

S2 File: Supplementary Data:

Sample Q/C analysis. Erythrocyte lysis is an important source of miRNA contamination in plasma samples [1, 2]. Therefore, as published previously [3], we assessed the quality of the plasma samples and derived RNA in four ways. Firstly, we assessed plasma samples for free hemoglobin content as well as bound oxy- and de-oxy hemoglobin by spectrometric analysis. We found no evidence for whole erythrocyte contamination (i.e., no detectable oxy and deoxyhemoglobin **S1 Fig., a**) in plasma samples, and minimal hemolysis (spectral absorbance at 414nm), equal to or less than previously published [3]. There was no significant effect of recruitment site ($P=0.88$) or exposure group ($P=0.76$) on hemolysis. Interestingly, there was a small but statistically significant decline in free hemoglobin at the end of pregnancy compared to the mid-pregnancy period (ANOVA, $F_{(1,125)}=6.67$, $P<0.01$, **S1 Fig., b**), indicative perhaps of pregnancy-associated anemia in this cohort. Secondly, none of the assessed plasma samples amplified the mRNA transcript for Band-3 protein (SLC4A1, **S1 Fig., c**) which is abundant in erythrocytes. Thirdly, the mean sample ΔCq for $_{\text{plasma_stable}}\text{miR23a-erythrocyte_abundant}\text{miR451a}$, -0.88 ± 0.677 (Mean \pm SEM), was below the identified threshold for hemolysis ($\Delta Cq>7$, [4], and was not significantly altered due to recruitment site ($P=0.94$), pregnancy stage ($P=0.71$) or exposure group ($P=0.65$) indicating that there was not a systematic variable-dependent effect on hemolysis. These data collectively eliminate hemolysis as a significant contributor to sample miRNA content.

References:

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