

Role of UPF1-LIN28A interaction during early differentiation of pluripotent stem cells

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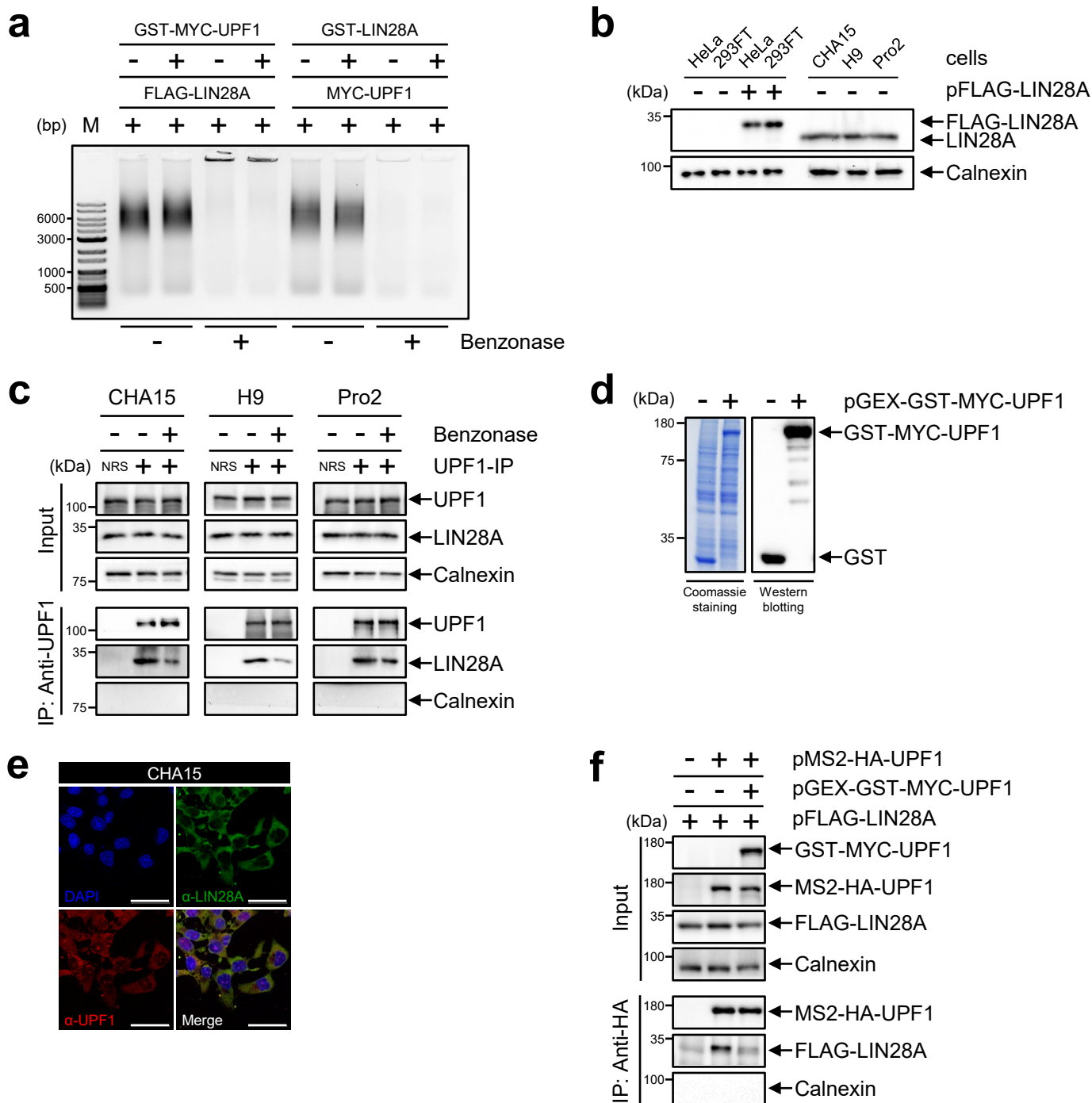
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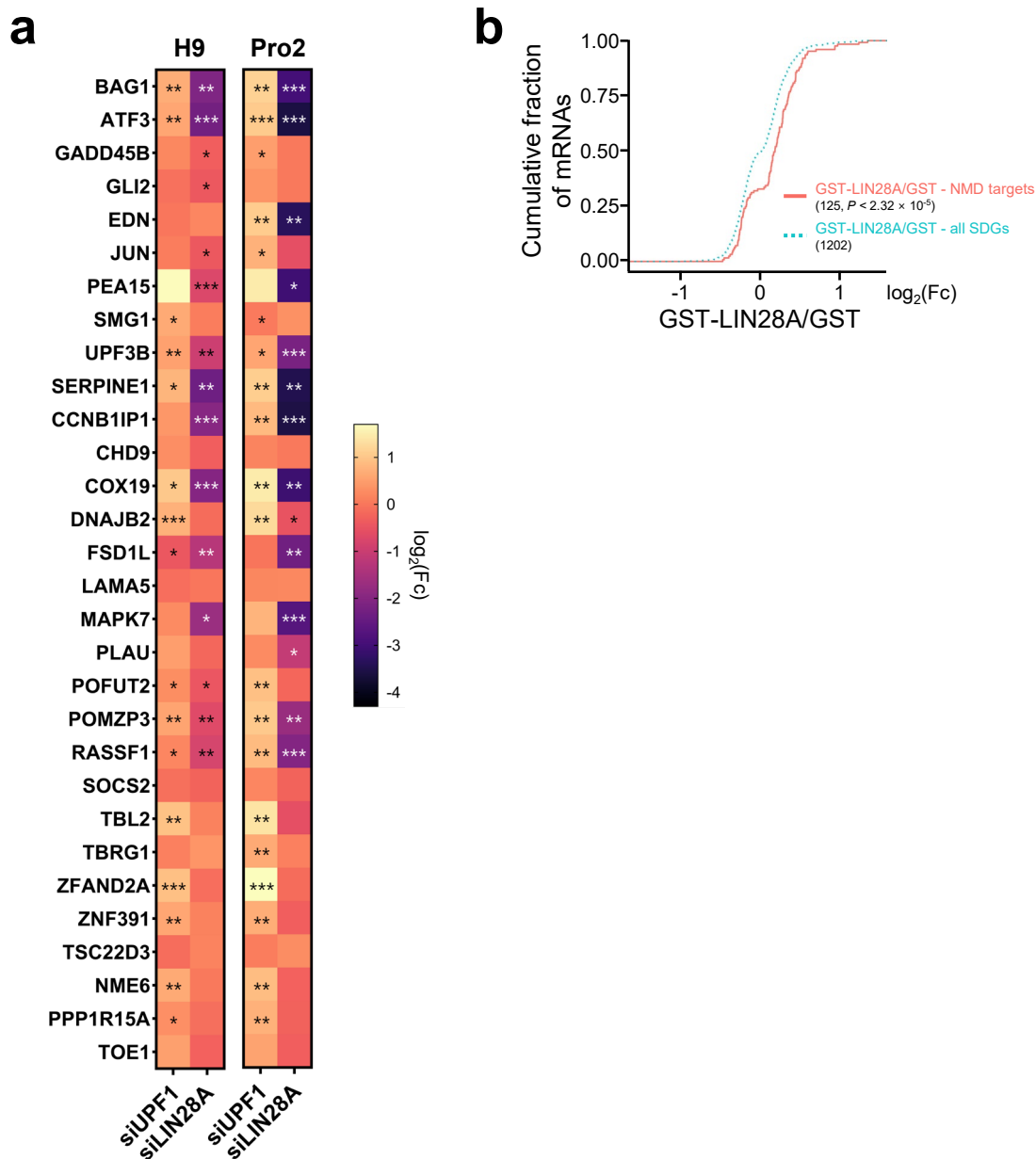
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- Supplementary Figure 1-7

