The dark matter of the genome and blood pressure regulation – modeling non-coding genetic mechanisms in cellular models and rats

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Department of Physiology
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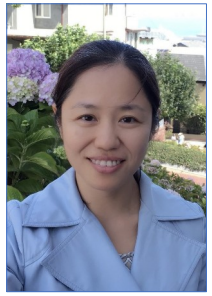
Acknowledgements

National Heart Lung and Blood Institute
People Science Health

P01HL149620





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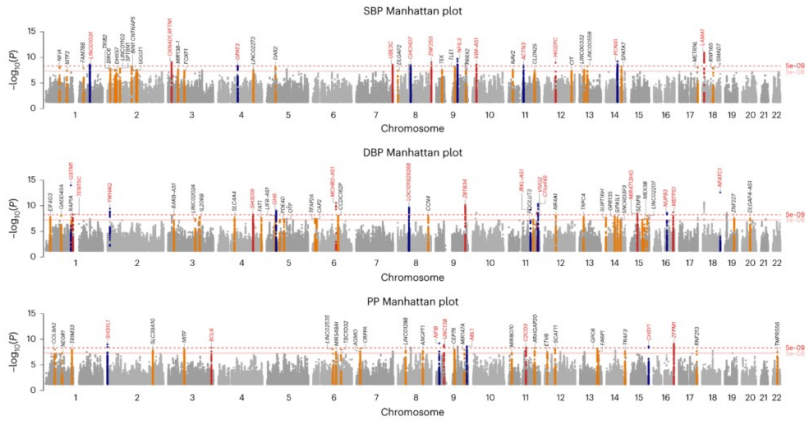
Human BP SNPs -> rat orthology & human iPSC-derived cell types

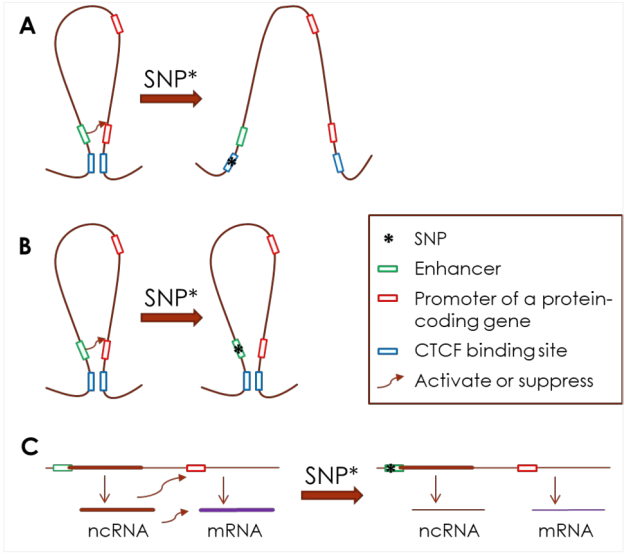
Overall hypothesis: Non-coding, non-transcribed SNPs must exert their effects on phenotypes by modifying expression of gene(s) in some tissue(s) to modify one or more physiological mechanisms relevant to the trait.

•PMCID: PMC11096100



<1% missense or nonsense variants,
>20% >10-kbp from nearest gene

•PMCID: PMC7035167



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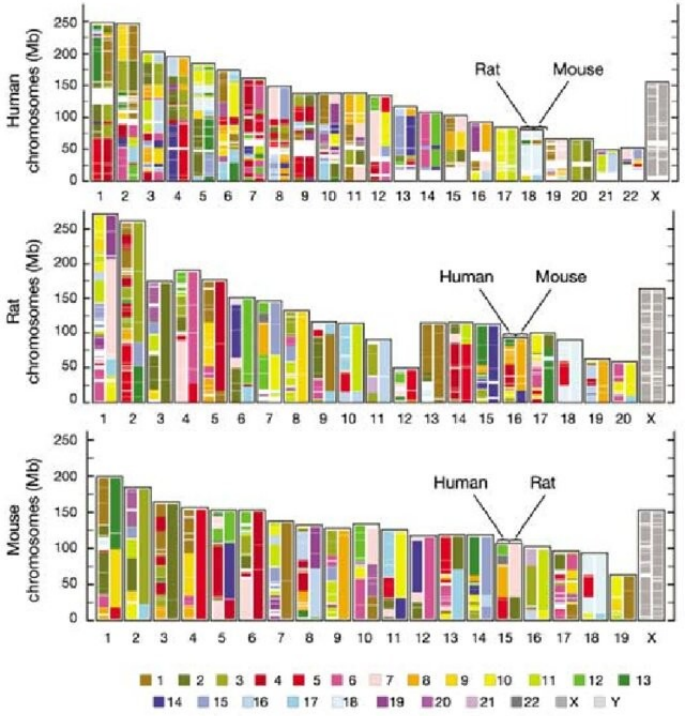
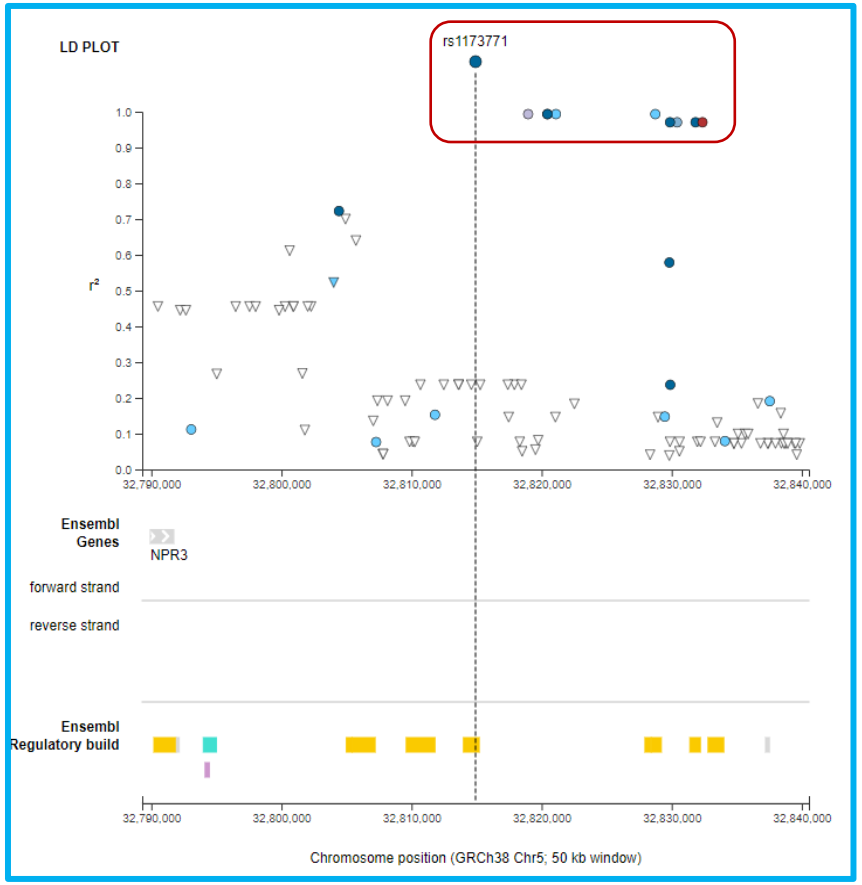
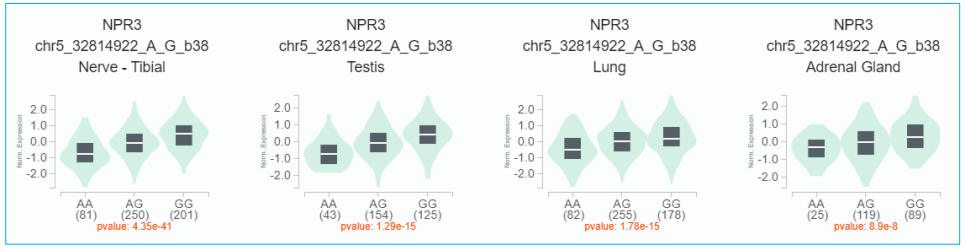


