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1 **Long non-coding RNA *PCAT19* safeguards DNA in quiescent endothelial cells by preventing**  
2 **uncontrolled phosphorylation of replication protein A2**

3

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39 **Summary**

40 In healthy vessels, endothelial cells maintain a stable, differentiated and growth-arrested phenotype  
41 for years. Upon injury, a rapid phenotypic switch facilitates proliferation to restore tissue perfusion.  
42 Here we report the identification of the endothelial cell-enriched long non-coding RNA (lncRNA)  
43 *PCAT19*, which contributes to the proliferative switch and acts as a safeguard for the endothelial  
44 genome. *PCAT19* is enriched in confluent, quiescent endothelial cells and binds to the full replication  
45 protein A (RPA) complex in a DNA damage and cell cycle-related manner. Our results suggest that  
46 *PCAT19* limits the phosphorylation of RPA2, primarily on the serine 33 (S33) residue, and thereby  
47 facilitates an appropriate DNA damage response while slowing cell cycle progression. Reduction in  
48 *PCAT19* levels, either in response to loss of cell contacts or knockdown, promotes endothelial  
49 proliferation and angiogenesis. Collectively, *PCAT19* acts as a dynamic guardian of the endothelial  
50 genome and facilitates rapid switching from quiescence to proliferation.

51

52 **Keywords**

53 Long non-coding RNA, endothelial cells, replication protein A, quiescence, checkpoint control, Ataxia  
54 telangiectasia and Rad3-related



## 55 Introduction

56 Endothelial cells (ECs) form the innermost layer of blood vessels and are indispensable for vascular  
57 patterning and homeostasis. This patterning is required for vascular development and includes  
58 sprouting and branching, with the density of the vascular network being further adjusted by vessel  
59 regression<sup>1, 2</sup>. To maintain a functional monolayer, ECs must switch from a proliferative to quiescent  
60 state while remaining primed for re-entry into the cell cycle<sup>3</sup>. Contact inhibition and quiescence of the  
61 cell cycle is triggered by the contact of cell-to-cell junctions, through VE-cadherin clustering in  
62 particular<sup>4-6</sup>. VE-cadherin is a transmembrane protein linked to p120-catenin and  $\beta$ -catenin which are  
63 retained with VE-cadherin in the cytoplasm under confluent conditions thereby preventing their  
64 transcriptional activity at genes involved in cell cycle progression. VE-cadherin also interacts with  
65 VEGFR2 to prevent its proliferative signalling<sup>4</sup>. Ultimately, multiple signalling pathways converge to  
66 halt the cell cycle in a controlled and coordinated fashion upon endothelial cell monolayer confluence.  
67 Conversely, upon vascular injury or loss of contact inhibition due to vessel outgrowth, the endothelial  
68 cell cycle is rapidly reinstated. In addition to the cell cycle control in response to environmental cues,  
69 extensive intrinsic cell cycle mechanisms have evolved to coordinate, safeguard and potentially correct  
70 the individual steps of the cell cycle<sup>7</sup>.

71

72 A central regulator of the genome maintenance machinery is the ssDNA-binding Replication Protein A  
73 (RPA) complex which acts during the initiation and elongation steps of DNA replication and during DNA  
74 damage<sup>8</sup>. The complex consists of RPA1, RPA2 and RPA3. Of these, RPA2 is the most important with  
75 regards to RPA regulation as it is heavily controlled by post-translational modifications, particularly  
76 phosphorylation<sup>9</sup>. RPA2 is sequentially phosphorylated by three phosphoinositide 3-kinase (PI3K)-like  
77 protein kinases (ATR, ATM and DNA-PK) in response to varying degrees of DNA damage.  
78 Phosphorylation of the serine 33 (S33) residue by ATR occurs during S-phase in response to replicative  
79 stress while signalling the progression of cell cycle<sup>10, 11</sup>. If DNA damage is extensive, subsequent  
80 hyperphosphorylation of RPA2 is mediated by ATM and DNA-PK, particularly at the S4/8 residue<sup>12</sup>. This  
81 triggers the cell cycle checkpoints and the DNA damage response. Following S33 phosphorylation by  
82 ATR, RPA2 can also be phosphorylated at its two cyclin-CDK sites by cyclin B-Cdk1 during mitosis<sup>13</sup> and  
83 by cyclin A-Cdk2 at the G1/S boundary<sup>14</sup>.

84

85 RPA is involved in multiple DNA repair pathways such as nucleotide excision repair (NER), base excision  
86 repair (BER), mismatch repair (MMR) and homologous recombination (HR). Mutations in RPA are  
87 known to cause DNA damage accumulation due to faulty G1, S and G2/M checkpoint signalling which  
88 is in part a consequence of insufficient loading of the ATR kinase onto DNA<sup>9</sup>. ATR is normally activated

89 on RPA-coated ssDNA to activate proteins such as Chk1, p53 and downstream cyclins to trigger cell  
90 cycle arrest and promote DNA repair. As such, problems with RPA activation and loading onto ssDNA  
91 disrupt ATR signalling and predispose the cell to faulty checkpoint signalling and genome instability.  
92 Importantly, hyperphosphorylation of free RPA2 not bound to DNA hinders its subsequent loading  
93 onto DNA and thereby reduces the effectiveness of the DNA damage response<sup>15, 13</sup>. While the main  
94 proteins involved in this fundamental pathway have been characterised, a growing body of evidence  
95 suggests that RNAs, and in particular long non-coding RNAs (lncRNA), act on the cell cycle and  
96 contribute to cellular proliferation, the DNA damage response and the maintenance of DNA integrity<sup>16-</sup>  
97 <sup>18</sup>.

98  
99 lncRNAs are RNAs longer than 200 nucleotides that do not have an apparent protein coding potential<sup>19</sup>.  
100 They are now believed to contribute to numerous cellular processes both within and outside the  
101 nucleus. In the nucleus, lncRNAs can control processes such as transcription, chromatin organisation  
102 and the maintenance of genome integrity<sup>20</sup>. With respect to the RPA complex, a recent study identified  
103 the lncRNA *Discn* as being crucial for the regulation of RPA availability in stem cells<sup>21</sup>. *Discn* is induced  
104 under genotoxic stress to prevent the nuclear translocation of nucleolin, a protein that sequesters RPA,  
105 thereby preventing RPA exhaustion. The lncRNA *TERRA* (Telomeric-repeat-containing RNA) prevents  
106 the displacement of RPA from telomeric ssDNA during the early to mid S phase by sequestering  
107 heterogenous nuclear ribonucleoproteins (hnRNPs)<sup>22</sup>. When *TERRA* expression declines towards the  
108 end of S phase, hnRNPs displace RPA from ssDNA to reduce ATR activation and allow ssDNA coating by  
109 POT1 (protection of telomeres 1) until the next round of DNA replication. This highlights a tightly  
110 controlled cell cycle-dependent function of RPA that is mediated through the expression of a single  
111 lncRNA. Given the great importance of the cell cycle and DNA damage response and considering that  
112 the human genome codes for more than 30,000 lncRNAs, it is evident that the lncRNAs characterised  
113 so far only represent the tip of the iceberg.

114  
115 Here we set out to uncover endothelial-enriched lncRNAs that play a role in cell cycle regulation and  
116 angiogenesis and which therefore might offer a therapeutic target in vascular disease. This led to the  
117 identification of the lncRNA Prostate Cancer Associated Transcript 19 (*PCAT19*), which is highly  
118 enriched in the confluent endothelium. Our study revealed that *PCAT19* is induced by endothelial  
119 quiescence to protect RPA2 from uncontrolled phosphorylation, primarily on its S33 residue. This  
120 permits the proper and timely loading of RPA2 onto DNA and results in a safeguarding function by  
121 *PCAT19* that maintains the human endothelial cell resting state.

## 122 Results

### 123 *PCAT19 is highly enriched in endothelial cells and is differentially expressed in vascular diseases*

124 When screening for endothelial lncRNAs in the FANTOM5 CAGE (Cap Analysis of Gene Expression)  
125 database<sup>23</sup>, we identified *PCAT19* as one of the most highly expressed lncRNAs in endothelial cells,  
126 with limited expression in other cell types (**Figure 1A**). Interestingly, *PCAT19* is listed in PanglaoDB as  
127 an endothelial marker<sup>24</sup>. Owing to its high endothelial expression, *PCAT19* is expressed in all human  
128 tissues listed in the GTEx database<sup>25</sup> (GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2)).  
129 Tissues such as lung and spleen with a relatively dense vasculature, and therefore more endothelial  
130 cells, have the highest *PCAT19* expression compared to other tissues (**Figure 1B**). This tissue expression  
131 pattern was similar for other highly endothelial-enriched endothelial genes such as *CDH5* and *PECAM1*  
132 (**Figure S1A**). Given the initial identification of *PCAT19* in prostate tissue<sup>26</sup> and the elucidation of its  
133 role in prostate cancer<sup>27</sup>, we analysed the expression of *PCAT19* in the prostate gland in more detail  
134 by interrogating publicly available data from a prostate single-cell RNA-seq experiment (GSE172357)<sup>28</sup>.  
135 In this unbiased dataset, *PCAT19* was highly enriched in the endothelial cell cluster with limited  
136 expression in other cell types (**Figure 1C and D**). The remarkable endothelial enrichment of *PCAT19*  
137 can also be observed in the Tabula Sapiens dataset<sup>29</sup>. We show the expression of *PCAT19*, *CDH5* and  
138 *PECAM1* across human cell types where all three genes are enriched in the endothelial cell cluster  
139 (**Figure S1B**). When looking at *PCAT19*, *CDH5* and *PECAM1* expression in endothelial cells only and  
140 clustering by tissue, there is a clear widespread expression of each gene across endothelial cells (**Figure**  
141 **S1C**).

142  
143 We next searched for fluctuations in *PCAT19* expression as a possible indicator of its involvement in  
144 vascular disease by analysing relevant RNA-seq datasets. Diseases of the vasculature often result in, or  
145 are caused by, differential rates of endothelial proliferation. *PCAT19* was significantly lower expressed  
146 in haemangioma<sup>30</sup>, a malformation of blood vessels largely characterised by increased endothelial cell  
147 proliferation<sup>31</sup> (**Figure 1E**). Additionally, in advanced carotid artery disease (characterised by plaque  
148 accumulation), *PCAT19* expression was significantly higher than in healthy or early disease samples  
149 (**Figure 1F**) but was unchanged between stable and unstable plaques from the advanced carotid artery  
150 samples (**Figure 1G**). Due to the previous description of *PCAT19* in cancer, we checked whether the  
151 expression of *PCAT19* differed between healthy and cancerous tissues in the GEPIA database<sup>32</sup> which  
152 returned a differential expression in most of the listed cancers, the majority of which displayed a  
153 downregulation of *PCAT19* in cancerous tissue compared to the respective healthy tissue (**Figure S1D**).  
154 This was most obvious in lung cancer samples (lung adenocarcinoma (LUAD) and lung squamous cell  
155 carcinoma (LUSC)) and highly intriguing for us since we identified lung tissue as having the highest  
156 *PCAT19* expression in the GTEx data. We also checked whether *PCAT19* expression differed specifically

157 in the endothelial cells that formed a cancer compared to healthy endothelial cells. From publicly  
158 available lung single-cell RNA-seq data<sup>33</sup> we observed that *PCAT19* was indeed expressed in fewer  
159 cancerous endothelial cells (squamous cell carcinoma (SCC) and large cell carcinoma (LCC)) compared  
160 to normal endothelial cells (**Figure 1H**). These data not only demonstrate a strong enrichment of  
161 *PCAT19* in endothelial cells but also its differential expression in vascular diseases and cancerous  
162 endothelial cells. This raises the question of what the functional significance of *PCAT19* is in endothelial  
163 cells.

#### 164 *PCAT19* represses proliferation, sprouting and vascularisation

165 Due to the enrichment of *PCAT19* in endothelial cells and its previously reported link to prostate  
166 cancer<sup>27</sup>, we wondered whether the perturbation of *PCAT19* would impact endothelial cell cycle or  
167 growth. As determined by EdU incorporation, the knockdown of *PCAT19* with LNA-GapmeRs increased  
168 the rate of endothelial cell proliferation; 6 hours after EdU application, three times as many cells had  
169 incorporated EdU after *PCAT19* knockdown compared to control cells (**Figure 2A**). Conversely, *PCAT19*  
170 overexpression by electroporation reduced endothelial cell proliferative capacity (**Figure 2B**). The  
171 *PCAT19* knockdown and overexpression efficiencies are provided in **Figure S1E**. *PCAT19* knockdown  
172 using LNA-GapmeRs was also performed in other endothelial cell types: human microvascular  
173 endothelial cells (HMEC), human carotid artery endothelial cells (HCAEC), human aortic endothelial  
174 cells (HAoEC) and human dermal lymphatic endothelial cells (HDLEC). Knockdown significantly  
175 promoted proliferation in HMEC and HAoEC but not in HCAEC or HDLEC, the latter of which did not  
176 proliferate well in general (**Figure S2A**). The effect of *PCAT19* perturbation on endothelial proliferation  
177 could also be confirmed using a CRISPRi and CRISPRa approach. *PCAT19* CRISPRi was able to promote  
178 endothelial proliferation while *PCAT19* CRISPRa had the opposite effect in attenuating proliferation  
179 (**Figure S2B**). To further measure the relevance of *PCAT19* in endothelial growth and its potential  
180 impact on angiogenic sprouting, a three-dimensional endothelial spheroid outgrowth assay was  
181 performed. The knockdown of *PCAT19* promoted sprouting under both basal and VEGF-A-stimulated  
182 conditions (**Figure 2C**) while the overexpression of *PCAT19* attenuated sprouting under basal  
183 conditions (**Figure 2D**). Since *PCAT19* knockdown enhanced both endothelial proliferative and  
184 sprouting capacity, we hypothesised that reduction of *PCAT19* levels may promote vascularisation.  
185 This was studied in a three-dimensional organoid system, which involved the differentiation of induced  
186 pluripotent stem cells (iPSCs) into cardiomyocytes and endothelial cells to form functioning cardiac  
187 organoids. In this system, endothelial cells sprout and form contacts with neighbouring endothelial  
188 sprouts, eventually forming a vascular network with some vessels even containing a lumen<sup>34, 35</sup>. All  
189 cardiac organoids formed a vascular network but those subsequently transfected with *PCAT19* LNA-  
190 GapmeRs produced a denser network, as measured by the cumulative vascular network length (**Figure**

191 **2E)**. As *PCAT19* knockdown promoted cell cycle progression and proliferation we wondered whether  
192 the expression of *PCAT19* itself is dependent on the cell proliferative state. Strikingly, *PCAT19*  
193 expression was strongly induced with cell density (as cells become more confluent and cell cycle-  
194 arrested) (**Figure 2F**). These data demonstrate that *PCAT19* acts as an anti-proliferative and anti-  
195 angiogenic lncRNA that is induced during contact-mediated inhibition of the endothelial cell cycle.

196

197 To gain a deeper insight into how *PCAT19* may enact these anti-proliferative and anti-angiogenic  
198 effects, RNA-seq was performed after *PCAT19* knockdown. *PCAT19* itself was significantly lower  
199 expressed, confirming a successful knockdown (**Figure 2G and H, Table S1**). After filtering for  
200 differentially regulated genes (P-adjusted value <0.05), 186 genes were analysed for KEGG (Kyoto  
201 Encyclopedia of Genes and Genomes) pathways. The top significant terms (P-adjusted value <0.05)  
202 were “Cell cycle” and “Cellular senescence” (**Figure 2I**), followed by “Progesterone-mediated oocyte  
203 maturation” and “Human T-cell leukemia virus 1 infection” (**Figure S2C and S2D**). Some of the next  
204 terms such as “MAPK signaling pathway”, “transcriptional misregulation in cancer” and “p53 signaling  
205 pathway” are interesting and relevant but were not significantly enriched with a P-adjusted value  
206 <0.05. We therefore decided to focus on the top term “Cell cycle”. The same group of 186 differentially  
207 regulated genes was used to identify their potential upstream regulators using the QuaternaryProd  
208 package. The top 10 predicted regulators were mapped according to their number of significant  
209 downstream targets and whether the regulators themselves were up- or down-regulated (**Figure 2J**  
210 **and S2E**). Most of these such as *CCNB1*, *E2F3*, *PLK1* and *CDK1* are strongly involved in cell cycle and  
211 senescence<sup>36</sup>, confirming that *PCAT19* indeed has a profound impact on cell cycle. Since *PCAT19* seems  
212 to be important for endothelial proliferation and angiogenic sprouting, coupled with the associated  
213 expression changes of cell cycle genes upon *PCAT19* knockdown, we chose to further investigate the  
214 role of *PCAT19* in cell cycle regulation given the indication from the RNA-seq experiment that “Cell  
215 cycle” is impacted to some degree by *PCAT19* knockdown.

