The American Journal of Human Genetics, Volume *104*

Supplemental Data

HNRNPR Variants that Impair Homeobox Gene Expression Drive Developmental Disorders in Humans

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Supplemental Note: Case reports

Individual 1 (P1) is a 12-year-old female (Figure 1A). Birth measurements at 39 weeks of gestation were normal with a weight of 3610 gram (75th percentile), a length of 50 cm (50th percentile), and a head circumference of 34 cm (50th percentile). During the neonatal period, she presented with poor weight gain and feeding difficulties, first thought to be a severe gastroesophageal reflux. At 3 months of age the individual was referred to the French clinic because of hypotonia, acquired microcephaly (below 1st percentile at 3 months while at 50th percentile at birth), and significant strabismus. At the age of 3 months rough hair was also observed and at the age of 18 months she experienced febrile seizure and esophagitis. At age 9 years and 6 months the patient developed precocious puberty and still had feeding problems, mainly bulimia, and regurgitation. She has a severe global developmental delay. She could sit unsupported at age 11 months and gait was acquired at 4 years of age. She had progressive valgus and flat feet that impaired walking. She has not developed any spoken language but can communicate with hand movements, and comprehension skills for simple tasks are correct. She is able to undress and eat alone but requires assistance for other daily tasks. Behavioral disturbances are characterized by hand stereotypies, attention disorder, and tantrums. Upon clinical examination she showed a narrow forehead, up-slanted palpebral fissures, convergent strabismus, ear lobule hypoplasia, wide nasal bridge, short and broad thumbs and halluces, and clinodactyly of the fifth fingers and toes (Figures 1A, 2A, and S4A). She had some degree of limb stiffness. Her primary teeth had to be extracted because of loss failure and dental crowding with permanent tooth growth.

Additional investigations were performed and hand X-rays show hypodense lesions on distal phalanges and the first metacarpal, as well as brachymesophalangy of the fifth finger (Figure 2D). MRI of the brain shows microcephaly and a short corpus callosum (but with conserved proportions with microcephaly). Ultrasound investigation of the kidneys, vertebral x-rays and audiometry are normal. Genetic DNA-tests such as karyotyping, array-CHG and DNA-investigations for Angelman syndrome were normal; as was the metabolic screening. Trio

whole exome sequencing (WES) identified *HNRNPR* as a candidate when a *de novo* frameshift variant chr1: g.23637248_23637249insC, NM_001102398.1: c.1609dupG, p.(Ala537Glyfs*10) was found.

Individual 2 (P2) is a 3-year old boy that presented at birth with multiple congenital abnormalities, breathing problems, and dysmorphic features (Figures 1B and S1). He was born with a severe laryngomalacia, for which wedge excision of the adenoids and a tracheostomy was performed. He also had a few small, muscular ventral septum defects (VSDs) that closed spontaneously. Furthermore, he had cryptorchidism, a micropenis, and is suspected to have a precocious puberty. He was diagnosed with nightly hypoventilation at the age of 9 months for which nocturnal respiratory support was started. He has had a few epileptic seizures, but this is not one of his main clinical problems. He was surgically operated on for severe hip dysplasia. Physical examination revealed a boy with characteristic facial features including bitemporal narrowing, short and narrow palpebral features, almond-shaped eyes, telecanthus, low set ears with thickened helix, and some degree of retrognathia (Figures 1B and S1). He also has a short and thick neck with excess skin folds. The hands appear short, with a combination of short middle phalanges and ulnar deviation of the distal phalanx of the second digit on both hands (Figures 2B, S3, and S4B). The distal bending folds of these fingers are absent. Clinodactyly of the fifth digit was also apparent and the feet show a chaotic implant of the toes (Figures 2B and S3). During his first year he had remarkable hair (Figure S1B) and he was diagnosed with hirsutism at 21 months of age. The boy was very cheerful in the first two years of life. After this his behavioral problems have become increasingly clear with attention deficit disorder, head banging, repeated removal of his cannulas and stereotypic behavior. Developmental delay was present from the beginning; however, the degree of developmental delay has also become more apparent in the last year. At the age of 3 years and 5 months his motor development is estimated at approximately 11 months but this may underestimate his developmental potential because of his severe hip dysplasia. He can now stand upright with

support but is unable to walk. He cannot speak, but this is probably due to his tracheostomy. He also does not understand sign language or pictograms yet. The only signal he uses is for his own name. This signal is named after his favorite position laying on this back with both hands behind the head and frog-like leg position.

Additional investigations that have been performed are an MRI-scan of the brain showing underdevelopment and atrophy of the frontal lobes, a hypoplastic corpus callosum, and small cerebellar hemispheres and vermis (Figure 1D). Thoracic x-rays show 11 rib pairs (Figure S5A). X-rays of the hands and feet show a remarkable pattern of brachydactyly, delayed ossification, and clinodactyly (Figure 2E. DNA diagnostics showed a small maternally inherited CNV by SNP-array (arr[hg19] 1p34.1(44,680,009-44,686,395)x1 mat), which is probably innocent. Trio-based exome sequencing revealed a heterozygous variant c.1652dupG p.(Pro552Serfs*34) in *HNRNPR*.

Individual 3 (P3) is a 10-year old female (Figures 1C and S2). At 2½ months of age she presented as a symmetrically small infant with a height and weight in the 3rd percentile and head circumference of at the 50th percentile. She has generalized hypotonia and swallowing problems for which she still requires tube feeding. At the first clinical examination she showed brachycephaly with prominent frontal bossing. Later a short, sloping forehead, with mild facial asymmetry, arched eyebrows, a narrow nasal bridge, depressions in the nasal tip, a low placed columella, short philtrum, angular lower facies, and mild micrognathia became apparent (Figure 1C). Her eyes are almond-shaped and her ears are small and cup-shaped (Figure 1C). Her left ear is smaller and shows an abnormal lobule (Figure 1C). The incisors are widely spaced, while the rest of the teeth are crowded. She has sparse, fine scalp hair and excess skin folds in the neck. More recently she still shows sparse scalp hair, but generalized hirsutism and a low posterior hairline. She has symmetrically small, puffy hands and feet (Figure 2C). There was bilateral clinodactyly of the 5th digits, proximal placed short thumbs and brachydactyly (more specific marked hypoplasia of the middle and distal phalanges;

