

1 **Relationship Between Neuroendocrine and Immune Gene Expression in Small Cell Lung Cancer**

2 Ling Cai^{1,2,3,*}, Hongyu Liu^{1,4}, Fang Huang², Junya Fujimoto⁵, Luc Girard^{3,6,7}, Jun Chen^{4,8}, Yongwen Li⁴, Yu-
3 an Zhang⁶, Dhruva Deb⁶, Victor Stastny⁶, Christin S. Kuo⁹, Gaoxiang Jia¹, Chendong Yang², Wei Zou¹⁰,
4 Adeeb Alomar⁶, Kenneth Huffman⁶, Mahboubeh Papari-Zareei⁶, Lin Yang¹¹, Benjamin Drapkin^{3,6,12}, Esra
5 Akbay¹³, David S. Shames¹⁰, Ignacio I. Wistuba⁵, Tao Wang^{1,3}, Guanghua Xiao^{1,3,14}, Ralph J.
6 DeBerardinis^{2,3,15}, John D. Minna^{3,6,7,12,*}, Yang Xie^{1,3,14,*}, Adi F. Gazdar^{3,6,13, v}

7

8 **Affiliations**

9

10 ¹Quantitative Biomedical Research Center, Department of Population and Data Sciences, UT Southwestern
11 Medical Center, Dallas, TX 75390, USA.

12

13 ²Children's Research Institute, UT Southwestern Medical Center, Dallas, TX 75390, USA.

14

15 ³Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX 75390, USA.

16

17 ⁴Tianjin Key Laboratory of Lung Cancer Metastasis and Tumor Microenvironment, Tianjin Lung Cancer
18 Institute, Tianjin Medical University General Hospital, Tianjin 300052, People's Republic of China.

19

20 ⁵Department of Translational Molecular Pathology, University of Texas MD Anderson Cancer Center,
21 Houston, TX 77054, USA.

22

23 ⁶Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center,
24 Dallas, TX 75390, USA.

25

26 ⁷Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX 75390, USA.

27

28 ⁸Department of Lung Cancer Surgery, Tianjin Lung Cancer Institute, Tianjin Medical University General
29 Hospital, Tianjin 300052, People's Republic of China.

30
31 ⁹Department of Pediatrics, Stanford University, CA 94305, USA

32
33 ¹⁰Department of Oncology Biomarker Development, Genentech Inc., South San Francisco, CA 94080, USA

34
35 ¹¹Department of Pathology, National Center/Cancer Hospital, Chinese Academy of Medical Sciences and
36 Peking Union Medical College, Beijing, China

37
38 ¹²Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390,
39 USA.

40
41 ¹³Department of Pathology, UT Southwestern Medical Center, Dallas, TX 75390, USA.

42
43 ¹⁴Department of Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

44
45 ¹⁵Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390,
46 USA.

47
48
49 † Deceased

50
51 *Correspondence to Ling.Cai@UTSouthwestern.edu, John.Minna@UTSouthwestern.edu and
52 Yang.Xie@UTSouthwestern.edu

53

54 **ABSTRACT**

55 Small cell lung cancer (SCLC) is classified as a high-grade neuroendocrine (NE) tumor, but a subset of
56 SCLC has been termed “variant” due to the loss of NE characteristics. In this study, we computed NE
57 scores for patient-derived SCLC cell lines and xenografts, as well as human tumors. We aligned NE
58 properties with transcription factor-defined molecular subtypes. Then we investigated the different immune
59 phenotypes associated with high and low NE scores. We found repression of immune response genes as a
60 shared feature between classic SCLC and pulmonary neuroendocrine cells of the healthy lung. With loss of
61 NE fate, variant SCLC tumors regain cell-autonomous immune gene expression and exhibit higher tumor-
62 immune interactions. Pan-cancer analysis revealed this NE lineage-specific immune phenotype in other
63 cancers. Additionally, we observed MHC I re-expression in SCLC upon development of chemoresistance.
64 These findings provide a new framework to guide design of treatment regimens in SCLC.

65

66 **INTRODUCTION**

67 Small cell lung cancer (SCLC), accounting for 15% of lung cancer cases, with a 5-year survival of 6%, is
68 designated by the US Congress as a “recalcitrant cancer” (1, 2). SCLC is classified as a high-grade
69 neuroendocrine (NE) tumor (3). A large fraction of SCLC tumors are driven by *ASCL1*, a lineage oncogene
70 also important for pulmonary neuroendocrine cell (PNEC) fate determination (4, 5). In healthy lung tissue,
71 PNECs are rare and dormant (6), whereas upon lung injury, some act as stem cells to regenerate
72 surrounding epithelial cells (7). SCLC occurs primarily in heavy smokers, but despite the very high mutation
73 burden (8-10) from SCLC genomes predicted to contribute an ample supply of neoantigens, SCLCs express
74 low levels of major histocompatibility complex class I (MHC I) proteins to present tumor-specific antigens
75 (11, 12). This could explain why, among various types of cancer, checkpoint-blockade immunotherapy (CBI)
76 underperforms in SCLC (13, 14).

77

78 Thirty-five years ago, it was observed that by contrast to “classic” SCLC cell lines (which grew in tissue
79 culture as floating cell aggregates), a subset of patient-derived SCLC lines behaved differently - growing as
80 adherent monolayers in culture, with morphologically larger cells, more prominent nucleoli, and expressed
81 few or no NE markers (15, 16). These characteristics led such tumors to be termed “variant” or “non-NE”

82 SCLC. Many of these variant SCLC lines were established from patients whose tumors had acquired
83 resistance to chemotherapy and clinically relapsed, a context in which genomic *MYC* amplification was also
84 noted to be more frequent (17). Notch activation had been shown to mediate the transition from “classic” to
85 “variant” subtypes and accounts for the intratumoral heterogeneity commonly seen in SCLC (18).

86

87 Recently, extending the concepts of “classic” and “variant” SCLC, both intertumoral, and intratumoral
88 heterogeneity in SCLC has been documented and has been associated with expression of lineage-specific
89 transcription factors (TFs) *ASCL1*, *NEUROD1*, *YAP1*, and *POU2F3*, and these various subtypes express
90 different levels of NE markers (19-21).

91

92 We have previously defined a 50-gene NE signature that helps us quantify the NE properties as a
93 continuous NE score ranging from -1 to 1, with a more positive score indicating higher NE properties (22). In
94 the current study we applied this NE scoring method to SCLC samples from preclinical models and patient
95 tumors. We first assessed the relationship between NE scores and SCLC molecular subtypes. Then, we
96 investigated the immune phenotypes associated with variable NE scores in SCLC and other cancer types.

97

98 **RESULTS**

99 Relationship between NE scores and SCLC molecular subtypes

100 Using the 50-gene NE signature updated with all available SCLC-related RNA-seq data (**Table S1**), we
101 computed NE scores for patient-derived SCLC lines and xenografts (PDXs) as well as four independent
102 patient tumor datasets (including one newly generated for this study) (**Tables 1 and S2**). We examined the
103 relationship between NE scores and expression of SCLC molecular subtype-specific TFs as proposed by
104 Rudin et al (**Figure 1a-b**). Our findings are largely consistent with the previous proposal that assigns
105 *ASCL1*⁺ and *NEUROD1*⁺ SCLCs to NE subtypes and *POU2F3*⁺ and *YAP1*⁺ SCLCs to non-NE subtypes.
106 However, we note some discrepancies. First, we found that while expression of *ASCL1* and *NEUROD1*
107 seems to be mutually exclusive in cell lines, they seem to co-express in many of the tumor samples;
108 secondly, in “George_2015”, “Jiang_2016” and our own dataset, we have observed rare *POU2F3*⁺ samples
109 that have high NE scores.

110

111 With serially sectioned formalin fixed paraffin embedded (FFPE) slides from 9 out of the 18 tumors for which
112 we had performed expression profiling, we examined the tumors with hematoxylin and eosin (H&E) staining
113 as well as immunohistochemistry (IHC) staining of *ASCL1*, *NEUROD1* and *POU2F3* (**Figures 1c-f**). The
114 high NE-score tumors exhibited predominantly classic SCLC morphology with dark nuclei, scant cytoplasm
115 and inconspicuous nucleoli. Notably, this was not only seen in *ASCL1*+ tumors (for example, SCLC-04, NE
116 score 0.4) but also in the *POU2F3*+ tumor with a positive NE-score (SCLC-15, NE score 0.26) (**Figure 1c**).
117 On the other hand, while we observed variant morphology in tumors with low NE scores, we noticed
118 intratumoral heterogeneity. In a tumor weakly positive for *ASCL1* (SCLC-20, NE score -0.05), the *ASCL1*-
119 high regions were found to be more “classic”-like whereas the *ASCL1*-low regions were more “variant”-like
120 (**Figure 1d**). Our IHC-based quantifications largely agree with the microarray gene expression assessments
121 (**Figure 1e**). Tumors that were found to express both *ASCL1* and *NEUROD1* stained positive for both
122 markers as well. In addition, intratumoral heterogeneity was commonly found within such tumors, where
123 there are areas with high expression of both TFs but also areas with expression of only one TF (**Figure 1f**).

124

125 Immune gene repression is a NE lineage-specific property

126 We performed correlation between NE scores and SCLC transcriptomic data to identify gene expression
127 changes associated with the NE program. Not surprisingly, gene ontology (GO) analyses revealed genes
128 related to the neuronal system as highly expressed in high NE-score samples (**Figures S1a-b**). By contrast,
129 genes negatively associated with NE score were enriched for GO terms related to immune response, and
130 this was observed in both the cell line and human tumor datasets (**Figure S1c-d**). We also performed gene
131 set enrichment analysis (GSEA) (23) with a variety of gene set libraries collected by Enrichr (24). Consistent
132 with previous report that Notch signaling dependent REST (Neuron-Restrictive Silencer Factor) activation
133 represses neuronal gene expression in variant SCLC (18), we found REST targets (i.e. repressed by REST)
134 are abundantly expressed in high NE-score SCLCs. On the other hand, interferon-stimulated genes (ISGs)
135 are found to highly express in the low NE-score (variant) SCLC samples (**Figures 2a-b**). As NF κ B signaling
136 mediates activation of ISGs, we examined reverse phase protein array (RPPA) data from the cancer cell

137 line encyclopedia (CCLE) (25) and found higher levels of activating serine 536 phosphorylation on p65 (26)
138 in low NE-score SCLC lines (**Figure 2c**).

139

140 Our 50-gene signature derived from lung cancer cell line mRNA data (**Table S1**) contains several genes
141 with immune related functions that were found to highly express in variant SCLC. Some are involved in
142 cytokine signaling; for example, *IL18* encodes for a proinflammatory cytokine (27), and *OSMR* encodes for
143 a receptor for oncostatin M and IL-31 (28). Furthermore, many of these genes are involved with
144 immunosuppressive processes, including *NT5E* (29), *TGFBR2* (30), *ANXA1* (31), *EPHA2* (32), *HFE* (33)
145 and *LGALS3* (34). Our pathway analyses indicates that beyond these genes included in the NE expression
146 signature, there is a broad immune program concertedly upregulated in low NE-score SCLC samples. We
147 extended our analysis to a few immune gene sets that were previously identified to express cell
148 autonomously in cancer. These gene sets include the following: SPARCS genes (stimulated 3 prime
149 antisense retroviral coding sequences) reported to express in mesenchymal tumors and mediate interferon-
150 gamma signal amplification (35); “parainflammation” (PI) genes in epithelial tumor cells (36); and
151 senescence-associated secretory phenotype (SASP) genes (37) that reinforce the senescence arrest, alter
152 the microenvironment, and trigger immune surveillance of the senescent cells (38). We observed that the
153 expression these genes also negatively correlate with NE scores in SCLC despite little overlap among
154 genes in these various sets (**Figure S2**).

155

156 While the expression of neuronal program genes in in high NE-score SCLCs can be attributed to the NE
157 lineage, we examined single-cell RNA-seq (scRNA-seq) data from the healthy human lung epithelial cells
158 (39) to check whether the expression repression of ISGs is also a lineage-specific phenomenon that could
159 be observed in PNECs rather than being cancer-specific. Consistent with the previous report that *ASCL1*
160 negatively regulate *YAP1* during neuronal differentiation (40), the highest expression of *ASCL1* and lowest
161 expression of *YAP1* were observed in PNECs, relative to other cell types. We confirmed that while PNECs
162 have increased expression of REST target genes, ISGs are indeed repressed as well (**Figure 2d and S3a**).

163 Additionally, we specifically examined interferon receptors in PNECs and found that they also have the
164 lowest expression in PNECs (**Figures 2d**), suggesting that besides repression of basal ISG expression, in

165 the presence of interferon stimulation, PNECs would also be less primed for further activation of ISGs. It
166 has been estimated that 10% of the genes in the human genome have the potential to be regulated by IFN,
167 many ISGs work in immune defense against viral infection, but some could be hijacked by viruses (41). As
168 some PNECs are rare stem cells, we reason that ISG repression could lower their risk from viral infection. In
169 the context of the current COVID-19 pandemic, we examined scRNA-seq data from Ouadah et al., who
170 performed lineage tracing with an *Ascl1*^{CreERT2}; *Rosa26*^{LSL-ZsGreen} mouse model to show that some PNECs
171 can transdifferentiate into other cell types (7). **Figure S3b** generated with their data shows that AT2 and
172 ciliated cells originated from PNECs in this model have lost *Ascl1* but increased *Yap1* expression. *Ly6e* and
173 *Tmprss2*, genes involved in coronavirus defense (42) and hijacked entry (43) respectively, were also
174 upregulated.

