SUPPLEMENTARY NOTE

1. Reprogramming of global epigenetic state during the evolution of distant metastasis

Rapid autopsy samples were ideal to study tumor evolution

We previously collected matched primary and metastatic PDAC lesions from individual patients by rapid autopsy[1,2], and reported the genetic progression of subclonal evolution by whole exome and sanger sequencing for mutations[3], paired-end sequencing for rearrangements[4], and whole genome sequencing[5] in a subset of these samples. These samples represent a unique resource especially suited to study tumor evolution, since they were collected from matched primary and metastatic tumors from the same patient(s), each has been deep sequenced, individual subclones have been identified, and no metastasis-specific driver mutations are present[3,5,6]. From these patients, we selected a large panel of diverse PDAC samples to test for global epigenomic reprogramming during subclonal evolution. As summarized in Supplementary Table 1, these samples were chosen because they represented the diversity of PDAC evolution (different regions of primary tumor paired to peritoneal and distant metastases), each sample represented a sequence-verified (sub)clonal population, patients were both treated and untreated, driver mutations were shared by all subclones in each patient in the absence of metastasis-specific drivers, formalin-fixed tissue was available for immunoassays, frozen tissue was available for whole-genome bisulfite sequencing, and cell lines were available for all other experiments. As such, this panel of diverse rapid autopsy samples represented an ideal format to test the nature and extent of large-scale (global) epigenetic reprogramming during the evolution of PDAC progression.

2. The epigenomic landscape of PDAC progression

Reprogramming was targeted to large chromatin domains across the PDAC genome

Thousands of heterochromatin domains were detected in each sample (range: 2,008-3,166) and these were organized into large block-like segments (median lengths: 232Kb-311Kb) that

collectively occupied more than half of the genome in each subclone (average: 61.7% of the genome, range: 54.1-71.7%, Supplementary Table 3). In addition to reprogramming of H3K9me2 in LOCKs (outlined in the main text), we also detected patient-specific reprogramming of H3K9me3 from these same LOCK regions (Supplementary Fig. 4). In patient A38, H3K9me3 was largely absent from A38Lg LOCKs relative to A38Per (loss of H3K9me3 from 184/208Mb, 88.5%, p<2.2e-16). Loss of LOCK-wide H3K9me3 was also detected between the paired primary tumor subclones in patient A13 (106Mb/118Mb, 90.0% in A13Pr2 vs. A13Pr1, p<2.2e-16), and similar to western blot findings (Supplementary Fig. 1f) this change was inherited in the distant metastatic subclone (152Mb/169Mb, 90.2% in A13Lg vs. A13Pr1, p<2.2e-16).

Similar to heterochromatin, we also detected thousands of large ECDs in each sample (range: 1,935-2,318). ECDs were also partitioned into large, block-like segments (median lengths: 207Kb-277Kb) that occupied similar lengths of the genome across subclones (average: 29% of the genome; range: 23.5%-32.0%, Supplementary Table 3).

Finally, we also detected patient-specific reprogramming targeted to a unique subset of very large LOCK domains. Although these regions were situated within DNA hypomethylated blocks similar to other LOCKs[7,8], they differed in several other respects. First, these domains were substantially larger (median lengths: 730Kb-1,340Kb vs. 232-311Kb for other LOCKs, Supplementary Table 3). Second, they were strongly enriched with H3K9me3[9] yet depleted of H3K9me2/H3K27me3 (Supplementary Fig. 7). Third, their abundance was patient-specific: subclones from patient A13 possessed very few of these domains (range: 50-111 domains covering 1.4-3.5% of the genome) while they occupied a much higher fraction of the A38 genome (range: 226-344 domains covering 14.5-20.6% of the genome). Finally, unlike reprogramming changes detected in other LOCKs (loss of H3K9me2/3 and DNA methylation), reprogramming in these LOCKs was characterized by loss of H3K9me3 coupled to increased

H3K9me2 and DNA methylation (Supplementary Fig. 7b-e). Finally, large LOCK domains were also prominent in the genome of normal (immortalized) human pancreatic ductal epithelial (HPDE) cells, similar to A38Per (Supplementary Fig. 7d).

