

Identification and characterization of novel factors in the **DNA damage response**Singh, J.K.

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CHAPTER 5

Characterization of the exonuclease ERI1 in replication fork stability and R-loop formation

ABSTRACT

The DNA in our cells is particularly vulnerable in S-phase, as many types of endogenous and exogenous sources can cause lesions that block replication fork progression and impact cell division. It is therefore essential for cells to activate replication stress pathways that stabilize and rescue stalled replication forks, allowing the completion of faithful DNA replication. Here, we describe the RNA processing factor ERI1 to play a role in the replication stress response. ERI1 depletion causes an increase in the levels of DNA-RNA hybrids (R-loops), which may enhance replication fork stalling and collapse. Indeed, ERI1 knockdown cells display increased phospho-RPA (S4/S8) levels after hydroxyurea (HU), which is indicative of replication fork instability. Accordingly, we found that ERI1 loss renders cells hypersensitive to HU-induced replication fork damage. Finally, we found that ERI1 is recruited to (stalled) replication forks, suggesting that ERI1 plays a direct role in the replication stress response. Future research will investigate how ERI1 functions at stalled replication forks and whether its function at these structures relies on its role in RNA-processing.

Keywords: Replication stress (RS), R-loops, ERI1, RNA processing

INTRODUCTION

DNA replication is a tightly regulated process in which the replisome rapidly copies billions of DNA bases during each cell division. This process is, however, constantly challenged by replication stress (RS) [1], which is defined as the slowing or stalling of replication fork progression and/or DNA synthesis, and can be caused by a multitude of impediments including limiting nucleotides, ribonucleotide incorporation, repetitive DNA elements, transcription-replication conflicts, DNA secondary structures, fragile sites, and oncogene-induced stress [2]. Fortunately, cells have evolved sophisticated S-phase and mitotic checkpoint pathways that help DNA replication to complete upon RS. Defects in these checkpoint pathways can lead to the stalling or collapse of replication forks, which in turn can cause mutations, chromosome rearrangements or the missegregation of chromosomes, thereby contributing to the development of diseases such as cancer [1].

ATR is the major kinase involved in dealing with RS. It is is recruited to RPA-coated single-stranded DNA (ssDNA) generated by uncoupling of the replicative MCM helicase from DNA polymerases at stalled replication forks, or for instance at replicationdependent DSBs induced by the topoisomerase I inhibitor camptothecin (CPT) [3, 4]. This is followed by its activation via two parallel pathways mediated by either ETAA1 or TOPBP1. ETAA1 stimulates ATR signaling by binding RPA on ssDNA, whereas TOPBP1 does so via its interaction with the RAD17 and 9-1-1 complexes at singlestrand/double-strand DNA junctions [5, 6]. Once activated, ATR phosphorylates a wide variety of substrates, including CHK1 [7]. ATR-CHK1 then promote the restart of stalled replication forks, while enabling the slowdown or arrest of cell cycle progression until replication is completed [2], and controlling the firing of new origins to preserve the RPA pool [3]. Another failsafe mechanism that ensures fork integrity is replication fork reversal. During this process, annealing of newly synthesized and parental DNA occurs to form a fourth regressed arm, which becomes actively converted into a Holliday junction (HJ)-like structure [8]. Several key players are involved in fork reversal such as the SWI/ SNF chromatin remodelers SMARCAL1 and ZRANB3, the helicase HLTF, as well as PARP1. PARP1 stabilizes reversed forks by inhibiting the activity of ATP-dependent DNA helicase Q1 (RECQ1), which can otherwise restart the fork [9]. Reversed forks are substrates for degradation by the nuclease MRE11 and must be properly protected by BRCA1, BRCA2 and Fanconi anemia (FA) proteins FANCA, FANCD2 and FANCI to prevent fork collapse and ensure fork restart [10]. Alternatively, replication forks

that stall at DNA lesions can restart downstream of the lesion by PrimPol-dependent re-priming on the leading strand, leaving unreplicated ssDNA gaps to be filled post-replicatively by translesion synthesis (TLS) polymerases such as POL ŋ and REV1, or by template switching (TS), during which an undamaged homologous template on the sister chromatid is used to bypass DNA lesions [11].

Since DNA replication and transcription both utilize DNA, it is unavoidable that the two processes will interfere with each other, giving rise to transcription-replication conflicts (TRCs) [2]. TRCs can occur in a head-on (HO) or co-directional orientation (CD), depending on the orientation of the genes relative to the replication fork directionality. In mammalian cells the majority of replication forks progress through genes in a CD orientation to avoid the more deleterious HO collisions [12]. However, TRCs that occur in an HO orientation are frequently associated with the formation and persistence of R-loops. These are structures in which RNA is annealed to genomic DNA to create an RNA:DNA hybrid surrounded by a loop of non-templated single-stranded DNA which is protected by RPA [13, 14]. While R-Loops have several regulatory and topological roles in genome regulation, their persistence and association with TRCs can cause replication-stress associated genome instability [15].

RNA binding proteins have emerged as important players in preventing R-loops and genome stability [16]. RnaseH1 and RNaseH2 are evolutionary conserved enzymes that specifically degrade the RNA in R-loops. In addition, RNaseH2 removes single ribonucleotides mis-incorporated during DNA replication [17]. Interestingly, studies have implicated several other RNA processing factors in R-loop associated genome instability. For instance, the THO/TREX complex functions in transcription elongation and mRNP biogenesis to prevent the accumulation of R-loops [18], whereas SRSF1, a protein involved in RNA metabolism, is recruited to nascent transcripts by RNA polymerase II and prevents the formation of mutagenic R-loops [19]. However, despite several studies linking RNA binding proteins to R-loops and genome instability, the function of many RNA binding proteins in R-loop processing remains uncharacterized.

Here, we describe an important regulatory role of the exonuclease ERI1 in preventing R-loop formation following replication stress. ERI1 was found to be enriched at (stalled) replication forks and protects cells against replication stress. Our results suggest that ERI1 may resolve R-loop induced TRCs that lead to replication fork stalling and/or collapse.

RESULTS

ERI1 was among the candidate genes found whose loss causes sensitivity to Hydroxyurea (HU) treatment, which induced RS by dNTP depletion (Fig. 1A and [20], To validate this finding, we performed clonogenic cell survival assays in the presence and absence of HU. We found that ERI1 loss rendered cells sensitive to HU, an effect that was similar to that observed after depletion of RPA-interacting protein ETAA1 (Fig. 1B and Fig. S1A [5]. To better understand how the absence of ERI1 sensitizes cells to replication stress (RS), we examined whether ERI1 is involved in ATR signaling following HU-induced helicase-polymerase uncoupling. ATR is the major kinase, which preserves the nuclear RPA pool by restraining the firing of new origins in order to prevent replication for instability. Inhibition of ATR in HU-treated cells leads to excessive double-strand break (DSB) formation and RPA hyperphosphorylation [3]. We found that ERI1-depleted cells showed increased levels of RPA2 phosphorylation at Serine 4 and 8 (p-RPA (S4/8) and Fig. 1C-D), as well as a mild increase in vH2AX levels (Fig. 1E). Interestingly, we also observed an increase in p-RPA (S4/8) levels upon camptothecin (CPT) treatment, which induces replication-dependent DSBs (Fig. S1B). Importantly, RPA levels remained unchanged in HU-treated ERI1-depleted cells (Fig. S1C-D). Finally, we employed the Flp-In/T-Rex system to establish U2OS cells stably expressing inducible and siRNAresistant GFP-tagged ERI1 (Fig. 1F). Confirming our previous data (Fig. 1D), we found that ERI1 knockdown resulted in an increase of p-RPA (S4/8). Importantly, expression of siRNA-resistant GFP-ERI1 almost fully rescued the increase in p-RPA (S4/S8) observed after ERI1 knockdown (Fig. 1G-H), ruling out off-target effects of the siRNA against ERI1. Taken together, these results suggested that ERI1 protects cells against HU-induced RS likely by promoting replication fork stability.

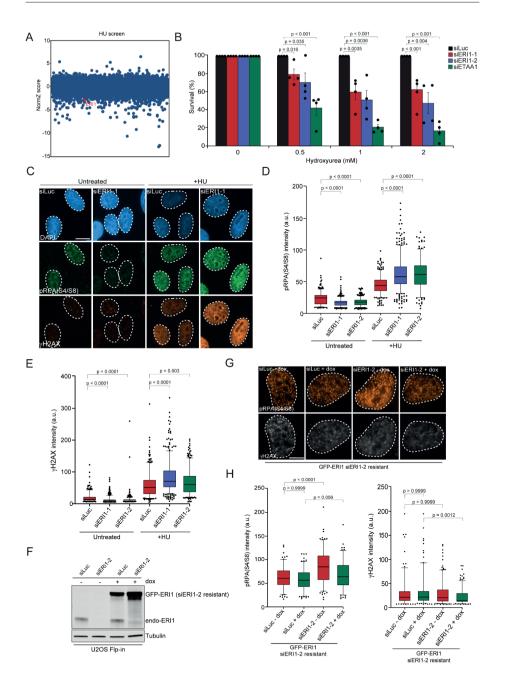


Figure 1. ERI1 promotes cell survival and ATR signaling upon replication stress. (A) CRISPR dropout screen results for RPE cells exposed to 2 mM HU. HU was added for 16 h every 3 days for a period of 12-14 days. (B) Relative survival efficiency in HCT116 cells transfected with the indicated siRNAs and treated with 0.5, 1 and 2 mM HU. The mean ±SEM of 3 independent experiments is shown. Data were normalized to un-irradiated conditions and set to 100%. Statistical significance was calculated using Student's t test. (C) Phosphorylated RPA at Ser4/8 and γH2AX foci formation in U2OS cells transfected with the indicated siRNAs. Cells were exposed to 2 mM of HU and foci intensity was measured after 4 hours. (D) Quantification of RPA (\$4/8) foci intensity in cells from C. Mean foci intensity was quantified in immuno-stained p-RPA (\$4/8) cells. The mean of 3 independent experiments is shown. (E) As in D, except that γH2AX foci intensity was measured. (F) Expression levels of endogenous ERI1 and dox-inducible siRNA-resistant GFP-ERI1 in U2OS Flp-In/TRex cells. Tubulin is a loading control. (G) Effect of inducible expression of siRNA resistant GFP-tagged ERI1 on phosphorylated RPA at Ser4/8 and \(\gamma H2AX \) foci formation in U2OS cells transfected with the indicated siRNAs. Cells were exposed to 2 mM of HU and foci intensity was measured after 4 hours. (H) Quantification of RPA (\$4/8) and \(\gamma H2AX \) foci intensity in cells from G. Mean foci intensity was quantified in immuno-stained p-RPA (S4/8) and yH2AX cells. The mean of 3 independent experiments is shown. P-values were derived from Kruskal-Wallis ANOVA Dunn's post test.

ERI1 interacts with PARP1

To gain insight into ERII's potential role in maintaining fork stability, we aimed to identify possible interaction partners of ERI1. To this end, we generated U2OS Flp-In/T-Rex cells stably expressing inducible GFP-tagged ERI1 or GFP-NLS, and performed GFP-trap-based pulldowns followed by label-free mass spectrometry (MS) (Fig. 2A). Our analysis revealed that ERI1 interacts with 173 proteins that were at least 2-fold enriched in GFP-ERI1 pulldowns when compared to those of GFP-NLS (Fig. 2A and Table S1). Although the majority of the interactors were ribosomal proteins, we also found additional interaction partners including RNA binding proteins (RNPS1 and PNN), RNA helicases (DHX15), chromatin remodelers (SMARCA5 and CHD4), histone proteins (HP1BP3, H1F0 and H2AFY) and RNA splicing factors (THRAP3). Interestingly, we found that ERI1 interacts with PARP1, a protein that is implicated in replication fork reversal [21], and TOP2A, a DNA topoisomerase involved in DNA replication [22]. Since ERI1 maintains fork integrity upon RS, we also performed the interaction analysis of GFP-ERI1 in the presence of HU and compared it to that of untreated cells (Fig. 2C and Table S1). In this analysis we found that ERI1 interacts with DHX9, a protein that recruits BRCA1 to RNA to promote DNA end-resection and homologous recombination (HR) repair [23]. Moreover, GNL3, a protein involved in the RS response, was also found to interact with ERI1 under these conditions [24]. Since PARP1 has several roles in the stabilization of stalled replication forks including fork reversal and the regulation of replication fork speed [25], we sought to validate this interaction. GFP pulldowns coupled to western blot analysis confirmed that GFP-tagged ERI1 interacts with endogenous PARP1 (Fig. 2B), manifesting a possible role of this interaction in RS.

