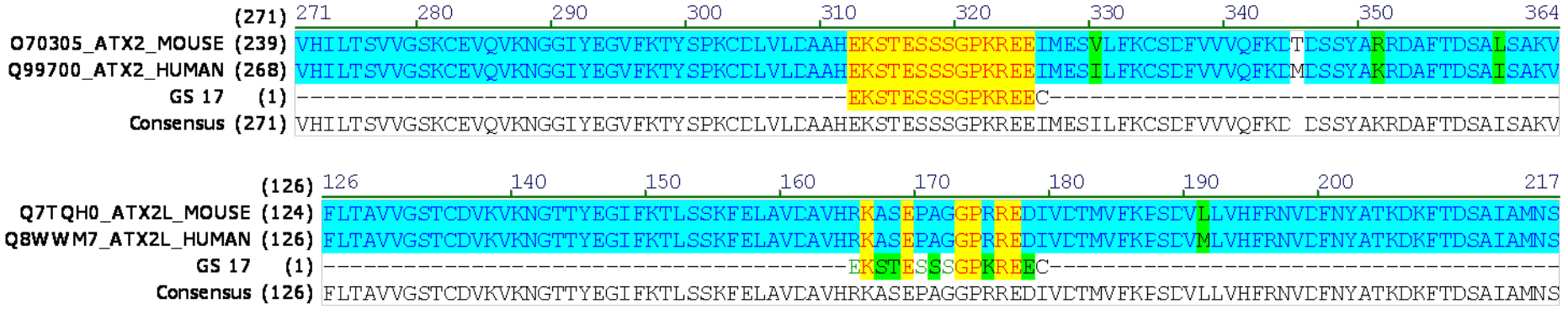
Affinity purified polyclonal SCA-2 antibodies

SCA2	280	Genscript
SCA2	1080	Genscript
SCA2	714	Proteintech Group
SCA2	790	Pacific Immunology

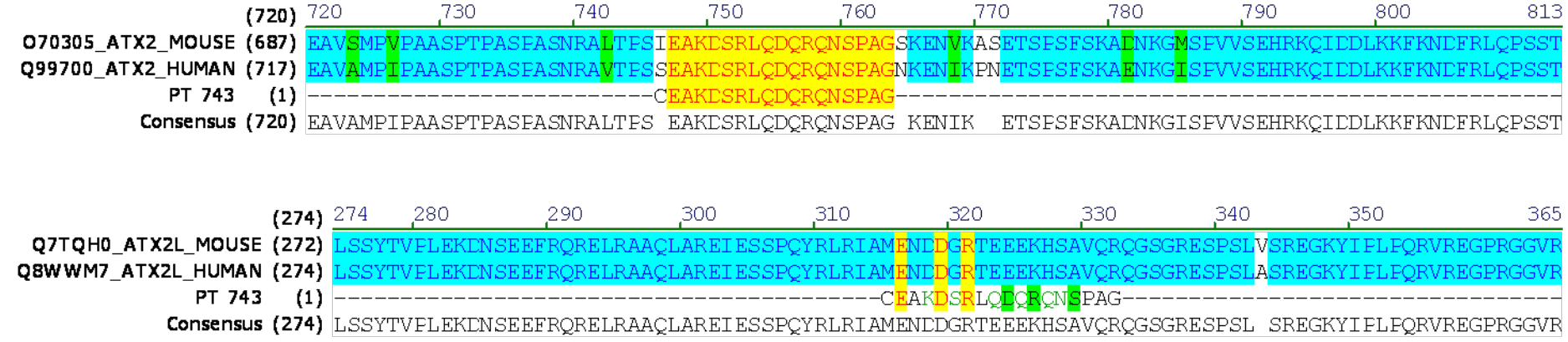
mouse 280-293 / human 309-322

SCA2 280



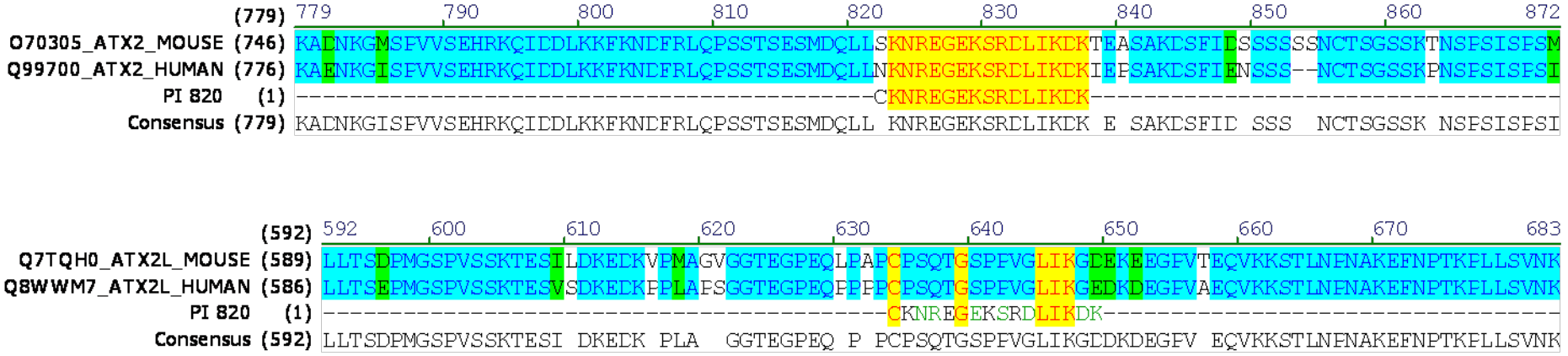
mouse 714-730 /human 748-764

SCA2 714



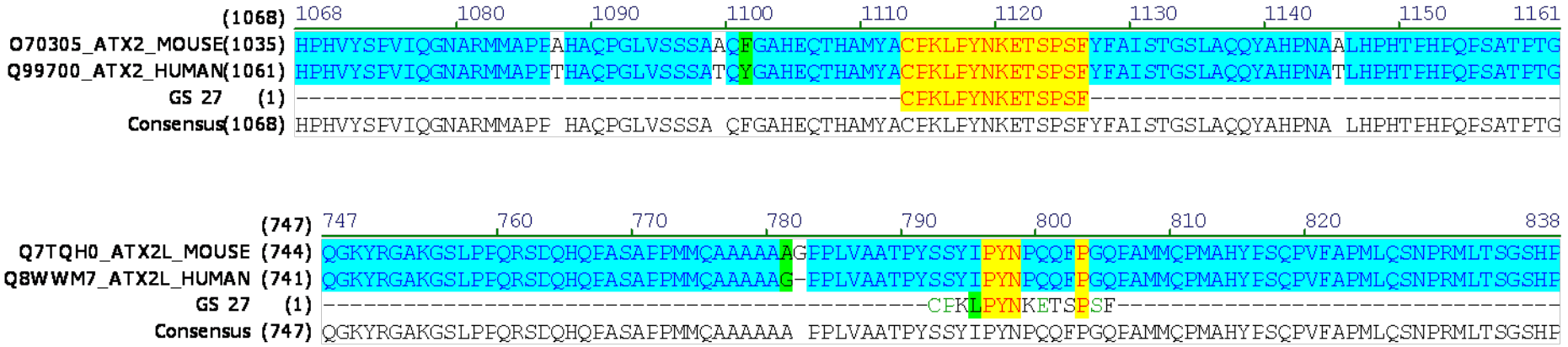
mouse 790-804 / human 820-834

SCA2 790



mouse 1080-1093 / human 1106-1119

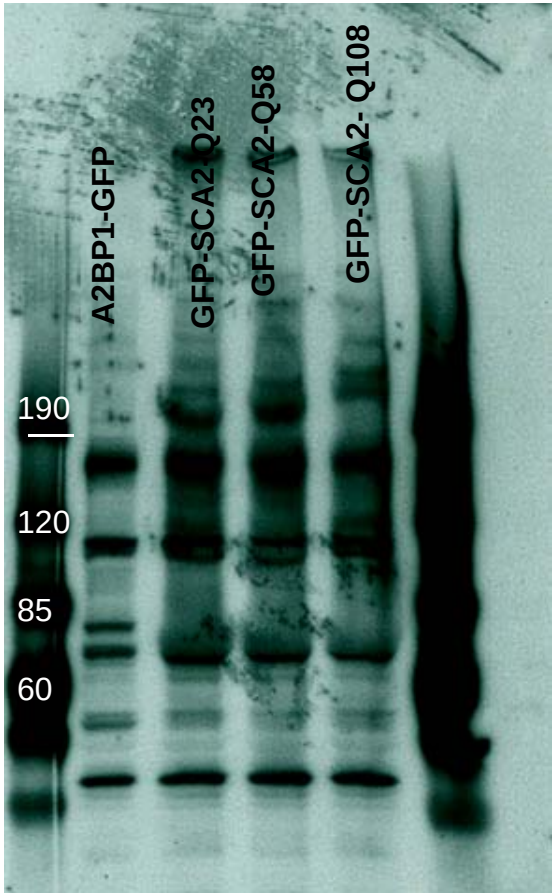
SCA2 1080



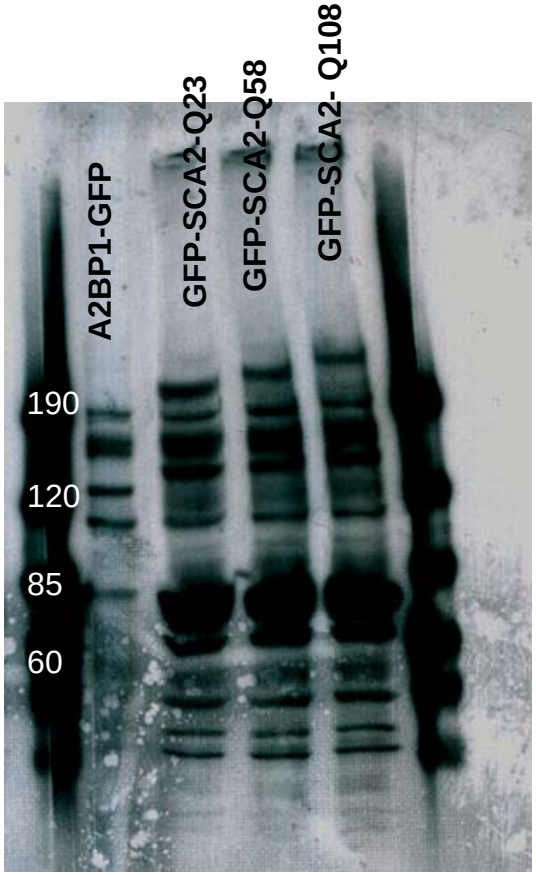
	Genscript	Genscript	Pacific Immunology	Proteintech Group	Proteintech Group
	SCA2 280	**SCA2 1080**	**SCA2 790**	**SCA2 714-1**	**SCA2 714-2**
boosts	3	3	3	4	4
Elisa rabbit 1	A 1: 128.000	1: 512.000	2213 1: 200.000	1: 10.000	
Elisa rabbit 2	B 1: 64.000	1: 512.000	2214 1: 500.000		1: 1,000.000
affinity purified	60 ml	60 ml	25 ml	30 ml	30 ml
amount of antib.	9 ml	6 ml	4.2 ml	6.5 ml	10 ml
conc. of antib.	**0.552µg/µl**	**0.46µg/µl**	**0.60µg/µl**	**0.75µg/µl**	**0.75µg/µl**
left over bleeds	30ml final (rab.A)	30ml final (rab.A)	70ml final (rab.2213)	1ml prod.bleed (P2)	1ml prod bleed(P2)
	30ml final (rab.B)	30ml final (rab.B)	60ml final (rab.2214)		
				extended protocol	extended protocol
total boosts				6	6
amount of antib.				17 ml	29 ml
conc. of antib.				**0.60µg/µl**	**0.60µg/µl**
left over bleeds				1ml final bleed	1 ml final bleed

	SCA2 antibodies		
aa on ATXN2 (mouse)	280 - 293	714 -730	790 - 804
epitope N- …	EKSTESSSGPKREEC	CEAKDSRLQDQRQNSPAG	CKNREGEKSRDLIKDK
antib. concentration	0.552 µg/µl	0.75µg/µl, 0.6µg/µl(ext.)	0.6µg/µl
conc. for westernblot	0.5 µg/ml	0.5 µg/ml	0.1 - 0.5 µg/ml
results on westernblot	no	yes	yes
antibody-conc. for IHC	4µg/ml	8µg/ml	9-10µg/ml
IHC staining	yes	yes	no
cereb.staining results with antibody	staining of Purk.cells and dentr.spines, contrast to rest of tissue	staining of Purk.cells and dentr.spines, contrast to rest of tissue	staining of all tissue layers, weaker contrast
antiserum-conc .for ICH	1:350, 1: 1000 (rb.A)	1:1000 (rb.2)	1:100, 1:350 (rb.2214)
cereb.staining results with final antiserum	staining of Purk.cells and dentr.spines, contrast to rest of tissue	staining of Purk.cells and dentr.spines, contrast to rest of tissue	staining of Purk.cells and dentr.spines, contrast to rest of tissue

