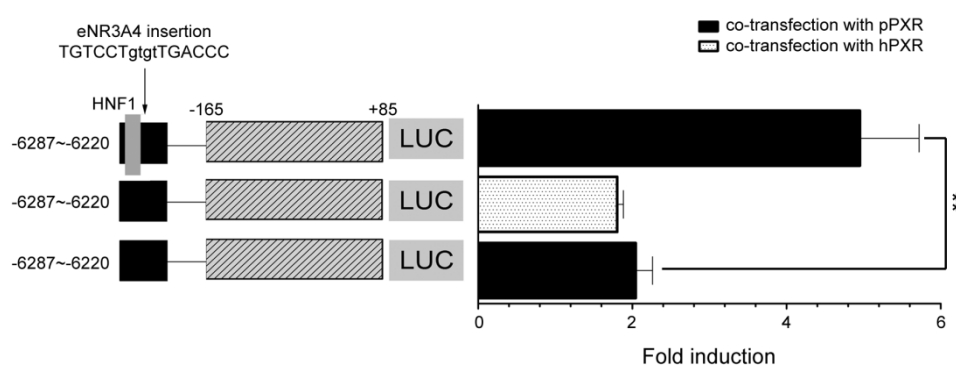# Role of Sp1, HNF1α, and PXR in the Basal and Rifampicin Induced Transcriptional Regulation of Porcine Cytochrome P450 3A46

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Supplemental Figure 1. eNR3A4 insertion enhanced the response of the (-6287~-6220)-(-165~+85)-luc construct to the RIF induction.

Human PXR binding site eNR3A4 was inserted into the site -6256 of the (-6287~-6220)-(-165~+85)-luc construct adjacent to the HNF1 binding site. The gray box indicates the HNF1 binding site. The (-6287~-6220)-(-165~+85)-luc construct or the eNR3A4 inserted (-6287~-6220)-(-165~+85)-luc construct was co-transfected with pcDNA-hPXR or pcDNA-pPXR. Transfected HepG2 cells were then cultured in the presence of RIF (10 μM) or DMSO (0.1%) for 24 h before harvest. The luciferase activities were detected and normalized as described in the legend of Fig. 2A. Each column represents the fold induction of the normalized luciferase activities in the

RIF-treated cells relative to the DMSO-treated cells. The experiment was performed four times independently, and similar results were obtained. The data shown are derived from a representative experiment expressed as the mean ± S.D. Statistical significance was calculated by one-way ANOVA, and significance was defined as **$p<0.01$.

Supplemental Table 1   Primers used for site-directed mutagenesis

Sequences for all forward primers are shown below, which are reverse complement counterpart of the reverse primers. Putative binding sites are underlined. Mutated bases are shown in bold.

Target	Sequence (5'-3')	Mutation
DR4	CACAGAATATG**TT**TTCAAAGGA**AA**TCAAACTTGAGTGTCC	**AG-TT** and **GG-AA**
DR1	GAGTTCAAAGGAGGTC**CTC**CTTGAGTGTCCATGG	**AAA-CTC**
C/EBP	GTCCATGGTGCTTTG**AGGT**CTCTCAAGGGGGGA	**CCAA-AGGT**
Distal GC box	ACTCTCAAGGGGGGA**CTC**CTCCTGCAGGAAAAG	**GGG-CTC**
Middle GC box	TTTTCACTGGCTGCAA**GAG**CAGCTCCACCCACTT	**CCC-GAG**
Proximal GC box	ACCCCAGCTCCACC**AGA**TTCCCAGCATATAAATC	**CAC-AGA**
HNF1 (CYP3A46)	CATAGTCAACAATGAT**AGGA**TCAGGATTCACTTCC	**TAAT-AGGA**
HNF1 (CYP3A4)	AATTATTTCAGTGATTAT**GCCT**CCTTGTCCTGTGTTG	**TAAA-GCCT**
eNR3A4 (insertion)	CAACAATGATTAATTCATTGTCCTGTGTTGACCCCGGATTCACTTCCCCT	---------------