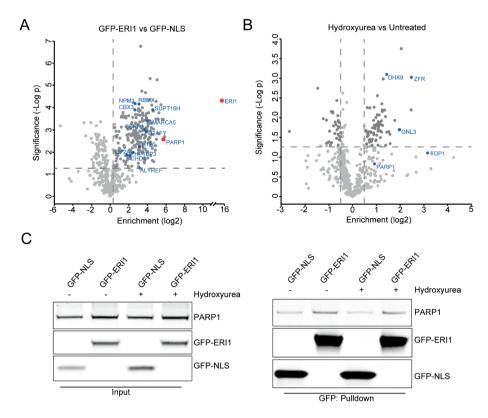


Figure 2. ERI1 interacts with PARP1. (A) Volcano plot depicting the statistical differences of the MS analysis on GFP-KANSL3 versus GFP-NLS pulldowns. The enrichment is plotted on the x-axis and the significance (t-test -log2 p-value) is plotted on the y-axis (see also Supplementary Table 1). (B) As in A, except that cells were treated with 2 mM HU for 4 hours before pulldowns. (C) Pull-down of the indicated GFP fusion proteins in the presence and absence of HU in U2OS Flp-In/T-Rex cells. Blots were probed for GFP and PARP1.

ERI1 is recruited to sites of DNA damage in a manner dependent on PARP activity

Next, we addressed whether ERI1 acts at sites of DNA damage induced by multi-photon laser micro-irradiation (Fig. 3A). Under these conditions, ectopically expressed GFP-tagged ERI1 co-expressed with the DNA damage sensor mCherry-NBS1 in U2OS cells, was rapidly recruited (within 30 s) to DNA damage tracks (Fig. 3B-C). Because of ERI1's interaction with PARP1, we analyzed whether it is recruited to sites of DNA damage via the activity of PARP. To this end, we treated cells with the PARP1 and PARP2 inhibitor (PARPi) Olaparib. This treatment almost completely abrogated the recruitment of GFP-

ERI1 (Fig. 2D-E). Thus, ERI1 is rapidly, but transiently recruited to sites of DNA damage in a manner dependent on the activity of PARP.

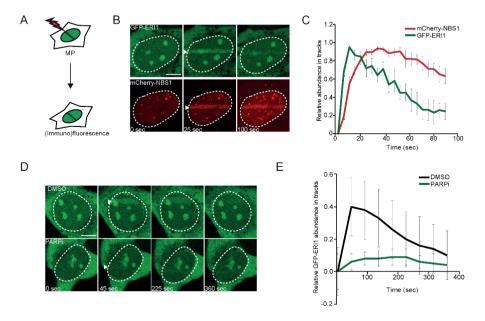


Figure 3. ERI1 is recruited to DNA damage sites via PAR/PARP. (A) Schematic representation of the laser micro-irradiation approach to study protein accumulation at sites of DNA damage. An 800 nm multiphoton (MP) laser was used. (B) Recruitment of GFP-ERI1 to 800 nm multiphoton tracks in U2OS Flp-In/T-Rex cells. mCherry-NBS1 was used as a DNA damage marker. White triangles indicate irradiated regions. (C) Quantification of B. The data are plotted on a timescale as relative abundance in tracks. Peak values were set to 1. The graph represents the mean ± SD of 2 independent experiments. (D) GFP-ERI1 recruitment to 800 nm multiphoton tracks in stable U2OS Flp-In/T-Rex cells treated with PARP inhibitor (PARPi) for 1 h before micro-irradiation. (E) Quantification of D. The data is presented as the mean ± SD.

ERI1 is enriched at (stalled) replication forks

Since multiphoton laser micro-irradiation may induce a variety of lesions other than damaged replication forks, we next investigated the presence of ERI1 on damaged forks [26]. We carried out the isolation of proteins on nascent DNA (iPOND) in unperturbed and replication stress conditions (Fig. 4A). Consistent with previous reports we observed an increase in replication stress proteins, including MRE11 and RPA phosphorylated at Serine 33 (Fig. 4B and [27]. Strikingly, we also found ERI1 enriched at unperturbed forks and increased at HU-induced stalled replication forks (Fig. 4B). This suggests that ERI1 associates with stalled replication forks to maintain their stability.

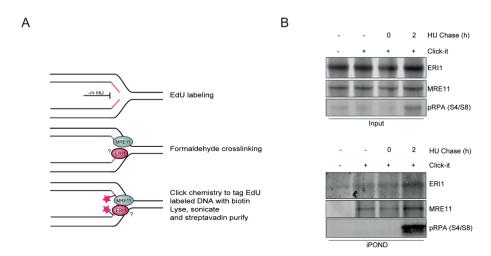


Figure 4. ERI1 is recruited to (stalled) replication forks. (A) Schematic representation of isolation of proteins on nascent DNA (iPOND) procedure. B) iPOND analysis of ERI1 in HEK293T cells after replication stress induced by 2mM HU for the indicated time points. Blots were probed for ERI1, MRE11 and p-RPA (S4/S8).

ERI1-depleted cells suffer from aberrant RNA-DNA hybrid formation

Given the role of ERI1 in several RNA-processing pathways [28], we hypothesized that ERI1 may be involved in the regulation of RNA-DNA hybrid structures (R-loops). R-loops are often associated with transcription-replication conflicts (TRCs), and might be responsible for the observed RS in ERI1-depleted cells. We therefore tested the impact of ERI1 on R-loop formation using the S9.6 antibody for immunofluorescence, which mostly recognizes R-loops, but also has low affinity for dsRNA structures [29]. In order to validate our S9.6 immunofluorescence staining, we depleted a well-known R-loop processing factor AQR and found, as expected, that this led to an increase in R-loop levels (Fig. S2A-B and [30]. In addition, we employed the Flp-In/T-Rex system to establish U2OS cells stably expressing inducible GFP-tagged RNaseH1 (Fig. S2C). Expression of GFP-RNaseH1 almost fully abolished R-loop formation, confirming the specificity of our S9.6 staining (Fig. S2D-E). Next, we measured the accumulation of R-loops in ERI1-depleted cells in both undamaged and CPT-treated cells. We found that loss of ERI1 increased the accumulation of R-loops most clearly following CPT treatment (Fig. 5A-C). Together, these results suggest that ERI1 suppresses R-loop formation, most prominently under RS conditions.

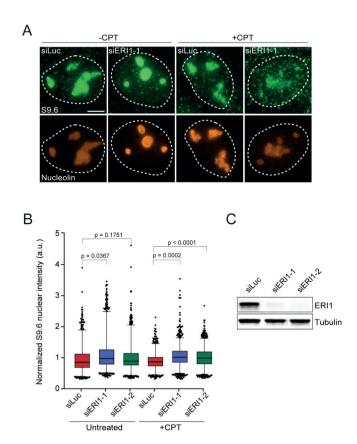


Figure 5. ERI1 suppresses the formation of R-loops. (A) S9.6 nuclear intensity was measured in U2OS cells transfected with the indicated siRNAs. Cells were exposed to 1 μM of CPT and foci intensity was measured after 2 hours. The S9.6 signal intensity per nucleus was determined by subtracting the S9.6 staining with that from nucleoli in each nucleus. (B) Quantification of S9.6 intensity in cells from A. Mean intensity was quantified in immuno-stained S9.6 cells. *P*-values were derived from Kruskal–Wallis ANOVA Dunn's post test. (C) Western blot analysis of ERI1 expression in cells from Figure 5A. Tubulin is a loading control.

The exonuclease domain of ERI1 is critical to suppress replication stress

Since ERI1 is a 3'exonuclease that possesses SAP and exonuclease domains required for binding and processing of RNA, respectively (Fig. 6A and [31, 32]. We next asked whether the decrease in fork stability as measured by phosphorylated RPA at Serine 4/8 relates to ERI1's role in RNA processing (Fig. 1D). Re-expression of siRNA-resistant GFP-tagged wildtype (Fig. 1G), but not exonuclease-dead (D130G and E132G) ERI1 largely suppressed p-RPA (S4/8) intensity in these cells (Fig. 6B-D and [33]. These data

suggest that replication fork stability is strictly dependent on the catalytic activity of ERI1. We speculate that ERI1 prevents the formation of TRCs by the resolution of R-loops and the subsequent stalling/collapse of replication forks (Fig. 6E).

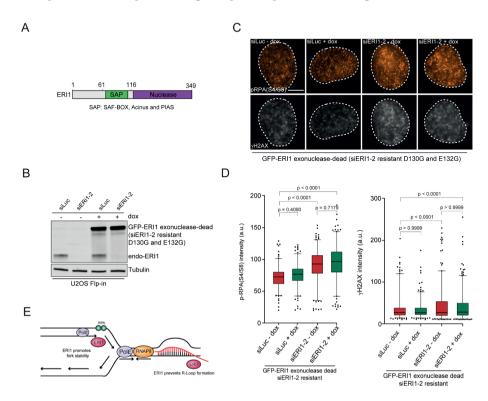


Figure 6. The catalytic activity of ERI1 is critical to suppress replication stress. (A) Schematic representation of full-length ERI1 protein with its indicated domains (SAP and nuclease domain). (B) Expression levels of endogenous ERI1 and dox-inducible siRNA-resistant GFP-ERI1 (exonuclease dead D130G and E132G) in U2OS Flp-In/TRex cells. Tubulin is a loading control. (G) Effect of inducible expression of siRNA resistant GFP-tagged ERI1 exonuclease dead on phosphorylated RPA at Ser4/8 and γH2AX foci formation in U2OS cells transfected with the indicated siRNAs. Cells were exposed to 2 mM of HU and foci intensity was measured after 4 hours. (H) Quantification of RPA (S4/8) and γH2AX foci intensity in cells from G. Mean foci intensity was quantified in immuno-stained p-RPA (S4/8) and γH2AX cells. The mean of 3 independent experiments is shown. *P*-values were derived from Kruskal–Wallis ANOVA Dunn's post test.

DISCUSSION

In this study, we uncover an important role of the exonuclease ERI1 in the RS response. First, we demonstrate that ERI1 protects cells from HU-induced RS and maintains fork stability under these conditions. Second, we show that ERI1 interacts with PARP1 and

is recruited to sites of DNA damage in a manner dependent on PARP activity. Third, iPOND analysis revealed that ERI1 associates with stalled replication forks. Finally, ERI1 suppresses the formation of R-loops and maintains fork stability through its activity in RNA-processing. Thus, ERI1 may ensure fork stability by preventing TRC collisions that are causally linked to R-loop formation and induce replication fork collapse (Fig. 6F).

ERI1 protects cells from HU-induced replication stress and maintains fork stability

In agreement with previous results we confirmed that ERI1-depleted cells are sensitive to HU-induced RS [20]. In response to HU-induced helicase-polymerase uncoupling, ATR kinase is recruited to and activated via its direct interaction with ssDNA at stalled replication forks [34]. This is followed by the ATR-dependent phosphorylation of RPA (at Serine 33), which prevents fork breakage by the suppression of new origin firing, thereby preserving the nuclear pool of RPA [3]. Consequently, inhibition of ATR leads to excessive amounts of ssDNA and limits the available amount RPA molecules by a process referred to as 'RPA exhaustion'. This phenomenon in turn leads to replication fork catastrophies and the formation of DSBs [5, 34]. RPA levels, however, remained unaffected in ERI1-depleted cells, ruling out RPA exhaustion. Instead, we found increased p-RPA (S4/S8) and γ H2AX levels upon RS in ERI1-depleted cells. This suggests that ERI1 loss results in the conversion of stalled replication forks into collapsed forks, which may ultimately be converted into DSBs. To investigate this further, DNA fiber analysis may provide insights into whether ERI1 loss affects fork stability/recovery following replication stress.

ERI1 interacts with PARP1 and is enriched at (stalled) replication forks

Our mass spectrometry experiments revealed that the majority of ERI1 interactors are ribosomal proteins. However, we also found several additional interactors such as RNA binding proteins (RNPS1 and PNN), RNA helicases (DHX15), chromatin remodelers (SMARCA5 and CHD4), histone proteins (HP1BP3, H1F0 and H2AFY), RNA splicing factors (THRAP3) and the DNA repair protein PARP1 (Table S1). Moreover, we validated PARP1 as an ERI1 interactor by GFP-pulldowns coupled to western blot analysis, although this needs to be confirmed endogenously and reciprocally. Given that PARP1 is implicated in normal DNA replication and the stabilization of stalled replication forks, it may be possible that ERI1's role in these processes involves its interaction with

PARP1 [35]. Indeed, our findings reveal that ERI1 is recruited to sites of DNA damage via the activity of PARP. However, whether this involves the binding of ERI1 to PAR chains or to PARP1 itself needs to be further investigated [28]. Moreover, our iPOND analysis revealed that ERI1 is enriched at unperturbed forks and increased at HU-induced stalled replication forks. Similarly, PARP1 is activated and binds to HU-induced stalled replication forks [36]. Thus, it seems logical to next assess by iPOND whether ERI1's recruitment to stalled forks is dependent on PAR/PARP1. However, since ERI1 has affinity to bind RNA via its SAP domain, its recruitment to stalled forks may also rely on its interaction with RNA or its exonuclease activity (e.g. to process RNA in R-loops). Further work, including domain mapping analysis, should identify the PARP1 and RNA binding regions in ERI1 to resolve the functional relevance of interplay between ERI1 and PARP1 or RNA in response to replication fork stalling.

