

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

	(271)	271	280	290	300	310	320	330	340	350	364
O70305_ATX2_MOUSE (239)		VHILTSVVGSKCEVQVKNGGIYEGVF	FKTYS	SPKCDLVLDAAHEKSTESSSGPKREEIMESVLFKC	SDFVWQFKITDSSYAKRDAFTDS	SAISAKV					
Q99700_ATX2_HUMAN (268)		VHILTSVVGSKCEVQVKNGGIYEGVF	FKTYS	SPKCDLVLDAAHEKSTESSSGPKREEIMESVLFKC	SDFVWQFKITDSSYAKRDAFTDS	SAISAKV					
GS 17 (1)		-----	-----	-----	EKSTESSSGPKREEC	-----	-----	-----	-----	-----	-----
Consensus (271)		VHILTSVVGSKCEVQVKNGGIYEGVF	FKTYS	SPKCDLVLDAAHEKSTESSSGPKREEIMESVLFKC	SDFVWQFKITDSSYAKRDAFTDS	SAISAKV					

	(126)	126	140	150	160	170	180	190	200	217
Q7TQH0_ATX2L_MOUSE (124)		FLTAVVGSTCDVKVKN	GTTYEGIFKTLSSK	FELAVLAVHRKASEPAGGPRREDIV	CTMVFKESVLLVHFRNV	CFNYATKDKFTDS	AIAMNS			
Q8WWM7_ATX2L_HUMAN (126)		FLTAVVGSTCDVKVKN	GTTYEGIFKTLSSK	FELAVLAVHRKASEPAGGPRREDIV	CTMVFKESVLLVHFRNV	CFNYATKDKFTDS	AIAMNS			
GS 17 (1)		-----	-----	-----	EKSTESSSGPKREEC	-----	-----	-----	-----	-----
Consensus (126)		FLTAVVGSTCDVKVKN	GTTYEGIFKTLSSK	FELAVLAVHRKASEPAGGPRREDIV	CTMVFKESVLLVHFRNV	CFNYATKDKFTDS	AIAMNS			

mouse 714-730 /human 748-764

SCA2 714

	(720)	720	730	740	750	760	770	780	790	800	813
O70305_ATX2_MOUSE (687)		EAVSMFVPAASPTPAS	FASNRALTPTSEAKDSRLQDQRQNSPAGSKENIKASE	TSPFSKALNKGISFVVSEHRKQIDDLKKFKNDFRLQPSST							
Q99700_ATX2_HUMAN (717)		EAVAMPIPAASPTPAS	FASNRALTPTSEAKDSRLQDQRQNSPAGNKENIKPNETSPFSKALNKGISFVVSEHRKQIDDLKKFKNDFRLQPSST								
PT 743 (1)		-----	-----	CEAKDSRLQDQRQNSPAG	-----	-----	-----	-----	-----	-----	-----
Consensus (720)		EAVAMPIPAASPTPAS	FASNRALTPTSEAKDSRLQDQRQNSPAG	NKENIK	ETSPFSKALNKGISFVVSEHRKQIDDLKKFKNDFRLQPSST						

	(274)	274	280	290	300	310	320	330	340	350	365
Q7TQH0_ATX2L_MOUSE (272)		LSSYTVPLEKCNSEEF	RQRELRAAQLAREIES	SPQYRLRIAMENDDGRTEEEKHSAVCRQGS	GRESPSLV	SREGKYIFLFQRVREGPRGGVR					
Q8WWM7_ATX2L_HUMAN (274)		LSSYTVPLEKCNSEEF	RQRELRAAQLAREIES	SPQYRLRIAMENDDGRTEEEKHSAVCRQGS	GRESPSLV	SREGKYIFLFQRVREGPRGGVR					
PT 743 (1)		-----	-----	-----	CEAKDSRLQDQRQNSPAG	-----	-----	-----	-----	-----	-----
Consensus (274)		LSSYTVPLEKCNSEEF	RQRELRAAQLAREIES	SPQYRLRIAMENDDGRTEEEKHSAVCRQGS	GRESPSLV	SREGKYIFLFQRVREGPRGGVR					

SCA2 790

(592) 592 600 610 620 630 640 650 660 670 683
 Q7TQH0_ATX2L_MOUSE (589) LLTSDPMGSPVSSKTESI LDKEDKVPMA GVG GTEGPEQLP APCPSQTGSPFVGLIKGDEKEEGFVTEQVKKSTLNENAKEFNPTKFLLSVNVK
 Q8WWM7_ATX2L_HUMAN (586) LLTSEPMGSPVSSKTESI VSDKECKFPLA FSG GTEGPEQLP FPCPSQTGSPFVGLIKGDEKDEGEFVAEQVKKSTLNENAKEFNPTKFLLSVNVK
 PI 820 (1) -----CKNREGEKSRDLTKDK-----
 Consensus (592) LLTSDPMGSPVSSKTESI DKECK PLA GTEGPEQ P PCPSQTGSPFVGLIKGDKDEGEV ECVKKSTLNENAKEFNPTKFLLSVNVK

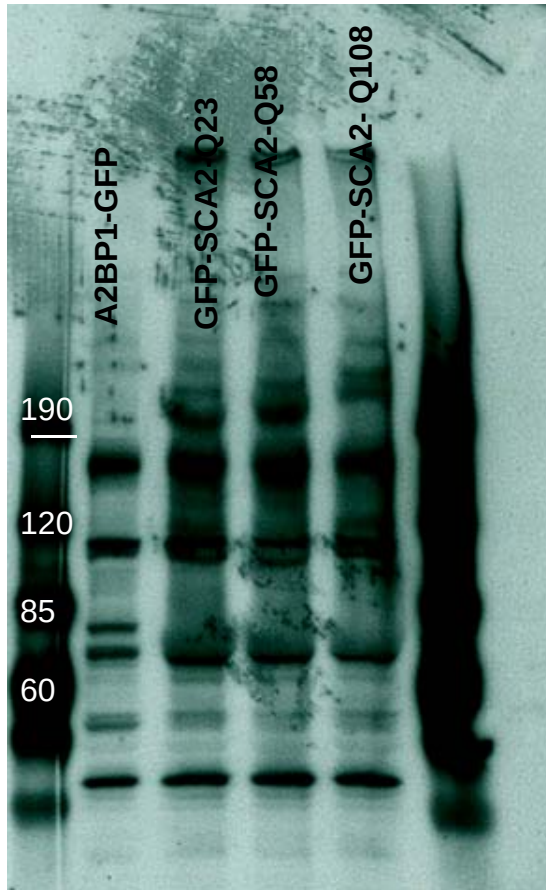
SCA2 1080

	(747)	747	760	770	780	790	800	810	820	838
Q7TQH0_ATX2L_MOUSE	(744)	QGKYRGAKGSLPEQRSDQHQFA	SAPFMMQAAAAAG	EPLVAATPYSSYI	YPYN	PCQF	PGQFAMMC	PMAHY	FSQPVFAPMLC	SNPRMLTSGSHE
Q8WWM7_ATX2L_HUMAN	(741)	QGKYRGAKGSLPEQRSDQHQFA	SAPFMMQAAAAAG	EPLVAATPYSSYI	YPYN	PCQF	PGQFAMMC	PMAHY	FSQPVFAPMLC	SNPRMLTSGSHE
GS 27	(1)	-----CFKIYPYNKETSSSF-----								
Consensus	(747)	OGKYRGAKGSLPEQRSDQHQFA	SAPFMMQAAAAAA	EPLVAATPYSSYI	YPYN	PCQF	PGQFAMMC	PMAHY	FSQPVFAPMLC	SNPRMLTSGSHE

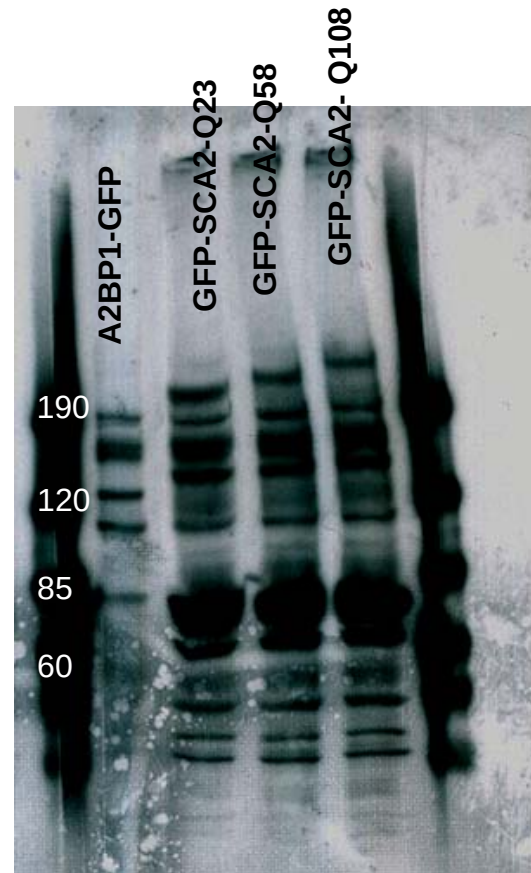
	Genscript	Genscript	Pacific Immunology	Proteintech Group	Proteintech Group
	SCA2 280	SCA2 1080	SCA2 790	SCA2 714-1	SCA2 714-2
boosts	3	3	3	4	4
Elisa rabbit 1	A 1: 128.000	1: 512.000	2213 1: 200.000	1: 10.000	
Elisa rabbit 2	B 1: 64.000	1: 512.000	2214 1: 500.000		1: 1,000.000
affinity purified	60 ml	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

