NetNorM: capturing cancer-relevant information in somatic exome mutation data with gene networks for cancer stratification and prognosis

Marine Le Morvan, Andrei Zinovyev, Jean-Philippe Vert

NetNorM: capturing cancer-relevant information in somatic exome mutation data with gene networks for cancer stratification and prognosis

Marine Le Morvan[1,2,3], Andrei Zinovyev[2,3,1] and Jean-Philippe Vert[1,2,3]

[1]MINES ParisTech, PSL Research University, CBIO-Centre for Computational Biology, 77300 Fontainebleau, France
[2]Institut Curie, 75248 Paris Cedex 5, France
[3]INSERM, U900, 75248 Paris Cedex 5, France

Abstract

Genome-wide somatic mutation profiles of tumours can now be assessed efficiently and promise to move precision medicine forward. Statistical analysis of mutation profiles is however challenging due to the low frequency of most mutations, the varying mutation rates across tumours, and the presence of a majority of passenger events that hide the contribution of driver events. Here we propose a method, NetNorM, to represent whole-exome somatic mutation data in a form that enhances cancer-relevant information using a gene network as background knowledge. We evaluate its relevance for two tasks: survival prediction and unsupervised patient stratification. Using data from 8 cancer types from The Cancer Genome Atlas (TCGA), we show that it improves over the raw binary mutation data and network diffusion for these two tasks. In doing so, we also provide a thorough assessment of somatic mutations prognostic power which has been overlooked by previous studies because of the sparse and binary nature of mutations.

1 Introduction

Tumourigenesis and cancer growth involve somatic mutations which appear and accumulate during cancer progression. These mutations impair the normal behaviour of various cancer genes, and give cancer cells an often devastating advantage to proliferate over normal cells [1–3]. Systematically assessing and monitoring somatic mutations in cancer therefore offers the opportunity not only to better understand the biological processes involved in the disease, but also to help rationalise patient treatment in a clinical setting. Rationalising treatment involves finely characterising the genomic abnormalities of each given patient to discover which may be treatable by a targeted therapeutic agent, as well as improving prognosis using molecular information [4–6]. The development of fast and cost-effective technologies for high-throughput sequencing in the last decade has triggered the launch of numerous data collection projects such as The Cancer Genome Atlas (TCGA) [7] or the International Cancer Genome Consortium (ICGC) [8], aiming at characterising at the molecular level, including genome-wide or exome-wide somatic mutations, thousands of cancer samples of multiple origins. By systematically comparing the molecular portraits of the resulting cohorts, one might expect to be able to detect frequently mutated genes or groups of genes, and find associations between particular mutations and cancer phenotypes, response to treatment, or survival [9–12].

The analysis of somatic mutation profiles is however challenging for multiple reasons. First, most somatic mutations detected by systematic sequencing are likely to be irrelevant for biological or clinical applications. This is due to the fact that only a few driver mutations are required to confer a growth advantage to the cancer cell, and therefore most somatic mutations are likely to be passenger mutations which do not contribute to the cancer phenotype [3, 13]. Second, sequencing efforts have shown that while a few genes are significantly mutated, the vast majority of genes are mutated in only a handful of patients [14, 15]. As a result, the mutation profiles of two tumours often only share a few if any genes in common. Third, tumours even originating from the same tissue exhibit widely varying mutation rates. Patient mutation rates constitute a strong and informative signal [16–18] but can however complicate the retrieval of more subtle signals. Combined with the inherent high dimensionality of somatic mutation datasets, this makes any statistical analysis of cohorts of whole-exome somatic mutation profiles extremely challenging.

In order to make somatic mutation profiles more amenable to statistical analysis, several studies have used gene networks as prior knowledge [19, 20]. Considering genes in the context of networks instead of analysing them independently allows sharing mutation information among neighbouring genes and identifying disruptions at the level of pathways or protein complexes instead of single genes. A popular method to leverage this prior knowledge consists in using a diffusion process on the gene network. This technique first appeared for the analysis of gene expression and GWAS data [21–25], and has more recently been used for mutation profiles [26–30]. Diffusion processes allow smoothing binary vectors of somatic gene mutations into dense vectors where the mutation status of a gene is increased when it is close to mutated genes in the network. This approach led to state-of-the-art methods for the discovery of driver pathways or complexes [29] and for the stratification of patients into clinically relevant subtypes [30] using whole-exome mutation profiles.

In this work we propose NetNorM, a new method to enhance mutation data with gene networks. NetNorM transforms a patient's binary mutation profile by either removing mutations or creating "proxy" mutations based on the gene network topology, until all patients reach a consensus number of mutations. The resulting mutation matrix is binary like the initial one, nonetheless we establish that it encodes new information relative to genes' local neighbourhood mutational burden and patient mutation rates. Compared to diffusion-based methods, NetNorM exhibits several advantages. First, diffusion relies on a parameter which controls the distance that a mutation signal is allowed to travel on the gene network. Choosing such a parameter is often difficult in practice, and it is unclear how this can be done systematically. By contrast, NetNorM does not require such parameter. Second, while diffusion-based methods transform a very sparse binary mutation matrix into a dense matrix, NetNorM allows integrating somatic mutations with gene networks while keeping the mutation matrix binary and sparse. This feature allows a better interpretability of the data by humans and preserves it from unwanted noise. Finally, current methods for whole exome analysis do not account for the varying background mutation rates of tumours, which can hamper statistical analysis. NetNorM fills this gap by normalising mutation profiles.

We evaluate the relevance of NetNorM on two tasks: survival prediction and patient stratification from exome somatic mutation profiles. In doing so, we also provide a thorough assessment of somatic mutations prognostic power which has been overlooked by previous studies because of the sparse and binary nature of mutations [31]. We show that NetNorm produces state-of-the-art results for these two tasks compared to the raw binary mutation data and to diffusion processes. By comparing results obtained with real versus randomised networks, we further show that the increase in relevance is actually partly driven by the gene's network prior knowledge. However, we observe that considering interactions between mutated genes and their neighbours only is enough do achieve state-of-the-art results, thereby shedding light on the actual radius of influence that can be modelled with current methods.

2 Results

2.1 Overview of NetNorM

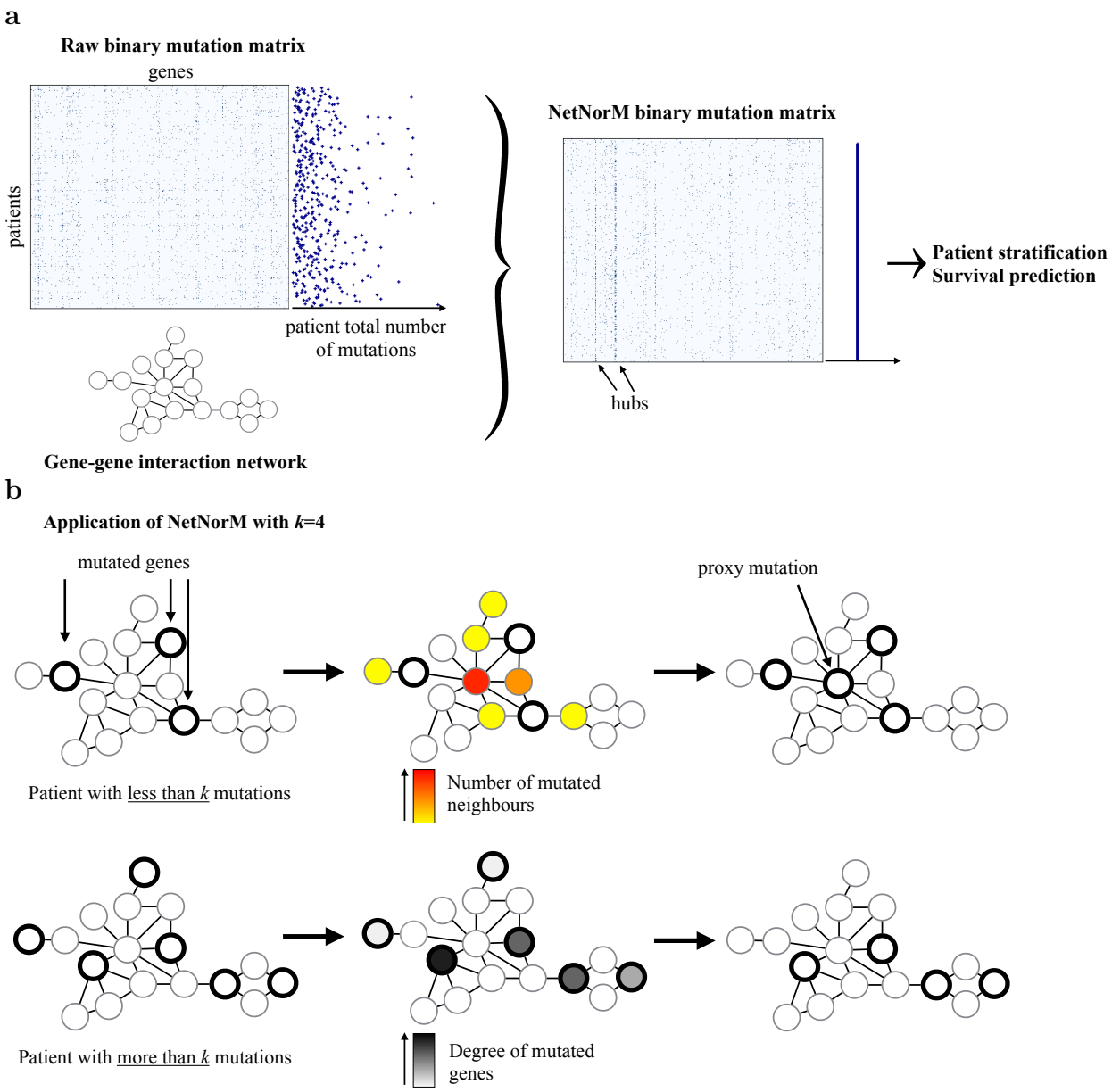

Figure 1 – Overview of NetNorM. (**a**) Using a gene network as background knowledge (lower left), NetNorM normalises each mutation profile in a collection of somatic mutation profiles (upper left) into a new, binary representation (right) which encodes additional information relative to patient mutation rates and hubs' neighbourhood mutational burden. This new representation allows performing patient stratification with unsupervised clustering techniques, or survival analysis. (**b**) NetNorM normalises every patient mutation profile to k mutations. Patients with less than k mutations get 'proxy' mutations in their genes with the highest number of mutated neighbours until they reach k mutations. Patients with more than k mutations have mutations 'removed' in their genes with lowest degree until they reach k mutations.

