Supporting Information

Non-Acidic Free Fatty Acid Receptor 4 Agonists with Antidiabetic Activity

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Contents

Synthetic protocols

3-(3-Fluoro-5-nitrophenoxy)pyridine (3a). 3-Hydroxypyridine (598 mg, 6.2 mmol), 3,5-difluoronitrobenzene (1.0 g, 6.2 mmol) and K_2CO_3 (1.72 g, 12.4 mmol) in dry DMF (10 mL) under an inert atmosphere was heated at 100 ºC for 16 h. The reaction was allowed to cool to room temperature and partitioned between water and EtOAc, the aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (EtOAc/PE, 1:1) to give **3a** as a yellow oil that solidified upon standing (859 mg, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$): 8.55 (dd, *J* = 4.4, 1.7 Hz, 1H), 8.49 (dd, *J* = 2.5, 0.6 Hz, 1H), 7.70 (dt, *J* = 8.0, 2.2 Hz, 1H), 7.65 – 7.61 (m, 1H), 7.47 – 7.38 (m, 2H), 7.06 (dt, *J* = 8.9, 2.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 163.0 (d, *J* = 252.4 Hz), 158.9 (d, *J* = 11.0 Hz), 151.5, 149.9 (d, *J* = 10.9 Hz), 146.8, 142.5, 127.3, 124.7, 111.4 (d, *J* = 25.1 Hz), 108.7 (d, *J* = 3.4 Hz), 106.4 (d, *J* = 26.9 Hz); HRMS (ESI) calcd for $C_{11}H_8FN_2O_3$ [M+H$^+$] 235.0513, found 235.0515. The ^{1}H NMR spectrum is in accordance with the literature.[1]

1-Fluoro-3-nitro-5-phenoxybenzene (3b). The title compound was obtained as described for **3a** using phenol (148 mg, 1.6 mmol) and 3,5-difluoronitrobenzene (250 mg, 1.6 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:1) to provide **3b** as a white solid (61.4 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.63 – 7.58 (m, 2H), 7.47 – 7.40 (m, 2H), 7.26 (ddd, *J* = 8.4, 7.5, 1.0 Hz, 1H), 7.10 – 7.06 (m, 2H), 7.00 (dt, *J* = 9.3, 2.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 162.9 (d, *J* = 251.0 Hz), 159.9 (d, *J* = 11.2 Hz), 154.7, 149.7 (d, *J* = 10.8 Hz), 130.5, 125.6, 120.3, 111.1 (d, *J* = 25.1 Hz), 108.6 (d, *J* = 3.4 Hz), 105.3 (d, *J* = 27.0 Hz).

1-(Benzyloxy)-3-fluoro-5-nitrobenzene (3c). The title compound was obtained as described for **3a** using benzyl alcohol (340 mg, 3.14 mmol) and 3,5-difluoronitrobenzene (400 mg, 3.14 mmol) as

starting materials. The crude product was purified by flash chromatography (EtOAc/PE,1:1) to give **3c** as a light yellow oil (234 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.64 (dd, *J* = 3.0, 2.2 Hz, 1H), 7.52 (dt, *J* = 8.2, 2.2 Hz, 1H), 7.46 – 7.33 (m, 5H), 7.00 (dt, *J* = 9.7, 2.2 Hz, 1H), 5.12 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 162.9 (d, *J* = 249.8), 160.2 (d, *J* = 11.5), 149.6 (d, *J* = 11.3), 128.9, 128.7, 127.6, 109.2 (d, *J* = 2.5), 106.0 (d, *J* = 3.1), 104.0 (d, *J* = 27.2), 71.1.

3-Fluoro-5-(pyridin-3-yloxy)aniline (4a). A vial charged with a stir bar, **3a** (737 mg, 3.147 mmol), ammonium formate (992 mg, 15.7 mmol), 10% Pd/C (100 mg) and EtOH (4 mL) was sealed and heated for 10 minutes at 90 ºC in a microwave oven. The reaction was filtered through a pad of celite. The pad was washed with EtOAc and the combined organic phases were concentrated to yield a quantitative amount of **4a** as light yellow oil which was used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): 8.41 (d, *J* = 2.7 Hz, 1H), 8.37 (dd, *J* = 4.6, 1.5 Hz, 1H), 7.33 (ddd, *J* = 8.4, 2.7, 1.5 Hz, 1H), 7.27 (dd, *J* = 8.4, 4.6 Hz, 1H), 6.15 (dt, *J* = 10.4, 2.1 Hz, 1H), 6.11 – 6.04 (m, 2H), 4.03 (br s, 2H).

3-Fluoro-5-phenoxyaniline (4b). The title compound was obtained as described for **4a** using **3b** (114 mg, 0.489 mmol) as starting material, yielding **4b** as a colorless oil (99.2 mg, 99.8%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.38 – 7.31 (m, 2H), 7.13 (t, *J* = 7.4 Hz, 1H), 7.07 – 7.01 (m, 2H), 6.16 – 6.02 (m, 3H), 3.77 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 164.4 (d, *J* = 243.0 Hz), 159.7 (d, *J* = 13.8 Hz), 156.3, 148.78 (d, *J* = 13.3 Hz), 129.8, 123.9, 119.7, 100.5 (d, *J* = 2.6 Hz), 96.8 (d, *J* = 25.0 Hz), 96.1 (d, *J* = 25.3 Hz). HRMS (ESI) calcd for $C_{12}H_{11}FNO$ $[M+H]^+$ 204.0819, found 204.0815.

