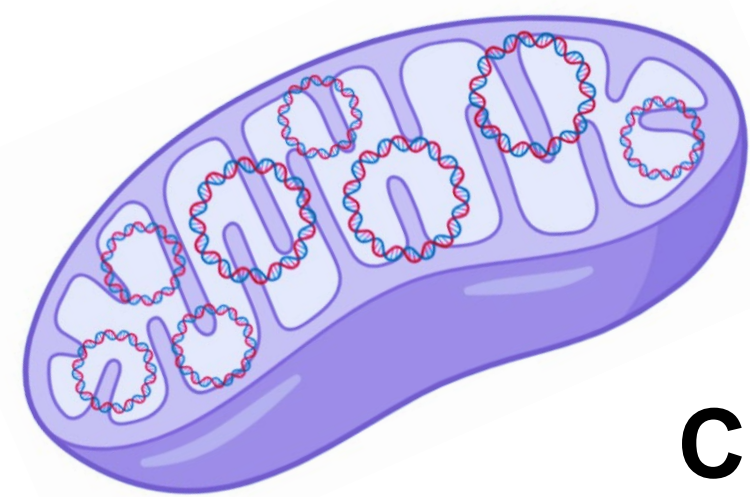


Saliva and blood cell-free mtDNA reactivity to acute psychosocial stress

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Picard Lab

Introduction

Cell-free mitochondrial DNA (cf-mtDNA) – an emerging disease biomarker



Chronic

Physiological

- Cancer ▲
- Heart failure ▲
- Inflammatory conditions ▲
- Conditioning ▼

Psychological

- Major depressive disorder ●
- Bipolar disorder ●

Acute

- Exercise ▲
- Trauma ▲
- Sepsis ▲

- Suicide attempt ▲
- Psychosocial stress test ▲

▲ Higher

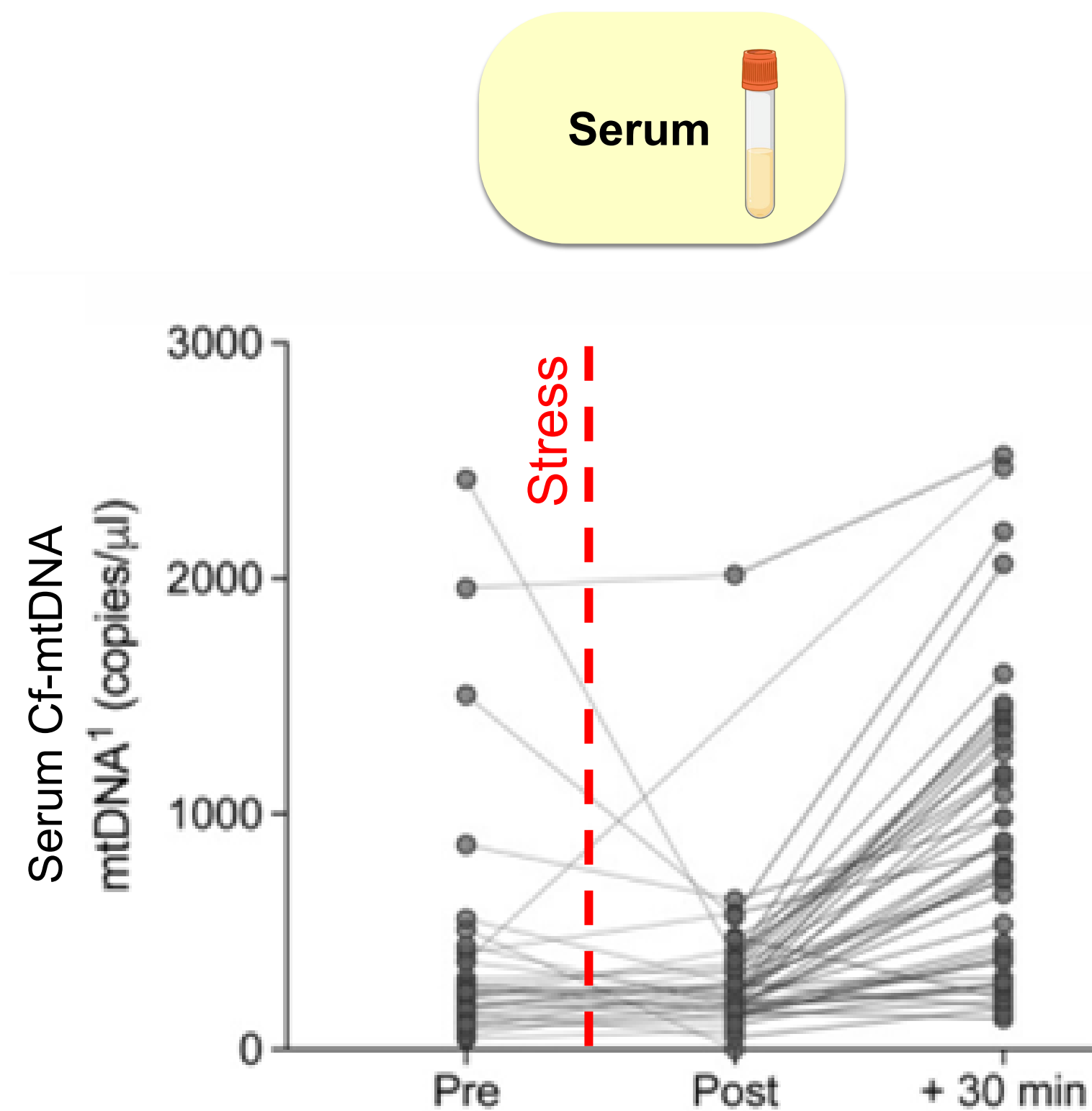
▼ Lower

● Mixed Results

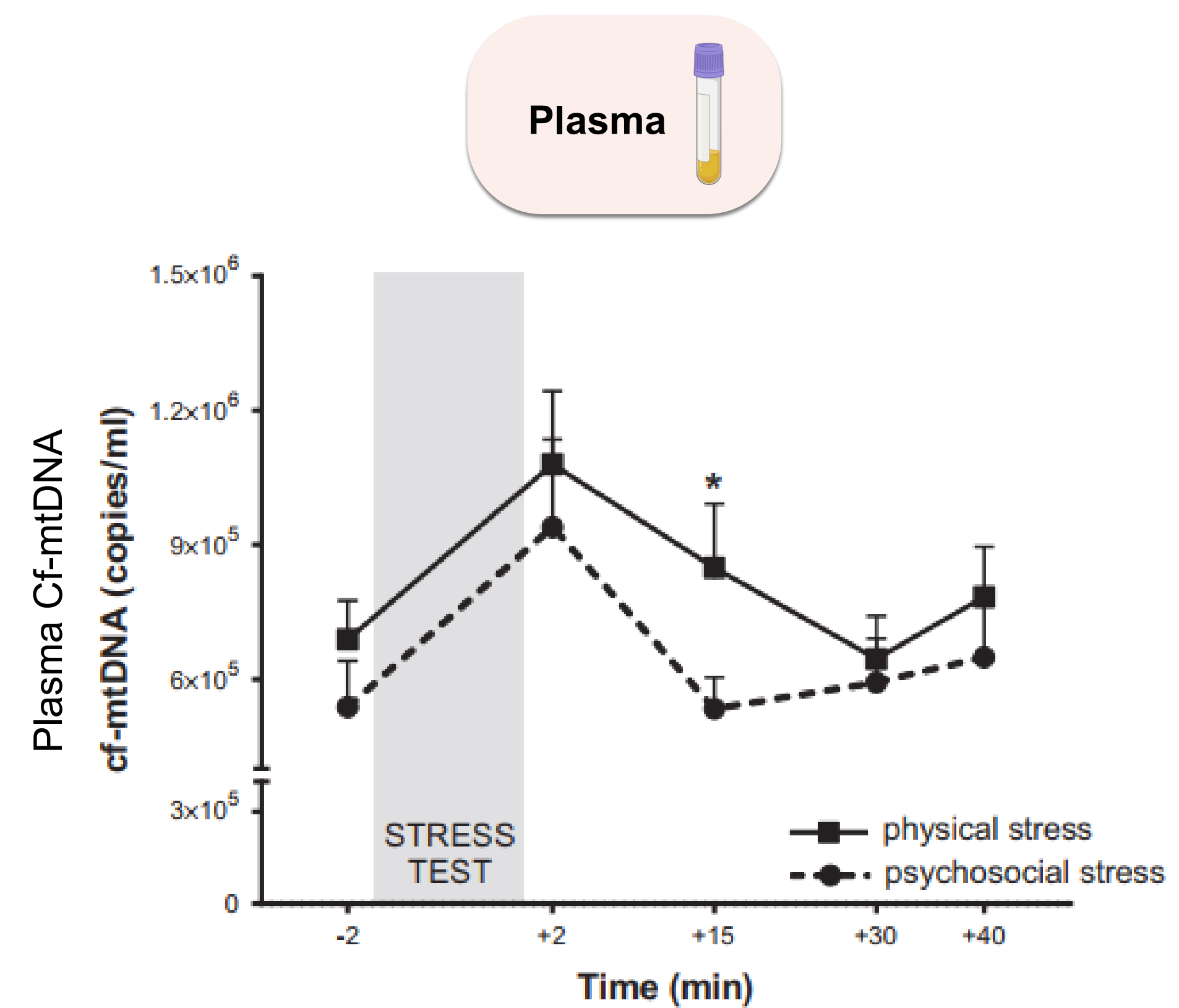
- cf-mtDNA is elevated under conditions of acute and chronic physiological stress
 - Studies of cf-mtDNA in relation to psychopathology have mixed results

Introduction

Evidence of cf-mtDNA reactivity to acute psychological stress in blood



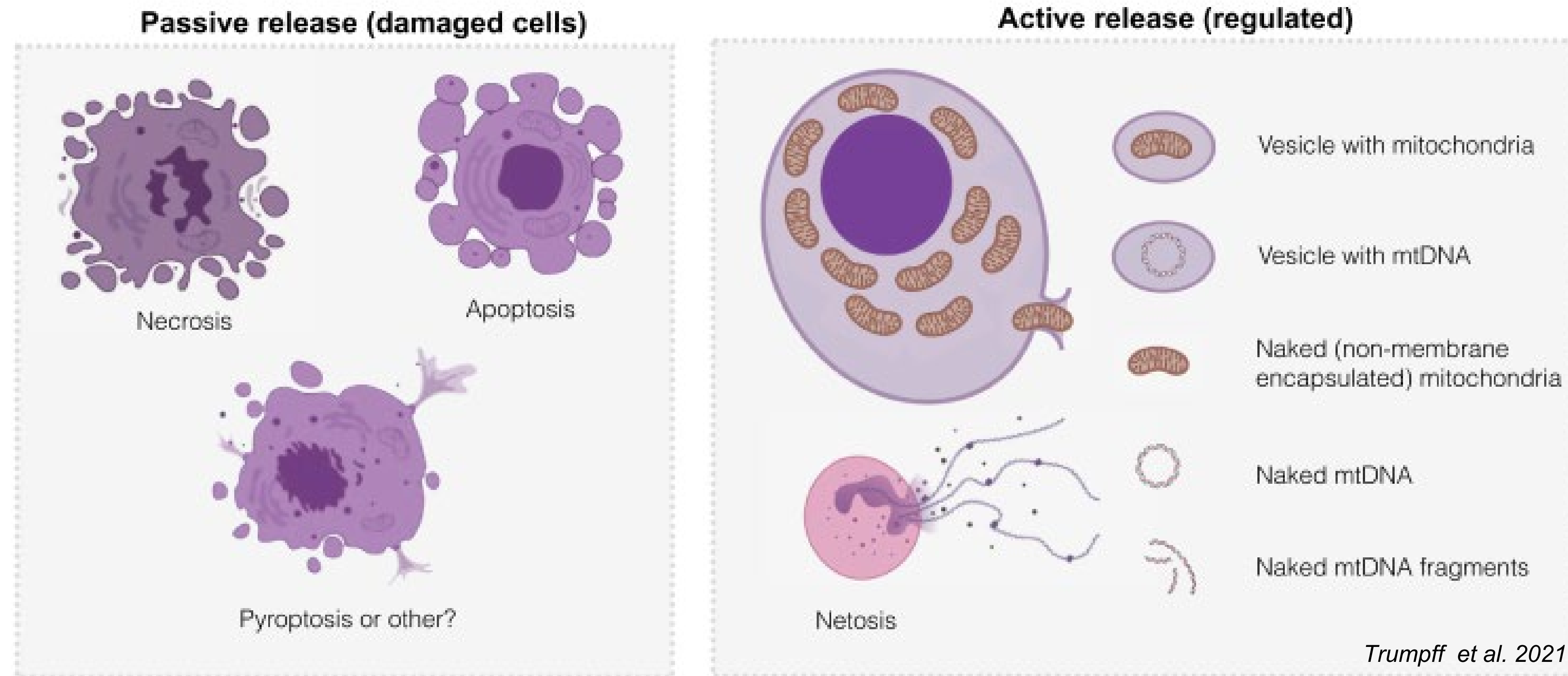
Trumpff et al. 2019



Hummel et al. 2018

Introduction

Hypothetical forms of cf-mtDNA in circulation



The majority of cf-mtDNA in circulation is thought to be contained within a membrane, not 'naked' DNA

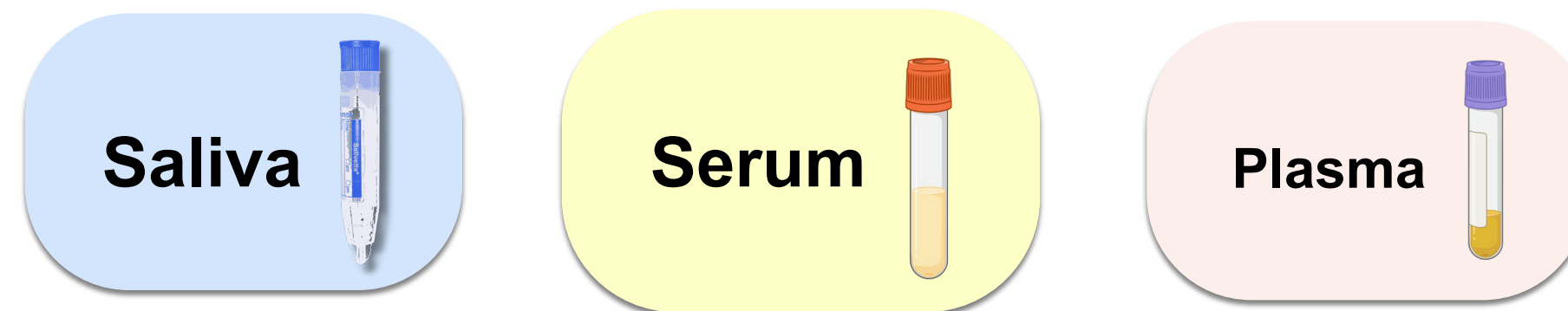
Introduction

Objectives

- Evaluate whether **saliva** cf-mtDNA levels change in response to acute psychosocial stress



