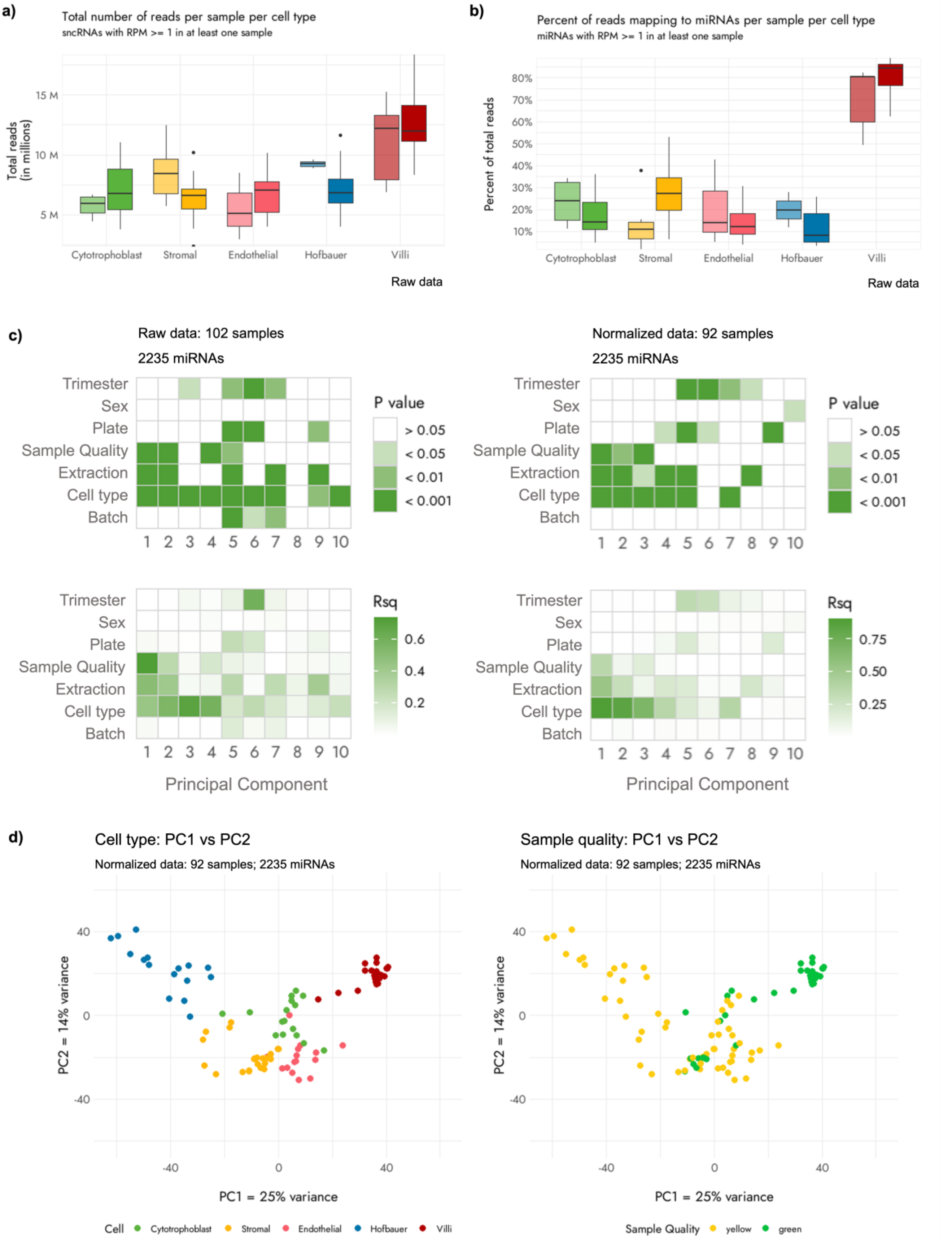
Supplementary Methods

Summary of sample processing and quality control

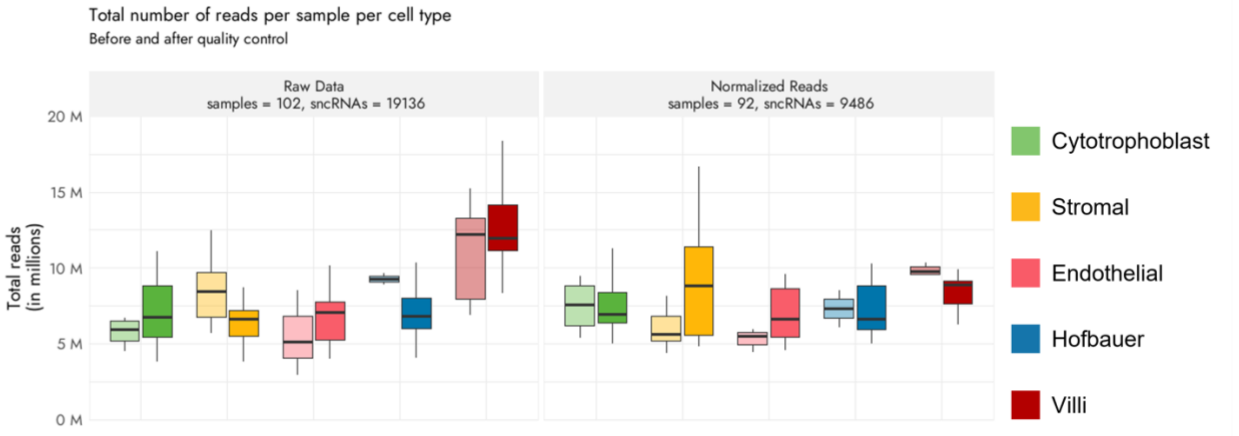
As the first exploratory study of the sncRNA transcriptome of sample-matched placental cells and chorionic villi (CV), we undertook an in-depth examination of sample quality. From the raw, unfiltered data, 9485 distinct sncRNAs had expression in at least one sample (**Supplementary Methods Table 1**). Overall, the total number of reads and the total percentage of mapped reads were variable across the sorted cell types (**Supplementary Methods Figure 1a and 1b**), and CV samples showed about twice as many total reads than the sorted-cells (**Supplementary Methods Figure 2**). As bulk tissue sequencing captures the average gene expression across several different cell types including the added contribution of syncytiotrophoblast, a higher number of sequences are detected and sequenced in bulk tissue, and can lead to a higher total read count as compared to individual cell types. The expected mapping rate of 30-50% was observed for the majority of samples, in consensus with the sequencing chemistry for this project (**Supplementary Methods Table 2**).

Supplementary Methods Table 1: Characteristics of human sncRNAs**.** All databases accessed 20 January 2025. Abbreviations - miRNA: microRNA; mRNAs: messenger RNA; RISC: RNA-induced silencing complex; siRNA: small interfering RNA; piRNA: piwi-interacting RNA; piRISC: piRNA-induced silencing complex; rRNAs: ribosomal RNA; snRNA: small nuclear RNAs; snRNP: small nuclear ribonucleoproteins; snoRNA: small nucleolar RNAs; tRNA: transfer RNA; snoRNP: small nucleolar ribonucleoproteins; shRNA: small hairpin RNAs; TEs: transposable elements.

sncRNA species	**Nucleotide length**	**Targets**	**Complex of action**	**Number of species expressed in humans**	**Number of sncRNAs identified in this study of *n* = 9485** (% of those identified)	**Percent of all human sncRNAs identified in this study**
miRNA	19 - 22	Several mRNAs	RISC	2,887 (miRBase)	2,235 (23.6%)	77%
piRNA	23 - 36	TEs, mRNAs	piRISC	8,592,949 (piRBase)	4,380 (46.2%)	0.05%
5S / 5.8S rRNA	< 200	rRNA	RISC	435 (RNACentral)	38 (0.4%)	9%
