

# Association of Maternal Serum Per- and Polyfluoroalkyl Substances with Offspring Mesenchymal Stem Cell Transcriptome and DNA Methylation

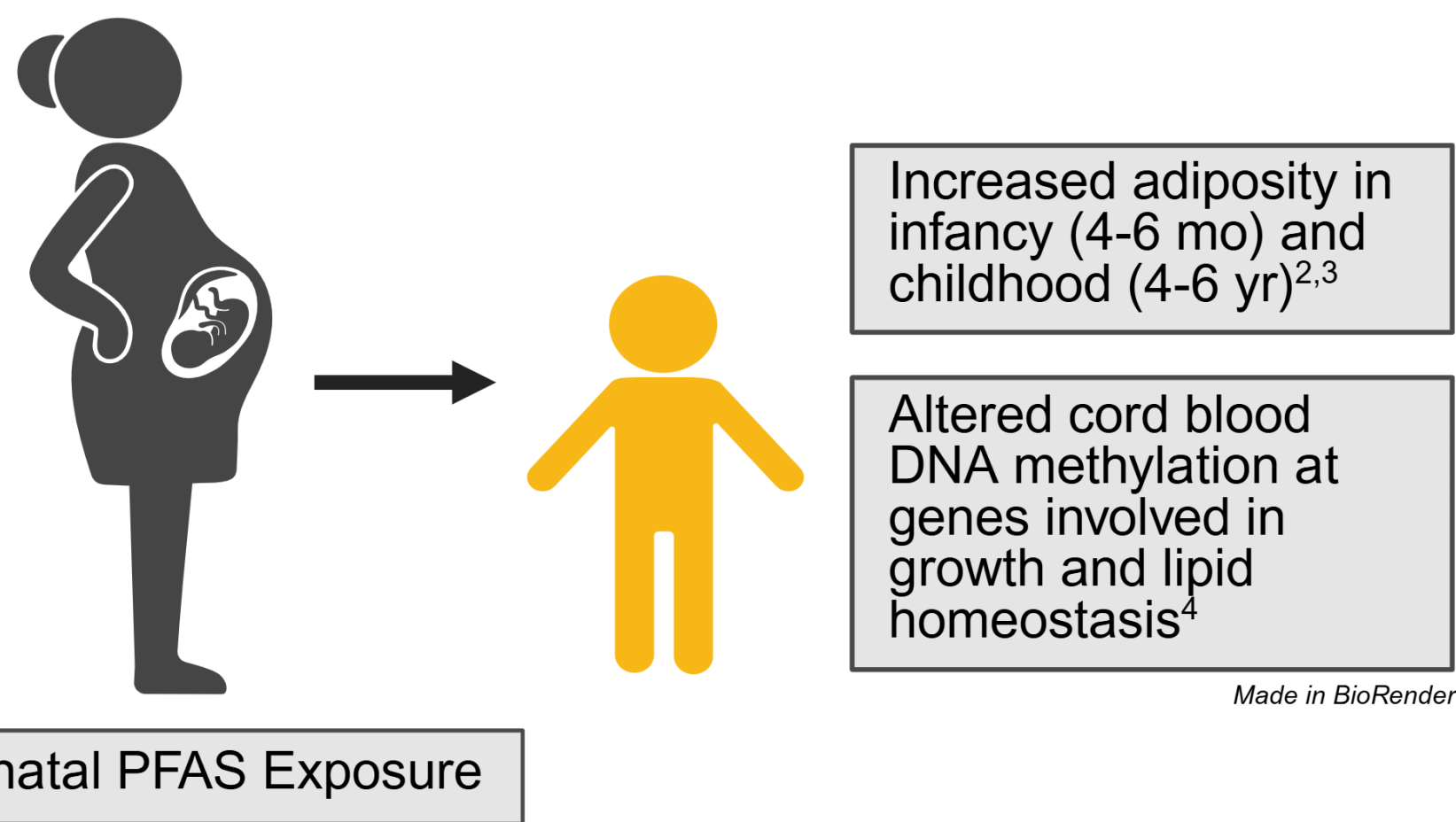


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## Background

- Per- and Polyfluoroalkyl Substances (PFAS) are persistent and ubiquitous environmental pollutants.
- PFAS can cross the placenta during pregnancy and can therefore directly impact fetal development.<sup>1</sup>
- Prenatal PFAS exposure is associated with adverse cardiometabolic outcomes in offspring, as previously reported in the Healthy Start Cohort.

