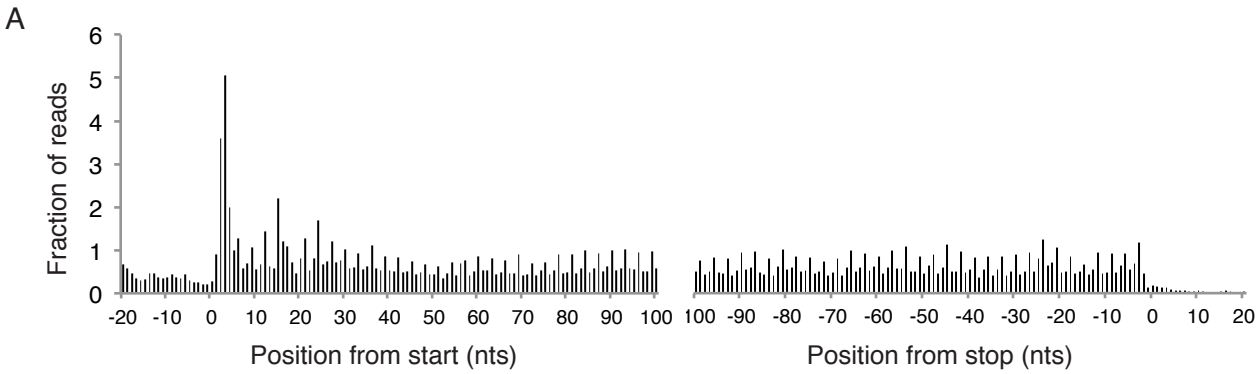
Figure S1. Analysis of ribosome footprint distribution on RefSeq mRNAs relative to annotated start and stop codons A, Ribosome footprint sequences aligning to RefSeq mRNAs that had CDS longer than 1000 nts and UTRs greater than 100 nts were analyzed in the Wt 0.1 ppm dietary Se sample. The first nucleotide of the A-site for each ribosome footprint (RPF) was determined based on the following rule: The position of the 5' end of RPFs less than or equal to 34 nts and RPFs greater than 34 nts in length were offset +17 and +18 nts, respectively. The number of RPFs mapping to each nt position within an mRNA was then determined as a fraction of the total RPFs mapping to that mRNA. This nucleotide fractional count was then summed at each position relative to the start and stop codons for all mRNAs with greater than 1000 total mapped reads. Nucleotide '0' corresponds to the first nucleotide of the start and stop codon, respectively. B, Same as in A, except the positions shown are the location of the 5' ends of RPFs sequences (no offset) binned by size between 32 and 36 nts. These data were used to establish the offset rule to determine the position of first nucleotide of the A-site.

Figure S2. Quantitative analysis of selenoprotein ribosome footprint and mRNA levels Selenoprotein mRNA levels were determined by RNA-Seq (RPKM, RNA reads per kilobase per million mapped reads) and qRTPCR. The values for each were normalized to *Gapdh* levels. Data from Wt (black bars) and $Trsp^{A37G}$ (white bars) mice fed diets supplemented with 0, 0.1 and 2.0 ppm Se are shown. Ribosome profiling RPFKM (ribosome footprint reads per kilobase per million mapped reads) values were determined for all selenoproteins 3' of the UGA-Sec codon (3' Ribosome Footprints) with the exception of *Sels, Txnrd1, Selk, Selo, Txnrd2,* and *Txnrd3* that have UGA-Sec codons located near the C-terminus. For these selenoproteins, CDS ribosome footprinting RPFKMs are reported (*Sels-CDS, Txnrd1-CDS, Selk-CDS, Selo-CDS, Txnrd2-CDS, and Txnrd3-CDS).* For *Sepp1*, the region downstream of the first UGA-Sec codon to the termination codon was considered. The red box indicates the selenoprotein mRNAs most affected by dietary Se levels. The correlation coefficient between qRTPCR and RPKM measurements of selenoprotein mRNA levels were determined and found to be as follows: Wt 0 ppm Se, r = 0.99; Wt 0.1 ppm Se, r = 0.95; Wt 2.0 ppm Se, r = 0.95; $Trsp^{A37G}$ 0 ppm Se, r = 0.98; $Trsp^{A37G}$ 0.1 ppm Se, r = 0.99; $Trsp^{A37G}$ 2.0 ppm Se, r = 0.99.