#### 216 *PCAT19 binds the DNA replication protein A complex*

217 The biological effects of lncRNAs are often mediated through their interaction with other RNAs, DNA  
218 or proteins. Since *PCAT19* had a profound effect on the cell cycle, we wondered whether this resulted  
219 from a potential interaction of *PCAT19* with cell cycle-related proteins. We first determined the  
220 subcellular localisation of *PCAT19* using RNA fluorescent *in situ* hybridisation (FISH) and noticed a large  
221 fraction of *PCAT19* localised to the nucleus (**Figure S3A**) which would at least place it within close  
222 proximity to cell cycle proteins. Cytoplasmic and nuclear fractionation of endothelial cells revealed an  
223 equal distribution of *PCAT19* between the cytoplasm and nucleus under subconfluent conditions  
224 (**Figure S3B**). Surprisingly, there was significantly more *PCAT19* localised to the nucleus compared to

225 the cytoplasm under confluent conditions, again highlighting the potential importance of *PCAT19* in  
226 the nucleus. To determine whether *PCAT19* indeed interacts with proteins, the endogenously  
227 expressed *PCAT19* was pulled down using biotinylated antisense oligonucleotides (AS-oligos)  
228 containing a *PCAT19*-specific targeting sequence. Mass spectrometry identified eight significantly  
229 enriched ( $P < 0.05$ ) proteins in the *PCAT19* pulldown versus scramble control pulldown (**Figure 3A and**  
230 **3B, Table S2**). Using the  $\log_{10}(\text{iBAQ})$  value, the most abundant of the eight *PCAT19*-enriched proteins  
231 were RPA1, RPA2 and RPA3 – the three members of the replication protein A (RPA) complex. DNA  
232 ligase 3 (LIG3) and its known interaction partner X-Ray Repair Cross Complementing 1 (XRCC1) were  
233 also enriched with *PCAT19*. In addition, Ubiquitin Like With PHD And Ring Finger Domains 1 (UHRF1)  
234 and UHRF2 as well as Polynucleotide Kinase 3'-Phosphatase (PNKP) were identified as *PCAT19*  
235 interaction partners. Each of these proteins is involved in the DNA damage response, DNA replication  
236 or cell cycle<sup>37-40</sup>. Given that these proteins interact with DNA and, in some cases, with one another  
237 (**Figure 3C**), the primary *PCAT19* interactor could not be inferred from this experiment alone. The  
238 proteins of the RPA complex (RPA1, 2 and 3) were the most abundant of the enriched interactors. Of  
239 these, RPA2 can be considered the central target of regulatory pathways, as it is subject to extensive  
240 regulation through dynamic and sequential context-dependent phosphorylation on several sites<sup>9</sup>. A  
241 potential interaction between RPA2 and *PCAT19* was therefore investigated in more detail. The  
242 *PCAT19*-RPA2 interaction was confirmed with AS-oligo pulldown from endothelial cell lysates and  
243 Western blotting (**Figure 3D**). The interaction was further confirmed with an immunoprecipitation of  
244 RPA2 followed by RNA isolation and RT-qPCR (RIP-qPCR) for *PCAT19* (**Figure 3E**). 18S rRNA and U4  
245 snRNA were not enriched with RPA2, as expected. To exclude that these findings were a consequence  
246 of an indirect interaction through other proteins tightly bound to RPA2, a fully *in vitro* approach was  
247 used with purified His-tagged RPA2 incubated with or without *in vitro*-transcribed biotinylated *PCAT19*.  
248 Pulldown of biotinylated *PCAT19* recovered His-tagged RPA2, demonstrating that the interaction  
249 between the two molecules is indeed direct (**Figure 3F**). The central role of RPA2 in DNA repair and  
250 synthesis processes and the role of *PCAT19* in limiting cellular proliferation may suggest that *PCAT19*  
251 mediates its effects through RPA2 in a cell cycle- or DNA damage-dependent manner.

#### 252 *Loss of PCAT19 predisposes and sensitises DNA to damage*

253 Given that the RPA complex and the other *PCAT19*-interacting proteins are involved in the DNA  
254 damage and repair response, the potential contribution of *PCAT19* to this process was determined.  
255 Interestingly, after *PCAT19* knockdown, cells displayed a positive Terminal deoxynucleotidyl  
256 transferase dUTP nick end labeling (TUNEL) signal indicative of DNA double-strand breaks which could  
257 not be detected in the cells transfected with control LNA-GapmeR (**Figure 4A**). Upon treatment with  
258 camptothecin (CPT), an inducer of DNA double-strand breaks, cells displayed a noticeable increase in

259 TUNEL signal and this was exacerbated by LNA directed against *PCAT19*. This suggests that the loss of  
260 *PCAT19* may lead to an accumulation of DNA damage. A comet assay confirmed these findings as  
261 *PCAT19* knockdown resulted in a significantly longer tail olive moment compared to cells transfected  
262 with control LNA (**Figure 4B**). In line with this, knockdown of *PCAT19* enhanced the DNA damage-  
263 induced accumulation of p53 and  $\gamma$ H2AX (**Figure 4C and 4D**), whereas *PCAT19* overexpression had the  
264 opposite effect (**Figure 4E and 4F**). We also observed the LNA GapmeR-mediated knockdown of  
265 *PCAT19* to increase p53 levels in HCAEC, HAoEC, HMEC and HDLEC (**Figure S3C**). Again this was more  
266 pronounced after treatment with CPT. The same was true for *PCAT19* knockdown on S33-pRPA2 levels  
267 in these other endothelial cell types (**Figure S3D**). The effects on p53 levels in the presence and absence  
268 of *PCAT19* could also be confirmed with the CRISPRi and CRISPRa approach. *PCAT19* CRISPRi was able  
269 to increase p53 levels after CPT stimulation (**Figure 4G**), while CRISPRa reduced p53 levels after CPT  
270 stimulation (**Figure 4H**). We next performed an RNA in situ hybridisation followed by a proximity  
271 ligation assay (rISH-PLA) to determine whether *PCAT19* co-localised with  $\gamma$ H2AX and where in the cell  
272 this co-localisation occurs. The biotin-tagged antisense-oligonucleotides specific to *PCAT19*, an  
273 antibody against biotin and an antibody against  $\gamma$ H2AX was used. A conventional PLA was performed  
274 with secondary antibodies against the primary biotin and  $\gamma$ H2AX antibodies. *PCAT19* and  $\gamma$ H2AX indeed  
275 co-localised in nuclear foci as visualised by positive PLA signals and the numbers of interaction sites  
276 significantly increased after treatment with CPT (**Figure 4I and S3E**). RPA is central in DNA synthesis  
277 and homologous recombination where it binds single-stranded DNA (ssDNA) and prevents the  
278 formation of secondary DNA structures that could impede DNA replication or repair<sup>38</sup>. To determine  
279 whether *PCAT19*, via its interaction with RPA2, had an impact on DNA replication, a DNA fibre assay  
280 was performed. Despite reducing the rate of cell proliferation, *PCAT19* overexpression had no effect  
281 on DNA replication speed, as indicated by similar DNA tract lengths between *PCAT19* and pcDNA3.1+  
282 control overexpressed cells (**Figure S2F**).

283

284 Given the increased rate of endothelial cell proliferation and accumulation of DNA damage after  
285 *PCAT19* knockdown we wondered whether cell cycle transitions themselves were affected by *PCAT19*  
286 knockdown. A BrdU incorporation and propidium iodide staining followed by FACS analysis was  
287 performed after *PCAT19* knockdown but these cells did not display a difference in cell cycle phase  
288 profiles (**Figure 4J**). However, treatment with hydroxyurea (HU), which causes replication stress, led to  
289 a significantly greater accumulation of cells in early S phase (S1) and significantly fewer cells in the  
290 G2/M phase after *PCAT19* knockdown compared to control cells (**Figure 4J**). Given that there was no  
291 difference between control and *PCAT19* knockdown cells in mid and late S phase, the increased  
292 accumulation of *PCAT19* knockdown cells in early S phase presumably arises from the G2/M  
293 population. This highlights the faster transitioning through cell cycle after *PCAT19* knockdown, and in

294 this case from G2/M back to G1, and thereby a sensitisation of *PCAT19* knockdown cells to DNA  
295 damage.

#### 296 *PCAT19 protects RPA2 from uncontrolled phosphorylation*

297 The phosphorylation of RPA2 on its serine 33 (S33) residue is a tightly controlled process mediated by  
298 the ATR kinase that precedes cell cycle transition from S phase into G2 phase<sup>9</sup> (**Figure 5A**). S33-pRPA2  
299 is required for the efficient repair of ssDNA that may have been produced from damaged DNA during  
300 replication<sup>41</sup>. As RPA2 phosphorylation in endothelial cells has not been studied, S33-pRPA2 levels  
301 were compared between proliferating subconfluent and non-proliferating confluent HUVEC.  
302 Proliferating cells exhibited higher S33-pRPA2 levels than growth-arrested cells and, as expected, ATRi  
303 massively reduced S33-pRPA2 levels in both conditions (**Figure 5B**). Surprisingly, *PCAT19* knockdown  
304 cells exhibited significantly elevated S33-pRPA2 levels (**Figure 5C**) while the overexpression of *PCAT19*  
305 reduced S33-pRPA2 levels (**Figure 5D**). In all knockdown and overexpression conditions, additional ATR  
306 inhibition markedly reduced S33-pRPA2 levels, as expected (**Figure 5B to D**). To determine whether  
307 S33 phosphorylation impacts the interaction between RPA2 and *PCAT19*, a semi-*in vitro* binding assay  
308 was performed with recombinant His-RPA2 and *in vitro*-transcribed *PCAT19* in the presence of ssDNA.  
309 HUVEC lysate was added to the mixture to permit RPA2 phosphorylation by kinases. RPA2 and *PCAT19*  
310 were again found to strongly interact and, unexpectedly, this interaction could be blocked by ATR  
311 inhibitor or phosphatase treatment (**Figure 5E**). Since the phosphorylation of S4/8-RPA2 occurs after  
312 the phosphorylation of S33-RPA2 we tested whether S4/8-pRPA2 levels would be altered in the  
313 presence of *PCAT19*. Another semi-*in vitro* assay was performed, this time in HEK293 lysate, where  
314 Flag-ATR and His-RPA2 were added with or without *in vitro*-transcribed *PCAT19*. With the addition of  
315 Flag-ATR to the lysate containing His-RPA2, more S4/8-pRPA2 was formed (since ssDNA was also  
316 present in the mixture to promote RPA2 loading and phosphorylation). Importantly, both the His-  
317 tagged RPA2 and endogenous RPA2 were phosphorylated on S4/8. As expected, *PCAT19* was able to  
318 strongly attenuate both the endogenous and His-tagged RPA2 S4/8 phosphorylation levels (**Figure 5F**).  
319 These results suggest that *PCAT19* can bind to and modulate S33-pRPA2 and prevent the sequential  
320 hyperphosphorylation of RPA2, as measured by lower S4/8-pRPA2 levels. This particular assay was also  
321 performed in HEK293T lysate, where *PCAT19* should not be present, to confirm the molecular action  
322 of *PCAT19* on RPA2. Since *PCAT19* seems to have an effect primarily on ATR-dependent RPA2 S33  
323 phosphorylation, we wondered whether *PCAT19* mediates the interaction between ATR and RPA2.  
324 Indeed, *PCAT19* overexpression attenuated the interaction between RPA2 and ATR, as determined by  
325 proximity ligation assay (PLA) (**Figure 5G**). Additionally, the ATRi was able to reverse *PCAT19*  
326 knockdown-induced proliferation (**Figure 5H**) and angiogenic sprouting (**Figure 5I**), indicating that this  
327 growth phenotype after *PCAT19* knockdown is related to elevated p-RPA2. Taken together, these



328 results demonstrate a regulatory role for *PCAT19* in endothelial S33-RPA2 phosphorylation which  
329 ultimately controls the state of downstream sequential RPA2 hyperphosphorylation. *PCAT19* reduces  
330 the degree of RPA2-ATR interaction and the levels of phosphorylation of the ATR target, S33-RPA2.  
331 S33-pRPA2 is required for proliferation; thus, the hyperproliferation resulting from *PCAT19* knockdown  
332 is a consequence of increased ATR-dependent S33-pRPA2 phosphorylation. Importantly, premature  
333 hyperphosphorylation of RPA2 which is not bound to DNA prevents its subsequent loading onto DNA  
334 and thereby an inability to efficiently repair DNA damage<sup>13, 15</sup>. Therefore, depletion of *PCAT19*  
335 promotes the uncontrolled hyperphosphorylation of RPA2 rendering it unable to repair DNA damage  
336 or signal for cell cycle arrest; this leads to the observed phenotype of endothelial hyperproliferation  
337 and DNA damage accumulation.

338 **Discussion**

339 We have identified *PCAT19* as a highly enriched endothelial lncRNA that is induced by quiescence to  
340 fine-tune and protect RPA2 from excessive phosphorylation. In doing so, *PCAT19* aids in the slowing of  
341 the cell cycle and inhibition of angiogenic sprouting while safeguarding the DNA of endothelial cells  
342 during the proliferation-quiescence switch. When *PCAT19* is knocked down, RPA2 can be prematurely  
343 and excessively phosphorylated, which impacts its cell cycle and DNA damage condition-dependent  
344 functionality. This ultimately results in cell cycle promotion and hyperproliferation with an overall  
345 reduced DNA stability.

346  
347 More than 100 lncRNAs were originally identified as being strongly associated with prostate cancer  
348 and subsequently termed the prostate cancer associated transcripts (PCATs)<sup>26, 42</sup>. A handful of studies  
349 have characterised some of the PCATs in more detail, one of which reported the importance of *PCAT19*  
350 in the development of cancer<sup>27, 43</sup> but did not address its physiological function in health. We were  
351 surprised to find that such a prominent cancer-related lncRNA was so highly enriched in healthy  
352 endothelial cells. The strong induction of *PCAT19* with endothelial contact inhibition of the cell cycle,  
353 taken together with its previously described roles in cancer, suggested that *PCAT19* could maintain  
354 certain aspects of endothelial quiescence. This quiescent state is particularly important for long-lived  
355 endothelial cells to maintain the functioning inner monolayer of blood vessels<sup>44</sup>. Our data indicate that  
356 *PCAT19* facilitates DNA integrity and repair which is required for long-lived, non-dividing cells. On the  
357 other hand, endothelial cells require the ability to rapidly re-enter the cell cycle and proliferate under  
358 conditions that either damage the blood vessels or promote angiogenesis. This behavior of the  
359 endothelium is a somewhat unique cellular feature. For example, in epithelial cells, healing is facilitated  
360 by increased proliferation of progenitor cells while mesenchymal cellular activation results in an  
361 expansion of an undifferentiated cell pool (like fibroblasts) rather than a transient activation. Thus,  
362 *PCAT19*, which is differentially expressed between single and confluent cells may therefore have  
363 specifically evolved to address the conflicting needs of rapidly proliferating and long-lived endothelial  
364 phenotypes. This not only explains its endothelial-specific expression but may also help to explain why  
365 *PCAT19* is a human-specific lncRNA. Humans have a relatively long lifespan compared to other  
366 mammals and therefore have a need to balance cell proliferation, repair and maintenance of DNA  
367 integrity. Indeed, genomic instability is one of the main causative factors of vascular aging, which itself  
368 is a risk factor for cardiovascular disease.

369  
370 While the study of Hua *et al.*<sup>27</sup> highlighted a SNP risk region within the *PCAT19* locus that ultimately  
371 mediates prostate cancer progression, we have identified a specific role for *PCAT19* in the quiescent-  
372 proliferative switch of human endothelial cells. Hua *et al.* demonstrated a reduced proliferation of

373 cancer cells after *PCAT19* knockdown, while we observed an increased proliferation of endothelial cells  
374 accompanied by the accumulation of spontaneous DNA damage. It is becoming abundantly clear that  
375 lncRNAs have evolved cell type-specific functions and mechanisms of action; this includes certain  
376 lncRNAs that are highly and ubiquitously expressed. The lncRNA *H19*, for example, interacts with HuR  
377 in epithelial cells to regulate barrier function<sup>45</sup>; with methyl-CpG-binding domain protein 1 (MBD1) in  
378 mouse embryonic fibroblasts to mediate embryonic growth<sup>46</sup>; and with p53 to inhibit apoptosis in  
379 gastric cancer cells<sup>47</sup>. The exact regulatory mechanisms between lncRNAs and their protein interaction  
380 partners in different cell types are not completely understood. This is likely due to a complex interplay  
381 between cell type-specific transcription factors, the expression of lncRNAs themselves and their  
382 downstream molecular targets. We uncovered the RPA complex as the strongest *PCAT19* interactor in  
383 endothelial cells, again pointing towards a fundamental role in cell cycle regulation, specifically in DNA  
384 stability and cell cycle checkpoints. This goes hand-in-hand with our RNA-seq of HUVEC that returned  
385 “Cell cycle” as the top term after *PCAT19* knockdown; this supports the finding that *PCAT19* is  
386 upregulated with cell cycle arrest, and its removal promotes cell cycle re-entry.

