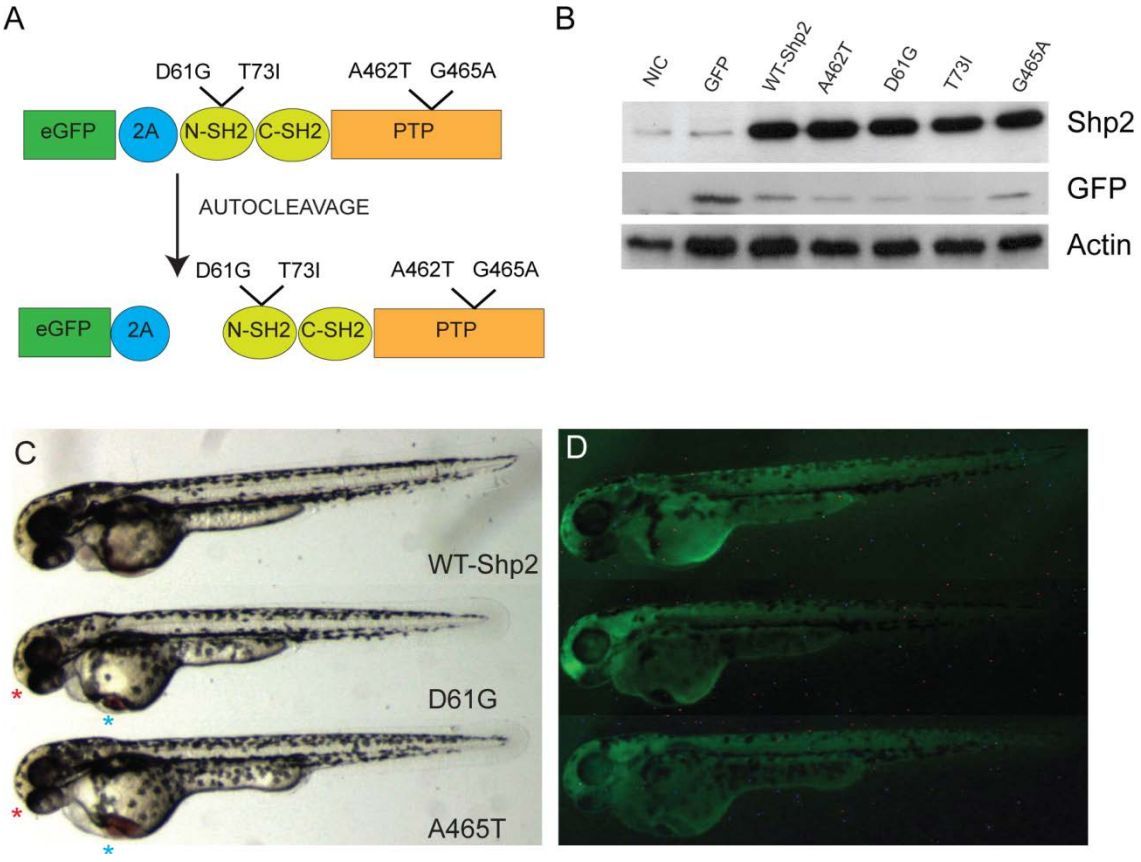
Supplementary Material

Noonan and LEOPARD syndrome Shp2 variants induce heart displacement defects in zebrafish

Monica Bonetti, Jeroen Paardekooper Overman, Federico Tessadori, Emily Noël, Jeroen Bakkers, Jeroen den Hertog



