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Supplemental information

TRACE-seq: A transgenic system for unbiased and non-invasive transcriptome profiling of living cells

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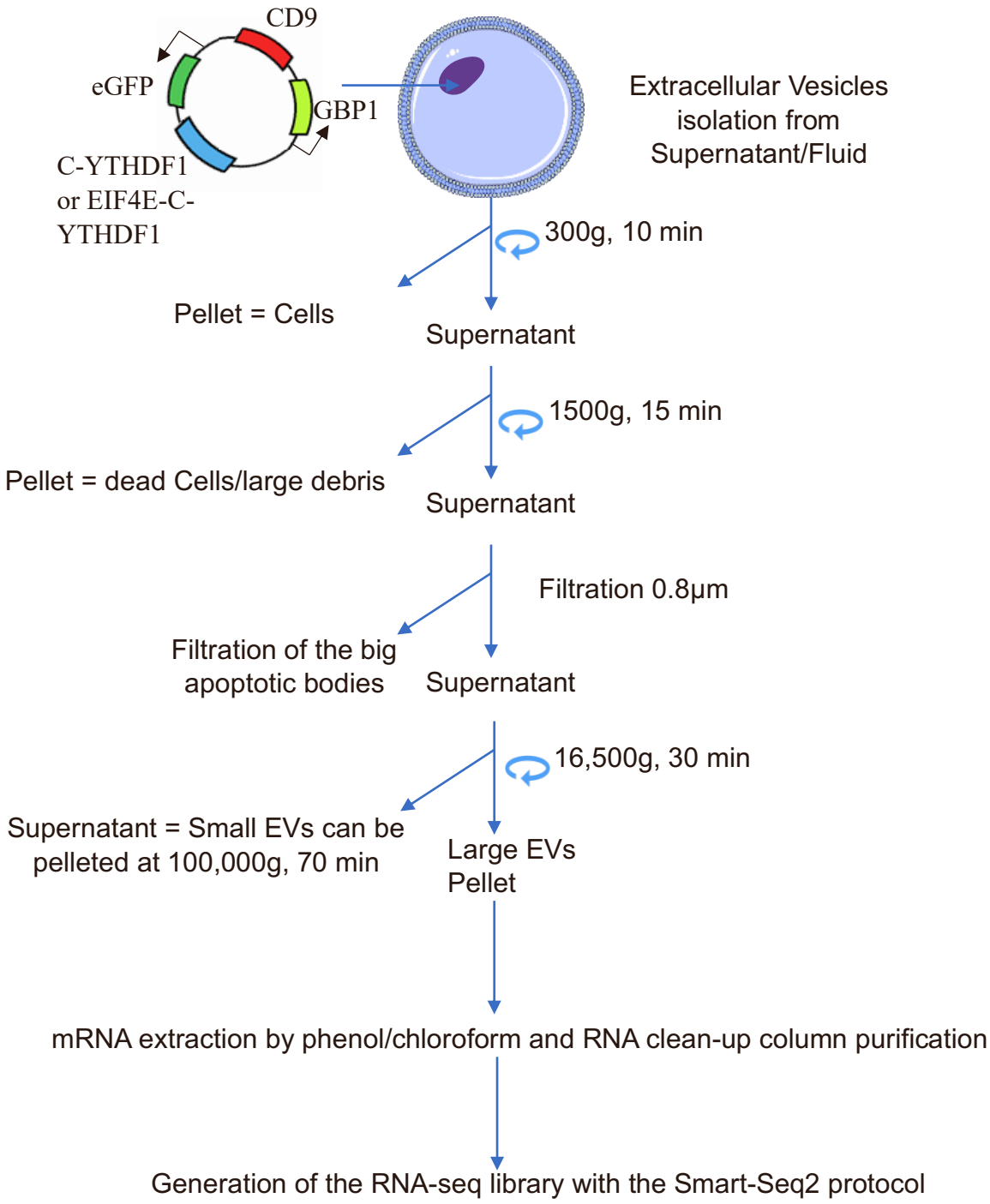
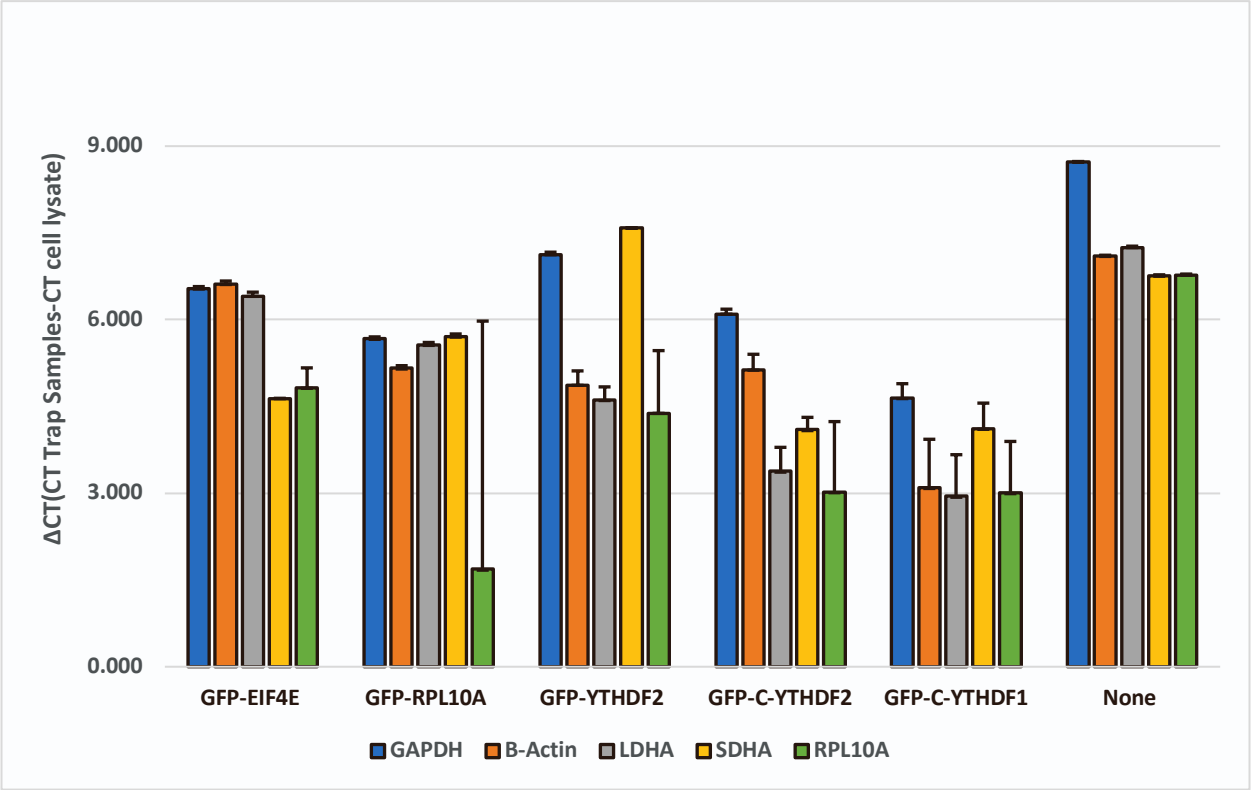
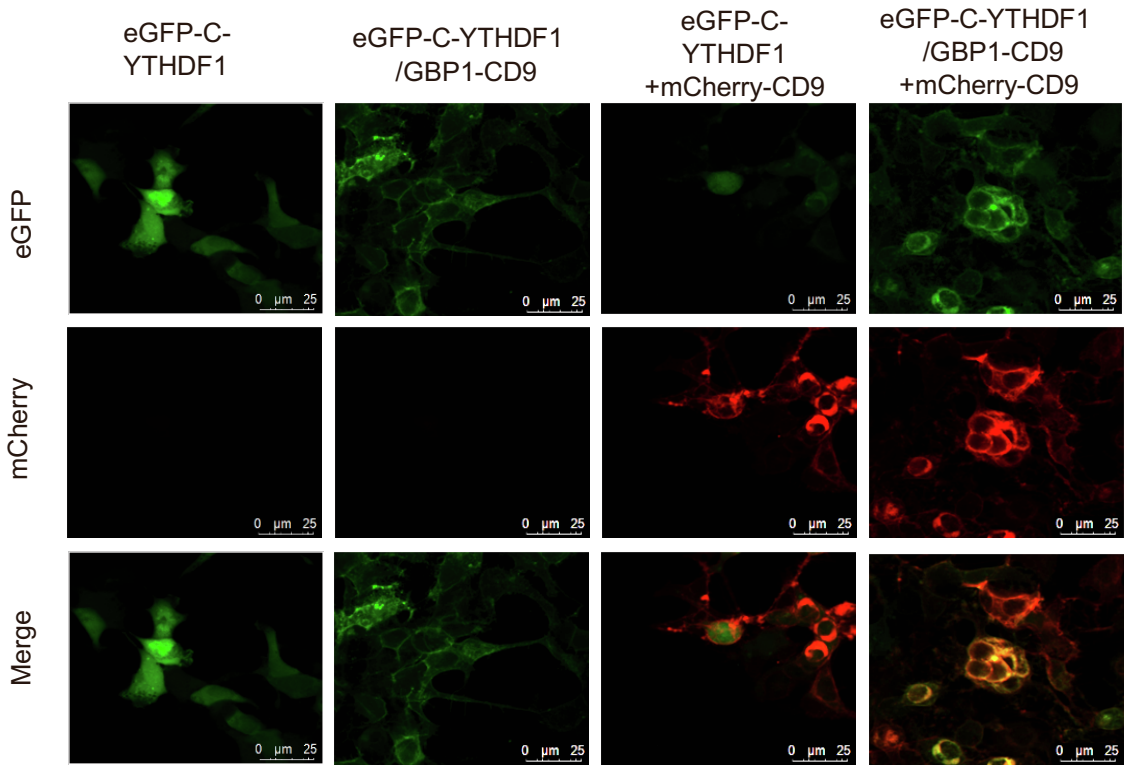


