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Investigating Ubiquitin Like Modifier, UBD, Linking Genetic Variation to Weight Loss After Exercise

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Investigating Ubiquitin Like Modifier, UBD, Linking Genetic Variation to Weight Loss After Exercise

A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science in Physiological Science

by

Brayden K. Leyva

2022

ABSTRACT OF THE THESIS:

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by

Brayden K. Leyva

Master of Science in Physiology

University of California. Los Angeles, 2022

Professor Mark Frye, Co-Chair

Professor Andrea Hevener, Co- Chair

Obesity incidence worldwide continues to rise annually and is considered a major contributor to the pathobiology of other life-threatening diseases including cardiometabolic disorders and cancers. Globally, obesity is responsible for over 67.5 billion dollars of healthcare expenditure and over 4 million days lost to absenteeism in the workforce. Therapeutics targeting increased adiposity besides prescribed exercise are minimal. Additionally, mechanisms that underlie adipose tissue weight loss in response to exercise training remain inadequately understood. We utilized mouse genetics and a human Genome Wide Association Study (GWAS) to identify candidate genes driving reductions in adiposity associated with daily exercise. In a 100-strain mouse panel, we performed Ribonucleic Acid (RNA) sequencing on gonadal and inguinal fat pads from sedentary (SED) vs exercise trained (TRN) animals. We identified transcripts associated with fat pad mass after 30 days of volitional activity. We cross-referenced putative exercise-responsive and adipocyte-regulatory transcripts against other mouse panels from our laboratory, as well as human exercise studies. Adipose tissue RNA sequencing data from the METabolic Syndrome In Men (METSIM) study of 10,000 Finnish men, was plotted against self-reported activity level. We performed caloric modification studies in 5 strains of mice to determine whether TRN-impacted targets were differentially expressed in adipose tissue in response to caloric intake. We mined adipose tissue

RNA sequencing data from the 4-core genotype mouse panel to determine target regulation by sex (chromosomes vs hormones). Adipose Ubiquitin D1 (UBD1) transcript was inversely associated with exercise-induced adipose tissue weight loss ($P=5.2*10^{-13}$). Moreover, adipose tissue UBD1 expression was elevated in females compared to males ($P<0.01$), and its expression was impacted by caloric intake and X chromosome-linked mechanism(s). Gene dosing studies in adipocytes to interrogate molecular actions of UBD1 on adipocyte metabolism are underway. UBD1 is an exercise-responsive transcript with sexually dimorphic expression. Reduction in adipose tissue expression following 30 days of wheel running was highly associated with exercise-induced adipose tissue weight loss in a large panel of mice, and this relationship was confirmed in human subjects. These data suggest that repression of specific targets in adipose tissue, including UBD1, may in part underlie the remarkable remodeling that occurs in adipose tissue depots as a consequence of daily physical activity. Identification of the molecular transducers that deliver the health benefits associated with exercise training will improve clinical care of individuals suffering from cardiometabolic related diseases.

The thesis of Brayden K. Leyva is approved

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University of California, Los Angeles

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Thank you to my family and friends for providing me with unconditional support throughout my academic journey. A special thank you to my cousins Sydney Dowling, Marlene Alcantar, and Lauren Adams, and best friend Alexia Joseph for encouraging me to never give up. Thank you to my committee members and research mentor, Dr. Hevener, without whom this endeavor would not have been possible.

Introduction

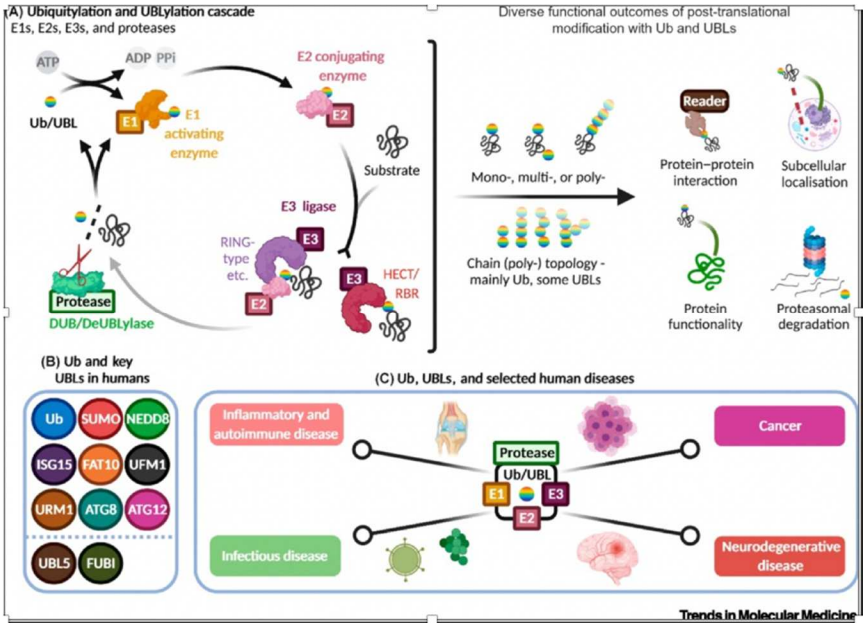
The National Institute of Health (NIH) has made a significant investment through the Office of the Director Common Fund, to investigate the molecular transducers of physical activity (MoTrPAC) to determine the exercise-induced adaptations that are critical for driving improvements in human health. Exercise remains an important clinical intervention to combat metabolic dysfunction and obesity however mechanisms that underlie adipose tissue weight loss in response exercise training remain inadequately understood. Obesity incidence continues to rise annually, and new data from the Center for Disease Control and Prevention (CDC) indicates that the percentage of adults in America who are currently overweight is 69% while ~36% are obese. The National Institute of Health (NIH) estimates over 40% of adults living in the United States are clinically obese, and over 40% of adults worldwide are at least overweight. Increased adiposity is strongly associated with increased risk of morbidity and mortality as obesity is an underlying factor in the pathobiology of cardiometabolic disease and certain cancers. This level of adiposity has devastating consequences with negative physiological and economic implications. In fact, inadequate levels of physical activity are estimated to cost at least $67.5 billion globally in health-care expenditure and lost productivity per year. Weight loss generally occurs in rodents and humans following chronic exercise and may be partially unrelated to the temporary increase in energy demand during and shortly after exercising[31-35]. While sustained physical activity over long periods of time is known to reduce disease burden and improve general well-being, the molecular transducers, especially those related to the well-known exercise induced reduction in weight loss, are not well understood. Remarkably, to date, there are few effective therapeutics for human weight loss, and exercise/sustained physical activity is one of the only proven interventions to reduce disease burden and improve general well-being. However, the molecular transducers, especially those related to the well-known exercise induced reduction in adipose mass, are not well understood. Moreover, the role genetic background and biological sex play in modulating these mechanism(s) driving exercise reduction in adiposity remains inadequately studied. Our laboratory has developed a mouse resource called the University of California, Los Angeles (UCLA) exercise (Exc) Hybrid Mouse