Supplemental Figure S1. EGFP-Peptide 2A-Shp2 fusions induced defects in zebrafish embryos. (A) Schematic representation of eGFP-Peptide 2A-Shp2 with D61G, T73I (NS) and A462T, G465A (LS) mutations indicated. The full length fusion protein is produced and is then cleaved autoproteolytically, resulting in eGFP and (mutant) Shp2. **(B)** One- or two-cell stage embryos were injected with *in vitro* transcribed mRNAs encoding the indicated proteins. Immunoblotting analysis reveals that autoproteolytic cleavage of the fusion proteins is highly efficient in zebrafish embryos. The injected embryos were lyzed at 10 hpf, proteins were separated on SDS-polyacrylamide gels, blotted and probed with antibodies specific for Shp2, GFP and β-actin as a loading control**. (C-D)** Two representative injected embryos (D61G and A462T) show craniofacial defects (red asterisks), a decrease in body length and cardiac edema (blue asterisks). These defects are absent in WT-Shp2 injected control embryos. Injection efficiency was checked by fluorescence microscopy with a GFP filter.

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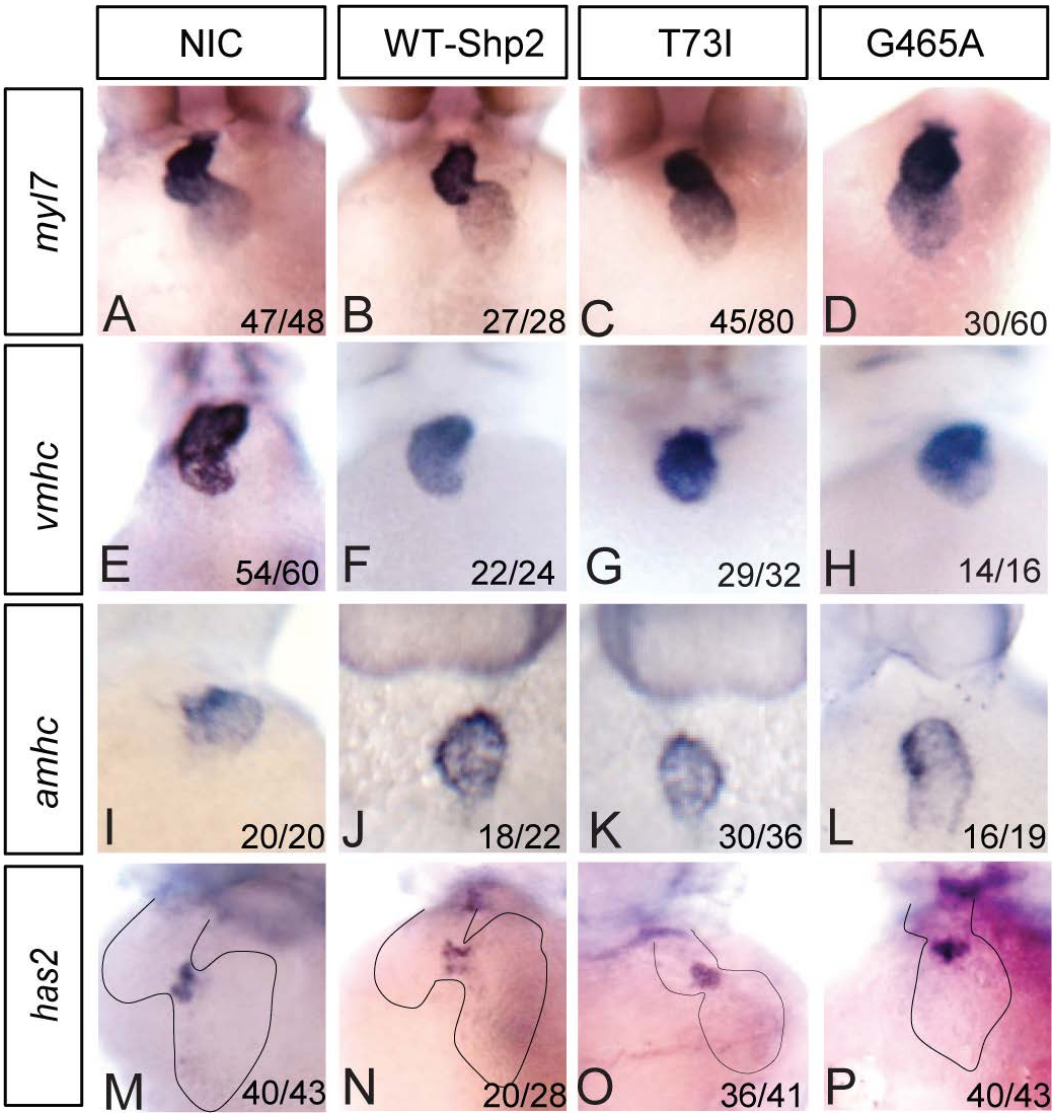

