# Supplemental data

## Methods

### Immunohistochemistry

Staining was performed manually or using the Ventana BenchMark Ultra automated IHC/ISH slide staining system (Ventana Medical Systems Inc., Tucson, USA) (suppl. table 1a). The cytoplasmic expression of TFF3 and MUCL3 was evaluated using the immunoreactivity scoring system (IRS) based on staining intensity (0: negative, 1: mild, 2: moderate, 3: intense) and the percentage of stained cells (0: no positive cells, 1: <10% positive cells, 2: 10-50% positive cells, 3: 51-80% positive cells, 4: >80% positive cells). The final score (0-12) was found by multiplying the positive cells proportion score (0-4) and the staining intensity score (0-3). The mean value of Ki67 proliferation rate of five randomly selected high power (40x) fields (HPFs) was calculated using the percentage of stained cells.

### DNA/ RNA Isolation from FFPE samples

For genomic DNA or total RNA Isolation, 5-8 8-µm-thick tissue sections were prepared, and lesions were dissected manually or by laser-capture microdissection (LMD), depending on their size to ensure adequate cellularity (>80%) for subsequent molecular analysis. For LMD, cresyl violet staining was done before using the Palm Microbeam System (Carl Zeiss, Oberkochen, Germany) according to the manufacturer's instructions. In some samples containing larger lesions, manual microdissection was used, as previously described.[1] The obtained cell clusters were isolated using the QIAamp DNA micro Kit or the GeneRead FFPE DNA Kit for DNA and the RNeasy FFPE Kit for RNA (all from Qiagen, Hilden, Germany) following the manufacturer's instructions. The genomic DNA quality control was performed by quantitative PCR using the Power SYBR™ Green PCR Master Mix on a StepOnePlus™ Real-Time PCR System. Quantification was performed with a self-designed primer assay (HML-2 for: 5' AAACGCCAATCCTGAGTGTC-3'; HML-2 rev: 5' CATAGCTCCTCCGATTCCAT-3'). These primers are complementary to long terminal repeats (LTRs) of the HML 2 human endogenous retroviruses and have a length of about 115 bp.



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**Targeted NGS**

A PDAC-Panel with two primer pools was created by the Ion AmpliSeqTM Designer (v5.6, ThermoFisher Scientific, Dreieich, Germany). The panel consists of 217 amplicons of 21 genes covering hot-spot mutational sites of 18 and the whole coding sequence of 3 (*ARID1A*, *TP53* and *RNF43*) additional genes relevant for PDAC (suppl. table 2). Barcoded libraries from gDNA (up to 10 ng per pool) were prepared using the Ion AmpliSeq Library kit 2.0 with Ion Xpress™ Barcode adapters. The Ion library TaqMan™ Quantitation Kit was used for quantification of the libraries. The libraries were pooled and amplified in an emulsion PCR reaction using the Ion 520™ & Ion 530™ Kit-OT2. The resulting Ion Sphere particles (ISPs) were loaded on a 520™ or 530™ Chip and sequenced on the Ion S5™ system (all reagents from ThermoFisher).

The results of the next generation sequencing from the Ion S5™ system were aligned to the human reference genome (GRCh37/hg19) using the S5 Ion Torrent Server VM (ThermoFisher). The Ion Reporter software (Version 5.12.0.0) was used for variant calling and annotations of the DNA panel sequencing. The parameters for variant calling were set equal for all samples. Following thresholds were defined: 3% allele frequency with a minimum coverage of 500 and a Phred Score of ≥ 30. Detected variants were validated using the Integrative Genomics Viewer (IGV), ClinVar database from National Institutes of Health (NIH) and University of California Santa Cruz (UCSC) Genome Browser. Variants not present in the above mentioned databases were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines using the ACMG database (varsome.com; v7.3.7).[2]

**Fusion transcript analysis**

50 ng of isolated RNA were used for cDNA synthesis by QuantiTect Reverse Transcription Kit (Qiagen) and were subsequently subjected to library preparation using the Oncomine Comprehensive Assay Plus RNA (ThermoFisher) targeting over 1,300 isoforms of 49 tumor driver genes including approximately 200 known *BRAF* fusion transcripts. NGS was performed (as described above) and data analysis was done using the Oncomine Comprehensive Plus w2.1 - Fusion workflow implemented within the IonReporter Software package (V5.18; ThermoFisher).

**Isolation of epithelial cells from the main pancreatic duct and from peripheral (branch) ducts**



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Specimens were obtained fresh from the operating theater and immediately subjected to gross examination. The main pancreatic duct was probed, and the specimen dissected by a pathologist along the probe. The main duct was then carefully dissected with a scissor and then fixed in 10% buffered formalin and embedded in paraffin. Peripheral tissue blocks were prepared, and branch-ducts were isolated by LMD, as described above. DNA extraction was performed as described above.

**Generation of β-cells**

β-cell populations from FFPE tissue were generated from 50-µm-thick sections. Tissue sections were dewaxed with xylol and rehydrated in descending ethanol concentrations. Antigen retrieval was done at 80°C for one hour in a pressure cooker before tissue was digested with 1% (w/v) collagenase Ia (Sigma, Steinheim, Germany) and 1%(w/v) dispase (Gibco, Grand Island, USA) for 45 min at 37°C to obtain single cells. The cell suspension was subsequently filtered (30 µm mesh) and the cells were collected by centrifugation. Single cells were stained Insulin (Abcam, Cambridge, UK; 1:200). The stained cells were sorted with a BD FACS AriaTM III System. DNA was isolated from the sorted cells as described above.

**Transcriptome analysis**

After total RNA isolation, the samples were shipped to Macrogen (Seoul, Korea) for sequencing. Libraries from total RNA were prepared using the Illumina TruSeq™ Stranded mRNA Library Prep kit and sequenced with 2 x 100 bp on the Illumina NovaSeq 6000 (Illumina Inc, San Diego, USA). The raw data processing of the transcriptome data was performed by Macrogen. Briefly, adapter and low-quality base trimming was carried out with Trimmomatic (v0.38).[3] Trimmed reads were mapped against the GRCh38/hg38 human reference genome using the Bowtie2 (v2.3.4.1) aligner.[4] Afterwards, the aligned reads were assembled with Cufflinks (v2.2.1).[5] After assembly the abundance of gene was calculated in read counts per gene. Before differential gene expression analysis lowly expressed genes were filtered from the data set. Therefore, genes which showed a lower read count as 0.5 transcripts per million reads and were missing in more than one sample per group were excluded from further analysis. The filtered raw count matrix was normalized and batch-corrected using the DESeq2 package (v3.14).[6] Finally, differentially expressed genes were calculated pairwise and defined as followed $\log_2$ fold change of < -1 and > 1, respectively, and the significance level of

the adjusted p-value was set to < 0.05. PCA, heatmap and expression plots were calculated based on the variance stabilizing transformation output of DESeq2

**Pathway analysis**

Gene set enrichment analysis

For methylation data, enrichment of KEGG terms was estimated for all differentially methylated probes (DMP) in a pair-wise manner. DMPs were defined as displaying a beta value change of 0.4 and an adjusted p-value < 0.05. Gene set enrichment was calculated with the gometh function of the missMethyl package (v.1.26.1).[7]

The single sample Gene Set Enrichment Analysis (ssGSEA) was performed only for RNA seq derived data. Briefly, the normalized enrichment scores (NES) were calculated on the variance stabilized transformation data with the GSVA package (v.1.40.1).[8] Differentially activated gene sets were calculated between the different precursor lesions as described by Larsen *et al.* with a p-value of < 0.05.[9]

VIPER analysis

The activation of transcription factors was calculated with the VIPER algorithm (v1.26.0).[9] For the analysis, the paad regulon was taken from the arcane.networks package (1.18.0). Activated transcription factors were defined as displaying a p-value < 0.005 and a NES score of >3 or >-3.

