Anti-opioid Antibodies in Individuals Using Chronic Opioid Therapy for Lower Back Pain

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SUPPLEMENTAL INFORMATION

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SYNTHESIS

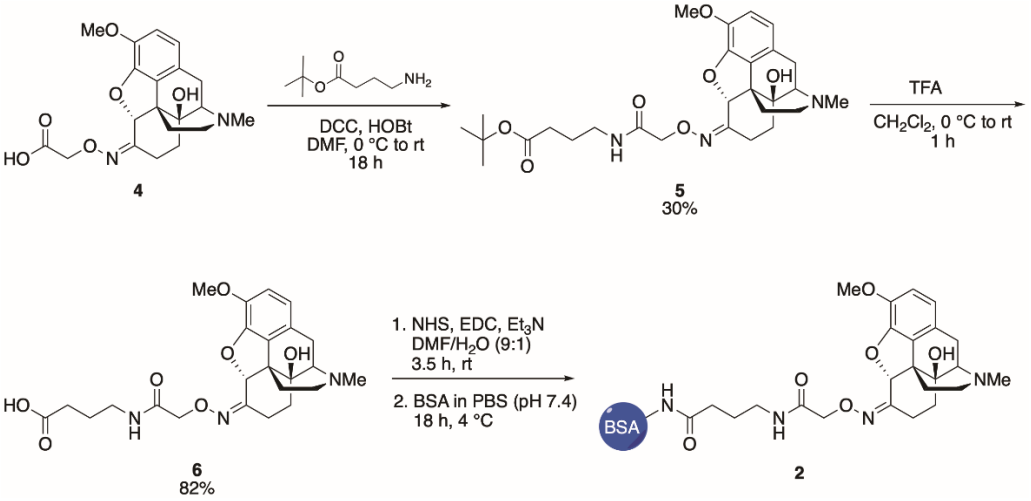
Chemistry

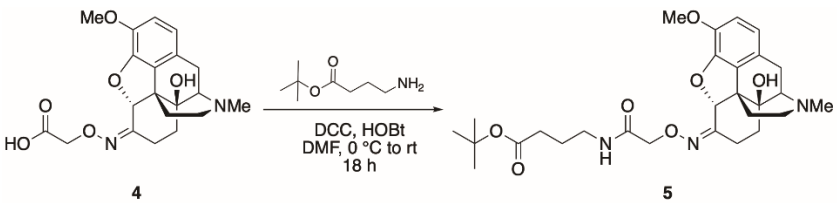
Nuclear magnetic resonance (^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz)) spectra were obtained on a Bruker Avance III HD instrument unless otherwise noted. Chemical shifts for ^{1}H NMR are reported in parts per million upfield from chloroform (7.26 ppm) unless otherwise specified. Chemical shifts for ^{13}C NMR were reported in ppm relative to the center line of the triplet at 77.0 ppm for deuterated chloroform unless otherwise specified. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry performed using Bruker MaXis time-of-flight spectrometer. LC/MS analysis performed on an Agilent 1290 UHPLC with 6120 Single Quad MS using electrospray atmospheric pressure chemical ionization (ES-APCI).

Chemicals and Reagents

Cyanogen bromide obtained from Thermofisher. 1-*tert*-butoxycarbonyl-2-pyrrolidinone from sigma. DIBAL from Sigma. $NaBH(OAc)_3$ from Sigma. Sodium sulfate from sigma. DCE from Sigma. TFA from Fluka. Succinic anhydride from Sigma. Dioxane from sigma. Sulfo-NHS and EDC from Thermofisher. BSA powder for hapten synthesis obtained from FisherScientific. Pierce Zeba Desalt spin columns used for purification of BSA conjugate.

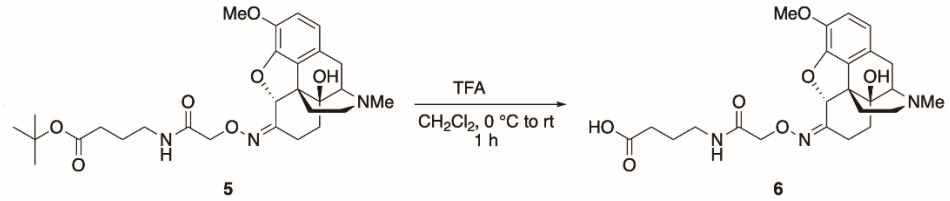
Synthesis of OxyBSA **2**



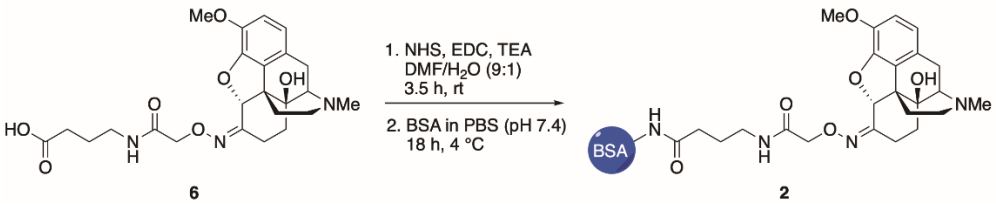


***tert*-Butyl 4-(2-((((4a*S*,7a*R*,12b*S*,*Z*)-4a-hydroxy-9-methoxy-3-methyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-ylidene)amino)oxy)acetamido)butanoate (5)**: To a solution of **4**[1] (43 mg, 0.11 mmol) in dry *N,N*-

dimethylformamide (1.5 mL) were added dicyclohexylcarbodiimide (30 mg, 0.14 mmol) and hydroxybenzotriazole hydrate (18 mg, 0.13 mmol) at 0 °C. After stirring for 15 min at 0 °C, 4-aminobutaoinc acid *tert*-butyl ester (15 mg, 0.11 mmol) was added to the reaction mixture. The resulting mixture was allowed to warm to room temperature and stirred for 18 h. The reaction mixture was quenched by an addition of water, and the resulting mixture was diluted with ethyl acetate. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, dichloromethane/methanol, 10/1) to afford **5** (17 mg, 30%) as a white solid. ^{1}H NMR (500 MHz, MeOD) δ 6.79 (d, *J* = 8.2 Hz, 1H), 6.68 (d, *J* = 8.2 Hz, 1H), 4.93 (s, 1H), 4.50 (s, 2H), 3.83 (s, 3H), 3.30–3.17 (m, 4H), 2.97 (d, *J* = 6.1 Hz, 1H), 2.76–2.57 (m, 4H), 2.48 (s, 3H), 2.40–2.33 (m, 2H), 2.25 (t, *J* = 7.4 Hz, 2H), 1.73 (p, *J* = 7.1 Hz, 2H), 1.62 (ddd, *J* = 13.8, 6.8, 2.9 Hz, 1H), 1.57–1.54 (m, 1H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, MeOD) δ 174.4, 172.3, 159.8, 146.3, 144.0, 131.7, 127.2, 120.6, 116.7, 88.2, 81.6, 73.8, 71.6, 66.2, 57.6, 46.7, 42.9, 39.4, 33.7, 32.4, 29.3, 28.4, 25.8, 23.3, 21.7, 19.3;HRMS (ESI) *m/z* found 530.2862 [(M+H)$^{+}$ calcd for $C_{28}H_{39}N_3O_7$ 530.2861].



4-(2-((((4a*S*,7a*R*,12b*S*,*Z*)-4a-Hydroxy-9-methoxy-3-methyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-ylidene)amino)oxy)acetamido)butanoic acid (6): To a solution of **5** (17 mg, 0.03 mmol) in dichloromethane (0.5 mL) was added trifluoroacetic acid (0.1 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Upon complete disappearance of the starting material, the solvent was removed under reduced pressure. The crude reaction mixture was subjected to azeotropic drying by using toluene. The residue was purified by flash column chromatography (silica gel, dichloromethane/methanol, 10/1 to 5/1) to afford **6** (12.5 mg, 82%). ^{1}H NMR (600 MHz, MeOD) δ 6.86 (d, *J* = 8.2 Hz, 1H), 6.77 (d, *J* = 8.2 Hz, 1H), 5.03 (s, 1H), 4.52 (s, 2H), 3.84 (s, 3H), 3.49–3.38 (m, 2H), 3.29–3.19 (m, 2H), 3.09–2.94 (m, 2H), 2.82 (s, 3H), 2.78–2.57 (m, 4H), 2.30–2.25 (m, 2H), 1.85–1.64 (m, 4H), 1.43 (ddd, *J* = 13.5, 11.2, 7.1 Hz, 1H). ^{13}C NMR (150 MHz, MeOD) δ 158.9, 157.2, 146.5, 144.5, 130.5, 125.0, 121.1, 117.3, 115.9, 87.7, 83.4, 73.9, 71.4, 67.6, 57.3, 57.0, 47.8, 47.6, 42.0, 40.4, 30.7, 29.0, 24.4, 18.9; HRMS (ESI) *m/z* found 474.2241 [(M+H)$^{+}$ calcd for $C_{24}H_{31}N_3O_7$ 474.2235].



Hapten Conjugation to Bovine Serum Albumin (BSA) (OxyBSA 2)

To a solution of **6** (1.1 mg, 2.3 μmol) in *N,N*-dimethylformamide (100 μL) and water (10 μL) were added triethylamine (1 μL, 6.9 μmol), *N*-hydroxysuccinimide (1.6 mg, 13.9 μmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (2.7 mg, 13.9 μmol) in one portion at room temperature. The solution was allowed to stir for 2 h and was monitored by LC/MS. Another

5 equiv of *N*-hydroxysuccinamide and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride were added to the reaction mixture. After 1.5 h, LCMS indicated the completion of the reaction. MS *m/z* 570.2 [$(M+H)^+$ calcd for $C_{28}H_{34}N_4O_9$ 570.2].
The above mixture was added to a solution of BSA (1 mg/mL in pH 7.4 PBS buffer). The activated hapten was allowed to react with the proteins for 18 h at 4 °C using gentle end-over-end mixing. The reaction solution was dialyzed against pH 7.4 PBS buffer using a Slide-A-Lyzer 10K MWCO dialysis cassette (Thermo Scientific) at room temperature. The buffer was exchanged every 1 h for 3 h, and then dialysis was continued for 12 h at 4 °C. The conjugates were quantified by BCA assay. The hapten conjugate **2** was analyzed by MALDI-ToF for the hapten:carrier protein conjugation number.

