The American Journal of Human Genetics, Volume *93*

Supplemental Data

SCRIB and *PUF60* Are Primary Drivers of the Multisystemic Phenotypes of the 8q24.3 Copy-Number Variant

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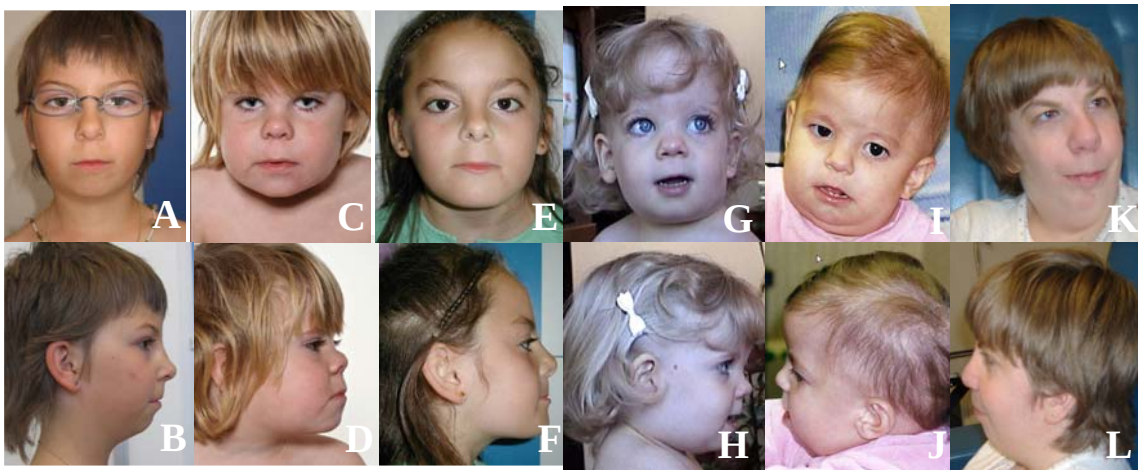

Figure S1: Frontal and profile views of the 5 individuals with 8q24.3 deletion and Case 6 with the PUF60 p.His169Tyr mutation.
A, B: Frontal and profile of Case 1. C, D: Frontal view and profile of Case 2. E, F: Frontal view and profile of Case 3. G, H: Frontal view and profile of Case 4. I, J: Frontal view and profile of Case 5. K, L: Frontal view and profile of Case 6.

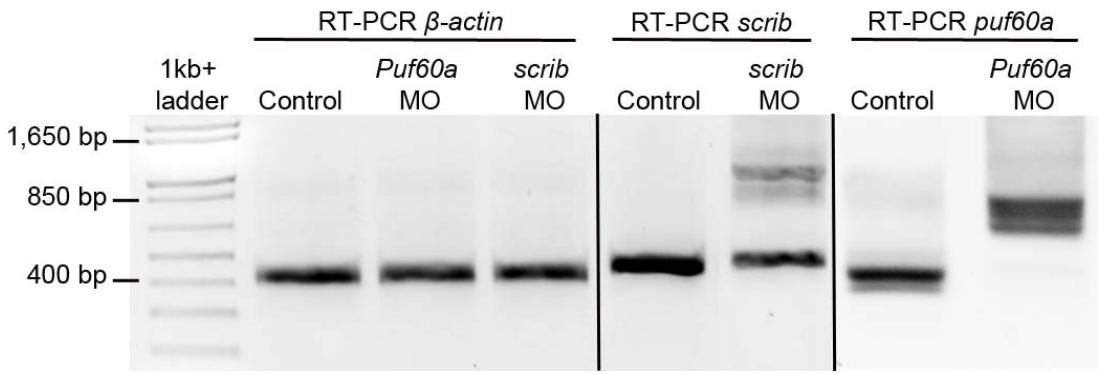

Figure S2: Both *scrib* and *puf60a* MOs efficiently disrupt the splicing of their respective zebrafish endogenous messages.
Injection of *scrib* and *puf60a* splice-blocking morpholinos results in abnormal splicing and the retention of the next intron as shown by PCR amplification of cDNA reverse transcribed from extracted total mRNA. β-actin was used as a control.

