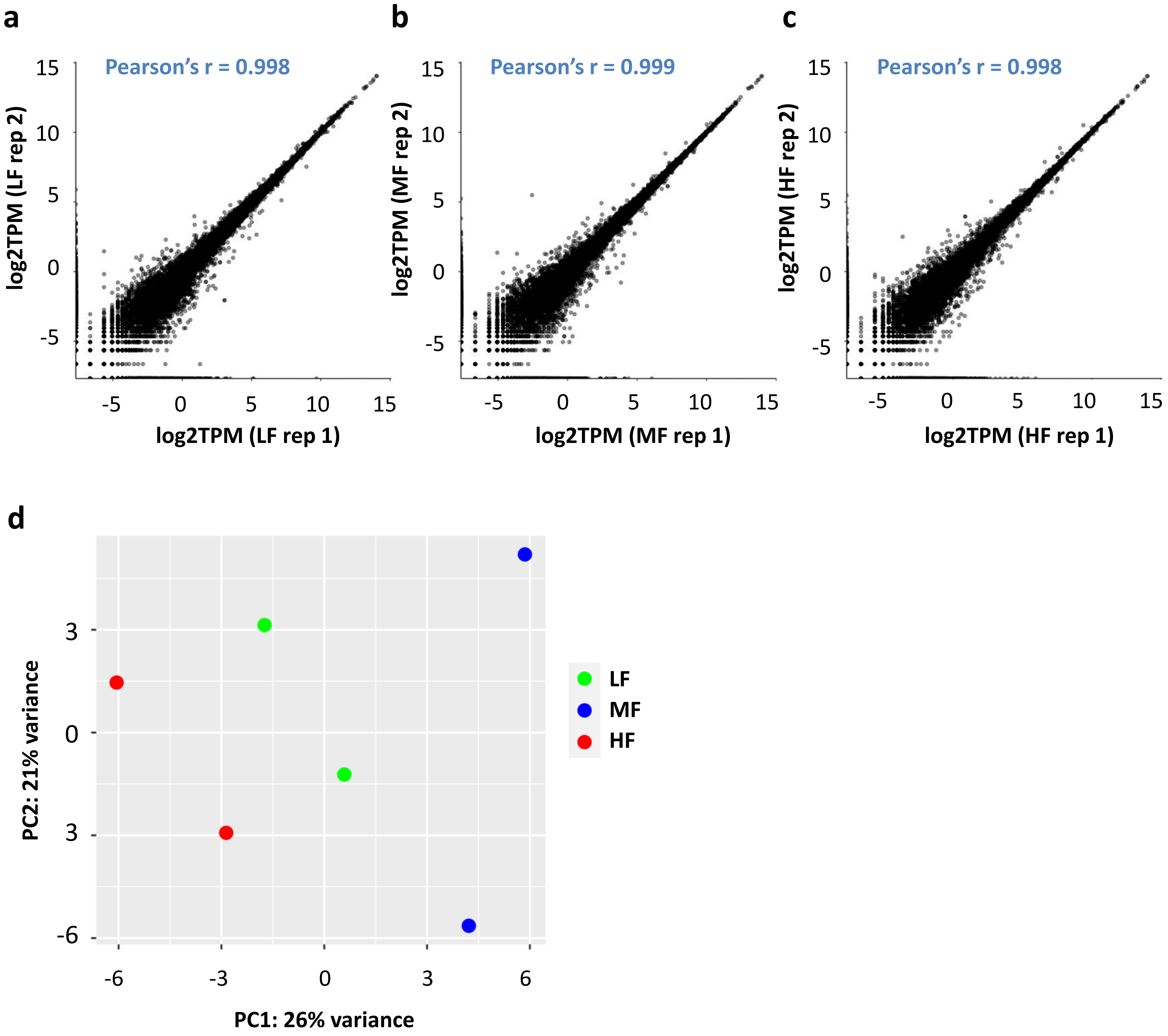


Fig. S1. Quality control of total mRNA-seq datasets. (a, b, c) Scatter plot showing the Pearson correlation between two biological replicates in total mRNA-seq datasets in NSCs. (d) Principal component analysis of total mRNA-seq datasets.

