Title

Poly(A) binding protein is required for mRNP remodeling to form P-bodies in mammalian cells

Running title

Poly(A) binding protein and P-body formation

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Keywords

PABPC1, P-bodies, stress granules, mRNP remodeling, GW182

Summary statement

A novel role of poly(A) binding protein is reported in P-body formation

Abstract

Compartmentalization of mRNA through formation of RNA granules is involved in many cellular processes, yet it is not well understood. mRNP complexes undergo dramatic changes in protein compositions, reflected by markers of P-bodies and stress granules. Here, we show that PABPC1, albeit absent in P-bodies, plays important role in P-body formation. Depletion of PABPC1 decreases P-body population in unstressed cells. Upon stress in PABPC1 depleted cells, individual P-bodies fail to form and instead P-body proteins assemble on PABPC1-containing stress granules. We hypothesize that mRNP recruit proteins via PABPC1 to assemble P-bodies, before PABPC1 is displaced from mRNP. Further, we demonstrate that GW182 can mediate P-body assembly. These findings help us understand the early stages of mRNP remodeling and P-body formation.

32 **Introduction**

33

34 Cytoplasmic messenger ribonucleoproteins (mRNPs) are suggested to cycle among
35 polysomes, stress granules (SGs) and P-bodies (PBs). In this cycle, mRNAs exist in
36 different functional states from translating, non-translating to degradation. While
37 polysomes consist of translating mRNAs and SGs paused mRNAs with translation
38 initiation components, PBs contain mRNA decay machineries.

39

40 PBs are present in unstressed cells, and further induced upon inhibition of translation
41 initiation (Teixeira et al., 2005, Kedersha et al., 2005). PBs are closely related to control
42 of translation and mRNA degradation. Proteins at PBs are involved in mRNA decay and
43 translation repression, including decapping enzyme complex Dcp1/Dcp2; decapping
44 activator hedls/GE-1; translation repressor and decapping activator DDX6/RCK, and the
45 CCR4/NOT deadenylase complex (Anderson and Kedersha, 2006, Eulalio et al., 2007a).

46

47 SGs can be juxtaposed with PBs in animal cells(Kedersha et al., 2005, Stoecklin and
48 Kedersha, 2013), which suggests that they are close in origin. SGs share some common
49 components with PBs, but distinctively contain translation initiation factors like
50 PABPC1, eIF4G, eIF4A, eIF3 and eIF2 etc. (Decker and Parker, 2012).

51

52 Assembly of PBs and SGs may start from non-translating mRNPs, which aggregate into
53 microscopic granules through certain protein-protein interactions. In yeast, a self-
54 interacting domain of Edc3 protein and prion-like glutamine/asparagine (Q/N) rich region
55 of Lsm4 can facilitate the aggregation (Reijns et al., 2008, Decker et al., 2007). However,
56 in metazoans, Edc3 is not required for PB assembly (Eulalio et al., 2007b). Instead,
57 depletion of GW182 or hedls/GE-1, two proteins containing low-complexity and Q/N
58 rich regions, leads to decreased PBs in unstressed animal cells (Eulalio et al., 2007b, Liu
59 et al., 2005a, Yu et al., 2005, Kato et al., 2012).

60

61 One interesting issue is the absence of most translation initiation factors, including
62 poly(A) binding protein cytoplasmic 1 (PABPC1), in P-bodies of mammalian cells.

63 Components of RNA granules are in dynamic exchange with cytoplasmic proteins
64 (Kedersha et al., 2005, Andrei et al., 2005). The association and dissociation of proteins,
65 such as PABPC1, to and from the cap and tail of mRNA may be an important step in the
66 transitions of mRNAs from translating to non-translating or decay states.
67
68 mRNAs have a poly(A) tail of about 50-100 nucleotides in mammalian cells (Chang et
69 al., 2014, Subtelny et al., 2014). PABPC1 occupies around 27-residues on the poly(A)
70 tail and forms repeating structures (Baer and Kornberg, 1983). PABPC1 is the major
71 isoform out of four known in mammals. PABPC1 consists of four RNA binding domains
72 (RRM 1-4) followed by a poorly conserved linker region and a protein-protein interaction
73 (MLLE) domain at the C-terminus. The RRM domains are pivotal for circularization of
74 mRNA through the binding of the poly(A) tail and eIF4G (Kahvejian et al., 2005, Deo et
75 al., 1999, Imataka et al., 1998, Safaee et al., 2012). The MLLE domain recognizes a
76 conserved PAM2 peptide motif, found in a number of proteins including PB components
77 GW182, Pan3 and Tob1/2 (Xie et al., 2014).
78
79 PABPC1 plays important roles in decay besides translation of mRNA. Yeast poly(A)
80 binding protein (Pab1) can inhibit Ccr4/Pop2/Not deadenylase complex (Tucker et al.,
81 2002), possibly due to increased association and protection of Pab1 to the poly(A) tail. It
82 was recently shown that PABPC1 helps recruiting microRNA-induced silencing complex
83 (miRISC) through GW182, a major component of miRISC (Moretti et al., 2012).
84 GW182, in turn, facilitates PABPC1 dissociation from silenced mRNA without
85 deadenylation (Zekri et al., 2013).
86
87 Although PABPC1 displacement is a critical step in mRNA decay and can act as a
88 scaffold protein on mRNA to recruit PB proteins GW182 and Pan3 etc. through PAM2
89 motifs, it is not known what role PABPC1 plays in assembling PBs. Here, we report that
90 depletion of PABPC1 affects PB numbers in unstressed cells and causes fusion of PB and
91 SG components upon cell stress. Further, we found that the availability of PABPC1, but
92 not the presence of SG structures, is critical in PB genesis. By engineering GW182 to

93 strengthen its binding to PABPC1, we simulated the fusion of SGs and PBs. Overall, we
94 conclude that PABPC1 serves as a platform for early mRNP remodeling to form PBs.
95
96 **Results**
97 **PABPC1 protein depletion decreases PBs in unstressed cells**
98 We were able to knock-down PABPC1 using siRNAs as previously shown (Yoshida et
99 al., 2006). The number of PBs decreased significantly upon PABPC1 knock-down in
100 unstressed HeLa and MEF cells (Fig. 1A). PABPC1 depletion affects bulk translation
101 only mildly and doesn't perturb the levels of the major translation factors (Yoshida et al.,
102 2006). However, Paip2, a PABPC1-interacting protein, decreases in cells following
103 PABPC1 depletion (Yoshida et al., 2006). To exclude a role of Paip2 in PB formation,
104 we knocked down both isoforms, Paip2a and Paip2b, by siRNA and found little effect on
105 the number of PBs (Fig. 1B). It was reported that depletion of hedls or GW182 decreased
106 PBs in cells (Eulalio et al., 2007b, Liu et al., 2005b, Yu et al., 2005). However, PABPC1
107 depletion didn't lower hedls or GW182 protein levels (Fig. 1C), which suggests that
108 PABPC1 is affecting PBs independently of changes in hedls or GW182.
109
110 **PBs cannot be re-induced in PABPC1 protein depleted cells**
111 PB population may be reduced by siRNAs unrelated to their silencing activities and PBs
112 can often be re-induced by stress (Serman et al., 2007). Therefore, we checked whether
113 PBs could be re-induced upon cell stress. In mammalian cells, PBs and SGs can be
114 clearly distinguished. PBs are relatively compact and dense, while SGs are bigger, loose
115 and more irregular (Stoecklin and Kedersha, 2013, Souquere et al., 2009, Kedersha et al.,
116 2005). When we treated PABPC1-depleted cells with arsenite to induce PBs and SGs, we
117 were surprised to find that compact dense PBs failed to form. Instead, the PB component
118 hedls was relocalized to SGs (Fig. 2). The colocalization was found across the cytoplasm
119 by 3D confocal microscopy. This suggests that PB assembly is affected by depletion of
120 PABPC1.
121 To our knowledge, only DDX6, a RNA helicase also known as RCK/p54, can trigger
122 PB/SG fusions and prevent PB formation when depleted (Mollet et al., 2008, Serman et
123 al., 2007, Ayache et al., 2015). However, DDX6 protein levels remained unchanged after

