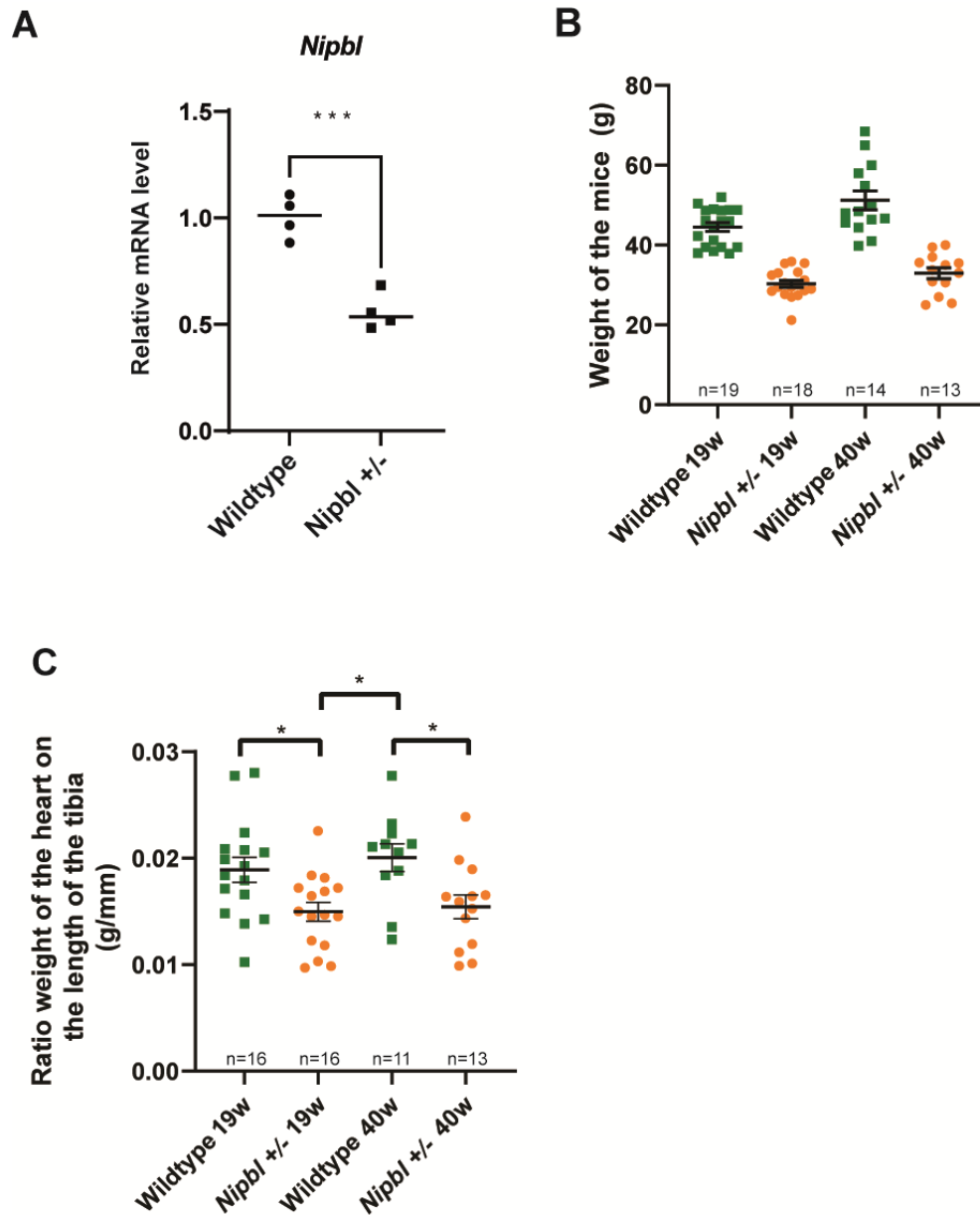