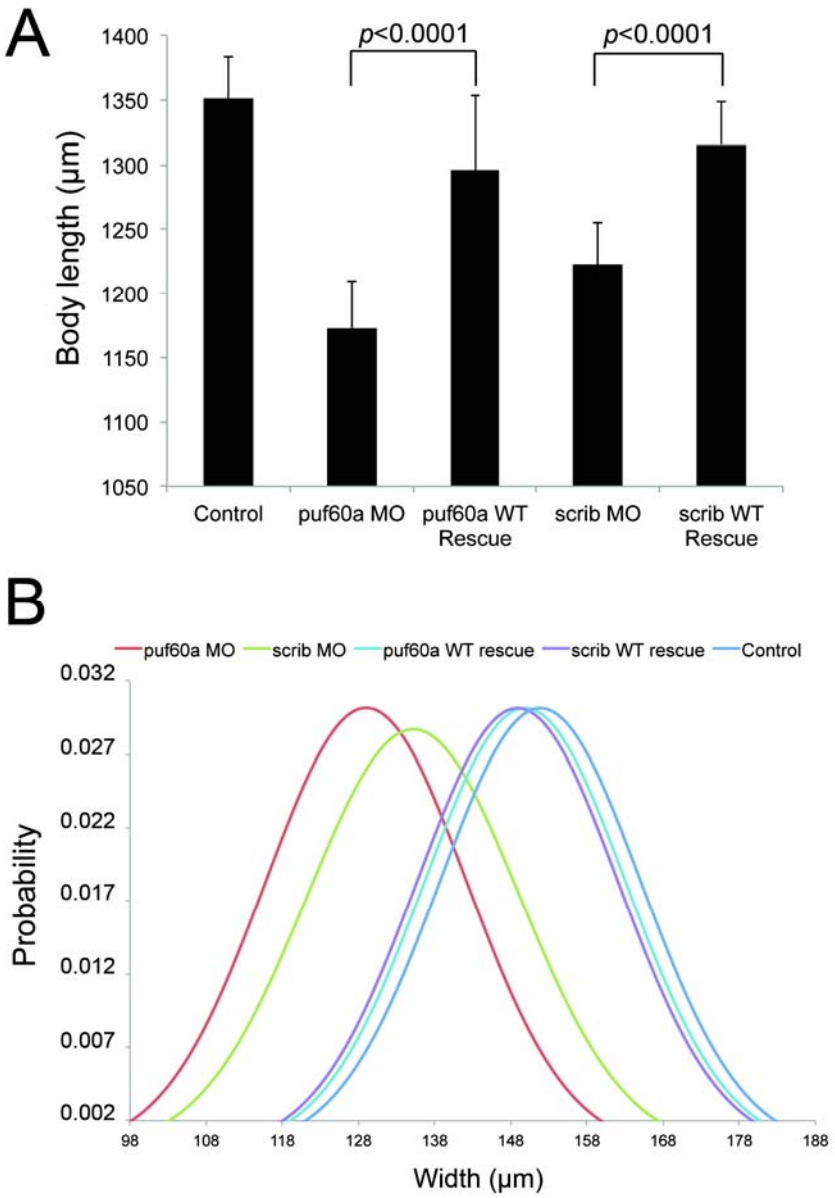

Figure S3: Rescue of MO phenotypes.
Embryos injected with either 4 ng of *scrib* MO or 4.5 ng of *puf60* MO with 100 pg of human wild-type (WT) *SCRIB* or *PUF60* mRNA respectively rescue body length (A) and head size (B) phenotypes seen in each of MO-alone injections. (A) Control denotes embryos from the same clutch injected with a control MO at two-cell stage and scored as in Figure 2. Bars represent the mean length of 80 embryos at 3 d.p.f. per condition, which were scored blind to injection cocktail. The corresponding *p*-values are denoted on the bar graphs (two-tailed *t*-test comparisons). (B) Head size measurements are represented as a normal probability distribution curve as in Figure 2. Significant differences were observed for the microcephaly phenotype; $p<0.0001$ between *puf60a* morphants and WT rescue and $p<0.0001$ between *scrib* morphants and WT rescue (n=80; 3 independent experiments; two-tailed *t*-test comparisons).

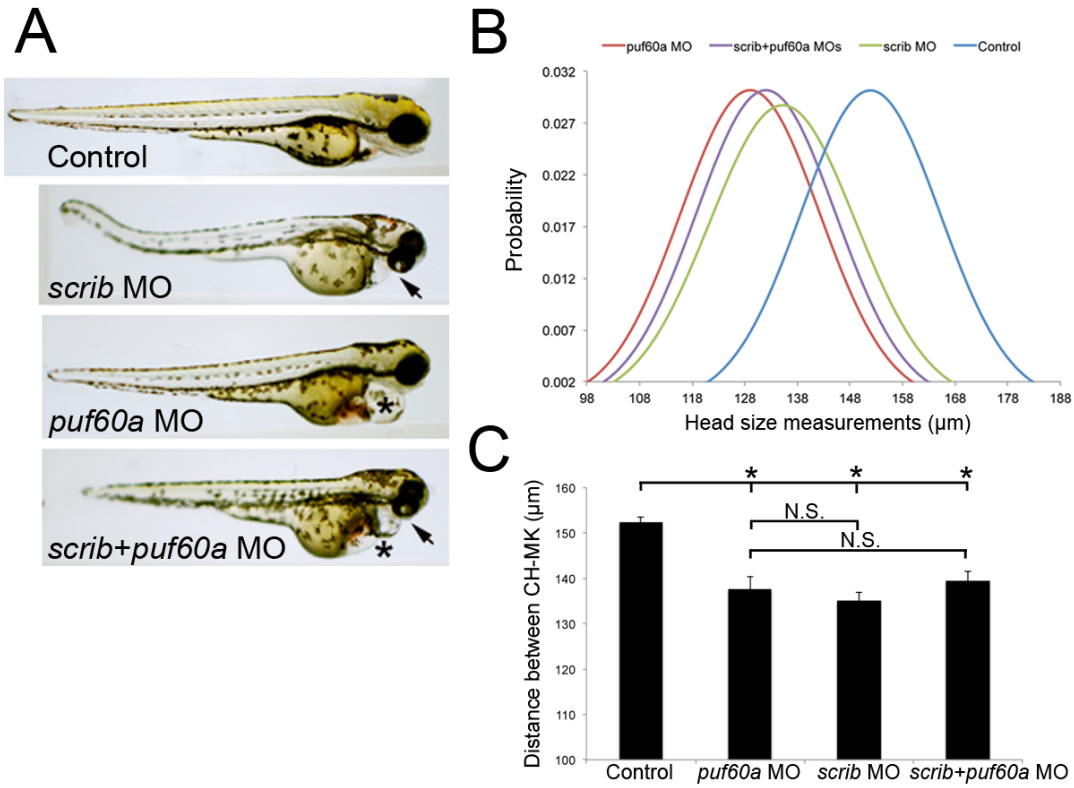

Figure S4: Suppression of both *scrib* and *puf60a* do not increase the severity or the penetrance of the microcephaly and craniofacial defects.
(A) Lateral views of representative sham-injected embryos and those injected with *scrib, puf60a,* and double MOs at 3 d.p.f.. Coloboma was observed in *scrib* and double morphants (black arrow) and cardiac edema was observed in *puf60a* and double morphants (black asterisk). (B) Quantification of microcephaly was performed on 4.5 d.p.f. embryos injected with *scrib*, *puf60a*, and double MOs. (C) Quantification of craniofacial defects was performed in control and embryo batches injected with *scrib*, *puf60a,* and double MOs, by measuring distance between ceratohyal (CH) and Meckel's cartilages (MK). No significant difference was observed between double morphants and either *scrib* or *puf60a* morphants for the microcephaly and craniofacial defects. N.S, nonsignificant. **p*<0.0001 (n=80 per condition; 3 independent experiments; two-tailed *t*-test comparisons).