Figure 2C). The digits are tubular in shape (Figure 2C). There are bilateral unusual palmar creases and hyperkeratosis pilaris. Her toes reveal a cutaneous syndactyly from the second to the fourth digit and short, curved 5th toes (Figures 2D and S4C). She has hypoplastic labia majora and a normal clitoris. Developmentally at 8 months, poor eye contact was noted. She was rolling, sitting, reaching, transferring objects, and babbling by this age. She crawled at 13 months and stood with support by 14 months. She walked with assistance by 20 months and walked independently by 33 months. A Denver II developmental assessment performed at 24 months revealed age appropriate personal-social skills. Fine motor-adaptive skills were delayed to about 13-17 months. Spoken language was at the approximate 16-month level but the patient knew body parts and could point to 4 pictures age-appropriately. Gross motor skills were delayed as she was not able to jump or climb stairs. A formal developmental evaluation was performed at an outside center at the age of 9 8/12 years. This revealed speech delay with the ability to use single words, use of some signs, and ability to use a tablet. Fine motor development was delayed; she was ambidextrous, could dress herself slowly, but not use zippers or buttons. She was working on utensils. She could write the first letter of her name and pick her name out from distractors. Gross motor development was delayed as she continued to need help with stairs. She was not completely toilet trained. By our last evaluation at 10 9/12 years, she carried a diagnosis of moderate developmental delay (IQ tested 52), pervasive developmental disorder and exhibited stereotypic behavior. She often rubs her knuckles into her bones, wants to touch her elbows or the elbows of others and chews on her hands or necklace. She was still not fully toilet trained.

Simple, focal seizures are present. Her visual maturation was delayed and she has myopic astigmatism, intermittent esotropia, and anisometropia. A heart exam revealed variable systolic murmur along the left sternal midline without abnormalities on ultrasound.

Other additional investigations were performed as well and brain MRI shows microcephaly, but no other abnormalities. Radiographs show 11 rib pairs (Figure S5B), abnormal articulation at the costovertebral junction of the ribs, subluxation of the radial head, subtle narrowing of the

interpediculate distance at the lumbosacral region. X-rays of the hands demonstrate hypoplastic phalanges with abnormal epiphyses and delayed ossification of the thumb (Figure 2F). The big toe of the foot shows shortening and broadening of both phalanges (Figure S4C). Trio exome sequencing revealed a de novo heterozygous variant in *HNRNPR* c.1652dupG p.(Pro552Serfs*34). This is the same variant identified in P2.

Individual 4 (P4) is an 8-year old male. Birth measurement at 39 weeks of gestation were normal with a weight of 3340g (50th percentile), a length of 50 cm (50-75th percentile) a head circumference of 34 cm (50th percentile). During the first year he presented with hypotonia, severe gastroesophageal reflux, feeding difficulties and constipation. He had Nissen fundoplication and was fed via percutaneous gastrostomy. Later a partial achalasia of the esophagus was diagnosed.

He was referred to clinical genetic at 6 years for severe global developmental delay, acquired microcephaly and growth delay. He could sit unsupported at two years and walked with ataxia at 4 years. At six years, the language was at a 9-month-old level. He could use three signs. He had no behavioral disturbances.

Clinical examination showed a high forehead, micrognathia, wide palpebral fissures, low columella, low-set and anteverted ears. He had short hands and brachydactyly, clinodactyly of fifth fingers, fetal pads and syndactyly of the fingers. He had a sacrococcygeal fistula.

Additional investigations performed included a brain MRI showing cerebellar hypoplasia, mega cisterna magna and a short corpus callosum (Figure 1E and S6). Medullary MRI showed normal medullary signs and arachnoidal cyst. Ultrasound of the kidneys and heart, vertebral X-Ray, and audiometry tests were normal. Ophthalmological examination showed normal vision and albinoid retina.

Karyotype, CGH-array, DNA investigations for Fragile X syndrome (FXS; MIM:300624) and Angelman syndrome (AS; MIM: 105830) were normal. Trio exome sequencing revealed a heterozygous missense variant in *HNRNPR* c.1763G>A p.(Arg588His).

