Supplementary Information for:

Fetal Growth Restriction Promotes Physical Inactivity and Obesity in Female Mice

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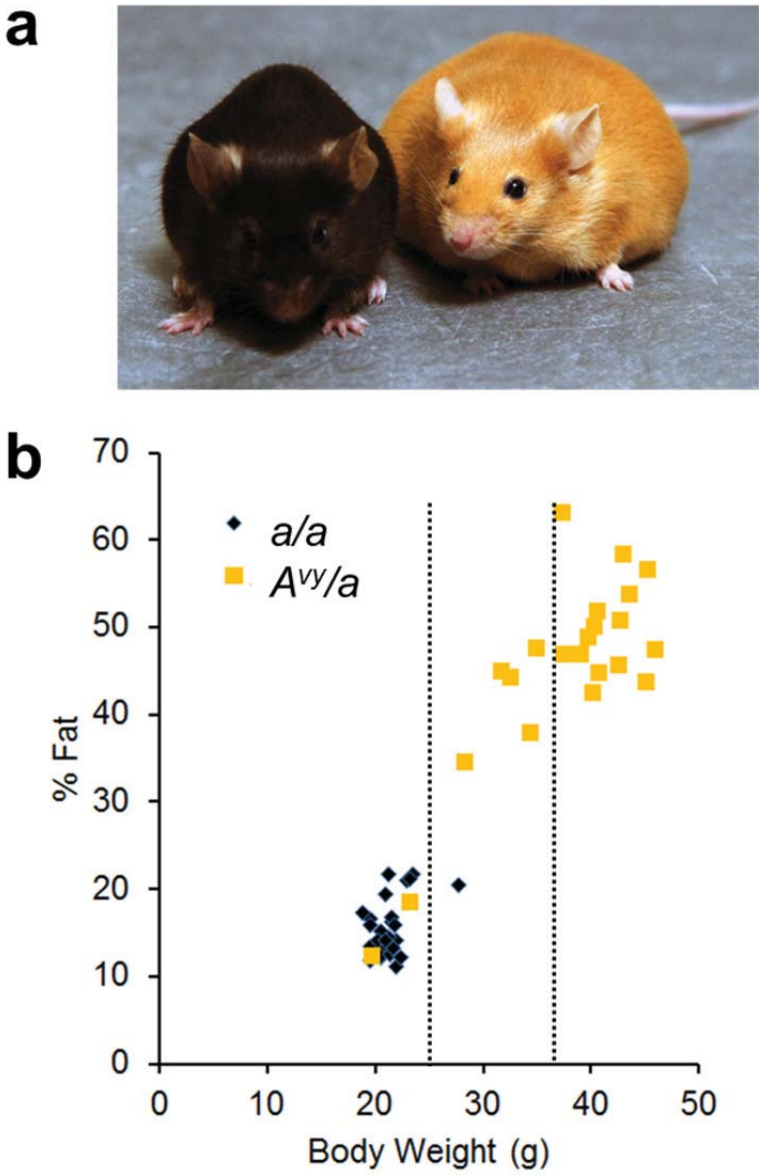
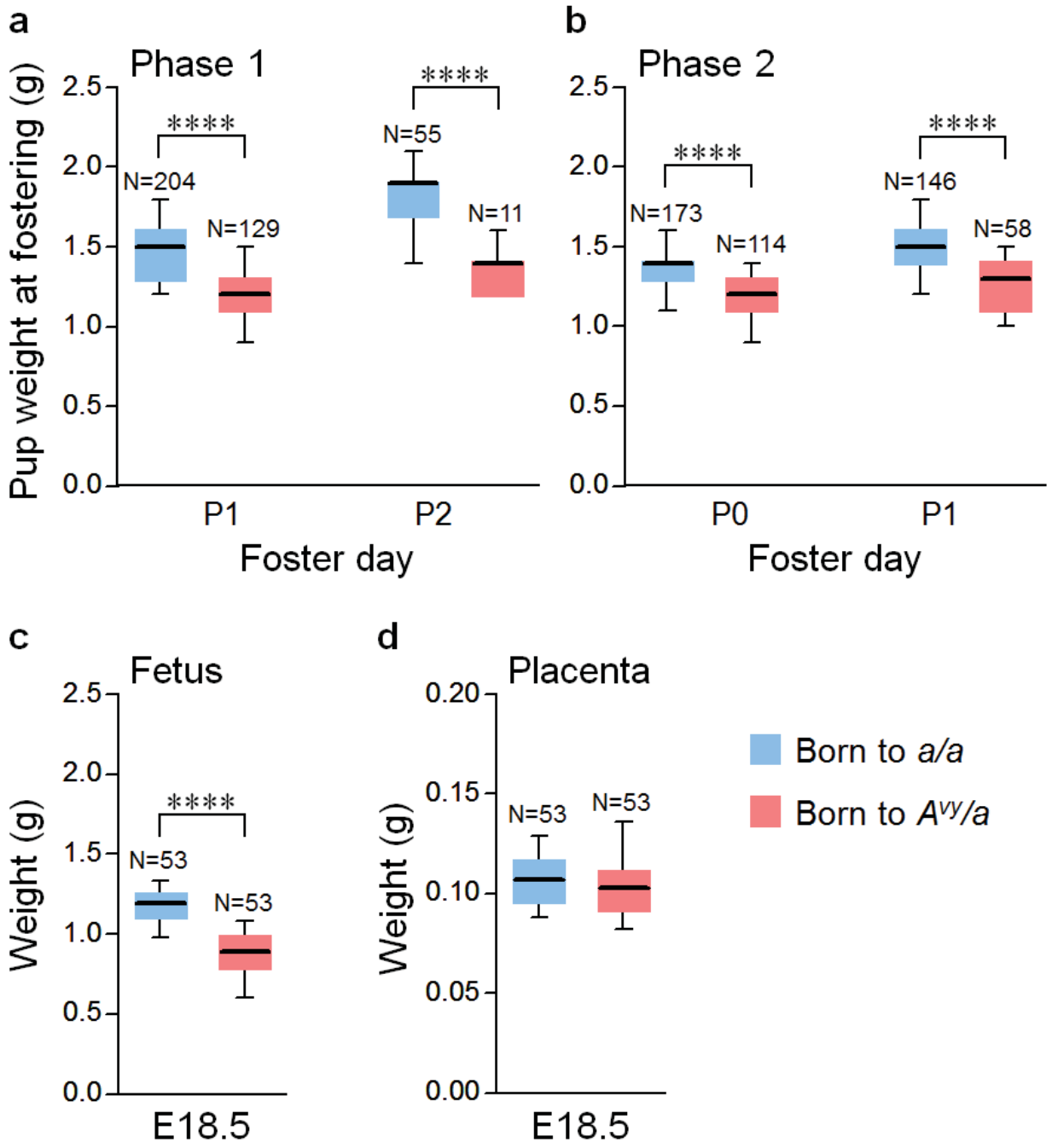
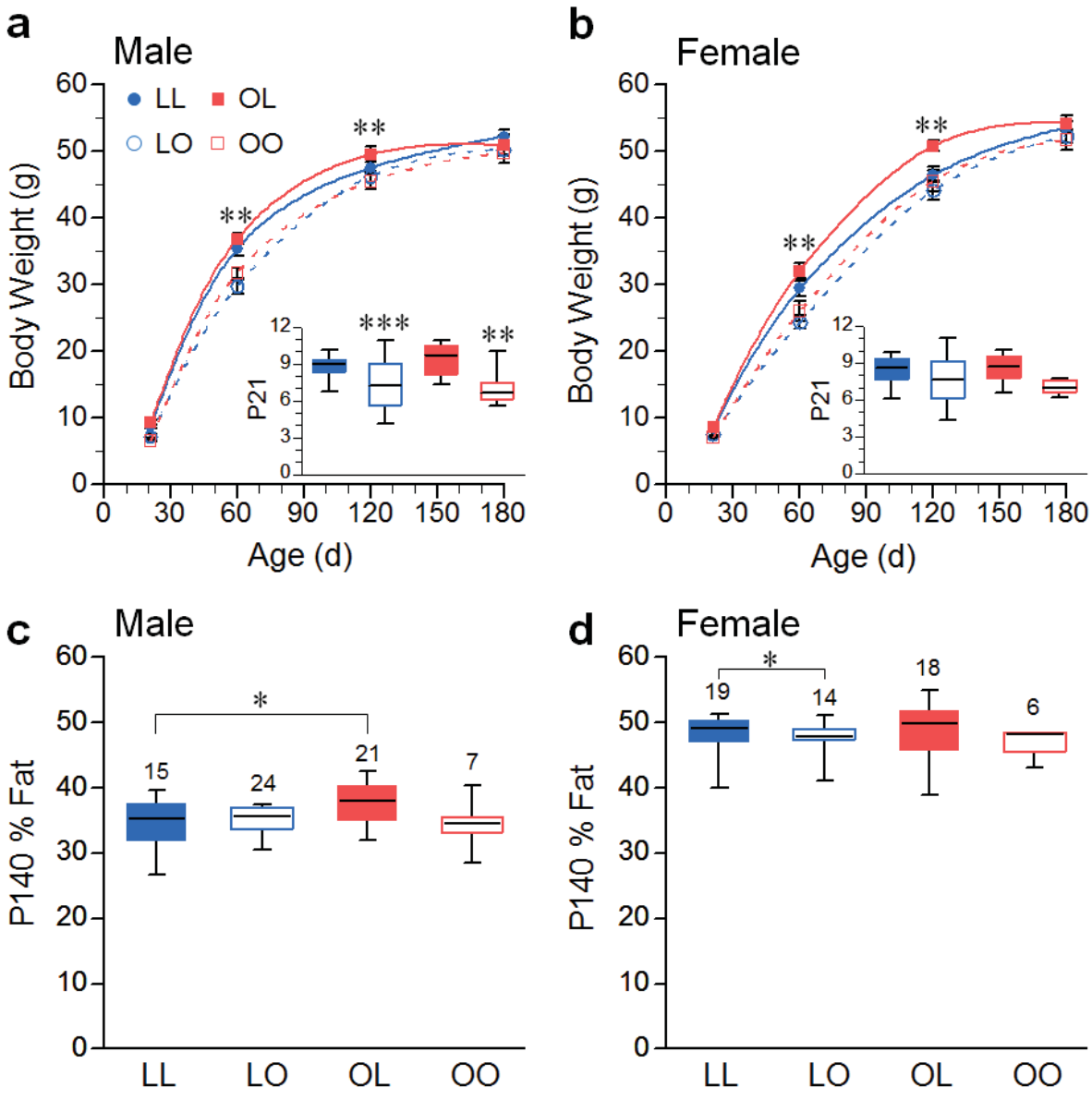