Supplemental Fig. S1 (Related to main Fig 1 and main Fig. 2). TRACE isolation method and RNA-Seq library generation steps



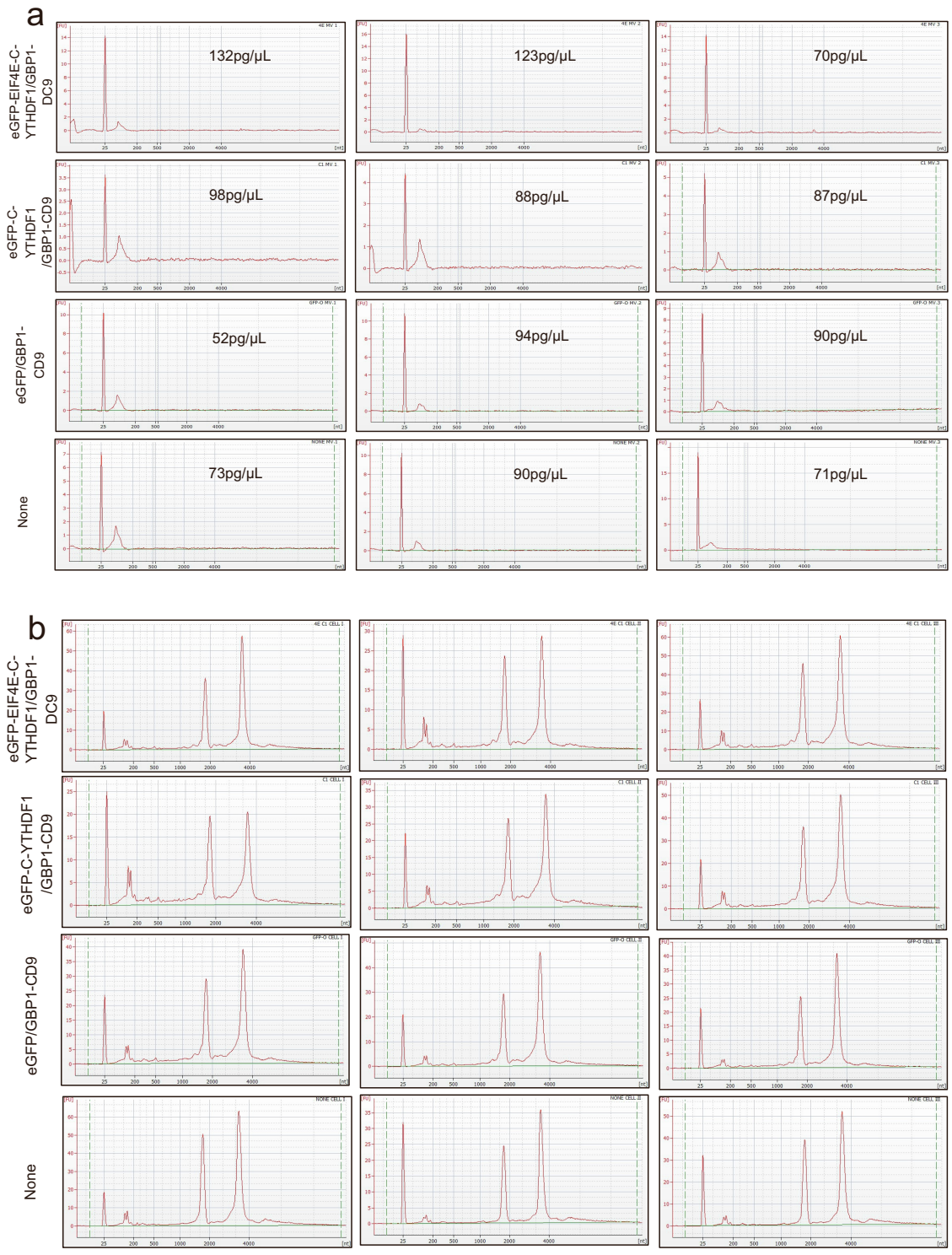
Supplemental Fig. S2 (related to main Figure 1). Relative efficacy of mRNA capture for five different GFP-RNA binding protein fusion constructs.

RNA binding protein (RBP) immunoprecipitation results of different HEK 293T populations transfected with five eGFP-RBP constructs. For each of them, cells were lysed, mRNA was purified and immunoprecipitated using an anti-GFP antibody. These trapped genomic populations were normalized against the remaining expression level of their own whole lysate. Data are represented as mean ± SEM.