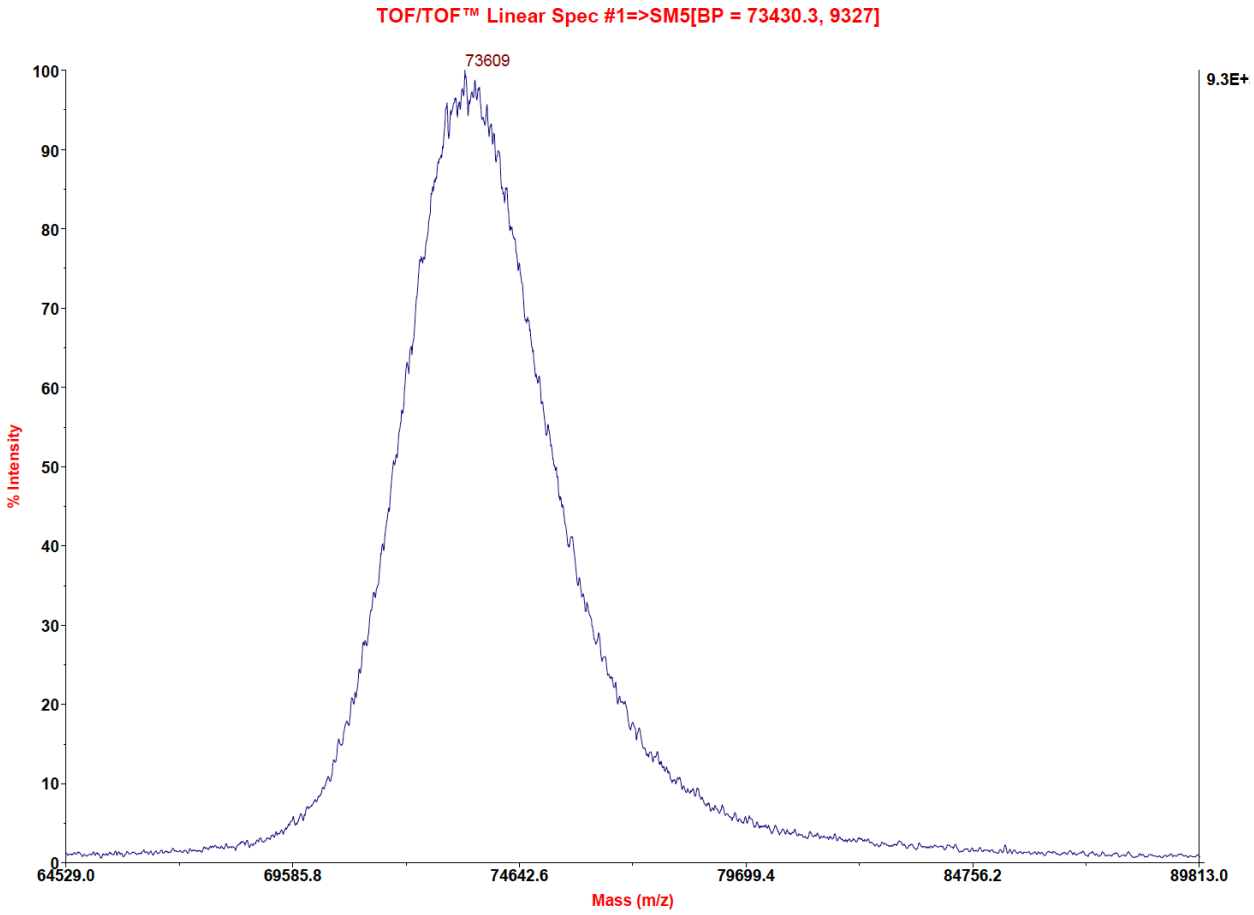
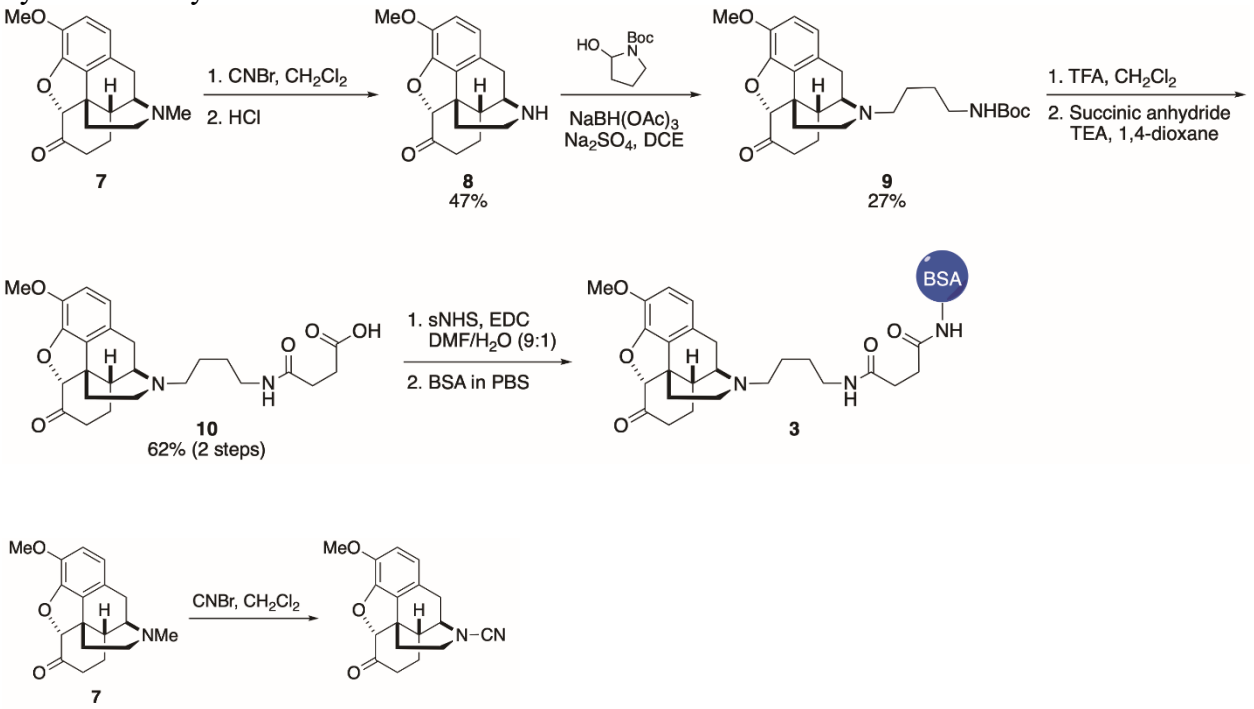
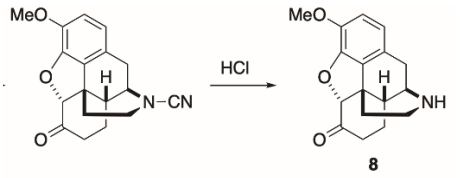


Figure S1. MALDI-ToF analysis of OxyBSA **2**.

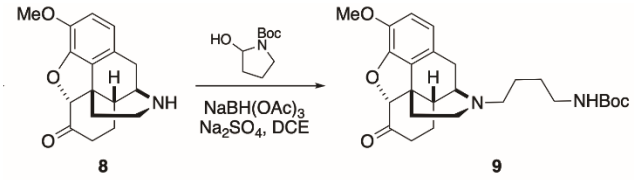
Synthesis of HydroBSA **3**



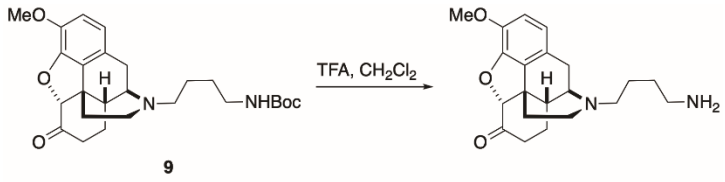
(4*R*,4a*R*,7a*R*,12b*S*)-9-methoxy-7-oxo-1,2,4,4a,5,6,7,7a-octahydro-3*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline-3-carbonitrile. Hydrocodone bitartrate (0.4 g) was first converted to the free base.[2] The starting material was dissolved in deionized water (3 ml) and heated to 35 ºC. To the resulting solution, a 20% aqueous solution of sodium hydroxide was added dropwise (0.3 mL). The mixture was stirred for 3 hours, then cooled to room temperature. The reaction mixture was extracted 3x with dichloromethane. The organic fractions were combined, dried over sodium sulfate, and condensed *in vacuo* to provide a white solid. The crude material was then combined with cyanogen bromide (2 equiv) in dichloromethane (5 mL) and stirred for 6 hours.[3] An additional 3 equiv of cyanogen bromide were added. The reaction mixture was stirred overnight prior to the addition of a third aliquot of cyanogen bromide (2 equiv) and allowed to stir for 8 h. The reaction mixture was washed with 1 M HCl. The mixture was extracted 3 times with dichloromethane and purified by flash column chromatography (silica gel, 0-50% ethyl acetate in hexanes) to provide the desired beige solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 – 6.64 (m, 2H), 4.67 (s, 1H), 3.92 (d, J = 1.1 Hz, 2H), 3.27 (dd, *J* = 13.0, 5.4 Hz, 1H), 3.17 – 3.02 (m, 2H), 2.89 (dd, *J* = 18.7, 5.5 Hz, 1H), 2.70 (dt, *J* = 12.8, 3.8 Hz, 1H), 2.52 – 2.32 (m, 2H), 2.13 (td, *J* = 12.6, 5.3 Hz, 1H), 1.91 (dt, *J* = 12.9, 4.5 Hz, 2H), 1.24 – 1.12 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.0, 145.6, 143.5, 125.3, 123.6, 120.5, 115.4, 91.0, 57.6, 56.8, 46.4, 43.7, 40.8, 39.5, 34.0, 29.7, 28.1, 25.1. LCMS ES-APCI *m/z* found 311.7 [$(M+H)^+$ calcd for $C_{18}H_{19}N_2O_3$ 311.1].



(4*R*,4a*R*,7a*R*,12b*S*)-9-methoxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (8). *N*-cyanonorhydrocodone was combined with 30% HCl and heated to reflux overnight.[3] After cooling to room temperature, the reaction mixture was made alkaline with 1 M NaOH to pH 12. The mixture was extracted 3 times with dichloromethane and purified by reverse phase flash column chromatography (C18, water/acetonitrile/trifluoroacetic acid). The desired product was obtained as a white solid in 47% yield over 2 steps. LCMS ES-APCI *m/z* found 286.1 $[(M+H)]^+$ calcd for $C_{17}H_{20}NO_3$ 286.1].

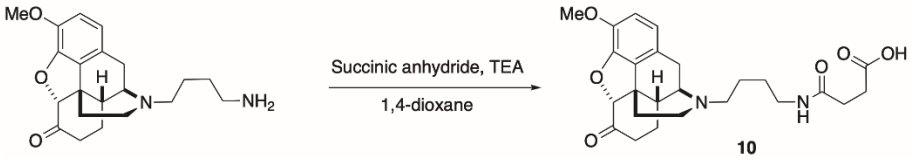


***tert*-butyl (4-((4*R*,4a*R*,7a*R*,12b*S*)-9-methoxy-7-oxo-1,2,4,4a,5,6,7,7a-octahydro-3*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-3-yl)butyl)carbamate (9). 8** (0.089 g, 0.31 mmol) was dissolved in 1,2-dichloroethane (1 mL). Sodium sulfate (0.133 g, 0.94 mmol, 3 equiv), *N*-Boc-pyrrolidin-2-ol (0.175 g, 0.94 mmol, 3 equiv), and sodium triacetoxyborohydride (0.132 g, 0.62 mmol, 2 equiv.) were added to the solution at room temperature.[4] After stirring at room temperature for 1 h, the reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate and extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate, filtered, and condensed *in vacuo*. The resulting butylamine was purified by reverse phase flash column chromatography (C18, water/acetonitrile/trifluoroacetic acid) to provide the desired oil in 27% yield (0.039 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (d, J = 8.2 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 5.14 (s, 1H), 4.64 (s, 1H), 3.90 (s, 3H), 3.29 – 3.06 (m, 3H), 2.95 (d, J = 18.4 Hz, 1H), 2.70 – 2.28 (m, 8H), 2.11 (dtd, J = 29.2, 13.7, 13.0, 4.7 Hz, 3H), 1.92 – 1.72 (m, 3H), 1.45 (s, 9H), 1.35 – 1.20 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.9, 156.1, 145.4, 142.9, 127.3, 126.1, 119.8, 114.6, 91.4, 79.0, 57.3, 56.8, 54.3, 47.4, 45.0, 42.3, 40.5, 40.2, 35.3, 28.5, 27.9, 25.6, 24.2, 20.8. LCMS ES-APCI *m/z* found 457.1 $[(M+H)]^+$ calcd for $C_{26}H_{37}N_2O_5$ 457.3].

