Role of IGF2BP2 and HMGA2 in adipocyte differentiation and Type 2 Diabetes

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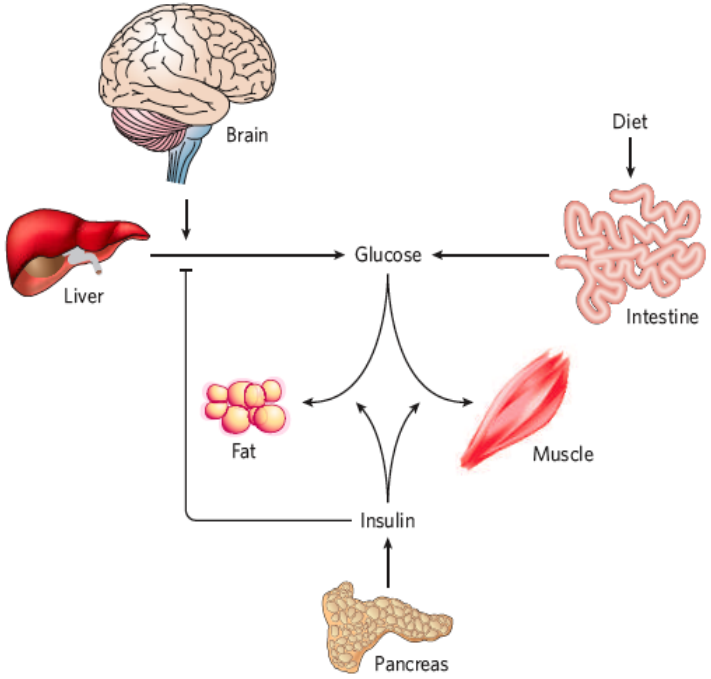
Adipose tissue is necessary for proper glucose regulation, which protects against T2D

