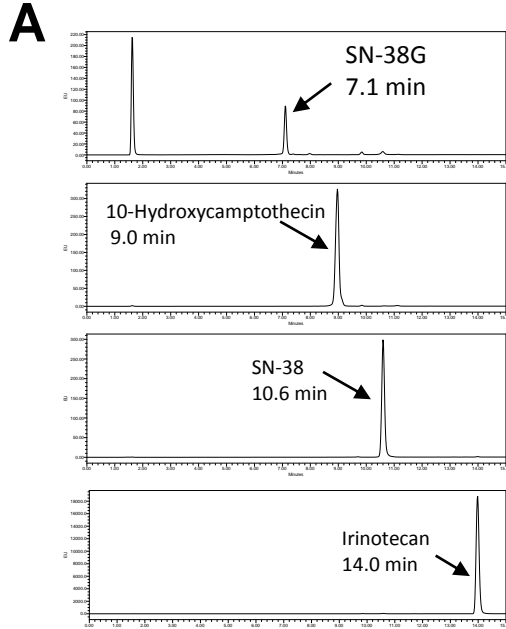


Figure S1. Evaluation of the procedure used to quantitate the constitutive products derived from irinotecan or IMMU-132. (A) Reversed-phase HPLC separation of the 4 products of interest, SN-38, SN-38G, and irinotecan, as well as the internal standard, 10-hydroxycamptothecin. (B) Example of standard curve derived for SN-38 showing a linear range from 10 to 10,000 ng/mL.

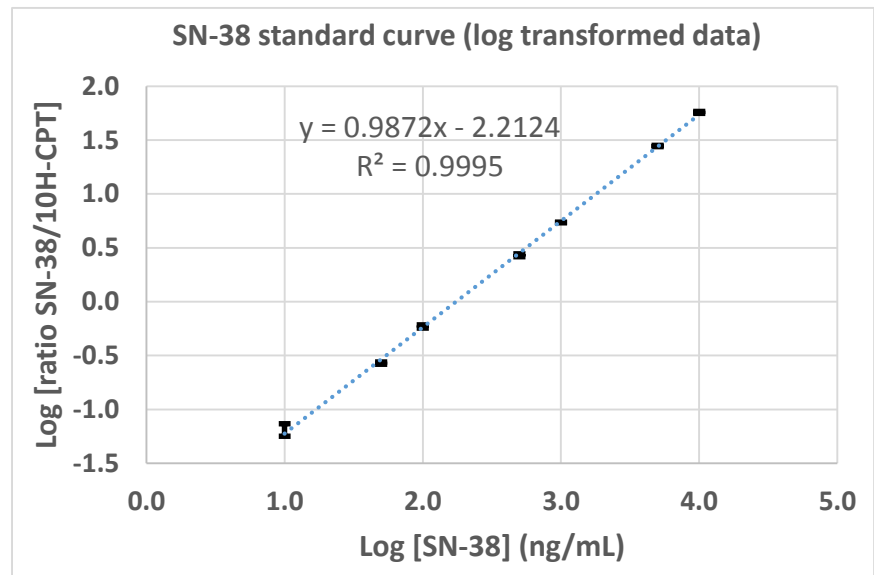


Blank samples of serum, liver, tumor, and feces processed in the same manner as samples for analysis were run to assess if there were background fluorescence peaks co-migrating at these times. Some background subtraction required for serum and feces required.

C: 50 mM KH_2PO_4 and 4mM Na-1decanesulfonate in water, pH 3.5
D: 600 mL buffer C and 400 mL acetonitrile
Linear gradient, starting with 85:15 C/D to 100% D over 15 min

B

SN-38 Standards (ng/mL)	Mean SN-38/10-OH-CPT ratio
10	0.0647
50	0.2691
100	0.5897
500	2.6978
1000	5.4443
5000	27.8591
10000	57.1465



Lower limit of sensitivity for all products = 10 ng/mL

- Serum is diluted 1:2 so minimum sensitivity = 20 ng/mL
- Tissue homogenates are a 11:1 dilution, therefore minimum sensitivity is 110 ng/mL