Supplementary Information to the manuscript:

TAR Syndrome-associated Rbm8a deficiency causes hematopoietic defects and attenuates Wnt/PCP signaling by Kocere et al.

This Supplementary Information contains information about the Supplementary Data 1-12 (excel tables), as well as Supplementary Figures 1-4.

Supplementary Data 1: Rescue experiment data for Supplementary Fig. 1E-K

Supplementary Data 2: Counts of *cd41:EGFP* for Fig. 2J,K, Supplementary Fig. 2E-J

Supplementary Data 3: Differential gene expression (DGE) results filtered budstage for Fig. 3B

Supplementary Data 4: Differential gene expression (DGE) results filtered budstage for Fig. 3C

Supplementary Data 5: Metascape results for Supplementary Fig.3A,B

Supplementary Data 6: DEXSeq results filtered differential intron usage for Fig. 3D

Supplementary Data 7: DEXSeq results filtered differential exon usage for Supplementary Fig. 3C

Supplementary Data 8: Metascape results for Supplementary Fig.3D,E

Supplementary Data 9: Values of SPIM area and volume for Fig. 4

Supplementary Data 10: Measurements of axis length, somite width, and neural plate width for Fig. 5

Supplementary Data 11: Counts of morpholino injections and phenotype analysis for Fig. 6

Supplementary Data 12: Measurements of ISH signal intensity for Fig. 7

**A close-up of a microscope

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**Supplementary Fig. 1: Human *RBM8A* mRNA rescues the *rbm8a* morpholino phenotype.**

(**A-D**) Uncropped images of western blots displayed in Fig. 1B. Dashed lines represent frames included in Fig. 1B. MW: molecular weight marker. 50 kDa a-Tubulin: loading control. (**A**) Ten tailbud stage embryos per well. 20 kD a-Rbm8a shows 2 bands at this timepoint, with the lower one presumably being the specific one, the upper one phosphorylated protein. (**B**) Ten 10 somite stage embryos per lane. Box indicates rbm8a mutant embryos genotyped individually prior to pooling ten embryos of respective genotype. (**C**) Lanes 2-4: 30 17 hpf embryos per lane; lanes 5-9: Ten 17 hpf embryos per lane; lane 10: Ten 26 hpf embryos per lane; lanes 4-10 loading control 50 kDa a-Tubulin. (**D**) Ten 26 hpf embryos per lane.

(**E-J**) Representative confocal images of *drl:EGFP; myl7:mCerulean;lmo2:mCherry* wildtype, morphant, rescued and control injected zebrafish embryos at 48 hpf with brightfield images for reference on the right. (**E**) Representative image of wildtype embryo. (**F**) Representative image of MO-*rbm8a*ATG injected embryo. (**G**) Representative image of *EGFP* mRNA injected control embryo. (**H**) Representative image of MO-*rbm8a*ATG and *EGFP* mRNA co-injected control embryo. (**I**) Representative image of *RBM8A* mRNA injected control embryo. (**J** Representative image of MO-*rbm8a*ATG and *RBM8A* mRNA co-injected (rescued) embryo.

(**K**) Overview of rescue. Note how nearly 100% of MO-*rbm8a*ATG groups and only 25% of the rescued embryos have a phenotype. Datapoints (%) shown with mean and standard deviation (see **Supplementary Data 1** for details).

(**L**) Compound heterozygosity for *1q21.1* microdeletions and polymorphisms in human *RBM8A* as observed in TAR syndrome patients, and *rbm8a* mutant allele combinations in zebrafish. Created with Biorender.com (Subscription: Individual; Agreement number: TT25WRFMB0). Scale bar in **E**: 200 μm (applies to panels **E-J** and corresponding brightfield images).

