**Human gene function publications that describe wrongly identified nucleotide sequence
reagents are unacceptably frequent within the genetics literature**

Short title: Wrongly identified nucleotide sequences in gene function papers

Yasunori Park¹, Rachael A West^{1,2}, Pranujan Pathmendra¹, Bertrand Favier³, Thomas
Stoeger^{4,5,6}, Amanda Capes-Davis^{1,7}, Guillaume Cabanac⁸, Cyril Labbé⁹, Jennifer A
Byrne^{1,10,*}

¹Faculty of Medicine and Health, The University of Sydney, NSW, Australia

²Children's Cancer Research Unit, Kids Research, The Children's Hospital at Westmead,
Westmead, NSW, Australia

³Univ. Grenoble Alpes, TIMC, Grenoble, France

⁴Successful Clinical Response in Pneumonia Therapy (SCRIPT) Systems Biology Center,
Northwestern University, Evanston, United States.

⁵Department of Chemical and Biological Engineering, Northwestern University, Evanston,
United States.

⁶Center for Genetic Medicine, Northwestern University School of Medicine, Chicago, United
States

⁷CellBank Australia, Children's Medical Research Institute, Westmead, New South Wales,
Australia

⁸Computer Science Department, IRIT UMR 5505 CNRS, University of Toulouse, 118 route
de Narbonne, 31062 Toulouse Cedex 9, France

⁹Univ. Grenoble Alpes, CNRS, Grenoble INP, LIG, Grenoble, France

¹⁰NSW Health Statewide Biobank, NSW Health Pathology, Camperdown, NSW, Australia

*Corresponding author

24

25 **Keywords:** cancer; gene function; miRNA; non-coding RNA's; nucleotide sequence reagent;

26 paper mill; protein-coding gene

27

28 **Abstract**

29 Nucleotide sequence reagents underpin a range of molecular genetics techniques that have
30 been applied across hundreds of thousands of research publications. We have previously
31 reported wrongly identified nucleotide sequence reagents in human gene function
32 publications and described a semi-automated screening tool Seek & Blastn to fact-check the
33 targeting or non-targeting status of nucleotide sequence reagents. We applied Seek & Blastn
34 to screen 11,799 publications across 5 literature corpora, which included all original
35 publications in *Gene* from 2007-2018 and all original open-access publications in *Oncology*
36 *Reports* from 2014-2018. After manually checking the Seek & Blastn screening outputs for
37 over 3,400 human research papers, we identified 712 papers across 78 journals that described
38 at least one wrongly identified nucleotide sequence. Verifying the claimed identities of over
39 13,700 nucleotide sequences highlighted 1,535 wrongly identified sequences, most of which
40 were claimed targeting reagents for the analysis of 365 human protein-coding genes and 120
41 non-coding RNAs, respectively. The 712 problematic papers have received over 17,000
42 citations, which include citations by human clinical trials. Given our estimate that
43 approximately one quarter of problematic papers are likely to misinform or distract the future
44 development of therapies against human disease, urgent measures are required to address the
45 problem of unreliable gene function papers within the literature.

46

47 **Author summary**

48 This is the first study to have screened the gene function literature for nucleotide sequence
 49 errors at the scale that we describe. The unacceptably high rates of human gene function
 50 papers with incorrect nucleotide sequences that we have discovered represent a major
 51 challenge to the research fields that aim to translate genomics investments to patients, and
 52 that commonly rely upon reliable descriptions of gene function. Indeed, wrongly identified
 53 nucleotide sequence reagents represent a double concern, as both the incorrect reagents
 54 themselves and their associated results can mislead future research, both in terms of the
 55 research directions that are chosen and the experiments that are undertaken. We hope that our
 56 research will inspire researchers and journals to seek out other problematic human gene
 57 function papers, as we are unfortunately concerned that our results represent the tip of a much
 58 larger problem within the literature. We hope that our research will encourage more rigorous
 59 reporting and peer review of gene function results, and we propose a series of responses for
 60 the research and publishing communities.

61

62 **Introduction**

63 The promise of genomics to improve the health of cancer and other patients has resulted in
 64 billions of dollars of research investment which have been accompanied by expectations of
 65 similar quantum gains in health outcomes (1, 2). Since the first draft of the human genome
 66 was reported (3, 4), a series of increasingly rapid technological advances has permitted the
 67 routine sequencing of human genomes at scale (1, 2), and the increasing application of
 68 genomics to inform clinical care (1, 2, 5). Despite the now routine capacity to sequence the
 69 human genome, genomics research relies upon results produced by other research fields to
 70 translate genome sequencing results to patients (5-7). For example, while whole genome
 71 sequencing demonstrates that thousands of human genes are mutated or deregulated in human
 72 cancers (1), knowledge of human gene function is required to prioritise individual gene
 73 candidates for subsequent pre-clinical and translational studies (5-7).

74

75 A first step in triaging and prioritising gene candidates for further analysis is the
 76 consideration of available knowledge of predicted and/or demonstrated gene functions (5-8).
 77 High quality, reliable information about gene function is important to select the most
 78 promising gene candidates and to then progress these candidates through pre-clinical and
 79 translational research pipelines (8), which is supported by drug candidates with genetically
 80 supported targets being significantly more likely to progress through phased clinical trials (9,
 81 10). However, in contrast to the sophisticated platforms that produce genomic or
 82 transcriptomic sequence data at scale, gene function experiments typically analyse single or
 83 small numbers of genes through the application of more ubiquitous molecular techniques (6),
 84 some of which have been in routine experimental use for 15-30 years. For example, gene
 85 knockdown approaches have been widely employed to assess the consequences of reduced

86 gene expression in model systems (6). Similarly, RT-PCR is frequently used to analyse the
87 transcript levels of small groups of genes, either to confirm the effectiveness of gene
88 knockdown experiments or in association with other experimental techniques. The
89 widespread use and reporting of gene knockdown and PCR approaches reflect their low cost
90 and accessibility, in terms of the necessary reagents, laboratory equipment and facilities, and
91 the availability of technical expertise within the research community. As a consequence, the
92 results of experiments employing gene knockdown and/or PCR in the context of human
93 research have been described in hundreds of thousands of publications that are retrievable
94 through PubMed or Google Scholar.

95

96 Experiments that analyse the functions of individual genes typically require nucleotide
97 sequence reagents as either targeting and/or control reagents, with the sequences
98 corresponding to these reagents being disclosed to indicate the exact experiments that were
99 conducted (8, 11). As nucleotide sequence identities cannot be deduced by eye, DNA or RNA
100 reagent sequences must be paired with text descriptions of their genetic identities and
101 experimental use (8, 11-13). The integrity of reported experiments therefore requires both the
102 identities of nucleotide sequence reagents and their text descriptions to be correct (11, 12).
103 Accurate reporting of nucleotide sequence reagents is also critical to permit reagent reuse
104 across different experiments and publications (11).

105

106 The ubiquitous description of nucleotide sequence reagents within the biomedical and
107 genetics literature, combined with the routine pairing of nucleotide sequences and text
108 identifiers, are likely to contribute to tacit assumptions that reported nucleotide sequence
109 reagents are correctly identified. However, as nucleotide sequences cannot be understood by

eye, we have proposed that nucleotide sequence reagents are susceptible to different types of errors (8, 11, 12). These error types represent the equivalent of spelling errors (12, 14, 15), as well as identity errors, where a correct sequence is replaced by a different and possibly genetically unrelated sequence (11-13, 16-21).

The problem of wrongly identified nucleotide sequences was recognised in the context of DNA microarrays in the early 2000's, where wrongly identified sequence probes affected the reliability and reproducibility of data from particular microarray platforms (22, 23). Our team subsequently identified frequent wrongly identified sh/siRNA's and RT-PCR primers in studies that reported the effects of knocking down single human genes in cancer cell lines (12, 13). Some incorrect sh/siRNA's invalidated the experimental results reported, through for example the repeated use of "non-targeting" shRNA's that were in fact indicated to target specific human genes (11-13). Our discovery of incorrect and indeed impossible results associated with wrongly identified nucleotide sequence reagents led us to develop a semi-automated tool Seek & Blastn (S&B) to fact-check the reported identities of nucleotide sequence reagents in human research publications (12, 24). The S&B tool scans text to identify and extract nucleotide sequences and their associated text descriptors, submits extracted nucleotide sequences to blastn analysis (25) to predict their genetic identities and hence their targeting or non-targeting status, and then compares the predicted status of each nucleotide sequence reagent with the claimed status within the text (12). The blastn results for each extracted nucleotide sequence are then reported and any sequence whose text identifier contradicts its blastn-predicted targeting/ non-targeting status is flagged as being potentially incorrect (12). Flagged nucleotide sequences are then subjected to manual verification, as described in our original publication (12) and an expanded online protocol (26).

135

136 Our initial application of S&B identified 77/203 (38%) screened papers with incorrect
 137 nucleotide sequence reagents, with our focus being the description of the S&B tool (12), as
 138 opposed to its application. We have now employed S&B to screen original research papers
 139 across 5 literature corpora, representing 3 targeted and two journal corpora. The 3 targeted
 140 corpora included papers that were identified through literature searches that employed
 141 specific keywords, and in some cases, PubMed similarity searches of index papers. The two
 142 journal corpora included all original and original open-access papers in *Gene* and *Oncology*
 143 *Reports*, respectively, as examples of journals that have published papers with wrongly
 144 identified nucleotide sequences (11-13). S&B screening of 11,799 papers flagged 3,423
 145 papers for further analysis, which required manually verifying the identities of over 13,700
 146 nucleotide sequences. Our combined results provide very worrying evidence that human
 147 research papers with wrongly identified nucleotide sequences are unacceptably frequent
 148 within the literature.

149

Results

Analysis of targeted publication corpora

To extend our previous results from employing S&B to screen gene function papers (11, 12), we used S&B to screen 3 targeted publication corpora (Fig 1) that were identified through literature searches that employed specific keywords, and in some cases, PubMed similarity searches of index papers (12, 13). In all cases, the keywords that were used to derive targeted corpora did not refer to author affiliations, such as institution type or country of origin (see Methods).

Single gene knockdown (SGK) corpus

We have previously identified frequent wrongly identified nucleotide sequences in papers that reported the effects of knocking down a single human gene in cancer cell lines that corresponded to a single cancer type, which we referred to as single gene knockdown (SGK) papers (12, 13). As a subset of SGK papers analysed the same human genes across multiple papers and cancer types (12, 13), we sought to identify all SGK papers for a set of 17 human genes (*ADAM8*, *ANXA1*, *EAG1*, *GPR137*, *ICT1*, *KLF8*, *MACC1*, *MYO6*, *NOB1*, *PP4R1*, *PP5*, *PPM1D*, *RPS15A*, *TCTN1*, *TPD52L2*, *USP39*, and *ZFX*), most of which had been analysed in previously reported papers (12, 13).

