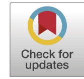
Stem Cell Research 85 (2025) 103696

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Single Cell Line



Derivation of the IGGi006-A stem cell line from a patient with *CAPRIN1* haploinsufficiency

Giuseppina Conteduca [a], Chiara Baldo [a], Alessia Arado [a], Joana Soraia Martinheira da Silva [b], Renata Bocciardi [b,c], Barbara Testa [a], Simona Baldassari [c], Maria Margherita Mancardi [d], Federico Zara [b,c], Michela Malacarne [a], Domenico Coviello [a,*]

[a] *Laboratory of Human Genetics, IRCCS Istituto Giannina Gaslini, Genova, Italy*
[b] *Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Child and Maternal Sciences (DINOGMI), University of Genoa, Genoa, Italy*
[c] *Unit of Medical Genetics, IRCCS Istituto Giannina Gaslini, Genoa, Italy*
[d] *Child Neuropsychiatry Unit Member of the ERN EpiCARE, IRCCS Istituto Giannina Gaslini, 16147 Genoa, Italy*

ABSTRACT

CAPRIN1 gene encodes a RNA-binding protein, abundant in the brain where it plays a crucial role, regulating the transport and translation of mRNAs of synaptic proteins. *CAPRIN1* haploinsufficiency causes a neurodevelopmental disorder characterized by language impairment/speech delay, intellectual disability, attention deficit, hyperactivity disorder, and autism spectrum disorder. To understand the pathogenesis of this disorder and in view of future treatment, we generated human induced pluripotent stem cells (iPSCs) from a patient carrying the c.1744C>T *CAPRIN1* variant. The line show marker expression for the pluripotency and the capacity to differentiate into the three germ layers.

Resource table

Unique stem cell lines identifier	IGGi006-A
Alternative name(s) of stem cell lines	*N/A*
Institution	*IRCCS Istituto Giannina Gaslini*
Contact information of distributor	*geneticbiobank@gaslini.org*
Type of cell lines	*iPSC*
Origin	*Human*
Additional origin info required for human ESC or iPSC	*Age: 10* *Sex: F* *Ethnicity: Caucasian*
Cell Source	*Fibroblasts*
Clonality	*Clonal*
Method of reprogramming	*Non-integrating vector, transgenes used: SeV, COS, klf4, c-Myc*
Genetic Modification	*Yes*
Type of Genetic Modification	*De novo germline nonsense variant*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*RT-/q-PCR*
Associated disease	Neurodevelopmental disorders (OMIM# 620636 and 620782)
Gene/*locus*	GRCh38/hg38, chr11:34,051,731-34,102,610
Date archived/stock date	*24/03/2023*
Cell line repository/bank	https://hpscreg.eu/cell-line/ IGGi006-A
Ethical approval	This study was approved by the Ethics Committee of the Liguria Region, Italy (Approval #GTB12101 n∘8/2015, 14 September 2015).

(*continued on next column*)

(*continued*)

1. Resource utility

CAPRIN1 loss of function causes autism spectrum disorders associated with reduced neuronal processes, overall disruption of the neuronal organization and increased neuronal degeneration. The generation of iPSCs will be a powerful tool to elucidate the underlying pathogenic mechanism and a disease-relevant model to investigate innovative therapeutic approaches (Table 1).

2. Resource details

Cell cycle associated protein 1 (*CAPRIN1*; OMIM*601178) encodes a ubiquitously expressed protein in postsynaptic granules in dendrites in the hippocampus. Recently studies showed that immunoprecipitates of Caprin-1 from lysates of Xenopus brain cells contain proteins involved in synaptic plasticity. The N-terminal part of CAPRIN1 harbors a

* Corresponding author at: Laboratory of Human Genetics, IRCCS Istituto Giannina Gaslini, via Gerolamo Gaslini, 5, 16147 Genoa, Italy.
E-mail address: domenicocoviello@gaslini.org (D. Coviello).

