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1 **Regulation of the conserved 3'-5' exoribonuclease**

2 **EXOSC10/Rrp6 during cell division, development and cancer**

3

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16

17 **ABSTRACT**

18 The conserved 3'-5' exoribonuclease EXOSC10/Rrp6 processes and degrades RNA, regulates
19 gene expression and participates in DNA double-strand break repair and control of telomere
20 maintenance *via* degradation of the telomerase RNA component. EXOSC10/Rrp6 is part of
21 the multimeric nuclear RNA exosome and interacts with numerous proteins. Previous
22 clinical, genetic, biochemical and genomic studies revealed the protein's essential functions
23 in cell division and differentiation, its RNA substrates and its relevance to autoimmune
24 disorders and oncology. However, little is known about the regulatory mechanisms that

25 control the transcription, translation and stability of EXOSC10/Rrp6 during cell growth,
26 development and disease and how these mechanisms evolved from yeast to human. Herein,
27 we provide an overview of the RNA- and protein expression profiles of EXOSC10/Rrp6
28 during cell division, development and nutritional stress, and we summarize interaction
29 networks and post-translational modifications across species. Additionally, we discuss how
30 known and predicted protein interactions and post-translational modifications influence the
31 stability of EXOSC10/Rrp6. Finally, we explore the idea that different EXOSC10/Rrp6
32 alleles, which potentially alter cellular protein levels or affect protein function, might
33 influence human development and disease progression. In this review we interpret
34 information from the literature together with genomic data from knowledgebases to inspire
35 future work on the regulation of this essential protein's stability in normal and malignant
36 cells.

37

38 *Key words:* EXOSC10, Rrp6, RNA exosome, loss-of-function allele, 5-fluorouracil.

39

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61

62 **I. INTRODUCTION**

63 When eukaryotic cells divide and differentiate they synthesize, process and degrade
64 messenger RNAs (mRNAs) and non-coding RNAs (ncRNAs) in a highly coordinated
65 fashion. The conserved 3'-5' exoribonuclease ribosomal RNA-processing protein 6 (Rrp6,
66 termed exosome component 10 (*EXOSC10*) in mammals) participates in these processes as a
67 catalytic and RNA-binding subunit of the nuclear RNA exosome (Kilchert, Wittmann &
68 Vasiljeva, 2016; Wasmuth & Lima, 2017) and in cooperation with other regulatory RNA
69 binding proteins (Wagschal *et al.*, 2012; Eberle *et al.*, 2015). There is also evidence that Rrp6
70 acts independently of the RNA exosome (Callahan & Butler, 2008; Wang *et al.*, 2020).
71 Extensive work in yeast, fly and mouse model organisms has identified a wide range of
72 protein-coding and non-coding *EXOSC10*/Rrp6 substrates (Pefanis *et al.*, 2015; Xu *et al.*,
73 2009; Neil *et al.*, 2009; Kuai, Das & Sherman, 2005; Gudipati *et al.*, 2012; Schneider *et al.*,
74 2012; Lardenois *et al.*, 2011; Davidson *et al.*, 2019), revealed a mechanism for how the

75 protein selects its substrates (Axhemi *et al.*, 2020), and identified numerous protein–protein
76 interactors (Meldal *et al.*, 2015). Moreover, the three-dimensional structure of the enzyme
77 associated with the RNA exosome, its co-factors and RNA substrates has been resolved using
78 samples from bacteria, yeast and humans (Makino, Baumgartner & Conti, 2013; Midtgaard *et*
79 *al.*, 2006; Wasmuth, Januszyk & Lima, 2014). The structure and subunit composition of the
80 RNA exosome is remarkably conserved between yeast and humans (Fig. 1A) (Burley *et al.*,
81 2021).

82 EXOSC10/Rrp6 contains several conserved functional domains [see www.ebi.ac.uk/interpro/
83 (Mitchell *et al.*, 2019)] (Fig. 1B). In yeast Rrp6, the polycystin 2 N-terminal (PMC2NT)
84 domain is bound by Rrp47 (C1D in mammals) (Stead *et al.*, 2007). The central region of
85 Rrp6 is highly homologous to that of *E. coli* RNase D and includes the exoribonuclease
86 (EXO) domain and the regulatory helicase and RNase D carboxy terminal (HRDC) domain.
87 The EXO domain harbours the DEDD active site (carrying aspartate and glutamate residues)
88 that is conserved in a variety of enzymes, such as the Klenow fragment of *E. coli* DNA
89 polymerase I (Derbyshire, Grindley & Joyce, 1991). Negatively charged DEDD residues
90 coordinate two metal ions in the active site, which deprotonate and activate a water molecule
91 that carries out a nucleophilic attack on the RNA phosphodiester bond (Steitz & Steitz,
92 1993). Rrp6 is also classified as a DEDD-Y enzyme, because an additional tyrosine residue
93 (Y361) is employed near the active site to orient the nucleophilic water and stabilize the
94 transition state intermediate (Phillips & Butler, 2003). The HRDC domain interacts *via* a
95 conserved aspartic acid residue (D457) within the EXO domain and regulates its activity
96 (Midtgaard *et al.*, 2006). The C-terminal domain formed by Rrp6 residues 518–623
97 comprises the exosome associating region (EAR) domain, which interacts with the RNA
98 exosome (Wasmuth *et al.*, 2014; Wasmuth & Lima, 2017; Makino *et al.*, 2013). The highly
99 basic and unstructured Rrp6 C-terminal tail (termed Lasso; Fig. 1B) comprises 100 amino

100 acids. It binds RNA, activates the exosome and contains a putative bipartite nuclear
101 localization signal (NLS) (Wasmuth & Lima, 2017; Phillips & Butler, 2003). There are no
102 structural data showing direct contacts between the human EXOSC10 catalytic domain and
103 the RNA exosome core subunits; however, Weick *et al.* (2018) proposed that mammalian
104 EXOSC10 is located at a similar position to yeast Rrp6, given that the orthologous proteins
105 show similar ultra violet (UV) light-crosslinking patterns and are inhibited by an altered
106 exosome core central channel [Fig. 1A; (Weick *et al.*, 2018) and references therein].
107 Despite playing important and highly diverse cellular roles and evolutionary conservation,
108 budding yeast *RRP6* is not essential for mitotic cell division at permissive temperature;
109 however, *rrp6* mutant cells display temperature-sensitive (*ts*) phenotypes (Assenholt *et al.*,
110 2008; Briggs, Burkard & Butler, 1998). Recent publications reveal a novel role for Rrp6 in
111 regulating protein glycosylation and maintenance of cell wall stability; these findings
112 elegantly explain why *rrp6* mutant cells fail to divide at elevated temperatures (Wang *et al.*,
113 2020; Novacic *et al.*, 2021). Furthermore, *rrp6* mutant cells show decreased growth rates as
114 compared to wild-type cells even at the permissive temperature; a phenotype that is
115 exacerbated on media containing ethanol or glycerol as carbon sources and on synthetic
116 complete medium (Qian *et al.*, 2012; Briggs *et al.*, 1998). It is interesting that slow growth
117 and temperature sensitivity appear to be at least partially unrelated phenotypes, since *rrp6*
118 mutants lacking catalytic activity also grow slowly but show a much weaker *ts* phenotype
119 (Assenholt *et al.*, 2008; Phillips & Butler, 2003). Finally, diploid yeast cells lacking Rrp6 fail
120 to proceed normally through meiosis and gametogenesis (Lardenois *et al.*, 2011). Genetic
121 data indicate that the gene is essential for development in *Drosophila melanogaster*
122 (Nakamura *et al.*, 2008). More specifically, fly Rrp6 is important for cultured cell
123 proliferation, mRNA splicing and maintenance of heterochromatin (Graham, Kiss &
124 Andrulis, 2009; Eberle *et al.*, 2010, 2015). Consistently, very recent work on the malaria

125 parasite *Plasmodium falciparum* also revealed a role for Rrp6 in the epigenetic control of
126 heterochromatic gene expression *via* a long non-coding RNA (lncRNA) termed RNA of
127 unknown function 6 (RUF6) (Fan *et al.*, 2020). The mouse ortholog is required for
128 spermatogonial cells, oocyte maturation and long-range enhancer function in B-cells and
129 pluripotent embryonic stem cells (Jamin *et al.*, 2017; Wu & Dean, 2020; Pefanis *et al.*, 2015).
130 In the case of human *EXOSC10*, initial evidence argued against an essential role in cell
131 division but later work showed that the gene is required for cell division in mitotically
132 growing cultured cells (Blomen *et al.*, 2015; van Dijk, Schilders & Pruijn, 2007). The initial
133 study by van Dijk *et al.* (2007) was based on small interfering RNA (siRNA)-mediated
134 mRNA knock-down experiments that revealed strong inhibition of cell division for *EXOSC2*
135 (*RRP4*), *EXOSC4* (*RRP41*), and *EXOSC9* (PM/Scf-75), while only moderate inhibition was
136 observed following depletion of *EXOSC10* (PM/Scf-100) (see fig. 3C in (van Dijk *et al.*,
137 2007). van Dijk *et al.* concluded that this phenomenon was reminiscent of the non-essential
138 growth phenotype initially reported for yeast mutants lacking *RRP6* (Briggs *et al.*, 1998) but
139 they pointed out that residual *EXOSC10* mRNA levels may have prevented a more
140 pronounced effect on mitosis. Later work by Blomen *et al.* (2015) employed a high-
141 throughput gene-trap retrovirus mutagenesis screen using cultured haploid cells to identify
142 loci important for optimal cell division, which clearly identified human *EXOSC10* as being
143 important for progression through the mitotic cell cycle.
144 More recently, the protein was also shown to be involved in the repair of DNA double-strand
145 breaks (DSBs) by homologous recombination (HR) in a cancer cell line. These studies
146 revealed that recruitment of radiation damage 51 (RAD51) to DSBs requires the 3'-5'
147 exoribonucleolytic activity of *EXOSC10* and the authors proposed that this might occur by
148 processing of small non-coding RNAs [sncRNAs, also called damage-induced RNAs
149 (diRNAs)] (Marin-Vicente *et al.*, 2015; Domingo-Prim *et al.*, 2019). This interesting finding

150 implicates EXOSC10 in a ncRNA-based DNA repair mechanism, which might be relevant
151 for cancer cell resistance against DNA-damage-inducing chemotherapeutical agents, such as
152 5-fluorouracil (5-FU), which was proposed to decrease the efficiency of HR repair (Srinivas
153 *et al.*, 2015). This is in keeping with inhibition of the exoribonucleolytic activity of
154 EXOSC10 by 5-FU (Silverstein, Gonzalez de Valdivia & Visa, 2011; Kammler, Lykke-
155 Andersen & Jensen, 2008), and recent work that revealed EXOSC10 to be a prognostic
156 marker for liver and thyroid cancer (Uhlen *et al.*, 2017) and a cancer driver gene with
157 tumour-suppressive potential in bladder urothelial carcinoma (Wang *et al.*, 2018).
158 Clinical interest in EXOSC10 also includes autoimmune disorders because the gene is
159 associated with polymyositis/scleroderma overlap (PM/Scl) syndrome, which is why the
160 protein also referred to as PM/Scl-100. PM/Scl syndrome affects skeletal muscle and skin
161 and involves the production of antibodies against EXOSC10 (Bluthner & Bautz, 1992; Ge *et*
162 *al.*, 1992; Mahler & Raijmakers, 2007). Finally, recent work has demonstrated a role for the
163 protein (as part of the RNA exosome) in frontotemporal lobar degeneration (FTLD) and
164 amyotrophic lateral sclerosis (ALS) *via* degradation of pathogenic repeat RNA and telomere
165 maintenance *via* degradation of the telomeric RNA component (hTERC) (Kawabe *et al.*,
166 2020; Kroustallaki *et al.*, 2019; Shukla *et al.*, 2016).
167 Previous excellent reviews have covered the cellular functions and catalytic activity of
168 EXOSC10/Rrp6, its role in autoimmune conditions and its three-dimensional structure when
169 associated with the RNA exosome (Fox & Mosley, 2016; Mahler & Raijmakers, 2007; Staals
170 & Pruijn, 2011; Januszyk & Lima, 2014; Chlebowski *et al.*, 2010). Here, we focus on what is
171 known about, or points to transcriptional, translational and post-translational mechanisms
172 controlling the expression and stability of EXOSC10/Rrp6 across species from yeast to
173 humans. We elaborate on possible effects of the protein's cellular concentration on mitotic
174 cell division, meiotic cell differentiation, response to nutritional signalling and cancer. We

175 interpret data from the primary and secondary scientific literature, general and species-
176 specific genome annotation and RNA expression databases and genome viewers that
177 accompany individual RNA/protein/network profiling studies. These sources are referenced
178 in the text and summarized in Table 1; in important cases report pages from databases are
179 provided as annotated and/or visually enhanced figures or supporting information. The reader
180 is referred to annotation databases for gene and protein nomenclature in different species.

181

182 **II. YEAST *RRP6***

183 **(1) Budding yeast *RRP6* mRNA transcription during the mitotic and meiotic cell cycles**

184 The *RRP6* 5'-region contains a core promoter (Chromosome XV: 326787–326828, + strand)
185 located immediately upstream of the single-exon open reading frame. An adenine at position
186 326792 is thought to be the consensus transcription start site (TSS) determined in several
187 growth conditions, as annotated in the Yeast Transcription Start Site database [Fig. 2A;
188 www.yeastss.org (McMillan *et al.*, 2019)]. A nucleosome-depleted region (NDR) upstream
189 of the core promoter contains multiple transcription factor (TF) binding sites at its boundary
190 (Venters *et al.*, 2011). Consistently, the epigenetic marks tri-methylation of lysine 4 on
191 histone H3 (H3K4me3) and acetylation of lysine 14 on histone H3 (H3K14ac) that are
192 associated with active transcription are enriched within the *RRP6* promoter and the gene's 5'-
193 region (Kirmizis *et al.*, 2007; Pokholok *et al.*, 2005).

