Supplementary Information

Overexpression of *Fto* leads to increased food intake and results in obesity

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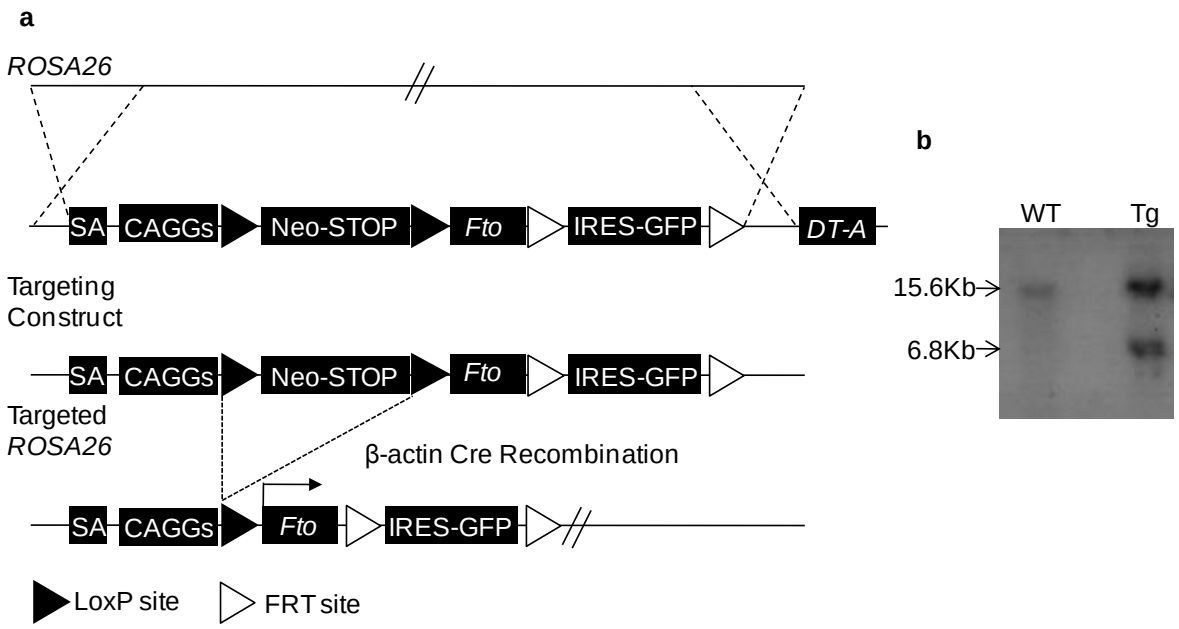
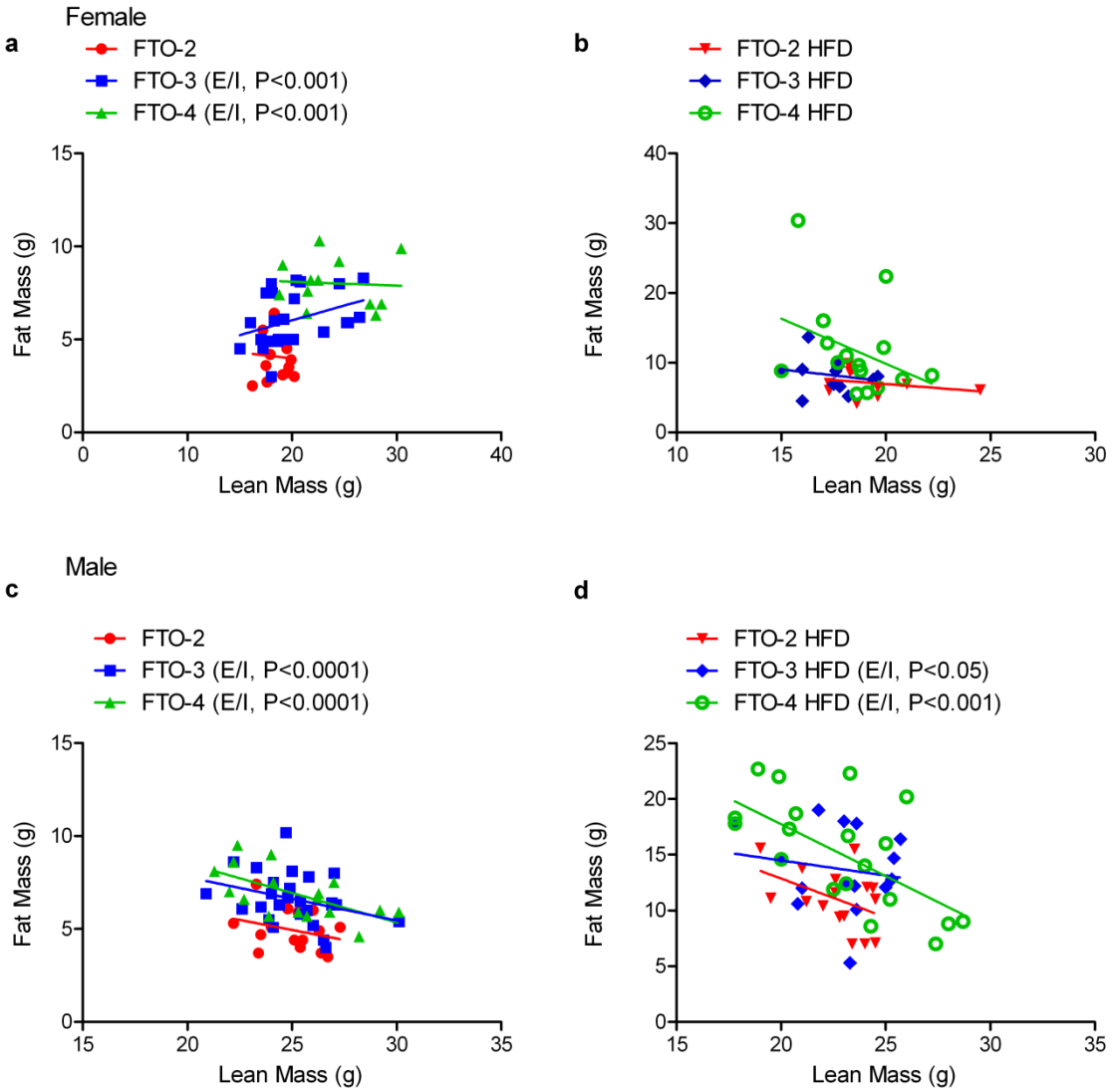


