

1 **Cancer archetypes co-opt and adapt the transcriptional programs of existing cellular states**

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11

12 **ABSTRACT**

13

14 Tumors evolve as independent systems comprising complex survival-ensuring functions,
15 however the nature of these distinct processes and their recurrence across cancers is not clear.
16 Here we propose that melanoma cancer-cells can be classified to three ‘archetypes’ that co-opt
17 the neural crest, mature melanocytes, and stress gene expression programs, respectively, have a
18 unique subclonal structure, and are conserved between zebrafish and human melanomas.

19 Studying the natural history of a zebrafish melanoma tumor at the single-cell level, we found that
20 one archetype exclusively exhibits the signature of the Warburg effect, suggesting that a shifting
21 balance in energy production occurs differentially in the tumor. Deconvolving bulk human
22 melanomas, we found that patients with a dominant fraction of the neural crest archetype show
23 worse survival rates, indicating a clinical relevance for the composition of archetypes. Finally,
24 we provide evidence that extending our approach to other cancer types can reveal universal and
25 cancer-specific archetypes.

1 INTRODUCTION

2

3 Cancer is an evolutionary process occurring at the level of an individual's cells – rather than
4 individuals within a species where it is traditionally studied – by which driver mutations
5 accumulate and increase tumor 'fitness' (Nowell, 1976). Indeed, a universal feature of cancer is
6 the genomic accumulation of mutations to a recurring set genes, including *p53*, *BRCA1/2*, and
7 telomerase (Goode, Ulrich, & Potter, 2002; Hollstein, Sidransky, Vogelstein, & Harris, 1991;
8 Vogelstein & Kinzler, 2004). All tumors also show some degree of genetic heterogeneity
9 (McGranahan & Swanton, 2017; Navin et al., 2011; Wang et al., 2014), which may be usefully
10 classified into mutational signatures (Alexandrov et al., 2013). Less well understood, however, is
11 the functional consequences of such heterogeneity – whether stemming from cooperation or
12 competition among the clones of a tumor. Traditionally, tumor heterogeneity is interpreted as a
13 snapshot in the rise to fixation of driver alleles and their linked sites (Hanahan & Weinberg,
14 2000; Vogelstein et al., 2013), while evidence for the cooperation model is not abundant, though
15 one pioneering study examining sub-clonal heterogeneity did find strong evidence for the notion
16 of clonal cooperation during tumorigenesis (Marusyk et al., 2014). Distinguishing between these
17 two explanations of tumor heterogeneity thus constitutes an important problem in cancer
18 biology.

19

20 Single-cell RNA-Seq (scRNA-Seq) has revolutionized the study of tumors by enabling an
21 examination of the composing cell types, defining both the microenvironment and the cancer
22 cells themselves. Exploring the tumor microenvironment at the single-cell level, has revealed
23 exhaustion program of T-cells in melanoma (Tirosh, Izar, et al., 2016), diversity of tissue
24 resident and normal immune cells and the trajectories of activation of T-cells shaped by TCR
25 diversity in breast cancer (Azizi et al., 2018), and a highly complex structure containing 52
26 stromal cell types in lung cancer (Lambrechts et al., 2018). Transcriptional programs of cancer
27 cells have also been identified in human melanoma, glioblastoma, oligodendrogloma, breast and
28 head and neck cancer (Chung et al., 2017; Patel et al., 2014; Puram et al., 2017; Tirosh, Izar, et
29 al., 2016). For example in glioblastoma, multiple transcriptional programs co-exist according to
30 classical, proneural, neural, and mesenchymal cell states (Patel et al., 2014). However, the
31 plasticity of these transcriptional states is not known. Moreover, the relationship to the

1 hypothesized cancer stem cell theory (Meacham & Morrison, 2013) has not been established and
2 thus it is not clear if transcriptional states are hierarchically related to one another.

3
4 The genetics of melanoma have been well characterized in terms of recurring genes and
5 pathways across nevus and invasive bulk tumors (Akbani et al., 2015; Tsao, Chin, Garraway, &
6 Fisher, 2012). However, in order to understand how heterogeneity is distributed across the cells
7 that compose the tumor, it is necessary to study the natural history of this process. A system
8 allowing for sampling the cells of individual tumors over time is thus required. This is difficult to
9 achieve in human patients, however a zebrafish model for cancer has been introduced in which
10 the expression of the human BRAF^{V600E} gene in a *p53*-deficient background leads to 100% fish
11 developing melanoma (Figure 1A) (White et al., 2011). These tumors resemble the human
12 disease at histological (Patton et al., 2005), genomic (Yen et al., 2013), and gene expression
13 levels (Kansler et al., 2017), suggesting that studying these tumors can reveal universal
14 principles of melanoma.

15
16 Here, we study melanoma at the level of the transcriptomes of thousands of cells by repeatedly
17 sampling a melanoma tumor over the course of its evolution without treatment. Analyzing the
18 resulting dataset led us to introduce ‘archetypes’ as a theory for understanding cancer cell-types.
19 An archetype is a cell type that co-opts an existing transcriptional program and adapts it over the
20 progression of the tumor. We propose that each melanoma archetype manifests a distinct
21 function – the neural crest, differentiated, and stress transcriptional programs – in distinct
22 anatomic locations in the tumor. We find t very same archetypes in human melanoma suggesting
23 their universality to melanomas. Over the natural history of the tumor, we detected changes in
24 how the archetypes adapt their co-opted transcriptional program. In particular, we detected
25 activation of the Warburg effect – in terms of turning ‘on’ glycolysis and turning ‘off’ oxidative
26 phosphorylation – perhaps due to its central location in the tumor, revealing that the Warburg
27 effect may occur separately for distinct tumor archetypes. Reanalyzing tumors from the TCGA
28 project we found that patients show a worse prognosis if their tumors are dominated by the
29 neural crest archetype. Finally, we report evidence for the recurrence of one of the detected
30 archetypes in breast cancer and pancreatic cancer tumors. Collectively, our results lead to the

1 hypothesis that tumors of each cancer type will be composed of a coherent set of archetypes, as
2 well as that universal archetypes may span all cancers.