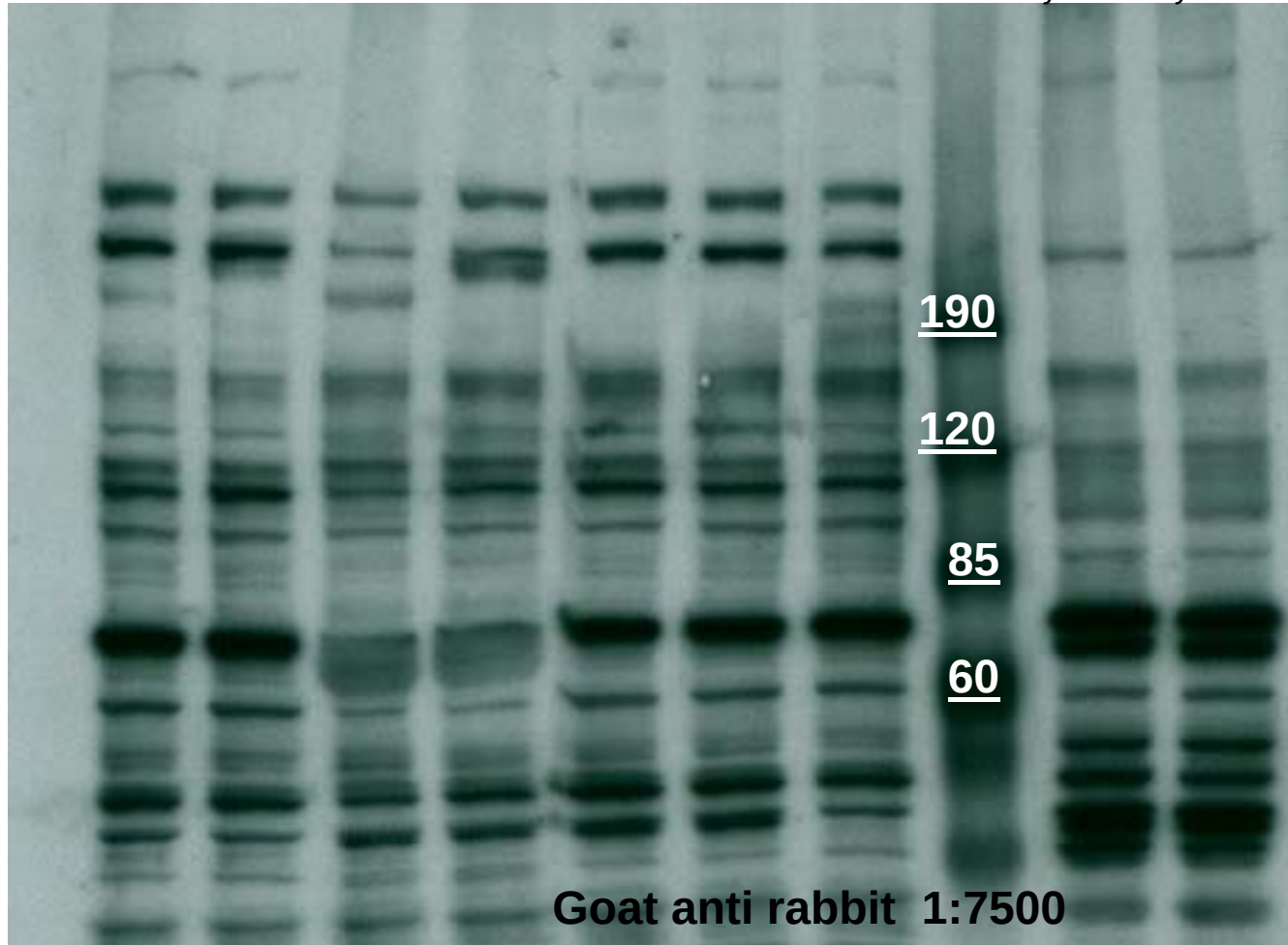


SCA2 714 rabbit 2 0.5 µg/ml

Sec.antib.: donkey anti rabbit 1:7500

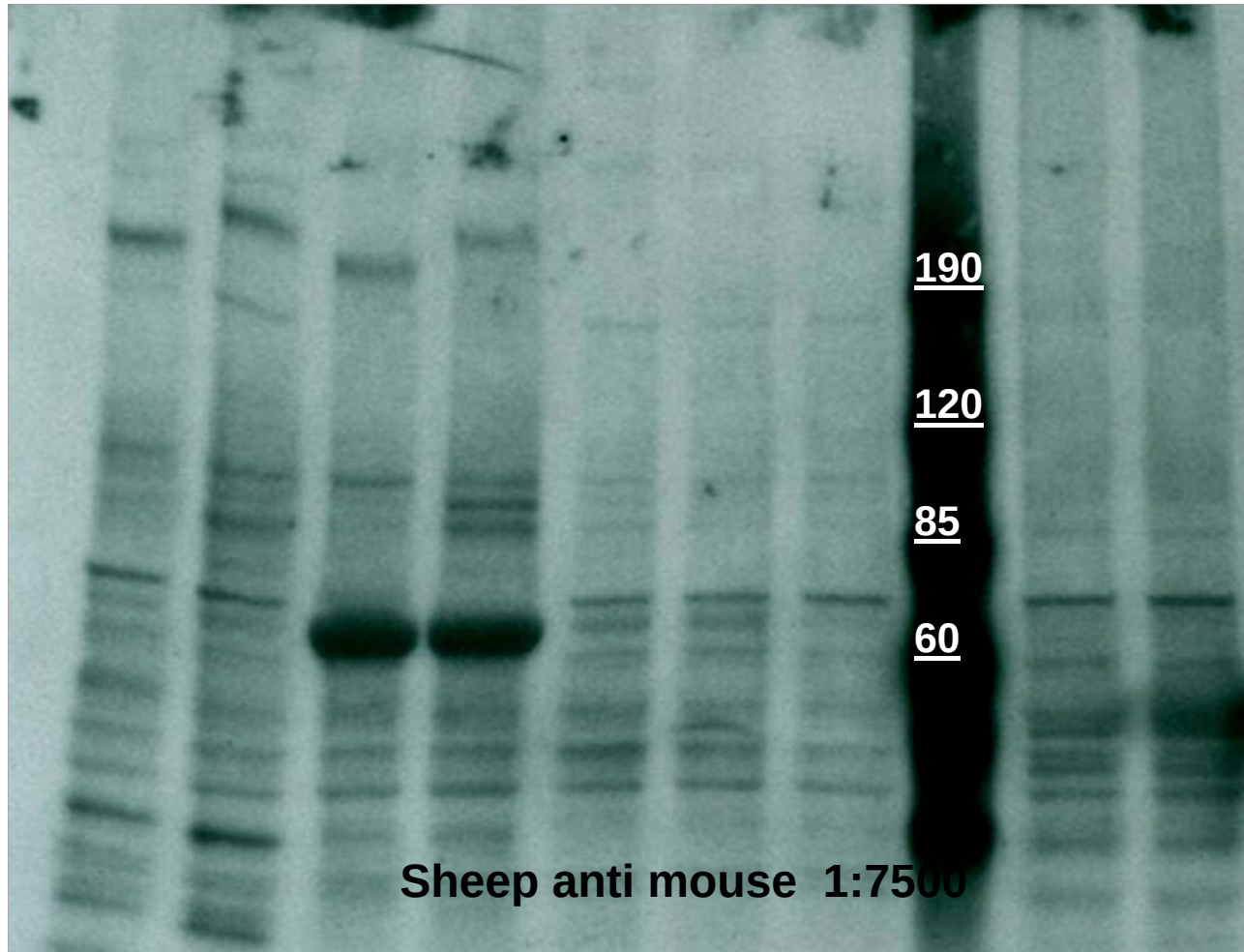
SCA 2 790 0.5 µg/ml HEK 293 cells

GFP-SCA2 Q23	GFP-SCA2 Q108	GFP-SCA2 Q23	GFP-SCA2 Q108	pCMV -HA control	pCMV-SCA2-HAQ23	pCMV-SCA2-HAQ108	Cereb. 5B lysate	Cereb. WT lysate
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anti-GFP (Chemicon) 1:750 (HEK 293 lysates)

GFP-SCA2 Q23	GFP-SCA2 Q108	GFP-SCA2 Q23	GFP-SCA2 Q108	pCMV -HA control	pCMV-SCA2-HAQ23	pCMV-SCA2-HAQ108	Cereb. 5B lysate	Cereb. WT lysate
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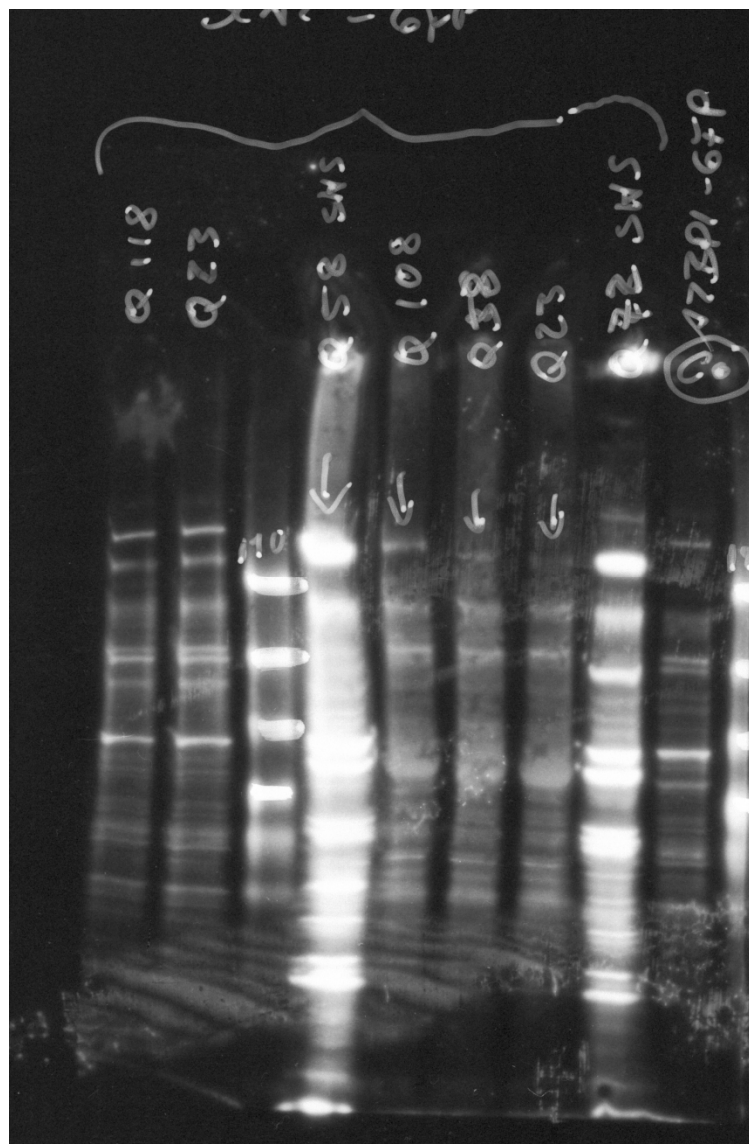


SCA2 790 0.5 ug/ml

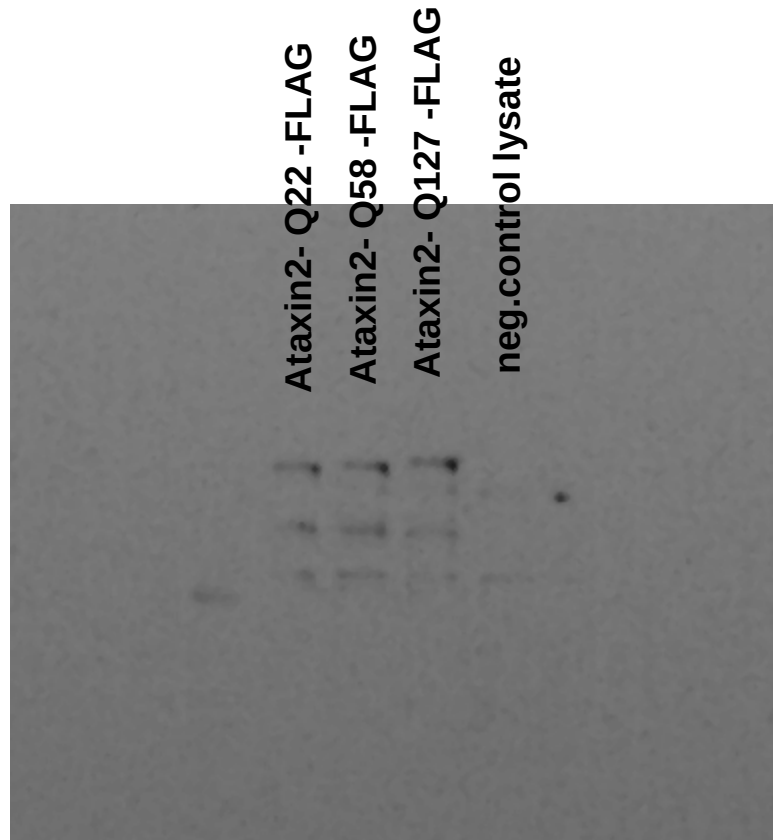
HEK 293 cells

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**

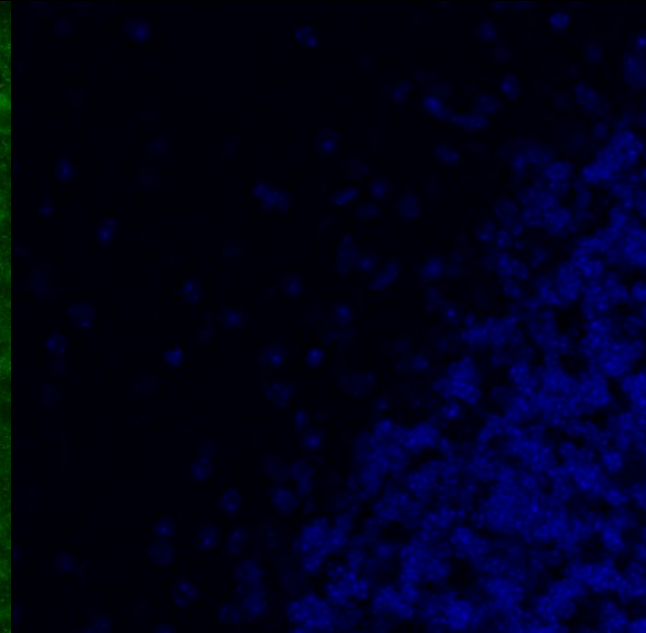
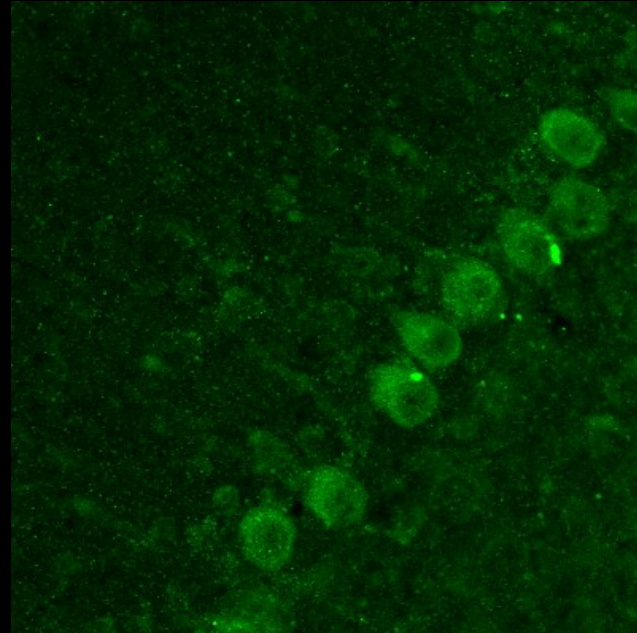
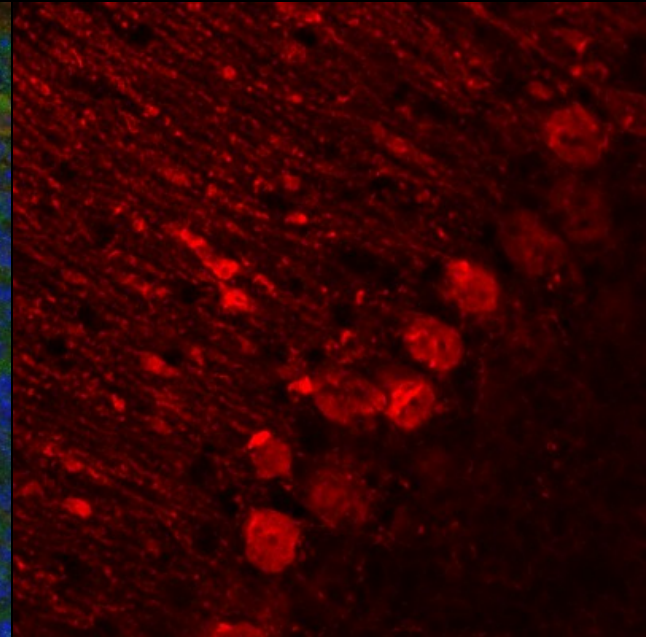
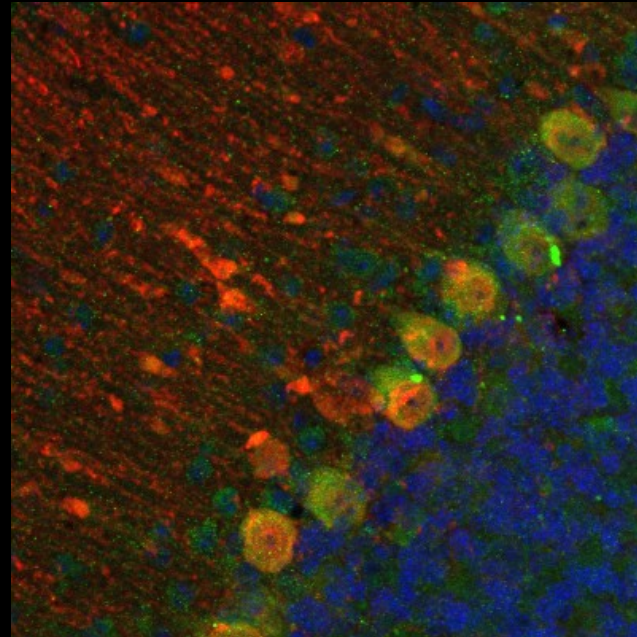
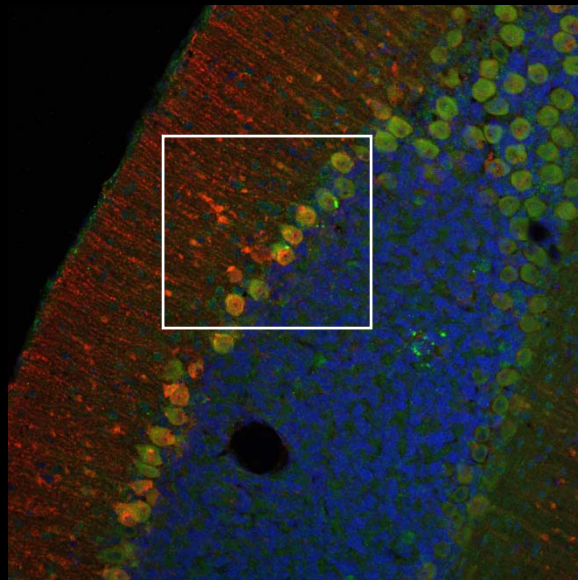
Primary-and secondary antib. Sol.: **Triton PBS containing 5% Normal Serum**

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.

