

Characterization of SCA2 antibodies

Spinocerebellar Ataxia Type 2 (SCA2)
autosomal dominant neurodegenerative disease



The more repeats mean the earlier the onset of the disease.

A2BP1/Fox1 interact with ATX2 (Shibata et al Hum Mol Genet, 2000)

Mice expressing mutant ATXN2 [ataxin 2 (Q58)] results in the loss of Purkinje cells (Huynh et al Nat Genet, 2000)

Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6, a component of P-bodies and stress granules. Ataxin-2 regulates the intracellular concentration of the poly(A)-binding protein (stress granule component and a key factor for translational control) [Nonhoff et al Mol Biol Cell, 2007]

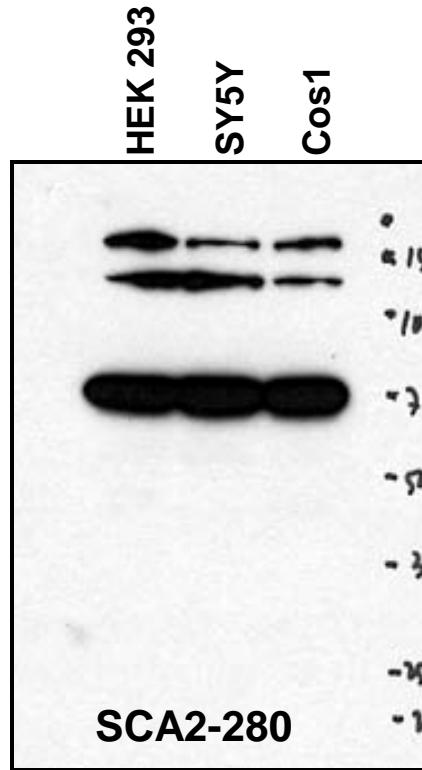
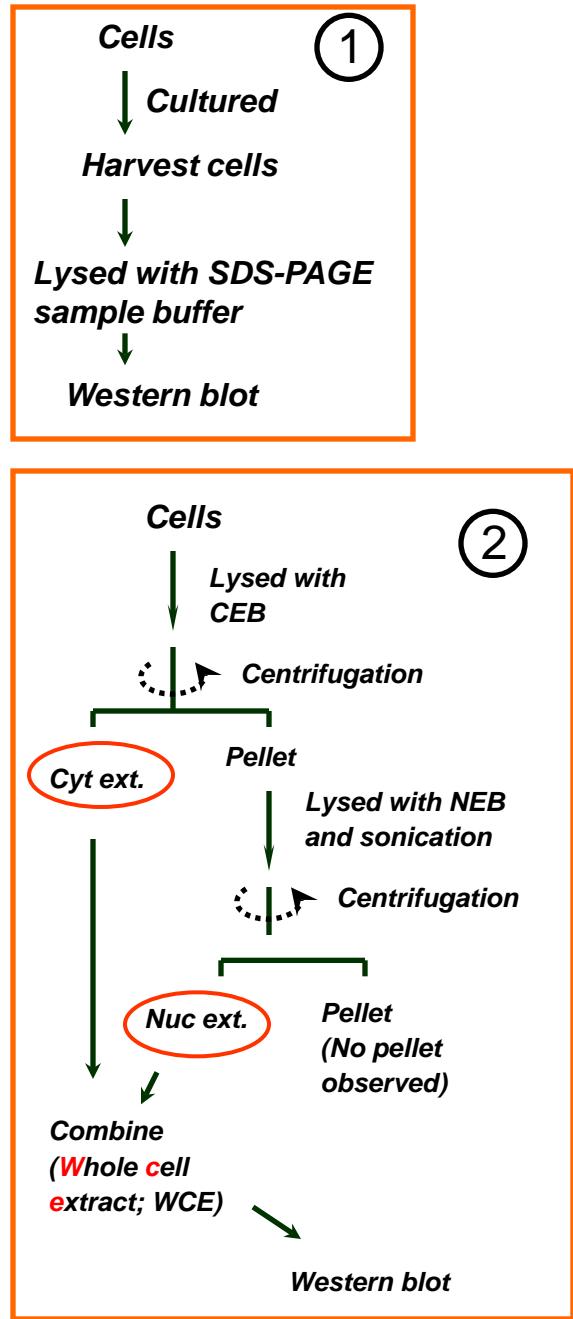
SCA2 antibodies