194 *RRP6* shows a mitotic expression pattern that peaks during the G2/M phase similar to B-type
195 cyclin *CLB2* [Fig. 2B; <https://cyclebase.org/> (Santos, Wernersson & Jensen, 2015)], while its
196 mRNA concentration decreases during meiotic M-phase and then increases again during later
197 post-meiotic stages [Fig. 2C, upper panel; see also Saccharomyces Genomics Viewer (SGV)
198 at www.germonline.org (Lardenois *et al.*, 2010)]. *RRP6* is broadly expressed in all haploid
199 and diploid yeast mating types under various growth, stress-response and developmental

200 conditions [for an overview, see Saccharomyces Genome Database (SGD) at
201 www.yeastgenome.org (Wong *et al.*, 2019)]. As one would expect based on these diverse
202 expression patterns, a search for DNA binding regulators that participate in the transcriptional
203 control of *RRP6* using Yeastract yielded 26 transcription factors, including some that are
204 known to act during mitosis and meiosis [(Reimand *et al.*, 2010; Lardenois *et al.*, 2011);
205 www.yeastract.com (Teixeira *et al.*, 2018)]. The presence of DNA binding motifs recognized
206 by the G2/M phase cell cycle regulator forkhead 2 (Fkh2) is consistent with fluctuating *RRP6*
207 mRNA expression levels observed in haploid cells undergoing the mitotic cell cycle
208 (Lardenois *et al.*, 2011; Cho *et al.*, 1998; Granovskaia *et al.*, 2010; Spellman *et al.*, 1998;
209 Brar *et al.*, 2012) www.yeastract.com). A deeper understanding of forkhead transcription
210 factors Fkh1 and Fkh2 or other DNA binding activators in the transcriptional regulation of
211 *RRP6* – and the biological significance of this control for cell division and differentiation –
212 remains to be established.

213 The sense *RRP6* mRNA overlaps an antisense meiotic unannotated transcript (MUT). This
214 RNA is up-regulated in meiosis and peaks during spore formation but its function is as yet
215 unknown (Fig. 2C; see also SGV at www.germonline.org). *MUT1312* is considered to be a
216 ncRNA, confirmed by ribosome profiling data that show only weak ribosome binding to the
217 extreme 5'-end of the transcript (Brar *et al.*, 2012). ncRNAs are important for the onset of the
218 meiotic developmental pathway (Moretto *et al.*, 2018; van Werven *et al.*, 2012). It currently
219 remains poorly understood how and why lncRNAs are induced during meiosis and spore
220 formation in budding yeast and what roles they might play.

221 In fission yeast, genome-wide RNA profiling studies using microarrays show little change of
222 wild-type *rrp6* mRNA levels during the mitotic cell cycle (see online Supporting
223 Information, Fig. S1A). However, the *rrp6* transcript is down-regulated during chemical and
224 temperature stress (Fig. S1B). The mRNA also decreases approximately twofold in

225 sporulating as compared to vegetatively growing wild-type cells but not in a mutant strain
226 lacking the serine/threonine protein kinase Pat1, which is a negative regulator of meiosis
227 [Fig. S1C; see also <http://bahlerweb.cs.ucl.ac.uk/cgi-bin/SPGE/geexview> (Mata *et al.*, 2002;
228 Rustici *et al.*, 2004; Chen *et al.*, 2003)]. These data indicate that fission yeast *rrp6* mRNA
229 levels are affected by environmental cues. It is currently unclear if this is due to
230 transcriptional repression or altered mRNA stability. Given the importance of RNA
231 processing and degradation in response to environmental cues that alter cell fate from
232 division to differentiation, studies that provide further insights into the transcriptional
233 mechanisms that control *rrp6* in fission yeast are warranted.

234

235 **(2) Budding yeast *RRP6* mRNA and Rrp6 protein levels are affected by nutritional**
236 **signals**

237 Diploid budding yeast cells enter the meiotic developmental pathway in the absence of
238 nitrogen and a fermentable carbon source (Neiman, 2011). Rrp6 is important for efficient
239 meiosis and spore formation and exerts its function associated with the RNA exosome during
240 early meiosis (Frenk, Oxley & Houseley, 2014; Lardenois *et al.*, 2011). *RRP6* mRNA peaks
241 during fermentation in rich medium in the presence of glucose (YPD), decreases during
242 respiration in pre-sporulation medium where glucose is replaced by acetate (YPA) and
243 further diminishes during the onset of meiosis, before it increases again during late meiosis
244 and spore formation (Fig. 2D) (Becker *et al.*, 2017; Brar *et al.*, 2012; Lardenois *et al.*, 2011).
245 It is noteworthy that the carbon source-dependent decrease, but not the late-meiotic increase
246 of *RRP6* mRNA is observed in a sporulation-deficient *ume6* mutant, which lacks the DNA
247 binding subunit of a trimeric complex containing the conserved histone deacetylase Rpd3 and
248 the co-repressor Sin3 (Fig. 2D) (Kadosh & Struhl, 1997; Strich *et al.*, 1994).

249 The level of Rrp6 protein decreases accordingly when cells switch from fermentation to
250 respiration but then drops below the threshold level of detection by Western blot as cells
251 progress through later stages of meiosis and spore formation (Frenk *et al.*, 2014; Lardenois *et*
252 *al.*, 2011). This pattern coincides with the meiotic ncRNA expression program where early,
253 middle and late ncRNAs accumulate to peak levels akin to the classical expression profile
254 observed for meiotic mRNAs, including stable/cryptic unannotated transcripts (SUTs/CUTs)
255 and a novel subclass of meiotic unannotated transcripts (MUTs) (Lardenois *et al.*, 2011; Chu
256 *et al.*, 1998; Primig *et al.*, 2000); for review, see (Mitchell, 1994). Taken together, the data
257 indicate that Rrp6 is important for normal progression through meiotic development and that
258 the protein becomes unstable during later stages of gametogenesis (Frenk *et al.*, 2014;
259 Lardenois *et al.*, 2011).

260 These observations raise three questions. First, what renders Rrp6 unstable in late-
261 differentiating diploid cells? A plausible answer to this question is that Rrp6 is targeted for
262 late meiotic destruction *via* the ubiquitin and/or the small ubiquitin-like modifier (SUMO)
263 pathway, in a similar way to how Ume6 is targeted by the anaphase-promoting
264 complex/cyclosome (APC/C) during the onset of meiosis (Lardenois *et al.*, 2011; Mallory,
265 Cooper & Strich, 2007).

266 Second, what prevents the protein's re-accumulation during spore formation even though the
267 mRNA continues to be detectable and appears to associate with ribosomes until the end of the
268 process (Fig. 2C) (Brar *et al.*, 2012)? It is unclear why – in contrast to Ume6 – Rrp6 is not
269 detectable during advanced stages of gametogenesis. Perhaps the proteolytic activities
270 targeting Ume6 and Rrp6 are not identical. We note that Ume6 and Rrp6 are both
271 ubiquitinated but Rrp6 is also SUMOylated (see Section II.3 for more details).

272 Third, why did meiotic protein down-regulation of Rrp6 evolve, that is, do yeast cells need to
273 degrade Rrp6 to progress through meiosis and spore formation rapidly and efficiently? Two

274 possibilities that are not mutually exclusive are conceivable. First, in the absence of Rrp6
275 activity, a subset of non-coding substrate RNAs may be able to accumulate to greater levels
276 and fulfil certain regulatory roles (Davis & Ares, 2006; Gudipati *et al.*, 2012; Wyers *et al.*,
277 2005; Schneider *et al.*, 2012). Second, the production of ribosomes may be attenuated
278 because ribosomal RNAs are not processed and ribosomes are not efficiently assembled any
279 more (Briggs *et al.*, 1998). The latter would make sense given that the endpoint of
280 sporulation is the formation of transcriptionally and translationally inert gametes, as opposed
281 to the rapidly growing and dividing cells that are generated during mitosis.
282 Growth under suboptimal conditions is also a negative regulator of protein translation, since
283 this process consumes a lot of energy. Therefore, Rrp6 protein levels should be negatively
284 affected by limiting growth conditions. A recent meta-analysis of protein profiling data by
285 Ho *et al.* (2018) quantified the number of yeast proteins per cell in the S288C reference strain
286 cultured in rich medium (YEPD; (Webb *et al.*, 2013; Lee *et al.*, 2011; Nagaraj *et al.*, 2012),
287 synthetic defined (SD; (de Godoy *et al.*, 2008; Thakur *et al.*, 2011), synthetic complete (SC;
288 (Peng *et al.*, 2012) and minimal C-limiting medium (F1; (Lawless *et al.*, 2016) (Ho,
289 Baryshnikova & Brown, 2018). The level of Rrp6 protein was highest in three studies that
290 measured the protein in YEPD [9205, 9194 and 7640 proteins/cell (p/c)], progressively lower
291 in SD (6865 and 4681 p/c) and SC (2296 p/c) and at its lowest level during starvation in F1
292 (880 p/c) (Fig. 2E; see also www.yeastgenome.org). It would be interesting to determine if
293 the nutritional signal that controls Rrp6 protein levels acts *via* transcriptional or post-
294 translational mechanisms (or a combination of both).
295 These findings are consistent with the roles of Rrp6 in ribosome biogenesis and accumulating
296 evidence that the control of protein translation is different in dividing, starving and
297 differentiating cells (Jin & Neiman, 2016). The hypothesis that persistent Rrp6 protein levels
298 might perturb gametogenesis is verifiable by monitoring meiotic landmarks in cells that over-

299 express *RRP6* and strains that harbour stable *rrp6* mutant alleles that fail to be degraded as
300 efficiently as the wild-type protein.

301

302 **(3) Yeast Rrp6 is subject to multiple post-translational modifications**

303 Rrp6 undergoes at least four post-translational modifications. First, by protein
304 phosphorylation and de-phosphorylation, which are catalysed by kinases and phosphatases,
305 respectively. These enzymes are often involved in regulating a variety of functions and
306 physical properties of their target proteins; large-scale analyses of this type of modification
307 by phosphoproteomics have identified numerous substrates (reviewed in (Ribeiro *et al.*, 2017;
308 Offley & Schmidt, 2019). Specifically, Rrp6 is phosphorylated at serine (S110, S412, S417,
309 S640, S645, S709), threonine (T410, T520) and tyrosine (Y406) residues as shown by mass
310 spectrometry-based analyses (Synowsky *et al.*, 2006; Albuquerque *et al.*, 2008; Swaney *et al.*,
311 2013; Holt *et al.*, 2009). However, in the case of Rrp6, the roles of amino acid
312 phosphorylation have not yet been functionally analysed, except in *Schizosaccharomyces*
313 *pombe* where the *rrp6*^{S112A} mutant allele did not show a measurable effect on RNA
314 degradation or processing (Telekawa, Boisvert & Bachand, 2018).

315 Second, Synowsky *et al.* (2006) reported the presence of an N-terminal acetyl group in Rrp6.
316 The acetyl group (provided by acetyl-coenzyme A) is covalently attached either to an N-
317 terminal α -amino group or to the ϵ -amino group of lysines. Protein acetylation and
318 deacetylation is catalysed by acetyltransferases and deacetylases that typically act in a
319 balanced fashion to control numerous biological processes (Drazic *et al.*, 2016). This
320 modification could be relevant for the roles of Rrp6 since acetylation is critical for protein
321 function and stability.

322 Third, Rrp6 is a substrate for protein SUMOylation and Rrp6 interacts directly with
323 suppressor of mif two 3 (Smt3), a protein of the SUMO family (Gonzales-Zubiate *et al.*,

2017; Pabst *et al.*, 2019; Wohlschlegel *et al.*, 2004). During this modification process SUMO proteins are attached to their targets by multi-component complexes. Global SUMOylation of proteins occurs under conditions of genotoxic stress, hypoxia, heat shock and hypothermia (Enserink, 2015). SUMOs are conserved in eukaryotes and play roles in development and disease by affecting their target protein's function, stability, interactions and localization. Importantly, SUMOs are also involved in the DNA damage response, which is particularly relevant for cancer therapies that introduce DNA lesions (Jalal, Chalissery & Hassan, 2017; Flotho & Melchior, 2013) and the assembly of messenger ribonucleoproteins (mRNPs), which is a critical step in the regulation of gene expression (Bretes *et al.*, 2014). SUMOylation may therefore be relevant for the role of EXOSC10/Rrp6 in the response to chemotherapy by DNA damage-inducing drugs such as 5-FU and the quality control of mRNP assembly (Marin-Vicente *et al.*, 2015; Domingo-Prim *et al.*, 2019; Mosrin-Huaman, Honorine & Rahmouni, 2009; Stuparevic *et al.*, 2013).

Fourth, Rrp6 is ubiquitinated at as yet undetermined lysine (K) residues (Kolawa *et al.*, 2013). Ubiquitylation requires so-called E3 ligases (writers), ubiquitin-binding effectors (readers), and deubiquitylases (erasers) that work together to establish the protein concentration, conformation and localisation needed for robust cell growth and development (Oh, Akopian & Rape, 2018). Rrp6 physically interacts with binds ubiquitin ligase 2 (Bul2), a subunit of the reverses spt phenotype 5 (Rsp5) E3-ubiquitin ligase important for growth during stress (Frattini *et al.*, 2017; Kaida, Toh-e & Kikuchi, 2003), the ubiquitin-activating enzyme (E1) Uba1, the ubiquitin conjugating enzyme (E2) Ubc1, which is associated with the APC/C (Gonzales-Zubiate *et al.*, 2017; Girard, Tenthorey & Morgan, 2015) and Grr1, a glucose-responsive subunit of the Skp1-Cullin-F-box protein (SCF) ubiquitin-ligase complex (Gonzales-Zubiate *et al.*, 2017; Fey & Lanke, 2007).