By combining each of the 17 human gene identifiers with keywords previously used to identify SGK papers (13), we identified 174 SGK papers published between 2006-2019 across 83 journals (Table 1). As most gene identifiers were selected from previously reported SGK papers (12, 13), the SGK corpus consisted of 41 (24%) previously reported papers and

132 (76%) additional papers (Table 2). All 174 SGK papers analysed gene function in a single human cancer type (Table 2). Across the 17 queried genes, we identified a median of 8 papers/ gene (range 3-20) that analysed a median of 8 human cancer types (range 3-11) (Table 2, S1 Data). Most (136/174, 78%) SGK papers named a single queried gene in their titles, with the remaining titles also referring to other human gene(s) and/or drugs, most frequently cisplatin (S1 Data). Most (159/174, 91%) SGK papers were published by authors from mainland China, almost all of which were affiliated with hospitals (147/159, 92%) (see Methods) (Table 1). In contrast, less than half (6/15, 40%) SGK papers from 5 other countries were affiliated with hospitals (Table 1, S1 Data).

S&B screening (24) flagged 144/174 (83%) SGK papers for further analysis (Fig 1). Manual verification of the identities of all nucleotide sequences in flagged papers (see Methods) confirmed that 75/174 (43%) SGK papers included 1-8 wrongly identified sequences/ paper (Table 1, S1 Data). The 75 problematic SGK papers analysed 24 human cancer types, most frequently brain cancer, where 1-9 problematic SGK papers were identified per queried gene (Table 2). Whereas 31/75 (41%) problematic SGK papers have been reported in earlier studies (12, 13), the remaining 45 SGK papers have not been previously analysed (Table 2). The 75 problematic SGK papers were published across 42 journals, where Spandidos Publications published the highest proportion (20/75, 27%) (S1 Data). Problematic SGK papers described 115 wrongly identified sequences (Table 3), where half of these sequences (57/115) targeted a gene or genomic sequence other than the claimed target, followed by incorrect “non-targeting” reagents (44/115, 38%) (Fig 2, Table 3). The 71 incorrect targeting sequences were claimed to interrogate 20 protein-coding genes (S1 Table). Most (67/115, 58%) incorrect sequences recurred across at least 2 SGK papers (Fig 3, S1 Table), where the most frequent incorrect reagent was a previously described “non-targeting” shRNA that is

predicted to target *TPD52L2* (11-13). This shRNA or highly similar variants were employed as “non-targeting” controls in 41% (31/75) problematic SGK papers (S1 Table).

miR-145 corpus

PubMed similarity searches employing individual SGK papers identified numerous papers that analysed the functions of different human miR’s in cancer cell lines. We selected *miR-145* from many possible candidates to define a single miR corpus to be screened by S&B. Papers that focussed upon *miR-145* were identified using PubMed similarity searches of index papers (12, 13) and keyword searches of the Google Scholar database (see Methods). A total of 163 *miR-145* papers were then screened by S&B to flag 50 *miR-145* papers for further analysis (Fig 1). The 50 flagged *miR-145* papers were published between 2009-2019 across 35 journals (Table 1), and examined 18 human cancer types, where a single cancer type was analysed in each paper (S2 Data). All flagged papers examined *miR-145* in combination with 1-5 other human gene(s) that were named in publication titles, with a minority of titles (5/50, 10%) also naming a single drug (S2 Data). Most (44/50, 88%) flagged *miR-145* papers were published by authors from China, where most papers (40/44, 91%) were also affiliated with hospitals (Table 1, S2 Data). The 6 *miR-145* papers from 5 other countries were affiliated with institutions other than hospitals (Table 1, S2 Data).

Manual verification of S&B results revealed that most (31/50, 62%) flagged *miR-145* papers described at least one wrongly identified sequence, with a median of one (range 1-5) incorrect sequence/ paper (Table 1, S2 Data). The 31 problematic *miR-145* papers were published from 2009-2019 across 25 journals, with the highest proportion published by Wiley (S2 Data). The 31 problematic *miR-145* papers analysed 12 human cancer types, most

frequently colorectal or lung cancer, and described 49 wrongly identified sequences, most of which (38/49, 78%) targeted a different gene or target from that claimed (Fig 2, Table 3). The 47 incorrect targeting sequences (Table 3) were claimed to interrogate 13 protein-coding and 4 non-coding RNA's (ncRNA's) (S1 Table). In contrast to SGK papers, most incorrect sequences in *miR-145* papers were employed as (RT)-PCR primers (Table 3) and were identified only once within the corpus (Fig 3). All problematic *miR-145* papers were published by authors from China, where almost all papers (29/31, 94%) were affiliated with hospitals (Table 1, S2 Data).

Cisplatin and Gemcitabine (C+G) corpus

We noted examples of SGK and *miR-145* papers that analysed the effects of drugs in cancer cell lines (S1 Data, S2 Data). PubMed similarity searches of index papers (12, 13) combined with keyword searches of the Google Scholar database were therefore employed to identify papers that described either cisplatin or gemcitabine treatment of human cancer cell lines and/or cancer patients (see Methods) (Fig 1). A total of 258 papers were screened by S&B to flag 100 papers (n=50 papers for each drug) for further analysis as a combined cisplatin + gemcitabine (C+G) corpus (Fig 1). The 100 flagged C+G papers were published between 2008-2019 across 48 journals (Table 1) and referred to a median of 2 (range 0-4) human genes across their titles (S3 Data). The 100 flagged C+G papers examined 13 human cancer types, where most (96/100, 96%) examined a single cancer type, typically pancreatic (35/100, 35%) or lung (22/100, 22%) cancer (S3 Data), reflecting the clinical use of cisplatin and gemcitabine (27, 28). Most (90/100, 90%) C+G papers were published by authors from China, where most (82/90, 91%) were also affiliated with hospitals (Table 1, S3 Data). In contrast, 1/10 C+G papers from 8 other countries was hospital-affiliated (Table 1, S3 Data).

246

247 Approximately half (51/100, 51%) the flagged C+G papers were found to include a median
 248 of 2 (range 1-8) wrongly identified sequences/ paper (Table 1). The 51 problematic C+G
 249 papers were published between 2009-2019 across 31 journals (Table 1), where Springer
 250 Nature published the highest proportion (15/51, 29%), followed by Elsevier (12/51, 24%) (S3
 251 Data). The 51 problematic C+G papers examined 13 human cancer types, most frequently
 252 pancreatic cancer, and described 109 wrongly identified nucleotide sequences, most of which
 253 (79/109, 72%) targeted a gene or genomic sequence other than the claimed target (Fig 2,
 254 Table 3, S1 Table). The 103 incorrect targeting sequences (Table 3) were claimed to
 255 interrogate 31 protein-coding genes and 16 ncRNA's (S1 Table). As in *miR-145* papers, most
 256 incorrect sequences in problematic C+G papers represented (RT)-PCR primers (Table 3) and
 257 were identified once within the corpus (Fig 3). Almost all (50/51, 98%) problematic C+G
 258 papers were published by authors from China, where almost all (48/50, 96%) were affiliated
 259 with hospitals (Table 1, S3 Data).

260

261 ***Analysis of Gene and Oncology Reports corpora***

262 As papers in targeted corpora were selected using known features of human research papers
 263 with incorrect nucleotide sequences, we complemented these analyses by screening original
 264 research papers in the journals *Gene* and *Oncology Reports*. These journals were selected as
 265 examples of journals that have published papers with incorrect nucleotide sequences (11-13),
 266 where *Oncology Reports* also published the highest number of problematic SGK papers (S1
 267 Data). S&B was employed to screen all original articles published in *Gene* from 2007-2018,
 268 and all open-access articles published in *Oncology Reports* from 2014-2018 (Table 4).

269

Screening 7,399 original *Gene* articles from 2007-2018 flagged 742 (10%) papers for further analysis (Fig 1) (see Methods). Manual verification of S&B outputs found that 17% (128/742) flagged papers described a median of two (range 1-36) wrongly identified sequences/ paper (Table 4, S4 Data). These 128 problematic papers referred to 186 human genes (n=146 protein-coding, n=40 ncRNA's) across their publication titles (S4 Data). Approximately half (65/128, 51%) the problematic *Gene* papers analysed gene function in research contexts other than human cancer, most frequently by examining gene polymorphisms in patient cohorts (16/65, 25%) (S4 Data). The remaining 60 papers analysed 17 different human cancer types, most frequently lung cancer (12/60, 20%) (S4 Data). A minority of problematic *Gene* papers (7/128, 5%) referred to drugs within their titles. Manual verification of over 5,200 sequences highlighted 284 wrongly identified sequences across the 128 problematic *Gene* papers. Almost all (275/284, 97%) incorrect sequences represented targeting reagents (Fig 2, Table 3) for the analysis of 92 protein-coding genes and 24 ncRNA's (S1 Table). Most (261/279, 92%) incorrect sequences were described once within the *Gene* corpus (Fig 3).

As *Oncology Reports* published many more papers per year than *Gene* from 2007-2018, we employed S&B to screen open-access *Oncology Reports* articles from 2014-2018 (n=3,778 papers, 99% *Oncology Reports* articles) (Fig 1, Table 4). Almost half (1,709/ 3,778, 45%) screened papers were flagged for further analysis (Fig 1), and over one quarter (436/1,709, 26%) of flagged papers were confirmed to describe a median of 2 (range 1-15) wrongly identified sequences/ paper (Table 4, S5 Data). Almost all (432/436, 99%) problematic *Oncology Reports* papers studied gene function in human cancer, most frequently lung (54/432, 13%) or liver cancer (46/432, 11%). A subset (51/432, 12%) of problematic papers referred to 42 different drugs across their titles, most frequently cisplatin or 5-fluorouracil (S5

Data). Manual verification of over 5,100 sequence identities confirmed 995 wrongly identified sequences (S1 Table). Almost all (965/995, 97%) incorrect sequences represented targeting reagents (Fig 2, Table 3) for the analysis of 262 protein-coding genes and 86 ncRNA's (S1 Table). Most (816/965, 85%) incorrect sequences were described once across the *Oncology Reports* corpus (Fig 3).

Geographic, institutional and temporal distributions of problematic Gene and Oncology Reports papers

The 128 problematic *Gene* papers were authored by teams from 19 countries (S1A Fig) (see Methods). Just over half (69/128, 54%) problematic *Gene* papers were authored by teams from China (Table 4, Fig 4, S1B Fig), followed by India (10/128, 8%) and Iran (9/128, 7%) (S1A Fig). Similar results were obtained for the 95 problematic *Gene* papers from 2014-2018, where teams from China authored 66% (63/95) papers (Fig 4). A significantly greater proportion of problematic *Gene* papers from China were affiliated with hospitals (54/69, 78%), compared with papers from other countries (5/59, 8%) (Fisher's exact test, $p < 0.001$, $n = 128$ papers) (Fig 4). This difference was also noted for problematic *Gene* papers from 2014-2018 (Fisher's exact test, $p < 0.001$, $n = 98$ papers) (Fig 4).

In contrast to the broader geographic distribution of problematic *Gene* papers, the 436 problematic *Oncology Reports* papers were authored by teams from just 13 countries (S2A Fig). Most (393/436, 90%) problematic *Oncology Reports* papers were authored by teams from China (Table 4, Fig 4), followed by much smaller proportions from South Korea (14/436, 3%) and Japan (12/436, 3%) (S2A Fig). As noted for problematic *Gene* papers, a significantly greater proportion of problematic *Oncology Reports* papers from China were

319 affiliated with hospitals (342/393, 87%) compared with papers from other countries (5/43,
320 12%) (Fisher's exact test, $p < 0.001$, $n = 436$ papers) (Fig 4).