124 PABPC1 depletion (Fig. 1C). Thus, PABPC1 affects PB formation independently of
125 DDX6.
126
127 **SG assembly is not affected by PABPC1 depletion and is independent of PB**
128 **assembly**
129 Previously, it was observed that PABPC1 depletion induced SG in a low percentage of
130 unstressed cells (Mokas et al., 2009). We checked SG assembly in stressed cells, and
131 found SGs marked by G3BP, HuR (Fig. 3A & B) or Ago2 were not significantly affected
132 by PABPC1 depletion. This implies that SGs can form in the presence of low levels of
133 PABPC1 protein.
134 The assembly of PBs and SGs are independent processes (Kedersha et al., 2005). PBs
135 exist in unstressed cells in the absence of SGs. Meanwhile, PBs can be increased by
136 arsenite stress in MEF cells expressing non-phosphorylated eIF2α mutant, which prevents
137 SG formation (Kedersha et al., 2005). We treated HeLa cell with PP242, an mTOR
138 inhibitor inhibiting SG assembly (Fournier et al., 2013, Feldman et al., 2009), and found
139 PBs were increased by arsenite without SG formation (Fig. 3C). This implies that PBs do
140 not require SGs to form in agreement with previous studies (Kedersha et al., 2005).
141
142 **Endogenous PABPC1 can be substituted by over-expressed PABPC1-GFP**
143 We have shown that PB components can be found at PABPC1-rich SG (Fig. 2), and that
144 only a fraction of the SGs contain PABPC1 in knocked-down cells (Fig. 3). Thus PBs
145 require PABPC1, but not other SG components, for assembly.
146 It is known that overexpression of PABPC1 down-regulates the endogenous PABPC1
147 translation and protein abundance through self-regulation (Hornstein et al., 1999b, de
148 Melo Neto et al., 1995, Wu and Bag, 1998, Hornstein et al., 1999a). This provides an
149 opportunity to create cells where endogenous PABPC1 is replaced by exogenous
150 PABPC1-GFP. We took advantage of an antibody (Abcam ab21060) that recognized the
151 tail region of PABPC1 but not the chimeric PABPC1-GFP protein. Overexpression of
152 PABPC1-GFP reduced endogenous PABPC1 to a very low level in cells (Fig. s1).
153 However, the PBs assembled as normal, which implies that the over-expressed PABPC1-

154 GFP can substitute endogenous PABPC1 in maintaining the PB population in unstressed
155 cells.
156
157 **Separate PBs can be induced after SG inhibition**
158 PBs assembly is closely related to PABPC1-containing SGs at low PABPC1 levels. We
159 then asked whether structures of SG were required or not in such process. With PP242
160 inhibiting SG assembly, we stressed PABPC1-depleted cells with arsenite and individual
161 PBs began to form (Fig. 3D). This shows that the low level of PABPC1 dissipated in
162 cytoplasm can support PB assembly under stresses. It also confirms that SGs are not
163 required for PB assembly. On the contrary, when the low level of PABPC1 was packed
164 into SGs, it results in fusion of PBs and SGs. It is likely the tightly packed PABPC1 in
165 SGs will not easily dissociate for mRNP remodeling, and thus lead to fusion of PBs and
166 SGs.
167
168 **Overexpressed Dcp1a, TNRC6A and DDX6 can induce PB formation in PABPC1-**
169 **depleted cells, and are found associated with the fusions of PBs and SGs**
170 Overexpression of PB proteins can generate PBs that are not functional (Cougot et al.,
171 2004). In PABPC1-depleted cells, overexpression of Dcp1a or DDX6 could induce PBs
172 (Fig. 4A). When cells were stressed with arsenite, sorbitol, or heat shock, we found
173 Dcp1a-GFP or HA-TNRC6A were present in PB/SG fusions (Fig. 4B & C). This
174 confirms that multiple PB components were assembling at the fusion structures.
175 Meanwhile, overexpression of Dcp1a or DDX6 could induce PB-like structures in
176 stressed cells, as observed by other groups. Therefore, depletion of PABPC1 did not
177 prevent the aggregation of overexpressed PB proteins into granules.
178
179 **Creating PB/SG fusions without PABPC1 depletion**
180 We next asked how PABPC1, a protein absent from PBs, could affect their formation.
181 One hypothesis is that PB assembly may initiate on PABPC1-containing mRNPs. When
182 the residual PABPC1 after depletion is packed into SGs, PB components are recruited
183 there and thus a PB/SG fusion emerges. We speculated that certain PB proteins

184 interacting with PABPC1 might mediate the processes. We used a protein engineering
185 approach to test our hypothesis.
186 GW182 proteins (TNRC6A/B/C in human) interact with PABPC1 through the MLLE
187 domain and are required for PB assembly (Eulalio et al., 2007b; Liu et al., 2005a). The
188 native affinity of the GW182 PAM2 motif for MLLE of PABPC1 is about 6 μM (Kozlov
189 et al., 2010, Jinek et al., 2010). We engineered the PAM2 motif in GW182 to increase its
190 affinity by including features from Paip2 PAM2 motif. The engineered GW182 protein
191 contained a super-PAM2, binding PABPC1 about 300 times tighter (Fig. 5A, construct
192 information in Materials and Methods). The super-PAM2 motif could efficiently localize
193 fused GFP protein to stress granules (Fig. 5B). Introduction of super-PAM2 greatly
194 increased association of GW182 and endogenous PABPC1 (Fig. 5C). When we stressed
195 cells overexpressing super-GW182-GFP, the super-GW182 along with other PB
196 components located to SGs to make PB/SG fusions (Fig. 6). This fusion is the combined
197 result of the increased affinity PAM2/MLLE interaction and tightly packed PABPC1 at
198 SG. It indicates that PABPC1 interacting proteins, like GW182, could play an important
199 role in PB assembly through interaction with PABPC1.
200
201 **Ago-binding region of super-GW182 is involved in recruiting other components**
202 Both the N-terminal Ago-binding and mid Q/N region of GW182 were required for PB
203 localization (Lazzaretti et al., 2009, Behm-Ansmant et al., 2006). We generated deletions
204 of super-GW182 (TNRC6A) and checked their capability to bring PB/SG components
205 together. Under arsenite stress, super-GW182ΔQ/N could induce fusion of PB/SG (Fig.
206 7B), while super-GW182ΔAgo was less efficient in fusing PB/SG (Fig. 7A). This
207 suggests that the Ago-binding region is critical in subsequent PB components recruiting
208 and assembly. This agrees with previous studies that showed that deletion of Q/N region
209 in TNRC6A affects its PB localization much less than deletion of Ago-binding region
210 (Lazzaretti et al., 2009). This indicates that the redirection of PB components to SGs by
211 super-GW182 uses a similar mechanism to TNRC6A assembly into PBs.
212
213