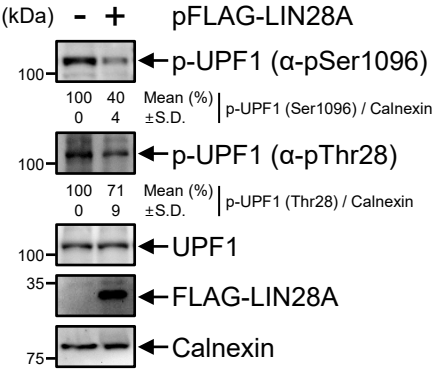


Supplementary Fig. 1 **a** To determine whether the nuclease (benzonase) completely removed the DNA and RNA, 293T cell lysates that were co-transfected with the indicated plasmid DNA were treated with nuclease and loaded onto an agarose gel. The DNA and RNA were visualised using EtBr staining. **b** The levels of overexpressing LIN28A in HeLa and 293T cells by transfection of FLAG-LIN28A were confirmed by WB to be comparable with the endogenous LIN28A in hPSCs. **c** hPSCs lysates were used for IP using UPF1-antibody in the presence or absence of nuclease (benzonase). **d** Cell lysates expressing GST-MYC-UPF1, or GST before GST pull-down were observed using Coomassie staining and WB. **e** Endogenous UPF1 (red), LIN28A (green), and nucleus (blue, DAPI) in CHA15 cells were visualised using confocal microscopy. Scale bars, 50 μ m. **f** 293T cell lysates that were transfected with pFLAG-LIN28A and pMS2-HA-UPF1 or pGEX-GST-MYC-UPF1 were employed for IP using anti-HA antibody. WB was performed to detect coimmunoprecipitated FLAG-LIN28A with HA-UPF1. The minimum number of independent biological replicate experiments was **a-f** $n = 3$. The experiments were conducted three times, each iteration producing consistent results.

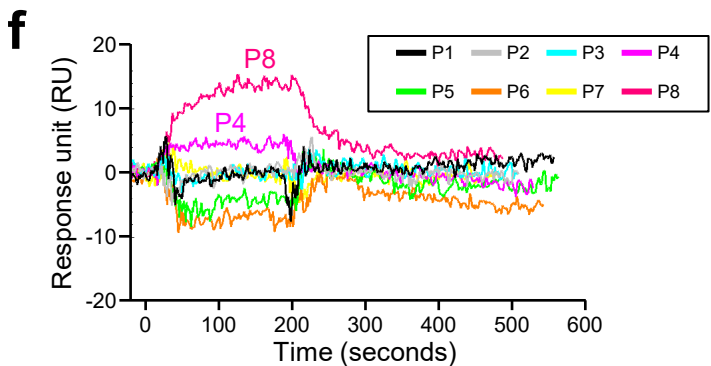
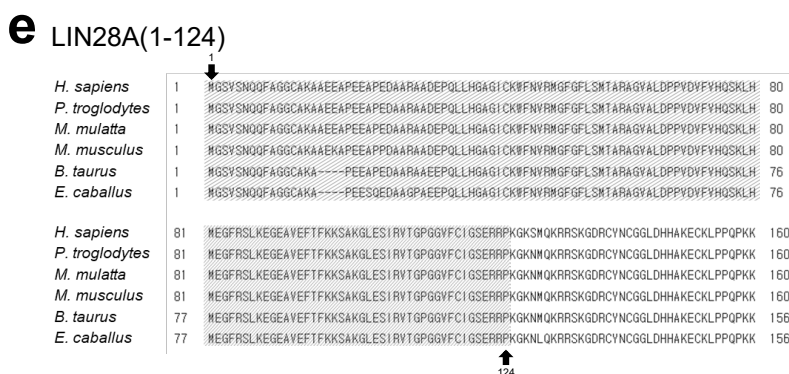
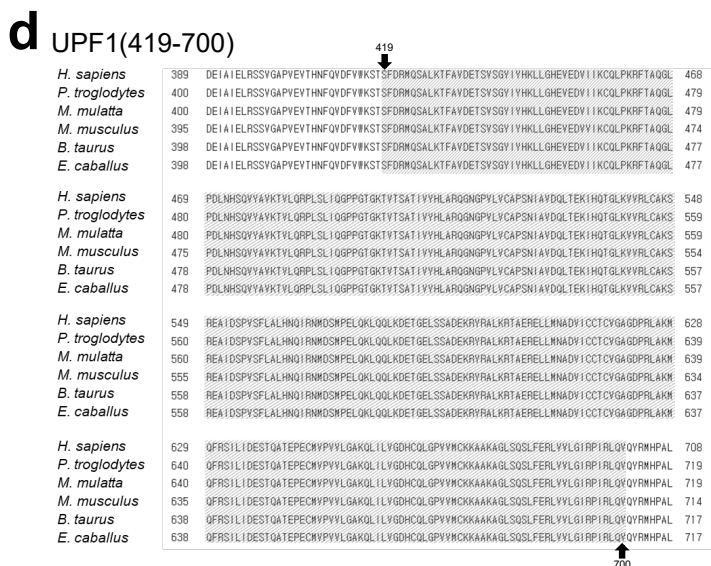
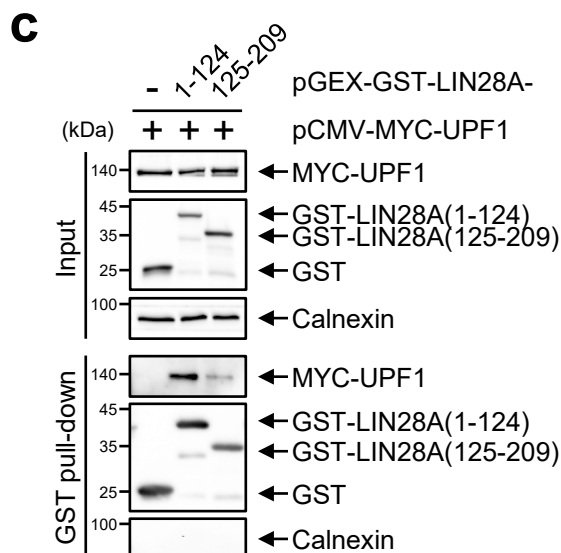
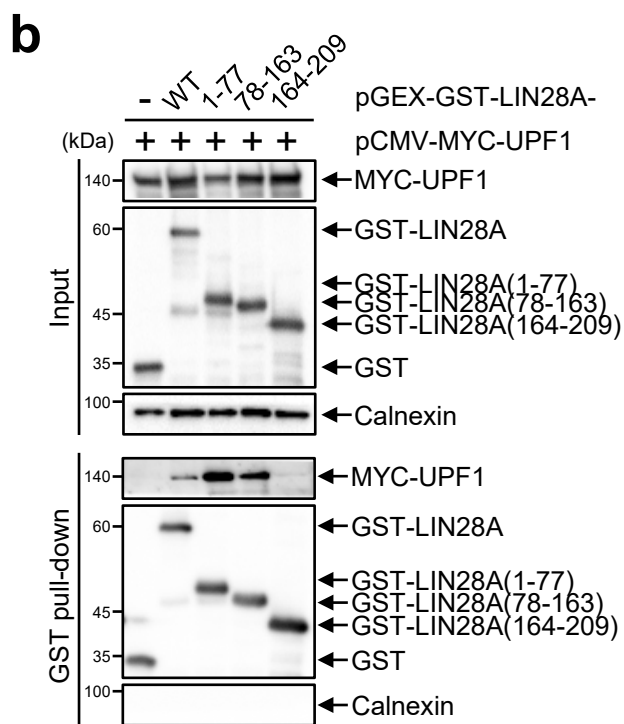
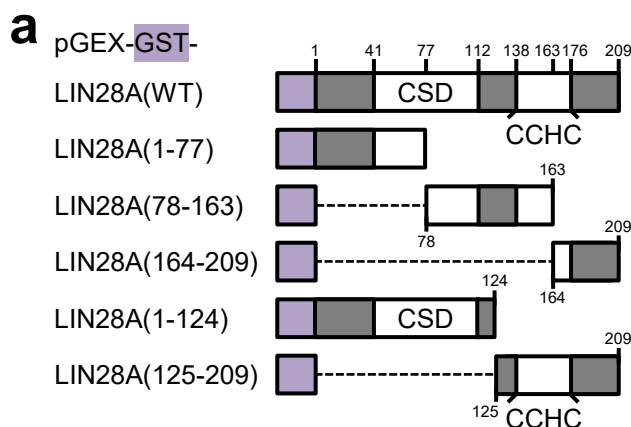


