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Supplemental Data

A Syndromic Neurodevelopmental Disorder Caused by Mutations in *SMARCD1*, a Core SWI/SNF Subunit Needed for Context-Dependent Neuronal Gene Regulation in Flies

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Figure S1

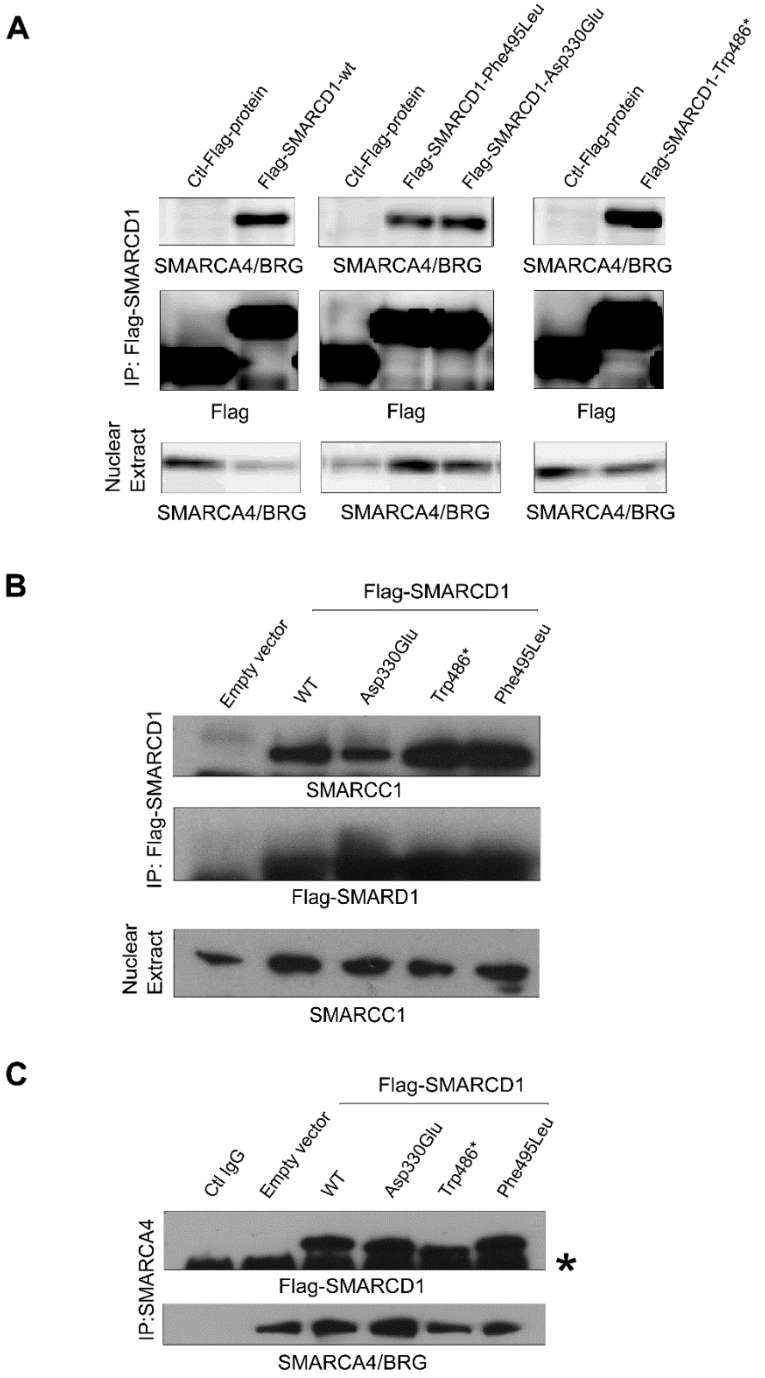

Figure S1: Mutations in SMARCD1 do not impair its binding to SMARCA4 and SMARCC1. Co-Immunoprecipitation of SMARCA4 (BRG1) **(A)**, SMARCC1 **(B),** and reciprocally, of SMARCD1 **(C)** following immunoprecipitation of FLAG-SMARCD1 **(A-B)** or endogenous SMARCA4 (BRG1) **(C)** respectively.

