SUPPLEMENTARY INFORMATION FOR

Optoacoustic imaging of GLP-1 Receptor with a near-infrared exendin-4 analog

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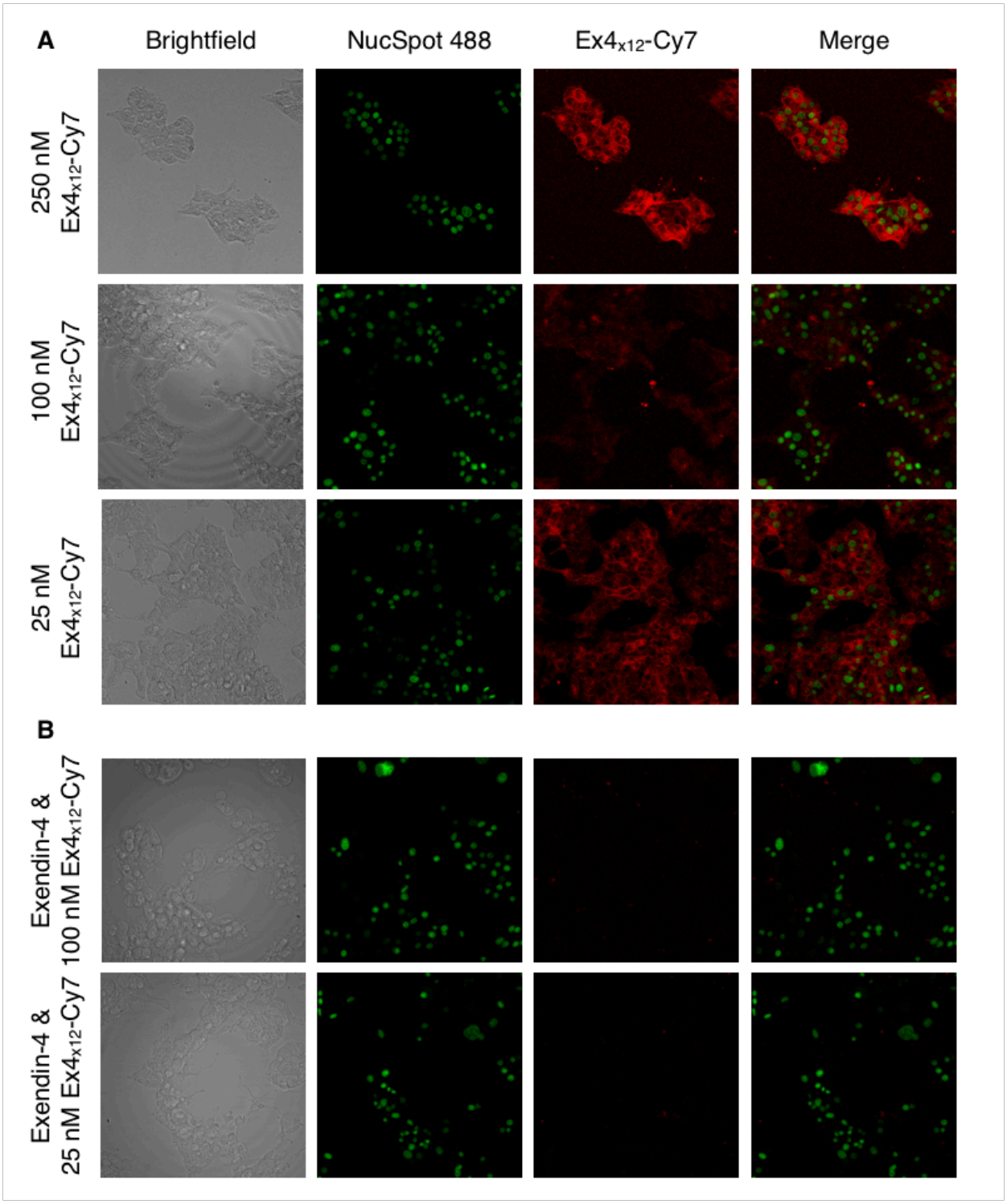

Figure S1. *In vitro* binding and inhibition studies. (A) Confocal microscopy imaging experiments using MIN6 cells following the addition of $E4_{x12}$-Cy7 (red) at different concentrations: 250 nM (*top row*), 100 nM (*middle row*) and 25 nM (*bottom row*).

Columns from *left* to *right:* brightfield (1st column), nuclear staining with NucSpot 488 (green, 2nd column), $E4_{x12}$-Cy7 staining (red, 3rd column) and the corresponding composite image (4th column). (B) Coincubation of $E4_{x12}$-Cy7 at 100 nM (*top*) and 25 nM (*bottom*) with excess exendin-4.

