**Supplementary Figure 1: Comparison of candidates identified by previous global transcriptome analyses after UPF1 depletion in HeLa cells.** (A) Heatmap comparing the fold-change of expression of all 118 UPF1 targets on protein (pSILAC) or RNA level (5, 7, 8, 10) that have been identified by pSILAC and at least one RNA screen. (B-E) Correlation analyses between RNA screens (5, 7, 8, 10). The degree of correlation does not depend on the type of RNA analysis. R = Pearson correlation coefficient.

Supplementary Figure 2: mRNAs that direct increased protein synthesis following UPF1 depletion are not enriched for those with uORFs or 3'UTR introns. (A) The principal transcripts of the 30 most up- or downregulated and the 30 least regulated genes from the group of significant UPF1 targets were analyzed for the presence of uORFs (green) and introns in their 3'UTR (blue). Sequence information was taken from the Ensembl database. No significant differences could be detected between up- and downregulated genes. (B) Same analysis as in (A) with 28 most up and downregulated from the list of targets that overlap between pSILAC screen and RNA-seq (8). mRNA equivalents of only 28 downregulated proteins were found in the RNAseq, hence also the 28 most upregulated transcripts were used for the comparison. Fisher exact test was applied for statistical analysis.

**Supplementary Figure 3: Low dosage of DTT causes downregulation of NMD factors.** Protein synthesis of the NMD factors UPF1, UPF2, UPF3B, and SMG8 and the EJC component Y14 is downregulated by DTT. Bars represent the average fold change of expression of two independent biological replicates, +/- SD.

## Supplementary Figure 1:







C microarray Viegas et al. (12) vs. microarray Yepiskoposyan et al. (14)



microarray Viegas et al. (12) vs. RNAseq Tani et al. (11) 4.0 R=0.88 3.0 p=3.88E-20 .... . 2.0 log2-fold change Tani siUPF1 RNAseq . 1.0 0.0 -3.0 -2.0 -1.0 -1.0 00 3.0 1.0 2.0 ••• • -2.0 : :: . -3.0 -4.0 -5.0 log2-fold change Viegas siUPF1 microarray

D

F microarray Mendell et al. (9) vs. RNAseq Tani et al. (11)





G RNAseq Tani et al. (11) vs. microarray Yepiskoposyan et al. (14)

## Supplementary Figure 2:



## Supplementary Figure 3:

