Supplementary data.

In silico simulation experiments for validating the deconvolution method on inferring placenta fractions.

We started with 5820 methylation markers from Sun et al [8] that were originally selected to deconvolute 14 tissues. We narrowed down the marker set by filtering out those biomarkers which frequently reported high placenta percentage with our read-based method. We used 32 normal plasma samples from Chan et al [42], and if a biomarker reported the placenta percentage more than 20% in at least 4 normal samples, it was filtered out. Finally, 1767 (out of 5820) biomarkers were kept as a new set of biomarkers.

We simulated the mixed methylation data by sampling and merging the methylation sequencing reads of two samples, a white blood cell and a solid placenta. The bisulfite sequencing data of six white blood cell samples were generated in-house and the bisulfite sequencing data of 5 solid placenta samples were collected from Jensen, et al [41], so that we would have 30 combinations. For each combination, we mixed the samples at 11 different placenta fractions (theta=0, 1%, 2%, ..., 10%), and 5 different sequencing coverages (c=0.1, 0.5, 1, 2 and 5X). This experiment setting generated 30*11*5=1650 mixed samples.

We compared the read-based method to the commonly used Quadratic Programming (QP) method [8] using the simulation data. For each mixed percentage at every sequencing coverage, we calculated the median predicted placenta percentage over all 30 combinations, its 25^{th} quartile and 75^{th} quartile. We used Pearson's correlation coefficient and mean square error between the input and predicted placenta percentages to evaluate its accuracy. To compare the sensitivity of two

methods in identifying a minor trace of placental DNA, we defined a metric namely, low input detection rate (denoted as LDR) for these samples with low input (= 1%, 2%, 3% or 4%).

$LDR = 1 - \frac{\text{\# of undetectable samples}}{\text{\# of total samples}}$, where a sample is undetectable if its predicted placental fraction is close to zero (<1e-5).

As shown in Supplementary Fig. 1a, the predicted placental percentages by our read-based method were highly consistent with the input values and showed low variability. The PCC reached was 0.990 at coverage=0.1X and increased to 0.999 for other higher coverages. The MSE decreased with the sequencing coverage. More importantly, the LDR of our method is 0.867 for coverage= 0.1X, and increased to 1 for other higher coverages. All the results show that our method works with low-coverage sequencing data yielding high accuracy that demonstrate high detection rate of minor traces of placental input even when the coverage is 0.1X. QP method (Supplementary Fig. 1b) gave a good correlation between the input and predicted percentage (achieved PCC=0.97 at 0.1X and larger for higher coverage), but inferior to the read-based method. The MSE of the QP method is larger when compared to that of the read-based method for all coverages. Note that it is difficult for QP method to detect placenta when the input percentage is low (below 4%), where the resulting LDR varied from 0.59 to 0.68 across different sequencing coverages. In summary, the read-based method can sensitively detect a minor trace of placental tissue in mixed samples even at low sequencing coverage, and the prediction accuracy increased with the sequencing coverage.