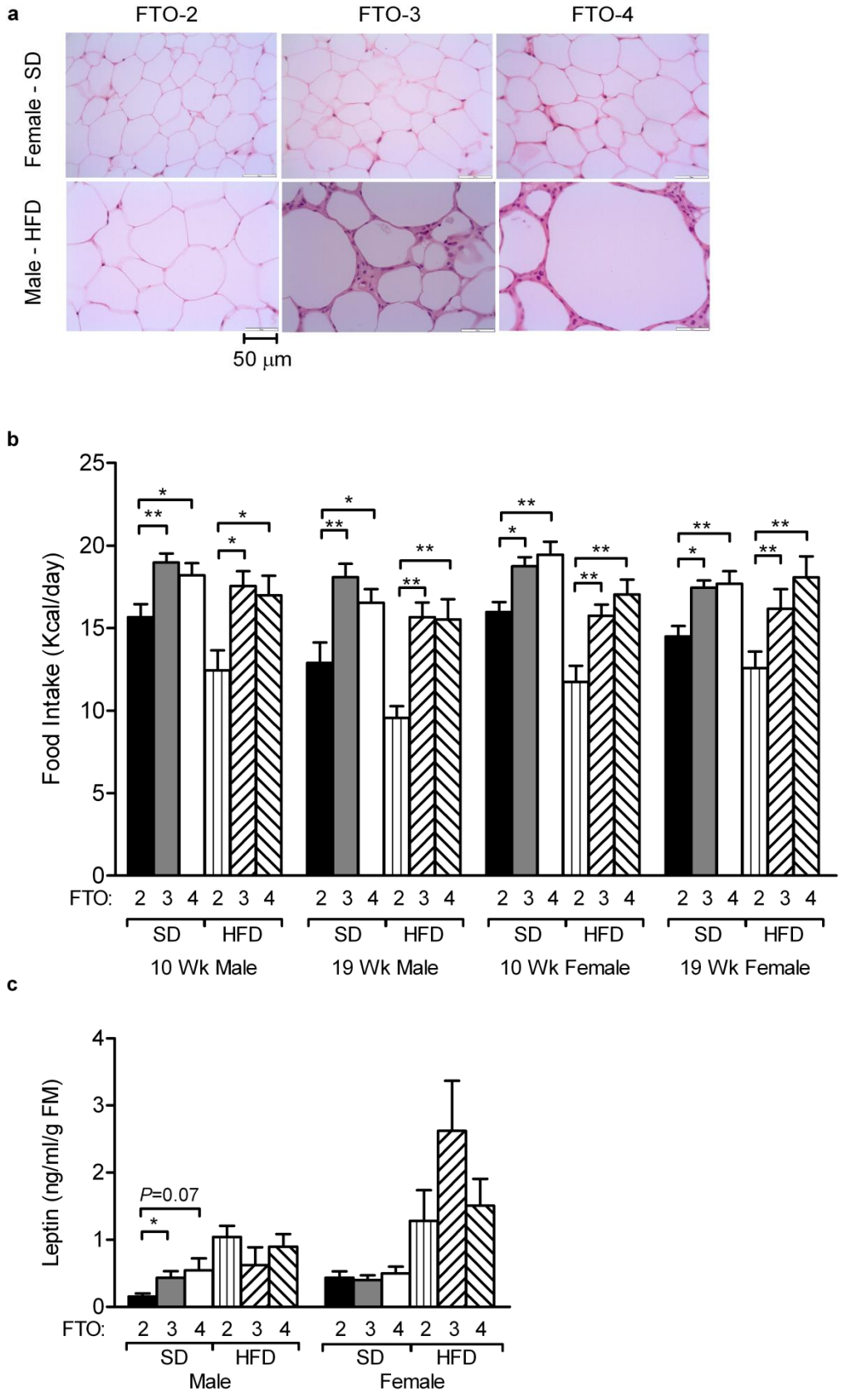
Supplementary Figure 1: *Generation of a mouse model overexpressing Fto*

(a) Strategy for generating a conditional *Fto* overexpression allele. *Top*, the targeting construct consisted of *Fto* cDNA, downstream of a LoxP-flanked neomycin resistance gene and a transcriptional stop signal and followed by an IRES eGFP reporter. This construct was placed under the control of a CAGGs promoter and inserted into the ROSA26 locus. *Bottom*, mice carrying the floxed allele were crossed to mice expressing Cre-recombinase under the control of the beta-actin promoter. Cre recombination excises the stop cassette, activating expression of *Fto* in all tissues. Neo, neomycin resistance gene; Stop, transcriptional stop signal; IRES, Internal Ribosome Entry Site; DT-A, diphtheria toxin A. SA, splice acceptor; CAGGS chicken beta-actin promoter; slashed lines, not to scale.

(b) Southern blot analysis of targeted ES clones. Genomic DNA was digested with EcoRI and probed with a 5'-external ROSA probe to confirm targeting of the ROSA26 locus. WT, wild-type; Tg, transgenic.



