**Supplemental Information**

**Supplementary Table 1.** Urinary and adipose tissue catecholamines in *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice fed a high–fat diet for 8 weeks.

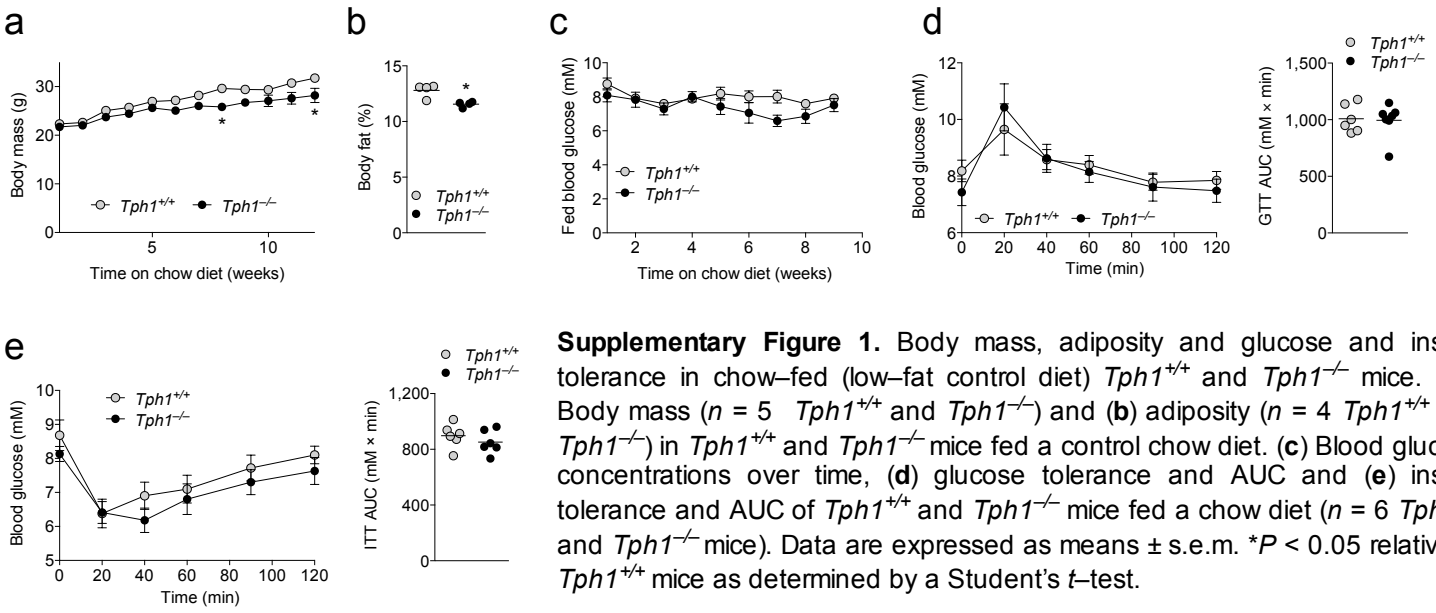
| | *Tph1*$^{+/+}$ | | | *Tph1*$^{-/-}$ | | |
|---|---|---|---|---|---|---|
| **Analyte** | Urine (ng/ml) | eWAT (pg/mg) | iBAT (pg/mg) | Urine (ng/ml) | eWAT (pg/mg) | iBAT (pg/mg) |
| Epinephrine | 29.7 ± 3.95 | 113±8.6 | 170±9.8 | 16.4 ± 3.37* | 106±4.7 | 286±78 |
| Norepinephrine | 174 ± 4.8 | 8.7±2.0 | 18.6±1.0 | 170 ± 3.3 | 8.5±2.0 | 18.6±0.9 |
| Dopamine | 306 ± 7.8 | 1.5±0.5 | 12.1±0.7 | 270 ± 4.2* | 1.4±0.4 | 15.4±2.8 |

Data are expressed as means ± s.e.m. $n = 6$. *$P < 0.05$ versus *Tph1*$^{+/+}$ as determined using a Student's *t*–test. A Welch's *t*–test was used for the iBAT epinephrine analyses.