https://doi.org/10.1016/j.scr.2025.103696
Received 18 January 2025; Received in revised form 6 March 2025; Accepted 9 March 2025
Available online 13 March 2025
1873-5061/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

dimerization domain and the binding sites for the RBPs G3BP1 and FMR1 promoting the formation of a complex that colocalizes in cytoplasmic RNA granules on microtubules (Solomon et al., 2007). Patients with *CAPRIN1* variants, show a neurodevelopmental phenotype characterized by language impairment/speech delay, intellectual disability, attention deficit, hyperactivity disorder and autism spectrum disorder (Pavinato et al., 2023). *CAPRIN1* loss causes reduced neuronal processes, disruption of the neuronal organization and an increased neuronal degeneration. In order to establish a patient-derived cellular model to study the effect of *CAPRIN1* haploinsufficiency in patients, we reprogrammed into iPSCs primary fibroblasts cells from a female patient with intellectual disability, absent speech, generalized epilepsy and severe hyperactive behaviour, carrying a c.1744C>T (p.Gln582X) nonsense mutation located in exon 16 of *CAPRIN1* (Fig. 1, panel F). Fibroblasts were obtained from skin biopsies punch and fibroblasts at passage 3 (P3) were transfected with CytoTune-iPS 2.0 Sendai reprogramming kit (Conteduca et al., 2022). The generated iPSC lines presented the typical stem cell morphology with tightly packed colonies and high nuclear/cytoplasmic ratio (Fig. 1, panel A). Twenty days after transduction iPSC colonies were picked. Normal female karyotype (46, XX) without structural chromosomal aberrations was confirmed by Cytogenetic analysis (Fig. 1, panel B). RT-PCR analysis confirmed the absence of exogenous Sendai vectors (SeV genome and the transgenes Klf4, KOS and c-Myc), in the IGGi006-A line (P14) (Supplementary Fig. 1A). We performed a Short tandem repeat (STR) analysis to confirm that parental fibroblasts and iPSC (P14) clone were both from the same patient (Supplementary Table 1). We excluded the presence of genomic rearrangement, as deletion or duplication, by Comparative Genomic Hybridisation analysis (CGH) (Table 3). The pluripotent markers OCT4, SSEA4, TRA-1–60 and SOX2 were confirmed to be present by immunofluorescence staining (Fig. 1, panels C1, C2), and we tested the expression of pluripotency markers, *NANOG, SOX2, OCT4, DPPA2, DPPA4, KFLA4, FGF4, REX, RUNX1*, and *TGDF1* by Real-Time PCR in iPSC at 20 days after the transduction, compared to the cell 1 day before the transduction and we used iPSC cell line validated in our recently work as positive control (Conteduca et al., 2022) (Fig. 1 panel E). In Table 2 we reported all antibodies and primers used. Also, high quality cells were differentiated into the three embryonic germ layers, in order to evaluate the iPSCs pluripotency. Specific markers for each layer were analysed by Real Time PCR (Fig. 1, panel D) and immunofluorescence (Fig. 1, panel G). Moreover, the established iPSC line was negative for Mycoplasma (Supplementary Fig. 1C). This cell line will serve as a valuable in vitro model resource for both basic and translational studies in *CAPRIN1* haploinsufficiency.

3. Materials and methods

3.1. Skin biopsy and fibroblasts culture

This study was approved by the Liguria Region Ethical Committee (Approval # GTB12101 n∘8/2015, 14 September 2015) and we obtained a written informed consent from parents of the patient.

We received the fibroblast cell line by the Biobank of the Laboratory of Human Genetics – IRCCS istituto Gaslini, member of the Telethon Network of Genetic Biobanks (project no. GTB18001).

Using a dermal puncher tool we collected a skin biopsy from patient's forearm. Also, we established a primary culture of fibroblasts with Dulbecco's modified Eagles (DMEM) with the addition of 10 % fetal bovine serum and 2 mM of L-glutamine and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin).