SCA2 (280-293) 0.552 ug/ul

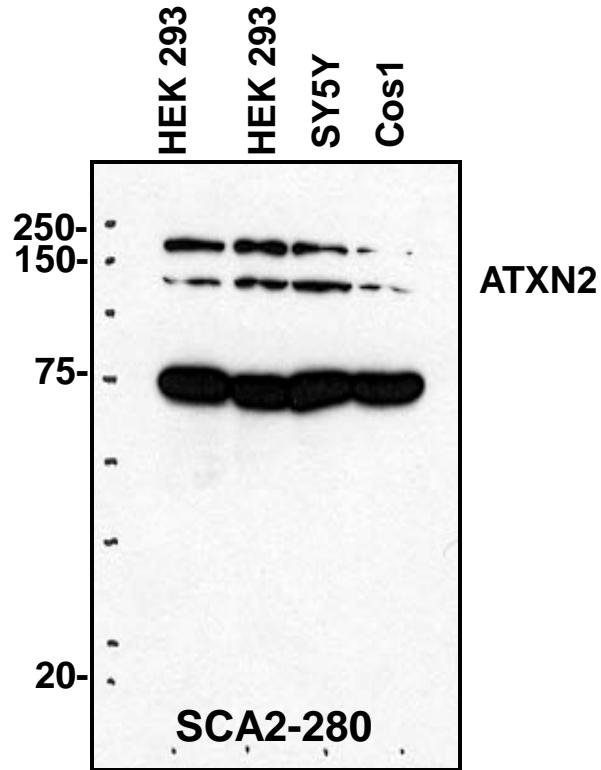
SCA2 (714-730) [714-2] 0.75 ug/ul

SCA2 (790-804) 0.6 ug/ul

Characterization of SCA2 Antibodies

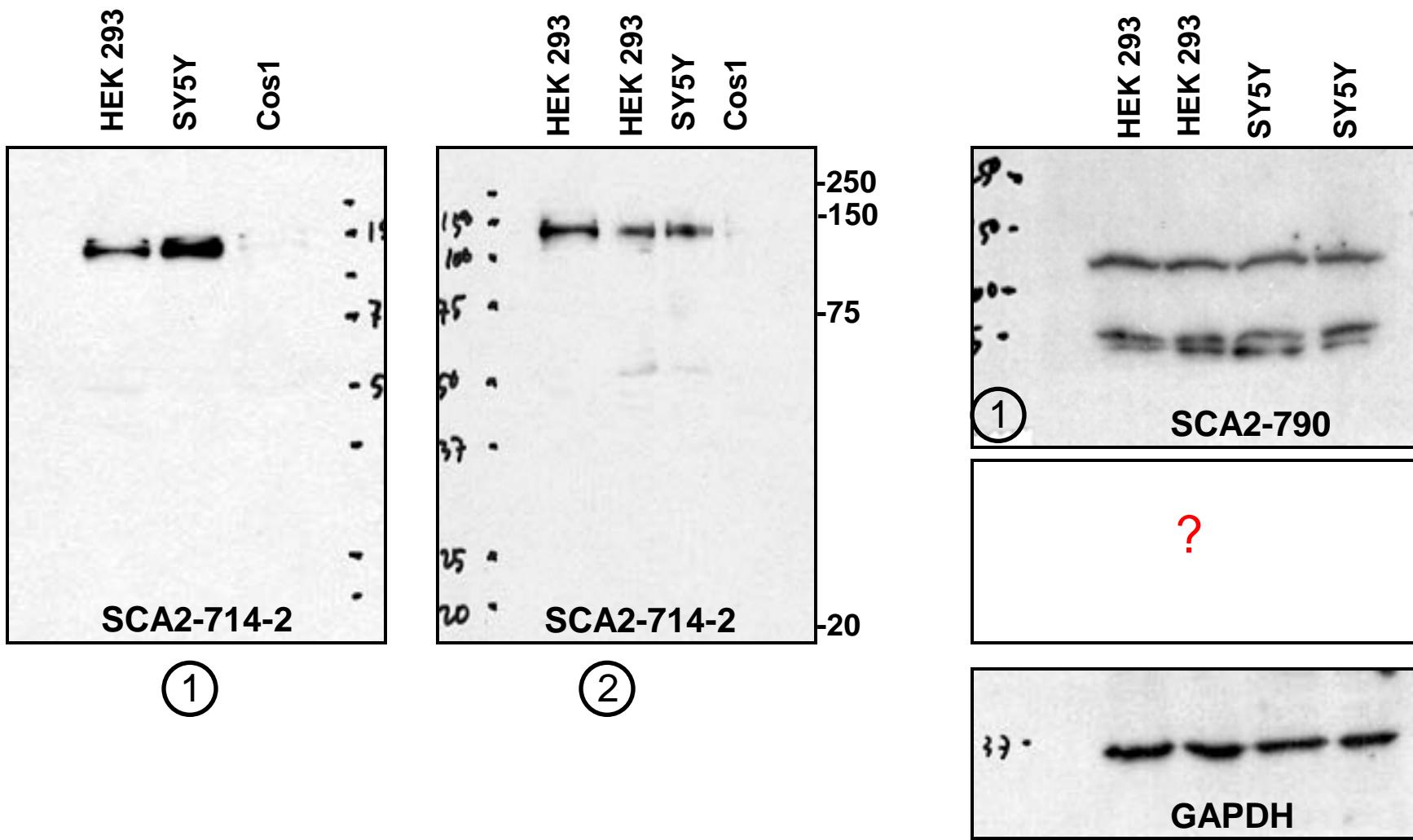


①

