SUPPLEMENTARY INFORMATION

Plasmid constructions

For yeast two-hybrid screening, a Krox20 bait was prepared by cloning a *Bam*HI fragment encoding amino acids 184 to 470 from plasmid pSCT-Krox20 (Vesque, unpublished) into the *Msc*I site of pDBLeu (Invitrogen). Other constructs were generated as follows: for plasmids encoding amino acids 184 to 332 and 332 to 470, *Bam*HI-*Apa*LI and *Apa*LI-*Xba*I fragments obtained from plasmid pSCT-Krox20 were cloned into the *Not*I and *Stu*I sites of pDBLeu, respectively; for plasmids encoding amino acids 329 to 425 and 422 to 470, *Sma*I-*Spe*I fragments to be cloned in pDBLeu were obtained by digestion of PCR fragments obtained with the primers 5'-CAGCCCGGGCAAAACGCCAGTGCACG-3' and 5'-AGACTAGTCTGCTCTTCCGTTCCTTC-3' or 5'-CCTCCCGGGGAAGGAACGGAAGAGCA-3' and 5'-TGACTAGTAGAGCTTCATCTCACGGT-3', respectively; for plasmids containing the R409W and S382RD383Y mutations, the *Psh*AI-*Sfi*I fragment of the wild-type construct was substituted by mutant fragments from plasmids pAdRSVKrox20R409W (Giudicelli et. al. 2001) and pAdRSVKrox20S382R/D383Y (see below), respectively. For Krox20 *in vitro* coupled transcription and translation (TNT), the cDNA was cloned in plasmid pBluescriptII KS+ (Stratagene) and transcribed from the T7 promoter. The GST-Krox20 fusion was obtained by cloning the *Sty*I-*Xba*I fragment from *Krox20* cDNA into the *Sma*I site of pGEX-4T-1 (Amersham Biosciences, Inc.). TNT Krox20R409W and GST-Krox20R409W constructs were derived from TNT Krox20 and GST-Krox20 constructs by substituting the wild-type *Psh*AI-*Sfi*I fragment for that of plasmid pAdRSVKrox20R409W. The expression constructs for electroporation

pAdRSVSp, pAdRSVKrox20 and pAdRSVKrox20R409W were previously described (Giudicelli et. al. 2001). The pAdRSVKrox20S382R/D383Y was derived from pAdRSVKrox20 by site directed mutagenesis using the oligonucleotide 5'AACTTCAGCCGAAG**AT**ACCACCTTACTACTCAC-3' (the mutated residues are in bold) using the Transformer Site-Directed Mutagenesis Kit (Clontech). Construction of the *PIASxβ* TNT construct involved the transfer of the two-hybrid clone into pBluescriptII SK+ (Stratagene) and substitution of the 5' end by a PCR fragment obtained with the primers 5'-GACCATGGCGGATTTCGAGGAG-3' and 5'-GAAGGCAACGAGTGGATCCCAG-3'. The other TNT *PIASxβ* constructs were prepared as follows: 1-489, 1-339 and 1-101 were C-terminal deletions from *Nsi*I, *Spe*I and *Bam*HI sites, respectively; 486-621 and 336-621 were N-terminal deletions from *Bam*HI and *Spe*I sites, respectively; 1-286 was a 3' replacement by a PCR fragment obtained with primers 5'-CATCACCCGTAGAACCTGACT-3' and 5'-AGTAACTAGTTCCAATTTCAGATGC-3'; 132-621 was a 5' replacement by a PCR fragment obtained with primers 5'-AAGCCCACGCTAGCCATGCAGCAGCCGT-3' and 5'-AGACTAGTTGTAGCAATTTCACTA-3'(SPE); 101-286 was a N-terminal deletion to the *Bam*HI site of the construct 1-286. Two-hybrid constructs of *PIASxβ* were as follows: Δ102-162 and Δ102-131 were substitutions of the *Bam*HI-*Spe*I fragment of the original clone by the PCR fragments obtained with primers 5'-TTCGGATCCAGCCCACGAGTTTAGTTCA-3' and SPE or 5'-CCAGGATCCAGATGCAGCAGCCGTCTCCG-3' and SPE, respectively; Δ133-162 was a substitution of the *Sal*I-*Spe*I fragment of the original clone by a PCR fragment obtained with primers 5'-TATAACGCGTTTGGAATCACT-3' and 5'-GGACTAGTCGGGCGCGCCATCTCAAAGGTGGGCTTA-3', followed by cloning of a PCR fragment obtained with primers 5'-

TTGGCGCGCCCCACGAGTTTAGTTCAA-3' and 5'-GCACATCAAGGACACTCGAAG-3' in the *Bss*HII-*Spe*I sites. The *PIASy* two-hybrid construct was obtained by cloning a *Xho*I fragment from plasmid CMV-T7-PIASy (Sachdev et. al. 2001) into the *Sma*I site of pPC86. *PIASxß* expression constructs were cloned into plasmid pAdRSVSp-HA (Garcia-Dominguez et al. 2003) to generate N-terminal HA-tags. For expression of full-length *PIASxß*, a *Nco*I fragment from the TNT construct was cloned into the *Pml*I site of pAdRSVSp-HA. Expression constructs 1-489 and 132-621 were derived from the corresponding TNT constructs. Expression construct of Δ102-162 was derived from the corresponding two-hybrid construct. Expression construct of Δ340-396 was generated by substitution of the *Spe*I-*Nsi*I fragment of the full-length construct by a PCR fragment obtained with primers 5'-TGCCTATACTAGTCTGATACTAGA-3' and 5'-TTGGACTGCTTTGTGTTTCTGACA-3'. The GST-PIASxß fusion construct was obtained by cloning the *Nco*I fragment from the full-length TNT construct in the *Sma*I site of pGEX-4T-1. The Par4 positive clone isolated in the two-hybrid screening encoded the 158 C-terminal amino acids. The two electroporation reporter constructs consisted of the *lacZ* gene driven by a minimal ß-globin promoter associated with the following enhancer elements: a 470 bp SacI-BglII fragment carrying the mouse *EphA4*-r3/r5 enhancer (construct#10 of Theil et al., 1998) or a PstI-HindIII 2130 bp fragment carrying the mouse *Hoxb1*-r4 enhancer (Studer et. al. 1994).

References

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