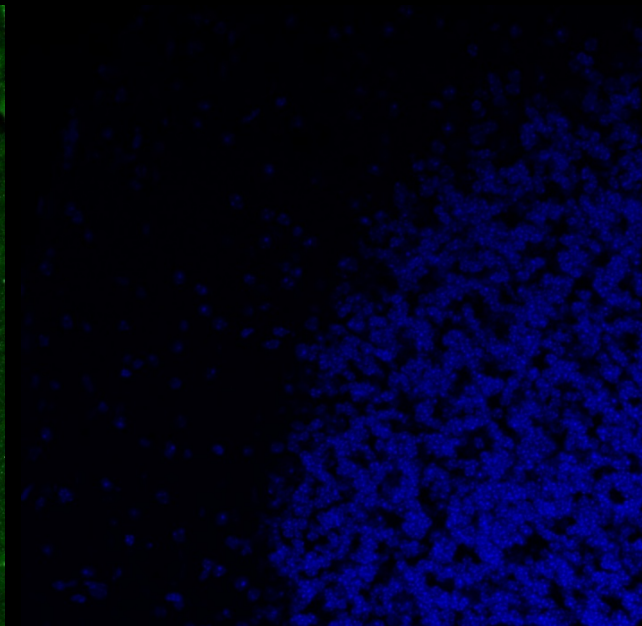
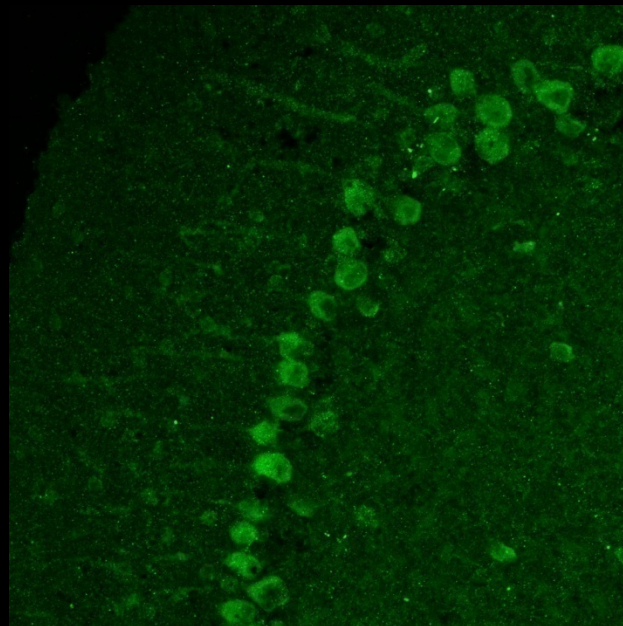
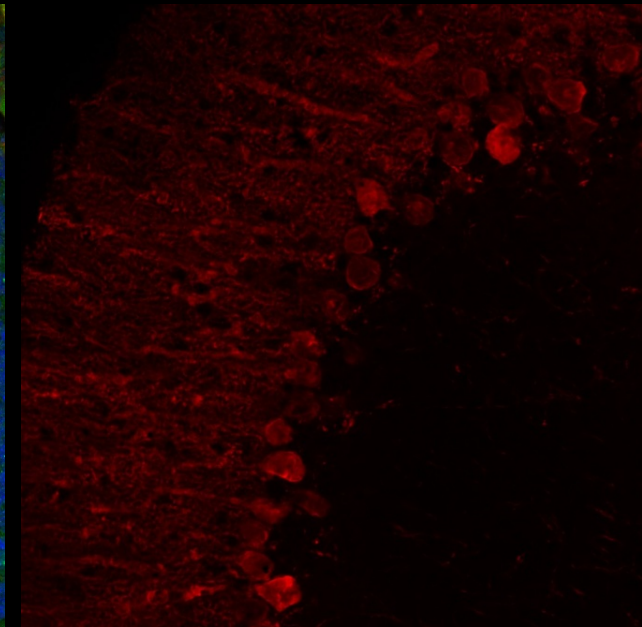
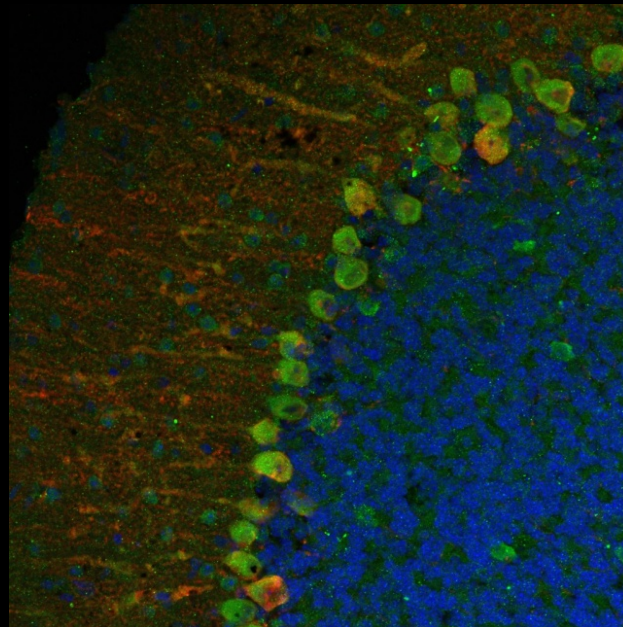
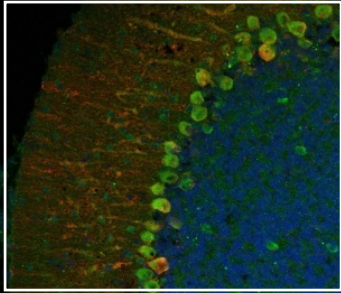
SCA2 280 on B6 wt



SCA2 280 6 μ g/ml
Calbindin (mse) 1:600

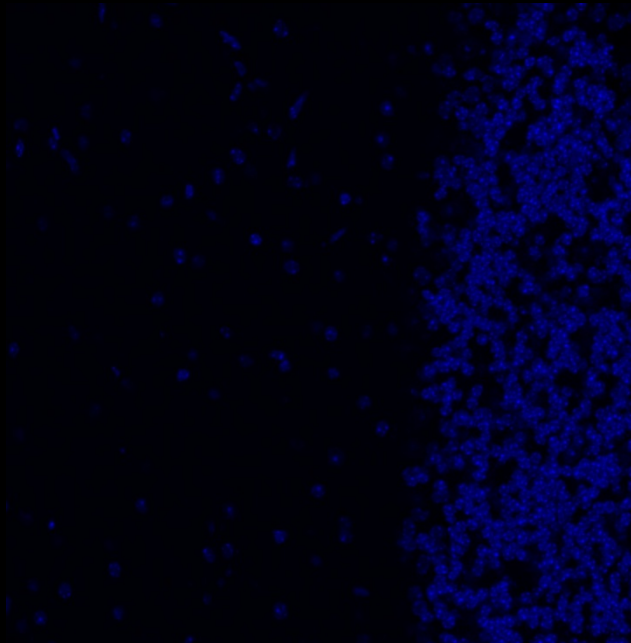
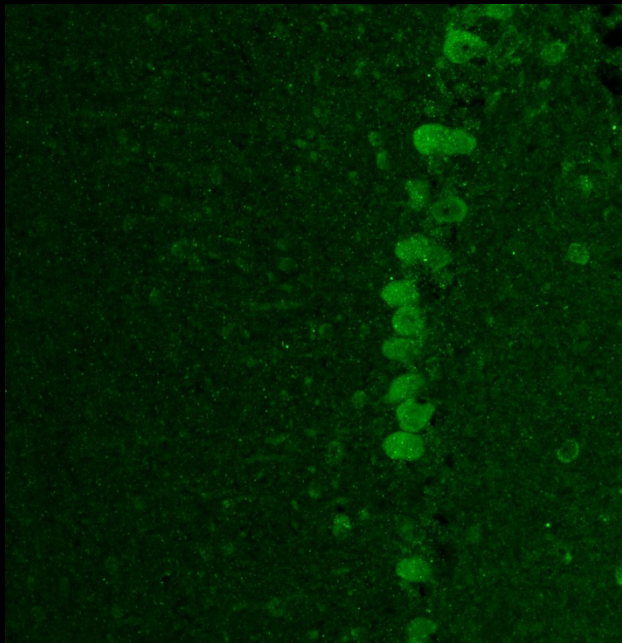
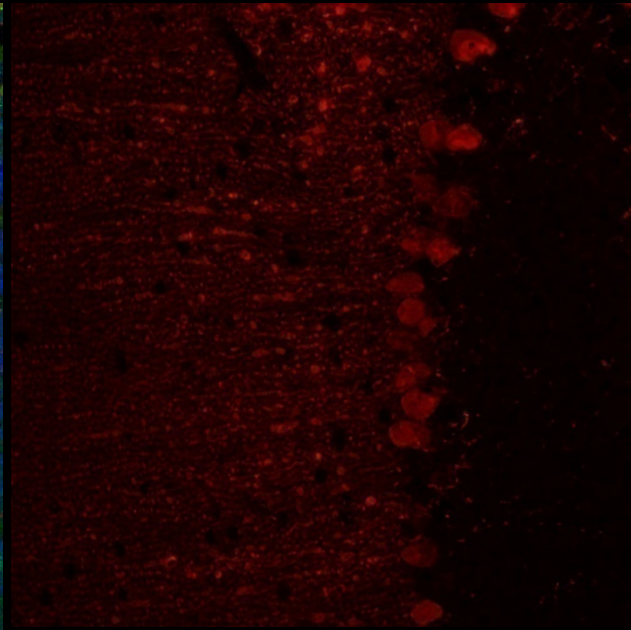
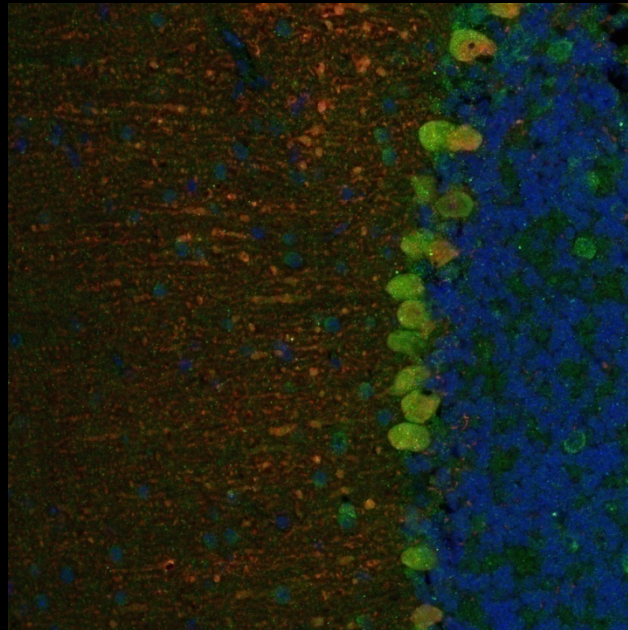
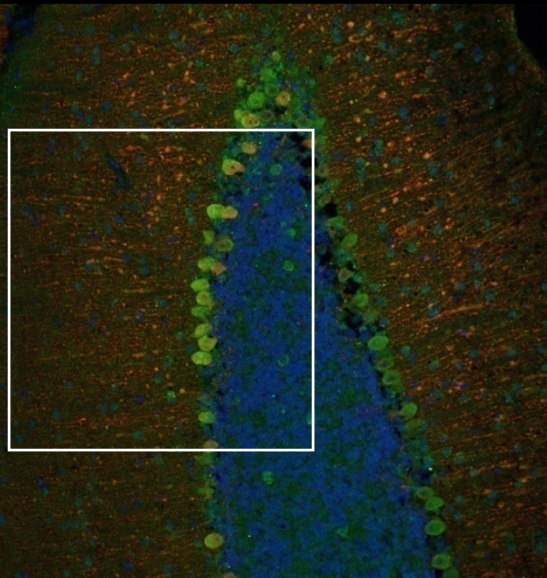
Cy 2 1:200
Cy 3 1:600
Dapi 1:7000