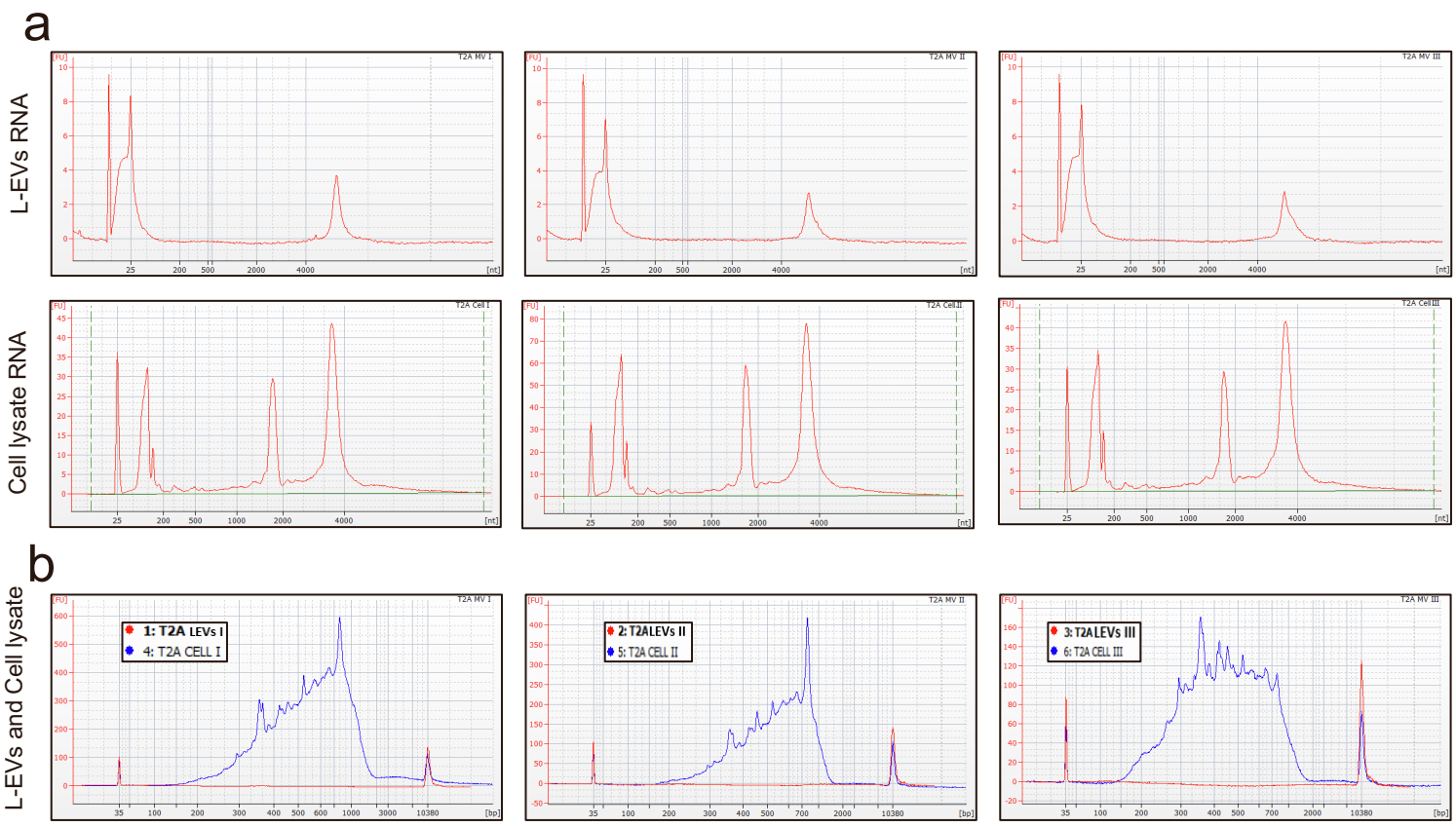
Supplementary Fig S3 (related to Fig 1). Localization of eGFP-C-YTHDF1 on cell membranes in the presence of GBP1-CD9 construct.

Confocal images after transfection of different HEK 293T populations with two constructs: GFP control (eGFP-C-YTHDF1), C1 (eGFP-C-YTHDF1/GBP1-CD9) and mCherry-CD9. mCherry CD-9 is used to demonstrate localization to the cell membrane. Image taken 36h post transfection.



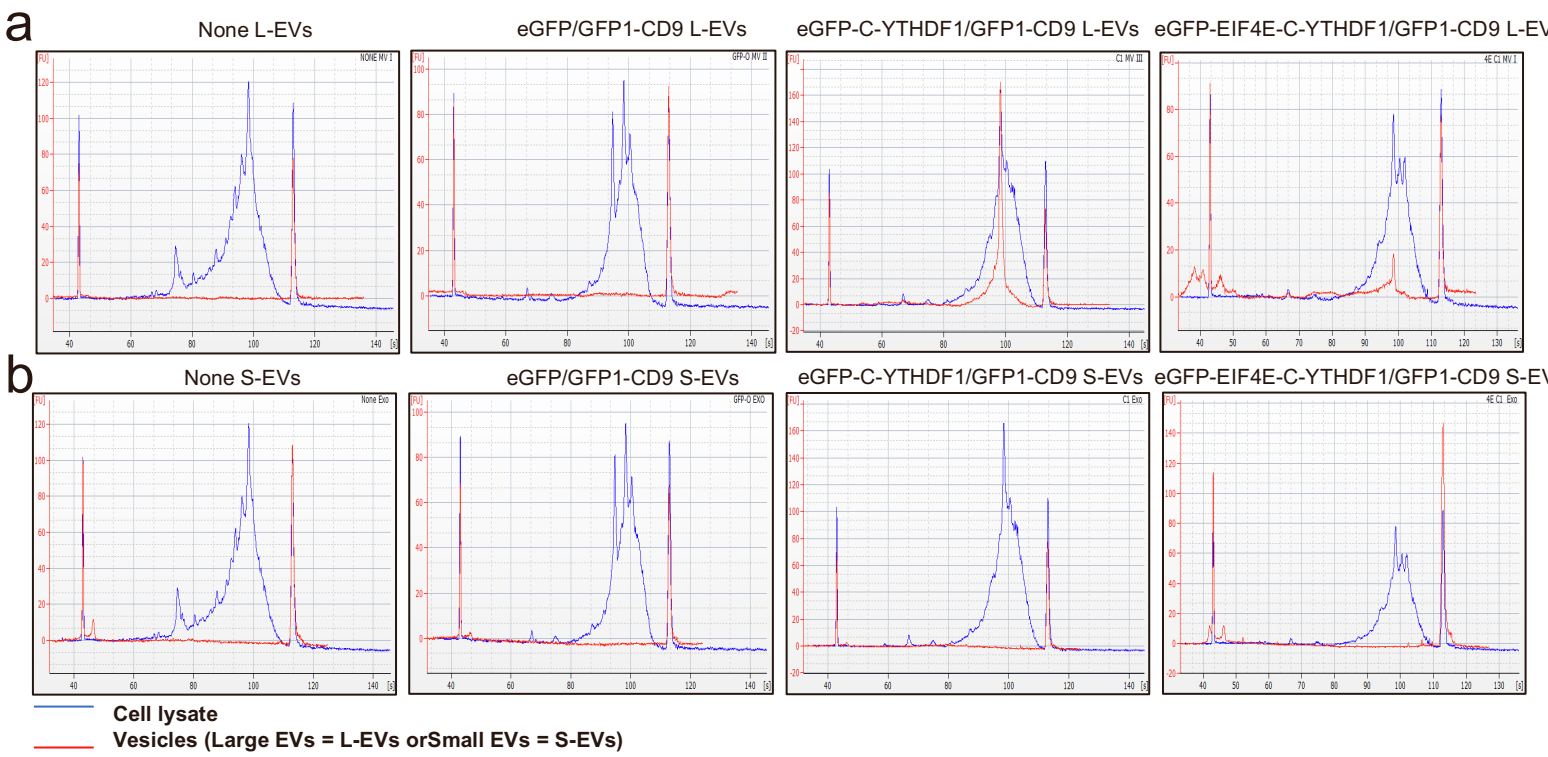
Supplementary Fig. S4 (Related to main Figure 2). RNA content of L-EVs and corresponding cellular lysates for C1, and 4EC1 constructs.

Bioanalyzer analysis of RNA content from Large EVs and their corresponding RNA from cell lysates isolated from different HEK 293T populations (Triplicate of 4M cells per condition): untransfected cells (None), negative GFP control (eGFP/GBP1-CD9) and the two types of TRACE construct C1 and E4C1 (eGFP-C-YTHDF1/GBP1-CD9 and eGFP-EIF4E-C-YTHDF1/GBP1-CD9). **a** Bioanalyzer of RNA content from Large EVs. **b** Bioanalyzer of RNA content from cell lysate.