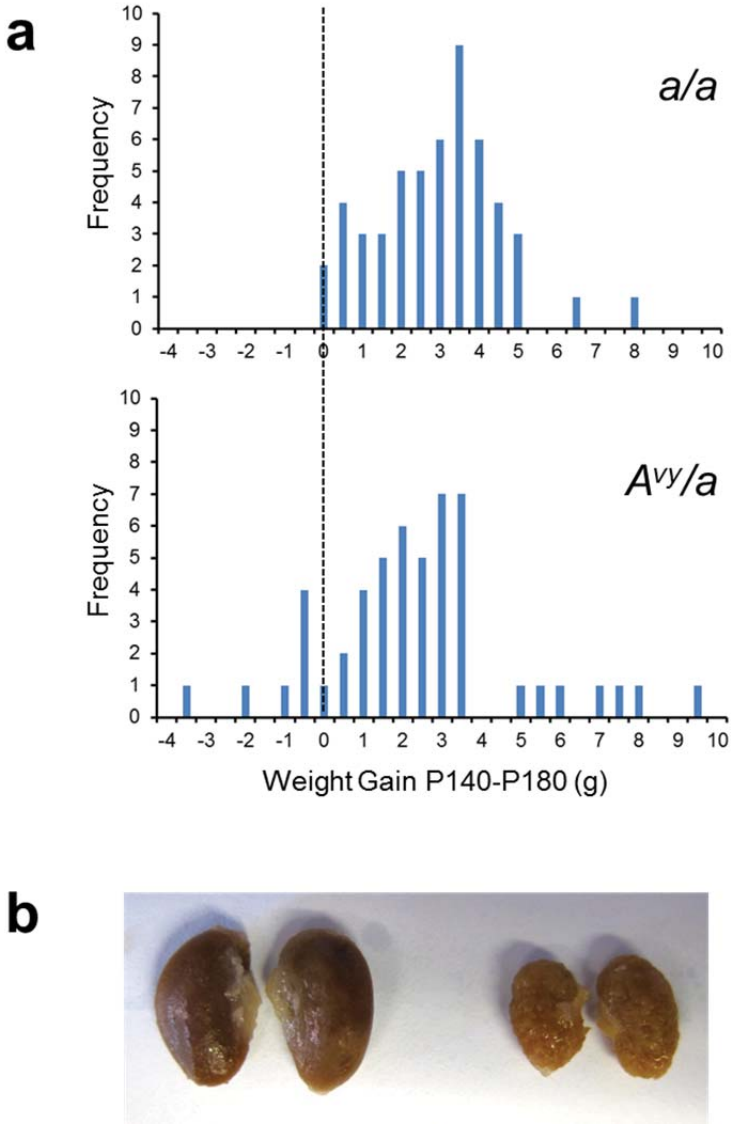
Supplemental Figure S1. Establishment of dam body weight cutoffs for inclusion in the study. (**a**) Representative *a/a* (left) and A^{vy}/a (right) dams (age 11 wk), illustrating the severe obesity caused by the A^{vy} mutation. (**b**) Percent body fat (by dual energy X-ray absorptiometry) vs. body weight in 11-wk old *a/a* and A^{vy}/a females. Body weight cutoffs of <25g and >37g correspond to <22 and >40% fat, respectively (i.e. A^{vy}/a dams included in the study were essentially twice as fat as the *a/a* dams). Note: The two A^{vy}/a mice with body weight <25g are pseudoagoutis (brown coat color, protected from obesity), and were not included in the study.