Figure S3. A-site assignment A, The A-site positions of ribosome footprints mapping near the AUG start codons of RefSeq mRNAs with coding sequences longer than 33 codons are shown as total reads adjusted for the total number of mapped reads in each sample (RPM). B, Same as in A for ribosome footprints mapping at the 3' end of mRNAs with UGA termination codons. C, Ribosome footprints across the *Xbp1* coding sequence in the Wt mouse fed a diet supplemented with 0.1 ppm Se. D, Ribosome footprints mapping near Asn256 of *Xbp1* shown as the total reads adjusted for the total number of mapped reads in each sample (RPM). E, Histogram of ribosome footprints sizes identified in A, B, C and in Figure 5.

Figure S1


A
Fraction of reads
Position from start (nts)
Position from stop (nts)

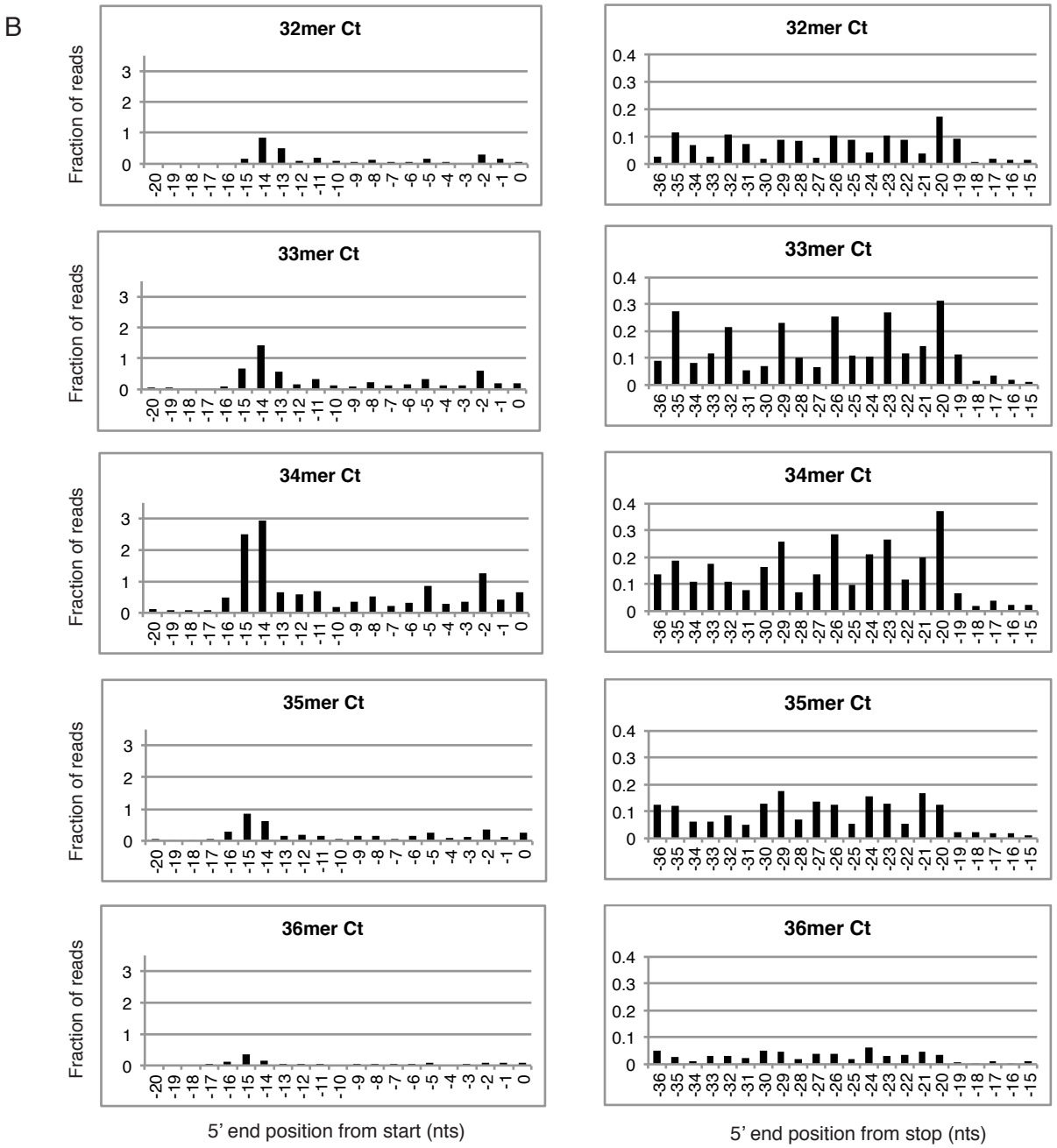

B
32mer Ct
33mer Ct
34mer Ct
35mer Ct
36mer Ct
Fraction of reads
5' end position from start (nts)
5' end position from stop (nts)