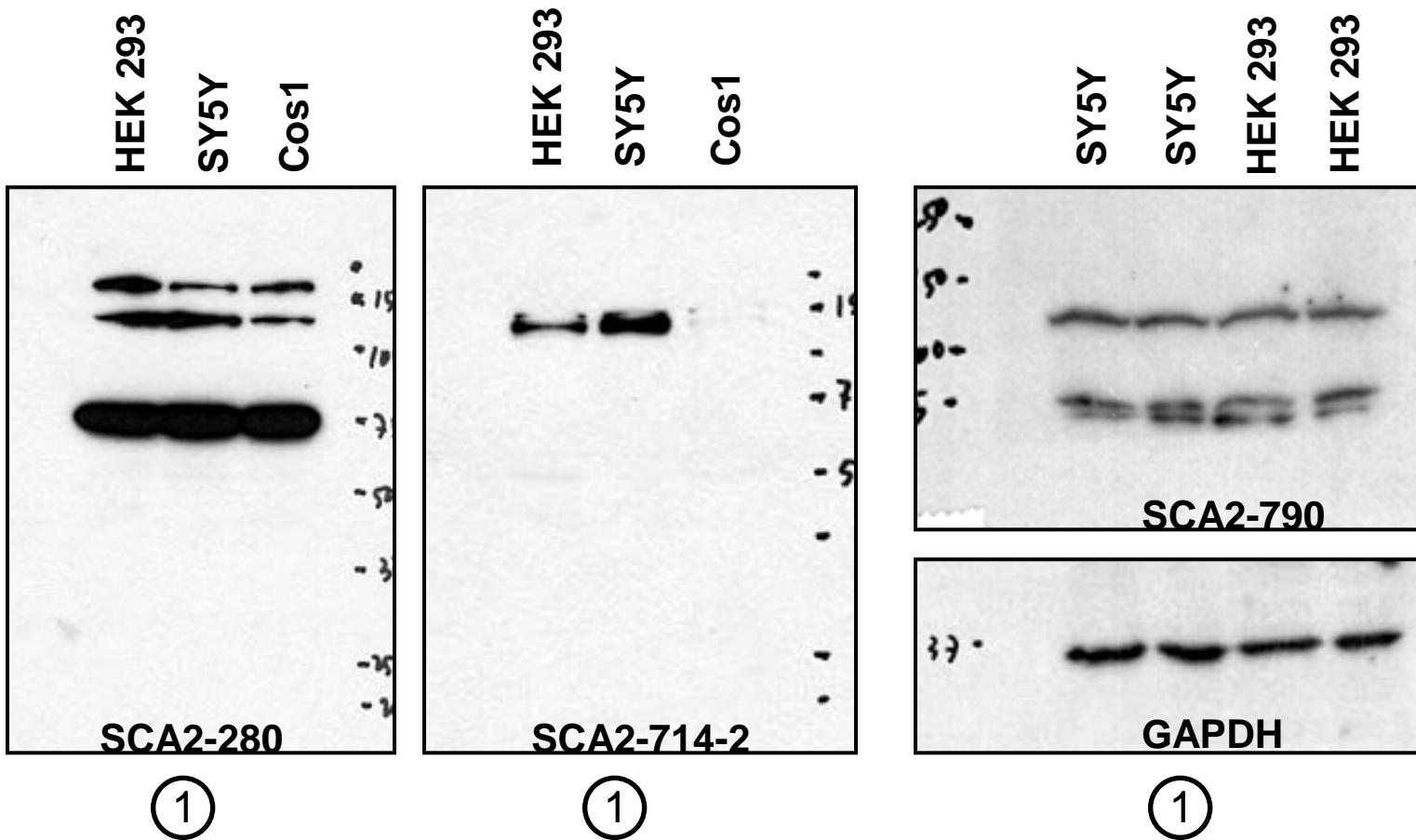


②

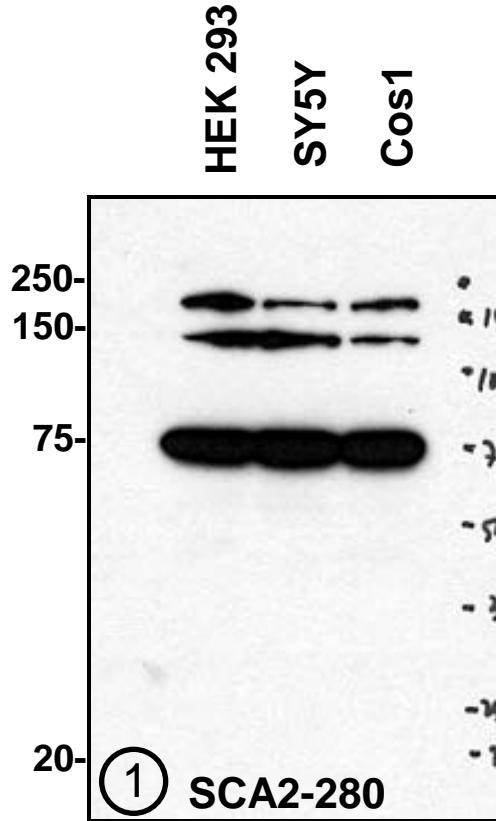
Characterization of SCA2 Antibodies



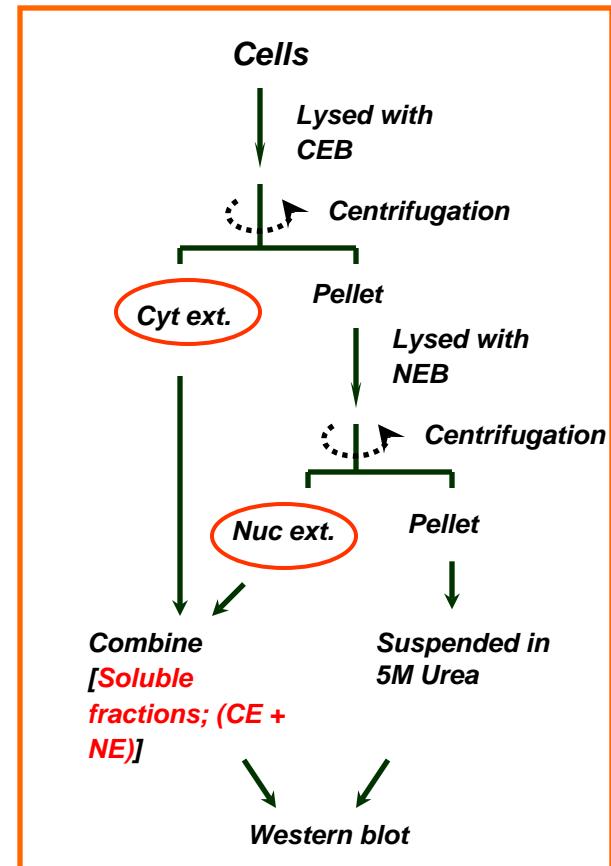
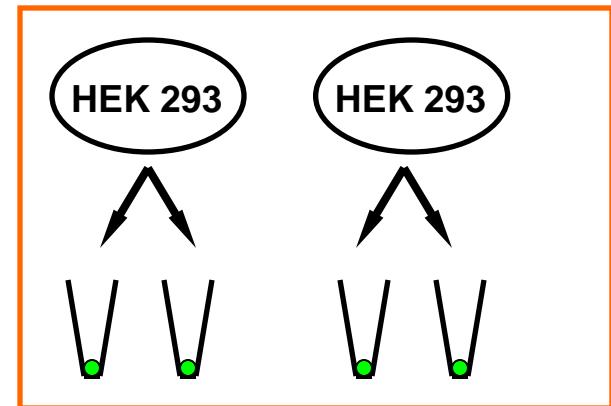
Summary of SCA2 Antibodies in Western blot