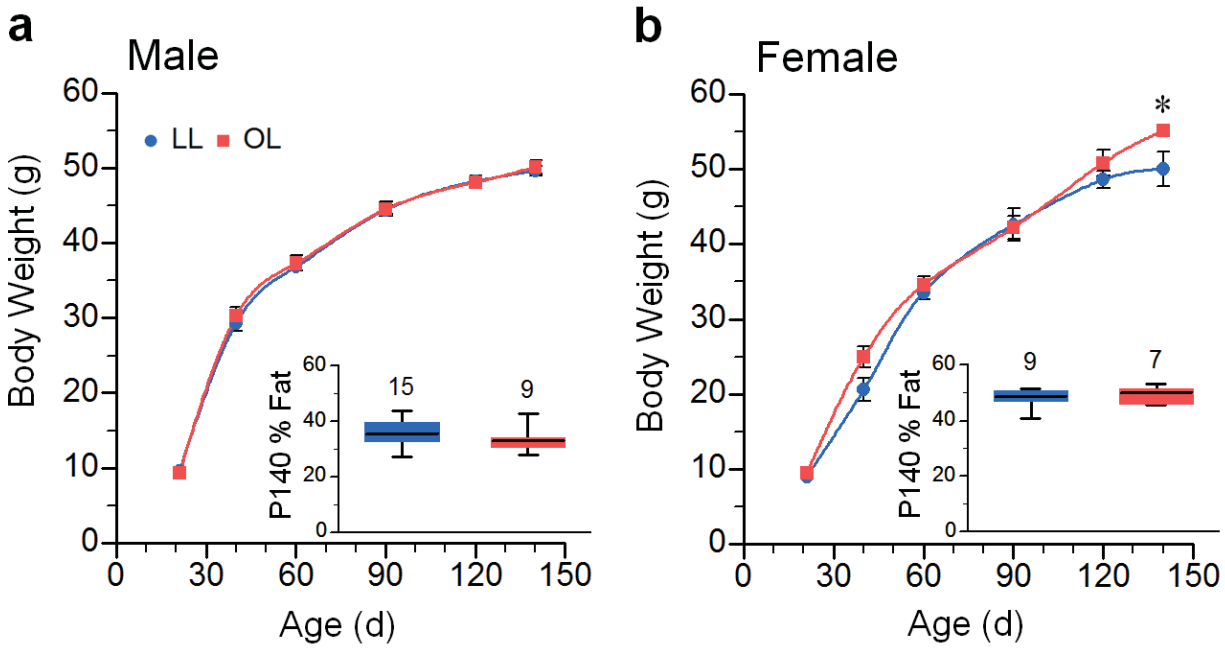
Supplemental Figure S2. Offspring of obese *A^{vy}/a* dams experience fetal growth restriction. (**a**) In Phase 1, most offspring were cross-fostered at P1. At both P1 and P2, offspring of *A^{vy}/a* dams are lighter than offspring of *a/a* dams. (Number of offspring indicated above each box.) (**b**) In Phase 2, most offspring were cross-fostered at P0. Again, at P0 and P1, offspring of *A^{vy}/a* dams are lighter than offspring of *a/a* dams. (**c**) Measuring E18.5 fetal weight confirms growth restriction in offspring of *A^{vy}/a* dams. (**d**) E18.5 placental weight is not affected by maternal genotype. ****P<0.0001 by repeated measures ANOVA (dam as unit of analysis).