387

388 Once bound to ssDNA, RPA acts as a platform to recruit and regulate multiple other protein factors  
389 essential for DNA stability and maintenance. Since the genome is constantly exposed to different  
390 sources of DNA damage, the coordination of cell cycle and DNA damage response proteins is of  
391 paramount importance for an appropriate and measured response. Faulty checkpoint activation can  
392 result in uncontrolled growth and irreparable DNA damage which often triggers cell apoptosis.  
393 However, if the damage occurs within oncogenes, tumour-suppressor genes, or genes that control the  
394 cell cycle then cancer can develop<sup>48</sup>. Knockdown of *PCAT19* promoted proliferation and angiogenic  
395 sprouting and this was accompanied by a heightened sensitivity to DNA damaging agents such as CPT  
396 and HU. Owing to the strong binding of *PCAT19* to the RPA complex we hypothesised that the role of  
397 *PCAT19* in endothelial quiescence and apparent safeguarding of the genome could be mediated  
398 directly through its interaction with RPA2.

399

400 RPA2 is phosphorylated on the S33 residue at the beginning of S phase and is then dephosphorylated  
401 upon the successful completion of mitosis. RPA2 can also undergo sequential hyperphosphorylation  
402 by three phosphoinositide 3-kinase (PI3K)-like protein kinases (ATR, ATM and DNA-PK) depending on  
403 the type and level of DNA damage. For example, the resection of DNA double-strand breaks promotes  
404 the phosphorylation of RPA2 by ATR on the S33 residue; this then permits the subsequent  
405 phosphorylation at S4/8 by DNA-PKs. If functional ATR is missing, ssDNA accumulates from DNA  
406 resection and leads to the exhaustion of RPA pools. As such, ATR phosphorylation of RPA2 S33 aids in  
407 the prevention of ssDNA accumulation<sup>9</sup>. If hyperphosphorylation of DNA-bound RPA occurs, a

408 signalling cascade is activated that ultimately leads to cell cycle arrest and activation of the DNA  
409 damage response. However, it is important to note that if premature hyperphosphorylation of RPA2  
410 occurs, the RPA complex does not bind as efficiently to DNA and therefore damage can accumulate<sup>13</sup>,  
411 <sup>15</sup>. This offers a potential explanation for the increased levels of DNA damage following *PCAT19*  
412 knockdown: depletion of *PCAT19* promotes the uncontrolled hyperphosphorylation of RPA2 which  
413 could prevent its efficient binding to DNA in the repair response and a potential lack of cell cycle arrest  
414 signals. This may then lead to cell cycle progression and the accumulation of DNA damage.

415

416 It has also already been shown that the phosphorylation of RPA2 is a dynamic process rather than a  
417 simple “on-off” phosphorylative switch. Lee and colleagues showed that RPA2 must undergo  
418 phosphorylation followed by rapid de-phosphorylation by human protein phosphatase 4 (PP4) for the  
419 successful repair of DSBs<sup>49</sup>. Depletion of PPR4 leads to an extended G2-M checkpoint and the  
420 accumulation of DNA damage. This lends support to the hypothesis that *PCAT19* could also function as  
421 a mediator that fine-tunes RPA2 phosphorylation. *PCAT19* knockdown heightened the levels of DNA  
422 damage and promoted S33 phosphorylation by increasing the interaction between RPA2 and ATR, the  
423 kinase responsible for S33 phosphorylation. These results suggest that *PCAT19* binds RPA2 and  
424 protects it from excessive S33 phosphorylation by ATR in a cell cycle-dependent manner. We  
425 confirmed that subconfluent proliferating endothelial cells have heightened levels of S33-pRPA2, as  
426 would be expected in cycling cells. However, when *PCAT19* is knocked down, S33-pRPA2 levels increase  
427 further to maintain genome stability during a faster cell cycle progression as seen with the  
428 hyperproliferative response. This excessive S33 phosphorylation could equally disable RPA2 and  
429 negatively impact on DNA damage responses. Interestingly, our *in vitro* binding experiments revealed  
430 that addition of an ATR inhibitor or a phosphatase could abolish the *PCAT19*-RPA2 interaction. This  
431 suggests a model in which *PCAT19* may bind RPA2 to fine-tune the levels of S33 phosphorylation in the  
432 presence of phosphorylated and active ATR. S33 phosphorylation is a pre-cursor to S4/8  
433 phosphorylation, the hallmark of RPA2 phosphorylation, which should therefore be dependent on  
434 *PCAT19*-S33-pRPA2 modulation. Indeed, S4/8-pRPA2 levels were markedly reduced in the presence of  
435 *PCAT19*. Importantly, this semi-*in vitro* assay for S4/8-pRPA2 was performed using HEK293 lysate,  
436 indicating that the molecular mechanism would be ubiquitous. However, the endothelial-enrichment  
437 of *PCAT19* ensures that this particular regulation of RPA2 is restricted to endothelial cells. Of course,  
438 inhibition or removal of ATR prevents S33 phosphorylation and so *PCAT19* may be removed from RPA2  
439 to permit S33 phosphorylation and avoid faulty DNA damage repair responses. Importantly, ATR  
440 inhibition was able to rescue the *PCAT19* knockdown-induced increase in proliferation and angiogenic  
441 sprouting, supporting the idea that the heightened S33-pRPA2 levels permit cell cycle.

442

443 Under conditions that damage the vessel or promote new vessel growth, endothelial cells re-enter the  
444 cell cycle to reach confluence again. This quiescent-proliferative switch is central in many vascular  
445 diseases. For example, infantile haemangioma which is the most common type of tumour in infants,  
446 results from increased proliferation of endothelial cells and pericytes<sup>31</sup>. It was therefore interesting to  
447 find a significant and marked reduction in *PCAT19* expression in haemangioma samples. The opposite  
448 endothelial proliferative scenario is often observed in carotid artery restenosis and atherosclerosis<sup>50</sup>,  
449 <sup>51</sup>, characterised by damage to the endothelium, reduced proliferation and formation of a neointima  
450 which is essentially scar tissue on the inner blood vessel. In this scenario, *PCAT19* is significantly higher  
451 expressed; this again correlates with the endothelial proliferative rate. At first glance, the concept that  
452 endothelial cells favour rapid proliferation over tight control of DNA integrity is surprising. It is,  
453 however, important to mention that endothelial proliferation due to faulty contact inhibition is a  
454 somewhat rare event in mature vessels and only occurs at sites of vessel damage.

455

456 *PCAT19* could play a role in many vascular diseases that depend on the proliferation of endothelial  
457 cells, as well as in tumour angiogenesis which is crucial in supporting cancer growth. It is therefore  
458 tempting to speculate that by targetting *PCAT19* and thereby impacting the fine-tuning of the S33-  
459 pRPA2 switch, the endothelial quiescence-proliferation transition could be controlled to positively  
460 alter the outcome of vascular disease. In conclusion, with the present work, we identified the  
461 endothelial-enriched lncRNA *PCAT19* which safeguards the endothelial genome by interacting with and  
462 modulating RPA2. Upon loss of contact inhibition, for example during vascular injury, *PCAT19*  
463 expression decreases and thereby facilitates rapid endothelial monolayer repair by permitting normal  
464 RPA2 phosphorylation.

## 465 **Limitations**

466 The current study highlights the cell type- and condition-specific functions of lncRNAs by providing new  
467 insights into the fine-tuning of RPA2 phosphorylation in endothelial cells by the lncRNA *PCAT19*. Our  
468 data demonstrates that *PCAT19* binds RPA2, alters the RPA2 phosphorylation state and impacts cell  
469 cycle progression and DNA damage responses. Details on the precise *PCAT19*-RPA2 interaction are  
470 missing; the exact binding site of *PCAT19* on RPA2 and whether binding at this site physically prevents  
471 kinase accessibility is unclear and would require mutagenesis experiments and structural analyses. The  
472 dynamics of this interaction and in particular the promotion of binding factors and the subsequent  
473 inhibition of binding is unknown. We propose that the endothelial-enrichment of *PCAT19* is what  
474 confers this endothelial-specific mechanism of RPA2 regulation. It is unclear as to whether this  
475 mechanism exists between RPA2 and lncRNAs in other cell types. Although we provide data on the  
476 differential expression of *PCAT19* in vascular diseases and in cancer endothelial cells, exactly how  
477 *PCAT19* is involved in these diseases is so far unknown. Evidence in human vascular disease cohorts is

478 sparse and the use of an *in vivo* model is not possible due to the lack of *PCAT19* conservation between  
479 species. The transcriptional regulation of *PCAT19* needs to be clarified, since it has been reported in  
480 cancer cells and we report its enrichment in healthy endothelial cells.

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500 **Declaration of interests**

501 The authors declare no competing interests.

502 **Main figure titles and legends**

503 **Figure 1: *PCAT19* is highly enriched in endothelial cells and is differentially expressed in vascular**  
504 **diseases. A.** FANTOM5 CAGE expression of the 30 highest expressed endothelial lncRNAs across  
505 different cell types. Z-score across cell types for each lncRNA. **B.** *PCAT19* expression ( $\log_{10}(\text{TPM}+1)$ ) in  
506 normal human tissues from The GTEx Portal (GTEx Analysis Release V8 (dbGaP Accession  
507 phs000424.v8.p2). TPM, transcripts per million. **C, D.** Uniform Manifold Approximation and Projection  
508 (UMAP) plot (C) and violin plot (D) of published scRNA-seq from healthy prostate tissue (Joseph *et al.*,  
509 2021). Cell types and respective normalised *PCAT19* expression displayed. **E.** *PCAT19* expression  
510 (relative Fragments per Kilobase of transcript per Million mapped reads) in healthy vessel (CTL) and  
511 haemangioma (HA). *PCAT19* expression normalised to PECAM1 expression. **F.** *PCAT19* expression  
512 relative to PECAM1 expression in healthy/early carotid artery plaque vessel samples (CTL) (n=10) or  
513 advanced carotid artery plaque (CAP) samples (n=12) from the Munich Vascular Biobank (30781475)  
514 **G.** *PCAT19* expression relative to PECAM1 expression in stable (St) (n=6) or unstable (Unst) (n=5)  
515 carotid artery plaque samples. Munich Vascular Biobank (30781475). **H.** Proportion of endothelial cells  
516 expressing *PCAT19* in lung sc-RNA-seq data (Goveia *et al.*, 2020). Healthy endothelial cells (Norm);  
517 squamous cell carcinoma endothelial cells (SCC); and large cell carcinoma endothelial cells (LCC). Data  
518 are represented as mean +/- SD. \*\* signifies  $P < 0.01$ ; \*\*\* signifies  $P < 0.001$ .

519

520 **Figure 2: *PCAT19* represses endothelial cell proliferation, angiogenic sprouting and cardiac organoid**  
521 **vascularisation. A.** Endothelial cell proliferation measured by percentage EdU-positive cells after LNA  
522 GapmeR-mediated knockdown of *PCAT19* (P19) or negative control (CTL). Scale bars indicate 100  $\mu\text{m}$ .  
523 n=3 biological replicates, unpaired t-test. Representative shown. **B.** Endothelial cell proliferation  
524 measured by percentage EdU-positive cells after overexpression (OE) of *PCAT19* (P19) or pcDNA3.1+  
525 control (CTL). Scale bars indicate 100  $\mu\text{m}$ . n=6 biological replicates, unpaired t-test. Representative  
526 images shown. **C, D.** Endothelial cell spheroid outgrowth assay after LNA GapmeR-mediated  
527 knockdown of *PCAT19* (P19) or negative control (CTL) LNA GapmeR (C) or overexpression of *PCAT19*  
528 (P19) or pcDNA3.1+ control (CTL) (D). Spheroids were treated with and without VEGF-A stimulation.  
529 Scale bars indicate 100  $\mu\text{m}$ . n=3 biological replicates, One-way ANOVA. Representative images shown.  
530 **E.** Vascularisation of cardiac organoids after LNA GapmeR-mediated knockdown of *PCAT19* (P19) or  
531 negative control (CTL). Scale bars indicate 200  $\mu\text{m}$ . n=3 biological replicates, unpaired t-test.  
532 Representative images with maximum projection of the full z-stack. **F.** *PCAT19* expression in HUVEC  
533 seeded at various densities n=3, One-way ANOVA. **G.** Heatmap of top 50 differentially expressed genes  
534 after *PCAT19* knockdown. Z-score displayed (n=3). **H.** Relative expression of all genes after *PCAT19*  
535 knockdown. Dashed line indicates a threshold of  $P\text{-adjusted} < 0.05$ . (n=3). **I.** KEGG pathway fold



536 enrichment over background from differentially expressed genes ( $P$ -adjusted $<0.05$ ) after *PCAT19*  
537 knockdown. **J.** Prediction of upstream regulators of differentially expressed genes ( $P$ -adj $<0.05$ ) after  
538 *PCAT19* knockdown using QuaternaryProd R package. Colour of outer circles indicates up- or down-  
539 regulation for that upstream regulator. Size of circle indicates number of downstream targets.  
540 Thickness of line connecting inner and outer circles indicates significance level of that upstream  
541 regulator. Data are represented as mean  $\pm$  SD. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ , \*\*\*\* $P<0.0001$ .

542

543 **Figure 3: RPA2 is a *PCAT19* interaction partner.** **A.** Biotin-tagged antisense-oligo (AS-oligo) RNA  
544 pulldown of *PCAT19* and its interacting proteins from endothelial cell lysate measured by mass  
545 spectrometry. Scramble AS-oligos were used as negative control (CTL).  $\log_{10}$ iBAQ representation of  
546 enriched proteins (sum of all peptide intensities/number of observable peptides) against the  $\log_2$  fold  
547 difference of *PCAT19*/CTL ( $n=6$ ). Proteins highlighted indicate enrichment with *PCAT19* ( $P<0.05$ ;  
548  $q<0.05$ ). iBAQ, intensity based absolute quantitation. **B.** Table of significantly enriched *PCAT19*-  
549 interacting proteins ( $P<0.05$ ,  $q<0.05$ ). **C.** Schematic depicting the proteins pulled down with *PCAT19*  
550 and potential interaction map based on literature searches. RPA2 is central to the RPA complex and  
551 reportedly functions alongside most of the proteins identified with mass spectrometry. **D.** AS-oligo  
552 RNA pulldown of *PCAT19* (P19) or control AS-oligos (CTL) and western blotting with antibodies against  
553 RPA2 and PKNP. Lamin B1 served as negative control. **E.** RNA immunoprecipitation (RIP) in HUVEC cell  
554 extract with an antibody against RPA2 followed by RT-qPCR for *PCAT19*. Percentage of input recovery  
555 of *PCAT19* versus a non primary-antibody control (IgG) is shown. 18S rRNA and U4 snRNA served as  
556 negative controls. **F.** *In vitro* binding assay of RPA2 and *PCAT19*. His-tagged RPA2 was combined with  
557 *in vitro*-transcribed and biotinylated *PCAT19* or pcDNA3.1+ control RNA (biotin-CTL). Streptavidin  
558 beads were used to pull down the biotin-tagged RNAs and blots stained for RPA2. Data are represented  
559 as mean  $\pm$  SD. \*\* $P<0.01$ .

560

561 **Figure 4: *PCAT19* maintains genomic stability and limits RPA2-ATR signalling.** **A.** HUVEC were  
562 transfected with LNA Gapmers against *PCAT19* or negative control LNA and treated with 1  $\mu$ M  
563 camptothecin or DMSO for 16 hours. TUNEL assay was performed and cells imaged for DNA double-  
564 strand breaks. Double strand breaks are shown in red (AlexaFluor 580 nm). DAPI was used to stain  
565 nuclei (blue). Scale bar indicates 100  $\mu$ m. Quantification of TUNEL signal mean intensity per nucleus is  
566 shown. One-way ANOVA. Error bars defined as mean  $\pm$  SD.  $n=3$  biological replicates. **B.** HUVEC were  
567 transfected with LNA Gapmers against *PCAT19* or negative control LNA and treated with 10  $\mu$ M  
568 camptothecin or DMSO for 16 hours. Comet assay was performed and cells imaged. Quantification of  
569 comets and tail olive moment shown. ( $n=3$ ). Scale bar indicates 100  $\mu$ m. **C.** HUVEC were transfected

570 with *PCAT19* (P19) LNA or negative control (CTL) LNA and then treated with or without CPT. Western  
571 blot staining for p53 and GAPDH or **D.**  $\gamma$ H2AX and H2A (n=3 for all panels). **E.** HUVEC were transduced  
572 with either *PCAT19* (P19) overexpression (OE) plasmid or pcDNA3.1+ backbone control (CTL) plasmid  
573 and then treated with or without camptothecin (CPT). Western blot staining for p53 and GAPDH or **F.**  
574  $\gamma$ H2AX and H2A (n=3 for all panels). **G.** HUVEC transfected with *PCAT19* CRISPRi or respective negative  
575 controls and treated with and without camptothecin (CPT) for 16 h. Western blot staining for p53 and  
576 GAPDH. n=3 biological replicates. **H.** HUVEC transfected with *PCAT19* CRISPRa or respective negative  
577 controls and treated with and without camptothecin (CPT) for 16 h. Western blot staining for p53 and  
578 GAPDH. n=3 biological replicates. **I.** RNA *In Situ* Hybridization Proximity Ligation Assay (rISH-PLA)  
579 between *PCAT19* and  $\gamma$ H2AX treated with DMSO or CPT. Biotin-tagged *PCAT19* antisense  
580 oligonucleotides and antibodies against biotin and  $\gamma$ H2AX were added to fixed cells. Cells which  
581 received only *PCAT19* oligonucleotides/biotin antibody or  $\gamma$ H2AX antibody served as negative controls.  
582 Red signal indicates PLA signal (546nm) between *PCAT19* and  $\gamma$ H2AX, blue indicates DAPI. Upper panels  
583 scale bar indicates 100  $\mu$ m, lower panels scale bar indicates 25  $\mu$ m. **J.** HUVEC were transfected with  
584 LNA Gapmers against *PCAT19* (P) or negative control LNA (C) and treated with and without 2mM HU  
585 for 16 h. Cells were analysed by FACS after BrdU incorporation and propidium iodide staining. Cell cycle  
586 phases are indicated. Quantification for percentage cells in each phase (G1, S1, S2, S3 and G2/M) is  
587 displayed (n=3). Data are represented as mean +/- SD. \* signifies  $P < 0.05$ , \*\* signifies  $P < 0.01$ , \*\*\*  
588 signifies  $P < 0.001$ , \*\*\*\* indicates  $P < 0.0001$ .

