Figure S1

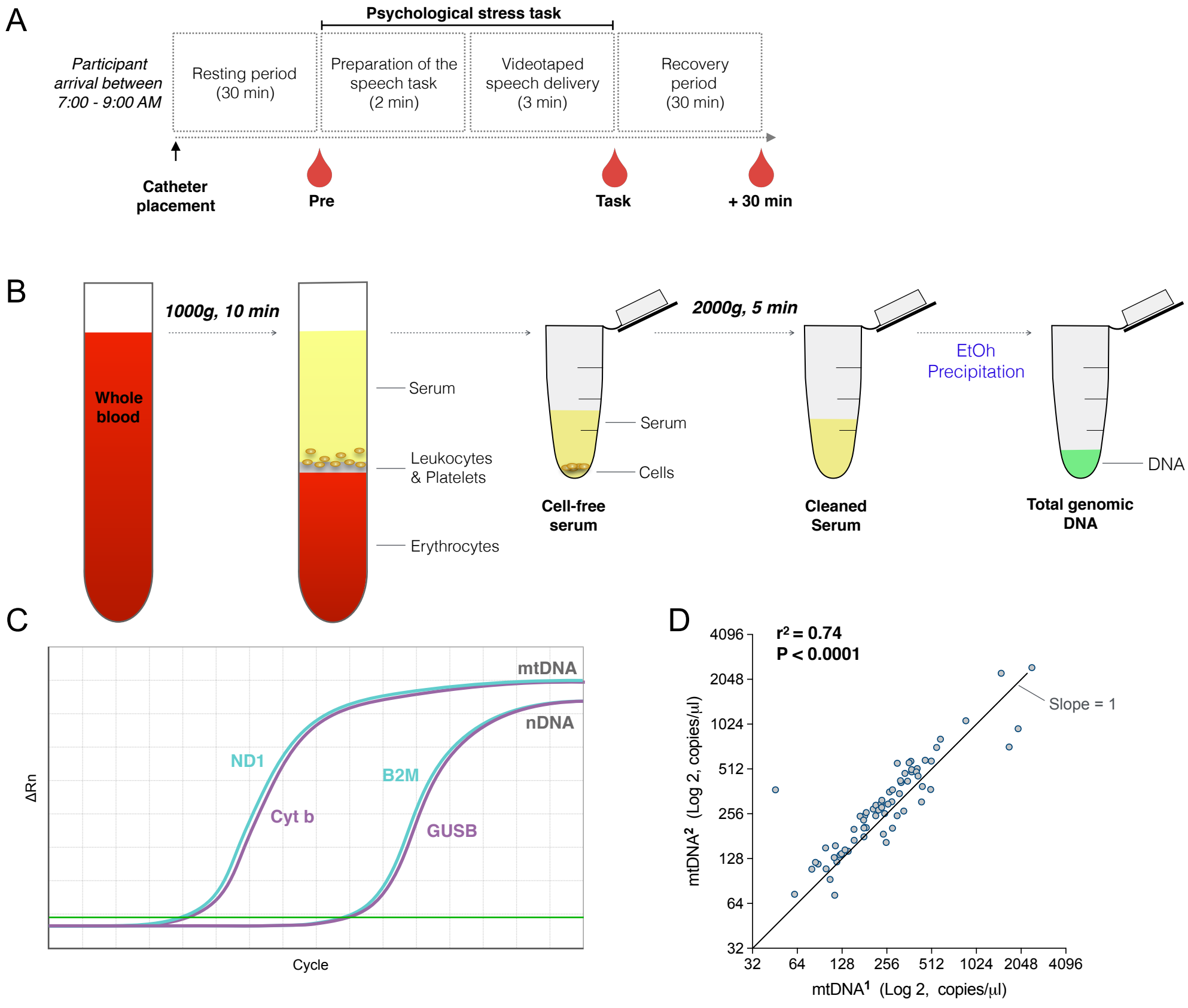
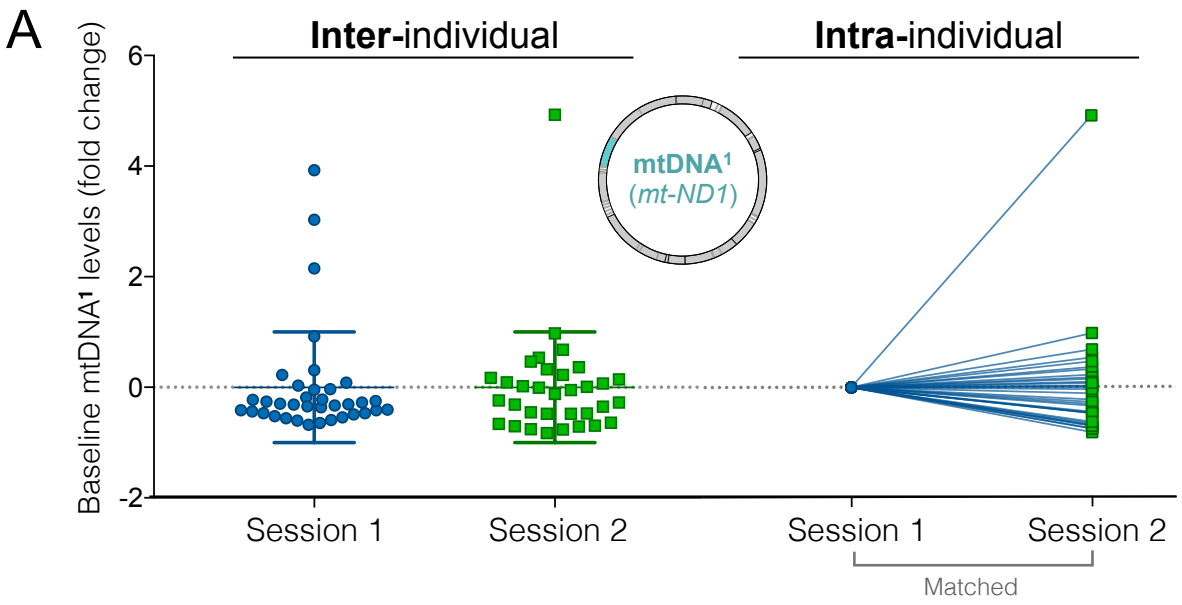
