Supplementary Information

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Supplementary Table 3. Pathway enrichment analysis of genes associated with injury and/or mutant Kras-sensitive ATAC-seq clusters.

Supplementary Table 4. Differentially expressed genes (DEGs) between Kras wild-type or Kras-mutant pancreatic epithelial cells expressing shBrd4.1448 vs shRen.713 (control) triggered to undergo regenerative metaplasia (C^{sh}:*Injury*) or injury-accelerated neoplastic transformation (KC^{sh}:*Kras*+Injury*), respectively, as identified by RNA-seq analyses in lineage-traced (mKate2+;GFP+) cells. See Extended Data Figure 4a for experimental details.

Supplementary Table 5. DEGs between regenerating (*Injury)*, early neoplastic (*Kras**, *Kras*+Injury)* or malignant (*PDAC*) epithelial states versus healthy normal counterparts (*Normal*), identified by RNA-seq analyses in pancreatic epithelial cells isolated from C, KC, $KP^{fl}C$-GEMMs. Upregulated (UP), downregulated (DN) or non-differentially expressed (NS) genes in each tissue state are annotated depending whether they exhibit parallel by accessibility-*GAIN*, -*LOSS* or no accessibility change (NC) at associated loci in that same condition vs *Normal*.

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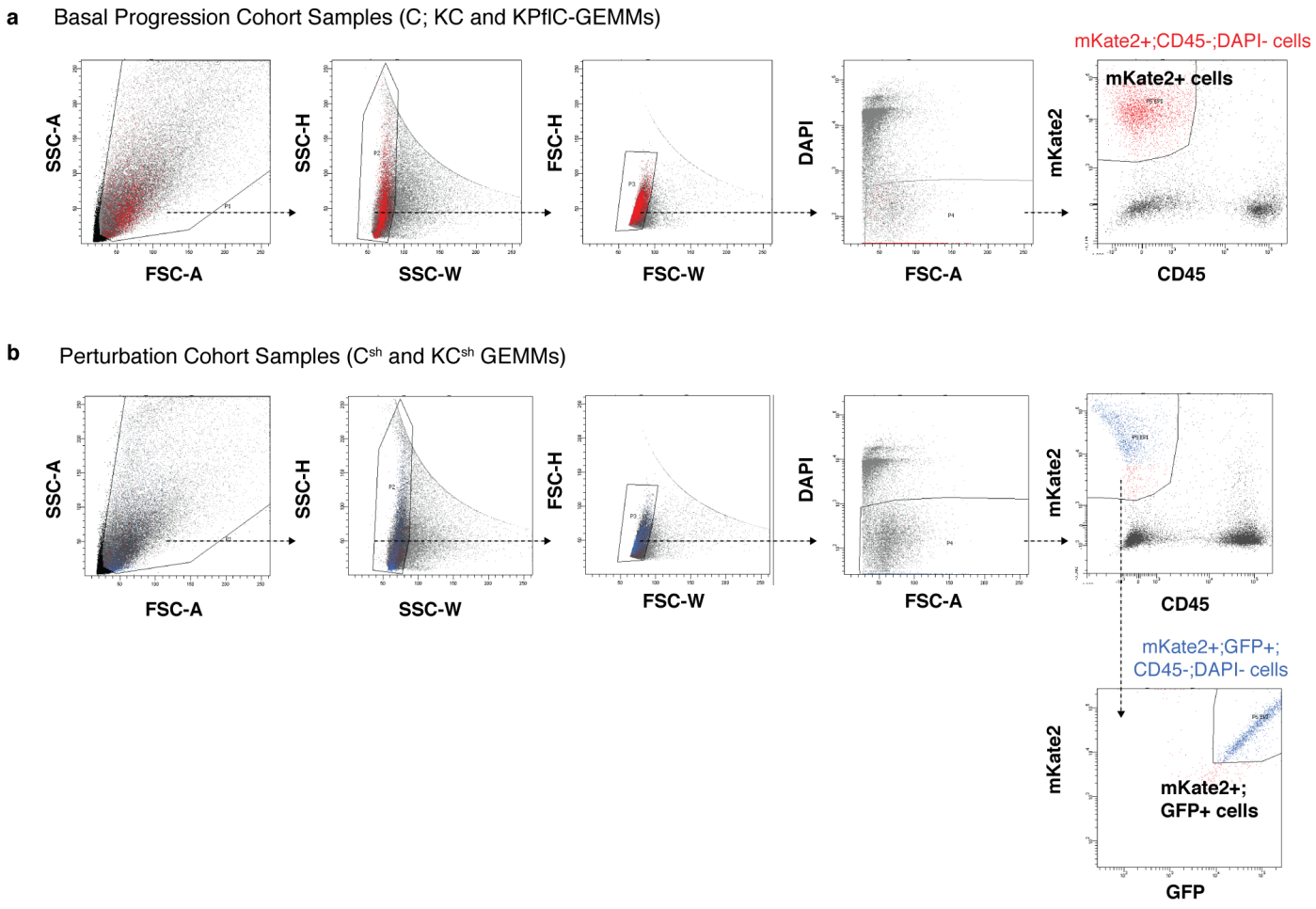
Supplementary Table 7. HOMER analyses of motifs enriched in dynamic cis-regulatory elements of 'chromatin-dynamic DEGs' between the indicated experimental conditions vs *Normal*. Numbers in brackets indicate the total number of peaks per category.

