Supplemental Materials and Methods

Cell culture, transfection, and generation of DIS3L2-deficient cell lines

Igrov1 cells were a gift from R. Gregory. All other cell lines were obtained from ATCC and grown under standard conditions. E14tg2a cells were grown without feeder cells. A TALEN pair was used to knockout *DIS3L2* in human cell lines and was designed using ZiFit Targeter v4.1 (Sander et al. 2010) and constructed using the restriction enzyme and ligation (REAL) assembly method (Sander et al. 2011) with Addgene Kit #1000000017. CRISPR/Cas9 was used to knockout mouse *Dis3l2* in E14tg2a cells using pX330 (Addgene plasmid #42230). Sequences used for TALEN and pX330 cloning are provided in Supplemental Table S4. Cell lines were transiently transfected with genome-editing plasmids using Xfect (Clontech) for E14tg2a, Lipofectamine (Invitrogen) for Igrov1, or FuGENE HD (Promega) for HeLa, HCT116, HEK293T, and Huh7. Single-cell clones were generated and screened by TOPO-TA cloning (Invitrogen) and western blot.

Nephron progenitor cell culturing

NPCs were isolated as previously described (Brown et al. 2015) with minor modifications to the protocol. Embryos were genotyped prior to enzymatic digestion of the kidneys. Individual kidney pairs of the genotypes of interest were processed separately and digested in 600 µl of enzymatic solution. Magnetic depletion was carried out with MS Columns on a MiniMACS Separator (Miltenyi Biotec). All data shown are from RNA collected within the first two passages of NPC cultures.

Western blots

Cell lysates were prepared by harvesting in Laemmli buffer. Antibodies used for western blotting were anti-LIN28A (Cell Signaling, 8641), anti-LIN28B (Cell Signaling, 11965), anti-DIS3L2 (Sigma, HPA035797 and Novus, NBP2-38264), anti-α-tubulin (Sigma, T6199), anti-GAPDH (Cell Signaling, 2118), and anti-Actin (Santa Cruz, sc-1616).

RNA isolation, qRT-PCR, and Northern blot

RNA isolations were performed according to manufacturers' protocols with the miRNeasy Mini Kit with On-Column DNase Digestion (Qiagen) for qRT-PCR and RNA-seq or, for northern blot, TRIzol Reagent (Invitrogen). For mature let-7 and U6 snRNA quantification, pre-designed TaqMan primers and probes (Applied Biosystems) were used according to the manufacturer's protocol. Relative expression of mature let-7 normalized to U6 was calculated using the ΔCt method [$2^{-(Ct\_let\text{-}7\text{-}Ct\_U6)}$]. For all other qRT-PCR assays, RNA was reverse transcribed with either SuperScript III or IV (Invitrogen). Exon 11-containing *Dis3l2* mRNA was quantified with a custom-made

TaqMan Gene Expression Assay, designed with Primer Express software v3.0.1, and normalized using a pre-designed 18S rRNA Taqman assay, using TaqMan Universal Master Mix II, no UNG (all Applied Biosystems). All other transcripts were quantified using Power SYBR Green PCR Master Mix (Applied Biosystems). Pre-let-7 quantification (Supplemental Fig. S3) was performed as described (Chang et al. 2013), with isolation of <200-nucleotide RNA using the miRvana kit (Thermo Fisher Scientific) and reverse transcription with either a gene-specific primer (for total pre-let-7g or U6) or an oligo$(dA)_{12}$ (for selective priming of uridylated species). Northern blot analysis was performed as previously described (Hwang et al. 2007) with 100 µg of total RNA from parental or *Dis3l2* knockout E14tg2a cells using a probe complementary to the terminal loop of pre-let-7g. Sequences of all probes and primers provided in Supplemental Table S4.

Phenotypic and histologic analyses

Phenotyping of *Dis3l2*$^{\Delta 11/\Delta 11}$ and *Dis3l2*$^{\Delta 10/\Delta 10}$ embryos was performed at E18.5 after delivery by caesarean section. Lungs were removed and placed directly into 10% formalin. Sectioning and staining were performed at the UT Southwestern Histopathology core. Images of kidneys and urogenital systems were acquired on a Nikon SMZ800N, and NIS-Elements Documentation version 4.12 (Nikon) was used to measure cross-sectional area of kidneys. Low power images of kidneys and lungs (Figs. 2G, 3H; Supplemental Figs. S4, S6B) were acquired using a Zeiss Axio Observer Z1 microscope with a 5X objective and the MosaiX/Stitching function of AxioVision, which produces a composite image of multiple adjacent fields.

RNA-sequencing and analysis

NPC cultures were derived and RNA isolated from 2 *Dis3l2*$^{+/+}$ and 3 *Dis3l2*$^{\Delta 11/\Delta 11}$ embryos from the same litter. Sequencing libraries were prepared using the TruSeq Stranded Total RNA LT Sample Prep Kit (Illumina) and 50 base pair single-end sequencing was performed at the McDermott Center Next Generation Sequencing core at UT Southwestern. Reads were aligned to the mm10 reference genome with GENCODE M16 annotation (Mudge and Harrow 2015) using HISAT2 version 2.1.0 (Kim et al. 2015). Differential expression analysis was performed on uniquely mapped reads with edgeR version 3.20.1 (Robinson et al. 2010).

Igf2/H19 allelic expression assay

Dis3l2$^{+/\Delta 11}$ mice maintained on a C57BL/6J background were crossed with BALB/cJ, producing *Dis3l2*$^{+/\Delta 11}$ offspring with defined heterozygous SNPs in exons of both *Igf2* and *H19*. These mice were then intercrossed, yielding *Dis3l2*$^{\Delta 11/\Delta 11}$ and *Dis3l2*$^{+/+}$ embryos, half of which were heterozygous for the *Igf2* and *H19* SNPs. NPC cultures from these embryos were then derived and RNA was isolated. RT-PCR followed by

Sanger sequencing of SNPs allowed determination of monoallelic or biallelic expression of *Igf2* and *H19*. Primers used for PCR amplification provided in Supplemental Table S4.

Supplemental References

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