Diversity Panel (HMDP) which serves as a rich phenotypic and multi-omics resource for studying exercise and its effects including ~8,500 tissue samples, five transcriptomics data sets, fecal metagenomics, and sixty metabolic, clinical, and cellular traits. In assessing gene-trait relationships we identified UBD1 as one of the top genes differentially expressed in adipose tissue in response to exercise training. A reduction in expression of UBD1 was strongly associated with adipose tissue weight loss after 30 days of volitional wheel running. The post-translational modification of proteins with ubiquitin or ubiquitin-like modifiers (ULMs) is a central mechanism to regulate the stability, protein-protein interactions, and cellular localization and function of these modified proteins. How UBD1 is regulated during exercise training is poorly understood and identifying the mechanisms by which UBD1 controls adiposity will determine UBD1's potential therapeutic value to improve human health.

Human leukocyte antigen F adjacent transcript 10 (FAT10) also known as Ubiquitin D (UBD1) is a member of the ubiquitin-like modifier (ULM) family[61]. UBD1 is present exclusively in mammals and localized in the MHC class 1 locus, directly targeting proteins for proteasomal degradation. Protein expression is mainly concentrated in the immune system as well as adipose tissue, skeletal muscle, and liver. UBD1 is one of the only ULMs which directly targets its substrates for degradation by the 26S proteasome, and is ubiquitously induced in response to IFN-y or TNF-a. In fact, UBD1 is one of the most differentially expressed genes (DGEs) in models of inflammation, and regulates pathways involved in cancer. Structurally, FAT10 is unique in that it is composed of two UBL domains that are connected by a short link of five amino acids. FAT10 is relatively small and contains a molecular weight of 18.3 kilodaltons (kDa)[61]. Aichem et al., and Hipp et al., discovered FAT10's short half-life in cells (1 hour) due to the absence of recycling of ubiquitin at the proteasome via deubiquitylating enzymes (DUBs), but instead found it is degraded with its substrates by the proteasome. NEDD8- ultimate buster 1 (Nub1L), the FAT10 non-covalent long isoform partner speeds up this process eight-fold and is responsible for this increased rate of degradation[62-63].

FAT10 plays a major role in both adaptive and innate immune responses. Briefly, FAT10 expression alters MHC class 1 molecule peptide presentation in medullary thymic epithelial cells (mTECS) which function to aid in T-cell selection. Buerger et al. found that when FAT10 is N-terminally infused to pp65, MHC class I presentation of the human cytomegalovirus (HCMV)- derived antigen pp65 is enhanced in Henrietta Lacks (HeLa) cells, while Schliehe et al. discovered enhancement of presentation and degradation rate of lymphocytic choriomeningitis virus (LCMV) epitopes on MHC class I molecules fused to the N-terminus of the LCMV nucleoprotein[64-65]. FAT10 is also thought to play a part in autophagy, and Aichem et al. demonstrate Fat10 is shown to promote changes in substrate binding partners, cellular localization, and efficacy of transcriptional factors[61]. Most notably however, FAT10 is known to target hundreds of proteins within the 26S proteasome for degradation, and more recently Roverato et al. published a distinct interplay between FAT10 and Parkin, an E3 ubiquitin ligase whose mutations via the PARK2 gene are associated with Parkinson's disease[66]. FAT10 has been shown to conjugate to Parkin while regulating its degradation dependent of the proteasome. Parkin binds to FAT10's E2 enzyme where it auto-FAT10ylates regulating FAT10 ylation of Mitofusin2 indicating it as a FAT10 E3 ligase and signifying its role in mitochondria. This FAT10ylation of Parkin inhibits its mitochondrial depolarization activation causing mitophagy impairment[66]. Our laboratory has made the observation that there is poor correlation between mRNA and protein abundance especially for nuclear encoded genes that modulate mitochondrial form and function. Additionally, we observed that Parkin regulates adiposity by coordinating mitophagy. Canaan et al. developed a UBD1 KO mouse that not only demonstrated leanness with reduced body fat compared to control, but also showed a reduction of the development of age-associated obesity, and an increased lifespan[54]. Collectively these data support our hypothesis that FAT10 is linked with the regulation of the mitochondrial control E3 Ubiquitin ligase Parkin in the control of adiposity and exercise-induced adipose tissue weight loss. These data are supportive of the notion that UBD1 plays a role in the regulation of adiposity. Additionally, Parkin is a UBD1 substrate and Parkin too is directly correlated with adiposity and inversely associated with mitochondrial DNA (mtDNA) copy number (CN). We

have previously shown a strong inverse correlation between adiposity and mtDNA CN, a surrogate for mitochondrial density.



*Schematic showcasing Ubd actions and highlighting its action in disease pathobiology[68]

