Inherited defects in natural killer cells shape tumor immune microenvironment, clinical outcome and immunotherapy response

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Abstract

Tumor immune microenvironment (TIME) plays an important role in metastasis and immunotherapy. However, it has been not much known how to classify TIMEs and how TIMEs are genetically regulated. Here we showed that tumors were classified into TIME-rich, -intermediate and -poor subtypes which had significant differences in clinical outcomes, abundances of tumor-infiltrating lymphocytes (TILs), the degree of key immune programs' activation, and immunotherapy response across 13 common cancer types (n= ~6,000). Furthermore, TIME-intermediate/-poor patients had significantly more inherited genetic defects (i.e., functional germline variants) in natural killer (NK) cells, antigen processing and presentation (APP) and Wnt signaling pathways than TIME-rich patients, and so did cancer patients than non-cancer individuals (n=4,500). These results suggested that individuals who had more inherited defects in NK cells, APP and Wnt pathways had a higher risk of developing cancers. Moreover, in the 13 common cancers the number of inheritably defected genes of NK cells was significantly negative-correlated with patients' survival, TILs' abundance in TIMEs and immunotherapy response, suggesting that inherited defects in NK cells alone were sufficient to shape TILs' recruitment, clinical outcome, and immunotherapy response, highlighting that NK cell activation was required in the 13 cancer types to drive the recruitment of immune troops into TIMEs. Thus, we proposed that cancer was a disease of NK cell inherited deficiencies. These results had implications in identifying of high-risk individuals based on germline genomes, implementing precision cancer prevention by adoptive transfer of healthy NK cells, and improving existing immunotherapies by combining of adoptive NK cell transfer (i.e., converting TIME-intermediate/-poor tumors into TIME-rich tumors) and anti-PD-1 or CAR-T therapy.

50 **Introduction**

51 In the past two decades, classification of tumors based on omic data has resulted in distinct tumor
52 molecular subtypes for each cancer type and then provided a framework to study the molecular
53 mechanisms such as oncogenic pathways and discover drug targets for tumors. Moreover, tumor
54 molecular subtypes enable to inform clinical outcomes and treatments. For example, treatment options
55 can be made based on either Her2+, luminal or basal subtypes of breast cancer ([1, 2, 3, 4]). However, the
56 enormous complexity of cancer subtypes, tumor microenvironment, subclones, and somatic genomic
57 alteration landscapes has been reported. For example, tumor molecular subtypes and patient
58 stratifications based on tumor gene expression profiles showed that each cancer type has its own
59 subtypes which were often unique and not commonly shared between any 2 cancer types. Each subtype
60 has its own oncogenic pathways and somatic mutating drivers.

61 Tumor microenvironments often interact with tumor cells to affect metastasis and clinical outcomes. In
62 the past few years, immune-checkpoint therapy (ICT) has been able to successfully eliminate tumors
63 in 10-40% of patients with melanoma and other cancer types, however, in the majority of patients,
64 ICT failed to have their intended effect ([5, 6]). It has been believed that understanding of tumor
65 infiltrated immune cells (TILs) in tumor immune microenvironment (TIME) could help in getting
66 insights into ICT response, resistance and might improve existing immunotherapies ([7, 8]). Infiltrating
67 T cells are a critical component for ICT response. However, recently it has been shown that NK
68 (natural killer) cell activation is required in melanoma to recruit CD103+ DC (dendritic cell) and then
69 CD8+ effector T cells. Except for the TIL-T cell recruitment function, the best-known role of NK cells
70 is cancer cell killing and tumor immunosurveillance. To fulfill this function, distinct from T and B
71 cells, NK cell is not mediated by antigen specificity but through multiple germline-encoded activating
72 and inhibiting receptors ([9, 10, 11]), the complex balance of inhibitory and activating signals promotes
73 self-tolerance or drives potent effector function of NK cells.

74 Historically, tumor molecular subtypes have generated lots of insights into the underlying molecular
75 mechanisms of tumorigenesis, metastasis and informing of treatments. With advances of cancer ICT,
76 CAR-T and other immunotherapies, it has a strong interest in stratifying TIMEs into TIME subtypes.
77 Further stratification of patients on the basis of their TIMEs could discover better insight into overall
78 survival and underlying molecular mechanisms for ICT response and help identify new
79 immunotherapeutic targets. However, so far, we have no idea how to study TIMEs and lack of efficient
80 tools to classify TIMEs. Here we conducted an analysis of the omic data (i.e., tumor RNA-seq and

81 whole-exome sequences of germline genomes) of TCGA (The Cancer Genome Atlas) cancer patients
82 (n=~6,000) representing 13 common cancer types, and a non-cancer cohort (n=4,500) to show that
83 tumors were classified into three universally distinct TIME subtypes across the 13 common cancer
84 types. They were different in prognosis, TILs' abundance and degree of immune programs' activation,
85 regardless of cancer type. Inherited defects of NK cells, antigen processing and presentation (APP) and
86 Wnt pathways in patients' germline genomes modulated TIME subtypes and ICT response.
87 Importantly, we showed that inherited defects in NK cells alone were sufficient to regulate TILs'
88 abundance in TIMEs, clinical outcomes and immunotherapy response. Further, individuals who have
89 inherited defects in NK cells, APP and Wnt pathways bear high-risk of developing cancers.

90 **Results**

91 **Three universal TIME subtypes across 13 common cancers**

92 To classify tumors into TIME subtypes, we applied the unsupervised clustering of gene expression
93 data (i.e., melanoma data from TCGA) based on a set of genome-wide CRISPR–Cas9 screen-
94 determined essential genes (i.e., ICT essential genes, n=1,294) from a previous study ([12]) and other
95 known tumor immune-related genes ([13, 14]) (see Methods). We found that melanoma tumors were
96 classified into three TIME subtypes (Fig 1a). Similarly, the three TIME subtypes were repeatedly
97 obtained in the 13 common cancer types (Supplementary Fig 1): bladder urothelial carcinoma (BLCA),
98 breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical
99 adenocarcinoma (CESC), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma
100 (HNSC), kidney renal clear cell carcinoma (KIRC), lower grade glioma (LGG), lung adenocarcinoma
101 (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PRAD), skin cutaneous
102 Melanoma (SKCM), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA) and uterine corpus
103 endometrial carcinoma (UCEC). These results suggested that TIME subtypes were much simpler than
104 tumor molecular subtypes, each of which was unique and couldn't be shared by any two cancer types.
105 Thus, the universal TIME subtypes provided a means to identify subtypes' unifying features and to
106 understand the underlying common molecular mechanisms of each TIME subtype.
107
108 To discover the common features and critical differences which defined the 3 distinct TIME subtypes,
109 we compared their gene expression profiles, TILs' abundance in TIMEs and clinical outcomes. The
110 abundance of TILs in TIMEs were significantly decreased from TIME-rich ('immune-hot' tumors), -
111 intermediate ('immune-cold' tumors) to TIME-poor subtype ('immune-desert' tumors) (Fig 1b,
112 Supplementary Table 1). It has been known that TILs are associated with tumor prognosis, thus we

113 hypothesized that clinical outcomes could be different between TIME subtypes. In fact, both TIME-
114 poor and -intermediate tumors had significantly poorer clinical outcomes than TIME-rich tumors (Fig
115 1c), however, patient survival differences were not significant between TIME-poor and -intermediate
116 tumors. Pathway enrichment analysis of the significantly modulated genes between subtypes showed
117 that the degree of the activated (i.e., represented by gene expression of each pathway or program)
118 immune programs such as APP, NK cell-mediated cytotoxicity, T cell receptor singling pathways were
119 significantly decreased from TIME-rich, -intermediate to -poor subtype (Fig 1d and Supplementary Fig
120 2). These results suggested that TIME-poor and -intermediate tumors had a lower degree of activated
121 immune programs so that they could have a better chance to escape immunosurveillance. For example,
122 APP is an immunological process that prepares and presents antigens to T cells. NK effector and T cell
123 receptor signaling pathways function as tumor cell killing. Therefore, lower activity of APP, NK cells
124 and T cell receptor singling allow tumor cells to have fewer immune constraints. These results were
125 repeatedly observed across the 13 common cancer types, suggesting that the TIME subtypes are not
126 only universal subtypes across cancer types but also share common cellular and clinical features.
127
128 In general, TIME-rich, -intermediate and -poor tumors represent 25.35%, 32.94%, and 41.70% of the
129 tumors, respectively, depending on cancer types (Supplementary Table 2). These results indicated that
130 TIME-rich patients represent only a small fraction of tumors, and more than 70% of the tumors are
131 either TIME-poor or -intermediate tumors, most of which are known to non-respond to ICT. These
132 results explain why only 10-40% of tumors (i.e., depending on the cancer type) respond to ICT.

133 **Inherited defects in NK cells shape TIME subtypes and clinical outcomes**

134 The discovery of the universal TIME subtypes across the 13 common cancer types triggered us to
135 hypothesize that the TIME subtypes could be modulated by common genetic regulators. We previously
136 proposed that pre-existing inherited variants in germline genomes of cancer patients could play an
137 important role in shaping somatic mutations, CNVs and oncogenic pathways in their paired tumor
138 genomes ([15]). Furthermore, we have shown that inheritably functional variants of breast cancer patients
139 significantly predicted tumor recurrence ([16, 17]), and the risk for breast, brain and other cancers ([18]).
140 Moreover, inheritably functional variants of patients could be used to predict key somatic-mutated
141 genes in their paired tumor genomes (Zaman et al., unpublished data). Finally, cancer patients'
142 germline genomes contain specific genomic patterns which are associated with cancer risk and clinical
143 outcomes (Feng et al., unpublished data). These results raised a question whether cancer patients'
144 germline genomes modulated TIME subtypes.

