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ANNUAL MEETING

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March 12, 2016

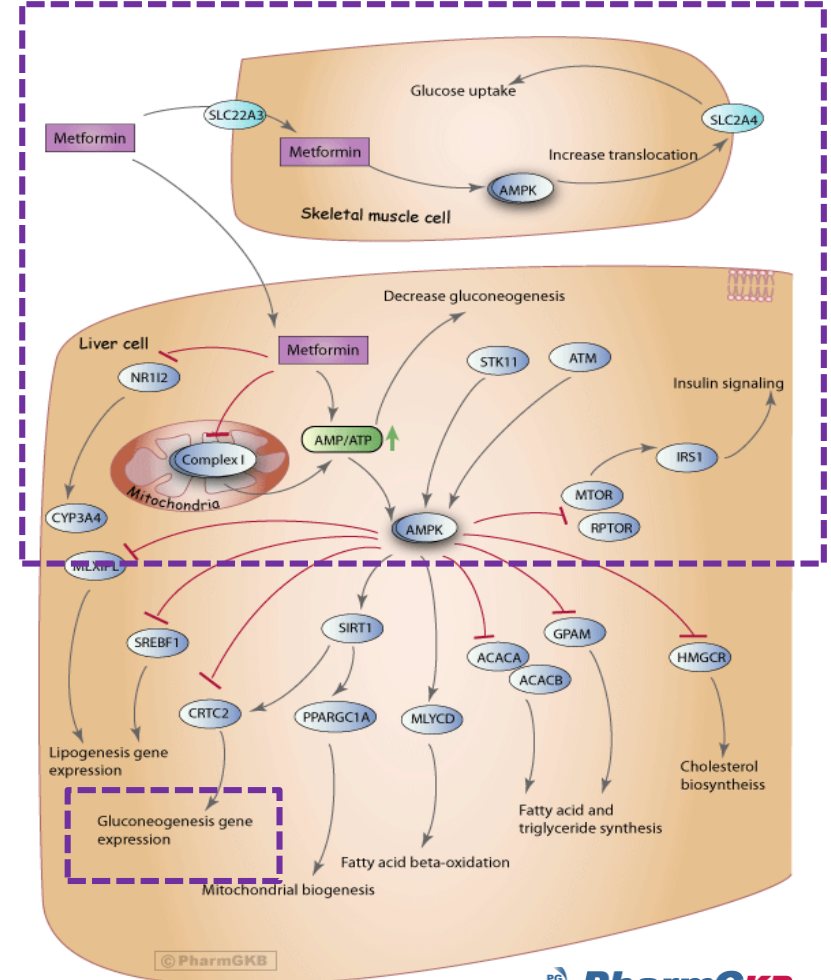
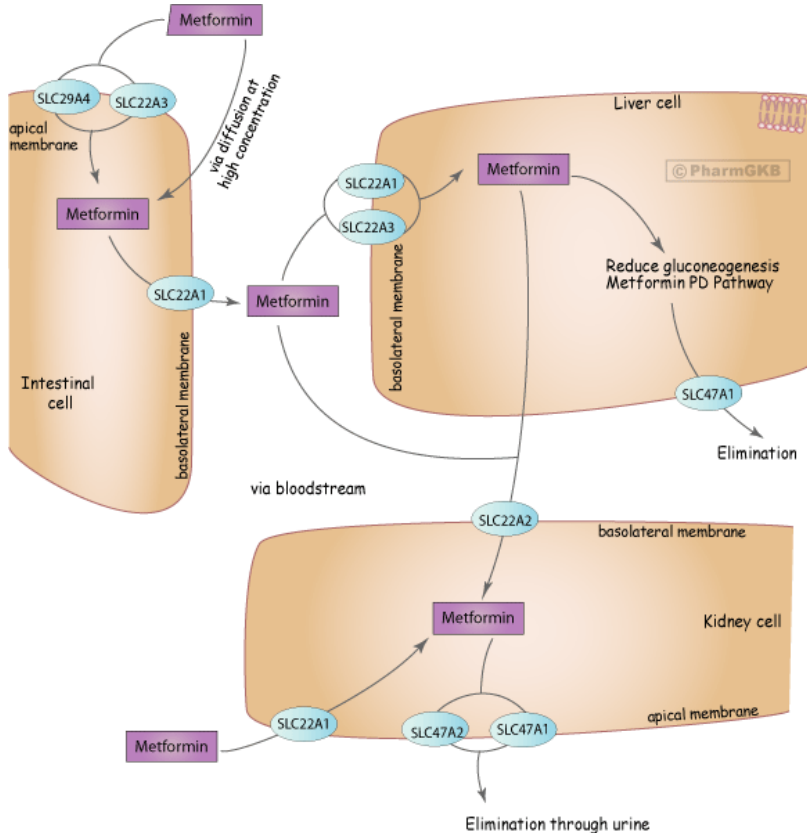
Session “Pharmacogenomics: From  
Discovery to Implementation”

# GENOMIC IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF METFORMIN-RESPONSIVE REGULATORY ELEMENTS

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R. P. Smith, L. Lin, S. Yee, K. M. Giacomini, N. Ahituv**