- Compare cf-mtDNA response in different biofluids

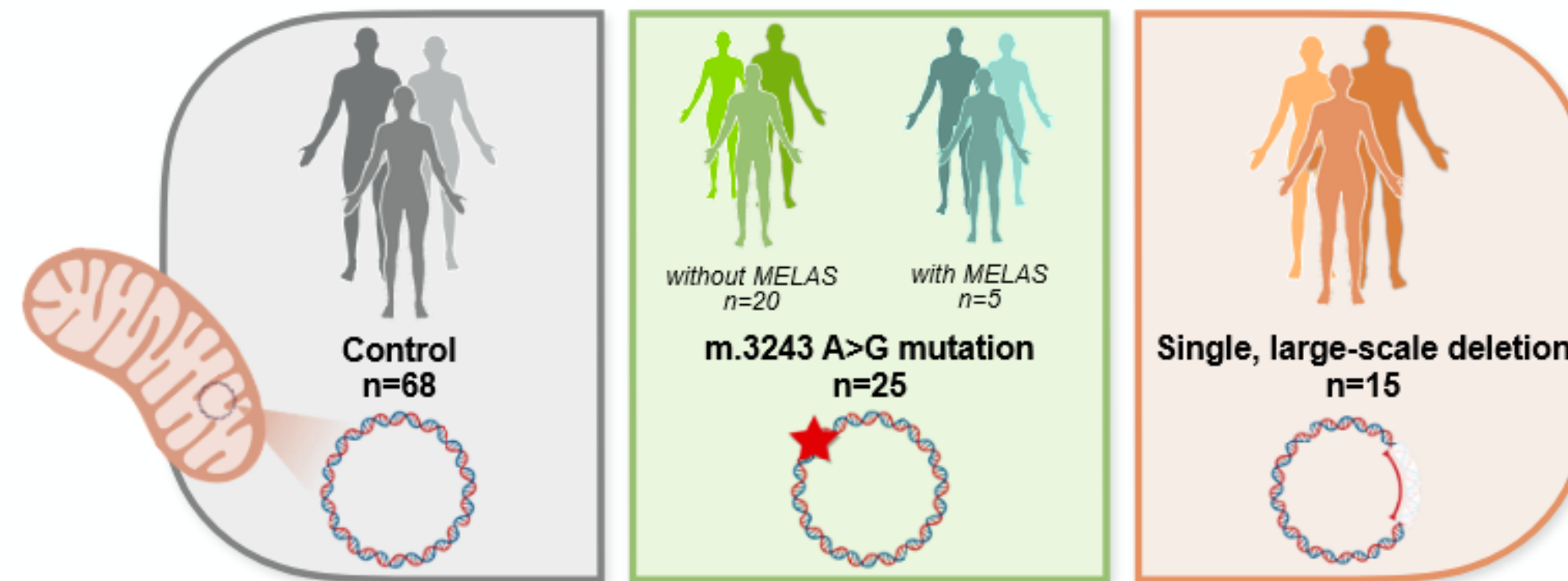


- Compare cf-mtDNA levels and reactivity in mitochondrial disease patients

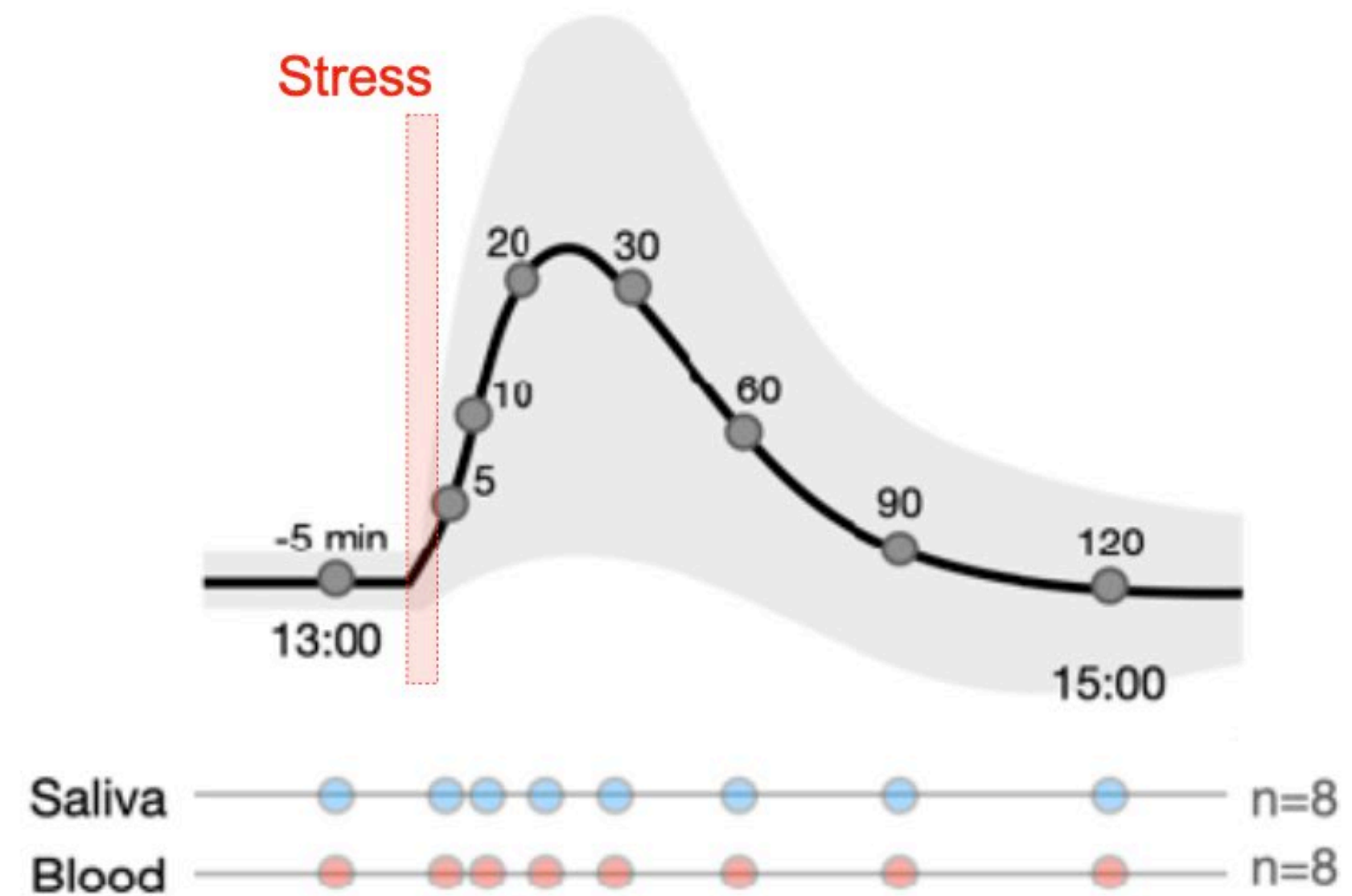


Methods

Study cohort and psychosocial stress experiment (TSST)

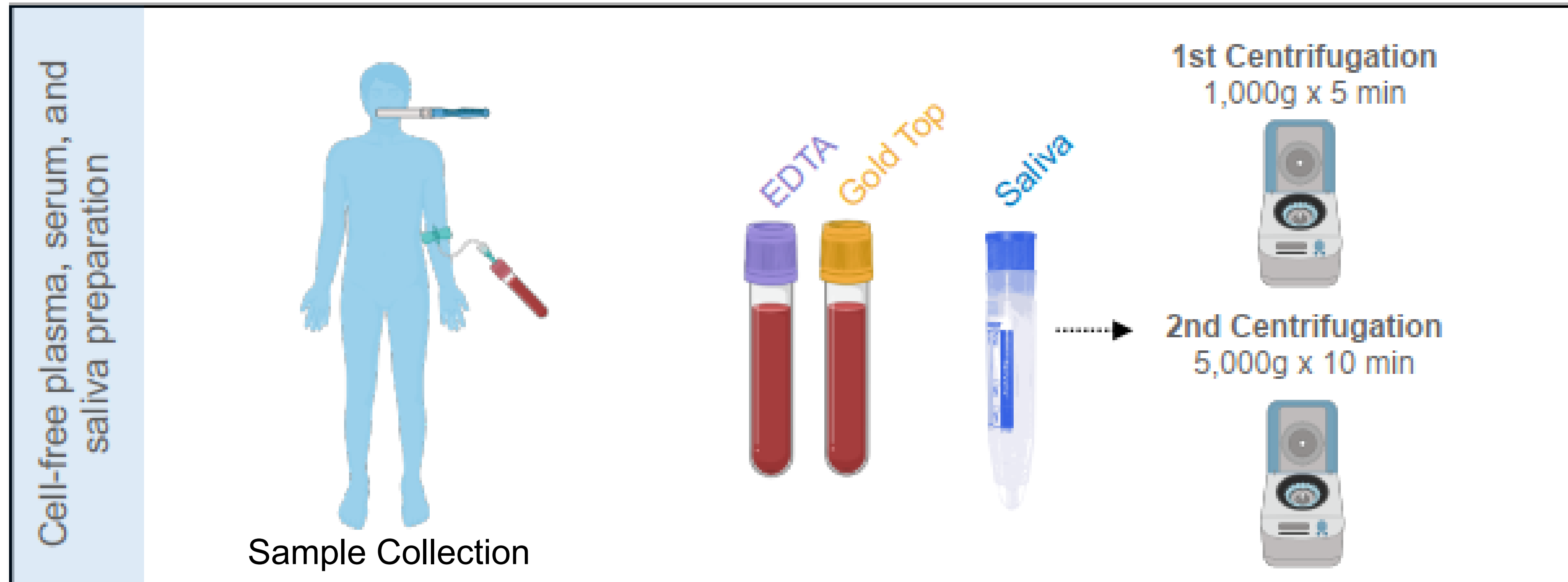


Speech task



Methods

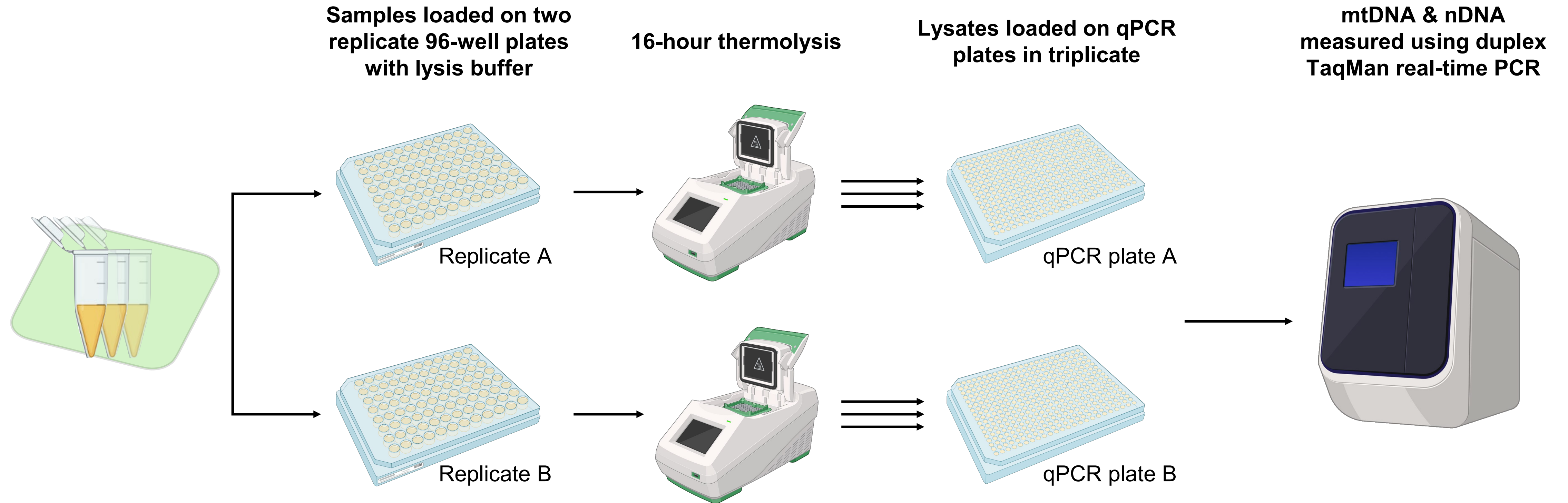
Sample processing for cf-DNA analysis



After collection, biofluids are cleared of cells, platelets, and similarly sized bodies by two stages of centrifugation