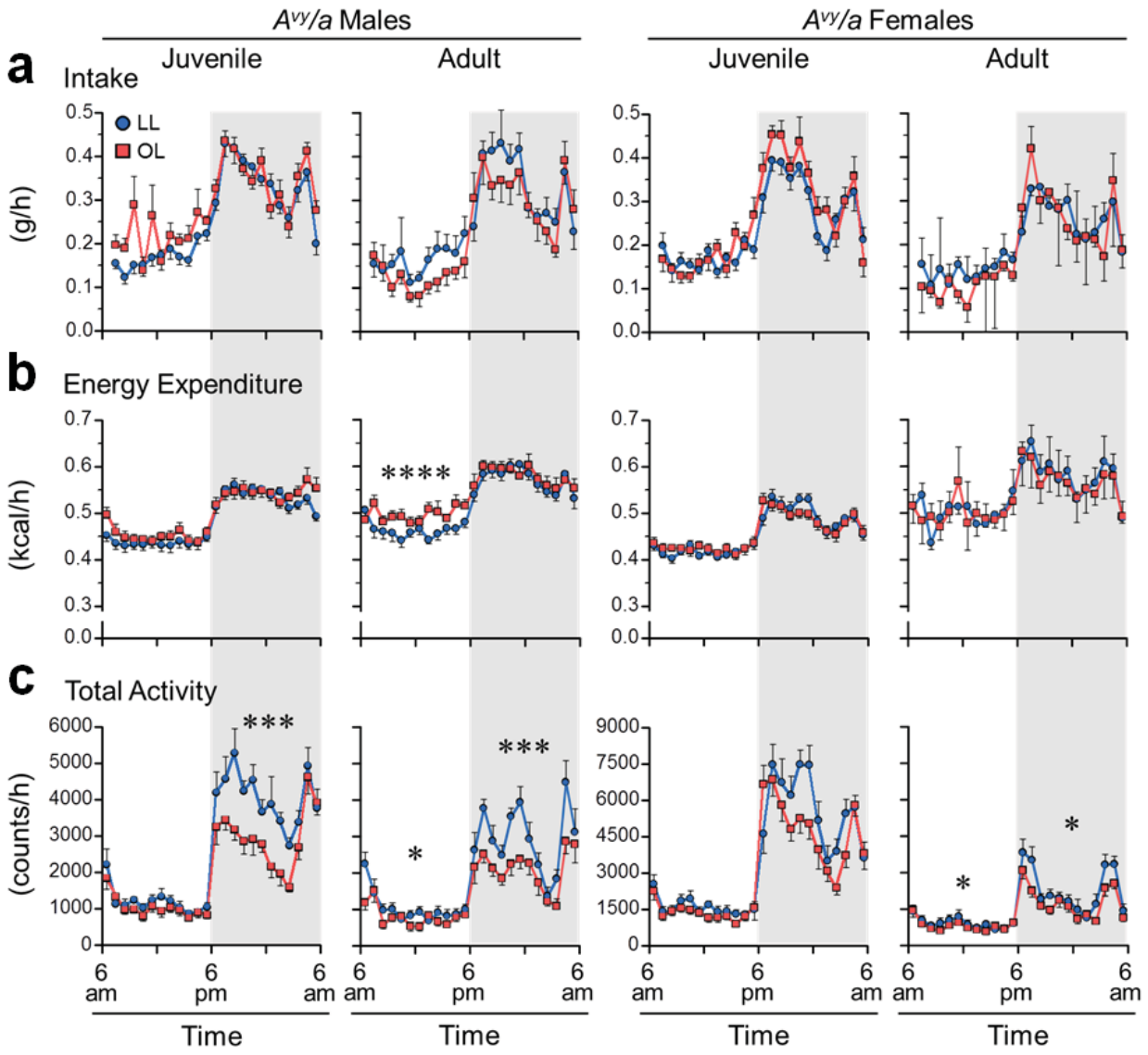
Supplemental Figure S3. Effects on *A^vy/a* offspring of being born to or fostered by an obese *A^vy/a* dam. Body weight vs. age of male (**a**) and female (**b**) *A^vy/a* offspring cross fostered among lean *a/a* and obese *A^vy/a* dams. Each plot represents mean ± SEM of 25-26 LL offspring from 14-15 litters, 19-41 LO offspring from 10-31 litters, 18-22 OL offspring from 11-12 litters, and 8-11 OO offspring from 5 litters. (Detailed sample size tabulation in Table S1A-B.) Asterisks in main plots indicate significance of OL vs. LL differences only. (P values for all group comparisons in Table S1C.) Insets are box plots of body weight at P21, each indicating median, 25th-75th percentiles, and 5th-95th percentiles. (**c**) and (**d**) are box plots illustrating group differences in body composition (% fat) in P140 *A^vy/a* male and female offspring, respectively. Number of mice studied is indicated above each box. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 relative to LL mice of same age and sex.



