# Proteomic survey of the DNA damage response in *Caulobacter crescentus*

Running Title: Proteomics survey of *Caulobacter* DNA damage response

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## ABSTRACT

The bacterial DNA damage response is a critical, coordinated response to endogenous and exogenous sources of DNA damage. Response dynamics are dependent on coordinated synthesis and loss of relevant proteins. While much is known about its global transcriptional control, changes in protein abundance that occur upon DNA damage are less well characterized at the system level. Here, we perform a proteome-wide survey of the DNA damage response in *Caulobacter crescentus*. We find that while most protein abundance changes upon DNA damage are readily explained by changes in transcription, there are exceptions. The survey also allowed us to identify the novel DNA damage response factor, YaaA, which has been overlooked by previously published, transcription-focused studies.

A similar survey in a Δ*lon* strain was performed to explore *lon's* role in DNA damage survival. The Δ*lon* strain had a smaller dynamic range of protein abundance changes in general upon DNA damage compared to the wild type strain. This system-wide change to the dynamics of the response may explain this strain's sensitivity to DNA damage. Our proteome survey of the DNA damage response provides additional insight into the complex regulation of stress response and nominates a novel response factor that was overlooked in prior studies.

## IMPORTANCE

The DNA damage response helps bacteria to react to and potentially survive DNA damage. The mutagenesis induced during this stress response contributes to the development of antibiotic resistance. Understanding how bacteria coordinate their

39 response to DNA damage could help us to combat this growing threat to human health.
40 While the transcriptional regulation of the bacterial DNA damage response has been
41 characterized, this study is the first to our knowledge to assess the proteomic response
42 to DNA damage in *Caulobacter*.

43 **INTRODUCTION**

44 The bacterial DNA damage response is a major driver of antibiotic resistance
45 development (1, 2) and the horizontal gene transfer that spreads resistance and
46 pathogenicity from cell to cell (3–6). The canonical DNA damage response was first
47 characterized in *Escherichia coli*. During DNA damage ssDNA accumulates in the cell
48 and is bound by the cell's main recombinase RecA to form a nucleoprotein filament that
49 induces the autocleavage of the global transcriptional repressor LexA (7, 8). Cleavage of
50 LexA allows transcription of more than 40 genes related to DNA damage tolerance and
51 repair, including *recA* and *lexA* themselves (9, 10). The energy-dependent protease
52 ClpXP then degrades the DNA binding N-terminal domain of LexA to prevent residual
53 repression (11).

54 As DNA damage is repaired, accumulation of uncleaved LexA represses transcription of
55 DNA damage response genes. Shutdown of the response is also aided by proteolysis of
56 certain response factors (e.g. UmuD' (12) and UvrA (13) by ClpXP and UmuD (14) and
57 SulA (15) by Lon). In fact, both ClpXP (11) and Lon (16, 17) contribute to the survival of
58 DNA damage in *E. coli*.

59 The LexA/RecA-based transcriptional control of the response is conserved in *Caulobacter*
60 *crescentus*, as are many of the genes in the *E. coli* LexA regulon (18). A second, LexA-

61 independent DNA damage response pathway that is dependent on global transcriptional
62 regulator DriD has also been identified and characterized in *Caulobacter* (19, 20). While
63 the energy-dependent protease Lon is conserved in *Caulobacter* and contributes to
64 survival of DNA damage (21), Lon's confirmed DNA damage-related substrates (UmuD
65 (14) and SulA (15)) are not. Lon has been shown to contribute to DNA damage survival
66 in *Caulobacter* but its role in response to DNA damage remains unclear.

67 Transcriptome measurements upon treatment with various DNA damaging agents and a
68 bioinformatic search for LexA binding sites have nominated a set of genes that are
69 upregulated upon damage in *Caulobacter* (18, 19, 22). While transcription and proteolysis
70 both play roles in DNA damage response induction and shut-off, relatively little is known
71 about how well transcriptional changes correspond to protein abundance changes at the
72 proteome level.

73 Here, we present a proteomic survey to investigate the dynamics of protein abundance
74 during the *Caulobacter* DNA damage response. First, we measured protein abundance
75 changes upon DNA damage treatment. Next, we measured changes following translation
76 shutoff to characterize potential proteolysis substrates. We found that while most changes
77 in protein abundance upon DNA damage are explained by changes in transcription, there
78 are exceptions to this rule. By combining our proteomics and previously measured
79 changes in transcript levels upon mitomycin C (MMC) treatment (19), we were able to
80 identify the novel DNA damage response factor YaaA, which has previously been
81 overlooked in surveys that considered transcriptional changes alone. Our validation
82 experiments showed this factor to be important during DNA damage, demonstrating the
83 utility of our approach.

84 In the second stage of this study, we attempted to identify novel Lon substrates to explain
85 the importance of this protease to DNA damage survival. While we were unable to identify
86 any novel Lon substrates that sufficiently explain this phenotype, we did characterize
87 system-wide changes to the DNA damage response in a Δ*lon* strain. The magnitude of
88 protein abundance changes upon DNA damage are dampened in the Δ*lon* strain
89 compared to those in the wild type strain. This effect can be attributed in part to the
90 elevated basal levels of DNA damage proteins in the untreated Δ*lon* strain (23). This
91 suggests Lon may be of wider importance to the regulation of the DNA damage response
92 than simply degrading of one or a few key substrates.

93 This study provides new insights into Lon's broader importance to protein dynamics in
94 *Caulobacter's* DNA damage response and identifies a novel factor that is important for
95 survival to DNA damage.

96 **RESULTS**

97 **Proteomics yields novel insights into the DNA damage response**

98 To characterize protein abundance changes during the DNA damage response, we first
99 used the sentinel protein RecA to determine the timescale of an acute MMC treatment in
100 *Caulobacter* (Figure 1A and S1). Cells were treated with MMC for 1 hour and RecA levels
101 were monitored through recovery (Figure 1A and S1). RecA levels peaked 3 hours after
102 the addition of MMC (Figure 1A and S1), which is similar to what was previously observed
103 during chronic MMC treatment (24).

104 This same timeline was used to obtain samples before and after MMC treatment for a
105 proteomics survey (timescale in Figure 1B, results in Table S1, S2, and Figure 1C). In

106 order to determine where post-translational regulation (e.g., proteolysis) may be affecting
107 protein levels during DNA damage recovery, we treated cells with chloramphenicol at 3
108 hours to shut off translation and sampled cells for proteomics at 5 hours (timeline in Figure
109 1B, results in Figure 1C).

