

Supplementary Tables

Supplementary Table 1: Clinical characteristics of eighteen affected individuals with biallelic *SUPV3L1* variants.

Supplementary Table 2: MRI features of patients with *SUPV3L1* variants.

Supplementary Figures

Supplementary Figure 1: Conservation of SUV3 missense variants in orthologs.

Supplementary Figure 2: Collated MRI imaging of patients with *SUPV3L1* variants.

Supplementary Figure 3: Representative images from the optimisation and validation of the dsRNA complementation assay.

Supplementary Figure 4: Interferon signature in patients with biallelic *SUPV3L1* mutations.

Supplementary Table 1: Clinical characteristics of the 19 patients with biallelic *SUPV3L1* variants

	Family 1			Family 2	Family 3
	P1	P2	P3	P4	P5
Variant 1	c.1093C>T, p.Arg365Trp;	c.1093C>T, p.Arg365Trp;	c.1093C>T, p.Arg365Trp;	c.458-2A>G, p.(splice);	c.1321dup, p.Tyr441Leufs*10;
Variant 2	c.1093C>T, p.Arg365Trp	c.1093C>T, p.Arg365Trp	c.1093C>T, p.Arg365Trp	c.1070C>A, p.Ala357Glu	c.1924A>C, p.Ser642Arg
Background details					
Gender	F	F	M	F	M
Ethnicity	South Asian	South Asian	South Asian	Caucasian	Slavic
Consanguinity	Y	Y	Y	N	N
Current age	15y	13y	20y	5y 9m	3y
Age at last examination	15y	13y	13y	6y	2y 1m
Age at death	NA	NA	NA	NA	NA
Presentation					
Age at onset	<12m	<12m	UK	5w	<6m
Presenting features	Developmental delay, hypotonia	Developmental delay, hypotonia	Speech and motor delay	Feeding issues, weight loss	Hypotonia, motor delay
Additional features					
Microcephaly	Y	Y	Y	Y (0.4 th – 2 nd percentile)	Y (-0.5 SD)
Spasticity	Y	Y	Y	Y – LL>UL	Y
Seizures	N	Y – from 12y	N	Y – aged 2m, resolved	N
Skin changes	Y – vitiligo	Y – vitiligo (progressive)	NK	Y – hypopigmented lesions	Y – hypopigmented lesions
Feeding difficulties	N	Y – NGT since 12y	N	Y – GOR	N
Other	Hypermobility, swan-neck deformity of fingers	Intermittent nystagmus, brisk DTRs	LL weakness, contractures	Central hypotonia, laryngomalacia, small ASD, strabismus, hypersensitivity to sound	Central hypotonia
Development & progress					
Intellectual impairment	Y – severe	Y – severe	Y – non-verbal	Y	NK
Developmental delay	Y – severe	Y – severe	Y – speech and motor	Y – speech and motor	Y – speech and motor
Highest motor milestone	Commando crawls	Head control	Walk with significant support	Walked with support 2y	Walked 1y 7m – unsteady
Regression	N	Y – 12y with seizure onset	N	Y – no longer walks/crawls	N
Abnormal IFN signature	NK	NK	NK	Y	NK

	Family 4		Family 5	Family 6	Family 7
	P6	P7	P8	P9	P10
Variant 1 Variant 2	c.854-3A>G, p.(splice); c.854-3A>G, p.(splice)	c.854-3A>G, p.(splice); c.854-3A>G, p.(splice)	c.329G>A, p.Gly110Asp; c.329G>A, p.Gly110Asp	c.2215C>T, p.Gln739Ter; c.2215C>T, p.Gln739Ter	c.2215C>T, p.Gln739Ter; c.2215C>T, p.Gln739Ter
Background details Gender Ethnicity Consanguinity Current age Age at last examination Age at death	M Pakistani Y 12y 11y NA	F Pakistani Y 3y 3y NA	M Afghan Suspected genetically 10y 9y 11m NA	F Arab N 17y 12y NA	M Arab Y 11y 6y NA
Presentation Age at onset Presenting features	Birth Neonatal hypoglycaemia and thrombocytopenia	<12m Acquired microcephaly, developmental delay	<12m Hypotonia, areflexia, nystagmus	10m Global developmental delay	5m Global developmental delay
Additional features Microcephaly Spasticity Seizures Skin changes Feeding difficulties Other	Y Y Y - myoclonus N Y – PEG/J Neonatal thrombocytopenia, hypoglycaemia, dyskinesia	Y – acquired Y N N Y – PEG Pre-term birth 27/40 – chorioamnionitis, ASD	N N Y N Y NA	Y Y N N Y Facial dysmorphism, axial hypotonia, retinal dystrophy	Y Y N N Y Ambiguous genitalia, failure to thrive
Development & progress Intellectual impairment Developmental delay Highest motor milestone Regression	Y – severe Y - non-verbal Walk with support N	Y Y – severe, non-verbal Stands with support N	Y Y Crawls Y	Y – severe Y – severe Walked with support Y – no longer mobile	Y – severe Y – severe Sitting (supported) N
Abnormal IFN signature	Y	Y	NK	NK	NK