348 The ubiquitination of Rrp6 is intriguing because it resembles the case of Ume6, which
349 represses meiosis-specific genes and meiotic mRNA isoforms with extended 5'-untranslated
350 regions (5'-UTRs) during mitosis in cooperation with Rpd3 and Sin3 (Lardenois *et al.*,
351 2015*b*; Strich *et al.*, 1994). In the absence of glucose, Ume6 is acetylated by the Spt-Ada-
352 Gcn5 acetyltransferase (SAGA)-dependent complex, which stimulates the protein's
353 ubiquitination by the cell division cycle 20 (Cdc20)-activated ubiquitin ligase APC/C during
354 the onset of meiosis (Mallory *et al.*, 2012; Law *et al.*, 2014; Mallory *et al.*, 2007). Contrary to
355 Rrp6, Ume6 is degraded during meiosis but subsequently re-accumulates at later stages of
356 spore maturation and ascus formation; this is likely due to its important role during spore
357 germination (Lardenois *et al.*, 2011; Mallory *et al.*, 2007; Strich, Khakhina & Mallory, 2011).
358 Given the collective evidence, it is a distinct possibility that Rrp6 might be controlled in part
359 by regulated and targeted proteolysis, especially under stress conditions which typically
360 involve extensive remodelling of the transcriptome. The precise mechanisms and functional
361 implications of ubiquitination and SUMOylation in fine-tuning Rrp6 protein levels during
362 mitotic growth, meiotic development and stress responses remain to be determined.

363

364 **(4) Rrp6 is a hub protein and its stability depends on protein interactions**

365 Protein networks based on direct or indirect physical contacts have been established using
366 yeast two-hybrid (Y2H) screens and tandem affinity purification (TAP) tagging (Williamson
367 & Sutcliffe, 2010). The former is based on *in vivo* binding of two fusion proteins that are
368 tethered to DNA binding and gene activation domains, respectively. The latter works by co-
369 immunoprecipitating (Co-IP) a given target protein with its interactors and identifying them
370 by mass spectrometry. Initial studies based on these methods revealed that the majority of
371 yeast proteins can form complexes (Goll & Uetz, 2006). More recent work in the field of
372 computational biology has addressed the crucial question of how relevant such interactions

373 are under physiological conditions and if it is possible to predict the robustness of network
374 architecture under different conditions using systems biological approaches (Rizzetto &
375 Csikasz-Nagy, 2018). While protein networks built from Y2H and Co-IP data reveal
376 interactions that are physically possible, they do not prove that these complexes are stable *in*
377 *vivo*; they rather inspire further *in vivo* work to gain insights into a given protein's functions.
378 One particularly interesting aspect of network biology focusses on the extent to which
379 interactions of multi-subunit complexes (notably hub proteins) are conserved. The
380 extraordinary degree of functional and structural conservation of the RNA exosome and the
381 positioning of its core- and catalytic subunits makes it a prime example for an essential
382 protein complex (Weick *et al.*, 2018; Wasmuth *et al.*, 2014).

383 The frequency of protein–protein interactions varies from at least one to large numbers in the
384 case of so-called hub proteins that tend to be essential for cell growth and development
385 (Ekman *et al.*, 2006; Song & Singh, 2013). The BioGrid database currently references for
386 Rrp6 502 direct and indirect physical interactions of which 74% were identified by high-
387 throughput methods [Fig. 3A; www.thebiogrid.org v. 3.5.184 (Oughtred *et al.*, 2019); the
388 reader is referred to the Saccharomyces Genome Database (SGD; www.yeastgenome.org) for
389 full gene names]. This vast network reflects a wide variety of known, putative and possibly
390 novel functions attributed to Rrp6 in RNA processing and degradation (Csl4|EXOSC1, Dis3,
391 Mpp1, Mtr3|EXOSC6, Mtr4, Nab2, Nab3, Pab1, Pab2, Rrp4|EXOSC2, Rrp40|EXOSC3,
392 Rrp42|EXOSC7, Rrp43|EXOSC8, Rrp45|EXOSC9, Rrp46|EXOSC5, Rrp47|Lrp1|C1D, Ski7,
393 Ski6|EXOSC4), transfer RNA (tRNA) synthesis and maturation (Cdc60, Dps1, Dtd1, Gln4,
394 Grs1, Gus1, Hts1, Ils1, Krs1, Lhp1, Ths1, Hyp2, Yhr020w, Ynl247w), ribosome biogenesis
395 and translation (Eft1, Gis2, Krr1, Nop4, Nop15, Nop53, Pno1, Rps8a, Rrb1, Sbp1, Ssb2,
396 Urb1, Utp14, Utp21), chromatin assembly and modification (Hht1, Hht2, Isw1, Rtt109,
397 Tra1), mitotic and meiotic chromosome cohesion (Hop1, Irr1), transcription (Rpc40, Rpo21),

398 mitotic and meiotic recombination (Exo1, Hpr1, Mei5, Mnd1, Rad54), and the onset of
399 meiosis and gametogenesis (Emi2) (Fig. 3A; www.thebiogrid.org v. 3.5.184).

400 Similar experiments in *Schizosaccharomyces pombe*, *Drosophila melanogaster* and humans
401 unsurprisingly also identified interactions between EXOSC10/Rrp6 and RNA exosome
402 subunits and associated co-factors (Fig. 3B–D). Interestingly, in fission yeast Rrp6 protein
403 interacts with Mmi1, Red1 and Red5, which are components of a regulatory complex that
404 actively degrades meiotic RNAs in mitotically growing cells (Fig. 3B) (Harigaya *et al.*, 2006;
405 Sugiyama & Sugioka-Sugiyama, 2011; Sugiyama *et al.*, 2013; Shichino *et al.*, 2020; Lee *et al.*,
406 *et al.*, 2020). These data highlight the role of Rrp6 as a suppressor of meiosis by targeting
407 transcripts that are involved in this conserved developmental pathway.

408 While certain interactions, for example with the RNA exosome core, mediate the
409 exoribonucleolytic activity of Rrp6 in budding yeast, others mostly affect its stability. For
410 example, Rrp6 and Rrp47 form a heterodimer after they are independently imported into the
411 nucleus (Feigenbutz *et al.*, 2013a; Feigenbutz *et al.*, 2013b; Kumar *et al.*, 2002). The N-
412 terminal domains of Rrp6 and Rrp47 assemble into a globular heterodimer formed mostly by
413 intertwining alpha helices. This complex ensures mutual stabilization of the proteins and
414 forms a conserved surface groove, which tethers the helicase Mtr4 to the core RNA exosome
415 (Feigenbutz *et al.*, 2013a; Feigenbutz *et al.*, 2013b; Stead *et al.*, 2007; Stuparevic *et al.*, 2013;
416 Dedic *et al.*, 2014; Schuch *et al.*, 2014). The interaction is conserved between EXOSC10 and
417 the human ortholog of Rrp47 (C1D), which is implicated in DNA repair and RNA processing
418 (Fig. 3D) (Schilders, van Dijk & Pruijn, 2007).

419 Deleting either *RRP6* or *RRP47* mutually destabilizes the proteins: in *rrp6* mutant cells,
420 Rrp47 is undetectable and in *rrp47* cells the level of Rrp6 is reduced by up to 90%
421 (Stuparevic *et al.*, 2013; Feigenbutz *et al.*, 2013a; Feigenbutz *et al.*, 2013b; Stead *et al.*,
422 2007). This effect is predominantly due to altered protein stability, because *RRP6* and *RRP47*

423 mRNA levels in general do not decrease under the experimental conditions used to determine
424 protein concentrations (Stuparevic *et al.*, 2013; Feigenbutz *et al.*, 2013a). In the absence of
425 Rrp6, Rrp47 is degraded *via* a proteasome-mediated pathway, since treating *rrp6* mutant cells
426 with the proteasome inhibitor MG132 increases Rrp47 protein levels (Feigenbutz *et al.*,
427 2013b). Rrp6 interacts with Rrp47's functionally important N-terminal Sas10/C1D domain
428 (amino acids 10–100) (Costello *et al.*, 2011; Mitchell, 2010) *via* its N-terminal PMC2NT
429 (amino acids 13–102) domain that was shown to be necessary and sufficient for normal
430 expression of Rrp47 protein (Stead *et al.*, 2007). Rrp47 is thought to function specifically to
431 support the activities of Rrp6, since *rrp47* mutant cells show the same temperature-sensitive
432 and RNA-processing phenotypes as *rrp6* cells (Mitchell *et al.*, 2003; Erdemir *et al.*, 2002;
433 Phillips & Butler, 2003). In line with their non-redundant functions, a *rrp47-rrp6* double-
434 mutant strain is viable and displays a growth rate similar to that of the *rrp6* single-mutant
435 background (Mitchell *et al.*, 2003). Finally, Rrp47 levels are not determined solely by Rrp6
436 since its overexpression does not increase the cellular Rrp47 protein concentration
437 (Feigenbutz *et al.*, 2013b).

438 In summary, Rrp6 is a highly interactive protein that associates with a wide variety of co-
439 factors, which mediate its stability and influence its enzymatic activity when exerting diverse
440 cellular functions in RNA processing and degradation, chromatin modification, gene
441 expression and DNA recombination.

442

443 **III. MAMMALIAN *EXOSC10***

444 **(1) *EXOSC10* expression varies among tissues, during mitotic cell cycle stages and** 445 **within cancer/control sample pairs**

446 Human *EXOSC10* is located on chromosome 1 and spans 33 kb. The gene comprises 22
447 exons and encodes 11 transcripts [Fig. 4A; www.ensembl.org (Yates *et al.*, 2020)].

448 Transcriptional data for *EXOSC10* indicate that the full-length transcript
449 (ENST00000376936.8) is expressed in all tissues assayed [www.gtexportal.org (Yizhak *et*
450 *al.*, 2019); Fig. S2A]. Expression levels determined by RNA sequencing are fairly
451 homogenous and vary approximately within a twofold range among somatic and reproductive
452 organs, with the exception of a peak signal in the cerebellum [www.proteinatlas.org (Uhlen *et*
453 *al.*, 2016); Fig. S2B].
454 *EXOSC10* mRNA levels fluctuate moderately during the mitotic cell cycle and show a profile
455 reminiscent of B-type cyclin (*CCNB1*) with a G2/M peak (www.cyclebase.org; Fig. 4B). The
456 biological significance of this variation is unclear but the timing of transcriptional mRNA
457 induction is consistent with the role of *Drosophila* Rrp6 in mitotic chromosome segregation
458 where the protein shows a dynamic pattern of localisation (Graham *et al.*, 2009).
459 If *EXOSC10* plays a role in human cell cycle progression it should be deregulated in
460 abnormal cells that undergo rapid and uncontrolled cell divisions. Indeed, expression patterns
461 in healthy *versus* tumour cells available at the Tumor Immune Estimation Resource (TIMER,
462 <https://cistrome.shinyapps.io/timer/> (Li *et al.*, 2017)] show a significant difference in the
463 signal distributions. *EXOSC10* expression is elevated in bladder urothelial carcinoma,
464 cholangiocarcinoma, colon adenocarcinoma, oesophageal carcinoma, head and neck
465 squamous cell carcinoma, liver hepatocellular carcinoma and lung and stomach
466 adenocarcinoma samples. The only exception is renal cancer (kidney chromophobe) that
467 shows the opposite pattern (Fig. S3). Increased expression in liver cancer is associated with
468 *EXOSC10* being an unfavourable prognostic factor (www.proteinatlas.org).
469 Why do certain tumours contain more *EXOSC10* mRNA than healthy cells? An obvious
470 reason might be that normal differentiated post-mitotic cells express the gene at lower levels
471 than actively growing and dividing cancer cells, because differentiated cells do not need
472 *EXOSC10* activity to be at peak levels, notably as far as ribosome biogenesis is concerned.

473 However, the pattern observed in renal cancer argues against this possibility. Other
474 explanations might be that epigenetic or regulatory alterations occurring in cancer cells affect
475 the transcriptional activation of *EXOSC10* or that its mRNA is destabilized, for example by
476 pairing with regulatory microRNAs (MIRs).

477 TarBase [www.microrna.gr/tarbase (Karagkouni *et al.*, 2018)], a resource that provides
478 experimentally validated miRNA/mRNA interactions, annotates 37 miRNAs for *EXOSC10*
479 that were analysed in 13 cell lines from eight different tissues (bone marrow, cervix, embryo,
480 intestine, kidney, mammary gland, pancreas, pleura). The majority of these interactions
481 (19/37) were observed in three kidney cell lines (HEK293, HEK293T, 293S) (Karginov &
482 Hannon, 2013; Grosswendt *et al.*, 2014; Krishnan *et al.*, 2013). Among them, hsa-miR-182-
483 5p is associated with anti-cancer drug resistance and hsa-miR-17-5p is an oncogenic MIR
484 (Dhawan *et al.*, 2018; Uhr *et al.*, 2019). The RNA interactome database RISE
485 [<http://rise.life.tsinghua.edu.cn> (Gong *et al.*, 2018)] provides two experimentally validated
486 interactions with MIRs for *EXOSC10* mRNA: hsa-miR-222 and hsa-miR-193b. The former is
487 relevant to cancer radiotherapy and the metastasis of oral tongue squamous cell carcinoma
488 (Shi *et al.*, 2019; Liu *et al.*, 2009). Down-regulation of the latter MIR affects blood cancer
489 (Gonzalez-Gugel *et al.*, 2013).

490 These examples illustrate a promising route for further analyses that aim at a better
491 understanding of the post-transcriptional regulation of *EXOSC10* by MIRs in normal and
492 cancer cells.