Methods

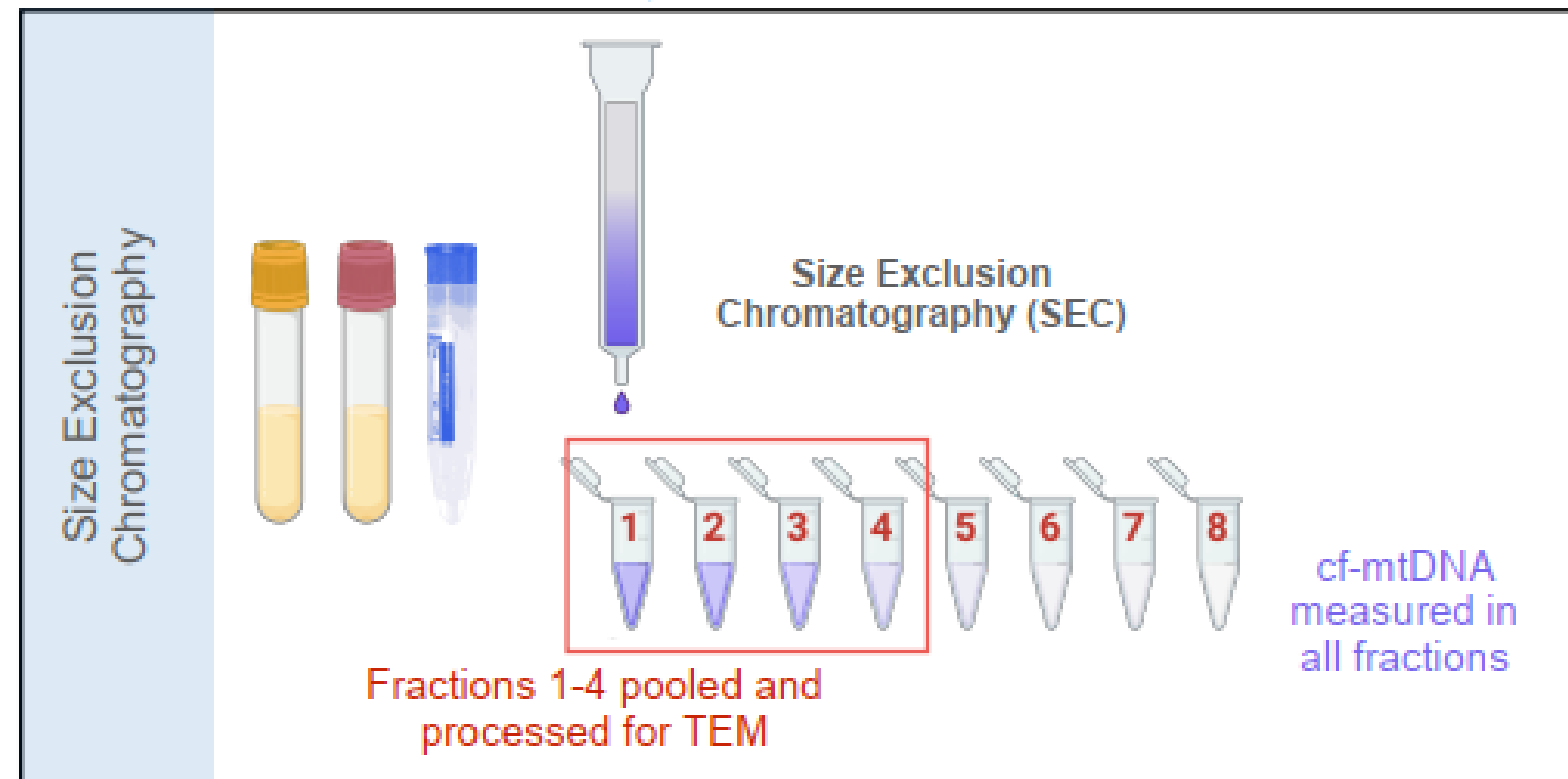
Thermolysis and qPCR



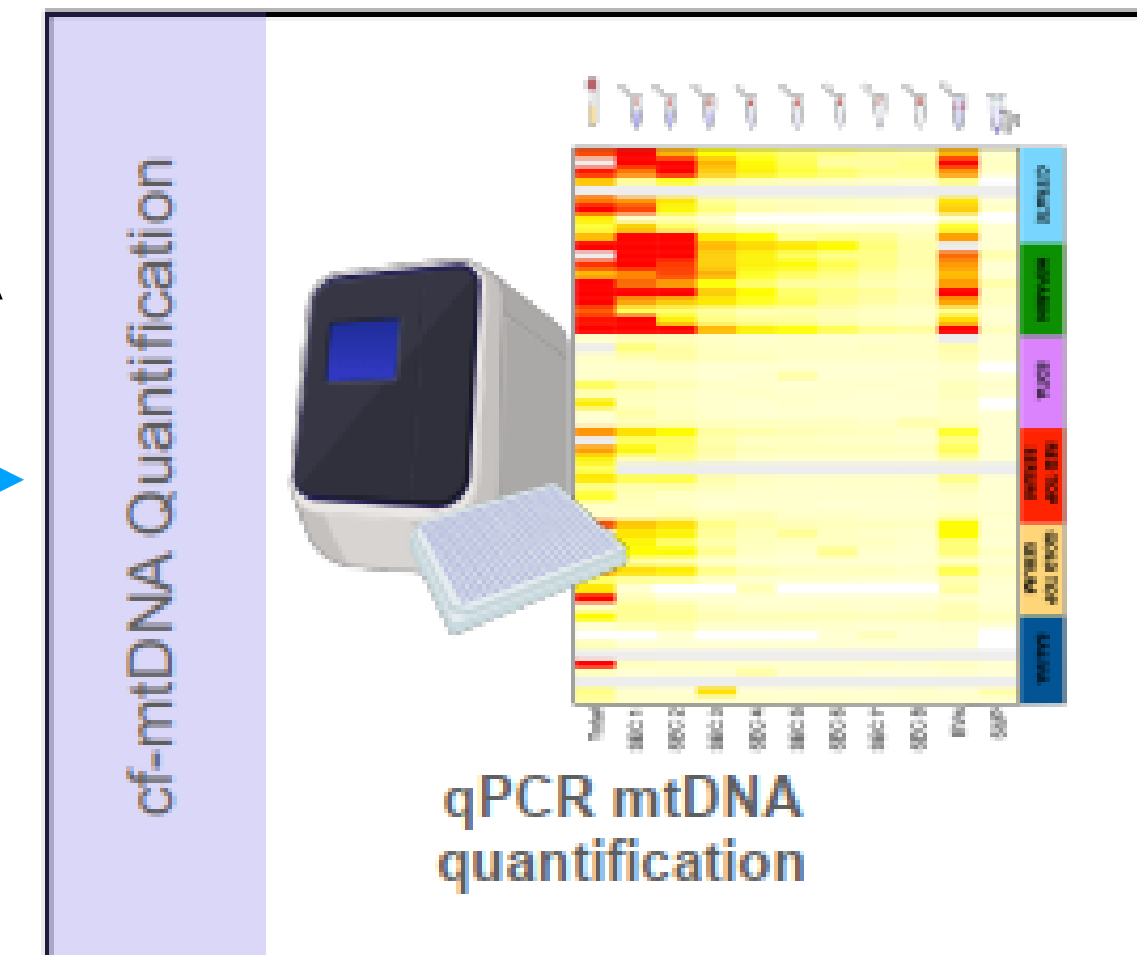
Methods

Size exclusion chromatography, qPCR, and electron microscopy

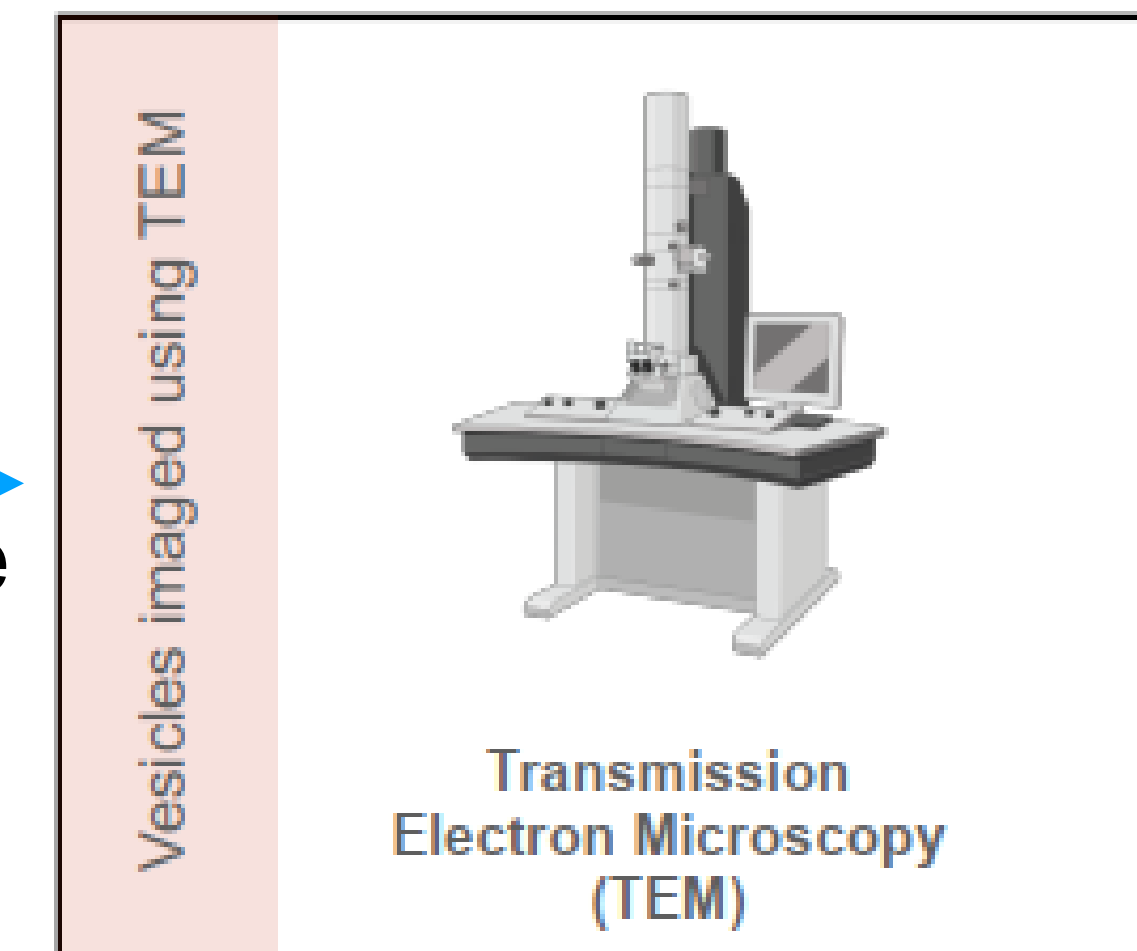
Cell-free biofluids are fractionated using size exclusion chromatography



Measure cf-mtDNA In all fractions

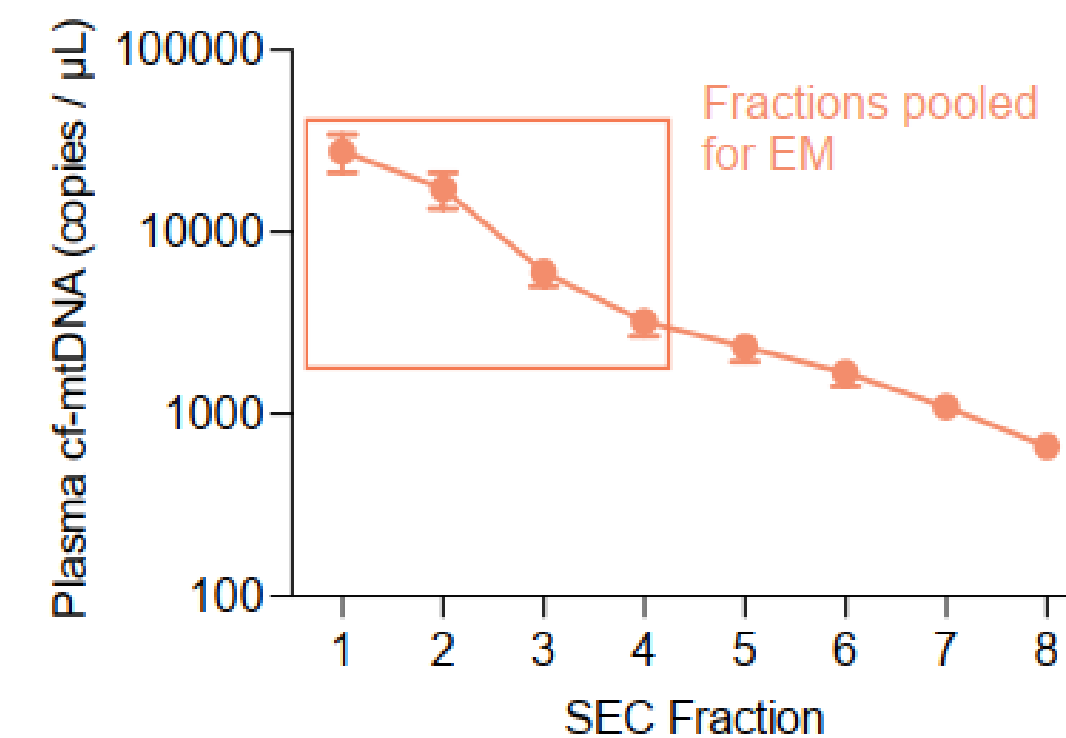
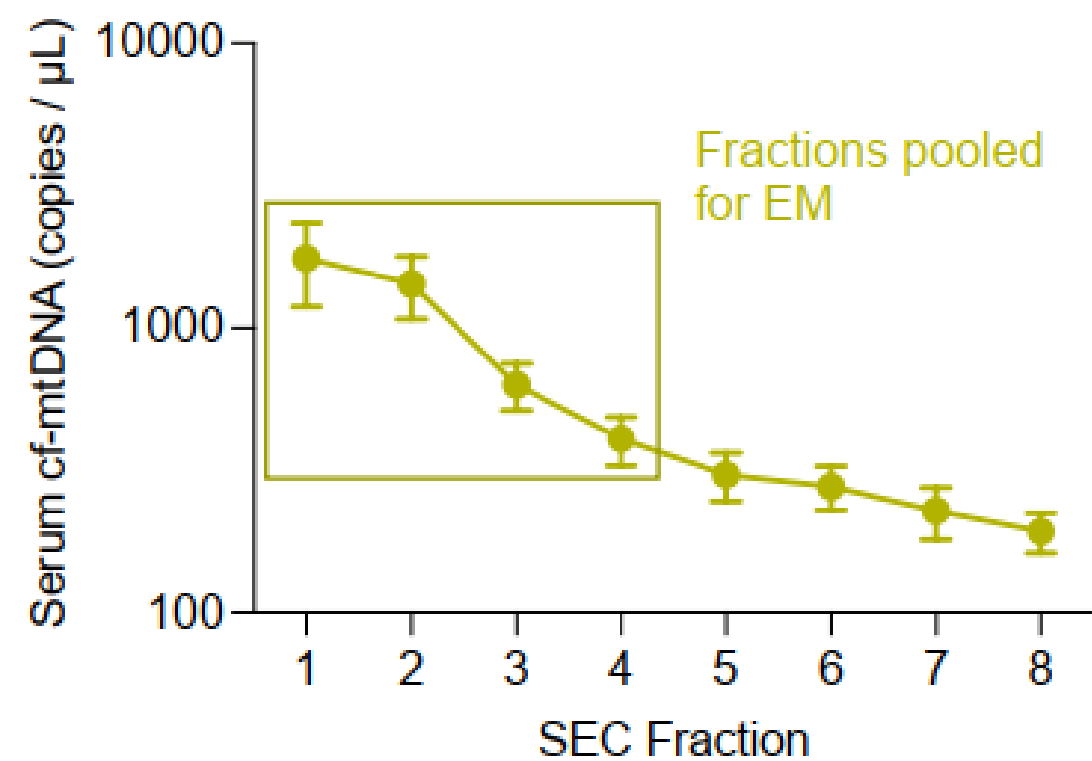
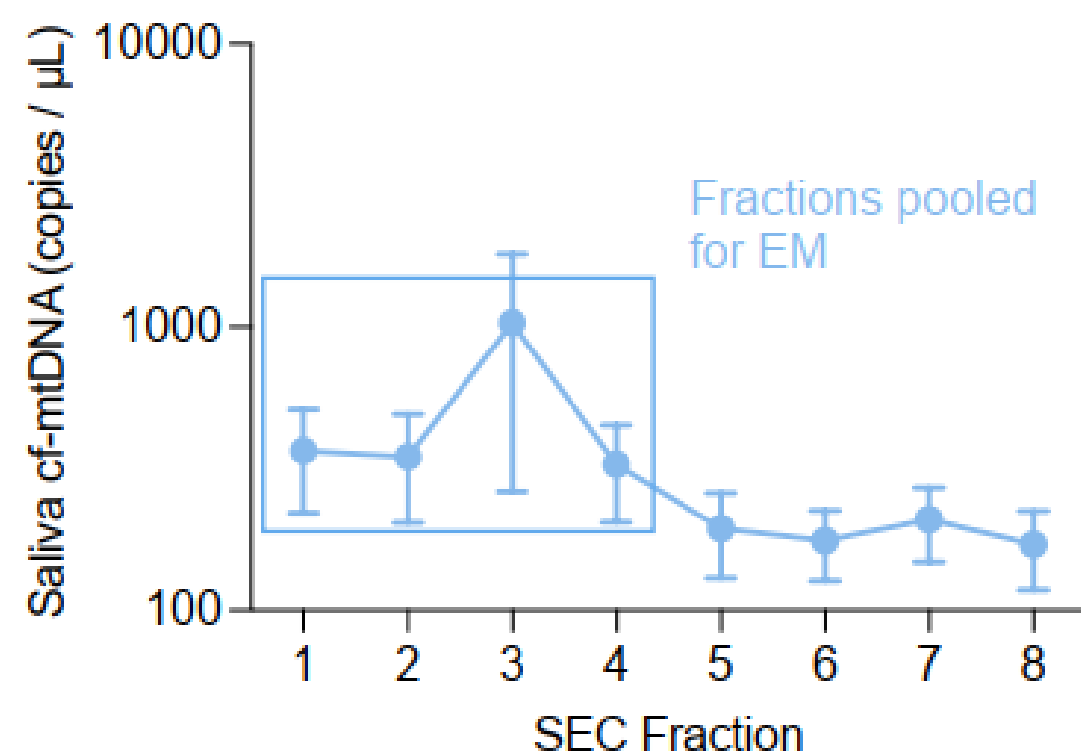


