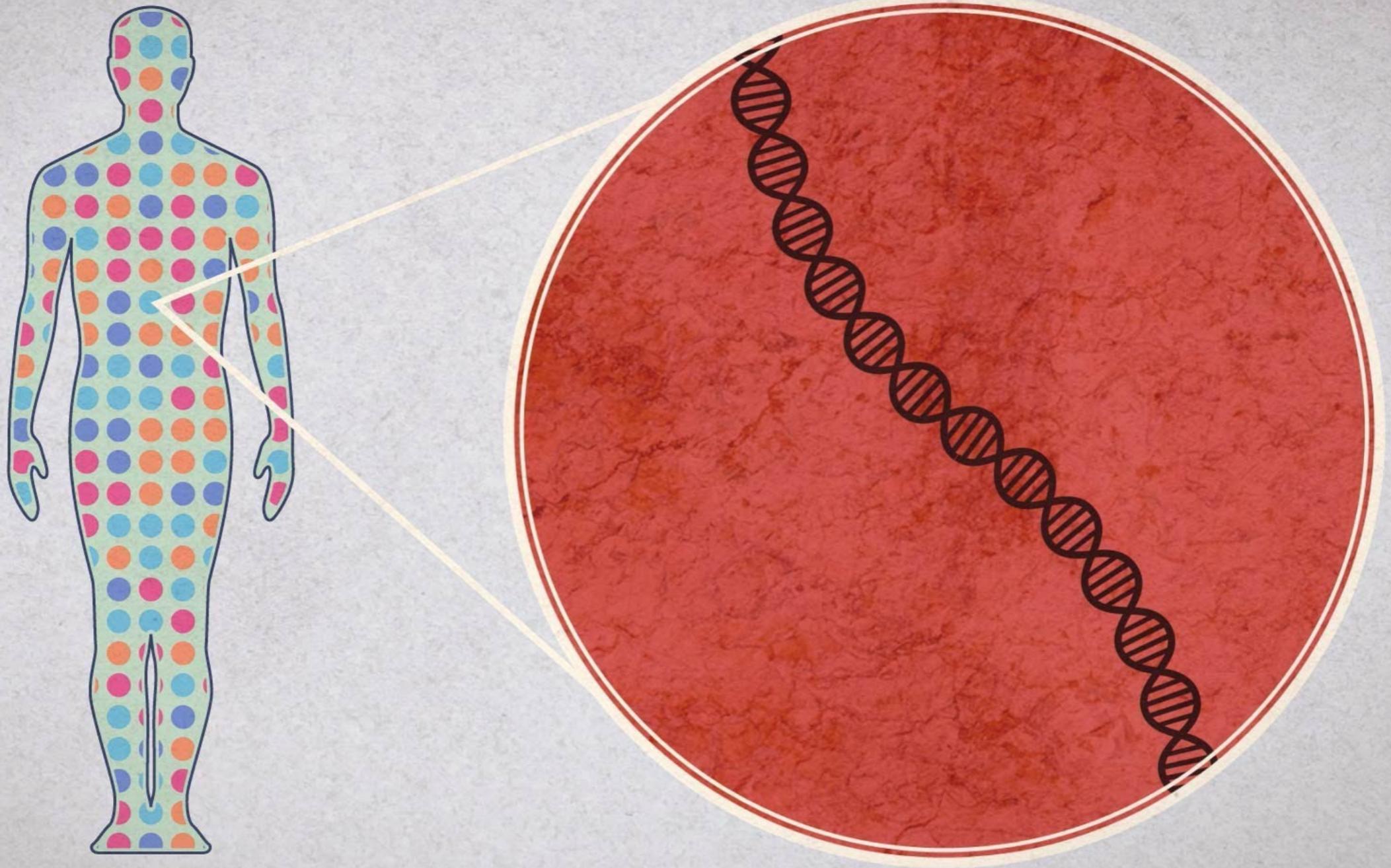
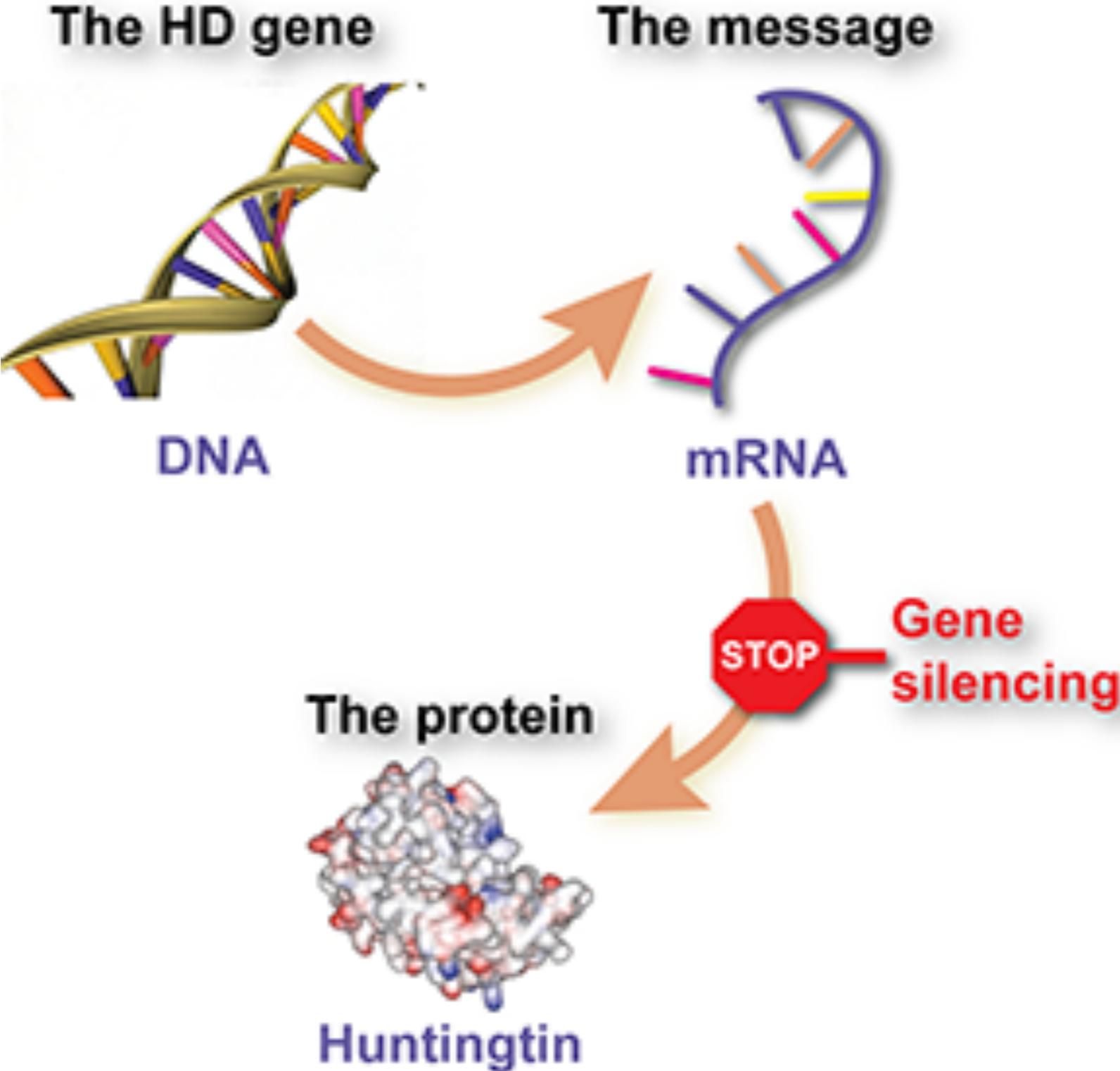


Genome-wide RNAi Screening and Obesity

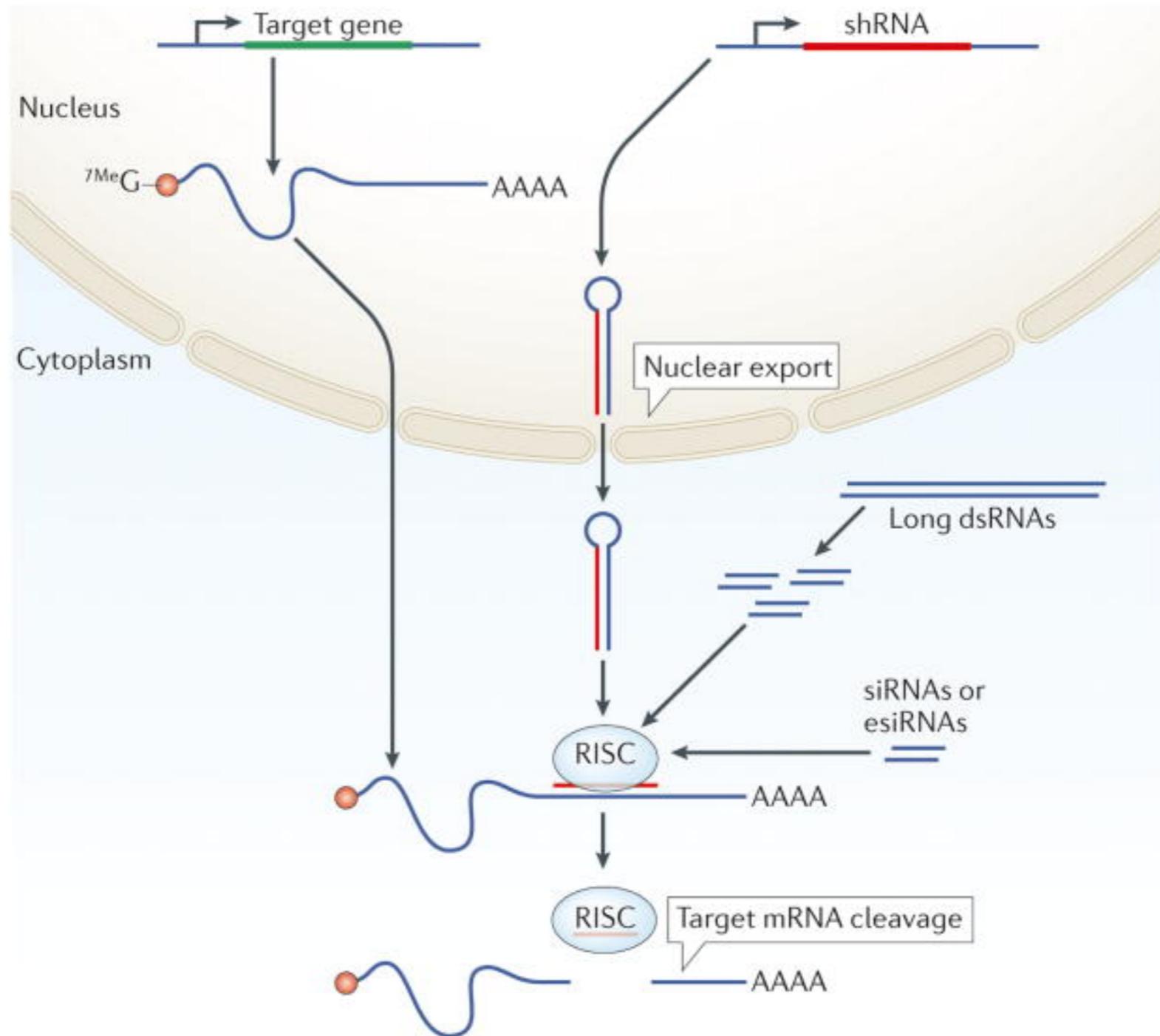


Madison Sanderson & Erika Nelson

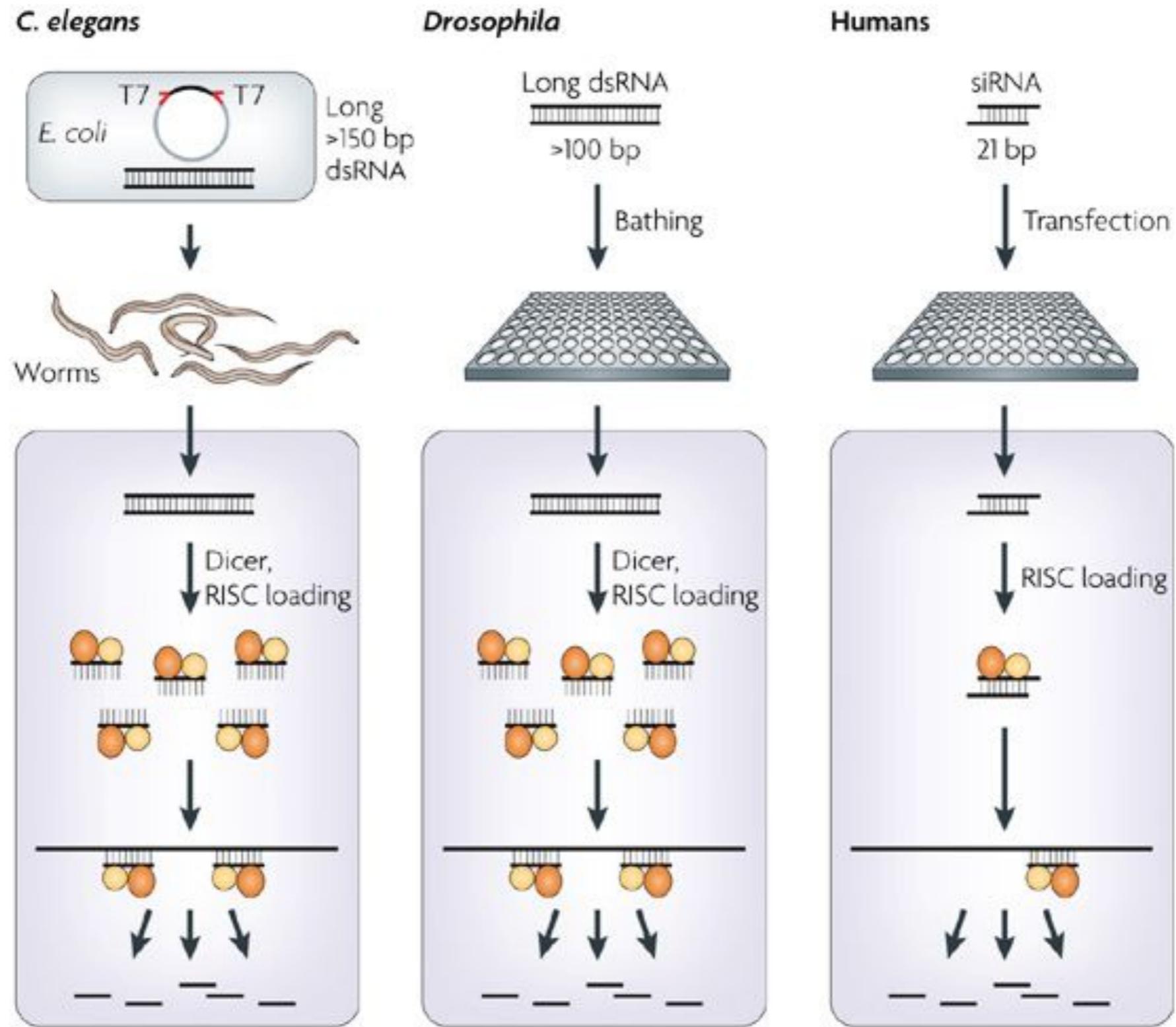
What is gene silencing?



What is RNAi?

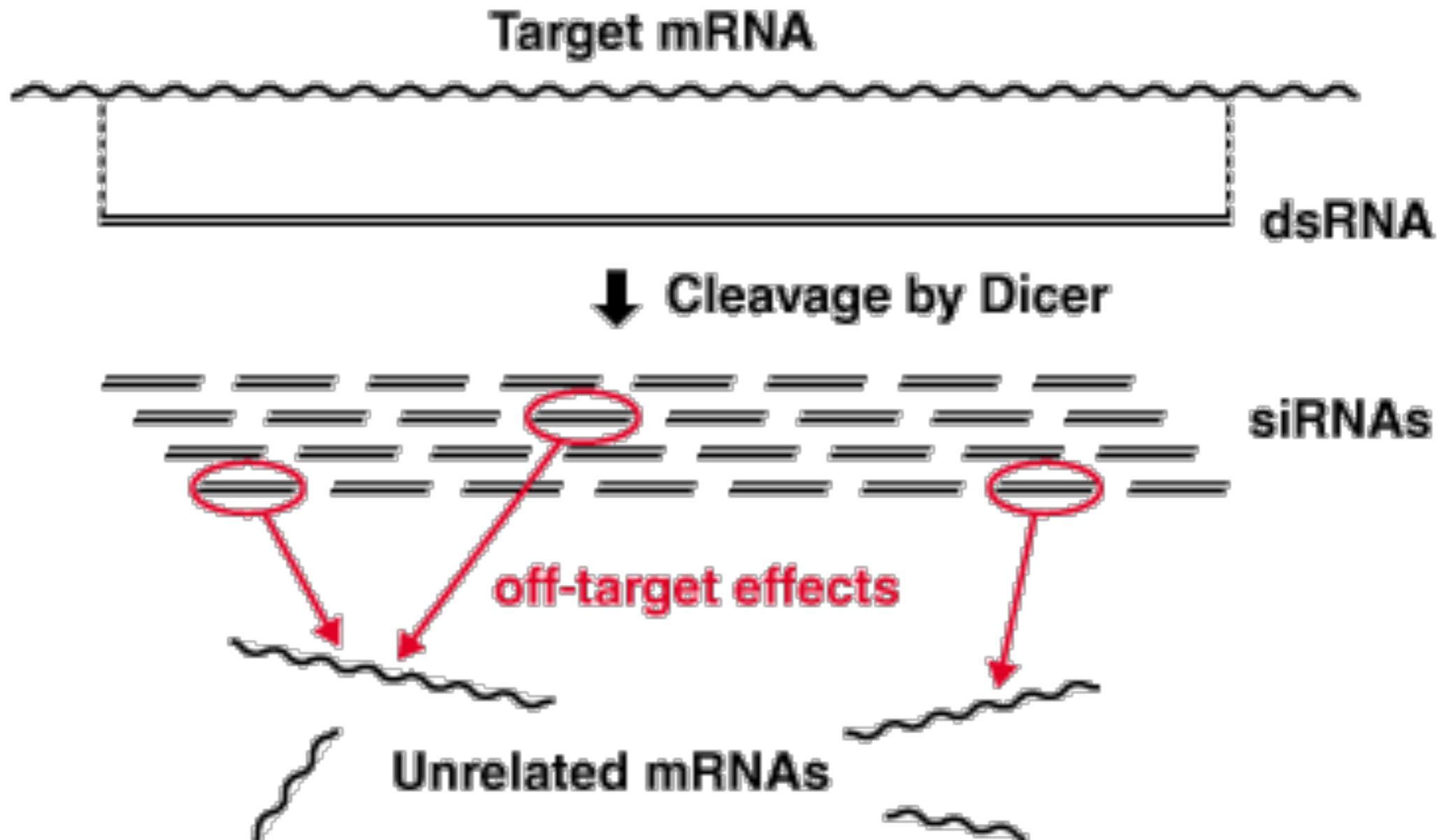


What are the benefits of RNAi screens?