493

494 **(2) The promoter architecture of *EXOSC10* associates the gene with transcription**
495 **factors involved in cell division, development and cancer**

496 The *EXOSC10* promoter region is poorly characterized and no enhancer regions have been
497 described. Regulatory motif predictions associate 11 DNA-binding transcription factors (TFs)

498 with the up-stream promoter region of *EXOSC10* [www.swissregulon.org (Pachkov *et al.*,
499 2013); Fig. S4; please refer to NextProt (www.nextprot.org) for full gene names]. This group
500 includes KLF4, which is a transcriptional regulator that reprograms differentiated cells into
501 induced pluripotent stem (iPS) cells when co-expressed with POU5F1/OCT4, SOX2 and
502 MYC (reviewed in (Takahashi & Yamanaka, 2016). KLF4 was also shown to play a role in
503 cell proliferation and early cerebellar development (Zhang *et al.*, 2015). Critically, the
504 predicted KLF4 motif is confirmed by *in vivo* protein/promoter DNA interaction data from
505 normal skin cells, keratinocytes and in a pancreatic ductal adenocarcinoma (PDAC) cell line
506 [see ChIP-Atlas, which provides access to a comprehensive set of published chromatin
507 immunoprecipitation and sequencing (ChIP-Seq) data sets; <https://chip-atlas.org> (Oki *et al.*,
508 2018)]. These results argue in favour of a role for KLF4 in the activation of *EXOSC10*,
509 notably in brain cells, where the exoribonuclease is present at high levels (see Fig. S2B and
510 <http://proteinatlas.org>).

511 The remaining 10 TFs are potentially interesting candidates for regulators contributing to the
512 transcriptional control of *EXOSC10* because of their association with cell division,
513 development and cancer (Table 2).

514

515 **(3) EXOSC10 protein levels vary among healthy tissues, fluctuate during mitotic cell** 516 **division and decrease progressively during meiotic development**

517 Currently, three human EXOSC10 protein isoforms are known. They comprise 885
518 (Q01780), 860 (Q01708-2) and 679 (B4DKG8) amino acids [www.proteomicsdb.org
519 (Samaras *et al.*, 2020)]. Two large-scale mass spectrometry-based protein-profiling analyses
520 of the human proteome across several tissues and cell lines, show that EXOSC10 levels vary
521 approximately twofold between cytotoxic T-lymphocytes [5.2 expression units (eu)] and the
522 adult spinal cord (2.8 eu) (Fig. 5A,B; (Wilhelm *et al.*, 2014; Kim *et al.*, 2014);

523 www.proteomicsdb.org; www.humanproteomemap.org). The protein expression studies show
524 the same results apart from bladder, heart and rectum samples where the protein was detected
525 in only one of the analyses (Fig. 5A,B). These protein data are concordant with similar
526 patterns observed at the mRNA level and concur with the genes' important role in
527 mammalian embryonic and adult cell growth and division (Wu & Dean, 2020; Jamin *et al.*,
528 2017).

529 In asynchronously growing and non-transformed mammalian cells, EXOSC10 proteins are
530 localized in the nucleus, particularly to distinct nucleolar-like structures in all cells except
531 those in mitosis (Fig. 5C). Nucleolar localization is consistent with earlier reports using
532 immunofluorescence, cell fractionation and transfection experiments (reviewed by
533 (Raijmakers, Schilders & Pruijn, 2004) and data from a proteomic profiling experiment
534 designed to identify nucleolar proteins (Tafforeau *et al.*, 2013). Furthermore, we and others
535 have reported that yeast Rrp6 colocalizes with the nucleolar RNA polymerase Rpa190 [see
536 fig. 9 in (Okuda *et al.*, 2020)] and that mouse and human EXOSC10 colocalize with the
537 nucleolar markers nucleophosmin (B23) (Jamin *et al.*, 2017), nucleolin (C23) (von Kopylow
538 *et al.*, 2012) and fibrillarin (FBL; Fig. 5D). Finally, a nuclear/nucleolar distribution is also at
539 least partially confirmed by immunohistochemical assays provided by the Human Protein
540 Atlas (HPA), although we note that the HPA also shows localization in the cytoplasm
541 (www.proteinatlas.org). The possibility that EXOSC10 is also present in the cytoplasm was
542 raised by Lejeune, Li & Maquat (2003), who showed that nonsense-mediated mRNA decay
543 in mammalian cells involves decapping, deadenylating, and cytoplasmic exonucleolytic
544 activities. This is contradictory to the presence of a putative nuclear localization signal in
545 EXOSC10 (Raijmakers *et al.*, 2004). We have been unable to confirm any cytoplasmic
546 localization of EXOSC10 in a range of normal and transformed cells. In a more detailed
547 analysis using non-chemically induced cell synchrony, the nucleolar localisation is observed

548 weakly in cells in G0 (at quiescence) but rapidly rises and then remains constant in nucleoli
549 throughout G1, S-phase and G2, in which yeast Rrp6 and mammalian EXOSC10 associated
550 with the RNA exosome and the ribonuclease Dis3 exert roles in nucleolar ribosomal RNA
551 (rRNA) processing (Davidson *et al.*, 2019; Okuda *et al.*, 2020; Tafforeau *et al.*, 2013; Briggs
552 *et al.*, 1998).

553 However, as cells enter mitosis, in prophase EXOSC10 begins to redistribute from nucleoli
554 within the nucleus; by prometaphase nucleolar staining is lost completely and the protein
555 distributes equally between mitotic cytoplasm and near the condensed but not aligned
556 chromatin. It also clearly does not localize with CCNB1 at any point in the mitotic process
557 although the mRNA expression profiles of these genes are similar (Figs 4B and 5C). At
558 metaphase, EXOSC10 surrounds the chromosomes aligned on the metaphase plate and
559 segregates with the chromosomes during anaphase A and B. No nucleolar staining for
560 EXOSC10 protein can be detected until late telophase and the formation of the midbody.

561 These findings suggest that with the loss of nucleolar integrity, human EXOSC10 localizes to
562 the prophase nucleus and then the mitotic cytoplasm after nuclear envelope breakdown.

563 However, a proportion of EXOSC10 remains associated with the condensed DNA although
564 not in the form of specific association with the chromosomes since the staining around the
565 condensed DNA is diffuse. These localization patterns raise the possibility that EXOSC10
566 exerts molecular functions in the mitotic cytoplasm and on condensed mitotic chromosomes
567 that are independent from the nuclear RNA exosome.

568 During mouse meiosis and gametogenesis, *Exosc10* mRNA is highly expressed in mitotic,
569 meiotic and early post-meiotic germ cells (peaking in spermatocytes and then diminishing in
570 round spermatids); the protein's cellular concentration decreases in early round spermatids
571 and subsequently drops below the threshold level for detection at late post-meiotic stages of
572 the developmental pathway (Jamin *et al.*, 2017; Chalmel *et al.*, 2007) (www.germonline.org).

573 This is reminiscent of the pattern observed for budding yeast Rrp6 in diploid cells, which
574 undergo meiosis and spore formation, and points to an evolutionarily conserved mechanism
575 of regulation of Rrp6 and EXOSC10 during gametogenesis (Lardenois *et al.*, 2011).

576

577 **(4) EXOSC10 undergoes extensive protein modifications**

578 According to PhosphoSite, EXOSC10 is monomethylated on arginine 79 and tri-methylated
579 on lysines 754 and 835 [Fig. 6A; www.phosphosite.org (Hornbeck *et al.*, 2015); (Larsen *et*
580 *al.*, 2016; Santos *et al.*, 2015; Cao, Arnaudo & Garcia, 2013)]. Arginines are methylated by
581 protein methyltransferases (PRMTs) that transfer methyl groups from S-adenosyl methionine
582 to the side chains of arginine residues. Arginine methylation is important for protein–protein,
583 protein–RNA, and protein–DNA interactions, and is therefore of considerable clinical
584 interest, especially in oncology (reviewed in (Guccione & Richard, 2019).

585 EXOSC10 contains 28 serine, threonine and tyrosine residues that are phosphorylated (Fig.
586 6A; www.phosphosite.org). These amino acids include tyrosine 90, which is located in the N-
587 terminal PMC2NT domain, serines 370/402 and tyrosines 419/448 in the DNA_pol_A_exo1
588 3'5'-exoribonuclease domain and serine 530 in the HDRC domain (Fig. 6A). Human
589 EXOSC10 is also phosphorylated on serine 821, which is conserved in the mouse (Zhou *et*
590 *al.*, 2013). However, we are unaware of any data to date on the functional significance of
591 these phosphorylation events.

592 Four lysines in EXOSC10 are acetylated, including lysine 109 located in the PMC2NT
593 domain; the remaining residues are at the extreme N- (lysine 37) and C-termini (lysines
594 851/865) (Fig. 6A). Again, nothing is known about the roles of these modifications during
595 cell division, differentiation and in malignant tissues.

596 21 lysines modified by ubiquitin are referenced in the PhosphoSite database (Fig. 6A;
597 www.phosphosite.org). In this context it is noteworthy that the proteasome, a multi-subunit

598 protease that normally degrades ubiquitinated proteins but that can also act as an
599 endonuclease within mRNA surveillance, co-localizes with EXOSC10 in cultured HeLa
600 cancer cells (Brooks, 2010). EXOSC10 is in fact ubiquitinated by the APC/C and therefore
601 likely is targeted for proteolytic destruction during the mitotic cell cycle (Kim *et al.*, 2011).
602 Given the classical role of the APC/C as a cell cycle regulator that targets cyclins (regulatory
603 subunits of cell division kinases) it is conceivable that EXOSC10 is subject to cell cycle
604 stage- or developmental stage-specific proteolytic destruction (Watson *et al.*, 2019). This has
605 interesting implications regarding the protein's RNA exosome-dependent and independent
606 roles during the mitotic cell cycle and meiotic differentiation (Graham *et al.*, 2009).
607 Earlier mass spectrometry-based studies identified EXOSC10 and other RNA exosome
608 subunits as SUMO targets (Zhao *et al.*, 2004; Golebiowski *et al.*, 2009; Impens *et al.*, 2014;
609 Lamoliatte *et al.*, 2014) and 10 SUMOylated lysines are currently referenced in the
610 PhosphoSite database (Fig. 6A; www.phosphosite.org). Indeed, EXOSC10 has several
611 glycy-l-lysine isopeptides, which are implicated in interactions with the C-terminus of
612 SUMO2. Several lines of evidence show that EXOSC10 protein stability is directly
613 controlled by SUMOylation. First, culturing mammalian cells at a low temperature results in
614 increased conjugation of SUMO1 to EXOSC10, which decreases the cellular concentration of
615 EXOSC10 and thereby leads to incomplete 3' pre-rRNA processing and a reduced ratio of
616 40S to 60S ribosomal subunits. Second, overexpression of SUMO1 decreases EXOSC10
617 levels. Third, a mutant allele of EXOSC10 lacking three putative SUMO target lysines
618 (K168, K201 and K583) located between the PMC2NT domain and the catalytic domain,
619 within the core catalytic domain and at the end of the HRDC domain, respectively, is
620 stabilized under physiological and cold-stress conditions (Knight *et al.*, 2016). Upon cooling,
621 expression of exosome core proteins EXOSC3, EXOSC5 and EXOSC8 is reduced in a

622 manner similar to EXOSC10, while expression of DIS3 does not change (Knight *et al.*,
623 2016).

624 In summary, an array of high-throughput studies provide solid evidence for extensive protein
625 modifications in EXOSC10 and are consistent with the notion that this protein is subject to
626 targeted proteolysis during cell division and differentiation. Mutant alleles that change target
627 residues involved in protein stability are therefore of particular interest because they might
628 explain variable EXOSC10 protein levels in normal and malign tissues.

629

630 **(5) EXOSC10 mutant alleles associated with cancer affect amino acids undergoing post-** 631 **translational modifications**

632 Major advances in DNA sequencing technologies have spawned projects aiming to decipher
633 the allelic composition of genomes from diverse human populations and the mutation load of
634 malign tumours, from whole organs and tissues down to the single-cell level. Much work has
635 focused on the protein-coding part of the human genome (exome sequencing) but data for
636 entire genomes have been generated at an increasing pace (Mallick *et al.*, 2016; Rozenblatt-
637 Rosen *et al.*, 2020).

638 The Catalog of Somatic Mutations in Cancer [COSMIC, <https://cancer.sanger.ac.uk/cosmic/>
639 (Tate *et al.*, 2019)] provides data on single nucleotide variants/polymorphisms (SNVs/SNPs)
640 associated with protein-coding genes in cancer samples. A total of nine mutations affect
641 EXOSC10 amino acids subject to PTMs (Fig. 6A; Table 3). Four alleles that alter
642 phosphorylated amino acids were discovered in liver (S370P missense mutation), colon
643 (S402T), lung (Y448C) and intestinal (S785I) cancers. The S402T mutation replaces the
644 serine with a threonine and therefore effects of this mutation on EXOSC10 should be due to
645 structural changes rather than an altered phosphorylation pattern. Serines 402 and 370 are
646 located at the extreme ends of alpha-helices, while tyrosine 448 is within an alpha helix (Fig.

647 6B). Three out of four mutations affecting amino acids modified by kinases and phosphatases
648 are found within the catalytic exoribonuclease domain and consequently might alter the
649 enzyme's activity (see Fig. 6A; <https://cancer.sanger.ac.uk/cosmic/>). Four alleles that affect
650 lysines subject to ubiquitination were found in brain (K136N), oesophagus (K136*
651 nonsense), thyroid (K218E) and breast (K592N) cancer. Finally, a lysine that is both
652 ubiquitinated and SUMOylated is mutated in bone cancer (K583E) (Fig. 6A;
653 <https://cancer.sanger.ac.uk/cosmic/>).

654 It is not known if these mutant alleles are homo- or heterozygous in the sample tissues, but a
655 mutation that affects protein stability could have a dominant effect on cellular EXOSC10
656 protein levels. These tumour-linked mutations are intriguing, given that cancer samples
657 assayed by immunohistochemistry for EXOSC10 show large variations in intensity levels,
658 indicating that altered protein concentrations are a frequent event in malign tissues
659 (www.proteinatlas.org). EXOSC10 mutant alleles that escape normal proteolytic degradation
660 because their structure is altered, their protein-protein interactions are affected or their
661 destruction box motifs are changed may deregulate cell growth, division and differentiation.
662 This important question clearly merits further investigation using engineered cell lines and
663 corresponding transgenic mouse models.

