Cell

Tracking cancer evolution reveals constrained routes to metastases: TRACERx Renal

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Abstract:	Clear-cell renal cell carcinomas (ccRCC) exhibit a broad range of metastatic phenotypes, which have not been systematically studied to date. Here we analysed 589 primary and 336 metastatic biopsies, across 100 metastatic ccRCC patients, including two cases profiled in a post-mortem setting. Metastatic competence was afforded by chromosome complexity and we identify 9p loss as a highly selected event driving metastasis and ccRCC related mortality (HR=7.7, p=0.0014). Distinct patterns of metastatic dissemination were observed, including rapid progression to multiple tissue sites seeded by primary tumours of monoclonal structure. By contrast we observe attenuated progression in cases characterised by high primary tumour heterogeneity, with metastatic competence acquired gradually and initial progression to solitary metastasis. Finally, we observe early divergence of primitive ancestral clones and protracted latency of up to two decades as a feature of pancreatic metastases.
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5th March 2018

Dr Yiaying Tan

Senior Editor

Cell

Dear Dr Tan,

Re: Tracking renal cancer evolution reveals constrained routes to metastases, results from the TRACERx Renal study

I am pleased to enclose our revised manuscript on the evolutionary dynamics of metastatic clear cell renal cell carcinomas (ccRCC). We would like to thank reviewers for their constructive feedback and suggestions which have resulted in a significantly improved manuscript. We have also taken this opportunity to clarify the presentation of both text and figures, ensuring consistency throughout. The major changes to the manuscript are:

1. We present a meta-analysis of the metastasising clone across three cohorts of primary metastasis pairs: TRACERx Renal (n=38), HUC (n=26) and MSK (N=34); in total, the meta-analysis is based on 580 primary and 255 metastatic biopsies across 98 patients with metastatic ccRCC. This is the largest study of its kind to date, offering broad insight into the diverse spectrum of modes of progression from primary to metastatic disease. We identify the selection of loss of 9p as a potent driver of both metastases and mortality risk, even after adjustment for established clinically prognostic indicators (HR=7.7, p=0.0014).
2. We stratified the mode of disease progression as “rapid” or “attenuated” according to the metastatic disease tempo and show that these clinical phenotypes are underpinned by distinct evolutionary features.

3. In the previous version of the manuscript we presented one case sampled at post-mortem and in the revised version we present an additional case; adding to a total of 9 primary and 81 metastatic regions obtained at post-mortem. Critically the two cases illustrate the contrasting patterns of progression we observed in the TRACERx Renal cohort from a punctuated evolution in the primary tumour and rapidly spreading metastases in a patient who survived 6 months; to a primary tumour which evolved gradually into multiple subpopulations in which metastases were seeded over the course of 17 years.
4. In an exploratory analysis, we show that in the context of intravascular tumour growth seeding from the ancestral clone is associated with improved clinical outcome; that lymph nodes are seeded by the same primary tumour clones as visceral metastases; and that pancreatic metastases lack the genomic features that confer an aggressive clinical phenotype. Although descriptive in nature these findings reconcile a number of clinical observations in ccRCC

Overall, we have emphasised the preliminary nature of our results which will be explored further in the ongoing analyses in TRACERx Renal and PEACE studies. Nevertheless, our data provide the first large-scale resolution of the metastasising clones and offer a biomarker opportunity in ccRCC.

We have included our point by point rebuttal and actions in response to reviewer comments in a separate document.

Thank you for your consideration

Yours sincerely,

Charles Swanton

On behalf of the TRACERx Renal consortium

Response to Reviewers

The previous version of this paper was not sent out for peer review.

Tracking cancer evolution reveals constrained routes to metastases: TRACERx Renal

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Summary

Clear-cell renal cell carcinomas (ccRCC) exhibit a broad range of metastatic phenotypes, which have not been systematically studied to date. Here we analysed 589 primary and 336 metastatic biopsies, across 100 metastatic ccRCC patients, including two cases profiled in a post-mortem setting. Metastatic competence was afforded by chromosome complexity and we identify 9p loss as a highly selected event driving metastasis and ccRCC related mortality (HR=7.7, p=0.0014). Distinct patterns of metastatic dissemination were observed, including rapid progression to multiple tissue sites seeded by primary tumours of monoclonal structure. By contrast we observe attenuated progression in cases characterised by high primary tumour heterogeneity, with metastatic competence acquired gradually and initial progression to solitary metastasis. Finally, we observe early divergence of primitive ancestral clones and protracted latency of up to two decades as a feature of pancreatic metastases.

Introduction

Large-scale sequencing initiatives such as the cancer genome atlas (TCGA) and the international cancer genome consortium (ICGC) have profiled thousands of primary tumours, across many cancer types. Large-scale studies of metastases have been limited (Robinson et al., 2017; Zehir et al., 2017), and, critically have not included matched primary tumours. Understanding evolution of metastatic disease requires simultaneously analysis of the primary tumour in order to distinguish clones with and without metastatic potential. To date, a limited a number of primary-metastasis(es) pairs have been analysed retrospectively (Shah et al., 2009; Campbell et al., 2010; Yachida et al., 2010; Ding et al., 2010; Haffner et al., 2013; Bashashati et al., 2013; Lee et al., 2014; Gerlinger et al., 2014; Brastianos et al., 2015; Gundem et al., 2015; Schwarz et al., 2015; McPherson et al., 2016; Zhao et al., 2016; Casuscelli et al., 2017; Yates et al., 2017).

The well-established molecular landscape of primary clear cell Renal Cell Carcinoma (ccRCC) defined by the loss of 3p and *VHL* mutations/methylation as early events, provides an excellent model for study of cancer evolution. Metastatic ccRCC, with its variable clinical presentation and natural history, is a compelling model for understanding the clonal evolution of metastases. CcRCC metastases spread by both lymphatic and haematogenous routes and colonise a range of sites including, lung, bone, liver, brain, pancreas, adrenal, parotid and thyroid glands, as well as muscle, skin and soft tissue (Bianchi et al., 2012). Liver metastases confer a worse prognosis (McKay et al., 2014), while low volume lung metastases are associated with a more indolent disease course. Some ccRCCs also grow intravascularly forming a tumour thrombus in the renal vein/inferior vena cava. Approximately one third of patients have metastases detected at pre-operative screening or surgery, termed synchronous metastases. Up to 50% develop metastases after the removal of the primary tumour (at least 3 months and as late as 30 years after primary surgery), termed metachronous metastases. The spatial distribution of metastatic disease in ccRCC ranges from solitary (a single metastasis in a single location), to oligo

(limited in number and location; usually defined as ≤5 or ≤3 metastases (Weichselbaum and Hellman, 2011)), to widespread (multiple metastases in multiple sites).

The clinical relevance of solitary and oligometastases is that they can be managed by local strategies (surgery, stereotactic radiotherapy and other ablative therapies) rather than systemic therapy. For metachronous metastases the outcome of this approach generally depends on the time interval since the initial surgery (Dabestani et al., 2014). For patients presenting with synchronous solitary or oligometastases, a multi-modal strategy that involves cytoreductive nephrectomy (removal of the primary tumour), metastasectomy (complete resection of the metastasis) and systemic therapy can achieve an improved outcome (Bex et al., 2016). However, 20% of patients progress as early as one month following surgery (A. Bex, 2017), sometimes failing to receive systemic therapy due to rapid disease pace (Kutikov et al., 2010). Thus, there is a pressing need to understand which patients harbour more widespread occult metastases and therefore do not benefit from surgery.

In our analyses of 100 primary ccRCCs in the prospective longitudinal cohort study, TRAcking renal cell Cancer Evolution through Therapy (TRACERx Renal, clinical trials no NCT03226886) we used conserved patterns of evolution to classify tumours into 7 distinct evolutionary subtypes (Companion paper, Turajlic et al., 2018). Primary tumours with low intratumour heterogeneity (ITH) and a low fraction of the tumour genome affected by somatic copy number alterations (SCNAs), had an overall low metastatic potential. Primary tumours with high ITH were associated with an attenuated pattern of progression, whereas primary tumours with low ITH but elevated SCNAs were associated with a rapid progression at multiple sites (Companion paper, Turajlic et al. 2018). A pre-defined endpoint in the TRACERx Renal study was to explore the contribution of subclonal dynamics to ccRCC metastasis. To distinguish metastasis-competent from incompetent clones, and examine the routes and timing of metastases across multiple anatomic sites, we analysed 463 primary and 169 matched metastatic regions from a subset of 38/100 patients in the TRACERx Renal Cohort (Companion paper, Turajlic et al., 2018); 80 primary regions and 54 metastatic regions in an extension cohort of 26 patients; 34

matched primary-metastasis pairs in a further validation cohort; and finally, 9 primary and 81 metastatic regions obtained at post-mortem in 2 patients. In total, we analysed 100 primary-metastasis pairs derived from 589 primary and 336 metastatic biopsies.

Results

Overview of the cohorts under study

ccRCC tumours exhibit a variety of progressive phenotypes including invasion of the peri-renal and renal sinus fat (T3a), direct invasion through the renal capsule (Gerota's fascia) and into the adrenal gland (T4), intravascular tumour growth (T3a-T3c); and lymph node (N1/N2) and visceral metastases (M1), including indirect spread to the adrenal gland. In 38 patients whose primary tumours were profiled in the TRACERx Renal cohort (**Table S1A**), we profiled multiple regions from matched tumour thrombi, lymph node or visceral metastases using a bespoke gene panel (**STAR Methods**: Driver Panel).

The overall number of driver events (mutations and SCNAs as presented in **Figure 1A**) was lower in metastases (mean=9), compared to primary tumours (mean=12, p=0.05, adjusted for the varying number of profiled regions, **STAR methods**) (**Figure 1A**). Consistent with evolutionary bottlenecking, metastases were significantly more homogeneous (proportion of clonal variants = 0.87) compared to primary tumours (proportion of clonal variants = 0.33, p=6.6x10-13, adjusted for the varying number of profiled regions, **Figure 1B**). Across the 56 primary-metastasis pairs 456 driver events were shared between primary and metastases, 230 were private to primary tumours and 39 driver events were private to metastases (**Figure 1C**). Driver phylogenies were reconstructed to infer clonal relationships between primary tumours and metastases (**STAR methods**).

The TRACERx Renal cohort was enriched for synchronous metastases (**Figure 1D**), and to widen our investigation we analysed two additional cohorts. Using the Driver Panel (**STAR methods**) we multi-region profiled the "HUC" (Hospital Unversitario Cruces) cohort of archived formalin fixed paraffin embedded (FFPE) primary ccRCCs and matched synchronous (6 cases), and metachronous metastases

(23 cases) (**STAR methods; Table S1B**). We successfully profiled 80 primary tumour regions and 54 metastatic regions in 26 patients (two patients contributed multiple metastases, **Figure S2**). For the second cohort, "MSK" (Memorial Sloane Kettering), we re-analysed the sequencing data from a study of primary-metastasis pairs (Becerra et al., 2017) (**STAR methods**), to obtain both mutational and SCNA events in a total of 34 cases, including 19 synchronous, and 15 metachronous metastases (**Table S1C**, **Figure S2**). As expected, we noted a difference in the overall frequency of driver events in the HUC and MSK cohorts owing to the increased sensitivity for detecting subclonal alterations in the TRACERx Renal cohort (**Table S2**). There was a wide temporo-spatial representation of metastases across the three cohorts encompassing 18 distinct metastatic sites (**Figure 1E**), and presenting 0-17 years after the removal of the primary tumour (**Tables S1A-C**). Finally, we profiled a wide range of metastatic tissues sampled at post-mortem in the context of the Posthumous Evaluation of Advanced Cancer Environment (PEACE) study (NCT03004755) in two cases of metastatic ccRCC (**Table S1A**).

Characterisation of the metastasising clone(s)

Taking advantage of the dense spatial sampling and phylogenetic reconstruction conducted in the TRACERx Renal cohort (Companion paper, Turajlic et al. 2018), we analysed the progression of individual clones from primary to metastatic sites. Across the 38 patients we observed 250 distinct tumour clones which we categorised into three groups based on the evidence of selection in the metastasis/metastases: i) clones that are not selected ("not selected", n=129 clones, defined as subclonal in the primary and absent in metastasis), ii) clones that are maintained ("maintained", n=38 clones, defined as the most recent common ancestor (MRCA) clones, clonal in both primary and metastasis), iii) clones that are selected ("selected", n=83 clones, defined as subclonal in the primary and clonal in metastasis; or absent in the primary and present in metastasis) (**Figure 2A, STAR Methods**). Clones that were private to the metastasis may have evaded detection as a minor subclone in the primary tumour, or arisen de novo in the metastasis. The ability to differentiate the clones that appear to be selected versus not on a matched patient/tumour specific background across the whole

cohort, allowed us to characterise the features associated with metastasis. We observed no difference in the number of non-synonymous mutations between the two groups (based on Driver Panel profiling median value = 4 for both, p=0.5295), however wGII was significantly elevated in selected clones (median “selected” = 0.29 vs “not selected” = 0.17, p<0.001, **Figure 2B**). This was further supported by ploidy (determined by regional fluorescence activated cell sorting, FACS, **STAR methods**) also being significantly elevated in selected clones (mean DNA index “selected” = 1.29, “not selected” = 1.16, p<0.001, **Figure 2B**). Multi-region immunohistochemistry staining for Ki67 (**STAR Methods**) demonstrated higher proliferation index in the clones that were selected, compared to those that were not (median Ki67 +40% higher in selected versus non-selected clones, p=0.0317, **Figure 2B**). Finally, we observed increased allelic imbalance at the human leukocyte antigen (HLA) locus in selected versus non-selected clones (HLA allelic imbalance observed in n=12 “selected”, versus n=2 “not selected” clones, **Table S3**), in concordance with the findings in non-small cell lung cancer (McGranahan et al., 2017).

Next, we considered the individual driver events, mutational or SCNAs, that are selected during progression to metastasis, by comparing, for each event, the proportion of times it was found in “selected” versus “not selected” clones (**Figure 2C**). We conducted this analysis across TRACERx Renal (n=38), HUC (n=26) and MSK (n=34) cohorts, providing a total dataset of 98 matched primary-metastasis pairs. Significance was calculated by comparing event selection proportions, to null background rates as observed across all passenger events in each cohort (**STAR Methods**). "Selected” event frequencies were compared to "not selected", and one event was found to be significantly enriched in "selected" clones: loss of chromosome 9p21.3 (p=0.0026, padj<0.1 after adjustment for multiple testing, **Figure 2C**). We also note loss of chromosome 14q31.1 reached significance in the meta-analysis before correction for multiple testing (p=0.0275, padj=0.303), suggesting this and other driver events may also contribute to metastasis. We acknowledge the risk of illusion of clonality (*i.e.* subclonal events appearing clonal within a single region of a primary tumour) limited our power to detect metastatic selection in the MSK, and to a lesser extent HUC, cohorts. For example, 53% of

events in the TRACERx Renal cohort were subclonal, compared to only 31% in HUC and 11% in MSK cohorts.

Metastatic ccRCC has a variable spectrum of survival outcomes, with overall survival (OS) times ranging from short (<6 months), to prolonged (>5 years). Accordingly, we conducted OS analysis for the two events that were enriched in metastasising clones (P<0.05 from **Figure 2C**), to understand if they were also driving early ccRCC-related mortality, based on their presence/absence within the metastasising clone(s) of each case. OS data were not available for the MSK cohort. Hazard ratios (HR) were observed as follows (**Figure 2D**): 9p loss (HUC cohort HR=7.7, [2.8-20.8] 95% confidence interval, TRACERx cohort HR=Infinity [no events in wild type group], p=0.0014 log-rank test across both cohorts, with study included as a term in the cox model) and 14q loss (HUC cohort HR=1.5, [0.6-3.9] 95%, TRACERx cohort HR=2.0, [0.5-8.2], non-significant). We note the strong association between reduced survival and 9p loss in the metastasising clone remained significant after correction for known clinical variables (p=0.046, adjusted for stage, grade and study) (**Figure 2E**). 9p deletions have been reported to confer a poor prognosis (El-Mokadem et al., 2014; La Rochelle et al., 2010), however the hazard ratios in our analysis (HR=7.7 and HR=infinity) are higher than reported in those studies (HR=4.3 (El-Mokadem et al., 2014), HR=1.7 (La Rochelle et al., 2010)), which may reflect the greater sensitivity of profiling events within the metastasising clones.

Evolution of tumour thrombus

Intravascular tumour growth and formation of tumour thrombus (TT) is observed in ~15% of ccRCCs either in the renal vein (level I), extending to the infrahepatic inferior vena cava (IVC) (level II), retrohepatic or suprahepatic IVC (level III) or reaching the right atrium (level IV) (Psutka and Leibovich, 2015) (**Figure 3**). Untreated TT is associated with a poor outcome (Reese et al., 2013), but aggressive surgical management involving a thrombectomy can result in long-term survival in some patients (Psutka and Leibovich, 2015). In the TRACERx Renal cohort 33/100 ccRCC cases presented with venous tumour extension (Companion paper, Turajlic et al., 2018), only one of which was classified as a "*VHL* monodriver" tumour which harboured 9p loss (K253, **Figure 3**). Median survival in patients with TT

was 17.8 months (**Table S1D**) with three patients dying within 6 months of surgery due to disease progression (K328, K263, K390); classified as "multiple clonal" driver (2 cases) and "*VHL* wt" (1 case) subtypes (**Table S1D**).