3.2. iPSC reprogramming

Fibroblast cells were reprogrammed using CytoTune-iPS 2.0 Sendai kit (Thermo Fisher). iPSCs were maintained in feeder-free conditions on plates coated with Vitronectin (VTN-N, Thermo Fisher) using Essential 8 Flex Medium (Thermo Fisher) at 37 °C and 5 % CO2. Every 3–4 days, when the cells reached the 85–90 % of confluence we used 0.5 mM Versene (Thermo Fisher Scientific, Waltham, MA, USA) to harvest the cells, and to split them with a ratio of 1:5–1:8. We performed RT-PCR to check the loss of the exogenous reprogramming factors (primers in Table 2). In order to prevent cell death, after passaging we added the ROCK inhibitor (10 μM Y27632, Voden Medical) to the culture for 24 h. We cryopreserved the mycoplasma-free cells in the liquid nitrogen for further usage.

3.3. Immunocytochemistry

At passage 6 and 20 the pluripotency of iPSCs where evaluated with Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Thermo Fisher). Briefly, cells were fixed with 4 % formaldehyde for 15 min at room temperature (RT), incubated with permeabilization solution for

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panels A1-A2
Phenotype	Qualitative analysis by Immunocytochemistry	Both iPCS lines express pluripotency markers: OCT4, SSEA4, TRA-1–60 and SOX2	Fig. 1 panels C1-C4
	Quantitative analysis by RT-qPCR	Both iPSC lines express pluripotency markers: OCT4, NANOG, SOX2, DPPA2, DPPA4, KFLA4, FGF4, REX, RUNX1, and TGDF1	Fig. 1 panel D1-D2
Genotype	Karyotype (G-banding) and resolution	46 XX, Resolution 450–500	Fig. 1 panels B1-B2
Identity	STR analysis	10 markers tested-matched	Submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous for c.1744C > T(p.(Gln582Ter)nonsense mutation in *CAPRIN1* gene	Fig. 1 panel F
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR. Culture Negative	Supplementary Fig. 1 panel B
Differentiation capacity	Direct differentiation	Three germ layers formation: ectoderm, mesoderm and endoderm	Fig. 1, panel E1-E2
List of recommended germ layer markers	Expression of these markers needs to be demonstrated at mRNA (RT PCR) and protein (IF) levels, at least 2 markers *per* germ layer are required	Expression of germ layer specific markers: ectoderm (*PAX6, TUBB3*), mesoderm (*BRACHYURY, NCAM*) and endoderm (*FOXA2, GATA4*).	RT-PCR with reference gene(s): Fig. 1 panel E1-E2
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