Figure S2

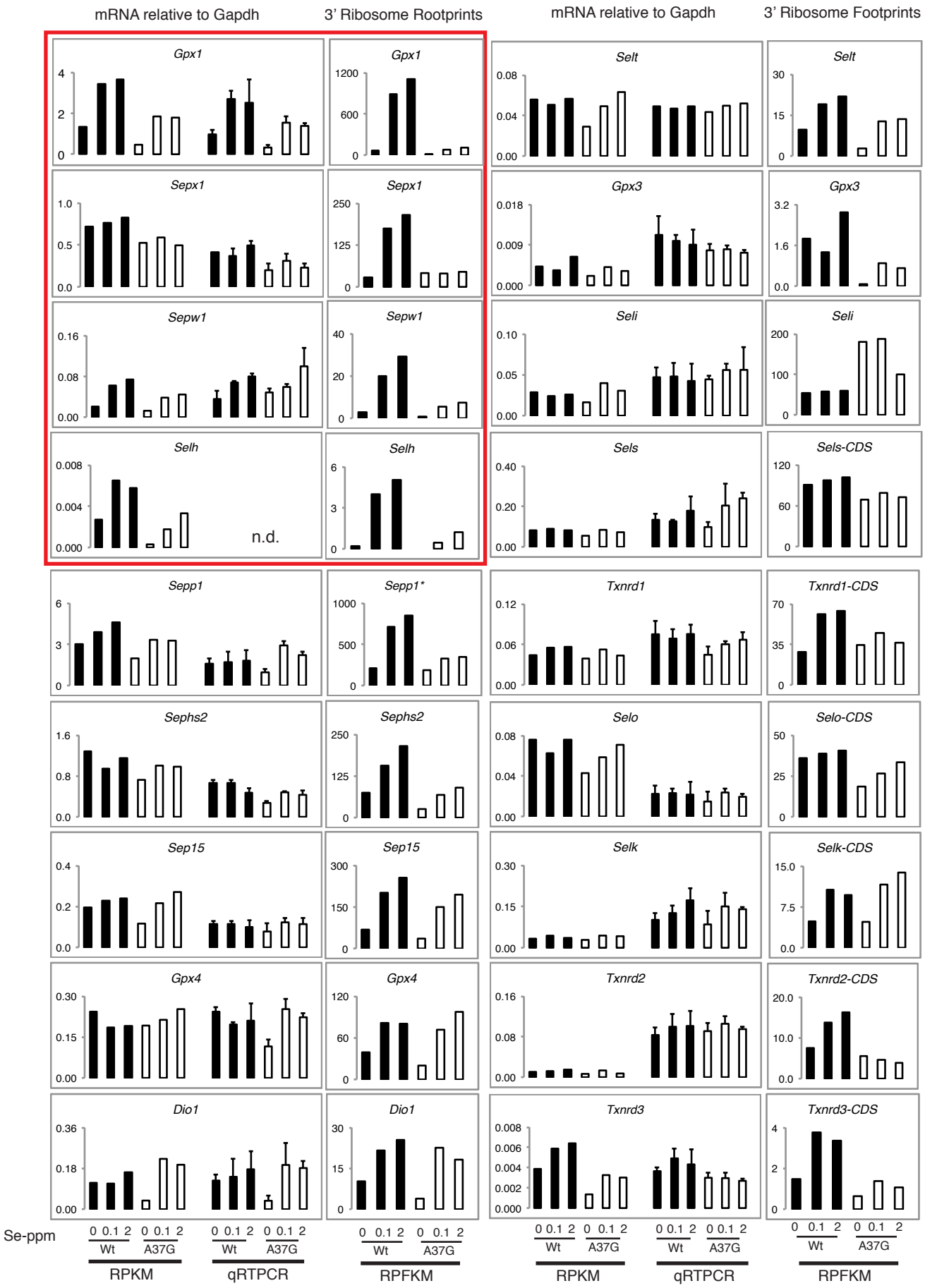