110 To validate our proteomics survey, we looked at the protein abundance changes of known
111 DNA damage proteins after MMC treatment. As expected, several known DNA damage
112 response proteins were more abundant after MMC treatment (e.g., RecA, UvrA, RuvB,
113 ImuA, and MmcA, which are highlighted in red in Figure 1C). In the translation shutoff
114 portion of the experiment, we observed a decrease in protein abundance of several
115 known proteolysis substrates, including ClpXP substrates DnaX (25), CtrA (25), FtsZ (26),
116 UvrA (13), and LexA(11), ClpAP substrates FtsA (26) and Lon (27), and Lon substrates
117 DnaA (28) and IbpA (29) (all highlighted in red in Figure 1D). These data validated that
118 our proteomics analysis could be used to identify known DNA damage response factors
119 and proteolysis substrates.

120 Previous genome-wide surveys of the *Caulobacter* DNA damage response have focused
121 on transcriptional regulation (18, 19, 22). We sought to determine how well the changes
122 in protein abundance correlate to changes in RNA levels. To do this, we compared our
123 proteomics data to previously published microarray data (19). In our western blot, we
124 found that that RecA levels peak approximately 3 hours after MMC treatment and this
125 data was consistent with a previous study (24). The same study indicated that RNA levels
126 for *recA* and several other DNA damage response genes (e.g., *lexA, uvrA, ruvB, bapE*)
127 peak at 60-80 minutes after MMC treatment. Based on this timeline and the consistency
128 with our experimental design we used the study performed by Modell, et al. to compare

129 our measurement of protein abundance changes to their published changes in RNA levels
130 upon MMC treatment in PYE (19). In this study, transcript levels were monitored using
131 microarrays over several time points (20-80 min) after MMC treatment (or hydroxyurea or
132 ultra-violet light) in rich medium (or minimal medium) (19).

133 To discern between genes that are transcriptionally upregulated upon MMC treatment
134 and those that are not, we used the maximum $\log_2$(Fold Change of RNA) from these
135 timepoints for each gene and treated genes that were induced more than one standard
136 deviation above the mean of these as "upregulated". Genes that fell below this cutoff were
137 considered “not transcriptionally upregulated.” This strategy highlighted 615 upregulated
138 genes of which 146 could be identified as peptides in our proteomics survey. Of these,
139 43 show significant increase in protein abundance upon DNA damage (Table S4, $\log_2$(3
140 hrs/0 hrs) > 0, FDR < 0.05). As expected, the known DNA damage proteins mentioned
141 above that showed increased protein abundances are included in this set (e.g., RecA,
142 UvrA, RuvB, ImuA, and MmcA, which are highlighted in red in Figure 1C, all data in Table
143 S4).

144 We identified 81 proteins that significantly increased in abundance upon DNA damage
145 ($\log_2$(Fold Change in Protein) > 0, FDR < 0.05) that did not show a corresponding
146 significant increase in RNA level by microarray as defined above ((19), Table S5). To
147 further validate these hits, we compared them to a RNA-seq dataset that measured
148 expression of genes following chronic MMC treatment (30). Of our list of 81 proteins, 76
149 showed no significant increase in expression after chronic MMC treatment (this study
150 defined upregulated as a 3-fold increase in RNA levels after 4 hours (30)).

151 We followed up on three of the most induced proteins in this list to determine if they were
152 important for survival to MMC, but deletion of these genes (CCNA_02118, CCNA_00591,
153 and CCNA_00276; highlighted in purple in Figure 2A) had no effect on survival to MMC
154 (Figure S2). These data indicate that while there are examples of proteins that are
155 upregulated upon DNA damage that are not induced at the RNA level, not all of these are
156 physiologically relevant to MMC survival.

157 Of this list of 81 proteins (Table S4) CCNA_03496 was also of particular interest. This
158 gene is not upregulated upon MMC treatment in microarray (19) but does show a 17%
159 increase in expression during chronic MMC treatment by RNA-seq (30). Due to its minor
160 transcriptional induction upon DNA damage, YaaA had previously been overlooked as a
161 potential DNA damage response factor. It has not previously been shown to be important
162 for DNA damage survival and does not appear to be regulated by the LexA/RecA system
163 (18, 22). CCNA_03496 is a homolog of *E. coli*'s *yaaA* ((31), we will refer to this gene as
164 *yaaA* henceforth), which is important for regulating free iron levels during oxidative stress
165 conditions (32). Our Tn-seq experiments identified it as potentially important for survival
166 of MMC (Table S3) and published RB-Tn-seq results suggest that it is important for
167 survival of the DNA damaging agent cisplatin (33). For these reasons, we decided to
168 further characterize the connection between *yaaA* and MMC survival.

169 Deletion of *yaaA* had no effect on growth of isolated cultures treated with MMC (Figure
170 S3A). We turned to more sensitive competition assays to quantify fitness defects. In these
171 assays, we grew mKate2-expressing test strains with a Venus-expressing control strain
172 and measured ratios of mKate2 to Venus fluorescence before and after several
173 generations of growth. A Δ*yaaA* test strain expressing the mKate2 is less able than the

174 wild type mKate2-expressing strain to compete in co-culture with the wild type Venus-
175 expressing control (Figure 2C). This competitive disadvantage worsens in the presence
176 of MMC (Figure 2B). To confirm that these results are not an artifact of the fluorescent
177 strains, we reversed the markers to observe Venus-expressing test strains competed
178 against a mKate2-expressing control and saw similar results (Figure S3B).

179 Because MMC activation can generate hydrogen peroxide ($H_2O_2$) in the cell (34), we
180 sought to determine whether the sensitivity of the Δ*yaaA* strain to MMC was due to a
181 sensitivity to DNA damage or to a secondary effect of possible $H_2O_2$ generation. We find
182 that the Δ*yaaA* strain is indeed sensitive to $H_2O_2$ (Figure 2C and reciprocal experiment in
183 Figure S3C). Importantly, the Δ*yaaA* strain is also sensitive to ultra-violet (UV) light, a
184 non-chemical DNA damaging agent (Figure 2D). Taking these results together, the
185 simplest explanation is that *yaaA* is important for survival to DNA damage resulting from
186 photochemistry (UV), crosslinking/intercalation (MMC), or oxidative modification ($H_2O_2$).

187 We next looked at those genes downregulated following MMC treatment. As before, we
188 considered a change in RNA levels of more than one standard deviation below the mean
189 of all values as “downregulated.” Based on these criteria, we identified 87 proteins that
190 became significantly less abundant upon DNA damage without a significant decrease in
191 RNA abundance (Table S7). We performed further experiments on two genes that our
192 Tn-seq results identified as more likely to contain transposon insertions upon MMC
193 damage: *fzlC* and *purD*. However, deletion of these genes did not result in any change to
194 MMC sensitivity (Supplemental Figure S4).

195 In the second stage of the proteomics survey, we compared protein level changes before
196 and after translation shutoff (timeline in Figure 1B). We identified 320 proteins that
197 significantly decreased in protein abundance (Table S8). Among these are the DNA
198 damage response factors LexA and UvrA, which are known to be proteolyzed by ClpXP
199 in *E. coli* (11, 13). These data suggest that this proteolysis may be conserved in
200 *Caulobacter*. Others in this list may be substrates for proteolysis by ClpXP or other
201 *Caulobacter* proteases.