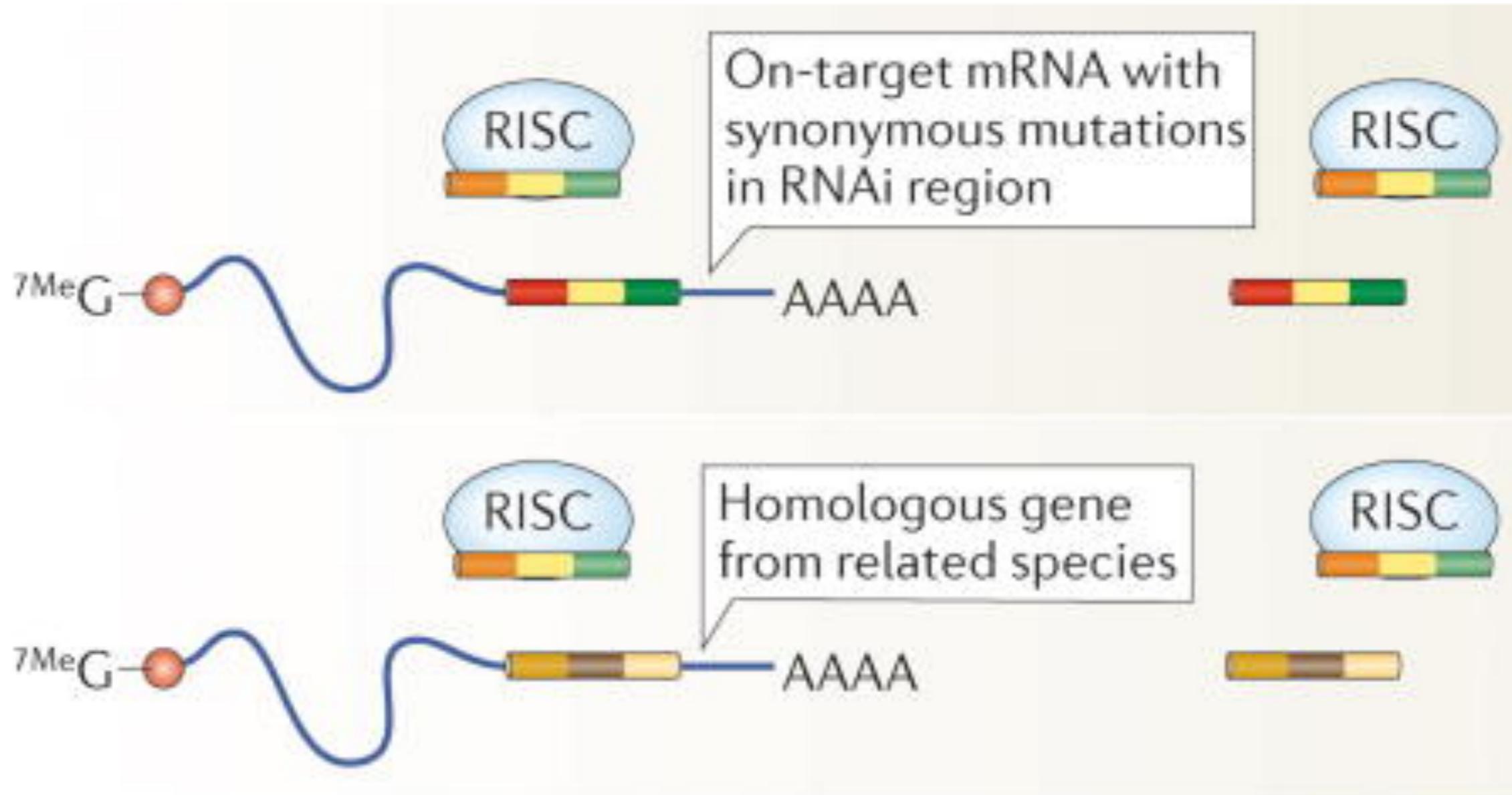


**What are the disadvantages
of RNAi screens?**

What are off-target effects?

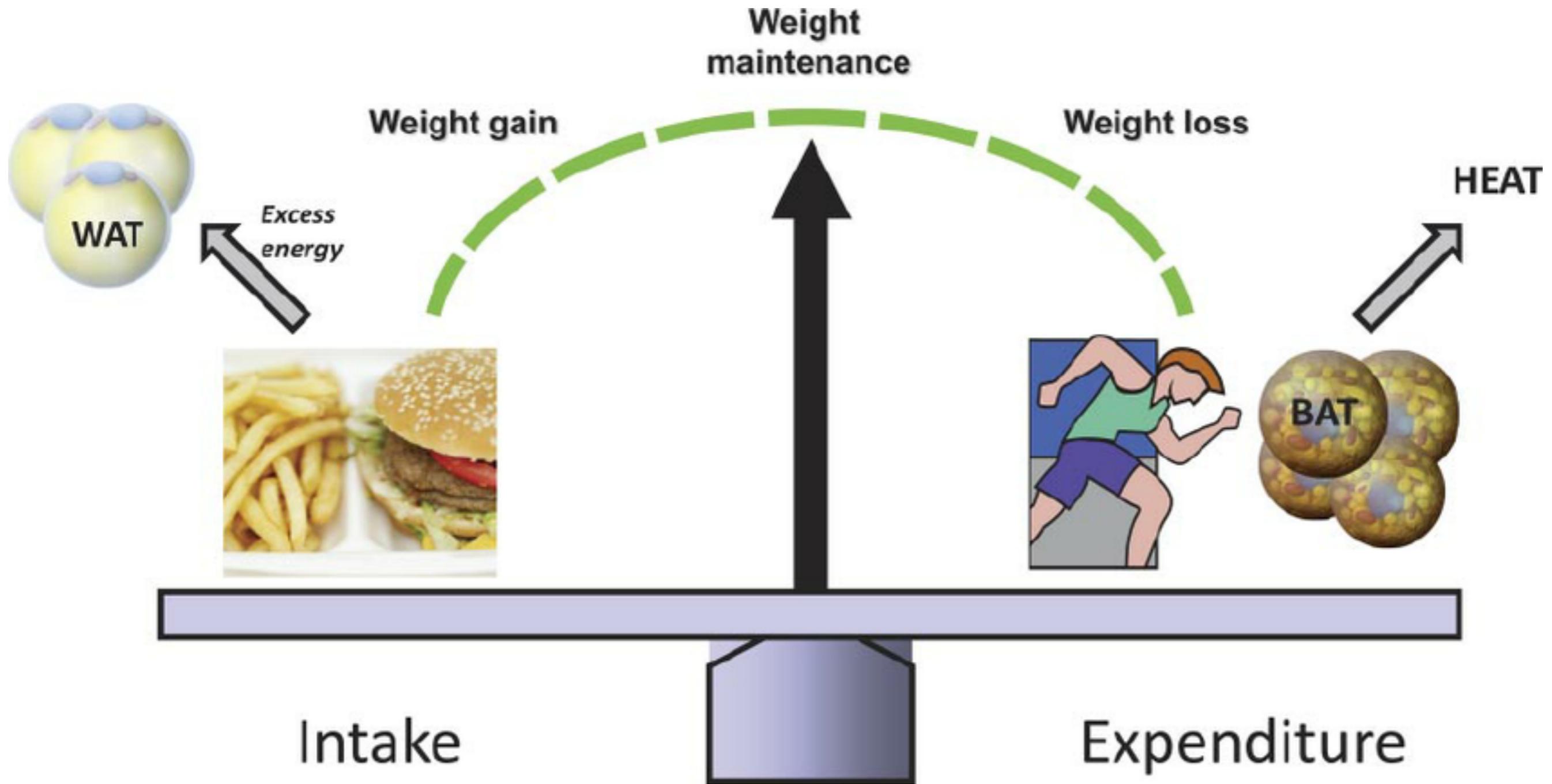


How can RNAi screen results be validated?

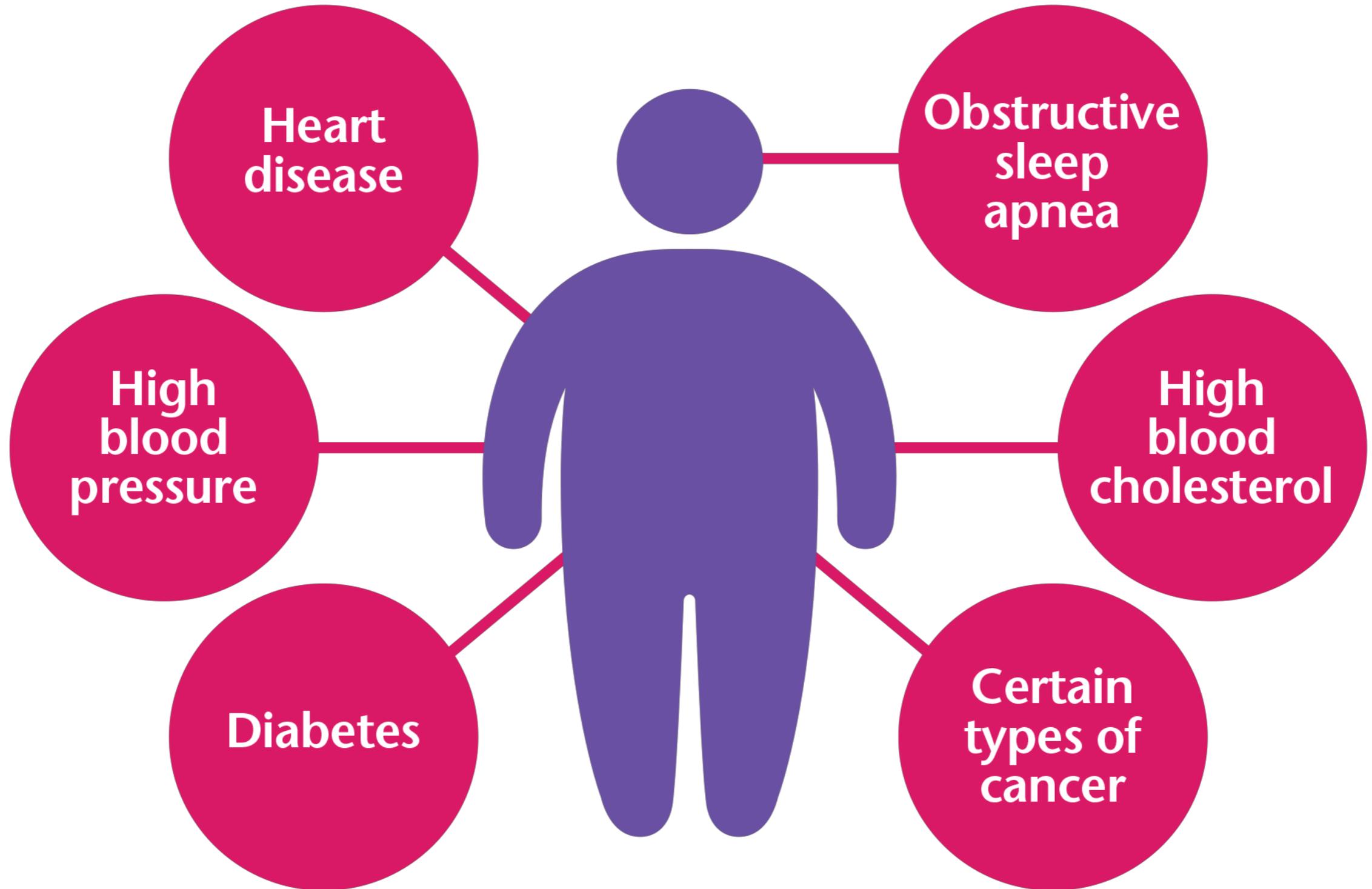


How did this study use RNAi screens?

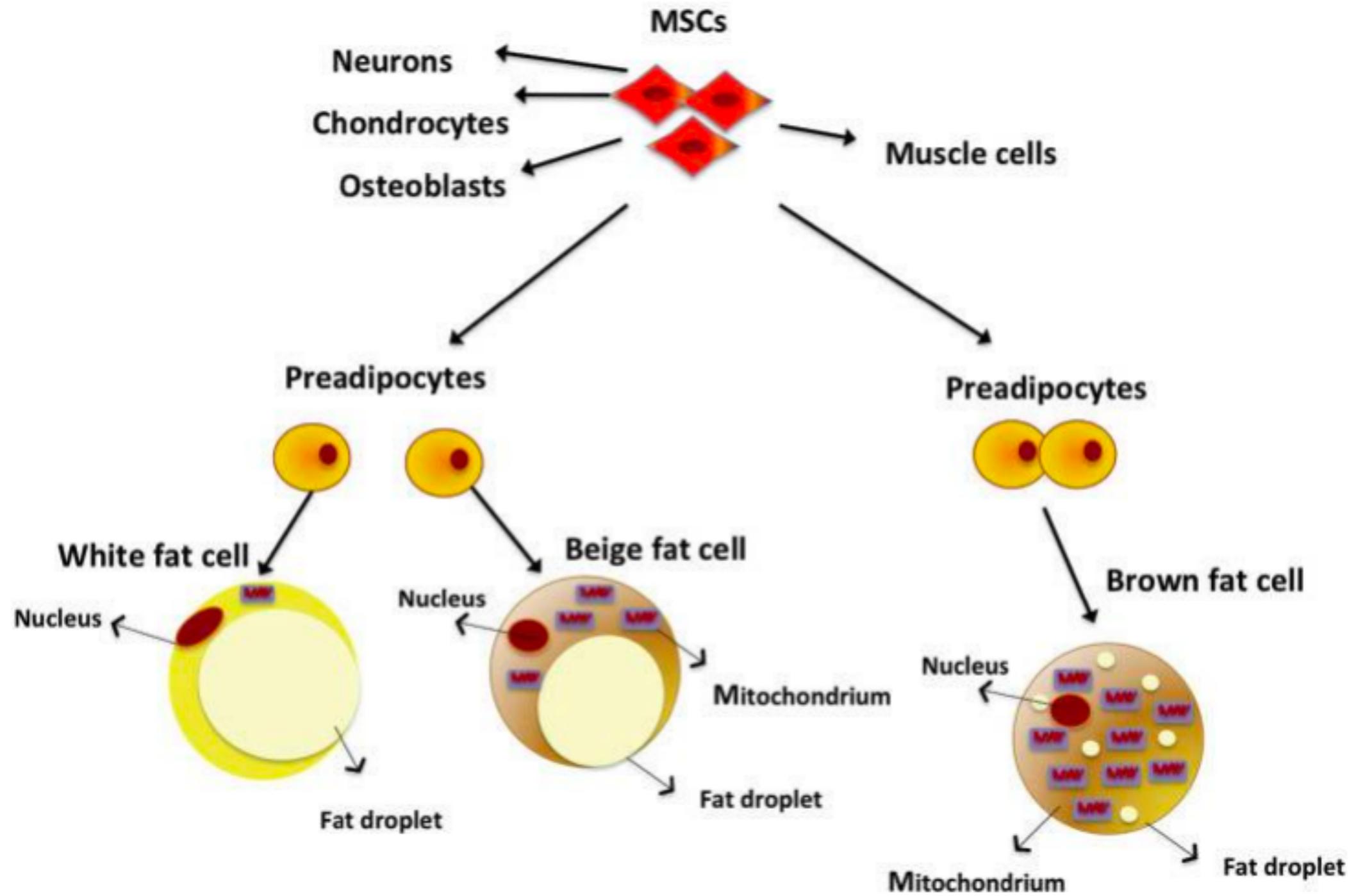
What is obesity?



What are the risks of obesity?



How do adipocytes differentiate?



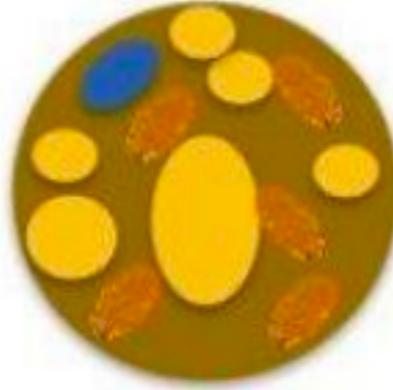
What are the functions of adipocytes?