NetNorM takes as input an undirected gene network and raw exome somatic mutation profiles and outputs a new representation of mutation profiles which allows better survival prediction and patient stratification from mutations (Figure 1). Here and in what follows, the "raw" mutation profiles refer to the binary patients times genes matrix where 1s indicate non-silent point mutations or indels in a patient-gene pair and 0s indicate the absence of such mutations. The new representation of mutation profiles computed with NetNorM also take the form of a binary patients times genes mutation matrix, yet with new properties. While different tumours usually harbour different number of mutations, with NetNorM all patient mutation profiles are normalised to the same number k of genes marked as mutated. The final number of mutations k is the only parameter of NetNorM, which can be adjusted by various heuristics, such as the median number of mutations in the original profiles, or optimised by cross-validation for a given task such as survival prediction. In order to represent each tumour by k mutations, NetNorM adds "missing" mutations to samples with less than k mutations, and removes "non-essential" mutations from samples with more than k mutations. The "missing" mutations added to a sample with few mutations are the non-mutated genes with the largest number of mutated neighbours in the gene network, while the "non-essential" mutations removed from samples with many mutations are the ones with the smallest degree in the gene network. These choices rely on the simple ideas that, on the one hand, genes with a lot of interacting neighbours mutated might be unable to fulfil their functions and, on the other hand, mutations in genes with a small number of interacting neighbours might have a minor impact compared to mutations in more connected genes. In this study, we compare NetNorM to the raw mutation data and to network smoothing (NS) [32] (also called network diffusion, or network propagation) of the mutation profiles over the gene network followed by quantile normalisation (QN) as implemented in [30]. We refer to this method as NSQN below. While both NetNorM and NSQN leverage gene network prior knowledge to enhance mutation data, the two methods have fundamental differences. First, NetNorM leverages only direct neighbourhood information while NSQN spreads mutation information at a more global scale on the gene network. Second, with NetNorM the normalised profiles all have the same value distribution by construction, since they are all binary vectors with k ones, removing the need for further quantile normalisation which, as we discuss below, is critical for NSQN.

2.2 NetNorM gives state-of-the-art prognosis from mutation profiles

To assess the relevance of NetNorM, we first explore the capacity of somatic mutations to predict cancer survival. We collected a total of 3,278 full-exome mutation profiles of 8 cancer types from the TCGA portal (Table 1), censored survival information and clinical data. In parallel we retrieved a gene network to be used as background information for NSQN and NetNorM : Pathway Commons, which integrates a number of pathway and molecular interaction databases [33]. For each cancer type, we use these data to assess how well survival can be predicted from somatic mutations. For that purpose, we perform survival prediction with a sparse survival SVM (see Methods) using either the raw mutation profiles or the profiles normalised by NSQN or NetNorM, respectively, and assess their performance by cross-validation using concordance index (CI) on the test sets as performance metric.

Figure 2 summarises the performance of the survival prediction for the 8 cancer types, when the sparse survival SVM is fed with the raw mutation profile, or with the mutation profiles modified by NSQN or NetNorM using Pathway Common as gene network. For three cancers (LUSC, HNSC, OV), none of the methods manages to outperform a random prediction, questioning the relevance of the mutation information in this context. For BRCA, KIRC and GBM, all three methods are significantly better than random, although the estimated CI remains below 0.57, and we again

Cancer type	Patients	Genes	Deaths	Download date
LUAD (Lung adenocarcinoma)	430	20 596	110	6/22/2015
SKCM (Skin cutaneous melanoma)	307	17 461	129	11/18/2015
GBM (Glioblastoma multiform)	265	14 748	195	11/18/2015
BRCA (Breast invasive carcinoma)	945	16 806	97	11/25/2015
KIRC (Kidney renal clear cell carcinoma)	411	10 608	136	11/25/2015
HNSC (Head and Neck squamous cell carcinoma)	388	17 022	140	11/25/2015
LUSC (Lung squamous cell carcinoma)	169	13 589	70	11/25/2015
OV (Ovarian serous cystadenocarcinoma)	363	10 192	172	11/24/2014

Table 1 – Summary of the full exome mutation profiles used in this study. We analysed a total of $3,278$ samples from 8 cancer types, downloaded from the TCGA portal.

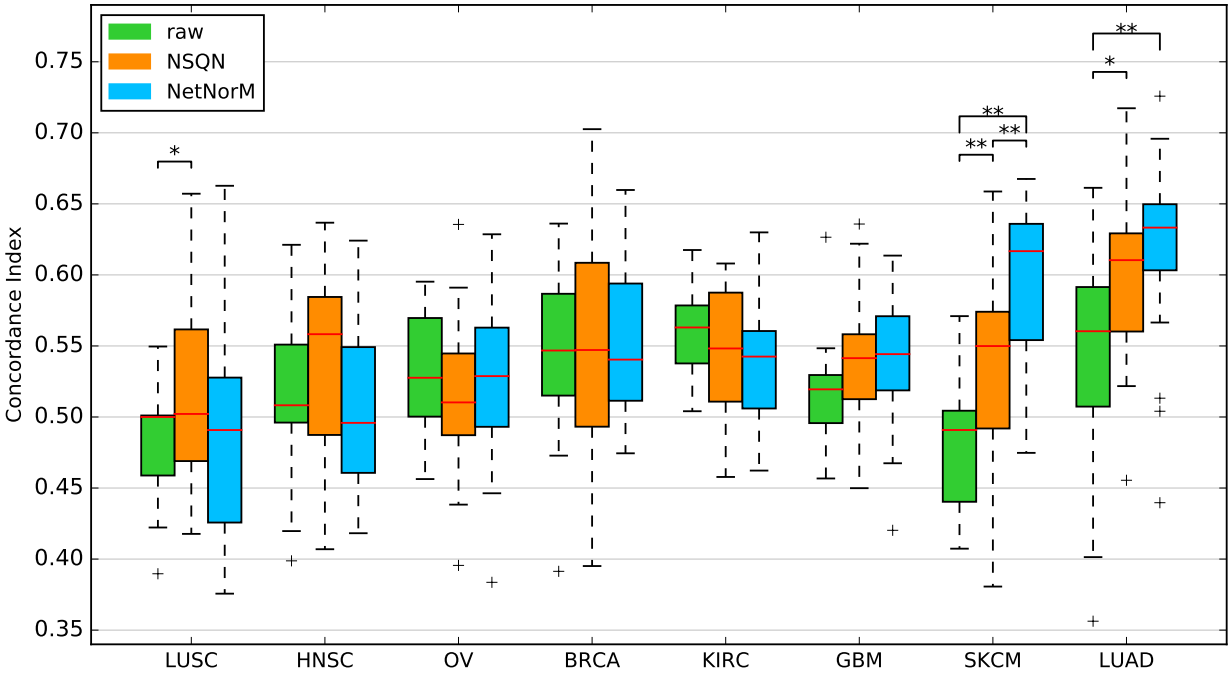

Figure 2 – Comparison of the survival predictive power of the raw mutation data, NSQN and NetNorM (with Pathway Commons as gene network) for 8 cancer types. For each cancer type, samples were split 20 times in training and test sets (4 times 5-fold cross-validation). Each time a sparse survival SVM was trained on the training set and the test set was used for performance evaluation. The presence of asterisks indicate when the test CI is significantly different between 2 conditions (two-sided t-test, $P < 5 \times 10^{-2}$ (*) or $P < 1 \times 10^{-2}$ (**)).

observe no significant difference between the raw data and the data transformed by NSQN or NetNorM. Finally, the last two cases, SKCM and LUAD, are the only ones for which we reach a median CI above 0.6. In both cases, normalising the mutation data with either NSQN or NetNorM significantly improves the performance of models based on the raw data, and in both cases NetNorM outperforms NSQN. More precisely, for SKCM the median CI increases from 0.49 for the raw data to 0.55 for NSQN, and to 0.62 for NetNorM. Both improvements are statistically significant ($P < 0.01$). In the case of LUAD, the median CI increases from 0.56 for the raw data to 0.61 for NSQN and 0.63 for NetNorM. In that case, although the improvement of NetNorM over NSQN is not significant, they are both significantly better than the raw data.

In summary, these results show that for at least 5 out of 8 cancer investigated, somatic mutation profiles have a prognostic value, and that for two of them (SKCM and LUAD) it is possible to improve the prognostic power of mutations by using gene networks and to reach a CI above 0.6. In both cases, NetNorM is at least as good (LUAD) or significantly better (SKCM) than NSQN.

2.3 The biological information encoded in the gene network contributes to the prognosis

To test whether the biological information contained in the gene network plays a role in the improvement of survival predictions, we evaluate again NetNorM and NSQN using 10 different randomised versions of Pathway Commons on SKCM and LUAD. Random networks were obtained by shuffling the nodes of the real network while keeping the structure unchanged. The results, shown on Figure 3, demonstrate that NetNorM performs significantly better with real networks ($P = 3.5 \times 10^{-2}$ for LUAD, and $P = 1 \times 10^{-4}$ for SKCM, two-sided Welsh t-test) and so does NSQN ($P = 2 \times 10^{-3}$ for LUAD, and $P = 1.2 \times 10^{-2}$ for SKCM, two-sided Welsh t-test). This indicates that NetNorM and NSQN do benefit from the biological information encoded in the network.

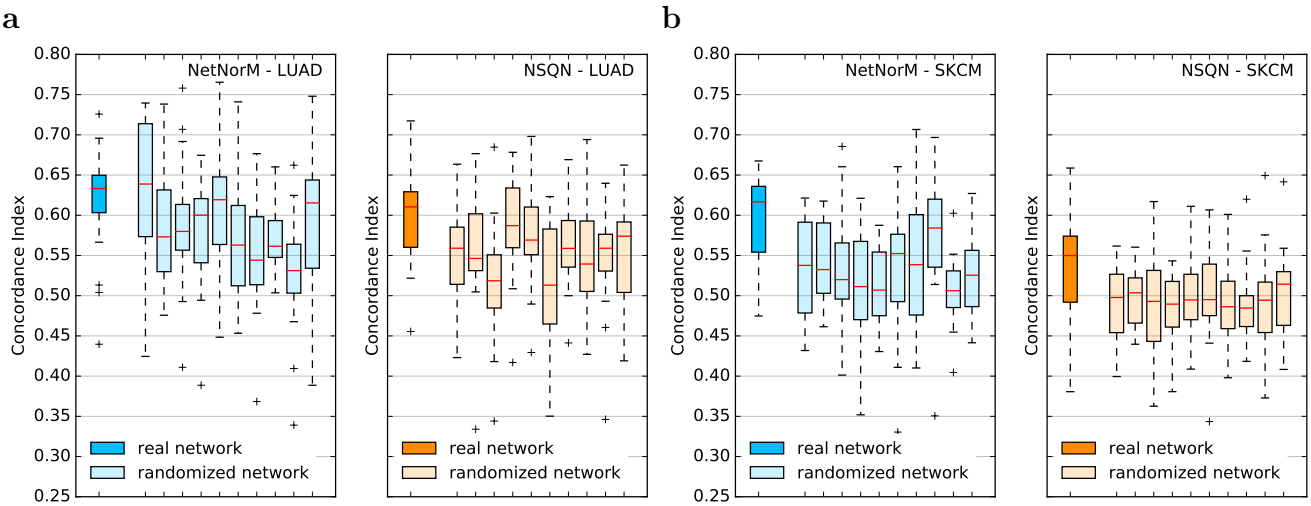

Figure 3 – Effect of network randomisation on survival prediction performances. (**a**-**b**) Performances obtained for 20 cross-validation folds with Pathway Commons (real network) and 10 randomised versions of Pathway Commons (randomised network) with NetNorM (left) and NSQN (right) for LUAD (**a**) and SKCM (**b**).

