# Expanded View Figures

**Figure EV1. Validation of translational offsetting by RNA sequencing.**

A Number of RNAseq reads sequenced and after each preprocessing step.

B A principal component analysis, using per gene-centered expression data, was performed on samples from the transcriptome-wide polysome profiling of ERα depletion (normalized RNAseq data, $N = 4$). Scores were plotted for the first two principal components.

C Shown is a scatter plot of RNAseq quantification of polysome-associated mRNA vs. cytosolic mRNA $\log_2$ fold change (shERα vs. shCtrl). Genes are colored according to their identified mode of regulation (i.e., from Fig 1E). Confidence ellipses (based on RNAseq data, level 0.7) are overlaid for each mode of regulation. Selected targets (also quantified using Nanostring, Western blotting) are indicated.

D–F A similar analysis as Fig 1B–E was performed on RNAseq data instead of DNA microarrays data. Distributions (quantified by kernel density estimation) of *P*-values (D) and FDR-adjusted *P*-values (E) for differential expression between shERα and shCtrl BM67 cells using data from polysome-associated mRNA (orange) or cytosolic mRNA (purple), for analysis of differences in translational efficiencies leading to altered protein levels (red) and analysis of translational offsetting (blue). (F) A scatter plot similar to (Fig 1E) where genes are colored according to their mode of regulation derived from anota2seq analysis on RNAseq data.