321

322 We considered the distributions of problematic *Gene* and *Oncology Reports* papers according
323 to year of publication, country of origin, and affiliated institution type (Fig 5, S1 Fig, S2 Fig).
324 Problematic *Gene* papers were infrequent from 2007-2011 (1-4 papers/ year), rising to 8-38
325 papers/ year from 2012-2018, where the highest number of problematic papers was identified
326 in 2018 (Fig 5). These numbers correspond to 1.0%-4.2% of all original *Gene* papers
327 published per year from 2012-2018. Papers from China represented the majority of
328 problematic *Gene* papers from 2015-2018 (Fig 5), where most papers were also affiliated
329 with hospitals (S1B Fig). Compared with *Gene*, *Oncology Reports* published higher numbers
330 of problematic papers per year, corresponding to 8.3-12.6% original *Oncology Reports* papers
331 from 2014-2018 (Fig 5). Across all 5 years, most (87-93%) problematic *Oncology Reports*
332 papers were authored by teams from China, corresponding to 11% original *Oncology Reports*
333 articles in 2015-2017 (Fig 5), most of which were also affiliated with hospitals (S2B Fig).

334

335 ***Analysis of all problematic human gene function papers***

336 After adjusting for 9 duplicate papers across the 5 corpora, we identified 712 problematic
337 papers with wrongly identified sequences (Fig 1) that were published by 78 journals and 31
338 publishers (S6 Data). The 712 problematic papers included 1,535 wrongly identified
339 sequences, most of which were (RT-)PCR reagents (1,301/1,535, 85%), followed by
340 si/shRNA's (226/1,535, 15%) (Table 3).

341

As most incorrect reagents represented (RT-)PCR primers which are employed as paired reagents, we considered the verified identities of primer pairs that were found to include at least one wrongly identified primer (Fig 6). Problematic papers frequently paired one (RT-)PCR primer that targeted the claimed gene with a primer that was predicted to target a different gene (n=237 papers), or to be non-targeting in human (n=118 papers) (Fig 6). Many problematic papers (n=192) described primer pairs that were predicted to target the same incorrect gene (Fig 6). Problematic papers also combined one non-targeting primer with another that was predicted to target an incorrect gene (n=70 papers), two non-targeting primers (n=63 papers), and/or primers that were predicted to target two different incorrect genes (n=42 papers) (Fig 6). Notably, 21% (276/1,301) incorrect (RT-)PCR primers were predicted to target an orthologue of the claimed gene, typically in rat or mouse (S1 Table).

Bibliometric analysis of human genes analysed in problematic papers

Almost all (1,442/1,535, 94%) incorrect sequences represented targeting reagents that were claimed to target 365 protein-coding genes and 120 ncRNA's (S1 Table). The remaining 88 sequences represented incorrect "non-targeting" sequences that were instead predicted to target 35 genes, most of which (28/35, 80%) were protein-coding genes.

To count the numbers of papers in PubMed that are associated with protein-coding genes in n=709 problematic papers (Fig 7), we used the gene2pubmed service of the National Center for Biotechnology Information (29), restricting these analyses to protein-coding gene identifiers that mapped to official gene names. Primary protein-coding genes, which represented the first-listed genes in publication titles or abstracts, tended to be associated with more papers in PubMed than a randomly chosen human protein-coding gene (median

publication numbers: 167 vs 31, $P < 10^{-109}$, two-sided Mann-Whitney U test) (S3A Fig). Only two genes that were the primary focus of least two problematic papers (*TCTN1* and *GPR137*) (Table 2) have appeared in fewer publications in PubMed than a randomly chosen human protein-coding gene (Fig 7A). We repeated these analyses to examine the protein-coding genes that were claimed as targets by wrongly identified reagents. Again, most wrongly identified target genes have appeared in more papers than a randomly chosen protein-coding gene (median publication numbers: 238 vs 31, $P < 10^{-94}$, two-sided Mann-Whitney U test) (S3B Fig). The most frequent wrongly claimed gene targets were *GAPDH* and *ACTB* (Fig 7B), reflecting their widespread use as RT-PCR control genes. In summary, these analyses demonstrate that problematic papers can focus upon and/or employ reagents that are wrongly claimed to target highly-investigated human genes such as *BCL2*, *EGFR*, *PTEN*, *STAT3*, and *CCND1* (Fig 7).

Post-publication correction, citation and curation of problematic papers

We considered whether any problematic papers have been the subject of post-publication notices, such as retractions, expressions of concern or corrections (11). Only 2% (11/712) of problematic papers have been retracted, where most (8/11) retraction notices did not refer to wrongly identified sequence(s), and 3 problematic papers have been subject to expressions of concern (S2 Table). Although we excluded papers in which incorrect sequences had been subsequently corrected (see Methods), we noted 5 corrections to problematic papers that addressed issues other than incorrect sequences (S2 Table). Almost all (693/712, 97%) problematic papers therefore remain uncorrected within the literature.

We then considered how problematic papers have been curated within gene knowledgebases and cited within the literature. Between 1-207 problematic papers were found within 5 gene knowledgebases that rely upon text mining (30-34), where knowledgebases of miR functions contained the most problematic papers (S3 Table). In March 2021, the 712 problematic papers had been cited 17,183 times according to Google Scholar. Subsets of problematic C+G, *Gene* and *Oncology Reports* papers have also been cited by one or more clinical trials (Fig 8A). Given expected publication delays between pre-clinical and clinical research, we extended these data by considering the approximate potential to translate (APT) for problematic papers (35) according to publication corpus (Fig 8B). The APT metric uses the combination of concepts contained within a paper to infer the probability that the paper will be cited by future clinical trials or guidelines (35). The average APT for problematic papers in the 5 corpora ranged from 15-35% (Fig 8B), indicating that 15-35% of problematic papers in each corpus resemble papers that will be cited by clinical research. Without timely interventions, or without heuristics not captured by the models underlying the APT (35), around one quarter of problematic papers are likely to directly misinform or distract the future development of therapies against human disease.

Discussion

Experimental analyses of gene function require nucleotide sequence reagent identities to precisely match their published descriptions. Wrongly identified nucleotide sequence reagents therefore represent a threat to the continuum of genetics research, from population-based genomic sequencing to pre-clinical functional analyses, and the translation of these results to patients. While there have been previous reports of wrongly identified PCR and gene knockdown reagents in single papers or small cohorts (11-21), the present study is the first to systematically fact-check the identities of nucleotide sequences in over 3,400 papers. Our supported application of S&B (12, 24, 26) for both targeted corpora and journal screening identified 712 papers in 78 journals that describe 1-36 wrongly identified nucleotide sequences/ paper. These 712 papers described over 1,500 wrongly identified sequences, most of which were claimed targeting reagents for the study of over 480 human genes. The sheer number of problematic papers and incorrect reagents that we have uncovered from screening a very small fraction of the human gene function literature predicts a problem of alarming proportions that requires urgent and co-ordinated action.

Before outlining the possible significance of our results, we must recognise the limitations of the present study. Firstly, we recognise that by verifying the identities of over 13,700 sequences, we are likely to have committed some errors of our own. The most challenging incorrect reagents that we encountered are claimed human targeting sequences that appear to have no human target. As blastn is indicated to have a very low but measurable false-negative rate (36, 37), we acknowledge that a small fraction of what appear to be non-targeting reagents may in fact be targeting reagents, as claimed. Nonetheless, as we have taken extensive steps to verify the identities of individual nucleotide sequences (see Methods), we

reasonably expect that false-positives at both the sequence and publication level will be rare within our dataset. Many papers also described at least two wrongly identified nucleotide sequences, building in some protection against false-positives at the publication level.

We also recognise that we have screened three targeted corpora, of which two (the *miR-145* and C+G corpora) did not include all available papers. We therefore recognise that the rates of problematic papers within these corpora may not reflect the rates of problematic papers in other targeted corpora or in the wider literature. Nonetheless, the relative proportions of nucleotide sequence error types in problematic *miR-145* and C+G papers were similar to those identified by screening papers in both *Gene* and *Oncology Reports* (Fig 2). This indicates that features of problematic papers identified within the targeted corpora may extend to papers elsewhere, particularly as problematic *miR-145* and C+G papers were published across 47 journals.

Finally, we recognize that by only verifying the S&B outputs for a minority of papers published in *Gene* and *Oncology Reports*, we will not have described all papers with wrongly identified nucleotide sequences in these journals. Indeed, the identified numbers and proportions of problematic papers are very likely to represent underestimates, as these proportions were derived from verifying sequences in just 10% *Gene* papers from 2007-2018 and 45% *Oncology Reports* papers from 2014-2018. As S&B does not analyze papers where most species identifiers correspond to species other than human (26), and we did not verify the identities of sequences that were claimed to target non-human species, we also recognize that our results do not indicate whether wrongly identified nucleotide sequences occur in

453 papers that examine other species. Analyses of targeted corpora identified around reference
454 papers may begin to answer this question.

455

456 Despite these limitations, our results paint a worrying picture of the reliability of some
457 sections of the human gene-focussed literature. The consequences of many incorrect gene
458 function papers therefore deserve consideration. As previously discussed, papers that describe
459 incorrect nucleotide sequences could encourage the incorrect selection of genes for further
460 experimentation, possibly at the expense of more productive candidates (8). This could be
461 exacerbated where multiple problematic papers report similar results for the analysis of the
462 same gene (8, 13). For example, series of 3-20 single gene knockdown papers that universally
463 claim that the gene target plays a causal role in 3-11 different cancer types could collectively
464 encourage further research. Many incorrect gene function papers could also lead to the
465 overestimation of knowledge of gene function from text mining approaches (30-34),
466 particularly given the assumed reliability of published experimental results (38). Our results
467 demonstrate that problematic papers are already indexed within gene function knowledge
468 bases (30-34).

469

470 As experimental reagents, wrongly identified nucleotide sequences carry the additional risk
471 of being wrongly used in other studies (8, 11). Although siRNA's and shRNA's are
472 increasingly purchased from external companies as preformulated reagents, many researchers
473 continue to order custom-made PCR primers. The most frequent incorrect reagent type that
474 we have identified can therefore easily be reused from the literature. Experiments that either
475 attempt to replicate published results associated with incorrect reagents and/or unknowingly
476 reuse incorrect reagents are likely to generate unexpected results that may then remain

unpublished. As possible evidence of this, we could only identify one study that reported incorrect sequences based on the results of follow-up experiments (20) that our analyses also identified. Given that over 17,000 citations have been accumulated by the problematic papers that we have identified, it seems inevitable that unreliable gene function papers are already wasting time and resources.

Given the numbers of problematic papers and incorrect reagents that we have identified, we must consider possible explanations for both incorrect nucleotide sequences and the papers that describe them. As nucleotide sequences are prone to being affected by different error types, we believe that wrongly identified sequences represent unintended errors that persist when authors, editors and peer reviewers do not check sequence identities during manuscript preparation or review (8, 11, 12). We recognize that wrongly identified sequences in papers could also reflect some form of research sabotage. However, as workplace sabotage is typically directed towards known individuals (39, 40), this seems an unlikely explanation for wrongly identified sequences across hundreds of gene function papers published by many different authors.

