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1 **Will urbanisation affect the expression level of genes related to cancer of**
2 **wild great tits?**

3
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19

20 **Abstract**

21 Recent studies suggest that oncogenic processes (from precancerous lesions to
22 metastatic cancers) are widespread in wild animal species, but their importance for ecosystem
23 functioning is still underestimated by evolutionary biologists and animal ecologists. Similar to
24 what has been observed in humans, environmental modifications that often place wild
25 organisms into an evolutionary trap and/or exposes them to a cocktail of mutagenic and
26 carcinogenic pollutants might favor cancer emergence and progression, if animals do not up-
27 regulate their defenses against these pathologies. Here, we compared, for the first time, the
28 expression of 59 tumor-suppressor genes in blood and liver tissues of urban and rural great tits
29 (*Parus major*); urban conditions being known to favor cancer progression due to, among other
30 things, exposure to chemical or light pollution. Contrary to earlier indications, once we
31 aligned the transcriptome to the great tit genome, we found negligible differences in the
32 expression of anti-cancer defenses between urban and rural birds in blood and liver. Our
33 results indicate the higher expression of a single caretaker gene (i.e. BRCA1) in livers of rural
34 compared to urban birds. We conclude that, while urban birds might be exposed to an
35 environment favoring the development of oncogenic processes, they seem to not upregulate
36 their cancer defenses accordingly and future studies should confirm this result by assessing
37 more markers of cancer defenses. This may result in a mismatch that might predispose urban
38 birds to higher cancer risk and future studies in urban ecology should take into account this,
39 so far completely ignored, hazard.

40 **Introduction**

41 Over the last century, the size of urban centres has drastically increased all over the world
42 with considerable effects on natural ecosystems (Marzluff et al., 2008). Consequently, many
43 wild species are now experiencing extreme changes in their habitats with, among other things,
44 an increased exposure to a mixture of mutagenic pollutants, changes in habitat temperature
45 (i.e. heat-island effect) and/or access to novel types of food items through human waste or
46 intentional feeding. As these environmental modifications, as well as other characteristics of
47 our modern world, have increased cancer prevalence in humans (Kloog et al., 2010), similar
48 effects can also be expected in wild animals living in human-modified habitats, and especially
49 in urban environments (Giraudeau et al., 2018; Sepp et al., 2019). In line with this hypothesis,
50 it has now been shown that most animal species can develop cancer (Madsen et al., 2017), and
51 several studies proposed that, even if invasive metastatic cancer might be rare in wild animals,
52 most metazoan organisms should start to host early stages of oncogenic processes early in life
53 and thus host them for months, years or decades with some effects on their health and vigour
54 and an impact on intra- and inter-specific relationships (Vittecoq et al., 2013, Thomas et al.
55 2017). In addition, evidence is starting to accumulate showing that exposure to pollution
56 might strongly affect neoplasia development in wild populations (Martineau et al., 2002).
57 However, while cancer is one of the leading causes of mortality in humans and a research
58 topic of prime importance for scientists and funding agencies worldwide, our understanding
59 of oncogenic processes in wild populations is extremely limited due to the methodological
60 difficulties of measuring this pathology in wild animals. Even more important in terms of
61 conservation, the ability of wild organisms to limit cancer progression in “oncogenic
62 environments” (i.e. habitats that favour neoplasia development such as those polluted with
63 chemicals or artificial light at night) has never been studied in the field. In addition, longer
64 lifespan has been linked with higher cancer-related mortality in humans, and age is considered

65 to be one of the major risk factors for cancer (White et al., 2014). As it has been suggested
66 that many wild species also experience lower predation rates and increased survival to older
67 age in urban environments (e.g. see Sepp et al., 2018)), age-related increase in cancer risk in
68 wild animals in urban environment should also be considered.

