Supporting Information

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SI Materials and Methods

RNA-seq–Based Expression Analysis. The amplified cDNA was converted to sequencing library according to Illumina's protocol and quantified by real-time PCR. Sequencing was performed on the GAII and the HiSeq instruments. The raw reads of RNA-seq were first assessed for their quality using FastQC. Bad quality reads (phred score < 30) were trimmed. Then the illumina adaptor sequences at the end of reads were cut. The reads were aligned against the UCSC hg19 version of human genome reference sequence using TopHat software. The gene expression level was quantified as fragments per kilobase of transcript per million mapped reads (FPKM) value using Cufflinks software.

Microarray-Based Expression Analysis. The amplified cDNA (by Nugen) was fragmented using DNase I and labeled with biotin, following by hybridization to Affymetrix GeneChip ST 1.0 microarrays. The gene expression value was computed by Affymetrix Expression Console software using the robust multiarray average (RMA) algorithm.

Identification of Temporal Trends in Expression Data. Identification of genes with temporal variation was performed using a repeated ANOVA test across all genes with the ANOVA model using R and the Bioconductor package. The ANOVA model breaks down the variation of the quantified RNA transcripts into time, patients, and batches. Following ANOVA, correlation tests were performed. Because the ANOVA model only accounts for the variation, the additional correlation tests are required to pick out genes that match the required trends. In this study, we included nonpregnant samples and postpartum samples. The correlation tests will allow us to discover transcripts exhibiting low expression in nonpregnant controls and postpartum.

Selection of Fetal Tissue-Specific Transcripts Panel for Quantitative PCR. To detect the presence of these fetal tissue-specific transcripts, we selectively curate a list of known fetal tissue-specific genes from known literature (1) and databases. We validated the specificity for fetal tissues by cross referencing two main databases: Tissue-Specific Genes Database (TISGeD) (2) and BioGPS (3, 4) Most of these selected transcripts are associated with known fetal developmental processes. We further overlapped this list of genes with our RNA sequencing and microarray data to generate our panel of genes as shown in the results.

1. Hui L, Slonim DK, Wick HC, Johnson KL, Bianchi DW (2012) The amniotic fluid transcriptome: A source of novel information about human fetal development. *Obstet Gynecol* 119(1):111–118.
2. Xiao S-J, Zhang C, Ji Z-L (2010) TiSGeD: A database for tissue-specific genes. *Bioinformatics* 26(9):1273–1275.
3. Wu C, et al. (2009) BioGPS: An extensible and customizable portal for querying and organizing gene annotation resources. *Genome Biol* 10:R130.
4. Su AI et al. (2004) A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA* 101(6):6062–6067.

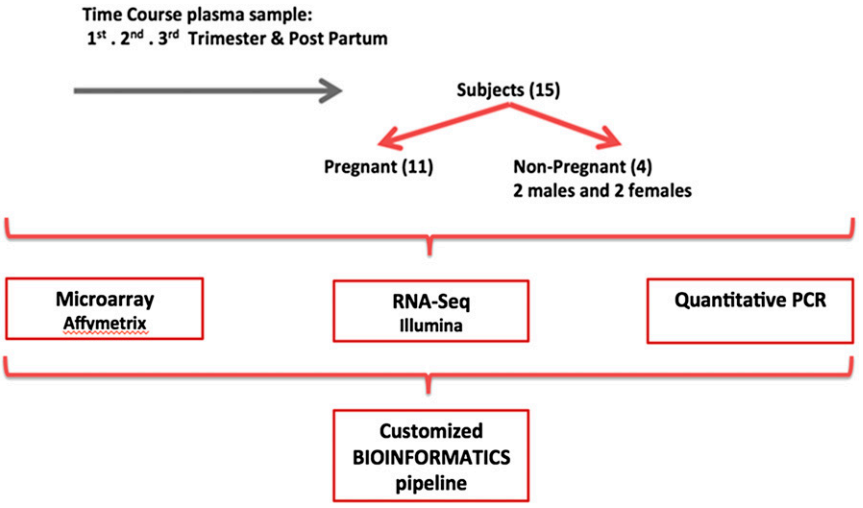

Fig. S1. Summary of study design. In the current cohort, 11 pregnant women and 4 nonpregnant control subjects were recruited. For all of the pregnant patients, blood was drawn at the first, second, and third trimesters and postpartum. The cell-free plasma RNA was then extracted, amplified, and characterized by Affymetrix microarray, Illumina sequencer, and quantitative PCR.