Supplemental Figure S4. Weight loss from P140-P180 in *A^vy/a* mice is correlated with kidney disease. (**a**) Distribution of body weight gain from P140-P180 in 52 *a/a* and 51 *A^vy/a* mice. None of the *a/a* mice lost substantial weight over this epoch, but 7 of the *A^vy/a* mice did (P=0.006, Chi-squared test). (**b**) Representative kidneys from P180 *A^vy/a* mice that gained (left) and lost weight (right) from P140-P180. At P180, necropsies were performed in 8 *A^vy/a* mice that lost weight and 7 that gained weight from P140-P180. In all those that had lost weight, but none of those that had not, a hypotrophic kidney disease was observed.



Supplemental Figure S5. Data on *A^{vy}/a* offspring in second experiment confirm female-specific effect on adult body weight. Body weight vs.age of *A^{vy}/a* male (**a**) and female (**b**) LL and OL offspring. Each plot represents mean ± SEM of 9-37 LL offspring from 4-18 litters, and 7-33 OL offspring from 7-17 litters. (Detailed sample size tabulation in Table S2a-b.) Insets are box plots of % body fat at P140, each indicating median, 25th-75th percentiles, and 5th-95th percentiles. Number of mice studied is indicated above each box. *P<0.05 relative to LL mice of same age and sex.