69

70 To fill this gap, we used a recently published transcriptome analysis which compared
71 gene expression levels between urban and rural great tits and focused our analysis on the
72 expression of 59 well-known tumour suppressor genes. In addition, the previous analyses
73 published by Watson et al. (2017) aligned the transcriptome to the best available genome at
74 the time of their publication (i.e. zebra finch). In order to perform a more accurate scrutiny of
75 differential expression of tumor-suppressor genes, we re-aligned the above transcriptomes to
76 the great tit genome and re-analysed the latter. Given that urban great tits up-regulate the
77 expression of genes related to immune activity, detoxification and repair machinery (Watson
78 et al., 2017), we predicted that they might also up-regulate the activity of genes related to
79 cancer defences.

80

81 **Methods**

82 Field and laboratory methods have been previously described in (Watson et al., 2017).
83 Briefly, six urban male great tits (Malmö, 55°35'N 12°59'E) and six rural great tits (Vombs
84 fure located 35 km northeast of Malmö, 55°39'N 13°33'E), were captured in late winter 2014.
85 At capture, birds were transported to the laboratory in Lund where biometrics were recorded,
86 and a blood sample was collected and immediately transferred to storage at -80 °C. Birds
87 were then euthanized and liver tissues were collected and transferred to storage at -80°C
88 within 5 min of death. RNA was isolated and sequenced using 100 bp paired-end RNA-seq on
89 Illumina HiSeq 2000. For the present study, we focused on the re-analysis of 59 tumor-

90 suppressor genes including 31 gatekeeper genes and 28 caretaker genes, following (Caulin
91 and Maley, 2011). By limiting DNA damage and being involved in DNA repair, caretaker
92 genes help to maintain genome integrity and thus limit cancer initiation. Gatekeeper genes are
93 involved in the control of cells at risk for neoplastic transformation in order to stop their
94 propagation (Caulin and Maley, 2011).

95 Read quality was first assessed using FastQC 0.11.5
96 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and quality trimming and
97 filtering were performed to remove low-quality reads and adapter contamination using
98 Trimmomatic (Bolger et al., 2014). Clean reads were aligned to the annotated regions of the
99 great tit genome (Parus_major1.1 RefSeq assembly accession GCF_001522545.2) using
100 HiSat2 2.1.0 (Kim et al., 2015) and sorted with SAMtools 1.5 (Li et al., 2009). Counts were
101 made using StringTie 1.3.3 (Pertea et al., 2015) in accordance with the protocol described by
102 Pertea et al. (Pertea et al., 2016). Counts were extracted for the 59 target genes. Following
103 (Watson et al., 2017), one rural bird was removed from the analysis of reads from blood, since
104 PCA revealed it to be an extreme outlier. All raw reads have been deposited in NCBI's
105 Sequence Read Archive (PRJNA314210).

106

107 Statistical analyses

108 Analyses of differences in the expression of various genes between urban and rural great tits
109 were performed separately for counts originating from blood and liver tissue, using the edgeR
110 3.24.3 package (Robinson et al., 2009) in R 3.5.2 (R Core Team 2018). Only genes with
111 expression greater than one count per million (CPM) in at least two samples were considered
112 for the differential expression analyses in order to filter out weakly expressed genes. As such,
113 a total of 52 and 51 genes were tested for differential expression in liver and blood
114 respectively (see Table S1 for the list of genes tested and excluded). Six of the target genes

115 have not been annotated in the great tit genome and there was no detectable expression of
116 PHOX2B. P-values obtained from the differential expression analyses were corrected for
117 multiple comparisons using the Benjamini–Hochberg (BH) procedure and only genes
118 determined to have a false discovery rate (FDR) < 0.05 were considered to be significant.

119 In order to visualize the expression profiles of the sampled individuals, we generated
120 heatmaps (see ESM) of the regularized log-transformed count data using the “pheatmap”
121 function (R., 2019). Counts of gene expression were clustered for the genes according to the
122 similarity of expression profiles across different samples.

123

124 This study was carried out in accordance with Swedish legislation and approved by the
125 Malmö-Lund animal ethics committee (Dnr M454 12:1).