**Statistical analysis**

Statistical analysis was performed using the GraphPad Prism 8 software (GraphPad Software Inc., San Diego, USA) or R v. 3.6.0 (R Core Team 2018). Statistical significance in immunohistochemistry was determined by Kruskal-Wallis test with Dunn's multiple comparison test. Results are presented as means ± standard error of the mean (SEM). P values less than 0.05 were considered statistically significant (* p< 0.05; ** p < 0.01; *** p<0.001).

## References


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# Supplementary tables

**Supplementary table 1a: Antibodies and protocols for immunohistochemistry.**

| Antibody | Type | Dilution | Antigen Demasking | Source |
|---|---|---|---|---|
| Anti-MUC1 | Mo Mono | 1:100 | CC1 | Biocare |
| Anti-MUC2 | Mo Mono | 1:100 | CC1 | Dako |
| Anti-MUC5AC | Mo Mono | 1:1000 | CC1 | Chemicon |
| Anti-CDX2 | Mo Mono | 1:40 | CC1 | BioGenex |
| Anti-MIB1 | Mo Mono | 1:100 | CC1 | Dako |
| Anti-TFF3 | Rb Mono | 1:2000 | EDTA buffer pH 9 | Abcam |
| Anti-MUCL3 | Rb Poly | 1:500 | Citrate buffer pH 6 | LSBio |

*Rb: rabbit. Mo: mouse Mono: monoclonal. Poly: polyclonal. CC1: Cell Conditioning 1 (Ventana Medical System, Tucson, AZ, USA).

**Supplementary table 1b: Tissue collective used for Ki67, TFF3 and anti-MUCl3 staining.**

| **Type of lesion** | **Number of lesions** |
|---|---|
| **PanIN** | **31** |
| Low grade | 26 |
| High grade | 5 |
| **Gastric IPMN** | **28** |
| Low grade | 20 |
| High grade | 8 |
| **Intestinal IPMN** | **20** |
| Low grade | 9 |
| High grade | 11 |
| **PDAC** | **24** |



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1 **Supplementary table 2: Genes and amplicons in targeted NGS.**

| Gene Symbol | Chr | Ion AmpliSeq Fwd Primer (5'-3') | Ion AmpliSeq Rev Primer (5'-3') | Amplicon ID |
|---|---|---|---|---|
| ALK | chr2 | TCTCTCGGAGGAAGGACTTGAG | GCCCAGACTCAGCTCAGTTAAT | CHP2_ALK_1 |
| ALK | chr2 | ACAGGGTACCAGGAGATGATGTAAG | GGAAGAGTGGCCAAGATTGGA | CHP2_ALK_2 |
| APC | chr5 | GAGAGAACGCGGAATTGGTCTA | GTATGAATGGCTGACACTTCTTCCA | CHP2_APC_1 |
| APC | chr5 | AGCACTGATGATAAACACCTCAAGTT | ATCTTCTTGACACAAAGACTGGCT | CHP2_APC_2 |
| APC | chr5 | TTCATTATCATCTTTGTCATCAGCTGAA | TTTGGTTCTAGGGTGCTGTGAC | CHP2_APC_3 |
| APC | chr5 | GCAGACTGCAGGGTTCTAGTT | GTGAACTGACAGAAGTACATCTGCT | CHP2_APC_4 |
| APC | chr5 | AGCCCCAGTGATCTTCCAGATA | CCCTCTGAACTGCAGCATTTACT | CHP2_APC_5 |
| APC | chr5 | AGAGGGTCCAGGTTCTTCCA | TCATTTTCCTGAACTGGAGGCATT | CHP2_APC_6 |
| APC | chr5 | ATGAAACAGAATCAGAGCAGCCTAAA | CGTGATGACTTTGTTGGCATGG | CHP2_APC_7 |
| ARID1A | chr1 | CAAAATGAACAACAAGGCAGATGGG | TCAGAGACTATCTAGTCCGGTGTC | ARID1A_10.112972 |
| ARID1A | chr1 | CAGCTAAACTTACTGGACTTGAGAATTTTT | GAGTCAAGACAAAAATCACTACCTTGG | ARID1A_10.135473 |
| ARID1A | chr1 | CATGATGGGAACTGGACCTCCTTA | TTAGCTGTGATGTGACTCTTGAAGAAAT | ARID1A_10.143283 |
| ARID1A | chr1 | CCCCCAGCCTACGGCTTC | CCCCGCGTAGGGCTCCA | ARID1A_1.1.15178 |
| ARID1A | chr1 | CCCTAGGCCCGCCCTGA | GGCTCCGGCCGTAGGGT | ARID1A_1.1.16654 |
| ARID1A | chr1 | CAGTCAAGAGACTTCTGAGACCCTTA | CAGATAACGGTCCACCCACATC | ARID1A_11.181180 |
| ARID1A | chr1 | CCGCTGGGAAAGGAGCTG | GCCTAGGGCCCGCGTTC | ARID1A_1.1.20289 |
| ARID1A | chr1 | CTATCGCCTCTATGTGTCTGTGAAG | GTACCACATGAAGCCAGTGAGTAC | ARID1A_11.248116 |
| ARID1A | chr1 | ACAACTCCTACTACCCCAACC | CTGCTGAGCGAAGGACGA | ARID1A_1.1.2481 |
| ARID1A | chr1 | CTTCCAGAAATCCAGTTCTTCTACTACA | ATAGAGGTCCAGAGGTTTCCTACC | ARID1A_11.279375 |
| ARID1A | chr1 | CTCAGCAGCGCTTCGGG | GGGCCCGCCACTGTAGT | ARID1A_1.1.36612 |
| ARID1A | chr1 | CTCGGAGCTGAAGAAAGCCG | GCTCTCGGCCCCGTCCT | ARID1A_1.1.38056 |
| ARID1A | chr1 | GAGCCCGTCTGCCGTCG | GGAGTTGTACTGGTGGTTGGG | ARID1A_1.1.42139 |
| ARID1A | chr1 | GGCCCCAGCAGAACTCTCAC | AGCCCGGAGTGCCACCTC | ARID1A_1.1.52554 |
| ARID1A | chr1 | GGCTGCCGGCTCCAAGC | GCTGGGCGACGTGAGCA | ARID1A_1.1.54514 |
| ARID1A | chr1 | GGGATCATGGCCGCGCA | CCGGCGGCTGCCTTCAT | ARID1A_1.1.54590 |
| ARID1A | chr1 | TTATCTGGCCTTCACTGAGGAGAA | CTCACCTGAGTCAATCCACCAAT | ARID1A_11.550938 |
| ARID1A | chr1 | AGCCGGACCTGAAGAACTCG | GGCCGCGGCTGAGTGAG | ARID1A_1.1.6484 |
| ARID1A | chr1 | CTCGCCCGGACCCCTCAG | GCCAGACAATGGCAGCTCC | ARID1A_1.2.19161 |
| ARID1A | chr1 | GGGCTACCAGGGCTACCC | GGGCTCATGGGCGCGTG | ARID1A_1.2.26067 |
| ARID1A | chr1 | GATATACCTCGACTCCTTTGGTTTGG | AGGGTCTTCTCCCCGTTCAAT | ARID1A_12.293039 |
| ARID1A | chr1 | GCCAGCTCCTTGAAAAAGCAGTATATC | GACCCCATCCTTACCAGGAGAG | ARID1A_12.311881 |
| ARID1A | chr1 | AGACATCTTTGCAGCTGCTGATT | CACAGATCCTTGGCATATCCTGTTG | ARID1A_12.73402 |
| ARID1A | chr1 | CCGGCGGACATGGCCTC | CCTCCCCACTCAGCTGTGTA | ARID1A_1.2.9363 |
| ARID1A | chr1 | CTCAACTTGTATCTCTGTCCACAGC | CTGCTCTTGGCCTTACCTCATG | ARID1A_13.224100 |
| ARID1A | chr1 | CTCCTGCGTGTCCTTTGTTATATTGG | TGGAGTCATGGAATTCCGCTT | ARID1A_13.228066 |
| ARID1A | chr1 | GAGGAGACTTAAAGCCACCAACTC | CAAGGAGTTCCCATGCACTTATCT | ARID1A_13.262576 |
| ARID1A | chr1 | GCCTTGTAGATCCTCTGCTAAGAAG | GCCCCTGCATAGATCCTGATCC | ARID1A_13.286741 |
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| ARID1A | chr1 | TGACTCCAAACCCTGGGTATCA | CATTTCACTGGCCCTGTCTTTACG | ARID1A_14.440936 |
| ARID1A | chr1 | GACCACGACAGCACTATCCCTA | TCATGTTTCCCTCAGGCCCTATT | ARID1A_15.209989 |
| ARID1A | chr1 | TCACCGCTTGCCTTTCTACG | TCACTCTGTCATAAGGACCTCCA | ARID1A_15.321878 |
| ARID1A | chr1 | CCAATTTTGTTTAGGACGGAGCCT | CACCGAGACCAGGCTTTACTC | ARID1A_15.99688 |

