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Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition

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Mutations of the tricarboxylic acid cycle (TCA cycle) enzyme fumarate hydratase (FH) cause Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC)[1]. FH-deficient renal cancers are highly aggressive and metastasise even when small, leading to an abysmal clinical outcome[2]. Fumarate, a small molecule metabolite that accumulates in FH-deficient cells, plays a key role in cell transformation, making it a bona fide *oncometabolite*[3]. Fumarate was shown to inhibit α-ketoglutarate (aKG)-dependent dioxygenases involved in DNA and histone demethylation[4,5]. However, the link between fumarate accumulation, epigenetic changes, and tumorigenesis is unclear. Here we show that loss of FH and the subsequent accumulation of fumarate elicits an epithelial-to-mesenchymal-transition (EMT), a phenotypic switch associated with cancer initiation, invasion, and metastasis[6]. We demonstrate that fumarate inhibits Tet-mediated demethylation of a regulatory region of the antimetastatic miRNA cluster[6] *miR-200ba429*, leading to the expression of EMT-related transcription factors and enhanced migratory properties. These epigenetic and phenotypic changes are recapitulated by the incubation of FH-proficient cells with cell-permeable fumarate. Loss of FH is associated with suppression of *miR-200* and EMT signature in renal cancer patients, and is associated with poor clinical outcome. These results imply that loss of FH and fumarate accumulation contribute to the aggressive features of FH-deficient tumours.

To identify oncogenic features associated with FH loss we performed unbiased proteomics analyses of mouse ($Fh1^{-/-}$) and human (UOK262) FH-deficient cells[7] (Extended Data Fig. 1). We found that vimentin, a known EMT marker, is the most overexpressed protein in these cells, compared to FH-proficient counterparts (Fig. 1a). Gene expression profiling (Fig. 1b) followed by Gene Set Enrichment Analysis (GSEA)[8] confirmed an enrichment of EMT-related genes in FH-deficient cells (Extended Data Fig. 2 and Extended Data Fig. 3a, respectively). The reintroduction of full-length *Fh1* (*pFh1*) in $Fh1^{-/-}$ cells (Extended Data Fig.

50 1a-e) was sufficient to rescue the EMT signature (Extended Data Fig. 2a and Extended Data
51 Fig. 2c), to abolish vimentin expression (Fig. 1c-e), and to restore expression of E-Cadherin
52 (Fig. 1c-d), a key epithelial marker. *Fh1*$^{-/-}$*+pFh1* cells acquired an epithelial morphology
53 (Extended Data Fig. 1e) and their motility was reduced compared to that of Fh1-deficient
54 cells (Fig. 1f-g). UOK262 cells exhibited a strong Vimentin expression (Extended Data Fig.
55 3b-d), and increased migration (Extended data Fig. 3e) compared to UOK262pFH. However,
56 localisation of E-Cadherin at the plasma membrane was not observed in UOK262pFH
57 (Extended Data Fig. 3d).

58 EMT is orchestrated by several transcription factors, including *Twist, Snai1, Snai2,*
59 *and Zeb1/2* (ref 9). *Twist*, which is activated by the Hypoxia-Inducible Factor HIF1 (ref 10), a
60 key player in FH-deficient tumours[11], was elevated in Fh1-deficient cells (Fig. 1h). The
61 silencing of HIF1β, the constitutively expressed subunit of HIFs required for their
62 transcriptional activity[12], failed to reduce the expression of EMT markers (Extended Data
63 Fig. 4a-b), suggesting that EMT in Fh1-deficient cells is likely HIF-independent. *Snai2, Zeb1*
64 and *Zeb2* were also induced in Fh1-deficient cells, and their expression was reverted by Fh1
65 re-expression in these cells (Fig. 1h-i). *Zeb2* expression was also decreased upon FH
66 restoration in UOK262 cells (Extended Data Fig. 3f). *Snai2* and *Zeb1*/2 are suppressed by
67 antimetastatic miRNAs *miR-200ba429* and the *miR-200c141* (ref 6). miRNA profiling
68 revealed that *miR-200* family members were among the most down-regulated miRNAs in
69 Fh1-deficient cells (Fig. 2a). Suppression of *MIR-200* was also observed in UOK262 cells
70 compared to the non-transformed counterpart HK2 and partially restored by FH re-expression
71 (Extended Data Fig. 3g-h). qPCR confirmed the miRNA profiling results and showed that the
72 reconstitution of Fh1 in Fh1-deficient cells restored the expression levels of *miR-200a* and
73 *miR-200b* and, in part, that of *miR-200c* and *miR-141* (Fig. 2b). We hypothesised that the
74 partial restoration of *miR-200c141* could be ascribed to the residual fumarate in *Fh1*$^{-/-}$*+pFh1*

75 cells (Extended Data Fig. 1c and Extended Data Fig. 5b), which could also explain the partial
76 recovery of the EMT gene signature (Extended Data Fig. 2a-c). Blunting fumarate levels by
77 re-expressing high levels of Fh1 in *Fh1*$^{-/-}$ cells rescued their phenotype (Extended Data Fig.
78 5b-g) and led to a full reactivation of the entire *miR-200* family (Extended Data Fig. 5h),
79 indicating that members of this family have a different susceptibility to fumarate. The
80 incomplete rescue of fumarate levels in UOK262pFH (ref 7) could also explain the partial
81 restoration of *MIRNAs* and some EMT markers in these cells.

82 Since *miR-200ba429* expression was fully restored in *Fh1*$^{-/-}$*+pFh1* and its expression
83 was sufficient to suppress *vimentin* and rescue *E-cadherin* expression in Fh1-deficient cells
84 (Fig. 2c), we investigated the role of this miRNA cluster in Fh1-dependent EMT. Repression
85 of *miR-200* is associated with its epigenetic silencing *via* CpG island hypermethylation[13],
86 which can also be caused by downregulation of Tets[14,15]. We hypothesised that fumarate
87 could cause suppression of *miR-200ba429* by inhibiting their Tets-mediated demethylation.
88 The combined silencing of *Tet2* and *Tet3*, the most abundant Tets isoform in *Fh1*$^{fl/fl}$ cells
89 (Extended Data Fig. 6a), but not the inhibition of aKG-dependent histone demethylases with
90 GSK-J4 (ref 16), decreased miRNAs and *E-Cadherin* expression (Extended Data Fig. 6b-e),
91 highlighting the role of Tets in regulating EMT, in line with previous findings[14,15]. Genome
92 Browser[17] view of an ENCODE dataset generated in mouse kidney cells revealed a conserved
93 CpG island at the 5' end of *miR-200ba429, CpG43,* that is enriched in binding sites for Tets
94 and for lysine-methylated histone H3 (Extended Data Fig. 7a). Chromatin
95 immunoprecipitation (ChIP) experiments showed that a region adjacent to *CpG43* is enriched
96 for the repressive marks H3K9me2 and H3K27me3 and depleted of the permissive marks
97 H3K4me3 and H3K27Ac in Fh1-deficient cells (Extended Data Fig. 7b) in the absence of
98 changes in H3K4 and H3K27 methylation among the four cell lines (Extended data Fig. 7c).
99 Chromosome Conformation Capture (3C) analysis[18] identified a physical association between

100 this regulatory region and the transcription starting site of *miR-200ba429,* which sits in the
101 intronic region of the gene *Ttl10* (Extended Data Fig. 7d). This region was hypermethylated
102 in Fh1-deficient cells and the re-expression of Fh1 restored its methylation levels (Fig. 2d and
103 Extended Data Fig. 7e). Binding of Tets to the *CpG43* was comparable among the cell line
104 tested (Extended Data Fig. 7f), suggesting that the changes in methylation of this region are,
105 at least in part, caused by inhibition of Tets enzymatic activity rather than by their differential
106 binding to chromatin. Consistently, 5-hydroxymethylcytosine (5hmc), the product of
107 oxidation of 5-methylcytosine by Tets[15], was significantly decreased in Fh1-deficient cells
108 (Extended Data Fig. 7g).

