Genome editing: from modeling disease to novel therapeutics

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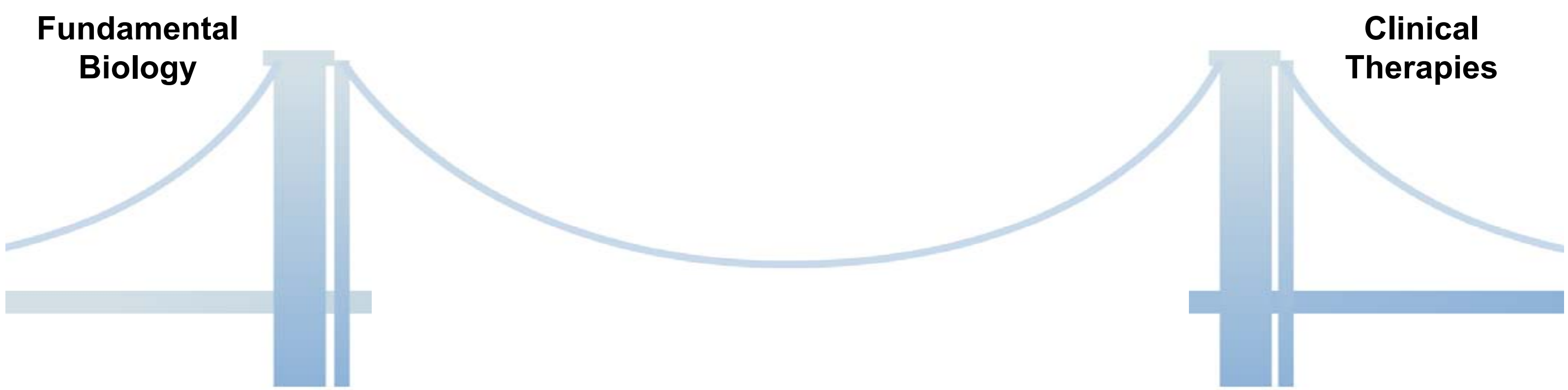
Disclosure:
CRISPR Therapeutics and Sana Biotechnology

How do we bridge the gap to understanding and treating metabolic disease?



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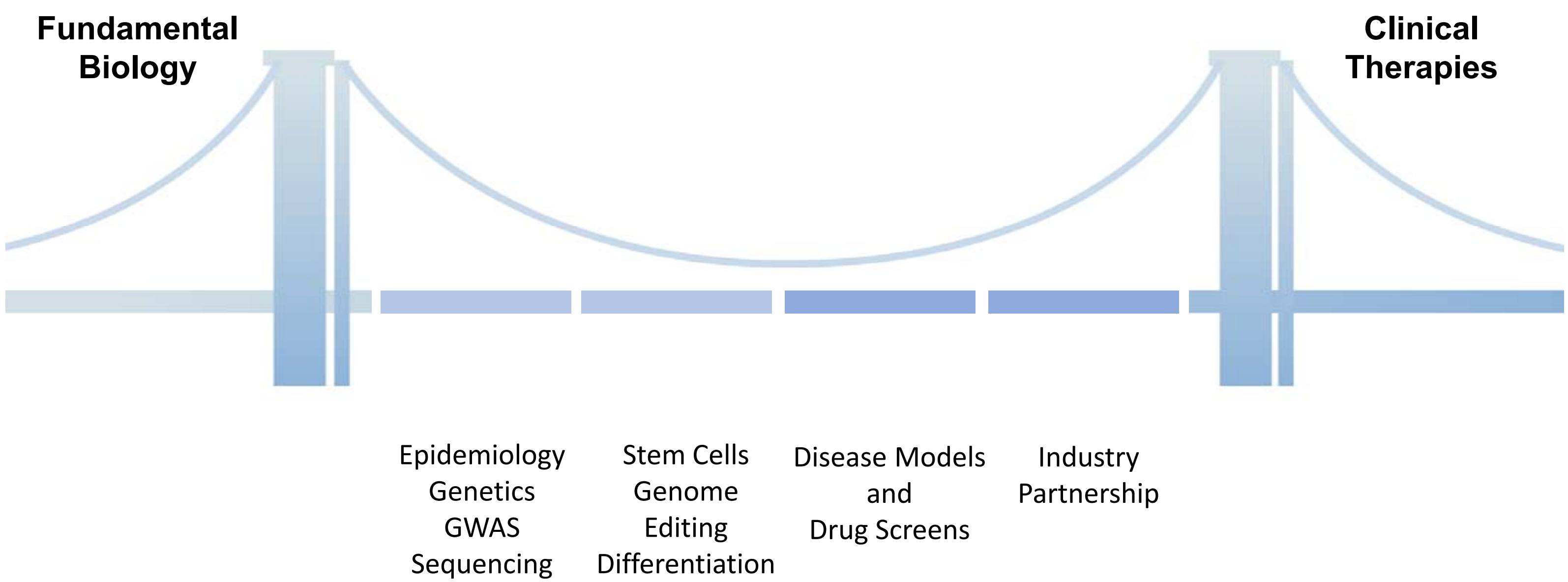
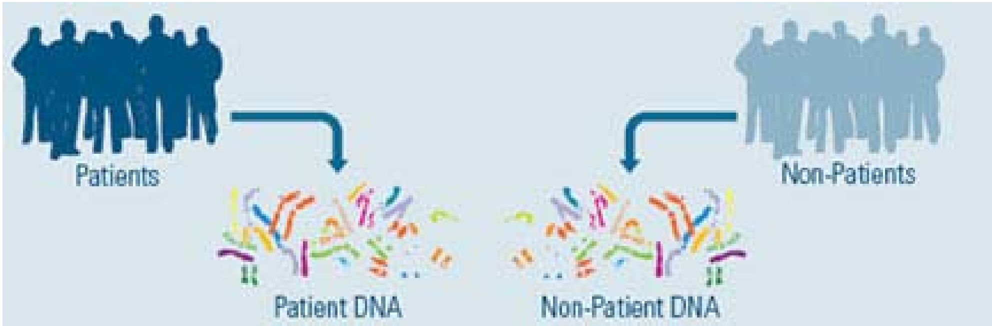
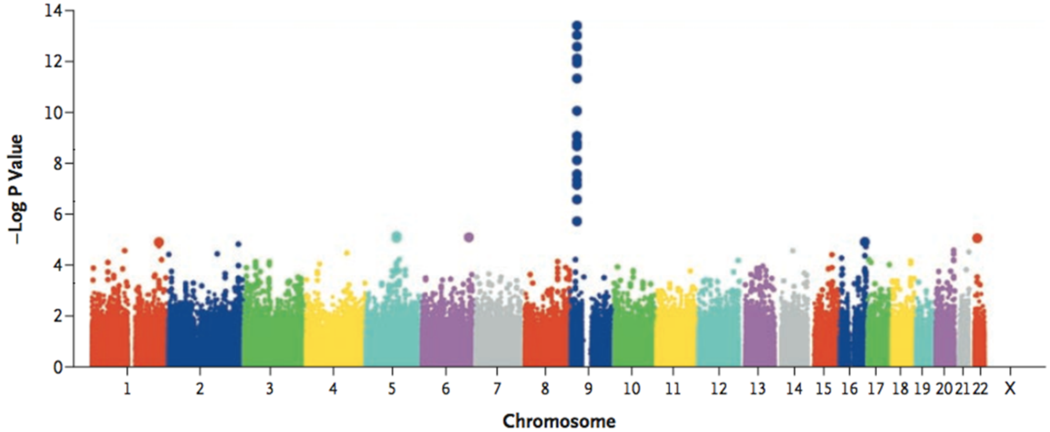