214 **GW182ΔPAM2 can aggregate in PABPC1 depleted cells and goes to SGs when**
215 **stressed**
216 We have shown that super-GW182 mimicked fusion of PB/SGs without depletion of
217 PABPC1. This led us to ask what if the PAM2/MLLE interaction was missing? We
218 transfected GW182ΔPAM2 (ΔT952–Q971) plasmid together with PABPC1 siRNA. HA-
219 GW182ΔPAM2 formed PB-like granules in unstressed cells (Fig. s2A) and could be
220 found in PB/SG fusions when stressed (Fig. s2B). The tendency of overexpressed PB
221 proteins to aggregate into granules makes it hard to dissect the role of PAM2/MLLE
222 interaction here.
223
224 **Discussion**
225 Here, we have shown that PABPC1 protein level is critical to PB assembly. PABPC1 is a
226 translation initiation factor and plays important role in mRNA metabolism. Depletion of
227 PABPC1 decreases the number of PBs in unstressed cells. When the PABPC1-depleted
228 cells are stressed, PB components are recruited to PABPC1-enriched SG. However, if SG
229 assembly is inhibited, individual PBs can form in stressed cells.
230 PB assembly may initiate on PABPC1-containing mRNPs (Fig. 8). PABPC1 might be
231 required in mRNP remodeling into P-bodies, thus PABPC1 depletion decreases PB
232 numbers in unstressed conditions. The requirement of PABPC1 for PB formation drives
233 PB components to certain SGs, where the residual PABPC1 is enriched after depletion.
234 The displacement of PABPC1 at SG is difficult so individual PBs fail to form. When SG
235 is inhibited by PP242, the same residual amount of PABPC1 in cytoplasm can support
236 normal PB formation. It implies that the ability of PABPC1 to disassociate from mRNPs
237 is required for formation of individual PBs. However, the mechanisms regarding how and
238 when PABPC1 disassociates from mRNP are still to be investigated.
239 The displacement of PABPC1 from microscope-visible PBs is a critical step in PB
240 assembly as was proposed by other colleagues (Chen and Shyu, 2013). PABPC1 plays a
241 double role regarding poly(A) tail of mRNA. mRNA is stabilized and protected by
242 PABPC1 from deadenylation by Ccr4/Not deadenylases (Tucker et al., 2002).
243 Meanwhile, PABPC1 is required for deadenylation by Pan2/Pan3 deadenylases (Lowell
244 et al., 1992, Zheng et al., 2008). The absence of PABPC1 in PBs is likely due to

245 shortening of poly(A) in remodeling, as suggested by the absence of a signal for poly(A)
246 RNA in PBs (Cougot et al., 2004).
247 Tendency of PB proteins to aggregate when overexpressed adds to the difficulty of PB
248 assembly studies. Using super-PAM2 to relocate proteins to SGs is a novel way to
249 evaluate their roles in PB assembly and dissect specific interactions. In all, we show that
250 PABPC1 functions in mRNP remodeling and PB formation. Many mechanistic details
251 need further studying. We look forward to future studies on roles of PABPC1 in mRNA
252 metabolism.
253
254
255 **Materials and methods**
256 Plasmids and siRNA
257 pT7-EGFP-C1-HsTNRC6A, pT7-EGFP-C1-HsTNRC6B, pT7-EGFP-C1-HsRCK
258 (DDX6), pT7-EGFP-C1-HsDcp1a, and pT7-EGFP-C1-HsDCP2 were gifts from Elisa
259 Izaurralde (Addgene plasmid # 25030, 25031, 25033, 25034, 25035)(Tritschler et al.,
260 2009). Sequence (agcaatctgaatccaaatgca) encoding amino acids SNLNPNA was inserted
261 between nucleotides 4827A and 4828C of TNRC6A, or 4431T and 4432C of TNRC6B to
262 create super-GW182. Reverse-PCR was used for constructing TNRC6AΔAgo (Δ7-300)
263 and TNRC6AΔQ/N (Δ360-402). Plasmids expressing HA-MBP, HA-TNTC6A and HA-
264 TNRC6AΔPAM2 were generous gifts from Drs. Eric Huntzinger and Elisa Izaurralde
265 (Huntzinger et al., 2010). PABPC1 was inserted between BamH I and Not1 of pCDNA3-
266 EGFP. siRNAs were synthesized at Dharmacon. The siRNA sequences used were
267 siPABPC1 (5'-AAGGUGGUUUGUGAUGAAAAU-3', 5'-
268 AAUCGCUCCUGAACCAGAAUC-3'), siPaip2 (5'-
269 GAGUACAUGUGGAUGGAAAUU-3', 5'- UGGAAGAUCUUGUGGUCAAUU-3').
270 Control siRNA was purchased from Qiagen (SI03650318).
271
272 Cell culture and transfections
273 HeLa S3 or MEF cells were cultured in DMEM supplemented with 10% fetal bovine
274 serum. 10^5 cells were plated per well in 24-well plate the day before transfection. 60-90
275 pmol siRNA or 0.8 μg DNA plasmid was mixed with 2 μl Lipofectamine 2000 in Opti-

276 MEM and then added to cells. After 48 hours, cells were trypsin digested and split onto
277 cover slides. Cells were treated and fixed after 24 hours. For western analysis, cells were
278 changed into fresh medium for another day and harvested in SDS loading buffer.
279
280 Immunofluorescence and confocal microscopy
281 Cells were fixed with cold methanol (-20°C) for 10 minutes and then treated with 0.1%
282 Triton X-100 in 1x PBS. Cells were blocked with 5% normal goat serum (Millipore S26)
283 in PBS. Cells were incubated in 1x PBS, supplemented with anti-PABPC1 (Abcam
284 ab21060; Santa Cruz sc32318)(1:200), anti-hedls (Santa Cruz sc8418)(1:1000), anti-
285 Paip2 (Sigma-Aldrich P0087)(1:500), anti-eIF4G1 (Sigma-Aldrich AB-1232)(1:200),
286 anti-Ataxin-2 (BD Biosciences 611378)(1:200), anti-HA (Covance MMS-101P)(1:200),
287 anti-Dcp1a (Abcam ab47811)(1:200), anti-DDX6 (Abcam ab40684)(1:200), anti-EDC4
288 (hedls)(Abcam ab72408)(1:200), anti-Ago1 (Santa Cruz sc53521)(1:200), anti-G3BP
289 (gift of Dr. Imed Gallouzi)(1:500) or anti-HuR (gift of Dr. Imed Gallouzi)(1:200). Cells
290 were washed in PBS 3 times, before incubated with corresponding second antibodies
291 conjugated with Alexa488 or Alexa647 (Sigma-Aldrich A31620, A31628, A31571) or
292 Dylight550 (Bethyl A120-101D3) or Rhodamine (Millipore 12-509, 12-510) at 1:200 –
293 1:500 dilutions. DAPI (Roche) was added to washing buffer at 0.5μg/ml to treat cells for
294 10 minutes. Cover slides were finally mounted in ProLong Gold antifade reagent (Life
295 technology P36930). Images were collected on Zeiss LSM 310 confocal microscope in
296 the McGill University Life Sciences Complex Advanced BioImaging Facility (ABIF).
297 PBs were counted manually in a sample size of 50 cells for each group. Sample deviation
298 was calculated within group.
299
300 Western blotting
301 Protein samples were heated at 95°C and separated in SDS-PAGE. Proteins were then
302 transferred to PVDF membrane in Tris/glycine buffer, with 20% methanol in cold room.
303 PVDF membrane was blocked in 1x TBS (pH 7.5), containing 0.05% Tween-20 and 5%
304 skim milk powder or bovine serum albumin. Besides antibodies above, anti-GW182
305 (Novus NBP1-88232)(1:200) and anti-tubulin (Sigma-Aldrich T9028) were also used for
306 detection of related proteins.