Supplemental Figure S2. Heart looping defects in embryos expressing Shp2-T73I (NS) and Shp2-G465A (LS). Non-injected control embryos (NIC) and embryos injected at the one-cell stage with mRNA encoding WT-Shp2, Shp2-T73I (NS) or Shp2-G465A (LS) were fixed at 48 hpf and *in situ* hybridization was performed using probes for the myosin genes *myl7* (A-D, cardiomyocytes), *vmhc* (E-H, ventricle), *amhc* (I-L, atrium), and for *has2* (M-P, endocardial cushions). Representative pictures are shown and the number of embryos showing this pattern as well as the total number of embryos that were analyzed is indicated in the bottom right corner of each panel. The outline of the heart is indicated with a dashed line in panels M-P.

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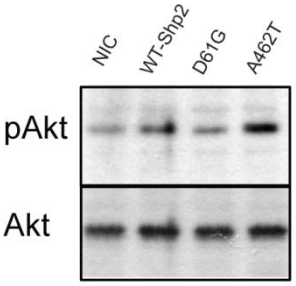

Supplemental Figure S3. Enhanced Akt signaling in Shp2-A462T (LS), but not Shp2-D61G (NS) expressing embryos. Zebrafish embryos were injected with WT-Shp2, Shp2-D61G (NS) or Shp2-A462T (LS) at the one-cell stage. Embryos were lysed at 10 hpf. Immunoblots of the zebrafish lysates were stained using antibodies specific for pAkt and Akt.

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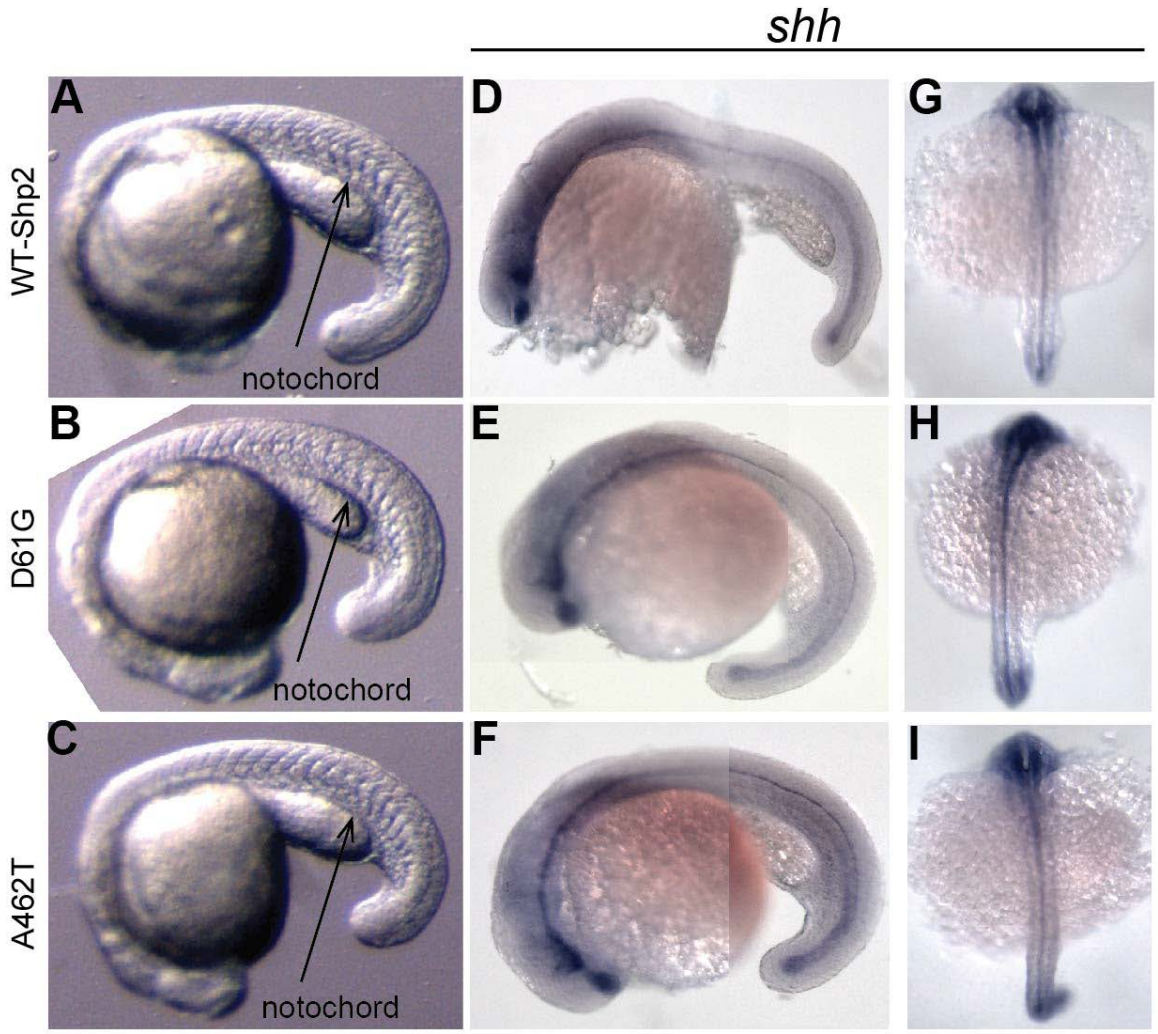