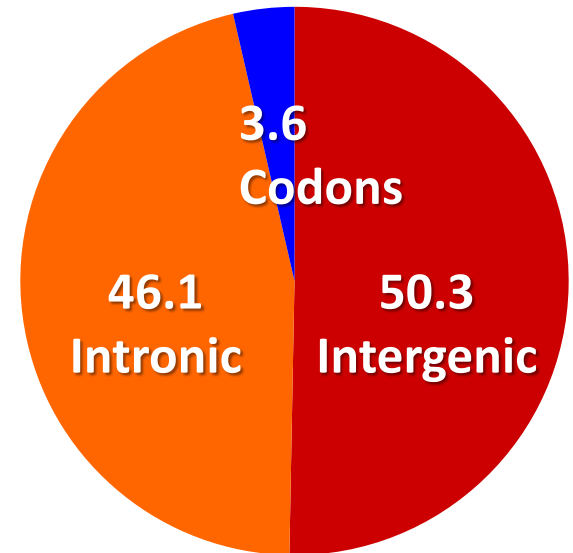
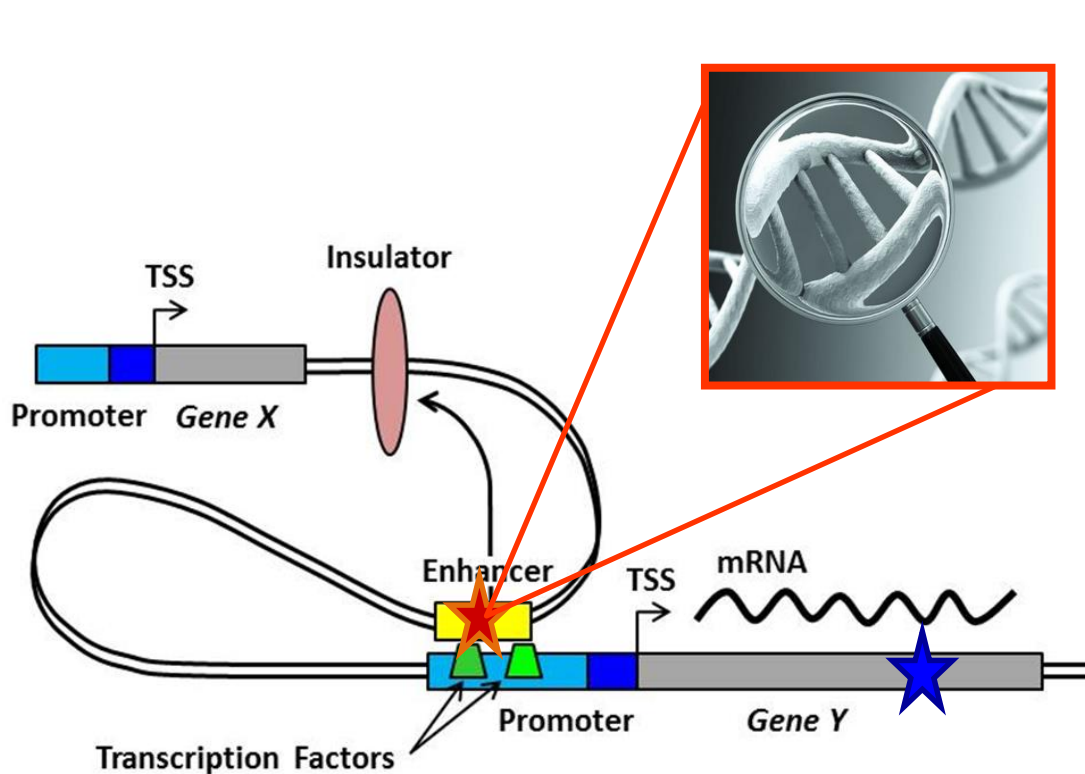
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# Metformin is the first-line therapy for Type 2 Diabetes, but its mechanisms of action in the liver are not fully known



Transporters are major determinants of the PK, and their genotypes explain part of the variance in metformin response

# Gene regulatory elements can have a major effect on interindividual differences in drug response



**Reviewed findings from  
108 Pharmacogenomic  
GWAS**

# Hypothesis

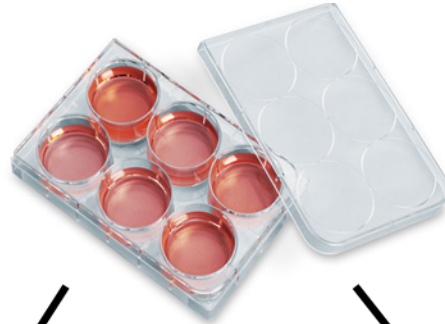
- Gene regulatory elements may explain a subset of the variation in metformin response.

# General AIM

- We carried out RNA-seq and ChIP-seq on human hepatocytes treated with and without metformin in order to better characterize the mechanisms of action of metformin.