SCA2 280 on B6 wt



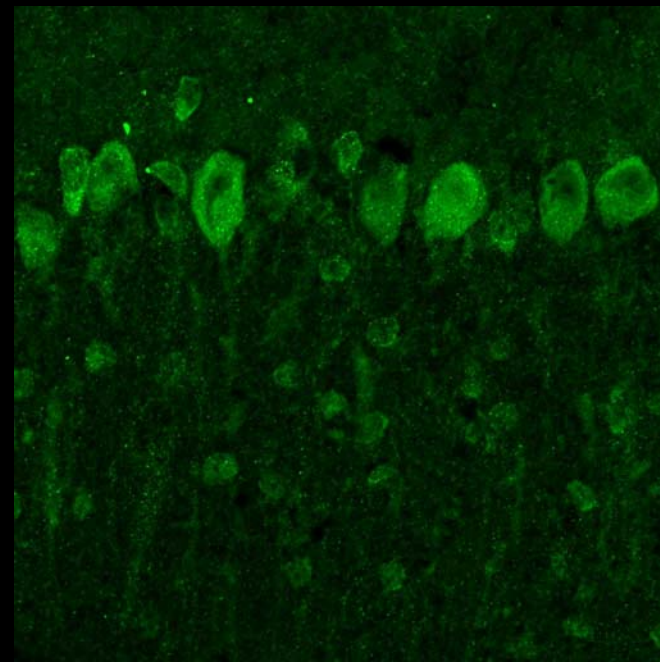
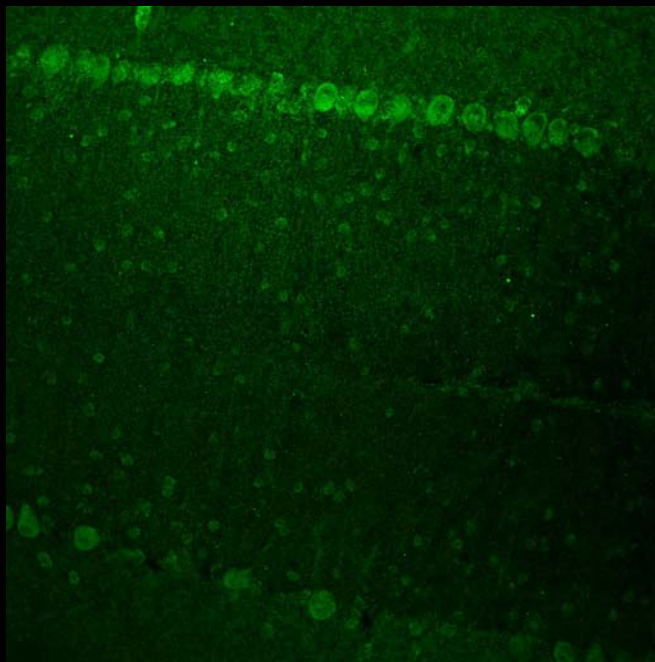
SCA2 280	6 μ g/ul
Calbindin (mse)	1:600
Cy 2	1:200
Cy 3	1:600
Dapi	1:7000

SCA2 280 on SCA2 knockout (-/-)

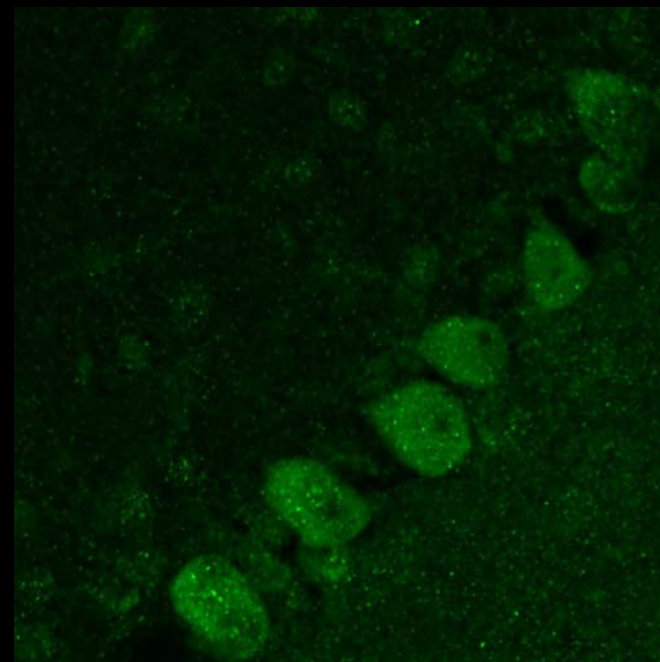
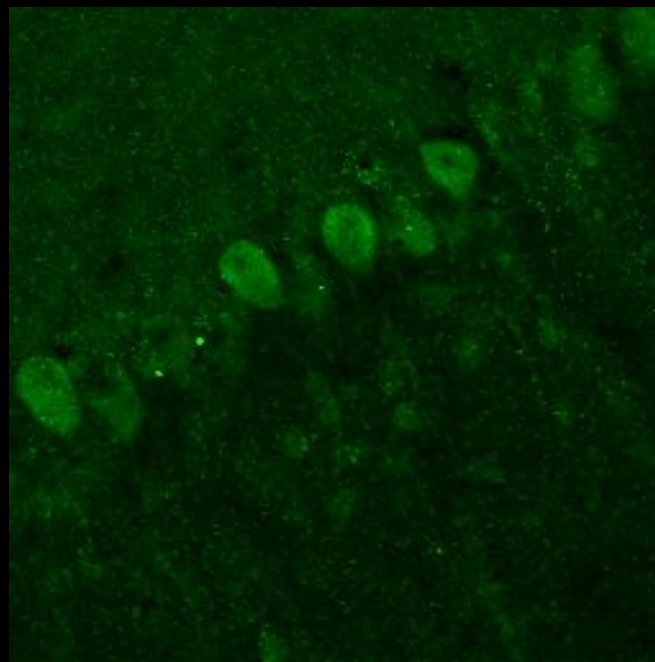


SCA2 280	6 μ g/ul
Calbindin (mse)	1:600
Cy 2	1:200
Cy 3	1:600
Dapi	1:7000

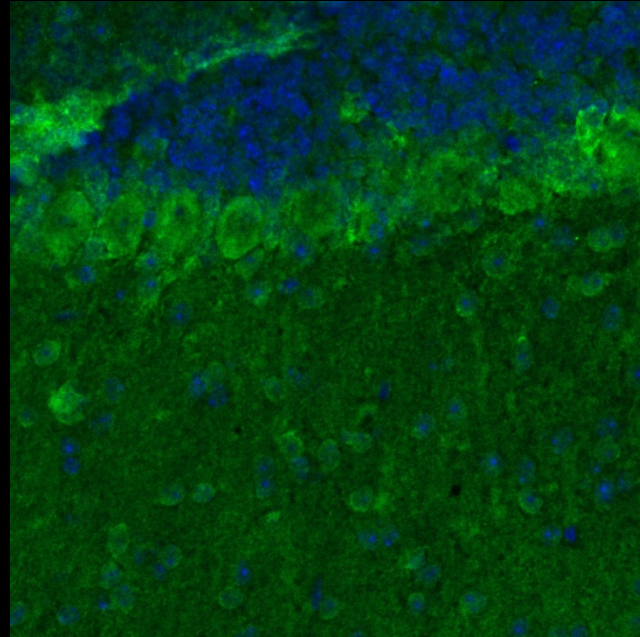
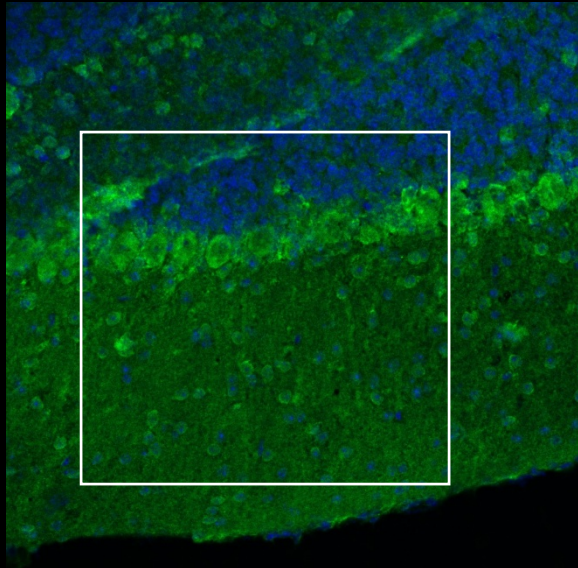
SCA2 280
4 μ g/ml
Cy2 1:200
on B6 wt



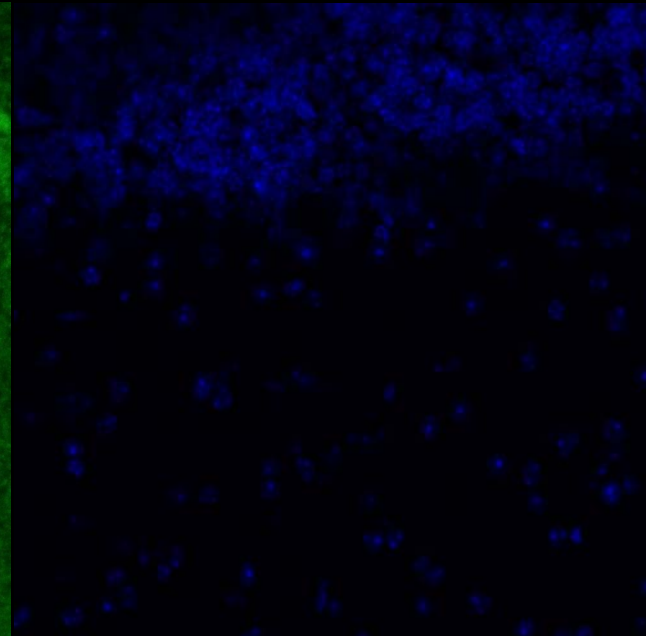
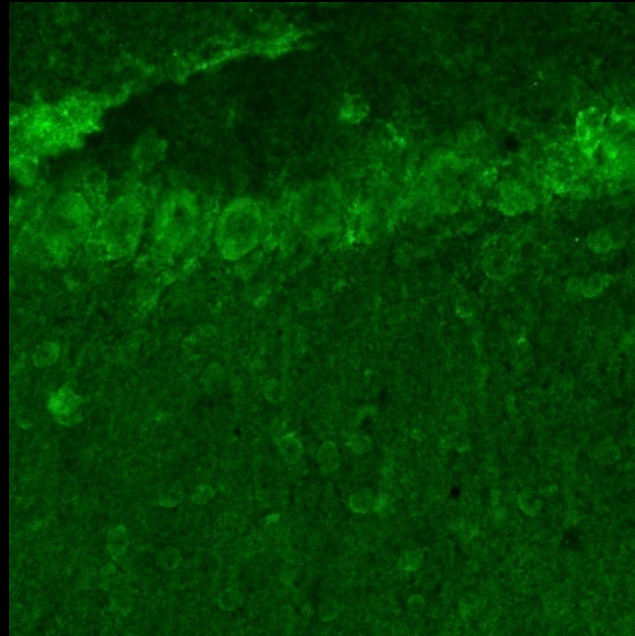
SCA2 280 4 μ g/ml
Cy2 1:200
on
SCA2 knockout (-/-)