664

665 **IV. CONCLUSIONS**

666 (1) *EXOSC10/RRP6* mRNA and protein are detected in dividing and differentiating cells
667 across the vast majority of samples assayed.

668 (2) *EXOSC10/RRP6* mRNA fluctuates during the mitotic cell cycle

669 (3) The gene is likely regulated by multiple mechanisms at the transcriptional, translational
670 and post-translational level.

671 (4) Altered EXOSC10/Rrp6 protein levels during normal cell division and differentiation and
672 in pathological tissues have implications for RNA exosome-dependent and independent
673 cellular functions in RNA processing and degradation.

674 (5) *EXOSC10/RRP6* alleles that affect protein stability will likely yield information on this
675 protein's roles during cell division, development and disease and will facilitate the design of
676 novel therapeutical concepts.

677

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683

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1306 VII. SUPPORTING INFORMATION

1307 Additional supporting information may be found online in the Supporting Information section
1308 at the end of the article.

1309 **Fig. S1.** *Schizosaccharomyces pombe* RNA profiling data for *rrp6*.

1310 **Fig. S2.** Human *EXOSC10* gene annotation and expression.

1311 **Fig. S3.** *EXOSC10* expression in normal versus cancer samples.

1312 **Fig. S4.** Regulatory motif predictions for the *EXOSC10* promoter region.

1313

1314 **Figure legends**

1315 **Fig. 1.** RNA exosomes and EXOSC10/Rrp6 structure and domain composition. (A) Overall
1316 structures of RNA exosomes from yeast [Protein Databank (PDB) identifier 6FSZ] and
1317 humans (6D6Q) are shown as examples. Rrp6 and Dis3 in yeast and EXOSC10 and DIS3 in
1318 humans are highlighted in purple and green, respectively. The structures were visualized
1319 using the Mol* viewer from PDB at www.rcsb.org. (B) Schematic summarizing structural
1320 motifs in the N-terminal (NTD), catalytic (CAT) and C-terminal (CTD) domains of Rrp6
1321 (Wasmuth & Lima, 2017). Structures of yeast Rrp6 (2HBM) and human EXOSC10 (3SAG)
1322 catalytic domains are shown at the bottom using the NGL (WebGL) viewer. Amino-acid
1323 coordinates are indicated for each protein fragment. EAR, exosome-associating region; EXO,
1324 exoribonuclease; HRDC, helicase and RNase D carboxy terminal; Lasso is the name of the
1325 domain; NLS, nuclear localization signal; PMC2NT, polycystin 2 N-terminal.

1326
1327 **Fig. 2.** Promoter architecture and gene expression data for *RRP6* in *Saccharomyces*
1328 *cerevisiae*. (A) Schematic showing the *RRP6*/MUT1312 locus on chromosome XV, with
1329 genome coordinates indicated. Data defining the transcription start site (TSS) were retrieved
1330 from the Yeast Transcription Start Site (YeastSS) database (www.yeastss.org). (B) Graph of
1331 log-transformed colour-coded expression data provided by CycleBase (<https://cyclebase.org>)
1332 against cell cycle phase. Horizontal red lines indicate zero expression. (C) Colour-coded
1333 diagram showing strand-specific RNA-sequencing expression data (in blue) and ribosome
1334 profiling data (in red) from Brar *et al.* (Brar *et al.*, 2012) for *RRP6* (top two panels, grey
1335 rectangle) and its antisense long non-coding RNA (lncRNA) *MUT1312* (bottom two panels,
1336 yellow rectangle). The data were visualized using the Integrated Genomics Viewer (IGV)
1337 2.8.0 (Thorvaldsdottir, Robinson & Mesirov, 2013). DNA rep, DNA replication; Meiotic rec,
1338 Meiotic recombination. (D) Colour-coded bar diagram showing normalized expression data

1339 in red (YPD rich medium), blue (YPA pre-sporulation medium) and green (4 h, 8 h and 10 h
1340 in S_{PII} sporulation medium) from (Lardenois *et al.*, 2015a). Error bars show standard
1341 deviation. Percentiles and linear signal intensities as shown on the *y*-axis. The SK1 triplicate
1342 wild-type and *ume6* mutant samples are indicated on the *x*-axis. Image retrieved from the
1343 GermOnline 4.0 database (www.germonline.org). (E) Colour-coded bar diagram showing
1344 Rrp6 protein molecules per cell (*y*-axis) for samples cultured in rich medium (YEPD, yellow)
1345 and synthetic defined (SD, blue), synthetic complete (SC, green) and minimal C-limiting
1346 media (F1, violet) from the different listed references included in a meta-analysis by Ho *et al.*
1347 (2018).

1348

1349 **Fig. 3.** Protein networks for EXOSC10/Rrp6 across species. (A) Protein–protein interaction
1350 data from the BioGrid database (www.thebiogrid.org) for *Saccharomyces cerevisiae*.
1351 Interactions were limited for readability using the ‘hide genetic experiments’ and ‘minimal
1352 evidence 2’ filters and the ‘circular layout’ option. Nodes are in blue and edges are in yellow.
1353 The thickness of the yellow edges represents the number of experiments (pieces of evidence)
1354 that demonstrate the interaction. Colour-coded halos mark proteins involved in certain
1355 biological processes or molecular functions as indicated in the legend. (B–D) Equivalent data
1356 using the same filtering criteria for *Schizosaccharomyces pombe* (B), *Drosophila*
1357 *melanogaster* (C) and humans (D). DSB, double strand break.

1358

1359 **Fig. 4.** *EXOSC10* mRNA levels in mitosis. (A) The mRNA isoforms and antisense long non-
1360 coding RNAs (lncRNAs) currently annotated by Ensembl. Full-length mRNAs are
1361 highlighted with yellow arrows. The legend shows the colour-coding for genome annotation.
1362 (B) Expression data compiled and processed by CycleBase for the two genes *EXOSC10* and
1363 *CCNBI*. Expression units (*y*-axis) are plotted against samples taken at different stages of the

1364 mitotic cell cycle. Horizontal red lines indicate zero expression. The colour-coded lines
1365 represent data reported by the publication shown in the legend (Whitfield *et al.*, 2002).

1366

1367 **Fig. 5.** Human EXOSC10 protein expression. (A) Graphical display showing quantitative
1368 mass spectrometry signal intensity values plotted for the samples identified on the left. Graph
1369 retrieved from www.proteomicsdb.org. Green arrows highlight reproducible protein detection
1370 data, while red arrows highlight proteins that were detected in only one of the protein
1371 profiling studies. (B) A heatmap retrieved from www.humanproteomemap.org (Kim *et al.*,
1372 2014) showing EXOSC10 protein levels in foetal and adult samples. (C)
1373 Immunofluorescence data for EXOSC10 and Cyclin B1 (CCNB1). Normal human fibroblasts
1374 (HS68) growing asynchronously on glass coverslips were fixed in formalin, extracted and
1375 incubated with a polyclonal anti-EXOSC10 antibody (Abcam) and a monoclonal anti-
1376 CCNB1 antibody (Santa Cruz Biotechnology) before visualization with affinity-purified
1377 Alexa-fluor-488 anti-mouse and Alexa-fluo-555 anti-rabbit antibodies (ThermoFisher). The
1378 images are fluorescence micrographs of a typical field stained for DNA (blue), EXOSC10
1379 (red), CCNB1 (green,) and merged (EXOSC10/DNA or all) images. (D)
1380 Immunofluorescence data obtained by staining normal fibroblasts, cultured and processed as
1381 in C, for EXOSC10 and the nucleolar marker fibrillarin (FBL). We used the anti-FBL
1382 monoclonal antibody from SantaCruz (sc-166001). DNA in C and D was stained using
1383 Hoechst 33342 (Sigma-Aldrich, Merck). Scale bars, 10 μ M.

1384

1385 **Fig. 6.** EXOSC10 alleles and cancer. (A) A lollipop blot retrieved from www.phosphosite.org
1386 showing the number of references for given post-translational modifications (PTMs) (y -axis)
1387 along the complete primary sequence of EXOSC10. Amino acids identified in COSMIC as
1388 related to somatic cancers are highlighted in violet. Colour code for PTMs and cancer-related

1389 residues is given in the key. EXO, exoribonuclease; HRDC, helicase and RNase D carboxy
1390 terminal; PMC2NT, polycystin 2 N-terminal. (B) Structure of human EXOSC10 catalytic
1391 domain containing a point mutation at position 313 (D313N) as provided by PDB. The
1392 positions of three amino acids known to be mutated in cancer are indicated.

1393

1394 **Figure legends for supporting information**

1395 **Fig. S1.** *Schizosaccharomyces pombe* RNA profiling data for *rrp6*. (A) Gene expression for a
1396 mitotic time course (*x*-axis) shown as ratios of data from asynchronous cells and time points
1397 from synchronized cells (exp asynch/timepoint, *y*-axis). (B) Gene expression for different
1398 stress conditions (*x*-axis) shown as ratios of data from untreated cells (0 minute time point)
1399 and treated cells (15 and 60 minute time points) indicated using colour-coded bars (*y*-axis,
1400 exp 0/time point). Cd, 0.5 mM cadmium sulphate CdSO₄; H₂O₂, oxidative stress by 0.5 mM
1401 hydrogen peroxide; Heat, temperature shift within two minutes from 30°C to 39°C in a water
1402 bath; MMS, alkylating agent methylmethane sulphonate at 0.02% (weight per volume); Sb,
1403 osmotic stress in 1M sorbitol. (C) Gene expression for mitosis and meiosis in wild-type (WT,
1404 blue) and *pat1* mutant cells (red, *x*-axis) shown as ratios of vegetatively growing mitotic cells
1405 and timepoints of meiotic cells (exp mitosis/meiosis).

1406

1407 **Fig. S2.** Human *EXOSC10* gene annotation and expression. (A) Expression data obtained in
1408 different tissues clustered according to signal intensities. Isoform identifiers are given on the
1409 right, and a schematic of RNA isoforms at the bottom. (B) Colour-coded chart showing
1410 expression levels in RNA-Seq normalized expression (NX) units for different tissues.

1411

1412 **Fig. S3.** *EXOSC10* expression in normal *versus* cancer samples. Expression data given as the
1413 log₂ of transcript count per million (log₂ TPM) from cancer (red) or healthy (blue) samples

1414 are shown for the samples indicated on the left, with sample names annotated in the box on
1415 the right. BRCA Basal, Luminal and Her2 are molecular subtypes of breast cancer defined by
1416 characteristic gene expression patterns in the basal (outer) layer of the mammary gland, the
1417 luminal (inner) cells lining the mammary ducts or the presence of epidermal growth factor
1418 receptor 2 (HER2). HNSC HPVpos and neg subtypes are positive or negative for human
1419 papilloma virus (HPV). The number of asterisks indicates the level of statistical significance
1420 for the observed differential gene expression between normal and cancer samples. Green
1421 arrows highlight examples of *EXOSC10* overexpression, while red arrows highlight samples
1422 where there was decreased expression in cancer.

1423

1424 **Fig. S4.** Regulatory motif predictions for the *EXOSC10* promoter region. The image shown is
1425 a screenshot from www.swissregulon.org, which was edited for clarity. The *EXOSC10* locus
1426 region is highlighted with the yellow arrow. Transcription factors that bind predicted motifs
1427 are marked by green arrows.

1428

1429

1430 Table 1. Data sources, including the type, name and web address for relevant databases. MIR,
 1431 microRNA.

Category	Species	Name	Uniform resource locator
Annotation	Budding yeast	Saccharomyces Genome Database (SGD)	www.yeastgenome.org
	Fission yeast	Pombase	www.pombase.org
	Fly	Flybase	www.flybase.org
	Mouse	Mouse Genome Database (MGD)	www.informatics.jax.org
Cancer	Human	Catalog of Somatic Mutations in Cancer (COSMIC)	https://cancer.sanger.ac.uk/cosmic
	Human	Tumor Immune Estimation Resource (TIMER)	http://timer.cistrome.org
Data viewers	Fission yeast	TranscriptomeViewer	http://bahlerweb.cs.ucl.ac.uk/TranscriptomeViewer
		Geexview	http://bahlerweb.cs.ucl.ac.uk/cgi-bin/SPGE/geexview
	Multiple	GermOnline	www.germonline.org
		Genotype Tissue Expression (GTEx) portal	www.gtexportal.org
Interactome	Multiple	BioGrid	www.thebiogrid.org
Knowledge	Multiple	GeneCards	www.genecards.org
Literature	All	PubMed	https://pubmed.ncbi.nlm.nih.gov
MIRs	All	miRcode	www.mircode.org
Protein	Human	NeXtprot	www.nextprot.org
	Human	Human Protein Atlas (HPA)	www.proteinatlas.org
	Multiple	Interpro	www.ebi.ac.uk/interpro
	Multiple	Proteomics Database	www.proteomicsdb.org
	Human	Human Proteome Map	http://www.humanproteomemap.org
	Multiple	PhosphoSite	www.phosphosite.org
	Multiple	The Protein Database	www.rcsb.org
Search engine	Multiple	Genevestigator	www.genevestigator.com
Transcription	Multiple	CycleBase	https://cyclebase.org

	Budding yeast	Yeast Search for Transcriptional Regulators And Consensus Tracking (Yeasttract)	www.yeasttract.com
	Budding yeast	Yeast Transcription Start Site (YeastTTS)	www.yeastts.org

