

# Pan-cancer image-based detection of clinically actionable genetic alterations

Jakob Nikolas Kather<sup>1,2,3</sup>, Lara R. Heij<sup>4,5,6</sup>, Heike I. Grabsch<sup>7,8</sup>, Loes F. S. Kooreman<sup>7</sup>, Chiara Loef-  
fler<sup>1</sup>, Amelie Echle<sup>1</sup>, Jeremias Krause<sup>1</sup>, Hannah Sophie Muti<sup>1</sup>, Jan M. Niehues<sup>1</sup>, Kai A. J. Sommer<sup>1</sup>,  
Peter Bankhead<sup>9</sup>, Jefree J. Schulte<sup>10</sup>, Nicole A. Cipriani<sup>10</sup>, Nadina Ortiz-Brüchle<sup>6</sup>, Akash Patnaik<sup>11</sup>,  
Andrew Srisuwananukorn<sup>12</sup>, Hermann Brenner<sup>2,13,14</sup>, Michael Hoffmeister<sup>13</sup>, Piet A. van den  
Brandt<sup>15</sup>, Dirk Jäger<sup>2,3</sup>, Christian Trautwein<sup>1</sup>, Alexander T. Pearson<sup>11,\*</sup>, Tom Luedde<sup>1,16,\*</sup>

<sup>1</sup> Department of Medicine III, University Hospital RWTH Aachen, Aachen, Germany

<sup>2</sup> German Cancer Consortium (DKTK), Heidelberg, Germany

<sup>3</sup> Applied Tumor Immunity, German Cancer Research Center (DKFZ), Heidelberg, Germany

<sup>4</sup> Department of Surgery and Transplantation, University Hospital RWTH Aachen, Aachen, Ger-  
many

<sup>5</sup> Department of Surgery, NUTRIM, School of Nutrition and Translational Research in Metabolism,  
Maastricht University, Maastricht, The Netherlands

<sup>6</sup> Institute of Pathology, University Hospital RWTH Aachen, Aachen, Germany

<sup>7</sup> Department of Pathology, GROW School for Oncology and Developmental Biology, Maastricht  
University Medical Center+, Maastricht, The Netherlands

<sup>8</sup> Pathology & Data Analytics, Leeds Institute of Medical Research at St James's, University of  
Leeds, Leeds, UK

22 <sup>9</sup> MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK

23 <sup>10</sup> Department of Pathology, University of Chicago Medicine, Chicago, IL, USA

24 <sup>11</sup> Department of Medicine, University of Chicago Medicine, Chicago, IL, USA

25 <sup>12</sup> Department of Medicine, University of Illinois – Chicago, Chicago, IL, USA

26 <sup>13</sup> Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ),  
27 Heidelberg, Germany

<sup>14</sup> Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center  
for Tumor Diseases (NCT), Heidelberg, Germany

28 <sup>15</sup> Department of Epidemiology, GROW School for Oncology and Developmental Biology, Maas-  
29 tricht University Medical Center+, Maastricht, The Netherlands

30 <sup>16</sup> Division of Gastroenterology, Hepatology and GI Oncology, University Hospital RWTH Aachen,  
31 Aachen, Germany

32 \* these authors contributed equally to this work

33 Correspondence should be addressed to [jkather@ukaachen.de](mailto:jkather@ukaachen.de),

34 [apearson5@medicine.bsd.uchicago.edu](mailto:apearson5@medicine.bsd.uchicago.edu) and [tluedde@ukaachen.de](mailto:tluedde@ukaachen.de)

35

36 **Precision treatment of cancer relies on genetic alterations which are diagnosed by molecular**  
37 **biology assays.<sup>1</sup> These tests can be a bottleneck in oncology workflows because of high turna-**  
38 **round time, tissue usage and costs.<sup>2</sup> Here, we show that deep learning can predict point muta-**  
39 **tions, molecular tumor subtypes and immune-related gene expression signatures<sup>3,4</sup> directly**  
40 **from routine histological images of tumor tissue. We developed and systematically optimized**  
41 **a one-stop-shop workflow and applied it to more than 4000 patients with breast<sup>5</sup>, colon and**  
42 **rectal<sup>6</sup>, head and neck<sup>7</sup>, lung<sup>8,9</sup>, pancreatic<sup>10</sup>, prostate<sup>11</sup> cancer, melanoma<sup>12</sup> and gastric<sup>13</sup> can-**  
43 **cer. Together, our findings show that a single deep learning algorithm can predict clinically ac-**  
44 **tionable alterations from routine histology data. Our method can be implemented on mobile**  
45 **hardware<sup>14</sup>, potentially enabling point-of-care diagnostics for personalized cancer treatment**  
46 **in individual patients.**

47 Clinical guidelines recommend molecular testing of tumor tissue for most patients with advanced  
48 solid tumors. However, in most tumor types, routine testing includes only a handful of altera-  
49 tions, such as KRAS, NRAS, BRAF mutations and microsatellite instability (MSI) in colorectal can-  
50 cer. While new studies identify more and more molecular features of potential clinical relevance,  
51 current diagnostic workflows are not designed to incorporate an exponentially rising load of  
52 tests. For example, in colorectal cancer, previous studies have identified consensus molecular  
53 subtypes (CMS) as a candidate biomarker, but sequencing costs preclude widespread testing.

54 While comprehensive molecular and genetic tests are hard to implement at scale, histological  
55 images stained with hematoxylin and eosin (H&E) are ubiquitously available. We hypothesized  
56 that these routine images contain information about established and candidate biomarkers and  
57 thus could be used for rapid pre-screening of patients, potentially alleviating the load of molec-  
58 ular assays. To test this, we developed, optimized and validated a deep learning algorithm to  
59 determine molecular features directly from histology images. Deep learning with convolutional  
60 neural networks has been used for tissue segmentation in cancer histology<sup>15-17</sup> or detecting mo-  
61 lecular changes in circumscribed use cases in a single tumor type<sup>18-22</sup>, but our aim was to use  
62 deep learning in a pan-molecular pan-cancer approach. Our method is a 'one-stop-shop' work-  
63 flow: we collected large patient cohorts for individual tumor types, partitioning each cohort into

64 three groups for cross-validation (**Fig. 1a**). Whole slide images were tessellated into an image  
65 library of smaller tiles<sup>20,21</sup> which were used for deep transfer learning (**Fig. 1b**). We chose predic-  
66 tion of microsatellite instability (MSI) in colorectal cancer as a clinically relevant benchmark task<sup>20</sup>  
67 and sampled a large hyperparameter space with different commonly used deep learning mod-  
68 els<sup>16,18,20,21</sup>. Unexpectedly, ‘inception’<sup>23</sup> and ‘resnet’<sup>24</sup> networks, which had been the previous de-  
69 facto standard, were markedly outperformed by ‘densenet’<sup>25</sup> and ‘shufflenet’<sup>14</sup> architectures, the  
70 latter demonstrating high accuracy at a low training time (raw data in Suppl. Table 1, N=426 pa-  
71 tients in the “Cancer Genome Atlas” [TCGA] cohort). Shufflenet is optimized for mobile devices,  
72 making this deep neural network architecture attractive for decentralized point-of-care image  
73 analyses or direct implementation in microscopes<sup>26</sup>. We trained a shufflenet on N=426 patients  
74 in the TCGA-CRC cohort<sup>20</sup> and validated it on N=379 patients in the DACHS cohort<sup>20</sup> cohort, reach-  
75 ing an AUC of 0.89 [0.88; 0.92] (**Fig. 1d**). This represents a marked improvement over the previous  
76 best performance of 0.84 in that dataset<sup>20</sup>. Subsequently, we tested the full workflow in breast  
77 cancer for detection of standard molecular pathology features which are usually measured by  
78 immunohistochemistry: Estrogen [ER] and progesterone [PR] receptor status and HER2 status  
79 were highly significantly detectable from histology alone, reaching AUCs of up to 0.82 in a three-  
80 fold patient-level cross-validation (**Fig. 1e**).

