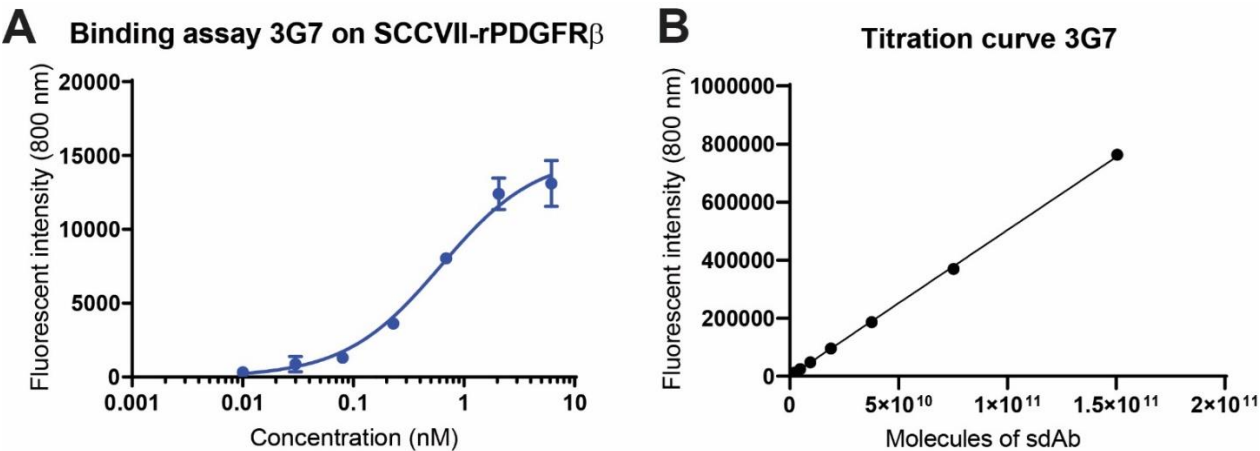


Figure S1. PDGF β receptor quantification on SCCVII-PDGFR β cells. (A). A binding assay with 3G7 conjugated to a fluorophore was performed in order to determine the B_{max} . (B). The B_{max} was then interpolated in the titration curve of 3G7 to determine the number of sdAb molecules at B_{max} .

Table S1. Calculated immune library sizes.

Animal	Library	Size
SNL154	rP-154	$9.6 * 10^6$
SNL155	rP-155	$4.8 * 10^6$

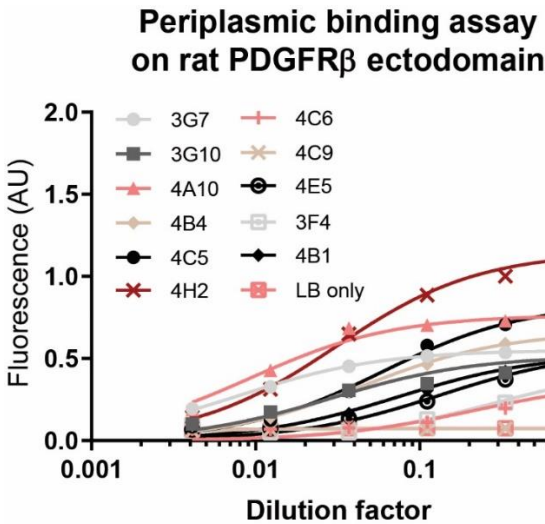


Figure S2. Serial dilutions of unpurified bacterial periplasm containing sdAbs binding to immobilized rat PDGFR β ectodomain.