307

308 Immunoprecipitation

309 Cells were lysed in 20 mM Hepes (pH 7.4), 150 mM NaCl, 0.5% NP-40, 2 mM DTT, 2
310 mM $MgCl_2$, 1 mM $CaCl_2$ and protease inhibitor tablet (Roche), and cleared by
311 centrifugation. Optimized amount of antibodies were added to cleared lysate for 2 hours.
312 Dynabeads protein A or protein G were washed and added to lysate for 0.5 hour.
313 Dynabeads were then washed with 1x PBS and boiled in SDS loading buffer for further
314 analysis.

315

316 Surface plasmon resonance

317 GST-MLLE protein was applied to Series S sensor chip CM5 bound with anti-GST
318 antibodies (GE Healthcare BR100223), in Biacore T100. After wash, various
319 concentrations of super-PAM2 (SNLNPNAPEFHPGVPWKGLQ) or super-PAM2-
320 Phe10Ala (SNLNPNAPEAHPGVPWKGLQ) were added to bind GST-MLLE captured
321 on chip. The corresponding steady states measured in relative units (RU) were plotted
322 versus concentrations of peptides in the flow system to estimate dissociate constants.

323

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328

329 **Competing interests**

330 No competing interests declared.

331

332 **Author contributions**

333 J.X. designed the experiments. Y.C. and X.W. assisted J.X. in carrying out the
334 experiments. G.K. designed the super-PAM2 sequence. J.X. and K.G. prepared the
335 manuscript.

336

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489

490

491

492

493 **Figure legends**

494

495 Figure 1. PB number and protein levels after PABPC1 or Paip2 depletion. (A-B) HeLa
496 cells transfected with scramble siRNA were mixed with siPaip2 or siPABPC1 treated
497 cells, to highlight knockdown effects and compare cells in the same environment.
498 Staining of PABPC1 or Paip2 (*green*) indicated knockdown effects in cells. PB number
499 was monitored by hedls (*red*). Visible P-bodies of 50 cells were counted manually and
500 sample deviation was calculated. The PB number in siPABP cells was significantly lower
501 at 0.01 significance level using two-tailed t-test for two samples with unequal variance.
502 (C) Levels of PABPC1, Paip2, hedls, DDX6, Dcp1a, G3BP, GW182 and tubulin 72
503 hours after siRNA knockdown. The Paip2 antibody recognized both Paip2a and Paip2b.

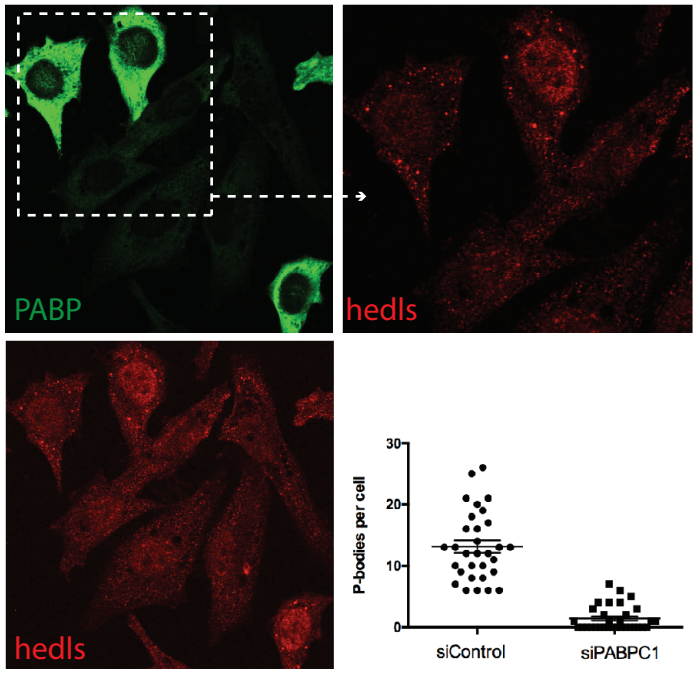
504

505 Figure 2. Relocalization of hedls or Dcp1a to stress granules in PABPC1-depleted cells.
506 (A-B) HeLa cells were treated with 0.5 mM sodium arsenite for 0.5 hour before fixation.

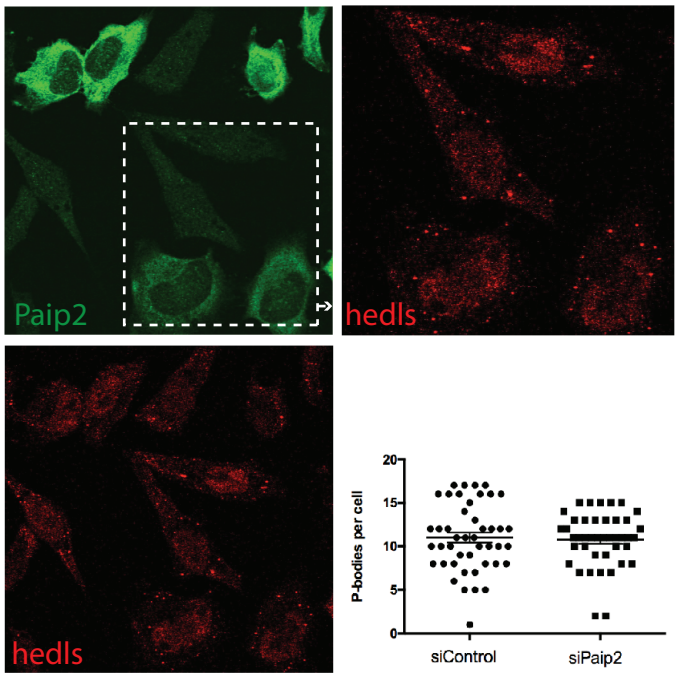
507 The PB components hedls (*red*) formed loose and irregular granules, colocalized to stress
508 granules (*green*).
509
510 Figure 3. (A-B) Stress granule assembly is not affected by PABPC1 depletion. PABPC1
511 depleted cells (Red) displayed similar stress granule pattern to control cells, stained by
512 anti-G3BP or anti-HuR (*green*). (C) P-bodies form independent of stress granules. HeLa
513 cells treated by PP242 overnight failed to form stress granules (*red*), but had normal PBs
514 present. (D) Separate PBs can be induced after SG inhibition in depletion of PABPC1.
515 HeLa cells were treated with 10μM PP242 for overnight before stressed in 0.5 mM
516 sodium arsenite for 0.5 hour. Individual PBs (*green*) formed in PABPC1 depleted cells
517 (*red*).
518
519 Figure 4. P-bodies can be induced in PABPC1 depleted cells. (A) Dcp1a or DDX6
520 overexpression (*green*) could induce P-body like granules in cells with low PABPC1
521 (*red*). (B-C) Dcp1a or TNRC6A (*green*) were partly localized to stress granules in
522 arsenite stressed PABPC1 depleted cells. HeLa cells were transfected with siRNA and
523 related plasmids simultaneously.
524
525 Figure 5. Engineering of super-GW182 to interact with PABPC1. (A) Super-PAM2
526 integrated sequences of Paip2-PAM2 and GW182-PAM2 to bind an extended surface on
527 MLLE domain (PDB: 3KUS and 3KTP). Super-PAM2 bound MLLE of PABPC1 at
528 around 20 nM measured by surface plasmon resonance using Biacore T100. The
529 Phe10Ala mutant was not binding MLLE and used as control. (B) Overexpressed super-
530 PAM2-GFP (*green*) localized to PABP or G3BP (*red*) marked stress granules upon
531 sodium arsenite treatment. (C) HA-tagged MBP, GW182 (TNRC6A), GW182ΔPAM2 or
532 super-GW182 were transfected in HeLa cells. Immunoprecipitation with anti-HA
533 antibodies showed stronger interaction of super-GW182 with endogenous PABPC1. The
534 weak association of natural GW182 and PABPC1 was not shown due to harsh washings.
535