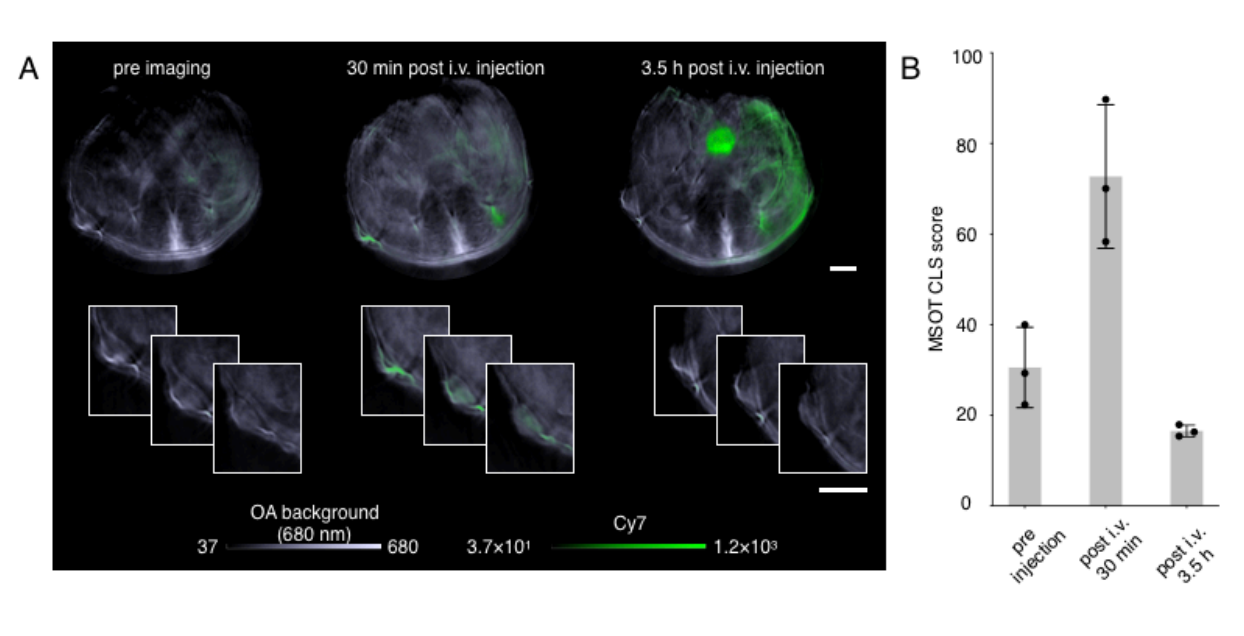

Figure S2. *In vivo* multi-spectral optoacoustic evaluation of $E4_{x12}$-Cy7 at different timepoints. (A) Optoacoustic image reconstruction showing transverse 2D projection of mice at the region of interest before and 30 min after intravenous injection of $E4_{x12}$-Cy7 (6.7 mg/kg) before (*left)*, 30 min post-i.v. injection (*middle*) and 3.5 h post-i.v. injection (*right*). Overall optoacoustic signal at 680 nm (*greyscale*) is overlaid on top of multi-spectrally unmixed signals showing, $E4_{x12}$-Cy7 channel (*green channel*). Both scale bars are 2.5 mm. (B) Optoacoustic signal quantification after multi-spectral unmixing.