175

176 Increased tumor-immune interaction in low NE-score SCLC tumor samples

177 It has been long observed that expression of MHC I is low in SCLC (11). Using single sample gene set
178 enrichment analysis (ssGSEA) (44), we derived the MHC I scores for MHC I genes. From studies that had
179 collected lung tumors of different histology, MHC I scores positively correlate with *PTPRC* (which encodes
180 for pan-leukocyte marker CD45) levels (**Figures 3a and S4a**). The lowest MHC I and *PTPRC* gene
181 expression were found in neuroendocrine tumors, including not only SCLC but also carcinoids (**Figure 3a**),
182 suggesting these NE tumors with decreased MHC I have fewer immune infiltrates. In SCLC datasets, low
183 NE-score samples exhibited upregulation of MHC I genes (**Figure 3b**) and were associated with higher
184 *PTPRC* expression in patient tumor datasets (**Figure S4b**). We also estimated immune cell infiltration by
185 deriving immune cell type-specific signature scores (45) and found that they negatively correlate with NE
186 scores in SCLC patient tumors, suggesting increased tumor-immune interaction in low NE-score tumors
187 (**Figure 3c**).

188

189 We saw higher expression of PD-L1 (CD274) in low NE-score SCLCs (**Figure S5a**). Furthermore, genes
190 from an IFN-gamma related signature that has been shown to predict PD-1 blockade response in multiple
191 cancer types (46) are highly expressed in low NE-score SCLC tumors across multiple datasets (**Figure 3c**).
192 We also examined a list of 21 immune checkpoint genes (47), immune suppressive cytokines (IL-10 and

193 TGF-beta), and their receptors (48), for their association with NE scores. We found that these genes also
194 have higher expression in low NE-score SCLC tumors (**Figure 3c**). Finally, the expression of 995
195 immunosuppressive genes from the Human Immunosuppression Gene Atlas (47) were assessed and again,
196 the majority of these genes exhibit negative correlation between mRNA expression and NE scores across
197 different SCLC tumor datasets (**Figure S5b** and **Table S3**).

198

199 Besides gene expression-based analyses, we also performed immunohistochemistry (IHC) with our 9 SCLC
200 tumor samples to quantify tumor infiltrating CD8+ and CD4+ T cells (**Table S2**). Of importance, both
201 intratumoral and intertumoral heterogeneity were observed in T cell infiltration. Within the same tumor,
202 areas with low tumor ASCL1 levels exhibited more CD8+ and CD4+ T cell infiltration, whereas areas with
203 high tumor ASCL1 levels showed fewer CD8+ or CD4+ T cells (**Figure 4a**). Across all the SCLC tumor
204 specimens assessed, CD8+ and CD4+ T cell per area cell count positively correlated with the T cell score
205 computed from gene expression data, and both IHC-based T cell counts and gene expression-based T cell
206 scores negatively correlated with NE scores (**Figure 4b**).

207

208 Pan-cancer analyses for NE score expression and immune response genes.

209 These findings had prompted us to examine other cancer types to see whether immune gene repression is
210 seen in other NE tumors and whether “variant” subtype from NE lineage loss could also be observed
211 (**Figure 4c**). A recent study identified “SCLC-like” epithelial tumors in pan-cancer samples using a principal
212 component analysis-based approach. They found that tumors across many lineages with a higher “SCLC-
213 like” score had lower immune gene expression (49). We applied our NE scoring method across all cancer
214 lineages (“pan-cancer” analysis) to compute NE scores and assess their relationship with immune
215 phenotypes. In pediatric (Therapeutically Applicable Research to Generate Effective Treatments – TARGET)
216 and adult (The Cancer Genome Atlas – TCGA) pan-cancer studies (50), neuroendocrine tumor
217 neuroblastoma (NBL), as well as pheochromocytoma & paraganglioma (PCPG) were identified as
218 containing the highest NE scores (**Figures 5a-b**). Tumors of glial origin, including Low Grade Glioma (LGG)
219 and Glioblastoma Multiforme (GBM) also have high NE scores. Besides these NE/glial tumors, a small
220 number of high NE-score samples were observed for bladder urothelial carcinoma (BLCA), breast invasive

221 carcinoma (BRCA), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic
222 adenocarcinoma (PDAC) and stomach adenocarcinoma (STAD), for which it is also known that
223 neuroendocrine tumors, while uncommon, still comprise a small proportion of the cases (**Figures 5a-b**).
224 Previous immunogenomic analysis had classified pan-cancer TCGA samples into six immune subtypes (51).
225 We found samples from the “immunologically quiet” subclass have the highest NE score, followed by the
226 “lymphocyte depleted” subclass (**Figure 5c**). We further assessed the relationship between NE scores and
227 the tumor-infiltrating lymphocytes and leukocyte regional fractions previously reported for the pan-cancer
228 samples (51), these immune metrics negatively correlate with NE scores across all samples (**Figure 5d**)
229 and also within specific tumor types (**Figure S6**).

230

231 We took a close examination of NBL using cell line expression data from CCLE (25) along with patient
232 tumor data from TARGET (52) for lineage factors *ASCL1* and *YAP1*, REST targets, ISGs, MHC I, immune
233 cell-specific signature scores (45), Ayer et al.’s PD-1 blockade response signature (46), immune
234 checkpoints (47) and suppressive cytokines and receptors (48). The pattern for NBL (**Figure 5e**) highly
235 resembles that of SCLC (**Figures 2a, 3b-c**) suggesting the existence of a “variant” NBL subset with
236 decreased neuroendocrine features, increased cell-autonomous expression of immune genes as well as
237 increased tumor-immune interaction. Like SCLC, we also found higher levels of NFkB-p65 phosphorylation
238 in the low NE-score variant NBL cell lines (**Figure 5f**).

239

240 MHC I re-expression in chemoresistant SCLC

241 As it was previously observed that variant SCLC cell lines were frequently derived from patients whose
242 tumors had relapsed on chemotherapy (17), we wondered if the development of chemo-resistance in
243 tumors was associated with the altered expression of immune genes, especially MHC I. Five sets of data
244 with origin-matched chemosensitive and chemoresistant samples were examined to address this question.
245 In 2017, using a genetically engineered mouse model (GEMM), Lim et al showed that Notch-active SCLC
246 cells were more chemoresistant (18). Using their data we found the Notch active SCLC cells had switched
247 from *ASCL1*⁺ to *YAP1*⁺, have reduced NE scores and increased expression of ISGs and MHC I genes
248 (**Figure 6a**). We next examined a series of preclinical models we and others have developed for human

249 SCLC. Classic, high NE-score SCLC cell lines predominantly grow as floating aggregates in culture, but
250 contain a small proportion of cells growing adherently in a monolayer. By selecting for adherent growth, we
251 generated an adherent subline H69-AD/(NCI-H69-AD) from parental, chemosensitive H69/(NCI-H69) cells
252 (**Figure 6b**). Increased resistance to Cisplatin (~10 fold) and Etoposide (~6 fold) was observed in H69-AD
253 compared to the parental H69 cells (**Figure 6c**). We found H69-AD had transitioned to become a low NE-
254 score (-0.02) YAP1+ variant line compared to the parental high NE-score (0.91) classic ASCL1+ line. Both
255 ISGs and MHC I genes were found to have increased expression in H69-AD (**Figure 6d**). In a previous
256 study, Cañadas et al. also derived sublines from H69. Hepatocyte growth factor treatment was used to
257 induce mesenchymal transition of H69 cells, resulting in H69-M lines that were found to be chemoresistant
258 both *in vitro* and *in vivo* (53). From their dataset, we also found MHC I expression increased in H69-M
259 compared to parental H69 cells. There was some increase in ISGs too, but less prominent compared to
260 H69-AD from us. Notably, although *YAP1* expression increased in H69-M, *ASCL1* levels did not change
261 (**Figure 6d**). As the fourth set of data, PDX models established sequentially from SCLC tumors
262 (“Drapkin_2018”) collected before and after chemotherapy from the same patient (54) were examined. In
263 PDXs from patient MGH1518 for which chemoresistance had developed in the relapsed sample, we found
264 upregulation of MHC I, but not ISGs (**Figure 6d**). Of note, this relapsed sample maintained a high NE score,
265 but expressed higher levels of *MYC*, consistent with previous findings that *MYC* mediates chemoresistance
266 (54). Lastly, we generated a set of subcutaneous xenograft models from a high NE-score human SCLC cell
267 line NCI-H1436 with or without selection for resistance to Cisplatin and Etoposide in the mice (**Figure 6e**).
268 Compared to the parental xenograft, the drug resistant xenografts maintained *ASCL1* expression but
269 exhibited increased *B2M* (MHC I complex subunit), *PSMB8* (immunoproteasome subunit) (**Figure 6e**), and
270 *MYC* (Fang Huang’s manuscript under review at JCI). Collectively, these findings suggest MHC I can re-
271 express upon development of chemoresistance - in some cases, with lineage transition; and in some other
272 cases, accompanied by an increase in *MYC* expression.

273

274 We also checked whether expression levels of MHC I and *MYC* differ by tumor source and anatomical site
275 based on the “NCI/Hamon Center” patient-derived SCLC lines dataset (**Figures S7**). Interestingly, the
276 lowest MHC I and *MYC* levels were both observed for cell lines derived from primary site lung tumor

277 specimens and they are all high-NE tumors, whereas higher MHC I levels were observed in SCLC lines
278 isolated from metastatic tumor samples especially those from lymph node and bone marrow. These
279 observations remain to be validated with primary and metastatic samples from the same patients.
280

281 **DISCUSSION**

282 In this study, we examined NE properties of patient-derived SCLC cell lines, PDXs and human tumors
283 based on NE scores estimated from a gene expression signature. Currently, it is believed that Notch
284 activation drives the lineage transition from ASCL1+ to NEUROD1+ to YAP1+ subtype (55) whereas
285 POU2F3+ SCLC is a standalone subtype originated from tuft cells (56). While we observed mutually
286 exclusive patterns of *ASCL1* and *NEUROD1* expression in cell lines, their co-expression was identified in
287 many patient tumors. Our IHC results further revealed intratumoral heterogeneity in such tumors,
288 suggesting ongoing lineage transition in primary treatment naïve tumors. From alignment of NE scores and
289 molecular subtype-specific TF expression, we observed rare high NE-score POU2F3+ tumors in three
290 independent datasets, raising the possibility that POU2F3+ tumors could also arise from NE lineage.

291
292 Our investigation of immune phenotypes associated with variable NE scores had identified repression of
293 ISGs in classic high NE-score SCLC. While it remains to be determined what other pulmonary cells besides
294 PNECs can function as “cells of origin” for SCLC (57-60), the gene expression similarities between PNECs
295 and SCLC suggests many of the SCLC properties could be tied to PNEC characteristics. We confirmed ISG
296 repression in PNECs relative to other lung epithelial cells through examination of scRNA-seq data from
297 healthy human lung. ISGs provide viral defense for cells but some can be hijacked by viruses (41). Since
298 PNECs assume stem cell roles for tissue regeneration after injury (7), lowering expression of ISGs and
299 other genes involved in viral entry presumably play some role in self-protection. Like SCLC, PNECs can
300 switch from ASCL1+ to YAP1+ through Notch activation, but in the context of tissue repair (7). Our findings
301 suggest that the increase of cell autonomous immune gene expression as high NE-score SCLCs transition
302 to low NE-score SCLCs is mirroring the changes that normally take place during the transdifferentiation of
303 PNECs to other lung epithelial cell types, and in SCLC, this had in turn led to an increased tumor-immune
304 interaction. Interestingly, our pan-cancer analysis had extended this finding of reciprocal relationship

305 between neuroendocrine and immune gene expression to other cancer types. For NBL, a cancer that had
306 not previously been classified based on NE properties, we were able to identify more inflammatory variant
307 tumors with loss of NE lineage gene expression. It would be interesting to explore more of such molecular
308 similarities between SCLC and other tumors with NE/neuronal lineage.