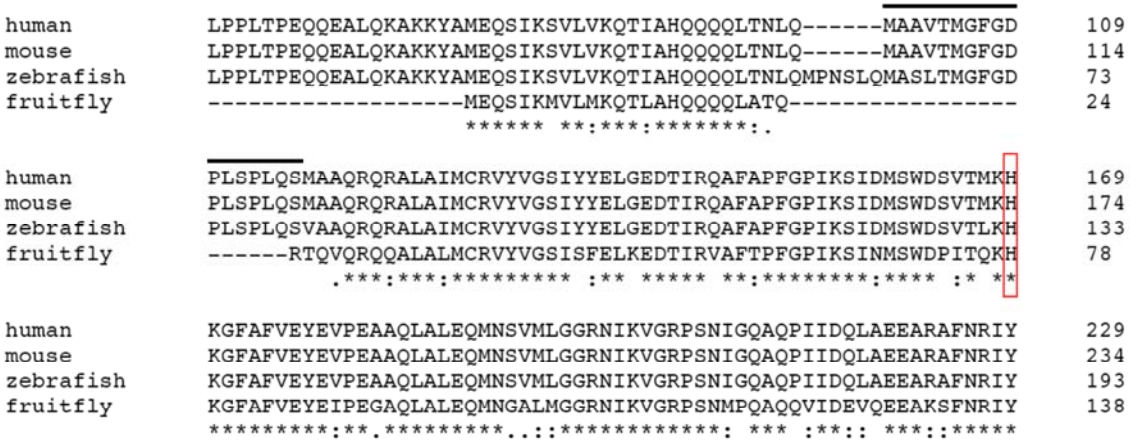

Figure S5: PUF60 His 169 is highly conserved.
Protein sequence alignment of PUF60 from four different species. Histidine 169 is boxed in red. The black horizontal line indicates amino acids encoded by the alternative exon 5.

Table S1: Sequences of the real-time PCR and mutagenesis primers.

RT-PCR Primers	Sequence
Puf60ex4F	5’-gcc aag aag tac gcc a-3’
Puf60ex7+12Rev	5-gga cct cat act cca cg-3’
SMNex6Fxho-A	5’-cga tct cga gat aat tcc ccc acc acc tcc c-3’
SMNex8not-d	5’-ata tgc ggc cgc cac ata cgc ctc aca tac a-3’
APPex6For	5’-tga aga caa agt agt aga agt ag-3’
APPex9Rev	5’-ctg gga cat tct ctc tcg gtg ctt g-3’
hBIN1e11-for	5’-cct cca gat ggc tcc cct gc-3’
hBIN1e14-Rev	5’-ccc ggg ggc agg tcc aag cg-3’
Zebrafish-puf60a-RT-F	5’-aat tta cgt ggc atc cgt tc-3’
Zebrafish-puf60a-RT-R	5’-gtc atc gcc cca aga ata ga-3’
Zebrafish-scrib-RT-F	5’-aac gac act gct cgc tca c-3’
Zebrafish-scrib-RT-R	5’-ccc tca gct cta gag tca cca-3’
QuikChange Primers	
Puf60His169Tyr-cDNA-F	5’-cac cat gaa gta caa ggg ctt tg-3’
Puf60His169Tyr-cDNA-R	5’-caa agc cct tgt act tca tgg tg-3’