3

4 **RESULTS**

5 **Single-cell RNA-Seq on zebrafish melanoma reveals eight distinct cell-types**

6 To understand the natural history of this process, a system is required that allows for sampling
7 individual tumor cells over time and space, which is difficult to achieve in human patients. To
8 overcome this limitation, we employed an isogenic zebrafish model for melanoma which arises
9 from expression of the human *BRAF^{V600E}* gene in a *p53*-deficient background (Figure 1A)
10 (White et al., 2011). 100% of these fish develop melanoma that resembles the human disease at
11 histological (Patton et al., 2005), genomic (Yen et al., 2013), and gene expression levels (Kansler
12 et al., 2017). To begin to study the composition and dynamics of tumorigenesis, we used this
13 experimental system to examine transcriptomes in individual cells using scRNA-Seq.

14 We processed ~3k cells from a tumor biopsy using the inDrop system (Klein et al., 2015),
15 thus comprehensively capturing the set of cell-types present in the tumor. After quality control
16 and filtering (see STAR Methods), we were left with 1957 cells, each with an average of
17 approximately 2,500 transcripts and 1,000 genes detected. Studying the transcriptomes, we
18 detected eight cell-types which form distinct clusters when visualized using t-distributed
19 stochastic neighbor embedding (tSNE) (Van Der Maaten & Hinton, 2008). To annotate these
20 clusters, we first identified the cancer cell population by the detection of *BRAF^{V600E}* transcripts.
21 The rest of the clusters were annotated using known markers: keratinocytes, fibroblasts,
22 erythrocytes, natural killer cells, neutrophils, macrophages, other immune cells, and the cancer
23 cell population (Figure 1B and S1). For example, a cluster was annotated as keratinocytes since
24 it was enriched in the expression of keratin 4 (*krt4*) and other genes (see Table S1).

25

26 **Principal component analysis on human and zebrafish malignant cells reveals three
27 conserved cancer cell-types**

28 We next examined the heterogeneity of the cancer cells using principal components analysis
29 (PCA), and found a triangle-shaped arrangement of transcriptomes, with a concentration of cells

1 near the vertices (Figure 1C). Studying the genes that most contribute to PC1 and PC2, we found
2 that the *sox2*, *dct*, and *jun* genes exhibit expression each restricted to one of the three vertices
3 (Figure 1D). Furthermore, we found the same configuration of transcriptional programs when
4 examining two additional zebrafish melanoma tumors (Figure S2).

5 These vertices may be unique to the zebrafish system or general to melanoma neoplasm.
6 To test for their universality, we studied previously reported human melanoma scRNA-Seq data
7 by Tirosh et al. (Tirosh, Izar, et al., 2016) who described the *MITF* and *AXL* transcriptional
8 programs of metastatic cancer cells, respectively corresponding to proliferating (Carreira et al.,
9 2005) and invasive features (Müller et al., 2014). Since our zebrafish tumor was induced by the
10 *mitfa* promoter, we focused on the human cells annotated with the *MITF*-transcriptional program
11 and found that these have a strikingly similar pattern of expression to the zebrafish tumor
12 expression (Figure 1E). This correspondence suggests that the transcriptional programs that we
13 observe in zebrafish are a conserved aspect of melanoma.

14

15 **Cancer cell-types as archetypes**

16 Recent work has shown that a population of individuals in ‘morphospace’ often reveals
17 geometrical shapes, whose vertices may be interpreted as ‘archetypes’, each capturing an
18 idealized state of the population (Shoval et al., 2012). The notion of archetypes has also been
19 extended to the study of bulk cancer samples (Hart et al., 2015). Here, we invoke archetypes to
20 distinguish cancer cell-types within an individual tumor, by identifying the cells most closely
21 associated with the vertices of the triangle shape in the PC analysis (Figure 1C and STAR
22 Methods). We proceeded to characterize each archetype by identifying the most uniquely-
23 expressed genes among the 500 cells closest to the corresponding vertex (Figure 2A). We found
24 that archetype 1 is enriched for the expression of neural crest genes, such as *sox2* and *sox10*,
25 suggesting that these cells co-opt this progenitor transcriptional program. In contrast, archetype 2
26 is enriched with the expression of genes associated with mature melanocytes, such as *dct*, *tyrp1b*,
27 and *pmela*, indicating the co-option of the differentiated melanocyte transcriptional program. To
28 test for the distinction between archetypes 1 and 2, we compared their expression profiles to a
29 dataset that measured the gene expression changes that accompany human melanocyte
30 differentiation from pluripotent stem cells (Mica, Lee, Chambers, Tomishima, & Studer, 2013).

1 As expected, we found that archetypes 1 and 2 best correlate in the expression to neural crest
2 cells and mature melanocytes, respectively (Figure 2B), further supporting the notion that these
3 archetypes capture distinct stages of the melanocyte differentiation process.

4 We found that archetype 3 is enriched in the expression of genes such as *jun*, *fosb*, *fosab*,
5 and eight ‘heat-shock protein’ genes, all involved in an apparent stress-activated transcriptional
6 program. Cells expressing this program have also been observed in other single-cell tumor
7 analyses (Puram et al., 2017; Tirosh, Izar, et al., 2016), where they were found to be in a distinct
8 – though uncharacterized – tumor region (Tirosh, Izar, et al., 2016). To test if archetype 3 also
9 occupies a distinct region in the zebrafish melanoma tumor, we invoked a spatial transcriptomics
10 approach. We sectioned a zebrafish tumor and placed it on a DNA microarray where each spot
11 queried the entire polyA complement of the transcriptomes of the surrounding cells. Delineating
12 three concentric rings in the tumor, we found that expression of archetype 3 genes is higher in
13 the outer ring of the tumor (Figure 2C, $P < 10^{-10}$, Wilcoxon rank-sum test, Figure S3). This
14 suggests that the cells of this archetype are enriched at the outer edges of the tumor, thus relating
15 the function of the archetype 3 cells to their spatial organization. Enriched expression of the
16 stress module in the outer ring also controls for the possibility that the stress-induced signals
17 follows from the cell dissociation protocol (Van Den Brink et al., 2017). Together, our inference
18 of the three archetypes suggests that cancer cells co-opt existing transcriptional programs
19 corresponding to neural-crest cells, mature melanocytes, and a stress response.