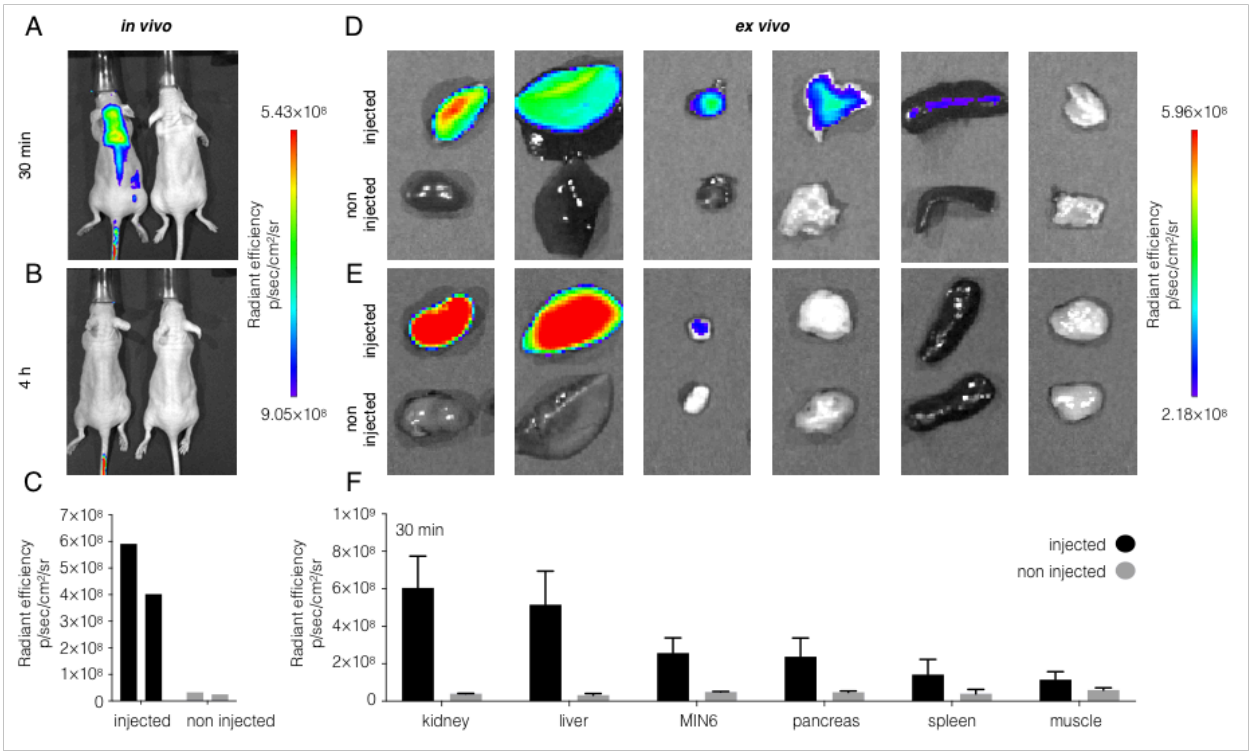

Figure S3. *In vivo* and *ex vivo* kinetics and biodistribution validation of E4$_{x12}$-Cy7 using fluorescence IVIS imaging. (A) Representative *in vivo* fluorescent images of E4$_{x12}$-Cy7 30 min post-i.v. injection, (B) 4 h post-i.v. injection and (C) quantifications (n = 3). (D) Corresponding *ex vivo* fluorescent images from left to right of kidney, liver, MIN6, pancreas, spleen and muscle tissues excised at 30 min and (E) 4 h post-i.v. injection of E4$_{x12}$-Cy7. (F) Quantification of the *ex vivo* organs by drawing regions of interest (ROIs) around the tissue outlines using the white field images.

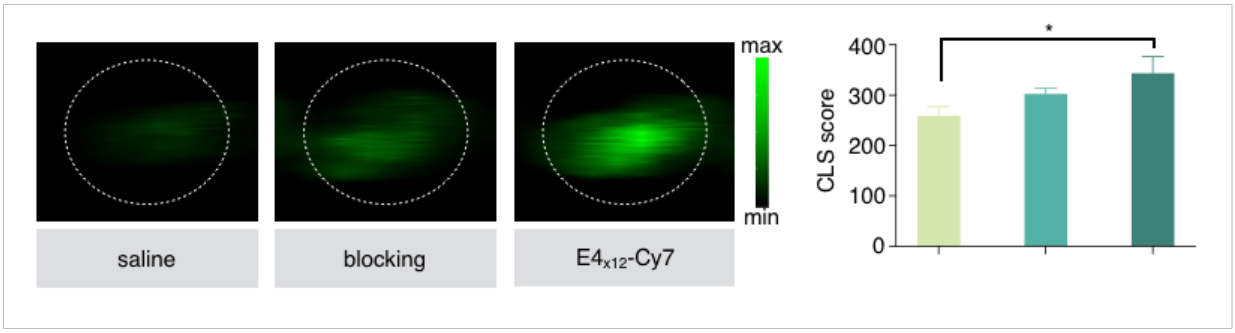

Figure S4. The ex vivo optoacoustic signal of kidneys between non injected, blocking and injected. From left to right are the MSOT images of the kidney from a mouse that was injected with saline, exendin-4 (151 µg) followed by the injection of E_{4x12}-Cy7 (57 µg, 20 mins time interval (blocking), and 57 µg of E_{4x12}-Cy7 (*left*) and the corresponding quantification (*right*). There is a statistically significant differences between mice that were injected with saline and $E4_{x12}$-Cy7 ($p^* = 0.0178$).