## SUPPLEMENTARY DATA

# Identification of FDA-approved drugs as modulators of UCP1 expression in brown adipose tissue

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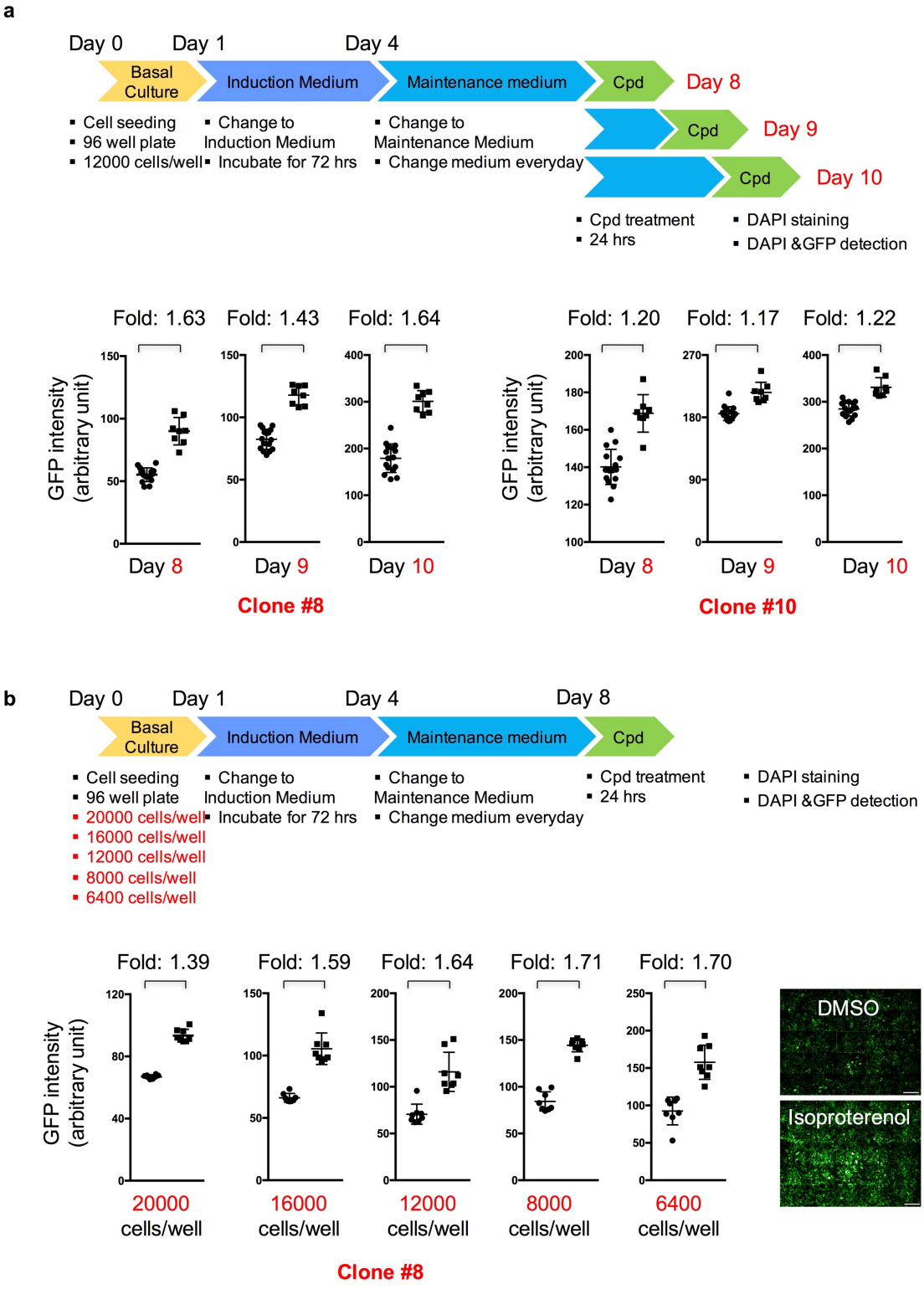
Inventory of Supplemental Information