3. Anabolic glucose metabolism controls epigenetic state and tumorigenicity

Selection of *CDH2* and *TOP2B* for validation studies

To functionally test the hypothesis that 6AN epigenetically regulated malignant gene expression, we selected two candidate genes for targeted in-depth validation experiments: N-cadherin (*CDH2*) and topoisomerase 2β (*TOP2B*). *CDH2* and *TOP2B* are both thought to be important for cancer progression[10], are not known to be mutated in PDAC[3,11], can be therapeutically targeted[10], were recurrently over-expressed across distant metastatic and primary tumor precursor subclones by RNA-seq (Supplementary Table 7), and were selectively repressed by 6AN which we confirmed with RT-PCR (compare y-axis values between A38Per and A38Lg from Fig. 7a-b and Supplementary Fig. 15a-b). Furthermore, *CDH2* was located within a reprogrammed LOCK targeted by 6AN, and *TOP2B* was located immediately adjacent to a LOCK boundary. *CDH2* and *TOP2B* thus represented strong candidate cancer genes for validation studies on control samples and subclones that over-expressed these genes.

4. DISCUSSION

Mechanistic questions that warrant further study

These findings also raise several important but complex questions, which we are pursuing in other studies. Perhaps the most complicated pertains to the extent of epigenetic and malignant heterogeneity between subclones across patients. Just to answer this in a single patient, a combination of whole-genome mapping, RNA-seq, bioinformatics, and several downstream experimental approaches were required. We hypothesize that such heterogeneity is a function of evolutionary time: patients who present with late-stage, widely metastatic disease may

possess more epigenetic and malignant divergence between subclones in their tumors than patients who present with early-stage disease. This possibility underscores the pressing need to detect cancers early, before such malignant heterogeneity arises.

Also unclear are the precise mechanisms whereby PGD/oxPPP activity controls global epigenetic state, which may also be complex. This could be mediated through any of the known oxPPP-dependent changes in cellular metabolism, including redox balance, fatty acid biosynthesis, and/or ribose biosynthesis, any of which can affect global epigenetic state through control of metabolite cofactors that activate or inhibit entire classes of chromatin modifying enzymes[12]. PGD activity itself is also complex and subject to several modes of regulation, including transcriptional over-expression[13], post-transcriptional repression[14], post-translational modification[15], protein:protein interactions[16], substrate availability[16], feedback inhibition[17], cross-talk with other pathways[18], and subcellular localization including a highly conserved yet uncharacterized nuclear fraction (C. Lyssiotis, personal communication). PGD-dependence may be selected for by any of these mechanisms during the evolution of distant metastasis in different patients. To illustrate, in rapid autopsy samples PGD is over-expressed in distant metastases from some patients but not in others. However, distant metastases possess hyperactive PGD activity by *in vitro* enzymatic assays irrespective of expression levels (OGM, unpublished observations), indicating that distinct routes to PGD hyperactivity are selected for during subclonal evolution in different patients.

A final question is how global epigenetic changes are targeted to specific chromatin domains that encode gene expression changes during subclonal evolution. We hypothesize that transcription factors and chromatin modifying enzymes that directly bind these regions play major roles in targeting the reprogramming events, and several candidates were recurrently over-expressed in our RNA-seq datasets. This includes the histone demethylase *KDM1A*

(LSD1), which could be particularly important since we previously found that this enzyme regulates LOCK reprogramming[19].

SUPPLEMENTARY NOTE REFERENCES

1. Embuscado, E.E. *et al.* Immortalizing the complexity of cancer metastasis: genetic features of lethal metastatic pancreatic cancer obtained from rapid autopsy. *Cancer Biol Ther* **4**, 548-54 (2005).
2. Iacobuzio-Donahue, C.A. *et al.* DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* **27**, 1806-13 (2009).
3. Yachida, S. *et al.* Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* **467**, 1114-7 (2010).
4. Campbell, P.J. *et al.* The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature* **467**, 1109-13 (2010).
5. Makohon-Moore et al. Limited heterogeneity of driver gene mutations among the metastases of individual pancreatic cancer patients. *Nature Genetics* (in press).
6. Jones, S. *et al.* Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* **321**, 1801-6 (2008).
7. Hansen, K.D. *et al.* Increased methylation variation in epigenetic domains across cancer types. *Nat Genet* **43**, 768-75 (2011).
8. Timp, W. *et al.* Large hypomethylated blocks as a universal defining epigenetic alteration in human solid tumors. *Genome Med* **6**, 61 (2014).
9. Hon, G.C. *et al.* Global DNA hypomethylation coupled to repressive chromatin domain formation and gene silencing in breast cancer. *Genome Res* **22**, 246-58 (2012).
10. Mariotti, A., Perotti, A., Sessa, C. & Ruegg, C. N-cadherin as a therapeutic target in cancer. *Expert Opin Investig Drugs* **16**, 451-65 (2007).
11. Bailey, P. *et al.* Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* **531**, 47-52 (2016).
12. Kaelin, W.G., Jr. & McKnight, S.L. Influence of metabolism on epigenetics and disease. *Cell* **153**, 56-69 (2013).
13. Shimomura, I., Shimano, H., Korn, B.S., Bashmakov, Y. & Horton, J.D. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J Biol Chem* **273**, 35299-306 (1998).
14. Singh, A. *et al.* Transcription factor NRF2 regulates miR-1 and miR-206 to drive tumorigenesis. *J Clin Invest* **123**, 2921-34 (2013).
15. Shan, C. *et al.* Lysine acetylation activates 6-phosphogluconate dehydrogenase to promote tumor growth. *Mol Cell* **55**, 552-65 (2014).
16. Hanau, S. *et al.* Evidence for dimer/tetramer equilibrium in Trypanosoma brucei 6-phosphogluconate dehydrogenase. *Biochim Biophys Acta* **1834**, 2647-52 (2013).
17. Rippa, M., Giovannini, P.P., Barrett, M.P., Dallocchio, F. & Hanau, S. 6-Phosphogluconate dehydrogenase: the mechanism of action investigated by a