SCA2 714 HEK 293 lysates



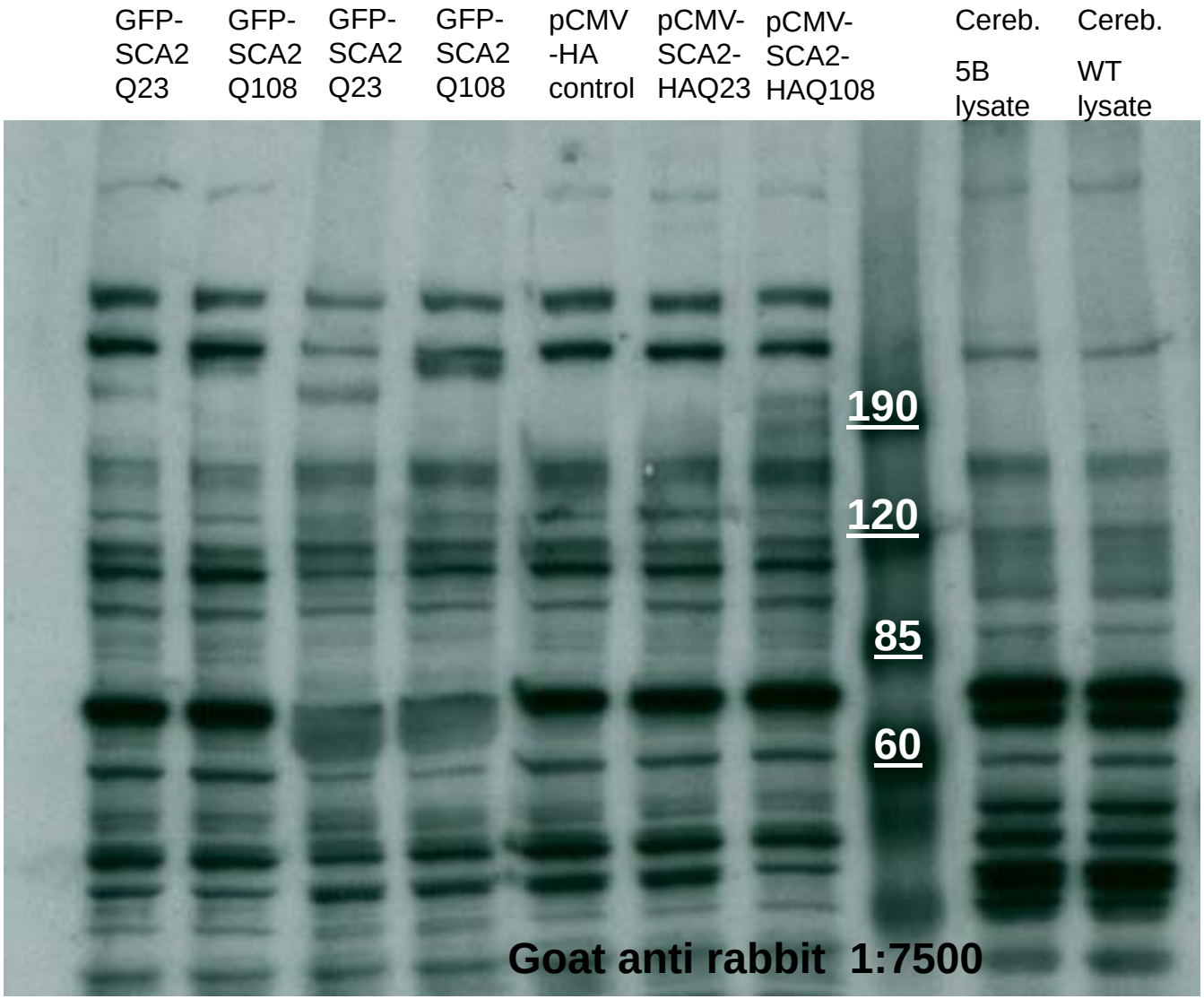
SCA2 714 rabbit 1 0.5 µg/ml



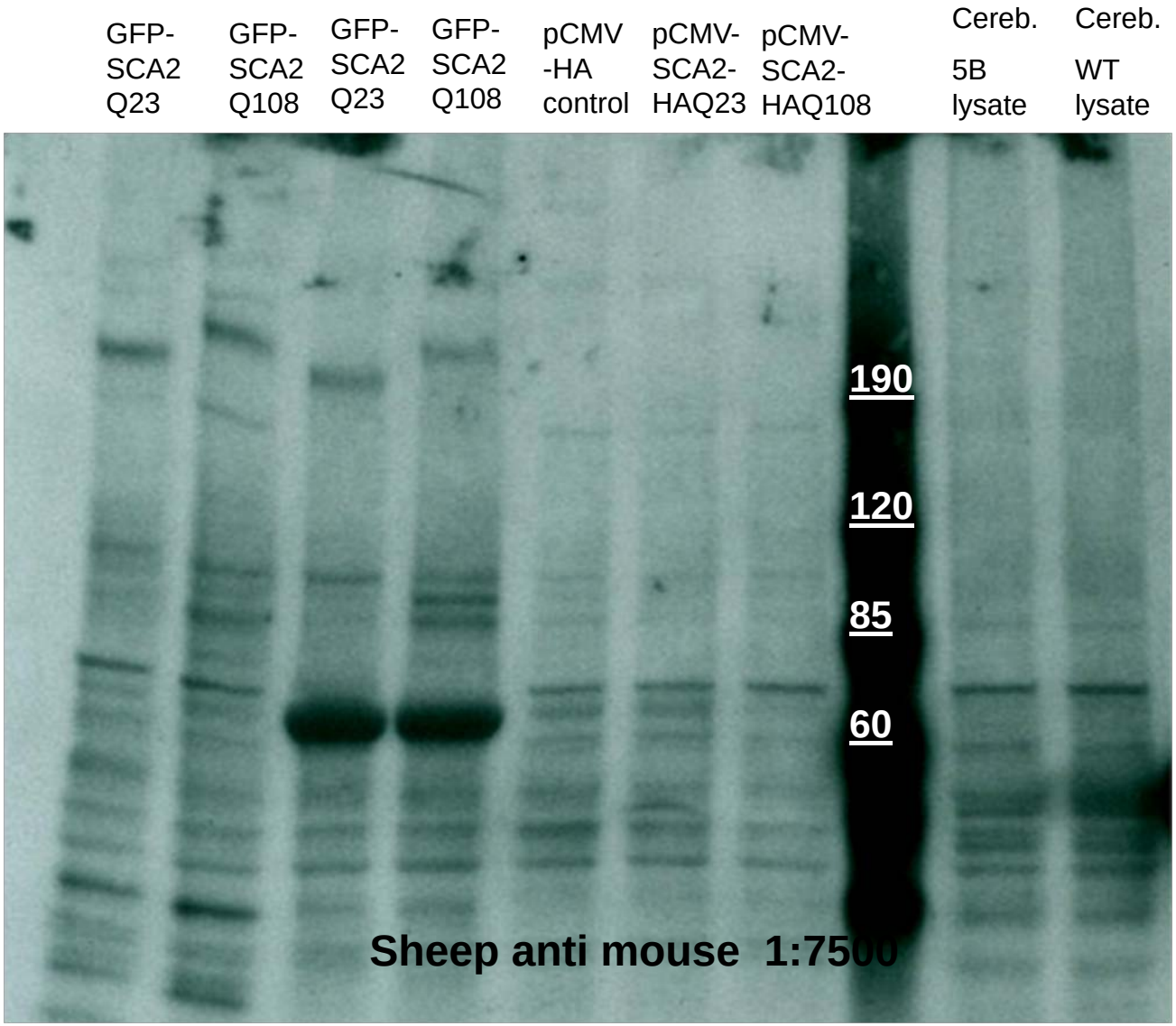
SCA2 714 rabbit 2 0.5 µg/ml

Sec.antib.: donkey anti rabbit 1:7500

SCA 2 790 0.5 µg/ml HEK 293 cells

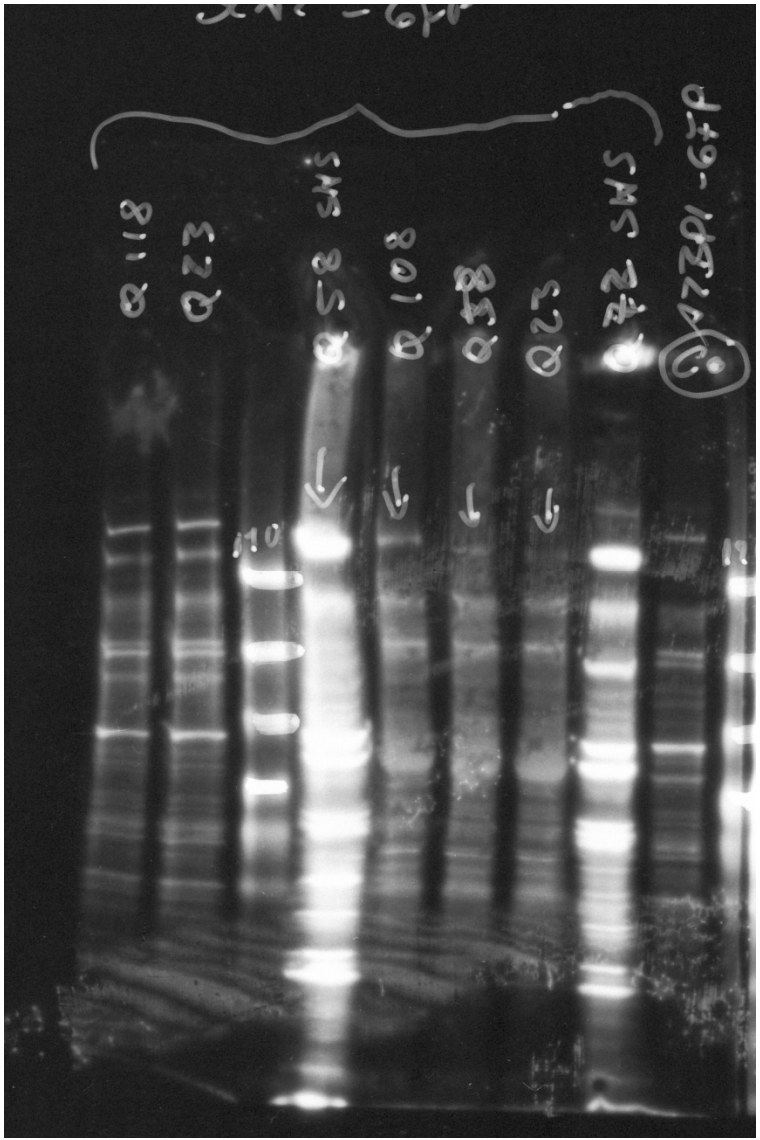


anti-GFP (Chemicon) 1:750 (HEK 293 lysates)

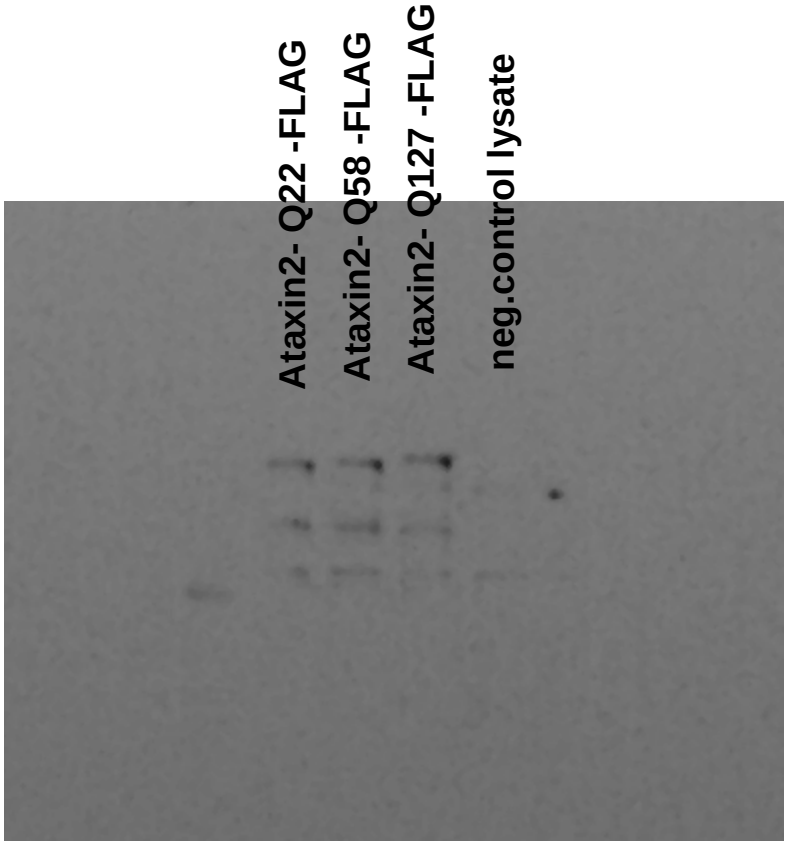


SCA2 790 0.5 ug/ml HEK 293 cells SCA2-GFP lysates

Sec.ab
goat anti
rabbit
1:7500



Westernblot SCA2 790 0.1 ug/ml HEK 293 cells



Sec.antibody: HRP anti rabbit 1:50.000

Olympus - Protocol for Confocal Microscopy - free floating staining

Solutions:

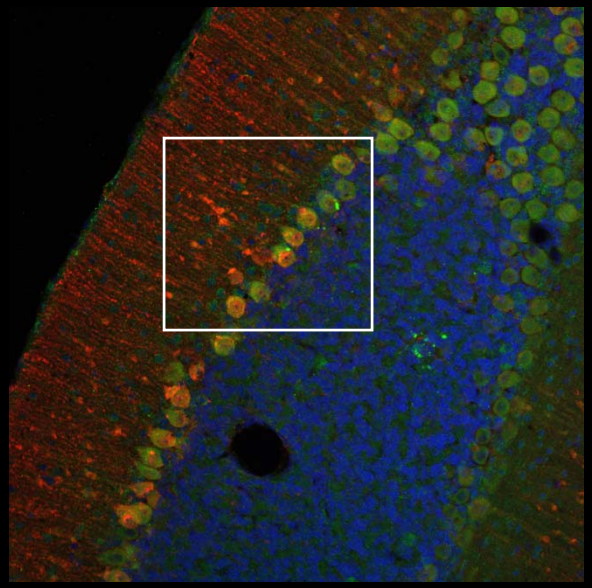
PBS
Triton –PBS(containing **0.05% Triton X-100**)
Permeabilization Sol.: **0.2% Triton in PBS, sonicated for 30-60 minutes**
Blocking solution: **Triton-PBS containing 10% Normal Serum**
Primary-and secondary antib. Sol.: **Triton PBS containing 5% Normal Serum**
Triton-PBS wash buffer : **0.05%Triton PBS containing 1% Normal Serum**
(after ab. incubation)

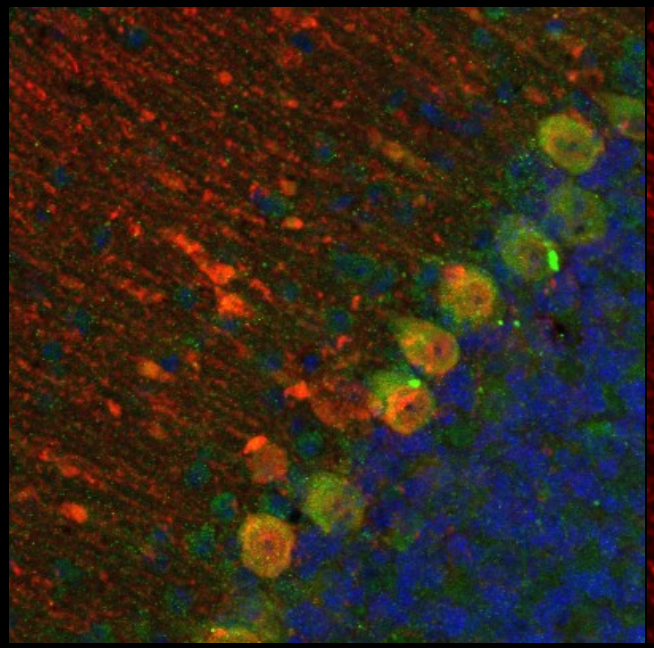
Staining:

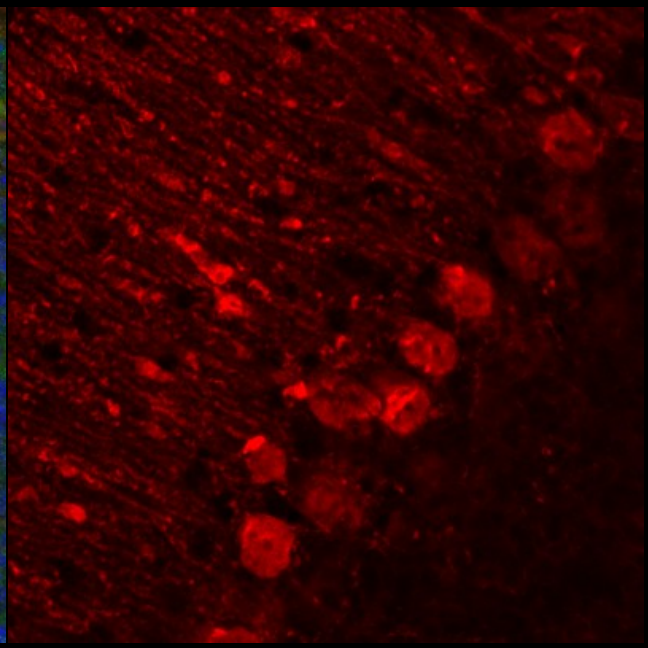
1.Wash **4 x 15 mins** with PBS on a orbital shaker
2.Permeabilize with 0.2% Triton-PBS for **2 h.**
3.Block for **2- 4 h at room temperature or at 37 C.**
4.Incubate 1st antib. in 5% serum Triton-PBS for 48 h at 4C.

