

SUPPLEMENTAL DATA

Transgene construction. Intestine-specific expression of human CYP3A4 was achieved by generating the following transgene construct. An SV40 polyadenylation signal was released from pGL3 (Promega Corporation, Madison, WI) by digestion with XbaI and BamHI and ligated into the XbaI and BamHI sites of pSL1180 (Amersham Pharmacia Biotech, Piscataway, NJ) yielding pSL1180-SV40. Wild-type human CYP3A4 cDNA was released as ~2.1 kb XbaI fragment from pUV1-CYP3A4 (Genbank acc. nr. M18907, kindly provided by Dr. F. Gonzalez (NIH, MD)) and ligated into the XbaI site of pSL1180-SV40. Subsequently, pSL1180-CYP3A4-SV40 was digested with Asp718 and SmaI, releasing CYP3A4-SV40 which was then ligated into the Asp718 and SmaI sites of pGL3 yielding pGL3-CYP3A4-SV40. The CYP3A4 open reading frame was cloned behind the murine villin promoter by PCR. In the first PCR, pUV1-CYP3A4, a forward 5'CCTCTAGGCTCGTCCACCAATGGCTCTCATCCCAGACTTGG3' primer with the last bases of the villin promoter and the first bases of CYP3A4 (including ATG-start sequence) and a reverse 5'CCCAAAGACGTCTTTCAAGG3' primer aligning with the CYP3A4 sequence at its AatII digestion site was used. Subsequently, the obtained PCR product was elongated at its 5' villin promoter side using pKS-2kbvill as a template (kindly provided by D. Louvard, Institut Curie, Paris, France) containing the last 2 kb 3' of the villin promoter (Pinto, D., Robine, S., Jaisser, F., El Marjou, F.E., Louvard, D. (1999) Regulatory sequences of the mouse villin gene that efficiently drive transgenic expression in immature and differentiated epithelial cells of small and large intestines. *J. Biol. Chem.* **274**: 6476-6482). This elongated product was used as the template for a subsequent PCR with forward 5'GCCTTAAGCCGGCTGTGATAGC3' primer, aligning with the villin promoter sequence at its AflII digestion site, and the earlier used reverse 5'CCCAAAGACGTCTTTCAAGG3' primer, aligning with the CYP3A4 sequence at its Aat II digestion site, and thereby a PCR product was generated spanning the last part of the villin promoter sequence connected with the ATG start

sequence and subsequently the first part of CYP3A4, flanked by AflIII and AatII digestion sites. The AflIII and AatII digested fragment was then inserted into the digested AflIII and AatII sites of pKS 9kbVill (kindly provided by D. Louvard) containing the full murine villin promoter that stops at the AatII site, yielding pKS 9kbVill-ATG-partialCYP3A4. The Asp718 and AatII fragment released from pKS 9kbVill-ATG-partialCYP3A4 was subsequently inserted into the Asp718- and AatII-digested pGL3-CYP3A4-SV40, yielding pGL3-9kbVill-CYP3A4-SV40 (Fig. 2A). An EcoRV-SmaI excised insert of this clone was used to inject pronuclei of FVB/N mice. Two-cell stage embryos were implanted into oviducts of pseudopregnant F1 fosters and carried to term. Transgenic founder lines were detected by initial PCR screen with forward 5'-CCATAGGAAGCCAGTTTCCC-3' and reverse 5'-CGTCTTTCAAGGTGACAGGC-3' primers located in the villin intron 1 and *CYP3A4* cDNA, respectively, to yield a 616 bp band. DNA was extracted from ear snips or tail tips of mice [16]. Southern analysis confirmed integration of the *Villin-CYP3A4*-polyA transgenic construct into the genome (data not shown). Two independent founder lines were generated (V1 and V2) and each was inbred to obtain homozygous lines as determined by Southern analysis. A ~2.1 kb XbaI *CYP3A4* cDNA fragment was used as probe. Transgenic strains with either ApoE-promoter-driven liver-specific [18] or villin-promoter-driven intestine-specific expression of human CYP3A4 were crossed to homozygosity into a *Cyp3a^{-/-}* background, and further referred to as *Cyp3a^{-/-}A* and *Cyp3a^{-/-}V*, respectively. Transgenic CYP3A4 expression was monitored over minimally 4 mouse generations and was found to be stable (data not shown).