Comparative gene expression in cells competent in or lacking DNA-PKcs kinase activity following etoposide exposure reveal differences in gene expression associated with histone modifications, inflammation, cell cycle regulation, Wnt signaling, and differentiation.

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Abstract

Maintenance of the genome is essential for cell survival, and impairment of the DNA damage response is associated with multiple pathologies including cancer and neurological abnormalities. DNA-PKcs is a DNA repair protein and a core component of the classical nonhomologous end-joining pathway, but it may have roles in modulating gene expression and thus, the overall cellular response to DNA damage. Using cells producing either wild-type (WT) or kinase-inactive (KR) DNA-PKcs, we assessed global alterations in gene expression in the absence or presence of DNA damage. We evaluated differential gene expression in untreated cells and observed differences in genes associated with cellular adhesion, cell cycle regulation, and inflammation-related pathways. Following exposure to etoposide, we compared how KR versus WT cells responded transcriptionally to DNA damage. Downregulation of pathways involved in biosynthesis were observed in both genotypes, but upregulated biological pathways

27 were divergent, again with KR cells manifesting a more robust inflammatory response compared
28 to WT cells. To determine what major transcriptional regulators are controlling the differences in
29 gene expression noted, we used pathway analysis and found that many master regulators of
30 histone modifications, proinflammatory pathways, cell cycle regulation, Wnt/β-catenin signaling,
31 and cellular development and differentiation were impacted by DNA-PKcs status. Overall, our
32 results indicate that DNA-PKcs, in a kinase-dependent fashion, decreases proinflammatory
33 signaling following genotoxic insult. As multiple DNA-PK kinase inhibitors are in clinical trial as
34 cancer therapeutics utilized in combination with DNA damaging agents, understanding the
35 transcriptional response when DNA-PKcs cannot phosphorylate downstream targets will inform
36 the overall patient response to combined treatment.
37
38 **Introduction**
39
40 Eukaryotic cells frequently experience genotoxic stress from various exogenous and
41 endogenous sources, including exposure to UV radiation, reactive oxygen/nitrogen species
42 formed during metabolism, genotoxic chemicals, and others, inducing myriad types of DNA
43 damage(1). DNA double strand breaks (DSB) are highly deleterious, which if unrepaired or
44 misrepaired can cause genomic instability associated with multiple diseases, including cancer.
45 In response to DSB, cells initiate a complex, coordinated set of pathways, collectively known as
46 DNA damage response (DDR) involving damage sensing, transferring signals via signal
47 transducers and many effector proteins to respond to damage. Ideally, the DDR serves to repair
48 DNA damage when possible through activation of repair proteins and upregulating transcription
49 of additional genes needed for a full response. However, if repair is not feasible, the DDR
50 initiates apoptotic death. In eukaryotes the majority of DSB are repaired by the nonhomologous
51 end-joining (NHEJ) and homologous recombination (HR) pathways. The DNA dependent
52 protein kinase catalytic subunit (DNA-PKcs), a member of the phosphatidylinositol 3-kinase

53 related kinase (PIKK) family of proteins including ATM and ATR, is directly involved in NHEJ-
54 mediated repair (2). DNA-PKcs is recruited to DSB by the Ku 70/80heterodimer, forming the
55 catalytically active DNA-PK holoenzyme, which in turn results in DNA-PKcs autophosphorylation
56 and phosphorylation of multiple other proteins (Ku, H2AX, RPA32). Additionally, DNA-PKcs
57 serves to tether the two broken DNA ends (3) and activates other NHEJ proteins (XRCC4, XLF,
58 Lig4 or PAXX) to facilitate DNA end processing and ultimately ligation (4, 5). DNA-PKcs
59 regulates proteins involved in HR, including ATM, WRN RPA32,cAbl and SMC1, facilitating
60 crosstalk between the DDR proteins and two dominant DSB repair pathways (2, 6-11).DNA-
61 PKcs phosphorylates KAP-1 immediately after DSB and promotes chromatin decondensation to
62 facilitate recruiting DDR proteins (12). Clearly, DNA-PKcs has multifaceted roles in regulating
63 the cellular response to DSB.
64
65 DNA-PKcs is dually involved in regulating proteins involved in cellular transcription. DNA-PKcs
66 phosphorylates Snail1, a zinc-finger transcription factor involved in the epithelial-to-
67 mesenchymal transition, which promotes its stability and function potentiation (8, 13).DNA-PKcs
68 is also involved in the activation of several metabolic genes. DNA-PKcs activation in hypoxic
69 condition protects HIF1α from degradation which promotes one of its target gene's, GluT1,
70 expression(14). DNA-PKcs promotes fatty acid biosynthesis in an insulin-dependent manner by
71 activating transcription factor USF-1 (15). During aging, DNA-PKcs phosphorylation of heat-
72 shock protein HSP90α decreases the mitochondrial function in skeletal muscles, thus
73 decreasing overall metabolism and fitness (16). DNA-PKcs was originally isolated as a
74 component of SP1 transcriptional complex (17) as well as co-eluted with the largest subunit of
75 RNA polymerase II (RNAPII) (18). DNA-PKcs phosphorylates RNAPII (19) as well as many
76 other transcription factors TFIIB, SP1, Oct1, c-Myc, c-Jun and TATA binding protein (TBP) (20).
77 The availability of DNA-PKcs at the active transcription sites modulates the function of many
78 transcription factors like, autoimmune regulator (AIRE), p53, erythroblast transformation-specific

related gene (ERG) by direct or indirect interactions (13). DNA-PKcs may mediate transcription initiation by interacting with TopoIIβ and PARP1 in the presence of DSB (21, 22).The effects of DNA-PK on global gene expression are yet unknown. In this study, we analyzed global gene expression with and without DNA damage in cell lines expressing wild-type or kinase-inactive DNA-PKcs, or cells lacking the protein to elucidate the roles DNA-PKcs has in regulating gene expression in kinase-dependent and -independent manners alone or following damage.

Materials and Methods

Cells culture:V3-derived Chinese Hamster Ovary (CHO) cell lines were kindly provided by Dr. Katherine Meek, complemented with either human wild-type (WT) or kinase inactive (K3753R) DNA-PKcs (KR) (23). Cells were cultured in complete media: α-MEM (LifeTechnologies, Waltham, MA) supplemented with 10% FBS (MilliporeSigma, St. Louis, MO), 1% penicillin/streptomycin (LifeTechnologies), 200 µg/mL G418 (LifeTechnologies) and 10 µg/ml puromycin (Santa Cruz Biotechnology, Santa Cruz, CA) at 37ºC with 5% CO_2 and 100% humidity. All chemicals were purchased from MilliporeSigma unless otherwise indicated.

Immunoblotting: Cells cultured to 80-90% confluence and whole cell lysate were prepared using RIPA buffer (50mM Tris (pH 7.4), 2mM EDTA, 150mM NaCl, 0.1% SDS, 1.0% Triton X-100) supplemented with Halt phosphatase and protease inhibitor cocktails (Fisher Scientific, Waltham, MA). Protein was quantified using the Pierce BCA Protein Assay (Fisher Scientific) per manufacturer's instructions. Immunoblotting was used to assess the production of DNA-PKcs in all cell lines. Briefly, 25µg of protein was subjected to SDS-PAGE, transferred to PVDF membrane, and blocked with 5% nonfat dried milk in 1X TBS-T (Tris buffer saline with 0.1% Tween-20) for 1 h at room temperature (RT), then primary antibody recognizing DNA-PKcs (Abcam, catalog # 70250) was added overnight at 4°C in blocking buffer. Membranes were

105 washed, then a HRP-conjugated secondary antibody (Jackson Immuno Research, catalog #
106 111035144, West Grove, Pennsylvania)in blocking buffer was added for 1h at RT. DNA-PKcs
107 protein was assessed using Clarity Western ECL Substrate (BioRad, Hercules, CA)and
108 captured using a ChemiDoc MP Imaging System (BioRad).Total vinculin levels (Santa Cruz,
109 catalog # 73614)were assessed after DNA-PKcs analysis following the same procedure.
110
111 **Clonogenic Survival Assay:** We assessed clonogenic survival as previously described (24).
112 Cells were plated onto 6 wells plates at 150 cells per well and allowed to attach for 4 hours and
113 then treated with etoposide (0.01 to 100 μM) or DMSO. After 24 hours incubation at 37°C the
114 treatment containing media was removed and the cells were re-incubated for 5 to 7 days with
115 fresh media to allow colony formation. Surviving colonies were fixed using 4%
116 paraformaldehyde and stained with 0.5% crystal violet in 20% methanol, washed with water and
117 air dried. Colonies with ≥ 50 cells were counted. Plating efficiency (PE) and surviving fraction
118 (SF) were calculated: PE = (number of colonies formed divided by the number of cells seeded)
119 x100; SF = PE of treated cells divided by PE of control cells. Data is the average of multiple
120 assays which each contained three experimental replicates.
121
122 **Cell Viability:** Cells were cultured in complete media in white-walled 96-well plates (CoStar,
123 Corning, NY) for 24 h and then treated with increasing concentrations of etoposide or DMSO
124 and incubated for 72h. Viability was quantified using the CellTiter-Glo Assay (Promega,
125 Madison, WI) per manufacturer's instructions.
126
127 **RNA Isolation:** Cells were cultured on 100 mm dishes overnight in complete medium and then
128 treated with DMSO or 20 μM etoposide for 24 h. Total RNA was isolated using Trizol reagent
129 (MilliporeSigma) isolation was according to manufacturer's instructions. RNA was dissolved in

130 nuclease-free water followed by spectrophotometric analysis of the RNA quantity; RNA was
131 subjected to electrophoresis on an agarose gel to assess RNA quality and purity.
132
133 **Transcriptome Library Preparation and Sequencing:** RNA libraries were constructed strictly
134 according to [kit or protocol credit needed] user's guide. The oligo (dT) beads were used to
135 purify poly-A mRNAs then fragmented in fragmentation buffer. The first-strand complementary
136 DNA was synthesized using random-hexamer primers. The second-strand cDNA was
137 polymerized using dNTPs, RNase H, DNA polymerase I, and buffer. After DNA library
138 quantification and validation steps, they amplified by PCR. The sequencing of amplified library
139 was conducted through Illumina paired-end runs (100 bp long reads) on HiSeq™ 2000
140 technology (Illumina Inc, USA).

141 **Differentially expressed gene analysis:** The low quality raw reads (fastq format) filtered
142 based on Q30 and GC content, then the Illumina adaptors were trimmed of reads for minimum
143 read length of 36 bases using *Trimmomatic* v0.34 (25). The index of the *Chinese hamster*
144 reference genome (CHOK1GS_HDv1) was built using HISAT2 v2.1.0.(26). Hisat2 v2.2.1 tool
145 was utilized to align the reads to the genome sequences in FASTA format and output aligned
146 reads in binary alignment map (BAM) format were translated into the transcriptomes of each
147 sample using stringtie v2.0 tool which uses a novel network flow algorithm as well as an optional
148 de novo assembly step to assemble and quantitate full-length transcripts representing multiple
149 splice variants for each gene locus (27). The stringtie outputs (GTF files) were merged to create
150 a single master transcriptome GTF with exact same naming and numbering scheme across all
151 transcripts. The *featureCounts* tool implemented under *subread v2.0* was utilized to quantify
152 transcripts assembled by stringtie mapped to each gene (28). Eventually, the differentially
153 expressed (DE) gene profiles were statistically analyzed through *edgeR* and *limma* R libraries
154 (29, 30).

155 **Biological Pathway Analyses:** The ClusterProfiler R tool was applied on the to demonstrate
156 the functional pathways and gene network enrichment analysis (31). This analysis provides
157 information related to the biological pathways significantly enriched in up- or down-regulated
158 genes through mediating the Kyoto Encyclopedia of Genes and Genomes (KEGG) database
159 and gene ontology (GO) terms (using a standard false discovery rate (FDR) < 0.05 and p-value
160 cutoff 0.05).

161 **Regulatory Network Analyses:** The upstream regulatory network analysis was performed
162 through Ingenuity Pathway Analysis (IPA, QIAGEN) platform. The IPA casual network approach
163 was applied on contrast matrices, by having gene symbol, log fold change, and p-value<0.05 of
164 each gene, to characterize the upstream regulators as well as master regulator (the root of
165 network) those responsible for driving a set of target genes(32).