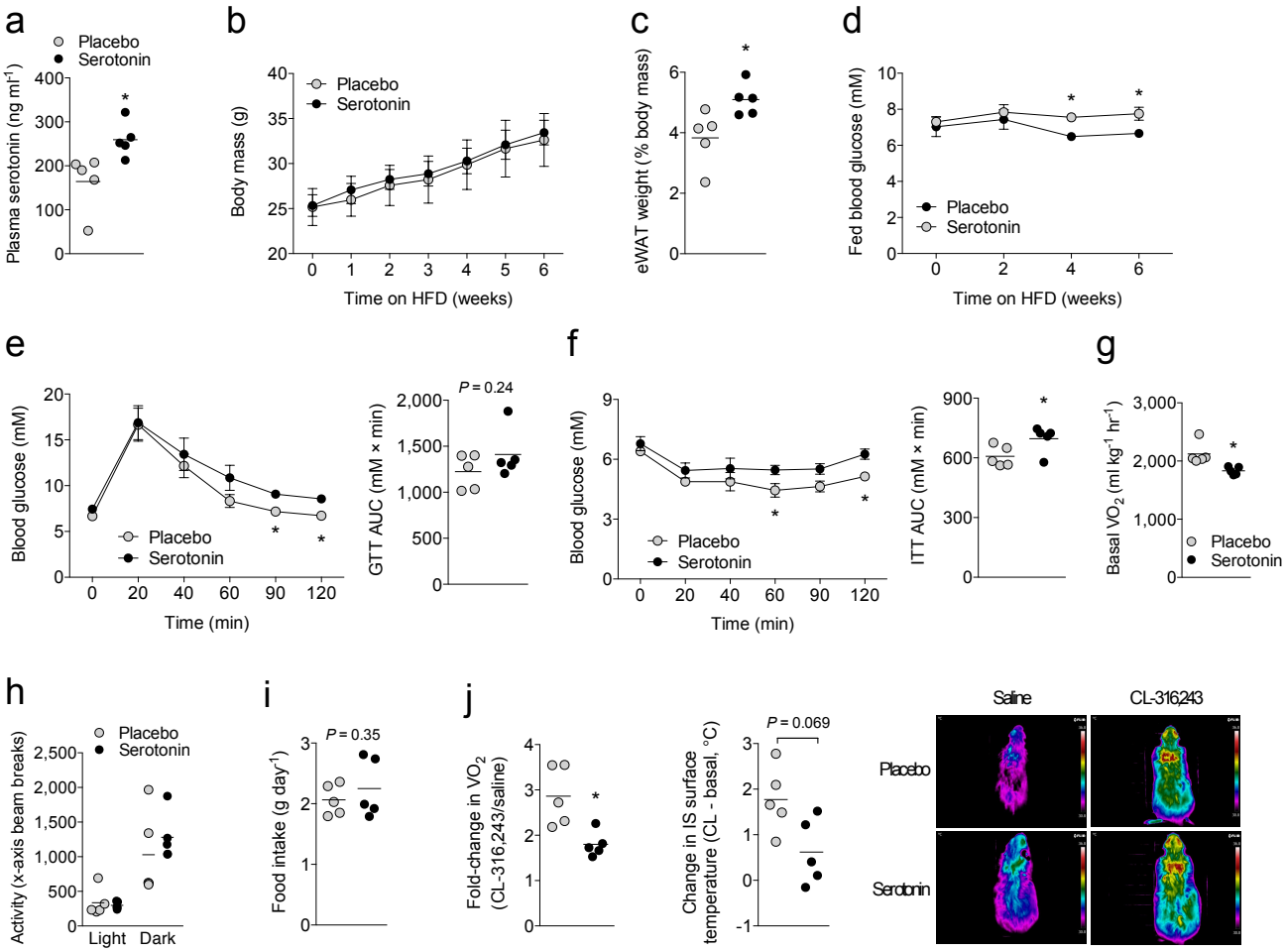
**Supplementary Table 2.** Tissue weights of HFD–fed C57Bl6 mice treated with vehicle or LP533401 for 10 weeks.

| **Tissue** | **Vehicle** | **LP533401** | ***P*–value** |
|---|---|---|---|
| Heart | 172 ± 4.03 | 153 ± 10.6 | 0.06 |
| Spleen | 116 ± 5.56 | 147 ± 23.3 | 0.45 |
| Retroperitoneal Fat Pad | 442 ± 30.5 | 265 ± 29.9* | 0.002 |
| Inguinal Fat Pad | 183 ± 21.5 | 117 ± 16* | 0.04 |
| Epididymal Fat Pad | 2075 ± 160 | 1157 ± 121* | 0.001 |

Tissue weights are in milligrams. Data are expressed as means ± s.e.m. $n = 9$ vehicle and $n = 8$ LP533401 treated. *P*–value was determined using a Student's *t*–test, except for heart and spleen weight, which required a Welch's *t*–test.