We also recognize that some of the wrongly identified sequences that we have described have almost certainly arisen in the context of genuine research. Nonetheless, some wrongly identified sequences were associated with results that appeared highly implausible. For example, the majority of incorrect (RT-)PCR primer pairs that we identified should have uniformly failed to generate (RT-)PCR products across all templates, and yet appeared to generate results that were consistent with primers targeting the claimed genes. As previously reported (11-13), we also identified papers where the non-targeting si/shRNA was verified to

501 target the gene of interest and yet still generated the expected negative or baseline results.

502 Based on the many discrepancies between verified sequence identities and their associated

503 results, it would appear that many experiments across hundreds of gene function papers were

504 not performed as described.

505

506 Although most analyses of experimental errors consider the context of genuine research (41-

507 43), errors can also arise within the context of fraudulent or fabricated research (43, 44). We

508 have previously proposed that incorrect nucleotide sequences could flag that some affected

509 papers are fraudulent (8, 11-13, 45, 46). Due to numerous unexpected similarities between

510 single gene knockdown papers with incorrect sequences, we initially proposed that these

511 papers may reflect the undeclared involvement of organisations such as paper mills (13)

512 which have been alleged to mass-produce fraudulent manuscripts for publication (47). The

513 production of fraudulent manuscripts at scale may involve the use of manuscript templates,

514 leading to unusual degrees of similarity between the resulting publications (8, 13, 46). Papers

515 with incorrect nucleotide sequences showed features consistent with the possible use of

516 manuscript templates, such as similarities in textual and figure organisation, outlier levels of

517 textual similarity, superficial explanations for the analysis of particular genes, and generic

518 experimental approaches regardless of predicted gene function (8, 13, 45, 46). In addition to

519 the use of manuscript templates, we have proposed that producing many gene-focused

520 manuscripts at minimal cost could involve writers with either an incomplete understanding of

521 the experiments that they are describing and/or limited time for quality control (8, 46). These

522 conditions could lead to incorrect nucleotide sequences being a feature of gene function

523 manuscripts from paper mills (8, 46) where incorrect sequences could also be reused across

524 different manuscripts (11-13). The description of errors which are incompatible with reported

results and the presence of multiple incorrect sequences across different techniques within single papers could therefore be features of experiments that were never actually performed.

We have proposed that human genes represent attractive publication targets for paper mills, with many under-studied human genes (48-50) that can be targeted in different cancer or disease types that can then be distributed across different authors and journals over many years (8, 45, 46). However, whereas we had previously hypothesised that under-studied genes might be preferentially targeted by paper mills (8), in the present analysis, problematic papers rarely focussed on under-studied human protein-coding genes, and instead either focussed on or employed incorrect reagents that were claimed to target protein-coding genes that had received prior attention. While under-studied genes may present more individual publication opportunities, papers that describe human genes with known functions and significance could carry more editorial and reader interest, increasing the likelihood of problematic manuscripts being accepted for publication and then cited by future research.

Our analyses of both targeted and journal corpora indicate that ncRNA's may provide a further layer of possibilities for the fabrication of gene-focussed papers. While we recognise that studying genes in different diseases and analysing the functions of ncRNA's in combination with other genes are features of genuine research, our results suggest that a focus upon ncRNA's such as miR's could allow the inclusion of more topic variables within manuscripts from paper mills, such as ncRNA's and protein-coding genes that are studied across different disease types, with or without drug or natural product treatments. Examining ncRNA's in combination with other gene(s) could allow larger and more diverse publication series to be created, compared with those that focus on single genes. Furthermore, as

549 ncRNA's possess largely numeric identifiers that may be more difficult to recognise and
550 recall than alphanumeric gene identifiers, any focus upon ncRNA's could contribute to large
551 publication series being less visible within the literature. As papers that describe miR
552 functions have also been shown to be highly cited (51), miR's and other ncRNA's could
553 represent attractive target genes for paper mills.

554

555 In both targeted and journal corpora, most problematic papers were authored by teams from
556 mainland China that were also overwhelmingly affiliated with hospitals. While researchers in
557 different countries may use paper mills to meet publication targets or quotas (49), paper mills
558 have been most widely discussed in the context of academics and medical doctors in China
559 (47, 53-59). Stringent publication requirements may represent a particular challenge for
560 hospital doctors in China, where some hospital doctors have described limited time, training
561 and/or opportunities to undertake research (47, 53-59). Large numbers of human gene
562 function papers with incorrect nucleotide sequences that list hospital affiliations in China
563 could reflect hospital doctors turning to paper mills to meet publication requirements,
564 whereas the contrasting institutional profiles of problematic papers from other countries
565 could highlight different publication pressures elsewhere. In summary, our results combined
566 with previous reports (47, 53-59) indicate that publication pressure upon hospital doctors in
567 China may be exerting measurable effects upon the human gene function literature.

568

569 In summary, we are concerned that the sheer number of human genes that are available for
570 analysis, combined with research drivers that favour the continued investigation of genes of
571 known function (48-50), are unwittingly providing an extensive source of topics around
572 which gene function papers can be fraudulently created. Furthermore, since genuine pre-

clinical gene research requires specialised expertise, time and material resources (13), the mass production of fraudulent gene function papers could be quicker and cheaper by orders of magnitude (8). Given the number of human genes which can be studied either singly or in combination with other genes and topics such as drugs and analysed across different cancer types or other diseases, combined with unrealistic demands for research productivity (46, 53-59), the publication of fraudulent gene function papers could potentially outstrip the publication of genuine gene function research.

Future directions

Our results indicate that the problem of incorrect gene function papers requires urgent action. Within the research community, this can take place in several ways. As the validation of nucleotide sequence identities using algorithms such as blastn (12, 25) represents a routine activity for teams that investigate gene function, we hope that our results will encourage researchers to unfailingly check the identities of published nucleotide sequences, both in the context of their own research and during peer review. Researchers encountering wrongly identified sequences can describe these to authors, journal(s) and/or PubPeer (60) using the reporting fields that we have proposed (11). Researchers can also compare the claims of gene-focused papers with those from high-throughput experimental studies (61) and/or predictive algorithms (38, 62). Professional societies can reinforce the importance of reagent verification through conference presentations, education programs, and journal editorials, and can advocate for tangible incentives to encourage further fact-checking of the genetics literature.

596 Our analysis of only a small proportion of human gene function papers, combined with the
597 discovery that most incorrect nucleotide sequences are unique within screened literature
598 corpora, highlights the need for further screening studies to identify other problematic gene
599 function papers. Journals that published problematic papers in targeted corpora represent
600 possible targets for future screening approaches. As incorrect nucleotide sequences are
601 unlikely to be found in all problematic gene function papers, future research should also
602 combine analyses of nucleotide sequences with other features of concern such as manipulated
603 or recurring experimental images and data (46, 63, 64).

604

605 Unfortunately, efforts from the research community alone will not solve the problem that we
606 have described. Similarly, recent changes to researcher assessment (65, 66) will not address
607 problematic papers that have already been published. The ability to alter the published record
608 relies upon the engagement and co-operation of journals and publishers (11). Over the past
609 year, growing numbers of journals have begun to recognize the issue of manuscripts and
610 publications from paper mills (67-77), including papers that analyse gene function and drug
611 treatment in cell lines (71, 74). While the described efforts to screen incoming manuscripts
612 are welcome and should be extended to all journals that publish gene function research,
613 screening incoming manuscripts must be coupled with addressing problematic papers that are
614 already embedded in the literature (71, 74-76). These efforts could be supported by gene
615 function experts who could explain the significance of incorrect nucleotide sequences and/or
616 provide training for editorial staff, particularly as the necessary researcher skills are already
617 widely available. To overcome the protracted timeframes that can be associated with journal
618 investigations of incorrect sequences (11), we have proposed the rapid publication of editorial
619 notes to transparently flag papers with verifiable errors while journal and institutional
620 investigations proceed (46).

621

622 *Summary and conclusions*

623 To fully extend the benefits of genomics towards patients and broader populations, it is
624 widely recognised that we must understand the functions of every human gene (1, 2).
625 However, the availability of many human genes for experimental analysis, combined with
626 research drivers that favour the continued investigation of genes of known function (46-49),
627 may unwittingly provide an extensive source of topics around which gene function papers
628 can be fraudulently created. Whereas genuine gene research requires time, expertise, and
629 material resources, the mass production of fraudulent gene function papers by paper mills
630 could be quicker and cheaper by orders of magnitude (8). Given the number of human genes
631 whose functions can analysed singly and/or in combination with other genes and/or drugs
632 across different cancer types or other diseases, combined with acute demands for research
633 productivity that may not always be matched by researcher capacity and training (78),
634 fraudulent gene function papers could unfortunately outstrip the publication of genuine gene
635 function research. Indeed, the possible extent of the problem of unreliable human gene
636 function papers is indicated by the lack of overlap between the problematic papers that we
637 have reported, and other papers of concern reported elsewhere (71, 74, 79). While publishers
638 and journals decide how to address this urgent problem, laboratory scientists, text miners and
639 clinical researchers must approach the human gene function literature with a critical mindset,
640 and carefully evaluate the merits of individual papers before acting upon their results.

641

642 **Materials and Methods**

643 *Identification of literature corpora*

644 *Single Gene Knockdown (SGK) corpus*

645 Single gene knockdown (SGK) papers were identified by combining each of 17 human gene
 646 identifiers (*ADAM8*, *ANXA1*, *EAG1*, *GPR137*, *ICT1*, *KLF8*, *MACC1*, *MYO6*, *NOB1*, *PP4R1*,
 647 *PP5*, *PPM1D*, *RPS15A*, *TCTN1*, *TPD52L2*, *USP39*, and *ZFX*) with the search string "cancer
 648 AND/OR knockdown AND/OR lentivirus" (13), to search PubMed and Google Scholar
 649 databases in June 2019 using the "allintext:" function for Google Scholar searches. No
 650 publication date ranges, country-specific or journal-based search terms were used to limit
 651 search results. Papers were visually inspected to confirm that papers described gene
 652 knockdown experiments that targeted one of the 17 human genes in human cancer cell lines.

653

654 *miR-145 corpus*

655 The *miR-145* corpus included papers that analysed human *miR-145* function in human cell
 656 lines. Two reference papers PMID 29749434 and PMID 29217166, where PMID 29217166
 657 was verified to describe incorrect nucleotide sequence reagents (24, 26, see below), were
 658 used in PubMed similarity searches conducted in September 2019 and October 2020.
 659 Additional papers were identified through Google Scholar searches employing the keywords
 660 "gene" + "miR-145" + "cancer" conducted in April 2019 and September 2020. Publication
 661 dates were limited to 2019 to broadly align with the SGK corpus. All identified papers were
 662 visually inspected to confirm the analysis of human *miR-145* function in human cell lines.