comparison of the enzyme from different species. *Biochim Biophys Acta* **1429**, 83-92 (1998).
18. Hitosugi, T. *et al.* Phosphoglycerate mutase 1 coordinates glycolysis and biosynthesis to promote tumor growth. *Cancer Cell* **22**, 585-600 (2012).
19. McDonald, O.G., Wu, H., Timp, W., Doi, A. & Feinberg, A.P. Genome-scale epigenetic reprogramming during epithelial-to-mesenchymal transition. *Nat Struct Mol Biol* **18**, 867-74 (2011).

Supplementary Table 1: PDAC sample characteristics with LOCK epigenetic changes

Sample (Clonal Relation[a])	**Sample Source**	**Patient Metastatic Sites**	**Patient Chemo Status**	**Driver Genes (in primary and mets)**	**Assays[b]** IHC, Western Blots, ChIP-seq, WGBS	**Results[b]** LOCK Methylation Change
A124PrF (Founder Clone)	Tissue: Primary Tumor	Regional	Untreated	*KRAS* *CDKN2A* *SMAD4* *ATM*	K9me2/3 IHC:	Diffusely Positive
A124PrS (Subclone)	Tissue: Primary Tumor	Regional	Untreated	*KRAS* *CDKN2A* *SMAD4* *ATM*	K9me2/3 IHC: WGBS:	Diffusely Positive High (75%, control)
A124Per (Metastasis)	Tissue: Peritoneum	Regional	Untreated	*KRAS* *CDKN2A* *SMAD4* *ATM*	K9me2/3 IHC: WGBS:	Diffusely Positive High (74%)
A141PrF (Founder Clone)	Tissue: Primary Tumor	Regional	Untreated	*KRAS* *CDKN2A* *TP53* *MLL3* *ARID1B*	K9me2/3 IHC:	Diffusely Positive
A141PrS (Subclone)	Tissue: Primary Tumor	Regional	Untreated	*KRAS* *CDKN2A* *TP53* *MLL3* *ARID1B*	K9me2/3 IHC:	Diffusely Positive
A141Per (Metastasis)	Tissue: Peritoneum	Regional	Untreated	*KRAS* *CDKN2A* *TP53* *MLL3* *ARID1B*	K9me2/3 IHC:	Diffusely Positive
A125PrF (Founder Clone)	Tissue: Primary Tumor	Distant metastases	Untreated	*KRAS* *CDKN2A* *TP53* *ARID1A*	K9me2/3 IHC: WGBS:	Diffusely Positive High (74%)
A125PrS (Subclone)	Tissue: Primary Tumor	Distant metastases	Untreated	*KRAS* *CDKN2A* *TP53* *ARID1A*	K9me2/3 IHC: WGBS:	Positive+Negative Reduced (51%)
A125Lv1 (Metastasis)	Tissue: Liver	Distant metastases	Untreated	*KRAS* *CDKN2A* *TP53* *ARID1A*	K9me2/3 IHC: WGBS:	Diffusely Negative Reduced (57%)
A125Lv2 (Metastasis)	Tissue: Liver	Distant metastases	Untreated	*KRAS* *CDKN2A* *TP53* *ARID1A*	K9me2/3 IHC: WGBS:	Diffusely Negative Reduced (59%)
A132PrF (Founder Clone)	Tissue: Primary Tumor	Distant metastases	Untreated	*KRAS* *CDKN2A* *TP53* *ATM*	K9me2/3 IHC:	Diffusely Positive

