Supplementary Materials for

**BRAF**\(^{V600E}\) drives dedifferentiation in small intestinal and colonic organoids and cooperates with mutant p53 and Apc loss in transformation

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1. Supplementary Methods

Establishment of organoids
The proximal small intestine or colon were isolated, flushed with ice-cold PBS and opened longitudinally. In the small intestine, the villi were scraped off using a glass slide. Afterwards, the intestine was put into a falcon tube containing 20 ml ice-cold PBS and vortexed for 5-10 sec. This washing was repeated 5-10 times until the supernatant was almost clear. The intestine was subsequently incubated in 30 ml ice-cold crypt isolation buffer (small intestine: 2 mM EDTA in PBS for 30 min; colon: 15 mM EDTA in PBS for 1 h) at 4 °C on a rotating mixer. The intestine was placed into 20 ml ice-cold PBS, inverted 10 times and then transferred into a fresh falcon tube with 10 ml ice-cold PBS and shaken about 10 times (= supernatant 1). Subsequently, the intestine was transferred into fresh 10 ml ice-cold PBS and shaken again. The crypt releasing procedure was repeated 10 times and each supernatant was inspected under the microscope. The crypt containing fractions were pooled, centrifuged at 100 x g for 10 min and the supernatant was discarded. Afterwards, the crypt pellet was resuspended with 10 ml Advanced DMEM/F12 containing 2 mM L-glutamine, 10 mM HEPES, 100 U/ml penicillin and 100 µg/ml streptomycin (all from PAN-Biotech GmbH), passed through a 70-µm cell strainer and collected in a 1% BSA-coated falcon tube. The crypts were spun down at 300 x g for 5 min, resuspended with Matrigel (Corning) and seeded in 50 µl onto a pre-warmed 24-well culture dish. After the Matrigel had solidified, the crypts were overlaid with 500 µl complete crypt culture medium (Advanced DMEM/F12 with 2 mM L-glutamine, 10 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin, 1xN2, 1xB27 (both from Thermo Fisher), 81.5 mg/ml N-Acetylcystein (Sigma-Aldrich)), supplemented with 100 ng/ml murine recombinant Noggin, 500 ng/ml human recombinant R-Spondin1, 50 ng/ml murine recombinant EGF (all from Peprotech) and 10 µM Rho kinase inhibitor Y27632 (TOCRIS). For colonic crypts 50 ng/ml recombinant murine Wnt3a (Peprotech) and 2.5 µM GSK3i CHIR99021 (Cayman Chemical) were added. Medium was changed every 3-4 days and crypts were passaged once a week to remove dead cell debris.

Global induction of Cre recombinase was achieved by treatment of the organoids with 1 µM (for small intestinal crypts) and 3 µM (for colonic crypts) 4-HT ((Z)-4-hydroxytamoxifen (Sigma-Aldrich)), respectively, for 24 h.
Cre mediated recombination of $Braf^{floxV600E}$ and $Apc^{flox}$ was assessed by genomic PCR using primers that were described previously [1, 2] and Cre mediated recombination of $Trp53^{LSL-R172H/+}$ was assessed using the following primers: 5'-AGCCTTAGACATAACACACGA-3', 5'-CTTGGAGACATAGCCACACTG-3', 5'-GCCACCATGGCTTGAGTAA-3'.

MTT staining of organoids
Organoids were incubated with 500 µg/ml MTT solution diluted in DMEM/F12 for 2 h at 37 °C.