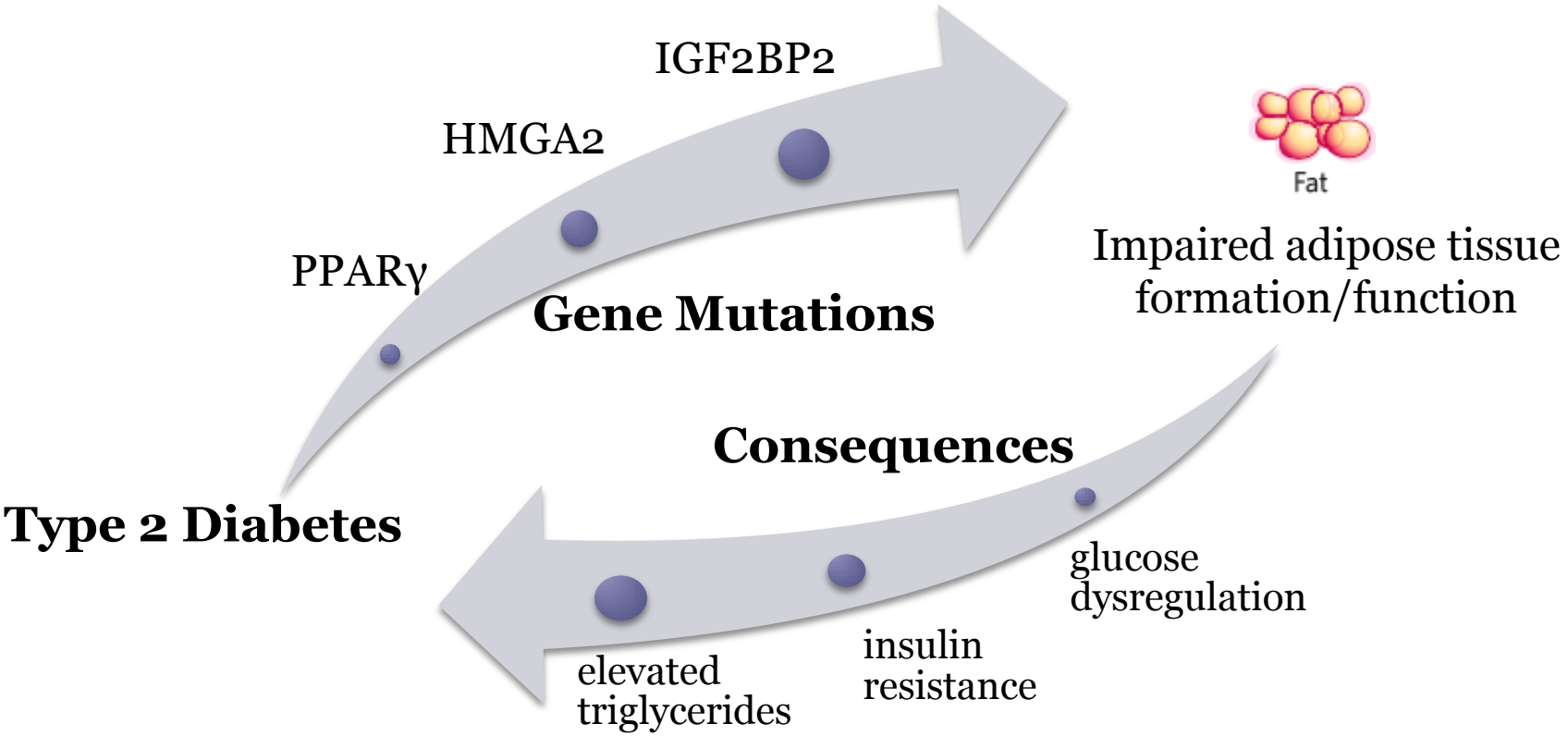
- Normal Glucose Homeostasis
  - Glucose enters the blood stream
  - Pancreatic β-cells secrete insulin
  - Adipocytes secrete hormones that increase insulin sensitivity, repress food intake and glucose production
- Type 2 Diabetes