Brown Adipocyte



Heat

Beige Adipocyte



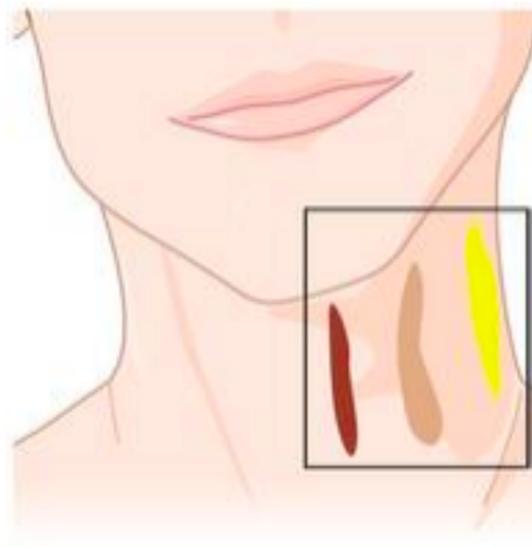
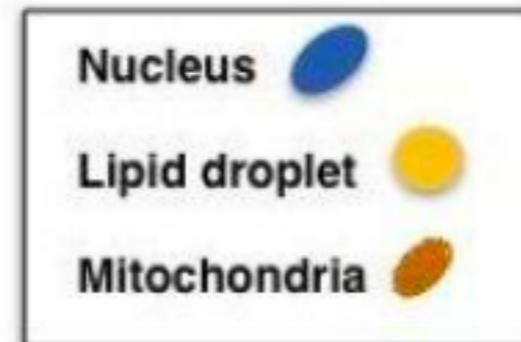
Heat

Thermogenic
Fat converted to heat

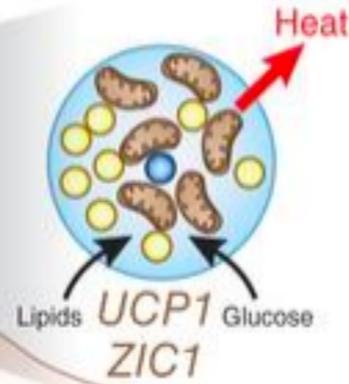
White Adipocyte



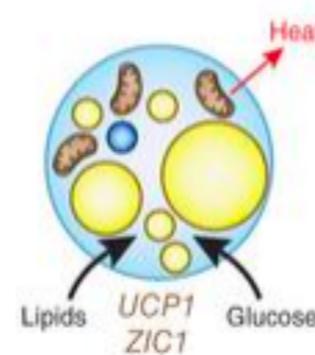
Fat storage



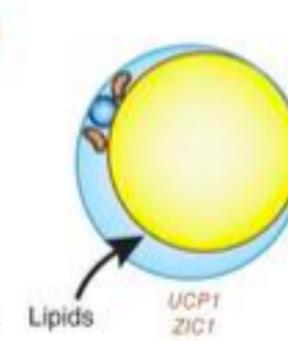
Classical brown



Brite/beige?



White



What model organism can be used for studying obesity?

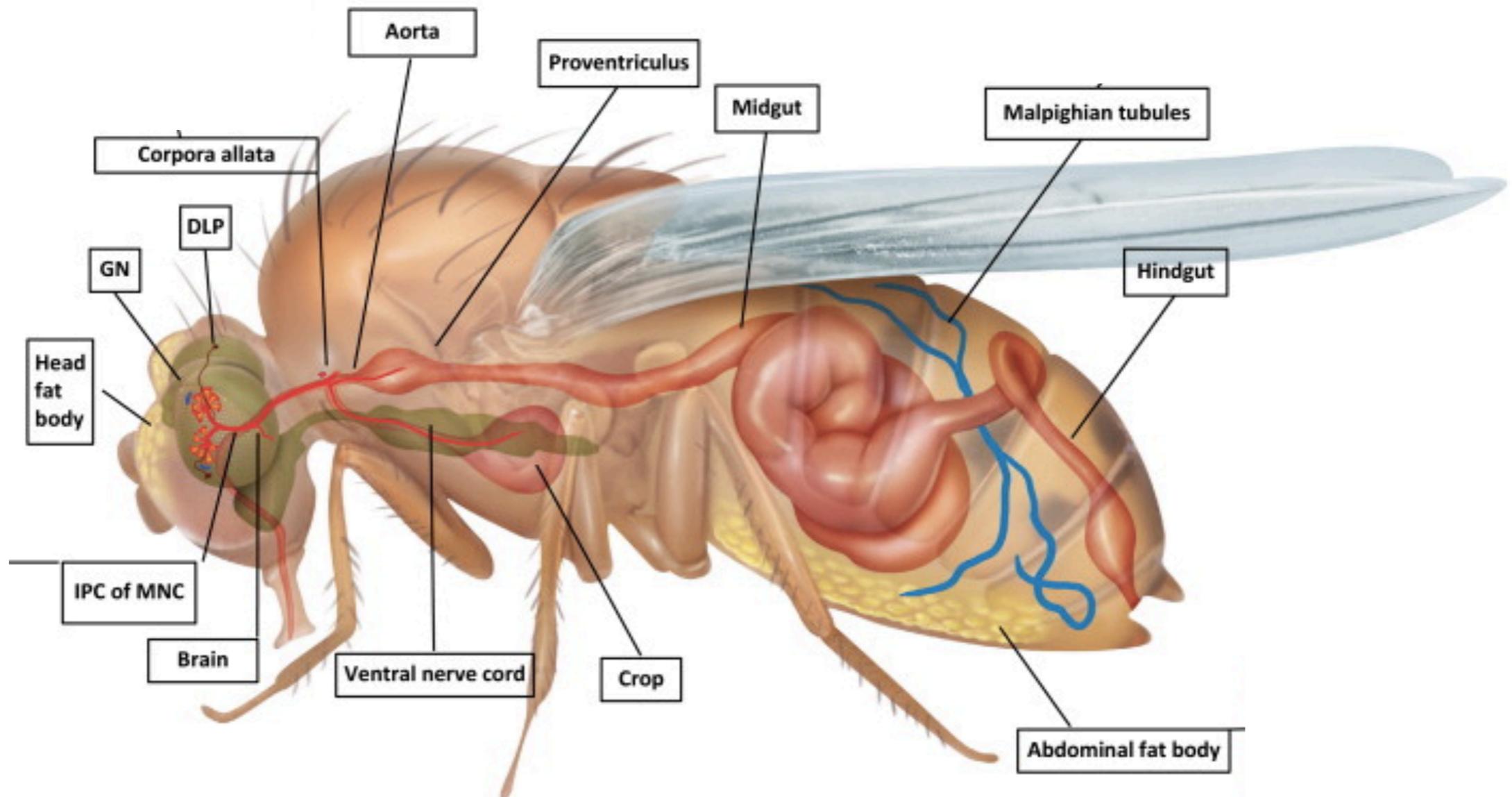
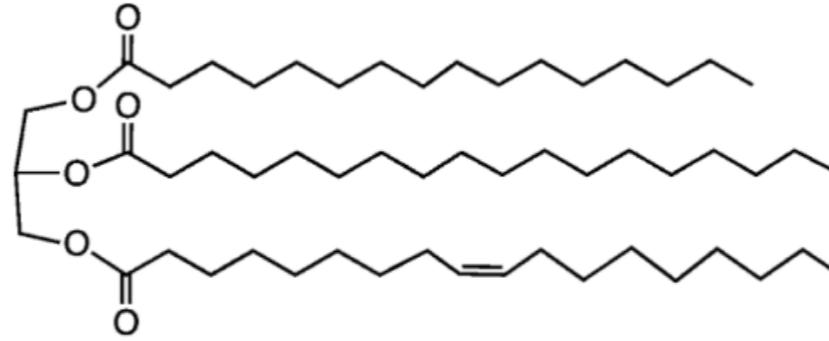


Do flies get fat?

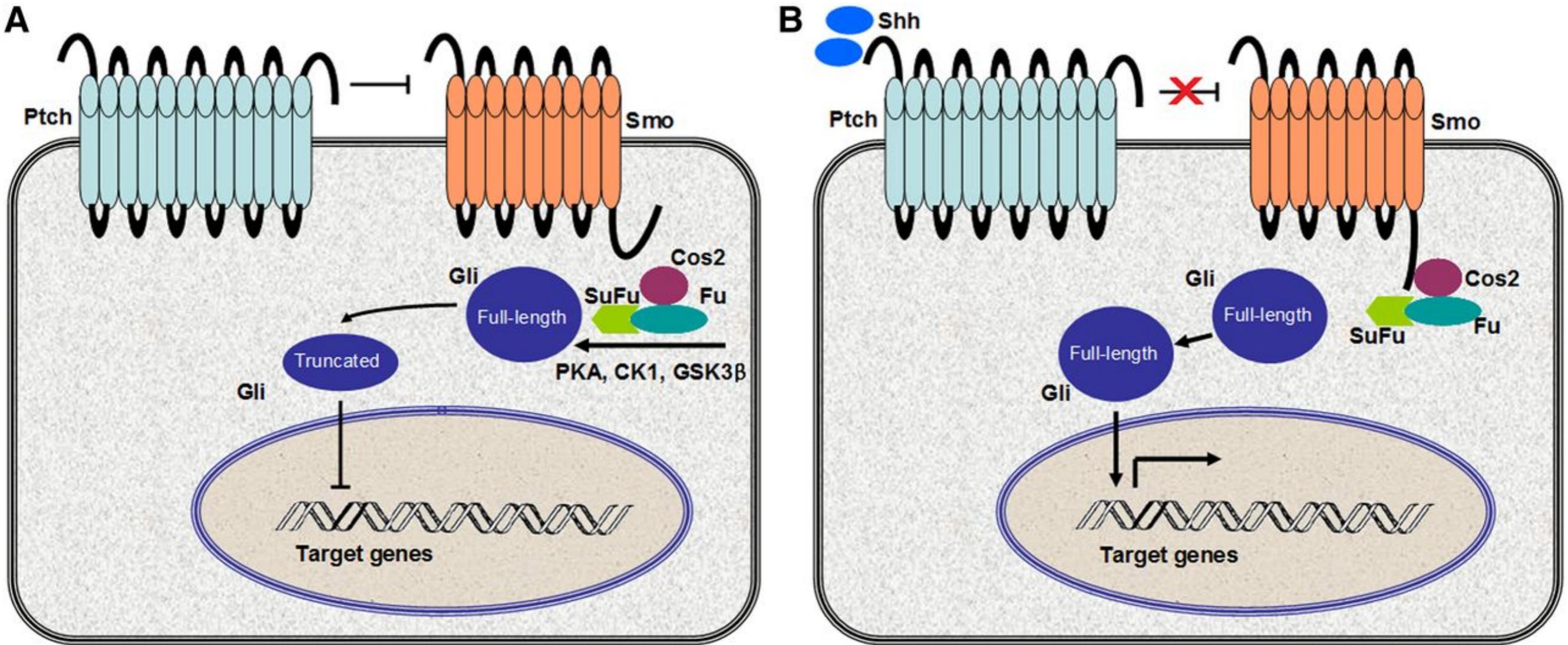
Do flies get fat?



How is fat stored in *Drosophila*?

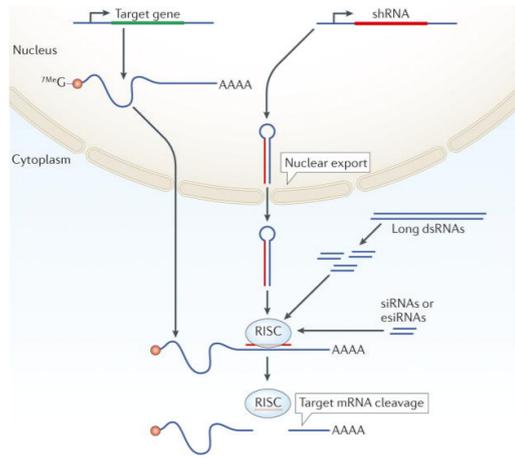


How is adipocyte differentiation regulated in Drosophila?

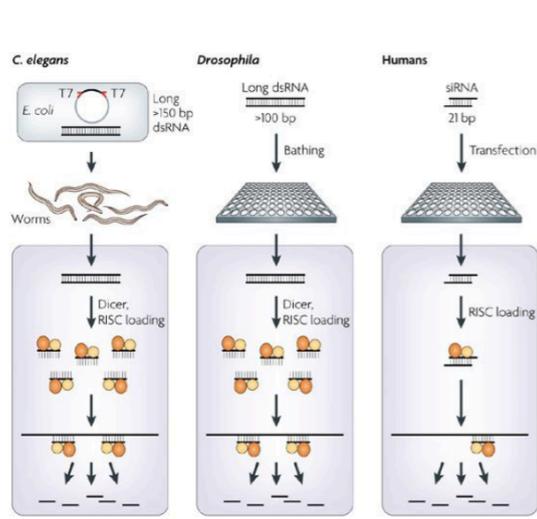


Adipocyte differentiation is regulated by the **Hedgehog** pathway

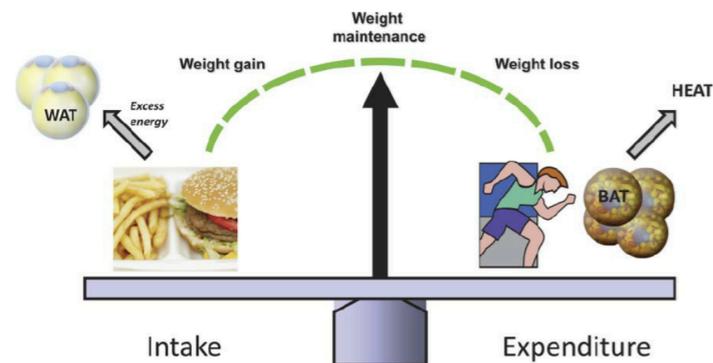
Summary



RNAi is used for gene silencing



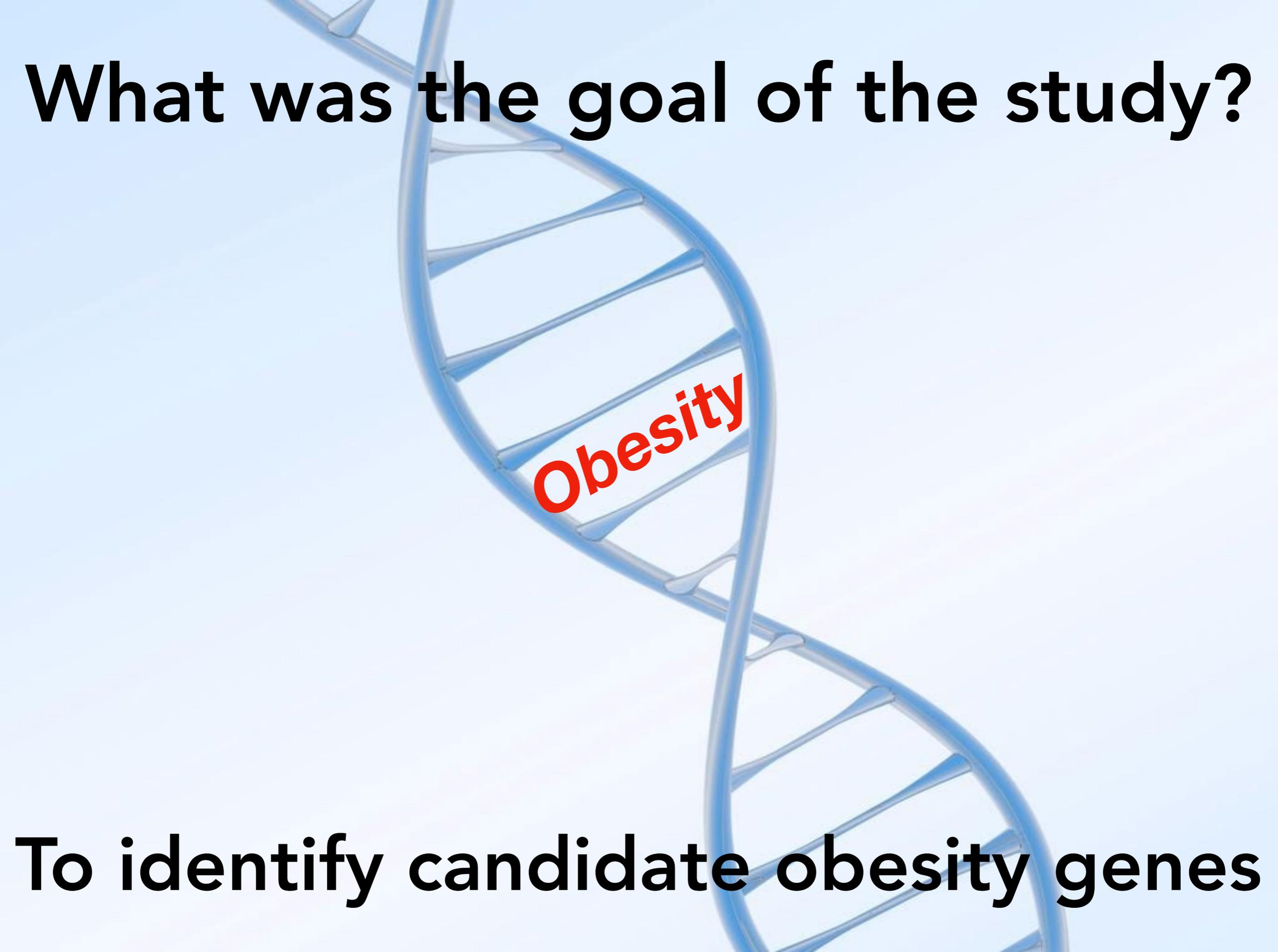
Screens can be used to identify candidate genes