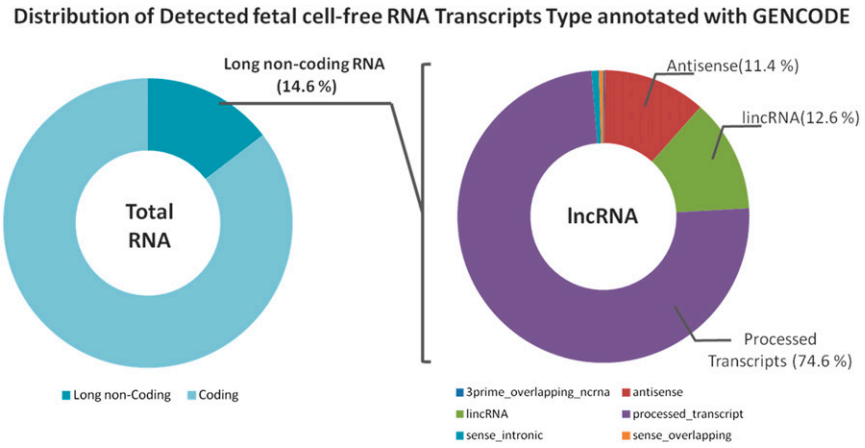

Fig. S2. Distribution of detected fetal cell-free RNA transcripts type as annotated with GENCODE. Using GENCODE as the annotation method for the transcripts present in the plasma cell-free RNA transcriptome, we were able to categorize the detected gene transcripts present in the plasma transcriptome. Approximately 15% of detected transcripts are long noncoding RNA, the majority of which are processed transcripts, transcripts that do not contain an ORF, and are placed in this category by ENCODE due to its complexity in structure. The relative proportions of different ncRNA categories remains relatively stable and consistent across all different patients and trimesters without huge deviations.

Sub	Gene	SNP	Chr	Position	Alleles	T1 -> T2 -> T3 -> Post	First Trimester	Second Trimester	Third Trimester	Post Partum	pvalues.cor	pvalues
P12	CASP1	rs199997208	11	104915231	G/A						0.001045004	1.86E-07
P12	FAM46C	rs2282456	1	118169463	G/A						0.003618119	6.43E-07
P12	SPTBN1	rs1052820	2	54886347	G/A						0.003618119	6.43E-07
P12	SOD2	rs7766006	6	160169258	G/T						0.005391283	9.57E-07
P12	PFKFB3	rs1539234	10	6276743	G/A						0.005391283	9.57E-07
P12	XPO7	rs7220	8	21863290	G/A						0.008711915	1.55E-06
P12	SEC14L1	rs62078342	17	75211870	C/A						0.009614964	1.71E-06
P12	STAT6	rs4559	12	57489648	C/T						0.009614964	1.71E-06
P12	CAST	rs754615	5	96086334	G/C						0.009614964	1.71E-06
P60	MARCKS	rs79053472	6	114182253	T/C						0.01100167	1.56E-06
P12	MMP8	rs4754871	11	102583279	T/C						0.011762435	2.09E-06
P36	UBB	rs17026960	17	16285266	T/G						0.016362781	2.32E-06
P12	DTX3L	rs74937187	3	122292965	G/A						0.017222568	3.06E-06
P12	SP110	rs1129411	2	231077725	A/G/C						0.017222568	3.06E-06
P12	CD300LF	rs191447419	17	72690630	C/T						0.020624523	3.66E-06
P12	STK4	rs17420378	20	43629135	G/A						0.024289977	4.31E-06
P36	GMCL1	rs114517801	2	70096862	T/C						0.02442936	3.47E-06
P12	NA	rs4779576	15	32792157	G/A						0.024668749	4.38E-06
P12	DDX17	rs5845381	22	38880098	A/-						0.02507075	4.45E-06
P12	SMC5	rs142489701	9	72897441	G/A						0.02507075	4.45E-06
P12	MARCKS	rs79053472	6	114182253	T/C						0.028553413	5.07E-06
P12	PDLIM5	rs2452600	4	95496882	C/T						0.029706215	5.28E-06
P12	EIF4EBP2	rs14761	10	72182975	G/A						0.029943528	5.32E-06
P58	LINC00152	rs28513497	2	87820915	C/T						0.030145531	5.15E-06
P12	AKNAD1	rs12030	1	109472813	T/C						0.036000945	6.39E-06
P12	HIST1H4B	rs115249469	6	26027286	G/A						0.036000945	6.39E-06
P36	PTBP3	rs7869523	9	114982549	C/T						0.036236421	5.15E-06
P12	RAB31	rs610532	18	9860755	G/A						0.036283416	6.44E-06
P12	HNRNPH2	rs3027574	X	100668296	C/T						0.036283416	6.44E-06
P12	DDX17	rs144704720	22	38880298	A/-						0.043220809	7.68E-06
P12	MARCKS	rs79053472	6	114182253	T/C						0.047135842	8.37E-06
P12	CASP1	rs55646419	11	104905047	G/A						0.001601177	2.84E-07
P12	HNRNPK	rs167203	9	86593314	G/C						0.001601177	2.84E-07
P12	C1orf123	rs1134688	1	53681699	T/G						0.002413974	4.29E-07
P12	NCF1	rs201802880	7	74193642	G/A						0.002929277	5.20E-07
P12	ELF1	rs7987185	13	41515371	C/T						0.005391283	9.57E-07
P12	NCF1	rs62475423	7	74193668	G/A						0.005391283	9.57E-07
P12	SYNPO	rs144429804	5	150036998	A/T						0.005391283	9.57E-07
P36	RPS25	rs11545855	11	118888103	T/C						0.007570142	1.08E-06
P12	SMARCD2	rs6919	17	61909485	T/A						0.007986616	1.42E-06
P12	FXYD6	rs138248978	11	117708626	G/A						0.007986616	1.42E-06
P36	SPC25	rs3931	2	169721381	A/G						0.0098939	1.41E-06
P36	HIST1H4H	rs2393593	6	26285683	T/C						0.009982206	1.42E-06
P12	FBXO7	rs9726	22	32887150	C/T						0.011561441	2.05E-06
P58	EFHD2	TMP_ESP_1_15755249	1	15755249	G/A						0.011824645	2.02E-06
P12	FKBP1A	rs79119937	20	1350026	A/G						0.012037173	2.14E-06
P12	EEF2	rs3170368	19	3976321	T/G						0.014123197	2.51E-06
P12	ADAR	rs2229857	1	154573967	T/C						0.014123197	2.51E-06
P58	SPI1	rs1057233	11	47376448	G/A						0.014685014	2.51E-06
P36	LUC7L2	rs9683	7	139107739	T/A						0.014701477	2.09E-06
P36	HSP90B1	rs116891695	12	104332224	C/T						0.014701477	2.09E-06
P12	SEC14L1	rs150757431	17	75212231	C/T						0.015418608	2.74E-06
P53	NA	rs80037846	1	789050	G/A						0.015796046	2.09E-06
P53	NA	rs10158938	1	789099	G/A						0.015796046	2.09E-06