109 Incubating cells with dimethyl aKG (DM-aKG), a cell-permeable derivative of aKG,
110 known to reactivate aKG-dependent dioxygenases[19], restored the expression *miR-200a* in
111 Fh1-deficient cells (Extended Data Fig. 6f). Conversely, treating *Fh1*$^{fl/fl}$ and human FH-
112 proficient epithelial kidney cells HK2 with monomethyl fumarate (MMF), a cell permeable
113 derivative of fumarate triggered profound phenotypical (Extended Data Fig. 8a) and
114 (epi)genetic (Fig. 3a-g) changes that resembled those of FH-deficient cells. However, we
115 could not observe induction of *Snai2* that we observed in *Fh1*$^{-/-}$ cells (Fig. 1h) and changes in
116 *Vimentin* in HK2 cells*,* which is expressed in these cells[22], despite their epithelial origin.
117 MMF did not cause mitochondrial dysfunction but lead to a typical fumarate-dependent
118 metabolic signature, characterised in both cell types by accumulation of fumarate and
119 fumarate-derived succinic-GSH (succGSH) and succinic-cysteine (2SC) that we and others
120 recently described[20,21] (Extended Data Fig. 8b-c and SI Table 3). To rule out the possibility
121 that by-products of fumarate accumulation, rather than fumarate itself, elicit EMT we
122 analysed the effects of accumulation of succinate, another metabolite that can inhibit Tets[3-5],
123 but cannot promote succination. Since we could not increase succinate levels with the cell
124 permeable dimethyl succinate (Extended Data Fig. 9a) we used succinate dehydrogenase b

125 (Sdhb)-deficient cell lines[23], which accumulate succinate but not fumarate by-products,
126 including succGSH (Extended Data Fig. 9b-c). These cells exhibited striking mesenchymal
127 features (Extended Data Fig. 9d-e), and epigenetic suppression of the *miR-200ba429* family
128 (Extended Data Fig. 9f-g), in line with the hypermethylation phenotype and EMT signature
129 recently observed in SDH-deficient cells[24].

130 We next investigated the link between FH loss, fumarate accumulation and EMT in
131 renal cancer samples. *Vimentin* was highly expressed and *E-Cadherin* was decreased in a
132 previously published dataset[25] of HLRCC tumour samples, when compared to normal tissue
133 (Extended Data Fig. 10a). Two HLRCC tumours that we profiled (Fig. 4a), exhibited
134 decreased 5hmC levels (Fig. 4b) despite comparable TETs levels (Extended Data Fig. 10b),
135 *MIR-200* suppression (Fig. 4c), a marked Vimentin staining and loss of E-Cadherin
136 (Extended Data Fig 10b), compared to matched normal tissue. We also took advantage of
137 data from a collection of papillary renal-cell carcinoma (KIRP), a tumour type associated
138 with loss of FH[26]. These tumours exhibited a partial EMT signature (Extended Data Fig. 10c)
139 and downregulation of *MIR-200* (Extended Data Fig. 10d). FH levels were positively
140 correlated with patients' survival (Extended Data Fig. 10e) in line with the poor prognosis
141 associated with EMT[6]. The five FH-mutant tumours in this cohort exhibited overexpression
142 of *Vimentin* and suppression of *E-Cadherin* (Extended Data Fig. 10f), hypermethylation and
143 suppression of *MIR-200A* and *MIR-200B* (Fig. 4d-e) in the absence of TETs mutations
144 (Extended Data Fig. 10g). These tumours were associated with the worst prognosis among
145 papillary cancers (Extended Data Fig. 10h). FH mRNA was also significantly decreased in a
146 panel of clear cell renal carcinoma (KIRC)[27] (Extended Data Fig. 10i) and its levels
147 negatively correlated with *Vimentin* (Pearson correlation coefficient of -0.5, p-value < 1e-5;
148 Fig. 4f) and positively with *E-Cadherin* (Pearson correlation coefficient of 0.22, p-value <

149 1e-5; Fig. 4g), and were positively correlated with patients' survival (Extended Data Fig.
150 10k), confirming the role of FH in tumour malignancy and patient outcome.

151 Our results report a novel link between the loss of FH and epigenetic suppression of
152 *miR-200* mediated by fumarate (see Extended Data Fig. 1f for a schematic). Although other
153 mechanisms could contribute to fumarate-driven EMT, our findings offer an explanation for
154 the suppression of *MIR-200* in papillary and clear-cell renal carcinoma and the expression of
155 EMT-related transcription factors, including *ZEB2,* in KIRC[28]. Our data imply that
156 dysregulation of FH activity and fumarate accumulation have roles in EMT induction and
157 may feature in other tumour types where FH loss has been reported, including
158 neuroblastoma[29], colorectal and lung cancer[30].

159 **Online Content.** Methods, along with additional Extended Data display items and Source Data, are available in
160 the online version of the paper; references unique to these sections appear only in the online paper.

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273 **Author Contributions** M.S. and C.F. conceived the study. M.S. performed and analysed all the experiments on
274 cell lines with the help of A.V.D.; S.A.; and S.J.T.; and prepared the figures. E.Go. performed the
275 bioinformatics analyses with the supervision on J.S-R.. I.T.J. helped M.S. with the invasion assays and
276 generation of constructs for miRNA and Fh1-GFP expression. V.Z. performed and analysed ChIP-PCR assays.

277 A.S.C. performed and analysed all the metabolomics analyses with the help of E.G.. M.T. performed the work
278 on human samples with input from P.H.M. A.W.; V.G.; P.H.M.; and E.M. provided the HLRCC samples. V.R
279 and P.C. performed the proteomics analyses. H.Y. and B.H. supervised and performed the 3C experiments. S.C.
280 and E.G provided Sdhb-deficient cells and generated the gene expression profile of these cells. S.F. and K.F.
281 performed cell motility assays. C.F. directed the research, prepared the figures and wrote the paper, with
282 assistance from all other authors.