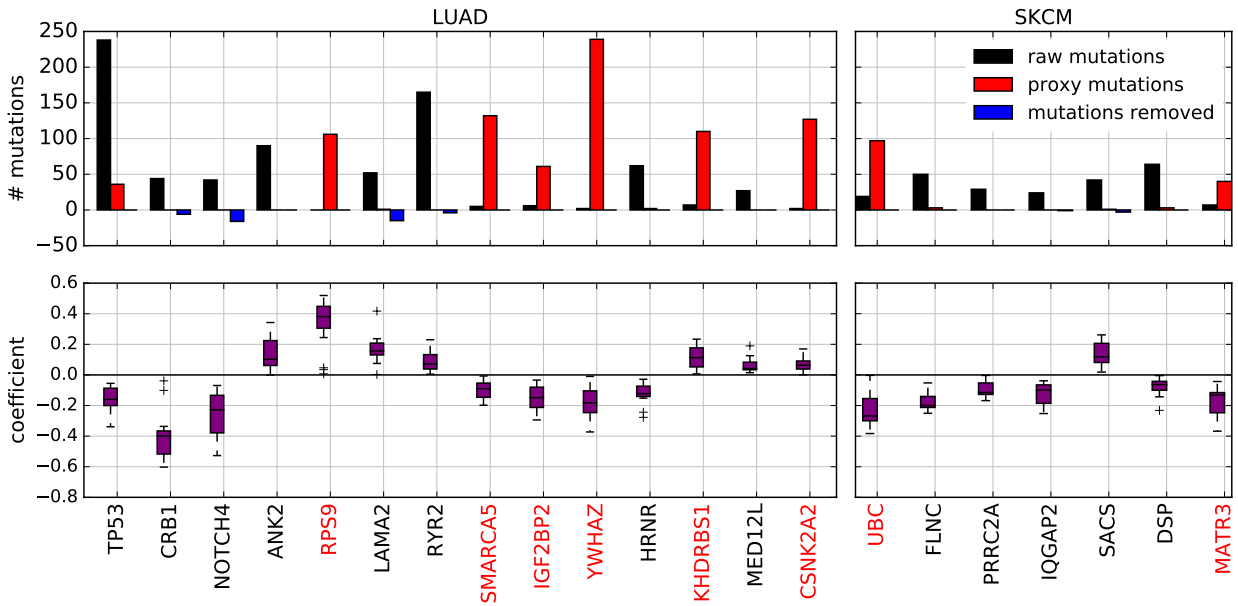

Figure 4 – Genes frequently selected in the survival prediction model for LUAD (left) and SKCM (right) learned using the NetNorM representation of mutations with Pathway Commons as gene network. The genes reported are those that were selected at least 10 times in 20 cross-validation folds. For each cancer, genes are ordered from the most frequently selected (left) to the least frequently selected (right). The top panel reports the number of raw mutations in the selected genes (black), as well as the number of 'proxy' mutations (red) and the number of mutations removed (blue) after application of NetNorM. The bottom panel reports the coefficients of a gene in the survival SVM model across the cross-validation folds where this gene was selected.

2.4 Analysis of predictive genes

In order to shed light on the reasons why NetNorM outperforms the raw data and NSQN on survival prediction for SKCM and LUAD, we now analyse more finely the normalisation carried out by NetNorM on the mutation profiles, and why they lead to better prognostic models. For that purpose, we focus on the genes that are selected at least 50% of the times by the sparse survival SVM during the 20 different train/test splits of cross-validation, after NetNorM normalisation. This leads to 14 frequently selected genes for LUAD and 7 for SKCM (Figure 4). Remembering that NetNorM either removes mutated genes for patients with many mutations, or adds proxy mutations for patients with few mutations, we can assess for each frequently selected gene whether it tends to have proxy mutations or whether it tends to be actually mutated in the tumour. This is done by comparing how frequently it is marked as mutated on the raw data and after NetNorM normalisation (Figure 4, top plot). For both cancers, we observe two clearly distinct groups of frequently selected genes: those that concentrate proxy mutations (which we will call *proxy genes*, in red in Figure 4), and those to which NetNorM brings only few modifications compared to the raw data, meaning they are usually actually mutated in the tumours (in black in Figure 4).

2.4.1 Genes with few modifications imputed by NetNorM

In the case of LUAD, 8 out of the 14 selected genes are non-proxy genes, meaning they tend to be really mutated when they are marked as mutated after NetNorM normalisation. Interestingly, mutations in half of these genes are predictive of an increased survival time (corresponding to a

positive coefficient in the sparse survival SVM) while mutations in the other half are predictive of a decreased survival time (corresponding to a negative coefficient) (Figure 4, bottom plot). The three most important predictors according to their weight in the model and their frequency of selection include TP53 (selected in 95% of the folds, median coefficient -0.16), CRB1 (selected in 90% of the folds, median coefficient -0.4) and NOTCH4 (selected in 85% of the folds, median coefficient -0.23) and are all predictive of a decreased survival time. TP53 is a well-known cancer gene and has been reported as significantly mutated in LUAD [34, 35]. NOTCH4 is part of the NOTCH signalling pathway which has been widely implicated in cancer and shown to act as both oncogene or tumour suppressor depending on the context [36]. Finally, CRB1 is known to localise at tight junctions but little is known about its role in carcinogenesis [37]. In the case of SKCM, 5 out of the 7 selected genes are genes with few modifications. This includes 4 genes whose mutations are predictive of a decreased survival time (FLNC, PRRC2A, DSP, IQGAP2), and one whose mutations are predictive of an increased survival time (SACS). Among these genes, Filamin C (FLNC) is a large actin-cross-linking protein which has been shown to inhibit proliferation and metastasis in gastric and prostate cancer cell lines [38]. Desmoplakin (DSP) is required for functional desmosomal adhesion which has been linked to cancer cells development and progression in several cancers [39, 40]. Moreover IQGAP2 has been identified as a tumour suppressor gene in hepatocellular carcinoma, gastric and prostate cancers [41].

2.4.2 Proxy genes

In addition to somatically mutated genes, several proxy genes, mutated by the NetNorM procedure, are often selected by the survival model. The proxy genes for LUAD are KHDRBS1, IGF2BP2, SMARCA5, YWHAZ, RPS9, CSNK2A2 and for SKCM they are UBC and MATR3. These genes are among the biggest hubs in the network. This is expected as proxy mutations are imputed in genes with a lot of mutated neighbours, which is more likely to occur for genes that simply have a lot of neighbours. The fact that these proxy genes were selected in the survival models means that they have some prognostic power. In particular for LUAD, the better prediction performances achieved by NSQN and NetNorM compared to the raw data are largely explained by better predictions made for the half of patients with fewer mutations, and therefore in the case of NetNorM by the proxy mutations that were created in these patients (Figure 5a).

The prognostic power of proxy genes in NetNorM comes from at least two types of information they capture. First, proxy mutations act as proxies for the total number of mutations in a patient. Patients harbouring proxy mutations are significantly less mutated than those without proxy mutations (Welsh t-test, $P \leq 1 \times 10^{-2}$) in a given proxy gene. This results from the fact that patients with few mutations receive as many proxy mutations as needed to reach the target number of mutations k, and therefore proxy mutations have a higher probability to be set in low mutation rate patients. Second, proxy mutations act as proxies for genes' neighbourhood mutational burden (NMB). When we look at which patients get mutated in a given gene after NetNorM normalisation (red dots in Figure 5b), we observe that they tend to have more mutations in the neighbours of this gene than what the pure mutation rate would predict (represented by the regression line in Figure 5b). In other words, among the hubs that could get mutated by NetNorM for patients with few mutations, the ones that get mutated tend to be the ones surrounded by more mutations than expected given the mutation rate of the person: NetNorM thus creates proxy mutations when a gene's NMB is higher than expected.

Among the proxy genes selected in LUAD (resp. SKCM), IGF2BP2, SMARCA5 and YWHAZ (resp. UBC and MATR3) define groups of patients with significantly different survival outcomes (log-rank test, $P \leq 1 \times 10^{-2}$) . Given the discussion in the previous paragraph, this may be due

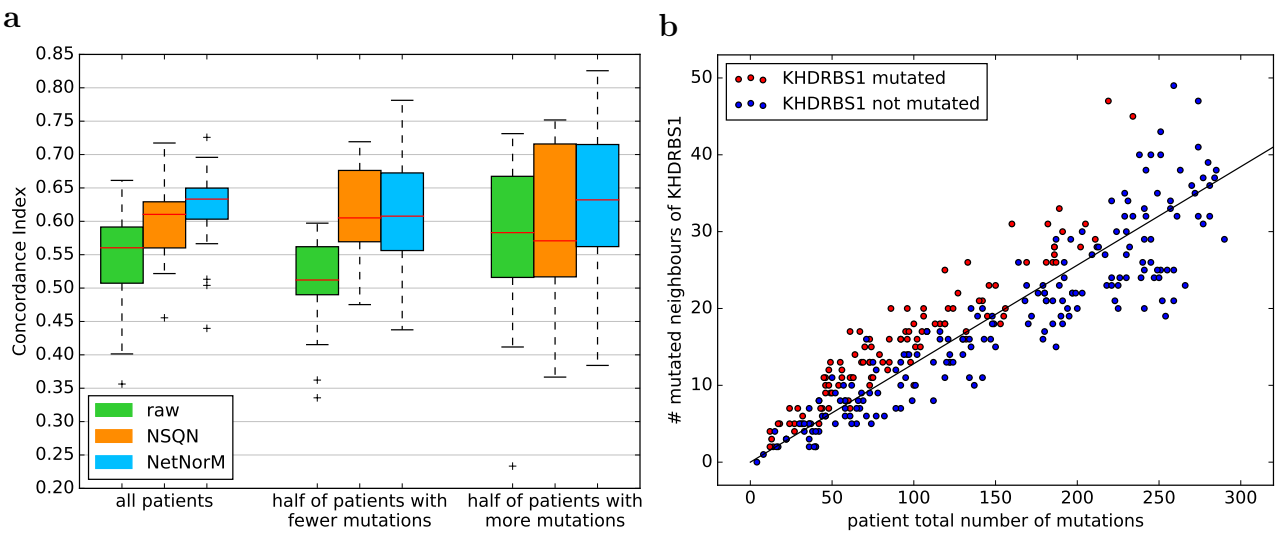

Figure 5 – Analysis of predictive genes. (**a**) Comparison of survival prediction performances according to patients' mutation rate for LUAD. Three different representations of the mutations are used to perform survival prediction using a ranking SVM: *raw* (the raw binary mutation data), *NSQN* (network smoothing with quantile normalisation) and *NetNorM*. Performances for half of the patients with fewer (resp. more) mutations are derived from the predictions made using the whole dataset. (**b**) Scatter plot of the total number of mutations in a patient of the LUAD cohort (x-axis) against the number of mutated neighbours of KHDRBS1 in a patient (y-axis). Only patients with less that $k_{med} = 295$ mutations are shown, where k_{med} is the median value of k learned across cross-validation folds. Red (resp. blue) indicate patients mutated (resp. non mutated) in KHDRBS1 after processing with NetNorM using $k = k_{med}$. The black line was fit by linear regression and by definition indicates the expected number of mutated neighbours of KHDRBS1 given the mutation rate of a patient.

to differences in global mutation rates between patients, to differences in NMB for some genes, or to both effects. To clarify the contributions of each effect, we investigate whether such distinct survival outcomes can be obtained with proxies for the total number of mutations only, regardless of NMBs. To this end, we simulate proxy mutations according to a probability depending on patients' total number of mutations only. By contrast, NetNorM mutates genes according to patients' total number of mutations and according to genes' NMB. Then for each gene we compare the survival outcomes of the obtained subgroups (patients which were imputed a proxy mutation versus those that were not) using a log-rank test and examine whether the log-rank statistic is higher with NetNorM than with the simulations (see Methods for more details). We find that all of IGF2BP2, SMARCA5, YWHAZ and MATR3 produce groups with a significantly higher log-rank statistic with NetNorM than with their simulated counterpart (log-rank test, $P \leq 1 \times 10^{-2}$). This clarifies that the prognostic information captured by proxy mutations with NetNorM combines the overall mutation rate of the patient with local mutation burden on the gene network.