166

167 **Statistical analysis:** Cell viability was analyzed using the non-linear regression function in
168 GraphPad Prism (version 8)comparing viability as a function of the log etoposide dose. The
169 area under the curve (AUC) for viability and colony forming assays was assessed using
170 GraphPad Prism (version 7; San Diego, CA), and the resultant total peak AUC and standard
171 error per sample were compared using a one-way ANOVA with an *ad hoc* Tukey's multiple
172 comparisons test.

173

174 **Results**

175

176 **DNA-PKcs promotes survival following exposure to etoposide.** We confirmed that DNA-
177 PKcs is produced in our WT and KR cells (Figure 1A). We assessed the effect of DNA-PKcs on
178 cell survivability after exposure to the topoisomerase II inhibitor, etoposide. Consistent with

179 previous reports, cells lacking DNA-PKcs kinase activity are more sensitive to etoposide (Figure
180 1B – E).
181

182 **Differential expression mediated by DNA-PKcs.** A total of 12,759 genes were of sufficient
183 quality to allow assessment of potential differential regulation in our samples (Table 1). In each
184 of our comparisons, approximately 7,000 genes were differentially expressed, either up or down
185 regulated. We assessed genotype specific differentially expressed genes (DEG) by comparing
186 DMSO-treated WT versus KR gene expression, etoposide-induced alterations within each
187 genotype compared to DMSO alone, and finally, differential expression due to drug treatment in
188 WT compared to KR cells. Each red dot is one single gene that has a significant ($p < 0.05$)
189 positive log -fold change and blue dot denotes gene with a significant negative log-fold
190 change(Figure 2A-D). Each black dot represents a gene with a p-value that was not significantly
191 altered($p \geq 0.05$).

192

193 Upon assessing DEG in KR following etoposide to those in WT, we found most genes, 2511 of
194 4333, are induced independently of DNA-PKcs kinase activity, as these were upregulated in
195 both KR and (Figure 3A), where 571 were discreetly induced in WT and 1251 were observed
196 only in KR cells. In comparing diminished expression, most, 2338 of 4466 genes were
197 downregulated independently of DNA-PKcs kinase in both KR and WT in response to etoposide
198 (Figure 3B).The number of privately downregulated DEGs are similar in WT (1088 DEGs) and
199 KR (1040 DEGs) cells (Figure 3B).

200

201 **DNA-PKcs alters the transcription of multiple genes involved in crucial cellular**
202 **processes.** DEG were analyzed with KEGG pathway database and were involved in diverse

203 cellular processes(supplemental figures S 1A – F).Upregulated genes are represented with a
204 blue to red dots and downregulated genes are represented in blue to green dots. Corresponding
205 pathways regulated by DEG are represented with a solid tangerine circle. The size of the
206 tangerine circle is correlated with the number of genes. We analyzed these cellular pathways
207 regulated by DEG to ascertain significantly altered biological processes within WT and KR cells
208 in the context of normal homeostasis (DMSO) or DNA damage (etoposide).

209 **Genotype specific alterations in biological processes.** We compared the biological
210 pathways populated by the DEG in WT and KR cells without etoposide treatment. A total of 14
211 biological pathways were enriched in upregulated DEG (Figure 4 A) and 7pathways were
212 associated with downregulation (Figure 4 B) in KR cells compared to WT.

213 We observed several genes involved in DNA damage repair pathways, including homologous
214 recombination, mismatch repair and Fanconi anemia pathways, were upregulated in KR
215 cells(Figure 4A and S1A). Genes upregulated in KR cells compared to WT were predominantly
216 involved in the cell cycle, cytokine signaling and associated anomalies, and cytoskeletal
217 regulation (Figure 4A).Genes upregulated in KR compared to WT were involved ARVC, HCM,
218 focal adhesion, axon guidance and cytokine signaling pathways which indicates DNA-PKcs
219 kinase dependent regulation of these pathways. Either these are suppressed by DNA-PKcs
220 endogenously or they are upregulated when DNA-PKcs cannot function as a kinase. In contrast,
221 genes involved in protein processing and degradation, autophagy, protein and sugar
222 metabolism were less abundant in KR cells compared to WT (Figure 4 B and Figure S1B).

223 **Etoposide-induced differentially regulated biological pathways within WT and KR cells.**
224 We analyzed the role of DNA damage in differentially regulated biological pathways between
225 cell lines. A total of 34 pathways were upregulated following etoposide treatment in WT cells
226 (Figure 5 A),whereas only two pathways were suppressed in WT cells (Figure 5 B).In KR cells,

227 37 pathways were upregulated (Figure 5 C)and 3 pathways were downregulated (Figure 5
228 D)after etoposide treatment. Comparing KR to WT following drug treatment, five pathways were
229 uniquely up regulated in WT, and 8 were observed only in KR following drug treatment. Most, 29
230 pathways of 37 were shared between genotypes (Figure 5A, 5C and 6A) indicating genes
231 represented here are upregulated independent of DNA-PKcs kinase activity. Few pathways,
232 3,decreased in response to etoposide, and 2 pathways were observed in both cell lines, again,
233 indicating they are regulated in a DNA-PKcs kinase independent manner (Figure 5B, 5D, and
234 6B).

235 Multiple DNA damage repair processes were commonly upregulated in both cell types after
236 etoposide treatment, whereas NHEJ was exclusively induced in WT cells (Figure 6A). Other
237 commonly upregulated pathways include cell cycle, DNA replication, cytokine signaling, calcium
238 signaling, and extracellular matrix (ECM)-receptor interactions are observed in both WT and KR
239 cells. The two common pathways that were downregulated in both WT and KR cells were
240 related to ribosome biogenesis and protein processing (Figure 6B).

241 **Differentially regulated biological processes between etoposide exposed WT and KR**
242 **cells.** We assessed the biological pathways impacted by differential gene regulation between
243 each genotype after exposure to etoposide to discern how DNA-PKcs regulates expression
244 changes following topoisomerase II inhibition. A total of 25 biological pathways were
245 upregulated in etoposide treated KR cells compared to WT cells, whereas only three pathways
246 were diminished (Figure 7A and B). When comparing the effects of etoposide on gene
247 expression between genotypes, we found many genes upregulated in KR cells compared to WT
248 are involved in multiple kinase-driven signaling pathways, including many involved in cancer
249 biology, TNF signaling, cell-cell and cell-matrix interactions, and DNA damage response
250 pathways. Interestingly genes involved in pathways related human papilloma virus infection
251 were also upregulated in etoposide treated KR cells compared to etoposide exposed WT cells

252 (Figure S1G). downregulated genes in KR cells compared to WT after etoposide exposure are
253 involved in mainly protein processing and carbon metabolism (Figure S1H).

254 **Analysis of global transcriptional regulators.** We evaluated our DEGs using Ingenuity
255 Pathway Analysis (IPA, © 2000-2020 QIAGEN) to determine principal transcriptional regulators
256 that drive gene expression changes observed in our samples (Table 2, Supplementary Tables
257 1-4). Master regulators controlling genes induced in KR without treatment include steroid-
258 responsive proteins such as the estrogen receptor, growth hormone, IGFBP2, corticotropin
259 releasing hormone receptor and others. We also note an enrichment in proteins involved in
260 proliferation, including some listed above, as well as, NOTCH1, and oncogenes including Raf,
261 KDR, MAP3K1FGR and RET. In addition, genes associated with the inflammatory response and
262 angiogenesis, including VEGF and its receptors were observed.

263 Master regulators controlling down regulation include ZFHX3, ETV6, SPARC, SAFB and
264 NEUROG2. Of note, many of these are potentially tumor suppressors and known modulators of
265 gross or intracellular morphology. IPA analysis reveals that drugs inhibiting VEGF and various
266 pro-growth pathways, including those induced by oncogenes such as Raf, KIT, ALK and ROS1
267 are diminished, consistent with what we observed based on upregulation. Overall, this indicates
268 that the cellular milieu induced by expressing DNA-PKcs without functional kinase activity varies
269 substantially compared to WT.

270 We also analyzed etoposide induced DEGs in all three DNA-PKcs variant cell types for
271 detecting the master transcriptional regulators that modify DNA damage related gene
272 expression. A total of 35 global regulator have been identified those were involved in the
273 upregulation of DEGs in WT after etoposide exposure (Table 2). Some of these activators are
274 HDAC2 (2.41E-15), TP63 (5.54E-15),CTNNB1 (2.23E-14), MITF (3.11E-14) andSMAD2 (6.76E-
275 13).In comparison, 30 global regulators are involved in downregulation of the DEG in etoposide
276 treated WT cells. Some of the transcriptional regulators are as follows: ETS1 (1.27E-23),

277 MEOX1 (1.94E-15), CDKN2A(3.06E-15), HDAC7 (3.07E-15), ZFHX3 (3.08E-15) and SOX11
278 (4.21E-15).In KR cells, after etoposide exposure, a total of 46 global regulators, includingETV7
279 (3.02E-19), SMAD5 (3.47E-19), CTNNB1 (3.5E-16), MEF2C (1.26E-15) and SMAD2 (6.06E-
280 15), were driving the upregulation of the genes. In contrast25 master regulators, including TOB1
281 (3.33E-19), ZGPAT (1.54E-17), MAFK (1.74E-17) and SOX11 (6.88E-17), were maintaining the
282 downregulation of the genes in etoposide treated KR cells.

283 Ten (CBX4, SMARCA4, EHF, MITF, TFDP2, SMAD2, NFIC, KDM3A, PURA and E2F2) and
284 five(RBL1, ETS1, CDKN2A, SOX11 and E2F6) master regulators were detected commonly in
285 etoposide treated WT and KR cells, up and down- regulating genes, respectively, indicating
286 these are regulated independently of DNA-PKcs. We analyzed the upstream master regulators
287 between etoposide treated WT and KR cells. Comparing the upregulated genes in etoposide
288 treated KR cells after with etoposide treated WT cells, we found14 global regulators, including
289 MRTFB (1.82E-09) and TGIF1 (3.04E-09)were involved in upregulation of genes In contrast,
290 only 4 upstream global regulators, includes HOXA4 (1.43E-09) and NAB2 (1.14E-06), were
291 driving the repression of down regulated genes in the KR cells after etoposide treatment.

292 A complete summary of IPA-designated master regulators is presented in Supplementary
293 Tables 1-4; we opted to assess the transcription regulators modified in WT versus KR cells with
294 and without drug treatment. A total of five (CCNE1, HDAC2, SMARCE1, UHRF1 and ZNF281)
295 and four (FOXP3, HDAC1, HDAC7 and NUPR1) master regulators involving in, respectively,
296 upregulation and down regulation of histone modifying genes were found in WT cells (Table 2).
297 Two (SCML2 and SNAI1) or four master transcriptional regulators (AHRR, CBFA2T3, CDKN2C
298 and TRIM24) were involved in altering transcription of histone modifying genes in KR cells.
299 Three (CBX4, KDM3A and SMARCA4) master regulators inducing histone modifying genes
300 were detected in both WT and KR cells while two regulators (DNMT3L and HDAC11) in both
301 WT and KR cells are associated with down regulation of genes controlling histone modifications.