Fig. S3. List of identified gene transcripts with identified fetal SNPs and the captured temporal dynamics. The bar plot reflects the relative contribution of fetal SNPs as reflected in the sequencing data. The red color bar reflects the extent of the relative fetal SNP contribution.

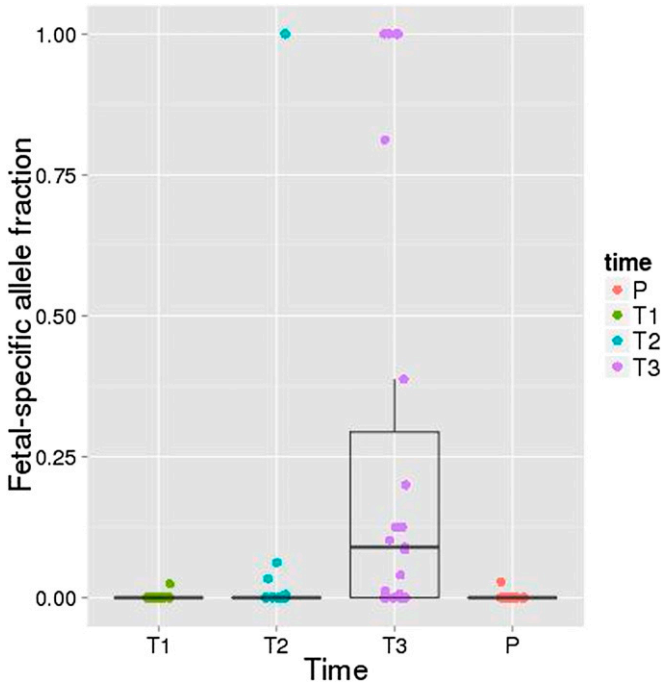

Fig. S4. The distribution of fetal-specific allele fraction at different SNPs loci over the time course of pregnancy. These SNPs loci are homozygous in mother's genome and heterozygous in fetus' genome. The fetal-specific allele fraction was calculated by the RNA-seq data of P58 at four time points (T1, first trimester; T2, second trimester; T3, third trimester; P, postpartum).

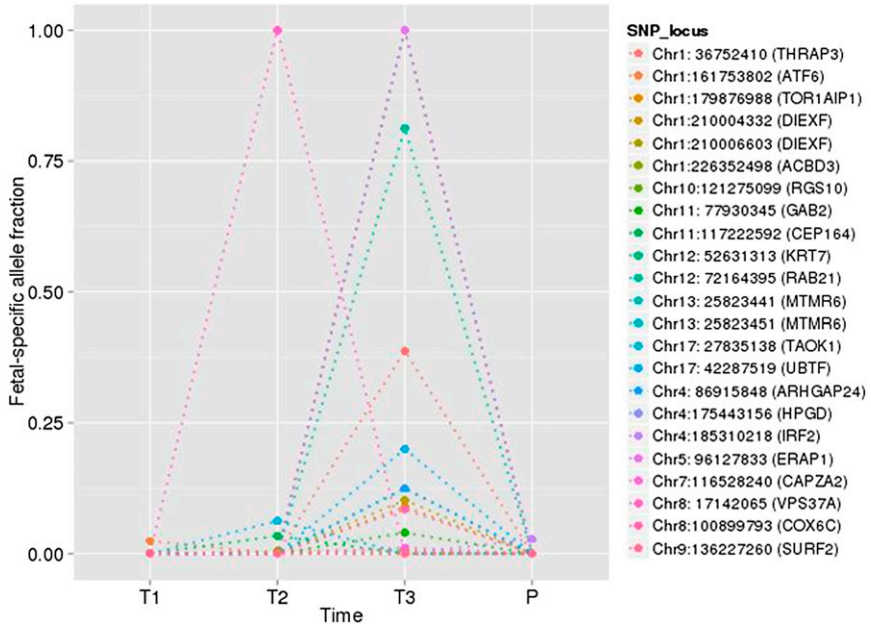

Fig. S5. The change of fetal-specific allele fraction at different SNPs loci over the time course of pregnancy. These SNPs loci are homozygous in mother's genome and heterozygous in fetus' genome. The fetal-specific allele fraction was calculated by the RNA-seq data of P58 at four time points (T1, first trimester; T2, second trimester; T3, third trimester; P, postpartum).