126

127 **Results**

128 None of the 51 genes on which our analysis was focused were differentially expressed in
129 blood and only one gene (BRCA1) was differentially expressed among the 52 genes tested in
130 liver tissue between urban and rural great tits following p-value adjustment (see Table 1a and
131 1b, respectively, for the differential expression statistics of each of these genes). BRCA1 was
132 expressed at significantly higher levels in rural, compared with urban birds in liver, but not
133 blood tissues.

134

135 **Discussion**

136 Despite the increasing occurrence of epizootics of cancer observed in wild species
137 (McAloose and Newton, 2009) and the broad distribution of oncogenic processes along the
138 tree of life (Athena Aktipis et al., 2015), wildlife cancer is, for the moment, a largely
139 unexplored topic (except for a few emblematic species such as the Tasmanian devil

140 (*Sarcophilus harrisi* (Ujvari et al., 2016)) or the sea turtle (*Chelonia mydas* (Duffy et al.,
141 2018)). This lack of interest from the scientific community is explained by the misconception
142 shared by many ecologists and evolutionary biologists until recently that only late-stage
143 visually detectable cancers affect wild organisms, mostly after the end of the reproductive
144 period. Many articles published over the last few years have challenged this view, and it is
145 now admitted that the first cancerous cells appear early in life and that the community of
146 cancer cells (i.e. oncobiota) is a major actor of evolutionary processes influencing host life-
147 history traits and strategies (Vittecoq et al., 2013, Thomas et al., 2017, Pesavento et al., 2018,
148 Giraudeau et al., 2018).

149 An intriguing question in this emerging field of study, with consequences for the conservation
150 of wild species, concerns the ability of wild organisms inhabiting environments impacted by
151 human activities to adjust their level of defenses against this pathology whose progression
152 should be stimulated by, among other things, exposure to many carcinogenic pollutants
153 (Vittecoq et al., 2018). Our study constitutes a first attempt to study this question in wild
154 urban birds, in a population known to be exposed to higher levels of urban pollutant (i.e. nitric
155 oxide, a marker of traffic-related air pollution) (Salmón et al., 2018a) known to be associated
156 with cancer in humans (Cohen et al. 2019). While examining the expression of a large number
157 of tumor-suppressor genes, we found negligible differences in the expression of tumor-
158 suppressor genes between urban and rural great tits examined in blood and liver tissues. The
159 only gene differentially expressed was BRCA1, with reduced levels of expression in urban
160 birds. BRCA1 is involved in the maintenance of genome integrity by repairing damaged DNA
161 and has been mainly associated with breast and prostate cancers so far in humans (Gorodetska
162 *et al.* 2019). Given our limited knowledge on the consequences of a reduced expression of
163 BRCA1 in liver, especially in wild birds, it's difficult to draw any conclusion based on this

164 result. However, we can hypothesize that this reduced expression might favor the
165 development of oncogenic processes in urban birds.

166 One can argue that the sample size used in this study was too small to detect differences
167 between populations, but due to the terminal nature of our sampling to get the liver samples
168 and for obvious ethical reasons, we preferred to limit the number of individuals euthanized for
169 this study. In addition, the sample size used here is common for this kind of study and
170 (Watson et al., 2017) found large differences in the expression of genes related to immune
171 activity, detoxification and repair machinery using the same transcriptome data and thus the
172 same sample size. We can also exclude the hypothesis that rural birds were exposed to
173 mutagenic substances, such as pesticides to explain the absence of significant differences
174 observed in our study since our rural site was a water reserve area without any agricultural
175 activity nearby.