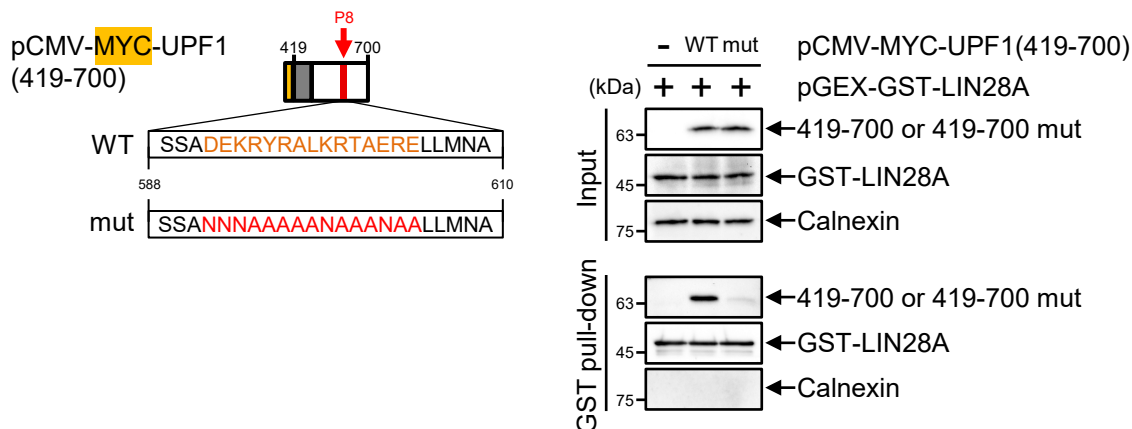
Supplementary Fig. 2 a Relative levels of NMD targets by RT-qPCR were presented as heat-maps upon depletion of UPF1 or LIN28A in H9 and Pro2. **b** RNA-seqs using GST-LIN28A- or GST-overexpressing HeLa cells were performed. Cumulative fractions of $\log_2$ changes in the expression of GST-LIN28A against GST were shown as CDF graphs using significantly changed 125 NMD targets and SDGs ($p < 0.05$). mRNA level was normalised to that of *U6* mRNA in **a**. Unpaired Student's t-test was used for **a**. K-S tests were used for **b**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact p-values were provided in Source data. Data are presented as mean values $\pm$ SEM. All statistical test used were two-sided. The minimum number of independent biological replicate experiments was **a** $n = 3$, **b** $n = 2$.