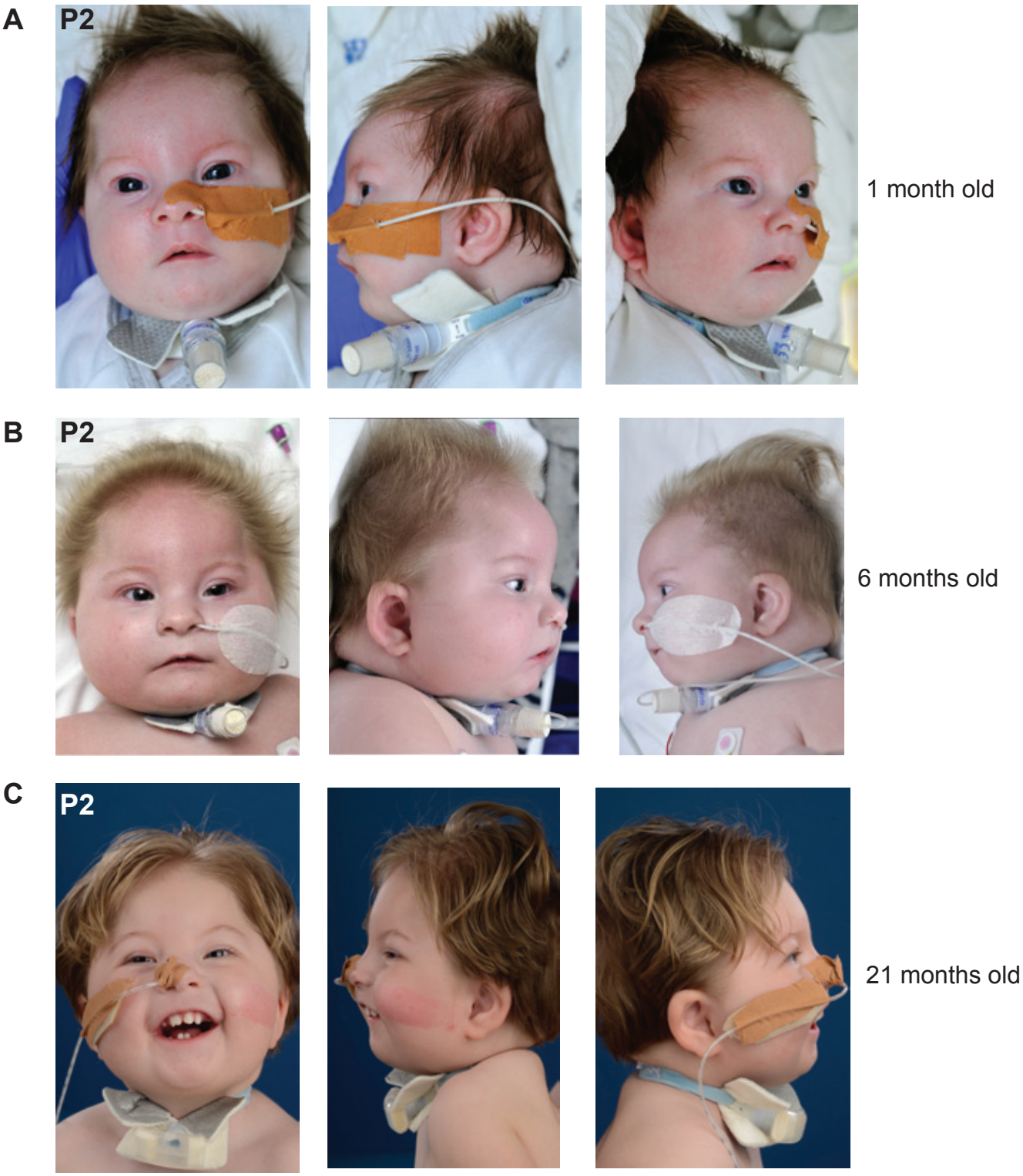


Figure S1. Photographs of P2 at different ages. Head and side portrait photographs of P2 at **A)** One month **B)** Six months and **C)** 21 months of age.

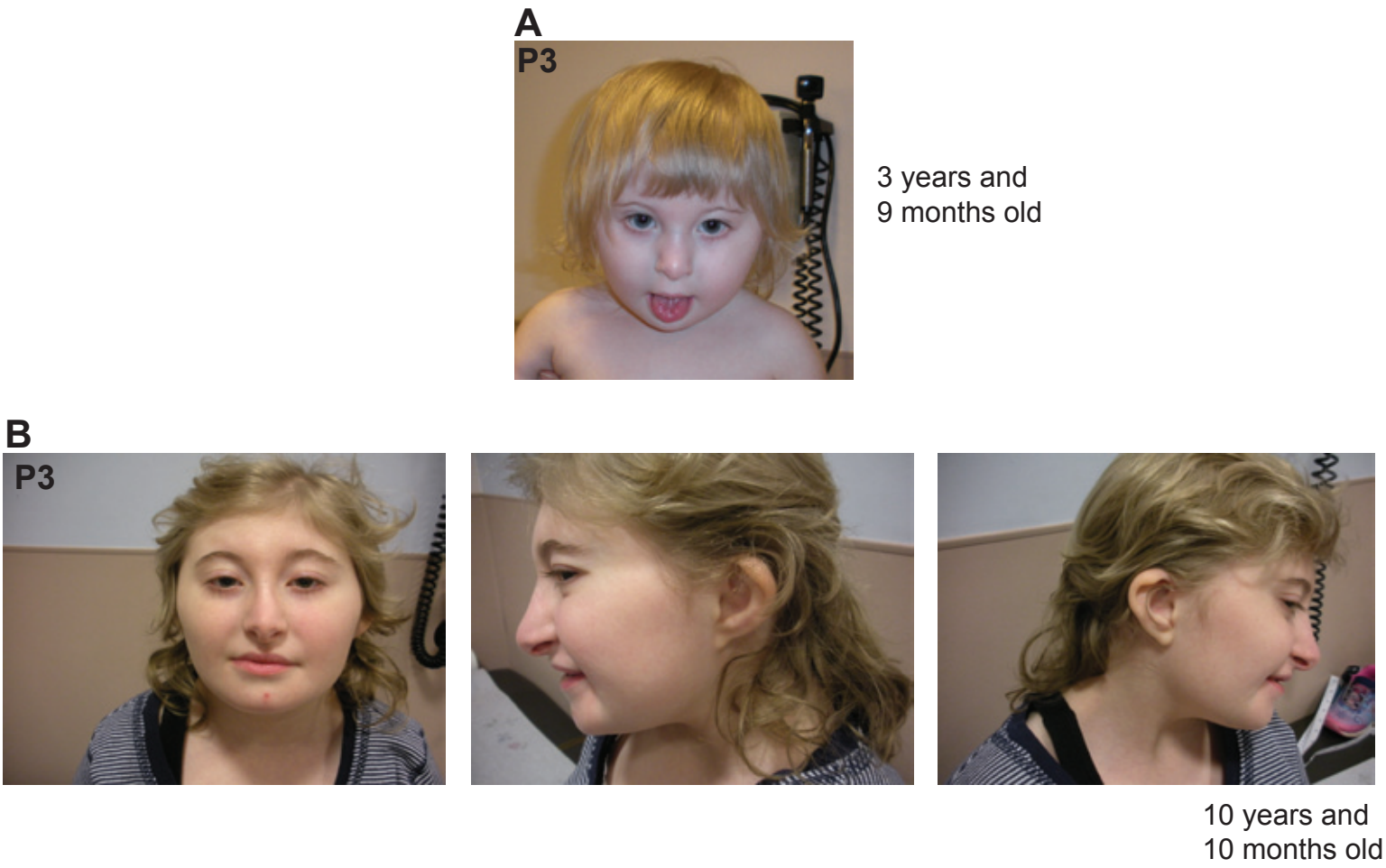
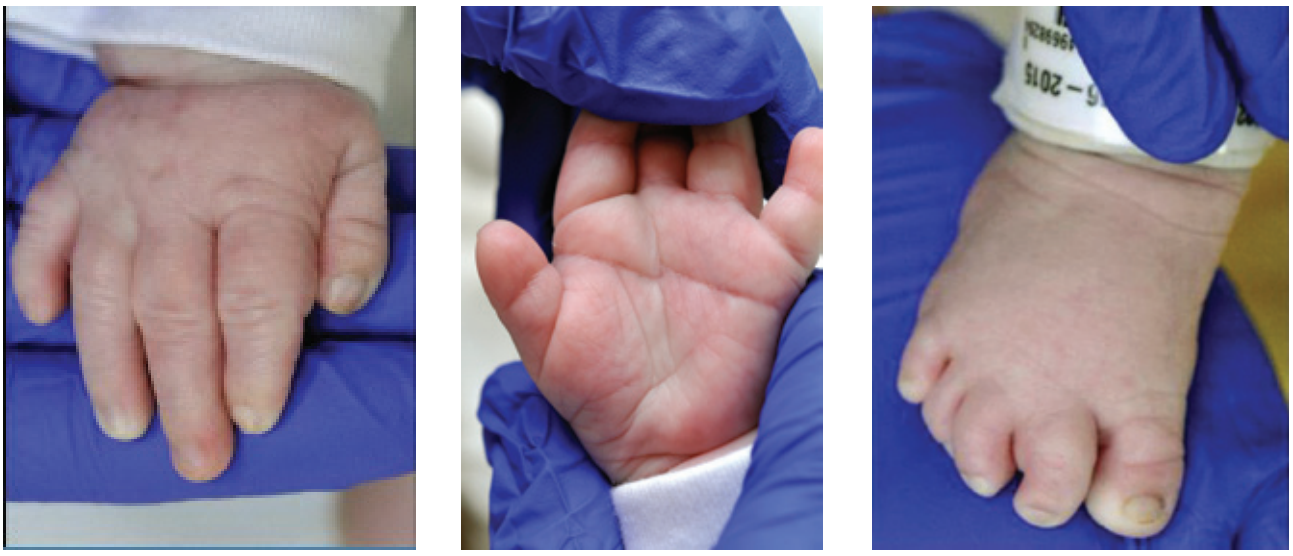