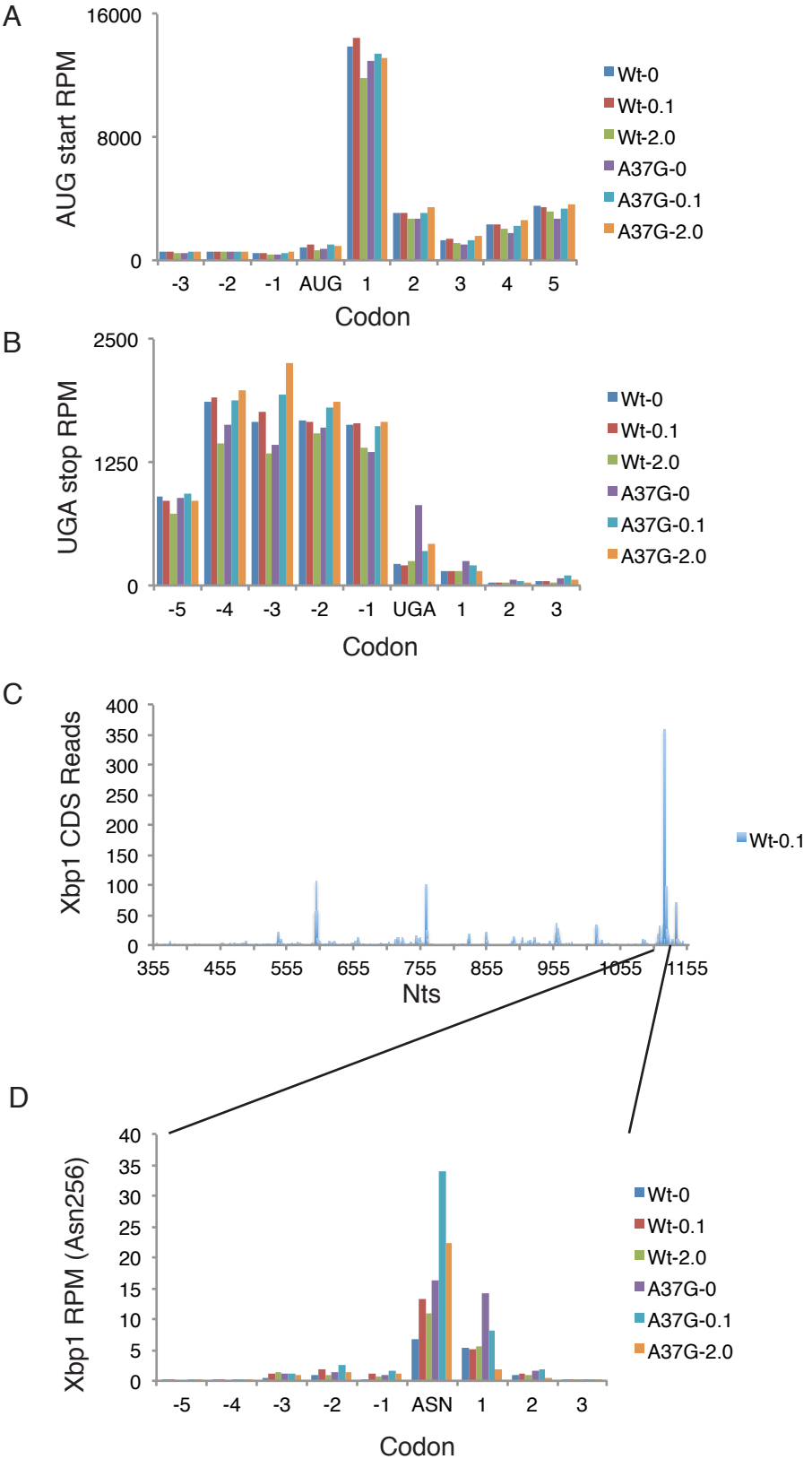
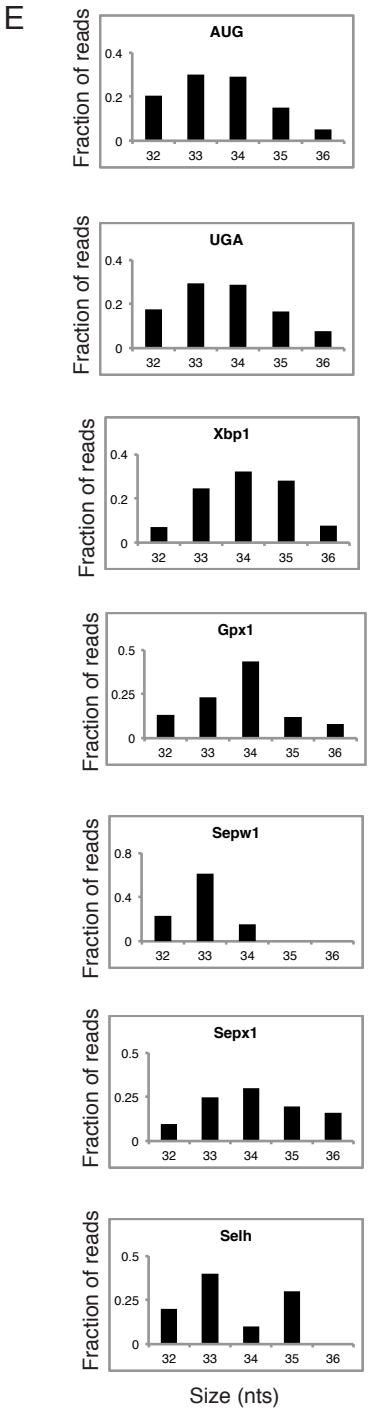

