

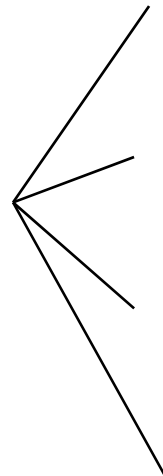
# **Affinity purification of SCA-2 antisera**

**Marion Schiffmann**

**06-21-2007**

## Purification of SCA-2 antibodies A,B,C,D

Purification of rabbit antisera - immunized with peptides:



**A**

**AKVNGEHKEKDLE**

aa 359-371 (human)

**B**

**EQVRKSTLNPNAKEFN**

aa 904-921

**C**

**LGRGRNSNKG**

aa 241-250

**D**

**ILSNTEHKGPEV**

aa 867-879

## Immunization of rabbits

| day | week |  |
|-----|------|--|
| 0   |      | <i>Conjugation of peptide to KLH</i><br>pre-immune bleed |
| 1   |      | Primary injection  |
| 28  | 4    | Boost 1  |
| 42  | 6    | Boost 2  |
| 56  | 8    | test bleed and Elisa for Titer                           |
| 60  | 8,5  | Boost 3  |
| 74  | 10,5 | Production bleed   |
| 78  | 11   | Boost 4  |
| 88  | 12,5 | Production bleed   |
| 102 | 14,5 | Final bleed  |

### Preparation of sera / bleeds for affinity purification:

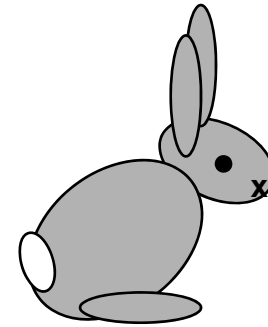
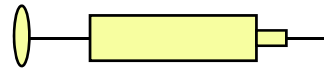
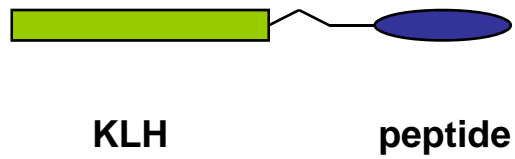
**Ammonium sulfate precipitation: Slowly addition of saturated ammonium sulfate solution to the antiserum. IgG is “salted out“ and can be centrifuged and collected as pellet. IgG concentration is measured with OD280:  $A_{280}$  of 1.35 = 1 mg/ml**

**KLH: ( Keyhole limpet hemocyanin) M.W.:  $4.5 \times 10^6$  –  $1.3 \times 10^7$  Da**

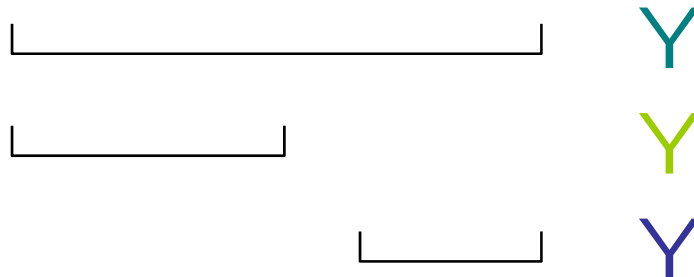
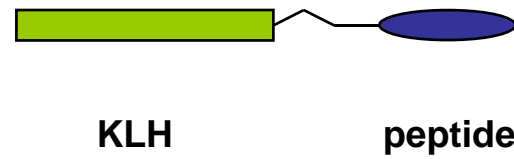


**Small molecules like peptides are not usually immunogenic. To generate an immune response it is necessary to conjugate them to a larger carrier protein. Hemocyanin is a high molecular weight respiratory protein found in mollusks and arthropods. Its large size makes it very immunogenic and the large number of lysine residues available for conjugation make it very useful as a carrier.**

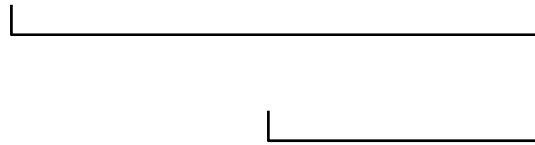
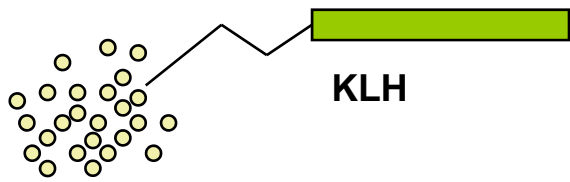
## Coupling antibody via KLH or peptide



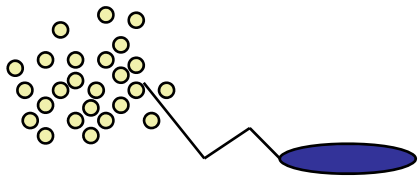
## Generation of antibodies against antigen



# Affinity-purification of antibody via KLH or peptide



**activated  
Sepharose**



**peptide**



## Protocol ligand-coupling to column

1) Ligand: KLH

2) Ligand: SCA2-peptide



Activated  
Sepharose

Sepharose is suspended and swollen to resin in cold 1 mM HCL, than added to column

Wash-steps with cold HCL

Wash-step with 0.1M NaHCO<sub>3</sub> pH 6.5

Antigen is suspended in 0.1 M NaHCO<sub>3</sub> pH 6.5

OD measurement of ligand-solution

1-2 hours incubation of ligand-solution to sepharose

Collection of ligand

OD measurement of collected ligand-solution

1-2 hours blocking-step with 0.1M TrisHCL, pH 8.0

Wash-step with cold NaHCO<sub>3</sub> (unbound ligand)

Wash-step with 0.05 M Tris-HCl ,0.5M NaCl pH 8.0

Wash-step with 0.05 M NaAcetate,0.5M NaCl, pH 4.0

Wash-step with a neutral buffer (0.1M PBS pH 7.0)

Incubation of antibody to ligand at 4°C (ON)

## Protocol affinity purification

### Collection of antibody-solution

Resin is washed with 0.1M PBS pH 7,5 until absorbance OD280 is lower than 0.01.

2 wash cycles of

a) 20mM Tris HCl, 2M NaCl, pH 7.5

b) 0.1 M Na borate pH 9.1

c) 0.1 M PBS pH 4.5

Elution of antibody fractions with 20mM glycine-HCL ,0.2M NaCl, pH 2.4

800ul-fractions are collected into 400 ul of 0.1 M Tris.HCl pH 8.8

pH of antibody fractions has to be controled.