309

310 The full repertoire of immune evasion strategies employed by SCLC remains to be elucidated. However, our
311 results tying together with current clinical treatment findings raise several important questions and
312 paradoxes. The first paradox is that we found a depletion of immune infiltrates in high NE-score SCLC
313 tumors and other neuroendocrine tumors (NETs), associated with down regulation of MHC I expression.
314 While this MHC I expression explains the presence of very few T cells, it raises the question of how high
315 NE-score NETs evade natural killer cells that normally would recognize the “missing self” that such MHC I
316 expression loss conveys (61). Thus, we feel the high NE-score low-MHC I expression pairing indicates we
317 need to understand how natural killer cell mechanisms are avoided in NET pathogenesis. The second
318 paradox is that low NE-score “variant” SCLCs appear to be associated with expression of MHC I and a
319 more immune infiltrated tumor microenvironment, yet clinical trials of immune checkpoint blockade do not
320 clearly show these are the tumors responding to such therapy. Since we found these tumors also express
321 many immunosuppressive genes it will be important to know which of these immunosuppressive gene
322 functions need to be targeted to achieve anti-tumor immune responses. Finally, we observed expression of
323 MHC I in selected SCLC samples with chemoresistance and increased *MYC* expression even without
324 changes in *ASCL1* expression. We need to know if immunosuppressive mechanisms are the same or
325 different in the high vs. low NE-score SCLC resistant to chemotherapy. We conclude, that some 30 years
326 after the first description of “classic” (high NE-score) and “variant” (low NE-score) SCLCs there are
327 important links between these NE phenotypes and the expression of immune phenotypes, and between
328 similar gene expression profiles of SCLC and pulmonary neuroendocrine cells. Importantly, these
329 correlations identify important problems to be solved of clinical therapeutic translational relevance.

330

Source	Name	Tissue Source	Sample Type	n	Reference
Human	SCLC cell lines/ NCI/Hamon Center	SCLC	Cell line	69	This study
Human	Cañadas_2014	SCLC	Cell line	6	(53)
Human	Drapkin_2018	SCLC	PDX	19	(54)
Human	Rudin_2012	SCLC	Tumor	29	(9)
Human	George_2015	SCLC	Tumor	81	(10)
Human	Jiang_2016	SCLC	Tumor	79	(62)
Human	SCLC tumors (this study)	SCLC	Tumor	18	This study
Human	expO	Lung cancer	Tumor	109	(63)
Human	Rousseaux_2013	Lung cancer	Tumor	286	(63, 64)
Human	CCLC	Pan-cancer	Cell line	-	(25)
Human	TCGA	Pan-cancer	Tumor	10535	(50)
Human	TARGET	Pan-cancer	Tumor	734	(50)
Mouse	Lim_2017	SCLC	Pooled FACS sorted tumor cells ^	6	(18)
Human	Travaglini_2020	Healthy lung	Single cell	9384*	(39)
Mouse	Ouah_2019	Healthy lung	Single cell	46*	(7)

Table 1. Datasets used for analyses

PDX, Patient-derived Xenografts; FACS, Fluorescence-activated cell sorting; scRNA-seq, Single-cell RNA sequencing; * cells; - Note that CCLC datasets were used in multiple analyses with different numbers of cell lines; ^ In Lim_2017, *Rb1^{flox/flox}*; *p53^{flox/flox}*; *p130^{flox/flox}*; *R26^{mTmG}*; *Hes1^{GFP/+}* GEMM SCLC tumors were initiated by Ad-CMV-Cre, sorted by Tomato and GFP to obtain relatively pure tumor cells.

333 **FIGURE LEGENDS**

334 **Figure 1 NE score and SCLC molecular subtypes**

335 **a**, Heatmaps visualizing expression of molecular subtype-specific TFs and NE scores. Two heatmaps were
336 generated for each study, with one ordered by complete linkage hierarchical clustering of TFs and the other
337 ordered by NE scores. **b**, Pairwise associations among NE scores and molecular subtype-specific TFs.
338 Lower left panels are scatter plots, diagonal line panels are density plots and upper right panel shows
339 correlation coefficients from pairwise Pearson correlation. *, p-value < 0.05. **c**, H&E staining of two high NE-
340 score SCLC tumor samples showing classic SCLC morphology with dark nuclei, scant cytoplasm and
341 inconspicuous nucleoli. **d**, ASCL1 IHC staining and H&E staining of a low NE-score SCLC tumor, showing
342 variable morphology at different selected areas, where ASCL1-low areas appear to be more variant-like **e**,
343 Quantifications of TF expression from IHC staining or microarray profiling, samples are ordered by
344 increasing NE scores. **f**, IHC of ASCL1, NEUROD1 and POU2F3 in two tumors that express both ASCL1
345 and NEUROD1. Two areas per tumor were selected for showing intratumoral heterogeneity in ASCL1 and
346 NEUROD1 expression patterns.

347

348 **Figure 2 Repression of ISGs in high NE-score SCLC and PNECs**

349 **a**, GSEA enrichment plots for selected genesets. Results from SCLC cell lines, PDXs (Drapkin_2018) and
350 patient tumor datasets were superimposed. Normalized enrichment score (NES) were provided. *, multiple
351 comparison adjusted p-value < 0.05. **b**, Heatmaps for top 25 leading edge genes selected from genesets in
352 **(a)**. Gene expression matrix of each dataset was annotated with color-coded Pearson correlation coefficient
353 (from correlating NE score with gene expression) as a left-side column, and a top bar indicating NE scores.
354 **c**, Scatter plots showing negative correlation between NE score and Ser536 phosphorylation on NFkB-p65
355 in SCLC cell lines. Pearson correlation coefficient was provided. *, p-value < 0.05. **d**, Heatmap showing
356 relative expression of selected lineage factors (*ASCL1* and *YAP1*), REST targets and ISGs (same genes as
357 used in **b**, determined from **a**) as well as interferon receptor genes in healthy human lung epithelial cells
358 based on scRNA-seq experiments.

359

360 **Figure 3 Low NE-score variant SCLC have increased tumor-immune interaction**

361 **a**, Expression of MHC I genes and pan-leukocyte marker *PTPRC* in lung tumors from the
362 “Rousseaux_2013” dataset. Box whisker plots are filled with color reflecting the median NE score in
363 different histological subtypes. Color for scatterplot symbols reflects NE score for different samples. **b**,
364 Heatmaps visualizing expression of MHC I genes across multiple SCLC datasets. **c**, Heatmaps visualizing
365 expression of *PTPRC*, immune-cell-type-specific signature scores, PD-1 blockade response-predicting IFN-
366 gamma related signature genes (46), immune checkpoint genes and suppressive cytokines and receptors in
367 SCLC tumor datasets.

368

369 **Figure 4 Intertumoral and intratumoral heterogeneity in T cell infiltration from SCLC tumors with**
370 **variable NE features**

371 **a**, IHC of ASCL1, CD4 and CD8 in selected tumors. SCLC-04 is a SCLC tumor with NE score of 0.4. CD8
372 or CD4 T cells were few in the ASCL1-high regions but abundant in the ASCL1-low region; SCLC-20 is a
373 tumor with NE score of -0.05, similar reciprocal relationship of ASCL1 staining and T cell infiltration was
374 observed. Representative regions with high or low ASCL1 staining were shown. **b**, Relationship between
375 IHC-determined per area CD4 and CD8 T cell count, gene expression-based T cell score and NE score in
376 all 9 tumors assessed. Scatter plots and Pearson correlation coefficients were provided for assessment of
377 pairwise correlations. *, Pearson correlation with p-value < 0.05. **c**, Schematic diagram showing relationship
378 between neuroendocrine and immune gene expression in normal cells and neuroendocrine tumors (NETs).

379

380 **Figure 5 Relationship between NE scores and immune phenotypes in pan-cancer samples**

381 **a-b**, NE scores of pan-cancer samples in the TARGET pediatric cancer cohorts (**a**) and TCGA adult cancer
382 cohorts (**b**). **c**, NE scores by immune subtype in TCGA pan-cancer samples. **d**, Relationship between NE
383 scores and tumor-infiltrating lymphocytes regional fraction or leukocyte fraction in TCGA pan-cancer
384 samples. **e**, Heatmap visualizing expression of various genes and summary scores previously assessed for
385 SCLC and now in NBL with cell line and tumor datasets side-by-side. **f**, Scatter plots showing negative
386 correlation between NE score and Ser536 phosphorylation on NFkB-p65 in NBL cell lines. Pearson
387 correlation coefficient was provided. *, p-value < 0.05.

388

389 **Figure 6 MHC I upregulation in chemoresistant SCLC**

390 **a**, Heatmap visualizing increased expression of ISGs, MHC I genes as cells switch from Ascl1+ to Yap1+ in
391 SCLC GEMM tumors from the “Lim_2017” . GFP was expressed from endogenous promoter of a Notch
392 target gene *Hes1* in *Rb^{-/-}/p53^{-/-}/p130^{-/-}* (TKO) background. Using flow cytometry, the authors first sorted out
393 SCLC tumor cells and then further sorted by GFP to obtain relatively pure tumor cells with different Notch
394 activation status. Three biological replicates were each provided for GFP negative (Notch inactive, classic
395 high-NE) cells and GFP positive cells (Notch active, low-NE). **b**, Different morphology and culture
396 characteristics of adherent H69-AD and the parental H69. **c**, Dose response curves for Cisplatin and
397 Etoposide in the H69 cell line pair. Note that H69-AD, the adherent line, is more resistant with higher IC50s.
398 **d**, Expression changes of selected genes in: H69 cell line pair from this study, H69 and derived
399 mesenchymal H69M cell lines from “Canadas_2014” and autologous PDX samples before and after
400 chemotherapy from “Drapkin_2018”. PDX parameters: TTP, time to progression, defined by time to 2x initial
401 tumor volume; RESP, change in tumor volume between initial tumor volume and minimum of day 14-28.
402 Relapsed sample from MGH-1514 did not show increased chemoresistance based on the RESP and TTP
403 parameters. Note that unlike other heatmaps, due to the small number of samples in each dataset,
404 expression is not scaled by gene in this heatmap. **e**, qPCR measurement of normalized *ASCL1*, *B2M* and
405 *PSMB8* expression in naïve parental and chemoresistant H1436 xenograft tumors.

406

407 **SUPPLEMENTARY FIGURE LEGENDS**

408 **Figure S1 Gene ontology analyses of genes associated with NE scores in SCLC datasets**

409 Treemaps summarizing enriched Biological Process GO terms, with reduced redundancy, for genes
410 associated with NE scores in SCLC tumor dataset “George_2015” (**a** and **c**) or in SCLC cell lines (**b** and **d**).

411

412 **Figure S2 Expression of SPARCS, parainflammation and SASP genes in SCLC datasets**

413 **a**, Heatmaps visualizing expression of selected immune gene sets in multiple SCLC datasets. These gene
414 sets were previously reported in different studies to express cell autonomously in cancer. Genes within each
415 gene set were ordered by correlation with NE score from meta-analysis of all datasets. Gene expression
416 matrix of each dataset was annotated with a left-side column with color-coded Pearson correlation

417 coefficient (from correlating NE score with gene expression), and a top bar indicating NE scores. **b**, UpSet
418 plot showing gene counts in intersections of the three gene sets used in **(a)**. SPARCS genes are genes with
419 stimulated 3 prime antisense retroviral coding sequences; these genes have been shown to activate IFN-
420 mediated innate immune pathways (35). Parainflammation genes are innate immunity genes that were
421 found to express in cancer (36). SASP genes are senescence-associated secretory phenotype genes (65).

422

423 **Figure S3 Repression of viral defense/hijacked genes in PNECs compared to other lung epithelial**
424 **cell types**

425 **a**, Violin plots showing expression of selected lineage factor (*ASCL1* and *YAP1*) and ISGs (*TLR3* and *IFI44*)
426 in healthy human lung epithelial cells based on scRNA-seq experiments from “Travaglini_2020”. Note that
427 from research of Zika virus, *TLR3* has been shown to mediate the deleterious effect of Zika virus through
428 disruption of neurogenesis (66). **b**, Scatter plot showing relationship among selected lineage factor genes
429 (*Ascl1* and *Yap1*) and ISGs (*Ly6e* and *Tmprss2*) in scRNA-seq data (“Ouadah_2019”) of lung epithelial cells
430 isolated from a mouse model genetically engineered to enable lineage tracing of PNECs. AT2 and ciliated
431 cells transdifferentiated from PNECs have lost expression of *Ascl1* but upregulated *Yap1*, *Ly6e* and
432 *Tmprss2*. Note that from research of coronavirus, *LY6E* is implicated in viral defense (42) whereas
433 *TMPRSS2* mediates viral entry (43).

434

435 **Figure S4 Expression of MHC I gene is positively associated with pan-leukocyte marker CD45**
436 **(encoded by *PTPRC*) in lung tumors and SCLC tumors**

437 **a**, Expression of MHC I genes and *PTPRC* in lung tumors from Expression Project for Oncology (expO). **b**,
438 Positive correlation between MHC I expression scores and *PTPRC* in SCLC tumors but not cell lines or
439 PDXs (“Drapkin_2018”). *, p-value < 0.05.

440

441 **Figure S5 Upregulation of immunosuppressive genes in low NE-score SCLC tumors**

442 **a**, Correlation between CD274 (PD-L1) expression and NE score. Negative association was not observed in
443 SCLC cell line or PDX (“Drapkin_2018”) datasets but was observed in SCLC tumor datasets as well as NBL
444 cell line (CCLE_neuroblastoma) and tumor (TARGET_NB) datasets. *, Pearson correlation with p-value <

445 0.05. **b**, Ridgeline plot showing distribution of Pearson correlation coefficients from correlating NE score to
446 expression of 995 immunosuppressive genes (47) in SCLC cell line, PDX and patient tumor datasets. With
447 meta-analysis in the four SCLC tumor datasets, 562 out of the 995 genes were found to have significant
448 correlation with NE score and about 80% of those are negative correlations.

