

Expanded View Figures

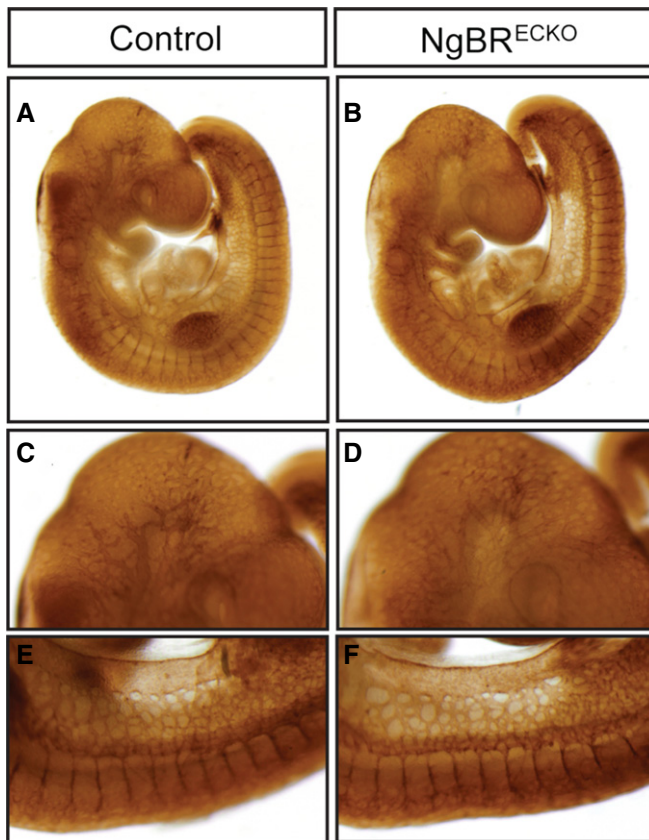


Figure EV1. NgBR endothelial KO embryos exhibit normal vascular development.

A–F Whole-mount immunohistochemistry of vasculatures with CD31 antibody in NgBR^{ECKO} and control embryos at E9.5. Properly developed vasculature was present in both mutant and control embryos. Note the similar CD31 staining pattern on head vasculature (C, D) and intersomitic vessels (E, F).