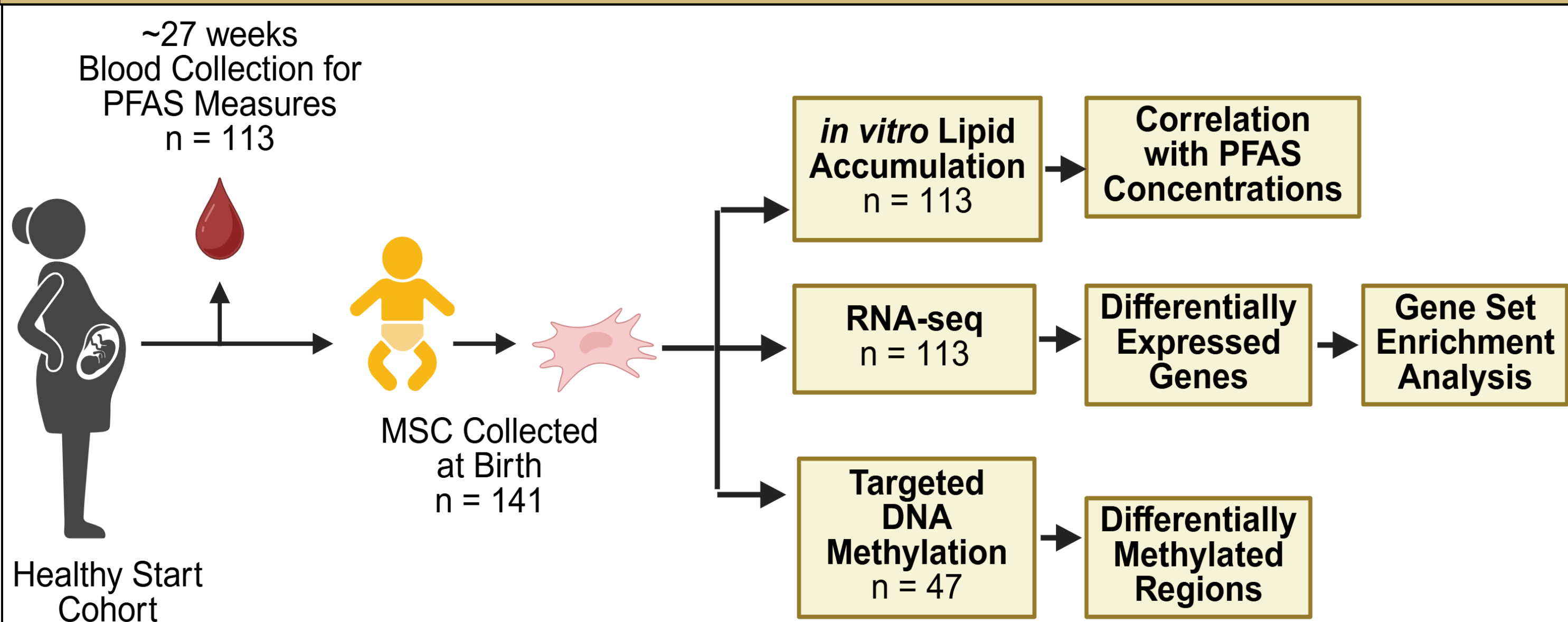


- To better understand how prenatal PFAS exposures may be linked to epigenetic signatures promoting long-term adiposity-related outcomes in offspring, this study utilized offspring MSC from the Healthy Start Cohort.
- Mesenchymal stem cells (MSC) are the precursor to adipocytes and myocytes, which are metabolically critical cell types.
- Offspring umbilical cord-derived MSC phenocopy the offspring from which they are derived, making them a useful tool to investigate the molecular mechanisms underpinning offspring metabolic phenotypes.

## Hypothesis

**Prenatal PFAS exposure is associated with transcriptomic and epigenetic changes in infant MSCs, primarily in genes related to lipid metabolism and cardiometabolic pathways.**