**Supplementary Figure 1.** Body mass, adiposity and glucose and insulin tolerance in chow–fed (low–fat control diet) *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice. (**a**) Body mass ($n$ = 5 *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$) and (**b**) adiposity ($n$ = 4 *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$) in *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice fed a control chow diet. (**c**) Blood glucose concentrations over time, (**d**) glucose tolerance and AUC and (**e**) insulin tolerance and AUC of *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice fed a chow diet ($n$ = 6 *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice). Data are expressed as means ± s.e.m. *$P$ < 0.05 relative to *Tph1*$^{+/+}$ mice as determined by a Student's $t$–test.




**Supplementary Figure 2.** Replacement of serotonin in *Tph1*$^{-/-}$ mice increases adiposity, reduces glucose tolerance and insulin sensitivity and suppresses basal metabolic rate and UCP1 mediated thermogenesis. (**a**) Plasma serotonin in HFD–fed *Tph1*$^{-/-}$ mice implanted with subcutaneous 60–day slow release placebo or serotonin (0.5 mg) producing pellet ($n$ = 5). (**b**) Body mass, (**c**) eWAT mass, (**d**) fed blood glucose over time, (**e**) glucose tolerance and (**f**) insulin sensitivity in HFD–fed *Tph1*$^{-/-}$ mice implanted with a subcutaneous placebo or serotonin pellet ($n$ = 5). (**g**) Basal oxygen consumption, (**h**) cage activity and (**i**) food intake in HFD–fed *Tph1*$^{-/-}$ mice implanted with subcutaneous placebo or serotonin producing pellet ($n$ = 5). (**j**) Change in oxygen uptake (left) and dorsal interscapular surface temperature (right) of HFD–fed *Tph1*$^{-/-}$ mice implanted with subcutaneous placebo or serotonin pellets following acute injection with saline or the β3–adrenergic activator CL–316,243 ($n$ = 5, representative thermal images at far right). Data are expressed as means ± s.e.m. *$P$ < 0.05 relative to placebo pellet mice as determined by a Student's $t$–test.