663

664 *Cisplatin + Gemcitabine (C+G) corpus*

665 The cisplatin + gemcitabine (C+G) corpus included papers that described either cisplatin or
666 gemcitabine treatment of human cancer cell lines and/or biospecimens from cisplatin or
667 gemcitabine-treated cancer patients, where most papers also investigated human gene
668 function. Two reference papers PMID's 30250547 and 26852750 that both described
669 incorrect sequences (24, 26) were used as reference papers in PubMed similarity searches
670 conducted in September 2019 and October 2020. Additional papers were identified using
671 Google Scholar searches with the search string "gene", "cancer", "cisplatin" +/- "miR"
672 conducted in September 2019 and October 2020. A PubMed similarity search for PMID
673 26852750 conducted in September 2019 also identified 5 papers that referred to gemcitabine
674 treatment (PMID's 18636187, 26758190, 28492560, 30117016, 31272718). Four of these
675 papers (PMID's 26758190, 28492560, 30117016, 31272718) described incorrect sequences
676 and were used as reference papers for PubMed similarity searches conducted in September
677 2019 and October 2020. Additional publications were identified through Google Scholar
678 searches with the query "gene", "cancer", "gemcitabine" +/- "miR" between September 2019
679 and October 2020. In all cases, publication dates were limited to 2019 to align with other
680 targeted corpora. Papers were visually inspected to confirm that they studied either cisplatin
681 or gemcitabine treatment in the context of human cancer cell lines or biospecimens, and to
682 exclude papers from other targeted corpora.

683

684 *Journal corpora*

685 *Gene* and *Oncology Reports* were selected for S&B screening as examples of journals that
686 have published papers with incorrect nucleotide sequences (11-13), where *Oncology Reports*
687 also published the highest number of problematic SGK papers (S1 file). Whereas *Gene*

688 (published by Elsevier) encompasses a broad range of gene research across different species,
689 *Oncology Reports* (published by Spandidos Publications) focusses on human cancer research.

690

691 *Gene* papers from January 2007 to December 2018 were retrieved using the Web of Science
692 search criteria: PY= "2007-2018" AND SO= "GENE" AND DT= ("Article" OR "Review").

693 *Oncology Reports* papers from January 2014 to December 2018 were retrieved using the Web
694 of Science search criteria: PY="2014-2018" AND SO= "ONCOLOGY REPORTS" AND

695 DT= ("Article" OR "Review"). In the case of *Gene* papers, DOI's were retrieved, and PDF
696 files were downloaded using the Elsevier Application Programming Interface with Crossref

697 Content negotiation (<http://tdmsupport.crossref.org>), whereas open-access *Oncology Reports*
698 articles were directly downloaded from www.spandidos-publications.com.

699

700 ***Seek & Blastn screening***

701 SGK, *miR-145* and C+G papers were named using PMID's or journal identifiers and
702 screened by S&B as described (12, 24, 26). All SGK papers identified for the 17 selected

703 human genes were screened by S&B. In the case of *miR-145* and C+G papers, S&B
704 screening was conducted until 50 *miR-145*, cisplatin and gemcitabine papers were flagged for

705 further analysis, either because S&B had flagged at least one wrongly identified reagent or
706 had failed to extract any sequences from the text. This required S&B screening of 163 *miR-*

707 *145* papers and 258 C+G papers. S&B screening was conducted in 2019 and/or 2020, with all
708 papers flagged by S&B in 2019 being rescreened by S&B in 2020.

709

Gene papers were labelled with PMID's, and batched pdf files were zipped into two compressed files according to publication dates (2007-2013 and 2014-2018). *Oncology Reports* papers were labelled by PMID's and journal identifiers. S&B screening was conducted between July-October 2019, with all papers rescreened in November 2020-February 2021. *Gene* and *Oncology Reports* papers were flagged for further analysis where S&B had either flagged at least one nucleotide sequence or had failed to extract any sequences from the text.

Visual inspection of papers following S&B screening

Papers were visually inspected to determine the claimed genetic and/or experimental identity of each sequence. If the claimed target or experimental use of any sequence was not evident, or if a sequence was claimed to target a species other than human, the sequence was excluded from further analysis. Papers that had been subject to post-publication corrections where wrongly identified nucleotide sequences had been corrected were also excluded. We included retracted papers, to align with previous descriptions of SGK papers (11-13), and in recognition of the possibility of retracted papers continuing to be cited (80).

Manual verification of nucleotide sequence reagent identities

Nucleotide sequence identities were manually confirmed for all sequences that were not (correctly) extracted and/or flagged as being possibly incorrect by S&B, as described (26). For the *Oncology Reports* and *Gene* corpora, this involved checking at least 34% and 54% of all sequences, respectively. Further verification steps were performed for particular reagents, as follows:

(i) For reagents that were claimed to target specific gene polymorphisms or mutant sequences and for which no sequence match could be identified by either blastn or blat (25, 80), manual sequence alignments were performed in Word with the query sequence in forward, forward complement, reverse and reverse complement orientations, against either the sequence corresponding to the accession number provided within the text, or to the most relevant genomic sequence found in NCBI GenBank, according to the text claim. Sequences were delineated using the R studio “stringr” library and accepted as targeting if the specified mutated base(s), when reverted to their original base(s) as described in NCBI dbSNP <https://www.ncbi.nlm.nih.gov/snp/>, allowed the reagent to target the wild-type sequence according to previously published targeting criteria (12).

(ii) If no significant matches were identified for reagents specified for the analysis of mutant or variant targets, mismatches within the nucleotide sequence were converted to the wild type sequence, either as described in the publication or according to dbSNP and reanalysed as described (26). Reagents that were indicated to target the claimed wild-type sequence were accepted as correct targeting reagents.

(iii) All flagged incorrect targeting sequences were double-checked through additional blastn searches against the database: “Homo sapiens (taxid:9606)”, optimized for “Somewhat similar sequences (blastn)”, using an expect threshold 1000, in February 2021.

Nucleotide sequence reagents that were verified to have been wrongly identified were assigned to one of 3 previously described error categories (11, 12):

(i) Reagents claimed to represent targeting reagents but verified to target a human gene or target other than that claimed within the text. This error category included miR-targeting reverse RT-PCR primers with incorrect gene targeting descriptions, as supported by sequence verification (26), and by having been employed to analyse gene(s) other than the claimed

miR and/or as a claimed universal primer according to Google Scholar searches (11, 13). While we recognise that some of these reagents could amplify the claimed miR target as described, their descriptions as specific targeting reagents were incorrect and could lead to incorrect RT-PCR primer reuse.

(ii) Reagents claimed to target a human gene or genomic sequence but verified to be non-targeting in human. These reagents included RT-PCR primers that targeted introns or other non-transcribed regions within claimed genes.

(iii) Reagents claimed to represent non-targeting reagents in human but verified to target a human gene or genomic sequence.

Additional publication analyses

For all papers subjected to S&B analysis, publication titles were visually inspected to identify human gene identifiers, human cancer types, and drug identifiers which were confirmed through Google searches. Human genes were categorized as either protein-coding or ncRNA's (miRs, lncRNA or circRNA) according to GeneCards (<https://www.genecards.org/>).

Journal publishers were identified via the SCImago database (<https://www.scimagojr.com/>).

The Journal Impact Factor corresponding to the (closest) publication year of each paper was obtained from the Clarivate Analytics Journal Citation Reports database

(<https://jcr.clarivate.com/>). Numbers of original articles published per year by *Gene* and

Oncology Reports were obtained from Clarivate InCites (<https://incites.clarivate.com>), under

Entity type= "Publication Sources", Publication Date= 2007-2018, DT= include only

"Article". The country of origin of each paper was assigned according to the affiliations of at least half of the listed authors. Papers were considered to be affiliated with hospital(s) if the institutional affiliations of at least half of the listed authors were associated with one or more of the keywords: "clinic", "health cent", "hosp", "hospital", "infirmary", "sanatorium", "surgery". Papers not meeting this criterion were considered to be affiliated with institutions other than hospitals. Proportions of problematic papers (from China versus all other countries, hospitals versus other institutions) were compared using the Fisher's Exact test (SPSS statistics 27).

Bibliometric analysis of human genes in problematic papers

Linkages of protein-coding genes to publications were obtained via gene2pubmed from NCBI NIH (<https://ftp.ncbi.nlm.nih.gov/gene/DATA/gene2pubmed.gz>) on 15 July, 2021 as described (29, 49). Two-sided Mann Whitney U tests were performed using SciPy (82). Post-publication notices linked with problematic papers were identified through PubMed and Google Scholar searches. PubMed ID's or other publication identifiers were employed as search queries of gene knowledgebases in May 2021 (30-34). Publication citation counts are those reported by Google Scholar in March, 2021. Problematic papers cited by clinical trials were cited by at least one publication within the NIH Open Citation collection (83), which in MedLine carried the annotation of a publication type of any "clinical trial" (without distinguishing clinical trial stage). The approximate potential to translate (APT) for problematic papers in each corpus was calculated as described (35) and obtained from iCite (83).

804 **Acknowledgements**

805 **Funding:** JAB and CL gratefully acknowledge funding from the US Office of Research
806 Integrity, grant ID ORIIR180038-01-00. JAB, CL and ACD gratefully acknowledge grant
807 funding from the National Health and Medical Research Council of Australia, Ideas grant ID
808 APP1184263. TS gratefully acknowledges funding from the National Science Foundation,
809 1956338, SCISIPBIO: A data-science approach to evaluating the likelihood of fraud and
810 error in published studies; K99AG068544, National Institutes on Aging, Integrative Multi-
811 Scale Systems Analysis of Gene-Expression-Driven Aging Morbidity; National Institute of
812 Allergy and Infectious Diseases, AI135964, Successful Clinical Response In Pneumonia
813 Therapy (SCRIPT) Systems Biology Center. The authors thank journal editorial staff for
814 discussions and support of this study.

815

816 **Authors' contributions (alphabetical order): Conceptualization:** Jennifer Byrne,
817 Guillaume Cabanac, Cyril Labbé, Thomas Stoeger; **Methodology:** Jennifer Byrne, Guillaume
818 Cabanac, Cyril Labbé, Thomas Stoeger; **Formal analysis:** Jennifer Byrne, Bertrand Favier,
819 Yasunori Park, Pranujan Pathmendra, Thomas Stoeger, Rachael West; **Writing - original**
820 **draft preparation:** Jennifer Byrne, Yasunori Park, Thomas Stoeger, Rachael West; **Writing**
821 **- review and editing:** Jennifer Byrne, Guillaume Cabanac, Amanda Capes-Davis, Bertrand
822 Favier, Cyril Labbé, Yasunori Park, Pranujan Pathmendra, Thomas Stoeger, Rachael West;
823 **Funding acquisition:** Jennifer Byrne, Amanda Capes Davis, Cyril Labbé, Thomas Stoeger;
824 **Supervision:** Jennifer Byrne

825

826 **Conflicts of Interest:** The authors have no relevant financial or non-financial interests to
827 disclose.