scaRNA	120 - 420	rRNAs (maybe mRNA, tRNA, snRNA)	snoRNP - spliceosome	51 (GeneCards)	46 (0.5%)	90%
snRNA	60 - 450	Pre-mRNA introns	snRNP - spliceosome	1,957 (GeneCards)	1,655 (17.4%)	85%
snoRNA	60 - 300	rRNAs (maybe mRNA, tRNA, snRNA)	snoRNP - spliceosome	1,266 (GeneCards)	863 (9.1%)	68%
tRNA	76 - 90	mRNAs, TEs	RISC	429 (GtRNAdb)	268 (2.8%)	62%



Supplementary Methods Figure 1: Read metrics and Principal Component Analysis (PCA) for miRNAs. a) Total number of reads (for all sncRNA species [n = 9485] and all samples) sequenced. The lighter coloured boxplots on the left are first trimester samples and the darker coloured boxplots are term samples. **b)** Total number of reads mapping to microRNAs (n = 2235). **c)** P-value significance and r-squared correlation of variables towards miRNA (n = 2235) expression for the first 10 principal components for raw (n = 102 samples) data and after qualitu control and normalization (n = 92 samples) expression. Sample Quality refers to the combined variable made using total reads sequenced and percentage of reads mapped, as described in Methods. Plate refers to the physical sequencing plate (either Plate 1 or Plate 2), and Batch refers to instance of sample submission for sequencing (either Batch 1 or 2). **d)** PCA of miRNA (2235) expression for variables related to sample quality and cell type for PC1 and PC2 after pre-processing, quality control, and normalization.



Supplementary Methods Figure 2: Total reads of all sorted-cell and whole chorionic villi samples. Number of reads that were sequenced per sample before pre-processing (raw data) and number of reads retained after quality control and normalization. Lighter coloured boxplots on the left are first trimester samples and and darker coloured boxplots are term samples.

Supplementary Methods Table 2: Sample characteristics and sequencing metrics of all samples (n = 102) that were selected for sequencing. Valid reads = reads which had a length of more than 17 nucleotides. Mapped reads = Valid reads which aligned to the genome based on the alignment criteria (described in Methods). Normalized Reads = Adjusted number of reads (increased or decreased) corrected for by the normalization technique.