Obesity is the result of a positive energy balance

***Drosophila* Genome-wide Obesity Screen Reveals Hedgehog as a Determinant of Brown versus White Adipose Cell Fate**

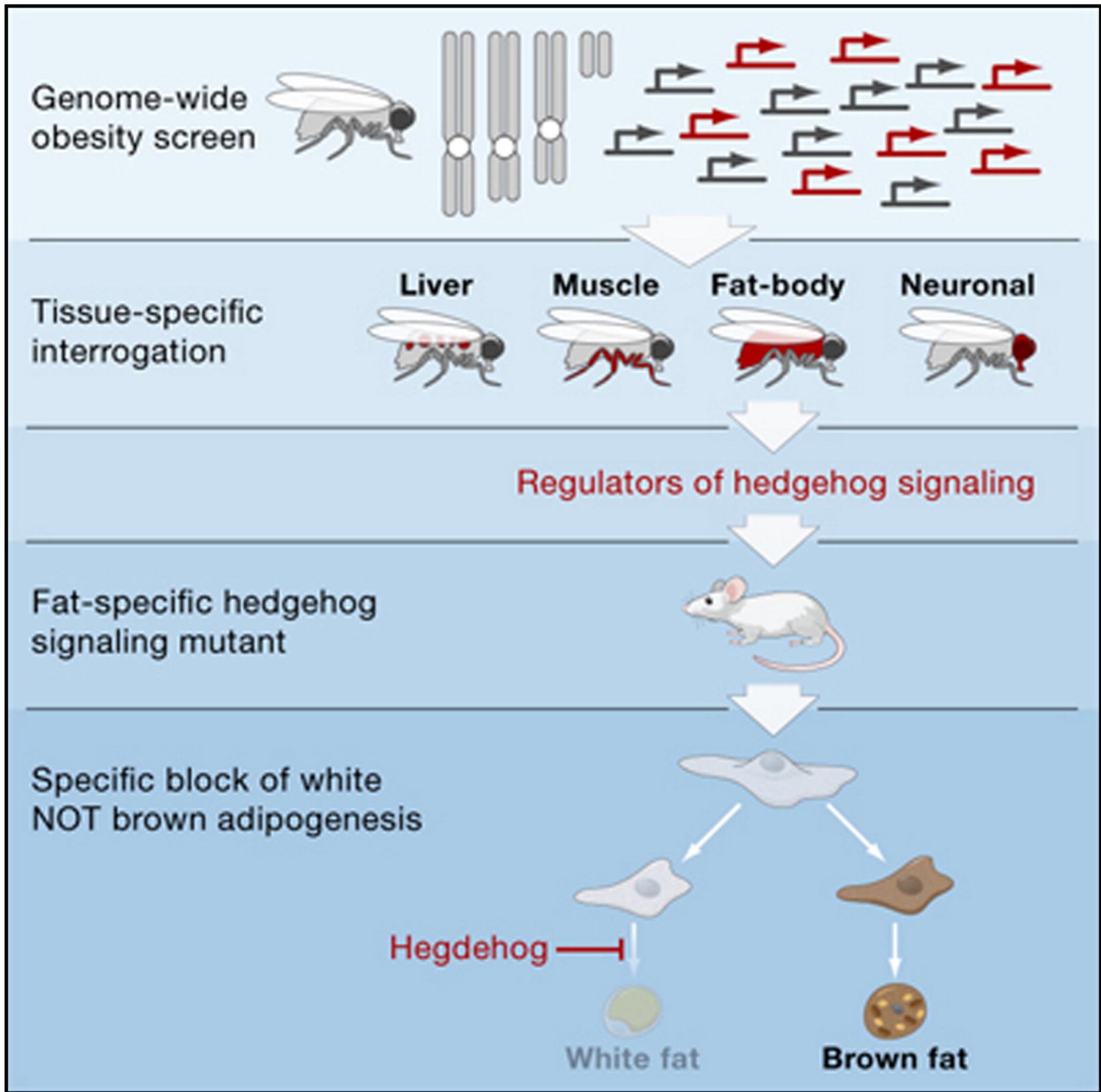
J. Andrew Pospisilik,^{1,12,13} Daniel Schramek,^{1,12} Harald Schnidar,² Shane J.F. Cronin,¹ Nadine T. Nehme,³ Xiaoyun Zhang,⁴ Claude Knauf,⁵ Patrice D. Cani,⁶ Karin Aumayr,¹ Jelena Todoric,⁹ Martina Bayer,⁹ Arvand Haschemi,⁹ Vijitha Puviindran,⁴ Krisztina Tar,⁹ Michael Orthofer,¹ G. Gregory Neely,¹ Georg Dietzl,⁷ Armen Manoukian,⁸ Martin Funovics,¹⁰ Gerhard Prager,¹¹ Oswald Wagner,⁹ Dominique Ferrandon,³ Fritz Aberger,² Chi-chung Hui,⁴ Harald Esterbauer,^{9,*} and Josef M. Penninger^{1,*}



What was the goal of the study?

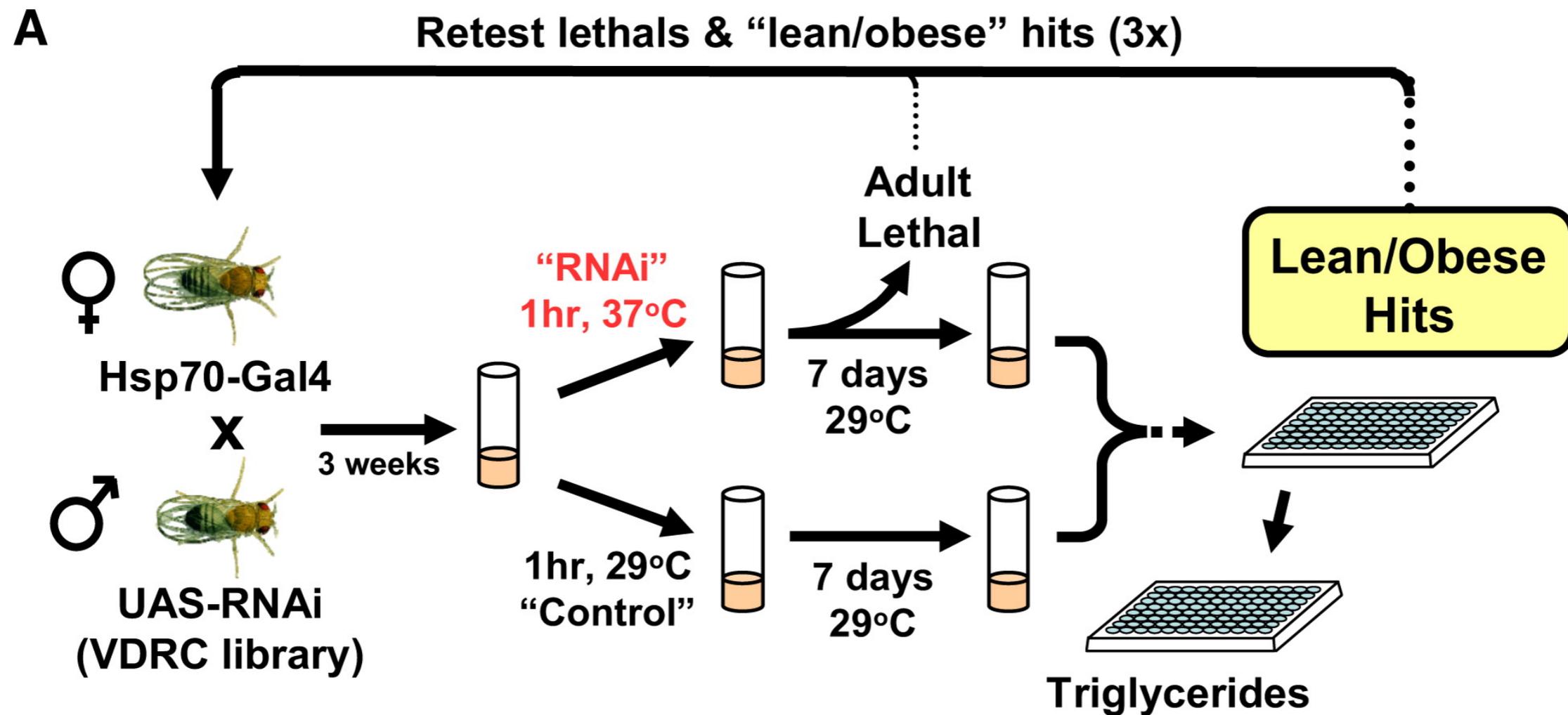
Obesity

To identify candidate obesity genes



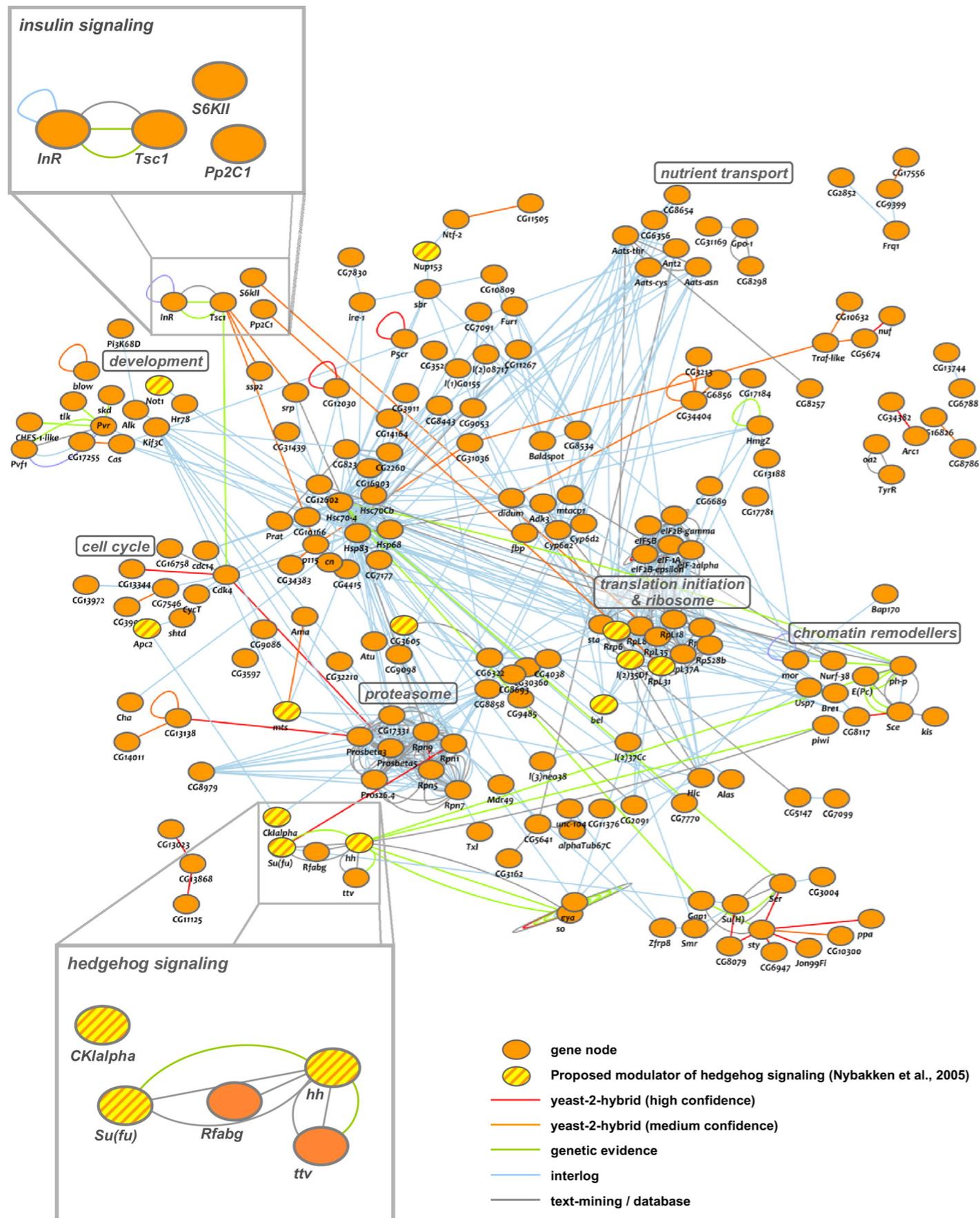
How did the experiment work?

How did they find candidate obesity genes?



They performed a genome-wide RNAi screen in *Drosophila*

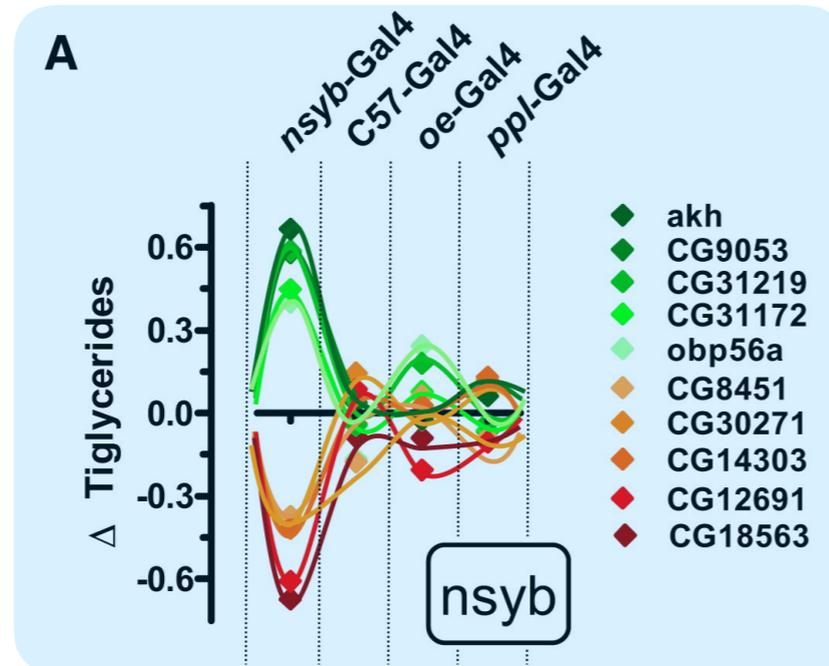
What did the genome-wide RNAi screen reveal?



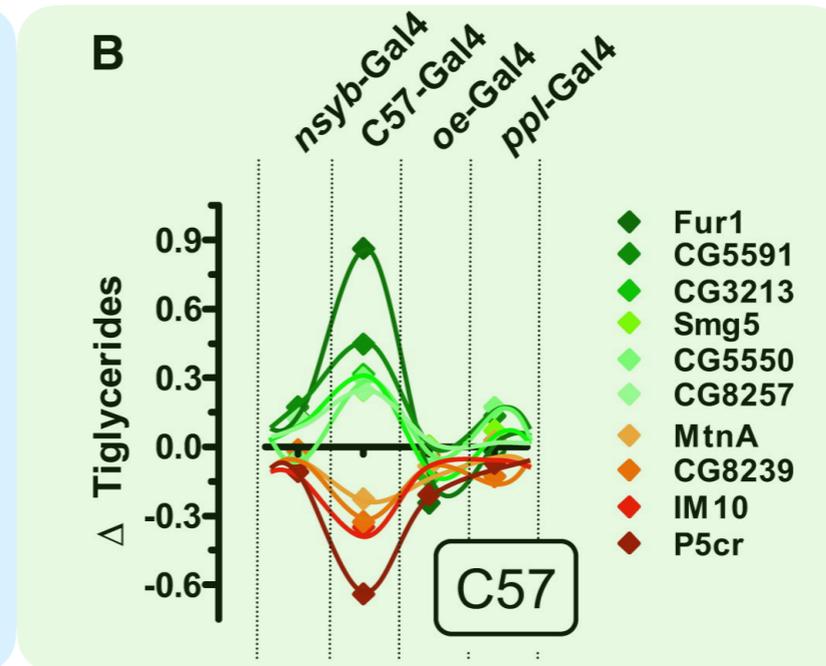
Many candidate genes not previously associated with obesity were found

What did they do next?

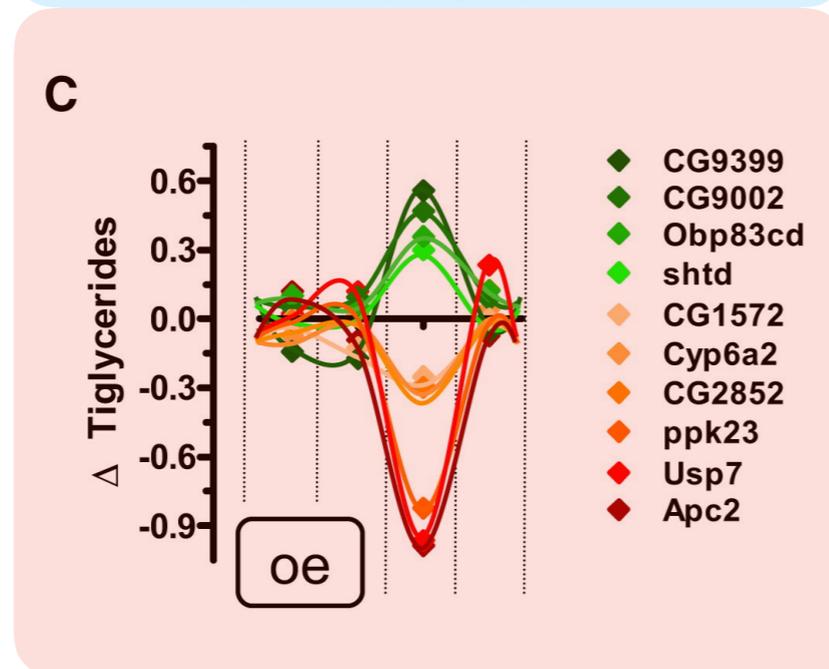
Neuronal



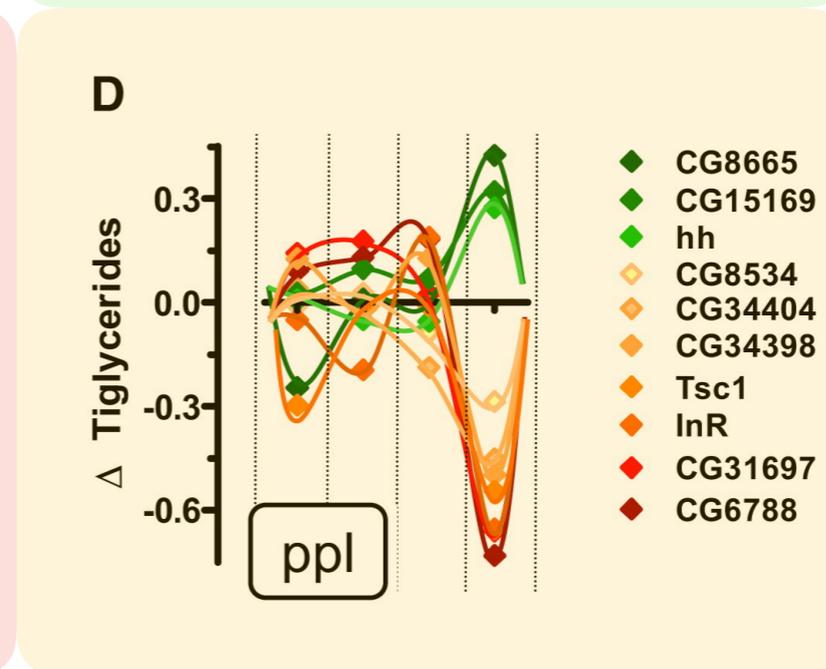
Muscle



Oenocyte



Fat-body

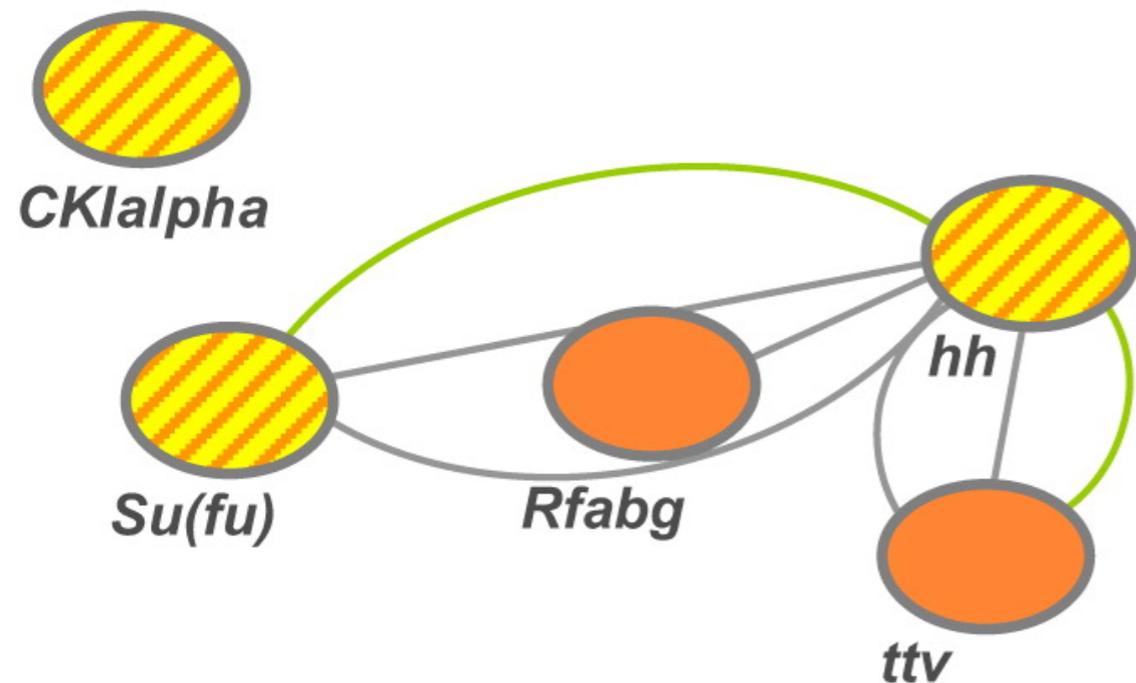


Considering the complexity of metabolism and diversity of tissue, they categorized lipid regulators by tissue specificity

What did gene ontology show?

