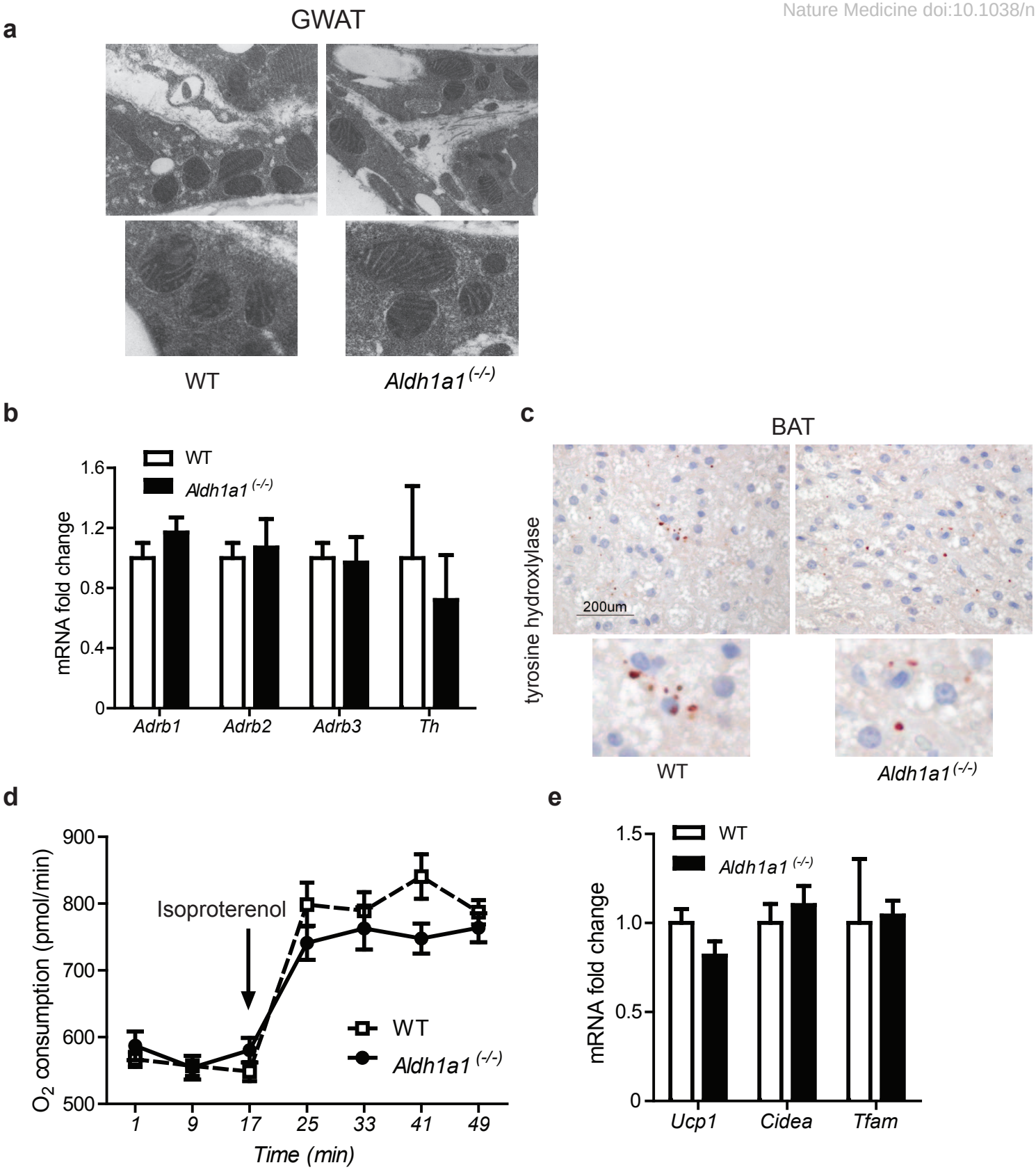