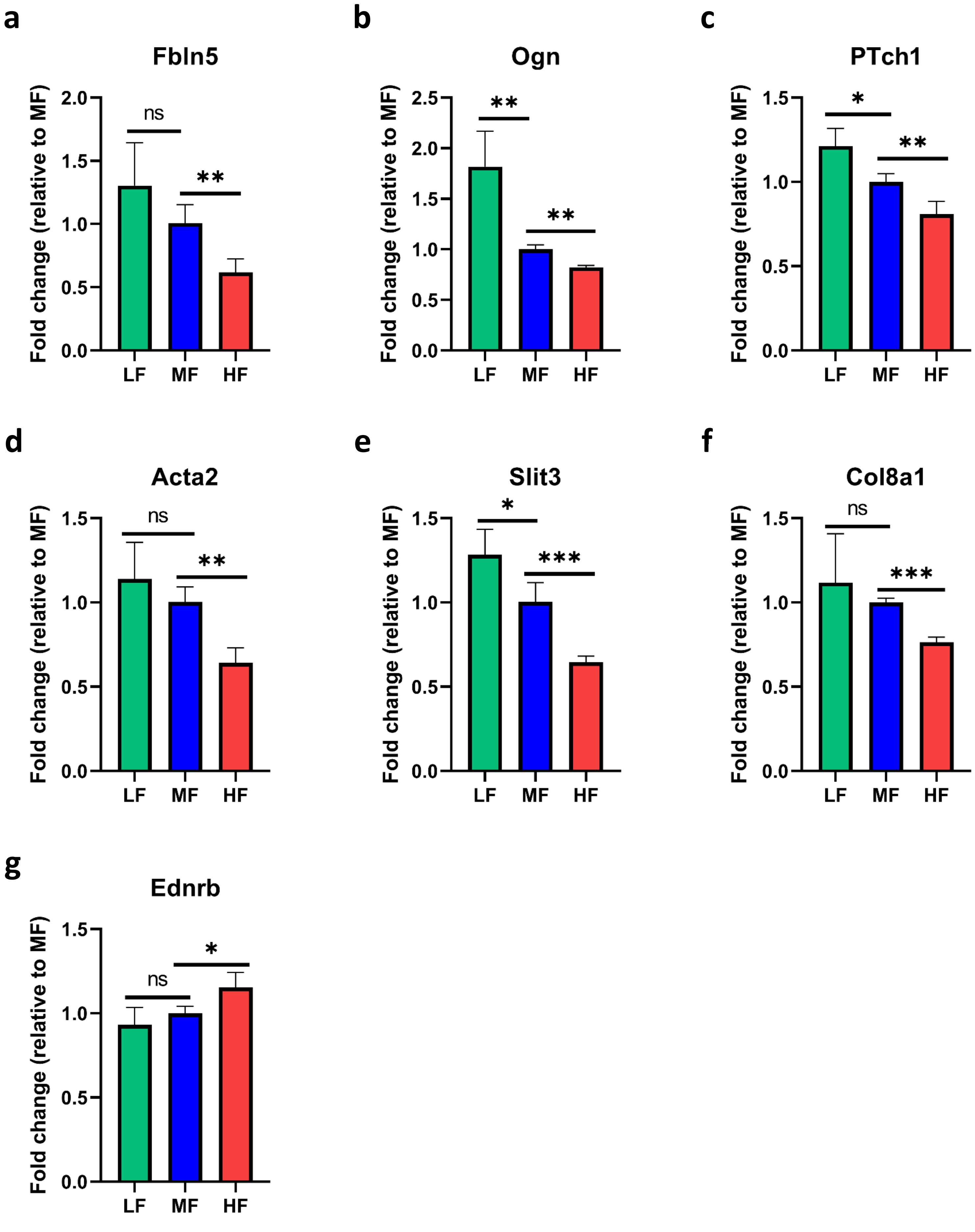


Fig. S2. Validation of differentially expressed genes (DEGs). (a-g) RT-qPCR was performed to validate the expression of DEGs identified by RNA-seq. (number of replicates = 3 or 4. *<0.05, **<0.01, ***<0.001.) Numerical data is provided in Additional file 7. The error bars indicate standard error of the mean (SEM).