In 24/33 cases we successfully profiled the TT along its length (**Table S1D**), and reconstructed driver phylogenies to infer the clonal relationship between primary tumour and the intravascular tumour extension (**Figure 3**). The TT was seeded directly by the most recent common ancestor (MRCA, the clone which harbours the full complement of alterations common to all the clones in the tumour; denoted by the first node in the phylogenetic tree) in ten cases (K239, K118, K250, K207, K059**,** K167, K276, K107, K253, K191**; Figure 3**)**.** In other cases, the TT emerged from the more advanced subclones in the primary tumour, which harboured additional drivers, including 9p loss. Cases where the TT was seeded by the MRCA, suggesting intravascular growth was an early event, had an improved clinical outcome compared to the cases where late emerging clone seeded the TT (**Figure S3A**). Whilst most primary tumours had evidence of ongoing evolution, tumour thormbi harboured limited additional alterations (94.9% of TT events were shared with primary). Consistent with the propensity of TT to progress rapidly (Woodruff et al., 2013), we observed an elevated proliferation index within primary tumours presenting with TT compared to those without (P = 0.00095, **Figure S3B).** Thus, the lack of fixation of new driver events in TTs may be due to their rapid extension and/or limited selective pressure in the intravascular space.

An interesting biological and clinical question relates to the ability of TT to act as a source of other metastases, and in this context, we profiled six patients with venous tumour extension and concurrent lymph node and/or visceral metastases (**Figure S3C**). In some cases, distinct clones in the primary tumour seeded the TT and the metastasis (K326 and K390; **Figure S3C**). Consistent with the worse prognosis conferred by lymph node involvement in ccRCC, in K390 the lymph node seeding clone harboured 9p loss while the TT clone did not. The same primary clone seeded both TT and metastasis in K096 and K427 (**Figure S7)**; whilst in K107 and K263 (**Figure S7**) the metastasising clone appeared to first seed the thrombus, and then lymph node and adrenal sites, respectively, suggesting TT may

act as a reservoir of metastases, consistent with the poor outcomes of untreated thrombus (Reese et al., 2013). The alternative explanation is that all the sites, including TT were seeded by a clone which evaded detection in the primary tumour.

Evolution of progressive disease

Within the 38 TRACERx Renal primary-metastasis cohort 25 patients developed progressive disease. The clinical outcomes in this group were variable, with overall survival time ranging from 1.5-54.4 months (**Table 1A**). Given that cytoreductive nephrectomy and metastasectomy are performed to achieve longer disease-free survival, we considered the evolutionary features of cases that progressed rapidly (*i.e.* multiple sites of disease progression within 6 months of surgery) versus those with attenuated progression (*i.e.* single site progression <6 months; or multi-site progression >6 months), capturing both the speed and the extent of metastatic spread (**Table S1E**, **Figure 4A**). One patient (K328) died from operative complications and was excluded from the analysis. Eight cases were classified as having "rapid progression": K376, K326, K263, K107, K153, K446, K390 and K066 (**Figure 4A**). This group was enriched for "multiple clonal driver", "*VHL* wild type" and "*BAP1* driven" evolutionary subtypes (**Figure 4B**) and associated with lower ITH and elevated wGII relative to the cases with attenuated progression (**Figure 4C**)**.** All primary tumours in this group harboured loss of 9p **(Figure 4A).** They were more likely to progress to liver metastases (6/8) compared to cases in the "attenuated progression" group (1/16) (p=0.0013), and had a short overall survival **(Figure 4A)**. Particularly notable in this group was case K153 in which lymph node and lung metastases were seeded from the same "*BAP1* driven" subclone, which had high WGII and harboured 9p loss, while the competing *"PBRM1-->SETD2"* subclone failed to metastasise (**Figure S4**).

16 cases were classified within the "attenuated progression" group: K379, K096, K208, K071, K243, K206, K520, K180, K029, K228, K427, K253, K229, K386, K276, K280 (**Figure 4A**). This group was enriched for "*PBRM1→SETD2"*, "*PBRM1→PI3K"*, "*PBRM1→*SCNA" and *"VHL* monodriver" evolutionary subtypes, with the primary tumours were characterised by higher ITH index and lower

wGII, as compared to the "rapid progression" group (**Figure 4C**). Disease progression interval was longer and often limited to a single metastatic site. Consequently, in some patients, metastatic disease was successfully controlled with further surgery (K029) or radiotherapy (K096, K228, K208, K243), consistent with the lack of other occult metastases. For example, case K029 ("*PBRM1->PI3K*") presented with spatially separate bone metastases three years apart. The metastasising clone harboured a *PBRM1* mutation, but not 9p loss (**Figure S4**). Thus, although ITH is associated with metastatic disease, the pattern of metastases suggests a reduced metastatic efficiency, possibly due to increased clonal competition. This observation is consistent with the notion that heterogenous tumours harbour clones with a wide range of metastatic competence.

Evolution of latent metastases

We compared the time from primary tumour to metastasis, by tissue site, across the combined TRACERx/HUC/MSK cohorts. In keeping with the known modes of late recurrence in ccRCC, we observed the pancreatic metastases to have the longest time to presentation (median 15 years, compared to 3 years for all other tissue sites, **Figure 5A**). Intriguingly, pancreatic metastases were found to have significantly lower wGII, as compared to all other metastatic tissue sites (p=0.0489, **Figure 5B**). A shared clonal ancestry was confirmed between primary and metastatic sites in all 3 pancreatic metastasis cases, and we observed a strikingly low number of additional driver alterations in pancreatic metastases, despite the extended latency time (**Figure 5C**). In the case of SP006, the pancreatic metastasis occurred 17 years after the primary tumour was resected, and the latent clone mapped directly back to the founding MRCA clone, suggesting early divergence of a primitive ancestral clone. Similarly, in SP023, a case with pancreatic metastasis at 15 years, the latent clone derived from the primary MRCA and only acquired one additional driver mutation in *MTOR* (**Figure 5C, Figure S5**). Finally, SP058 presented with pancreatic metastasis at 8 years, with a single additional driver event (*SETD2* mutation) in metastasis, while we detected alternative subclones with a greater number of driver events in the primary tumour (**Figure 5C**). The seeding by the ancestral clone and the lack of 9p loss suggests that the pancreas may be a more permissive metastatic niche for ccRCC. The reasons for

the characteristic latency of pancreatic metastases remain unknown, but are likely to involve interactions with the tumour microenvironment, the immune system and altered epigenetic states (Giancotti, 2013).

Spatial resolution of metastases through post-mortem sampling

To explore the clonal dynamics of multiple metastases we sampled them at post-mortem in two cases (**Table S1A**, **Figure 6**) through the PEACE study (NCT03004755). Case K548 presented with a primary ccRCC which had already disseminated to multiple sites including adrenal, loco-regional and mediastinal lymph nodes, liver, and pleura (**Table S1F**). All disease sites, including the primary tumour, were sampled at post-mortem (**Figure 6A**). Clonal mutations were detected in *VHL*, *PBRM1* and *SETD2* genes, and accordingly this case was categorised as a "multiple clonal driver" subtype. The primary tumour had low ITH and high wGII, and all 13 metastatic sites sampled were seeded by the dominant clone which was characterised, in addition, by 9p loss **Figure 6A**). We note this patient progressed rapidly through two lines of systemic therapy and died six months after the diagnosis of ccRCC (**Table S1F**). The evolutionary features of the primary tumour are in keeping with those we observe in the TRACERx Renal cases with "rapid progression" (**Figure 4A**).

In case K489 the patient presented with a primary ccRCC and underwent a nephrectomy with curative intent (**Figure 6B**). 7 years following surgery two pancreatic metastases were detected on imaging and the patient underwent a complete metastasectomy (**Table 1F, Figure 6B**). 4 years later they presented with lymph node and lung metastases (**Figure 6B**). They received multiple lines of systemic therapy, subsequently developing metastases at additional sites including liver and bone, and succumbing to their disease 17 years after the original diagnosis (**Table 1F, Figure 6B**). We obtained fresh samples at post-mortem from multiple lymph node sites, liver, lung, and contralateral kidney metastases; and we accessed the primary tumour and the resected pancreatic metastases from archived FFPE material. The primary tumour harboured a clonal *VHL* mutation and 3p loss, and a subclonal *PBRM1* and

multiple *SETD2* mutations, indicating parallel evolution. These features were consistent with the "*PBRM1→SETD2*" evolutionary subtype (Companion paper, Turajlic et al., 2018). In accordance with our observations in the TRACERx renal cohort (**Figure 4**), the pattern of disease spread was consistent with "attenuated progression". The two pancreatic metastases were seeded by separate clones (indicating potentially distinct waves of metastatic spread) neither of which harboured 9p loss . By contrast, subsequent metastases to the lymph nodes, liver, lung and kidney were seeded by an advanced clone harbouring additional SCNA events, including loss of 9p.

Discussion

We present an integrated analysis of 580 primary and 255 metastatic biopsies across 98 patients with metastatic ccRCC, in addition to the first report of two ccRCC cases profiled in a post-mortem setting. To our knowledge this is the largest study of its kind to date, offering broad insights into the diverse spectrum of modes of progression from primary to metastatic disease. A key objective of the TRACERx Renal study is to reduce sampling bias and provide clonal resolution of the primary tumour, such that the metastasis-seeding clones can be distinguished from metastasis incompetent clones more reliably. Clonal resolution facilitates an improved understanding of the genomic events, and broader clonal characteristics, that drive metastasis and mortality risk. In addition, the wide range of metastatic tissue sites sampled in this study allows detailed analysis of the varying metastatic phenotypes in ccRCC.

First, in characterising metastases, we show profound evidence of evolutionary bottlenecking, with metastatic sites being both more homogeneous (median proportion of clonal variants = 0.87) and harbouring fewer somatic alterations (median=9), compared to their matched primary tumours (0.33 and 12). Furthermore, only a minority of driver events (5.4%) were found to be private (or *de novo*) in metastases, indicating that the majority of driver diversity accumulated at the primary tumour site, which then serves as the substrate for selection of metastasis-competent populations. Tumour clones which were "selected" and progressed from primary to metastatic sites of disease were characterised by elevated levels of somatic copy number alterations, increased proliferation and evidence of

immune evasion (in the form of HLA LOH), but not by increased SNV/INDEL counts. 9p loss was found to be a potent driver of both metastasis and ccRCC mortality risk, even after adjustment for established clinically prognostic indicators. Loss of 14q also showed a trend towards significance, and taken together these two events represent hallmark genomic alterations in ccRCC metastasis (overall 36 of 38 TRACERx Renal cases had loss of at least one of these chromosome arms). Furthermore 71% (n=27) of the metastatic cases in the TRACERx renal cohort had loss of both 9p and 14q, compared to only 35% (n=22) of cases without metastatic disease at presentation (n=62, Companion paper, Turajlic et al., 2018), suggesting these events interact to drive metastatic risk. While investigation of functional mechanisms is beyond the scope of this study, we note that p16 (encoded by *CDKN2A* on 9p) has been shown to modulate VEGF expression via its interaction with HIF-1alpha, encoded by *HIF1A* on 14q (Zhang et al., 2010). Critically, in the context of their potential utility as biomarkers both 9p and 14q loss were predominantly subclonal in our multi-regional analysis of primary ccRCC (Companion paper, Turajlic et al., 2018) and may be missed by single biopsy approaches.

Secondly, our analyses highlight distinct modes of metastatic dissemination (**Figure 7**). In primary tumours characterised by low ITH and high wGII, metastatic competence is acquired within the most recent common ancestor, which drives rapid dissemination, leading to surgical failure, poor response to systemic therapy and early death from disease. These observations are consistent with the presence of occult micrometastases at the time of surgery. The multiclonal driver case K548, examined at post-mortem, is an exemplar of the disseminated metastatic seeding from such tumours. In this context, we note that these tumours are a minority in the TRACERx Renal cohort which was weighted towards operable patients; hence low ITH / high wGII pattern may be prevalent in patients who are deemed inoperable. Acquisition of the metastatic potential at the early stage of tumour evolution has been reported in pancreatic (Notta et al., 2016) and breast cancers (Gao et al., 2016) as well as uveal melanoma (Field et al., 2018). These observations are consistent with the tendency of

some tumours to metastasise rapidly and improving outcomes in such cases presents a significant challenge.

We observed a contrasting phenotype in primary tumours characterised by high ITH and "attenuated progression" (**Figure 4**). Metastatic competence was acquired gradually and was limited to certain subpopulations in the primary tumour at the time of surgical resection. The clinical course was characterised by an initial solitary or oligometastatic pattern, with metastatic capacity increasing over time resulting in more efficient and widespread metastases, as exemplified by case K489. The marked latency of metastases in case K489 that may have been mediated by effective immune surveillance, a notion supported by the subsequent disease control with interferon. Our observations in this group underpin the significance of cytoreductive nephrectomy and removal of an "evolutionary sink of diversity" (Gerlinger et al., 2012) in minimising the risk of future metastatic seeding from evolving primary tumours harbouring clones of variable metastatic potential. We note that the evolutionary trajectories in this group are underpinned by *PBRM1* alterations, in keeping with the observation by Brugorolas and colleagues that loss of *PBRM1* expression is associated with an increased risk of metastasis but not with decreased survival (Joseph et al., 2016). Further OS analysis in larger metastatic cohorts will be required to comprehensively contrast the drivers of metastasis from the drivers of early mortality.

In an exploratory analysis of intravascular tumour growth, we observe that TT is associated with improved prognosis when seeded from the ancestral clone. In this context, the TT conceivably formed shortly after the clonal sweep in the primary tumour, rapidly ascending through the IVC, leading to clinical diagnosis (majority of patients presented with symptoms). This notion is consistent with the fact TT is not prognostic in the absence of nodal or metastatic disease (Wszolek et al., 2008) and that thrombectomy can be curative. In contrast to primary tumours presenting with TT, isolated lymph node involvement portends an extremely poor prognosis in ccRCC, significantly worse than in other solid tumour types (Gershman et al., 2017). We observe that lymph node metastases are characterised by similar levels of wGII compared to distant metastases (no significant difference,

P=0.21) and frequently harbour 9p loss (21/22 cases), indicating that lymphatic and haematogenous spread require comparable metastatic competence. Our observations contrast the divergent lymph node/distant metastasis patterns reported in other tumour types (Brastianos et al., 2015; Yates et al., 2017); however, are consistent with the frequent presentation of lymph node metastases with visceral metastases and the lack of therapeutic benefit from lymph node clearance (Bhindi et al., 2018). Finally, in our limited analyses of late recurrences in the pancreas (4 cases) the metastasis-establishing clone diverged early from the primary tumour and harboured fewer additional events, consistent with protracted latency. The metastasising clones lacked 9p loss, suggesting that less aggressive clones establish pancreatic metastasis in isolation in keeping with the excellent clinical outcome in these patients.

In summary, we demonstrate that the fitness attribute common to metastases and tumour thrombus-seeding (sub)clones is chromosomal complexity. Chromosome level alterations that simultaneously affect the expression of 100s of genes (Santaguida and Amon, 2015) can support the complex metastatic cascade, by altering many functional phenotypes and potentially impacting immune evasion (Davoli et al., 2017). The onset of chromosomal complexity in ccRCC provides a permissive genomic background for selection of 9p loss, previously linked to poor outcomes (El-Mokadem et al., 2014; Klatte et al., 2009; La Rochelle et al., 2010). While preliminary in nature, our collective observations point to the deterministic nature of the ccRCC evolutionary subtypes and their association with the mode and tempo of metastatic progression. The “rapid progression” group has echoes of the punctuated equilibrium model of rapid speciation events (Eldredge and Gould, 1997) and subsequent clonal stasis. In contrast, the evolution of the “attenuated progression” group is analogous to Darwin’s phyletic gradualism. Continuing longitudinal and post-mortem sampling opportunities in the TRACERx Renal and PEACE studies aim to address this question.

In conclusion, evolutionary classification of tumours could serve as an important biomarker for stratification of patients for surgical intervention (e.g. cytoreductive nephrectomy/metastasectomy) in the presence of metastatic disease; to aid management of patients following surgery with curative

intent, including decisions on surveillance schedule and adjuvant therapy; and in the context of active surveillance programme at the earliest stages of disease by identifying metastatic potential.

Consortia

TRACERx Renal

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Figure Legends

Figure 1 - Overview

Panel A is an overview of somatic alterations detected in matched primary and metastatic tumours across a subset of 38 TRACERx Renal patients. The top panel shows the proportion of clonal and subclonal alterations. In the middle panel alterations in primary tumours are indicated in a lighter shade of colour, those in metastases in darker. Clonal alterations are shown as rectangles and subclonal alterations as triangles. Parallel evolution is indicated in orange with a split indicating multiple events. Abbreviation for tumour sites: P - primary; TT - tumour thrombus; AD - adrenal gland, indirect metastasis; AD(D) – direct invasion of adrenal gland; AD(CL) – contralateral adrenal gland; Renal(CL) – contralateral kidney; Pr – perirenal invasiona; Pf – peri-nephric fat and gerota fascia invasion. **Panel B** shows the number of clonal and subclonal somatic alterations in primary and metastatic tumours. **Panel C** shows the number of somatic alterations 1) detected in both primary tumour and the matched metastatic tumour; 2) detected in primary tumour but not the matched metastatic tumour and 3) detected in metastatic tumour but not the matched primary tumour. **Panel D** shows the proportions of synchronous and metachronous metastatic tumours profiled in the TRACERx Renal, HUC and MSK cohorts. **Panel E** shows the range of the metastatic sites sampled across the TRACERx, HUC and MSK cohorts. The total number of metastases sampled (n), and the number from each study (Tx represents TRACERx Renal; HUC and MSK are extension cohorts) are shown in brackets.