3-(Benzyloxy)-5-fluoroaniline (4c). To a solution of **3c** (233 mg, 0.942 mmol) in water (0.5 mL), MeCN (15 mL) and EtOH (15 mL) was added $SnCl_2$ (2.13 g, 9.42 mmol) in one portion. The solution was heated to reflux for 1 h under a nitrogen atmosphere. The reaction was allowed to reach room temperature and quenched with sat. aq. Na_2CO_3. The aqueous phase was extracted three times with

CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (EtOAc/PE, 1:4) to give **4c** as a colorless oil (175 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.43 – 7.28 (m, 5H), 6.11 (dt, *J* = 10.8, 2.1 Hz, 1H), 6.07 (t, *J* = 2.1 Hz, 1H), 6.00 (dt, *J* = 10.4, 2.1 Hz, 1H), 4.98 (s, 2H), 3.73 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 164.5 (d, *J* = 249.8), 160.9 (d, *J* = 13.6), 148.6 (d, *J* = 13.5), 136.7, 128.6, 128.1, 127.5, 97.5 (d, *J* = 2.4), 95.2 (d, *J* = 25.1), 92.8 (d, *J* = 25.6), 70.1. HRMS (ESI) calcd for $C_{13}H_{13}FNO$ $[M+H]^+$ 218.0976, found 218.0971.

Methyl 2-(*N*-(3-fluoro-5-(pyridin-3-yloxy)phenyl)sulfamoyl)benzoate (5a). To a solution of **4a** (591 mg, 2.89 mmol) in pyridine (10 mL) and under nitrogen atmosphere was added methyl 2-(chlorosulfonyl)benzoate (1.03 g, 4.34 mmol). The reaction was stirred for 16 h at room temperature before quenching with NH_4Cl (sat. aq.) and water. The aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (EtOAc/PE, 3:2) to yield the desired compound **5a** as a white solid (973 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.42 (dd, *J* = 4.5, 1.5 Hz, 1H), 8.29 (d, *J* = 2.4 Hz, 1H), 8.26 (br s, 1H), 7.91 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.85 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.64 (td, *J* = 7.6, 1.4 Hz, 1H), 7.59 (td, *J* = 7.6, 1.4 Hz, 1H), 7.29 (dd, *J* = 8.4, 4.5 Hz, 1H), 7.25 (ddd, *J* = 8.4, 2.4, 1.5 Hz, 1H), 6.75 (dt, *J* = 9.6, 2.1 Hz, 1H), 6.67 – 6.65 (m, 1H), 6.42 (dt, *J* = 9.5, 2.1 Hz, 1H), 4.02 (s, 3H). The ^{1}H NMR spectrum is in accordance with the literature.[1]

Methyl 2-(*N*-(3-fluoro-5-phenoxyphenyl)sulfamoyl)benzoate (5b). The title compound was obtained as described for **5a** using **4b** (85 mg, 0.42 mmol) and methyl 2-(chlorosulfonyl)benzoate (147 mg, 0.63 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:9) to provide **5b** as a white solid (148 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.13 (br s, 1H), 7.91 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.84 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.63 (td, *J* = 7.6, 1.1 Hz, 1H), 7.56

(td, *J* = 7.6, 1.3 Hz, 1H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.15 (t, *J* = 7.4 Hz, 1H), 6.91 (d, *J* = 7.7 Hz, 2H), 6.70 (dt, *J* = 9.6, 2.1 Hz, 1H), 6.62 (s, 1H), 6.39 (dt, *J* = 9.9, 2.1 Hz, 1H), 4.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 168.1, 166.0 (d, *J* = 262.1 Hz), 159.1 (d, *J* = 12.7 Hz), 155.8, 138.9 (d, *J* = 14.3 Hz), 137.8, 132.9, 131.7, 130.9, 130.6, 130.4, 129.9, 124.3, 119.5, 107.5 (d, *J* = 3.0 Hz), 103.80 (d, *J* = 25.4 Hz), 102.8 (d, *J* = 25.1 Hz), 53.6. HRMS (ESI) calcd for $C_{20}H_{16}FNNaO_5S$ $[M+Na]^+$ 424.0625, found 424.0633.

Methyl 2-(*N*-(3-(benzyloxy)-5-fluorophenyl)sulfamoyl)benzoate (5c). The title compound was obtained as described for **5a** using **4c** (170 mg, 0.78 mmol) and methyl 2-(chlorosulfonyl)benzoate (275 mg, 1.17 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:4) to provide **5c** as a light yellow oil (284 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.11 (br s, 1H), 7.81 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.80 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.56 (td, *J* = 7.6, 1.2 Hz, 1H), 7.48 (td, *J* = 7.7, 1.3 Hz, 1H), 7.38 – 7.30 (m, 5H), 6.63 (br s, 1H), 6.55 (dt, *J* = 9.6, 2.1 Hz, 1H), 6.42 (dt, *J* = 10.4, 2.1 Hz, 1H), 4.96 (s, 2H), 4.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 168.2, 163.6 (d, *J* = 244.9), 160.2 (d, *J* = 12.86), 138.8 (d, *J* = 13.2), 137.7, 136.1, 132.9, 131.7, 130.9, 130.5, 130.4, 128.7, 128.2, 127.5, 104.3 (d, *J* = 2.9), 101.8 (d, *J* = 25.5), 99.9 (d, *J* = 25.1), 70.3, 53.6. HRMS (ESI) calcd for $C_{21}H_{18}FNNaO_5S$ $[M+Na]^+$ 438.0782, found 438.0785.