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284 authors declare no competing financial interests. Correspondence and requests for materials should be addressed
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287 cells are deposited at Array Express (www.ebi.ac.uk/arrayexpress, accession number A-AFFY-130).

288 Figure Legends

289 **Figure 1. FH-deficient cells display mesenchymal features. a**, **b**, Volcano plots of
290 proteomics (**a**) and RNA-seq (**b**) experiments. FDR = false discovery rate. **c**, **d**, mRNA
291 expression measured by qPCR (**c**) and protein levels measured by western blot (**d**) of EMT
292 markers. **e**, Immunofluorescence staining for vimentin and E-cadherin. Scale Bar = 25 µm. **f**,
293 Cells migration assay. Data indicate cell index at 17 hours. Results were obtained from 4
294 (*Fh1* $^{-/-}$+*pFh1*) or 3 replicate wells and presented as mean ± S.D. p-value was calculated
295 using One way-ANOVA. **g**, Average speed of cells. p-value was calculated using Mann-
296 Whitney test. Results were obtained from 3 independent cultures. **h**, mRNA expression of
297 EMT-related transcription factors measured by qPCR. **i**, Western blot analysis of Zeb1.
298 Calnexin was used as loading control. All qPCR results were obtained from 3 independent
299 cultures and presented as RQ with max values, normalised for *β-actin*. p-values was
300 calculated using unpaired t-test. *P ≤0.05, ***P* ≤0.01, ****P* ≤0.001, *****P*≤0.0001. For
301 western blot source data, see Supplementary Figure 1. For Raw data see SI Table 2.

302 **Figure 2. Loss of Fh1 triggers epigenetic suppression of *miR-200*. a**, Volcano plot of
303 miRNA profiling. **b**, miRNAs expression measured by qPCR. Date were normalised to
304 *Snord95*. **c**, miRNAs and EMT markers expression in *Fh1*$^{-/-}$ cells expressing *miR-200ba429*.

305 *β-actin* and *Snord95* were used as endogenous control for mRNA and miRNA, respectively.
306 NTC= non-targeting control. **d**, Methylation-specific PCR of *CpG43*. U = un-methylated; M
307 = methylated CpG island. The *miR-200ba429* cluster (blue) and *CpG43* (green) are
308 represented in the schematic. qPCR results were obtained from at least 3 independent cultures
309 and presented as RQ with max values. p-values was calculated using unpaired t-test. *P
310 ≤0.05, ***P* ≤0.01, ****P* ≤0.001, *****P*≤0.0001. For gel source data, see Supplementary
311 Figure 1. For Raw data see SI Table 2.

312 **Figure 3. Fumarate triggers EMT in FH-proficient cells.** miRNA methylation (**a**) and
313 expression (**b**, **e**); EMT transcription factors (**c**, **f**) and EMT markers (**d**, **g**) levels from MMF-
314 treated cells. Results were obtained from 3 independent cultures. qPCRs are presented as RQ
315 with max values, normalised for *Snord95* (mouse) or *SNORD95* (human) for miRNAs, and
316 for *β-actin* for mRNA. p-values were calculated using unpaired t-test. *P ≤0.05, ***P* ≤0.01,
317 ****P* ≤0.001, *****P*≤0.0001. For gel source data, see Supplementary Figure 1. For Raw data
318 see SI Table 2.

319 **Figure 4. Loss of FH correlates with EMT signature in renal cancers. a-c,** Metabolomic
320 analysis (**a**), 5hmc levels in DNA (**b**), and MIRNAs expression (**c**) in tumour samples from
321 two HLRCC patients. Results were obtained from 4 technical replicates per sample. qPCRs
322 are presented as RQ with max values, normalised for *RNU6B* and *SNORD61*. **d**, **e**,
323 Expression levels (**d**), and promoter methylation (**e**) of the indicated *MIRNAs* in KIRP
324 patients **f**, **g**, *Vimentin* (**f**) and *E-Cadherin* (**g**) expression in clear cell renal cell carcinoma
325 (KIRC) patients. For Raw data see SI Table 2.

326 # METHODS

327 **No statistical methods were used to predetermine sample size.**

328 **Cell culture**

329 *Fh1*-proficient (*Fh1$^{fl/fl}$*), and the two Fh1-deficient clones (*Fh1$^{-/-CL1}$*, and *Fh1$^{-/-CL19}$*) cells were
330 obtained as previously described[7]. *Fh1$^{-/-}$+pFh1* were single clones generated from *Fh1$^{-/-CL19}$*
331 after stable expression of a plasmid carrying mouse wild-type *Fh1* gene (Origene,
332 MC200586). Mouse cells were cultured using DMEM (Gibco-41966-029) supplemented with
333 10% heat inactivated serum (Gibco-10270-106) and 50 μg x mL^{-1} uridine. Genotyping of
334 cells was assessed as previously described[7]. Human FH-deficient (UOK262) and FH-restored
335 (UOK262pFH) were obtained as previously described[7] and cultured in DMEM (Gibco-
336 41966-029) supplemented with 10% serum heat inactivated (Gibco-10270-106). HK2 cells
337 were a gift from the laboratory of E.R.M. These cells were authenticated by Short Tandem
338 Repeat and cultured in DMEM (Gibco-41966-029) supplemented with heat inactivated 10%
339 serum. All cell lines have been tested for mycoplasma contamination using MycoProbe®
340 Mycoplasma Detection Kit (R&D Systems CUL001B), and were confirmed mycoplasma-
341 free.

342 **Generation of *Fh1$^{-/-}$+pFh1-GFP* cells**

343 *Fh1*-GFP vector was generated by amplifying wild-type *Fh1* sequence using cDNA
344 generated from *Fh1$^{fl/fl}$* cells by PCR. Restriction overhangs (KpnI, EcoRI) were included in
345 the primer sequence allowing for restriction enzyme cloning of *Fh1* into the backbone vector
346 pEF1α-V5/His (Life Technology). We then used a two-step PCR “restriction-free” method to
347 swap the V5-His sequence within pEF1α with the AcGFP sequence to yield a fusion protein,
348 Fh1-GFP. $1x10^5$ *Fh1$^{-/-\ CL1}$* cells were plated onto 6-well plate and the day after transfected
349 with *Fh1-GFP* vector using Lipofectamine 2000 following manufacturer’s instructions. After
350 2 weeks, cells were sorted for GFP expression and the medium-expressing population was

351 maintained in culture and amplified. pEF1α-GFP empty vector was used as control. Primers
352 for cloning are listed in SI Table 1.