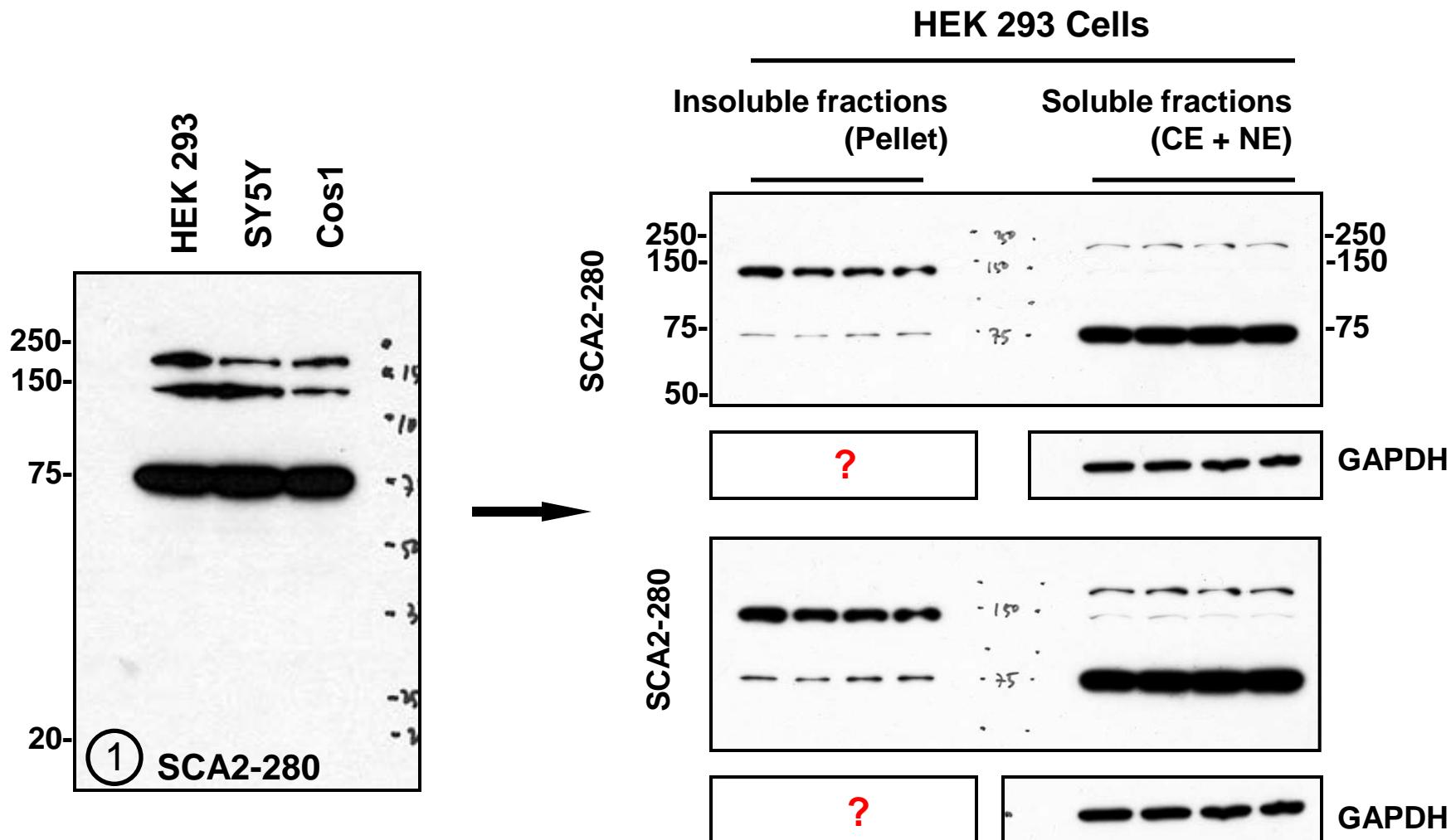
Proteins distribution in sub-cellular fractions



The protein
bands are
separable



Proteins distribution in sub-cellular fractions



1) Major portion of ATXN2 (?) remain in the insoluble fractions