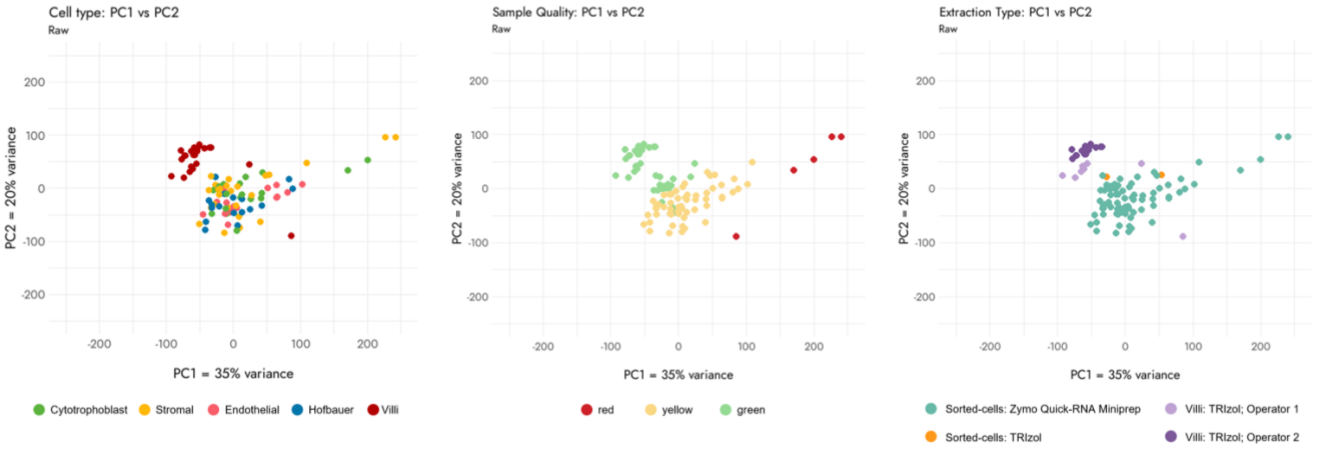
Sample	**Case ID**	**Gestational age**	**Original reads**	**Valid reads**	**Total reads mapped to genome**	**Normalized reads**	**Sample quality flag**
PL289_endo	PL289	6.6	7,310,442	4,602,488	4,259,142	Excluded after QC	green
PL289_strom	PL289	6.6	12,475,813	6,386,959	4,371,873	4,356,187	yellow
PL289_v	PL289	6.6	17,299,822	15,717,785	11,018,167	Excluded after QC	red
PM367_endo	PM367	39.1	10,150,676	5,254,189	4,594,263	4,520,029	yellow
PM367_troph	PM367	39.1	11,513,839	7,728,913	6,094,248	Excluded after QC	red
PM367_strom	PM367	39.1	5,789,625	3,346,094	3,187,489	5,360,729	green
PM367_hofb	PM367	39.1	11,652,924	6,370,755	5,836,249	6,483,768	yellow
PM367_vc	PM367	39.1	17,805,397	12,347,937	12,129,379	6,218,236	green
PL292_strom	PL292	7.1	9,381,539	4,913,735	4,314,259	5,322,644	yellow
PL292_v	PL292	7.1	15,252,678	7,940,964	7,724,970	5,682,366	green
PL281_hofb	PL281	9	7,981,703	5,129,029	4,622,794	Excluded after QC	green
PL281_strom	PL281	9	6,152,121	3,580,982	2,278,221	Excluded after QC	yellow
PL281_v	PL281	9	8,859,396	1,859,447	1,798,643	Excluded after QC	green
PL290_endo	PL290	10.7	2,944,820	2,039,875	1,159,261	4,404,379	yellow
PL290_troph	PL290	10.7	5,386,050	3,409,661	3,185,987	5,366,662	yellow
PL290_strom	PL290	10.7	5,699,330	3,873,989	2,881,473	5,618,481	yellow
PL290_v	PL290	10.7	13,289,913	6,458,828	6,343,215	9,549,301	green
PL294_endo	PL294	11.9	5,123,044	4,105,779	3,725,173	5,503,336	green
PL294_hofb	PL294	11.9	8,881,679	6,294,789	5,967,460	6,042,503	yellow