Elevated glucose levels in blood
Insulin Resistance
Impaired insulin secretion
Over production of glucose



Rosen & Spiegelman, Nature 2006

Identify what is the potential role of genes in GWAS loci in adipocytes to understand association with T2D

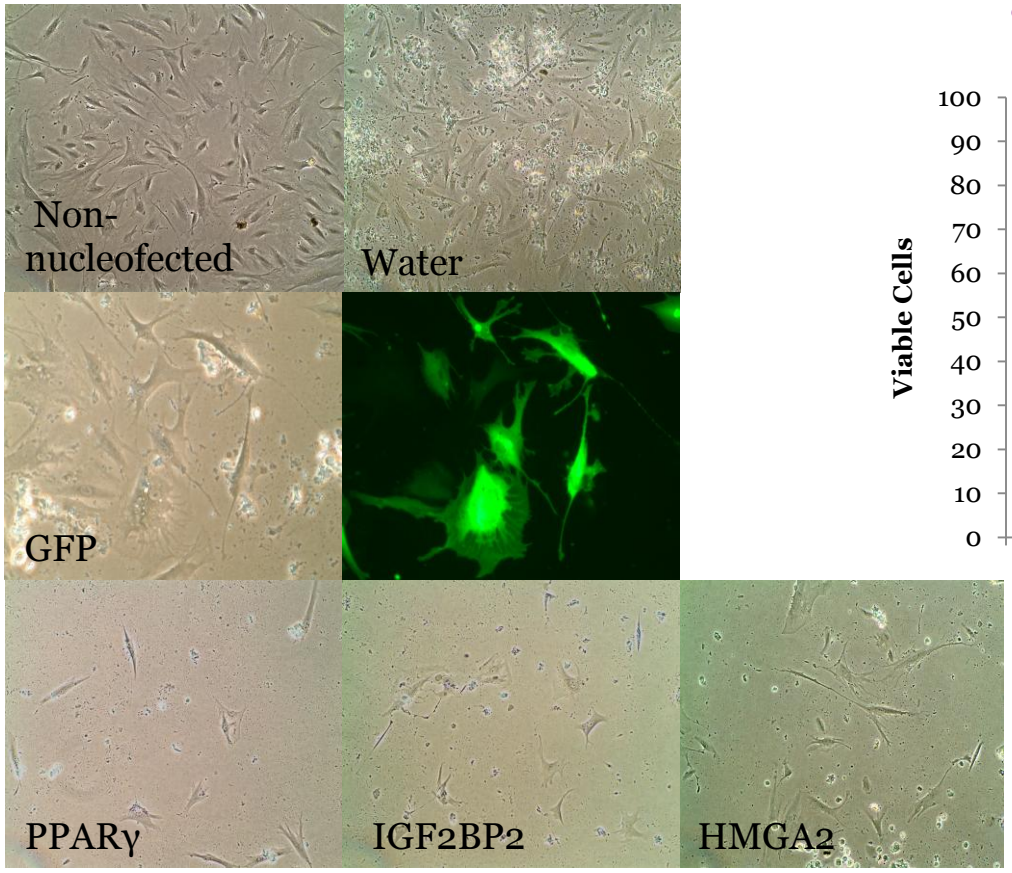


Aims

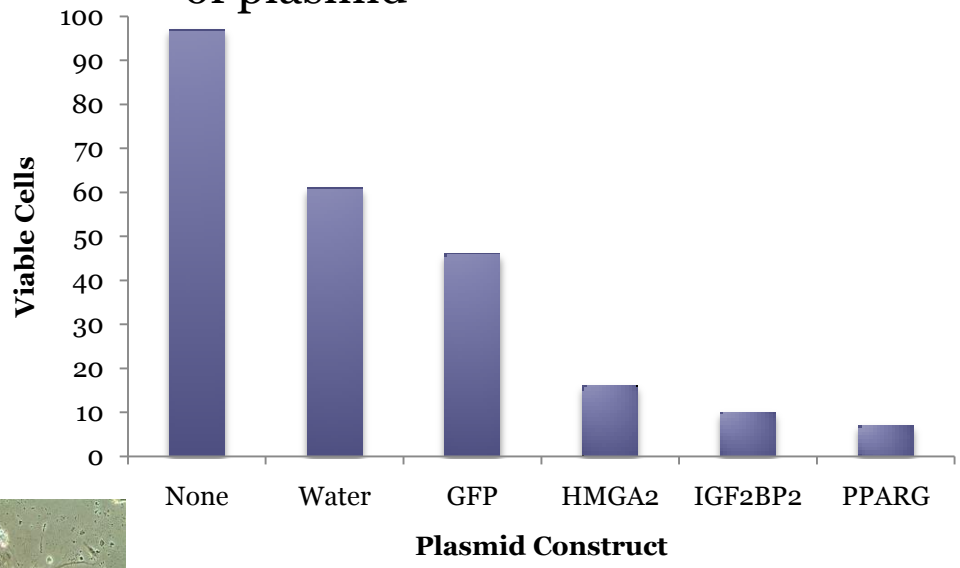
1. To identify the optimal method for manipulating gene expression in Human preadipocytes
2. Differentiate genetically altered Human preadipocytes into adipocytes