Supplementary Table 8. Subpopulation-defining peaks identified in single-cell ATAC-seq (scATAC-seq) analysis. Significantly scATAC-seq enriched peaks in the indicated cell subpopulations of mKate2+ cells isolated from from *Kras** and *Kras*+Injury* tissue states analyzed together. Subpopulations are labeled S1-S7, with numbers matching those shown in Extended Data Fig. 8b.

Supplementary Information

Supplementary Figure 1



Supplementary Figure 1. Gating strategies for flow cytometric experiments from genetically-engineered mice a, Gating strategy for FACS sorting of mKate2+ pancreatic epithelial cells subjected to omics analyses presented in Fig. 1b-f; Fig. 3f; Fig. 4a,b,e-g; Fig. 5a,c-d; Extended Data Fig. 1c-e; Extended Data Fig. 2a-g; Extended Data Fig. 3a-c; Extended Data Fig. 7a-c, e, f; Extended Data Fig. 8a-l; Extended Data Fig. 9a-g; and Extended Data Fig. 10b-j, l. **b,** Gating strategy for FACS sorting of mKate2;GFP double-positive pancreatic epithelial cells expressing doxycycline-inducible shRNAs, for omics analyses presented in Fig. 2c; Fig. 3f; Fig. 4d; Fig. 5a; Extended Data Fig. 3f,g; Extended Data Fig. 6b-d, f-I, l.m; and Extended Data Fig. 7d.

Supplementary Information

Supplementary Discussion

Cancer initiation results from a complex interaction of genetic and environmental insults that triggers changes in cell identity and tissue state that resemble regenerative processes yet paradoxically lead to a neoplastic cell fate[89-91]. Despite its importance, the interplay between cancer-predisposing mutations and tissue damage has proven difficult to study, owing in part to the lack of experimentally tractable model systems. For example, while *in vitro* culture systems and transplantation models have provided valuable information on the genetic, genomic and epigenomic aberrations of cancer[17,77,92], these systems cannot fully capture effects of environmental cues or tissue context in promoting (or restraining) neoplastic transformation. Alternatively, genome-wide profiling of tissue samples can provide molecular snapshots of tumors[23,30,93], however the resulting data are correlative and do not establish functional significance[94]. To address these issues, we combined genomics, single-cell chromatin assays and spatiotemporally- controlled functional perturbations in autochthonous mouse models to dissect how normal epithelial homeostasis is subverted during carcinogenesis. Our approach revealed a cooperative interaction between gene mutation (oncogenic *Kras*) and environment insult (tissue injury) in shaping chromatin accessibility that produces an epigenetic state that is not accessible by injury alone, unleashes mutant Kras driven-pancreatic transformation, and defines advanced PDAC.