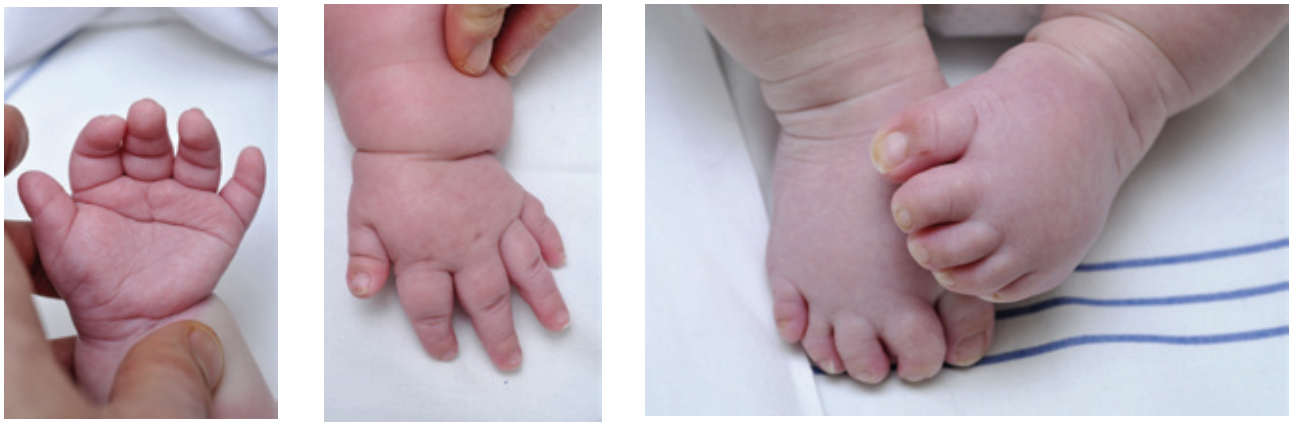
Figure S2. Photographs of P3 at different ages. Head and side portrait photographs of P3 at **A)** Three years and 9 months of age and **B)** 10 years and 10 months of age.

A



1 month old

B



6 months old

C

21 months old

Figure S3. Photographs of P2 at different ages. Photographs of the hands and feet of P2 at **A)** One month **B)** Six months and **C)** 21 months of age.

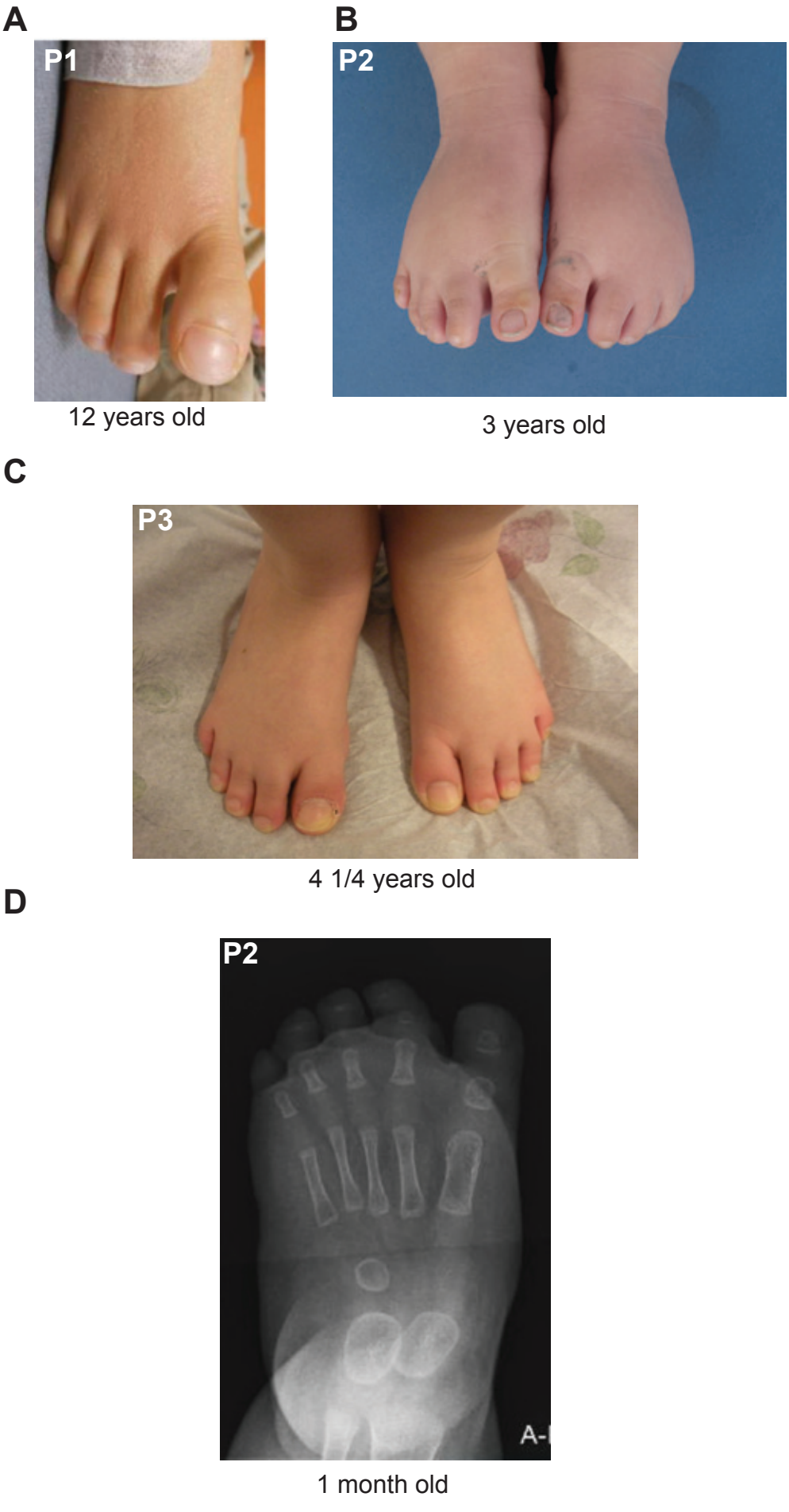


Figure S4. Foot features of individuals carrying variants in *HNRNPR.* Photographs of the feet of P1 **(A)**, P2 **(B)**, and P3 **(C)** taken at the ages of 12 years, 3 years, and 10 years, respectively showing broad 1st digit in P1 and P3 and small 5th digits with clinodactyly. **D)** X-ray of the foot of P2 taken at one month of age showing hypoplastic midphalanges of digits 2-3, hypoplastic endphalanges of digits 2-4 and absence of ossification of the mid phalanx of digits 4-5 and end phalanx of digit 5.