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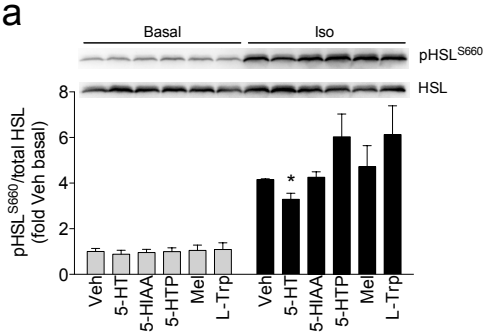
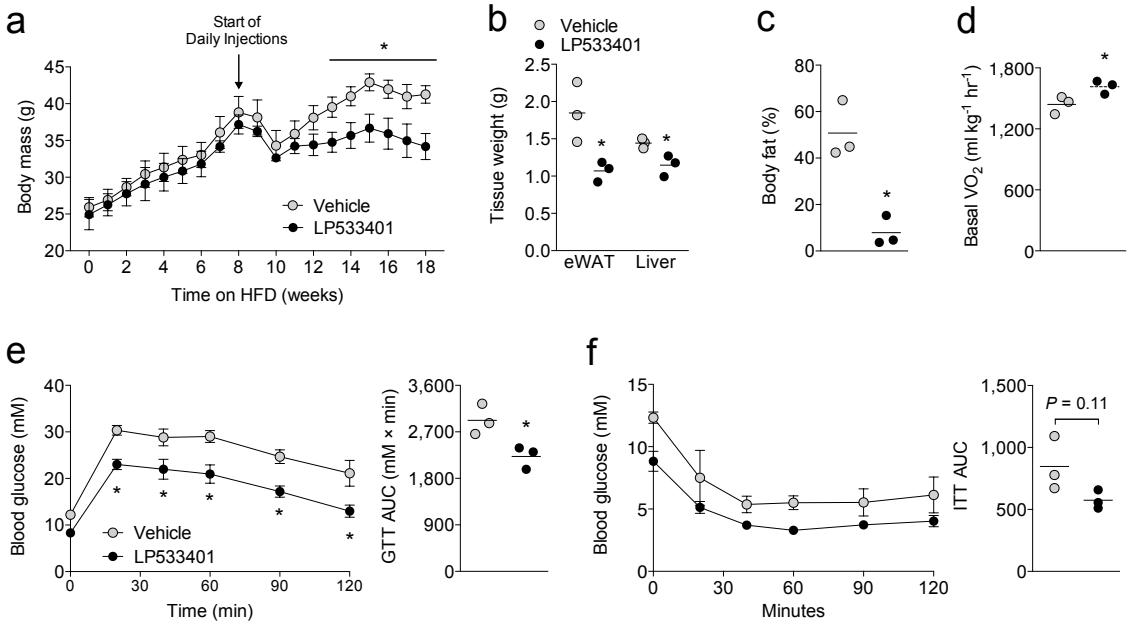
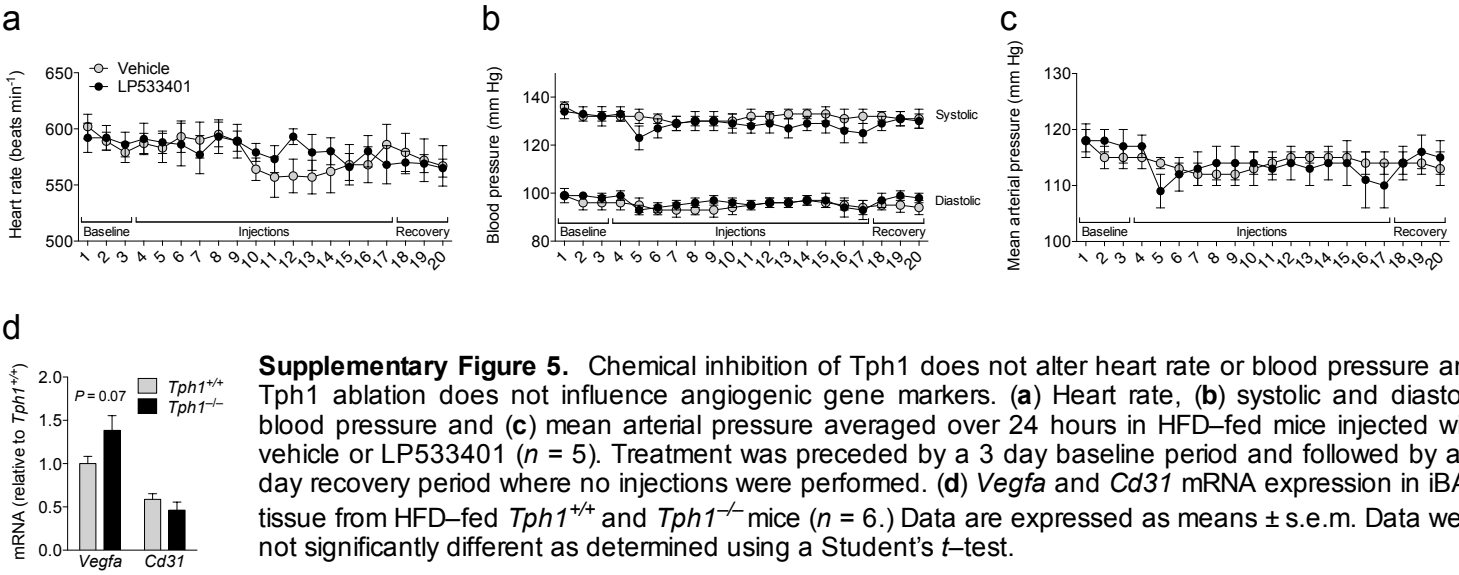