202 These data suggest that while most protein abundance increases during the DNA
203 damage response are explained by changes in gene expression, there are some
204 exceptions. These exceptions may be instances where protein levels are controlled by
205 post-transcriptional or post-translational regulation, but it is unclear how important these
206 are to MMC survival. In our survey, we were able to identify and characterize a novel DNA
207 damage response factor, YaaA, which is important to the survival of oxidative damage as
208 well as the DNA damaging agents MMC and UV light. Finally, we found evidence to
209 suggest that the proteolytic regulation of LexA and UvrA that was discovered in *E. coli* is
210 conserved in *Caulobacter*.

211 **Investigation of the effect of *lon* deletion on the DNA damage response**

212 The Lon protease is important for DNA damage survival in several bacterial species (16,
213 17, 35, 36), including *Caulobacter* (21). To investigate *lon's* role in *Caulobacter's* DNA
214 damage response we performed the same proteomics experiment as above in a *Δlon*
215 strain (full dataset in Table S1 and S2). Most of the known DNA damage-related proteins
216 that were found to increase in abundance upon MMC treatment in the wild type strain

217 also increase in the Δ*lon* strain (See RecA, RuvB, and MmcA in Figure 3A). The
218 exceptions to this are UvrA, which did not increase in abundance upon MMC treatment
219 in the *Δlon* strain (Figure 3A), and ImuA, which was not identified in the *Δlon* strain
220 proteomics.

221 In the translation shutoff portion of the proteomics experiment known Lon substrates
222 DnaA and IbpA, which were shown to decrease in abundance in the wild type strain upon
223 shutoff, showed a far smaller decrease in abundance in the *Δlon* strain (Figure 3B). As
224 expected, decreases in protein abundance upon shutoff in the Δ*lon* strain are largely
225 unchanged for ClpXP and ClpAP substrates (see examples for FtsZ, FtsA, and DnaX in
226 Figure S5).

227 To identify potential Lon substrates from our list of potential protease substrates (Table
228 S8), we compared the change in protein abundance after translational shutoff for each
229 protein in the wild type and Δ*lon* strains. The 10% of proteins with the greatest difference
230 in $\log_2$(5 hrs/3 hrs) between the two strains were considered potential Lon substrates (29
231 candidates; Table S8). The only known DNA damage factor identified in this list was UvrD;
232 however, loss of *uvrD* is synthetic lethal with the deletion of *lon* (21, 37), suggesting that
233 accumulation of UvrD is unlikely to underlie the Δ*lon* sensitivity to DNA damage. Overall,
234 we consider it unlikely that stabilization of a single known DNA damage related protein is
235 the primary driver for the increased sensitivity to DNA damage for the Δ*lon Caulobacter*
236 strain. However, we did observe global changes to the DNA damage response in the Δ*lon*
237 strain, which may be responsible for the MMC sensitivity.

238 Similar to the wild type strain, we monitored RecA levels through induction and recovery
239 after MMC treatment in the Δ*lon* strain by western blot (Figure 4A and S1). RecA levels
240 are about 3-fold higher in the Δ*lon* strain before MMC treatment (average RecA
241 quantification normalized to ClpP quantification in wild type is 0.09±0.02 and in the Δ*lon*
242 strain is 0.30±0.02, Figure S1). This is consistent with a previous observation that DNA
243 damage response genes are upregulated in the Δ*lon* strain even in the absence of
244 exogenous DNA damaging agents (23). In addition, peak RecA induction in the Δ*lon*
245 strain occurs at a similar time point as the wild type (Figure 4A and S1) and both strains
246 reach similar maximum RecA concentration at 3 hours (average RecA quantification
247 normalized to ClpP quantification in wild type is 0.9±0.2 and in the Δ*lon* strain is 0.8±0.3,
248 Figure S1). Together, this means that the fold-increase of RecA in the Δ*lon* strain is about
249 3-fold smaller of that observed in the wild type strain (Figure 4A and S1).

250 We found it to be a general trend that protein changes upon MMC treatment in Δ*lon* strain
251 were less extreme than in the wild type strain (Figure 4B). Similarly, changes in protein
252 abundance were dampened in general upon translation shutoff in Δ*lon* strain (Figure S6).
253 Furthermore, previous studies have found evidence that highly expressed DNA damage
254 response proteins are most rapidly degraded (38). We see a similar negative correlation
255 between protein abundance changes upon MMC treatment (0-3 hours) and upon
256 translational shutoff (3-5 hours) in the wild type strain (Figure 4C) that is absent in the
257 Δ*lon* strain (Figure 4D). These proteome-level changes that we observe in the Δ*lon* strain
258 may be related to *lon's* importance to DNA damage survival in *Caulobacter*.

259 These data indicate that protein changes during the DNA damage response are generally
260 explained by changes in RNA levels, but that there are some proteins that do not follow

261 this trend. In our survey, we identified a novel DNA damage response factor, YaaA, which
262 had been overlooked in previous transcription-focused surveys. The proteomics survey
263 of the Δ*lon* strain was not able to identify a single critical Lon substrate that sufficiently
264 explained the protease's importance to MMC survival. However, we did observe global
265 changes in protein dynamics that may be responsible for the MMC sensitivity of the strain.

266 **DISCUSSION**

267 **Novel insights into the *Caulobacter* DNA damage response**

268 By comparing our proteomics survey to previously published microarray data (19), we
269 found that most protein abundance changes upon DNA damage are explained by
270 changes at the RNA level, though there are exceptions (Figure 2A). Not all genes
271 upregulated at the transcription or protein level were important for survival, which is
272 consistent with the observations that genes upregulated during a stress response do not
273 necessarily overlap with those genes important for tolerating that stress (39–41).

274 In addition, proteins encoded by three essential genes related DNA replication (*dnaQ,*
275 *gyrA,* and *gyrB*) showed changes in protein abundance that are not explained by changes
276 in RNA levels according to microarray (19) or RNA-seq (30). The *dnaQ* gene encodes the
277 proofreading ε subunit of DNA polymerase III and its expression is upregulated upon
278 MMC treatment in *E. coli* in a *recA*-dependent manner (42). Our proteomics survey
279 suggests that its regulation may be post-transcriptional or post-translational in
280 *Caulobacter*. Since its protein abundance decreases after translational shutoff (Table S2),
281 proteolysis may play a role. Levels of another replisome component, DnaX, is also
282 regulated differently in *E. coli* and *Caulobacter.* In *E. coli* a truncated form of DnaX (γ)

283 that is important during DNA damage (43) is created through a ribosomal frameshift (44),
284 while in *Caulobacter* similar DNA damage-related γ forms (45) are created through partial
285 proteolysis by ClpXP (46). It is possible that proteolysis regulates the concentrations of
286 both of these replisome components in *Caulobacter*.