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| ARID1A | chr1 | CCAATGCCAACTACCCCAGTG | GGCCATGTTAGGGCCATAAGG | ARID1A_8.229785 |
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| ARID1A | chr1 | AGGATGAGTCACGCCTCCATG | GGCCTTACCTGTTTTGGATAGAGTTG | ARID1A_8.91537 |
| ARID1A | chr1 | AGCACTATTTGGCTCCAGTTCAAATC | GGTTGATCATGCCAGCCATACTATTAA | ARID1A_9.65769 |
| BRAF | chr7 | CATACTTACCATGCCACTTTCCCTT | TTTCTTTTTCTGTTTGGCTTGACTTGA | CHP2_BRAF_1 |
| BRAF | chr7 | CCACAAAATGGATCCAGACAACTGT | GCTTGCTCTGATAGGAAAATGAGATCTA | CHP2_BRAF_2 |
| CDKN2A | chr9 | CACCAGCGTGTCCAGGAA | CCCTGGCTCTGACCATTCTGT | CHP2_CDKN2A_1 |
| CDKN2A | chr9 | CATCTATGCGGGCATGGTTACT | CGCTGGTGGTGCTG | CHP2_CDKN2A_2 |
| CTNNB1 | chr3 | ACTGTTTCGTATTTATAGCTGATTTGATGGA | CCTCTTCCTCAGGATTGCCTTT | CHP2_CTNNB1_1 |
| EGFR | chr7 | CCTCATTGCCCTCAACACAGT | TCAGTCCGGTTTTATTTGCATCATAGTT | CHP2_EGFR_1 |
| EGFR | chr7 | CACCACGTACCAGATGGATGT | CCCAAAGACTCTCCAAGATGGGATA | CHP2_EGFR_2 |
| EGFR | chr7 | AGACATGCATGAACATTTTTCTCCAC | TCCAGACCAGGGTGTTGTTTTC | CHP2_EGFR_3 |
| EGFR | chr7 | TGTGGAGCCTCTTACACCCA | GTGCCAGGGACCTTACCTTATAC | CHP2_EGFR_4 |
| EGFR | chr7 | ACGTCTTCCTTCTCTCTCTGTCA | CTGAGGTTCAGAGCCATGGA | CHP2_EGFR_5 |
| EGFR | chr7 | CATGCGAAGCCACACTGAC | ACATAGTCCAGGAGGCA | CHP2_EGFR_6 |
| EGFR | chr7 | GACTATGTCCGGGAACACAAAGA | CCCCATGGCAAACTCTTGCTA | CHP2_EGFR_7 |
| EGFR | chr7 | CGCAGCATGTCAAGATCACAGAT | GCATGTGTTAAACAATACAGCTAGTG | CHP2_EGFR_8 |
| FBXW7 | chr4 | TGACAATGTTTAAAGGTGGTAGCTGTT | ACTCATTGATAGTTGTGAACCAACACA | CHP2_FBXW7_1 |
| FBXW7 | chr4 | CCTGTGACTGCTGACCAAACTTTTA | CACATCTTTCTTATAGGTGCTGAAAGG | CHP2_FBXW7_2 |
| FBXW7 | chr4 | CCCAACCATGACAAGATTTTCCC | GGTCATCACAAATGAGAGACAACATCA | CHP2_FBXW7_3 |
| FBXW7 | chr4 | ACTAACAACCCTCCTGCCATCATA | TCTGCAGAGTTGTTAGCGGTT | CHP2_FBXW7_4 |
| FBXW7 | chr4 | GTAGAATCTGCATTCCCAGAGACAA | TCTCTTGATACATCAATCCGTGTTTGG | CHP2_FBXW7_5 |
| FGFR2 | chr10 | CATCACTGTAAACCTTGCAGACAAAC | TGGTCTCTCATTCTCCCATCCC | CHP2_FGFR2_1 |
| FGFR2 | chr10 | CATCCTCTCTCAACTCCAACAGG | AGTGGATCAAGCACGTGGAAAA | CHP2_FGFR2_2 |
| FGFR2 | chr10 | GCTTCTTGGTCGTGTTCTTCATT | CTCCTCCTGTGATCTGCAATCT | CHP2_FGFR2_3 |
| FGFR2 | chr10 | TGGAAGCCCAGCCATTTCTAAA | GATGATGAAGATGATTGGGAAACACAAG | CHP2_FGFR2_4 |
| GNAS | chr20 | TTGGTGAGATCCATTGACCTCAATTT | TGAATGTCAAGAAACCATGATCTCTGTT | CHP2_GNAS_1 |
| GNAS | chr20 | CCTCTGGAATAACCAGCTGTCC | TGATCCCTAACAACACAGAAGCAA | CHP2_GNAS_2 |