Pool 1st four fractions and image

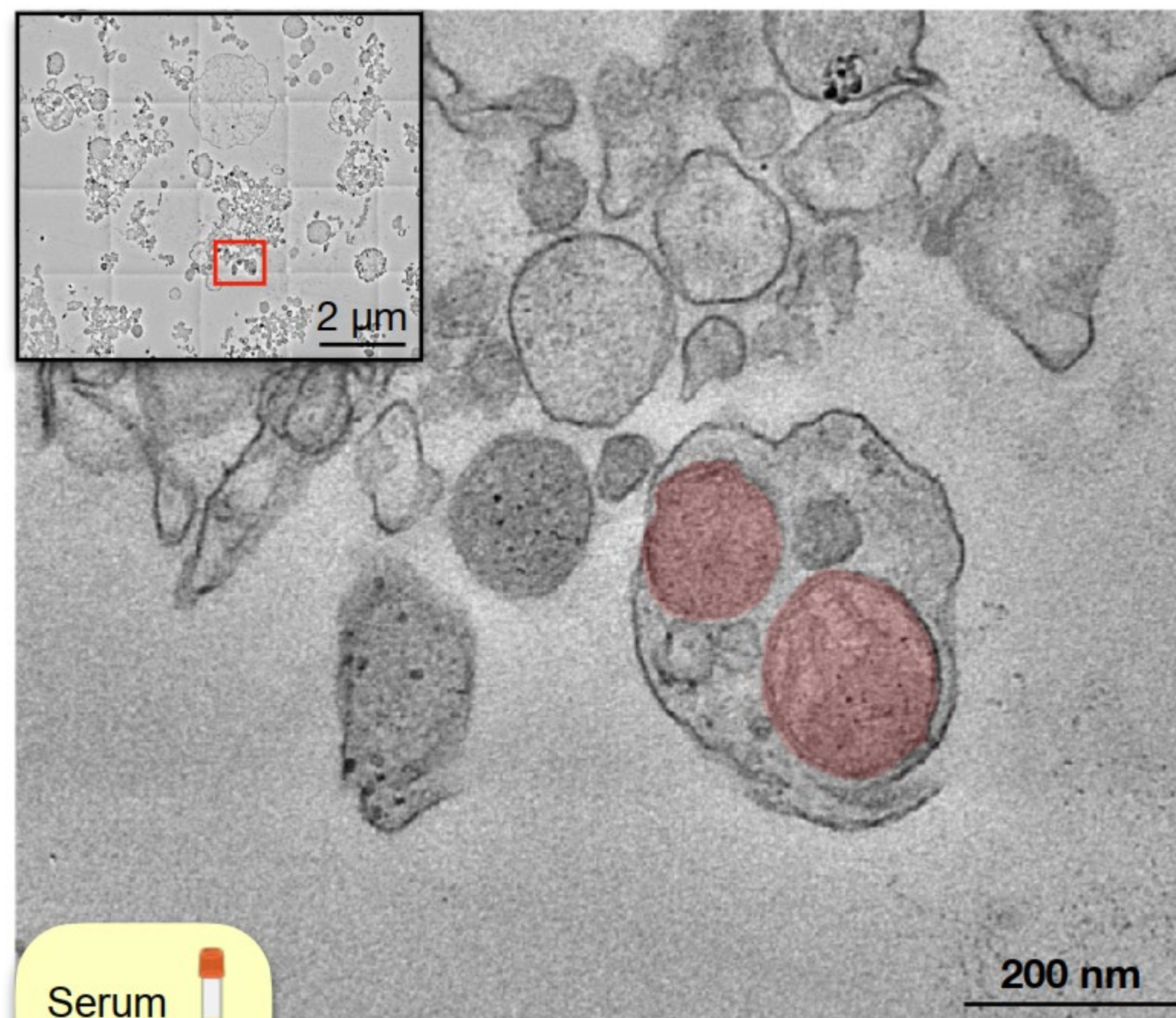


Results

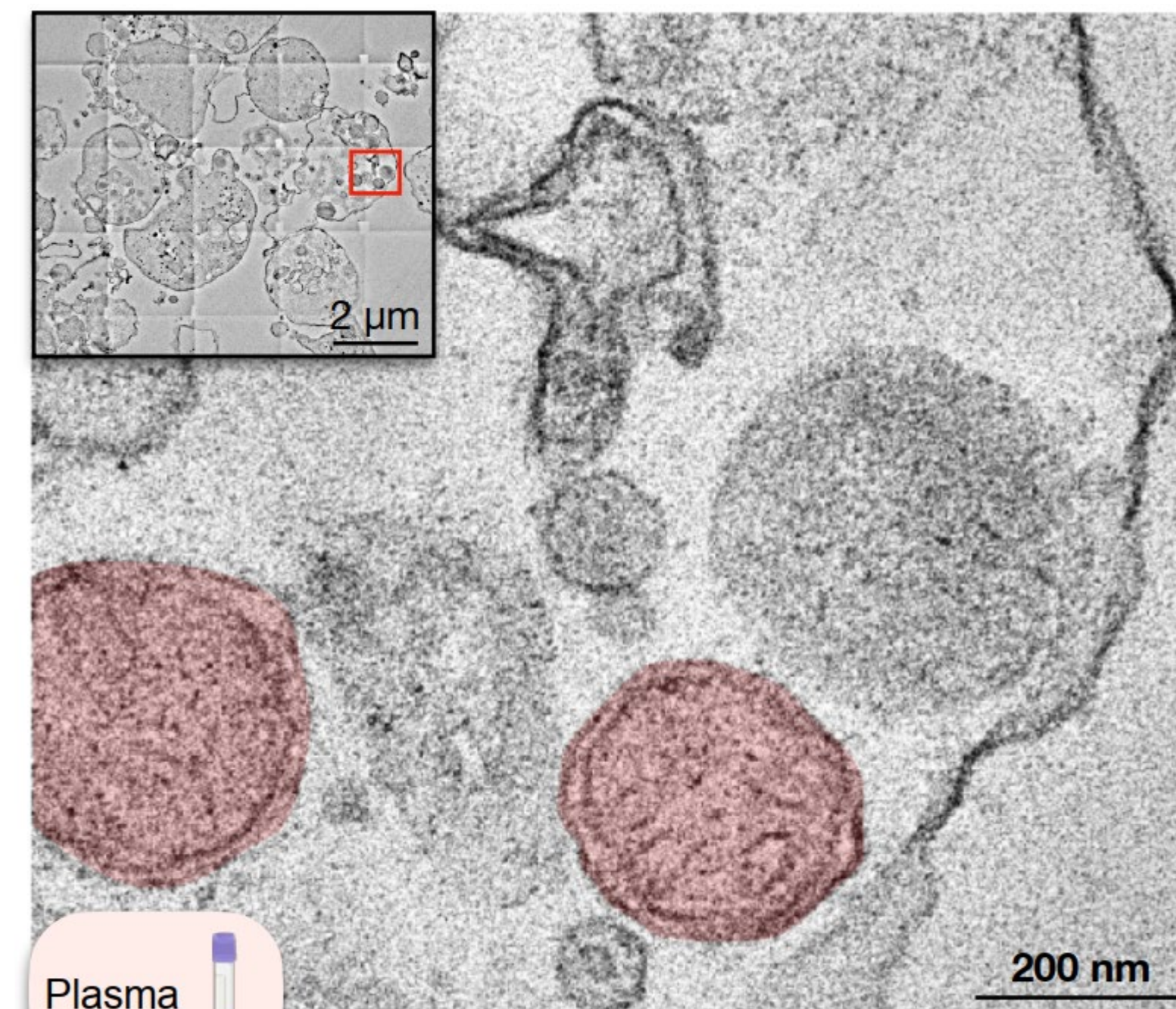
Observation of electron-dense, double-membrane bound objects in cell-free samples



Saliva



Serum

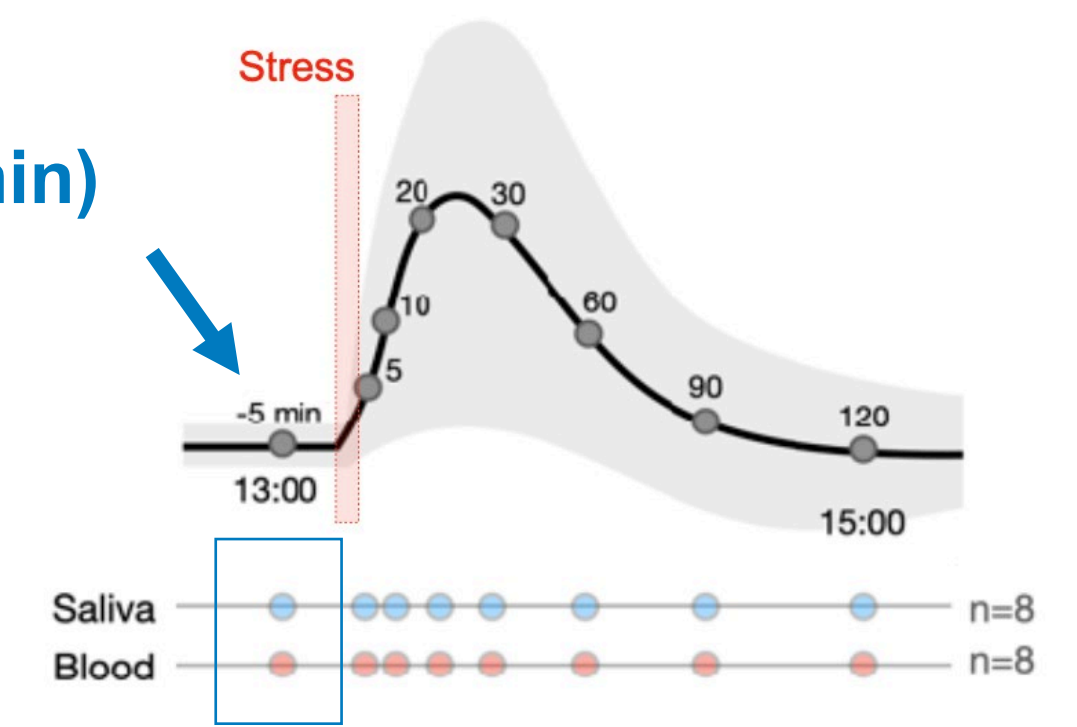


Plasma

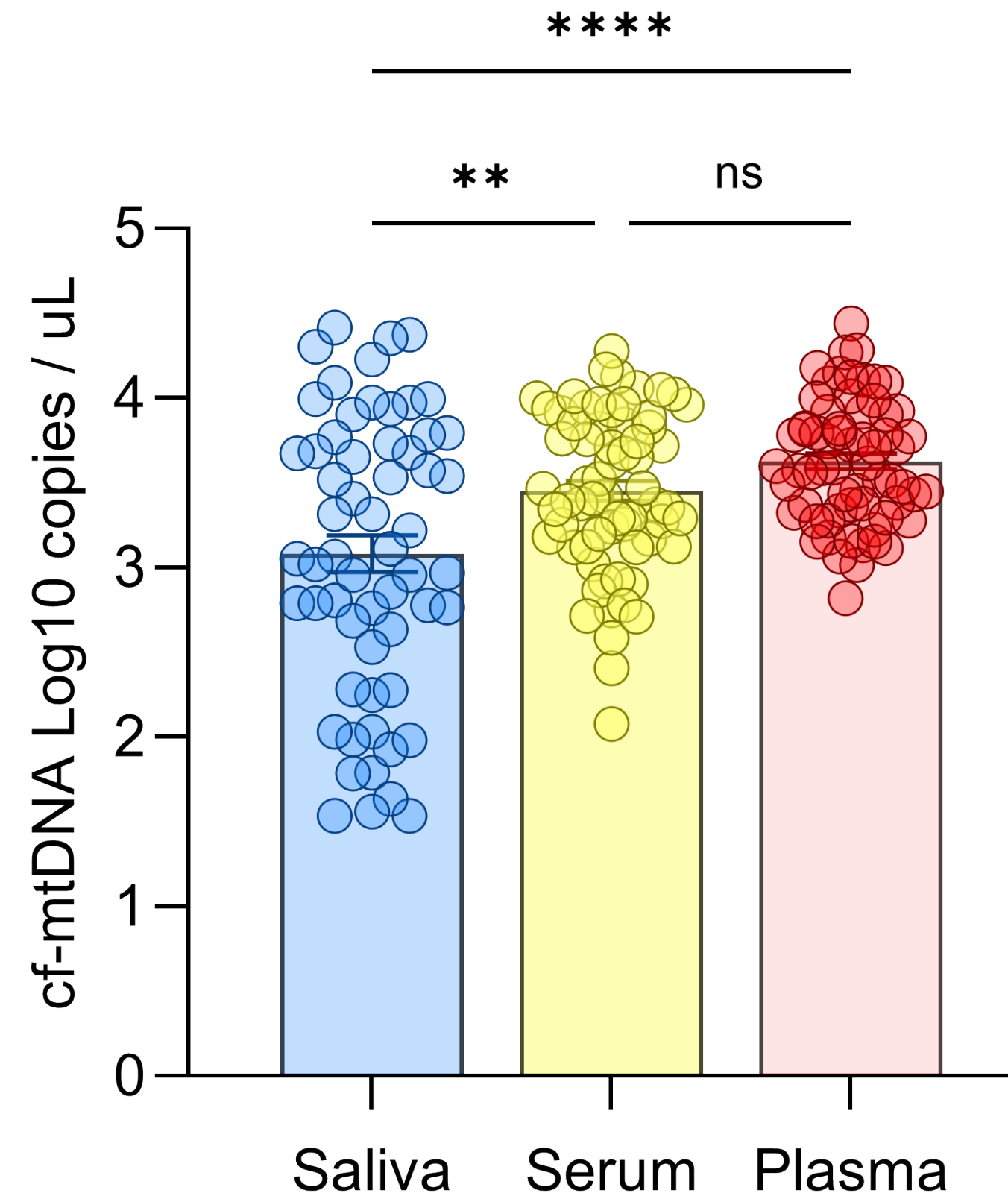
Results

Baseline measurements

Baseline (-5 min)



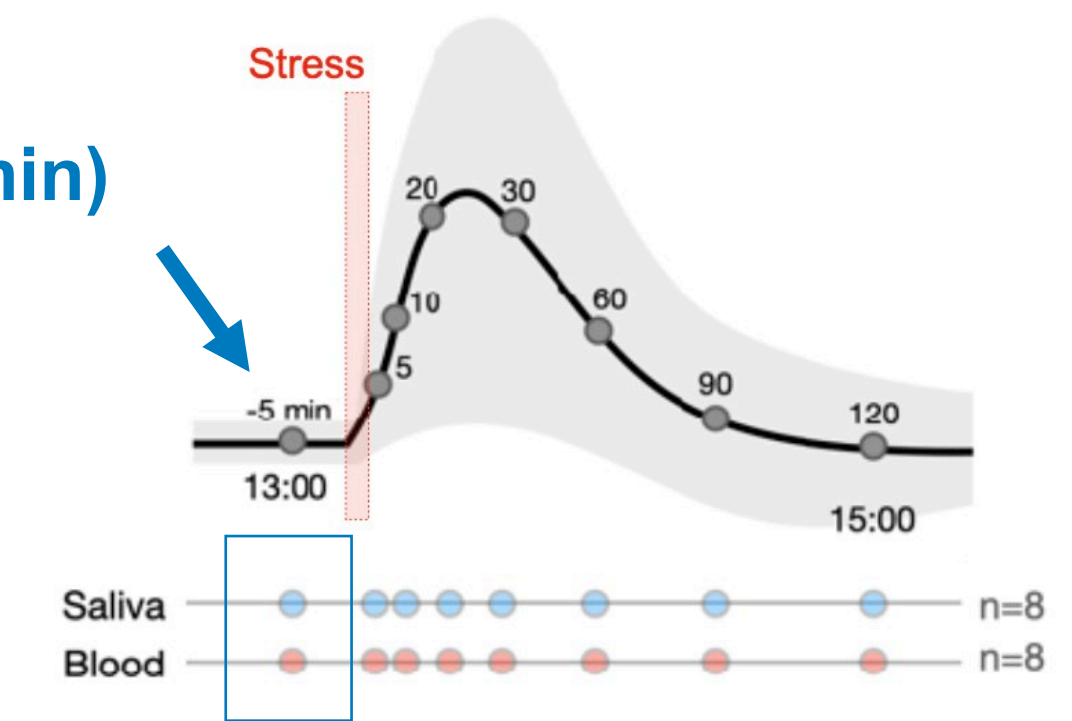
Baseline cf-mtDNA levels
(control participants)



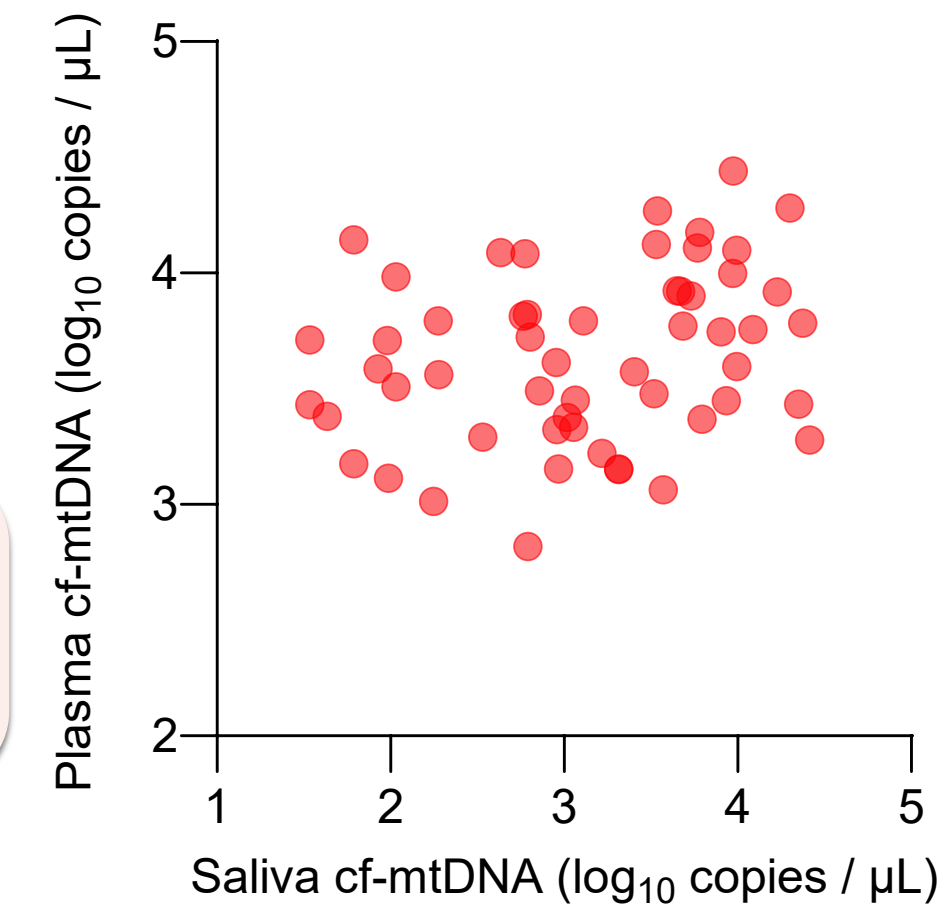
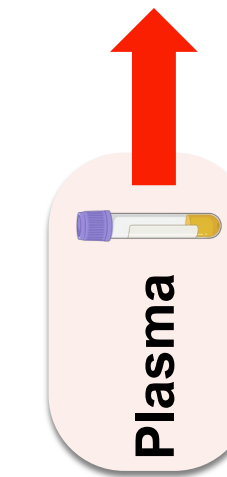
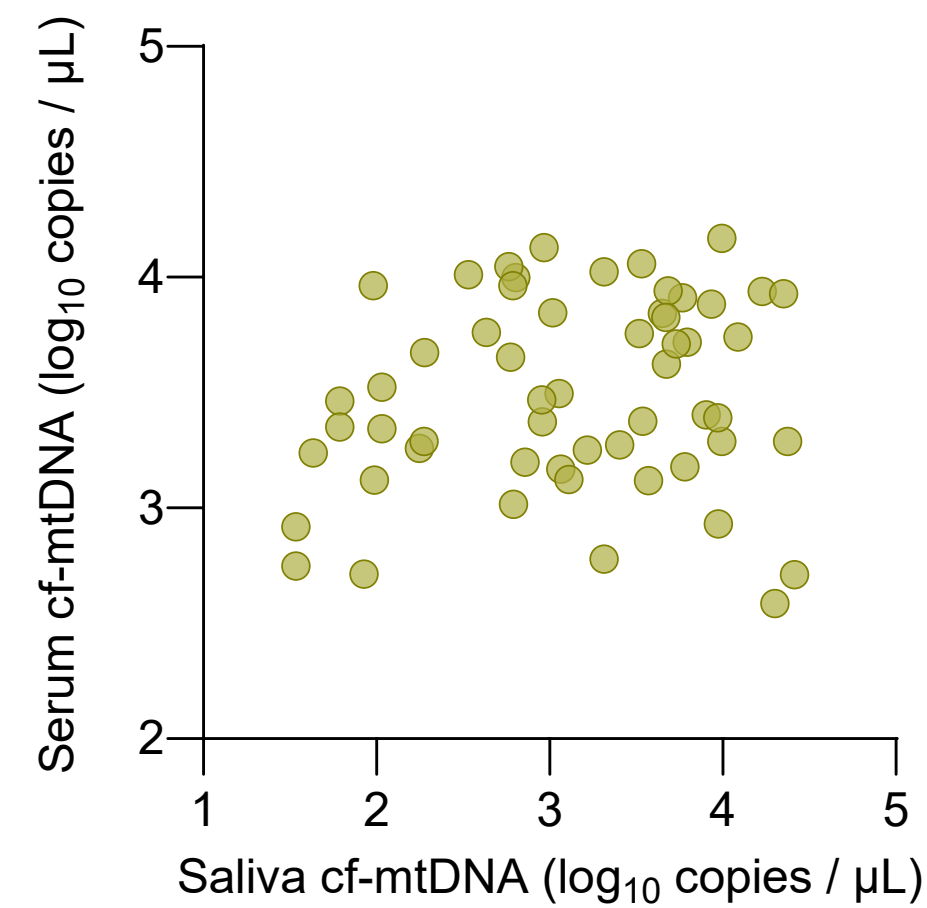
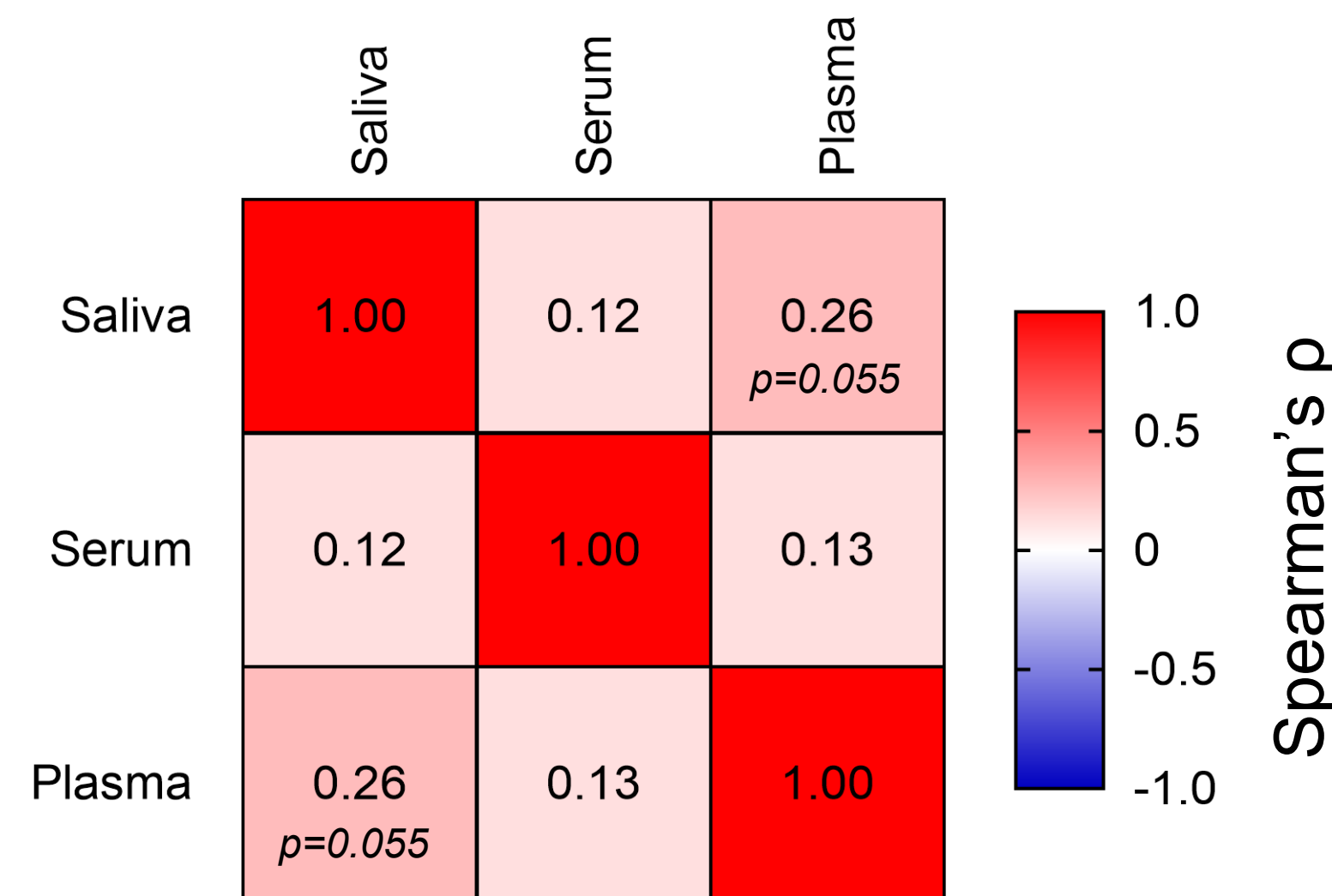
Results

