#### (SUPPLEMENTAL DATA)

# CeFra-seq reveals broad asymmetric mRNA and non-coding RNA distribution profiles in *Drosophila* and human cells.

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Table S1: RNA-seq read statistics for Human HepG2 and Drosophila D17 subcellular fractions.									
Library	Species	Fraction	Replicate	Number of reads	Duplicate (%)	Number of Aligned reads	Alignment (%)	Multiple alignment	Average FPKM
rRNA-depletion	Drosophila	Cytosolic	1	20,698,062	68.32	18,876,632	91.2	53.4	4.00.406
rRNA-depletion	Drosophila	Cytosolic	2	24,285,303	71.73	22,488,190	92.6	55.2	1.80x10°
rRNA-depletion	Drosophila	Membrane	1	21,843,697	43.58	19,069,547	87.3	17.1	0.04.406
rRNA-depletion	Drosophila	Membrane	2	21,134,180	44.12	18,513,541	87.6	18.4	0.61X10
rRNA-depletion	Drosophila	Insoluble	1	22,607,476	30.85	19,781,541	87.5	23.3	0.04.406
rRNA-depletion	Drosophila	Insoluble	2	24,059,152	32.19	20,979,580	87.2	24.4	0.34X10
rRNA-depletion	Drosophila	Nuclear	1	22,996,271	47.03	18,902,934	82.2	56.8	0.44.406
rRNA-depletion	Drosophila	Nuclear	2	24,902,681	43.5	20,694,127	83.1	52.8	0.44x10°
rRNA-depletion	Human	Cytosolic	1	32,552,634	64.76	27,344,212	84	24.6	2 20-406
rRNA-depletion	Human	Cytosolic	2	30,564,649	67.55	25,246,400	82.6	24.3	3.20X10
rRNA-depletion	Human	Membrane	1	35,909,087	40.04	30,774,087	85.7	17.5	0.00.406
rRNA-depletion	Human	Membrane	2	35,061,741	39.84	30,819,270	87.9	16.5	0.62810
rRNA-depletion	Human	Insoluble	1	34,097,076	20.53	31,539,795	92.5	8.1	0.00.406
rRNA-depletion	Human	Insoluble	2	35,451,428	22.57	32,757,119	92.4	7.6	0.26x10°
rRNA-depletion	Human	Nuclear	1	35,747,174	10.16	32,065,215	89.7	7	0.04.406
rRNA-depletion	Human	Nuclear	2	37,390,540	11.56	33,913,219	90.7	6.8	0.24x10
PolyA+	Drosophila	Cytosolic	1	22,668,517	53.28	20,628,350	91	7.4	0.40.406
PolyA+	Drosophila	Cytosolic	2	19,499,648	53.13	17,569,182	90.1	8	2.10x10
PolyA+	Drosophila	Membrane	1	25,331,304	55.33	22,139,559	87.4	7.8	4.00.406
PolyA+	Drosophila	Membrane	2	21,260,027	51.94	18,474,963	86.9	9.8	1.30X10
PolyA+	Drosophila	Insoluble	1	20,515,878	42.19	17,664,170	86.1	34.7	0.40.406
PolyA+	Drosophila	Insoluble	2	20,678,208	41.61	17,783,258	86	32.9	0.42x10
PolyA+	Drosophila	Nuclear	1	23,076,204	48.43	18,460,963	80	55.7	0.25x10 <sup>6</sup>
PolyA+	Drosophila	Nuclear	2	21,195,707	47.52	16,998,957	80.2	54.5	
PolyA+	Human	Cytosolic	1	31,719,232	53.46	30,101,551	94.9	6.8	0.00.406
PolyA+	Human	Cytosolic	2	24,755,754	49.65	22,948,583	92.7	6.4	0.99x10°
PolyA+	Human	Membrane	1	24,235,894	37.53	21,885,012	90.3	13.5	0.40.406
PolyA+	Human	Membrane	2	20,236,753	35.82	18,840,417	93.1	6.4	0.46x10°
PolyA+	Human	Insoluble	1	25,382,072	17.75	22,996,157	90.6	12.6	0.21x10 <sup>6</sup>
PolyA+	Human	Insoluble	2	23,337,888	17.33	21,354,167	91.5	12.2	
PolyA+	Human	Nuclear	1	21,059,227	11.22	19,795,673	94	6.5	0.10×10 <sup>6</sup>
PolyA+	Human	Nuclear	2	19,884,506	12.69	18,731,204	94.2	6.6	0.19X10