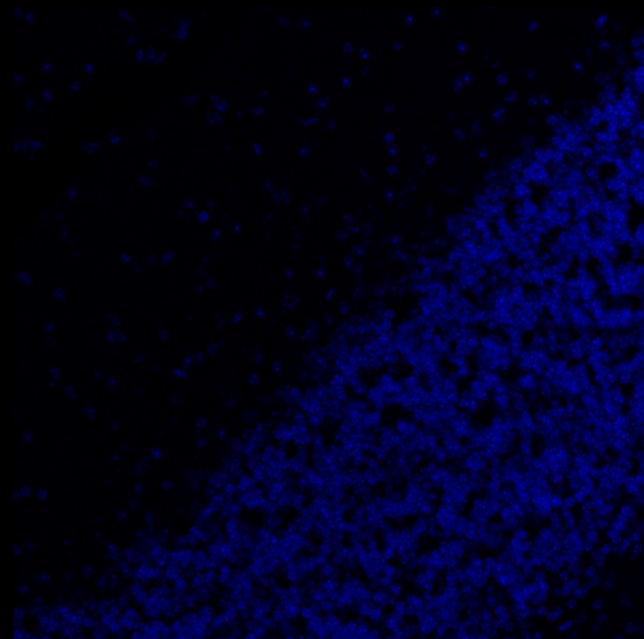
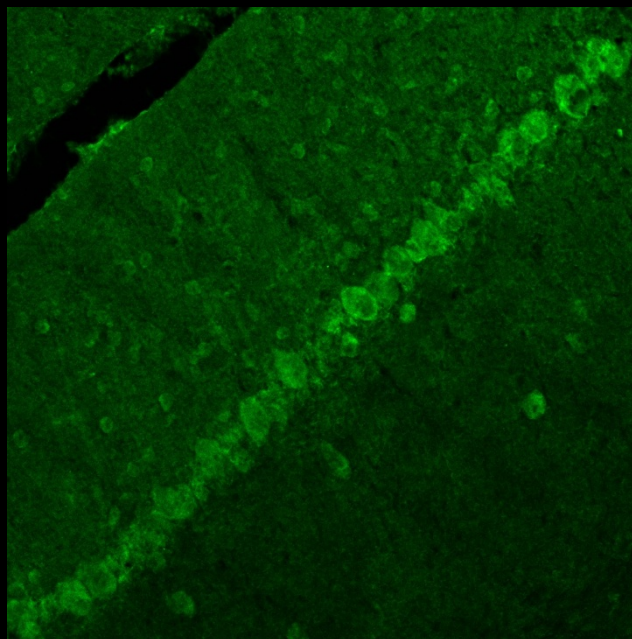
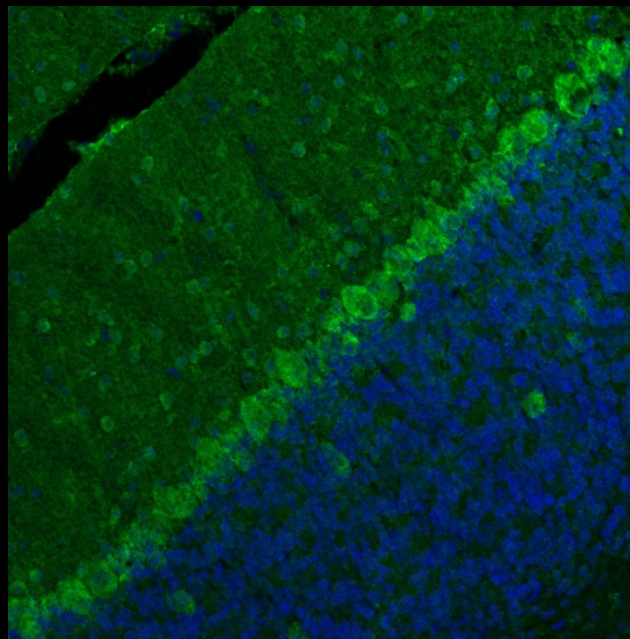
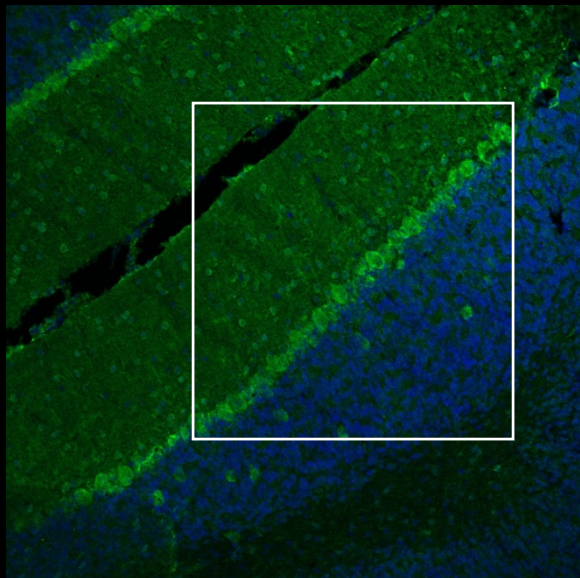
SCA2 280 on SCA2 kout (-/-)



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



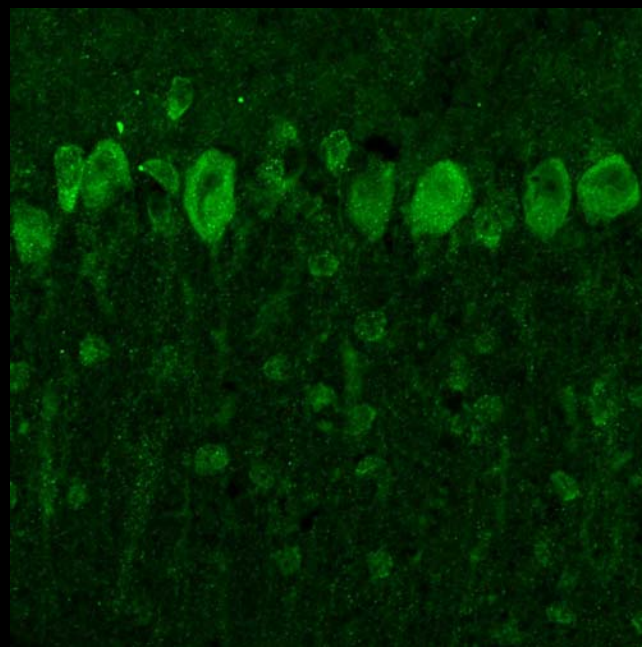
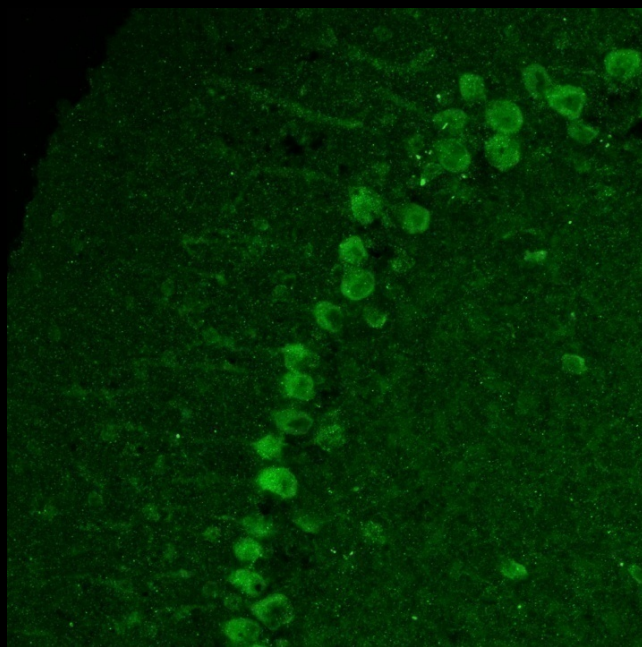
SCA2 280 on SCA2 kout (-/-)



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

B6 WT

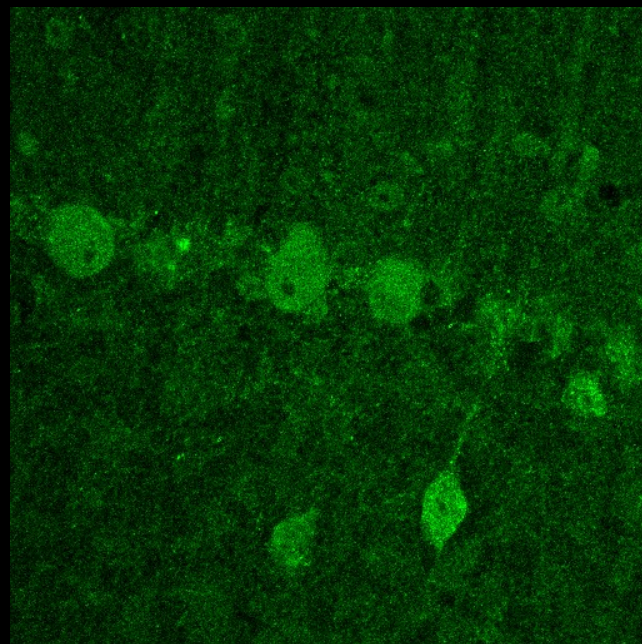
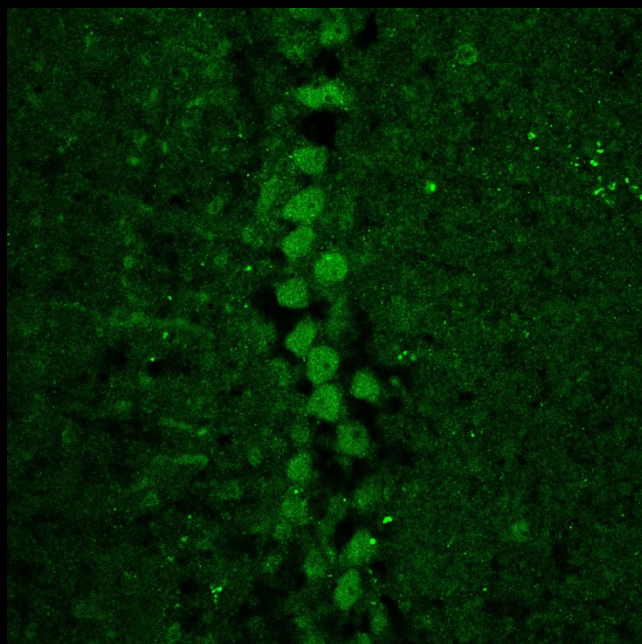
SCA2 280
4 μ g/ml
Cy2 1:200



SCA2 280
4 μ g/ml
Cy2 1:200

Final bleed
SCA2 280
1: 350

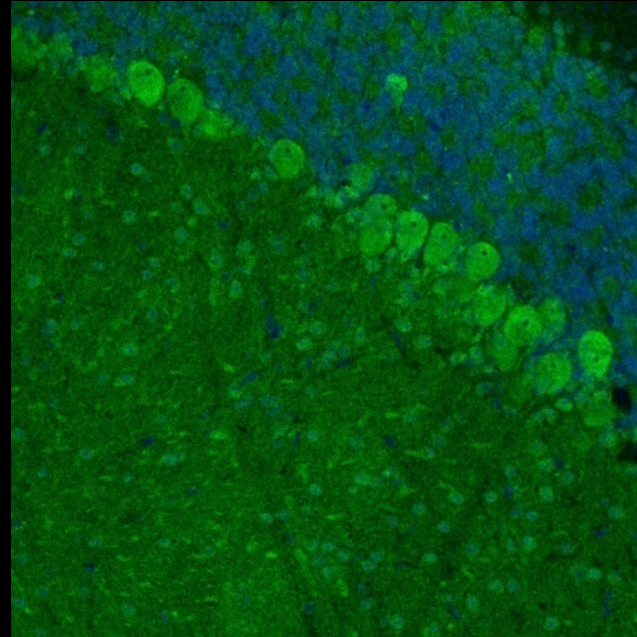
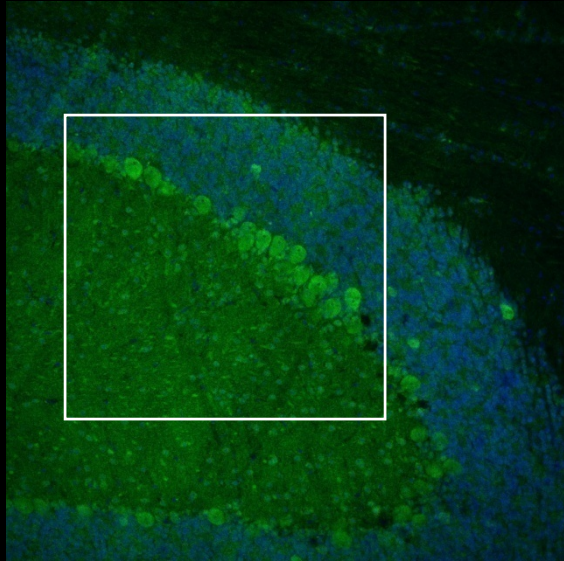
Cy2 1:200



Final bleed
SCA2 280
1: 1000

Cy2 1:200

Final bleed SCA2 280 on B6 wt

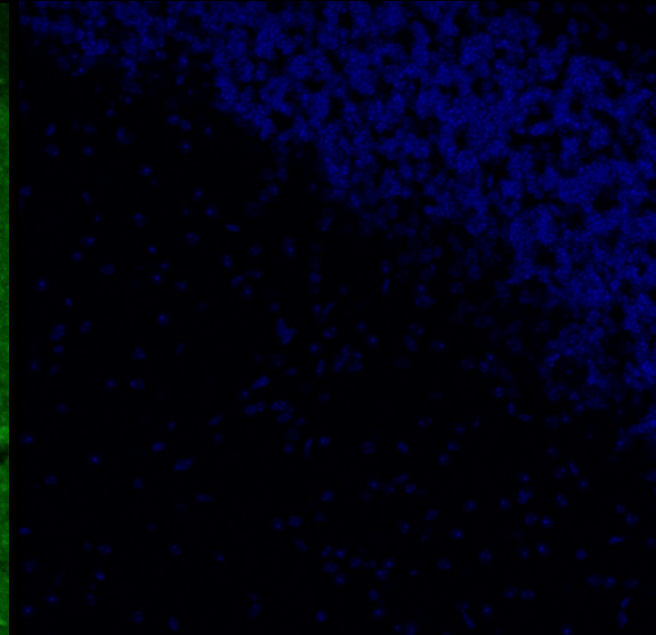
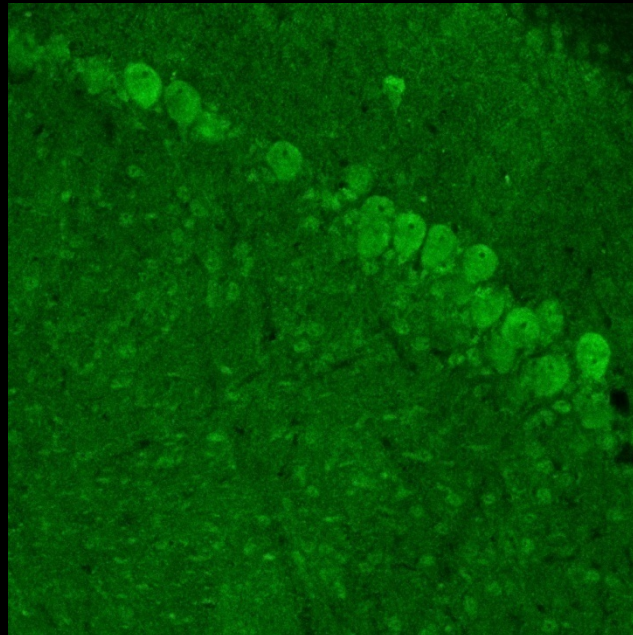