RNA-sequencing
For the RNA-Seq of small intestine (SI) and colon (COL), RNA was extracted from freshly isolated SI and COL crypts of four (two female and male, respectively) mice. For the RNA-Seq of organoids, the organoid cultures of three donor mice ($Trp53^{LSL-R172H/+;Villin::CreER_T2}$, $Braf^{floxV600E/+;Villin::CreER_T2}$, $Braf^{floxV600E/+;Trp53^{LSL-R172H/+;Villin::CreER_T2}}$) were induced with 4-HT (0.5 µM for SI organoids, 3.5 µM for COL organoids) in duplicates for 24 h. SI crypt RNA was isolated after 8 days and COL crypt RNA was isolated after 3 days. Isolated RNA, from 24 organoid- and 8 crypt samples, was sequenced on an Illumina HiSeq4000. Paired-end reads were filtered to remove bad quality reads and adapter sequences using Trimmomatic [3]. The selected reads were aligned to the mouse reference genome mm10 and read count per gene was quantified with STAR aligner [4]. A linear model-based algorithm, limma [5], was used to calculate the differentially regulated genes on the following contrasts: $Braf^{V600E}$ on vs. off, $Trp53^{R172H}$ on vs. off, $Braf^{V600E}$ and $Trp53^{R172H}$ on vs. off for colonic and small intestinal organoids separately. For wildtype crypts we compared the small intestine vs. colon replicates. The same significant threshold was used for all comparisons, i.e. adjusted p-value (Benjamini-Hochberg) below 0.05. The gene-set enrichment analysis was performed using the fgsea R package [6], where genes were ranked according to the on vs. off log2 fold-change and tested against the MSigDB [7] gene-sets (Hallmark, chemical and genetic perturbations, Biocarta, KEGG, PID, Reactome, miRNA, transcription factor targets, cancer modules, Gene Ontology, oncogenic signature and immunologic signature) as well as the SSAs UP and DOWN signature lists (GSE45270), the $Cdx1/Cdx2$ double knockout signature (GSE24633) and the mouse fetal intestinal spheroid signature [8].
Data availability
RNA-Seq data were uploaded to Gene Expression Omnibus database.
To review GEO accession GSE132551, go to:
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132551
Enter token avgfcaecltkpbfm into the box.
To review GEO accession GSE132546:
Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132546
Enter token mlyzwysyluhfoj into the box.

TCGA data
RNA-Seq raw counts were downloaded from TCGA using TCGAbiolinks R package [9]. BRAF$^{V600E}$ mutant colorectal adenocarcinoma were selected (n=54), as well as healthy colon samples (n=41). Counts were converted into log2 count per million (CPM) for further analysis. GSEA analysis was performed on the average log2 foldchange between COAD and healthy colon using fgsea R package.
2. Supplementary Figure legends

Fig. S1. Confirmation and effects of Villin::CreER\textsuperscript{T2}-activated recombination within SI and COL organoids.

(a) Schematic models of Villin::CreER\textsuperscript{T2}-activated recombination. Following 4-HT induction, BRAF\textsuperscript{V600E} or dominant negative acting p53\textsuperscript{R172H} are expressed under the control of their own promoters.

(b) Confirmation of successful recombination within 4-HT-treated organoids by genomic PCR. DNA of donor mice serve as controls and EtOH serves as vehicle control for 4-HT.

(c) Western blot (WB) of SI crypts. Treatment with 4-HT induces expression of BRAF\textsuperscript{V600E} and downstream signaling, indicated by the phosphorylation of MEK and ERK and increased expression of their target gene DUSP6. The tight control of CreER\textsuperscript{T2} is further demonstrated by a BRAF\textsuperscript{V600E} specific antibody [10], showing immunoreactivity only in lysates of 4-HT-treated organoid cultures.

(d) Time lapse images of disintegrating organoids following 4-HT induction. The Paneth cells (white arrows) disappear within SI organoids after oncogene expression.

(e) Quantification of organoid disintegration time after 4-HT induction. Data are presented as mean ± SD. **P ≤ 0.01; ****P ≤ 0.0001 (one-way ANOVA, corrected for multiple comparison by Bonferroni) (n ≥ 3 independent 4-HT inductions).

(f) BRAF\textsuperscript{V600E}-mutant COL organoids were induced with 3 µM 4-HT or left untreated. After 4 days, organoids were stained with 1.5 µM DRAQ7 for 10 min to visualize dead cells. Fluorescent and bright-field images are shown.

(g) WB of COL crypt lysates three days following 4-HT induction. BRAF\textsuperscript{V600E} and BRAF\textsuperscript{V600E}/p53\textsuperscript{R172H} samples show increased presence of the large fragment (17/19 kDA) of activated CASPASE-3. Detection of 14-3-3 serves as loading control.

Figure information: In (d,f), scale bars: 50 µm.

Fig. S2. BRAF\textsuperscript{V600E} expression leads to profound changes in organoid organization.

(a) Induction of $Trp53^{R172H/+}$ neither leads to disintegration of SI nor COL organoids, and Paneth cells (white arrow) remain visible within $Trp53^{R172H/+}$ mutant SI crypts. Scale bar: 50 µm.
(b) Control SI organoids, which are unable to express BRAF<sub>V600E</sub> due to the lack of floxed alleles or cre recombinase, do not show any morphological changes after treatment with the indicated 4-HT concentrations. Scale bars: 50/100 µm.

