Supplementary Information

METHODS

Plasmid constructs. Deletion constructs of Krox20 were performed by standard PCR techniques. All expression constructs except GFP-sumo1 were derived from vector pAdRSV-Sp (RSV) (Giudicelli *et al*, 2003), with flag, HA or His tail tags. Mouse *Nab1*, *Nab2*, *Ubc9*, *CHD4, Aos1,* and *Uba2*, and human *sumo1* and *sumo2* were cloned by RT-PCR reactions with RNA isolated from mouse P19 or human 293T cells. The Krox20 I268F, Nab2 Q64RH95Q and the Ubc9 C93S mutations, and the HA-neuroM expression and *EphA4-lacZ* reporter constructs have been described elsewhere (Garcia-Dominguez *et al*, 2006; Garcia-Dominguez *et al*, 2003; Giorgino *et al*, 2000; Svaren *et al*, 1998). The RanGAP1-C-ter expression construct, corresponding to the C terminus of RanGAP1, was derived from plasmid pET28RanGAP1-C2 (Uchimura *et al*, 2004), and was used as a control for sumoylation (Lee *et al*, 1998). Mutation of Lys379 and Lys517 of Nab2 to Arg was conducted by standard PCR techniques. The GFP-sumo1 construct was based on the pEGFP-C2 plasmid (Clontech). GST constructions were derived from plasmid pGEX-6P-3 (GE Healthcare). The pET28a plasmid (Novagen) was used for bacterial production of Nab2.

Protein purification and in vitro sumoylation assay. Mouse Krox20, Ubc9, Aos1 and Uba2, and matured human sumo1, were produced in *E. coli* DH5α at 20 °C as GST fusions and purified with Glutathione Sepharose 4B beads (GE, Healthcare) as indicated by manufacturer. Except for Aos1 and Uba2, the GST moiety was excised by using the PreScission protease (GE, Healthcare). His and flag-tagged mouse Nab2 was produced in *E. coli* BL21(DE3) at 4 °C and purified with His-Select Nickel Affinity Gel (Sigma) as indicated by manufacturer. In vitro sumoylation assays with purified Nab2 and Krox20 were performed at 30 °C for 2 h in 20 µl of 20 mM Hepes pH 7.5, 50 mM NaCl, 4 mM $MgCl_2$ and 1 mM DTT buffer, containing 0.2 µg of Aos1/Uba2 mix (E1), 0.25 µg of Ubc9 and 1 µg of sumo1. Reactions were initiated with 250 µM ATP and stopped with SDS and ß-mercaptoethanol-containing Laemmli buffer.

Western blot. Antibodies and dilutions were as follows: mouse monoclonal anti-flag M2 (Sigma-Aldrich, 1:2000), rat monoclonal anti-HA (Roche, 1:2000), rabbit anti-Krox20 (Covance, 1:1000), mouse anti-Nab2 1C4 (Santa Cruz Biotecgnology sc-23867, 1:1000), goat anti-mouse HRP, anti-rat HRP and anti-rabbit HRP (Sigma-Aldrich, 1:10,000).

Quantitative PCR. For gene expression analysis total RNA was isolated with the RNAsy kit (QIAGEN). For retrotranscription of RNA we used the Superscript III enzyme (Invitrogen). Quantitative PCR reactions, in triplicate, were performed with the SensiMix SYBR Low-ROX kit (BIOLINE) in the Applied Biosystems 7500 FAST Real-Time PCR System. *GAPDH* was used for normalization. Primers for gene expression analysis and ChIP are described in Table S1. Algorisms for calculation of relative units and normalization of values according to oligonucleotide efficiencies are described in (Pfaffl, 2001).

Immunofluorescence and in situ hybridization. Antibodies and dilutions were as follows: rabbit anti-EphA4 S-20 (sc-921, Santa Cruz biotechnology, 1:100), mouse anti-Ubc9 (BD Bioscience, 1:100), donkey anti-rabbit DyLight549 and DyLight488, and donkey anti-mouse DyLight649 (Jackson ImmunoResearch, 1:800). Full-length *Ubc9* was cloned in pBluescript SK+ for synthesis of antisense RNA probe. cDNA was obtained by PCR with primers based on available EST sequences. Other probes were previously described: *Krox20* (Giudicelli *et al*, 2001), *EphA4* (Sajjadi and Pasquale, 1993), *Hoxb1* (Guthrie *et al*, 1992).