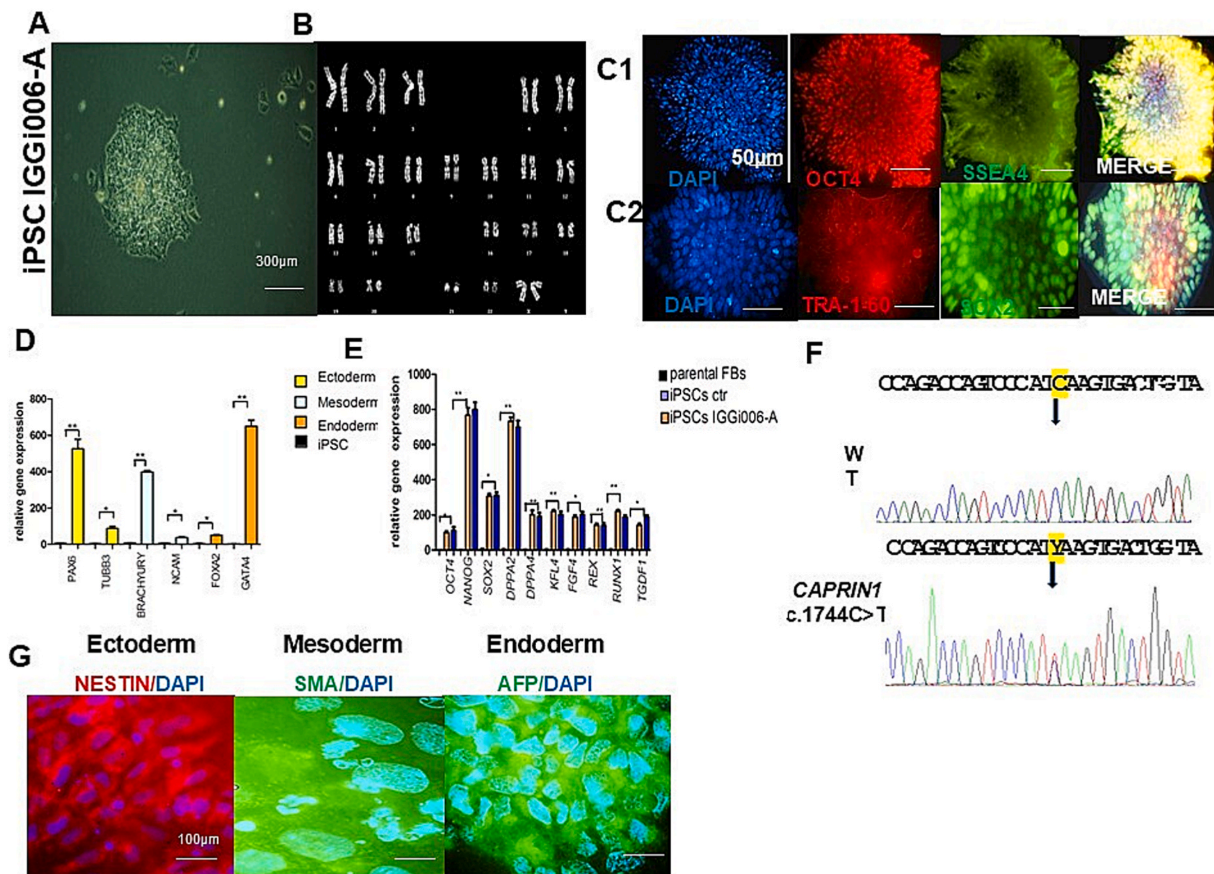


Fig. 1.

15 min at RT, and sequentially with blocking solution for 30 min at RT. Specific primary antibodies for OCT4, SSEA4, TRA1-60, SOX2 were applied at 4 °C for 3 h. Secondary antibodies were then incubated for 1 h at RT. To identify *nuclei* we used DAPI 0,1 µg/mL. Images were realized with Leica SP8 Confocal microscope and analysed with ImageJ (white bar = 50 µm). We showed in Table 2 detailed information of antibodies used.

3.4. Real Time PCR

We performed a total RNA extraction using Trizol (Thermo Fisher) at passage 6 and 20. RNA was reverse transcribed into cDNA using the Advantage RT cDNA Kit (Clontech). LightCycler 480 SYBR Green I Master (Roche) and specific primers used for Real Time PCR were listed in Table 2. Data were normalized to the housekeeping gene GAPDH using the $2^{-\Delta\Delta Ct}$ method and we considered $p < 0.05$ as statistically significant and $p < 0.001$ as highly statistically significant.

3.5. Karyotyping

iPSCs cultures (between passages 10–15) were analysed with quinacrine (Q) −banding and a minimum of 15 metaphase were analysed with Cytovision 3.93.2 analytical system.

3.6. Comparative genomic hybridisation (CGH)

The presence of and chromosomal imbalances such as aneuploidy, deletions and duplications of the genome, were analysed by CGH analysis using Sureprint G3 Human CGH Microarray Kit (8 × 60 K format) and scanned with the Agilent Scanner C and analysed using CytoGenomics software (Agilent Technologies).

3.7. DNA profiling

We performed a genomic DNA extraction from iPSCs and parental fibroblasts using the QIAamp DNA Mini Kit (Qiagen) and analysed using the Geneprint 10 system (Promega, B9510) to acquire permanent genetic identification of the cell lines.

3.8. Analysis of differentiation capacity

STEMdiff™ Trilineage Differentiation Kit (Stem Cell Technologies) was used to differentiate iPSC cells to all three germ layers, (ectoderm, mesoderm and endoderm). qPCR was performed as above described with specific primers (Table 2).