	Family 8	Family 9	Family 10		Family 11
	P11	P12	P13	P14	P15
Variant 1 Variant 2	c.1177G>A, p.Ala393Thr; c.1177G>A, p.Ala393Thr	c.196C>T, p.Pro66Leu; c.1240C>T, p.Pro414Ser	c.1469A>G, p.Asp490Gly; c.1469A>G, p.Asp490Gly	c.1469A>G, p.Asp490Gly; c.1469A>G, p.Asp490Gly	c.1240C>T, Phe124Ser; c.1240C>T, Phe124Ser
Background details Gender Ethnicity Consanguinity Current age Age at last examination Age at death	M Turkish Y Deceased 3d 25d	M Mexican American N 5y 5y NA	M Iranian (Arab) Y 5y 8m 5y 8m NA	F Iranian (Arab) Y 4y 3m 4y 3m NA	M Pakistani Y 2y 2y NA
Presentation Age at onset Presenting features	Birth Prenatal brain anomalies, neonatal respiratory distress	4 y Ataxia, encephalopathy, drooling, dystonia, dysarthria following illness	4m Developmental delay, hypotonia	<6m Developmental delay, hypotonia	NK Global developmental delay
Additional features Microcephaly Spasticity Seizures Skin changes Feeding difficulties Other	Y Y Y – neonatal NK Y – from birth, NGT and GOR Lissencephaly, neonatal RDS	N N N N N	Y (49.5 cm) Y N Y – hypopigmented lesions N Toe walking, flat foot	Y (48 cm) Y N Y – hypopigmented lesions N Strabismus	Y (45cm) Y N N N BL optic disc pallor
Development & progress Intellectual impairment Developmental delay Highest motor milestone Regression	NA Y None N	N Y – mild speech (articulation only) Walking Y	Y - moderate delay Y- speech and motor Walk slowly without support N	Y - moderate delay Y- speech and motor Walks with significant support N	Y Y- global, non-verbal NK N
Abnormal IFN signature	NK	Y	NK	NK	NK

	Family 12			Family 13	Family 14
	P16	P17	P18	P19	P20
Variant 1 Variant 2	c. 1469A>G, p.Asp490Gly; c. 1469A>G, p.Asp490Gly	c. 1469A>G, p.Asp490Gly; c. 1469A>G, p.Asp490Gly	c. 1469A>G, p.Asp490Gly; c. 1469A>G, p.Asp490Gly	c. 1924A>C, p.Ser642Arg; c. 926T>G, p.Leu309Arg	c.2215C>T, p.Gln739Ter; c.2215C>T, p.Gln739Ter
Background details Gender Ethnicity Consanguinity Current age Age at last examination Age at death	F Egyptian Y 8y6m 8y Alive	M Egyptian Y 7y6m 7y Alive	M Egyptian Y 32y9m 32y Alive	M Russian-Caucasian N 34y4m 34y3m Alive	F Omani N 7m 6m Alive
Presentation Age at onset Presenting features	4m Developmental delay, hypotonia, nystagmus	6m Developmental delay, nystagmus	Childhood Intellectual impairment and abnormal gait	6m Developmental delay	2m Focal seizures, irritability
Additional features Microcephaly Spasticity Seizures Skin changes Feeding difficulties Other	Y (HC 49cm) Y N Y - hypopigmented areas Y N	Y (HC: 47.5cm) Y - mild N Y - hypopigmented areas Y Behavioural concerns – autistic features	N Y - mild, asymmetry of lower limbs N N N Dysarthria, cataracts and retinal changes (operated)	N Y N Y – hypopigmented areas in groin Y – dysphagia with solids Dystonia (oro-facial), oculomotor apraxia, ataxia	Y (below 3 rd centile) Y – upper > lower limbs Y – focal N Y – disordinated swallow and choking episodes Dysmorphic – narrow, elongated face, high palate
Development & progress Intellectual impairment Developmental delay Highest motor milestone Regression	Y Y – single words only Walks supported N	Y Y Walk supported N	Y N Walk with limp Y – visual deterioration following febrile illness aged 22y	Y Y Walked 4y N – progression motor disorder led to loss of walking aged 16y	N/A Y Partial head control 6 months N
Abnormal IFN signature	NK	NK	NK	NK	NK

Abbreviations: WT = wild type; F = female; M = male; Y = yes; N = no; d = day; w = week; m = month; y = year; NA = not applicable; LL = lower limb; UL = upper limb; FTT = failure to thrive; NK = not known; ASD = atrial septal defect; PEG/J = percutaneous endoscopic gastro-jejunostomy, NGT = nasogastric tube, GOR = gastro-oesophageal reflux, BL = bilateral, IFN = interferon.

Supplementary Table 2: MRI features of patients with *SUPV3L1* variants

	P1	P2	P3	P4	P5	P6	P7	P9
Age at last MRI	5y	32m	22m	6y	23m	10m	4m	2y
Abnormal myelination	Y	Y	Y	Y - improving	Y	Y	Y	N
Temporal horn changes	Y – SC cysts	Y – SC cysts	Y – SC cysts	Y – SC cysts	NK	NK	NK	N
Structural abnormality	N	N	N	Y – thin CC	Y – thin CC	N	NK	Y- dysmorphic CC
Atrophy Cerebral Cerebellar	N Y	N Y	N Y	N Y	Y Y - mild	N N	Y NK	N N
Calcification	N	N	N	N	N	Y - BG	Y - BG	N
Other features	N	N	N	N	N	N	NK	N

	P11	P12	P13	P14	P16	P17	P18	P19
Age at last MRI	3d	5y	6y	4y	4y	3y	32y	16y
Abnormal myelination	NK	N	Y	Y - mild	Y	Y	N	N
Temporal horn changes	NK	N	N	N	N	Y	N	N
Structural abnormality	Y – partial CC agenesis, vermian/pons hypoplasia, lissencephaly	N	N	N	N	N	Y – dysmorphic CC	N
Atrophy Cerebral Cerebellar	N Y	N N	N Y	N Y	Y Y	Y NK	Y Y	N Y – moderate
Calcification	NK	N	N	N	NK	NK	N	N
Other features	Ventriculomegaly Post. fossa/SC WM cysts	N	N	N	N	N	N	N

Abbreviations: Y = yes; N = no; m = month; y = year; NK = not known; CC = corpus callosum; SC = subcortical; BG = basal ganglia

Supplementary Figure 1: Conservation of SUV3 missense variants in orthologs.