Genetic Studies of Lipids and Heart Attack

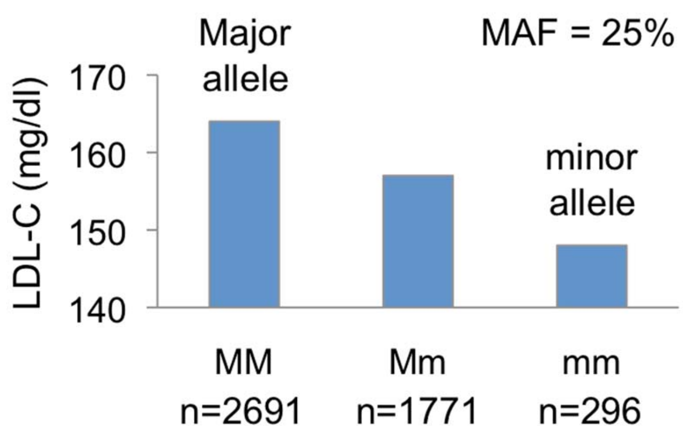




Teslovich, Musunuru, et al.
Nature 2010; 466:707-13

1p13 minor allele is associated with ↓ LDL-C, ↓ MI risk

Chr	SNP (genes)	Combined *P* value
1p13	rs599839 (?????)	8×10^{-160}
19p13	rs4420638 (*APOE*)	3×10^{-140}
19p13	rs6511720 (*LDLR*)	2×10^{-110}
2p24	rs1367117 (*APOB*)	6×10^{-109}
2p21	rs6544713 (*ABCG5/ABCG8*)	4×10^{-47}
5q13	rs12916 (*HMGCR*)	1×10^{-45}