SCA2 280 final bleed

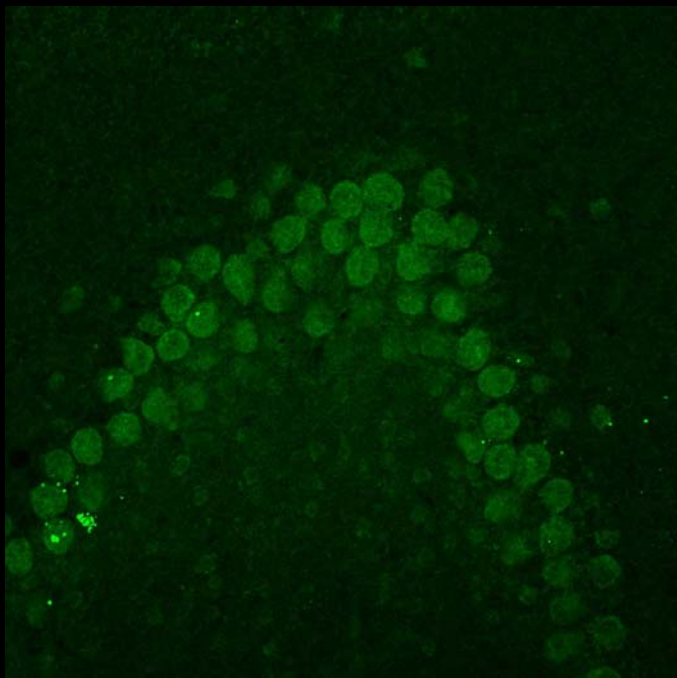
1:900

Dy Light 488 rb 1:1700

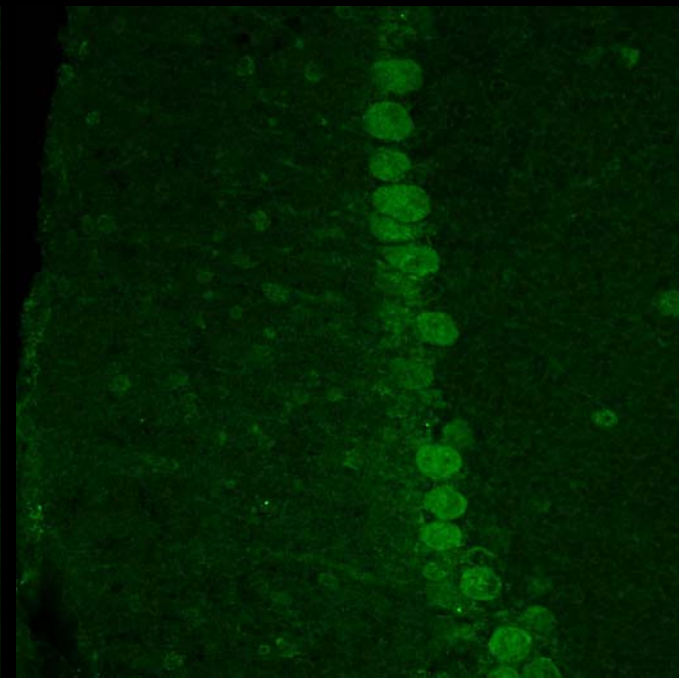
Dapi 1:10.000



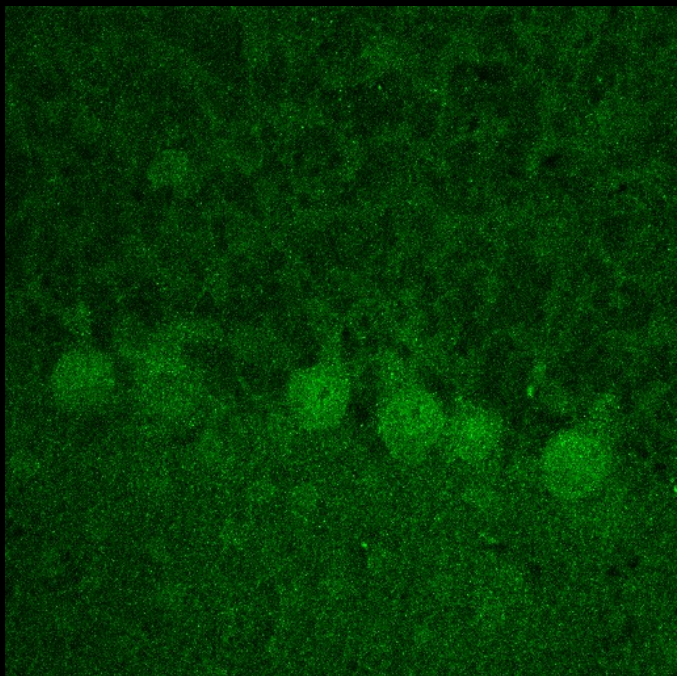
SCA2 714
8μg/ml
on B6 wt



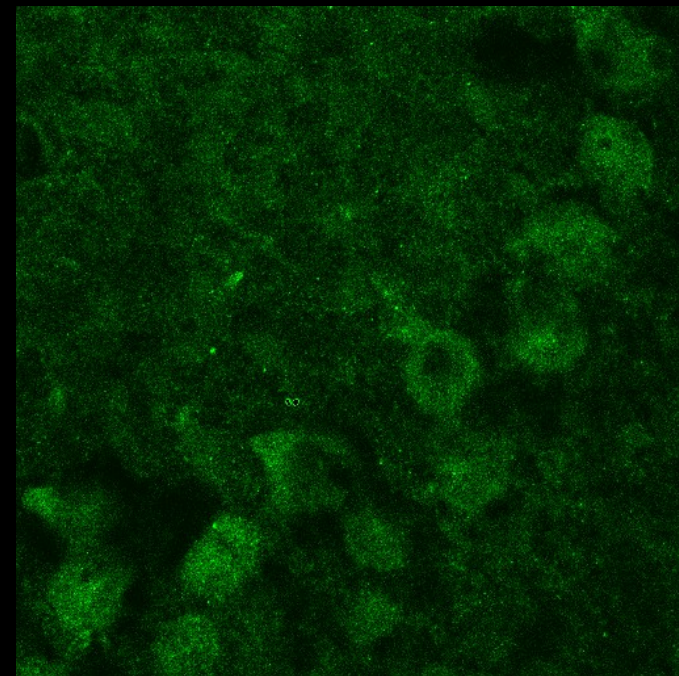
SCA2 714
8μg/ml
on B6 wt



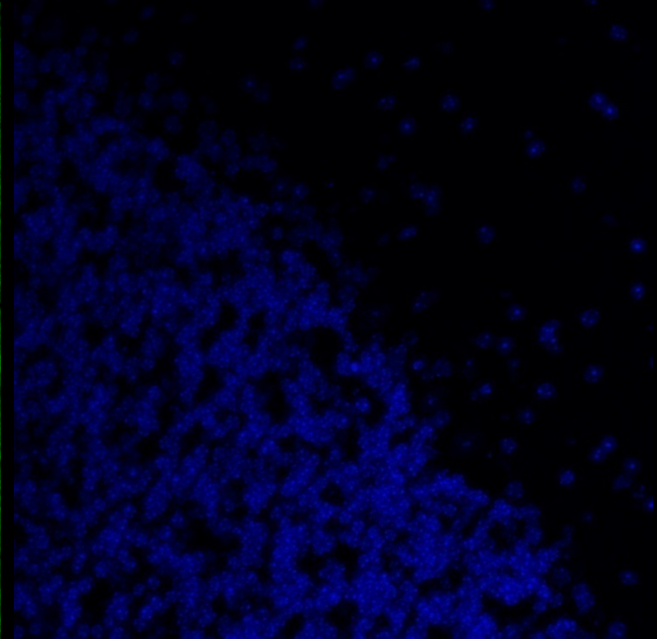
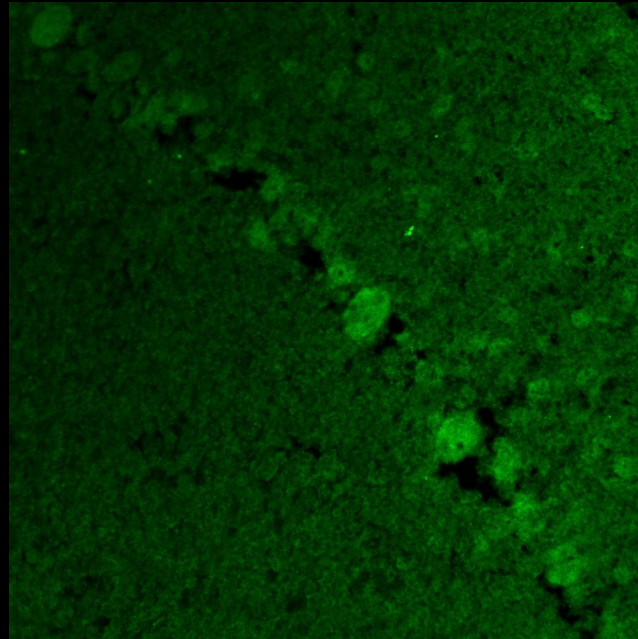
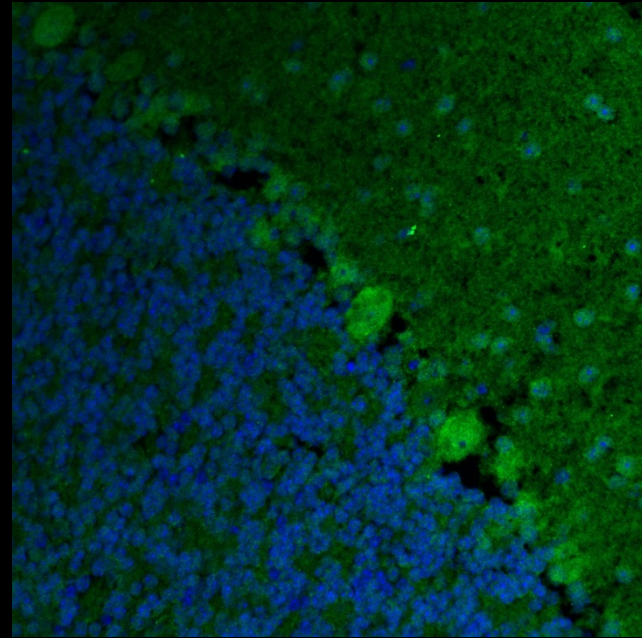
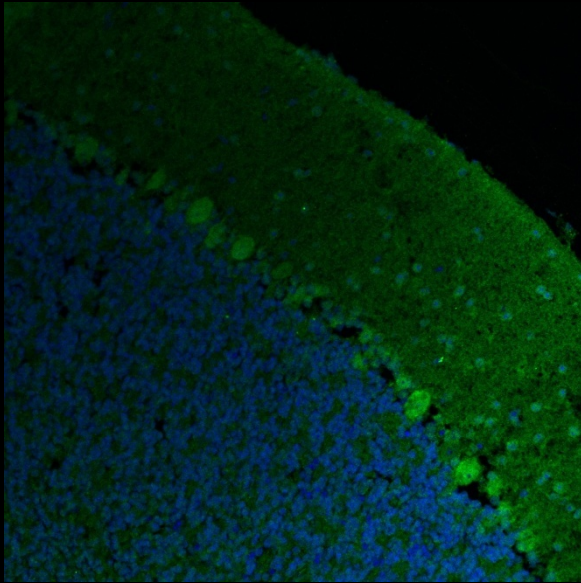
Final bleed
SCA2 714
on B6 wt
1: 1000



Final bleed
SCA2 714
on B6 wt
1: 1000



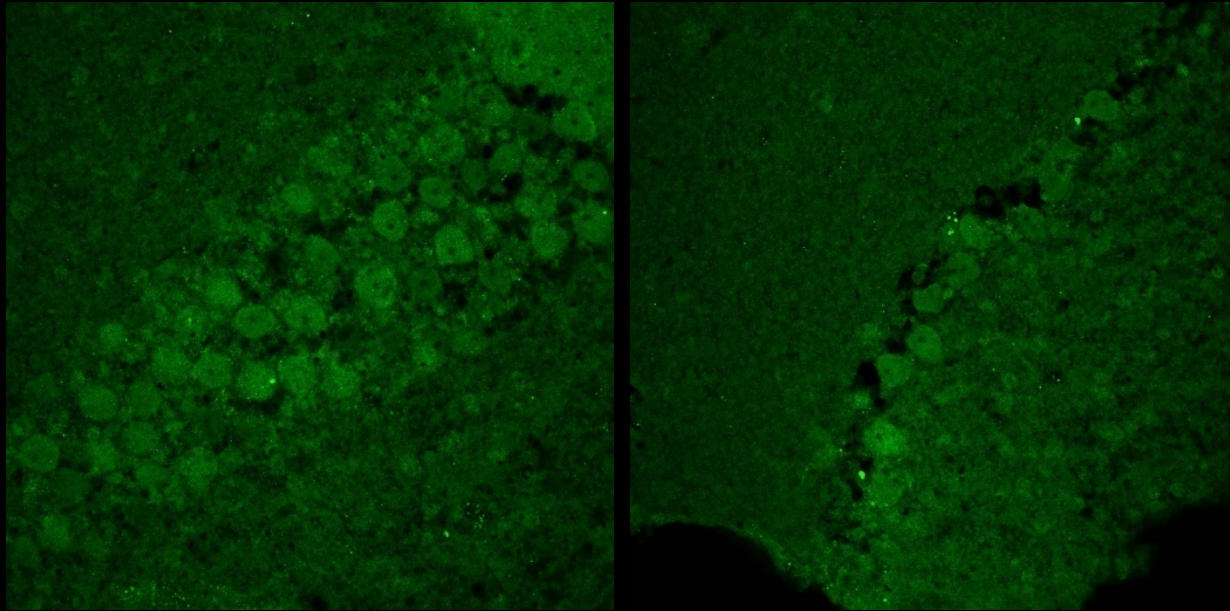
SCA2 714 on SCA2 kout (-/-)