Several features of the cancer-associated chromatin states induced in the Kras-mutant pancreatic epithelium upon injury support their contribution to the neoplastic process. From a temporal perspective, they emerge remarkably fast (within 48 hours), at the onset of ADM and well before the appearance of widespread PanIN lesions. From a molecular perspective, they impact cis-regulatory elements of many established regulators of pancreas lineage specification, tumorigenesis and metastasis that remain otherwise unaltered in normal epithelium undergoing physiological regeneration. From a functional perspective, these divergent chromatin states are coupled with a distinctive rewiring of transcriptional programs, gene regulatory networks and Brd4 outputs that identified novel effectors of pancreatic tumorigenesis. Additionally, the incorporation of single-cell ATAC-seq allowed us to link these early chromatin accessibility changes identified in analyses of bulk populations to *bona fide* chromatin remodeling events leading to the emergence of neoplasia-specific epigenetic states *in vivo* and gene programs that define the human disease. Thus, while chromatin remodeling may facilitate metastatic competence and other late-stage traits of PDAC cells[17,30,77], our results establish chromatin dysregulation as an early component of PDAC pathogenesis.

While normal differentiation programs restrain pancreatic metaplasia and are a potent tumor suppressive barrier for neoplasia and ultimately, malignancy[22,81,95], our results provide substantially more granularity to these transitions. We identify a chromatin remodeling program that alters the accessible landscape of known master regulators of pancreatic epithelial cell fate that is unique to the neoplastic process and unleashes Kras' oncogenic potential. While acquisition of additional events (eg. alteration of tumor suppressor genes and/or chromatin modifiers) may confer Ras-mutant cells to acquire or sustain such oncogenic chromatin states cell-autonomously during the transition to invasive cancer[37,92], inputs

from the tissue environment (damage, pancreatitis) are sufficient to induce such changes in pre-malignancy. Their net result is an acinar-to-neoplasia chromatin switch that redirects reparative injury responses towards neoplasia.

Consistent with recent roles for the AP-1 dimeric complex in firing injury[96] and mutant Ras-responsive enhancers[97], this altered response to injury is associated with a complex the transcriptional regulatory network characterized by a marked enrichment of AP-1 motifs in both regeneration- and cancer-associated loci. However, the differential expression of AP-1 subunits (FOS, ATF, JUN and MAF subfamilies) in normal, regenerating, pro-neoplastic and malignant epithelia inferred from our *in vivo* expression analyses suggest distinct AP-1 dimer configurations likely underlie regenerative[98,99] and pro-oncogenic[13,100] effects. While the activity of specific TFs that underlie these transitions currently relies on correlative results, a priority for future work will be to dissect both the *in vivo* composition of AP-1 dimers and their functional interplay with master lineage-specifying transcription factors during physiological versus pathological injury responses to identify the individual and/or collaborative TF pioneer activities directing each outcome. Along these lines, new drugs inhibiting specific of bromodomain chromatin readers that are differentially required for maintenance vs inducible gene expression[101] may serve as strategies to selectively perturb injury/Kras-responsive states without compromising normal, tumor suppressive differentiation programs.

Thus, while pancreatic carcinogenesis has long been viewed as a defect in regenerative processes[10,102], our study provides molecular detail on what makes the neoplastic process unique. It reveals that gene-environment interactions act, at least in part, by promoting a large-scale reorganization of chromatin accessibility that explains why (and how) many aberrantly activated cell fate programs become engaged during PDAC development. It also uncovers new chromatin-activated effectors of mutant Kras and tissue damage such as the alarmin cytokine IL-33, which may, in turn, contribute to the poorly understood epithelial cell-autonomous inflammation that drives tumorigenesis[103]. IL-33 has both nuclear chromatin-binding and extracellular activities[26]. As the latter can elicit potent stromal and immune inflammatory reactions that are known to shape mutant Kras pancreatic epithelial cell states[4,20], it is likely such tissue regulatory roles contribute to its effects on early neoplasia. Further study of gene – environment interactions relevant for cancer initiation will lay the groundwork for rational early diagnosis and interception strategies.

Supplementary Discussion References

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