***N*-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-2-(hydroxymethyl)benzenesulfonamide (6a).** To a suspension of $LiAlH_4$ (40 mg, 1.05 mmol) in THF (8 mL), at 0 ºC and under nitrogen atmosphere was added dropwise a solution of **5a** (201 mg, 0.5 mmol) in THF (5 mL). The reaction was stirred for 2 h at 0 ºC and then quenched with EtOAc (1 mL) followed by a slurry of Na_2SO_4 and water. The reaction was filtered through celite and washed with EtOAc. The combined organic phases were dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (EtOAc/PE, 7:3) to give **6a** as a colorless oil that solidified upon standing (110 mg, 59%). ^{1}H NMR (400 MHz, DMSO-*d*6): 10.81

(br s, 1H), 8.47 (dd, *J* = 4.4, 1.5 Hz, 1H), 8.36 (d, *J* = 2.6 Hz, 1H), 7.82 (d, *J* = 7.7 Hz, 1H), 7.75 (dd, *J* = 7.7, 1.0 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.48 (ddd, *J* = 8.3, 4.4, 0.7 Hz, 1H), 7.46 – 7.40 (m, 2H), 6.63 (dt, *J* = 10.5, 2.1 Hz, 1H), 6.58 (dt, *J* = 10.0, 2.1 Hz, 1H), 6.47 (s, 1H), 5.53 (br s, 1H), 4.88 (s, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6): 162.9 (d, *J* = 243.6 Hz), 158.3 (d, *J* = 13.7 Hz), 151.6, 145.7, 141.8, 141.7, 140.2 (d, *J* = 13.4 Hz), 134.6, 133.3, 128.8, 127.4, 127.0, 126.8, 124.8, 102.9 (d, *J* = 2.7 Hz), 100.2 (d, *J* = 25.3 Hz), 100.1 (d, *J* = 26.2 Hz), 59.0. HRMS (ESI) calcd for $C_{18}H_{16}FN_2O_4S$ $[M+H]^+$ 375.0809, found 375.0828. The ^{1}H NMR is in accordance with the literature.[1]

***N*-(3-Fluoro-5-phenoxyphenyl)-2-(hydroxymethyl)benzenesulfonamide (6b).** The title compound was obtained as described for **6a** using **5b** (140 mg, 0.35 mmol) and $LiAlH_4$ (28 mg, 0.7 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 3:7) to provide **6b** as a yellowish oil (62 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.07 (br s, 1H), 7.80 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.52 (td, *J* = 7.5, 1.1 Hz, 1H), 7.44 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.39 – 7.28 (m, 3H), 7.14 (t, *J* = 7.4 Hz, 1H), 6.94 – 6.84 (m, 2H), 6.59 (dt, *J* = 9.7, 2.1 Hz, 1H), 6.50 (br s, 1H), 6.36 (dt, *J* = 9.9, 2.1 Hz, 1H), 5.06 (s, 2H), 3.20 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 163.5 (d, *J* = 246.2 Hz), 159.2 (d, *J* = 12.7 Hz), 155.7, 139.1 (d, *J* = 12.9 Hz), 137.8, 136.9, 133.6, 131.7, 130.2, 130.0, 128.6, 124.4, 119.6, 106.8 (d, *J* = 3.1 Hz), 103.2 (d, *J* = 25.6 Hz), 102.5 (d, *J* = 25.0 Hz), 64.0. HRMS (ESI) calcd for $C_{19}H_{16}FNNaO_4S$ $[M+Na]^+$ 396.0676, found 396.0679.

***N*-(3-(Benzyloxy)-5-fluorophenyl)-2-(hydroxymethyl)benzenesulfonamide (6c)**. The title compound was obtained as described for **6a** using **5c** (270 mg, 0.67 mmol) and $LiAlH_4$ (53 mg, 1.4 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 3:7) to provide **6c** as a light yellow oil (247 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.95 (br s, 1H), 7.76 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.42 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.39 – 7.28 (m, 6H), 6.52 (s, 1H), 6.45 (dt, *J* = 9.7, 2.2 Hz, 1H), 6.39 (dt, *J* = 10.4, 2.2 Hz, 1H), 5.06 (s, 2H), 4.93 (s, 2H),

3.04 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 163.5 (d, *J* = 244.9), 160.2 (d, *J* = 13.0), 138.8 (d, *J* = 13.1), 137.7, 137.1, 136.1, 133.5, 131.7, 130.2, 128.7, 128.6, 128.2, 127.5, 103.9 (d, *J* = 2.5), 101.4 (d, *J* = 25.6), 99.7 (d, *J* = 25.2), 70.3, 64.2.

2-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-2,3-dihydrobenzo[*d*]isothiazole-1,1-dioxide (7). To a solution of **6a** (142 mg, 0.38 mmol) in THF (5 mL) at 0 ^{o}C under a nitrogen atmosphere was added PBr_3 (180 µL, 1.90 mmol) dropwise. The reaction was stirred for 5 min at 0 ºC and 25 min at room temperature, and then quenched by the addition of a Na_2CO_3 (sat. aq,) and stirred for 1 h. The aqueous phase was extracted 3 times with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated. The crude product was purified by silica gel flash chromatography (EtOAc/PE, 1:1 to 7:3) to give **7** as a white solid (60 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.48 (s, 1H), 8.46 (d, *J* = 4.1 Hz, 1H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.70 (td, *J* = 7.7, 1.1 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.40 (ddd, *J* = 8.4, 2.7, 1.4 Hz, 1H), 7.34 (dd, *J* = 8.4, 4.1 Hz, 1H), 7.00 (dt, *J* = 10.2, 2.1 Hz, 1H), 6.92 (dd, *J* = 3.0, 2.1 Hz, 1H), 6.47 (dt, *J* = 9.5, 2.1 Hz, 1H), 4.83 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 164.0 (d, *J* = 247.1 Hz), 158.8 (d, *J* = 12.6 Hz), 152.5, 145.6, 142.2, 139.72 (d, *J* = 13.0 Hz), 134.7, 133.6, 131.3, 129.8, 126.5, 124.5, 124.3, 121.7, 103.8 (d, *J* = 3.2 Hz), 101.2 (d, *J* = 25.2 Hz), 100.8 (d, *J* = 26.7 Hz), 49.2. HRMS (ESI) calcd for $C_{18}H_{14}FN_2O_3S$ $[M+H]^+$ 357.0704, found 357.0697. HPLC t_R = 10.9 min, 99.0%. The data is in accordance with the literature.[1]