### Table S2. Cell component gene ontology (GO) enrichments of HepG2 cellfraction-specific proteins.

Gene Category cytoplasmp-ValueGene Category p-Valuep-Valuecytoplasm6.015 57bounding membrane system3.345-30extracellular vesicle2.485-50endoplasmic reticulum4.406-37vesicle2.485-50extracellular exosome2.185-22vesicle8.386-34extracellular vesicle3.061-27uceloplasmic part1.055-34extracellular vesicle3.061-22nucleoplasm1.656-05cytoplasm5.346-13nucleoplasm1.657-05cytoplasm5.346-13nuclear part7.356-05extracellular region part2.786-13intracellular organelle1.646-03integral component of membrane2.521-13intracellular organelle1.646-03integral component of membrane2.521-03intracellular organelle1.646-03intrinsic component of membrane2.521-03veroule6.522-07vacuole5.382-165veroules complex4.866-08muclear part3.78-92ribonucleoprotein complex4.866-08muclear part3.78-92ribonucleoprotein complex4.866-08muclear part3.78-92intracellular nhonucleoprotein complex4.866-08muclear part3.78-92ribonucleoprotein complex4.866-08muclear part3.78-92ribonucleoprotein complex4.866-08muclear part3.78-92ribonucleoprotein complex4.866-08muclear part3.78-92ribonucleoprotein complex4.866-08muclear part3.82-52 <th>Cytosolic</th> <th colspan="4">Membrane</th>	Cytosolic	Membrane			
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			PcG protein complex	4.45E-06	

transcriptional repressor complex

methyltransferase complex

transferase complex

5.11E-06

5.11E-06

7.19E-06

Cytosolic				
Gene Category	p-Value			
ribosome	3.50E-50			
cytosolic part	3.20E-46			
ribonucleoprotein complex	5.80E-35			
small ribosomal subunit	2.10E-28			
large ribosomal subunit	5.70E-27			
cytosol	6.10E-19			
mitochondrion	5.30E-15			
mitochondrial part	3.90E-11			
mitochondrial membrane	4.90E-10			
mitochondrial envelope	1.10E-09			
mitochondrial ribosome	3.80E-05			
non-membrane-bounded organelle	1.00E-04			
respiratory chain	4.10E-04			

Table S3. Cell component	gene ontology (GO) enrichments	of HepG2 cell
fraction-specific mRNAs.		

Insoluble				
Gene Category	p-Value			
nucleoplasm	1.20E-43			
nucleolus	1.00E-31			
chromosome	3.00E-24			
microtubule cytoskeleton	2.50E-22			
nucleoplasm part	2.10E-20			
nuclear body	3.00E-20			
spliceosome	1.10E-19			
spindle	3.70E-19			
chromosomal part	6.50E-18			
condensed chromosome	1.10E-17			
ribonucleoprotein complex	9.10E-16			
cytoskeleton	1.20E-15			
kinetochore	7.10E-14			
nuclear speck	1.70E-12			
nuclear chromosome	2.60E-12			
nuclear pore	3.00E-10			
microtubule organizing center	6.20E-10			
centrosome	9.30E-10			
nuclear envelope	1.90E-08			
proteasome complex	5.50E-08			

Membrane			
Gene Category	p-Value		
cytosolic ribosome	3.90E-31		
ribosomal subunit	3.00E-22		
cytosolic part	7.20E-22		
ribosome	1.40E-18		
cytosol	3.50E-13		
large ribosomal subunit	5.00E-13		
small ribosomal subunit	2.30E-09		
ribonucleoprotein complex	5.50E-09		
endoplasmic reticulum	3.40E-05		
endosome	8.10E-05		
transcription factor complex	4.30E-04		
nucleoplasm part	8.70E-04		
extrinsic to membrane	1.10E-03		
Golgi apparatus part	7.10E-03		
organelle subcompartment	7.10E-03		
Nuclear			
Gene Category	p-Value		

Gene Category	p-Value
extracellular matrix part	5.40E-06
nuclear lumen	6.60E-06
membrane-enclosed lumen	1.40E-05
basement membrane	2.20E-05
nuclear speck	2.70E-05
nucleoplasm	6.50E-05
endomembrane system	8.40E-05
endoplasmic reticulum part	9.30E-05
collagen	1.00E-04
nucleoplasm part	2.90E-04
anchoring collagen	2.30E-03
cell-cell adherens junction	2.40E-03
nuclear body	3.40E-03
anchoring junction	4.50E-03
microtubule cytoskeleton	4.60E-03
nuclear envelope	4.80E-03
cell-cell junction	7.30E-03



#### Figure S1: Fractionation validation and inter-replicate correlations.

(A) RT-qPCR of RNAs sample controls show fractionation efficiency.

The accumulation of the indicated RNA fraction-specific markers was assessed in HepG2 and D17 cells.

(B) Summary table of inter-replicates Pearson correlations of transcript per million (TPM) values within each fractions for HepG2 and D17 cell RNA-seq samples. RD= rRNA-depletion, PA= poly-A+.



Figure S2: Distinctive transcript composition of subcellular fractions in poly-A+ dataset.