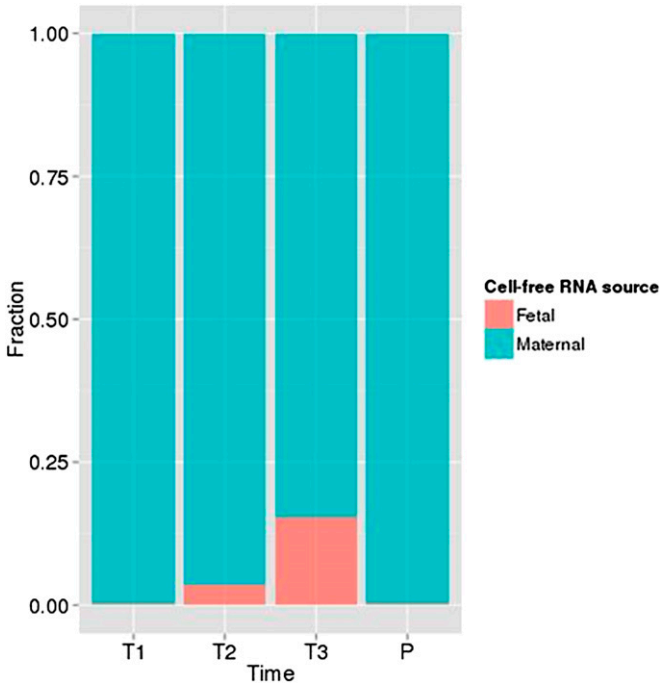

Fig. S6. The fraction of fetus-originated cell-free RNA in maternal plasma over the time course of pregnancy. The fraction of fetus-originated cell-free RNA was calculated by the RNA-seq data of P58 at four time points (T1, first trimester; T2, second trimester; T3, third trimester; P, postpartum).