3.9. Mycoplasma detection

We evaluated the absence of mycoplasma contamination in supernatant of cell culture (between passages 8–15) with mycoplasma assay under the guideline of the manufacturer's instructions (Lonza's MycoAlert ® Mycoplasma Detection Kit).

CRediT authorship contribution statement

Giuseppina Conteduca: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chiara Baldo:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Alessia Arado:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Joana Soraia Martinheira da Silva:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation. **Renata Bocciardi:** Writing –

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	*Rabbit anti-OCT4*	1:200	Thermo Fisher Cat# A24867	RRID: AB_2650999
	Mouse anti-SSEA4	1:100	Thermo Fisher Cat# A24866	RRID: AB_2651001
	Mouse anti-TRA-1–60	1:100	Thermo Fisher Cat# A24868	RRID: AB_2651002
	Rat anti-SOX2	1:100	Thermo Fisher Cat# A24759	RRID: AB_2651000
Differentiation Markers	*Mouse anti- αSMA*	1:100	Thermo Fisher Cat# 14–9760–82	RRID: AB_2572996
	Mouse anti-AFP	1:500	Thermo Fisher Cat#MA514666	RRID: AB_10987005
	Mouse anti-NESTIN	1:100	Thermo Fisher Cat# 14–9843–82	RRID: AB_1548837
Secondary antibodies	Alexa Fluor® 594 conjugated donkey anti-rabbit IgG H&L	*1:500*	Thermo Fisher Scientific Cat# A24869	RRID:AB_2651006
	Alexa Fluor® 488 conjugated goat anti-mouse IgG H&L	*1:500*	Thermo Fisher Scientific Cat# A24877	RRID:AB_2651008
	Alexa Fluor®488 conjugated donkey anti-rat IgG H&L	*1:500*	Thermo Fisher Scientific Cat# A24876	RRID:AB_2651007
	Alexa Fluor® 594 conjugated goat anti-mouse IgM	*1:500*	Thermo Fisher Scientific Cat# A24872	Not available

Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Pluripotency Markers (qPCR)	NANOG	70bp	Fw: TGTCTTCTGCTGAGATGCCT Rv: AATAAGCAGATCCATGGAGGA
	OCT4	100bp	Fw: GAGAAGGATGTGGTCCGAGT Rv: GTGCATAGTCGCTGCTTGAT
	SOX2	90bp	Fw: ACCAGCTCGCAGACCTACAT Rv: CCTGCTGCGAGTAGGACAT
	TDGF1	115bp	Fw: GGATACCTGGCCTTCAGAGA Rv: CAGGCAGCAGGTTCTGTTTA
	FGF4	95bp	Fw: CTCTATGGCTCGCCCTTCT Rv: TGTAGGACTCGTAGGCGTTG
	REX	120bp	Fw: GGCCTTCACTCTAGTAGTGCTCA Rv: CTCCAGGCAGTAGTGATCTGAGT
	KFL4	98bp	Fw: CGAACCCACACAGGTGAGAA Rv: GAGCGGGCGAATTTCCAT
	DPPA2	103bp	Fw: CATGCTTACCCTGAACAACG Rv: GAAGCCTTGCTCTCTTGGTC
	DPPA4	110bp	Fw: GAAGAGGATCAGCAGGCTTC Rv: GTTGTCAGTGTGCTCTGCCT
	RUNX1	90bp	Fw: GAGGATTTGGTCAGAATGCAG Rv: ACACTGTTCTGAAGTCCTGCTTT
House-Keeping Genes (qPCR)	GAPDH	120bp	Fw:AGCAAGAGCACAAGAGGAAGAG Rv: TAACTGGTTGAGCACAGGGTAC
	PPIA	89bp	Fw: GGAGGCTTTGAGGTTTTGCAA Rv: CCTGACATCTAACTGCCAGCA
Differentiation Markers	PAX-6	88bp	Fw: GATAACATACCAAGCGTGTCATCAATA Rv: TGCGCCCATCTGTTGCT
	TUBB3	71bp	Fw: GGCCAAGTTCTGGGAAGTCA Rv: CCGAGTCGCCCACGTAGTT
	BRACHYURY	96bp	Fw: GGGTCCACAGCGCATGAT Rv: ATTTTAAGAGCTGTGATCTCCTCGTT
	NCAM	107bp	Fw: TCCTGGGAACTGCAGTTTCTCT Rv: TTTGGCATCTCCTGCCACTT
	FOXA2	60bp	Fw: TTCAGGCCCGGCTAACTCT Rv: ACCCCCACTTGCTCTCTCACT
	GATA4	69bp	Fw: AGCTGGGTAGTTTAGCCAAACG Rv: TGTGTGACACGGTGAACGAA
Sendai viral genome (PCR)	SeV	181 bp	Fw: GGATCACTAGGTGATATCGAGC Rv: ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	528pb	Fw: ATGCACCGCTACGACGTGAGCGC Rv: ACCTTGACAATCCTGATGTGG
	Klf4	410bp	Fw: TTCCTGCATGCCAGAGGAGCCC Rv: AATGTATCGAAGGTGCTCAA
	c-Myc	532bp	Fw: TAACTGACTAGCAGGCTTGTCG Rv: TCCACATACAGTCCTGGA TGATGATG
	CAPRIN1	387bp	Fw: GGTTTGGAATTGTGGTTGGAG Rv: TCATCAAGCCTCTAGCACCA