Supplementary Figure 2: *Association of 20 week lean mass with fat mass.*
(a) Females SD, FTO-2 (n=16), FTO-3 (n=25), FTO-4 (n=12).
(b) Females HFD, FTO-2 (n=12), FTO-3 (n=12), FTO-4 (n=15).
(c) Males SD, FTO-2 (n=15), FTO-3 (n=27), FTO-4 (n=16).
(d) Males HFD, FTO-2 (n=15), FTO-3 (n=15), FTO-4 (n=19).
All graphs were obtained by linear regression with comparison of slope (S) and elevation or intercept (E/I) to FTO-2 mice (GraphPad Prism). The lines are the best fit of a straight line through the data using linear regression analysis. P values for differences in the slope (S) and the intercept (I) of the lines are against FTO-2 mice. (GraphPad Prism). Regression analysis of fat mass against lean mass shows that there is significant difference in the intercept between FTO-3 (and FTO-4) mice and FTO-2 mice, for female mice on a standard diet and for male mice on standard and high fat diets. Although the slope of the relationship between fat and lean mass in individual animals is similar between the genotype groups, overexpression mice have a different body composition, with a higher fat to lean tissue ratio.



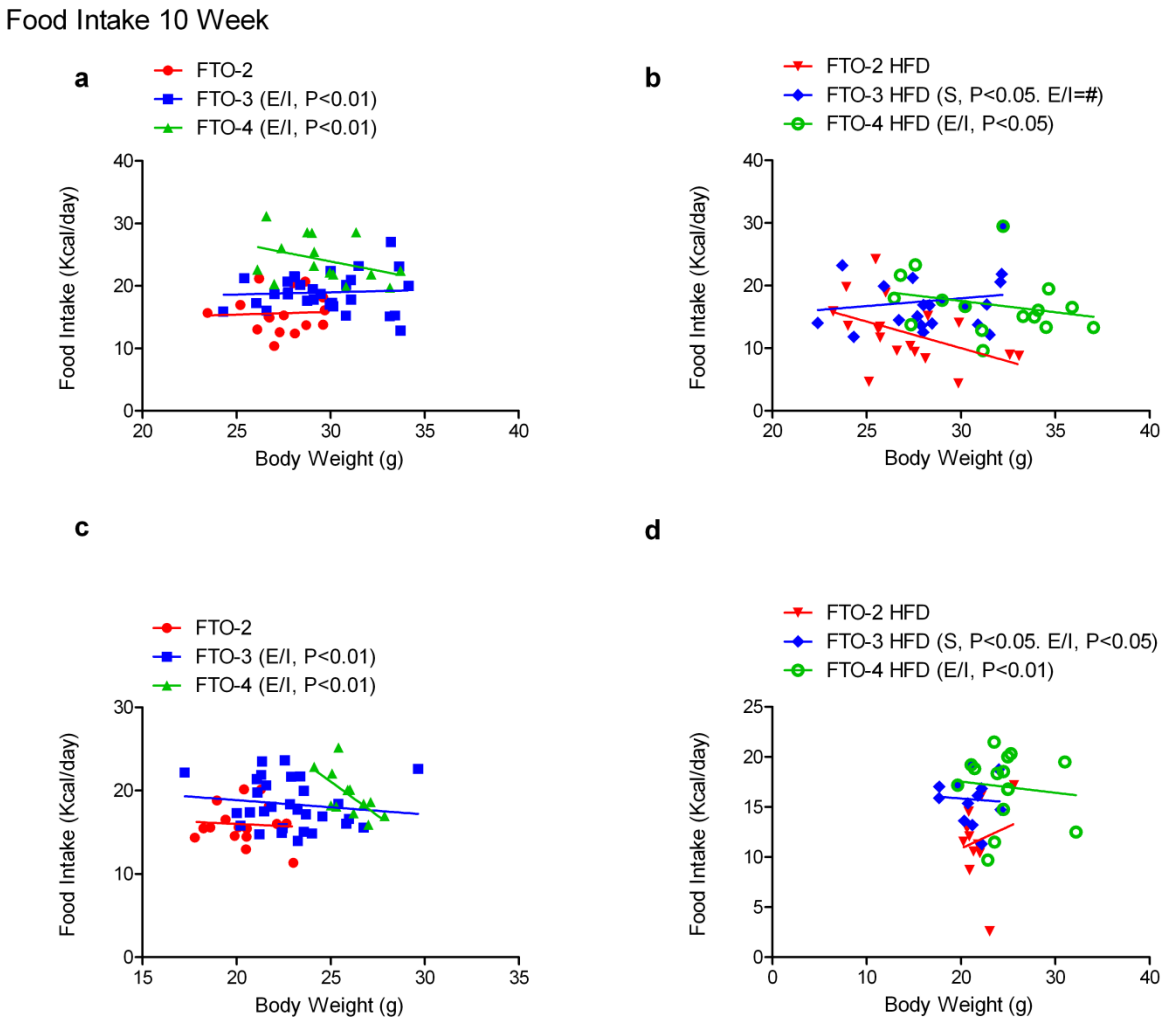
Supplementary Figure 3: *Epigondal WAT, food intake and plasma leptin*

(a) Epigondal WAT dissected from 20 week-old female FTO-2, FTO-3 and FTO-4 mice on a SD and male FTO-2, FTO-3 and FTO-4 mice on a HFD, stained with haematoxylin and eosin to show adipocyte size. Scale bar, 50 μm.

(b) Food intake over 24 hours measured at 10 and 19 weeks in male and female mice on a standard or high fat diet. **Males: SD**, FTO-2 (n=16), FTO-3 (n=30), FTO-4 (n=15); **HFD**, FTO-2 (n=12), FTO-3 (n=14), FTO-4 (n=15). **Females: SD**, FTO-2 (n=16), FTO-3 (n=30), FTO-4 (n=15); **HFD**, FTO-2 (n=15), FTO-3 (n=14), FTO-4 (n=16).