Conservation analysis of SUP3L1 variant residues, including 10 amino acids flanking either side, across 11 vertebrate species: Human (NP_003162.2), Mouse (NP_852088.1), Rat (NP_001012480.1), Zebrafish (NP_001077033.1), Chicken (NP_001006498.1), Chimp (XP_521497.2), Dog (XP_546136.2), Pig (XP_001928933.2), Cow (XP_005226398.1), Horse (XP_001918024), Cat (XP_003994050.3).

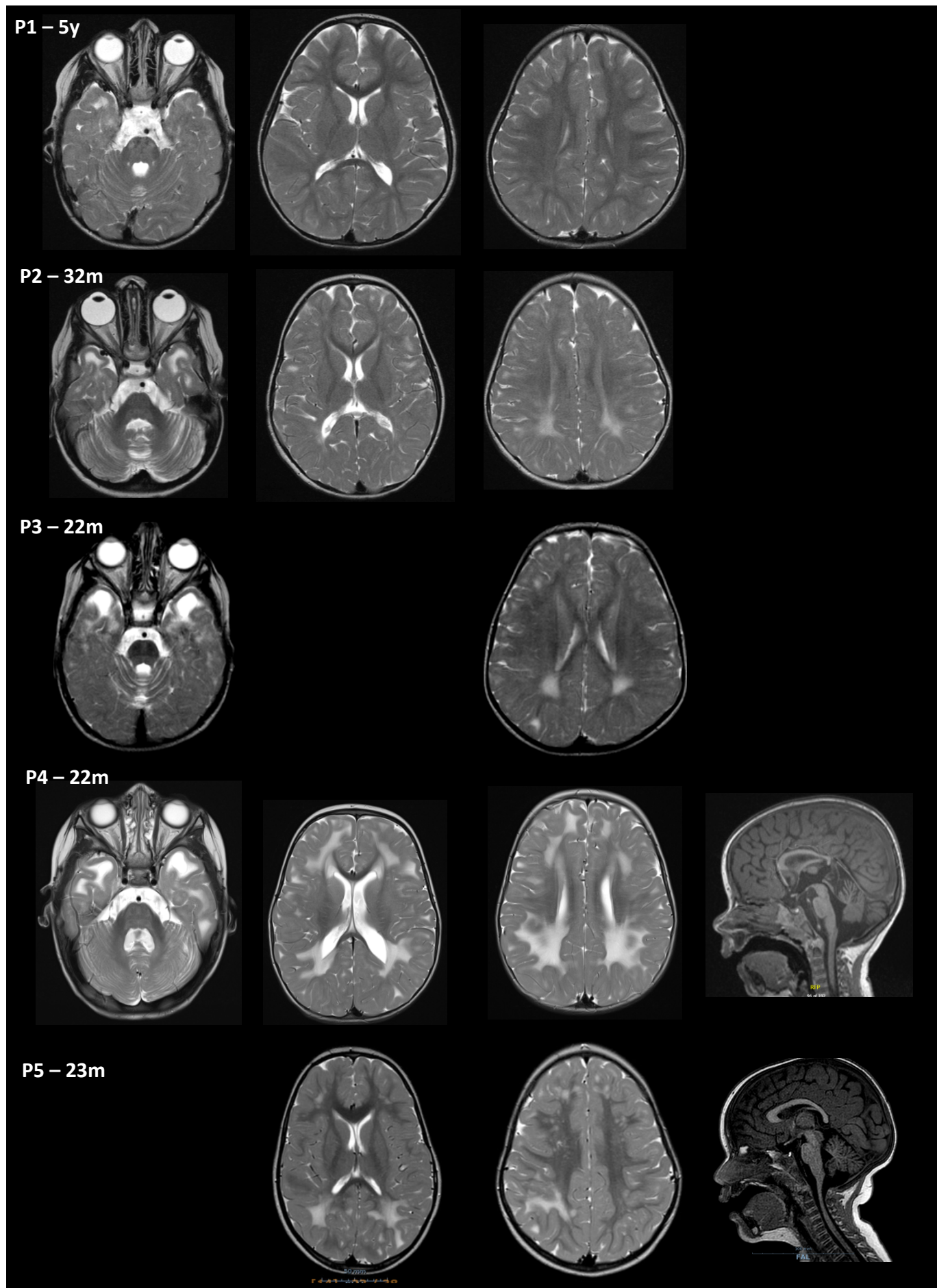
		Pro66Leu			Gly110Asp		Phe124Ser	
HUMAN	56	SKIPNTSLFVPLTVKPGGPSA	76	100	FYKRKEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	120
CHIMP	56	SKIPNTSLFVPLTVKPGGPSA	76	100	FYKRKEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	120
MOUSE	56	SKAPNTSLFVPLTVKPGGPSA	76	100	FYKRQEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	120
RAT	56	SKAPNTSLFVPLTVKPGGPSA	76	100	FYKRQEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	120
DOG	58	SKAPNTSLFVPLTVKPGGPSA	78	102	FYKRKEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	122
CAT	59	SKAPNTSLFVPLTVKPGGPSA	79	103	FYKRKEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	123
PIG	58	SKAPNTSLFVPLTVKPGGPSA	78	102	FYKRKEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	122
HORSE	56	SKAPNTSLFVPLTVKPGGPSA	76	100	FYKRKEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	120
COW	57	GKAPNTSLFVPLTVKPGGPSA	77	101	FYKRKEIQKL	GADYGLDARLFHQAF	ISFRNYIMQS	121
CHICKEN	54	LRAPDTSLFVPLKPEVEGAA	64	88	FYKRKEIQRL	GTENGLDARLFHQAF	ISFRKYIMES	108
ZEBRAFISH	53	SRPLDTSLFIPFVKTDEDA-	74	98	FYKRKEMQKL	ASDQGLDARLFHQAF	VSFRKYVLEM	118