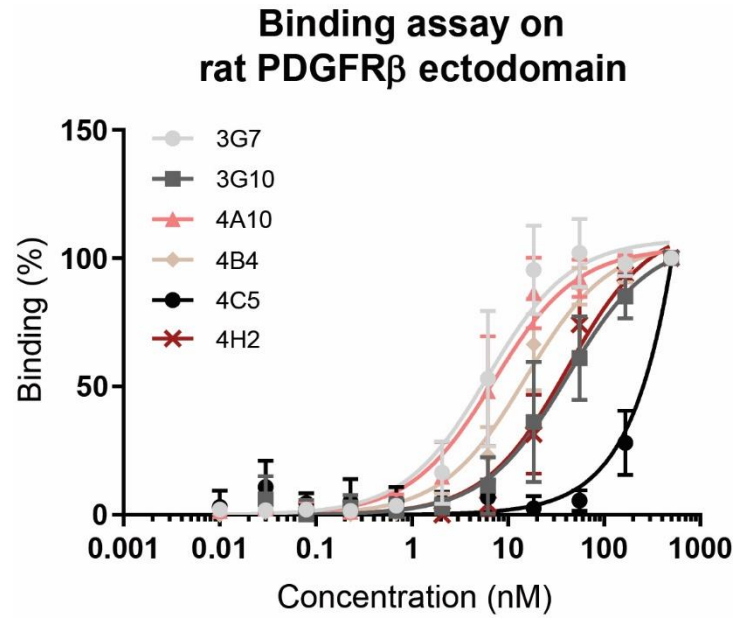


Figure S3. Serial dilutions of monomeric sdAbs binding to immobilized rat PDGFR β ectodomain.

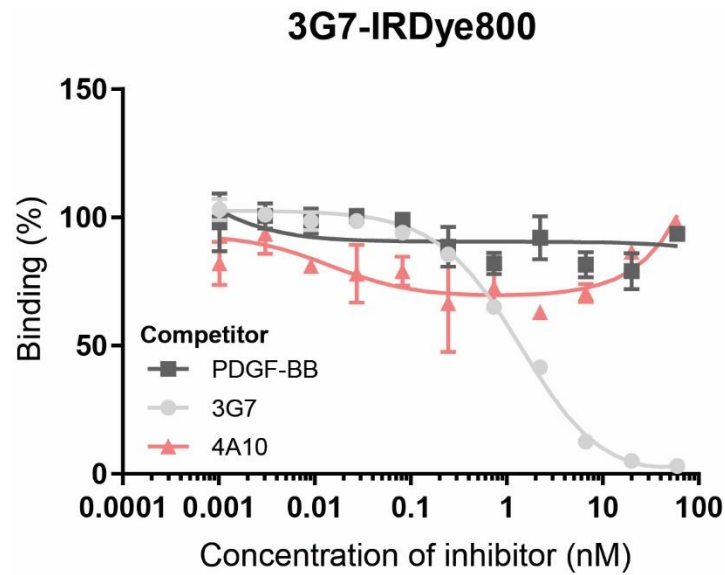


Figure S4. Competition assay in which 3G7 conjugated to IRDye800 (3G7-IRDye800) was incubated on immobilized rat PDGFR β -ECD in the presence or absence of excess unconjugated competitor (3G7 or PDGF-BB).

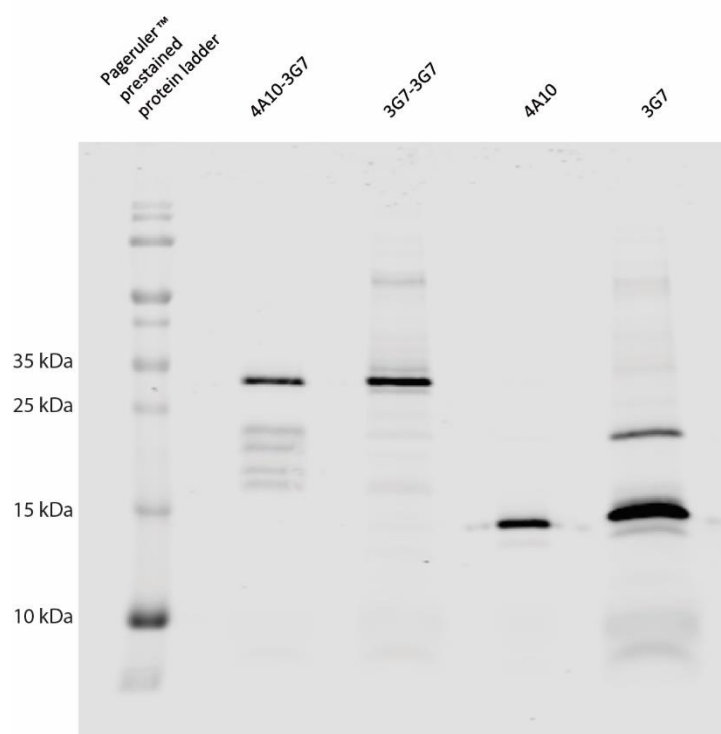


Figure S5. Purity of IRDye800 conjugated sdAbs on a SDS-PAGE gel. Signal shown is the IRDye800 signal.