2) Proteins; 75 and >150 kDa proteins are in soluble fractions

Future Plan

A) Test the specificity of this abs

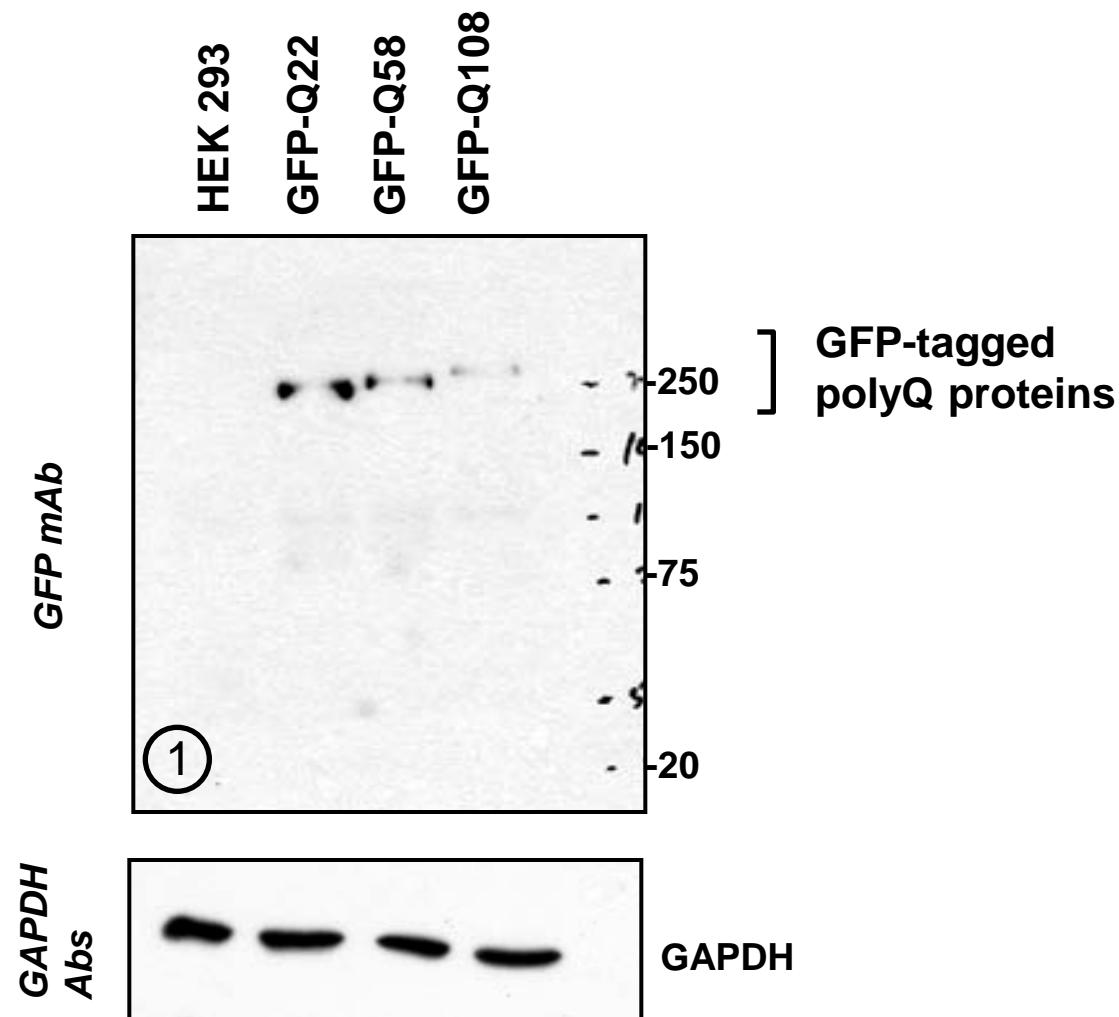
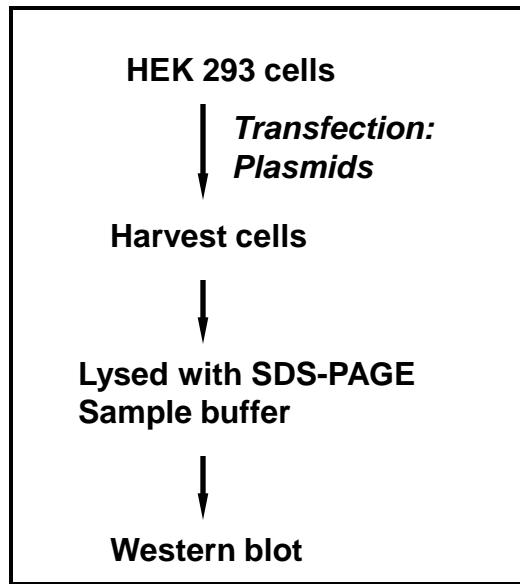
- 1) Determine the ATXN2 levels in cells where ATXN2 will be depleted using siRNA**