Supplementary Figure 1

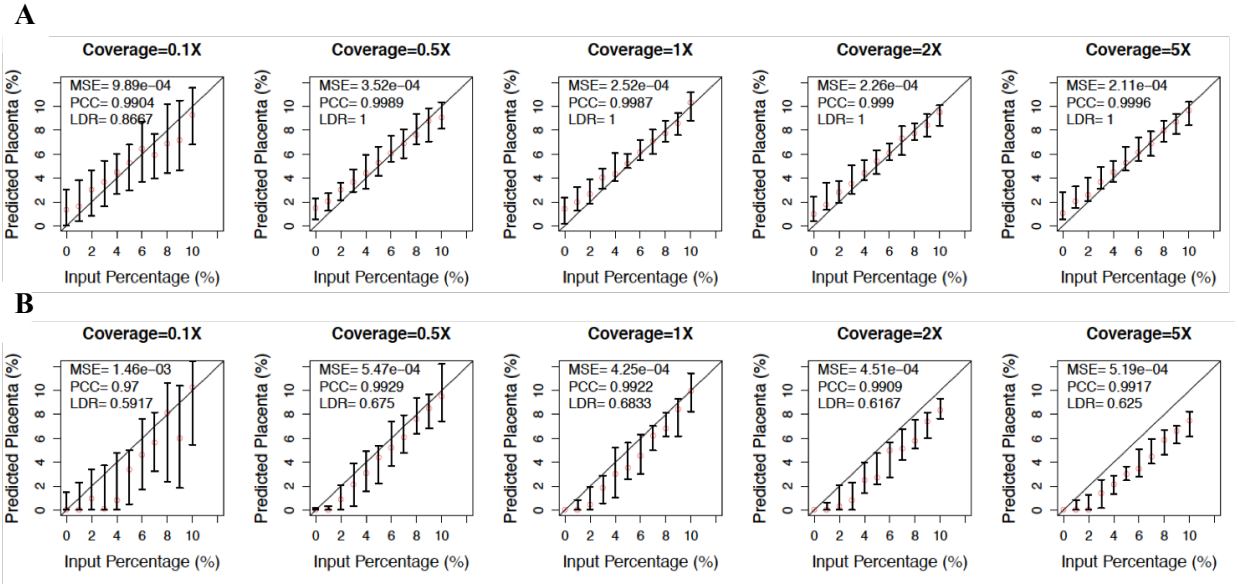

Figure S1. **In silico simulation experiments for validating the deconvolution method on inferring placental fractions.** Deconvolution of the placental fraction from the mixed samples, generated by subsampling and merging sequencing reads from one out of the six white blood cell samples and one out of the five solid placental samples, at 11 different placental percentages (theta=0, 1%, 2%, ..., 10%), and 5 different sequencing coverages (c=0.1, 0.5, 1, 2 and 5X). The x-axis is the true placental percentage and the y-axis is the predicted percentage. For each input percentage, the red dot is the median value of the predicted placental percentages, the bars are 25th quartile and 75th quartile respectively. At each sequencing coverage, PCC is the Pearson's correlation coefficient between the input percentages and the mean values of the predicted placental percentage. MSE is the average of the squares of the error between input and predicted placental percentages. LDR is the detection rate for samples with low input percentage (from 1% to 4%). (**A**) The results from the read-based method (**B**) The results from QP method.