		Glu339Val			Ala357Glu		Arg365Trp	
HUMAN	329	VMELMYTTGEEVEVRDYKRLTPISVLDHALES	LDNLR	PGDCIVCF	SK	375		
CHIMP	329	VTELMYTTGEEVEVRDYKRLTPISVLDHALES	LDNLR	PGDCIVCF	SK	375		
MOUSE	329	VSELLYTTGEEVEVQKYERLTPISVLDHALES	LDNLR	PGDCIVCF	SK	375		
RAT	329	VTELLYTTGEEVEVQKYERLTPISVLDHALES	LDNLR	PGDCIVCF	SK	375		
DOG	331	VTELMYTTGEEVEVRNYKRLTPISVLDHALES	LDNLR	PGDCIVCF	NK	377		
CAT	332	VTELMYTTGEEVEVRNYKRLTPISVLDHALES	LDNLR	PGDCIVCF	SK	378		
PIG	331	VTELMYTTGEEVEVRTYKRLTPISVLDHALES	LDNLR	PGDCIVCF	SK	377		
HORSE	329	VTELMYTTGEEVEVRTYKRLTPITVLDHALES	LDNLR	PGDCIVCF	SK	375		
COW	330	VTELMYTTGEEVEVRTYKRLTPISVLDHALES	LDNLR	PGDCIVCF	SK	376		
CHICKEN	317	VTELMYTTGEEVEVRNYKRLTPLTVLDYALE	SLDNLR	PGDCIVCF	SK	363		
ZEBRAFISH	327	ITELMFTTGEEVEVHNYKRLTPFSISNHA	VESLDNLR	PGDCIVCF	SK	373		

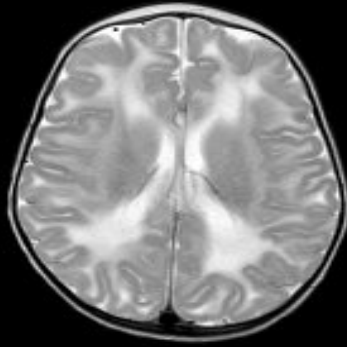
		Ala393Thr					Pro414Ser				
HUMAN	383	RQIEIRGLES	AVIYGSLPPGT	403	404		KLAQAKKFND	PNDPCKILVAT	424		
CHIMP	383	RQIEIRGLES	AVIYGSLPPGT	403	404		KLAQAKKFND	PNDPCKILVAT	424		
MOUSE	383	RQIEIRGLES	AVIYGSLPPGT	403	404		KLAQARKFND	PNDPCKILVAT	424		
RAT	383	RQIEIRGLES	AVIYGSLPPGT	403	404		KLAQARKFND	PNDPCKILVAT	424		
DOG	385	RQIEIRGLES	AVIYGSLPPGT	405	406		KLAQAKKFND	PDDPCKILVAT	426		
CAT	307	RQIEIRGLES	AVIYGSLPPGT	407	408		KLAQAKKFND	PDDPCKILVAT	428		
PIG	385	RQIEIRGLES	AVIYGSLPPGT	405	407		KLAQAKKFND	PDDPCKILVAT	427		
HORSE	383	RQIEIRGLES	AVIYGSLPPGT	403	404		KLAQAKKFND	PDDPCKILVAT	424		
COW	384	RQIEIRGLES	AVIYGSLPPGT	404	407		KLAQAKKFND	PDDPCKILVAT	427		
CHICKEN	371	RQIEARGLECA	VIYGSLPPGT	391	392		KLEQAKKFND	PNDPCKILVAT	412		
ZEBRAFISH	381	RQIEIRGLECA	VIYGSLPPGT	401	402		KLAQAKKFND	PDDPCKILVAT	422		