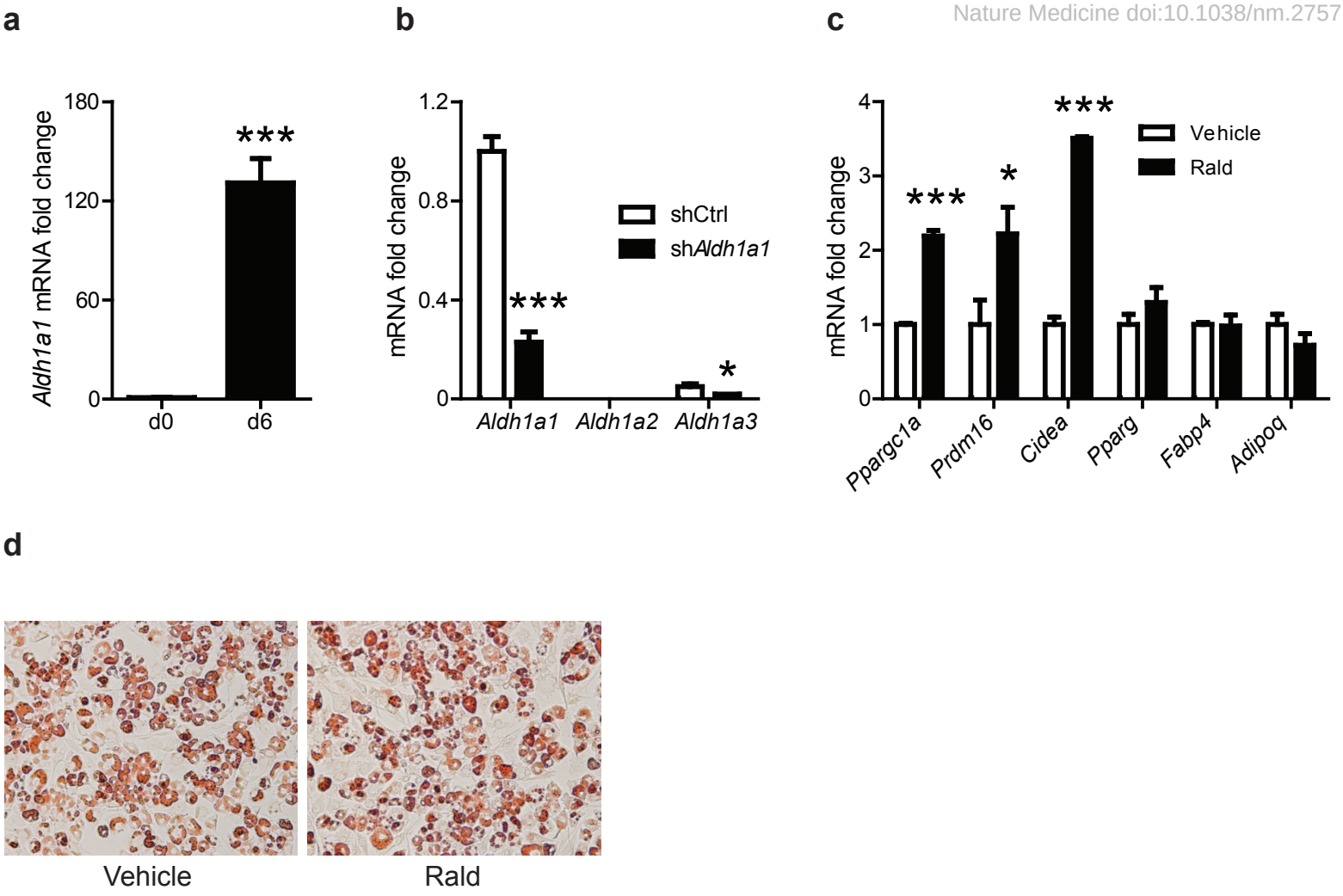