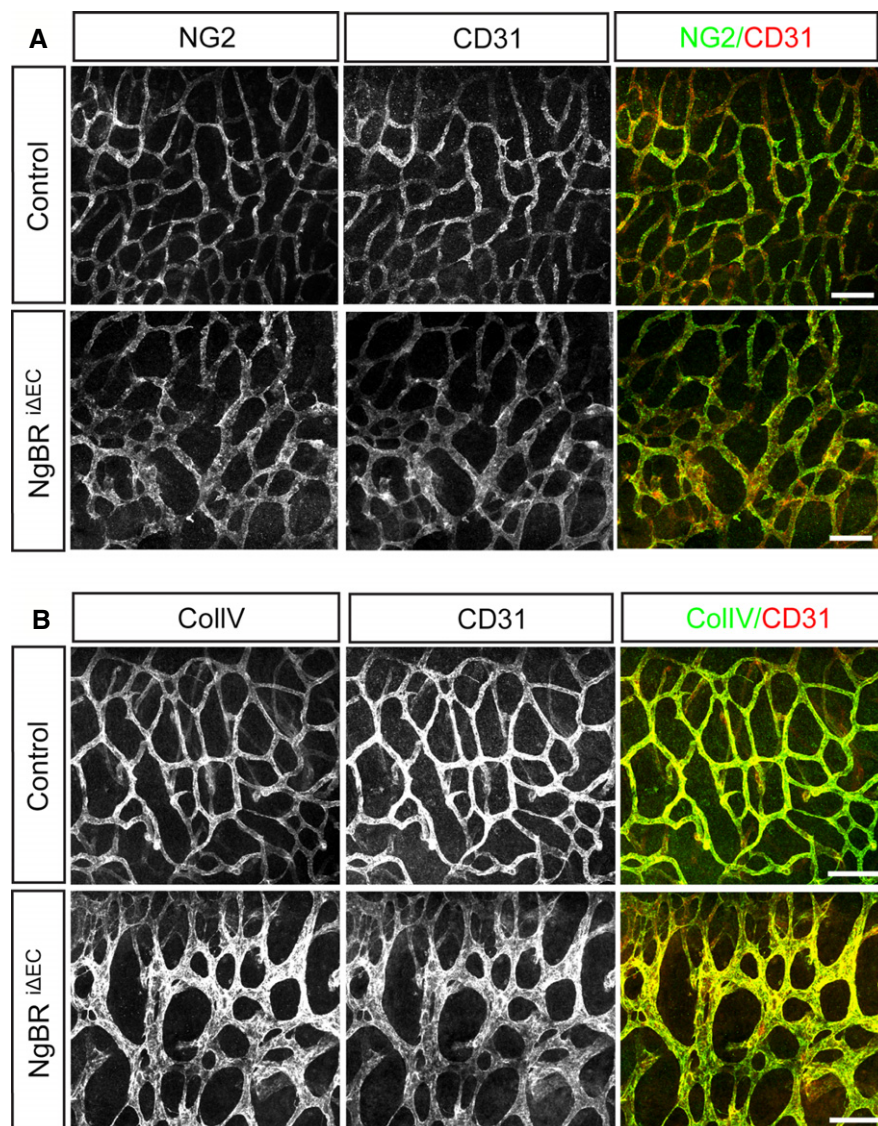


Figure EV2. Normal pericyte coverage and no vessel regression in the NgBR^{ΔEC} embryo.

A NG2 immunostaining with CD31 on the hindbrain of NgBR^{ΔEC} embryos at E12.5. There was no obvious difference in NG2-positive pericyte distribution between control and NgBR^{ΔEC}.

B Immunodetection of collagen IV and CD31 in the hindbrain of NgBR^{ΔEC} embryos at E12.5. Vessel regression that is associated with the presence of empty basement membrane sleeves was not detected in NgBR^{ΔEC}.

Data information: Scale bar, 100 μ m.

