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Supplemental Data

Recurrent CNVs and SNVs at the *NPHP1* Locus Contribute Pathogenic Alleles to Bardet-Biedl Syndrome

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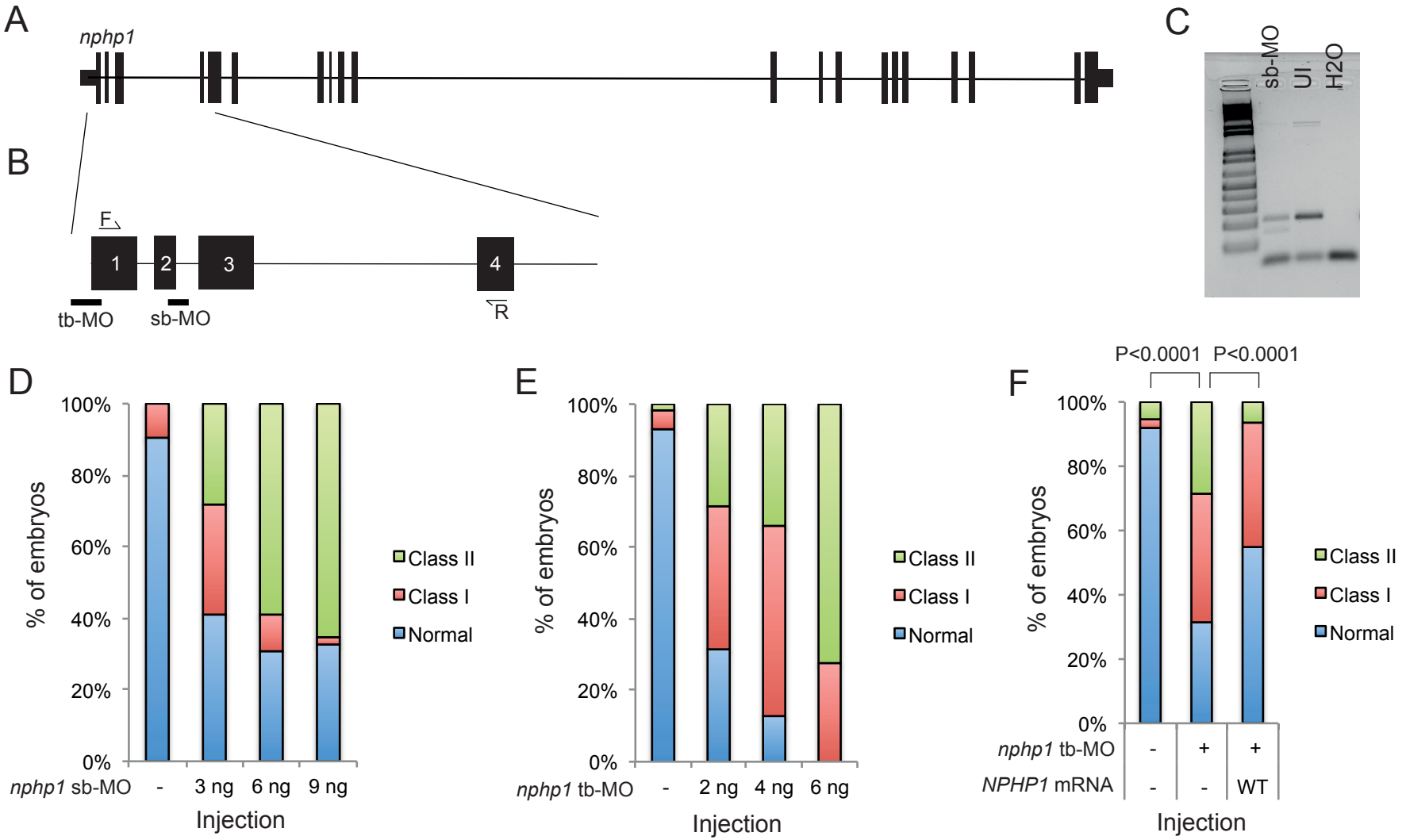

Figure S1: Knockdown efficiency of the *nphp1* morpholinos in zebrafish embryos

(A) Schematic illustration of the *D. rerio nphp1* (NM_001077170.1) locus.

(B) A zoomed schematic of exon 1 through exon 4 illustrating the position of the translational blocking (tb) and splice blocking (sb) MOs and RT-PCR primer target location. Total RNA was extracted from 10-somite stage embryos injected with 3ng of *nphp1* sb-MO (targeting exon2/intron2-3). After cDNA synthesis RT-PCR was performed with primers flanking the sb-MO target sites (F and R).