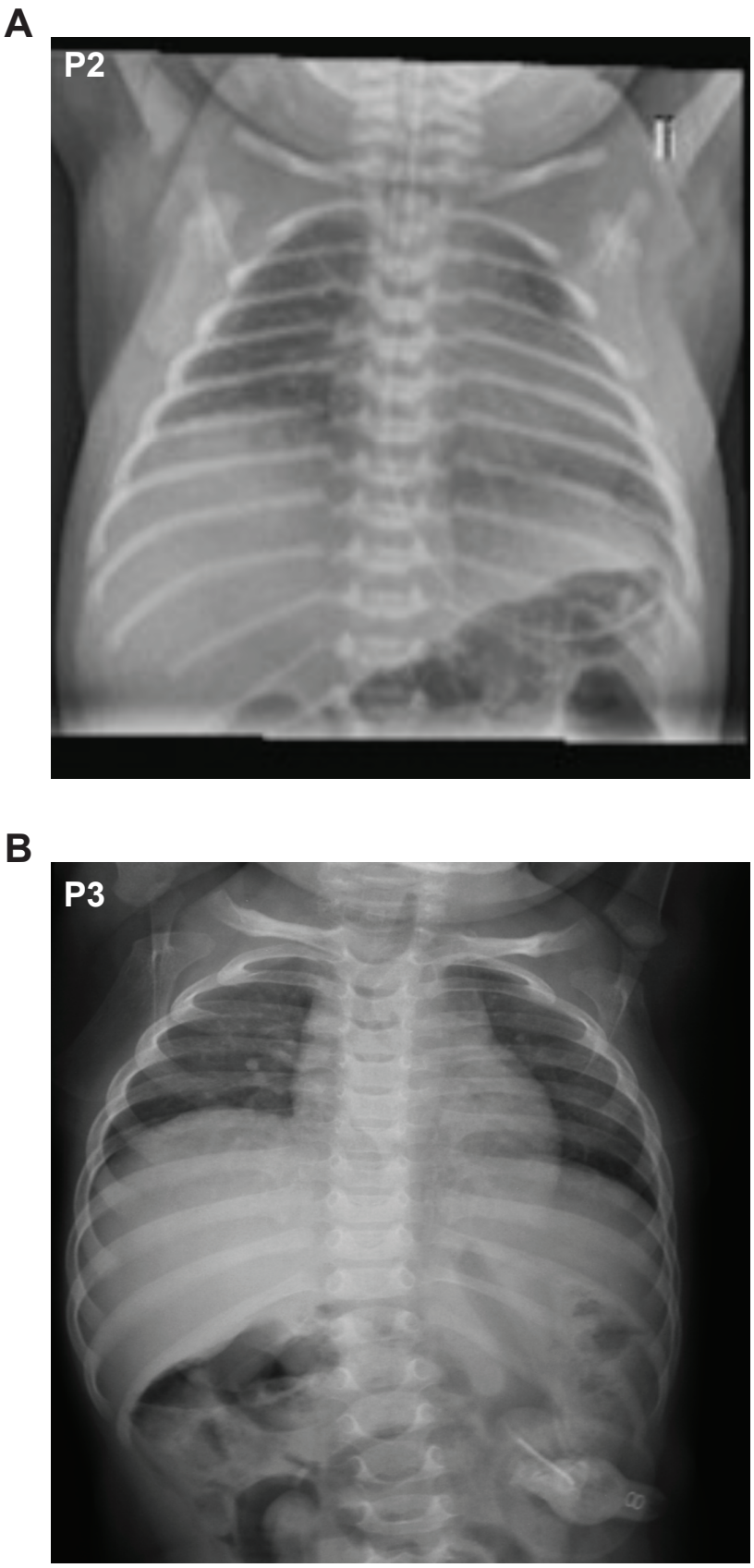


Figure S5. Skeletal features of individuals carrying variants in *HNRNPR.* **A)** X-ray of P2 at one week of age showing only 11 rib pairs. **B)** X-ray of P3 at 20.5 months of age showing only 11 rib pairs.

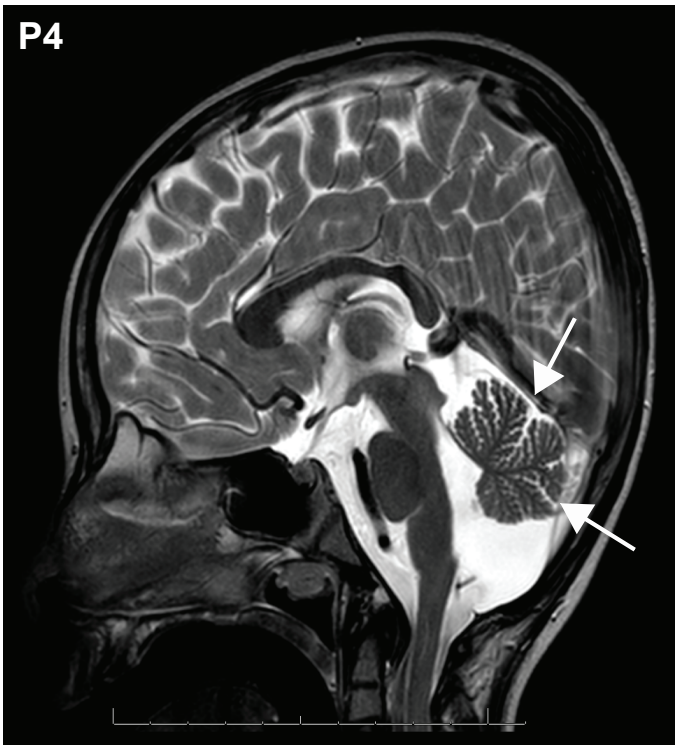


Figure S6. MRI of P4. MRI taken at 7 years and 10 months of age in an individual carrying the missense variant p.Arg588His in hnRNPR revealing cerebellar vermis hypoplasia (arrows).