PL294_strom	PL294	11.9	9,957,207	7,457,374	6,948,035	5,080,046	yellow
PL294_v	PL294	11.9	7,935,918	6,629,296	6,533,834	10,104,011	green
PL295_endo	PL295	11.9	6,805,750	5,018,217	4,726,659	Excluded after QC	yellow
PL295_hofb	PL295	11.9	9,615,382	6,952,994	6,591,438	8,533,360	yellow
PL295_troph	PL295	11.9	4,465,211	3,163,081	3,051,424	9,474,538	green
PL295_strom	PL295	11.9	7,006,115	4,533,009	4,232,017	8,120,028	yellow
PL295_vc	PL295	11.9	6,881,621	6,161,227	6,083,596	10,352,538	green
PL296_strom	PL296	11.5	6,539,043	5,233,594	4,865,149	6,447,332	yellow
PL296_v	PL296	11.5	12,197,705	10,111,209	9,976,730	9,779,851	green
P128_endo	P128	12.3	8,493,656	5,094,075	4,013,622	5,938,388	yellow
P128_troph	P128	12.3	6,451,585	4,637,884	4,086,903	6,490,982	green
P128_strom	P128	12.3	8,411,518	6,040,633	5,583,961	7,179,924	green
P131_strom	P131	13	16,172	9,253	7,771	Excluded after QC	red
P131_troph	P131	13	6,658,774	4,332,187	3,995,143	8,639,881	yellow
PM359_hofb	PM359	39.9	4,025,590	3,034,275	2,412,552	5,541,321	yellow
PM359_troph	PM359	39.9	2,003,028	1,380,950	562,185	Excluded after QC	red
PM359_strom	PM359	39.9	6,436,908	4,437,147	3,642,898	5,659,992	yellow
PM359_vc	PM359	39.9	18,331,501	7,318,903	7,179,112	6,933,433	green
PM364_endo	PM364	39.3	6,927,834	5,303,712	4,930,331	4,851,748	yellow
PM364_troph	PM364	39.3	4,269,979	3,087,536	2,210,676	4,977,494	yellow
PM364_strom	PM364	39.3	4,871,489	3,879,050	3,671,521	4,939,998	yellow
PM364_vc	PM364	39.3	12,523,737	7,340,460	7,268,523	6,751,345	green
PM366_endo	PM366	39	5,313,724	4,090,259	3,637,058	5,346,974	yellow
PM366_hofb	PM366	39	10,334,298	6,528,740	6,058,671	5,017,060	yellow
PM366_troph	PM366	39	9,091,839	6,593,851	5,839,514	5,005,449	yellow
PM366_strom	PM366	39	4,979,070	3,845,149	3,734,409	5,823,129	yellow
PM366_vc	PM366	39	16,534,580	8,326,473	8,225,723	6,944,638	green
PM368_endo	PM368	38.7	7,173,553	5,585,657	5,359,996	8,492,282	green
PM368_troph	PM368	38.7	3,789,263	2,642,338	2,446,012	11,293,075	yellow
PM368_strom	PM368	38.7	8,843	4,412	3,410	Excluded after QC	red
PM368_vc	PM368	38.7	8,564,839	7,815,155	7,772,953	8,479,146	green
PM369_strom	PM369	39.6	6,989,066	5,123,693	4,610,299	4,830,327	yellow
PM369_vc	PM369	39.6	11,370,019	9,362,410	9,275,340	9,123,476	green
PM370_endo	PM370	39	5,032,466	3,600,628	2,796,968	21,401,994	yellow
PM370_hofb	PM370	39	5,614,050	3,901,888	3,543,304	40,558,748	yellow
PM370_troph	PM370	39	8,745,640	6,550,601	6,100,575	8,172,723	green
PM370_strom	PM370	39	6,909,645	4,933,872	4,523,867	10,628,811	yellow