20

21 **Copy number variations distinguish among the archetypes**

22 Beyond transcriptional programs, scRNA-Seq data allows us to study copy number variations
23 (CNVs) at the level of individual cells. As previously introduced (Patel et al., 2014) and used
24 (Darmanis et al., 2017; Kim et al., 2018; Tirosh, Izar, et al., 2016; Tirosh, Venteicher, et al.,
25 2016), by mapping gene expression levels to their chromosomal locations, large-scale CNVs can
26 be inferred. Analyzing our scRNA-Seq data, we found striking evidence for at least two distinct
27 clones (Figure 3A). Both clones were found to lack one or two copies of chromosome 3. We
28 detected an amplification and a loss of chromosome 19 and 16, respectively, in clone A, while
29 clone B cells showed an amplification of chromosomes 10 and 23. These observations may be
30 driven by the transcriptional levels and not actual DNA changes, and so we performed three

1 controls to mitigate this possibility. First, we performed bulk whole-genome sequencing on a
2 sample of the same single-cell suspension used to generate the scRNA-Seq data and detected a
3 pattern of deletions and amplifications that is consistent with the RNA-inferred CNVs (Figure
4 3A, lower panel). Second, we excluded from the RNA-CNV analysis the 235 genes whose
5 expression is archetype-specific (Figure 2A) in order to eliminate the effect of uniquely
6 expressed genes. Finally, we studied two zebrafish melanoma cell lines cultured in two
7 conditions and found that while gene expression of the cells clusters by condition, the RNA-
8 CNV analysis retains the strain-specific pattern (Figure S4), thus indicating the robustness of the
9 RNA-CNV approach. Together these results support the existence of two genetically distinct
10 clones present in the tumor.

11 Projecting clones A and B cells to the same PCA-space, we found that each clone
12 localized to a specific region (Figure 3B). Clone A cells matched archetype 1, while clone B
13 cells matched both archetypes 2 and 3. This dichotomy of two clones with distinct archetype
14 suggests that the clones are adapted for different functions. Since our data indicates that
15 archetype 1 (clone A) cells are enriched for the interior of the tumor (Figure 2C), we
16 hypothesized that a fundamental functional distinction between clones A and B is the increased
17 interaction of clone B cells with the micro-environment. We sought to test this by studying the
18 zebrafish melanoma cell lines in two conditions: 1. *in vitro*, where cells are cultured in a dish and
19 do not interact with non-cancer cell types and 2. *in vivo*, where cells were injected into zebrafish
20 embryos resulting in a tumor, whose cells are naturally exposed to other cell types (Figure 3C).
21 For each of the two cell lines (ZMEL1 and ZMEL2), we found that cells from the *in vitro*
22 condition clustered with archetype 1 (clone A) while cells from the *in vivo* condition clustered
23 with archetype 3 (clone B). This supports the hypothesis that clones A and B may indeed reflect
24 functions involved in proliferation and tumor-microenvironment interaction, respectively. These
25 results also highlight the notion that cancer clones may specialize in a particular archetype and
26 achieve a distinct genotype over time (Figure 3A-B), though the same genotype may also adopt
27 different archetypes through transcriptional plasticity (Figure 3C-D).

28

29

30

1 **Natural history of clonal gene expression during tumor progression**

2 To study changes over time in the gene expression of the two clones, we exploited the zebrafish
3 system's ability to repeatedly query the same tumor. We thus performed three additional weekly
4 biopsies for the same analyzed tumor to produce a 4-week period time-course dataset (Figure
5 4A). We detected both clones in similar proportions at each biopsy (Figure 4B). In order to
6 identify functional properties that change over time in the tumor, we queried for KEGG
7 pathways that contain genes significantly varying across the biopsies (see STAR Methods). For
8 clone A, we found that glycolysis genes increase in expression over time ($P < 10^{-7}$,
9 hypergeometric test, Figure 4C), suggesting activation of the Warburg effect (Warburg, 1956) of
10 shifting balance in energy production. Consistently, oxidative phosphorylation genes exhibited
11 a correlated decrease in expression ($P < 10^{-10}$, hypergeometric test, Figure 4C). Furthermore, this
12 effect was restricted to clone A (Figure 4D), suggesting that clone A / archetype 1's internal
13 location in the tumor (Figure 2C) may selectively activate the Warburg effect.

14 In clone B, we found that MAPK signaling significantly increases in expression ($P < 10^{-5}$,
15 hypergeometric test, Figure 4D), while glycolysis genes decrease, suggesting adaptation
16 beyond the co-opted differentiation program. Expanding this analysis, Figure 4E shows the
17 expression profiles of 18 KEGG pathways over time, revealing the distinct clonal trajectories,
18 with the exception of FoxO signaling and focal adhesion which increase in expression for both.
19 Overall, this analysis suggests that signaling pathways and metabolic pathways are selected for
20 dynamic expression in clone A and B, respectively. We hypothesized that as the clones diverge
21 into these complementary functions, they would resemble the idealized expression of functions
22 of cells growing under *in vitro* or *in vivo* conditions. To test this, we again turned to our zebrafish
23 melanoma cell lines (Figure 3C) and identified for each KEGG pathway the level of enrichment
24 for differential expression between the *in vitro* and *in vivo* conditions. We found that pathways
25 that increase in clone A are generally significantly enriched for expression in the *in vivo*
26 condition. In contrast, clone B adaptive pathways are enriched for increased expression in the *in*
27 *vitro* condition ($P < 10^{-2}$, Wilcoxon rank-sum, Figure 4E). We conclude from this analysis that
28 clone-specific changes to gene expression implicate distinct and independent functional
29 requirements.

1 **Archetype frequency is correlated with clinical prognosis**

2 To study the effect of the archetypes on clinical outcome we extended our analysis to the TCGA-
3 Skin Cutaneous Melanoma dataset which contains bulk RNA-Seq data for 472 melanoma tumors
4 (Akbani et al., 2015). For each tumor, we inferred the fraction of each archetype by
5 deconvolving the expression profile using the expression of archetype markers (see STAR
6 Methods). As expected, we found that most bulk tumors are composites of all three archetypes
7 (Figure 5A), and archetype 3 was not a dominant archetype in any of the tumors. We
8 distinguished four groups of tumors according to their archetype frequencies: Group 1 containing
9 tumors comprising exclusively archetype 1 (neural crest), group 2 containing tumors comprising
10 all archetypes but dominated mostly by archetype 1, group 3 containing tumors comprising all
11 archetypes but dominated mostly by archetype 2 (mature melanocyte) and group 4 containing
12 tumors comprising only archetype 2 and 3 (stress) (Figure 5A).