A132PrS (Subclone)	Tissue: Primary Tumor	Distant metastases	Untreated	*KRAS* *CDKN2A* *TP53* *ATM*	K9me2/3 IHC:	Positive+Negative
A132Lv (Metastasis)	Tissue: Liver	Distant metastases	Untreated	*KRAS* *CDKN2A* *TP53* *ATM*	K9me2/3 IHC:	Positive+Negative
A38PrF (Founder Clone)	Tissue: Primary Tumor	Regional + Distant metastases	Gem. Bev.	*KRAS* *TP53* *SMAD4*	K9me2/3 IHC:	Diffusely Positive
A38PrS1 (Peritoneal Precursor Subclone)	Tissue: Primary Tumor	Regional + Distant metastases	Gem. Bev.	*KRAS* *TP53* *SMAD4*	K9me2/3 IHC:	Diffusely Positive
A38PrS2 (Liver/Lung Precursor Subclone)	Tissue: Primary Tumor	Regional + Distant metastases	Gem. Bev.	*KRAS* *TP53* *SMAD4*	K9me2/3 IHC:	Positive+Negative
A38Lg1 (Metastasis)	Tissue: Lung	Regional + Distant metastases	Gem. Bev.	*KRAS* *TP53* *SMAD4* *SMARCA2*[c]	K9me2/3 IHC:	Diffusely Negative
A38Per (Metastasis)	Cell Line: Peritoneum	Regional + Distant metastases	Gem. Bev.	*KRAS* *TP53* *SMAD4*	K9me2 Western Blot: K9me2 ChIP-seq: WGBS:	High (100%, cont.) High (100%, cont.) High (79%, cont.)
AsPC1[d] (N/A)	Cell Line: Ascites	Regional	Gem.	*KRAS* *CDKN2A* *TP53* *SMAD4*	K9me2 Western Blot:	High (102%)
HPAFII[d] (N/A)	Cell Line: Ascites	Regional	Gem.	*KRAS* *TP53*	K9me2 Western Blot:	High (94%)
Capan2[d] (N/A)	Cell Line: Primary Tumor	Regional	Gem.	*KRAS* *CDKN2A* *TP53*	K9me2 Western Blot:	High (93%)
A2Lg (Metastasis)	Cell Line: Lung	Regional + Distant metastases	Taxoprex. Gem.	*KRAS* *TP53*	K9me2 Western Blot:	Reduced (40%)
A2Lv (Metastasis)	Cell Line: Liver	Regional + Distant metastases	Taxoprex. Gem.	*KRAS* *TP53*	K9me2 Western Blot:	Reduced (50%)
A6Lv (Metastasis)	Cell Line: Liver	Regional + Distant metastases	Gem. Trox.	*KRAS* *TP53* *MLL3*	K9me2 Western Blot:	Reduced (47%)
A10Lv (Metastasis)	Cell Line: Liver	Distant metastases	Untreated	*KRAS* *TP53* *MLL3*	K9me2 Western Blot:	Reduced (61%)
A13Pr1 (Subclone)	Cell Line: Primary Tumor	Distant metastases	Untreated	*KRAS* *CDKN2A* *MYC* *TP53*	K9me2 Western Blot: K9me2 ChIP-seq: WGBS:	Reduced (64%) Reduced (25%) Reduced (72%)

A13Pr2 (Subclone)	Cell Line: Primary Tumor	Distant metastases	Untreated	*KRAS* *CDKN2A* *MYC* *TP53*	K9me2 Western Blot: K9me2 ChIP-seq: WGBS:	 Reduced (51%) Reduced (81%) Reduced (73%)
A13Lg (Metastasis)	Cell Line: Lung	Distant metastases	Untreated	*KRAS* *CDKN2A* *MYC* *TP53*	K9me2/3 Western Blot: K9me2 ChIP-seq: WGBS:	 Reduced (53%) Reduced (86%) Reduced (71%)
A32O (Metastasis)	Cell Line: Omentum[e]	Regional + Distant metastases	5-FU	*KRAS* *TP53*	K9me2 Western Blot:	 Reduced (58%)
A38Lv (Metastasis)	Cell Line: Liver	Regional + Distant metastases	Gem. Bev.	*KRAS* *TP53* *SMAD4*	K9me2 Western Blot: WGBS:	 Reduced (47%) Reduced (67%)
A38Lg (Metastasis)	Cell Line: Lung	Regional + Distant metastases	Gem. Bev.	*KRAS* *TP53* *SMAD4* *SMARCA2*[c]	K9me2 Western Blot: K9me2 ChIP-seq: WGBS:	 Reduced (58%) Reduced (48%) Reduced (72%)

Supplementary Table 1: PDAC sample characteristics with LOCK epigenetic changes. Samples from patients with regional spread (peritoneal/ascites) showed relatively high global H3K9/DNA methylation as indicated by multiple assays (right two columns), while samples from patients with distant metastases showed reduced methylation across all assays, which initiated in primary tumors as indicated.