PM370_vc	PM370	39	10,477,925	8,715,471	8,604,785	9,094,391	green
PM371_endo	PM371	36.4	4,609,941	3,367,823	3,005,108	9,551,838	yellow
PM371_hofb	PM371	36.4	5,964,830	5,194,297	4,837,449	8,694,422	yellow
PM371_troph	PM371	36.4	11,057,665	8,304,190	7,629,059	9,223,922	green
PM371_strom	PM371	36.4	2,378,385	1,476,979	1,153,373	12,696,265	yellow
PM371_vc	PM371	36.4	13,835,413	10,789,711	10,688,288	7,631,261	green
PM372_endo	PM372	39.6	8,546,252	5,171,925	4,427,685	5,849,557	yellow
PM372_hofb	PM372	39.6	6,112,215	5,177,853	4,959,348	6,505,362	yellow
PM372_troph	PM372	39.6	5,535,731	3,843,271	3,241,030	6,955,929	yellow
PM372_strom	PM372	39.6	6,688,313	5,615,325	5,410,366	10,383,118	green
PM372_vc	PM372	39.6	9,150,153	7,487,433	7,424,539	8,994,745	green
PM373_endo	PM373	39.3	9,481,361	6,670,238	5,760,418	5,553,997	yellow
PM373_hofb	PM373	39.3	7,165,309	4,849,249	4,311,952	6,077,210	yellow
PM373_troph	PM373	39.3	10,450,756	7,883,386	7,405,653	6,468,183	yellow
PM373_strom	PM373	39.3	7,701,305	6,583,156	6,338,921	8,172,071	green
PM373_vc	PM373	39.3	14,135,443	10,557,633	10,476,339	8,865,716	green
PM374_endo	PM374	39.3	7,516,286	5,111,033	4,526,331	7,836,284	yellow
PM374_hofb	PM374	39.3	5,166,485	3,549,132	3,016,407	22,388,924	yellow
PM374_troph	PM374	39.3	5,884,808	4,470,155	4,045,937	7,180,186	yellow
PM374_strom	PM374	39.3	8,678,909	6,626,347	6,220,152	10,921,949	green
PM374_vc	PM374	39.3	15,421,805	13,004,515	12,880,972	8,226,840	green
PM375_endo	PM375	39.3	7,316,546	5,592,700	5,168,773	6,631,397	yellow
PM375_hofb	PM375	39.3	6,530,707	5,354,164	4,843,912	5,021,846	yellow
PM375_troph	PM375	39.3	8,325,037	6,361,001	6,021,960	8,951,016	yellow
PM375_strom	PM375	39.3	10,209,722	7,008,512	6,605,523	8,647,628	yellow
PM375_vc	PM375	39.3	11,154,673	9,606,888	9,540,600	8,493,949	green
PM376_endo	PM376	39.1	3,978,482	2,829,920	2,628,430	19,119,192	yellow
PM376_hofb	PM376	39.1	6,815,165	4,941,789	3,900,554	10,269,915	yellow
PM376_troph	PM376	39.1	7,648,664	5,772,369	5,361,954	5,977,325	yellow
PM376_strom	PM376	39.1	3,810,950	2,534,158	2,172,027	9,046,877	yellow
PM376_vc	PM376	39.1	11,722,277	9,654,090	9,582,650	9,396,202	green
PM377_hofb	PM377	39.6	8,237,808	6,187,672	5,722,359	6,791,315	yellow
PM377_troph	PM377	39.6	5,775,116	4,983,467	4,620,671	6,912,233	yellow
PM377_strom	PM377	39.6	5,673,384	4,589,807	4,189,117	5,358,957	yellow
PM377_vc	PM377	39.6	11,975,444	9,998,681	9,917,692	9,869,151	green
PM378_endo	PM378	39.1	5,968,924	4,224,369	3,740,679	8,725,499	yellow
PM378_hofb	PM378	39.1	7,195,880	5,966,263	5,631,556	8,461,217	yellow

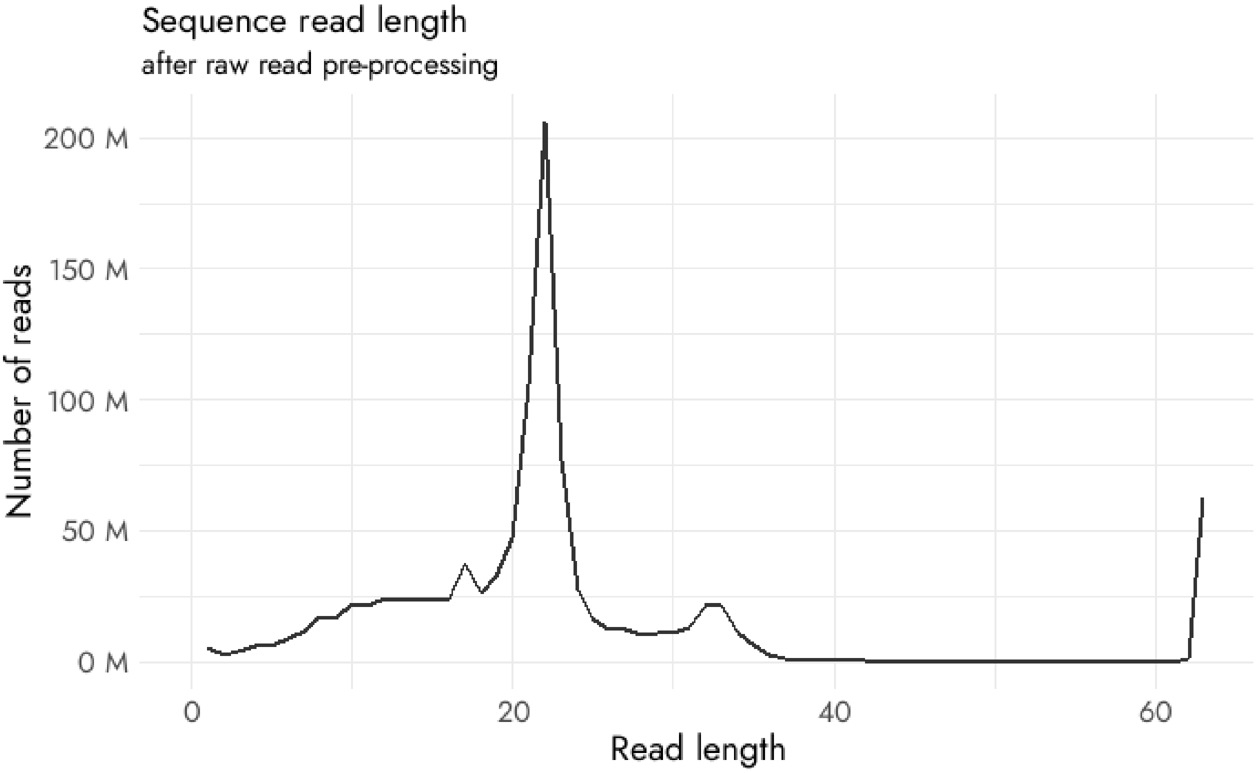
PM378_strom	PM378	39.1	6,574,558	5,447,999	5,181,047	14,506,438	green
PM378_vc	PM378	39.1	12,452,895	10,746,327	10,685,073	8,882,254	green
PM379_hofb	PM379	39.3	6,851,351	4,740,931	4,395,791	10,013,253	yellow
PM379_strom	PM379	39.3	8,122,631	7,512,541	7,376,564	15,056,518	green
PM379_vc	PM379	39.3	8,326,658	7,324,804	7,246,429	9,653,642	green
PM381_hofb	PM381	40.4	9,881,192	7,549,014	7,189,681	9,178,888	yellow
PM381_troph	PM381	40.4	5,232,368	4,074,077	3,671,151	6,752,808	yellow
PM381_strom	PM381	40.4	6,693,696	5,488,500	4,641,624	16,675,624	green
PM381_vc	PM381	40.4	11,522,934	9,973,673	9,910,839	11,746,192	green