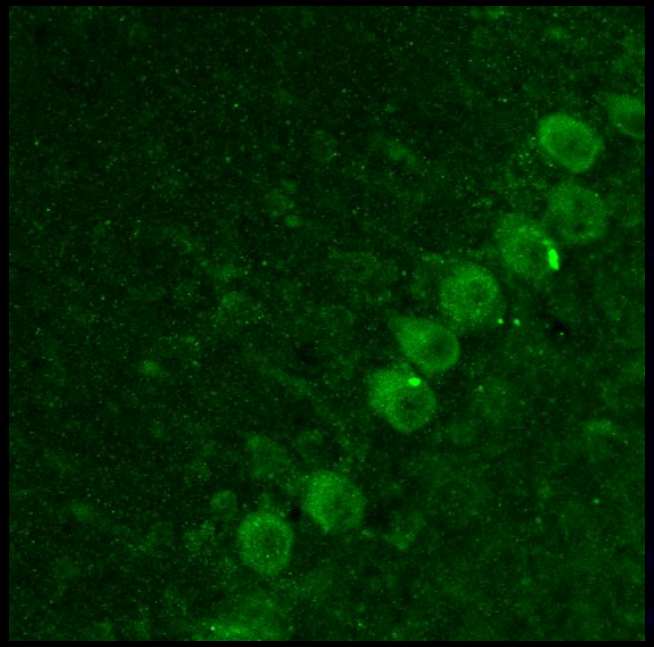
5.Wash sections **4 x 30mins** in 1% Serum wash buffer
6.Incubate 2nd antib. in 5% Triton-PBS for 48 h at 4C.
7.Wash sections **4 x 30 mins** in 1% Normal Serum wash buffer
8.Add additional **3 x 15 mins** wash in PBS to remove serum and Triton.

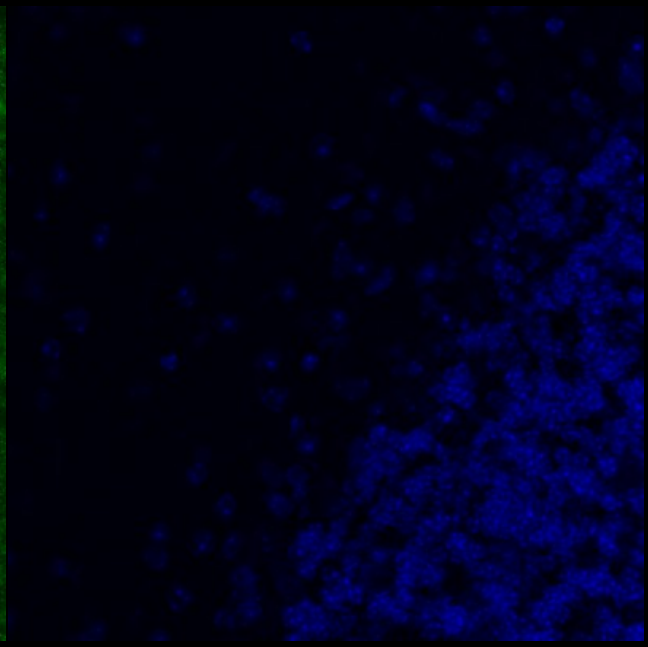
SCA2 280 on B6 wt





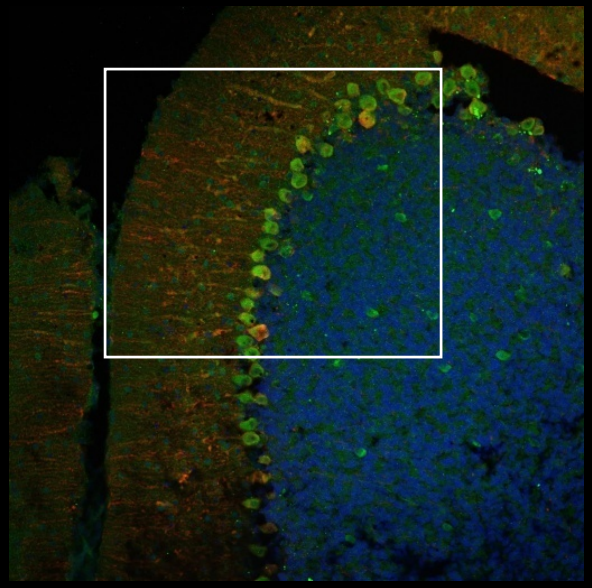


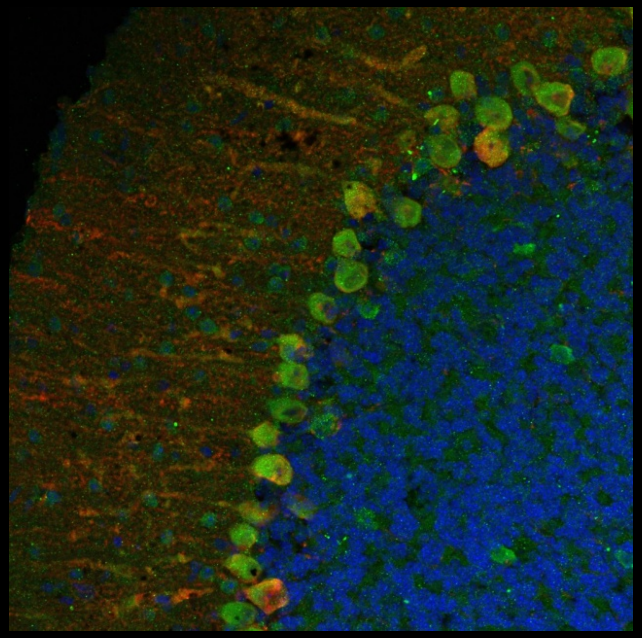


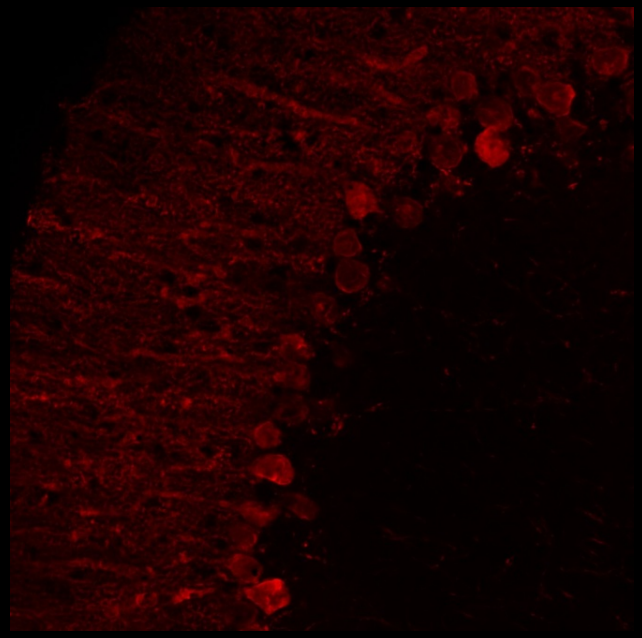


SCA2 280	6µg/ml
Calbindin (mse)	1:600
Cy 2	1:200
Cy 3	1:600
Dapi	1:7000

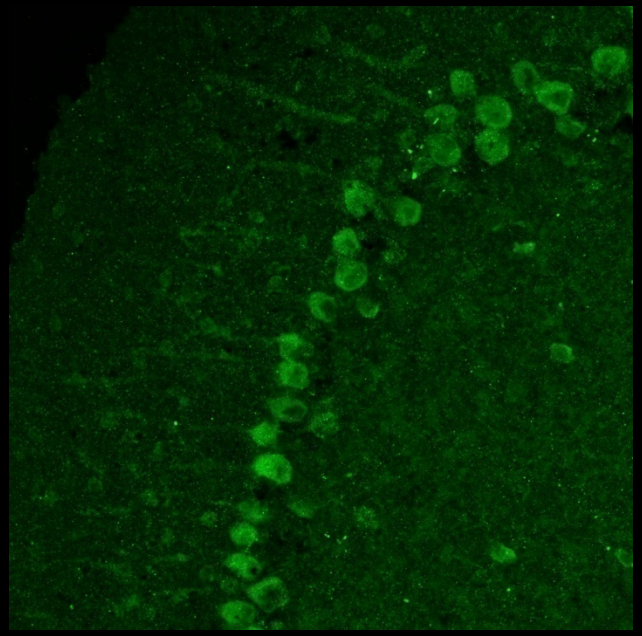
SCA2 280 on B6 wt

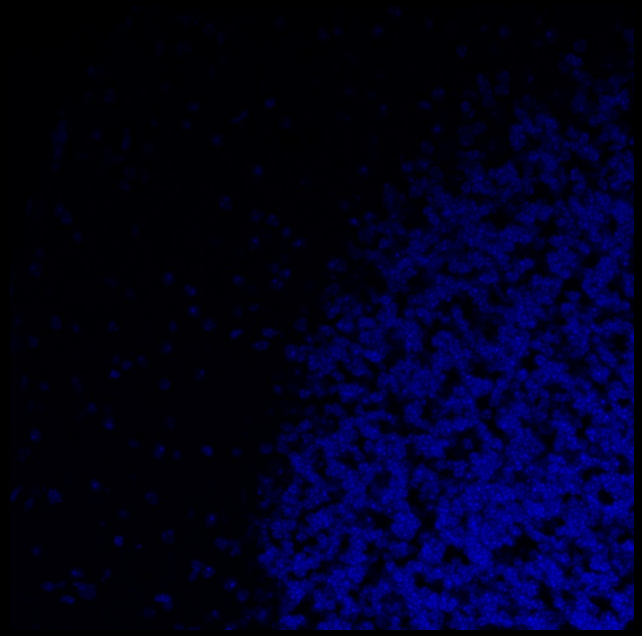




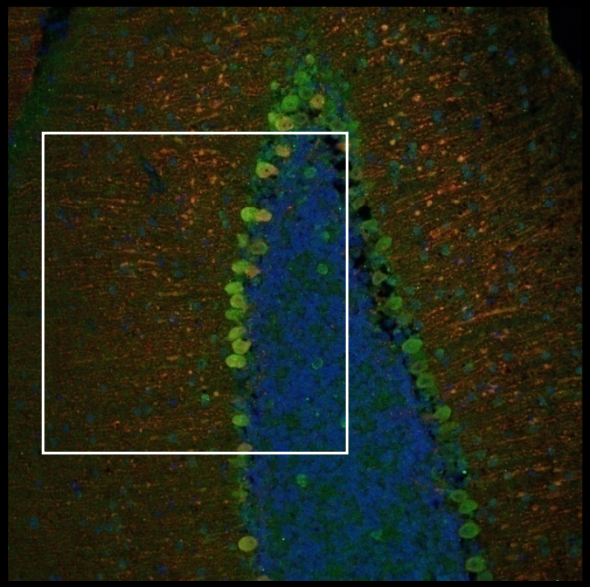


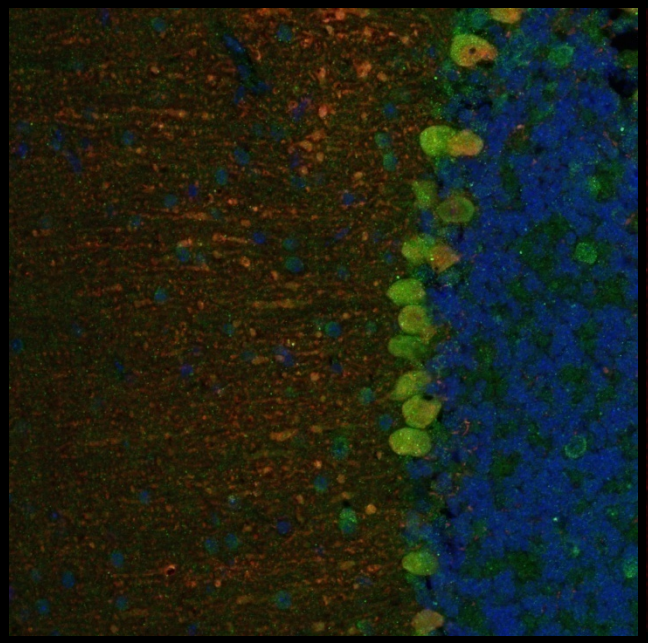
SCA2 280	6µg/ul
Calbindin (mse)	1:600
Cy 2	1:200
Cy 3	1:600
Dapi	1:7000

