Supplementary Figure 1. Molecular structure of everolimus (A) and putative molecular structure of metabolite A (B).
Supplementary Figure 2. Liver expression of Ces1 and Ces2 genes in male wild-type, Abcb1a/1b(-/-), Abcg2(-/-) and Abcb1a/1b;Abcg2(-/-) mice. Expression levels of Ces1b (A), Ces1c (B), Ces1d (C), Ces1e (D), Ces1f (E), Ces1g (F) and Ces2a (G) mRNA in livers of wild-type, Abcb1a/1b(-/-), Abcg2(-/-) and Abcb1a/1b;Abcg2(-/-) mice, as determined by real-time RT-PCR. Data are normalized to GAPDH expression. Values represent mean fold change ± SD (n = 3), compared to wild-type mice with low Ces expression.
Supplementary Figure 3. Inhibitory effect of everolimus on the conversion of irinotecan to SN-38 by Ces1c in male wild-type and knockout mouse plasmas in vitro. Concentrations of irinotecan (A) and SN-38 (B) in wild-type, Abcb1a/1b(-/-), Abcg2(-/-) and Abcb1a/1b;Abcg2(-/-) individual plasmas 30 min after spiking with 5 µM irinotecan. All data are presented as mean ± SD (n = 3-5; *, P < 0.05; **, P < 0.01; ***, P < 0.001 when compared with plasmas of the same genotype without inhibitor). ND, not detectable; detection limits were 0.0148 µM and 0.0026 µM for irinotecan and SN-38, respectively.
Supplementary Figure 4. Blood concentration-time curves of everolimus in male wild-type, Abcb1a/1b(-/-), Cyp3a(-/-) and Abcb1a/1b;Cyp3a(-/-) mice receiving oral everolimus (2 mg/kg). All data are presented as mean ± SD (n = 3-5). Blood concentrations of “low” wild-type mice at 12 hr and 24 hr were below LLQ and therefore for calculation purposes replaced with the LLQ value (10 ng/ml).
Supplementary Figure 5: Schematic model of the envisaged effect of Ces1c on the stability and degradation of everolimus in plasma. Everolimus (red squares) is added to wild-type or knockout (e.g., Abcb1a/1b(-/-)) plasma with, respectively, low or high concentrations of Ces1c (blue semicircles). The situation shortly after addition is shown on the left, the situation several hours after addition is shown on the right. Over time, an everolimus-degrading enzyme (green oval) irreversibly metabolizes free everolimus, but not bound everolimus, to an everolimus metabolite (yellow triangles). The rapid and apparently tight binding of everolimus to Ces1c, as experimentally evident from the very slow loss of everolimus in knockout plasma, is reflected in the quantitative binding of everolimus to Ces1c. The situation rendered can, with modification, also be applied to the in vivo i.v. administration of everolimus. Note, however, that, as only about 10% of the i.v. administered dose of everolimus can be effectively bound by Ces1c in knockout plasma, the overall amount of everolimus available for tissue distribution very shortly after everolimus administration is not very different between wild-type and knockout strains (~99% versus ~90% of the dose). These amounts of free everolimus quickly distribute over various organs, explaining the similar liver concentrations observed for wild-type and knockout mice. The extensive binding of ~10% of the everolimus dose by the high concentration of Ces1c in knockout plasma explains the prolonged retention of everolimus in blood of the knockout animals, as it can neither be metabolized by the everolimus-degrading enzyme, nor freely distribute to tissues.
Supplementary Figure 6. Semi-synthetic diet does not prevent altered everolimus pharmacokinetics. In Cyp3a(-/-) mice altered pharmacokinetics of some drugs were due to upregulation of some detoxifying proteins relative to wild-type mice by inducing compounds present in the diet, and this upregulation was reversed on a semi-synthetic diet (39). To test whether a similar process might be responsible for the altered everolimus pharmacokinetics, blood concentration-time curves of everolimus were determined in male wild-type, Abcb1a/1b(-/-), Abcg2(-/-) and Abcb1a/1b;Abcg2(-/-) mice that were given either a control (AM-II) (A) or semi-synthetic diet (B) for 8 weeks prior to oral administration of everolimus (2 mg/kg). All data are presented as mean ± SD (n = 2-5). Data for the standard- and semi-synthetic-diet fed mice were virtually superimposable, except for a few “high” wild-type mice present in the semi-synthetic diet group, suggesting that diet is not a determining factor of the altered everolimus pharmacokinetics in these knockout strains.