(c-e) Side-by-side comparison of epithelial features in SI and COL organoids expressing BRAF<sub>V600E</sub> and/or p53<sup>R172H</sup>. Different time points of Trp53<sup>R172H/+</sup> mutant COL organoids are shown to illustrate that p53<sup>R172H</sup> by itself does not disturb epithelial organization, neither at an early nor at a later time point following oncogene induction.

(c) KI-67 and E-Cadherin immunofluorescence (IF) staining of formalin-fixed paraffin-embedded (FFPE) organoid sections. Clusters positive for the S-phase marker KI-67 were confined to the stem cell niches of control organoids and those expressing p53<sup>R172H</sup>. In contrast, organoids expressing BRAF<sub>V600E</sub>, either singly or in combination with p53<sup>R172H</sup>, displayed extended stretches of KI-67 positive cells that often occurred outside of morphologically defined crypt bases. Note that the organization of the single-layered polarized epithelium appeared disturbed and the nuclei no longer exhibited their basal deposition.

(d) The establishment of an apico-basal-polarity axis represents a defining moment in epithelial organogenesis and for the sealing of epithelia by the proper deposition of tight junctions. Therefore, localization of PKCζ, a widely used marker for the identification of the apical surface of epithelia [11], was assessed by IF. Note that PKCζ is deposited at the luminal (apical) plasma membranes in control and p53<sup>R172H</sup> expressing SI and COL organoids, while its typical localization is absent in the presence of BRAF<sub>V600E</sub>.

(e) Choleratoxin (CTX; 0.1 µg/ml) was used to assess the integrity of tight junctions in spheroid culture, as this toxin triggers ion mediated water efflux into the spheroid lumen [11]. Only if tight junctions are sealed, the lumen will expand in response to CTX. In line with our previous data showing that BRAF<sub>V600E</sub> impairs the seal of tight junctions in human CRC cell line spheroids [10, 12], we demonstrate that CTX triggers luminal expansion in control and p53<sup>R172H</sup> expressing SI and COL organoids but not in those expressing BRAF<sub>V600E</sub>. This indicates that BRAF<sub>V600E</sub> impairs epithelial sealing.

(f) IF staining for Lysozyme C in SI organoids. The niche of SI stem cells is organized by Paneth cells, which supply the crypt base with growth factors and can be visualized by lysozyme expression [13]. In agreement with the loss of
morphologically defined crypts and the disappearance of Paneth cells in bright-field micrographs (Fig. S1d), lysozyme positive Paneth cells were almost completely absent in organoids expressing BRAFV600E. This suggests that Paneth cells are lost upon BRAFV600E expression, which agrees with previous findings that ERK promotes Goblet cell generation at the expense of Paneth cells [14].

Figure information: In (c-f), scale bars: 50 µm.

**Fig. S3. Validation of RNA-Seq analysis.**

(a) PCA of RNA-Seq of SI (circles) and COL (triangles) crypts. Color code represents the different conditions where “off” corresponds to EtOH-treated control organoids and “on” corresponds to 4-HT induced organoids.

(b) IF staining of FFPE organoid sections indicates increased membranous β1-Integrin (ITGB1) within BRAFV600E and double-mutant organoids.

(c) CTSE IF staining of FFPE organoid sections. BRAFV600E mutant SI and COL organoids show increased Cathepsin E production, which is even more enhanced by the addition of p53R172H.

(d) WB analyses and densitometric quantification of NR2E3 and Ephrin type-A receptor 2 (EPHA2) expression in COL organoids. Protein expression was normalized to corresponding loading control and EtOH-treated control organoids. N=3 independent biological replicates.

(e) Densitometric quantification of the WB analyses of CTSE and CAV1 shown in Fig. 1c and four and two additional biological replicates, respectively. Protein expression was normalized to corresponding loading control and EtOH-treated control organoids.

Figure information: In (b,c), scale bars: 50 µm. In (d,e), data are presented as mean ± SD. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001 (one-way ANOVA, corrected for multiple comparison by Bonferroni) (n ≥ 3 independent experiments).

**Fig. S4. BRAFV600E mutant organoids resemble the gene signature of SSAs.**

BrafV600E/+ and double-mutant COL organoids show enrichment and depletion of genes that are expressed at high and low levels (top 500), respectively, in SSAs when compared to tubular adenomas (TAs) from familiar adenomatous polyposis patients, who harbor an APC germline mutation (GSE45270).
Fig. S5. GSEA of the delta log2 fold-changes of the BRAF^{V600E}-only and double-mutant organoids.
Barplots of the top 50 (sorted by NES) UP (left panel) and DOWN (right panel) regulated MSigDB gene sets. Enrichment was performed on the calculated delta log2 fold-change between the double-mutant and BRAF^{V600E}-only in the SI (a) and COL (b) organoids.