2.5 NetNorM allows stable unsupervised stratification of patients with significantly different survival curves

We now assess the possibility to stratify patients into a small number of groups in an unsupervised way, meaning without using survival information, in order to identify distinct subgroups of patients in terms of mutational profiles. For that purpose, we use a standard unsupervised clustering pipeline based on nonnegative matrix factorisation (NMF), and apply it to the different cohorts of patients

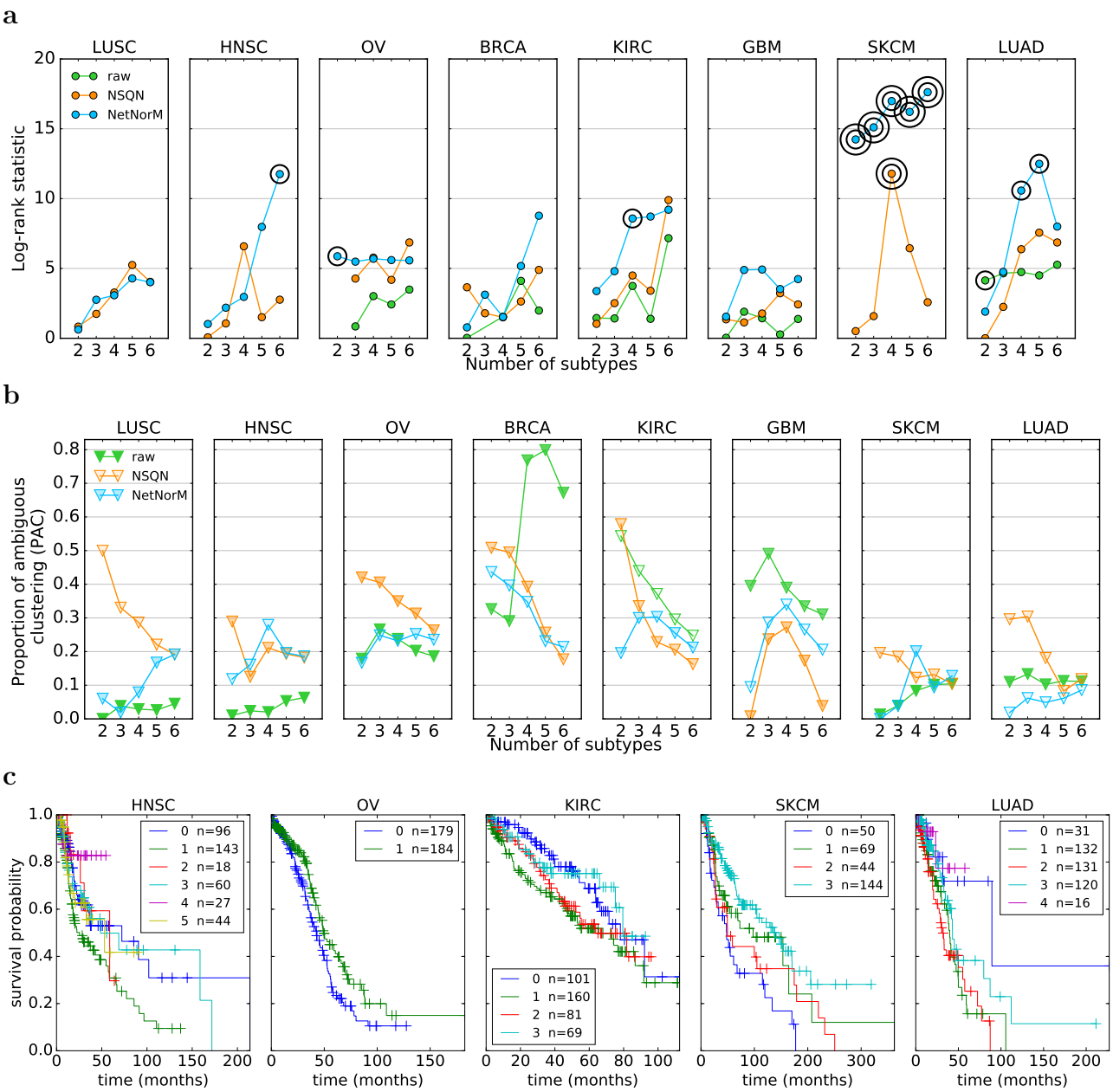

Figure 6 – Comparison of patient stratifications obtained with the raw mutation data, NSQN (Pathway Commons) and NetNorM (Pathway Commons) for 8 cancer types. (**a**) Association of patient subtypes with survival time. One circle indicates $P \leq 0.05$ and two concentric circles indicate $P \leq 0.01$ (log-rank test). Cases where clusters were too unbalanced (95% of the patients in one single cluster) are not shown. (**b**) Evaluation of the clustering stability as measured by the proportion of ambiguous clustering (PAC). The transparency of the triangles indicate the percentage of patients in the largest cluster. The scale ranges from 100% (totally opaque) to $\frac{1}{N}$ % (totally transparent) where N is the number of subtypes. Therefore opacity (resp. transparency) indicate unbalanced (resp. balanced) clusters. (**c**) Kaplan Meir survival curves for NetNorM subtypes with significantly distinct survival outcomes. In the legend are indicated the subtype number followed by the number of patients in the subtype.

represented by the raw mutation profiles, or the profiles normalised by NSQN or NetNorM. As we have no ground truth regarding "true" groups of patients, we assess the quality of clustering by two factors: (i) the stability of the clusters, assessed by the proportion of ambiguous clustering (PAC) which is the rate of discordant cluster assignments across 1,000 random subsamples of the full cohort; and (ii) the significance of association between clusters and survival.

With the raw data, NMF tends to stratify patients into very unbalanced subtypes with typically one subtype gathering the majority of patients (Figure 6b). LUSC, HNSC and SKCM are extreme cases where one cluster contains 95% of the patients, whatever the number of clusters. In addition, in cases where the obtained clusters are reasonably balanced as for KIRC, the clustering stability is low. These results are coherent with [30] who highlighted the difficulty to cluster raw mutation profiles. These undesirable behaviours disappear with both NSQN and NetNorM (Figure 6). For both methods the obtained clusters are reasonably balanced across all cancers and the clusters are pretty stable ($PAC \leq 30\%$) for most cancers. However for small number of subtypes (between 2 and 4), the clustering stability is generally better with NetNorM than with NSQN. To assess the clinical relevance of the obtained subtypes, we test whether they are associated with significantly distinct survival outcomes (Figure 6a). With the raw data, patient stratification is never significantly associated with clinical data. With NetNorM, significant associations of patient subtypes with survival times are achieved for HNSC, OV, KIRC, SKCM and LUAD (Figure 6c), while with NSQN, a significant association is only achieved for LUAD. The stratification based on NetNorM remains prognostic beyond clinical data for LUAD and SKCM (Likelihood ratio test, $P = 1.5 \times 10^{-2}$ (LUAD, $N = 5$), $P = 5.5 \times \times 10^{-2}$ (SKCM, $N = 4$)). Overall, these results confirm the findings of [30] that network-based normalisation with NSQN allows stratifying patients better than the raw mutation profiles, and also show that the stratification obtained from NetNorM normalisation is both more stable and more clinically relevant than the one obtained with NSQN.

2.6 Patient stratification with randomised networks

We now assess whether the biological information contained in Pathway Commons is crucial to obtain subtypes with significantly distinct survival outcomes. For that purpose, we carry out patient stratification with NSQN and NetNorM using 10 randomised versions of Pathway Commons for both LUAD and SKCM. As for the survival prediction experiment, the randomisation involves shuffling the vertices of the network so as to keep its structure unchanged. For SKCM with Pathway Commons, subtypes with significantly different survival times occur for any number of subtypes with NetNorM and for 4 subtypes with NSQN. In these cases, network randomisation lowers the log-rank statistic obtained with NSQN but, surprisingly, not that obtained with NetNorM (Figure 7). This suggests that although NetNorM generates subtypes with more distinct survival times than NSQN, it does not benefit from Pathway Commons gene-gene interaction knowledge in the case of SKCM, but rather exploits the global mutation rate of each patient captured by proxy mutations. Regarding LUAD with Pathway Commons, only NetNorM produces subtypes with significantly different survival times with 4 and 5 clusters. In these two cases, the non-randomised network yields the subtypes with the most distinct survival times (Figure 7). This indicates that for LUAD, NetNorM takes advantage of gene-gene interaction knowledge to stratify patients into clinically relevant subtypes.