81 Having optimized our method in these use cases, we applied it to more than 4000 patients across  
82 ten of the most prevalent solid tumor types from the TCGA reference database. We aimed to  
83 predict all clinically and/or biologically relevant mutations with a prevalence above 2% and af-  
84 fecting at least four patients. The list of candidate mutations (Suppl. Table 2) also included all  
85 point mutations targetable by FDA-approved drugs ([www.oncokb.org](http://www.oncokb.org)). We found that in multiple  
86 major cancer types, the genotype of point mutations was predictable directly from images. For  
87 example, in lung adenocarcinoma (TCGA-LUAD<sup>8</sup>, N=464 patients), significant AUCs were achieved  
88 for TP53 mutational status (AUC 0.71, **Fig. 2a**) and EGFR mutational status (AUC 0.60), which is  
89 targetable by clinically approved treatments. Also in colon and rectal cancer (TCGA-COAD and  
90 TCGA-READ<sup>27</sup>, N=590 patients), standard-of-care genetic biomarkers<sup>28</sup> BRAF (AUC 0.66) and KRAS  
91 (AUC 0.60) were significantly detectable, as were oncogenic driver mutations linked to tumor  
92 aggressiveness, including CDC27<sup>29</sup> (AUC 0.70, **Fig. 2b**). Similarly, in breast cancer (TCGA-BRCA<sup>5</sup>,

93 N=1007 patients), gene mutations of TP53 (AUC 0.75), MAP2K4 (which is a potential biomarker  
94 for response to MEK inhibitors<sup>30</sup>, AUC 0.66) as well as PIK3CA (which is directly targetable by a  
95 small molecule inhibitor<sup>31</sup>, AUC 0.63) were significantly detectable (**Fig. 2c**). In gastric cancer  
96 (TCGA-STAD<sup>13</sup>, N=363 patients), mutations of MTOR – a candidate for targeted treatment<sup>32</sup> –  
97 were significantly detectable with a high AUC of 0.80 (**Fig. 2d**) as were a range of driver mutations  
98 including BRCA2 (AUC 0.67), PTEN (AUC 0.66), PIK3CA (AUC 0.65) among others. In head and neck  
99 squamous cell carcinoma (TCGA-HNSC<sup>7</sup>, N=424 patients), genotype of CASP8, which is linked to  
100 resistance to cell death<sup>33</sup>, was significantly detected with a high AUC of 0.72 (**Suppl. Fig. 1a**). In  
101 other tumor types such as melanoma (TCGA-SKCM<sup>12</sup>, N=429 patients), or lung squamous cell car-  
102 cinoma (TCGA-LUSC<sup>9</sup>, N=412 patients), few mutations were significantly detected (**Suppl. Fig. 1b-**  
103 **c**). Lung squamous cell carcinoma is known for its difficulty in molecular diagnosis and few mo-  
104 lecularly or genetically targeted treatment options even in clinical trials. Thus, it is plausible that  
105 tumor histomorphology was not well correlated to mutations. In pancreatic adenocarcinoma  
106 (TCGA-PAAD<sup>10</sup>, N=166 patients), identifying KRAS wild type patients is of high clinical relevance  
107 because these patients are potential candidates for targeted treatment. Our method significantly  
108 identified KRAS genotype with AUC 0.66 (**Suppl. Fig. 1d**). Lastly, in prostate cancer (TCGA-PRAD<sup>11</sup>,  
109 N=402 patients), our method detected targetable mutations from histology – most remarkably  
110 PIK3CA, which was significantly detected with an AUC of 0.75 (**Suppl. Fig. 1e**). Furthermore,  
111 CDK12, which is linked to immune evasion in prostate cancer<sup>34</sup> was detected with an AUC of 0.71.  
112 Together, these data show that deep learning can detect a wide range of targetable and poten-  
113 tially targetable point mutations directly from histology across multiple prevalent tumor types.

114 Next, we applied our method to a broader set of molecular signatures beyond single mutations.  
115 We chose features with known biological and potential clinical significance which are currently  
116 not part of clinical guidelines in most solid tumors. A major group of such features are immune-  
117 related gene expression signatures<sup>3</sup> of CD8-positive lymphocytes, macrophages, proliferation, in-  
118 terferon-gamma (IFNg) signaling and transforming growth factor beta (TGFb) signaling. These bi-  
119 ological processes are involved in response to cancer treatment, including immunotherapy. De-  
120 tecting their morphological correlates in histology images could facilitate the development of  
121 more nuanced treatment strategies. Indeed, in lung adenocarcinoma signatures of proliferation,