Supplementary Figure 1. White and brown fat characteristics in *Aldh1a1* deficiency

(a) Mitochondrial ultrastructure was analyzed in gonadal white adipose tissue (GWAT) of wild type (WT) and retinaldehyde dehydrogenase deficient (*Aldh1a1-/-*) mice (n=4/group) using electron microscopy. Representative images at 10,000-fold magnification are shown. **(b)** mRNA expression of the beta adrenergic receptors (*Adrb1-3*) and tyrosine hydroxylase (*Th*) in brown adipose tissue (BAT) from WT and *Aldh1a1-/-* mice (n = 8/group). **(c)** Representative tyrosine hydroxylase immunohistochemistry in BAT from WT and *Aldh1a1-/-* mice. **(d)** Oxygen consumption in stromal-vascular cell-derived brown adipocytes from WT and *Aldh1a1-/-* BAT at baseline and after isoproterenol stimulation (1μM). **(e)** mRNA expression of classic BAT markers in isoproterenol stimulated WT and *Aldh1a1-/-* brown adipocytes. (n = 5/condition).



Supplementary Figure 2. Manipulation of the retinaldehyde pathway in C3H10T1/2 adipocytes
(a) *Aldh1a1* mRNA expression in C3H10T1/2 (10T1/2) cells before (d0) and after adipogenic (d6) differentiation. **(b)** *Aldh1a1* mRNA expression in 10T1/2 cells stably transfected with shRNA against *Aldh1a1* (sh*Aldh1a1)* or control shRNA (shCrtl). **(c)** mRNA expression of indicated genes in 10T1/2 cells following retinaldeyhde (Rald) stimulation (1μM) during adipocyte differentiation. **(d)** Oil-Red-O staining in Rald- or vehicle- stimulated differentiated 10T1/2 cells. n=5/condition, *p<0.05, ***p<0.001.

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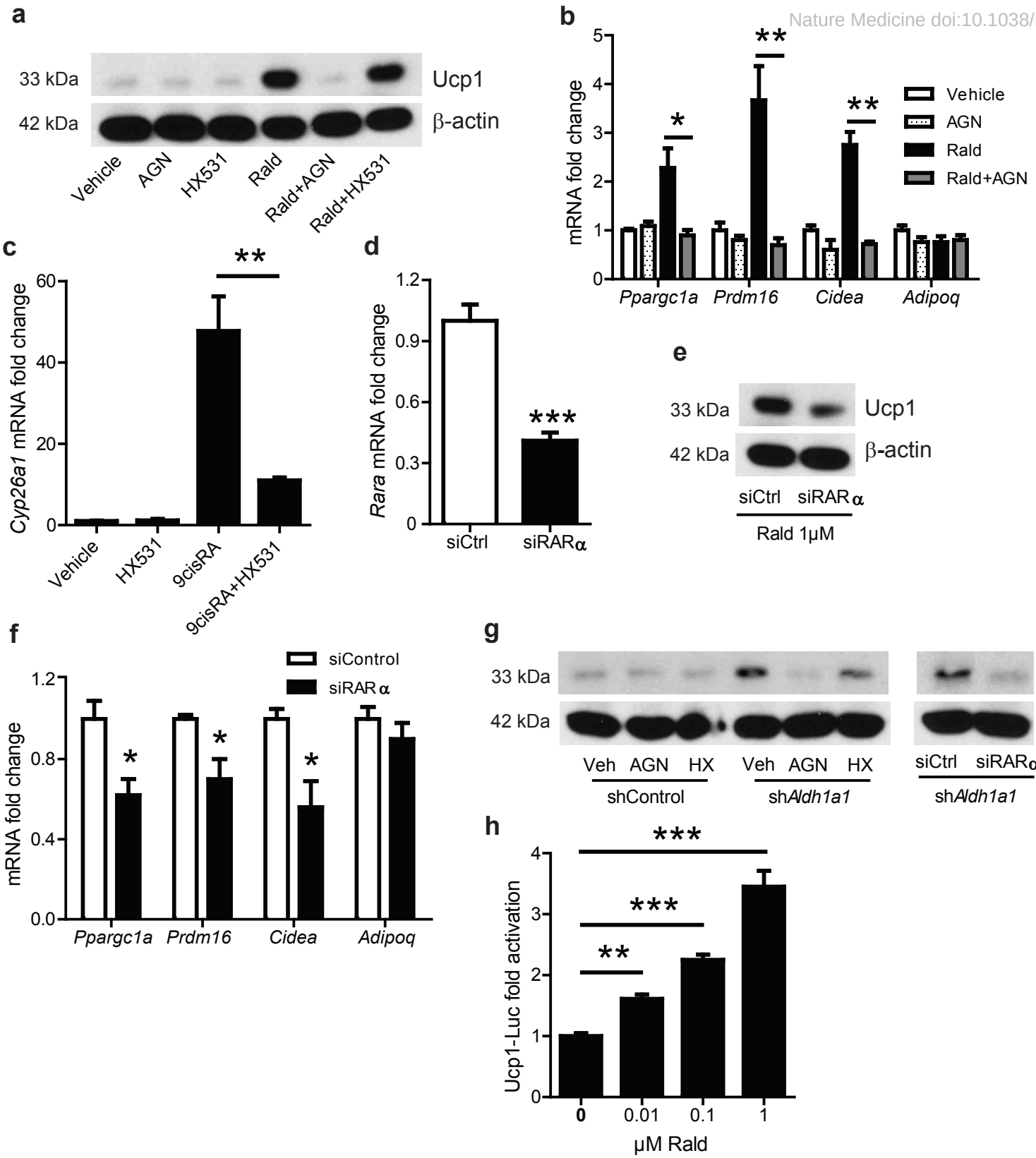