(C) Aberrant splicing was clearly visible by agarose gel electrophoresis following PCR amplification.

(D) Dose response curve for *nphp1* sb-MO. Wild type zebrafish embryos were injected at the 1 to 4 cell stage with the indicated dose. Embryos were kept at 23° C and scored live for gastrulation defects 24h post fertilization, mutant embryos were subdivided into class I or class II depending on the severity of the phenotype.

(E) Dose response curve for *nphp1* tb-MO as in (D).

(F) Co-injections of *nphp1* tb-MO with wild type (WT) human *NPHP1* mRNA result in a significant decrease in the number of morphant class I and class II embryos compared to tb-MO injected batches (P<0.0001).

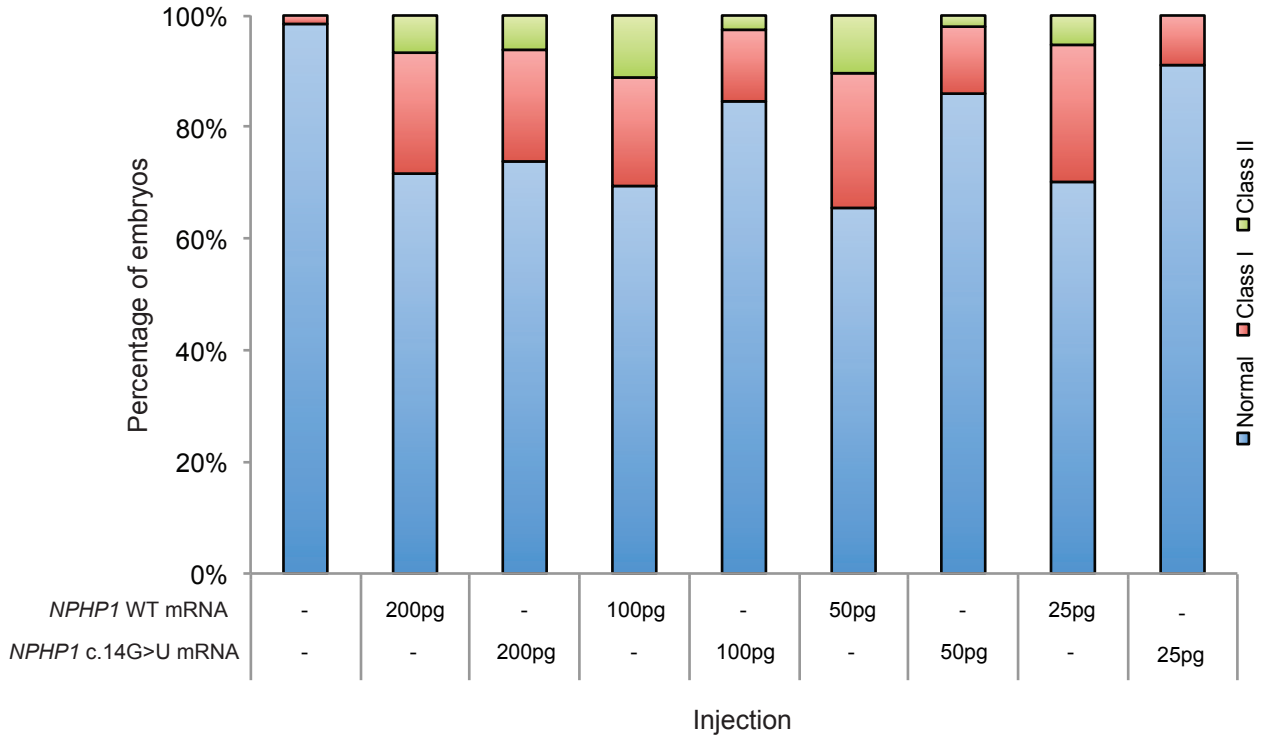

Figure S2: Dose response curve of *NPHP1* wild-type and c.14G>U mRNA.
Zebrafish embryos were injected with progressively decreasing concentrations of either wild-type (WT) or mutant (c.14G>U) human *NPHP1* mRNA and scored for gastrulation defects at the midsomite stage (see Figure 3A for representative images of each phenotypic class). WT mRNA results in a persistent, mild phenotype affecting ~30% of embryo batches; c.14G>U mRNA produces a progressive decrease in the number of affected embryos, supporting the notion that this is a loss-of-function variant (n=29-66 embryos/injection batch, repeated at least twice, with masked scoring.