Supplemental Figure S4. Midline structures are intact in NS and LS Shp2-variants. (A-C) Bright-field images of (A) WT-Shp2 control, (B) Shp2-D61G and (C) Shp2-A462T expressing embryos, showing normal notochord formation. (D-I) *In situ* hybridization analysis showed contiguous *shh* expression in the floorplate. (D-F) Composite pictures of lateral views. (G-I) Dorsal views. The number of embryos showing the depicted expression pattern/ total number of embryos is: WT-Shp2, *n*=20/20; Shp2-D61G, *n*=30/30; Shp2-A462T, *n*=30/30.

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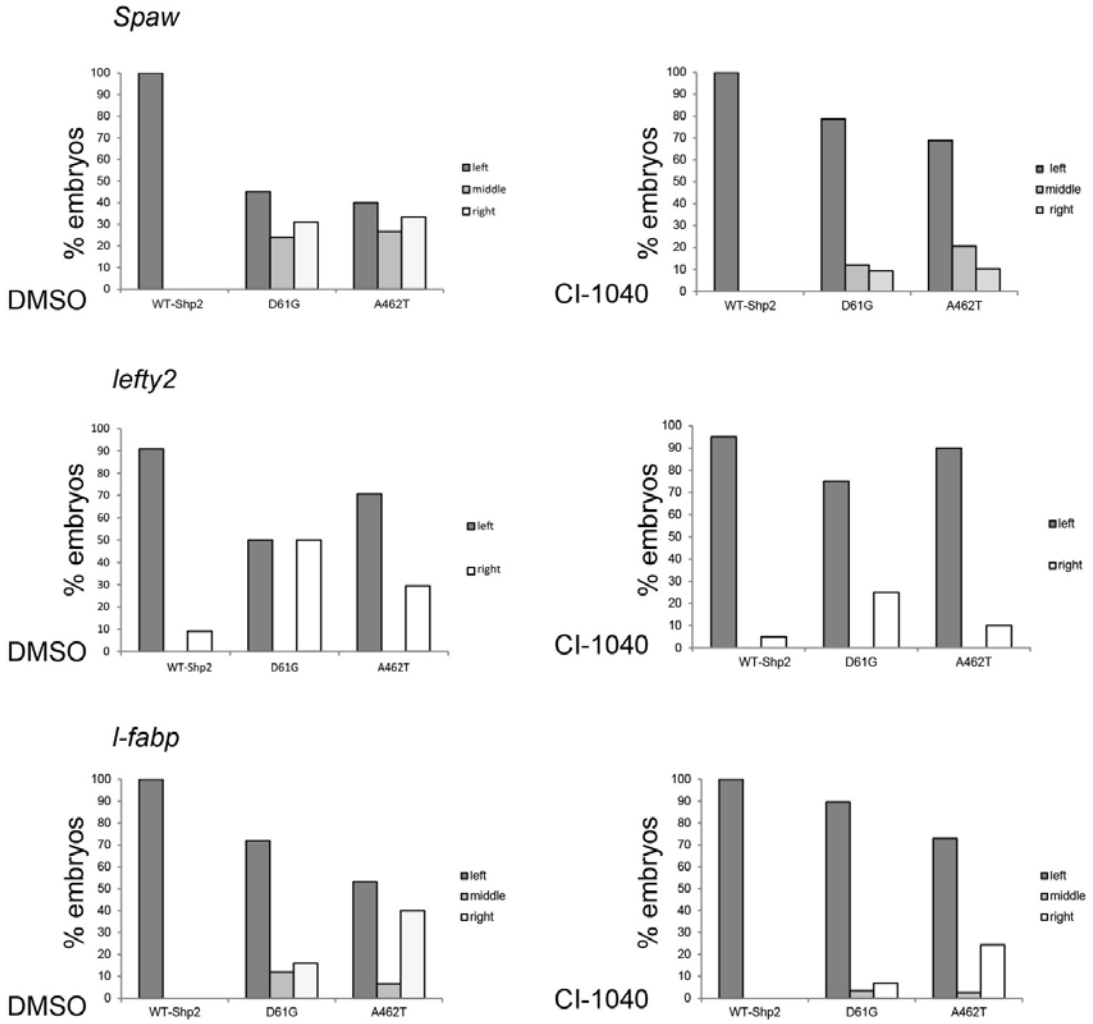

Supplemental Figure S5. Early treatment with the MEK-inhibitor, CI-1040, rescued L/R asymmetry in embryos expressing NS and LS Shp2-variants. Embryos were injected at the one-cell stage with mRNA encoding WT-Shp2, Shp2-D61G (NS) or Shp2-A462T (LS). Embryos were treated with