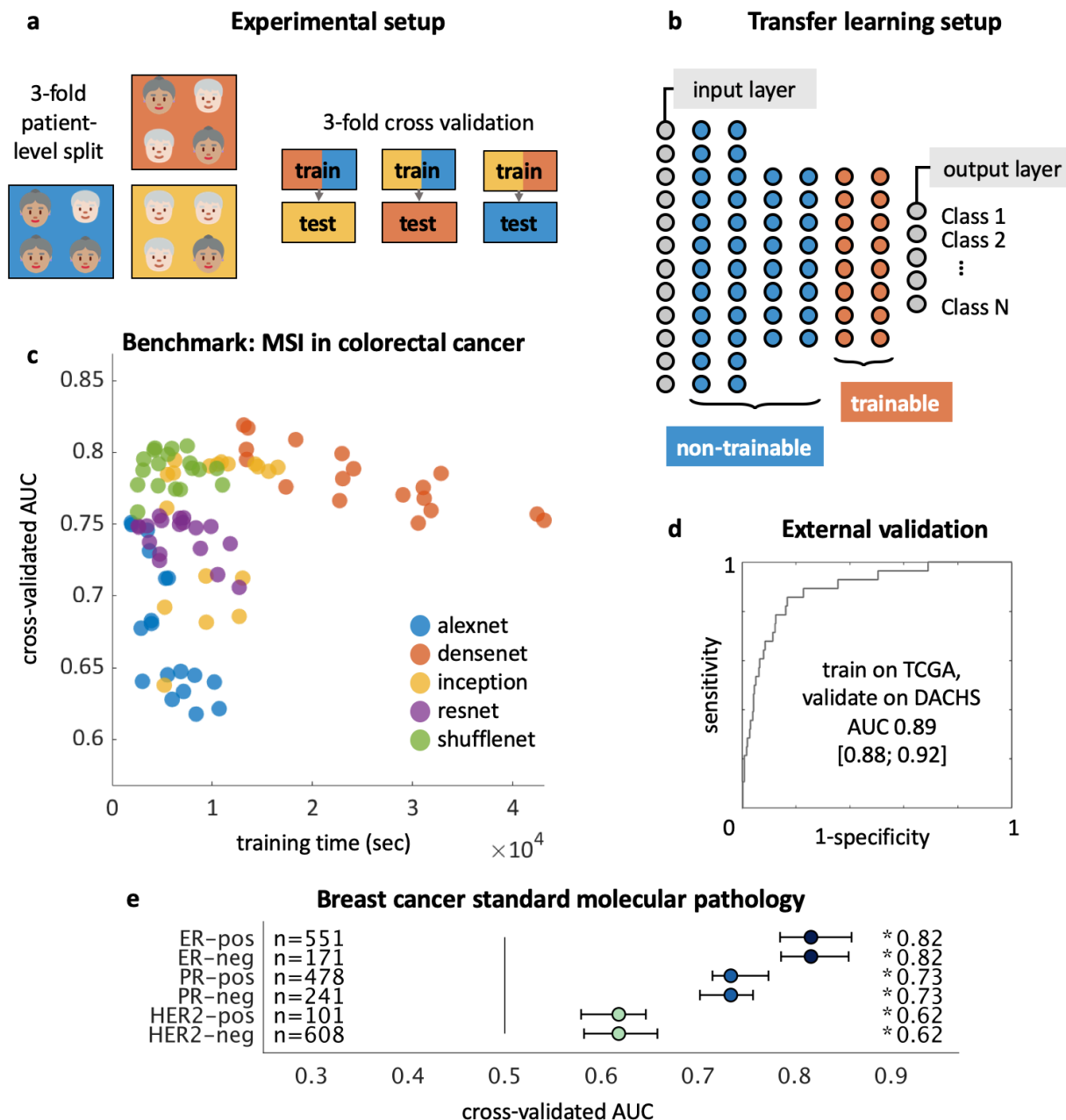
122 macrophage infiltration and T-lymphocyte infiltration were significantly detectable from images  
123 with high AUCs (**Fig. 3a**). Similarly, significant AUCs for these biomarkers were achieved in colo-  
124 rectal cancer (**Fig. 3b**), breast cancer (**Fig. 3d**) and gastric cancer (**Fig. 3d**). In gastric cancer, we  
125 additionally investigated a signature of stem cell properties (stemness) which was highly detect-  
126 able in images (AUC 0.76, **Fig. 3d**). Recent studies have clustered tumors into comprehensive  
127 ‘immune subtypes’<sup>3</sup>, but again this classification system relies on deep molecular profiling una-  
128 vailable in a clinical setting. We found that our method could detect these immune subtypes with  
129 up to AUC 0.75 in lung adenocarcinoma (**Fig. 3a**), up to AUC 0.72 in colorectal cancer (**Fig. 3b**)  
130 and up to AUC 0.71 in breast cancer (**Fig. 3c**). Together, these findings show that immunological  
131 processes that are quantifiable by molecular profiling are also accessible to deep-learning-based  
132 histology image analysis.

133 Finally, we investigated the use of deep learning on conserved molecular classes of tumors such  
134 as recently identified TCGA subtypes<sup>3</sup>, pan-gastrointestinal subtypes<sup>4</sup> and consensus molecular  
135 subtypes of colorectal cancer<sup>6</sup>. Few of these classification systems are currently incorporated in  
136 clinical workflows, mainly because of the high cost and logistic effort associated with sequencing  
137 technology. In our experiments, TCGA molecular subtypes LUAD1-6 were highly detectable in  
138 histology images of lung adenocarcinoma (**Fig. 3a**) with AUCs of up to 0.74. In colorectal cancer  
139 (**Fig. 3b**) and gastric cancer (**Fig. 3d**), the pan-gastrointestinal (GI) subtypes GI-hypermutated-  
140 indel (GI-HM-indel), GI genome stable (GI-GS), GI-chromosomally unstable (GI-CIN), GI-hypermu-  
141 tated-single-nucleotide variant predominant (GI-HM-SNV) and GI Epstein-Barr-Virus-positive (GI-  
142 EBV) were significantly detectable from histology. Correspondingly, in colorectal cancer, ‘consen-  
143 sus molecular subtypes’<sup>6</sup> were detectable by deep learning (**Fig. 3b**). These findings could open  
144 up fundamentally new options for clinical trials of cancer: While accumulating evidence shows  
145 that molecular clusters of tumors are correlated to biologically and clinical outcome, deep mo-  
146 lecular classification of these tumors is usually not available to patients in clinical routine or to  
147 patients within clinical trials. Detecting these subtypes merely from histology would immediately  
148 allow for these subtypes to be analyzed in clinical trials directly from routine material, potentially  
149 helping to identify new biomarkers for treatment response. A full description of the methods is  
150 available in the “Extended Methods” section.

151 Together, our results demonstrate the feasibility of pan-cancer deep learning image-based test-  
152 ing. We show that a unified workflow yields reliably high performance across multiple clinically  
153 relevant scenarios. Compared to conventional genetic tests, our methodology enables detailed  
154 prediction of the spatial heterogeneity of genotypes which is not possible in molecular bulk test-  
155 ing of tumor tissue. An example of this visualization is shown in (**Fig. 4a-g**): Based only on a rou-  
156 tine histological image of colorectal cancer (**Fig. 4a**), deep learning classifiers correctly predicted  
157 CDC27 mutational status (**Fig. 4b-c**) and consensus molecular subtype (**Fig. 4d-g**) with a high prob-  
158 ability, while assigning a low probability to competing classes.

159 Image-based genotyping could be used for definitive testing once performance surpasses previ-  
160 ous tests, potentially disrupting clinical workflows **Suppl. Fig. 3a-c**. A limitation of our method is  
161 the low AUC values for some molecular features, but re-training on larger cohorts with up to  
162 10,000 patients per tumor type is expected to increase performance.<sup>16</sup> Another limitation is that  
163 for very unbalanced features – for scarce molecular features – the uncertainty of the AUC esti-  
164 mate is high. Thus, before clinical implementation, multicenter validation is essential, requiring  
165 collaborative efforts. Together, our results show that deep learning can consistently unlock  
166 dormant patterns in widely available histology images, potentially improving current workflows  
167 for molecularly targeted therapy of cancer.

