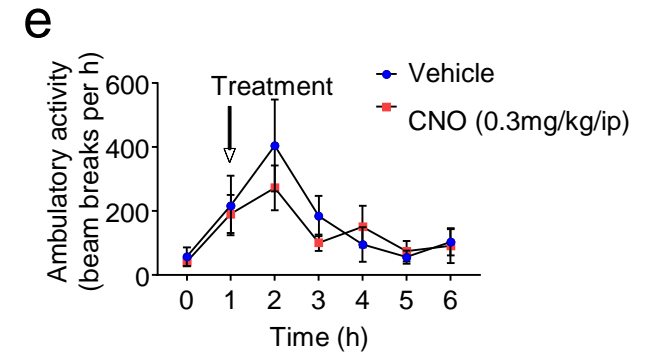
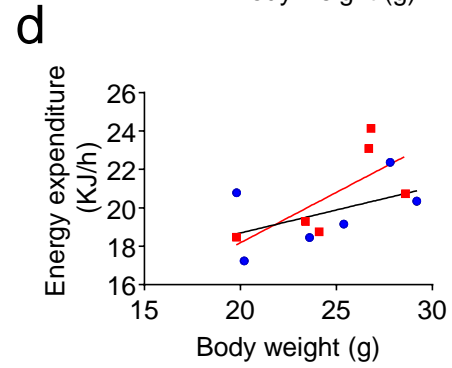
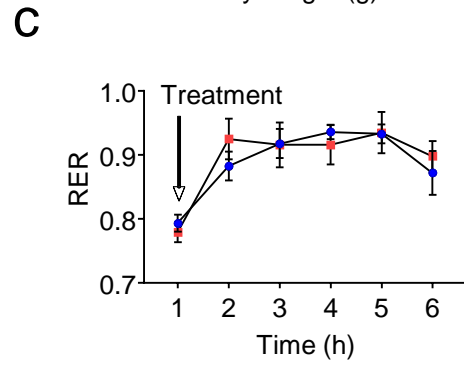
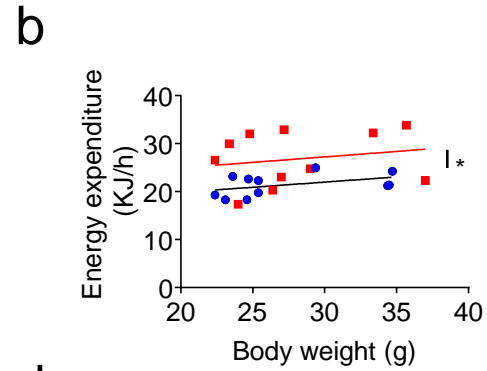
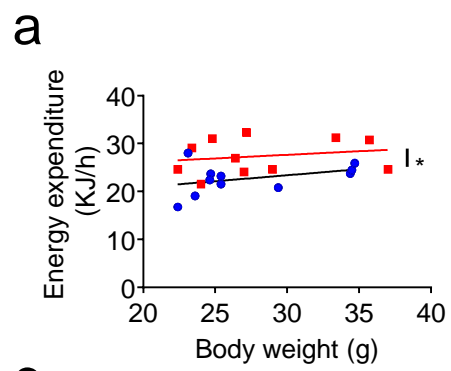
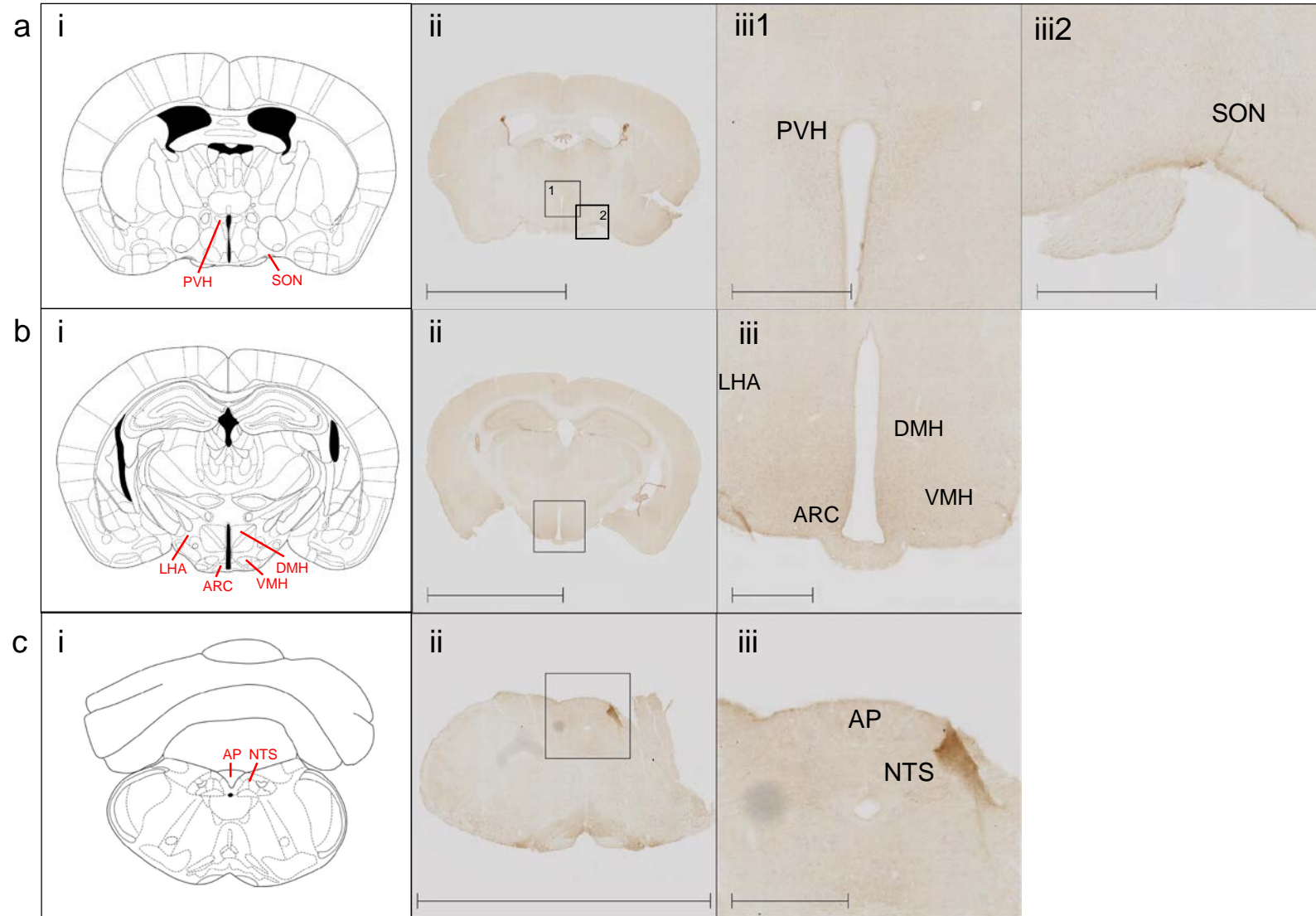