| | | | | |
|---|---|---|---|---|
| IDH1 | chr2 | CCAACATGACTTACTTGATCCCCAT | ATCACCAAATGGCACCATACGA | CHP2_IDH1_1 |
| IDH2 | chr15 | ACCCTGGCCTACCTGGTC | AGTTCAAGCTGAAGAAGATGTGGAA | CHP2_IDH2_1 |
| KRAS | chr12 | CAAAGAATGGTCCTGCACCAGTAATAT | AGGCCTGCTGAAAATGACTGAATATAA | CHP2_KRAS_1 |
| KRAS | chr12 | TCCTCATGTACTGGTCCCTCATT | GTAAAAGGTGCACTGTAATAATCCAGACT | CHP2_KRAS_2 |
| KRAS | chr12 | CAGATCTGTATTTATTTCAGTGTTACTTACCT | GACTCTGAAGATGTACCTATGGTCCTA | CHP2_KRAS_3 |
| NRAS | chr1 | CCTCACCTCTATGGTGGGATCATAT | GTTCTTGCTGGTGTGAAATGACTG | CHP2_NRAS_1 |
| NRAS | chr1 | TTCGCCTGTCCTCATGTATTGG | CACCCCCAGGATTCTTACAGAAAA | CHP2_NRAS_2 |
| NRAS | chr1 | GCACAAATGCTGAAAGCTGTACC | CAAGTGTGATTTGCCAACAAGGA | CHP2_NRAS_3 |
| PIK3CA | chr3 | CCATAAAGCATGAACTATTTAAAGAAGCAAGA | GGTTGAAAAAGCCGAAGGTCAC | CHP2_PIK3CA_1 |
| PIK3CA | chr3 | TGGAATGCCAGAACTACAATCTTTTGAT | AAGATCCAATCCATTTTTGTTGTC | CHP2_PIK3CA_10 |
| PIK3CA | chr3 | TGGATCTTCCACACAATTAAACAGCAT | TGCTGTTCATGGATTGTGCAATTC | CHP2_PIK3CA_11 |
| PIK3CA | chr3 | CCCTTTTTAAAAGTAATTGAACCAGTAGGC | TTTAAGATTACGAAGGTATTGGTTTAGACAGAA | CHP2_PIK3CA_2 |
| PIK3CA | chr3 | GACGCATTTCCACAGCTACAC | AGCATCAGCATTTGACTTTACCTTATCA | CHP2_PIK3CA_3 |
| PIK3CA | chr3 | CATAGGTGGAATGAATGGCTGAATTATG | TCAATCAGCGGTATAATCAGGAGTTTTT | CHP2_PIK3CA_4 |
| PIK3CA | chr3 | TCCCATTATTATAGAGATGATTGTTGAATTTTCCT | CAAACAAGTTTATATTTCCCCATGCCA | CHP2_PIK3CA_5 |
| PIK3CA | chr3 | GCTTTGAATCTTTGGCCAGTACCT | CATAAGAGAGAAGGTTTGACTGCCATA | CHP2_PIK3CA_6 |
| PIK3CA | chr3 | CAGAGTAACAGACTAGCTAGAGACAATGA | GCACTTACCTGTGACTCCATAGAAA | CHP2_PIK3CA_7 |
| PIK3CA | chr3 | CACGATTCTTTTAGATCTGAGATGCACA | CCTTTTGTGTTTCATCCTTCTTCTCCTG | CHP2_PIK3CA_8 |
| PIK3CA | chr3 | GATGCAGCCATTGACCTGTTTAC | AGAAAACCATTACTTGTCCATCGTCT | CHP2_PIK3CA_9 |
| PTEN | chr10 | GCCATCTCTCTCCTCCTTTTTCTT | GCCGCAGAAATGGATACAGGTC | CHP2_PTEN_1 |
| PTEN | chr10 | TGTTAATGGTGGCTTTTTGTTTGTTTGT | TCTACCTCACTCTAACAAGCAGATAACT | CHP2_PTEN_2 |
| PTEN | chr10 | CCATAACCCACCACAGCTAGAA | TGCCCCGATGTAATAAATATGCACAT | CHP2_PTEN_3 |
| PTEN | chr10 | GGCTACGACCCAGTTACCATAG | TGCCACTGGTCTATAATCCAGATGAT | CHP2_PTEN_4 |
| PTEN | chr10 | TGAGATCAAGATTGCAGATACAGAATCC | ACCTTTAGCTGGCAGACCAC | CHP2_PTEN_5 |
| PTEN | chr10 | AGGTGAAGATATATTCCTCCAATTCAGGAC | TTGGATATTTCTCCCAATGAAAGTAAAGTAC | CHP2_PTEN_6 |
| PTEN | chr10 | CACTTTTGGGTAAATACATTCTTCATACCAGGA | TATACTGCAAATGCTATCGA | CHP2_PTEN_7 |
| PTEN | chr10 | GCAGTATAGAGCGTGCAGATAATGA | CATCACATACATACAAGTCAACAACCC | CHP2_PTEN_8 |
| RNF43 | chr17 | CCAAACACATCTGGAGCACACT | GCCTGACCCTCAATGACCTCTT | RNF43_1.100174 |
| RNF43 | chr17 | CCGCTTTTTGTAGTGGTGGT | TGACTTTGACCCCCTAGTGTACT | RNF43_2.1.213215 |
| RNF43 | chr17 | ACAACCACACTGGCTGTGAA | GCACCCAGCTTGCCAGATT | RNF43_2.1.22754 |
| RNF43 | chr17 | CGGTGTCAGAACTCCATTCAGAAG | GACAAGAGGCTGCTACCAGAAA | RNF43_2.1.264668 |
| RNF43 | chr17 | CTCTCCCTACCACACCCACTT | GTGGTTGTGCCTGACTCCTC | RNF43_2.1.277654 |
| RNF43 | chr17 | CTGGGTGCACAGTTGCATC | CCCTGGCCCAGTTGACG | RNF43_2.1.308947 |
| RNF43 | chr17 | GAAACCTGGGTTTCCCCTGT | GGGTCCATGGCAGCAGTTC | RNF43_2.1.342797 |
| RNF43 | chr17 | AAAGTCACTGCTTAGGGAGCT | AGAAAGCTATTGCACAGAACGC | RNF43_2.1.4249 |
| RNF43 | chr17 | GGGACCAAGGATATGCCACACT | TGCAAAAATCCAGCCTCTCTGC | RNF43_2.1.479773 |
| RNF43 | chr17 | GGGCACTGTGGGTTAGAGAG | AAAAGCGGTTCCAGTGGCA | RNF43_2.1.483268 |
| RNF43 | chr17 | GTGACTTGCTGATCAGGAGAAGGT | GTTTCCAGCCATGTCCACTACC | RNF43_2.1.554471 |
| RNF43 | chr17 | TTTTTGCAAGTTGAACAGACTGCT | CAAGTCACCAGATCCAACTCAGC | RNF43_2.1.716452 |
| RNF43 | chr17 | CTCCAGATCCACTGCTGTCA | TTCCCCAGAGCTGCACATC | RNF43_2.2.168519 |
| RNF43 | chr17 | GTAGGCTGATGTCCGTGCAG | GCTTGCCCAGTGCCCCTA | RNF43_2.2.335644 |
| RNF43 | chr17 | GTGCTGTGAGGTGGATTGGAG | CCCACGACCTGGTCCCTT | RNF43_2.2.348341 |
| RNF43 | chr17 | GTGATGCCGAGGGCCCAT | CAGGTCGAAGACTCCACCTC | RNF43_2.2.359855 |
| RNF43 | chr17 | AGGTGGTAGTGGGCATGGC | TGTCTTTCTGAATGCATTCTCTGTAGG | RNF43_2.2.62203 |
| RNF43 | chr17 | CCTCCTACCTGTGATGTTGAACATG | CCTGATTCCTGGCAATTCCTATGG | RNF43_3.144168 |