- 2) Determine the ATXN2 levels in ATXN2 KO mice**

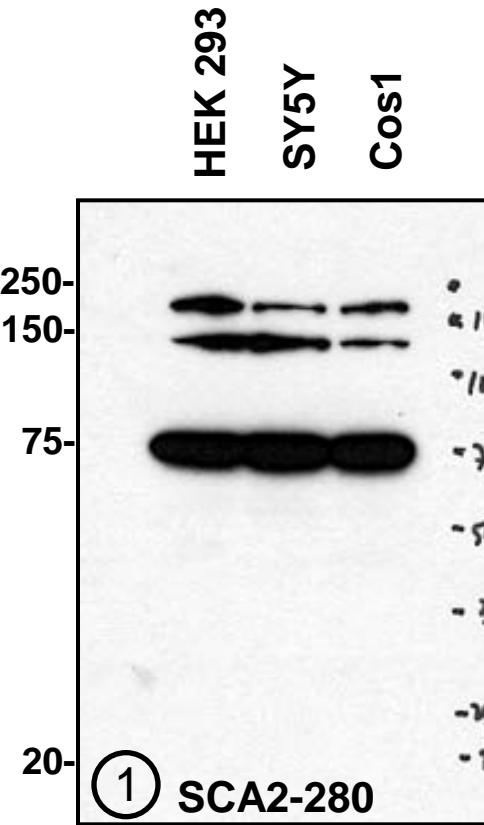
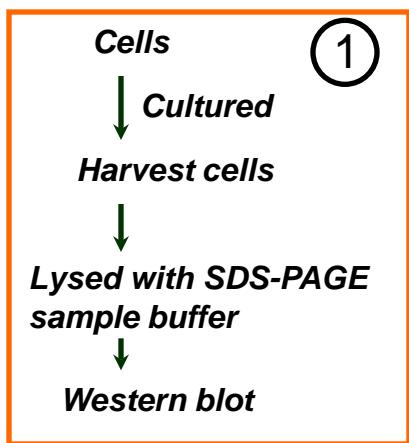
B) Test this abs in IP

C) Purification of ATXN2 associated proteins under normal and stress conditions in brain cells (SY5Y cells)