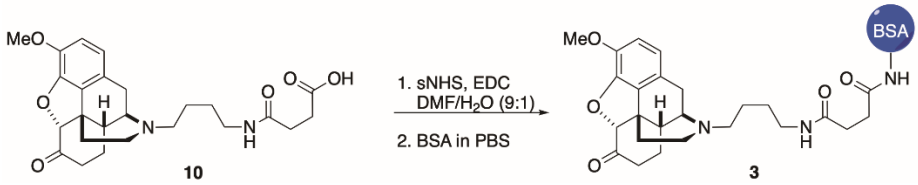


(4*R*,4a*R*,7a*R*,12b*S*)-3-(4-aminobutyl)-9-methoxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one. *N'*-Boc-*N*-butylaminenorhydrocodone was dissolved in dichloromethane.[4] Trifluoroacetic acid was added at room temperature and the reaction mixture was stirred at room temperature for 2 h. The mixture was then co-evaporated with

toluene *in vacuo*. The residue was dissolved in methanol and purified by reverse phase flash column chromatography (C18, water/acetonitrile/trifluoroacetic acid). LCMS ES-APCI *m/z* found 357.1 $[(M+H)]^+$ calcd for $C_{21}H_{29}N_2O_3$ 357.2].



4-((4-((4*R*,4a*R*,7a*R*,12b*S*)-9-methoxy-7-oxo-1,2,4,4a,5,6,7,7a-octahydro-3*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-3-yl)butyl)amino)-4-oxobutanoic acid (10). Butylamine (0.025 g, 0.071 mmol) was dissolved in 1,4-dioxane (1 mL). Succinic anhydride (0.071 mmol, 7.0 mg, 3 equiv) and triethylamine (29 µL, 0.21 mmol, 3 equiv) were added.[4] The reaction mixture was heated to 100 ºC for 2 h. The reaction mixture was condensed *in vacuo* and the subsequent residue was dissolved in methanol and purified via reverse phase flash column chromatography (C18, water/acetonitrile/trifluoroacetic acid) to provide the desired compound as a yellow amorphous solid in 62% yield (20 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (d, J = 8.2 Hz, 1H), 6.72 (d, J = 8.3 Hz, 1H), 6.59 (t, J = 6.1 Hz, 1H), 4.79 (s, 1H), 4.10 – 4.03 (m, 1H), 3.92 (s, 3H), 3.43 (dt, J = 13.0, 6.3 Hz, 2H), 3.25 (dd, J = 13.7, 5.9 Hz, 1H), 3.11 (td, J = 16.3, 14.7, 6.9 Hz, 3H), 3.02 (s, 1H), 2.90 (dd, J = 19.5, 5.8 Hz, 1H), 2.72 – 2.55 (m, 3H), 2.55 – 2.39 (m, 5H), 2.07 – 1.98 (m, 1H), 1.98 – 1.91 (m, 1H), 1.57 (p, J = 7.1 Hz, 2H), 1.35 – 1.08 (m, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 206.0, 173.1, 165.7, 145.6, 143.9, 125.6, 121.6, 120.4, 115.9, 90.4, 57.8, 56.9, 53.7, 46.6, 45.5, 39.4, 38.5, 37.6, 32.57, 32.56, 32.1, 26.4, 24.7, 21.3, 20.8. HRMS ESI *m/z* found 457.2348 $[(M+H)]^+$ calcd for $C_{25}H_{33}N_2O_6$ 457.2333].



Conjugation of hapten 10 with BSA HydroBSA 3. Succinate **10** (1.0 mg, 0.0022 mmol) was combined with sulfo-*N*-hydroxysuccinamide (1.43 mg, 0.0066 mmol, 3 equiv) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.26 mg, 0.0066 mmol, 3 equiv) and dissolved in a 9:1 *N,N*-dimethylformamide:water solution.[4] After the reaction mixture was stirred for 5 h, it was transferred to an Eppendorf tube containing BSA (10 mg/mL in PBS, 50 µL, 0.5 mg) and rocked overnight at room temperature. Reaction mixture was diluted to 1 mL with PBS, then purified using a Pierce ZEBA Desalting spin column at 4 ºC. Protein concentration determined using BCA assay and copy number was evaluated using MALDI-ToF.

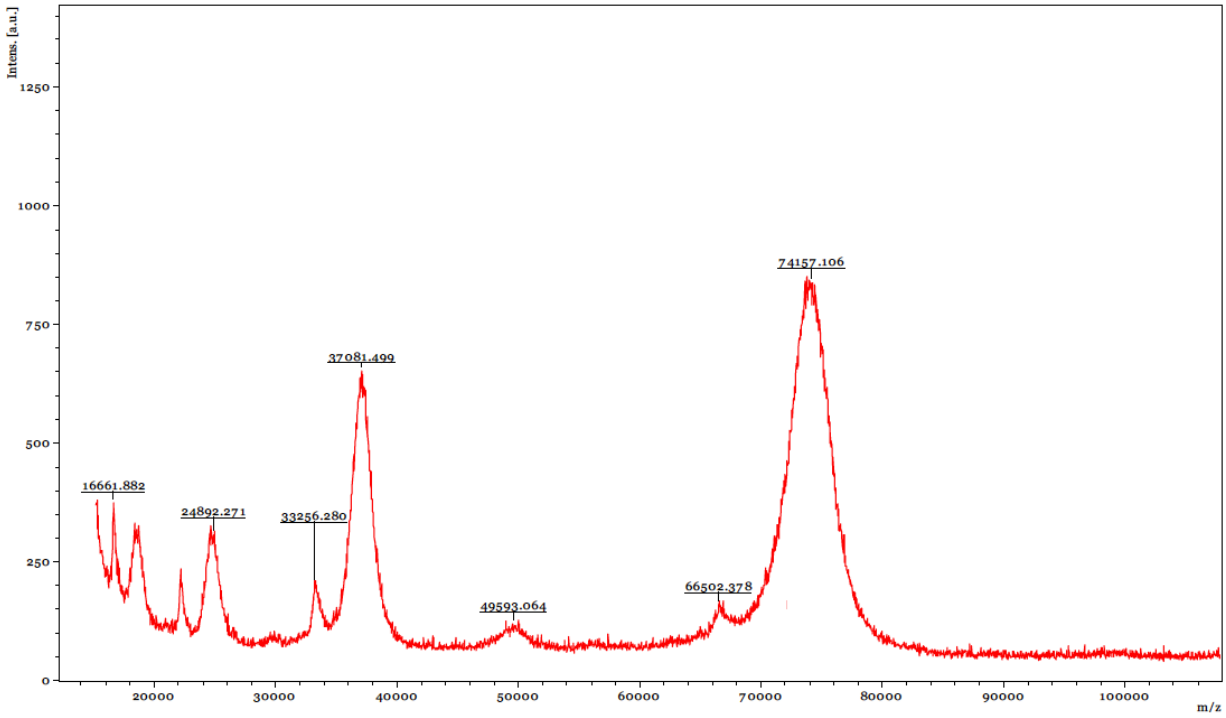
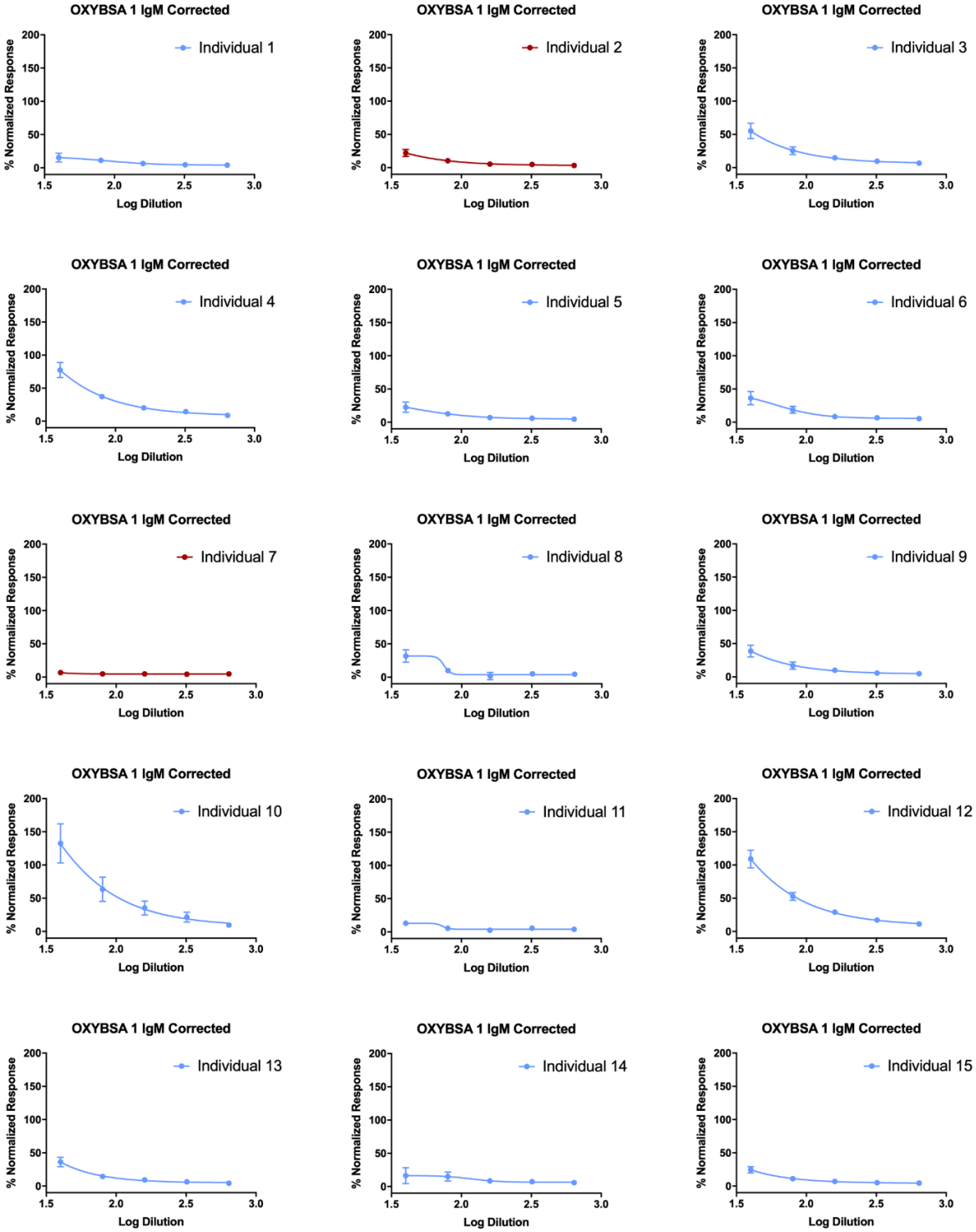


Figure S2. MALDI-ToF analysis of HydroBSA **3**.