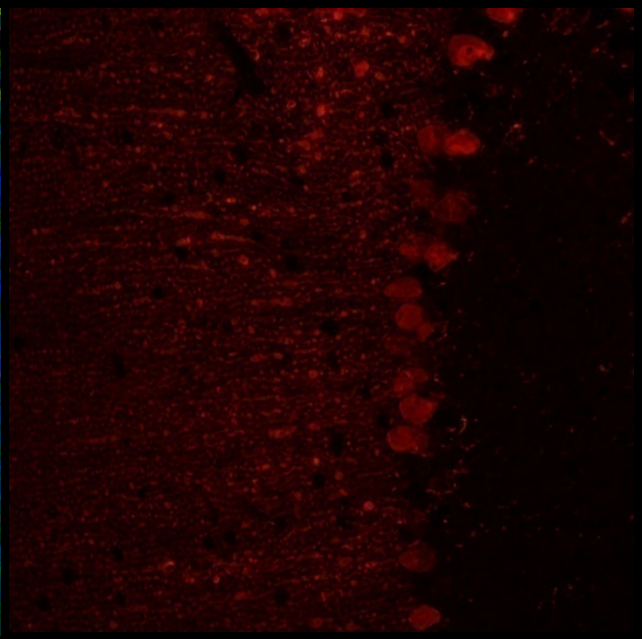


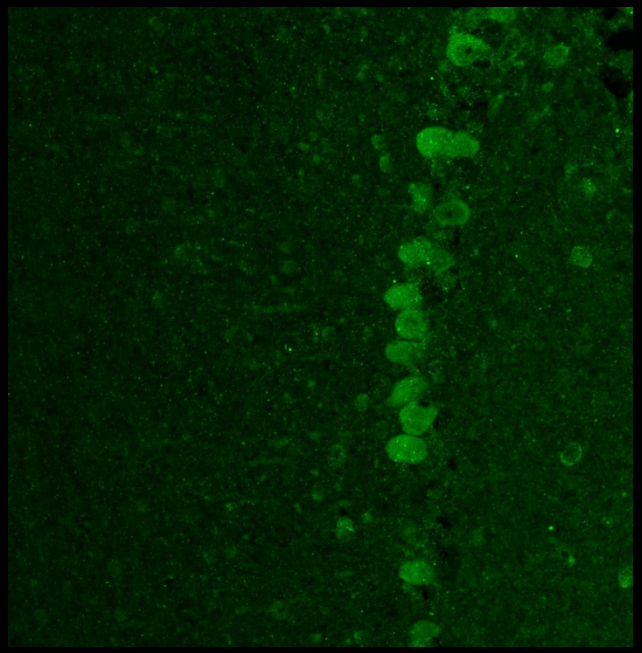


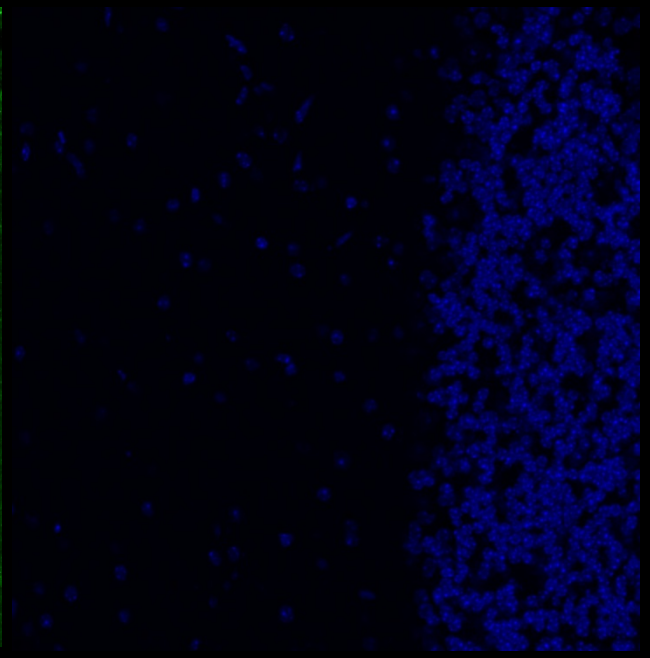
SCA2 280 on SCA2 knockout (-/-)





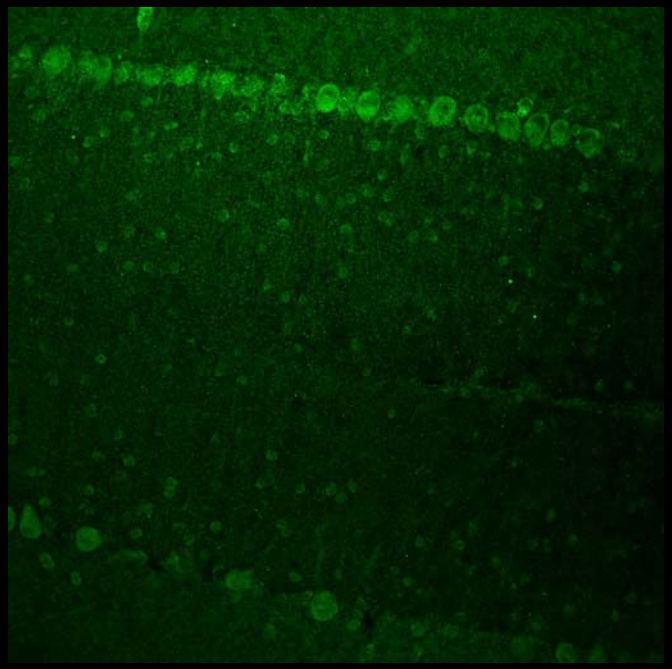


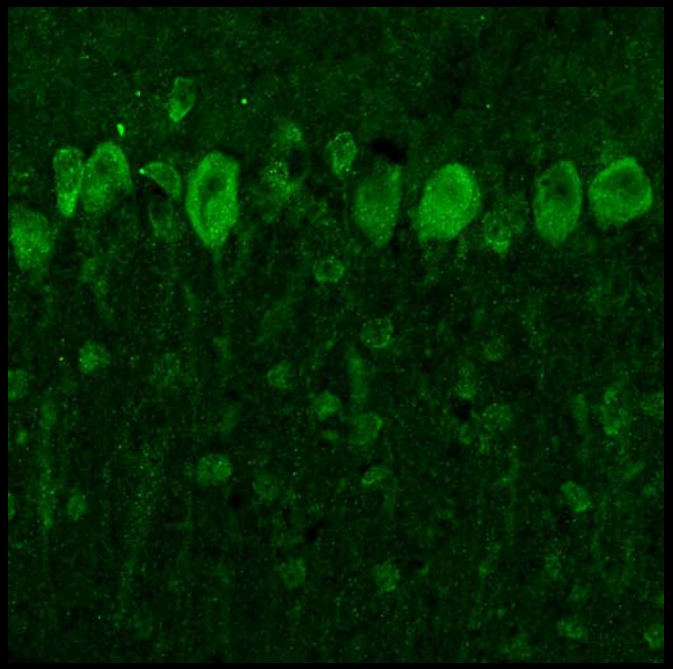




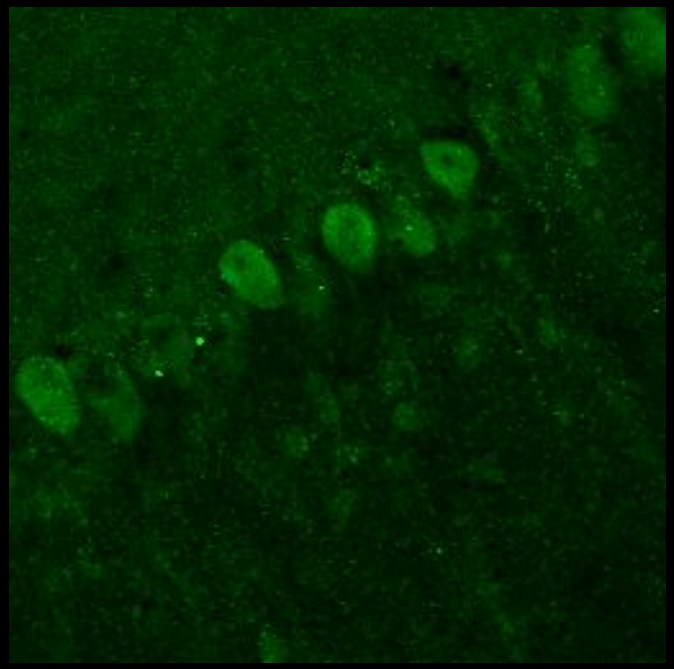
SCA2 280	6µg/ul
Calbindin (mse)	1:600
Cy 2	1:200
Cy 3	1:600
Dapi	1:7000

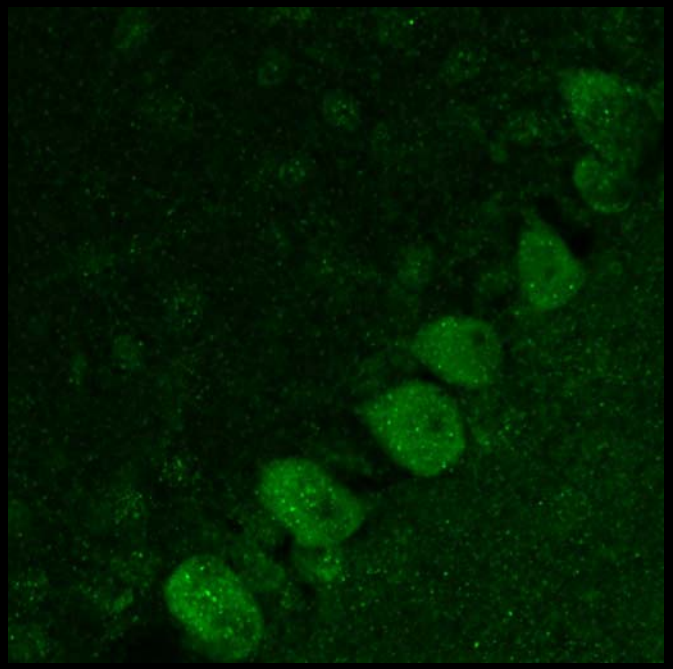
SCA2 280
4µg/ml
Cy2 1:200
on B6 wt



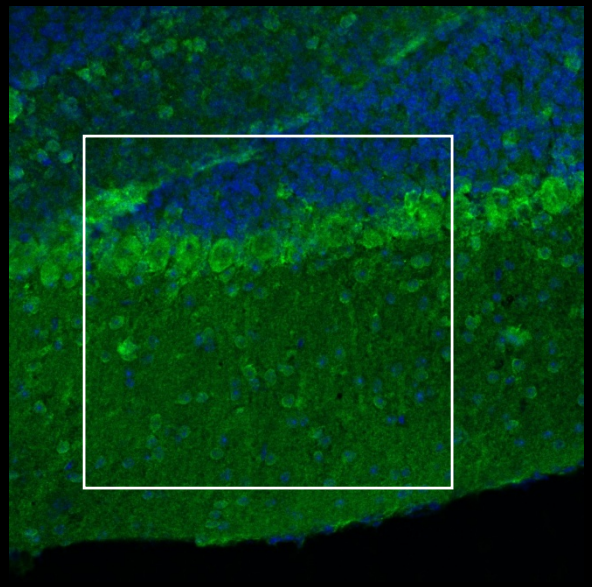


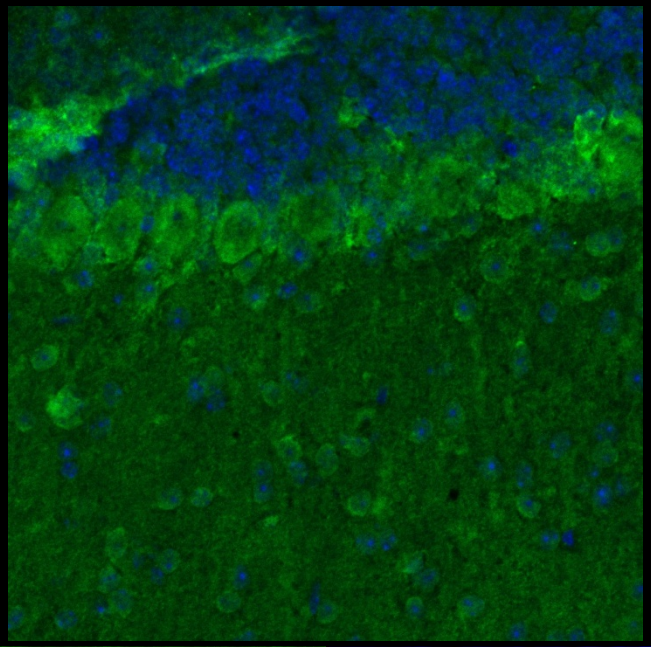
SCA2 280 4µg/ml
Cy2 1:200
on
SCA2 knockout (-/-)

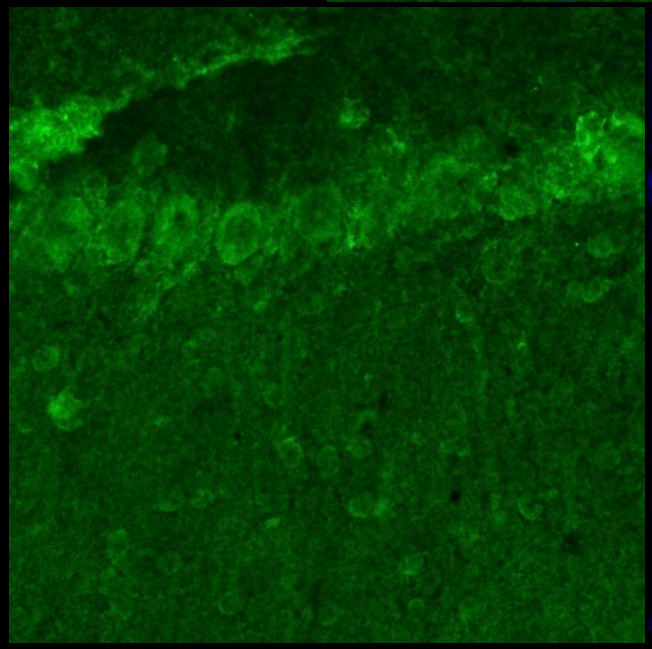


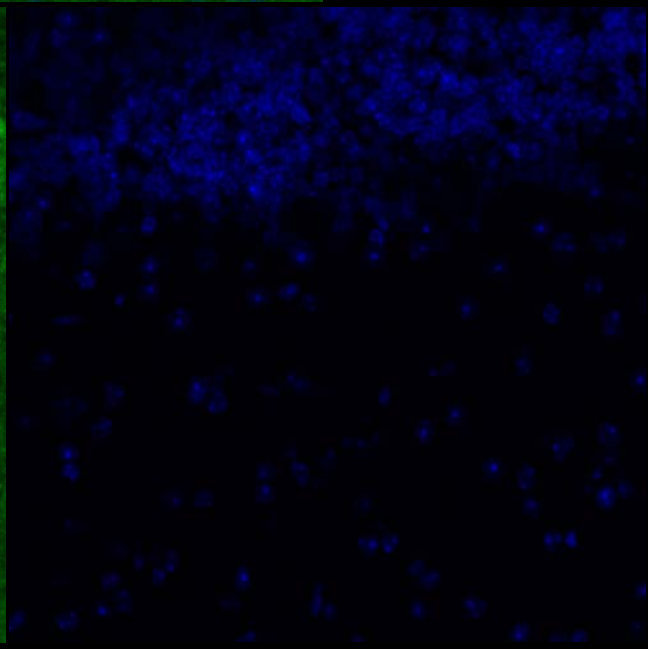


SCA2 280 on SCA2 kout (-/-)