What did gene ontology show?

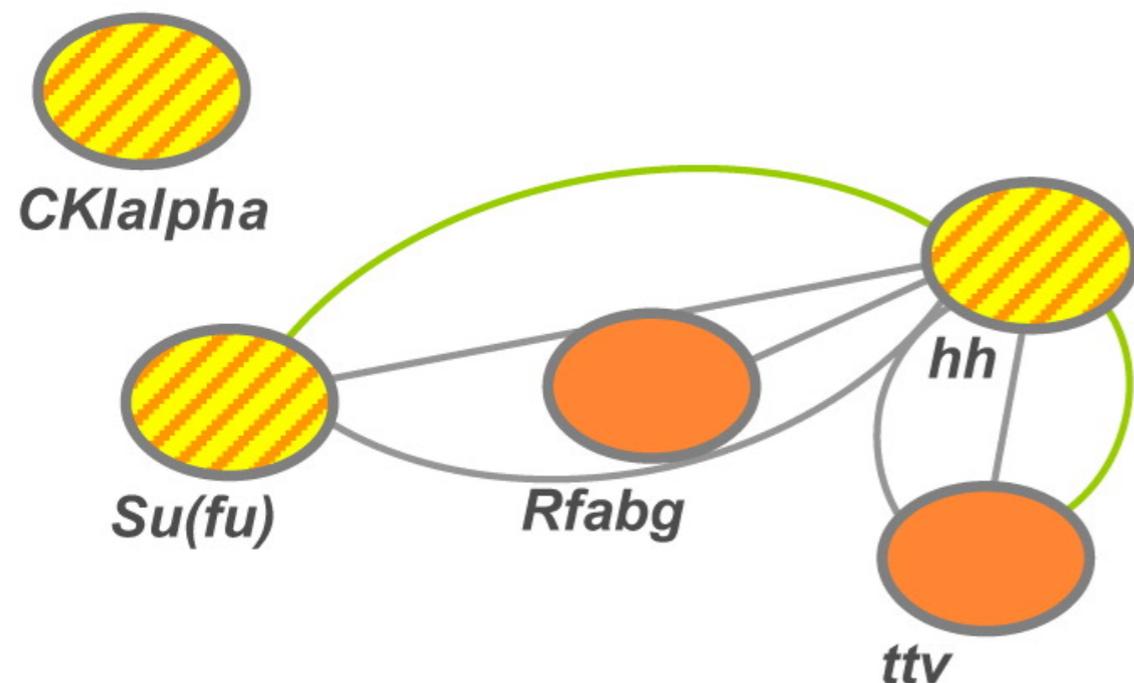
hedgehog signaling



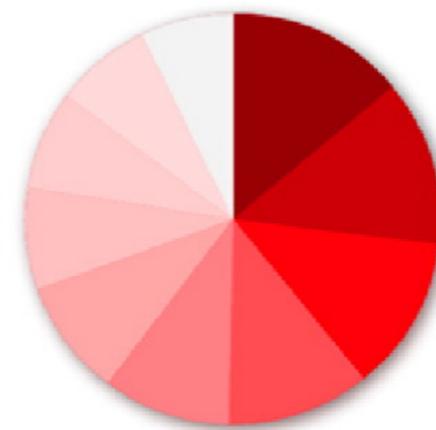
Hedgehog signaling was the top-scoring pathway in the primary screen

What did gene ontology show?

hedgehog signaling



fat-body

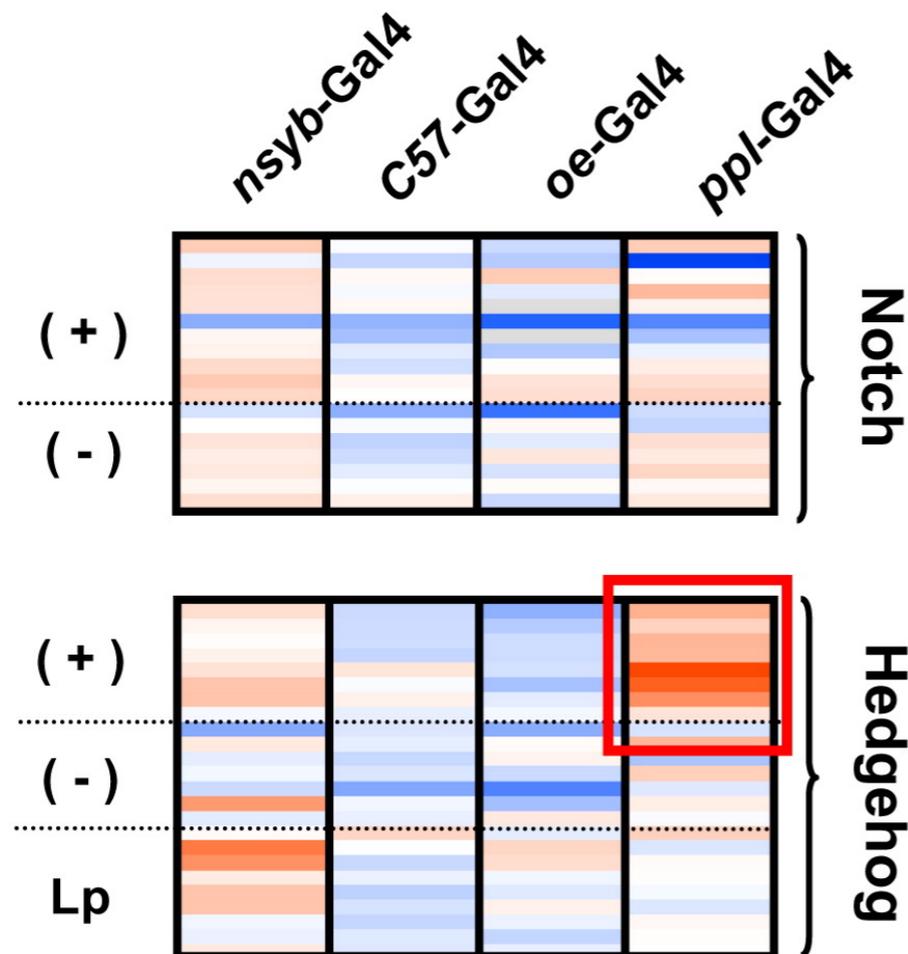


- imaginal disc lineage restriction
- stalk formation (Insecta)
- **reg. of smoothed signaling**
- response to nutrients
- neg. regulation of cell fate
- germ-band shortening
- regulation of organ size
- germ cell migration
- hematopoiesis
- epithelial cell migration

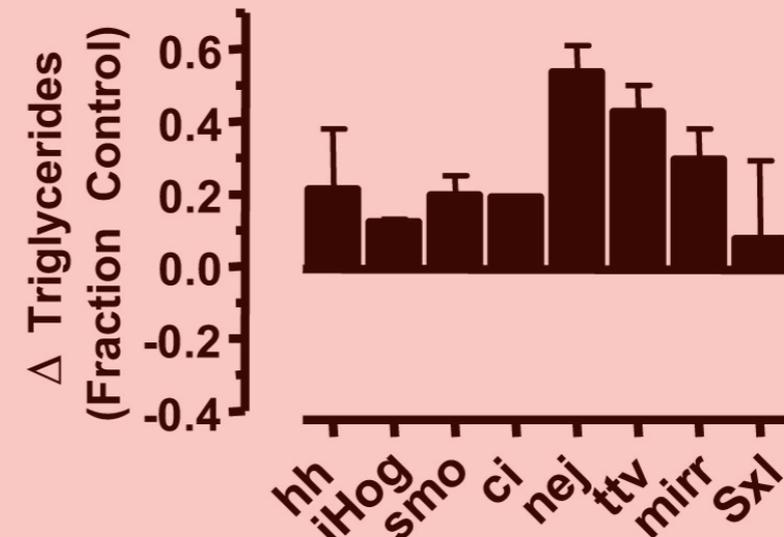
Hedgehog signaling was the top-scoring pathway in the primary screen and **third for fat-body-specific responsive pathways**

How does Hedgehog signaling impact triglyceride content?

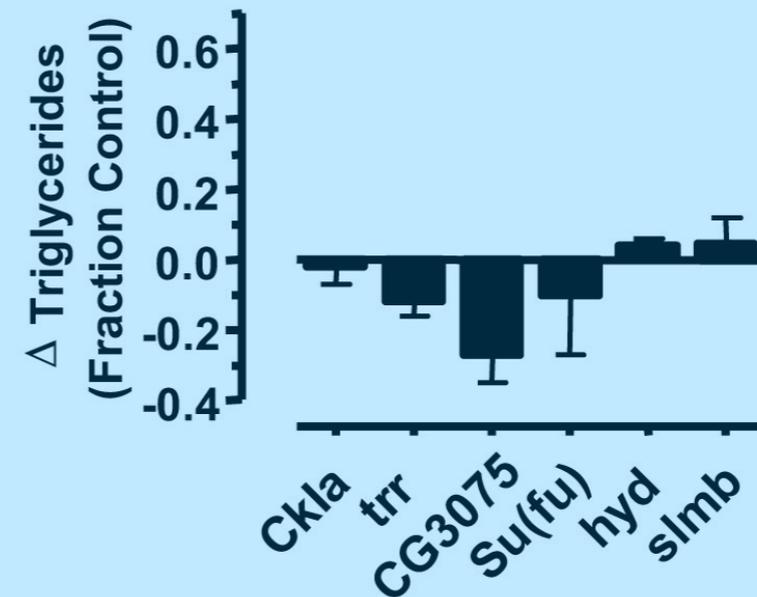
E



F

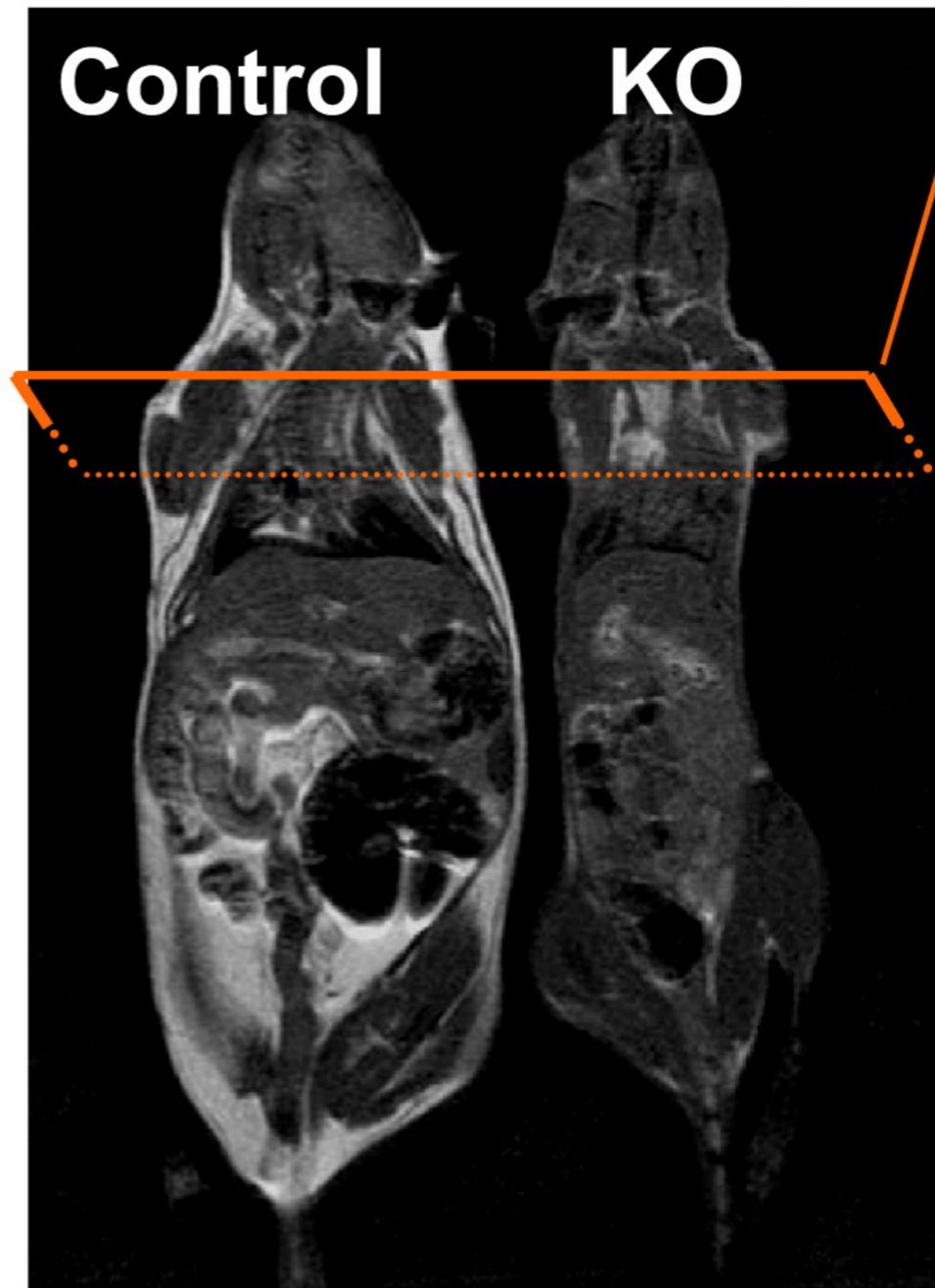


G



KD of Hedgehog activating factors resulted in **increased triglycerides**
KD of Hedgehog inhibiting factors resulted in **decreased triglycerides**

How was the Hedgehog pathway studied?



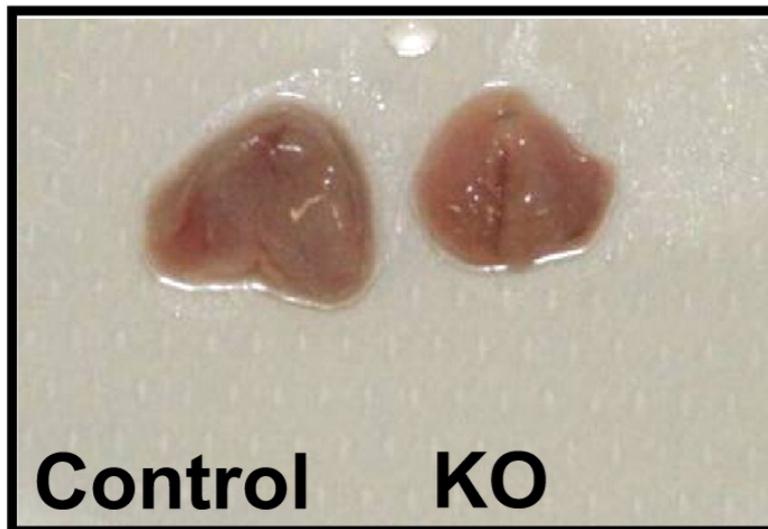
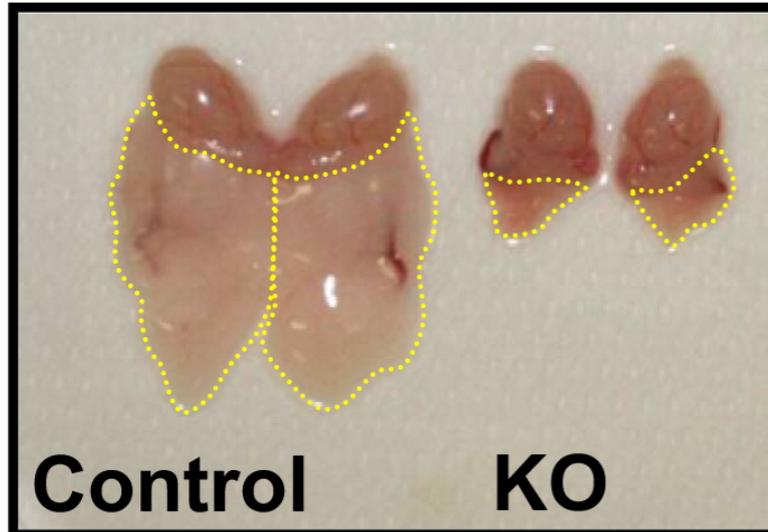
Sufu knock-out mice were used

What did the study reveal about adipose tissue?

