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## Conformational buffering underlies functional selection in intrinsically disordered protein regions

Nicolás González-Foutel, Juliana Glavina, Wade Borchers, Matías Safranchik, Susana Barrera-Vilarmau, Amin Sagar, Alejandro Estaña, Amelie Barozet, Nicolás Garrone, Gregorio Fernandez-Ballester, et al.

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1                   **Conformational buffering underlies functional selection in intrinsically**  
2                                           **disordered protein regions**

3  
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29

30 **ABSTRACT**

31 **Many disordered proteins conserve essential functions in the face of extensive sequence**  
32 **variation, making it challenging to identify the mechanisms responsible for functional**  
33 **selection. Here, we identify the molecular mechanism of functional selection for the**  
34 **disordered adenovirus early gene 1A (E1A) protein. E1A competes with host factors to**  
35 **bind the retinoblastoma (Rb) protein, subverting cell cycle regulation. We show that two**  
36 **binding motifs tethered by a hypervariable disordered linker drive picomolar affinity Rb**  
37 **binding and host factor displacement. Compensatory changes in amino acid sequence**  
38 **composition and sequence length lead to conservation of optimal tethering across a large**  
39 **family of E1A linkers. We refer to this compensatory mechanism as conformational**  
40 **buffering. We also detect co-evolution of the motifs and linker, which can preserve or**  
41 **eliminate the tethering mechanism. Conformational buffering and motif-linker coevolution**  
42 **explain robust functional encoding within hypervariable disordered linkers and could**  
43 **underlie functional selection of many disordered protein regions.**

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48 **Running title:** Entropic tethering underlies viral hijack in a minimal viral model system

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## 54 INTRODUCTION

55

56 Intrinsically disordered proteins and protein regions (IDRs) [1,2] use short linear motifs  
57 (SLiMs) to bind cellular partners. These conserved interaction modules play essential roles in cell  
58 biology [3]. In contrast, the regions connecting SLiMs often have lower sequence conservation  
59 and a high frequency of insertions and deletions [4]. Under the classical structure-function  
60 paradigm, these features indicate weak evolutionary restraints, leading to the view that these  
61 IDRs might play the roles of passive “spacers”, stringing together ordered domains and disordered  
62 SLiMs. However, recent progress in the quantitative description of sequence-ensemble  
63 relationships (SERs) in IDR conformations [5] indicates that specific features in these less  
64 conserved regions are required for function [6,7,8,9]. The fact that IDRs with different sequence  
65 characteristics have conserved SERs that are responsible for function [10], suggests that SERs  
66 are under natural selection. There is growing evidence that IDRs which function as flexible tethers  
67 that physically join ordered domains and/or disordered SLiMs fall into this category [11,12,13].

68 Tethering is essential for kinase signaling [8,14,15], gene silencing [7], enzyme catalysis [16],  
69 transcriptional regulation [13,17,18] and the formation of biomolecular condensates [9,19]. Tethering  
70 allows intra- or intermolecular coupling between ordered domains and/or SLiMs [20]. This coupling  
71 can increase the effective concentrations of interacting partners [21], and relatively simple polymer  
72 models such as the Worm Like Chain (WLC) [22,23,24] can estimate the affinity enhancement from  
73 tethering [22,25,26,12]. An emerging hypothesis is that SERs that encode IDR dimensions -as  
74 determined by sequence length, composition and patterning- [27,28,29,30,5] play an important role in  
75 tethered interactions by determining the effective concentrations of binding modules around  
76 binding partners either in *cis* or in *trans* [12,13,31,32,16,33]. This leads to the expectation that  
77 evolutionary pressure will preserve these dimensions in spite of large-scale sequence variation.

78 However, the lack of a well-defined model system in which molecular function is unambiguously  
79 conserved in the face of a hypervariable tether has hampered the ability to test this hypothesis.

80 In order to establish a model system for quantitatively understanding tethering  
81 mechanisms and their evolution, we chose the intrinsically disordered adenovirus early region 1A  
82 (E1A) protein. Viruses are under constant selection pressure from a changing environment, and  
83 many viral proteins utilize protein disorder to acquire novel traits [<sup>34,35,36,37,38</sup>]. This makes them  
84 robust as model systems to investigate functional selection of IDRs. E1A is a multifunctional  
85 signaling hub that employs multiple SLiMs [<sup>36,37,38</sup>] tethered by disordered linkers to hijack cell  
86 signaling [<sup>39</sup>]. Here we test the central hypothesis that conserved SERs drive functional selection  
87 of the disordered E1A protein. Our results demonstrate that IDRs with dramatic changes in the  
88 linear sequence have a conserved tethering function. We also found evidence for compensatory  
89 co-evolution between disordered tethers and SLiMs. Taken together, our findings have broad  
90 implications for understanding IDR function and evolution.

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92

93 **MAIN TEXT**

94

95 ***Tethering enhances Rb binding and promotes E2F displacement***

96 The subversion of cell cycle regulation by E1A involves essential interactions with the  
97 retinoblastoma (Rb) tumor suppressor, which displaces E2F transcription factors, triggering S-  
98 phase entry and viral genome replication (**Fig. 1a, b**). To identify the molecular mechanisms  
99 responsible for E2F displacement, we performed detailed structural and biophysical binding  
100 studies using the central RbAB domain of Rb (hereafter referred to as Rb) and the minimal Rb  
101 binding region from the adenovirus E1A protein (hereafter referred to as E1A<sub>WT</sub>). Rb contains the  
102 binding sites for the E2F and LxCxE SLiMs [40,41] and E1A<sub>WT</sub> harbors the E1A<sub>E2F</sub> and E1A<sub>LxCxE</sub>  
103 SLiMs [42] connected by a 71-residue disordered linker [43]. This linker contains additional SLiMs  
104 for the CREB binding protein (CBP) TAZ2 domain [43] and the BS69 transcriptional repressor  
105 MYND domain [39], which mediate the formation of ternary complexes [44] (**Fig.1b**).

106 To assess the affinity of E1A<sub>WT</sub> and the relative contributions of the two motifs, we also  
107 tested E1A constructs comprising the individual SLiMs or fragments where the E2F (E1A<sub>ΔE</sub>) or  
108 LxCxE (E1A<sub>ΔL</sub>) motifs were mutated to poly-alanine (**Extended Data Fig. 1 and Fig. 1b**) and the  
109 E2F SLiM (E2F2) taken from the host transcription factor E2F2 (**Fig. 1b**). Isothermal titration  
110 calorimetry (ITC) (**Extended Data Fig. 2 and Supplementary Data Table 1**) and size exclusion  
111 chromatography coupled to static light scattering (SEC-SLS) experiments (**Supplementary Data**  
112 **Table 2**) confirmed that all E1A constructs bound to Rb with 1:1 stoichiometry. To quantify binding  
113 affinities, we performed fluorescence polarization measurements using fluoresceine 5-  
114 isothiocyanate (FITC)-labelled constructs (**Extended Data Fig. 3 and Supplementary Data**  
115 **Tables 1 and 3**). While the host-derived E2F2 SLiM bound to Rb with high affinity ( $K_D = 1$  nM),  
116 the E1A<sub>E2F</sub> SLiM had a  $K_D = 119$  nM, suggesting it would be a weak competitor of E2F2 (**Fig. 1c**).  
117 Based on a previous study showing the E2F and LxCxE motifs bind simultaneously to Rb [45] we  
118 suspected that a protein containing both SLiMs and the linker (E1A<sub>WT</sub>) would increase the binding

119 affinity of the individual SLiMs by enhancing local concentrations. In support of this, we found that  
120 E1A<sub>WT</sub> had picomolar binding affinity ( $K_D = 24$  pM), conferring a 4000-fold enhancement compared  
121 to the individual E1A SLiMs and a 40-fold enhancement compared to E2F2, consistent with a role  
122 of tethering in affinity enhancement (**Fig. 1c**).

123 To further test the role of tethering in E2F displacement, we carried out competition  
124 assays. Synthetic peptides corresponding to the E1A<sub>LXCxE</sub> and E1A<sub>E2F</sub> motifs as well as the E1A<sub>ΔL</sub>  
125 mutant were unable to effectively displace E2F from Rb (**Fig. 1d**). However, E1A<sub>WT</sub> was a strong  
126 competitor, disrupting the [E2F2:Rb] complex at low nanomolar concentration (**Fig. 1d**). The  
127 agreement among ITC, direct titration and competition experiments confirmed that tethering was  
128 required for high affinity Rb binding and E2F displacement (**Fig. 1e, Supplementary Data Table**  
129 **1**).

130 We anticipated that tethering the two SLiMs would play a prominent role in the affinity  
131 enhancement between the independent and linked SLiMs of E1A by increasing the effective  
132 concentration ( $C_{eff}$ ) of the second motif once a primary interaction is established (**Fig. 1f, Model**  
133 **A**). However, alternative mechanisms that are not mutually exclusive with tethering could also  
134 contribute to the stability of the complex. The E1A linker could enhance affinity by establishing  
135 additional stabilizing interactions with Rb (**Fig. 1f, Model B**). Alternatively, a primary interaction  
136 by the E1A<sub>E2F</sub> or E1A<sub>LXCxE</sub> SLiMs could induce an allosteric change in Rb that enables the  
137 complementary motif to bind with higher affinity (**Fig. 1f, Model C**). We tested each of these  
138 mechanisms using a combination of structural biophysics and thermodynamic analysis.

139

#### 140 ***Linker-mediated interactions do not stabilize binding to Rb***

141 We first sought to confirm the disordered nature of E1A<sub>WT</sub> using nuclear magnetic  
142 resonance (NMR) spectroscopy. The transverse optimized relaxation (TROSY) spectrum of <sup>15</sup>N-  
143 labeled E1A<sub>WT</sub> revealed narrow chemical shift dispersion in the <sup>1</sup>H-dimension. This is a

144 characteristic signature of disordered regions and is consistent with previous work on E1A  
145 fragments (**Fig. 2a**) [43,46,47]. Further, the  $^{13}\text{C}_\alpha$  secondary chemical shifts ( $\Delta\delta\text{C}_\alpha$ ) showed minimal  
146 deviation from random coil values obtained from disordered proteins (**Fig. 2b I**) and negative  $^1\text{H}$ -  
147  $^{15}\text{N}$  nuclear overhauser effect (NHNOE) values observed for E1A<sub>WT</sub> indicated fast backbone  
148 dynamics (**Fig. 2b II**). Finally, sequence analysis also predicted that E1A<sub>WT</sub> is globally disordered  
149 (**Fig. 2b IV**). These results confirmed that the conformational ensemble of E1A<sub>WT</sub> is characterized  
150 by high heterogeneity (disorder) and with fast interconversion between distinct conformations on  
151 the nanosecond to picosecond timescale (flexibility).

152         Next, we used NMR spectroscopy to determine the structural basis for E1A<sub>WT</sub> binding to  
153 Rb. For this, we dissected the relative contributions of the SLiMs, their flanking regions, and the  
154 linker. Previous NMR work mapped binding of E1A fragments containing individual Rb binding  
155 motifs [43] but did not examine the structural details of E1A bivalently tethered to Rb. The TROSY  
156 spectrum of labeled E1A<sub>WT</sub> in complex with unlabeled Rb (MW=54.6 kDa, **Supplementary Data**  
157 **Table 2**) reveals a complete loss of peaks for residues corresponding to the E2F and LxCxE  
158 SLiMs (L43 to Y47 and L122 to E126) (**Fig. 2a, 2b III and Extended Data Fig. 4**) consistent with  
159 slow exchange expected from the high affinities of the untethered motifs and the tethered complex  
160 [48]. The E1A<sub>ΔL</sub> and E1A<sub>ΔE</sub> constructs retain binding of the wild type motif (**Fig. 2b III**), consistent  
161 with independent binding of each motif to Rb. Based on previous reports [49], we anticipated that  
162 the regions flanking the canonical E1A<sub>E2F</sub> or E1A<sub>LxCxE</sub> motifs contribute stabilizing interactions to  
163 the complex. In agreement with this expectation, the peaks corresponding to the flanking residues  
164 (E39 to T52 and V119 to E135) disappeared upon binding, yielding near-zero  $I/I_0$  ratios (**Fig. 2b**  
165 **III**).

166         Binding experiments using fluorescence polarization and ITC confirmed the stabilizing role  
167 of the flanking regions: the affinity of the E1A<sub>LxCxE</sub> motif increased incrementally upon adding the  
168 acidic stretch following the motif (E1A<sub>LxCxE-AC</sub>), and with Ser132 phosphorylation (E1A<sub>LxCxE-ACP</sub>)



169 (Fig. 1b and Supplementary Data Table 1) leading to an overall 5-fold increase in binding affinity.  
170 ITC revealed different origins for thermodynamic stability in each core motif and the flanking  
171 regions (Supplementary Data Table 1). While binding of E1A<sub>E2F</sub> is entropically driven, suggesting  
172 complex stabilization is dominated by the desolvation of apolar surfaces, binding of E1A<sub>LxCxE</sub> is  
173 enthalpically driven, likely due to the contribution from hydrogen bonds between the LxCxE motif  
174 and Rb.

175 The N-terminal linker region (residues 50-85) encompassing the TAZ2 binding motif is  
176 highly conserved and has a lower disorder propensity due to its hydrophobic nature (Fig. 2b IV-  
177 V). This region showed a decrease in peak intensities (Fig. 2b III) that a previous report suggested  
178 was due to weak interactions with Rb [43]. Consistent with this observation, the N-terminal linker  
179 region does not show increased chemical shift dispersion or large chemical shift changes upon  
180 binding Rb (Fig. 2a and Extended Data Fig. 4a-d). The C-terminal linker region (residues 86-  
181 120) shows no changes in chemical shifts or resonance intensities, indicating this region remains  
182 globally disordered and flexible when bound to Rb. These interpretations are supported by the  
183 lack of change in secondary structure upon binding Rb, as measured by circular dichroism (CD)  
184 (Fig. 2c).

185 Additional ITC studies using an isolated fragment from the N-terminal linker region that  
186 showed the largest decrease in peak intensities (E1A<sub>60-83</sub>) did not show any detectable association  
187 to Rb (Extended Data Fig. 2i). Further, E1A constructs that include the linker did not show higher  
188 binding affinities when compared to isolated E1A motifs (Fig. 2d, Supplementary Data Table 1).  
189 Taken together, these data rule out the presence of a high affinity binding site. To test for weak  
190 interactions that depend on bivalent tethering, we also designed a construct where the TAZ2  
191 binding region (<sub>71</sub>MLAVQEGID<sub>79</sub>), which showed the largest reduction in  $I/I_0$ , was replaced by a  
192 GS stretch (E1A<sub>WTΔHyd</sub>). The binding affinity of this mutant actually increased by 1.5-fold compared  
193 to E1A<sub>WT</sub> in fluorescence competition experiments (Supplementary Data Table 4), revealing a  
194 weak destabilizing effect of the TAZ2 binding site.