Detection of IgM anti-OxyBSA **1** antibodies via indirect ELISA



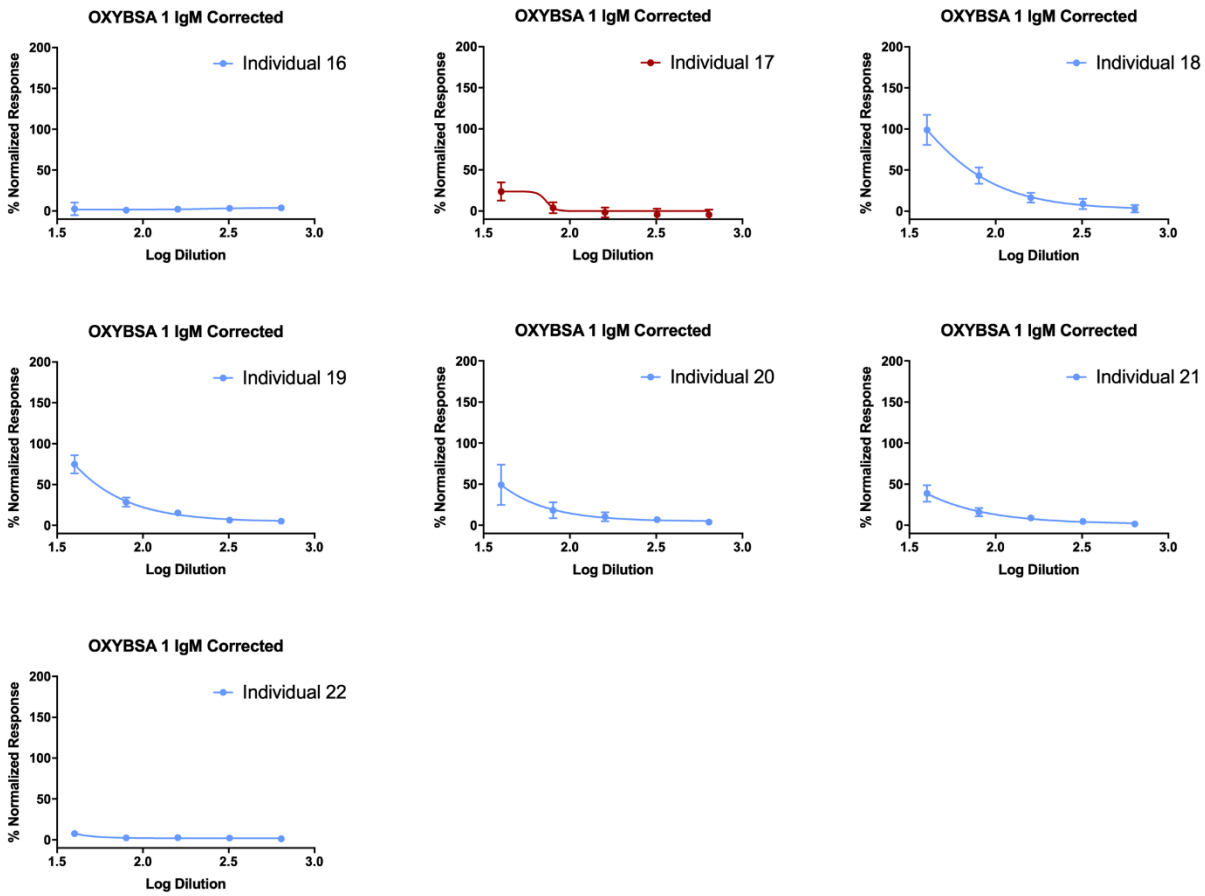


Figure S3. Detection of OxyBSA **1** specific response determined through subtraction of baseline BSA IgM response from OxyBSA **1** IgM response, normalized to 2º antibody positive control response for each individual subject. Exposed patients shown in blue; naïve patients shown in red. All data represented as mean ± SEM, n = 3.

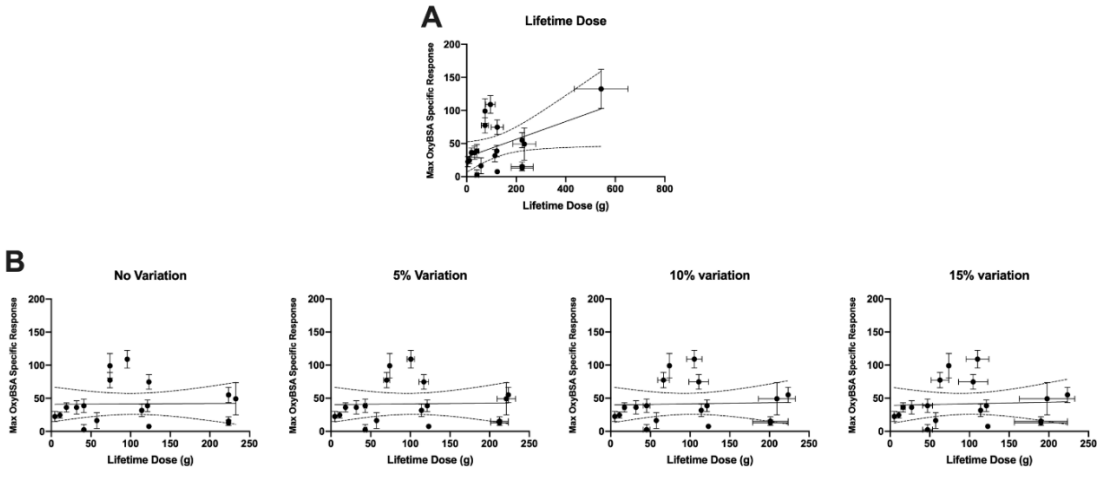
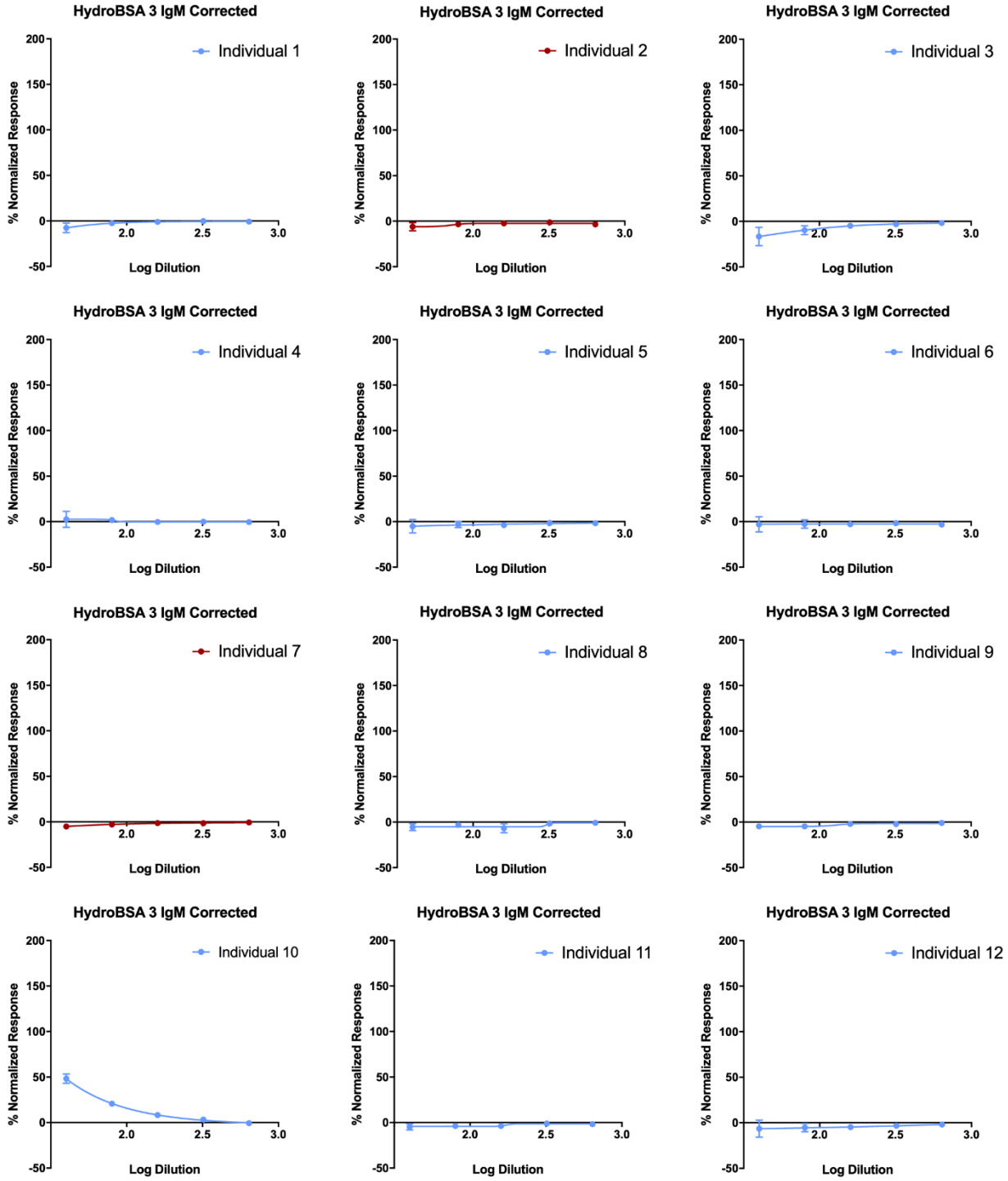


Figure S4. Correlation of maximum specific OxyBSA **1** response with estimated lifetime opioid dose. A) Outlier included, with assumed lifetime dose error of ±20%, $R^2 = 0.2167$, $p = 0.04$. B) Sensitivity analysis using assumed errors of ±0-15% variation for lifetime dose for subjects

reporting dosage changes (one outlier removed). ±0%: $p = 0.95$, $R^2 = 0.00025$. ±5%: $p = 0.91$, $R^2 = 0.00075$. ±10%: $p = 0.88$, $R^2 = 0.0016$. ±15%: $p = 0.83$, $R^2 = 0.0029$. Linear regression with 95% confidence bands shown. All data shown at mean ± SEM, n = 3 replicates.