13 Studying the gene expression profiles of the bulk tumors using PCA revealed a triangle
14 shape. Interestingly, archetype frequency is correlated with PC2: high PC2 scores corresponded
15 to patients with high fractions of archetype 1 and low PC2 corresponded to patients with high
16 fraction of archetype 2 (Figure 5B). However, other aspects available from the clinical data such
17 as gender, invasiveness, and survival did not correlate with the PCs (Figure S5). We next asked
18 if patients with tumors consisting of different archetype frequencies experienced significantly
19 different clinical outcomes. Focusing on the two groups of tumors that exhibited a mixed
20 combination of archetypes (groups 2 and 3) we found that the group with higher fractions of
21 archetype 1 has a significantly worse prognosis than the group with higher fractions of archetype
22 2 ($P<0.05$). This result indicates that while a mixture of archetypes is a feature of most tumors,
23 heterogeneity in their frequencies may be a predictor of tumor fate.

24

25 **The stress archetype is conserved in breast and pancreatic cancers**

26 To study the generality of the notion of archetypes across cancer types we applied our analytic
27 approach to two previously published scRNA-Seq tumor datasets: triple negative breast cancer
28 (TNBC) (Kim et al., 2018) and pancreatic cancer adenocarcinoma (PDAC) (Moncada et al.,
29 2018). For each cancer type, we analyzed the transcriptomes of only the cancer cells: 388 single

1 cells, from one TNBC patient and 462 single cells from another PDAC patient, both before
2 treatment. Studying these cancer cells using PCA, we found a triangle-shaped distribution of
3 cells for each cancer type (Figure 6), reminiscent to that found for melanoma (Figure 1). This
4 recurrent distribution of cells of a tumor according to their gene expression indicates that
5 transcriptional heterogeneity across cancer cells is not unique to melanoma.

6 Querying for the expression of key melanoma archetype genes in the TNBC and PDAC
7 datasets, we found that the expression of *JUN* is restricted to a single vertex of the triangle-
8 shaped distribution in both cancers (Figure 6A,B, left panels). Since *JUN* was identified as a
9 marker gene for the stress archetype (Figure 1D and E, right panel), we asked if expression of the
10 entire gene signature of that archetype (Table S2) is also enriched for expression in this vertex.
11 Indeed, we detected expression of the stress archetype signature for each of the tumors in the
12 vertex where *JUN* is enriched for expression (Figure 6A and B, right panels). These results
13 provide evidence for the generality of the archetype concept across cancers and moreover that
14 the stress archetype may be conserved across diverse cancer types.

1 **DISCUSSION**

2 Here we have studied the gene expression of individual cancer cells in zebrafish and human
3 melanomas. We detected three recurring gene expression patterns across melanoma cancer cells
4 which we refer to as ‘archetypes’ because they appear to be co-opted cellular states. Over the
5 temporal progression of the tumor, we detected the presence of all three archetypes in similar
6 frequencies (Figure 4), though their spatial locations in the tumor were distinct (Figure 2). An
7 analysis of the tumor heterogeneity revealed that archetype 1 was encoded by a unique clone
8 while a second clone encoded two archetypes (Figure 3), suggesting a greater transcriptional
9 plasticity for that clone. In this section, we discuss the notion of archetypes as a coherent theory
10 for understanding cancer cell-types, the relationships between the archetype notion and central
11 concepts in cancer biology, and the potential clinical relevance of archetypes.

12

13 We defined a cancer archetype as a transcriptional program that is co-opted from that of an
14 existing cell-type or cell-state. Strikingly, we found that cancer cells between human and
15 zebrafish melanomas exhibit the same transcriptional programs. We further provided evidence
16 that one of the archetypes – that of the stress cell state (archetype 3) – is present across other
17 cancers (Figure 6). The other two archetypes account for two states (pluripotent and
18 differentiated, respectively) along the development of melanocytes, the melanoma cell of origin.
19 Tsoi *et al.* also detected that bulk melanoma tumors show different states along the trajectory of
20 differentiation (Tsoi et al., 2018). Together, these results led us to propose that the archetypes
21 present in a particular cancer type will include those that co-opt states along the development of
22 the cell-of-origin of that cancer. This may result from those particular states being more
23 accessible from the particular epigenetic state of the cell that initiates tumorigenesis. It will be
24 interesting to further explore the dynamics by which archetype adapt over time. Since in our
25 study we examined a relatively later stage in tumorigenesis in which the tumor is already fully
26 formed, we did not gain purview into the origin of archetypes (Figure 4). It should also be
27 possible to provide a dictionary of all cancer archetypes – whereby each tumor is a collection of
28 a few of these – allowing for a deeper functional understanding of tumors of any given cancer
29 type.

30

1 A central concept in cancer biology posits that hallmarks are universal neoplastic features
2 (Hanahan & Weinberg, 2000). In our analysis, we captured evidence for the appearance of one
3 hallmark, the Warburg effect: over time, the glycolysis pathway was activated while oxidative
4 phosphorylation decreased in expression (Figure 4). Interestingly, however, we observed this
5 pattern only in archetype 1, suggesting that distinct hallmarks may accumulate across the
6 archetypes rather than in all of them. This observation, together with the result that archetypes
7 co-opt distinct transcriptional programs, leads us to propose that cooperation among the
8 archetypes in the tumor is vital to its overall functional. Previous work has indeed revealed a
9 form of cooperation across cancer cell lines as they form a tumor *in vivo* (Marusyk et al., 2014).
10 The coexistence of the archetypes in tumors also accounts for the observed genetic heterogeneity
11 universal to cancers (Anderson et al., 2011; Lawrence et al., 2013; Mroz et al., 2013; Sottoriva et
12 al., 2013). While natural selection leads to an increase in the frequency of certain alleles,
13 substantial variation may also be maintained as cooperating coexisting archetypes. Previous
14 work has also suggested a hierarchical model for cancer cells in the tumor, with cancer stem cells
15 occupying a key role in the generation of cancer cell-types (Meacham & Morrison, 2013). Our
16 results indicating the presence of distinct clones (Figure 3) suggest that – while one of our
17 archetypes co-opts a stem cell module – these cells are not hierarchically related to other cells in
18 the tumor.

19
20 The notion of archetypes as tumor building blocks has clinical implications. Since different drugs
21 likely lead to distinct impacts across the different archetypes, a tumor may resist a particular drug
22 by such transcriptional plasticity. Indeed, we observed that two archetypes may follow from the
23 same clone (Figure 3), suggesting that if a tumor is depleted of the function of one archetype, it
24 may be able to recover it using transcriptional plasticity followed by gradual evolution. By
25 defining the drugs that best target each specific archetype, it may be possible to develop a more
26 powerful chemotherapy consisting of a combination of drugs that match the archetypes
27 composing the particular tumor. Collectively, we have proposed here a theory for studying a
28 tumor through the archetypes that compose it. Future insight may follow by uncovering the
29 spatial-temporal nature of cancer archetypes in the tumor and their specific interaction with the
30 microenvironment.