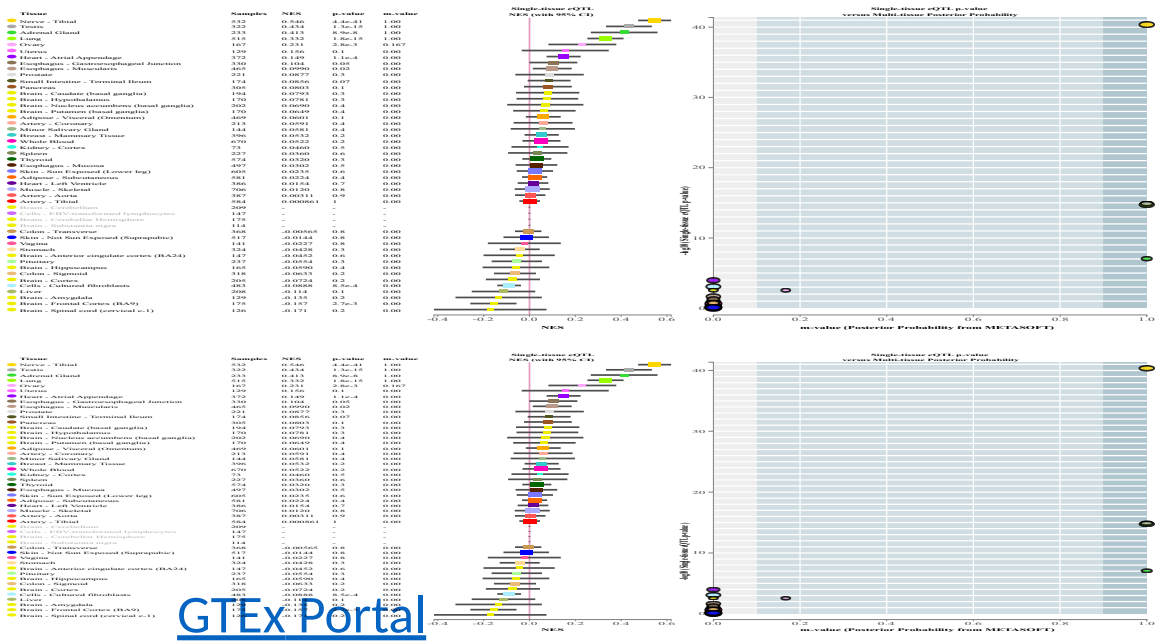
Synteny and sequence-level conservation

rs1173771 (rs771)



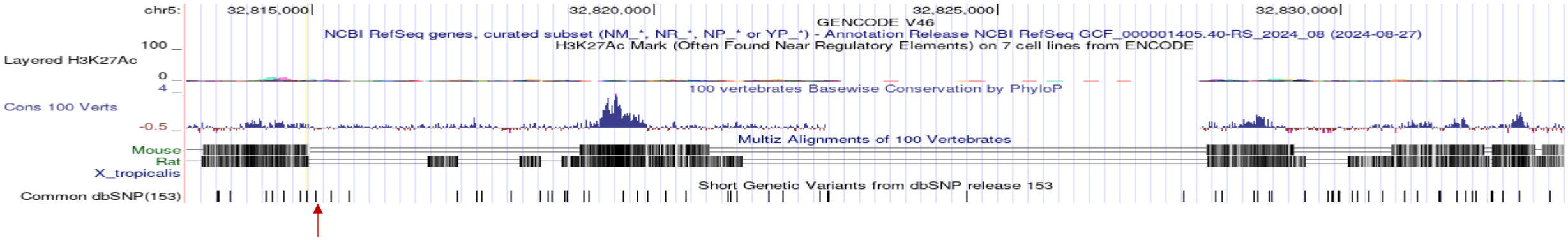
GWAS Catalog (ebi.ac.uk)