METHODS

We utilized mouse genetics and human GWAS to identify candidate genes driving reductions in adiposity that are associated with daily exercise. Because previous studies interrogating the benefits of exercise have been performed predominantly on a limited number of tissues and pathways, we employed an unbiased assessment of whole animal trait and genome wide responses to exercise training with a focus on adipose tissue. Mice from the Hybrid Mouse Diversity Panel (HMDP) performed voluntary wheel running for 30 days. The HMDP is a powerful and unique genetic resource consisting of 100 diverse inbred strains of mice. We have previously used the HMDP to perform molecular dissection of complex traits, especially related to cardiometabolic disorders[17-19]. The HMDP enables high-resolution genome wide association studies (GWAS) and assessment of gene-by-environment interactions (in this instance physical activity). The HMDP has been particularly powerful when integrated with multi-omics analyses. In the current study 12 tissues were harvested from each animal of the exercise (Exc)HMDP providing quantification of 30 distinct whole-body and tissue-specific physiological traits; however, herein we focused on findings in adipose tissue. Furthermore, we integrated our ExcHMDP data with the human METSIM Study (deep phenotyping of 10,000 men from Finland) and Skeletal Muscle, Myokines and Glucose Metabolism (MyoGlu) study, which includes longitudinal collection of clinical traits and multiple biopsies of tissues following acute (one session of exercise training) and long-term (12 weeks) exercise intervention[20]. MyoGlu, METSIM and ExcHMDP are complementary data sets where both human and mouse samples of several tissues were subjected to multi omic analysis and these data were subsequently integrated with a variety of phenotypic traits. To address the role of genetics in the exercise response, we used a 100-strain panel of mice, the Hybrid Mouse Diversity Panel (HMDP)[39]. All HMDP strains are inbred allowing for reliable comparisons to be made between different HMDP studies; thus, the HMDP is an expandable data resource. In our study, termed the ExcHMDP, mice from each strain were randomly divided into two groups, exercise trained (TRN), or sedentary (SED), no exercise. Mice were allowed to exercise train using an in-cage running wheel for thirty days, which is considered a sufficient time to induce

exercise training adaptations[40-41]. Following the experimental time frame, wheels were locked, and mice were euthanized 30 hours later to avoid the confounding effects of the last exercise bout[42]. Food was withdrawn from the cage and mice were fasted during the last 6 hours of the 30-hour recovery period from the last exercise bout to ensure a postabsorptive state. We examined the translational relevance of our findings in mice by integrating our results with a retrospective longitudinal exercise study including aerobic exercise training, clinical parameters, and molecular measurements in human subjects. The Skeletal Muscle, Myokines and Glucose Metabolism (MyoGlu) study consisted of twenty-six Norwegian male subjects as described previously[21]. The biopsy schedule employed allowed us to examine acute (a single session of exercise training) and chronic (long-term exercise training) exercise responsive genes.

Metabolic Syndrome in Men (METSIM) was interrogated for the study of adipose tissue insulin sensitivity including 8460 nondiabetic participants from an ongoing population based cross-sectional METSIM study were included. Tissue-specific expression data were obtained from GeneSapiens, version IST4, containing expression data of 16 adipose tissue samples from healthy human tissue, measured with Affymetrix gene expression microarrays. METSIM adipose array data are available from Gene Expression Omnibus (GSE70353). Gene-trait relationships presented here were obtained from 770 male participants[4,11,13].

RNA sequencing was performed on fat pads from sedentary vs exercise trained (TRN) animals of the ExcHMDP (n=200 per condition). mtDNA copy number was determined by qPCR (n=100 per condition). We identified transcripts associated with fat pad mass after 30 days of volitional activity. We cross-referenced putative exercise-responsive, adipocyte-regulatory transcripts against other mouse panels from our laboratory, as well as human exercise studies. Adipose tissue RNA sequencing data from the METSIM study, was plotted against self-reported activity level. We performed caloric modification studies in 5 common strains (C57Bl/6J, AJ, DBA, CHC3, and BalbC) of mice to confirm whether TRN-impacted targets were differentially expressed in adipose tissue in response to caloric intake. We mined adipose tissue RNA sequencing data from the 4-core genotype mouse panel to determine target regulation by sex (chromosomes vs hormones).

Floxed *Kdm5c* mice were backcrossed to C57BL/6J to generation N11 and then crossed with C57BL/6J Ella:*Cre* mice (Jackson Laboratory) to generate heterozygous knockouts of the floxed allele through Cre-lox recombination. Subsequent generations were crossed with C57BL/6J mice to eliminate Cre and the floxed allele. Because male $Kdm5^{-}/Y$ mice were not viable on a C57BL/6 inbred background, female XX $Kdm5c^{+/+}$ and $Kdm5c^{+/-}$ mice produced by mating $Kdm5c^{+/-}$ females with WT males were studied[67].

FCG mice were housed 2 per cage with identical genotypes, and mice of the *Kdm5c* gene dosage model were housed 2–4 per cage. All mice were maintained under a 12-hour light/12-hour dark cycle. Mice were initially fed a Purina Rodent Laboratory Chow diet containing 5%–6% fat from calories (Purina 5001). Where indicated, adult FCG and *Kdm5c* mice were fed a high-fat diet (S3282, Bio-Serve; 60% calories from fat, 26% from carbohydrate, 15% from protein)[67].

RESULTS

Utilizing the excHMDP, we discovered that 80 of the 100 strains showed a reduction in adiposity with exercise training, weight loss varied based upon genetic background. Weight loss was more strongly associated with genetic background (% of weight loss variance explained by genetics = 68.2%) than exercise volume (% of weight loss variance explained by exercise volume = 3.1%).

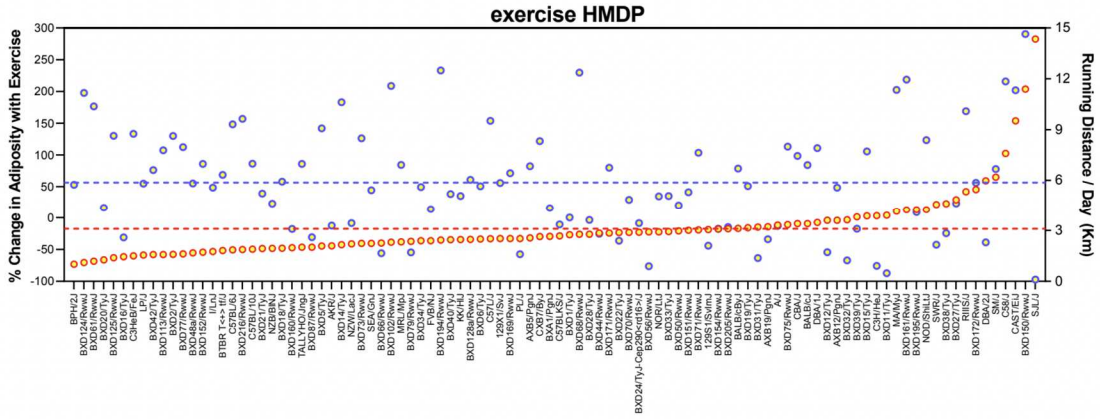


Figure1. Percent change in adiposity with exercise and running distance across 100 strains of the UCLA eHMDP.

