

Material S1: RT-PCR protocol sample set 1 and sample set 2

- *mRNA isolation and cDNA synthesis*

Total RNA was isolated from the tissue using a Nucleospin[®] RNA II kit (Machery-Nagel, Düren, Germany) for sample set 1 and RNeasy Mini Kit (Qiagen, Valencia, CA) for sample set 2, according to the manufacturer's instructions. Approximately 5-30 mg of frozen tissue was manually homogenized on ice in an Eppendorf tube using pellet pestles to yield 22 – 570 ng/UI (range) RNA. Quality Control standards were applied to all RNA samples in this study. Purity was assessed both with A260nm/A280nm 1.9-2.1. Absorbance measurements at 260 nm in water were used to adjust the stock concentrations of all RNA samples to 1 Ug/UI.

For reverse transcription, samples were treated with DNase to digest contaminating DNA. For sample set 1 cDNA was obtained following local protocol, and for sample set 2 using SuperScript VILO cDNA Synthesis Kit (Life Technologies) per manufacturer's protocol.

- *Quantitative RT-PCR*

For sample set 1, expression was measured by a SYBR green (SensiMix SYBR Hi-ROX kit; Biorline) quantitative RT-PCR using a 7900 Sequence detector (Applied Biosystems) on a 96 well optical reaction plate (Applied Biosystems). In house designed primer sequences, with confirmed specificity in appropriate melting curves for each PCR, can be found in Table S1 and were derived from Eurogentec (Eurogentec Netherlands, Maastricht, Netherlands). PCR efficiency of each primer pair was determined using serial dilutions of cDNA from the Caco-2 (colon carcinoma) cell line and from peripheral blood mononuclear cells. Non template controls confirmed the absence of exogenous contaminated DNA.

For sample set 2 RT-PCR was carried out in 384-well reaction plates using 2X Taqman Fast Universal Master Mix (Applied Biosystems, Foster City, CA), 20X Taqman specific gene expression probes and 10ng of the cDNA template. The reactions were carried out on an Applied Biosystems 7500 Fast Real-Time Polymerase Chain Reaction System (Applied

Biosystems).

Transporter mRNA expression levels for all samples were normalized to *GAPDH* mRNA expression levels (ratio transporter/*GAPDH*) and relative expression was compared across the age range. Quality was assessed by measuring the RNA integrity number (RIN) by microfluidic capillary electrophoresis on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA), whereby RIN's below 5 were to be excluded from the analysis.