

The S100 calcium binding protein A3 binds directly and specifically to RAR α and PML-RAR and modulates the activity of APL cells



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Aim of the work

- Identification of novel RAR α interacting proteins using a differential proteomic approach

Experimental design

- Flag-vector and Flag-RAR α were introduced in breast MDA-MB453 cells and stable cell lines named respectively FL53 and RA53 were selected
- Cells were treated 1hr with DMSO (vehicle) or ATRA
- Protein extracts of these cell lines were immunoprecipitated with agarose-flag.
- To screen for RAR α binding proteins, a differential proteomic approach based on the quantitative SILAC (Stable Isotope Labelling with Amino acids in Cell culture) was applied

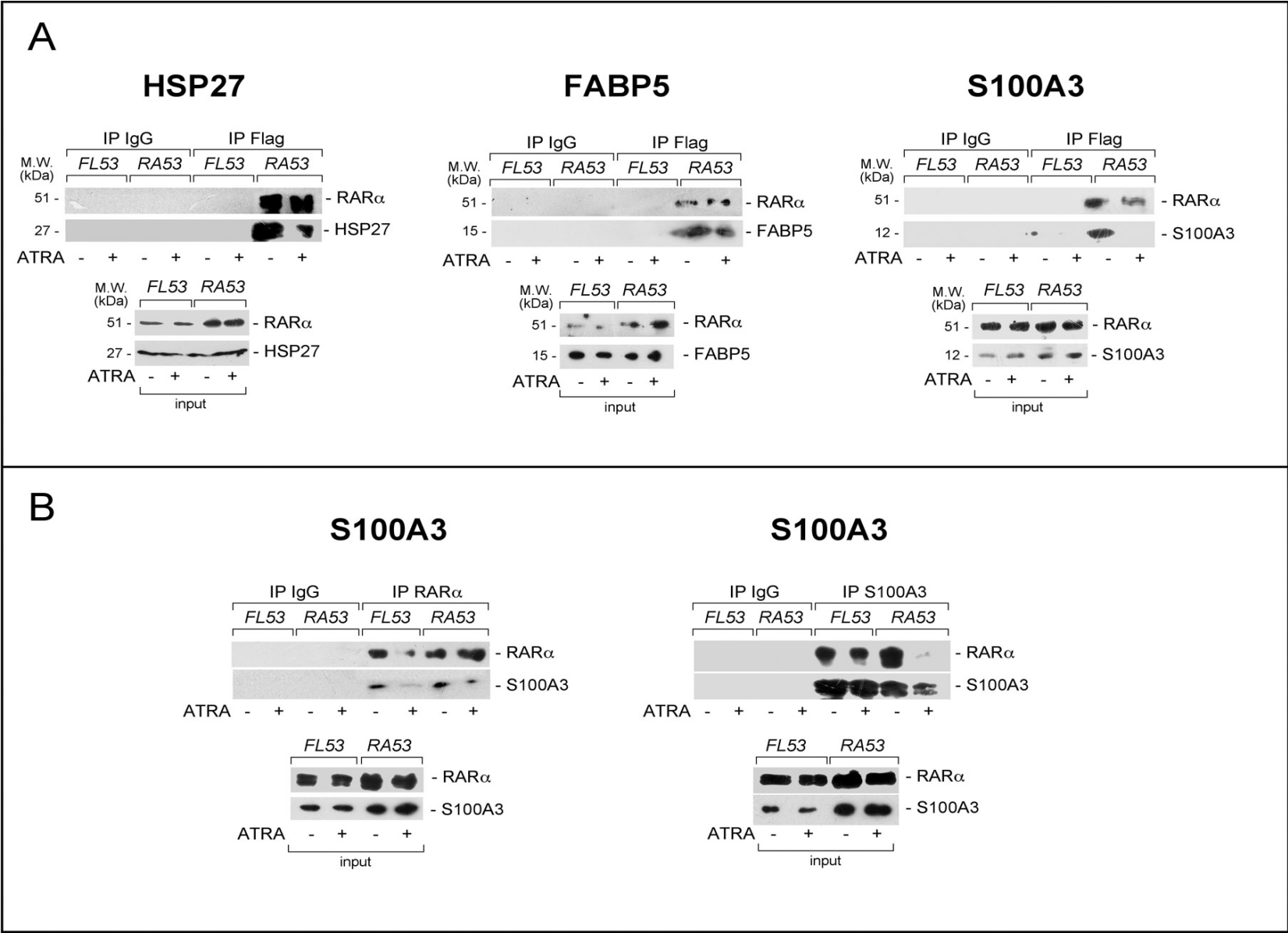
RAR α interacting proteins: ATRA-dependent increase of the binding