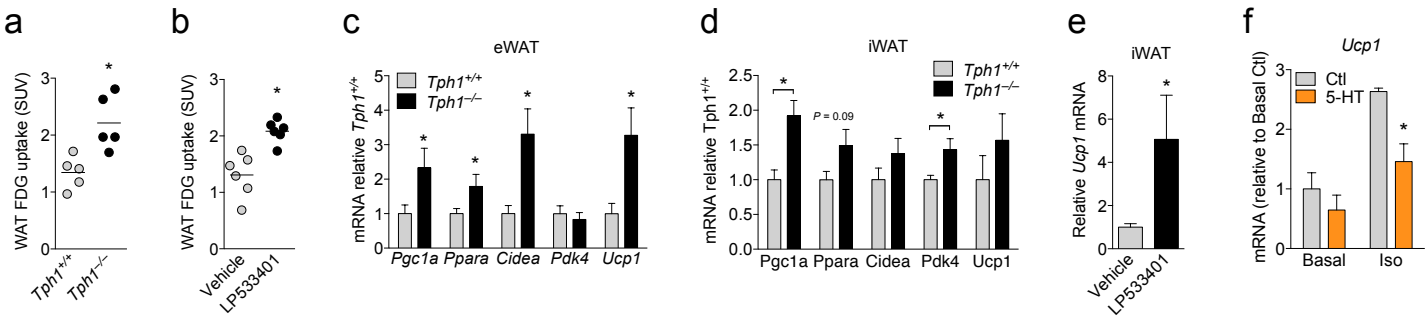

**Supplementary Figure 3.** Serotonin precursors and end products do not inhibit PKA signaling in BAT cells. (**a**) HSL phosphorylation (S660) in differentiated brown adipocytes treated with and without isoproterenol following treatment with serotonin related metabolites ($n$ = 3 experiments). Data are expressed as means ± s.e.m. *$P$ < 0.05 versus corresponding vehicle condition as determined using a Student's $t$–test. 5–HT, 5–hydroxytryptamine; 5–HIAA, 5–hydroxyindoleacetic acid; 5–HTP, 5–hydroxytryptophan; Mel, melatonin; L–try, L–tryptophan.