Figure 2 – Characterisation of metastasising clone(s)
Panel A illustrates the method used to categorising tumour clones. **Panel B** shows four violin plots summarising (starting top left and working clockwise): i) non-synonymous mutation count, ii) wGII, iii) ploidy and iv) Ki67. Values are compared between tumour clones “not selected” and “selected” for metastasis, with all region/clone values plotted per tumour (excluding MRCA “maintained” clones – see STAR methods). A linear mixed effect (LME) model was used to determine significance, to account for the non-independence of multiple observations from individual tumours. **Panel C** shows for each driver event the proportion of times it was observed in “not selected” and “selected” clones, for TRACERx, HUC and MSK cohorts. The far right panel shows the log10 p-value for each event, for enrichment in “selected” versus “not selected” clones, testing using a binomial test with meta-analysis conducted using Fisher's method of combining p values from independent tests, and p-values corrected for multiple testing using Benjamini–Hochberg procedure. **Panel D** shows overall survival hazard ratios for events with $P<0.05$ in panel C analysis. Data is shown for TRACERx and HUC cohorts separately, with the circle representing the hazard ratio value, and lines corresponding to the 95% confidence interval estimate. **Panel E** shows overall survival results for TRACERx and HUC cohorts (combined), split into two groups based on SCNA status at chr 9p21.3 (either copy number loss at chr 9p21.3, or normal wildtype copy number).

Figure 3 – Tumour thrombus

Figure 3 shows tumour thrombus (TT) driver trees with primary clones in the bottom panel; level I, level II, level III and level IV tumour thrombus clones in light green, blue, orange and red, respectively. Tumour TNM stage and driver events leading to TT are annotated. Where no uniquely identifiable primary clone was found to seed the TT, a dotted circle is used to represent the notional seeding clone at the TT level. Length of branches connecting clones is not informative.

Figure 4 – Lymph node and distant metastases

Panel A shows driver trees and the clinical course for cases with lymph node and distal metastases. Cases were grouped into those with "rapid progression" and "attenuated progression". Annotated for each case are the primary tumour evolutionary subtype, primary tumour ITH/wGII classification, select driver events on the tree (VHL, BAP1, PBRM1, MTOR, SETD2, TSC1, TSC2, chr 9p loss and chr 14q loss). Metastasis-seeding subclones and any subclones private to metastasis are highlighted in blue. Clinical course is shown from the time of nephrectomy to death or last follow up. Pattern of disease progression is classified as multiple new metastases (multiple circles), solitary new metastasis (single circle) and progression of existing metastases ("PD"). Progression and follow up times are shown in months. Systemic treatments are indicated. Synchronous and metachronous metastatic sites are listed under corresponding time points. Profiled metastases are highlighted in blue boxes. Abbreviation for tumour sites: P - primary; TT - tumour thrombus; AD - adrenal gland; AD(D) – direct invasion of adrenal gland; AD(CL) – contralateral adrenal gland; Renal(CL) – contralateral kidney; Pr – perirenal invasion; Pf – peri-nephric fat and gerota fascia invasion. **Panel B** shows the number of cases with "rapid progression" or "attenuated progression" in each evolutionary subtype. **Panel C** shows the maximum wGII and ITH in cases with "rapid progression" and "attenuated progression".

Figure 5 – Latent metastases

Panel A shows the distribution of times from nephrectomy to metastasis resection, split by site of metastasis. The circle represents the median value, and grey lines depicts the median average deviation (MAD) value (i.e. plus/minus one MAD). Far right in brackets are the range [min to max] values. **Panel B** shows wGII values per region split by site of metastasis. All regions are shown per metastasis, and a linear mixed effect (LME) model was used to determine significance (for pancreas versus all other), to account for the non-independence of multiple observations from individual tumours. **Panel C** shows fishplot progression patterns for the three cases (SP006, SP023, SP058) with latent pancreatic metastases.

Figure 6 – Spatial resolution of metastases through post-mortem sampling

Figure 6 shows cases K548 (top) and K489 (bottom) which were sampled at post-mortem with the extent of sampling and the clinical course shown. Metastatic progression is illustrated using fish plots with the select driver events annotated (*VHL, BAP1, PBRM1, MTOR, SETD2, TSC1, TSC2*, chr 9p loss and chr 14q loss). Metastasising clone colour matches that of the corresponding metastatic site.

Figure 7

Summary figure describing key conclusions from the study.

Supplementary figure legends

Figure S1: Consort diagram for the selection of the metastatic samples.
Related to Star methods.

Figure S2: Driver events in HUC and MSK cohorts.
Figure S2 shows driver mutations and driver SCNAs detected in matched primary and metastatic tumours in HUC (panel A) and MSK (panel B) cohorts. Clonal alterations are shown as rectangles and subclonal alterations as triangles. Parallel evolution is indicated in orange with a split indicating multiple events. Related to Figure 1

Figure S3: Analysis of tumour thrombus.
Figure S3 Panel A shows the survival analysis for cases where tumour thrombus had early (*Group C1_TT_early*, from founding most recent common ancestor clone) or late (*Group C2_TT_late*, from a later subclone) evolutionary divergence. Survival probability is measured as cancer related death P=0.018 (log-rank test). Panel B shows Ki67 proliferation index data (mean % of cells staining positive for Ki67 across all primary tumour regions) for cases presenting with and without TT. Panel C shows cases with TT and distal metastases. Related to Figure 3.

Figure S4: Fish-plot summary of selected cases.
Figure S4 shows 2 example cases with distal metastases. Diagrams of the primary tumour and the involved tissue sites are illustrated. Fishplots are used to show disease evolution. Driver events are annotated on each fishplot. Related to Figure 4

Figure S5: Driver tree for case SP58.
Related to Figure 5.

Table S1 Clinical and tumour characteristics of TRACERx, HUC and MSK Primary-Met pair patients, and evolutionary subtypes, progression patterns, treatment of disease and survival outcomes of TRACERx and PEACE patients.
Related to Figure 1, Figure 4 and Figure 6.

Table S6 Details of custom driver panels (v3, v5 and v6) and somatic alterations in TRACERx cohort.
Related to Figure 1 and Star methods.

STAR Methods

Contact for reagent and resource sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Charles Swanton (Charles.swanton@crick.ac.uk).

Experimental model and subject details

Patients were recruited into TRACERx Renal, an ethically approved prospective cohort study (National Health Service Research Ethics Committee approval 11/LO/1996). The study sponsor is the Royal Marsden NHS Foundation Trust. The study is coordinated by the Renal Unit at the Royal Marsden Hospital NHS Foundation Trust. The study is open to recruitment at the following sites: Royal Marsden Hospital NHS Foundation Trust, Guy's and St Thomas' Hospital NHS Foundation Trust, Royal Free Hospital NHS Foundation Trust and Western General Hospital (NHS Lothian). Patients were recruited into the study according to the following eligibility criteria:

Inclusion criteria

- Age 18- years or older
- Patients with histologically confirmed renal cell carcinoma, or suspected renal cell carcinoma, proceeding to nephrectomy/metastectomy
- Medical and/or surgical management in accordance with national and/or local guidelines
- Written informed consent (permitting fresh tissue sampling and blood collection; access to archived diagnostic material and anonymised clinical data)

Exclusion criteria

- Any concomitant medical or psychiatric problems which, in the opinion of the investigator, would prevent completion of treatment or follow-up
- Lack of adequate tissue

Further eligibility criteria were applied to the cohort presented in this paper (it therefore follows that these patients do not have consecutive study ID numbers from 001 to 100):

- Confirmed histological diagnosis of clear cell renal cell carcinoma.
- No family history of renal cell carcinoma.

- No known germline renal cell carcinoma predisposition syndrome (including VHL).
- At least three primary tumour regions available for analysis.

The cohort was representative of patients eligible for curative or cytoreductive nephrectomy. Full clinical characteristics are provided in Table S1. Demographic data include: Sex, Age and Ethnicity. Clinical data include: Presenting symptoms, Smoking status, BMI, History of Previous RCC, Family History of RCC, Bilateral or Multi-focal RCC, Neoadjuvant therapy (6 patients received systemic therapy prior to nephrectomy). Histology data include: overall TNM Stage (based on Version 7 classification), Location of nephrectomy, Number of harvested and involved lymph nodes, presence of Microvascular Invasion, presence of Renal Vein Invasion, presence of IVC tumour thrombus, Size of primary tumour, Leibovich score, Fuhrman Grade, Time to nephrectomy (days). Clinical status of patients included: Relapse -free survival (months), Total follow up (months), Survival Outcome.

Extension cohort of primary and metastatic (P-M) pairs was accessed under the approval of Basque Country Research Ethics Committee, Hospital Universitario Cruces (Ref CEIC-Euskadi PI2015101).

Post-mortem sampling was performed in the context of the PEACE study (National Health Service Research Ethics Committee approval 13/LO/0972/AM05); https://clinicaltrials.gov/ct2/show/NCT03004755.

Method details

Sample collection (TRACERx cohort and post-mortem sampling)

All surgically resected specimens were reviewed macroscopically by a pathologist to guide multi-region sampling for this study and to avoid compromising diagnostic requirements. Tumour measurements were recorded and the specimen were photographed before and after sampling. Primary tumours were dissected along the longest axes and spatially separated regions sampled from the “tumour slice” using a 6 mm punch biopsy needle. The punch was changed between samples to avoid contamination. The total number of samples obtained reflects the tumour size with a minimum of 3 biopsies that are non-overlapping and equally spaced. However, areas which are obviously

fibrotic or haemorrhagic are avoided during sampling and every attempt is made to reflect macroscopically heterogeneous tumour areas. Primary tumour regions are labelled as R1, R2, R3…and locations are recorded. Normal kidney tissue was sampled from areas distant to the primary tumour and labelled N1. Each biopsy was split into two for snap freezing and formalin fixing respectively, such that the fresh frozen sample has its mirror image in the formalin-fixed sample which is subsequently paraffin embedded. Fresh samples were placed in a 1.8 ml cryotube and immediately snap frozen in liquid nitrogen for >30 seconds and transferred to -80 C for storage. Peripheral blood was collected at the time of surgery and processed to separate buffy coat.

Nucleic acid isolation from tissue and blood (TRACERx and PEACE cohorts)

DNA and RNA were co-purified using the AllPrep DNA/RNA mini kit. (Qiagen). Briefly, a 2mm^3 piece of tissue was added to 900ul of lysis buffer and homogenised for five seconds using the TissueRaptor (Qiagen) with a fresh homogenisation probe being used for each preparation. Each lysate was applied to a QiaShredder (Qiagen) and then sequentially purified using the DNA and RNA columns according to the manufacturers protocol. Germline control DNA was isolated from whole blood using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturers protocol. DNA quality and yield was measured and accessed using the TapeStation (Agilent) and Qubit Fluorometric quantification (ThermoFisher Scientific).

Purification of DNA from Formalin Fixed Paraffin Embedded (FFPE) tissue

For a minority of TRACERx Renal cases (n=8), tumour material was obtained from FFPE material (**Table S5**). An H&E section from all patient FFPE blocks is reviewed by a pathologist and tumour rich regions are identified for DNA purification. Either a 20uM sections is cut and the area of interest scraped from the slide using a blade alternatively a 2mm core is directly punched from the block. DNA is purified using the GeneRead DNA FFPE kit (Qiagen) with yields and quality being determined by Qubit quantification and TapeStation analysis.

Micro-dissection and nucleic acid isolation (HUC extension cohort)

H&E slides from each case were annotated by pathologists for regions of interest (ROI). Multiple ROIs within the primary tumour were selected on the bases of good tissue preservation avoiding areas of necrosis and haemorrhage, and to reflect microscopically distinct areas with regards to grade (high vs low) and morphology (clear vs. granular/eosinophilic), and sarcomatoid differentiation, where present, as well as areas of normal tissue. The annotated H&E was then used as a reference to guide the dissection of ROIs from serial sections. All tissue sections were cut to 10 µm thickness and deparaffinized with three, five minute incubations in xylene prior to dissection using the alpha AVENIO Millisect System (Roche Diagnostics, Indianapolis, IN) (Adey et al., 2013). The milling tip blade size for the dissection was selected based on the estimated area of the ROI, where small ROIs less than 200mm2 used small blade sizes (200 or 400µm) and ROIs larger than 200mm2 used larger blade sizes (800 µm). The milling buffer for all dissections was 1x TE buffer with 2% SDS, pH 7.5. Genomic DNA was isolated from each of the dissected FFPE tissue samples using a High Pure FFPE DNA Isolation kit (Roche).

Methylation specific PCR

Methylation of the VHL promoter was detected after bisulphite treatment of 500ng of patient DNA using the EZ DNA Methylation-Direct kit (Zymo Research). Bisulphite treated DNA was amplified in the PCR using methylation specific oligonucleotides (oligonucleotide sequences are detailed in **Table S6**), followed by Big Dye terminator Sanger sequencing. Methylation was confirmed by comparing and contrasting patient tumour and normal renal tissue for methylation protected CpG sequences.

Regional staining by Immunohistochemistry and Digital Image Analysis of Ki67

Tissue sections of 4µm were mounted on slides and immunohistochemical staining for Ki67 was performed using a fully automated immunohistochemistry (IHC) system and ready-to-use optimized reagents according to the manufacturer´s recommendations (Ventana Discovery Ultra, Ventana, Arizona, USA). Primary antibody used was rabbit anti-Ki67 (AB16667, Abcam, Cambridge, UK) and secondary antibody was Discovery Omnimap anti-rabbit HRP RUO (760-4311, Roche, Rotkreuz, Switzerland). DAB kit was Discovery Chromomap DAB RUO (760-4311, Roche). After IHC procedure, slides were first evaluated for Ki67 staining quality using mouse intestine tissue as positive control. Regions containing tumour tissue were identified and marked by a pathologist and subsequently scanned in brightfield at 20x magnification using Zeiss Axio Scan.Z1 and ZEN lite imaging software (Carl Zeiss Microscopy GmbH, Jena, Germany). Digital images were then subjected to automated image analysis using StrataQuest version 5 (TissueGnostics, Vienna, Austria) for Ki67 quantification. Three different gates were set to quantify low, medium and high intensity DAB staining which corresponded to Ki67 expression levels. Results were depicted as total percentage of Ki67-positive nuclei.

Flow Cytometry Determination of DNA Content (FACS)

Fresh frozen tumour tissue samples, approximately 4mm^3 in size, were mechanically disrupted and incubated in 2ml of 0.5% pepsin solution (Sigma, UK) at 37 ºC for 40 minutes to create a suspension of nuclei. The nuclei were washed with phosphate-buffered saline (PBS) and then fixed with 70% ethanol for a minimum of 90 minutes. The nuclei were washed again with PBS and stained with 200µl of propidium iodide (50µg/ml) overnight. Flow cytometric analysis of DNA content was performed using the LSR Fortessa Cell Analyzer (Becton Dickinson, San Jose, USA), BD Facs Diva™ software and FlowJo software (FlowJo LLC, Oregon, USA. A minimum of 10,000 events were recorded (typically up to 20,000 and up to 100,000 in complex samples). Analysis was performed using methods derived from the European Society for Analytical Cellular Pathology DNA Consensus in Flow Cytometry guidelines. Gating of forward and side scatter was applied to exclude debris and cell clumping. Samples with <7,500 events after gating were excluded from further analysis. The coefficient of variation (CV) was measured on each G1 peak. Samples with a CV>10% were excluded from further

analysis. Each tumour sample was assumed to contain normal cells to act as internal standard. Where possible the position of the diploid peak was calculated with reference to the peak of diploid cells in a case matched normal tissue sample. The DNA index (DI) of any aneuploid peak present was calculated by dividing the G1 peak of the aneuploid population by the G1 peak of the normal diploid cells. Diploid samples were defined as having DI of 1.00. Any additional peak was defined as aneuploid. A tetraploid peak was defined as having a DI of 1.90-2.10 and containing >15% of total events unless a second peak corresponding to G2 was clear on the histogram. Similarly, aneuploid peaks near to G1 (DI 0.90-1.10) were only considered if there was a clear second peak containing >15% of total events.

Detection of allelic imbalance at the HLA locus

Allelic imbalance was detected using two polymorphic Sequence-Tagged Site (STR) markers located on the short arm of chr 6, close to the HLA locus - (D6S248 and ATA12D05), six STR markers located downstream of the HLA locus on the short arm of chr 6p - (D6S1960, GATA143B11, D6S1714, D6S1573, D6S438 and D6S257), and six STR markers located upstream of the HLA locus on the short arm of chr 6p – (D6S410, D6S2257, D6S1034, D6S202, D6S1617, D6S1668). 20ng of patient germline and tumor region DNA was amplified using the PCR. The PCR comprised of denaturing at 950C for 5mins, then 35 cycles of denaturing at 950C for 1min, followed by an annealing temperature of 550C for 1min, 720C for 1min and then a PCR extension at 720C for 10min. PCR products were separated on the ABI 3730xl DNA analyzer. Fragment length and area under the curve of each allele was determined using the Applied Biosystems software GeneMapper v5. When two separate alleles were identified for a particular marker, the fragments could be analyzed for allelic imbalance using the formula (Atumor/Atumor)/(Anormal/Anormal). The output of this formula was defined as the normalized allelic ratio.