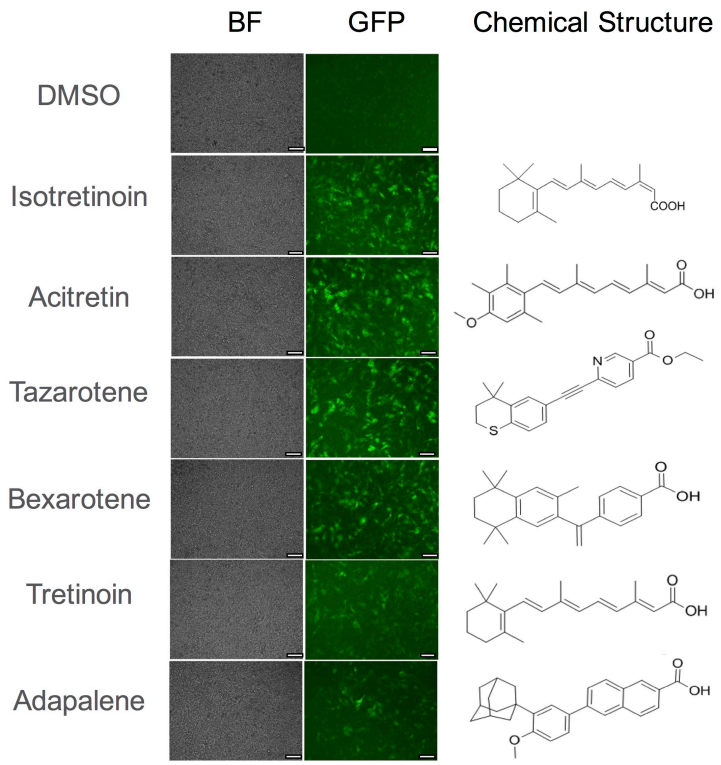
## SUPPLEMENTARY FIGURES

Supplementary Figure 1



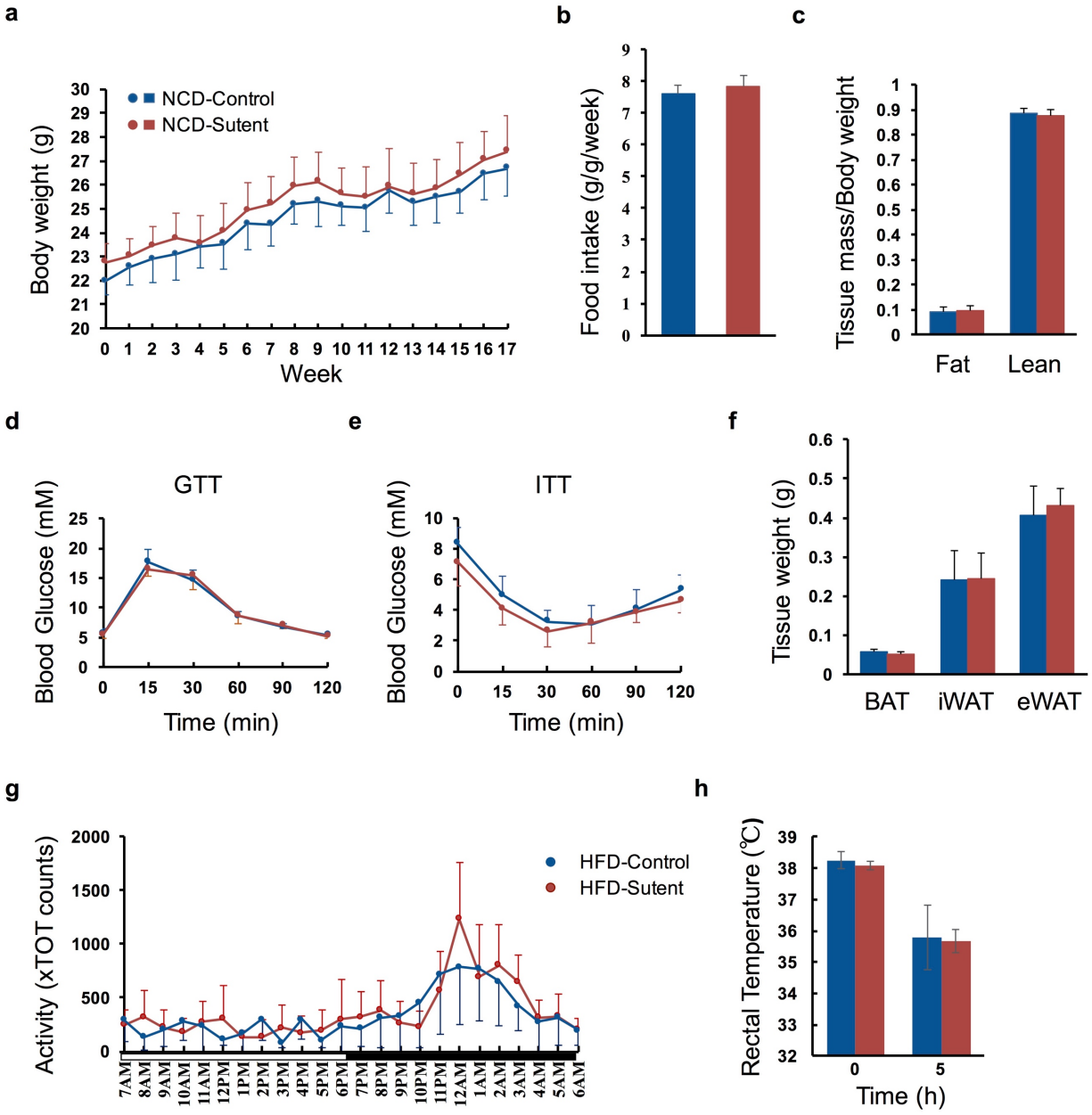
**Supplemental figure 1. Optimization of the culture condition in the screening platform. (a)** Schematic view of the optimization of the treatment timeline (upper). Analysis of the relative GFP intensity change upon isoproterenol treatment at different days (day 8, day 9, day 10) after differentiation using clone #8 or clone #10 (lower). (n=16 for DMSO, n=8 for isoproterenol). (**b**) Schematic view of the optimization of cell seeding density (upper). Analysis of the relative GFP intensity change upon isoproterenol treatment with different seeding densities using clone #8 (lower left). (n=16 for DMSO, n=8 for isoproterenol). Representative images with DMSO or isoproterenol treatment using optimized culture condition in 96-well plates (lower right). Scale bar = 50 μm. Cpd, compoud. Results are means with SD. * $P<0.05$, ** $P<0.01$ (unpaired, two-tailed Student's t test).

Supplementary Figure 2




**Supplemental figure 2. Retinoid receptor agonists increase UCP1 expression in brown adipocytes.** Representative images of GFP intensity in differentiated *Ucp1-2A-GFP* brown adipocytes treated with DMSO or different retinoid receptor agonists. Scale bar = 200 μm. BF, bright field.