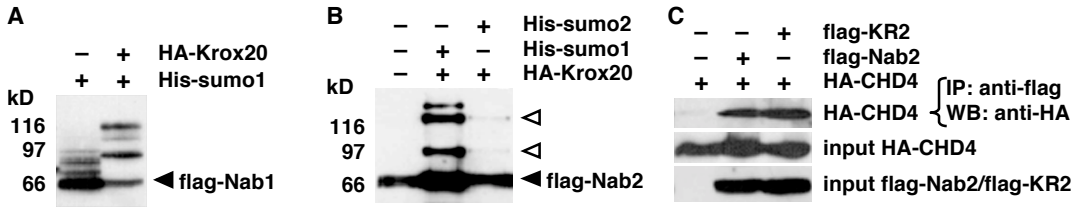

Fig S1. Analysis of Nab sumoylation. 293T cells were transfected with flag-Nab1 (A) or flag-Nab2 (B) and the constructs indicated at the top of each panel. Flag-tagged proteins were detected by Western blot. Black arrowheads indicate non-modified proteins while open arrowheads indicate modified proteins. Nab1 was sumoylated in 293T cells (A). Nab2 was more efficiently sumoylated by sumo1 than by sumo2 (B). (C) To test interaction of Nab and the KR2 mutant with CHD4, 293T cells were transfected with expression constructs of these molecules as indicated. For CHD4 we expressed the Nab-interacting domain tagged to the HA epitope (Srinivasan *et al*, 2006). Cell extracts were subjected to immunoprecipitation experiments with anti-flag antibodies and precipitates were analyzed by Western blot with anti-HA antibodies. 10% of input proteins are also shown.

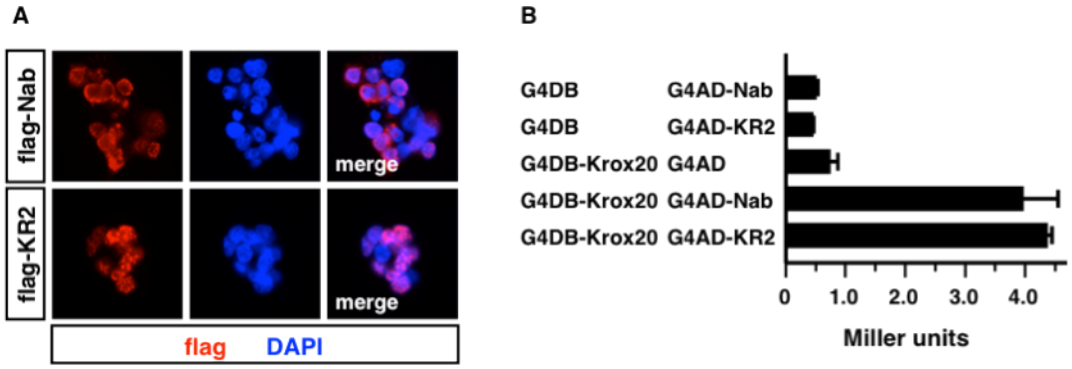

Fig S2. The KR2 mutant localizes to the nucleus and interacts with Krox20. (A) 293T cells transfected with expression constructs corresponding to flag-tagged wild type Nab (Fl-Nab) and the KR2 mutant were analyzed by immunofluorescence using an anti-flag antibody (red). DNA was visualized by DAPI staining (blue). (B) ß-galactosidase assays were used to analyze the interaction between the indicated proteins by yeast two-hybrid. Bait and prey constructions were based on Gal4 DNA binding domain (G4DB) and Gal4 activation domain (G4AD) vectors, respectively. Activities are indicated in Miller units and correspond to means of three independent experiments ± s.d.

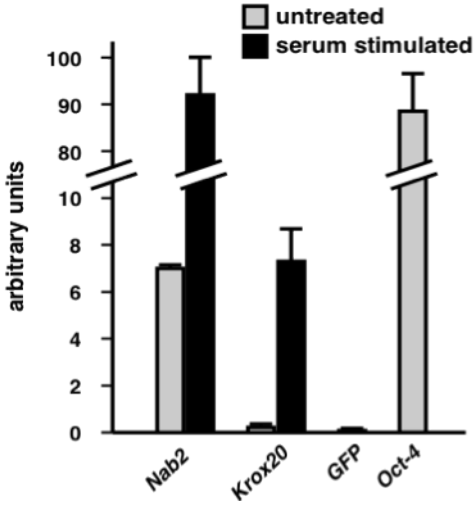

Fig S3. Expression levels of *Nab2* and *Krox20* genes in P19 cells. Expression levels of *Nab2* and *Krox20* in proliferating P19 cells (grey bars) were determined by quantitative PCR after retrotranscription of the isolated RNA. Levels of well-expressed and not expressed genes such as *Oct-4* and *GFP*, respectively, were determined as controls. Levels of *Nab2* and *Krox20* were also measured after serum stimulation (black bars). Means of arbitrary units from three independent experiments ± s.d. are represented.

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Table S1. Sequence of primers and siRNAs

Primer	*Sequence*
Nab2-F	AGGAAGAGGAGATCCGGAAG
Nab2-R	GTGTTGTCCCTCATGCAGAA
Krox20-F	CAGGAGTGACGAAAGGAAGC
Krox20-R	GACCAGAGGCTGAAGACTGG
GAPDH-F	AACTTTGGCATTGTGGAAGG
GAPDH-R	GGATGCAGGGATGATGTTCT
Id4prom-F	GCGCGGCTCTACAAATACTGC
Id4prom-R	AACCGCGCCTCCCAGCTCAAC
GFP-F	CAAGATCCGCCACAACATCG
GFP-R	GTCCATGCCGAGAGTGATCC
Oct4-F	CCAATCAGCTTGGGCTAGAG
Oct4-R	CTGGGAAAGGTGTCCCTGTA
siRNA Krox20#1	CGCCAAGGCCGUAGACAAA
siRNA Krox20#2	GCCCUUCCAGUGUCGGAUC
siRNA GFP	GGCACAAGCUGGAGUACAA