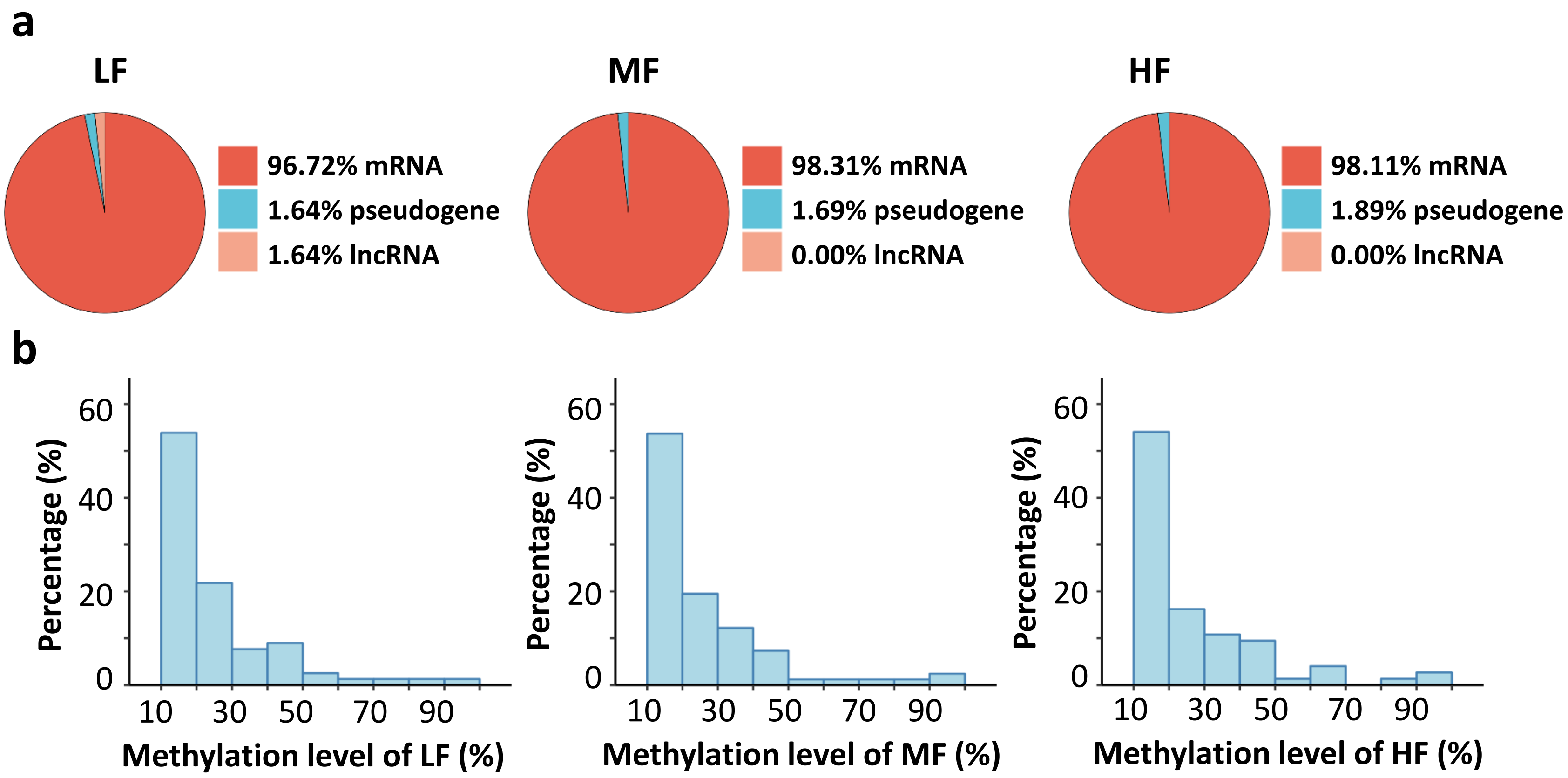


Fig. S3. Distribution of m^5C sites in total mRNA BS-seq datasets. (a) Pie chart showing the categories of RNA molecules with m^5C modification. (b) Histogram showing the distribution of methylation level for each condition.

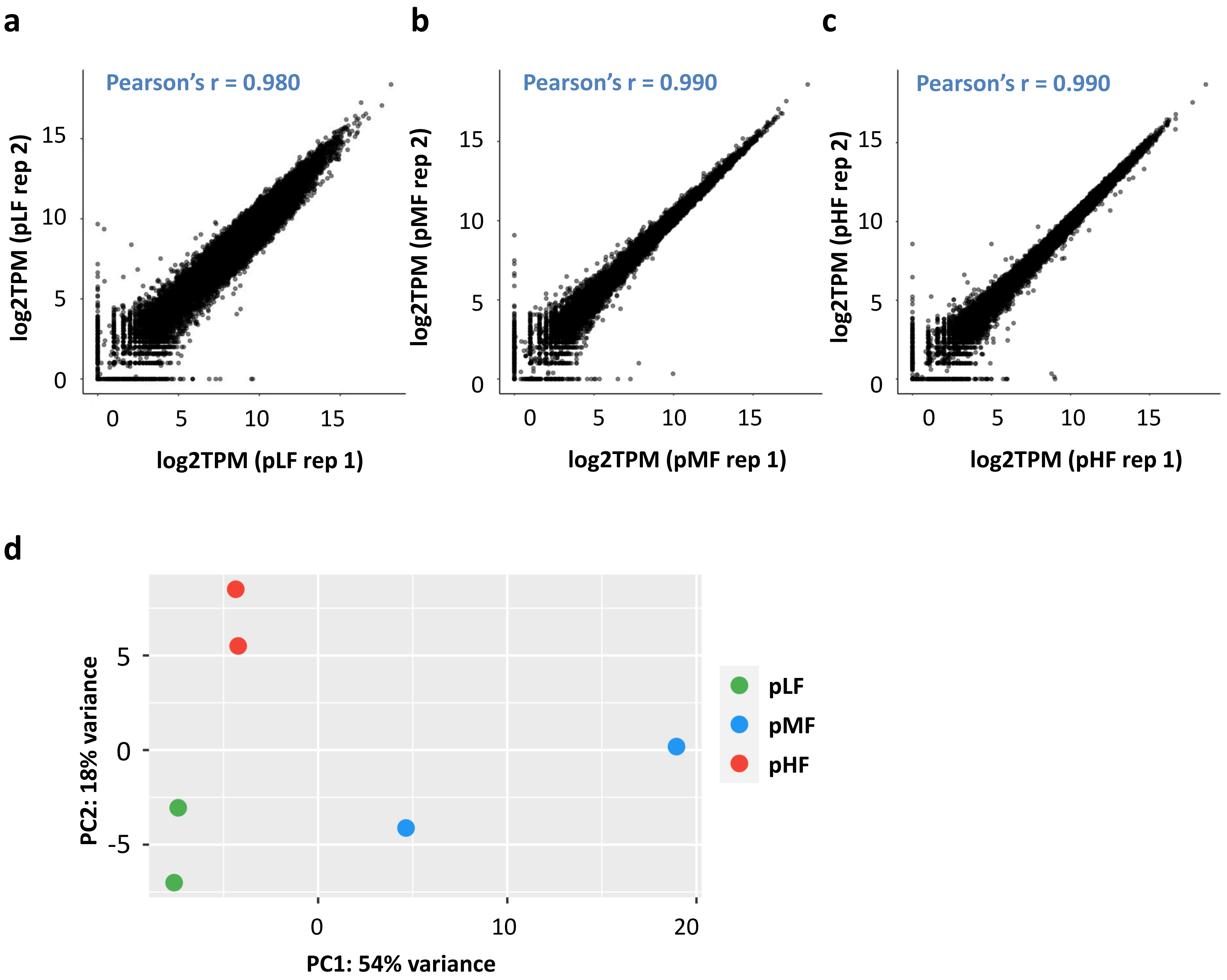


Fig. S4. Quality control of polysome mRNA-seq datasets. (a, b, c) Scatter plot showing the Pearson correlation between two biological replicates in polysome mRNA-seq datasets in NSCs. (d) Principal component analysis of polysome mRNA-seq datasets.