353 **Short hairpin RNA (shRNA) interference experiments**

354 Lentiviral particles for shRNA delivery was obtained as previously described[7] from the
355 filtered growth media of $2x10^6$ HEK293T transfected with 3 µg psPAX, 1 µg pVSVG and 4
356 µg of the plasmid of interest using Lipofectamine 2000/3000 (Life Technology). $1x10^5$ cells
357 of the indicated genotype were then plated onto 6-well plates and infected with the viral
358 supernatant in the presence of 4 µg x mL^{-1} polybrene. After two days, the medium was
359 replaced with selection medium containing 1 µg x mL^{-1} puromycin. pGIPZ vectors for
360 shRNA against mouse *HIF1β* (RMM4532-EG11863), *Tet2* (RMM4532-EG214133), and
361 *Tet3* (RMM4532-EG194388) were purchased from GE Healthcare UK. pLenti 4.1 Ex for
362 expression of microRNAs was purchased from Addgene (Plasmid #35533 and #35534).
363 pLenti 4.1 Ex scrambled vector was generated cloning a scrambled DNA sequence taken
364 from a commercially available vector (pCAG-RFP-miR-Scrint Addgene no. 198252) into the
365 empty backbone.

366 **RNA extraction and real time PCR**

367 Cells were plated the day before the experiments onto 6-well plates ($3x10^5$) or 12-well plates
368 ($1x10^5$). Total RNA was isolated using RNeasy Kit (Qiagen). miRCURY™ RNA Isolation
369 Kit (Exiqon, Denmark) was used for microRNAs extraction. RNA isolation was carried
370 following manufacturer's protocols. RNA was quantified using the fluorimeter Qubit 2.0
371 (Life Technologies) following manufacturer's instructions or Nanodrop (Thermo). Reverse
372 transcription of RNA was performed using Quantitect-Reverse transcription kit (Qiagen) or
373 miScript PCR kit (Qiagen) using 300-500 ng of total RNA. Real time qPCR was performed
374 using Quantitect Syber Green master mix (Qiagen) or Taqman universal mix (Life
375 Technology) on a Step One Plus real-time PCR system (Life Technology). Experiments were

376 analysed using the software Expression Suite (Life Technology) and StepOne software 2.3
377 and Relative quantification (RQ) with max and min values (RQ max and RQ min) were
378 calculated using S.D. algorithm. Statistical analysis was performed using Expression Suite
379 software on at least three independent cultures. Housekeeping genes used for internal
380 normalisation are *β-Actin* for mRNA and *Snord95 Snord61 and RNU6B,* for miRNAs. The
381 primers were designed using ProbeFinder- Roche or purchased by Qiagen and are listed in SI
382 Table 1.

383 **miRNA methylation analyses**

384 5x10^5 cells were plated onto 6-cm dishes. Their genomic DNA was extracted using DNeasy
385 kit (Qiagen), and purified using DNA Cleaning and Concentrator kit (Zymo Research)
386 following manufacturer's instructions. 20 ng/well of genomic DNA, quantified using Qubit,
387 were digested using OneStep qMethyl kit (Zymo Research) following manufacturer's
388 protocol. Primers used are listed in the SI Table 1.

389 For methyl specific PCR (MSP) assay 500 ng of purified DNA were bisulphate converted
390 using the EZ-DNA Methylation-direct kit (Zymo Research) following manufacturer's
391 datasheet. 50 ng of bisulphate-converted DNA, quantified using Nanodrop
392 spectrofluorimeter, were used for PCR reaction with AmpliTaq Gold (Life Technology)
393 following manufacturer's protocol. The number of amplification cycles used was thirty.
394 Methylation specific primers were designed using MethPrimer[31]
395 (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi) and are listed in the SI Table 1.

396 **Migration assay**

397 Migration experiments were performed using xCELLigence instrument (ACEA Biosciences).
398 In brief, 5x10^4 cells were plated onto CIM plates in medium supplemented with 1% FBS.
399 Complete medium with 20% FBS was used as chemo attractant. Migration was registered in

400 real time for at least 24 hours and cell index was calculated using the appropriate function of
401 the xCELLigence software.

402 **Motility assay**

403 $5x10^4$ mouse cells of the indicated genotype were plated the day before the experiment onto
404 6-cm dishes. The day after**,** medium was replaced with fresh medium containing Hoechst
405 (Sigma-Aldrich) and cells were incubated for 15 minutes at 37°C with 5% CO_2 before
406 starting recording. Images were collected every minute for 3 hours using a Zeiss Axiovert
407 200M microscope with a 10x objective. Analysis of cells movement was performed using cell
408 tracker (www.celltracker.website) implemented in MATLAB (MATLAB R2013b, The
409 MathWorks Inc., 2013) as previously described[32]. Three replicates were analysed for each
410 cell type. All tracks were examined and those belonging to non-isolated cells deleted.
411 Average speed for each cell was calculated as the sum length of the cell's trajectory divided
412 by the total time over which the trajectory was measured. Since the data were not normally
413 distributed (Shapiro-Wilk test), a Mann-Whitney test was used to compare the average speeds
414 of the cells.

415 **Oxygen consumption rate and Extracellular acidification rate measurements**

416 Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) were
417 measured using the real time flux analyser XF-24e (Seahorse Bioscience) as previously
418 described[7]. In brief, $4x10^4$ cells were left untreated and then treated with 1 µM Oligomycin, 2
419 µM Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), Rotenone and Antimycin
420 A (both 1 µM) (all purchased from Sigma-Aldrich). At the end of the run cells were lysed
421 using RIPA buffer (25 mM Tris/HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium
422 deoxycholate, 0.1% SDS). Protein content for each well was measured using BCA kit

(Pierce) following manufacturer's instruction. OCR and ECAR are normalised to total protein content were indicated.

Immunofluorescence experiments

$5x10^4$ cells were plated onto chamber slides (Lab Tech), cultured in standard condition overnight and then fixed using 100% methanol for 2 minutes at –20°C. After two washes in PBS, cells were permeabilised and incubated with blocking solution (BSA 2%, 0.1% Triton X-100, 0.1% Tween 20 in PBS) for 30 minutes at room temperature. Cells were then incubated with the primary antibody (overnight at 4°C). For 5hmc staining, cells were grown on coverslips onto a 12-well plate. Cells were then fixed with 4% PFA in PBS for 15 minutes at room temperature, washed three times in PBS and then incubated for 15 minutes with 0.4% Triton X-100 in PBS. After three washes in PBS, cells were denaturated using a solution of 2 M HCl for 15 minutes at room temperature and neutralised using 100 mM Tris pH.8, for 5 minutes. After three washes in PBS, cells were incubated with blocking solution (5% FBS, 0.1% Triton X-100, 0.1% Tween 20 in PBS) for 1 hour and then primary antibody was added at 4°C overnight. After three washes in PBS, cells were incubated with secondary antibody during 2 hours at room temperature and then slides or coverslips were mounted (Vectashield with DAPI) and images taken using Leica confocal microscope TCS SP5 using 20X or 40X objectives. Laser intensity, magnification, and microscope settings per each channel were maintained equal throughout the different experimental conditions. Antibodies used are listed in SI Table 1.