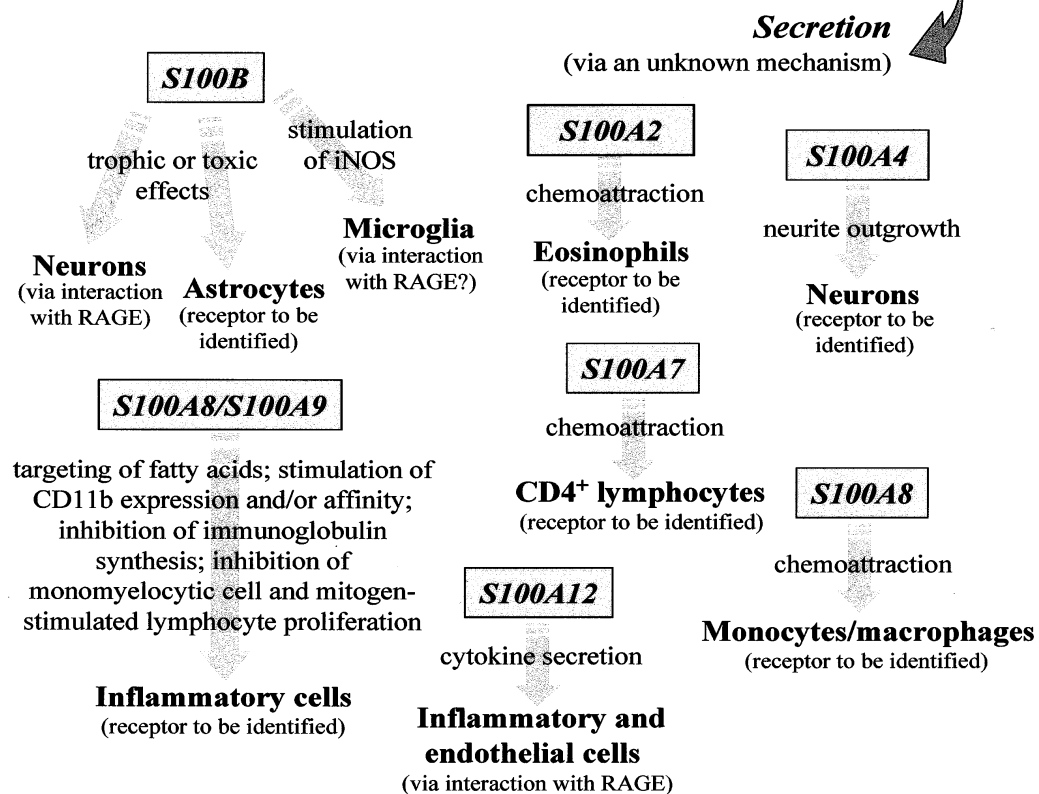
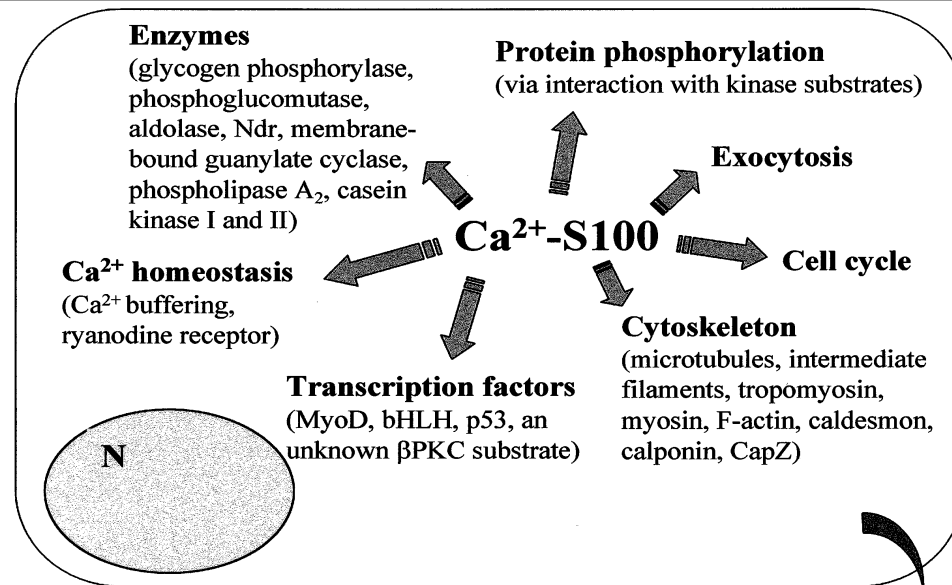
Accession	Description	Mass (kDa)	H/L ratio	unique peptides
<i>DB-fraction</i>				
ROA0_HUMAN	Heterogeneous nuclear ribonucleoprotein A0	31.0	1.09	1
H2A1A_HUMAN	Histone H2A type 1-A	14.2	1.55	2
<i>HIS-fraction</i>				
CEP83_HUMAN	Centrosomal protein of 83 kDa	82.2	1.80	2
H2AW_HUMAN	Core histone macro-H2A.2	40.7	2.03	1
H10_HUMAN	Histone H1.0	20.9	1.60	6
H2A1C_HUMAN	Histone H2A type 1-C	14.1	1.74	7
H2A2B_HUMAN	Histone H2A type 2-B	14.2	1.77	4
H2AV_HUMAN	Histone H2A.V	13.5	1.79	9
H2B1C_HUMAN	Histone H2B type 1-C	13.9	1.85	10
H31T_HUMAN	Histone H3.1t	15.6	1.81	20
RL1D1_HUMAN	Ribosomal L1 domain-containing protein 1	55.9	1.75	1