Measurement of IgG concentration with OD280: 1 Abs. = 1.35 mg/ml  
IgG



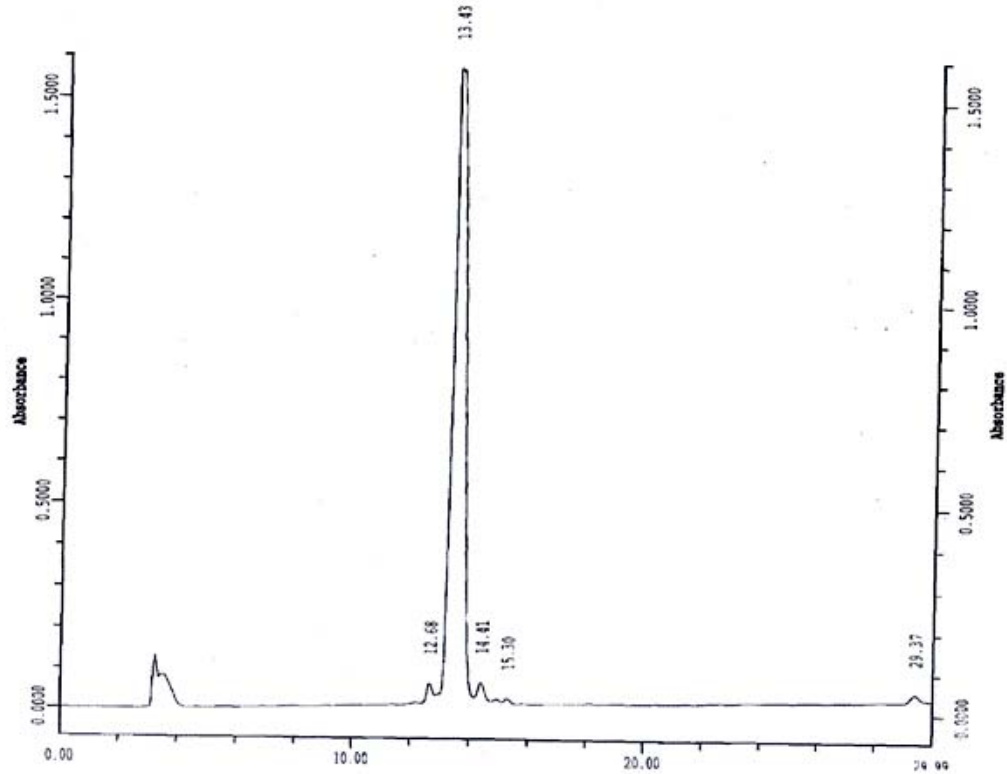
## Trouble shooting

Was purity of peptide not high enough ? Did peptide degrade ?

NAME: CERN LAY REP TYPE: DIENSTORT  
COLLECTION DATA: 22044921 A 1 1 Orig C:\GOLDENSYSTEMA  
METHOD: 10-40-30 C:\GOLDENSYSTEMA\MICROAZA

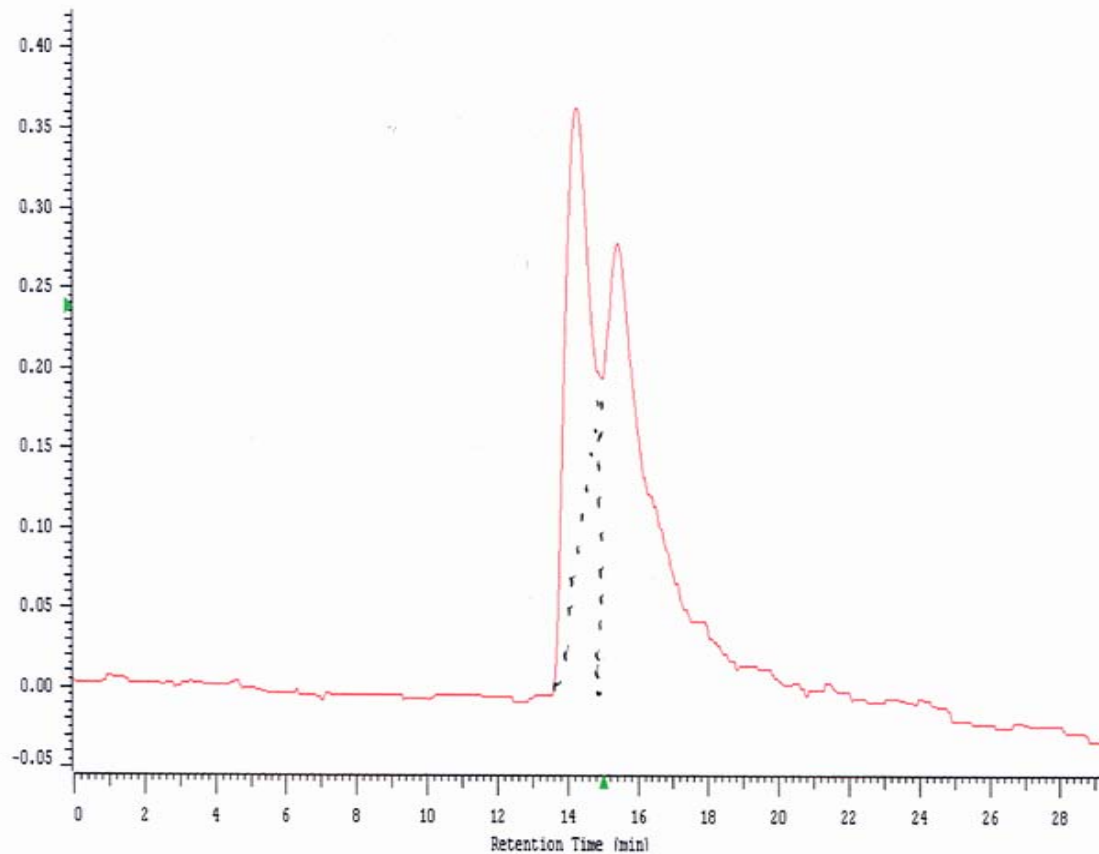
TIME: 09:45:21 22 APR 2007  
INJECTION: 10:19:22 22 APR 2007  
REPORT: 08:36:54 23 MAY 2007

SAMPLE NAME: none  
SYSTEM 1: SYSTEM1



## Trouble shooting

Was purity of peptide not high enough ? Did peptide degrade ?