145 To answer this question, we compared the functional germline variants (termed as inherited defects
146 here, see Methods) of the patients between TIME subtypes and showed that genetic makeups of the
147 patients were significantly different between them (Supplementary Fig 3): TIME-poor/-intermediate
148 and TIME-rich patients had significantly differentially inherited genetic defects. Further pathway
149 enrichment analysis showed that more than 30% of the defected or modulated pathways were
150 associated with NK cell deficient phenotypes, NK cell-associated virus infections, and the NK cell-
151 mediated cytotoxicity pathway (Fig 2a, Supplementary Fig 4). For example, among the significantly
152 differential pathways and phenotypes, 7 of them were known NKD (NK cell deficiency) phenotypes
153 ([19, 20, 21]) such as Epstein-Barr virus (EBV) infection, Herpes simplex infection, Leishmaniasis,
154 Rheumatoid arthritis, Type I diabetes mellitus, long-term depression and viral myocarditis, while 7 of
155 them were NK cell-related virus infections such as Graft-versus-host disease, HTLV-I infection,
156 Hepatitis B and C, Influenza A, Asthma and IBD (Fig 1d, 2a, and 2b). These results strongly suggested
157 that TIME-poor/-intermediate patients might have more inherited defects in their NK cells than TIME-
158 rich patients.
159
160 NK cells are the 'first-line immune cells' which enable to quickly detect and attack tumor cells and
161 viruses. NK cells are able to control tumorigenesis by accurately regulating the distinct germline
162 encoded inhibitory and activating NK cell surface receptors. In general, an excess of activating over
163 inhibitory signals triggers the production and release of effector molecules, which can lead to the death
164 of the infected or transformed cancer cell ([22]). Thus, to further explore the association between NK cell
165 inherited defects with cancer progression and metastasis, we compiled a comprehensive set of NK cell
166 genes including NK cell receptors and ligands, genes in NK cell signaling pathways (i.e., KEGG NK
167 cell-mediated cytotoxicity pathway ([23, 24, 25]), and then conducted Fisher's tests to identify genes which
168 had significantly differential frequencies between TIME-rich group and TIME-poor/-intermediate
169 group. For a given cancer type, we found 15-20 defected NK cell genes which had significantly higher
170 frequencies in TIME-poor/-intermediate group than TIME-rich group (FDR-corrected $p < 0.25$). The
171 gene list for each cancer type was different, which could be associated with the diversity of tissue-
172 resident NK cells, although many of the significantly defected NK cell genes of the 13 cancer types
173 were shared. In total there were 103 such genes (i.e., termed as NK-genes here), 70 of which appeared
174 in at least two cancer types. Among the 70 NK-genes, 37.2%, 25.8%, 24.3%, and 12.7% are known
175 NKD genes ([19, 20, 21]), NK cell receptors and ligands, NK cell singling genes but also expressed in cell
176 signaling in T or other immune cells (i.e., mainly innate immune cells, termed as 'global immune cell

177 genes' here) and universal genes (i.e., expressed in many cell types), respectively (Supplementary
178 Tables 3 and 4).
179
180 We further asked if inherited defected NK-genes were correlated with clinical outcomes. To answer
181 this question, for each cancer type, we top-to-bottom ranked the patients based on the number of
182 inheritably defected NK-genes. We defined top 40% and bottom 40% of the ranked patients as high-
183 and low-NK cell defected groups, respectively, to conduct survival analysis. In 10 of the 12 cancer
184 types (i.e., no survival data were available for THCA) except LGG and KIRC, the high-NK cell
185 defected group had significantly shorter survival than the low-NK cell defected group (Fig 3a).
186 Similar results were obtained when using top 30% and bottom 30% of the ranked patients as high-
187 and low-NK cell defected groups, respectively. These results suggested that NK cells inherited
188 defects could play an important role in tumor recurrence and overall survival in most of the cancer
189 types. Given the fact that TILs' abundance was different between TIMEs, we hypothesized that NK
190 cell defected gene number could be negatively correlated with the TILs' abundance in TIMEs.
191 Indeed, it was true that for all the 13 cancer types (Fig 3b, Supplementary Fig 5), the abundance of
192 immune cell troops such as NK, NKT, CD103+ DC (cDC), activated CD8+ T, CD4+ T, activated B, all
193 kinds of T cells and 23 other types of immune cells were negatively correlated with the defected gene
194 number in NK cells, suggesting that NK cells could be an important factor in recruiting other immune
195 cells into TIMEs.
196
197 NKD genes and NK cell receptors/ligands are more preferentially involved in either NK cell-mediated
198 cytotoxicity, NK cell development or proliferation ([11, 26]), while the global immune cell genes could
199 influence the immune response of not only NK cells but also other immune cells. On the other hand,
200 the universal genes play important roles in cell signaling of not only NK cells but also other cell types
201 including tumor cells. Therefore, NKD genes and NK receptors/ligands are more NK cell-specific (i.e.,
202 representing 63% of the NK-genes, termed as NK-specific-genes here), while the global immune cell
203 genes (i.e., representing 24.3% of the NK-genes) and the universal genes (i.e., representing 12.7% of
204 the NK-genes) are expressed in multiple immune cells and non-immune cell types, respectively. Thus,
205 we raised a question whether the correlations observed in Fig 3a, 3b and Supplementary Fig 5 were
206 truly from NK cell defected genes. By extending the same analyses in Fig 3a and 3b using only the
207 universal genes, there were not any correlation as shown in Fig 3a and 3b, suggesting that inherited
208 defects in the universal genes alone are insufficient to modulate either TILs in TIMEs or clinical
209 outcomes. Next, we re-did the same analyses by combining of the universal genes and the global

210 immune cell genes (i.e., representing 37% of the NK-genes, termed as NK-immune-universal-genes
211 here) and the NK-specific-genes, respectively. For the NK-immune-universal-genes, correlations, as
212 shown in Fig 3a (i.e., survival), were not observed, on the other hand, for the NK-specific-genes, such
213 correlations were reproduced across the 10 cancer types. These results suggested that the NK-specific-
214 genes are sufficient to shape clinical outcomes, but the NK-immune-universal-genes were not.
215
216 Similarly, significantly negative correlations between TILs' abundance in TIMEs and the number of
217 the inheritably defected NK-specific-genes were reproduced in 10 cancer types including BLCA,
218 BRCA, KIRC, LAUD, LUSC, THCA, UCEC, HNSC, LGG, and SKCM (Fig 3b, Supplementary Fig
219 5). In particular, the correlations derived from the NK-specific-genes were much stronger than those
220 derived from the NK-genes in 7 cancer types (i.e., BLCA, BRCA, KIRC, LAUD, LUSC, THCA and
221 UCEC, Fig 3b, Supplementary Fig 5). On the other hand, weaker negative correlations (i.e., correlation
222 co-efficiencies were 2-10 times less than those derived from the NK-genes) and weaker statistical
223 significances (i.e., p values in the range of 0.01-0.05) were observed for some immune cells in some
224 cancer types when using the NK-immune-universal-genes (Fig 3b, Supplementary Fig 5). These results
225 suggested that inherited defects of the NK-specific-genes are sufficient to impair communications
226 between NK cells and other immune cells and then block TILs' recruitment into TIMEs. Of note, in
227 COAD, STAD, and PRAD, both NK-specific-genes and NK-immune-universal-genes did not
228 reproduce the negative correlation (i.e., for TILs in TIMEs) derived from the NK-genes. PRAD has
229 dense stroma ([27]) which could need the whole NK gene set (i.e., NK-genes) for TILs' recruitment. NK
230 cells surrounding both COAD and STAD are directly interacting with environmental factors such as
231 microbiome, food, drinks, and others so that NK cells associated with both COAD (colon cancer) and
232 STAD (gastric cancer) are much more complex. These data highlighted that inherited defected in NK
233 cells are one of the key genetic factors in shaping TIME subtypes, TILs' abundances and clinical
234 outcomes.
235
236 Based on these insights, we hypothesized that NK cell was a critical factor for recruiting immune
237 troops into TIMEs. This hypothesis is partially supported by two recent studies which had no idea
238 about NK cell inherited defects ([27, 28]), but showing that depletion of NK cells resulted in failed
239 recruitment of CD8+ T cells to the tumor microenvironment in melanoma mice. They further showed
240 that NK cells recruited CD103+ DC, which in turn were required for the recruitment of effector T
241 cells. Our results here suggested that NK cells could be able to recruit not only CD103+ DC (cDC) and
242 CD8+ T cells but also 30 other immune cells. Because NK cells act as first responders to detect and

243 kill cancer cells, it is reasonable to believe that NK cells could sequentially recruit many other
244 immune cells including CD103+ DC and T cells into the tumor microenvironment. Thus, NK cells
245 could recruit a whole immune troop but not only one or two immune cell types into TIMEs.
246
247 This insight provides a potential opportunity that adoptive cell transfer of NK cells from healthy donors
248 could convert a TIME-poor tumor into a TIME-rich tumor. Adoptive NK cell was safe and does not
249 cause a graft-versus-host disease attack on the recipients. Thus, we hypothesized that adoptive NK cell
250 transfer of healthy donors (i.e., allogeneic) to a patient could have better clinical outcomes than the
251 adoptive transfer of NK cells from the patients themselves (i.e., allogeneic) because cancer patients'
252 own NK cells are defected and don't function very effectively. This hypothesis is supported by a recent
253 clinical trial showing that allogenic adoptive NK cell transfer was much better than autogenic NK cell
254 immunotherapy for breast cancer outcomes ([29]). TIME-rich tumors are more suitable to conduct
255 immunotherapy than TIME-poor tumors, while TIME-rich tumors can gain significantly more survival
256 benefit than TIME-poor tumors for neoadjuvant chemotherapy as well. For example, a meta-analysis of
257 13 studies showed that higher abundance of TILs in pre-treatment tumor biopsy was correlated with
258 better pathological complete response rates to neoadjuvant chemotherapy([30, 31, 32, 33, 34]). Therefore, it is
259 a great interest to improve patient outcomes by applying adoptive transfer of healthy, functional NK
260 cells to convert TIME-poor tumors into TIME-rich tumors which could be in turn treated with
261 chemotherapy or immunotherapy. A recent clinical trial partially supported this hypothesis: adoptive
262 transfer of NK cells in combination with chemotherapy in stage IV colon cancers significantly
263 prevented recurrence and prolonged survival than chemotherapy alone. Most importantly, they found
264 that poorly differentiated colon cancers were more susceptible to adoptive NK cell transfer than well-
265 differentiated ones ([35]). Poorly and well-differentiated cancers are shorter and longer survival patients,
266 respectively, which are corresponding to TIME-rich and -poor tumors, respectively. Therefore, these
267 results agree with our above hypothesis. Another study also supported this hypothesis: the combination
268 therapy of anti-PD-1 antibodies and activated autologous NK cells significantly delayed tumor
269 progression in glioma-bearing animals as compared to the monotherapy regimens of anti-PD-1 or
270 stimulated NK cells alone ($P<0.001$)([36]).
271
272 **Tumorigenesis and metastasis are not random events and cancer patients are highly selected**
273 As shown in Fig 2a and Supplementary Fig 4, except NK cell inherited defects, TIME-poor/-
274 intermediate patients had significantly more defected APP and Wnt pathway genes than TIME-rich
275 patients. APP is a biological process which presents antigens to T cells, functional defects in APP could