Detection of IgM anti-HydroBSA **3** antibodies via indirect ELISA



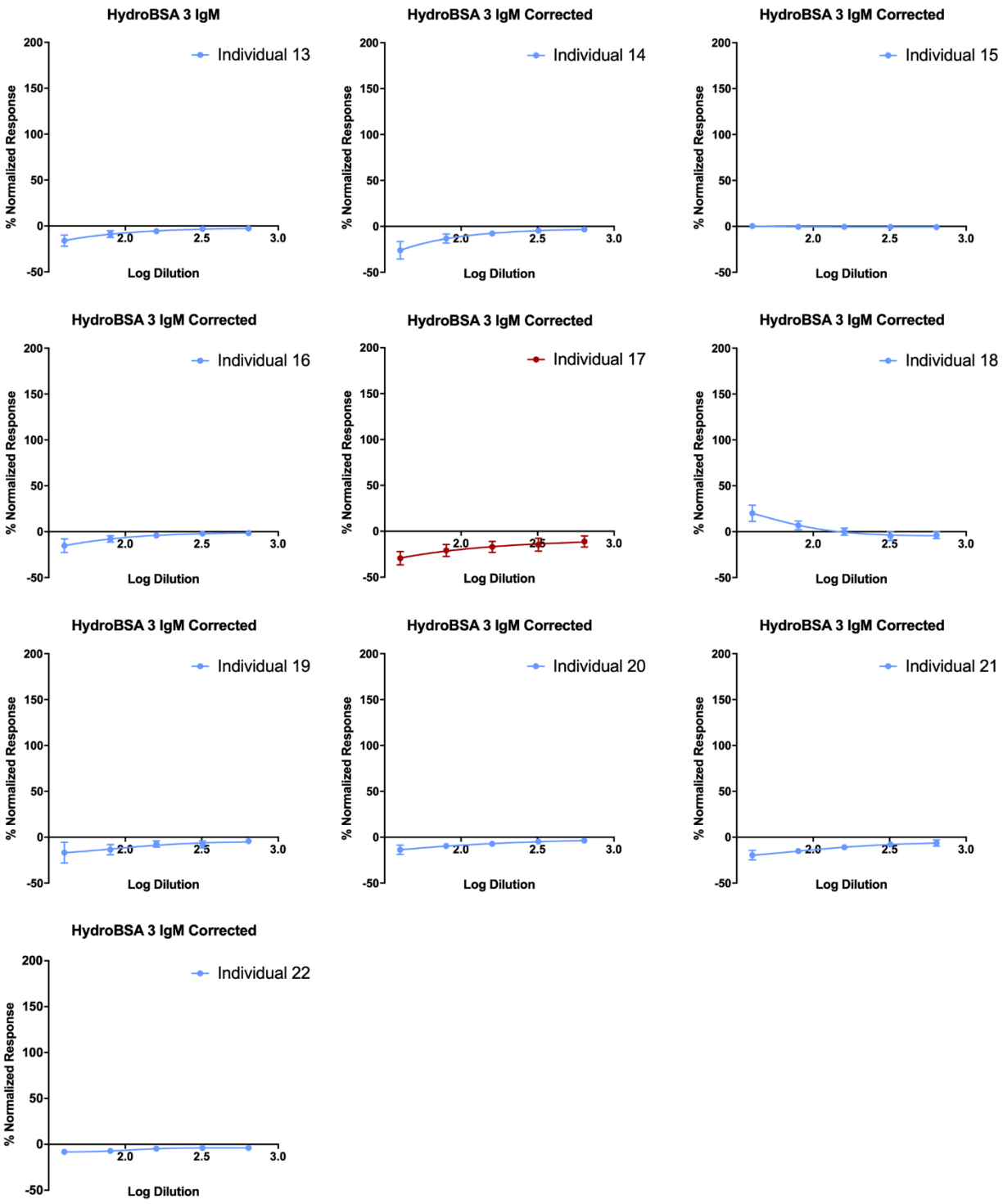
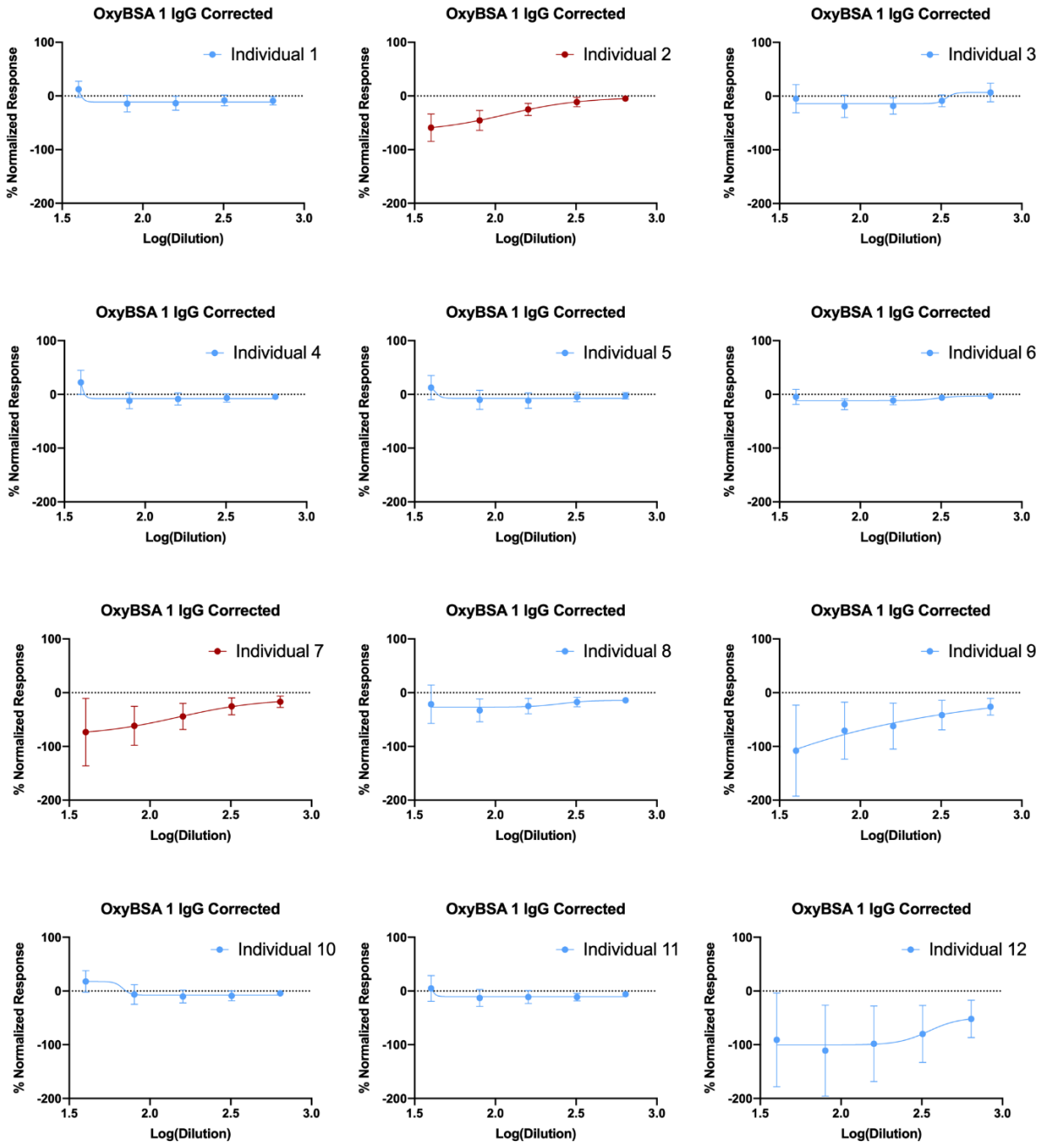


Figure S5. Detection of HydroBSA **1** specific response determined through subtraction of baseline BSA IgM response from HydroBSA **1** IgM response, normalized to 2º antibody positive control response for each individual subject. Exposed patients shown in blue; naïve patients shown in red. All data represented as mean ± SEM, n = 3.

Detection of IgG anti-OxyBSA **1** antibodies via indirect ELISA



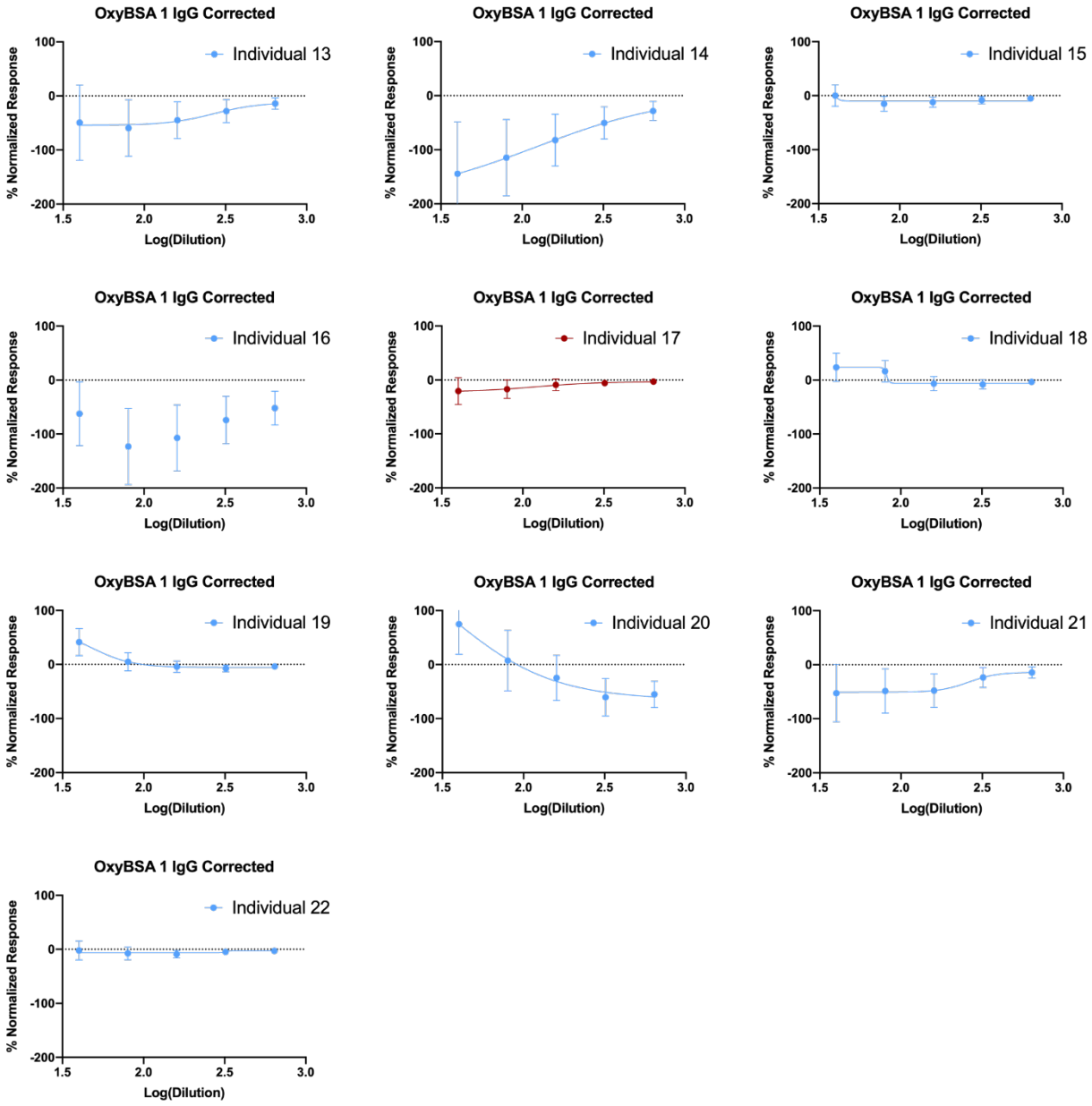


Figure S6. Detection of OxyBSA **1** specific response determined through subtraction of baseline BSA IgG response from OxyBSA **1** IgG response, normalized to 2º antibody positive control response for each individual subject. Exposed patients shown in blue; naïve patients shown in red. All data represented as mean ± SEM, n = 3.