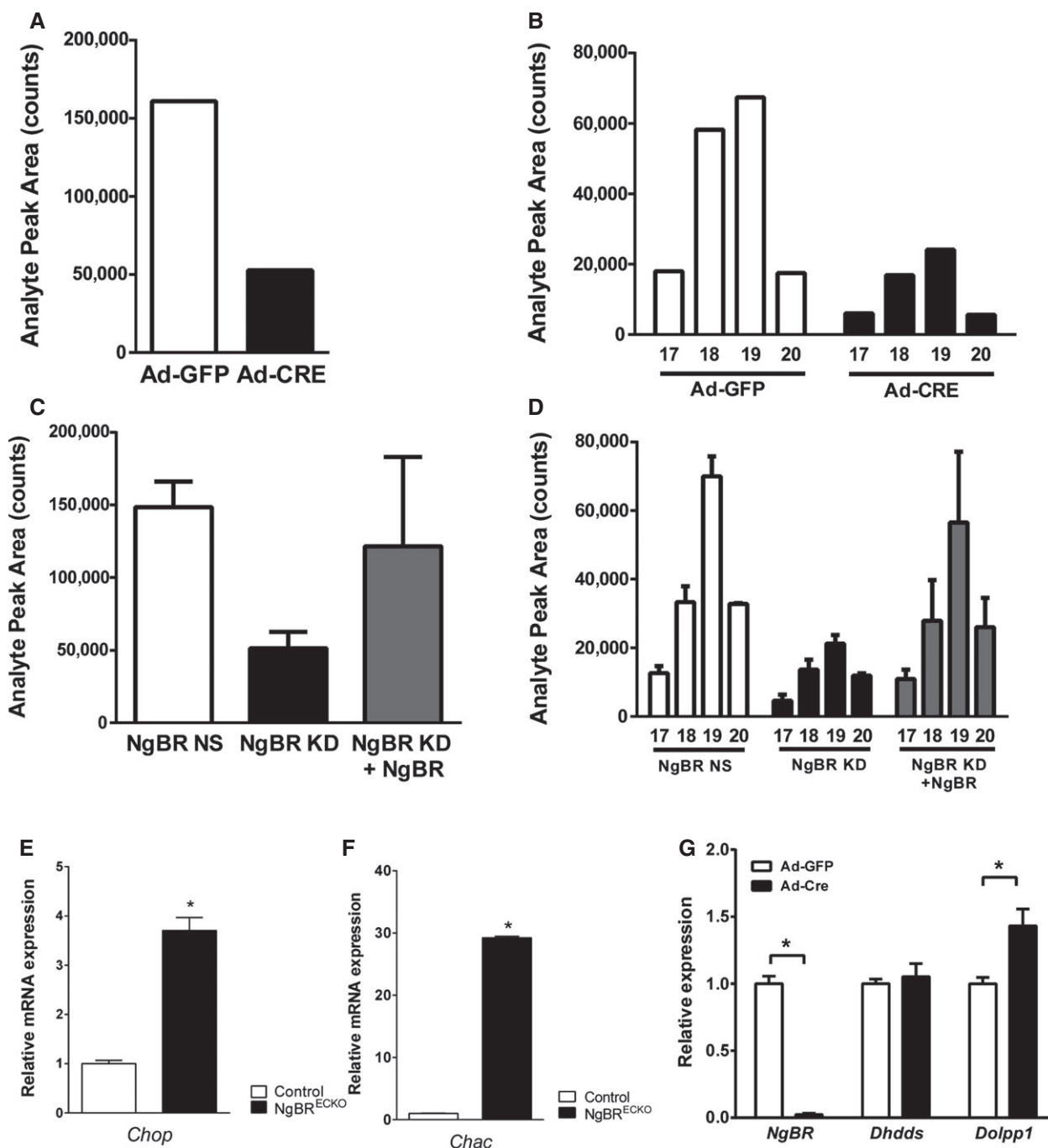


Figure EV3. Dolichol level measurement and gene expression analysis in NgBR-depleted tissues and cells.

A, B Total dolichol levels (A) and distribution of dolichol-17, 18, 19, and 20 (B) measured from primary MLEC treated with Ad-GFP or Ad-Cre.

C, D Total dolichol levels (C) and distribution of dolichol-17, 18, 19, and 20 (D) measured from HeLa cells with stable knockdown of NgBR by shRNA (NgBR KD), control (NgBR NS), and pcDNA-NgBR-transfected NgBR KD cells showing conservation of the role of NgBR. Analysis of dolichol species by liquid chromatography and mass spectroscopy was performed as described in [4].

E mRNA expression levels of *Chop* in the NgBR^{ECKO} yolk sac detected by qPCR.

F mRNA expression levels of *Chac* in the NgBR^{ECKO} yolk sac detected by qPCR.

G Primary MLEC were treated with Ad-GFP or Ad-Cre, and mRNA expression levels were determined after 5 days of infection by qPCR. In Ad-Cre-infected cells, almost no *NgBR* mRNA expression was detected. *Dolpp1* mRNA expression was upregulated in NgBR-deleted MLEC.

Data information: * $P < 0.05$. P -values were calculated by unpaired Student's t -test. Data are mean \pm SEM from 3–5 independent experiments.