589

590 **Figure 5: *PCAT19* limits RPA2 serine 33 (S33) phosphorylation.** **A.** Depiction of RPA2 phosphorylation  
591 sites. **B.** HUVEC were seeded at subconfluent or confluent levels and treated with 10  $\mu$ M ATRi or DMSO  
592 for 16 hours. Western blot staining for RPA2 and S33-pRPA2 (n=3) **C.** HUVEC were transfected with  
593 LNA Gapmers against *PCAT19* or negative control LNA and treated with 10  $\mu$ M ATRi or DMSO for 16  
594 hours. Western blot staining for RPA2 and S33-pRPA2 (n=3). **D.** HUVEC were transduced with either  
595 *PCAT19* overexpression (OE) plasmid or pcDNA3.1+ backbone control plasmid and then treated with  
596 10  $\mu$ M ATRi or DMSO for 16 hours. Western blot staining for RPA2 and S33-pRPA2 (n=6). **E.** *in vitro*  
597 binding assay for various combinations of His-RPA2, Biotin-*PCAT19*, Biotin-CTL RNA, ATRi and  
598 phosphatase. Staining of S33-pRPA2 or RPA2 in biotin pulldown and 5% input samples. **F.** *in vitro*  
599 phosphorylation assay of endogenous RPA2 and recombinant His-RPA2. Combinations of His-RPA2,  
600 Flag-ATR, *in vitro*-transcribed *PCAT19*, ATR inhibitor, phosphatase (CIP), phosphatase inhibitor and  
601 ATP. Flag, His and S4/8-pRPA2 antibodies used for staining. **G.** HUVEC were transduced with either  
602 *PCAT19* overexpression (OE) plasmid or pcDNA3.1+ backbone control plasmid. Duolink proximity  
603 ligation assay for RPA2-ATR. Red signal indicates duolink PLA signal (546nm), blue indicates DAPI. **H.**  
604 EdU proliferation assay after *PCAT19* LNA-GapmeR-mediated knockdown or control LNA and with or  
21

605 without treatment with 10  $\mu$ M ATRi for 16h (n=3). I. Spheroid outgrowth assay after *PCAT19* LNA-  
606 GapmeR-mediated knockdown or control LNA and with or without treatment with 10  $\mu$ M ATRi for 16h  
607 (n=3). Data are represented as mean +/- SD. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001.

608 **STAR★Methods**

609 **Resource availability**

610 *Lead contact*

611 Further information and requests for resources and reagents should be directed to and will be fulfilled  
612 by the lead contact, Ralf P. Brandes (Brandes@vrc.uni-frankfurt.de).

613 *Materials availability*

614 Plasmids generated in this study are available from the [lead contact](#).

615 *Data and code availability*

616 RNA-seq data have been deposited at NCBI GEO datasets and are publicly available as of the date of  
617 publication under the accession number: [GSE199091](#).

618 Mass spectrometry data have been deposited under ProteomeXchange Consortium  
619 (<http://proteomecentral.proteomexchange.org>) via the PRIDE partner repository with the dataset  
620 identifier and are publicly available as of the date of publication under the accession number:  
621 [PXD032669](#).

622 This paper analyzes existing, publicly available data: *PCAT19* expression across organs was analysed  
623 using the GTEx database<sup>25</sup> (GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2). FANTOM5  
624 CAGE expression data was obtained from the FANTOM5 website (gencode v19)<sup>23,52,53</sup>. Prostate tissue  
625 scRNA-seq data was obtained from GSE172357<sup>28</sup>. Haemangioma RNA-seq data was obtained from<sup>30</sup>.  
626 Lung endothelial scRNA-seq data was obtained from ArrayExpress (E-MTAB-6308)<sup>33</sup>. The GEPIA  
627 database was used to analyse *PCAT19* expression between normal and cancerous tissues<sup>32</sup>. Tabula  
628 Sapiens data was used for gene expression analysis<sup>29</sup>.

629 This paper does not report original code.

630 Any additional information required to reanalyze the data reported in this paper is available from the  
631 lead contact upon request.

632

633 **Experimental model and subject details**

634 *Primary cell cultures and cell lines*

635 Pooled human umbilical vein endothelial cells (HUVEC, purchased from PromoCell, #C-12203), human  
636 microvascular endothelial cells (HMEC, from CDC, 98247, male), human coronary artery endothelial  
637 cells (HCAEC, from PeloBiotech, PB-CH-182-2011, QC06814F10) and human aortic endothelial cells  
638 (HAoEC, purchased from PeloBiotech, 304K-05a, Lot No. 2366, male) were cultured on gelatine-coated  
639 plates in endothelial growth medium (EGM) containing 12% (for HUVEC, HMEC, HCAEC) or 20% (for  
640 HAoEC) fetal calf serum (FCS, S0113, Biochrom, Germany), penicillin (50 U/mL) and streptomycin (50  
641 µg/mL) (15140-122, Gibco/ Lifetechnologies, USA) in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C. The  
642 different batches of HUVEC were all commercial pools of cells obtained from umbilical cord/ umbilical  
643 vein of caucasians (474Z010: 2 males, 1 female; 408Z014: 2 males, 1 female; 471Z011: 2 males, 2  
644 females; 466Z022: 2 males, 1 female). HUVEC that had been frozen and stored at passage two, were  
645 seeded for passage three and used for experiments after seeding to passage four. The seeding density  
646 was dependent on the experiment to be performed. Standard seeding conditions (50,000 cells/cm<sup>2</sup>)  
647 were used for experiments such as protein or chromatin immunoprecipitation. Experiments involving  
648 RNA interference required a cell seeding density of 25,000 cells/cm<sup>2</sup> for next day transfection. Cell  
649 cycle-related experiments also required a low seeding density to ensure continued cycling. For each  
650 experiment, at least three different batches of HUVEC from passage 3 were used.

651 Human dermal lymphatic endothelial cells (HDLEC, C-12217; Lot No. 394Z027.3, 4092401.3, both  
652 female) were purchased from Promocell (Heidelberg, Germany) and cultured in a humidified  
653 atmosphere of 5% CO<sub>2</sub> at 37°C in endothelial cell growth medium MV2 (Promocell, Heidelberg,  
654 Germany). HEK-293 (293, ATCC, CRL-1573) and HEK293T (293T/17 [HEK 293T/17], ATCC, CRL-11268)  
655 cells were cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS) and 1% Pen-  
656 Strep in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C.

657 Human induced pluripotent stem cells (hiPSCs, WSTli081-A, EbiSC, male) were used for the generation  
658 of cardiac organoids. In brief, 500 hiPSCs were cultured for 2 d on ultra-low-attachment surface in  
659 TeSR™-E8™ medium (#05990, STEMCELL™ Technologies) at 37 °C and 5 % CO<sub>2</sub> in a humidified  
660 atmosphere to form iPSC-aggregates.

661

662

## 663 **Method details**

### 664 *Cell stimulations*

665 HUVEC were seeded the day before stimulation and cultured as described above. The following  
666 chemicals were used in cell stimulation experiments: Human recombinant VEGF-A 165 (50 and 100  
667 ng/mL; R&D, 293-VE), camptothecin (1 µM and 10 µM), ATR inhibitor (10 µM, VE-821, Selleckchem)  
668 and hydroxyurea (2mM, Sigma-Aldrich). Stimulations were performed in either EGM (12% FCS) or in

669 EBM (6% FCS) (e.g. spheroid VEGF-A stimulations). The duration of stimulations varied between  
670 experiments and is therefore indicated in the individual figure legends.

#### 671 *LNA GapmeR-mediated knockdown*

672 Cells were seeded at a density of 25,000 cells/cm<sup>2</sup> one day before transfection with LNA GapmeRs  
673 (Qiagen). Cells were transfected with LNAs using the RNAiMAX transfection reagent according to the  
674 manufacturer's protocol (Qiagen). A final LNA concentration of 30 nM was used for 48-72h before  
675 stopping cells with either RNA lysis buffer or protein lysis buffer. In some cases, cells were re-seeded  
676 for further experiments. LNA GapmeRs were designed with the Qiagen/Exiqon LNA probe designer and  
677 had the following sequences: *PCAT19* 5'-AAT TCG GCT CTT ACA A-3' and as a negative Control 5'-AAC  
678 ACG TCT ATA CGC-3'.

#### 679 *Overexpression*

680 700,000 cells were resuspended and electroporated in E2 buffer with the NEON electroporation  
681 system (Invitrogen) (1,400 V, 1x 30 ms pulse). 7 µg of plasmid was used for each overexpression. A full  
682 medium exchange was performed every 24 h and cells were incubated for a total of 48 h. The following  
683 plasmids were used: pcDNA3.1+ vector containing *PCAT19* and pcDNA3.1+ as a negative control.

#### 684 *EdU proliferation assay*

685 Cells were seeded at a density of 10,000 cells/cm<sup>2</sup> in ibidi 8-well plates. After 24 h a 2X working solution  
686 of EdU (C10337, ThermoFisher) in EGM was added to the cells for 6 h. 4% paraformaldehyde (PFA) was  
687 added to the cell medium for 15 min before washing with 3% BSA in PBS and then 0.5% TritonX for 20  
688 min. Cells were washed again with 3% BSA before the addition of a Click-iT<sup>®</sup> reaction cocktail (Click-iT  
689 reaction buffer, CuSO<sub>4</sub> (Component E), Alexa Fluor Azid and Click-iT<sup>®</sup> buffer additive) for 30 min at RT.  
690 Cells were washed and incubated in Hoechst 33342 (Component G) solution 1:2000 in PBS (5 µg/mL)  
691 for a further 30 min at RT before washing with PBS. Cells were imaged for Hoechst and EdU (488 nm)  
692 with a laser scanning confocal microscope (LSM800, Zeiss) and images quantified with FIJI/ImageJ<sup>54</sup>.

#### 693 *Spheroid outgrowth assay*

694 HUVEC spheroid outgrowth assays were performed as described previously<sup>55</sup>. Spheroids were  
695 stimulated in EBM (6% FCS) containing 50 ng/mL VEGF-A 165 for 16 h before the addition of 4% PFA  
696 to the medium. Images of 10 spheroids per condition and replicate were acquired using an Evos XL  
697 Core microscope (Life technologies) and outgrowth length and numbers quantified using ImageJ.

#### 698 *Human cardiac organoid formation*

699 iPSC-aggregates were differentiated to cardiac organoids (hCOs) using the STEMdiff™ Cardiomyocyte  
700 Differentiation Kit (#05010, STEMCELL™ Technologies) following the instructions from the supplier.  
701 hCOs were then maintained in mixed medium of STEMdiff™ Cardiomyocyte Maintenance Basal  
702 Medium (#05020, STEMCELL™ Technologies) and Endothelial Cell Growth Medium 2 (#C-22111,  
703 PromoCell) at a ratio of 4:1, with medium changes every second day for a further 28 d. Medium was  
704 then changed to medium supplemented with 140 nM CTL LNA or *PCAT19* LNA for 48 h. hCOs were  
705 then fixed with 4% PFA overnight at 4 °C. Whole mount staining was then performed by incubating  
706 hCOs in 1% Triton X-100 for 1 h, followed by blocking in 5% horse serum for 1 h. hCOs were  
707 immunostained with primary antibody solution (1:200 anti- alpha actinin (#A7811, Sigma Aldrich),  
708 1:200 anti-VE-Cadherin (#2500, Cell Signaling Technologies)) at 4°C overnight and secondary antibody  
709 solution (1:500 anti-mouse AlexaFlour488 (Invitrogen, A11017) and 1:500 anti-rabbit AlexaFluor 647  
710 (Invitrogen, A21246)) at RT for 3 h, followed by 2 h of washing in 1X PBST. Nuclei were counterstained  
711 with DAPI. The stained hCOs were transferred onto glass slides and imaged with the Leica SP8 Confocal  
712 System. The whole hCO was imaged using a z-stack between two ends of the organoid. Organoids were  
713 quantified for cumulative vascular network length and organoid diameter using the Leica LAS X  
714 software.

#### 715 *DNA fibre assay*

716 HUVEC were sequentially labelled with 5-Chloro-2'-deoxyuridine (CldU, 50 µM) and 5-Iodo-2'-  
717 deoxyuridine (IdU, 50 µM) for 15 min. After labelling, cells were trypsinised, resuspended in cold PBS,  
718 diluted to  $1.75 \times 10^5$ /ml and mixed 1:1 with unlabelled cells. 7.5 µl lysis buffer (200 mM Tris-HCl pH  
719 7.5, 50 mM EDTA, 0.5% SDS) was mixed with 4 µL of the cell suspension on a SuperFrost Plus  
720 microscopy slide (ThermoFisher), incubated horizontally for 9 min and tilted, allowing the solution to  
721 spread to the bottom of the slide. Following air-drying, DNA spreads were fixed with 3:1  
722 methanol:acetic acid overnight at 4 °C. The spreads were then rehydrated 3 × 3 min in PBS, denatured  
723 in 2.5 M HCl for 1.5 h at RT, then washed 5 × 2.5 min in PBS. The slides were blocked for 40 min in  
724 blocking solution (2% BSA in PBS-T), followed by incubation with primary antibodies (mouse anti-BrdU,  
725 1:100, BD Bioscience and rat anti-BrdU, 1:100, Abcam) at RT for 2.5 h. After 3 × 5 min washes with PBS-  
726 T, the slides were incubated with secondary antibodies (goat anti-mouse Alexa Fluor 647, 1:500,  
727 Thermo Scientific and goat anti-rat Alexa Fluor 488, 1:500, Thermo Scientific) at RT for 1 h. The slides  
728 were then washed 3 × 5 min with PBS-T, air-dried and mounted with Prolong Gold AntiFade Mountant  
729 (Thermo Scientific). Images of DNA fibers were acquired with a Widefield Fluorescence Microscope  
730 (Thunder, LASX software, Leica) (magnification: 100x, NA 1.44 HC PL APO oil immersion objective; LED  
731 illumination and the corresponding emission filters: 635 nm, 642/80 and 475 nm, 535/70). Lengths of  
732 DNA fibers were quantified using the Fiji/ImageJ software.

733 *Overexpression and purification of RPA2 proteins*

734 Recombinant overexpression of full-length RPA2 protein was achieved using Turbo E.coli chemically  
735 competent cells (NEB, catalog number: C2984H). Recombinant plasmid (pVM\_MBP) was transformed  
736 by heat-shock on Luria broth agar plates and colonies were inoculated the next day in fresh Luria broth  
737 medium supplemented with 100 µg/mL ampicillin and cultured overnight at 37 °C. Overexpression was  
738 induced at OD<sub>600</sub>=0.7 using a final concentration of 0.4 mM isopropyl-β-D-1-thiogalactopyranoside  
739 (IPTG) and the cultures were further left to grow at 18 °C overnight. Cells were harvested the next day  
740 by centrifugation and lysed by sonication in lysis buffer (50 mM Tris pH 7.5, 500 mM NaCl, 5% (v/v)  
741 Glycerol, 15 mM imidazole) supplemented with an EDTA-free protease inhibitor cocktail tablet (Roche  
742 Applied Science) and 30 µg/mL DNase I. The lysate was cleared by centrifuging at 10,000 rpm for 1 h  
743 and filtered using a 0.22 µm filter membrane, before applying the lysate to Nickel-NTA metal affinity  
744 agarose resin beads (Cube Biotech) pre-equilibrated in lysis buffer. The lysate was left to incubate on  
745 the beads at 4 °C for 1 h, and the flow-through was removed after gentle centrifugation at 300 g for 2  
746 min. The beads were washed 5 times with column volume of lysis buffer and an incubation time was  
747 10 min at 4 °C with subsequent gentle centrifugation during each step. Elutions were performed using  
748 lysis buffer supplemented with increasing imidazole concentrations of 50 mM, 100 mM, and 200 mM  
749 and 500 mM and an incubation time of 10 min and subsequent gentle centrifugation. The purest  
750 fractions, determined by SDS-PAGE, were concentrated using Merck-Millipore centricons by  
751 centrifugation at 4,500 rpm and loaded onto a HiLoad Superdex S200 10/300 GL (GE Healthcare)  
752 column previously equilibrated in gel filtration buffer (25 mM Tris pH 7.5, 200 mM NaCl, 0.5 mM TCEP)  
753 for size exclusion chromatography. Concentrated proteins were used later for all *in vitro* or semi-*in-*  
754 *vitro* interaction studies.