Baseline measurements

Baseline (-5 min)

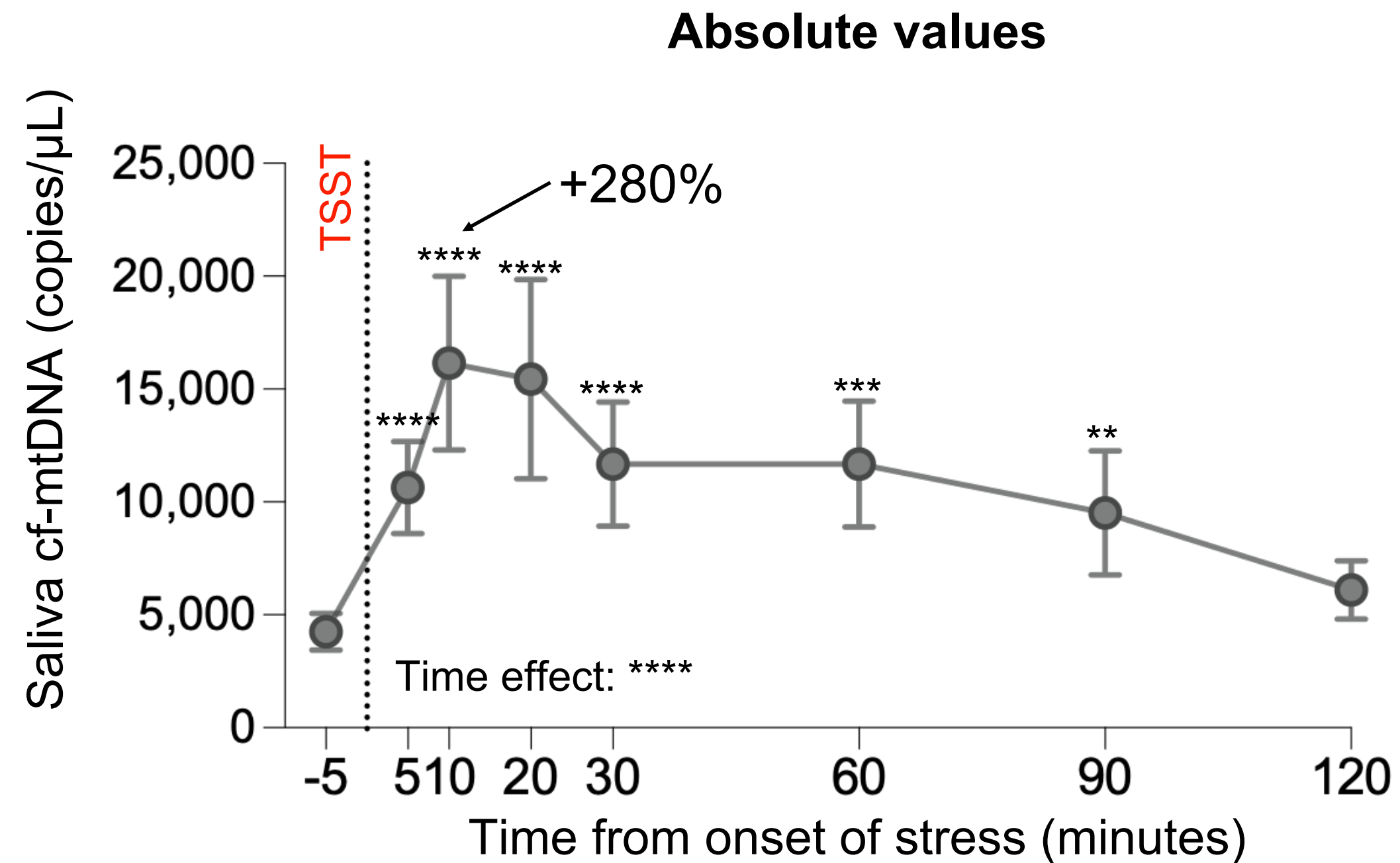
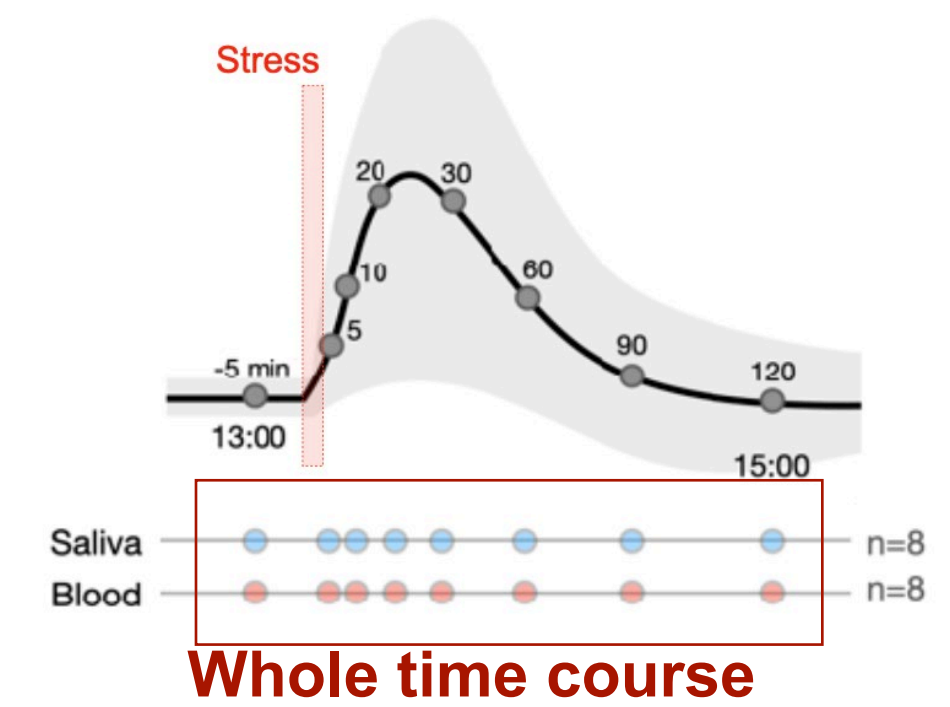


Correlations of baseline values between sample types (control participants)



Results

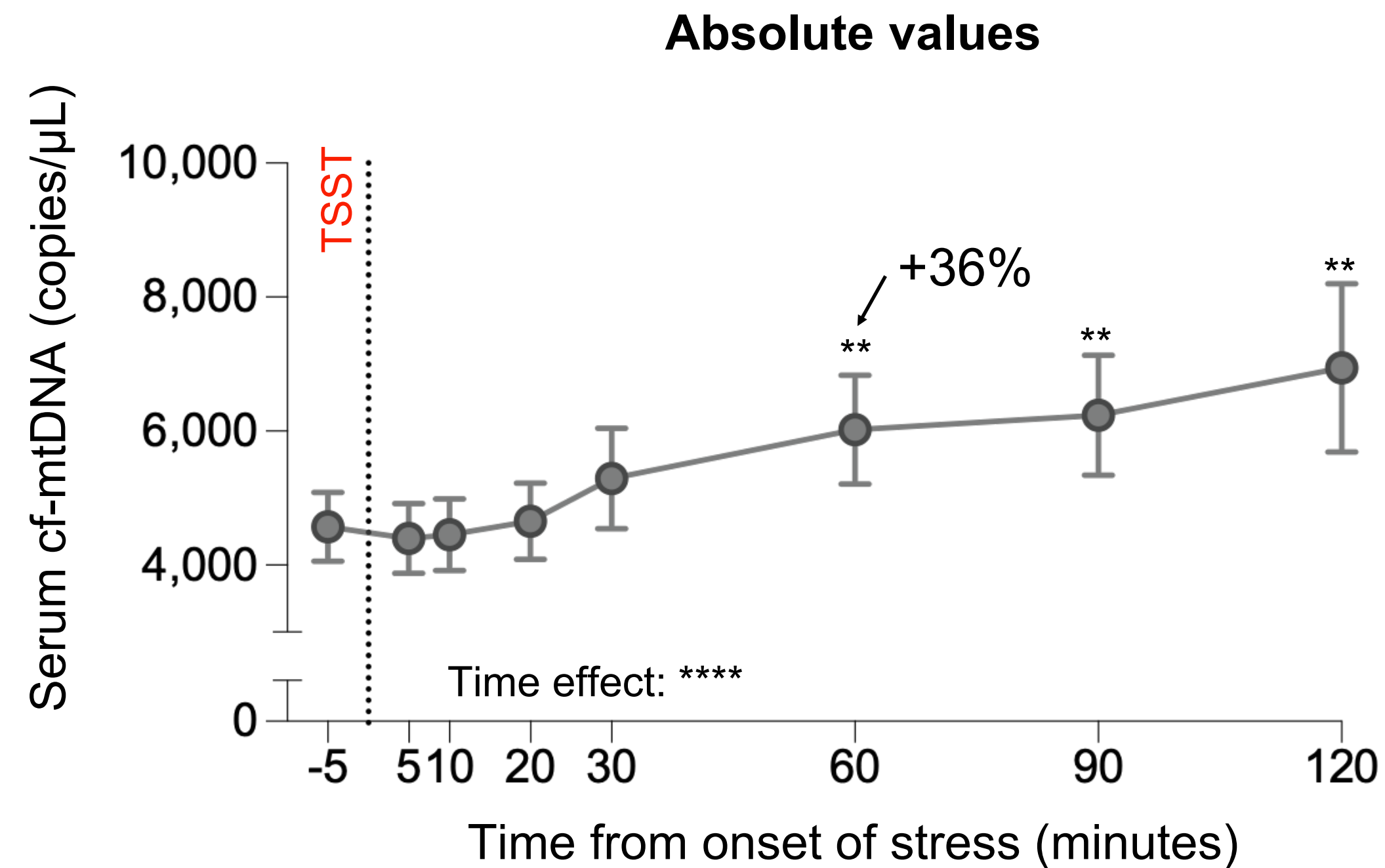
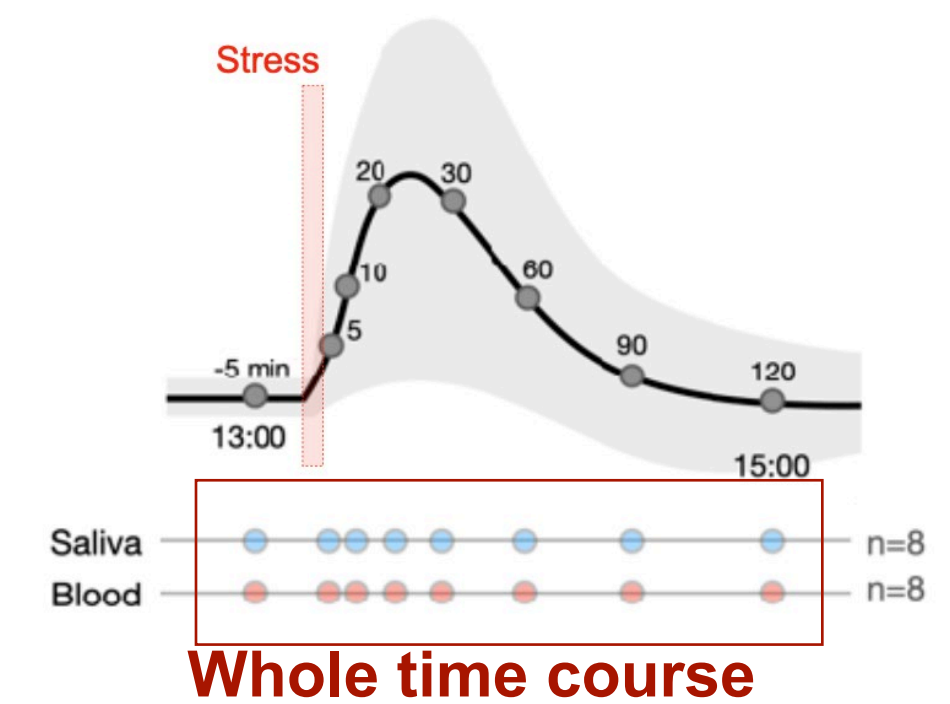
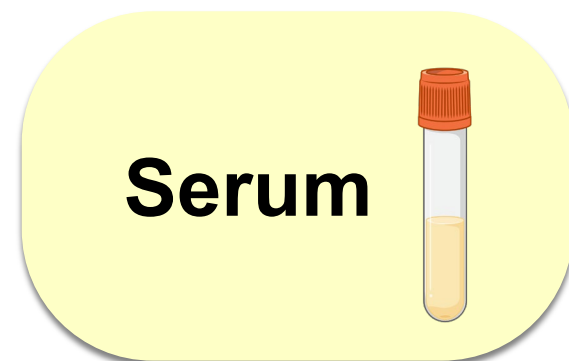
Saliva cf-mtDNA trajectories (control participants)