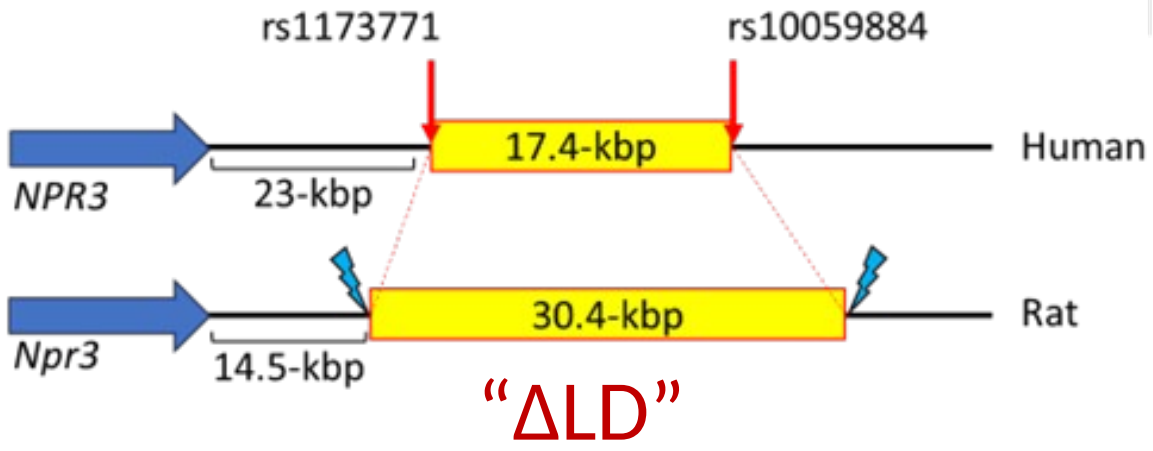


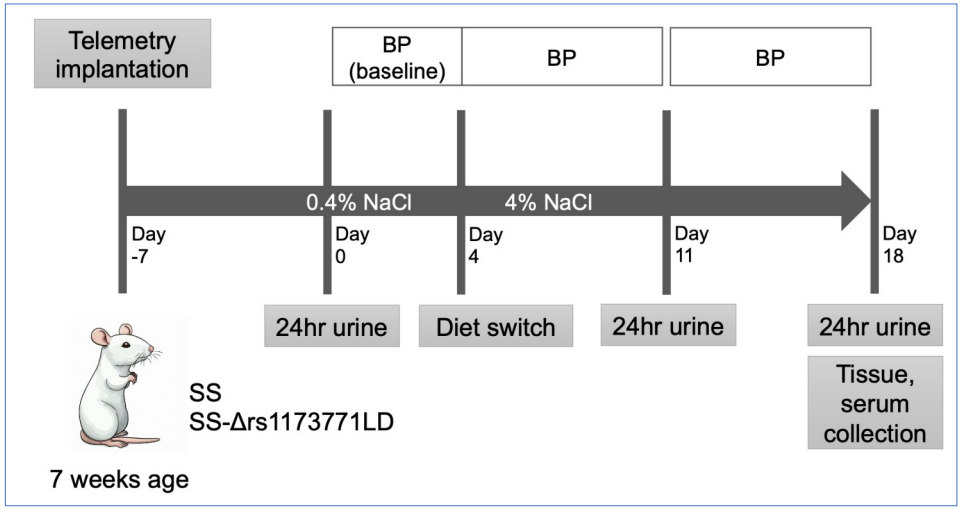


GTEx Portal

We deleted the entire homologous non-coding region in SS using CRISPR-Cas9

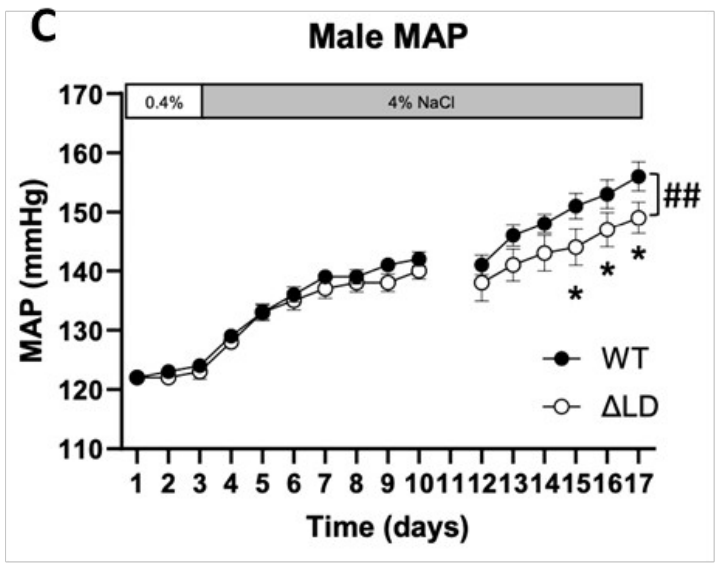


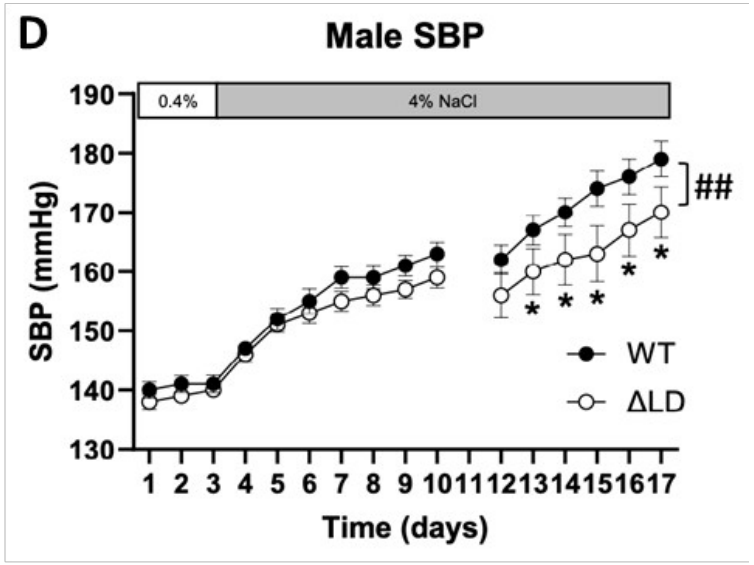




Hong Xue

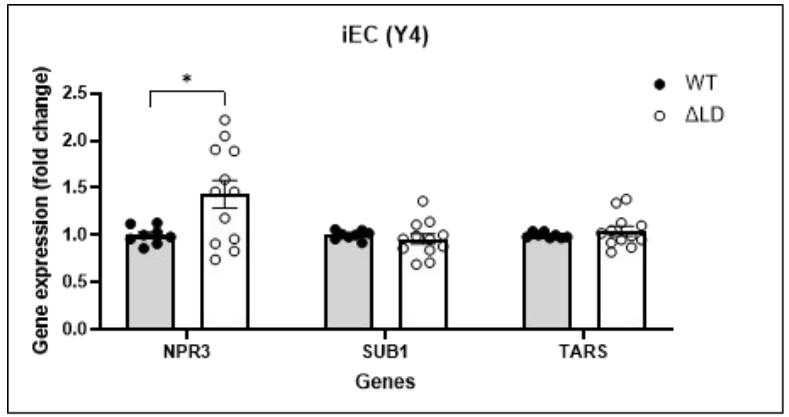
Systolic blood pressure was reduced in ΔLD male rats