Supplementary Figure 2

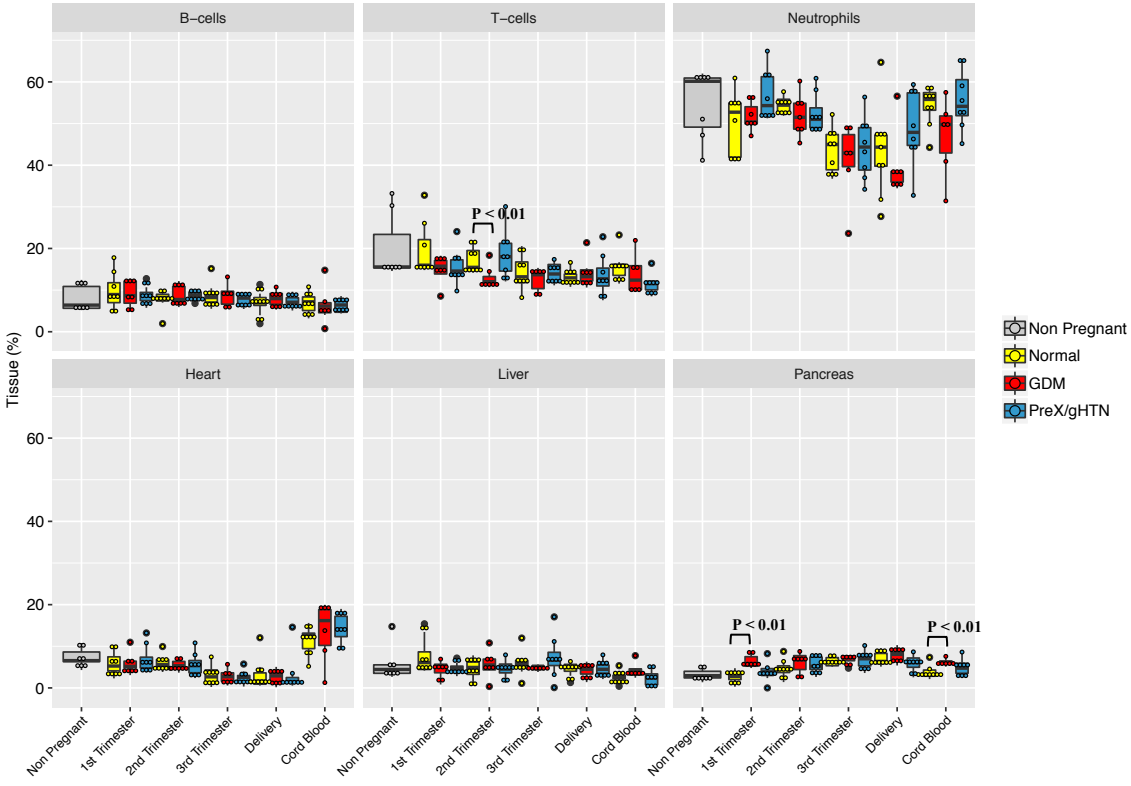

Figure S2. Deconvolution analysis results in subjects with adverse pregnancy outcomes (APOs) and subjects that did not develop any adverse outcomes (Normal). The box plots show the deconvolution results expressed in percentage for every single tissue in which the cell- free DNA from maternal plasma has been deconvoluted. The placental contribution is described in detail in Figure 2. Each dot represents a single subject and the gray bar represents the non-pregnant subjects who served as controls. All the P-values represented in the figure are based on the non-parametric Mann-Whitney U test.