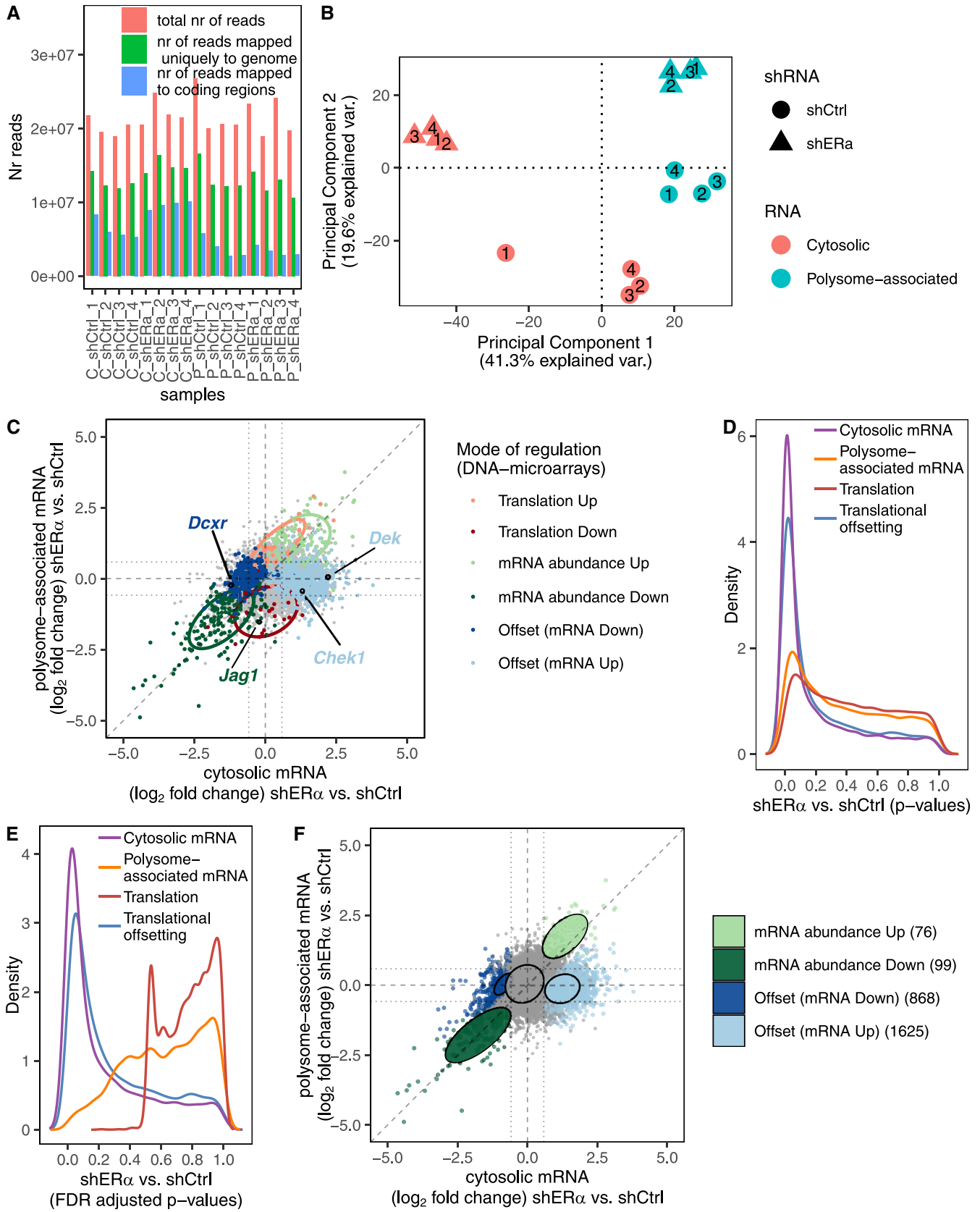
A
total nr of reads
nr of reads mapped uniquely to genome
nr of reads mapped to coding regions
Nr reads
3e+07
2e+07
1e+07
0e+00
C_shCtrl_1
C_shCtrl_2
C_shCtrl_3
C_shCtrl_4
C_shERa_1
C_shERa_2
C_shERa_3
C_shERa_4
P_shCtrl_1
P_shCtrl_2
P_shCtrl_3
P_shCtrl_4
P_shERa_1
P_shERa_2
P_shERa_3
P_shERa_4
samples
B
Principal Component 2 (19.6% explained var.)
Principal Component 1 (41.3% explained var.)
shRNA
shCtrl
shERa
RNA
Cytosolic
Polysome–associated
C
polysome–associated mRNA (log2 fold change) shERα vs. shCtrl
cytosolic mRNA (log2 fold change) shERα vs. shCtrl
Dcxr
Dek
Jag1
Chek1
Mode of regulation (DNA–microarrays)
Translation Up
Translation Down
mRNA abundance Up
mRNA abundance Down
Offset (mRNA Down)
Offset (mRNA Up)
D
Cytosolic mRNA
Polysome–associated mRNA
Translation
Translational offsetting
Density
shERα vs. shCtrl (p–values)
E
Cytosolic mRNA
Polysome–associated mRNA
Translation
Translational offsetting
Density
shERα vs. shCtrl (FDR adjusted p–values)
F
polysome–associated mRNA (log2 fold change) shERα vs. shCtrl
cytosolic mRNA (log2 fold change) shERα vs. shCtrl
mRNA abundance Up (76)
mRNA abundance Down (99)
Offset (mRNA Down) (868)
Offset (mRNA Up) (1625)