Targeted Driver Panel (DP) design and validation

Driver gene panels (Panel_v3, Panel_v5 and Panel_v6) were used in this study. Panel_v3 was designed in 2014, including 110 putative driver genes. Panel_v5 and Panel_v6 were designed in 2015, including 119 and 130 putative driver genes respectively. Driver genes were selected from genes that were frequently mutated in TCGA (accessed in April 2015) or highlighted in relevant studies (Arai et al., 2014; Sato et al., 2013; Scelo et al., 2014). Only alterations in driver genes represented in all three panels were considered in the overall driver mutation analyses. All panels targeted potential driver SCNA regions. To prevent inter-patient samples swaps, we included the 24 SNPs that were previously identified by Pengelly et al in Panel_v5 and Panel_v6. Details of the 3 panels can be found in Supplementary table (**Table S8**).

Driver Panel Library Construction and Targeted Sequencing

Following isolated gDNA QC, depending on the available yield, samples were normalised to either 1-3 µg or 200 ng for the Agilent SureSelectXT Target Enrichment Library Protocol; standard or low input sample preparation respectively. Samples were normalised using a 1X Low TE Buffer. Samples were sheared to 150-200bp using a Covaris E220 (Covaris, Woburn, MA, USA), following the run parameters outlined in the Agilent SureSelectXT standard 3 µg and low input 200 ng DNA protocols. Library construction of samples was then performed following the SureSelectXT protocols, using 6 pre-capture PCR cycles for the standard input samples and 10 pre-capture PCR cycles for the 200 ng low input samples. Hybridisation and capture were performed for each individual sample using the Agilent custom Renal Driver Panel target-specific capture library (versions 3, 5 & 6). The same version of the capture library being used for all samples from the same patient case. Captured SureSelect-enriched DNA libraries were PCR amplified using 14 post-capture PCR cycles in PCR reactions that included the appropriate indexing primer for each sample. Amplified, captured, indexed libraries passing final QC on the TapeStation 4200 were normalised to 2nM and pooled, ensuring that unique indexes were allocated to all final libraries (up to 96 single indexes available) in the pool. QC of the final library pools was performed using the Agilent Bioanalyzer High Sensitivity DNA Assay. Library pool QC results were

used to denature and dilute samples in preparation for sequencing on the Illumina HiSeq 2500 and NextSeq 500 sequencing platforms. The final libraries were sequenced 101bp paired-end multiplexed on the Illumina HiSeq 2500 and 151bp paired-end multiplexed on the NextSeq 500, at the Advanced Sequencing Facility at the Francis Crick Institute. Equivalent sequencing metrics, including per sample coverage, was observed between platforms. Single nucleotide variants (SNVs), dinucleotide variants (DNVs), small insertions and deletions (INDELs) and somatic copy number alterations (SCNAs) were derived from 463 primary tumour regions and 169 matched metastatic regions from 56 primary-metastasis pairs in 38 patients (with some patients providing multiple metastases, **Figure S1**, **Figure 1A**). Median sequencing coverage was 613x (range 166-1479x) across primary tumour regions and 567x (range 273-2661x) across metastatic regions.

Targeted DP library construction and sequencing (HUC cohort)

DP targeted hybrid-capture panel- Solution-based hybridization capture probes (Roche Sequencing Solutions) were selected from a genome-wide database of pre-scored probes, which varied in size from 50 to 100 nucleotides. Probes were filtered for repetitiveness in the human genome by building a 15-mer histogram from the entire human genome, and then calculating the average 15-mer frequency of the probe by sliding a 15 bp window across the length of each probe. Probes with a score greater than 100 were filtered as repetitive. The remaining probes were scored for uniqueness in the human genome, using SSAHA (http://www.sanger.ac.uk/science/tools/ssaha). A match in the genome was defined as any 30-mer match in the genome, allowing up to 5 mismatches or indels along the length of the match. Additional scoring parameters included penalties for simple sequence repeats and penalties for deviation from a target Tm of 80 C. Target regions of interest were increased to a minimum size of 100 bp, and then tiled with an average overlap of 35 bp, allowing the probes to overhang the ends of the target regions. These tiled probes were selected from the aforementioned pre-scored database of probes by choosing the best scoring probe starting in a 15 bp window, moving 20 bp in the 3' direction, and repeating. Probes were allowed to have up to 20 possible matches in the

genome, though for this panel 99.5% of the probes had 5 or fewer matches. Selected probe sequences were manufactured into biotinylated sequence capture probe pools by Roche Sequencing Solutions – Madison.

Library construction. Libraries were constructed using the SeqCap EZ HyperCap Workflow User's Guide, v1.0 (Roche Sequencing Solutions). The extracted DNA was enzymatically fragmented using the KAPA HyperPlus library prep kit according to manufacturer's instructions (Roche Sequencing Solutions). Fragmentation time for DNA isolated from FFPE was linked to the mass of input DNA, and varied from 12 to 22 minutes depending on input amount (10 to 100 ng). To increase the efficiency of library prep, adapter volume was reduced to 3 l and the adapter ligation reaction was extended to 3 hours at 20oC for cases with 100ng of input DNA, and at 16 hours at 16oC for libraries with less than 100ng of input DNA.

Sequencing- Captured samples were pooled following post-capture amplification, and sequenced using an Illumina HiSeq 2500 instrument. Dual HiSeq SBS v4 (Illumina) runs at 101 base-paired-end reads generated the data for analysis.

SNV, and INDEL calling from multi-region DP sequencing

Paired-end reads (2x100bp) in FastQ format sequenced by Hiseq or NextSeq were aligned to the reference human genome (build hg19), using the Burrows-Wheeler Aligner (BWA) v0.7.15. with seed recurrences (-c flag) set to 10000 (Li and Durbin, 2009). Intermediate processing of Sam/Bam files was performed using Samtools v1.3.1 and deduplication was performed using Picard 1.81 (http://broadinstitute.github.io/picard/) (Li and Durbin, 2009). Single Nucleotide Variant (SNV) calling was performed using Mutect v1.1.7 and small scale insetion/deletions (INDELs) were called running VarScan v2.4.1 in somatic mode with a minimum variant frequency (--min-var-freq) of 0.005, a tumour purity estimate (--tumor-purity) of 0.75 and then validated using Scalpel v0.5.3 (scalpel-discovery in --somatic mode) (intersection between two callers taken)(Cibulskis et al., 2013; Fang et al., 2016; Koboldt et al., 2009). SNVs called by Mutect were further filtered using the following criteria: i) ≤5

alternative reads supporting the variant and variant allele frequency (VAF) ≤ 1% in the corresponding germline sample, ii) variants that falling into mitochondrial chr, haplotype chr, HLA genes or any intergenic region were not considered, iii) presence of both forward and reverse strand reads supporting the variant, iv) >5 reads supporting the variant in at least one tumour region of a patient, v) variants were required to have cancer cell fraction (CCF)>0.5 in at least one tumour region (see **Subclonal deconstruction of mutations** section for details of CCF calculation) , vi) variants were required to have CCF>0.1 to be called as present in a tumour region, vii) sequencing depth in each region need to be >=50 and ≤3000. Finally, suspected artefact variants, based on inconsistent allelic frequencies between regions, were reviewed manually on the Integrated Genomics Viewer (IGV), and variants with poorly aligned reads were removed. Dinucleotide substitutions (DNV) were identified when two adjacent SNVs were called and their VAFs were consistently balanced (based on proportion test, *P*>=0.05). In such cases the start and stop positions were corrected to represent a DNV and frequency related values were recalculated to represent the mean of the SNVs. To reduce sequencing artefacts from FFPE samples, we further filtered out variants that were significantly enriched for presence in FFPE compared with fresh frozen samples (Fisher's exact test, *P*<0.001). Variants were annotated using Annovar (Wang et al., 2010). Variants were annotated using Annovar (Wang et al., 2010). Deleterious mutations were defined if two out of three algorithms - SIFT, PolyPhen2 and MutationTaster - predicted the mutation as deleterious. Individual tumour biopsy regions were judged to have failed quality control and excluded from analysis based on the following criteria: i) sequencing coverage depth below 100X, ii) low tumour purity such that copy number calling failed. Mutations detected in high-confidence driver genes (*VHL, PBRM1, SETD2, PIK3CA, MTOR, PTEN, KDM5C, CSMD3, BAP1, TP53*) were defined as *driver mutations*.

SCNA calling from multi-region DP sequencing

To estimate somatic copy number alterations, CNVkit v0.7.3 was performed with default parameter on paired tumour-normal sequencing data (Talevich et al., 2016). Outliers of the derived log2-ratio

(logR) calls from CNVkit were detected and modified using Median Absolute Deviation Winsorization before case-specific joint segmentation to identify genomic segments of constant logR (Nilsen et al., 2012). Tumour sample purity, ploidy and absolute copy number per segment were estimated using ABSOLUTE v1.0.6 (Carter et al., 2012). In line with recommended best practice all ABSOLUTE solutions were reviewed by 3 researchers, with solutions selected based on majority vote. Copy number alterations were then called as losses or gains relative to overall sample wide estimated ploidy. Arm gain or loss was called when >50% of the chromosomal have copy number gain or loss. Driver copy number was identified by overlapping the called somatic copy number segments with putative driver copy number regions identified by Beroukhim and colleagues (Beroukhim et al., 2009). For a subset of TRACERx Renal patients, we compared SCNA calls between targeted panel and WGS datasets, and SCNA concordance was 87% (Companion paper, Turajlic et al., 2018). The average proportion of the genome with aberrant copy number, weighted on each of the 22 autosomal chromosomes, was estimated as the weighted genome instability index (wGII).

MSK validation cohort

Matched tumour and normal aligned sequencing files (BAM format) for the MSK cohort were obtained directly from the authors (Becerra et al., 2017) and were then converted into FASTQ format files using bam2fastq in bedtools package (Quinlan and Hall, 2010). SNVs, INDELs and SCNAs were called using the same methods as TRACERx Renal data (**STAR Methods:** SNV, and INDEL calling from multi-region DP sequencing, SCNA calling from multi-region DP sequencing). Of the 49 cases with ccRCC histology, 15 cases (Pair 8, Pair 9, Pair 13, Pair 17, Pair 22, Pair 35, Pair 38, Pair 42, Pair 43, Pair 44, Pair 48, Pair 52, Pair 56, Pair 58, Pair 59) were excluded from the study as the ABSOLUTE v1.0.6 algorithm failed to find a stable SCNA solution. Clonality of SNVs and SCNAs were estimated using ABSOLUTE v1.0.6. Cancer cell fraction for INDELs were calculated using method described in **STAR Methods:** Subclonal deconstruction of mutations. INDELs with CCF>0.5 were called clonal. ITH index for each patient was

calculated as the measure of intratumour heterogeneity (ITH index = # subclonal drivers / # clonal drivers).

Quantification and statistical analysis

R 3.3.2 was used for all statistical analyses. We tested for difference in driver event count between primary and metastatic samples using linear regression, including biopsy number per sample as an independent term in the regression model. The comparison of wGII, DNA index and Ki67 scores between "not selected" and "selected" clones was assessed using region values per case. Regions were classified as being within "not selected" or "selected" clones based on the clustering solution for each tumour. Regions found to be only within the founding MRCA clone, or polyclonal with both "not selected" and "selected" clones, were excluded. The comparison of non-synonymous mutation counts between "not selected" and "selected" clones was based directly on clonal clustering solution obtained for each case, again with founding MRCA clones excluded. For all "not selected" versus "selected" comparisons a linear mixed effect (LME) model was used to determine significance, to account for the non-independence of multiple observations from individual tumours. The comparison of maximum wGII (defined as the maximum regional wGII value per primary tumour) between "Rapid" and "Attenuated" metastatic progression groups was assessed using Mann-Whitney test. Comparison of ITH values (again one score per tumour) between "Rapid" and "Attenuated" metastatic progression groups was determined using Mann-Whitney test. The comparison of wGII between pancreatic and all other metastatic tissue sites was assessed conducted using region values per case, with significance determined using a LME model.

Subclonal deconstruction of mutations

To estimate the clonality of a mutation in a region, we used the following formula:

$$vaf = \frac{CN_{mut} * CCF * p}{CN_n * (1-p) + CN_t * p}$$

where vaf is the variant allele frequency at the mutation base; p is estimated tumour purity; CN_t and CN_n are the tumour locus specific copy number and the normal locus specific copy number which was

assumed to be 2 for autosomal chromosomes; and CCF is the fraction of tumour cells carrying the mutation. Consider CN_{mut} is the number of chromosomal copies that carry the mutation, the possible CN_{mut} is ranging from 1 to CN_t (integer number). We then assigned CCF with one of the possible value: 0.01, 0.02, ..., 1, together with every possible CN_{mut} to find the best fit cancer cell fraction of the mutation. Since we focused on driver genes in this study and the accuracy of the estimated CCF is limited by the size of the panel, we call mutations with CCF>0.5 as clonal mutations, mutations with CCF≤0.5 and CCF>0.1 are subclonal. To determine the clonality of a mutation in a tumour, we ask for the mutation to be clonal in all regions in a tumour. Exceptions were made for long INDELs that affect >6 bp of the genome, due to VAF under estimation. If a long INDEL is present in all regions of a tumour, we called it as clonal. To determine the clonality of a SCNA in a tumour, we ask for the SCNA to be presence in all tumour regions, otherwise it is called subclonal.

Driver tree reconstruction

A matrix with presence and absence of nonsynonymous and synonymous point mutations, DNVs, INDELs and arm level SCNAs was created for each tumour, and all the events were clustered based on the following rule: a valid cluster has to have at least two arm level SCNAs or one non-synonymous mutation. The driver events clusters were then ordered into a clonal hierarchy using TRONCO and presented as driver trees (De Sano et al., 2016).

In terms of limitations, we recognise that our Driver Panel phylogenies are based on fewer clonal markers, as compared to whole exome or genome derived phylogenetic trees. As a consequence, some tumour clones are based on only a limited number of genomic markers, and similarly the inferred modes of metastatic seeding (e.g. monoclonal vs polyclonal) are also based on a limited set of markers. However, two contingency measures are in place to mitigate against phylogenetic misconstruction: i) ultra-deep 500x sequencing coverage, which ensures stably derived cancer cell

fraction estimates, ii) a bespoke gene panel which is enriched for driver events, increasing the likelihood that mutational markers are driving genuine clonal expansion.

Enrichment of events in metastases

All tumour clones were categorised into three groups based on evidence of selection in the metastasis/metastases: i) clone that are not selected ("no selection", defined as subclonal in the primary and absent from metastasis), ii) clones that are maintained ("maintained", defined as the most recent common ancestor (MRCA) clones, clonal in both primary and metastasis), iii) clones that are selected ("selection", defined as subclonal in the primary and clonal in metastasis; or absent in the primary and present in metastasis). In addition, we observed a small number of clones with alternative selection patterns: a) being subclonal in both primary and in metastases (*i.e.* polyclonal metastases), which we categorised as "maintained", and b) being clonal in primary and sublconal in metastases categorised as "maintained" c) clonal in primary but absent in metasteses (*i.e.* illusion of clonality or events lost by secondary somatic changes), which we categorised as "no selection". For each driver event (mutational or SCNA), the proportion of times it was found in "not selected", "maintained" and "selected" clones was calculated for each of the TRACERx, HUC and MSK cohorts. For comparison purposes, a background null distribution of proportions was determined for both mutations and SCNAs, based on all passenger events in each cohort. The proportion of "selected" clones was compared to the "not selected" proportion, using a Binomial test, with probability of selection taken from the null model, and number of trials based on event counts in each cohort. Meta-analysis across the three cohorts was conducted using Fisher's method of combining p values from independent tests, and p-values were corrected for multiple testing using Benjamini–Hochberg procedure.

Survival analysis

Survival analysis was conducted using the Kaplan-Meier method, with p-value determined by a log-rank test. Hazard ratio and multivariate analysis adjusting for clinical parameters was determined through a Cox proportional hazards model.

Data and Software Availability

Sequencing data that supports this study will been deposited at the European Genome-phenome Archive (EGA), which is hosted by the European Bioinformatics Institute (EBI); accession number EGAS00001002793.
Additional resources

Clinical trial registry numbers:
https://clinicaltrials.gov/ct2/show/NCT03226886;
https://clinicaltrials.gov/ct2/show/NCT03004755

TRACERx Renal study website, detailing investigators, sponsors and collaborators: http://tracerx.co.uk/studies/renal/

Declaration of interest

S.T., H.X., K.L. and C.S. have a patent on renal cell carcinoma biomarkers pending.

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References

A. Bex, P.M., M.A.S. Jewett, J. Wagstaff, R. van Velthoven, P.M. Laguna Pes, L. Wood, H.H.E. van Melick, P. Soetekouw, J.B. Lattouf, T. Powles, E. Boleti, I-J. de Jong, S. Rottey, B. Tombal, S. Marreaud, L. Collette, S. Collette, C.U. Blank, J.B. Haanen (2017). Immediate versus deferred cytoreductive nephrectomy (CN) in patients with synchronous metastatic renal cell carcinoma (mRCC) receiving sunitinib (EORTC 30073 SURTIME). Ann Oncol.

Arai, E., Sakamoto, H., Ichikawa, H., Totsuka, H., Chiku, S., Gotoh, M., Mori, T., Nakatani, T., Ohnami, S., Nakagawa, T., et al. (2014). Multilayer-omics analysis of renal cell carcinoma, including the whole exome, methylome and transcriptome. Int J Cancer 135, 1330-1342.