a

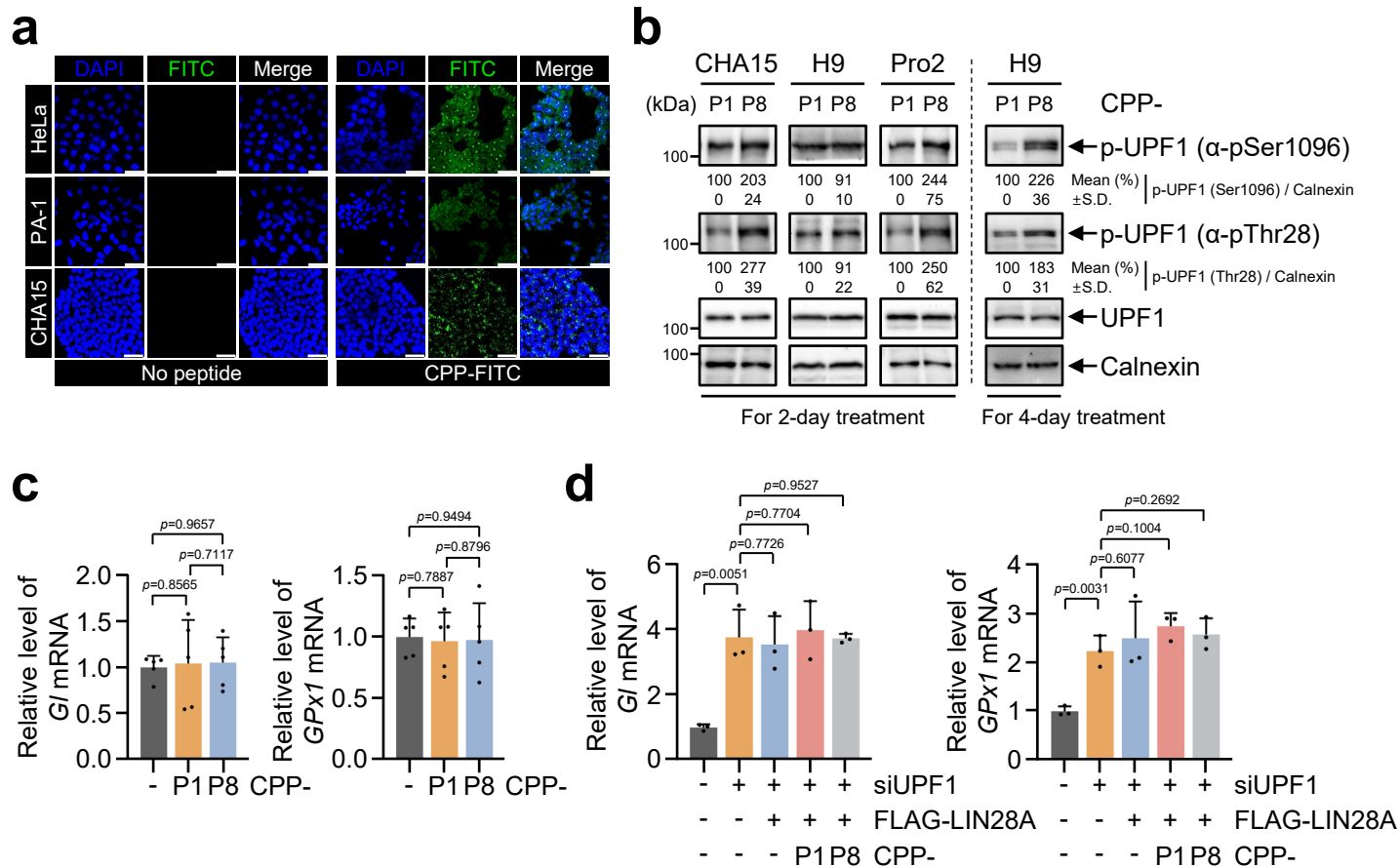


Supplementary Fig. 3 a HeLa cells were transfected with pFLAG-LIN28A or pFLAG. WB was performed to detect phosphorylated UPF1 at serine 1096 or threonine 28. Data are presented as mean values $\pm$ S.D. All statistical test used were two-sided. The minimum number of independent biological replicate experiments was **a** $n = 3$. The experiments were conducted three times, each iteration producing consistent results.

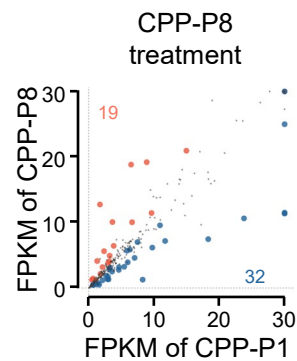
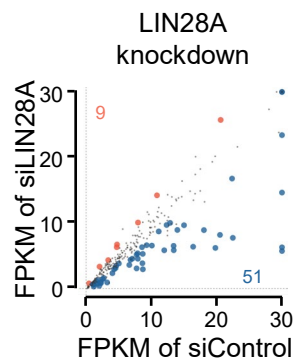
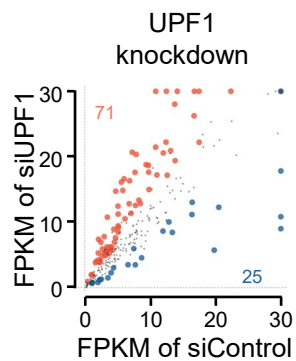
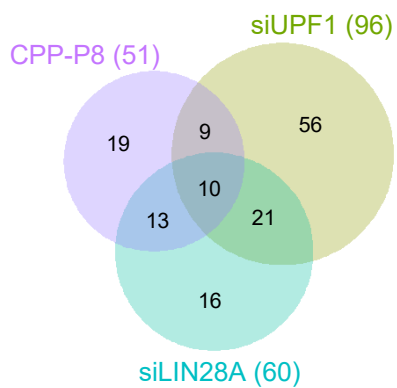
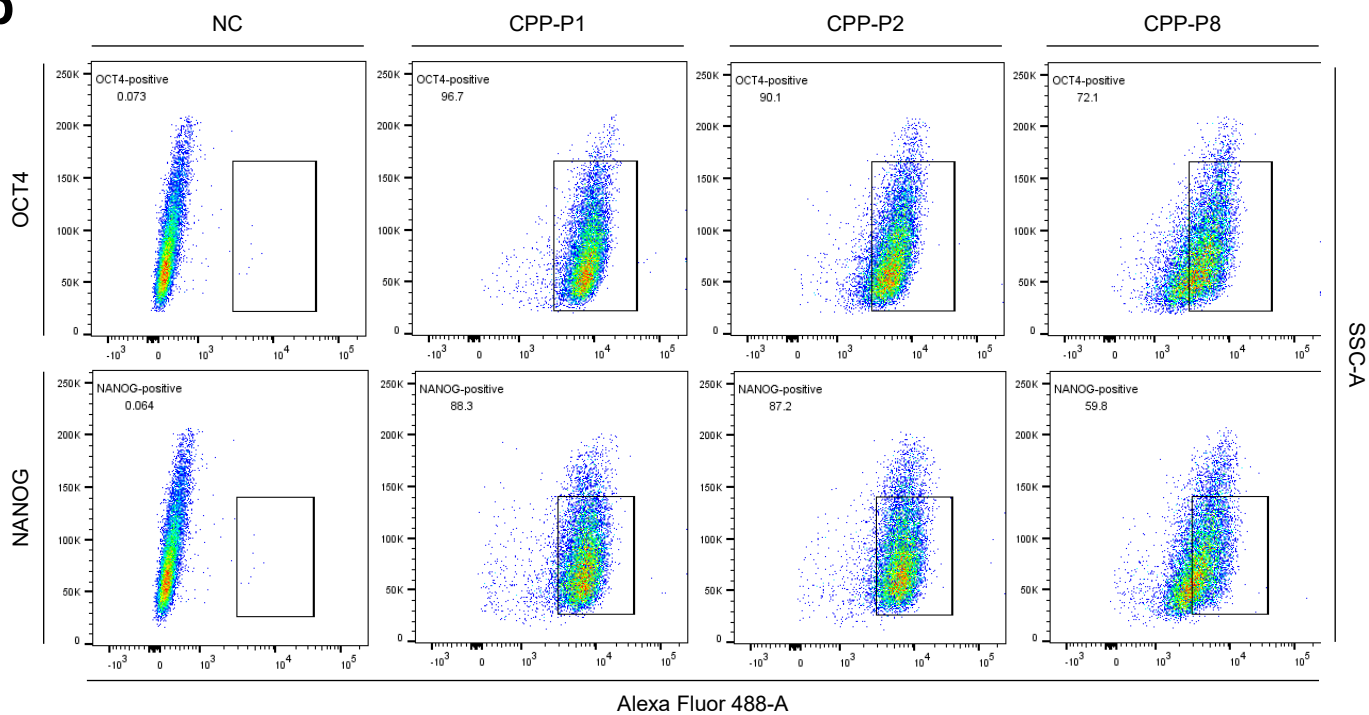