31

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5

6 **Author contributions.** I.Y. conceived the project. M.B. led the collection and sequencing of the
7 single-cell RNA-Seq data collection, with contributions from I.S.K. and N.R.C.. R.M.
8 contributed the spatial transcriptomics data. M.B. provided the WGS and Y.Y. contributed to its
9 analysis. M.B. led the analysis of the data, with significant contribution from I.Y.. I.Y. and
10 R.M.W. provided project coordination. I.Y. and M.B. drafted the manuscript on which all
11 authors commented.

12

13 **Declaration of interests.** The authors declare no competing interests.

14

15 **Data and materials availability:** The complete data that support the findings of this study have
16 been deposited in NCBI GEO database with the accession code GSE115140.

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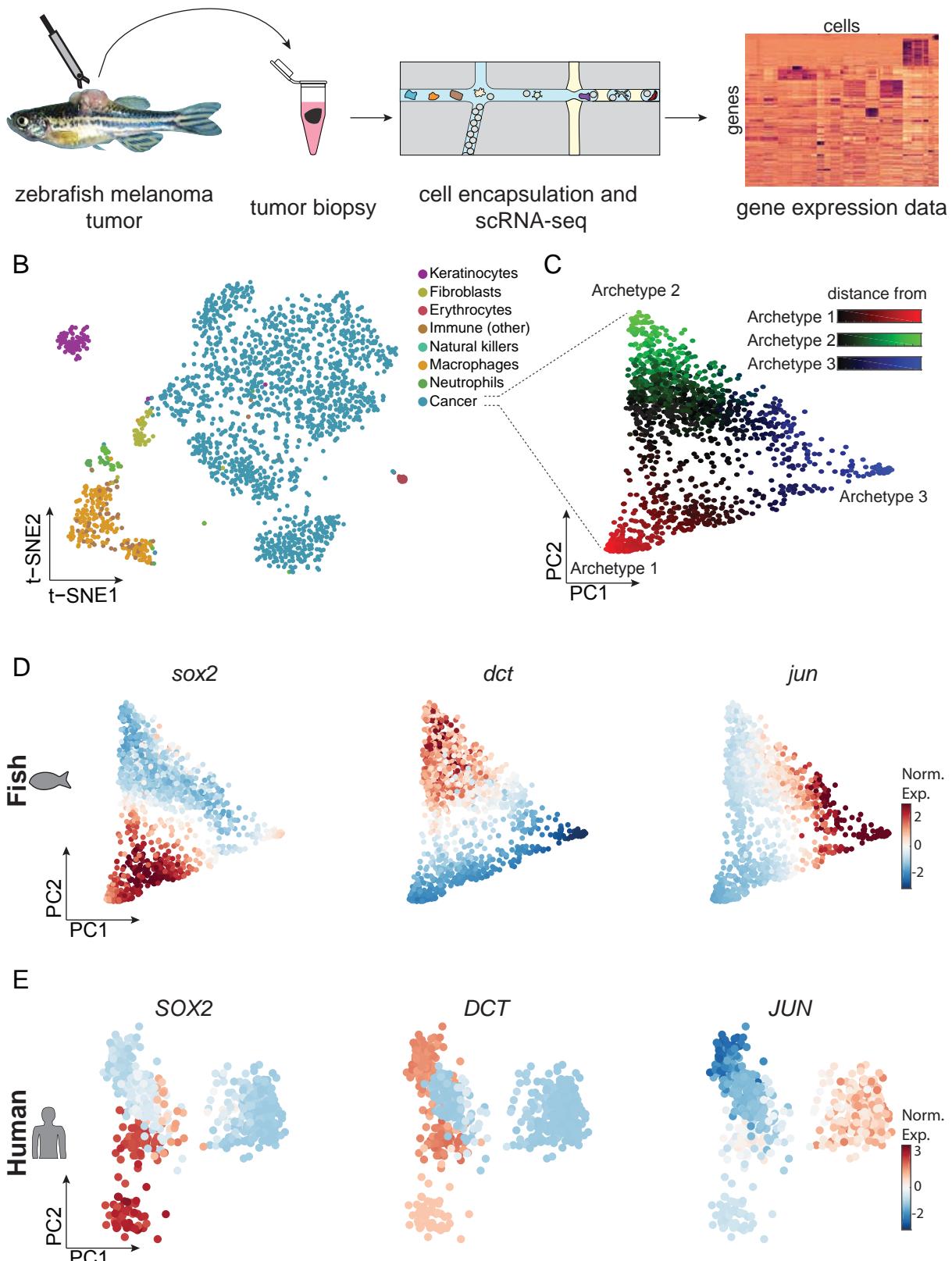


Figure 1. Single-cell RNA-Seq on zebrafish melanoma

- (A) Tumor biopsy was processed using scRNA-Seq, producing a gene expression matrix.
- (B) tSNE analysis of 1957 tumor cells from the biopsy. Color indicates the inferred cell-type.
- (C) PCA on the tumor cancer cells. Color indicates distance from the three vertices.
- (D) Gene expression levels for the indicated genes, mapped onto the PCA shown in (C).
- (E) Analysis of human melanoma data indicating expression levels across the cancer cells for the indicated genes.