**Figure EV1.**

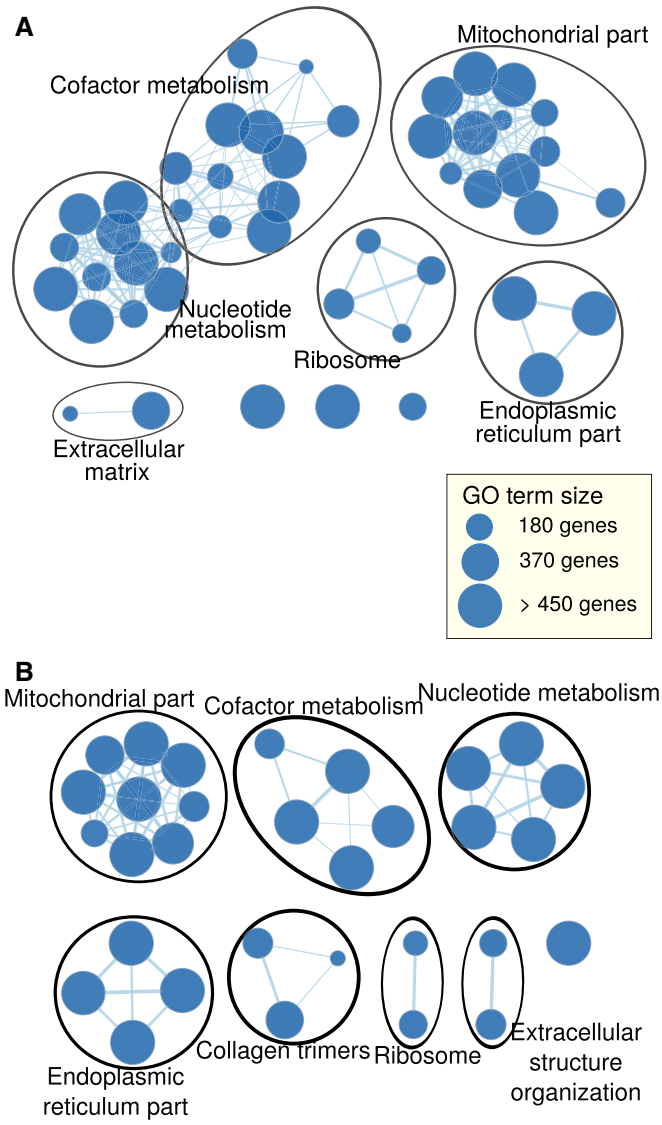


**Figure EV2. ERα depletion-associated translational offsetting modulates expression of genes belonging to distinct cellular functions.**

A, B GO terms enriched among proteins encoded by mRNAs suppressed but offset upon ERα depletion with FDR < 0.15 (A) and enriched among proteins encoded by cytosolic mRNAs downregulated (FDR < 0.15) (B) are visualized as a network. Each node is a GO term connected to other GO terms whose enriched genes overlap. The size of each node reflects the number of genes associated with its corresponding term and the width of the edge illustrates the size of the overlap between two connected nodes.

**Figure EV3. Analyzes of codon usage.**

A Boxplots (plotted as in Fig 3B) of mRNAs under all modes for regulation of gene expression comparing six measurements of codon usage: Codon Adaptation Index (CAI), Codon Bias Index (CBI), Frequency of optimal codons (Fop), GC content at the codon third position (GC3s), Effective number of codons (Nc), tRNA adaptation index (tAI). The numbers of mRNAs in each group are indicated in the figure.

B CDS are divided into sextiles, and the mean tRNA adaptation index is plotted for each mode of regulation at these CDS positions.

C For each codon, the average frequency (per thousand) is compared between transcripts suppressed but offset vs. non-offset upon ERα depletion. Codons coding for the same amino acid are connected by a gray line.

D Left panel shows average frequency (percent) of amino acids encoded by codons previously identified as over-represented among induced or suppressed but offset mRNAs (colored in light or dark blue, respectively; leucine (L, black) is encoded by codons in both groups). Middle panel shows average frequency (percent) of all other amino acids. Right panel shows boxplots (plotted as in Fig 3B) for all mRNAs within each mode for regulation of gene expression comparing number of amino acids ($\log_2$, L_aa, right panel). The numbers of mRNAs in each group are indicated in the figure.

E, F For each codon, the codon usage normalized by amino acid count is compared between transcripts induced (E) or suppressed (F) but offset vs. non-offset (i.e., abundance mode of regulation) upon ERα depletion. Codons coding for the same amino acid are connected by a gray line.

G Unsupervised clustering of a Spearman correlation matrix of codon frequency of regulated mRNAs. Codons identified as over-represented among induced but offset and suppressed but offset mRNAs are indicated in light and dark blue, respectively.