(c) Plasma leptin levels adjusted for fat mass (FM) determined by ELISA at 20 weeks of age following a 6-hour light phase fast. **Males: SD,** FTO-2 (n=14) FTO-3 (n=24) and FTO-4 (n=14); **HFD**, FTO-2 (n=16) FTO-3 (n=14) and FTO-4 (n=14). **Females: SD,** FTO-2 (n=14) FTO-3 (n=16) and FTO-4 (n=13); **HFD,** FTO-2 (n=12) FTO-3 (n=14) and FTO-4 (n=9).

Data (b-c) are expressed as mean±SEM, Statistical analysis was performed using Student's t-test. * $P<0.05$, ** $P<0.01$.



Supplementary Figure 4: *Association of body weight with food intake at 10 weeks.*

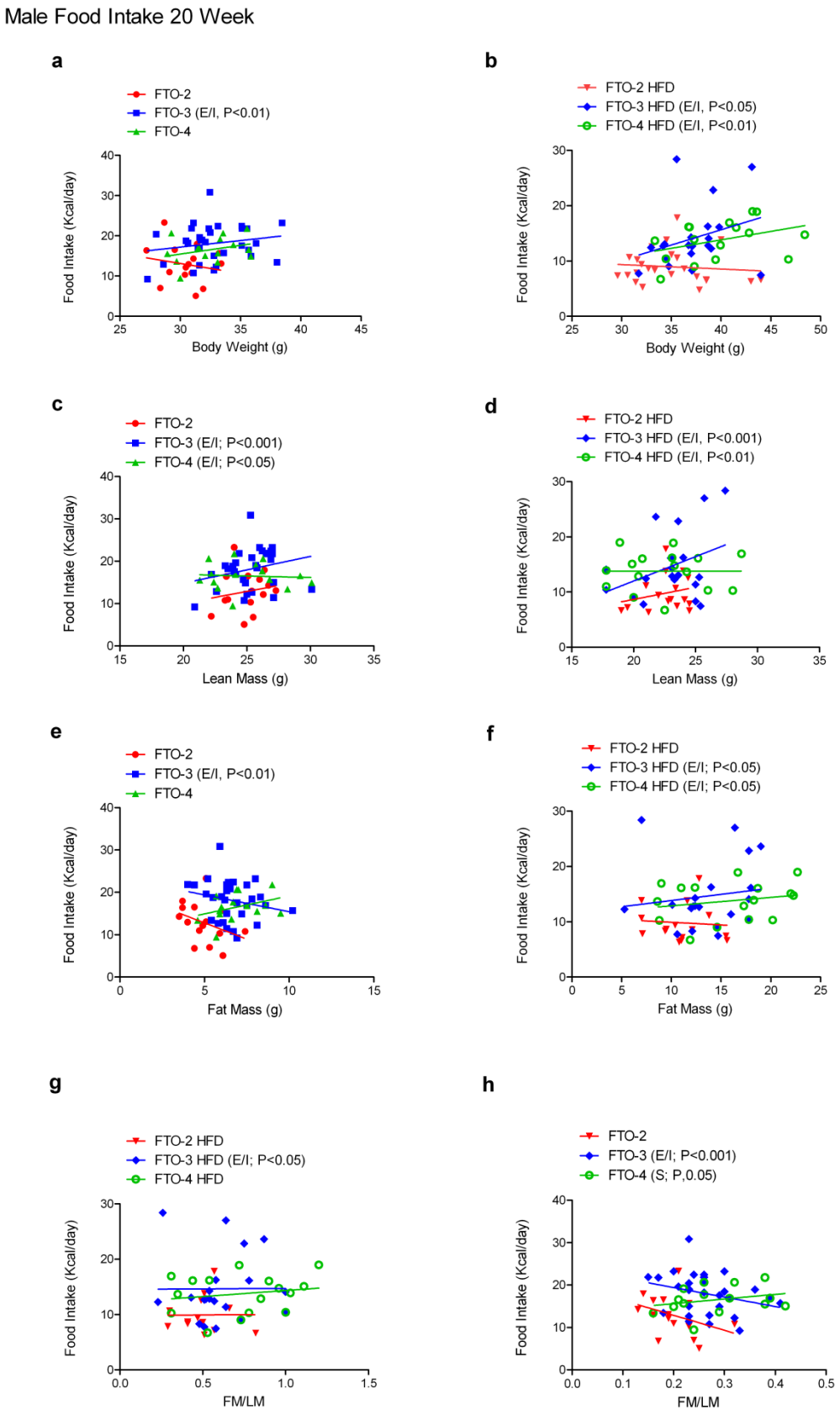
(a) Males: SD, FTO-2 (n=13), FTO-3 (n=28), FTO-4 (n=14).

(b) Males: HFD, FTO-2 (n=13), FTO-3 (n=15), FTO-4 (n=15).

(c) Females: SD, FTO-2 (n=16), FTO-3 (n=25), FTO-4 (n=12).

(d) Females: HFD, FTO-2 (n=12), FTO-3 (n=12), FTO-4 (n=15).

The lines are the best fit of a straight line through the data using linear regression analysis. *P* values for differences in the slope (S) and the elevation or intercept (E/I) of the lines are against FTO-2 mice (GraphPad Prism). #, slope differs too much to test intercept or elevation (GraphPad Prism).