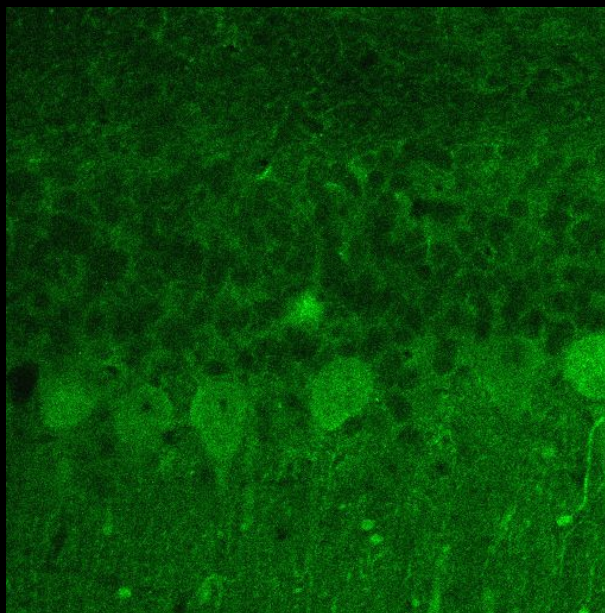
SCA2 714	8μg/ml
Dy Light 488 rb	1:1700
Dapi	1:10.000

B6 wt

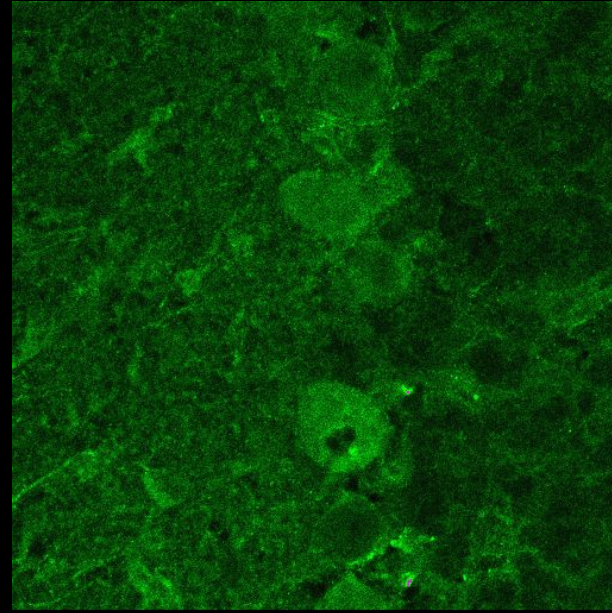
SCA2 790
10 μ g/ μ l



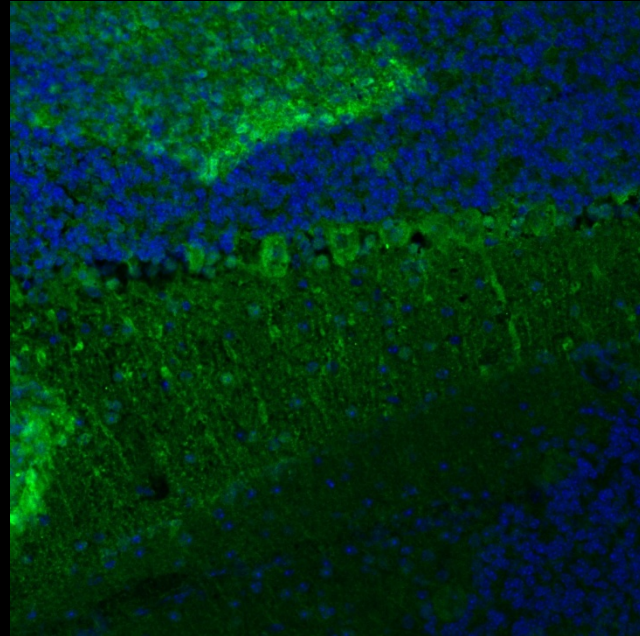
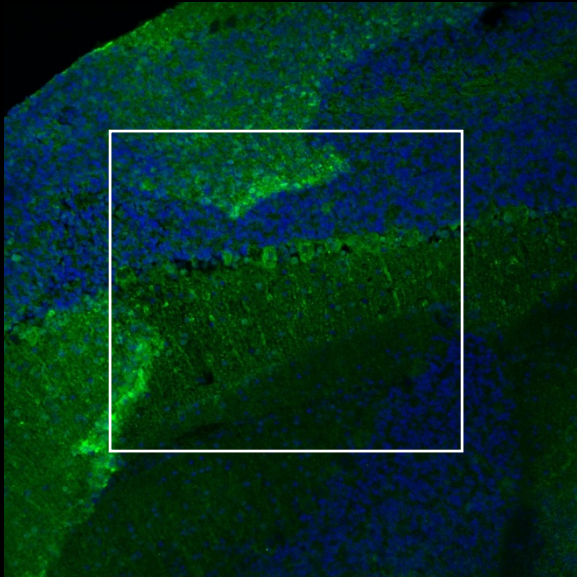
Final bleed
SCA2 790
1: 350



Final bleed
SCA2 790
1: 1000



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

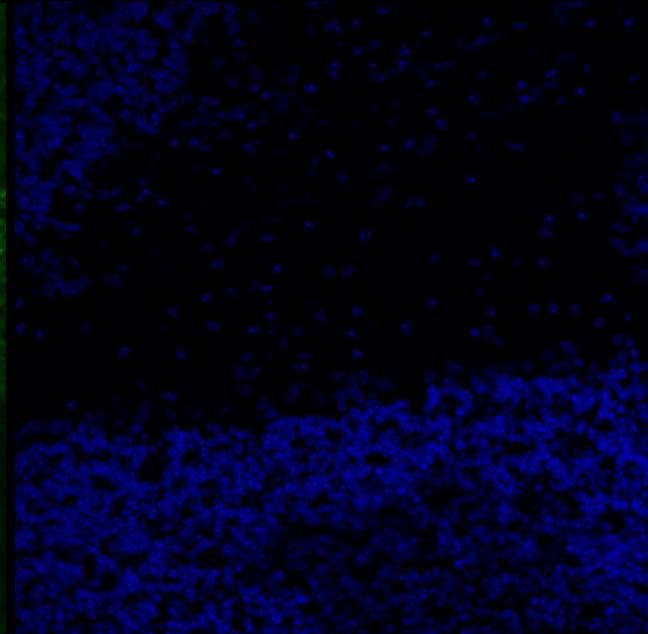
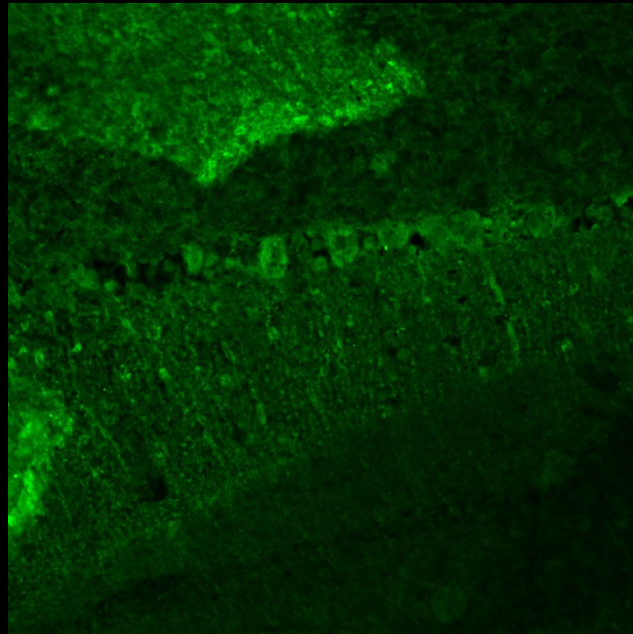