# Methods

Primary human  
hepatocytes



Vehicle (Non-Treated), 8h

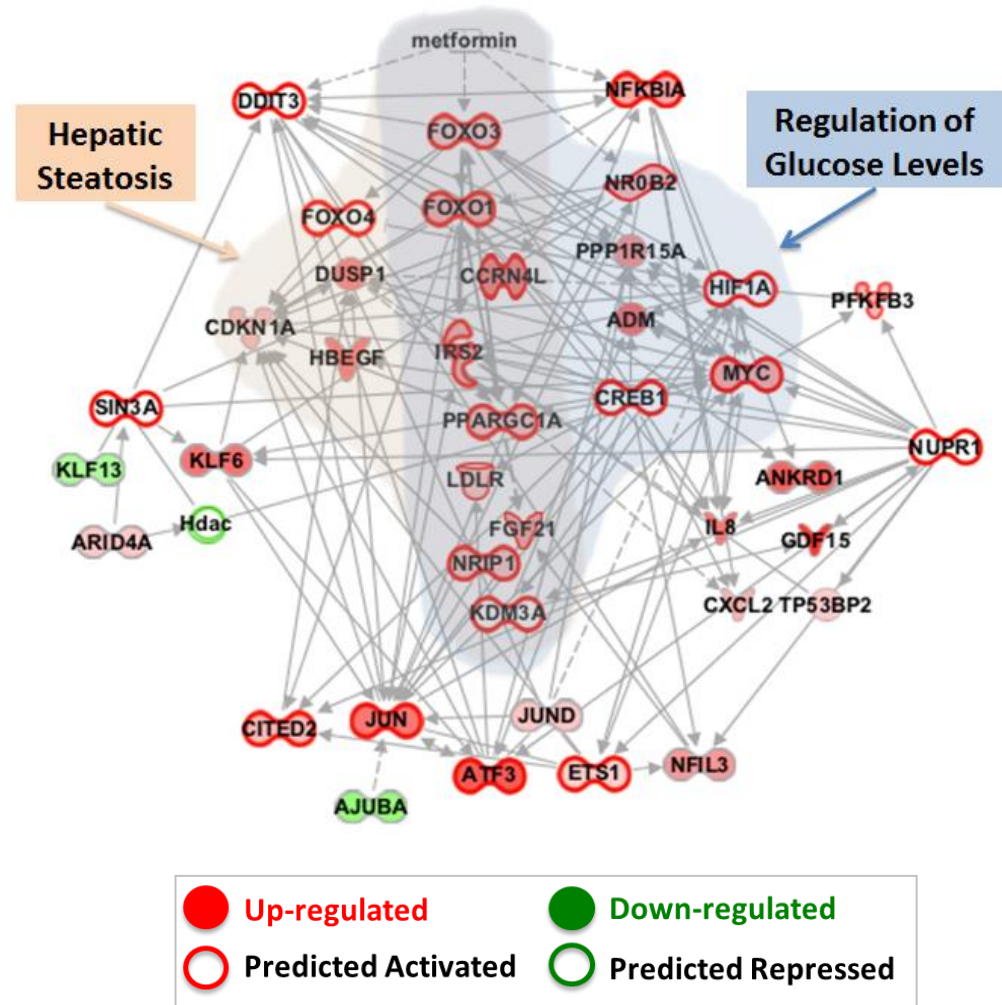
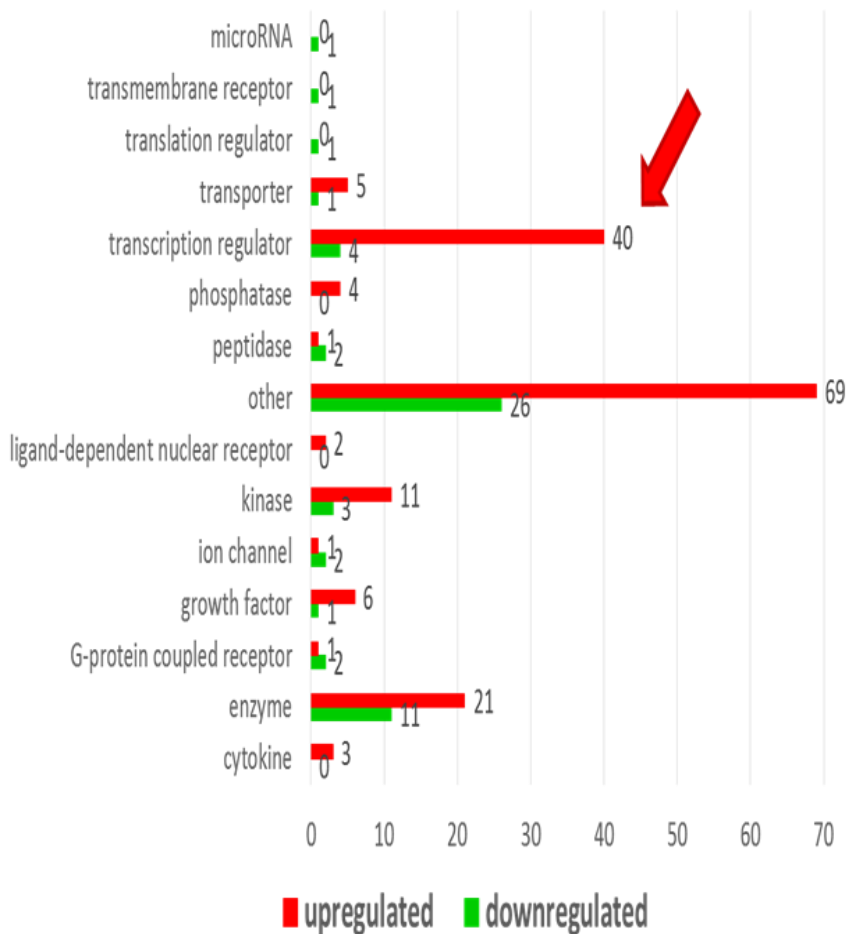
2.5mM Metformin, 8h