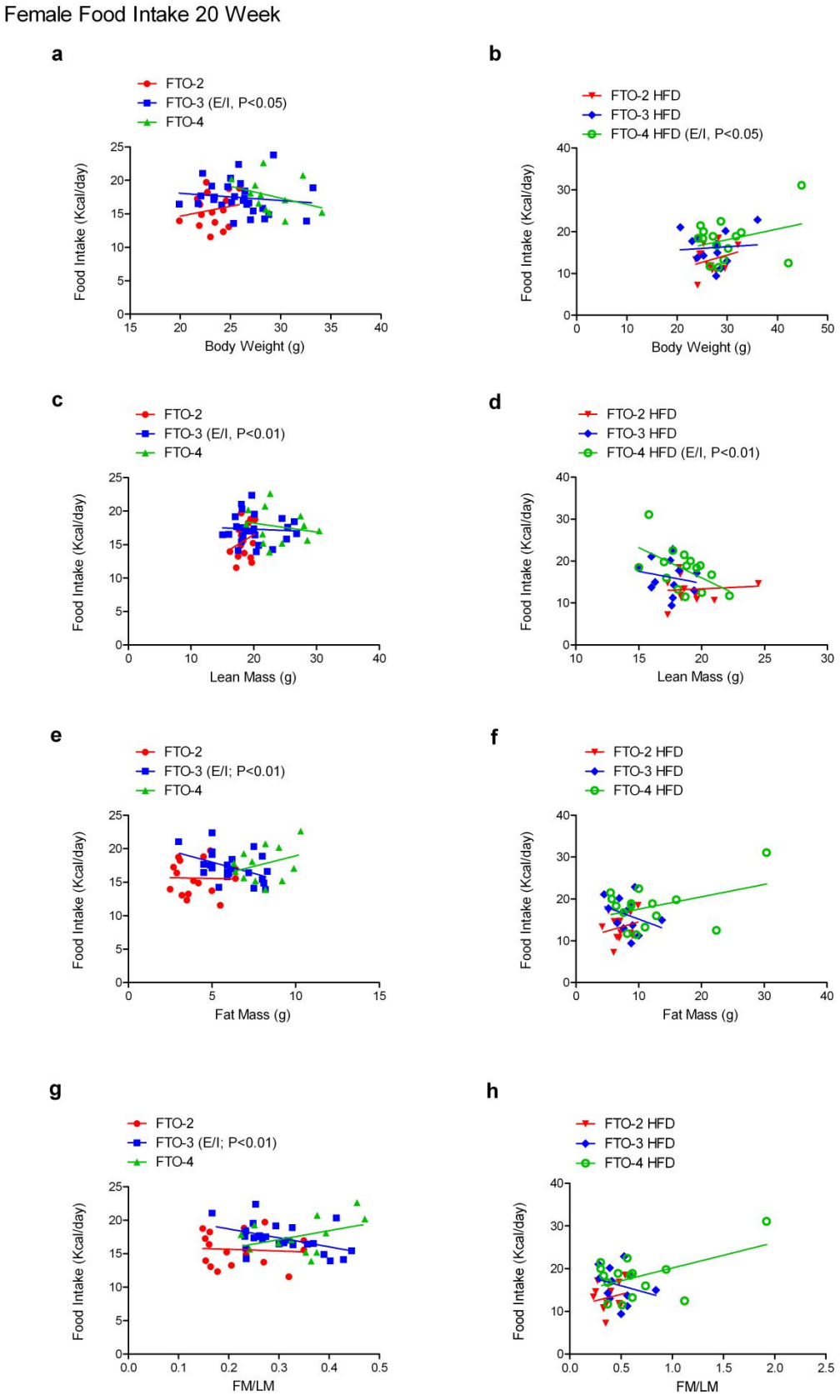


Supplementary Figure 5: *Association of body weight **(a,b)**, lean mass **(c,d)**, fat mass **(e,f)** and the ratio of fat mass/lean mass (FM/LM) **(g,h)** with food intake at 19 weeks in males.*

(a,c,e,g) Males: SD, FTO-2 (n=14), FTO-3 (n=28), FTO-4 (n=14).

(b,d,f,h) Males: HFD, FTO-2 (n=18), FTO-3 (n=15), FTO-4 (n=15).

The lines are the best fit of a straight line through the data using linear regression analysis. P values for differences in the slope (S) and the elevation or intercept (E/I) of the lines are against FTO-2 mice. (GraphPad Prism).



Supplementary Figure 6: *Association of body weight **(a,b)**, lean mass **(c,d)**, fat mass **(e,f)** and the ratio of fat mass/lean mass (FM/LM) **(g,h)** with food intake at 19 weeks in females.*

(a,c,e,g) Females: SD, FTO-2 (n=16), FTO-3 (n=25), FTO-4 (n=12).

(b,d,f,h) Females: HFD, FTO-2 (n=12), FTO-3 (n=12), FTO-4 (n=15).

The lines are the best fit of a straight line through the data using linear regression analysis. P values for differences in the slope (S) and the elevation or intercept (E/I) of the lines are against FTO-2 mice. (GraphPad Prism).