276 lead tumor cells to escape T cell surveillance. However, careful analyses showed that APP pathway
277 genetic defects were not significantly associated with clinical outcomes and TILs' abundance. These
278 results suggested that APP pathway defects alone were not sufficient to shape either clinical outcomes
279 or TIME subtypes. In addition, it has been shown that activated Wnt signaling pathway in tumors
280 excluded the recruitment of T cells into TIMEs ([37]). Furthermore, somatic mutations of the Wnt
281 pathway in tumors could activate the pathway to prevent T cells to be recruited into TIMEs ([38]). Here
282 we showed that in most of the cancer types the number of inherited defects in Wnt pathway in germline
283 genomes had a positive correlation with the gene expression of the Wnt pathway (i.e., pathway
284 activation) in their paired tumors, suggesting that a positive correlation between inherited defects in the
285 Wnt pathway and activation of the pathway, which is similar to the somatic mutations in genes of the
286 pathway.
287
288 The number of the inheritably defected genes in the Wnt pathway had weakly negative correlations
289 (i.e., with marginally significant p values in the range of 0.02-0.05) with clinical outcomes in 8 cancer
290 types (i.e., BLCA, BCRA, HNSC, LAUD, LUSC, PRAD, STAD and UCEC) and no such correlation
291 with other cancer types (Supplementary Fig 6). Similarly, Wnt pathway inheritably defected gene
292 number had negative correlations with the abundance of TILs in TIMEs (Supplementary Fig 7) in eight
293 cancer types (i.e., HNSC, KIRC, LGG, LUAD, LUSC, SKCM, STAD and THCA), however, the
294 correlations were weaker (i.e., in terms of correlation co-efficiencies and correlation significance
295 represented by p values) than those derived from NK cells. These results suggested that inheritably
296 defected in Wnt pathway could shape TILs' recruitment in some cancer types, and the influences were
297 much weaker than NK cells. Next, we asked if inheritably defects of the Wnt pathway and NK cells
298 worked in a synergy manner. To answer this question, we conducted the correlation analysis using the
299 genes combined from the NK cells and the Wnt pathway and showed that negative correlations
300 between the inheritably defected gene number and the TILs' abundance in TIMEs remained in eight
301 cancers but much weaker than the correlations derived from either NK cells or Wnt pathway alone.
302 These results suggested that both NK cells and Wnt pathway were parallel and complementary
303 modulators to shape TILs recruitment, implying that to reverse immune exclusion in TIMEs, Wnt
304 signaling inhibitors could be better than adoptive NK cell transfer in some tumors but not most of the
305 tumors.
306
307 Together with the discovery that TIME-rich tumors have significantly longer survival than TIME-
308 poor/-intermediate patients, we concluded that inherited defects in NK cells and Wnt pathways were

309 associated with metastasis and clinical outcomes. These results highlighted that metastasis is affected
310 by inherited defects and is not a random process. Similarly, significantly more inherited defects in NK
311 cells, APP and Wnt pathways were observed between non-cancer individuals and cancer patients in the
312 13 common cancer types (Supplementary Fig 8). They were even more significant between TIME-
313 poor/-intermediate patients and non-cancer individuals (Fig 2b and Supplementary Fig 9). These results
314 suggested that individuals who bear inherited defects in NK cells, APP and Wnt pathways have a
315 higher risk to get cancer. This hypothesis is indirectly supported by a study showing that individuals
316 with lower NK cytotoxic activity in peripheral blood had a higher incidence of cancer (n=3,500 with
317 11-year follow-up)[39]). Similarly, previous studies showed that impaired NK cell activity was found in
318 family members of patients with different types of cancer ([40, 41, 42]). Importantly, such inherited defects
319 in NK cells, APP and Wnt pathways were repeatedly observed between non-cancer and cancer
320 individuals (Fig 2b, Supplementary Fig 9), between TIME-rich and TIME-intermediate/-poor patients
321 (Fig 2a, Supplementary Fig 4, Fig 1d), suggesting that these individuals were highly selected for
322 tumorigenesis and metastasis. The immune programs (defects in NK cells, APP and Wnt pathways) are
323 carried from germline genomes to their paired tumors. In fact, the number of inherited defects in the
324 NK cells, APP and Wnt pathways was gradually increased from the non-cancer cohort, TIME-rich, -
325 intermediate to -poor subtypes, suggesting that inherited defects in the NK cells, APP and Wnt
326 pathways have a profound impact on cancer risk, TIME subtypes and clinical outcomes. These insights
327 provided a potential opportunity to identify high-risk cancer subpopulation based on the inherited
328 defects in NK cells, APP and Wnt pathways, further, adoptive NK cell transfer from healthy donors to
329 high-risk individuals could postpone or prevent cancer development. This hypothesis is partially
330 supported by a study showing that NK cell depletion in melanoma mice resulted in substantial
331 metastasis, but the adoptive transfer of NK cells protects NK cell-deficient mice from tumor
332 establishment ([43]).
333
334 In addition, as shown in Fig 2b, Type I diabetes and long-term depression phenotypes were linked to
335 some cancers, suggesting that they were genetic diseases as well. Diabetes and obesity have been
336 shown to be a risk factor for some cancers, for example, a meta-analysis of 121 cohorts including 20
337 million individuals and one million events confirmed that diabetes is a risk factor for all-site cancer
338 ([44]), while obesity can increase cancer incidences of 13 cancer types ([45]). A 24-year follow-up study
339 showed that depression increases the risk of cancer ([46]), Moreover, a meta-analysis of 16 studies
340 (n=163,000) showed that cancer patients with anxiety and depression had a greater risk of dying from
341 all types of cancer ([47]). Impairing of the NK cell function is one of the common factors behind these

342 links. For example, obesity has been known to impair NK cell function and then lead to an increased
343 risk for severe infections and several cancer types ([48, 49]), while chronic family stress is consistently
344 associated with decreases in NK cell cytotoxicity ([50]). These results indicated that NK function
345 impaired by either genetic defects or regulatory factors such as depression, obesity and diabetes could
346 increase cancer incidence.
347
348 **TIME subtypes could guide in immunotherapies**
349 As shown above, both NK cell-mediated cytotoxicity and Wnt signaling pathways became the
350 hallmarks which enabled to significantly distinguish TIME-rich and TIME-intermediate/-poor
351 subtypes. From the ICT clinical trials ([51,52]), we obtained 49 melanoma (SKCM, 10 responders) and 47
352 gastric (STAD, 12 responders) samples which had tumor RNA-Seq data before administrating of ICT.
353 We used the significantly modulated genes of both pathways between TIME subtypes to assign the
354 ICT-clinical trial samples into either TIME-rich or TIME-intermediate/-poor group based on the k-
355 nearest neighbor algorithm (KNN, see Methods). By doing so, 70% and 30% of the ICT-responding
356 melanoma were assigned into TIME-rich and TIME-intermediate/-poor group, respectively. In contrast,
357 56% and 44% of the ICT non-responding melanoma were assigned into TIME-rich and TIME-
358 intermediate/-poor group, respectively. Similarly, 58% and 42% of the ICT-responding gastric tumors
359 were assigned into TIME-rich and TIME-intermediate/-poor group, respectively. In contrast, 31% and
360 69% of the ICT non-responding gastric tumors were assigned into TIME-rich and TIME-intermediate/-
361 poor group, respectively. Not surprisingly, these results indicated that TIME-rich patients were
362 enriched with ICT responders. Pathway enrichment analysis of tumor gene expression profiles showed
363 that TIME-rich non-responders had significantly higher gene expression in Wnt pathway (FDR-
364 corrected $p=5.3x10^{-12}$ and $4.0x10^{-5}$ for melanoma and gastric tumors, respectively) than TIME-rich
365 non-responders, suggesting that using of Wnt inhibitors could improve ICT treatment for TIME-rich
366 non-responders. Similarly, genes of the NK cell-mediated cytotoxicity pathway (FDR-corrected
367 $p=2.5x10^{-3}$ and $2.7x10^{-3}$ for melanoma and gastric tumors, respectively) and APP (FDR-corrected
368 $p=7.1x10^{-4}$ for melanoma) were significantly lower expressed in TIME-intermediate/-poor non-
369 responders than TIME-intermediate/-poor responders, suggesting that adoptive NK cell transfer in
370 combination with CAR-T or ICT could improve the existing immunotherapies for TIME-intermediate/-
371 poor non-responders. However, it should be cautious about these conclusions due to the small number
372 of clinical trial samples (n=96) here, more samples are needed to further validate these discoveries in
373 the future.
374

375

376 **Discussion**

377 **A unified view of the tumor immune microenvironment**

378 Tumor molecular subtypes could inform not only prognosis but also treatment. Here we showed that
379 TIMEs can be classified into three universal subtypes across 13 common cancer types. Different from
380 previous observations of the complex tumor molecular subtypes, each of which often has its own
381 unique features, the universal TIME subtypes were commonly shared by multiple cancer types,
382 providing a framework to get better insight into the unifying features of each TIME subtype and the
383 differences between the distinct TIME subtypes, and then understand why some tumors respond to
384 immunotherapy and some don't. Furthermore, this framework allows exploring genetic factors to
385 regulate TIMEs and could help in identifying new druggable targets. Regardless of cancer types, TIME
386 subtypes have unifying features: (1) TIME-rich patients have significantly longer survival than other
387 TIME subtypes, because (2) the abundance of the TILs in TIME-rich, -intermediate and -poor tumors is
388 gradually decreased. These features recaptured the known immune-hot, -cold and -desert tumors
389 described previously based on immunohistochemistry ([53, 54]). It has been well-known that a lower
390 abundance of TILs in TIMEs is associated with more cancer recurrence and shorter survival ([55, 56]). (3)
391 signaling pathways associated with key immune programs such as APP, NK, and T cell signaling were
392 more highly activated in TIME-rich tumors than TIME-intermediate/-poor tumors. The degree of the
393 activated immune programs (i.e., represented by the expression levels of the pathway genes) was
394 gradually decreased from TIME-rich, -intermediate to -poor tumors. Lower level expression of the
395 genes in these immune-programs and pathways will allow tumors to escape from immune attack. (4)
396 finally, ICT-responders were more enriched in TIME-rich tumors.