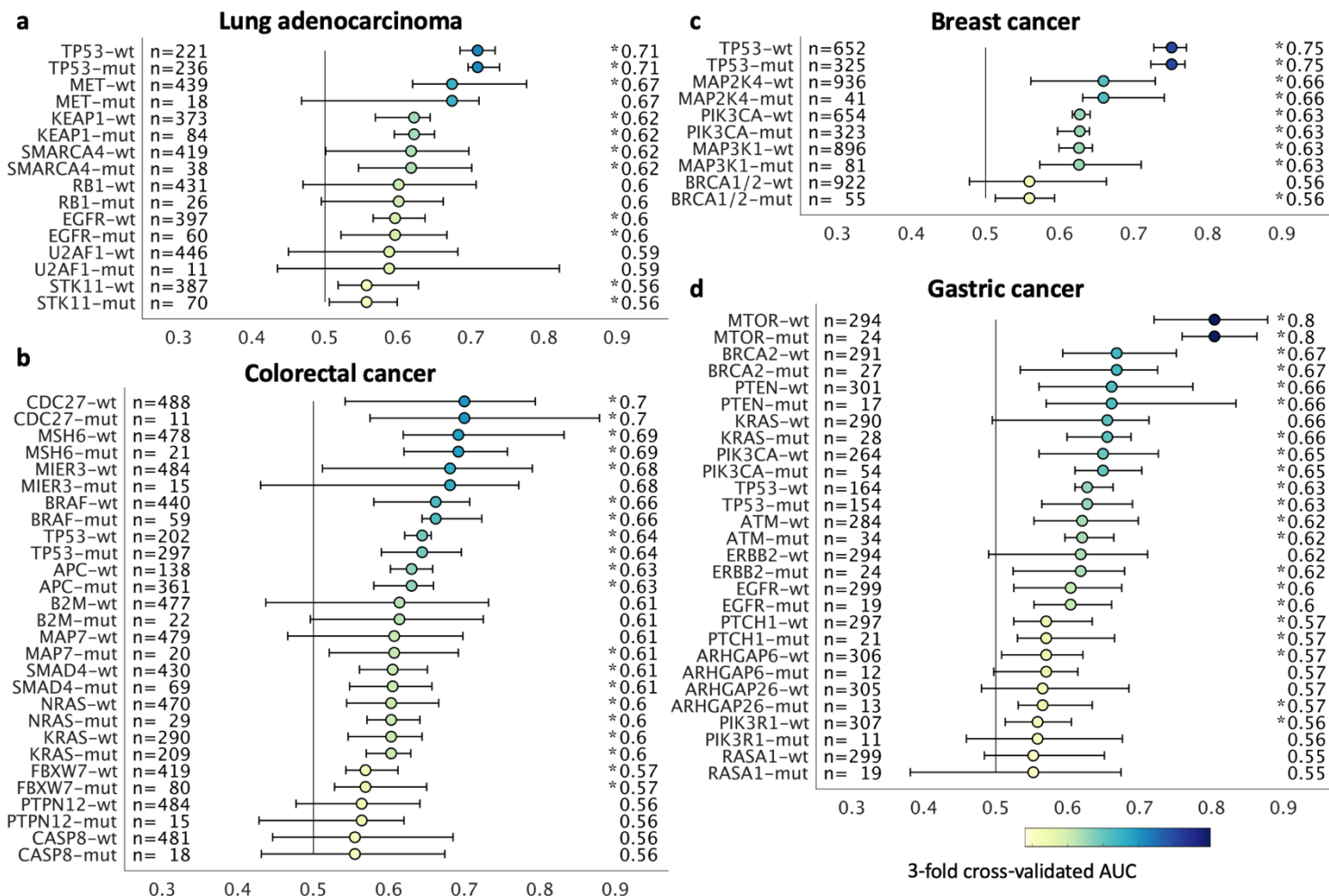
168



169

170 **Fig. 1: Transfer learning workflow for histology images.** (a) Patient cohorts are split into three  
 171 partitions for cross-validation of deep classifiers (b) Pre-trained networks re re-trained with only  
 172 the deepest layers trainable, speeding up computation while enabling state-of-the-art perfor-  
 173 mance. (c) A hyperparameter sweep with multiple networks shows that shufflenet consistently  
 174 yields high accuracy and speed for detection of microsatellite instability (MSI) in colorectal cancer  
 175 (N=426 patients), raw data in Suppl. Table 1. (d) External validation of the best shufflenet on the  
 176 DACHS cohort (N=379 patients). (e) Validation of the workflow by prediction of estrogen receptor  
 177 (ER), progesterone receptor (PR), HER2 status and tumor mutational burden (TMB) in breast can-  
 178 cer, assessed by cross-validated area under the receiver operating curve (AUC).