ERI1 depletion results in increased R-Loop formation

Our data reveal that ERI1 depletion leads to increased R-loop levels mostly following RS. We used the S9.6 antibody for immunofluorescence (IF) to measure R-loop levels. As expected, we found AQR depletion to increase the formation of R-loops, and overexpression of RnaseH1 to decrease the formation of R-loops, which validates our S9.6 IF [37]. However, because the S9.6 antibody also recognizes double-strand RNA [30, 38], it is of utmost importance to further validate our S9.6 IF experiments using RnaseIII treatment, as well as treatment with recombinant wildtype and catalytic inactive version of human RNaseH1 to further validate the specificity of our S9.6 IF [38]. Since ERI1 is involved in the processing of multiple RNA species [28], and our data reveal that the exonuclease domain of ERI1 is critical to suppress RS as measured by p-RPA (S4/8), it may be interesting to investigate whether the exonuclease domain of ERI1 has affinity to process the RNA in R-loops. Future *in vitro* R-loop formation assays using purified ERI1 wildtype and exonuclease dead will determine whether ERI1 processes R-loops and if this is dependent on its exonuclease domain [39].

The unscheduled formation of R-loops has been linked to TRCs and RS [40]. This raises the question whether the aberrant R-loops observed in ERI1-depleted cells, are a potential mechanism driving RS. Future endeavors including RNaseH1 overexpression rescue experiments in ERI1-depleted cells will give insight into whether the increase in fork stalling measured by p-RPA (S4/8) and DNA breaks measured by γ H2AX levels, as well as the HU-sensitivity observed in ERI1-depleted cells is caused by R-loops.

Furthermore, R-loops may pose an obstacle for replication fork progression and thereby cause defects in replication fork instability upon HU-induced RS in ERI1-depleted cells [41]. To investigate this possibility, proximity ligation assay (PLA) between PCNA, as a marker for replication, and RNAPII may evaluate the occurrence of TRCs in absence of ERI1 [42].

A proper balance of histones and DNA during DNA replication is crucial, as dysregulation of the amount of histone levels is associated with chromosomal loss and genome instability [43]. ERI1 is required for the degradation of replication-dependent histone mRNAs [44, 45]. This raises the question whether this function of ERI1 is linked to R-loops and is associated replication stress. It would be important to decipher the genomic location in which R-loops are enriched in ERI1 depleted cells, and whether this correlates with changes in histone incorporation levels at these loci during replication. In depth analysis of R-loop distribution by DRIP-sequencing in both control and ERI1-depleted cells, as well as ChIP-sequencing, including e.g. chromatin occupancy after replication-seq (ChOR-seq) [46], to determine changes in histone levels at these sites will be required in the future to address these questions.

MATERIAL AND METHODS

Cell lines

U2OS and HCT116 cells were cultured in 5% CO $_2$ at 37°C in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal calf serum and antibiotics. U2OS Flp-In/T-Rex cells, which were generated using the Flp-In/T-REx system (Thermo Fisher Scientific), were a gift of Geert Kops (University Medical Center Utrecht, the Netherlands) and Stephen Taylor (University of Manchester, UK). These cells were used to stably express inducible versions of GFP-NLS, as well as GFP-ERI1 by co-transfection of pCDNA5/FRT/TO-Puro plasmid encoding GFP or GFP-tagged ERI1 (5 μ g), together with pOG44 plasmid encoding the Flp recombinase (1 μ g). After selection on 1 μ g/mL puromycin, single clones were isolated and expanded. U2OS Flp-In/T-Rex were incubated with 2 μ g/mL doxycycline for 24h to induce expression of cDNAs. All cells were authenticated by STR profiling and tested negative in routinely performed mycoplasma tests.

Chemicals

Cells were treated with 2mM Hydroxyurea (Sigma) at the indicated timepoints and collected for further analysis. The PARP inhibitor olaparib (Selleck Chemicals) was used at a final concentration of 10 μ M. Camptothecin (Sigma-Aldrich) was used at a final concentration of 1 μ M.

Transfections, siRNAs and plasmids

Cells were transfected with siRNAs using RNAiMAX (Invitrogen) according to the manufacturer's instructions. Cells were transfected twice with siRNAs at 0 and 24 h at a concentration of 40 nM and were analyzed 48 h after the second transfection unless otherwise indicated. siRNA sequences are listed in Table 1. Cells were transfected with plasmid DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions and analyzed 24-48 hours after transfection. The expression vector for full length human ERI1 (ORFeome collection), was amplified and cloned into pCDNA5/FRT/TO-Puro as a *BspEI/Acc65I* fragment (Table 2). Mutant constructs were generated by site-directed mutagenesis PCR (Table 2). siERI1-resistant cDNA was generated by introducing the underlined mutations GTTATCGATTGGATGAAGTTA by overlap PCR and cloned as *BspEI/Acc65I* fragment into pCDNA5/FRT/TO-Puro-ERI1-WT (Table 2). All ERI1 expression constructs were verified using Sanger sequencing.

Multiphoton laser micro-irradiation

Cells grown were grown on 18 mm coverslips. For micro-irradiation, cells were placed in a Chamlide CMB magnetic chamber and the growth medium was replaced by CO₂-independent Leibovitz's L15 medium supplemented with 10% FCS and antibiotics. Laser micro-irradiation was performed on a Leica SP5 confocal microscope equipped with an environmental chamber set to 37°C. DNA damage-containing tracks (1.5 µm width) were generated with a Mira mode locked titanium-sapphire (Ti:Sapphire) laser (l = 800 nm, pulse length = 200 fs, repetition rate = 76 MHz, output power = 80 mW) using a UV-transmitting 63x 1.4 NA oil immersion objective (HCX PL APO; Leica). Confocal images were recorded before and after laser irradiation at 5 seconds time interval over a period of 3-5 minutes. Images after multi-photon micro-irradiation of living cells were recorded using LAS-AF software (Leica) and analyzed with Image J as described previously [47]. The average pixel intensity of laser tracks was measured within the locally irradiated area (Idamage), in the nucleoplasm outside the locally irradiated area (Inucleoplasm)

and in a region not containing cells in the same field of view (Ibackground). The level of protein accumulation relative to the protein level in the nucleoplasm was calculated as follows: ((Idamage – Ibackground)/(Inucleoplasm – Ibackground) – 1).

Immunofluorescence analysis

Cells were either directly fixed with 2% formaldehyde in PBS for 20 minutes at room temperature (RT), or pre-extracted with 0.5% Triton-X100 (Serva) in PBS on ice for 2-5 minutes prior to fixation. Alternatively, cells were fixed, post-extracted with 0.25% Triton-X100 (Serva) in PBS and treated with 100 mM glycine in PBS for 20 minutes to block unreacted aldehyde groups. Cells were then rinsed with PBS and equilibrated in wash buffer (PBS containing 0.5% BSA). Antibody incubation steps and washes were in wash buffer. Primary antibodies were incubated for 1-2 hours at room temperature. Detection was done using goat anti-mouse or goat anti-rabbit Ig coupled to Alexa 488, 555 or 647 (1:1500; Invitrogen Molecular probes). All antibodies are listed in Table 3. Samples were incubated with 0.1 μ g/mL 4',6-Diamidino-2-Phenylindole Dihydrochloride (DAPI) and mounted in Polymount.

Sample preparation and mass spectrometry

For mass spectrometry, U2OS Flp-In/T-Rex cells expressing GFP-NLS and GFP-ERI1^{WT} were treated with 2 mM Hydroxyurea for 4 hours or left untreated. Cell pellets were lysed in EBC-1 buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 0.5% NP-40, 2 mM MgCl, protease inhibitor cocktail tablets) with 500 units benzonase. Samples were incubated for 1 hour at 4°C under constant mixing followed by high speed centrifugation for 10 minutes at 4°C. Protein concentration was measured by Qubit in the cleared lysates, equalized and transferred to tubes containing GFP-Trap beads (Chromotek). After 90 minutes of incubation at 4°C under rotating condition, the beads were washed 4 times with EBC-2 buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, and protease inhibitor cocktail tablets) and 3 times with 50 mM ammonium bicarbonate followed by overnight digestion using 2.5 µg trypsin at 37°C under constant shaking. Digestion was terminated with 1% trifluoroacetic acid and centrifuged for 5 minutes at high speed to precipitate insoluble fractions. Consequently, C18 cartridges were prepared by washing 2 times with acetonitrile followed by 2 times with 0,1% Acetic acid. Peptides were loaded on the cartridge, while bound peptides were washed 2 times with 0.1% acetic acid and eluted with 1 mL 80% acetonitrile / 0.1% acetic acid and lyophilized.

Mass spectrometry was performed essentially as previously described [48]. Samples were nalysed on a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, Germany) coupled to an EASY-nanoLC 1000 system (Proxeon, Odense, Denmark). Digested peptides were separated using a 15 cm fused silica capillary (ID: 75 Um, OD: 375 Um, Polymicro Technologies, California, US) in-house packed with 1.9 µm C18-AQ beads (Reprospher-DE, Pur, Dr. Maisch, Ammerburch-Entringen, Germany). Peptides were separated by liquid chromatography using a gradient from 2% to 95% acetonitrile with 0.1% formic acid at a flow rate of 200 nl/minute for 65 minutes. The mass spectrometer was operated in positive-ion mode at 2.8 kV with the capillary heated to 250°C, and in a Data-Dependent Acquisition (DDA) mode with a top 7 method. Full scan MS spectra were obtained with a resolution of 70,000, a target value of 3x106 and a scan range from 400 to 2.000 m/z. Maximum Injection Time (IT) was set to 50 ms. Higher-Collisional Dissociation (HCD) tandem mass spectra (MS/MS) were recorded with a resolution of 35,000, a maximum IT of 20 ms, a target value of 1x105 and a normalized collision energy of 25%. The precursor ion masses selected for MS/MS analysis were subsequently dynamically excluded from MS/MS analysis for 60 seconds. Precursor ions with a charge state of 1 and greater than 6 were excluded from triggering MS/MS events. Three replicates were included per condition with two technical repeats each.

Mass Spectrometry data analysis

Raw mass spectrometry files were analysed with MaxQuant software (v1.5.3.30) as described [49] with the following modifications from default settings: the maximum number of mis-cleavages by trypsin/p was set to 3, Label Free Quantification (LFQ) was enabled disabling the Fast LFQ feature. Match-between-runs feature was enabled with a match time window of 0.7 minutes and an alignment time window of 20 minutes. We performed the search against an in silico digested UniProt reference proteome for Homo sapiens (8th June 2020). Analysis output from MaxQuant was further processed in the Perseus (v 1.5.5.3) computational platform [49]. Proteins identified as common contaminants, only identified by site and reverse peptide, were filtered out, and then all the LFQ intensities were log2 transformed. Different biological repeats of each condition were grouped and only protein groups identified in all three biological replicates in at least one condition were included for further analysis. Missing values were imputed using Perseus software by normally distributed values with a 1.8 downshift (log2) and a randomized 0.3 width (log2) considering total matrix values. Volcano plots were

generated, and Student's T-tests were performed to compare the different conditions. Spreadsheets from the statistical analysis output from Perseus were further processed in Microsoft Excel for comprehensive visualization and analysis of the data (Table S1).

Western blot analysis

Cells were lysed in 2x Laemmli buffer and proteins were separated by Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) using 4-12% pre-cast polyacrylamide gels (BioRad or Invitrogen) and MOPS running buffer (Invitrogen). Next, proteins were transferred onto nitrocellulose membranes (Millipore). Protein expression was analyzed by immunoblotting with the indicated primary antibodies (Table 3) and secondary CF680 goat anti-rabbit or CF770 goat anti-mouse Ig antibodies (1:5000, Biotium). Membranes were scanned and analyzed using a Licor Odyssey scanner (LI-COR Biosciences).