302 We detected comparatively higher numbers of master regulators involve in development or cells
303 differentiation related DEG in WT as well as KR cells. In WT, seven regulators (E2F1, ETV1,
304 HDAC2, HOXA9, NOTCH3, TAL1 and ZNF281) induced genes involve in cells differential,
305 whereas, eight (FOXP3, GLIS2, HDAC7, HOXA4, MEOX1, NUPR1, PSIP1 and ZFHX3)
306 mediate the downregulation of DEG of similar function. Fourteen regulators (ETV7, FOXF1,
307 FOXL2, GATA2, LIMD1, MEF2C, SCML2, SNAI1, SOX2, SOX7, MRTFB, MYB, NFAT5 and
308 NOTCH4) were found in KR cells maintains the upregulation of genes involved in cell
309 differentiation. While eight master regulators (AHRR, ETV6, HOXA4, IKZF2, MAFK, NEUROG1,
310 NEUROG2 and TP73) were detected in downregulating similarly functioning in KR cells. Nine
311 (CBX4, CTNNB1, EHF, MITF, NFIC, PURA, REL, TBX2 and YAP1) and five (DNMT3L, ETS1,
312 HDAC11, SOX11 and ZFP36) master regulators were respectively mediate up and down
313 regulation of cell differentiation genes commonly in both WT and KR cells. We found 11 master
314 regulators (CCNE1, E2F1, EE2F3, FOXM1, HDAC2, NOTCH3, SERTAD1, SP3, TAL1, UHRF1
315 and ZNF217) in WT cells were maintain the upregulation of genes involved in cell cycle or
316 proliferation, while only 4 master regulators were detected which to downregulates genes from
317 the similar function. On the other hand, nine (CARL, EHMT1, ELK1, ETV7, FOXL2, LIMD1,
318 MYB, NOTCH4 and THAP12) and seven (CBFA2T3, CDKN2C, ETV6, TOB1, TP73, TRIM24
319 and ZGPAT) master regulators were involved in respectively upregulating and down regulating
320 cell cycle and proliferation mediated genes in KR cells. We also found nine master regulators
321 (CCND1, E2F2, EHF, PURA, REL, SMAD2, SMARCA4, TFDP2 and YAP1) involved in
322 upregulating cell cycle related genes commonly in both genotypes and eight (CDKN2A, E2F6,
323 HDAC11, RBL1, SOX11, SPDEF and ZFP36) were involved in down regulating. Interestingly,
324 eight master regulators (FOXP3, GLIS2, HDAC7, HOXA4, MEOX1, NUPR1, PSIP1 and ZFHX3)
325 involved in down regulating WNT signaling pathways genes were found only in WT cells,
326 whereas only one regulator, TCF7L2, were found in upregulation of genes in the same signaling
327 pathway. In KR cells two regulators (EBF2 and TAF4) were involved in upregulated genes

328 expression and only one master regulator, NEUROG1, is found controlling the down regulated
329 genes in KR cells.

330

331 **Discussion**

332

333 DNA-PKcs is a key regulator in two major DNA double strand break repair pathways, HR and
334 NHEJ (2). In parallel to its role in DNA damage response, DNA-PKcs is also involved in
335 phosphorylation of many other cellular proteins including zinc finger transcription factor (Snail1),
336 heat-shock proteins (HSP90α), RNA polymerase II, and other transcription factors including
337 SP1, Oct1, c-Myc, c-Jun (11, 33, 34) In this study, we use high throughput RNA sequence
338 technology to analyze the total mRNA transcripts isolated from DNA-PKcs WT and kinase
339 inactivated KR CHO cells after exposure to DNA damaging agent etoposide. We applied
340 bioinformatic tools to analyze our RNA sequence data to analyze the differentially regulated
341 biological pathways depend on DNA-PKcs kinase activity with or without DNA damage
342 response.

343 We found a high number of DEG in all samples compare to each other. Previously, using
344 subtractive hybridization technique of cDNA, a study demonstrated a limited role of DNA-PKcs
345 in transcription after DNA damage (35). Similarly, low differential expression of other DNA repair
346 genes was observed in DNA-PKcs deficient human glioblastoma cell line M059J cells (36).
347 Microarray based gene expression analysis identified upregulation of 15 genes in DNA-PKcs
348 depleted HeLa cells using targeted siRNA, most of the genes were associated with interferon-
349 mediated signal transduction and cell proliferation (37). They also found eight downregulated
350 genes, involved as nuclear factors of activated T lymphocytes. Recently a microarray based
351 analysis of DEG showed that DNA-PKcs depletion induced upregulation of 418 and
352 downregulated 1316 genes in human prostate cancer cell line, whereas Inhibition of DNA-PKcs

353 by chemical inhibitor upregulated 1571 genes and downregulated 3270 genes (33). We
354 conducted DEG analysis on DNA-PKcs wildtype and stable kinase inactivated CHO cell lines
355 using sophisticated high through-put RNAseq technology. We detected more than 7000 DEG in
356 KR cells compared to WT, while, etoposide treatment alone changed the expression of more
357 than 6500 and 7100 genes in WT and KR respectively.

358 Differentially expressed genes were subjected to enrichment analysis using KEGG pathway
359 database. We prepared bar plots demonstrating the over-expressed or repressed biological
360 processes related to the upregulated or downregulated genes in WT or KR cells with or without
361 etoposide treatment. Upregulated genes in DMSO treated KR cells compared to WT were
362 enriched three DNA damage repair pathways including HR. DNA-PKcs is a core component of
363 NHEJ mediated DSB repair, and as KR cells express a catalytically inactive DNA-PKcs, these
364 cells rely more heavily on HR to repair endogenously formed DSB. We also observed
365 upregulation of several cell genes involved in cell cycle regulation and proliferation, in
366 agreement with our previous work indicating DNA-PKcs regulates replication in a kinase-
367 dependent fashion (6, 8). Genes from two DNA repair clusters, HR and Fanconi anemia
368 predictably connected to the cell cycle via ATR (Figure S1).

369 Other genes upregulated in KR cells compared to WT were predominantly enriched cell cycle,
370 cytokine signaling and associated anomalies, including arrhythmogenic right ventricular cardio
371 myopathy (ARVC) and hypertrophic cardiomyopathy (HCM), and cytoskeletal regulation (Figure
372 and S1). The upregulation of these genes in KR compared to WT without etoposide exposure
373 indicates DNA-PKcs kinase dependent regulation of these pathways in unstressed conditions.
374 Therefore, fully functional endogenously expressed DNA-PKcs would normally suppress these
375 pathways via its kinase activity. Induction of apoptosis in cardiomyocytes is associated with
376 ARVC. N226S mutation in human *DSG2* gene, which is important in structural integrity of
377 cardiac discs by Ca^{2+} binding in intracellular cadherins molecule for cell-cell interaction, has

378 been associated with ARVC (44). High inflammatory infilters and coagulative necrosis as well as
379 apoptotic cardiomyocytes are observed in cardiac restricted over-expression of N271S mutation
380 (mice homolog of human N22S mutation) containing transgenic mice (45). Mitochondrial
381 dysfunction related oxidative stress in also associated with ARVC (reviewed in (46)). More in-
382 depth investigation of the role of DNA-PKcs on ARVC is required to understand its progression.

383 Down regulated genes in KR compared to WT cells can be clustered into two larger groups of
384 biological pathways, protein metabolism (includes protein processing, degradation, autophagy
385 and lysosome) and sugar metabolism (amino and nucleotide sugar metabolism) (Figure 4 B and
386 Figure S1B). Interestingly, Goodwin et. al. previously reported that genes involve in HR,
387 mismatch repair, cell cycle and DNA replication pathways were repressed in DNA-PKcs
388 inhibited prostate cancer cell lines (35). The same study showed over-expression of various
389 metabolic pathways in DNA-PKcs depleted cancer cells (35). Our observation did not follow the
390 previously reported result where multiple genes associated with metabolic pathways were
391 repressed because of the downregulation of related genes in absence functional DNA-PKcs in
392 KR cells. These discrepancies may be attributable to our assessment in kinase inactivated CHO
393 cells compared using DNA-PKcs inhibitor or siRNA mediated DNA-PKcs depletion.
394

395 We observed that 29 biological pathways were commonly upregulated in both WT and KR cell
396 (Figure 6A). The genes involved in these pathways are upregulated in a DNA-PKcs kinase
397 independent manner, which may indicate ATM-mediated regulation. ATM plays major roles in
398 the DDR, namely assisting in pathway choice between NHEJ and HR. In addition, ATM and
399 DNA-PKcs share many protein targets, often phosphorylating the same proteins. Previous
400 studies consistently indicate altering DNA-PKcs reduces the level of ATM in only in absence of
401 the DNA-PKcs protein, whereas the ATM levels remain same in WT in kinase inactivated KR
402 cells (37-39). Similar ATM expression in WT and KR cells after etoposide exposure coupled with

403 a lack of DNA-PKcs kinase activity may modify gene expression differences noted between WT
404 and KR cells (40). Several genes involved in HR, hepatitis B signaling, rheumatoid arthritis,
405 p53 signaling pathway and IL-17 signaling pathway were upregulated following etoposide
406 treated KR cells alone, indicating their transcription may regulated by DNA-PKcs kinase and
407 other DNA damage response proteins, including ATM. HR differences are noted in the WT
408 versus KR cells following exposure to other genotoxic agents, as we demonstrated the KR cells
409 promote higher levels of recombination compared to WT cells or cells lacking DNA-PKcs (8).
410 Future studies will be aimed at determining whether ATM may also be promoting alterations in
411 gene expression in the context of DNA-PK inhibition.

412

413 To assess the influence DNA-PKcs exerts the cellular transcriptome following DNA damage, we
414 treated cells with etoposide (topoisomerase II inhibitor, a widely used DNA damaging agent)
415 and analyzed the transcriptomes. Genes involved in NHEJ are discretely upregulated in WT
416 cells in presence of fully functional DNA-PKcs. Etoposide exposure modified the global
417 transcription of several genes in all three genotypes. in both genotypes, transcripts involved in
418 ECM – receptor interaction, HR, Fanconi Anemia and cell cycle regulation are upregulated
419 following etoposide exposure, and genes involved to ribosome biogenesis are downregulated
420 independently of DNA-PKcs. Previous report suggested several DNA repair proteins have
421 defined role in ribosome biogenesis and vice versa (41). In WT cells, genes involved in calcium
422 signaling, mismatch repair and DNA replication were upregulated in WT and KR cells in
423 response to etoposide only in presence of DNA-PKcs protein. Upregulation of these genes in
424 both WT and KR cells indicate their transcriptional regulation might be independent to DNA-
425 PKcs kinase activity in response to etoposide exposure. Genes associated with various DNA
426 repair pathways including HR, mismatch repair, nucleotide excision repair (NER), base excision
427 repair (BER) and FA along with DNA replication a were upregulated in WT and KR cells after

428 treatment with etoposide. Genes involved to HIF-1 signaling pathways were repressed in WT
429 after etoposide exposure which support the fact that DNA-PKcs positively regulates transcription
430 factor HIF1(14).In response to etoposide, genes modulating NFκB signaling were upregulated
431 KR cells; previous studies suggested ATM may contribute to secretion of signaling molecules
432 like NFκB (42) and inflammatory cytokines like interleukin-6 and interleukin-8 following DNA
433 damage(43). Consistent with our data, adipocyte degeneration due to over activation of
434 transcription of chronic pro-inflammatory factors IL-6, TNF and KC (murine homolog to IL-8)
435 were correlated with persistent DNA damage in mice (44). DNA damage induces secretion of
436 NFκB, which can activate interferon (IFN) signaling and induce the expression of IFN-α and IFN-
437 λ genes (45). Etoposide induced genes associated with cardiovascular disease in KR cells.
438 Induction of apoptosis in cardiomyocytes is associated with ARVC. N226S mutation in human
439 *DSG2* gene, which is important in structural integrity of cardiac discs by Ca^{2+} binding in
440 intracellular cadherins molecule for cell-cell interaction, has been associated with ARVC (46).
441 High inflammatory infilters and coagulative necrosis as well as apoptotic cardiomyocytes are
442 observed in cardiac restricted over-expression of N271S mutation (mice homolog of human
443 N22S mutation) containing transgenic mice (47). Mitochondrial dysfunction related oxidative
444 stress in also associated with ARVC (reviewed in (48)). Many genes associated with amino
445 acid biosynthesis and protein metabolism were repressed in KR cells after etoposide exposure
446 suggesting their expression controlled either by DNA-PKcs kinase activity.

447 We analyzed the upstream regulators those are involved in globally regulating the transcription
448 of the genes with or without etoposide treatment. Among genotypes the changes in gene
449 expression were dominantly regulated by Akt signaling pathway. A large percentage of the
450 master regulators are fall within the category of transcription regulators, chemical drugs,
451 enzymes and other biological drugs. Among others, upstream regulators included cytokines,
452 growth factors, (e.g. VEGF), G-protein coupled receptors, various kinase and transporters.