Fig. S6. Analysis of the expression of EMT marker genes in Brat^{V600E+/+} (VE) single and Brat^{V600E+/+},Trp53^{R172H/++} (VE,p53) double mutant COL organoids. Heatmap of the log2 fold-changes (4-HT induced vs. non-induced, color coded) of the genes listed in the EMT hallmark gene set.

Fig. S7. Brat^{V600E+/+},Trp53^{R172H/++}-mutant COL organoids show enhanced invasive morphology.
Bright-field images at a higher magnification of the invaded organoids shown in Fig. 4 G/H. Double-mutant COL organoids present with faster and increased attachment to the plastic surface and with pseudopodia formation (marked with white arrows). Scale bars: 50 µm.

Fig. S8. Analysis of 4-HT induced APC deficiency in COL organoids.
(a) Oncogene expression was induced and COL organoids were simultaneously treated with 7 µM of GSK inhibitor (GSK3i). Bright-field images are shown 4 days after oncogene induction. Scale bars: 50 µm.
(b) Confirmation of successful recombination of the Apc^{flox} allele within 4-HT-treated COL organoids by genomic PCR. DNA of donor mouse serves as control and EtOH serves as vehicle control for 4-HT.
(c) WB of COL organoids demonstrating ERK pathway activation and OLFM4 preservation within APC deficient organoids
(d) Schematic model of the Rosa26::mTOM/mGFP reporter allele, which produces a membrane-tethered tdTomato (mTOM) protein and a membrane-bound green fluorescent protein (mGFP) prior to and following Cre mediated recombination, respectively.
(e) Bright-field and fluorescent microscopy images of COL organoids treated with 0.1 µg/ml CTX for 24 hours. Brat^{V600E+/+},Apc^{ΔΔ} mutant organoids do not show CTX-
induced luminal expansion, indicating impaired tight junction formation (see Fig. S2e for more details). Scale bars: 50 µm.

**Fig. S9. WB analysis of trametinib-treated COL organoids.**
(a) COL organoids with the indicated genotypes (numbers 1-3 refer to different triple-mutant donor mice) were induced with 3 µM 4-HT. They were treated with 25 nM trametinib for 24 h before they were lysed and analyzed by WB with the indicated antibodies. Detection of GAPDH or Vinculin serves as loading control.
(b) Densitometric quantification of the WB analyses shown in (a) and two additional biological replicates. Protein expression was normalized to GAPDH/Vinculin and DMSO-treated double-mutant organoids. Data are presented as mean ± SD. **P ≤ 0.01 (one-way and two-way ANOVA, respectively, corrected for multiple comparison by Bonferroni) (n=3 independent experiments).

**Fig. S10. BRAF\(^{V600E}\) upregulates YAP/TAZ targets in murine organoids.**
Analysis of Hippo pathway activity by YAP/TAZ transcriptional targets identified by Wang and colleagues in SI and COL organoids. Color code represents the log2 fold-change (induced vs. non-induced). \(Braf^{V600E/+}\) (VE); \(Braf^{V600E/+}, Trp53^{R172H/+}\) (VE,p53).

**Videos S1,2.**
Time lapse videos (every 4h) of SI (Video S1) and COL (Video S2) organoids at days 1-6 after oncogene induction. Note the fast proliferation of mutant organoids, especially of COL organoids in video supplement 2. Scale bars: 250 µm.

**Video S3.**
Time lapse videos (every 1 h) of COL organoids with the indicated genotypes at days 2-5 after oncogene induction. Scale bars: 250 µm.
### Table S1. Summary of studies using BRAF<sup>V600E</sup> mutant SI and COL organoids.