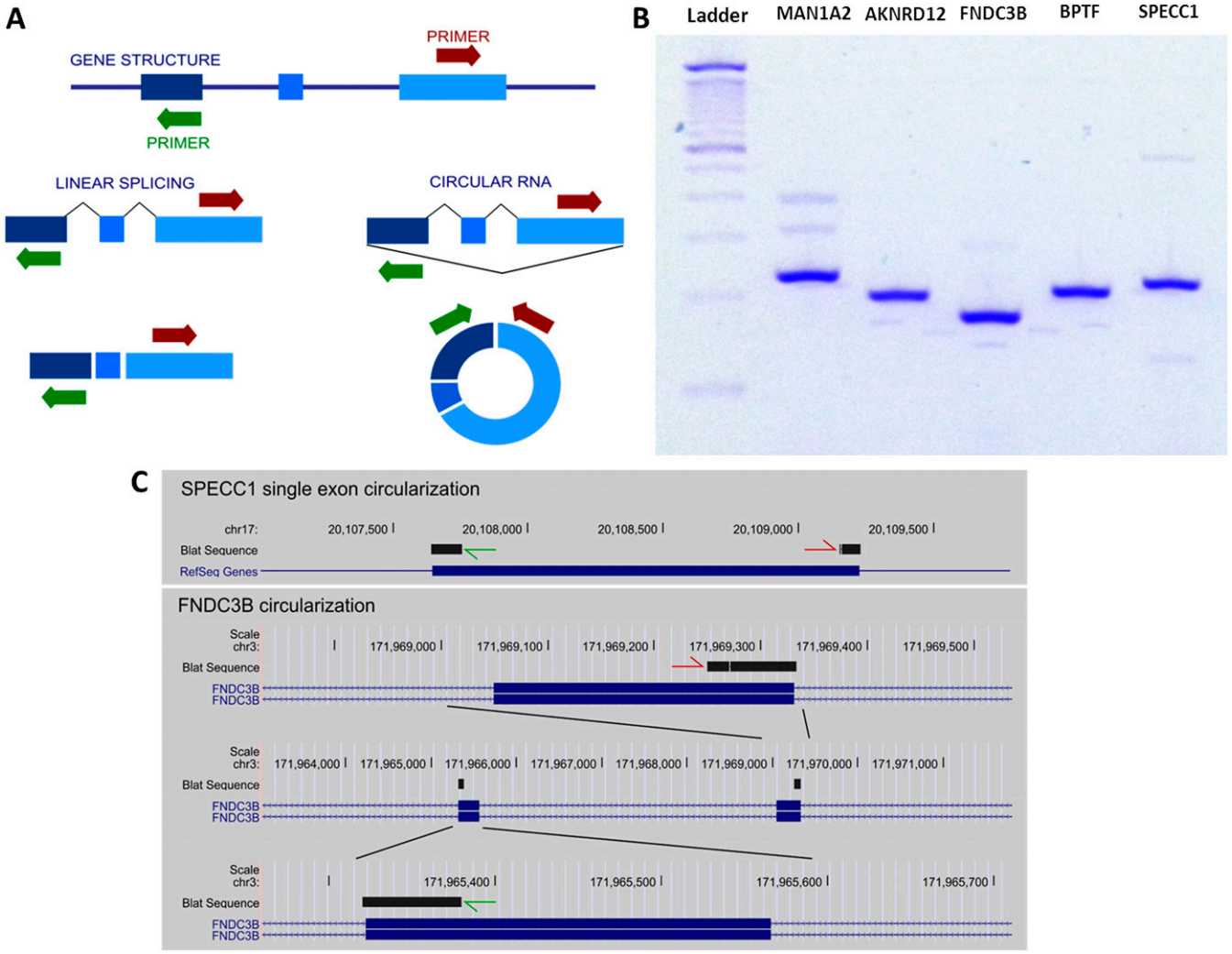

Fig. S7. (*A*) Outward facing primers were designed such that amplification would only occur specifically in the presence of circular RNA identified from our sequencing data. (*B*) Gel picture of amplicons indicating the presence of these circRNA in the plasma. (*C*) Amplified sequences for SPECC1 and FNDC3B were Sanger sequenced and mapped back onto genome where the amplicons reveal the circular RNA junction.

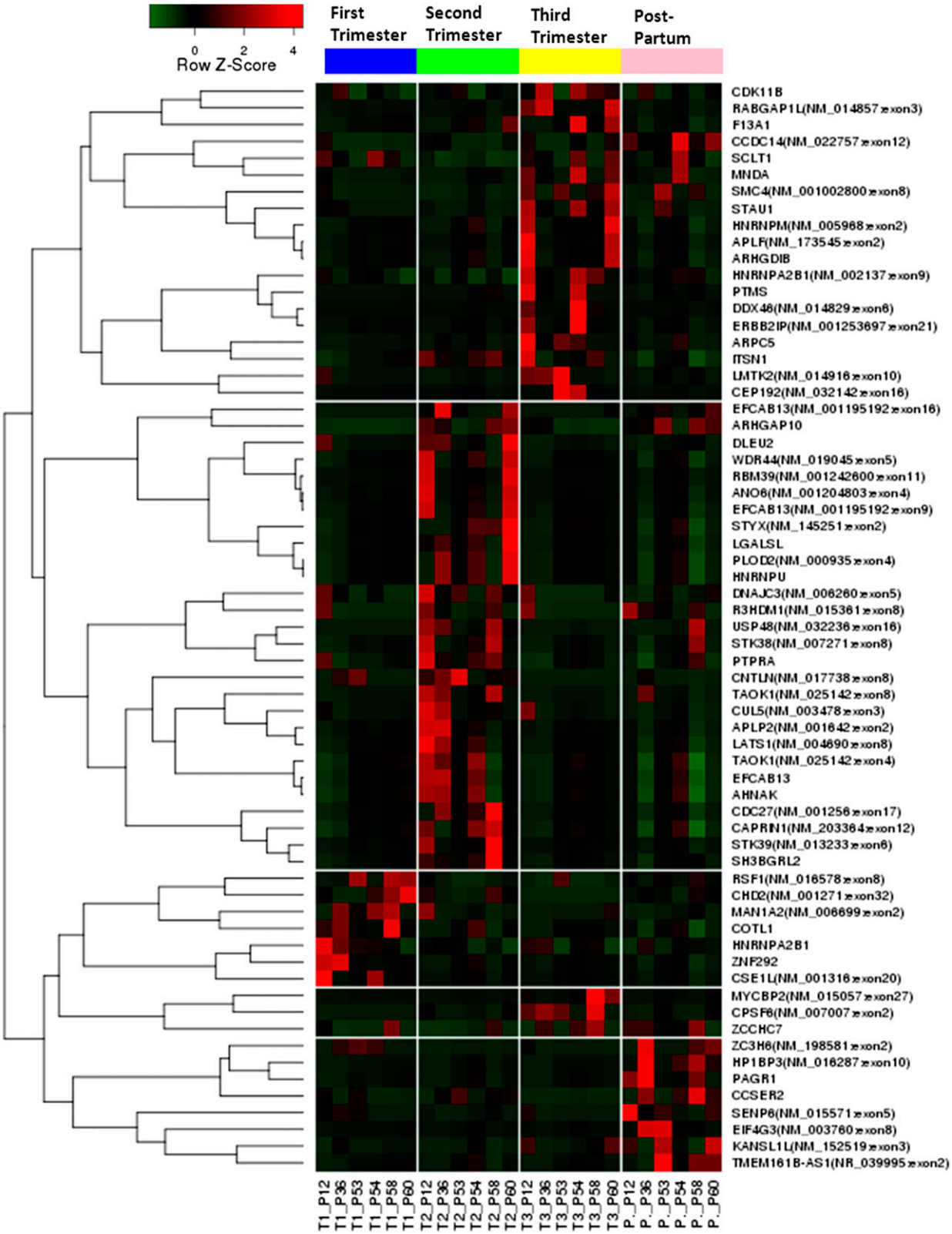

Fig. S8. Heatmap of time-varying circular RNA transcripts identified from sequencing data. The color bar corresponds to different time points in pregnancy. Each row represents a transcript that showed specificity to a particular time point.