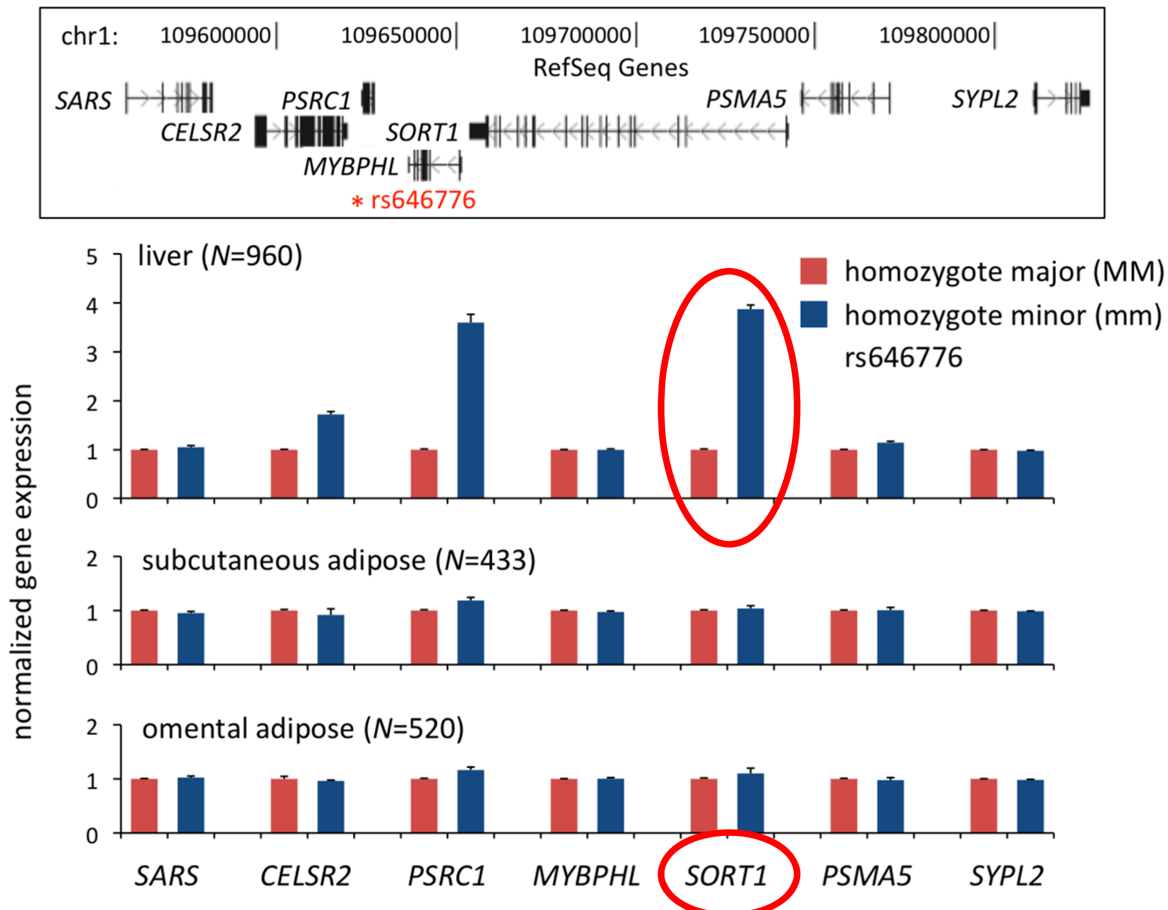


rs599839

mm: 16 mg/dL lower LDL-C
$P = 1 \times 10^{-14}$

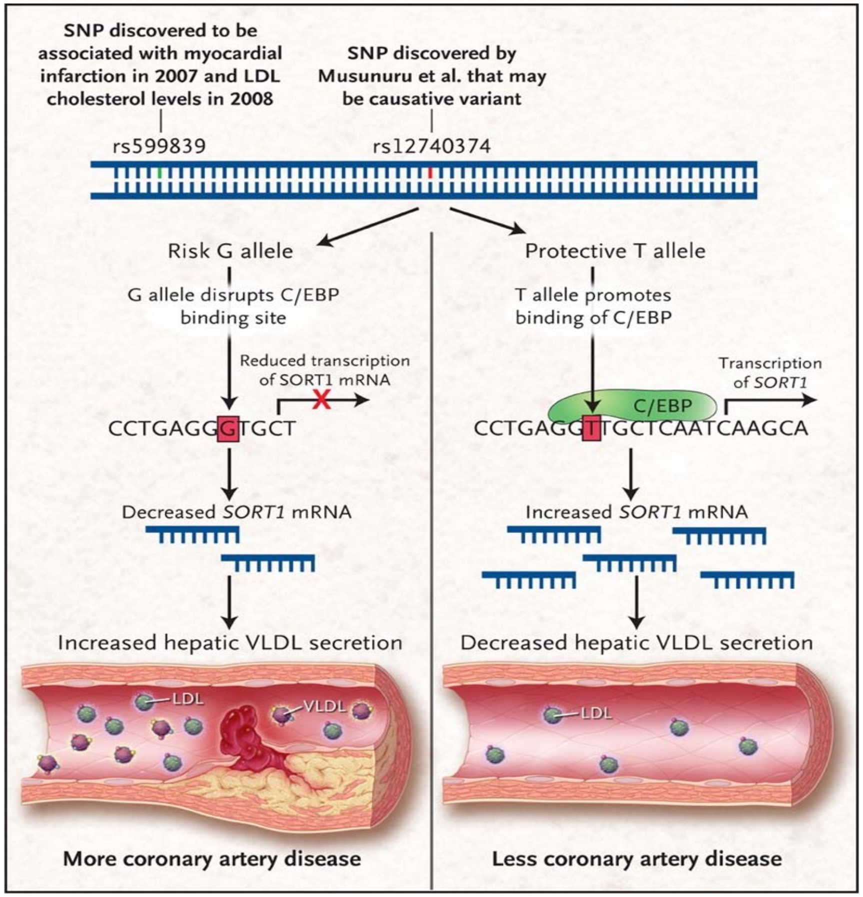
mm: 40% lower risk of coronary disease

1p13 Minor Allele is Associated with ↑ Gene Expression, Specific to Liver



Musunuru, Strong, et al. *Nature* 2010; 466:714-19

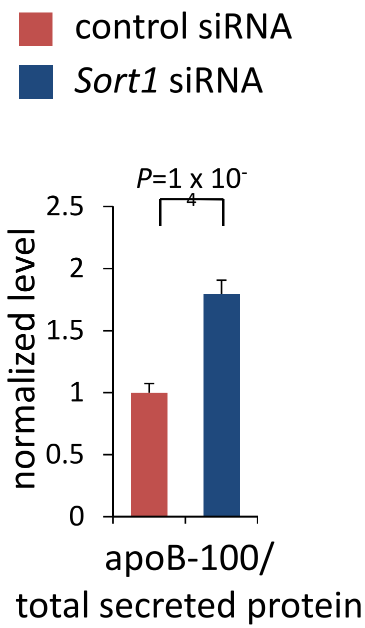
Proposed model



Linset-Nitschke et al., *NEJM* 2010

Contradictory conclusions from mouse studies of Sort1

Sort1 knockdown in mouse hepatocytes



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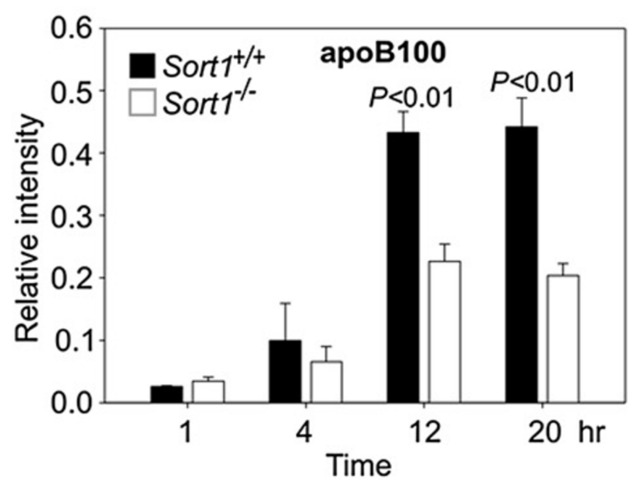
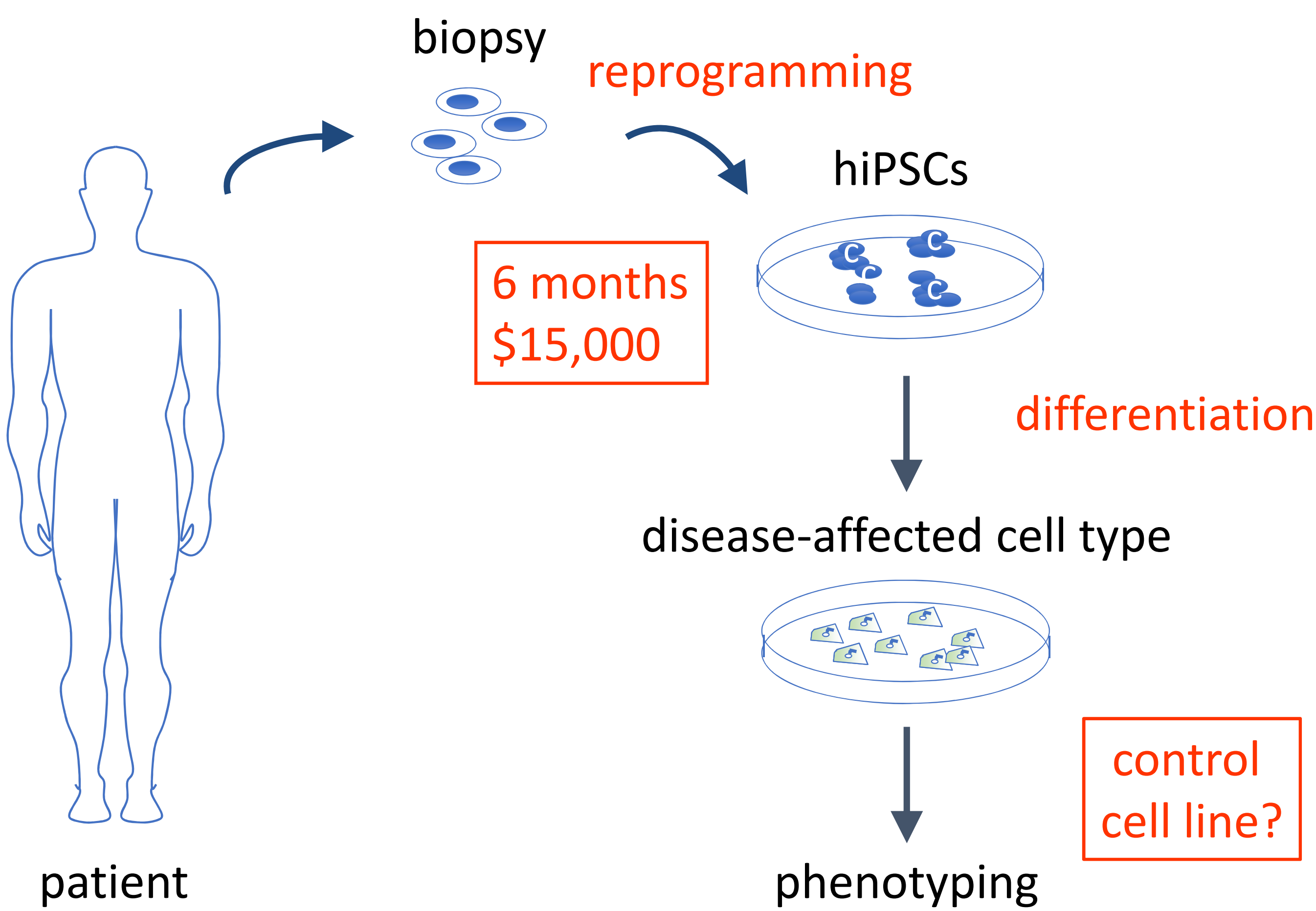
Sort1–/– mouse hepatocytes



Kjolby et al. *Cell Metab* 2010; 12:213-23

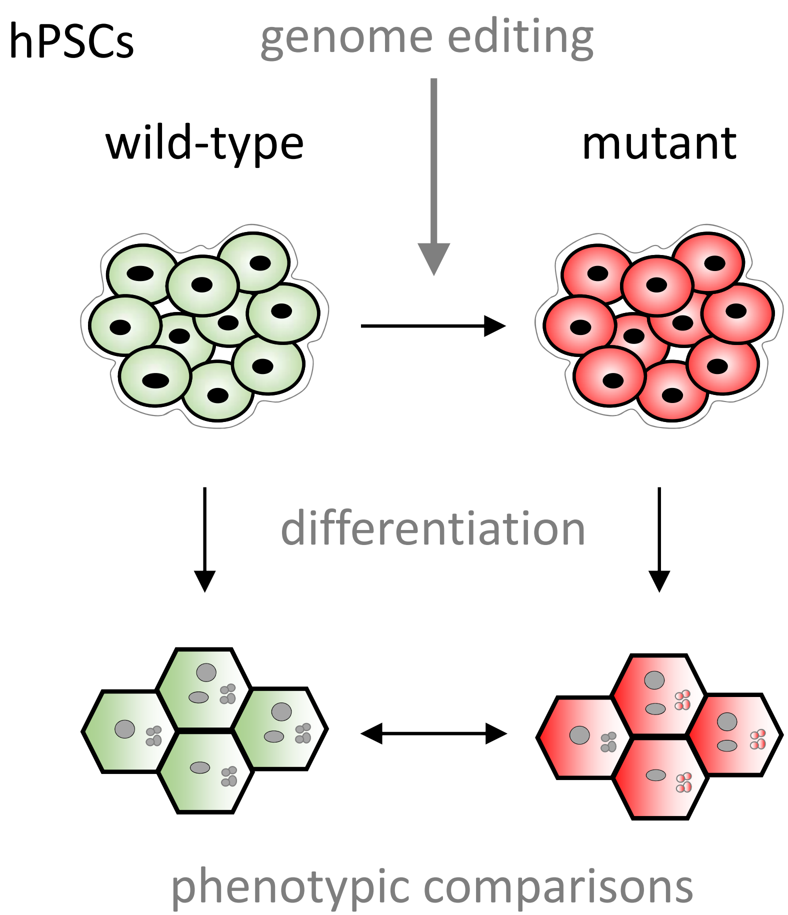
Disease modeling: human induced pluripotent stem cells