449

450 **Figure S6 Cancer type-specific association between NE score and immune cell fractions**

451 Cancer type-specific scatter plots of NE scores with tumor-infiltrating lymphocytes regional fraction (**a**) or
452 leukocyte fraction (**b**) estimated by Thorsson, Gibbs, et al. (51) in TCGA samples.

453

454 **Figure S7 Expression of MYC and MHC I genes (summarized as MHC I scores) in SCLC cell lines** 455 **derived from primary lung tumors and metastatic tumors.**

456 **a**, Scatterplots of MHC I score and MYC expression in lung cancer cell lines by samples types and
457 anatomical sites of origin. **b**, MYC and MHC I gene expression score compared by anatomical site. For
458 each gene, p-values for pairwise comparisons were calculated based on two-sided t-test followed by
459 adjustment for multiple comparisons using the Bonferroni method.

460

461 **SUPPLEMENTARY TABLES**

462

463 **Table S1** NE signature based on SCLC cell line RNA-seq data

464 **Table S2** Patient characteristics, data availability and quantification of CD4 and CD8 T cells based on IHC

465 **Table S3** Correlation between 995 immunosuppressive genes and NE scores from SCLC datasets

466

467 **METHODS**

468 Computation of NE score

469 The construction of the original NE signature has been described by Zhang, Girard et al.(22). In this study
470 this signature has been updated with expression data from RNA-seq experiments. A quantitative NE score
471 can be generated from this signature using the formula: NE score = (correl NE – correl non-NE)/2 where
472 correl NE (or non-NE) is the Pearson correlation between expression of the 50 genes in the test sample and

473 expression of these genes in the NE (or non-NE) cell line group. This score has a range of -1 to +1, where
474 a positive score predicts for NE while a negative score predicts for non-NE cell types. The higher the score
475 in absolute value, the better the prediction.

476

477 Pathway enrichment analysis with GO terms

478 Gene Ontology enRiChment anaLysis and visualizAtion tool (GORilla (67), <http://cbl-gorilla.cs.technion.ac.il/>)
479 was used to identify enriched GO terms (68) related to biological processes (BP) from gene lists ranked by
480 increasing or decreasing Pearson correlation with NE scores in cell line datasets or “George_2015” tumor
481 dataset. P-value threshold was set at 10^{-3} for resulting GO terms. The output was visualized by Treemap
482 R scripts generated from “reduce + visualize gene ontology” (REViGO (69), <http://revigo.irb.hr/>) and further
483 customized with modified color scheme.

484

485 Gene Set Enrichment Analysis (GSEA)

486 Gene set libraries were downloaded from Enrichr (24) (<https://amp.pharm.mssm.edu/Enrichr/>). Fast GSEA
487 based on gene label permutation from R package “fgsea” (70) was first used for a fast screening across a
488 large number of gene set libraries. After reviewing the results for SCLC cell lines, sample label permutation-
489 based GSEA (23) was run for selected gene set libraries to obtain normalized enrichment scores and
490 multiple comparison adjusted p-values. Pearson correlation was used as the ranking metric from correlating
491 gene expression with NE scores.

492

493 Visualization

494 All heatmaps were generated by R package “ComplexHeatmap”(71). Other R packages used for
495 visualization include “ggplot2” (72), “ggridges” (73), “ggrepel” (74), “ggpubr” (75), “treemap” (76),
496 “RColorBrewer” (77), “jcolors” (78) and “patchwork” (79).

497

498 Expression data

499 . Drapkin_2018 (54) was downloaded from GEO with accession id GSE110853; Rudin_2012 (9) was
500 obtained from the authors; George_2015 (10) was obtained from a supplementary table of the original

501 publication; Jiang_2016 (62) was downloaded from GEO with accession id GSE60052; IGC's Expression
502 Project for Oncology - expO (GSE2109) and Rousseaux_2013 (GSE30219) (64) was processed
503 previously for the lung cancer explorer (LCE) (63). Pan-cancer data from TCGA and TARGET was
504 downloaded from Toil xena hub (50). For data from GEO, R package GEOquery (80) was used for
505 extracting the expression and phenotype data. Quantile normalization was performed for bulk expression
506 data by running the “normalize.quantiles” function from R package “preprocessCore” (81). Library size
507 normalization was performed for author-processed scRNA-seq data by running the “library.size.normalize”
508 function from R package “phateR” (82). Travaglini_2020 (39) was downloaded from Synapse with
509 accession id syn21041850. FACS-sorted SmartSeq2 data was used. Cell types with less than 10 cells were
510 removed from analyses.

511

512 Gene Signatures

513 SPARCS gene set is from a study by Cañadas et al (Figure 1S in original article) (35). Parainflammation
514 gene set is from a study by Aran et al (Figure 1C in original article) (36). SASP gene set is from a study by
515 Ruscetti et al (Figure 2C in original article) (65).

516

517 Gene set “REST ENCODE” is from the “ENCODE_and_ChEA_Consensus_TFs_from_ChIP-X” library; and
518 “IFNA-BT2” and “IFNG-BT2” are from the “LINCS_L1000_Ligand_Perturbations_up” library. Both libraries
519 were downloaded from Enrichr (24). Top 25 genes from leading edge and are common to all SCLC datasets
520 were selected for heatmap visualization. For ISGs, the leading edge genes from “IFNA-BT2” and “IFNG-
521 BT2” were first combined and then the top 25 genes were selected.

522

523 Mouse ISGs, from Cilloniz et al. (83), was identified from interferome (84) by specifying “mouse” as the
524 species of interest and “lung” as the organ of interest. An unfiltered ISG set was used for Figure 6A.

525

526 Human MHC I gene set is a combination of genes under GO terms
527 “GO_MHC_CLASS_I_PROTEIN_COMPLEX” and “GO_MHC_CLASS_I_PEPTIDE_LOADING_COMPLEX”
528 from Molecular Signatures Database (MSigDB) (23, 85). Mouse MHC I genes were selected from GO:

529 0019885, “antigen processing and presentation of endogenous peptide antigen via MHC class I” based on
530 the Mouse Genome Informatics (MGI) database (86). Immune-cell-specific gene sets in human are from
531 DisHet (45). Interferon-gamma signature that predicts response to PD-1 blockade is from Ayers et al. (46).
532 The 21-gene immune checkpoint set and 995-gene immunosuppressive set are from HisgAtlas, a human
533 immunosuppression gene database (47).

534

535 MHC I and Immune infiltrate scores

536 R package GSVA (87) was used to compute immune infiltrate scores by single sample GSEA (ssGSEA)
537 method (23, 44).

538

539 Patients and tissue specimens

540 Study participants included 18 patients who were diagnosis with SCLC and underwent surgical resection of
541 lung cancer between 2006 and 2010 at the Department of Lung Cancer Surgery, Tianjin Medical University
542 General Hospital. Written informed consent was obtained, and the institutional ethics committee of Tianjin
543 Medical University General Hospital approved the study. The cases were selected based on the following
544 criteria: (1) diagnosis of primary lung cancer clinical stage I to IV (pTNM); (2) undergoing surgical resection.
545 Pathologic diagnosis was based on WHO criteria. Lung cancer staging for each patient was performed
546 according to the AJCC Cancer Staging Manual, 8th edition, and was based on findings from physical
547 examination, surgical resection, and computed tomography of the chest, abdomen, pelvis, and brain. The
548 following information was collected from the patients' medical records: age, gender, clinical stage,
549 pathologic diagnosis, differentiation, lymph node status, metastasis, smoking status, and overall survival
550 time. Resected lung and lymph node tissues were immediately immersed in liquid nitrogen until RNA
551 extraction.

552

553 Immunohistochemistry - histology and immunohistochemistry

554 Tissue blocks, once collected, were reviewed by staff thoracic pathologist to confirm SCLC histology.
555 Consecutive four-micrometer-thick tissue sections were cut for immunohistochemistry. IHC staining was
556 performed with a Bond Max automated staining system (Leica Microsystems Inc., Vista, CA) using IHC

557 parameters optimized previously. Antibodies used in this study included ASCL1 (dilution 1:25; Clone
558 24B72D11.1, BD Biosciences, Catalog # 556604), NEUROD1 (dilution 1:100; Clone EPR20766, Abcam,
559 ab213725), POU2F3 (dilution 1:200; polyclonal, Novus Biologicals, NBP1-83966), CD4 (dilution 1:80; Leica
560 Biosystems, CD4-368-L-CE-H) and CD8 (dilution 1:25; Thermo Scientific, MS-457s) in a Leica Bond Max
561 automated stainer (Leica Biosystems Nussloch GmbH). The expression of proteins was detected using the
562 Bond Polymer Refine Detection kit (Leica Biosystems, Cat# DS9800) with diaminobenzidine as chromogen
563 (88). The slides were counterstained with hematoxylin, dehydrated and cover-slipped. FFPE cell lines
564 pellets with known expression of ASCL1, NEUROD1 and POU2F3 were used to establish and optimize IHC
565 conditions and assess sensitivity and specificity for each antibody.

566

567 Immunohistochemistry - Image analysis

568 The stained slides were digitally scanned using the Aperio ScanScope Turbo slide scanner (Leica
569 Microsystems Inc.) under $\times 200$ magnification. The images were visualized by ImageScope software (Leica
570 Microsystems, Inc.) and analyzed using the Aperio Image Toolbox (Leica Microsystems Inc.). Different
571 intensity levels of ASCL1, NEUROD1 or POU2F3 nuclear expression were quantified using a 4-value
572 intensity score (0, none; 1, weak; 2, moderate; and 3, strong) and the percentage (0-100%) of the extent of
573 reactivity. A final expression score (H-score) was obtained by multiplying the intensity and reactivity
574 extension values (range, 0–300) as previously described (89).

575 The lymphocyte cells expressing CD4⁺ and CD8⁺ were counted by a pathologist using Aperio Image
576 Toolbox analysis software (Aperio, Leica Biosystems) and expressed as cell density (CD4⁺ and CD8⁺
577 cells/mm² of analyzed tissue) (88, 90).

578

579 Microarray Assay

580 The Human Genome U133 Plus 2.0 microarray with 54,000 probe sets was purchased from the Affymetrix
581 (Lot #: 4032359). Total RNA was extracted with the Trizol reagent (Invitrogen) from the tissue samples. The
582 extracted RNA was purified using the Oligotex mRNA Midi kit (Qiagen). Then double-strand cDNA synthesis
583 was made using one-cycle cDNA synthesis kit (Affymetrix) and purified again by column followed by the
584 synthesis of complementary RNA (cRNA) with in vitro transcription (IVT) kit (Affymetrix). The cRNA was

585 fragmented after purification by column and the quality was verified by ultraviolet spectrophotometer and
586 1.2% denaturing agarose gel. After the test gene-chip (Lot#: 4020852, Affymetrix) was affirmed satisfactory,
587 the real chip hybridization of cRNA fragmentation was performed and then stained and washed. Finally the
588 real chip was scanned in Affymetrix scanner and the data was collected by GCOS (gene-chip operation
589 software). CEL files were read into an AffyBatch object by "AffyBatch" function under the "affy" (91) R
590 package. Alternative cdf package (92) "hgu133plus2hsentrezg" was downloaded from
591 "http://mbni.org/customcdf/22.0.0/entrezg.download/hgu133plus2hsentrezg.db_22.0.0.zip" and was
592 specified in the function so that the resulting expression data was processed to gene level rather than the
593 original probe level. (Probe name follows format concatenating Entrez ID for the gene and "_at". For
594 example "3939_at" corresponds to gene *LDHA*). The AffyBatch object was then converted to an expression
595 set using robust multi-array average (RMA) expression measure by running the "rma" function under R
596 package "affy". Quantile normalization was performed by running "normalize.quantiles" function from R
597 package "preprocessCore" (81)

598

599 RNA-seq

600 RNA samples from SCLC cell lines (n = 69) were prepared at UT Southwestern (Dallas TX) and sent to
601 Baylor College of Medicine (David Wheeler, Houston TX) for paired-end RNA sequencing. Analysis was
602 then performed at UT Southwestern: Reads were aligned to the human reference genome GRCh38 using
603 STAR-2.7(93) (<https://github.com/alexdobin/STAR>) and FPKM values were generated with cufflinks-2.2.1
604 (94) (<http://cole-trapnell-lab.github.io/cufflinks/>). All data were then pooled, upper-quartile normalized (95),
605 and log-transformed.

606

607 Cell culture

608 All SCLC cell lines used in these studies were originally established in the John D. Minna and Adi F. Gazdar
609 laboratories. The cultured Small Cell Lung Cancer (SCLC) cell lines were obtained from both the National
610 Cancer Institute (NCI) and Hamon Cancer Center (HCC) libraries. Cells were cultured in RPMI-1640 media
611 (Sigma Life Science, St. Louis, MO) supplemented with 5% Fetal Bovine Serum (FBS). RPMI-1640
612 supplemented with 5% FBS will be referred to as R5. All cells were incubated in NuAire (NuAire, Plymouth,

613 MN) humidified incubators at 37°C at 5% CO₂. All cell lines were regularly tested for mycoplasma
614 contamination (Bulldog Bio, Portsmouth, NH) and fingerprinted using a PowerPlex 1.2 kit (Promega,
615 Madison, WI) to confirm the cell line identity.