536 Figure 6. Simulation of P-body/Stress granule fusion using super-GW182. HeLa cells
537 overexpressing super-GW182-GFP (*green*) were stressed by sodium arsenite and stained
538 with anti-hedls (*cyan*) for P-bodies and anti-PABPC1 (*red*) for stress granules.
539
540 Figure 7. Ago-binding region of GW182 (TNRC6A) is important for recruitment of PB
541 components. (A-B) Super-GW182ΔAgo or super-GW182ΔQ/N (*green*) was co-
542 transfected with siPABPC1. PBs were labeled by anti-hedls (*cyan*) and stress granules
543 were by anti-PABPC1 (*red*). Super-GW182ΔAgo was less efficient in redirecting PB
544 components to SGs (A) compared with super-GW182ΔQ/N (B).
545
546 Figure 8. P-body formation on mRNPs or stress granules. PB assembly may initiate on
547 PABPC1-containing mRNPs. The requirement of PABPC1 in PB formation drives PB
548 components to stress granules, where the residual PABPC1 is enriched after depletion.
549 However, the displacement of PABPC1 is difficult in SG and then individual PBs fail to
550 form.
551
552
553
554
555
556

A


PABP
hedls
hedls
P-bodies per cell
30
20
10
0
siControl
siPABPC1

B


Paip2
hedls
hedls
P-bodies per cell
20
15
10
5
0
siControl
siPaip2

C

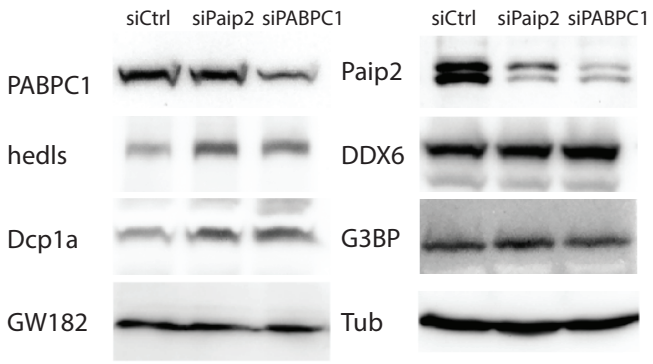
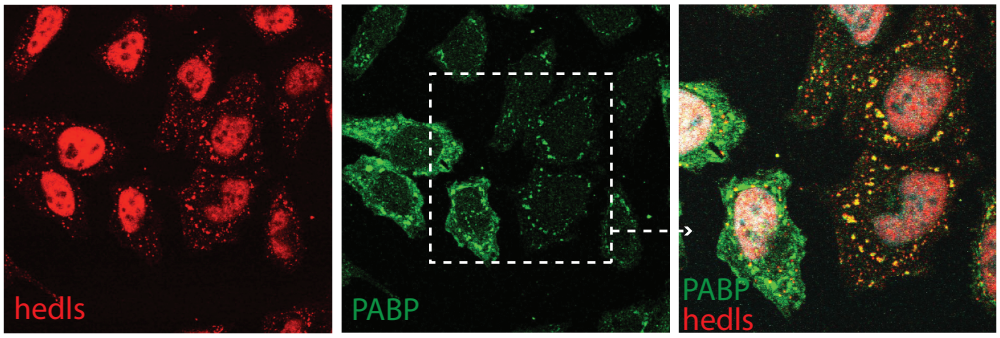

siCtrl
siPaip2
siPABPC1
siCtrl
siPaip2
siPABPC1
PABPC1
hedls
Dcp1a
GW182
Paip2
DDX6
G3BP
Tub

Figure 2

A



B

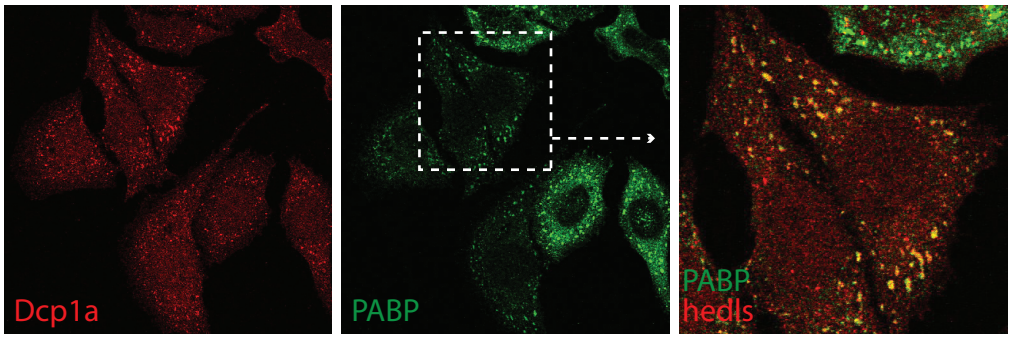
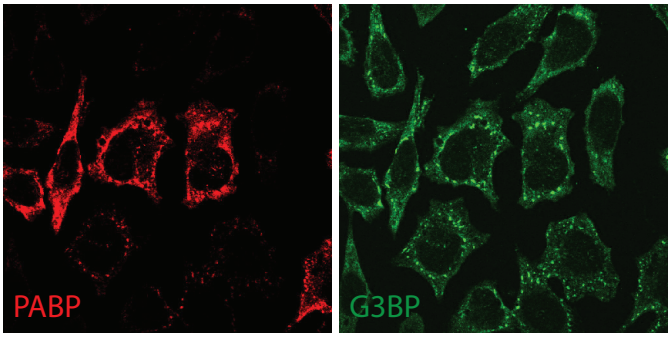
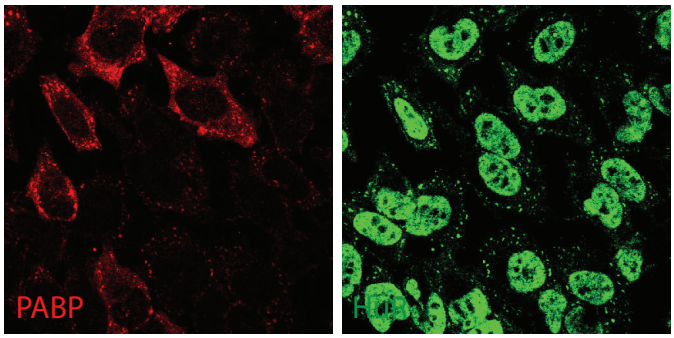