Supplemental Figure S6. In *A^vy/a* offspring, fetal growth restriction does not induce the same sex-specific physical activity and energy expenditure changes as seen in *a/a* offspring. (**a**) Dietary intake, (**b**) energy expenditure, and (**c**) total physical activity of *A^vy/a* LL and OL mice, by sex and age. (Dietary intake and energy expenditure data have been least-squares normalized for lean mass and fat mass.) As in *a/a* mice, there are no group differences in food intake. The only effect that was detected in both juvenile and adult mice is reduced dark-period physical activity (C) in OL males. This is not, however, associated with changes in energy expenditure (B). For each mouse, three days of metabolic cage data were collapsed into 24 1-hour averages. Each plot represents mean ± SEM of 8-10 LL offspring from 5-6 litters, and 9-12 OL offspring from 8-10 litters. Statistical analyses were performed separately by light and dark periods (indicated by shading). *P<0.05, ***P<0.001, ****P<0.0001 relative to LL mice of same age and sex.

Supplemental Table S1

(**a**) Number of F1 offspring represented in each group, related to Fig. 1.

Group	*a/a* Males	A^{vy}/a Males	*a/a* Females	A^{vy}/a Females
LL	26-37	25	20-30	26
LO	26-41	24-50	41-72	19-52
OL	19-24	23-25	22	18
OO	9	4	11	8

(**b**) Number of litters represented in each group, at each age, related to Fig. 1.

	a/a Males				A^{vy}/a Males				*a/a* Females				A^{vy}/a Females			
Age (d)	LL	LO	OL	OO	LL	LO	OL	OO	LL	LO	OL	OO	LL	LO	OL	OO
21	15	32	12	5	15	31	11	5	14	28	11	3	14	27	12	5
60	13	13	9	5	15	15	11	5	12	11	10	3	14	10	12	5
120	13	13	9	5	15	15	11	5	12	11	10	3	14	10	12	5
180	13	13	9	5	15	15	11	5	12	11	10	3	14	10	12	5

(**c**) P values for all group comparisons, related to Fig. 1. Significant group differences are highlighted.

	a/a Males			A^{vy}/a Males			*a/a* Females			A^{vy}/a Females		
Age (d)	OL vs. LL	LO vs. LL	OO vs. LL	OL vs. LL	LO vs. LL	OO vs. LL	OL vs. LL	LO vs. LL	OO vs. LL	OL vs. LL	LO vs. LL	OO vs. LL
21	0.59	0.003	0.005	0.28	0.0009	0.005	0.89	0.012	0.6	0.75	0.11	0.07
60	0.41	0.0002	0.003	0.008	<.0001	0.17	0.63	0.0004	0.26	0.009	0.0003	0.11
120	0.2	0.027	0.0105	0.005	0.23	0.77	0.001	0.03	0.73	0.005	0.007	0.98
180	0.099	0.0004	0.039	0.41	0.16	0.9	0.031	0.0005	0.81	0.44	0.011	0.75

SupplementalTable S2

(**a**) Number of F1 offspring represented in each group, related to Fig. 2.

Group	*a/a* Males	A^{vy}/a Males	*a/a* Females	A^{vy}/a Females
LL	30-36	18-36	21-35	9 to 37
OL	19-34	9 to 24	13-28	7 to 33

(**b**) Number of litters represented in each group, at each age, related to Fig. 2.

	a/a Males		A^{vy}/a Males		*a/a* Females		A^{vy}/a Females	
Age (d)	LL	OL	LL	OL	LL	OL	LL	OL
21	18	17	18	14	18	14	14	17
40	14	13	9	10	9	10	8	15
60	15	13	10	10	10	10	6	11
90	14	13	9	10	9	10	4	11
120	15	13	10	9	10	9	4	8
140	15	13	10	9	10	9	4	7