MEK inhibitor CI-1040 (0.25 µM) for 1 h at 4.5 hpf or mock-treated with DMSO and fixed at the appropriate stage. *In situ* hybridization was done using probes specific for *southpaw (spaw*), *lefty2* and *l-fabp*. Representative embryos are depicted in Fig. 5. Asymmetry of the markers was scored for embryos injected with WT-Shp2, Shp2-D61G and Shp2-A462T. Percentages of left, middle/bilateral and right expression of the markers are depicted. The total number of embryos analyzed is: DMSO *spaw* (WT-Shp2 *n*=40, Shp2-D61G *n*=50, Shp2-A462T *n*=35); CI-1040 *spaw* (WT-Shp2 *n*=50, Shp2-D61G *n*=64, Shp2-A462T *n*=30); DMSO *lft2* (WT-Shp2 *n*=50, Shp2-D61G *n*=42, Shp2-A462T *n*=34); CI-1040 *lft2* (WT-Shp2 *n*=30, Shp2-D61G *n*=35, Shp2-A462T *n*=30); DMSO *l-fabp* (WT-Shp2 *n*=26, Shp2-D61G *n*=28, Shp2-A462T *n*=35) and CI-1040 *l-fabp* (WT-Shp2 *n*=37, Shp2-D61G *n*=34, Shp2-A462T *n*=38).