## A2RP with SCA2 peptides A-D:

**A**

|                    |       |  |     |     |     |     |     |     |     |     |     |  |
|--------------------|-------|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|                    | (181) | 181  | 190 | 200 | 210 | 220 | 230 | 240 | 250 | 260 | 272 |  |
| Q7TQH0_ATX2L_MOUSE | (179) | DTMVFKPSDVLVHFRNVDFNYATKDKFTDSAIAMNSKVNGEHKEKVLQRWEGGDSNSDDYDLESMSNGWDPNEMFKFNEENYGVKTTYDS |     |     |     |     |     |     |     |     |     |  |
| Q8WWM7_ATX2L_HUMAN | (181) | DTMVFKPSDVLVHFRNVDFNYATKDKFTDSAIAMNSKVNGEHKEKVLQRWEGGDSNSDDYDLESMSNGWDPNEMFKFNEENYGVKTTYDS |     |     |     |     |     |     |     |     |     |  |
| SCA2-A             | (1)   | -----AKVNGEHKEKDL-----   |     |     |     |     |     |     |     |     |     |  |
| Consensus          | (181) | DTMVFKPSDVLVHFRNVDFNYATKDKFTDSAIAMNSKVNGEHKEKVLQRWEGGDSNSDDYDLESMSNGWDPNEMFKFNEENYGVKTTYDS |     |     |     |     |     |     |     |     |     |  |

**B**

|                    |       |   |     |     |     |     |     |     |     |     |     |  |
|--------------------|-------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|                    | (622) | 622   | 630 | 640 | 650 | 660 | 670 | 680 | 690 | 700 | 713 |  |
| Q7TQH0_ATX2L_MOUSE | (619) | GGTEGPEQLFAPCPSQTGSPVVGLIKGDDEKDEGPVTEQVKKSTLNPNAKEFNPTKPLLSVNKSTSTPTSPGPRTHSTPSIPVLTAGQSGLYS |     |     |     |     |     |     |     |     |     |  |
| Q8WWM7_ATX2L_HUMAN | (616) | GGTEGPEQLFAPCPSQTGSPVVGLIKGDDEKDEGPVTEQVKKSTLNPNAKEFNPTKPLLSVNKSTSTPTSPGPRTHSTPSIPVLTAGQSGLYS |     |     |     |     |     |     |     |     |     |  |
| SCA2-B             | (1)   | -----EQVKKSTLNPNAKEFN-----  |     |     |     |     |     |     |     |     |     |  |
| Consensus          | (622) | GGTEGPEQ P PCPSQTGSPVVGLIKGDDEKDEGPV EQVKKSTLNPNAKEFNPTKPLLSVNKSTSTPTSPGPRTHSTPSIPVLTAGQSGLYS |     |     |     |     |     |     |     |     |     |  |

**C**

|                    |      |   |    |    |    |     |     |     |     |     |     |  |
|--------------------|------|---|----|----|----|-----|-----|-----|-----|-----|-----|--|
|                    | (61) | 61  | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 152 |  |
| Q7TQH0_ATX2L_MOUSE | (61) | FAAAAGSGLRRGAESILAASAPP--QHQERPGAVAIGSVRGQTTGKGPQSPVFEVYNNRMLHFLTAVVGSTCDVKVKNGTTYEGIFKTL |    |    |    |     |     |     |     |     |     |  |
| Q8WWM7_ATX2L_HUMAN | (61) | FVAAAGSGLRRGAEGILAPQPPPPQHQERPGAAIGSARGQSTGKGPQSPVFEVYNNRMLHFLTAVVGSTCDVKVKNGTTYEGIFKTL   |    |    |    |     |     |     |     |     |     |  |
| SCA2-C             | (1)  | -----LGRGRNSKGR-----  |    |    |    |     |     |     |     |     |     |  |
| Consensus          | (61) | P AAGSGLRRGAE ILA PP QHQERPGA AIGSARGQSTGKGPQSPVFEVYNNRMLHFLTAVVGSTCDVKVKNGTTYEGIFKTL     |    |    |    |     |     |     |     |     |     |  |

**D**

|                    |       |  |     |     |     |     |     |     |     |     |  |
|--------------------|-------|--|-----|-----|-----|-----|-----|-----|-----|-----|--|
|                    | (608) | 608  | 620 | 630 | 640 | 650 | 660 | 670 | 680 | 699 |  |
| Q7TQH0_ATX2L_MOUSE | (605) | SILDKEDKVPMAAGVGGTEGPEQLFAPCPSQTGSPVVGLIKGDDEKDEGPVTEQVKKSTLNPNAKEFNPTKPLLSVNKSTSTPTSPGPRTHSTP |     |     |     |     |     |     |     |     |  |
| Q8WWM7_ATX2L_HUMAN | (602) | SVSDKEDKPPPLAPSGGTEGPEQLFAPCPSQTGSPVVGLIKGDDEKDEGPVTEQVKKSTLNPNAKEFNPTKPLLSVNKSTSTPTSPGPRTHSTP |     |     |     |     |     |     |     |     |  |
| SCA2-D             | (1)   | -----LISNTEHHRGPEV-----  |     |     |     |     |     |     |     |     |  |
| Consensus          | (608) | SI DKEDK PLA GGTGPEQ P PCPSQTGSPVVGLIKGDDEKDEGPV EQVKKSTLNPNAKEFNPTKPLLSVNKSTSTPTSPGPRTHSTP    |     |     |     |     |     |     |     |     |  |

## Criteria of new SCA-2 peptides:

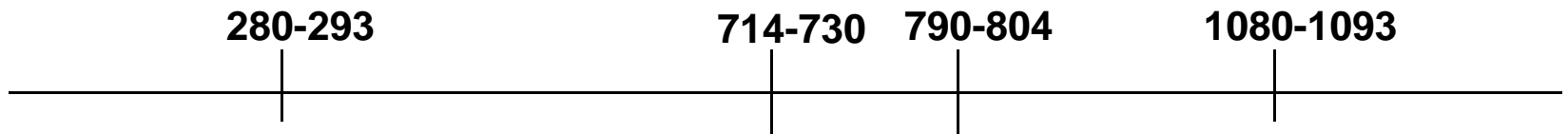
No crossreactivity with other proteins especially with A2RP(Blast)

Immunogenicity (Average of Jameson and Wolf method)