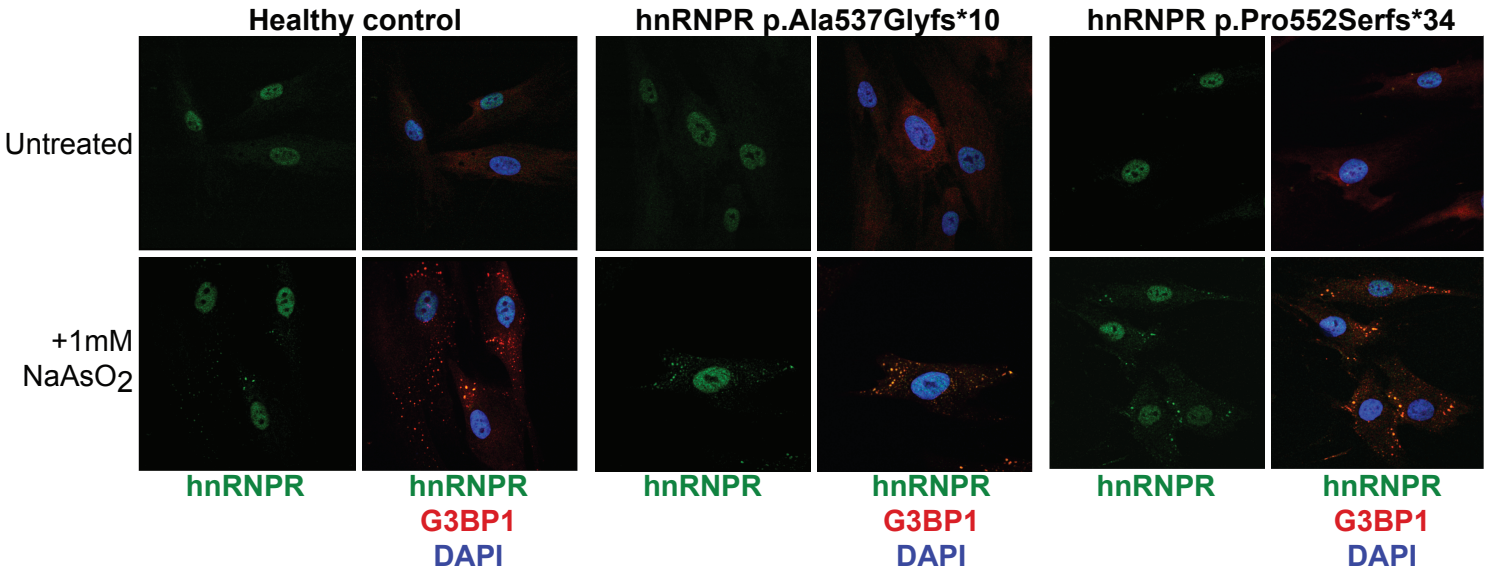


Figure S7. Truncated hnRNPR proteins do not drive stress granule formation at basal levels. Immunofluorescence of primary fibroblasts from healthy control individuals or those carrying variants truncating hnRNPR (P1 and P2). Cells were untreated (top) or treated with 1mM $NaAsO_2$ for 60' then stained with antibodies against hnRNPR (green), the stress granule marker G3BP1 (red), and DAPI (blue).

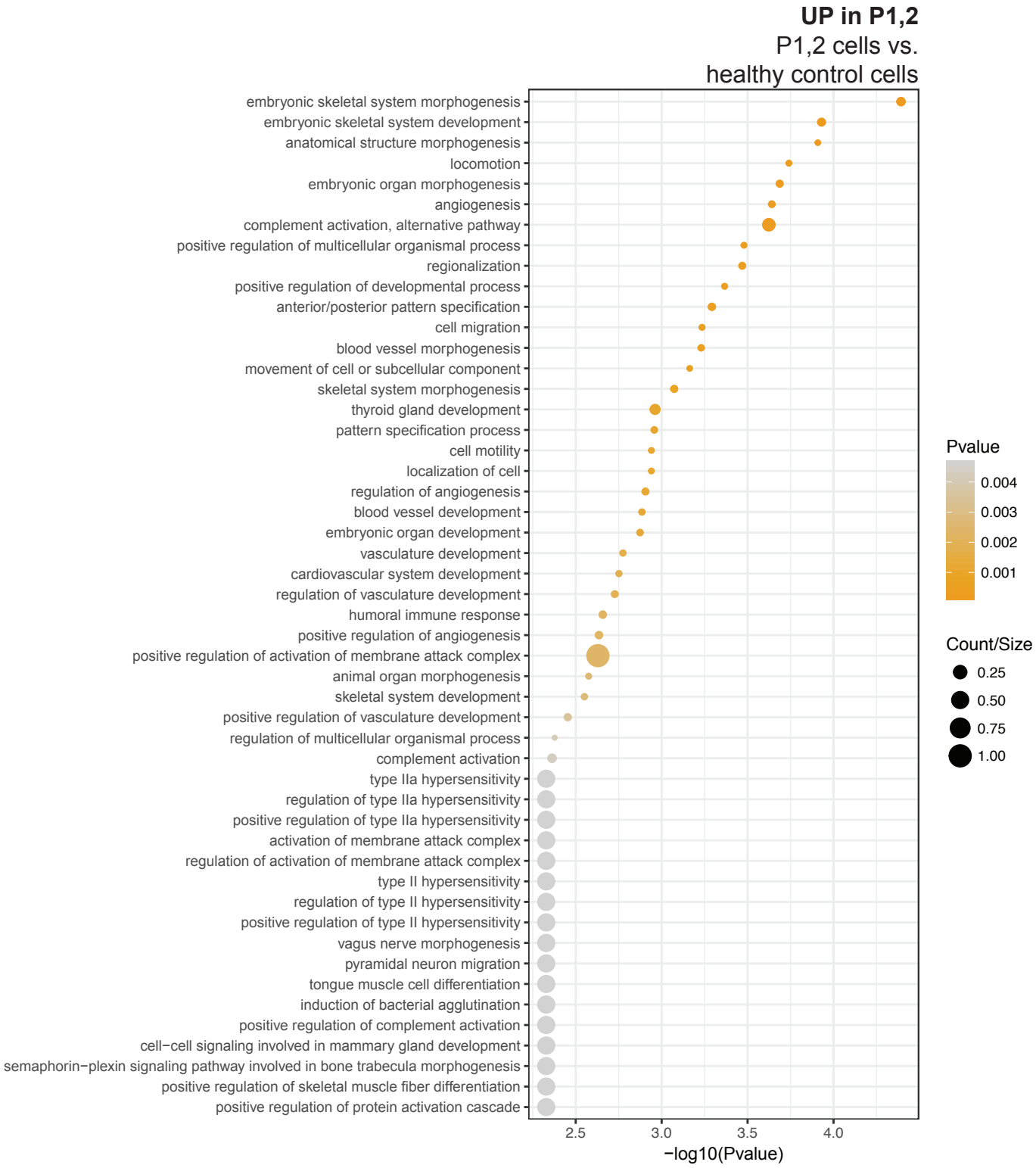


Figure S8. GO terms associated with upregulated RNAs in *HNRNPR* mutant cells. GO analysis of the differentially expressed RNAs (adjusted *p* value <0.05) deteremined by RNA-seq of untreated fibroblasts derived from individuals carrying *HNRNPR* mutations (P1 and P2) and healthy controls.