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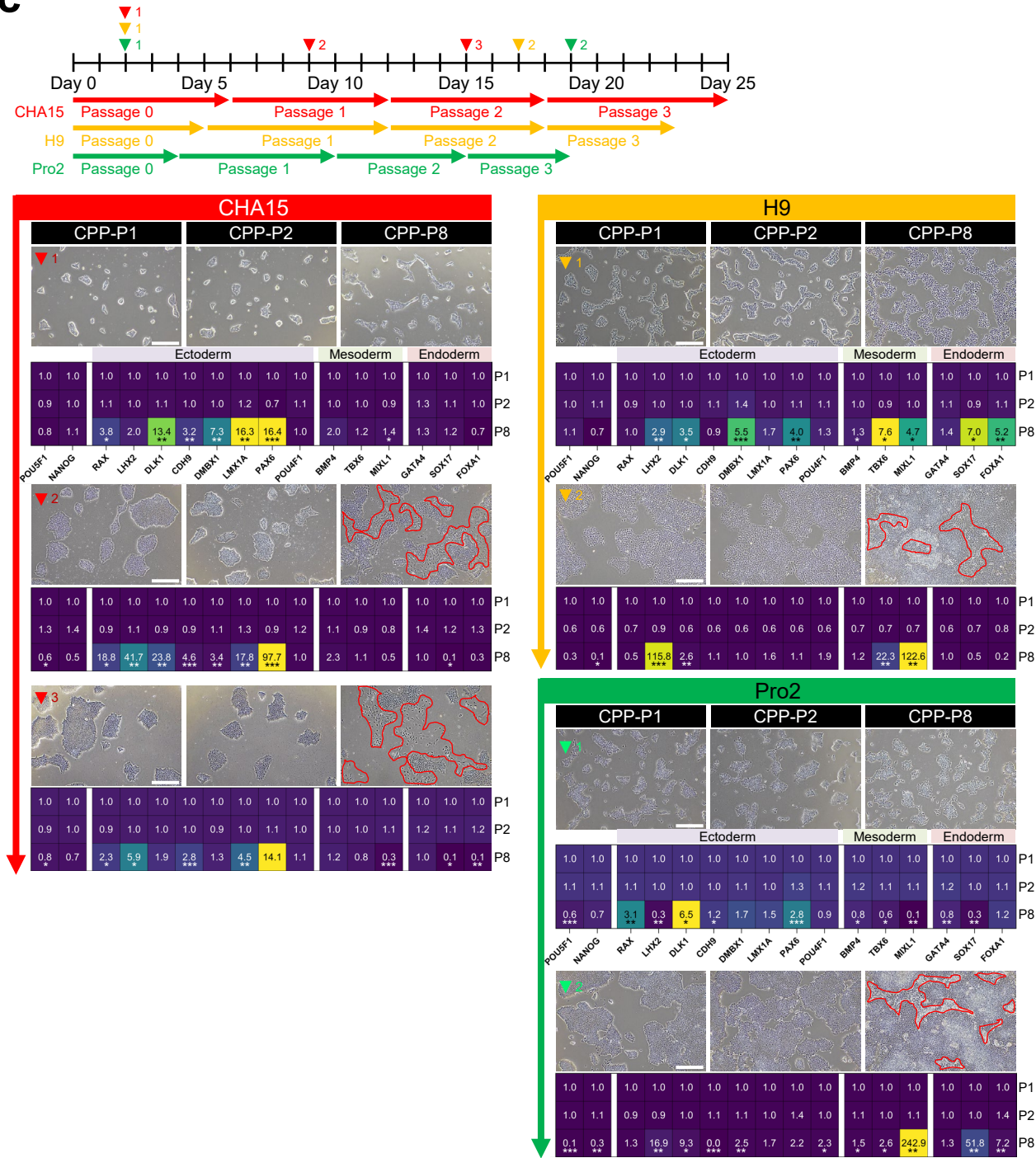
Supplementary Fig. 4 a Schematic representation of GST-tagged LIN28A variants. LIN28A is composed of two domains: cold shock domain (CSD) and Cys-Cys-His-Cys (CCHC)¹. **b** 293T cell lysates that were transfected with pGEX-GST-LIN28A variants (WT, 1-77, 78-163, and 164-209) and pCMV-MYC-UPF1 were employed for GST pull-down. WB was performed to evaluate the expression levels of LIN28A variants and eluates. **c** Same as in (**b**); however, 293T cell lysates were transfected with pGEX-GST-LIN28A variants (1-124 and 125-209). **d–e** Conserved amino acid sequences of putative interacting domain in UPF1 (**d**) and LIN28A (**e**). **f** SPR analysis using the eight peptide analytes as depicted in Fig. 4c, where His-LIN28A protein was used as the ligand. **g** Schematic representation of MYC-UPF1(419-700) and mutation (mut). The fifteen amino acids (red) in the P8 region were substituted with asparagine (N) or alanine (A). 293T cell lysates that were transfected with pGEX-GST-LIN28A and pCMV-MYC-UPF1(419-700) variants (WT or mut) were used for GST pull-down in the presence of nuclease. WB was performed to evaluate the expression levels of UPF1(419-700) variants and eluates. The minimum number of independent biological replicate experiments was **b**, **c**, and **g** $n = 3$. The experiments were conducted three times, each iteration producing consistent results.