		Asp490Gly			Ser642Arg	
HUMAN	480	EGEVTTMNHEDLSLLKEILKR	500	632	HDVLDLYLWLSYRFMDMFPDA	652
CHIMP	480	EGEVTTMNHEDLSLLKEILKR	500	632	HDVLDLYLWLSYRFMDMFPDA	652
MOUSE	480	EGQVTTMHRDDLALLKDILNR	500	632	HDVFDLYLWLSYRFIDMFPDS	652
RAT	480	EGEVTTMHRDDLALLKEILNR	500	632	HDVFDLYLWLSYRFIDMFPDS	652
DOG	482	EGEVTTMNRDLSLLKEILKR	502	634	HDVLDLYLWLSYRFIDMFPDA	654
CAT	483	EGEVTTMNRDNLNLLKEILNR	503	635	HDVLDLYLWLSYRFIDMFPDA	655
PIG	482	EGEVTTMHRDNLNLLKEILSR	502	634	HDVLDLYLWLSYRFIDMFPDA	654
HORSE	480	EGEVTTMNRDNLNLLREILNR	500	632	HDVLDLYLWLSYRFIDMFPDA	652
COW	481	EGEVTTMNRDLSLLKEILNR	501	633	HDVLDLYLWLSYRFLDMFPDA	653
CHICKEN	468	QGEVTAMHRDDLQLKEILSE	488	620	HDVFDLYLWLSYRFMDMFPDA	640
ZEBRAFISH	478	EGEVTTMHRDDLPLVLEILGK	498	630	HDVLDLYLWLSYRFMDMFPDS	650

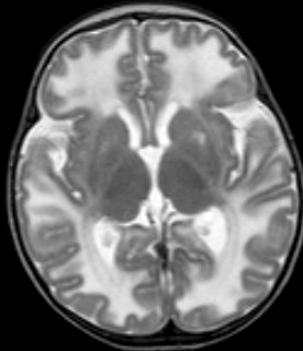
Supplementary Figure 2: Collated MRI imaging of patients with *SUPV3L1* variants.