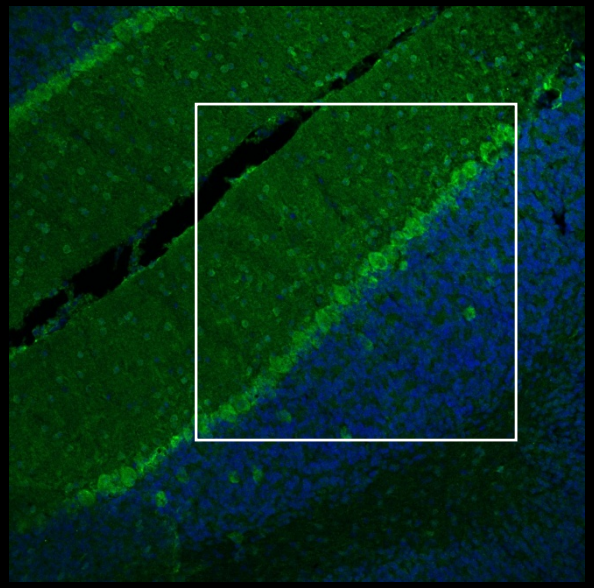


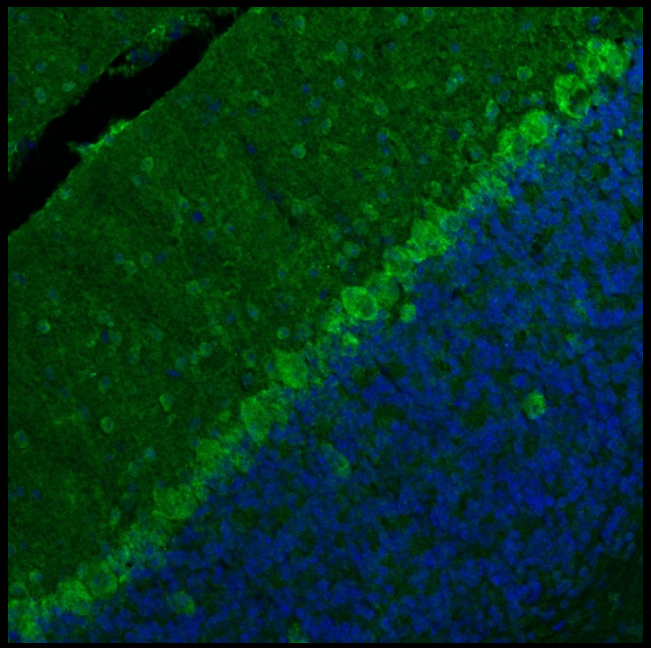




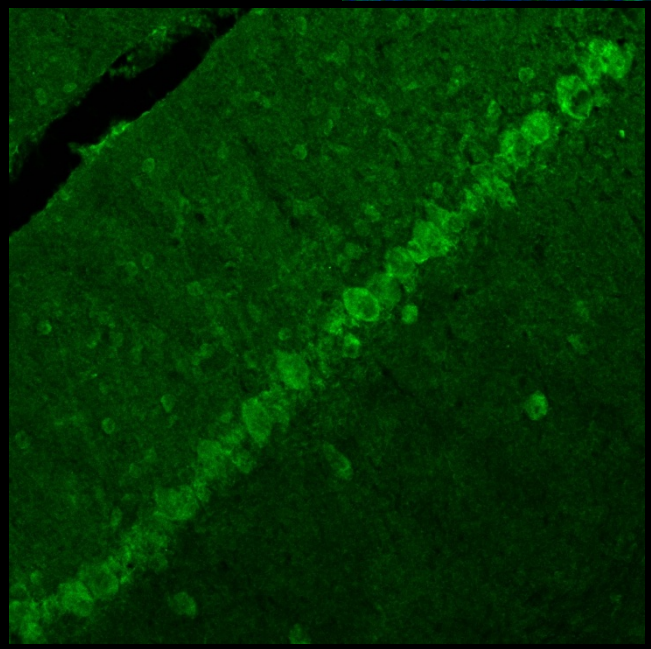
SCA2 280	4µg/ml
Dy Light 488 rb	1:1700
Dapi	1:10.000

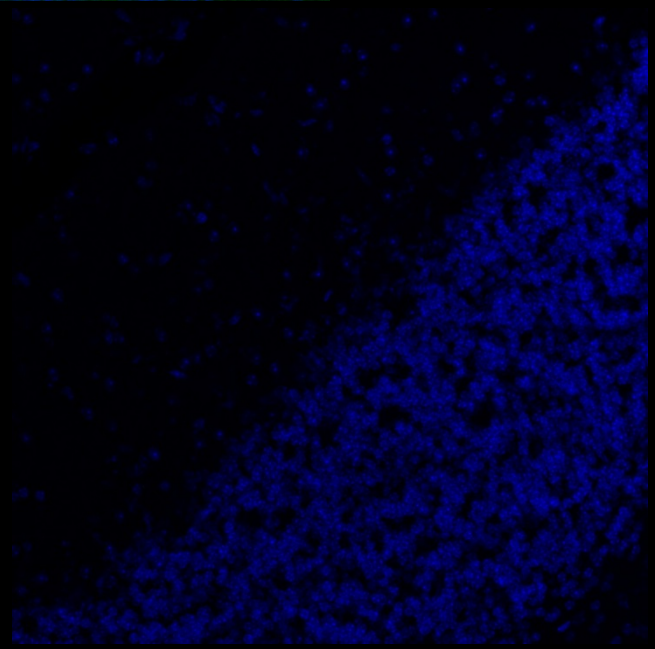
SCA2 280 on SCA2 kout (-/-)





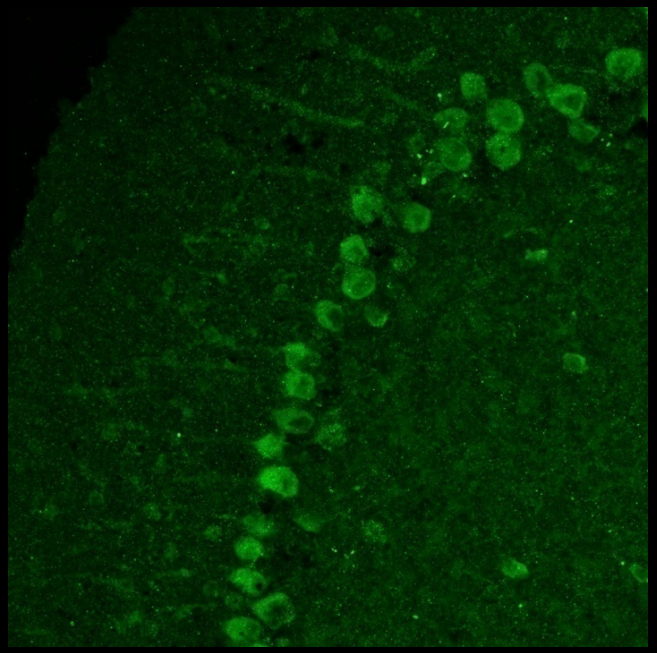
SCA2 280	4µg/ml
Dy Light 488 rb	1:1700
Dapi	1:10.000