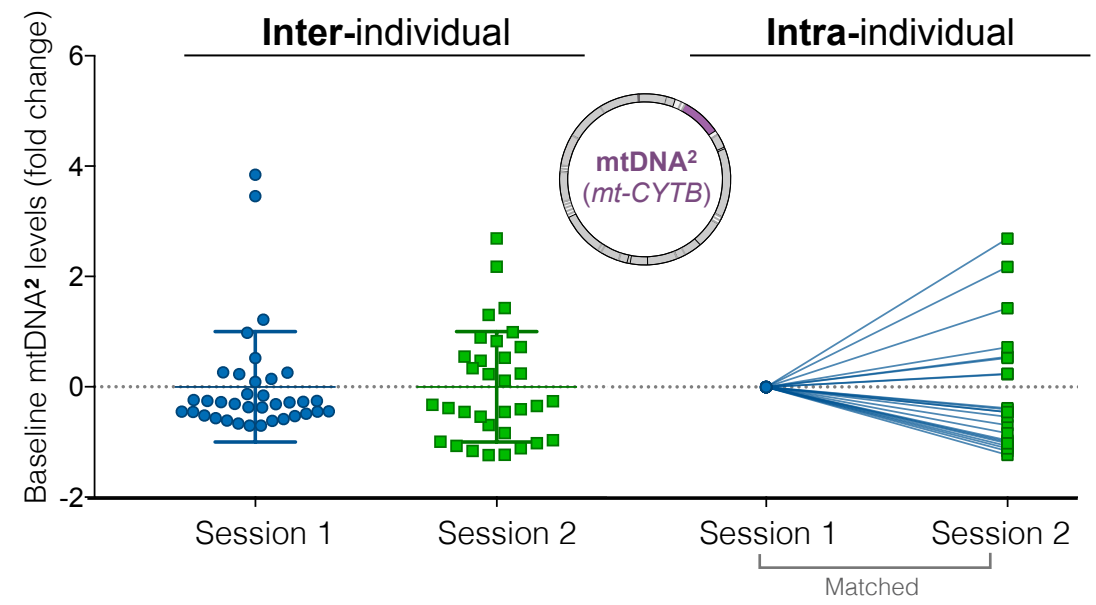
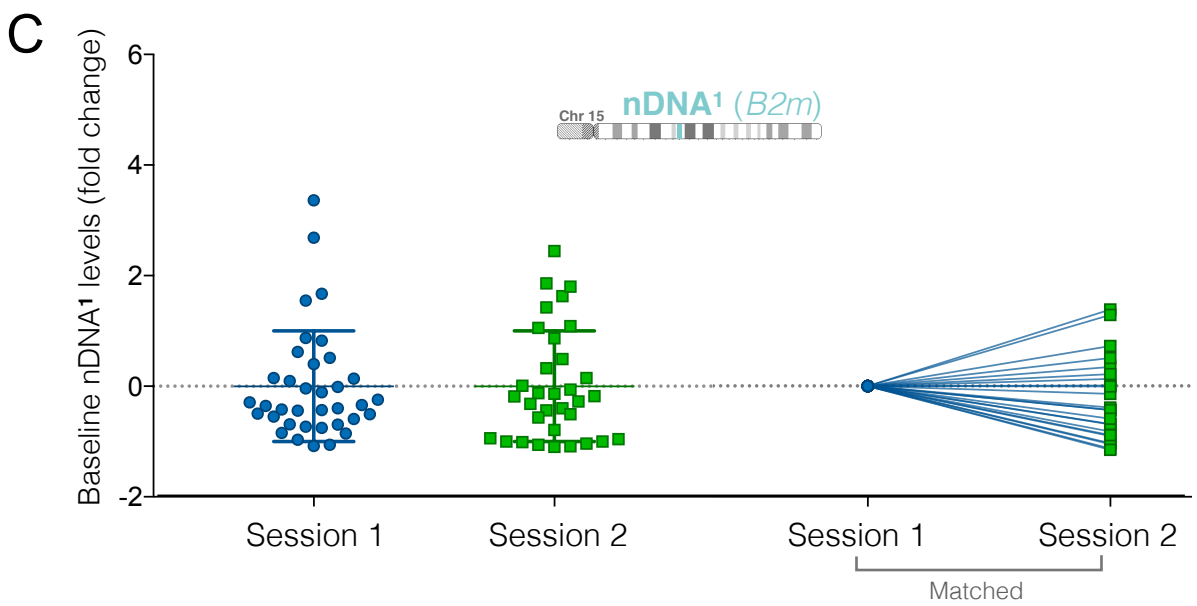