Distribution of peptides on mouse ataxin 2-protein sequence

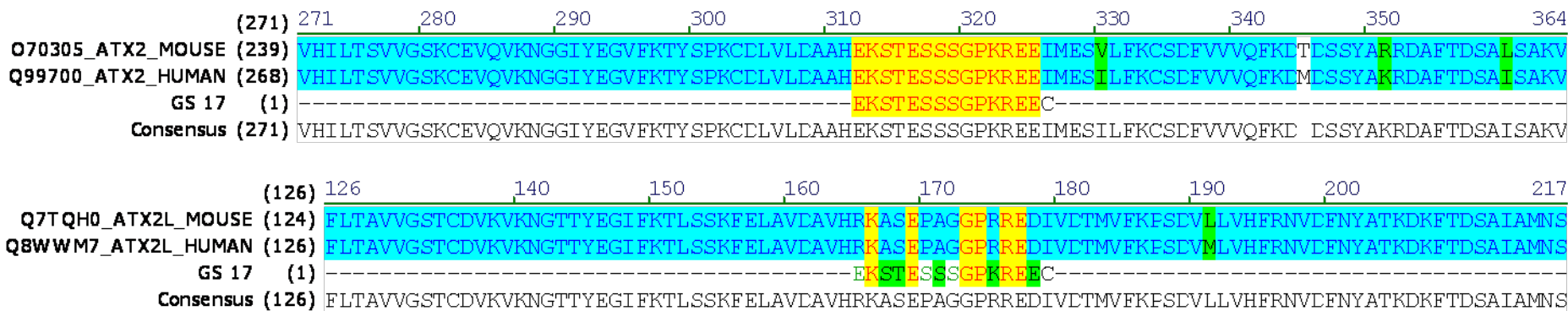
( N-terminal, C-terminal and `middle` position )

### Ataxin-2 mouse



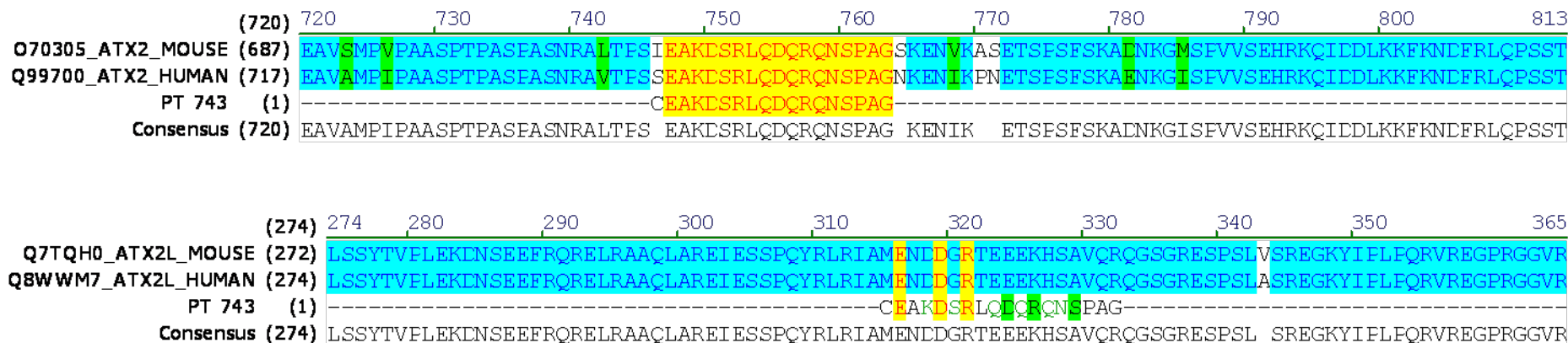
mouse 280-293 / human 309-322

SCA2 280



mouse 714-730 / human 748-764

SCA2 714



mouse 790-804 / human 820-834

SCA2 790

|                   |       |       |       |     |        |     |       |       |       |      |      |                 |     |       |     |    |    |     |       |    |    |    |    |  |
|-------------------|-------|-------|-------|-----|--------|-----|-------|-------|-------|------|------|-----------------|-----|-------|-----|----|----|-----|-------|----|----|----|----|--|
|                   | (779) | 779   | 790   | 800 | 810    | 820 | 830   | 840   | 850   | 860  | 872  |                 |     |       |     |    |    |     |       |    |    |    |    |  |
| O70305_ATX2_MOUSE | (746) | KA    | LNKGM | SFV | VEHRKQ | IDD | LKKFK | NDFRL | QPSST | SESM | QLLS | KNREGEKSRDLIKDK | TEA | SAK   | SFT | SS | SS | NCT | SGSSK | TN | SF | SF | SM |  |
| Q99700_ATX2_HUMAN | (776) | KA    | ENKGI | SFV | VEHRKQ | IDD | LKKFK | NDFRL | QPSST | SESM | QLLN | KNREGEKSRDLIKDK | IE  | SAK   | SFT | SS | -- | NCT | SGSSK | PN | SF | SF | SI |  |
| PI 820            | (1)   | ----- |       |     |        |     |       |       |       |      |      |                 | C   | ----- |     |    |    |     |       |    |    |    |    |  |
| Consensus         | (779) | KAD   | NKGI  | SFV | VEHRKQ | IDD | LKKFK | NDFRL | QPSST | SESM | QLL  | KNREGEKSRDLIKDK | E   | SAK   | SFT | SS |    | NCT | SGSSK | N  | SF | SF | SI |  |

|                    |       |       |       |     |         |     |     |     |     |       |      |     |      |       |      |      |     |    |      |    |     |      |    |      |      |      |      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|--------------------|-------|-------|-------|-----|---------|-----|-----|-----|-----|-------|------|-----|------|-------|------|------|-----|----|------|----|-----|------|----|------|------|------|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|                    | (592) | 592   | 600   | 610 | 620     | 630 | 640 | 650 | 660 | 670   | 683  |     |      |       |      |      |     |    |      |    |     |      |    |      |      |      |      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Q7TQH0_ATX2L_MOUSE | (589) | LLT   | SDPMG | SPV | SSKTESI | LDK | EKL | VPM | AGV | GGTEG | PEQL | PAP | PCPS | QTG   | SPFV | GLIK | GED | KE | EGFV | TE | CVK | STLN | FN | NAKE | FNPT | KELL | SVNK |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Q8WWM7_ATX2L_HUMAN | (586) | LLT   | SEPMG | SPV | SSKTESV | SDK | EKL | PLA | ES  | GGTEG | PEQ  | PPP | PCPS | QTG   | SPFV | GLIK | GED | KE | EGFV | AE | CVK | STLN | FN | NAKE | FNPT | KELL | SVNK |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| PI 820             | (1)   | ----- |       |     |         |     |     |     |     |       |      |     | C    | ----- |      |      |     |    |      |    |     |      |    |      |      |      |      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Consensus          | (592) | LLT   | SDPMG | SPV | SSKTESI | D   | K   | E   | 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 | E | D | K | E | G | F | V | E | C | V | K | S | T | L | N | F | N | A | K | E | F | N | P | T | K | E | L | L | S | V | N | K |