Protein lysates and Western Blot

Cell lysates were prepared in RIPA buffer. Protein content was measured using BCA kit (Pierce) following manufacturer's instructions. 50-100 µg of proteins were heated at 70°C for 10 minutes in presence of Bolt Loading Buffer 1x supplemented with 4% β-mercaptoethanol (Sigma). Samples were then loaded onto Bolt Gel 4-12% Bis-Tris (Life Technology) and run

448 using MOPS 1x or MES 1x buffer at 165 V constant for 40 minutes. Dry transfer of the gels
449 was carried using IBLOT2 system (Life Technology). Membranes were then incubated in
450 blocking buffer (5% BSA or 5% milk in TBS 1x + 0.01 % Tween 20) for one hour at room
451 temperature. Primary antibodies in blocking buffer were incubated overnight at 4°C.
452 Secondary antibodies (conjugated with 680 or 800 nm fluorophores from Li-Cor) were
453 diluted 1:2000 in blocking buffer and incubated for one hour at room temperature. Images
454 were acquired using Odyssey software (Li-Cor). Primary antibodies are listed in SI Table 1.

455 **Chronic treatment of mouse and human cells**

456 *Fh1*$^{fl/fl}$ cells were cultured either with 200 µM monomethyl-fumarate (MMF, Sigma-Aldrich)
457 for 2 weeks and then with 400 µM MMF for the following 6 weeks, or with 4 mM
458 monomethyl-succinate (MMS, Sigma-Aldrich) for 8 weeks. HK2 cells were cultured with
459 MMF 400 µM for 8 weeks. *Fh1*$^{-/-}$ cells were treated with the indicated doses of dimethyl
460 aKG (DM-aKG, Sigma-Aldrich). *Fh1* $^{fl/fl}$ cells were treated with histone demethylase
461 inhibitor GSKJ4 (Tocris) 1 µM for 8 weeks. MMF, MMS and GSKJ4 were added twice a
462 week after passaging the cells.

463 **Chromatin immunoprecipitation (ChIP)-real time PCR (ChIP-PCR)**

464 ChIP was performed as previously described[33]. Enrichment was determined by Real-time
465 PCR and ChIP signal was normalised to input, IgG only ChIP and negative control (genomic
466 region devoid of histone markers). For Tets ChIP-PCR, the signal was normalised over input
467 and IgG ChIP, as Tet-specific genomic negative controls are not as readily identifiable.
468 Antibodies and primers for ChIP-PCR are indicated in SI Table 1.

469 **Chromatin Conformation Capture assay (3C)**

470 3C assay coupled with quantitative PCR (qPCR) was performed as previously described[18]. In
471 brief, 10^7 cells were crosslinked with 1% formaldehyde for 10 minutes at room temperature

472 and were quenched with glycine. Cells were then lysed by dounce homogenization in ice-cold
473 lysis buffer (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 0.2% Igepal CA-630, all from Sigma)
474 supplemented with protease inhibitor (Roche). Cells were then washed in 1.2x NEB buffer 2
475 (New England Biolabs). Non-crosslinked proteins were removed with SDS (Sigma- Aldrich)
476 and were then quenched with Triton X-100. Chromatin was digested overnight with EcoR I
477 restriction enzyme (New England Biolabs). Afterwards EcoR I was inactivated by heating at
478 65˚C for 20 minutes. In-nuclear DNA ligation was performed at 16˚C for 4 hours in the
479 mixture containing 1x T4 DNA ligase buffer (New England Biolabs), 10 mg/ml BSA (New
480 England Biolabs), and 1U/μL T4 DNA ligase (Invitrogen). Ligation mixture was then
481 incubated with Proteinase K (Roche) at 65˚C overnight to reverse the crosslinking and was
482 incubated with RNase A (Roche) at 37˚C for 1 hour. DNA was purified with Phenol (pH 8.0,
483 Sigma) once and then with Phenol:Chloroform:Isoamyl Alcohol (25:24:1, pH 8.0, Sigma),
484 followed by ethanol precipitation by adding 2.5 volume of ice-cold 100% ethanol and 1/10
485 volume of 3 M sodium acetate (pH 5.2, Lonza). DNA pellet was washed with 70% ethanol
486 twice and was eventually dissolved in 100 μL distilled water. The concentration of 3C DNA
487 was determined by Qubit dsDNA HS assays (Invitrogen). 100 ng DNA was taken to run
488 qPCR in duplicate wells for each 3C sample, using Taqman Universal PCR Master Mix
489 (Applied Biosystems) and specific Taqman primers and probes on ABI 7900 (Applied
490 Biosystems) following manufacturer's instruction. Data were analysed as recommended[18] and
491 were normalized to the internal loading control of *Gapdh* locus. Calculation of primers
492 location was based on the transcription start site (TSS) of *Ttll10* transcript
493 (ENSMUST00000097731). Oligo sequences are listed in the SI Table 1.

494 **Metabolomic analyses**

495 $3x10^5$ cells were plated onto a 6-well plate and cultured in standard conditions for 24 hours.
496 Medium was replenished with fresh one and, after 24 hours, intracellular metabolites were

497 extracted as previously described[20]. LCMS analysis was performed on a QExactive Orbitrap
498 mass spectrometer coupled to Dionex UltiMate 3000 Rapid Separation LC system (Thermo).
499 The liquid chromatography system was fitted with either a SeQuant Zic-HILIC column
500 (column A, 150 mm × 4.6 mm, internal diameter 3.5 µm), or a SeQuant Zic-pHilic (column
501 B, 150 mm × 2.1 mm, internal diameter 3.5 µm) with guard columns (20 mm × 2.1 mm,
502 internal diameter 3.5 µm) both from Merck (Darmstadt, Germany). With column A, the
503 mobile phase was composed by 0.1% aqueous formic acid (solvent A) and 0.1% formic acid
504 in acetonitrile (solvent B). The flow rate was set at 300 µL x min^{-1} and the gradient was as
505 follows: 0-5 min 80 % B, 5-15 min 15 min 30% B, 15-20 min 10 % B, 20-21 min 80% B,
506 hold at 80% B for 9 minutes. For column B, the mobile phase was composed of 20 mM
507 ammonium carbonate and 0.1% ammonium hydroxide in water (solvent C), and acetonitrile
508 (solvent D). The flow rate was set at 180 µL x min^{-1} with the following gradient: 0 min 70%
509 D, 1 min 70% D, 16 min 38% D, 16.5 min 70% D, hold at 70% D for 8.5 minutes. The mass
510 spectrometer was operated in full MS and polarity switching mode. Samples were
511 randomised, in order to avoid machine drift, and were blinded to the operator. The acquired
512 spectra were analysed using XCalibur Qual Browser and XCalibur Quan Browser softwares
513 (Thermo Scientific) by referencing to an internal library of compounds. Calibration curves
514 were generated using synthetic standards of the indicated metabolites.

515 **Proteomics analysis**

516 Proteomics experiments were performed using mass spectrometry as reported before[34,35]. In
517 brief, cells were lysed in urea lysis buffer (8 M urea, 10 mM Na_3VO_4, 100 mM β-Glycerol
518 phosphate and 25 mM $Na_2H_2P_2O_7$ and supplemented with phosphatases inhibitors-Sigma)
519 and proteins reduced and alkylated by sequential addition of 1 mM DTT and 5 mM
520 iodoacetamide. Immobilised trypsin was then added to digest proteins into peptides. After
521 overnight incubation with trypsin, peptides were desalted by solid phase extraction (SPE)

522 using OASIS HLB columns (Waters) in a vacuum manifold following manufacturer's
523 guidelines with the exception that the elution buffer contained 1 M glycolic acid.