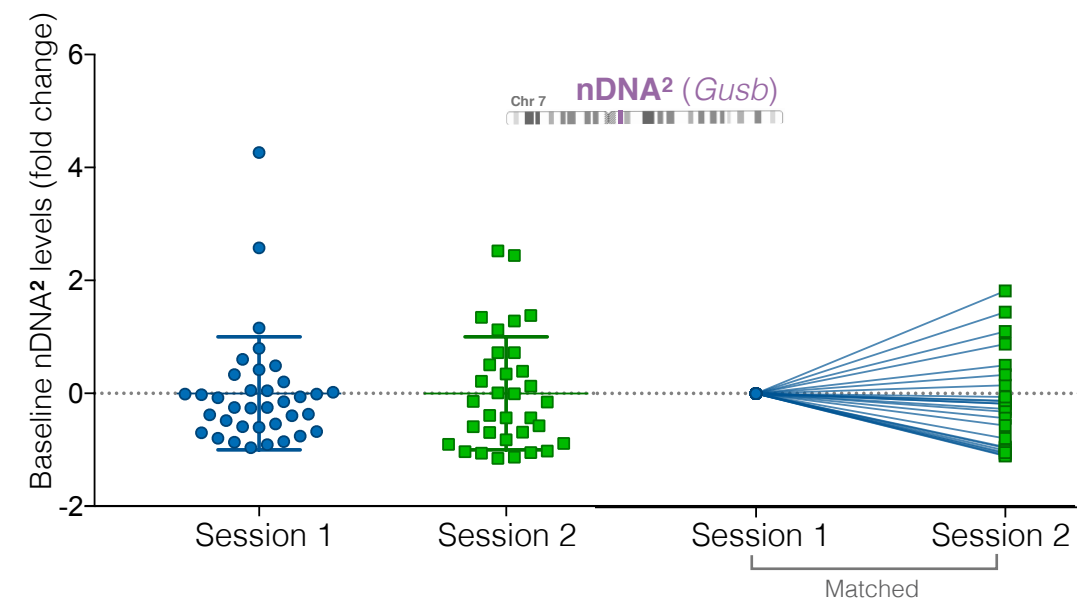
Fig. S1. Human cell-free serum contains circulating mitochondrial and nuclear DNA (**A**) Detailed study timeline. (**B**) Workflow used to obtain cell-free serum. (**C**) Schematic of amplification plot for duplex Taqman qPCR reactions for mitochondrial and nuclear amplicons. *mt-ND1/B2m* and *mt-CYTB/Gusb* are run as pairs in duplex reactions. (**D**) Comparison of abundance for mtDNA amplicons 1 (*mt-ND1*, mtDNA¹) and 2 (*mt-CYTB*, mtDNA²) at *pre* for sessions 1 and session 2. R-squared from linear regression on non-transformed data, n = 69 for both sessions.