Supplementary Figure 3. Rald regulates a transcriptional BAT program in white adipocytes through RAR
(a) Uncoupling protein 1 (Ucp1) protein analysis and **(b)** mRNA expression of classic BAT markers in 10T1/2 adipoyctes, stimulated as in Fig. 5a. **(c)** *Cyp26a1* mRNA expression in differentiated 10T1/2 cells stimulated with the RXR antagonist HX513, 9cis retinoic acid (9cisRA) or both (1μM each, 24h). **(d)** Retinoic acid receptor α (RARα, *Rara*) mRNA expression in 10T1/2 cells 48h after transient transfection with *Rara* siRNA (siRARα) or scrambled (siCtrl). **(e)** Ucp1 protein analysis and **(f)** mRNA expression of classic BAT markers in 10T1/2 cells transfected with siCtrl or siRARα and stimulated as in Fig. 5b. **(g)** Ucp1 protein analysis in shControl and sh*Aldh1a1*-transfected 10T1/2 cells stimulated as in Fig. 5c,d. **(h)** Undifferentiated 10T1/2 cells were transfected with a murine 3.1kb Ucp1 promoter luciferase construct (Ucp1-Luc) and subsequently stimulated (24h) with increasing concentrations of Rald. Normalized luciferase activities are shown as fold change. n=5/condition, *p<0.05, **p<0.01, ***p<0.001

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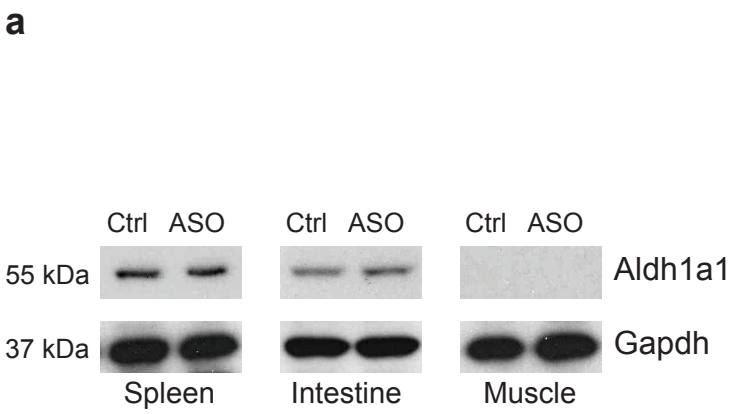

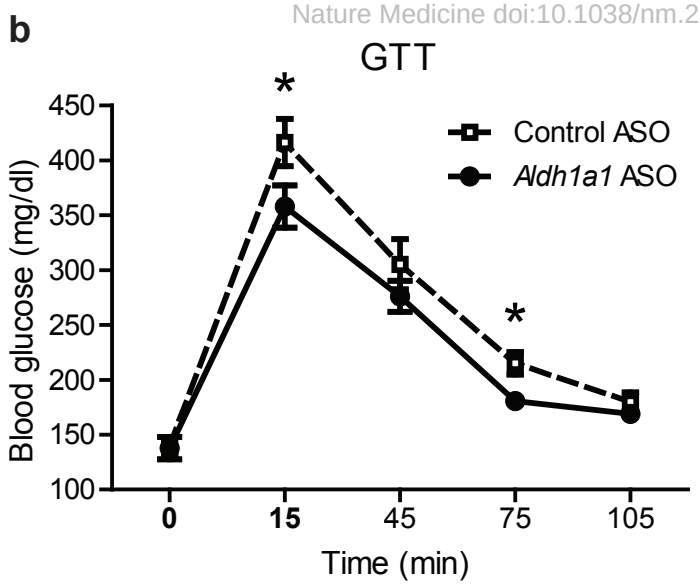

Supplementary Figure 4. *Aldh1a1* antisense treatment

(a) Aldh1a1 protein expression in indicated tissues of *Aldh1a1* antisense oligonucleotide (*Aldh1a1* ASO) versus control ASO (Ctrl ASO) treated chow-fed mice. Representative western blots are shown. **(b)** Glucose tolerance test in *Aldh1a1* versus Ctrl ASO-treated C56Bl/6 mice on high-fat diet (n=8/group). *p<0.05.