755 *In vitro* transcription and RNA 3'end biotinylation.

756 pcDNA3.1+PCAT19 or control pcDNA3.1+ plasmid DNA were linearised with SmaI (ThermoFisher) and  
757 purified. DNA was *in vitro* transcribed according to the manufacturer's protocol with T7 Phage RNA  
758 Polymerase (NEB). Afterwards, the remaining DNA was digested with RQ DNase I (Promega). The *in*  
759 *vitro* transcribed RNA was purified with the RNeasy Mini Kit (Qiagen) and biotinylated at the 3'end with  
760 the Pierce RNA 3'end biotinylation kit (ThermoFisher).

761 *PCAT19-RPA2 in vitro* assays

762 For the *in vitro* interaction assay, purified recombinant RPA2 protein (5 µg) was mixed with *in vitro*-  
763 transcribed Biotin-PCAT19 (300 ng) in a reaction containing 1 µL/mL (20 units /ml) SUPERaseIN  
764 inhibitor for 2 hours at RT. For the *in vitro* phosphorylation assay, purified RPA2 protein (10 µg), *in vitro*  
765 transcribed Biotin-PCAT19 (300 ng) and HUVEC crude cell lysate (200 µg) were mixed in kinase reaction



766 buffer (20 mM HEPES (pH 7.5), 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, and 0.3 μM ATP) containing 1 μL/mL  
767 (20 units/mL) SUPERaseIN inhibitor (ThermoFisher) and ssDNA from salmon sperm (100 μg/mL)  
768 (ThermoFisher). The mixture was incubated for 30 min at 37°C and alternatively, 20 μM ATR inhibitor  
769 (VE-821) (Selleckchem) or phosphatase (100 U/mL) (Merck) were added to the mixture before  
770 incubation. Importantly, biotin-PCAT19 RNA or biotin-pcDNA3.1+ control RNA was previously folded  
771 and added to the respective mixtures (*in vitro* phosphorylation assay and *in vitro* protein interaction  
772 experiment) at equimolar concentrations. Lastly, biotinylated labelled substrates were captured with  
773 20 μL Streptavidin Magnetic Beads (NEB) and incubating the mixture overnight at 4 °C. Beads were  
774 washed 4 times with cold PBS-T (0.1% Tween20) and then boiled in 20 μL 1x Laemmli SDS sample buffer  
775 (ThermoFisher) for 10 min. Samples were applied to SDS-PAGE and Western Blotting and the detection  
776 of biotinylated-proteins was performed with the Odyssey CLx Imaging System.

777

778 For the semi-*in vitro* phosphorylation assay in HEK293T lysate, purified RPA2 protein (10 μg) , *in vitro*  
779 transcribed Biotin-PCAT19 (300 ng) and HEK293T crude cell lysate (200 μg) (of transfected cells the day  
780 before with 10 μg CMV Flag ATRwt (gift from Stephen Elledge (Addgene plasmid #41909;  
781 <http://n2t.net/addgene:41909> ; RRID:Addgene\_41909) <sup>56</sup> with PEI (Polyethylenimine, linear, MW  
782 25000, Polysciences, Cat# 23966)) were mixed in kinase reaction buffer (20 mM HEPES (pH 7.5), 10  
783 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, and 0.3 μM ATP) containing 1 μL/mL (20 units/mL) SUPERaseIN  
784 inhibitor (ThermoFisher) and ssDNA from salmon sperm (100 μg/mL) (ThermoFisher). The mixture was  
785 incubated for 30 min at 37 °C and alternatively, 20 μM ATR inhibitor (VE-821) (Selleckchem) or  
786 phosphatase (100 U/mL) (Merck) were added to the mixture before incubation. Samples were applied  
787 to SDS-PAGE and Western Blotting and the detection of biotinylated-proteins was performed with the  
788 Odyssey CLx Imaging System.

#### 789 *RNA isolation, reverse transcription and RT-qPCR*

790 Total RNA was isolated and purified from HUVEC using the RNA Mini Kit according to the  
791 manufacturer's protocol (Bio&SELL). Purified RNA was reverse transcribed with SuperScript III Reverse  
792 Transcriptase (Thermo Fisher) and oligo(dT)23 together with random hexamer primers (Sigma). cDNA  
793 was quantified with RT-qPCR using ITaq Universal SYBR Green Supermix with ROX as reference dye  
794 (Bio-Rad, 1725125) in an AriaMX cycler (Agilent). Human target genes were normalised to GAPDH.  
795 Relative expressions were calculated using the ΔΔCt method with the AriaMX qPCR software (Agilent).  
796 Primers used in this study are listed in **Table S3**.

#### 797 *Protein isolation and Western blot by SDS-PAGE*

798 HUVECs washed in Hanks solution (Applichem) were lysed with buffer A (10 mM Hepes pH 7.9, 10 mM  
799 KCl, 0.1 mM EDTA, 0.1 mM EGTA, protein inhibitor mix (PIM), Phenylmethylsulfonylfluoride (PMSF),  
800 and DTT). After 10 min incubation at 4 °C, 0.75% nonidet was added to the lysate, vortexed for 10 s  
801 and centrifuged for 1 min at 16,000 g. Nuclear pellets were resuspended in buffer C (20 mM Hepes pH  
802 7.9, 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, protein inhibitor mix (PIM), Phenylmethylsulfonylfluoride  
803 (PMSF), and DTT) for 15 min at 4 °C before centrifugation for 1 min, 16,000 g. Protein concentrations  
804 of the supernatant were determined with the Bradford assay and the cell extract was boiled in Laemmli  
805 buffer. Equal amounts of protein were separated with SDS-PAGE and the gels were blotted onto a  
806 nitrocellulose membrane and blocked in Rotiblock (Carl Roth, Germany). After incubation with the first  
807 antibody, infrared-fluorescent-dye-conjugated secondary antibodies (Licor, Bad Homburg, Germany)  
808 were used and signals detected with an infrared-based laser scanning detection system (Odyssey  
809 Classic, Licor, Bad Homburg, Germany). Images were acquired with the Image Studio Ver 5.2 software  
810 (Licor). The following antibodies were used: RPA2 (ab2175, Abcam) RPA2 (A300-244A, Bethyl), S33-  
811 pRPA2 (A300-246A, Bethyl), T21-pRPA2 (ab61065, Abcam), S4/8-pRPA2 (A700-009, Bethyl), p53 (sc-  
812 6243, Santa Cruz), Lamin B1 (sc-20682, Santa Cruz), His6 (11922416001, Roche), DYKDDDDK Tag  
813 (D6W5B) (FLAG, 14793, Cell Signaling Technology), GAPDH (G8795, Sigma-Aldrich), Beta-actin (A1978,  
814 Sigma-Aldrich), PNKP (A300-257A, Bethyl), yH2AX (MABE205, Millipore), H2A (3636, Cell Signalling).

#### 815 *RNA immunoprecipitation*

816  $3 \times 10^6$  HUVEC were grown to 80% confluence and washed once with Hanks buffer. 6 mL Hanks buffer  
817 was added to the cells on ice and irradiated with 0.150 J/cm<sup>2</sup> 254 nm UV light (BIO-LINK, BLX-254,  
818 Vilber). Cells were scraped twice in Hanks buffer and centrifuged at 1,000 g at 4°C for 4 min. Isolation  
819 and lysis of the nucleus was performed as outlined above for protein isolation and  
820 immunoprecipitation. 10% of the nuclear lysate served as the “input”. 4 µg anti-RPA2 (A300-244A,  
821 Bethyl) or anti-IgG (ab37415, Abcam) negative control antibody were pre-coupled to 50 µL protein A  
822 magnetic beads (ThermoFisher) in buffer C for 1 h at RT then washed once with high salt buffer (1 M  
823 NaCl) and twice with buffer C3. The antibody-coupled beads were added to the nuclear lysate and  
824 rotated for 1 h at 4 °C. Samples were placed on a magnetic bar and the lysate discarded. The beads  
825 were washed three times in high salt buffer (4°C for 10 min). Beads were then washed twice in buffer  
826 PNK (350 mM Tris-HCl pH 6.5, 50 mM MgCl<sub>2</sub>, 5 mM DTT). For elution of RNA, all PNK buffer was  
827 removed and RNA isolation performed with QIAzol (Qiagen) according to the manufacturer’s protocol.

#### 828 *Antisense-oligonucleotide pulldown of RNA*

829 Antisense oligonucleotides containing a 5'-biotin tag were designed with the online GeneGlobe tool  
830 (QIAGEN) using the target RNA sequence as input. HUVEC were UV-crosslinked on ice (0.150 J/cm<sup>2</sup> 254

831 nm UV light (BIO-LINK, BLX-254, Vilber)) and scraped. Cell pellets were flash frozen and thawed to  
832 disrupt the nuclei. Cells were resuspended in 200  $\mu$ L buffer L (50 mM Tris/HCl pH8, 50 mM NaCl, 0.5%  
833 NP-40, 1 mM EDTA, protein inhibitor mix (PIM), Phenylmethylsulfonylfluoride (PMSF), DTT and  
834 superase 1 $\mu$ L/mL), incubated on ice for 30 min and centrifuged at 10,000 g at 4 °C for 3 min. 1mL buffer  
835 L and 20  $\mu$ L MyOne Streptavidin C1 beads were added to the lysate for 30 min at 4 °C. The beads were  
836 discarded and 200 pmol of *PCAT19* antisense-oligonucleotide (5'-AAG CAG ACA TGA GAC CTC ACT-3')  
837 or scramble control oligonucleotide (5'-GTG TAA CAC GTC TAT ACG CCC A-3') added to the pre-cleared  
838 lysate for rotation overnight at 4°C. The next day, 50  $\mu$ L MyOne Streptavidin C1 beads were added to  
839 the samples for rotation at 4°C for 2 h. Beads were then washed and used for mass spectrometry or  
840 cooked in Laemmli buffer for Western blotting as described above.

#### 841 *CRISPR/dCas9 activation (CRISPRa) and inactivation (CRISPRi)*

842 Guide RNAs (gRNA) were designed with the help of the web-interfaces of CRISPick GPP sgRNA designer  
843 <sup>57</sup>. For CRISPRa, a catalytically inactive Cas9 (dCas9) fused to the transcription activator VP64 (pHAGE  
844 EF1 $\alpha$  dCas9-VP64) was used. For CRISPRi, a dCas9 fusion to the KRAB repressive domain (pHAGE EF1 $\alpha$   
845 dCas9-KRAB) was used. Either of them was transfected in HUVEC together with a sgRNA(MS2) vector  
846 containing the individual guide RNA (gRNA) using the NEON electroporation system (Invitrogen).  
847 pHAGE EF1 $\alpha$  dCas9-VP64 and pHAGE EF1 $\alpha$  dCas9-KRAB were a gift from Rene Maehr and Scot Wolfe  
848 (Addgene plasmid # 50918, # 50919) <sup>58</sup> and sgRNA(MS2) cloning backbone was a gift from Feng Zhang  
849 (Addgene plasmid # 61424) <sup>59</sup>. The following oligonucleotides were used for cloning of the guide RNAs  
850 into the sgRNA(MS2) vector: For CRISPRa, 5'-CACCGAATGTGCAGGACTCATCAAC-3' and 5'-  
851 AAACGTTGATGAGTCCTGCACATTC-3', and for CRISPRi 5'-CACCGAGTGTTATTTGACTGGAGTG-3' and 5'-  
852 AAACCACTCCAGTCAAATAACACTC-3'. After cloning, plasmids were purified and sequenced.

#### 853 *Mass spectrometry*

854 Immunoprecipitation was performed as above but with the final wash of IP beads in wash buffer  
855 without protease inhibitors. Beads were transferred to fresh low-binding tubes in order not to disrupt  
856 protein digestion and to remove sticky proteins. Beads were flash frozen in liquid nitrogen and  
857 subjected to mass spectrometry. Briefly, samples underwent digestion with trypsin (Promega,  
858 Walldorf, Germany) overnight at 37°C and stopped with trifluoroacetic acid (Sigma-Aldrich). Peptides  
859 were purified with multi-stop-and-go tips (StageTips) <sup>60</sup>. Liquid chromatography/mass spectrometry  
860 (LC/MS) was performed on Thermo Scientific™ Q Exactive Plus equipped with an ultra-high  
861 performance liquid chromatography unit (Thermo Scientific Dionex Ultimate 3000) and a Nanospray  
862 Flex Ion-Source (Thermo Scientific). Peptides were loaded and separated using gradient phases.  
863 MaxQuant 1.6.1.0132 <sup>61</sup> and Perseus 1.6.1.3 <sup>62</sup> were used for data analysis. The human reference

864 proteome set (Uniprot) was used to identify peptides and proteins with a false discovery rate (FDR) of  
865 less than 1%. Reverse identifications and common contaminants were removed and the dataset was  
866 reduced to proteins that were identified in at least 4 of 6 samples in one experimental group. Missing  
867 LFQ values were replaced by random background values. Significant interacting proteins were  
868 determined by permutation-based false discovery rate (FDR) calculation and students t-test. The  
869 abundance of each protein was determined using the iBAQ value, which is measured by dividing the  
870 sum of peptide intensities the number of theoretically observable peptides<sup>63</sup>.

871 A detailed description and the mass spectrometry proteomics data have been deposited to the  
872 ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) via the PRIDE partner  
873 repository with the dataset identifier PXD032669. (Reviewer account details: Username:  
874 reviewer\_pxd032669@ebi.ac.uk, Password: xRv4QWmB).

#### 875 *Proximity ligation assay (PLA)*

876 The PLA was performed similarly as described in the manufacturer's protocol (Duolink II Fluorescence,  
877 OLink, Upsalla, Sweden). After fixation in phosphate buffered formaldehyde solution (4%), HUVEC  
878 were permeabilized with Triton X-100 (0.2%) and blocked with serum albumin solution (3%) in  
879 phosphate-buffered saline. After incubation overnight with anti-RPA2 (A300-244A, Bethyl) and anti-  
880 ATR (sc-515173, Santa Cruz), samples were washed and incubated with the respective PLA-probes for  
881 1 h at 37 °C. After washing and ligation for 30 min (37 °C), the amplification with polymerase was  
882 performed for 100 min (37 °C). The nuclei were stained using DAPI. Images (with Alexa Fluor, 546 nm)  
883 were acquired with a confocal microscope (LSM 800, Zeiss) and the number of PLA signals was  
884 normalised to the number of nuclei per image.

#### 885 *RNA-fluorescent in situ hybridisation (FISH)*

886 RNA-FISH was performed to determine the subcellular localisation of RNAs of interest. Cells that had  
887 been grown on 8-well culture plates (Ibidi) were fixed in 4% PFA for 7 min at RT and washed 3 times  
888 with PBS. Cells were permeabilised in 0.5% TritonX-100 containing 1 µL/mL SuperaseIN on ice for 10  
889 min. Cells were washed three times in PBS for 5 min each and rinsed with 2XSSC buffer. Hybridisation  
890 was then performed overnight at 37°C in hybridisation buffer containing 100 µM antisense  
891 oligonucleotide probes with a 5'-TYE tag. *PCAT19* antisense-oligonucleotide (5'-AAG CAG ACA TGA GAC  
892 CTC ACT-3') or scramble control oligonucleotide (5'-GTG TAA CAC GTC TAT ACG CCC A-3'). The next  
893 day, cells were washed four times for 20 min each in 2XSSC buffer containing 50% formamide at 37°C.  
894 DAPI staining (1:200) was included in the second wash step. Cells were imaged with a laser scanning  
895 confocal microscope (LSM800) and images quantified with FIJI/ImageJ.

#### 896 *RNA in situ hybridization-proximity ligation assay (rISH-PLA)*

897 10,000 HUVECs were grown on 8-well ibidi slides, treated as indicated, and were fixed using 4%  
898 paraformaldehyde for seven minutes. To confirm the interaction between *PCAT19* and  $\gamma$ H2AX, the  
899 rISH-PLA assay was performed as described elsewhere<sup>64</sup> with the biotinylated *PCAT19* oligonucleotide,  
900 an anti-biotin antibody (Anti-Biotin antibody [Hyb-8] (ab201341, Abcam)) and an anti- $\gamma$ H2AX antibody  
901 (MABE205, Millipore).

#### 902 *Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL)*

903 The TUNEL assay was used to detect single- and double-stranded DNA breaks according to the  
904 manufacturer's protocol (TMR red, Sigma-Aldrich). Briefly, cells that had been grown on 8-well culture  
905 plates (Ibidi) were fixed in 4% PFA for 1 h at RT. Cells were rinsed with PBS and incubated in 0.1%  
906 TritonX-100 containing 0.1% sodium citrate for 2 min on ice. Cells were then incubated in 1:2 TUNEL  
907 reaction mixture for 60 min at 37 °C in the dark. Cells were rinsed 3 times with PBS and DAPI staining  
908 (1:200) included in the second wash. Cells were imaged with a laser scanning confocal microscope  
909 (LSM800) and images quantified with FIJI/ImageJ.