453 Some peptidase and phosphatases are also found as master regulator in etoposide treated WT
454 and KR cells. Notably, alterations in histone modifiers, cancer signaling/proliferation/apoptosis,
455 Wnt/β-catenin signaling, and regulators of development and differentiation were altered in our
456 samples (Table 2). Wnt signaling was more frequently observed in WT cells, indicating DNA-
457 PKcs may regulate this pathway, as we have previously reported (49). The abundance of
458 regulators associated with development, differentiation, and steroid signaling are likely due to
459 the origin of the cells as oocytes. However, we have previously indicated a role for DNA-PK in
460 differentiation and stemness (49), so these observations warrant additional investigation.
461 Though common regulators were observed, so were discreet regulators altered in either the WT
462 or KR cells, suggesting DNA-PKcs, in a kinase-dependent fashion, modifies a subset of
463 transcriptional regulators following DNA damage. The KR cells produce a more robust
464 inflammatory response to DNA damage, which may induce further secondary tumor formation if
465 a patient is treated with a DNA-PK inhibitor in combination with chemo- or radiotherapy. This
466 would a significant modification of the tumor microenvironment and may lead to secondary
467 tumor formation. Further investigations into the clinical ramifications of this data will clarify if
468 DNA-PKcs mitigates the inflammatory response following DNA damage.

469 Overall this global transcriptome study will help us to identify role of DNA-PKcs in transcriptional
470 regulation with or without DNA damage response. We anticipate our results will provide
471 additional knowledge about how DNA-PKcs might modify the transcriptional response to DNA
472 damage, leading to alterations of many essential cellular pathways, that ultimately dictate
473 genomic stability. Multiple DNA-PKcs inhibitors are in clinical trials as potential therapeutic
474 interventions in multiple cancer subtypes coupled with traditional chemotherapy and/or
475 radiotherapy; however, we lack a full appreciation for how inhibition of DNA-PKcs kinase will
476 alter the cellular transcriptional response to DNA damage. Our results indicate impairing DNA-
477 PKcs kinase activity may profoundly impact the transcriptional response to DNA damage,

478 modulating the proclivity for secondary tumor formation both via impairing traditional DNA
479 damage signaling and repair, as well as impairing normal transcriptional changes aimed at
480 maintaining genomic stability.

481

482

483

484 **Table and Figure Legends**

485

486 **Table 1. Summary of differentially expressed genes in cells expressing wild-type DNA-**
487 **PKcs compared to two mutant cell lines with and without exposure to etoposide.**

488

489 **Table 2. Summary of transcriptional regulators dictating differential gene expression in**
490 **cells expressing wild-type (WT) or kinase inactive (KR) DNA-PKcs with and without**
491 **exposure to etoposide.**

492

493 **Supplementary Table 1. Genotypic master regulators controlling the gene expression**
494 **differences observed in cells producing wild-type (WT) or kinase inactive (KR) DNA-**
495 **PKcs.**

496

497 **Supplementary Table 2. All master regulators controlling the gene expression differences**
498 **observed in cells producing wild-type (WT) DNA-PKcs following etoposide treatment.**

499

500 **Supplementary Table 3. Master regulators controlling the gene expression differences**
501 **observed in cells producing kinase-inactive (KR) DNA-PKcs following etoposide**
502 **treatment.**
503
504 **Supplementary Table 4. Genotypic master regulators controlling the gene expression**
505 **differences observed in cells producing wild-type (WT) or kinase inactive (KR) DNA-PKcs**
506 **after etoposide exposure.**
507
508
509 **Figure 1: DNA-PK protects cells from etoposide toxicity via its kinase domain.** (A)
510 Evaluation of DNA-PK proteins in CHO variants by western blot. (B, C) Cells survival profile of
511 CHO cells assessed using the CellTiter Glo (CTG) assay with increasing doses (0.003 – 1 μM)
512 of etoposide at 72 h post-exposure with associated area under the curve analysis. (D, E)
513 Clonogenic survival of DNA-PKcs WT, Null, or KR cells following exposure to etoposide (0.003
514 – 1 μM) with associated area under the curve analysis.
515
516 **Figure 2: Differentially expressed genes in DNA-PK wild-type (WT) versus kinase-inactive**
517 **(KR) cells.** Each red dot is one single gene that has a significant ($p < 0.05$) positive log -fold
518 change and blue dot denotes gene with a significant negative log-fold change. Each black dot
519 defines a gene with a p-value that was not significantly altered ($p > 0.05$). A – C, represent the
520 genes differentially expressed between DNA-PK variant genotypes. D – F, represent the
521 differentially expressed genes following etoposide treatment in different genotypes. G – I,
522 represent the differentially expressed genes within etoposide treated genotypes.
523
524 **Figure 3: Comparison of shared versus discreet differentially regulated genes in DNA-PK**
525 **wild-type (WT) versus kinase-inactive (KR) cells.** A, Assessment of differentially regulated

526 genes between genotype. B, Assessment of differentially regulated genes following etoposide
527 treatment. Comparisons are made to DMSO-treated controls within genotype. C, Assessment of
528 genotype-specific differentially expressed genes following etoposide treatment.
529
530 **Figure 4: Genotype-specific biological pathways altered by differential gene regulation in**
531 **DNA-PKcs wild-type (WT) versus kinase-inactive (KR) without drug exposure.** A,
532 Genotypic specific upregulated biological processes. B, Genotypic specific downregulated
533 biological processes.
534
535 **Figure 5: Etoposide-induced biological pathways altered by differential gene regulation**
536 **in DNA-PKcs wild-type (WT) and kinase-inactive (KR) cells.** A, Etoposide induced
537 downregulated biological processes within WT cells. B, Etoposide induced upregulated
538 biological processes in WT cells. C, Etoposide induced downregulated biological processes
539 within KR cells. D, Etoposide induced upregulated biological processes in KR cells.
540
541 **Figure 5: Comparison of shared versus divergent etoposide-induced biological pathways**
542 **altered by differential gene regulation in DNA-PKcs wild-type (WT) compared to kinase-**
543 **inactive (KR) cells.** A, Etoposide induced downregulated biological processes within WT cells.
544 B, Etoposide induced upregulated biological processes in WT cells. C, Etoposide induced
545 downregulated biological processes within KR cells. D, Etoposide induced upregulated
546 biological processes in KR cells.
547
548 **Figure 7: A comparative assessment of etoposide-induced biological pathways altered**
549 **by differential gene regulation in DNA-PKcs wild-type (WT) and kinase-inactive (KR) cells.**
550 A, Genotypic specific upregulated biological processes. B, Genotypic specific downregulated
551 biological processes. C, Etoposide induced upregulated biological processes within genotypes.

552 D, Etoposide induced downregulated biological processes within genotypes. E, Etoposide
553 induced upregulated biological processes between genotypes. F, Etoposide induced
554 downregulated biological processes between genotypes.
555
556 **Supplemental Figure 1 (A – Q) :** Bar-plots represented differentially regulated biological
557 pathways in DNA-PKcs variants genotypes followed by etoposide exposure.
558
559 **Supplemental Figure 2 :** Nearest neighbor plots represents major differentially regulated
560 biological pathways in DNA-PKcs variants genotypes followed by etoposide exposure.
561
562 **Supplemental Figure 3 :** Differentially regulated genes with major biological pathways in DNA-
563 PKcs variants genotypes followed by etoposide exposure.
564
565
566
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Comparisons	# Down	# Up	Sum changed	# Unchanged	Sum All
Genotype-specific alterations (DMSO only)					
WT vs KR	3818	3798	7616	5143	12759
Etoposide-induced alterations by genotype					
WT	3426	3082	6508	6251	12759
KR	3378	3762	7140	5619	12759
Genotype-specific alterations (Etoposide treatment)					
WT vs KR	3285	3604	6889	5870	12759

Table 1.

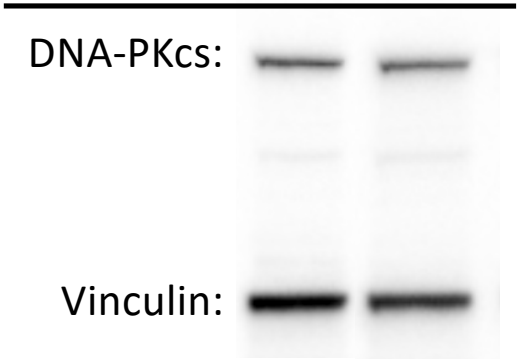
Activated in response to etoposide		
KR	WT	SHARED
CALR	CCNE1	CBX4
CAND1	E2F1	CCND1
CREB1	E2F3	CTNNB1
EBF2	ETV1	E2F2
EHMT1	FOXM1	EHF
ELK1	HDAC2	KDM3A
ETV7	HOXA9	MITF
FOXF1	NOTCH3	NFIC
FOXL2	SERTAD1	PURA
GATA2	SMARCE1	REL
HES1	SP3	SMAD2
LIMD1	TAL1	SMARCA4
MEF2C	TCF7L2	TBX2
MRTFB	TP63	TFDP2
MYB	UHRF1	TP63
NFAT5	WBP2	YAP1
NOTCH4	ZNF217	
SCML2	ZNF281	
SMAD5		
SNAI1		
SOX2		
SOX7		
TAF4		
TAF4B		
THAP12		

Inhibited in response to etoposide		
KR	WT	SHARED
AHRR	FOXH1	CDKN2A
CBFA2T3	FOXP3	DNMT3L
CDKN2C	GLIS2	E2F6
ETV6	HDAC1	ETS1
HOXA4	HDAC7	HDAC11
IKZF2	HEYL	RBL1
MAFK	HOXA4	SOX11
NEUROG1	MEOX1	SPDEF
NEUROG2	NFX1	ZIC2
NONO	NOSTRIN	
TOB1	NUPR1	
TP73	PSIP1	
TRIM24	PTTG1	
ZFP36	TRERF1	
ZGPAT	ZFHX3	
	ZFP36	

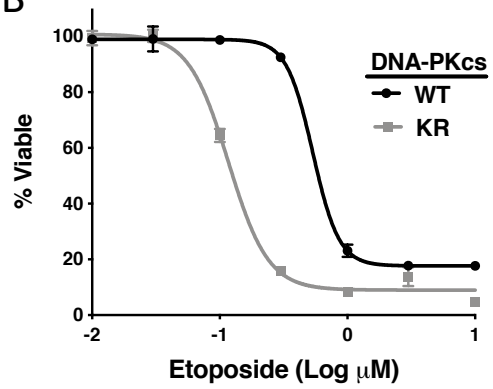
Table 2.

Fig 1

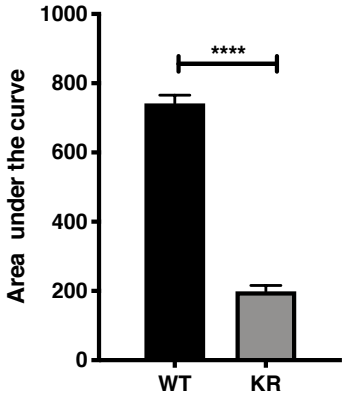
A


Genotype: WT KR
DNA-PKcs:
Vinculin:

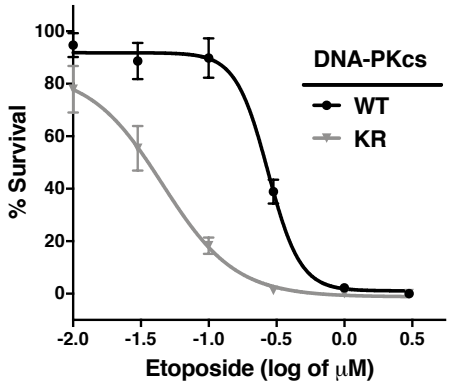
B


% Viable
Etoposide (Log μM)
DNA-PKcs
WT
KR

C


Area under the curve
****
WT
KR

D


% Survival
Etoposide (log of μM)
DNA-PKcs
WT
KR

E

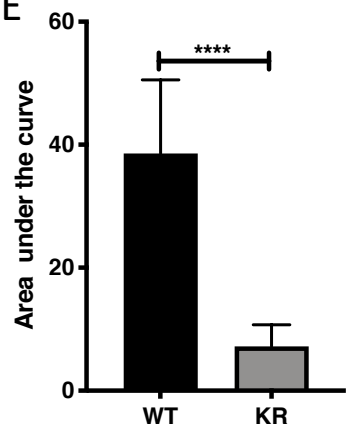

Area under the curve
****
WT
KR

Fig 2

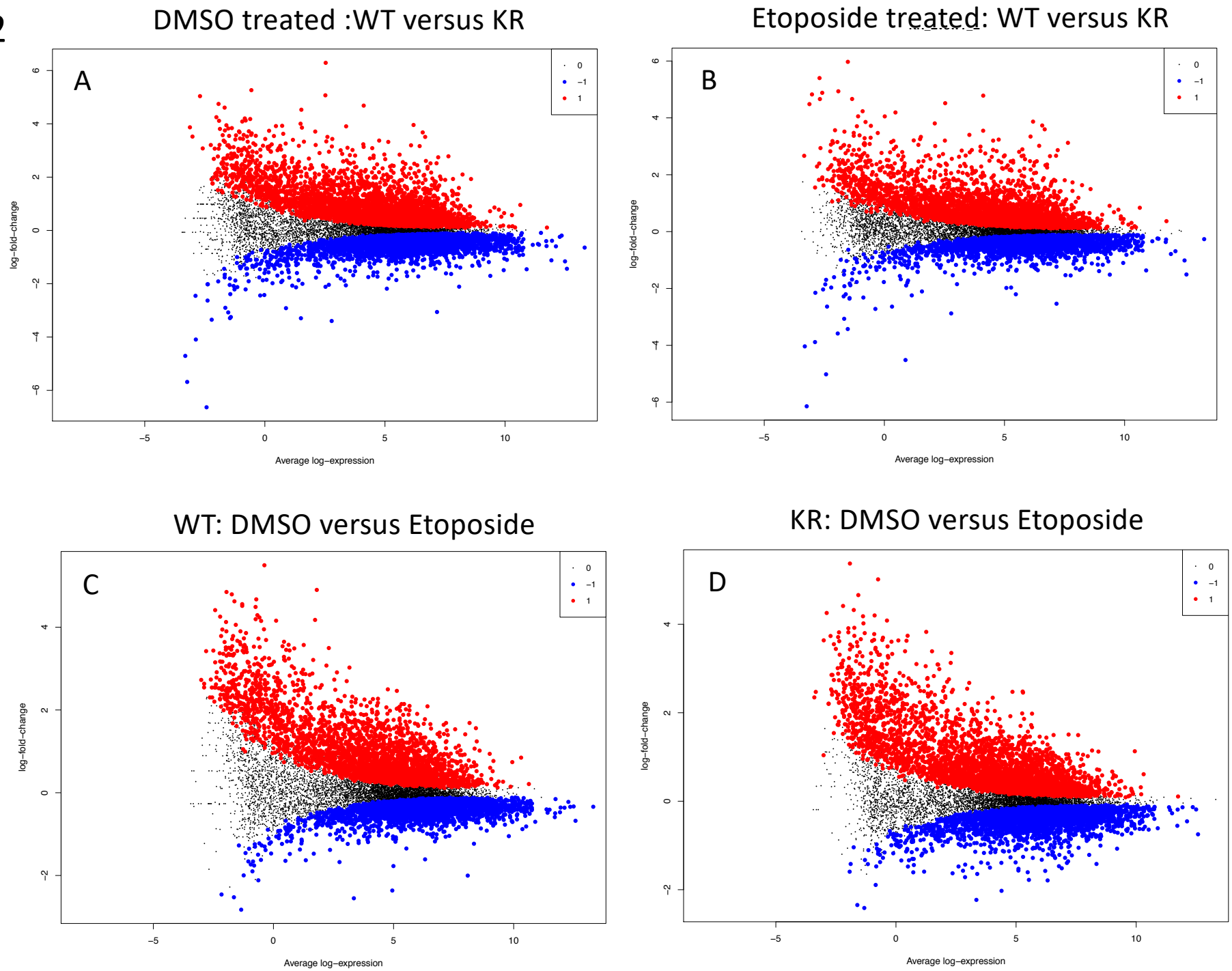
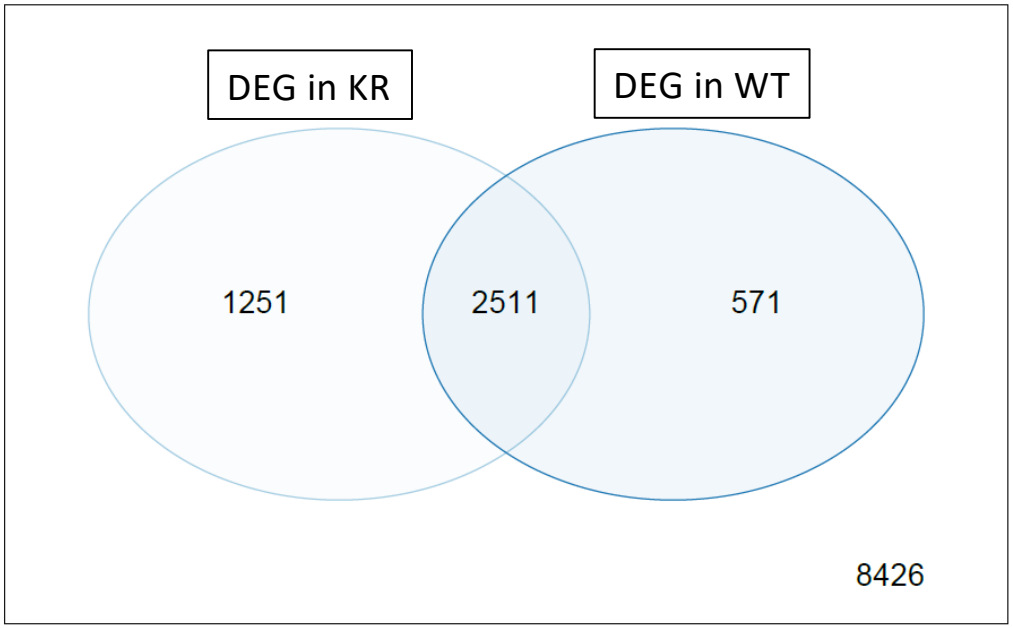
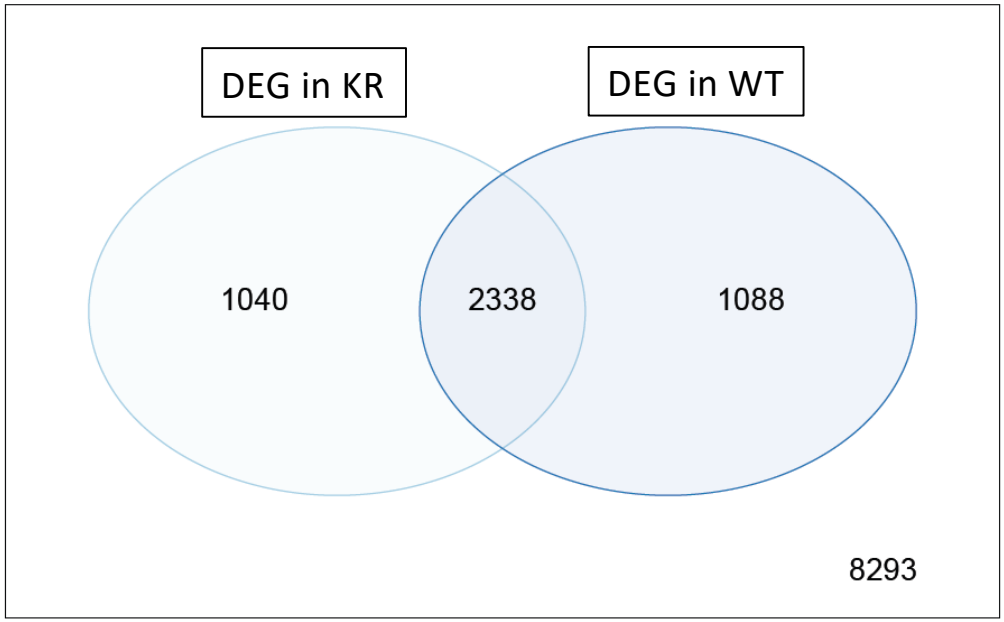

DMSO treated :WT versus KR
A
Etoposide treated: WT versus KR
B
WT: DMSO versus Etoposide
C
KR: DMSO versus Etoposide
D
log−fold−change
Average log−expression
0
−1
1

Fig 3



Up only



Down only

Fig 4A

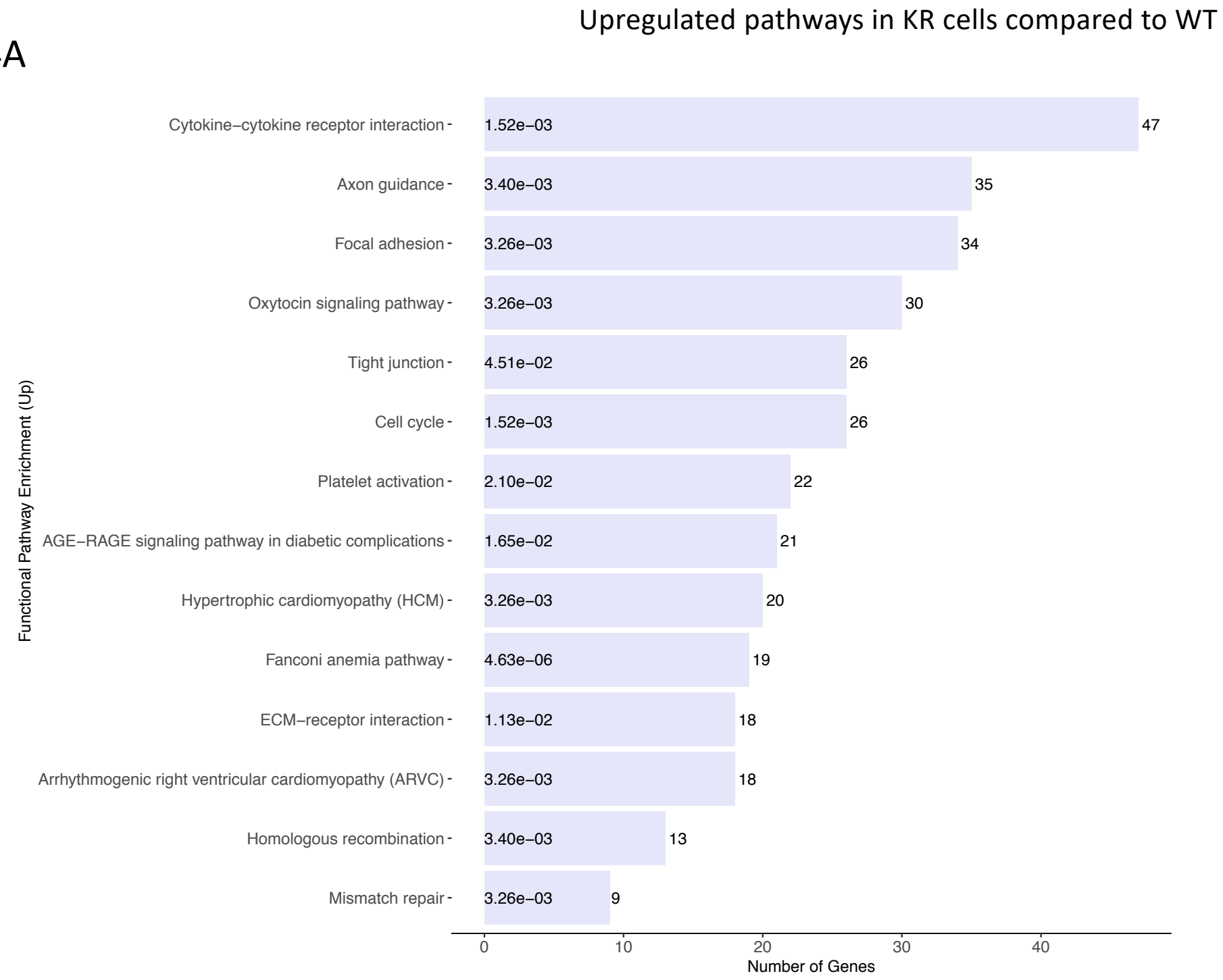

Upregulated pathways in KR cells compared to WT
Functional Pathway Enrichment (Up)
Cytokine−cytokine receptor interaction
1.52e−03
47
Axon guidance
3.40e−03
35
Focal adhesion
3.26e−03
34
Oxytocin signaling pathway
3.26e−03
30
Tight junction
4.51e−02
26
Cell cycle
1.52e−03
26
Platelet activation
2.10e−02
22
AGE−RAGE signaling pathway in diabetic complications
1.65e−02
21
Hypertrophic cardiomyopathy (HCM)
3.26e−03
20
Fanconi anemia pathway
4.63e−06
19
ECM−receptor interaction
1.13e−02
18
Arrhythmogenic right ventricular cardiomyopathy (ARVC)
3.26e−03
18
Homologous recombination
3.40e−03
13
Mismatch repair
3.26e−03
9
0
10
20
30
40
Number of Genes

