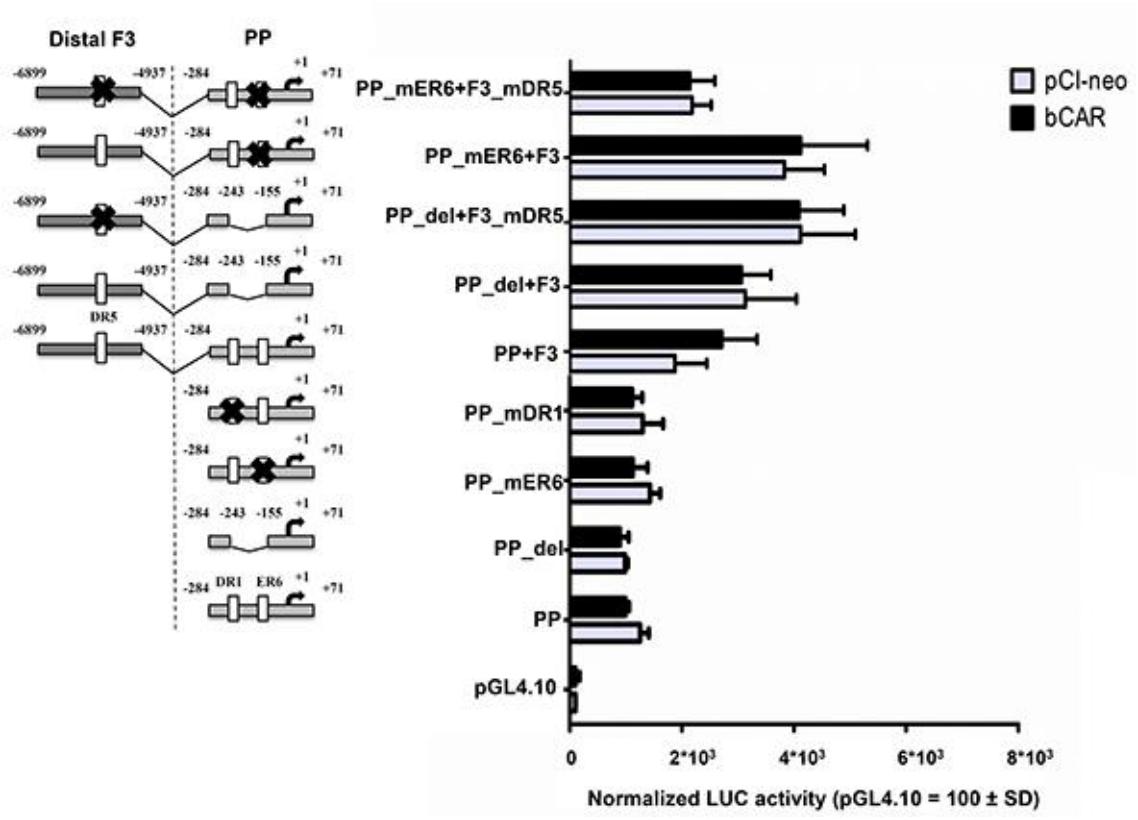
Title: DNA Elements for Constitutive Androstane Receptor- and Pregnane X Receptor-mediated Regulation of Bovine *CYP3A28* Gene

Authors: Mery Giantin, Jenni Küblbeck, Vanessa Zancanella, Viktoria Prantner, Fabiana Sansonetti, Axel Schoeniger, Roberta Tolosi, Giorgia Guerra, Silvia Da Ros, Mauro Dacasto, Paavo Honkakoski

Journal: Plos One



S3 Fig. Identification of bCAR-responsive elements in the proximal promoter and fragment 3 in *CYP3A28* promoter. Several constructs were produced to study the binding elements identified in the *CYP3A28* proximal promoter (PP) and the contribution of the binding motif DR5 identified in F3. The parental PP was deleted of the whole putative region containing several TF binding-sites resulting in the PP_del; through site-direct direct mutagenesis the ER6 (PP_mER6) and DR1 (PP_mDR1) motifs were inactivated. The parental PP+F3 was deleted of the whole putative region containing several TF binding-sites resulting in the PP_del+F3; through site-direct direct mutagenesis the ER6 (PP_mER6+F3), the DR5 motif (PP+F3_mDR5 and PP_del+F3_mDR5) or both (PP_mER6+F3_mDR5) were inactivated. Details are reported in S1 File. Numbers indicate the positions relative to the transcriptional start site. C3A cells were transfected with the control reporter pCMVβ (150 ng/well), each reporter plasmid or PBREM-tk-luc (50 ng/well) and either bCAR expression plasmid or pCI-neo empty vector (25 ng/well). After transfection, cells were treated with vehicle (0.1% DMSO) for 24 hours, and reporter activities were measured. Firefly luciferase activities were normalized with β-galactosidase activities. Data are expressed as relative activities to those in pGL4.10 transfected cells (= 100) for each condition (pCI-neo empty or bCAR co-transfection). Data are the mean ± SD (n = 3 or 4). Results shown are representative of three independent assays.