Abbreviations: Gem. (Gemcitabine), Bev. (Bevacizumab), Taxoprex. (Taxoprexin), Trox. (Troxacitabine).

Superscript Notes

[a] Clonal origins represent phylogenetic estimates from previously collected whole-exome and whole-genome sequencing data.

[b] Western blot data reflect densitometry percentages of H3K9me2 signals relative to A38Per controls (cont.). Western blots are shown in Supplementary Fig. 1 and the absolute densitometry values are graphed together in Fig. 1d. ChIP-seq data reflect percent of LOCK Mb with reduced H3K9me2 relative to A38Per controls (cont.), as detailed with Mb and RPKM values in Supplementary Table 3. WGBS data reflect percent of DNA methylation within LOCKs relative to A124Pr controls (tissues) and A38Per controls (cell lines) as shown in Supplementary Tables 5 and 6.

[c] This metastasis from a chemotherapy-treated patient had a missense mutation in *SMARCA2* of unclear significance.

[d] These cell lines were not from the rapid autopsy cohort and rely on previously published genotyping data which may underestimate the driver mutations.

[e] The A32O cell line was isolated from an omental mass lesion in a patient with very aggressive disease including widespread lung metastases, and showed findings similar to the other distant (lung/liver) metastatic subclones.

Samples **Name** **Source**	**Global LOCK Methylation** **% CpG Methylation in LOCKs by WGBS**	**Significant Difference** **p-value vs. A38Per**
A38Per Peritoneal Met	78.58%	N/A (Highest Methylation)
A38Lv Liver Met	67.39%	2.20E-16
A38Lg Lung Met	71.67%	2.20E-16
A13Pr1 Primary Tumor 1 Subclone	72.46%	2.18E-07
A13Pr2 Primary Tumor 2 Subclone	73.48%	2.14E-06
A13Lg Lung Met	71.30%	2.20E-16

Supplementary Table 5. Percent CpG Methylation levels across LOCK domains detected in cell lines by WGBS. DNA methylation levels were highest for the peritoneal subclone A38Per across cell lines. Methylation was significantly reduced in distant metastases from the same patient (A38Lv, A38Lg) and in primary tumor precursors (A13Pr1/2) and the matched lung metastasis from patient A13. P-values were calculated with paired wilcox tests using a 3% threshold.

Samples **Sample name** **Sample source**	**Global LOCK Methylation** **% CpG Methylation in LOCKs by WGBS**	**Significant Difference** **p-value vs. A124Pr**
A124PrF Primary Tumor	75.37%	N/A (Highest Methylation)
A124Per Peritoneal Met	74.17%	1
A125PrF Primary Tumor #1 (Founder Clone)	73.65%	1
A125PrS Primary Tumor #2 (Subclone)	51.12%	2.20E-16
A125Lv1 Liver Met #1	56.95%	2.20E-16
A125Lv2 Liver Met #2	59.47%	2.20E-16
Normal Pancreas	67.75%	2.20E-16

Supplementary Table 6. Percent CpG Methylation levels across LOCK domains detected in frozen tissue samples by WGBS. DNA methylation levels were relatively high in both primary tumor and metastatic tumors from patient A124, who presented with peritoneal carcinomatosis. Similar high levels of DNA methylation were also detected in the founder clone from patient A125, which were significantly reduced in the primary tumor subclone that seeded distant metastases and in the liver metastases themselves. P-values were calculated with paired wilcox tests using a 3% threshold.

GO Terms	# of Genes	% of Genes	P-value
Oxidation-reduction	64	6.0	3.9e-6
Oxidoreductase	56	5.2	4.7e-6
EGF-like domain	28	2.6	6.4e-5
Transferase	105	9.8	1.0e-4
NADP	21	2.0	1.7e-4
Cell Adhesion	41	3.8	1.8e-4
Cell Migration	31	2.9	2.7e-4
Cell Morphogenesis	29	2.7	3.8e-4
Mitochondrion	67	6.3	4.0e-4
Acetylation	174	16.3	5.0e-4

Supplementary Table 8: GO analysis of DE genes that were up-regulated from reprogrammed LOCKs in A38Lg, relative to A38Per. Genes involved in redox balance (oxidation-reduction, NADP) and EMT (cell adhesion, migration) were up-regulated from reprogrammed DE genes in LOCKs.