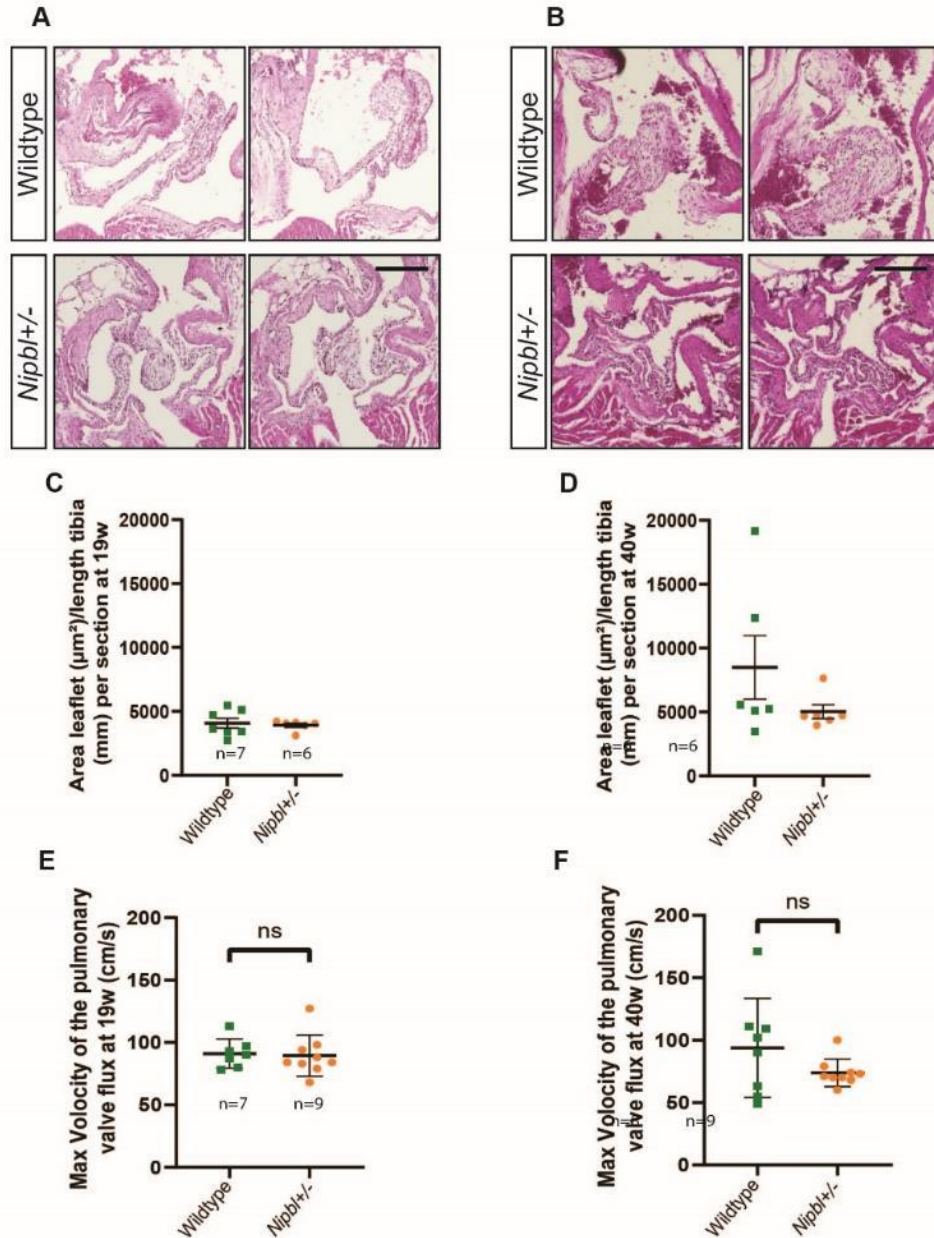