Decreased WAT cell size & number, but normal BAT

What did the study reveal about adipose tissue?

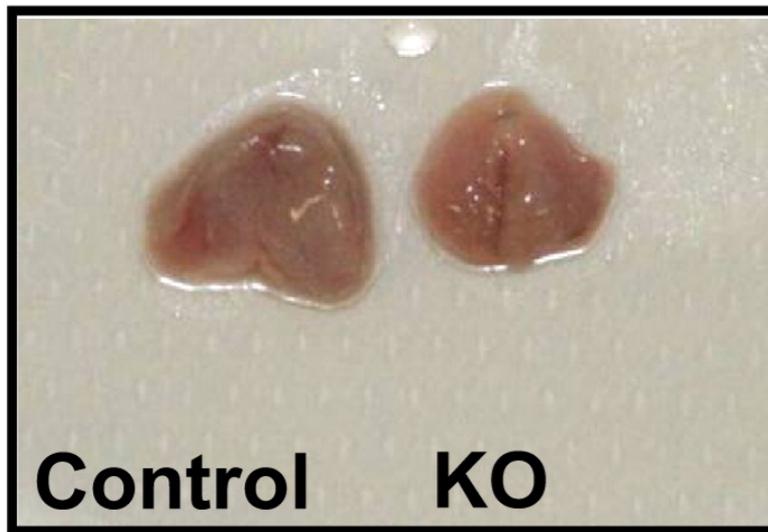
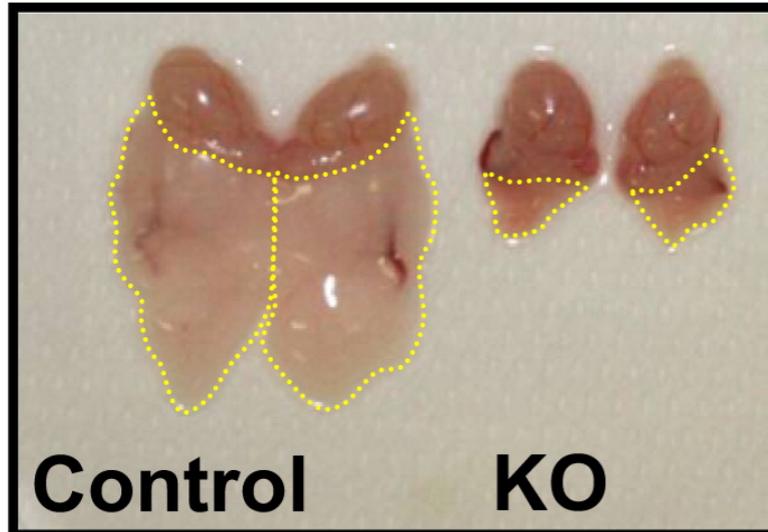
E



Decreased WAT cell size & number, but normal BAT

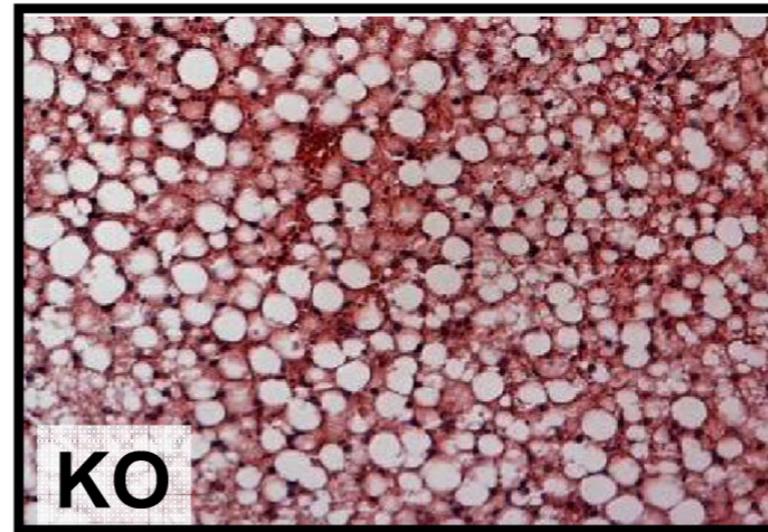
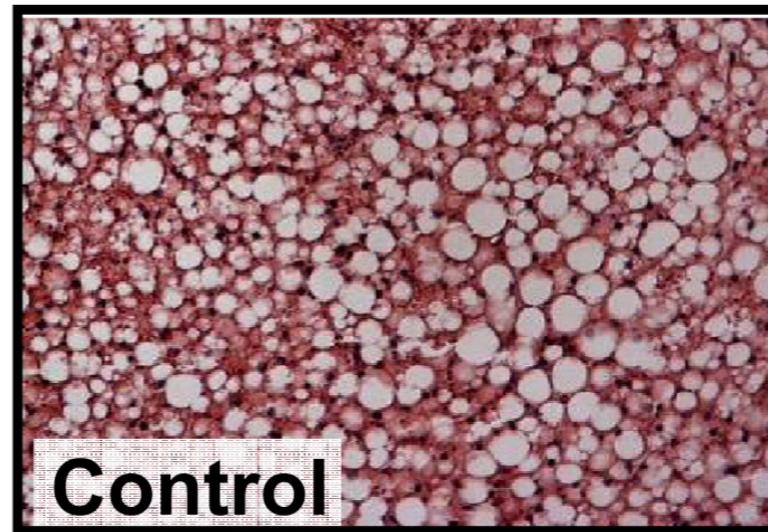
What did the study reveal about adipose tissue?

E



F

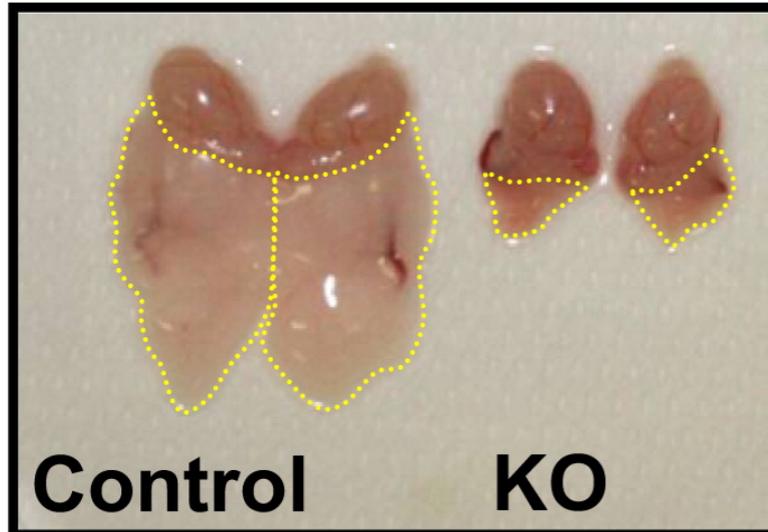
BAT



Decreased WAT cell size & number, but normal BAT

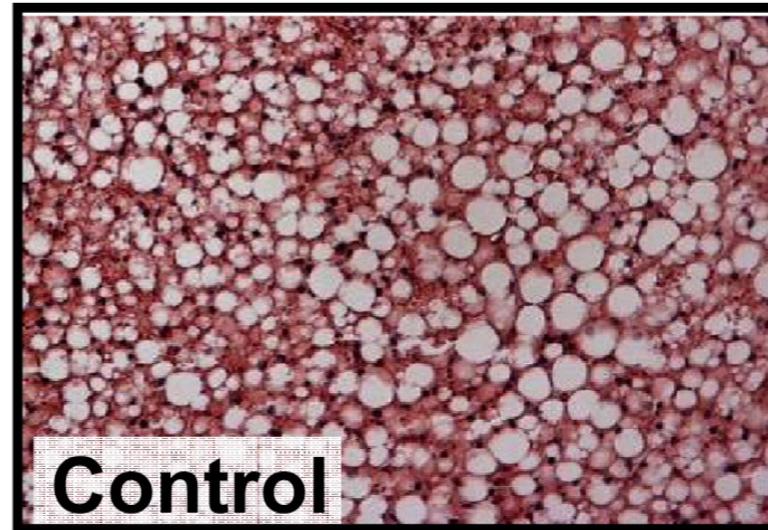
What did the study reveal about adipose tissue?

E



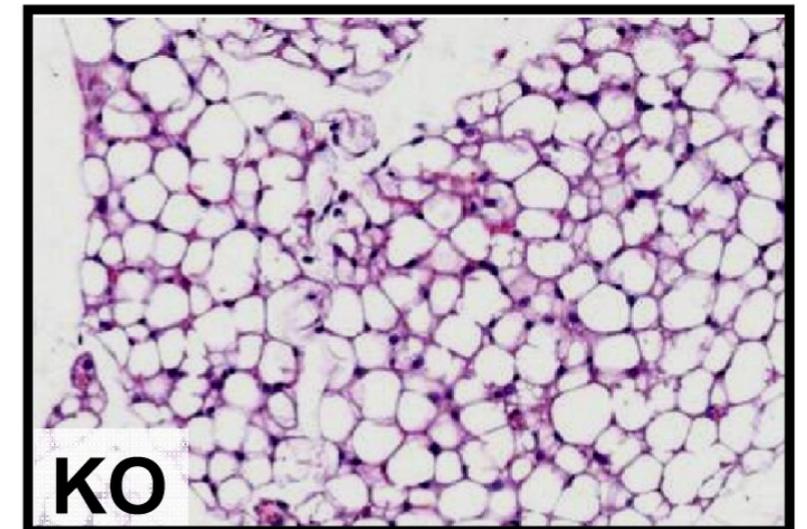
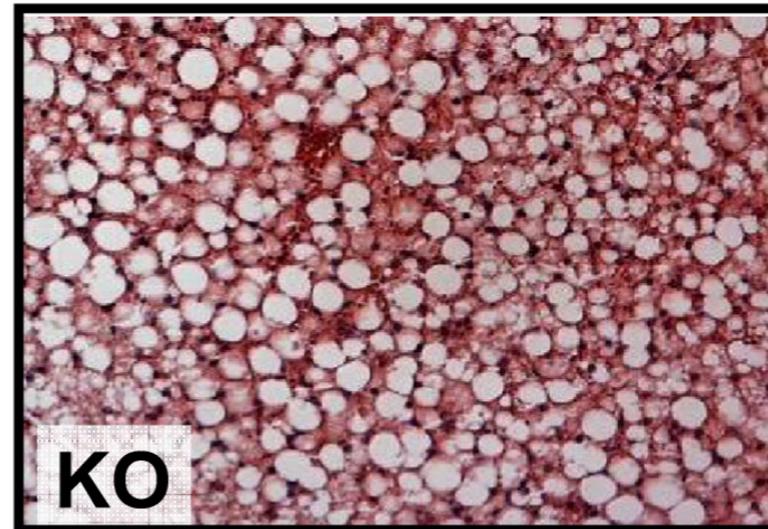
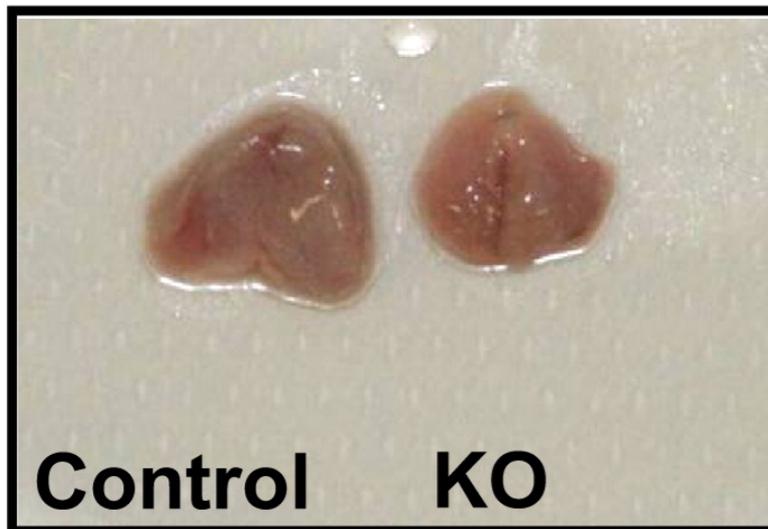
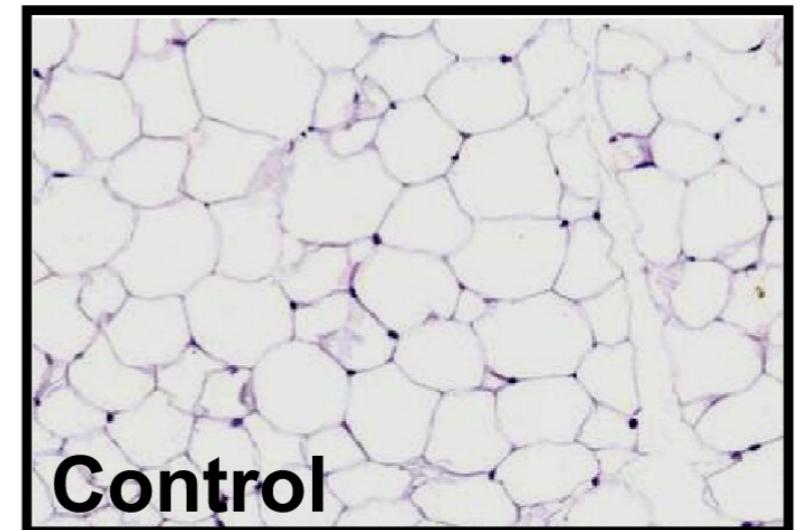
F

BAT



G

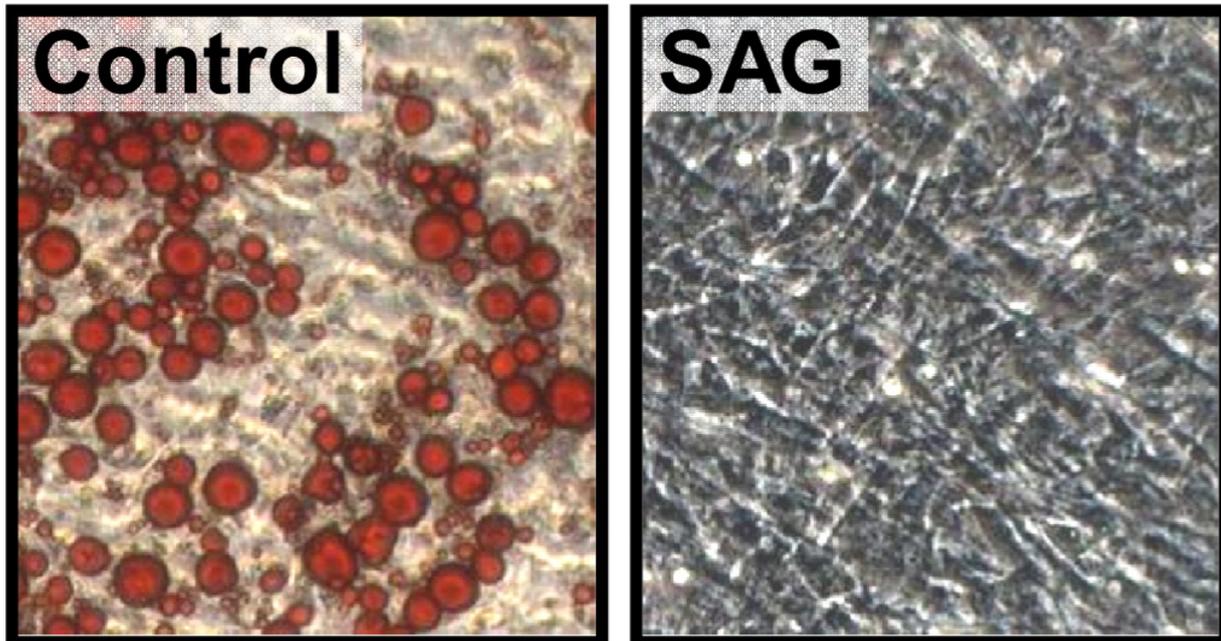
WAT



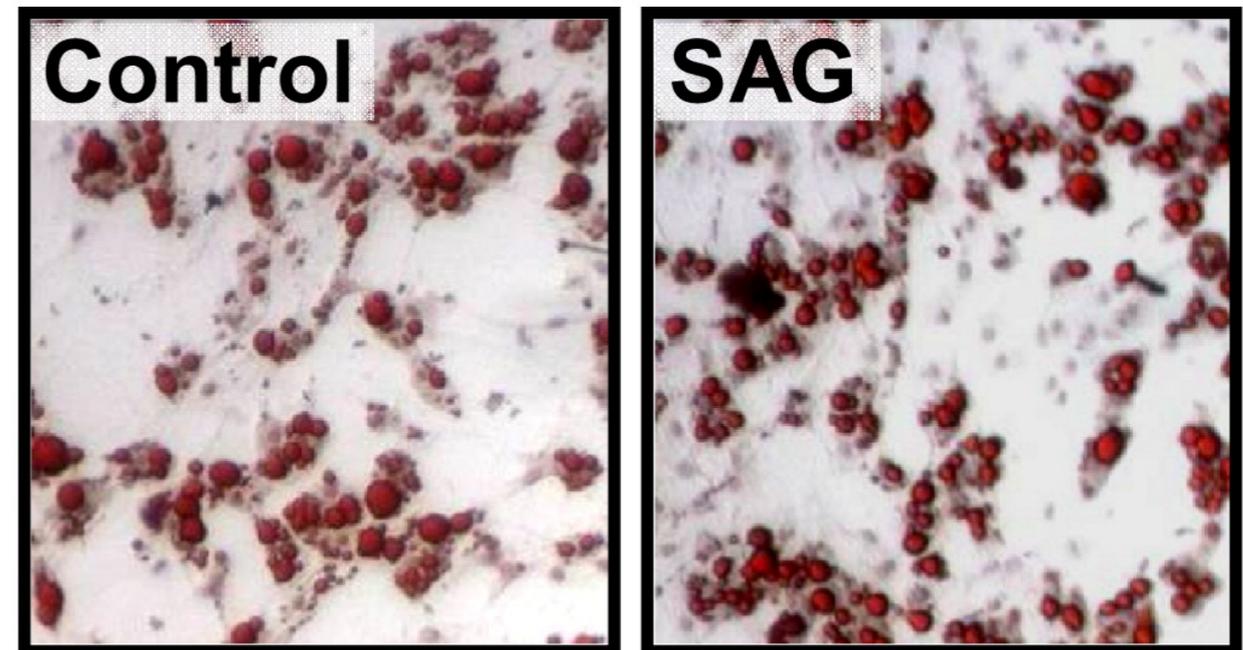
Decreased WAT cell size & number, but normal BAT

What did they do next?