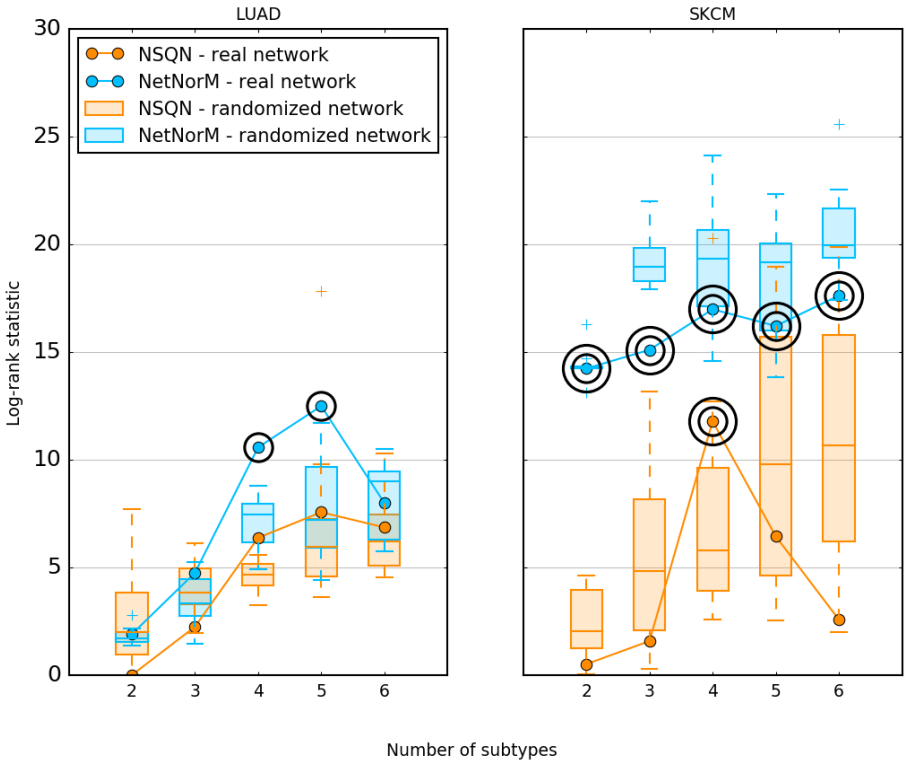

Figure 7 – Effect of network randomisation on patient stratification. Log-rank statistic obtained with Pathway Commons (curve) and 10 randomised versions of Pathway Commons (boxplots) with NetNorM (blue) and NSQN (orange) for LUAD (left) and SKCM (right). One circle indicate a P-value $P \leq 5 \times 10^{-2}$ and two concentric circles indicate $P \leq 1 \times 10^{-2}$.

2.7 Patient subtypes obtained with NetNorM are characterised by distinct pathways

To interpret biologically the subgroups of patients identified by automatic stratification after NetNorM normalisation, we look at differentially mutated genes and pathways across subtypes. We focus on LUAD as a proof of principle with N=5 groups, which is when subtypes have the most distinct survival times according to the log-rank test. As the basis vectors or "metapatients" yielded by the NMF summarise the mutational patterns found in the different subtypes, we analyse genes in terms of their weight in the different metapatients, and restrict our attention to the approximately 900 genes displaying highest variance (variance greater than 0.01) across basis vectors since these genes are expected to be the most differentially mutated across subtypes. Interestingly, this gene list comprises most significantly mutated genes in LUAD including to TP53, KRAS, KEAP1, EGFR, LRP1B, NF1, RB1 [34, 35]. To analyse these genes we cluster them into groups with similar weights across basis vectors using hierarchical clustering (Figure 8b), and we test for enrichment in known biological pathways the 20 gene clusters (GCs) obtained.

One first observation is that the 5 patient subtypes have distinct mutation rates with groups 4 and 5 (resp. 1 and 2) gathering high (resp. low) mutation rates patients (Figure 8a). This confirms the fact that NetNorM-normalised profiles contain information about the initial number of mutations, although they are normalised to a fixed number of mutations. More importantly, most GCs exhibit high weights in one metapatient and low weights in others, suggesting that they are mainly enriched in mutations in one single patient subtype (Figure 8b). χ^2 contingency tests

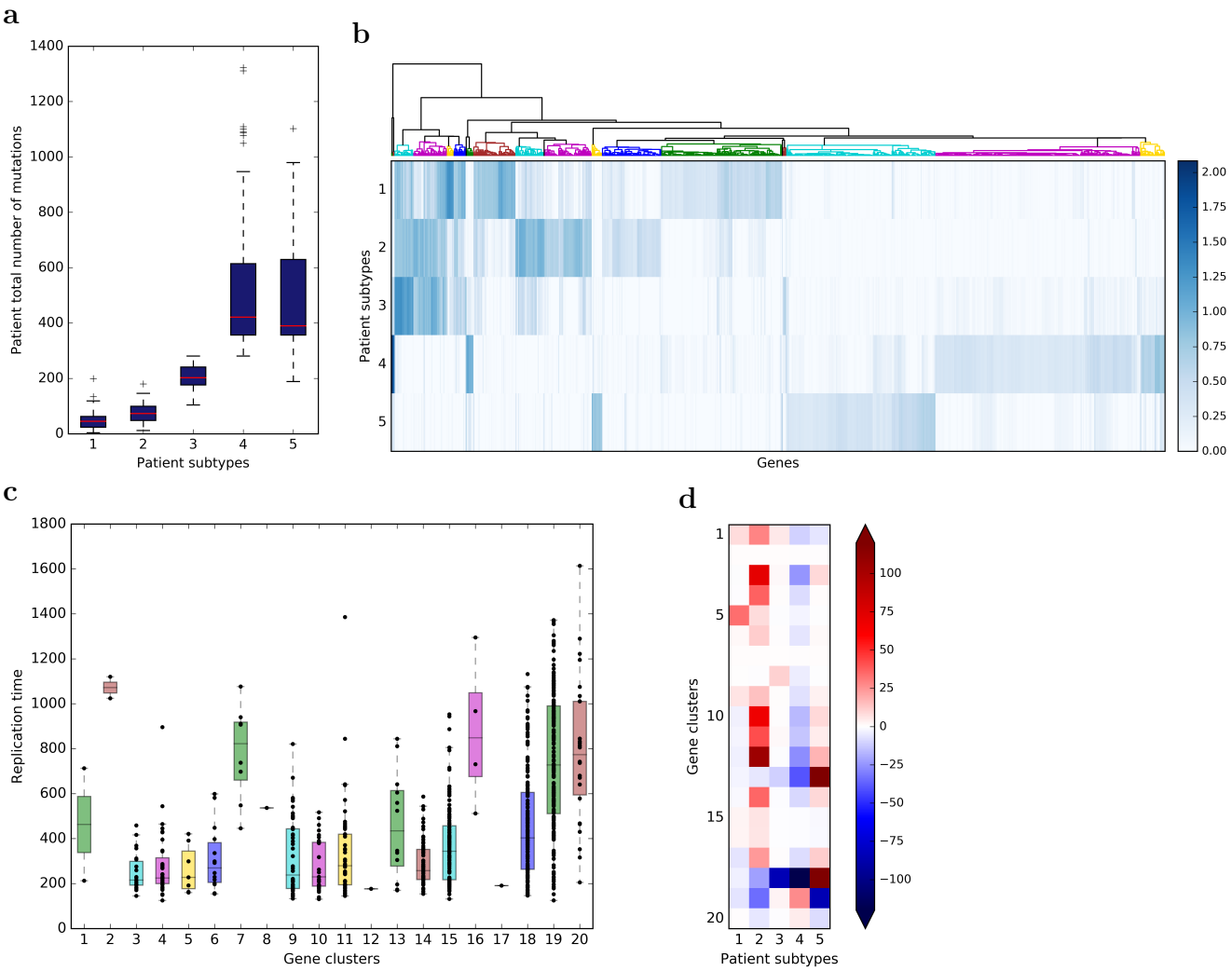

Figure 8 – Characterisation of LUAD patient subtypes obtained with NetNorM ($k = 5$ groups, Pathway Commons). (**a**) Distribution of patients' total number of (raw) mutations across patient subtypes. (**b**) Metapatients matrix obtained by applying NMF to mutation profiles processed with NetNorM. The matrix shown is restricted to the genes with highest variance across metapatients. The genes (columns) are clustered via hierarchical clustering. Clusters are numbered from 1 to 20 from left to right. (**c**) Distribution of gene replication times across gene clusters. (**d**) A χ^2 contingency test was performed for each gene cluster to test its enrichment (or depletion) in mutations across patient subtypes given the subtypes' marginal number of mutations. The value represents the contribution of a subtype to the test statistic, and the colour indicates an enrichment (red) or a depletion (blue) in mutations.

(see methods) for each GC confirms that for most of them (18/20), the distribution of the mutations across patient subtypes is not that expected according to subtypes' mutational rates ($P < 5 \times 10^{-2}$). The contribution of each subtype to the test statistic for each GC also confirms that GCs are often enriched in mutations in mainly one patient subtype (Figure 8d). Subtypes could thus easily be associated with one or several GCs, and therefore pathways through pathway enrichment analysis using the KEGG database [42] (see Methods). Consequently, subtype 1 is characterised by an enrichment in mutations in genes involved in endocytosis, cell adhesion and vesicular transport (GC 5). Subtype 2 is enriched in genes associated with ribosomes and spliceosomes (GCs 3, 4, 10, 11, 12, 14). Subtype 4 is mainly enriched in late replicating genes (GC 19, 20) (Figure 8c). This reflects the fact that subtype 4 is enriched in high mutation rate patients as there exists a positive correlation between somatic mutation frequency and genes replication time [16]. Finally, although subtype 5 also include high mutation rate patients, it is not enriched in late replicating genes, indicating that the high mutational load carried by subtype 5 is not distributed similarly to that of subtype 4 throughout the genome. Rather, subtype 5 is very strongly associated with gene clusters 18 and 13. Gene cluster 18 is enriched in genes from the ABC transporters pathway. Interestingly, ABC transporters act as efflux pumps and their expression is known to confer multi-drug resistance by increasing drug efflux from the cell [43]. Gene cluster 13 could not be significantly associated to a known biological pathway. However it contains FANCD2 (Fanconi Anemia Complementation Group D2) which is involved in double-strand breaks DNA repair and the maintenance of chromosomal stability [44]. We note that 12 of the 16 patients in subtype 4 present the same 4-nucleotides splice site deletion in FANCD2, whereas across the rest of the 430 patients FANCD2 is mutated in 6 patients only, and only one of these 6 mutations is the same as that observed in subtype 4 patients.

3 Discussion

Exploiting the wealth of cancer genomic data collected by large-scale sequencing efforts is a pressing need for clinical applications. Somatic mutations are particularly important since they may reveal the unique history of each tumour at the molecular level, and shed light on the biological processes and potential drug targets dysregulated in each patient. Standard statistical techniques for unsupervised classification or supervised predictive modelling perform poorly when each patient is represented by a raw binary vector indicating which genes have a somatic mutation. This is both because the relevant driver mutations are hidden in the middle of many irrelevant passenger mutations, and because there is usually very little overlap between the somatic mutation profiles of two individuals. NetNorM aims to increase the relevance of mutation data for various tasks such as prognostic modelling and patient stratification by leveraging gene networks as prior knowledge.

One important aspect of NetNorM is the property that, after normalisation, all patients have the same number of 1's in their normalised mutation profile. Although there is no biological rational for this constraint, we believe that the fact that all normalised samples have the same distribution of values is an important property for many high-dimensional statistical methods such as survival models or clustering techniques to work properly. To support this claim, we notice that the Network-based stratification (NBS) method proposed in [30] performs a quantile normalisation step after network smoothing. To investigate whether the quantile normalisation step in NSQN plays an important role, we applied network smoothing without quantile normalisation (called NS) and performed survival prediction and patients stratification with this representation of the mutations. Regarding survival predictions for LUAD, surprisingly NS does not improve over the raw mutation profiles, while for SKCM the performance of NS is between those of the raw data and NSQN (Figure 9c). Moreover just as the raw data, NS is unable to stratify patients into approximately

balanced clusters (Figure 9b). This suggests that quantile normalisation plays a crucial role in the performances obtained with NSQN, in spite of non obvious biological justification for this step.