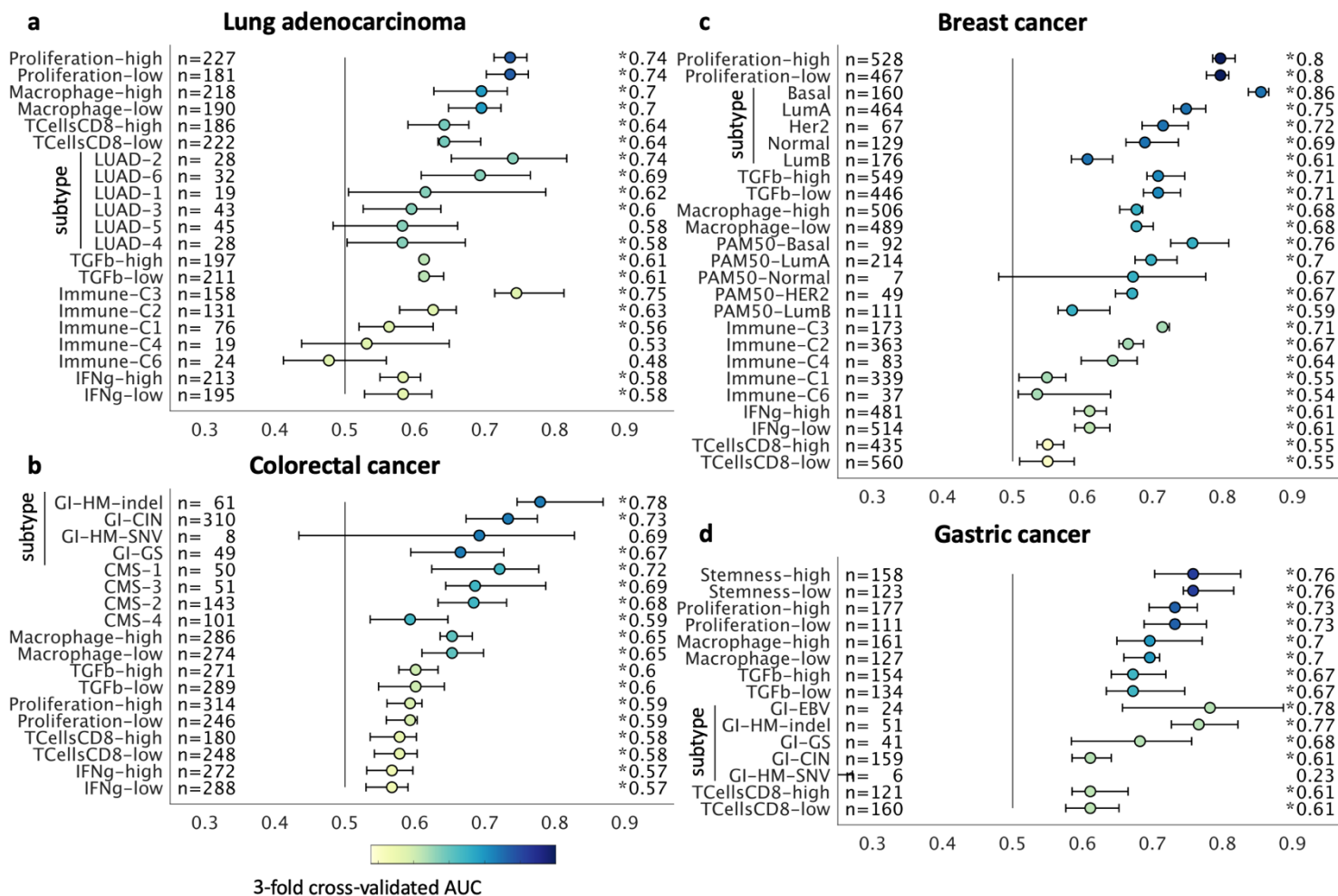




179

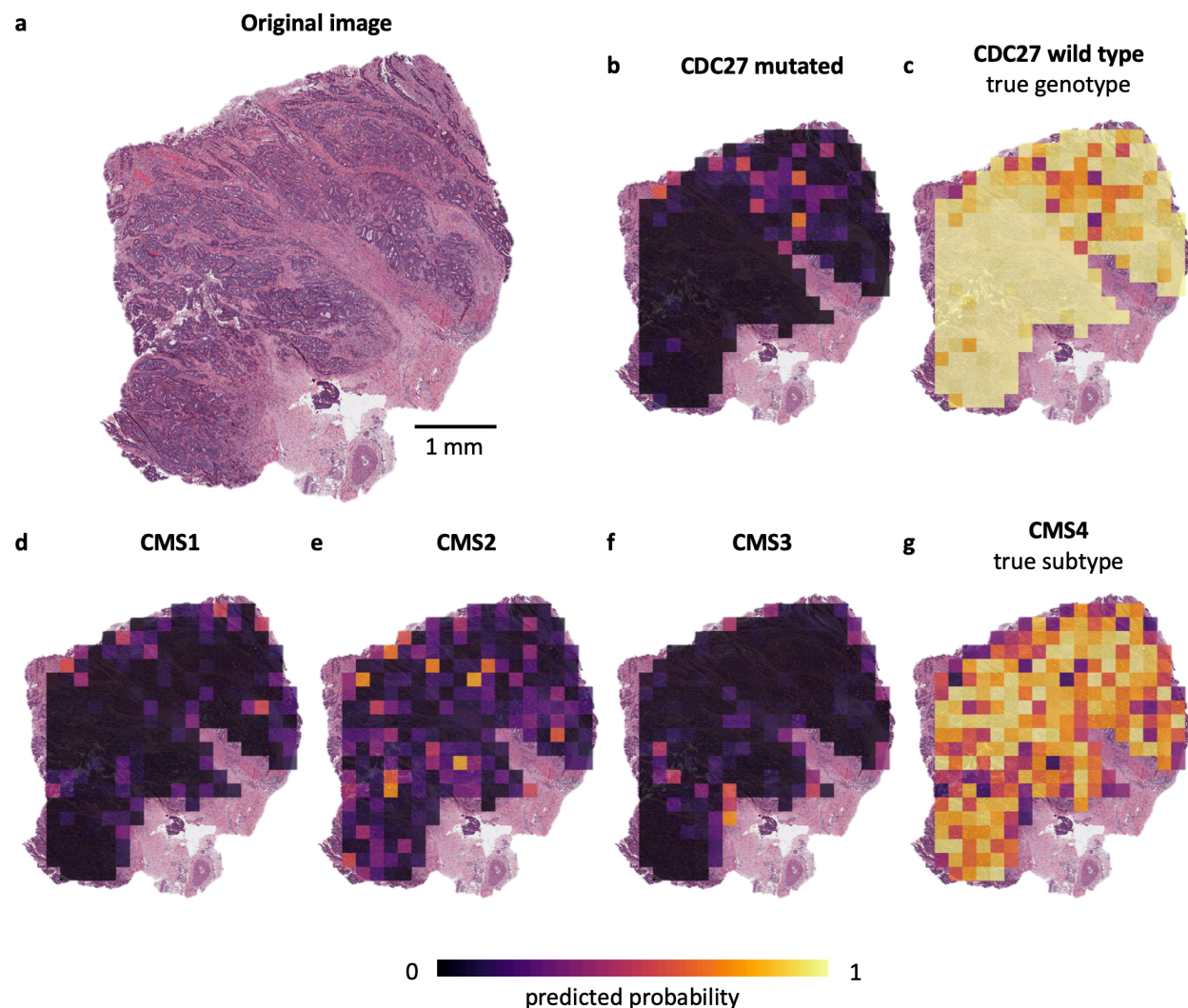
180 **Fig. 2: Prediction of point mutations directly from histological images.** Deep networks predicted  
 181 genotype directly from histological images in (a) lung adenocarcinoma, (b) colorectal, (c) breast  
 182 cancer and (d) gastric cancer. Patient cohorts were randomly split for cross validation and classi-  
 183 fiers were assessed by the area under the receiver operating curve (AUC, horizontal axis) with a  
 184 95% bootstrapped confidence interval. Genotype was predicted from histology with a high AUC  
 185 for multiple clinically actionable mutations. (\*) denotes all cases where the lower confidence  
 186 bound exceeds a random classifier (AUC 0.5). "n" denotes the number of patients. Mutations  
 187 with an AUC<0.55 are not shown. For a full list of all tested alterations, see Suppl. Table 2.

188



189  
 190 **Fig. 3: Prediction of gene expression signatures directly from histology.** Deep networks were  
 191 trained to predict clinically relevant gene expression signatures directly from histological images  
 192 in (a) lung adenocarcinoma, (b) colorectal, (c) breast cancer and (d) gastric cancer. Classifiers  
 193 were assessed by the cross-validated area under the receiver operating curve with bootstrapped  
 194 confidence intervals (AUC under ROC, horizontal axis). Continuous signatures were binarized at  
 195 the mean. Variables with an average AUC<0.55 are not shown. (\*) denotes all cases where the  
 196 lower confidence bound exceeds a random classifier (AUC 0.5). “n” denotes the number of pa-  
 197 tients. For a full list of all tested alterations, see Suppl. Table 2. “subtype” denotes TCGA molec-  
 198 ular subtypes.

199



200

201 **Fig. 4 Multiplex genotype maps with local predictability uncovered by deep learning.** (a) A  
202 whole slide image of a colorectal cancer from the TCGA cohort was used for genotype prediction  
203 by deep learning classifiers. (b) A prediction map for CDC27 wild type status and (c) a prediction  
204 map for CDC27 mutated status, correctly predicting that this particular patient is mutated. Simi-  
205 larly, prediction maps for consensus molecular subtype (CMS) classes (d) CMS1, (e) CMS2, (f)  
206 CMS3 and (g) CMS4 correctly show that deep learning robustly predicts CMS from histology alone  
207 while highlighting potential intratumor heterogeneity.