524 Dried peptide extracts were dissolved in 0.1% TFA and analysed by nanoflow LCMS/MS in
525 an LTQ-orbitrap as described before[34,35]. Gradient elution was from 2% to 35% buffer B in
526 90 minutes with buffer A being used to balance the mobile phase (buffer A was 0.1% formic
527 acid in water and B was 0.1% formic acid in acetonitrile). MS/MS was acquired in multistage
528 acquisition mode. MS raw files were converted into Mascot Generic Format using Mascot
529 Distiller (version 1.2) and searched against the SwissProt database (version 2013.03)
530 restricted to human entries using the Mascot search engine (version 2.38). Allowed mass
531 windows were 10 ppm and 600 mmu for parent and fragment mass to charge values,
532 respectively. Variable modifications included in searches were oxidation of methionine, pyro-
533 glu (N-term) and phosphorylation of serine, threonine and tyrosine. Results were filtered to
534 include those with a potential for false discovery rate less than 1% by comparing with
535 searches against decoy databases. Quantification was performed by obtaining peak areas of
536 extracted ion chromatographs (XICs) for the first three isotopes of each peptide ion using
537 Pescal [36,37]. To account for potential shifts in retention times, these were re-calculated for
538 each peptide in each LCMS/MS run individually using linear regression based on common
539 ions across runs (a script written in python 2.7 was used for this retention time alignment
540 step). Mass and retention time windows of XICs were 7 ppm and 1.5 minutes, respectively.

541 **Toray miRNA array**

542 Initial sample quality control was performed using a Bioanalyzer 2200 system in conjunction
543 with the Total RNA Nano chip (Agilent, Cheadle UK). 250 ng total RNA were labelled using
544 the miRCURY LNA microRNA Hy5 Power labelling kit (Exiqon, Vedbæk Denmark)
545 according to the Toray array protocol. Samples were hybridized to the Human/Mouse/Rat

546 miRNA 4-plex miRBase v17 array (Toray, London UK) and subsequently scanned using the
547 3D-Gene Scanner 3000 (Toray) according the manufacturer's instructions. Data was
548 normalized according to instructions provided by Toray. Briefly, presence or absence of
549 signals was determined using a cut off defined as the mean of the middle 90% of the blank
550 control intensities (background average intensity) + 2σ. Positive control signals were
551 removed and the background average intensity subtracted from the signal intensities to give
552 the background subtracted signal intensities (y). Normalised signal intensities (NSI) were
553 then calculated as follows: NSI = $25y/(y)$. Raw data are presented in SI Table 4.

554 **Mass spectrometry-based analysis of methylated DNA of HLRCC tumours**

555 DNA from healthy and tumour tissue was extracted using DNeasyKit (Qiagen) following
556 manufacturer's instructions. 0.5-1 µg of DNA resuspended in 25 µL of water was first
557 denatured at 100°C for 30 seconds, cooled on ice, and then added of 2 µL of 20 mM $ZnSO_4$.
558 DNA was digested at 50°C for 16 hours using 1 µL Nuclease P1 (200 units x mL^{-1}, Sigma
559 Aldrich) and dephosphorylated at 65°C for 2 hours by adding 1 µL of Bacterial alkaline
560 phosphatase BAP (150 U x μL^{-1}, Life Technology). pH was then adjusted using 30 µL of 0.5
561 M Tris-HCl pH 7.9 for one hour at 37°C.

562 Analysis of global levels of C, 5hmC and 5mC was performed on a QExactive Orbitrap mass
563 spectrometer coupled to a Dionex UltiMate 3000 Rapid Separation LC fitted with an Acquity
564 UHPLC HSS T3 column (100 x 2.1 mm, 1.8 µm particle size). The mobile phase consisted of
565 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a
566 flow rate of 300 µl x min^{-1}. Calibration curves were generated using synthetic standards for
567 2'-deoxycytidine, 5-methyl- and 5-hydroxymethyl-2'-deoxycytidine (Berry&Associates).
568 The mass spectrometer was set in a positive ion mode and operated in parallel reaction
569 monitoring. Ions of masses 228.10, 242.11, and 258.11 were fragmented and full scans were

570 acquired for the base fragments 112.0505, 126.0661, and 146.0611 ± 5ppm (corresponding to
571 C, 5mC and 5hmC, respectively). The extracted ion chromatogram (EIC) of the
572 corresponding base-fragment was extracted using the XCalibur Qual Browser and XCalibur
573 Quan Browser software (Thermo Scientific), and used for quantification. Quantification was
574 performed by comparison with the standard curve obtained from the pure nucleoside
575 standards running with the same batch of samples. The level of 5hmC present in the sample
576 was expressed as a percentage of total cytosine content.

577 **Immunohistochemistry on HLRCC tumours**

578 Specimens were formalin fixed and embedded in paraffin wax; 3-μm serial sections mounted
579 on Snowcoat X-tra slides (Surgipath, Richmond, IL) were dewaxed in xylene and rehydrated
580 using graded ethanol washes. For antigen retrieval, sections were immersed in preheated
581 DAKO target retrieval solution (DAKO) and treated for 90 seconds in a pressure cooker.
582 Sections analysed contained both tumour and adjacent normal renal parenchyma acting as an
583 internal control; in addition, substitution of the primary antibody with antibody diluent was
584 used as a negative control. Antigen/antibody complexes were detected using the Envision
585 system (DAKO) according to the manufacturer's instructions. Sections were counterstained
586 with hematoxylin for 30 seconds, dehydrated in graded ethanol washes, and mounted in DPX
587 (Lamb, London, United Kingdom). Antibodies used were: E-cadherin (HECD1, CRUK) and
588 vimentin (clone V9, Dako). TET1 (SAB 2501479) and TET2 (HPA 019032) antibodies were
589 purchased by Sigma Aldrich.

590 **miRNA expression on HLRCC tumours**

591 Total RNA was extracted from tumour and healthy tissue using miRCURY kit (Exiqon,
592 Denmark) following manufacture's protocols. RNA reverse-transcription and real-time qPCR

593 were obtained as described above. Data are normalised to healthy tissue using both
594 *SNORD61* and *RNU6B* as endogenous controls.

595 **Clinical details of HLRCC patients**

596 The patients consented to use of tissues for study approved by the National Research Ethics
597 Committee London (REF number 2002/6486 and 03/018). FH mutations in HLRCC Patient
598 A is c.1300T>C, and in Patient B is c.1189G>A

599 **Bioinformatics and statistical analyses**

600 Volcano plots were generated using the log10 fold-change on the x-axis and the -log10 of the
601 multi hypothesis corrected p-value (false-discovery rate) on the y-axis generated by Limma[38]
602 differential analysis. The Epithelial–Mesenchymal Transition gene signature was extracted
603 from Taube and colleagues[39]. Signature enrichment was performed with the commonly used
604 Gene-Set Enrichment Analysis (GSEA)[8] test. Signature significance was calculated by
605 randomizing the genes signatures 10000 times.