Variability among different human pluripotent stem cell (hPSC) lines

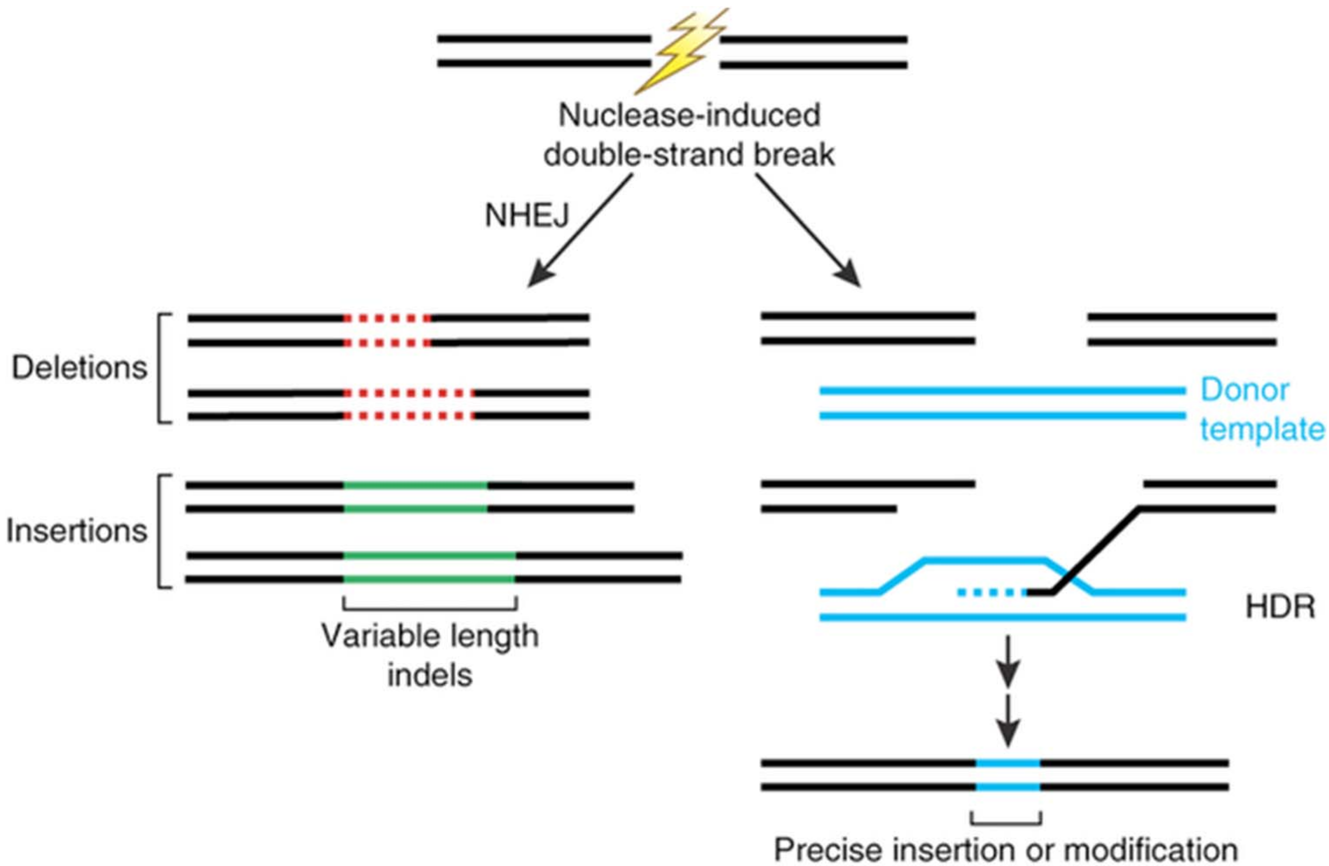
- Differences in genetic background
- Differences in epigenetic state
- Differences in pluripotency/differentiation capacity

Disease modeling: genome editing

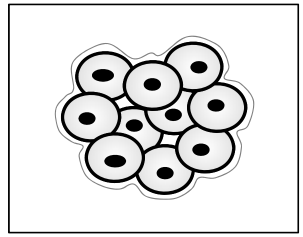


- no patient recruitment
- no need for quality control of pluripotency of iPSC clones
- allows for studies of multiple gene variants side-by-side
- more efficient (and cheaper)

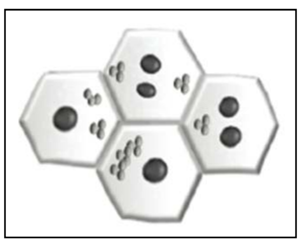
How do engineered nucleases create or repair mutations?



hPSCs

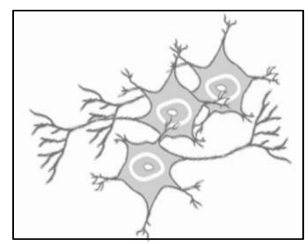


Genetic studies of *SORT1*



hepatocytes

adipocytes

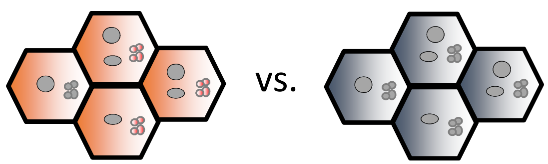


motor neurons

SORT1 has functions in multiple cell types

- Discovered by GWAS to be involved in hepatic lipoprotein processing
- Insulin-responsive glucose transport in adipocytes
- Mediates growth factor-induced neuronal apoptosis

SORT1–/– hepatocytes have increased secreted apoB



wild-type HLCs

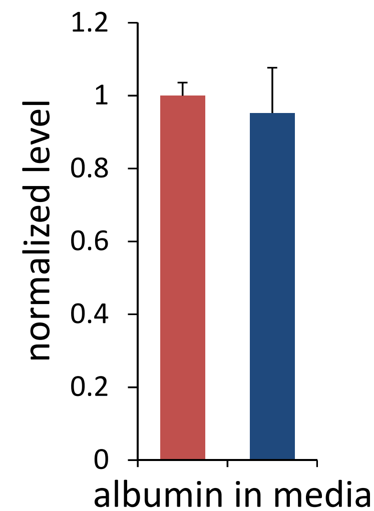
SORT1–/– HLCs

wild-type clones

SORT1–/– clones

2 clones vs.
2 clones

ELISA of media



SORT1–/– hepatocytes have increased secreted apoB

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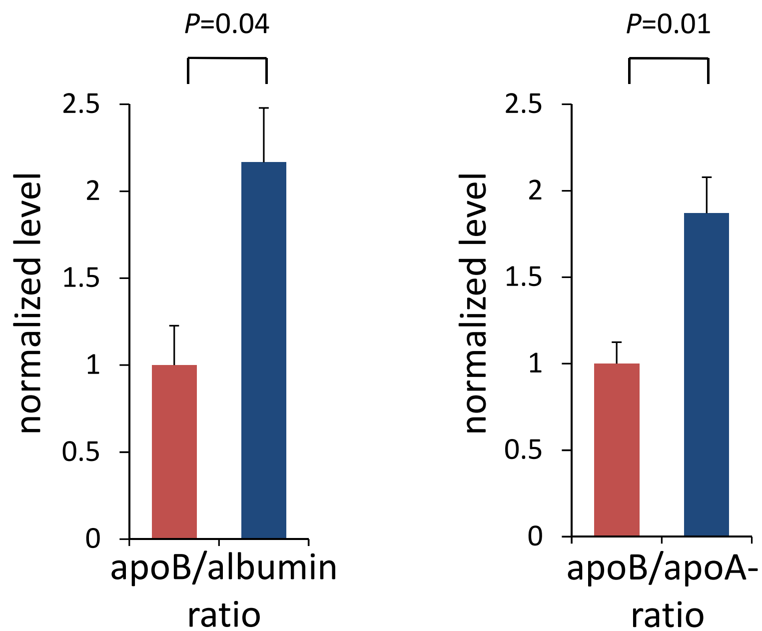
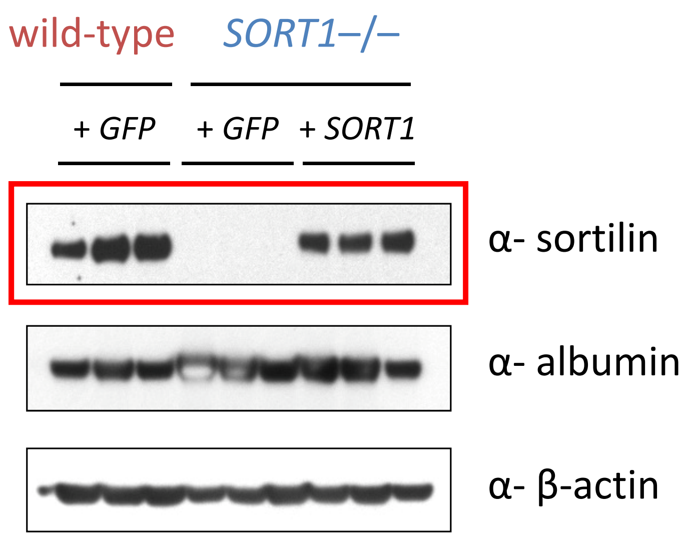
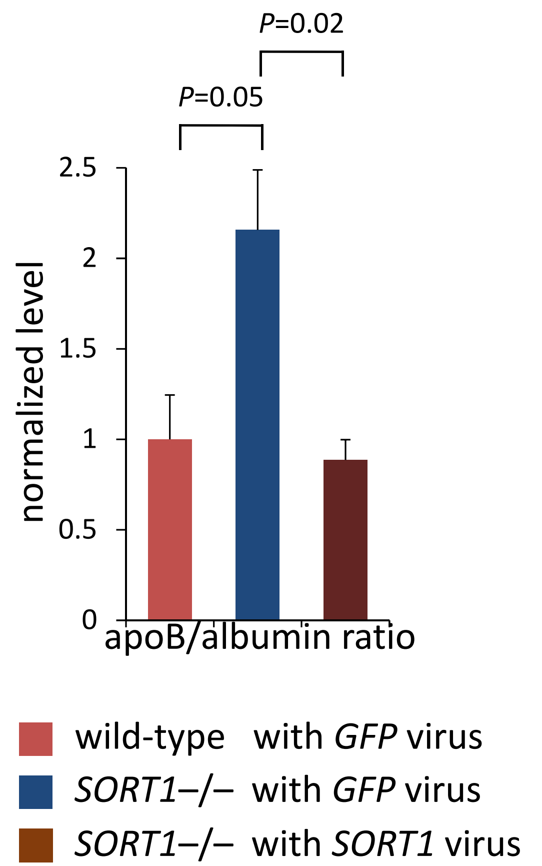