References

1. Shendure J, Findlay GM, Snyder MW. Genomic Medicine-Progress, Pitfalls, and Promise. *Cell*. 2019;177(1):45-57. PMID: 30901547
2. Green ED, Gunter C, Biesecker LG, Di Francesco V, Easter CL, Feingold EA, et al. Strategic vision for improving human health at The Forefront of Genomics. *Nature*. 2020;586(7831):683-692. PMID: 33116284
3. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. *Nature*. 2001;409(6822):860-921. PMID: 11237011.
4. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. *Science*. 2001;291(5507):1304-13051. PMID: 11181995.
5. Boutros PC. The path to routine use of genomic biomarkers in the cancer clinic. *Genome Res*. 2015(10);25:1508-1513. PMID: 26430161
6. Kaelin WG Jr. Common pitfalls in preclinical cancer target validation. *Nat Rev Cancer*. 2017;17(7):425-440. PMID: 28524181
7. Hahn WC, Bader JS, Braun TP, Califano A, Clemons PA, Druker BJ, et al. An expanded universe of cancer targets. *Cell*. 2021;184(5):1142-1155. PMID: 33667368
8. Byrne JA, Grima N, Capes-Davis A, Labbé C. The possibility of systematic research fraud targeting under-studied human genes: causes, consequences and potential solutions. *Biomarker Insights* 2019;14:1177271919829162. PMID: 30783377
9. Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, et al. The support of human genetic evidence for approved drug indications. *Nat Genet*. 2015;47(8):856-860. PMID: 26121088

- 851 10. King EA, Davis JW, Degner JF. Are drug targets with genetic support twice as likely to
852 be approved? Revised estimates of the impact of genetic support for drug mechanisms on
853 the probability of drug approval. PLoS Genet. 2019;15(12):e1008489. PMID: 31830040
- 854 11. Byrne JA, Park Y, West RA, Capes-Davis A, Cabanac G, Labbé C. The thin ret(raction)
855 line: biomedical journal responses to reports of incorrect non-targeting nucleotide
856 sequence reagents in human gene knockdown publications. Scientometrics.
857 2021;126:3513-3534.
- 858 12. Labbé C, Grima N, Gautier T, Favier B, Byrne JA. Semi-automated fact-checking of
859 nucleotide sequence reagents in biomedical research publications: the Seek & Blastn
860 tool. PLoS ONE. 2019;14(3):e0213266. PMID: 30822319
- 861 13. Byrne JA, Labbé C. Striking similarities between publications from China describing
862 single gene knockdown experiments in human cancer cell lines. Scientometrics.
863 2017;110:1471-1493.
- 864 14. Habbal W, Monem F, Gärtner BC. Errors in published sequences of human
865 cytomegalovirus primers and probes: do we need more quality control? J Clin Microbiol.
866 2005;43:5408-5409. PMID: 16208034
- 867 15. Shannon BA, Cohen RJ, Garrett KL. Influence of 16S rDNA primer sequence
868 mismatches on the spectrum of bacterial genera detected in prostate tissue by universal
869 eubacterial PCR. The Prostate. 2008;68(14):1487-1491. PMID: 18651564
- 870 16. Chiarella P, Carbonari D, Iavicoli S. Utility of checklist to describe experimental
871 methods for investigating molecular biomarkers. Biomarkers Med. 2015;9(10):989-995.
872 PMID: 26439607
- 873 17. Katavetin P, Nangaku M, Fujita T. Wrong primer for rat angiotensinogen mRNA. Am J
874 Physiol Renal Physiol. 2005;288(5):F1078. PMID: 15821251

- 875 18. Kocemba KA, Dudzik P, Ostrowska B, Laidler P. Incorrect Analysis of MCAM Gene
876 Promoter Methylation in Prostate Cancer. *The Prostate*. 2016;76(15):1464-1465. PMID:
877 27418327
- 878 19. Tamm A. Incorrect primer sequences in the article on methylprednisolone treatment.
879 *Acta Neurol Scand*. 2016;134(1):90. PMID: 27251935
- 880 20. Khoshi A, Sirghani A, Ghazisaeei M, Mahmudabadi AZ, Azimian A. Association
881 between TPO Asn698Thr and Thr725Pro gene polymorphisms and serum anti-TPO
882 levels in Iranian patients with subclinical hypothyroidism. *Hormones*. 2017;16:75-83.
883 PMID: 28500830
- 884 21. Bustin S, Nolan T. Talking the talk, but not walking the walk: RT-qPCR as a paradigm
885 for the lack of reproducibility in molecular research. *Eur J Clin Invest*. 2017;47(10):756-
886 774. PMID: 28796277.
- 887 22. Harbig J, Sprinkle R, Enkemann SA. A sequence-based identification of the genes
888 detected by probesets on the Affymetrix U133 plus 2.0 array. *Nucleic Acids Res*.
889 2005;33(3):e31. PMID: 15722477
- 890 23. Draghici S, Khatri P, Eklund AC, Szallasi Z. Reliability and reproducibility issues in
891 DNA microarray measurements. *Trends Genet*. 2006;22(2):101-109. PMID: 16380191
- 892 24. Labbé C, Cabanac G, West RA, Gautier T, Favier B, Byrne JA. Flagging errors in
893 biomedical papers: to what extent does the leading publication format impede automatic
894 error detection? *Scientometrics*. 2020;124:1139-1156.
- 895 25. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search
896 tool. *J Mol Biol*. 1990;215:403-410. PMID: 2231712
- 897 26. <https://www.protocols.io/view/seek-amp-blastn-standard-operating-procedure-bjhpj5n>
- 898 27. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action.
899 *Eur J Pharmacol*. 2014;740:364-378. PMID: 25058905

- 900 28. de Sousa Cavalcante L, Monteiro G. Gemcitabine: metabolism and molecular
901 mechanisms of action, sensitivity and chemoresistance in pancreatic cancer. Eur J
902 Pharmacol. 2014;741:8-16. PMID: 25084222
- 903 29. Maglott D, Ostell J, Pruitt KD, Tatusova T. Entrez Gene: gene-centered information at
904 NCBI. Nucleic Acids Res. 2011;39:D52-D57. PMID: 2111545
- 905 30. Li G, Ross KE, Arighi CN, Peng Y, Wu CH, Vijay-Shanker K. miRText: A Text Mining
906 System for miRNA-Gene Relation Extraction. PLoS Comput Biol.
907 2015;11(9):e1004391. PMID: 26407127
- 908 31. Vergoulis T, Kanellos I, Kostoulas N, Georgakilas G, Sellis T, Hatzigeorgiou A, et al.
909 mirPub: a database for searching microRNA publications. Bioinformatics.
910 2015;31(9):1502-1504. PMID: 25527833
- 911 32. Lever J, Zhao EY, Grewal J, Jones MR, Jones SJM. CancerMine: a literature-mined
912 resource for drivers, oncogenes and tumor suppressors in cancer. Nat Methods.
913 2019;16(6):505-507. PMID: 31110280
- 914 33. Jang YE, Jang I, Kim S, Cho S, Kim D, Kim K, et al. ChimerDB 4.0: an updated and
915 expanded database of fusion genes. Nucleic Acids Res. 2020;48(D1):D817-D824. PMID:
916 31680157
- 917 34. Roychowdhury D, Gupta S, Qin X, Arighi CN, Vijay-Shanker K. emiRIT: A text-mining
918 based resource for microRNA information. bioRxiv. 2020.11.05.370593; doi:
919 <https://doi.org/10.1101/2020.11.05.370593>
- 920 35. Hutchins BI, Davis MT, Meseroll RA, Santangelo GM. Predicting translational progress
921 in biomedical research. PLoS Biol. 2019;17(10):e3000416. PMID: 31600189.
- 922 36. Koonin EV, Galperin MY. Principles and Methods of Sequence Analysis. In: Sequence-
923 Evolution- Function: Computational Approaches in Comparative Genomics. Kluwer
924 Academic, Boston, 2003.

925 37. Buhler JD, Lancaster JM, Jacob AC, Chamberlain RD. Mercury BLASTN: Faster DNA
926 Sequence Comparison Using a Streaming Hardware Architecture. In: Proceedings of
927 Reconfigurable Systems Summer Institute, July 2007.

928 38. Pinkney HR, Wright BM, Diermeier SD. The lncRNA Toolkit: Databases and In Silico
929 Tools for lncRNA Analysis. *Noncoding RNA*. 2020;6(4):49. PMID: 33339309

930 39. Anderson MS, Ronning EA, De Vries R, Martinson BC. The perverse effects of
931 competition on scientists' work and relationships. *Sci Eng Ethics*. 2007;13:437-461.
932 PMID: 18030595

933 40. Serenko A. Knowledge sabotage as an extreme form of counterproductive behaviour:
934 conceptualisation, typology and empirical demonstration. *J Knowledge Production*.
935 2019;23(7):1260-1288.

936 41. Brown AW, Kaiser KA, Allison DB. Issues with data and analyses: Errors, underlying
937 themes, and potential solutions. *Proc Natl Acad Sci USA*. 2018;115(11):2563-2570.
938 PMID: 29531079

939 42. Wren JD. Algorithmically outsourcing the detection of statistical errors and other
940 problems. *EMBO J*. 2018;37(12):e99651. PMID: 29794111

941 43. Allchin D. Error types. *Perspectives on Science*. 2001;9(1):38-58.

942 44. Stroebe W, Postmes T, Spears R. Scientific misconduct and the myth of self-correction
943 in science. *Perspect Psychol Sci*. 2012;7:670-688. PMID: 26168129

944 45. Byrne J. We need to talk about systematic fraud. *Nature*. 2019;566(7742):9. PMID:
945 30728525

946 46. Byrne JA, Christopher J. Digital magic, or the dark arts of the 21st century-how can
947 journals and peer reviewers detect manuscripts and publications from paper mills? *FEBS*
948 *Lett*. 2020;594(4):583-589. PMID: 32067229

949 47. Else H, Van Noorden R. The fight against fake-paper factories that churn out sham
950 science. *Nature*. 2021;591(7851):516-519. PMID: 33758408

951 48. Pfeiffer T, Hoffmann R. Temporal patterns of genes in scientific publications. *Proc Natl*
952 *Acad Sci U S A*. 2007;104(29):12052-12056. PMID: 17620606

953 49. Stoecker T, Gerlach M, Morimoto RI, Nunes Amaral LA. Large-scale investigation of the
954 reasons why potentially important genes are ignored. *PLoS Biol*. 2018;16(9):e2006643.
955 PMID: 30226837

956 50. Gates AJ, Gysi DM, Kellis M, Barabási AL. A wealth of discovery built on the Human
957 Genome Project - by the numbers. *Nature*. 2021;590(7845):212-215. PMID: 33568828

958 51. Fire M, Guestrin C. Over-optimization of academic publishing metrics: observing
959 Goodhart's Law in action. *Gigascience*. 2019;8(6):giz053. PMID: 31144712

960 52. Hu Z, Wu Y. An empirical analysis on number and monetary value of ghostwritten
961 papers in China. *Curr Sci*. 2013;105:1230-1234.

962 53. Liu X, Chen X. Journal Retractions: Some Unique Features of Research Misconduct in
963 China. *J Schol Pub*. 2018;49(3):305-319.

964 54. Han J, Li Z. How Metrics-Based Academic Evaluation Could Systematically Induce
965 Academic Misconduct: A Case Study. *East Asian Sci Tech Soc*. 2018;12(2):165-179.

966 55. Zhao T, Dai T, Lun Z, Gao Y. An Analysis of Recently Retracted Articles by Authors
967 Affiliated with Hospitals in Mainland China. *J Schol Pub*. 2021;52(2):107-122.

968 56. Li Y. Chinese doctors connecting to the English publishing world: literature access,
969 editorial services, and training in publication skills. *Publications*. 2014;2:1-13.

970 57. Li Y. Chinese medical doctors negotiating the pressure of the publication requirement.
971 *Iberica*. 2014;28:107-126.

972 58. Tian M, Su Y, Ru X. Perish or publish in China: pressures on young Chinese scholars to
973 publish in internationally indexed journals. *Publications*. 2016;4:9.