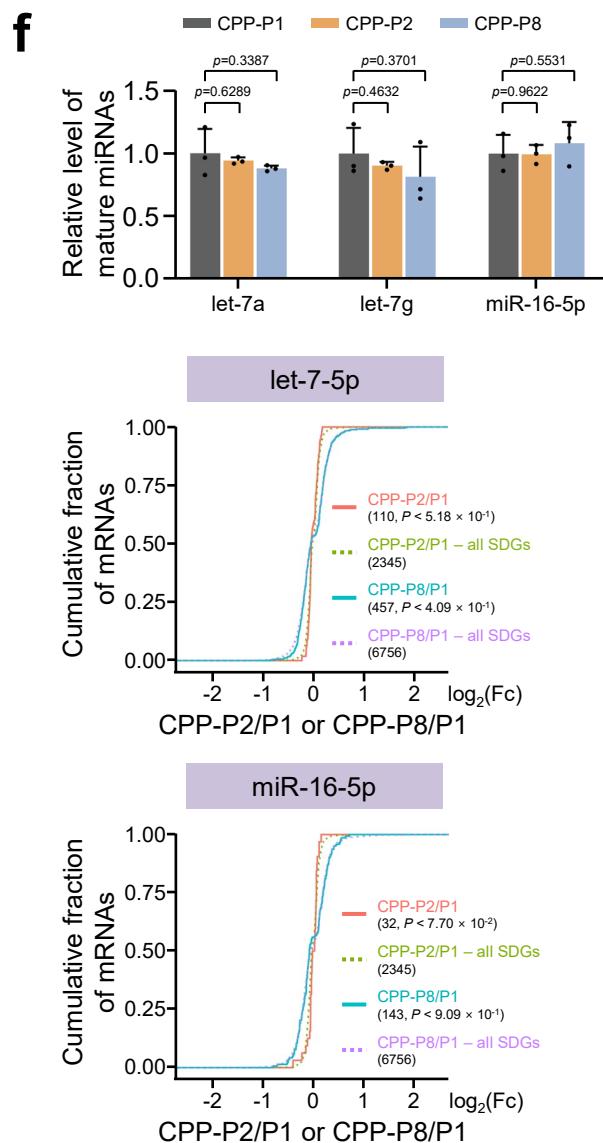
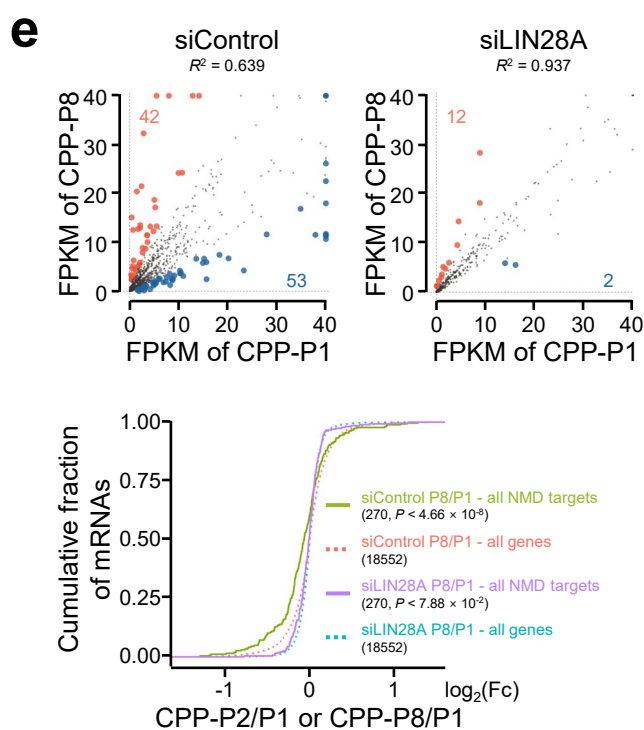
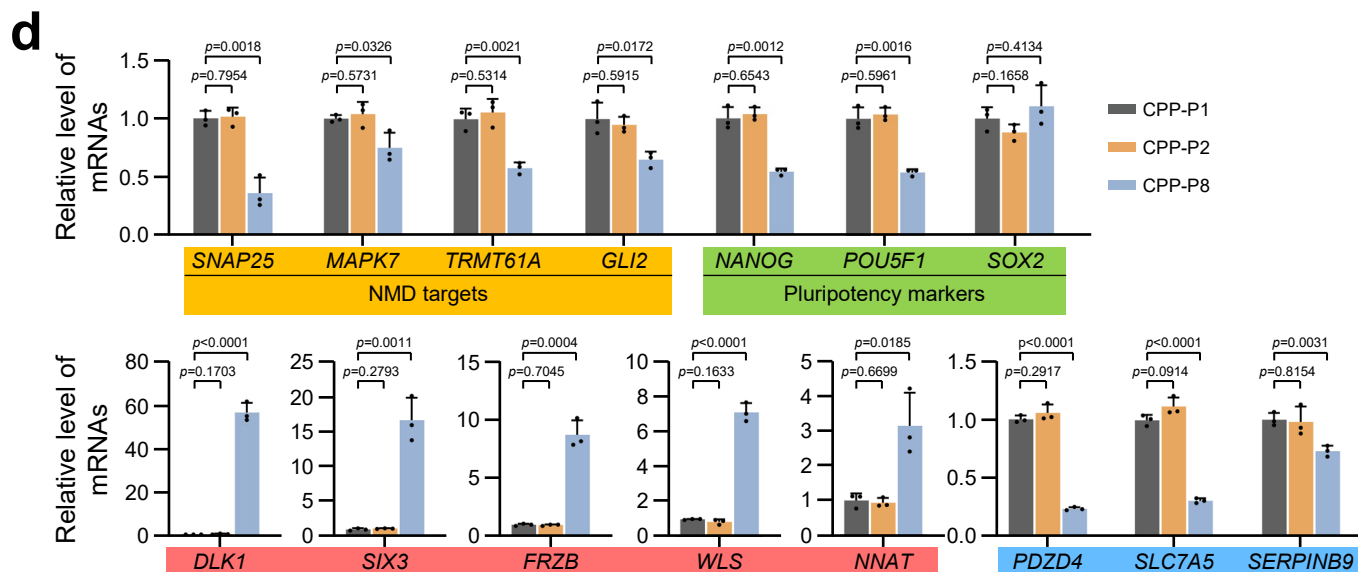


Supplementary Fig. 5 **a** HeLa, PA-1, and CHA15 were treated with CPP-FITC. FITC and DAPI (nucleus) were visualised using confocal microscopy. Scale bars, 50 μ m. **b** hPSCs lysates that were treated with CPP-P1 or -P8 were used for WB to detect the levels of p-UPF1. **c** HeLa cells where LIN28A was not expressed were transfected with NMD reporter plasmids and incubated with 2 μ M of the indicated CPP-conjugated peptides. RT-qPCR was performed to measure NMD efficiency. **d** Same as (c); however, the UPF1-depleted condition was established by siRNA in the presence of FLAG-LIN28A in HeLa cells. mRNA level was normalised to that of *MUP* mRNA in (c and d). Unpaired Student's t-test was used. Data are presented as mean values $\pm$ S.D (b) and mean values $\pm$ SEM (c and d). All statistical test used were two-sided. The minimum number of independent biological replicate experiments was **a**, **b**, and **d** $n = 3$, **c** $n = 5$. The experiments were conducted three times, each iteration producing consistent results.