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1433

1434 Table 2. Transcription factors (TFs) predicted to bind the EXOSC10 promoter.

Symbol	Summary	Reference
<i>MTF1</i>	Activates its target genes by binding the metal-responsive element (MRE). The gene's up-regulation in ovarian cancer is associated with poor patient survival	Ji <i>et al.</i> , (2018)
<i>MYBL1</i>	Master regulator of male meiosis, was associated with cutaneous adenocystic carcinoma	Kyrpychova <i>et al.</i> , (2018); Li <i>et al.</i> , (2013)
<i>PLAGL1</i>	Acts as a suppressor for cell growth, paralog of the <i>PLAG1</i> oncogene	for review, see Van Dyck <i>et al.</i> , (2007)
<i>PATZ1</i>	Involved in embryogenesis, stem cell biology and cell proliferation, was reported to act either as a tumour suppressor or as an oncogene	for review, see Fedele, Crescenzi & Cerchia, (2017)
<i>GATA3</i>	Important for immune- and inflammatory responses and associated with metastatic breast cancer	Bertucci <i>et al.</i> , (2019)
<i>GABPA</i>	Involved in nuclear control of mitochondrial function and plays roles in bladder and hepatocellular cancers	Guo <i>et al.</i> , (2020)
<i>ELK4</i>	Regulator that interacts with the serum response factor (SRF) on the <i>FOS</i> proto-oncogene	Treisman, (1994)
<i>ERF</i>	Erythroblast transformation specific (ETS) domain-containing repressor implicated in cell proliferation	for review, see Mavrothalassitis & Ghysdael, (2000)
<i>ERG</i>	ETS domain TF associated with a variety of cancers, including prostate cancer, Ewing's sarcoma and acute myeloid leukemia, as a fusion gene	Adamo & Lodomery, (2016)
<i>ETV5</i>	Belongs to an oncogenic subfamily of ETS TFs	Oh, Shin & Janknecht, (2012)

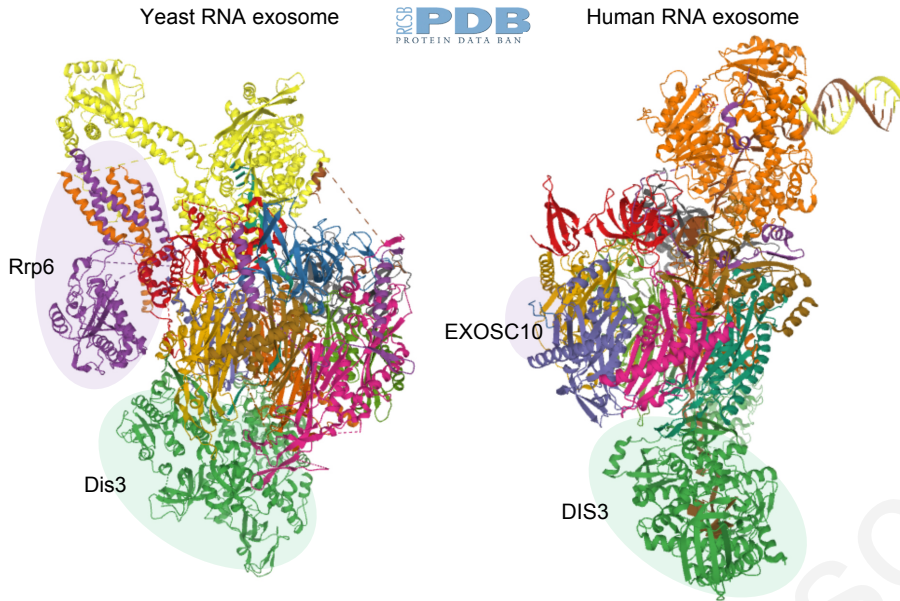
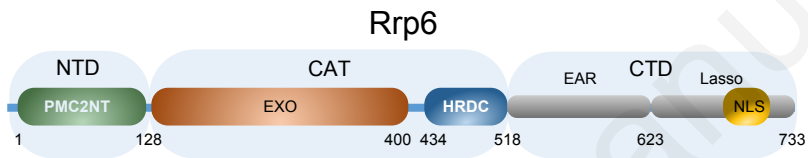
1435

1436

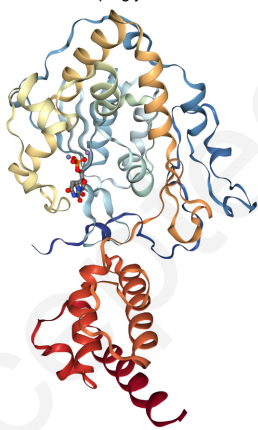
1437 Table 3. Point mutations in EXOSC10; the mutation, its localization within known domains
 1438 and the cancer type in which the mutation was detected are indicated.

Mutation	Post-translational modification	Domain	Cancer type
S370P	Phosphorylation	Catalytic	Liver
S402T			Colon
Y448C			Lung
S785I		CTD	Intestine
K136N	Ubiquitination		Brain
K136*			Oesophagus
K218E			Thyroid
K592N			Breast
K583E	Ubiquitination, SUMOylation		Bone

1439

A**B**

Yeast Rrp6 gly127-asn516



Human EXOSC10 ser179-ala588

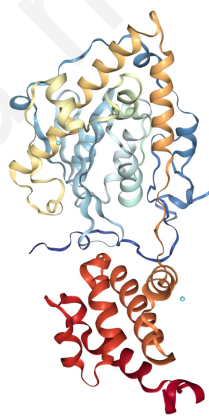


Figure 1

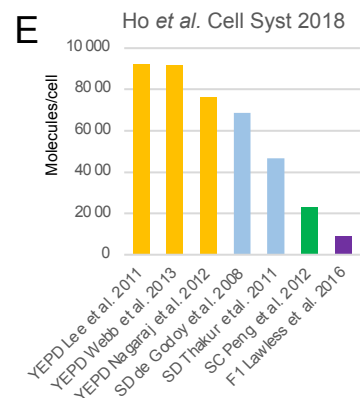
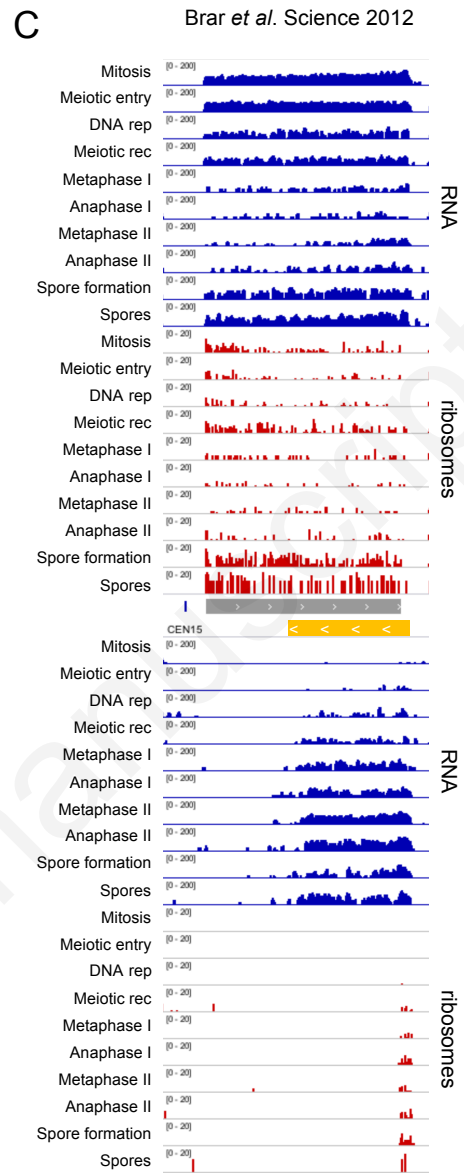
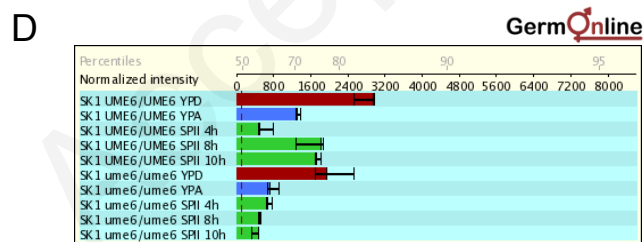
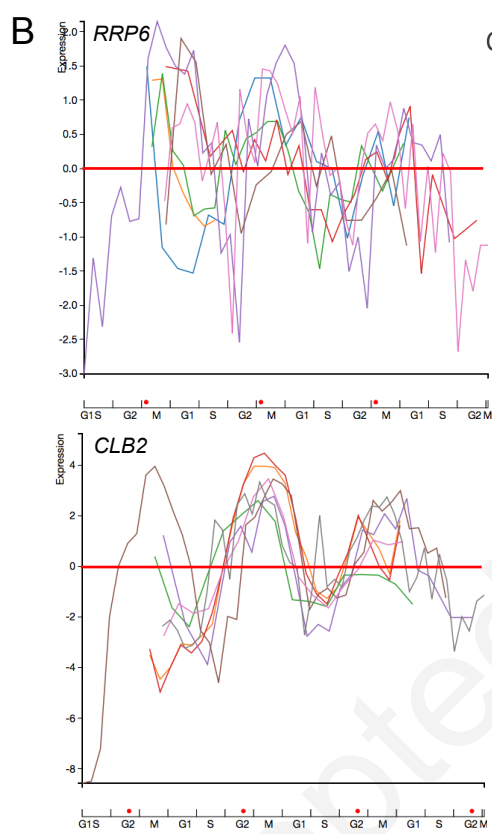
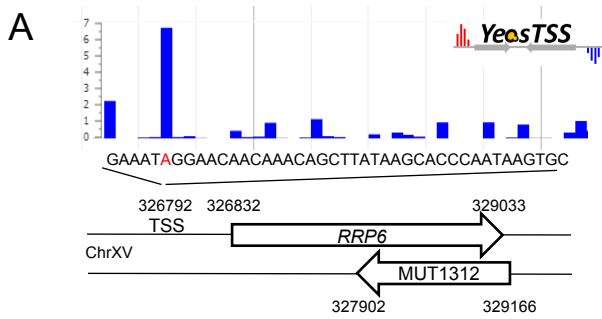


Figure 2

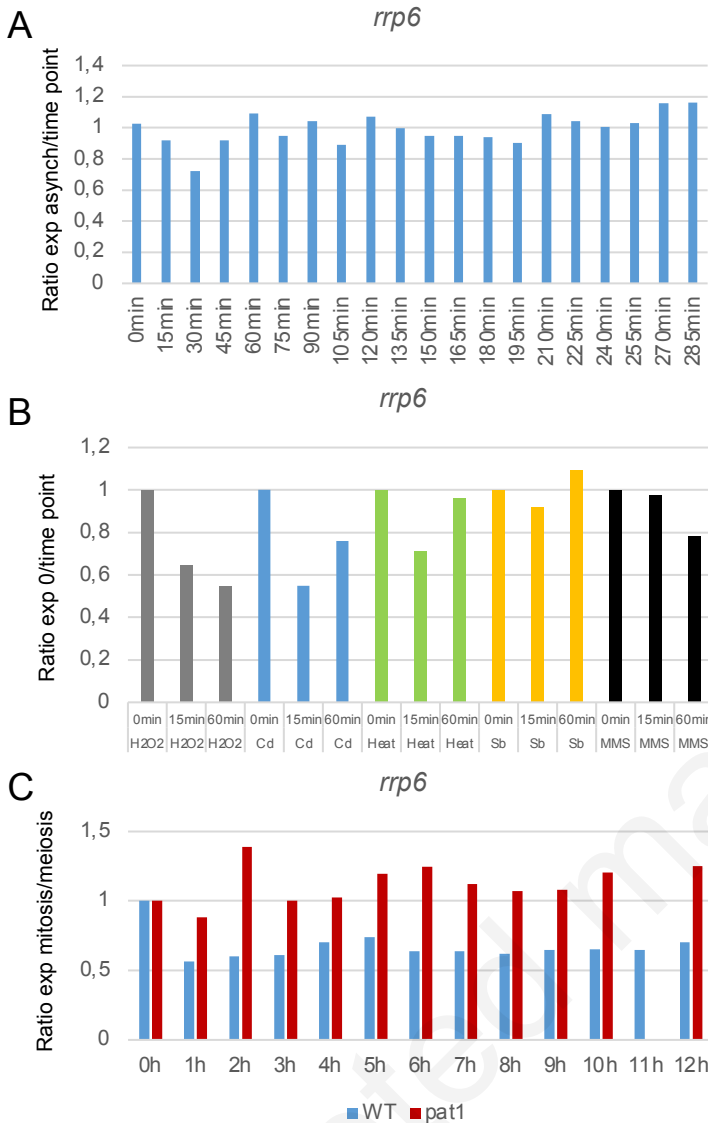
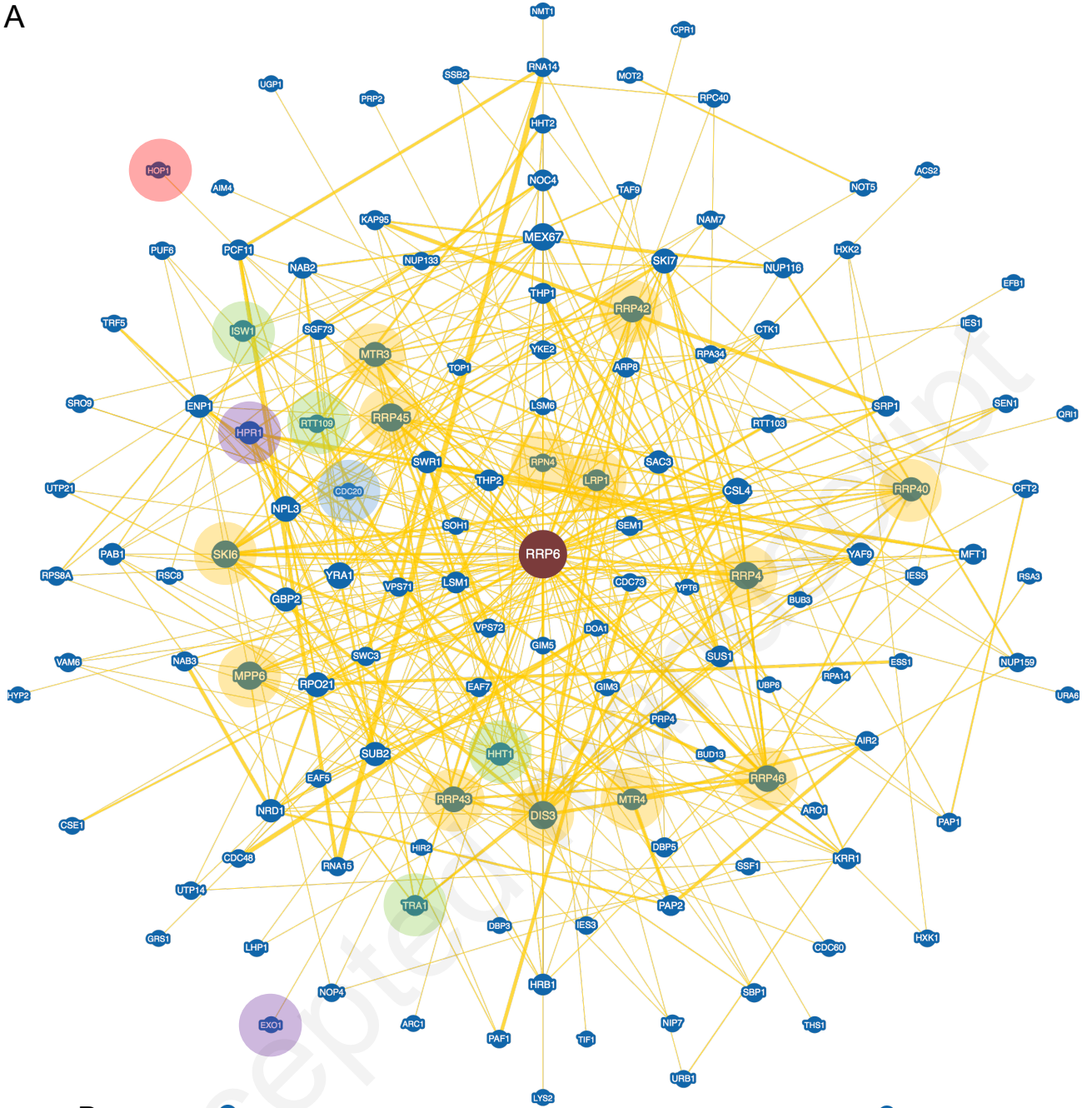
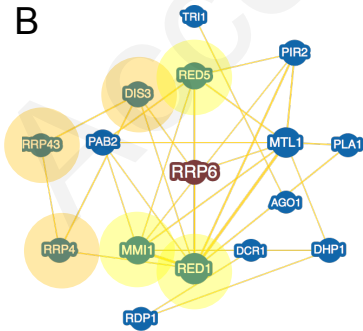