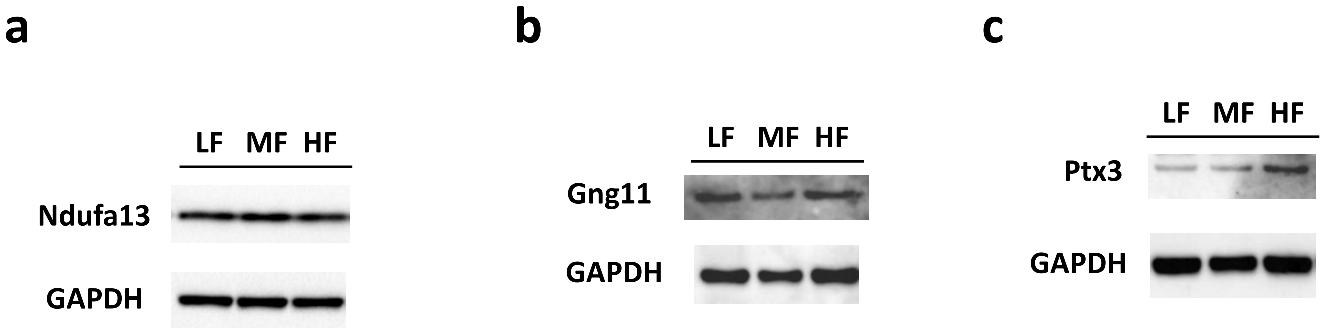


Fig. S5. Validation of differentially translated genes (DTGs). (a, b) Western blot was performed to validate the expression of DTGs identified by polysome profiling. Ndufa13 showed decreased translation efficiency in LF (a), Gng11 showed increased translation efficiency in LF (b), and Ptx3 showed increased translation efficiency in HF (c). Original blot images were provided in Additional file 8.