Expression of GFP-polyQ proteins in HEK 293 cells

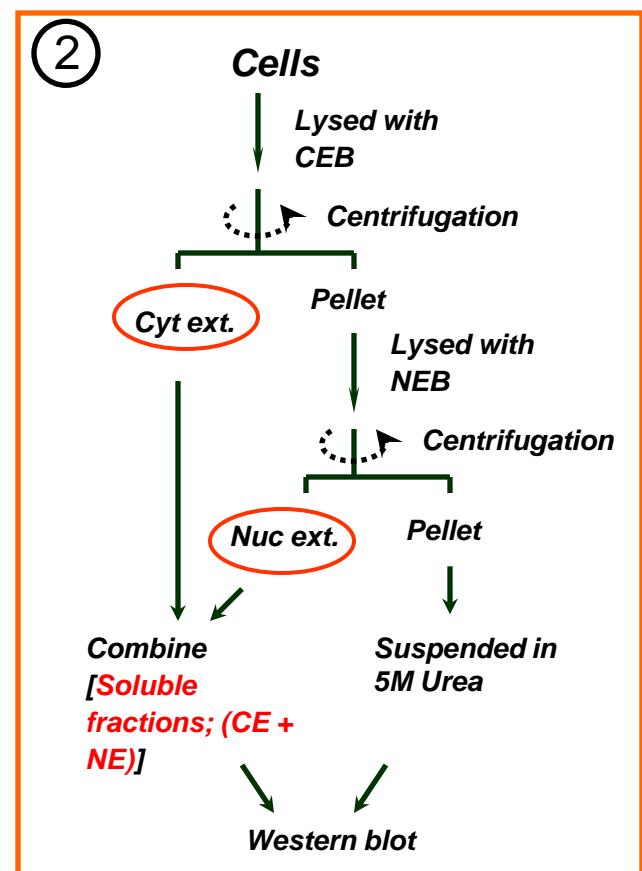


Characterization of SCA2-280 Antibodies



Proteins distribution in sub-cellular fractions

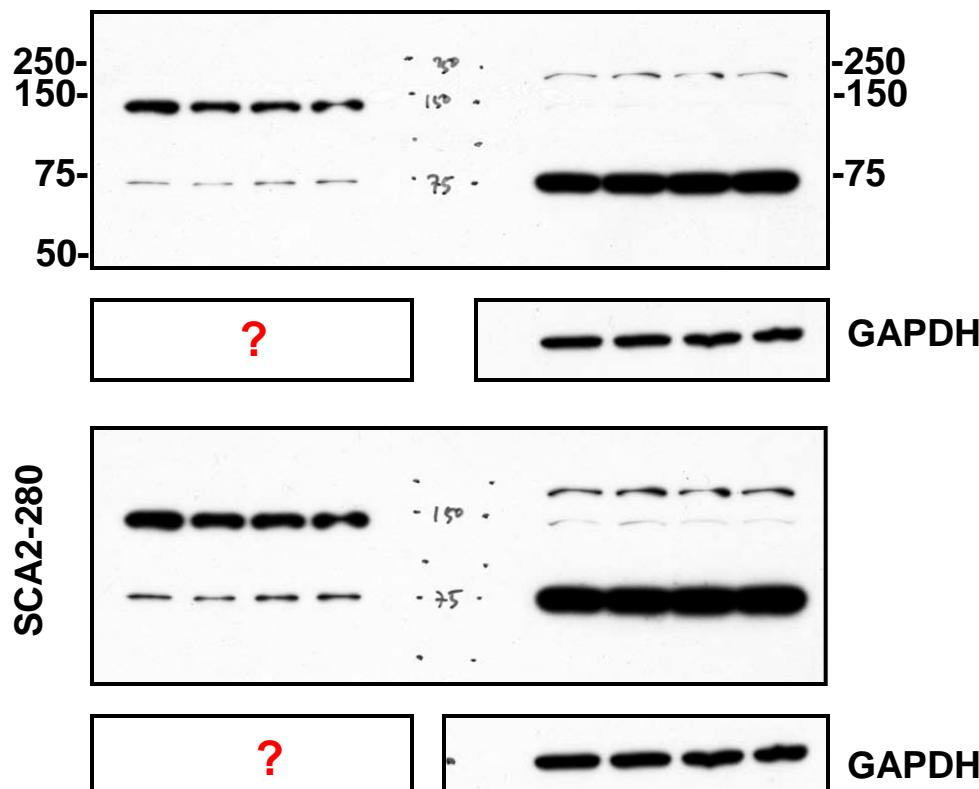
②



HEK 293 Cells