195 In order to identify thermodynamic contributions of the linker binding to Rb, temperature  
196 dependent  $\Delta H$  measurements were used to infer changes in accessible surface area ( $\Delta ASA_T$ )  
197 and the number of residues ( $X_{res}$ ) that fold upon binding to Rb (**Extended Data Fig. 5** and  
198 **Supplementary Data Tables 5 and 6**) (See **Methods**).  $\Delta ASA_T$  values calculated using  
199 conventional and IDP-specific models [<sup>50,51</sup>] failed to reveal an increase in  $\Delta ASA_T$  of the motif-  
200 linker construct (E1A<sub>ΔL</sub>) compared to the individual motif (E1A<sub>E2F</sub>) (**Fig. 2d** and **Supplementary**  
201 **Data Table 6**), demonstrating that the linker did not contribute to additional surface desolvation.  
202 The IDP-specific method yielded  $X_{res} = 33$  residues for the 16-mer E1A<sub>E2F</sub> binding to Rb, indicating  
203 a similar number of Rb residues fold at the E1A-binding interface. However,  $X_{res}$  did not increase  
204 for E1A<sub>ΔL</sub> compared to E1A<sub>E2F</sub>, suggesting that no additional linker residues were involved in  
205 coupled folding and binding.

206 Collectively, these results demonstrate that the linker does not contribute to the  
207 thermodynamics of complex formation through coupled folding and binding or through persistent  
208 molecular interactions with Rb. While the hydrophobic TAZ2-binding region may establish  
209 transient, weak interactions with Rb that have a minor destabilizing effect and result in the  
210 resonance intensity reductions we observe, our results do not support a model that invokes linker-  
211 mediated interactions (**Fig. 1f, Model B**) as a source for affinity enhancement.

212

### 213 ***Allosteric coupling in Rb does not increase E1A-Rb affinity***

214 To assess whether allosteric coupling between the E2F and LxCxE binding sites in Rb  
215 play a role in affinity enhancement (**Fig. 1f, Model C**), we saturated Rb with the E1A<sub>E2F</sub> or E1A<sub>LxCxE</sub>  
216 motifs and performed ITC titrations with the complementary motif (**Extended Data Fig. 5**). If a  
217 positive allosteric effect is at play, E1A<sub>LxCxE</sub> should bind more tightly to Rb when E1A<sub>E2F</sub> is already  
218 bound, and vice versa. This was measured as the change in Gibbs free energy  $\Delta\Delta G =$   
219  $\Delta G_{SATURATED} - \Delta G_{UNSATURATED}$ , where a negative value for  $\Delta\Delta G$  indicates positive cooperativity. For  
220 both motifs, the values of  $\Delta\Delta G$  were in the range +/- 0.25 kcal/mol (**Supplementary Data Table**

221 7). In E1A<sub>LXCXE</sub> binding assays, saturation with E1A<sub>ΔL</sub> instead of E1A<sub>E2F</sub> did not change the  
222 outcome, indicating that neither the motif nor the motif + linker arrangement behaved as an  
223 allosteric effector on the complementary site. Therefore, our results suggest that allosteric  
224 coupling in Rb (**Fig. 1f, Model C**) does not make a major contribution to affinity enhancement.

225

### 226 ***Entropic tethering optimizes affinity of E1A for Rb***

227 Our results indicate the positive cooperativity of the tethered E1A<sub>E2F</sub> and E1A<sub>LXCXE</sub> motifs  
228 binding to Rb results from an increase in the effective concentration ( $C_{eff}$ ) of one motif once the  
229 other motif is bound [<sup>45</sup>] (**Fig. 1f, Model A**). It is well established that this form of cooperativity can  
230 be described using a simple Worm Like Chain (WLC) model [<sup>23,24,22, 33</sup>] that treats the linker as an  
231 entropic tether (**Fig. 3a,b**) wherein the dimensions of the linker will determine the degree of the  
232 affinity enhancement. A short linker would be unable to straddle the distance between the two  
233 binding sites and lead to low affinity enhancement (**Fig. 3a,b I**), an optimal linker would maximize  
234  $C_{eff}$ , leading to maximal positive cooperativity (**Fig. 3a,b II**), and a longer than optimal linker would  
235 decrease  $C_{eff}$  (**Fig. 3a,b III**). Application of the WLC model to the E1A linker predicts a  $C_{eff}$  value  
236 of 0.92 mM, which is close to the optimal value (**Fig. 3b**) and within a factor of two of the  $C_{eff}$  ( $0.52$   
237  $\pm 0.09$  mM) obtained from the affinities of E1A<sub>WT</sub> and the isolated motifs (**Supplementary Data**  
238 **Table 1**). For E1A<sub>WTΔHyd</sub>, where the destabilizing effect of the linker region is removed, the  
239 agreement with the WLC model improves ( $C_{eff} = 0.78 \pm 0.24$  mM), indicating that this mutated  
240 linker behaves more like an entropic tether optimized to bind Rb with near-maximal affinity.

241 To further test the tethering model, we performed Small Angle X-ray Scattering (SAXS)  
242 on Rb, E1A<sub>WT</sub>, and the [E1A<sub>WT</sub>:Rb] complex (**Fig. 3c, Extended Data Fig. 6**). The experimental  
243 SAXS profile of the Rb domain could be fit to the theoretical SAXS profile derived from its crystal  
244 structure ( $\chi^2 = 1.3$ ) and further refined (RMSD = 1.7 Å) using a SAXS-driven modelling approach  
245 ( $\chi^2 = 0.82$ ) (**Fig. 3c, Extended Data Fig. 6a**), indicating that Rb in solution retained its folded  
246 structure. Alternatively, the Kratky plots of E1A<sub>WT</sub> were characteristic of an IDP. Fitting of the

247 SAXS profiles using the Ensemble Optimization Method (EOM) [52] indicated that E1A<sub>WT</sub> adopts  
248 highly expanded conformations (**Extended Data Fig. 6b**). To analyze the conformation of the  
249 linker in the [E1A<sub>WT</sub>:Rb] complex, we applied a sampling method [53] to generate a pool of 10250  
250 realistic conformations [54] and computed theoretical SAXS profiles that were selected using EOM  
251 analysis. The SAXS profile of the complex was best described by sub-ensembles where the linker  
252 sampled expanded conformations (**Fig. 3c-e, Extended Data Fig. 6c**) with hydrodynamic radius  
253 ( $R_h$ ) values ( $R_{h,EOM} = 3.36$  nm) in good agreement with those obtained from SEC-SLS experiments  
254 ( $R_{h,SEC} = 3.20 \pm 0.12$  nm) (**Fig. 3f-g, Extended Data Fig. 6d and Supplementary Data Table 2**)  
255 and  $R_g/R_h$  ratios consistent with bivalent tethering (**Supplementary Data Table 2**).

256 Our structural and thermodynamic dissection establishes E1A as a quantitative model  
257 system for entropic tethering, demonstrating that other mechanisms have a negligible contribution  
258 to affinity enhancement (with linker interactions having  $\Delta G \sim +200$  cal.mol<sup>-1</sup> over a total  $\Delta G_{BINDING}$   
259 = -14240 cal.mol<sup>-1</sup>). Our ability to isolate tethering as the key determinant of binding affinity is  
260 unparalleled and provides us with a unique opportunity to test how tethering operates in biological  
261 systems. A longstanding question is whether the sequences of regions that encode tethering have  
262 any unique relationships with the conformational ensembles they form. If they do not it is difficult  
263 to imagine that a family of linkers with extensive variation in sequence and length could function  
264 in the same way. The model we present below is a comprehensive assessment of how this is  
265 possible.

266

### 267 ***Hypervariable E1A linkers have a conserved functional length***

268 Inspection of selected linker sequences representative of mastadenoviruses that infect a  
269 wide range of mammalian hosts (**Fig. 4a**) revealed that while the N- and C-terminal acidic  
270 extensions and the aromatic/hydrophobic TAZ2 binding region were highly conserved, the linker  
271 lengths and compositions vary considerably within the central region enriched predominantly with  
272 polar, hydrophobic and proline residues (**Fig. 4a and 2b V**). To understand how function is

273 conserved in the face of these extensive differences in linker length and sequences, we performed  
274 all atom simulations [9] and generated conformational ensembles of 27 E1A linker sequences with  
275 linker lengths from 27 to 75 residues (**Fig. 4a**). While the shortest linkers from Bovine/Ovine E1A  
276 proteins had smaller end-to-end distances, the average end-to-end distance of linkers 41 to 75  
277 residues long remained roughly constant despite almost doubling the length (**Fig. 4b**). This  
278 suggested that the linkers have a conserved functional length [55] that is determined by a joint  
279 contribution of sequence length, amino acid composition, and sequence patterning as  
280 determinants of end-to-end distances. To test the feasibility of this hypothesis, we performed  
281 simulations for 140 random synthetic sequences of variable length that matched the amino acid  
282 composition of one of the shortest linkers (HF\_HAdV40). In sharp contrast to natural sequences,  
283 the synthetic sequences showed the expected monotonic increase in end-to-end distance with  
284 chain length ( $R_{\text{natural}} = 0.37$ ,  $R_{\text{synt}} = 0.99$ , **Extended Data Fig. 7a**). To examine the sequence-  
285 encoded origins of this compensation we analyzed various statistical properties (**Extended Data**  
286 **Fig. 7 b,c**). Net charge per residue (NCPR) had the strongest positive correlation with normalized  
287 end-to-end distance, with more expanded chains having a higher NCPR (**Extended Data Fig.**  
288 **7b**). This is in agreement with previous findings that net charge and patterning are major  
289 determinants of IDR dimensions in natural [55,13,30,29,11] and synthetic [30,27,32] sequences. Longer  
290 chains also tend to have higher proline content with fewer hydrophobic and charged residues  
291 (**Extended Data Fig. 7c**).

292 The results of the simulations suggest that the functional length of the linkers is conserved  
293 and the linker dimensions are key to providing optimal affinity enhancement by tethering. Based  
294 on these results, we hypothesize that the end-to-end distances of disordered linkers are under  
295 functional selection through compensatory covariations in sequence length and composition, an  
296 adaptive mechanism that we term conformational buffering.

297

298 ***Conformational buffering preserves optimal tethering***

299           The conformational buffering mechanism predicts that linker dimensions and optimal  
300 tethering will be conserved across E1A proteins with very different linker sequences. To test this  
301 prediction, we constructed a series of E1A chimeras by grafting different linker sequences with  
302 the E1A<sub>E2F</sub> and E1A<sub>LxCxE</sub> motifs (**Fig. 4c**) and determined Rb binding affinity using the competition  
303 assay of **Fig.1d (Supplementary Data Table 4 and Extended Data Figure 8)**. We selected  
304 linkers from E1A types infecting a wide range of mammalian hosts (**Fig.4a,c**). These sequences  
305 cover a wide range of linker lengths (27-75), amino acid composition, and sequence patterning  
306 (**Fig. 4a and Extended Data Fig. 7c**). The E1A variants were expressed as MBP fusion proteins  
307 (**Extended Data Fig. 1e**) and we verified that MBP-E1A<sub>WT</sub> had the same binding affinity as  
308 cleaved E1A<sub>WT</sub> (**Supplementary Data Table 4**).

309           The sequence and structure of the Rb domain that binds to E1A is highly conserved across  
310 the host range covered in our experiments (> 95% sequence identity and RMSD < 1.2 Å). The  
311 residues that make up the E2F and LxCxE binding clefts and the spacing between the sites are  
312 also highly conserved suggesting that functional length is under selection (**Extended Data Fig. 9**  
313 and **Supplementary Fig. 1**). This conservation implies that human Rb is an excellent proxy for  
314 the mammalian Rb proteins.

315           We predict that optimal tethering depends mainly on the linker dimensions and variants  
316 with conserved end-to-end distances will confer similar affinity to E1A<sub>WT</sub>. In accordance with this  
317 prediction, human and simian E1A linkers ranging from 41 to 75 residues have similar binding  
318 affinities, with  $K_D/K_{D,E1A_{WT}}$  ratios between 0.4 and 1.2 (**Fig. 4c,d**). E1A linkers with the highest  
319 affinities (Hum-2 and Sim-1) had polar residues interrupting the weakly destabilizing hydrophobic  
320 interactions in the TAZ2 SLiM (**Fig. 4a**). To directly assess linker dimensions we used SEC to  
321 measure  $R_h$  for selected E1A variants after MBP cleavage (**Extended Data Fig. 1f,g and 7d**).  
322 Both  $K_D$  and  $R_h$  agreed closely with those predicted from the atomistic simulations (**Fig. 4d,**  
323 **Supplementary Data Table 4 and Extended Data Fig. 7d**). We also created a tandem repeat

324 where the Hum-2 linker was duplicated (Hum-2-2x, **Fig. 4c,d**).  $K_{D,Hum-2-2x}/K_{D,E1AWT}$  was 1.1,  
325 suggesting its dimensions are still optimized.

326 E1A linkers appear to be under strong functional selection to preserve optimal tethering  
327 using a mechanism that requires compensatory covariations in sequence length and composition  
328 (i.e. conformational buffering). These results underscore the functional implications of preserving  
329 sequence-ensemble-relationships (SERs), which in the case of E1A is achieved by preserving  
330 the dimensions of the disordered linkers, which is necessary for hijacking the eukaryotic cell cycle.

331

### 332 ***Linker-motif coevolution modulates conformational buffering***

333 The shorter bovine linkers (**Fig. 4a**) had  $K_D/K_{D,E1AWT}$  ratios between 13 and 20 (**Fig. 4c,d**).  
334 This weaker than predicted affinity (**Fig. 4d**) was not due to the linkers being less expanded since  
335 the predicted and experimental  $R_h$  values for Bov-1 were similar (**Extended Data Fig. 7d**).  
336 Instead, it suggests that a minimal sequence length, not predicted by WLC, is necessary to  
337 overcome entropic effects required for proper orientation of the SLiMs to bind Rb. This highlights  
338 a limitation of the WLC model which is not unexpected since this simplified homopolymer model  
339 does not include excluded volume or local changes in the chain stiffness.