PCA for raw data was conducted for each sncRNA species independently to assess sample variability, and highlighted that sample/cell type (villi and sorted-cells), sample quality, and extraction type contributed significantly to PC1 (observed for all sncRNA species), with sample quality being most highly correlated with sncRNA expression for the raw data; **Supplementary Methods Figure 1c** depicts the PCA for miRNAs only, as an example. Samples also showed grouping by these three variables for PC1 against PC2 (**Supplementary Methods Figure 3**). This variation also held true when PCA was performed separately for each sncRNA species and underscores the importance of accounting for technical variables in any bioinformatic analyses. As the overall expression profile for each species differs significantly, reads were normalized for each sncRNA species independently and then combined - i.e., after retaining sncRNAs with expression of at least 1 read in at least one sample, sncRNAs were subset by species (miRNAs, piRNAs, rRNAs, scaRNAs, snoRNAs, snRNAs, tRNAs) and normalized using the RLE method and then scaled per million (detailed in Methods). After normalization, PCA was re-run, and in the normalized data, samples clustered prominently by cell type along PC1 and PC2 **(Supplementary Methods Figure 1c and 1d)**. We saw that sample quality was no longer the variable most strongly associated with PC1 and PC2 after normalization and preprocessing, demonstrating that those steps are important to reduce technical variation between samples.