RAR α interacting proteins: ATRA-dependent decrease of the binding

Accession	Description	Mass (kDa)	H/L ratio	unique peptides
DB-fraction				
GTF2I_HUMAN	General transcription factor II-I	112.9	0.85	2
HNRPK_HUMAN	Heterogeneous nuclear ribonucleoprotein K	51.2	0.71	1
KHDR1_HUMAN	KH domain-containing, RNA-binding, signal transduction-associated protein 1	48.3	0.71	1
LRC15_HUMAN	Leucine-rich repeat-containing protein 15	65.2	0.01	2
PSPC1_HUMAN	Paraspeckle component 1	59.5	0.74	1
RBM3_HUMAN	Putative RNA-binding protein 3	17.2	0.87	1
RU17_HUMAN	U1 small nuclear ribonucleoprotein 70 kDa	51.6	0.76	1
HIS-fraction				
DSG4_HUMAN	Desmoglein-4	115.5	0.01	1
FABP5_HUMAN	Fatty acid-binding protein 5	15.5	0.01	2
HSP7C_HUMAN	Heat shock cognate 71 kDa protein	71.1	0.01	1
HSPB1_HUMAN	Heat shock protein beta-1 or HSP27	22.8	0.01	1
ROA0_HUMAN	Heterogeneous nuclear ribonucleoprotein A0	31.0	0.72	1
AT8B2_HUMAN	Phospholipid-transporting ATPase	138.6	0.01	1
PKP3_HUMAN	Plakophilin-3	87.5	0.78	2
S10A3_HUMAN	Protein S100-A3	12.3	0.01	1
SBP1_HUMAN	Selenium-binding protein 1	52.9	0.01	3
RS27A_HUMAN	Ubiquitin-40S ribosomal protein S27a	18.3	0.01	2