616

617 Establishing adherent H69 (H69-AD)

618 The early passage of parental H69 cell line grew as a mixture of floating and adherent cells. To enrich for
619 adherent cells, the floating population of H69 was washed off during growth media replacement and fresh
620 media was provided for expansion of the remaining adherent cells. This was repeated until every passage
621 grew as adherent cells with few to no suspension cells. This derived adherent subline was designated as
622 H69-AD.

623

624 Drug response assay

625 Cisplatin and Etoposide were obtained from Selleck Chemicals LLC, USA. 5,000 cells of H69 and H69-AD
626 were cultured in 100 uL R5 growth media per well in ultra-low adherent, clear, round bottom, 96 well plates
627 (BD Biosciences, USA) for 48 hours. An additional 100 µl R5 plus either a control (DMSO) or drug was
628 added to the plate. 96 hours after drug treatment, each cell line was assayed using the Cell-Titer-Glo
629 reagent (Promega, Inc.). The fluorescence intensity was recorded at 570 nM. A standard 4-parameter log-
630 logistic fit between the survival rate and the dosage was generated by the “drm” function from the R
631 package “drc”(96).

632

633 Xenograft models for parental and chemoresistant SCLC tumors

634 Subcutaneous xenograft in NSG mice was derived from direct implantation of untreated H1436 cells or re-
635 implantation of chemoresistant tumors after 4 cycles of Cisplatin and Etoposide (EC), or plus 4 cycles of
636 Cisplatin (reduced from EC due to toxicity). Specifically, a million H1436 cells were resuspended in 100 µl
637 mixture of serum-free RPMI 1640 and Matrigel (BD Bioscience #356237) at 1:1 ratio and immediately
638 injected in the flank of 6-8-week-old female NSG mouse (Jackson Laboratory #005557). Mice were
639 randomized after tumor cell injection. Treatment starts after a week when the tumor becomes palpable. 5
640 mg/kg/w Cisplatin (Sigma P4394) in saline, 10 mg/kg/w Etoposide (Sigma E1383) in 30% PEG 300 (Sigma

641 202371) were freshly prepared and administered by intraperitoneal injection, for 4 cycles in total to obtain
642 the first group of chemoresistant tumors. An additional 4 cycles of Cisplatin were administered in a second
643 group of mice to obtain tumors with further potentiated chemoresistance. To harvest the tumor, 10 ml
644 digestion media was used per mouse. This was prepared freshly by supplementing 9 ml HBSS with 1 ml
645 type IV collagenase, 50 µl DNase II and 50 µl 1 M CaCl₂. Tumors were collected and placed in HBSS
646 immediately following dissection. A fraction of the tumor was cut into a few pieces and flash-frozen in liquid
647 nitrogen to be saved in aliquots for molecular assays. The remaining chunk was finely minced with a sterile
648 scalpel blade. For re-implantation, the minced tissue was resuspended in digestion media, rotated at 37 °C
649 for 20 min, filtered through a 40 µm filter, centrifuged at 300x g for 5 min.

650

651 Quantitative reverse transcription PCR

652 ~20 mg flash-frozen tumor fragments were weighed out and homogenized in 1 ml TRIzol (Invitrogen
653 #15596-026) in Precellys tissue homogenizing mixed beads kit (Cayman Chemical #10409). 0.2 ml
654 chloroform (Fisher #S25248) was added to the TRIzol lysate and the mixture was vortexed for 10 s and
655 centrifuged at 12,000 x g for 15 min at 4 °C for phase separation. 450 µl aqueous phase was collected,
656 mixed well with 0.5 ml isopropanol (Fisher #A451-1) and precipitated RNA was collected by centrifugation at
657 12,000 x g for 10 min at 4 °C, The RNA pellet was rinsed in 1 ml 75% ethanol, then dissolved in 100 µl
658 deionized water by incubating at 55 °C for 5 min. 500 ng total RNA was reverse-transcribed to cDNA in a 20
659 µl reaction with 4 µl iScript reverse transcription supermix (Bio-Rad #1708841) at 25 °C for 5 min, 46 °C for
660 20 min, and 95 °C for 1 min. The mixture was then 1:5 diluted with deionized water. Target sequences in
661 cDNA library were amplified in 10 µl qPCR reaction (5 µl SYBR Green supermix (Bio-Rad #1725121), 0.675
662 µl 2.5 µM primer mix and 0.45 µl diluted cDNA) at 95 °C 10 s, 60 °C 30 s, for 40 cycles. All procedures were
663 performed under RNase-free condition unless specified. For data analysis, median was taken from
664 triplicates, normalized by Ct values of control gene *PPIA*, exponentiated with base 2 then divided by the
665 median of parental samples.

666

667 Study Approval

668 The protocol of collecting human SCLC tumor tissue for research was approved by the Ethics Committee of
669 Tianjin Medical University General Hospital. Written informed consent was received from participants prior
670 to inclusion in the study. Specimen collection did not interfere with standard diagnostic and therapeutic
671 procedures. All mouse procedures were performed with the approval of the University of Texas
672 Southwestern Medical Center IACUC.

673

674 Data availability

675 Scripts used for this manuscript are available upon request. The RNA-seq gene expression data from
676 UTSW SCLC has been added to dbGaP (accession phs001823.v1.p1) (97). SCLC tumor microarray data
677 used in this study has been deposited to GEO with accession id GSE149507.

678

679 **ACKNOWLEDGEMENT**

680 Support for this work comes from the National Institutes of Health [1R35GM136375, 5P30CA142543,
681 5U01CA213338-04, 3P50CA070907, and R01GM115473], and the Cancer Prevention Research Institute of
682 Texas [RP180805]. We dedicate this work to Dr. Adi F. Gazdar, who had initially conceptualized and led this
683 project until his passing in December 2018. We thank Ms. Jessie Norris for proofreading the manuscript.

684

685 **AUTHOR CONTRIBUTIONS**

686 Conception and design, A.F.G. and L.C.; Development of methodology, L.C., L.G. and T.W.; Acquisition of
687 data, L.C., H.L., F.H., J.F., L.G., J.C., Y.L., Y.Z., D.D., V.S., C.S.K., C.Y., A.A., K.H., M.P. and B.D.;
688 Analysis and interpretation of data, L.C., J.F., G.J., L.Y. and W.Z.; Writing, review and/or revision of the
689 manuscript, L.C., H.L., E.A., R.J.D and J.D.M.; Study supervision, D.S.S., I.I.W., G.X., J.D.M., Y.X. and
690 A.F.G.

691

692 **COMPETING INTERESTS**

693 J.D.M. receives licensing fees from the NCI and UT Southwestern to distribute cell lines. R.J.D is on the
694 advisory board for Agios Pharmaceuticals. D.S.S and W.Z. are currently employed by Genentech Inc. and
695 own stock in Roche Holdings. I.I.W is speaker at Medscape, MSD, Genentech/Roche, PlatformQ Health,

696 Pfizer, AstraZeneca, Merck; receives research support from Genentech, Oncoplex, HTG Molecular,
697 DepArray, Merck, Bristol-Myers Squibb, Medimmune, Adaptive, Adaptimmune, EMD Serono, Pfizer,
698 Takeda, Amgen, Karus, Johnson & Johnson, Bayer, Iovance, 4D, Novartis, and Akoya; and is on the
699 advisory boards for Genentech/Roche, Bayer, Bristol-Myers Squibb, AstraZeneca/Medimmune, Pfizer, HTG
700 Molecular, Asuragen, Merck, GlaxoSmithKline, Guardant Health, Oncocyte, and MSD.

701

702 REFERENCE

- 703 1. Gazdar AF, Bunn PA, Minna JD. Small-cell lung cancer: what we know, what we need to know
704 and the path forward. *Nat Rev Cancer*. 2017;17(12):765. Epub 2017/11/11. doi:
705 10.1038/nrc.2017.106. PubMed PMID: 29123245.
- 706 2. H.R.733 UC. Recalcitrant Cancer Research Act of 2012 2012. Available from:
707 <https://www.congress.gov/bill/112th-congress/house-bill/733>.
- 708 3. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, Chirieac LR, Dacic S,
709 Duhig E, Flieder DB, Geisinger K, Hirsch FR, Ishikawa Y, Kerr KM, Noguchi M, Pelosi G, Powell CA, Tsao
710 MS, Wistuba I, Panel WHO. The 2015 World Health Organization Classification of Lung Tumors: Impact
711 of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. *J Thorac Oncol*.
712 2015;10(9):1243-60. Epub 2015/08/21. doi: 10.1097/JTO.0000000000000630. PubMed PMID:
713 26291008.
- 714 4. Augustyn A, Borromeo M, Wang T, Fujimoto J, Shao C, Dospoy PD, Lee V, Tan C, Sullivan JP,
715 Larsen JE, Girard L, Behrens C, Wistuba, II, Xie Y, Cobb MH, Gazdar AF, Johnson JE, Minna JD. ASCL1 is a
716 lineage oncogene providing therapeutic targets for high-grade neuroendocrine lung cancers. *Proc Natl*
717 *Acad Sci U S A*. 2014;111(41):14788-93. Epub 2014/10/01. doi: 10.1073/pnas.1410419111. PubMed
718 PMID: 25267614; PMCID: PMC4205603.
- 719 5. Borges M, Linnoila RI, van de Velde HJ, Chen H, Nelkin BD, Mabry M, Baylin SB, Ball DW. An
720 achaete-scute homologue essential for neuroendocrine differentiation in the lung. *Nature*.
721 1997;386(6627):852-5. Epub 1997/04/24. doi: 10.1038/386852a0. PubMed PMID: 9126746.
- 722 6. Boers JE, den Brok JL, Koudstaal J, Arends JW, Thunnissen FB. Number and proliferation of
723 neuroendocrine cells in normal human airway epithelium. *Am J Respir Crit Care Med*. 1996;154(3 Pt
724 1):758-63. Epub 1996/09/01. doi: 10.1164/ajrccm.154.3.8810616. PubMed PMID: 8810616.
- 725 7. Ouadah Y, Rojas ER, Riordan DP, Capostagno S, Kuo CS, Krasnow MA. Rare Pulmonary
726 Neuroendocrine Cells Are Stem Cells Regulated by Rb, p53, and Notch. *Cell*. 2019;179(2):403-16 e23.
727 Epub 2019/10/05. doi: 10.1016/j.cell.2019.09.010. PubMed PMID: 31585080; PMCID: PMC6782070.
- 728 8. Pleasance ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, Jones D, Lin ML, Beare D, Lau KW,
729 Greenman C, Varela I, Nik-Zainal S, Davies HR, Ordóñez GR, Mudie LJ, Latimer C, Edkins S, Stebbings L,
730 Chen L, Jia M, Leroy C, Marshall J, Menzies A, Butler A, Teague JW, Mangion J, Sun YA, McLaughlin SF,
731 Peckham HE, Tsung EF, Costa GL, Lee CC, Minna JD, Gazdar A, Birney E, Rhodes MD, McKernan KJ,
732 Stratton MR, Futreal PA, Campbell PJ. A small-cell lung cancer genome with complex signatures of
733 tobacco exposure. *Nature*. 2010;463(7278):184-90. Epub 2009/12/18. doi: 10.1038/nature08629.
734 PubMed PMID: 20016488; PMCID: PMC2880489.
- 735 9. Rudin CM, Durinck S, Stawiski EW, Poirier JT, Modrusan Z, Shames DS, Bergbower EA, Guan Y,
736 Shin J, Guillory J, Rivers CS, Foo CK, Bhatt D, Stinson J, Gnad F, Haverty PM, Gentleman R, Chaudhuri S,
737 Janakiraman V, Jaiswal BS, Parikh C, Yuan W, Zhang Z, Koeppen H, Wu TD, Stern HM, Yauch RL,
738 Huffman KE, Paskulin DD, Illei PB, Varella-Garcia M, Gazdar AF, de Sauvage FJ, Bourgon R, Minna JD,
739 Brock MV, Seshagiri S. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene

740 in small-cell lung cancer. *Nat Genet.* 2012;44(10):1111-6. Epub 2012/09/04. doi: 10.1038/ng.2405.
741 PubMed PMID: 22941189; PMCID: PMC3557461.