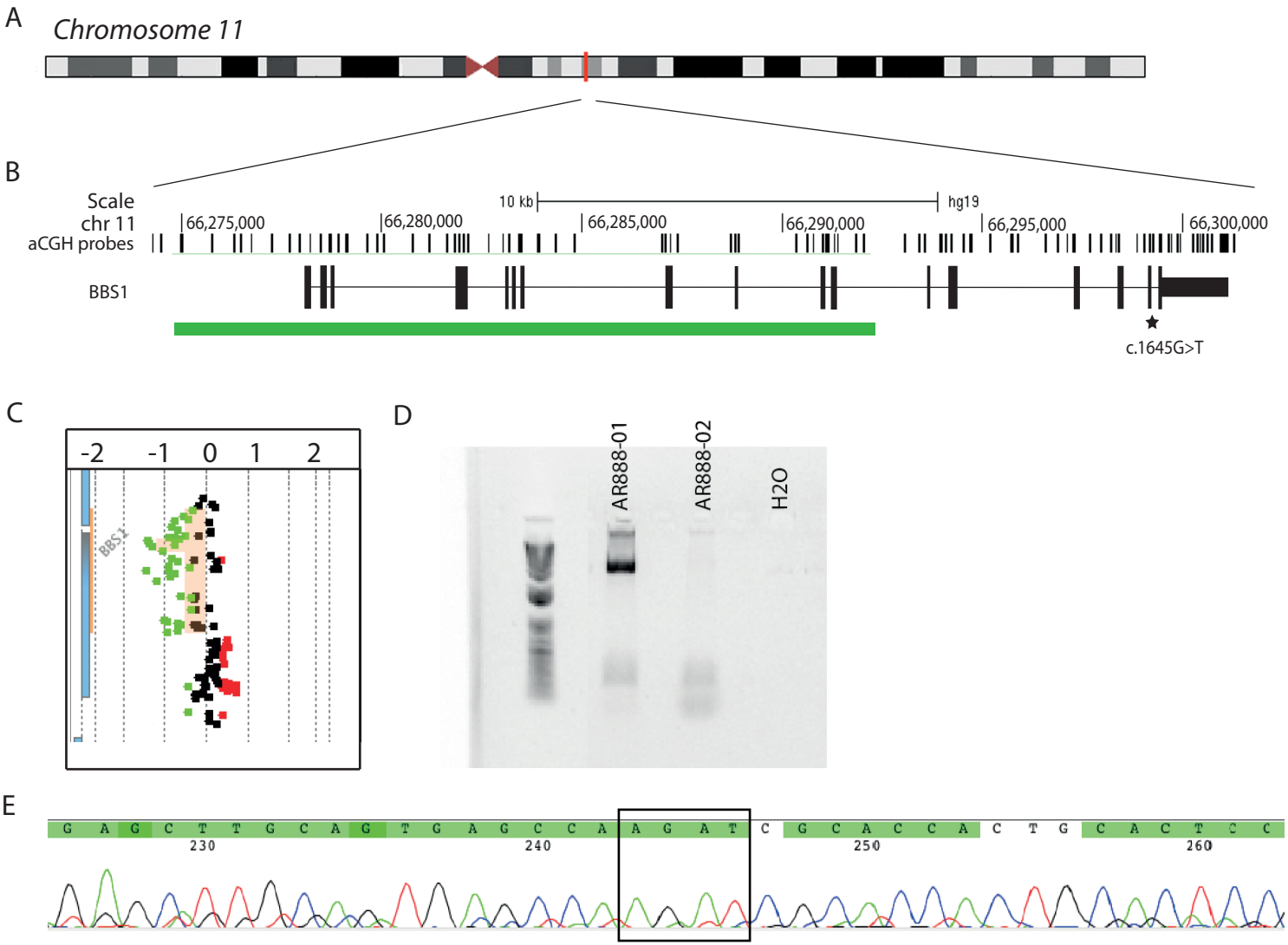

Figure S3: Characterization of the 17.7kb *BBS1* deletion in family AR888

(A) Schematic of human chromosome 11. A vertical red line indicates the position of *BBS1* (NM_024649.4) at 11q13.2.

(B) A zoomed depiction of the *BBS1* locus with a schematic illustration of exons (vertical blue bars). The genomic location on chromosome 11 (in Mb) is shown and the distribution of array comparative genomic hybridization (aCGH) probes. The solid green bar below the gene represents the genomic location of the deletion identified in AR888-03. The location of the heterozygous c.1645G>T (p.Glu549*) mutation is marked by a star.

(C) High-resolution results from the aCGH analyses in individual AR888-03 are visualized as a plot. The horizontal blue bar represents *BBS1*. Individual dots represent specific oligonucleotide probes and are indicated as black (normal copy number), red (copy number gain), and green (copy number loss) compared to a reference sample. The normalized log2 ratios of the Cy5/Cy3 intensity values for cases versus controls are shown on the Y-axis.