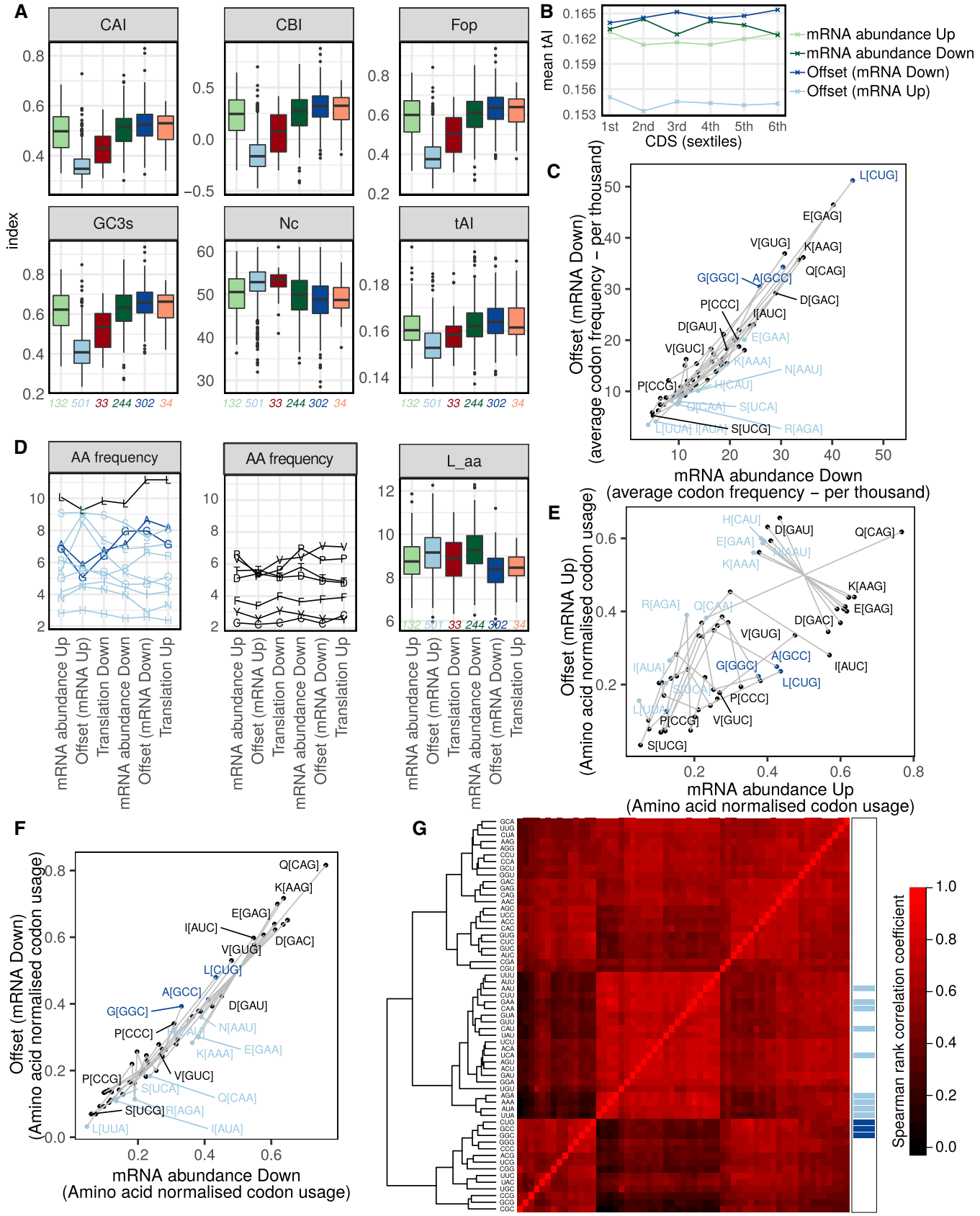
A
CAI
CBI
Fop
GC3s
Nc
tAI
index
132 501 33 244 302 34
B
mean tAI
CDS (sextiles)
mRNA abundance Up
mRNA abundance Down
Offset (mRNA Down)
Offset (mRNA Up)
C
Offset (mRNA Down)
(average codon frequency – per thousand)
mRNA abundance Down
(average codon frequency – per thousand)
D
AA frequency
L_aa
mRNA abundance Up
Offset (mRNA Up)
Translation Down
mRNA abundance Down
Offset (mRNA Down)
Translation Up
E
Offset (mRNA Up)
(Amino acid normalised codon usage)
mRNA abundance Up
(Amino acid normalised codon usage)
F
Offset (mRNA Down)
(Amino acid normalised codon usage)
mRNA abundance Down
(Amino acid normalised codon usage)
G
Spearman rank correlation coefficient