We observed a ~20% reduction in adiposity ($P=5.2*10^{-13}$) following one month of exercise training across the exercise Hybrid Mouse Diversity Panel (excHMDP) irrespective of genetic background.

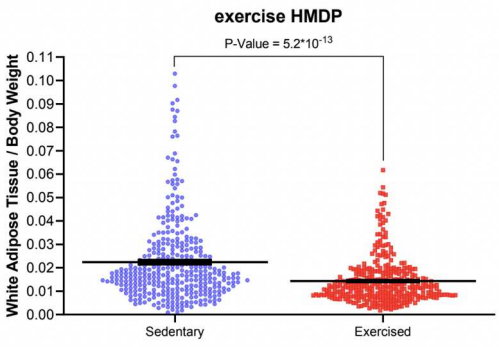


Figure 2. Adiposity was significantly different between TRN vs SED ($P=5.2*10^{-13}$) irrespective of genetic background.

These data suggest some mouse strains are predisposed to more exercise induced weight loss. We exploited the natural variation within the excHMDP by performing a genome wide association study (GWAS) and identified a region on chromosome (chr) 17 that significantly associated with exercise induced weight-loss.

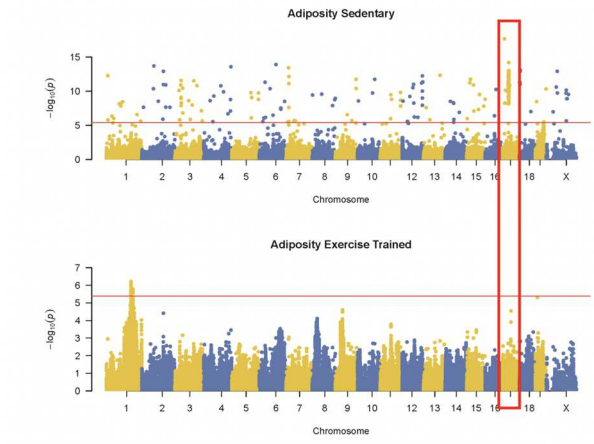


Figure 3. GWAS for adiposity. Red line= significance threshold (P-Value: Log_{10} 4.1*10^{41}.

Correlation of all chr 17 region genes with adiposity, exercise induced-weight loss, and running volume each displayed a significant association with one gene, FAT10 (human leukocyte antigen-F adjacent transcript 10) in adipose tissue (P=0.001). Adipose *Fat10* expression is also negatively correlated with exercise volume (P=0.003).

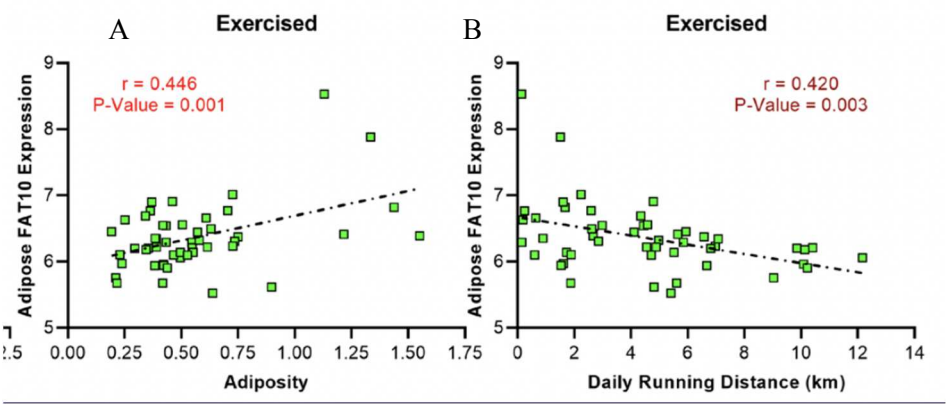


Figure 4. Adipose FAT10 correlates with adiposity. (A) Adipose FAT10 gene expression correlates with adiposity in sedentary or exercised animals respectively (P=0.001). (B) Adipose *Fat10* expression correlates with daily running distance (P=0.003). Dotted lines indicate linear model with significance.

Furthermore, the exercise-induced change in adiposity and adipose *UBD1* expression were positively correlated (P=0.006). METSIM).

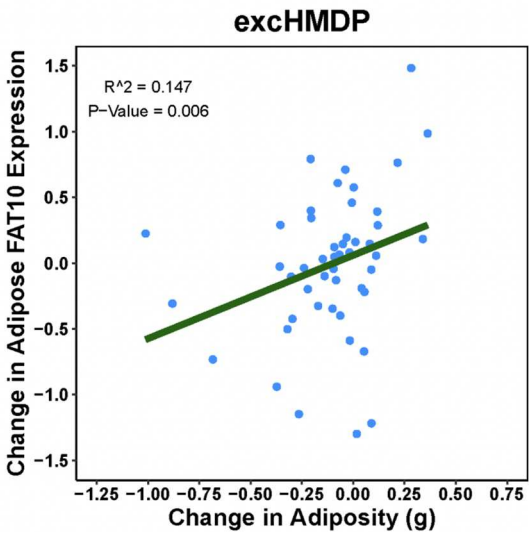


Figure 5. Change in adiposity and change in Adipose FAT10 expression demonstrate a positive correlation (P=0.006). METSIM).

Adipose UBD1 transcript was inversely associated with exercise-induced adipose tissue weight loss (P=5.2*10^{-13}).

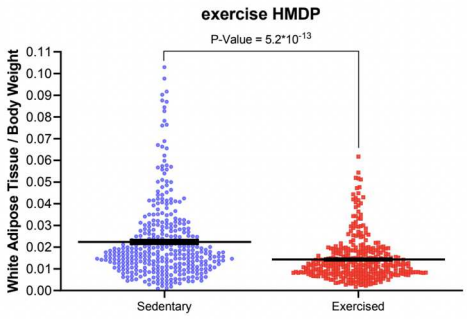


Figure 6. Exercise induced Adipose tissue weight loss is inversely correlated with Adipose UBD1 (P=5.2*10^{-13}).

In humans, self-reported physical activity from METSIM, when stratified for meeting recommended physical activity guidelines, adipose *UBD1* expression was reduced in adipose tissue for physically fit vs sedentary (P=0.008).