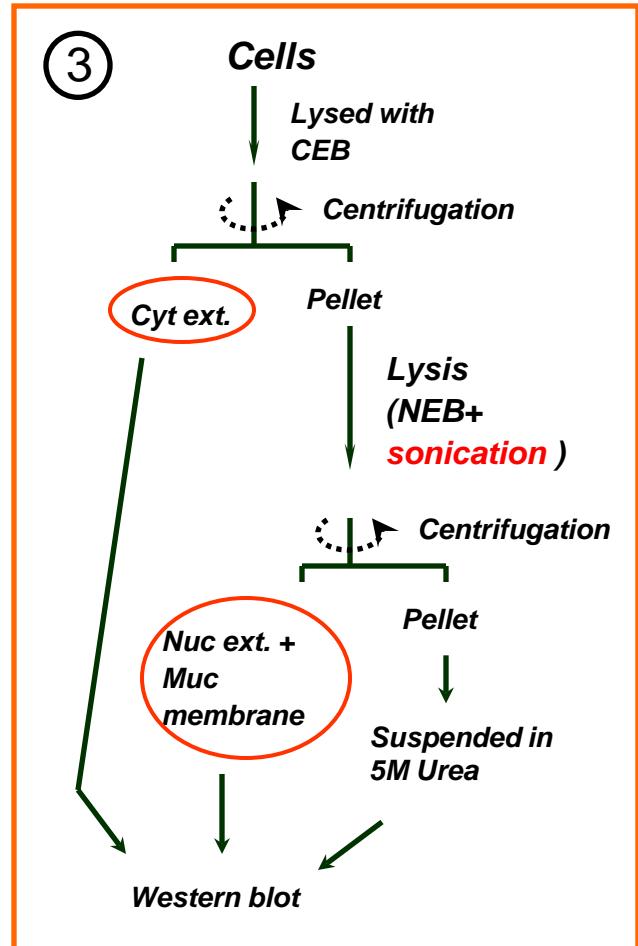
Insoluble fractions
(Pellet)

Soluble fractions
(CE + NE)

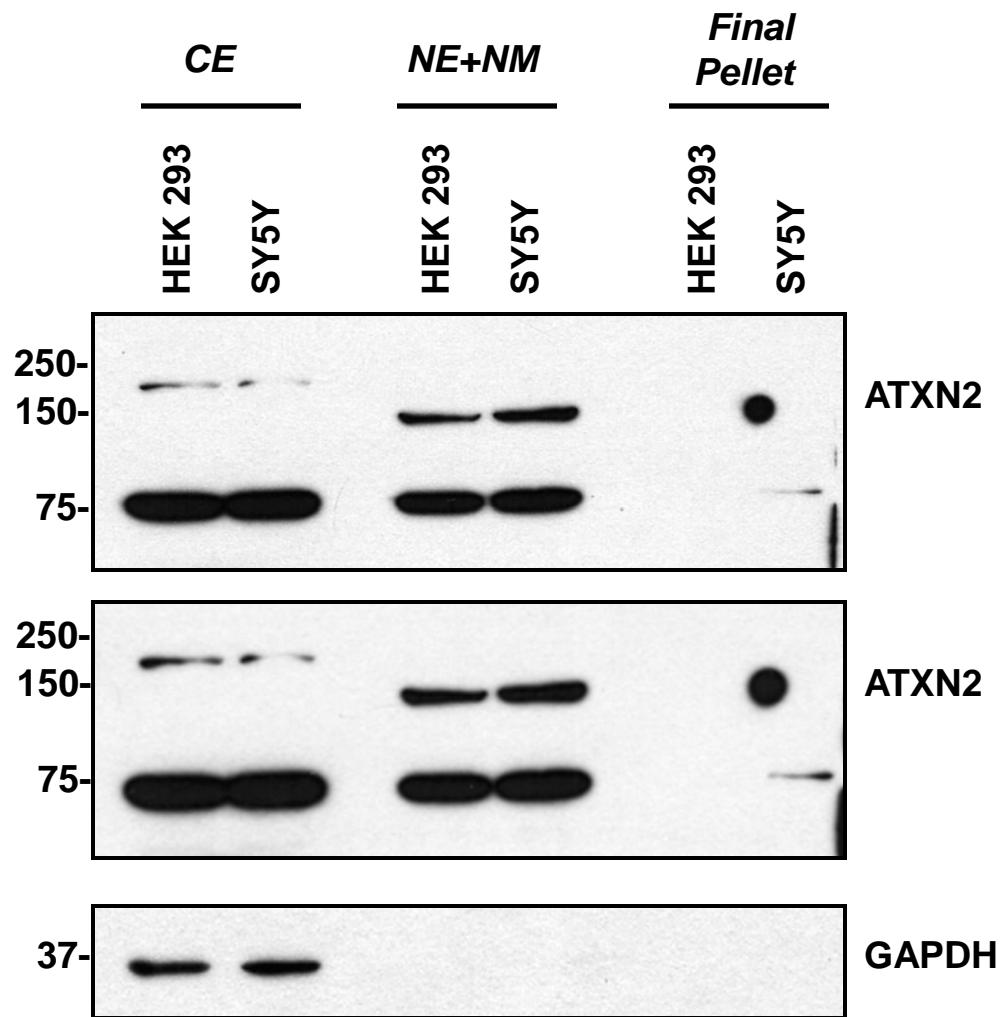


Proteins distribution in sub-cellular fractions

(3)



SCA2-280



CE, Cytoplasmic Ext.; NE+NM, Nuclear Ext. + Nuclear membrane