(A) Histograms depicting the RNA biotype content, in TPM, detected via PA sequencing of cytosolic (C), membrane (M), insoluble (I), and Nuclear (N) fractions or whole-cell RNA (T=total) from HepG2 (upper panel) and D17 cells (lower panel). (B) Histograms of the RNA biotype content of HepG2 and D17 cell fractions, binned according to the length of the longest annotated isoform of detected RNA species, following a log10 scale from 1.5-5 (i.e. ranging from 31-100,000 nt). The expected lengths for mRNA and total RNA populations are indicated for each fraction. For A and B, biotypes accounting for less than 1% of the overall TPMs were grouped as "other". (C) Boxplots showing the fraction distribution profiles of different RNA biotypes in percent FPKM (pFPKM) for HepG2 (upper plots) and D17 (lower plots) cells. The number (n) of transcripts analyzed for each biotype is indicated. TPM: Transcripts Per Millions; FPKM: Fragment per kilobase per million; IncRNA: long noncoding RNA; mRNA: messenger RNA; miRNA: microRNA; miscRNA: miscellaneous other non-coding RNA; snoRNA: small nucleolar RNA; snRNA: small nuclear RNA; rRNA: ribosomal RNA.



#### Figure S3: RNA-seq read distribution profiles varies between fractions.

Fraction of the total number of aligned bases that mapped to eiter the protein coding regions of genes, untranslated regions (UTR) of genes, gene introns, or intergenic regions of genomic DNA in HepG2 and D17 cells either assessed from polyA-enriched (PA) or rRNA-depleted (RD) sequencing datasets. C= Cytosolic, M= Membrane, I= Insoluble, N= Nuclear.



#### Figure S4: pFPKM values are strongly correlated with the results of RT-qPCR.

Scatter plot of RT-qPCR cycle threshold (CT) values and pFPKM for fraction-specific RNA markers demonstrate that CT values are strongly correlated with pFPKM values. Similar results were obtained with rRNA-depletion and poly-A+ RNA sequencing datasets. Pearson correlations are indicated on each graph.

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#### Figure S5: Distribution profile of mRNAs in rRNA-depleted sequencing datasets.

(A-B) Histograms showing the percent of asymmetrically distributed and fraction-specific mRNAs in HepG2 (A) and D17 (B) cells.

(C-D) Simplex graphs (left panels) and pie charts (right panels) depicting the relative distribution and proportion of fraction-specific mRNAs, coloured according to the fraction they are enriched in, relative to the total mRNA population in HepG2 (C) or D17 (D) cells.

C= Cytosolic, M= Membrane, I= Insoluble, N= Nuclear.



**Figure S6**: **Human and fly cell mRNA localization is strongly correlated between the same fraction.** Scatter plot of mRNA relative localization in pFPKM between the same fraction in HepG2 and D17 cells. Pearson correlations are indicated on each graph. All p-values  $<2.2x10^{-16}$ .



## Figure S7: LncRNAs from rRNA-depleted sequencing datasets are asymmetrically distributed and exhibit preferential polarization towards the nucleus and cytosol.

(A-B) Histogram showing the percent of asymmetrically distributed and fraction-specific IncRNAs, obtained from RD-sequencing, expressed in HepG2 (A) and D17 (B) cells, either using a standard expression threshold (≥1FPKM) or focusing on highly expressed transcripts (≥10 FPKM).
(C-D) Simplex graphs (C) and pie charts (D) depicting the relative distribution and proportion of fraction-specific IncRNAs, coloured according to the fraction they are enriched in, relative to the total IncRNA populations detected in HepG2 or D17 cells at the expression thresholds indicated in (A-B).



## Figure S8: CircRNA exhibit distinct distribution compared to other intronic regions or their host mRNAs.

(A) Boxplot of the expression, in FPKM, of 1,040,283 introns as annotated by Ensembl in HepG2 cells. (B) Zoomed view of the boxplot described in (A).

(C) The relative localization distance between a circRNA and its cognate mRNA is similar to that of a random pairing of an equal number of cirRNA and a mRNA encompassing a putative circRNA for both back-spliced and intronic circRNA in both human and fly cells.



#### Figure S9: Proteome distribution of each fraction.

(A) Venn diagram depicting the relative distribution of proteins in HepG2 (upper panel) and D17 (lower panel) cell fractions following tandem mass spectrometry (LC-MS/MS), assessed by measuring their total spectrum count.

(B) Boxplot showing the relative distribution, in percent spectrum count, of all proteins identified in our LC-MS/MS datasets for HepG2 (upper panel) and D17 (lower panel) cell fractions. C= Cytosolic, M= Membrane, I= Insoluble, N= Nuclear.

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#### Figure S10: mRNA bearing various motifs exhibit asymmetric disributions.

(A) Boxplots showing the fraction distribution profiles of mRNAs bearing a signal peptide cleavage sites, in pFPKM, for HepG2 (upper plots) and D17 (lower plots) cells.

(B) Boxplots showing the fraction distribution profiles of mRNAs canonical histones, in pFPKM, for HepG2 (upper plots) and D17 (lower plots) cells.

The number (n) of transcripts analyzed and Kruskal-Wallis p-value is indicated for each motifs.