Isolation of proteins on nascent DNA (iPOND)

iPOND was performed as described by [27] with minor modifications. Briefly, 80-100*106 HEK293T cells were pulse labelled with 10 µM 5-ethynyl-2'-deoxyuridine (EdU; Invitrogen) for 20 min. Next, cells were treated with 2 mM HU for 5 hr to induce stalled forks. After treatment, cells were cross-linked with 1% formaldehyde without methanol in PBS for 15 min at room temperature, quenched with 0.125 M glycine and collected by scraping. Fixed cells were spun down for 5 min at 4 °C and 900 g, washed two times with PBS. Next, cells were permeabilized with 0.25% Triton X-100 in PBS for 30 min at room temperature, followed by incubation with click-iT reaction buffer (10 µM biotin azide (life technologies), 10 mM sodium-L-ascorbate, and 2 mM CuSO₂) or control buffer for 3 hr at room temperature. The cells were then washed with 0.5% BSA/PBS, and resuspended in lysis buffer (0.5% SDS, 50 mM Tris-HCl pH 8.0, protease inhibitors (Roche)). Samples were then sonicated using a Branson 250 SONIFIER with microtip for 1 s pulse for 10 cycles, repeated 3 times, with 90 s pause between repetition. For input samples, an aliquot of the lysate was taken. The rest of the samples were incubated with pre-washed streptavidin coated beads (Life Technologies) for 1 hr at room temperature. Beads were washed twice with high salt buffer (1% Triton X-100, 20 mM Tris [pH 8.0], 2 mM EDTA, 500 mM NaCl) and twice with lithium chloride salt wash buffer (100 mM Tris [pH 8.0], 500 mM LiCl and 1% Igepal [NP-40]), followed by elution of the captured proteins by boiling the beads in 2X Laemmli for 25 min at 95°C. Proteins were separated on a 4-12% CriterionTM XT Bis-Tris gel (Bio-Rad) and detected by western blot.

Cell survival assays

HCT116 cells were transfected with siRNAs, trypsinized, seeded at low density and exposed to HU for 24 hours. For HCT116 Flp-In/T-Rex, cDNAs were expressed by adding Dox for 24 hours after siRNA transfection. After 12 days, the cells were washed with 0.9% NaCl and stained with methylene blue (2.5 g/L in 5% ethanol, Sigma-Aldrich). Colonies of more than 20 cells were scored.

AUTHOR CONTRIBUTION

J.S. performed western blot analysis, immunofluorescence experiments, clonogenic survival assays. J.L. and W.W.W. performed iPOND assay. A.J.L.G. prepared mass-spectrometry samples. R.P. and A.C.O.V. performed mass-spectrometry experiments and data analysis. H.v.A. conceived and supervised the project.

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Table 1. List of siRNAs

Target	Sequence (5'-3')	
ERI1-1	ACGAAUGGCUGUAUUAAUA	
ERI1-2	GUAAUUGACUGGAUGAAAU	
XRCC4	AUAUGUUGGUGAACUGAGA	
ETAA1	GAGCAAAACAAGAGGAAUUUU	
Luciferase	CGUACGCGGAAUACUUCGA	
AQR	SMARTpool (siGENOME)	

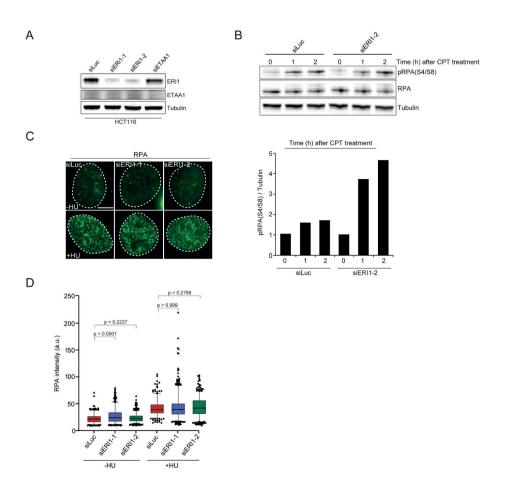
Table 2. List of primers

Name	Sequence (5'-3')
HindIII FW	TAAAAAGCTTATATGGAAGAATCTCACTTCAATTCTAAC
KpnI RV	TAATAATGGTACCCTAAGAGCTGGCCAGGTGC
ERI1_siRNAres_HindII Rv	ACTCTCGGCATGGACGAGCTG
ERI1_siRNAres_KpnI FW	GTTAAACCCGCTGATCAGCCTCGA
M13 FW	GTAAAACGACGGCCAGT
M13 RV	CAGGAAACAGCTATGAC
ER1_Catalytic_dead_Fw	GTTATTATGACTACATTTGTATTATTGGCTTTGGAG CCACTTGTGAAGAAGG
ERI1_Catalytic_dead_Rv	CCTTCTTCACAAGTGGCTCCAAAGCCAATAATACAA ATGTAGTCATAATAAC

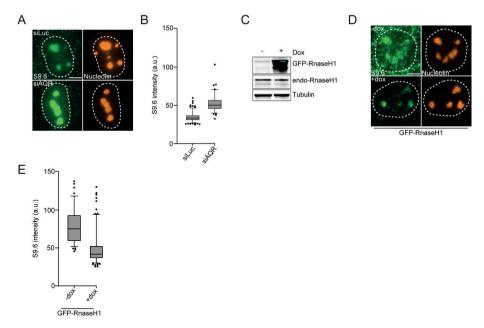
Table 3. List of primary antibodies

Protein	Host	Company	IF	WB
GFP	Mouse	Roche (11814460001)		1:1000
α-Tubulin	Mouse	Sigma (cloneDM1A, T6199)		1:5000
MRE11	Rabbit	Kind gift of Roland Kanaar		1:3000
ERI1	Rabbit	Cell signaling (405SS)		1:2000
p-RPA (S4/S8)	Rabbit	Bethyl Laboratories (A300-245A)	1:1000	1:1000
ETAA1	Rabbit	Kind gift of Niels Mailand		1:400
RPA	Mouse	Abcam (ab2175)	1:1000	1:1000

Supplementary Figures and Tables - Heading



Supplementary Figure. 1 – related to Figure. 1. ERI1 protects cells against HU-induced RS. (A) Western blot analysis of ERI1 expression in cells from Figure 1B. Blots are probed for ERI1, ETAA1 and Tubulin. Tubulin is a loading control. (B) Western blot analysis of phosphorylated RPA32 (S4/S8) and RPA32 expression in cells transfected with the indicated siRNAs. Cells were examined after the indicated timepoint upon 1μM CPT treatment. Tubulin is a loading control (upper panel). Quantification of phosphorylated RPA32 (S4/S8) expression Tubulin is a loading control, which was used for normalization of phosphorylated RPA32 (S4/S8) expression. Data were normalized to siLuc UT, which was set to 1 (lower panel). (C) RPA foci formation in U2OS cells transfected with the indicated siRNAs. Cells were exposed to 2 mM of HU and foci intensity was measured after 4 hours. (D) Quantification of RPA intensity in cells from B. Mean foci intensity was quantified in immuno-stained RPA cells. The mean of 3 independent experiments is shown.



Supplementary Figure. 2 – related to Figure. 5. Validation of S9.6 staining. (A) S9.6 nuclear intensity was measured in U2OS cells transfected with the indicated siRNAs. The S9.6 signal intensity per nucleus was determined by subtracting the S9.6 staining with that from nucleoli in each nucleus. (B) Quantification of S9.6 intensity in cells from A. Mean intensity was quantified in immuno-stained S9.6 cells. *P*-values were derived from Kruskal–Wallis ANOVA Dunn's post test. (C) Expression levels of endogenous RnaseH1 and dox-inducible GFP-RnaseH1 in U2OS Flp-In/TRex cells. Tubulin is a loading control. (D) Effect of inducible expression of GFP-tagged RnaseH1 on S9.6 nuclear intensity U2OS Flp-In/TRex cells. The S9.6 signal intensity per nucleus was determined by subtracting the S9.6 staining with that from nucleoli in each nucleus. (E) Quantification of S9.6 intensity in cells from D. Mean intensity was quantified in immuno-stained S9.6 cells. *P*-values were derived from Kruskal–Wallis ANOVA Dunn's post test.