2-(3-Fluoro-5-phenoxyphenyl)-2,3-dihydrobenzo[*d*]isothiazole-1,1-dioxide (8). The title compound was obtained as described for **7** using **6b** (57.5 mg, 0.15 mmol) and PBr_3 (73 µL, 0.77 mmol) as starting materials. The crude product was purified by silica gel flash chromatography (EtOAc/PE, 3:7) and the title compound **8** was isolated as a colorless oil that solidifies upon standing (42 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.85 (d, *J* = 7.7 Hz, 1H), 7.67 (td, *J* = 7.7, 1.1 Hz, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.41-7.36 (m, 2H), 7.18 (ddd, *J* = 8.4, 7.5, 1.0 Hz,

1H), 7.08-7-05 (m, 2H), 6.97 (dt, *J* = 10.3, 2.1 Hz, 1H), 6.88 – 6.83 (m, 1H), 6.43 (dt, *J* = 9.8, 2.1 Hz, 1H), 4.79 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 164.0 (d, *J* = 246.0 Hz), 159.9 (d, *J* = 12.8 Hz), 155.7, 139.4 (d, *J* = 13.1 Hz), 134.8, 133.4, 131.4, 130.0, 129.6, 124.5, 124.5, 121.6, 119.8, 103.7 (d, *J* = 3.1 Hz), 101.0 (d, *J* = 25.2 Hz), 100.1 (d, *J* = 26.9 Hz), 49.3. HRMS (ESI) calcd for $C_{19}H_{15}FNNaO_3S$ $[M+Na]^+$ 356.0751, found 356.0742. HPLC t_R = 12.8 min, 99.0%.

2-(3-(Benzyloxy)-5-fluorophenyl)-2,3-dihydrobenzo[*d*]isothiazole-1,1-dioxide (9). The title compound was obtained as described for **7** using **6c** (234 mg, 0.6 mmol) and PBr_3 (287 µL, 3.02 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 3:7) to provide **9** as a white solid (185 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.86 (d, *J* = 7.6 Hz, 1H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.50 – 7.31 (m, 6H), 6.90 (s, 1H), 6.78 (d, *J* = 10.3 Hz, 1H), 6.50 (d, *J* = 10.4 Hz, 1H), 5.07 (s, 2H), 4.80 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 164.1 (d, *J* = 244.6 Hz), 160.8 (d, *J* = 13.2 Hz), 139.1 (d, *J* = 13.3 Hz), 136.1, 134.9, 133.4, 131.6, 129.6, 128.7, 128.3, 127.7, 124.5, 121.6, 101.1 (d, *J* = 2.6 Hz), 98.41 (d, *J* = 23.4 Hz), 98.15 (d, *J* = 22.1 Hz), 70.5, 49.3. HRMS (ESI) calcd for $C_{20}H_{16}FNNaO_3S$ $[M+Na]^+$ 392.0727, found 392.0743. HPLC t_R = 12.8 min, 98.1%.

2-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)isoindoline-1,3-dione (12). To **4a** (82 mg, 0.4 mmol) in acetic acid (2 mL) was added phtalic anhydride (40 mg, 0.27 mmol) and the reaction was heated for 16 h at 100 ºC under argon atmosphere. The reaction was cooled to room temperature and quenched with $NaHCO_3$ (sat. aq.). The aqueous phase was extracted 3 times with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (EtOAc/PE, 3:2) to give **12** as a white solid (61 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.50 (d, *J* = 2.8 Hz, 1H), 8.45 (dd, *J* = 4.7, 1.4 Hz, 1H), 7.98 – 7.92 (m, 2H), 7.84 – 7.78 (m, 2H), 7.43 (ddd, *J* = 8.4, 2.8, 1.4 Hz, 1H), 7.34 (ddd, *J* = 0.5, 4..7, 8.4 Hz, 1H), 7.06 (dt, *J* = 9.2, 2.2 Hz,

1H), 7.00 (dd, *J* = 2.9, 2.1 Hz, 1H), 6.75 (dt, *J* = 9.5, 2.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.5, 163.3 (d, *J* = 247.8 Hz), 158.1 (d, *J* = 12.2 Hz), 152.4, 145.7, 142.3, 134.8, 134.0 (d, *J* = 12.9 Hz), 131.4, 126.6, 124.3, 124.0, 112.0 (d, *J* = 3.6 Hz), 109.0 (d, *J* = 25.0 Hz), 105.4 (d, *J* = 24.9 Hz). HRMS (ESI) calcd for $C_{18}H_{13}FN_2NaO_3S$ $[M+Na]^+$ 357.0704, found 357.0697. HPLC t_R = 11.4 min, 97.4%.