Bashashati, A., Ha, G., Tone, A., Ding, J., Prentice, L.M., Roth, A., Rosner, J., Shumansky, K., Kalloger, S., Senz, J., et al. (2013). Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. J Pathol 231, 21-34.

Becerra, M.F., Reznik, E., Redzematovic, A., Tennenbaum, D.M., Kashan, M., Ghanaat, M., Casuscelli, J., Manley, B., Jonsson, P., DiNatale, R.G., et al. (2017). Comparative Genomic Profiling of Matched Primary and Metastatic Tumors in Renal Cell Carcinoma. Eur Urol Focus.

Beroukhim, R., Brunet, J.P., Di Napoli, A., Mertz, K.D., Seeley, A., Pires, M.M., Linhart, D., Worrell, R.A., Moch, H., Rubin, M.A., et al. (2009). Patterns of gene expression and copy-number alterations in von-hippel lindau disease-associated and sporadic clear cell carcinoma of the kidney. Cancer Res 69, 4674-4681.

Bhindi, B., Wallis, C.J.D., Boorjian, S.A., Thompson, R.H., Farrell, A., Kim, S.P., Karam, J.A., Capitanio, U., Golijanin, D., Leibovich, B.C., et al. (2018). The role of lymph node dissection in the management of renal cell carcinoma: a systematic review and meta-analysis. BJU Int.

Bianchi, M., Sun, M., Jeldres, C., Shariat, S.F., Trinh, Q.D., Briganti, A., Tian, Z., Schmitges, J., Graefen, M., Perrotte, P., et al. (2012). Distribution of metastatic sites in renal cell carcinoma: a population-based analysis. Ann Oncol 23, 973-980.

Brastianos, P.K., Carter, S.L., Santagata, S., Cahill, D.P., Taylor-Weiner, A., Jones, R.T., Van Allen, E.M., Lawrence, M.S., Horowitz, P.M., Cibulskis, K., et al. (2015). Genomic Characterization of Brain Metastases Reveals Branched Evolution and Potential Therapeutic Targets. Cancer Discov 5, 1164-1177.

Campbell, P.J., Yachida, S., Mudie, L.J., Stephens, P.J., Pleasance, E.D., Stebbings, L.A., Morsberger, L.A., Latimer, C., McLaren, S., Lin, M.L., et al. (2010). The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature 467, 1109-1113.

Carter, S.L., Cibulskis, K., Helman, E., McKenna, A., Shen, H., Zack, T., Laird, P.W., Onofrio, R.C., Winckler, W., Weir, B.A., et al. (2012). Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol 30, 413-421.

Casuscelli, J., Weinhold, N., Gundem, G., Wang, L., Zabor, E.C., Drill, E., Wang, P.I., Nanjangud, G.J., Redzematovic, A., Nargund, A.M., et al. (2017). Genomic landscape and evolution of metastatic chromophobe renal cell carcinoma. JCI Insight 2.

Cibulskis, K., Lawrence, M.S., Carter, S.L., Sivachenko, A., Jaffe, D., Sougnez, C., Gabriel, S., Meyerson, M., Lander, E.S., and Getz, G. (2013). Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 31, 213-219.

Dabestani, S., Marconi, L., Hofmann, F., Stewart, F., Lam, T.B., Canfield, S.E., Staehler, M., Powles, T., Ljungberg, B., and Bex, A. (2014). Local treatments for metastases of renal cell carcinoma: a systematic review. Lancet Oncol 15, e549-561.

Davoli, T., Uno, H., Wooten, E.C., and Elledge, S.J. (2017). Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science 355.

De Sano, L., Caravagna, G., Ramazzotti, D., Graudenzi, A., Mauri, G., Mishra, B., and Antoniotti, M. (2016). TRONCO: an R package for the inference of cancer progression models from heterogeneous genomic data. Bioinformatics 32, 1911-1913.

Ding, L., Ellis, M.J., Li, S., Larson, D.E., Chen, K., Wallis, J.W., Harris, C.C., McLellan, M.D., Fulton, R.S., Fulton, L.L., et al. (2010). Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature 464, 999-1005.
El-Mokadem, I., Fitzpatrick, J., Bondad, J., Rauchhaus, P., Cunningham, J., Pratt, N., Fleming, S., and Nabi, G. (2014). Chr 9p deletion in clear cell renal cell carcinoma predicts recurrence and survival following surgery. Br J Cancer 111, 1381-1390.
Eldredge, N., and Gould, S.J. (1997). On punctuated equilibria. Science 276, 338-341.
Fang, H., Bergmann, E.A., Arora, K., Vacic, V., Zody, M.C., Iossifov, I., O'Rawe, J.A., Wu, Y.Y., Barron, L.T.J., Rosenbaum, J., et al. (2016). Indel variant analysis of short-read sequencing data with Scalpel. Nat Protoc 11, 2529-2548.
Field, M.G., Durante, M.A., Anbunathan, H., Cai, L.Z., Decatur, C.L., Bowcock, A.M., Kurtenbach, S., and Harbour, J.W. (2018). Punctuated evolution of canonical genomic aberrations in uveal melanoma. Nat Commun 9, 116.
Gao, R., Davis, A., McDonald, T.O., Sei, E., Shi, X., Wang, Y., Tsai, P.C., Casasent, A., Waters, J., Zhang, H., et al. (2016). Punctuated copy number evolution and clonal stasis in triple-negative breast cancer. Nat Genet 48, 1119-1130.
Gerlinger, M., Horswell, S., Larkin, J., Rowan, A.J., Salm, M.P., Varela, I., Fisher, R., McGranahan, N., Matthews, N., Santos, C.R., et al. (2014). Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet 46, 225-233.
Gerlinger, M., Rowan, A.J., Horswell, S., Math, M., Larkin, J., Endesfelder, D., Gronroos, E., Martinez, P., Matthews, N., Stewart, A., et al. (2012). Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 366, 883-892.
Gershman, B., Moreira, D.M., Thompson, R.H., Boorjian, S.A., Lohse, C.M., Costello, B.A., Cheville, J.C., and Leibovich, B.C. (2017). Renal Cell Carcinoma with Isolated Lymph Node Involvement: Long-term Natural History and Predictors of Oncologic Outcomes Following Surgical Resection. Eur Urol 72, 300-306.
Giancotti, F.G. (2013). Mechanisms governing metastatic dormancy and reactivation. Cell 155, 750-764.
Gundem, G., Van Loo, P., Kremeyer, B., Alexandrov, L.B., Tubio, J.M.C., Papaemmanuil, E., Brewer, D.S., Kallio, H.M.L., Hognas, G., Annala, M., et al. (2015). The evolutionary history of lethal metastatic prostate cancer. Nature 520, 353-357.
Haffner, M.C., Mosbruger, T., Esopi, D.M., Fedor, H., Heaphy, C.M., Walker, D.A., Adejola, N., Gurel, M., Hicks, J., Meeker, A.K., et al. (2013). Tracking the clonal origin of lethal prostate cancer. J Clin Invest 123, 4918-4922.
Joseph, R.W., Kapur, P., Serie, D.J., Parasramka, M., Ho, T.H., Cheville, J.C., Frenkel, E., Parker, A.S., and Brugarolas, J. (2016). Clear Cell Renal Cell Carcinoma Subtypes Identified by BAP1 and PBRM1 Expression. J Urol 195, 180-187.
Klatte, T., Rao, P.N., de Martino, M., LaRochelle, J., Shuch, B., Zomorodian, N., Said, J., Kabbinavar, F.F., Belldegrun, A.S., and Pantuck, A.J. (2009). Cytogenetic profile predicts prognosis of patients with clear cell renal cell carcinoma. J Clin Oncol 27, 746-753.
Koboldt, D.C., Chen, K., Wylie, T., Larson, D.E., McLellan, M.D., Mardis, E.R., Weinstock, G.M., Wilson, R.K., and Ding, L. (2009). VarScan: variant detection in massively parallel sequencing of individual and pooled samples. Bioinformatics 25, 2283-2285.
Kutikov, A., Uzzo, R.G., Caraway, A., Reese, C.T., Egleston, B.L., Chen, D.Y., Viterbo, R., Greenberg, R.E., Wong, Y.N., Raman, J.D., et al. (2010). Use of systemic therapy and factors affecting survival for patients undergoing cytoreductive nephrectomy. BJU Int 106, 218-223.
La Rochelle, J., Klatte, T., Dastane, A., Rao, N., Seligson, D., Said, J., Shuch, B., Zomorodian, N., Kabbinavar, F., Belldegrun, A., et al. (2010). Chr 9p deletions identify an aggressive phenotype of clear cell renal cell carcinoma. Cancer 116, 4696-4702.
Lee, S.Y., Haq, F., Kim, D., Jun, C., Jo, H.J., Ahn, S.M., and Lee, W.S. (2014). Comparative genomic analysis of primary and synchronous metastatic colorectal cancers. PLoS One 9, e90459.

Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754-1760.
McGranahan, N., Rosenthal, R., Hiley, C.T., Rowan, A.J., Watkins, T.B.K., Wilson, G.A., Birkbak, N.J., Veeriah, S., Van Loo, P., Herrero, J., et al. (2017). Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. Cell 171, 1259-1271 e1211.
McKay, R.R., Kroeger, N., Xie, W., Lee, J.L., Knox, J.J., Bjarnason, G.A., MacKenzie, M.J., Wood, L., Srinivas, S., Vaishampayan, U.N., et al. (2014). Impact of bone and liver metastases on patients with renal cell carcinoma treated with targeted therapy. Eur Urol 65, 577-584.
McPherson, A., Roth, A., Laks, E., Masud, T., Bashashati, A., Zhang, A.W., Ha, G., Biele, J., Yap, D., Wan, A., et al. (2016). Divergent modes of clonal spread and intraperitoneal mixing in high-grade serous ovarian cancer. Nat Genet 48, 758-767.
Nilsen, G., Liestol, K., Van Loo, P., Vollan, H.K.M., Eide, M.B., Rueda, O.M., Chin, S.F., Russell, R., Baumbusch, L.O., Caldas, C., et al. (2012). Copynumber: Efficient algorithms for single- and multi-track copy number segmentation. Bmc Genomics 13.
Notta, F., Chan-Seng-Yue, M., Lemire, M., Li, Y., Wilson, G.W., Connor, A.A., Denroche, R.E., Liang, S.B., Brown, A.M., Kim, J.C., et al. (2016). A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns. Nature 538, 378-382.
Psutka, S.P., and Leibovich, B.C. (2015). Management of inferior vena cava tumor thrombus in locally advanced renal cell carcinoma. Ther Adv Urol 7, 216-229.
Quinlan, A.R., and Hall, I.M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841-842.
Reese, A.C., Whitson, J.M., and Meng, M.V. (2013). Natural history of untreated renal cell carcinoma with venous tumor thrombus. Urol Oncol 31, 1305-1309.
Robinson, D.R., Wu, Y.M., Lonigro, R.J., Vats, P., Cobain, E., Everett, J., Cao, X., Rabban, E., Kumar-Sinha, C., Raymond, V., et al. (2017). Integrative clinical genomics of metastatic cancer. Nature 548, 297-303.
Santaguida, S., and Amon, A. (2015). Short- and long-term effects of chr mis-segregation and aneuploidy. Nat Rev Mol Cell Biol 16, 473-485.
Sato, Y., Yoshizato, T., Shiraishi, Y., Maekawa, S., Okuno, Y., Kamura, T., Shimamura, T., Sato-Otsubo, A., Nagae, G., Suzuki, H., et al. (2013). Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet 45, 860-867.
Scelo, G., Riazalhosseini, Y., Greger, L., Letourneau, L., Gonzalez-Porta, M., Wozniak, M.B., Bourgey, M., Harnden, P., Egevad, L., Jackson, S.M., et al. (2014). Variation in genomic landscape of clear cell renal cell carcinoma across Europe. Nat Commun 5, 5135.
Schwarz, R.F., Ng, C.K., Cooke, S.L., Newman, S., Temple, J., Piskorz, A.M., Gale, D., Sayal, K., Murtaza, M., Baldwin, P.J., et al. (2015). Spatial and temporal heterogeneity in high-grade serous ovarian cancer: a phylogenetic analysis. PLoS Med 12, e1001789.
Shah, S.P., Morin, R.D., Khattra, J., Prentice, L., Pugh, T., Burleigh, A., Delaney, A., Gelmon, K., Guliany, R., Senz, J., et al. (2009). Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 461, 809-813.
Talevich, E., Shain, A.H., Botton, T., and Bastian, B.C. (2016). CNVkit: Genome-Wide Copy Number Detection and Visualization from Targeted DNA Sequencing. Plos Comput Biol 12.
Wang, K., Li, M.Y., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38.
Weichselbaum, R.R., and Hellman, S. (2011). Oligometastases revisited. Nat Rev Clin Oncol 8, 378-382.
Wszolek, M.F., Wotkowicz, C., and Libertino, J.A. (2008). Surgical management of large renal tumors. Nat Clin Pract Urol 5, 35-46.
Yachida, S., Jones, S., Bozic, I., Antal, T., Leary, R., Fu, B., Kamiyama, M., Hruban, R.H., Eshleman, J.R., Nowak, M.A., et al. (2010). Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature 467, 1114-1117.

Yates, L.R., Knappskog, S., Wedge, D., Farmery, J.H.R., Gonzalez, S., Martincorena, I., Alexandrov, L.B., Van Loo, P., Haugland, H.K., Lilleng, P.K., et al. (2017). Genomic Evolution of Breast Cancer Metastasis and Relapse. Cancer Cell 32, 169-184 e167.
Zehir, A., Benayed, R., Shah, R.H., Syed, A., Middha, S., Kim, H.R., Srinivasan, P., Gao, J., Chakravarty, D., Devlin, S.M., et al. (2017). Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 23, 703-713.
Zhang, J., Lu, A., Li, L., Yue, J., and Lu, Y. (2010). p16 Modulates VEGF expression via its interaction with HIF-1alpha in breast cancer cells. Cancer Invest 28, 588-597.
Zhao, Z.M., Zhao, B., Bai, Y., Iamarino, A., Gaffney, S.G., Schlessinger, J., Lifton, R.P., Rimm, D.L., and Townsend, J.P. (2016). Early and multiple origins of metastatic lineages within primary tumors. Proc Natl Acad Sci U S A 113, 2140-2145.

KEY RESOURCES TABLE

The table highlights the genetically modified organisms and strains, cell lines, reagents, software, and source data **essential** to reproduce results presented in the manuscript. Depending on the nature of the study, this may include standard laboratory materials (i.e., food chow for metabolism studies), but the Table is **not** meant to be comprehensive list of all materials and resources used (e.g., essential chemicals such as SDS, sucrose, or standard culture media don't need to be listed in the Table). **Items in the Table must also be reported in the Method Details section within the context of their use.** The number of **primers and RNA sequences** that may be listed in the Table is restricted to no more than ten each. If there are more than ten primers or RNA sequences to report, please provide this information as a supplementary document and reference this file (e.g., See Table S1 for XX) in the Key Resources Table.

Please note that ALL references cited in the Key Resources Table must be included in the References list. Please report the information as follows:

- **REAGENT or RESOURCE:** Provide full descriptive name of the item so that it can be identified and linked with its description in the manuscript (e.g., provide version number for software, host source for antibody, strain name). In the Experimental Models section, please include all models used in the paper and describe each line/strain as: model organism: name used for strain/line in paper: genotype. (i.e., Mouse: $OXTR^{fl/fl}$: B6.129(SJL)-$Oxtr^{tm1.1Wsy/J}$). In the Biological Samples section, please list all samples obtained from commercial sources or biological repositories. Please note that software mentioned in the Methods Details or Data and Software Availability section needs to be also included in the table. See the sample Table at the end of this document for examples of how to report reagents.

- **SOURCE:** Report the company, manufacturer, or individual that provided the item or where the item can obtained (e.g., stock center or repository). For materials distributed by Addgene, please cite the article describing the plasmid and include "Addgene" as part of the identifier. If an item is from another lab, please include the name of the principal investigator and a citation if it has been previously published. If the material is being reported for the first time in the current paper, please indicate as "this paper." For software, please provide the company name if it is commercially available or cite the paper in which it has been initially described.

- **IDENTIFIER:** Include catalog numbers (entered in the column as "Cat#" followed by the number, e.g., Cat#3879S). Where available, please include unique entities such as RRIDs, Model Organism Database numbers, accession numbers, and PDB or CAS IDs. For antibodies, if applicable and available, please also include the lot number or clone identity. For software or data resources, please include the URL where the resource can be downloaded. Please ensure accuracy of the identifiers, as they are essential for generation of hyperlinks to external sources when available. Please see the Elsevier list of Data Repositories with automated bidirectional linking for details. When listing more than one identifier for the same item, use semicolons to separate them (e.g. Cat#3879S; RRID: AB_2255011). If an identifier is not available, please enter "N/A" in the column.
    - ***A NOTE ABOUT RRIDs:*** We highly recommend using RRIDs as the identifier (in particular for antibodies and organisms, but also for software tools and databases). For more details on how to obtain or generate an RRID for existing or newly generated resources, please visit the RII or search for RRIDs.

Please use the empty table that follows to organize the information in the sections defined by the subheading, skipping sections not relevant to your study. Please do not add subheadings. To add a row, place the cursor at the end of the row above where you would like to add the row, just outside the right border of the table. Then press the ENTER key to add the row. You do not need to delete empty rows. Each entry must be on a separate row; do not list multiple items in a single table cell. Please see the sample table at the end of this document for examples of how reagents should be cited.