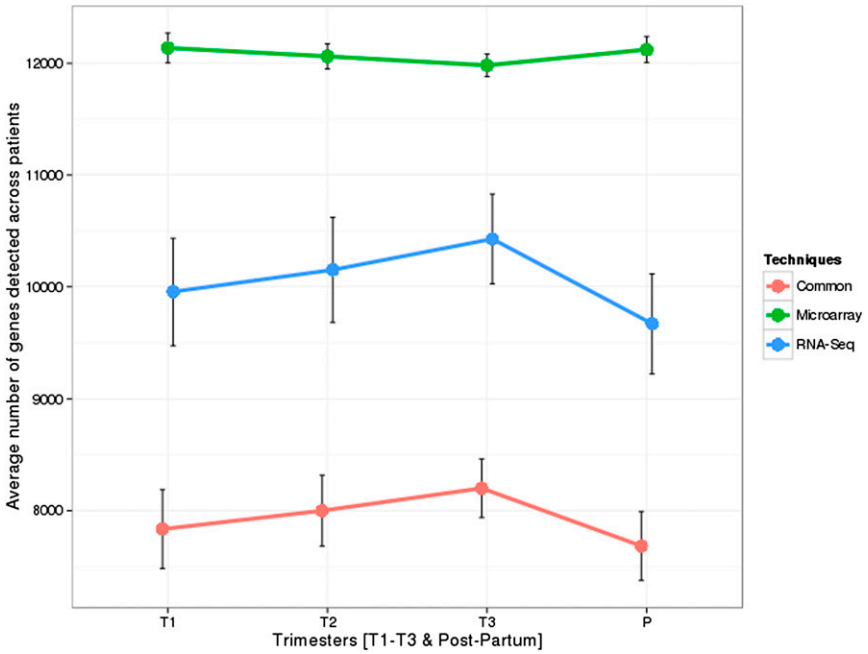

Fig. S9. Average number of genes detected by each technique across different trimesters. There is a general increasing trend for the number of genes detected across the different trimesters using RNA-seq and also for the common genes found in both the microarray and RNA-seq.

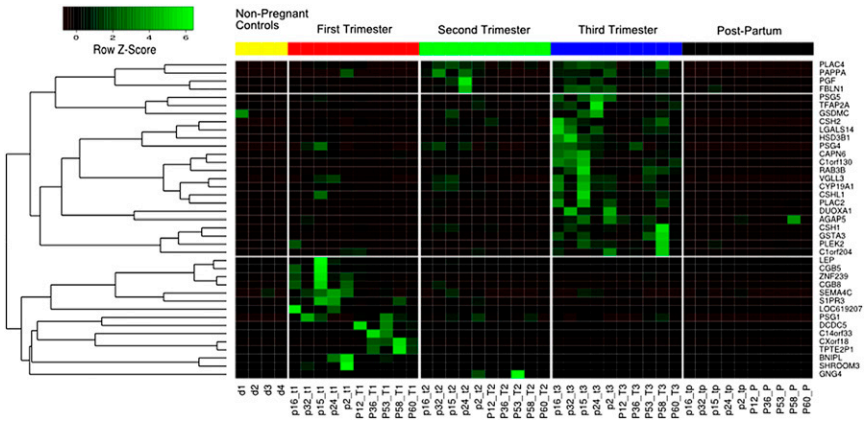

Fig. S10. Heatmap of time-varying genes identified from RNA-seq. The color bar on the top of the heatmap corresponds to different time points during pregnancy. Each row of the heatmap refers to a gene, and each column is a sample taken at a particular time point: D, nonpregnant controls (yellow); T1, first trimester (red); T2, second trimester (green); T3, third trimester (blue); and postpartum (black). Unsupervised clustering was performed on the genes across the different trimesters to find genes that exhibit similar temporal trends.