287 We find that DNA gyrase levels (subunits A and B) decrease upon MMC treatment in
288 *Caulobacter* (Table S2) but RNA levels do not appear to decrease when measured by
289 microarray (19, 22) or RNA-seq (30). DNA gyrase levels continue to decrease after
290 translational shutoff, indicating that the abundance of this protein may be regulated by
291 proteolysis (Table S2). In *E. coli* overexpression of a chromosomally encoded DNA
292 gyrase inhibitor *gyrI* increases survival to MMC (47, 48). It's possible that *Caulobacter*'s
293 downregulation of DNA gyrase is filling a similar role.

294 We also identified a novel DNA damage response factor, YaaA, which has previously
295 been shown to be important for survival to oxidative damage in *E. coli* (32). In
296 *Caulobacter*, we show that *yaaA* is important to the survival of the DNA damaging agents
297 MMC and UV light (Figure 2B-2D), indicating that its physiological relevance is not limited
298 to oxidative damaging conditions. It's unclear if *yaaA's* phenotypes in *Caulobacter* are
299 related to its role in regulating iron levels as seen in *E. coli* (32).

300 Previous work has suggested a correlation between level of expression during the DNA
301 damage response and rate of proteolysis (38). This proteomics survey supports this
302 model, as we see a negative correlation between protein abundance changes upon MMC
303 treatment and those upon translation shutoff (Figure 4C). Several DNA damage repair
304 proteins have been identified as proteolysis substrates in *E. coli,* including the LexA N-

305 terminal domain, UvrA, RecA, RuvB, and RecN (38). Of these, our proteomics data
306 provides evidence to support this categorization for the LexA N-terminal domain, UvrA,
307 and RecA but RuvB and RecN do not decrease in protein abundance upon translation
308 shutoff in our survey (Table S2).

309 This proteomics survey has identified examples of proteins that are regulated
310 independently of transcription during the DNA damage response, though it is unclear how
311 many of these are physiologically relevant to the response. We have also identified
312 instances where regulation of certain proteins during DNA damage in *E. coli* appears to
313 be conserved in *Caulobacter*, however the mechanisms of regulation appear to differ in
314 some of these cases. Finally, we have identified a novel DNA damage response factor
315 that has been overlooked in previous surveys that have focused on transcription alone.
316 This highlights the utility of combining transcriptomic and proteomic approaches to screen
317 for novel factors.

318 **The Lon protease and the DNA damage response**

319 Since DNA damage related Lon substrates UmuD (14) and SulA (15) discovered in *E.*
320 *coli* are not conserved in *Caulobacter*, it is unclear why *lon* is important to MMC survival
321 (21). We attempted to identify novel Lon substrates but did not find any potential
322 substrates that sufficiently explain this phenotype (Table S8).

323 The Δ*lon* strain exhibits global differences from the wild type strain in protein dynamics
324 that may account for the strain's sensitivity to MMC (Figure 4 and S6). We find that protein
325 abundance changes during the DNA damage response are dampened in the Δ*lon* strain
326 (Figure 4B and S6), which is at least partially due to the higher basal level expression of

327 DNA damage response genes in the strain (23). We also find that the correlation between
328 changes in protein abundance upon DNA damage treatment and those upon translation
329 shutoff is absent in the Δ*lon* strain (Figure 4C and 4D). These proteome-wide changes to
330 the DNA damage response may be at least partially responsible for the strain's sensitivity
331 to MMC, possibly due to a diminished range of response dynamics.

332 **Concluding thoughts**

333 In our proteomics analysis, we identified and quantified protein abundances for about
334 30% of the *Caulobacter* proteome. While some of the missing proteins are likely not
335 expressed during the growth conditions of our assays, some key DNA damage response
336 proteins are missing from our dataset. Some of these are known to be membrane-
337 associated (e.g. SidA (19) and DidA (22)), which may reduce our detection, and others
338 likely have relatively low abundance, even during DNA damage induction. Future work
339 using protocols designed specifically to observe these proteins could reveal additional
340 novel factors involved in DNA damage survival or reveal novel mechanisms of regulation
341 for known factors.

342 Our survey of protein dynamics during the DNA damage response allowed us to confirm
343 that, in general, protein abundance changes during the response are explained by
344 changes in RNA level. We also cataloged exceptions to this general rule and characterize
345 the novel DNA damage response factor, YaaA. Finally, though we did not identify novel
346 Lon substrates that explain this protease's importance to DNA damage survival, we did
347 identify systems-level differences in the size of protein abundance changes during the
348 response in the absence of *lon* which may play a role in this phenotype.

349 **METHODS**

350 **Bacterial strains and growth conditions**

351 Bacterial strains and plasmids used in this work are listed in Tables S9 and S10,
352 respectively. All *Caulobacter* strains used in this work are NA1000 derivatives.
353 *Caulobacter* strains were grown in PYE broth (2 g/L peptone, 1 g/L yeast extract, 1 mM
354 $MgSO_4$, and 0.5 mM $CaCl_2$). Solid media was made with 1.5% agar. Knockout strains
355 were created by replacing the native gene with a gentamycin resistance cassette using
356 the pNPTS138 non-replicating vector (45). Venus- and mKate2-expressing strains were
357 created by integrating pXGFPC-2 (49) based plasmids encoding constitutively expressed
358 fluorescent proteins driven by a *lac* promoter (50) at the xylose locus.

359 **Bacterial growth curves**

360 An overnight culture was diluted into PYE at an OD600 = 0.05. A volume of 200 µL of
361 cells was added to wells of a 96-well plate. Plate was shaken continuously at 30ºC and
362 OD600 was read every 20 minutes in a BioTek Epoch 2 Microplate Spectrophotometer
363 (Agilent, Santa Clara, CA). MMC (0.5 µg/ml; Millipore Sigma, St. Louis, MO) was used to
364 treat cells with DNA damage.

365 **Western blot**

366 Western blots were performed using a commercially available rabbit anti-*E. coli* RecA
367 primary antibody (1:10,000 dilution; Abcam, Cambridge, MA, 16 hours). Blots were
368 visualized using the goat anti-rabbit Alexa Fluor™ Plus 800 secondary antibody (1:10,000
369 dilution; Licor, Lincoln, NE, 1 hour). Bands were quantified using ImageJ (51).