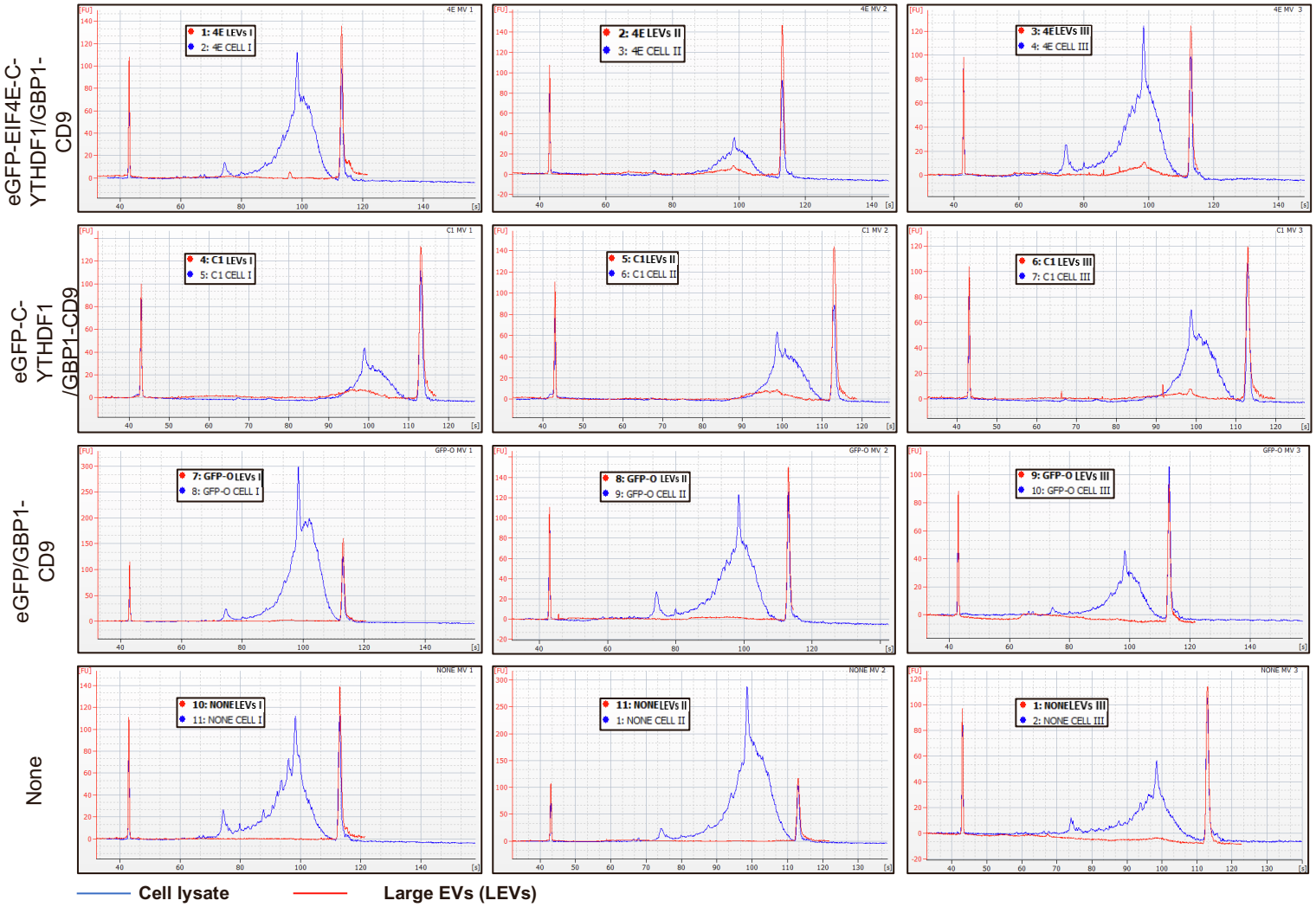


Supplementary Fig. S5 (Related to main Figure 2). RNA content and corresponding reverse transcribed cDNA of L-EVs and corresponding cellular lysates for T2A construct.

a Bioanalyzer analysis of RNA content from Large EVs (L-EVs) and their corresponding RNA from cell lysates isolated from HEK 293T populations (Triplicate of 4M cells per condition) transfected with GBP1-CD9-T2A-eGFP-C-YTHDF1 (T2A). **b** Bioanalyzer analysis of cDNA generated from Large EVs and their corresponding cDNA generated from cell lysates, isolated from HEK 293T populations (Triplicate of 4M cells per condition) transfected with GBP1-CD9-T2A-eGFP-C-YTHDF1 (T2A).

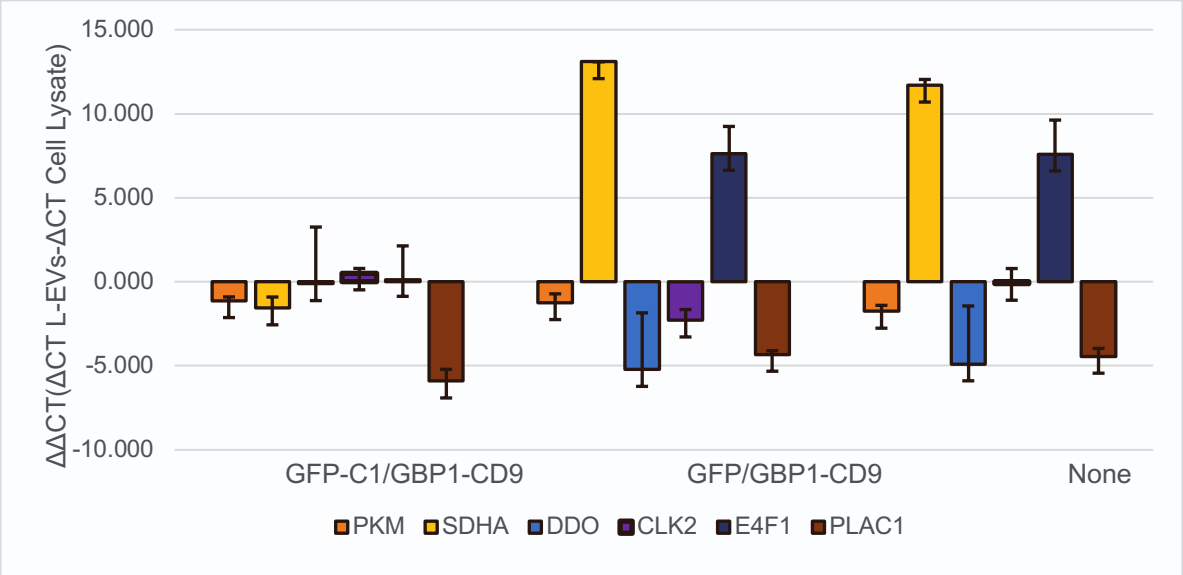


Supplementary Fig. S6 (related to main Fig 2). Presence of non-fragmented mRNAs in L-EVs versus S-EVs in the C1 and 4EC1 constructs as assayed by measurement of reverse transcribed cDNA from the isolated RNA. **a**. Bioanalyzer analysis of cDNA generated from Large EVs produced by different HEK 293T populations (4M each): untransfected cells (None), negative GFP control (eGFP/GBP1-CD9) and the two types of TRACE construct C1 and E4C1 (eGFP-C-YTHDF1/GBP1-CD9 and eGFP-EIF4E-C-YTHDF1/GBP1-CD9).



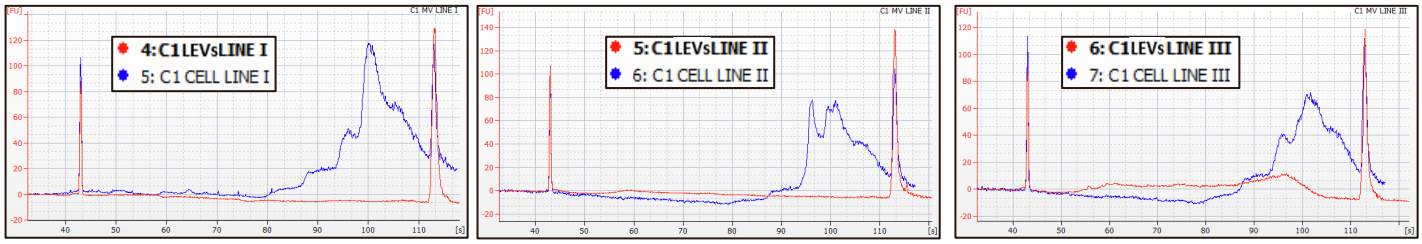
Supplementary Fig. S7 (related to main Fig 2). Assessment of unfragmented mRNA in L-EVs from cells transiently transfected with C1 and 4EC1 constructs (lower cell number).

Bioanalyzer analysis of cDNA generated from Large-EVs and their corresponding cDNA from cell lysates isolated from different HEK 293T populations (Triplicate of 1M cells per condition): untransfected cells (None), negative GFP control (eGFP/GBP1-CD9) and the two types of TRACE construct C1 and 4EC1 (eGFP-C-YTHDF1/GBP1-CD9 and eGFP-EIF4E-C-YTHDF1/GBP1-CD9).