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TABLE FOR AUTHOR TO COMPLETE

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Bacterial and Virus Strains		
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Critical Commercial Assays		
Deposited Data		
Human reference genome NCBI build 37, GRCh37	Genome Reference Consortium	http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/

Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		
Oligonucleotides		
Oligonucleotide sequences for VHL exon amplification and methylation specific PCR	This paper	Table S6
Primer, D6S248; Forward:TGCAGTGAGCCGAGATCAA	https://genome.ucsc.edu	D6S248_FAM_F
Primer, D6S248; Reverse: GACAATATCAAAAAGAACTGCCAAA	https://genome.ucsc.edu	D6S248_R
Primer, ATA12D05 Forward: AAAGTGAGACTCCGCCTCAT	https://genome.ucsc.edu	ATA12D05_HEX_F
Primer, ATA12D05 Reverse: CACCTCAGCCTCTTTGGTAG	https://genome.ucsc.edu	ATA12D05_R
Recombinant DNA		
Software and Algorithms		
Burrows-Wheeler Aligner (BWA) v0.7.15	Li and Durbin, 2009	http://bio-bwa.sourceforge.net/
Samtools v1.3.1	Li et al., 2009	http://samtools.sourceforge.net/
Picard 1.81		http://broadinstitute.github.io/picard/
Mutect v1.1.7	Cibulskis et al., 2013	http://archive.broadinstitute.org/cancer/cga/mutect
VarScan v2.4.1	Koboldt et al., 2009	http://varscan.sourceforge.net/
Scalpel v0.5.3	Fang et al., 2016	https://github.com/hanfang/scalpel-protocol
Annovar	Wang et al., 2010	http://annovar.openbioinformatics.org/en/latest/
CNVkit v0.7.3	Talevich et al., 2016	https://cnvkit.readthedocs.io/en/stable/cp

R package 'Copynumber'	Nilsen et al., 2012	http://bioconductor.org/packages/release/bioc/html/copynumber.html
ABSOLUTE v1.0.6	Carter et al., 2012	http://archive.broadinstitute.org/cancer/cga/absolute
bedtools package	Quinlan and Hall, 2010	http://bedtools.readthedocs.io/en/latest/
R package 'TRONCO'	De Sano et al., 2016	http://www.bioconductor.org/packages/release/bioc/html/TRONCO.html
AlleleCounter		github.com/cancerit/alleleCount
ASCAT	Van Loo et al., 2010	https://github.com/Crick-CancerGenomics/ascat
Battenberg	Nik-Zainal et al., 2012	https://github.com/cancerit/cgpBattenberg
Other		

TABLE WITH EXAMPLES FOR AUTHOR REFERENCE

REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Antibodies		
Rabbit monoclonal anti-Snail	Cell Signaling Technology	Cat#3879S; RRID: AB_2255011

Mouse monoclonal anti-Tubulin (clone DM1A)	Sigma-Aldrich	Cat#T9026; RRID: AB_477593
Rabbit polyclonal anti-BMAL1	This paper	N/A
Bacterial and Virus Strains		
pAAV-hSyn-DIO-hM3D(Gq)-mCherry	Krashes et al., 2011	Addgene AAV5; 44361-AAV5
AAV5-EF1a-DIO-hChR2(H134R)-EYFP	Hope Center Viral Vectors Core	N/A
Cowpox virus Brighton Red	BEI Resources	NR-88
Zika-SMGC-1, GENBANK: KX266255	Isolated from patient (Wang et al., 2016)	N/A
Staphylococcus aureus	ATCC	ATCC 29213
Streptococcus pyogenes: M1 serotype strain: strain SF370; M1 GAS	ATCC	ATCC 700294
Biological Samples		
Healthy adult BA9 brain tissue	University of Maryland Brain & Tissue Bank; http://medschool.umaryland.edu/btbank/	Cat#UMB1455
Human hippocampal brain blocks	New York Brain Bank	http://nybb.hs.columbia.edu/
Patient-derived xenografts (PDX)	Children's Oncology Group Cell Culture and Xenograft Repository	http://cogcell.org/
Chemicals, Peptides, and Recombinant Proteins		
MK-2206 AKT inhibitor	Selleck Chemicals	S1078; CAS: 1032350-13-2
SB-505124	Sigma-Aldrich	S4696; CAS: 694433-59-5 (free base)
Picrotoxin	Sigma-Aldrich	P1675; CAS: 124-87-8
Human TGF-β	R&D	240-B; GenPept: P01137
Activated S6K1	Millipore	Cat#14-486
GST-BMAL1	Novus	Cat#H00000406-P01
Critical Commercial Assays		
EasyTag EXPRESS 35S Protein Labeling Kit	Perkin-Elmer	NEG772014MC
CaspaseGlo 3/7	Promega	G8090
TruSeq ChIP Sample Prep Kit	Illumina	IP-202-1012
Deposited Data		
Raw and analyzed data	This paper	GEO: GSE63473
B-RAF RBD (apo) structure	This paper	PDB: 5J17
Human reference genome NCBI build 37, GRCh37	Genome Reference Consortium	http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/
Nanog STILT inference	This paper; Mendeley Data	http://dx.doi.org/10.17632/wx6s4mj7s8.2

Affinity-based mass spectrometry performed with 57 genes	This paper; and Mendeley Data	Table S8; http://dx.doi.org/10.17632/5hvpvspw82.1
Experimental Models: Cell Lines		
Hamster: CHO cells	ATCC	CRL-11268
D. melanogaster: Cell line S2: S2-DRSC	Laboratory of Norbert Perrimon	FlyBase: FBtc0000181
Human: Passage 40 H9 ES cells	MSKCC stem cell core facility	N/A
Human: HUES 8 hESC line (NIH approval number NIHhESC-09-0021)	HSCI iPS Core	hES Cell Line: HUES-8
Experimental Models: Organisms/Strains		
C. elegans: Strain BC4011: srl-1(s2500) II; dpy-18(e364) III; unc-46(e177)rol-3(s1040) V.	Caenorhabditis Genetics Center	WB Strain: BC4011; WormBase: WBVar00241916
D. melanogaster: RNAi of Sxl: y[1] sc[*] v[1]; P{TRiP.HMS00609}attP2	Bloomington Drosophila Stock Center	BDSC:34393; FlyBase: FBtp0064874
S. cerevisiae: Strain background: W303	ATCC	ATTC: 208353
Mouse: R6/2: B6CBA-Tg(HDexon1)62Gpb/3J	The Jackson Laboratory	JAX: 006494
Mouse: OXTRfl/fl: B6.129(SJL)-Oxtr$^{tm1.1Wsy}$/J	The Jackson Laboratory	RRID: IMSR_JAX:008471
Zebrafish: Tg(Shha:GFP)t10: t10Tg	Neumann and Nuesslein-Volhard, 2000	ZFIN: ZDB-GENO-060207-1
Arabidopsis: 35S::PIF4-YFP, BZR1-CFP	Wang et al., 2012	N/A
Arabidopsis: JYB1021.2: pS24(AT5G58010)::cS24:GFP(-G):NOS #1	NASC	NASC ID: N70450
Oligonucleotides		
siRNA targeting sequence: PIP5K I alpha #1: ACACAGUACUCAGUUGAUA	This paper	N/A
Primers for XX, see Table SX	This paper	N/A
Primer: GFP/YFP/CFP Forward: GCACGACTTCTTCAAGTCCGCCATGCC	This paper	N/A
Morpholino: MO-pax2a GGTCTGCTTTGCAGTGAATATCCAT	Gene Tools	ZFIN: ZDB-MRPHLNO-061106-5
ACTB (hs01060665_g1)	Life Technologies	Cat#4331182
RNA sequence: hnRNPA1_ligand: UAGGGACUUAGGGUUCUCUCUAGGGACUUAGGGUUCUCUCUAGGGA	This paper	N/A
Recombinant DNA		
pLVX-Tight-Puro (TetOn)	Clonetech	Cat#632162
Plasmid: GFP-Nito	This paper	N/A
cDNA GH111110	Drosophila Genomics Resource Center	DGRC:5666; FlyBase:FBcl0130415
AAV2/1-hsyn-GCaMP6- WPRE	Chen et al., 2013	N/A
Mouse raptor: pLKO mouse shRNA 1 raptor	Thoreen et al., 2009	Addgene Plasmid #21339

Software and Algorithms		
Bowtie2	Langmead and Salzberg, 2012	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
Samtools	Li et al., 2009	http://samtools.sourceforge.net/
Weighted Maximal Information Component Analysis v0.9	Rau et al., 2013	https://github.com/ChristophRau/wMICA
ICS algorithm	This paper; Mendeley Data	http://dx.doi.org/10.17632/5hvpvspw82.1
Other		
Sequence data, analyses, and resources related to the ultra-deep sequencing of the AML31 tumor, relapse, and matched normal.	This paper	http://aml31.genome.wustl.edu
Resource website for the AML31 publication	This paper	https://github.com/chrisamiller/aml31SuppSite

Figure 1

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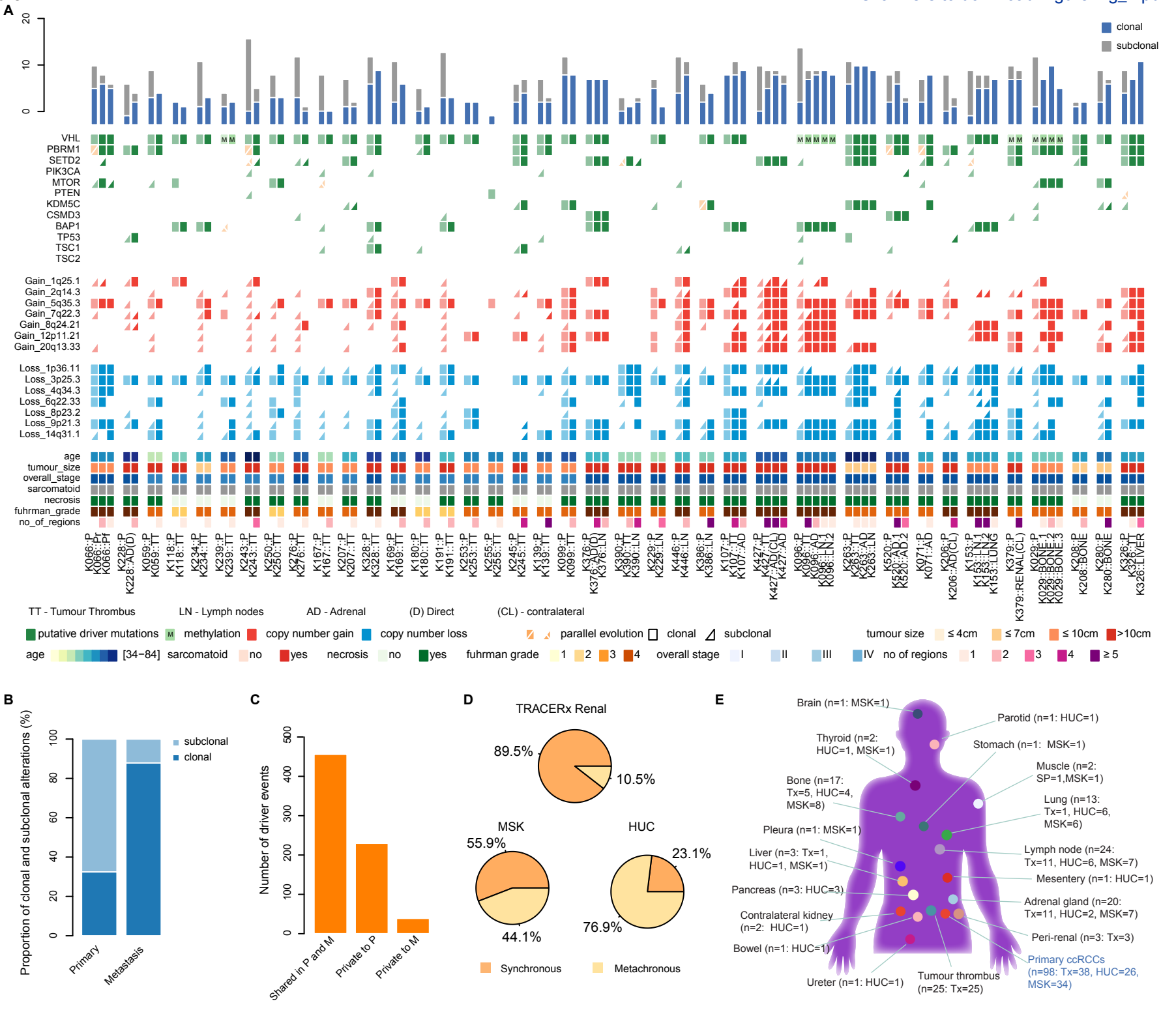

Figure 2

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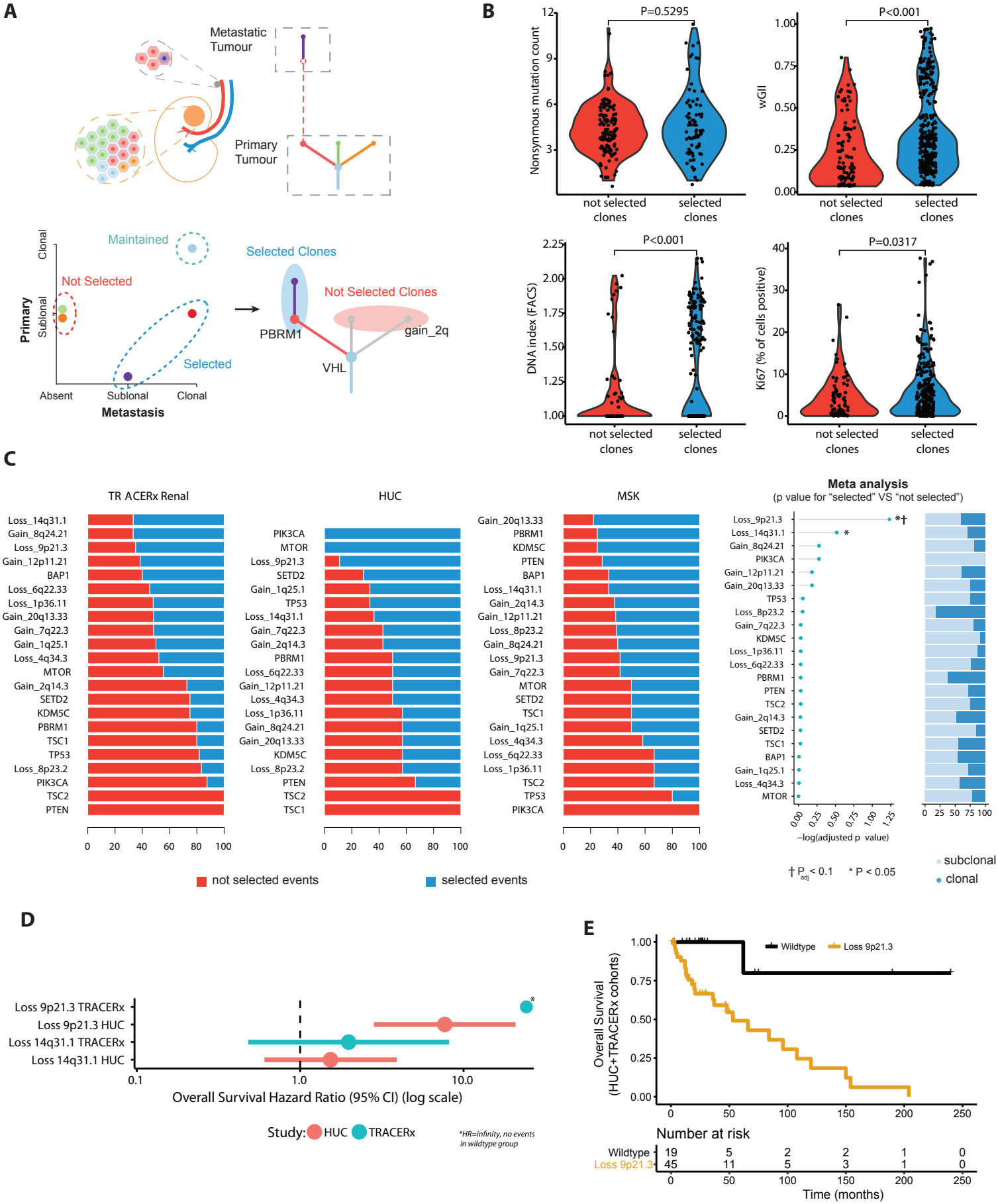