Final bleed SCA2 790

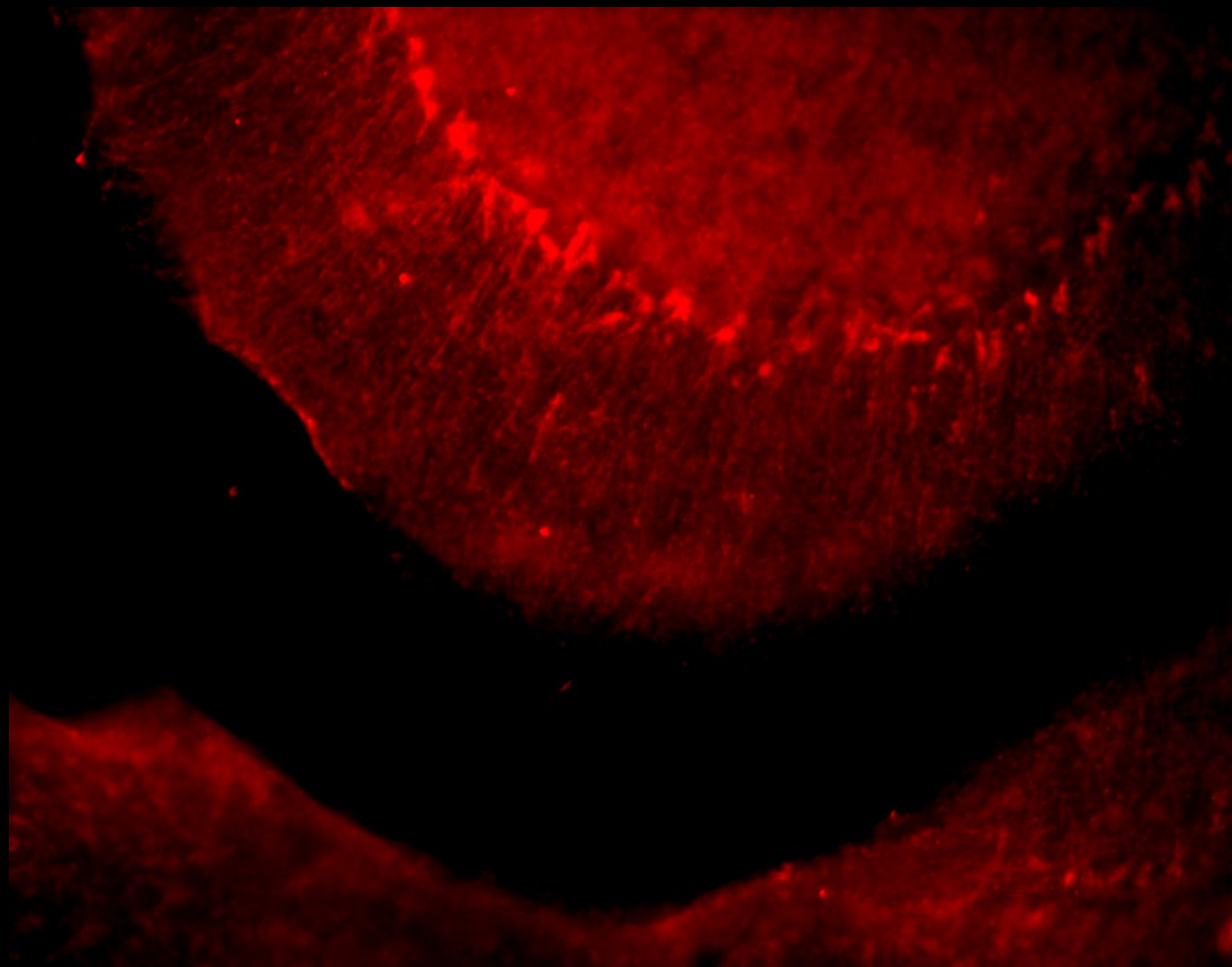
1:500

Dye light 488 rb 1:1700

Dapi 1:10.000



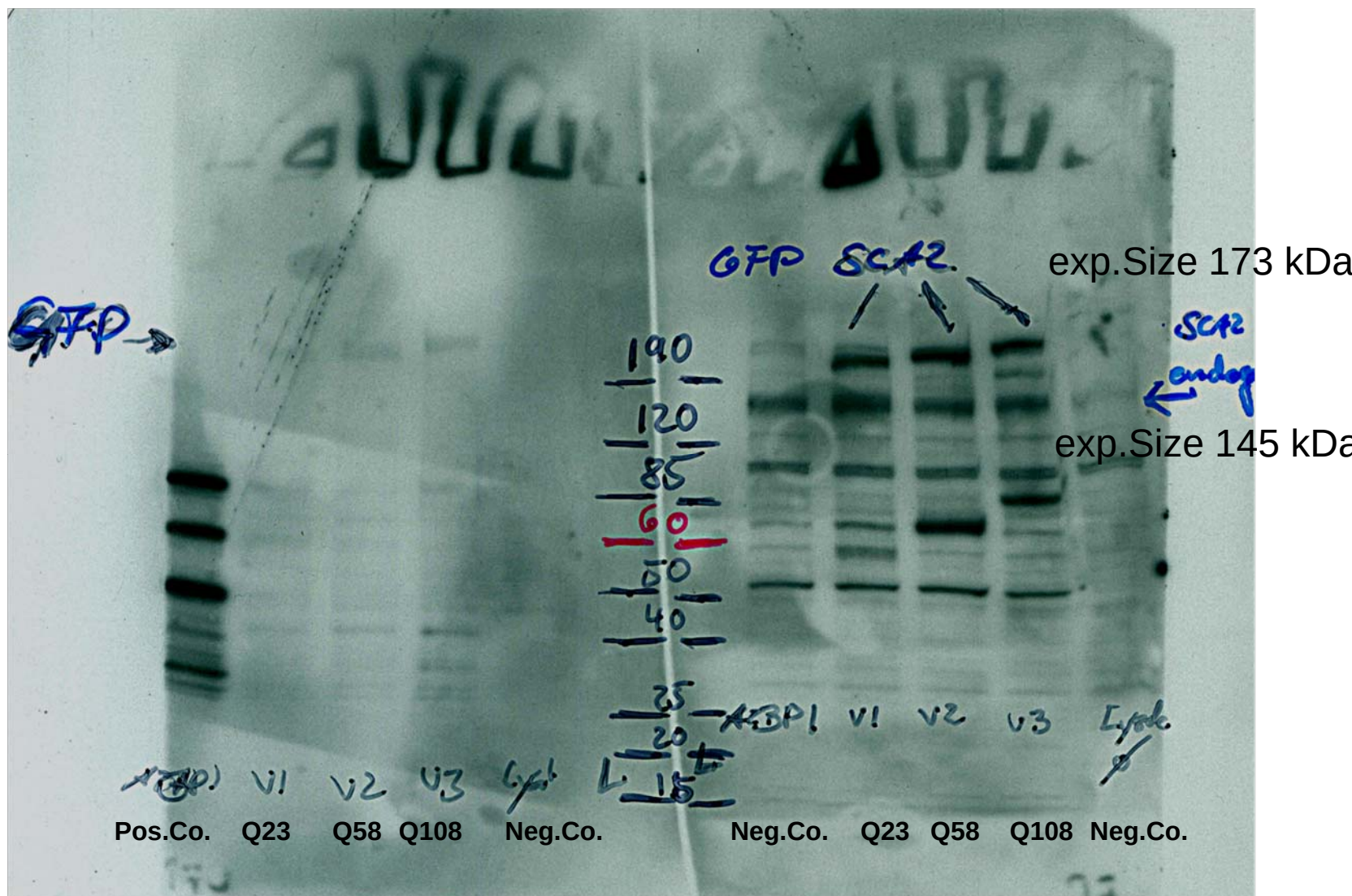
SCA2-D on 5B (Q58) wt



SCA2-D 10 μ g/ml

Cy3 1:500

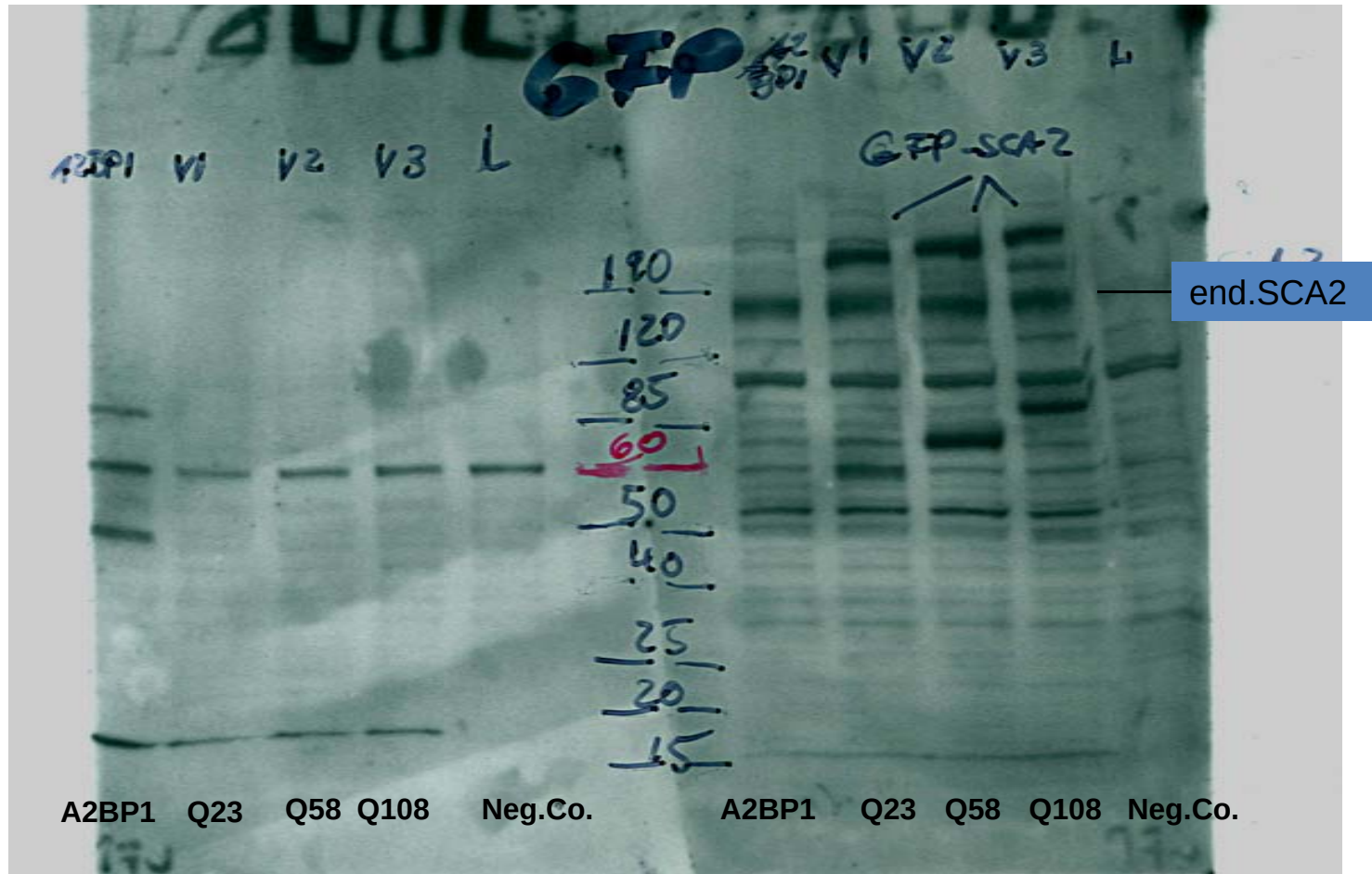
SCA2-GFP- Lysates (HEK 293 cells)



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-A (1) (299)

O70305_ATX2_MOUSE (299) LFKCSD FVVVQFKD T DSSYAK RDAFTCSA I SAKVNGEHKEKDLE FWDAGELTA SEELE LENDVSN GWD FNL MFRYNEEN YGVVSTYDSSLSSY

SCA2-A (1) LFKCSD FVVVQFKD T DSSYAK RDAFTCSA I SAKVNGEHKEKDLE FWDAGELTA SEELE LENDVSN GWD FNL MFRYNEEN YGVVSTYDSSLSSY

Q99700_ATX2_HUMAN (328) LFKCSD FVVVQFKD T DSSYAK RDAFTCSA I SAKVNGEHKEKDLE FWDAGELTA SEELE LENDVSN GWD FNL MFRYNEEN YGVVSTYDSSLSSY

Consensus (328) LFKCSD FVVVQFKD T DSSYAK RDAFTCSA I SAKVNGEHKEKDLE FWDAGELTA SEELE LENDVSN GWD FNL MFRYNEEN YGVVSTYDSSLSSY

SCA2-A (1) (186)

Q7TQH0_ATX2L_MOUSE (184) KFSCLV L VHFRNV DFN YATKCKFTCSA I AMNSKVNGEHKEKVLQRWEGGDSNSDDYDLES DMSNGWDFNEMFKENEEN YGVKTTYDSSLSSY

SCA2-A (1) KFSCLV L VHFRNV DFN YATKCKFTCSA I AMNSKVNGEHKEKVLQRWEGGDSNSDDYDLES DMSNGWDFNEMFKENEEN YGVKTTYDSSLSSY

Q8WWM7_ATX2L_HUMAN (186) KFSCLV L VHFRNV DFN YATKCKFTCSA I AMNSKVNGEHKEKVLQRWEGGDSNSDDYDLES DMSNGWDFNEMFKENEEN YGVKTTYDSSLSSY

Consensus (186) KFSCLV L VHFRNV DFN YATKCKFTCSA I AMNSKVNGEHKEKVLQRWEGGDSNSDDYDLES DMSNGWDFNEMFKENEEN YGVKTTYDSSLSSY

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

(875) 875 880 890 900 910 920 930 940 950 968
 SCA2-B (1) ----- EQVRKSTLNENAKEFN -----
 Q99700_ATX2_HUMAN (873) HKRGFEVTSQGVCTSSPACKCEKDDKEEKLA EQVRKSTLNENAKEFN ERSFSQPKFSTTFTSPRPQACPSPSMVGHQCP PVYTQFVCFAPN
 O70305_ATX2_MOUSE (845) HKRGFEVTSQGVCTSSPACKCEKDDREEKKT EQVRKSTLNENAKEFN ERSFSQPKFSTTFTSPRPQACPSPSMVGHQCPA PVYTQFVCFAPN
 Consensus (875) HKRGFEVTSQGVCTSSPACKCEKDDKEEKD EQVRKSTLNENAKEFN ERSFSQPKFSTTFTSPRPQACPSPSMVGHQCP PVYTQFVCFAPN

(635) 635 640 650 660 670 680 690 700 710 726
 Q7TQH0_ATX2L_MOUSE (632) PSQTGSFPVGLIKGDEKEEGFV EQVKKSTLNENAKEFN PTKFLLSVNKSTSTFTSPGPRTHSTPSIFVLTAGCSGLYSFOYISYIPQIHMG
 Q8WWM7_ATX2L_HUMAN (629) PSQTGSFPVGLIKGEDKDEGFV EQVKKSTLNENAKEFN PTKFLLSVNKSTSTFTSPGPRTHSTPSIFVLTAGCSGLYSFOYISYIPQIHMG
 SCA2-B (1) ----- EQVRKSTLNENAKEFN -----
 Consensus (635) PSQTGSFPVGLIKGEDKDEGFV EQVKKSTLNENAKEFN PTKFLLSVNKSTSTFTSPGPRTHSTPSIFVLTAGCSGLYSFOYISYIPQIHMG

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

	(205)	205	210	220	230	240	250	260	270	280	298																														
Q99700_ATX2_HUMAN	(205)	AS	FAAA	SPSS	SVSS	SAT	APSS	VVA	AT	SGGR	FG	LGRGR	NS	NKG	LPQ	STIS	FDGI	YAN	MR	MV	HL	TS	SV	GS	KE	CV	QV	KN	GG	II	YE	GV	FK	TY	SE						
Q70305_ATX2_MOUSE	(176)	EG	AA	FA	SA	AV	TS	SA	SV	VF	FA	AP	VA	SS	AA	AGGR	FG	LGRGR	NS	NKG	LPQ	ETIS	FDGI	YAN	VR	MV	HL	TS	SV	GS	KE	CV	QV	KN	GG	II	YE	GV	FK	TY	SE
SCA2-C	(1)	-----LGRGRNSNKG-----																																							
Consensus	(205)	A A A S S A A P A A A G G R F G L G R G R N S N K G L P Q T I S F D G I Y A N M R M V H I L T S V G S K C E V Q V K N G G I Y E G V F K T Y S E																																							

	(61)	61	70	80	90	100	110	120	130	140	152																																																																			
Q7TQH0_ATX2L_MOUSE	(61)	P	AAAG	SGLR	RGA	E	S	I	LA	A	S	P	---	Q	H	Q	E	R	P	G	A	A	I	G	S	A	R	G	O	T	T	G	K	G	P	Q	S	F	V	F	E	G	V	Y	N	N	S	R	M	L	H	F	L	T	A	V	G	S	T	C	E	V	K	V	K	N	G	T	T	Y	E	G	I	F	K	T	L	
Q8WWM7_ATX2L_HUMAN	(61)	P	AAAG	SGLR	RGA	E	G	I	LA	F	Q	P	P	---	Q	H	Q	E	R	P	G	A	A	I	G	S	A	R	G	O	T	T	G	K	G	P	Q	S	F	V	F	E	G	V	Y	N	N	S	R	M	L	H	F	L	T	A	V	G	S	T	C	E	V	K	V	K	N	G	T	T	Y	E	G	I	F	K	T	L
SCA2-C	(1)	-----LGRGRNSNKG-----																																																																												
Consensus	(61)	P AAAGSGLRRGAE I LA P P Q H Q E R P G A A I G S A R G O T T G K G P Q S F V F E G V Y N N S R M L H F L T A V G S T C E V K V K N G T T Y E G I F K T L																																																																												

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

(827) 827 840 850 860 870 880 890 900 910 920
Q99700_ATX2_HUMAN (827) S R D L I K D K I E P S A K D S F I E N S S S -- N C T S G S S K N S P S I S P S I L S N T E H K R G P E V T S Q G V Q T S S P A C K Q E K D D K E E K K D A A E Q V R K S T L N P N A K
O70305_ATX2_MOUSE (797) S R D L I K D K T E A S A K D S F I D S S S S S N C T S G S S K T N S P S I S P S M L S N A E H K R G P E V T S Q G V Q T S S P A C K Q E K D D K E E K K D T T E Q V R K S T L N P N A K
SCA2-D (1) ----- I L S N T E H K R G P E V -----
Consensus (827) S R D L I K D K E S A K D S F I D S S S N C T S G S S K N S P S I S P S I L S N T E H K R G P E V T S Q G V Q T S S P A C K Q E K D D K E E K K D E Q V R K S T L N P N A K

(615) 615 620 630 640 650 660 670 680 690 706
Q7TQH0_ATX2L_MOUSE (612) K V P M A G V G G T E G P E Q L P A P C P S Q T G S P F V G L I K G D E K E E G P V T E Q V K K S T L N P N A K E F N F T K P L L S V N K S T S T F T S P G P R T H S T P S I F V L T A
Q8WWM7_ATX2L_HUMAN (609) K P L A F S G G T E G P E Q P P P C P S Q T G S P F V G L I K G E L K E G P V A E Q V K K S T L N P N A K E F N F T K P L L S V N K S T S T F T S P G P R T H S T P S I F V L T A
SCA2-D (1) ----- I L S N T E H K R G P E V -----
Consensus (615) K P L A G G T E G P E Q P P C P S Q T G S P F V G L I K G D E K E G P V E Q V K K S T L N P N A K E F N F T K P L L S V N K S T S T F T S P G P R T H S T P S I F V L T A