Figure 3

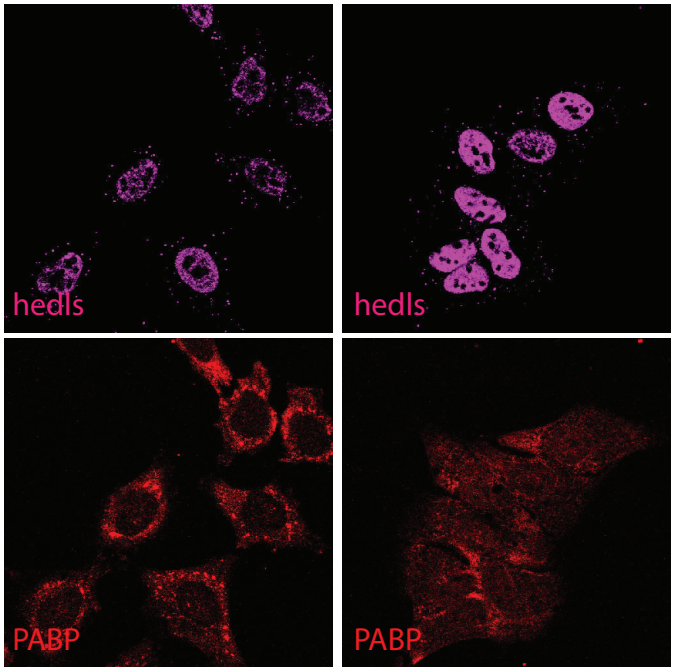
A



B



C



D

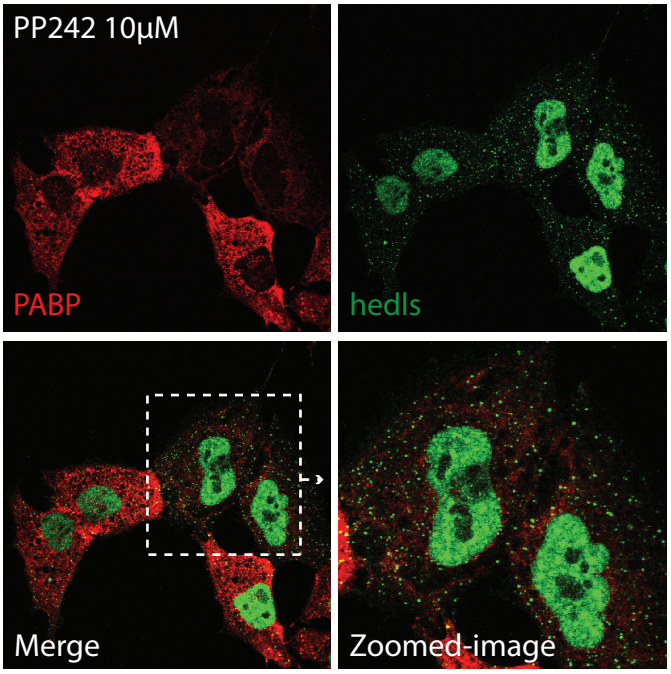


Figure 4

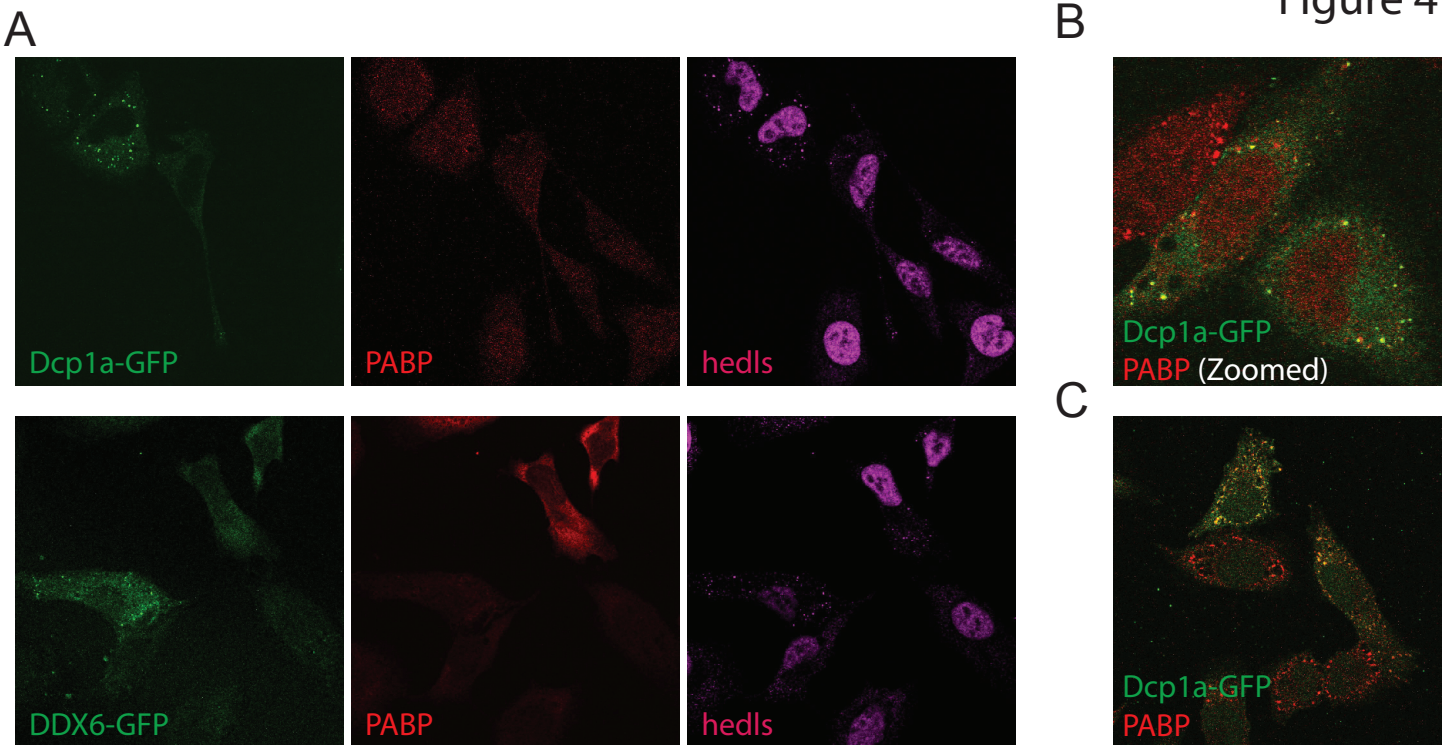
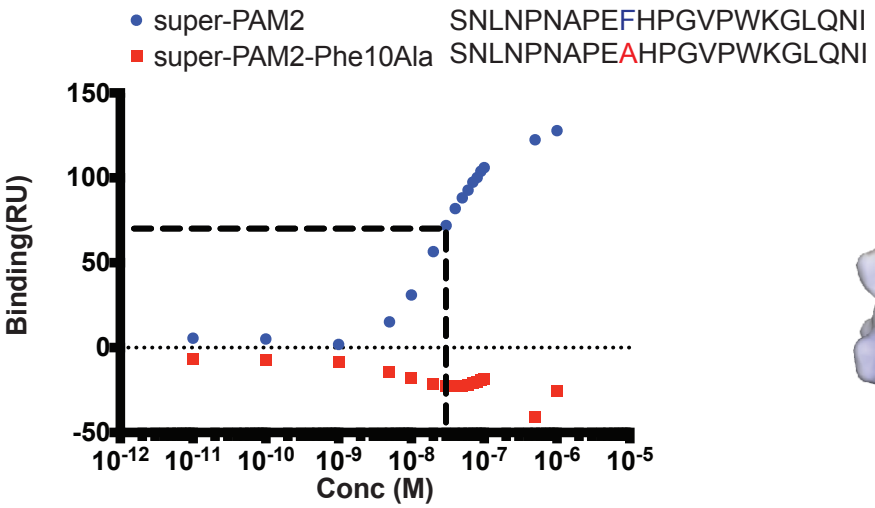
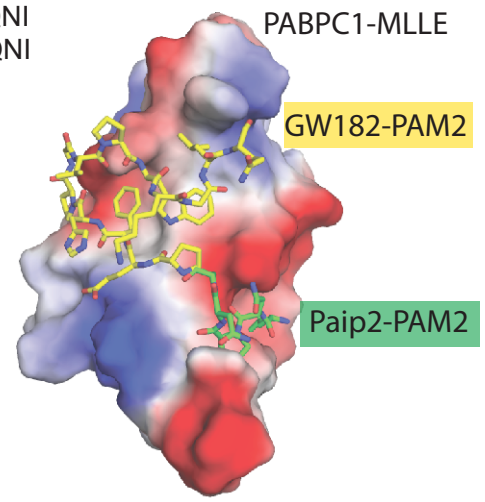

A
Dcp1a-GFP
PABP
hedls
DDX6-GFP
PABP
hedls
B
Dcp1a-GFP
PABP (Zoomed)
C
Dcp1a-GFP
PABP