4-Fluoro-*N*-(3-fluoro-5-(pyridin-3-yloxy)phenyl)benzenesulfonamide (13). The title compound was obtained as with **5a** using compound **4a** (130 mg, 0.64 mmol) and 4-fluorobenzenesulfonyl chloride (185 mg, 0.96 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:1) to yield **13** as a yellowish oil that crystallize upon standing (166 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.80 (br s, 1H), 8.41 (dd, J = 4.0, 1.8 Hz, 1H), 8.34 (s, 1H), 7.85 – 7.80 (m, 2H), 7.36 – 7.28 (m, 2H), 7.15 (t, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 9.7 Hz, 1H), 6.52 (s, 1H), 6.43 (dd, *J* = 9.5, 1.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 165.4 (d, *J* = 256.2 Hz), 163.8 (d, *J* = 247.6 Hz), 162.6, 158.5 (d, *J* = 12.8 Hz), 152.6, 145.2, 139.6 (d, *J* = 12.9 Hz), 134.9 (d, *J* = 3.2 Hz), 130.0 (d, *J* = 9.5 Hz), 127.1, 124.6, 116.6 (d, *J* = 22.8 Hz), 105.4 (d, *J* = 3.1 Hz), 102.7 (d, *J* = 25.9 Hz), 102.1 (d, *J* = 25.2 Hz). HRMS (ESI) calcd for $C_{17}H_{13}F_2N_2O_3S$ $[M+H]^+$ 363.0609, found 363.0611. HPLC t_R = 10.8 min, 98.7%.

4-Fluoro-*N*-(3-fluoro-5-(pyridin-3-yloxy)phenyl)-*N*-methylbenzenesulfonamide (14). To a suspension of NaH (60% in mineral oil, 7 mg, 0.18 mmol) in DMF (2 mL), at 0 ^{o}C and under nitrogen atmosphere was added **13** (54 mg, 0.15 mmol) solubilized in DMF (2 mL). The resulting thick mixture was warmed to room temperature and stirred for 30 min. The reaction mixture was cooled to 0 °C and iodomethane (14 µL, 0.22 mmol) was added dropwise, warmed to room temperature and stirred for 1 h. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted three times with diethyl ether. The combined organic phases were dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (EtOAc/PE, 1:1) to give **14** as a light yellow oil (36 mg, 65%). ^{1}H NMR (400

MHz, $CDCl_3$): 8.45 (s, 1H), 8.39 (s, 1H), 7.69 – 7.52 (m, 2H), 7.32 (d, *J* = 2.5 Hz, 2H), 7.17 (t, *J* = 8.5 Hz, 2H), 6.79 – 6.47 (m, 3H), 3.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 165.4 (d, *J* = 255.9 Hz), 163.1 (d, *J* = 248.5 Hz), 161.9, 157.9 (d, *J* = 12.6 Hz), 152.4, 145.7, 143.8 (d, *J* = 12.0 Hz), 141.9, 132.2 (d, *J* = 3.3 Hz), 130.4 (d, *J* = 9.4 Hz), 126.4, 124.3, 116.3 (d, *J* = 22.6 Hz), 112.0 (d, *J* = 3.5 Hz), 108.7 (d, *J* = 23.9 Hz), 105.0 (d, *J* = 25.0 Hz), 37.9. HRMS (ESI) calcd for $C_{19}H_{15}F_2N_2O_3S$ $[M+H]^+$ 377.0766, found 377.0767. HPLC t_R = 11.4 min, 99.1%.

***N*-(4-Butylphenyl)-4-fluorobenzenesulfonamide (15).** To 4-butylaniline (150 μL, 0.95 mmol) solubilized in CH_2Cl_2 (2 mL) and pyridine (2 mL) and under argon atmosphere, was added in one portion 4-fluorobenzene-1-sulfonyl chloride (239 mg, 1.2 mmol) stirred at room temperature for 16 h. The reaction was partioned between water and CH_2Cl_2, and the aqueous phase extracted twice with CH_2Cl_2. The combined organic phases were washed with water, 2N HCl, dried ($MgSO_4$) and concentrated. The crude mixture was purified by flash chromatography (EtOAc/PE, 2:3) to provide **15** as an orange solid (284 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.73 (m, 2H), 7.13 – 7.02 (m, 4H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.87 (s, 1H), 2.56 – 2.51 (m, 2H), 1.57 – 1.49 (m, 2H), 1.30 (dq, *J* = 14.5, 7.3 Hz, 1H), 0.90 (t, *J* = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3 (d, *J* = 255.2 Hz), 141.0, 135.2 (d, *J* = 3.2 Hz), 133.6, 130.2 (d, *J* = 9.4 Hz), 129.4, 122.7, 116.4 (d, *J* = 22.6 Hz), 35.1, 33.657, 22.4, 14.0. HRMS (ESI) calcd for $C_{16}H_{18}FNNaO_2S$ $[M+Na]^+$ 330.0934, found 330.0952. HPLC t_R = 13.0 min, >99.9%.

4-Fluoro-*N*-(4-phenoxyphenyl)benzenesulfonamide (16). The title compound was obtained as described for **5a** using 4-phenoxyaniline (101 mg, 0.55 mmol) and 4-fluorobenzene-1-sulfonyl chloride (118 mg, 0.60 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:4) to provide **16** as a white solid (73 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.80 – 7.71 (m, 2H), 7.37 – 7.30 (m, 2H), 7.16 – 7.09 (m, 3H), 7.04 – 6.99 (m, 2H), 6.98 – 6.94 (m, 2H), 6.91 –

6.86 (m, 2H), 6.59 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 165.4 (d, J = 255.5 Hz), 156.9, 155.9, 135.1 (d, J = 3.1 Hz), 130.9, 130.2 (d, J = 9.4 Hz), 130.0, 125.2, 123.8, 119.5, 119.2, 116. 5 (d, J = 22.6 Hz). HRMS (ESI) calcd for $C_{18}H_{14}FNNaO_3S$ $[M+Na]^+$ 366.0571, found 366.0571. HPLC t_R = 12.3 min, 99.8%.