RNA-seq

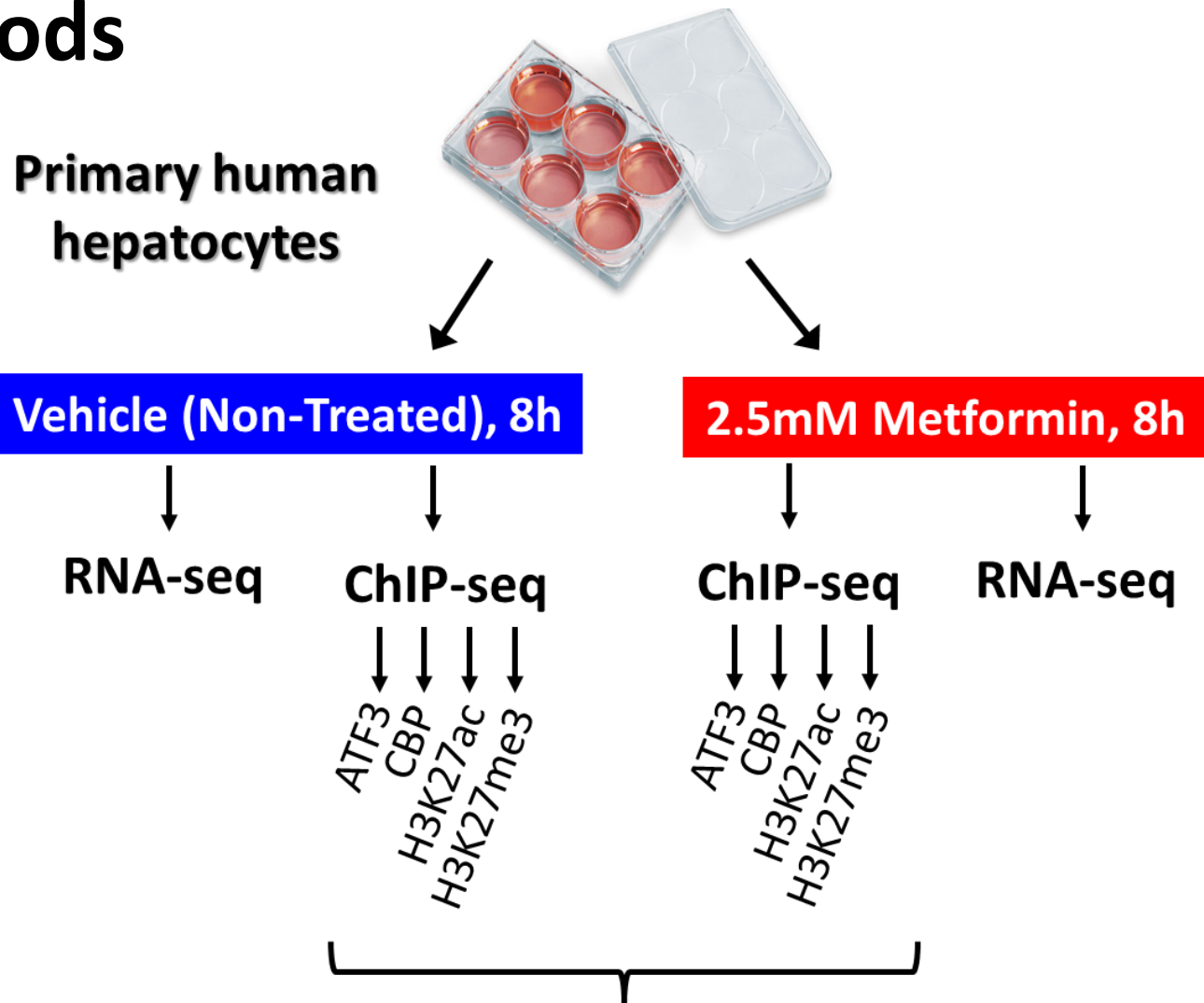
RNA-seq

# RNA-seq Results

We identified novel metformin-induced transcriptional regulators



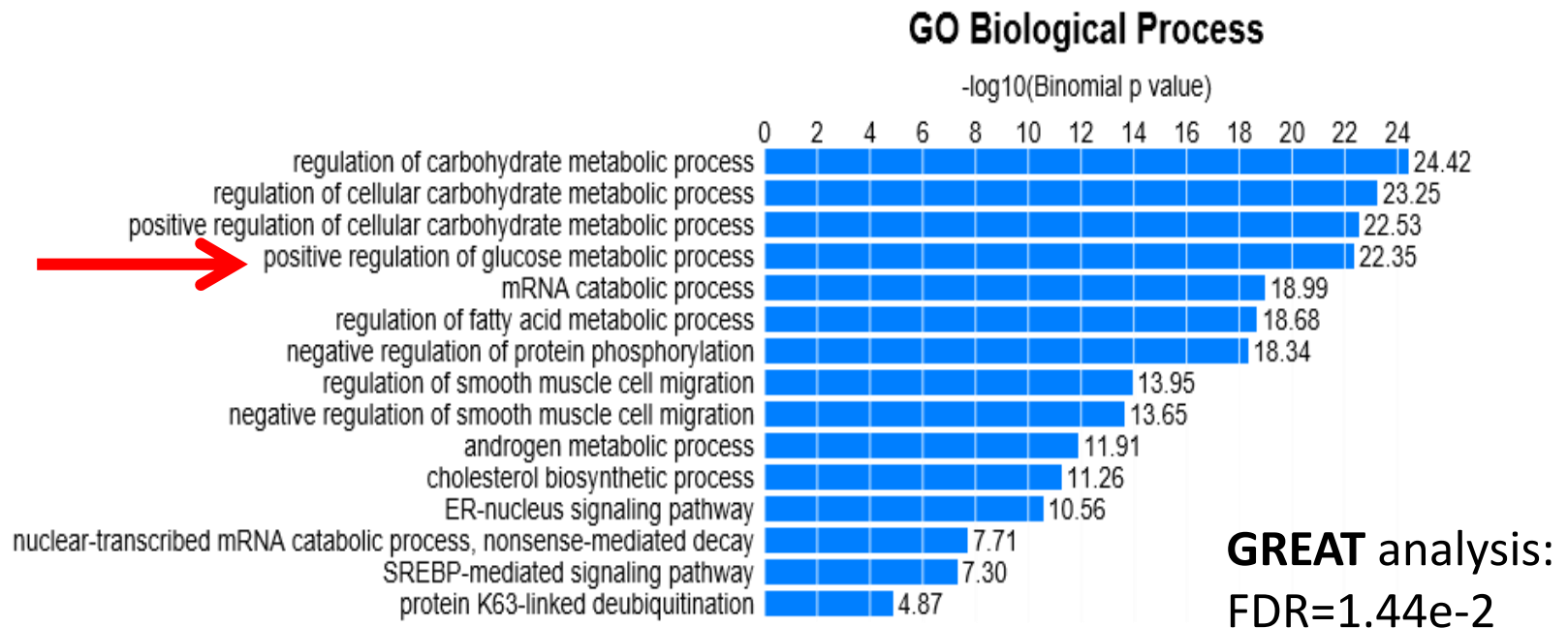
# Methods



**Candidate regions bearing a conditional ATF3 or CBP occupancy and the H3K27ac active enhancer mark**

# Results

ChIP-seq for H3K27ac identified metformin-induced peaks near genes associated with *positive regulation of glucose metabolic processes*



agctagctagctagctagctacta

## Genomic Regions Enrichment of Annotations Tool

McLean *et al.*, Nat Biotechnol. 2010

*Genes X, Y, Z...*



# Methods

Primary human hepatocytes



Vehicle (Non-Treated), 8h

2.5mM Metformin, 8h

RNA-seq

ChIP-seq

ChIP-seq

RNA-seq

ATF3  
CBP  
H3K27ac  
H3K27me3

ATF3  
CBP  
H3K27ac  
H3K27me3

Metformin-treated  
or NT liver cells  
(Huh-7)