340 We expect that the E1A linkers and SLiMs are co-evolving in a way that may not be  
341 represented in the chimeras. For instance, the canine and bat chimeras had similar predicted and  
342 experimental  $R_h$  values (**Extended Data Fig. 7d**) but showed reduced binding affinity, with  
343  $K_D/K_{D,E1AWT}$  ratios between 5 and 6 (**Fig. 4c,d**) even though FoldX predicted the bat SLiMs to have  
344 higher affinity for Rb than E1A<sub>WT</sub> motifs. This reduction in binding could be due to additional  
345 destabilizing interactions of the bat/canine linkers with Rb, implying the possibility of  
346 compensatory changes that optimize but do not maximize binding affinity. To test this hypothesis,  
347 we measured the affinity of a variant containing the endogenous SLiMs and linker from bat (Bat-  
348 ED), which recovered high affinity binding with Rb ( $K_D/K_{D,E1AWT} = 1.5$ ). This is a clear signature of

349 coevolution whereby linker mutations that weaken affinity enhancement by tethering are  
350 compensated by SLiM mutations that directly increase Rb affinity.

351 The Bovine linker is predicted to have a smaller than optimal end-to-end distance  
352 compared with other E1A linkers (**Fig. 4b**) and the bovine SLiMs are predicted by FoldX to bind  
353 Rb with lower affinity (**Fig. 5a**), suggesting that the SLiMs and linker for Bovine are suboptimal.  
354 To test this prediction, we measured the affinity of a variant with the endogenous Bov-1 SLiMs  
355 and linker (Bov-1-ED). Bov-1-ED was unable to displace E2F in our competition experiments  
356 ( $K_D/K_{D,E1AWT} > 20,000$ ) (**Fig. 4 c,d** and **Extended Data Fig. 8**). Taken together, these results  
357 suggest that Bovine E1A cannot displace E2F to hijack the host cell cycle.

358

### 359 ***Evolutionary conservation of E1A tethering***

360 Our results suggest that conformational buffering is a selection mechanism that conserves  
361 end-to-end distances and affinity enhancement by tethering for E1A, and that motifs and linkers  
362 co-evolve. To test these hypotheses on a larger family of sequences<sup>56</sup>, we predicted global  
363 binding affinities for 110 distinct E1A SLiMs and linkers. We used FoldX to predict SLiM affinities  
364 and we predicted  $C_{eff}$  using either the WLC model with a single persistence length (E1A WLC), or  
365 the sequence-specific persistence length (E1A Lp-Sim) from the simulations of the 27 linkers in  
366 **Fig. 4a (Extended Data Fig. 10)**. The results are shown in **Figure 5a** together with the measured  
367 affinities from the grafting experiments (E1A Graft) or from endogenous variants (E1A ED).

368 The conservation of affinity enhancement by tethering is predicted across E1A proteins  
369 from adenoviruses infecting human (HA-G), simian (SA/B/F), canine (CA), bat (BtA/B) and equine  
370 (EA) species. These results are in agreement with our binding affinity measurements for human,  
371 simian and bat E1A proteins. The structural conservation of the p107/p130 paralogs that harbor  
372 the same SLiM binding sites (**Extended Data Fig. 9**) suggests that E1A uses the same  
373 mechanism to displace E2F factors bound to all Rb paralogs.



374 In contrast, in a divergent branch of E1A proteins infecting rodents (MA/B/C), treeshrew  
375 (TSA) and artiodactyls (including bovine, sheep and pig OA/BA/PA), binding to Rb seems  
376 impaired or lost completely due to the presence of short linkers coupled to low affinity (PC/OA/BA)  
377 or missing SLiMs. For instance, E1A proteins from rodents retain the LxCxE motif but lose the  
378 E2F motif. These E1A proteins could interfere with host factors binding to the LxCxE cleft but  
379 would be unable to displace E2F. These results suggest that the SLiMs and the linker are under  
380 co-evolutionary selection, such that either the SLiMs and linker are jointly optimized, or selection  
381 pressure is lost on both elements, leading to a loss of E2F displacement and possibly a loss of  
382 E1A's ability to hijack the eukaryotic cell cycle (**Fig. 5a**). This branch of divergent adenoviruses is  
383 likely to employ alternative mechanisms to induce host cell proliferation.

384 In summary, we demonstrate that tethering is the main mechanism that allows E1A to bind  
385 Rb with picomolar affinity and displace E2F transcription factors. We show that the functional  
386 length of the linkers is conserved and fine-tuned through conformational buffering to enable  
387 maximal affinity enhancement in the face of extensive changes in sequence composition and  
388 length. We also uncover a previously unknown linkage between the evolution of linkers and their  
389 tethered motifs. This study shows that strong functional selection can operate both on the motifs  
390 and on the physical properties of an IDR linker, providing important insights regarding the  
391 evolution of sequence features and tethering functions in IDRs.

392

## 393 **DISCUSSION**

394 Here, we demonstrate how E1A hijacks the eukaryotic cell cycle using two SLiMs tethered  
395 by a flexible linker with conserved dimensions [<sup>12,26</sup>]. The proposed docking and displacement  
396 mechanism is conserved across divergent E1A proteins by conformational buffering and  
397 coevolution of the SLiMs and tether. Conformational buffering promotes robust encoding of a core  
398 function (**Fig. 5b, upper**) while supporting the extensive sequence variation necessary to rewire  
399 the E1A interactome (**Fig.5b, lower**) and adapt to different hosts by gaining or losing additional

400 SLiMs [56-58], as we show for several SLiMs in **Fig.5a** [39,43,56,59]. Our work challenges the view  
401 that IDRs with extensive sequence variation evolve neutrally. We also demonstrate that  
402 conserved SERs that encode for IDR dimensions -as determined by sequence length,  
403 composition and patterning- can be detected with atomistic simulations even if they are obscured  
404 by naïve sequence alignments.

405 Conformational buffering results in the conservation of tethering for any sequence solution  
406 that preserves the functional length. Our experimental validation using a collection of E1A linkers  
407 largely supports this hypothesis, demonstrating that linkers with a broad range of sequence  
408 compositions and lengths are functionally equivalent (**Fig. 4 a,d**). However, the molecular  
409 evolution of tethered systems will be constrained by competing evolutionary pressures [6,2]. In the  
410 case of the E1A linkers, correlated changes in NCPR and proline content (**Fig. 4 a,b**) maintain  
411 linker extension and prevent folding. The linker can contain additional SLiMs that mediate the  
412 formation of higher order complexes [43] and impose restrictions on sequence variation that could  
413 prevent optimal tethering, analogous to the frustrated energy landscapes in protein folding [60]. By  
414 dissecting the contribution of linker versus SLiMs, we found signatures of these competing forces:  
415 E1A<sub>WT</sub> confers optimal tethering and harbors a hydrophobic SLiM (TAZ2 motif) that plays a minor  
416 destabilizing role, while the Bat E1A linkers evolved stronger destabilizing interactions with Rb  
417 that are compensated by mutations that restore optimal tethering by increasing the affinity of the  
418 SLiMs for Rb. Thus it appears that competition between linker tethering and SLiM binding  
419 constrains IDR evolution due to different contributions from conformational buffering and coupled  
420 folding and binding. This can result in linker sequence conservation patterns ranging from highly  
421 variable [this work,<sup>11,13</sup>] to highly conserved [12]. Other systems such as the intrinsically disordered  
422 Notch RAM region show similar mixed contributions from optimal tethering and sequence-specific  
423 effects [61]

424 Our work establishes E1A as an example of optimal tethering. The low picomolar affinity  
425 of E1A for Rb and the 4000-fold affinity enhancement enforced by the E1A linker is amongst the

426 highest reported positive cooperativity produced by tethering in a natural system, similar to the  
427 POU domain activator Oct-1 binding to DNA ( $K_D = 71$  pM and 2100-fold enhancement) [22]. E1A  
428 is the first adenoviral gene expressed, and the picomolar affinity is likely required for E1A to bind  
429 stably to Rb and efficiently displace E2F and hijack the cell cycle at low expression levels during  
430 early infection [62]. As a comparison, intramolecular MdmX inhibition, exhibits optimal tethering  
431 with a 400-fold enhancement and  $K_{INTRA} = 250$  [12] and other bivalently tethered systems show  
432 variable degrees of enhancement with affinities in the nanomolar range [63,64,65]. At the opposite  
433 extreme, multiple low affinity interactions tethered by short and/or non-optimal linkers might  
434 promote the dynamic binding required for multivalent binding or liquid-liquid phase separation  
435 [66,17]. Our work suggests that conformational buffering can tune the functional length of linkers to  
436 produce an optimal functional output. Thus, conformational buffering may be a widespread  
437 mechanism driving dimensional compensation among IDRs.

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466

467 **Author Contribution Statement**

468 LBC, GWD, ASH and RVP designed research and conceived the study. NSGF WMB and MS  
469 produced reagents. NSGF and WMB performed FP, ITC and NMR experiments and WB, NSGF,  
470 GWD and LBC analyzed data. JG designed and conducted bioinformatic analyses of E1A variants

471 and Rb proteins. MS purified E1A protein variants and NAG performed SEC experiments. AS  
472 and PB performed and analyzed SAXS experiments. AE, AB and JC produced and analyzed E1A  
473 conformational ensembles. SBV and ASH performed and analyzed all atom simulations of E1A  
474 linkers. GFB, CBM and IES computed and analyzed FOLDX matrices. NSGF, JG, AS and ASH  
475 produced figures. LBC, GWD, PB, JC, GdPG, IES, ASH and RVP Supervised research. LBC,  
476 NSGF, JG, RVP, ASH and GWD wrote the paper with critical feedback from all authors.

477

#### 478 **Competing Interests Statement**

479 A.S.H. is a scientific consultant with Dewpoint Therapeutics Inc. and R.V.P. is a member of the  
480 scientific advisory board of Dewpoint Therapeutics Inc. This work has not been influenced by the  
481 affiliation with Dewpoint. The rest of the authors have no competing interests.

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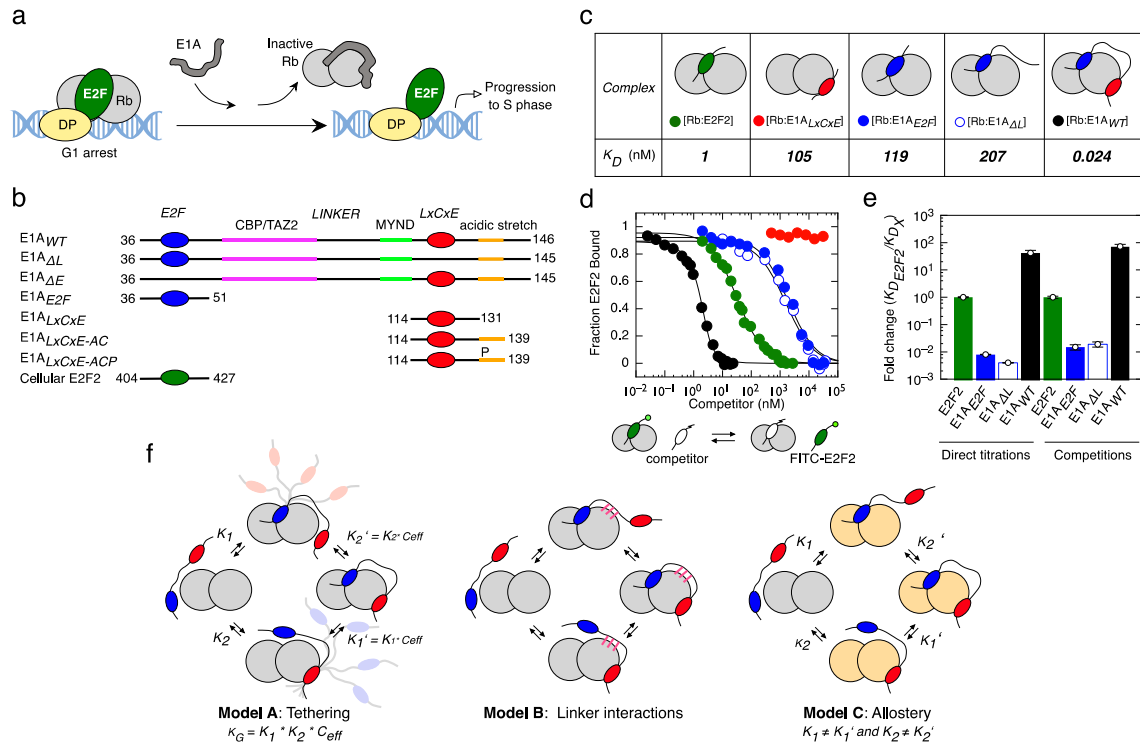
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490 **FIGURE LEGENDS**



491

492 **FIGURE 1. Tethering is required for high affinity Rb binding and E2F displacement by E1A.**

493 **a)** Model for disruption of the repressive Rb-E2F complex by E1A. **b)** Schematic representation

494 of E1A and E2F2 constructs used in this study. Color coding for the E2F, LxCxE, TAZ2 and MYND

495 SLiMs, the acidic stretch and S132 phosphorylation are maintained throughout figures. **c)**

496 Representative interactions tested using fluorescence spectroscopy (**Extended Data Fig. 3 and**

497 **Supplementary Data Tables 1 and 3**). **d)** E2F competition titrations. Color code is as in panel c.