#### 910 *Comet Assay*

911 The comet assay was used to detect DNA damage by single-cell gel electrophoresis according to the  
912 manufacturer's protocol (CometAssay Single Cell Gel Electrophoresis Assay, 4250-050-K, R&D  
913 Systems). Briefly, cells were treated with and without DNA damaging agents (as indicated in figure  
914 legends). Cells treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 20 min at 4 °C served as a positive control. Cells were  
915 trypsinised, counted and  $1 \times 10^5$  cells mixed with low-melting agarose before being placed on  
916 prewarmed comet slides. Slides were stored in the dark at 4 °C for 30 min then immersed in lysis  
917 solution for 60 min at RT. Slides were then immersed in alkaline unwinding solution for 20 min at RT.  
918 Slides were placed in an electrophoresis chamber and 21 V applied for 30 min before immersing slides  
919 twice in distilled H<sub>2</sub>O for 5 min, then 70% ethanol for 5 min. Slides were dried for 15 min at 37 °C and  
920 100  $\mu$ L SYBR added to the cells for 30 min at RT. Slides were then briefly rinsed in distilled H<sub>2</sub>O and  
921 dried completely at 37 °C. Cells were imaged with a laser scanning confocal microscope (LSM800) and  
922 images quantified with CometScore 2.0 (TriTek Corp).

#### 923 *BrdU/PI FACS*

924 Cells were grown on 6cm culture plates and incubated with 10 $\mu$ M BrdU (10280879001, Roche) for 30  
925 min before washing in 3% BSA and centrifugation at 500 g for 10 min. Cells were resuspended in 70%  
926 ethanol while vortexing and then incubated on ice for 30 min. Cells were centrifuged again at 500 g for  
927 10 min and resuspended in 2 mM HCl containing 0.5% TritonX-100 for 30 min at RT. Cells were then  
928 resuspended in 0.1 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> for 2 min. Cells were centrifuged again and resuspended in PBS/BSA +

929 0.05% Tween 20 with 1:100 antibody (rat anti-BrdU (ab6326, Abcam)) overnight at 4 °C. Cells were  
930 then incubated with 1:500 secondary antibody (anti-rat 488nm) for 30 min at 4 °C. Before washing and  
931 staining with 10 µg/mL Propidium Iodide in 1% BSA containing 20 µg/mL RNase (00552782,  
932 ThermoFisher) for 20 min at 4 °C. Cells were then resuspended in 1% BSA containing containing 10  
933 µg/mL Propidium Iodide for FACS analysis. Cells were subjected to FACS analysis (SH800, Sony) using  
934 the FL2 (500-550 nm) and FL3 (570-630 nm) filters for BrdU and propidium iodide detection. Data was  
935 analysed using the FlowJo™ v10.8 Software (BD Life Sciences).

### 936 *RNA-sequencing*

937 RNA-sequencing was performed as described previously<sup>65</sup>. Briefly, Total RNA and library integrity were  
938 verified and 600 ng of total RNA used as input for SMARTer Stranded Total RNA Sample Prep Kit - HI  
939 Mammalian (Takara Bio). Sequencing was performed on the NextSeq2000 instrument (Illumina) using  
940 a P2 flowcell with v3 chemistry, resulting in an average of 36M reads per library with 1x72bp single  
941 end setup. The resulting raw reads were assessed for quality, adapter content and duplication rates  
942 with FastQC<sup>66</sup>. Trimmomatic version 0.39<sup>67</sup> was employed to trim reads after a quality drop below a  
943 mean of Q20 in a window of 10 nucleotides. Only reads between 30 and 150 nucleotides were cleared  
944 for further analyses. Trimmed and filtered reads were aligned to the Ensembl human genome version  
945 hg38 (ensembl release 104) using STAR 2.74.9a<sup>68</sup> with the parameter "--outFilterMismatchNoverLmax  
946 0.1" to increase the maximum ratio of mismatches to mapped length to 10%. The number of reads  
947 aligning to genes was counted with featureCounts 2.0.2<sup>69</sup> tool from the Subread package. Only reads  
948 mapping at least partially inside exons were admitted and aggregated per gene. Reads overlapping  
949 multiple genes or aligning to multiple regions were excluded. Differentially expressed genes were  
950 identified using DESeq2 version 1.30.1<sup>70</sup>. Further analysis of RNA-seq data was performed with  
951 QuaternaryProd<sup>71</sup>, ClusterProfiler<sup>72</sup> and ReactomePA<sup>73</sup> and visualised with ggplot2<sup>74</sup>.

### 952 *Human carotid artery plaques*

953 Human carotid artery plaque specimens were harvested during carotid endarterectomies (CEA)  
954 performed in the Department for Vascular and Endovascular Surgery at the Klinikum rechts der Isar of  
955 the Technical University Munich. The study was approved by the local Ethics Committee, and all  
956 patients provided their written informed consent in accordance with the Declaration of Helsinki. Two  
957 types of analysis were performed as described previously: stable (n=6) vs. unstable (n=5) plaques<sup>75</sup>  
958 based on the Rothwell/Redgrave criteria<sup>76</sup> (fibrous caps >200µm are considered stable, fibrous caps  
959 <200µm are rendered unstable or ruptured); as well as late stage, advanced atherosclerotic plaques  
960 (n=12) compared to early diseased/healthy control (n=10) specimens stemming from the same  
961 individual<sup>77</sup>. Plaque samples underwent basic stains to assess and characterise plaque morphology

962 using hematoxylin & eosin (HE) as well as Elastica van Giesson (EvG) protocols. For molecular analysis,  
963 plaques were placed in RNA later (Qiagen) for 24h, before being frozen at -80°C for further analysis.  
964 Both of the plaques settings were sent for bulk RNA-sequencing, as described previously<sup>75, 77</sup>.

965

#### 966 **Quantification and statistical analysis**

967 Results are presented as mean ± standard deviation (SD). Statistical significance was calculated using  
968 GraphPad Prism 9.3.1. For multiple comparisons testing One-way ANOVA with Tukey multiple  
969 comparisons test was employed. The students t-test (paired or unpaired) was performed for  
970 experiments where only two conditions were included. Statistical analysis for RNA-sequencing  
971 experiments were performed with the DESeq2 and Diffbind packages respectively. P-value and number  
972 of replicates (n) are displayed with each result.

973

#### 974 **Supplemental information**

975 **Table S1.** Differentially expressed genes after *PCAT19* LNA GapmeR-mediated knockdown in HUVEC,  
976 related to Figure 2.

977 **Table S2.** Mass spectrometry data to identify PCAT19 interaction partners, related to Figure 3.

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## Key resources table

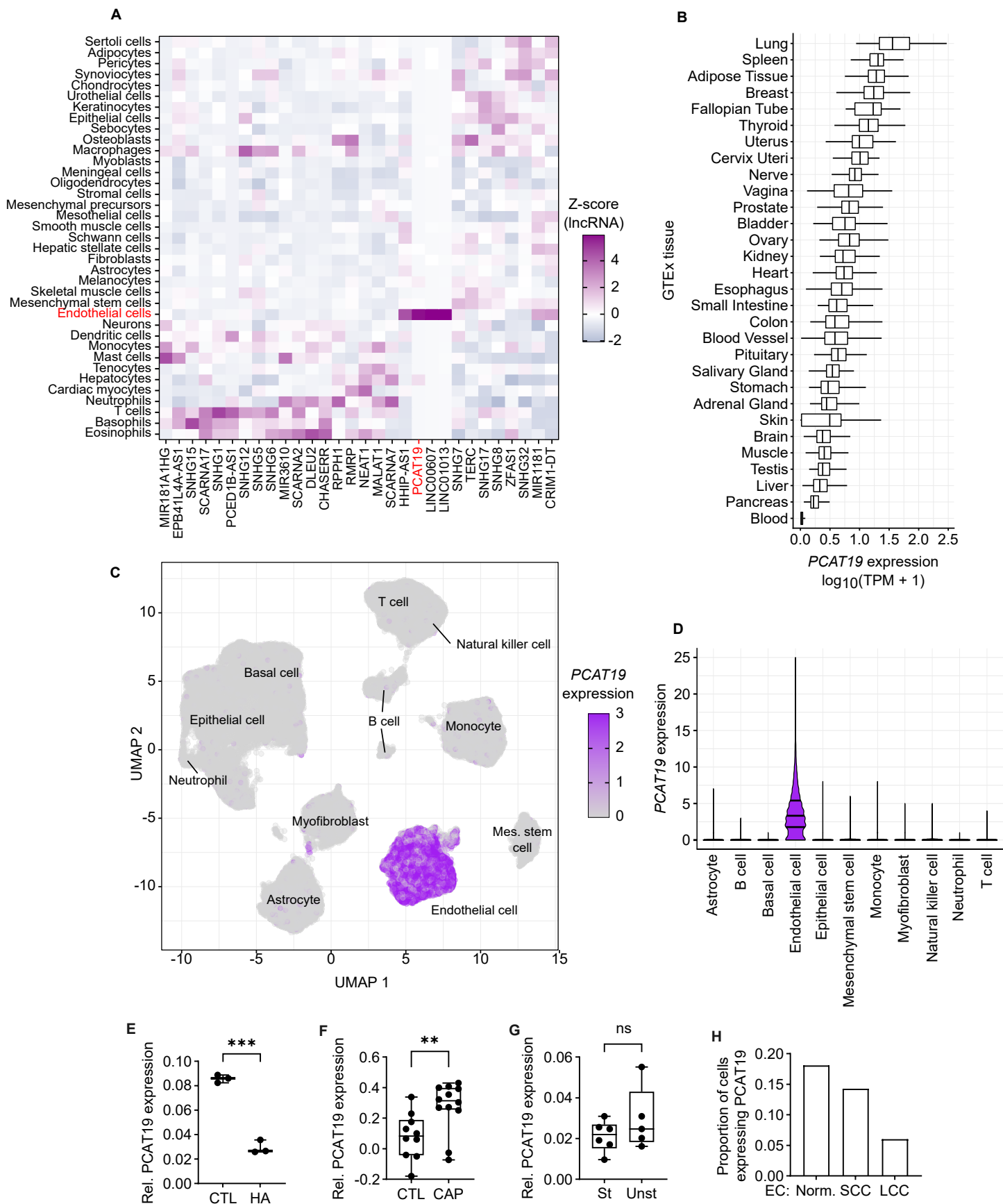
REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
anti-BrdU, mouse	BD Biosciences	347580; RRID:AB_400326
anti-BrdU, rat	Abcam	ab6326; RRID:AB_305426
Anti-RPA32/RPA2 antibody [9H8], mouse	Abcam	ab2175; RRID:AB_302873
Rabbit anti-RPA32 Antibody	Bethyl	A300-244A; RRID:AB_185548
Anti-phospho-RPA32 (Ser33), rabbit	Bethyl	A300-246A; RRID:AB_2180847
Anti-RPA32/RPA2 (phospho T21) antibody, rabbit	Abcam	ab61065; RRID:AB_946322
Anti-phospho-RPA32 (Ser4/Ser8) Recombinant Monoclonal, rabbit	Bethyl	A700-009; RRID:AB_2765278
ATR antibody (C-1)	Santa Cruz	sc-515173; RRID:AB_2893291
p53 antibody (FL-393), rabbit	Santa Cruz	sc-6243; RRID:AB_653753
Lamin B1 antibody (H-90), rabbit	Santa Cruz	sc-20682; RRID:AB_2136308
PNK1 Polyclonal Antibody (PNKP), rabbit	Bethyl	A300-257A; RRID:AB_263356
Anti-phospho-Histone H2A.X (Ser139) Antibody, rabbit	Millipore	MABE205; RRID:AB_10851746
Histone H2A (L88A6) Mouse mAb	Cell Signaling Technology	3636; RRID:AB_2118801
Monoclonal Anti- $\alpha$ -Actinin (Sarcomeric) antibody produced in mouse	Sigma-Aldrich	A7811; RRID:AB_476766
VE-Cadherin (D87F2) XP <sup>®</sup> Rabbit mAb	Cell Signaling Technology	2500; RRID:AB_10839118
DYKDDDDK Tag (D6W5B) Rabbit mAb (Binds to same epitope as Sigma's Anti-FLAG <sup>®</sup> M2 Antibody)	Cell Signaling Technology	14793; RRID:AB_2572291
Anti-His6	Roche	11922416001; RRID:AB_514486
Anti-GAPDH antibody, Mouse monoclonal	Sigma-Aldrich	G8795; RRID:AB_1078991
Anti- $\beta$ -Actin antibody, Mouse monoclonal	Sigma-Aldrich	A1978; RRID:AB_476692
Anti-Biotin antibody [Hyb-8]	Abcam	ab201341; RRID:AB_2861249
<b>Bacterial and virus strains</b>		
NEB Turbo Competent E. coli (High Efficiency)	NEB	C2984H
<b>Biological samples</b>		
Pooled human umbilical vein endothelial cells (HUVEC)	PromoCell	C-12203; Lot No.: 474Z010, 408Z014, 471Z011, 466Z022
human coronary artery endothelial cells (HCAEC)	PeloBiotech	PB-CH-182-2011; Lot No. QC06814F10



human aortic endothelial cells (HAoEC)	PeloBiotech	304K-05a; Lot No. 2366
Human dermal lymphatic endothelial cells (HDLEC)	Promocell	C-12217; Lot No. 394Z027.3, 4092401.3
Human cardiac organoids (hCOs)	This study	N/A
Chemicals, peptides, and recombinant proteins		
Recombinant Human VEGF 165 Protein	R&D	293-VE; Accession # NP_001165097
(S)-(+)-Camptothecin	Sigma-Aldrich	C9911; CAS: 7689-03-4
VE-821 ATR inhibitor	Selleckchem	S8007; CAS: 1232410-49-9
Hydroxyurea	Sigma-Aldrich	H-8627; CAS: 127-07-1
Phosphatase, Alkaline, Calf Intestine	Merck	524572; CAS: 9001-78-9
5-Bromo-2-deoxyuridine (BrdU)	Roche	10280879001; CAS: 59-14-3
Critical commercial assays		
STEMdiff™ Cardiomyocyte Differentiation Kit	STEMCELL Technologies	05010
Pierce RNA 3'end biotinylation kit	ThermoFisher	20160
CometAssay Single Cell Gel Electrophoresis Assay	R&D Systems	4250-050-K
SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian	Takara	634873
Deposited data		
RNA-Seq PCAT19 knockdown data	This paper	GEO: GSE199091
Raw mass spectrometric data of PCAT19 protein interaction partners	This paper	PRIDE: PXD032669
Experimental models: Cell lines		
Human microvascular endothelial cells (HMEC)	CDC	98247
293T/17 [HEK 293T/17] (HEK293T)	ATCC	CRL-11268; RRID:CVCL_1926
293 [HEK-293]	ATCC	CRL-1573; RRID:CVCL_0045
Human induced pluripotent stem cells (hiPSCs)	EbiSC	WSTIi081-A
Oligonucleotides		
LNA GapmeR <i>PCAT19</i> 5'-AAT TCG GCT CTT ACA A-3'	This study	N/A
Primers for 18S rRNA, GAPDH, PCAT19 and U4 snRNA, see Table S1	This study	N/A
<i>PCAT19</i> antisense-oligonucleotide (5'-Biotin-AAG CAG ACA TGA GAC CTC ACT-3')	This study	N/A
scramble control oligonucleotide (5'-Biotin-GTG TAA CAC GTC TAT ACG CCC A-3')	This study	NA/
<i>PCAT19</i> antisense-oligonucleotide (5'-TYE665-AAG CAG ACA TGA GAC CTC ACT-3')	This study	N/A
scramble control oligonucleotide (5'-TYE665-GTG TAA CAC GTC TAT ACG CCC A-3')	This study	NA/
Recombinant DNA		
Plasmid: pcDNA3.1+ <i>PCAT19</i>	This study	N/A
Plasmid: pcDNA3.1+	ThermoFisher	V79020