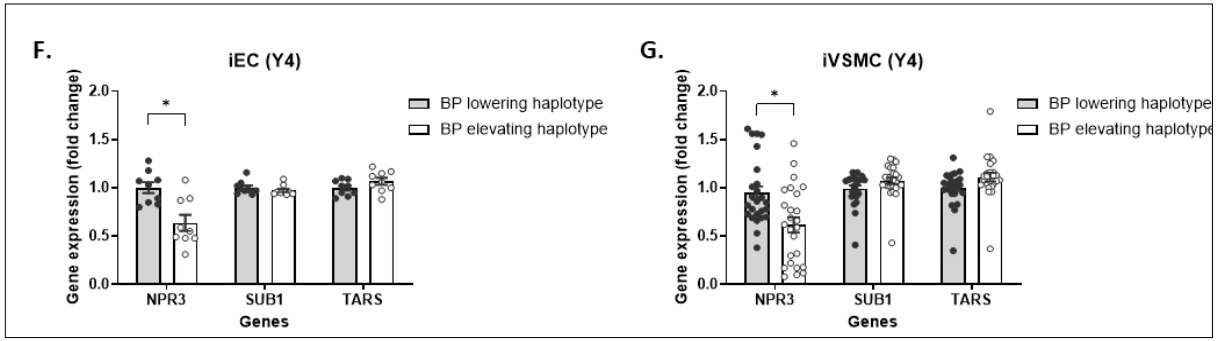


Manoj Mishra, Yong Liu

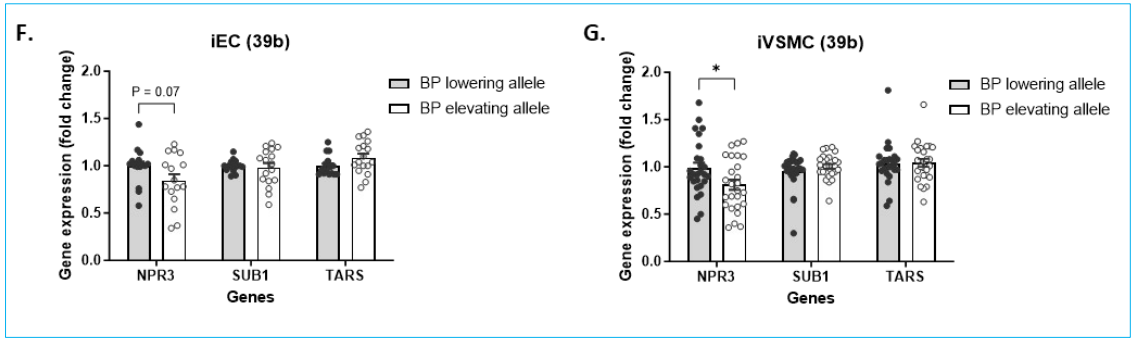
CRISPR-SpCas9 gene edited iPSC models reveal similar effects of the haplotype and single rs771 on Npr3



Deletion model



Haplotype reconstituted models



Single rs1173771 edited model

NPR3 encodes NPR-C, a multifunctional receptor

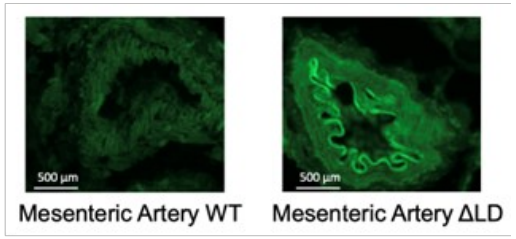
- In the vasculature, NPR-C binds C-type natriuretic peptide (CNP), mediates the effect of CNP on vascular function and structure, and plays an important role in preserving vascular homeostasis in vivo.
  - *Moyes, et al. J Clin Invest. 2014; PMID: 25105365*
  - *Villar, et al. Cardiovasc Res. 2007; PMID: 17391657*

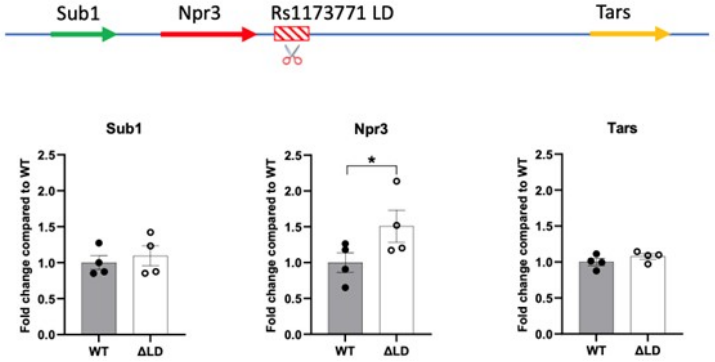
- In the kidney, NPR-C facilitates the clearance of atrial, B-type, and C-type natriuretic peptides (ANP, BNP, CNP) from the circulation via endocytosis.
  - Maack, et al. *Science*, 1987; PMID: 2823385
  - Almeida, et al. Am J Physiol. 1989; PMID: 2537040

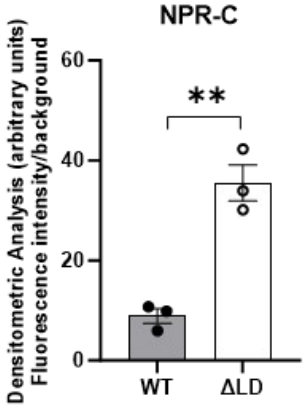
ΔLD has tissue-specific effects on Npr3 expression and function