| | | | | |
|---|---|---|---|---|
| RNF43 | chr17 | AAGCCACATTCTAGACCTGTCTG | CTCTTTTTCTCCAGGAGCTACGG | RNF43_3.9926 |
| RNF43 | chr17 | TCCTCCAGACAGATGGCACA | CCCAATCTGAGCCCCATTCCT | RNF43_4.332752 |
| RNF43 | chr17 | TTCAATCTCCCCAGTCTGGTCAT | AGCTGGCCACCAGGAGGTA | RNF43_4.381754 |
| RNF43 | chr17 | TACTCCTTCCTTCTCCCTAACCAC | ATGATGTGTGGATCCTAATGACAGT | RNF43_5.252108 |
| RNF43 | chr17 | AAGCCAGGATGATCACAAAGATGG | CTCAAGGGAACCTCCAGTTAGCTAT | RNF43_5.9080 |
| RNF43 | chr17 | CCCTGAGAGCTTTATCTTCCTCCATC | GACCTCAGCCCAACCTCTACT | RNF43_6.126819 |
| RNF43 | chr17 | GTCTGGAGGTCTAGTGTGCT | TGGGCACTTTCCCCCTGTA | RNF43_6.251138 |
| RNF43 | chr17 | ATCAGCTTCTCAGCGTCATTACC | CTGGATGGAGGAAGATAAAGCTCTCA | RNF43_6.62210 |
| RNF43 | chr17 | CACAGGACAAAGTAGGGCTAAGTG | AAGCTGATGGAGTTTGTGTACAAGAA | RNF43_6.83735 |
| RNF43 | chr17 | CCAGCTTGACGATGCTGATGAAT | TGGTACCTCCCTAGAAAAATGGAGAG | RNF43_8.145848 |
| RNF43 | chr17 | GTGTAGGGCGAAGTGTGAGTC | CCTAACCCAAGTCTGTCTCTCTCTG | RNF43_8.334246 |
| RNF43 | chr17 | ATTTCCACTTCTCTCAGACCAGTCAT | CCTGTCACTGGCTAGCAAGGTA | RNF43_8.99948 |
| RNF43 | chr17 | GCAAACACACCTTCCAAAGTGAGATT | TGGACGCACAGGACTGGTAC | RNF43_9.251835 |
| RNF43 | chr17 | ACAAAAGAAGAAAGACATATTTCAAACAGATG | TTATCAGAGTGATCCCCTTGAAAATGG | RNF43_9.43347 |
| RNF43 | chr17 | AGCTTTCTGTTCTGCTGATCTTTCA | GTATGTATGGTTGAAGTGCATTGCTG | RNF43_9.83487 |
| SMAD4 | chr18 | CTCATGTGATCTATGCCCGTCT | AGTCTACTTACCAATTCCAGGTGATACA | CHP2_SMAD4_1 |
| SMAD4 | chr18 | TGCTACTTCTGAATTGAAATGGTTCA | GATTACCTACCATTACTCTGCAGTGTT | CHP2_SMAD4_2 |
| SMAD4 | chr18 | ATGGTGAAGGATGAATATGTGCATGA | GCTGGTAGCATTAGACTCAGATGG | CHP2_SMAD4_3 |
| SMAD4 | chr18 | GTGAAGGACTGTTGCAGATAGCAT | AAGGCCCACATGGGTTAATTTG | CHP2_SMAD4_4 |
| SMAD4 | chr18 | TTTCTTTAGGGCCTGTTCACAATGA | CTGAGAAGTGACCCCATAATTCCATT | CHP2_SMAD4_5 |
| SMAD4 | chr18 | GCTCCTGAGTATTGGTGTTCCAT | CCTGTGGACATTGGAGAGTTGA | CHP2_SMAD4_6 |
| SMAD4 | chr18 | TGTAATTTCTTTTTTCTTCCTAAGGTTGCACATAG | ACTTGGGTAGATCTTATGAACAGCAT | CHP2_SMAD4_7 |
| SMAD4 | chr18 | AGGTCTTTGATTTGCGTCAGTGT | GCTGGAGCTATTCCACCTACTG | CHP2_SMAD4_8 |
| SMAD4 | chr18 | GCTGCTGGAATTGGTGTTGATG | AGTACTTCGTCTAGGAGCTGGAG | CHP2_SMAD4_9 |
| STK11 | chr19 | GAGCTGATGTCGGTGGGTAT | CTCCGAGTCCAGCACCTC | CHP2_STK11_1 |
| STK11 | chr19 | CTCCCAGGCAGCTGCAA | CCGGTGGTGAGCAGCAG | CHP2_STK11_2 |
| STK11 | chr19 | CCGGTGGCACCCTCAAA | CTGGTCCGGCAGGTGTC | CHP2_STK11_3 |
| STK11 | chr19 | AACATCACCACGGGTCTGTAC | GATGAGGCTCCCACCTTTCAG | CHP2_STK11_4 |
| STK11 | chr19 | GAAGAAACATCCTCCGGCTGAA | ACCGTGAAGTCCTGAGTGTAGA | CHP2_STK11_5 |
| TP53 | chr17 | TCCACTCACAGTTTCCATAGGTCT | GTTGGAAGTGTCTCATGCTGGAT | CHP2_TP53_1 |
| TP53 | chr17 | GGCTGTCCCAGAATGCAAGAA | GATGAAGCTCCCAGAATGCCA | CHP2_TP53_2 |
| TP53 | chr17 | TGCACAGGGCAGGTCTTG | CCGTCTTCCAGTTGCTTTATCTGT | CHP2_TP53_3 |
| TP53 | chr17 | ACCAGCCCTGTCGTCTCT | GTGCAGCTGTGGGTTGATTC | CHP2_TP53_4 |
| TP53 | chr17 | CCAGTTGCAAACCAGACCTCA | AGGCCTCTGATTCCTCACTGAT | CHP2_TP53_5 |
| TP53 | chr17 | GGCTCCTGACCTGGAGTCTT | CTCATCTTGGGCCTGTGTTATCTC | CHP2_TP53_6 |
| TP53 | chr17 | CGCTTCTTGTCCTGCTTGCT | TTCTCTTTTCCTATCCTGAGTAGTGGT | CHP2_TP53_7 |
| TP53 | chr17 | GGAAGGGGCTGAGGTCACT | CCCCTCCTCTGTTGCTGC | CHP2_TP53_8 |
| VHL | chr3 | CTCCCAGGTCATCTTCTGCAAT | GTACCTCGGTAGCTGTGGATG | CHP2_VHL_1 |
| VHL | chr3 | GTGGCTCTTTAACAACCTTTGCT | GTCAGTACCTGGCAGTGTGATA | CHP2_VHL_2 |
| VHL | chr3 | GGCAAAGCCTCTTGTTCGTTC | TGACGATGTCCAGTCTCCTGTAAT | CHP2_VHL_3 |