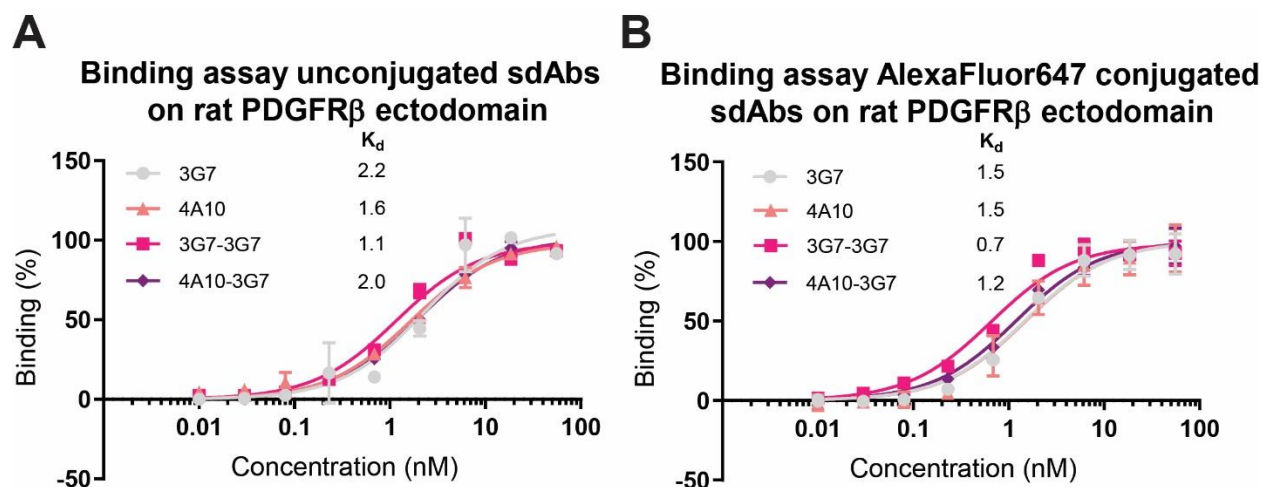


Figure S6. Serial dilutions of sdAbs binding to immobilized rat PDGFR β ectodomain. (A). Binding assay of unconjugated sdAbs binding to immobilized rat PDGFR β ectodomain detected indirectly. (B). Binding assay of AlexaFluor647 conjugated sdAbs binding to immobilized rat PDGFR β ectodomain detected indirectly.