## Methods

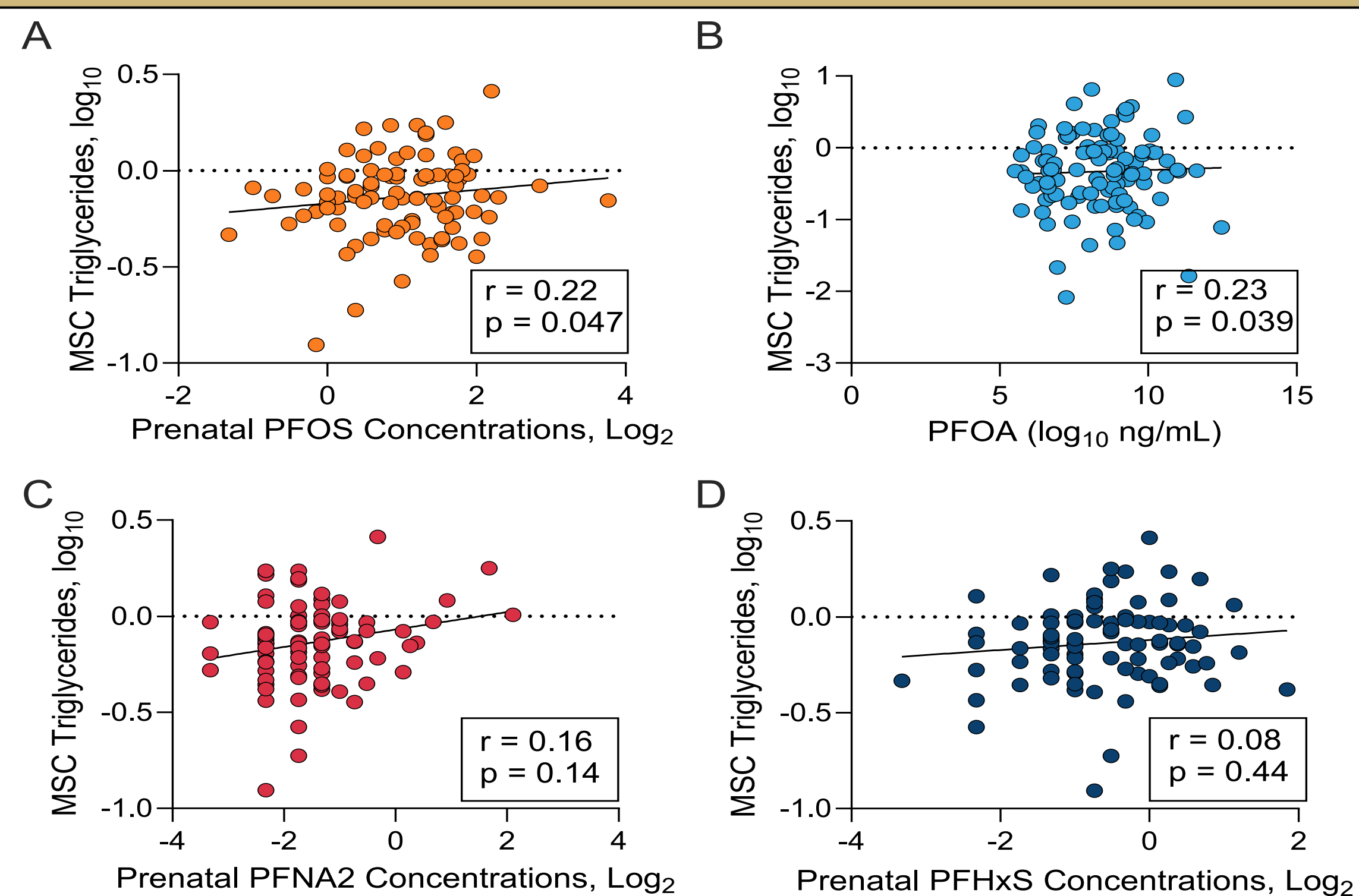


**Figure 1.** Depiction of Healthy Start cohort and downstream applications of samples collected and utilized for this study. Created in BioRender.