Figure 3

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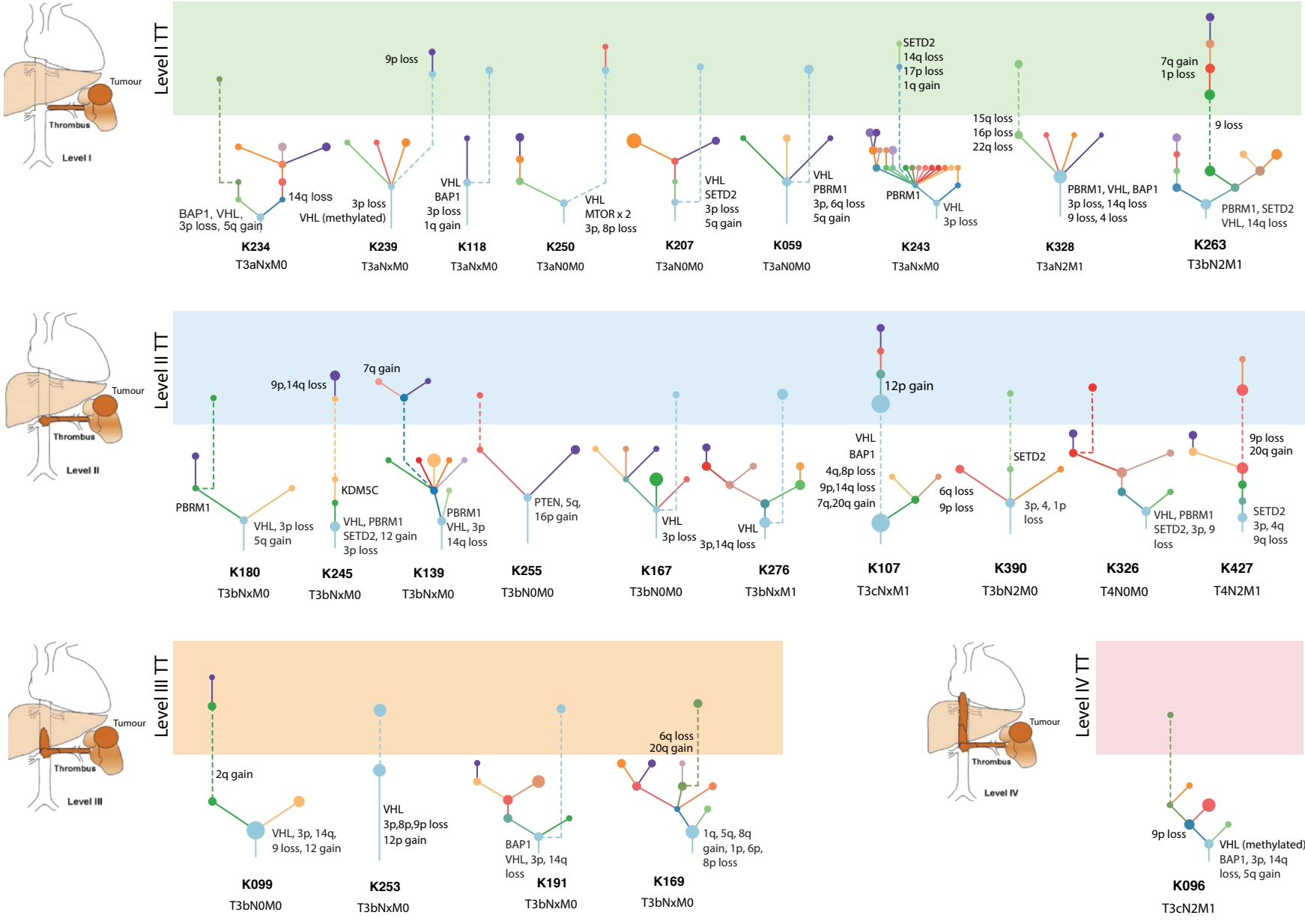

Figure 4

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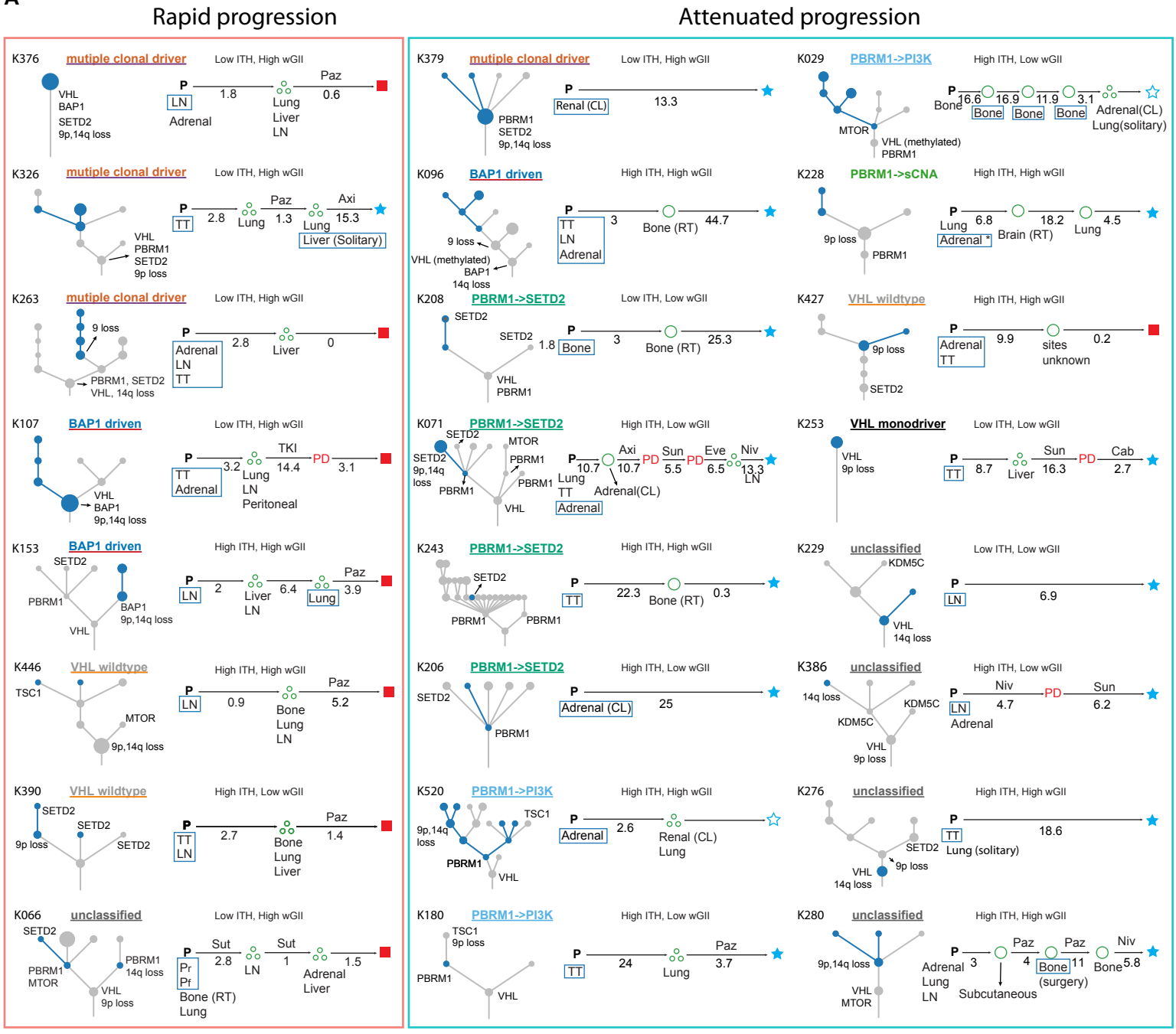

Figure 5

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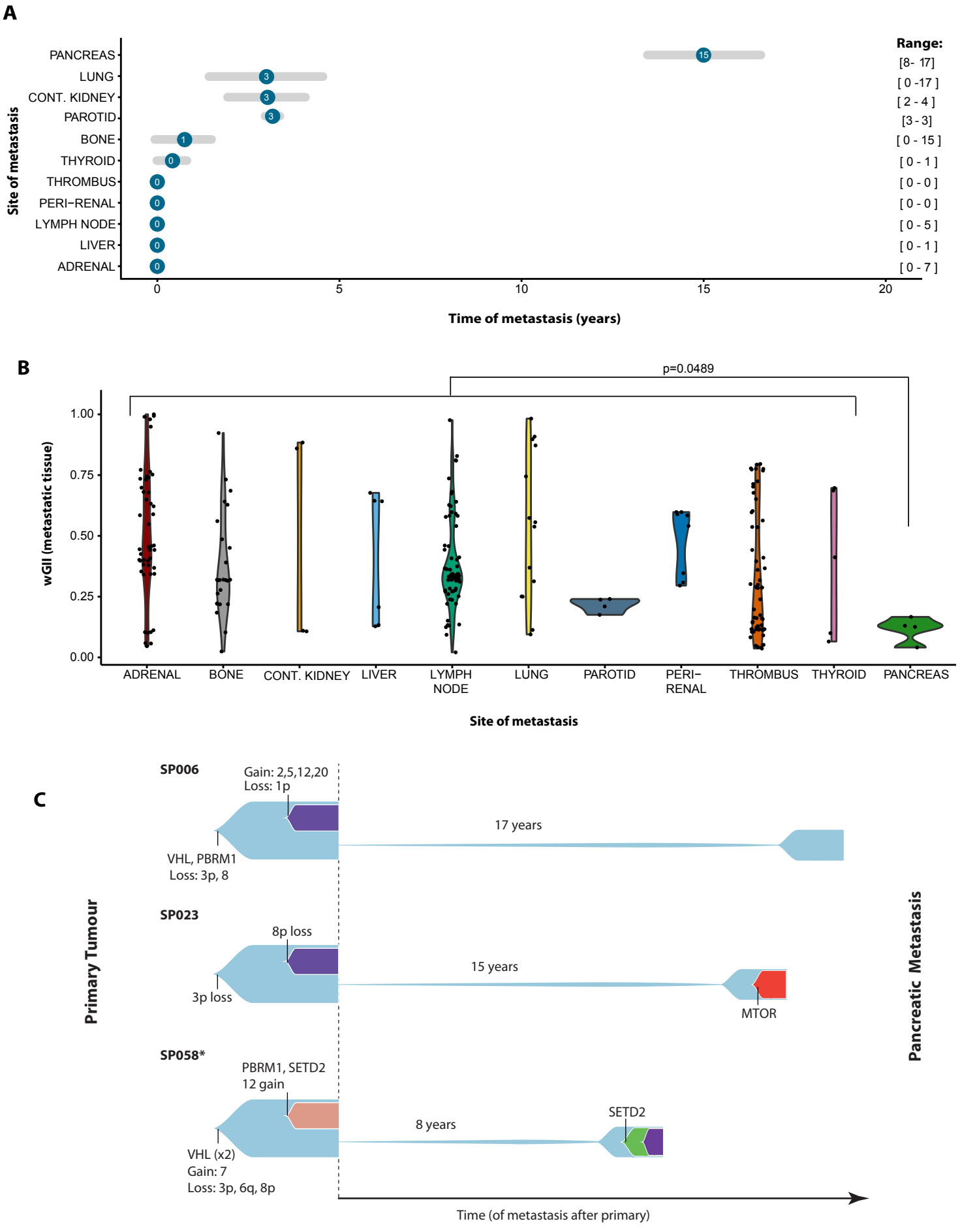

** Case SP058 had additional metastases to skeletal muscle (time=0) and the small bowell (time=7), see supplementary data.*

Figure 6

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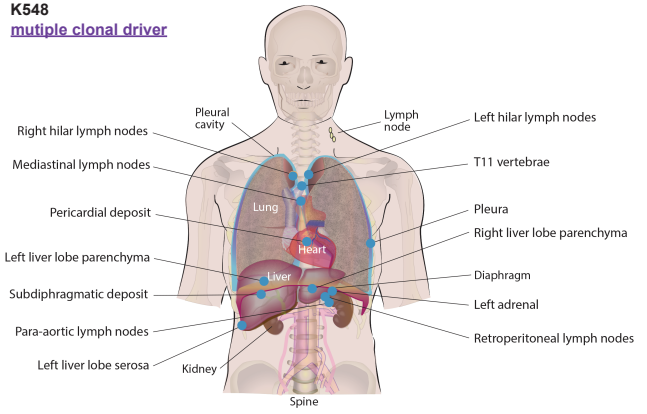
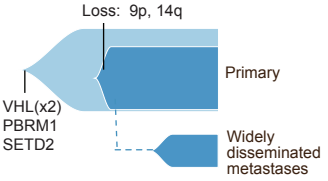
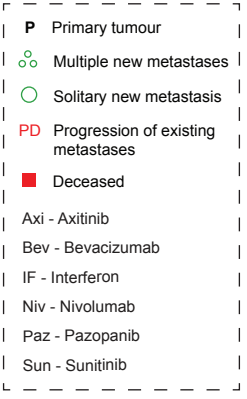
A



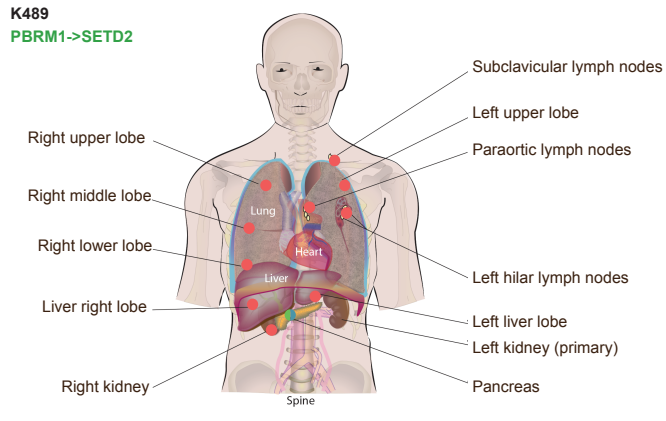


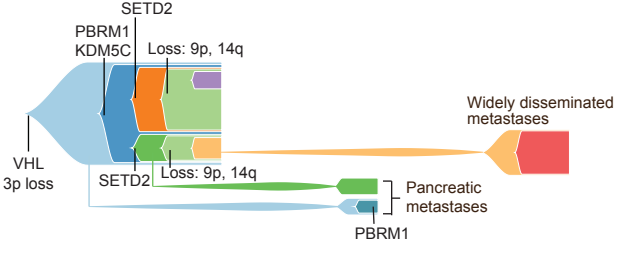
P (in situ)
Bone
LN
Liver
Adrenal
Pericardial effusion

Paz 5 — PD — Niv 2



B





P — 86.2 — Pancreas (surgery) — 48.8 — LN Lung (solitary) — Bev & IF 34 — LN Liver — Sun 13.3 — PD — Axi 15.5 — Bone — Niv 2 — PD — 5.2

Figure 7

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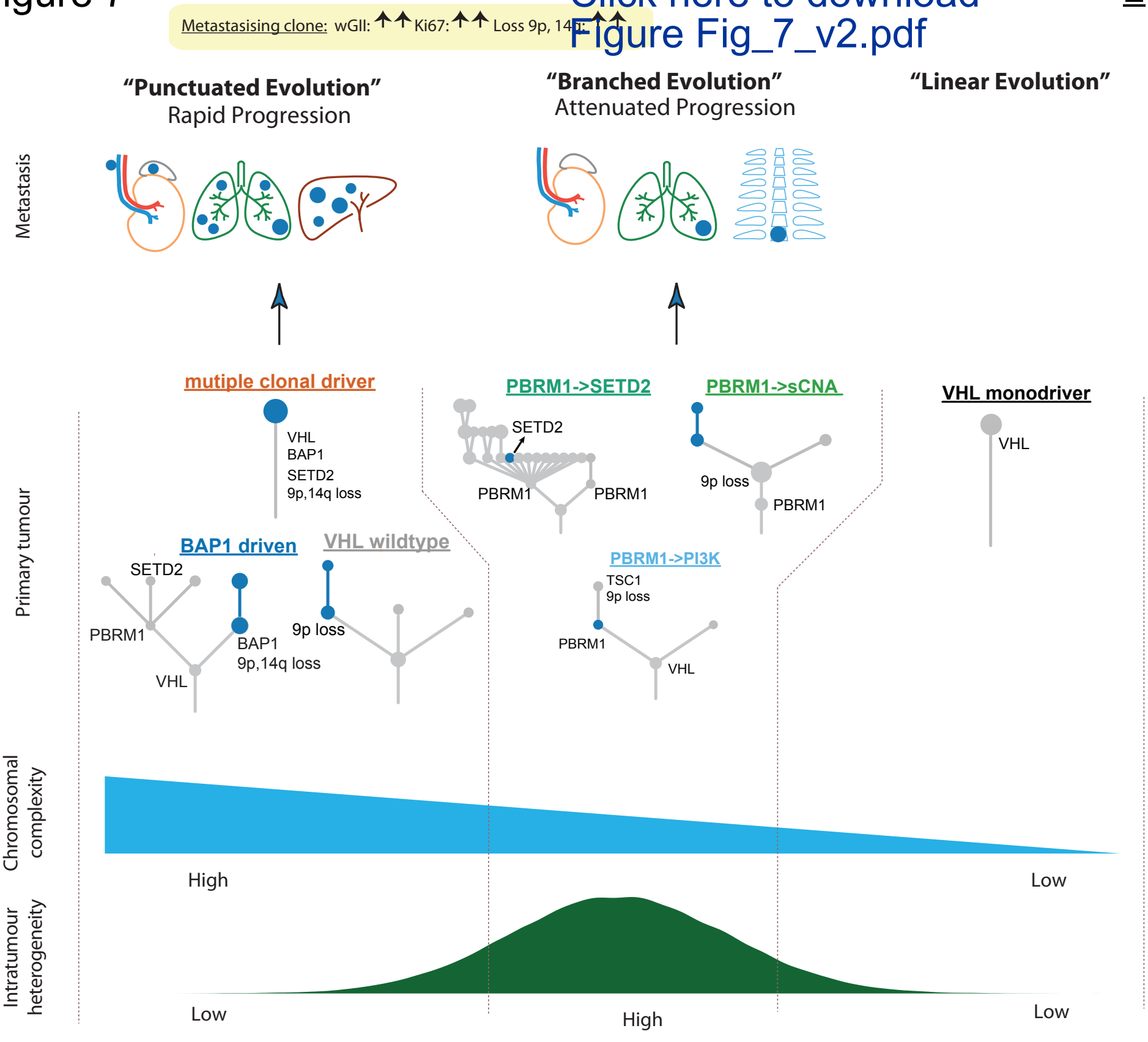