498 **e)** Comparison of the fold-change in binding affinity from direct titrations versus competition

499 assays. The height of the bar is obtained by dividing the  $K_D$  of E2F2 by each  $K_D$  ( $n=1$ ), and values

500 higher than unity indicate an increase in binding affinity with respect to E2F2. For direct titrations,

501 each  $K_D$  value was obtained by averaging (global fitting) over several independent binding

502 isotherms (E2F2:  $n=5$ , E1A<sup>E2F</sup>:  $n=3$ , E1A<sup>ΔL</sup>:  $n=3$ , E1A<sup>WT</sup>:  $n=3$ ) containing 16-22 points each (see

503 **Source Data**). For competition experiments, each  $K_D$  was obtained by fitting of a single binding

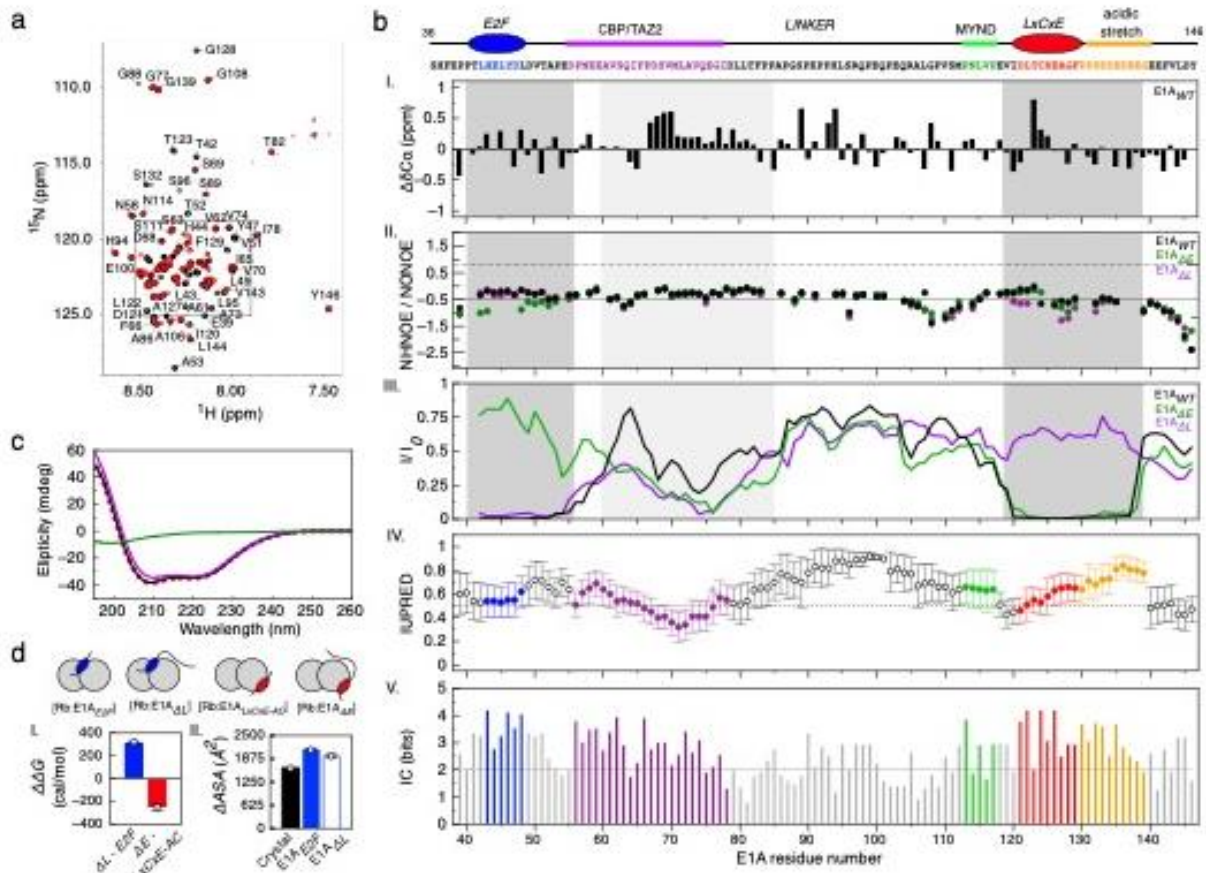
504 isotherm ( $n=1$ ). Error bars correspond to the propagated standard deviation of the averaged  $K_D$

505 values. **f)** Three models that account for affinity enhancement in the Motif-Linker-Motif E1A

506 arrangement (See main text for details).

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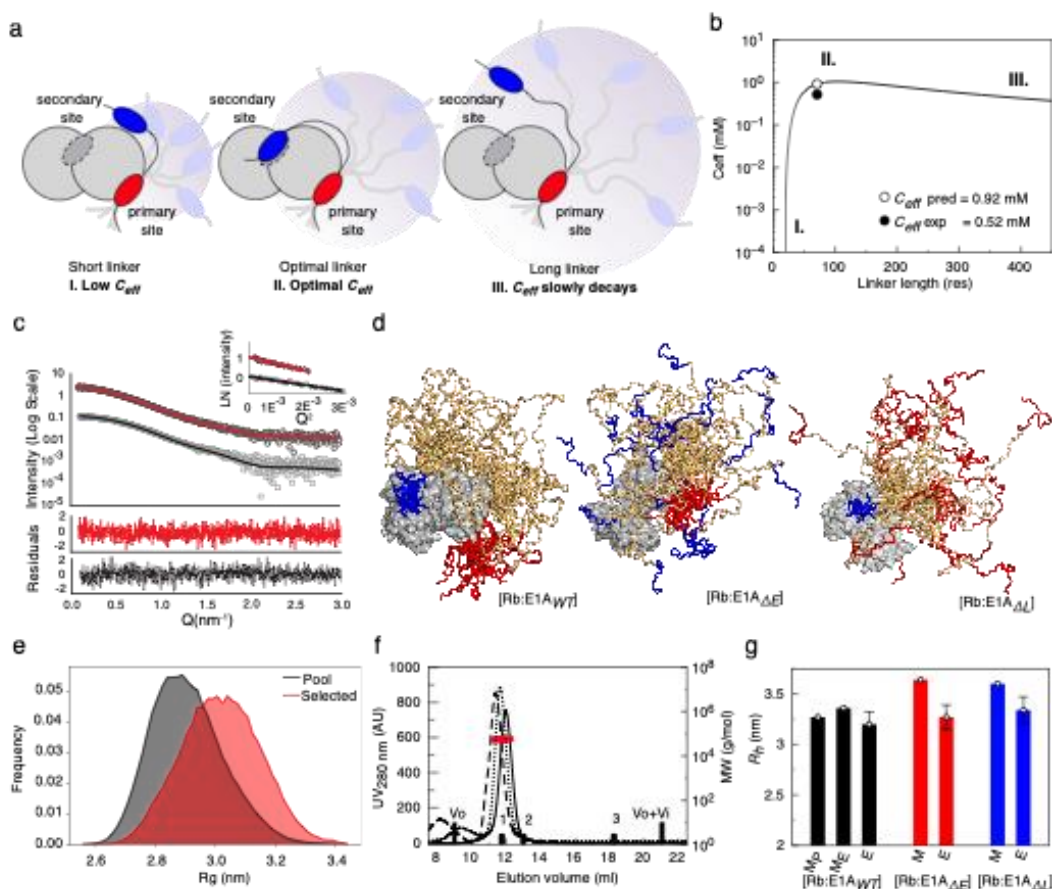
510 **FIGURE 2. NMR and ITC analysis of the [E1A<sub>WT</sub>:Rb] complex.** **a)** <sup>1</sup>H-<sup>15</sup>N TROSY spectra of free  
 511 <sup>15</sup>N-E1A<sub>WT</sub> (black) and <sup>15</sup>N-E1A<sub>WT</sub> bound to unlabeled Rb (red). <sup>15</sup>N-E1A<sub>WT</sub> peak assignments for  
 512 the inset are shown in **Extended Data Fig. 4.** **b)** I. <sup>13</sup>Cα secondary chemical shift (ΔδCα) of <sup>15</sup>N-  
 513 E1A<sub>WT</sub>. II. NHNOE/NONOE ratio for <sup>15</sup>N-E1A<sub>WT</sub>. Dashed line: reference value for rigid backbone.  
 514 III. Intensity ratio plots of bound state (*I*) with respect to the free state (*I*<sub>0</sub>) for E1A<sub>WT</sub>, E1A<sub>ΔL</sub> and  
 515 E1A<sub>ΔE</sub>. Dark gray: E2F/LxCxE SLiMs and flanking regions; Light gray: N-terminal linker region.  
 516 **IV-V.** Disorder propensity and residue conservation (information content: IC) were predicted from  
 517 an alignment of E1A sequences (n=110) (**Supplementary Data File 1**). For disorder prediction,  
 518 data points represent the mean IUPred value at each position and error bars represent the  
 519 standard deviation of the mean. The number of residues averaged at each position is variable  
 520 depending on the number of gaps in the alignment. For the conservation plot, the height of each  
 521 bar represents the IC value at each position. **c)** Far-UV CD spectra for E1A<sub>WT</sub> (green line), Rb  
 522 (violet line), the [E1A<sub>WT</sub>:Rb] complex (black line) and the arithmetic sum of the Rb and E1A<sub>WT</sub>  
 523 spectra (red dashed line). The latter CD spectra largely overlap. While it is possible the low salt  
 524 concentration of the CD experiments might mask hydrophobic interactions occurring at the higher  
 525 salt concentration used for NMR and other binding experiments, such effects are unlikely to

526 prevail for the types of monovalent salts used in our binding experiments. **d)** Left: Plot of the  
527 change in free energy of binding ( $\Delta\Delta G$ ) for E1A fragments containing or lacking the linker region,  
528 measured by ITC. The bar height results from the subtraction between mean  $\Delta G$  values obtained  
529 by averaging several independent binding experiments:  $\Delta G$  E1A $_{\Delta L}$  (n=3),  $\Delta G$  E1A $_{E2F}$  (n=1),  $\Delta G$   
530 E1A $_{\Delta E}$  (n=3) and  $\Delta G$  E1A $_{LXCxE-AC}$  (n=3) (**Supplementary Data Table 1**). Right: Plot of the change  
531 in  $\Delta ASA$  for E1A fragments containing or lacking the linker region. The height of the bar represents  
532 the  $\Delta ASA$  value from PDB structure 2R7G (n=1, black bar) or that derived from ITC experiments  
533 using parameters from Murphy & Freire for [E1A $_{E2F}$ :Rb] (n=1, blue bar) and [E1A $_{\Delta L}$ :Rb] (n=1,  
534 empty blue bar) (**Supplementary Data Table 6**).  $\Delta ASA$  was calculated by ITC measurements at  
535 several temperatures (n=4 [E1A $_{E2F}$ :Rb], n=3 [E1A $_{\Delta L}$ :Rb]). Error bars correspond to the propagated  
536 mean standard errors of the  $\Delta ASA$  value.

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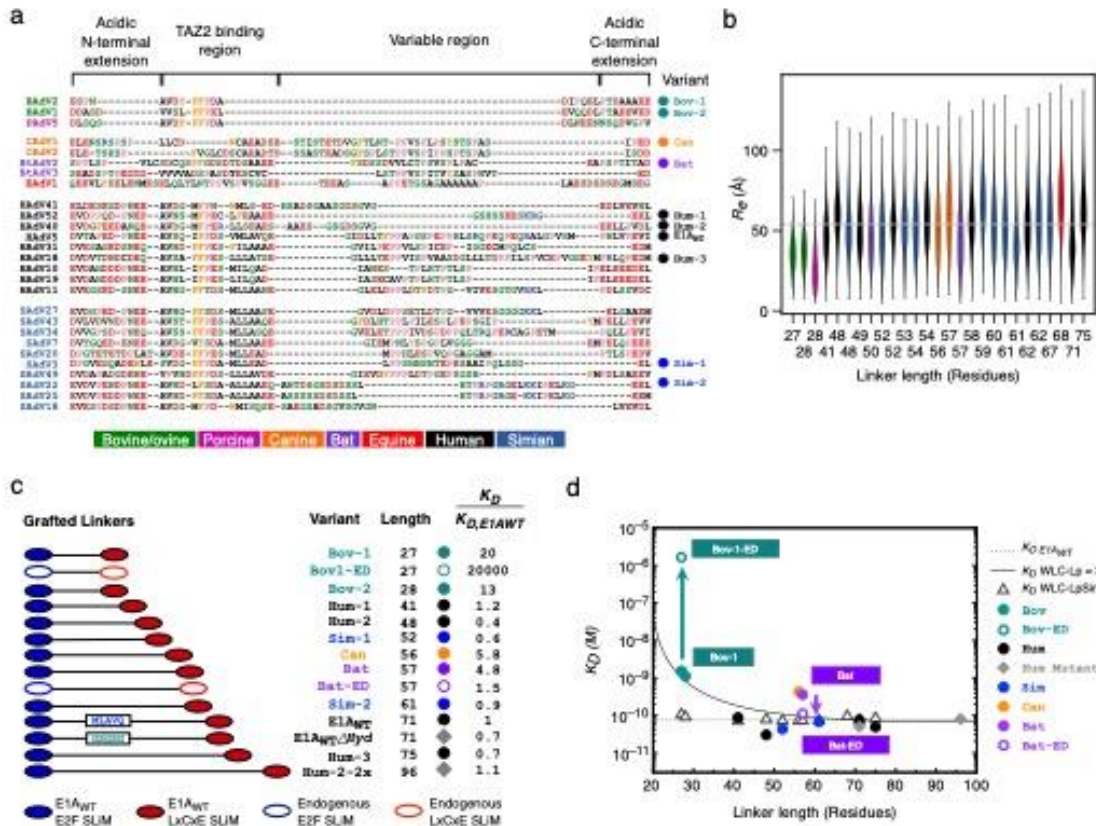
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540 **FIGURE 3: The E1A linker behaves as an entropic tether.** **a)** Schematic representation of how  
541  $C_{eff}$  depends on linker length. **b)**  $C_{eff}$  curve from the WLC model. The scenarios depicted in a) are  
542 shown as regions (I, II, III). **c)** SAXS intensity profile of: Rb (gray squares) with best fit to the  
543 theoretical profile derived from the Rb crystal structure (RbAB domain, black line); and the  
544 [E1A<sub>WT</sub>:Rb] complex (black circles) with best fit from the EOM method (red line). Inset: Guinier  
545 plots for Rb and [E1A<sub>WT</sub>:Rb]. **d)** SAXS-selected [E1A<sub>WT</sub>:Rb] EOM ensemble (both motifs bound)  
546 and simulated ensembles for [E1A<sub>ΔE</sub>:Rb] and [E1A<sub>ΔL</sub>:Rb] (one motif bound). **e)**  $R_g$  distribution of  
547 the ensemble pool for [E1A<sub>WT</sub>:Rb] (black) and the EOM ensemble (red). The linker samples  
548 conformations more extended than the random-coil model of the pool. **f)** SEC-SLS of [E1A<sub>WT</sub>:Rb]  
549 (solid line), [E1A<sub>ΔE</sub>:Rb] (dotted line) and [E1A<sub>ΔL</sub>:Rb] (dashed line). Black bars: BSA 66 kDa (1),  
550 MBP 45 kDa (2) and Lysozyme 14.3 kDa (3). Black line: SEC profile, Red line: MW value (g/mol).  
551 **g)** Comparison between the hydrodynamic radius ( $R_h$ ) of modeled ( $M_P$  = pool,  $M_E$  = EOM) and  
552 experimental (E) ensembles for [E1A<sub>WT</sub>:Rb] (black bars), [E1A<sub>ΔE</sub>:Rb] (red bars) and [E1A<sub>ΔL</sub>:Rb]  
553 (blue bars). The height of each bar represents the  $R_h$  value. Modeled  $R_h$  values ( $n=1$ ) have no  
554 associated error. For Experimental  $R_h$  values ( $n=1$ ) error bars represent the propagated error  
555 obtained from estimation of the  $R_h$  parameter (see **Methods**).



