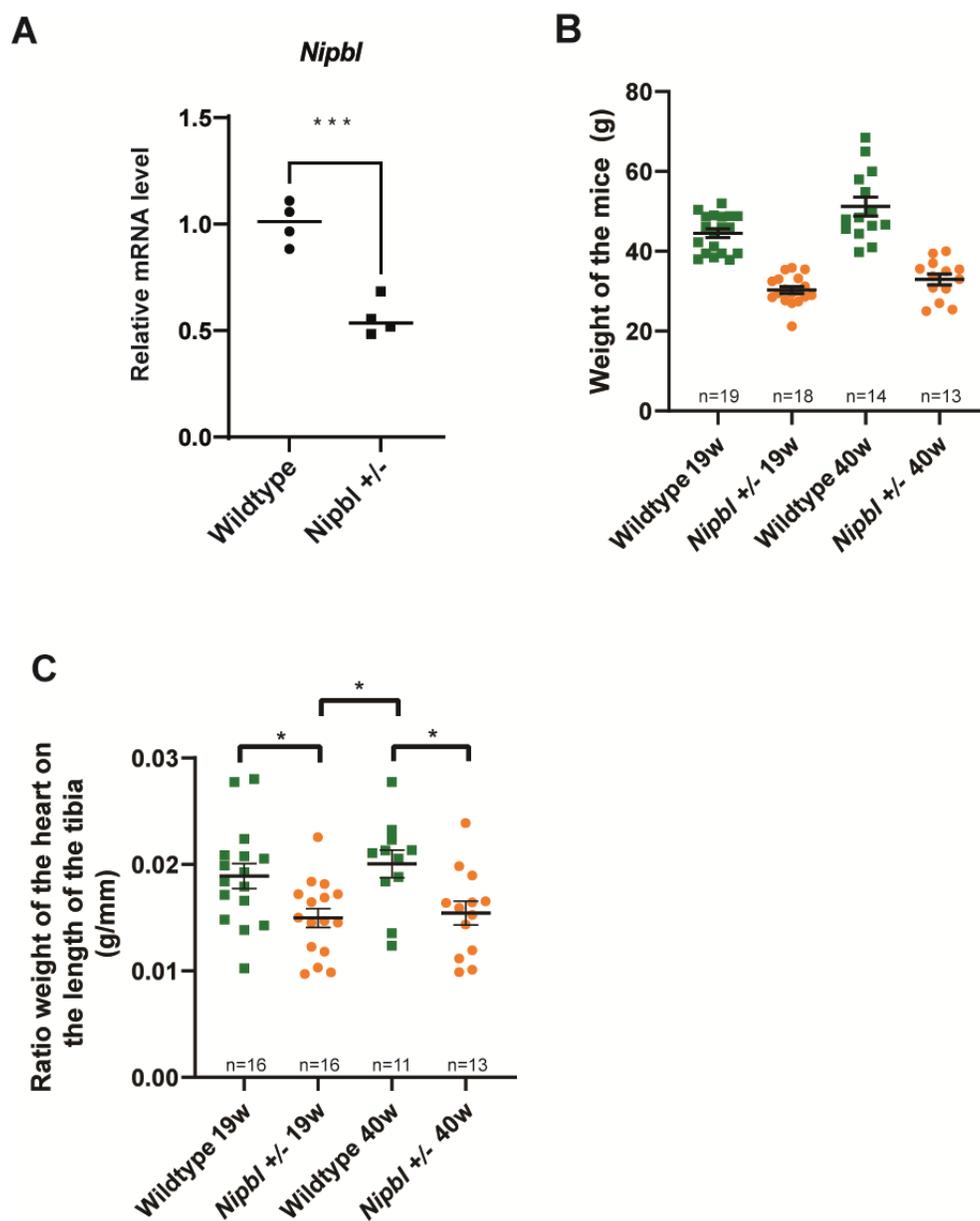
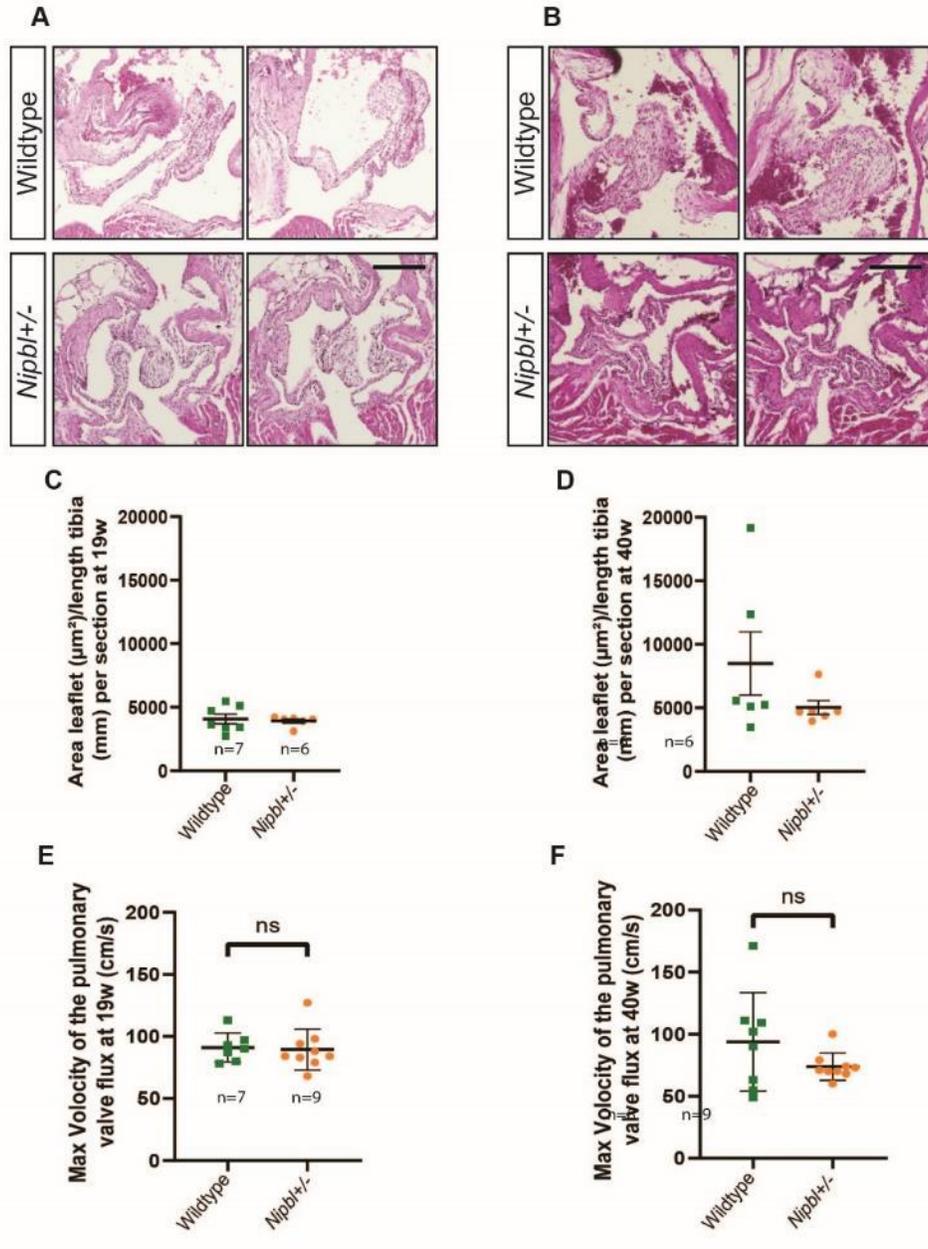


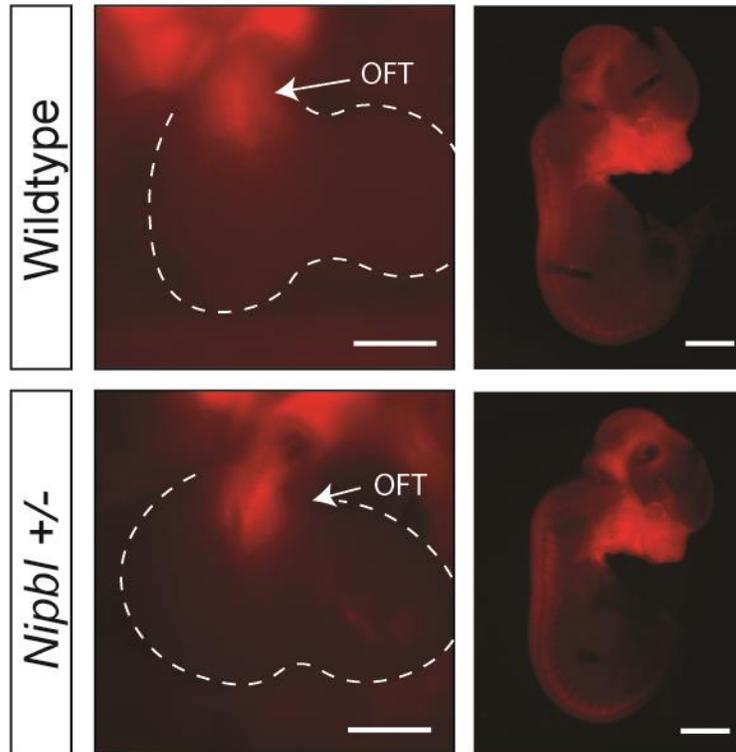
## Supplementary Materials



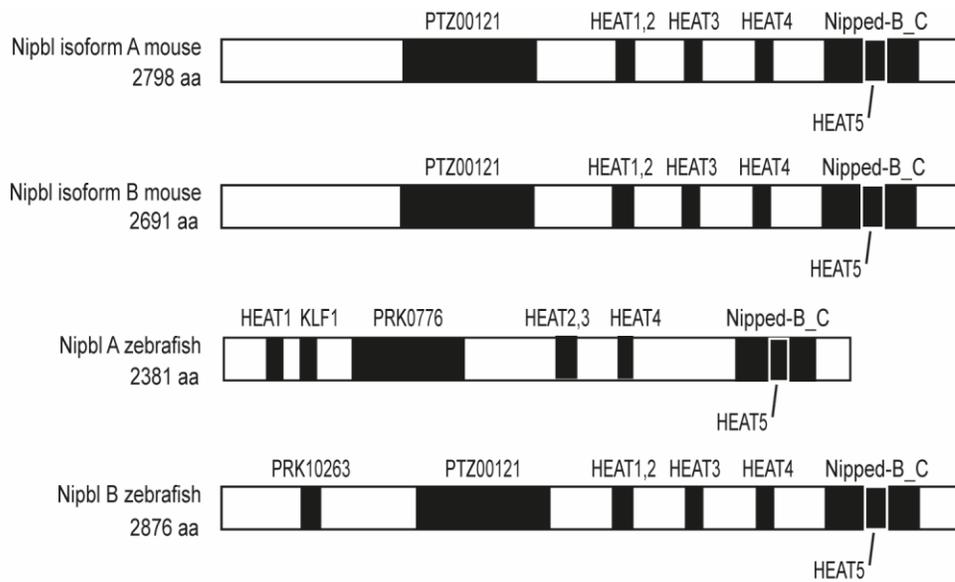
**Supplemental Figure S1.** **A** *Nipbl* transcript levels in whole E9.5 embryos. \*\*\* $p < 0.001$ , two-sided and unpaired t test. **B** Weight in grams of *Nipbl*<sup>+/-</sup> and wild-type littermate controls at 19 and 40 weeks. **C** Heart weight over tibia length ratios at 19 and 40 weeks. \* $p < 0.05$ , 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*<sup>+/-</sup> 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*<sup>+/-</sup> mice at 19 and 40 weeks. ns = not significant,  $p > 0.05$ ; two-sided and unpaired t test. Scale bar 200 $\mu\text{m}$ .



**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.

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