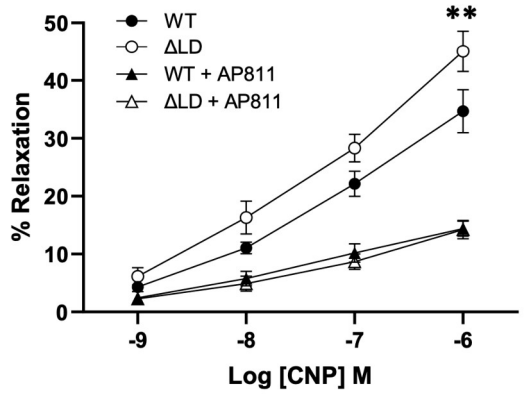
Hong Xue
Abdel Alli

Mesenteric Artery

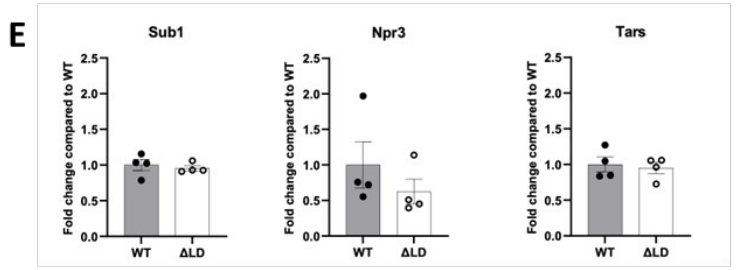


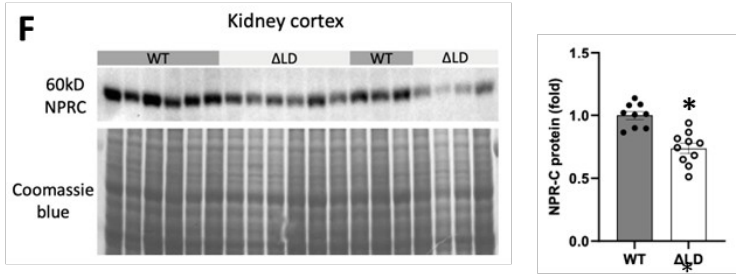


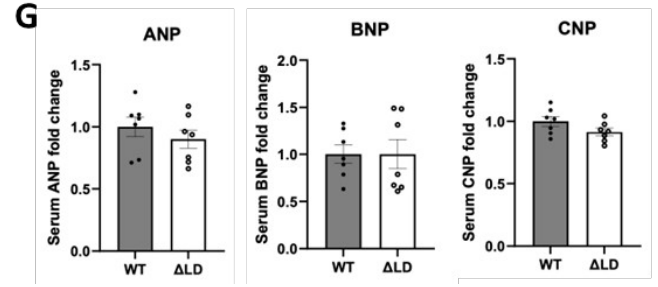




Kidney



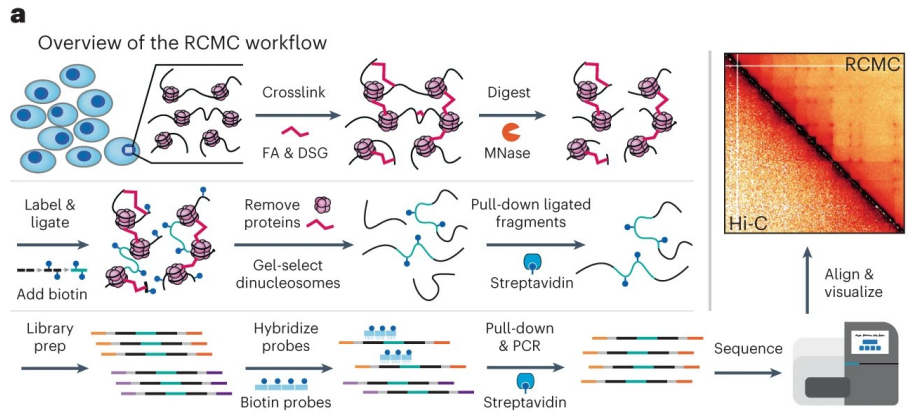


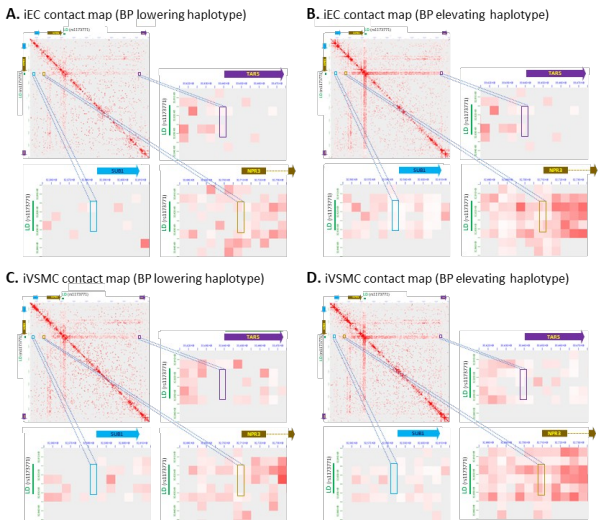


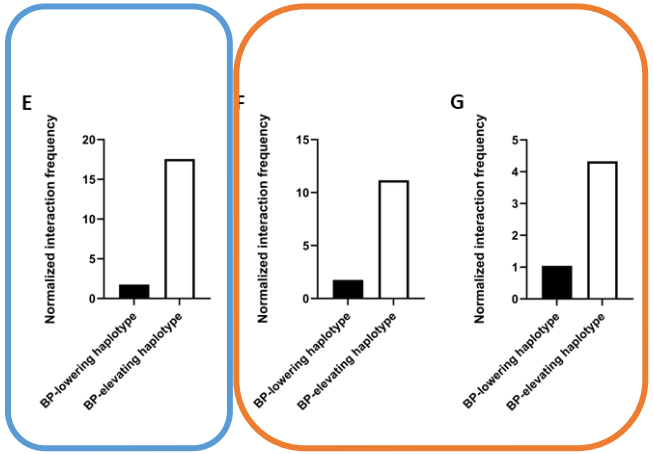
Yong Liu

The BP elevating haplotype increases contact frequency between the LD region and the Npr3 promoter

- [Region Capture Micro-C reveals coalescence of enhancers and promoters into nested microcompartments | Nature Genetics]



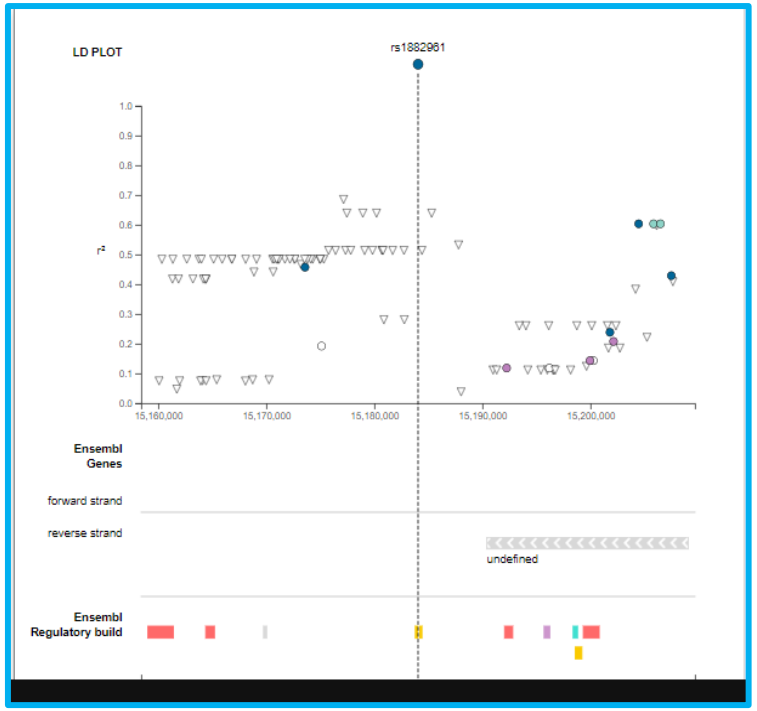




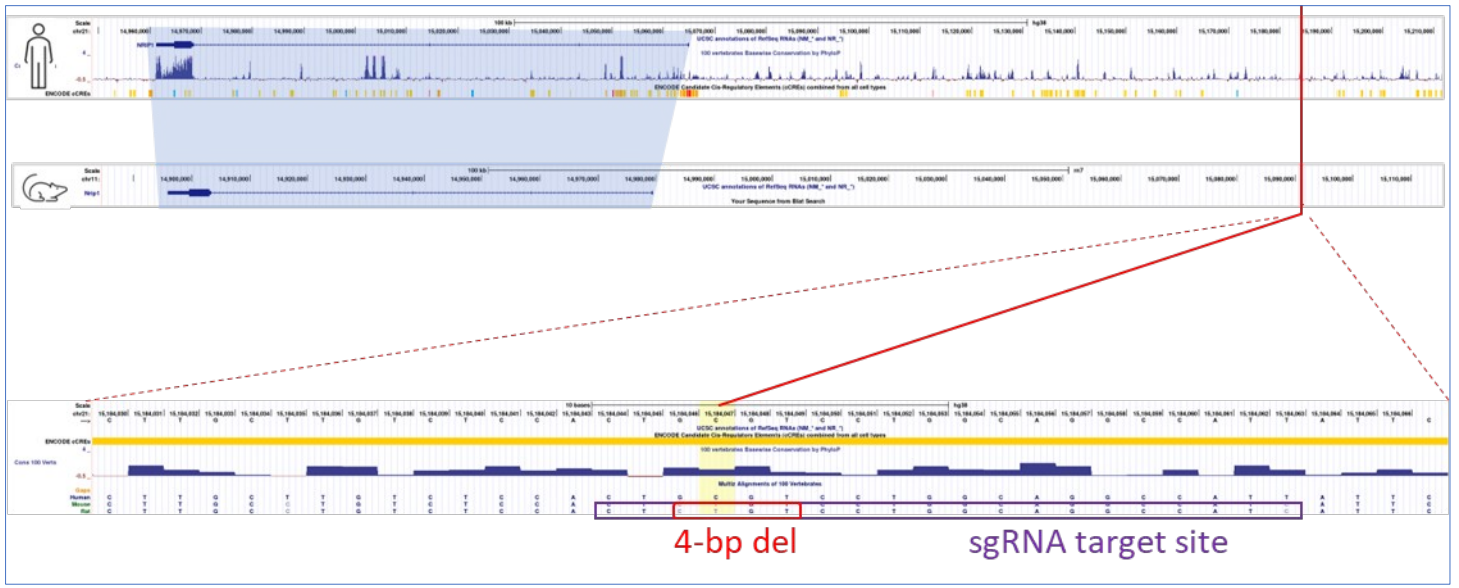
rs1882961 C->T (rs961)