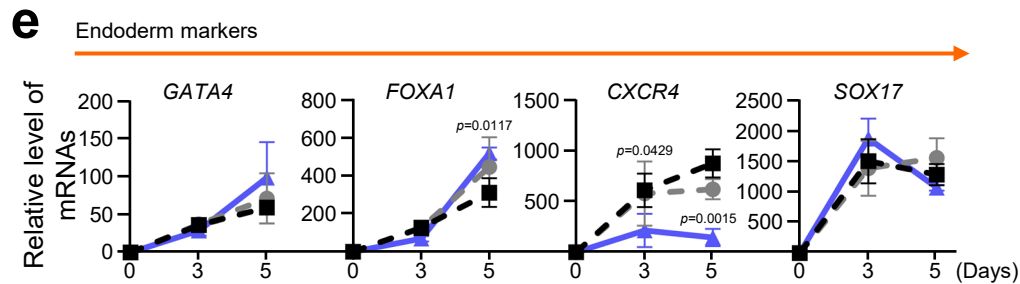
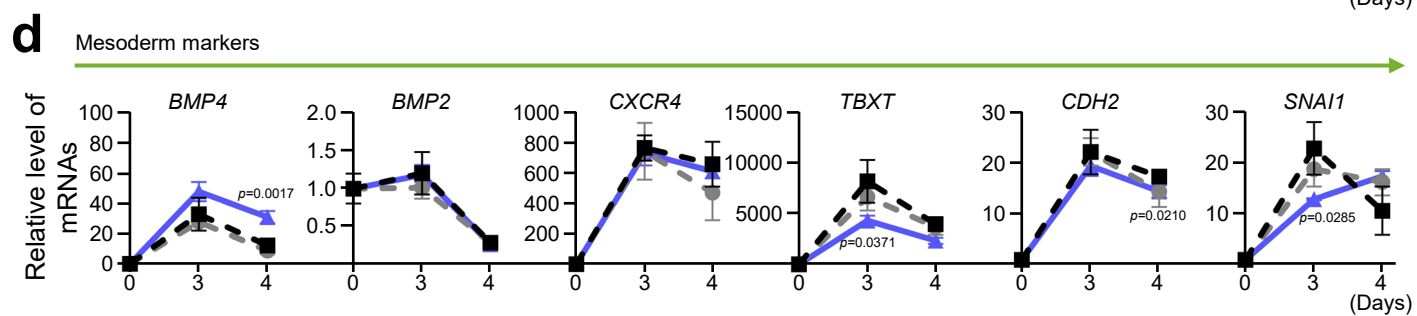
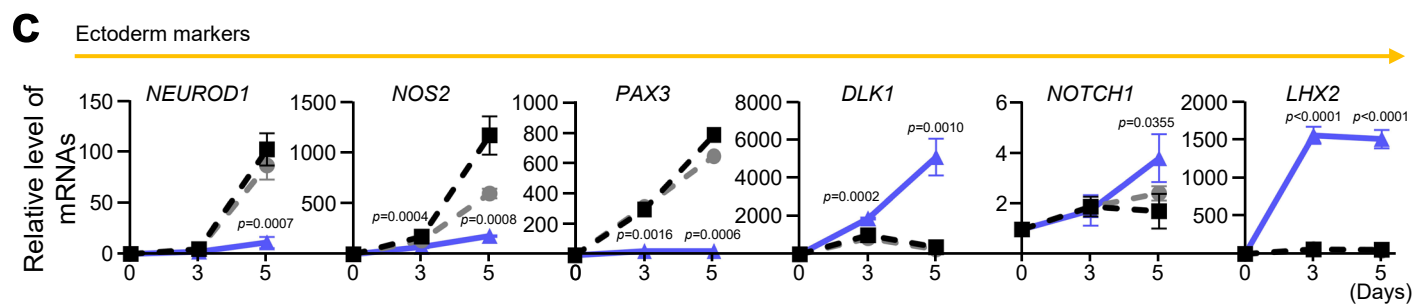
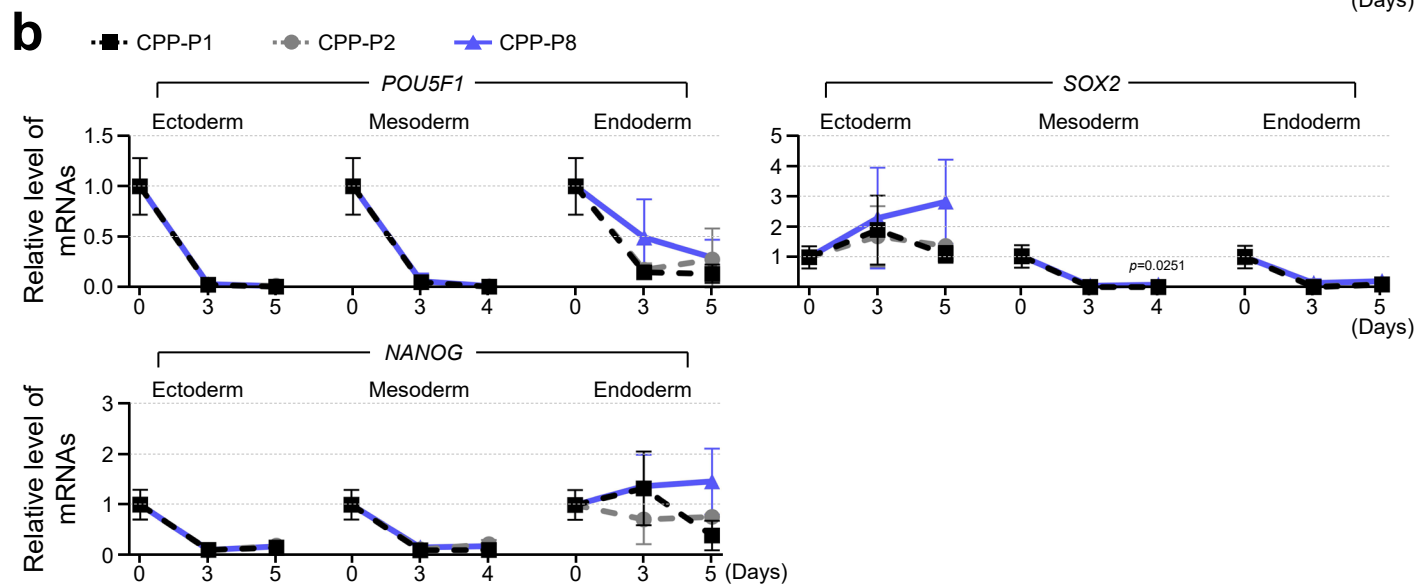
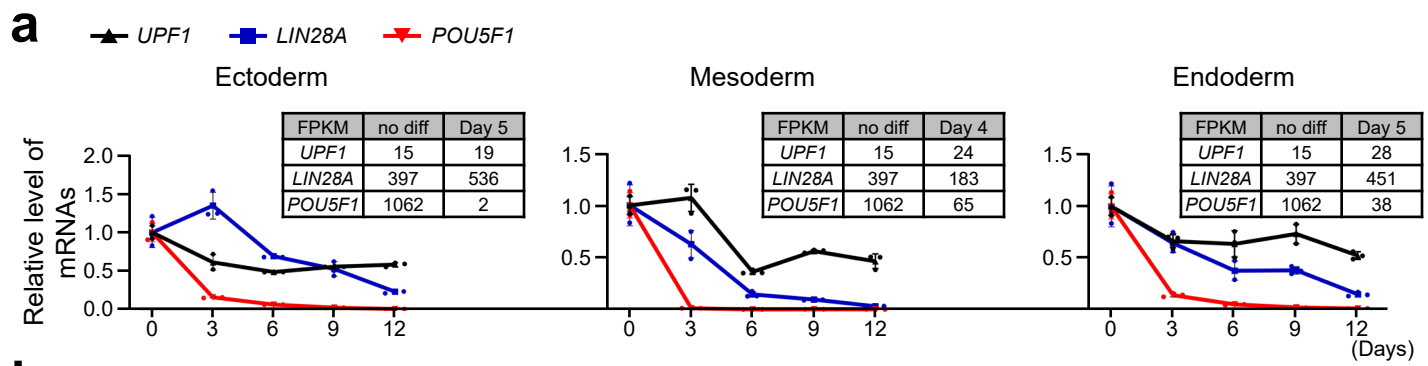
a**b**