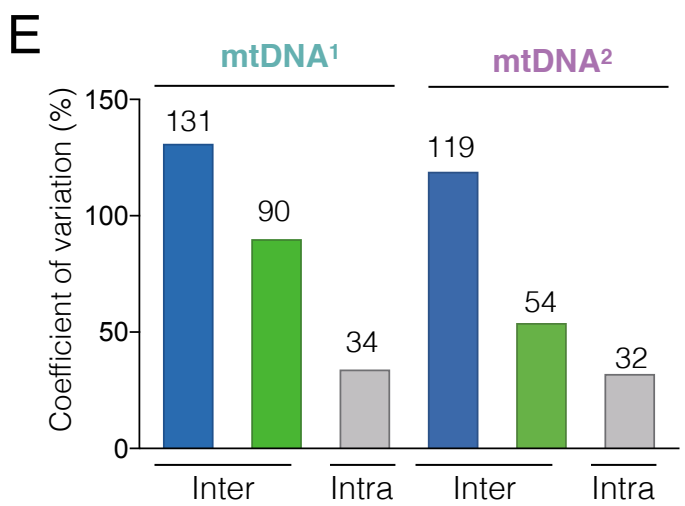
Figure S2

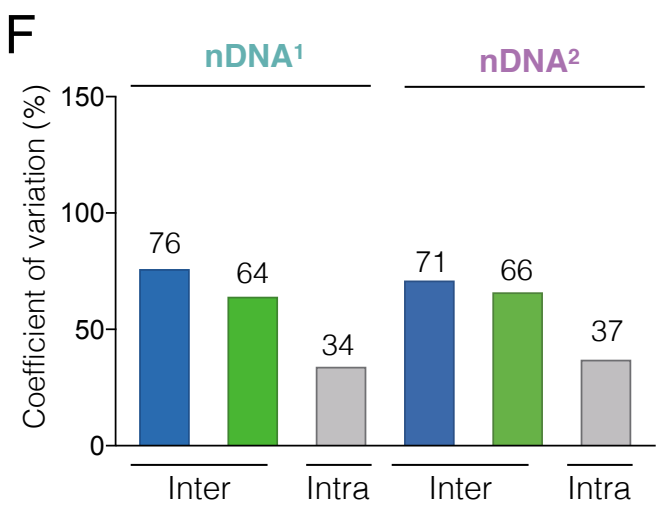


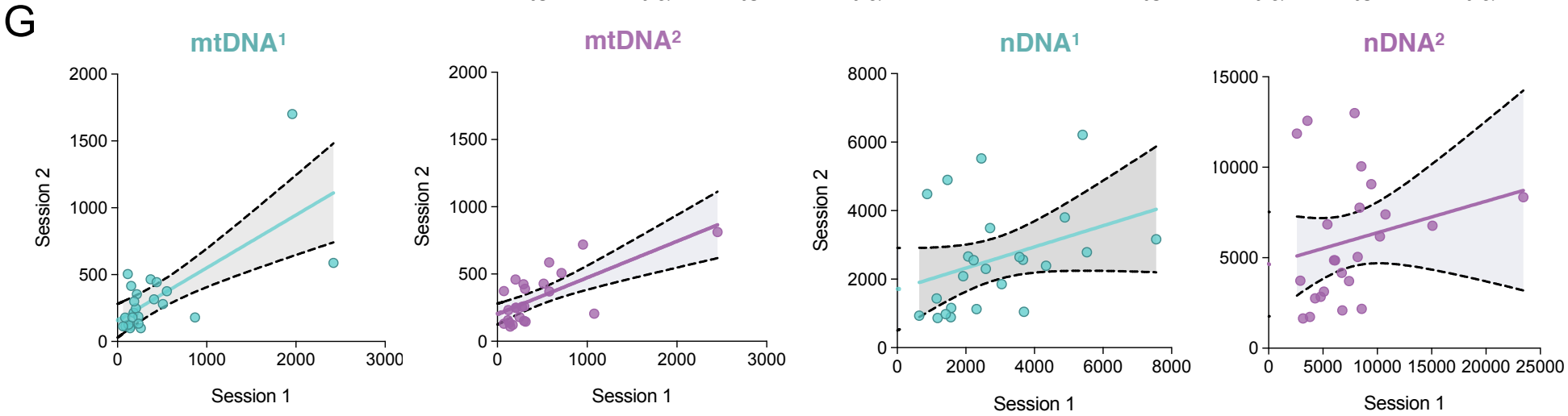












H

R-squared for *pre* at session 1 session 2

	N	r^2	P
mtDNA¹	24	**0.49**	**<0.001**
mtDNA²	24	**0.50**	**<0.001**
nDNA¹	24	0.12	0.094
nDNA²	24	0.05	0.291

Fig. S2. Inter- and intra-individual variability in baseline ccf-mtDNA. (**A**) Baseline "pre" ccf-mtDNA¹ (*mt-ND1*) levels for each participant at both sessions (left) illustrating *inter*-individual variability, and expressed relative to session 1 values for each participant (right), illustrating *intra*-individual variability. Values are centered at the group mean. (**B**) Same as in (A) for mtDNA² (*mt-CYTB*), showing similar results for both mtDNA amplicons. (**C**) Same as in (A) but with ccf-nDNA¹ (*B2m*), and (**D**) with nDNA² (*Gusb*). (**E**) Coefficient of variation of *inter*-individual variability at session 1 (blue) and two (green) and *intra*-individual variability between sessions (grey) of "pre" ccf-mtDNA¹ and ccf-mtDNA². Coefficient of variation were determined on non-transformed data. (**F**) Same as in (E) for ccf-nDNA¹ and ccf-nDNA². (**G**) Correlations of the abundance of mtDNA and nDNA amplicons for sessions 1 and session 2. (**H**) Results of linear regressions from (G), R-squared calculated from non-transformed data, n = 24.