riskAllele	pValue	pValueAnnotation	riskFrequency	orValue	beta	ci	Mapped Genes	traitName	Traits	bkgTraits	accessionId	locations	pubmedId	author
rs1882961-?	3.00E-13	-	NR	-	-	-	LINC02920,CYCSP42	Systolic blood pressure	systolic blood pressure	-	GCST007087	21:15184047	30595370	Kichaev G

GWAS Catalog (ebi.ac.uk)



No evidence of eQTL, 120-kb away from nearest gene



Hong Xue

Editing this conserved region in SS rats results in sex-divergent effects on blood pressure traits

Fig 1

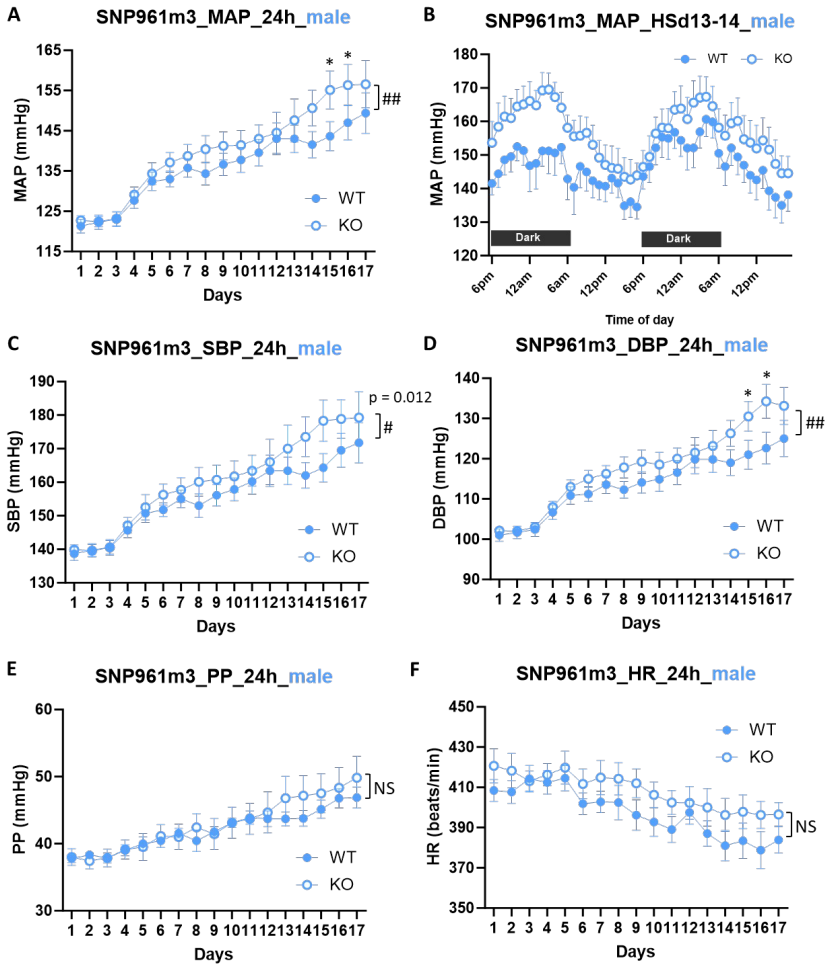
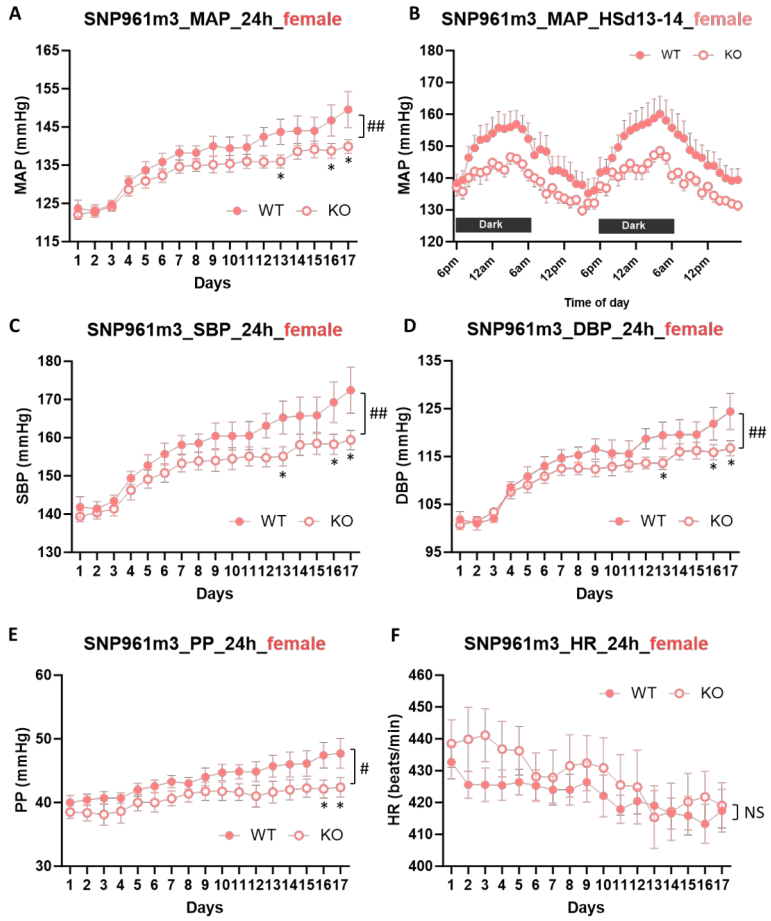
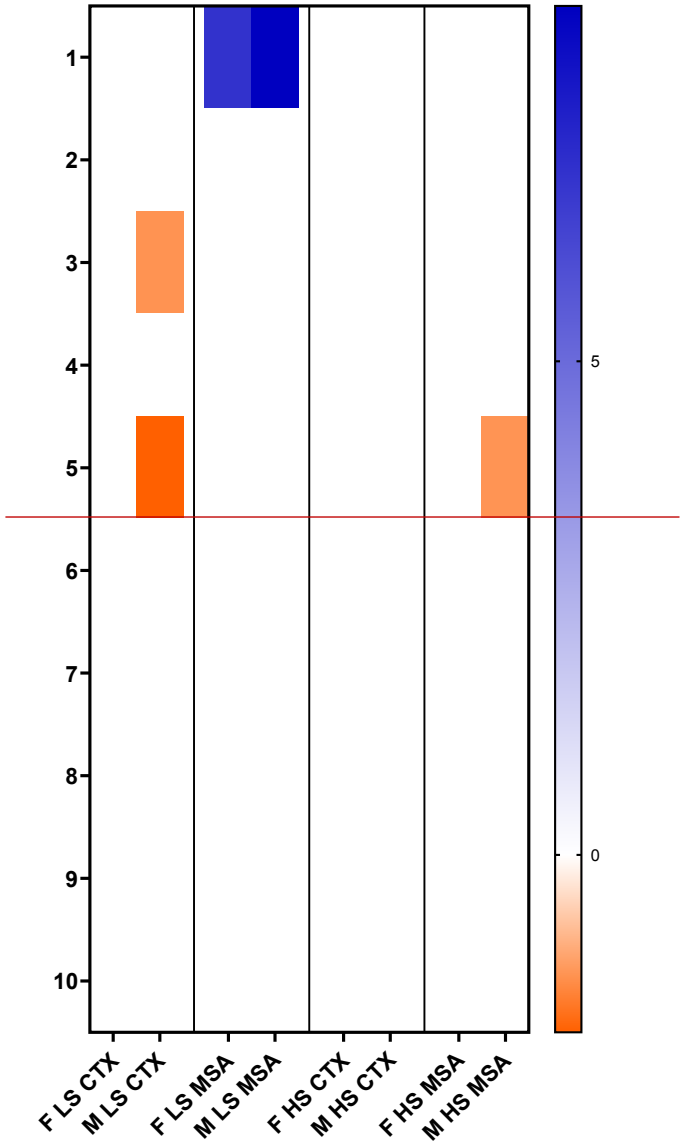