Another important difference between NSQN and NetNorM is the fact that NetNorM only exploits mutation information about direct neighbours in the network, while NSQN can potentially diffuse a mutation further than the direct neighbours. However, we found that NSQN does not benefit from this possibility. Indeed, we tested a simplified version of NSQN where the network propagation is stopped after one iteration, and assessed the performance of the corresponding method which we call SimpNSQN. For survival prediction, we observe no significant difference between NSQN and SimpNSQN (Figure 9c). For patient stratification, SimpNSQN produces subtypes that are vey similar to that produced by NSQN (Figure 9d), notably for LUAD. Therefore the subtypes generated by both methods associate equally well to clinical data, and even slightly better for SimpNSQN in the case of LUAD (Figure 9a). Overall, these pieces of information indicate that the useful information created by NSQN is mostly concentrated on shared mutated order 1 neighbourhoods, and explain why we observe no loss in performance with NetNorM which explicitly restricts the diffusion of mutations to direct neighbours only. More generally, these elements also indicate that diffusion to indirect neighbours is still difficult with current methods. This is a likely consequence of the small world property of biological graphs [45]. Because the path between any two genes is usually short, diffusion even to order-2 neighbours reaches a substantial number of genes, and therefore the resulting signal observed for one gene is the superposition of a large number of signals originating from close mutations.

NetNorM encodes information about patients' total number of mutations in the raw data, and potentially can exploit it if this information is relevant for the problem at hand. However we found that the raw number of mutations is a poor predictor or survival (Figure 9c), and a poor feature for LUAD patient stratification (Figure 9a). This confirms that NetNorM conserves useful information regarding both the total mutation rate of a patient and the distribution of the mutations on the gene network, and manages to leverage both types of information. In addition to mutation rates, NetNorM also encodes information about genes' NMB which proved to carry some prognostic power. The fact that NMB might reveal new insights into mutation profiles is an emerging idea supported by this study. Further support has been formalised with two recently published methods [46, 47] which rely on NMB to achieve state-of-the-art performances for cancer gene discovery.

We emphasize that randomised gene networks lead to significantly worse performances than the real network for survival prediction as well as for patient stratification in the case of LUAD. While it is not always clear whether incorporating gene networks as prior knowledge does help for a given task, this provides a sound argument that such prior knowledge is effectively leveraged with NetNorM. This raises the question of how the choice of the gene network impacts the performances. Besides Pathway Commons, we tested NetNorM with the Human Protein Reference Database (HPRD) [48] and BioGRID [49] and observed decreased performances in the context of survival prediction. As Pathway Commons is 1 to 2 orders of magnitude denser than the other 2 networks, we hypothesise that a gene network with a high sensitivity is better than one with a high specificity for NetNorM.

Increasing the relevance of mutation data to various tasks is a broad project and NetNorM could be extended in many ways. First, although NetNorM was successful for LUAD and SKCM, we note that the method brings few improvements compared to the raw data for the remaining cancer types. Therefore extensive efforts are needed to determine whether it is possible to design representations of mutations that would increase the statistical power of models learned on these datasets. Second, NetNorM does not integrate further information about mutations such as their predicted functional impact. A possible extension could therefore include this type of information. Finally, the final distribution of values for the normalised profiles is defined as the mean distribution

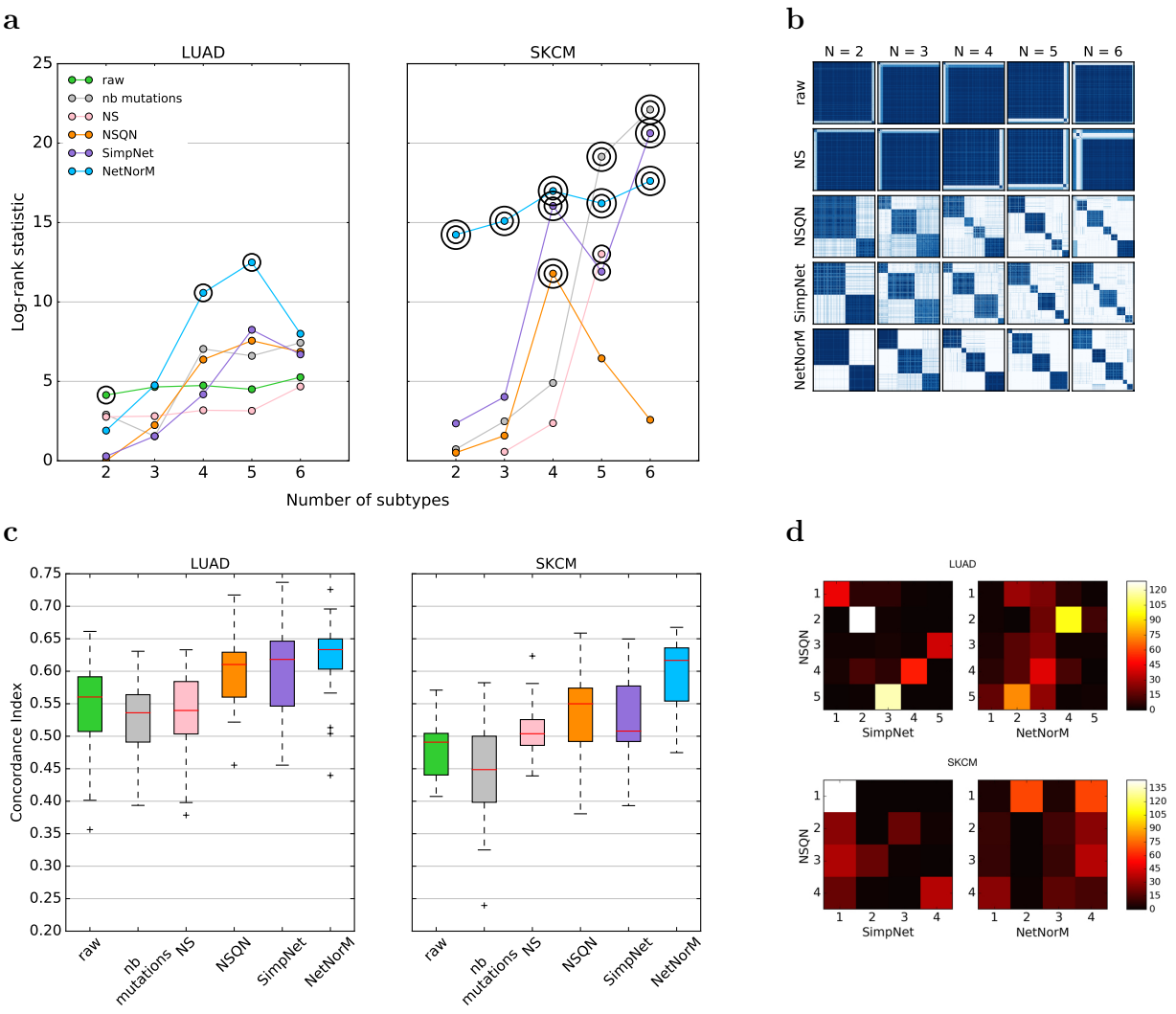

Figure 9 – Exploring NSQN and NetNorM performances levers. (**a**) Subtypes log-rank statistic obtained for LUAD (left) and SKCM (right). One circle indicate a P-value $P \leq 5 \times 10^{-2}$ and two concentric circles indicate $P \leq 1 \times 10^{-2}$ (log-rank test). (**b**) Consensus clustering matrices for LUAD. (**c**) Survival prediction performances for LUAD (left) and SKCM (right). (**d**) Confusion matrices for LUAD (top) and SKCM (bottom) comparing the subtypes obtained with NSQN and SimpNet on the one hand, and NSQN and NetNorM on the other hand. (**a**, **b**, **c**, **d**) were obtained with Pathway Commons.

of the original profiles in the case of NSQN, and simply a binary vector with a fixed number of 1's in the case of NetNorM, however these choices are empirical. This suggests that an interesting future work may be to assess more precisely the effect of this distribution and, perhaps, optimise it for each specific task.

4 Methods

4.1 Patient mutation profiles preprocessing

Whole exome somatic mutation calls (MAF files) were downloaded from TCGA data portal (`https://tcga-data.nci.nih.gov/tcga`) for 8 cancer types (LUAD, SKCM, GBM, BRCA, KIRC, HNSC, LUSC, OV) (Table 1). The data include point mutations (single nucleotide polymorphism as well as di/tri/oligo-nucleotide polymorphism) and indels. Silent mutations were filtered out and mutations profiles were defined as binary vectors with ones whenever a patient is mutated in a given gene and zeros otherwise.

4.2 Gene-gene interaction network

Pathway Commons (http://www.pathwaycommons.org/pc2/downloads) was used throughout this work (Pathway Commons v6, SIF format). It integrates gene networks from several public databases and aggregates both genetic and protein-protein interactions (PPIs). PPIs refer to physical contacts established between proteins while genetic interactions refer to interactions through regulatory and signalling pathways. To remove interactions involving small molecules in Pathway Commons, the following interaction types were filtered out: "consumption-controlled-by", "controls-production-of", "controls-transport-of-chemical", "chemical-affects", "reacts-with", "used-to-produce", "SmallMoleculeReference", "ProteinReference;SmallMoleculeReference", "ProteinReference". We obtained a network with 16,674 nodes (genes) and 2,117,955 edges (interactions).

4.3 Network based Normalisation of Mutation profiles (NetNorM)

NetNorM is a method that integrates patients mutation profiles with a gene network to produce normalised mutation profiles where all patients have the same number k of mutations. The target number of mutations k is a tuning parameter. In the context of survival prediction (supervised setting), it is learned by cross-validation while for patient stratification (unsupervised setting), it is set as the median best k learned across cross-validation folds for survival prediction. Concretely, NetNorM defines a ranking over genes separately for each patient and then use this ranking to normalise mutation profiles. The ranking defined in NetNorM is obtained with a simple two-step procedure. First, genes are ranked according to their mutation status with mutated genes ranked higher than non mutated genes. Then, mutated genes are ranked according to their degree (i.e. their number of neighbours) and non mutated genes are ranked according to their number of mutated neighbours. The normalisation is then obtained by considering the k highest ranked genes as mutated while the rest of the genes will be considered non mutated. By construction, mutated genes are always ranked higher than non-mutated genes. Therefore patients with a lot of mutations will have mutations removed while patients with few mutations will be artificially mutated. Note that when the obtained ranking contains ties, all genes are given distinct ranks according to the order in which they occur in the mutation matrix.