Figure S3

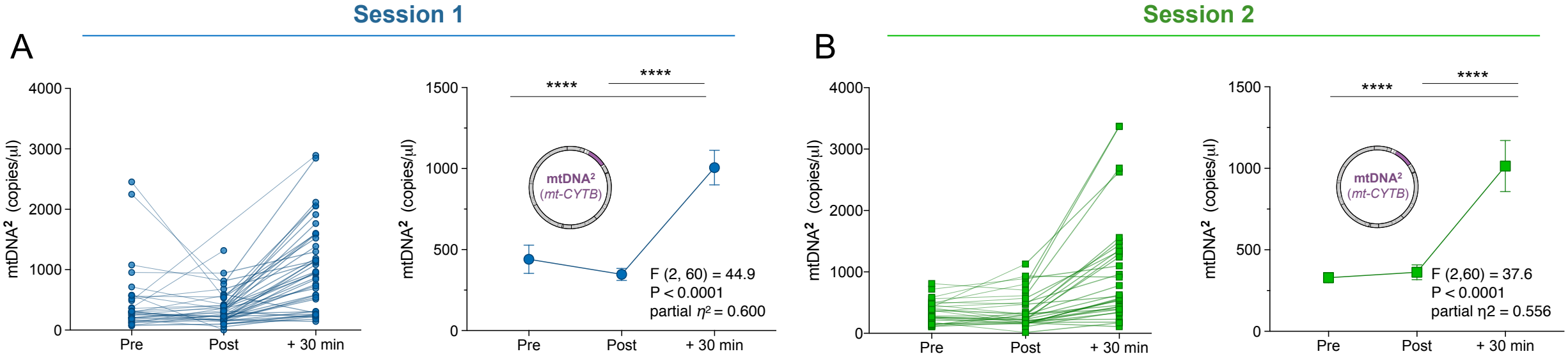


Fig. S3. Psychological stress selectively increases serum circulating cell-free mitochondrial DNA. (**A**) Individual (left) and group mean (right) ccf-mtDNA² (*mt-CYTB*) responses to acute psychological stress at session 1. (**B**) Validation of the results in (A) in a repeat session 2 one month later for ccf-mtDNA². Data are means and SEM. Repeated measure ANOVA on log transformed data and Least Significant Difference (LSD) pairwise comparisons, n = 31 per session, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Figure S4

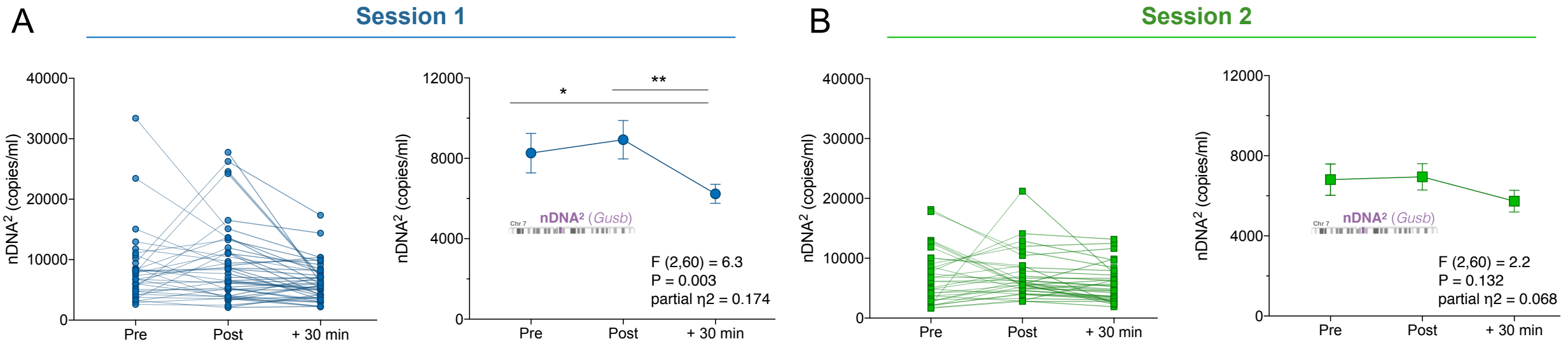


Fig. S4. Psychological stress does not increase serum circulating cell-free nuclear DNA. (**A**) Responses to acute psychological stress at session 1 for the nuclear DNA (ccf-nDNA^2, *Gusb*) and (**B**) at the validation session 2. Data are means and SEM. Repeated measure ANOVA on log transformed data and Least Significant Difference (LSD) pairwise comparisons, n = 31 per session, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Figure S5