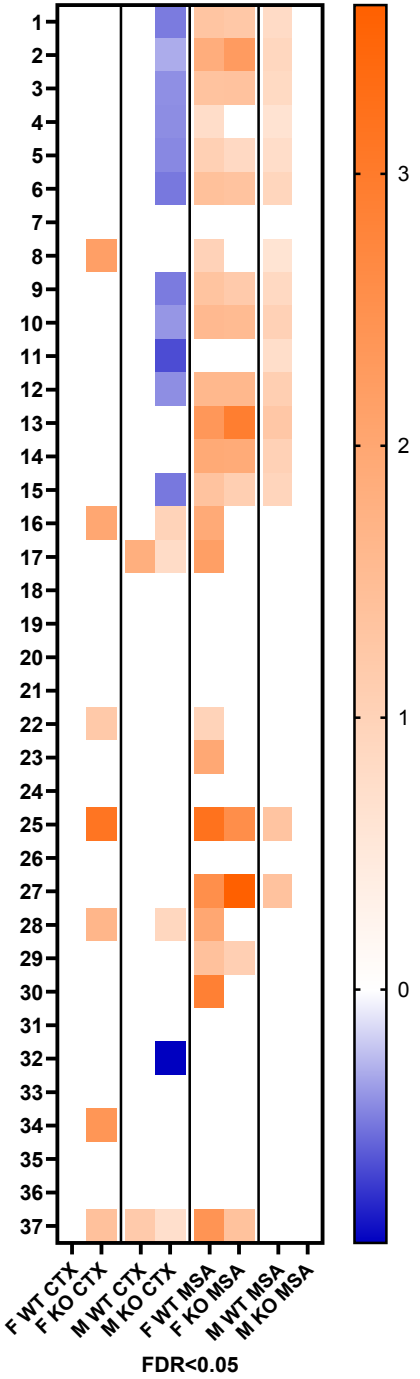
Fig 2



Effects on local genes (very preliminary data)

log2FC KO/WT, FDR<0.05





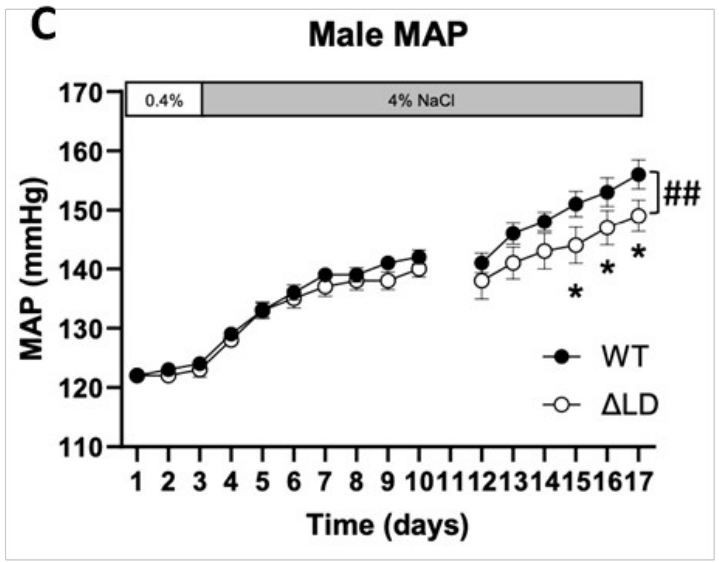
Overall conclusions

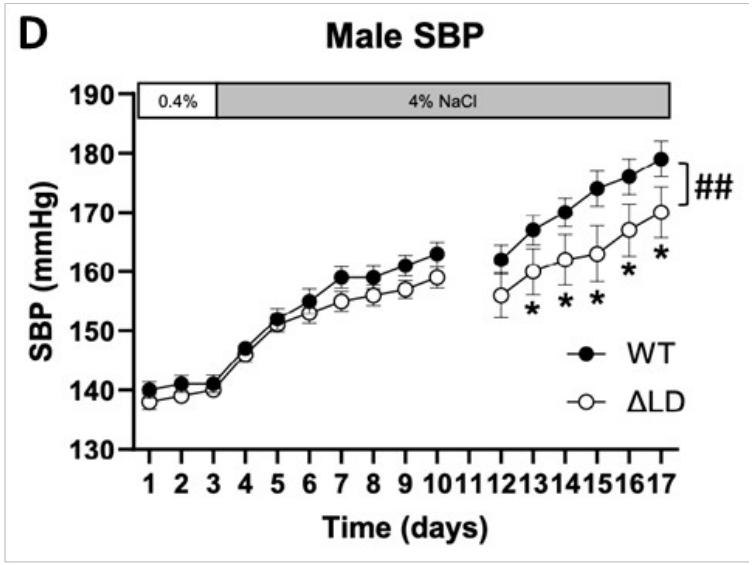
- The majority of loci (now >2,000) associated with blood pressure are non-coding and hundreds of functional SNP may be quite distant from the genes they regulate
- Human iPSCs can be engineered to harbor blood pressure elevating and lowering alleles and haplotypes then differentiated to study their effects on gene expression
- At least some human non-coding loci can be manipulated in animal models to provide functional evidence of their role in complex traits
- Unlike knockout models, each engineered SNP model will have unique tissue(s)-specific (and sex-specific!) effects on gene(s) expression and phenotypes, leading to complex hypotheses about their mechanisms