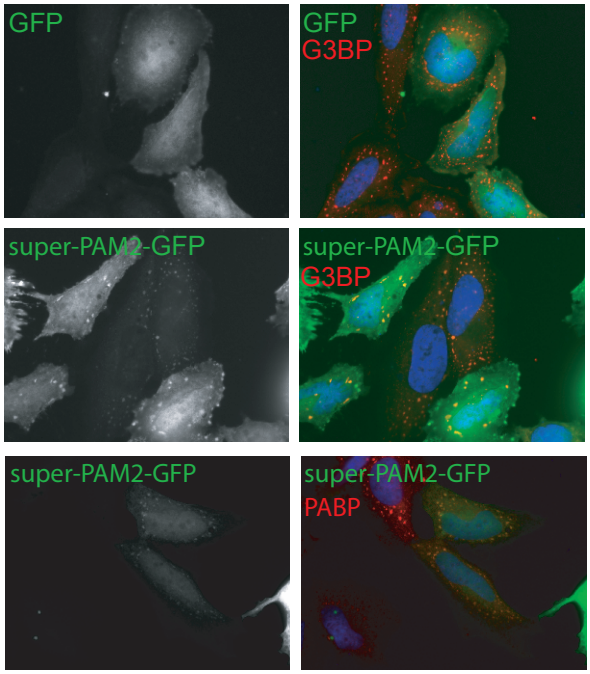
Figure 5

A


super-PAM2 SNLNPNAPEFHPGVPWKGLQNI
super-PAM2-Phe10Ala SNLNPNAPEAHPGVPWKGLQNI
Binding(RU)
150
100
50
0
-50
10-12 10-11 10-10 10-9 10-8 10-7 10-6 10-5
Conc (M)


PABPC1-MLLE
GW182-PAM2
Paip2-PAM2

B


GFP
GFP
G3BP
super-PAM2-GFP
super-PAM2-GFP
G3BP
super-PAM2-GFP
super-PAM2-GFP
PABP

C

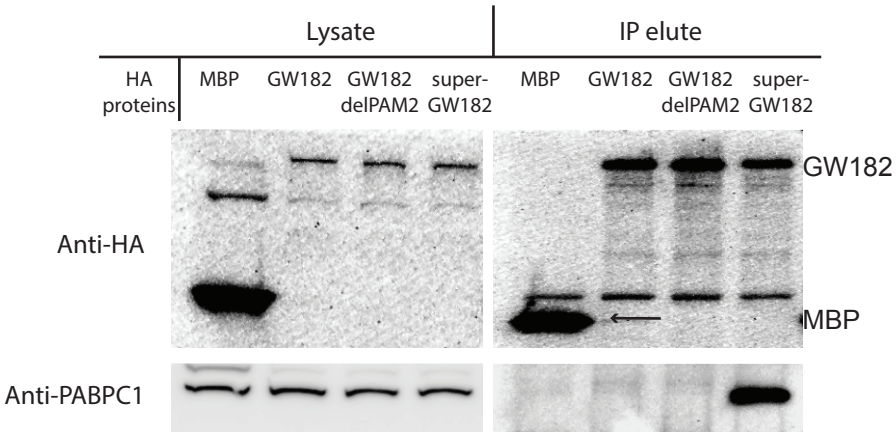

Lysate
IP elute
HA proteins
MBP
GW182
GW182 delPAM2
super-GW182
MBP
GW182
GW182 delPAM2
super-GW182
Anti-HA
GW182
MBP
Anti-PABPC1

Figure 6

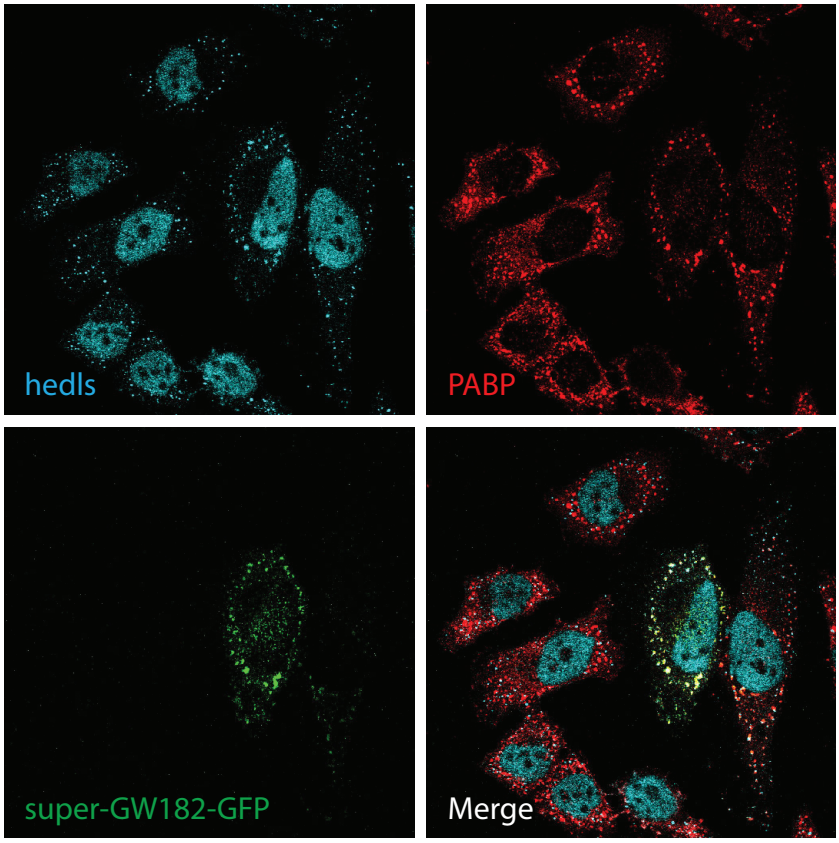
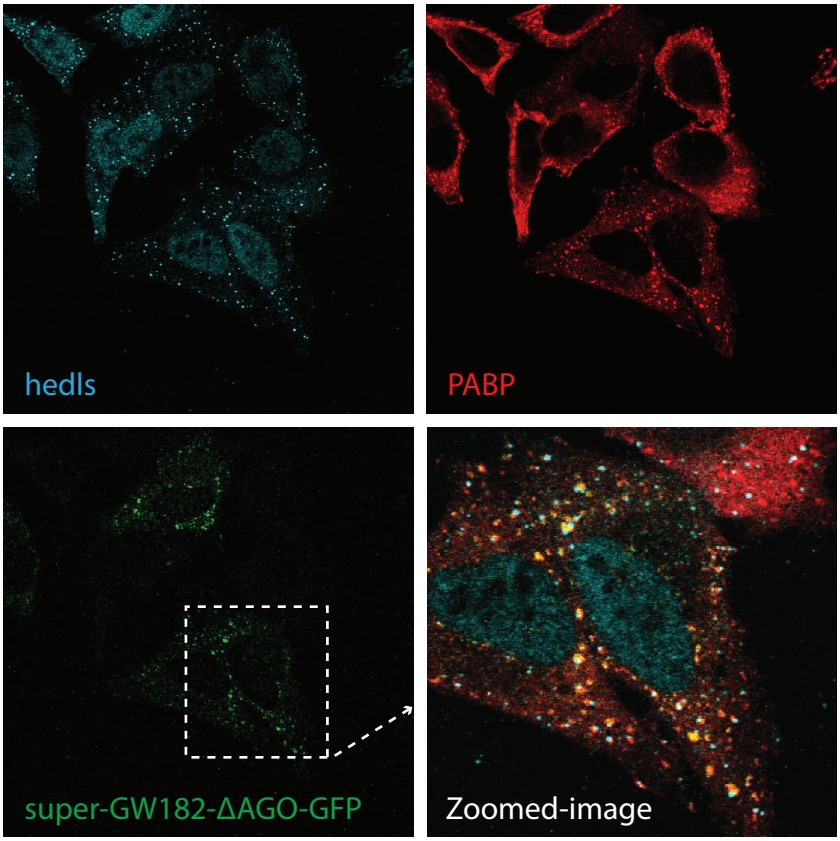