| Reference         | Model                                                                 | Time point of 4-HT (or dox) treatment | Culture conditions | Organoid type | Observation on organoids                                                                 |
|-------------------|-----------------------------------------------------------------------|---------------------------------------|-------------------|---------------|------------------------------------------------------------------------------------------|
| Riemer et al., 2015 | dox inducible Braf<sup>V600E</sup> transgene knocked into Rosa26 locus | in vivo (dox) via drinking water and in vitro | (Sato et al., 2011) | SI            | no organoid survival after isolation of dox treated mice; cell death of dox treated organoids; loss of stem cell niche; rescue by BRAF and MEK inhibition or Wnt pathway activation |
| Fessler et al., 2016 | introduction of BRAF<sup>V600E</sup> in human COL organoids by homologous recombination | Not applicable | (Prasetyanti et al., 2013; Sato et al., 2011) | COL           | no disintegration observed                                                                      |
| Tong et al., 2017  | Villin-CreER<sup>VT</sup> + Braf<sup>V600E</sup> mice (Dankort et al., 2007) | in vivo via intraperitoneal injection | 2.5% R-Spondin conditioned-media | SI            | no organoid survival after isolation; loss of Paneth cells; reduction of Cdx2 gene, loss of SMAD4 or elevated WNT signaling rescue organoids |
| Sakamoto et al., 2017 | Cdx2P-CreER<sup>VT</sup> + Braf<sup>V600E</sup> mice (Dankort et al., 2007) | in vivo via intraperitoneal injection | (Yui et al., 2012); 10% R-spondin2 (Bell et al., 2008), 10% Wnt3A (Barker et al., 2010) conditioned-media; 50 ng/ml mouse HGF; 10 µM Y-27632 | COL           | no disintegration observed                                                                      |
| Bond et al., 2018  | Villin-CreER<sup>VT</sup> + Braf<sup>V600E</sup> mice (Dankort et al., 2007) | 1 µM in vitro | (Sato et al., 2011); Matrigel “sandwich” | COL           | no disintegration observed                                                                      |
| Lannagan et al., 2019 | Villin-CreER<sup>VT</sup> + Braf<sup>V600E</sup> mice (Dankort et al., 2007) | 1 µM in vitro | Matrigel “sandwich” (Onuma et al., 2013); 20% R-spondin2, 50% Wnt3A conditioned-media | COL           | no disintegration observed                                                                      |
| Tao et al., 2019   | Lentiviral Cre delivery in organoids derived from Braf<sup>V600E</sup> mice (Dankort et al., 2007) | Not applicable | (Weeber et al., 2015); 10% Noggin, 20% R-Spondin1, 50% Wnt3A conditioned-media | COL           | cell death upon BRAF<sup>V600E</sup> induction in organoids at low passage number. Long-term cultured organoids are less sensitive to oncogene induction |
| Brandt et al., 2019 | dox inducible Braf<sup>V600E</sup> transgene knocked into Rosa26 locus | in vitro (dox) | R-Spondin conditioned-media | SI            | cell death upon BRAF<sup>V600E</sup> induction; epithelial disorganization; rescue of organoid death by MEK or ERK inhibitors |
4. Table S2. Summary of selected BRAF$^{V600E}$ regulated genes that have been validated by WB or IHC and their relevance in human CRC and other cancers.

| Gene | Response to BRAF$^{V600E}$ induction in murine organoids | Alteration in human colorectal and other cancers | Prognostic relevance |
|------|--------------------------------------------------------|-------------------------------------------------|----------------------|
| AMACR | downregulated | Strongly expressed in differentiated CRC, but weakly expressed in anaplastic CRC as assessed by IHC [30-32]. Low expression correlates with mucinous histology [33] and right-sided tumors [34]. | Potential marker for tumor differentiation/neoplastic change and significant association with prognostic factors such as tumor grade, tumor stage and tumor location [30-32, 34]. |
| CAV1 | upregulated | Increased expression in high grade tumors with greater depth of invasiveness [35]. | Potential tumor suppressor gene [36] and potential marker for selecting low- and high-stage CRC patients [35]. |
| CTSE | upregulated | Upregulated, in particular in SSAs as shown by IHC and quantitative RT-PCR [37, 38]. | Potential marker for SSA [37]. |
| EPHA2 | upregulated | High mRNA and protein expression are associated with poor overall survival in stage II/III CRC (shown using a CRC microarray dataset and IHC) [39]. High expression correlates with metastatic spread [40]. | Poor prognostic marker in stage II/III CRC and EPHA2-targeted agents as potential treatment strategy [39]. |
| ITGB1 | upregulated | High expression in advanced tumors and liver metastases as shown by IHC. High expression correlates with shortened OS and DFS [41]. | Potential biomarker for disease progression [41]. |
| MUC2 | upregulated | Increased expression of MUC2 in mucinous CRC. Significantly higher incidence of BRAF mutations in mucinous CRC [42, 43]. | Mucinous CRCs often present at an advanced stage and correlate with poor survival [43, 44]. |
| MYOF | upregulated | Overexpressed in human CRC as shown by IHC staining and TCGA gene expression data sets [45]. | High expression is associated with lower survival in CRC patients [45]. |
| NR2E3 | downregulated | **No data for CRC so far.** Low expression in ERα-positive breast cancer patients associated with worse recurrence-free survival [46]. Low expression in liver tumors as assessed by IHC [47]. | Potential tumor suppressor in breast and liver cancer [46, 47]. |
| OLFM4 | downregulated | IHC and proteomic approaches show upregulation of OLFM4 in adenoma and early CRC stages compared to normal epithelium. There is no difference between OLFM4 expression in stage III/IV CRC vs. normal tissue [48, 49]. | Reduced OLFM4 (also known as hGC-1) expression as potential marker for poor differentiation and malignant progression of CRC [49]. |
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**Reischmann_Supplementary Figure 3**