mouse 1080-1093 / human 1106-1119

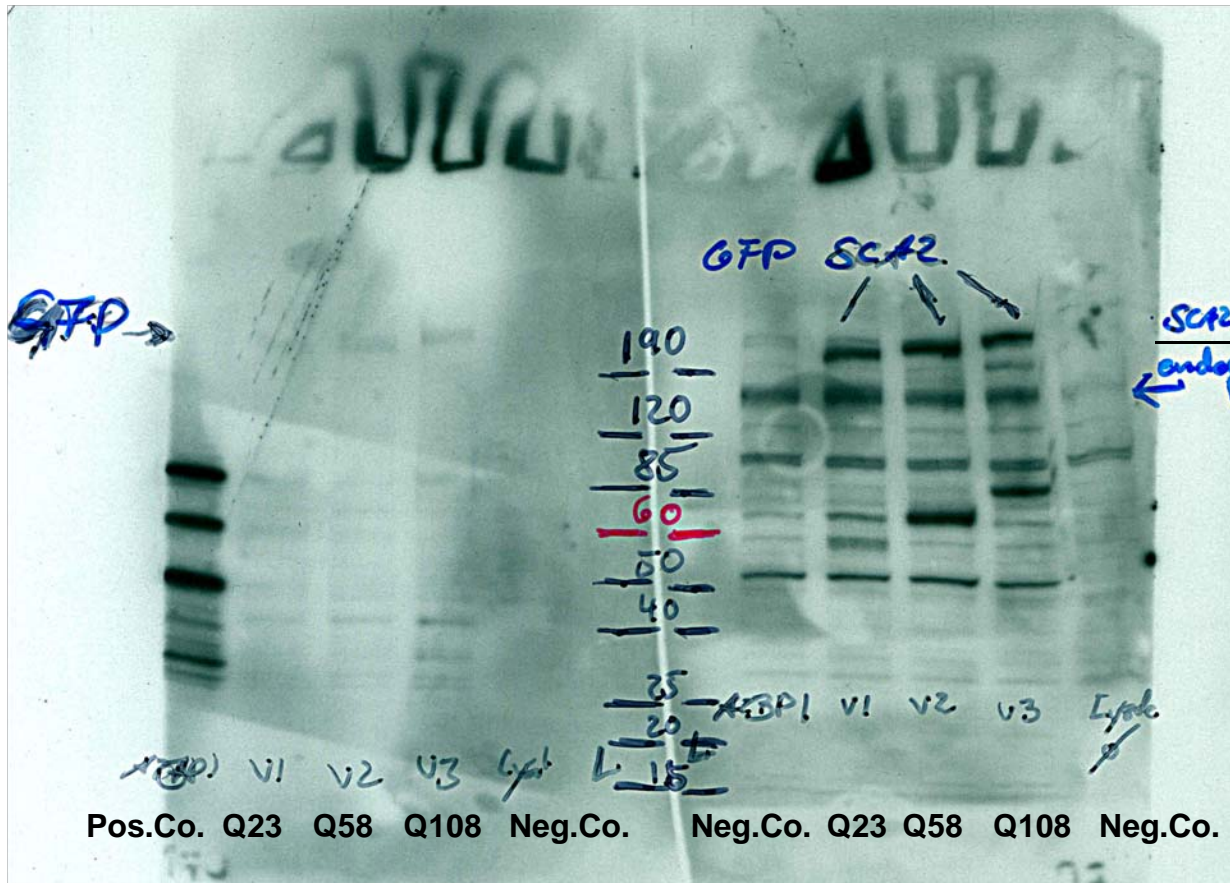
SCA2 1080

|                   |        |       |      |      |      |      |      |      |      |      |      |     |    |       |    |    |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|-------------------|--------|-------|------|------|------|------|------|------|------|------|------|-----|----|-------|----|----|-----|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|                   | (1068) | 1068  | 1080 | 1090 | 1100 | 1110 | 1120 | 1130 | 1140 | 1150 | 1161 |     |    |       |    |    |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| O70305_ATX2_MOUSE | (1035) | HPH   | VYSF | VIQ  | GNAR | M    | APFA | HA   | CPGL | VSS  | SAAC | FGA | HE | Q     | TH | AM | YAC | PKL | F   | Y | N | K | E | T | S | P | S | F | Y | F | A | I | S | T | G | S | L | A | Q | Q | Y | A | H | P | N | A | L | H | P | H | T | E | H | F | Q | E | S | A | T | F | T | G |   |   |   |   |   |   |   |   |   |   |
| Q99700_ATX2_HUMAN | (1061) | HPH   | VYSF | VIQ  | GNAR | M    | APFT | HA   | CPGL | VSS  | SAT  | CG  | GA | HE    | Q  | TH | AM  | YAC | PKL | F | Y | N | K | E | T | S | P | S | F | Y | F | A | I | S | T | G | S | L | A | Q | Q | Y | A | H | P | N | A | T | L | H | P | H | T | E | H | F | Q | E | S | A | T | F | T | G |   |   |   |   |   |   |   |   |
| GS 27             | (1)    | ----- |      |      |      |      |      |      |      |      |      |     | C  | ----- |    |    |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Consensus         | (1068) | HPH   | VYSF | VIQ  | GNAR | M    | APF  | HA   | CPGL | VSS  | S    | Q   | F  | G     | A  | H  | E   | Q   | T   | H | A | M | Y | A | C | P | K | L | F | Y | N | K | E | T | S | P | S | F | Y | F | A | I | S | T | G | S | L | A | Q | Q | Y | A | H | P | N | A | L | H | P | H | T | E | H | F | Q | E | S | A | T | F | T | G |