- 974 59. Yi N, Nemery B, Dierickx K. Perceptions of research integrity and the Chinese situation:
975 In-depth interviews with Chinese biomedical researchers in Europe. *Account Res.*
976 2019;28:405-426. PMID: 31379202.
- 977 60. Barbour B, Stell BM. PubPeer: Scientific assessment without metrics. In: Biagioli M,
978 Lippman A, editors. *Gaming the metrics: Misconduct and manipulation in academic*
979 *research*. MIT Press; 2020. pp. 149-155.
- 980 61. Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, et al.
981 *Defining a Cancer Dependency Map*. *Cell*. 2017;170(3):564-576. PMID: 28753430
- 982 62. Chen L, Heikkinen L, Wang C, Yang Y, Sun H, Wong G. Trends in the development of
983 miRNA bioinformatics tools. *Brief Bioinform*. 2019;20(5):1836-1852. PMID: 29982332
- 984 63. Fanelli D, Costas R, Fang FC, Casadevall A, Bik EM. Testing Hypotheses on Risk
985 Factors for Scientific Misconduct via Matched-Control Analysis of Papers Containing
986 Problematic Image Duplications. *Sci Eng Ethics*. 2019;25(3):771-789. PMID: 29460082
- 987 64. Acuna DE, Brookes PS, Kording KP. Bioscience-scale automated detection of figure
988 element reuse. *bioRxiv* 2018;269415. doi: <https://doi.org/10.1101/269415>
- 989 65. Wang L, Liu Z. Keeping a clean research environment: Addressing research misconduct
990 and improving scientific integrity in China. *Cancer Lett*. 2019;464:1-4. PMID: 31430529
- 991 66. Zhang L, Sivertsen G. The New Research Assessment Reform in China and Its
992 Implementation. *Schol Assess Reports*. 2020;2(1):3.
- 993 67. Miyakawa T. No raw data, no science: another possible source of the reproducibility
994 crisis. *Mol Brain*. 2020;13(1):24. PMID: 32079532
- 995 68. Pinna N, Clavel G, Roco MC. The *Journal of Nanoparticle Research* victim of an
996 organized rogue editor network! *J Nanopart Res*. 2020;22:376.
- 997 69. Pines J. Image integrity and standards. *Open Biol*. 2020;10(6):200165. PMID: 32574547

- 998 70. Hackett R, Kelly S. Publishing ethics in the era of paper mills. Biol Open.
999 2020;9(10):bio056556. PMID: 33115756
- 1000 71. Seifert R. How Naunyn-Schmiedeberg's Archives of Pharmacology deals with fraudulent
1001 papers from paper mills. Naunyn Schmiedebergs Arch Pharmacol. 2021;394(3):431-436.
1002 PMID: 33547901
- 1003 72. Frederickson RM, Herzog RW. Keeping Them Honest: Fighting Fraud in Academic
1004 Publishing. Mol Ther. 2021;29(3):889-890. PMID: 33581045
- 1005 73. Calver M. Combatting the rise of paper mills. Pacific Conserv Biol. 2021;27:1-2.
- 1006 74. Fisher L, Cox R. *RSC Advances* Editorial: retraction of falsified manuscripts. RSC Adv.
1007 2021;11:4194.
- 1008 75. Behl C. Science integrity has been never more important: It's all about trust. J Cell
1009 Biochem. 2021;22(7):694-695. PMID: 33559144
- 1010 76. Heck S, Bianchini F, Souren NY, Wilhelm C, Ohl Y, Plass C. Fake data, paper mills, and
1011 their authors: The International Journal of Cancer reacts to this threat to scientific
1012 integrity. Int J Cancer. 2021;149(3):492-493. PMID: 33905542
- 1013 77. Cooper CDO, Han W. A new chapter for a better Bioscience Reports. Biosci Rep.
1014 2021;41(5):BSR20211016. PMID: 33942870
- 1015 78. Memon AR, Rathore FA. The rising menace of scholarly black market Challenges and
1016 solutions for improving research in low- and middle-income countries. J Pak Med Assoc.
1017 2021;71(6):1523-1526. PMID: 34111064
- 1018 79. Bik E. The Tadpole paper mill. [https://scienceintegritydigest.com/2020/02/21/the-](https://scienceintegritydigest.com/2020/02/21/the-tadpole-paper-mill/)
1019 [tadpole-paper-mill/](https://scienceintegritydigest.com/2020/02/21/the-tadpole-paper-mill/)
- 1020 80. Schneider J, Ye D, Hill AM, Whitehorn AS. Continued post-retraction citation of a
1021 fraudulent clinical trial report, 11 years after it was retracted for falsifying data.
1022 Scientometrics. 2020;125:2877-2913.

1023 81. Lee CM, Barber GP, Casper J, Clawson H, Diekhans M, Gonzalez JN, et al. UCSC
1024 Genome Browser enters 20th year. Nucleic Acids Res. 2020;48(D1):D756-D761. PMID:
1025 31691824

1026 82. Virtanen P, Gommers R, Oliphant TE, Haberland M, Reddy T, Cournapeau D, et al.
1027 SciPy 1.0: fundamental algorithms for scientific computing in Python. Nat Methods.
1028 2020;17(3):261-272. PMID: 32015543

1029 83. Hutchins BI, Baker KL, Davis MT, Diwersy MA, Haque E, Harriman RM, et al. The
1030 NIH Open Citation Collection: A public access, broad coverage resource. PLoS Biol.
1031 2019;17(10):e3000385. PMID: 31600197

1032

Figure and Supporting Information Captions

Fig 1. Diagram describing the five literature corpora screened by S&B. For each corpus (top row), the diagram shows the numbers of articles that were (i) screened by S&B (white), (ii) flagged by S&B with sequences manually verified (grey), and (iii) found to be problematic by describing at least one wrongly identified nucleotide sequence (dark grey). Total numbers of problematic papers and wrongly identified sequences are indicated below the diagram, corrected for duplicate papers between the corpora.

Fig 2. Percentages of sequence identity error types in each corpus. Percentages of wrongly identified nucleotide sequence reagents that correspond to the 3 identity error types (Y axis) in each corpus (X axis). Percentages corresponding to each error type are indicated, rounded to the nearest single digit. The numbers of incorrect sequences in each corpus are shown below the X axis.

Fig 3. Percentages of wrongly identified nucleotide sequences that were either unique or repeated within each corpus. Percentages of wrongly identified sequences that were identified at least twice in any single corpus (black) are shown above each image, rounded to the nearest single digit. All other wrongly identified sequences were unique in the indicated corpus (grey). Numbers of wrongly identified sequences identified in each corpus are shown below each image.

Fig 4. Percentages of problematic *Gene* and *Oncology Reports* papers according to hospital affiliation status and country of origin. Percentages of problematic *Gene* and

Oncology Reports papers according to hospital affiliation status (Y axis) from either China or all other countries (X axis). The journal and relevant date ranges of problematic papers are shown above each panel. Problematic papers that were (not) affiliated with hospitals are shown in blue (grey), respectively. Percentages shown have been rounded to the nearest single digit. Numbers of problematic papers from China or all other countries are indicated below the X axis. For the comparisons shown in each panel, significantly higher proportions of problematic papers from China were affiliated with hospitals versus problematic papers from other countries (Fisher's Exact test, $p < 0.001$).

Fig 5. Percentages and numbers of problematic *Gene* and *Oncology Reports* papers per year. Percentages of all *Gene* or *Oncology Reports* papers that were found to be problematic (Y axis) per publication year (X axis). The journal and relevant publication year ranges are shown above each panel. Problematic papers from China or all other countries are shown in orange or grey, respectively. Percentages shown are rounded to one decimal place. Total numbers of problematic papers per year are shown below each graph.

Fig 6. Summary of (RT-)PCR primer pairings that involved at least one wrongly identified primer. For $n=851$ primer pairs that were claimed to target particular genes/sequences (gene X) (left panel), one or both primers were predicted to be incorrect (right panel), either by targeting unrelated genes or sequences (gene Y or gene Z), or by having no predicted human target (no target). Numbers of primer pairs and affected papers are indicated below each incorrect primer pair category. Some problematic papers described more than one (category of) incorrect primer pairing. Left- or right-hand primers are not intended to indicate forward or reverse primer orientations.

Fig 7. Numbers of past research papers that have studied human protein-coding genes in problematic papers. Numbers (log base 10) of problematic papers (Y axis) versus past research papers (X axis) for A: primary protein-coding genes in problematic papers and B: claimed protein-coding gene targets of wrongly identified reagents. Vertical dashed lines indicate the median number of research papers for protein-coding genes, with the associated interquartile range shown in grey. Subsets of protein-coding genes are highlighted in each panel.

Fig 8. Clinical trial citations and approximate potential to translate for problematic papers. A: Percentages of problematic papers that are cited at least once according to the NIH Open Citation Collection (Y axis), according to publication corpus (X axis). Error bars indicate 95% confidence intervals of bootstrapped estimates of percentages. Numbers of problematic papers with at least one clinical citation are shown below the X axis for each corpus. B: Average approximate potential to translate for problematic papers (Y axis) according to publication corpus (X axis). Error bars indicate bootstrapped 95% confidence intervals. Numbers of problematic papers for which the approximate potential to translate was computed by iCite are shown below the X axis for each corpus.

S1 Fig. Problematic *Gene* papers per year, according to country of origin and institutional affiliation type. Total numbers of problematic papers for each country/ group of countries are shown in the upper left corner of each panel. A: Numbers of problematic *Gene* papers (Y axes) per publication year (X axes) according to country of origin, shown above each graph. Countries are shown in alphabetical order, from top left. B: Numbers of

problematic *Gene* papers (Y axis) per publication year (X axis) from China (right panel) or all other countries (left panel). Papers affiliated with hospitals or other institution types are shown in blue or grey, respectively. Numbers of problematic papers per year are shown below each stacked bar graph.

S2 Fig. Problematic *Oncology Reports* papers per year, according to country of origin and institutional affiliation type. Total numbers of problematic papers for each country/group of countries are shown in the upper left corner of each panel. A: Numbers of problematic *Oncology Reports* papers (Y axes) per publication year (X axes) according to country of origin, shown above each graph. Countries are shown in alphabetical order, from top left. B: Numbers of problematic *Oncology Reports* papers (Y axis) per publication year (X axis) from China (right panel) or all other countries (left panel). Papers affiliated with hospitals or other institution types are shown in blue or grey, respectively. Numbers of problematic papers per year are shown below each stacked bar graph.

S3 Fig. Human protein-coding genes in problematic papers appear frequently in PubMed. Numbers (log base 10) of PubMed papers (Y axis) for primary protein-coding genes in problematic papers (A) (green) or claimed protein-coding gene targets of wrongly identified reagents (B) (green), versus all other human protein-coding genes (A, B) (grey). Horizontal lines within box plots indicate median values, with box plots showing percentiles according to letter-proportions, ie +/- 25% percentile, +/- (25 + 25/2)% percentile, +/- (25 + 25/2 + 25/4)% percentile.

S1 Table. Wrongly identified nucleotide sequence reagents. Wrongly identified sequences are listed for each corpus in separate tabs, as well as a combined list of all wrongly identified sequences. Primary human protein-coding and ncRNA's refer to the first-mentioned gene in each category in each publication title.