mRNA relative to Gapdh
3' Ribosome Rootprints
mRNA relative to Gapdh
3' Ribosome Footprints
Gpx1
Sepx1
Sepw1
Selh
n.d.
Selt
Gpx3
Seli
Sels
Sels-CDS
Sepp1
Sepp1*
Txnrd1
Txnrd1-CDS
Sephs2
Selo
Selo-CDS
Sep15
Selk
Selk-CDS
Gpx4
Txnrd2
Txnrd2-CDS
Dio1
Txnrd3
Txnrd3-CDS
Se-ppm
0 0.1 2
Wt
A37G
RPKM
qRTPCR
RPFKM

Figure S3


A
AUG start RPM
16000
8000
0
-3 -2 -1 AUG 1 2 3 4 5
Codon
Wt-0
Wt-0.1
Wt-2.0
A37G-0
A37G-0.1
A37G-2.0
B
UGA stop RPM
2500
1250
0
-5 -4 -3 -2 -1 UGA 1 2 3
Codon
C
Xbp1 CDS Reads
400
350
300
250
200
150
100
50
0
355 455 555 655 755 855 955 1055 1155
Nts
Wt-0.1
D
Xbp1 RPM (Asn256)
40
35
30
25
20
15
10
5
0
-5 -4 -3 -2 -1 ASN 1 2 3
Codon
Wt-0
Wt-0.1
Wt-2.0
A37G-0
A37G-0.1
A37G-2.0


E
Fraction of reads
AUG
UGA
Xbp1
Gpx1
Sepw1
Sepx1
Selh
32 33 34 35 36
Size (nts)