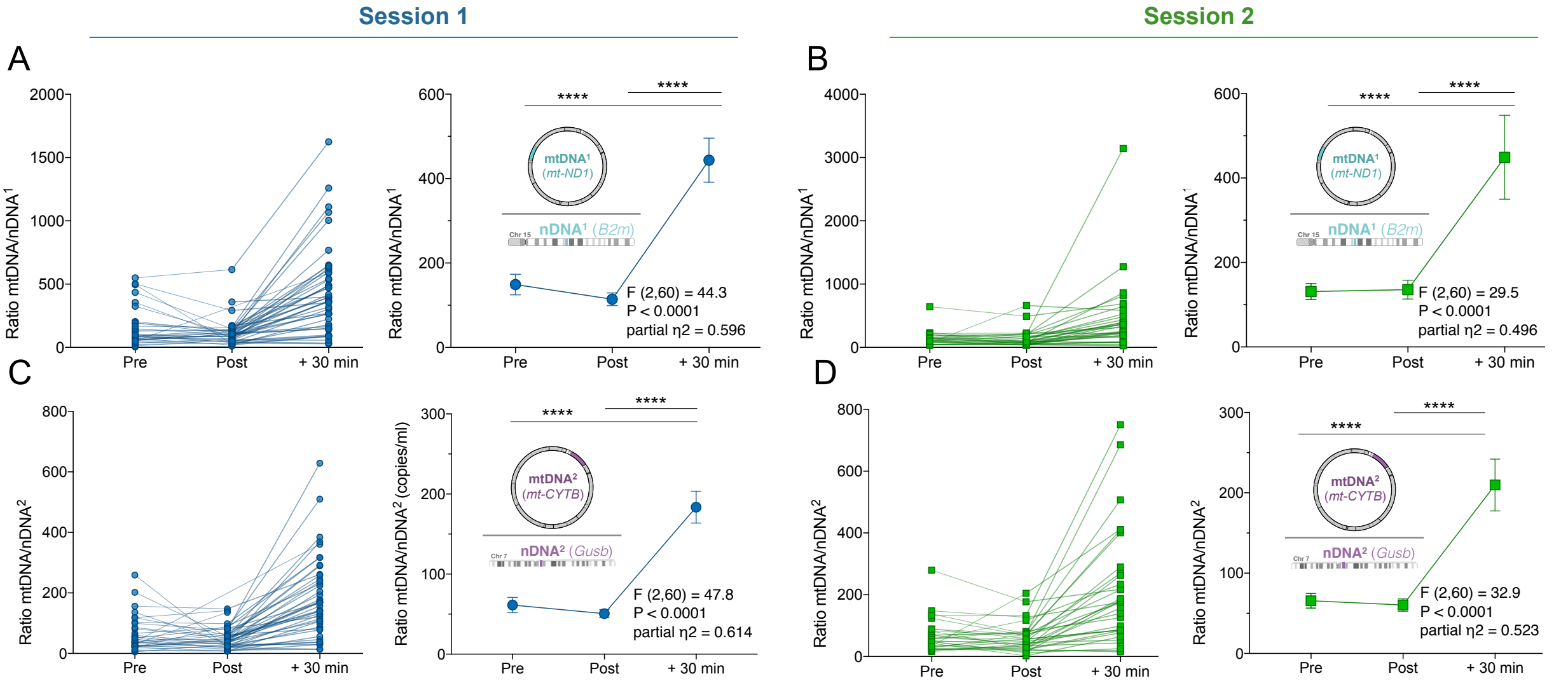


Fig. S5. The mtDNA/nDNA ratio increases in response to acute psychological stress. (**A**) Responses to acute psychological stress at session 1 for the ratio of ccf-mtDNA¹/DNA¹ for each individual (left) and the group average (right) at Session 1 and (**B**) at the validation session 2. (**C**) Same as in (A) for ccf-mtDNA²/ DNA² at session 1 and (**D**) at session 2. Data are means and SEM. Repeated measure ANOVA on log transformed data and Least Significant Difference (LSD) pairwise comparisons, n = 31 per session, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Figure S6