Asterisks: sig. diff. from -5 min time point

Results

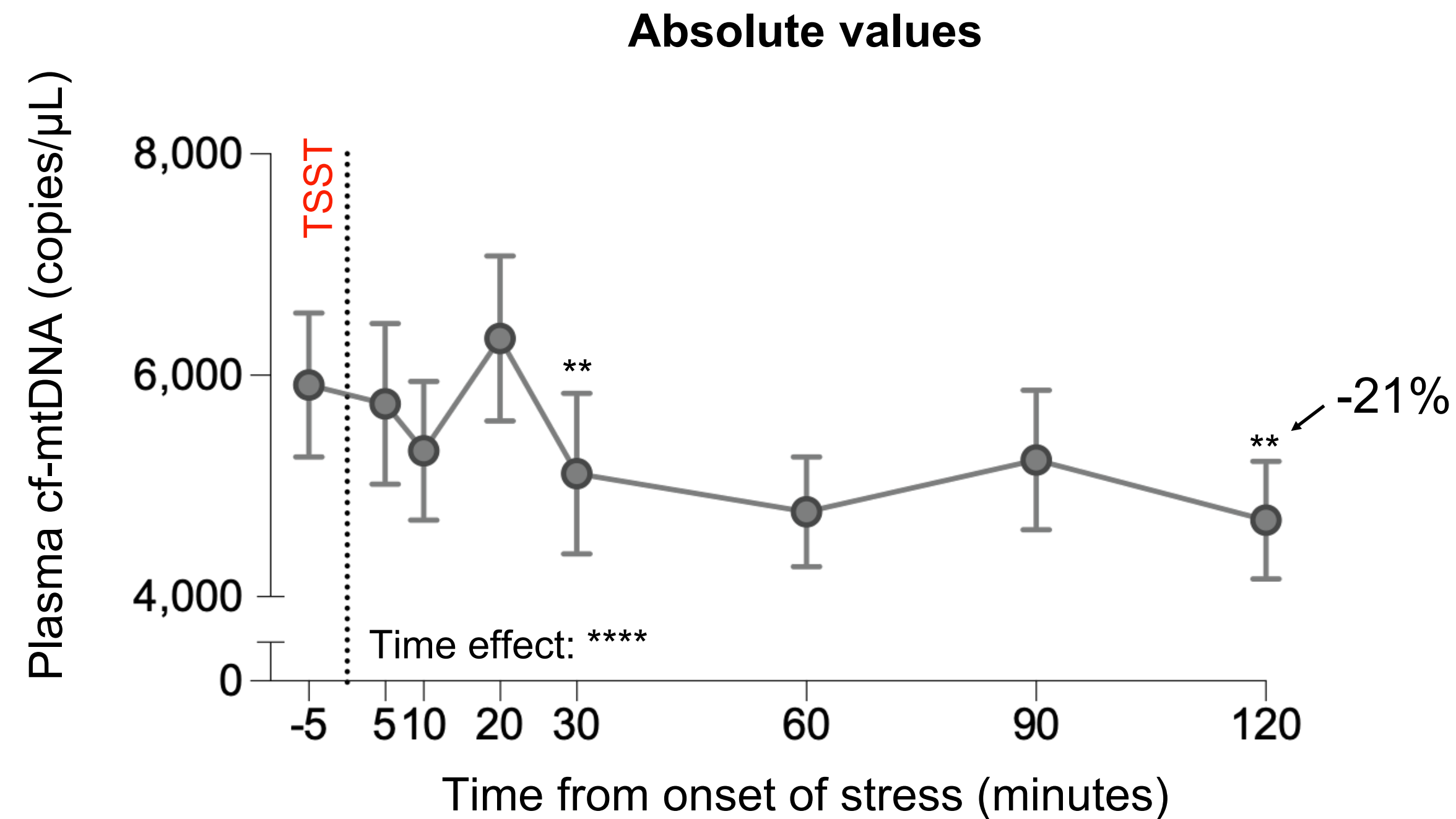
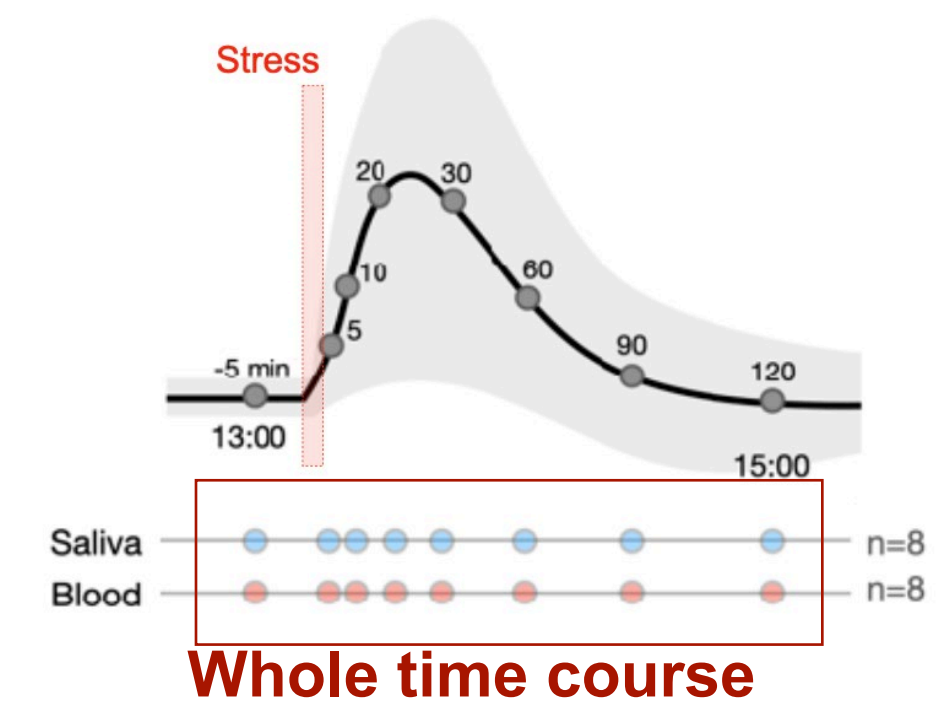
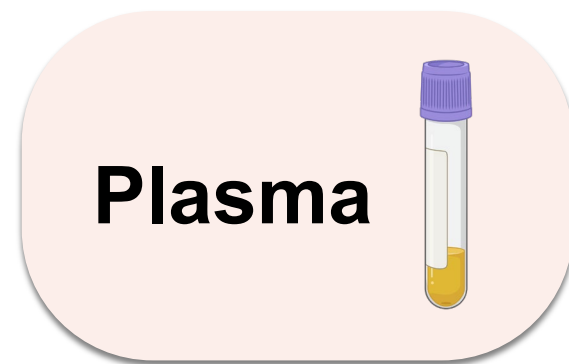
Serum cf-mtDNA trajectories (control participants)



Asterisks: sig. diff. from -5 min time point

Results

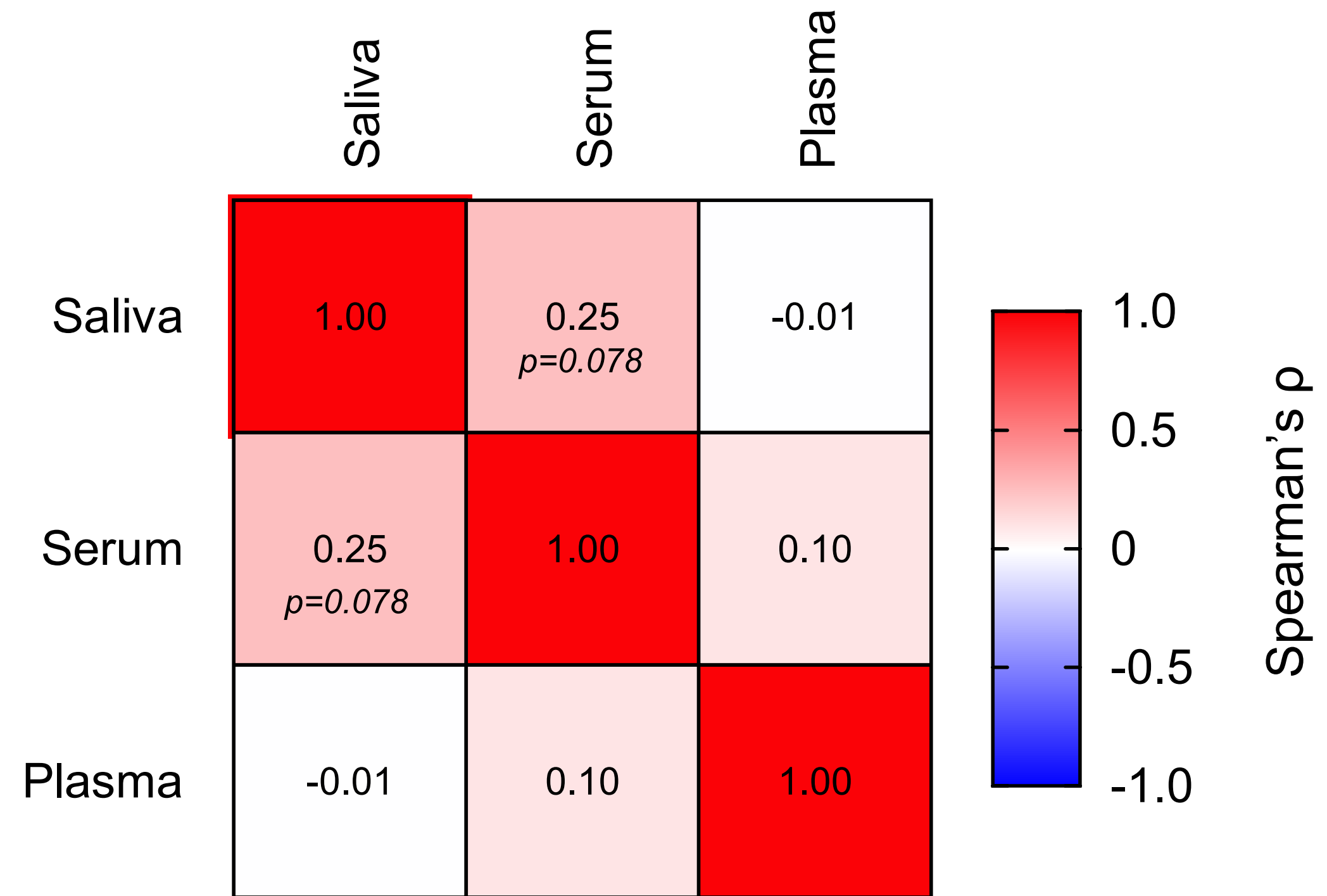
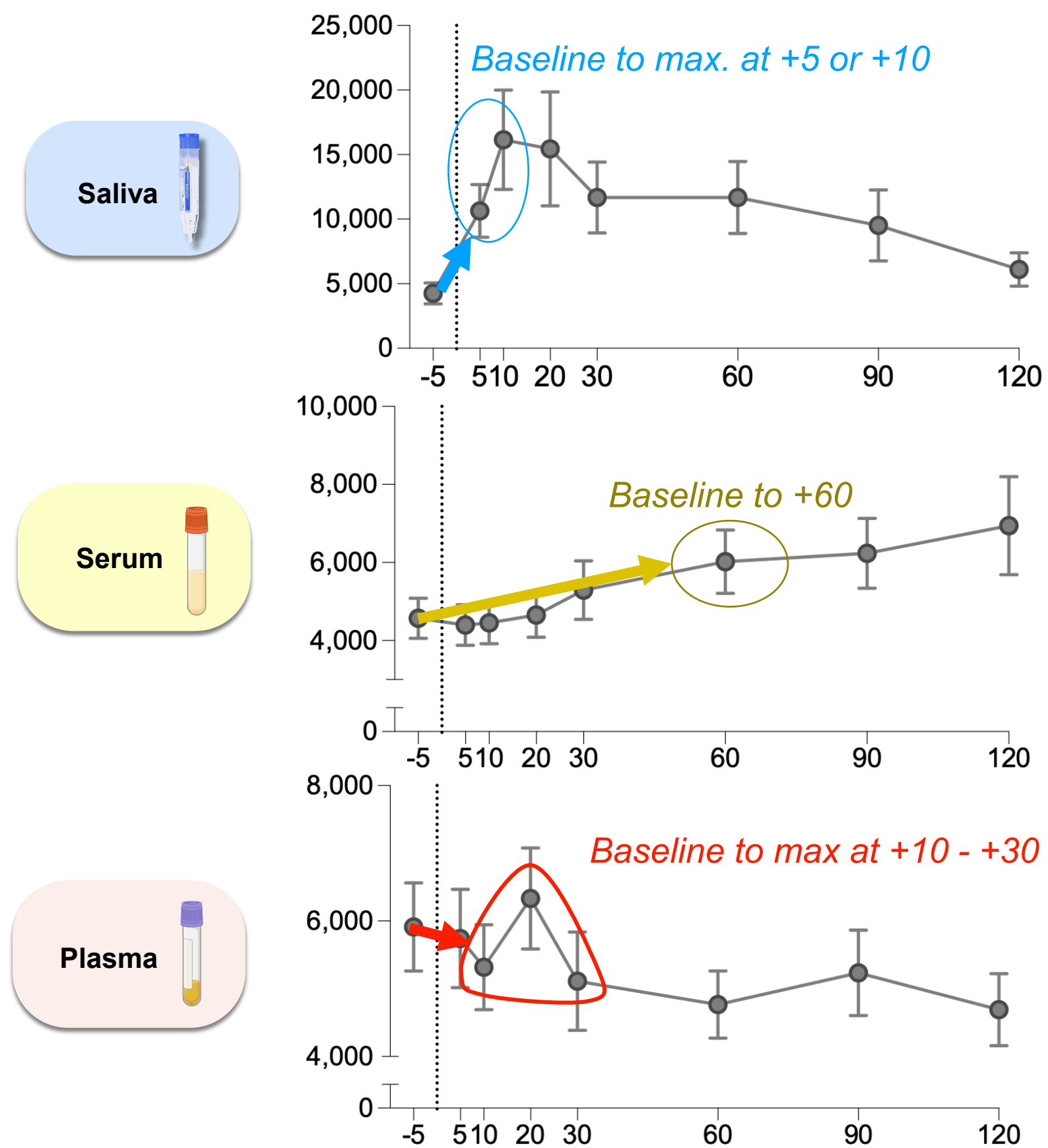
Plasma cf-mtDNA trajectories (control participants)