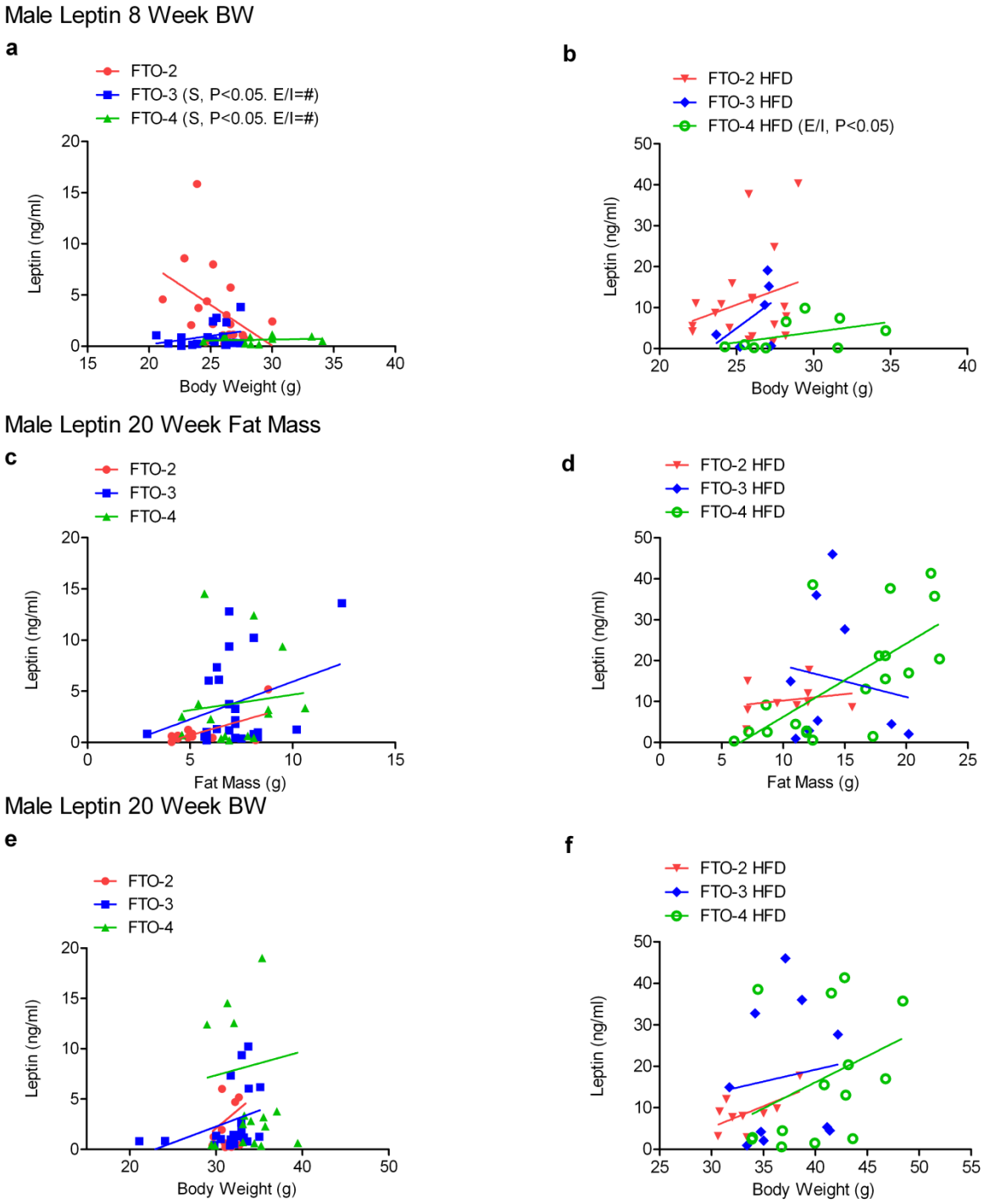
Supplementary Note:

Note on food Intake at 20 weeks: Supplementary Figures 4-6

The relationships between food intake at 20 weeks and body weight, lean mass, fat mass or the ratio of fat mass to lean mass (FM/LM), all show significantly elevated food intake in FTO-3 and FTO-4 males relative to FTO-2 males, on both diets (apart from FT0-4 on a standard diet against body weight and fat mass which only showed a trend; *P*=0.051 and *P*=0.0615 respectively) **(Supplementary Fig. 5)**.

Female FTO-3 mice show a significant increase in food intake on a standard diet but not on a high fat diet when plotted against body weight **(Supplementary Fig. 6)**. Data for FTO-4 female mice did not reach significance on either diet, apart from in the regression against body weight and lean mass on a high fat diet, for elevated food intake in the analysis at 20 weeks **(Supplementary Fig. 6)**.

Analysis of the variance r^2 (correlation coefficient squared) in food intake that was accounted for by either body weight, fat mass, lean mass or FM/LM did not show any significant effects (data not shown) in males within any genotype. This was also largely true in females, with the following exceptions: FTO-3 SD, fat mass explains 21% of food intake *P*=<0.05; FTO-3 SD, FM/LM explains 20% of food intake *P*<0.05; FTO-4 HFD, lean mass explains 29% of food intake *P*<0.05. Overall it seems clear that elevated food intake is not strongly determined by body composition or weight but rather by genotype.



Supplementary Figure 7: *Association of body weight **(a,b)** with leptin at 8 weeks and fat mass and body weight **(c,d,e,f)** with plasma leptin levels at 20 weeks in males.*

8 Week body weight and leptin:

(a) Males: SD, FTO-2 (n=18), FTO-3 (n=19), FTO-4 (n=10).