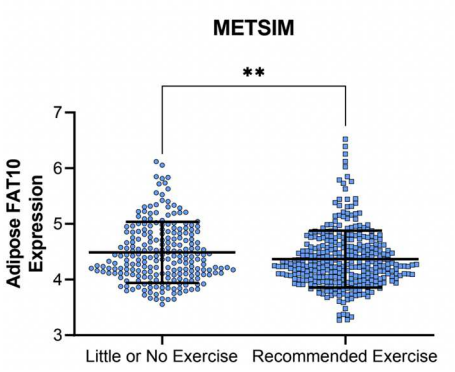


Figure 7. Adipose *UBD1* expression was reduced in adipose tissue for physically fit vs sedentary (*P*=0.008)(METSIM).

We identified a significant positive correlation between gonadal white adipose tissue mass and adipose *UBD1* gene expression (r^2=0.751, *P*=7.6*10^{-23}).

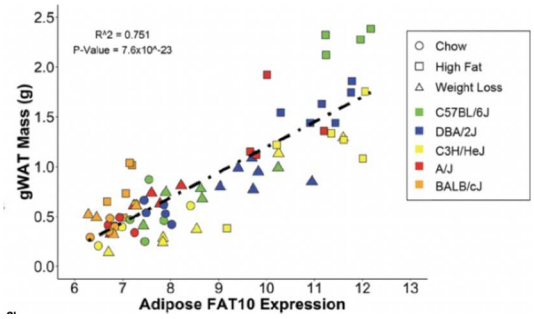


Figure 8. A positive correlation between gonadal white adipose tissue mass and adipose UBD1/FAT10 expression

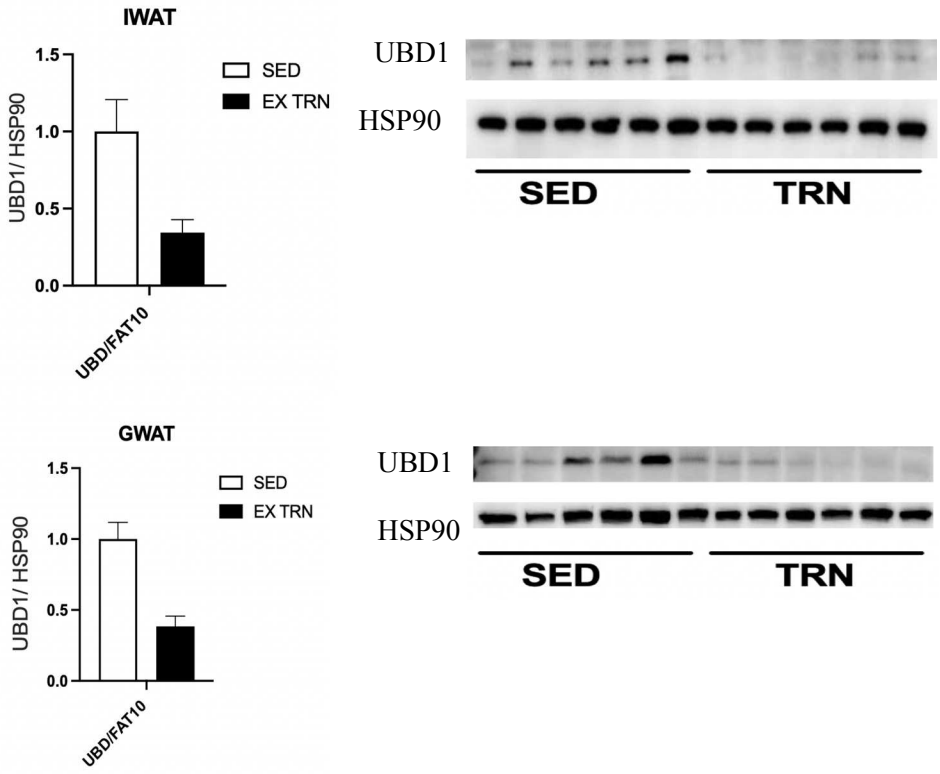


Figure 9. Quantification of male Gonadal and Inguinal White Adipose Tissue (GWAT/IWAT) indicate a reduction in UBD1 expression after exercise. iWAT (Protein P-Value: 0.014935). gWAT (Protein P-Value: 0.001226).

With respect to sex as a biological variable, UBD1 expression was elevated in females compared to males, and its expression was impacted by caloric intake (HFD $P<0.0001$) and X chromosome-linked mechanism(s) (*Kdm5c*).

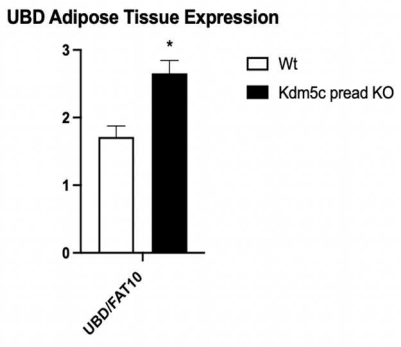


Figure 10. UBD1 expression is regulated via the x chromosome escape gene KDM5C, not estradiol. Significant increase in UBD1 expression in KDM5C Knock-Out mice.

DISCUSSION

Gene dosing studies in adipocytes to interrogate molecular actions of UBD1 on adipocyte metabolism are underway. Molecular targets of UBD1 include Parkin and P53 which we have previously shown to regulate mitochondrial content and adipocyte size and number. UBD1 might stabilize Parkin expression directly or indirectly by modifying some other Parkin binding partner. Stabilization of Parkin is permissive for fat accumulation. This is likely achieved by a larger adipogenic/lipogenic program that Parkin plays a role in but includes the downregulation of mitochondrial number. This is consistent with our data showing that UBD1 is increased in expression in adipose tissue following HFD-feeding. In contrast during repetitive bouts of activity, chronic exercise training, we show reduced expression of UBD1 and Parkin and this may underlie the increase in mtDNA copy number and reduction in adiposity. The increase in caloric expenditure seen in exercise training leading to reduced UBD1 expression is in alignment conceptually with the reduction in UBD1 seen in caloric restriction – thus overall shifts in caloric intake and expenditure seem to impact UBD1 expression to modulate adiposity.