4.4 Network smoothing with quantile normalisation (NSQN)

Network smoothing propagates the influence of mutations over gene-gene interaction networks. It was implemented according to the following update function [30]:

$$\boldsymbol{X_{t+1}} = \alpha \boldsymbol{X_t} \boldsymbol{D}^{-\frac{1}{2}} \boldsymbol{A} \boldsymbol{D}^{-\frac{1}{2}} + (1-\alpha)\boldsymbol{X_0}$$

where $\boldsymbol{X_t}$ is the patient $\times$ genes mutation matrix at iteration t, $\boldsymbol{X_0}$ is the initial binary mutation matrix, $\boldsymbol{A}$ is the adjacency matrix representing the network used and $\boldsymbol{D}$ is the diagonal degree matrix where $D_{ii} = \sum_j A_{ij}$. α is a tuning parameter controlling the length of diffusion paths over the network. Similarly to the parameter k in the context of NetNorM, it is learned by cross-validation for survival prediction (supervised task) while for patient stratification (unsupervised task) it is set as the median best α learned across survival prediction cross-validation folds. The update function is applied until convergence, and the resulting smoothed matrix is then quantile normalised so that all patients have the same mutation distribution.

4.5 Simplified version of NSQN (SimpNSQN)

The simplified version of NSQN does not propagate mutations further than to order 1 neighbours in the network. More precisely, the SimpNSQN score of a gene is equal to its number of mutated neighbours normalised by its degree and by the degrees of its neighbours, plus a constant C if the gene is mutated. This constant is chosen to ensure that the scores obtained for mutated genes always remain higher than those of non mutated genes. This is obtained by computing:

$$\boldsymbol{X} = \boldsymbol{X_0} \boldsymbol{D}^{-\frac{1}{2}} \boldsymbol{A} \boldsymbol{D}^{-\frac{1}{2}} + C\boldsymbol{X_0}$$

where $\boldsymbol{X_0}$ is the initial binary mutation matrix, $\boldsymbol{A}$ is the adjacency matrix representing the network used, $\boldsymbol{D}$ is the diagonal degree matrix where $D_{ii} = \sum_j A_{ij}$ and $C \in \mathbb{R}$ is equal to $max(\boldsymbol{X_0} \boldsymbol{D}^{-\frac{1}{2}} \boldsymbol{A} \boldsymbol{D}^{-\frac{1}{2}})$. Note that SimpNSQN does not involve any tuning parameter.

4.6 Sparse survival SVM

To estimate a survival model from high-dimensional mutation profiles, we use a survival SVM model [50] combined with a sparsity-inducing regularisation to automatically perform gene selection. Let $\delta_i = 1$ (resp. $\delta_i = 0$) if patient i is deceased (resp. censored), and $y_i \in \mathbb{R}$ be the observed survival time of patient i. It corresponds to either a failure or a censoring time depending on whether the patient is deceased or censored. Define $Z \in \{0,1\}^{n \times n}$ which indicates whether a pair of patients is comparable, i.e,

$$Z_{ij} = \begin{cases} 1 \text{ if } (y_i < y_j \text{ and } \delta_i = 1) \text{ or } (y_j < y_i \text{ and } \delta_j = 1)\,, \\ 1 \text{ if } (y_i = y_j \text{ and } (\delta_i = 1 \text{ or } \delta_j = 1))\,, \\ 0 \text{ otherwise }. \end{cases}$$

Finally, let $\boldsymbol{x_i} \in \{0,1\}^p$ be the mutation profile of patient i. The survival time of patient i is modelled as $s_i = \boldsymbol{w^T x_i}$ where $\boldsymbol{w} \in \mathbb{R}^p$ is the model parameter learned using ranking Support Vector Machines (rSVM) as in [50]. However to get a sparse $\boldsymbol{w}$ we introduce an ℓ_1 regularisation instead of the ℓ_2 regularisation in [50] and thus solve the following optimisation problem:

$$\underset{w}{\text{minimise}}\ \frac{1}{2}||w||_1 + C\sum_{i,j} Z_{ij}\ \ell_{hinge}(w^T(\boldsymbol{x_j} - \boldsymbol{x_i}))\,,$$

Cancer type	k_{min}	k_{max}	step size
LUAD (Lung adenocarcinoma)	50	375	10
SKCM (Skin cutaneous melanoma)	100	1000	15
GBM (Glioblastoma multiform)	50	250	10
BRCA (Breast invasive carcinoma)	15	200	10
KIRC (Kidney renal clear cell carcinoma)	20	90	5
HNSC (Head and Neck squamous cell carcinoma)	40	400	10
LUSC (Lung squamous cell carcinoma)	90	400	10
OV (Ovarian serous cystadenocarcinoma)	20	100	5

Table 2 – Summary of the grid of values used to learn the parameter k (NetNorM) by cross-validation for survival prediction for each cancer type. k_{min} and k_{max} are the minimum and maximum values tested for k respectively.

where $\ell_{hinge}(u) = \max(1-u, 0)$ is the hinge loss and $C \in \mathbb{R}$ is the regularisation parameter. To solve this problem we used the support vector classification algorithm *svm.LinearSVC* from the Python package *scikit learn* [51]. This optimisation problem maximises a convex relaxation of the Concordance Index (CI) which measures how well the predicted survival times $\boldsymbol{s}$ are in accordance with the observed survival times $\boldsymbol{y}$ for the comparable pairs of patients. Formally, $\text{CI} = \frac{1}{|Z|} \sum_{y_i \leq y_j} Z_{ij} I(s_j - s_i)$ where

$$I(x) = \begin{cases} 1 \text{ if } x > 0\,, \\ \frac{1}{2} \text{ if x} = 0\,, \\ 0 \text{ otherwise}\,, \end{cases}$$

and $|Z| = \sum_{y_i \leq y_j} Z_{ij}$. To evaluate the CI obtained on a given dataset, samples were split in 80% train and 20% test sets 20 times using 4 five-fold cross-validation. Each time, a model was learned on the train set and tested on the test set. The CI was computed according to the a python implementation of the function *estC* from the R package *compareC*. Hyperparameters were learned thanks to an inner 5-fold cross-validation on the train set. The values tested for C were 5×10^{-4}, 7.5×10^{-4}, 1×10^{-3}, 2.5×10^{-3}, 5×10^{-3}. The values tested for α ranged from 0.1 to 0.9 included with steps of 0.1. Finally the values tested for k were chosen separately for each cancer type as a linear grid that spans most of the observed total number of mutations across patients (Table 2).

4.7 Patient stratification

Let $\boldsymbol{X} \in \mathbb{R}^{n \times p}$ be the matrix with patient mutations profiles as rows. To cluster the patients we perform a non-negative matrix factorisation (NMF) on $\boldsymbol{X}$, i.e., solve the following optimisation problem:

$$\underset{\boldsymbol{W}, \boldsymbol{H} > 0}{\text{minimise}} \; ||\boldsymbol{X} - \boldsymbol{W}\boldsymbol{H}||_2^2\,,$$

where $\boldsymbol{H} \in \mathbb{R}^{N \times p}$ defines N basis vectors or "metapatients" and $\boldsymbol{W} \in \mathbb{R}^{n \times N}$ defines basis vectors loadings. Patient i was then assigned to the group $j \in \{1..N\}$ that represents him best i.e. $\underset{j}{argmax}\, W_{ij}$.

Whenever the mutation profiles used for stratification were preprocessed with NSQN or NetNorM, the parameters k and α used were chosen as the median best k and α obtained across the 4 5-fold

Cancer type	k (NetNorM)	α (NSQN)	α (NS)
LUAD (Lung adenocarcinoma)	295	0.25	0.4
SKCM (Skin cutaneous melanoma)	167	0.35	0.8
GBM (Glioblastoma multiform)	50	0.5	0.55
BRCA (Breast invasive carcinoma)	55	0.2	0.7
KIRC (Kidney renal clear cell carcinoma)	52	0.4	0.45
HNSC (Head and Neck squamous cell carcinoma)	200	0.9	0.85
LUSC (Lung squamous cell carcinoma)	280	0.25	0.4
OV (Ovarian serous cystadenocarcinoma)	45	0.2	0.3

Table 3 – Summary of the values of k (NetNorM) and α (NS and NSQN) learned by cross-validation for survival prediction. The values given are the medians obtained over 20 cross-validation folds performed for each dataset and each method.

cross validations for survival prediction (Table 3). Different number N of groups were tested for each representation of the mutation profiles (from 2 to 6 groups included) and the best N was chosen by looking at the log-rank statistic.
To assess the stability of the obtained clusters, we computed the proportion of ambiguous clustering (PAC) which is the proportion of discordant cluster assignments obtained through consensus clustering. To compute the PAC, NMF was applied 1000 times to subsamples of the dataset composed of 80% of the samples and 80% of the features chosen at random without replacement. A consensus matrix $\boldsymbol{C} \in \mathbb{R}^{n \times n}$ was then derived from the 1000 cluster assignments obtained where each entry C_{ij} corresponds to the frequency at which two patients where clustered in the same group over all samplings where both patients were retained. Cluster assignments for a pair of patients (i, j) were considered discordant when $0.25 \leq C_{ij} \leq 0.75$.
In the case where only the total number of mutations was used for stratification, NMF is not applicable and kMeans was used instead with 1000 restarts and initialisation by kMeans++ [52].

4.8 Analysis of the proxy genes selected by the sparse survival SVM with NetNorM

Several proxy genes have a prognostic power according to log-rank tests performed for each gene separately and which compare patients with mutations (proxy or not) versus those without ($P \leq 1 \times 10^{-2}$). This prognostic power may be due to at least two types of information encoded in proxy genes: patients' mutation rate and genes' neighbourhood mutational burden (NMB). To clarify the contributions of each effect, we investigate whether such distinct survival outcomes can be obtained with proxies for the total number of mutations only, regardless of NMBs. To this end, we simulate proxy mutations for each gene separately according to a model that only depends on patient mutation rates. Let $T_i \in \mathbb{N}$ be the total number of mutations of patient i, $i \in \{1, ..., n\}$. Let $\mathcal{M}_o \subset \{1, ..., n\}$ and $\mathcal{M}_p \subset \{1, ..., n\}$ indicate which patients have original and proxy mutations respectively. For a given proxy gene whose mutations are described by the sets M_o and M_p, we leave the original mutations untouched and reallocate the proxy mutations according to

$$P(i \in \mathcal{M}_p | T_i) = \begin{cases} 0 \text{ if } (T_i \geq k) \text{ or } (i \in \mathcal{M}_o) \\ \frac{k - T_i}{\alpha} \text{ otherwise} \end{cases}$$

where α is chosen so that the probabilities sum to 1. Proxy mutations are drawn from this model 1000 times. Each time we compute the log-rank statistic between the mutated and non mutated

patients which yields a distribution of the log-rank statistic under the null hypothesis. The actual log-rank statistic obtained using NetNorM is then compared to this distribution to accept or reject the null hypothesis. Rejecting the null hypothesis means that the difference in survival outcomes observed between the patients with and without artificial mutations is not only driven by patients' total number of mutations.

4.9 Survival analysis using patient subtypes and clinical data

To determine whether the obtained patient subtypes are predictive of survival beyond clinical data, we fitted a Cox proportional hazards regression model to the clinical data and to the clinical data augmented with a variable describing patients' subtypes. We then performed a likelihood ratio test to compare the two models. The clinical variables used were downloaded from TCGA. It includes age, gender, stage, extent of spread to the lymph nodes, presence of metastasis, histology for both LUAD and SKCM and further variables such as smoking history, history of prior malignancy, residual tumour after surgery, tumour dimensions for LUAD and clark level at diagnosis, primary melanoma mitotic rate, new tumour event after initial treatment (yes/no), primary melanoma tumour ulceration (yes/no), primary melanoma known (yes/no) for SKCM.