Fig 4B

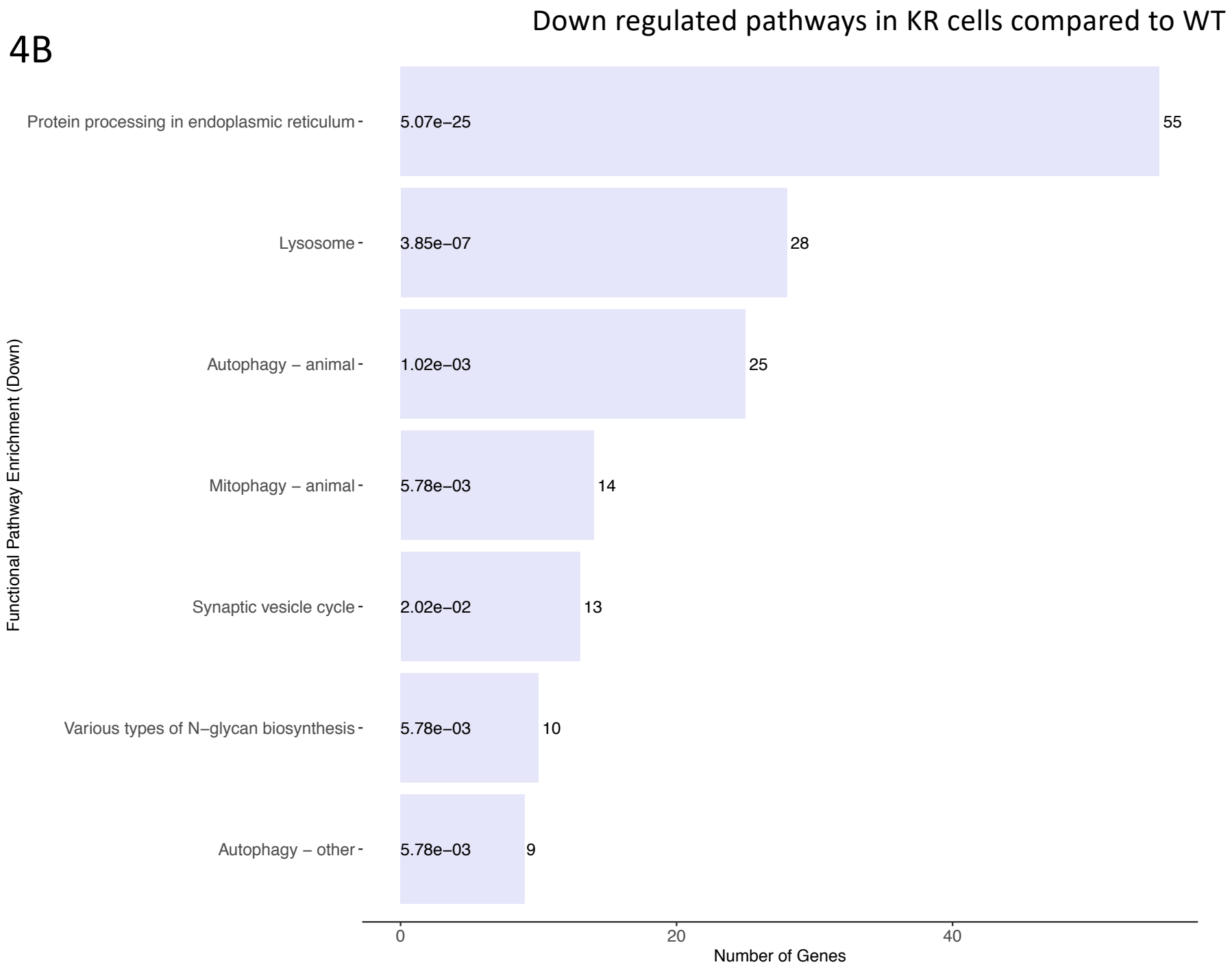

Down regulated pathways in KR cells compared to WT
Functional Pathway Enrichment (Down)
Protein processing in endoplasmic reticulum
5.07e−25
55
Lysosome
3.85e−07
28
Autophagy – animal
1.02e−03
25
Mitophagy – animal
5.78e−03
14
Synaptic vesicle cycle
2.02e−02
13
Various types of N−glycan biosynthesis
5.78e−03
10
Autophagy – other
5.78e−03
9
0
20
40
Number of Genes

Fig 5A

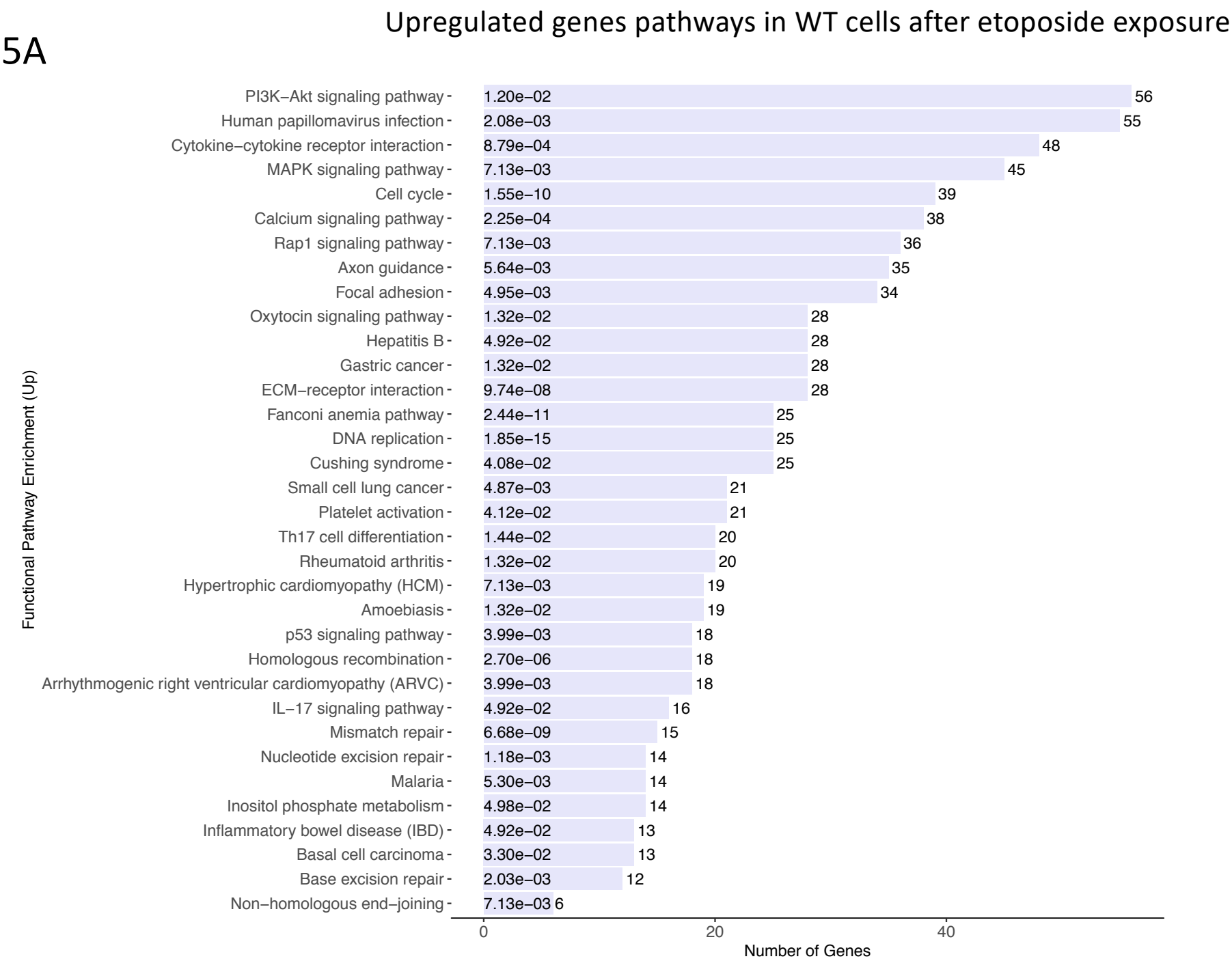
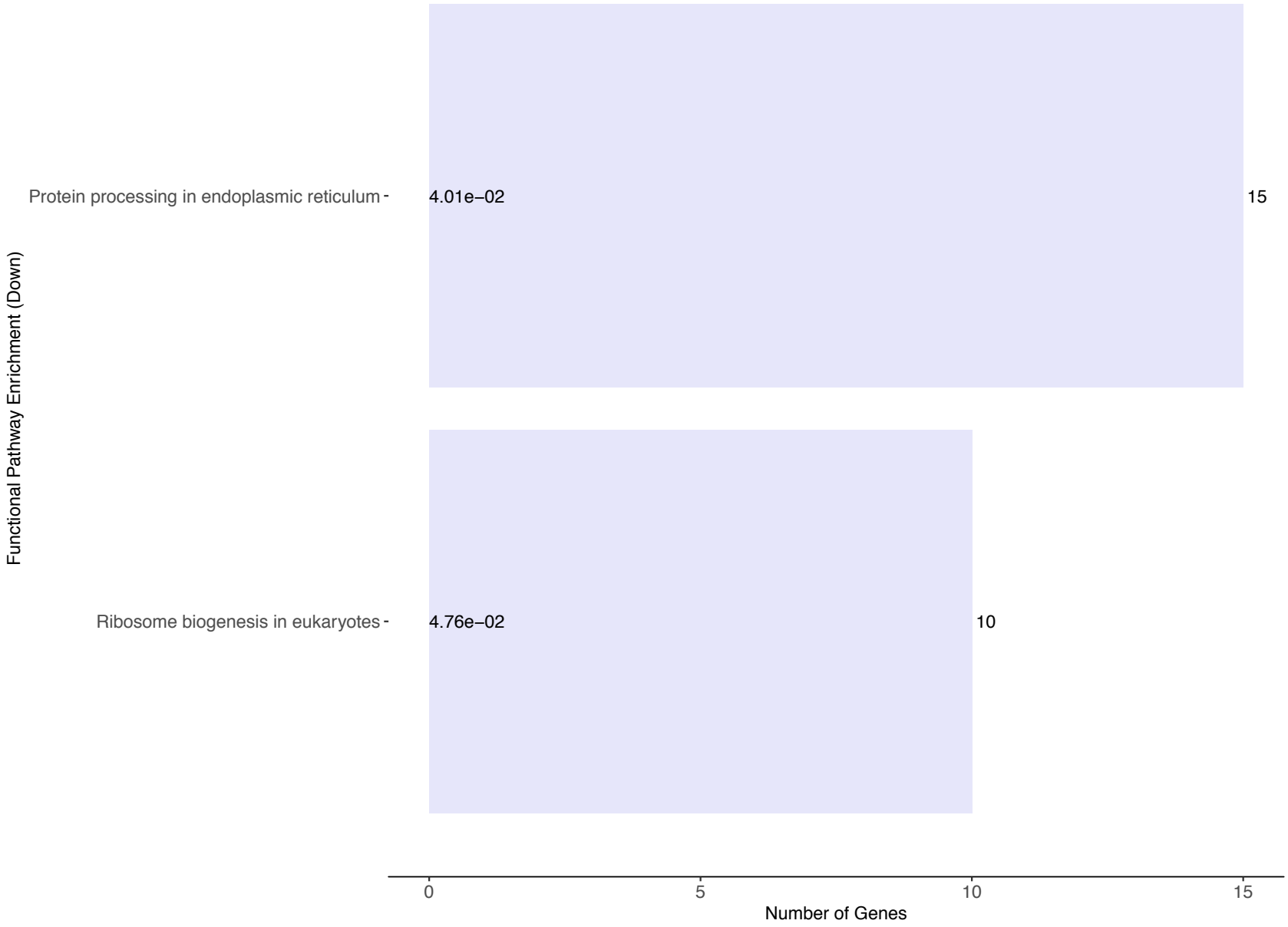