4-Fluoro-*N*-methyl-*N*-(4-phenoxyphenyl)benzenesulfonamide (17). The title compound was obtained as described for **14** using **16** (89.9 mg, 0.26 mmol) as starting material. The crude product was purified by flash chromatography (EtOAc/PE, 5:1) to give **17** as a clear oil (73 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.60 (dd, J = 8.8, 5.1 Hz, 2H), 7.36 (t, J = 7.9 Hz, 2H), 7.19 – 7.11 (m, 3H), 7.02 (d, J = 8.7 Hz, 4H), 6.91 (d, J = 8.9 Hz, 2H), 3.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4 (d, J = 254.9 Hz), 156.9, 156.6, 136.2, 132.7 (d, J = 2.9 Hz), 130.7 (d, J = 9.3 Hz), 130.1, 128.4, 124.1, 119.5, 118.8, 116.2 (d, J = 22.5 Hz), 38.5. HRMS (ESI) calcd for $C_{19}H_{16}FNNaO_3S$ $[M+H]^+$ 380.0727, found 380.0721. HPLC t_R = 12.9 min, 98.9%.

***N*-Mesityl-4-methoxybenzenesulfonamide (2).** The title compound was obtained as described for **5a** using 2,4,6-trimethylaniline as (200 µL, 1.42 mmol) and 4-methoxybenzene-1-sulfonyl chloride (329 mg, 1.59 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:4 to 1:2) and subsequent recrystallization (EtOH) gave **2** as a white solid (218 mg, 50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.9 Hz, 2H), 6.82 (s, 2H), 5.95 (br. s, 1H), 3.86 (s, 3H), 2.24 (s, 3H), 2.01 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 137.7, 137.6, 132.8, 130.2, 129.6, 129.5, 114.2, 55.7, 21.0, 18.8; HRMS (ESI) *m/z*: calculated $C_{16}H_{19}NNaO_3S$ $[M+Na^+]$ 328.0978, found 328.0983. HPLC t_R = 12.1 min, 98.7%. The data is in accordance with the literature.[2]

4-Methoxy-*N*-(4-phenoxyphenyl)benzenesulfonamide (18). The title compound was obtained as described for **5a** using 4-phenoxyaniline (101 mg, 0.55 mmol) and 4-methoxybenzene-1-sulfonyl

chloride (124 mg, 0.59 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:9) to provide **18** as a white solid (135 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.71 – 7.66 (m, 2H), 7.34 – 7.29 (m, 2H), 7.10 (t, *J* = 7.4 Hz, 1H), 7.04 – 7.00 (m, 2H), 6.95 (d, *J* = 7.8 Hz, 2H), 6.95--6.91 (m, 2H), 6.90 – 6.85 (m, 2H), 6.67 (br s, 1H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 163.3, 157.1, 155.4, 131.6, 130.7, 130.0, 129.6, 124.8, 123.6, 119.6, 119.0, 114.3, 55.7. HRMS (ESI) calcd for $C_{19}H_{17}NNaO_4S$ $[M+Na]^+$ 378.0770, found 378.0763. HPLC t_R = 12.0 min, >99.9%.

4-Methyl-*N*-(4-phenoxyphenyl)benzenesulfonamide (19). The title compound was obtained as described for **5a** using 4-phenoxyaniline (101 mg, 0.55 mmol) and 4-methylbenzene-1-sulfonyl chloride (115 mg, 0.59 mmol) as starting materials. The crude product was purified by flash chromatography (EtOAc/PE, 1:9) to provide **19** as a white solid (136 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.68 – 7.61 (m, 2H), 7.35 – 7.29 (m, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = ddd, *J* = 8.1, 7.4, 1.0 Hz), 7.04 – 7.00 (m, 2H), 6.97 – 6.93 (m, 2H), 6.90 – 6.85 (m, 2H), 6.68 (br s, 1H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 157.1, 155.4, 144.0, 136.1, 131.5, 130.0, 129.8, 127.5, 124.8, 123.6, 119.6, 119.0, 21.7 HRMS (ESI) calcd for $C_{19}H_{17}NNaO_3S$ $[M+Na]^+$ 362.0821, found 378.0811. HPLC t_R = 12.4 min, >99.9%.

2,3-Dihydrobenzo[*d*]isothiazole-1,1-dioxide (20). To a suspension of $LiAlH_4$ (2.18 g, 57.3 mmol) in THF (50 mL) at 0 ^{o}C and under nitrogen atmosphere was added dropwise a solution of saccharin (5.0 g, 27 mmol) in THF (20 mL). The reaction was allowed to stir for 2½ h at 0 ºC and 30 min at room temperature. The reaction was placed again at 0 ºC and quenched with EtOAc and solution of Rochelle's salt (sat. aq.). The aqueous phase was extracted with EtOAc (3x). The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (EtOAc/PE, 1:1) to give **20** as a white solid (3.6 g, 78%) . ^{1}H NMR (400 MHz,

$CDCl_3$): 7.76 (d, *J* = 7.8 Hz, 1H), 7.60 (td, *J* = 7.6, 0.9 Hz, 1H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.38 (d, *J* = 7.7 Hz, 1H), 5.17 (br s, 1H), 4.52 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 136.8, 135.4, 133.1, 129.2, 124.8, 121.3, 45.7. The data is in accordance with the literature.[3]

Procedure for determination of solubility

PBS (0.01 M after dilution with MilliQ water) was purchased from Sigma-Aldrich and FaSSIF (prepared according to provider's instructions) were purchased from biorelevant.com. The test compound in a 10 mM DMSO stock solution (20 μL) was added to PBS or FaSSIF (980 μL) and shaken at 25 ^{o}C (800 rpm) for 24 hours (PBS) or 1 hour (FaSSIF). The samples were centrifuged for 5 min at 10.000 rpm, filtered (45 μm, PFTE) and analyzed by HPLC. The solubility was calculated based on reference samples in duplicates (200 μM samples, prepared from 10 mM DMSO stocks diluted with 60% MeOH in water).