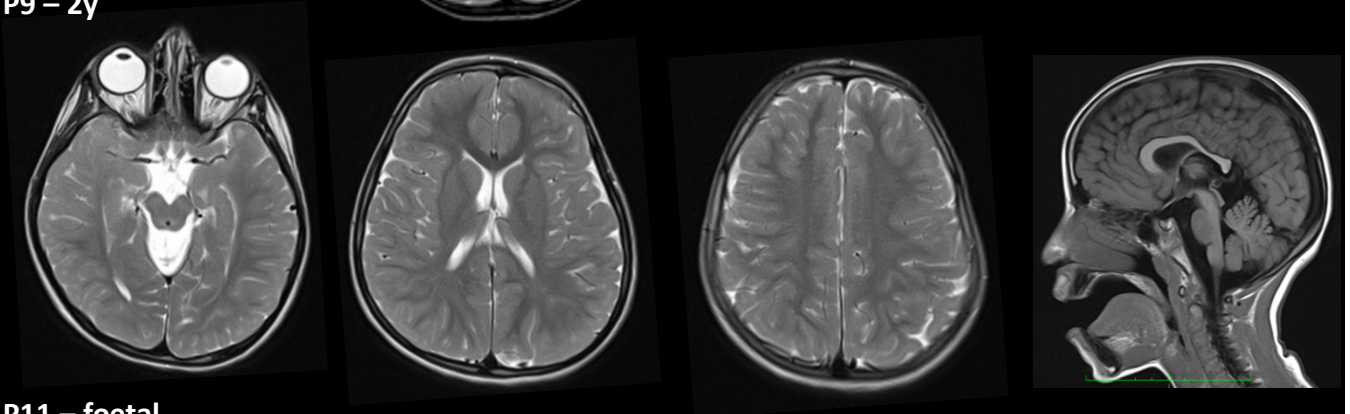
P6 – 10m



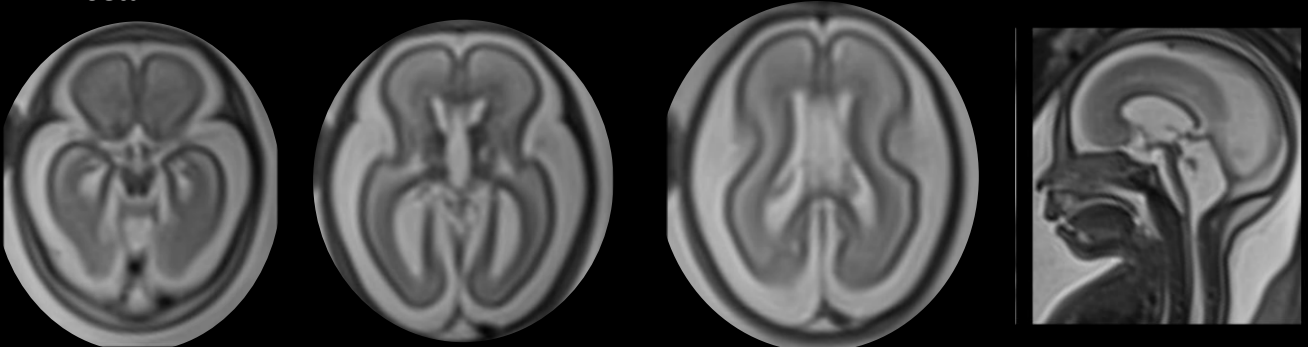
P7 – 4m



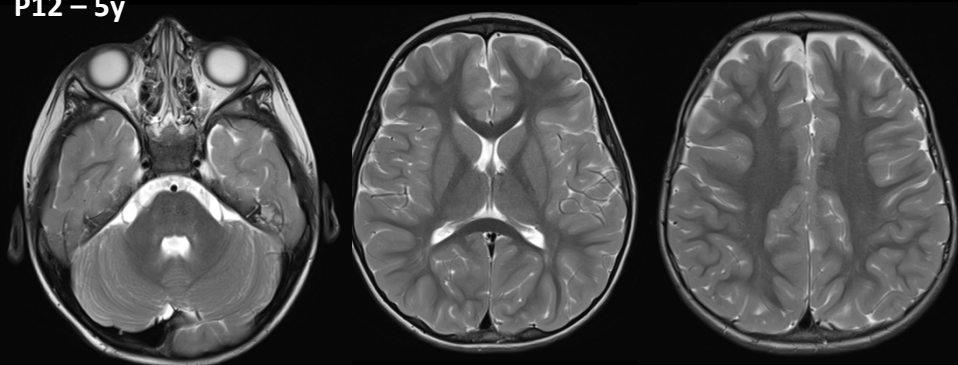
P9 – 2y



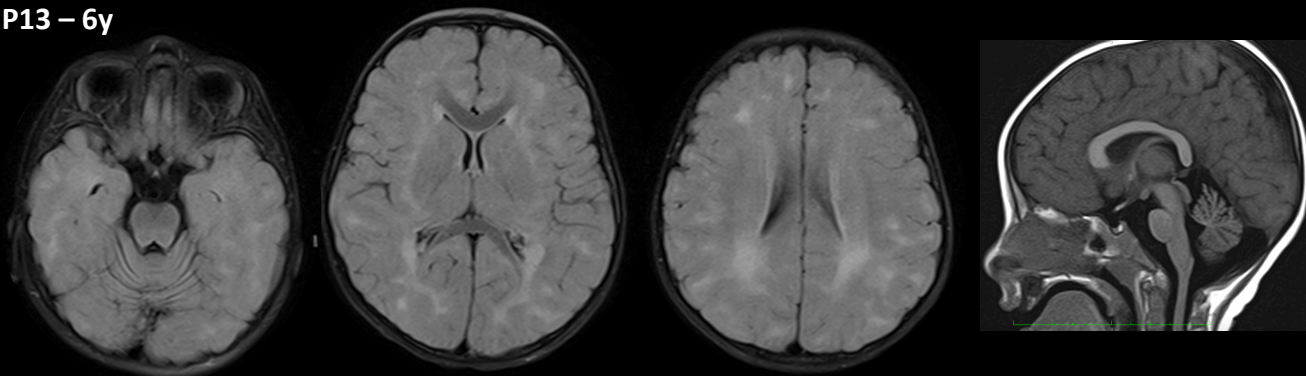
P11 – foetal



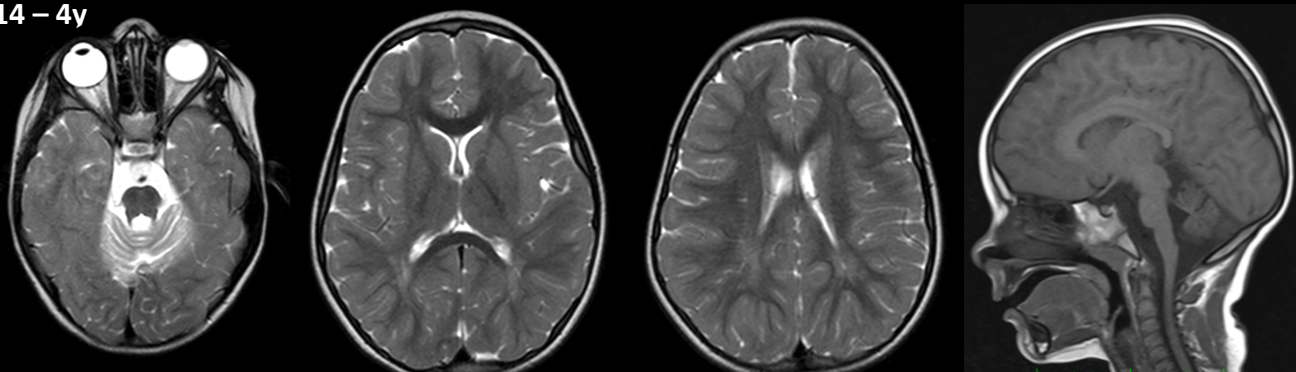
P12 – 5y



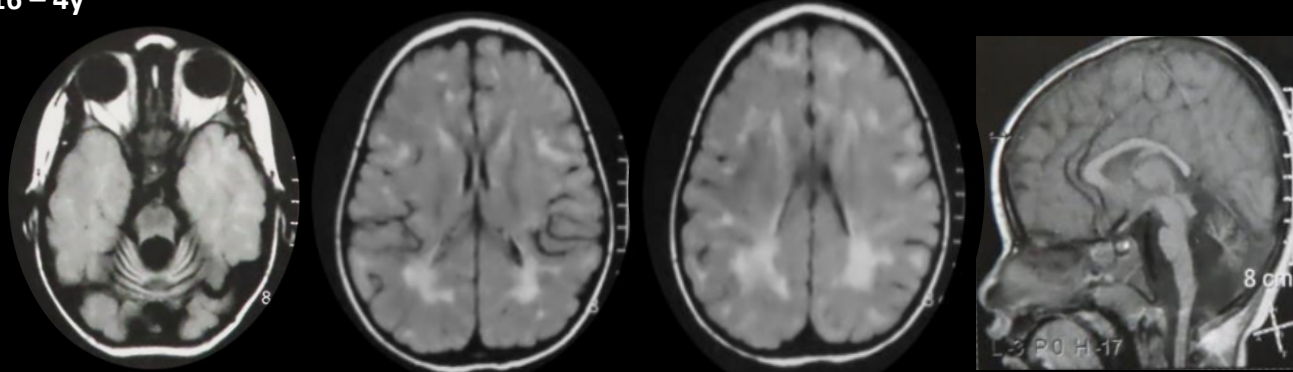
P13 – 6y



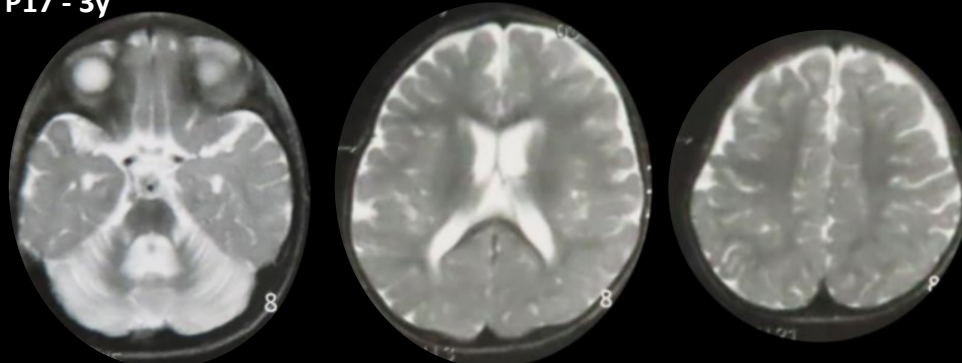
P14 – 4y



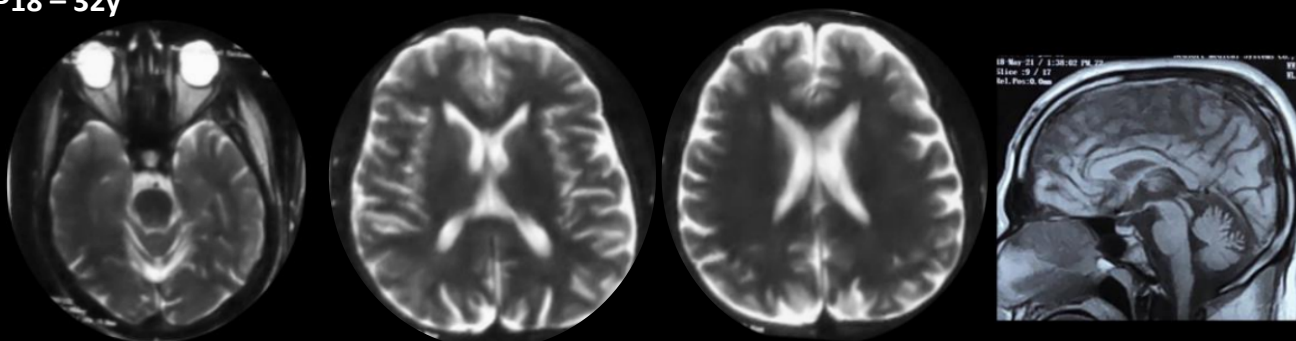
P16 – 4y



P17 – 3y

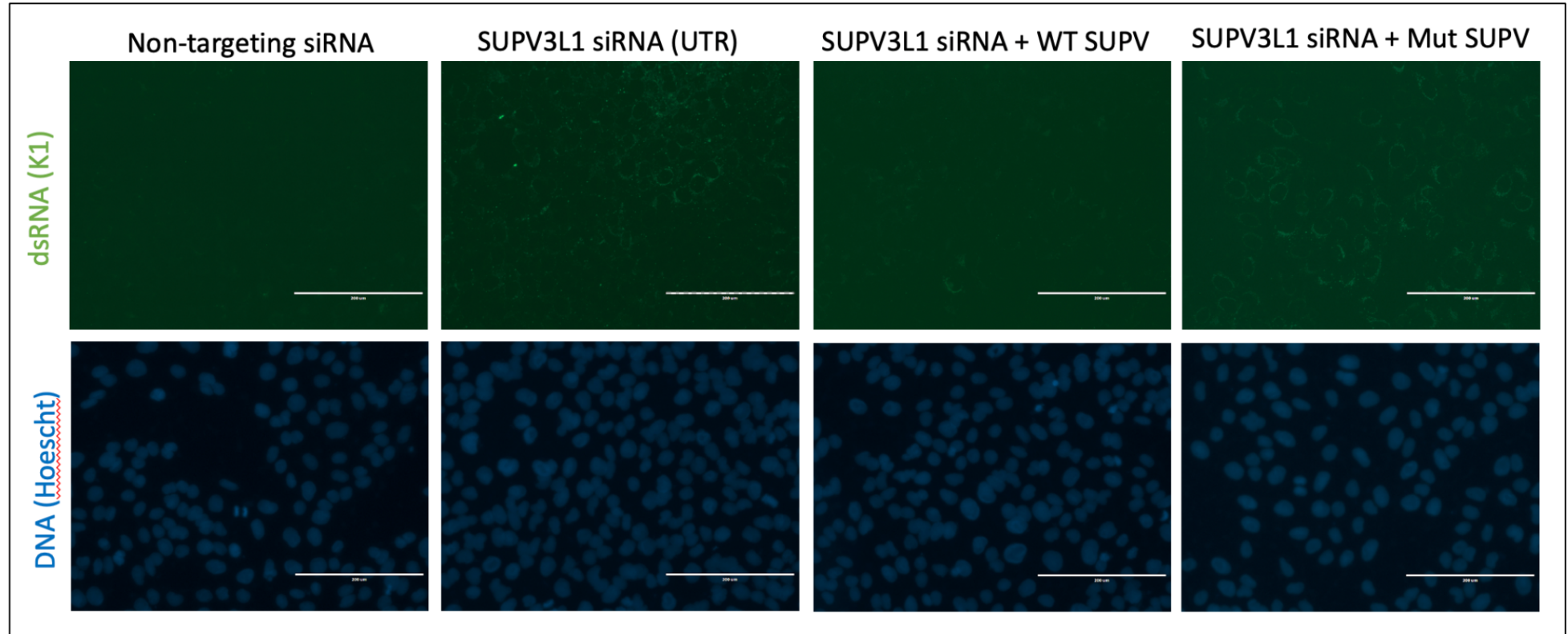


P18 – 32y

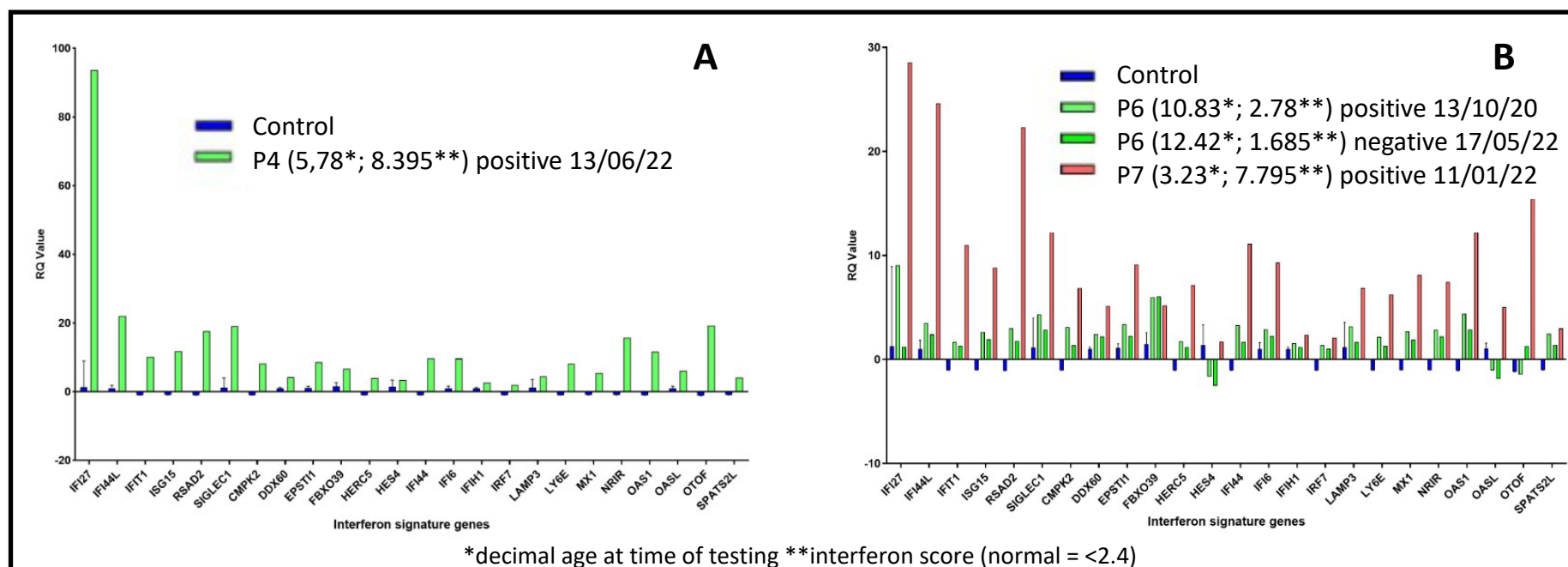


Supplementary Figure 3: Representative images from the optimisation and validation of the dsRNA complementation assay.