397

398 **Cancer is an NK cell deficient disease**

399 The hypothesis of cancer immunosurveillance suggests that tumor cell transformation occurs
400 frequently, but is under constant control by the immune system. The immune system is able to identify
401 transformed cells that have escaped cell-intrinsic tumor-suppressor mechanisms and eliminate them
402 before they can establish malignancy ([57]). Thus, if the individuals are genetically immunodeficient,
403 they could have a markedly increased incidence of cancer. In general, NK cells have a large repertoire
404 of germline-encoded inhibitory and activating receptors to sense 'danger' in the cell surfaces. The
405 germline-encoded receptors which recognize ligands associated with viral infection or cancer cell
406 transformation ([10]). Genetic defects in NK-genes including NK cell receptors and NKDs could impair
407 NK cell functions. By conducting an unbiased scanning of germline genomes of cancer patients, we

408 provided a clearer view of the spectrum of malignancies associated with impaired NK cells and showed
409 that inherited defects affecting NK cell functions were sufficient to accomplish cancer
410 immunosurveillance of the immune system.
411
412 Among highly related pathways (i.e., NK cells, APP and Wnt pathways) examined, only NK-genes'
413 defects were correlated with both survival and the abundance of TILs in TIMEs. Most importantly, in
414 10 out of the 12 common cancers (i.e., no survival data were available for THCA), defects of the NK-
415 specific-genes (i.e., NKDs + NK cell receptors) alone were sufficient to establish these correlations
416 (i.e., survival and TILs' abundance). Traditionally, the hypothesis of cancer immunosurveillance
417 mainly focuses on T cell or other immune cells for their cell-killing function. Here we demonstrated
418 that to implement cancer immunosurveillance, NK cells could play a critical role in communicating
419 with many immune cells to recruit the whole immune troops into the cancer-transformed cell or
420 TIMEs. Specifically, our results suggested that NK cells could have two functions for controlling tumor
421 progression and metastasis: (1) cancer cell killing (2) recruiting immune troops into TIMEs. Here we
422 proposed a working model for NK cell-based immunosurveillance: NK cells are the first to arrive in the
423 tumor microenvironment and recruit CD103+ DC through the secretion of chemokines. TIL-DCs then
424 recruit effector T cells. Activated NK cells produce numerous cytokines, communicate with other 30
425 immune cells, and then recruiting the immune troops into the cancer cell or TIMEs. Anti-tumor
426 and anti-metastasis activity (i.e., survival differences between TIME subtypes) is thus the result of the
427 collaboration of distinct innate and adaptive immune cell types. Thus, inheritably defected NK-genes
428 could impair NK cell function and then block to recruit the immune troops to the cancer cell or into
429 TIMEs to conduct the anti-tumor activity. Thus, we proposed that cancer is largely a disease of NK cell
430 deficiencies. This working model unraveled the cellular and molecular determinants (i.e., NK cells) of
431 multiple immune cell recruitment to and retention in solid tumors' TIMEs. Thus, allogeneic adoptive
432 NK cell therapy (i.e., collecting healthy NK cells which have no inherited defects from donors and
433 infusing them into the patient) could convert immunotherapy-resistant, TIME-poor tumors into
434 ICT/CAR-T-sensitive, TIME-rich tumors. This NK cell therapy could be a universal approach which is
435 independent of cancer types. Given the fact that more than 70% of the cancer patients are immune-cold
436 and -desert subtypes, successfully validating of this hypothesis will be a crucial step toward improving
437 the existing immunotherapies.
438
439 In addition, our results suggested that inherited defects in NK cells, APP and Wnt pathways were
440 positively selected in tumorigenesis and metastasis. Therefore, cancer patients are highly selected, and

441 thus, it provides an explanation for the fact that only 12-20% of the heavy smokers develop lung cancer
442 in their lifetime, although 85% of the lung cancer patients are heavy smokers ([58, 59]). Cancer causally
443 environmental factors play an important role in tumorigenesis, however, without a susceptible germline
444 genome (i.e., genetic defects in NK cells, APP and Wnt pathways), they still cannot induce cancer. We
445 proposed that germline genome is the most important factor in determining if a person will get cancer
446 in one's lifetime, and cancer is the result of the interactions between high-risk germline genomes and
447 risk-factors (i.e., environmental factors and lifestyles).

448 Thus, the discoveries in this study could open a new window to explore NK cell biology and lead to
449 novel thinking about identifying high-risk individuals for early cancer detection, precision cancer
450 prevention, and immunotherapy. Strategies to harness and augment NK cells for cancer therapy are
451 relatively new and rapidly developing field and have not been used for cancer prevention yet. The
452 concept that cancer is an NK cell deficient disease could lead this field to new directions: (1)
453 identifying of high-risk population based on NK cell, APP and Wnt pathways' inherited defects, so that
454 early cancer detection or precision prevention (i.e., by avoiding exposure to smoking, UV lights and
455 other risk factors) could be implemented. (2) Preventing or postponing of cancer development for the
456 high-risk population by adoptive NK cell transfer. (3) Converting TIME-poor/-intermediate tumors into
457 TIME-rich tumors by manipulating NK cells, adoptive NK cell transfer or using Wnt signaling
458 inhibitors so that ICT or CAR-T therapy could be applicable. Finally, many open questions still
459 remained, for example, defected genes in NK cells were largely shared by different cancer types,
460 however, each cancer type has some unique NK cell gene defects. Given the fact that tissue/organ-
461 resident NK cells are different and diverse ([60]), additional studies will be needed to elucidate if defected
462 genes are dominantly expressed in tissue/organ-resident NK cells. If so, adoptive transfer of tissue-
463 resident NK cells could be considered to be more efficient in cancer prevention and TIL's recruitments.
464 NK cells share lots of expression programs with NKT cells, which is a subset of innate-like T cells.
465 Both NK cells and NKT cells are cytotoxic cells, which trigger innate immune responses, provide the
466 first level of defense against infected cells and tumor cells, produce cytokines and trigger immune
467 responses without a prior sensitization by the immune system. Along with this long, we suspected that
468 the inherited defects in NKT cells could also play similar roles which were discussed in this study,
469 although the cell number of the NK cells is nearly 200 times of the NKT cells.

470

471 **Methods**

472 **Datasets**

473 Based on the availability of the whole exome sequencing (WES) of germline gnomes, their paired
474 tumor genomes, and paired RNA-seq data, 14 common cancer types were selected from TCGA (n=200
475 at least for each cancer type). To remove the effects of virus-infection for the NK cell study, we
476 removed the virus-infected tumors. Only 15% of the liver tumors (i.e., this number is too small to
477 conduct analysis) without virus infection, thus, we excluded this cancer type for further analysis. Thus,
478 13 common cancers used in this study were BLCA, BRCA, COAD, LGG, HNSC, KIRC, LUAD,
479 LUSC, PRAD, SKCM, STAD, THCA, and UCEC. Because the primary melanoma samples were not
480 many, only metastatic SKCM samples were included for analysis. Distinct clinical subtypes have been
481 reported in breast cancer, thus we took only the ER+ breast tumors in this study because the sample
482 numbers of HER2+ and triple negatives are small in TCGA. The WES files derived from buffy coats
483 (normal samples) of these cancer patients (n=5,883) were downloaded from TCGA, and the normalized
484 RNA-sequencing data with Root Mean Square Error (RMSE) across 13 types of cancers (n=5,373)
485 were downloaded from FireBrowse. The WES files of 4,500 non-cancer individuals (phs000473.v2.p2,
486 phs000806.v1.p1, phs001194.v2.p2) were downloaded from The database of Genotypes and
487 Phenotypes (dbGaP). RNA-sequencing data of 49 ICT-clinical trial melanoma sample and 47 ICT-
488 clinical trial gastric samples were collected from GSE91061 and PRJEB25780, respectively, and were
489 processed based on the mRNA analysis pipeline in TCGA.

490

491 **Variant calling and germline variant determination**

492 For TCGA WES files, variant calling was performed using Varscan (version 2.3.9). The thresholds for
493 germline variant calls required variant allele fraction (VAF) between 45% and 55%, and >90%.
494 Functional variants were examined and annotated using the Combined Annotation Dependent
495 Depletion (CADD) using the default parameters. To keep the consistency with the TCGA pipeline for
496 processing WES data of the non-cancer individuals, BWA (version 0.7.15) was used to align with
497 default parameters, piping into Samtools (version 0.1.8) to sort. Additional adding read groups and
498 duplicate removal were processed with Picard-tools (version 2.6.0). The resulted BAM files were
499 processed with GATK (version 4.0.11.0) for realignment and base recalibration. Varscan (version
500 2.3.9) and CADD were used to call and annotate functional germline variants.

501

502

503

504 **Immune gene set and clustering analysis**

505 Immune-related genes (n=1,384) including MHC system-related genes [61], immunophenoscore-related
506 genes [14], ICT essential genes for immunotherapy [12] and cytotoxic T cell-resistant genes [62] were
507 collected and identified as critical immune-related genes (the gene pool G). RNA-sequencing data of
508 melanoma patients in TCGA were used to screen the key genes.

509

510 Step#1 Initialize the candidate set of key genes, that is, $G_{candidate} = \phi$

511 Step#2 Randomly select 30% genes fro G m the gene set G_{random}.

512 Step#3 Replace the features of elements in the patient set P with G_{random} to form the sample set S_{random}.

513 Step#4 Group the samples S_{random} by using the hierarchical clustering method. For each of the clustering,
514 clValid (63) was used to evaluate the clustering stability and the most stable clustering number was
515 recorded.

516 Step#5 Repeat Steps 2-4 100,000 times. Rank the most stable clustering numbers and select the most
517 suitable clustering number 3.

518 Step#6 Extract the genes when clustering number is 3, and rank the genes. Select the most informative
519 genes and record them as the final set of key-gene candidates (1,294 genes).

520 We used the 1,294 genes to conduct unsupervised clustering analyses of the RNA-seq data for each
521 cancer type to define TIME subtypes.

522

523 **TIL abundance calculation and pathway enrichment analysis**

524 A deconvolution approach (14) was used to extract the abundance of each immune cell based on their
525 gene markers from a tumor RNA-Seq data. Pathway enrichment analysis was conducted using DAVID
526 (https://david.ncifcrf.gov).