Supplementary Figure 3

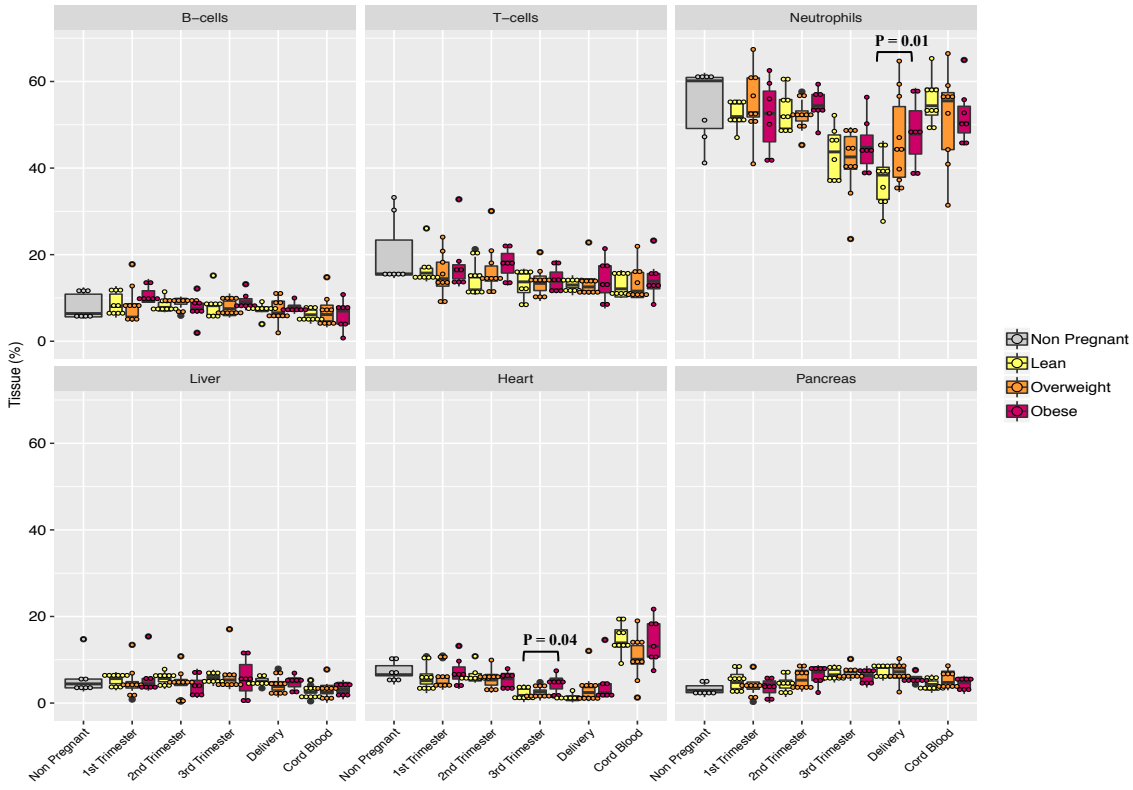

Figure S3. Deconvolution analysis results in subjects grouped according to their pre-pregnancy BMI. The box plots are the whole picture of the results obtained from the deconvolution analysis for the different BMI categories. The placental contribution is described in detail in Figure 3. Each dot represents a single subject and the gray bar represents the non-pregnant subjects that are represented in the graph as a baseline threshold. No information regarding their BMIs was available. All the P-Values represented in the figure were based on the Mann-Whitney U test.