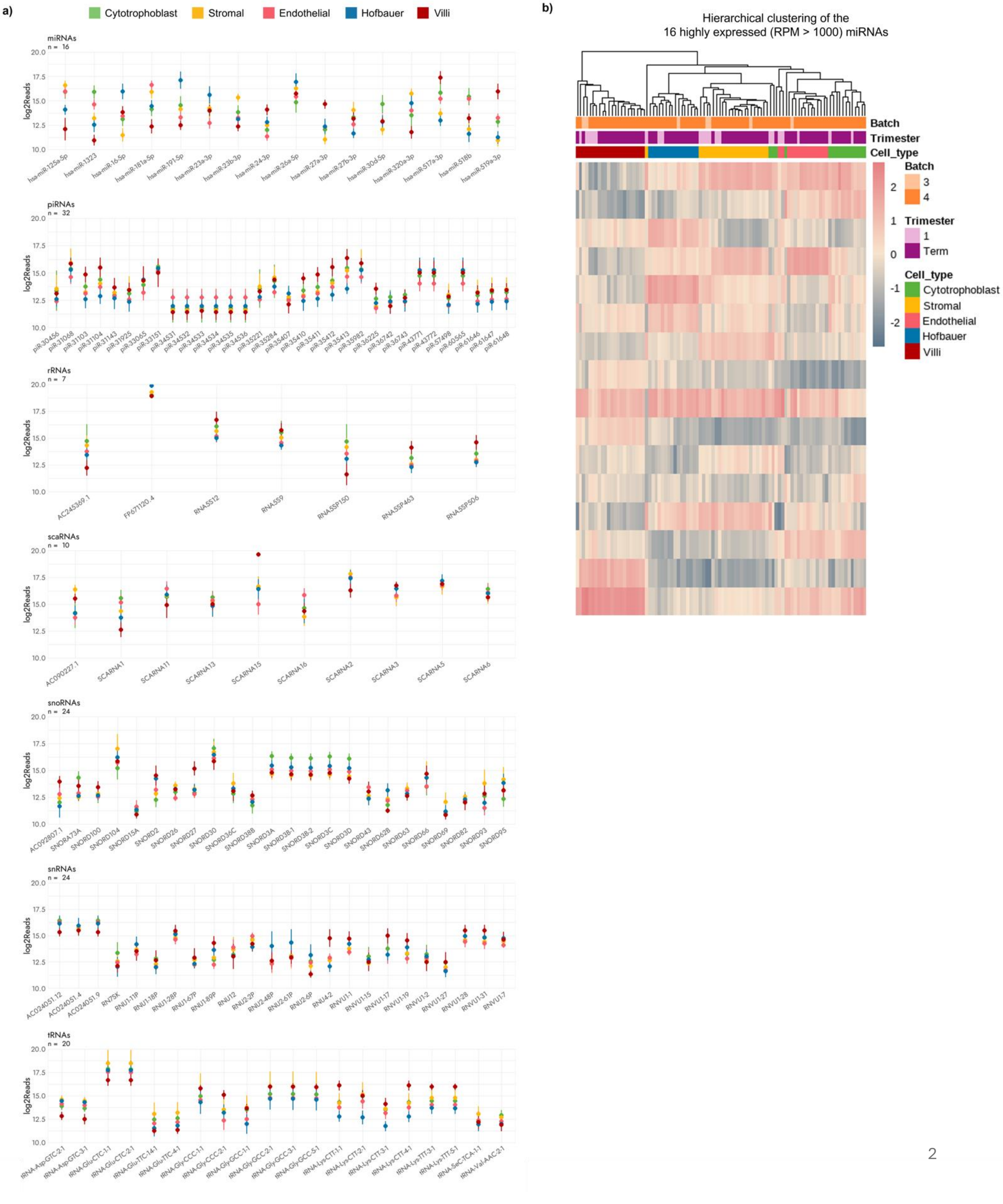


Supplementary Methods Figure 3: PC1 vs PC2 for raw data. PCA for miRNA expression (n = 2235) for raw data (102 samples). Samples showed clustering primarily by sample quality, followed by cell type, and then extraction type.

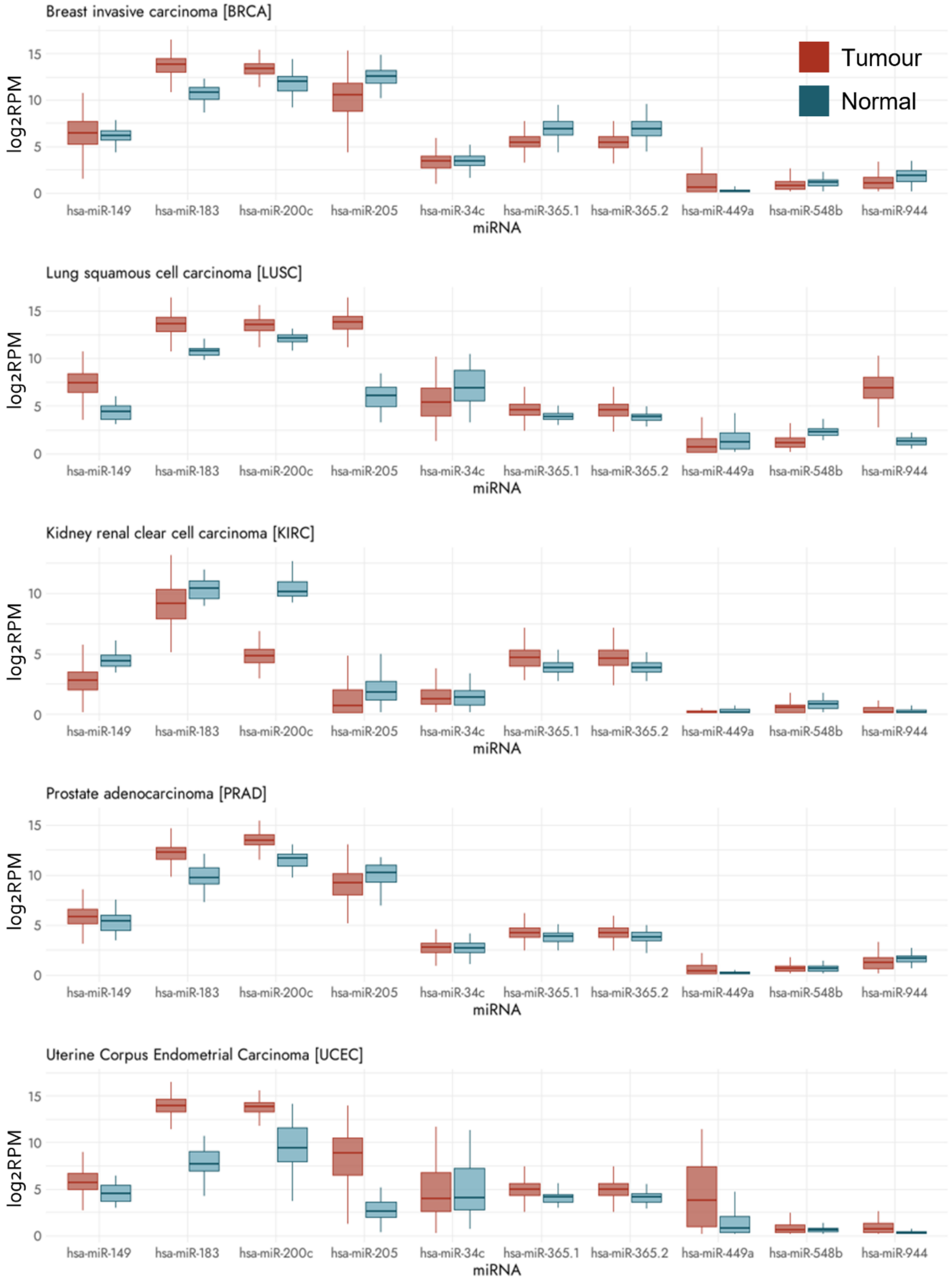
SF1: Distribution of sequence read length. Read length (in nucleotides) of raw data (all 102 samples and all 19136 sncRNAs) after trimming to remove adaptors and index primers.