527

528 **Assigning ICT trial samples into TIME subtypes**

529 To assign an ICT-clinical trial sample into a TIME subtype, t-test statistics were first conducted in TIME-
530 rich vs TIME-intermediate and TIME-intermediate vs TIME-poor tumors' RNA-seq data for the genes
531 derived from the NK cell-mediated cytotoxicity pathway and the Wnt signaling pathway. The
532 significantly differential genes ($p<0.05$) were used for conduct K-nearest neighbors (KNN, k=5) to assign
533 the sample to a TIME subtype. Spearman's rank correlation was conducted between the sample and the

534 samples in each TIME subtype based on the differential genes. TCGA-SKCM and TCGA-STAD samples
535 were used for assigning the ICT-clinical trial samples of SKCM and STAD, respectively.
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770 **Figure Legends**
771
772 **Figure 1. Clinical and cellular characteristics of the TIME subtypes.** (a) Representative heatmaps
773 derived from the gene expression of the immune-checkpoint therapy (ICT) essential genes, showing the
774 universal TIME subtypes in breast invasive carcinoma (BRCA), lung adenocarcinoma (LUAD) and
775 skin cutaneous Melanoma (SKCM). The heatmaps for other cancer types are shown in Supplementary
776 Figure 1; (b) Abundance of infiltrated immune cells in TIME subtypes across 12 types of cancers; (c)
777 Kaplan–Meier curves of the patient groups between the TIME-rich subtype and the combined TIME-
778 intermediate and -poor subtypes, revealing that the survival time for the patients in the TIME-rich
779 subtype is significantly longer than those in the TIME-intermediate and -poor subtypes; and (d)
780 Pathway enriched analysis derived from the significantly differential genes of RNA-seq data between
781 the TIME-rich subtype and the TIME-intermediate and -poor subtypes. The digital numbers represent
782 FDR-corrected p values.
783
784 **Figure 2**. **Heatmaps representing the enrichment pathways derived from functional germline**
785 **genomic variants.** (a) A heatmap shows the significantly enriched pathways derived from the
786 significantly differential germline variants between TIME-rich and TIME-intermediate-poor subtypes
787 in 13 cancer types. (b) A heatmap shows the significantly enriched pathways derived from the
788 significantly differential germline variants between non-cancer individuals and TIME-intermediate-
789 poor patients in 13 cancer types. The digital numbers represent FDR-corrected p values.
790
791 **Figure 3. The associations between the inherited detected NK cells and clinical outcomes, and the**
792 **abundance of infiltrated immune cells in TIMEs.** (a) Kaplan–Meier curves of the patient groups of
793 the high- and low-number of functionally inherited variants in the NK cells for disease-free survival.
794 Patients were top-to-bottom ranked based on the number of functionally inherited variants in NK cells.
795 Top 40% and bottom 40% of the ranked patients were defined as high- and a low number of the NK
796 cell defected patient groups, respectively. (b) Negative correlations between the number of the NK cell
797 inheritable defected and the abundance of the infiltrated immune cells in TIMEs. Breast invasive
798 carcinoma (BRCA), lung adenocarcinoma (LUAD) and skin cutaneous Melanoma (SKCM). The
799 survival differences for other types of cancer are shown in Supplementary Figure 6. *p-value >0.05;
800 **0.05>p-value >0.001; and ***p-value<0.001. The NK-specific-genes included NKD genes and NK

801 receptors/ligands of the 103 defected NK cell genes, while the NK-immune-universal-genes included
802 the global immune cell genes and the universal genes of the 103 defected NK cell genes.
803

Fig 1a

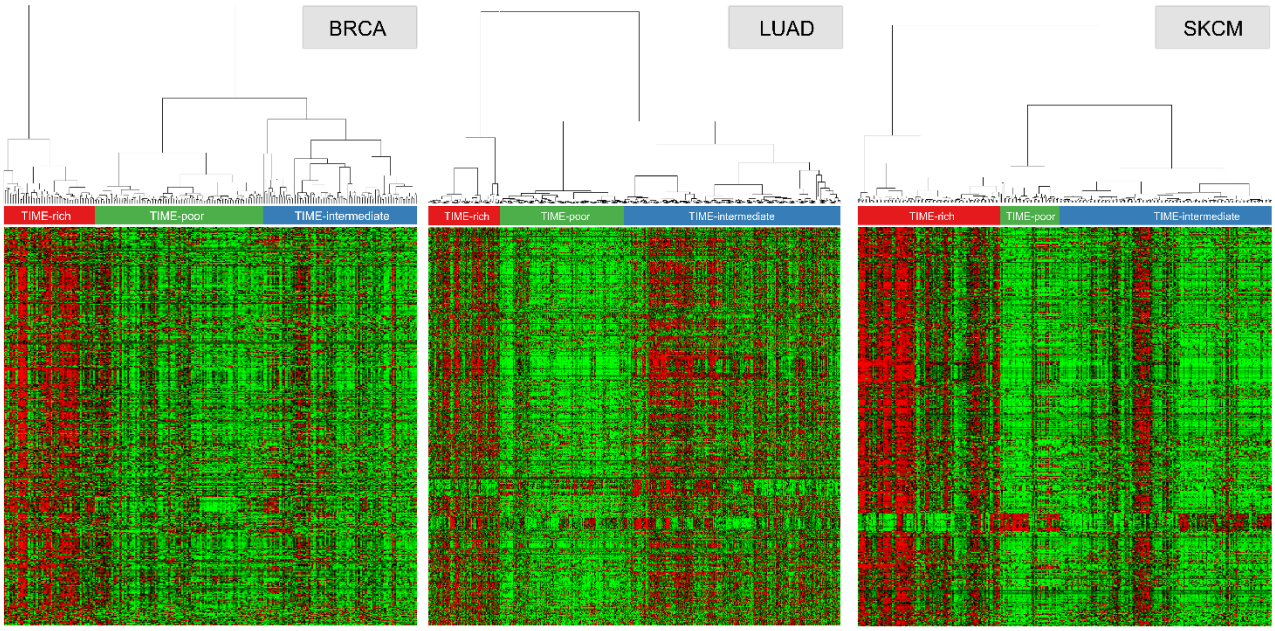
BRCA
LUAD
SKCM
TIME-rich
TIME-poor
TIME-intermediate

Fig 1b

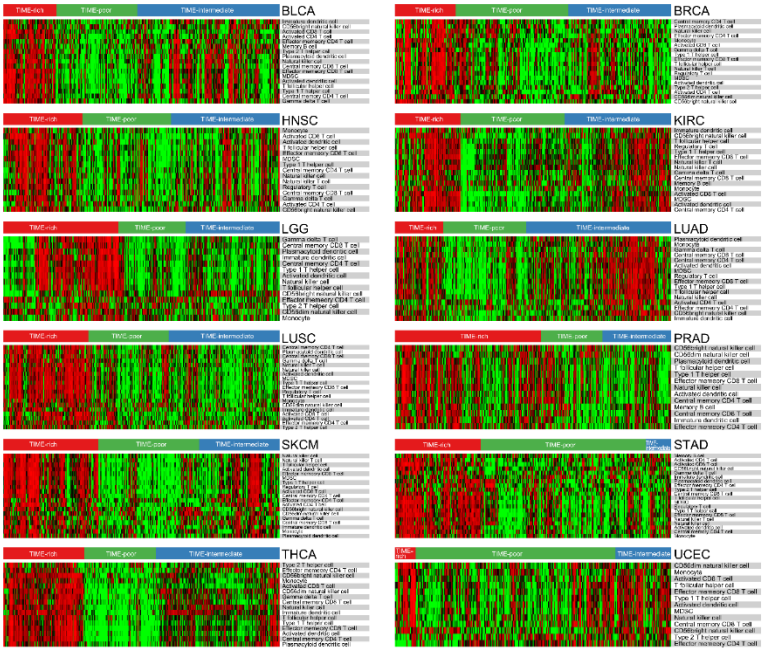
BLCA
BRCA
HNSC
KIRC
LGG
LUAD
LUSC
PRAD
SKCM
STAD
THCA
UCEC

Fig 1c

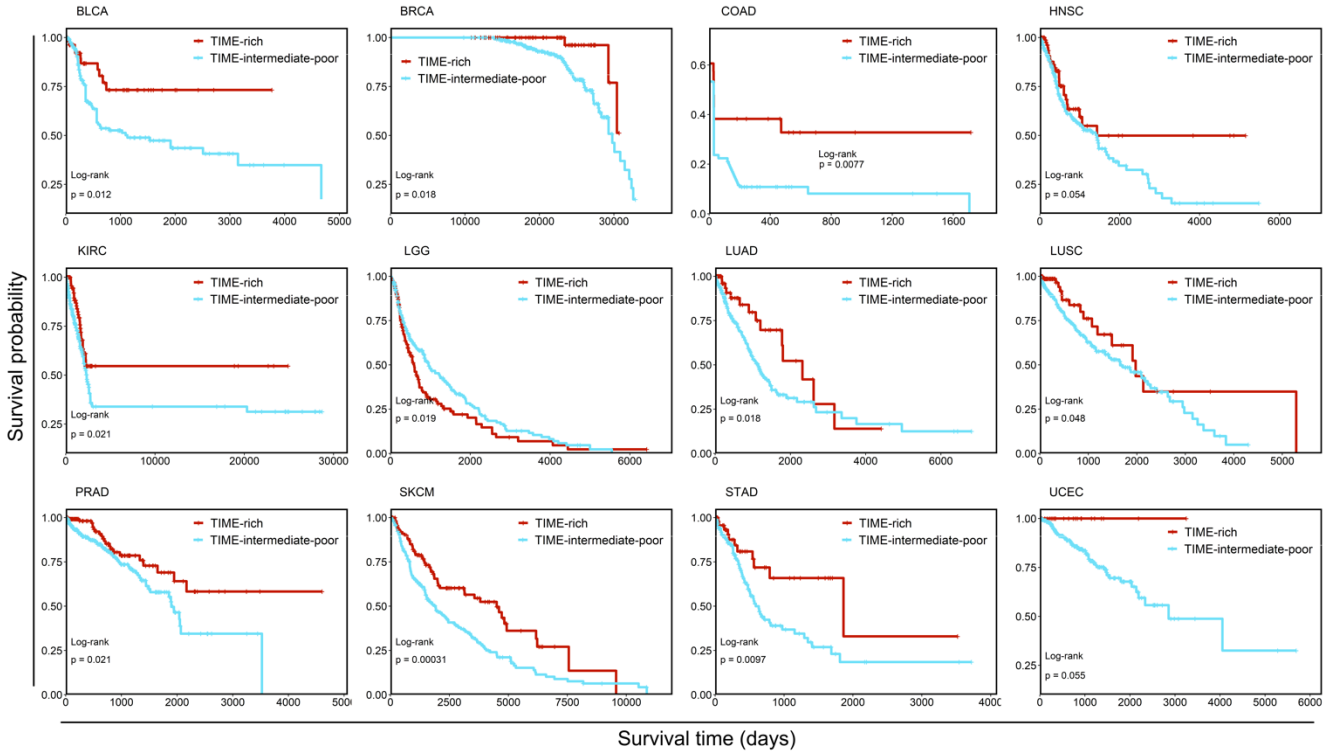
BLCA
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.012
BRCA
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.018
COAD
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.0077
HNSC
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.054
KIRC
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.021
LGG
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.019
LUAD
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.018
LUSC
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.048
PRAD
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.021
SKCM
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.00031
STAD
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.0097
UCEC
TIME-rich
TIME-intermediate-poor
Log-rank
p = 0.055
Survival probability
Survival time (days)