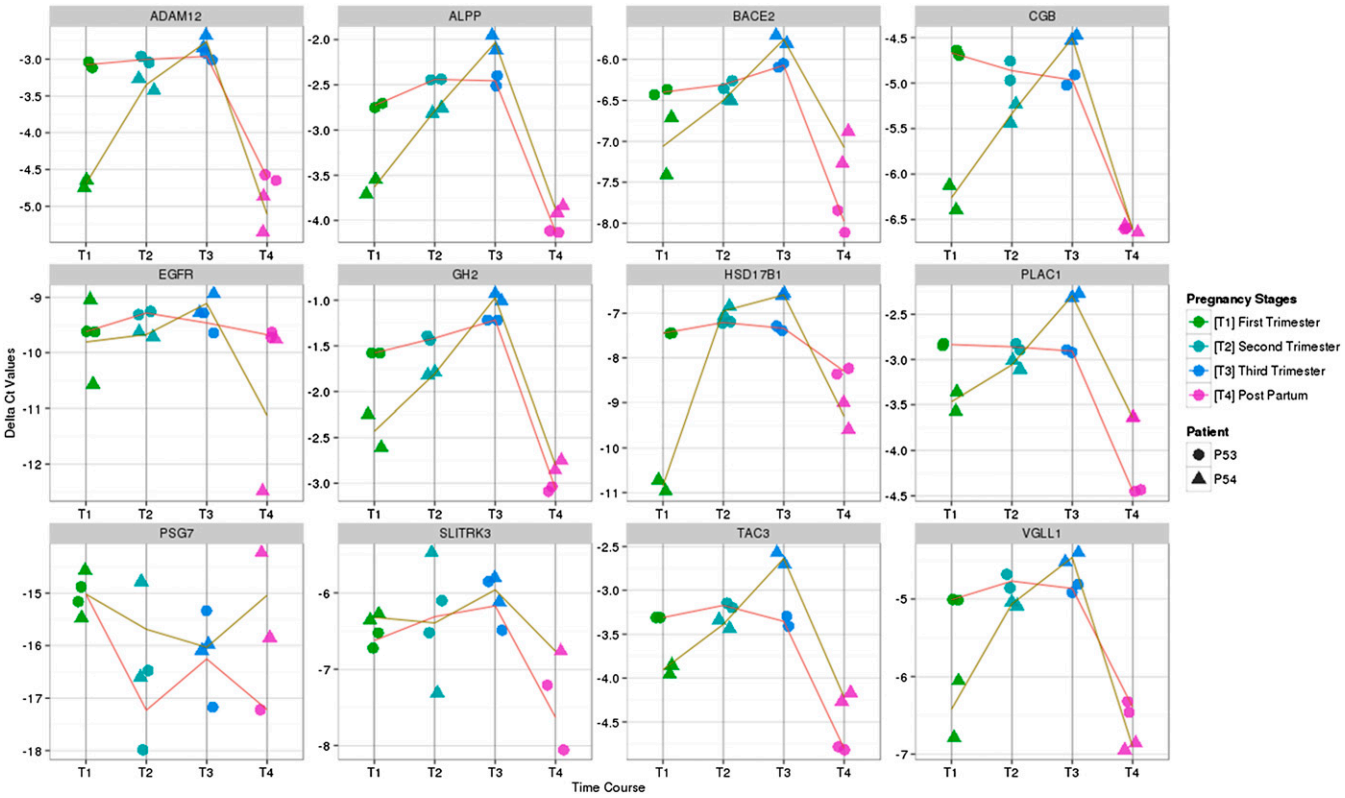

Fig. S11. Placental-specific genes. Plot showing the ΔCt value with respect to the housekeeping gene *ACTB* across the different trimesters of pregnancy including after birth. Time points across each patient is shown connected by the lines. Two replicates were performed for each patient at each time point. The general trends show elevated levels during the trimesters with a decline to low levels after the baby is born in concordance with the notion that fetal specific transcripts increased into the pregnancy followed by rapid clearance after birth.

