SUPPLEMENTARY METHODS

Validation of antibodies and analysis of SphK1 expression on FFPE colorectal cancer tissues. Three different antibodies (Abs), one monoclonal (abcam, Cambridge, UK) and two polyclonal (Santa Cruz Biotechnology and abcam) were tested following manufacturer's instruction to select the most efficient on FFPE material. The monoclonal Ab did not yield specific signal even after antigen retrieval procedures. Conversely, specific staining was obtained by both polyclonal Abs; particularly the abcam antibody, that yielded a better signal intensity and background noise ratio (Pan J, J Transl Med 2011), successfully completed a validation protocol described by Bordeaux et al. (Bordeaux J, Biotechniques 2010) and was selected to stain the case series. Briefly, the selected Ab was tested through Western blot analysis on cell line lysates with known levels of target expression. Therefore, 100 consecutive samples of colon cancer were selected from the files of the Histopathology Department. The corresponding FFPE tissue blocks were retrieved from the archives with their matched haematoxylin and eosin (H&E) sections. Using the H&E stained slides as a template, representative areas of carcinoma were identified and marked. A tissue microarray (TMA) was constructed by sampling four cores (0.6 mm diameter) from each block, as previously described (Bordeaux J, Biotechniques 2010). Four cores were sampled from different tumor areas to avoid heterogeneity effects and to minimize the occurrence of lost cores during the TMA processing. Five Sections (5 µm thick) of the TMA were cut, mounted on slides and dried overnight at 55˚C. Each TMA section was stained at five different antibody dilutions (1:100, 1:500, 1:1000, 1:2000) to establish the lowest threshold of anti-SphK1 sensitive detection. Moreover, the same TMA was stained with three different lots of the Ab. The results were visualized by the alkaline phosphatase system. The expression level was evaluated on a semiquantitative score (0: Absence of expression; 1: Low expression; 2: Intermediate expression; 3: High expression) by two different pathologists, independently, and results were reported on TMA-based spreadsheet file.

Patients. 50 metastatic colorectal cancer patients treated in our Institution in the last four years (33 males, 17 females), with a median age at diagnosis of 64 years (range 39-78 years) were analyzed for SphK1 staining, *K-Ras* status and clinical/pathological features. Twenty-nine patients had a metastatic stage at diagnosis time and were treated with cetuximab based chemotherapies in first (20 patients), second (16 patients) and third line (14 patients). Cetuximab was administered at a weekly dose of 250 mg/msq after an initial dose of 400mg/msq associated to irinotecan based regimens (FOLFIRI, 28 patients; irinotecan, 15 patients), or with oxaliplatin (FOLFOX, 4 patients), or in monotherapy (3 patients).

***K-Ras* mutational assessment.** PCR for *K-Ras* exon 2, codon 12 and 13, was carried out as previously described (Troncone G, Diagn Cytopathol 2010; http://kras.eqascheme.org/info/public/kras/assays.xhtml). Molecular Pathology Lab of our Institution is an accredited Italian reference center for *K-Ras* testing (http://kras.eqascheme.org/info/public/kras/assays.xhtml). Through a dedicated website, upon patient consent, the oncologist and the primary pathologist submitted the clinical-pathological data. Then tissue sample for each patient was mailed for *K-Ras* mutational assessment. The amplified fragments were purified by QiaQuick DNA Purification Kit (Qiagen) according to the manufacturer's instructions. Big Dye Terminator v1.1 (Applied Biosystems) sequencing reactions were performed for both DNA strands with a total of 10 ng PCR products, according to the manufacturer's instructions. Dye purification was done by alcohol/sodium acetate precipitation. Sequence analysis was carried out on Applied Biosystems 310 genetic analyzer. The obtained files were aligned and examined for mutations in codons 12 and 13 of the *K-Ras* gene by CodonCode software (CodonCode Inc.).

Labelling of ^{99m}Tc-Hynic-annexin V and micro-single photon emission computed tomography imaging. Hynic-rh-annexin V conjugate and stannous tricine were kindly supplied by

Biological Research Branch, NCI-Frederick Cancer Research and Development Center (Frederick, MD, USA). Radio-labelling was performed following the supplier's protocol. Briefly, 50 mCi (1,850 MBq) of ^{99m}Tc-pertechnetate was added to the HYNIC-rh-annexin V conjugate. Then an aliquot of 0.3 ml of stannous tricine solution was added to the Hynic-rh-annexin V. The reagents were mixed gently and allowed to incubate at room temperature for 15 min. Radiochemical purity of the ^{99m}Tc-Hynic-annexin V was greater than 90% as determined by thin-layer chromatography (ITLC). Untreated and treated tumor-bearing mice underwent a micro-single photon emission computed tomography (SPECT) study, as previously described (Zannetti A, Clin Cancer Res 2008). Briefly, mice were anaesthetized and ^{99m}Tc-Hynic-Annexin V was i.v. injected through the tail vein. After 4 hours, microSPECT images were obtained using the YAP-(S)PET scanner (ISE, Pisa, Italy). The data were acquired in list mode from 256 views over an angle of 360 degrees. Images were then reconstructed using an iterative reconstruction algorithm that provided transaxial, coronal and sagittal slices.