Fig 1d

	BLCA	BRCA	COAD	LGG	HNSC	KIRC	LUAD	LUSC	PRAD	SKCM	STAD	THCA	UCEC
Natural killer cell mediated cytotoxicity	1.3e-32	9.6e-39	1.8e-30	1.4e-56	3.3e-46	1.2e-37	2.2e-35	8.2e-45	2.4e-36	7.6e-41	5.1e-48	4.5e-40	6.4e-32
Wnt signaling pathway	7.1e-24	3.8e-24	2.0e-39	2.2e-62	1.1e-39	4.6e-41	8.8e-49	4.5e-33	6.4e-49	7.8e-33	7.6e-38	1.6e-57	1.0e-38
Antigen processing and presentation	2.9e-35	6.7e-42	8.1e-12	1.1e-38	3.9e-36	1.4e-36	6.6e-29	7.2e-40	1.0e-14	5.9e-34	3.6e-32	1.1e-43	4.9e-19
Epstein-Barr virus infection	1.9e-18	2.3e-11	4.1e-18	7.9e-21	2.9e-15	4.1e-14	3.3e-12	1.9e-12	8.2e-22	5.3e-18		4.4e-19	6.2e-17
Herpes simplex infection	3.6e-20	9.4e-23	1.3e-14	9.0e-23	7.1e-20	8.1e-22	1.3e-20	1.2e-17	1.0e-16	2.5e-26		6.7e-25	9.0e-22
Viral carcinogenesis	6.4e-06	9.0e-03	2.4e-05	9.5e-05	1.5e-04	3.1e-03	3.6e-04	9.0e-03	2.0e-07	5.9e-04		1.9e-04	1.3e-07
Viral myocarditis	6.5e-20	2.8e-18	7.9e-05	1.2e-18	1.8e-14	3.4e-20	2.8e-14	2.4e-18	7.1e-06	6.6e-16	2.4e-10	1.4e-18	2.9e-13
Inflammatory bowel disease (IBD)	1.4e-13	3.7e-15	2.1e-01	2.9e-13	1.3e-12	1.4e-13	1.4e-11	3.2e-16	3.8e-04	3.4e-17		2.3e-17	2.8e-06
Long-term depression			1.0e-03		5.5e-04								4.2e-03
Rheumatoid arthritis	6.3e-10	2.1e-10		7.2e-07		3.8e-09		5.9e-02		4.0e-10		5.7e-10	2.3e-02
Staphylococcus aureus infection	4.8e-05	7.6e-05		1.7e-03	4.7e-04	1.4e-04	3.5e-05	4.6e-05		5.3e-05		2.6e-03	
Type I diabetes mellitus	1.2e-17	4.5e-20	1.2e-01	1.4e-13	3.0e-17	3.3e-18	1.5e-11	1.1e-17	2.4e-01	1.4e-17	4.5e-16	3.5e-17	1.4e-08
Graft-versus-host disease	5.4e-19	6.0e-22	8.0e-03	2.6e-18	6.3e-19	7.7e-20	1.0e-14	2.5e-19	9.7e-02	7.3e-21		3.3e-19	5.2e-10
HTLV-I infection	7.4e-22	2.2e-30	2.3e-12	1.7e-34	3.3e-31	3.7e-22	3.8e-23	2.8e-25	3.0e-23	8.7e-23		7.8e-32	3.1e-28
Influenza A	3.5e-18	8.8e-17	3.0e-14	4.4e-26	1.2e-19	4.3e-19	8.6e-18	4.1e-15	2.8e-20	6.8e-22		2.4e-02	1.2e-01
Hepatitis B	4.5e-11	8.7e-13	3.3e-18	1.3e-22	6.7e-11	7.2e-13	1.0e-17	2.1e-09	8.2e-22	7.3e-16		2.9e-13	3.0e-15
Hepatitis C	1.6e-07	3.8e-03	1.4e-13	2.1e-06	5.5e-11	2.5e-03	1.8e-06	1.8e-02	5.8e-13	2.5e-07		1.2e-05	3.5e-12
Adherens junction		1.1e-01	1.5e-02	1.6e-04	9.3e-03	2.8e-03	2.3e-01		2.3e-05	6.3e-03		1.6e-01	
AMPK signaling pathway			6.1e-03	1.2e+00									
Apoptosis	3.6e-07	4.0e-03	1.8e-06	2.1e-07	9.8e-04	1.4e-03	3.9e-04	5.6e-04	3.9e-10	1.2e-04	1.1e-03	5.3e-09	8.8e-06
Asthma	8.7e-07	1.0e-07		1.0e-05	7.1e-05	2.3e-06	6.7e-07	2.6e-08		4.1e-07	3.3e-06	2.9e-07	2.1e-03
Autoimmune thyroid disease	1.2e-16	1.0e-18		5.1e-17	7.6e-15	8.2e-16	3.2e-12	2.0e-16	3.5e-02	9.2e-19	6.8e-11	7.3e-17	2.6e-09
B cell receptor signaling pathway	1.2e-08	4.2e-10	2.7e-14	8.2e-17	1.8e-15	1.3e-10	1.0e-16	3.1e-09	1.7e-17	3.9e-09	1.3e-09	4.6e-11	1.5e-16
Cell adhesion molecules (CAMs)	1.6e-14	1.9e-12	2.1e-01	7.2e-11	1.4e-10	4.2e-11	1.7e-03	2.0e-10	8.2e-03	2.2e-12	3.3e-09	4.0e-10	6.7e-06
Chemokine signaling pathway	1.9e-08	8.9e-06	1.1e-06	7.9e-09	1.5e-08	7.9e-06	2.4e-03	2.2e-07	1.9e-08	3.5e-06	6.5e-06	8.1e-07	2.0e-10
Cytokine-cytokine receptor interaction	2.3e-06	4.0e-09		6.0e-04	1.6e-03	2.1e-06		3.1e-07		8.9e-10	2.5e-05	8.3e-08	
ErbB signaling pathway	3.5e-06	1.1e-02	1.3e-11	5.0e-15	5.8e-10	2.9e-06	7.4e-08	7.6e-06	1.5e-11	1.9e-04	4.1e-03	4.0e-05	2.8e-09
Estrogen signaling pathway	1.5e-02		4.0e-06	4.8e-08	7.2e-05	3.6e-03	4.3e-05	7.7e-03	1.3e-09			2.0e-04	1.2e-07
Fc epsilon RI signaling pathway	1.1e-09	1.7e-08	1.5e-11	3.8e-12	1.0e-14	3.8e-08	4.1e-08	1.0e-07	1.1e-16	1.8e-08	9.1e-07	3.7e-14	1.0e-11
Fc gamma R-mediated phagocytosis	1.5e-02	8.2e-02	3.6e-03	1.8e-07	1.5e-04	3.2e-05	2.8e-03	7.3e-02	8.0e-06	2.4e-02		4.9e-03	1.1e-02
FoxO signaling pathway	2.1e-04	4.0e-04	1.1e-07	3.4e-05	3.7e-08	7.3e-05	1.8e-02	6.0e-02	7.9e-07	2.7e-03		5.5e-03	5.3e-06
GnRH signaling pathway			1.2e-05	5.5e-04	2.1e-06		1.0e-02		2.4e-06			2.1e-02	8.7e-06
Intestinal immune network for IgA production	3.2e-12	1.0e-16	2.2e-02	2.1e-15	3.9e-14	1.4e-13	3.4e-09	3.0e-18		8.4e-21	3.4e-17	7.1e-25	4.7e-19
Jak-STAT signaling pathway	2.5e-08	1.1e-06	6.1e-05	5.3e-08	8.6e-05	1.8e-07	2.5e-02	1.7e-07	2.2e-06	1.2e-08	9.7e-03	4.0e-09	1.5e-02
MAPK signaling pathway	1.0e-01	8.9e-02	5.3e-07	4.6e-06	2.0e-07	9.2e-02	1.2e-05	2.3e-02	2.6e-07	1.4e-01		2.6e-04	9.8e-08
mTOR signaling pathway			2.5e-01	3.2e-01					1.4e-01				
Neurotrophin signaling pathway	6.2e-05	5.5e-03	5.9e-10	3.5e-09	2.1e-08	7.1e-05	5.0e-07	7.6e-03	6.6e-12	2.3e-04		9.0e-06	6.9e-11
NF-kappa B signaling pathway	6.4e-11	4.2e-15	9.3e-05	2.5e-06	1.6e-05	9.8e-08	1.2e-03	4.3e-12	2.5e-08	1.2e-15		2.0e-14	1.7e-08
NOD-like receptor signaling pathway	2.2e-01	1.6e-02	4.1e-05	4.3e-02		2.6e-02	8.9e-03	4.4e-02	7.0e-04	4.3e-04	6.9e-02	2.4e-05	4.9e-06
PI3K-Akt signaling pathway	5.9e-02	1.2e-01	9.6e-05	1.3e-03	2.0e-03	7.9e-02	7.5e-02	1.3e-01	4.9e-04	8.4e-04		3.4e-02	4.1e-03
Primary immunodeficiency	6.1e-08	5.2e-10		1.3e-05	6.5e-07	1.0e-09		3.7e-07		2.8e-09		3.5e-08	1.8e-04
Proteasome	8.0e-11	1.2e-11	5.2e-08	8.1e-08	1.2e-11	2.7e-11	8.0e-08		2.0e-09	5.6e-05		1.8e-08	3.3e-06
Ras signaling pathway			3.0e-03		3.6e-04		6.6e-02	1.9e-01	4.3e-04	4.4e-02		1.9e-02	
T cell receptor signaling pathway	3.1e-24	1.2e-23	1.1e-17	2.2e-26	1.1e-28	7.3e-22	1.7e-18	4.8e-24	3.3e-20	7.8e-21	1.6e-19	5.3e-25	7.5e-23
Thyroid hormone signaling pathway	1.4e-02		7.6e-08	2.3e-07	8.0e-06	1.3e-02	1.0e-01	9.2e-03	1.1e-07	9.4e-04			2.5e-07
TNF signaling pathway	1.3e-10	5.5e-08	6.9e-12	8.3e-10	4.9e-08	6.3e-05	4.2e-07	9.5e-08	6.6e-16	1.2e-07		6.1e-15	7.2e-13
Toll-like receptor signaling pathway	9.3e-16	2.8e-15	6.5e-20	6.9e-18	5.2e-16	1.4e-13	1.7e-12	6.3e-10	1.8e-21	3.6e-20	1.4e-09	2.8e-20	2.4e-17
VEGF signaling path...	9.9e-03	2.5e-01	3.2e-09	5.1e-06	4.3e-06	2.1e-04	8.2e-06	4.2e-04	1.5e-08	1.1e-02	4.8e-03	7.0e-02	1.4e-07