GO Terms	# of Genes	% of Genes	P-value
Signal	399	32.4	1.5e-31
Glycoprotein	488	40.0	1.0e-30
Disulfide Bond	352	28.5	1.0e-25
Secreted	217	17.6	1.5e-18
Membrane	582	47.2	1.7e-15
Polymorphism	951	77.1	1.5e-13
Immune Response	106	8.6	1.6e-13
Immunoglobin Domain	75	6.1	8.5e-11
Cytokine-Cytokine Receptor Interaction	54	4.4	2.3e-10
Ion Channel	55	4.5	2.1e-9
Inflammatory Response	55	4.5	7.6e-9
Cell Adhesion	93	7.5	1.4e-8
Developmental Protein	98	7.9	4.4e-8
Cell Motion	69	5.6	4.7e-8
Transmembrane Protein	82	6.7	3.4e-7
Protease Inhibitor	25	2.0	3.9e-7
Response to Wounding	71	5.8	7.0e-7
Extracellular Matrix	40	3.2	1.2e-6
Epithelial Cell Differentiation	28	2.3	1.6e-6

Supplementary Table 9: GO analysis of DE genes that were down-regulated from reprogrammed LOCKs in A38Lg, relative to A38Per. Genes involved in differentiation state (cell adhesion, development, epithelial genes), immune regulation (immune response, cytokines, inflammation), and response to environmental cues (transmembrane signaling, extracellular matrix, secretion, locomotion) were down-regulated from reprogrammed LOCKs.

GO Terms	# of Genes	% of Genes	P-value
Acetylation	622	21.7	1.1e-59
Phosphoprotein	1290	45.0	5.1e-53
Cell Cycle	154	5.4	3.4e-30
Mitotic Cell Cycle	137	4.8	4.9e-30
Organelle Fission	95	3.3	3.7e-25
DNA Metabolic Process	157	5.5	9.4e-25
DNA Repair	95	3.3	1.4e-17
Response to DNA Damage Stimulus	113	3.9	3.8e-17
DNA Replication	68	2.4	2.7e-14
Cellular Response to Stress	144	5.0	3.1e-14
Protein Biosynthesis	62	2.1	2.8e-12
ATP Binding	256	8.9	1.3e-11
Ribonucleoprotein	78	2.7	4.1e-11
Nucleotide Binding	309	10.7	4.3e-11
Translation	86	3.0	2.5e-9
ncRNA Metabolic Process	66	2.3	3.5e-9
Mitochondrion	167	5.8	4.3e-9
DNA Recombination	39	1.4	4.6e-9
Microtubule-based Process	70	2.4	5.9e-9

Supplementary Table 10: GO analysis of DE genes that were up-regulated from reprogrammed ECDs in A38Lg, relative to A38Per. Genes involved in post-translational modifications, cell cycle control, DNA repair, response to stress, and DNA/RNA/protein biosynthesis were up-regulated from reprogrammed ECDs.

GO Terms	# of Genes	% of Genes	P-value
Alternative Splicing	1195	49.0	1.5e-16
Sh3 Domain	65	2.7	6.8e-10
Phosphoprotein	1112	45.0	1.3e-9
Membrane	964	39.4	1.4e-9
Response to Wounding	117	4.8	9.0e-8
Pleckstrin Homology	71	2.9	2.8e-7
Cell Migration	49	2.0	3.4e-7
Small GTPase Signal Transduction	65	2.7	3.9e-7
Locomotion	52	2.1	1.4e-6
Intracellular Signaling Cascade	227	9.3	3.5e-6
Tyrosine Protein Kinase	34	1.4	5.4e-6
Regulation of Apoptosis	153	6.2	1.1e-5
Protein Kinase Cascade	81	3.3	1.4e-5
Protein Amino Acid Phosphorylation	130	5.3	1.8e-5
Ankyrin Repeat	56	2.3	3.7e-5
Ras Protein Signal Transduction	51	2.1	4.6e-5

Supplementary Table 11: GO analysis of DE genes that were down-regulated from reprogrammed ECDs in A38Lg, relative to A38Per. Genes involved in oncogenic signal transduction cascades (Sh3 domains, transmembrane proteins, kinases, Ras signaling), cell motion (wounding, migration, locomotion), and cell death control (apoptosis) were down-regulated from reprogrammed ECDs.