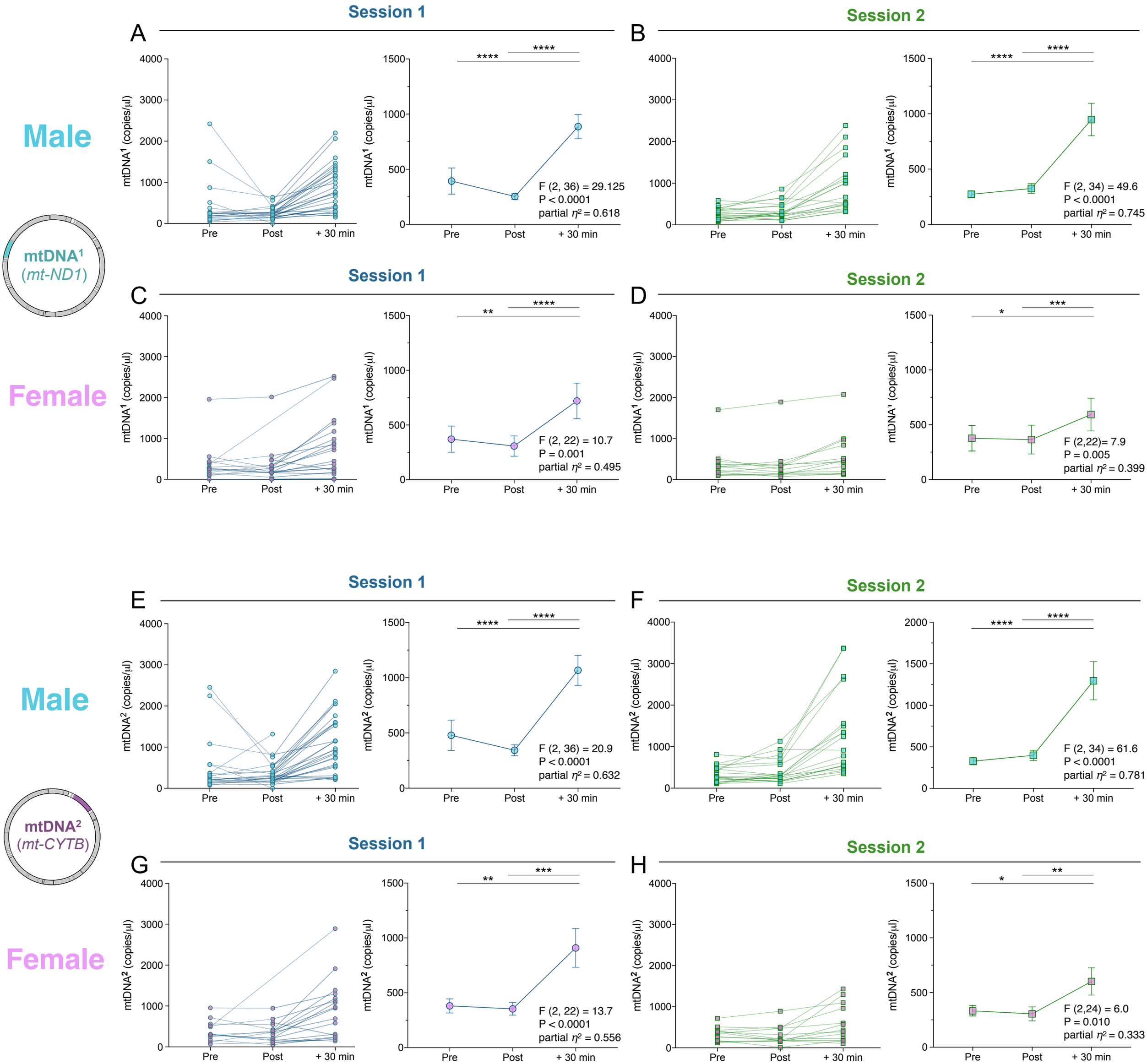


Fig. S6. Free circulating mtDNA in response to acute psychological stress in men and women. (**A**) ccf-mtDNA1 (*mt-ND1*) responses to acute psychological stress in male shown (left) for each individual and (right) for the group average on session 1 (**B**) and session 2, n = 19. (**C**) Same in female on session 1 (**D**) and session 2, n = 13. (**E**) Same as in (A) but for ccf-mtDNA2 (*mt-CYTB*) on session 1 (**F**) and session 2, n = 19. (**G**) Same in female on session 1 (**H**) and session 2, n = 13. Repeated measure ANOVA on log transformed data and Least Significant Difference (LSD) pairwise comparisons. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Figure S7

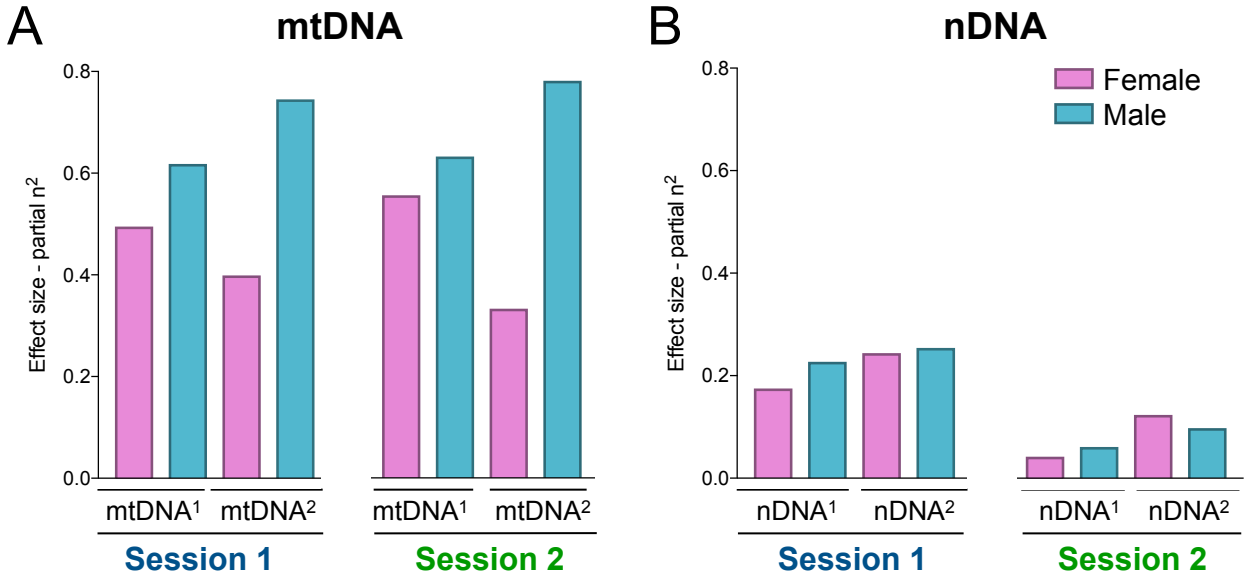
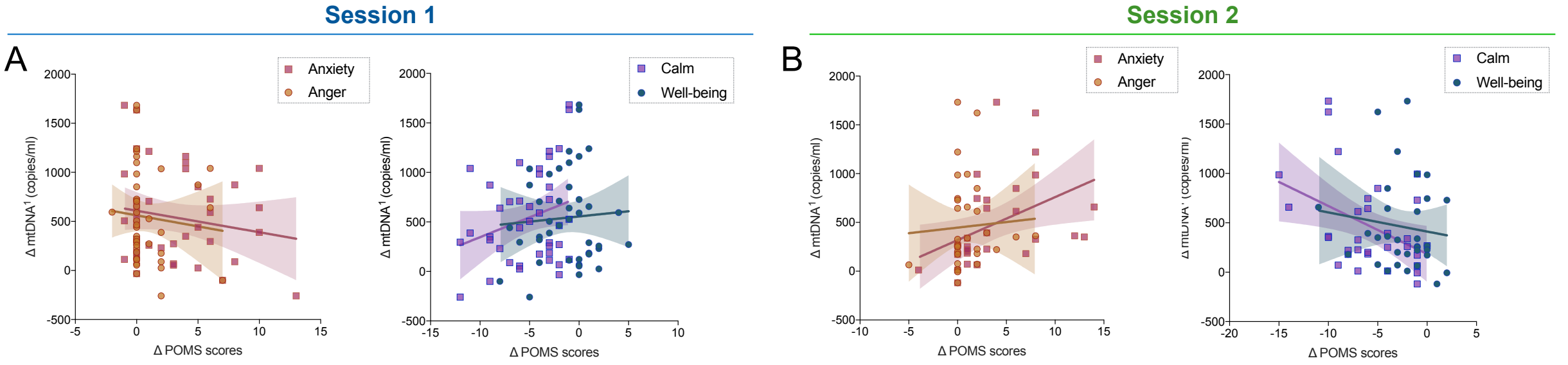


Fig S7. Sex differences in stress-induced elevations in serum mtDNA and nDNA levels. (**A**) Effect size (partial η^2) of the ccf-mtDNA responses to acute psychological stress in female and male (full analysis presented in ***SI*, Fig. S6**). Repeated measure ANOVA performed on log-transformed data. (**B**) Same as (A) for nuclear DNA (nDNA).