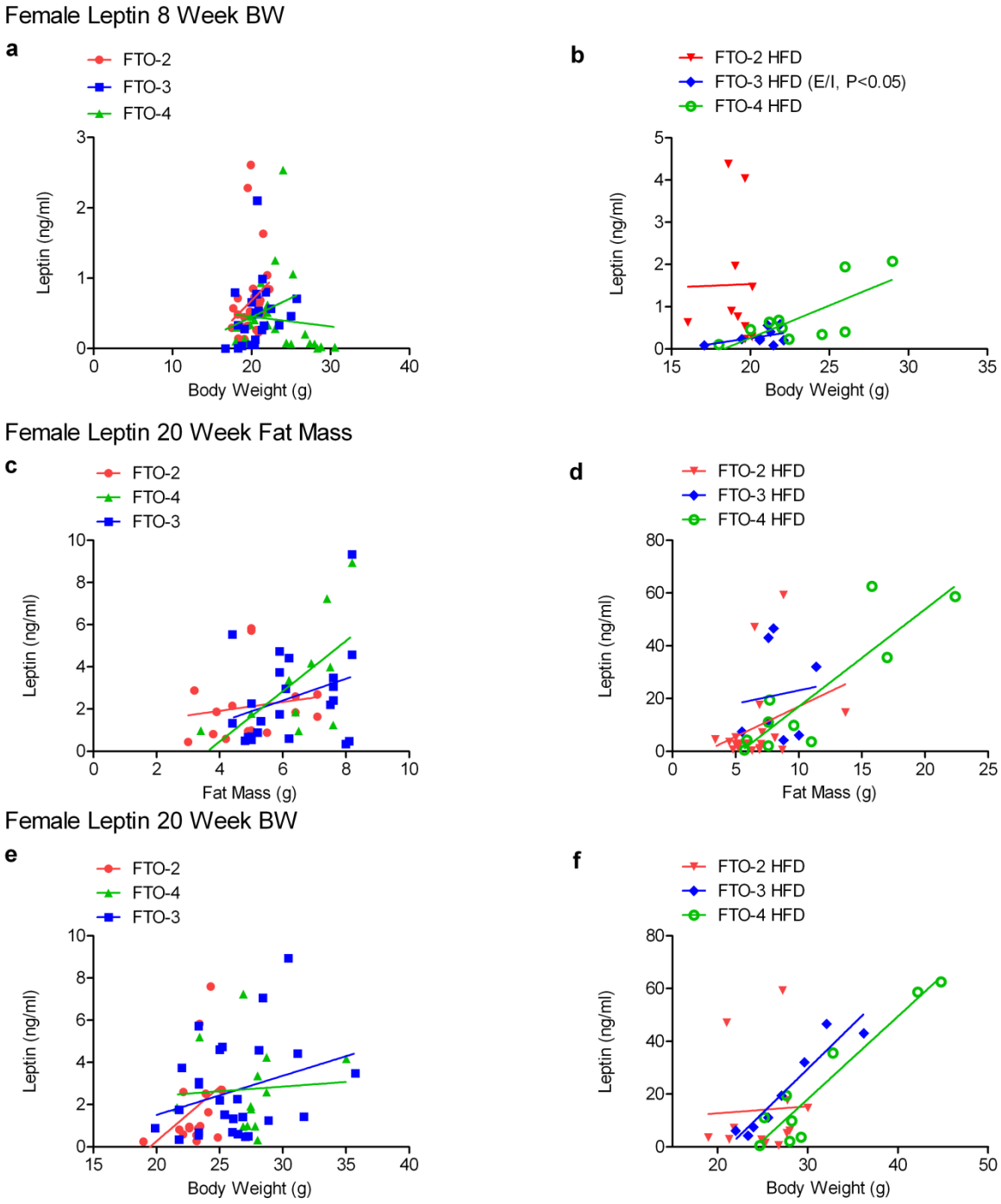
(b) Males: HFD, FTO-2 (n=18), FTO-3 (n=6), FTO-4 (n=10).

20 Week fat mass and body weight and leptin:

(c,e) Males: SD, FTO-2 (n=11), FTO-3 (n=25), FTO-4 (n=15).

(d,f) Males: HFD, FTO-2 (n=8), FTO-3 (n=6), FTO-4 (n=11).

The lines are the best fit of a straight line through the data using linear regression analysis. P values for differences in the slope (S) and the elevation or intercept (E/I) of the lines are against FTO-2 mice (GraphPad Prism). #, slope differs too much to test intercept or elevation (GraphPad Prism).



Supplementary Figure 8: *Association of body weight **(a,b)** with leptin at 8 weeks and fat mass and body weight **(c,d,e,f)** with plasma leptin levels at 20 weeks in females.*

8 Week body weight and leptin:

(a) Females: SD, FTO-2 (n=24), FTO-3 (n=22), FTO-4 (n=20).

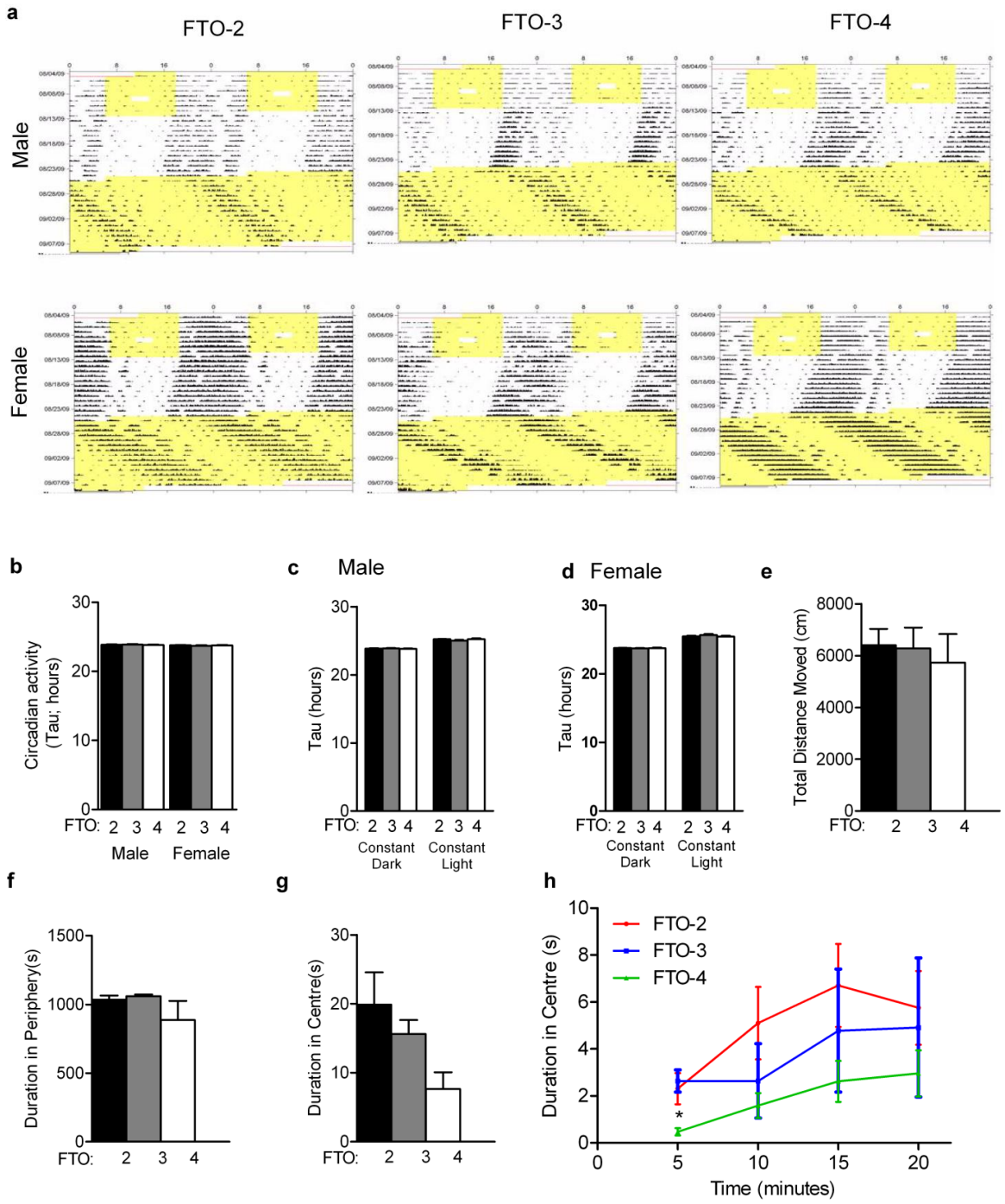
(b) Females: HFD, FTO-2 (n=10), FTO-3 (n=9), FTO-4 (n=10).