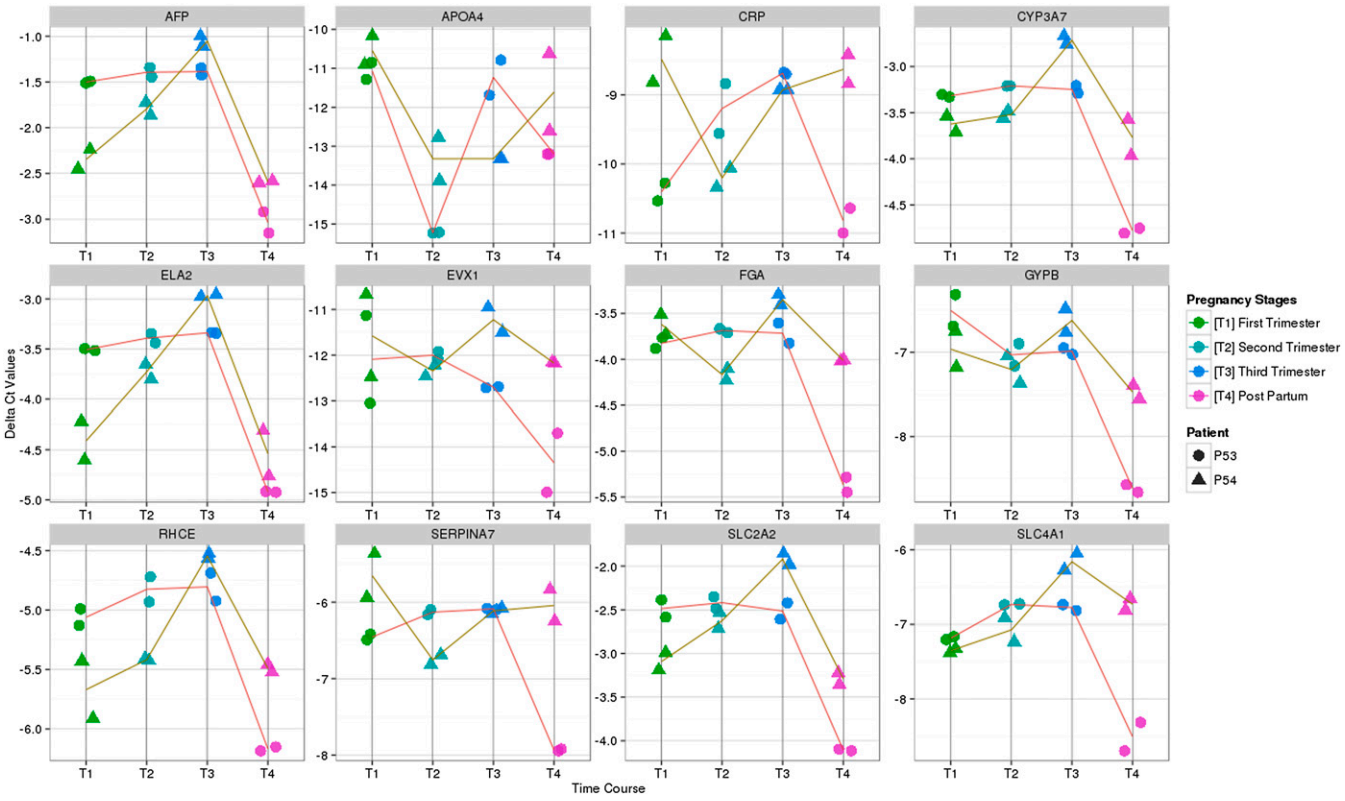

Fig. S12. Fetal liver-specific genes. Plot showing the ΔCt value with respect to the housekeeping gene *ACTB* across the different trimesters of pregnancy including after birth. Time points across each patient is shown connected by the lines. Two replicates were performed for each patient at each time point. The general trends show elevated levels during the trimesters with a decline to low levels after the baby is born in concordance with the notion that fetal-specific transcripts increased into the pregnancy followed by rapid clearance after birth.

Table S1. Number of genes detected by RNA-seq and microarray for all samples

	First trimester			Second trimester			Third trimester			Postpartum		
Sample ID	RNA-seq	Microarray	Intersection	RNA-seq	Microarray	Intersection	RNA-seq	Microarray	Intersection	RNA-seq	Microarray	Intersection
P12	9,582	12,197	7,683	9,309	12,345	7,502	10,842	12,026	8,527	7,576	12,578	6,299
P58	6,638	12,739	5,539	8,117	12,674	6,596	9,061	12,445	7,355	9,023	12,552	7,262
P53	9,217	12,214	7,417	7,442	12,602	6,119	7,946	12,589	6,566	10,072	12,002	7,882
P60	8,985	12,893	6,606	11,110	11,867	8,549	10,593	11,811	8,252	11,103	11,899	8,518
P36	9,516	12,320	7,657	9,473	11,977	7,616	9,437	11,919	7,485	10,023	12,011	7,966
P2	11,140	11,813	8,690	10,928	11,972	8,559	11,142	11,563	8,662	10,603	11,767	8,361
P15	10,620	11,749	8,319	11,424	11,719	8,855	11,735	11,745	9,029	10,370	11,842	8,234
P16	11,932	12,008	9,303	11,761	11,969	9,090	12,020	11,831	9,217	6,995	12,738	5,759
P24	11,030	11,718	8,561	11,224	11,670	8,697	11,164	11,790	8,709	11,151	11,738	8,769
P32	10,892	11,720	8,573	10,719	11,821	8,413	10,335	12,094	8,207	9,777	12,099	7,787
Average	9,955	12,137	7,835	10,151	12,062	8,000	10,428	11,981	8,201	9,669	12,123	7,684

Table S2. Correlation coefficient (Pearson) between the RNA-seq and microarray for all samples

Patient ID	First trimester	Second trimester	Third trimester	Postpartum
P12	0.747	0.741	0.778	0.685
P58	0.619	0.672	0.713	0.698
P53	0.726	0.655	0.668	0.764
P60	0.471	0.778	0.781	0.764
P36	0.73	0.76	0.744	0.762
P2	0.781	0.77	0.805	0.772
P15	0.771	0.799	0.805	0.762
P16	0.795	0.778	0.795	0.56
P24	0.776	0.789	0.782	0.786
P32	0.784	0.763	0.739	0.715
Average				0.739

Table S3. Table of detected circular RNA transcripts in maternal plasma

Chromosome	Start	End	Start_gene	Normalized number of reads supporting circular junction within gene
17	20107645	20109226	*SPECC1*	26.70971874
18	9182379	9221998	*ANKRD12*	22.47875554
1	117944807	117963272	*MAN1A2*	19.16673656
17	65941524	65972075	*BPTF*	15.41139919
3	171965322	171969332	*FNDC3B*	14.58824622
7	11021998	11030475	*PHF14*	9.705013355
14	31404368	31425449	*STRN3*	8.974938985
1	117944807	117957454	*MAN1A2*	7.997175657
4	129857809	129891624	*SCLT1*	5.851566103
2	40655612	40657445	*SLC8A1*	5.212403724
4	129857809	129880933	*SCLT1*	4.804552673
1	117944807	117984948	*MAN1A2*	4.634693803
5	89791493	89802492	*POLR3G*	4.350910726
1	1586822	1650895	*CDK11B*	4.268166302
X	76907603	76912144	*ATRX*	4.129867024
14	45587230	45599994	*FKBP3*	3.314747709
2	113057425	113069515	*ZC3H6*	3.127492386
1	117944807	117948268	*MAN1A2*	3.091640232
17	65941524	65944423	*BPTF*	3.089342947