siRNA knockdown of SUPV3L1 results in an increase in double stranded RNA (dsRNA) that is not observed with the non-targeting siRNA. Subsequent transfection of wild-type SUPV3L1 leads to a reduction in dsRNA, suggestive of a rescue of the cellular phenotype. Transfection with a known mutation (p.Gln739Ter) did not show a corresponding rescue of the dsRNA levels to that of the wild-type. Scale bar represents 200µM.



Interferon signature for P4, 6 and 7 was performed, comparing expression of 24 interferon stimulated genes (ISGs) compared to control datasets (blue). A normal interferon score is <2.4 . P4 (A green bars) and P7 (B red bars) demonstrated marked up-regulation when tested. P6 (B green bars), though also demonstrating a positive result with up-regulation of 21/24 ISGs at first testing, the result was much less marked. Repeat testing 19 months later was negative. Testing was performed at the MRC Human Genetics Unit, Edinburgh. P12 had assessment of interferon signature in the Cincinnati Children's hospital via a research study (C). Expressed as Z-scores calculated from comparison with healthy controls, this demonstrates a marked up-regulation of type 1 interferon response when compared with control and the probands mother.



	Control	Proband (P12)	Probands mother
Type 1	4.27	397.89	-2.75
Gamma	-0.54	6.36	-2.66
NK-kB	0.97	0.82	0.26

12