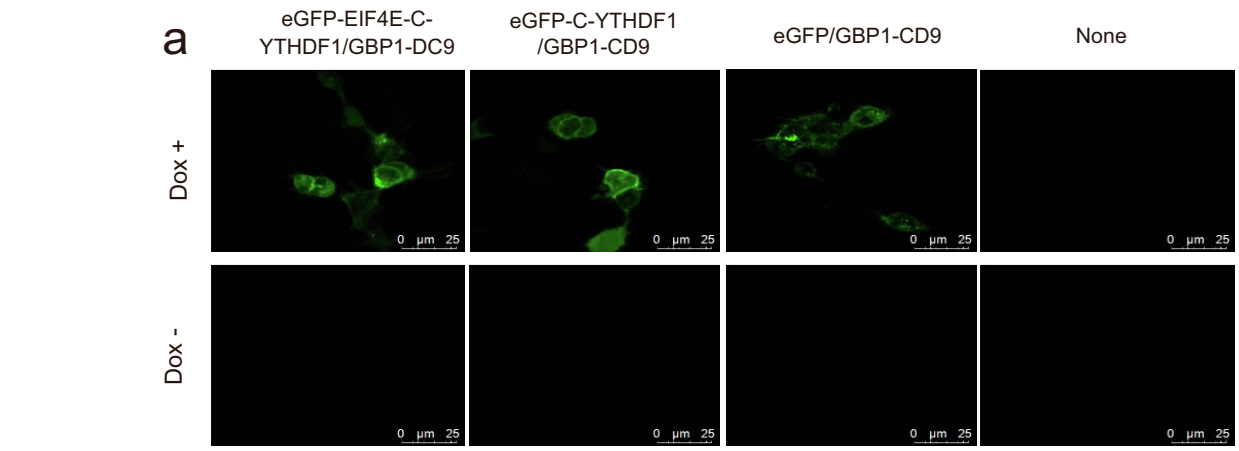
Supplementary Fig. S8 (related to main Fig. 2). Partial validation of bio-analyzer results using qRT-PCR for candidate genes in L-EVs isolated from cells transfected with C1 construct. RT-qPCR results generated from Large-EVs mRNA isolated from untransfected cells (None), negative GFP control (eGFP/GBP1-CD9) and the TRACE C1 construct (eGFP-C-YTHDF1/GBP1-CD9). 8 million cells were used for each experiment. These cDNA samples were normalized against the remaining expression level of their own whole lysate. All samples were made in triplicate, 8M cells were transfected per condition. Each gene CT value from each group were normalized against their GAPDH gene CT value. Notably PLAC1 mRNA appears to be present in L-EVs without constructs and may reflect its m^6A status. Data are represented as mean ± SEM.

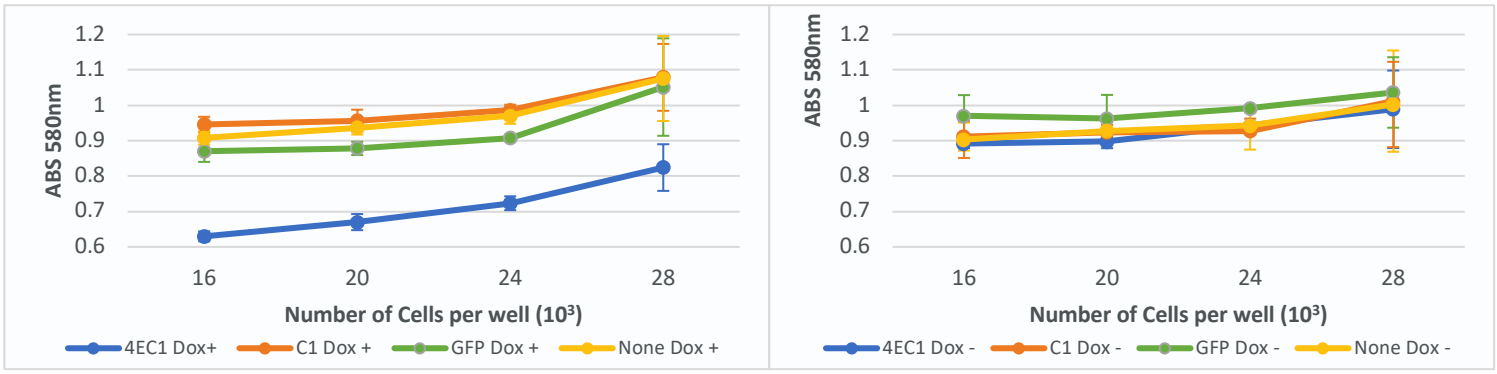
eGFP-C-YTHDF1
/GBP1-CD9

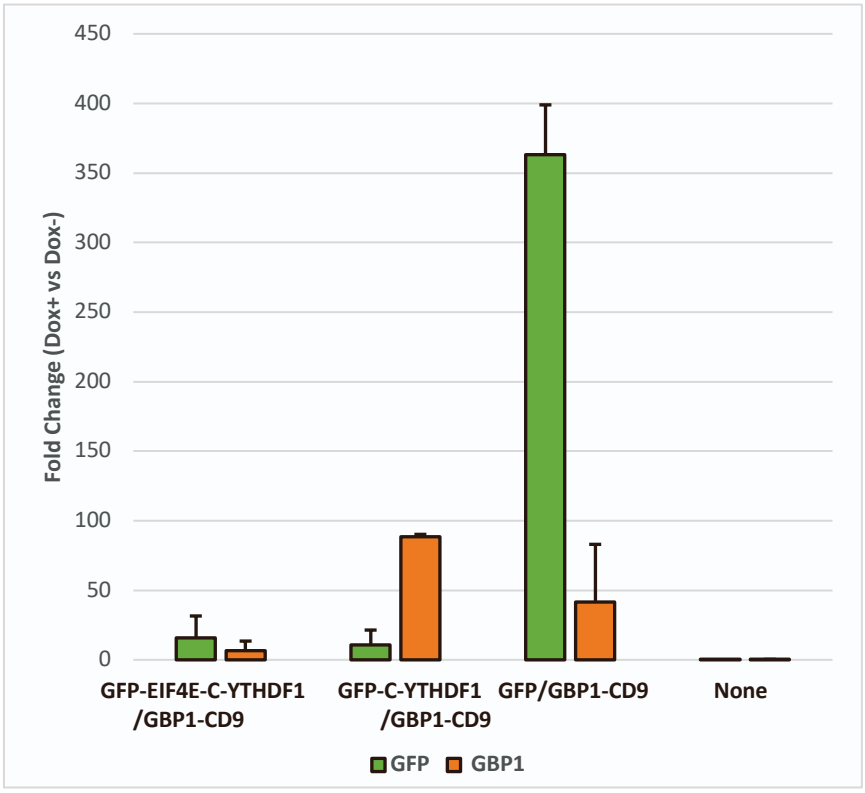


Supplementary Fig. S9 (related to main Figure 3). mRNA content (assayed by measurement of reverse-transcribed cDNA from isolated RNA) in cells stably transfected by C1.

Bioanalyzer analysis of cDNA generated from Large EVs (LEVs) and their corresponding cDNA generate from cell lysate from stable cell line generated with the construct C1: eGFP-C-YTHDF1/GBP1-CD9 (Triplicate of 10M cells per condition).