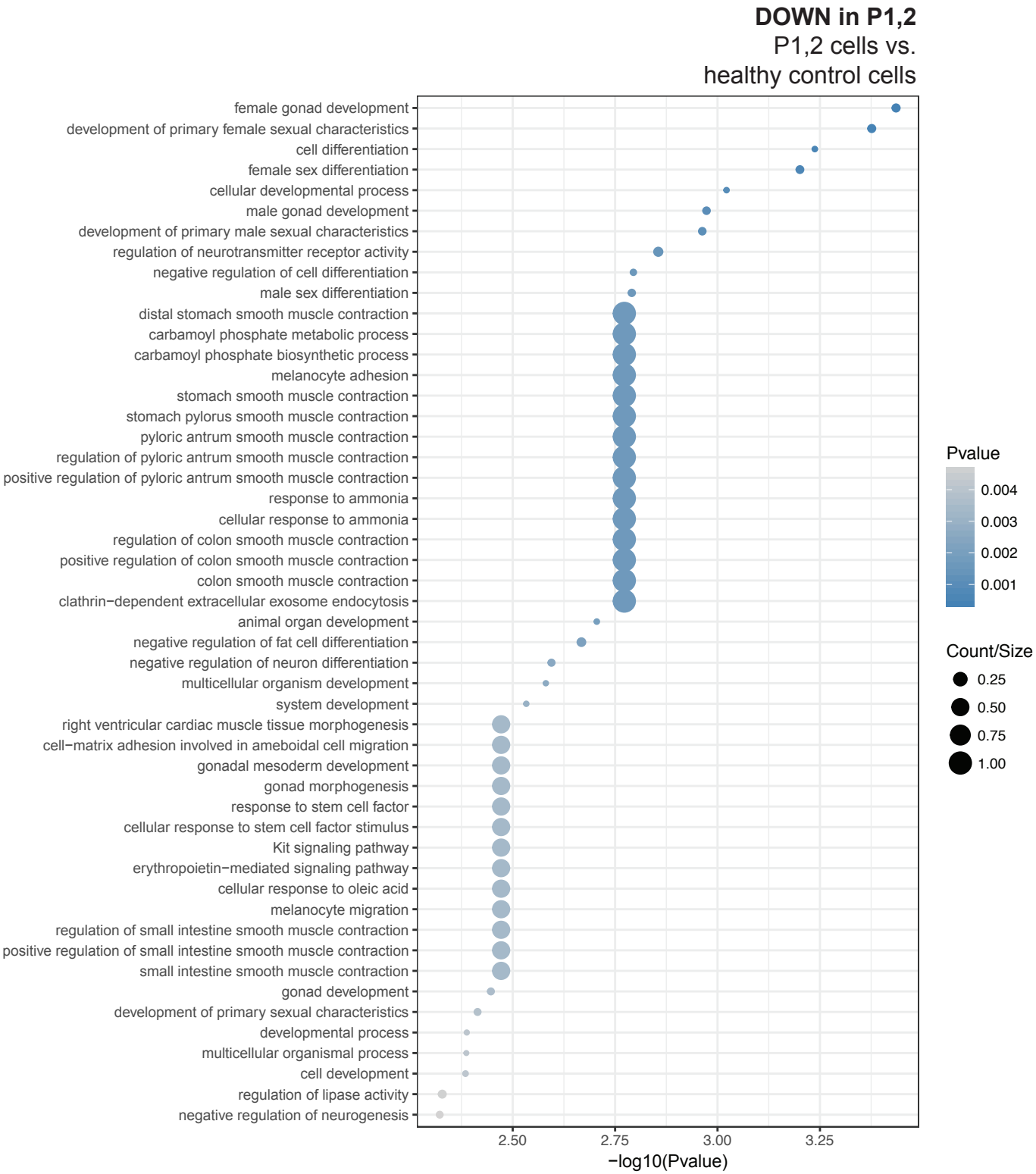


Figure S9. GO terms associated with downregulated RNAs in *HNRNPR* mutant cells. GO analysis of the differentially expressed RNAs (adjusted *p* value <0.05) deteremined by RNA-seq of untreated fibroblasts derived from individuals carrying *HNRNPR* variants (P1 and P2) and healthy controls.