Figure S4

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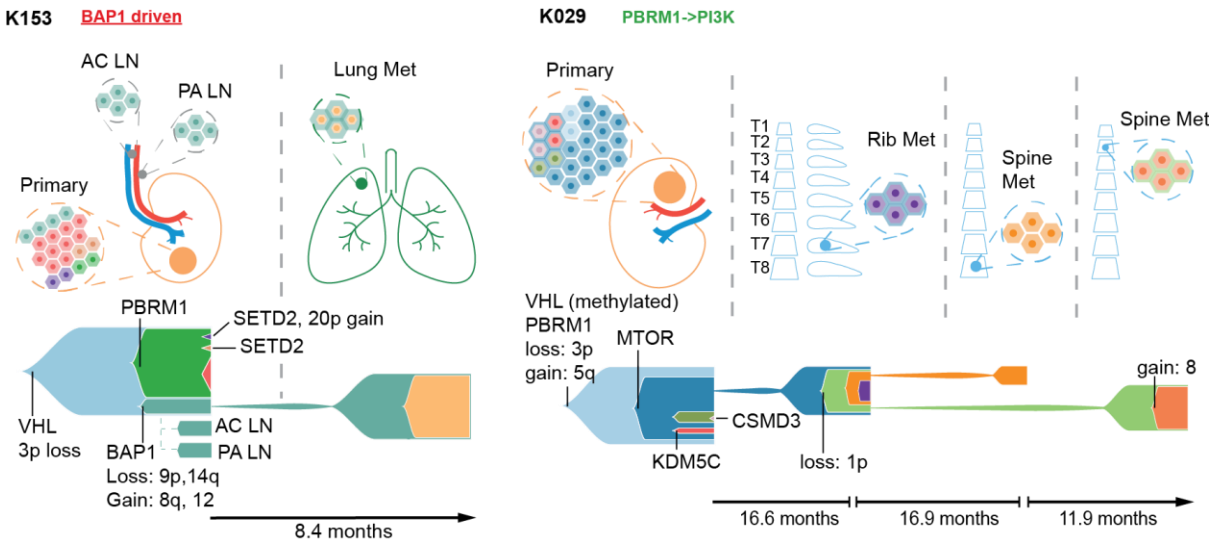

Figure S3

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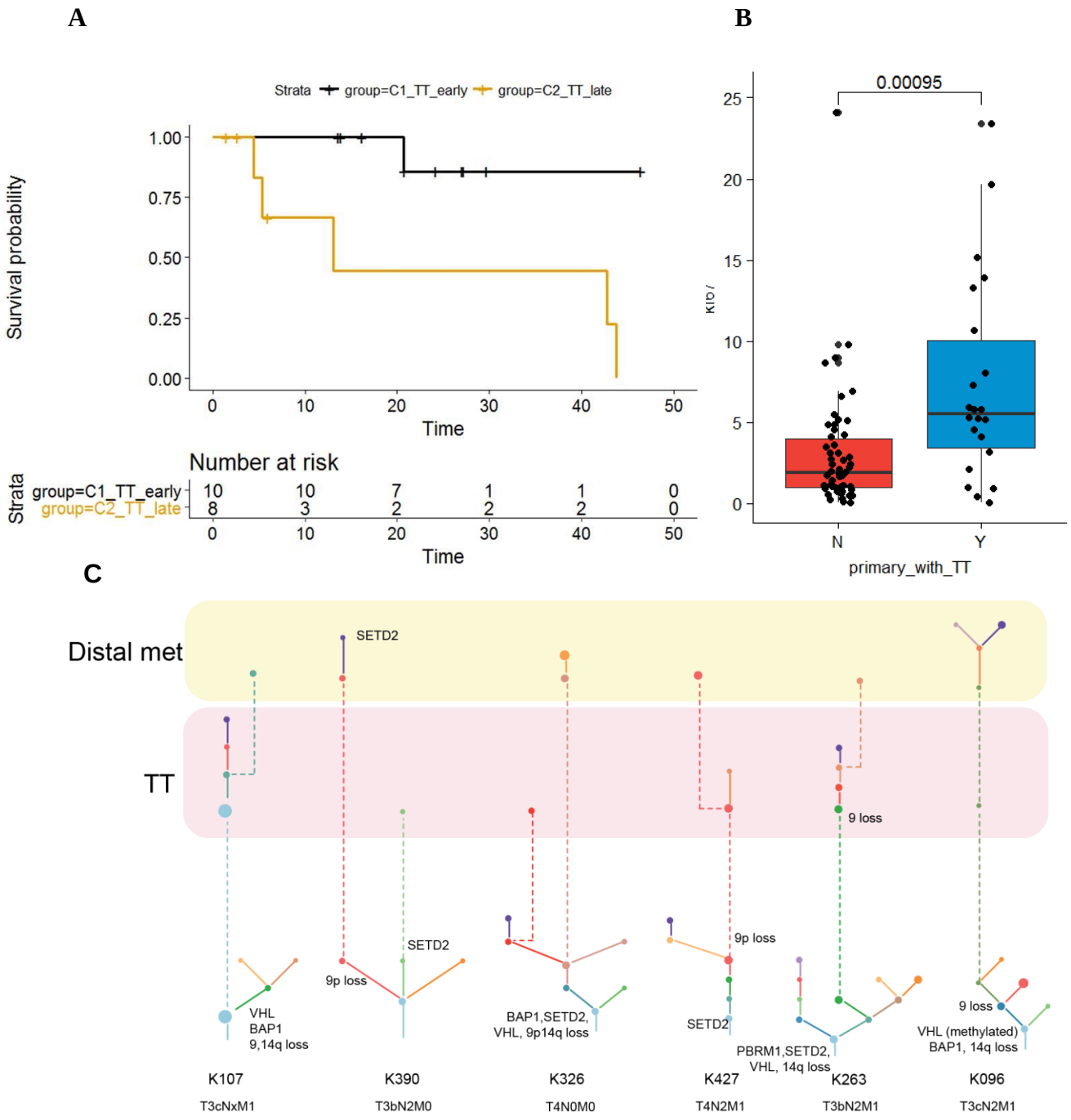

Figure S2

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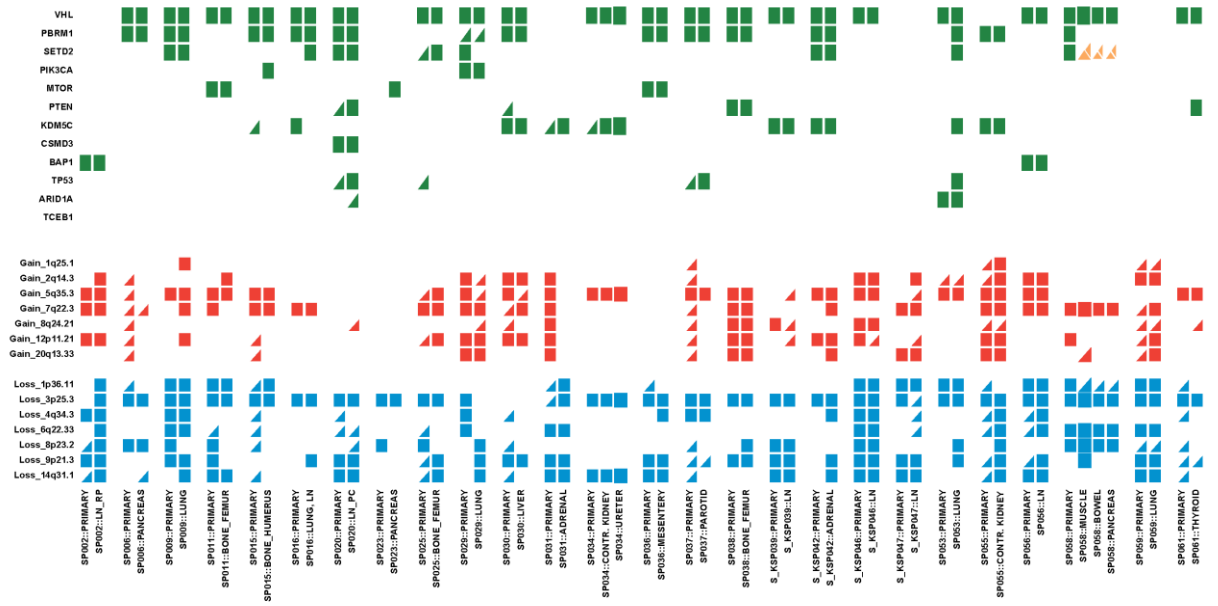
A



B

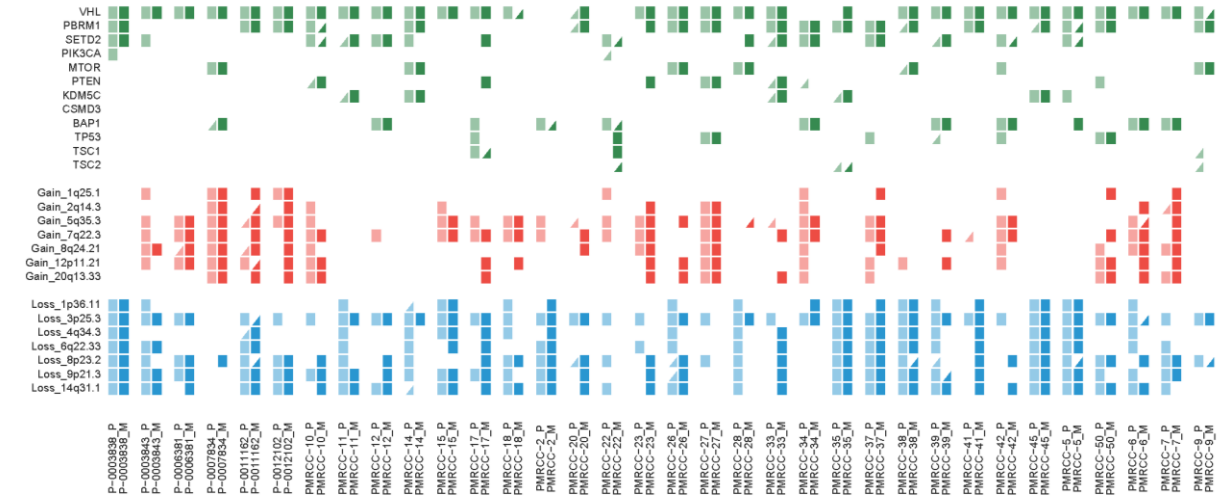
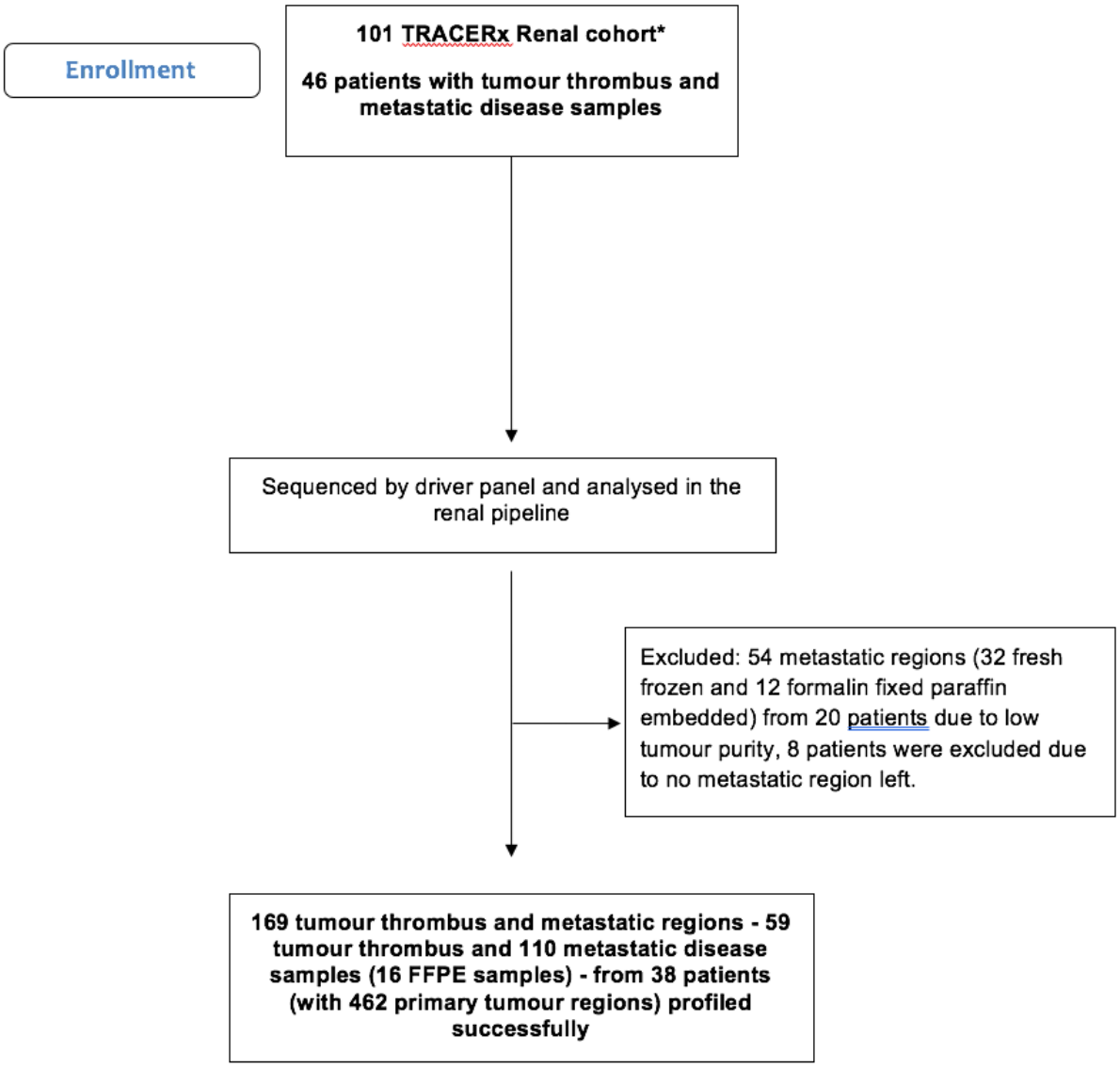


Figure S1



*See STAR methods and companion paper Turajlic et al (submitted)

Figure S5 Click here to download Supplemental Figure Figure S5.pdf

M11=skeletal muscle metastasis, M12=skeletal muscle metastasis

M21=small bowel metastasis, M31= pancreas metastasis

P=Primary tumour region

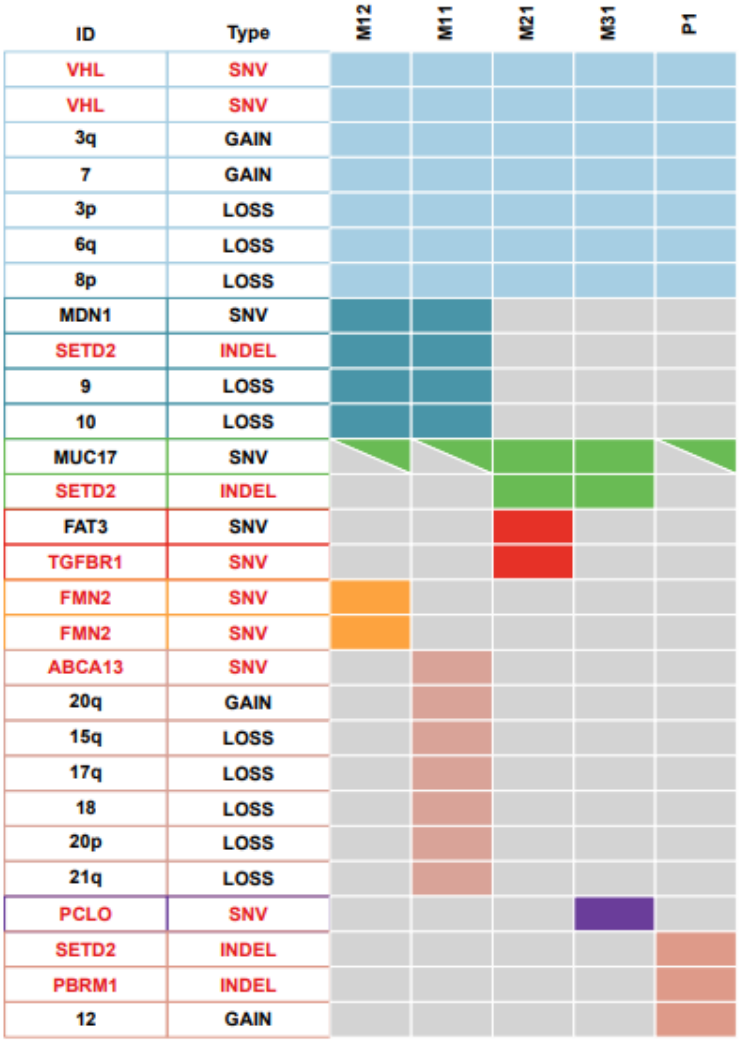


Table S6

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Supplemental Videos and Spreadsheets

Table S6.xlsx

Table S1

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Table S1.xlsx

Table S2

Table S3

Table S4

Table S5