Supplementary data

- *Statistical methods*

Statistical tests were performed using SPSS. Summary statistics include mean and standard deviation for continuous variables and frequency and percentage for categorical variables.

- *^{1}H-MRS data acquisition*

All ^{1}H-MRS studies were performed on a 3T MRI system (MR750, GE Healthcare, Waukesha, WI), with body coil transmission and reception coils tailored to the tumour location. Routine T_1- and T_2-weighted images were acquired and a single voxel sized between 2.2 and 100 ml was prescribed within a tumour. Automated adjustment of transmitter frequency and power and magnetic field homogeneity was performed on all voxels prior to acquisition. Spectra with an echo time of 144 ms were acquired from the tumour with and without chemical shift selective (CHESS) water suppression pulses, using respiratory gating when the location was in the upper abdomen. The spectral acquisition time was 15-20 min on average for each patient but the actual time and the number of averages varied depending on the location of the tumour and the respiratory rate of the patient when respiratory triggered (Supplementary Table 1).

- Germline genetic analysis:

DNA was extracted from peripheral blood samples according to standard protocols. Next generation sequencing of *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MAX*, *TMEM127*, *VH*L, *RET*, *FH*, *KIT*, *PDGFRA*, was performed using the Trusight One sequencing panel (Illumina

Inc., UK). An average coverage depth of >20 fold was achieved for 98% of the regions sequenced. All detected variants were confirmed by Sanger sequencing. Whole exon deletions and duplications and large rearrangements are not detected using this method and multiple ligation probe analysis (MLPA) was performed for *VHL*, *SDHB*, *SDHC* and *SDHD*, in cases in whom a *SDHx* mutation was suspected but no mutation was detected by next generation sequencing.

- *SDHB Immunohistochemistry*

SDHB immunohistochemistry (IH) was performed on 4 µm sections of formalin-fixed paraffin-embedded tissue, after appropriate selection of tissue blocks by experienced pathologists (AM and OG). Commercially available SDHB polyclonal rabbit antibody was used at a dilution of 1:300. Heat-induced epitope retrieval was carried out using a Leica heat retrieval solution (HPA002867, Sigma Aldrich, UK).

- *SDHC* hypermethylation analysis

Tumour slides were reviewed by an experienced pathologist (OG) and FFPE blocks suitable for molecular analysis selected. The tumour area was marked on a H+E stained slide. 5 mm thick tissue sections were mounted on glass slides, de-waxed with xylene and tumours macro dissected with a sterile scalpel blade. The dissected tissue was re-suspended in 70% EtOH and a minimum of 500 ng of DNA extracted using a commercial kit (Qiagen, UK). 500 to 1000 ng of DNA were used for bisulphite conversion using Qiagen Epitect Bisulfite kit (Cat 59104). In order to assess the methylation level of the SDHC promoter region DNA was

amplified as previously described by Anderson *et al.*[28] and the methylation level assessed by pyrosequencing®

- **Primers used for SDHC promoter region amplification:**

SDHC F: 5' GAGGAGGAGATTAAAAAATTAGAAAATAAT

SDHC R: 5' Bio-CCACTAAAATCACCTCAACAACAA

SDHC Seq: 5' GTTATATGATATTTTTAATTT

- **Measurement of succinate in ex-vivo tissue samples**

HRMAS (high resolution magic angle spinning) 1H NMR data acquisition was performed on a 600 MHz instrument with a 4 mm HRMAS probe (Bruker, Germany). All the spectra were obtained using the TOPSPIN 2.3 software (Bruker, Germany) and at a spin rate of 3000 Hz and a sample temperature of 4°C. A water-suppressed pulse sequence with a repetition time of 8 s, 128 transients and 64K time domain points was used to acquire the metabolite spectrum. The corresponding water spectrum was acquired with 8 s repetition time, 8 transients and 64K time domain points. A water-suppressed Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used with a T2 filter to acquire metabolite spectra with suppression of the broad lipid and macromolecule signals; acquisition parameters: 8 s repetition time, 128 transients, 64K time domain points, T2 filter times 50/100/200 ms, total analysis time per sample approximately 90 min. Metabolite concentrations were estimated by fitting the metabolite signals in the water-suppressed HRMAS 1H NMR spectrum in LCModel and USING tissue water signal as internal standard for absolute concentration[19]

- **Table S1**: Characteristics of the 15 tumours analysed by ^{1}H-MRS. TF: technical failure, defined as an estimated uncertainty (%SD) > 15% in automated peak fitting of Choline using LCModel. ND: not detected. NA: not applicable.

Case no.	Tumour site analysed	Maximum tumour diameter	Succinate: Choline ratio	Water FWHM	%SD Choline	%SD succinate	Succinate detected or technical failure	Convincing succinate seen
1	Liver	2.6cm	0.74	13	2	4	+	Y
2	Lymph node	8cm	1.32	6	11	47	+	Y
3	Liver	2.3cm	0.74	10	8	23	+	Y
4	GIST	3.6cm	0.07	18	4	113	-	N
5	Liver	2.5cm	0.15	12	5	27	+	Y
6	PA	1.8cm	ND	12	4	ND	-	N
7	PC	12cm	0.18	12	3	24	+	Y
8	Glomus PGL	9cm	1.71	29	6	8	+	N
9	Abdomin al PGL	8cm	2.49	12	7	9	+	Y
10	PC	9cm	0.9	12	8	18	+	N
11	Liver	3cm	0.80	8	9	20	+	Y
12	Liver	2.2cm	ND	18	16	NA	TF	N
13	Rib metastase s	1.8cm	ND	42	36	18	TF	N
14	Liver	2.3cm	ND	15	32	27	TF	N
15	PC	5cm	ND	19	73	NA	TF	N

- **Table S2**: Characteristics of the two patients in whom H-MRS was repeated on during the same examination to evaluate test reproducibility.

Case no.	Tumour site on 1st scan	Tumour site on 2nd scan	Maximum tumour diameter on 1st scan	Maximum tumour diameter on 2nd scan	Succinate: choline ration on 1st scan	Succinate: choline ratio on 2nd scan	Succinate detected on 1st scan	Succinate detected on 2nd scan
1	Liver	Liver	2.6	2.6	0.74	0.72	Y	Y
5	Liver	Liver	2.5	2.3	0.15	0.17	Y	Y

- **Figure S1:** (A) Coronal MRI image of a large left sided glomus paraganglioma from case 8 demonstrated by the white arrow. (B) Spectra processed with LCModel from the same patient showing a broad unreliable peak at 2.4 ppm, which was not convincing for succinate.