176 Avian antitumor defenses have been mainly described in poultry, with a focus on
177 commercially relevant diseases like the avian leucosis virus, which induces various neoplasias
178 in chickens (Sironi et al., 2006). In this model system, transcriptome analyses have indicated a
179 significant decrease of the expression of genes related to both immune defenses and antitumor
180 pathways in spleens and tumors of susceptible birds (Zhang et al., 2016, Qiu et al., 2018),
181 indicating that both mechanisms play an important role in avian cancer defenses. While in our
182 study, antitumor genes were not expressed in higher levels in birds living in a potentially
183 oncogenic environment, the role of increased expression of immune-related genes shown in
184 the same individuals (Watson et al., 2017) could act as a first line of defense against the
185 effects of oncogenic stressors. In accordance with life-history theory, in a short-lived animal
186 such as the great tit, selection should favor investment in physiological defenses that increase
187 short-term survival, at the expense of later-life performance and survival (Stearns, 2006).
188 Additionally, in other studies of the same population (but with larger sample size) reveal

189 increased antioxidant defenses (Salmón et al., 2018a, 2018b), shorter telomeres of developing
190 nestlings and selective disappearance of birds with short telomeres during their first year of
191 life (Salmón et al., 2016, 2017). Together, these results provide evidence that the present
192 urban environment is physiologically challenging and that only individuals with a “strong”
193 physiological response survive. However, whether this also protect the surviving urban birds
194 from developing cancer or if resources have been allocated to combat current physiological
195 threats rather than to more long-term anti-cancer defenses, requires further studies.

196 Unfortunately, we do not know the movements between the studied populations and their
197 surroundings. Thus, we cannot exclude that bird immigration influence the present findings
198 and thereby prevent local adaptation against the proliferation of neoplasia.

199 In any cases, given that lifespan of urban birds can be prolonged due to reduced predation
200 (Sepp et al., 2018), the lack of anti-cancer responses may be a greater cause of death in the
201 future than previously acknowledged.

202

203 While future studies should confirm our results with more tumor-suppressor genes (and thus
204 more anti-cancer mechanisms) examined and other tissues sampled (for example the kidney
205 due to its important contribution in detoxification or the spleen for its role in the production of
206 immune agents), our results suggest that wild birds do not up-regulate their anti-cancer
207 defenses in urban environments. As repeatedly suggested over the last few years (Thomas et
208 al. 2017, Vittecoq et al., 2018, Giraudeau et al., 2018, Sepp et al., 2019), this supports the idea
209 that wild animals living in human impacted habitats might be at higher risk of developing
210 oncogenic processes. An important methodological step allowing the detection of this
211 pathology in non-terminal samples is now urgently needed to confirm this hypothesis and
212 measure the potential unexplored consequences of pollution on wildlife cancer. Collaborative
213 projects between different fields (medicine, animal ecology, evolution) appear to be the

214 necessary approach to use the extensive knowledge accumulated in human oncology in order
215 to develop new cancer biomarkers usable with wild animals.

216 It is now admitted that oncogenic processes are widespread among metazoan species
217 with potential consequences on their life history strategies and, in some cases, on their
218 population dynamics as well as on ecosystem functioning. The worldwide modifications of
219 natural environments and in particular the release of mutagenic pollutants might exacerbate
220 this process, and wildlife neoplasia should thus be considered as an important player in the
221 reorganization of ecosystems currently happening under the pressure of human activities.

222

223

224 Data accessibility. All data are available in Dryad.

225 Authors' contributions. H.W., C.I., T.S. and M.G. conceived and designed the study. C.I.
226 and H.W. performed post-mortems and H.W. isolated RNA. D.P. carried out the alignment
227 and generation of read counts. H.W. and O.V. carried out downstream analyses. M.G.
228 wrote the manuscript with input from all other authors. All authors gave final approval for
229 publication.

230 Competing interests. We declare we have no competing interests.

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338 Table 1 Results of differential expression analyses of tumor suppressor genes in liver (A) and blood
339 (B) tissues of urban and rural great tits. LogFC = log2 fold-change (negative values indicate high
340 expression in rural birds), LogCPM = average log counts per million; LR = likelihood ratio statistic; P-
341 value = unadjusted P value; FDR = false discovery rate, Mean Expr. = average expression level in
342 urban and rural birds respectively. Genes with missing data were either not included in the annotated
343 gene set or had no mapped reads. Genes marked in italics are differentially expressed following p-
344 value adjustment.