SF2: sncRNAs with high expression (RPM >= 1000) in all cell types and bulk villi (n = 133). a) Expression of all 133 sncRNAs by species (miRNA, piRNA, rRNA, scaRNA, snoRNA, snRNA, tRNA). The dots represent the mean, and the vertical lines on either side of the dot represent the standard deviation. **b)** Hierarchical clustering of expression Z-scores of the 16 miRNAs with RPM >= 1000 in all cell types and matched villi.



SF3: Expression of cytotrophoblast cell-associated miRNAs in The Cancer Genome Atlas (TCGA) cohorts. Expression of each of the 10 cytotrophoblast cell-associated miRNAs in some of the TCGA cohorts.



Breast invasive carcinoma [BRCA]

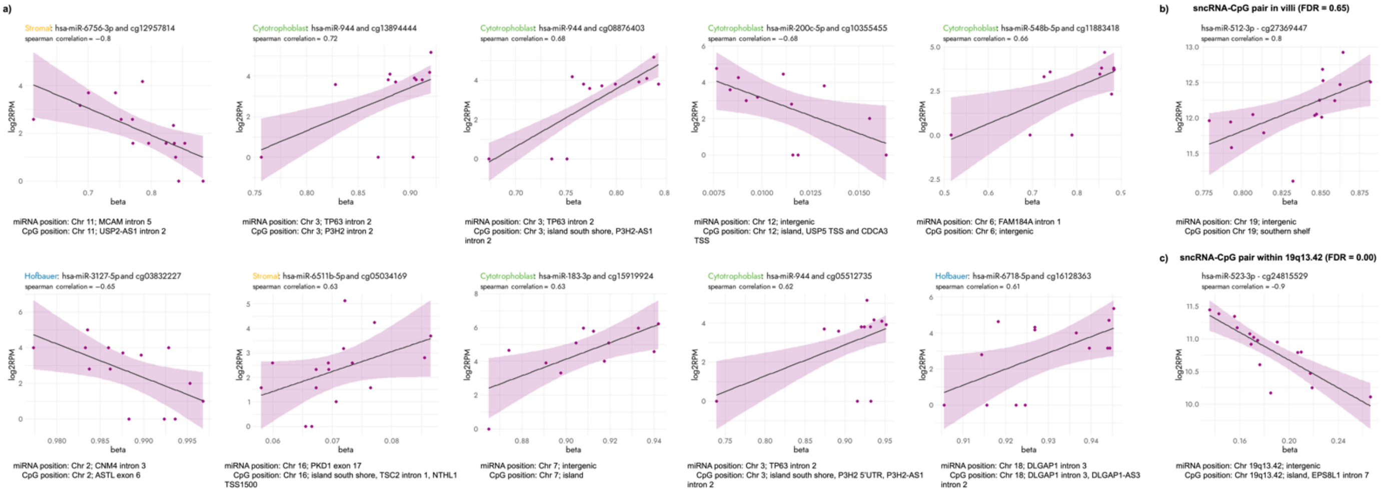
Lung squamous cell carcinoma [LUSC]

Kidney renal clear cell carcinoma [KIRC]

Prostate adenocarcinoma [PRAD]

Uterine Corpus Endometrial Carcinoma [UCEC]

SF4: Expression-methylation correlation for sncRNA-CpG pairs. a) sncRNAs and CpGs (CpGs within +/-10Kb of that sncRNA) which showed a correlation (R^2) greater than ±0.6 in term sorted-cell placental samples (x-axis = DNA methylation beta values; y-axis = sncRNA expression for log-2 transformed normalized expression counts). Genomic location of both the sncRNA and CpG are also provided. **b)** The one miRNA-CpG pair that showed high correlation (FDR = 0.65) in term bulk villi samples. **c)** The one miRNA-CpG pair that showed high correlation (FDR = 0.00) when C14MC and C19MC analysed separately.



SF5. Gene set enrichment for all cell type-associated sncRNAs. Significant gene sets for **a)** stromal cell-associated **b)** Hofbauer cell-associated **c)** endothelial cell-associated sncRNAs