742 10. George J, Lim JS, Jang SJ, Cun Y, Ozretic L, Kong G, Leenders F, Lu X, Fernandez-Cuesta L, Bosco
743 G, Muller C, Dahmen I, Jahchan NS, Park KS, Yang D, Karnezis AN, Vaka D, Torres A, Wang MS, Korbel
744 JO, Menon R, Chun SM, Kim D, Wilkerson M, Hayes N, Engelmann D, Putzer B, Bos M, Michels S, Vlastic I,
745 Seidel D, Pinther B, Schaub P, Becker C, Altmuller J, Yokota J, Kohno T, Iwakawa R, Tsuta K, Noguchi M,
746 Muley T, Hoffmann H, Schnabel PA, Petersen I, Chen Y, Soltermann A, Tischler V, Choi CM, Kim YH,
747 Massion PP, Zou Y, Jovanovic D, Kontic M, Wright GM, Russell PA, Solomon B, Koch I, Lindner M,
748 Muscarella LA, la Torre A, Field JK, Jakopovic M, Knezevic J, Castanos-Velez E, Roz L, Pastorino U,
749 Brustugun OT, Lund-Iversen M, Thunnissen E, Kohler J, Schuler M, Botling J, Sandelin M, Sanchez-
750 Cespedes M, Salvesen HB, Achter V, Lang U, Bogus M, Schneider PM, Zander T, Ansen S, Hallek M, Wolf
751 J, Vingron M, Yatabe Y, Travis WD, Nurnberg P, Reinhardt C, Perner S, Heukamp L, Buttner R, Haas SA,
752 Brambilla E, Peifer M, Sage J, Thomas RK. Comprehensive genomic profiles of small cell lung cancer.
753 *Nature.* 2015;524(7563):47-53. Epub 2015/07/15. doi: 10.1038/nature14664. PubMed PMID:
754 26168399; PMCID: PMC4861069.

755 11. Doyle A, Martin WJ, Funa K, Gazdar A, Carney D, Martin SE, Linnoila I, Cuttitta F, Mulshine J,
756 Bunn P, et al. Markedly decreased expression of class I histocompatibility antigens, protein, and mRNA
757 in human small-cell lung cancer. *J Exp Med.* 1985;161(5):1135-51. Epub 1985/05/01. doi:
758 10.1084/jem.161.5.1135. PubMed PMID: 2580935; PMCID: PMC2187608.

759 12. Burr ML, Sparbier CE, Chan KL, Chan YC, Kersbergen A, Lam EYN, Azidis-Yates E, Vassiliadis D,
760 Bell CC, Gilan O, Jackson S, Tan L, Wong SQ, Hollizeck S, Michalak EM, Siddle HV, McCabe MT, Prinjha
761 RK, Guerra GR, Solomon BJ, Sandhu S, Dawson SJ, Beavis PA, Tothill RW, Cullinane C, Lehner PJ,
762 Sutherland KD, Dawson MA. An Evolutionarily Conserved Function of Polycomb Silences the MHC
763 Class I Antigen Presentation Pathway and Enables Immune Evasion in Cancer. *Cancer Cell.*
764 2019;36(4):385-401 e8. Epub 2019/10/01. doi: 10.1016/j.ccell.2019.08.008. PubMed PMID:
765 31564637; PMCID: PMC6876280.

766 13. Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1
767 Inhibition. *N Engl J Med.* 2017;377(25):2500-1. Epub 2017/12/21. doi: 10.1056/NEJMc1713444.
768 PubMed PMID: 29262275; PMCID: PMC6549688.

769 14. Iams WT, Porter J, Horn L. Immunotherapeutic approaches for small-cell lung cancer. *Nat Rev*
770 *Clin Oncol.* 2020. Epub 2020/02/15. doi: 10.1038/s41571-019-0316-z. PubMed PMID: 32055013.

771 15. Gazdar AF, Carney DN, Nau MM, Minna JD. Characterization of variant subclasses of cell lines
772 derived from small cell lung cancer having distinctive biochemical, morphological, and growth
773 properties. *Cancer Res.* 1985;45(6):2924-30. Epub 1985/06/01. PubMed PMID: 2985258.

774 16. Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW, Zweig MH, Minna JD.
775 Establishment and identification of small cell lung cancer cell lines having classic and variant features.
776 *Cancer Res.* 1985;45(6):2913-23. Epub 1985/06/01. PubMed PMID: 2985257.

777 17. Johnson BE, Russell E, Simmons AM, Phelps R, Steinberg SM, Ihde DC, Gazdar AF. MYC family
778 DNA amplification in 126 tumor cell lines from patients with small cell lung cancer. *J Cell Biochem*
779 *Suppl.* 1996;24:210-7. Epub 1996/01/01. doi: 10.1002/jcb.240630516. PubMed PMID: 8806103.

780 18. Lim JS, Ibaseta A, Fischer MM, Cancilla B, O'Young G, Cristea S, Luca VC, Yang D, Jahchan NS,
781 Hamard C, Antoine M, Wislez M, Kong C, Cain J, Liu YW, Kapoun AM, Garcia KC, Hoey T, Murriel CL,
782 Sage J. Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer.
783 *Nature.* 2017;545(7654):360-4. Epub 2017/05/11. doi: 10.1038/nature22323. PubMed PMID:
784 28489825; PMCID: PMC5776014.

785 19. Gazdar AF, Bunn PA, Minna JD. Small-cell lung cancer: what we know, what we need to know
786 and the path forward. *Nat Rev Cancer.* 2017;17(12):725-37. doi: 10.1038/nrc.2017.87. PubMed PMID:
787 29077690.

788 20. Rudin CM, Poirier JT, Byers LA, Dive C, Dowlati A, George J, Heymach JV, Johnson JE, Lehman JM,
789 MacPherson D, Massion PP, Minna JD, Oliver TG, Quaranta V, Sage J, Thomas RK, Vakoc CR, Gazdar AF.

790 Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nat Rev*
791 *Cancer*. 2019;19(5):289-97. Epub 2019/03/31. doi: 10.1038/s41568-019-0133-9. PubMed PMID:
792 30926931; PMCID: PMC6538259.

793 21. Gazdar AF. Morphologic and Other Forms of Heterogeneity in Small Cell Lung Cancer: What Can
794 We Learn from Them? *J Thorac Oncol*. 2018;13(2):148-50. Epub 2018/02/10. doi:
795 10.1016/j.jtho.2017.11.004. PubMed PMID: 29425612.

796 22. Zhang W, Girard L, Zhang YA, Haruki T, Papari-Zareei M, Stastny V, Ghayee HK, Pacak K, Oliver
797 TG, Minna JD, Gazdar AF. Small cell lung cancer tumors and preclinical models display heterogeneity of
798 neuroendocrine phenotypes. *Transl Lung Cancer Res*. 2018;7(1):32-49. Epub 2018/03/15. doi:
799 10.21037/tlcr.2018.02.02. PubMed PMID: 29535911; PMCID: PMC5835590.

800 23. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A,
801 Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based
802 approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*.
803 2005;102(43):15545-50. Epub 2005/10/04. doi: 10.1073/pnas.0506580102. PubMed PMID:
804 16199517; PMCID: PMC1239896.

805 24. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL,
806 Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen GW, Ma'ayan A. Enrichr: a
807 comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*.
808 2016;44(W1):W90-7. Epub 2016/05/05. doi: 10.1093/nar/gkw377. PubMed PMID: 27141961;
809 PMCID: PMC4987924.

810 25. Ghandi M, Huang FW, Jane-Valbuena J, Kryukov GV, Lo CC, McDonald ER, 3rd, Barretina J,
811 Gelfand ET, Bielski CM, Li H, Hu K, Andreev-Drakhlin AY, Kim J, Hess JM, Haas BJ, Aguet F, Weir BA,
812 Rothberg MV, Paoletta BR, Lawrence MS, Akbani R, Lu Y, Tiv HL, Gokhale PC, de Weck A, Mansour AA,
813 Oh C, Shih J, Hadi K, Rosen Y, Bistline J, Venkatesan K, Reddy A, Sonkin D, Liu M, Lehar J, Korn JM,
814 Porter DA, Jones MD, Golji J, Caponigro G, Taylor JE, Dunning CM, Creech AL, Warren AC, McFarland JM,
815 Zamanighomi M, Kauffmann A, Stransky N, Imielinski M, Maruvka YE, Cherniack AD, Tsherniak A,
816 Vazquez F, Jaffe JD, Lane AA, Weinstock DM, Johannessen CM, Morrissey MP, Stegmeier F, Schlegel R,
817 Hahn WC, Getz G, Mills GB, Boehm JS, Golub TR, Garraway LA, Sellers WR. Next-generation
818 characterization of the Cancer Cell Line Encyclopedia. *Nature*. 2019;569(7757):503-8. doi:
819 10.1038/s41586-019-1186-3. PubMed PMID: 31068700.

820 26. Sakurai H, Chiba H, Miyoshi H, Sugita T, Toriumi W. IkappaB kinases phosphorylate NF-kappaB
821 p65 subunit on serine 536 in the transactivation domain. *J Biol Chem*. 1999;274(43):30353-6. Epub
822 1999/10/16. doi: 10.1074/jbc.274.43.30353. PubMed PMID: 10521409.

823 27. Dinarello CA. Interleukin-18, a proinflammatory cytokine. *Eur Cytokine Netw*. 2000;11(3):483-
824 6. Epub 2001/02/24. PubMed PMID: 11203186.

825 28. Cornelissen C, Luscher-Firzlaff J, Baron JM, Luscher B. Signaling by IL-31 and functional
826 consequences. *Eur J Cell Biol*. 2012;91(6-7):552-66. Epub 2011/10/11. doi:
827 10.1016/j.ejcb.2011.07.006. PubMed PMID: 21982586.

828 29. Kordass T, Osen W, Eichmuller SB. Controlling the Immune Suppressor: Transcription Factors
829 and MicroRNAs Regulating CD73/NT5E. *Front Immunol*. 2018;9:813. Epub 2018/05/04. doi:
830 10.3389/fimmu.2018.00813. PubMed PMID: 29720980; PMCID: PMC5915482.

831 30. Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the
832 tumor microenvironment and progression. *Trends Immunol*. 2010;31(6):220-7. Epub 2010/06/12.
833 doi: 10.1016/j.it.2010.04.002. PubMed PMID: 20538542; PMCID: PMC2891151.

834 31. D'Acquisto F, Perretti M, Flower RJ. Annexin-A1: a pivotal regulator of the innate and adaptive
835 immune systems. *Br J Pharmacol*. 2008;155(2):152-69. Epub 2008/07/22. doi: 10.1038/bjp.2008.252.
836 PubMed PMID: 18641677; PMCID: PMC2538690.

837 32. Markosyan N, Li J, Sun YH, Richman LP, Lin JH, Yan F, Quinones L, Sela Y, Yamazoe T, Gordon N,
838 Tobias JW, Byrne KT, Rech AJ, FitzGerald GA, Stanger BZ, Vonderheide RH. Tumor cell-intrinsic EPHA2

839 suppresses anti-tumor immunity by regulating PTGS2 (COX-2). *J Clin Invest*. 2019;130:3594-609. Epub
840 2019/06/05. doi: 10.1172/JCI127755. PubMed PMID: 31162144; PMCID: PMC6715369.

841 33. Reuben A, Chung JW, Lapointe R, Santos MM. The hemochromatosis protein HFE 20 years later:
842 An emerging role in antigen presentation and in the immune system. *Immun Inflamm Dis*.
843 2017;5(3):218-32. Epub 2017/05/06. doi: 10.1002/iid.3158. PubMed PMID: 28474781; PMCID:
844 PMC5569368.

845 34. Kouo T, Huang L, Pucsek AB, Cao M, Solt S, Armstrong T, Jaffee E. Galectin-3 Shapes Antitumor
846 Immune Responses by Suppressing CD8+ T Cells via LAG-3 and Inhibiting Expansion of Plasmacytoid
847 Dendritic Cells. *Cancer Immunol Res*. 2015;3(4):412-23. Epub 2015/02/19. doi: 10.1158/2326-
848 6066.CIR-14-0150. PubMed PMID: 25691328; PMCID: PMC4390508.

849 35. Canadas I, Thummalapalli R, Kim JW, Kitajima S, Jenkins RW, Christensen CL, Campisi M, Kuang
850 Y, Zhang Y, Gjini E, Zhang G, Tian T, Sen DR, Miao D, Imamura Y, Thai T, Piel B, Terai H, Aref AR, Hagan
851 T, Koyama S, Watanabe M, Baba H, Adeni AE, Lydon CA, Tamayo P, Wei Z, Herlyn M, Barbie TU,
852 Uppaluri R, Sholl LM, Sicinska E, Sands J, Rodig S, Wong KK, Paweletz CP, Watanabe H, Barbie DA.
853 Tumor innate immunity primed by specific interferon-stimulated endogenous retroviruses. *Nat Med*.
854 2018;24(8):1143-50. Epub 2018/07/25. doi: 10.1038/s41591-018-0116-5. PubMed PMID: 30038220;
855 PMCID: PMC6082722.

856 36. Aran D, Lasry A, Zinger A, Biton M, Pikarsky E, Hellman A, Butte AJ, Ben-Neriah Y. Widespread
857 parainflammation in human cancer. *Genome Biol*. 2016;17(1):145. Epub 2016/07/09. doi:
858 10.1186/s13059-016-0995-z. PubMed PMID: 27386949; PMCID: PMC4937599.

859 37. Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, Nelson PS, Desprez PY, Campisi J.
860 Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS
861 and the p53 tumor suppressor. *PLoS Biol*. 2008;6(12):2853-68. Epub 2008/12/05. doi:
862 10.1371/journal.pbio.0060301. PubMed PMID: 19053174; PMCID: PMC2592359.