**A**

![Plot showing PCA analysis](image)

**B**

|          | Control | Trp53R172H/+ | BrafV600E/+ | BrafV600E/+;Trp53R172H/+ |
|----------|---------|--------------|-------------|--------------------------|
| Si crypts| Day 3   | Day 4        | Day 3       | Day 4                    |
| COL crypts| Day 8   | Day 4        | Day 4       | Day 4                    |

**C**

|          | Control | Trp53R172H/+ | BrafV600E/+ | BrafV600E/+;Trp53R172H/+ |
|----------|---------|--------------|-------------|--------------------------|
| Si crypts| Day 3   | Day 3        | Day 3       | Day 3                    |
| COL crypts| Day 8   | Day 4        | Day 4       | Day 4                    |

**D**

|          | COL crypts |
|----------|------------|
| pERK     |            |
| NR2E3    |            |
| HSP90    |            |

**E**

- **pERK**
- **NR2E3**
- **HSP90**
- **EPHA2**
- **CTSE**
- **DAPI**
Reischmann_Supplementary Figure 4

NES = 2.099
padj = 0.0003

NES = -1.509
padj = 0.0086

NES = 1.921
padj = 0.0004

Braf
V600E/+

Trp53
R172H/+

Braf
V600E/+

Trp53
R172H/+

NES = -1.586
padj = 0.0007

NES = -1.222
padj = 0.0651

NES = -1.475
padj = 0.0086

UP in SSA

DOWN in SSA
Reischmann_Supplementary Figure 7

Day 7 - Invasion assay

| Braf^V600E/+ | Braf^V600E/+; Trp53^R172H/+ |
|-------------|-----------------------------|
| ![Image 1]  | ![Image 2]                   |
| ![Image 3]  | ![Image 4]                   |
| ![Image 5]  | ![Image 6]                   |
Reischmann_Supplementary Figure 8

A

| Braf^V600E/+ | Braf^V600E/+ Trp53^R172H/+ |
|--------------|---------------------------|
| - GSK3i      | - GSK3i                   |
| + GSK3i      | + GSK3i                   |

B

- Gapdh
- Olfm4
- Braf

D

| Control | Braf^V600E/+ | Braf^V600E/+ Trp53^R172H/+ |
|---------|--------------|---------------------------|
| BRAF    | 1            | 1                         |
| pERK    | 3.5          | 9.6                       |
| OLFM4   | 0.1          | 0.2                       |
| GAPDH   | 0.8          | 0.8                       |

C

- mTOM/mGFP::VillinCreER^T2

E

- Control
- Braf^V600E/+ Apc^ΔΔ

Day 4 (24h CTX)
Reischmann_Supplementary Figure 9

A

BRAF<sup>V600E</sup>

pMEK

pERK

p-c-FOS

DUSP6

pFRA1

GAPDH

c-FOS

VINCLIN

B

pERK/GAPDH

p-c-FOS/GAPDH

DUSP6/GAPDH

c-FOS/Vinculin

DMSO 25 nM trametinib

1 1.5 1.6 1.1 1.3 1.4 1.3 0.7

1 2.2 2.7 1.6 0.3 0.4 0.2 0.2

1 2.7 1.9 4.6

1 0.6 0.5 1.4

1 1.4 1.4 2.3 0.5 0.5 0.5 0.3

1 1.5 1.6 1.1 1.3 1.4 1.3 0.7

1 2.2 2.7 1.6 0.3 0.4 0.2 0.2

1 2.7 1.9 4.6

1 0.6 0.5 1.4

1 1.4 1.4 2.3 0.5 0.5 0.5 0.3

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