|                    |       |       |     |     |     |     |     |     |     |     |   |   |   |       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|--------------------|-------|-------|-----|-----|-----|-----|-----|-----|-----|-----|---|---|---|-------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|                    | (747) | 747   | 760 | 770 | 780 | 790 | 800 | 810 | 820 | 838 |   |   |   |       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Q7TQH0_ATX2L_MOUSE | (744) | Q     | G   | K   | Y   | R   | G   | A   | K   | G   | S | L | P | F     | Q | R | S | D | Q | H | Q | F | A | S | A | F | P | M | Q | A | A | A | A | A | A | A | A | G | P | P | L | V | A | A | T | P | Y | S | S | Y | I | P | Y | N | P | Q | Q | F | P | G | Q | F | A | M | Q | P | M | A | H | Y | F | S | Q | P | V | F | A | P | M | L | Q | S | N | P | R | M | L | T | S | G | S | H | E |   |
| Q8WWM7_ATX2L_HUMAN | (741) | Q     | G   | K   | Y   | R   | G   | A   | K   | G   | S | L | P | F     | Q | R | S | D | Q | H | Q | F | A | S | A | F | P | M | Q | A | A | A | A | A | A | A | A | A | G | P | P | L | V | A | A | T | P | Y | S | S | Y | I | P | Y | N | P | Q | Q | F | P | G | Q | F | A | M | Q | P | M | A | H | Y | F | S | Q | P | V | F | A | P | M | L | Q | S | N | P | R | M | L | T | S | G | S | H | E |
| GS 27              | (1)   | ----- |     |     |     |     |     |     |     |     |   |   | C | ----- |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Consensus          | (747) | Q     | G   | K   | Y   | R   | G   | A   | K   | G   | S | L | P | F     | Q | R | S | D | Q | H | Q | F | A | S | A | F | P | M | Q | A | A | A | A | A | A | A | A | G | P | P | L | V | A | A | T | P | Y | S | S | Y | I | P | Y | N | P | Q | Q | F | P | G | Q | F | A | M | Q | P | M | A | H | Y | F | S | Q | P | V | F | A | P | M | L | Q | S | N | P | R | M | L | T | S | G | S | H | E |   |

# Westernblot with antibodies SCA2-C and - D

## SCA2-GFP- Lysates



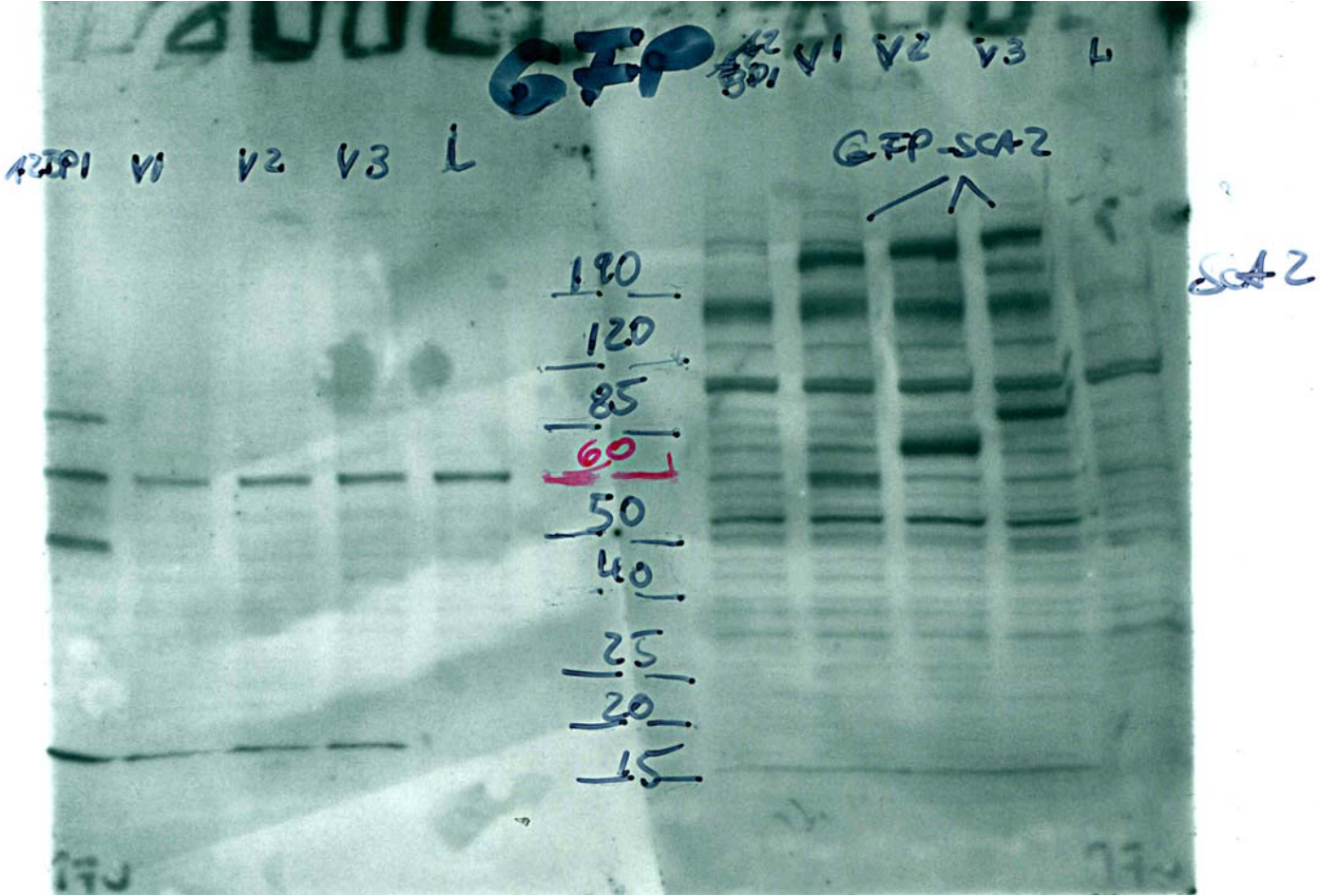
exp.Size 173 kDa

exp.Size 145 kDa

$\alpha$ -GFP

$\alpha$ -SCA2-D

# SCA2-GFP- Lysates



A2BP1 Q23 Q58 Q108 Neg.Co.