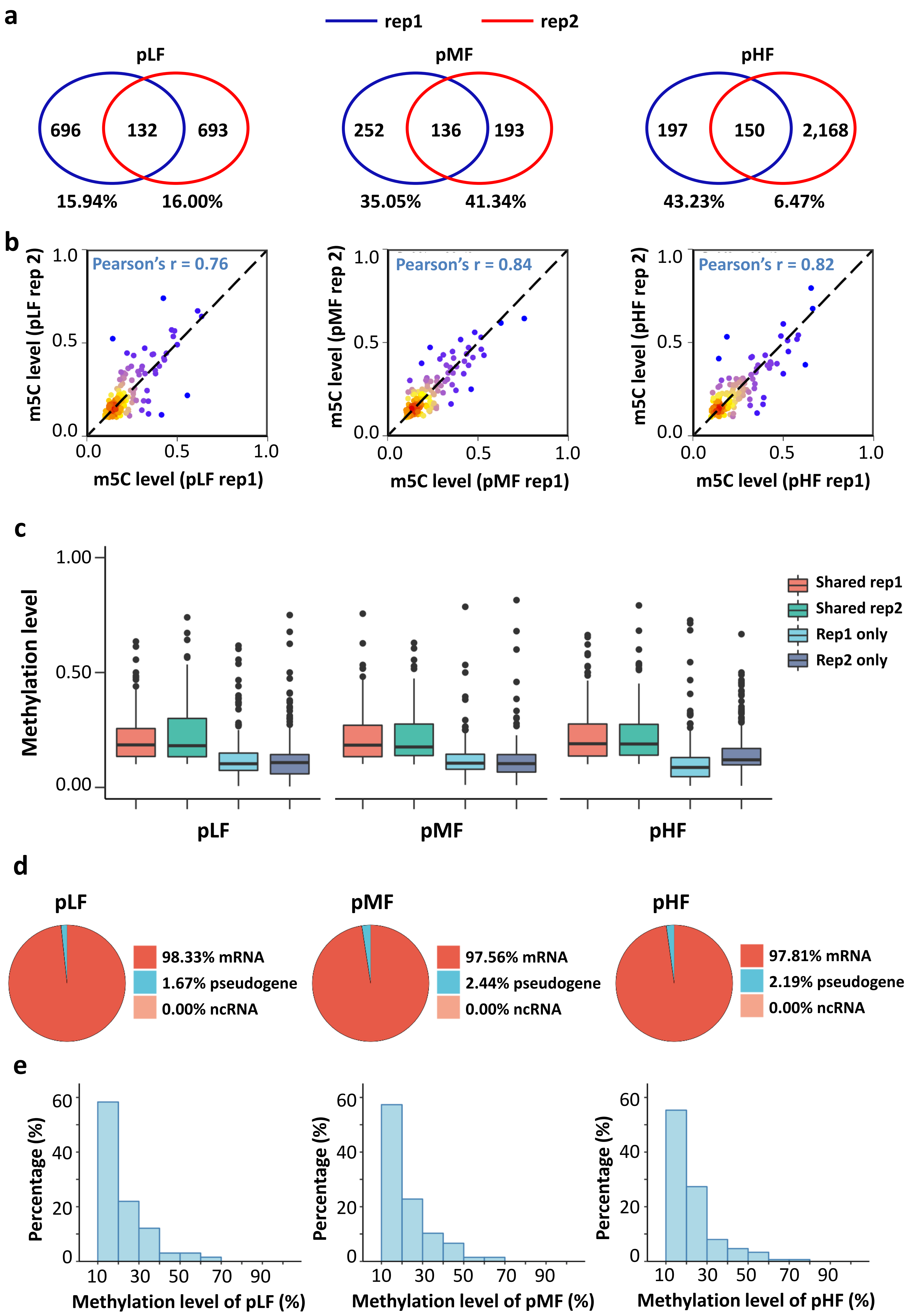

a
rep1
rep2
pLF
696
132
693
15.94%
16.00%
pMF
252
136
193
35.05%
41.34%
pHF
197
150
2,168
43.23%
6.47%
b
Pearson's r = 0.76
m5C level (pLF rep 2)
m5C level (pLF rep1)
Pearson's r = 0.84
m5C level (pMF rep 2)
m5C level (pMF rep1)
Pearson's r = 0.82
m5C level (pHF rep 2)
m5C level (pHF rep1)
0.0
0.5
1.0
c
Methylation level
1.00
0.50
0.00
Shared rep1
Shared rep2
Rep1 only
Rep2 only
pLF
pMF
pHF
d
pLF
98.33% mRNA
1.67% pseudogene
0.00% ncRNA
pMF
97.56% mRNA
2.44% pseudogene
0.00% ncRNA
pHF
97.81% mRNA
2.19% pseudogene
0.00% ncRNA
e
Percentage (%)
60
40
20
0
10
30
50
70
90
Methylation level of pLF (%)
Methylation level of pMF (%)
Methylation level of pHF (%)