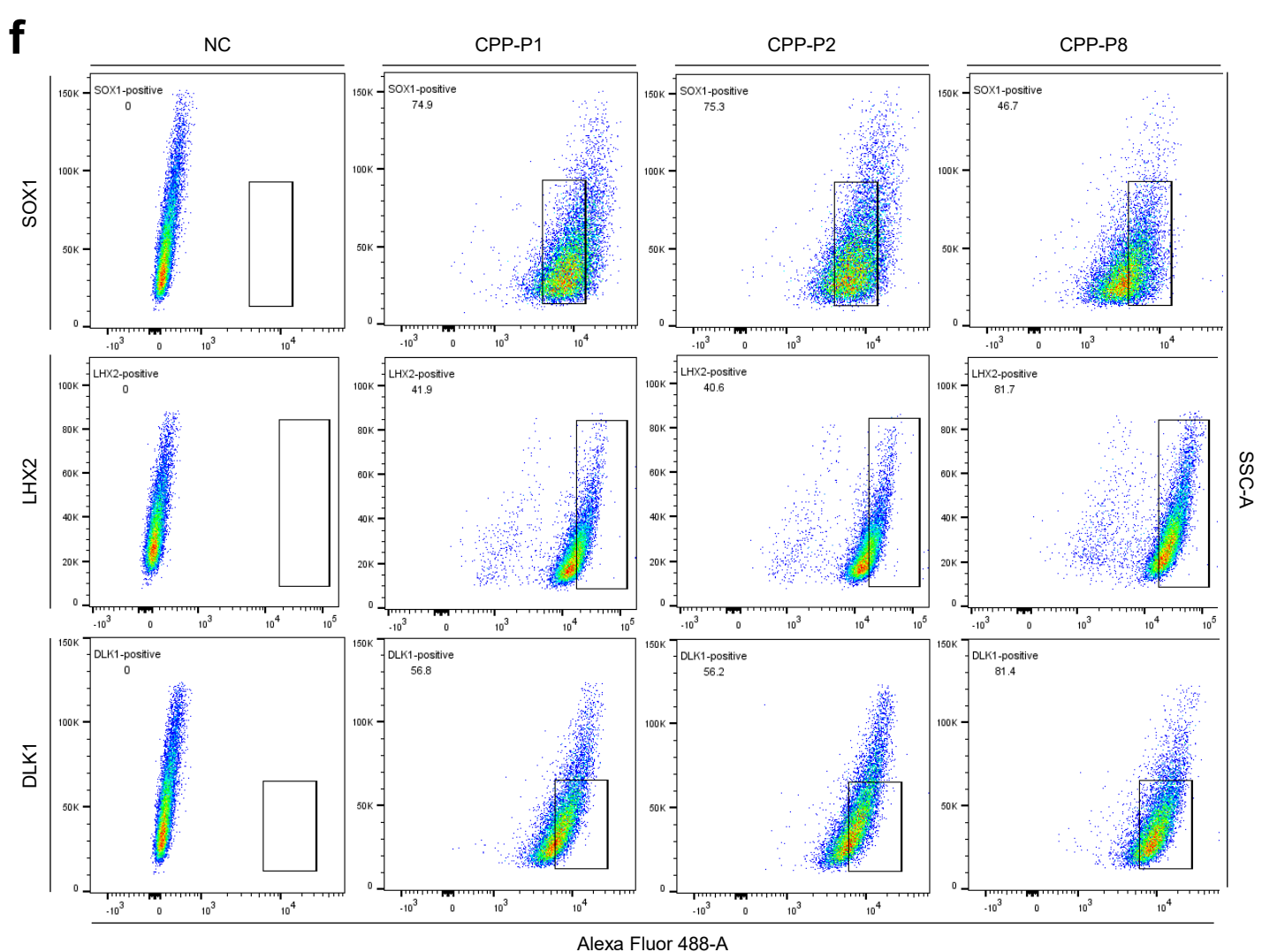
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Supplementary Fig. 6 a Scatter plots and Venn diagram shows the number of NMD targets that were significantly changed over 1.2-fold upon UPF1-depletion, LIN28A-depletion, or CPP-P8-treatment in CHA15 cells. **b** (Corresponding to Fig. 6c) Flow cytometry analysis was performed to determine the levels of OCT4 and NANOG in CPP-P1, -P2, or -P8-treated CHA15 cells. The primary antibody that detected the cellular proteins was labelled with the Alexa Fluor 488-conjugated antibody. The relative positive cells were indicated. **c** The schematic representation shows proliferation and passages day point of hPSCs. hPSCs that were treated with CPP-P1, -P2, or -P8 were expanded for the indicated day point with passages. Cell morphology was visualised by phase contrast microscopy. The red boundary indicated the spontaneously differentiated hPSCs. RT-qPCR was performed to measure the relative levels of each lineage marker. Scale bars, 100 μm . **d** (Corresponding to Fig. 6d) RT-qPCR was performed to evaluate the level of indicated transcripts upon the indicated peptide treatment in CHA15 cells. **e** Transcriptome analysis was performed using significantly changed transcripts ($p < 0.05$) from siLIN28A or siControl in the indicated CPP-peptide treated CHA15 cells. Scatter plots and CDFs employed SDGs over a 2.0-fold change (upper panel) and significantly changed the NMD targets (bottom panel), respectively. **f** Levels of mature endogenous miRNAs in CHA15 cells, which were regulated by LIN28A, were assessed using RT-qPCR. Cumulative fraction of $\log_2$ fold change in the expression of genes regulated by let-7-5p and miR-16-5p was shown as CDF graphs. The relative level of mRNA was normalised to that of *U6* snRNA in (**c**, **d**, and **f**). K-S tests were used for **e** and **f**. Unpaired Student's t-test was used for **c**, **d**, and **f**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact p -values were provided in Source data. Data are presented as mean values $\pm$ SEM. All statistical test used were two-sided. The minimum number of independent biological replicate experiments was **a** and **e** $n = 2$, **b–d** and **f** $n = 3$. The experiments were conducted three times, each iteration producing consistent results.





Supplementary Fig. 7 **a** Levels of UPF1, LIN28A, and POU5F1 in hPSCs were assessed using RT-qPCR during each germ layer differentiation for 12 days. FPKM of each gene was presented from RNA-seq analysis. **b–e** hPSCs were treated with the indicated peptide during each germ layer differentiation. Relative amounts of pluripotency markers (*POU5F1*, *SOX2*, and *NANOG*) and three germ layers markers were measured using RT-qPCR. mRNA levels were normalised to those of *U6* snRNA in **(a–e)**. **f** (Corresponding to Fig. 7b) Flow cytometry analysis was performed to detect the levels of OCT4 and NANOG in CPP-P1 or -P8-treated CHA15 cells. The primary antibody that detected the cellular proteins was labelled with the Alexa Fluor 488-conjugated antibody. The relative positive cells were indicated. An unpaired Student's t-test was used for **a–e**. Data are presented as mean values $\pm$ SEM. All statistical test used were two-sided. The minimum number of independent biological replicate experiments was **a–f** $n = 3$. The experiments were conducted three times, each iteration producing consistent results.

Reference

1. Heo, I. *et al.* TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. *Cell* **138**, 696-708 (2009).