556

557 **FIGURE 4. Conformational buffering leads to conserved functionality of E1A proteins. a)**

558 Global alignment of 27 selected E1A linker sequences. *Mastadenovirus* types are indicated on

559 the left and the color coding (bottom panel) indicates the host range. The variants used for the

560 design of chimeras are shown to the right, with three letter codes indicating the host range. Amino

561 acids color code: acidic (red), basic (blue), polar (green), hydrophobic (black), aromatic (orange)

562 and proline (pink). **b)** End-to-end distance calculated from all-atom simulations using the set of

563 E1A linkers from panel a. Violin plots are colored by host range as in panel a. For each sequence,

564 n=15 independent simulations were run (see **Methods**). The horizontal line within each violin plot

565 represents the median end-to-end distance ( $R_e$ ) value and the ends of the whiskers indicate the

566 maximal and minimal values. Horizontal dotted line: mean  $R_e$  value (53.39 Å) obtained by

567 averaging the median  $R_e$  values of all sequences excluding Bov-1, Bov-2 and Porcine. **c)** Motif-

568 Linker-Motif constructs used in the E1A linker grafting experiment. Filled circles: grafting of linkers

569 into the HAdV5 E1A<sub>E2F</sub> and E1A<sub>LxCxE</sub> motifs. Diamonds: Mutant where the hydrophobic

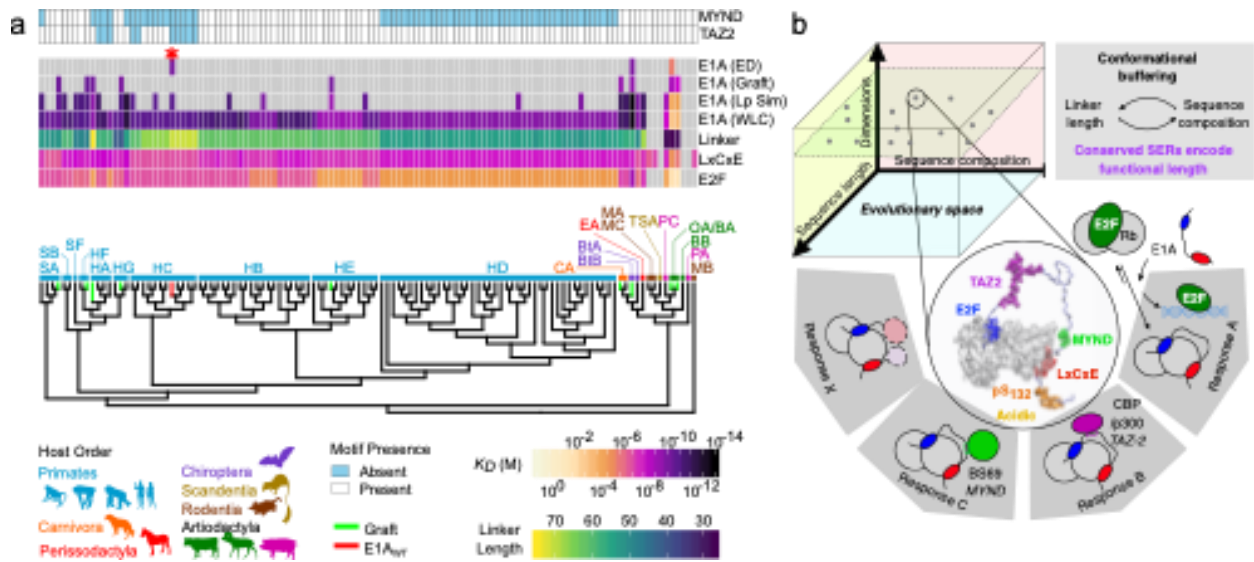
570 MLAVQEGID region was replaced by a GS stretch (E1A<sub>WTΔHyd</sub>) or where the HAdV40 linker

571 sequence was duplicated (Hum2-2x). Empty circles: Variants harboring endogenous linker and

572 motifs (ED). **d)** Global  $K_D$  as a function of linker length for the Motif-Linker-Motif constructs.  $K_D$  for

573 each variant was measured using an E2F displacement experiment (symbols as in c) or predicted

574 using the WLC model. The  $K_D$  values  $\pm$  errors for all measurements are reported in  
575 **Supplementary Data Table 4**. The predicted value of the  $K_D$  for the grafted linkers was calculated  
576 as  $K_D = (K_{D,E2F} * K_{D,LxCxE}) / C_{eff}$  (see **Methods**) using the known affinity of the E1A<sub>E2F</sub> and E1A<sub>LxCxE</sub>  
577 motifs from E1A<sub>WT</sub> (**Supplementary Data Table 1**) and  $C_{eff}$  values obtained using a sequence  
578 independent (Straight line: WLC-Lp=3) or sequence-dependent (Empty triangles: WLC-LpSim)  
579 persistence length ( $L_p$ ) parameter (see **Extended Data Fig. 10 and Methods**). Dotted line:  
580 Experimental  $K_D$  value of the E1A<sub>WT</sub> construct ( $75 \pm 17$  pM). Under the sequence-independent  
581 WLC model the  $K_D$  is expected to increase gradually with decreasing linker length, while LpSim  
582 predicts the  $K_D$  to remain constant in the 41-75 linker length range. Experimental  $K_D$  values are in  
583 good agreement with both models for longer linker lengths, but are closer to LpSim for shorter  
584 linker lengths (41, 48 and 52).  
585  
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587

588 **FIGURE 5. Evolutionary conservation of tethering by E1A proteins.** a) Phylogenetic tree of  
 589 mastadenovirus E1A proteins with species denoted by two letter codes. The affinity of the  
 590 E2F/LxCxE SLiMs and E1A<sub>WT</sub>, and linker length are indicated by color scales. E1A (WLC): Global  
 591  $K_D$  for E1A proteins predicted by the WLC model with standard  $L_p$  values ( $L_p = 3$ ); E1A (LpSim):  
 592  $K_D$  for E1A proteins predicted by the WLC model with sequence-dependent  $L_p$  values; E1A (Graft):  
 593 Experimental  $K_D$  measured for the grafted linkers of **Fig. 4d**; E1A (ED): Experimental  $K_D$   
 594 measured for the variants harboring endogenous linker and motifs of **Fig. 4d**. Gray box: absent  
 595 motif/linker. Light/blue box: present TAZ2/MYND SLiMs. The E1A<sub>WT</sub> protein is marked as a red  
 596 asterisk and as a red terminal branch in the tree and all other sequences used in the experiments  
 597 are marked as green terminal branches in the tree. b) Upper: E1A sequences evolved a  
 598 multiplicity of solutions in the sequence length-composition space to achieve conserved SERs  
 599 through conformational buffering. Lower: The model represents one pose of the conformational  
 600 [E1A<sub>WT</sub>:Rb] ensemble with E2F/LxCxE SLiMs bound to Rb. The evolvable E1A interaction  
 601 platform performs highly conserved functions (E2F activation) while allowing adaptive changes in  
 602 functionality (TAZ2, MYND and other protein binding).

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767 **METHODS**  
768

769 ***Protein purification and peptide synthesis and labeling***

770 *Protein expression and purification:* The human Retinoblastoma protein (Uniprot  
771 ID: P06400) AB domain with a stabilizing loop deletion (372-787Δ582-642), named Rb,  
772 was recombinantly expressed from a pRSET-A vector in *E. coli* BL21(DE3). Briefly, Rb  
773 cultures were induced with 1 mM IPTG and grown at 28 °C overnight. Rb was purified  
774 from the soluble fraction using a Ni<sup>2+</sup>-nitrilotriacetic acid immobilized metal affinity  
775 chromatography resin, followed by a purification with a sulfate cation exchange (SP-  
776 Sepharose) resin and size exclusion (Superdex 75) chromatography [67]. The  
777 adenovirus serotype 5 (HAdV5) Early 1A protein fragment (36-146) (Uniprot ID:  
778 P03255), named E1A<sub>WT</sub>, was subcloned into *Bam*HI/*Hind*III sites of a modified pMalC2x  
779 vector (NewEnglandBioLabs, Hitchin, UK). E1A<sub>ΔE</sub> (43-LHELY-47Δ43-AAAA-46) and  
780 E1A<sub>ΔL</sub> (122-LTCHE-126Δ122-AAAA-125) variants were obtained by site-directed  
781 mutagenesis of the wild type vector. E1A proteins were expressed as MBP fusion  
782 products in *E. coli* BL21(DE3). Unlabeled and single (<sup>15</sup>N) and double (<sup>15</sup>N/<sup>13</sup>C) labeled  
783 samples were obtained from 2TY medium and M9-minimal medium supplemented with  
784 <sup>15</sup>NH<sub>4</sub>Cl and <sup>13</sup>C-glucose respectively. Cultures were induced with 0.8 mM IPTG at 0.7  
785 OD<sub>600</sub> and grown at 37 °C overnight in 2TY medium or for 5 h after induction in M9-  
786 minimal medium. Harvested cells were lysed by sonication and proteins isolated  
787 performing amylose affinity chromatography of the soluble fraction, followed by Q-  
788 HyperD Ion exchange and size exclusion (Superdex 75) chromatography. The MBP tag  
789 was cleaved with Thrombin (Sigma-Aldrich, USA) at 0.4 unit per mg of protein. Synthetic  
790 MBP-E1A fusion constructs (construct sequences available in the **Source File for**  
791 **Figure 4**) subcloned into the pMalC4x vector (GenScript, USA) were expressed in *E.*  
792 *coli* BL21(DE3) followed by Amylose purification and Superdex 75 chromatography as

793 described above. All E1A protein stocks were stored at -80 °C in buffer containing 20mM  
794 Sodium Phosphate pH 7.0, 200mM NaCl, 20mM DTT and 2mM PMSF. Protein purity (>  
795 90%) and conformation were assessed by SDS-PAGE, SEC-SLS and circular dichroism  
796 analysis (**Extended Data Fig. 1**).

797 Peptide synthesis: Peptides corresponding to individual E1A or E2F2 binding  
798 motifs were synthesized by FMoc chemistry at >95% purity (GenScript, USA) and  
799 quantified by Absorbance at 280 nm or by quantitation of peptide bonds at 220 nm in  
800 HCl -when Tryptophan or Tyrosine residues were absent. The peptide sequences are:

801

802	E1A <sub>E2F</sub>	36-SHFEPPTLHELYDLDV-51
803	E1A <sub>LxCxE</sub>	116-VPEVIDLTCHEAGFPP-131
804	E1A <sub>LxCxE-AC</sub>	116-VPEVIDLTCHEAGFPPSDDDEDEEG-139
805	E1A <sub>LxCxE-ACP</sub>	116-VPEVIDLTCHEAGFPP <sub>p</sub> SDDDEDEEG-139
806	Human E2F2	404-SPSLDQDDYLWGLEAGEGISDLFD-427

807 FITC labeling: Proteins and peptides were labeled at their N-terminus with  
808 Fluorescein 5-Isothiocyanate (FITC, Sigma), purified and quantified following a  
809 described protocol [67]. F/P (FITC/Protein) ratio was above 0.8 in all cases.

### 810 **Circular Dichroism (CD)**

811 Far-UV CD spectra were measured on a Jasco J-810 (Jasco, Japan)  
812 spectropolarimeter equipped with a Peltier thermostat using 0.1 or 0.2 cm path-length  
813 quartz cuvettes (Hellma, USA). Five CD scans were averaged from 195 to 200 nm at  
814 100nm/min scan speed, and buffer spectra were subtracted from all measurements. All  
815 spectra were measured in 10mM Sodium Phosphate buffer pH 7.0 and 2mM DTT at 20  
816 ± 1 °C and 5 µM protein concentration.

817

818 **Size Exclusion Chromatography, Hydrodynamic radii calculations and Light**  
819 **Scattering Experiments**

820 Analytical size exclusion chromatography (SEC) was performed on a Superdex  
821 75 column (GE Healthcare) calibrated with globular standards: BSA (66 kDa), MBP (45  
822 kDa) and Lysozyme (14.3 kDa). All runs were performed by injecting 100  $\mu$ l protein  
823 sample (E1A<sub>WT</sub> and E1A<sub>ΔL</sub> at 270  $\mu$ M and E1A<sub>ΔE</sub> at 540  $\mu$ M) in 20 mM Sodium  
824 Phosphate buffer pH 7.0, 200 mM NaCl, 2 mM DTT. For each protein or complex a  
825 partition coefficient ( $K_{av}$ ) was calculated and apparent molecular weights were  
826 interpolated from the  $-\log MW$  vs  $K_{av}$  calibration curve. Experimental hydrodynamic radii  
827 ( $R_h$ ) were calculated following empirical formulations developed by Uversky and col. [68]:

$$828 \log R_h = -0.204 + 0.357 \log MW \quad (1)$$

829 Where  $MW$  is the apparent molecular weight derived from SEC experiments.  
830 The predicted  $R_h$  for E1A<sub>WT</sub> was calculated following the formulation developed by Marsh  
831 and Forman-Kay [3].

832 The exponent  $\nu$  was calculated from  $R_h = R_o \cdot N^\nu$  using the experimental  $R_h$  values,  
833 with  $R_o = 2.49$  nm for E1A<sub>WT</sub> and  $R_o = 4.92$  nm for Rb, following [28]. For E1A<sub>WT</sub>,  $\nu$  was  
834 calculated from  $R_g = R_o \cdot N^\nu$  using  $R_g$  obtained from SAXS measurements and  $R_o = 2.1$   
835 nm, following [69]. In both cases,  $N$  is the number of residues in the chain  
836 (**Supplementary Data Table 2**).

837 Static Light Scattering (SLS) coupled to SEC was carried out to determine the  
838 average molecular weight of individual protein peaks and the stoichiometry of [E1A:Rb]  
839 complexes using a PD2010 detector (Precision Detectors Inc, China) coupled in tandem  
840 to an HPLC system and an LKB 2142 differential refractometer. The 90° light scattering  
841 (LS) and refractive index (RI) signals of the eluting material were analyzed with  
842 Discovery32 software (Precision Detectors).