Figure 7

A


hedls
PABP
super-GW182-ΔAGO-GFP
Zoomed-image

B

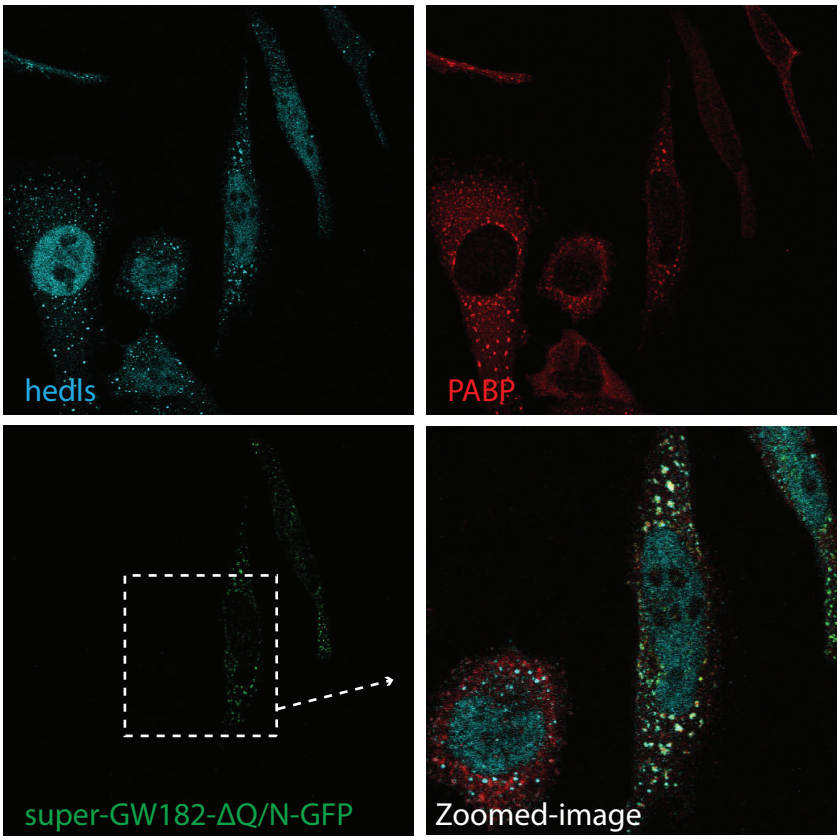
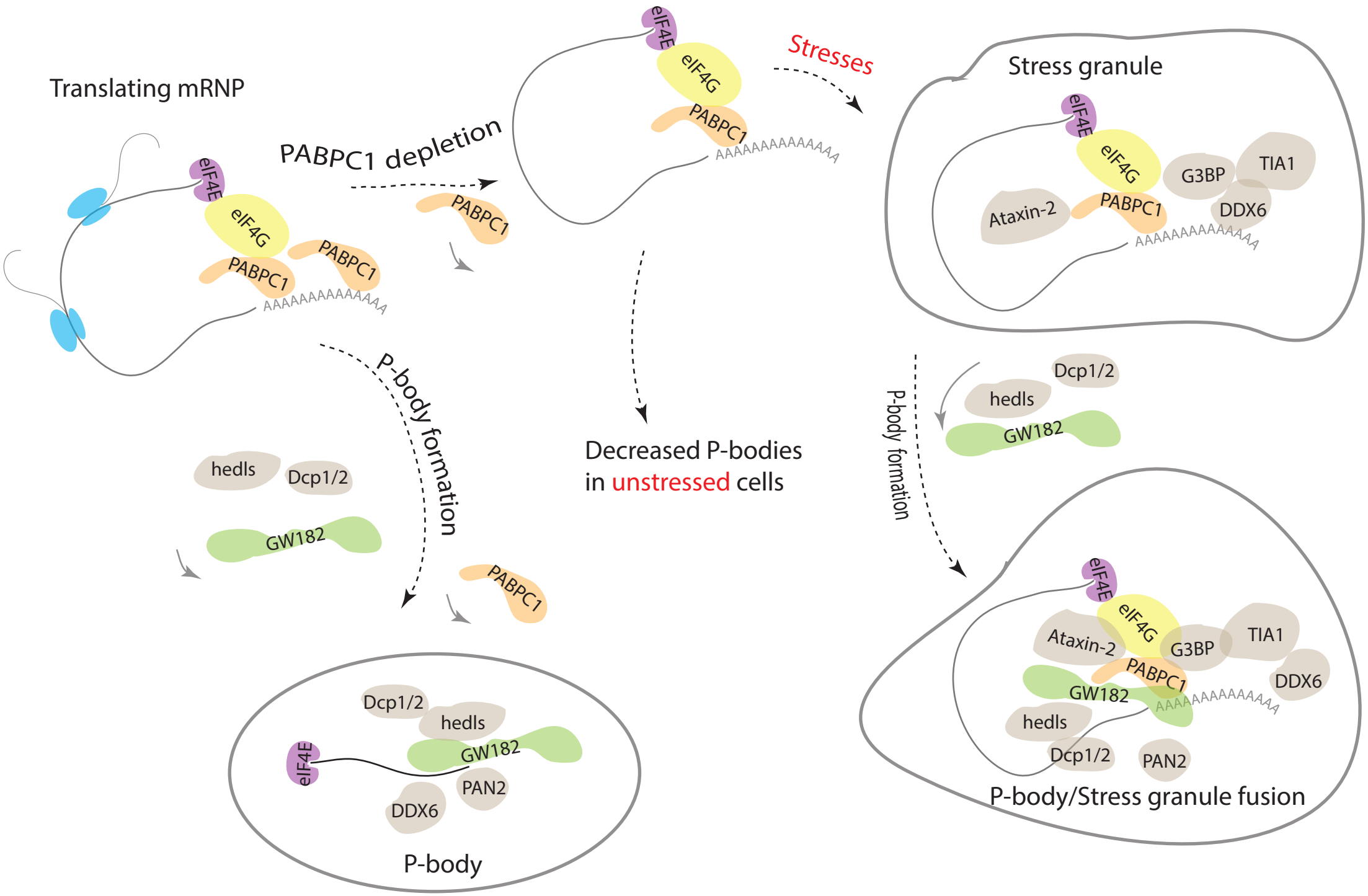

hedls
PABP
super-GW182-ΔQ/N-GFP
Zoomed-image

Figure 8


Translating mRNP
eIF4E
eIF4G
PABPC1
PABPC1
AAAAAAAAAAAAAA
PABPC1 depletion
PABPC1
eIF4E
eIF4G
PABPC1
AAAAAAAAAAAAAA
Stresses
Stress granule
eIF4E
eIF4G
G3BP
TIA1
Ataxin-2
PABPC1
DDX6
AAAAAAAAAAAAAA
P-body formation
hedls
Dcp1/2
GW182
PABPC1
Decreased P-bodies
in unstressed cells
P-body formation
Dcp1/2
hedls
GW182
Dcp1/2
hedls
eIF4E
GW182
PAN2
DDX6
P-body
eIF4E
eIF4G
Ataxin-2
G3BP
TIA1
PABPC1
DDX6
GW182
AAAAAAAAAAAAAA
hedls
Dcp1/2
PAN2
P-body/Stress granule fusion