S2 Table. Author corrections, expressions of concern and retractions associated with problematic papers.

S3 Table. Problematic papers curated within gene knowledgebases. Problematic papers identified within CancerMine, ChimerDB, emiRIT, miRText, and mirPub are shown in separate tabs.

S1 Data. Single gene knockdown (SGK) corpus. All SGK papers screened by S&B and problematic SGK papers are shown in separate tabs. Primary human genes refer to the first-mentioned gene in the publication title or abstract.

S2 Data. *miR-145* corpus. All *miR-145* papers flagged by S&B screening and problematic *miR-145* papers are shown in separate tabs. Primary human genes refer to the first-mentioned gene(s) in the publication title or abstract.

S3 Data. Cisplatin + gemcitabine (C+G) corpus. All C+G papers flagged by S&B screening and problematic C+G papers are shown in separate tabs. Primary human genes refer to the first-mentioned gene(s) in the publication title or abstract.

S4 Data. Problematic *Gene* papers. Primary human genes refer to the first-mentioned gene(s) in the publication title or abstract.

S5 Data. Problematic *Oncology Reports* corpus. Primary human genes refer to the first-mentioned gene(s) in the publication title or abstract.

1150 **S6 Data. Journals and publishers that have published problematic papers.** Journals and
 1151 publishers of problematic SGK, *miR-145* and C+G papers and all problematic papers are
 1152 shown in separate tabs.

Table 1: Descriptions of the targeted corpora screened by Seek & Blastn with manual verification of nucleotide sequence reagent identities

	Single gene knockdown (SGK)		<i>miR-145</i>		Cisplatin + Gemcitabine (C+G)	
	Corpus	Problematic	Corpus	Problematic	Corpus	Problematic
Number of papers (% of corpus)	174 (100%)	75 (43%)	50 (100%)	31 (62%)	100 (100%)	51 (50%)
Number of journals	83	42	35	25	48	31
Publication year median (range)	2015 (2006-2019) ^a	2015 (2010-2019)	2017 (2009-2019)	2017 (2009-2019)	2017 (2008-2019)	2017 (2009-2019)
Journal Impact Factor at publication year median (range)	2.204 (0.098-8.459)	1.778 (0.098-5.712)	3.34 (0.700-9.050)	3.23 (0.700-8.278)	3.571 (1.099-10.391)	3.041 (1.099-8.579)
Number of sequences/ paper median (range)	6 (0-24)	6 (1-24)	11 (4-46)	10 (4-46)	11 (2-71)	12 (2-70)
Number of incorrect sequences/ paper median (range)	ND	1 (1-8)	ND	1 (1-5)	ND	2 (1-8)
Papers from China proportion (%)	159/174 (91%)	73/75 (97%)	44/50 (88%)	31/31 (100%)	90/100 (90%)	50/51 (98%)
Papers from China affiliated with hospitals proportion (%)	147/159 (92%)	68/73 (93%)	40/44 (91%)	28/31 (90%)	82/90 (91%)	48/50 (96%)
Papers from all other countries affiliated with hospitals proportion (%)	6/15 (40%)	0/2 (0%)	0/6 (0%)	0/3 (0%)	1/10 (10%)	0/1 (0%)
Papers with post-publication notices ^b proportion (%)	20/174 (12%)	13/75 (17%)	1/50 (2%)	1/31 (3%)	1/100 (1%)	1/51 (2%)

^aSGK papers were published until June 2019

^bPost-publication notices include retractions, expressions of concern and corrections

Table 2: Cancer types studied in the Single Gene Knockdown (SGK) corpus, where each cancer type corresponds to a single paper

Gene	Previously reported SGK papers	New SGK papers
<i>ADAM8</i>	Liver^a	Breast, Breast, Colorectal, Gastric, Liver, Lung, Pancreatic
<i>ANXA1</i>	N/A	Breast , Breast, Breast, Esophageal, Leukemia, Liver, Lung, Prostate
<i>EAG1</i>	Liposarcoma, Osteosarcoma	Brain, Osteosarcoma , Osteosarcoma, Ovarian , Sarcoma
<i>GPR137</i>	Bladder, Brain, <u>Colorectal</u>^b, Pancreatic	Brain, Gastric, Leukemia , Liver, Osteosarcoma, Ovarian , Prostate
<i>ICT1</i>	<u>Brain</u>	Breast, Gastric, Leukemia, Lung, Lymphoma, Prostate
<i>KLF8</i>	Osteosarcoma	Bladder, Brain , Brain, Brain, Breast, Colorectal, Colorectal, Gastric, Gastric , Gastric, Gastric, Liver, Liver, Nasopharyngeal, Oral , Ovarian, Pancreatic, Renal
<i>MACC1</i>	Ovarian	Bladder , Brain, Cervical , Cervical, Colorectal, Colorectal, Esophageal , Esophageal, Gallbladder, Gastric, Liver, Liver, Lung, Oral, Oral , Nasopharyngeal, Ovarian , Ovarian, Skin
<i>MYO6</i>	<u>Brain</u>, Colorectal, Liver, Lung	Breast , Gastric, Oral, Prostate
<i>NOB1</i>	Brain, Breast , Colorectal, Liver, Osteosarcoma , Ovarian, Prostate	Laryngeal, Lung, Lung , Oral, Osteosarcoma, Renal, Thyroid, Thyroid
<i>PP4R1</i>	<u>Breast, Liver</u>	Lung
<i>PP5</i>	Colorectal, Ovarian	Bladder, Brain, Leukemia , Liver, Osteosarcoma, Pancreatic, Prostate
<i>PPM1D</i>	Bladder, Lung	Brain, Brain, Breast, Breast, Liver, Pancreatic
<i>RPS15A</i>	Brain, Lung	Brain, Gastric, Leukemia , Liver, Lung, Osteosarcoma, Renal, Thyroid
<i>TCTN1</i>	<u>Brain</u> , Brain, Pancreatic	Brain, Colorectal, Gastric, Thyroid
<i>TPD52L2</i>	<u>Brain, Breast, Gastric, Liver, Oral</u>	Brain
<i>USP39</i>	<u>Liver</u>, Thyroid	Breast, Colorectal , Colorectal, Gastric, Liver, Liver , Lung, Oral , Osteosarcoma, Renal, Skin
<i>ZFX</i>	Brain, Breast	Brain, Brain, Gallbladder, Laryngeal, Leukemia, Lung, Lung, Oral, Oral, Osteosarcoma , Pancreatic, Prostate, Renal

^aCancer types shown in bold correspond to problematic papers with wrongly identified nucleotide sequence(s)

^bUnderlined cancer types correspond to papers that have been retracted or assigned an expression of concern

Table 3: Wrongly identified nucleotide sequences summarized according to experimental technique and identity error type

Corpus	Technique	“Non-targeting” yet targeting proportion (%)	“Targeting” yet non-targeting proportion (%)	Targeting wrong gene/ sequence proportion (%)	Total per corpus proportion (%)
SGK (n = 115 reagents in n = 75 papers)	PCR ^a	0/45 (0)	7/14 (50)	45/57 (79)	52/115 (45)
	Gene knockdown ^b	44/44 (100)	7/14 (50)	12/57 (21)	63/115 (55)
	Other ^c	0/45 (0)	0/14 (0)	0/57 (0)	0/115 (0)
	Total (Error type)	44/44 (100)	14/14 (100)	57/57 (100)	115/115 (100)
miR-145 (n = 49 reagents in n = 31 papers)	PCR	0/2 (0)	8/9 (89)	33/38 (87)	41/49 (84)
	Gene knockdown	2/2 (100)	1/9 (11)	5/38 (13)	8/49 (16)
	Other	0/2 (0)	0/9 (0)	0/38 (0)	0/49 (0)
	Total (Error type)	2/2 (100)	9/9 (100)	38/38 (100)	49/49 (100)
C+G (n = 109 reagents in n = 51 papers)	PCR	0/6 (0)	23/24 (96)	73/79 (93)	96/109 (88)
	Gene knockdown	4/6 (67)	1/24 (4)	5/79 (6)	10/109 (9)
	Other	2/6 (33)	0/24 (0)	1/79 (1)	3/109 (3)
	Total (Error type)	6/6 (100)	24/24 (100)	79/79 (100)	109/109 (100)
Gene (n = 284 reagents in n = 128 papers)	PCR	0/9 (0)	35/42 (83)	218/233 (94)	253/284 (88)
	Gene knockdown	9/9 (100)	7/42 (17)	15/233 (6)	31/284 (11)
	Other	0/9 (0)	0/42 (0)	0/233 (0)	0/284 (0)
	Total (Error type)	9/9 (100)	42/42 (100)	233/233 (100)	284/284 (100)
Oncology Reports (n = 995 reagents in n = 436 papers)	PCR	0/36 (0)	296/335 (88)	573/630 (91)	869/995 (87)
	Gene knockdown	30/30 (100)	37/335 (11)	54/630 (8)	121/995 (12)
	Other	0/36 (0)	2/335 (1)	3/630 (1)	5/995 (1)
	Total (Error type)	30/30 (100)	335/335 (100)	630/630 (100)	995/995 (100)
Total (n = 1,535 reagents in n = 712 papers)	PCR	0/89 (0)	364/416 (87)	937/1,030 (90)	1,301/1,535 (84)
	Gene knockdown	87/89 (98)	50/416 (12)	89/1,030 (9)	226/1,535 (15)
	Other	2/89 (2)	2/416 (1)	4/1,030 (1)	8/1,535 (1)
	Total (Error type)	89/89 (100)	416/416 (100)	1,030/1,030 (100)	1,535/1,535 (100)

^aPCR = Human gene or genomic targeting primers for PCR, RT-PCR or methylation-specific PCR

^bGene knockdown = siRNA or shRNA

^cOther = Claimed Ribozyme, Talen, mimic sequences, other oligonucleotide sequences

Table 4: Summary of features of *Gene* and *Oncology Reports* journals and problematic papers

Feature	<i>Gene</i>	<i>Oncology Reports</i>
Publication years screened by Seek & Blastn	2007-2018	2014-2018
Journal Impact Factor (range during years screened)	2.082-2.871	2.301-3.041
Flagged/ screened papers proportion (%)	742/7,399 (10%)	1,709/3,778 (45%)
Problematic/ flagged papers proportion (%)	128/742 (17%)	436/1,709 (26%)
Incorrect sequences/ problematic paper median (range)	2 (1-36)	2 (1-15)
Problematic papers from China proportion (%)	69/128 (54%)	393/436 (90%)
Problematic papers from all other countries proportion (%)	59/128 (46%)	43/436 (10%)
Problematic papers from China affiliated with hospitals proportion (%)	54/69 (78%)	342/393 (87%)
Problematic papers from all other countries affiliated with hospitals proportion (%)	5/59 (9%)	5/43 (12%)
Retracted or corrected problematic papers proportion (%)	2/128 (2%)	2/436 (0.5%)

Fig 1

Fig 2

Error Types 'Non-targeting' yet targeting 'Targeting' yet non-targeting Targeting wrong gene or genomic sequence

Fig 3

Fig 4

Fig 5

Fig 6**Claimed primer identities****Verified primer identities**

Fig 7

A

B

Fig 8

A

B