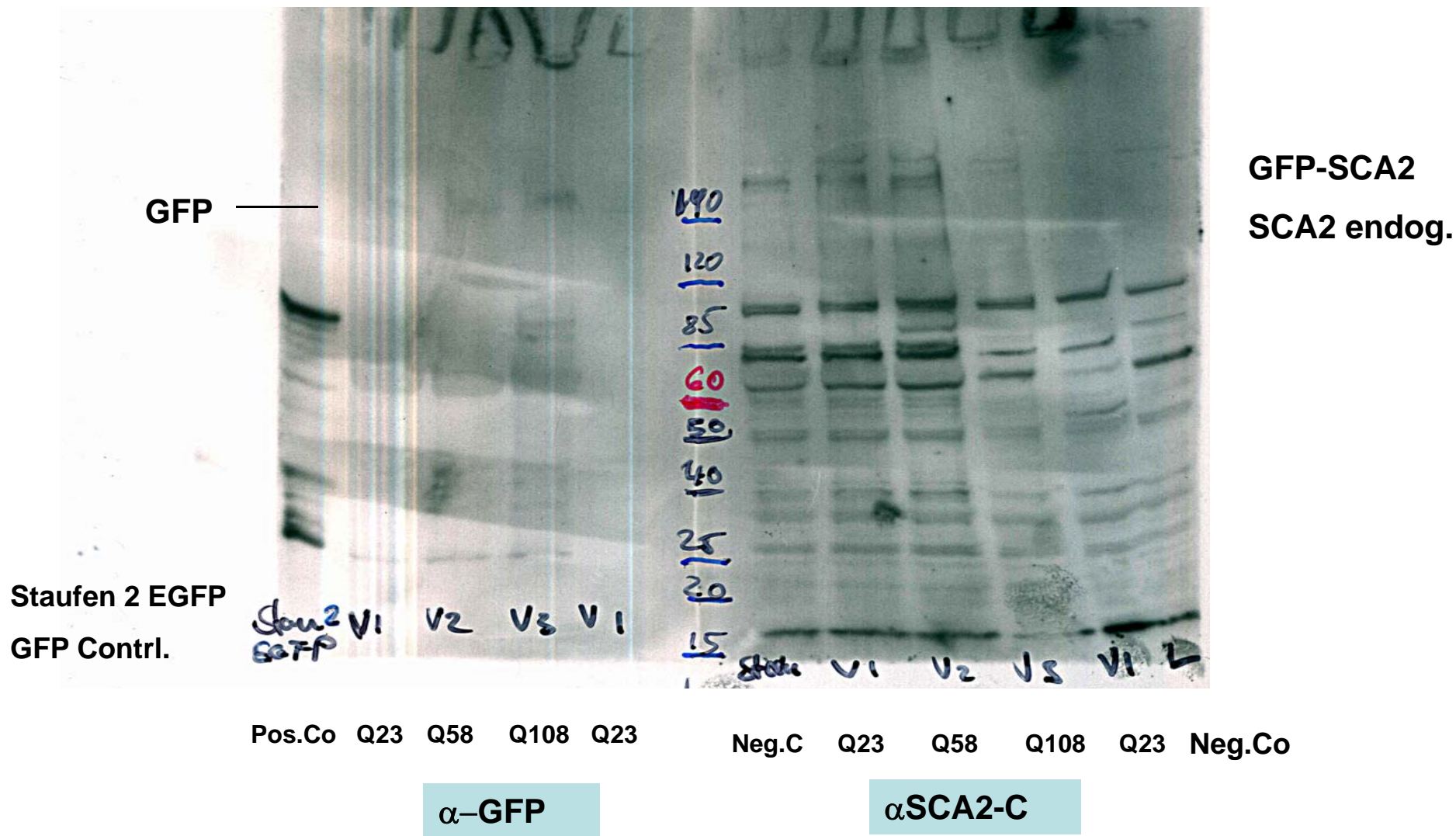
A2BP1 Q23 Q58 Q108 Neg.Co.