A collage of images of embryos

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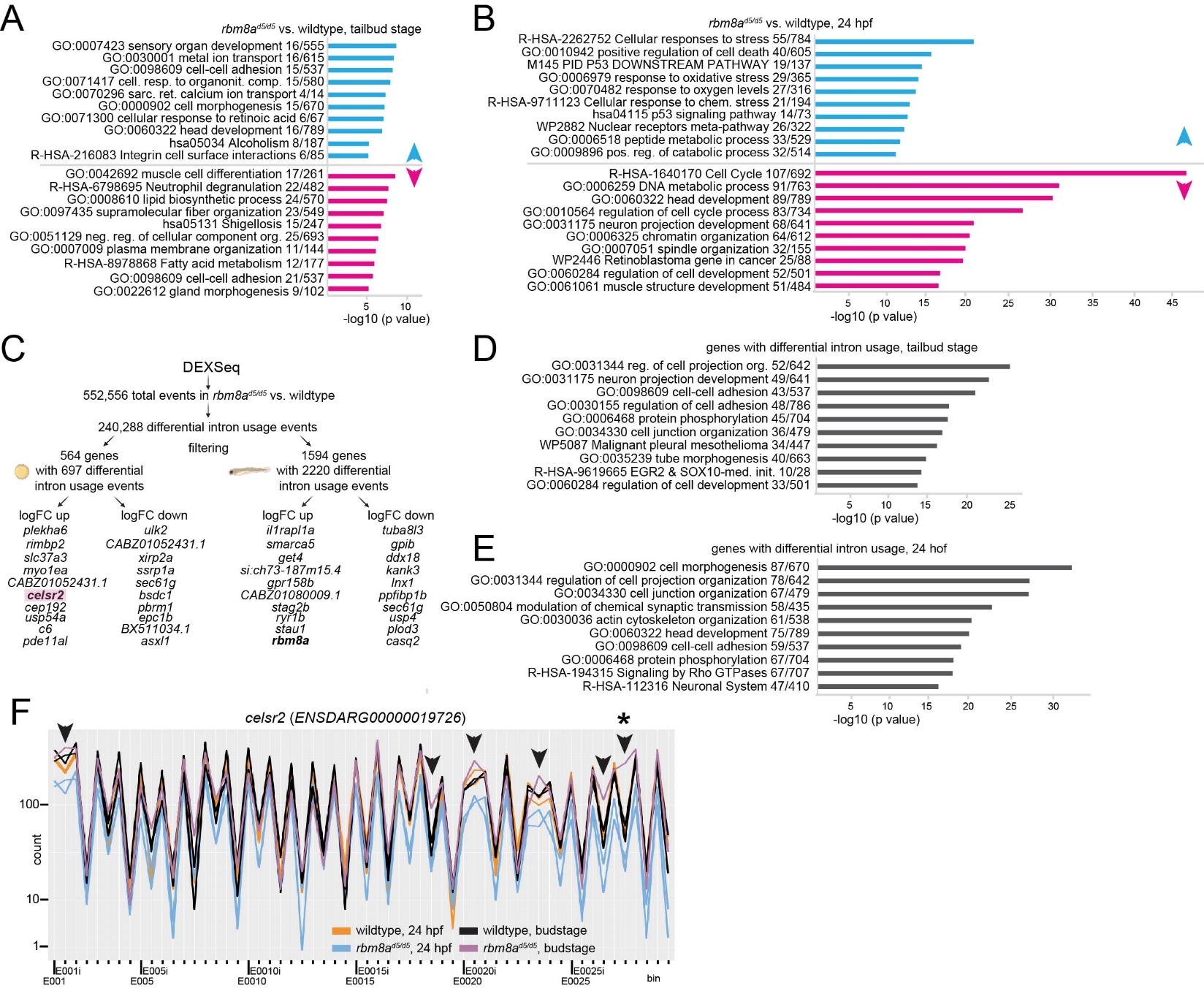
**Supplementary Fig. 2: *cd41*:EGFP-expressing thrombocyte quantification using video capture and laser injury assay.**

(**A**) Hypothesized connection of the human TAR syndrome phenotypes with early lateral plate mesoderm (LPM). Created with Biorender.com (Subscription: Individual; Agreement number: AO25WQRSMG).