Reconstitution of *SORT1* rescues phenotype

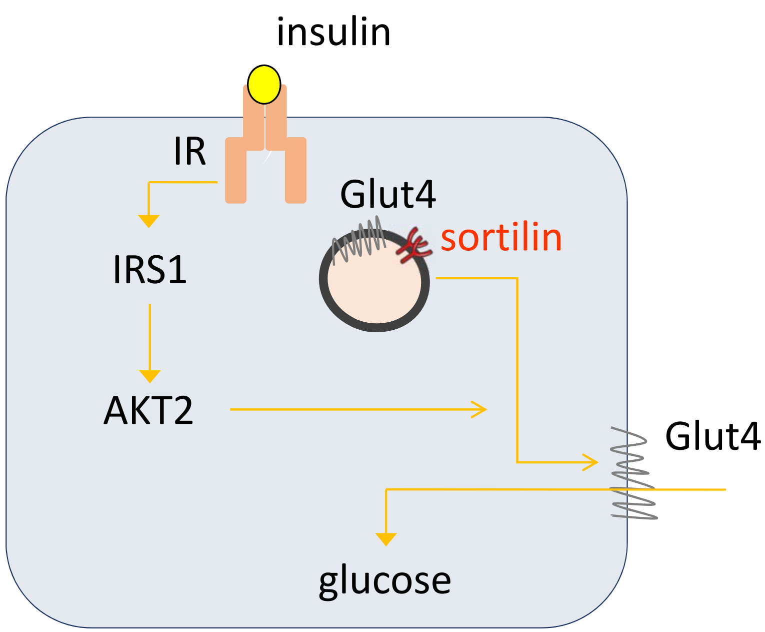


HLC lysate Western blotting



ELISA of media (1 clone each)

Sort1 in mouse adipocytes



Sortilin plays a role in the formation of Glut4 storage vesicles in 3T3-L1 adipoctyes

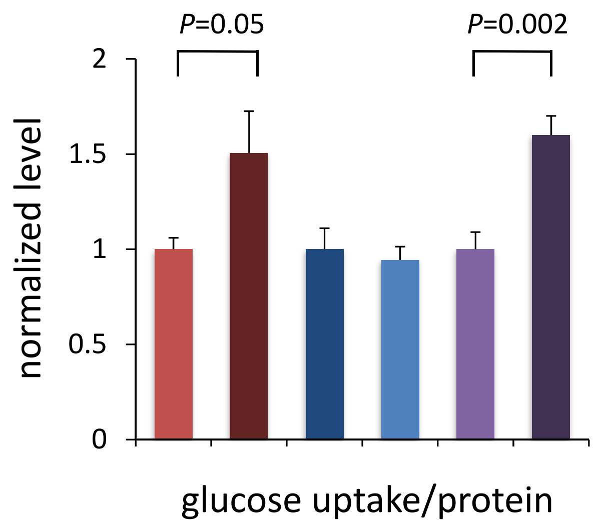
Shi et al. *Dev Cell* 2005; 9:99-108

SORT1–/– adipocytes have no insulin-stimulated glucose uptake

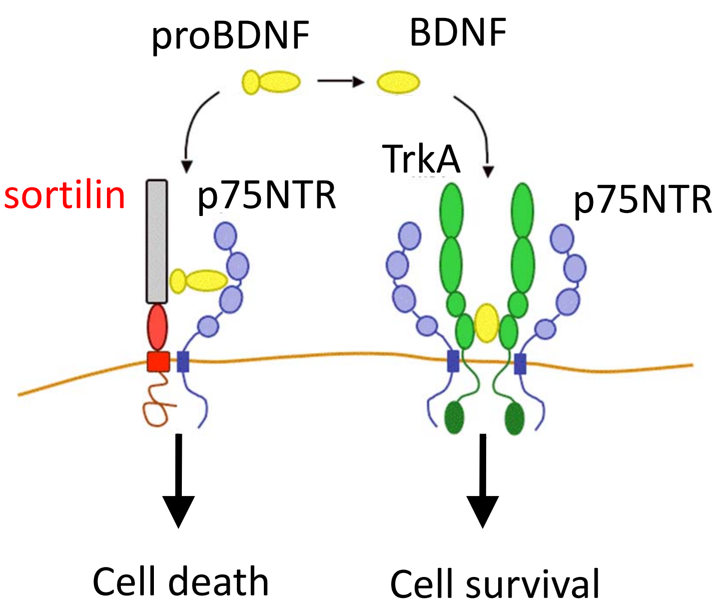
HUES 1 adipocytes

- wild-type + control virus – insulin
- wild-type + control virus + insulin
- *SORT1–/–* + control virus – insulin
- *SORT1–/–* + control virus + insulin
- *SORT1–/–* + *SORT1* virus – insulin
- *SORT1–/–* + *SORT1* virus + insulin

2 clones each



Sort1 in mouse motor neurons (MNs)



Sortilin is essential for proNGF or proBDNF-induced neuronal cell death

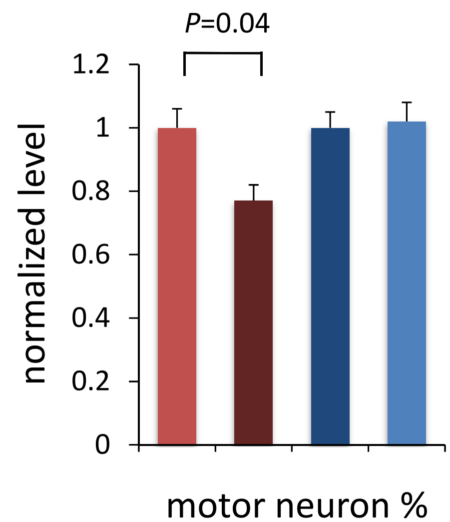
Taylor,et al. *Dev Neurobiol* 2012; 72:699-712
Nykjaer, et al. *Nature* 2004; 427:843-8

SORT1–/– MNs are resistant to proBDNF apoptosis

HUES 9 motor neurons

- wild-type + GDNF + BDNF
- wild-type + GDNF + proBDNF
- *SORT1–/–* + GDNF + BDNF
- *SORT1–/–* + GDNF + proBDNF