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1 **Supplementary table 3: Mutation profile of precursor lesions detected by targeted NGS.**

| Grade | Gene | Sample | Variant | VAF [%] | Variant Effect | Transcript |
|---|---|---|---|---|---|---|
| low-grade PanIN | *ARID1A* | **113** | Gln802fs | 4.83 | frameshift/insertion | NM_006015.5 |
| | *CDKN2A* | 74 | Arg58Ter | 3.48 | nonsense | NM_001195132.1 |
| | *GNAS* | 127 | Arg201His | 20.17 | missense | NM_000516.5 |
| | *KRAS* | 52 | Gly12Val | 15.38 | missense | NM_033360.3 |
| | | **55** | Gly12Val | 9.66 | missense | NM_033360.3 |
| | | **56** | Gly12Asp | 6.13 | missense | NM_033360.3 |
| | | 69 | Gln61His | 4.08 | missense | NM_033360.3 |
| | | 74 | Gly12Asp | 12.05 | missense | NM_033360.3 |
| | | **111** | Gly12Val | 11.19 | missense | NM_033360.3 |
| | | 125 | Gly12Val | 4.29 | missense | NM_033360.3 |
| | | 127 | Gly12Asp | 18.24 | missense | NM_033360.3 |
| | | 128 | Gly12Asp | 3.42 | missense | NM_033360.3 |
| | | 129 | Gly12Asp | 3.7 | missense | NM_033360.3 |
| | | **113** | Gly12Arg | 4.18 | missense | NM_033360.3 |
| | | **113** | Gly12Val | 8.75 | missense | NM_033360.3 |
| | | **43** | Gly12Val | 12.88 | missense | NM_033360.3 |
| | *PIK3CA* | 68 | Arg349Ter | 3.74 | nonsense | NM_006218.3 |
| | | 127 | Phe83fs | 4.52 | frameshift/deletion | NM_006218.3 |
| | *PTEN* | 128 | Asn323fs | 22.37 | frameshift/deletion | NM_000314.6 |
| high-grade PanIN | *ARID1A* | 73 | Trp2091Ter | 7.46 | nonsense | NM_006015.4 |
| | *GNAS* | 96 | Arg201His | 3.42 | missense | NM_000516.5 |
| | *KRAS* | 73 | Gly12Asp | 8.29 | missense | NM_033360.3 |
| | | **80** | Gly12Val | 16.59 | missense | NM_033360.3 |
| | | 101 | Gly12Asp | 13.7 | missense | NM_033360.3 |
| | | **104** | Gly12Asp | 5.15 | missense | NM_033360.3 |
| | | **114** | Gly12Val | 18.3 | missense | NM_033360.3 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | *TP53* | **80** | Arg213Ter | 34.5 | nonsense | NM_000546.5 |
| | | **104** | Arg196Ter | 4.04 | nonsense | NM_000546.5 |
| low-grade IPMN gastric | *ARID1A* | 21 | Asp1850fs | 7.45 | frameshift/insertion | NM_006015.5 |
| | *GNAS* | **2** | Arg201His | 33.98 | missense | NM_000516.5 |
| | | **7** | Arg201Cys | 11.34 | missense | NM_000516.5 |
| | | **35** | Arg201His | 41.52 | missense | NM_000516.5 |
| | | **62** | Arg201Cys | 15.56 | missense | NM_000516.5 |
| | | **86** | Arg201His | 30.46 | missense | NM_000516.5 |
| | | 89 | Arg201His | 5.56 | missense | NM_000516.5 |
| | | **94** | Arg201His | 21.97 | missense | NM_000516.5 |
| | | **97** | Arg201Cys | 33.81 | missense | NM_000516.5 |
| | | **99** | Arg201His | 25.08 | missense | NM_000516.5 |
| | | **110** | Arg201Cys | 22.37 | missense | NM_000516.5 |
| | | **112** | Arg201His | 24.32 | missense | NM_000516.5 |
| | | **54** | Arg201Cys | 23.16 | missense | NM_000516.5 |
| | *KRAS* | 21 | Gly12Val | 18.86 | missense | NM_033360.3 |
| | | **2** | Gly12Val | 35.1 | missense | NM_033360.3 |
| | | **7** | Gly12Val | 12.14 | missense | NM_033360.3 |
| | | **35** | Gly12Asp | 38.57 | missense | NM_033360.3 |
| | | **39** | Gly12Val | 35 | missense | NM_033360.3 |
| | | **61** | Gly12Val | 18.03 | missense | NM_033360.3 |
| | | **62** | Gly12Val | 18.5 | missense | NM_033360.3 |
| | | 64 | Gly12Val | 9.01 | missense | NM_033360.3 |
| | | **86** | Gly12Val | 27.81 | missense | NM_033360.3 |
| | | 89 | Gln61His | 36.49 | missense | NM_033360.3 |
| | | **94** | Gly12Asp | 21.16 | missense | NM_033360.3 |
| | | **97** | Gly12Asp | 33.68 | missense | NM_033360.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | **99** | Gly12Val | 26.83 | missense | NM_033360.3 |
| | | **110** | Gly12Val | 24.76 | missense | NM_033360.3 |
| | | **57** | Gly12Arg | 6.96 | missense | NM_033360.3 |
| | *STK11* | **61** | Tyr60Ter | 61.89 | nonsense | NM_000455.4 |
| high-grade IPMN gastric | *ARID1A* | 130 | Gln2115Ter | 30.84 | nonsense | NM_006015.5 |
| | *GNAS* | 51 | Arg201Cys | 31.03 | missense | NM_000516.5 |
| | *KRAS* | 51 | Gly12Val | 33.12 | missense | NM_033360.3 |
| | | 65 | Gly12Asp | 16.51 | missense | NM_033360.3 |
| | | 66 | Gly12Asp | 33.64 | missense | NM_033360.3 |
| | | 130 | Gly12Asp | 22.65 | missense | NM_033360.3 |
| | | 131 | Gly12Asp | 23.98 | missense | NM_033360.3 |
| | *TP53* | 65 | Arg248Trp | 17.99 | missense | NM_000546.5 |
| low-grade IPMN intestinal | *GNAS* | **92** | Arg201Cys | 80.59 | missense | NM_000516.5 |
| | *KRAS* | **92** | Gly12Arg | 37.95 | missense | NM_033360.3 |
| high-grade IPMN intestinal | *GNAS* | **33** | Arg201Cys | 45.42 | missense | NM_000516.5 |
| | | **88** | Arg201Cys | 36.81 | missense | NM_000516.5 |
| | | **90** | Arg201His | 35.51 | missense | NM_000516.5 |
| | | 17 | Gln227Lys | 42.36 | missense | NM_000516.5 |
| | *KRAS* | **88** | Gly12Ser | 33.81 | missense | NM_033360.3 |
| | *TP53* | **88** | Met237Thr | 49.16 | missense | NM_000546.5 |