Go Terms	# of Genes	% of Genes	P-value
Nucleus	112	40.0	1.4e-19
Phosphoprotein	150	53.6	1.3e-18
Acetylation	80	28.6	2.7e-16
DNA Metabolic Process	36	12.9	1.2e-15
Cell Cycle	39	13.9	3.4e-12
M-phase	29	8.9	1.8e-11
DNA Repair	22	7.9	2.8e-10
DNA Replication	18	6.4	8.5e-10
Cellular Response to Stress	27	9.6	5.0e-8
Ribonucleotide Biogenesis	13	4.6	6.3e-6

Supplementary Table 12: GO analysis of genes that were both recurrently over-expressed in distant metastases and down-regulated by 6AN, detected by RNA-seq.

GO Terms	# of Genes	% of Genes	P-value
Acetylation	217	20.7	8.0e-28
Phosphoprotein	428	40.0	5.3e-24
Protein Biosynthesis	36	3.4	1.4e-14
Nucleus	256	24.4	1.8e-13
Ribonucleoprotein	41	3.9	1.4e-12
Translation	47	4.5	1.5e-12
DNA Metabolic Process	53	5.1	4.5e-9
Cell Cycle	65	6.2	3.4e-7
Mitochondria	64	6.1	5.4e-7
DNA Repair	31	3.0	5.2e-6
Nitrogen Compound Biosynthesis	33	3.1	1.1e-5
Nucleotide Binding	102	9.7	1.4e-6
M-phase	33	3.1	1.4e-5
Cellular Response to Stress	47	4.5	2.5e-5
Transit Peptide	39	3.7	3.3e-5
Nucleotide Biosynthetic Process	22	2.1	5.1e-5
ATP Binding	82	7.8	5.3e-5
DNA Replication	22	2.1	7.0e-5
WD40 Repeat	24	2.3	1.4e-4

Supplementary Table 13: GO analysis of recurrently over-expressed genes detected by RNA-seq in distant metastatic subclones and primary tumor precursors, relative to peritoneal carcinomatosis.

GO Terms	# of Genes	% of Genes	P-value
Cell Cycle	226	12.2	2.5e-57
Acetylation	465	25.2	5.6e-57
Phosphoprotein	935	50.6	2.5e-55
M-phase	135	7.3	1.2e-52
DNA Metabolic Process	153	13.2	2.6e-40
Nucleus	582	31.5	1.4e-34
DNA Replication	82	4.4	1.0e-33
Chromosome Segregation	45	2.4	3.1e-24
DNA Repair	88	4.5	5.6e-24
Cytoplasm	444	24.0	3.5e-23
ATP Binding	221	12.0	4.1e-22
Cellular Response to Stress	125	6.8	1.6e-19
Chromosome Organization	107	5.8	9.3e-17
Microtubule-based Process	70	3.8	3.6e-16
Nucleotide Binding	244	13.2	4.2e-16
Cytoskeleton	113	6.1	3.2e-15
Ubl Conjugation	103	5.6	9.5e-12
DNA Recombination	36	1.9	1.6e-11
Macromolecular Complex Assembly	115	6.2	3.0e-10

Supplementary Table 14: GO analysis of DE genes detected by RNA-seq that were down-regulated in response to 6AN, compared to DMSO control cells (A38Lg subclone).

Antibody	Source	Catalogue #	Western dilution	Immuno dilution
CDH1	Cell Signaling	4065	1:500	1:100, IF
Vimentin	NeoMarkers	Ms-129-P	1:500	1:100, IF
G6PD	Cell Signaling	8866S	1:500	N/A
PGD	Cell Signaling	13389S	1:500	N/A
TKT	Cell Signaling	8616S	1:500	N/A
TALDO1	Santa Cruz	Sc-134795	2 ug/ml	N/A
ERK1/2	Cell Signaling	4696S	1:500	N/A
p-ERK1/2	Cell Signaling	4370S	1:500	N/A
KRAS	Santa Cruz	Sc-30	2 ug/ml	N/A
RPE	Santa Cruz	Sc-162124	2 ug/ml	N/A
RPIA	Abcam	Ab181235	1:500	N/A
CD44	Cell Signaling	5640S	1:500	N/A
Epcam	Millipore	CBL251	1:500	N/A
CDH2	Cell Signaling	4061S	1:250	N/A
H3K9me2	Abcam	ab1220	1:5000-20000	1:5000 IHC, IF
H3K9me3	Abcam	ab8898	1:5000	1:5000 IHC
H3K27me3	Millipore	07-449	1:7500	N/A
H3K9ac	Millipore	07-352	1:10000	N/A
H3K36me3	Abcam	ab9050	1:10000	1:5000 IHC
H3K27ac	Abcam	ab4729	1:5000	N/A
H4K20me3	Millipore	07-463	1:1000	1:5000 IHC
H4K16ac	Millipore, Abcam	07-329, ab109463	1:2500-10000	N/A
total H3	Abcam	ab1791	1:30000	N/A
total H4	Abcam	ab10158	1:30000	N/A
γH2AX	Abcam	ab11174	1:5000	N/A