Gene Overexpression

- **Nucleofection**: ~70% efficiency but low survival rate



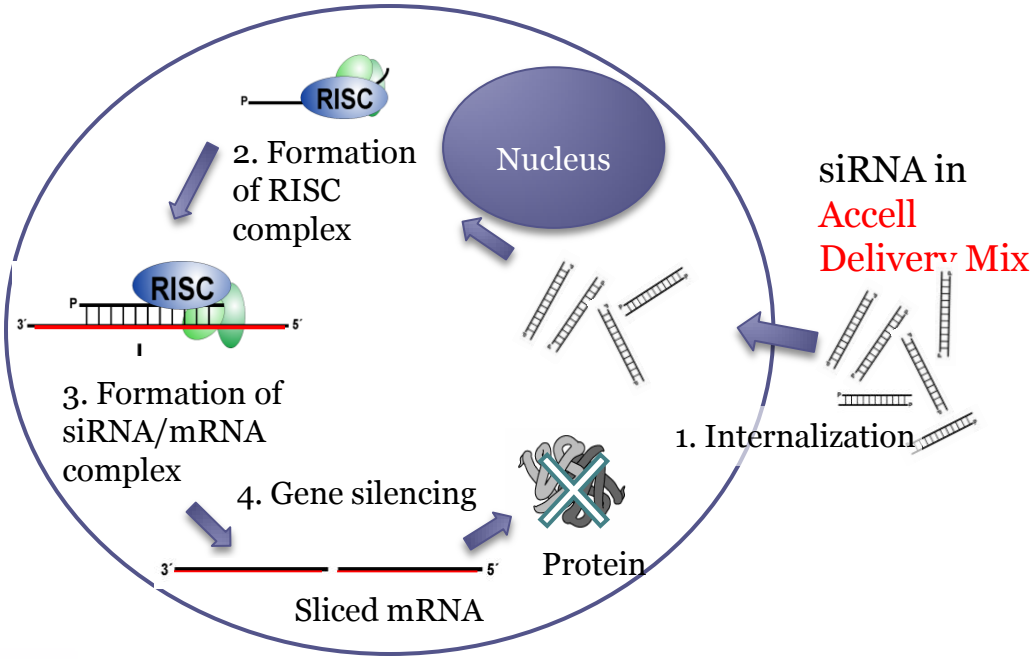
- Cell Survival decreased in presence of plasmid

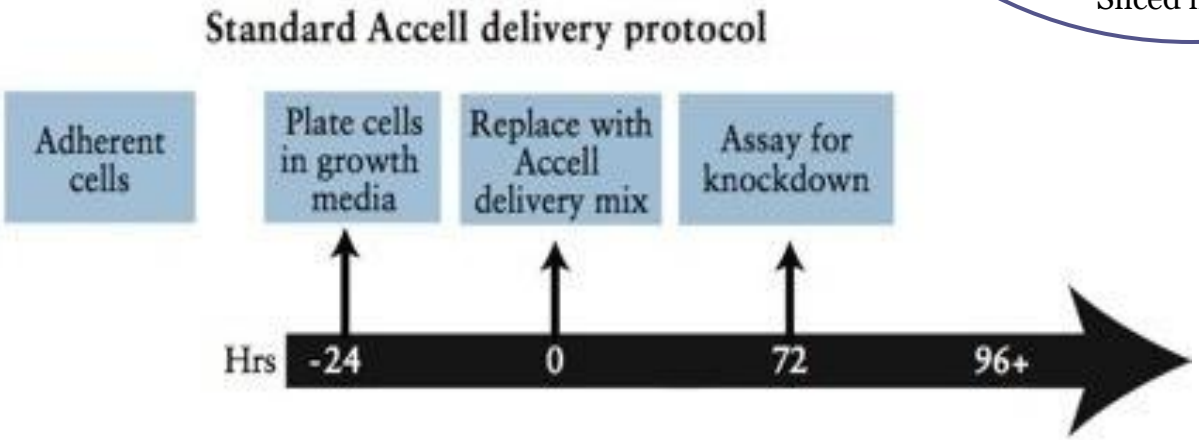


Gene Knockdown

Accell Delivery Media

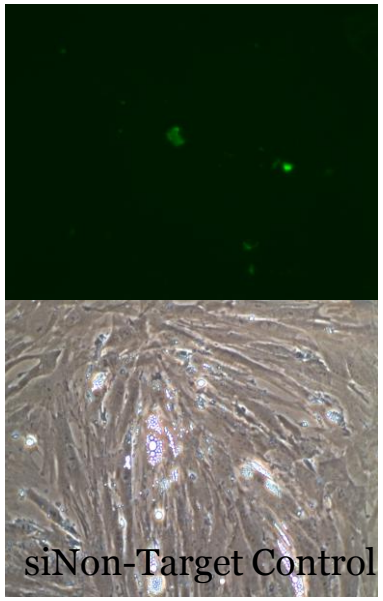
- Enhances siRNA, enabling passage through cell membrane