Plasmid: CMV Flag ATRwt	Cortez et al., 2001	Addgene plasmid #41909
Plasmid: pHAGE EF1 $\alpha$ dCas9-VP64	Kearns et al., 2014	Addgene plasmid #50918
Plasmid: pHAGE EF1 $\alpha$ dCas9-KRAB	Kearns et al., 2014	Addgene plasmid #50919
Plasmid: sgRNA(MS2) vector	Konermann et al., 2015	Addgene plasmid #61424
Plasmid: sgRNA(MS2) vector-CRISPRa-PCAT19_gRNA	This study	N/A
Plasmid: sgRNA(MS2) vector-CRISPRi-PCAT19_gRNA	This study	N/A
Software and algorithms		
FIJI/ImageJ	Schindelin et al., 2012	RRID:SCR_002285
Leica LAS X	Leica Microsystems	RRID:SCR_013673
Image Studio Ver 5.2	Licor	RRID:SCR_015795
CRISPick GPP sgRNA designer	Doench et al., 2016	<a href="https://portals.broadinstitute.org/gppx/crispick/public">https://portals.broadinstitute.org/gppx/crispick/public</a>
MaxQuant 1.6.1.0132	Tyanova et al., 2016	RRID:SCR_014485
Perseus 1.6.1.3	Tyanova et al., 2016	RRID:SCR_015753
CometScore 2.0	TriTek Corp	<a href="http://rexhoover.com/index.php?id=comet_score">http://rexhoover.com/index.php?id=comet_score</a>
FlowJo v10.8	BD Life Sciences	RRID:SCR_008520
FastQC	Andrews, 2010	RRID:SCR_014583
Trimmomatic 0.39	Bolger et al., 2014	RRID:SCR_011848
STAR 2.74.9a	Dobin et al., 2013	RRID:SCR_004463
featureCounts 2.0.2	Liao et al., 2014	RRID:SCR_012919
DESeq2 1.30.1	Love et al., 2014	RRID:SCR_015687
QuaternaryProd	Fakhry et al., 2016	<a href="https://www.bioconductor.org/packages/release/bioc/html/QuaternaryProd.html">https://www.bioconductor.org/packages/release/bioc/html/QuaternaryProd.html</a>
ClusterProfiler	Wu et al., 2021	RRID:SCR_016884
ReactomePA	Yu & He, 2016	RRID:SCR_019316
ggplot2	Wickham, 2016	RRID:SCR_014601
GraphPad Prism 9.3.1	GraphPad	RRID:SCR_002798

Figure 1



**Figure 2**

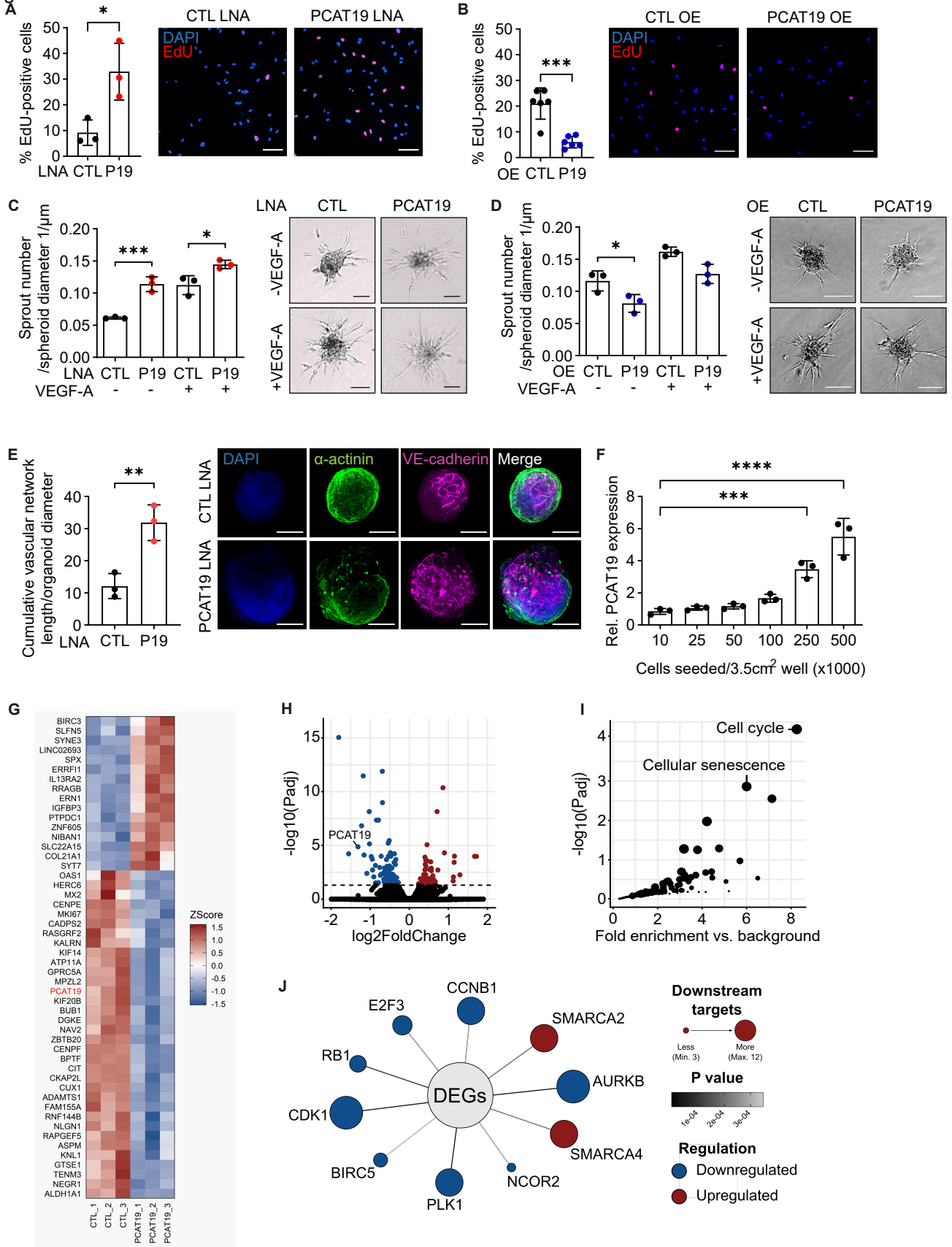


Figure 3

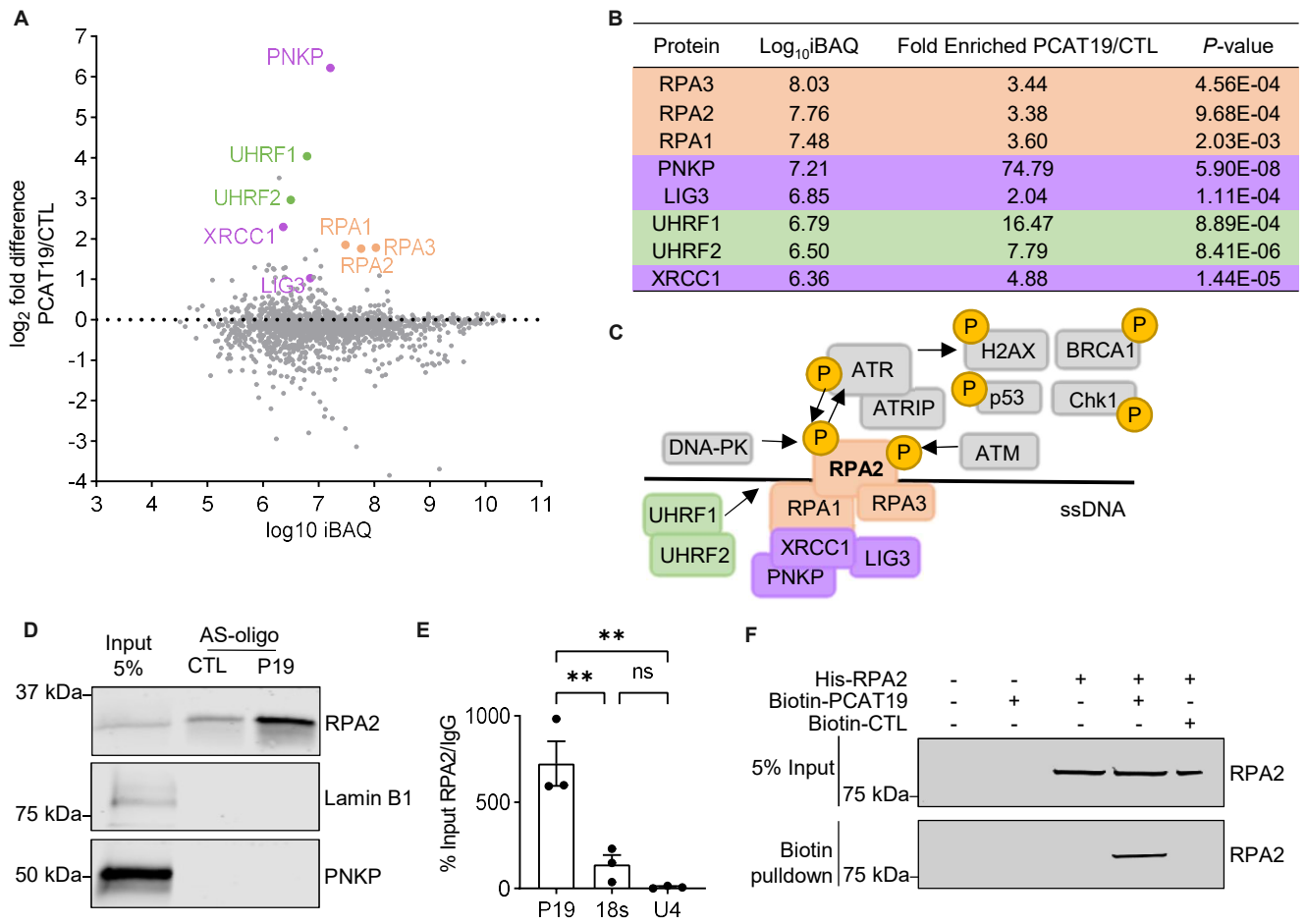


Figure 4

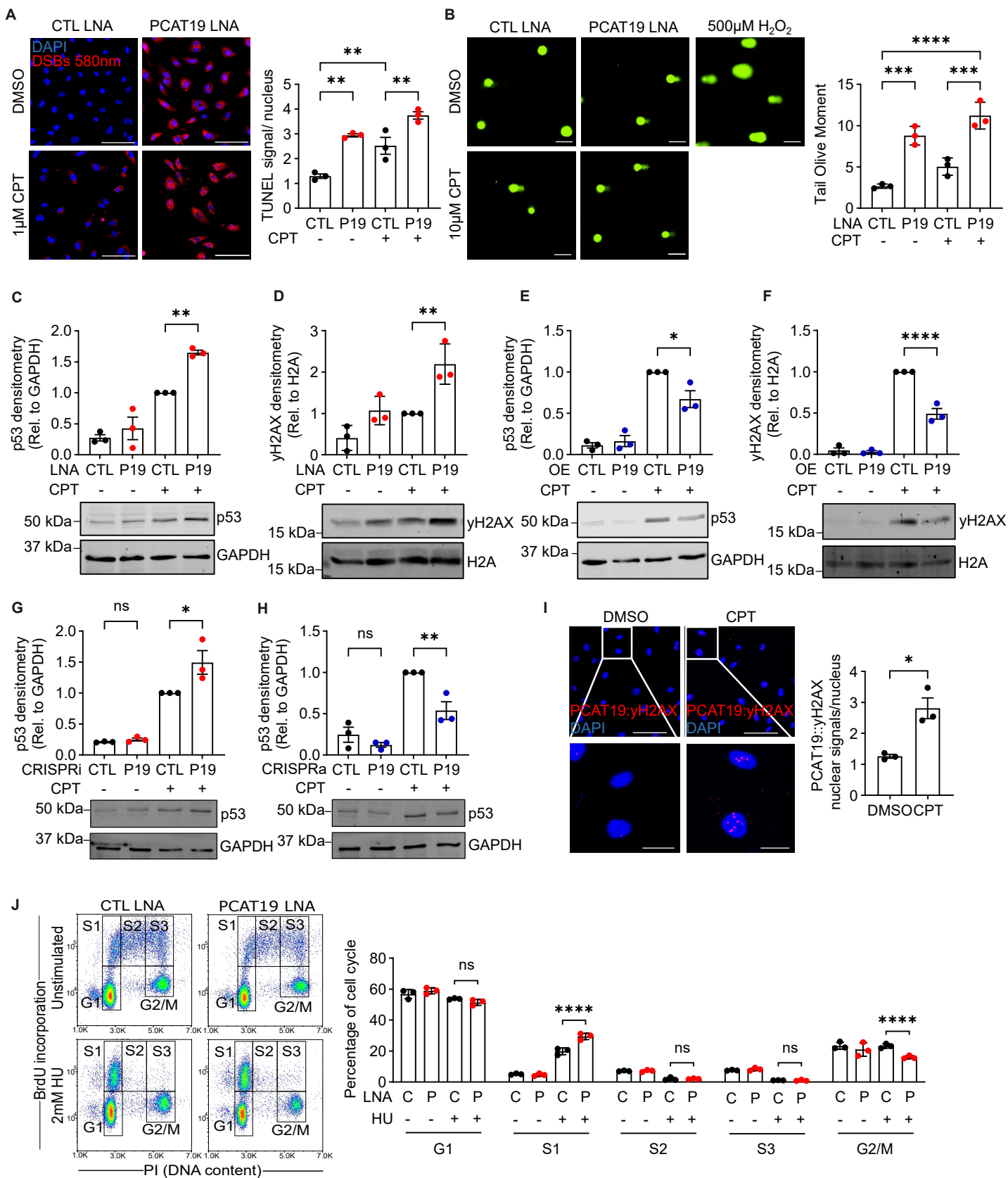
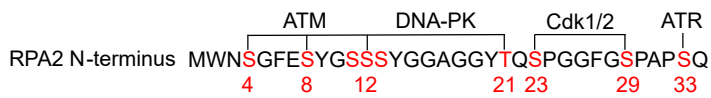
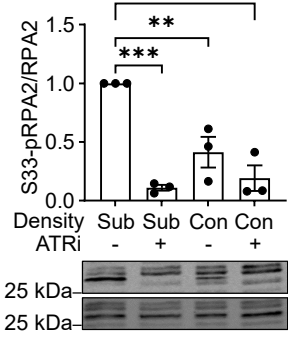