843 Dynamic Light Scattering (DLS) was used to measure the hydrodynamic size  
844 distribution of E1A, using a Wyatt Dynapro Spectrometer (Wyatt Technologies,  
845 USA). Data was fitted using Dynamics 6.1 software. All measurements were performed  
846 in 20 mM Sodium Phosphate buffer pH 7.0, 200 mM NaCl, 1 mM DTT at 2 mg/ml.  
847 Samples were filtered by 0.22 μM filters (Millipore) and placed into a 96 Well glass  
848 bottom black plate (In Vitro Scientific P96-1.5H-N) covered by a high performance cover  
849 glass (0.17+/-0.005mm) before measurements were taken.

850

### 851 ***Fluorescence Spectroscopy Experiments***

852 Measurements were performed in a Jasco FP-6200 (Nikota, Japan)  
853 spectropolarimeter assembled in L geometry coupled to a Peltier thermostat. Excitation  
854 and emission wavelengths were 495 nm and 520 nm respectively, with a 4 nm  
855 bandwidth. All measurements were performed in 20 mM Sodium Phosphate buffer pH  
856 7.0, 200 mM NaCl, 2 mM DTT and 0.1% Tween-20 at 20 ± 1 °C.

857 For direct titrations, a fixed concentration of FITC-labeled protein/peptide was  
858 titrated with increasing amounts of Rb until saturation was reached. Maximal dilution  
859 was 20% and samples equilibrated for 2 min ensuring steady state. Titrations performed  
860 at concentrations 10 times higher than the equilibrium dissociation constant ( $K_D$ ) allowed  
861 estimation of the stoichiometry of each reaction. Binding titrations performed at sub-  
862 stoichiometric concentrations allowed an estimation of  $K_D$ , by fitting the titration curves  
863 to a bimolecular association model:

$$864 \quad Y = Y_F + \frac{(Y_B - Y_F)}{P_0} * \frac{(x + P_0 + K_D) + \sqrt{(x - P_0 + K_D)^2 - (4 * P_0 * x)}}{2} + C * x \quad (2)$$

865 Where Y is the measured anisotropy signal,  $Y_F$  and  $Y_B$  are the free and bound  
866 labeled peptide signals,  $P_0$  is the total labeled peptide concentration, x is Rb  
867 concentration, and  $K_D$  is the equilibrium dissociation constant in Molar units. The  $[C * x]$

868 linear term accounts for slight bleaching or aggregation. Data was fitted using the Profit  
869 7.0 software (Quantumsoft, Switzerland), yielding a value for each parameter and its  
870 corresponding standard deviation. Titrations for each complex were performed in  
871 triplicate at least at three different concentrations of FITC-labeled sample, and  
872 parameters were obtained from fitting individual titrations or by global fitting of the  $K_D$   
873 parameter using normalized titration curves at different concentrations, obtaining an  
874 excellent agreement between individual and global fits (**Supplementary Data Table 3**  
875 **and Extended Data Fig. 3**).

876 Competition experiments were carried out by titrating the pre-assembled  
877 complex [Rb:FITC-E2F2] (1:1 molar ratio, 5 nM) with increasing amounts of unlabeled  
878 competitors and following the decrease in the anisotropy signal until the value  
879 corresponding to free FITC-E2F2 was reached. IC50 values were estimated directly  
880 from the curves as the concentration where the competitor produced a decrease in 50%  
881 of the maximal anisotropy value.  $K_D$  values were calculated by fitting the data  
882 considering the binding equilibrium of the labeled peptide and the unlabeled competitors  
883 according to [70], obtaining  $K_{D(comp)}$  values that differed only slightly (2 to 3-fold) from  
884 those obtained from direct titrations.  $K_D$  and  $K_{D(comp)}$  values also displayed similar fold  
885 changes in binding affinity relative to E2F2 within each method (**Supplementary Data**  
886 **Table 1**). The agreement between the  $K_D$  values obtained from fluorescence and ITC  
887 titrations (**Supplementary Data Table 1**) confirmed that FITC moiety did not cause  
888 significant changes in Rb binding affinity. MBP-E1A fusion protein affinities  
889 (**Supplementary Data Table 4 and Extended Data Fig. 8**) were determined by  
890 performing competition experiments assembling a [Rb:FITC-E2F2] complex at 10nM  
891 concentration, after verifying that MBP-E1A<sub>WT</sub> and E1A<sub>WT</sub> (cleaved and uncleaved  
892 HAdV5 proteins) had the same binding affinity (Extended Data Table 7). Measurements  
893 were performed on a PTI Quantamaster QM40 spectrofluorimeter (Horiba, Japan)



894 equipped with polymer film polarizers and coupled to a Peltier thermostat with excitation  
895 parameters as described above.

896

### 897 ***ITC Experiments***

898 Direct titrations. ITC experiments were performed on MicroCal VP-ITC and  
899 MicroCal PEAQ-ITC equipment (Malvern Panalytical) in 20 mM Sodium Phosphate pH  
900 7.0, 200 mM NaCl, 5mM 2-mercapto ethanol at  $20.0 \pm 0.1$  °C, unless stated otherwise.  
901 Prior to titrations, cell and titrating samples were co-dialyzed in the aforementioned  
902 buffer for 48 h at  $4 \pm 1$  °C and then de-gassed. Measurements performed in the MicroCal  
903 VP-ITC used 28 10- $\mu$ l injections at a flow rate of 0.5  $\mu$ l/s and those performed in the  
904 MicroCal PEAQ-ITC used 13 3- $\mu$ l injections. The concentration range of cell and titrating  
905 samples are detailed in **Extended Data Figs. 2 and 5**. Data were analyzed using the  
906 Origin software.

907 Allosteric coupling experiments. First, a pre-assembled [Rb:E1A<sub>LxCxE</sub>] complex  
908 (1:1 molar ratio, 30  $\mu$ M) was titrated with E1A<sub>E2F</sub> or E1A <sub>$\Delta$ L</sub> to assess whether binding of  
909 the LxCxE motif modified the binding affinity for the E2F site. Conversely, pre-  
910 assembled [Rb: E1A<sub>E2F</sub>] or [Rb: E1A <sub>$\Delta$ L</sub>] complexes were titrated with E1A<sub>LxCxE</sub> to assess  
911 whether binding of the E2F motif modified the binding affinity for the LxCxE site  
912 (**Supplementary Data Table 7**).

913 Calculation of  $\Delta C_p$  and  $\Delta ASA_T$  parameters from ITC data. A series of titrations  
914 were carried out at different temperatures (10.0, 15.0, 20.0 and  $30.0 \pm 0.1$  °C) and the  
915 change in binding heat capacity ( $\Delta C_p$ ) was obtained from the slope of the linear  
916 regression analysis of the plot of  $\Delta H$  vs temperature (**Extended Data Fig. 5**). The  
917 changes in accessible surface area ( $\Delta ASA_T$ ) and the number of residues that fold upon  
918 binding ( $X_{res}$ ) were estimated by solving semi-empirical equations from protein folding  
919 studies applied to protein-ligand binding [<sup>50</sup>] and from models that use parameters

920 derived for intrinsically disordered proteins (IDP-specific model) [<sup>51</sup>] (Details on the  
 921 model-specific parameter values are provided in **Supplementary Data Tables 5 and**  
 922 **6**). First we calculated  $\Delta H_{int(T)}$  from:

$$923 \quad \Delta H_{int(T)} = \Delta H_{int(T_H)} + \Delta C_p (T - T_H) \quad (3)$$

924 where  $\Delta H_{int(T)}$  is the change in enthalpy measured at experimental temperatures  
 925 (K),  $\Delta C_p$  is the change in heat capacity,  $\Delta H_{int(T_H)}$  is the change in enthalpy at the  
 926 temperature of enthalpic convergence and  $T_H$  is the temperature of enthalpic  
 927 convergence at which the apolar contribution is assumed to be zero (295.15 K). Then,  
 928  $\Delta ASA_T$  values were calculated as the sum of the contribution of changes in polar  
 929 ( $\Delta ASA_p$ ) and non-polar ( $\Delta ASA_{np}$ ) accessible surface areas, by solving the following set  
 930 of equations:

$$931 \quad \Delta H_{int(T_H)} = \Delta h_{np} \Delta ASA_{np} + \Delta h_p \Delta ASA_p \quad (4)$$

$$932 \quad \Delta C_p = \Delta c_{np} \Delta ASA_{np} + \Delta c_p \Delta ASA_p \quad (5)$$

933 where  $\Delta h_{np}$ ,  $\Delta h_p$ ,  $\Delta c_{np}$  and  $\Delta c_p$  are constants that assume different values  
 934 according to the model used [<sup>50,51</sup>] (See **Supplementary Data Table 6** for specific  
 935 values). Finally,  $X_{res}$  was calculated from:

$$936 \quad X_{res} = \Delta S_{config} / \Delta S_{residue} \quad (6)$$

937 Where  $\Delta S_{residue}$  is the change in configurational entropy per residue and the  
 938 change in configurational entropy ( $\Delta S_{config}$ ) was calculated as the sum of changes in  
 939 rotation-translation ( $\Delta S_{rt}$ ) and solvation ( $\Delta S_{solv}$ ) entropy:

$$940 \quad \Delta S_{config} = \Delta S_{rt} + \Delta S_{solv} \quad (7)$$

941 with  $\Delta S_{solv}$  defined as:

$$942 \quad \Delta S_{solv} = C_1 * \Delta C_p * \ln (T/T_S) \quad (8)$$

943 where  $T$  is the experimental temperature (K) and  $T_S$  is the temperature for  
944 entropic convergence (385 K).  $\Delta S_{rt}$ ,  $\Delta S_{residue}$  and the constant  $C_1$ , which depend on the  
945 relationship of apolar to polar surface area, assume different values depending on the  
946 model used [50,51] (see **Supplementary Data Table 6** for model-specific values).

947

### 948 ***NMR Experiments***

949 NMR experiments were carried out using a Varian VNMRs 800 MHz  
950 spectrometer equipped with triple resonance pulse field Z-axis gradient cold probe. A  
951 series of two-dimensional sensitivity-enhanced  $^1\text{H}$ - $^{15}\text{N}$  HSQC and three-dimensional  
952 HNCACB, HNCO and CBCA(CO)NH experiments [71,72] were performed for backbone  
953 resonance assignments on uniformly  $^{13}\text{C}$ - $^{15}\text{N}$ -labeled samples of E1A<sub>WT</sub>, E1A <sub>$\Delta$ E</sub> and  
954 E1A <sub>$\Delta$ L</sub> at 700  $\mu\text{M}$ , 975  $\mu\text{M}$  and 850  $\mu\text{M}$  respectively. All measurements were performed  
955 in 10 % D<sub>2</sub>O, 20 mM Sodium Phosphate pH 7.0, 200 mM NaCl, 2 mM DTT at 25 °C.  
956 The HSQC used 9689.9 Hz and 1024 increments for the  $t_1$  dimension and 2106.4 Hz  
957 with 128 increments for the  $t_2$ . The HNCACB used 9689.9, 14075.1, and 2106.4 Hz,  
958 with 1024, 128, and 32 increments for the  $t_1$ ,  $t_2$ , and  $t_3$  dimensions, respectively. The  
959 HNCO used 9689.9, 2010.4 Hz, and 2106.4 Hz with 1024, 64, and 32 increments for  
960 the  $t_1$ ,  $t_2$ , and  $t_3$  dimensions, respectively. The CBCA(CO)NH used 9689.9, 14072.6,  
961 and 2106.4 Hz, with 1024, 128, and 32 increments for the  $t_1$ ,  $t_2$ , and  $t_3$  dimensions,  
962 respectively. For E1A<sub>WT</sub> 88% of non-proline backbone  $^1\text{H}$  and  $^{15}\text{N}$  nuclei, 75% of  $^{13}\text{C}'$   
963 nuclei and 90% of  $^{13}\text{C}_\alpha$  and  $^{13}\text{C}_\beta$  of E1A nuclei were assigned (**Supplementary Data**  
964 **File 2**). For E1A <sub>$\Delta$ E</sub> and E1A <sub>$\Delta$ L</sub> 85% of non-proline backbone  $^1\text{H}$  and  $^{15}\text{N}$  nuclei, 72% of  
965  $^{13}\text{C}'$  nuclei and 87% of  $^{13}\text{C}_\alpha$  and  $^{13}\text{C}_\beta$  E1A nuclei were assigned.

966 NMRPipe and NMRViewJ software packages were used to process and analyze  
967 all the NMR spectra [73]. Residue-specific random coil chemical shifts were generated

968 for the three sequences using the neighbor-corrected IDP chemical shift library [74].  
969 Secondary chemical shifts ( $\Delta \delta$ ), were calculated by subtracting random coil chemical  
970 shifts from the experimentally obtained chemical shifts.

971 Two-dimensional  $^1\text{H}$ - $^{15}\text{N}$  TROSY experiments were performed on single  $^{15}\text{N}$ -  
972 labeled samples of free E1A<sub>WT</sub>, E1A <sub>$\Delta$ E</sub> and E1A <sub>$\Delta$ L</sub> and on each E1A protein bound  
973 stoichiometrically to Rb (1:1 molar ratio) at 525  $\mu\text{M}$  (E1A<sub>WT</sub>), 300  $\mu\text{M}$  (E1A <sub>$\Delta$ E</sub>) and 315  
974  $\mu\text{M}$  (E1A <sub>$\Delta$ L</sub>). The ratio between the peak intensity in the bound state ( $I$ ) and the peak  
975 intensity in the free state ( $I_0$ ) was calculated, allowing interacting residues to be  
976 determined together with additional data.

