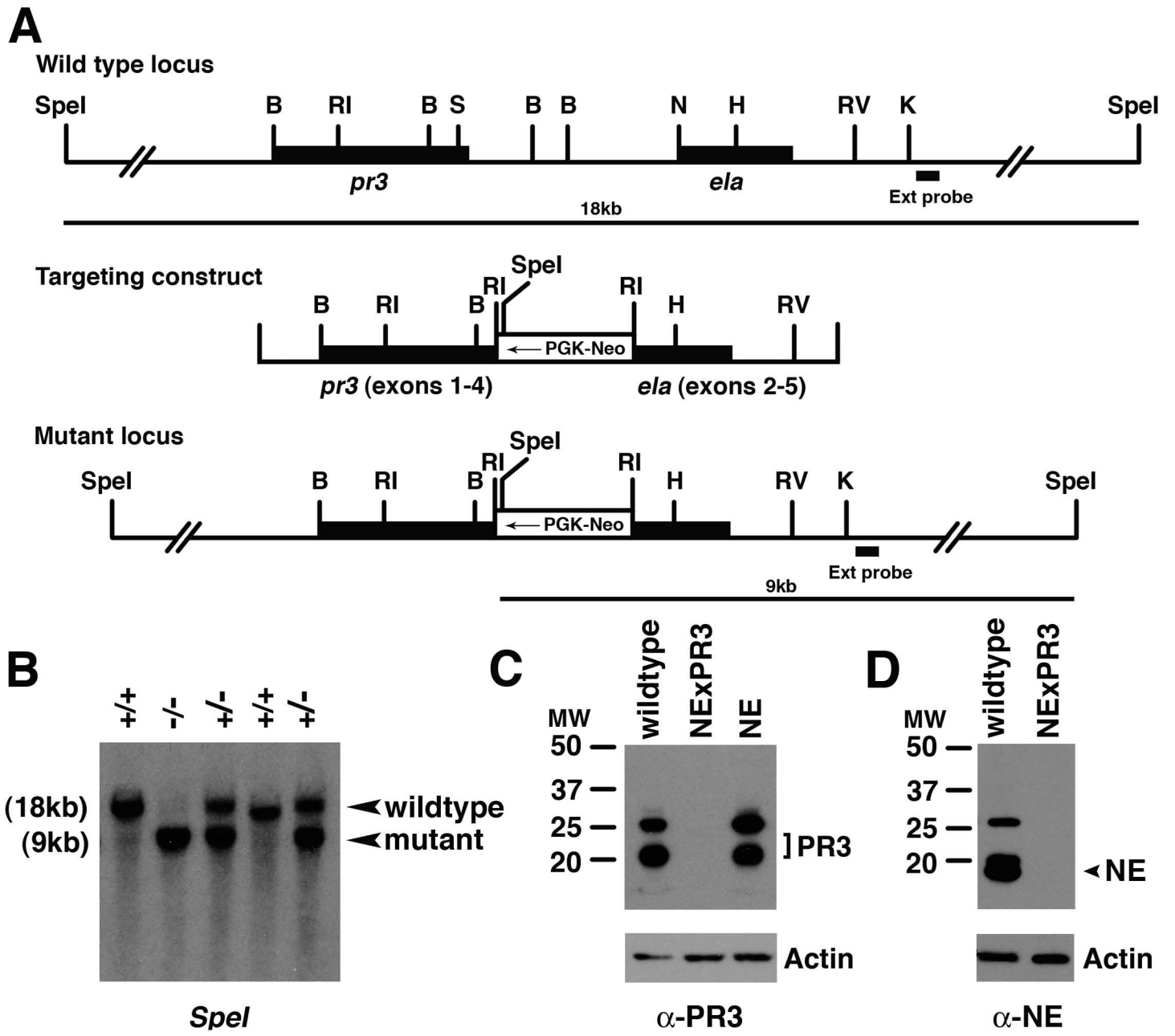

Generation of NExPR3-deficient mice. (**A**) A 4.4kb fragment that contains the 5'UT sequence and exons 1-4 of the *pr3* gene was amplified by PCR and used as the 5'-arm of the targeting construct. A 3.6kb fragment that contains exons 2-4 and the 3'UT of the *ne* gene (*ela*) was also amplified by PCR and used as the 3'-arm of the targeting construct. To assemble the targeting construct a 1.8kb EcoRI fragment containing the neomycin phosphotransferase gene, driven by the phosphoglycerate kinase 1 gene (PGK) promoter and flanked by LoxP sites, was subcloned in the antisense orientation into a pCR2.1 vector containing the 5'- and 3'-arms. The targeting construct was designed to replace exon 5 of the *pr3* gene, the intervening sequences, and exon 1 of the *ela* gene with the PGK-Neo reporter cassette. The complete vector was linearized with *SalI* and electroporated into SCC10 (129/SvJ) ES cells. Correctly targeted ES clones were confirmed by Southern blot analysis and microinjected into C57BL/6 blastocysts and transferred into pseudopregnant female mice. Chimeric males were bred to C57BL/6 females. B, *BamHI*; H, *HindIII*; K, *KpnI*; N, *NcoI*; RI, *EcoRI*; RV, *EcoRV*; S, *SalI*. (**B**) Germline transmission of the disrupted *pr3/ela* allele was confirmed by Southern blot analysis. Heterozygous animals were intercrossed to generate mutant mice. Tail DNA from a representative liter was digested with the restriction enzyme *SpeI* and analyzed by Southern blot analysis using an external probe. *SpeI* digest of wildtype locus resulted in a 18kb fragment while the mutant locus resulted in a 9kb fragment due to the introduction of a new *SpeI* site in the PGK-Neo gene. The heterozygous mice were completely backcrossed to C57BL/6 strain for 9 generations by speed congenics. Complete absence of PR3 (**C**) and NE (**D**) protein was confirmed by Western blot analysis.