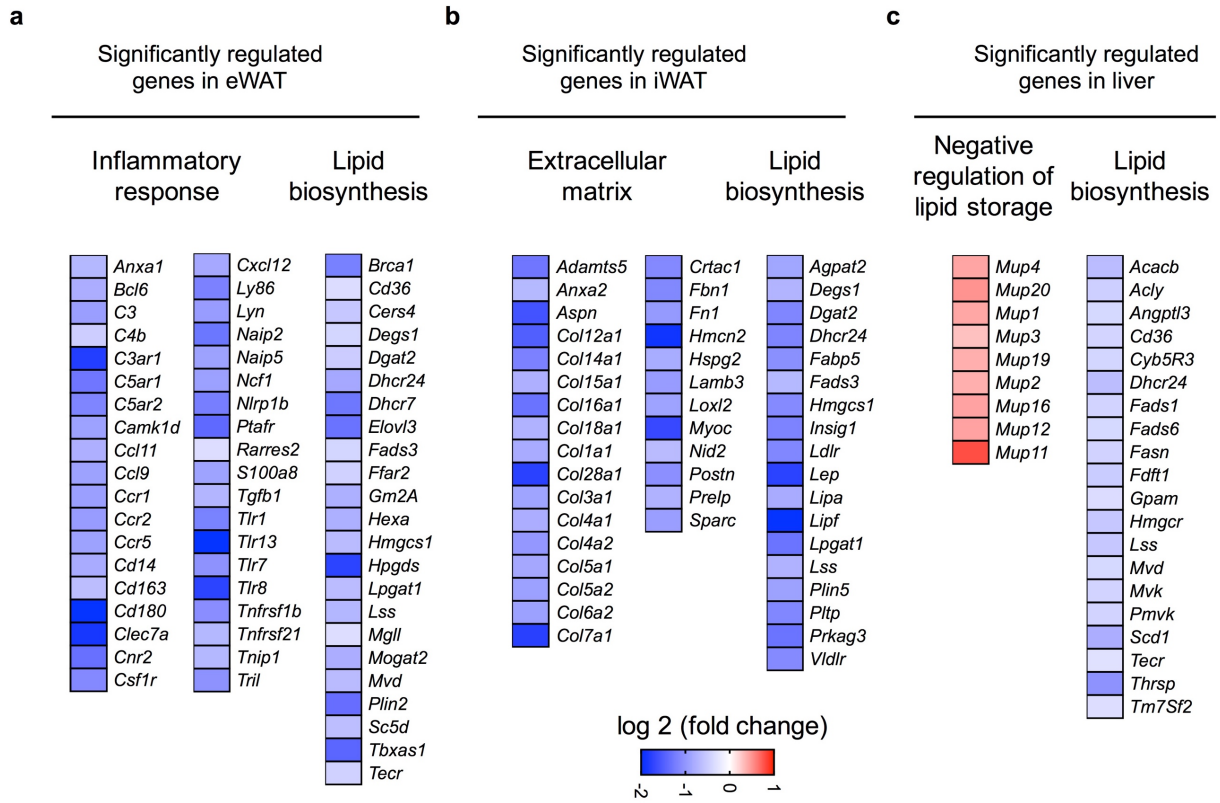
Supplementary Figure 3




**Supplemental figure 3. Sutent treatment causes no significant changes in mice under NCD. (a)** Body weights of mice treated with sutent or vehicle. (n=10 for each treatment). (**b)** Food intake per mouse treated with sutent or vehicle measured over 16-week normalized by body weight. (n=10 for each treatment). **(c)** Body lean and fat composition of mice determined by NMR after 16-week treatment with sutent or vehicle. (n=10 for each treatment). (**d**) Glucose tolerance test (GTT) performed in mice after 11-week treatment with sutent or vehicle. (n=10 for each treatment). (**e**)

Insulin tolerance test (ITT) performed in mice after 12-week treatment with sutent or vehicle. (n=10 for each treatment). **(f)** Weights of different mouse adipose tissues after 16-week treatment with sutent or vehicle. (n=10 for each treatment). (**g)** Physical activity measurement of mice in metabolic cages after 15-week treatment with sutent or vehicle under HFD. (n=8 for each treatment). (**h**) Rectal temperatures of mice exposed in 4 ℃ for the indicated periods after 15-week treatment with sutent or vehicle under HFD. (n=5 for each treatment). Results are means with SD. * $P<0.05$, ** $P<0.01$(unpaired, two-tailed Student's t test).

Supplementary Figure 4




**Supplemental figure 4. Sutent treatment reduces lipid synthesis, inflammation and fibrosis in white adipose tissues and liver tissue. (a)** Heatmap depicting down-regulated genes identified in Gene Ontology analysis in eWAT. **(b)** Heatmap depicting down-regulated genes identified in Gene Ontology analysis in iWAT. **(c)** Heatmap depicting up- or down-regulated genes identified in Gene Ontology analysis in liver tissue.