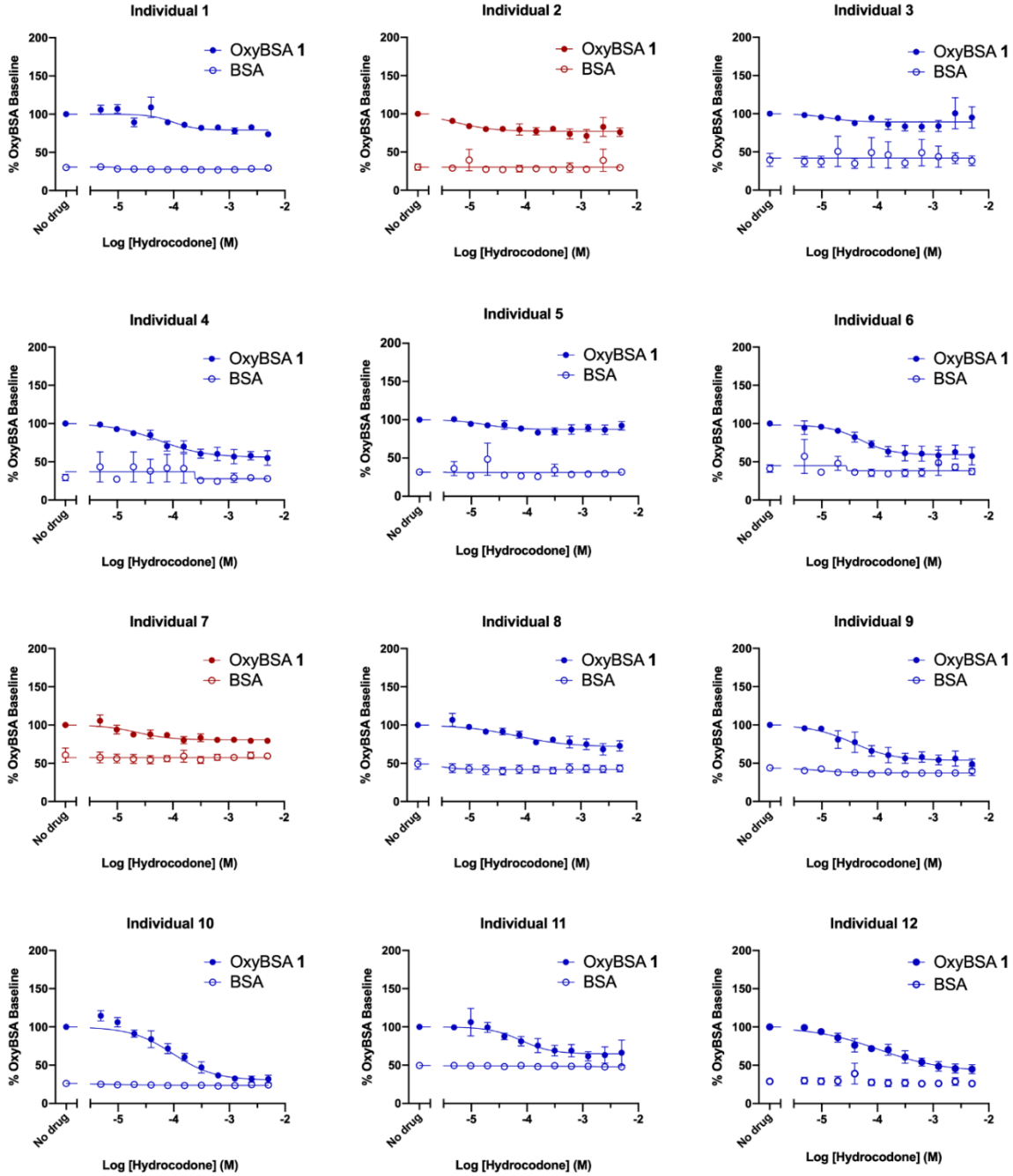
Evaluation of pooled anti-opioid IgM antibody selectivity via competitive ELISA

Table S1. pIC_{50}s determined for anti-opioid IgM antibodies via competitive ELISA against OxyBSA 1 and HydroBSA 3 for pooled samples of naïve or exposed subjects

	OxyBSA 1			HydroBSA 3		
	Hydrocodone	**Oxycodone**	**Methyl nicotinate**	**Hydrocodone**	**Oxycodone**	**Methyl nicotinate**
Exposed	4.016 ± 0.1337	4.288 ± 0.2093	0.3540 ± 209.8	4.588 ± 0.1445	4.631 ± 0.1914	3.272 ± 0.4879
Naïve	4.247 ± 0.1419	4.159 ± 0.2650	3.587 ± 229.4	4.616 ± 0.2624	4.301 ± 0.2711	3.010 ± 0.3670

pIC_{50}s provided as mean ± SEM

Evaluation of individual anti-OxyBSA **1** IgM antibody selectivity via competitive ELISA



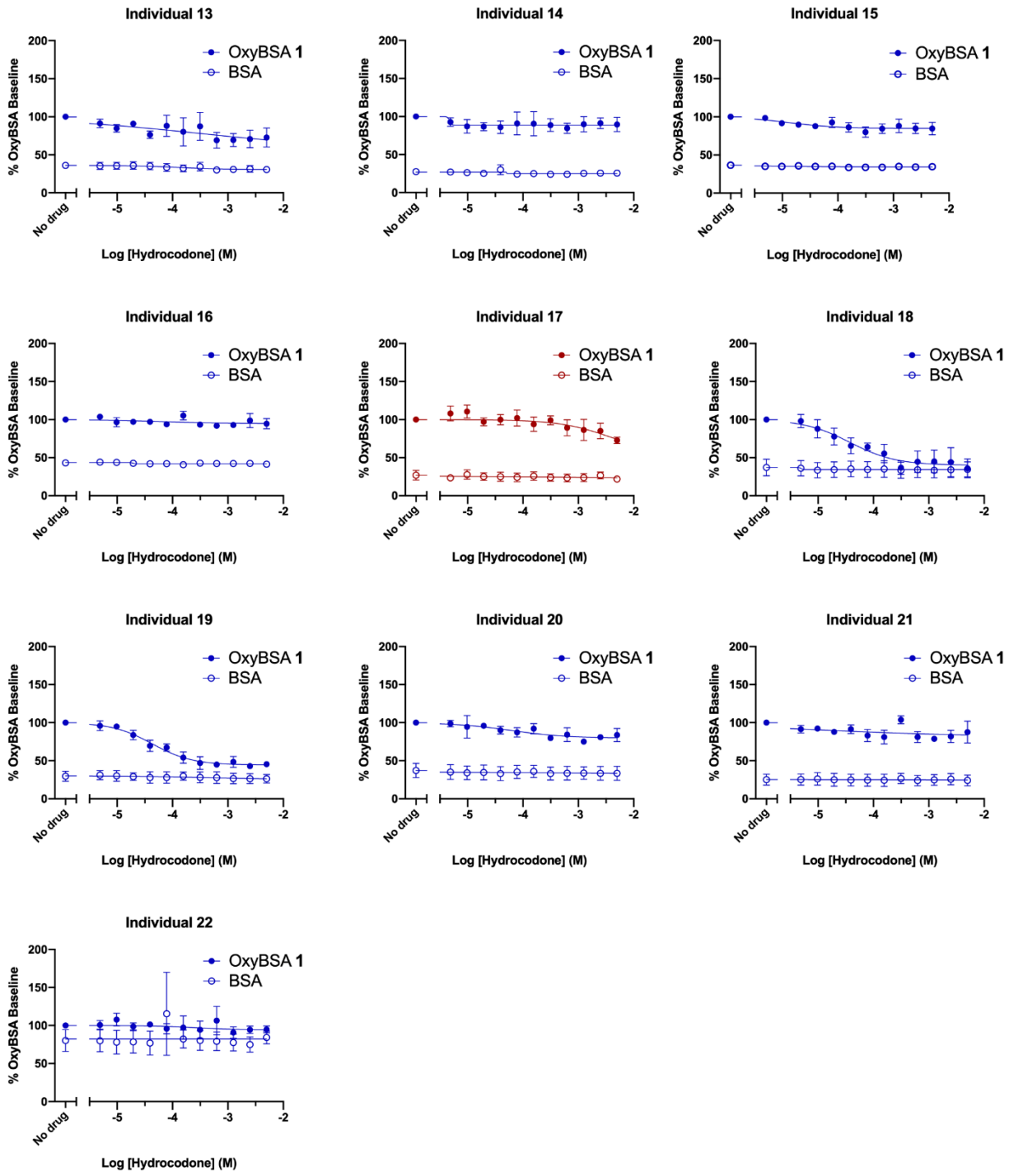


Figure S7. Determination of anti-opioid IgM antibody selectivity via competitive ELISA vs OxyBSA **1** (filled circles) and BSA (open circles) against hydrocodone for individual subjects Exposed patients shown in blue; naïve patients shown in red. All data represented as mean ± SEM, n = 3 replicates.

Table S2. pIC_{50}s of hydrocodone determined via competitive ELISA against OxyBSA 1 and BSA

	OxyBSA 1		BSA	
Patient	**pIC_{50}**	**span**	**pIC_{50}**	**span**
1	4.011	4.323 to und.	5.035	n/a
2	5.232	8.319 to und.	117.1	n/a
3	4.876	n/a	4333	n/a
4	4.252	4.547 to 3.782	3.606	n/a
5	4.758	5.430 to 4.024	5.451	n/a
6	4.306	4.655 to 3.913	4.551	n/a
7	4.641	5.058 to 3.957	Und.	n/a
8	4.153	4.561 to 2.290	5.516	n/a
9	4.448	4.755 to 3.932	5.013	47.25 to und.
10	4.019	4.176 to und.	5.153	n/a
11	4.107	4.501 to 3.398	-3.267	n/a
12	3.988	4.324 to und.	n/a	n/a
13	3.542	9.390 to und.	3.859	n/a
14	5.322	und.	4.297	n/a
15	4.887	7.555 to und.	5.226	n/a
16	4.192	n/a	4.719	n/a
17	2.411	4.462 to 2.194	-4.581	n/a
18	4.423	4.823 to 3.575	5.304	n/a
19	4.367	4.553 to 4.134	3.286	n/a
20	4.215	5.225 to ???	-15.9	n/a
21	5.064	115.7 to 1.709	-2.636	n/a
22	3.567	n/a	und.	n/a
Und. = Undefined Value (No Best Fit)				

Synthesis of nornicotine Amadori intermediate

The nornicotine Amadori intermediate was synthesized according to literature precedent.[5] ^{13}C NMR (100 MHz, Deuterium Oxide) δ 186.3, 148.6, 148.5, 148.0, 136.9, 124.5, 124.3, 96.8, 70.4, 69.9, 69.4, 69.1, 69.0, 68.9, 67.6, 63.0, 58.9, 55.7, 32.8, 22.7, 22.6.^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.55 – 8.42 (m, 2H), 7.93 (ddt, *J* = 10.8, 8.2, 2.0 Hz, 1H), 7.47 (dddd, *J* = 6.7, 5.7, 4.9, 0.9 Hz, 1H), 4.09 – 3.37 (m, 8H), 2.52 (q, *J* = 9.1 Hz, 1H), 2.45 – 2.19 (m, 1H), 2.18 – 1.90 (m, 3H), 1.90 – 1.67 (m, 1H).