2 clones each



Sort1-/- mouse cells vs. *SORT1-/-* human cells

- Knock down or knock out *Sort1* in mouse hepatocytes show contradictory results in terms of apoB secretion
- *SORT1-/-* human hepatocytes show increased apoB secretion
- knock down *Sort1* in 3T3-L1 adipocytes show decreased insulin stimulated glucose uptake
- *SORT1-/-* human adipocytes show no insulin stimulated glucose uptake
- Sortilin blockade enhances mouse motor neuron survival in the presence of proBDNF
- *SORT1-/-* human motor neurons show enhanced survival in the presence of proBDNF

Musunuru, Strong, et al. *Nature* 2010; 466:714-19
Kjolby et al. *Cell Metab* 2010; 12:213-23
Taylor,et al. *Dev Neurobiol* 2012; 72:699-712
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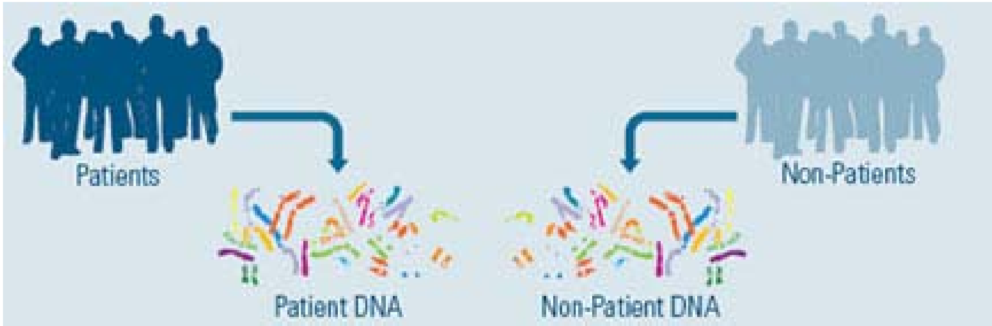

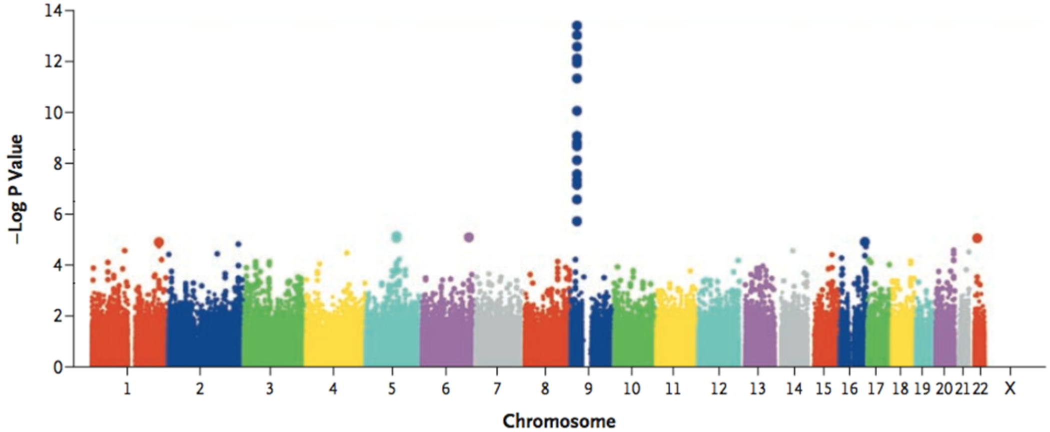

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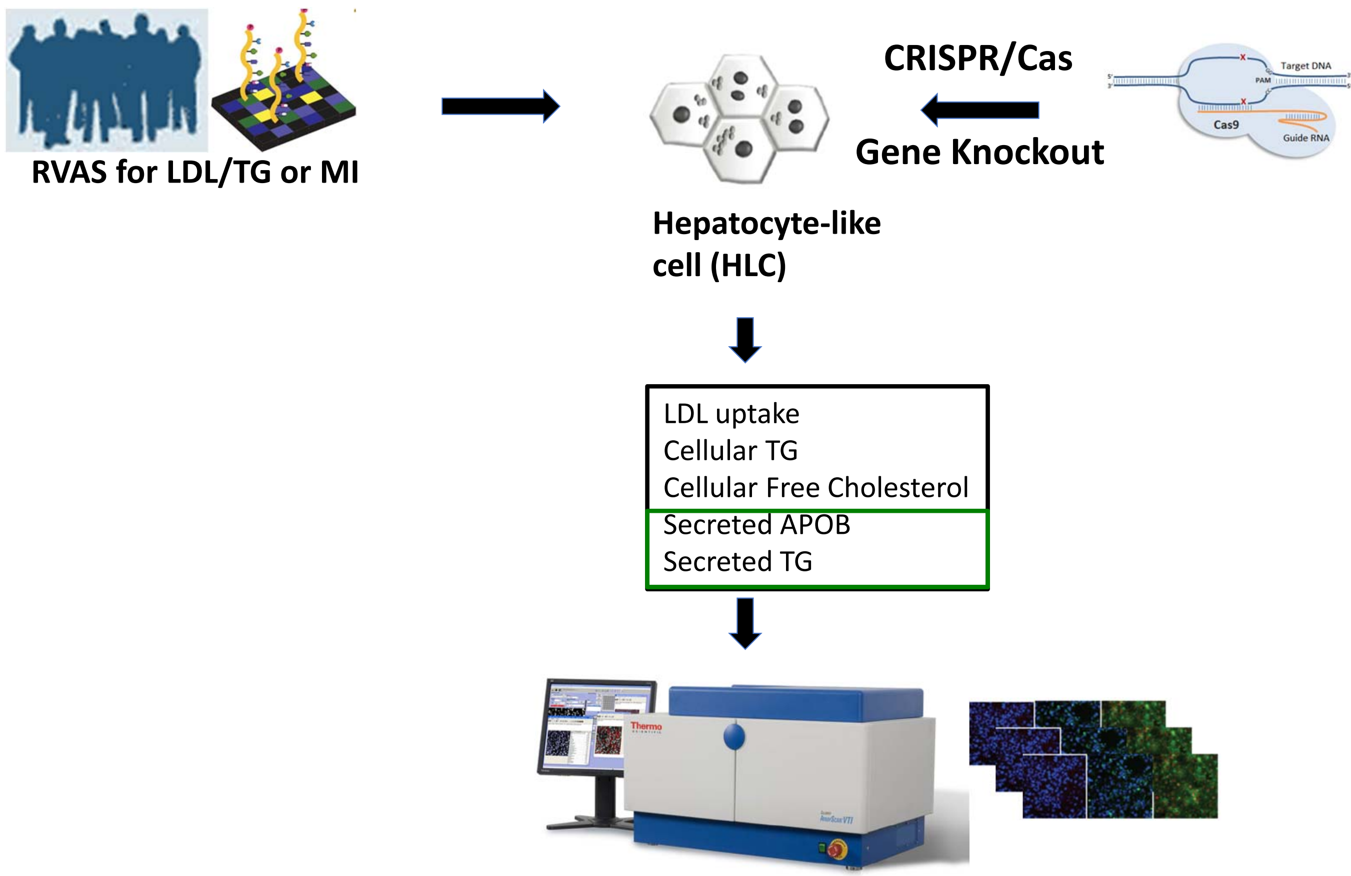
100s of genes and loci identified by genetics studies