1 PanIN/IPMN in cases without associated/concomitant PDAC are indicated in bold.

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**Supplementary table 4. Distribution of CNV-positive and negative precursor lesions according to the degree of dysplasia.**

| | | *CNV pos* | *CNV neg* |
|---|---|---|---|
| *PanIN* | **Low-grade** | 16 (57%) | 12 (43%) |
| | **High-grade** | 6 (75%) | 2 (25%) |
| *Gastric IPMN* | **Low-grade** | 22 (76%) | 7 (24%) |
| | **High-grade** | 6 (75%) | 3 (25%) |
| *Intestinal IPMN* | **Low-grade** | 8 (100%) | 0 |
| | **High-grade** | 13 (100%) | 0 |

Percentages refers to the total number of cases in each group



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**Supplementary table 5: Overview of log2 copy number ratios sorted in ascending order. Values of genomic alterations were detected by low-coverage sequencing (n=28) and DNA methylation data (n=67), respectively.**

| Genomic location | affected samples (n) | PanIN (n=36) | gIPMN (n=38) | | | | iIPMN (n=21) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **deleted regions** | | | | | | | | | | | |
| chr01:010875000-013052998 | 3 | | | | | | -0.26 | -0.25 | -0.24 | | |
| chr01:015375000-016825000 | 3 | | | | | | -0.26 | -0.25 | -0.20 | | |
| chr06:074175000-074375000 | 5 | | -0.29 | -0.22 | -0.21 | | -0.44 | -0.24 | | | |
| chr06:133664400-143100000 | 5 | | -0.37 | -0.28 | | | -0.50 | -0.45 | -0.28 | | |
| chr06:143620678-151100000 | 7 | -0.21 | -0.37 | -0.34 | -0.29 | | -0.50 | -0.45 | -0.28 | | |
| chr09:005958053-023802212 | 5 | -0.23 | -0.51 | -0.36 | -0.36 | -0.24 | | | | | |
| chr10:071075000-120925000 | 6 | | -0.45 | -0.22 | | | -0.36 | -0.30 | -0.28 | -0.22 | |
| chr10:120925000-125869472 | 5 | | -0.30 | | | | -0.41 | -0.30 | -0.28 | -0.22 | |
| chr11:057325000-058807232 | 4 | | | | | | -0.44 | -0.40 | -0.27 | -0.22 | |
| chr11:058807232-069089801 | 5 | | | | | | -0.44 | -0.40 | -0.28 | -0.27 | -0.22 |
| chr11:096437584-114325000 | 5 | | | | | | -0.51 | -0.44 | -0.40 | -0.28 | -0.22 |
| chr11:114325000-134898258 | 4 | | | | | | -0.51 | -0.44 | -0.28 | -0.22 | |
| chr17:006225000-009675000 | 4 | | -0.50 | -0.32 | -0.21 | | -0.23 | | | | |
| chr17:009675000-012500000 | 5 | | -0.53 | -0.31 | -0.21 | | -0.50 | -0.22 | | | |
| chr17:015792977-021566608 | 6 | | -0.58 | -0.23 | -0.21 | | -0.49 | -0.32 | -0.25 | | |
| **amplified regions** | | | | | | | | | | | |
| chr01:035225000-037325000 | 3 | | | | | | 0.25 | 0.33 | 0.61 | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| chr03:176225000-188875000 | 6 | 0.26 | 0.21 | 0.23 | 0.28 | 0.38 | 0.44 | | | | |
| chr05:028950000-044925000 | 5 | | | | 0.24 | 0.25 | 0.31 | 0.74 | 0.79 | | |
| chr06:024125000-033575000 | 5 | | 0.25 | 0.36 | 0.31 | 0.35 | 0.56 | | | | |
| chr06:033575000-042725000 | 4 | | 0.42 | | 0.32 | 0.34 | 0.56 | | | | |
| chr07:000282484-007150000 | 5 | | | | 0.22 | 0.30 | 0.32 | 0.36 | 0.41 | | |
| chr07:054725000-055775000 | 5 | | | | 0.21 | 0.26 | 0.27 | 0.33 | 0.36 | | |
| chr07:061967157-074715724 | 4 | | | | 0.27 | 0.32 | 0.36 | 0.37 | | | |
| chr07:112425436-130154523 | 5 | | | | 0.21 | 0.33 | 0.36 | 0.38 | 0.57 | | |
| chr07:139404377-142048195 | 5 | | | | 0.22 | 0.32 | 0.36 | 0.33 | 0.58 | | |
| chr07:143397897-154270634 | 5 | | | | 0.24 | 0.33 | 0.34 | 0.38 | 0.59 | | |
| chr08:086726451-089550000 | 3 | | | | 0.21 | 0.31 | 0.36 | | | | |
| chr08:127450000-129175000 | 7 | 0.42 | | | 0.21 | 0.27 | 0.29 | 0.36 | 0.56 | 0.66 | |
| chr09:001992685-035698318 | 3 | | | | 0.24 | 0.32 | 0.61 | | | | |
| chr09:070835468-092343416 | 4 | | | | 0.22 | 0.26 | 0.32 | 0.60 | | | |
| chr09:096718222-097575000 | 4 | | | | 0.21 | 0.24 | 0.32 | 0.60 | | | |
| chr09:097775000-114750000 | 4 | | | | 0.22 | 0.26 | 0.32 | 0.61 | | | |
| chr09:124994207-133073060 | 3 | | | | 0.22 | 0.32 | 0.61 | | | | |
| chr12:006475000-007169938 | 8 | 0.21 | | | 0.24 | 0.33 | 0.56 | 0.71 | 0.88 | 0.89 | 1.70 |
| chr12:024993545-028938805 | 4 | 0.21 | | | 0.21 | 0.38 | 1.32 | | | | |
| chr14:020700000-022050000 | 3 | | | | 0.21 | 0.28 | 0.33 | | | | |
| chr14:022800000-050175000 | 3 | | | | 0.22 | 0.25 | 0.35 | | | | |
| chr14:097258910-107289540 | 3 | | | | 0.25 | 0.22 | 0.36 | | | | |
| chr17:061125000-062410760 | 3 | | | | 0.32 | 0.32 | 0.74 | | | | |
| chr17:062775000-063525000 | 3 | | | | 0.32 | 0.34 | 0.49 | | | | |
| chr17:068117898-077546461 | 3 | | | | 0.21 | 0.32 | 0.38 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| chr20:008050000-016400000 | 7 | 0.24 | 0.22 | 0.23 | 0.24 | 0.26 | 0.33 | 0.84 |
| chr20:016625000-021300000 | 7 | 0.25 | 0.22 | 0.23 | 0.25 | 0.28 | 0.33 | 0.82 |
| chr20:030025000-034897085 | 7 | 0.26 | 0.22 | 0.22 | 0.23 | 0.31 | 0.33 | 0.81 |
| chr20:036958189-042991501 | 6 | 0.26 | 0.22 | 0.23 | 0.35 | 0.33 | 0.79 | |
| chr20:052650000-061091437 | 7 | 0.23 | 0.22 | 0.22 | 0.23 | 0.33 | 0.35 | 0.80 |
| chr21:032825000-034475000 | 3 | | 0.21 | 0.26 | 0.30 | | | |