345 Table 1A.

Gene	Classification	logFC	LR	P-Value	FDR	Mean Expr. urban	Mean Expr. rural
<i>BRCA1</i>	<i>Caretaker</i>	<i>-1.41</i>	<i>12.52</i>	<i>0.0004</i>	<i>0.0210</i>	<i>419.00</i>	<i>1093.17</i>
FLT3	Gatekeeper	1.23	6.26	0.0124	0.3212	1120.33	467.17
BLM	Caretaker	0.66	2.75	0.0970	0.8653	1209.17	738.00
BMPR1A	Gatekeeper	-0.45	1.70	0.1922	0.8653	888.33	1314.83
BRCA2	Caretaker	-0.32	1.26	0.2613	0.8653	1169.33	1480.00
CEBPA	Gatekeeper	0.27	1.66	0.1976	0.8653	9966.33	8444.33
ERCC4	Caretaker	-0.35	1.06	0.3034	0.8653	833.67	1095.50
EXT1	Gatekeeper	-0.33	1.01	0.3149	0.8653	1117.50	1408.50
EXT2	Gatekeeper	-0.27	0.62	0.4319	0.8653	5197.50	6708.00
FANCC	Caretaker	0.46	0.62	0.4299	0.8653	119.50	87.17
FANCD2	Caretaker	0.64	0.78	0.3777	0.8653	73.83	45.50
FANCE	Caretaker	0.36	1.03	0.3112	0.8653	3818.83	2979.67
FANCF	Caretaker	-0.85	2.40	0.1215	0.8653	73.00	130.33
FANCG	Caretaker	0.57	1.04	0.3075	0.8653	181.17	118.17
FH	Gatekeeper	0.54	2.43	0.1193	0.8653	23016.33	17341.00
GATA1	Gatekeeper	0.49	0.60	0.4383	0.8653	232.00	161.33
KLF6	Gatekeeper	1.02	1.87	0.1719	0.8653	669.17	388.00
NBN	Caretaker	0.36	1.46	0.2277	0.8653	1628.67	1267.83
NF1	Gatekeeper	0.46	1.21	0.2705	0.8653	3703.50	2694.17
NF2	Gatekeeper	0.28	0.53	0.4659	0.8653	843.00	691.33

PMS1	Caretaker	-0.32	0.93	0.3344	0.8653	2027.33	2692.83
PTEN	Gatekeeper	0.20	0.56	0.4552	0.8653	5434.83	4997.67
SDHB	Gatekeeper	0.22	0.82	0.3645	0.8653	3362.33	3018.67
SMARCB1	Gatekeeper	0.27	1.11	0.2926	0.8653	1986.17	1667.00
VHL	Gatekeeper	-0.20	0.56	0.4533	0.8653	5943.50	7199.83
WRN	Caretaker	0.34	1.44	0.2309	0.8653	1692.00	1330.17
XPA	Caretaker	-0.32	2.08	0.1496	0.8653	8957.50	11491.33
XPC	Caretaker	-0.16	0.63	0.4276	0.8653	3895.00	4426.50
APC	Gatekeeper	0.08	0.06	0.8040	0.9162	1400.00	1349.33
ATM	Caretaker	-0.18	0.43	0.5140	0.9162	1556.50	1802.17
CDH1	Gatekeeper	0.06	0.08	0.7805	0.9162	13177.50	13069.50
CHEK2	Caretaker	0.06	0.03	0.8709	0.9162	494.00	465.17
CYLD	Gatekeeper	0.09	0.16	0.6876	0.9162	2308.67	2173.00
DDB2	Caretaker	-0.08	0.02	0.8810	0.9162	406.33	414.67
ERCC3	Caretaker	0.06	0.07	0.7890	0.9162	2242.33	2166.33
FANCA	Caretaker	0.25	0.09	0.7669	0.9162	64.17	51.33
FLT4	Gatekeeper	-0.10	0.10	0.7541	0.9162	869.33	967.00
HNF1A	Gatekeeper	0.04	0.03	0.8615	0.9162	2428.00	2390.00
MAP2K4	Gatekeeper	-0.13	0.22	0.6419	0.9162	2903.00	3309.50
MEN1	Gatekeeper	0.21	0.33	0.5678	0.9162	624.83	527.67
MLH1	Caretaker	0.06	0.05	0.8229	0.9162	2089.33	2006.83
MSH2	Caretaker	0.19	0.37	0.5411	0.9162	721.33	651.00
MSH6	Caretaker	-0.06	0.02	0.8777	0.9162	238.17	245.67
MUTYH	Caretaker	-0.11	0.05	0.8156	0.9162	358.00	385.33
PMS2	Caretaker	-0.10	0.08	0.7726	0.9162	763.50	829.67
RB1	Gatekeeper	-0.14	0.16	0.6870	0.9162	4156.17	5007.67
SBDS	Gatekeeper	-0.12	0.21	0.6497	0.9162	3361.67	3856.17
SDHD	Gatekeeper	-0.13	0.31	0.5776	0.9162	19594.17	22325.83
SMAD4	Gatekeeper	0.34	0.15	0.6981	0.9162	123.67	96.17
STK11	Gatekeeper	0.24	0.23	0.6342	0.9162	775.17	637.00