Supplementary Figure 4

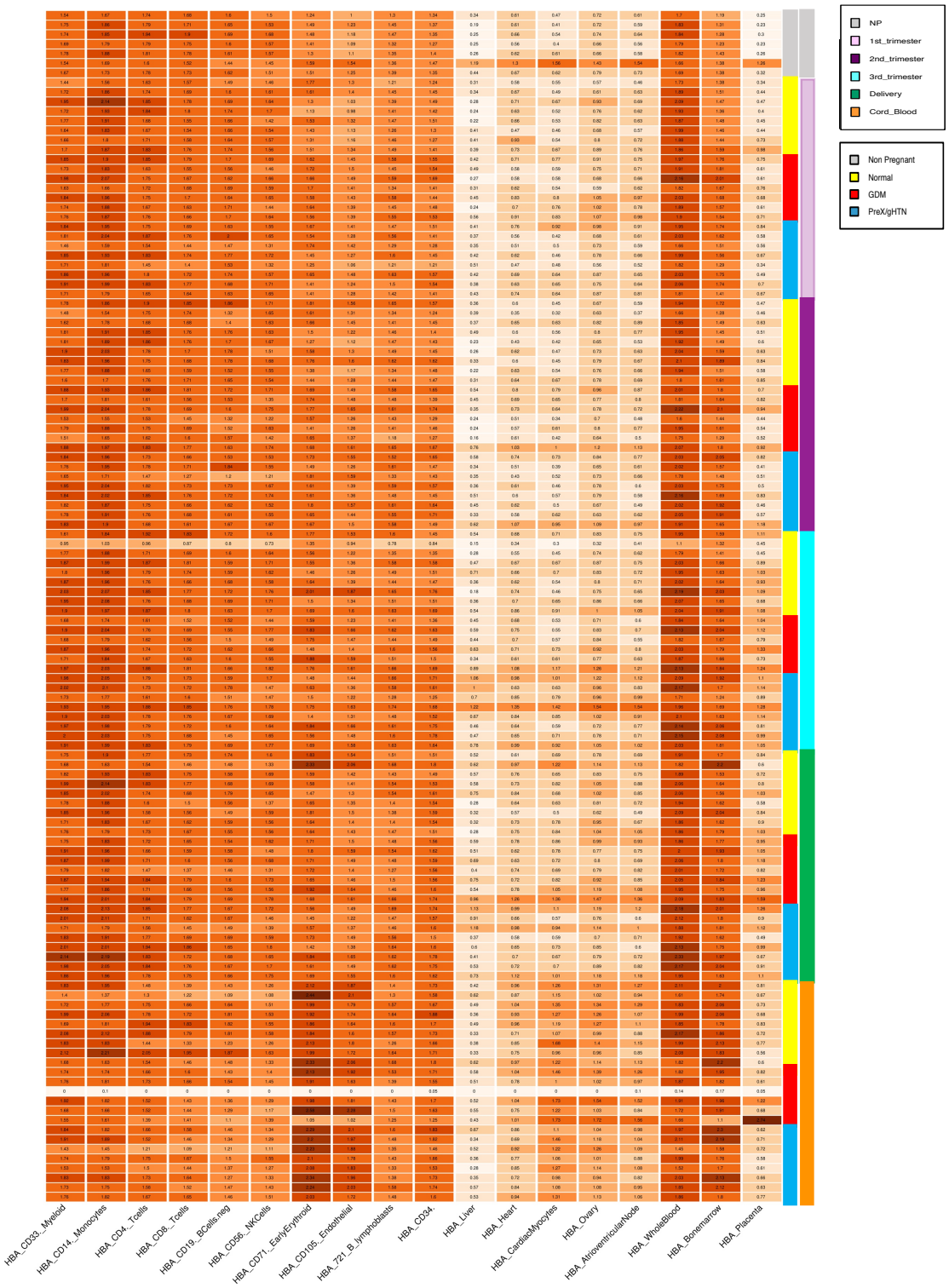

Figure S4. cfRNA deconstruction in sixteen tissues/cells of origins.

Heatmap showing in an increasing-red gradient the tissues/cells of origin signatures. Each row of the heatmap refers to a single subject while each column is a specific signature.