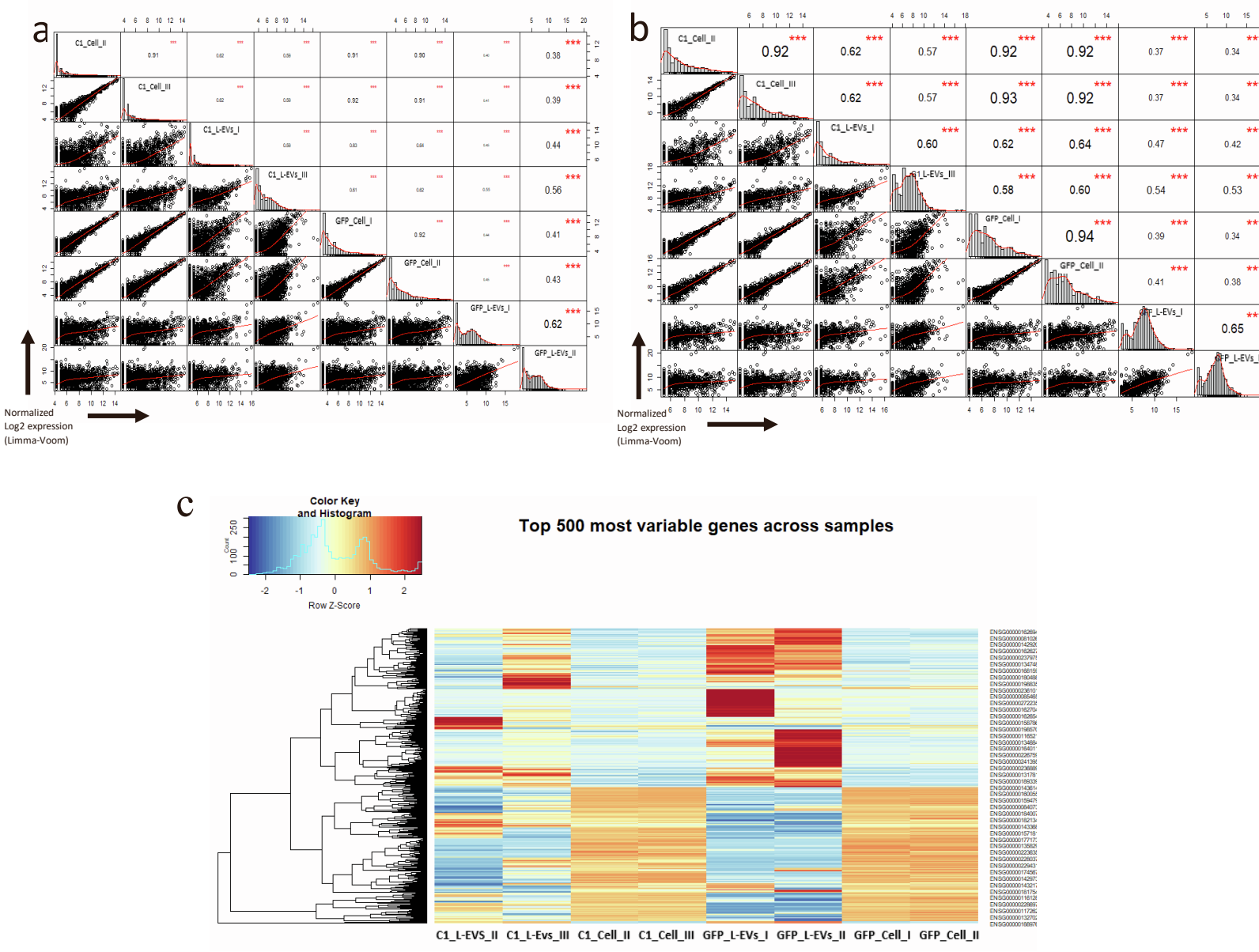




Supplementary Fig. S10 (related to main Figure 4). Characteristics of cells stably transfected with C1 or 4EC1. a Confocal image of the stable cell line. Different HEK 293T populations treated with and without doxycycline. Cells are untreated to maintain the stable cell line and treated for 48h with 2µl/ml doxycycline. Stable cell line generated with the two constructs: 4EC1 (eGFP-EIF4E-C-YTHDF1/GBP1-CD9) and C1 (eGFP-C-YTHDF1/GBP1-CD9), the negative GFP control: (eGFP/GBP1-CD9) and the regular HEK293T cells: None. Data are represented as mean ± SEM.

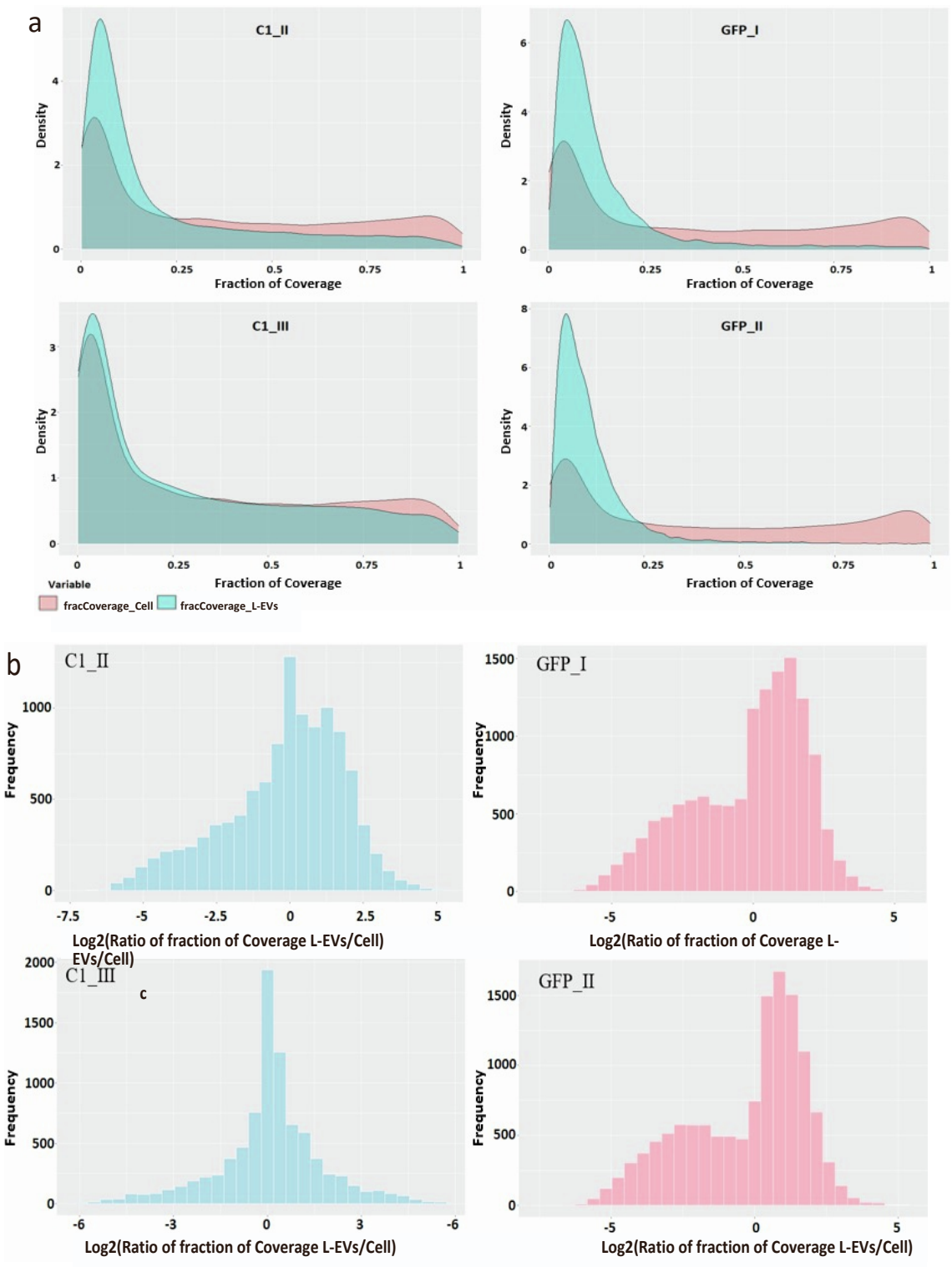
b Cell viability assay made with Reliablue on the same stable cell line as above, divided in two groups: treated or untreated with doxycycline. Cells were plated at 4 different concentrations and incubated at 37ºC, 5% CO_2 for 48 hours. After incubation, cells are incubated with Reliablue for 4 hours at 37ºC, 5% CO_2 and absorbance was measured 580nm.

c RT-qPCR results generated from total RNA isolated from the cell lysates of the same stable cell lines as above, divided in two groups of treated and untreated with doxycycline. Half of the plated stable cell line was treated for 48h with 2µl/ml doxycycline and the other not (each group made in triplicate).