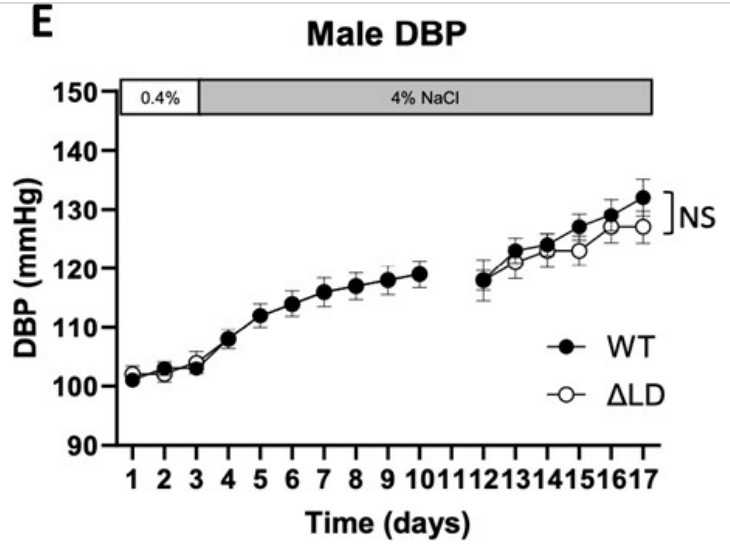
Thanks!
Questions?

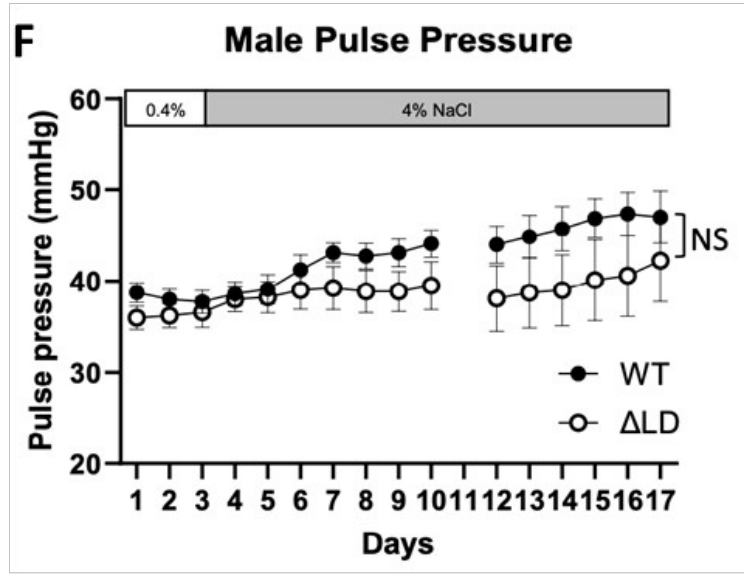
Hong Xue

Systolic blood pressure was reduced in ΔLD male rats

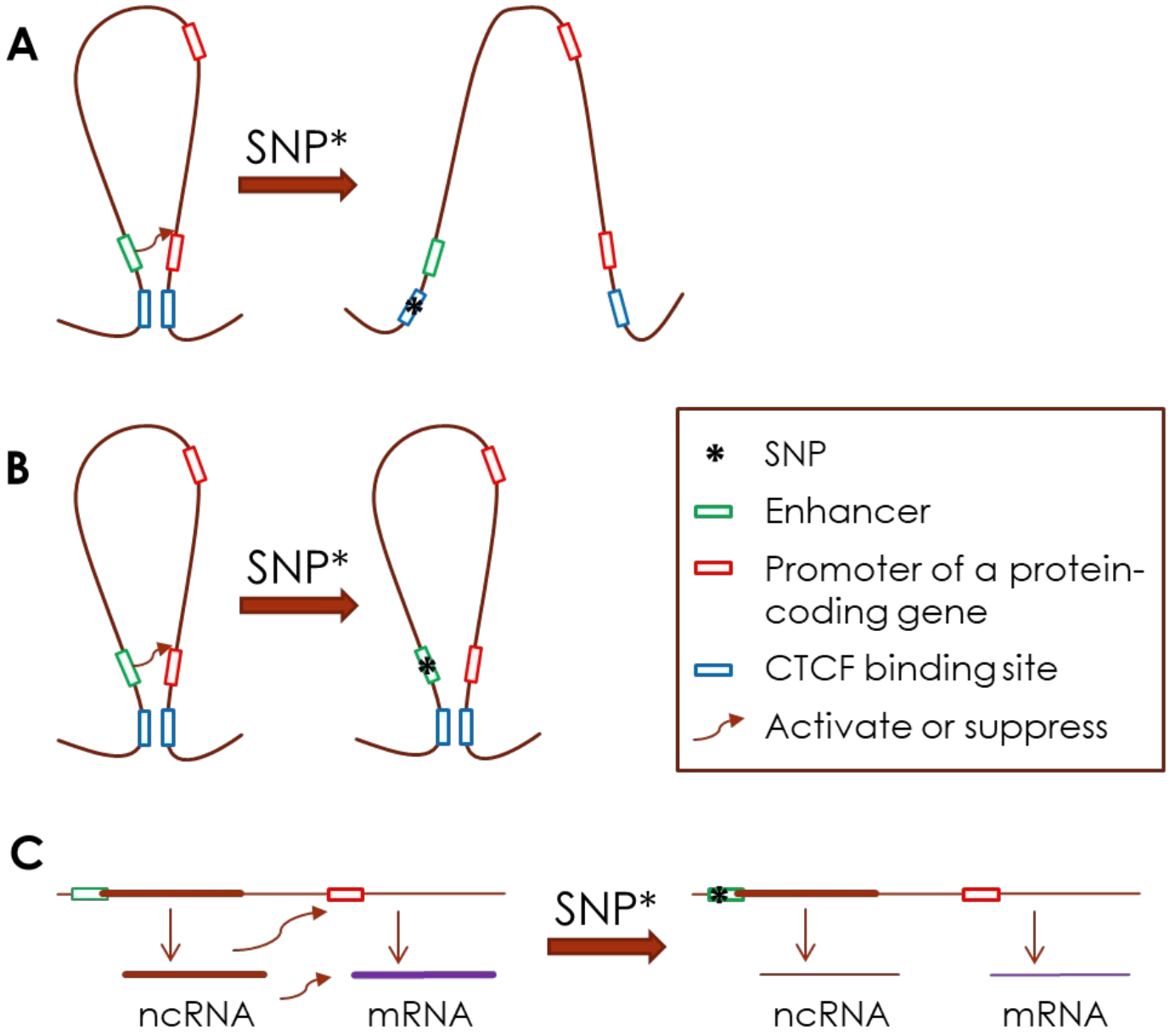








Proposed mechanisms by which non-coding SNPs could influence genes and blood pressure



[Lift Genome Annotations (ucsc.edu)]

Conservation in rats extends to a ~30.4-kbp region