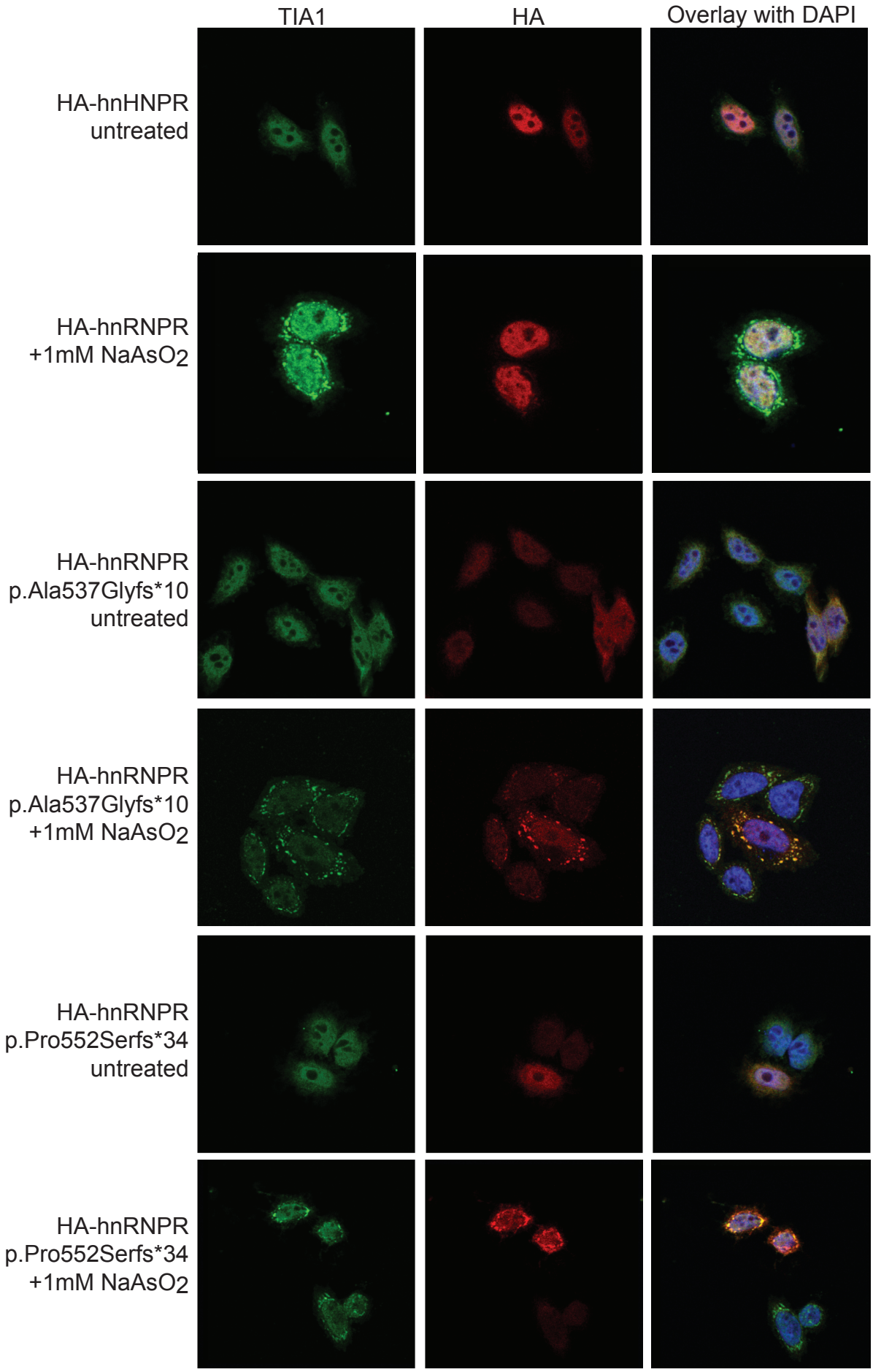


Figure S10. Truncated HA-hnRNPR variants co-localize more excessively with stress granules. HeLa cells transfected with HA-tagged hnRNPR contructs either untreated or exposed to 1mM $NaAsO_2$ for 30' and stained with antibodies against TIA1 (green), HA (red). DAPI (blue) is shown in the overlay.

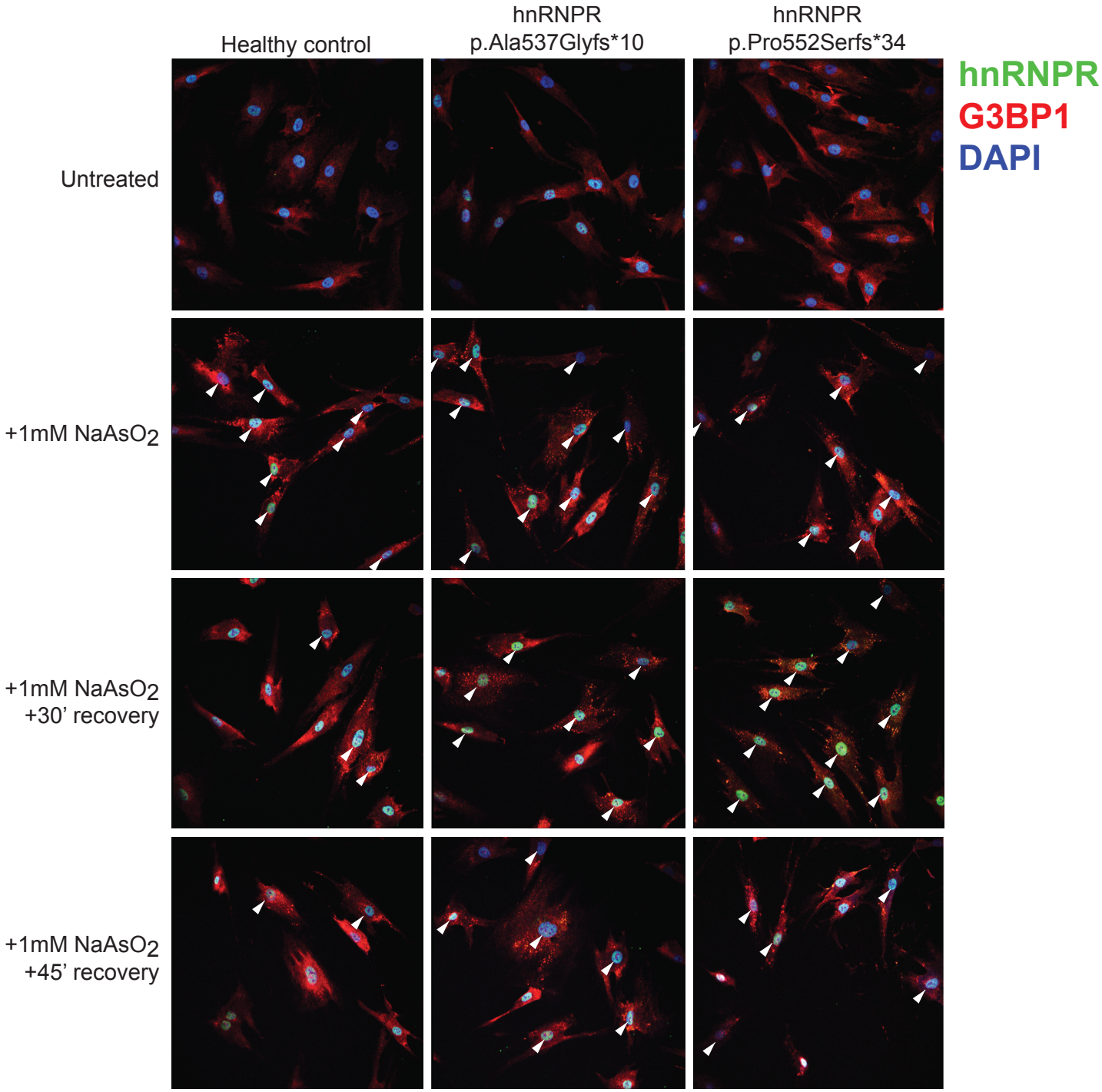


Figure S11. Stress granule disassembly is impaired in cells carrying truncated hnRNPR. Confocal microscopy of fibroblasts derived from a healthy control, P1 p.Ala537Glyfs*10, or P2 p.Pro552Serfs*34. Cells are untreated or treated with 1mM NaAsO2 for 30' and either immediately fixed or allowed to recover for 30' or 45'. Cells stained with DAPI (blue) and antibodies against hnRNPR (green) and the stress granule marker G3BP1 (red).Cells with stress granules are indicated with arrowheads.