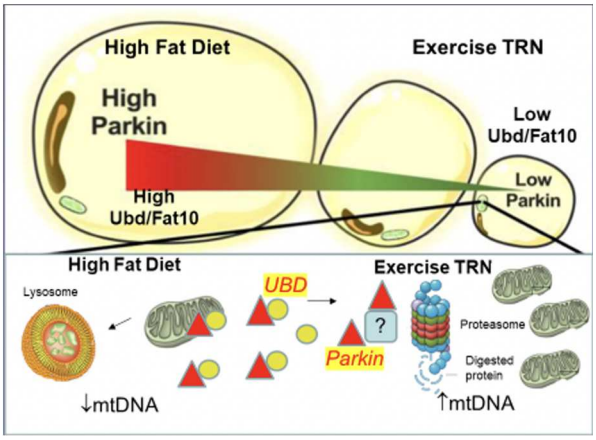


Figure 11. Image modified. Moore, T.M., Cheng, L., Wolf, D.M. *et al.* Parkin regulates adiposity by coordinating mitophagy with mitochondrial biogenesis in white adipocytes. *Nat Commun* 13, 6661 (2022). https://doi.org/10.1038/s41467-022-34468-2[60]

CONCLUSION

UBD1 is an exercise-responsive transcript with sexually dimorphic expression. Reduction in adipose tissue expression following 30 days of wheel running was highly associated with exercise-induced adipose tissue weight loss in a large panel of mice, and this relationship was confirmed in human subjects. These data suggest that repression of specific targets in adipose tissue, including UBD1, may in part underlie the remarkable remodeling that occurs in adipose tissue depots as a consequence of daily physical activity. Identification of the molecular transducers that deliver the health benefits associated with exercise training will improve clinical care of individuals suffering from cardiometabolic related diseases.

References

1. de Labra C, Guimaraes-Pinheiro C, Maseda A, Lorenzo T, Millán-Calenti JC. Effects of physical exercise interventions in frail older adults: a systematic review of randomized controlled trials. *BMC Geriatr*. Dec 2015;15:154. doi:10.1186/s12877-015-0155-4

2. McMullan RC, Kelly SA, Hua K, et al. Long-term exercise in mice has sex-dependent benefits on body composition and metabolism during aging. *Physiol Rep*. 11 2016;4(21)doi:10.14814/phy2.13011

3. Foulds HJ, Bredin SS, Warburton DE. Ethnic differences in vascular responses to aerobic exercise. *Med Sci Sports Exerc*. Feb 2015;47(2):280-8. doi:10.1249/MSS.0000000000000423

4. Lin X, Zhang X, Guo J, et al. Effects of Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *J Am Heart Assoc*. Jun 2015;4(7)doi:10.1161/JAHA.115.002014

5. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand J Med Sci Sports*. Dec 2015;25 Suppl 3:1-72. doi:10.1111/sms.12581

6. Bowen PG, Mankowski RT, Harper SA, Buford TW. Exercise is Medicine as a Vital Sign: Challenges and Opportunities. *Transl J Am Coll Sports Med*. Jan 2019;4(1):1-7.

7. Montero D, Lundby C. Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. *J Physiol*. 06 2017;595(11):3377-3387. doi:10.1113/JP273480

8. Ross R, Goodpaster BH, Koch LG, et al. Precision exercise medicine: understanding exercise response variability. *Br J Sports Med*. Sep 2019;53(18):1141-1153. doi:10.1136/bjsports-2018-100328

9. Liu Y, Wang Y, Ni Y, et al. Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention. *Cell Metab*. 01 2020;31(1):77-91.e5. doi:10.1016/j.cmet.2019.11.001

10. Bennett BJ, Farber CR, Orozco L, et al. A high-resolution association mapping panel for the dissection of complex traits in mice. *Genome Res*. Feb 2010;20(2):281-90. doi:10.1101/gr.099234.109

11. Norheim F, Hasin-Brumshtein Y, Vergnes L, et al. Gene-by-Sex Interactions in Mitochondrial Functions and Cardio-Metabolic Traits. *Cell Metab*. 04 2019;29(4):932-949.e4. doi:10.1016/j.cmet.2018.12.013 12. Ghazalpour A, Rau CD, Farber CR, et al. Hybrid mouse diversity panel: a panel of inbred mouse strains suitable for analysis of complex genetic traits. *Mamm Genome*. Oct 2012;23(9-10):680-92. doi:10.1007/s00335- 012-9411-5

13. Laakso M, Kuusisto J, Stančáková A, et al. The Metabolic Syndrome in Men study: a resource for studies of metabolic and cardiovascular diseases. *J Lipid Res*. 03 2017;58(3):481-493. doi:10.1194/jlr.O072629

14. Sanford JA, Nogiec CD, Lindholm ME, et al. Molecular Transducers of Physical Activity Consortium (MoTrPAC): Mapping the Dynamic Responses to Exercise. *Cell*. 06 2020;181(7):1464-1474. doi:10.1016/j.cell.2020.06.004

15. Stout NL, Baima J, Swisher AK, Winters-Stone KM, Welsh J. A Systematic Review of Exercise Systematic Reviews in the Cancer Literature (2005-2017). *PM R*. Sep 2017;9(9S2):S347-S384. doi:10.1016/j.pmrj.2017.07.074

16. Mikkelsen K, Stojanovska L, Polenakovic M, Bosevski M, Apostolopoulos V. Exercise and mental health. *Maturitas*. Dec 2017;106:48-56. doi:10.1016/j.maturitas.2017.09.003

17. Chekroud SR, Gueorguieva R, Zheutlin AB, et al. Association between physical exercise and mental health in 1·2 million individuals in the USA between 2011 and 2015: a cross-sectional study. *Lancet Psychiatry*. 09 2018;5(9):739-746. doi:10.1016/S2215-0366(18)30227-X

18. Northey JM, Cherbuin N, Pumpa KL, Smee DJ, Rattray B. Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. *Br J Sports Med*. Feb 2018;52(3):154-160. doi:10.1136/bjsports-2016-096587

19. da Silva FC, Iop RDR, de Oliveira LC, et al. Effects of physical exercise programs on cognitive function in Parkinson's disease patients: A systematic review of randomized controlled trials of the last 10 years. *PLoS One*. 2018;13(2):e0193113. doi:10.1371/journal.pone.0193113

20. Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA*. May 2009;301(19):2024-35. doi:10.1001/jama.2009.681

21. Ladenvall P, Persson CU, Mandalenakis Z, et al. Low aerobic capacity in middle-aged men associated with increased mortality rates during 45 years of follow-up. *Eur J Prev Cardiol*. 09 2016;23(14):1557-64. doi:10.1177/2047487316655466