Fig. S1. *Schizosaccharomyces pombe* RNA profiling data for *rrp6*. (A) Gene expression for a mitotic time course (x-axis) shown as ratios of data from asynchronous cells and time points from synchronized cells (exp asynch/timepoint, y-axis). (B) Gene expression for different stress conditions (x-axis) shown as ratios of data from untreated cells (0 minute time point) and treated cells (15 and 60 minute time points) indicated using colour-coded bars (y-axis, exp 0/time point). Cd, 0.5 mM cadmium sulphate CdSO₄; H₂O₂, oxidative stress by 0.5 mM hydrogen peroxide; Heat, temperature shift within two minutes from 30°C to 39°C in a water bath; MMS, alkylating agent methylmethane sulphonate at 0.02% (weight per volume); Sb, osmotic stress in 1M sorbitol. (C) Gene expression for mitosis and meiosis in wild-type (WT, blue) and *pat1* mutant cells (red, x-axis) shown as ratios of vegetatively growing mitotic cells and timepoints of meiotic cells (exp mitosis/meiosis).

A



B



C

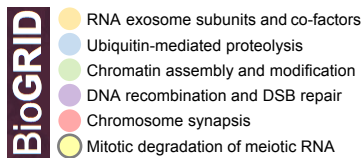
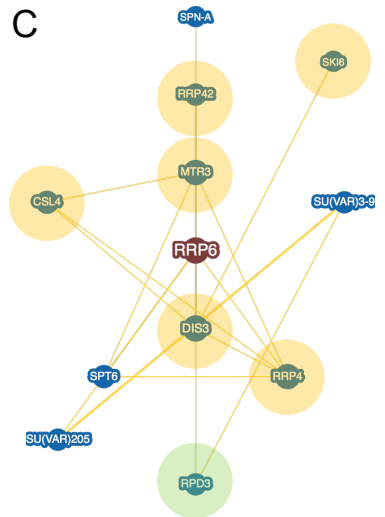


Figure 3

D

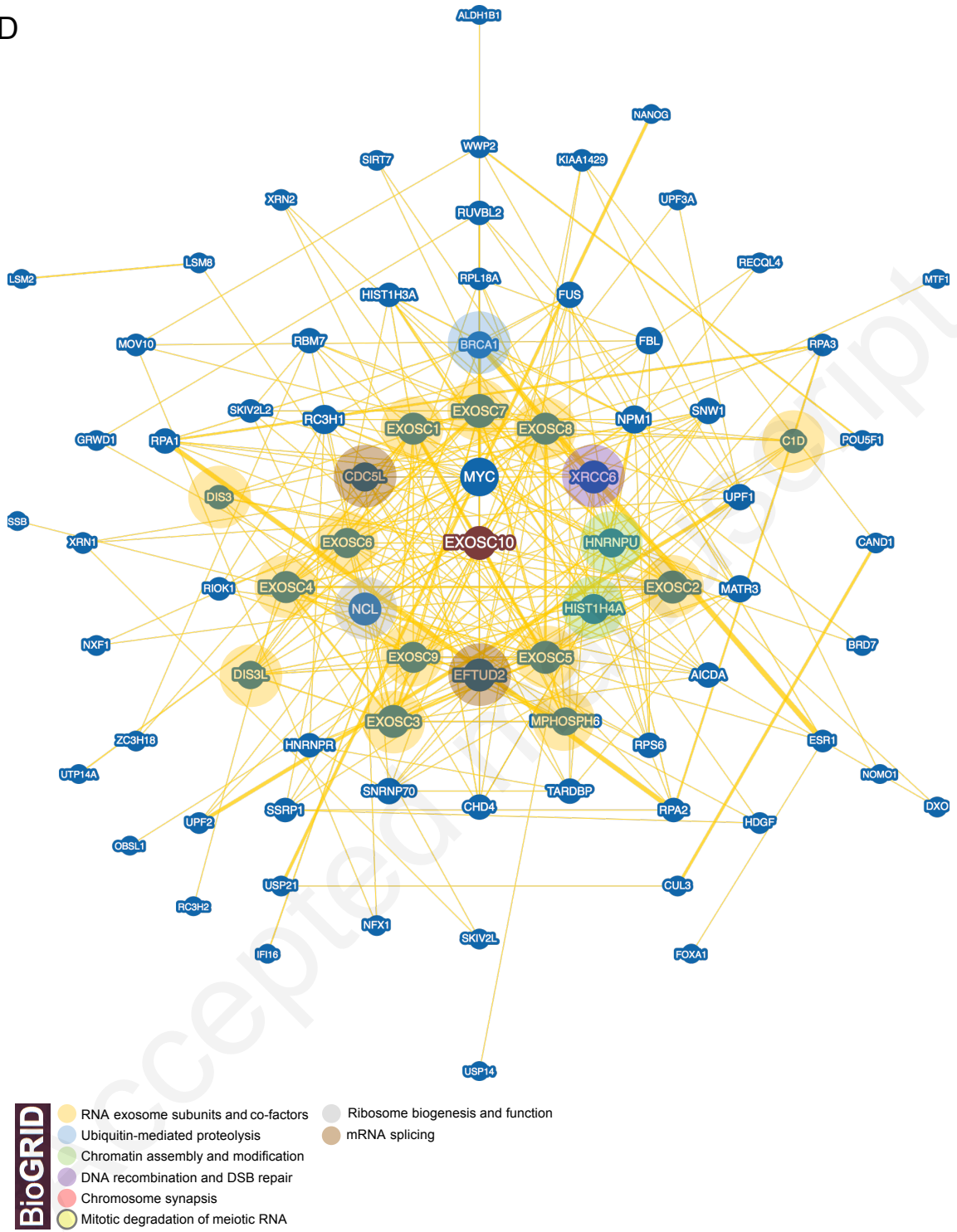
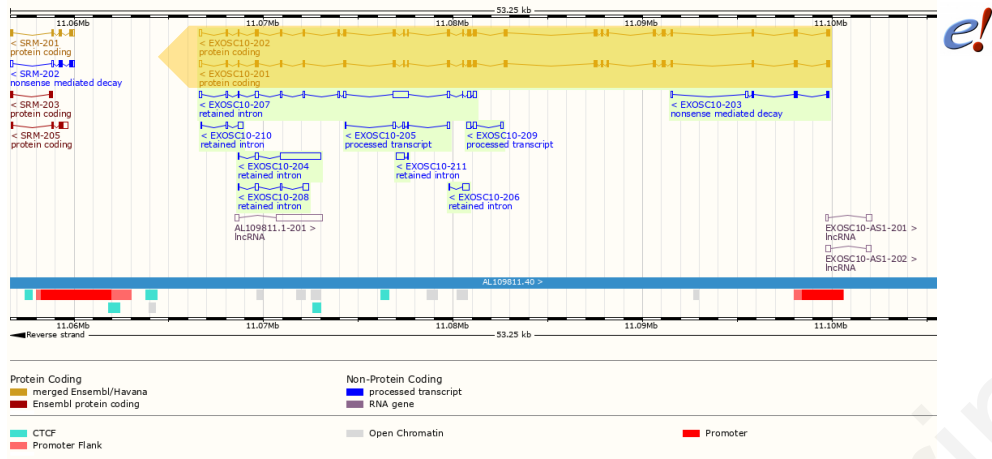


Figure 3

A



B

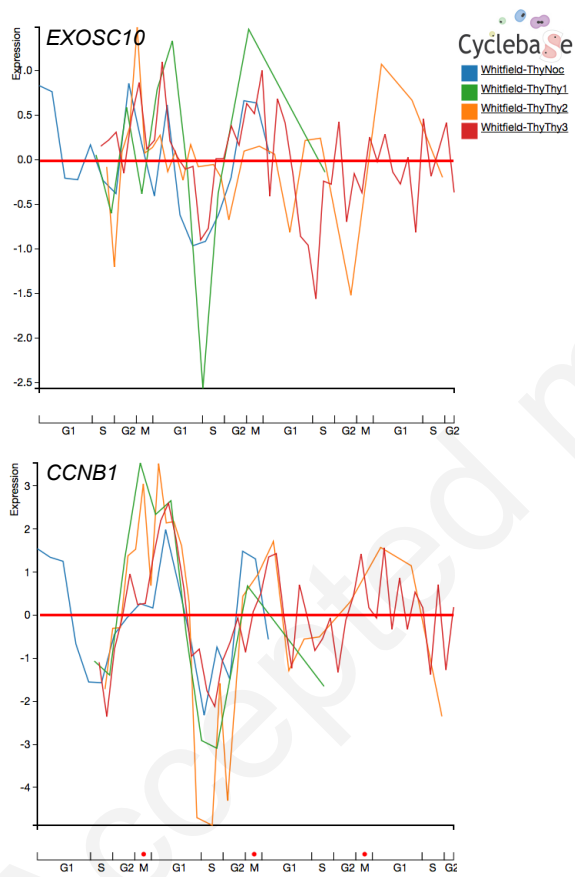


Figure 4

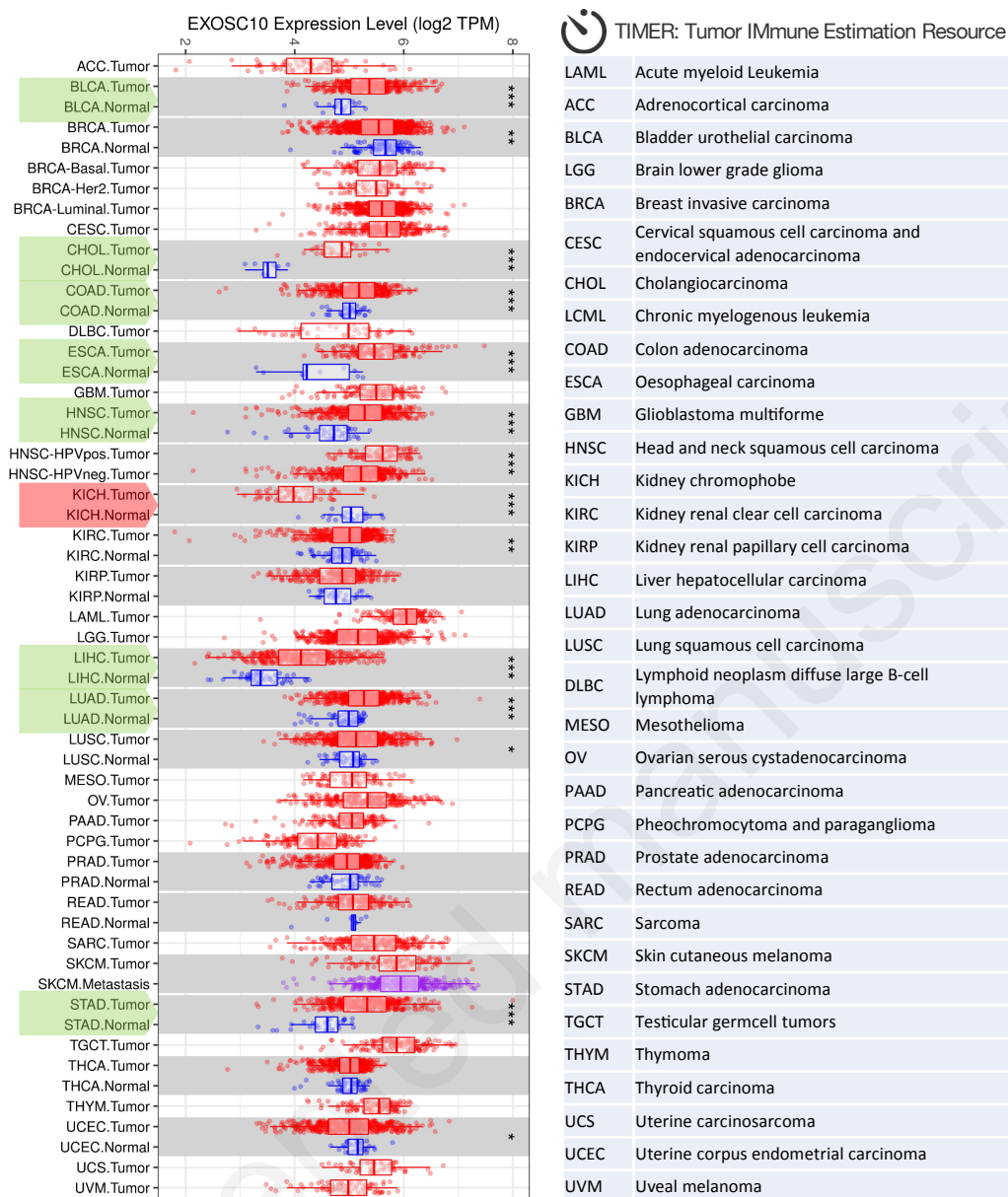


Fig. S3. EXOSC10 expression in normal versus cancer samples. Expression data given as the log2 of transcript count per million (log2 TPM) from cancer (red) or healthy (blue) samples are shown for the samples indicated on the left, with sample names annotated in the box on the right. BRCA Basal, Luminal and Her2 are molecular subtypes of breast cancer defined by characteristic gene expression patterns in the basal (outer) layer of the mammary gland, the luminal (inner) cells lining the mammary ducts or the presence of epidermal growth factor receptor 2 (HER2). HNSC HPVpos and neg subtypes are positive or negative for human papilloma virus (HPV). The number of asterisks indicates the level of statistical significance for the observed differential gene expression between normal and cancer samples. Green arrows highlight examples of *EXOSC10* overexpression, while red arrows highlight samples where there was decreased expression in cancer.