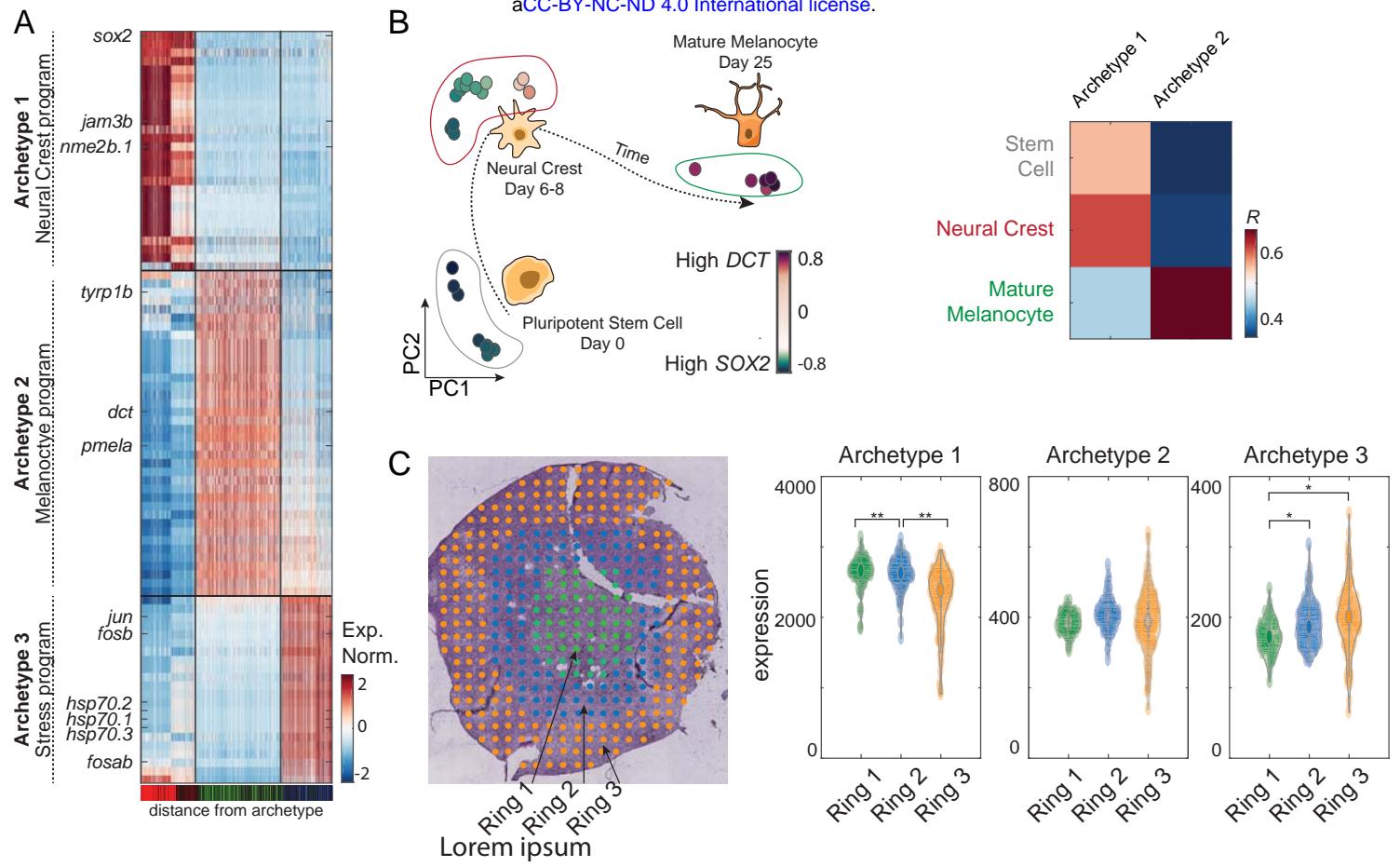


Figure 2. Functional and structural analysis of the cancer archetypes

(A) Expression levels of the differentially expressed genes across the cancer cells ordered by archetype proximity in Fig. 1C.

(B) PCA of bulk melanocyte differentiation from previously reported data (left panel). The color indicates expression levels of SOX2 (red) and DCT (green). Heatmap showing the Pearson's correlation levels between the human Archetype 1 & 2 and the developmental transcriptomes of stem cells, neural crest, and mature melanocytes (right panel).

(C) Spatial transcriptomics on a zebrafish p53-/BRAFV600E tumor. The micrograph shows H & E staining with three concentric rings indicated. Dots correspond to spatial transcriptomics array spots. The violin plots indicate the expression levels of neural-crest, mature melanocytes and stress transcription programs genes, respectively, across the three rings. *, P<10-10; **, P<10-20.