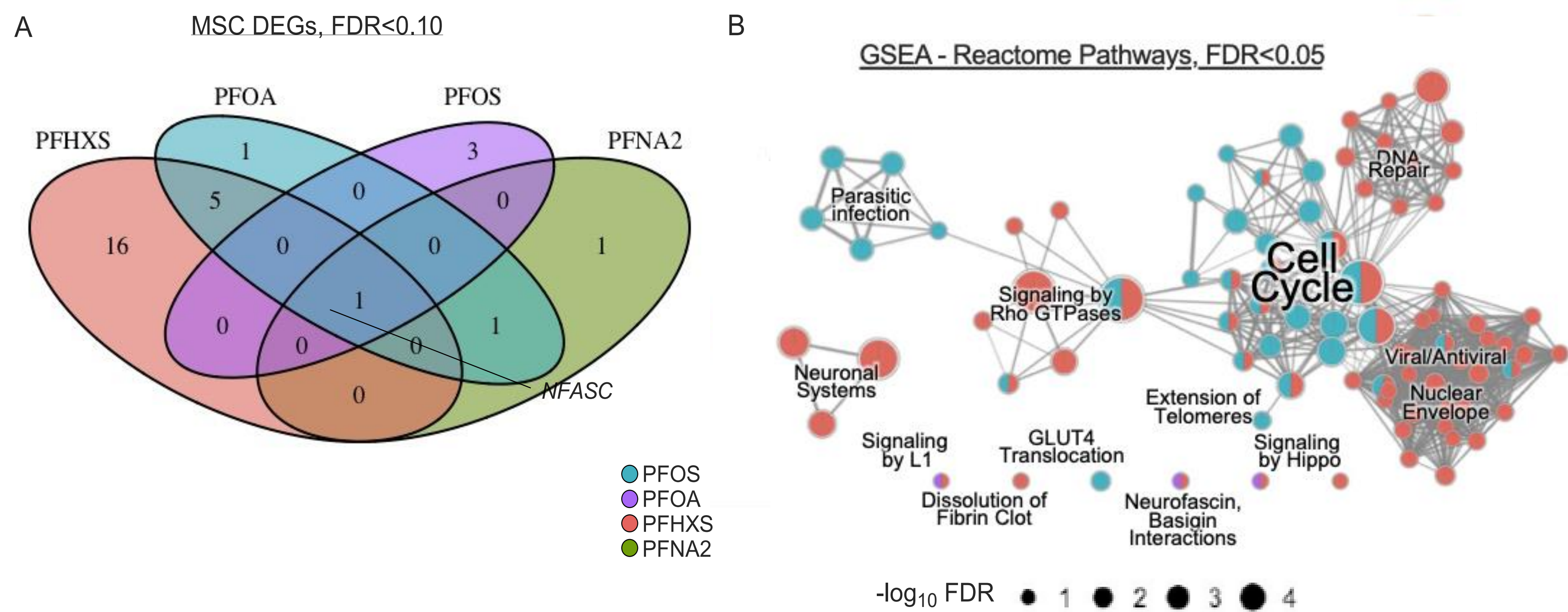
## Cohort Characteristics

Maternal Characteristics	
Maternal Age in Years, Mean (SD)	28.6 (6.1)
Pre-pregnancy BMI (kg/m <sup>2</sup> ), Mean (SD)	68 (61.8%)
Offspring Characteristics	
Females, n (%)	62 (44%)
Gestational age at birth, weeks, Mean (SD)	39.5 (1.2)
Birth weight, grams, Mean (SD)	3272 (429)

## MSC Triglyceride Content is Correlated with PFAS Concentrations



## Maternal PFAS Levels Are Associated with Changes in MSC Transcription



**Figure 3.** (A) Venn diagram of overlapping transcripts associated with maternal serum chemical concentrations at FDR < 0.10. (B) Reactome pathways associated with maternal PFOA n=35, PFHxS (n=70), and PFOS.

## Maternal PFOS and PFHxS Levels Are Associated With Changes in MSC DNA Methylation

	Gene	Region	Normalized Enrichment Score	Adjusted P-Value
PFOS	TRIM55	promoters	-1.8206	0.0342
	NFASC	gene	1.6532	0.0595
	L1CAM	promoters	-1.6221	0.0782
	WNT4	promoters	1.6516	0.0782
	NFASC	CGI	1.6513	0.3617
PFHxS	NFASC	promoters	1.7762	0.0064
	TRIM55	gene	-1.9041	0.0144
	TRIM55	CGI	1.7896	0.0221
	chr1:204950982-204951210 (FASC)	CGI	1.7697	0.0910
	TXNDC5	gene	1.6804	0.0289

**Figure 4.** Top 5 differentially methylated regions in association with PFOS and PFHxS.

## Conclusion

**Prenatal PFAS levels are associated with transcriptomic and DNA methylation changes in infant MSCs, which may contribute to increased metabolic risk.**

## Funding Sources

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## References

- Fromme H, Mosch C, Morovitz M, et al. Pre- and Postnatal Exposure to Perfluorinated Compounds (PFCs). *Environ Sci Technol.* 2010;44(18):7123-7129
- Starling AP, Adgate JL, Hamman RF, Kechris K, Calafat AM, Dabelea D. Prenatal exposure to per- and polyfluoroalkyl substances and infant growth and adiposity: the Healthy Start Study. *Environ Int.* 2019;131:104983.
- Starling AP, Friedman C, Boyle KE, et al. Prenatal exposure to per- and polyfluoroalkyl substances and early childhood adiposity and cardiometabolic health in the Healthy Start study. *Int J Obes.* 2024;48(2):276-283.
- Starling AP, Liu C, Shen G, et al. Prenatal Exposure to Per- and Polyfluoroalkyl Substances, Umbilical Cord Blood DNA Methylation, and Cardio-Metabolic Indicators in Newborns: The Healthy Start Study. *Environ Health Perspect.* 2020;128(12):127014.