review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis. **Barbara Testa:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Simona Baldassari:** Writing – review & editing, Visualization, Validation, Supervision, Investigation, Formal analysis, Data curation. **Maria Margherita Mancardi:** Writing – review & editing, Visualization, Validation, Supervision, Resources. **Federico Zara:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition. **Michela Malacarne:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Data curation. **Domenico Coviello:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Funding

This research was funded by Italian Ministry of Health, Ricerca Corrente IRCCS Istituto Giannina Gaslini 2023–2024 and Development

Table 3
CGH analysis summary; IGGi006-A (passage 10) reporting a female cell line and no abnormalities detected.

CGH summary	
Sample name	IGGi006-A
Date reported	12th February 2023
Hybridization	Balanced hybridization was observed for all chromosomes
Balance	Relative to reference DNA
Copy number change	No copy number changes above 400 kb were detected
Interpretation	Female cell line — no abnormalities detected

and Cohesion Plan of the Italian Ministry of Health, Trajectory 4 – Biotechnologies, bioinformatics and pharmaceutical development, project title: “Hybrid Hub (H2UB): Cellular and computational models, micro- and nanotechnologies for the personalization of innovative therapies”, project code T4-AN-10.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103696.

Data availability

Data will be made available on request.

References

Conteduca, G., Cangelosi, D., Coco, S., Malacarne, M., Baldo, C., Arado, A., Pinto, R., Testa, B., Coviello, D.A., 2022. NSD1 mutations in sotos syndrome induce differential expression of long noncoding RNAs, miR646 and genes controlling the G2/M checkpoint. Life (basel). 12 (7), 988. https://doi.org/10.3390/life12070988.

Pavinato, L., Delle Vedove, A., Carli, D., Ferrero, M., Carestiato, S., Howe, J.L., Agolini, E., Coviello, D.A., van de Laar, I., Au, P.Y.B., Di Gregorio, E., et al., 2023. CAPRIN1 haploinsufficiency causes a neurodevelopmental disorder with language impairment, ADHD and ASD. Brain 146 (2), 534–548. https://doi.org/10.1093/brain/awac278.

Solomon, S., Xu, Y., Wang, B., David, M.D., Schubert, P., Kennedy, D., Schrader, J.W., 2007. Distinct structural features of Caprin-1 mediate its interaction with G3BP-1 and its induction of phosphorylation of eukaryotic translation initiation factor 2α, entry to cytoplasmic stress granules, and selective interaction with a subset of mRNAs. Mol. Cell. Biol. 27 (6), 2324–2342. https://doi.org/10.1128/MCB.02300-06.