Figure 5

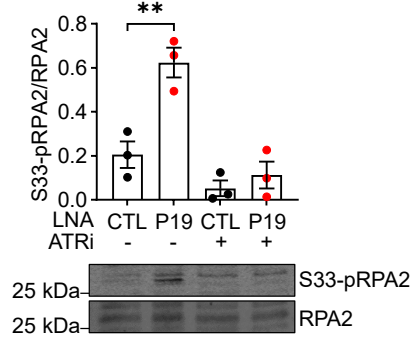
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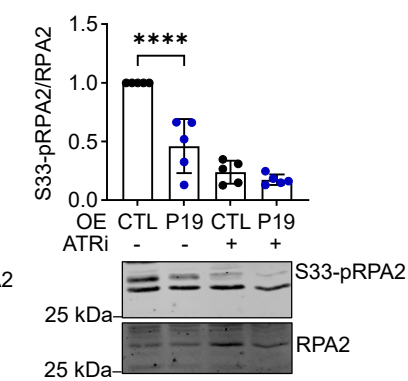
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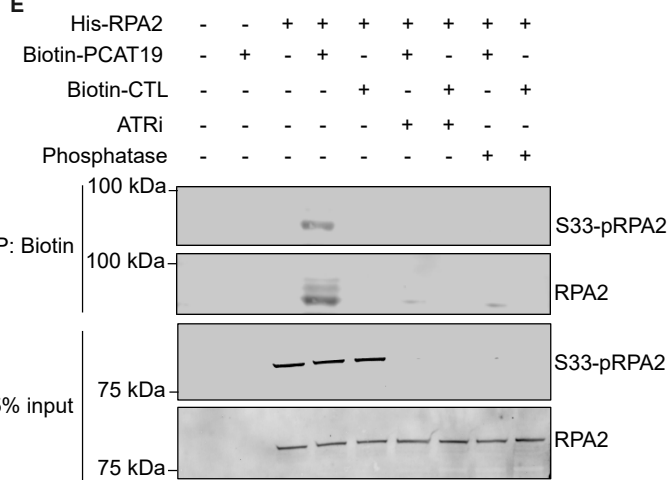
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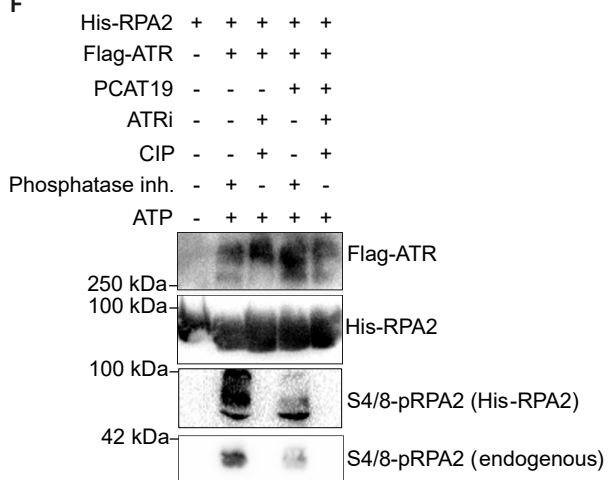
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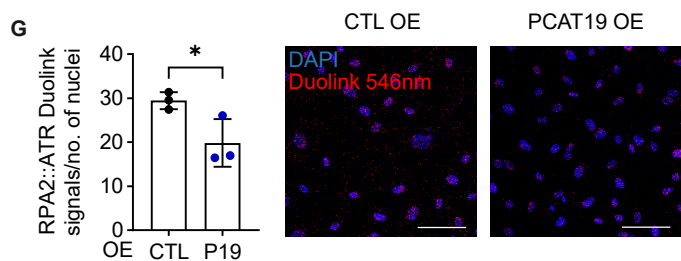
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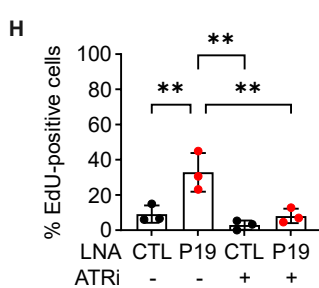
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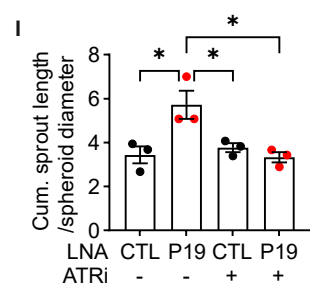
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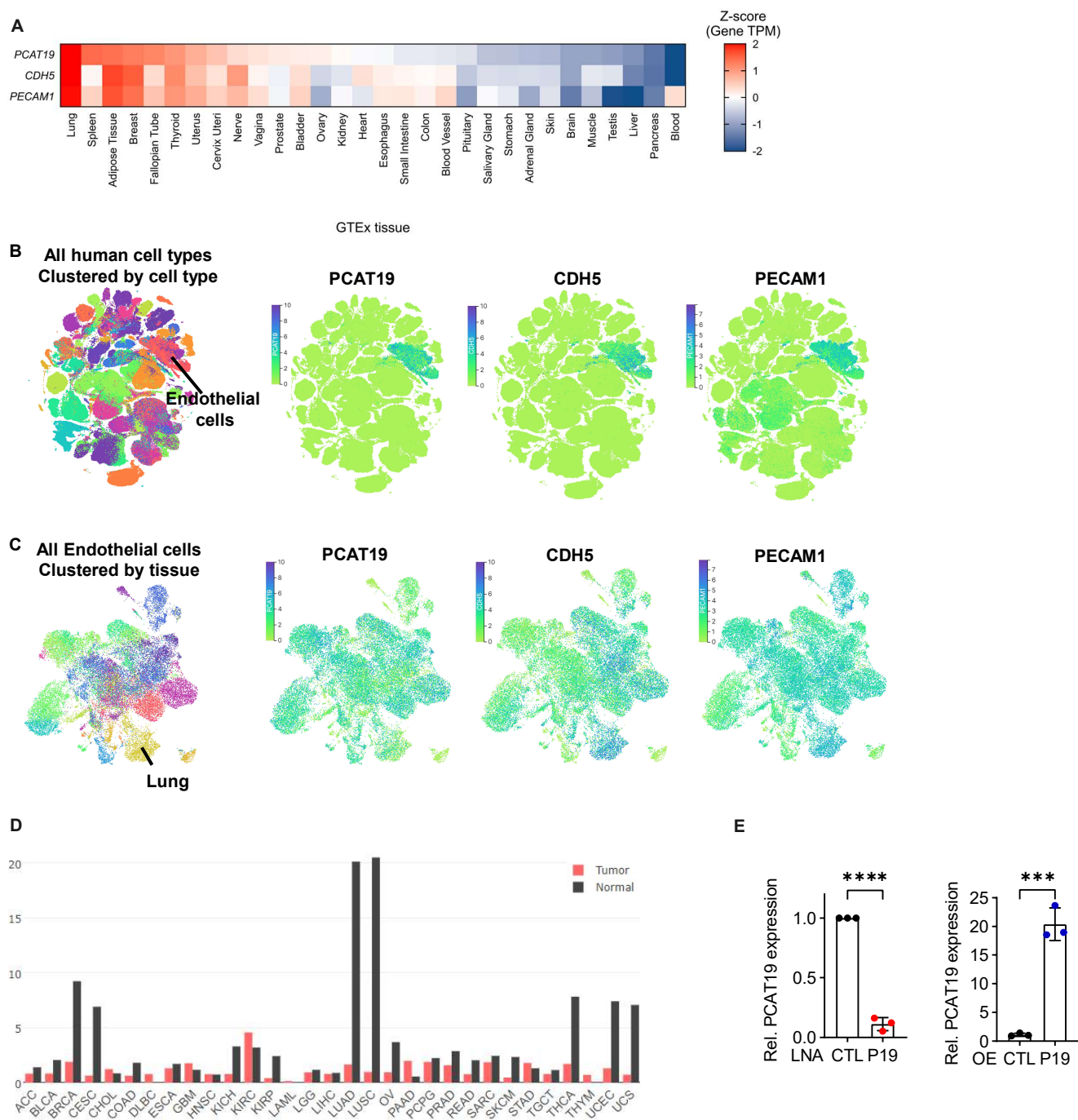
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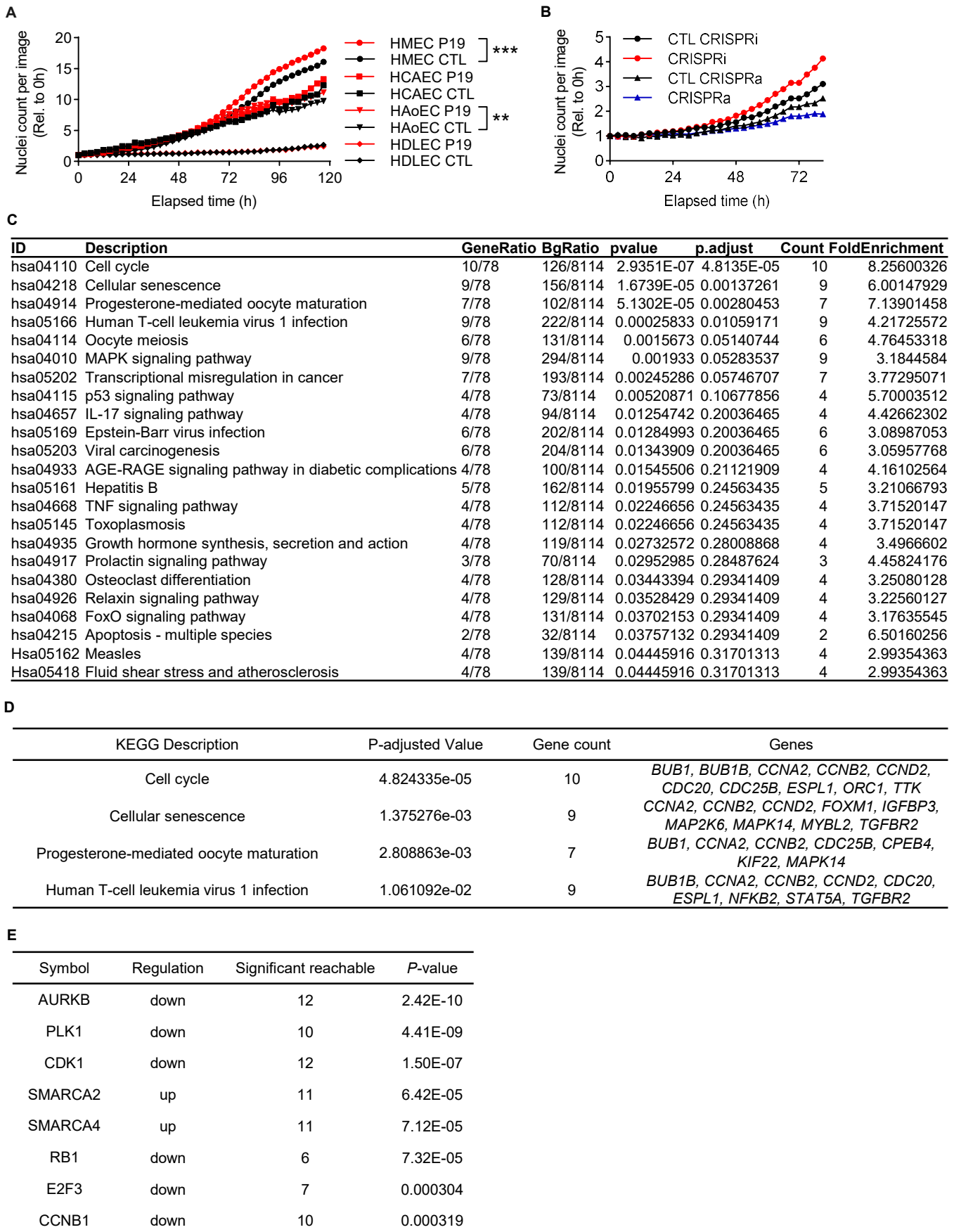




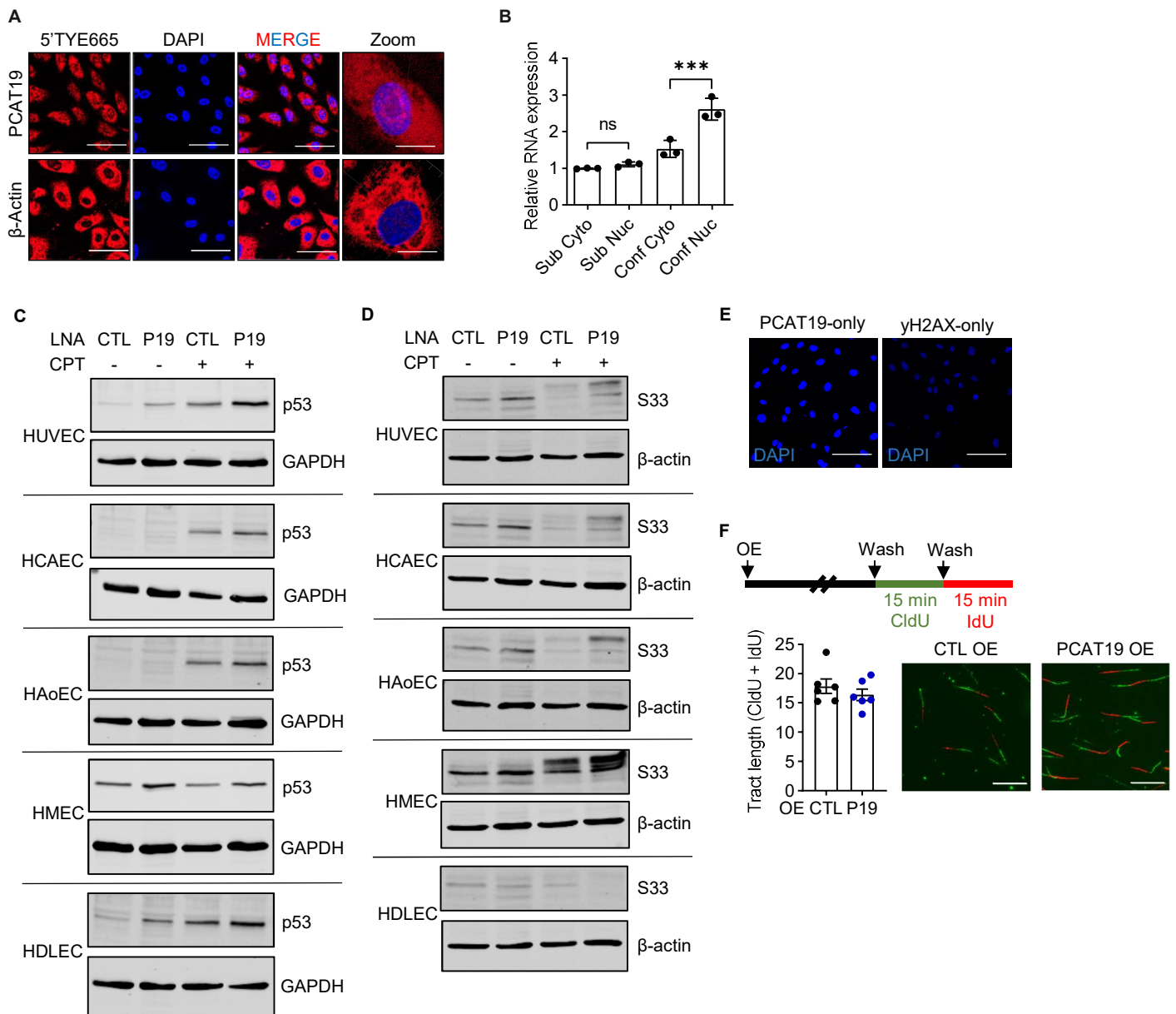


**Figure S1. PCAT19 expression, Related to Figures 1 and 2.** **A.** *PCAT19*, *CDH5* and *PECAM1* expression (Z-score of gene TPM) in normal human tissues from The GTEx Portal (GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2)). TPM, transcripts per million. **B.** *PCAT19*, *CDH5* and *PECAM1* expression in different human cell types. Clustered by cell type. Tabula Sapiens. **C.** *PCAT19*, *CDH5* and *PECAM1* expression in individual endothelial cells across all human tissues. Clustered by tissue. Tabula Sapiens. **D.** *PCAT19* expression in various tumour tissues and respective normal tissues, GEPIA database. **E.** RT-qPCR for *PCAT19* after *PCAT19* or control LNA GapmeR-mediated knockdown and *PCAT19* or pcDNA3.1+ control overexpression. HUVEC (n=3 biological replicates) for both. Unpaired t-test, \*\*\* signifies  $P < 0.001$ , \*\*\*\* signifies  $P < 0.0001$ .





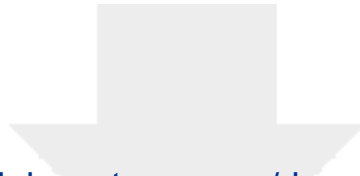
**Figure S2. PCAT19 on endothelial cell cycle, Related to Figure 2.** **A.** Proliferation measured with Incucyte imaging system after LNA GapmeR-mediated knockdown of PCAT19 (P19) compared to negative control (CTL) LNA GapmeR for HMEC, HCAEC, HAoEC and HDLEC. n=2 technical replicates, unpaired t-test of Area under the curve (AUC). \*\* signifies  $P < 0.01$ , \*\*\* signifies  $P < 0.001$ . **B.** Endothelial cell proliferation measured with Incucyte imaging system after PCAT19 CRISPRi or CRISPRa or respective negative controls. n=3 biological replicates. **C.** KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment list from 186 differentially regulated genes after PCAT19 knockdown. **D.** Differentially expressed genes associated with the top significant KEGG pathways ( $P_{adj} < 0.05$ ). **E.** Upstream regulator analysis (QuaternaryProd package). Top predicted regulators listed according to their number of significant downstream targets. \*\* signifies  $P < 0.01$ , \*\*\* signifies  $P < 0.001$ .



**Figure S3. PCAT19 subcellular localisation and effects on DNA damage markers and DNA replication, Related to Figures 1 and 4.** **A.** RNA-FISH for *PCAT19* and  $\beta$ -Actin localisation in HUVEC. Respective antisense-oligonucleotide probes tagged with 5'TYE665 (647nm). DAPI counterstaining. Scale bar represents 100  $\mu$ m and "Zoom" image scale bars represent 25  $\mu$ m. **B.** HUVEC fractionation into cytoplasm (Cyto) and nucleus (Nuc) after cell growth under both subconfluent (sub) and confluent (conf) conditions. RNA isolation and RT-qPCR for *PCAT19* and GAPDH. n=3, unpaired t-test, mean  $\pm$  SD. **C.** HUVEC, HCAEC, HAoEC, HMEC and HDLEC were transfected with *PCAT19* LNA (P19) or negative control LNA (CTL) and then treated with DMSO or camptothecin (CPT). n=1. Western blot staining for p53 and GAPDH or **D.** S33-pRPA2 (S33) and  $\beta$ -actin. **E.** RNA In Situ Hybridization Proximity Ligation Assay (rISH-PLA) *PCAT19*- and  $\gamma$ H2AX-only controls. Red signal indicates PLA signal (546nm) between *PCAT19* and  $\gamma$ H2AX, blue indicates DAPI. Scale bar represents 100  $\mu$ m **F.** HUVEC were transduced with *PCAT19* or pcDNA3.1+ control plasmids and pulsed with CldU and IdU for the DNA fibre assay. Quantification of fibres and representative images are displayed. (n=6) Scale bar represents 15  $\mu$ m. \*\*\* signifies  $P < 0.001$ .

**Table S3. Primers used in this study, Related to Figures 2 and 3.**

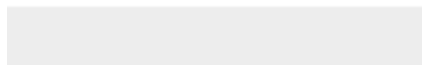
Name	Sequence (5'-3')
18S rRNA FP	CTT TGG TCG CTC GCT CCT C
18S rRNA RP	CTG ACC GGG TTG GTT TTG AT
GAPDH FP	TGC ACC ACC AAC TGC TTA GC
GAPDH RP	GGC ATG GAC TGT GGT CAT GAG
PCAT19 FP	ACC CTG CCC TTA GTC AAA TC
PCAT19 RP	TGG AAT CCC ACA CTG TTA CC
U4 snRNA FP	GCC AAT GAG GTT TAT CCG AGG
U4 snRNA RP	TCA AAA ATT GCC AAT GCC G



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**Supplemental Videos and Spreadsheets**

**Table S1- RNA-Seq.xlsx**





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**Supplemental Videos and Spreadsheets**  
**Table S2- Mass spectrometry.xlsx**