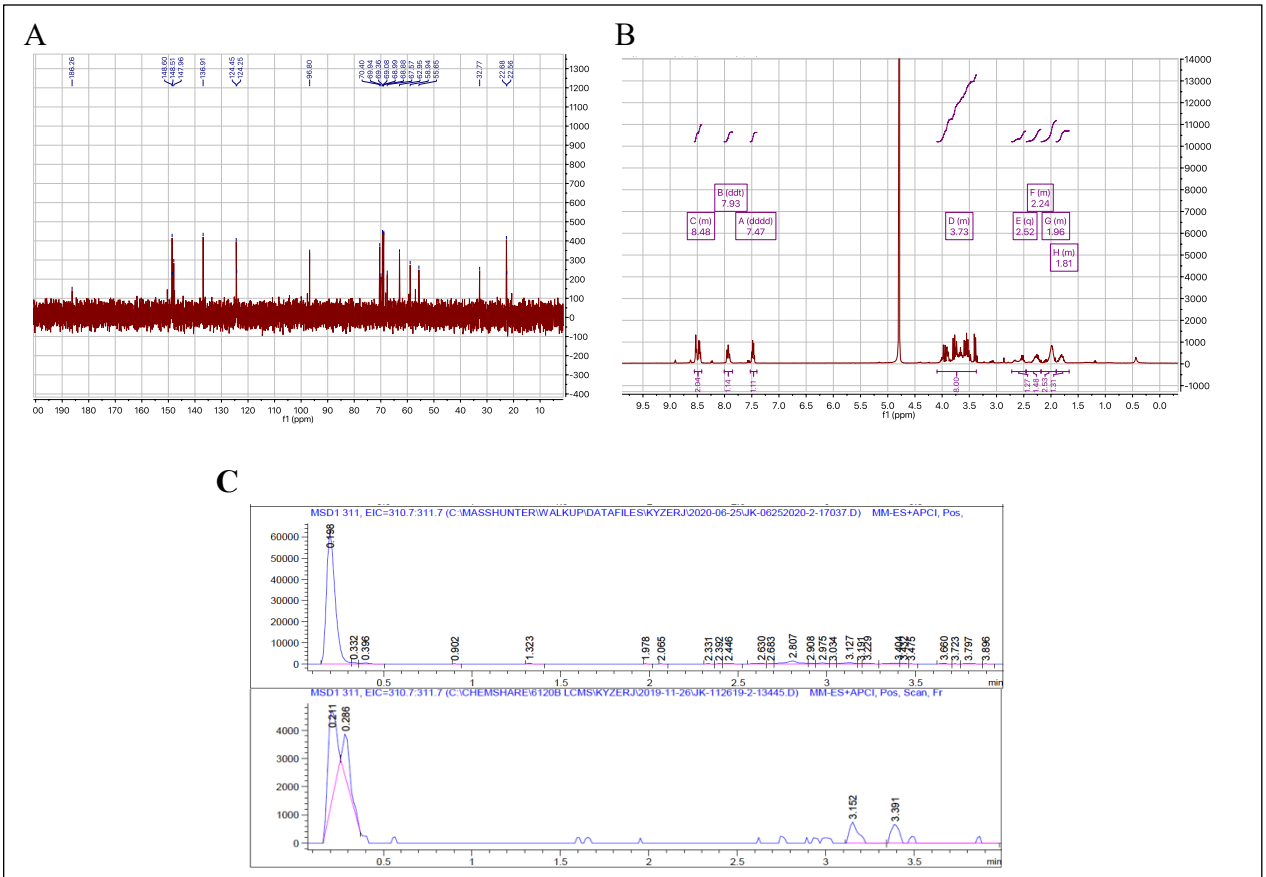


Figure S8. A)[13]CNMR and B)[1]HNMR of nornicotine Amadori intermediate. C) LCMS trace of m/z = 311 for nornicotine Amadori standard (top) and in vitro synthesis of nornicotine Amadori intermediate (bottom).

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(1) Pravetoni, M.; Le Naour, M.; Harmon, T. M.; Tucker, A. M.; Portoghese, P. S.; Pentel, P. R. An Oxycodone Conjugate Vaccine Elicits Drug-Specific Antibodies That Reduce Oxycodone Distribution to Brain and Hot-Plate Analgesia. *J. Pharmacol. Exp. Ther.* **2012**, *341* (1), 225–232. https://doi.org/10.1124/jpet.111.189506.

(2) Chopdekar, V. M.; Redkar, S. N.; Schlek, J. R. Opioid Tannate Compositions. US 20040157784A1, 2004.

(3) Iijima, I.; Minamikawa, J. ichi; Jacobson, A. E.; Brossi, A.; Rice, K. C.; Klee, W. A. Studies in the (+)-Morphinan Series. 5. Synthesis and Biological Properties of (+)-Naloxone. *J. Med. Chem.* **1978**, *21*, 398–400.

(4) Kimishima, A.; Wenthur, C. J.; Zhou, B.; Janda, K. D. An Advance in Prescription Opioid Vaccines: Overdose Mortality Reduction and Extraordinary Alteration of Drug Half-Life. *ACS Chem. Bio.* **2017**, *12*, 36–40.

(5) Dickerson, T. J.; Janda, K. D. A Previously Undescribed Chemical Link between Smoking and Metabolic Disease. *Proc. Natl. Acad. Sci.* **2002**, *99*, 15084–15088.

Appendix A. REDCap Survey

Participant ID _____

Before filling out this survey, please read the Informed Consent documents for the study, taking your time to fully understand their contents and to ask any questions you may have before signing.

If you have already agreed to participate in the study and signed the documents, please continue below.

I agree to participate.

◯ No
◯ Yes

(If you are unsure at this time, please select No. You may still indicate your consent to participate at a later date. By selecting Yes, you agree that this action is equivalent to and validation of your signature of the attached informed consent document.)

I have read and understood the explanation of the study. The study has also been explained to me by a member of the research team. I have had a chance to ask questions and have them answered to my satisfaction. I agree to take part in this study. I have not been forced or made to feel obligated to take part.

I have read the attached Experimental Subject's Bill of Rights and the Authorization to use my Private Health Information, which contain some important information about research studies. I must sign this consent form, the Experimental Subject's Bill of Rights and the Authorization to use my Private Health Information. I will be given a signed copy of each to keep.

Study ID _____

Date _____

Demographics Information

First Name _____

Last Name _____

Date of Birth _____

Age _____

Gender

◯ Female
◯ Male

Are you pregnant or planning to become pregnant in the next 3 months?

◯ Yes
◯ No

Height (in) _____

(Please round to the nearest inch (5 ft = 60; 5 ft,5.5 in = 66; 6 ft = 72))

Weight (lbs) _____

Body Mass Index _____

Race	◯ Caucasian ◯ African American ◯ Asian ◯ Other
Other Race	_____
Ethnicity	◯ Not Hispanic/Latino ◯ Hispanic/Latino
Phone number	_____ ((xxx)-xxx-xxxx)

Screening Questions

Do you have any medical or religious restrictions on the donation or receipt of blood or blood-derived products?	◯ Yes ◯ No
Have you been hospitalized in the last two months?	◯ Yes ◯ No
Please indicate whether you have been diagnosed with any of the following conditions.	☐ Rheumatoid Arthritis ☐ Lupus ☐ Crohn's Disease ☐ Irritable Bowel Syndrome ☐ Addison's Disease ☐ Celiac Disease ☐ Graves' Disease ☐ Multiple Sclerosis ☐ Myasthenia Gravis ☐ Sjogren Syndrome ☐ Type 1 Diabetes ☐ Type 2 Diabetes ☐ HIV/AIDS ☐ Hepatitis C ☐ Pernicious Anemia ☐ Chronic Renal Failure ☐ Alzheimer's Disease ☐ Other Autoimmune Disorder ☐ Any Blood Cancer (Leukemia, Lymphoma, Myeloma) ☐ Any Other Cancer (Breast, Prostate, Lung, Skin, etc.) (If you have NEVER been diagnosed with ANY of these conditions, please leave all the boxes unchecked for this question)

Please indicate if you have regularly taken any of the following medications in the past two months.

- ☐ coumadin (Warfarin)
- ☐ clopidogrel (Plavix)
- ☐ dabigatran (Pradaxa)
- ☐ rivaroxaban (Xarelto)
- ☐ enoxaparin (Lovenox)
- ☐ dalteparin (Fragmin)
- ☐ cilostazol (Pletal)
- ☐ prasugrel (Effient)
- ☐ ticagrelor (Brilinta)
- ☐ apixaban (Eliquis)
- ☐ predinsone (Orapred)
- ☐ methylprednisolone (Medrol)
- ☐ azathioprine (Imuran)
- ☐ mycophenolate (Cellcept)
- ☐ cyclosporine (Sandimmune)
- ☐ methotrexate (Rheumatrex)
- ☐ leflunomide (Arava)
- ☐ chlorambucil (Leukeran)
- ☐ Other anticoagulant (a.k.a. blood thinner)
- ☐ Other Immunosuppressant

(Regularly means MOST days in a week (4+). If you have NOT taken any of these medications regularly within the past two months, please leave all the boxes unchecked for this question)

Do you experience frequent lower back pain?

- ◯ Yes
- ◯ No

For how many months have you had frequent lower back pain?

- ◯ 0-3
- ◯ 3-6
- ◯ 6-12
- ◯ 12-18
- ◯ 18-24
- ◯ 24+

Brief Pain Inventory

In the LAST THREE MONTHS, what is the WORST level of back pain you have experienced?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, what is the LEAST amount of back pain you have experienced?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, what is the AVERAGE amount of back pain you have experienced?

0 5 10

(Place a mark on the scale above)

What is the amount of back pain you are experiencing RIGHT NOW?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how has your back pain interfered with your GENERAL ACTIVITY?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how has your back pain interfered with your MOOD?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how has your back pain interfered with your WALKING ABILITY?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how has your back pain interfered with your NORMAL WORK (work outside the home and housework)?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how has your back pain interfered with your RELATIONS WITH OTHER PEOPLE?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how has your back pain interfered with your SLEEP?