Mutational data

Table S1*. Potency and efficacy of aLA,* ***1*** *(TUG-891) and* ***7*** *(TUG-1096) on wild-type hFFA4 and mutations predicted to be in close proximity to the ligand binding pocket.*

		aLA[a]		1		7	
Mutant[b]	**Expression[c]**	**pEC_{50}[d]**	**Δ[e]**	**pEC_{50}[d]**	**Δ[e]**	**pEC_{50}[d]**	**Δ[e]**
WT	100 (100)	5.16 ± 0.08 (100)		7.14 ± 0.04 (98)		6.36 ± 0.03 (100)	
$F88^{2.53}A$	223 (92)	4.33 ± 0.14 (193)	-0.83(***)	5.98 ± 0.09 (134)	-1.16 (***)	5.58 ± 0.04 (114)	-0.78
$L94^{2.59}A$	55 (105)	5.07 ± 0.11 (89)	-0.09	6.90 ± 0.14 (80)	-0.25	6.29 ± 0.15 (72)	-0.07
$R99^{2.64}Q$	29 (70)	NR[f]		NR[f]		NR[f]	
$W100^{2.65}A$	34 (75)	4.88 ± 0.21 (8.8)	-0.29	6.55 ± 0.18 (6.5)	-0.59 (**)	6.24 ± 0.27 (6.0)	-0.12
$W104^{ECL1}A$	27 (72)	NR[f]		NR[f]		NR[f]	
$L114^{3.28}A$	31 (76)	5.30 ± 0.16 (36)	0.14	6.68 ± 0.07 (38)	-0.47 (*)	5.87 ± 0.08 (28)	-0.49 (*)
$F115^{3.29}A$	23 (76)	NR[f]		NR[f]		NR[f]	
$M118^{3.32}A$	36 (72)	5.09 ± 0.25 (4.5)	-0.07	6.96 ± 0.10 (7.8)	-0.19	5.99 ± 0.26 (5.4)	-0.37
$T119^{3.33}A$	89 (106)	5.24 ± 0.13 (95)	0.08	6.74 ± 0.14 (92)	-0.42 (*)	4.54 ± 0.19 (107)	-1.82 (***)
$G122^{3.36}A$	28 (38)	5.35 ± 0.09 (12)	0.18	6.66 ± 0.14 (6.3)	-0.49 (*)	5.93 ± 0.16 (7.2)	-0.43 (*)
$I126^{3.40}A$	87 (83)	4.03 ± 0.09 (94)	-0.14	6.56 ± 0.03 (65)	-0.59 (***)	5.72 ± 0.09 (70)	-0.64 (***)
$T195^{ECL2}A$	50 (94)	4.89 ± 0.13 (68)	-0.28	6.72 ± 0.05 (61)	-0.43	6.00 ± 0.05 (55)	-0.36
$I197^{ECL2}A$	73 (87)	4.94 ± 0.09 (60)	-0.22	6.86 ± 0.16 (56)	-0.29	6.34 ± 0.01 (55)	
$I201^{ECL2}A$	68 (72)	5.22 ± 0.11 (94)	0.06	7.20 ± 0.10 (35)	0.05	6.75 ± 0.07 (34)	0.39
$W207^{5.38}A$	41 (61)	NR[f]		NR[f]		NR[f]	
$F211^{5.42}A$	33 (68)	NR[f]		NR[f]		NR[f]	
$V212^{5.43}A$	116 (85)	5.12 ± 0.26 (75)	-0.04	6.65 ± 0.06 (73)	-0.50 (*)	6.47 ± 0.08 (75)	0.11
$N215^{5.46}A$	39 (82)	5.02 ± 0.19 (40)	-0.14	6.40 ± 0.03 (39)	-0.75 (***)	5.41 ± 0.11 (41)	-0.95 (***)
$F216^{5.47}A$	45 (85)	5.07 ± 0.30 (32)	-0.09	6.76 ± 0.07 (29)	-0.39	6.15 ± 0.07 (32)	-0.21
$W277^{6.48}A$	29 (88)	NR[f]		NR[f]		NR[f]	
$I280^{6.51}A$	68 (98)	5.05 ± 0.07 (29)	-0.12	5.90 ± 0.07 (26)	-1.25 (***)	<5	<-1.36
$I281^{6.52}A$	37 (93)	5.44 ± 0.17 (40)	0.28	7.33 ± 0.09 (29)	0.18	6.31 ± 0.09 (33)	-0.05
$I284^{6.55}A$	47 (98)	4.49 ± 0.18 (20)	-0.68 (*)	6.73 ± 0.07 (58)	-0.42	5.67 ± 0.02 (57)	-0.69 (***)
$F303^{7.35}H$	114 (75)	4.10 ± 0.17 (110)	-1.06 (***)	5.93 ± 0.12 (93)	-1.22 (***)	5.66 ± 0.08 (77)	-0.70 (***)
$F304^{7.36}A$	38 (80)	NR[f]		NR[f]		NR[f]	
$V307^{7.39}A$	70 (105)	5.00 ± 0.14 (130)	-0.16	7.22 ± 0.16 (123)	0.07	6.20 ± 0.22 (119)	-0.16
$T310^{7.42}A$	164 (86)	4.17 ± 0.14 (102)	-1.01 (***)	5.88 ± 0.06 (94)	-1.26 (***)	< 5	<-1.36
$F311^{7.43}A$	99 (100)	5.12 ± 0.10 (98)	-0.04	6.88 ± 0.09 (91)	-0.27	5.81 ± 0.05 (92)	-0.55 (*)