Asterisks: sig. diff. from -5 min time point

Results

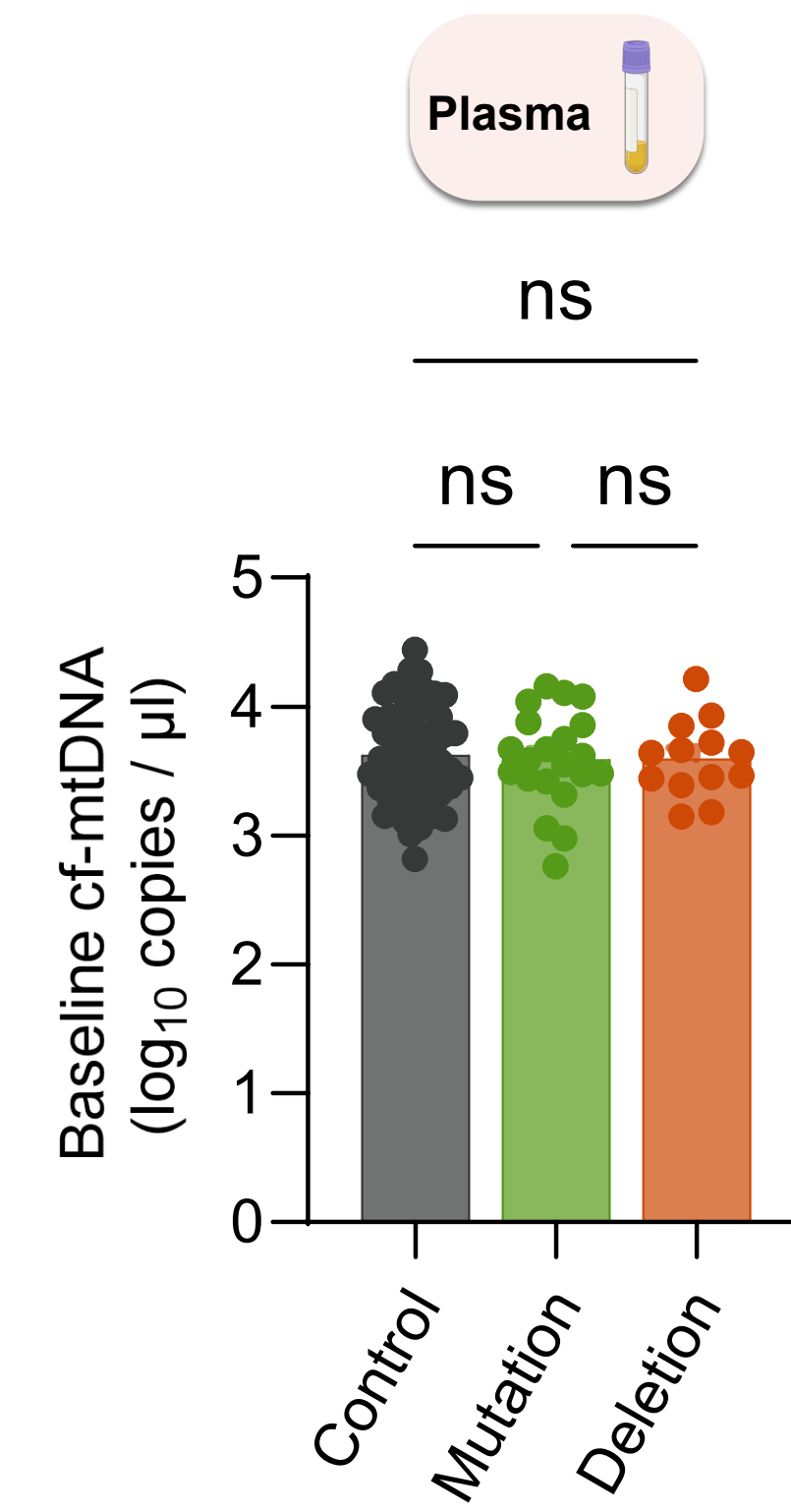
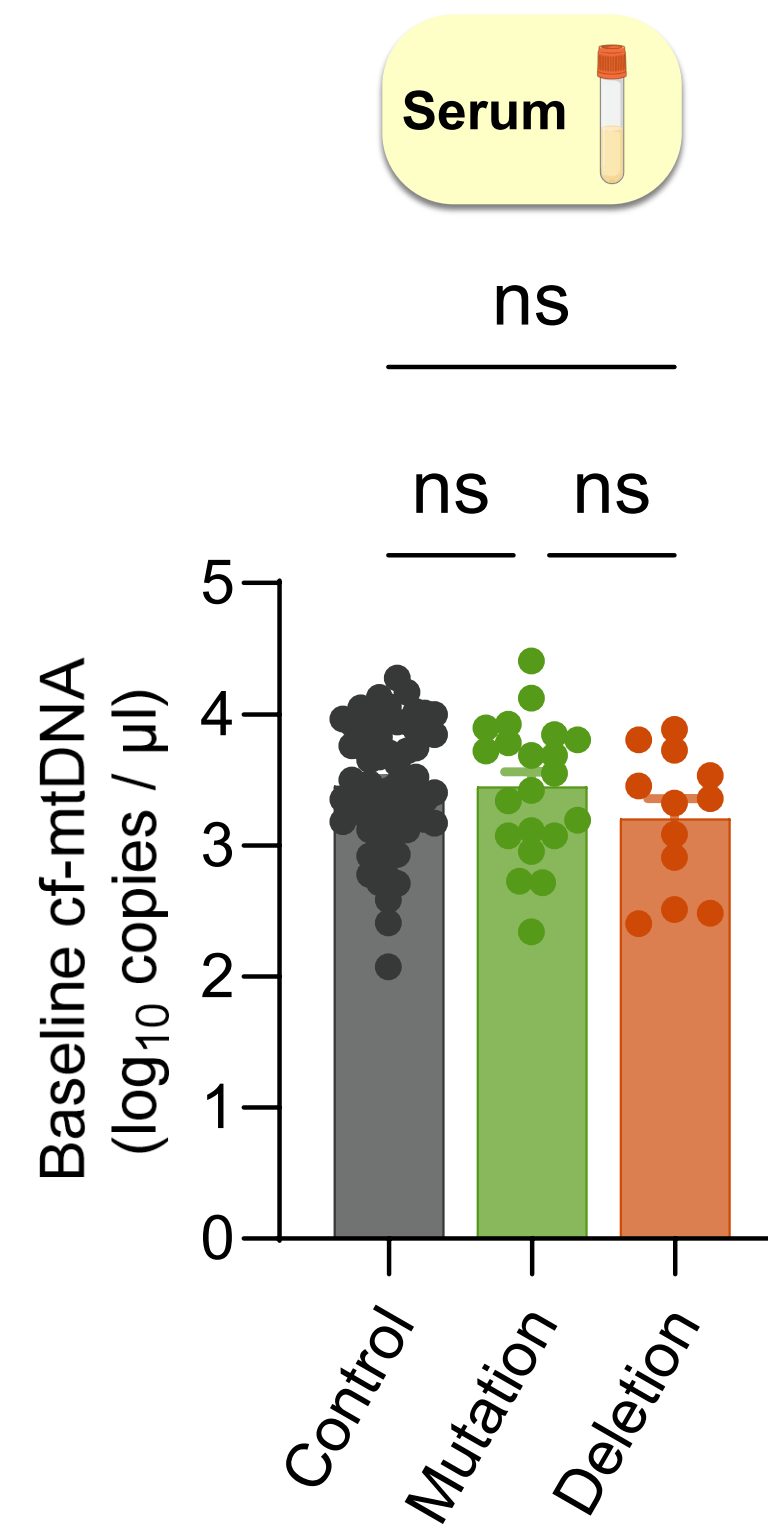
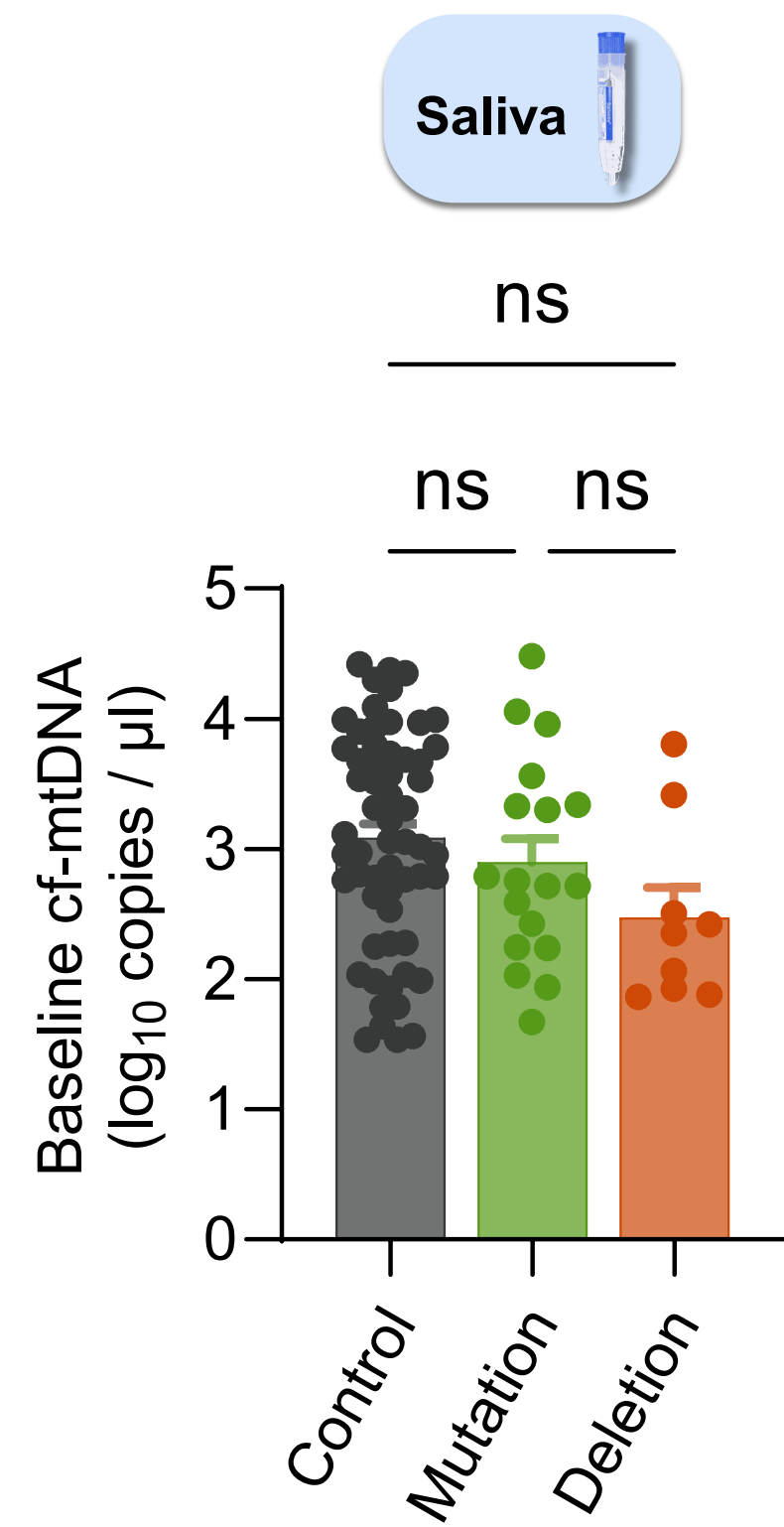
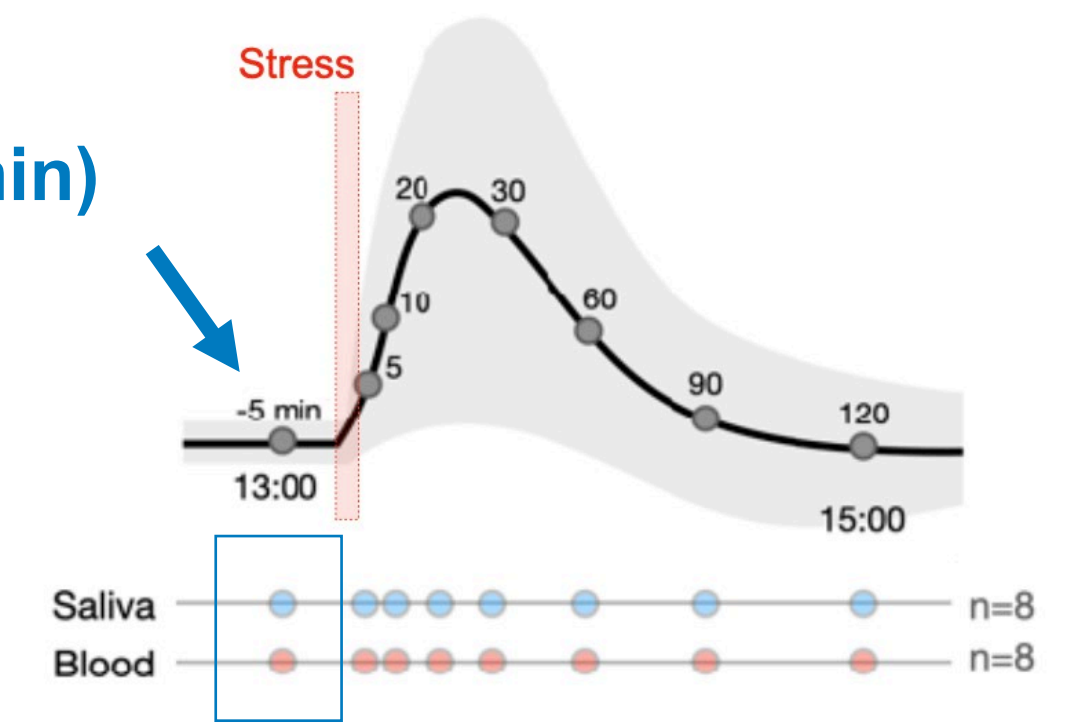
Comparison of cf-mtDNA reactivity in different biofluids (control participants)



Results

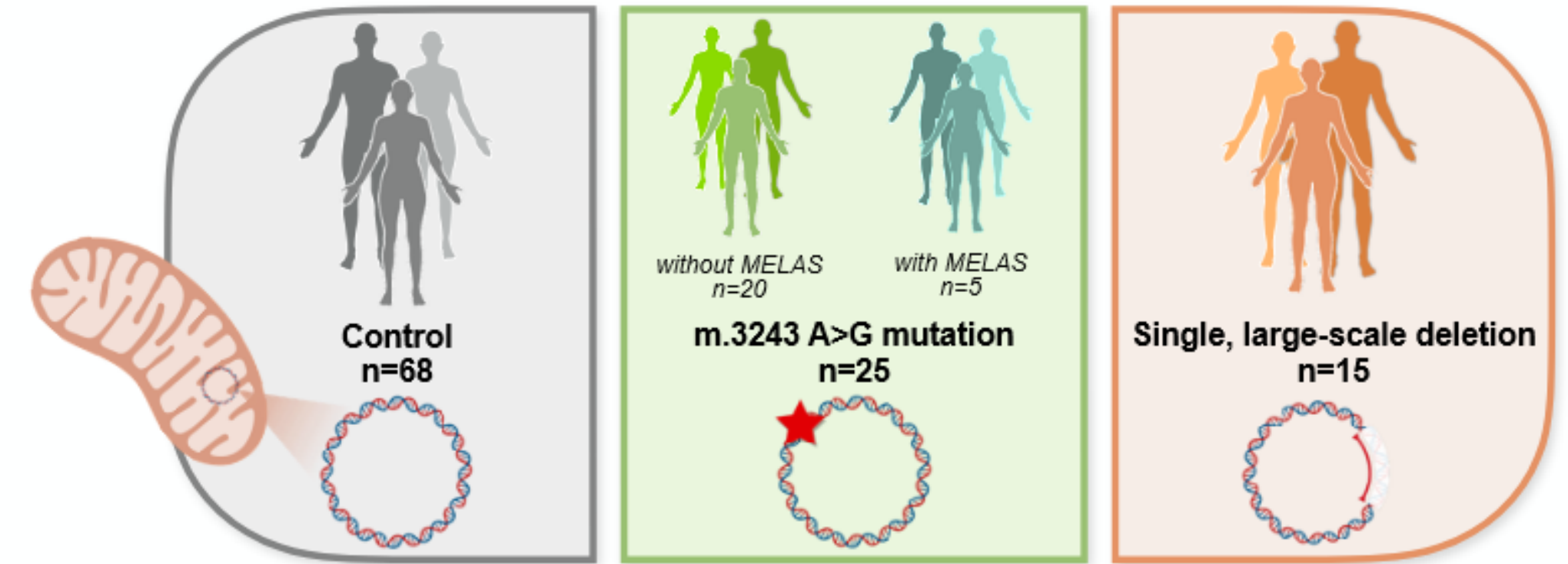
Comparison of baseline cf-mtDNA in control and mitochondrial disease participants

Baseline (-5 min)

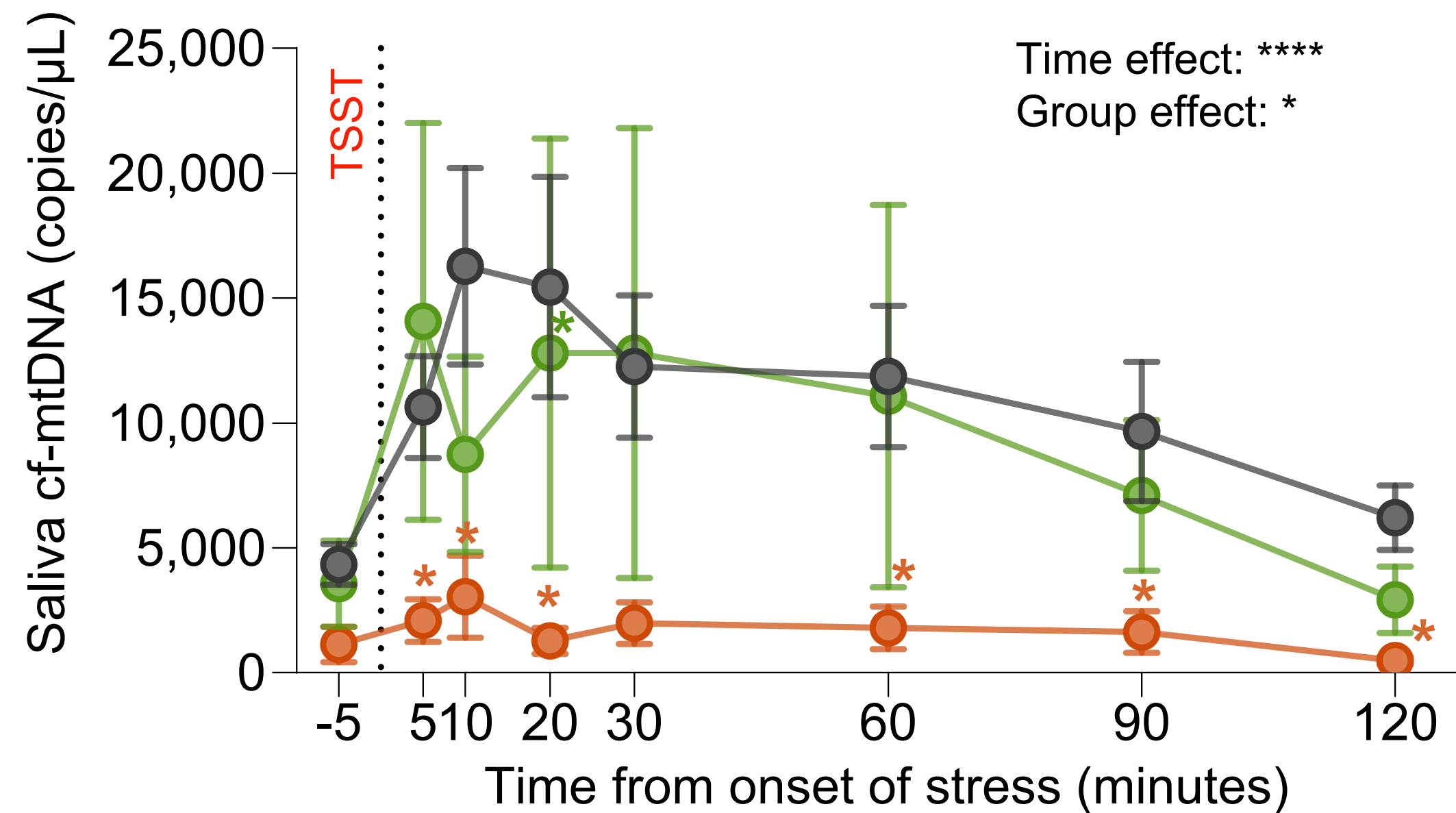


Results

Saliva cf-mtDNA trajectories (all groups)



