Report of the project “Targeting transmembrane ion balance to restore Purkinje cell functionality in ARSACS” by dr. F. Maltecca, Ospedale San Raffaele, Milan, Italy.

We have generated novel data indicating that the absence of sacsin alters calcium homeostasis in Purkinje neurons, and this event in turns affects Purkinje neurons firing, likely triggering ataxia in the *Sacs*$^{-/-}$ mouse. By performing proteomics on the cerebellum of *Sacs*$^{-/-}$ and *Sacs*$^{+/+}$ mice at post-symptomatic stage, we disclosed a huge deregulation of proteins related to two main categories: synaptic signalling and calcium transport across membranes. These results prompted us to investigate in deep sacsin localization in neurons. By immunofluorescence in primary Purkinje neurons, we found that sacsin localizes in the soma but is also distributed along the Purkinje neurons dendrites and particularly in spines. Combining different approaches of cellular fractionation in mouse cerebellum, we demonstrated that sacsin is enriched at the plasma membrane fraction (as compared to classical cytosolic proteins), especially in synapses.
Proteomics on cerebellar membranes revealed a significant increase of voltage-gated calcium channels, and accordingly electrophysiological patch clamp recordings in mouse cerebellar live sections revealed increased calcium current density in *Sacs*$^{-/-}$ PCs compared to the controls. This increased amplitude was not due to larger size of the *Sacs*$^{-/-}$ cells, as the average cell capacitance was comparable in the two genotypes Consistently with the well-established role of voltage-gated calcium channels in regulating action potential activity, we found a significantly slower pacemaking firing in *Sacs*$^{-/-}$ PCs.
These results enforce calcium deregulation as a potential target for ARSACS therapy.