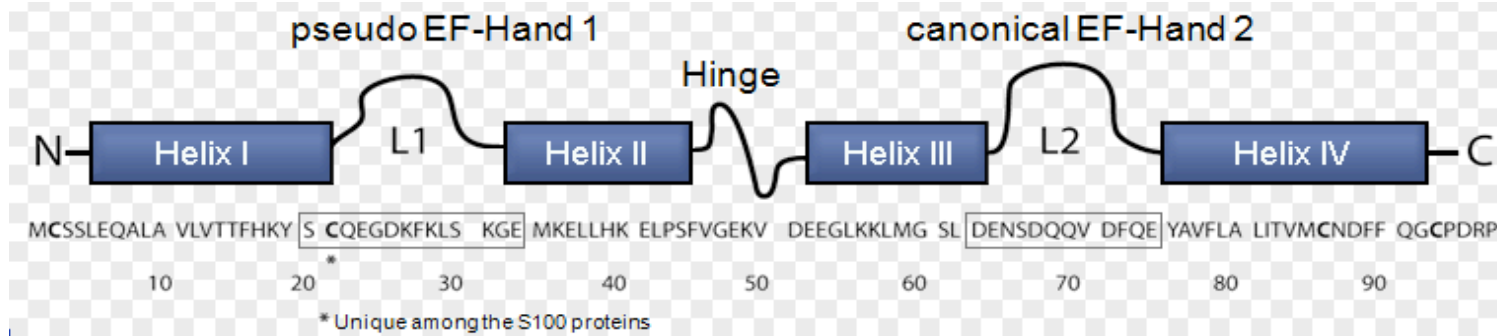
Validation of the interaction between HSP27, FABP5 and S100A3 with RAR α





Prof. Rosario Donato
The International Journal of Biochemistry &
Cell Biology 33 (2001) 637–668

S100: a multigenic family of calcium-modulated proteins of the EF - hand type with intracellular and extracellular functional roles



Schematic representation of the secondary structure of an S100 protein.

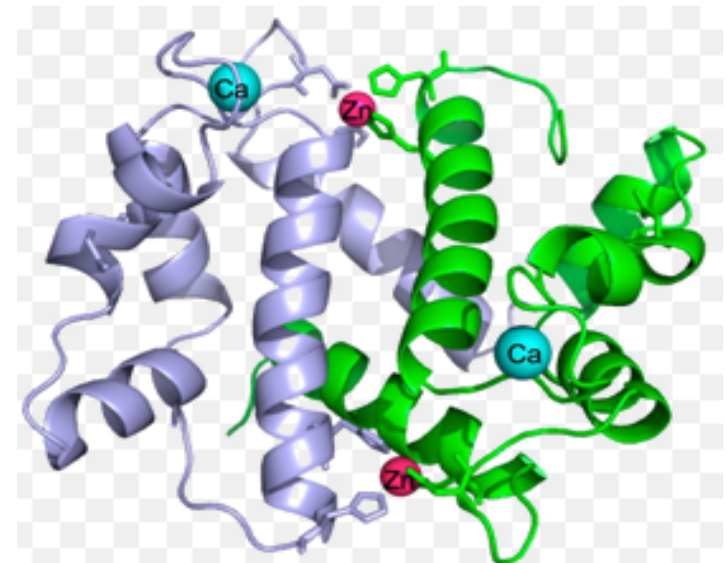
S100A3 primary sequence

10	20	30	40	50
MARPLEQAVA	AIVCTFQEYA	GRCGDKYKLC	QAELKELLQK	ELATWTPTEF
60	70	80	90	100
RECDYNKFMS	VLDTNKDCEV	DFVEYVRSLA	CLCLYCHEYF	KDCPSEPPCS