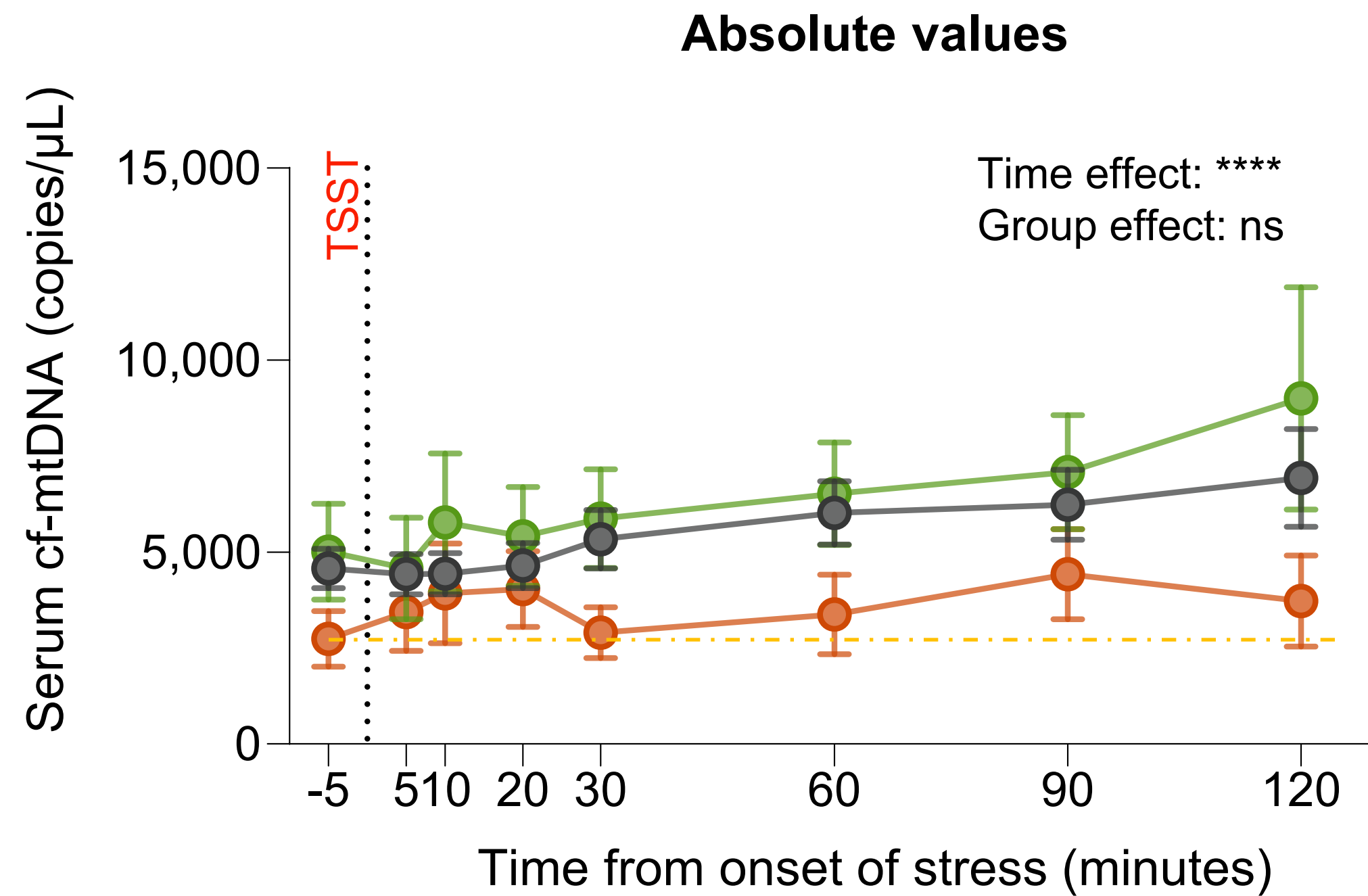
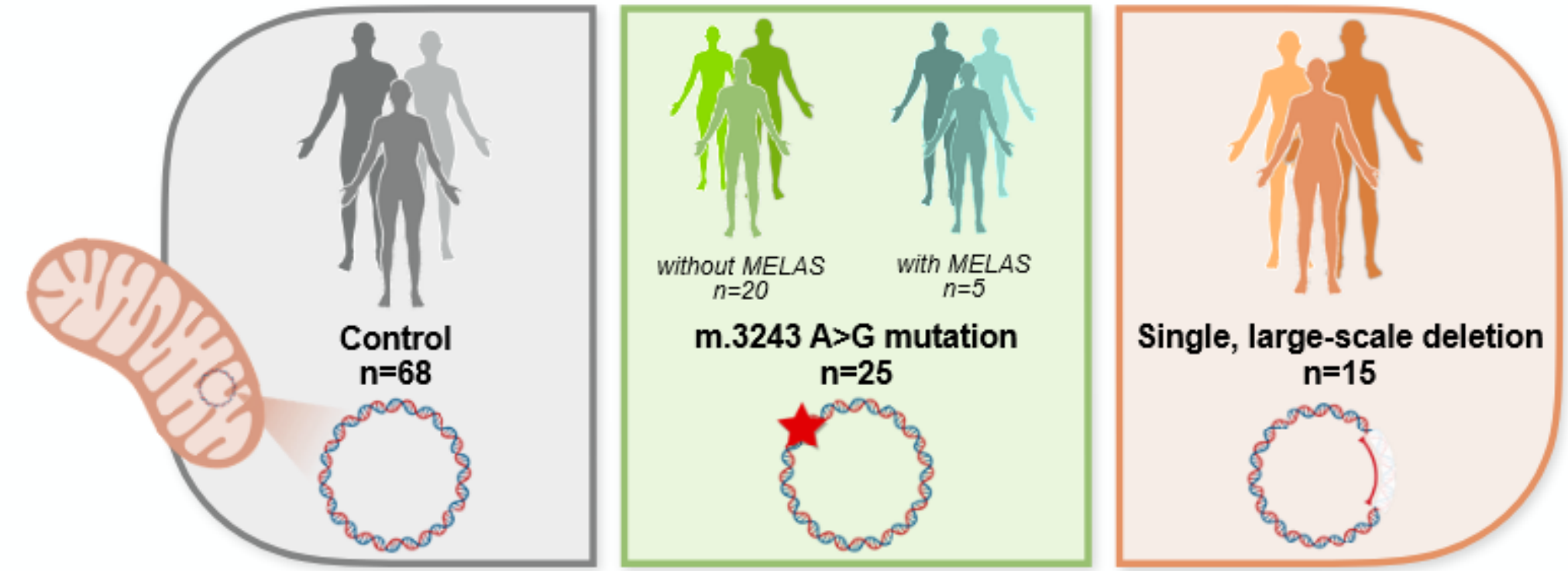
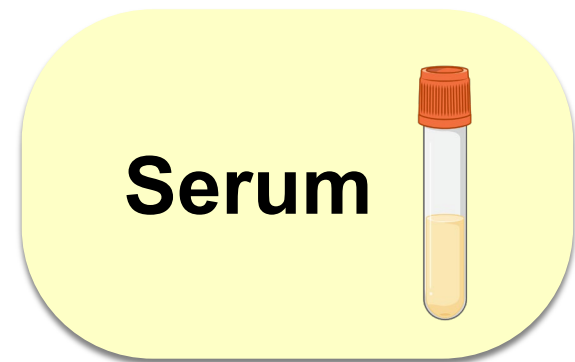
Absolute values



Asterisks: sig. diff. between control and mitoD groups

Results

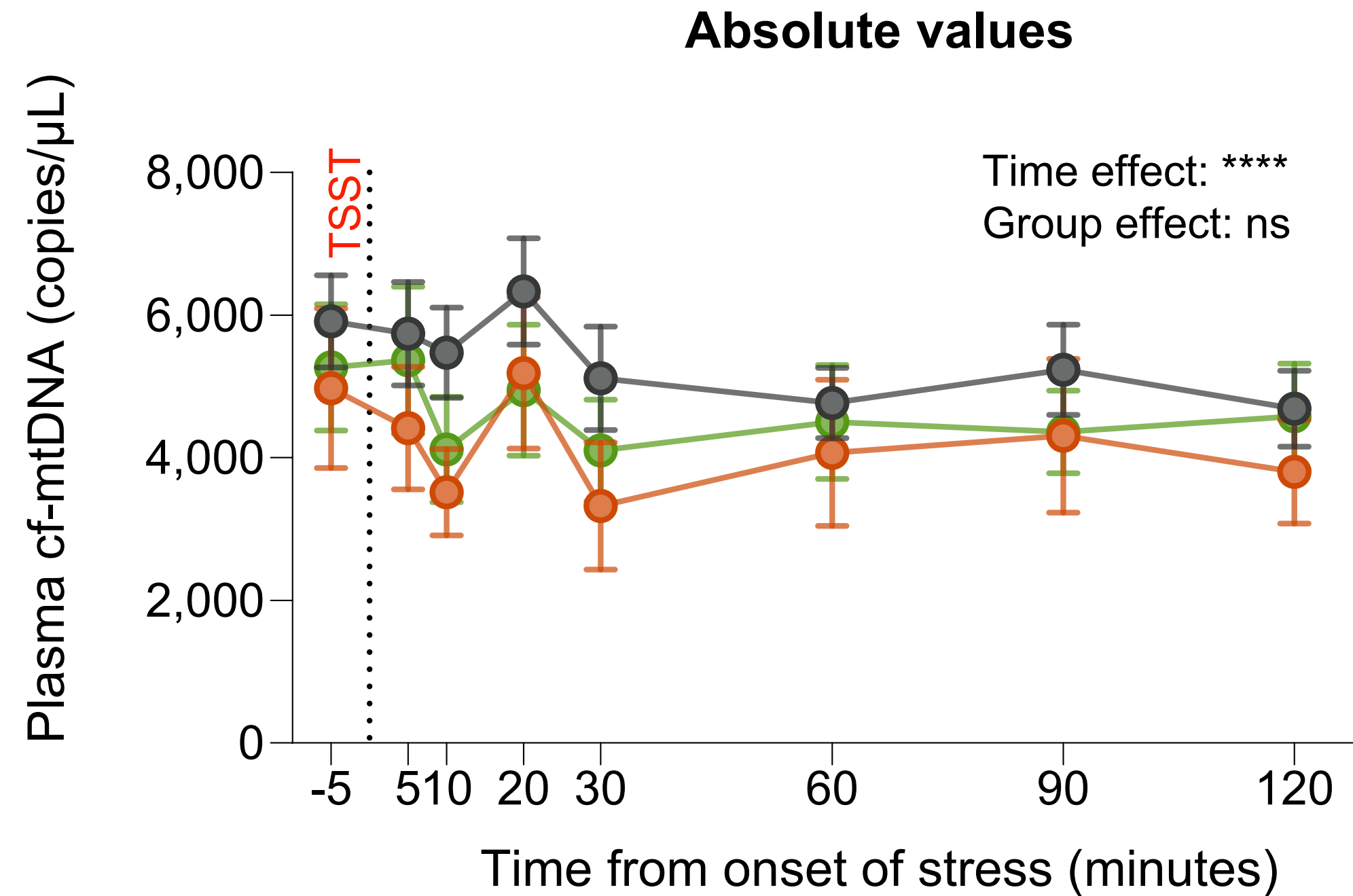
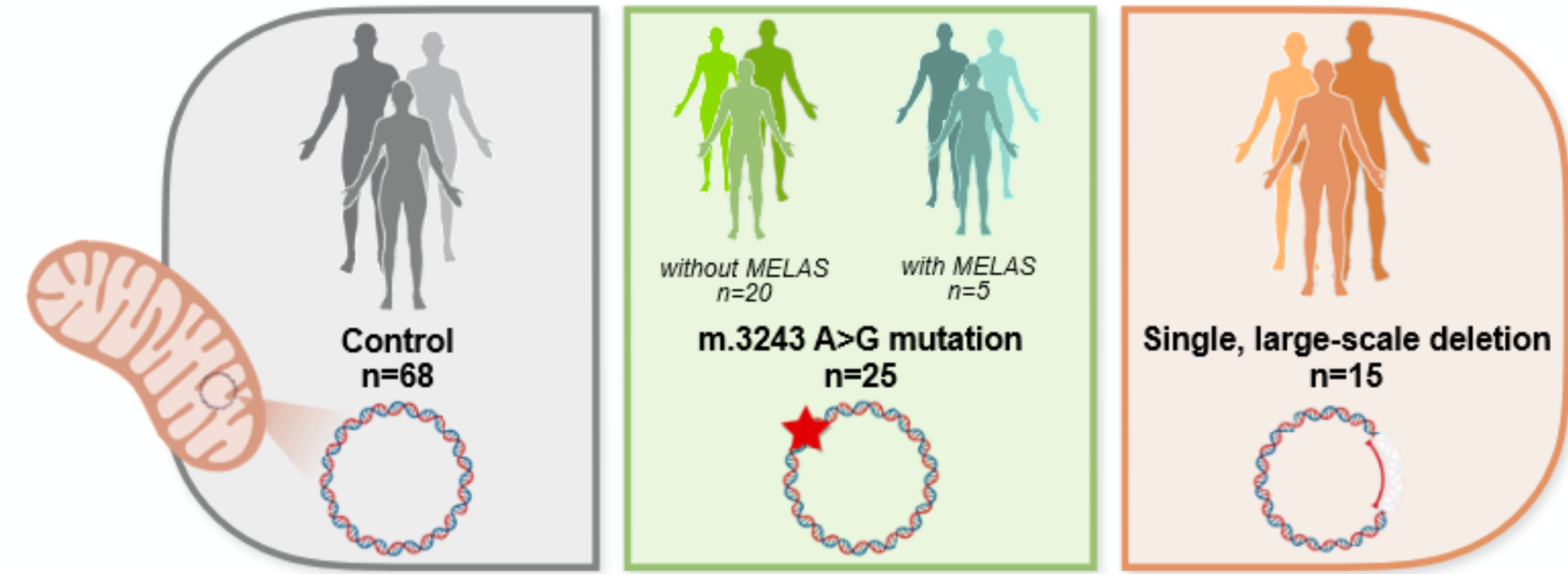
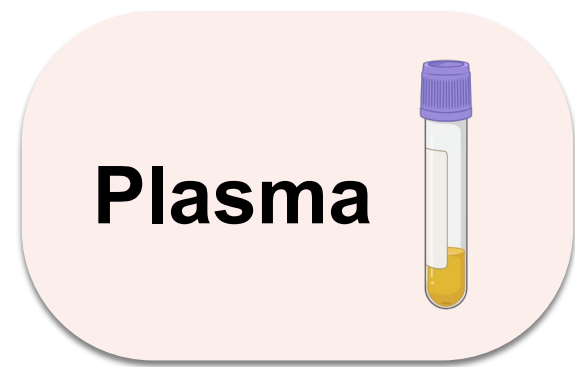
Serum cf-mtDNA trajectories (all groups)



Asterisks: sig. diff. between control and mitoD groups

Results

Plasma cf-mtDNA trajectories (all groups)



Asterisks: sig. diff. between control and mitoD groups

Discussion

Main findings

- Electron micrographs support evidence of cf-mtDNA in vesicles or whole mitochondria
- Acute psychosocial stress rapidly and robustly induces a cf-mtDNA in saliva that is distinct from plasma and serum
- The magnitude of cf-mtDNA reactivity in one biofluid does not reflect the reactivity in another
- Cf-mtDNA baseline values and trajectories were not dramatically different between healthy controls and mitochondrial disease patients

Discussion

Open questions

- What causes range of saliva cf-mtDNA baseline values & different responses to acute stress?
- Where does cf-mtDNA come from? Can we identify what vesicles or structures contain cf-mtDNA in different sample types?
- What is the fate of circulating cf-mtDNA? What physiological function does the increase in circulating cf-mtDNA following acute stress represent?

Thanks!



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