Figure S2

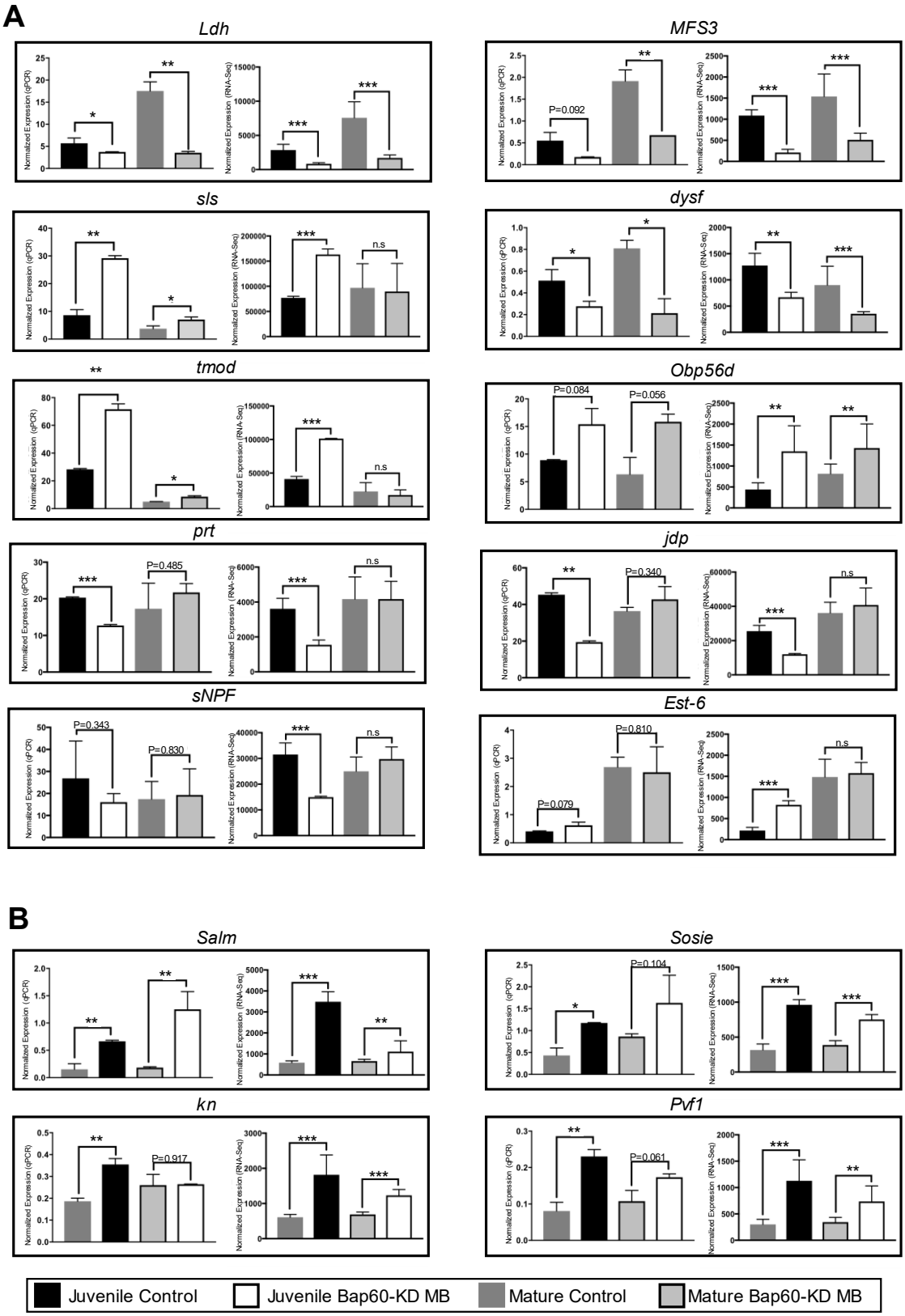
A
Ldh
MFS3
sls
dysf
tmod
Obp56d
prt
jdp
sNPF
Est-6
B
Salm
Sosie
kn
Pvf1
Normalized Expression (qPCR)
Normalized Expression (RNA-Seq)
P=0.092
P=0.084
P=0.056
P=0.485
P=0.340
P=0.343
P=0.830
P=0.810
P=0.079
P=0.104
P=0.917
P=0.061
n.s
Juvenile Control
Juvenile Bap60-KD MB
Mature Control
Mature Bap60-KD MB