Figure S8



C

R-squared for ΔmtDNA¹ and ΔPOMS at Session 1

	N	r	r^2	P
Anxiety	41	-0.17	0.03	0.273
Anger	41	-0.09	0.01	0.566
Calm	41	0.27	0.08	0.083
Well-being	41	0.06	0.00	0.690

D

R-squared for ΔmtDNA¹ and ΔPOMS at Session 2

	N	r	r^2	P
Anxiety	**30**	**0.41**	**0.17**	**0.023**
Anger	32	0.05	0.00	0.768
Calm	**31**	**-0.41**	**0.17**	**0.019**
Well-being	32	-0.11	0.01	0.519

Fig. S8. Association between change in psychological states (POMS scores) and the ccf-mtDNA response to induced psychological stress. (**A**) Pearson correlations of the change in POMS score (from baseline to task) and change in ccf-mtDNA (from task to +30 minutes) at session 1, for two negative (Anxiety, Anger) and two positive (Calm, Well-being) affective states. (**B**) Same as in (A), for session 2. (**C**) Pearson correlation statistics between change in POMS scores and changes in ccf-mtDNA. (**D**) Same as in (C), for session 2.

Figure S9

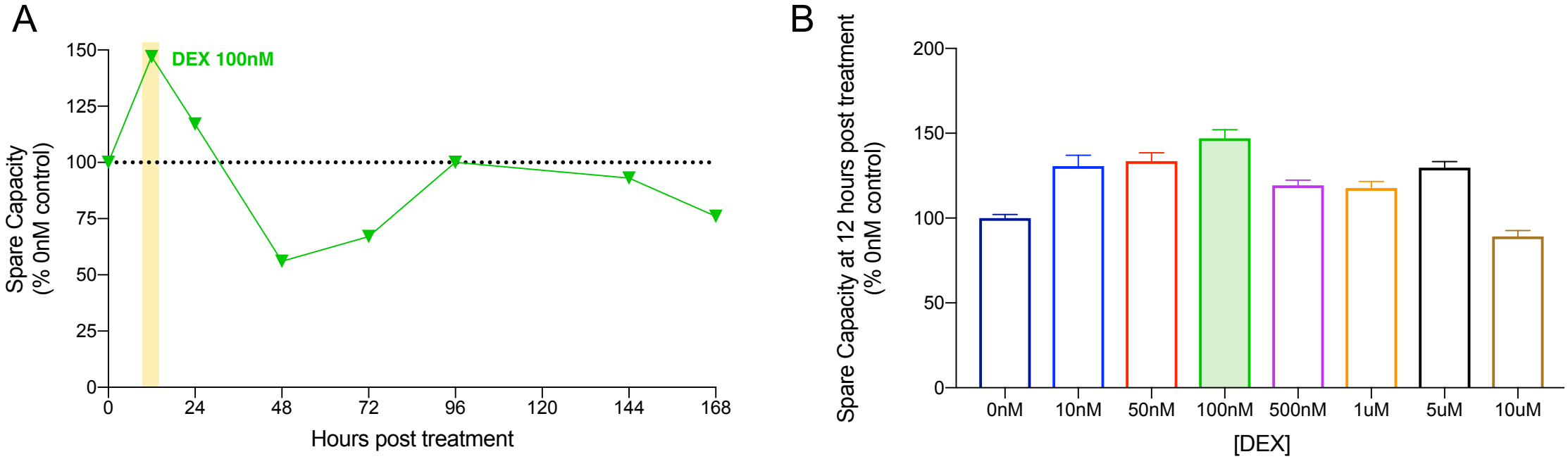


Fig. S9. Dexamethasone (DEX) titration and change in mitochondrial respiration capacity. (**A**) Mitochondrial spare respiratory capacity of primary human fibroblasts changes in response to 100nM dexamethasone, as previously used (Alm et al., 2012; Gerö and Szabo, 2016; Kim et al., 2013). Respiration was measured at different time points after exposure (12hr, 24hr, 48hr, 3d, 4d, 6d, 7d). Data shown as relative to 0nM control ran at each time point (n = 11 technical replicates). In this system, the maximal change in spare capacity was observed after 12hr exposure (highlighted in yellow). (**B**) Mitochondrial spare capacity of primary human fibroblasts with titration of dexamethasone (10nM, 100nM, 500nM, 1uM, 5uM, 10uM) at 12hr. Data shown as relative to 0nM control run at each time point (n = 8-12 technical replicates). The maximal change in spare capacity was observed at 100nM DEX (highlighted in green).