Fig 2a

	BLCA	BRCA	COAD	HNSC	KIRC	LGG	LUAD	LUSC	PRAD	SKCM	STAD	THCA	UCEC
Natural killer cell mediated cytotoxicity	8.7e-04	1.0e-07	3.3e-04	5.7e-03	6.8e-05	1.0e-05	7.6e-06	1.7e-01	2.4e-07	2.6e-10	1.5e-07	1.0e-04	8.9e-02
Wnt sigling pathway	1.9e-07	3.0e-10	9.7e-09	2.3e-02	3.0e-07	2.9e-08	6.6e-06	1.2e-05	1.9e-06	6.9e-04	6.1e-02	3.5e-12	3.9e-06
Antigen processing and presentation	1.4e-01	1.7e-05	5.2e-03		7.7e-03	5.3e-05	7.4e-07	2.2e-07	9.0e-03	2.6e-07	8.9e-04	2.9e-12	1.1e-02
Herpes simplex infection	1.9e-05	2.8e-05	1.7e-01		5.5e-03	1.0e-06	8.2e-05	1.9e-05	8.0e-02	8.0e-04	1.4e-02	2.3e-09	
Epstein-Barr virus infection		6.9e-03	6.7e-02	1.2e-03	3.7e-02	2.6e-03	3.9e-04	8.7e-04		2.8e-09	3.1e-03	1.0e-04	2.5e-03
Inflammatory bowel disease (IBD)					2.4e-02	3.6e-02	8.0e-02			5.7e-04		1.8e-03	
Rheumatoid arthritis					2.3e-02							3.3e-02	2.4e-01
Staphylococcus aureus infection					7.5e-03								
Type I diabetes mellitus							3.5e-02	2.0e-01		2.1e-01		2.7e-05	1.3e-01
Viral myocarditis		1.8e-01			1.1e-02			1.7e-02		1.9e-02		5.6e-03	
Graft-versus-host disease		3.1e-02			8.0e-02		8.0e-02	4.8e-02		5.1e-02	8.9e-02	6.3e-04	3.1e-02
Hepatitis B		1.5e-05	7.7e-05	3.1e-03		1.4e-04	1.4e-05	3.3e-02	2.8e-02	2.0e-04	9.8e-02	2.8e-02	7.9e-02
Hepatitis C						1.4e-03	1.8e-04		1.1e-02	2.7e-03	2.0e-01		
HTLV-I infection	4.6e-02	1.6e-09	1.2e-07		2.0e-04	9.6e-10	1.3e-04	1.2e-02	1.2e-03	1.1e-06	8.0e-04	6.5e-12	1.7e-04
Influenza A	3.3e-04	1.1e-05	4.9e-03		4.8e-04	2.4e-06	2.2e-09	8.5e-06	1.0e-02	2.2e-07	7.4e-03	7.6e-05	1.9e-02
Primary immunodeficiency		3.8e-02				2.2e-01							
Cell adhesion molecules (CAMs)		8.9e-03	1.2e-02		1.2e-01				2.2e-02	3.1e-02			
T cell receptor sigling pathway		1.1e-01	2.9e-06	4.4e-04		8.9e-03	2.4e-04		4.2e-04	1.1e-05	2.2e-03	1.0e-01	1.0e-01
B cell receptor sigling pathway			2.1e-03	1.1e-04		6.6e-02	1.4e-01		3.7e-03	6.6e-08	1.6e-01		
Toll-like receptor sigling pathway	1.2e-03	2.0e-04	6.6e-06	2.4e-02		9.0e-06	9.6e-08	8.4e-03	1.7e-01	2.7e-08	1.3e-01	8.2e-04	6.5e-05
Autoimmune thyroid disease		1.4e-04	1.3e-01		5.8e-03		1.4e-01	8.1e-02		9.5e-03	2.0e-03	2.4e-02	4.9e-02
Cytokine-cytokine receptor interaction		2.1e-04					8.3e-02						
ErbB sigling pathway		2.9e-02		5.8e-03			1.3e-01						
Jak-STAT sigling pathway		1.2e-02					1.7e-02			2.0e-04	4.1e-03		
NF-kappa B sigling pathway		4.8e-03								1.0e-02	2.4e-02	8.9e-07	
TNF sigling pathway		8.0e-03		4.1e-02			2.2e-02			1.1e-02	1.4e-01	9.2e-04	2.9e-02
Intestil immune network for IgA production			6.8e-02			2.6e-03							2.4e-01
Chemokine sigling pathway				6.9e-02						1.0e-01	3.7e-03		
Fc epsilon RI sigling pathway				5.1e-02			1.2e-01		2.5e-02	9.1e-03		1.7e-01	
Fc gamma R-mediated phagocytosis				3.7e-02			9.6e-02						
Neurotrophin sigling pathway				1.3e-02							7.8e-02		
Estrogen sigling pathway							1.6e-03				1.3e-02		
PI3K-Akt sigling pathway							5.3e-03			6.5e-02			

Fig 2b

	BLCA	BRCA	COAD	HNSC	KIRC	LGG	LUAD	LUSC	PRAD	SKCM	STAD	THCA	UCEC
Natural killer cell mediated cytotoxicity	1.1e-21	8.2e-31	3.3e-19	3.5e-21	1.9e-30	2.5e-30	9.8e-29	1.3e-12	2.8e-19	2.9e-13	8.4e-19	2.3e-32	1.8e-34
Antigen processing and presentation	3.9e-19	1.0e-20	2.7e-10	8.1e-12	3.4e-12	2.2e-17	1.8e-14	7.2e-05	1.4e-12	4.9e-10	1.1e-21	1.4e-13	7.9e-19
Wnt sigling pathway	1.4e-13	2.4e-27	2.9e-16	3.8e-15	1.4e-33	4.4e-23	7.4e-16	9.5e-15	4.0e-19	7.6e-06	1.8e-22	8.5e-23	1.4e-24
Inflammatory bowel disease (IBD)	1.9e-02	1.7e-04	2.6e-02	1.4e-08	7.6e-06	8.6e-07	7.9e-03		3.3e-02		3.1e-03	2.7e-04	6.4e-03
Herpes simplex infection	1.1e-16	6.8e-14	1.5e-07	2.0e-16	4.2e-16	1.0e-11	1.0e-10		7.3e-09	2.6e-04	7.8e-06	1.5e-14	3.2e-14
Epstein-Barr virus infection	4.7e-12	5.2e-10	3.5e-11	6.7e-10	1.4e-13	1.3e-12	3.1e-07	2.0e-02	1.8e-07	4.7e-06	4.3e-13	1.4e-13	5.8e-13
Long-term depression			1.4e-02										
Type I diabetes mellitus	8.6e-04	5.6e-05	3.1e-04	2.8e-10	1.9e-05	4.5e-08	3.8e-06		2.7e-04		9.2e-06	3.0e-06	1.4e-05
Viral carcinogenesis				2.1e-01	1.3e-02	1.8e-01	3.5e-02			4.8e-04	1.1e-01	2.4e-04	5.9e-02
Viral myocarditis	7.8e-04	3.2e-06	8.1e-03	1.2e-07	9.6e-03	1.0e-08	3.9e-05		2.1e-04	2.3e-02	1.5e-10	4.6e-05	6.7e-08
Rheumatoid arthritis		6.3e-03		4.2e-05		3.5e-05			2.0e-01		1.6e-03		
Graft-versus-host disease	4.2e-05	1.2e-05	2.4e-04	1.1e-12	2.4e-08	4.7e-10	1.7e-05		1.5e-04		1.4e-08	5.0e-08	2.4e-07
Hepatitis B	3.7e-12	2.2e-08	6.1e-11	2.1e-09	4.8e-14	1.5e-14	3.4e-14		1.7e-06	1.5e-08	6.4e-10	2.6e-15	6.0e-17
Hepatitis C	4.3e-10	8.5e-07	5.2e-04	1.2e-04	5.4e-07	1.8e-03	9.9e-04		1.1e-02	3.8e-06		1.0e-07	6.2e-08
HTLV-I infection	2.3e-17	2.7e-18	1.3e-08	8.2e-19	1.8e-19	7.1e-20	4.8e-19	4.0e-08	3.6e-17	2.8e-11	2.8e-23	5.3e-18	1.4e-13
Influenza A	4.3e-11	7.3e-15	2.2e-10	3.2e-18	4.0e-17	6.1e-11	3.3e-12	8.1e-03	9.0e-09	1.6e-07	9.0e-10	1.9e-15	2.1e-18
Primary immunodeficiency	5.3e-06	1.8e-04				3.1e-02		1.3e-01	2.2e-04	1.7e-03	4.0e-02	1.4e-03	4.9e-04
Apoptosis	6.7e-02			2.5e-04	2.7e-02	4.4e-02	1.5e-01		4.1e-03		6.0e-02	3.4e-06	7.6e-04
Autoimmune thyroid disease	2.7e-10	5.7e-11	3.6e-08	2.6e-15	7.2e-13	1.5e-09	5.9e-11		4.9e-10	1.4e-04	1.6e-11	9.8e-11	1.9e-15
B cell receptor sigling pathway	6.6e-06	8.7e-09	3.7e-06	2.8e-05	9.6e-04	5.7e-09	1.8e-06	1.1e-02		3.7e-03	5.5e-06	6.9e-09	6.2e-08
Cell adhesion molecules (CAMs)	9.9e-06	2.5e-07		5.1e-06	1.9e-04	3.5e-04	1.8e-06		2.5e-05	1.6e-03	1.2e-08	1.0e-02	3.6e-07
Chemokine sigling pathway	8.4e-03	9.7e-04	7.6e-02	4.0e-04	5.0e-03	3.2e-04			1.3e-01	5.6e-03	2.1e-02	1.1e-02	9.4e-03
Cytokine-cytokine receptor interaction	1.2e-01	2.9e-03		2.2e-06	3.7e-07	7.2e-05	1.0e-02		1.1e-06		2.4e-01	1.0e-02	5.3e-03
ErbB sigling pathway	3.2e-04	2.0e-06	4.3e-08	5.1e-03	4.0e-07	8.8e-04	5.7e-02		4.3e-02	3.4e-05	6.6e-03	1.0e-03	1.5e-06
Estrogen sigling pathway	4.2e-02	3.4e-05	3.6e-06	1.1e-01	7.3e-04	8.9e-06	6.4e-02	6.0e-02		3.6e-05	8.5e-05	1.7e-03	2.2e-05
Fc epsilon RI sigling pathway	1.4e-03	8.5e-05	3.3e-07	2.2e-05	7.7e-04	2.5e-06	7.6e-05	4.7e-02	6.2e-02	1.1e-01	3.0e-05	4.3e-08	1.6e-05
Fc gamma R-mediated phagocytosis	9.2e-02		7.5e-02		2.5e-01	5.2e-04					1.9e-02	2.1e-01	1.9e-01
FoxO sigling pathway	5.2e-02				1.0e-01					3.2e-03		1.4e-02	
Intestil immune network for IgA production	4.4e-04	6.8e-08		3.9e-07	1.5e-09	3.1e-05	9.8e-03		1.1e-08	3.7e-03	5.3e-06	4.4e-02	5.7e-04
Jak-STAT sigling pathway	2.8e-04	4.6e-07	2.0e-05	3.4e-07	1.3e-11	2.8e-05	1.5e-02		2.8e-03	9.4e-03	3.4e-03	1.9e-07	1.9e-04
MAPK sigling pathway	1.3e-02	7.7e-03	2.2e-01		1.1e-03	5.5e-03	2.7e-04	1.4e-03	4.6e-03		7.6e-02	5.9e-02	2.2e-04
Neurotrophin sigling pathway	1.3e-01	5.7e-06	2.4e-03	9.0e-02	1.4e-03		1.7e-01		1.3e-01	6.5e-04		8.7e-03	3.6e-03
NF-kappa B sigling pathway	6.8e-11	2.0e-06	2.2e-02	1.3e-07	6.9e-03	7.2e-07	2.7e-03		3.6e-04		2.9e-02	3.7e-05	1.5e-06
NOD-like receptor sigling pathway	1.2e-01			8.9e-02			1.0e-02				2.0e-02	2.4e-04	
PI3K-Akt sigling pathway	1.3e-02	8.0e-02	1.8e-03	8.9e-02	4.8e-03	2.9e-02	7.9e-02			4.9e-02	5.4e-04	8.2e-04	1.5e-04
Proteasome	6.7e-02	3.1e-02	3.5e-02			4.6e-02					6.1e-02		
Thyroid hormone sigling pathway	7.7e-02	6.9e-04	4.8e-05	5.2e-02	1.6e-04	1.2e-02			7.6e-02	1.7e-01	2.5e-04	1.1e-03	2.3e-02
TNF sigling pathway	8.2e-05	3.5e-02	5.0e-02	4.3e-08	3.6e-05	1.9e-04	1.1e-04	1.6e-01	2.2e-06	7.9e-02	6.7e-08	2.0e-09	1.9e-03
Toll-like receptor sigling pathway	5.0e-15	1.4e-09	7.0e-11	1.4e-13	3.3e-12	7.3e-11	4.2e-15		1.1e-05	5.8e-09	3.4e-07	6.2e-20	3.6e-19
GnRH sigling pathway		1.4e-01	1.4e-03		7.5e-04	1.4e-01							5.1e-04
VEGF sigling pathway		5.5e-02	1.6e-02		1.0e-01	6.7e-03			2.0e-02	6.6e-03	9.6e-03	8.2e-04	1.7e-02
Inflammatory mediator regulation			2.0e-05									9.4e-02	2.8e-02
Ras sigling pathway						4.2e-02						4.8e-02	