20 Week fat mass and body weight and leptin:

(c,e) Females: SD, FTO-2 (n=24), FTO-3 (n=22), FTO-4 (n=20).

(d,f) Females: HFD, FTO-2 (n=10), FTO-3 (n=9), FTO-4 (n=10).

The lines are the best fit of a straight line through the data using linear regression analysis. P values for differences in the slope (S) and the elevation or intercept (E/I) of the lines are against FTO-2 mice. (GraphPad Prism).



Supplementary Figure 9: *Physical Activity and Open Field*

(a) Representative records of locomotor activity of male and female FTO-2 (n=7), FTO-3 (n=7) and FTO-4 (n=6) mice. Short vertical bars represent bouts of wheel-running activity. Light periods are shown in yellow and dark periods are white. Each actogram is representative of those obtained during a 4-week period in a circadian chamber with free access to running wheels. Mice were tested from 12-16 weeks of age.

(b) Onset of activity (Tau) measured during a standard light/dark cycle (light period, 7am - 7pm). Tau was also determined during a constant 24-hour light or dark period in males **(c)** and females **(d)**.

(e-h) total distance moved **(e)**, duration of time spent in the periphery **(f),** or centre **(g)**, in an open field area during a 20-minute automatic tracking session in female FTO-2 (n=9), FTO-3 (n=12) and FTO-4 (n=8) mice. **(h)** Duration of time (seconds) spent in the centre of an open arena in a 5-minute time period (same mice as in **e**-**g**). FTO-4 mice spend less time in the centre of the arena over the total 20-minute test period (*P*=0.042) and during the first 5 minutes (*P*=0.025).

Data (a-h) are expressed as mean±SEM. * *P*<0.05.

Supplementary Table 1

Multiple regression analysis of Energy Expenditure using R

Multiple Regression

	Body Weight	Lean Mass	Fat Mass	FTO-3	FTO-4	Gender	Diet	Multiple R^2	Adjusted R^2	*P* value
***P*=**	0.1368	**0.0446**	0.8938	0.9789	0.4814	0.9654	**4.29×10^{-7}**	0.4529	0.4307	**2.20×10^{-16}**

AIC Optimised

	Body Weight	Lean Mass	Diet	Multiple R^2	Adjusted R^2	*P* value
***P*=**	**0.0077**	**0.0004**	**6.50×10^{-8}**	0.4505	0.4411	**2.20×10^{-16}**

Supplementary Table 2: *Body composition after 4 weeks access to running wheels.*

All data are given as mean±SEM. BMD: Bone mineral density, BMC; bone mineral content. Bold text *P*<0.05

	FTO-2	FTO-3	FTO-4	*t*-test	
	n=7	n=7	n=6	FTO-2 Vs FTO-3 (*P*=)	FTO-2 Vs FTO-4 (*P*=)
Male					
Body Weight (g)	29.5 ± 0.8	32.4 ± 1.3	32.5 ± 0.6	0.10	**0.015**
BMD (g/cm2)	0.0555 ± 0.001	0.0577 ± 0.001	0.0594 ± 0.002	0.17	0.09
BMC (g)	0.53 ± 0.02	0.58 ± 0.02	0.61 ± 0.02	0.10	**0.016**
Lean Tissue (g)	23.9 ± 0.6	24.9 ± 0.8	26.1 ± 0.5	0.40	**0.018**
Fat Tissue (g)	4.8 ± 0.2	7.2 ± 0.2	6.0 ± 0.2	**0.004**	**0.004**
Female					
Body Weight (g)	23.5 ± 0.9	25.6 ± 0.8	25.4 ± 0.3	**0.031**	**0.002**
BMD (g/cm2)	0.054 ± 0.010	0.0547 ± 0.001	0.0553 ± 0.001	0.50	0.44
BMC (g)	0.49 ± 0.01	0.52 ± 0.02	0.54 ± 0.02	0.11	**0.039**
Lean Tissue (g)	19.1 ± 0.7	20.9 ± 0.5	20.2 ± 0.2	**0.028**	**0.001**
Fat Tissue (g)	4.1 ± 0.29	4.7 ± 0.25	5.0 ± 0.2	0.08	**0.011**

Supplementary Table 3: *Primers for genotyping ROSACAGGs-Fto*

Primer Name	Sequence (5' to 3')	Product Size (bp)
*R26*WTF	TTCCCTCGTGATCTGCAACTC	
*R26*WTR	CCTTTAAGCCTGCCCAGAAGA	68
*R26*FTOF	TTATGGCGCGCCTAATGC	
*R26*FTOR	TCTTAGCTTCCCGCTCTCGTT	68