GPC3	Gatekeeper	-0.01	0.00	0.9865	0.9865	120.67	123.33
SUFU	Gatekeeper	-0.01	0.00	0.9708	0.9865	1089.17	1097.50
CDKN2A	Gatekeeper	NA	NA	NA	NA	NA	NA
ERCC2	Caretaker	NA	NA	NA	NA	NA	NA
ERCC5	Caretaker	NA	NA	NA	NA	NA	NA
PHOX2B	Gatekeeper	NA	NA	NA	NA	0.00	0.00
RECQL4	Caretaker	NA	NA	NA	NA	NA	NA
SDHC	Gatekeeper	NA	NA	NA	NA	NA	NA
TP53	Caretaker	NA	NA	NA	NA	NA	NA

346

347 Table 1B

348

Row.names	Classification	logFC	LR	P-Value	FDR	Mean Expr. urban	Mean Expr. rural
KLF6	Gatekeeper	1.32	6.83	0.0090	0.4573	7514.83	2699.00
ATM	Caretaker	0.54	1.63	0.2023	0.7723	326.00	199.20
CDH1	Gatekeeper	-2.21	3.86	0.0494	0.7723	74.67	347.40
CYLD	Gatekeeper	0.67	3.01	0.0826	0.7723	1027.33	569.40
ERCC4	Caretaker	0.64	2.16	0.1419	0.7723	219.17	121.60
EXT1	Gatekeeper	1.12	2.83	0.0926	0.7723	139.00	50.80
FANCA	Caretaker	1.12	1.46	0.2272	0.7723	14.33	5.40
FANCC	Caretaker	1.42	1.73	0.1882	0.7723	14.00	5.00
FH	Gatekeeper	-0.63	2.33	0.1266	0.7723	880.17	1324.80
FLT4	Gatekeeper	0.86	2.55	0.1103	0.7723	140.17	69.60
HNF1A	Gatekeeper	-0.61	2.52	0.1127	0.7723	249.50	364.40
MSH2	Caretaker	1.00	1.85	0.1742	0.7723	77.83	36.60
MSH6	Caretaker	0.74	1.54	0.2140	0.7723	33.67	17.80
NBN	Caretaker	0.53	1.96	0.1615	0.7723	484.00	291.60
PMS2	Caretaker	0.69	2.45	0.1178	0.7723	297.50	156.00
CHEK2	Caretaker	0.47	1.31	0.2525	0.8048	567.17	354.40
FANCF	Caretaker	-0.78	1.20	0.2738	0.8214	9.00	15.40
APC	Gatekeeper	0.33	0.99	0.3208	0.8739	3033.00	2056.40