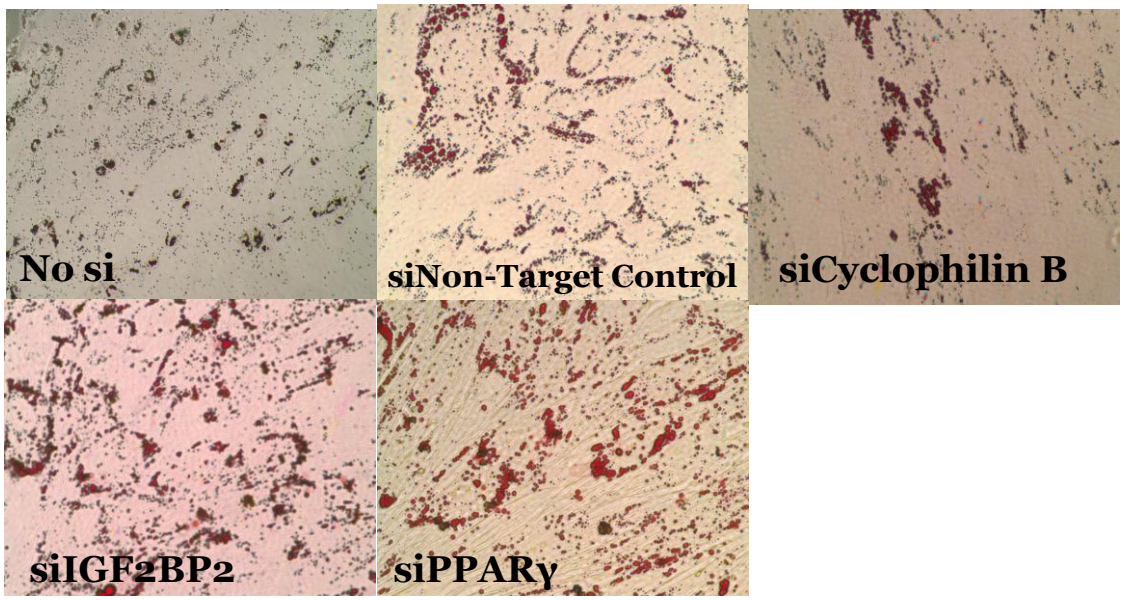


Experiment: Assess genes knockdown. Accell delivery protocol is staggered with preadipocyte differentiation

- Delivery efficiency was <1%

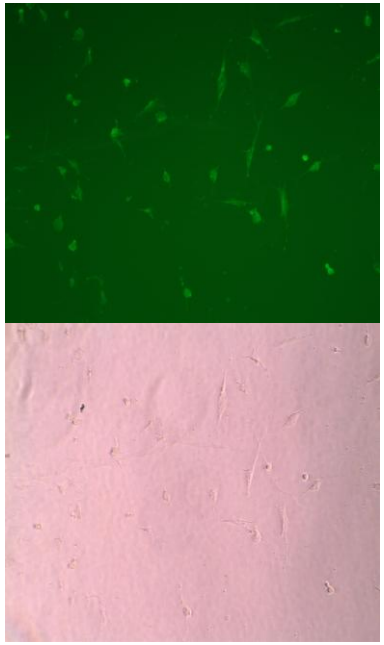


- Oil Red O Stains lipid droplets in cells red
- Lipid absorbtion was uninhibited.