(**B-D**) *rbm8a*-mutant zebrafish do not show overt LPM defects in early development. mRNA *in situ* hybridization (ISH) to document gene expression of individual LPM-associated genes that demarcate critical cell fates and structures. 3 dpf only shown for *rbm8aΔ3/Δ5* hypomorphs as *rbm8aΔ5/Δ5* do not survive until that timepoint. Numbers indicate embryos from depicted phenotype within a representative clutch, with confirmed genotyping as control. See text for details. *rbm8a*-mutant embryos show no appreciable gross anomalies in LPM formation, patterning, and individual structures as per these markers, indicating no pleiotropic LPM defects. Individual embryos with mutant *rbm8a* allele combinations showed small disruptions of the initially bilateral LPM stripes with ISH for *gata1* and *hand2* (arrowheads in **B**), but with variable penetrance. While starting functional blood circulation (*gata1*-positive erythrocytes over the yolk, **C**), *pu.1*-positive myeloid cells predominantly infiltrate the brain region with increasing age, consistent with previously reported onset of neuronal apoptosis and necrosis (**C**, asterisk). *rbm8a*-perturbed zebrafish form kidney progenitors from properly patterned *pax2a*-positive bilateral stripes (**B,C**). *rbm8a*-perturbed zebrafish also form pectoral fins and (despite circulation defects in *rbm8aΔ5/Δ5*) two-chambered, looped hearts with pericardia akin to wild type references (*tbx5a*, *hand2*, *bmp4*, **C**, and *hand2*, *bmp4*, **D**; black arrowheads: hearts, clear arrowheads: pectoral fins).

(**E,F**) Zebrafish with respective genotypes were incrossed and larvae collected. At 6 dpf, the number of circulating *cd41:EGFP*-positive thrombocytes was measured (video count). No statistically significant differences (Mann Whitneytest) were detected between the video count method and the semi-automated count method as documented in Fig. 2 (**E**). The number of circulating *cd41:EGFP*-positive thrombocytes demonstrated a trending yet not significant reduction in 6 dpf *rbm8a∆3/∆3* and *rbm8a∆3/∆5* zebrafish larvae, as shown with the semi-automated count method in Fig. 2K (**F**).

(**G-J**) Time to occlusion (TTO) after laser-mediated arterial endothelial injury and number of *cd41:EGFP*-labeled thrombocytes adhering to the site of occlusion. 6 dpf larvae were subjected to laser-mediated arterial endothelial injury, time to occlusion (TTO) was measured up to 120 seconds, and the number of *cd41:EGFP*-labeled thrombocytes adherent to the site of occlusion were counted over 120 seconds, followed by genotyping. The numbers above the x-axis indicate the number of larvae tested. There were no statistically significant differences in TTO of wildtype compared to heterozygous *rbm8a∆5/+* (**G**), homozygous *rbm8a∆3/∆3* or trans-heterozygous *rbm8a∆3/∆5* zebrafish larvae (**H**), or the number of adhering thrombocytes (**I,J**). Significance calculated by Mann-Whitney test (see **Supplementary Data 2** for details). Scale bar in **B:** 250 μm (applies to all panelsin **B**), scale bars in **C,D**: 200 μm (applies to all panelsin **C-D**).