370 **Competition assays**

371 Overnight cultures of each test strain and the control strain were diluted to an OD600 =
372 1.0, mixed 1:1, and serially diluted 30,000-fold. Undiluted cells were analyzed with a
373 SpectraMax M2 microplate reader (Molecular Devices, San Jose, CA). Measurements
374 were taken for cell density (OD600), Venus fluorescence (Excitation 490 nm, Cut off 515
375 nm, Emission 530 nm), and mKate2 fluorescence (Excitation 575 nm, Cut off 590 nm,
376 Emission 630 nm). Diluted samples were grown to stationary phase (~48 hours, shaking
377 at 30°C) and optical density and fluorescence measurements were taken again. The
378 competitive index was calculated as the $\log_2$[((ratio test/control)END / (ratio
379 test/control))START / ((average ratio wild type test/control)END/[(ratio wild type
380 test/control))START].

381 **Sensitivity testing to ultra-violet light**

382 Cells were grown to exponential phase and treated with 0 or 100 J/m$^2$ with a UV
383 Stratalinker (Strategene, San Diego, CA, USA). Cells were diluted $10^0$-$10^{-7}$ and dilutions
384 were plated on PYE agar. Plates were incubated for 48 hours at 30°C and then
385 photographed.

386 **Transposon Sequencing**

387 Transposon libraries were created as previously described (52, 53). A wild-type EZ-Tn5
388 library was treated in triplicate with mitomycin C (0.5 µg/mL) for 1 hour, outgrown for about
389 10 generations, and sequenced. Data was analyzed using edgeR (54). Differential
390 analysis of the counts in these libraries is available in Table S3.

391 **Sample collection and preparation for proteomics**

392 Pre-damage sample

393 Three biological replicates of overnight growth of each *Caulobacter* strain (wild type and
394 *Δlon)* were diluted to an OD600 = 0.0005 using PYE to a volume of 50 mL and grown for
395 approximately 10 generations to an OD600 of 0.5-0.6. All samples were normalized to an
396 OD600 of 0.5 using PYE to a volume of 25mL. A 5 mL pre-damage sample was taken,
397 and cells were collected by centrifugation (6,000 x g, 5min). Cell pellets were
398 resuspended in fresh lysis buffer (8 M urea, 50 mM HEPES pH7.5, 75 mM NaCl), flash
399 frozen, and stored at -80˚C.

400 Post-damage/pre-shutoff sample

401 Cells were treated with 0.5 µg/mL MMC for 1 hour before cells were collected by
402 centrifugation (6,000 x g, 10 min) and resuspended in fresh PYE broth to an OD600 = 0.5
403 to a volume of 25 mL. These treatment conditions are similar to those of the microarray
404 from the Laub lab (19) for simple comparison. Previous experiments indicate that the wild
405 type strain has about 55% survival to this treatment (45). After 2 hours, all samples were
406 normalized to an OD600 = 0.5 using PYE to a volume of 25 mL. A 5 mL post-damage
407 sample was taken, and cells were collected by centrifugation (6,000 x g, 5 min). Cell
408 pellets were resuspended in fresh lysis buffer, flash frozen, and stored at -80ºC.

409 Post-shutoff sample

410 Cells were treated with chloramphenicol (30 µg/mL) for 2 hours. A 5 mL post-shutoff
411 sample was taken, and cells were collected by centrifugation (6,000xg, 5 min). Cell pellets
412 were resuspended in fresh lysis buffer, flash frozen, and stored at -80˚C.

413 Sample preparation for proteomics

414 Samples were freeze-thawed three times in liquid nitrogen to aid cell lysis, then
415 centrifuged at 16,000 x g for 10 min at 4°C. Supernatant was carefully transferred to a
416 fresh 1.5mL tube. A Bradford assay was performed to quantify protein concentration.
417 Because the Bradford assay is sensitive to high urea concentrations, a small fraction of
418 each sample was diluted 4-fold for the Bradford assay. 50 µg of each sample was
419 transferred to a fresh 1.5mL tube. The final volume was adjusted to 50 µL using 100 mM
420 Triethylammonium bicarbonate (TEAB) buffer.

421 To reduce disulfide bonds, 5 µL 200 mM tris(2-carboxyethyl)phosphine (TCEP) was
422 added to each sample and incubated at 55˚C for 1 hour. To modify cysteine residues, 5
423 µL of 375 mM iodoacetamide was added to each sample and incubated 30 min at room
424 temperature in the dark. To precipitate protein, 6-volumes of cold acetone was added to
425 each sample, and they were stored overnight at -20˚C.

426 Samples were centrifuged at 8,000 x g for 10 min at 4˚C to collect precipitate. Supernatant
427 was carefully removed and discarded. Pellets were allowed to dry at room temperature
428 for 1 hour. Protein pelleted were resuspended in 100 µL 50 mM TEAB. Samples were
429 incubated overnight at 37˚C with 1.25 µg of trypsin (for a 2.5:100 ratio of trypsin to
430 protein).

431 **Tandem mass tagging for proteomics**

432 Samples were labelled using the TMT10plex™ Isobaric Label Reagent Set
433 (ThermoFisher Scientific, Waltham, MA, Catalog #90110) according to the product
434 instructions. Since each sample only contains 50 µg of peptides instead of the suggested
435 100 µg, half of each label was used per sample.

436 **Proteomics sample fractionation**

437 For each replicate experiment in each strain, equal volumes of each samples were
438 combined into a single tube and then dried in a SpeedVac. Pooled samples were
439 resuspended in 300 µL 0.1% TFA. Samples were dried in a SpeedVac a second time and
440 resuspended in 300 µL 0.1% TFA. The pH of each sample was measured to confirm that
441 it was between 2.5 - 3.5, which is critical for effective fractionation.

442 Pooled samples were fractionated using the Pierce™ High pH Reversed-Phase Peptide
443 Fractionation Kit (ThermoFisher Scientific, Waltham, MA, Catalog #84868) according to
444 the kit instructions. Fractions 1 and 5, 2 and 6, 3 and 7, as well as 4 and 8 were pooled
445 together for a final total of 4 fractions per sample. Samples were dried and resuspended
446 in 0.1% folic acid in water for mass spectrometry.

447 **Mass spectrometry for tandem mass tagging proteomics**

448 LC-MS analysis was performed, modified from (55) using an Easy-nLC 1000 nanoLC
449 chromatography system interfaced to an Orbitrap Fusion mass spectrometer
450 (ThermoFisher Scientific, Waltham, MA). Samples were pre-concentrated and desalted
451 on to a C18 trap column prior to separation over a 180 min gradient from 0% to 50%

452 mobile phase B (A:0.1% formic acid in water, B:0.1% formic acid in acetonitrile) at 300
453 nL/min flow rate with a 75 µm x 15 cm PepMap RLSC column (ThermoFisher Scientific,
454 Waltham, MA). Mass spectrometry parameters were as follows: ion spray voltage 2000V,
455 survey scan MS1 120k resolution with a 2 s cycle time, interleaved with data-dependent
456 ion trap MS/MS of highest intensity ions with charge state-dependent ion selection
457 window (z=2:1.2 m/z, z=3:0.7 m/z, z=4-6:0.5 m/z) and CID at 35% normalized collision
458 energy. Additionally, the top 5 (z=2) or 10 (z>2) product ions were synchronously selected
459 for HCD MS^3 at NCE 65% with Orbitrap detection to generate TMT tag intensities.