Supplementary Table 1

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Supplementary Table 1Log p-value and average: GENIE	Statistical old-enricht	analysis ment (log rid 17e G	comparir 2) are in: ED	g LFQ into Scated, Ad Ent	ensities o Iditional i	f proteins nformation Eri1	identified by LC-MI from the analysis	of identifie	P-pulldo d protein	evens from I s is also in	J208 Flp. cluded.	in/T-Rec	cells expr	essing eit	her GFP-EF	II1 or GFP-NLS in the prese	nce and a	bsence of	THU		Values				
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Facta Protein Gene headers names names	120.05t	-Log p- velve	e (hg))	120.05t	-Log p-	o Hegit	Razor + Peptides unique peptides	Unique peptides	Sequence coverage [%]	near sequence owerage	Oake raide	Mal. weight (s0-e)	Q-value	50010	Intensity	MS/MS Protein protein Count IOs IOs	E931-1	ERH-2	ERE-3	DOI-HU-1	E913-HU-2	D11-HU-2	GFP-1	GFP-2	GFF3
sa (PO425) Beretin, ty ERT68 sa (QS9GA Autotive & EPL3985)	2139	3,18 9,46	-5.36 1.56		1,09	4.25 3.32	54	2	613-518-618-618-618-618-618-618-618-618-618-6	1,8 19,6	1,8 19,6	63,066 6,3225 15,998	0	12,753	3616010010 739010010	5 NATES PAGES 3 OWNERS CONNECTED TO THE TEXT OF THE TE	30,90 22,05 21,16	22,71 25,92	21,33	22,64 26,41 22,19	26,35	29,36	26,88	17,01 20,07 20,34 11,81 20,34 31,91 20,34 31,91 20,31 20,41	27,46 30,91
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29 [PGDS1 405 risess 8957 29 [Q9897 Gastile + G4L]	•	2,74	2,23	:	1,75	2,05 1,97	7 3	13	47,9 5,6	47,9 5,6	47,9 5,6	22,127	9	147,34 19,425	4030400000 174040000	49 MG091,95 P62091,95 5 Q98492 2, Q98492-2	36,99 22,85	36,64 21,14	27,00 22,54	29,06 24,24	29,99	29,74	24,26 21,66	28,87 22,68	25,19
I/[HDYLRG GDS / Bossa RPL28 I/[AGA180GY/G [AGA180GV/G_H	MAN Kera	2,95 0,61	3,80 -1,56		0,66	1,79	7 3	- 4 - 5	27,1 47,7	37,1 32,7	27,1 22,7	9,657	9	22,555 22,406	3719400000 546530000	46 HOYUNG HE HOYUNG HE 21 ADALBOGY AGAING G 17 MARGO GT PRABO G	27,07 22,19	27,99 21,94	26,55 23,69	29,13 22,29	29,75 25,82	29,95 24,86	23,12	25,09	22,94 22,55
29 (P4276: 605 - 1000 FPL35 (+) (9 FB63 THO comp ALVREF	٠ ا	1,65	3,74	١.	1,07	1,64	3 3	5	32,5	32.5	32.5	14,551	9	36,147 170,68	1553400000 1709900000	29 M2766,F2 F42766,F2 55 E9FM1:GI E9FM61.GI	34,79 36,95	27,22	25,69	27,76	24,91 29,98 24,19 29,75 25,82 25,28 27,30 27,57	27,92	22,90	20,98	23,46
19 PG275C GG5 + Incom 8 P122A 1+1C5G188 GG5 + Incom 8 P122A	:	1,74	4,60 3,64	:	1,50	1,57	6 6	10 6 20 5 3 6 20 6 3 1 15 10 7	41,7	41,7 39	49,7	17,695	9	99,657 99,593	5256600000	29 NATINGS PLATINGS STATEMENT AND PROST PARTIES STATEMENT PARTIES AND PARTIES	27,54 36,97	29,64 29,19	27,49 26.95	29,05 29,11	29,39 29,77	29,95 28,82	22,87 24,13	22,25	34,96
se [P62886 605 risese RPL30 eLISQLES Small rud SNRPN:	NRPB	2,64 0,27	3,66 2,05	:	1,64	1.43	3 3	5	24,9	24,9 26,1 17.8	26.1 17.8	12,784	0	32,604 18,379	4039900000 6741700000 3696600000 309030000 3416400000	22 M2899.03 F63999.03 23 JSQLES P JSQLES P	25,99 25,99 24,48	36,56 24,90	25,92	27,41 25.23	27,10 2475	28,26 25,05	21,90	22,11 20,56	22,47
19 P18077 605 - Ibose FPL35A 19 Q1290 Isterleat ir ILF3	:	2,78 2,90	3,71 4,59	:	1,30 2,03	1,39	4 6 20 20	20	46,4 33,6	46,4 33,6	46.4 23.6	12,538 95,337	9	48,245 223,31		44 F18077;C9 F18077;C5 94 Q12906;Q Q12906;Q	36,23 27,36	27,65 29,19	26,80 27,57	29,74 29,37	27,78 29,81	29,34 29,09	23,50	22,77 23,51	23,68 23,91
19 01547 Melevore MAGES2 1/ ABAGAC legarine KSHG1: KS 19 19 19 19 19 19 19 19	G2:KSH64	1,14	3,42 -2,34 4.54	:	0,40 2,50	1,35	4 4	3	17,2	17,2	17,2 10,5	35,277 49,911 46,109	9	46,846 67,362	\$96040000 499910000 7945700000 1179500000 4395300000	45 015479;0 015479 24 ABADADA: ADADADAS 136 ESSESSAGE ESSESSAGE	25,14	25,89 22,05 29,09	25,27 24,43 29,59	26,94 22,25 30,00	26,55	26,86 23,66	25,33	21,90 25,06 21,67	22,74 34,47 25.98
I/(QSIW3/ Double-st/STAU1 I/(RWY7C 605 /lbass RPLID	:	2,16 2,59	3,07 4,99		0,92 1,91	1,29 1,26	15 15 10 10 7 3	10	22,1 29,2	22,1 29,2	22,1	54,708 18,545	9	121,43 94,524	1176500000 4395300000 4896700000	61 Q57W3QCQSW3QQ 82 RW7CQA FRW7CGA 58 M0429 QC M0429 QC	25,19 27,86	36,94 28,25	25,19 27,62	27,61 29,92	26,94 29,72	26,61 29,25	22,42	22,91 21,69	22,99 28,04
19 [P4042] 605 risese RPL13A,R 19 [P6224] 405 risese RP515A		2,36	2,97		2,16	1,25	3 3	3	31.5 45.4	31.5 45.4	31.5 45,4	14,839	9	77,045	3553650000	ET MODRACH BESTARD	27,24	27,63	27,65	29,46	29,21	29,00	24,13	23,65	25,22
19 07602 Riscon vial RS-LID1 19 P61254 605 Fiscon RP-L26-RP		3,14	3,29 4,65	:	1,92	1,22	2 1	11 2	4.5	4.5 37.9	4.5 37.9	54,972 17,258	9	20,755	603890000 3214900000	157 F8W60', PC F8W60', PC 16 076021,13 076021,13 37 PG125433 PG125413	21,95	25,41	24,99	26,75 29,14	26,11	26,04 28,56	21,72	21,30	22,36
19 POD 1G: Cyssonia v CYRSA 1- DERAM: GDS - Ibase FIFEB		0,80 3,96	1,26 3,04		1,82	1,20	1 4	6	34,7 51,4	34,7 51,4	34,7 51,4	11,269 20,775	9	15,295 118,78	239670000 1374700000	7 F00167-2; F00167-2; 50 DERANE; F DERANES	20,90 25,96	36,38 36,12	24,24	24,74 26,69	24,32 27,46	23,92 27,60	21,77	22,02 22,83	23,42
sa IPO7302 Hatave HI H1F0 sa IPO2305 Hatave HI H1F0	:	2,24 2,56	3,15 4,09	١.	1,27	1,19	1 1	7	27,8 41,6	27,8 41,6	27,8	20,863	0	52,924 71,83	1734910010 6123610010	54 107303.F0 F07303.F0 56 162899.H7 F62899.H7	36,10 36,16	27,37	26,05	27,64 29,62	27,64 29,29	27,77	23,56	22,60 29,74	23,91
spigswaa Nackoler DEXXI triESRHV5 Case vin ali CTNNAL	*	2,05 0,60	2,14 -1,68		1,49 0,94	1,17	13 12	12	20,0 24,9	20,8 26,8	18,7 26,8	79,656 13,449	9	84,517 19,879	1447900000 8546500000	49 Q9NR30-2 Q9NR30-2 17 ESRHV7;E: ESRHV7;E	35,36 39,79	36,57 27,26	25,59 26,91	27,70 29,50	27,00 29,34	27,32 29,59	24,24 29,50	22,38 21,45	34,49 27,95
19 P.26.275 605 r lease RPL12 10 PG2915 605 r lease RPL8 10 PG2915 605 r lease RPL8	:	1,73 2,41 0.27	4.10	:	1,99	1.14	10 10	7 12 2 10 6 6 3 14 4 4 11 12 12			22.2	28,024	9	96,839 73,903	555790000 60249000 321490000 129670000 137400000 1400000 612460000 144790000 844590000 1150390000 217710000 1795600000	3 ARABAM ARABAM 54 NOVASSED NOVASSED 56 NOVASSED NOVASSED 56 NOVASSED PROPRISE 49 CONTROL CONTROL 17 ESENTIS ESENTIS 17 ESENTIS PROPRISE 18 NOVASSED PROPRISE 10 COSTA COSTA 19 NOVASSED COSTA 19 NOVASSED COSTA 10	29,59	29,65 29,55 20,21	28,23	30,61 30,69 34,15	29,35	29,35	24,60	23,03	25,39 25,39
10 Q0287: 605 risese FPU6 10 PG292: 605 risese FPL22	:	2,36	4,39 2,65	:	2,07 1,90	1,12	14 14 4 4 11 11 13 12	14	44,8 29,3	44.8 29,3	44.8 29,2	32,728 14,965	9	323,31 29,161	179.56000000 25.23600000	163 002978;FI 002978 29 N62829;C9 F62829;C5	29,84 26,74	30,33 27,50	29,80 26,82	31,40 29,39	30,86 29,00	31,06 27,98	25,89	34,30 23,98	36,69 24,72
sa (P6131) 605 - inosa 8PL15 sa (P1812) 605 - inosa 8PL7	:	1,96	3,49	:	1,80	1,10	11 11	11	41.1	41.1	41.1	29,225	9	112,91	2522800000 7028700000 1207400000	103 M131347 F6131341 124 H312444 F1812444	29,50 29,17	29,10 29,70	29,20	29,96 30,74	29,48	29,79	25,85	22,46	25,99
1/(ADADAY 405 risess \$PL17)\$P 19 (P4920 405 risess \$PL14)	:	2,13 3,85	3,76 4,64		1,72	1,09	1 3	6	29,6	29,6 21,4	29,6 21,4	19,596	9	136,33	6590600000 3643400000	71 AGAGGTWC AGAGGTWC 42 M9207 P49207	29,32	29,92 27,57	29,29 27,01	29,91 29,61	29,24 27,91	29,59	24,54	22,61	26,09 22,78
59 (P457% 405 risess RP512 59 (P467% 405 risess RP121 59 (P467% 405 risess RP121	:	2,03	3,17 5,17	:	1,66	1,07	2 3	2	30,3 41,2	30,3 41,2	30,3 41,2	18,545	9	121,01 191,04 191,04 191,05 10	3410400000 4596600000 3643600000 5413900000 485970000 219720000	57 F25399 F25399 58 F46778;G1 F46778	29,04	29,15 29,45	27,70	29,54	193,10 19475 19475 19770 24475 197700 19770 19770 19770 19770 19770 19770 19770 19770 197700 19770 19770 19770 19770 19770 19770 19770 19770 197700 19770	29,64	23,16	21,79	25,49