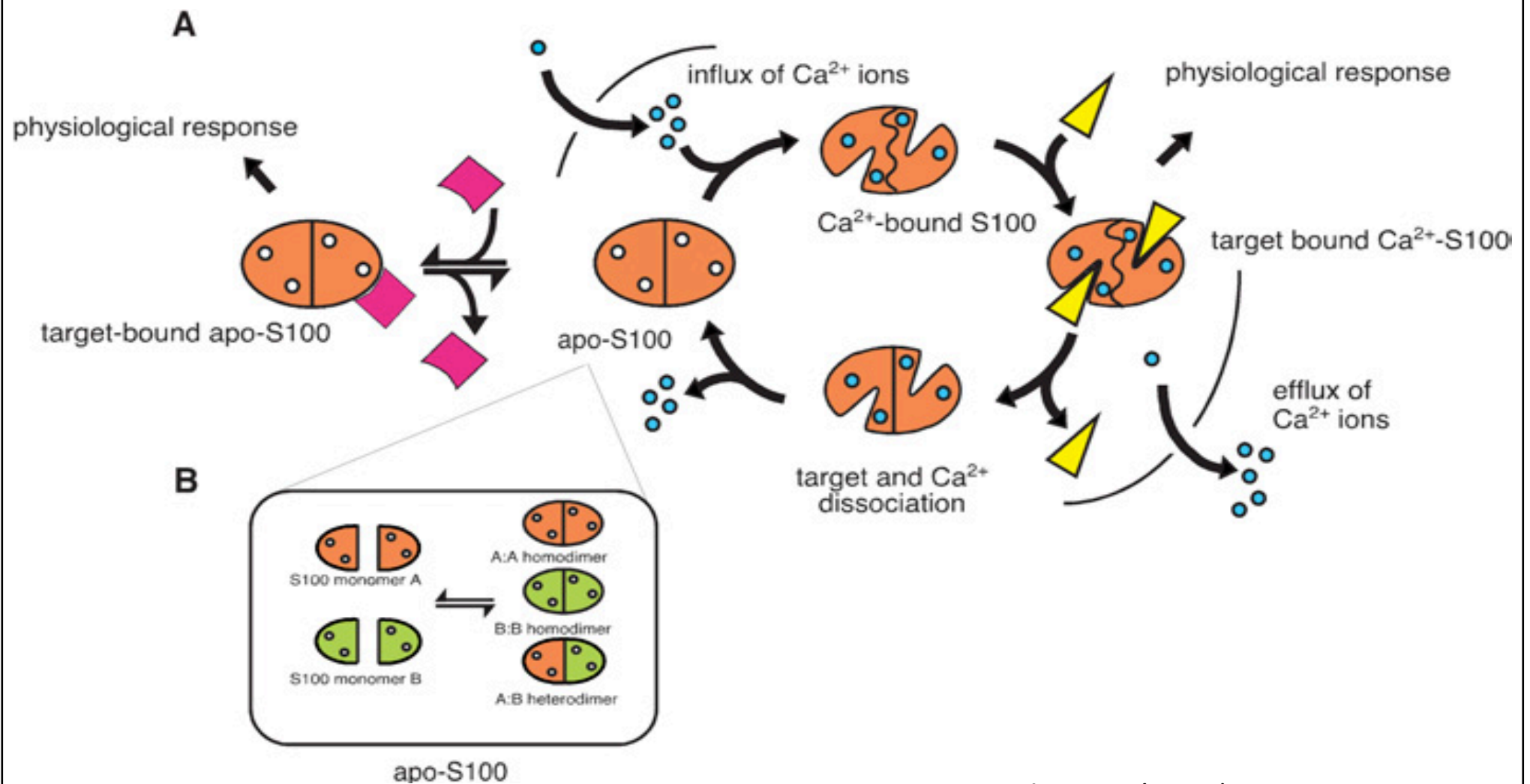
Q

- Ca⁺⁺ domain
- Zn⁺⁺ domain
- N-terminal non-canonical EF-hand
- C-terminal EF-hand