208

## 209 **Funding**

210 The results are in part based upon data generated by the TCGA Research Network: <http://cancergenome.nih.gov/>. Our funding sources are as follows. J.N.K.: RWTH University Aachen (START  
211 2018-691906). A.T.P.: NIH/NIDCR (#K08-DE026500), Institutional Research Grant (#IRG-16-222-  
212 56) from the American Cancer Society, and the University of Chicago Medicine Comprehensive  
213 Cancer Center Support Grant (#P30-CA14599). T.L.: Horizon 2020 through the European Research  
214 Council (ERC) Consolidator Grant PhaseControl (771083), a Mildred-Scheel-Endowed Professor-  
215 ship from the German Cancer Aid (Deutsche Krebshilfe), the German Research Foundation (DFG)  
216 (SFB CRC1382/P01, SFB-TRR57/P06, LU 1360/3-1), the Ernst-Jung-Foundation Hamburg and the  
217 IZKF (interdisciplinary center of clinical research) at RWTH Aachen.  
218

## 219 **Author contributions**

220 JNK, ATP and TL designed the study. LH, HIG, NAC, JJS, PAVDB, LFSK and AP oversaw the tumor  
221 annotation. CL, AE, JK, HSM, JMN and KAJS manually annotated all tumors. JNK, JK, JMN and PB  
222 designed and implemented the algorithm. JNK, CL, AS and NOB curated the list of molecular al-  
223 terations. HB and MH provided samples from the DACHS study and gave statistical advice. CT, DJ,  
224 ATP and TL provided infrastructure and supervised the study. All authors contributed to the data  
225 analysis and to writing the manuscript.

## 226 **Conflicts of interest**

227 The authors declare that no conflict of interest exists.

228

## 229 **Extended methods**

230 All experiments were conducted in accordance with the Declaration of Helsinki and the Interna-  
231 tional Ethical Guidelines for Biomedical Research Involving Human Subjects. Anonymized  
232 scanned whole slide images were retrieved from The Cancer Genome Atlas (TCGA) project  
233 through the Genomics Data Commons Portal (<https://portal.gdc.cancer.gov/>). Tissue samples  
234 from the DACHS trial<sup>35,36</sup> were retrieved from the tissue bank of the National Center for Tumor  
235 diseases (NCT, Heidelberg, Germany) as described before.<sup>20</sup>

236 Scanned whole slide images of tissue slides stained with hematoxylin and eosin were acquired in  
237 SVS format. Magnification was between 20x and 40x and corresponding resolution was between  
238 0.25 and 0.51 micrometers per pixel ( $\mu\text{m}/\text{px}$ ). All images were manually reviewed by a trained  
239 observer who discussed non-trivial cases with an expert pathologist. After review by the expert  
240 pathologist, only those images with tumor tissue on slide were used for downstream analysis.  
241 The observer manually delineated tumor tissue on the slide which in most cases included more  
242 than half of the total tissue. This region was then tessellated into square tiles of 256  $\mu\text{m}$  edge  
243 length. For the benchmark task, these images were resized 1.14  $\mu\text{m}/\text{pixel}$  to be consistent with  
244 a previous study<sup>20</sup>; for all subsequent tasks, images were processed at 0.5  $\mu\text{m}/\text{pixel}$ . Some pa-  
245 tients in the TCGA archive had more than one slide per patient and in these cases, tiles from all  
246 slides were pooled on a per-patient basis. From every slide, only a subset of tiles was used for  
247 neural network training and prediction (default 1000 tiles per slide; values explored in hyperpa-  
248 rameter sampling: 250, 500 and 750). A target variable (e.g. a particular mutation) was matched  
249 to each patient (see below) and all tiles corresponding to that patient inherited the label. The  
250 patient cohort was then randomly split in three parts in such a way that each part contained  
251 approximately the same number of patients with each label. These three parts of the patient  
252 cohort were then used for three-fold patient-level cross-validation. Before training, each cohort  
253 was randomly undersampled in such a way that the number of tiles per label was identical for  
254 each label. For training, we used on-the-fly data augmentation (random x-y-reflection and ran-  
255 dom horizontal and vertical shear of 5 px). No color normalization was used.

256 Molecular labels are listed in Suppl. Table 2 and were retrieved from the following sources: Basic  
257 clinical and pathological data was retrieved through <http://portal.gdc.cancer.gov>. Mutational  
258 status (wild type or mutated) and high-level amplification were acquired through [http://cbiopor-  
259 tal.org](http://cbioportal.org). In that database, we used “PanCancerAtlas” or “TCGA Provisional” project, whichever  
260 contained more patients in that particular tumor type. High-level data on gene expression signa-  
261 tures was retrieved from Thorsson et al. (10). For breast and endometrial cancer, additional data  
262 on tumor subtypes were retrieved from Berger et al. (27). For gastric and colorectal cancer, tumor  
263 subtype data was retrieved from Liu et al. (11).

264 Hyperparameter selection was performed for five deep neural networks which were pre-trained  
265 on ImageNet: resnet18, alexnet, inceptionv3, densenet201 and shufflenet. The sampled hyperpa-  
266 rameter space was as follows: learning rate (fixed) 5e-5 and 1e-4, maximum number of tiles per  
267 whole slide image: 250, 500 and 750, number of hot layers (**Fig. 1b**): 10, 20 and 30. The number  
268 of epochs was four with a mini batch size of 512, similar to previous experiments.<sup>20</sup>

269 All algorithms for whole slide image processing, including tessellation of images and visualization  
270 of spatial activation maps, were implemented in QuPath v0.1.2 in Groovy  
271 (<http://qupath.github.io>). All deep learning algorithms, including training and prediction, were  
272 implemented in Matlab R2018b (Mathworks, Natick, MA, USA).

273 All images from the TCGA cohort are available at <https://portal.gdc.cancer.gov/>. All source codes  
274 are available at <https://github.com/jnkather/DeepHistology>