863 38. Chien Y, Scuoppo C, Wang X, Fang X, Balgley B, Bolden JE, Premrurit P, Luo W, Chicas A, Lee CS,
864 Kogan SC, Lowe SW. Control of the senescence-associated secretory phenotype by NF-kappaB
865 promotes senescence and enhances chemosensitivity. *Genes Dev*. 2011;25(20):2125-36. Epub
866 2011/10/08. doi: 10.1101/gad.17276711. PubMed PMID: 21979375; PMCID: PMC3205583.

867 39. Travaglini KJ, Nabhan AN, Penland L, Sinha R, Gillich A, Sit RV, Chang S, Conley SD, Mori Y, Seita
868 J, Berry GJ, Shrager JB, Metzger RJ, Kuo CS, Neff N, Weissman IL, Quake SR, Krasnow MA. A molecular
869 cell atlas of the human lung from single cell RNA sequencing. *bioRxiv*. 2019.

870 40. Zhang H, Deo M, Thompson RC, Uhler MD, Turner DL. Negative regulation of Yap during
871 neuronal differentiation. *Dev Biol*. 2012;361(1):103-15. Epub 2011/11/01. doi:
872 10.1016/j.ydbio.2011.10.017. PubMed PMID: 22037235; PMCID: PMC3235039.

873 41. Schoggins JW. Interferon-Stimulated Genes: What Do They All Do? *Annu Rev Virol*.
874 2019;6(1):567-84. Epub 2019/07/10. doi: 10.1146/annurev-virology-092818-015756. PubMed
875 PMID: 31283436.

876 42. Pfaender S, Mar KB, Michailidis E, Kratzel A, Hirt D, V'kovski P, Fan W, Ebert N, Stalder H,
877 Kleine-Weber H, Hoffmann M, Hoffmann HH, Saeed M, Dijkman R, Steinmann E, Wight-Carter M,
878 Hanners NW, Pöhlmann S, Gallagher T, Todt D, Zimmer G, Rice CM, Schoggins JW, Thiel V. LY6E
879 impairs coronavirus fusion and confers immune control of viral disease. *bioRxiv*. 2020.

880 43. Shen LW, Mao HJ, Wu YL, Tanaka Y, Zhang W. TMPRSS2: A potential target for treatment of
881 influenza virus and coronavirus infections. *Biochimie*. 2017;142:1-10. Epub 2017/08/06. doi:
882 10.1016/j.biochi.2017.07.016. PubMed PMID: 28778717; PMCID: PMC7116903.

883 44. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E,
884 Scholl C, Frohling S, Chan EM, Sos ML, Michel K, Mermel C, Silver SJ, Weir BA, Reiling JH, Sheng Q, Gupta
885 PB, Wadlow RC, Le H, Hoersch S, Wittner BS, Ramaswamy S, Livingston DM, Sabatini DM, Meyerson M,
886 Thomas RK, Lander ES, Mesirov JP, Root DE, Gilliland DG, Jacks T, Hahn WC. Systematic RNA
887 interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*. 2009;462(7269):108-
888 12. Epub 2009/10/23. doi: 10.1038/nature08460. PubMed PMID: 19847166; PMCID: PMC2783335.

- 889 45. Wang T, Lu R, Kapur P, Jaiswal BS, Hannan R, Zhang Z, Pedrosa I, Luke JJ, Zhang H, Goldstein LD,
890 Yousuf Q, Gu YF, McKenzie T, Joyce A, Kim MS, Wang X, Luo D, Onabolu O, Stevens C, Xie Z, Chen M,
891 Filatenkov A, Torrealba J, Luo X, Guo W, He J, Stawiski E, Modrusan Z, Durinck S, Seshagiri S,
892 Brugarolas J. An Empirical Approach Leveraging Tumorgrafts to Dissect the Tumor Microenvironment
893 in Renal Cell Carcinoma Identifies Missing Link to Prognostic Inflammatory Factors. *Cancer Discov.*
894 2018;8(9):1142-55. Epub 2018/06/10. doi: 10.1158/2159-8290.CD-17-1246. PubMed PMID:
895 29884728; PMCID: PMC6125163.
- 896 46. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, Albright A, Cheng JD,
897 Kang SP, Shankaran V, Piha-Paul SA, Yearley J, Seiwert TY, Ribas A, McClanahan TK. IFN-gamma-
898 related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest.* 2017;127(8):2930-40.
899 Epub 2017/06/27. doi: 10.1172/JCI91190. PubMed PMID: 28650338; PMCID: PMC5531419.
- 900 47. Liu Y, He M, Wang D, Diao L, Liu J, Tang L, Guo S, He F, Li D. HisgAtlas 1.0: a human
901 immunosuppression gene database. *Database (Oxford).* 2017;2017. Epub 2017/01/01. doi:
902 10.1093/database/bax094. PubMed PMID: 31725860.
- 903 48. Lee S, Margolin K. Cytokines in cancer immunotherapy. *Cancers (Basel).* 2011;3(4):3856-93.
904 Epub 2011/01/01. doi: 10.3390/cancers3043856. PubMed PMID: 24213115; PMCID: PMC3763400.
- 905 49. Balanis NG, Sheu KM, Esedebe FN, Patel SJ, Smith BA, Park JW, Alhani S, Gomperts BN, Huang J,
906 Witte ON, Graeber TG. Pan-cancer Convergence to a Small-Cell Neuroendocrine Phenotype that Shares
907 Susceptibilities with Hematological Malignancies. *Cancer Cell.* 2019;36(1):17-34 e7. Epub
908 2019/07/10. doi: 10.1016/j.ccell.2019.06.005. PubMed PMID: 31287989; PMCID: PMC6703903.
- 909 50. Vivian J, Rao AA, Nothhaft FA, Ketchum C, Armstrong J, Novak A, Pfeil J, Narkizian J, Deran AD,
910 Musselman-Brown A, Schmidt H, Amstutz P, Craft B, Goldman M, Rosenbloom K, Cline M, O'Connor B,
911 Hanna M, Birger C, Kent WJ, Patterson DA, Joseph AD, Zhu J, Zaranek S, Getz G, Haussler D, Paten B. Toil
912 enables reproducible, open source, big biomedical data analyses. *Nat Biotechnol.* 2017;35(4):314-6.
913 Epub 2017/04/12. doi: 10.1038/nbt.3772. PubMed PMID: 28398314; PMCID: PMC5546205.
- 914 51. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF,
915 Plaisier CL, Eddy JA, Ziv E, Culhane AC, Paull EO, Sivakumar IKA, Gentles AJ, Malhotra R, Farshidfar F,
916 Colaprico A, Parker JS, Mose LE, Vo NS, Liu J, Liu Y, Rader J, Dhankani V, Reynolds SM, Bowlby R,
917 Califano A, Cherniack AD, Anastassiou D, Bedognetti D, Mokrab Y, Newman AM, Rao A, Chen K, Krasnitz
918 A, Hu H, Malta TM, Noushmehr H, Peadamallu CS, Bullman S, Ojesina AI, Lamb A, Zhou W, Shen H,
919 Choueiri TK, Weinstein JN, Guinney J, Saltz J, Holt RA, Rabkin CS, Cancer Genome Atlas Research N,
920 Lazar AJ, Serody JS, Demicco EG, Disis ML, Vincent BG, Shmulevich I. The Immune Landscape of Cancer.
921 *Immunity.* 2018;48(4):812-30 e14. Epub 2018/04/10. doi: 10.1016/j.immuni.2018.03.023. PubMed
922 PMID: 29628290; PMCID: PMC5982584.
- 923 52. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D, Carter SL, Cibulskis K, Hanna
924 M, Kiezun A, Kim J, Lawrence MS, Lichtenstein L, McKenna A, Peadamallu CS, Ramos AH, Shefler E,
925 Sivachenko A, Sougnez C, Stewart C, Ally A, Birol I, Chiu R, Corbett RD, Hirst M, Jackman SD, Kamoh B,
926 Khodabakshi AH, Krzywinski M, Lo A, Moore RA, Mungall KL, Qian J, Tam A, Thiessen N, Zhao Y, Cole
927 KA, Diamond M, Diskin SJ, Mosse YP, Wood AC, Ji L, Sposto R, Badgett T, London WB, Moyer Y, Gastier-
928 Foster JM, Smith MA, Guidry Auvil JM, Gerhard DS, Hogarty MD, Jones SJ, Lander ES, Gabriel SB, Getz G,
929 Seeger RC, Khan J, Marra MA, Meyerson M, Maris JM. The genetic landscape of high-risk
930 neuroblastoma. *Nat Genet.* 2013;45(3):279-84. Epub 2013/01/22. doi: 10.1038/ng.2529. PubMed
931 PMID: 23334666; PMCID: PMC3682833.
- 932 53. Canadas I, Rojo F, Taus A, Arpi O, Arumi-Uria M, Pijuan L, Menendez S, Zazo S, Domine M, Salido
933 M, Mojal S, Garcia de Herreros A, Rovira A, Albanell J, Arriola E. Targeting epithelial-to-mesenchymal
934 transition with Met inhibitors reverts chemoresistance in small cell lung cancer. *Clin Cancer Res.*
935 2014;20(4):938-50. Epub 2013/11/29. doi: 10.1158/1078-0432.CCR-13-1330. PubMed PMID:
936 24284055.
- 937 54. Drapkin BJ, George J, Christensen CL, Mino-Kenudson M, Dries R, Sundaresan T, Phat S, Myers
938 DT, Zhong J, Igo P, Hazar-Rethinam MH, Licausi JA, Gomez-Caraballo M, Kem M, Jani KN, Azimi R,

939 Abedpour N, Menon R, Lakis S, Heist RS, Buttner R, Haas S, Sequist LV, Shaw AT, Wong KK, Hata AN,
940 Toner M, Maheswaran S, Haber DA, Peifer M, Dyson N, Thomas RK, Farago AF. Genomic and Functional
941 Fidelity of Small Cell Lung Cancer Patient-Derived Xenografts. *Cancer Discov.* 2018;8(5):600-15. Epub
942 2018/02/28. doi: 10.1158/2159-8290.CD-17-0935. PubMed PMID: 29483136; PMCID: PMC6369413.
943 55. Ireland AS, Micinski AM, Kastner DW, Guo B, Wait SJ, Spainhower KB, Conley CC, Chen OS,
944 Guthrie MR, Soltero D, Qiao Y, Huang X, Tarapcsak S, Devarakonda S, Chalishazar MD, Gertz J, Moser JC,
945 Marth G, Puri S, Witt BL, Spike BT, Oliver TG. MYC Drives Temporal Evolution of Small Cell Lung
946 Cancer Subtypes by Reprogramming Neuroendocrine Fate. *Cancer Cell.* 2020. Epub 2020/06/01. doi:
947 10.1016/j.ccell.2020.05.001. PubMed PMID: 32473656.
948 56. Huang YH, Klingbeil O, He XY, Wu XS, Arun G, Lu B, Somerville TDD, Milazzo JP, Wilkinson JE,
949 Demerdash OE, Spector DL, Egeblad M, Shi J, Vakoc CR. POU2F3 is a master regulator of a tuft cell-like
950 variant of small cell lung cancer. *Genes Dev.* 2018;32(13-14):915-28. Epub 2018/06/28. doi:
951 10.1101/gad.314815.118. PubMed PMID: 29945888; PMCID: PMC6075037.
952 57. Park KS, Liang MC, Raiser DM, Zamponi R, Roach RR, Curtis SJ, Walton Z, Schaffer BE, Roake CM,
953 Zmoos AF, Kriegel C, Wong KK, Sage J, Kim CF. Characterization of the cell of origin for small cell lung
954 cancer. *Cell Cycle.* 2011;10(16):2806-15. Epub 2011/08/09. doi: 10.4161/cc.10.16.17012. PubMed
955 PMID: 21822053; PMCID: PMC3219544.
956 58. Semenova EA, Nagel R, Berns A. Origins, genetic landscape, and emerging therapies of small cell
957 lung cancer. *Genes Dev.* 2015;29(14):1447-62. Epub 2015/07/30. doi: 10.1101/gad.263145.115.
958 PubMed PMID: 26220992; PMCID: PMC4526731.
959 59. Yang D, Denny SK, Greenside PG, Chaikovskiy AC, Brady JJ, Ouadah Y, Granja JM, Jahchan NS, Lim
960 JS, Kwok S, Kong CS, Berghoff AS, Schmitt A, Reinhardt HC, Park KS, Preusser M, Kundaje A, Greenleaf
961 WJ, Sage J, Winslow MM. Intertumoral Heterogeneity in SCLC Is Influenced by the Cell Type of Origin.
962 *Cancer Discov.* 2018;8(10):1316-31. Epub 2018/09/20. doi: 10.1158/2159-8290.CD-17-0987.
963 PubMed PMID: 30228179; PMCID: PMC6195211.
964 60. Gazdar AF, Savage TK, Johnson JE, Berns A, Sage J, Linnoila RI, MacPherson D, McFadden DG,
965 Farago A, Jacks T, Travis WD, Brambilla E. The comparative pathology of genetically engineered mouse
966 models for neuroendocrine carcinomas of the lung. *J Thorac Oncol.* 2015;10(4):553-64. Epub
967 2015/02/13. doi: 10.1097/JTO.0000000000000459. PubMed PMID: 25675280; PMCID: PMC4523224.
968 61. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S.
969 Innate or adaptive immunity? The example of natural killer cells. *Science.* 2011;331(6013):44-9. Epub
970 2011/01/08. doi: 10.1126/science.1198687. PubMed PMID: 21212348; PMCID: PMC3089969.
971 62. Jiang L, Huang J, Higgs BW, Hu Z, Xiao Z, Yao X, Conley S, Zhong H, Liu Z, Brohawn P, Shen D, Wu
972 S, Ge X, Jiang Y, Zhao Y, Lou Y, Morehouse C, Zhu W, Sebastian Y, Czapiga M, Oganessian V, Fu H, Niu Y,
973 Zhang W, Streicher K, Tice D, Zhao H, Zhu M, Xu L, Herbst R, Su X, Gu Y, Li S, Huang L, Gu J, Han B, Jallal
974 B, Shen H, Yao Y. Genomic Landscape Survey Identifies SRSF1 as a Key Oncodriver in Small Cell Lung
975 Cancer. *PLoS Genet.* 2016;12(4):e1005895. Epub 2016/04/20. doi: 10.1371/journal.pgen.1005895.
976 PubMed PMID: 27093186; PMCID: PMC4836692.
977 63. Cai L, Lin S, Girard L, Zhou Y, Yang L, Ci B, Zhou Q, Luo D, Yao B, Tang H, Allen J, Huffman K,
978 Gazdar A, Heymach J, Wistuba I, Xiao G, Minna J, Xie Y. LCE: an open web portal to explore gene
979 expression and clinical associations in lung cancer. *Oncogene.* 2019;38(14):2551-64. Epub
980 2018/12/12. doi: 10.1038/s41388-018-0588-2. PubMed PMID: 30532070; PMCID: PMC6477796.
981 64. Rousseaux S, Debernardi A, Jacquiau B, Vitte AL, Vesin A, Nagy-Mignotte H, Moro-Sibilot D,
982 Bricchon PY, Lantuejoul S, Hainaut P, Laffaire J, de Reynies A, Beer DG, Timsit JF, Brambilla C, Brambilla
983 E, Khochbin S. Ectopic activation of germline and placental genes identifies aggressive metastasis-
984 prone lung cancers. *Sci Transl Med.* 2013;5(186):186ra66. Epub 2013/05/24. doi:
985 10.1126/scitranslmed.3005723. PubMed PMID: 23698379; PMCID: PMC4818008.
986 65. Ruscetti M, Leibold J, Bott MJ, Fennell M, Kulick A, Salgado NR, Chen CC, Ho YJ, Sanchez-Rivera
987 FJ, Feucht J, Baslan T, Tian S, Chen HA, Romesser PB, Poirier JT, Rudin CM, de Stanchina E, Machado E,
988 Sherr CJ, Lowe SW. NK cell-mediated cytotoxicity contributes to tumor control by a cytostatic drug