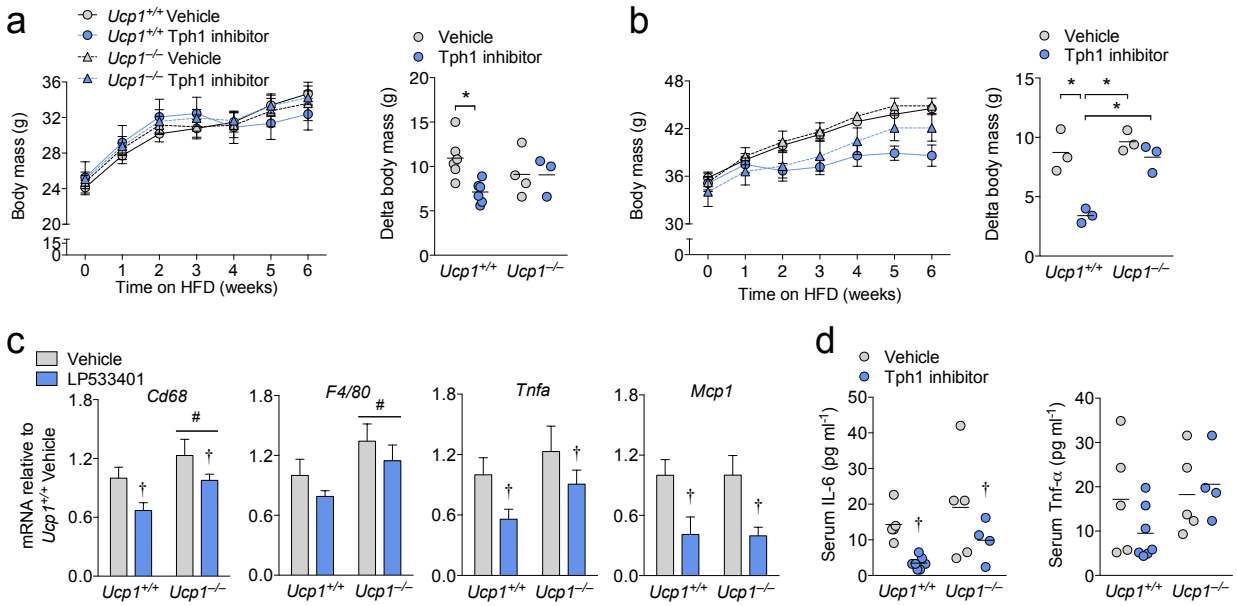

**Supplementary Figure 4.** Reversal of obesity and dysglycemia with chemical inhibition of Tph1. (**a**) Body mass over time, (**b**) eWAT pad and liver weights, (**c**) % body fat, and (**d**) basal oxygen consumption (during an absence of cage movement) in C57Bl6 mice fed a HFD for 8 wks before being treated with vehicle or LP533401 for 10 wks ($n$ = 3 Vehicle and LP533401 treated). (**e**) Glucose tolerance and (**f**) insulin sensitivity of C57Bl6 mice fed a HFD for 8 weeks before being treated with vehicle or LP533401 for 10 weeks ($n$ = 3 Vehicle and LP533401 treated). Data are expressed as means ± s.e.m. *$P$ < 0.05 versus vehicle as determined using a Student's $t$–test.




**Supplementary Figure 5.** Chemical inhibition of Tph1 does not alter heart rate or blood pressure and Tph1 ablation does not influence angiogenic gene markers. (**a**) Heart rate, (**b**) systolic and diastolic blood pressure and (**c**) mean arterial pressure averaged over 24 hours in HFD–fed mice injected with vehicle or LP533401 ($n$ = 5). Treatment was preceded by a 3 day baseline period and followed by a 3 day recovery period where no injections were performed. (**d**) *Vegfa* and *Cd31* mRNA expression in iBAT tissue from HFD–fed *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice ($n$ = 6.) Data are expressed as means ± s.e.m. Data were not significantly different as determined using a Student's $t$–test.




**Supplementary Figure 6.** Beige adipose tissue markers in HFD–fed LP533401 or vehicle treated mice. FDG uptake into WAT of HFD–fed (**a**) *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice ($n$ = 5) and (**b**) vehicle and LP533401 treated C57Bl6 mice ($n$ = 6 per treatment). Gene expression of brown adipose markers in (**c**) eWAT and (**d**) iWAT depots of HFD–fed *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$ mice ($n$ = 8 *Tph1*$^{+/+}$ and *Tph1*$^{-/-}$). (**e**) *Ucp1* mRNA expression from HFD–fed mice treated with vehicle or LP533401 ($n$ = 8 for vehicle and LP533401) and in (**f**) control and isoproterenol–stimulated differentiated inguinal stromal vascular cells ($n$ = 4 per treatment). 5-HT, 5-hydroxytryptamine. Data are expressed as means ± s.e.m. *$P$ < 0.05 versus corresponding *Tph1*$^{+/+}$, vehicle or control condition as determined using a Student's $t$–test.




**Supplementary Figure 7.** Body mass over time in HFD–fed *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ mice treated with a chemical Tph1 inhibitor. Weekly body weights (left) and the increase in body mass after 6 weeks of HFD (right) in (**a**) cohort 1 and (**b**) cohort 2 of *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ mice injected with vehicle or LP533401. Cohort 1: $n$ = 6 for *Ucp1*$^{+/+}$ vehicle and LP533401, $n$ = 4 for *Ucp1*$^{-/-}$ vehicle and $n$ = 3 for *Ucp1*$^{-/-}$ LP533401; cohort 2: ($n$ = 3 for all groups). (**c**) Inflammatory gene expression in eWAT of *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ mice injected with vehicle or LP533401 ($n$ = 9 for *Ucp1*$^{+/+}$ vehicle and LP533401, $n$ = 7 for *Ucp1*$^{-/-}$ vehicle and $n$ = 6 for *Ucp1*$^{-/-}$ LP533401). (**d**) Serum IL–6 and Tnf–α of *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ mice injected with vehicle or LP533401 ($n$ = 5 for *Ucp1*$^{+/+}$ vehicle, $n$ = 7 for *Ucp1*$^{+/+}$ LP533401, $n$ = 5 for *Ucp1*$^{-/-}$ vehicle and $n$ = 4 for *Ucp1*$^{-/-}$ LP533401). Data are expressed as means ± s.e.m. $^{*}P < 0.05$ compared to vehicle, $^{\dagger}P < 0.05$ as a main effect of LP533401 treatment and $^{\#}P < 0.05$ relative to *Ucp1*$^{+/+}$ as determined using a 2–way ANOVA.