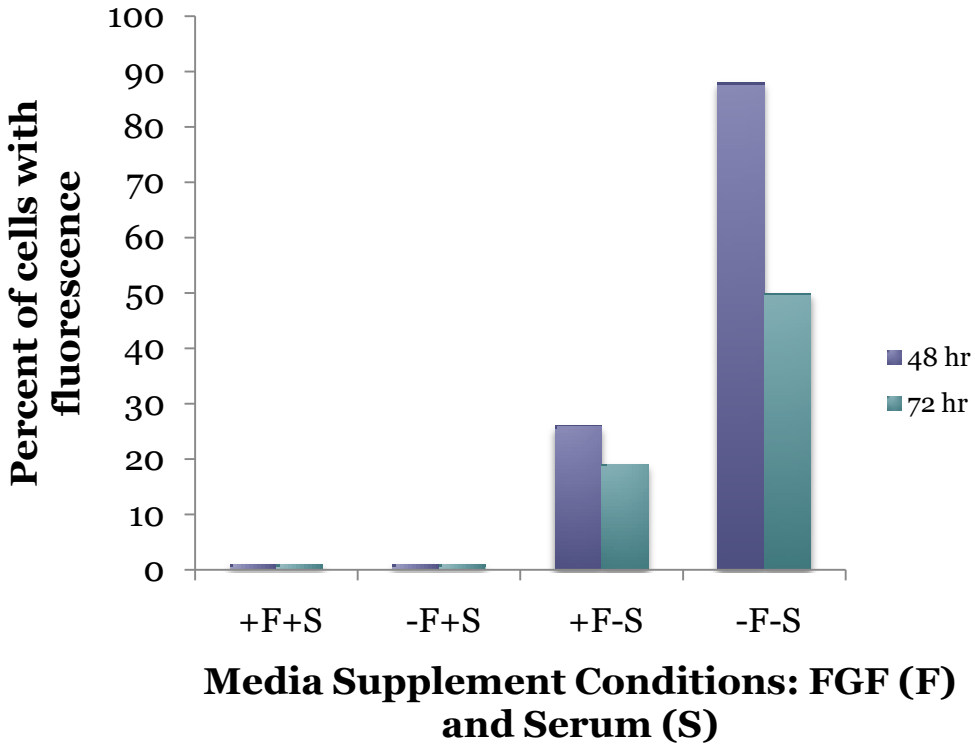
Experiment: Assess effect of FGF/Serum On Accell mediated gene knockdown