Fig. S6. Characterization of m^{5}C sites in polysome mRNA BS-seq datasets. (a) Venn diagram showing the overlap of m^{5}C sites between two replicates. (b) Dot plot showing the methylation level correlation of the overlapped m^{5}C sites between two replicates. (c) Box plot showing the methylation level of overlapped and non-overlapped m^{5}C sites in the two replicates. (d) Pie chart showing the categories of RNA molecules with m^{5}C modification. (e) Histogram showing the distribution of methylation level for each condition.

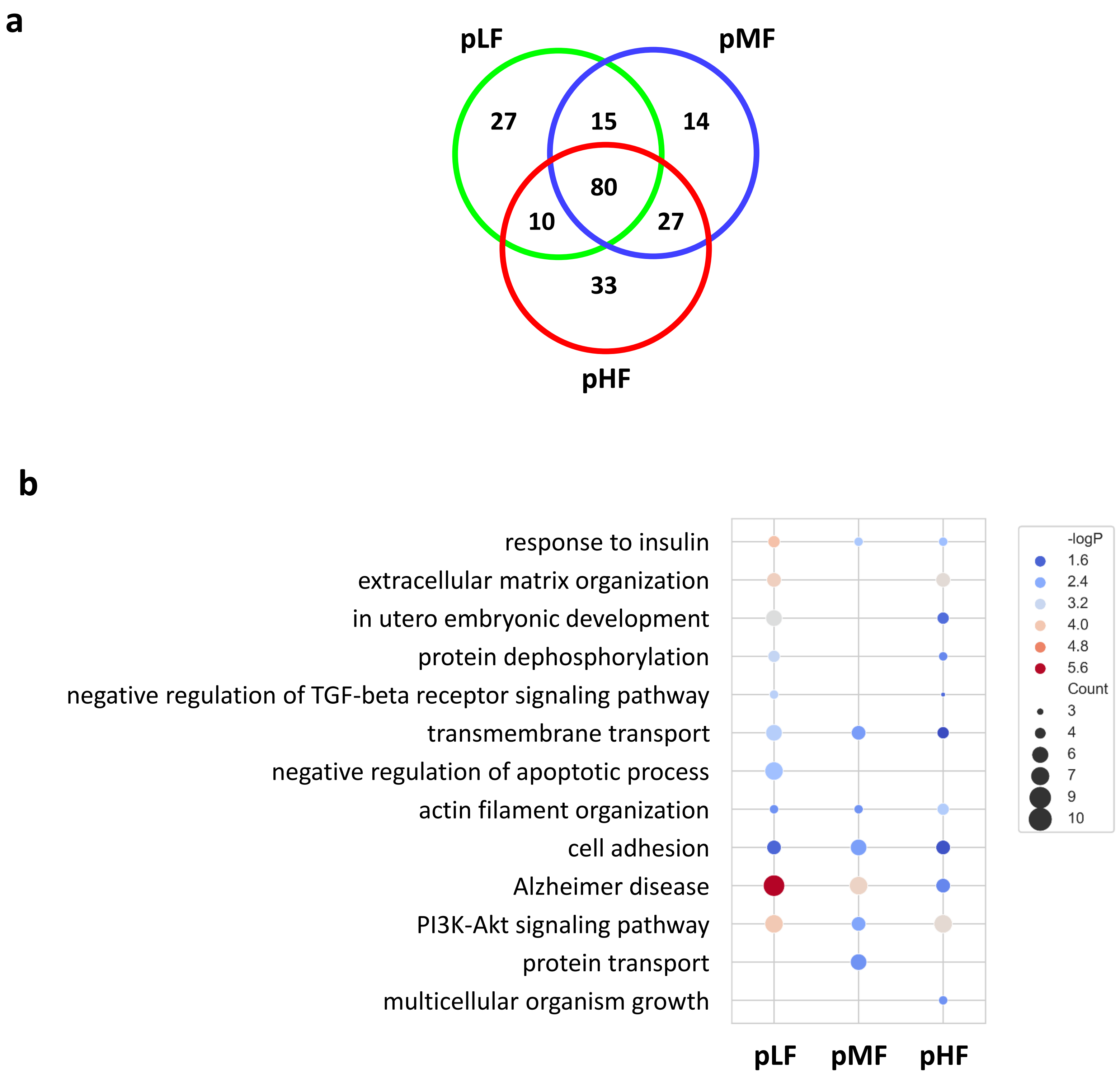


Fig. S7. Functional annotation of m^{5}C-modified mRNAs in polysome mRNAs. (a) Venn diagram showing the overlap of m^{5}C sites among the three conditions (pLF, pMF, pHF) (b) Bubble plot showing the GO terms of m^{5}C-modified mRNAs in pLF, pMF and pHF.