Supplementary Figure 5

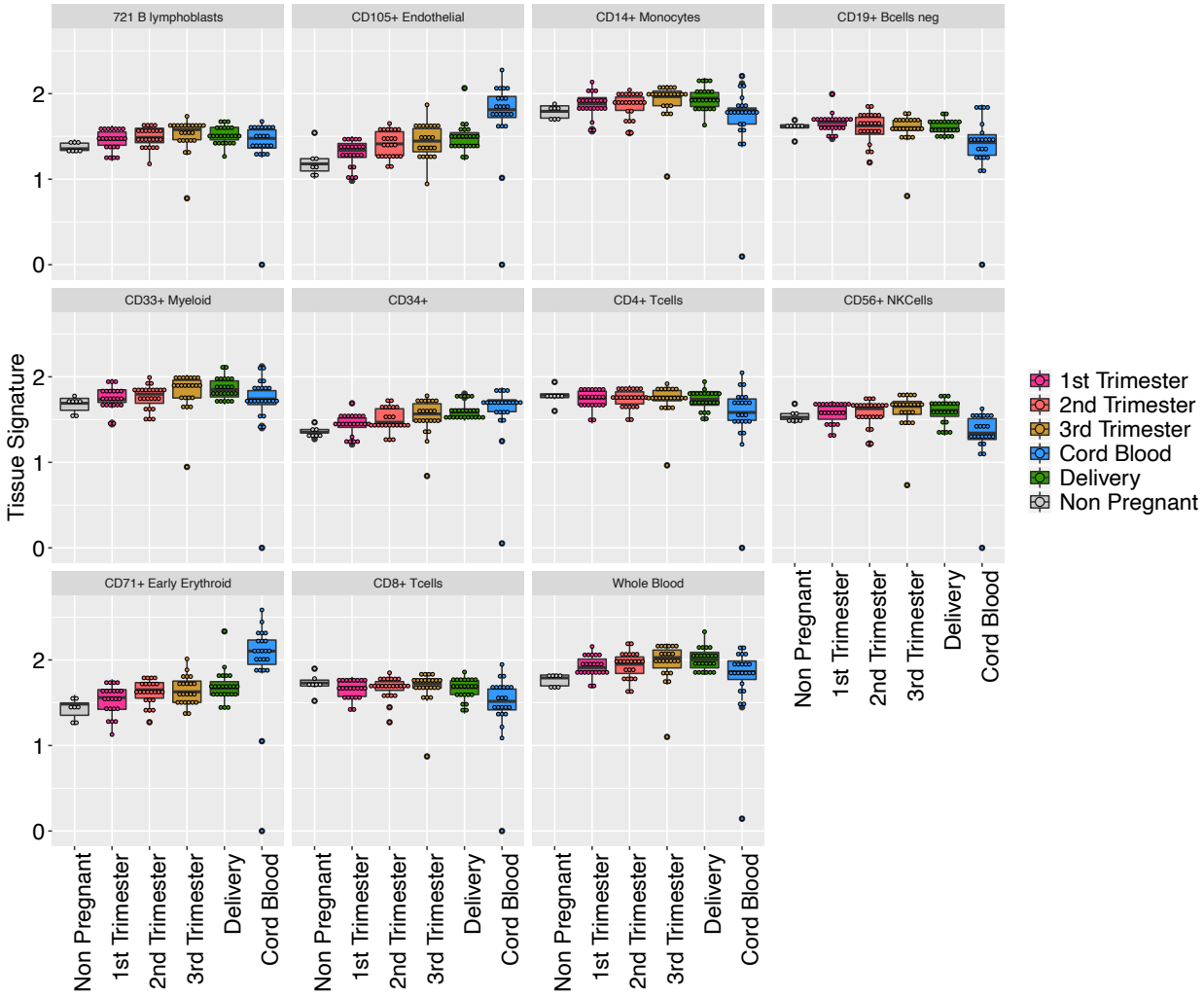

Figure S5. cfRNA Immune cell signature analysis during gestation. The box plots show the results from the SaVanT approach for every single mature immune cell that we analyzed within the cell-free RNA. The placental and bone marrow contribution are described in detail in Figure

4. Each dot represents a single subject and the gray bar represents the non-pregnant subjects who served as controls. All the P-values represented in the figure are based on the non-parametric Mann- Whitney U test.