275

## 276 Bibliography

- 277 1. Cheng, M.L., Berger, M.F., Hyman, D.M. & Solit, D.B. Clinical tumour sequencing for precision  
278 oncology: time for a universal strategy. *Nature Reviews Cancer* **18**, 527-528 (2018).
- 279 2. Rusch, M., *et al.* Clinical cancer genomic profiling by three-platform sequencing of whole genome,  
280 whole exome and transcriptome. *Nature Communications* **9**, 3962 (2018).
- 281 3. Thorsson, V., *et al.* The Immune Landscape of Cancer. *Immunity* **48**, 812-830.e814 (2018).
- 282 4. Liu, Y., *et al.* Comparative Molecular Analysis of Gastrointestinal Adenocarcinomas. *Cancer Cell*  
283 **33**, 721-735.e728 (2018).
- 284 5. The Cancer Genome Atlas Network, *et al.* Comprehensive molecular portraits of human breast  
285 tumours. *Nature* **490**, 61 (2012).
- 286 6. Guinney, J., *et al.* The consensus molecular subtypes of colorectal cancer. *Nature Medicine* **21**,  
287 1350 (2015).
- 288 7. The Cancer Genome Atlas Network, *et al.* Comprehensive genomic characterization of head and  
289 neck squamous cell carcinomas. *Nature* **517**, 576 (2015).
- 290 8. The Cancer Genome Atlas Network, *et al.* Comprehensive molecular profiling of lung  
291 adenocarcinoma. *Nature* **511**, 543 (2014).
- 292 9. Hammerman, P.S., *et al.* Comprehensive genomic characterization of squamous cell lung cancers.  
293 *Nature* **489**, 519-525 (2012).
- 294 10. The Cancer Genome Atlas Network. Integrated Genomic Characterization of Pancreatic Ductal  
295 Adenocarcinoma. *Cancer Cell* **32**, 185-203.e113 (2017).
- 296 11. The Cancer Genome Atlas Network. The Molecular Taxonomy of Primary Prostate Cancer. *Cell*  
297 **163**, 1011-1025 (2015).
- 298 12. Cancer Genome Atlas, N. Genomic Classification of Cutaneous Melanoma. *Cell* **161**, 1681-1696  
299 (2015).
- 300 13. The Cancer Genome Atlas Network, *et al.* Comprehensive molecular characterization of gastric  
301 adenocarcinoma. *Nature* **513**, 202 (2014).
- 302 14. Zhang, X., Zhou, X., Lin, M. & Sun, J. Shufflenet: An extremely efficient convolutional neural  
303 network for mobile devices. in *Proceedings of the IEEE Conference on Computer Vision and Pattern*  
304 *Recognition* 6848-6856 (2018).
- 305 15. Kather, J.N., *et al.* Predicting survival from colorectal cancer histology slides using deep learning:  
306 A retrospective multicenter study. *PLOS Medicine* **16**, e1002730 (2019).
- 307 16. Campanella, G., *et al.* Clinical-grade computational pathology using weakly supervised deep  
308 learning on whole slide images. *Nature Medicine* (2019).
- 309 17. Janowczyk, A. & Madabhushi, A. Deep learning for digital pathology image analysis: A  
310 comprehensive tutorial with selected use cases. *J Pathol Inform* **7**, 29 (2016).
- 311 18. Coudray, N., *et al.* Classification and mutation prediction from non-small cell lung cancer  
312 histopathology images using deep learning. *Nature Medicine* **24**, 1559-1567 (2018).
- 313 19. Schaumberg, A.J., Rubin, M.A. & Fuchs, T.J. H&E-stained Whole Slide Image Deep Learning  
314 Predicts SPOP Mutation State in Prostate Cancer. *bioRxiv*, 064279 (2018).
- 315 20. Kather, J.N., *et al.* Deep learning can predict microsatellite instability directly from histology in  
316 gastrointestinal cancer. *Nature Medicine* (2019).
- 317 21. Kather, J.N., *et al.* Deep learning detects virus presence in cancer histology. *bioRxiv*, 690206  
318 (2019).
- 319 22. Kim, R.H., *et al.* A Deep Learning Approach for Rapid Mutational Screening in Melanoma. *bioRxiv*,  
320 610311 (2019).

- 321 23. Szegedy, C., Vanhoucke, V., Ioffe, S., Shlens, J. & Wojna, Z. Rethinking the inception architecture  
322 for computer vision. in *Proceedings of the IEEE conference on computer vision and pattern*  
323 *recognition* 2818-2826 (2016).
- 324 24. He, K., Zhang, X., Ren, S. & Sun, J. Deep residual learning for image recognition. in *Proceedings of*  
325 *the IEEE conference on computer vision and pattern recognition* 770-778 (2016).
- 326 25. Huang, G., Liu, Z., Van Der Maaten, L. & Weinberger, K.Q. Densely connected convolutional  
327 networks. in *Proceedings of the IEEE conference on computer vision and pattern recognition* 4700-  
328 4708 (2017).
- 329 26. Chen, P.C., *et al.* An augmented reality microscope with real-time artificial intelligence integration  
330 for cancer diagnosis. *Nature Medicine* (2019).
- 331 27. The Cancer Genome Atlas Network, *et al.* Comprehensive molecular characterization of human  
332 colon and rectal cancer. *Nature* **487**, 330 (2012).
- 333 28. Kather, J.N., Halama, N. & Jaeger, D. Genomics and emerging biomarkers for immunotherapy of  
334 colorectal cancer. *Seminars in Cancer Biology* **52**, 189-197 (2018).
- 335 29. Qiu, L., *et al.* CDC27 Induces Metastasis and Invasion in Colorectal Cancer via the Promotion of  
336 Epithelial-To-Mesenchymal Transition. *J Cancer* **8**, 2626-2635 (2017).
- 337 30. Xue, Z., *et al.* MAP3K1 and MAP2K4 mutations are associated with sensitivity to MEK inhibitors in  
338 multiple cancer models. *Cell Research* **28**, 719-729 (2018).
- 339 31. André, F., *et al.* Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast  
340 Cancer. *New England Journal of Medicine* **380**, 1929-1940 (2019).
- 341 32. Fukamachi, H., *et al.* A subset of diffuse-type gastric cancer is susceptible to mTOR inhibitors and  
342 checkpoint inhibitors. *Journal of Experimental & Clinical Cancer Research* **38**, 127 (2019).
- 343 33. Li, C., Egloff, A.M., Sen, M., Grandis, J.R. & Johnson, D.E. Caspase-8 mutations in head and neck  
344 cancer confer resistance to death receptor-mediated apoptosis and enhance migration, invasion,  
345 and tumor growth. *Molecular oncology* **8**, 1220-1230 (2014).
- 346 34. Wu, Y.-M., *et al.* Inactivation of *CDK12* Delineates a Distinct Immunogenic Class of  
347 Advanced Prostate Cancer. *Cell* **173**, 1770-1782.e1714 (2018).
- 348 35. Hoffmeister, M., *et al.* Statin use and survival after colorectal cancer: the importance of  
349 comprehensive confounder adjustment. *J Natl Cancer Inst* **107**, djv045 (2015).
- 350 36. Brenner, H., Chang-Claude, J., Seiler, C.M. & Hoffmeister, M. Long-term risk of colorectal cancer  
351 after negative colonoscopy. *J Clin Oncol* **29**, 3761-3767 (2011).