989 combination. *Science*. 2018;362(6421):1416-22. Epub 2018/12/24. doi: 10.1126/science.aas9090.
990 PubMed PMID: 30573629; PMCID: PMC6711172.

991 66. Dang J, Tiwari SK, Lichinchi G, Qin Y, Patil VS, Eroshkin AM, Rana TM. Zika Virus Depletes
992 Neural Progenitors in Human Cerebral Organoids through Activation of the Innate Immune Receptor
993 TLR3. *Cell Stem Cell*. 2016;19(2):258-65. Epub 2016/05/11. doi: 10.1016/j.stem.2016.04.014.
994 PubMed PMID: 27162029; PMCID: PMC5116380.

995 67. Eden E, Navon R, Steinfeld I, Lipson D, Yakhini Z. GOrilla: a tool for discovery and visualization
996 of enriched GO terms in ranked gene lists. *BMC Bioinformatics*. 2009;10:48. Epub 2009/02/05. doi:
997 10.1186/1471-2105-10-48. PubMed PMID: 19192299; PMCID: PMC2644678.

998 68. The Gene Ontology C. The Gene Ontology Resource: 20 years and still GOing strong. *Nucleic
999 Acids Res*. 2019;47(D1):D330-D8. Epub 2018/11/06. doi: 10.1093/nar/gky1055. PubMed PMID:
000 30395331; PMCID: PMC6323945.

001 69. Supek F, Bosnjak M, Skunca N, Smuc T. REVIGO summarizes and visualizes long lists of gene
002 ontology terms. *PLoS One*. 2011;6(7):e21800. Epub 2011/07/27. doi: 10.1371/journal.pone.0021800.
003 PubMed PMID: 21789182; PMCID: PMC3138752.

004 70. Sergushichev A. An algorithm for fast preranked gene set enrichment analysis using cumulative
005 statistic calculation. *bioRxiv*. 2016.

006 71. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in
007 multidimensional genomic data. *Bioinformatics*. 2016;32(18):2847-9. Epub 2016/05/22. doi:
008 10.1093/bioinformatics/btw313. PubMed PMID: 27207943.

009 72. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York; 2016.

010 73. Wilke CO. *ggridges: Ridgeline Plots in 'ggplot2'*. 2020.

011 74. Slowikowski K. *ggrepel: Automatically Position Non-Overlapping Text Labels with
012 'ggplot2'*. 2019.

013 75. Kassambara A. *ggpubr: 'ggplot2' Based Publication Ready Plots*. 2020.

014 76. Tennekes M. *treemap: Treemap Visualization*. 2017.

015 77. Neuwirth E. *RColorBrewer: ColorBrewer Palettes*. 2014.

016 78. Huling J. *jcolors: Colors Palettes for R and 'ggplot2', Additional Themes for
017 'ggplot2'*. 2020.

018 79. Pedersen TL. *patchwork: The Composer of Plots*. 2019.

019 80. Davis S, Meltzer PS. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and
020 BioConductor. *Bioinformatics*. 2007;23(14):1846-7. Epub 2007/05/15. doi:
021 10.1093/bioinformatics/btm254. PubMed PMID: 17496320.

022 81. Bolstad B. *preprocessCore: A collection of pre-processing functions*. 2019.

023 82. Moon KR, van Dijk D, Wang Z, Gigante S, Burkhardt DB, Chen WS, Yim K, Elzen AVD, Hirn MJ,
024 Coifman RR, Ivanova NB, Wolf G, Krishnaswamy S. Visualizing structure and transitions in high-
025 dimensional biological data. *Nat Biotechnol*. 2019;37(12):1482-92. Epub 2019/12/05. doi:
026 10.1038/s41587-019-0336-3. PubMed PMID: 31796933; PMCID: PMC7073148.

027 83. Cilloniz C, Pantin-Jackwood MJ, Ni C, Carter VS, Korth MJ, Swayne DE, Tumpey TM, Katze MG.
028 Molecular signatures associated with Mx1-mediated resistance to highly pathogenic influenza virus
029 infection: mechanisms of survival. *J Virol*. 2012;86(5):2437-46. Epub 2011/12/23. doi:
030 10.1128/JVI.06156-11. PubMed PMID: 22190720; PMCID: PMC3302269.

031 84. Rusinova I, Forster S, Yu S, Kannan A, Masse M, Cumming H, Chapman R, Hertzog PJ.
032 Interferome v2.0: an updated database of annotated interferon-regulated genes. *Nucleic Acids Res*.
033 2013;41(Database issue):D1040-6. Epub 2012/12/04. doi: 10.1093/nar/gks1215. PubMed PMID:
034 23203888; PMCID: PMC3531205.

035 85. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular
036 Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst*. 2015;1(6):417-25. Epub
037 2016/01/16. doi: 10.1016/j.cels.2015.12.004. PubMed PMID: 26771021; PMCID: PMC4707969.

038 86. Eppig JT. Mouse Genome Informatics (MGI) Resource: Genetic, Genomic, and Biological
039 Knowledgebase for the Laboratory Mouse. *ILAR J.* 2017;58(1):17-41. Epub 2017/08/26. doi:
040 10.1093/ilar/ilx013. PubMed PMID: 28838066; PMCID: PMC5886341.

041 87. Hanzelmann S, Castelo R, Guinney J. GSEA: gene set variation analysis for microarray and RNA-
042 seq data. *BMC Bioinformatics.* 2013;14:7. Epub 2013/01/18. doi: 10.1186/1471-2105-14-7. PubMed
043 PMID: 23323831; PMCID: PMC3618321.

044 88. Parra ER, Behrens C, Rodriguez-Canales J, Lin H, Mino B, Blando J, Zhang J, Gibbons DL,
045 Heymach JV, Sepesi B, Swisher SG, Weissferdt A, Kalhor N, Izzo J, Kadara H, Moran C, Lee JJ, Wistuba, II.
046 Image Analysis-based Assessment of PD-L1 and Tumor-Associated Immune Cells Density Supports
047 Distinct Intratumoral Microenvironment Groups in Non-small Cell Lung Carcinoma Patients. *Clin*
048 *Cancer Res.* 2016;22(24):6278-89. Epub 2016/06/03. doi: 10.1158/1078-0432.CCR-15-2443. PubMed
049 PMID: 27252415; PMCID: PMC5558040.

050 89. Fujimoto J, Kadara H, Garcia MM, Kabbout M, Behrens C, Liu DD, Lee JJ, Solis LM, Kim ES, Kalhor
051 N, Moran C, Sharafkhaneh A, Lotan R, Wistuba, II. G-protein coupled receptor family C, group 5,
052 member A (GPRC5A) expression is decreased in the adjacent field and normal bronchial epithelia of
053 patients with chronic obstructive pulmonary disease and non-small-cell lung cancer. *J Thorac Oncol.*
054 2012;7(12):1747-54. Epub 2012/11/17. doi: 10.1097/JTO.0b013e31826bb1ff. PubMed PMID:
055 23154545; PMCID: PMC3622592.

056 90. Tang C, Hobbs B, Amer A, Li X, Behrens C, Canales JR, Cuentas EP, Villalobos P, Fried D, Chang JY,
057 Hong DS, Welsh JW, Sepesi B, Court L, Wistuba, II, Koay EJ. Development of an Immune-Pathology
058 Informed Radiomics Model for Non-Small Cell Lung Cancer. *Sci Rep.* 2018;8(1):1922. Epub
059 2018/02/02. doi: 10.1038/s41598-018-20471-5. PubMed PMID: 29386574; PMCID: PMC5792427.

060 91. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the
061 probe level. *Bioinformatics.* 2004;20(3):307-15. Epub 2004/02/13. doi:
062 10.1093/bioinformatics/btg405. PubMed PMID: 14960456.

063 92. Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H,
064 Watson SJ, Meng F. Evolving gene/transcript definitions significantly alter the interpretation of
065 GeneChip data. *Nucleic Acids Res.* 2005;33(20):e175. Epub 2005/11/15. doi: 10.1093/nar/gni179.
066 PubMed PMID: 16284200; PMCID: PMC1283542.

067 93. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR.
068 STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2013;29(1):15-21. Epub 2012/10/30. doi:
069 10.1093/bioinformatics/bts635. PubMed PMID: 23104886; PMCID: PMC3530905.

070 94. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ,
071 Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and
072 isoform switching during cell differentiation. *Nat Biotechnol.* 2010;28(5):511-5. Epub 2010/05/04.
073 doi: 10.1038/nbt.1621. PubMed PMID: 20436464; PMCID: PMC3146043.

074 95. Bullard JH, Purdom E, Hansen KD, Dudoit S. Evaluation of statistical methods for normalization
075 and differential expression in mRNA-Seq experiments. *BMC Bioinformatics.* 2010;11:94. Epub
076 2010/02/20. doi: 10.1186/1471-2105-11-94. PubMed PMID: 20167110; PMCID: PMC2838869.

077 96. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-Response Analysis Using R. *PLoS One.*
078 2015;10(12):e0146021. Epub 2015/12/31. doi: 10.1371/journal.pone.0146021. PubMed PMID:
079 26717316; PMCID: PMC4696819.

080 97. McMillan EA, Ryu MJ, Diep CH, Mendiratta S, Clemenceau JR, Vaden RM, Kim JH, Motoyaji T,
081 Covington KR, Peyton M, Huffman K, Wu X, Girard L, Sung Y, Chen PH, Mallipeddi PL, Lee JY, Hanson J,
082 Voruganti S, Yu Y, Park S, Sudderth J, DeSevo C, Muzny DM, Doddapaneni H, Gazdar A, Gibbs RA, Hwang
083 TH, Heymach JV, Wistuba I, Coombes KR, Williams NS, Wheeler DA, MacMillan JB, Deberardinis RJ,
084 Roth MG, Posner BA, Minna JD, Kim HS, White MA. Chemistry-First Approach for Nomination of
085 Personalized Treatment in Lung Cancer. *Cell.* 2018;173(4):864-78 e29. Epub 2018/04/24. doi:
086 10.1016/j.cell.2018.03.028. PubMed PMID: 29681454; PMCID: PMC5935540.

087