460 **Proteomics Data Analysis**

461 RAW data files were analyzed in Proteome Discoverer 2.4 (ThermoFisher Scientific,
462 Waltham, MA) using the SEQUEST search algorithm against *Caulobacter crescentus*
463 NA1000 (NCBI Reference Sequence: NC_011916.1) database downloaded from
464 uniprot.org. The search parameters used are as follows: 10 ppm precursor ion tolerance
465 and 0.4 Da fragment ion tolerance; up to two missed cleavages were allowed; dynamic
466 modifications of methionine oxidation and N-terminal acetylation. Peptide matches were
467 filtered to a protein false discovery rate of 5% using the Percolator algorithm. Peptides
468 were assembled into proteins using maximum parsimony and only unique and razor
469 peptides were retained for subsequent analysis. Protein quantitation based on TMT ion
470 abundance was performed using a co-isolation threshold of 75% and SPS match
471 threshold 65%. Each TMT channel was normalized to total peptide amount and then
472 abundance scaled to average 100% for all proteins.

473 Protein abundances were then normalized to the averages of the 0 hour samples (Table
474 S1). Protein ratios (Table S2) were calculated using edgeR (54).

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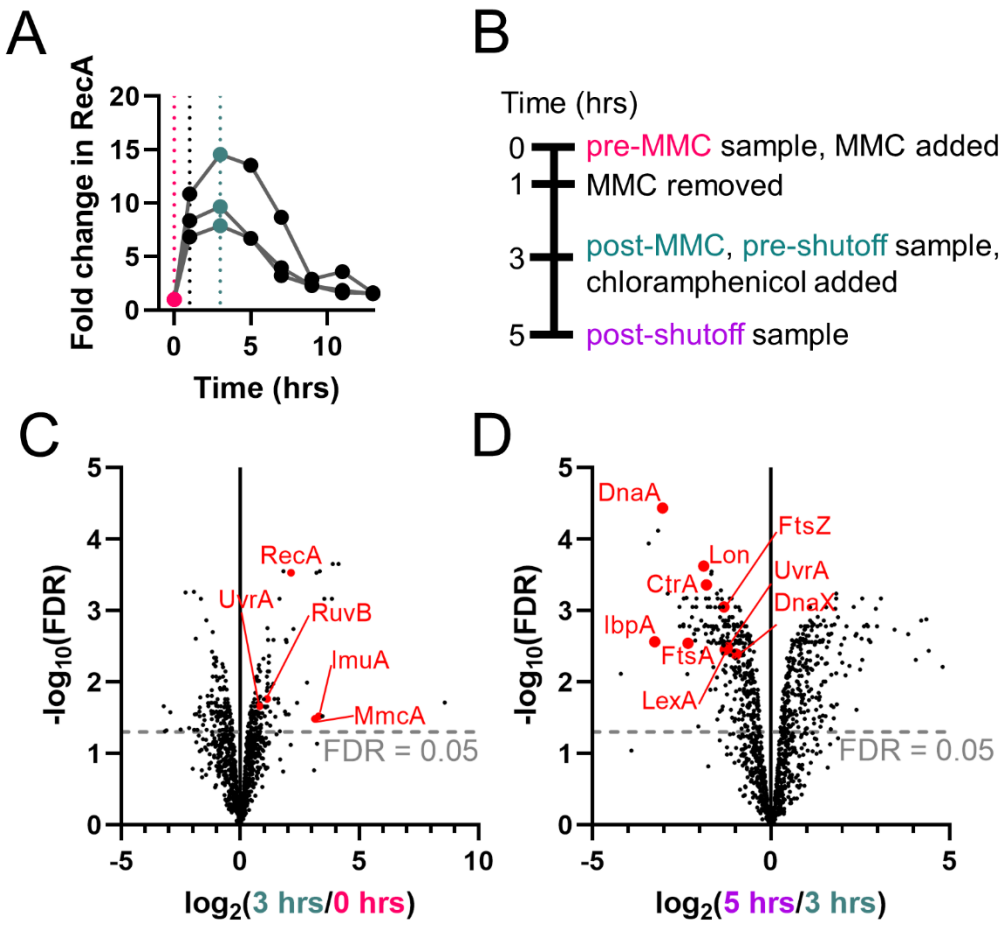


**Figure 1. Survey of *Caulobacter* DNA damage response distinguishes known DNA damage response factors and protease substrates.** (A) A western blot for RecA protein levels was used to determine peak protein induction (3 hours) to use for post-MMC timepoint for proteomics survey. Quantification of western blot for three replicates is shown, representative blot shown in Figure S1. RecA quantification was normalized to ClpP band and then to time 0 (Figure S1). (B) Samples for proteomics were taken before treatment (0 hours, pink line). Cells were treated with MMC for 1 hour and recovered in fresh medium for 2 additional hours before the post-treatment proteomics sample was taken (3 hours, teal line). At 3 hours chloramphenicol was added to shutoff translation and a post-shutoff sample was taken at 5 hours. (C) A volcano plot of proteomics data

593 compares the statistical confidence (-$\log_{10}$(false discovery rate)) versus the change in
594 protein abundance after MMC treatment $\log_2$(3 hrs/0 hrs). Several of the proteins that are
595 significantly upregulated after DNA damage treatment are known DNA damage response
596 proteins (highlighted in red). (D) A volcano plot of proteomics data compares the statistical
597 confidence (-$\log_{10}$(false discovery rate)) versus the change in protein abundance after
598 translation shutoff $\log_2$(5 hrs/3 hrs). Several of the proteins that are significantly
599 downregulated after chloramphenicol treatment are known protease substrates
600 (highlighted in red). FDR, false discovery rate.