Supplementary Figure 6

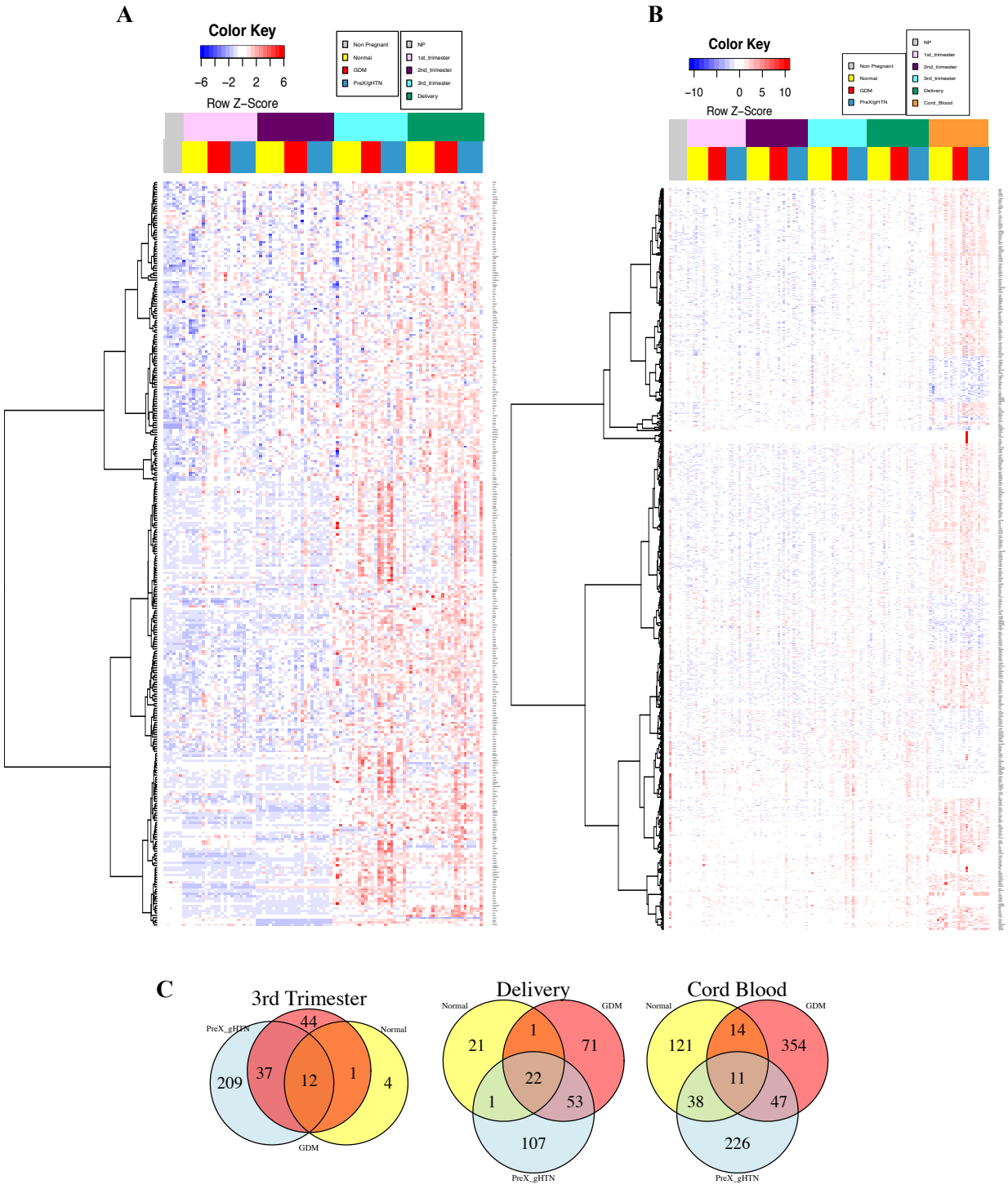

Figure S6. RNA sequencing: Differentially expressed genes in 3rd trimester of pregnancy, at delivery and in cord blood. (**A)** Heatmap showing in a blue-red gradient the expression level of all the DEGs during pregnancy when compared to non-pregnant subjects. All the genes that are differentially expressed in each trimester were grouped together, with an adjusted P-value ≤ 0.05 and a log2 fold-change ≥ 1 or ≤ -0.1. The p-adjusted value was calculated in DESeq that used Benjamin-Hochberg correction [19]. The horizontal color bars on the top of the heatmap corresponds to the different groups of subjects (the lower one) and different time points during pregnancy (the upper one). Each row of the heatmap refers to a gene and each column is a sample taken at a particular time point. (**B)** Heatmap shows in a blue-red gradient the expression level of all the DEGs in Cord blood samples when compared to all pregnancy-time points collectively in the respective study group. All the genes with a p adjusted value <0.05 and a log2 fold-change ≥ 1 or ≤ -0.1 were grouped together. (**C)** Venn diagram showing the numbers of differentially expressed genes for each group compared to non-pregnant controls. Yellow represents Normal pregnancy, red GDM and light blue PreX/gHTN. In addition, the Venn diagram shows the number of overlapping genes between the different groups during the 3rd trimester, at delivery and in cord blood.

Supplementary figure 7

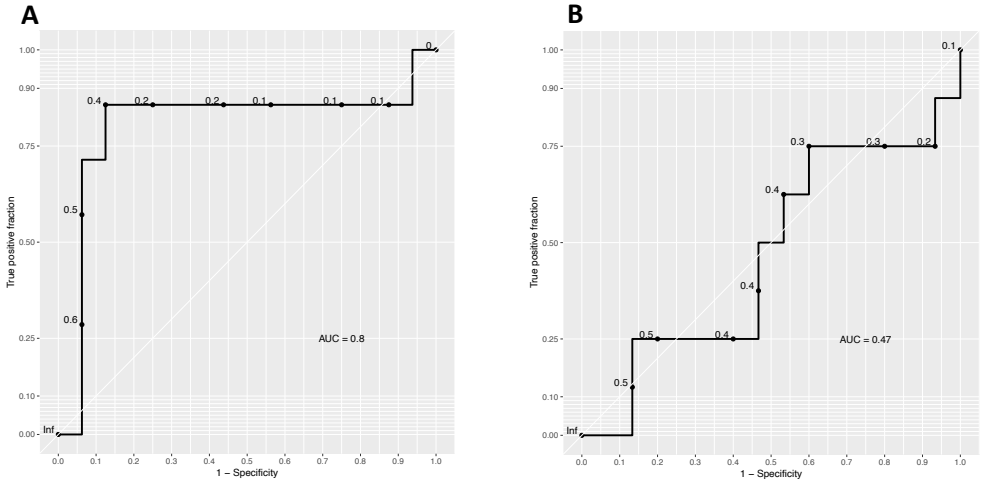

Figure S7. Receiver operator curves (ROC) of classification models with cfDNA deconvolution results in the first trimester. We used cfDNA-based deconvolution results for seven tissues as input; using elastic net regularization placenta and pancreas were selected as predictors. (A) First trimester of pregnancy with GDM n= 7, versus normal pregnancy n=8; AUC = 0.80. (B) First trimester of pregnancy with PreX/gHTN, n=8 versus normal pregnancy, n = 8; AUC = 0.47.