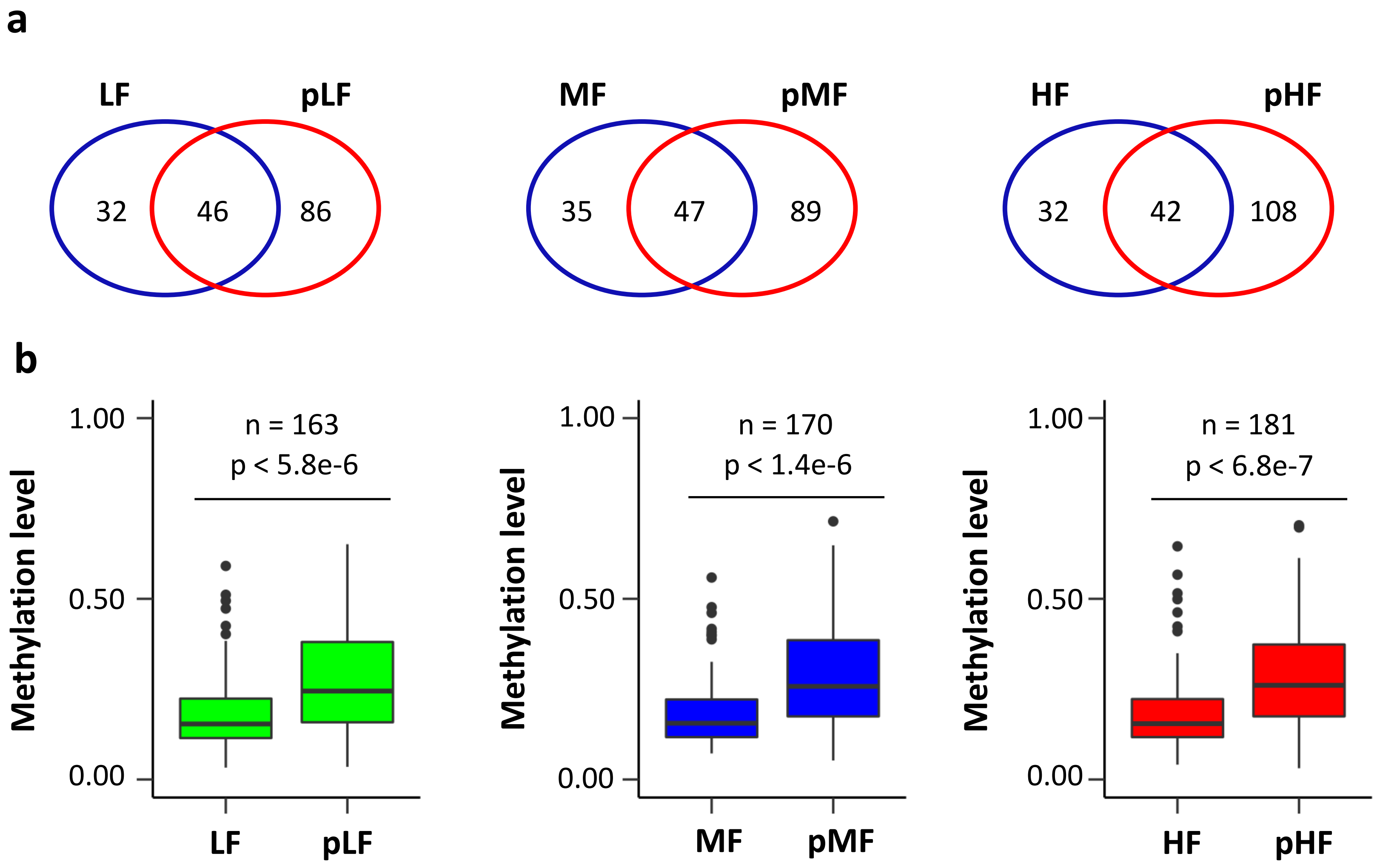
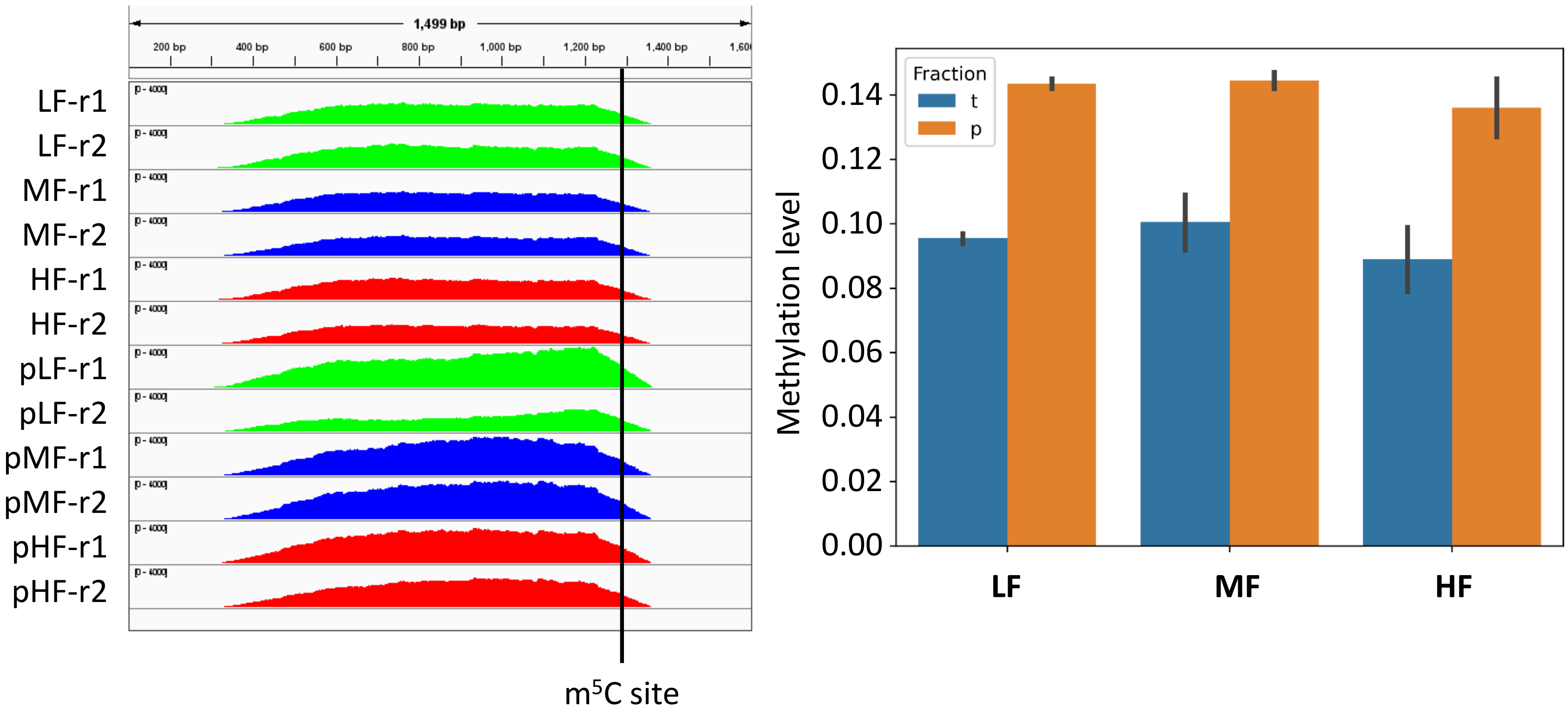