# Suppl Figure 1



# Suppl. Figure 2



488 Supplementary figure 1: Energy expenditure is increased by colonic L-cell stimulation. (a) Energy  
489 expenditure 1h and (b) 2h post administration of CNO in Dox-induced Tet-Cre<sup>rtTA</sup>-InsI5xDq mice. Values  
490 are group mean  $\pm$  SEM (n = 11, cross over design). \* p < 0.05 by ANCOVA (a,b). (c) RER, (d) energy  
491 expenditure and (e) activity were unaffected by CNO in wild-type mice. Values are group mean  $\pm$  SEM (n  
492 = 6).

493 Supplementary figure 2: GFP expression was undetected in the central nervous system of Dox-induced  
494 InsI5-rtTAxGCaMP6f mice. Coronal sections from mice stained for GCaMP6f (GFP). Drawings are based on  
495 the Paxinos Mouse Brain Atlas. (a) Paraventricular nucleus of the hypothalamus (PVN), supraoptic nucleus  
496 (SON). (b) Lateral hypothalamic area (LHA), dorsomedial hypothalamus (DMH), ventromedial  
497 hypothalamus (VMH), arcuate nucleus (ARC). (c) Area postrema (AP), nucleus tractus solitarius (NTS).  
498 Scale bars represent 5 mm in (ii) and 500  $\mu$ m in (iii). GFP-immunohistochemical staining was readily  
499 observable in the colon of these mice (not shown). Note: some small alterations were made during the  
500 preparation of coronal CNS section from the previously described method: Mice were anaesthetised with  
501 Dolethal (Vetoquinol, Towcester, UK) before being transcardially fixated with 4% PFA in PBS as described  
502 previously [21]. Tissue postfixed for 24h in 4% PFA overnight and a sucrose gradient (15% wt/vol for 6  
503 hrs, 30% overnight) was sectioned using a freezing sliding microtome. Sections were blocked for 1 h in 5%

504 donkey serum, 0.3% (v/v) Tween-20 in PBS, sequentially incubated with GFP antiserum (1:1000, Abcam  
505 #5450), biotinylated donkey anti-goat IgG (1:400, Millipore) and avidin-biotin complex (Vector  
506 Laboratories Inc.) and developed using DAB (Abcam), before being dehydrated with an ethanol gradient  
507 and mounted with Pertex mounting medium (Pioneer Research Chemicals Ltd, PRC/R/750).