19 (FIGS 60: 16:00 FELD 19:10 FIGS 60: 16:00 FIGS 19:10 FIGS 60: 16:00 FIGS 19:10 FIGS 1		0,79 3,10	-1.79 4.42	١.	0,27 2,07	1,06	1 1	14	11,5	11,5	11,5	42.1	9	30,427	219720000 2325400000	20 NELEGO NAZIONO NAZIONO NAZIONE NELEGIA NAZIONE NAZIONE NAZIONE NELEGIA NELE	22,12 36,95	29,97 27,45	23,07	20,96	25,17	22,20	24,02	24,02	22,51
sa (POP47K Huslachin IVI.	•	1,71	1,42	*	0,36	1,04	1	6	14,5	14.5	14.5	10,903 68,478	9	31,552 31,642 40,697 25,991 44,922 99,607 31,591 121,15	232540000 571780000 20044000 10442000 74778000 964520000	40 MC230434 PG220444 13 MC7476 PG7476	21,12	22,52	24,25 22,97	25,75	25,56 25,00	25,30	22,27	24,01	23,25
I/(FRWSX localin-lini IGF28F2 I/(CSIQVO Decharact C7ort50 I/(ISIQVO E4C6 risona EPIZA	1 :	3,63 2,86	4,04 4,32	:	1,03	1,03	3 3	3	28,6 33,1	28,6 33,1	28,6 23,1	21,883 29,995	0	44,022 99,607	747780000 9545200000	26 CROVOLO: CRIOVIDO 26 CROVOLO: CRIOVIDO 122 MEZEZAJO: PEZEZAJO	22,67 25,09 29,79	25,84 25,59	21,97 25,64 29,81	26,63 26,63	26,23 26,23 29,78	26,59 26,57	22,54 21,07 24,90	22,59 21,88 23,54	21,63
(+)1380/44 605 + lease FFL36A,F. (+)130/809 Risson et FFL19	:	3,44 2,29	3,77 4,39	:	1,43 1,80	0,59	7 7	2 7	17,6 28	17,6 28	. 7 28	16,378 23,134	9	31,591 121,15	1367910010 4034510010	42 13KQH4;K13KQH4;K 71 13QK99;3 13QK99;3	36,11 27,67	36,83 39,34	26,23 27,84	27,69 29,12	26,93 28,67	27,52 29,02	22,51 23,20	22,24 22,46	23,10 25,03
I/(DFIDE 605 risess RPL27X I/(H79XY) Austine & DR130 I/(M6RDR 405 risess RPS5	:	2,26 3,49 2,28	5,12 4,89	:	1,42	0,99 0,97 0,96	21 21	21	23,1	23,1 23,1 86,5	23,1 23,1 86,5	130,55	9	30,385 192,91 329,34	1340910010 2321910010	75 H78KY13LQ H78KY3LQ 59 M08QFQ F M08QFQ F	27,84 36,40 26,29	28,24 36,62 28,57	27,96 26,02 27,71	29,33 27,75 29,88	27,36 27,59	26,93 26,93 29,79	22,94	22,42 20,79 21,59	22,55 20,85 22,59
sa IP62906 605 risasa RPLIDA Ir(DERFAT Helenogen HYRNED	٠ ا	3,49 1,19	4,91 2,06		1,81	0.96 0.96	3 3	8	31,3 30,6	31,3 23,4	31,3 23,4	34,831 12,552	9	66,468 15,752	954520000 134590000 403450000 44240000 134990000 22790000 47721000 457240000 256900000	80 NG2906 PG2906 18 DERSHELK DERSHELK	27,37 24,52	29,10 22,60	27,89 24,65	28,93 24,53	28,61 25,02	28,69 25,09	22,84	22,25	23,52
Ir [NERGIZ Neterocky HF18F3 Ir [C7EP63 605 riseas EPL16 Ir [C7EP63 605 riseas EPL16	:	2,97	4,24		1,17	0,93	1 1	2	19,4	19,4	19,4	36,514 14,559	9	27,534 27,534	459240000 3549000000	46 X69032;Q2X69032;Q; 52 D7090;95 D7090;95	34,98 27,70	25,59 29,19	27,53	26,28 29,99	29,51	26,15	22,30	22,62	22,61 24,24
ICLIEGO Nº 400 T- HOUSE PELLON, I CITIZATOR I RESON THE IPELLON, I CITIZATOR I RESON THE IPELLON THE IPELLON, I CITIZATOR I RESON THE IPELLON THE IPELLON THE I CITIZATOR I RESON THE IPELLON	:	1,97	1,09		1,24	0.94	12 1	12	4.6	46	4.6 31.9	96,557 47,697	9	30,385 39,291 39,94 66,869 15,752 55,253 44,601 34,835 121,146 32,793 34,683 229,17 55,893 35,351 69,128 118,23	377690000 134220000 1747300000 216450000 216650000	42 DOWN-HI SUCKNING AND A STATE OF THE ADMINISTRATION OF THE ADMIN	22,79	23,22 30,45	23,19	24,63	23,52	23,96	22,37 26,45	21,79	21,79
ty PARCE Heating a HAL ty ESSEE Decorage MYO12	*	1,67 0,72	-1,97 1,01		0,56	0,94	5 3 7 7	5 7	9,7 7,5	9,7 7,5	9,7 7,5	72,697 129,49 35,494	9	32,836 52,793 36,639	216450000 216450000	10 M2357,P4 P42357,P4 10 E99EPG;C E99EPG;C	21,89	22,12 22,95	21,39 22,70 23,49	23,63	23,59 23,50 24,43	21,99 25,29 34.53	22,62	20,64 20,64	28,07 22,60
29 OEOSO Heterogen SYNCRIP 29 OENCS Heaming SERRP1	-	2,90	2,70 0,23		1,01	0,92	20 14	14	40,1	32,6 13,2	22,6 13,2	69,632 42,426	9	229,17 57,127	3022200000 340610000	90 060506;0 060506;0 23 089651-4 089651-4	27,18 34,28	27,79 34,47	27,42 22,68	29,21 24,77	29,66	29,26	25,32	24,55	34,41 34,45
19 (MDA) - Bellator a GA (MDA)	†	0,94	1,29 2,42		0.35	0.90	3 3	4	12.2	4.2	6.2	20,252 80,439	0	25,414	307660000 3022200000 340610000 7923000000 4202900000 2157300000 600520000	6 H8123-05 H38123-05	22,24	25,72	25,52	25,44	29,32	29,52	21,15	20,15	21,59
I/[840/09 Herieski ILF2 I/[8080C/ Delloy/HI DDOST	:	3,16	2,53		1,57	0.89		6	13,7	23	23	38,91	0	55,551 69,129	2157900000	95 NOVOS - ROPES 95 NOVOS - ROPUS - C 12 MINOCOS GARCERO 135 DERGILE PORSILIE 68 ETEMS - ROTUS - C 116 NOSSIS PROSISERS 116 NOSSIS - CONUCCIO - C 110 CONUCCIO - CONUCCIO	36,93 22,31	27,56	26,71	29,09	27,85	27,95	24,36 25,26	34,61	34,63
1/105RG1: 405 risese RPS1A 1/187EMS-405 risese RPS15		3,49	5,17 5,69	l . l	1,22	0,89	4 4	- 11	41,9 53,6	41,9 53,6	53,6	12,99	9	110,37 544,95	11343000000 4647400000		29,412	29,79	29,05	29,72	29,84	30,31 29,65	22,46	21,73	21,30
39 [PG539] 605 ecidir FPLPQ RP 39 [Q9NUC SPATS2-IR SPATS2].	1 :	2,36	2,90	:	1,93	0.88		3	94.5 34.7 11.5	34.7 11,5	34.7 11,5	34,273	0	141,41 28,818	1142000000 4647400000 7139400000 739900000 190590000 170900000	76 M1333/7 P61352/0 116 M3389/9 P63393/9 0 C9 NDQ6-1 C9NDQ6- 47 C9UM545 C9UM541 41 D68993/0 D68993/0	29,51 23,74	29,06	28,66	29,50 25,12	29,52	29,86 24,50	26,25	34,91 21,30	36,39 21,91
saliQSUAC PremitNA FRPF19 I/[DGS9F3 Heterogen HNENFAS	:	1,62 2,79	1,50 2,62		0,90 1,74	0.84 0.84	3 3	8	21.8 19.6	21.8 19.6	19,6	55,18 30,302	9	59,203 54,6	1208900000 1170900000 9836000000	47 Q9UMS4,FQ9UMS4,F 41 DER9F3;DI DER9F3;DI	25,67	36,85 36,59	25.59 26,14	27,69 27,17	26,59 26,95	26,96 27,00	24,35 23,37	34,74 24,14	34,52 23,23
19 [PGS305 GDS acidic RPLP2 19 [PGS305 GDS acidic RPLP2 19 [PSS311 Zino alpha AZGP1	:	2,79	2,63	1	1,45	0,83	7	7	77,4 14,4	77,4 14,4	77,4 14,4	11,665	9	293,75	13502000000 218500000	152 PGS387,9K PGS387 11 P25311,09 P25311	29,42 22,42	20,15 22,16	29,42 22,16	20,71 22,60	20,27	20,40 23,84	27,22 24,10	26,99	27,61
sa (00092) Heserogen HNRNPU sa (19938) Galectin 1 LGA131	•	3,02 0,59	1,91		1,00	0,80	23 22	4	28,6 31,1	28,6 31,1	31,1	90,593 14,716	9	223,31 27,466	1363200000	380 Q00839;Q Q00839 24 R09392 R09392	30,29 36,34	20,91 22,54	30,25 26,12	25,87	21,10 25,86	31,15 25,63	29,57 26,65	29,66 26,50	29,50 26,72
29 [PG2752 405 risess 8956 28 [PG2752 405 risess 8956 28 [OGUW Sananami SRSN	1 :	1,57	4,69		1,63	0.79			24.5 24.9	24.5 24.5	24.5	29,69	9	396,24 367,79 236,59	11585000000 11543000000	92 OSTWER OSTWER	29,23	29,92	25,20	20,44 20,44	20,02	27,91 30,24 26,91	25,86	22,04	25,28
Ir[130;067-605 risess RPL18 Ir[DL113] Mys-aindi MY88F18	:	2,09 2,54	4,92 1,94		0,97 1,63	0,77	3 3	5	34,3 4,5	36,3 4,5	26,2 4,5	21,729 140,13	9	90,769 32,808	7208700000 186610000	41 DERROCK DERROCK 73 NOSSELE PRISSE 122 NOSSELE PRISSE 123 NOSSELE PRISSE 131 NOSSELE PRISSE 144 NOSSEL PRISSE 144 NOSSEL PRISSE 144 NOSSEL PRISSE 145 NOSSELE PRISSE 145 NOSSELE 145 N	29,49 23,53	29,29	29,96 23,04	30,15 34,39	29,75 24,13	29,24	24,85 21,15	22,22 21,93	25,29 21,30
19 JOSEWY Protein PC POF18 1/1A0A019 Res GTFex POSAF1 18 JOSEPO 605 Financ EPLEGAL	:	0,18 2,93	-1,72 -0,44 0,94		0,29 0,54 1,29	0,76 0,75 0,75		5	14,5 4,2 21,6	4.2	16.5 5.5 9.4	136	9	72,558 47,159 12,595	193240000 193240000	20 ABADENG ABADENG 7 ORESON OSESSON	23,27	23,24 22,72	25,56 21,51 22,55	23,31	23,81	23,15	21,72	20,94 23,95 21,78	23,67
sa IP2748i Calmodalii CALMLII Ir MBQ2C 405 risosa IP541	.	0,14 3,89	-0.67 3.01	.	0.23 1.45	0,74 0,73		6	69,8 41,5	59,7 41,5	59,7 41,5	16,891 13,997	0	82,221 38,798	13617900000 13617000000 17367900000 15613900000 15613900000 13613900000 13723900000 1372390000 1372390000 137390000 137390000 137390000 137390000 137390000	28 BILILION DILLIUS 27 QBWWW, QBWWW, 28 ABADDYS ABADDYS 7 QBWQD QBBQD 20 K27492 F27492 91 MBQCCSP MBQCCS 83 QL3151 QL3151 247 GJWWG, GJWWG, 185 ABADDAM ABADDAM	21,30 28,90	21,96 29,20	24,82 29,69	24,52	29,82	22,15	22,25	26,18	21,66 36,20