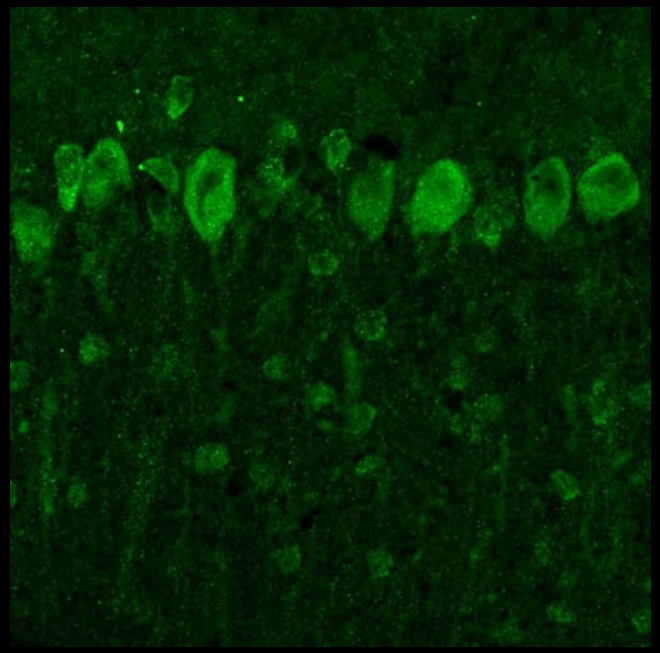




B6 WT

SCA2 280
4µg/ml
Cy2 1:200

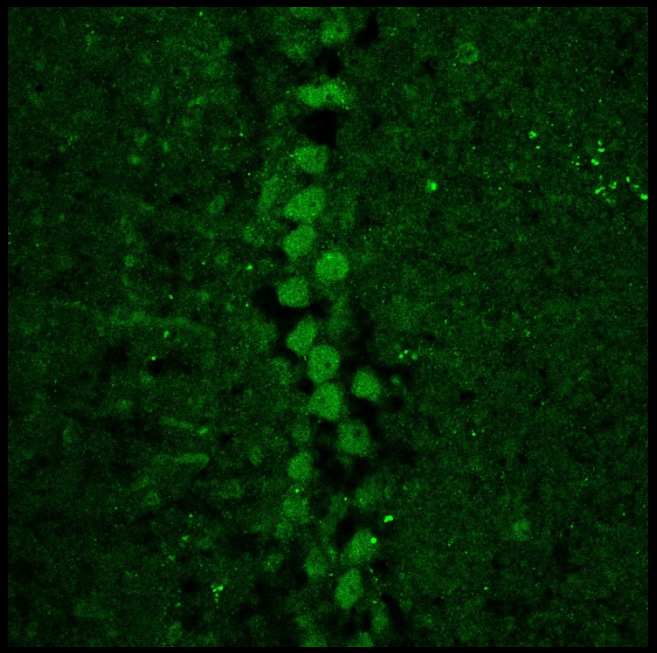


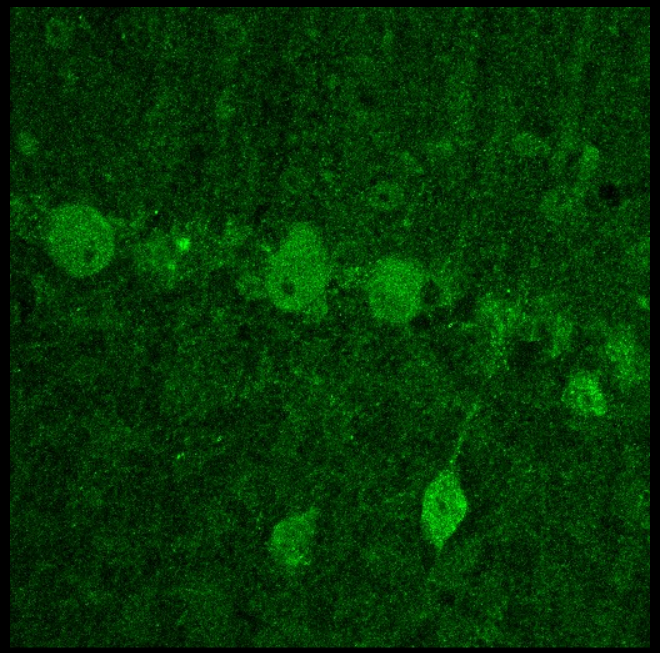


SCA2 280
4µg/ml
Cy2 1:200

Final bleed
SCA2 280
1: 350

Cy2 1:200

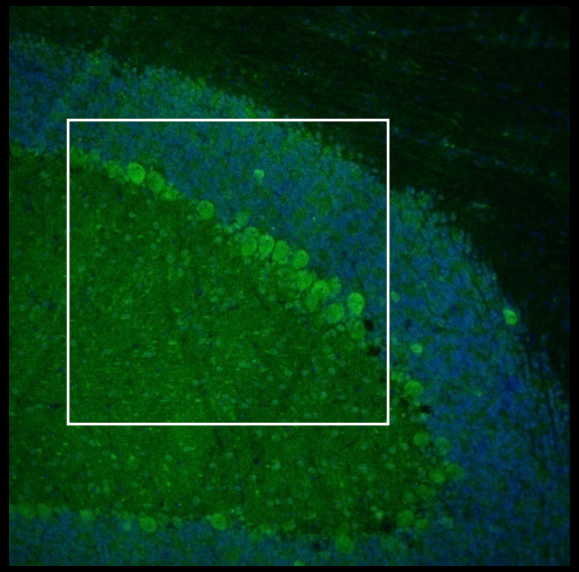


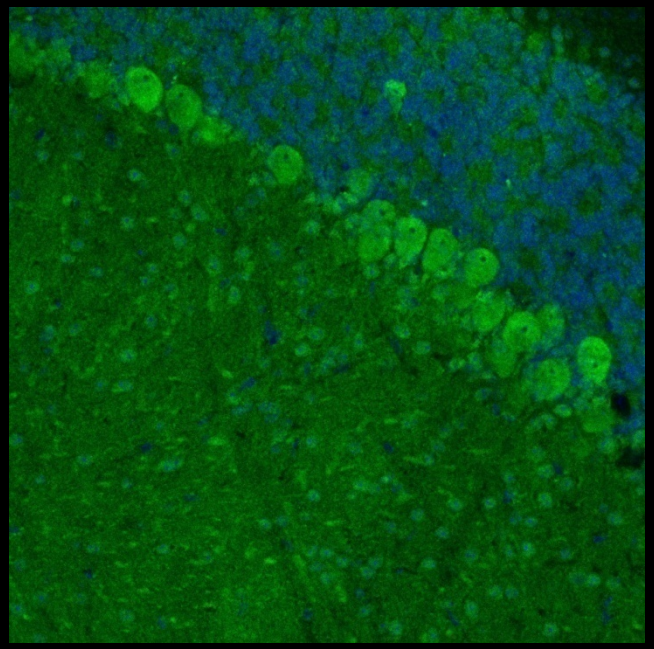


Final bleed
SCA2 280
1: 1000

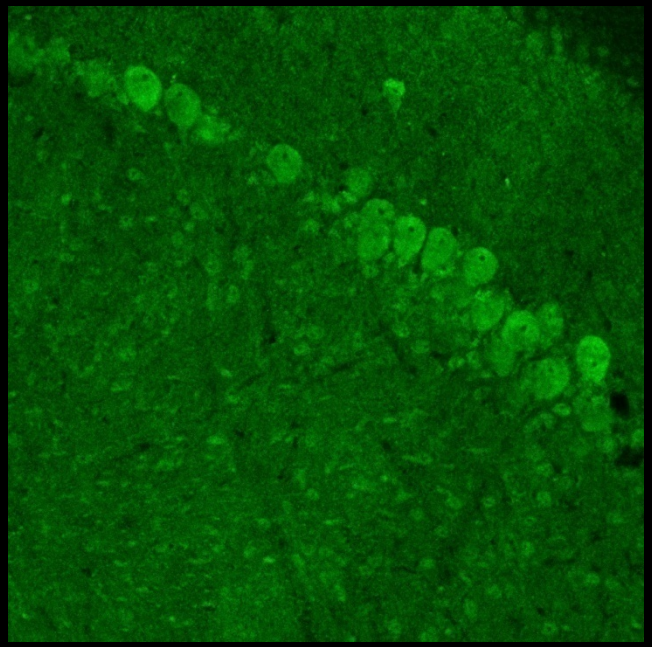
Cy2 1:200

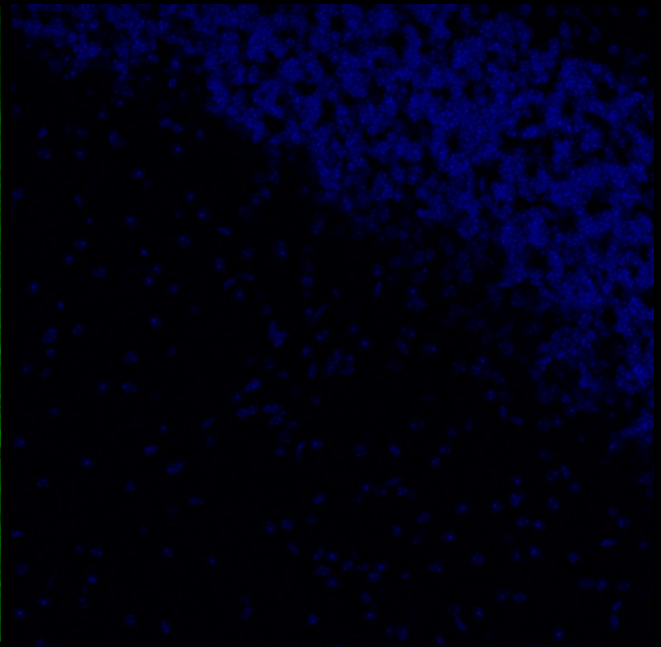
Final bleed SCA2 280 on B6 wt



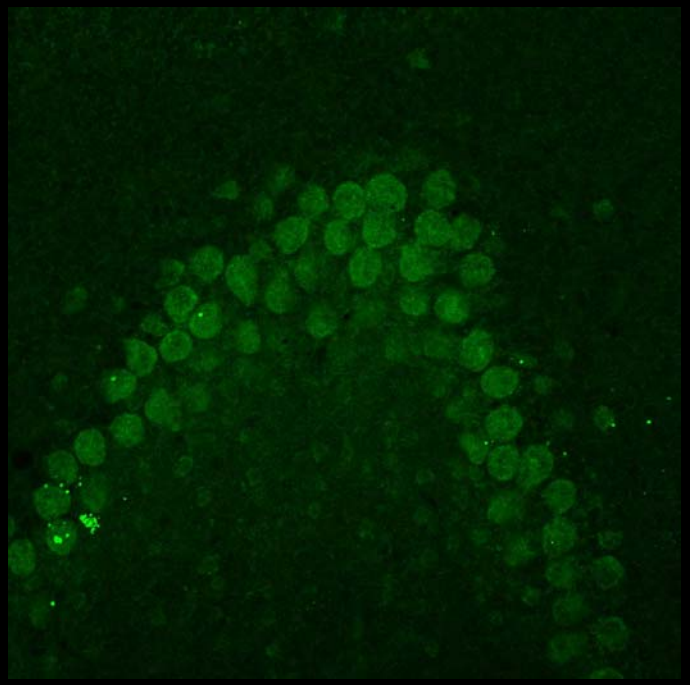


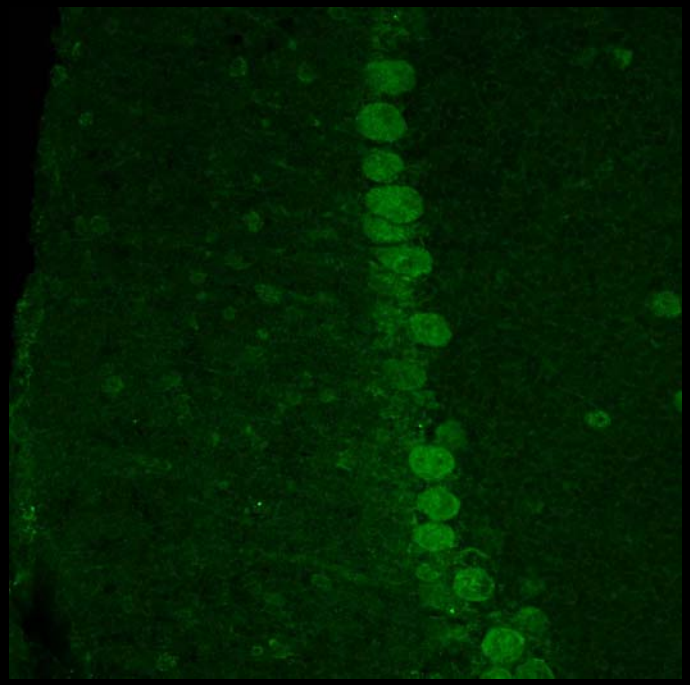
SCA2 280 final bleed		
1:900		
Dy Light	488 rb	1:1700
Dapi		1:10.000





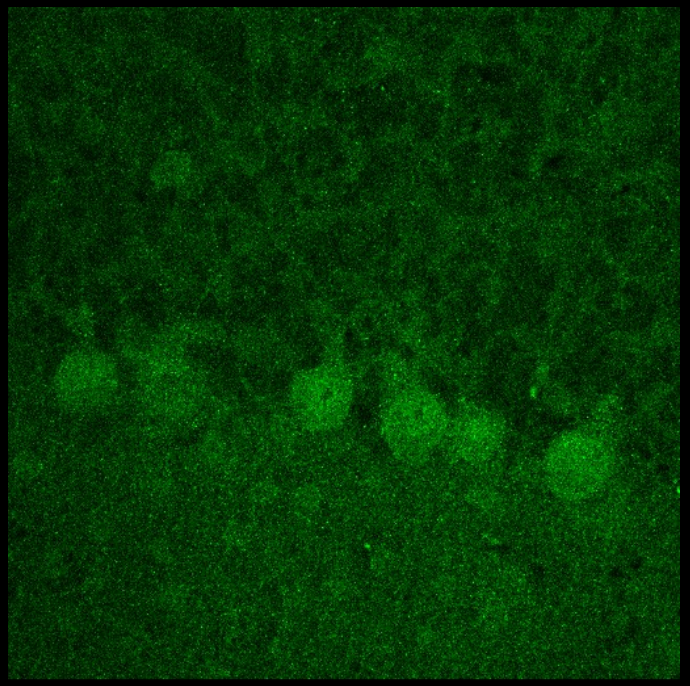
SCA2 714
8µg/ml
on B6 wt

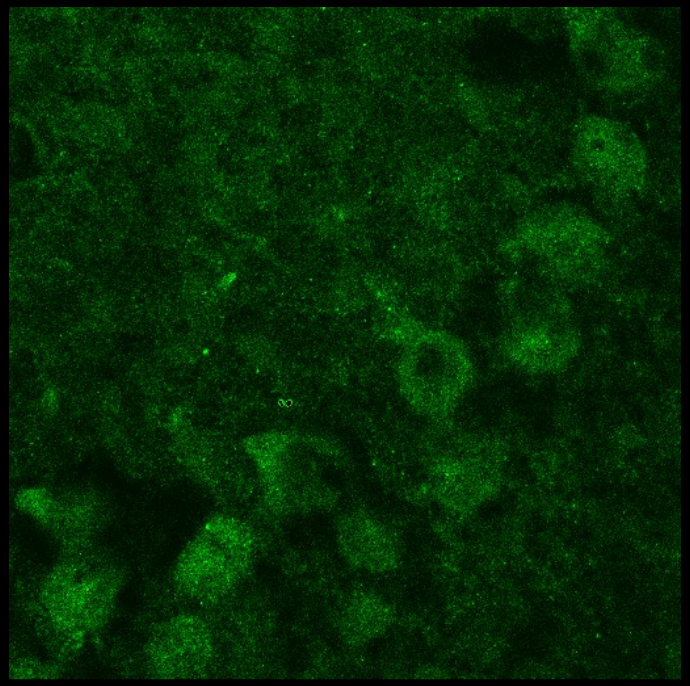




SCA2 714
8µg/ml
on B6 wt

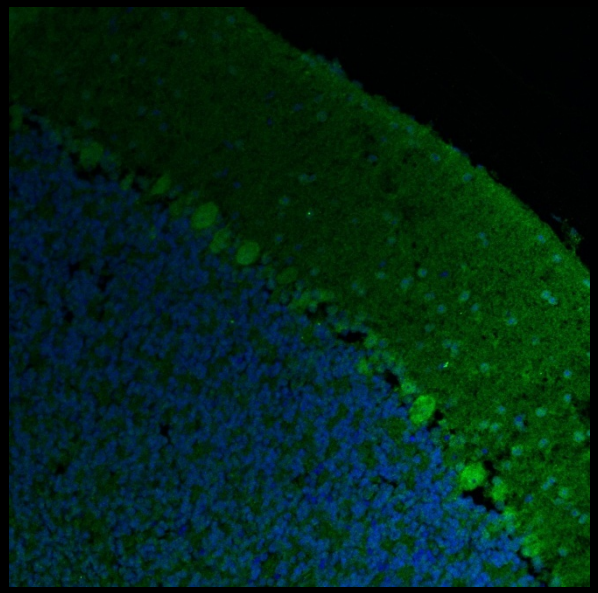
Final bleed
SCA2 714
on B6 wt
1: 1000

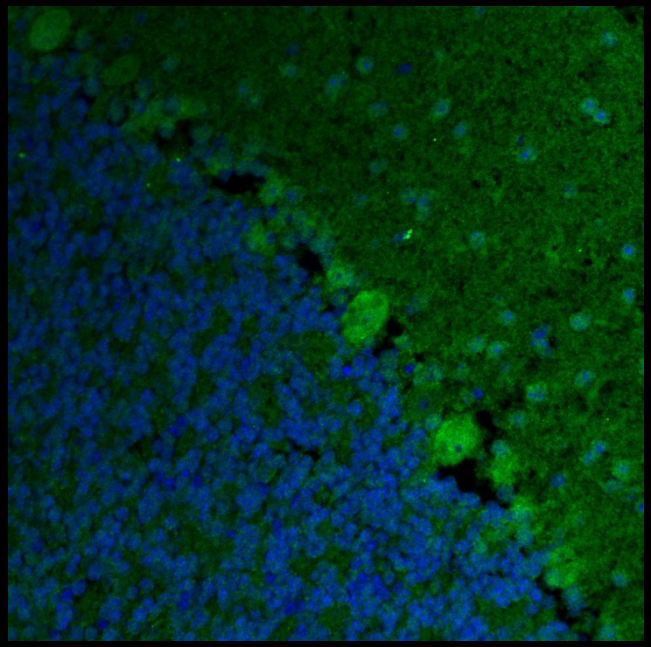


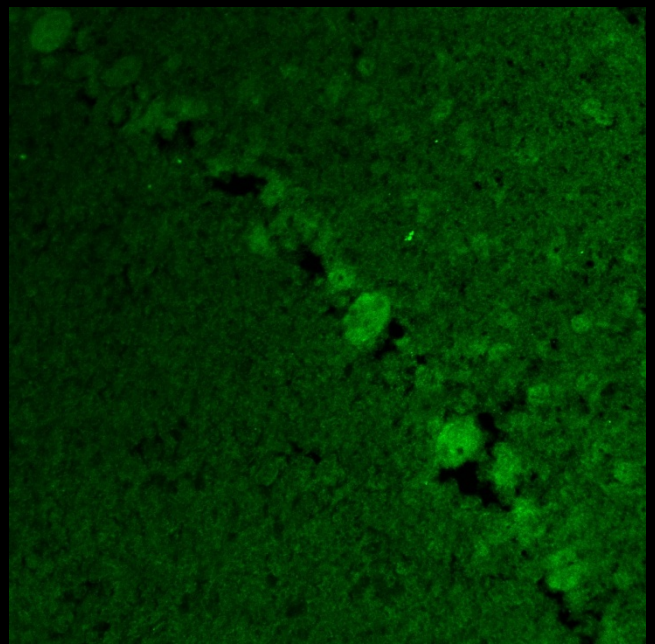


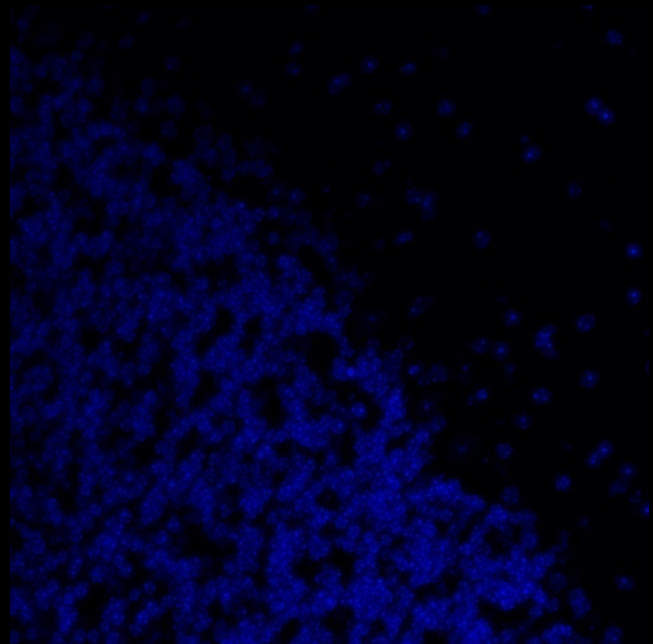
Final bleed
SCA2 714
on B6 wt
1: 1000

SCA2 714 on SCA2 kout (-/-)