Figure S2: qPCR validation of RNAseq gene expression changes. Average normalized gene expression ± SD of genes as measured through RT-qPCR (left panel) and RNA-Seq (right panel) in Bap60-KD MB and control mushroom body (MB) samples. RT-qPCR gene expression is normalized to the housekeeping genes *Eif2β* and *βcop* and RNA-Seq gene expression is normalized by DESeq2. (**A**) Validation of overall gene expression trends for *Ldh, MFS3, sls, dysf, tmod, Obp56d, prt, jdp, sNPF,* and *Est-6*. Comparisons were made between juvenile control and juvenile Bap60-KD MB and between mature control and mature Bap60-KD MB. Genes validated by RT-qPCR were: *Ldh*, *sls*, *dysf, tmod, prt,* and *jdp*. Genes that were showing similar trends between RT-qPCR and RNA-Seq were: *MFS3, Obp56d, and Est-6*. *sNPF* could not be validated by RT-qPCR due to variation between biological replicates. (**B**) Validation of gene expression trends of developmental genes: *Salm, Sosie, kn,* and *Pvf1* that were induced in juvenile control flies, but not juvenile Bap60-KD MB. Comparisons were made between mature control and juvenile control and between mature Bap60-KD MB and juvenile Bap60-KD MB. Genes validated were: *Kn* and *Pvf1*. *Sosie* is validated despite variability of the data. Not validated is: *Salm*. Significance for RT-qPCR is determined by Student's t-test; significance for RNA-Seq is determined by binomial Wald test (DESeq2); *p<0.05, **p<0.01, ***p<0.001.

Table S3: Additional clinical information.

Individual	1	2	3	4	5
Microarray (aCGH)	NA	normal	normal	normal	normal
Country of Origin and ethnicity	Japan, asian	France	UK, white	Northern Ireland, white	USA / European, German
Pregnancy issues		Antenatal diagnosis of agenesis of the corpus callosum	Reduced fetal movements, scans suggested large head	Delivery: Emergency CS for failure to progress	IUGR and small head size noted in 3rd trimester
Perinatal complications		Macrosomia at birth. Feeding difficulties with feeding tube during 15 days	very quiet baby, poor bowel movements with loose stools	Didn't cry at birth; stridor for several weeks	Mild jaundice at birth; gastroesophageal reflux as infant
Hypotonia	Yes	no	yes, still evident but milder	yes - congenital, severe	No
Intellectual disabillity	Yes	yes	Yes	Severe	Too young to determine
Details on development and IQ		4 years 9 m : WPPSI Performance IQ 75, verbal IQ 50 Has special educational needs	Has a statement of special educational needs and attends a special needs school	Profound disability	Receiving special instruction, physical and speech therapy No formal IQ testing at this time
Other diagnosis such as ADHD or Autism		Behavioral disorders, frustration intolerance	No formal diagnosis of autism but has obsessive traits.	No	No
Unusual anxieties			Anxiety of new things	NA	Very anxious with health

			and new situations and large places eg shopping centres		care providers
Medications		No	Domperipdone, lazoparole, antibiotics	Omeprazole	none
Seizures	No	no	none	No	none
Vision impaired	No	no	Yes, plus nystagmus	alternating divergent squint	No – mild astigmatism not requiring glasses/intervention
Frequent infections		no	frequent ear infections, tonsilitis. Necessitating hospititalisations	Yes; respiratory as baby	Frequent ear infection s/sp BMT
Surgeries		no	ENT- adeniods, tonsils removed and grommets inserted.	PEG, surgery for undesc testes	BMT for frequent ear infections
Further information			oral dyspraxia,	elevated T3 as baby; normalised frequent UTIs; mottled skin on legs & arms	

Table S4. Results of *in silico* pathogenicity prediction tools for identified *SMARCD1* variants.

	Conservation scores										Functional		General			
	Polyphen2[65] HDIV	Polyphen2[65] HVAR	LRT	MutationAssessor[66]	GERP++[67] RS rankscore	phyloP[68]100way vertebrate rankscore	phyloP[68]20way mammalian rankscore	phastCons[69]100way vertebrate rankscore	phastCons[69]20way mammalian rankscore	SiPhy[70]_29way logOdds rankscore	SIFT[71]	PROVEAN[72]	FATHMM[73]-MKL coding	MutationTaster[74]	DANN[75] rankscore	CADD[76] phred
p.Asp330Glu	D	D	D	M	0.393	0.26	0.165	0.424	0.75	0.537	D	D	D	D	0.816	26.8
p.Arg446Gly	B	B	N	M	0.173	0.389	0.044	0.715	0.625	0.36	D	D	D	D	0.698	20.9
p.Trp486*	.	.	D	.	0.693	0.997	0.605	0.715	0.697	0.934	.	.	D	D	0.559	39
p.Phe495Leu	B	B	D	M	0.693	0.886	0.626	0.715	0.888	0.744	T	D	D	D	0.182	14.24
p.Arg503*	.	.	D	.	0.6	0.502	0.391	0.715	0.75	0.684	.	.	D	D	0.732	41

D, deleterious or damaging; B, benign; M, medium; N, neutral; T, tolerated.