1997 1997	:	100 00 00 00 00 00 00 00 00 00 00 00 00	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	:	100 100 100 100 100 100 100 100 100 100	433 132 133 134 135	16 16 15 12 15 12	6 1 15		400 400 400 400 400 400 400 400 400 400	14 14 15 15 15 15 15 15	\$22,000 A	9			83 Q13151 Q13151 347 G3V4WQ1 G3V6WQ1 185 ADAQ35M AQAQ26M	200 A	32-20 (1997) (1998) (19	120 120	2055 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	2004 0 2010 0 20	20.11 (1.10	100 100	200 10 10 10 10 10 10 10	2040 A 2010 A 20
50 QSITH 88912-like 88912 50 P6006K 405 - 10030 89520	:	3,77 4,36	5.23 4,24		0,96 1,30	0.71 0.71	3 3	15 15 3	15.2 22.7	15.2 22.7	15.2 22.7	132,71 13,373	9	134,65	1362910010 5076310010	92 Q\$(TH9-2; Q;\$(TH9-2) 62 NO366,96 F60866,96	36,30 28,49	27,26 28,83	26,23	27,50 29,53	27,15	27,28 29,27	21,66 24,26	21,45 24,04	21,01 34,57
19 [P62206 405 +19330 FP523 19 [P08706 405 +19330 FP517 14 [P300/K 405 +1934 FP112	:	2,14 2,26 2,62	3,12 4,12 3,24	:	1,00	0,69	1 1	5	47,4 23,6	47,4 23,6	30,1 47,4 23,6	15,55 17,818	9	30,803 44,377 223,21 74,809 223,23 14,491	22152000000 1342900000 5094200000 5440400000 2924400000 3642000000	247 GUMMIG EGUMMIG 185 MIGOMM MOROMM 92 GETHR-2: CISTHH-2: 62 MORES PERCES PE 76 NG 206.01 FESCO 67 MOROM PERCES 58 HOROM PERCES 181 MOROM PERCES 5 MO	27,62	27,93 27,79	29,11	29,20 29,06 29,12	29,95 29,07 29,19	29,13 29,51 28,08	22,94	22,07	36,21 26,20 26,29
KU JPONENI PAN JADP PARPS IPJADAGAS ED ANIQUIS ZEPNS-CH	17:27991	2,57 0,74	5,74 0,37		0,73 0,96	0,69	26 26	26 2	29,7	29.7	29,7	113,08 59,57	9		4999900000 73225000	181 K09874 P09874 5 ADADASYY ADADASYY	27,96 22,23	29,19 22,64	29,31 22,16	29,50 23,61	29,05 22,68	29,87 22,77	22,62	21,43 21,86	24,10
19 (PK2277 405 risess 89512 19 (OSYZK Nackolar NOSSB 19 (OSYZK Nackolar NOSSB 19 (OSYZK Nackolar NOSSB	:	0,74 0,61	1,51		0,31	0,67	2 2	2	42 51	42 5.1	42 5.1	17,222 59,578 44,953	9	90,993 98,61 98,61 96,55 55,397 96,826 18,031 162,5	499900000 73225000 10912000000 121590000 2452500000 2106600000 669210000	5. MINOMENT MARDOWN 55. MINOMENT MARDOWN FASCON IN STREET, TO MARDOWN FASCON IN STREET, THE MINOMENT MARDOWN FASCON IN M	23,64	29,94 23,45 15.70	29,29	20,26 23,67 25.30	29,99 23,25 19,46	20,59 23,54 25,07	21,37	25,99	20,75
sa (C138). Katostilir PKP1 Ir (LIXCES) Myosin ve MYL134,4	YLIZEMY	1,51	-1.39 -0.42		0,28	0,65 0,65	14 14	14	21,0	21,0	32.2	90,496 20,457	9	336,55 55,297	2006600000 669210000	95 Q13035-2; Q13035-2 16 J3Q8SAP1 J3Q8SAP1	25,23 34,70	25,24 34,28	25,03	24,93	26,10	27,90 25,96	27,44	26,94 25,19	36,77 34,85
19 [PG270] 405 (1030) EP543; RF 19 [40,40;40;442] HV2 (GHV3-66	1 :	0,52	-1.69	1	0,30	0.62	2 1	14 4 11	37,3 25,9	37,3 19,8	19.8	29,597 12,698	9	18,031	9.25440000	114 M62701;C9 P62701;C5 22 ABAGC40+ AGAGC40+	29,87	29,79	29,26	23,09	24,73	20,00	26,73	26,62	22,56
19 (1970) 400 - 1980 1990 1990 1990 1990 1990 1990 1990		0,36 2,90	1,30		0,22	0,62	; ;	3 7	57,7 42	22,9 42	22,9 42	22,171	0	30,363 301,34	275450000 275450000 2004000000 5516900000 279660000 279660000 733020000 733020000	116 OSIBS NO OSISS N	34,37 30,38	34,89	21,40	24,51 21,24	24,11	23,90	20,76	24,24 25,91	21,75
sa 1043391 Heterogen HNRNFR sa (PG295/ 405 risosa RPS26/RP		3,83	3.12 1,64		1,64	0.61	24 24 4 4	18	40.8	40.8	33.3 40	20,942 13,015	9	323,31 36,37	9516900000 5219200000	183 043990 043990 88 M28540; M28540; 12 090402; 040402; 214 F239659 F239655	29,17 29,31	29,82 29,54	29,32	30.26 29.24	29.96 29.76	29,92	26,52	36,36 36,90	36,19
29 P222W 405 risess FP52 29 P222W 405 risess FP52 29 O7552 Berrier-to-BAHFL	:	1,97 3,09 2,36	2,23 2,24 3,55		1,29 0,63	0,60 0,59 0,59	1 1	12	19,2 59,2 50.6	19,2 59,2 50,6	18,2 59,2 50,6	20,699 10,059	0 0	37,976 213,49 70,555	279980000 20983200000 733020000	12 C9UNGES C9UNGES 216 F23396E9 F23396ES 24 C75531 C75531	23,93 20,26 25,15	25,01 20,75 26,16	24,06 20,11 25,27	25,26 21,20 25,76	24,71 20,85 26,02	24,72 20,95 26,69	27,21 27,21 21,83	21,46 26,46 21,20	22,69 27,52 22,99
19 (P622K 405 risosa 19516/2N 19 (P158K 405 risosa 1952	:	4,29 4,01	4,94 3,46		1,14 1,34	0,59	1 3		49,3 34,2	49,3 36,2	49,3 36,2	16,845 31,324	0	69,915 163,9	713020000 2000000 201200000 201200000 201200000 2012200000 20200000 202000000 202000000 202000000	218 F229659 F229655 24 C75231 C75231 223 M2299AF F6296M 204 F159059 F159055 221 F59019AF F29019A 22 M0ABT9 AGAMOT9 56 C02579 C0259 229 F1520479 F1520471	29,84 29,87	29,97 30,28	29,49 29,50	30,56 30,75	29.98 30.29	30,13 30,78	24,77 26,65	26,29 26,21	25,00 36,81
tri ADA140 Foulier 1 FLOT1 tri ADA240 Foulier 1 FLOT1 tra IO0252 House RI HETYLER	[0,20	4.59	١. ا	0,23	0,59 0,57 0,57	1 1	9 12 2	51.7 10.6	51.7 10.6	51,7 10,6 29,7	16.06 29,777 21.947	0	91,751 29,529 29,914	142340000 142340000	121 K9919,4C K39019,4C 12 ADAL4079 AGA14079 56 G92529 G00530	23,13	29,07 23,20 26,47	21,29	29,53 23,09 27,16	29,21 23,85 26,97	29,12 22,59 26,67	25,30 22,75 23,59	22,54 22,54	25,76 22,29 23,93
19 (P1592) Desmople DSP 19 (P2208) +RNA 2-0- FBL	-	2,13 0,49	-1,41		0,20	0,55 0,55	119 115	119	24.5	28,9 26,5	24,9	201,77 30,784	0	223,21 33,616	46114000000 284910000	1992 P15928-P1 P15928-P1 8 F22997-M F22097-M	30,11	29,97 34,26	20,75	29,72 24,19	20,61	25,25	31,95 23,84	23,27	21,45 34,18
uri ORSAN Small ruck SNEPG; SN 20 [PG287] Gaanine + GNN; GN uri E7 [PN9 Protein PR PROCEC	ELGNM	0,93 0,26	0,36 1,29		0,49	0,54 0,54 0,53	1 3	5	29,7 13,5	29,7 13,5 1,6	29,7 13,5 2,6	7,3012 37,331 309.77	0	12,479 32,139 52,695	343040000 405340000 153950000	9 QMANSE QMANSE 17 NO2879.09 F62879.05 22 E7ENS-0 E7ENS-0	34,30 34,30 36,19	34,63 34,45 36,94	24,57 23,84 26.29	24,92 24,76 27,55	25,14 23,95 26,47	25,31 25,49 26.97	22,19 23,85 26.74	21,90 23,81 22,21	22,96 23,84 26,56
so (PGS97) Gamine + GMR2;GM tr (CTEMO Protein Pt. PROCCC (FLAGALEC Calingsin CTS0 so (PGS96) 405 - House IPS18 so (1997) - 3 - Nydrany HS017811 so (PGS05) Proteine F NGP2	:	3,42	-2.64 4.21		0.27	0.53			48,7	17 49,7	17 49,7	43,688 17,718	9	49,758 72,673	353510000 1298300000	17 AGALBOGV AGALBOGV 104 NG2369,13 PG2369	22,97 30,04	21,99 30,59	22,21 29,88	21,84	22,63 30,42	24,28 30,63	25,44	34,99 25,54	34,76 36,57
pp (Q997): 3-hydraxy HS01761) pp (PAGOS) Propente 2 HOP2 pr (ESWER' Magnin to 1997)	i	1,02	-1.05 1.34	١. ا	1,25	0,51 0,51		9	28,6 14,6	28,6 14,6	28,6 14,6	25,984 99,972 16.20	9	28,276 69,642 36,722	290200000 715220000	14 Q997142; Q997142; 46 R6997-2; P6697-2; 46 R69187-1; F89187-1	25,55	34,49 36,00	22,47 25,64	24,49 26,21 25,43	24,44 26,29 25.57	24,23 26,00 25.00	25,03 24,94 24.77	23,13	34,49 34,99
sa (Q1661) Extracellul ECVII sa (Q7605- Signal rec: \$8972		0.26 1,27	-0.79 2,30		0.19	0,49	14 14	16	4.5 20	6.5 30	6.5	46,099 74,615	9	13,403	148380000 2911500000	15 016619-2 016619-2 85 0760940 0760940 213 F23026-92 F23026-91 52 050919/0 05099/0	22,90 27,51	30,30	22,20	23,04		21,03	24,34 25,26	21,69	21,70
sa (P2263): Neterogen HMENPA) Ur (OSOPIS ENA-bind): EALY		2,71 2,74	0,56 3,91	:	1,78	0,49	14 14	16 13 11	45,6	45,6	43,1	37,429 24,665	0	26,76	6057600000 2345600000	213 R2302692 R23626F0 52 Q5QF19;Q Q5QR9;Q	27,58 38,33 27,35 27,76 25,71	28,53 27,94	28,29	29,05	27,96 28,84 27,90 28,16 27,62	29,57	27,77	27,85	27,84
19 POS105 Protein SD 510048	ı : I	1,72	-1,54	L	0,27	0.46		6	42,9	40,9	40,9	10,834	9	41,06	2570700000	67 RGS109 FGS109	25,72	25,83	25,89	52,60	27,62	25,59	26,87	28,16	27,04