*Enhancer  
sequences?*

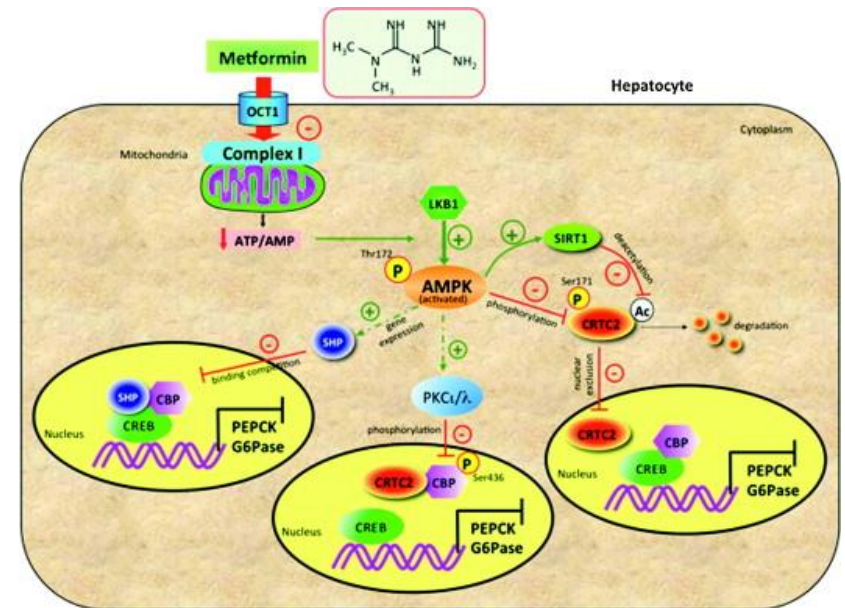
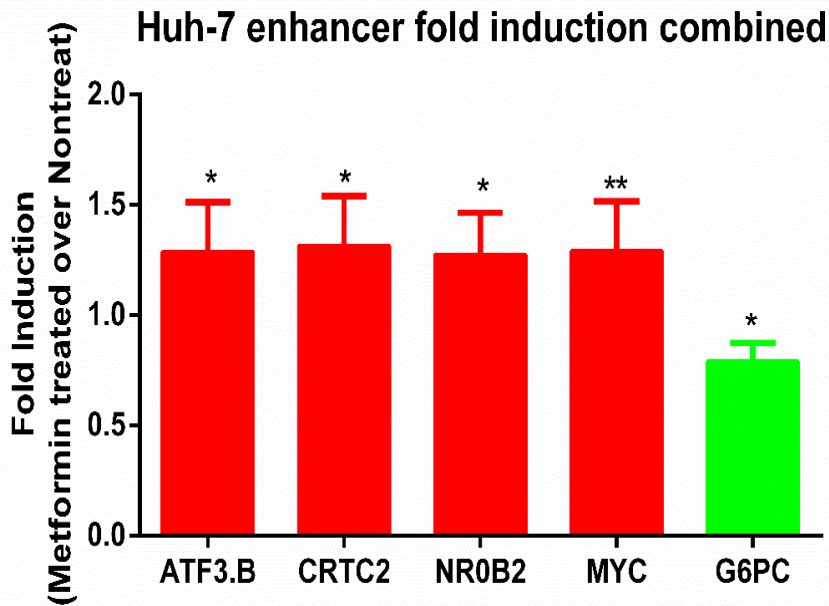
Minimal  
Promoter

Luciferase



# Results & Discussion

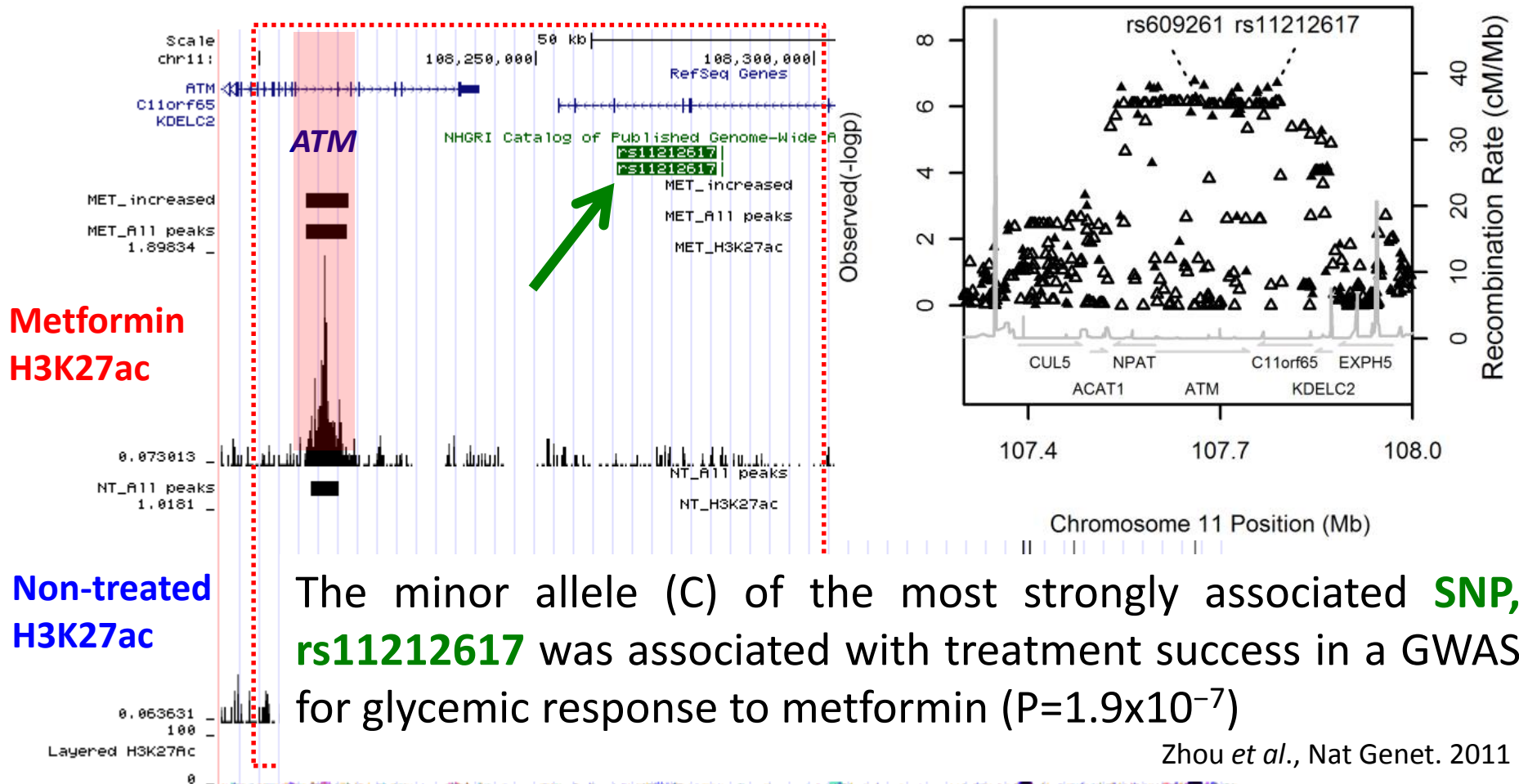
Enhancer assays for candidate sequences which were **induced** or **repressed** by metformin treatment on liver cells.