WAT



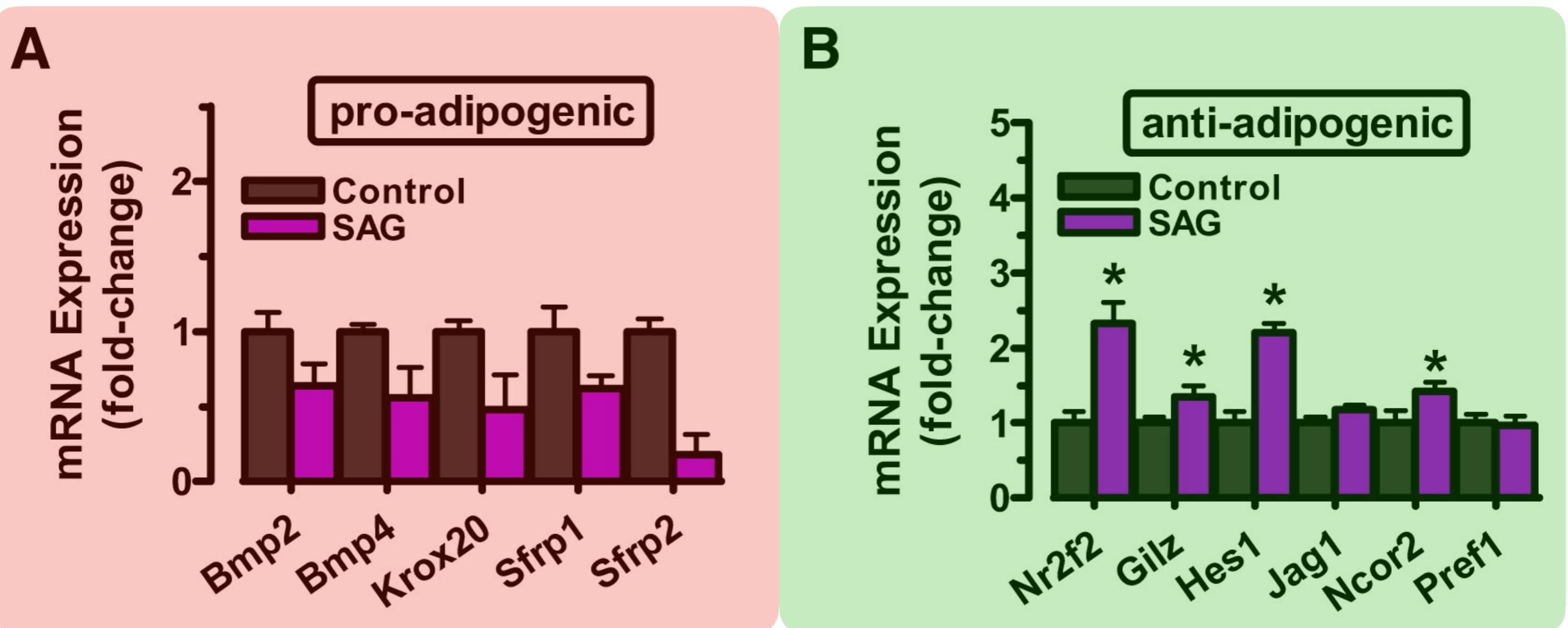
BAT



Stimulating SAG* in vitro blocked adipogenesis of WAT but not BAT

*SAG=Smoothened agonist

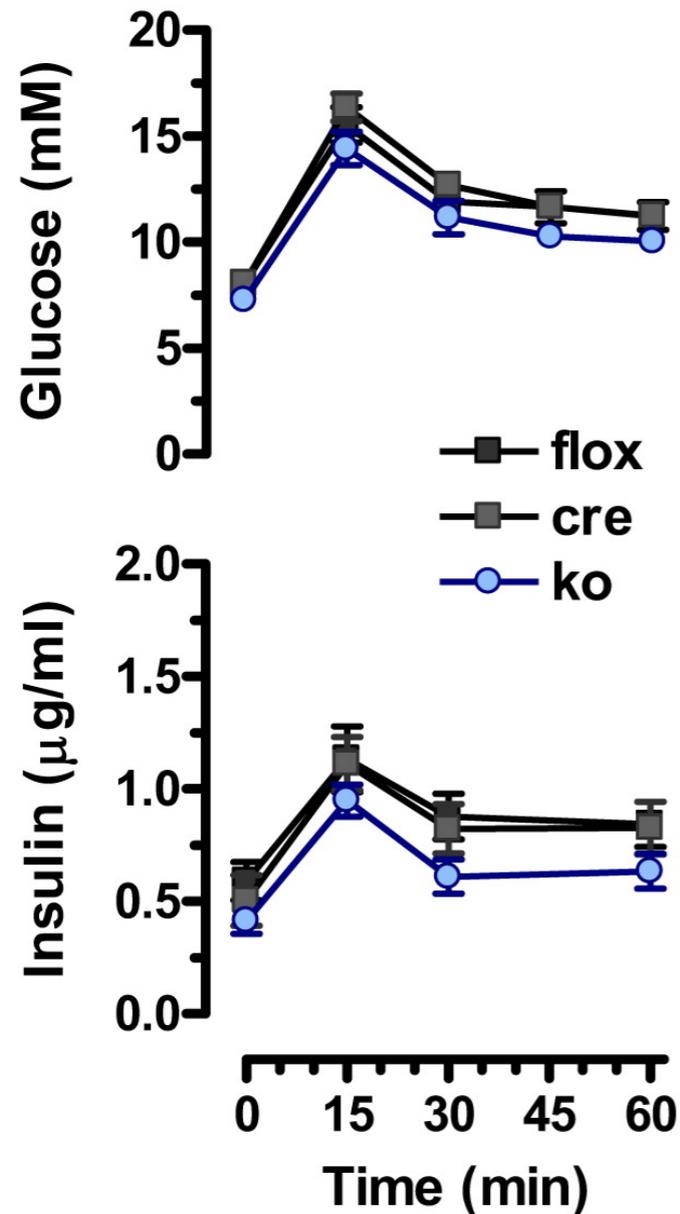
What effect does Hedgehog activation have on adipogenic factors?



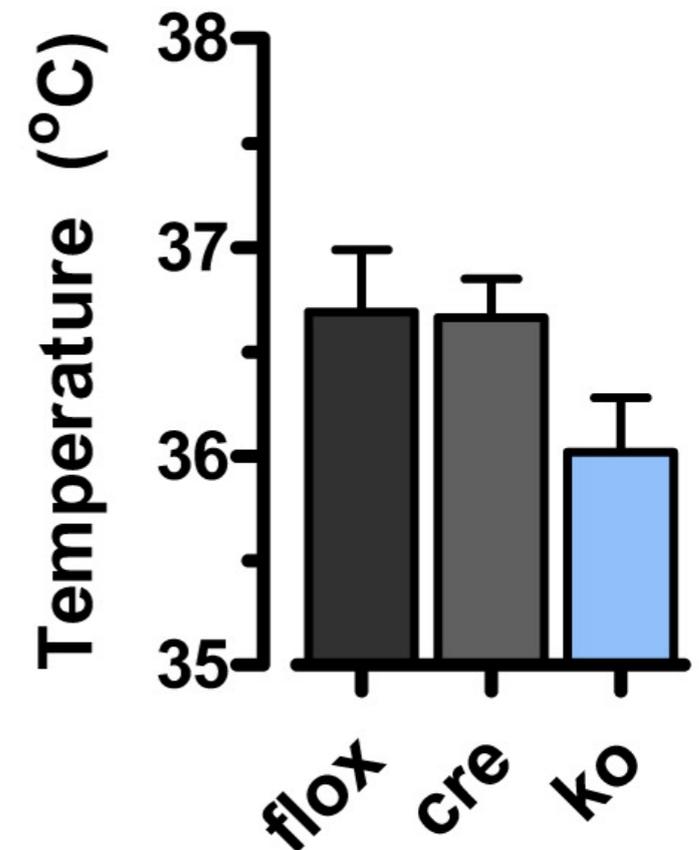
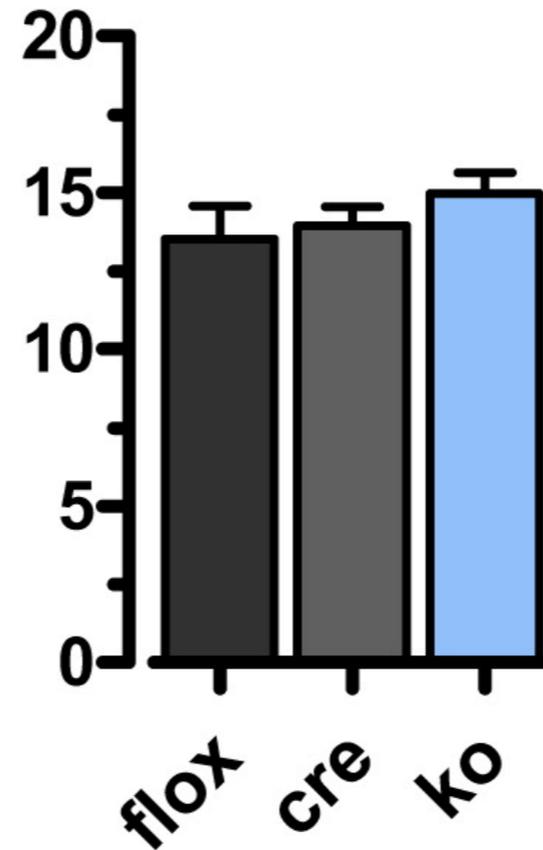
Downregulation of pro-adipogenic factors

Upregulation of anti-adipogenic factors

***Are there metabolic consequences as a result of WAT loss?**

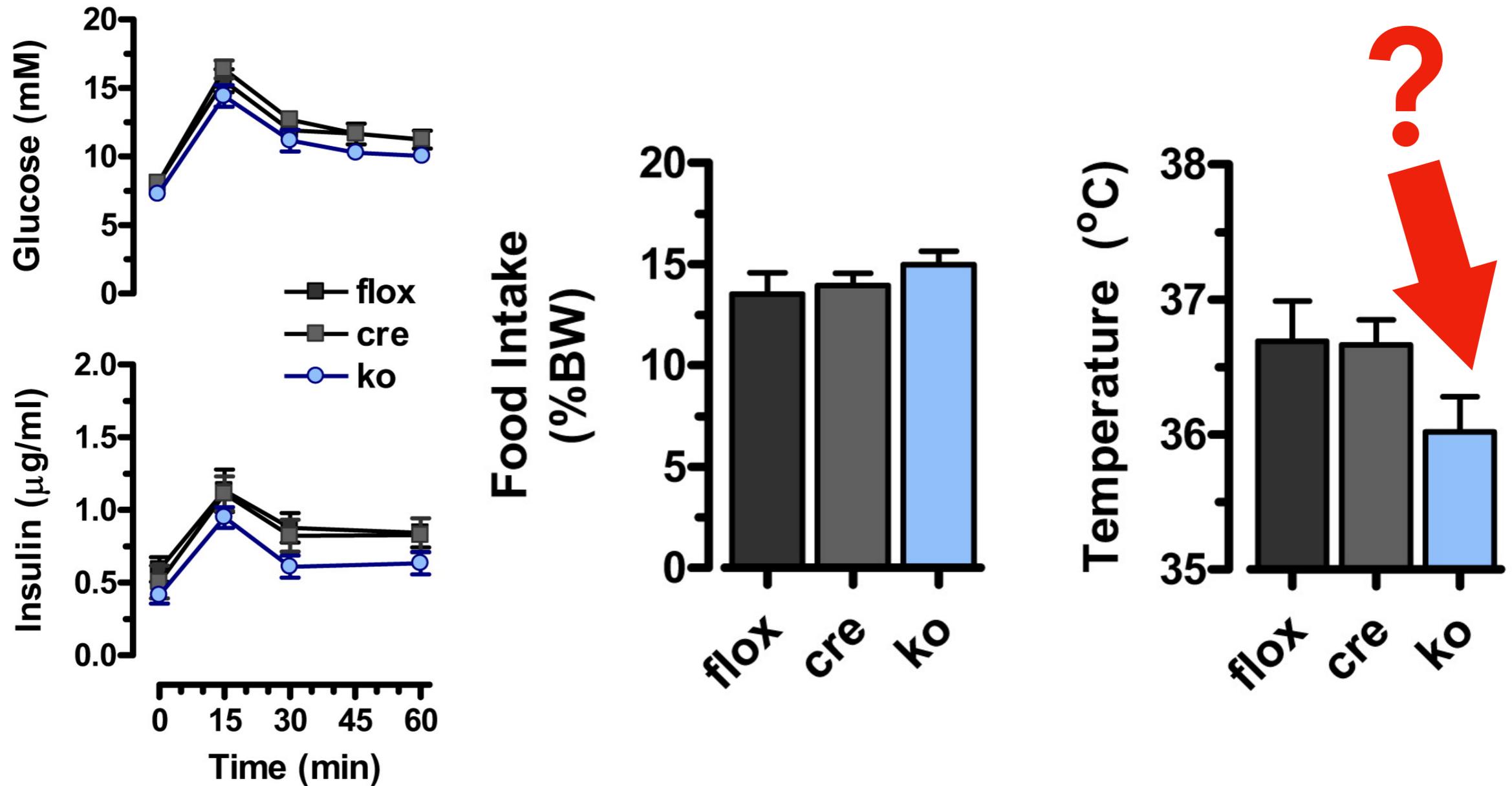


**Food Intake
(%BW)**



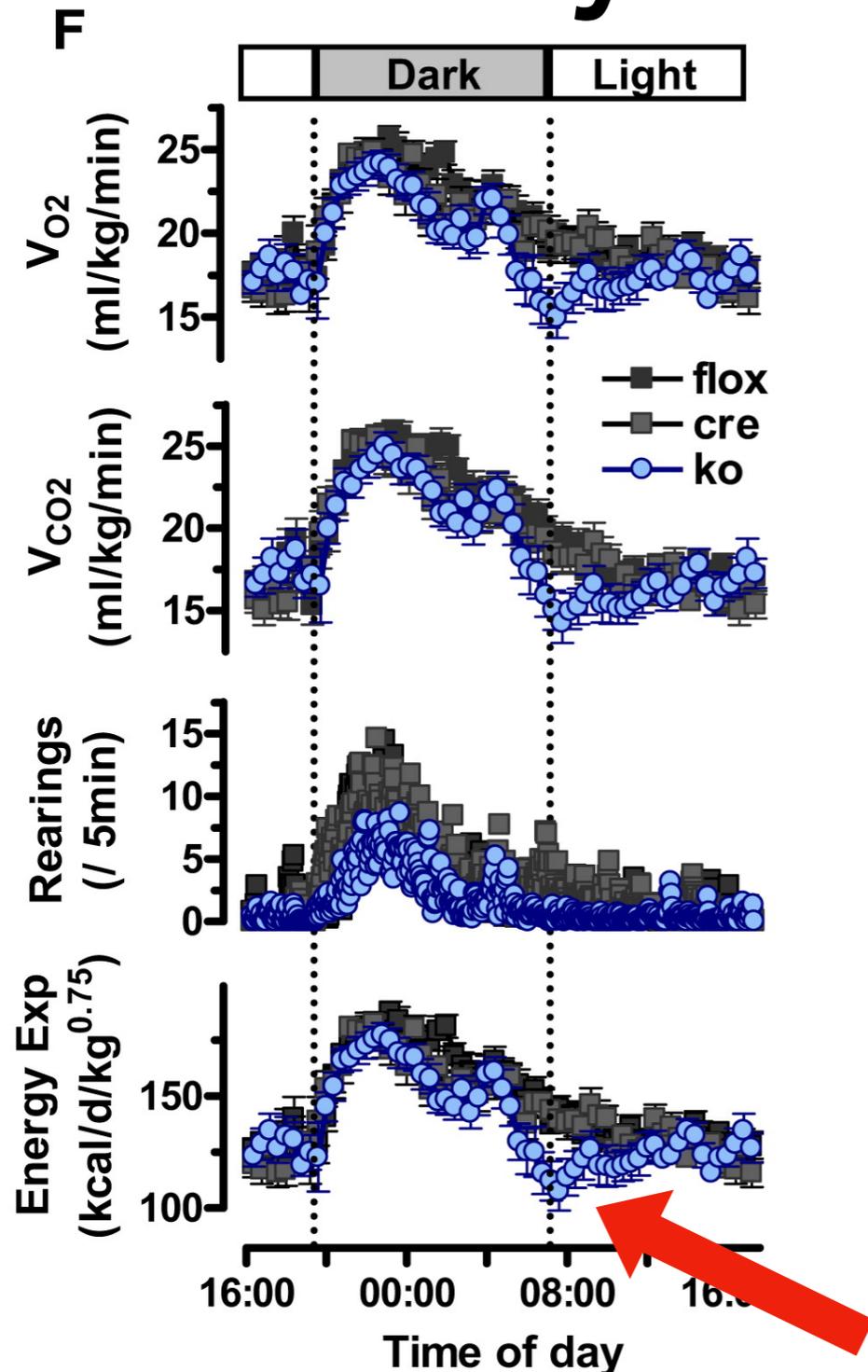
Normal levels of glucose homeostasis & food intake

Are there metabolic consequences as a result of WAT loss?