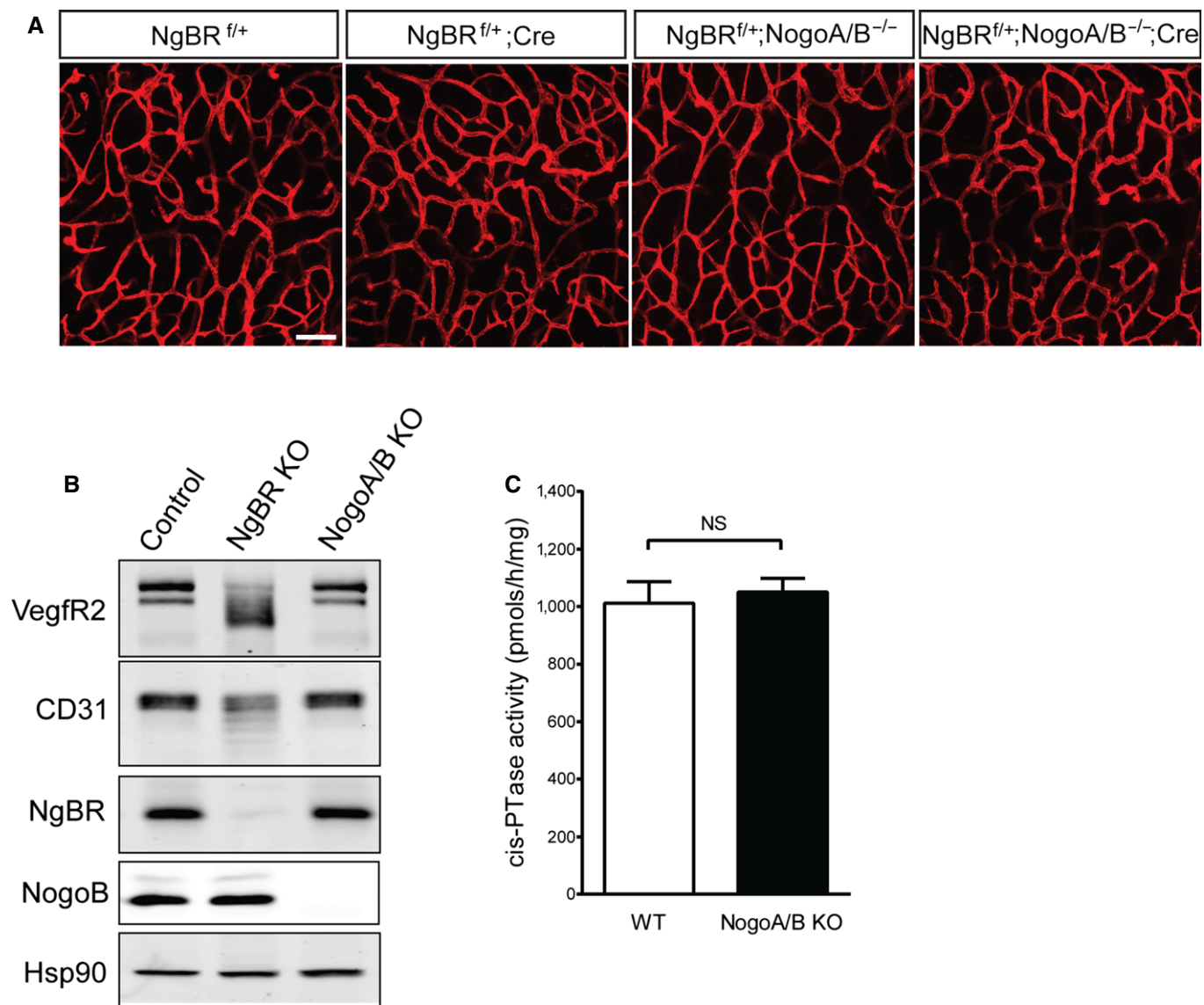


Figure EV4. Nogo-B is not required for NgBR functions in endothelial cells during embryogenesis.

A Staining with anti-CD31 on hindbrain of E12.5 embryos after tamoxifen injection at E8.5. Scale bar, 100 μ m.

B Western blot analysis for protein glycosylation in MLEC. Nogo-A/B KO MLEC were isolated from Nogo-A/B KO animals. Expression of VEGFR2 and CD31 was detected. Knockout cells were confirmed by detection with anti-NgBR and anti-Nogo-B antibodies. Hsp90 was used as a loading control.

C Microsomal cisPTase activity assay for Nogo-A/B KO MEF. No significant difference was detected between control and NogoA/B KO cells. cisPTase activity assay was performed as described in [4]. NS, not significant ($P > 0.05$). P -value was calculated by unpaired Student's t -test. Data are mean \pm SEM, $n = 3$ per group.