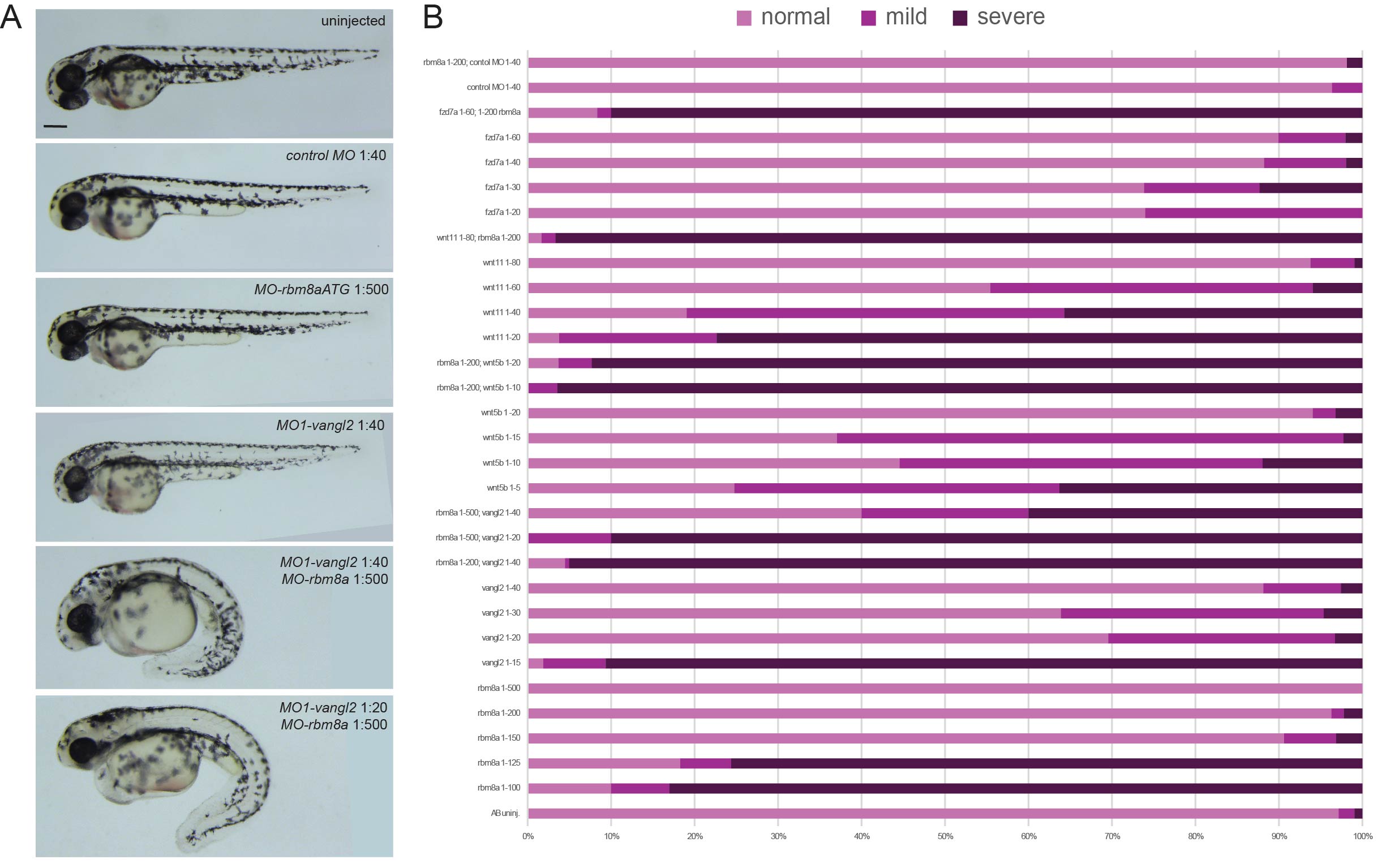
**Supplementary Fig. 3: Quantitative and qualitative transcriptome analysis of zebrafish *rbm8a* mutants.**

(**A,B**) Disrupted (up- and down-regulated) pathways and processes in *rbm8a*-mutant zebrafish embryos at tailbud stage (**A**) and at 24 hpf (**B**); blue indicates upregulation, magenta indicates downregulation (corresponding arrowheads genes (see **Supplementary Data 5** for details).

(**C**) DEXSeq analysis to identify genes with differential intron usage reveals transcripts with defects that are retained in rbm8a-mutant embryos. Workflow of the analysis, output, and top altered genes (see **Supplementary Data 6 and 7** for details)

(**D,E**) Disrupted (up- and down-regulated) pathways and processes of all genes with intron retention from (**C**) at tailbud stage (**D**) and at 24 hpf (**E**) (see **Supplementary Data 8** for details).

(**F**) Read count plot for *celsr2* as representative gene that shows retained intron events. Arrowheads indicate increased intron reads, asterisk indicates majority of transcript with retained individual intron.

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**Supplementary Fig. 4: Additional morpholino dosage schemes and extended data.**

(**A**) 2 dpf lateral views of representative zebrafish larvae analyzed for each genetic perturbation. Anterior to the left. Representative phenotypes following injection of highly suboptimal *MO-rbm8aATG* dose (1:500), suboptimal *MO1-vangl2*, and co-injection of both at different concentrations. (**B**) Quantification of observed phenotypes in percent of embryos of all morpholino conditions tested. Scale bar in **A:** 250 μm (applies to all panelsin **A**).