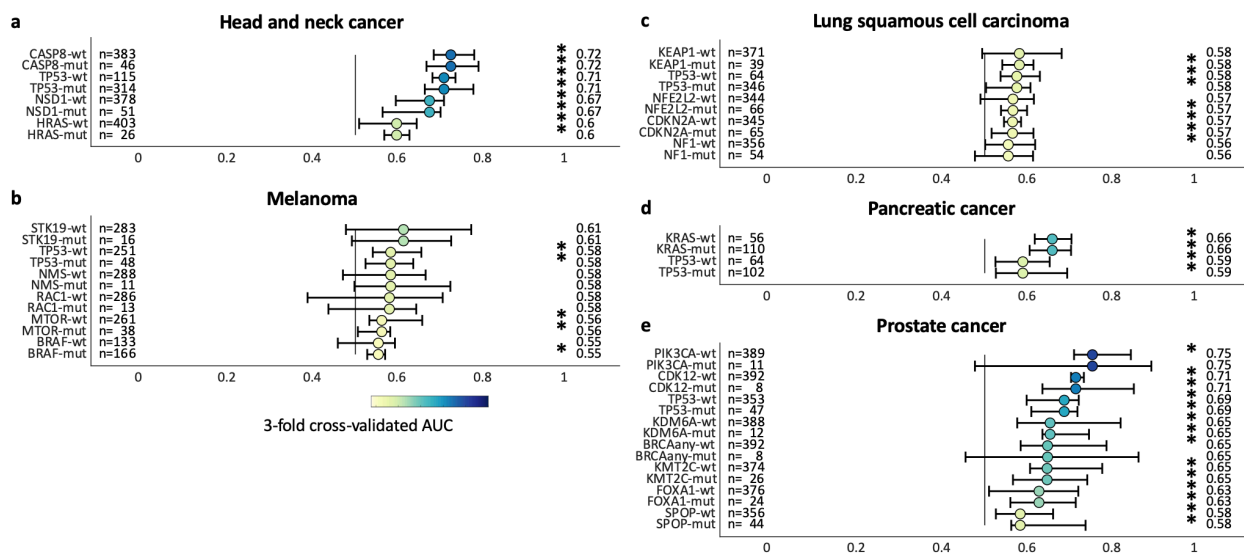
352

353



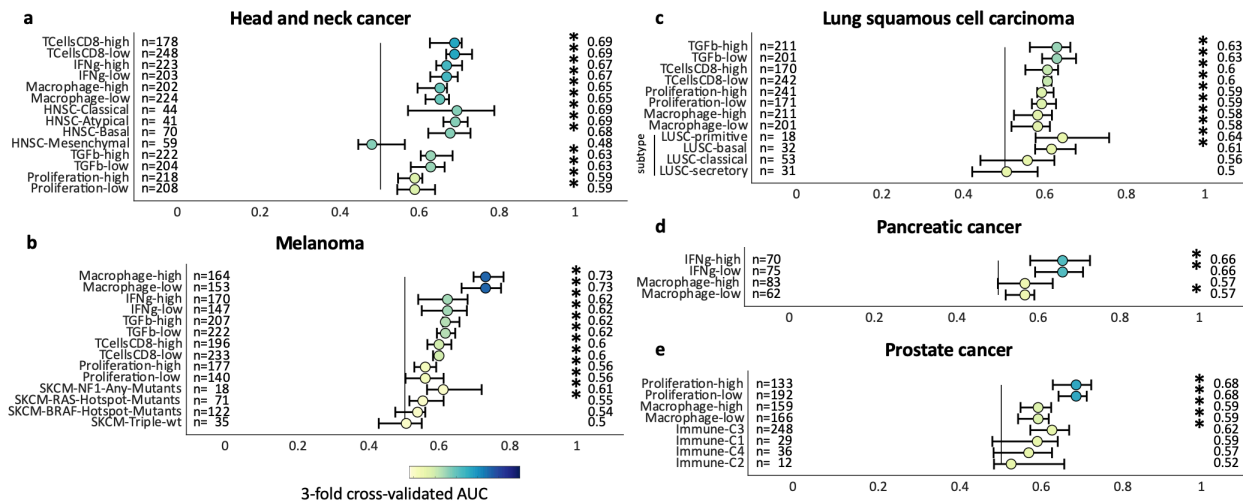
## 354 Supplementary Figures

355



356

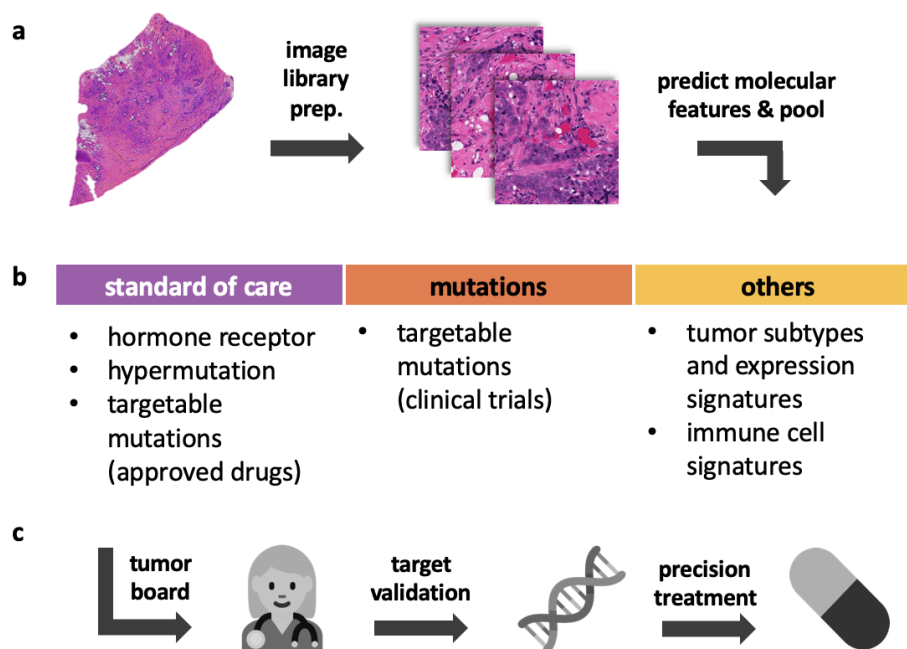
357 **Suppl. Fig. 1: Mutation prediction from histology in additional tumor types.** Our method signif-  
 358 icantly predicted oncogenic mutations from histology in (a) Head and neck squamous cell cancer,  
 359 (b) Melanoma, (c) Lung squamous cell carcinoma, (d) Pancreatic cancer and (e) Prostate cancer.  
 360 The horizontal axis shows three-fold cross-validated area under the receiver operating curve  
 361 (AUC) as mean +/- 95% bootstrapped confidence interval.



362

363 **Suppl. Fig. 2: Prediction of high-level gene expression signatures in additional tumor types.** Our  
 364 method significantly predicted high level gene expression signatures from histology in (a) Head  
 365 and neck squamous cell cancer, (b) Melanoma, (c) Lung squamous cell carcinoma, (d) Pancreatic  
 366 cancer and (e) Prostate cancer. The horizontal axis shows three-fold cross-validated area under  
 367 the receiver operating curve (AUC) as mean +/- 95% bootstrapped confidence interval.

368



369

370 **Suppl. Fig. 3: Proposed clinical workflow.** (a) Starting with ubiquitously available routine histol-  
371 ogy slides, our method relies on a tessellation of digitized images (“image library preparation”)  
372 which are passed to a deep convolutional neural network. The network predicts features on a  
373 tile level and the predictions are pooled on a patient level. (b) Histology-based testing can be  
374 applied to standard of care pathological biomarkers, driver mutations, and other features such  
375 as tumor expression subtypes. (c) We suggest that clinically meaningful findings of deep learn-  
376 ing networks could be discussed in a tumor board, validated by orthogonal methods and ulti-  
377 mately guide targeted treatment.