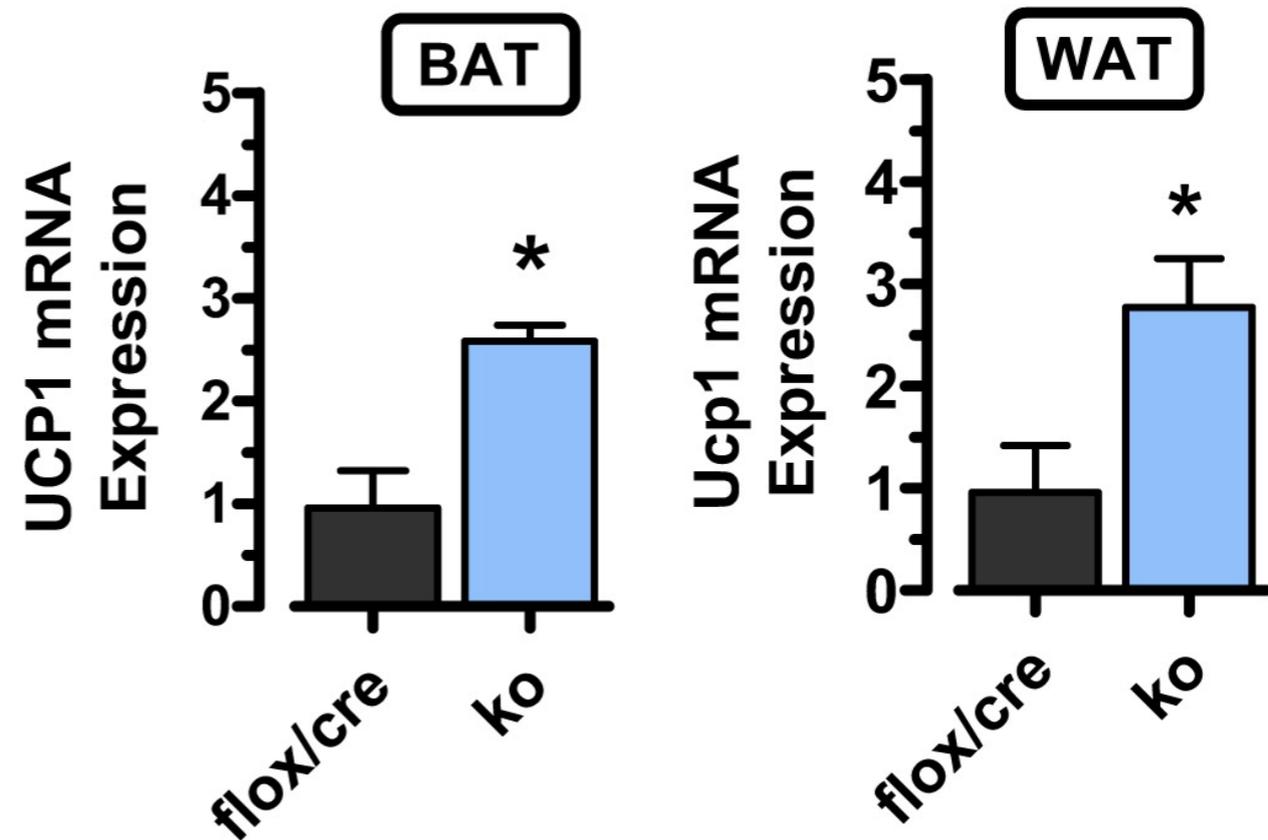


Normal levels of glucose homeostasis & food intake

Why the temperature drop?

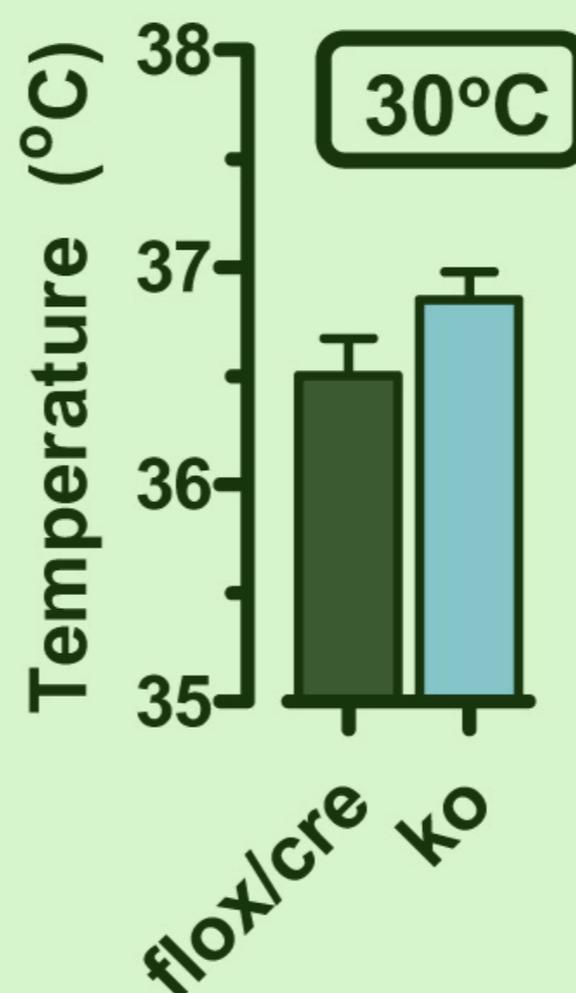
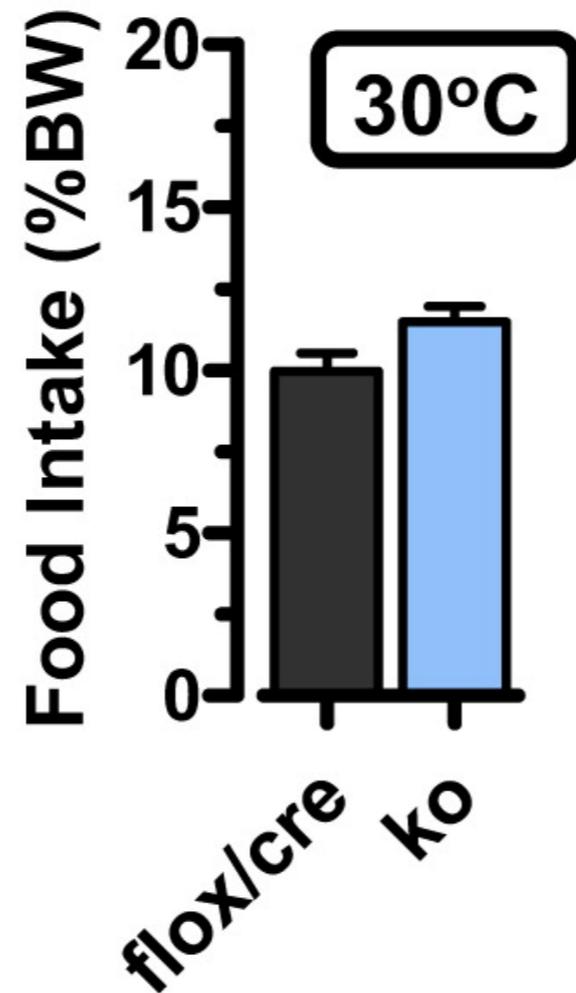


H



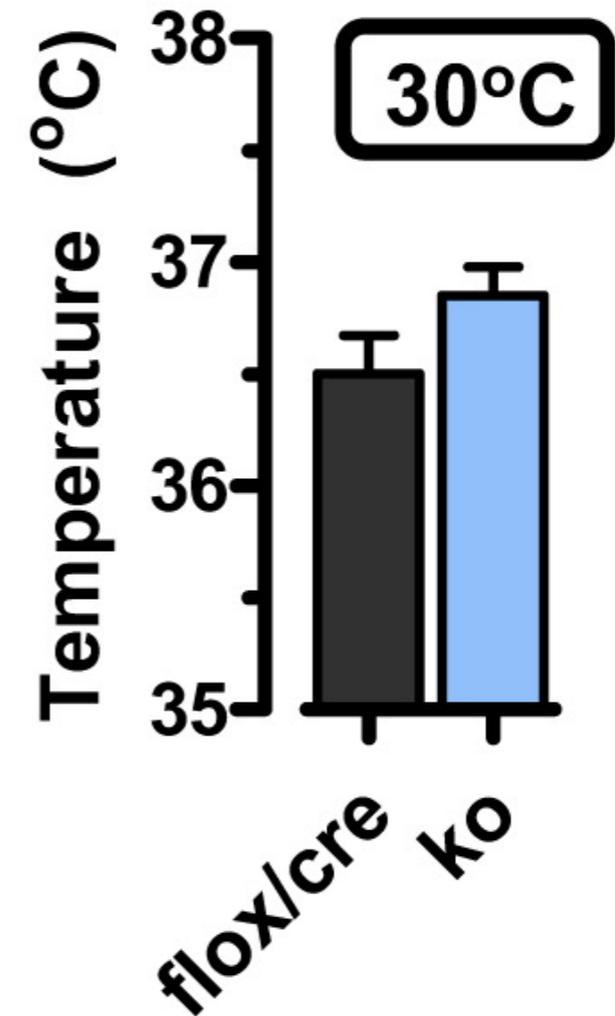
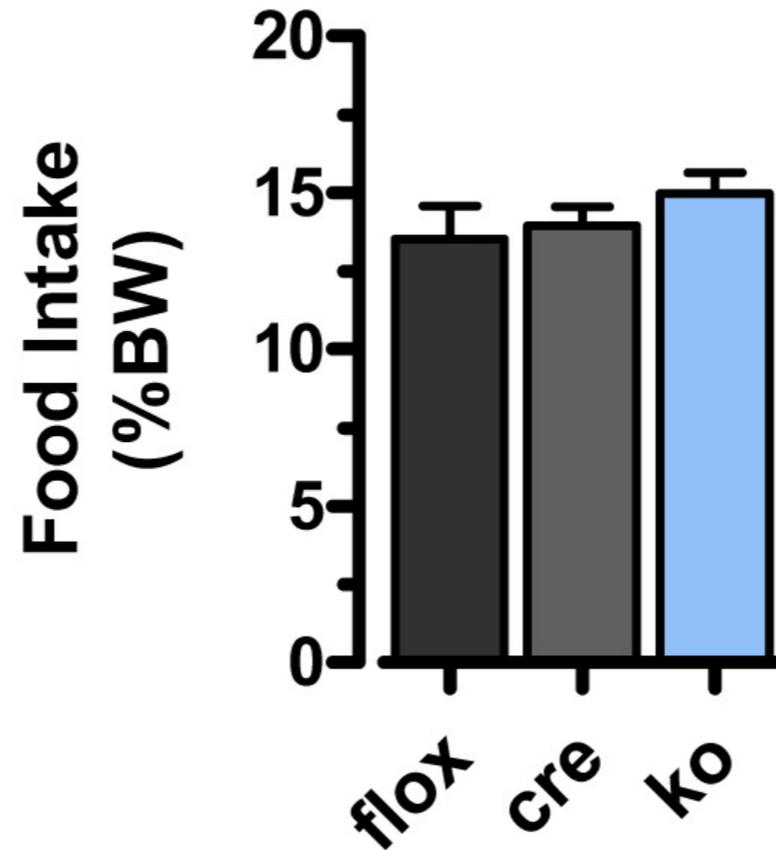
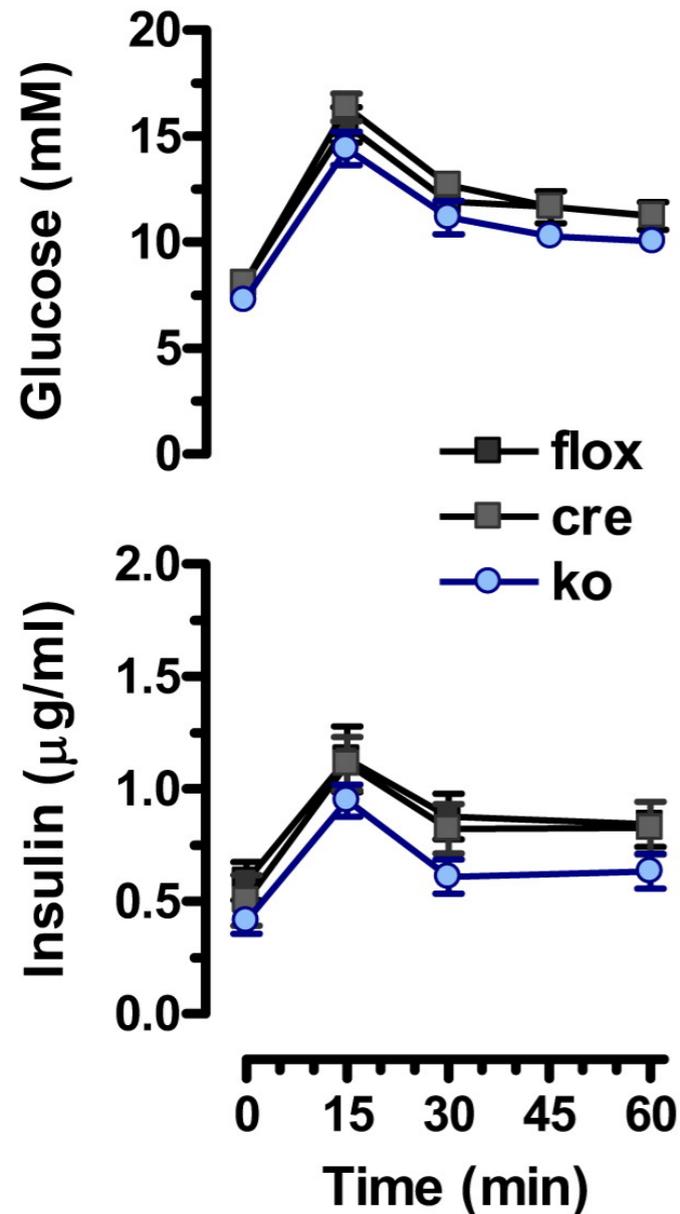
KO mice show **reduced activity** during dark cycle and **increased thermogenesis** suggesting thermal stress

What happened when the mice were kept at 30°C instead of 22°C?



Body temperature normalized after 1 day at 30°C suggesting energy expenditure on thermal regulation previously

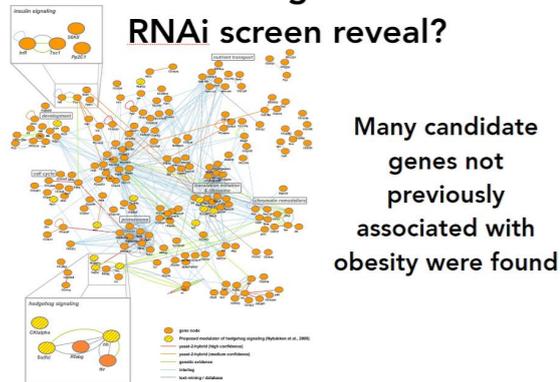
Are there metabolic consequences as a result of WAT loss? (take 2)



Normal levels of glucose homeostasis & energy expenditure

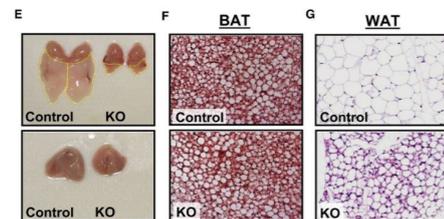
Summary

What did the genome-wide RNAi screen reveal?



RNAi screening revealed candidate obesity genes

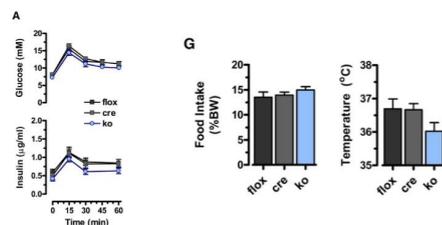
What did the study reveal about adipose tissue?



Decreased WAT cell size & number, but normal BAT

Activation of the Hedgehog pathway blocks WAT cell differentiation

Are there metabolic consequences as a result of WAT loss?



Normal levels of glucose homeostasis & energy expenditure

Sufu knock-out mice exhibit normal glucose tolerance & energy expenditure

Questions?



References

<https://en.hdbuzz.net/023>
[https://b110wiki.dkfz.de/confluence/display/rnaiwiki/RNA+interference+\(RNAi\)+screening-](https://b110wiki.dkfz.de/confluence/display/rnaiwiki/RNA+interference+(RNAi)+screening-)
<http://alps3.gi.k.u-tokyo.ac.jp/~dscheck/main/help/>
https://www.researchgate.net/figure/BAT-contributes-to-energy-expenditure-Weight-gain-and-obesity-are-caused-by-chronic_fig1_26328482
<https://esmmweighless.com/preventable-cost-obesity/>
https://openi.nlm.nih.gov/detailedresult.php?img=PMC3219668_2045-3701-1-35-1&req=4
<https://www.umassmed.edu/guertinlab/research/adipocytes/>
<https://www.benchfly.com/blog/model-organism-week-drosophila-melanogaster-the-fruit-fly/>
[https://www.cell.com/trends/endocrinology-metabolism/fulltext/S1043-2760\(14\)00042-3](https://www.cell.com/trends/endocrinology-metabolism/fulltext/S1043-2760(14)00042-3)
<https://docplayer.net/7687975-Pathway-del-signaling-dell-insulina-invecchiamento-e-longevita-michelangelo-barbieri.html>
<http://molpharm.aspetjournals.org/content/87/2/174>
<http://genchi.info/scientific-dna-wallpapers-2017>
<http://www.infovisual.co/sonic-x-sonic-screenshots.html>

Pospisilik JA, et al *Drosophila* genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. *Cell*. 2010 Jan 8;140(1):148-60. doi: 10.1016/j.cell.2009.12.027.

Review: Mohr SE, Smith JA, Shamu CE, Neumüller RA, Perrimon N. RNAi screening comes of age: improved techniques and complementary approaches. *Nat Rev Mol Cell Biol*. 2014 Sep;15(9):591-600. doi: 10.1038/nrm3860.