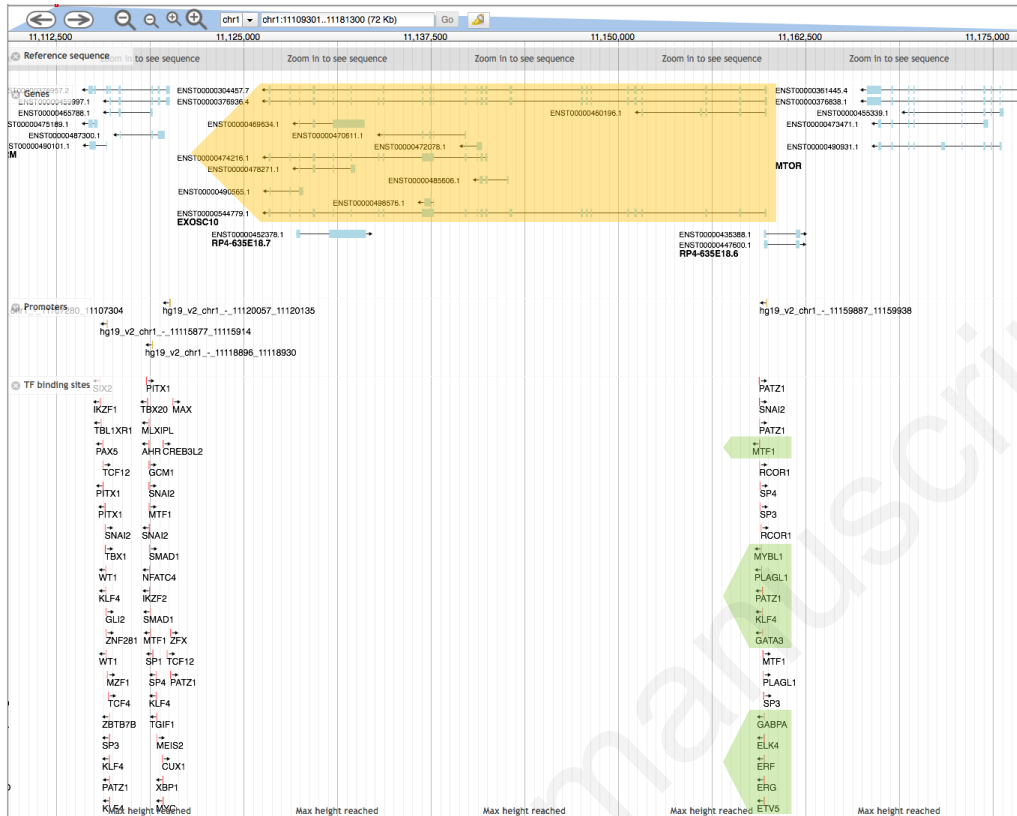


Fig. S4. Regulatory motif predictions for the *EXOSC10* promoter region. The image shown is a screenshot from www.swissregulon.org, which was edited for clarity. The *EXOSC10* locus region is highlighted with the yellow arrow. Transcription factors that bind predicted motifs are marked by green arrows.

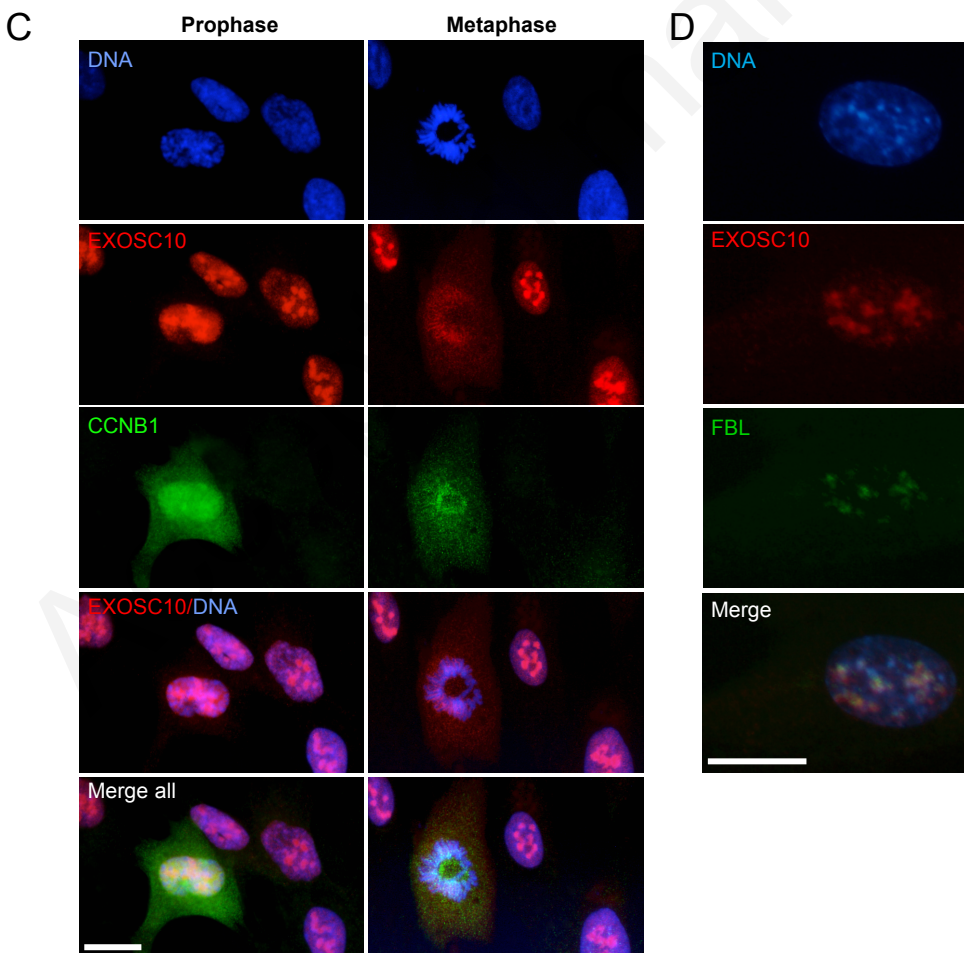
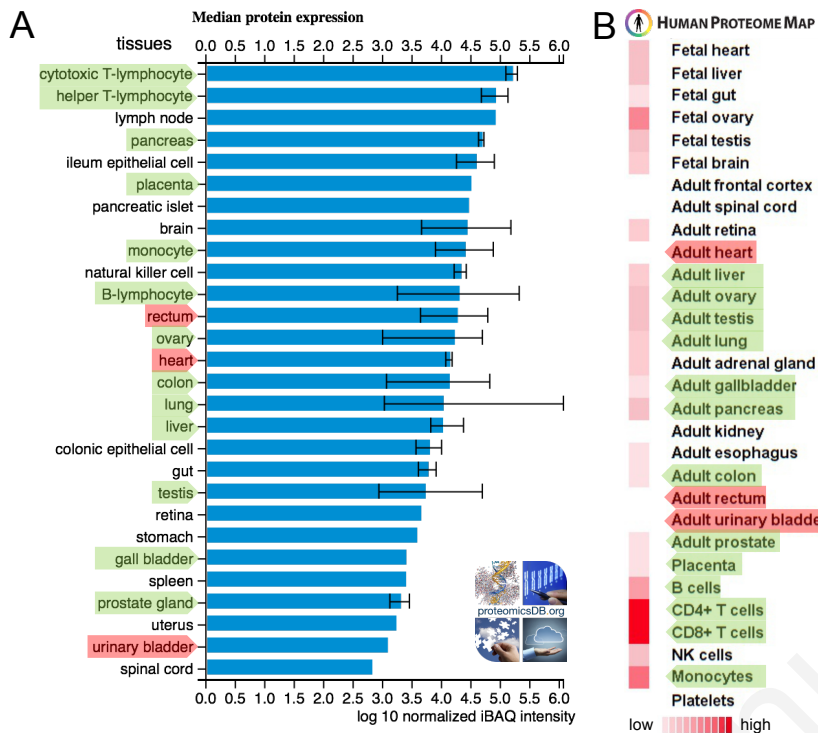


Figure 5

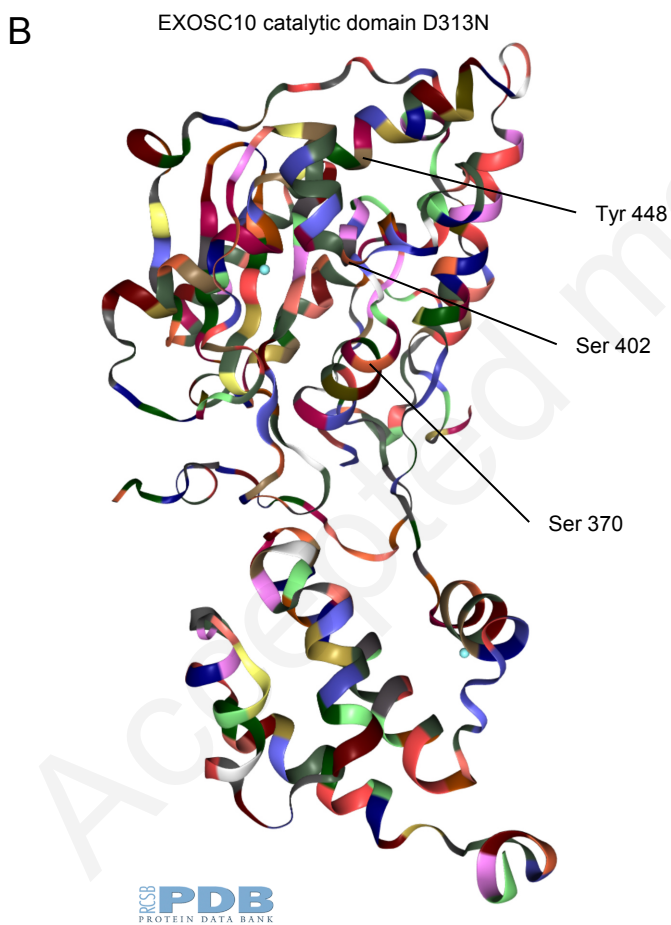
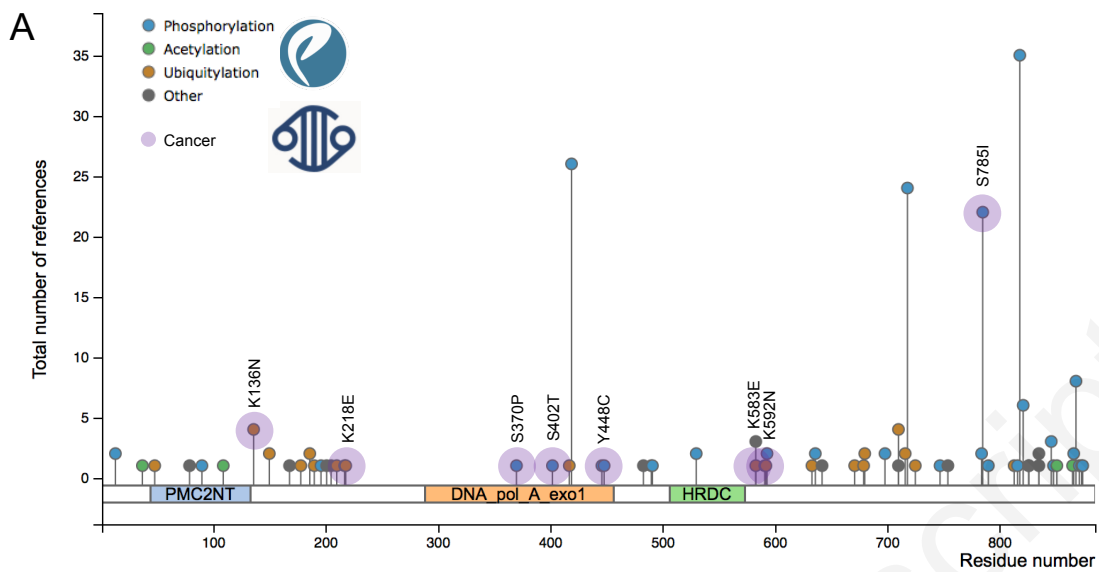


Figure 6