606 The TCGA RNA-seq and miRNA-seq data-sets for clear cell (KIRC) and papillary (KIRP)
607 renal carcinoma were downloaded from the Broad Firehose webpage
608 (http://gdac.broadinstitute.org/). Differential analysis was performed with R package
609 Limma[38] using voom[40] to transform the RNA-seq counts. Cancer patients were ranked
610 according to FH expression and survival analysis was performed by comparing the overall
611 survival time of upper vs. lower quartile of the FH-ranked list of patients. Kaplan Meier
612 curves were built using in-house R scripts and significance was calculated using the R
613 package Survival by applying a χ^2 test. Hive plots were generated using the R package
614 "HiveR".

615 Graphpad Prism 6 was used to generate graphs and perform statistical analysis (one-way
616 ANOVA test with Tukey's post hoc test for multiple comparisons was used unless otherwise
617 indicated). ChIP statistical analysis was generated using Excel (Microsoft). Except for
618 metabolomic experiments, no randomization or blinding was performed. No statistical
619 method or power analysis was used to predetermine sample size.

620 **Code availability**

621 The R and Python scripts for the analyses above can be found at
622 http://www.ebi.ac.uk/~emanuel/Sciacovelli_et_al/.

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650 **Extended Data Figure Legends**

651 **Extended Data Figure 1. Characterisation of Fh1-deficient and Fh1-rescued cells. a,**
652 PCR to assess *Fh1* recombination. The putative genotypes are indicated on the right and are

653 based on the expected size of the genomic PCR amplification products as from Frezza et al[7].
654 *Fh1*$^{fl/fl}$ = 470 bp and *Fh1*$^{-/-}$= 380 bp. **b**, Fh1 protein levels measured by western blot of cells
655 of the indicated genotype. Calnexin was used as loading control for western blot. **c**,
656 Intracellular fumarate levels measured by LCMS and normalised to total ion count. Results
657 were obtained from 4 independent cultures and are indicated as average ± S.D.. p-values were
658 calculated from one-way ANOVA. **d**, Oxygen Consumption rate (OCR) and Extracellular
659 Acidification rate (ECAR) assessed using the Seahorse Extracellular Flux Analyser. Results
660 were obtained from 5 replicate wells and are presented as average ± S.D.. **e**, Bright field
661 images of cells of the indicated phenotype. Bar = 400 µm. Western blot and gel sources are
662 presented in Supplementary Figure 1. Raw data are presented in SI Table 2. *P ≤0.05, ***P*
663 ≤0.01, ****P* ≤0.001, *****P*≤0.0001. **f**, Schematic representation of the proposed link
664 between loss of FH, fumarate accumulation, and epigenetic suppression of the antimetastatic
665 cluster of miRNA *miR-200*. Upon accumulation of fumarate as a result of FH inactivation,
666 the TET-mediated demethylation of the *miR-200ba429* cluster is inhibited, leading to their
667 epigenetic suppression. As a consequence, Zeb1/2 are de-repressed, eliciting a signalling
668 cascade that leads to EMT.

669 **Extended Data Figure 2. EMT signature in *Fh1*$^{-/-}$ cells. a**, Volcano plot of RNA-seq
670 analysis. Gene expression was normalised to *Fh1*$^{fl/fl}$ or *Fh1*$^{-/-}$*+pFh1* cells as indicated. **b**, **c**,
671 Gene set enrichment analysis (**b**) and EMT enrichment score (**c**) of the indicated cell lines.

672 **Extended Data Figure 3. EMT signature in UOK262 cells. a**, Gene set enrichment analysis
673 and EMT enrichment score of the indicated cell lines. Gene expression was normalised to
674 UOK262pFH. **b**, **c**, mRNA expression measured by qPCR (**b**) and protein levels measured by
675 western blot (**c**) of the indicated EMT markers. **d**, Immunofluorescence staining for Vimentin
676 and E-Cadherin. DAPI was used as marker for cell nuclei. Scale Bar = 25 µm. **e**, Cell

677 migration rate. Results were obtained from 14 replicate wells and presented as mean ± S.D..
678 **f**, mRNA expression of EMT-related transcription factors *ZEB1* and *ZEB2* from RNA-seq
679 data as in Fig. 1a. **g**, Expression levels of the indicated miRNAs measured by qPCR. **h**,
680 Volcano plot of miRNA profiling. All qPCR experiments were obtained from 3 independent
681 experiments and presented as RQ with max values, normalised to *β-actin* or
682 *RNU6B/SNORD61* as endogenous control for mRNA and miRNA analyses, respectively. *P
683 ≤0.05, ***P* ≤0.01, ****P* ≤0.001, *****P*≤0.0001. Western blot sources are presented in
684 Supplementary Figure 1. Raw data are presented in SI Table 2.

685 **Extended Data Figure 4. EMT features in Fh1-deficient cells are independent from HIF.**
686 mRNA levels of EMT genes (**a**) and HIF target genes (**b**) in *Fh1*$^{-/-}$ cells infected with shRNA
687 against HIF1β measured by qPCR. Results were obtained from 3 independent cultures and
688 presented as RQ with max values using *β-actin* as endogenous control. NTC = non-targeting
689 control. p-values from unpaired t-test are indicated in the graph. *LdhA* = lactate
690 dehydrogenase A; *Pdk1* = pyruvate dehydrogenase kinase 1; *Glut 1* = glucose transporter 1.
691 *P ≤0.05, ***P* ≤0.01, ****P* ≤0.001, *****P*≤0.0001. Raw data are presented in SI Table 2.

692 **Extended Data Figure 5. EMT signature in Fh1-reconstituted cells. a**, Fh1 protein levels
693 measured by western blot. Calnexin was used as loading control. **b**, Intracellular fumarate
694 levels the measured by LCMS. Data are presented as average ± S.D.. **c**, Representative bright
695 field images of cells of the indicated genotype. Scale Bar = 400 μm. **d**, **e**, mRNA expression
696 measured by qPCR (**d**) and protein levels measured by western blot (**e**) of the indicated EMT
697 markers. **f**, Average speed of cells calculated after tracking cells for 3 hours as in Fig. 1g.
698 Results were generated from 3 independent cultures. **g**, mRNA expression of EMT-related
699 transcription factors. *β-actin* was used as endogenous control. EV = empty vector. **h**,
700 Expression levels of the indicated miRNAs measured by qPCR and normalised to *Snord95*

701 and *Snord61* as endogenous control. All qPCR results were obtained from 3 independent
702 cultures and presented as RQ with max values. *P ≤0.05, ***P* ≤0.01, ****P* ≤0.001,
703 *****P*≤0.0001. Western blot sources are presented in Supplementary Figure 1. Raw data are
704 presented in SI Table 2.