$\alpha$ -SCA2-C

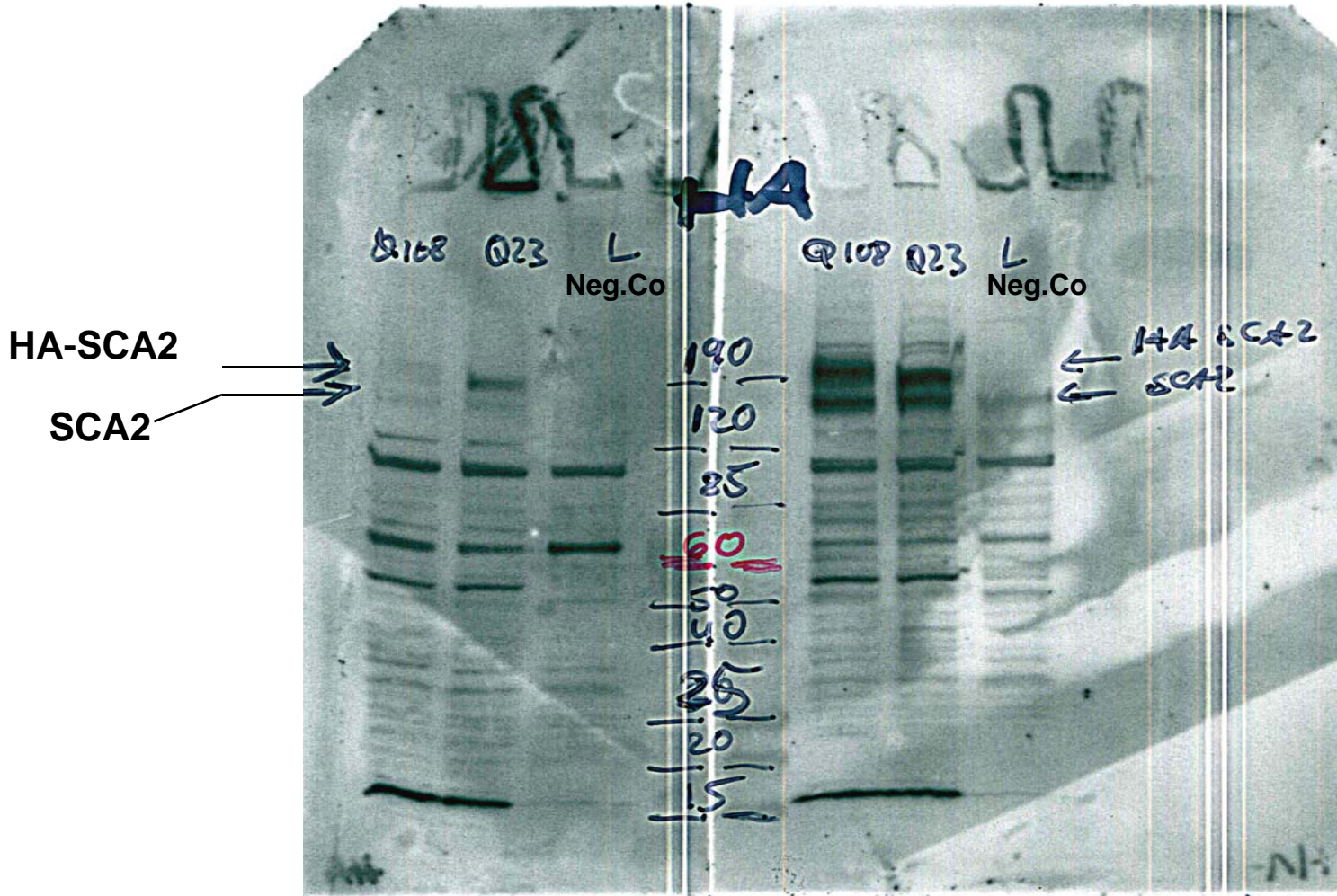
$\alpha$ -SCA2-D



# SCA2-GFP-lysates



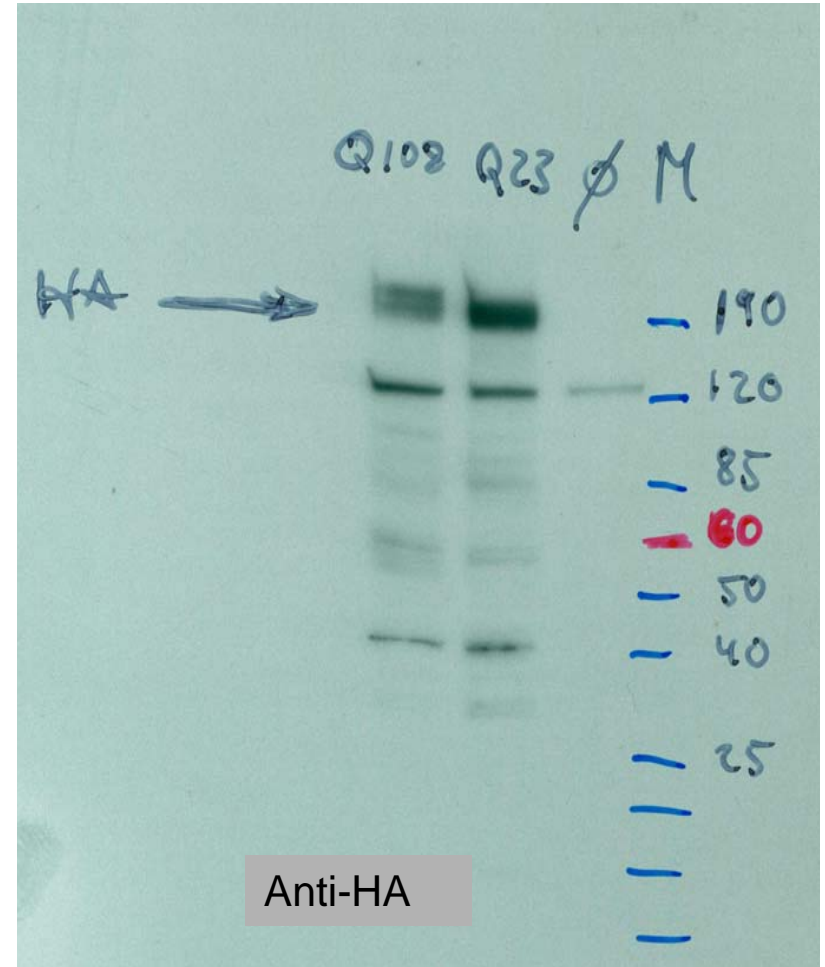
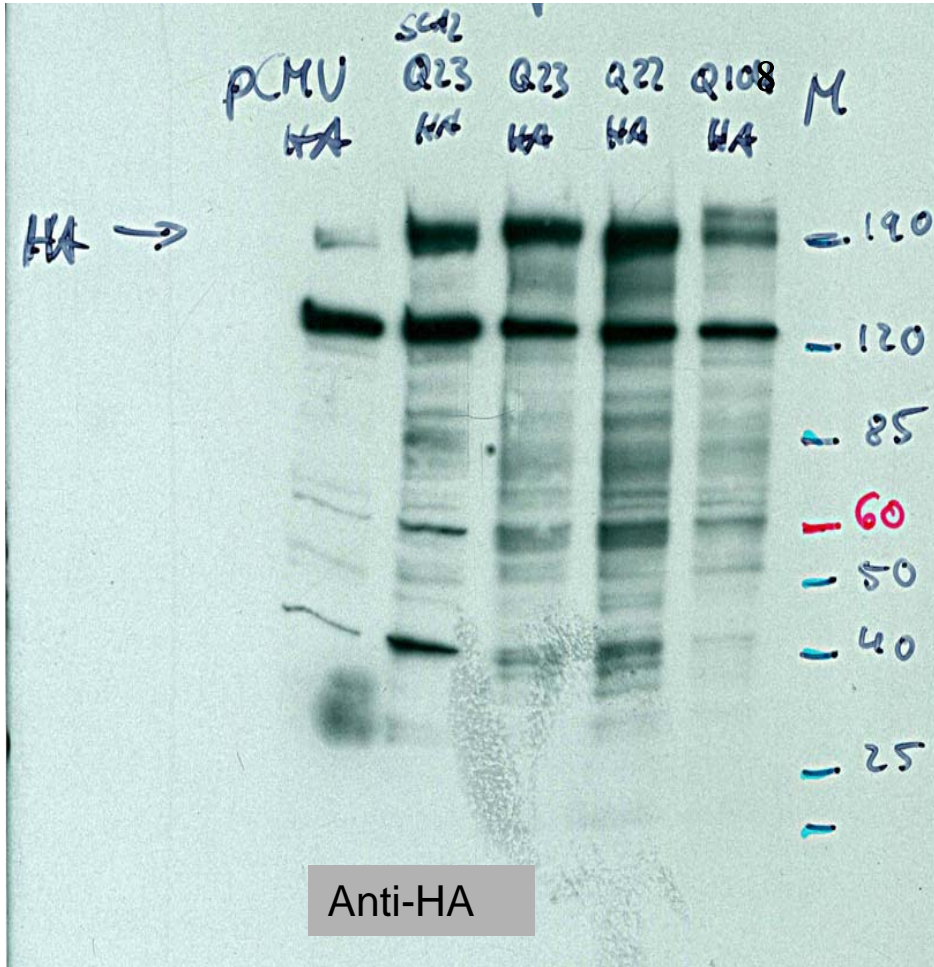
# SCA2-HA-lysates



$\alpha$  SCA2-C

$\alpha$  SCA2-D

# SCA- HA – lysates



# Testing SCA2 C+D Serum on HA-SCA2 Westernblotstripes