Supplementary Table 15. Antibodies and conditions used for western blots, immunostain, and ChIP experiments.

Locus/Assay	Primer Name: Sequence (5'-3')
CDH2 ChIP	Chr18_24.258F: GCTCAGCCCTGTATCAGCCAGC
CDH2 ChIP	Chr18_24.258R: GGGTTACAGGTATGAGCCACTGC
CDH2 ChIP	Chr18_24.506F: AATGGAGAAGTCAGGAATGTAGTCC
CDH2 ChIP	Chr18_24.506R: GTATTTCATTTATCAAGTTGCAGCTCC
CDH2 ChIP	Chr18_24.834F: TTTGCTTCTCACTCCAAGTTCATCC
CDH2 ChIP	Chr18_24.834R: CAACCTCAGGAACAATGCATCAGC
CDH2 ChIP	Chr18_25.125.6F: CGAAACAGTCCAGCTGCTATGG
CDH2 ChIP	Chr18_25.125.6R: CTTGGCTATTGTGACTGGTACTGC
CDH2 ChIP	Chr18_25.428.000F: CCAATGCACTAATTTAATGTCATGC
CDH2 ChIP	Chr18_25.428.000R: CGTGCTAATTTCTATGGTACACTGG
CDH2 ChIP	Chr18_25.632F: CCTAATCCAATATGCCTGGTGTCC
CDH2 ChIP	Chr18_25.632R: CTGGAAGTCTGAGATCAAGGTGC
CDH2 ChIP	Chr18_25.778F: AATAATCACGAAGCACTTCTGTATTGC
CDH2 ChIP	Chr18_25.778R: TCACCAGCAGACATAGTCATACTTCC
CDH2 ChIP	Chr18_25.808F: CCTTGGAGGTGGAGTCTACAGAGG
CDH2 ChIP	Chr18_25.808R: CTGCTAGCGTAGCCATCTGAGATCG
TOP2B ChIP	Chr3_25.398F: GCCCTGTCTTCCCAGAATCATTGC
TOP2B ChIP	Chr3_25.398R: CATGAAGCCTATGAAGATCATTATGG
TOP2B ChIP	Chr3_25.540F: TTTAGCCAGCAAGTATTCTAGCATGG
TOP2B ChIP	Chr3_25.540R: GTCAGTGTGATTCAGTAACAATGATGG
TOP2B ChIP	Chr3_25.622F: CCTGCTCAAGGCTGACATGTCACC
TOP2B ChIP	Chr3_25.622R: GTCGGACTCGATGGTCAGCACTGG
TOP2B ChIP	Chr3_25.733F: AACCCGAAACTTTCAATGCACTTGG
TOP2B ChIP	Chr3_25.733R: CTTCCTCTATAGTGAAGACCCTAGG
TOP2B ChIP	Chr3_25.812F: TATGGCCATTCTTGCAGCAGTAAGG
TOP2B ChIP	Chr3_25.812R: AAAGTTGGCTAAGGACATGAATAGGC
TOP2B ChIP	Chr3_25.973F: GGAGATTCCCTCAGGTGCCTATACC
TOP2B ChIP	Chr3_25.973R: CTGGTGTTCCAGGCACCACTGAGG
CDH2 RT-PCR	CDH2F: TTATTACTCCTGGTGCGAGT
CDH2 RT-PCR	CDH2R: GAGCTGATGACAAATAGCGG
TOP2B RT-PCR	TOP2BF: GTTACAGGTGGTCGTAATGGTT
TOP2B RT-PCR	TOP2BR: TTGGCTTCAGAAGTCTTCATCA

Supplementary Table 16. Real-time PCR primer sequences used for ChIP-qPCR and RT-PCR experiments.