705 **Extended Data Fig. 6. Role of Tets and Histone Demethylases in EMT induction. a,**
706 Expression levels of *Tet1-3* in *Fh1* $^{fl/fl}$ from RNA-seq data. **b**, **d**, Expression levels of *Tet2/3*
707 (**b**), *miRNA200* (**c**), and *E-cadherin* (**d**) in *Fh1* $^{fl/fl}$ cells upon combined silencing of *Tet2* and
708 *Tet3*. The results are presented as RQ with max values obtained from technical replicates. *β-*
709 *actin* and *Snord61* were used as endogenous control for mRNA and miRNA, respectively. **e**,
710 Expression levels of the indicated miRNAs upon inhibition of histone demethylases by GSK
711 J4. *Snord61* and *Snord95* were used as endogenous controls. **f**, Expression of the indicated
712 miRNAs in *Fh1*$^{-/-}$ cells incubated for 24 hours with 5 mM DM-aKG measured by qPCR.
713 Results were obtained from 4 (vehicle) or 5 (*Fh1*$^{-/-CL19}$) and 3 (*Fh1*$^{-/-CL1}$) (DM-aKG)
714 independent cultures and presented as RQ with max values, normalised to *Snord95* as
715 endogenous control. *P ≤0.05, ***P* ≤0.01, ****P* ≤0.001, *****P*≤0.0001.

716 **Extended Data Fig. 7. Characterisation of the regulatory CpG island *CpG43*. a**, Snapshot
717 of Genome Browser view of genomic DNA around the *miR200ba429* cluster taken from
718 NCBI37/mm9. Tet2 ChIP was obtained from GSE41720, sample GSM1023124. Shaded
719 rectangles indicate *miR-200ba429* and *CpG43*. **b**, ChIP-PCR of the indicated histone marks
720 in a region adjacent *CpG43*. Data were obtained from 3 independent cultures and are
721 presented as average ± S.D.. p-values from unpaired t-tests are indicated in the graph. **c**,
722 Expression levels of H3 histone marks in cells of the indicated genotypes measured by
723 western blot. H3 used as loading control. **d**, 3C data of the genomic region adjacent to *CpG43*
724 analysed in *Fh1*$^{fl/fl}$ cells. The position of *CpG30* and *CpG43,* and of the predicted restriction

725 sites are indicated in the graph. Results were generated from 2 independent cultures. **e**, DNA
726 methylation of the *CpG43* assessed by qPCR using OneStep qMethyl kit. Data were obtained
727 from 3 independent experiments and normalised to methylation levels of the region in $Fh1^{fl/fl}$.
728 Data are presented as average ± S.E.M.. **f**, ChIP-PCR of Tets binding to *CpG43*. Data were
729 obtained from three replicates and are presented as average ± S.D.. **g**, 5hmc nuclear staining
730 assessed by immunofluorescence using 5hmc antibody. Nuclear staining was quantified using
731 Image J and an average of 120 cells was used per genotype. p-values from One-way ANOVA
732 test. Representative images of 5hmc staining are shown. DAPI is used to indicate the nuclei.
733 Bar = 20 µm. *P ≤0.05, ***P* ≤0.01, ****P* ≤0.001, *****P*≤0.0001. Western blot sources are
734 presented in Supplementary Figure 1. Raw data are presented in SI Table 2.

735 **Extended Data Fig. 8. Monomethyl Fumarate (MMF) triggers EMT in FH-proficient**
736 **cells. a**, Bright field images of cells treated for 6 weeks with MMF. Arrows indicate the
737 typical protrusion of cells of mesenchymal phenotype. Bar = 400 µm. **b**, Oxygen
738 consumption rate of the indicated cell lines treated chronically with MMF (as in Fig. 3). See
739 Methods for drugs concentrations. OCR was normalised to total protein content. Results were
740 obtained from 6 (for mouse cells) or 8 (for human cells) wells ± SD.. **c**, Hive plot of
741 metabolomics data of mouse and human cells treated with MMF (as in Fig. 3). All identified
742 metabolites are included on the y-axis and grouped into human (pink) and mouse (green)
743 cells. Metabolites accumulated (right x-axis) or depleted (left x-axis) in MMF-treated cells
744 versus control are indicated by a connecting arc and their fold-change is colour-coded.
745 Metabolites accumulated commonly across the two cell lines are highlighted with a solid line.
746 2SC: 2-succinic-cysteine, succGSH: succinic-GSH. Raw data are presented in SI Table 2.
747 Raw metabolomic data are presented in SI Table 3.

748 **Extended Data Fig. 9. Succinate triggers EMT in Sdhb-deficient cells. a**, Intracellular
749 succinate levels after incubation with 4 mM MMS measured by LCMS. Data are presented as
750 average ±S.D.. **b**, **c**, Intracellular succinate (**b**) and succGSH (**c**) levels in Sdhb-deficient cells
751 measured by LMCS. Data are presented as average ±S.D.. **d**, Bright field images of cells of
752 the indicated genotype. Bar = 400 µm. **e**, Gene set enrichment analysis and EMT enrichment
753 score from expression analysis of the indicated cell lines. **f**, **g**, miRNA expression levels
754 normalised to *Snord61* and *Snord95* as endogenous control (**f**) and *CpG43* methylation (**g**).
755 Experiments were performed as in Fig. 2b and 2d, respectively. *P ≤0.05, ***P* ≤0.01, ****P*
756 ≤0.001, *****P*≤0.0001. Gel sources are presented in Supplementary Figure 1. Raw data are
757 presented in SI Table 2.

758 **Extended Data Fig. 10. Expression of FH and EMT markers in kidney cancer. a**,
759 Expression levels of *Vimentin* and *E-Cadherin* in HLRCC patients obtained from Ooi et al[25].
760 **b**, Immunohistochemistry staining of Vimentin and E-Cadherin (left), and TET1 and TET2
761 (right) in HLRCC patients obtained as in Fig. 4a. Bar = 100 µm. The insert in the left panel
762 indicate a 3X digital magnification, Bar = 50 µm. **c**, Gene set enrichment analysis and EMT
763 enrichment score from RNA-seq data of papillary renal cell carcinoma (KIRP) obtained by
764 Linehan et al[26]. **d**, Volcano plot of MIRNA expression in KIRP. **e**, Kaplan-Meier curve of
765 KIRP patients separated according to *FH* expression. **f**, *Vimentin* and *E-Cadherin* expression
766 in FH-mutant KIRP compared to normal renal tissue. **g**, Frequency of mutations in *FH* and
767 *TET1, TET2* and *TET3* in KIRP analysed using NCBO BioPortal. Only cancers with
768 mutations in the indicated genes are shown. **h**, Kaplan-Meier curve of FH-wild type and FH-
769 mutant KIRP. **i**, Expression levels of *FH*, *Vimentin,* and *E-Cadherin* in clear cell renal cell
770 carcinoma (KIRC) obtained from TCGA dataset[27]. **j**, Volcano plot of miRNA expression in
771 KIRC. **j**, Kaplan-Meier curve of KIRC patients separated according to *FH* expression.