Potential molecular mechanisms for the metformin action on gluconeogenesis

# Results & Discussion

Metformin induced peak in an intron of the ataxia telangiectasia mutated (*ATM*) gene near the **GWAS lead SNP rs11212617**

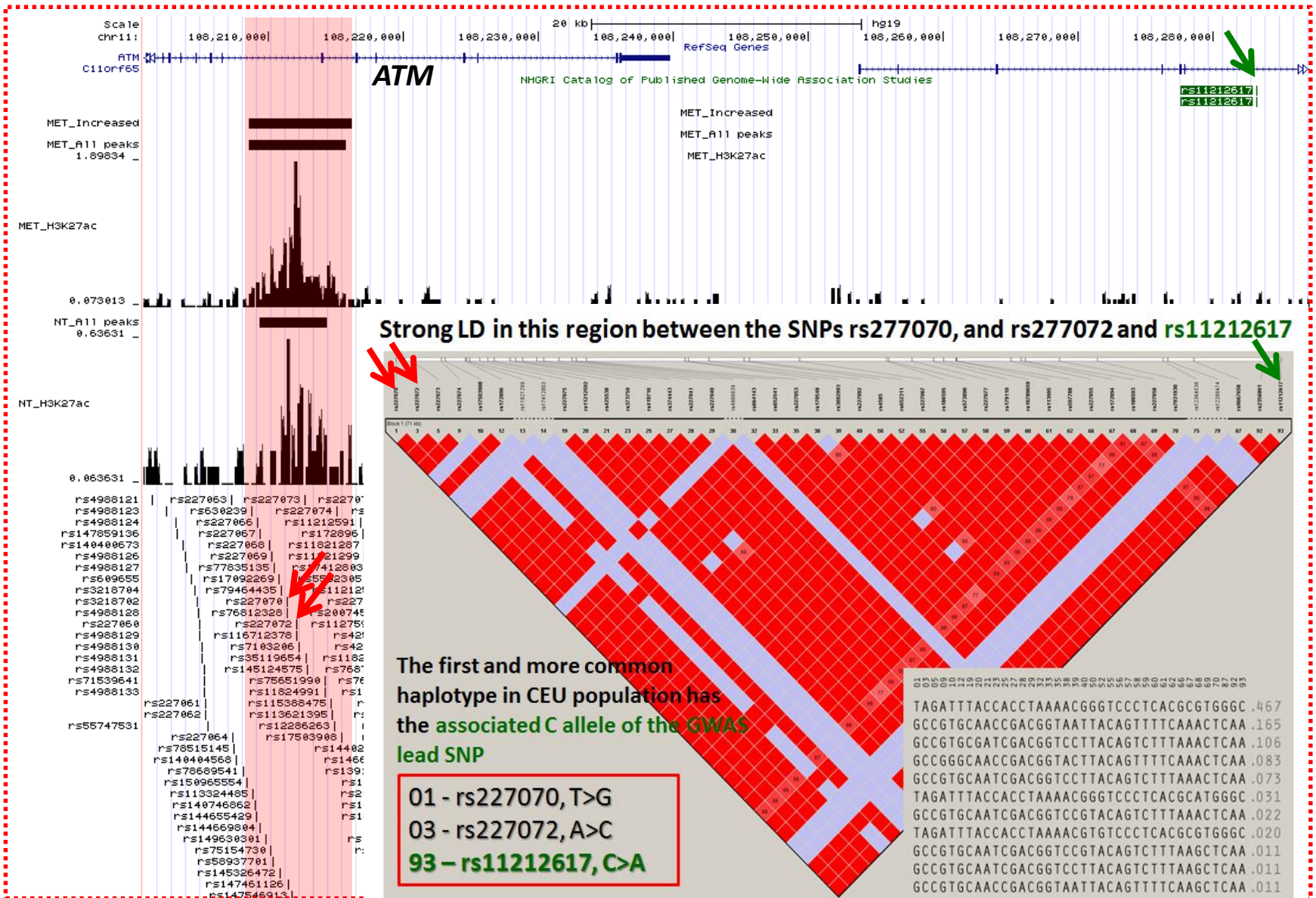


Zhou *et al.*, Nat Genet. 2011



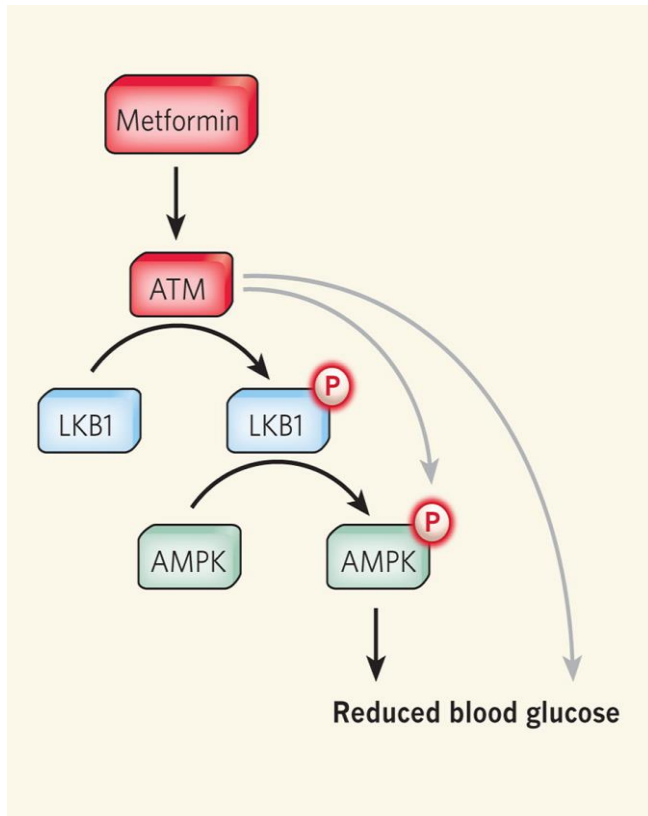
# Results

rs277070 and rs277072 in the *ATM* intron are linked to rs11212617

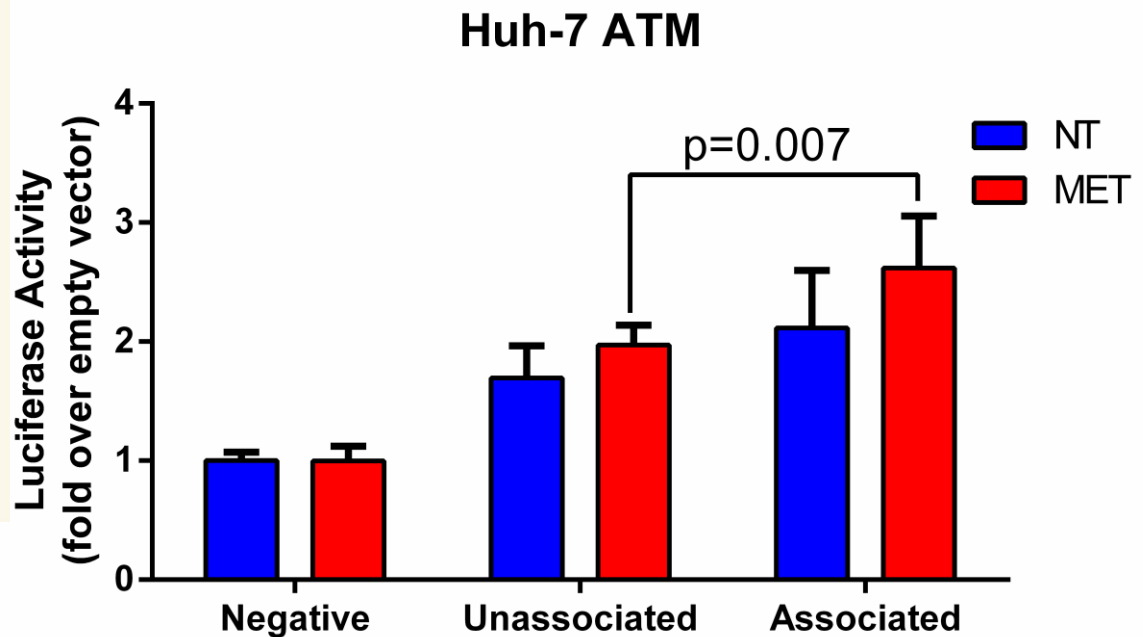


# Results & Discussion

Enhancer assays for **sequences with the associated haplotype** showed increased enhancer activity upon metformin response, suggesting that it might lead to elevated expression of *ATM*



01 03 95	<b>Haplotypes (50% each):</b>
GCA .500	<b>GCA – Unassociated</b>
TAC .500	<b>TAC – Associated haplotype</b>



**Possible mechanisms of the anti-diabetic effects of ATM**

# Conclusions

- We found several meformin up-regulated genes, including novel transcription factors using RNA-seq.
- We identified putative enhancer sequences induced by metformin using ChIP-seq.
- We found a metformin treatment success-associated haplotype in the *ATM* locus that showed increased enhancer activity following metformin treatment.
- Our findings provide for an increased understanding of mechanisms of action of metformin, and for the identification of novel candidates for T2D treatment.

# Acknowledgements

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(Bioinformatics)
- Yao Wang  
(Functional assays)



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- Sook Wah Yee

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Thanks for the attention!

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