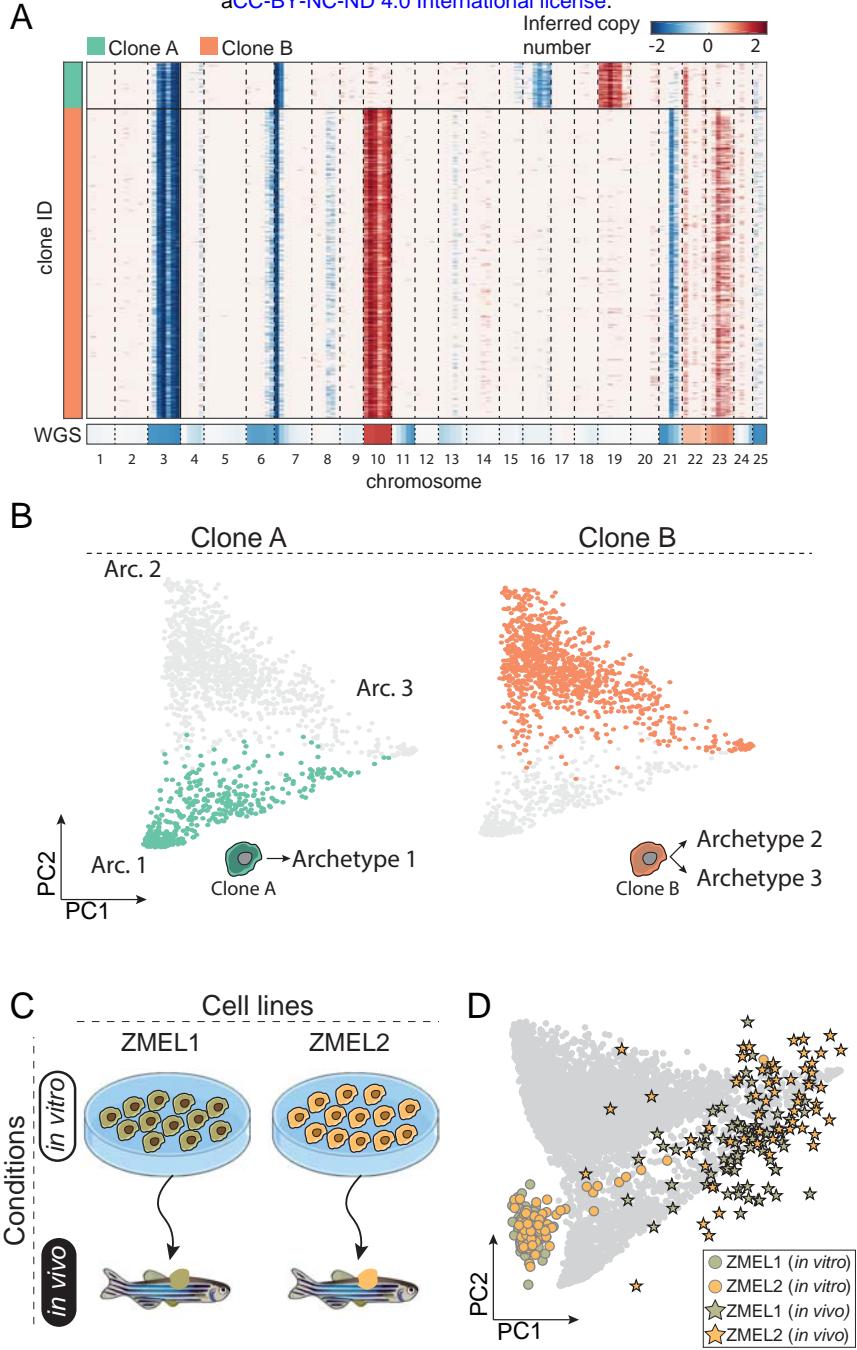


Figure 3. Genetic and non-genetic determinants of the transcriptional programs

(A) RNA and DNA CNV analysis. RNA-CNVs were inferred from the single-cell RNA-Seq data. Red and blue indicate chromosomal additions and deletions, respectively. Two clones (clusters) were identified. Inferred CNVs were globally validated by whole genome sequencing copy number analysis (lower bar).

(B) The locations of the cells of each clone (cluster), identified in (A), are mapped onto the PCA of cancer cells (shown in Fig. 2C). C) Schematic of two zebrafish melanoma cell lines cultured both *in vitro* and *in vivo*.

D) Projection of the expression of the cells represented in (C) onto the PCA shown in Figure 1C.

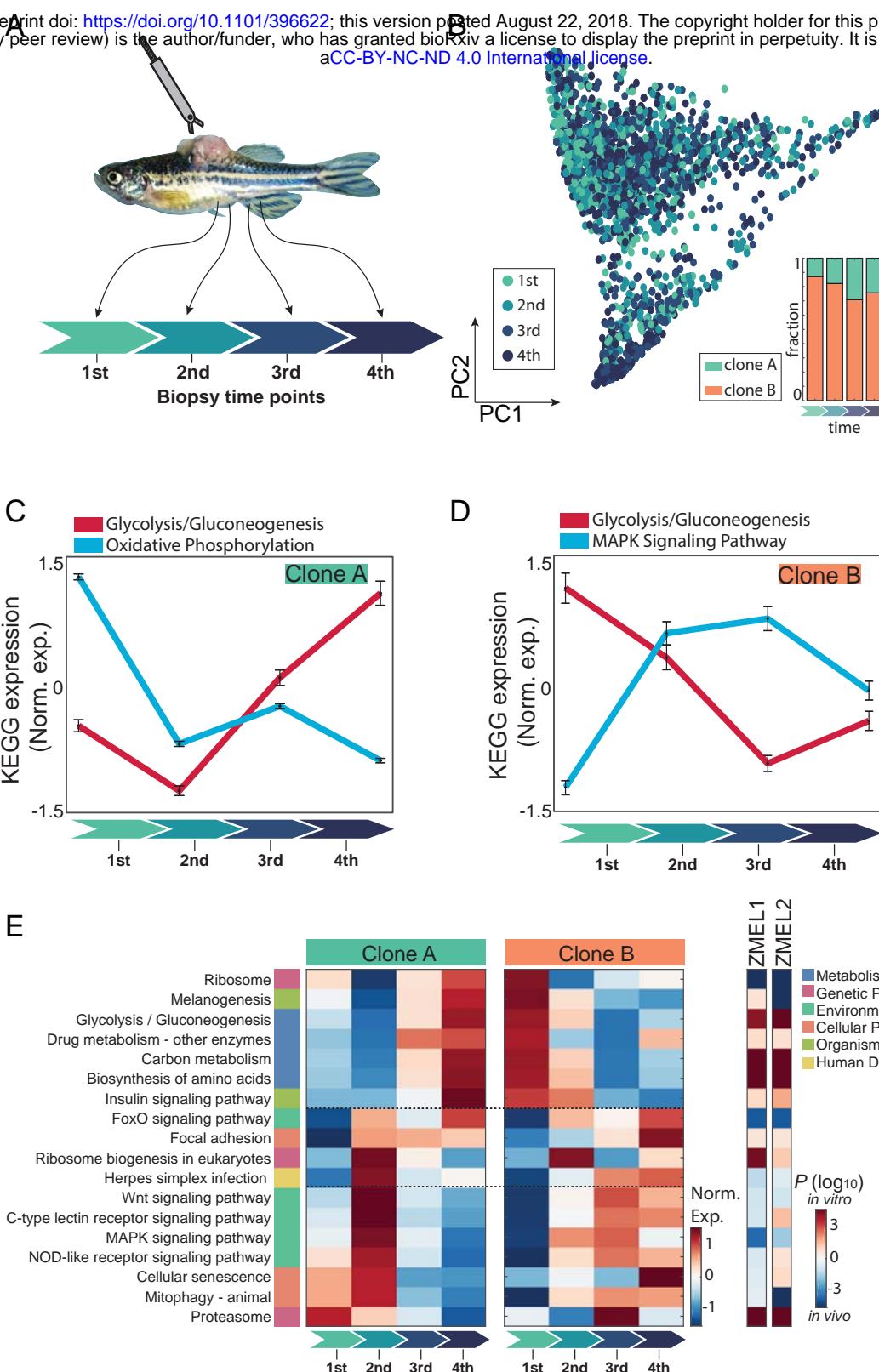


Figure 4. The natural history of clonal gene expression during tumor progression

- (A) Four tumor biopsies were collected in one-week intervals from the same individual fish and each processed using scRNA-Seq, as described in Fig. 1A.
- (B) PCA of the cancer cells, with biopsy time-point indicated by color. The inset shows the distribution of the inferred clone frequency over the biopsies.
- (C-D) For the indicated KEGG pathways, the mean and standard error of the normalized expression across all cells of the indicated clone are shown.
- (E) For the indicated KEGG pathways, the normalized mean expression is shown across the two clones over the four time-points. On the right, the P-values of the enrichment of expression of the genes between in vitro and in vivo culture are shown.