(D) Segregation of the *BBS1* deletion in the AR888 family. Deletion carriers are identified by clearly resolvable bands on an agarose gel electrophoresis following PCR amplification. Individual 01 harbors the deletion.

(E) Sequence traces from Sanger sequencing of the breakpoint PCR product shown in (D). A 4 bp micro-homology is present.

Table S1: Phenotypic scoring and statistical significance of interactions for zebrafish embryo injections.

In vivo complementation: Gastrulation defects scored live in midsomitic embryos							
Injection	**Normal**	**Class I**	**Class II**	**n=**	**% abnormal**	***p* vs *nphp1* MO**	***p* vs WT rescue**
nphp1 MO	27	29	4	60	55%	N/A	
nphp1 MO + *NPHP1* WT RNA	38	19	5	62	39%	0.0027	N/A
nphp1 MO + *NPHP1* c.14G>U RNA	29	20	10	59	51%	<0.0001	0.0017
nphp1 MO + *NPHP1* c.115C>A RNA	40	17	4	61	34%	0.0002	0.7126
nphp1 MO + *NPHP1* c.689C>U RNA	43	17	5	65	34%	<0.0001	0.5444
Injection	**Normal**	**Class I**	**Class II**	**n=**	**% abnormal**	***p* vs *nphp1* MO**	***p* vs WT RNA**
NPHP1 WT RNA	43	13	4	60	28%	<0.0001	N/A
NPHP1 c.14G>U RNA	48	13	4	65	26%	<0.0001	0.8842
NPHP1 c.115C>A RNA	47	12	3	62	24%	<0.0001	0.6114
NPHP1 c.689C>U RNA	41	11	4	56	27%	<0.0001	0.9697
In vivo complementation: Renal defects scored in 4 dpf larva stained with Na+/K+ ATPase antibody							
Injection	**Normal**	**Affected**		**n=**	**% abnormal**	***p* vs *nphp1* MO**	***p* vs WT rescue**
nphp1 MO	18	28		46	61%	N/A	
nphp1 MO + *NPHP1* WT RNA	32	14		46	30%	<0.0001	N/A
nphp1 MO + *NPHP1* c.14G>U RNA	24	25		49	51%	0.0403	<0.0001
nphp1 MO + *NPHP1* c.115C>A RNA	35	16		51	31%	<0.0001	0.8273
nphp1 MO + *NPHP1* c.689C>U RNA	34	17		51	33%	<0.0001	0.5127
Genetic interaction experiments: Gastrulation defects scored live in midsomitic embryos							
Morpholino target	**Normal**	**Class I**	**Class II**	**n=**	**% abnormal**	***p* vs *nphp1***	***p* vs bbs-gene**
nphp1	201	40	14	255	21%	N/A	N/A
bbs1	54	11	3	68	21%	N/A	N/A
bbs2	134	25	3	162	17%	N/A	N/A
bbs7	51	10	3	64	20%	N/A	N/A
bbs9	116	19	6	141	18%	N/A	N/A
bbs10	95	17	12	124	23%	N/A	N/A
bbs1+ nphp1	40	18	3	61	34%	0.0006	0.0005
bbs2+nphp1	89	43	16	148	40%	<0.0001	<0.0001
bbs7+nphp1	36	24	4	64	44%	<0.0001	<0.0001
bbs9+nphp1	110	41	11	162	32%	0.0248	0.0008
bbs10+nphp1	68	31	20	119	43%	<0.0001	<0.0001
Genetic interaction experiments: Renal defects scored in 4 dpf larva stained with Na+/K+ ATPase antibody							
Morpholino target	**Normal**	**Affected**		**n=**	**% abnormal**	***p* vs *nphp1***	***p* vs bbs-gene**
nphp1	70	25		95	26%	N/A	N/A
bbs1	39	16		55	29%	N/A	N/A
bbs2	35	12		47	26%	N/A	N/A
bbs7	54	10		64	16%	N/A	N/A
bbs9	13	3		16	19%	N/A	N/A
bbs10	34	11		45	24%	N/A	N/A
bbs1+ nphp1	35	25		60	42%	0.0003	0.0042
bbs2+nphp1	14	11		25	44%	<0.0001	<0.0001
bbs7+nphp1	32	24		56	43%	<0.0001	<0.0001
bbs9+nphp1	13	10		23	43%	<0.0001	<0.0001
bbs10+nphp1	31	25		56	45%	<0.0001	<0.0001

N/A=not applicable