- Delivery efficiency was ~23%

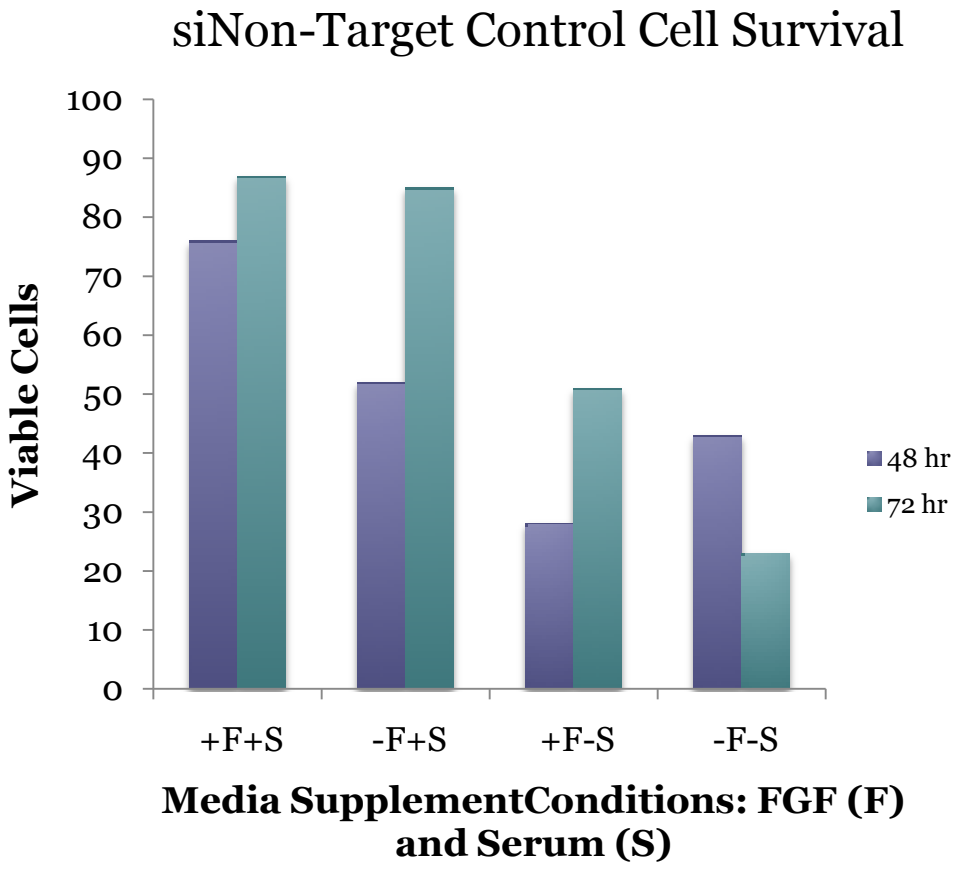


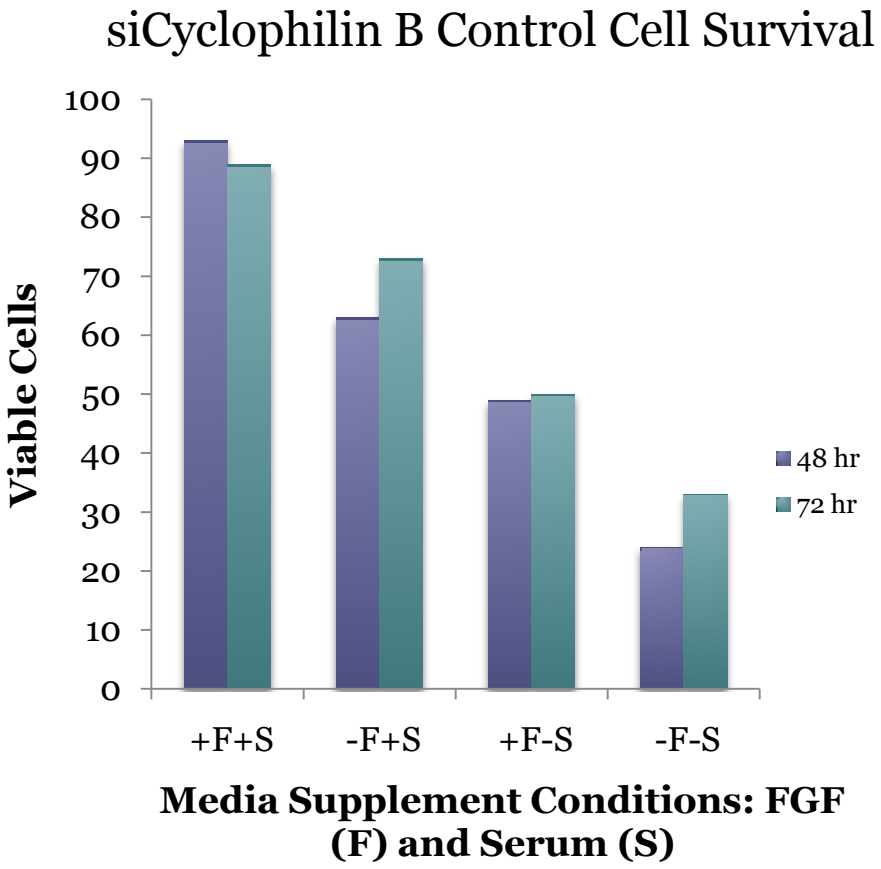
siNon-Target Control

- siRNA Delivery Efficiency increases in absence of serum and FGF



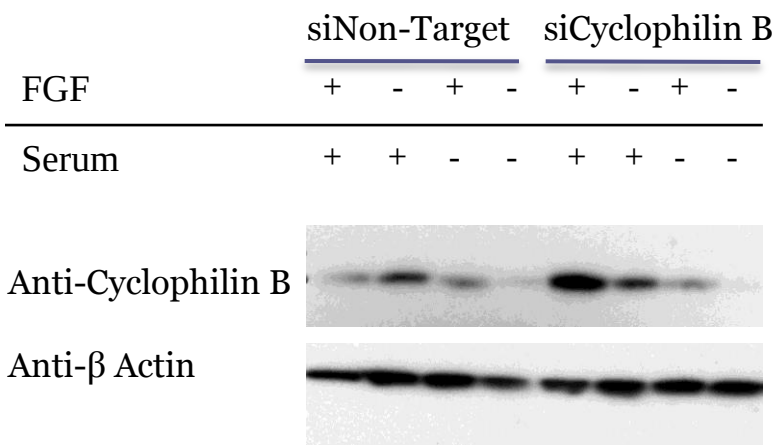
Absence of FGF/Serum decreases cell survival rate



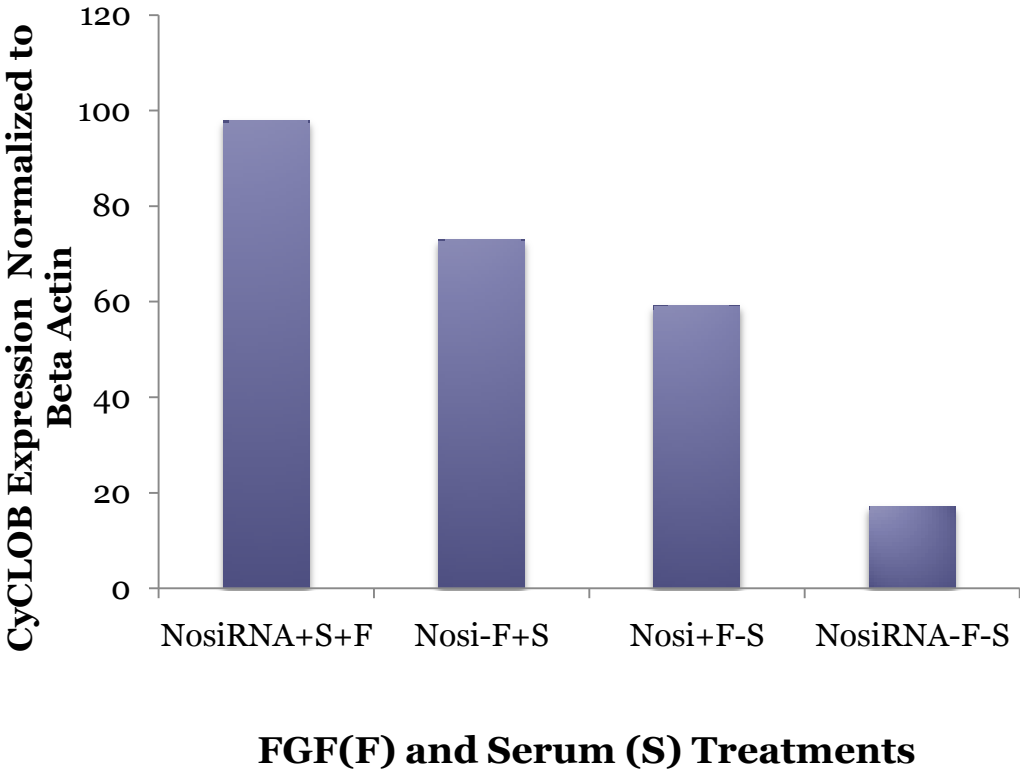


Two possibilities: FGF/serum interfere with knockdown or Cyclophilin B expression is FGF/serum dependent

- Western Blot



- Quantitative PCR on No si treated Samples



Conclusion

- Nucleofection yields greater efficiency than Lipofectamine treatment but higher cell mortality
- Accell mediated knockdown may not be suitable to assess differentiation of knockdown preadipocytes
- Use viral vectors knockdown and overexpress genes

Acknowledgments

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