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854 **Fig 3a**
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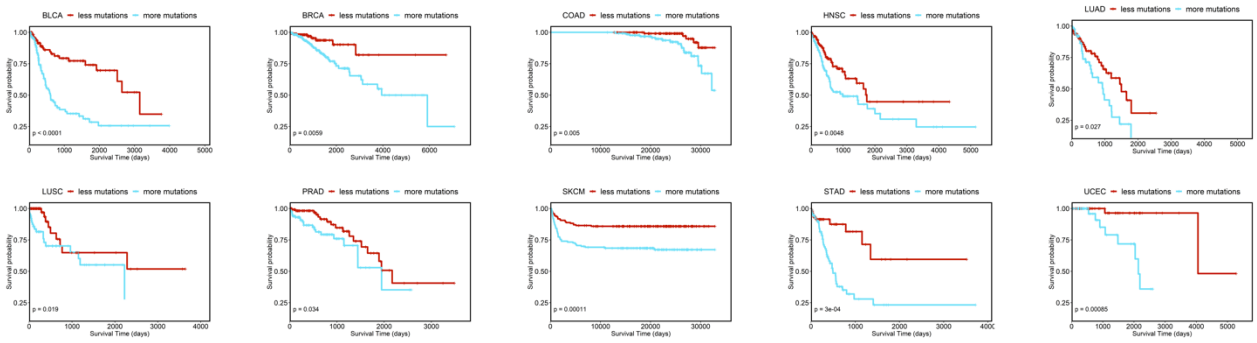

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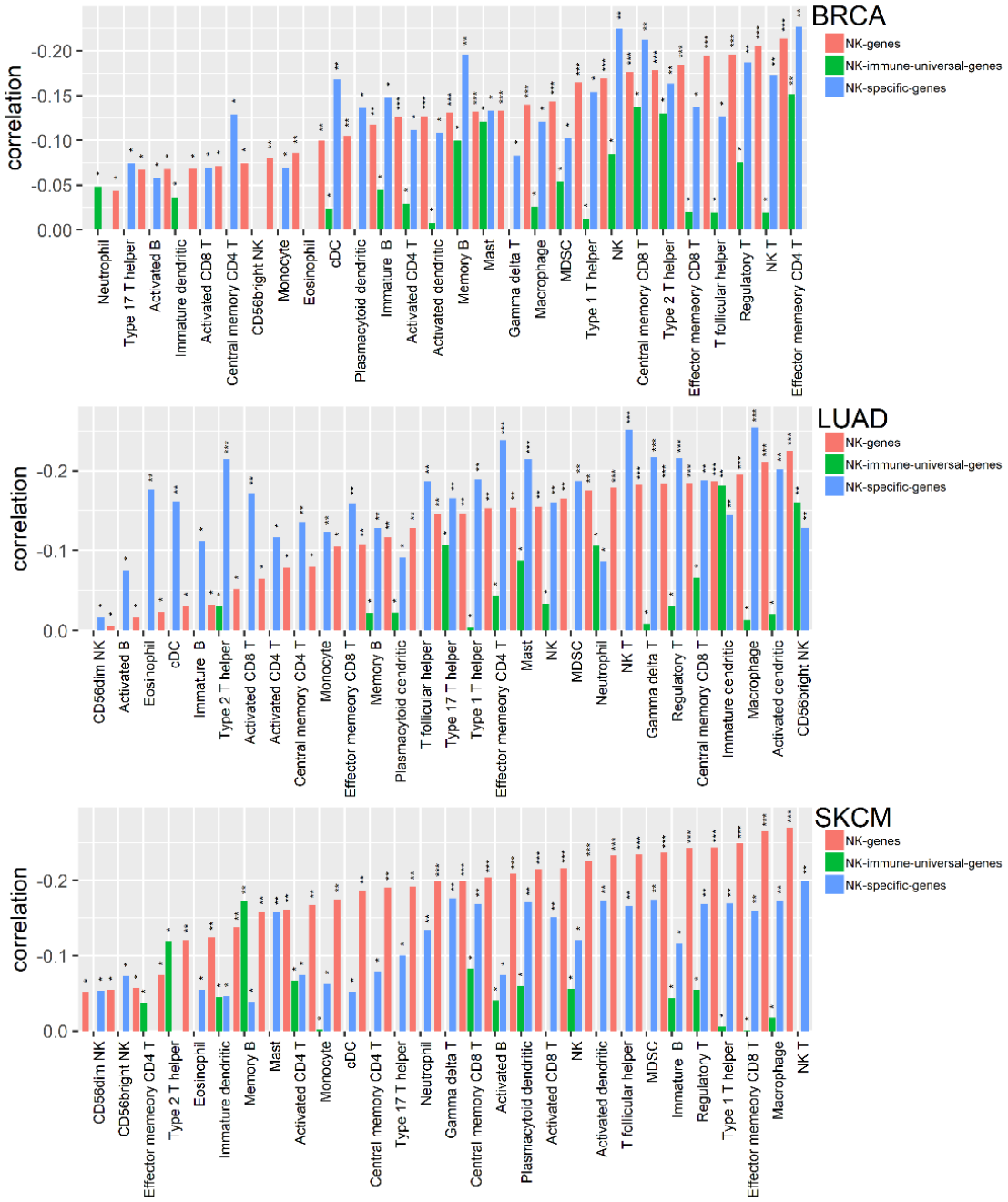
Fig 3b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NK-specific-genes
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-0.15
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Type 17 T helper
Activated B
Immature dendritic
Activated CD8 T
Central memory CD4 T
CD56bright NK
Monocyte
Eosinophil
cDC
Plasmacytoid dendritic
Immature B
Activated CD4 T
Activated dendritic
Memory B
Mast
Gamma delta T
Macrophage
MDSC
Type 1 T helper
NK
Central memory CD8 T
Type 2 T helper
Effector memeory CD8 T
T follicular helper
Regulatory T
NK T
Effector memeory CD4 T
LUAD
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CD56dim NK
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Eosinophil
cDC
Immature B
Type 2 T helper
Activated CD8 T
Activated CD4 T
Central memory CD4 T
Monocyte
Effector memeory CD8 T
Memory B
Plasmacytoid dendritic
T follicular helper
Type 17 T helper
Type 1 T helper
Effector memeory CD4 T
Mast
NK
MDSC
Neutrophil
NK T
Gamma delta T
Regulatory T
Central memory CD8 T
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Macrophage
Activated dendritic
CD56bright NK
SKCM
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NK-immune-universal-genes
NK-specific-genes
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Eosinophil
Immature dendritic
Memory B
Mast
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cDC
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Type 17 T helper
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Gamma delta T
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Activated CD8 T
NK
Activated dendritic
T follicular helper
MDSC
Immature B
Regulatory T
Type 1 T helper
Effector memeory CD8 T
Macrophage
NK T