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In ICSP712 Profile-2:1 PEN2	9,61	1 -0.20	1 . 1	1 121	ا مه ا	1 4	,	,	51.6	19.9	19.9	9.7992		12.91	199190000	25 (91712)92 (91712)93	25,19	25.29	25.15	25,02	24.44	24.25	25.83	25.19	25.22
sa IPGS782 Kerasin, by ERTER	9.23	0.16		1.00	-0.60	12	10	- i	21.9	28.1	24.4	49.057		202,49	1759200000	92 105792:59 105792:59	27.19	26.70	26.59	26.62	26.12	25.91	26.34	36.66	27.00
sa (09949) Protein de FARE7 4	2.76	11.29		193	-0.60		4	- 2	32.8	32.8	32.8	19,891		32.522	913500000	35 Q99497;K;Q99497;K	25.42	25.02	25.45	24.81	24.71	24.60	26,59	36.61	36,30
19 19926G 295 riboso MEPS25 +	3.10	3.94		1.77	-0.61		5		20.6	30.6	30.6	20.116		47.97	360060000	17 192663:57 192663:57	25.62	25.72	25.23	24.97	24.85	24.94	21.47	22.45	21.14
10 P3194/ Creame 1-CASP34 +	1.23	-1.90		0.44	-0.61	10	10	10	29.2	29.2	39.3	27,679	0	79.947	1223000000	76 931984 932944	20.52	31.25	26.09	24.44	24.57	24.03	27.16	26.87	36.24
I/(HOYAU, Blacessyn BLM	0.47	-0.38		0.96	-0.61	1	1	1	1.8	1.8	1.8	144,47	0.00129	7,3967	11125000000	24 HOYNUS P HOYNUS P	29.27	29,49	29.46	29.31	29.77	28.30	29,33	29.86	29.57
sp (Q9Y2Q 285 riboso MRP528 +	2,23	2,71		1.59	-0.61	1 3	5	á	22.5	23,5	19.2	20.942		29.569	260130000	28 091209:H 091209:H	20.97	25.24	24.64	24,34	28.28	24.23	22.36	21.32	22.95
29 IPGLAX Proving my MAGORIA +	2.34	2.11		2.06	-0.62	4	4	4	49.4	42.4	49.4	12,944	0	25.219	273290000	14 161326-2 161326-2	26.91	21.97	25.20	24.24	24.55	24.64	20.90	22.71	22.14
sal01473* Lamin Bire LBA.	2.05	0.94		1,26	-0.62	4	4	4	9.1	9.1	9.1	20,702	0	25,766	155920000	11 014739-01014739-0	23.71	23.75	23,46	23,39	22.65	23.03	22,67	23.02	22,42
10 109Y3D 285 risosa MEPS23 +	3.72	3.51		1.12	-0.62	1 4	4	4	25.2	25.2	25.3	21.77		25.175	393620000	26 091209:12 091209:13	25.22	26.02	25.39	24.92	25.05	24.90	22.06	21.86	22.20
19 IO96UP Kinecula ETN1	9.67	1.03		0.36	-0.62		à	- 1	2.2	2.2	2.2	149.61	0	19,944	279960000	7 QB6UF2-2 QB6UF2-2	26.16	23.92	23.90	21.92	22.82	24.26	22.09	20.24	22.21
UTC90171 Proffingly PEN2 4	2.12	-0.95		0.69	-0.63	s	5	2	52.7	52.7	20.9	9.8402	0	40.232	2282900000	65 C9017:65 C91017:65	27.11	36.57	26.67	26.87	26.01	25.59	27.82	27.81	27.55
sa IP49752 Transmer TMEDSD	9.36	0.22		9.87	-0.66	1 2	ä	á	12.7	12.7	12.7	28,976		29.544	194900000	15 M9755-G: M9755-G3	22.96	23.72	23.51	22.79	22.70	22.63	22,34	23.93	22.17
19 JQ7Z2E Pago trass POGZ +	1.35	3.05		1.69	-0.66	7	7	7	7.2	7.2	7.2	145.29	0	62,216	757650000	32 072313-5; 072313-5	25,72	36,22	25.97	25.51	25.25	25.19	22.20	21.59	31.99
(+140A084 Serins/are SRSF7 4	4.24	2.46		2.44	-0.67	- 6	6		43.1	43.1	43.1	15,763	0	61.24	2470300000	69 A0A084[1] A0A084[1]	29.25	28.17	27.93	27.35	27.45	27.54	25.83	25.49	35.64
sa 100159 Hydraxyn HMGCS1	9.07	0.12		0.44	-0.0	1 2	à	ā	10.6	10.6	10.6	57.292		29.621	254290000	15 001591 001591	26,23	23.60	23.94	22.69	24.06	22.02	24.25	24.47	22.69
19 Q1679, NADH den NDUFA9	9.22	0.29		1.07	-0.0	2	2	2		9		42,529	0	12.294	117920000	12 Q16795 Rt Q16795;R	22,11	23,07	23,15	22,69	22,61	22,02	23,23	23,14	22,12
sa IP31151 S-adenosy MATZA 4	1.70	1.46		0.49	0.68		a	4	13.2	13.2	13.2	43.66	0	25.019	199650000	20 101153-83 231153-83	22.50	22.83	23.45	21.19	22.84	22.72	24.67	24.65	23.84
spig9852 Endoplesy ER940	9.83	-0.94		0.75	-0.69	i i	á	á	8.4	8.4	8.4	46,971		21.507	304570000	18 098526 098526	24,19	23,96	23,00	23,30	23,17	22,61	24,01	25,31	34,64
I/[FSGWX Chromodo CHD&CHD +	1.94	2.10		1.92	-0.70		5	- 2	2.1	2.1	2.1	217.1	0	33,064	221210000	12 FSGW05;# FSGW05;#	20.64	25,02	24.52	24.15	22.89	24.06	22.25	22.04	23.59
sa 106120 PremiliNA FRRIB	1.99	2.21		0.64	-0.72	- 6	6	- 6	3.9	3.9	3.9	273.6	0	35.259	229650000	15 O6F209:E O6F209	24.28	34.84	24.45	2473	23.52	23.15	21.85	23.22	21.87
sa 1096IM Caramasa CH6MP1 +	2.90	2.99	I . I	1.99	-0.72	1 3	5		7.2	7.3	7.3	99,099		39.713	392250000	29 096443:5 096143	25.51	25,81	25.66	24.92	25.22	24.75	22.43	22.15	22.41
sa IR35996 365 proper PSMC2 +	1.31	0.34		1.03	-0.72	4	4	4	10.4	10.4	10.4	49.632	0	36.291	509700000	8 F35998 F3 F35998 F3	25.33	25.27	25.00	24.94	24.71	23.99	24.75	21.86	34.97
U-IE7EWK AT P-dege- DHS36	1.24	0.43		0.47	-0.74	1		- 1	5.0	5.9	5.8	91.43	0	18.74	80020000	6 DIWOLD DIWING	22.92	22.86	22.59	23.23	22.03	20.89	22.57	22.14	22,37
sa 10728Y Heassams HDGFRP2 +	2.19	2.36	I . I	1.60	-0.75	3	5	- 5	11.6	116	11.6	78.229		29.369	252810000	17 072895-2 072895-2	25.35	24.89	24.63	24.26	24.24	24.12	22.17	22.22	22.00
sa 1990-226 L-lectate d LDHA	9.36	0.37		0.99	-0.75		9		27.4	27.4	27.4	36,639	0	73.545	1143900000	62 P00339 P0 P00339 P0	25.64	25.80	26.64	25.51	25.39	24.92	25.31	26.25	25.42
OTHORNIC RINA sindi ENPSI. 4	3.00	3.99		0.98	-0.79		a	- 1	18.5	18.5	15.2	36 561	0	25.257	566590000	26 HORMON HORMON	25.92	X 18	25.63	24.46	25.27	25.63	21.63	21.35	22,78
1/1892254 30 tDate/ HSPE1	9.19	0.13		0.74	-0.90	2	2	2	86.8	46.9	46.8	5.1999		13.455	182570000	13 892254 NG 882258 NG	22.91	22.89	24.34	22.65	22.50	22.59	23.24	23.33	23.17
sp (Q1542: Scaffold at SAFR; SAFR +	5.32	4.29		1.79	-0.90		9		14.2	14.2	16.2	95.19	0	123.93	954170000	39 Q158262; Q15826-2	26.72	26.85	26.66	25,56	26.16	26.11	22.69	22.34	22,25
(-1K7CSCB Bleaviscin BLMH	1.13	1.68		0.27	-0.81		3	i i	13.9	13.9	13.9	36,738	0	33.874	373920000	29 170308-0 170308-0	22,41	23.59	24.75	24.04	20.79	23.49	25.34	25.54	34.92
saj07561 USswell v SNRNP200 +	3.57	1.19		2.15	-0.80		9		4.5	4.5	4.5	264.5	0	57.667	423040000	35 0756430 075649	25.05	25,26	25.30	24.49	28.55	24.09	24.03	20.11	23.90
spjQ96DY Perserko PTCD3 +	5,27	5,04		2.12	-0.98	7	2	2	13.2	13,2	13,2	29,549	0	56.577	539230000	40 094517;0 094517	25,86	26,31	26,10	25,34	25,39	25,05	20,96	21,11	21,14
sa IPQ7192 U leciale d LDWB	0.87	103		0.90	0.96				30.2	30.2	30.2	36.638	0	69.536	1351100000	51 107195-09 107195-09	26.67	26.06	27.18	26.52	25.32	25.09	24.78	X 12	25.72
sa (P7834) Ribonacki (PP30	1,22	104		0.94	-0.96	1 1	à	á	17.9	17.9	17.9	29,321		17.436	94662000	5 978346,97 978946,97	23,99	23,29	23,23	23,29	22,07	22,56	23,03	22,43	21,93
tr[138712 Serine/larg SRSF1 +	2.29	1.09		1.37	-0.99	, a	ä	ä	12.6	13.4	12.4	29.329	0	31.615	291050000	26 138712:00:138712:00	25.90	25,29	24.59	24.21	28.32	23.59	22.19	23.79	23.36
29 [P82931 285 riboso MRP56 4	1.06	1.71		2.16	-0.90	2	2	2	16	16	14	14,226	0	12,452	112700000	12 192502 192932	24.06	34.14	23.77	23.14	23.30	22.85	22.90	22.53	21.51
sa IP4975/ RNA-sindi RRAGS	0.34	-0.33	I . ∣	131	-0.94	1 1	à	á		5	- 5	100.19		17.52	529940000	15 R09756 HC R09756	25.29	25.76	25.09	25.19	24.32	24.22	26.59	25.41	25.52
prigSE972 Lemine-et: TMPO	9.03	0.01		0.52	-0.95	2	2	2	6.8	6.9	6.9	86,306	0	96.751	190390000	12 GSE972:N GSE972:N	22.24	23.62	23.76	22.21	23.43	21.03	22.59	23.53	22.03
(FIBIANDI DNA Nelice MCMS 4	1.49	-0.93		0.45	-0.97	2	7	2	14.2	14.3	14.3	77.592	0	42,718	489440000	10 81AH01:K 81AH01:P	24.75	34.29	24.60	24.60	21.77	24.37	25.83	25.60	34.99
sa (Q1336: Devidrage DHR52 +	2.63	104	I . ∣	166	-0.97		à		31.1	31.1	31,1	29,926		201.14	1303200000	63 Q13068(C)Q13368(C)	27,47	27,06	26,76	26,34	26,19	25,56	25,83	25,94	36,13
10100562' Denostion EEFIA2	9.89	1.89		0.70	-0.99	11		,	20.7	7.0	7.8	50.47	0	17.748	311190000	22 005639 005639	24.40	23.26	24.46	22,04	23.60	23.53	20.96	21.60	23.93
USARSHA Appelosic ACRS 4	2.66	4.22		0.89	-0.99	12	12	12	10.7	10.7	10.7	145,44	0	79,197	743329999	42 500000 540000	26.02	27,49	25.94	25.71	25.51	25.26	22.07	22.91	21.00
UTERPROP REFERENCE RCLAFS +	2.97	2.49		1.39	-1.00	- 6	6	- 6	10.2	10.2	10.2	89.134	0	49.263	540040000	33 595000 50 595600 50	25.85	36,09	25.90	2471	25.60	24.54	23.46	22.93	23.93
sa IP124% Ribonacki RHH1	0.23	0.72		0.43	-1.00	,		,	6.5	4.5	65	49.972	0	M 103	218240000	10 713409:09 713409:09	24.35	22.79	24.29	21.90	24.52	22.21	24.26	20.90	34.21
pp (Q1541' Celeanin-J CHH3)	0.14	0.11		0.61	-1.02	4	4	4	19.0	19.0	19.8	31.39	0	27.973	353640000	19 015417-3: 015417-3	24.77	24.44	24.37	24.60	23.83	22.09	23.89	21.63	34.74
19 (P\$1.296-285 riboso DAP3 +	2.92	3.94		1.15	-1.00		9	9	32.5	32.5	32.5	41.044	0	110.74	1003000000	39 851399-2; 851399-2;	27,34	27,13	26.23	26,71	26,01	25.37	23,13	23.99	22,55
28 109 129 285 r Boso MEPS23 4	2.05	2.20		1.37	-1.04	2	2	2	17	17	17	12,629	0	12.544	129120000	11 091291:01 091291:0	24.11	24.84	23.65	23.23	22.22	23.02	22.49	21.43	22.09
as IM0225 Troomales CCT6A	1.18	0.28		0.66	-1.06	4	4	4	10.9	10.9	10.9	58,024	0	36,172	500570000	32 140227:84 840227:84	25.30	25.27	25.23	24.85	25.02	22.76	25.00	25.16	34.78
sp (P31151 Protein 5 tr 5 100A7 +	2,42	-1,99		1,94	-1,30	5	5	5	30,7	30.7	30.7	11,471	0	32,923	1303100000	64 F91151,Q1 F91151	20,64	25,25	24,93	23,75	24.20	23,63	26,57	27,36	36,61
UTFSH2F4 C-1-letren MTHFD1 4	1.60	0.61		1.35	-1.30	6	6	4	7.1	7.1	7.1	110.61	0	29.921	510250000	26 F5H2F4:91 F5H2F4.91	25.34	25.19	25.30	24.74	24.32	23.46	24.44	21.99	31.55
19 01497- Protein on PPP1R12A 4	1,91	2,21		1,28	-1,33	5	5	5	6.4	4.4	6.4	111,10	0	31,224	181510000	10 0149742; 014974-2	24,47	31,67	24,73	24,01	23,83	22,74	21,47	23,18	22,60
salQ8M45 3-5 exoria- ERIS +	4.32	15,70		0,57	-1,30	20	20	20	54.4	54.4	54.4	40,063	0	321,94	4,75555+11	409 GBN49,ES GBN49	36,44	36,39	34,30	35,75	33.85	33,92	20,49	20,42	29,11
LYTINGREED Rep-value-RARM	9.92	-1.29		0.69	-1.22		2		23.9	23.9	22.9	22,429	0	27,024	250300000	12 NOTE US NO YORK US NO	24.09	21.93	22.01	21.97	21.74	20.62	24.19	21.09	23.59
IF ADADAC Nucleoler - NOSCI 4	4,19	2,69		1,97	-1,34	11	11	11	15,5	15.5	15.5	24,543	0	75,165	1493300000	MONDADA MONDADA 03	27,55	27,81	27,40	25,91	26,76	24,38	24,74	25,07	34,90
sa (P49455 Signal reg) 5899	0.95	-0.23		0,72	-1,25	3	3	- 3	30.2	30.2	30.2	10,112	0	34,605	316570000	15 849459,59 849459	24,41	24.14	24,26	24,53	22,66	21,89	24,57	24,24	34,59
PERSMOR GTP-bindi-TAN 4	1.65	-0.72		0.69	-1.22	4	4	4	23.2	23.2	22.2	26.224	0	25.236	499240000	32 85M0F5:1 85M0F5:1	26.81	31.59	24.91	24.62	23.99	21.72	25.14	25.64	25.70
19 QOJES Lamin-82 LMN82	1,23	-0.47	ı	1,28	-1,35	7	6	4	11,9	10,6	10,4	69,949	0	39,09	249570000	27 003252 003252	23,67	23,84	23,98	22,50	21,63	23,31	24,45	23,99	34,46
sa (P6103) Ras-velate: RAB30 +	2.80	2,28	ı	1,05	-1,41	4	3	- 3	20,5	15	1.5	22,541	0	30,221	206990000	16 R61026 R61026	23,63	24,41	24,36	22,01	23.86	22,30	22,07	21,94	21,55
I/1070096 Casele Kin-CSNK2AD	2,59	2,90		2,74	-1,46	- 5	5		18,7	18,7	19,7	45,31		52,062	399650000	29 E7EU96;Q E7EU96;Q	25,62	25,82	25,73	24,26	24,54	23,90	22,11	23,45	22,23
19 PS2996 Activated 15 UB1	0,43	0,65	1	0,91	-1,52	1 3	à	- 1	26	26	24	14,295	0	17,104	226400000	6 153999 153999	24,45	31,00	24,02	23,94	21,36	22,91	24,28	22,39	34,14
sa 106029. Zinc finger ZFC3HS	9,67	-4.19		0,21	-1,96	1	1	1	0.9	0.9	0.9	226,35	0,004762	6,5244	10100000000	34 060293 060299	29,59	20,46	29,99	21,73	29,15	22,49	31,19	30.15	29,14
(r)A0A075 285 riboso MRPS21 4	3,24	2,33	1	1,04	-1,09	2	2	2	29,9	29,9	29,9	10,610	0	15,999	159900000	12 AGAG7587 AGAG7587	20,56	31,91	24,29	24,26	21,76	21,99	22,47	22,02	22,27
I/(CSIRID Serins/arg SRSFDD;SF 4	1,92	3,01		0,74	-1,90	4	4	4	26,2	24,2	26,2	22,912	0	28,064	426790000	15 Q5IRIQ;07 Q5IRIQ;07	26,310	36,33	24,49	25,19	24,42	21,09	22,40	22,46	22,21
1/189FR30_405 r Bross_FAU +	4.13	5,46	ı	0,32	1,95	2	2	2	11,2	11.2	11.2	10,905	0	12,721	28666000000	16 E9 PR30; PG E9 PR30; PG	27,40	29.29	27,52	28,32	29,17	20,96	22,59	22,25	21,99
19 O4354 PremRNA DKN15 4	2,02	4,01	ı	0,91	-1,97	4	4	4	6	6	- 4	90,932		27,222	421910000	12 04343 043143	25,43	25,67	25,41	23,39	21,86	25,25	22,02	21,39	20,07
15 AUGUST Tabulis of TUBARC TUBARC	0,51	1,39		1,64	-2,66	12	2	2	41,1	5,2	5.3	49,959	0	15,764	492500000	25 Q13740;Q Q13740;Q	25,34	25,40	23,23	22,27	22,00	21,74	22,49	22,16	25,17