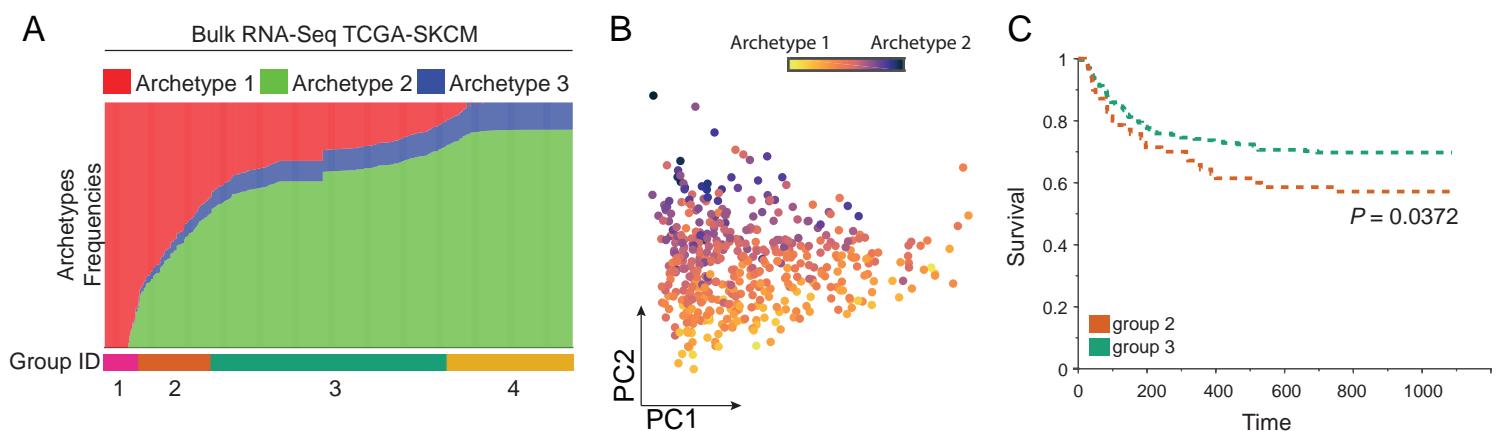


Figure 5. Archetype frequency is correlated with clinical prognosis

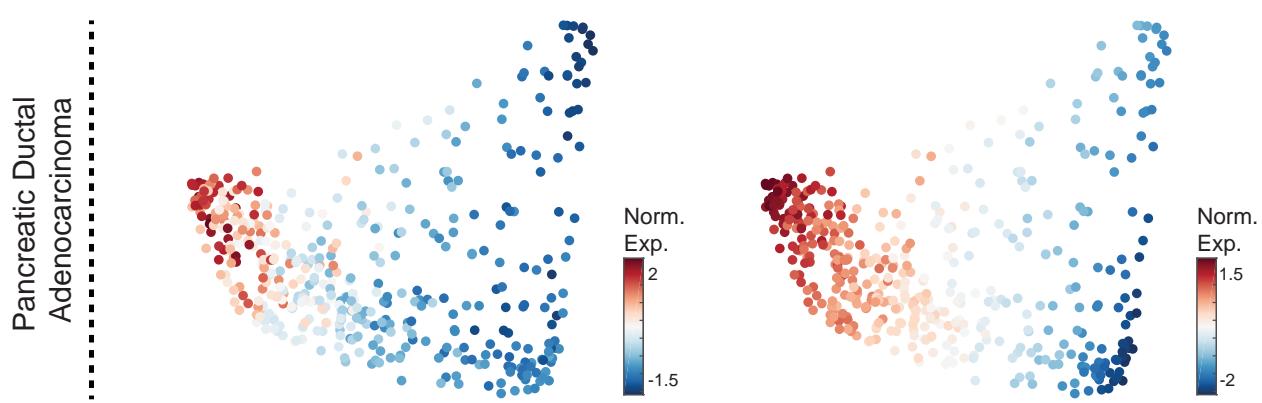
(A) Archetype frequencies in each of 472 bulk tumor from the TCGA Skin Cutaneous Melanoma project. Deconvolution was performed using expression of markers defined in Figure 2 (top panel). Samples were divided to four groups based on their archetype frequencies (lower panel).

(B) PCA of the bulk TCGA RNA-Seq data. Color indicates relative expression of Archetype 1 and 2 markers.
(C) Survival analysis of individuals with tumors high in archetype 1 (group 2) relative to those with tumors high in archetype 2 (group 3). Individuals with tumors high in Archetype 1 (group 2) showed a significantly worse prognosis compared to group 3 individuals (Kaplan-Meier estimate, $P = 0.0372$).

JUN expression

Stress Archetype Signature

A



B

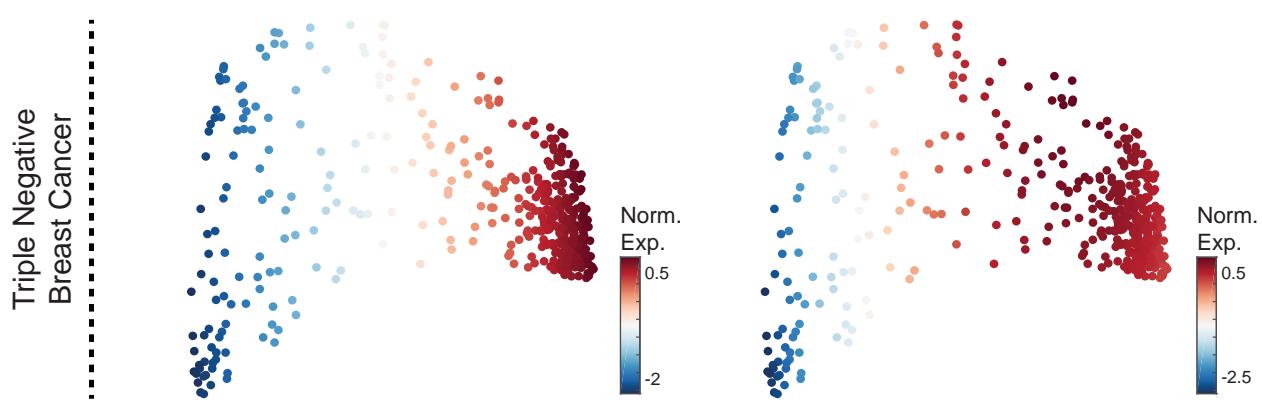


Figure 6. The stress archetype (archetype 3) is conserved across PDAC and TNBC tumors.

(A) PCA on cancer cells from a single PDAC tumor (Moncada et al., 2018). Color indicates normalized expression levels of JUN (left panel) and expression of the stress archetype signature (right panel).

(B) same as (A) for cells from a single TNBC tumor (C. Kim et al., 2018).