BMPR1A	Gatekeeper	-0.68	0.88	0.3483	0.8739	26.33	42.40
BRCA2	Caretaker	0.35	0.80	0.3708	0.8739	528.33	351.80
CEBPA	Gatekeeper	-0.41	0.66	0.4158	0.8739	269.67	336.00
FANCE	Caretaker	0.37	0.61	0.4355	0.8739	4627.17	3468.40
FANCG	Caretaker	0.27	0.51	0.4768	0.8739	907.50	741.80
GATA1	Gatekeeper	-0.20	0.43	0.5140	0.8739	17552.17	19833.20
MAP2K4	Gatekeeper	0.29	0.46	0.4987	0.8739	1504.67	1278.60
MLH1	Caretaker	0.36	0.57	0.4509	0.8739	567.50	411.20
NF1	Gatekeeper	-0.31	0.64	0.4237	0.8739	4124.83	4798.00
SMAD4	Gatekeeper	-0.45	0.77	0.3791	0.8739	344.83	436.40
STK11	Gatekeeper	-0.22	0.45	0.5003	0.8739	3353.00	3806.20
SUFU	Gatekeeper	-0.30	0.94	0.3315	0.8739	4010.83	4664.80
BLM	Caretaker	-0.16	0.22	0.6378	0.9393	5363.17	5824.00
ERCC3	Caretaker	0.15	0.28	0.5984	0.9393	4039.00	3470.40
FANCD2	Caretaker	-0.25	0.21	0.6499	0.9393	183.83	211.00
FLT3	Gatekeeper	0.27	0.19	0.6630	0.9393	58.67	45.00
SDHB	Gatekeeper	0.17	0.21	0.6470	0.9393	625.67	542.60
XPA	Caretaker	0.12	0.22	0.6389	0.9393	9170.33	7879.60
BRCA1	Caretaker	-0.19	0.14	0.7048	0.9715	121.50	121.00
DDB2	Caretaker	-0.05	0.01	0.9048	0.9950	471.00	439.00
EXT2	Gatekeeper	0.08	0.05	0.8291	0.9950	1278.83	1063.60
MEN1	Gatekeeper	0.07	0.02	0.8870	0.9950	191.50	158.20
MUTYH	Caretaker	0.06	0.01	0.9130	0.9950	88.00	71.60
NF2	Gatekeeper	0.15	0.11	0.7420	0.9950	169.83	134.60
PMS1	Caretaker	-0.04	0.01	0.9231	0.9950	274.50	252.40
PTEN	Gatekeeper	-0.07	0.07	0.7928	0.9950	4607.33	4593.00
RB1	Gatekeeper	0.02	0.01	0.9400	0.9950	9445.00	8756.80
SBDS	Gatekeeper	0.00	0.00	0.9950	0.9950	6287.00	6347.20
SDHD	Gatekeeper	0.06	0.03	0.8650	0.9950	4422.50	4121.80

SMARCB1	Gatekeeper	-0.01	0.00	0.9715	0.9950	1879.50	1788.80
VHL	Gatekeeper	0.06	0.05	0.8214	0.9950	8967.33	8265.60
WRN	Caretaker	0.06	0.04	0.8475	0.9950	2787.67	2467.40
XPC	Caretaker	-0.01	0.00	0.9757	0.9950	2009.33	1908.40
CDKN2A	Gatekeeper	NA	NA	NA	NA	NA	NA
ERCC2	Caretaker	NA	NA	NA	NA	NA	NA
ERCC5	Caretaker	NA	NA	NA	NA	NA	NA
GPC3	Gatekeeper	NA	NA	NA	NA	0.00	1.60
PHOX2B	Gatekeeper	NA	NA	NA	NA	0.00	0.00
RECQL4	Caretaker	NA	NA	NA	NA	NA	NA
SDHC	Gatekeeper	NA	NA	NA	NA	NA	NA
TP53	Caretaker	NA	NA	NA	NA	NA	NA

349