Upregulated genes pathways in WT cells after etoposide exposure
Functional Pathway Enrichment (Up)
PI3K−Akt signaling pathway 1.20e−02 56
Human papillomavirus infection 2.08e−03 55
Cytokine−cytokine receptor interaction 8.79e−04 48
MAPK signaling pathway 7.13e−03 45
Cell cycle 1.55e−10 39
Calcium signaling pathway 2.25e−04 38
Rap1 signaling pathway 7.13e−03 36
Axon guidance 5.64e−03 35
Focal adhesion 4.95e−03 34
Oxytocin signaling pathway 1.32e−02 28
Hepatitis B 4.92e−02 28
Gastric cancer 1.32e−02 28
ECM−receptor interaction 9.74e−08 28
Fanconi anemia pathway 2.44e−11 25
DNA replication 1.85e−15 25
Cushing syndrome 4.08e−02 25
Small cell lung cancer 4.87e−03 21
Platelet activation 4.12e−02 21
Th17 cell differentiation 1.44e−02 20
Rheumatoid arthritis 1.32e−02 20
Hypertrophic cardiomyopathy (HCM) 7.13e−03 19
Amoebiasis 1.32e−02 19
p53 signaling pathway 3.99e−03 18
Homologous recombination 2.70e−06 18
Arrhythmogenic right ventricular cardiomyopathy (ARVC) 3.99e−03 18
IL−17 signaling pathway 4.92e−02 16
Mismatch repair 6.68e−09 15
Nucleotide excision repair 1.18e−03 14
Malaria 5.30e−03 14
Inositol phosphate metabolism 4.98e−02 14
Inflammatory bowel disease (IBD) 4.92e−02 13
Basal cell carcinoma 3.30e−02 13
Base excision repair 2.03e−03 12
Non−homologous end−joining 7.13e−03 6
0
20
40
Number of Genes

Fig 5B

Down regulated pathways in WT cells after etoposide exposure



Upregulated pathways in KR cells after etoposide exposure

Fig 5C

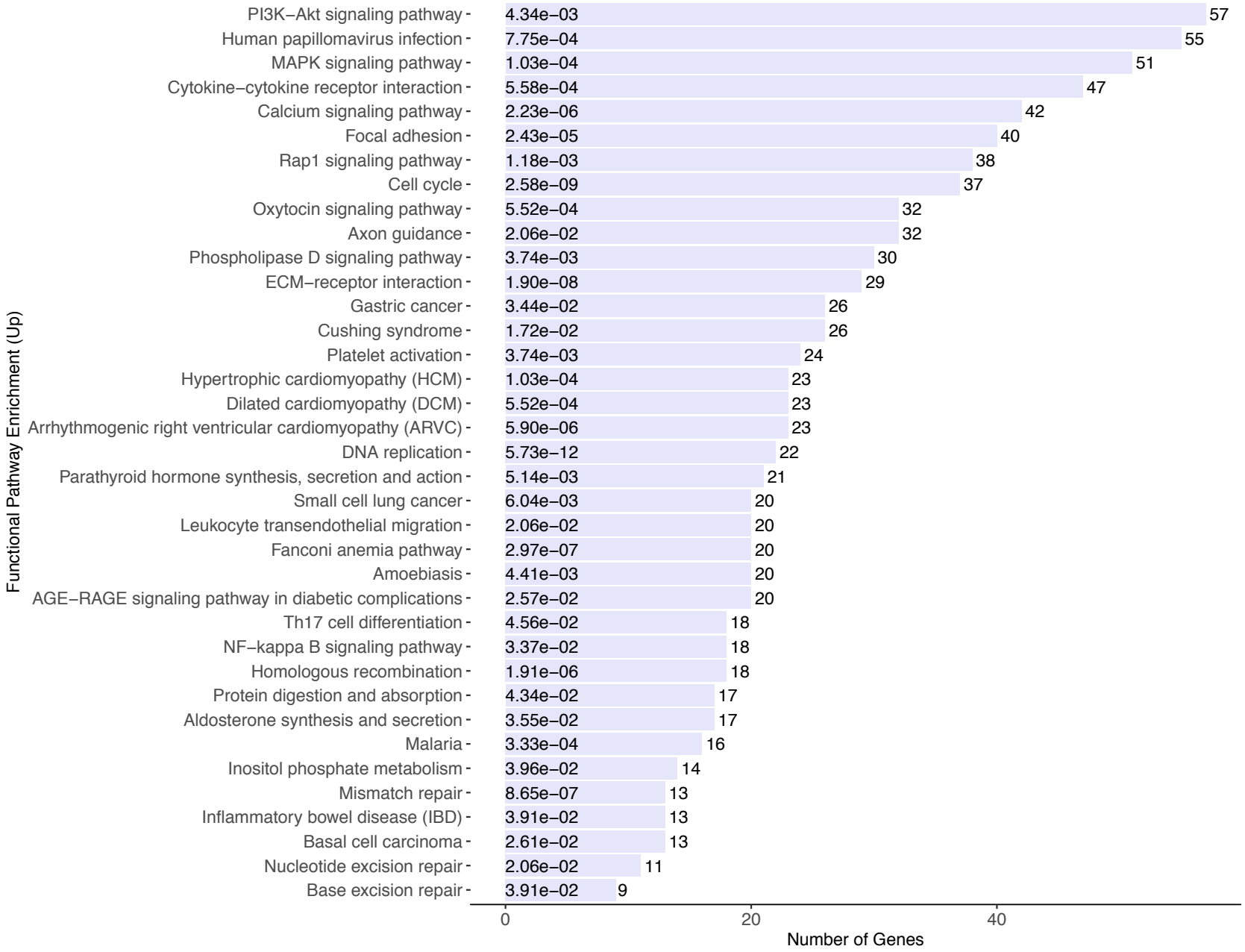


Fig 5D

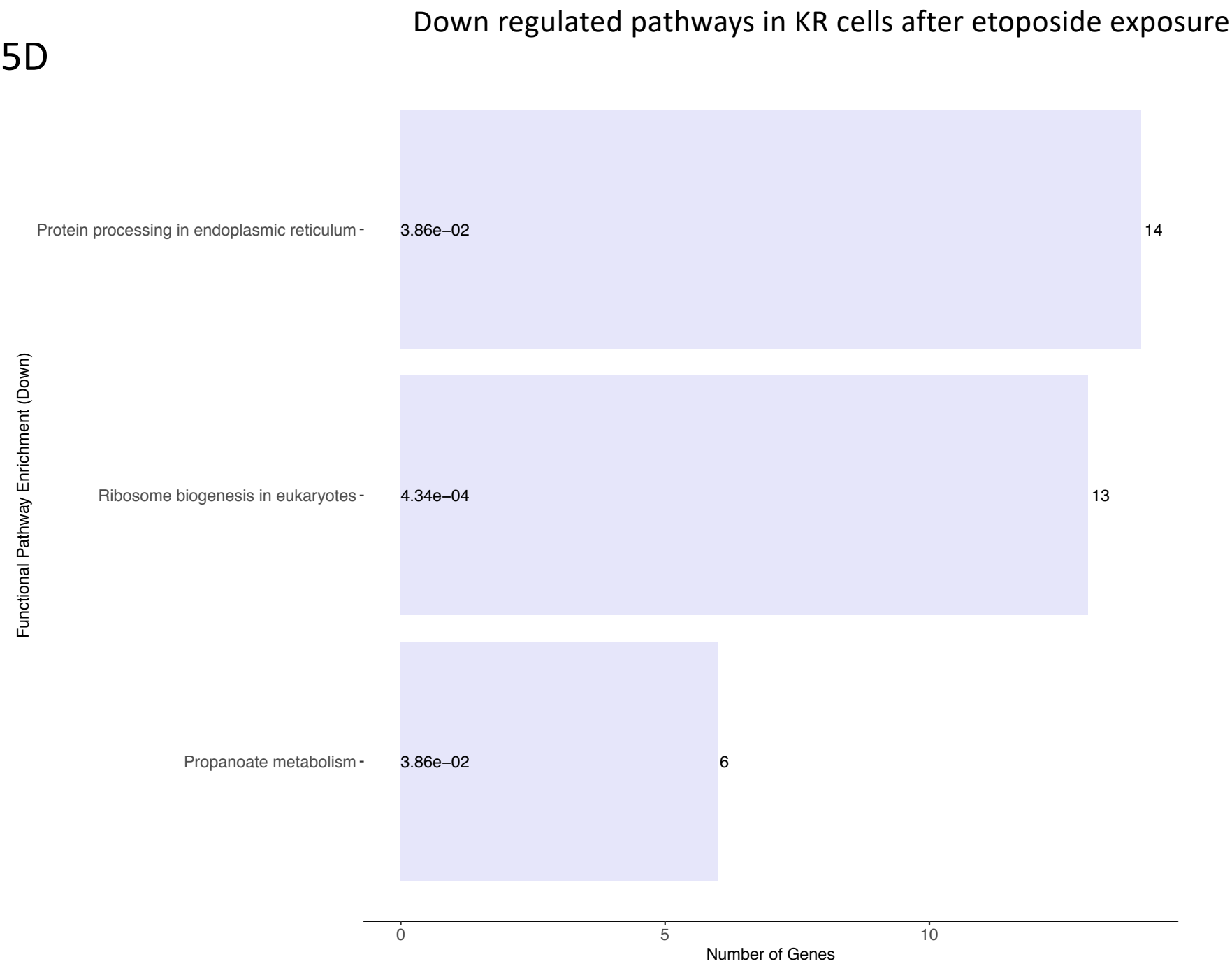
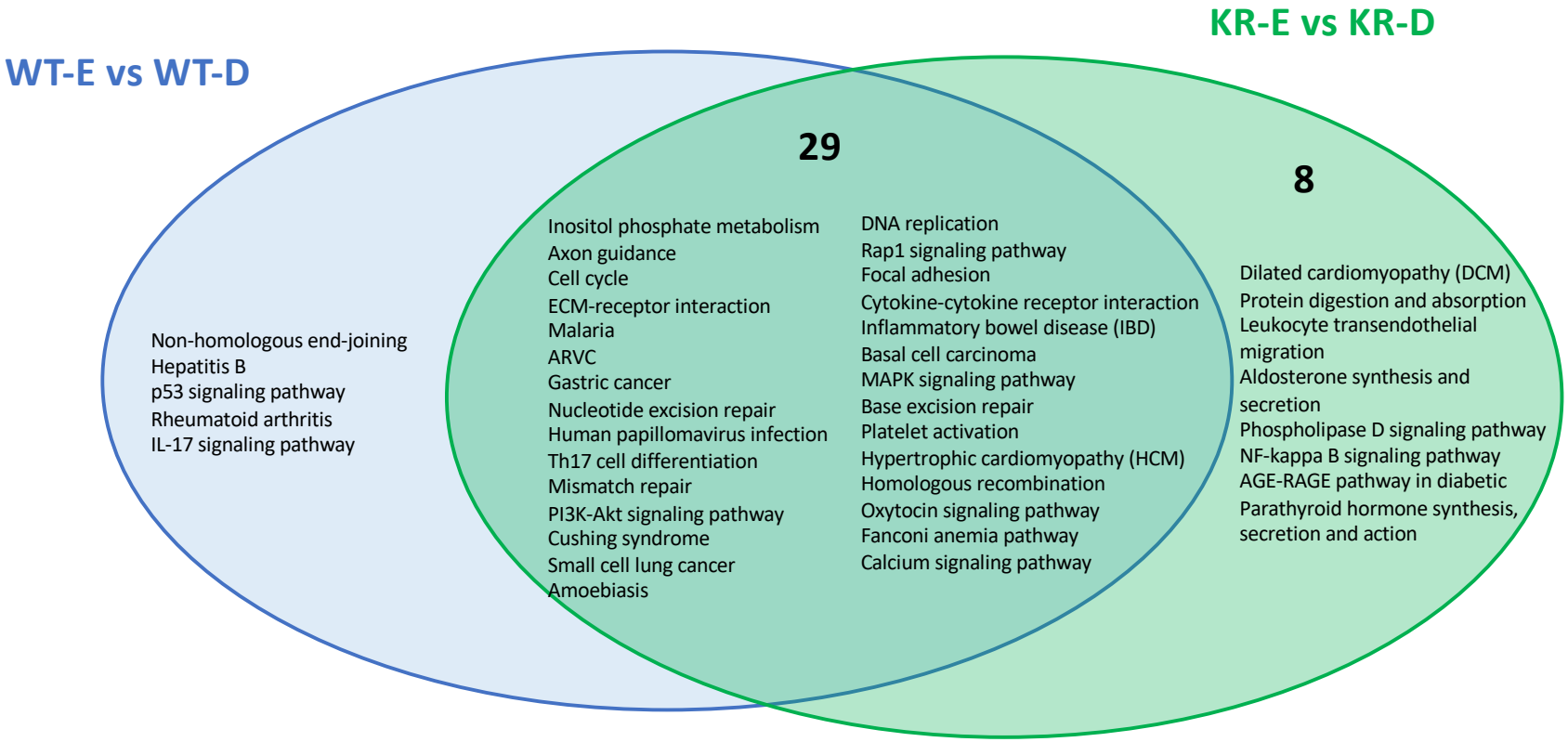