Fig. S8. Comparison between total and polysome methylomes. (a) Venn diagram showing the overlap of m^5C sites between total and polysome mRNAs. (b) Box plot showing the methylation level of the union of m^5C sites between total and polysome mRNAs with 20x coverage in both samples. A Wilcoxon signed-rank test was performed.

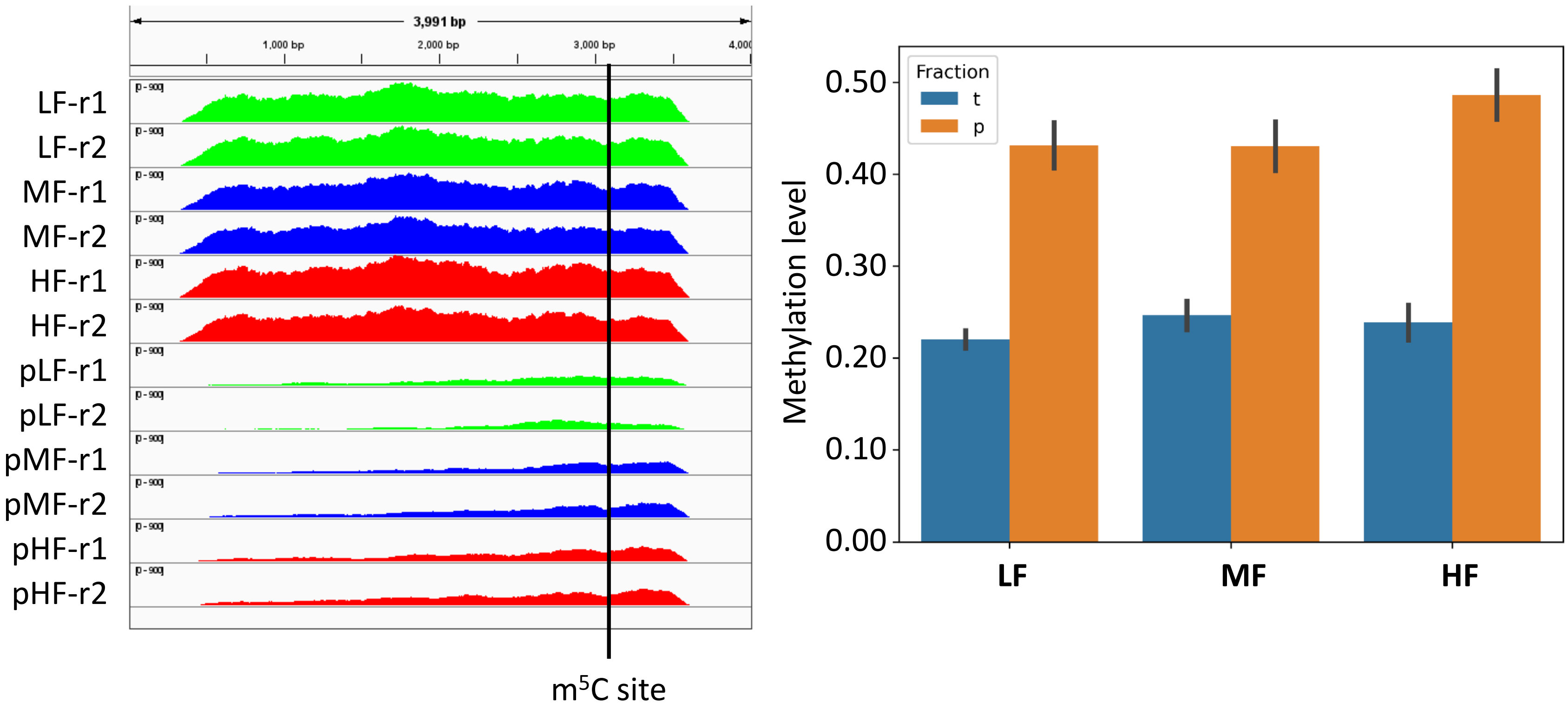
a

Hdgf m^{5}C site (positive correlation)
Chr3:87914991



b

Glg1 m^{5}C site (negative correlation)
Chr8:111155350



c

Dgcr2 m^{5}C site (neutral correlation)
Chr16:17841431

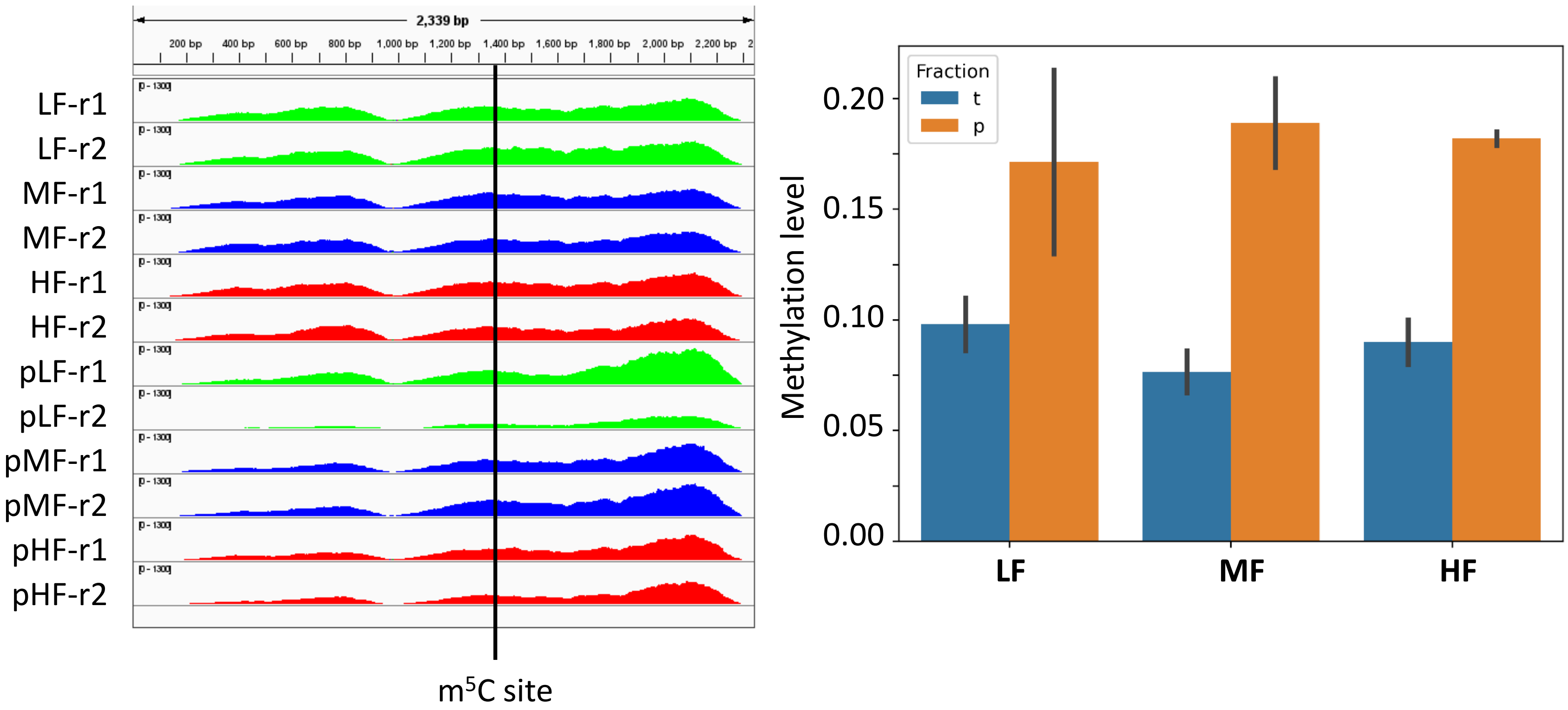


Fig. S9. Visualization of the correlation between m^{5}C methylation and mRNA translation. (a, b, c) Left panel depicts transcript-level read pileup of the mRNAs: Hdgf (a), Glg1 (b), and Dgcr2 (c) using the IGV browser. The total and polsyome mRNA-seq datasets were shown (green – LF, blue – MF, red –HF). Right panel depicted the methylation level of the m^{5}C site identified in the respective transcript in both total and polysome mRNAs (t: total, p: polysome).