[a]Published previously.[4]
[b]Primary amino acid residue number with Ballesteros and Weinstein position in parentheses.
[c]Cell surface expression is shown as a % of wild-type; total expression is in parentheses.
[d]pEC_{50} values with efficacy expressed as a % of the wild-type α-linolenic acid (aLA) response in parentheses.
[e](pEC_{50} mutant) – (pEC_{50} wt FFA4). Statistical significance is: * p<0.05, ** p<0.01, *** p<0.001.
[f]No response.

Activity of TUG-424 (10) on hFFA4

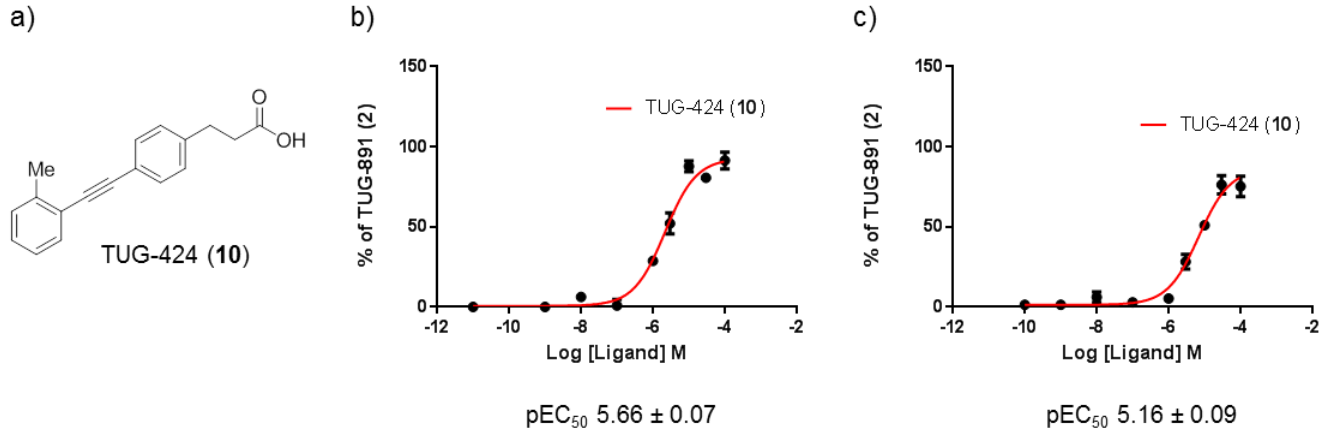

Figure S1. a) Structure of **10**. b) Activity in the β-arrestin-2 recruitment assay. c) Activity in the Ca^{2+} mobilization assay.

Relative blood glucose changes in the oral glucose tolerance test

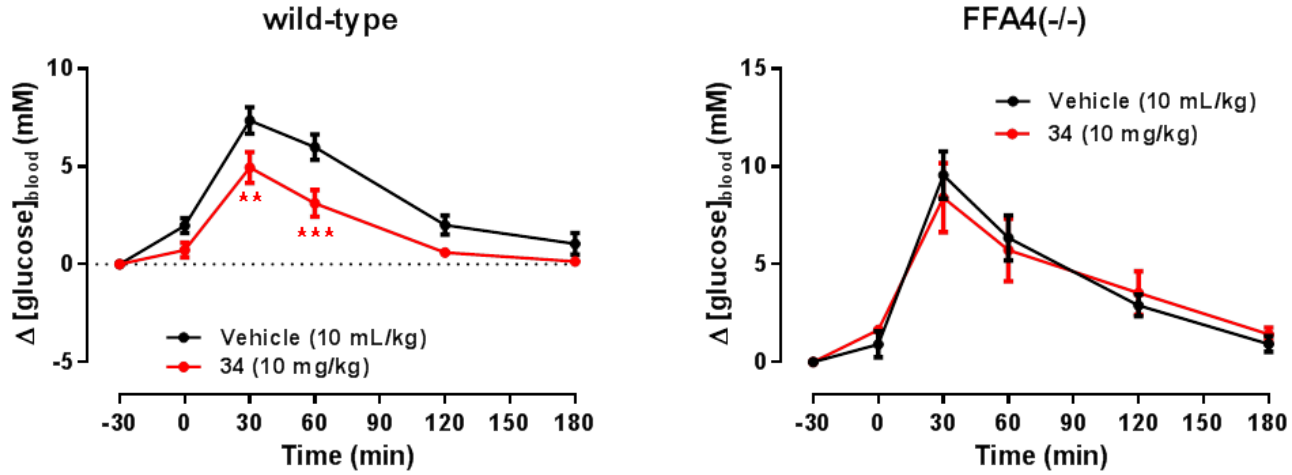

Figure S2. Acute oral glucose tolerance test on day 0 with data represented as change relative to t = -30 min (n=9 mice per group; *p=<0.05, **p=<0.01, ***p<0.001, two-way ANOVA with Bonferroni post hoc test).

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