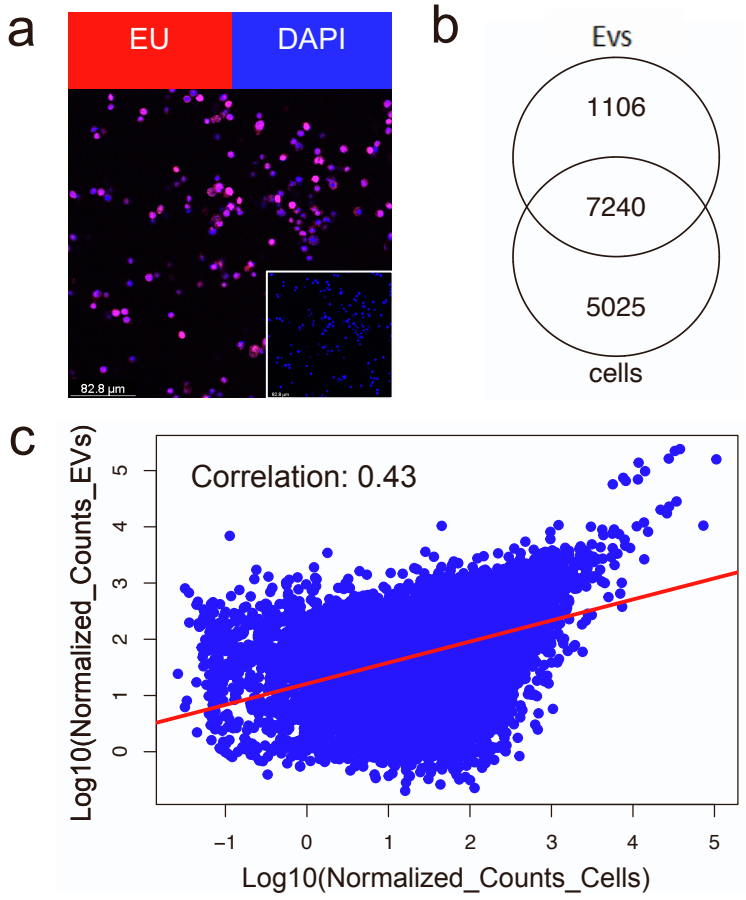
Supplementary Fig. S11 (related to main Figure 4). Correlations between L-EVs and cellular RNAs in cells transiently transfected with C1.

LIMMA-VOOM from the 8 selected samples. TRACE C1 (GFP-C-YTHDF1/GBP1-CD9) construct: duplicate L-EVs, duplicate cell lysates and GFP control (GFP/GBP1-CD9): duplicate Large EVs (L-EVs), duplicate cell lysates from transient transfection batch. **a.** Pearson correlation chart form the Limma-voom analysis between each 8 samples. **b.** Pearson correlation chart from the Limma-voom. DEseq2 analysis between each 8 samples. DEseq2 made on TRACE experiment vs POSTAR data base (methylome isolated with IP C-YTHDF1). **c.** Heat map for the most 500 variable genes across all 8 samples. Results from duplicate of 4M cells per condition from transient transfection batch.



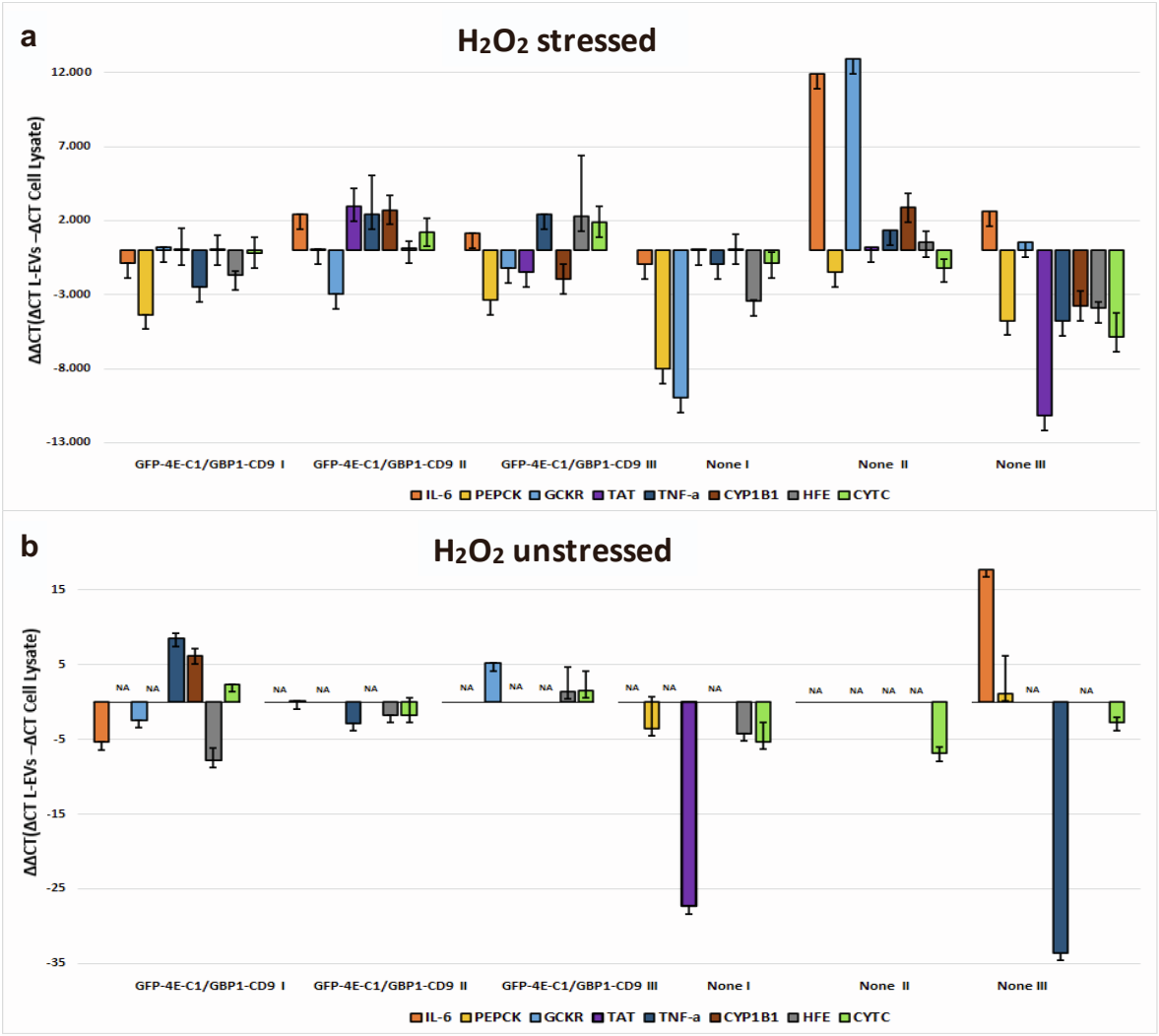
Supplementary Fig. S12 (related to main Figure 4). mRNA lengths as assessed by RNAseq read coverage in L-EVs corresponding to C1 transfected cells.

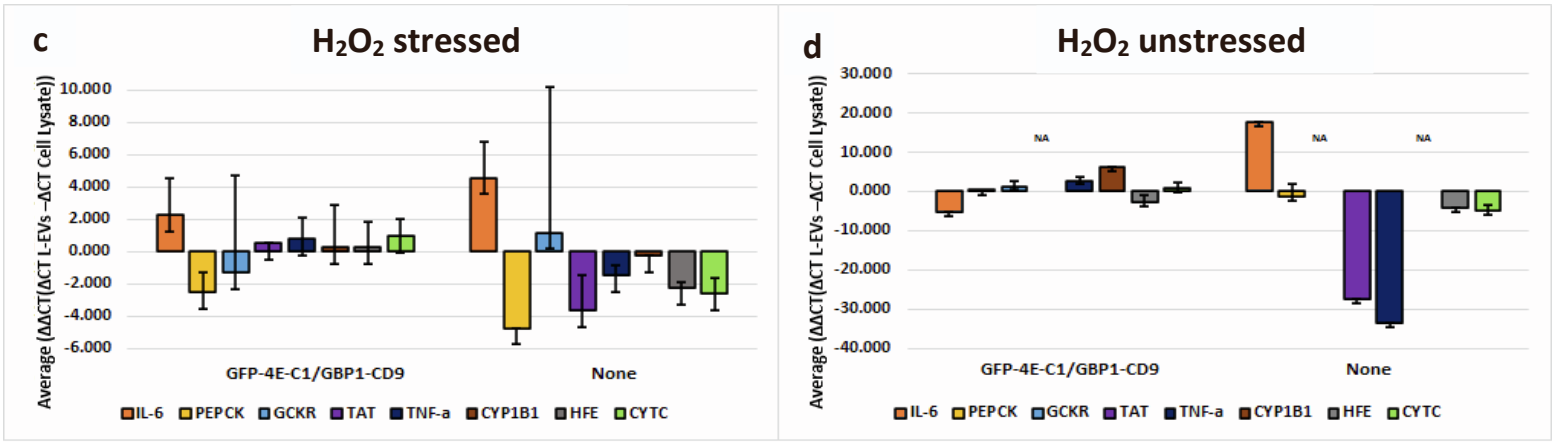
Mapping results generated for all 8 sequenced samples of TRACE C1 (GFP-C-YTHDF1/GBP1-CD9) construct: duplicate large EVs, duplicate cell lysates and GFP control (GFP/GBP1-CD9): duplicate large EVs, duplicate cell lysates from the transient transfection batch. **a.** Mapping of fraction of coverage distribution of large EVs and cell lysates on genes at ≥1000nt length. **b.** Mapping of Log2 ratio of fraction of coverage Large EVs/Cell distribution on genes at ≥1000nt length.