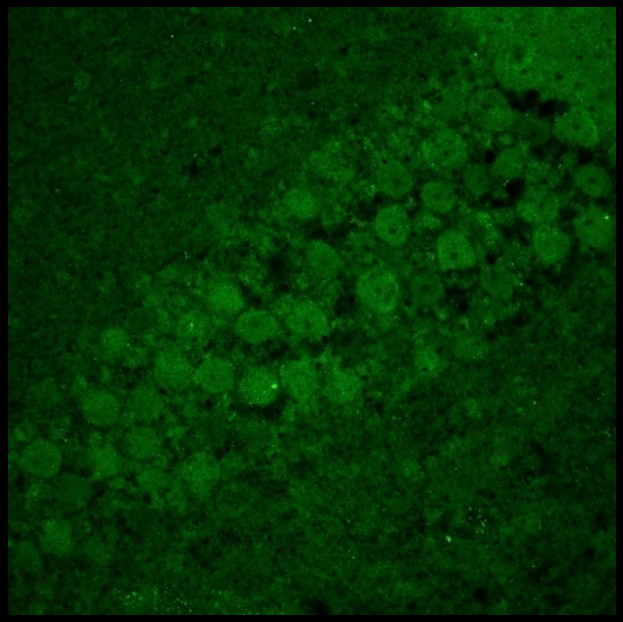


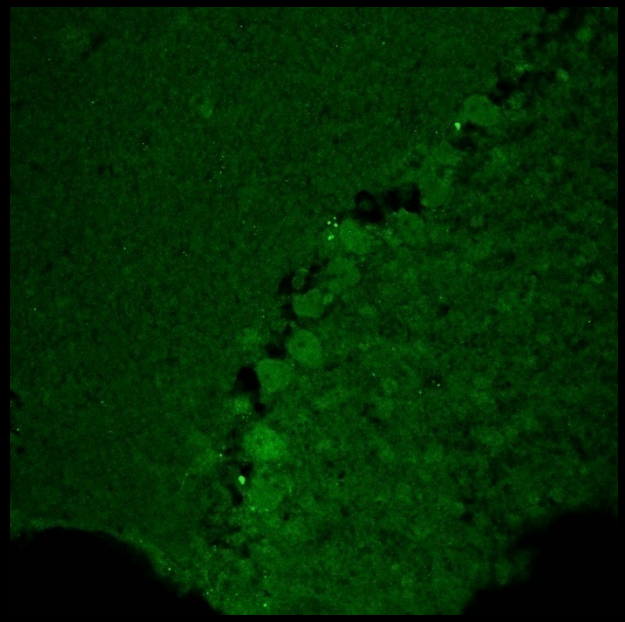


SCA2 714	8µg/ml
Dy Light 488 rb	1:1700
Dapi	1:10.000

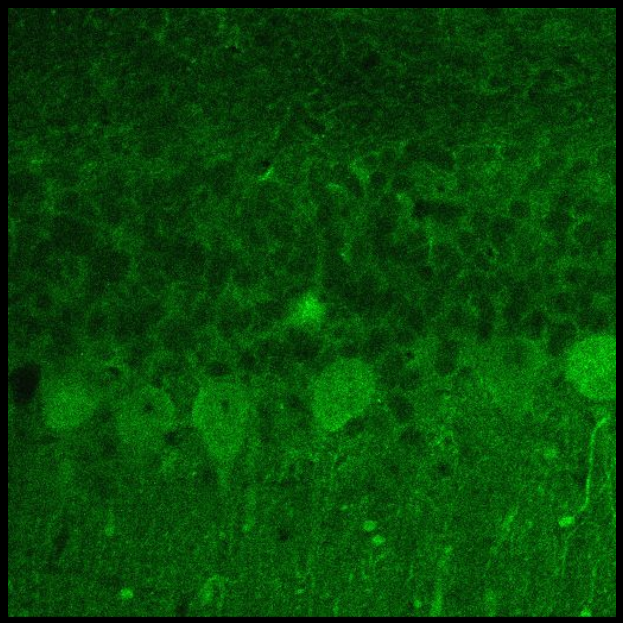
B6 wt

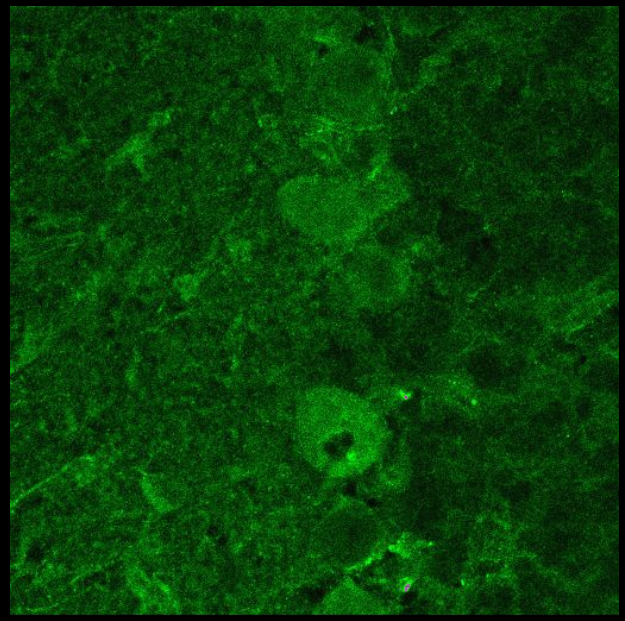
SCA2 790
10µg/ul





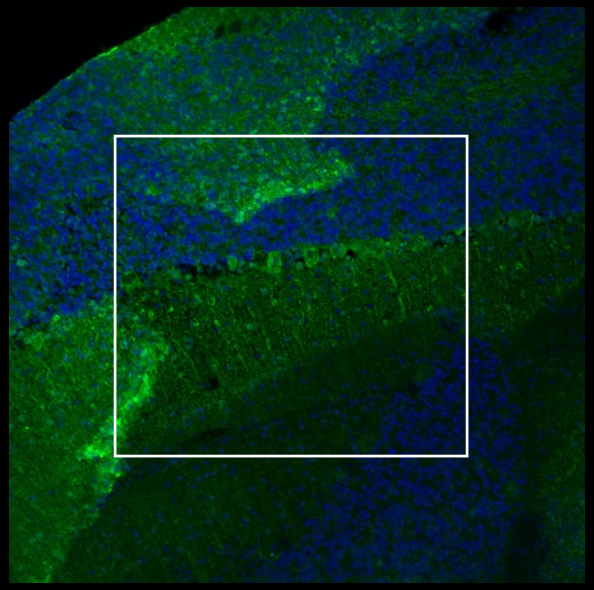
Final bleed
SCA2 790
1: 350

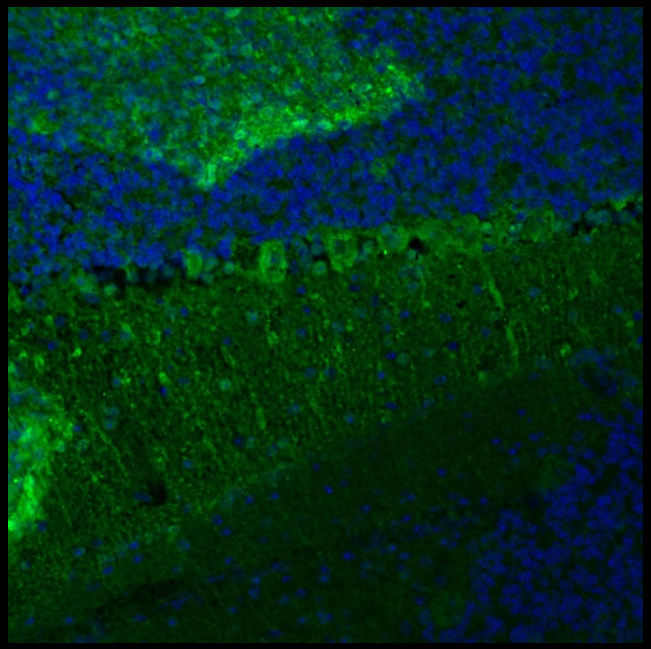




Final bleed
SCA2 790
1: 1000

Final bleed SCA2 790 on SCA2 kout (-/-)