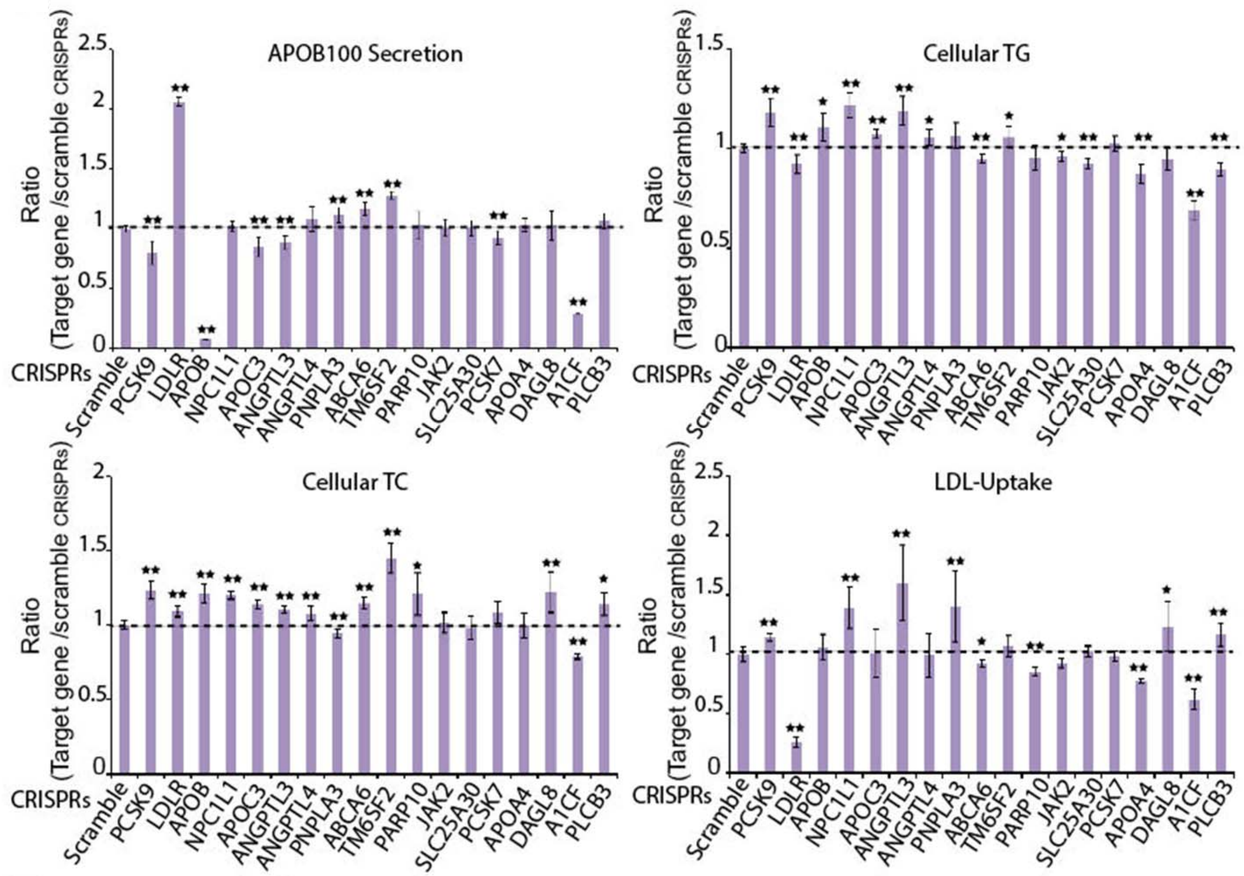
LDL-C			HDL-C			Triglycerides	
ABCG5/8	*HFE*	*SORT1*	*ABCA1*	*HNF4A*	*PDE3A*	*ACSS2*	*GALNT2*
ABO	*HMGCR*	*ST3GAL4*	*ABCA8*	*IRS1*	*PGS1*	*AFF1*	*GCKR*
ANGPTL3	*HNF1A*	*TIMD4*	*ADM*	*KLF14*	*PLTP*	*ANGPTL3*	*IRS1*
APOA	*HPR*	*TOP1*	*ANGPTL4*	*LACTB*	*PPP1R3B*	*ANKRD55*	*JMJD1C*
APOB	*IDOL*	*TRIB1*	*APOA*	*LCAT*	*SBNO1*	*APOA*	*LIPC*
APOE	*IRF2BP2*		*APOB*	*LILRA/B*	*SCARB1*	*APOB*	*LPL*
BRAP	*LDLR*		*APOE*	*LIPC*	*SLC39A8*	*APOE*	*LRP1*
BTNL2	*LDLRAP1*		*ARL15*	*LIPG*	*STARD3*	*BTNL2*	*MLXIPL*
CBLN3	*LPA*		*C6orf106*	*LPA*	*TRIB1*	*CAPN3*	*MSL2L1*
CETP	*MAFB*		*CETP*	*LPL*	*TRPS1*	*CETP*	*NAT2*
CILP2	*MOSC1*		*CITED2*	*LRP1*	*TTC39B*	*CILP2*	*PINX1*
CYP7A1	*NPC1L1*		*CMIP*	*LRP4*	*UBASH3B*	*COBLL1*	*PLA2G6*
DNAH11	*OSBPL7*		*COBLL1*	*MACF1*	*UBE2L3*	*CTF1*	*PLTP*
FADS	*PCSK9*		*DOCK6*	*MC4R*	*ZNF648*	*CYP26A1*	*TIMD4*
FRK	*PLEC1*		*FADS*	*MLXIPL*	*ZNF664*	*FADS*	*TRIB1*
GPAM	*PPP1R3B*		*GALNT2*	*MMAB*		*FRMD5*	*TYW1B*
							ZNF664

Total Cholesterol					
ERGIC3	*EVI5*	*FUT2*	*RAB3GAP1*	*RAF1*	*SPTY2D1*

High throughput phenotypic screens for understanding loci identified via genetics

