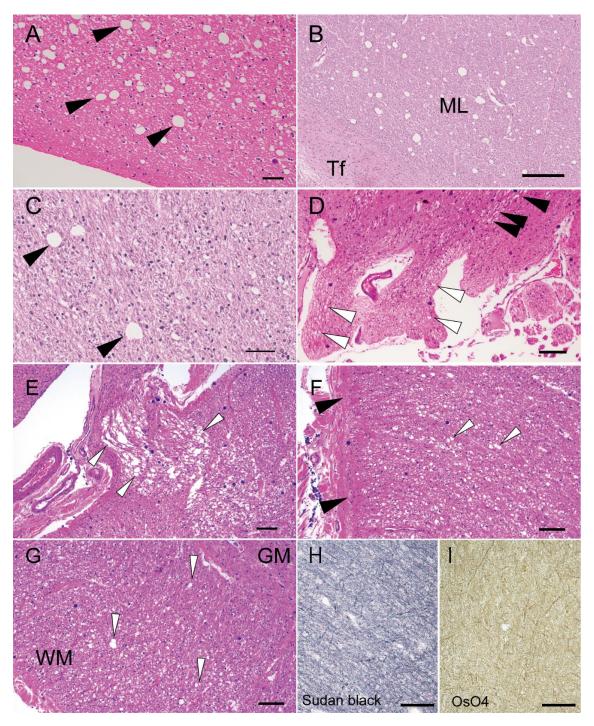
Supplementary Fig. 2
Vacuoles in the brain and spinal cord of the SCA34 patient



- A) Hematoxylin-Eosin (HE) staining of the optic tract showed massive vacuoles (arrowheads).
- B) HE staining of the pons showing vacuoles in the right pontine medial lemniscus (designated as ML). Tf indicates the area of transverse pontine fibers.

- C) HE staining of the pons showing a very small number of vacuoles in the pyramidal tract (arrowheads).
- D) Vacuoles in the right ophthalmic nerve root (white arrowheads) and intramedullary fibers (black arrowheads) of the midbrain stained with HE.
- E) HE staining of the lumbar spinal cord revealed vacuoles in bilateral intra-axial posterior roots (the right side is shown) (white arrowheads). In contrast, vacuoles were not observed in the extra-axial root (not shown).
- F) The right lateral column of HE-stained lumbar spinal cord showed fibrosis (black arrowheads) and vacuoles along neural fibers (white arrowheads).
- G) The right anterior column of HE-stained lumbar spinal cord showed fewer number and smaller sizes of vacuoles (white arrowheads) along the intramedullary anterior spinal root. Gliosis and fibrosis were observed in the anterior horn gray matter (GM).
- H and I) The vacuoles in the frontal white matter were not stained with Sudan black (H) and OsO4 (I), respectively.

Scale bar = 50  $\mu$ m (A), 250  $\mu$ m (B), 100  $\mu$ m (C, D, E, F, G, H, and I).