601

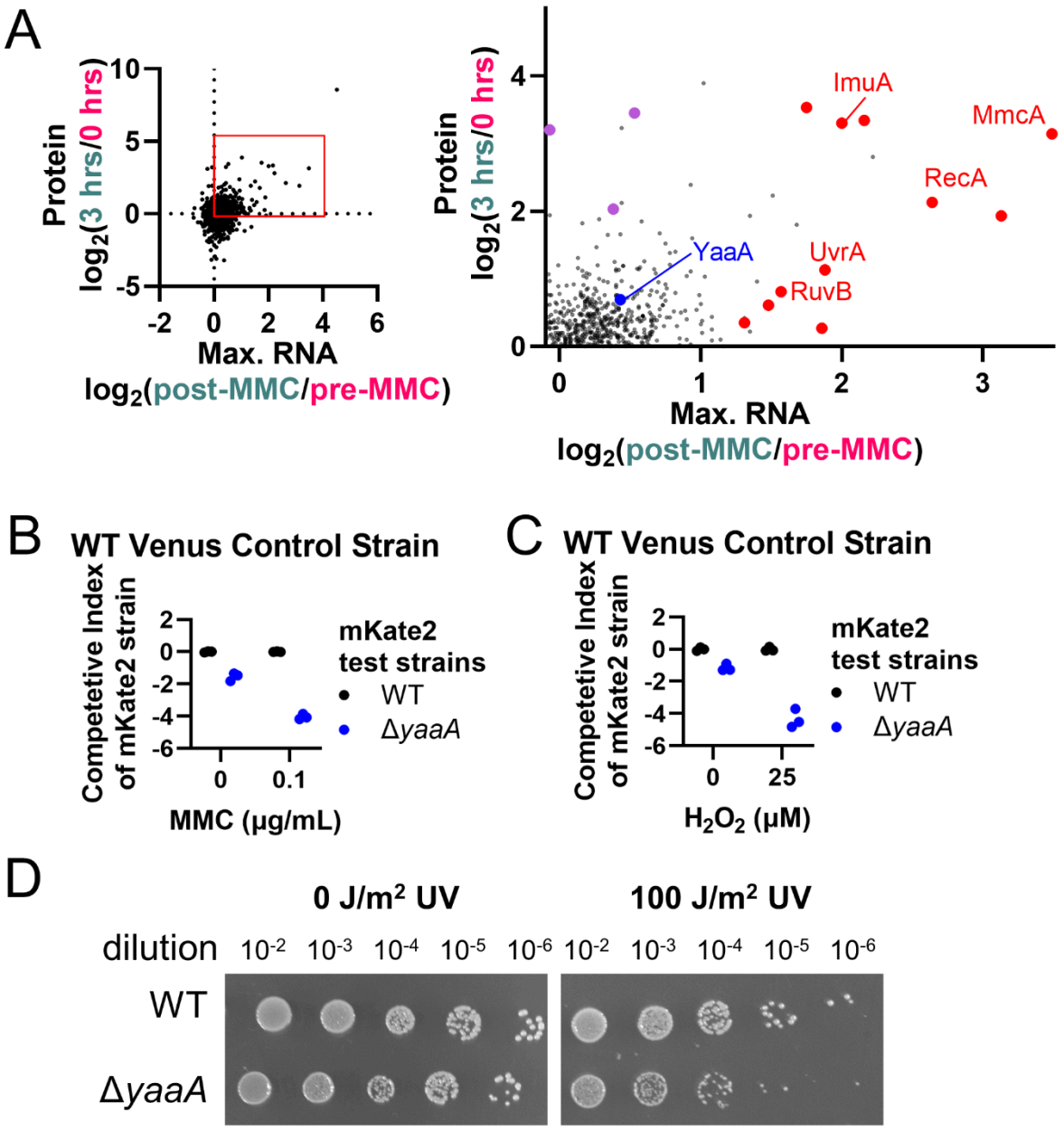


**Figure 2. YaaA is upregulated upon MMC treatment and is important for survival to MMC, $H_2O_2$, and UV light.** (A) A comparison between our proteomics and previously published microarray experiment that uses similar MMC treatment conditions (19). Graph on the right is an enlargement of the red box in the graph on the left. Proteins highlighted in red are known DNA damage response genes that are upregulated at the RNA and

608 protein levels. YaaA is highlighted in blue. CCNA_02118, CCNA_00591, and
609 CCNA_00276 are highlighted in purple. Graph cropped to upper right quadrant to
610 highlight proteins that are upregulated. One gene is excluded (CCNA_03825) due to its
611 extremely high induction to make other points more visible. (B and C) When each mKate2-
612 expressing test strain is competed in co-culture against a wild type Venus-expressing
613 fluorescent control strain, only the *ΔyaaA* mutant shows a competitive disadvantage that
614 is exacerbated by the presence of (B) 0.1 μg/mL MMC or (C) 25 μM hydrogen peroxide.
615 A reciprocal experiment in which test strains are marked with Venus and wild type control
616 is marked with mKate2 is shown in Figure S3B and S3C. (D) The wild type and Δ*yaaA*
617 strains were exposed to 0 or 100 J/m$^2$ of ultra-violet light and 10-fold dilutions were plated.
618 The Δ*yaaA* strain is more sensitive to ultra-violet light than the wild type strain.

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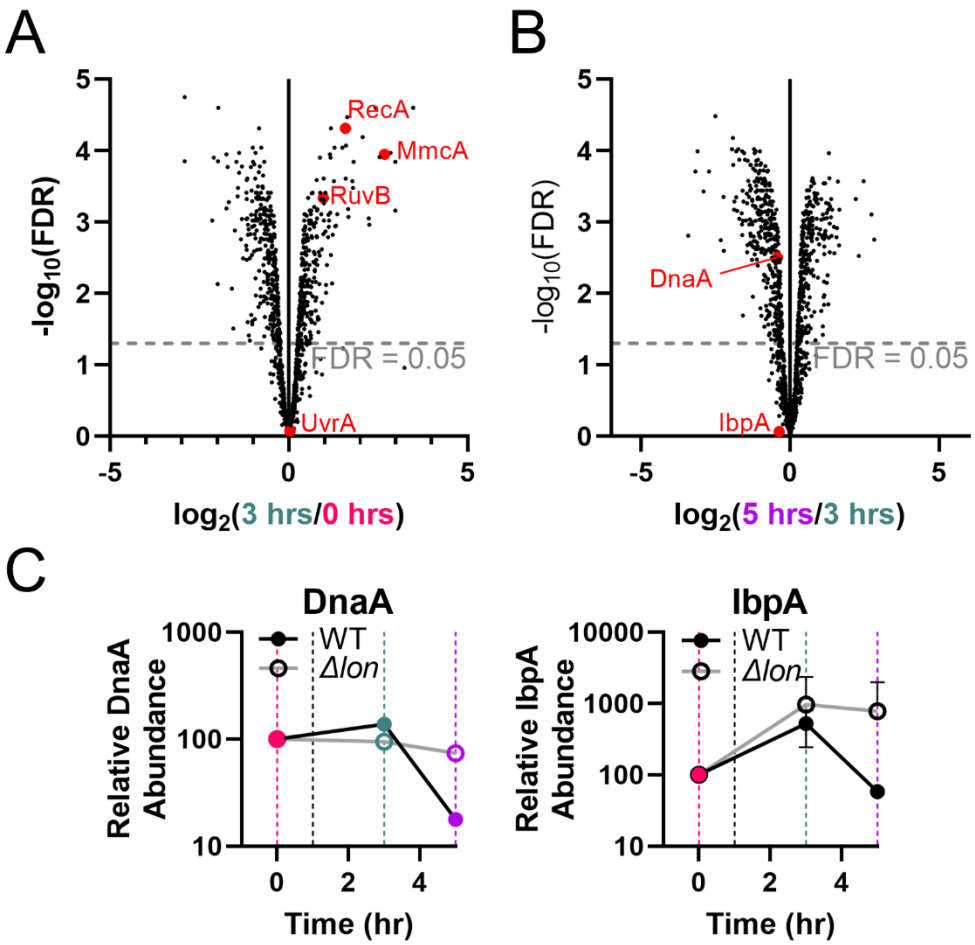