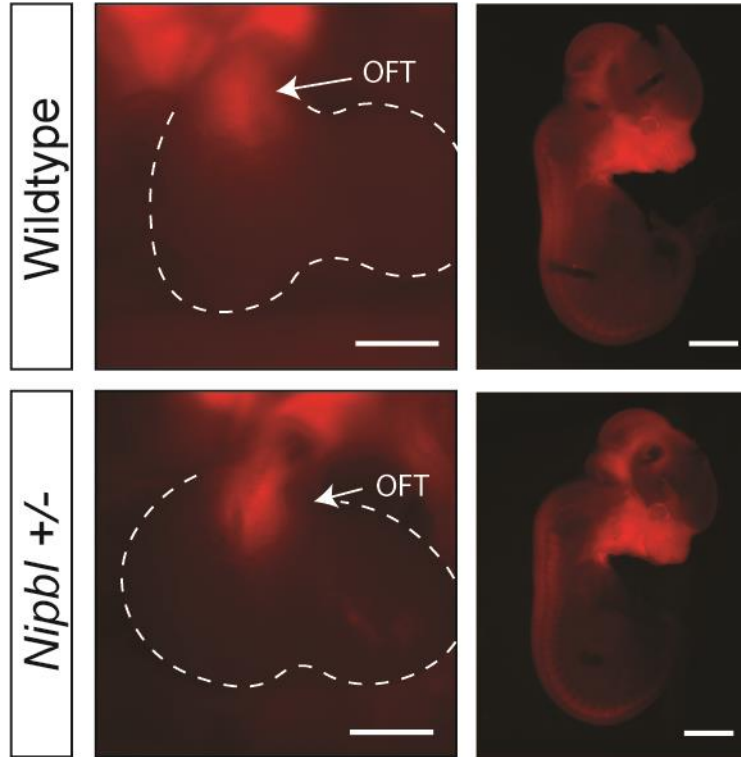
Supplementary Materials



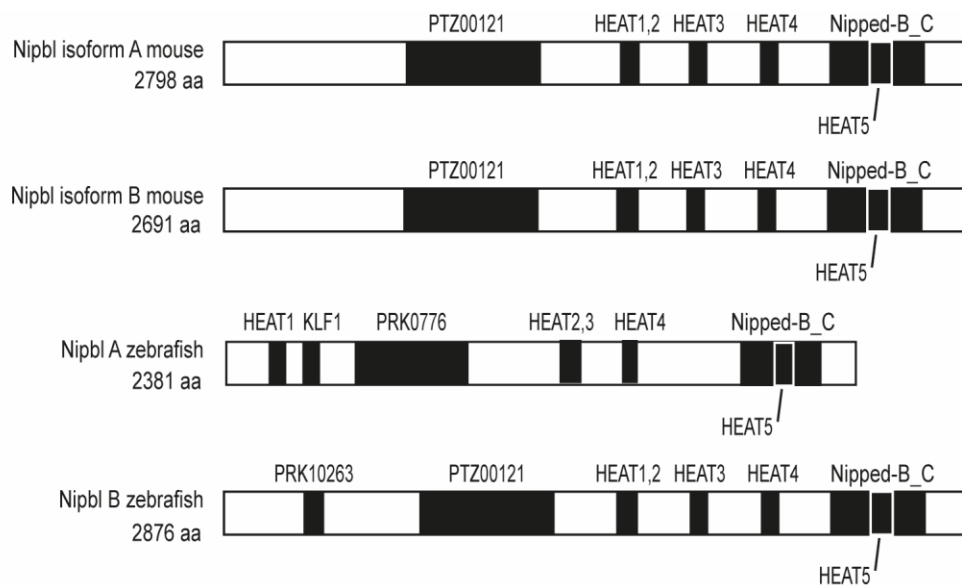
Supplemental Figure S1. A *Nipbl* transcript levels in whole E9.5 embryos. *** $p < 0.001$, two-sided and unpaired t test. **B** Weight in grams of *Nipbl*^{+/-} and wild-type littermate controls at 19 and 40 weeks. **C** Heart weight over tibia length ratios at 19 and 40 weeks. * $p < 0.05$, one way ANOVA with multiple comparisons.



Supplemental Figure S2. A-B. Hematoxylin eosin stained sections of pulmonary valves from representative 19 and 40 week-old male wild-type (WT) and *Nipbl*^{+/-} mice. **C-D.** Quantification of average pulmonary valve area over tibia length at 19 and 40 weeks. **E-F** Echocardiographic assessment of pulmonary valve function in adult wild-type control and *Nipbl*^{+/-} mice at 19 and 40 weeks. ns = not significant, $p > 0.05$; two-sided and unpaired t test. Scale bar 200 μm .



Supplemental Figure S3. Neural crest cell migration in *Wnt1-Cre+/-;ROSA-tdT+/-* and *Nipbl+/-;Wnt1-Cre+/-;ROSA-tdT+/-* embryos. Images show tdT+ (red) neural crest cell distribution in whole-mount heart (center) and whole-mount embryos (right). Dotted lines indicate the contour of the ventricles. Scale bars are 0.25mm (left) and 0.5mm (right).



Supplemental Figure S4. Nipbl protein architecture conservation in mice and zebrafish. There is a strong conservation of the C-terminal Nipped-B_C domain. C-terminal HEAT domains are involved in cohesin binding.

	Current study	<i>Schuster et al. [19]</i>
Mutant model species	Mouse	Zebrafish
Approach for cohesin targeting	Genetic deletion of Nipbl Exon 2	Morpholino knock down of Rad21
Neural crest invasion of heart	Normal	Neural crest cells present but unable to invade the heart
Defects in valve morphogenesis	Delayed outflow tract septation	Delay or absence of AV and/or VB development
Effects on valve progenitor proliferation/apoptosis	None detected	Not assessed
Endocardial EMT	Normal	Not assessed
Adult valve defects	Thickening of aortic valve	Not assessed*

AV, atrioventricular valve; VB, ventriculobulbar valve. * due to early lethality.

Supplemental Table S1. Comparison phenotypic changes observed during outflow tract development in *Nipbl*^{+/-} mice and following cohesin knockdown in zebrafish.