Table 1

PatientID	Baby gender	Delivery mode	Age	BMI	BMI category	Baby weight (gr)	baby length (cm)	baby head circumference (cm)	Placental weight (gr)	1st trimester collection week	2nd trimester collection week	3rd trimester collection week	Delivery
cHTN_1	F	spontaneous	40	34.75	Obese	3420	52.1	34.5	466.7	17	24	37	39
cHTN_2	M	spontaneous	32	25.69	overweight	3980	58.4	36.8	433.3	14	21	35	39
GDM_1	F	Spontaneous	36	33.95	obese	3402	50.8	34	570.7	16	22	36	39
GDM_2	F	spontaneous	32	21.26	Normal	3345	60.8	33.7	429.2	17	20	36	39
GDM_3	M	Vaginal-vacuum	33	18.69	Normal	2700	45.7	32.4	437.5	17	20	35	36
GDM_4	M	C_section	33	27.46	overweight	2620	47	31.8	309.7	16	20	37	38
GDM_5	M	spontaneous	31	26.85	overweight	3710	50.2	32.4	451.3	17	19	35	39
GDM_6	M	C_section	34	19.37	Normal	2900	49	34.5	403	15	22	35	39
GDM_7	F	C_section	37	28.34	overweight	3075	52.1	33	599.8	12	21	35	37
Normal1	M	C_section	32	26.09	overweight	3200	50	35	439.1	na	18	36	41
Normal2	M	Spontaneous	24	30.05	Obese	3720	50.8	35.6	450.6	15	19	36	38
Normal3	F	C_section	37	22.75	Normal	3560	51	34.5	450.8	14	19	36	40
Normal4	M	spontaneous	36	27.36	overweight	3118	50.8	34	530.5	13	21	35	37
Normal5	F	spontaneous	32	22.2	Normal	2800	51.5	32.5	403.1	12	19	35	37
Normal6	M	Spontaneous	31	28.94	overweight	3100	50.8	34.3	420.1	16	20	36	37
Normal7	F	C_section	29	30.15	Obese	4070	52.1	34.3	519.5	13	21	37	40
Normal8	F	spontaneous	35	20.83	normal	3600	52.1	34	475.6	13	21	36	40
Normal9	F	C_section	36	21.8	Normal	3520	50.8	34.3	554.6	16	20	35	39
PreX/gHTN_1	M	Spontaneous	30	48.57	extr_obese	3220	50.8	33	465.3	15	22	37	38
PreX/gHTN_2	F	Vaginal-vacuum	36	24.64	Normal	3830	52.1	36.2	503	14	20	36	37
PreX/gHTN_3	M	C_section	42	27.34	overweight	3190	48.9	33.7	406.2	12	18	37	38
PreX/gHTN_4	F	C_section	35	26.63	overweight	2510	45.1	33.7	336	13	19	36	37
PreX/gHTN_5	M	C_section	30	25	overweight	3650	52.1	34	524.9	12	20	36	39
PreX/gHTN_6	M	spontaneous	33	36.83	obese	3670	51	36.5	526.2	11	20	36	40
PreX/gHTN_7	M	C_section	33	22.13	normal	3290	53.3	35.6	411.5	12	19	37	40
PreX/gHTN_8	M	C_section	28	32.43	obese	3430	50.8	35.6	566.8	12	20	35	37
qRT-PCR only subjects													
GDM_8	F	Spontaneous	30	21.12	Normal	2930	60.8	33.5	489	11			
GDM_9	M	C_section	38	28.85	overweight	3530	52.7	33	465	12			
GDM_10	M	Spontaneous	39	26.79	overweight	3670	54.6	34.9	552.2	16			
GDM_11	M	C_section	38	23.47	normal	3515	50.8	33	532.4	10			
GDM_12	na	No_UCLA Hospital	38	29.68	overweight	na	na	na	na	16			
PreX/gHTN_9	F	C_section	50	29.42	overweight	2620	45.7	33.5	382.6	12			