Down regulated pathways in KR cells after etoposide exposure
Functional Pathway Enrichment (Down)
Protein processing in endoplasmic reticulum
3.86e−02
14
Ribosome biogenesis in eukaryotes
4.34e−04
13
Propanoate metabolism
3.86e−02
6
0
5
10
Number of Genes

Fig 6 (A) Up regulated pathways



(B) Down regulated pathways

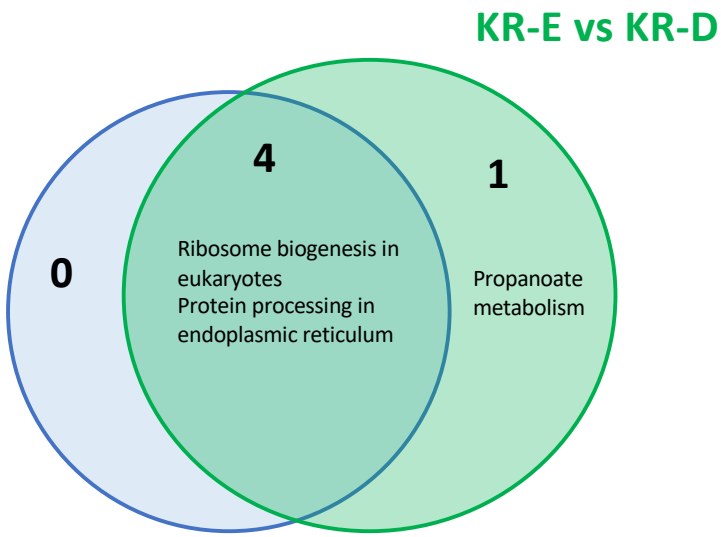


Fig 7A

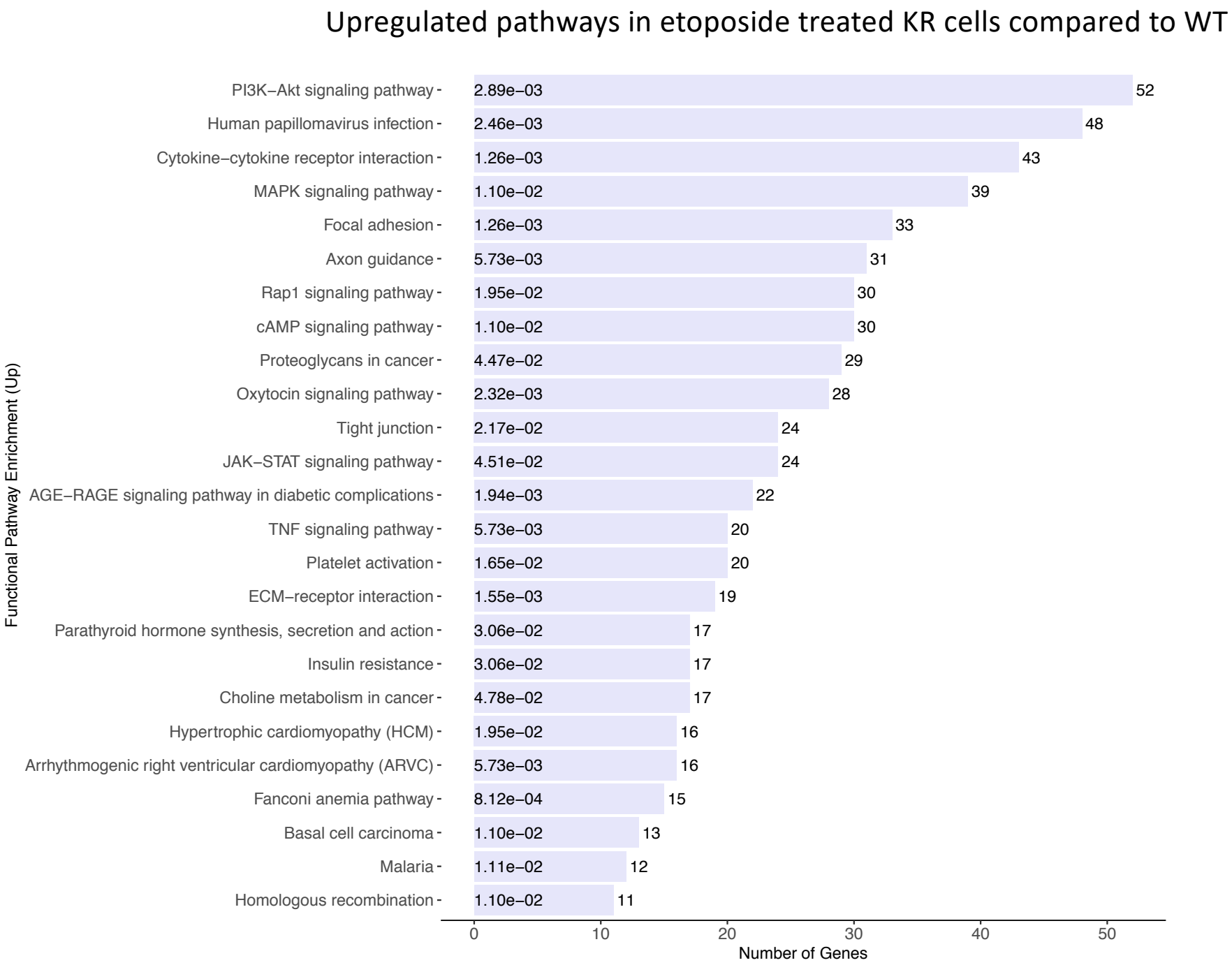
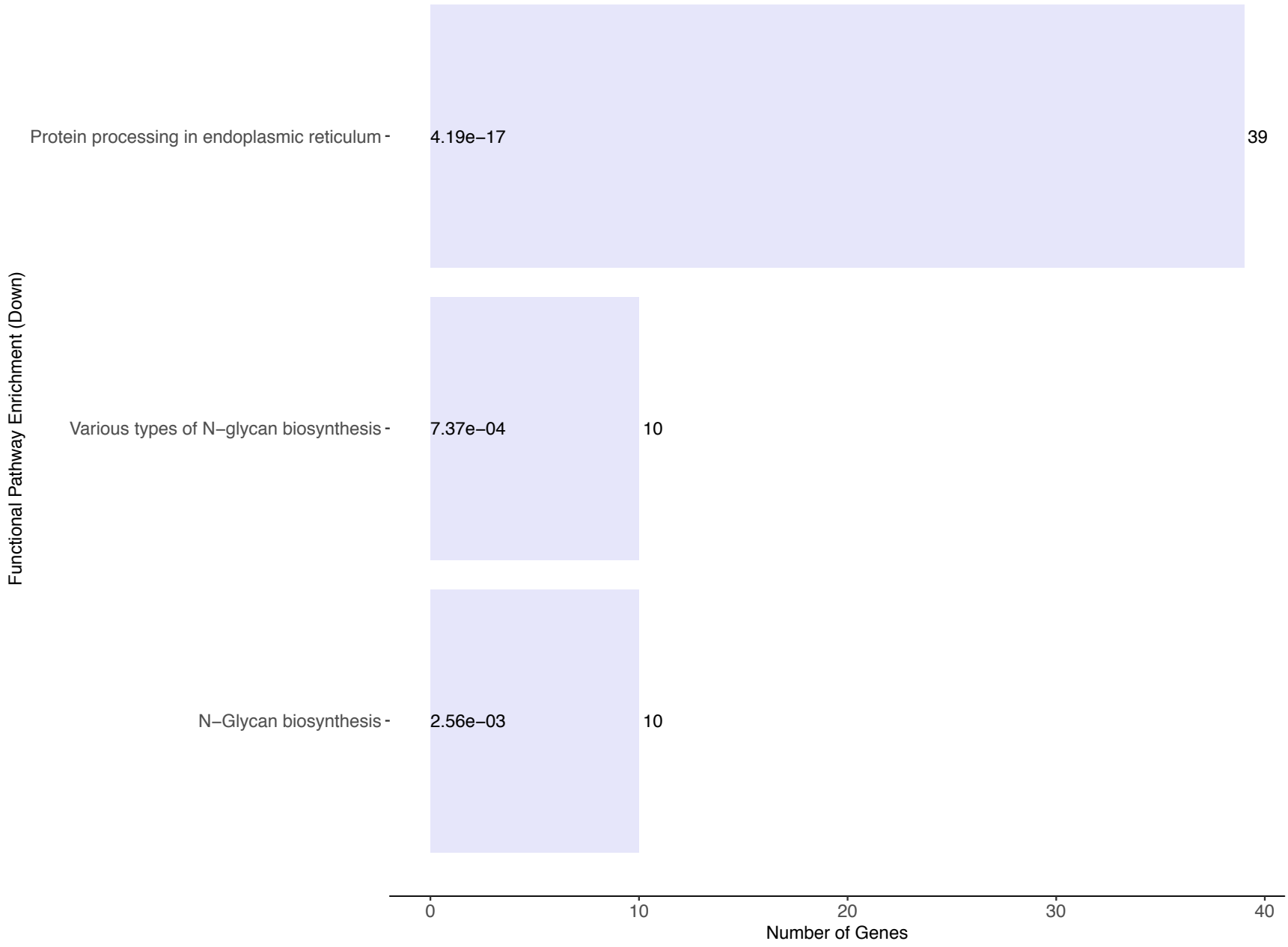

Upregulated pathways in etoposide treated KR cells compared to WT
Functional Pathway Enrichment (Up)
PI3K−Akt signaling pathway 2.89e−03 52
Human papillomavirus infection 2.46e−03 48
Cytokine−cytokine receptor interaction 1.26e−03 43
MAPK signaling pathway 1.10e−02 39
Focal adhesion 1.26e−03 33
Axon guidance 5.73e−03 31
Rap1 signaling pathway 1.95e−02 30
cAMP signaling pathway 1.10e−02 30
Proteoglycans in cancer 4.47e−02 29
Oxytocin signaling pathway 2.32e−03 28
Tight junction 2.17e−02 24
JAK−STAT signaling pathway 4.51e−02 24
AGE−RAGE signaling pathway in diabetic complications 1.94e−03 22
TNF signaling pathway 5.73e−03 20
Platelet activation 1.65e−02 20
ECM−receptor interaction 1.55e−03 19
Parathyroid hormone synthesis, secretion and action 3.06e−02 17
Insulin resistance 3.06e−02 17
Choline metabolism in cancer 4.78e−02 17
Hypertrophic cardiomyopathy (HCM) 1.95e−02 16
Arrhythmogenic right ventricular cardiomyopathy (ARVC) 5.73e−03 16
Fanconi anemia pathway 8.12e−04 15
Basal cell carcinoma 1.10e−02 13
Malaria 1.11e−02 12
Homologous recombination 1.10e−02 11
0
10
20
30
40
50
Number of Genes

Fig 7B

Down regulated pathways in etoposide treated KR cells compared to WT