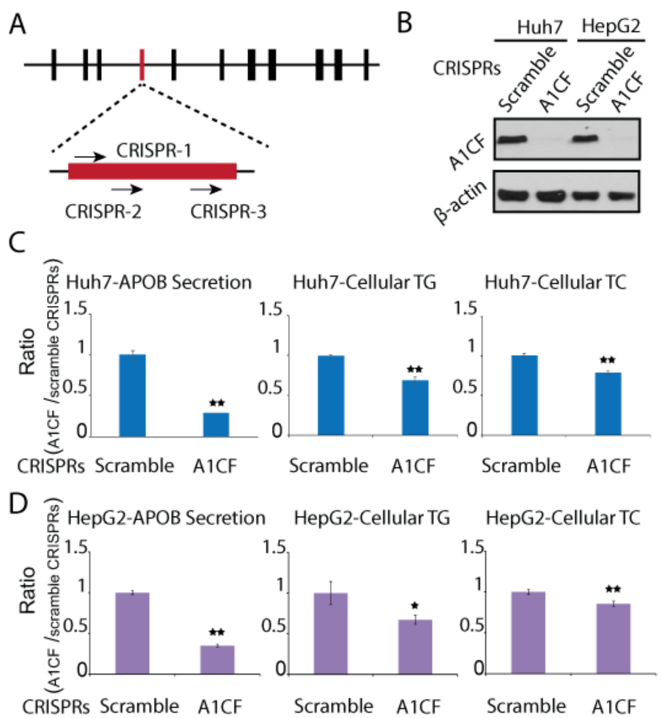


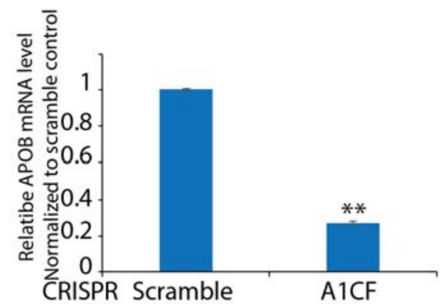
High-throughput phenotypic screening



Yu et al. *Nature Genetics* 2017

A1CF regulates hepatic and blood cholesterol and triglyceride levels

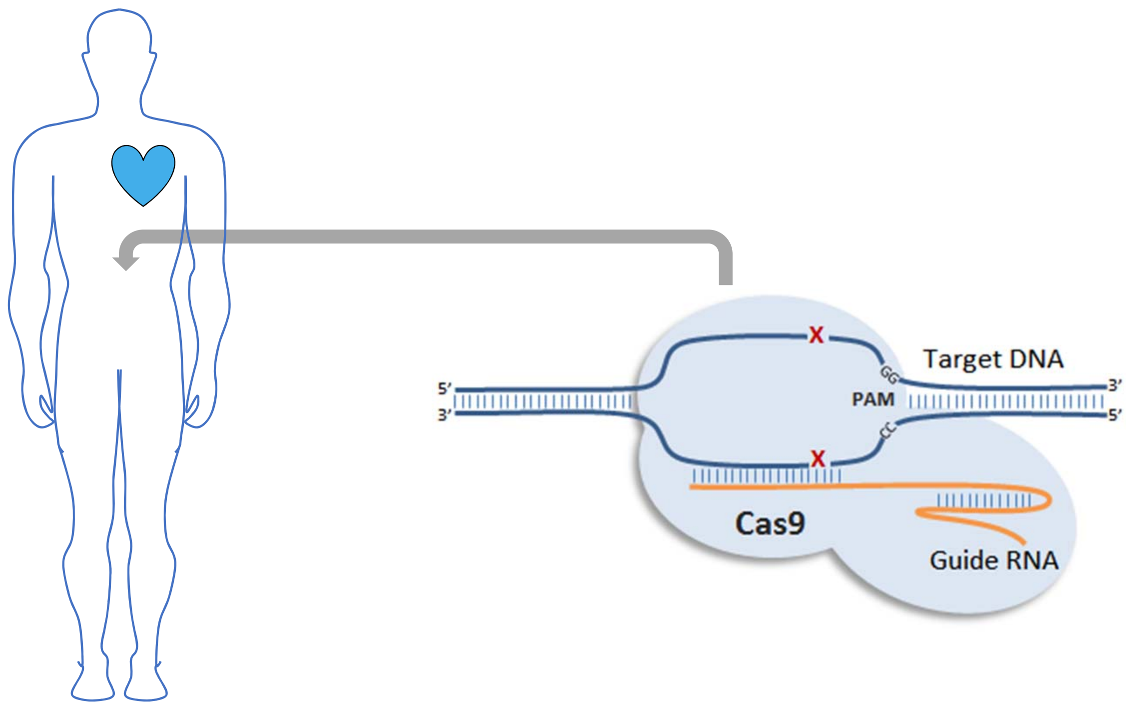




Gene Name	p-value	Fold Change (WT vs KO)
APOB100	0.002544	1.91
SREBF1	0.005796	1.53
FASN	0.013898	1.44
SCD	0.006216	1.36
ELOVL5	0.046486	1.33
ELOVL6	0.008088	1.82
PPARG	0.008356	1.54
MVD	0.007653	1.90

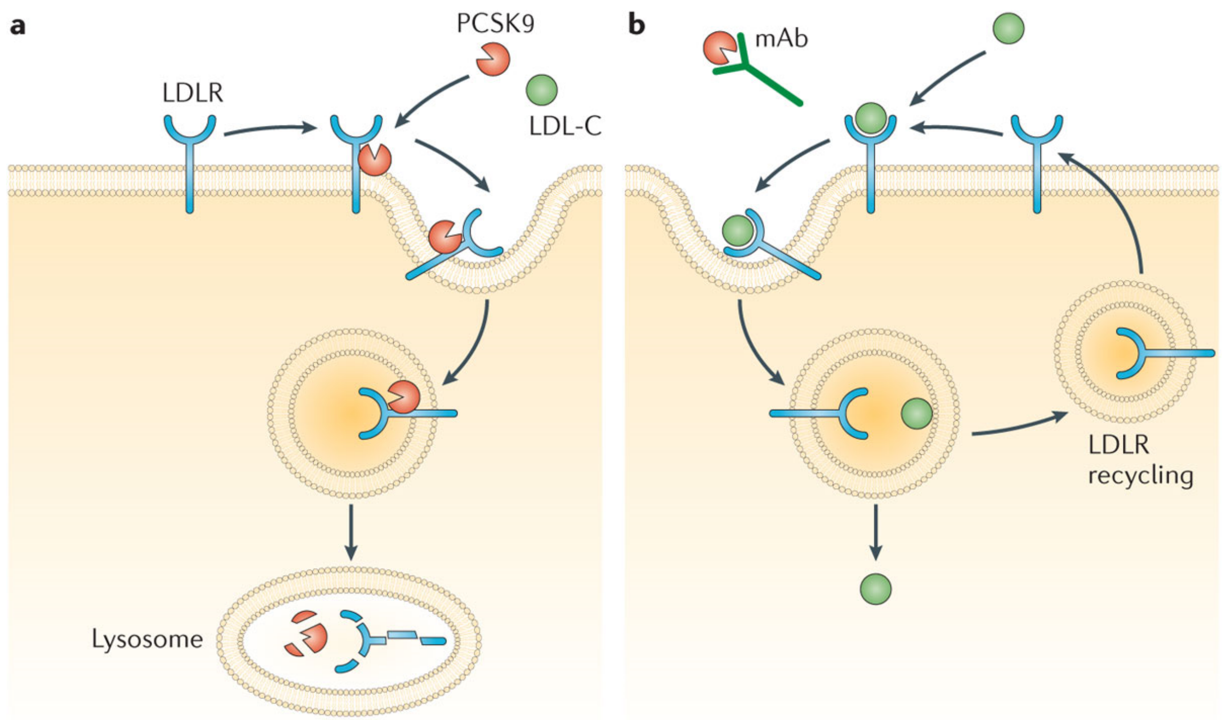
Yu et al. *Nature Genetics* 2017

In Vivo Gene Targeting



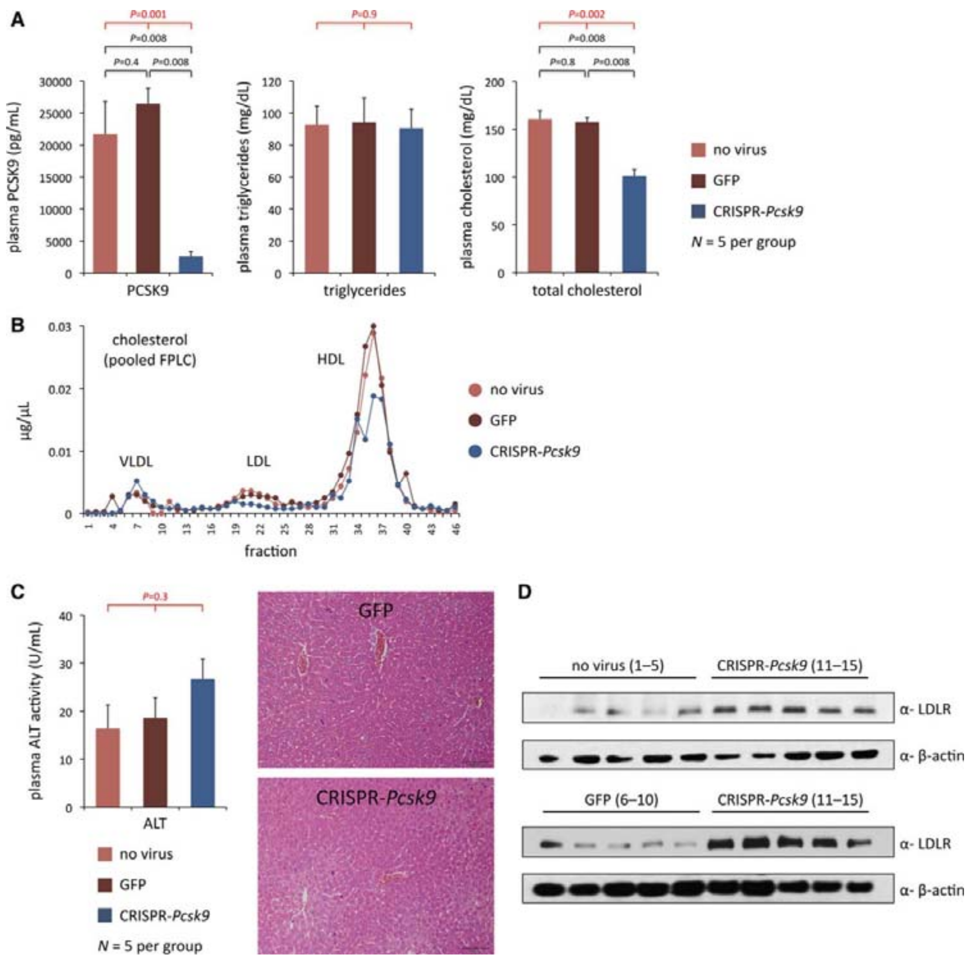
patient

PCSK9 inhibition lowers LDL Cholesterol



Nature Reviews | Drug Discovery

CRISPR/Cas9 ablation of PCSK9 *In Vivo* Reduces Plasma Cholesterol



Qiurong Ding et al. Circulation Research. 2014;115:488-492

Acknowledgements

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Alanna Strong