620

621 **Figure 3. Proteomics Survey in *Δlon* strain distinguishes known Lon substrates.**
622 (A) A volcano plot of proteomics data compares the statistical confidence ($-\log_{10}$(false
623 discovery rate)) versus the $\log_2$(3 hrs/0 hrs). Known DNA damage proteins shown in
624 Figure 1B are highlighted here in red. ImuA was not identified in the proteomics of the
625 Δ*lon* strain and so is absent. (B) A volcano plot compares the statistical confidence (-
626 $\log_{10}$(false discovery rate)) versus the $\log_2$(5 hrs/3 hrs) for the Δ*lon* strain. Known Lon
627 substrates DnaA and IbpA show a smaller decrease in protein abundance after translation
628 shutoff than in the wild type strain (compare to Figure 1D). (C) Protein abundance of
629 known Lon substrates DnaA and IpbA significantly increases upon MMC treatment (0-3

630 hours, pink and teal lines) and then decreases upon translation shutoff in a *lon*-dependent
631 manner (3-5 hours, teal and purple lines). Note that protein abundances are not directly
632 comparable between the two strains. FDR, false discovery rate.

633

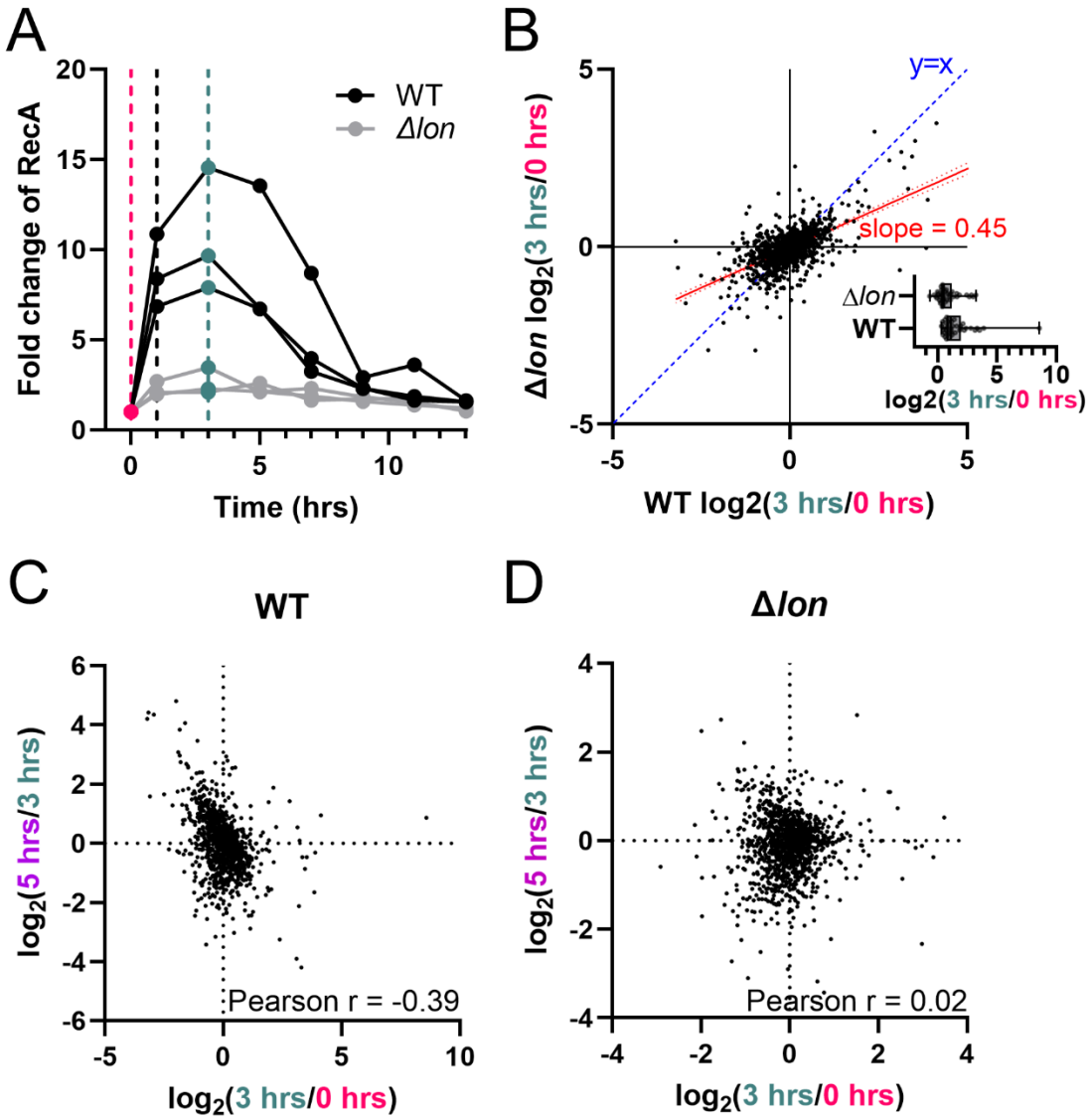


**Figure 4. Protein abundance changes upon DNA damage are generally smaller in the Δ*lon* strain compared to in the wild type strain.** (A) A western blot to determine RecA protein levels was used to determine when to sample for protein abundance changes after DNA damage treatment in the Δ*lon* strain. Note that the wild type data here is repeated from Figure 1A for easy comparison. Quantification of western blot for three replicates is shown, representative blot shown in Figure S1. Sampling for proteomics was performed as described in Figure 1B. RecA quantification was normalized to ClpP band

642 and then to time 0 (see Figure S1). (B) Comparison of the $\log_2$(3 hrs/0 hrs) for the Δ*lon*
643 versus wild type strain shows that fold changes of protein abundance are generally lower
644 in the Δ*lon* strain. Blue dashed line represents an y=x line. The red line represents the
645 trendline of the data with 95% CI drawn (slope = 0.45). The inset plots $\log_2$(3 hrs/0 hrs)
646 for all proteins that are significantly upregulated (FDR < 0.05). Comparison of protein
647 changes upon MMC treatment and translational shutoff indicates a negative correlation
648 in the wild type strain (C) that is absent in the (D) Δ*lon* strain.

649