Supplementary Fig. S13. (related to main Figure 4). Comparison of TRACE-seq with i-TAG based RNAseq to profile nascent mRNAs in EVs.

DEseq analysis generated from i-Tag RNA from EVs and Cell lysate from iTag THP1 Cells. **a.** Confocal microscopy on THP1 cells detection of the iTagRNA signal. **b.** Venn diagram from comparison matrix EVs vs Cell lysate. **c.** Correlation plot based on normalized counts of EVs vs Cell lysate. Cor function was used to calculate the Pearson correlation in the R package.





Supplementary Fig. S14 (related to Fig. 5). Stress pathway validation for 4EC1 construct using qRT-PCR approach.

RT-qPCR results generated from Large EVs mRNA isolated from control cells (None) and TRACE construct 4EC1 (eGFP-EIF4E-C-YTHDF1/GBP1-CD9) stable cell line. These cDNA samples (a,b) were normalized against the remaining expression level of their own cell lysate. Moreover, each gene CT value of each group was normalized against the GAPDH housekeeping gene CT value **a.** Batch of H_2O_2 stressed cells. **b.** Batch of unstressed cells. **c.** Average made with stressed cells triplicate (a). **d.** Average made with unstressed cells triplicate (b). Triplicate of 15M cells per condition. Data are represented as mean ± SEM.

NAME	SEQUENCE 5' TO 3'
GBP1 FOR	AGGAAGCTAGCGGCCCGGGATCCACCG
GBP1 REV	TCATGAAGCTTGACCTCCACCTCCTCCACCTCCTCCACCACCAGAACTAACAGTCACTTGTGTGCCCT
C-YTHDF1 FOR	CTGTTGCTCGAGTTTCAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGAGAATCCCACCCCGTCCTT
C-YTHDF1 REV	CTGTGTCAATTGAACGTTTCATTGTTTGTTTCGACTCTGCC
C-YTHDF2 FOR	TACTTGTCCGGATCAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGAACCCCACCCAGTGTT
C-YTHDF2 REV	CTGTACGGATCCTTATTTCCCACGACCTTGAC
YTHDF2 FOR	TACTTGTCCGGAACGGGTGGTGGTGGTGGTGGTGGTGGTGGAATGTCGGCCAGCAGCC
YTHDF2 REV	CTGTACGGATCCTTATTTCCCACGACCTTGACGTT
RPL10A FOR	TACAAGTCCGGAAGAACTCAGATCTCGTCCTCGTCCTTCGAATTCAGCAGCAAAGTCTCTCGCG
RPL10A REV	CTGTACGGATCCTTAATATAGGCGCTGGGGCTT
EIF4E FOR	TCTAGAAGATCTGGAGGAGGTGGTGGAGGTGGAGGTGGAGGTACTATGGCGACTGTCGAACC
EIF4E REV	CCCTCTCTCGAGTAACAACAAACCTATTTTTAGTGGTGG
CD9 T2A FOR	CATCAAAGAGGTCTTCGACAA
CD9 T2A REV	TGCATCGAATTCAGCGCTAATGGACCAGGGTTTTCTTCAACATCACCACAAGTGAGGAGAGAACCTCTACCTTCGACCATCTCGCGGTTCCT
TRE FOR	TAGTAATGCATGAGTTTACTCCCTATCAGTGATAGAGA
TRE REV	TAGTAACCGGTAGCCAATTCTCCAGGCGA
AFE1/MLU1 FRAG FOR	TTGATTGCTAGCTGAGCGCTTACCTTCCTTTATGAATTGTACAGTTGGAGACGTTATTCGTTCTTTTTGTCAAGACCTACTATGCTGCATATAGTCGATCACGCGTTACCGGTTTATCT
AFE1/MLU1 FRAG REV	AGATAAACCGGTAACGCGTGATCGACTATATGCAGCATAGTAGGTCTTGACAAAAAGAACGAATAACGTCTCCAACTGTACAATTCATAAAGGAAGGTAAGCGCTCAGCTAGCAAACGA

Supplemental Table S1 (related to Fig 1): Primer sequences for cloning RNA catching constructs.

NAME	SEQUENCE 5' TO 3'	NAME	SEQUENCE 5' TO 3'
GAPDH FOR	GGAGCGAGATCCCTCCAAAAT	**DDO For**	CACAGCACGGATTGCAGTTG
GAPDH REV	GGCTGTTGTCATACTTCTCATGG	**DDO Rev**	GCCATAGTGGTGGACTACAGG
PKM FOR	ATGTCGAAGCCCCATAGTGAA	**CLK2 For**	CGAGTTGCCCTGAAGATCA
PKM REV	TGGGTGGTGAATCAATGTCCA	**CLK2 Rev**	GACTGGAGTCCCACAACTTG
B-ACTIN FOR	AGAGCTACGAGCTGCCTGAC	**E4F1 For**	CCATGTCCTCAGTGCAGTGA
B-ACTIN REV	AGCACTGTGTTGGCGTACAG	**E4F1 Rev**	CAGGATCTCGATGTCCTCTGA
LDHA FOR	ATGGCAACTCTAAAGGATCAGC	**PLAC1 For**	CCTCCTCACCTCTGCGTTT
LDHA REV	CCAACCCCAACAACTGTAATCT	**PLAC1 Rev**	CTGTGTGAAGAGACCAATCCTC
SDHA FOR	ACTGTTGCAGCACAGCTAGAA	**GFP For**	GAACGGCATCAAGGTGAACTT
SDHA REV	GCTCTGTCCACCAAATGCAC	**GFP Rev**	TCCAGCAGGACCATGTGATC
RPL10A FOR	AGCAGCAAAGTCTCTCGCG	**GBP1 For**	CATGGCCGACGTGCAGCTC
RPL10A REV	TTAATATAGGCGCTGGGGCTT	**GBP1 Rev**	AGAACTAACAGTCACTTGTGTGCCCT
TNF-A FOR	ATGAGCACTGAAAGCATGATCC	**IL-6 For**	AGACAGCCACTCACCTCTTCAG
TNF-A REV	GAGGGCTGATTAGAGAGAGGTC	**IL-6 Rev**	TTCTGCCAGTGCCTCTTTGCTG
PEPCK FOR	AAGAGACACAGTGCCCATCC	**GCKR For**	GTTGGACCTTCGGATTAGCA
PEPCK REV	ACGTAGGGTGAATCCGTCAG	**GCKR Rev**	CCCAGAAACATGGGTTCACT
TAT FOR	TGGAGTTCACAGAGCGGTTG	**CYP1B1 For**	CACCGTTTTCCGCGAATTC
TAT REV	GGTACTCGAAGCACGTTGCTG	**CYP1B1 Rev**	CCTTCTTTTCCGCAGAGAGGAT
HFE FOR	ACTGATGAAGCTGCAGAACC	**Cyt C For**	AAGGGAGGCAAGCACAAGACTG
HFE REV	GTCACCCAATTCTTTGATGG	**Cyt C Rev**	CTCCATCAGTGTATCCTCTCCC

Supplemental Table S2. (related to main Fig 5 and Fig S2). Primers used for RT-qPCR.