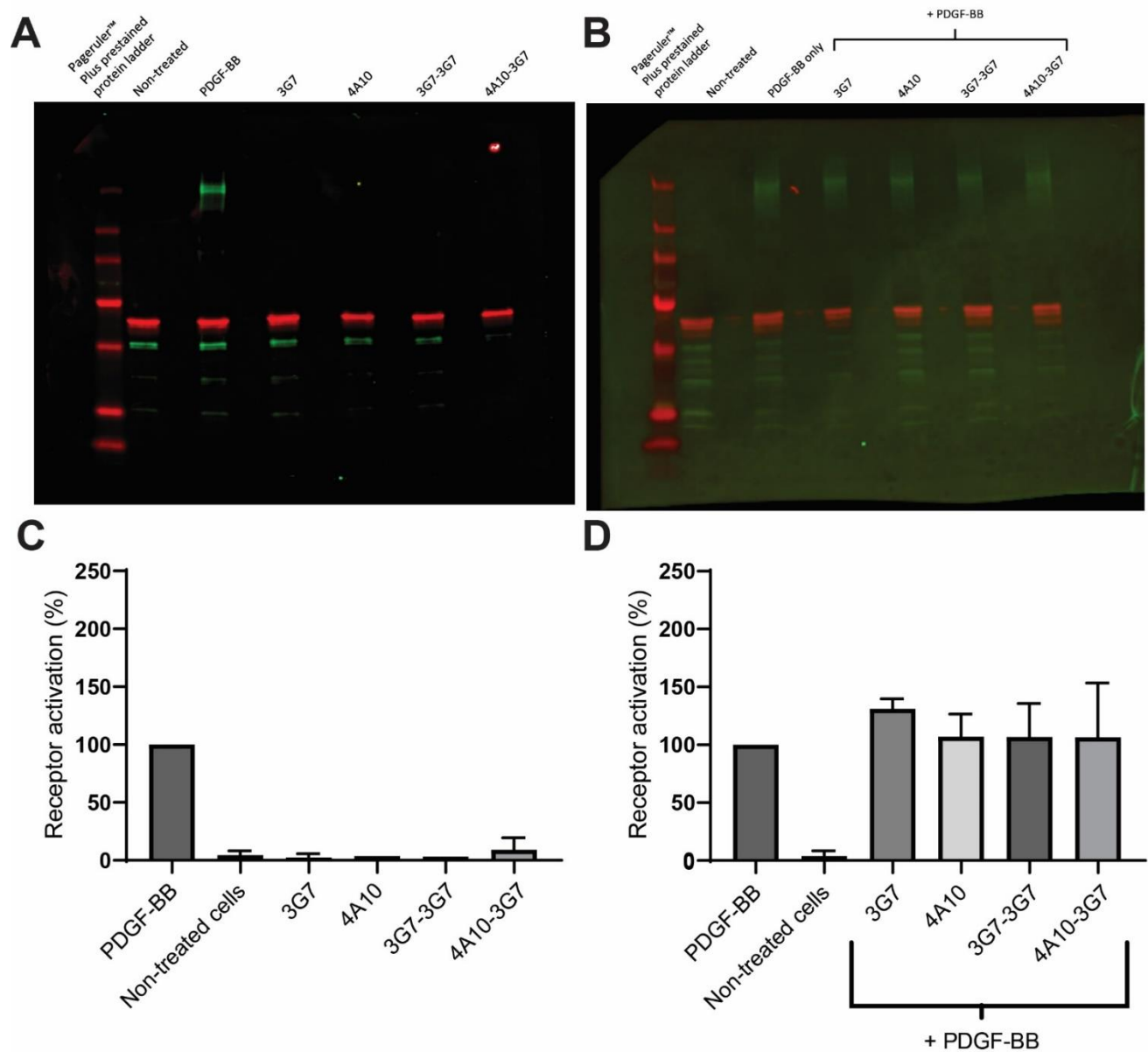


Figure S7. Original blots and quantification of Figure 4. Displayed in red is the IRDye700 signal (actin), and in green the IRDye800 signal (phospho-PDGFR β). (A). Untouched and uncropped blot of Figure 4A. (B). Untouched and uncropped blot of Figure 4B. (C). Quantification (phospho-PDGFR β / actin) of performed activation assays. (D). Quantification (phospho-PDGFR β / actin) of performed activation inhibition assays.