Supplemental Table 1. **Primer sequences.**

| | |
|---|---|
| **Primers used in donor construction in generation of *Ucp1-2A-GFP* reporter mice** | |
| *Ucp*1-5 arm-F | GCTTGATATCGAATTATTAACTTAAAATGGACAGACCAC |
| *Ucp*1-5 arm-R | AGTAGCTCCGCTACCTGTGGTACAATCCACTGTCTGTCT |
| 2A-GFP-F | GGTAGCGGAGCTACTAACTTCAGC |
| 2A-GFP-R | ACGATTGCTCCAGAACTCAAATTTACTTGTACAGCTCGTCCATGCC |
| *Ucp*1-3 arm-F | TTCTGGAGCAATCGTG CAACTTGGAGGAAGAGATACTGAA |
| *Ucp*1-3 arm-R | TAGAACTAGTGGATCAG CCCCTACAAGAGAAGCATGGCTA |
| **Primers used in genotyping of *Ucp1-2A-GFP* reporter mice** | |
| *Ucp*1-genotype-5-arm | TGCCATTTACTGTCAGCTCTTG |
| | AGTTCACC TTGATGCCGTTCTT |
| *Ucp1*-genotype-3-arm | GACAAGCAGAAGAACGGCATCA |
| | CTGGAACATTGGCTCCTCCCTA |
| **Primers used in qRT-PCR** | |
| *Ucp1* | GCATTCAGAGGCAAATCAGC |
| | GCCACACCTCCAGTCATTAAG |
| *Pgc1a* | TCACGTTCAAGGTCACCCTA |
| | TCTCTCTCTGTTTGGCCCTT |
| *Ppara* | CATTTCCCTGTTTGTGGCTG |
| | ATCTGGATGGTTGCTCTGC |
| *Cidea* | GAATAGCCAGAGTCACCTTCG |
| | AGCAGATTCCTTAACACGGC |
| *Prdm16* | CCGACTTTGGATGGGAGATG |
| | CACGGATGTACTTGAGCCAG |
| *Actin* | GGTGGGAATGGGTCAGAAG |
| | AGCTCATTGTAGAAGGTGTGG |

Supplemental Table 2. **Buffers and antibodies.**

| | |
|---|---|
| **Buffers used in immortalization of primary *Ucp1-2A-GFP* adipocytes** | |
| Isolation buffer | 123mM NaCl, 5mM KCl, 1.3mM CaCl2, 5mM Glucose, 50mM HEPES, 4% BSA, 1.5 mg/ml Collagenase A (Roche, 10103586001), 2mg/ml Dispase II (Sigma, D4693) |
| Primary culture medium | DMEM-high glucose, 20% FBS |
| **Buffers used in adipocyte differentiation** | |
| Induction medium | DMEM, 10% FBS, 1μg/mL insulin (Sigma, I3536), 1nmol/L T3 (Sigma, T2877), 0.125 mmol/L indomethacin (Sigma, I7378), 1μmol/L dexamethasone (Sigma, D4902), 0.5mmol/L isobutylmethylxanthine (Sigma, I5879) and 1μmol/L rosiglitazone (Santa Cruz, sc202795) |
| Maintenance medium | DMEM, 10% FBS, 1μg/mL insulin, and 1nmol/L T3. |
| **Antibodies used in western analysis** | |
| UCP1 | Abcam, ab10983, RRID: AB_2241462 |
| Tubulin | Sigma, T6557, AB_477584 |
| HSP90 | CST, 4874S, RRID: AB_10694856 |
| p-STAT3 (Tyr 705) | Santa Cruz, sc8059, RRID: AB_628292 |
| STAT3 | CST, 9139S, RRID: AB_331757 |
| p-p38 | ABclonal, AP0526 |
| p38 | ABclonal, A10832 |
| p-ERK1/2 | CST, 9106S, RRID: AB_331768 |
| ERK1/2 | ABclonal, A11116 |
| p-ATF2 | CST, 9221, RRID: AB_2561045 |
| ATF2 | ABclonal，A2155 |
| p-CREB1-S133 | ABclonal, AP0019 |
| CREB1 | ABclonal, A1189 |