**Figure EV3.**

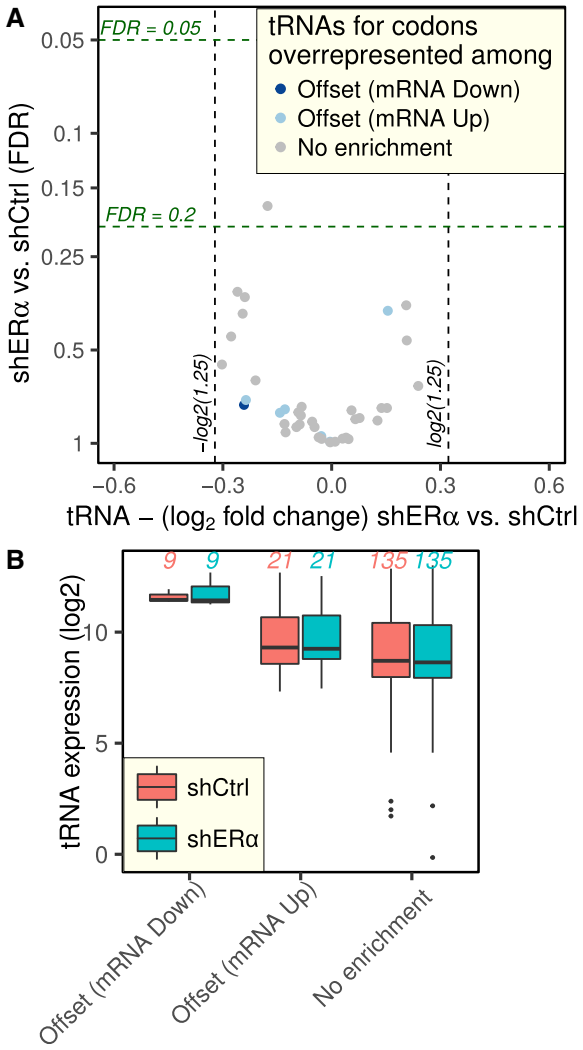


**Figure EV4. ERα depletion-associated translational offsetting cannot be explained by modulations of tRNA levels.**

A Volcano plot of changes in tRNA level (shERα vs. shCtrl). Each tRNA is colored according to the mode for regulation of gene expression it is enriched in.

B tRNAs were grouped according to the mode for regulation of gene expression they are enriched in, and their expression is compared using boxplots (plotted as in Fig 3B). The numbers of tRNAs in each group are indicated in the figure.

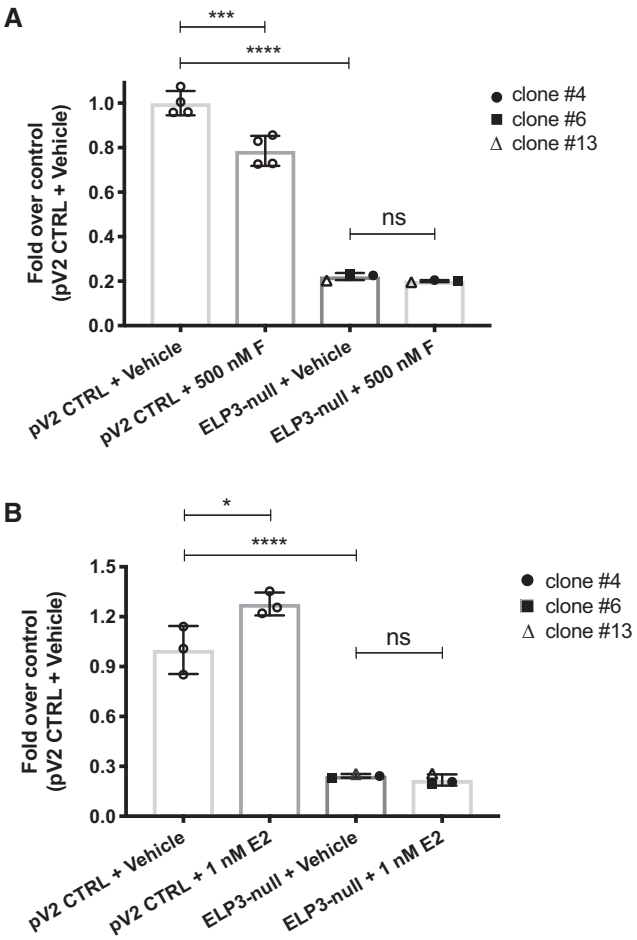


**Figure EV5. Selective estrogen receptor modulators (SERMs) treatment in parental and *Elp3*-null BM67 cells.**

A, B Total cell number analysis using the sulforhodamine B assay in BM67 cells transduced with pLentiCRISPRV2 empty vector vs. *Elp3*-null BM67 cells in response to (A) ICI-182780 (500 nM; 72 h) and (B) E2 (1 nM; 72 h). Graphs represent mean ± SD of $n = 3$ experiments. Data were analyzed by one-way ANOVA. $^{ns}P > 0.05$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; $^{****}P \leq 0.0001$.