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**Supplementary table 6a: Differentially methylated probes in low- and high-grade pancreatic cancer precursors.**

| | Low-grade samples | High-grade samples | DMPs (hypermethylated in high-grade) | DMP associated genes (hypermethylated in high-grade) |
|---|---|---|---|---|
| **iPMN** | **8** | **12** | **0** | **0** |
| **gIPMN** | **24** | **8** | **0** | **0** |
| **PanIN** | **20** | **7** | **86 (62)** | **59 (45)** |

iIPMN: intestinal IPMN; gIPMN: gastric IPMN

**Supplementary table 6b: Genes associated with significantly hyper- or hypomethylated CpG in PanIN high-grade**

| Genes associated with at least one significantly hypermethylated CpG | Genes associated with at least one significantly hypomethylated |
|---|---|
| TAC1 | GLRX |
| AKAP13 | BCL11B |
| POLR1D | ITFG3 |
| GLYATL3 | IFT140 |
| HOXA5 | CTNNA3 |
| ZIC2 | RUNX1 |
| SIM2 | SPARCL1 |
| ARID4B | PHLDB1 |
| MON2 | NINJ2 |
| CNKSR3 | METTL9 |
| SP8 | SLC51A |
| ADD2 | EMID2 |
| HOXB1 | CACNA1A |
| ST3GAL6 | C19orf35 |
| FBN2 | |
| ZIK1 | |
| LRP1B | |
| NTRK3 | |
| GLI3 | |
| NTM | |
| RASGRF1 | |
| FAM46C | |

| | |
|---|---|
| NXPH1 | |
| LBX2 | |
| LOC101929710 | |
| ADRA1A | |
| GRIK3 | |
| PARP8 | |
| KIAA1026 | |
| SLC6A15 | |
| IRX1 | |
| GRIA4 | |
| TLE4 | |
| DGKI | |
| PACSIN2 | |
| DOK5 | |
| ZIC4 | |
| MYLK | |
| DAPK1 | |
| C5orf66-AS1 | |
| AMER3 | |
| CPEB1 | |
| ST6GAL2 | |
| INA | |
| AP2A2 | |

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# Supplementary Figure Legends

**Supplementary figure 1: Overview of lesions and methods**

55 PanINs, 46 gastric IPMNs (gIPMN) and 21 intestinal IPMNs (iIPMN) were subjected to 4 main analyses: targeted next generation sequencing (n=52), low-coverage whole-genome sequencing (n=28), genome-wide DNA methylation analysis (n=79) and transcriptome analysis (n=34). Each circle of the diagram represents one of the mentioned methods and includes the number of samples used for related analyses. The samples that could not be placed into the diagram were shown at the right bottom corner of the figure. Pancreatobiliary and mixed-type IPMNs were excluded from further analyses due to small sample size.

**Supplementary figure 2: Allele frequency of *KRAS* and *GNAS* mutations in different precursor lesions.**

(A) VAF of *KRAS* mutations; (B) VAF of *GNAS* mutations; (C) scatterplot of the VAF of the *KRAS* mutations (G12) against the VAF of *GNAS* mutations (R201) detected in low- and high-grade gastric IPMN. The grey lines represent the 95% confidence interval of the Pearson's correlation coefficient r (n=9). (Kruskal-Wallis-test * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

**Supplementary figure 3: Morphology of lesions with different genetic status according to targeted NGS**

Representative HE images of low- and high-grade PanINs, gastric IPMNs and intestinal IPMNs with variable mutation profiles detected by targeted NGS are shown. No specific morphology was identified related to the mutation status between the samples in the same diagnostic group. In particular, gastric lesions with GNAS mutations (case 127, 96, 99 and 112) did not show relevant intestinal differentiation; only in case 112, focal (<5% of the cells) expression of MUC2 and CDX2 was observed (not shown). Scale bars represent 200 µm. Detailed mutation profile of the samples is provided in Suppl. Table 3.

**Supplementary figure 4: Quality control of DNA methylation data derived from normal pancreas cell preparations.**

(A) Multi-dimensional scaling of the 1000 most variable probes. (B) Hierarchical clustering of probes for known acinar and ductal marker genes.

**Supplementary figure 5: Proliferation activity, expression of TFF3 and of MUCL3 protein in PanIN, IPMN and PDAC.**

Representative images (A) and related graphs (B-D) of IHC staining performed in whole tissue sections in 31 PanIN, 28 gastric IPMN, 20 intestinal IPMN and 24 PDAC. Intestinal IPMNs and PDACs showed higher proliferation rates with Ki67 staining than PanIN and gastric IPMN. TFF3 was strongly expressed in intestinal IPMN. Gastric IPMN revealed higher expression of MUCL3 compared to PanIN. Scale bars represent 100 µm. (IRS: immunreactivity score) (*p<0.05).

**Supplementary figure 6: Hierarchical clustering of DNA methylation data based on published marker genes for distinct normal pancreas cell populations.** The mean methylation beta-value for all gene associated probes is displayed, respectively.

**Supplementary figure 7: Differentially activated gene sets.** Displayed pathways were detected by pairwise comparison between the indicated lesions. Odds ratios below 0.8 indicate the activation in the first listed lesion whereas 1.1 is associated with the second group. The analyzed gene sets based on the KEGG pathway (A) and hallmarks (B) from the MSigDB.



Liffers S-T, *et al. Gut* 2022;0:1–14. doi: 10.1136/gutjnl-2021-326550