22. Lee DC, Artero EG, Sui X, Blair SN. Mortality trends in the general population: the importance of cardiorespiratory fitness. *J Psychopharmacol*. Nov 2010;24(4 Suppl):27-35. doi:10.1177/1359786810382057

23. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med*. Mar 2002;346(11):793-801. doi:10.1056/NEJMoa011858

24. Bennie JA, De Cocker K, Teychenne MJ, Brown WJ, Biddle SJH. The epidemiology of aerobic physical activity and muscle-strengthening activity guideline adherence among 383,928 U.S. adults. *Int J Behav Nutr Phys Act*. 04 2019;16(1):34. doi:10.1186/s12966-019-0797-2

25. Avila JJ, Kim SK, Massett MP. Differences in Exercise Capacity and Responses to Training in 24 Inbred Mouse Strains. *Front Physiol*. 2017;8:974. doi:10.3389/fphys.2017.00974 26. Lightfoot JT, Turner MJ, Pomp D, Kleeberger SR, Leamy LJ. Interstrain variation in murine aerobic capacity.

27. Kelly SA, Nehrenberg DL, Peirce JL, et al. Genetic architecture of voluntary exercise in an advanced intercross line of mice. *Physiol Genomics*. Jul 2010;42(2):190-200. doi:10.1152/physiolgenomics.00028.2010

28. Bennett BJ, Davis RC, Civelek M, et al. Genetic Architecture of Atherosclerosis in Mice: A Systems Genetics Analysis of Common Inbred Strains. *PLoS Genet*. Dec 2015;11(12):e1005711. doi:10.1371/journal.pgen.1005711

29. Lusis AJ, Seldin MM, Allayee H, et al. The Hybrid Mouse Diversity Panel: a resource for systems genetics analyses of metabolic and cardiovascular traits. *J Lipid Res*. 06 2016;57(6):925-42. doi:10.1194/jlr.R066944

30. Moore TM, Zhou Z, Cohn W, et al. The impact of exercise on mitochondrial dynamics and the role of Drp1 in exercise performance and training adaptations in skeletal muscle. *Mol Metab*. Mar 2019;21:51-67. doi:10.1016/j.molmet.2018.11.012

31. Wedell-Neergaard AS, Lang Lehrskov L, Christensen RH, et al. Exercise-Induced Changes in Visceral Adipose Tissue Mass Are Regulated by IL-6 Signaling: A Randomized Controlled Trial. *Cell Metab*. 04 2019;29(4):844-855.e3. doi:10.1016/j.cmet.2018.12.007

32. Nayor M, Shah RV, Miller PE, et al. Metabolic Architecture of Acute Exercise Response in Middle-Aged Adults in the Community. *Circulation*. Nov 2020;142(20):1905-1924. doi:10.1161/CIRCULATIONAHA.120.050281

33. Melanson EL, Gozansky WS, Barry DW, Maclean PS, Grunwald GK, Hill JO. When energy balance is maintained, exercise does not induce negative fat balance in lean sedentary, obese sedentary, or lean

endurance-trained individuals. *J Appl Physiol (1985)*. Dec 2009;107(6):1847-56. doi:10.1152/japplphysiol.00958.2009

34. Gonzalez-Gil AM, Elizondo-Montemayor L. The Role of Exercise in the Interplay between Myokines, Hepatokines, Osteokines, Adipokines, and Modulation of Inflammation for Energy Substrate Redistribution and Fat Mass Loss: A Review. *Nutrients*. Jun 2020;12(6)doi:10.3390/nu12061899

35. Vidal P, Stanford KI. Exercise-Induced Adaptations to Adipose Tissue Thermogenesis. *Front Endocrinol (Lausanne)*. 2020;11:270. doi:10.3389/fendo.2020.00270

36. Hanssen H, Boardman H, Deiseroth A, et al. Personalized exercise prescription in the prevention and treatment of arterial hypertension: a Consensus Document from the European Association of Preventive Cardiology (EAPC) and the ESC Council on Hypertension. *Eur J Prev Cardiol*. Mar 2021;doi:10.1093/eurjpc/zwaa141

37. Zubin Maslov P, Schulman A, Lavie CJ, Narula J. Personalized exercise dose prescription. *Eur Heart J*. 07 2018;39(25):2346-2355. doi:10.1093/eurheartj/ehx686

38. Tuka V, Linhart A. Personalised exercise prescription: Finding the best for our patients. *Eur J Prev Cardiol*. 09 2020;27(13):1366-1368. doi:10.1177/2047487319884376

39. Seldin MM, Koplev S, Rajbhandari P, et al. A Strategy for Discovery of Endocrine Interactions with Application to Whole-Body Metabolism. *Cell Metab*. May 2018;27(5):1138-1155.e6. doi:10.1016/j.cmet.2018.03.015

40. O'Neill SM, Hinkle C, Chen SJ, et al. Targeting adipose tissue via systemic gene therapy. *Gene Ther*. Jul 2014;21(7):653-61. doi:10.1038/gt.2014.38

41. Chella Krishnan K, Sabir S, Shum M, et al. Sex-specific metabolic functions of adipose Lipocalin-2. *Mol Metab*. 12 2019;30:30-47. doi:10.1016/j.molmet.2019.09.009 42. Pamir N, Pan C, Plubell DL, et al.

Genetic control of the mouse HDL proteome defines HDL traits, function, and heterogeneity. *J Lipid Res*. 03 2019;60(3):594-608. doi:10.1194/jlr.M090555

43. Ghazalpour A, Bennett B, Petyuk VA, et al. Comparative analysis of proteome and transcriptome variation in mouse. *PLoS Genet*. Jun 2011;7(6):e1001393. doi:10.1371/journal.pgen.1001393

44. Edillor CR, Parks BW, Mehrabian M, Lusis AJ, Pellegrini M. DNA Methylation Changes More Slowly Than Physiological States in Response to Weight Loss in Genetically Diverse Mouse Strains. *Front Endocrinol (Lausanne)*. 2019;10:882. doi:10.3389/fendo.2019.00882

45. Orozco LD, Morselli M, Rubbi L, et al. Epigenome-wide association of liver methylation patterns and complex metabolic traits in mice. *Cell Metab*. Jun 2015;21(6):905-17. doi:10.1016/j.cmet.2015.04.025