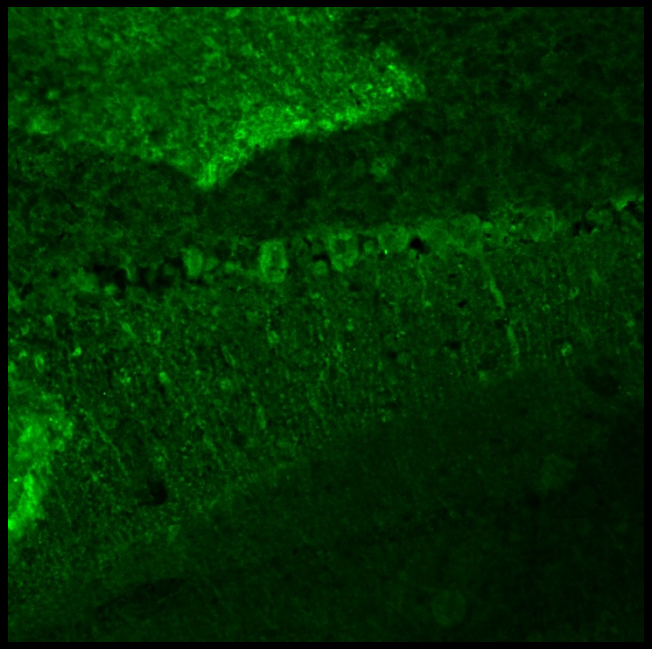


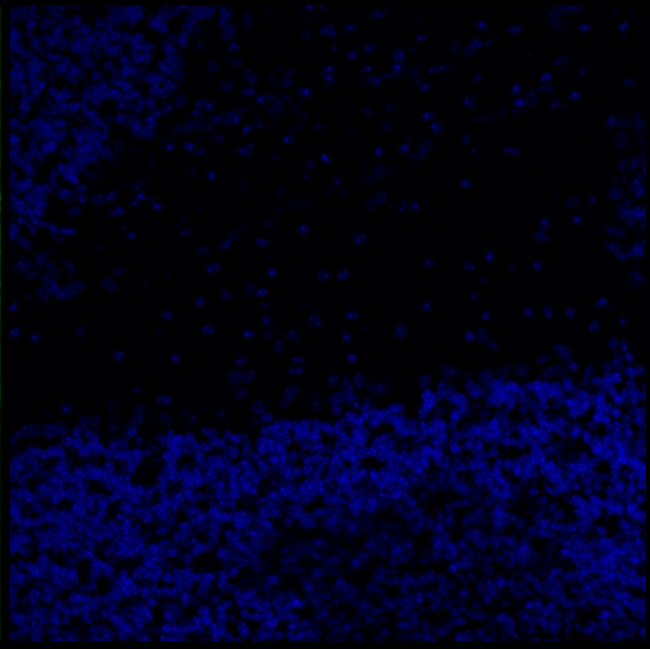
Final bleed SCA2 790

1:500

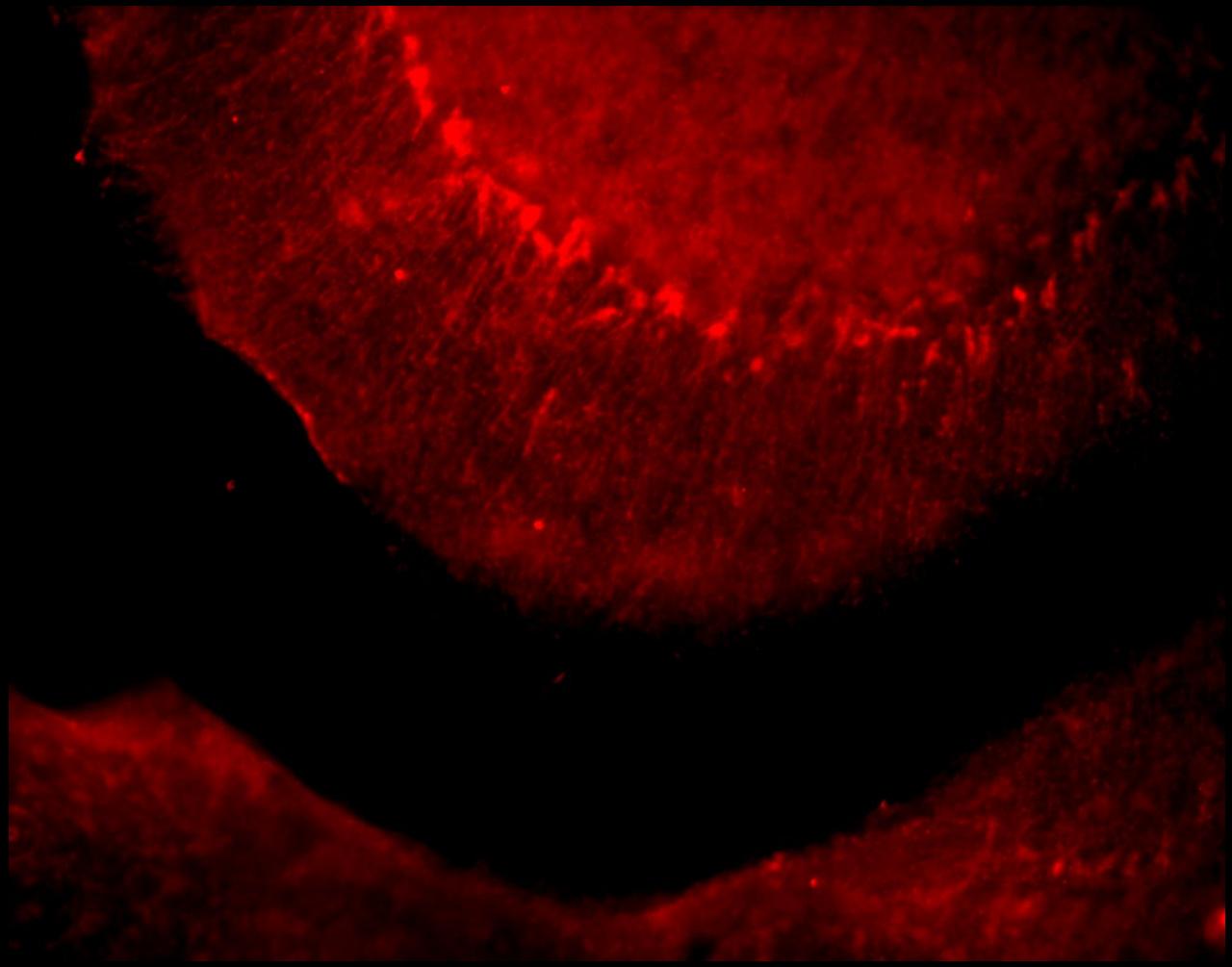
Dye light 488 rb 1:1700

Dapi 1:10.000



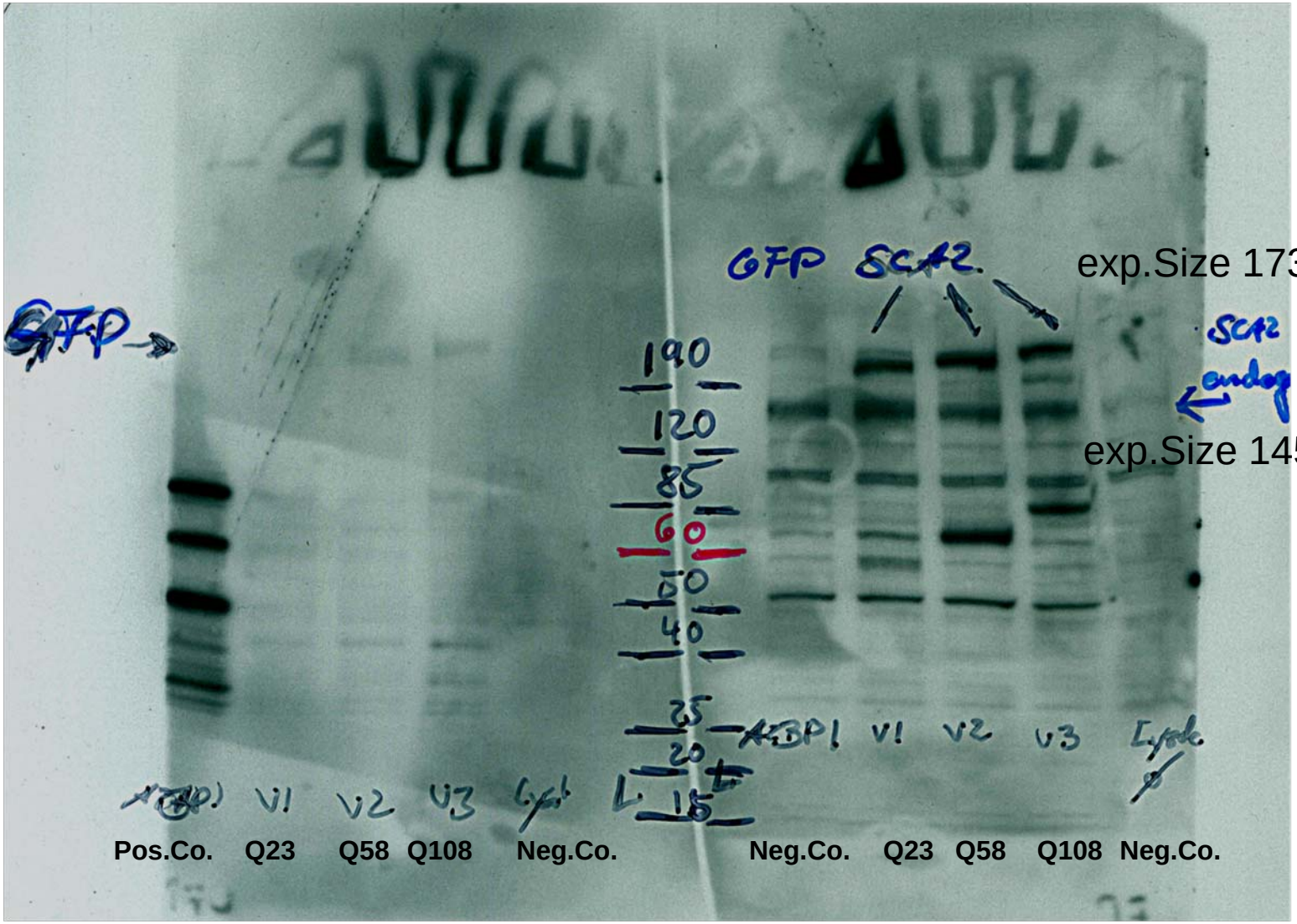


SCA2-D on 5B (Q58) wt



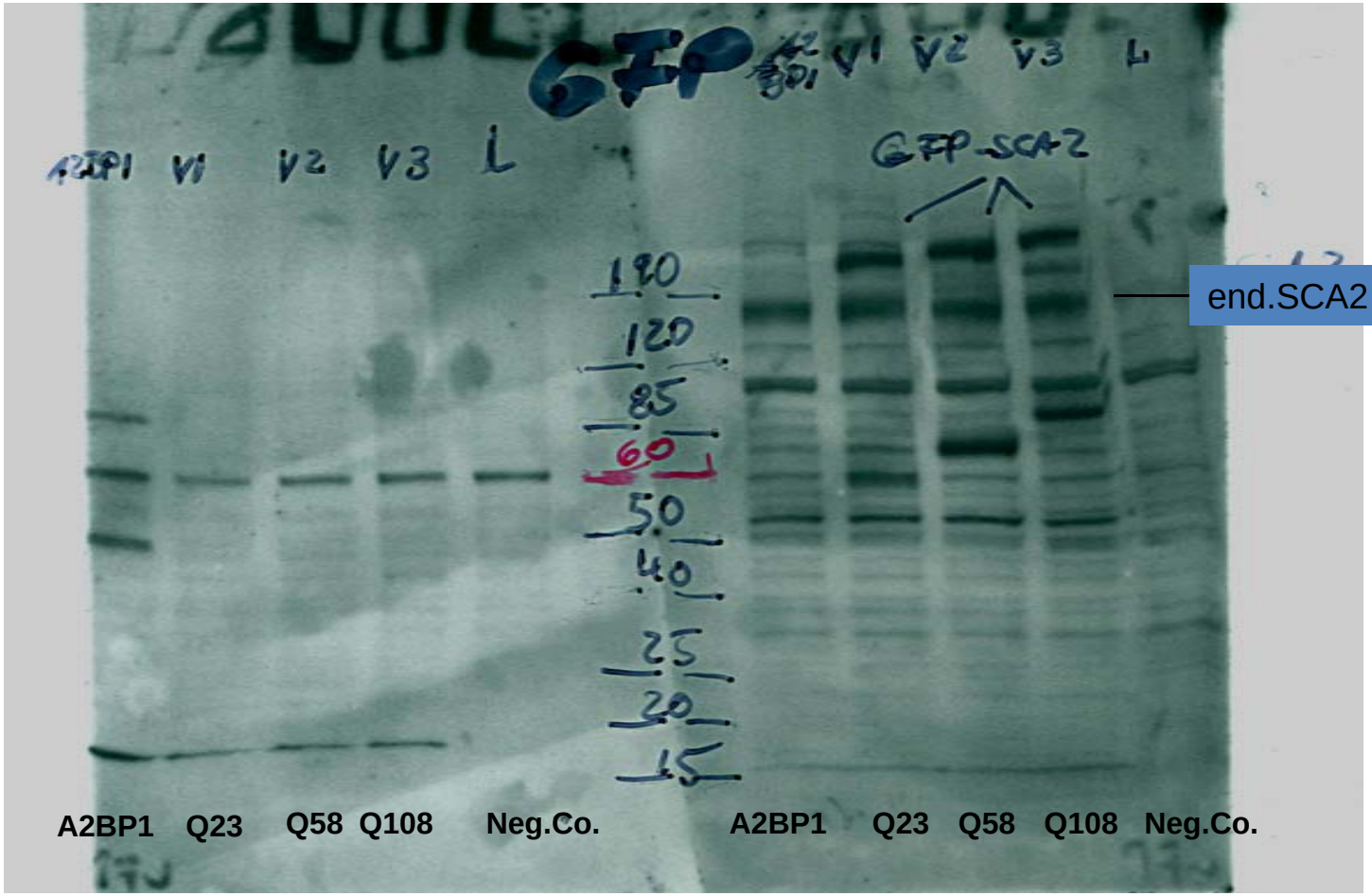
SCA2-D	10µg/ml
Cy3	1:500

SCA2-GFP- Lysates (HEK 293 cells)



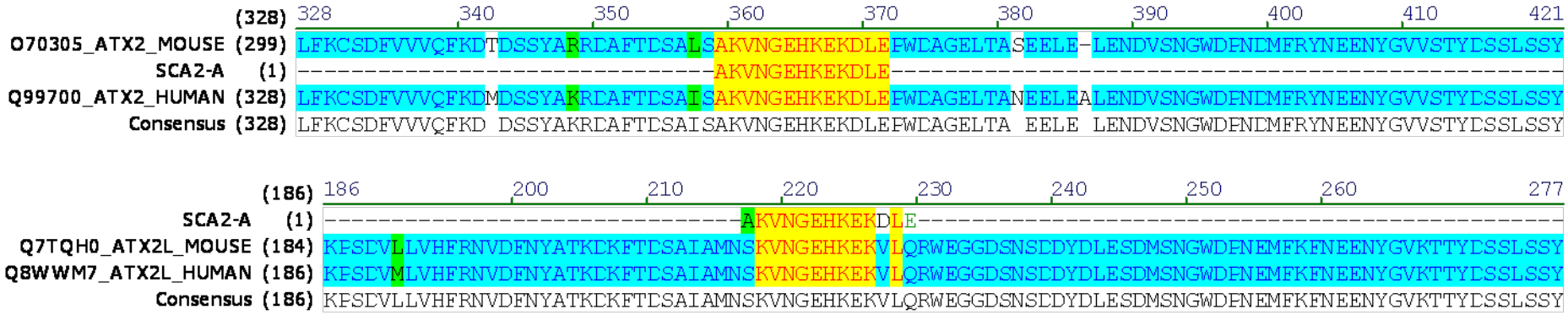
a-GFP **a-SCA2-D**

SCA2-GFP- lysates (HEK 293 cells)

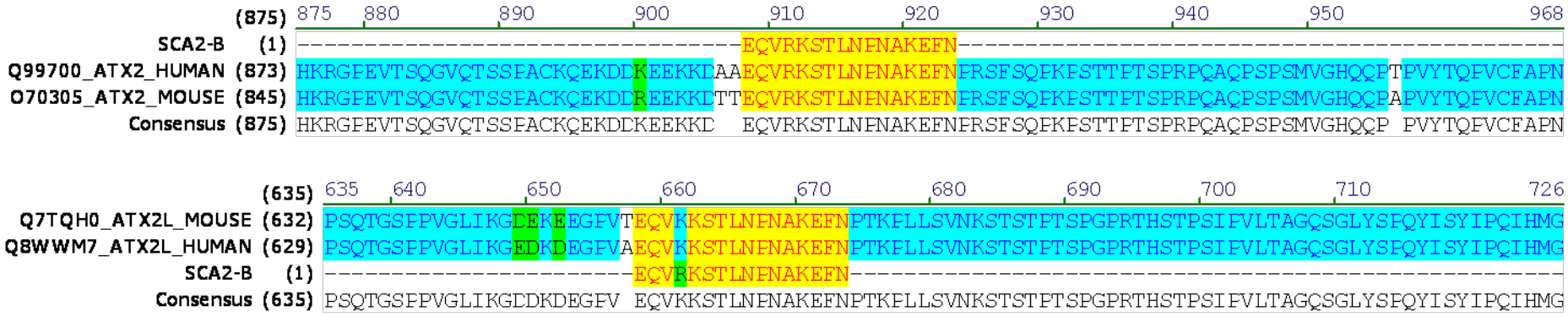


a-SCA2-C **a-SCA2-D**

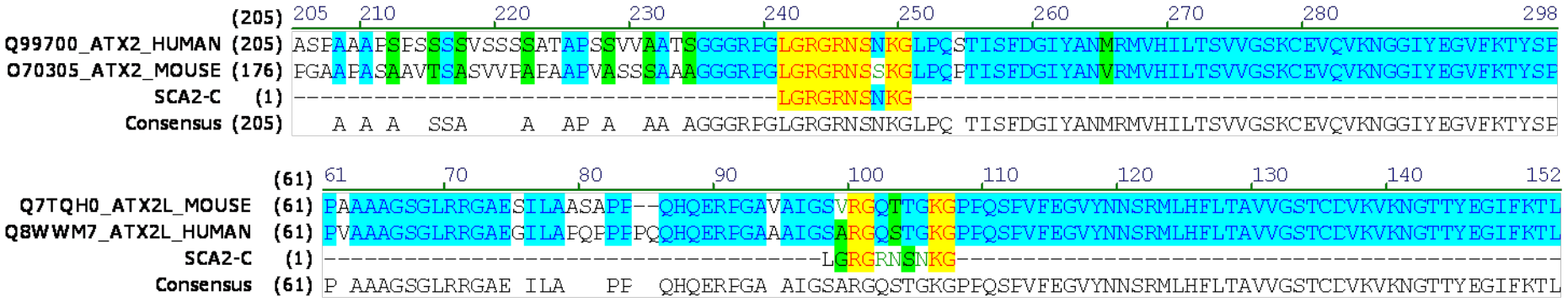
SCA2-A (359-371 human/ 330-342 mouse)



SCA2-B (904-921 human/ 878-893 mouse)



SCA2-C (241-250 human/ 212-221 mouse)



SCA2-D (867-879 human/ 839-851 mouse)