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how has your back pain interfered with your ENJOYMENT OF LIFE

0 5 10

(Place a mark on the scale above)

In the LAST THREE MONTHS, how often have you experienced breakthrough pain on AVERAGE (pain occurring even when you take your pain medication regularly)?

- ◯ Never
- ◯ Less than two days per MONTH
- ◯ One to three days per WEEK
- ◯ Four to six days per WEEK
- ◯ Daily

Opioid Use History

Please indicate whether you have used either of the following medications to treat your lower back pain.

- ◯ Hydrocodone Only
- ◯ Oxycodone Only
- ◯ Both Hydrocodone and Oxycodone
- ◯ Neither

(Common medications containing hydrocodone include: Lortab, Norco, Vicodin, Lorcet, Hysingla ER, Zohydro ER. Common medications containing oxycodone include: Percocet, Endocet, Roxicet, OxyContin, Oxecta, and Roxicodone)

In the LAST SIX MONTHS, how frequently have you taken medications containing either hydrocodone or oxycodone FOR ANY REASON?

- ◯ Never
- ◯ Two or less days TOTAL
- ◯ More than three days TOTAL

In the LAST SIX MONTHS, how frequently have you taken medications containing either hydrocodone or oxycodone?

- ◯ Less than four days per week
- ◯ Four or more days per week

In your LIFETIME, what is the total time period that you have used either Hydrocodone or Oxycodone at least once daily?

- ◯ 0-6 months
- ◯ 7-12 months
- ◯ 1-2 years
- ◯ 2-3 years
- ◯ 3-4 years
- ◯ 4-5 years
- ◯ 6-7 years
- ◯ 7-8 years
- ◯ 8-9 years
- ◯ 9-10 years
- ◯ 10-15 years
- ◯ 15-20 years
- ◯ 20+ years

What total dose (in mg) of Hydrocodone or Oxycodone do you currently take per day?

For example, taking one 5mg/325mg Norco every 6 hours would give a total daily dose of 20 mg (5mg X 4 pills daily)

- ◯ 1-10
- ◯ 11-20
- ◯ 21-30
- ◯ 31-40
- ◯ 41-50
- ◯ 51-60
- ◯ 61-70
- ◯ 71-80
- ◯ 80+

In the LAST SIX MONTHS, how frequently have you taken ANY of the following medications:

Morphine
Hydromorphone
Oxymorphone

- ◯ Two or less days TOTAL
- ◯ More than two days TOTAL

(Common medications that contain one of these compounds include: Astramorph, Avinza, Kadian, MS Contin, Oramorph SR, Dilaudid, Exalgo, Opana, Numorphan)

Over the following three months, are you interested in decreasing the amount of opioid pain medication that you take?

- ◯ Yes
- ◯ No

Please list ALL of the medications that you take regularly, AS OF TODAY, along with the dose and frequency that you take them.

Please include over-the counter and other non-prescription products, such as dietary supplements, herbals,vitamins, minerals, and topical products (creams/lotions).

If none, write 'NONE'

_____

(i.e. Simvastatin 20 mg Daily, Zoloft 50 mg Daily)

Please mark whether you are CURRENTLY using any of the following non-pharmacologic techniques to treat your back pain.

- ☐ Dietary supplements, vitamins, minerals, herbal medicine (oral and topical)
- ☐ Special Diet
- ☐ Chiropractic or osteopathic manipulation
- ☐ Massage
- ☐ Acupuncture
- ☐ Breathing Exercises
- ☐ Biofeedback
- ☐ Guided Imagery
- ☐ Hypnosis
- ☐ Meditation/Mindfulness Exercises
- ☐ Physical Exercise
- ☐ Tai Chi / Qi Gong
- ☐ Yoga
- ☐ Movement Therapies (Pilates, Alexander technique, Feldenkreis, Trager, etc.)
- ☐ Electrical Stimulation (TENS, Muscle Stimulator, Cefaly, Quell, etc.)
- ☐ Energy Medicine (Healing Touch, Reiki)
- ☐ Wearable for relief (Bracelets, Bands etc.)
- ☐ Biostimulation (Magnet therapy, light therapy, etc.)
- ☐ Prolotherapy
- ☐ Platelet Rich Plasma (PRP)
- ☐ Art Therapy
- ☐ Music Therapy
- ☐ Dance Therapy
- ☐ Spiritual/Religious practice (i.e. Prayer), any type
- ☐ Ayurveda
- ☐ Homeopathy
- ☐ Naturopathy
- ☐ Traditional Chinese Medicine
- ☐ Other

(If you are currently using NONE of the following techniques, please leave all boxes unchecked for this question)

What other non-pharmacologic technique are you using to treat your back pain?

_____

Please list any special diet that you are attempting for your back pain.

_____

Please list how many minutes you spend exercising EACH WEEK on average.

_____

Hydrocodone/Oxycodone Effectiveness

What was the effect of Hydrocodone/Oxycodone on your daily pain level...

	Much Improved	Somewhat Improved	No Change	Somewhat Worse	Much Worse
when you first started taking it?	○	○	○	○	○

when you take it now?	○	○	○	○	○

How frequently did you have breakthrough pain (pain occurring even when you take your pain medicine regularly)...

	Never	Rarely	Occasionally	Frequently	Constantly
when you first started taking Hydrocodone/Oxycodone?	○	○	○	○	○
when you take Hydrocodone/Oxycodone now?	○	○	○	○	○

Since starting hydrocodone or oxycodone treatment, has the prescription medication used to treat your lower back pain been changed?
- ○ Yes - Switched Hydrocodone to Oxycodone
- ○ Yes - Switched Oxycodone to Hydrocodone
- ○ Yes- Added Another Pain Medicine to Hydrocodone/Oxycodone
- ○ No

How did this medication change affect your daily back pain level?
- ○ Much Improved
- ○ Somewhat Improved
- ○ No Change
- ○ Somewhat Worse
- ○ Much Worse

How did this medication change affect how often you experience breakthrough pain?
- ○ Much Less Often
- ○ Somewhat Less Often
- ○ No Change
- ○ Somewhat More Often
- ○ Much More Often

From when you first started opioid treatment until now, how has the dosage of Hydrocodone or Oxycodone used to treat your lower back pain changed?
- ○ Dose has increased
- ○ Dose has decreased
- ○ No change

(If you started off taking only Hydrocodone or Oxycodone and now take both, please select 'Dose has increased')

How did this dosage change affect your daily back pain level?
- ○ Much Improved
- ○ Somewhat Improved
- ○ No Change
- ○ Somewhat Worse
- ○ Much Worse

How did this dosage change affect how often you experience breakthrough pain (pain occurring even when you take your pain medication regularly)?
- ○ Much Less Often
- ○ Somewhat Less Often
- ○ No Change
- ○ Somewhat More Often
- ○ Much More Often

Have you ever received a diagnosis of Opioid Induced Hyperalgesia?
- ○ Yes
- ○ No

Thank you very much for your time and interest in this study. Please speak with the study coordinator to get instructions on how to proceed from here.

Naive	◯ OK
Exposed	◯ OK
Null	◯ OK

Appendix B. What YOU can do to reduce chronic pain

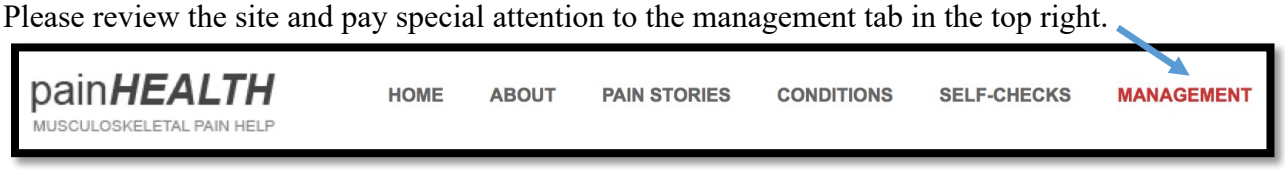
What *YOU* can do to reduce chronic pain

Robert Bonakdar MD Scripps Center for Integrative Medicine

You have been provided an excellent resource in Dr. Beth Darnall's book and CD for using mind-body techniques to help control your pain. In addition to this you are being provided this handout of additional self-management tools for managing your pain.

Many of these resources come from **painHEALTH!** a website of tools for improving pain developed by the Department of Health, Western Australia.
You can find the site at http://painhealth.csse.uwa.edu.au or by simply googling "*painhealth*"

Please review the site and pay special attention to the management tab in the top right.



Under the management tab you will find a number of section for managing pain. In addition to sections on understanding and approaching pain, there are a few that are especially helpful for helping to reduce pain:

1. **Move with Pain:**
   a. Movement is a very powerful tool for managing pain. Many times, when pain is present it can become difficult to enjoy movement. This section provides tips and exercises for how to effectively begin to use movement to manage your pain. Follow the guidance for starting exercise at the top of the page and at the bottom there are audio and video examples for relaxation and stretching programs. These example review techniques such as tai chi, yoga or simple stretching and breathing that will be helpful as you start your movement program. On the Move with Pain page (right side) there is a ***Get Moving Guide*** and a ***Movement, Exercise and Activities Sheet*** for tracking the progress you make with movement.

2. **Mindfulness and Pain**
   a. Mindfulness is a powerful technique which has been shown to reduce pain as well as improve brain function – especially in areas that control pain. Using the technique through the audio clips that are provided on this page can help to reduce the intensity of chronic pain as well as give you mental flexibility at times when pain or stress may be increased.

3. **Pacing and Goal Setting.** The techniques in this section can help you to:
   a. do more of what is important to you
   b. experience less pain flares
   c. reduce pain in the future

d. feel more in control of your life

This is done by examining your current activities and using tools such as **The pacing activity template** on the site to plan your day to minimize pain and maximize what is most important to you.

4. **Dietary approaches**
    a. We already know that dietary approaches can be an important way to improve your general wellbeing and manage conditions such as high cholesterol, obesity and diabetes. You may be surprised to know that for many of the same reasons, dietary approaches can also help manage your pain. The site www.TruRelief.org provides information on how your daily food and nutrition choices can influence your pain as well as simple ways to modify your diet to potentially reduce inflammation and pain. On the site you will find recipes and sample menus that may be utilized to shift in your diet and evaluate the effect on your pain

5. **Journaling**
    a. As part of the study you will be asked to complete a weekly diary of your pain as well as the treatments you are pursuing. Another way to monitor your progress is a daily journal such as the ones noted on the resource pages above. A journal can help you understand the daily factors, including your activities, thoughts and interactions that affect your pain and help your strategize ways to control your pain in the future. A number of studies have shown that journaling is an effective way to understand and reduce your pain

Summary:

There are many approaches to managing pain. Although medications play an important role in the management of pain, there are a host of self-management techniques as discussed above that may be worth considering to test if they may improve your pain or issues related to your pain including mood, sleep, energy and outlook. Review the various techniques and aim to spend at least 20-30 minutes a day trying those that may not have been tried before or which you are most interested in trying. After you try a technique journal about what you felt it did for your pain as well as other techniques that might compliment it. Also tell your friend and family about the techniques you are trying so they can provide their support along the way.

Note: The information on this handout and the resources mentioned do not replace professional medical advice. If you have any questions or concerns about your pain condition or treatments you are using or considering, please contact your treatment team.