46. Seldin M, Yang X, Lusis AJ. Systems genetics applications in metabolism research. *Nat Metab*. 11 2019;1(11):1038-1050. doi:10.1038/s42255-019-0132-x

47. Yu J, Seldin MM, Fu K, et al. Topological Arrangement of Cardiac Fibroblasts Regulates Cellular Plasticity. *Circ Res*. 06 2018;123(1):73-85. doi:10.1161/CIRCRESAHA.118.312589 48. Hui ST, Kurt Z, Tuominen I, et al. The Genetic Architecture of Diet-Induced Hepatic Fibrosis in Mice. *Hepatology*. 12 2018;68(6):2182-2196. doi:10.1002/hep.30113

49. Chella Krishnan K, Kurt Z, Barrere-Cain R, et al. Integration of Multi-omics Data from Mouse Diversity Panel Highlights Mitochondrial Dysfunction in Non-alcoholic Fatty Liver Disease. *Cell Syst*. Jan 2018;6(1):103- 115.e7. doi:10.1016/j.cels.2017.12.006

50. Committee PAGA. *Physical Activity Guidelines Advisory Committee Scientific Report*. Washington, DC: U.S. Department of Health and Human Services; 2018.

51. Raulerson CK, Ko A, Kidd JC, et al. Adipose Tissue Gene Expression Associations Reveal Hundreds of Candidate Genes for Cardiometabolic Traits. *Am J Hum Genet*. 10 03 2019;105(4):773-787. doi:10.1016/j.ajhg.2019.09.001

52. Aichem A, Groettrup M. The ubiquitin-like modifier FAT10 - much more than a proteasome-targeting signal. *J Cell Sci*. 07 21 2020;133(14)doi:10.1242/jcs.246041

53. Canaan A, Yu X, Booth CJ, et al. FAT10/diubiquitin-like protein-deficient mice exhibit minimal phenotypic differences. *Mol Cell Biol*. Jul 2006;26(13):5180-9. doi:10.1128/MCB.00966-05

54. Canaan A, DeFuria J, Perelman E, et al. Extended lifespan and reduced adiposity in mice lacking the FAT10 gene. *Proc Natl Acad Sci U S A*. Apr 08 2014;111(14):5313-8. doi:10.1073/pnas.1323426111

55. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol*. 05 2016;34(5):525-7. doi:10.1038/nbt.3519

56. Hao Y, Hao S, Andersen-Nissen E, et al. Integrated analysis of multimodal single-cell data. *Cell*. 06 24 2021;184(13):3573-3587.e29. doi:10.1016/j.cell.2021.04.048

57. Hirschey MD, Shimazu T, Goetzman E, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. *Nature*. Mar 04 2010;464(7285):121-5. doi:10.1038/nature08778

58. Ribas V, Nguyen MT, Henstridge DC, et al. Impaired oxidative metabolism and inflammation are associated with insulin resistance in ERalpha-deficient mice. *Am J Physiol Endocrinol Metab*. Feb 2010;298(2):E304-19. doi:10.1152/ajpendo.00504.2009

59. Poole DC, Copp SW, Colburn TD, et al. Guidelines for animal exercise and training protocols for cardiovascular studies. *Am J Physiol Heart Circ Physiol*. 05 01 2020;318(5):H1100-H1138. doi:10.1152/ajpheart.00697.2019

60. Moore, T.M., Cheng, L., Wolf, D.M. *et al.* Parkin regulates adiposity by coordinating mitophagy with mitochondrial biogenesis in white adipocytes. *Nat Commun* 13, 6661 (2022). https://doi.org/10.1038/s41467-022-34468-2

61. Aichem A, Groettrup M. The ubiquitin-like modifier FAT10 - much more than a proteasome-targeting signal. J Cell Sci. 2020 Jul 21;133(14):jcs246041. doi: 10.1242/jcs.246041. PMID: 32719056.

62. Aichem, A., Anders, S., Catone, N., Rö ßler, P., Stotz, S., Berg, A., Schwab, R., Scheuermann, S., Bialas, J., Schü tz-Stoffregen, M. C. et al. (2018). The structure of the ubiquitin-like modifier FAT10 reveals an alternative targeting mechanism for proteasomal degradation. Nat. Commun. 9, 3321. doi:10.1038/ s41467-018-05776-3

63. Hipp, M. S., Kalveram, B., Raasi, S., Groettrup, M. and Schmidtke, G. (2005). FAT10, a ubiquitin-independent signal for proteasomal degradation. Mol. Cell. Biol. 25, 3483-3491. doi:10.1128/MCB.25.9.3483-3491.2005

64. Buerger, S., Herrmann, V. L., Mundt, S., Trautwein, N., Groettrup, M. and Basler, M. (2015). The ubiquitin-like modifier FAT10 is selectively expressed in medullary thymic epithelial cells and modifies T cell selection. J. Immunol. 195, 4106-4116. doi:10.4049/jimmunol.1500592

65. Schliehe, C., Bitzer, A., van den Broek, M. and Groettrup, M. (2012). Stable antigen is most effective for eliciting CD8+ T-cell responses after DNA vaccination and infection with recombinant vaccinia virus in vivo. J. Virol. 86, 9782-9793. doi:10.1128/JVI.00694-12

66. Roverato ND, Sailer C, Catone N, Aichem A, Stengel F, Groettrup M. Parkin is an E3 ligase for the ubiquitin-like modifier FAT10, which inhibits Parkin activation and mitophagy. Cell Rep. 2021 Mar 16;34(11):108857. doi: 10.1016/j.celrep.2021.108857. PMID: 33730565.

67. Link JC, Wiese CB, Chen X, Avetisyan R, Ronquillo E, Ma F, Guo X, Yao J, Allison M, Chen YI, Rotter JI, El-Sayed Moustafa JS, Small KS, Iwase S, Pellegrini M, Vergnes L, Arnold AP, Reue K. X chromosome dosage of histone demethylase KDM5C determines sex differences in adiposity. J Clin

Invest. 2020 Nov 2;130(11):5688-5702. doi: 10.1172/JCI140223. PMID: 32701509; PMCID: PMC7598065.

68. Osborne HC, Irving E, Schmidt CK. The Ubiquitin/UBL Drug Target Repertoire. Trends Mol Med. 2020 Dec;26(12):1133-1134. doi: 10.1016/j.molmed.2020.08.009. Epub 2020 Sep 24. PMID: 32981839.