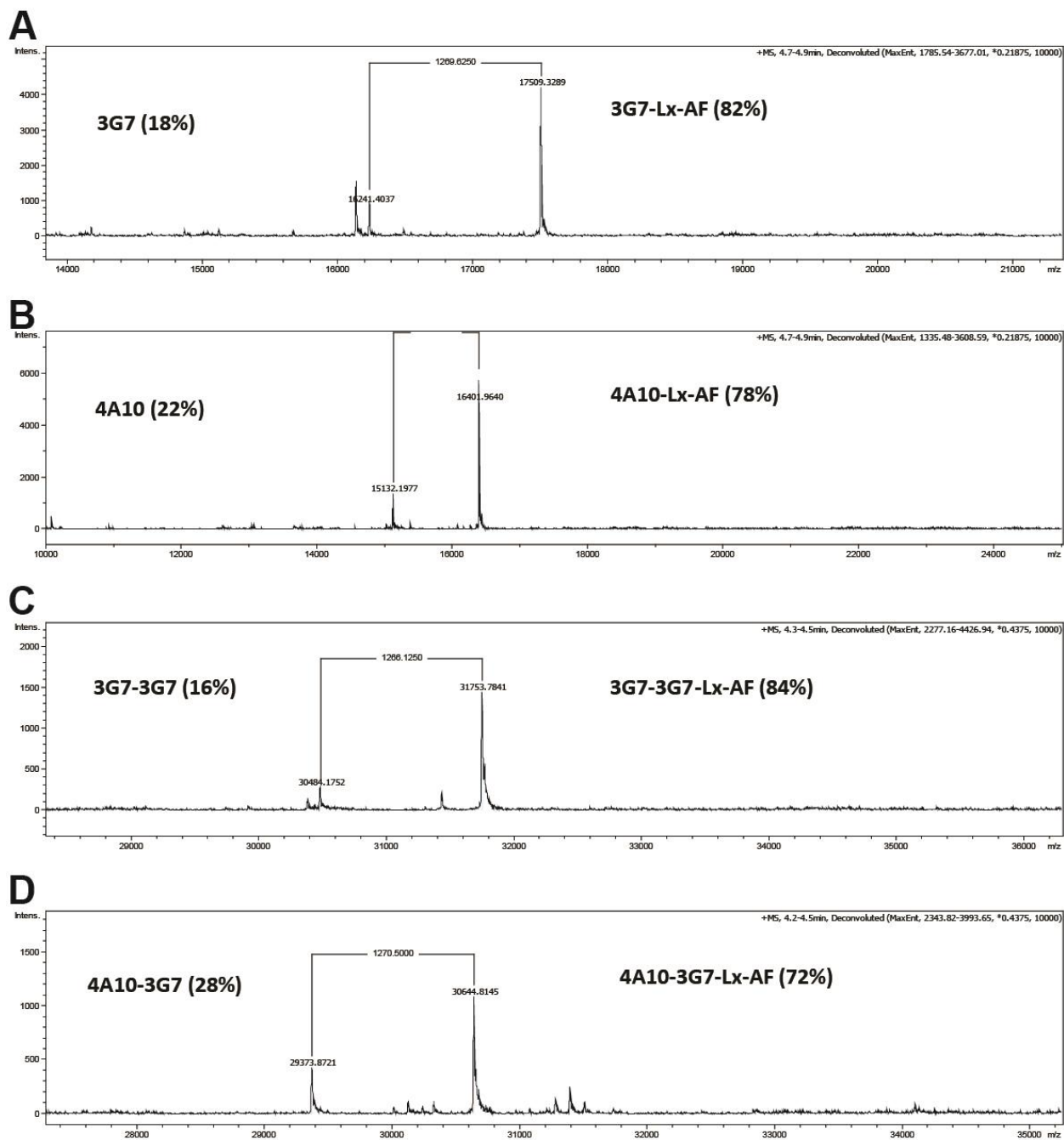


Figure S8. Deconvoluted mass spectra obtained upon SEC-MS analysis of the different auristatin F (AF) conjugates. (A). SEC-MS of 3G7 after conjugation to AF. (B). SEC-MS of 4A10 after conjugation to AF. (C). SEC-MS of 3G7-3G7 after conjugation to AF. (D). SEC-MS of 4A10-3G7 after conjugation to AF.

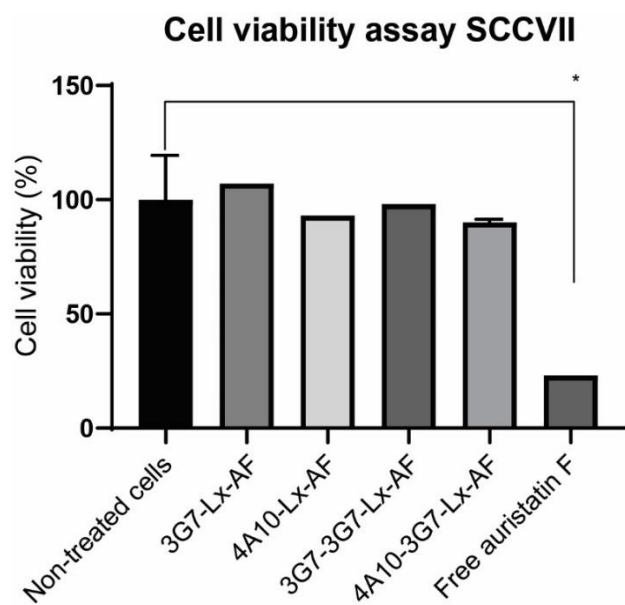


Figure S9. Cell viability assay on SCCVII cells. Cell viability is measured after 72 hours of treatment with 250nM conjugates or free Auristatin F. P values were determined using a one-way ANOVA test. * = $P \leq 0.05$ and ns = not significant.