977

### 978 ***Molecular modelling of Rb:E1A conformational ensembles***

979 Conformations of E1A<sub>WT</sub> bound to Rb were modeled using an extended version  
980 of a recently proposed method to generate realistic conformational ensembles of IDPs  
981 [53]. Conformational ensemble models of [E1A<sub>WT</sub>:Rb] were generated using a stochastic  
982 sampling algorithm implemented in the MoMA software suite (<https://moma.laas.fr>). This  
983 method exploits local, sequence-dependent structural information encoded in a  
984 database of three-residue fragments and builds conformations incrementally sampling  
985 dihedral angles values from the database, while avoiding steric clashes. In order to  
986 model the double-bound [Rb:E1A<sub>WT</sub>] complex, the E2F and LxCxE motifs were  
987 considered to be static, preserving the conformations extracted from experimentally  
988 determined structures (2R7G and 1GUX). The 71-residue fragment between these two  
989 motifs was considered as a long protein loop that adapts its conformation in order to  
990 maintain the two ends rigidly positioned. Conformational sampling considering such  
991 loop-closure constraints was performed using a robotics-inspired method [54] adapted  
992 to use dihedral angle values from the aforementioned database. For each feasible  
993 conformation of the central fragment, geometrically compatible conformations of the

994 short N- and C-terminal tails were sampled using the basic strategy explained in [53]. For  
995 singly bound models [E1A<sub>ΔL</sub>:Rb] and [E1A<sub>ΔE</sub>:Rb], only one of the two motifs were  
996 considered to be statically bound to Rb and the other motif behaved as the flexible linker.  
997 The loop sampling method used to model the linker between the two binding motifs can  
998 be used via a web server (<https://moma.laas.fr/applications/LoopSampler/>). Binaries  
999 can be provided upon request.

1000

### 1001 **SAXS Experiments**

1002 SAXS experiments for Rb and [E1A<sub>WT</sub>:Rb] were carried out at the European  
1003 Molecular Biology Laboratory beamline P12 of PETRAIII storage ring, using the X-ray  
1004 wavelengths of 1.24 Å and a sample-to-detector distance of 3.0 m [75]. The scattering  
1005 profiles measured covered a momentum transfer range of  $0.0026 < s < 0.73 \text{ \AA}^{-1}$ . SAXS  
1006 data for E1A was collected at the at the SWING beamline at the SOLEIL synchrotron,  
1007 France, on an Eiger 4M detector with a sample-to-detector distance of 2.0 m. SAXS  
1008 data were measured for Rb, E1A<sub>WT</sub> and the [E1A<sub>WT</sub>:Rb] complex at 10° C.  
1009 Concentrations used for E1A<sub>WT</sub> were 7.0, 5.6 and 4.2 mg/ml, for Rb were 4.0, 2.0, 1.0  
1010 mg/ml, and for and [E1A<sub>WT</sub>:Rb] were 2.7, 1.4, and 0.7 mg/ml, in 20 mM Sodium  
1011 Phosphate pH 7.0, 200 mM NaCl, 1mM DTT. The scattering patterns of the buffer  
1012 solution were recorded before and after the measurement of each sample. Multiple  
1013 repetitive measurements were performed to detect and correct for radiation damage.  
1014 The initial data processing steps including masking and azimuthal averaging were  
1015 performed using the SASFLOW version 3.0. pipeline for Rb and [E1A<sub>WT</sub>:Rb] and the  
1016 program FOXTROT version 3.5.2. [76] for E1A. Final curves at each concentration were  
1017 derived after the averaged buffer scattering patterns were subtracted from the protein  
1018 sample patterns. No sign of aggregation was observed in any of the curves. Final SAXS

1019 profiles for the systems were obtained by merging curves for the lowest and highest  
1020 concentrations to correct small attractive interparticle effects observed. The SAXS  
1021 profiles were analyzed using the ATSAS suite of programs version 2.8.4 [77]. The  
1022 forward scattering intensity,  $I(0)$ , and the radius of gyration,  $R_g$ , were evaluated using  
1023 Guinier's approximation [78], assuming that at very small angles ( $s < 1.3/R_g$ , the intensity  
1024 can be well represented as  $I(s) = I(0) \exp(-(sR_g)^2/3)$ ). The  $P(r)$  distribution functions  
1025 were calculated by indirect Fourier Transform using GNOM [79] applying a momentum  
1026 transfer range of  $0.01 < s < 0.33 \text{ \AA}^{-1}$  and  $0.013 < s < 0.27 \text{ \AA}^{-1}$  for Rb and [Rb:E1A],  
1027 respectively. For E1A<sub>WT</sub> a SEC-SAXS experiment was also performed which was  
1028 processed using the program CHROMIX [80] which is a part of ATSAS 2.8.4. to obtain  
1029 the SAXS profile from a highly monodisperse sample. This profile overlaid perfectly with  
1030 the E1A<sub>WT</sub> merged curve from the three batch experiments, discarding aggregation  
1031 problems.

1032 The fitting of the crystallographic structure of Rb (PDB: 3POM [81]) to the  
1033 experimental SAXS curve was performed with FOXS [82,83]. An optimal fit ( $\chi^2=0.86$ ) was  
1034 obtained after modelling the missing parts (loops, N- and C-termini) and a subsequent  
1035 refinement with the program AllosMod-FoXS [84]. SAXS data measured for [Rb:E1A]  
1036 were analyzed with the Ensemble Optimization Method (EOM) [52,85]. Briefly, theoretical  
1037 SAXS profiles of the 10250 structures of the complex were computed with CRY SOL [86].  
1038 200 different sub-ensembles of 20 or 50 conformations collectively describing the  
1039 experimental curve were collected with EOM and analyzed in terms of  $R_g$  distributions.  
1040 The experimental SAXS data of [E1A<sub>WT</sub>:Rb] complex is compatible with three distinct  
1041 scenarios: a 100% doubly-bound ensemble where the linker is highly expanded, a 100%  
1042 singly-bound ensemble where the linker is highly compact and thirdly, an ensemble with

1043 a combination of 76% doubly bound and :24% singly-bound species, which resulted  
1044 from the linear combination of a curve representing the ensemble average of all singly-  
1045 and all doubly-bound conformations. However, thermodynamic ( $K_D$  for  $E1A_{WT}$ ) data  
1046 strongly argue against the last two scenarios as it indicates an extremely low expected  
1047 population of the singly-bound forms at any concentration of the complex used in the  
1048 SAXS experiments.

1049

### 1050 ***Hydrodynamic radii for generated conformations***

1051 Hydrodynamic radii were calculated using the program HydroPro (version 10)  
1052 [87,88]. HydroPro was run on 1000 models selected by EOM for the doubly-bound  
1053 conformations and 1000 randomly selected conformations of N- and C-terminal bound  
1054 conformations. The calculations were done at temperatures of 20 and 25 °C with  
1055 corresponding solvent viscosities of 0.01 and 0.009 poise, respectively. The values of  
1056 atomic element radius (AER), Molecular Weight, Partial Specific Volume and Solvent  
1057 Density were set to 2.9 Å, 54590 Da, 0.702 cm<sup>3</sup>/g and 1.0 g/cm<sup>3</sup>, respectively. These  
1058 values have no associated error.

1059

### 1060 ***All-atom simulations of E1A Linker sequences***

1061 All-atom simulations were run using the CAMPARI simulation engine (V2)  
1062 Version 2.0 (<http://campari.sourceforge.net>) and ABSINTH implicit solvent model ABS-  
1063 OPLS3.2 [89,90]. All simulations were run at 320 K; while this is a slightly elevated  
1064 temperature compared to the experimental temperature, none of the terms the  
1065 Hamiltonian lacks temperature dependence such that this slightly high temperature  
1066 serves to improve sampling quality in a uniform way across all simulations. This  
1067 approach has been leveraged to great effect in previous studies and is especially  
1068 convenient in the case of simulating many different sequences that span a range of

1069 sequence properties and lengths [8]. A collection of Monte Carlo moves was used to  
1070 fully sample conformational space as previously described [91,92,13].

1071 For all simulations of natural sequences, 15 independent simulations were run  
1072 per sequence for a total of 90K conformations per sequence across 27 different  
1073 sequences (405 independent simulations,  $5.25 \times 10^8$  Monte Carlo steps per sequence).  
1074 Simulations were performed in 15 mM NaCl in a simulation droplet size sufficiently large  
1075 for each sequence, calibrated in a length dependent manner. Simulations were analyzed  
1076 using the MDTraj package version 1.9.5 [93] and SOURSOP version 0.1.3  
1077 (<https://soursop.readthedocs.io/>). Sequence analysis was performed using the local  
1078 CIDER software package [94] with all parameters reported in the **Source Data for**  
1079 **Extended Data Figure 7**. Normalized end-to-end distance was calculated as the  
1080 absolute end-to-end distance divided by the end-to-end distance expected for an  
1081 equivalently long Gaussian chain. Motif-linker-motif simulations were performed in a  
1082 manner analogous to the linker-only motifs. Each independent simulation was run for  $86$   
1083  $\times 10^6$  steps, with  $6 \times 10^6$  steps discarded as equilibration and conformations saved every  
1084 50,000 steps. Over 10 independent replicas, this approach generates ensembles of  
1085 16,000 conformations per sequence. To calculate the hydrodynamic radius we used the  
1086 approach of Nygaard et al. to convert the radius of gyration into the hydrodynamic radius  
1087 [95]. Code for this conversion is provided in the supporting GitHub repository.

1088

### 1089 ***Length titration Simulations***

1090 The linker from HF\_HAdV40 was used to determine the overall amino acid  
1091 composition and generate random sequences across a range of lengths that  
1092 recapitulated this composition. Specifically, for each length (45, 50, 55, 60, 65, 70, 75)  
1093 twenty random sequences were generated for a total of 140 randomly generated



1094 sequences. Each sequence was simulated under equivalent simulation conditions for  
1095  $35 \times 10^9$  simulation steps, with the goal of elucidating the general relationship between  
1096 sequence length and end-to-end distance for an arbitrary sequence of the composition  
1097 associated with HF\_HAdV40. The mean end-to-end distance for the collection of  
1098 sequences at a given length was determined, such that the mean value is a double  
1099 average over both conformational space and sequence space.

1100

### 1101 **WLC modelling**

1102 The worm like chain (WLC) model: A worm like chain (WLC) model [22] was used  
1103 to describe the end-to-end probability density distribution function of the E1A linker and  
1104 estimate the effective concentration term ( $C_{eff}$ ) used in the tethering model (**Fig. 1,**  
1105 **Model A and Fig. 3**). In this model, the disordered linker behaves as a random polymer  
1106 chain whose dimensions depend on the persistence length ( $L_p$ ), which represents the  
1107 chain stiffness, or the length it takes for the chain motions to become uncorrelated and  
1108 on the contour length ( $L_c$ ), which is the total length of the chain. For long peptides,  $L_p$   
1109 assumes a standard value of  $3\text{\AA}$  and  $L_c$  is  $L_c = N_{res} * b$ , where  $N_{res}$  is the number of linker  
1110 residues and  $b$  is the average unit size of one amino acid ( $3.8\text{\AA}$ ) [23]. Under this model,  
1111 the probability density function  $p(r)$  is defined by:

$$1112 \quad p(r) = 4\pi r^2 \left( \frac{3}{4\pi L_p L_c} \right)^{\frac{3}{2}} \exp \left( \frac{-3r^2}{4L_p L_c} \right) \zeta(r, L_p, L_c) \quad (3)$$

1113 Where  $p(r)$  is a function of distance  $r$  and depends on  $L_p$  and  $L_c$ . The last term in  
1114 the equation is expanded in [22, 23]. The end-to-end probability density function can be  
1115 related to the effective concentration in the bound state when the linker is restrained to  
1116 a fixed distance between binding sites,  $r_o$  [22]. In this case, the effective concentration  
1117  $C_{eff}$  is defined by:

1118 
$$C_{\text{eff}} = \frac{p(r_0) 10^{27} \text{\AA}^3 1^{-1}}{4\pi r^2 N_A} \quad (4)$$

1119 Where  $N_A$  is Avogadro's number and ( $r_0$ ) is the distance separating the binding  
 1120 sites obtained from the X-ray structure of the complex (49 Å calculated from PDB: 2R7G  
 1121 [40] and 1GUX [41]). Multiplying Eq. (4) by  $10^3$  yields  $C_{\text{eff}}$  in millimolar units.

1122

1123 ***Calculation of experimental and predicted  $C_{\text{eff}}$  values for the E1A<sub>WT</sub>:Rb interaction***

1124 Experimental  $C_{\text{eff}}$  values: In Model A the global dissociation constant is calculated as:

1125  $K_G = K_1 * K_2 * C_{\text{eff}}$  (**Fig. 1f**) where  $K_G$ ,  $K_1$  and  $K_2$  are equilibrium association constants ( $K =$   
 1126  $1/K_D$ ). Therefore, this relationship can be expressed equivalently as  $K_D =$   
 1127  $K_{D,E2F} * K_{D,LxCxE} * C_{\text{eff}}^{-1}$ . Here,  $K_{D,E2F}$  and  $K_{D,LxCxE}$  are the equilibrium dissociation constants  
 1128 of the E1A<sub>E2F</sub> and E1A<sub>LxCxE</sub> motifs respectively (reported in **Supplementary Data Table**  
 1129 **1**) and is the equilibrium dissociation constant for E1A<sub>WT</sub> (reported in **Supplementary**  
 1130 **Data Table 1**). The condition  $K_1 = K_1'$  and  $K_2 = K_2'$  (no allosteric coupling between sites)  
 1131 was met (**Extended Data Fig. 5 and Supplementary Data Table 7**). Therefore, the  
 1132 experimentally derived  $C_{\text{eff}}$  was calculated from the measured binding constants as:  $C_{\text{eff}}$   
 1133  $= (K_{D,E2F} * K_{D,LxCxE}) / K_D$  (**Fig. 3a**).

1134 Predicted  $C_{\text{eff}}$  values: The  $C_{\text{eff}}$  value predicted from the WLC model (**Fig. 3a**) was  
 1135 obtained by applying Eq. [4] with the designated  $L_p$  parameter (standard model  $L_p = 3\text{\AA}$   
 1136 and  $b = 3.8\text{\AA}$ ), using a linker length of 71 residues for HAdV5 E1A. The separation  
 1137 between binding sites,  $r_0$ , was 49 Å (from PDB:1GUX and PDB:2R7G).