Shaded are the damaging predictions, those with rank scores above 0.5, and CADD phred scores above 25.

Table S7: RT-QPCR primers

Gene	Forward Primer	Reverse Primer
Ldh	AGATCCTGACTCCCACCGAA	GCCTGGACATCGGACATGAT
MFS3	GCCTCCAATGTGACGGCTAA	GTAGCAGCTCAGCAGGGTTC
sls	ATCTCCTATTCGAGTGGAGTGG	CCCTGCAAATTCTCGGCAAG
dysf	CGGAGATAGCCAATCTGAGG	GCTTTCCGCACATAGACACA
tmod	GCAAGGATCTGAGTGAGTACGA	GCCAGTATGGTTATCTCCTCGG
Obp56d	TCCAGCCCGATGTCGTTCT	CCCTTGGTGGCATCACACT
prt	ATGTCGGAGAAATCGAACCGT	GGGGCATTCAGTTGAACAGC
jdp	GGAAACCTTGTGCGATCCC	AGCCACTGTTTGTAGCTCATC
sNPF	CGATCTGGGTGCCGACTAC	CCTCGAACTGAGGAACACTGC
Est-6	TGGGACTGGGACTTATCATTGT	CTGCACCAACAGAGGGTCATC
Salm	GAGCAAAGCACACCAGACCA	ATCGCCACTCTGTTGTTGTTAT
Sosie	ATGGTGTGCCAGTACGAGAAC	TCGCAGAGACACAGCTTGG
kn	CGCGCCCACTTTGAAAAGC	GTTGTCCAGCCCGATCATATAAG
Pvf1	CTGTCCGTGTCCGCTGAG	CTCGCCGGACACATCGTAG
Eif2β	CAGACCCTTAACTTTAGCTCCG	GATGGTCAAATCTGAGACCTGG
βcop	AGCGGGTAATCAAGTTGCTG	GGCAGGACGAAGCGTATGA

Supplementary Methods

Co-Immunoprecipitation

HEK293T and SK-N-AS were cultured in DMEM, 10% FBS (Wisent), 1X AA and 1X Glutamax all from ThermoFisher Scientific. SK-N-AS media was supplemented with 0.1mM NEAA (ThermoFisher Scientific) and 25mM HEPES (Wisent). pcDNA.3.1-Flag-SMARCD1 wild type (NM_003076.4) or SMARCD1-c.990C>G (p.Asp330Glu), c.1457G>A (p.Trp486*) and c.1483T>C (p.Phe495Leu) were transfected using jetPRIME (Polyplus-transfection) according to the manufacturer's instructions. After 48h, cells were washed once with ice-cold phosphate-buffered saline, lysed in a hypotonic buffer (25 mM HEPES pH 7.9, 25 mM KCL, 50mM EDTA, 5 mM MgCl2, 10% glycerol, 0.1% NP40, 1 mM DTT, complete mini protease inhibitors (Sigma-Aldrich)), and centrifuge at 3000 rpm for 3 minutes. The pelleted nuclei fraction (NE) was resuspended in nuclear lysis buffer (25 mM Tris, pH8, 150 mM NaCl, 1 mM EDTA, 1% triton, 5% glycerol, complete mini protease inhibitors), incubated 30 min at 4 degrees and centrifuge at 13 000 rpm for 20 min. For immunoprecipitation, nuclear extracts (100 to 300ug) were incubated with either pre-coupled M2 anti-FLAG (M8823, Sigma-Aldrich) or anti-Brg1 (H10) coupled to Dynabeads (ThermoFisher Scientific) magnetic beads. After 1.5 and 4 hours respectively, beads were washed 4 times and proteins eluted directly in 1X laemmli loading buffer supplemented with 50 mM DTT. Western blot was performed using the following antibodies: anti-SMARCC1 (PCRP-SMARCC1-1F1, DSHB), BRG-1 (H10) (sc-374197, Santa-Cruz), anti-FLAG M2 (368791, Sigma-Alrich), anti-BRG/BRM (J1) (a generous gift from J.Lessard, IRIC).

Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR)

Mushroom body nuclei were isolated from 3 biological replicates from both mature and juvenile Bap60-KD MB and control flies in an independent INTACT experiment followed by RNA isolation (see Methods). cDNA was synthesized using the SensiFAST cDNA Synthesis Kit (FroggaBio) following manufacturer's instructions. Quantitative PCR was performed on a BioRad CFX 384 using the SensiFAST SYBR No-ROX Kit (FroggaBio) following manufacturer's instructions using the primers listen in **Table S7**. Relative expression was determined using the ΔΔCt method normalized for the reference genes *eIF2β* and *βcop*.