4.10 Identifying differentially mutated genes and pathways across subtypes

We obtain gene clusters by applying hierarchical clustering with centroid linkage and Euclidean distance to the columns of the metapatients matrix (restricted to high variance genes). To obtain a reasonable number of gene clusters to analyse, we cut the hierarchical cluster tree at a distance threshold of 5.5, yielding 20 clusters. Gene clusters can be categorised into two types: those that contain a lot of proxy mutations ($\geq$ 80% of the total mutational load of the cluster) and whose genes form a dense subgraph, and those that have neither of these two features. The presence of dense subgraphs with many proxy mutations results from the fact that NetNorM tends to add proxy mutations to all genes in a dense subgraph or none since they all have roughly the same number of mutated neighbours. The association of a gene cluster with one subtype can therefore indicate two things: either the subtype is expected to be enriched in proxy mutations in the corresponding gene cluster, which in turn indicates that the subgraph in which the cluster lies is expected to be enriched in mutations, or the gene cluster itself is expected to be enriched in mutations in the corresponding subtype. The enrichment or depletion in mutations of one gene cluster across patient subtypes was therefore tested slightly differently according to the gene cluster type. In the first case, we first define the neighbourhood of the gene clusters as all genes lying in the same dense subgraph. Specifically, we include in the subgraph all genes sharing an edge with at least 90% of the genes in the cluster, thus keeping subgraphs very dense. The obtained set of genes is the one tested for enrichment in mutations across subtype. In the second case, the gene cluster is directly tested for enrichment. Enrichment is assessed with a χ^2 contingency test, where the contingency table is defined by the following marginals: the total number of raw mutations in each subtype, and the total number of raw mutations in and outside the gene cluster (generalised to the embedding dense subgraph if it is relevant).

Gene clusters are searched for pathway enrichment using DAVID online tool [53] (`https://david.ncifcrf.gov/summary.jsp`) with the KEGG database [42]. They are also tested for enrichment in late replicating genes thanks to a permutation test using data downloaded from `http://www.broadinstitute.org/cancer/cga/mutsig_run`. For each gene cluster c of length l_c, l_c genes are chosen uniformly at random without replacement from the list of genes with replication time information. This sampling is performed 1000 times and the null distribution was obtained

by computing the median replication time of these 1000 gene sets. The median replication time of cluster c is then compared to the null distribution to yield a p-value, i.e. the probability to observe a set of genes of length l_c with median replication time at least as extreme.

Acknowledgements

We thank Philippe Hupé, Isabel Brito, Claudia Rincon and Emmanuel Barillot for useful discussions. This work was supported by the European Research Council grant ERC-SMAC-280032 (MLM, JPV), and by fellowships from the Miller Institute for Basic Research in Science and from the Fulbright Foundation (JPV).

References

1. Stratton, M. R., Campbell, P. J. & Futreal, P. A. The cancer genome. *Nature* **458,** 719–724 (2009).
2. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **144,** 646–674 (2011).
3. Vogelstein, B. *et al.* Cancer genome landscapes. *Science* **339,** 1546–1558 (2013).
4. Chin, L. & Gray, J. W. Translating insights from the cancer genome into clinical practice. *Nature* **452,** 553–63 (2008).
5. Olivier, M. & Taniere, P. Somatic mutations in cancer prognosis and prediction: lessons from TP53 and EGFR genes. *Curr. Opin. Oncol.* **23,** 88–92 (2011).
6. Mardis, E. R. Genome sequencing and cancer. *Curr. Opin. Genet. Dev.* **22,** 245–250 (2012).
7. The Cancer Genome Atlas Research Network *et al.* The Cancer Genome Atlas Pan-Cancer analysis project. *Nat. Genet.* **45,** 1113–20 (2013).
8. Hudson, T. J., Anderson, W., Aretz, A. & Barker, A. D. International network of cancer genome projects. *Nature* **464,** 993–8 (2010).
9. The Cancer Genome Atlas. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **455,** 1061–8 (2008).
10. The Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature* **474,** 609–615 (2011).
11. The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature* **490,** 61–70 (2012).
12. Kandoth, C. *et al.* Mutational landscape and significance across 12 major cancer types. *Nature* **503,** 333–339 (2013).
13. Greenman, C. *et al.* Patterns of somatic mutation in human cancer genomes. *Nature* **446,** 153–8 (2007).
14. Wood, L. D. *et al.* The genomic landscapes of human breast and colorectal cancers. *Science* **318,** 1108–13 (2007).
15. Lawrence, M. S. *et al.* Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* **505,** 495–501 (2014).
16. Lawrence, M. S. *et al.* Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* **499,** 214–8 (2013).

17. Birkbak, N. J. *et al.* Tumor mutation burden forecasts outcome in ovarian cancer with BRCA1 or BRCA2 mutations. *PLoS ONE* **8** (2013).

18. Rizvi, N. A. *et al.* Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348,** 124–128 (2015).

19. Creixell, P. *et al.* Pathway and network analysis of cancer genomes. *Nat. Methods* **12,** 615–621 (2015).

20. Barillot, E., Calzone, L., Hupé, P., Vert, J.-P. & Zinovyev, A. *Computational systems biology of cancer* (Chapman & Hall/CRC, 2012).

21. Rapaport, F., Zinovyev, A., Dutreix, M., Barillot, E. & Vert, J.-P. Classification of microarray data using gene networks. *BMC Bioinformatics* **8,** 35 (2007).

22. Köhler, S., Bauer, S., Horn, D. & Robinson, P. N. Walking the Interactome for Prioritization of Candidate Disease Genes. *American Journal of Human Genetics* **82,** 949–958 (2008).

23. Vanunu, O., Magger, O., Ruppin, E., Shlomi, T. & Sharan, R. Associating genes and protein complexes with disease via network propagation. *PLoS Computational Biology* **6** (2010).

24. Qian, Y., Besenbacher, S., Mailund, T. & Schierup, M. H. Identifying disease associated genes by network propagation. *BMC systems biology* **8 Suppl 1,** S6 (2014).

25. Kuperstein, I. *et al.* The shortest path is not the one you know: Application of biological network resources in precision oncology research. **30,** 191–204 (2015).

26. Jia, P. & Zhao, Z. VarWalker: personalized mutation network analysis of putative cancer genes from next-generation sequencing data. *PLoS Comput. Biol.* **10** (2014).

27. Hou, J. P. & Ma, J. DawnRank: discovering personalized driver genes in cancer. *Genome Med.* **6,** 56 (2014).

28. Babaei, S., Hulsman, M., Reinders, M. & de Ridder, J. Detecting recurrent gene mutation in interaction network context using multi-scale graph diffusion. *BMC Bioinformatics* **14,** 29 (2013).

29. Leiserson, M. D. M. *et al.* Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes. *Nat. Genet.* **47,** 106–114 (2014).

30. Hofree, M., Shen, J. P., Carter, H., Gross, A. & Ideker, T. Network-based stratification of tumor mutations. *Nat. Methods* **10,** 1108–15 (2013).

31. Yuan, Y. *et al.* Assessing the clinical utility of cancer genomic and proteomic data across tumor types. *Nat. Biotechnol.* **32,** 644–652 (2014).

32. Zhou, D., Bousquet, O., Lal, T. N., Weston, J. & Schölkopf, B. in *Adv. Neural. Inform. Process Syst. 16* (eds Thrun, S., Saul, L. K. & Schölkopf, B.) 321–328 (MIT Press, 2004).

33. Cerami, E. G. *et al.* Pathway Commons, a web resource for biological pathway data. *Nucleic Acids Res.* **39,** D685–D690 (2011).

34. Collisson, E. A. *et al.* Comprehensive molecular profiling of lung adenocarcinoma. *Nature* **511,** 543–550 (2014).

35. Ding, L. *et al.* Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* **455,** 1069–1075 (2008).

36. Ranganathan, P., Weaver, K. L. & Capobianco, A. J. Notch signalling in solid tumours: a little bit of everything but not all the time. *Nat. Rev. Cancer* **11,** 338–351 (2011).

37. Roh, M. H. *et al.* The Maguk protein, Pals1, functions as an adapter, linking mammalian homologues of Crumbs and Discs Lost. *J. Cell Biol.* **157,** 161–172 (2002).

38. Qiao, J. *et al.* Filamin C, a dysregulated protein in cancer revealed by label-free quantitative proteomic analyses of human gastric cancer cells. *Oncotarget* **6,** 1171–1189 (2014).

39. Dusek, R. L. & Attardi, L. D. Desmosomes: new perpetrators in tumour suppression. *Nat. Rev. Cancer* **11,** 317–323 (2011).

40. Chidgey, M & Dawson, C. Desmosomes: a role in cancer? *Br. J. Cancer* **96,** 1783–7 (2007).

41. Xie, Y. *et al.* IQGAP2 Displays Tumor Suppression Functions. *J. Anal. Oncol.* **4,** 86–93 (2015).

42. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res.* **44,** D457–D462 (2016).

43. Fletcher, J. I., Haber, M., Henderson, M. J. & Norris, M. D. ABC transporters in cancer: more than just drug efflux pumps. *Nat. Rev. Cancer* **10,** 147–156 (2010).

44. Moldovan, G.-L. & D'Andrea, A. D. How the fanconi anemia pathway guards the genome. *Annu. Rev. Genet.* **43,** 223–249 (2009).

45. Watts, D. J. & Strogatz, S. H. Collective dynamics of 'small-world' networks. *Nature* **393,** 440–2 (1998).

46. Horn, H. *et al.* A comparative analysis of network mutation burdens across 21 tumor types augments discovery from cancer genomes. *bioRxiv.* doi:10.1101/025445 (2015).

47. Cho, A., Shim, J. E., Supek, F., Lehner, B. & Lee, I. MUFFINN: cancer gene discovery via network analysis of somatic mutation data. *Genome Biol.* **17,** 1 (2016).

48. Prasad, T. S. K. *et al.* Human Protein Reference Database - 2009 update. *Nucleic Acids Res.* **37,** D767–D772 (2009).

49. Stark, C. *et al.* BioGRID: a general repository for interaction datasets. *Nucleic Acids Res.* **34,** D535–D539 (2006).

50. Van Belle, V., Pelckmans, K., Suykens, J. & Van Huffel, S. Support vector machines for survival analysis. *Proceedings of the Third International Conference on Computational Intelligence in Medicine and Healthcare (CIMED2007),* 1–8 (2007).

51. Pedregosa, F. *et al.* Scikit-learn: Machine Learning in Python. *J. Mach. Learn. Res.* **12,** 2825–2830 (2011).

52. Arthur, D. & Vassilvitskii, S. k-means++: The advantages of careful seeding. *Proceedings of the eighteenth annual ACM-SIAM symposium on Discrete algorithms* **8,** 1027–1035 (2007).

53. Huang, D. W., Lempicki, R. a. & Sherman, B. T. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols* **4,** 44–57 (2009).