1138

1139 ***Calculation of predicted global binding affinity for grafted E1A linkers***

1140 We predicted the  $K_D$  values expected for each of the grafted linker variants of  
 1141 **Figure 4c,d** under a sequence-independent WLC model or accounting for sequence-

1142 dependent changes in the persistence length. We calculated  $K_D$  for E1A<sub>WT</sub> as  $K_D =$   
 1143  $(K_{D,E2F} * K_{D,LxCxE}) / C_{eff}$ , where  $K_{D,E2F}$  and  $K_{D,LxCxE}$  are the  $K_D$  values for the E1A<sub>E2F</sub> and  
 1144 E1A<sub>LxCxE</sub> motifs of E1A<sub>WT</sub> (reported in **Supplementary Data Table 1**) and  $C_{eff}$  was  
 1145 calculated using the WLC model as described below. Competition experiments for  
 1146 E1A<sub>WT</sub> typically yield  $K_D$  values ~3-fold higher than those obtained by direct titration  
 1147 (**Supplementary Data Tables 1 vs 4**). To correct for this effect, the predicted  $K_D$  values  
 1148 were corrected by a factor of three. Since the only element changing for each grafted  
 1149 variant in the grafting experiment is the linker (i.e. the  $C_{eff}$  value), we calculated  $K_{D,VARIANT}$   
 1150 as  $K_{D,VARIANT} = K_{DE1AWT} / (C_{eff}Ratio)$ , where  $C_{eff}Ratio = C_{eff.VARIANT} / C_{eff.E1AWT}$ . All predicted  
 1151  $C_{eff}$ ,  $L_{pSim}$  and  $K_D$  values for the grafting experiment are reported in the **Source Data**  
 1152 **Files for Figure 5 and Extended Data Figure 10**. The  $C_{eff}$  values were calculated as  
 1153 follows:

1154 WLC-Lp=3 model: For the standard assumption of a sequence-independent model  
 1155 (WLC-Lp=3) we calculated the  $C_{eff}$  function as a function of linker length ( $C_{eff(L)}$ ) using  
 1156 equations (3) and (4) with a standard value for the persistence length parameter ( $L_p =$   
 1157 3). We calculated the expected  $K_D$  as a function of linker length as  $K_D =$   
 1158  $(K_{D,E2F} * K_{D,LxCxE}) / C_{eff(L)}$  (Straight line, **Fig. 4d**).

1159 WLC-LpSim model: For the sequence-dependent model (WLC-LpSim) we calculated  
 1160  $C_{eff}$  for each linker using equations (3) and (4) applying the specific number of residues  
 1161 ( $N_{res}$ ) of each linker and an individual sequence-dependent  $L_p$  value for each linker  
 1162 ( $L_{pSim}$ ), which was obtained from the simulations.  $L_{pSim}$  values were calculated from  
 1163 the average end-to-end distance of each simulated ensemble using the equation  $\langle r^2 \rangle =$   
 1164  $2 * L_p * L_c$ , where  $L_c = N_{res} * b$  and  $b$  takes the value 3.8 Å. This equation is an approximation  
 1165 for the value of  $\langle r^2 \rangle$  for a worm like chain in the case where the contour length of the  
 1166 chain is much larger than its persistence length ( $L_c \gg L_p$ ) [23].

1167

1168 ***Calculation of predicted global binding affinity for a large family of E1A linkers***

1169 The WLC model was used to estimate the  $C_{eff}$  values and global Rb binding affinities of  
1170 a collection of 110 natural linker sequences of different length changing the length value  
1171 for each linker and keeping other parameters constant. All values are reported in **the**  
1172 **Source Data File for Figure 5 and Extended Data Figure 10.**

1173 Dataset: A previously reported alignment and phylogenetic tree of 116 Mastadenovirus  
1174 E1A sequences [56,58] was used to identify the E2F and LxCxE motifs as described 58,  
1175 collecting 110 sequences in which both motifs were present (**Supplementary Data File**  
1176 **1**). For all sequences, the length of the linker region between both motifs was recorded.  
1177 Individual motif binding affinities,  $C_{eff}$  values and E1A global affinity ( $K_{D,E1A}$ ) were  
1178 calculated as explained below (**Source Data File for Figure 5 and Extended Data**  
1179 **Figure 10**).

1180 Calculation of E1A binding affinity: The global binding affinity  $K_{D,E1A}$  (**Extended Data**  
1181 **Figure 10**) was calculated as  $K_{D,E1A} = (K_{D,E2F} * K_{D,LxCxE}) / C_{eff}$ , where  $C_{eff}$  is the  $C_{eff}$  value  
1182 predicted under a naïve or sequence-dependent assumption (see details below) and  
1183  $K_{D,E2F}$  and  $K_{D,LxCxE}$  are the predicted binding affinities of each motif calculated using  
1184 FoldX:

1185 Prediction of Motif binding affinities using FoldX: To estimate the binding affinity of  
1186 individual E2F and LxCxE motifs ( $K_{D,E2F}$  and  $K_{D,LxCxE}$ ) present in each sequence, FoldX  
1187 v5.0 [96] was used to build substitution matrices for all 20 amino acids at each position  
1188 (**Source Data File for Extended Data Figure 10**). Briefly, given a structural complex  
1189 the FoldX algorithm assesses the change in binding free energy produced by mutating  
1190 each position of the motif for each one of the 20 amino acids. For the E2F matrix, the  
1191 structure of the HAdV5 E1A<sub>E2F</sub> motif in complex with Rb (PDB: 2R7G) was used as input.

1192 For the LxCxE matrix, the structure used as input was a model of the HAdV5 E1A<sub>LxCxE</sub>  
 1193 motif in complex with Rb (**Supplementary Data File 3**), built using FlexPepDock [<sup>97</sup>]  
 1194 and the structure of the HPV E7 LxCxE motif bound to Rb (PDB: 1GUX). The total  
 1195 change in binding free energy with respect to the wild type sequence ( $\Delta\Delta G_{\text{FoldX}}$ ) was  
 1196 calculated by adding up the free energy terms for each residue at each matrix position  
 1197 (**Source Data File for Extended Data Figure 10**). The predicted equilibrium  
 1198 dissociation constant of the E2F and LxCxE motifs for each sequence ( $K_{D \text{ SEQ}}$ ) was  
 1199 calculated as:

$$1200 \quad \Delta\Delta G_{\text{FoldX}} = \Delta G_{\text{SEQ}} - \Delta G_{\text{WT}} = RT \ln(K_{D \text{ SEQ}}) - RT \ln(K_{D \text{ WT}}) \quad (5)$$

$$1201 \quad K_{D \text{ SEQ}} = \frac{K_{D \text{ WT}}}{e^{(-\Delta\Delta G_{\text{FoldX}}/RT)}} \quad (6)$$

1202 Where  $\Delta\Delta G_{\text{FoldX}}$  is the total predicted change in binding energy calculated using  
 1203 FoldX,  $RT$  is 0.582 kcal mol<sup>-1</sup>,  $K_{D \text{ WT}}$  is the experimentally measured binding affinity of  
 1204 the sequence (HAdV5 E1A) present in the model structure ( $K_{D,E2F}$  and  $K_{D,LxCxE}$  measured  
 1205 in this work, **Supplementary Data Table 1**).

1206 Prediction of  $C_{\text{eff}}$  values under the naïve WLC model: The  $C_{\text{eff}}$  value was calculated for  
 1207 the collection of 110 natural E1A linkers using Equations (3) and (4) with  $L_p = 3 \text{ \AA}$  ( $L_p$   
 1208 WLC, **Fig. 5a**) and the specific length (number of residues) of each linker, which defines  
 1209  $L_C$ .

1210 Prediction of  $C_{\text{eff}}$  values using a WLC model with sequence-dependent  $L_p$  parameters:

1211 For the subset of 27 natural E1A linkers used in all-atom simulations (**Fig. 4a**) we  
 1212 calculated sequence-specific  $L_p$  values from all atom simulations ( $L_{p\text{Sim}}$ ) in order to  
 1213 represent sequence-dependent changes in chain expansion. The details of these  
 1214 calculations are explained under the Methods section “**Calculation of predicted global**  
 1215 **binding affinity for grafted E1A linkers**”. New  $C_{\text{eff}}$  values were derived using the same

1216 parameters described above, but replacing the standard  $L_p$  value by the  $L_{p\ Sim}$  value. The  
1217  $L_p\ Sim$  values are reported in the **Source Data File for Extended Data Figure 10**.

1218 Statistical analysis. We used bootstrapping [98] to generate 99% confidence  
1219 intervals (CI) for  $K_{D,E2F}$ ,  $K_{D,LxCxE}$  and  $K_{D,E1A}$  average values, and compared the lower and  
1220 upper end points against the value of  $K_{D,E2F2}$  ( $1\ 10^{-9}\ M$ ). The lower bound of the 99% CI  
1221 for  $K_{D,E2F}$  and  $K_{D,LxCxE}$  is higher than  $K_{D,E2F2}$  and the upper bound of the 99% CI for all  
1222  $K_{D,E1A}$  are lower than  $K_{D,E2F2}$ . We also used permutation tests [98] to assess the null  
1223 hypothesis that the  $C_{eff}$ ,  $L_p$  and average  $K_D$  average values did not differ between all  
1224 pairs of groups. In order to control for the false discovery rate, the p-values were  
1225 corrected using the Benjamini-Hochberg [99] correction for multiple comparisons.

1226 Calculations of disorder propensity and conservation: All calculations were  
1227 performed on the dataset from **Supplementary Data File 1**, using the methods  
1228 described in [58]. For disorder propensity we recorded the mean IUPRED value  $\pm$  SD per  
1229 position using IUPRED 2a [100] and for residue conservation we recorded the information  
1230 content (IC) per position.

1231  
1232 **Sequence conservation and Evolutionary Scores**  
1233 We collected 77 mammalian orthologous sequences of the Retinoblastoma protein with no  
1234 unassigned residues within the pocket domain from the Ensembl Database [101].  
1235 Sequences were aligned using MUSCLE v3.8.1551 and manually curated according to  
1236 structural information. The evolutionary conservation scores were calculated with the  
1237 ConSurf 2016 webserver [102] using the E7-Rb complex (PDB: 1GUX) as the structural  
1238 model. The sequence analyses and alignment graphics were performed using Jalview  
1239 v2.11 [103]. The alignment was coloured according to residue identity and conservation  
1240 scores calculated according to [104]. We analyzed the conservation of residues making up

1241 the E2F and LxCxE motif binding sites according to previously reported contacts [40,41].  
1242 Results are presented in **Supplementary Fig. 1**.

1243

### 1244 **Structural Modeling**

1245 The structures of the human Rb (RbAB pocket domain) bound to E1A (PDB: 2R7G) and  
1246 E7 (PDB: 1GUX), and the structure from the human paralogue p107 pocket domain (PDB:  
1247 4YOZ) were collected from the protein data bank. Structural modeling of the human  
1248 paralogue p130, and the retinoblastoma pocket domains from Macaque (*Macaca mulatta*),  
1249 Chimpanzee (*Pan troglodytes*), Dog (*Canis lupus familiaris*), Microbat (*Myotis lucifugus*),  
1250 Sheep (*Ovis aries*), Pig (*Sus scrofa*), Cow (*Bos taurus*), Horse (*Equus caballus*) and Tree  
1251 shrew (*Tupaia belangeri*) were obtained by using AlphaFold v2.0 [105] implemented in  
1252 ColabFold v1.0 [106]. The template multiple sequence alignments were generated using  
1253 MMseqs2 [107] implemented within ColabFold v1.0. Template information and the predicted  
1254 structure relaxation using amber force fields, were included. The distance between the E2F  
1255 and LxCxE binding sites were measured between the alpha carbons of the C-terminal  
1256 anchor site of the E2F cleft and the N-terminal anchor site of the LxCxE cleft. Molecular  
1257 graphics and analyses were performed with UCSF Chimera v1.5 [108]. Results are  
1258 presented in **Extended Data Fig. 9**.

1259

### 1260 **Data availability**

1261 SAXS raw data for Rb, E1A<sub>WT</sub> and the [E1A<sub>WT</sub>:Rb] complex has been deposited in SASDB  
1262 (<https://www.sasbdb.org>) with codes SASDNK6 (Rb 1mg/ml), SASDNL6 (Rb 2mg/ml),  
1263 SASDNM6 (Rb 4mg/ml), SASDNN6 (E1A<sub>WT</sub> 4.2mg/ml), SASDNP6 (E1A<sub>WT</sub> 5.6mg/ml),  
1264 SASDNQ6 (E1A<sub>WT</sub> 7.0mg/ml), SASDNR6 ([E1A<sub>WT</sub>:Rb] 0.7mg/ml), SASDNS6 ([E1A<sub>WT</sub>:Rb]  
1265 1.4mg/ml), SASDNT6 ([E1A<sub>WT</sub>:Rb] 2.7mg/ml), SASDNU6 ([E1A<sub>WT</sub>:Rb] merged data),  
1266 SASDNV6 (E1A<sub>WT</sub>, SEC-SAXS). Refined conformational ensemble models for E1A<sub>WT</sub> and

1267 [E1A<sub>WT</sub>:Rb] have been deposited in the Protein Ensemble Database  
1268 (<https://proteinensemble.org/P03255>) with codes PED00175 (E1A<sub>WT</sub>) and PED00174  
1269 ([E1A<sub>WT</sub>:Rb]). Unfiltered conformational ensembles for the [E1A<sub>WT</sub>:Rb], [E1A<sub>ΔL</sub>:Rb] and  
1270 [E1A<sub>ΔE</sub>:Rb] complexes are available at (<https://moma.laas.fr/data/>) under the description  
1271 “Conformational ensemble models of the IDP E1A bound to Rb protein”. NMR assignments  
1272 of backbone resonances for E1A<sub>WT</sub>, E1A<sub>ΔE</sub> and E1A<sub>ΔL</sub> are provided in Supplementary Data  
1273 File 2. Trajectories for all E1A linker ensembles are provided at: Zenodo  
1274 (<https://zenodo.org/record/6332925>), and trajectory analysis results are provided at:  
1275 <https://github.com/holehouse->  
1276 [lab/supportingdata/tree/master/2021/Gonzalez\\_Foutel\\_2021](https://github.com/holehouse-lab/supportingdata/tree/master/2021/Gonzalez_Foutel_2021). PDB codes used in data  
1277 analysis and prediction are: PDB:1GUX, PDB:3POM, PDB:2R7G, PDB:4YOZ. Raw data  
1278 underlying Main Figures 1-5 and Extended Data Figures 1, 4, 7 and 10 are available as  
1279 Source Data Files.

1280

### 1281 **Code Availability**

1282 The loop sampling method used to model the linker between the two binding motifs can be  
1283 used via a web server (<https://moma.laas.fr/applications/LoopSampler/>), and binaries can  
1284 be provided upon request. All code used to analyze the E1A linker trajectories are provided  
1285 at [https://github.com/holehouse-](https://github.com/holehouse-lab/supportingdata/tree/master/2021/Gonzalez_Foutel_2021)  
1286 [lab/supportingdata/tree/master/2021/Gonzalez\\_Foutel\\_2021](https://github.com/holehouse-lab/supportingdata/tree/master/2021/Gonzalez_Foutel_2021).

1287



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