S100A3 dimer 3D structure

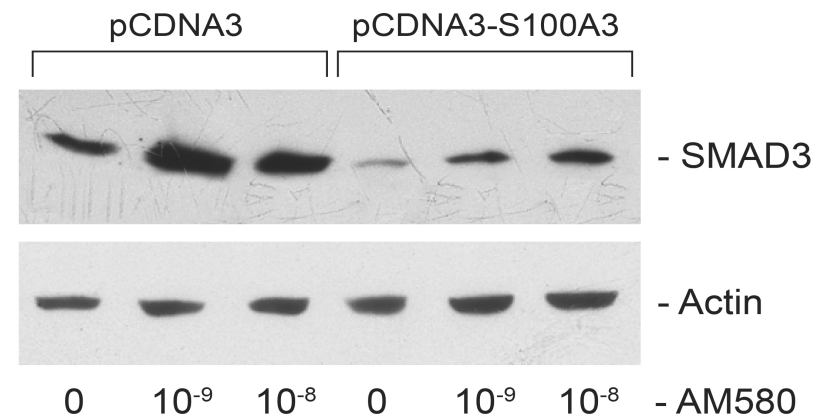
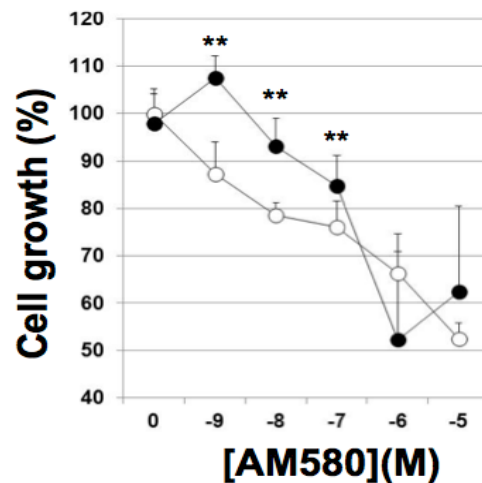
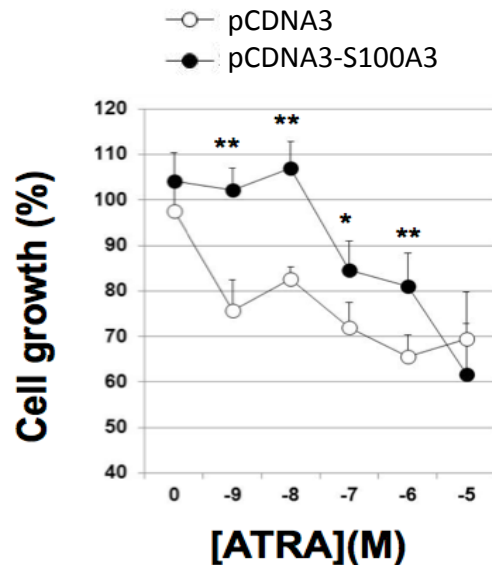
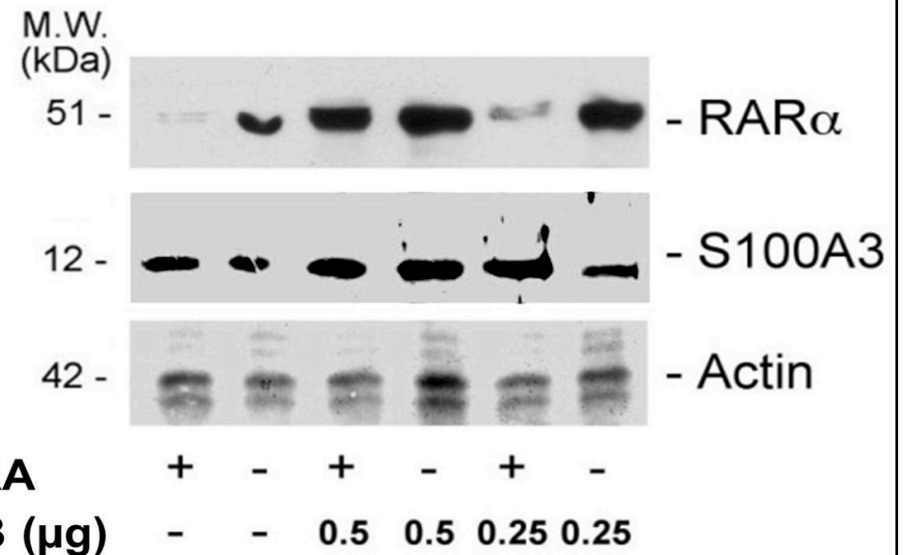


Calcium-dependent and -independent interactions of the S100 family



SKBR3 cells over-expressing S100A3 are characterized by:

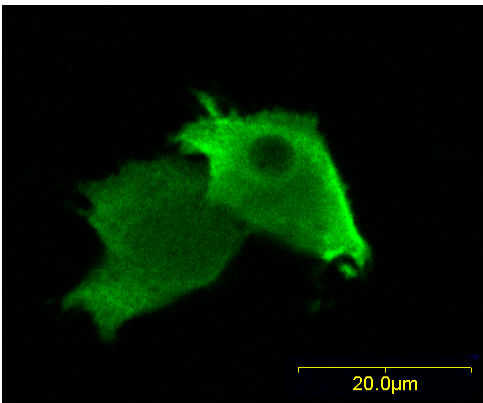
- increase in RAR α expression
- decrease responsiveness to ATRA



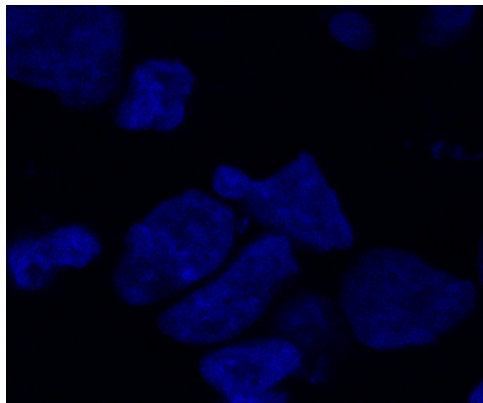
In COS-7 cells and in RA53 cells over-expressing RAR α and S100A3

- S100A3 signal is diffused in whole cell but it accumulates on cell membrane
- RAR α signal is present only in the nucleus

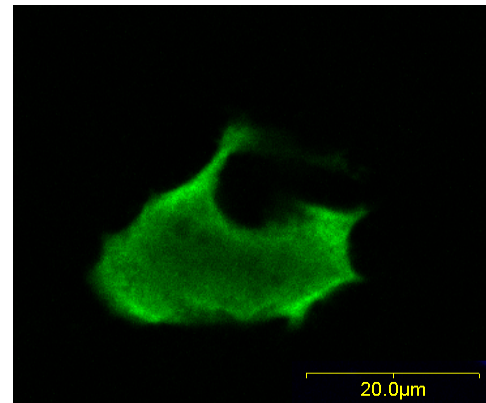
S100A3



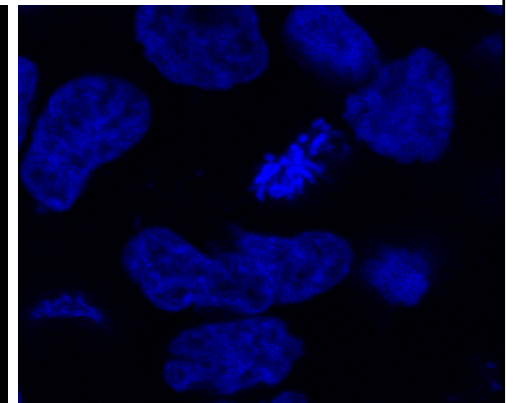
HOECHST



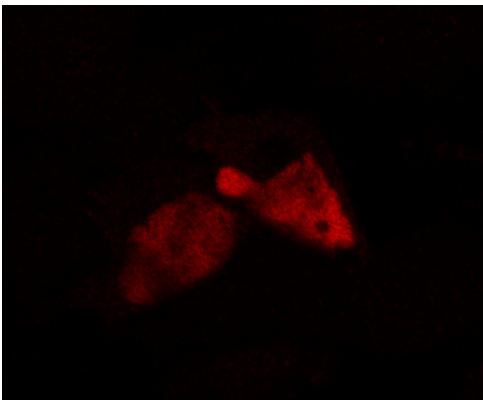
S100A3



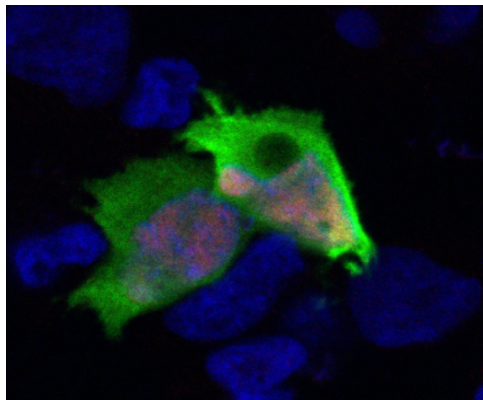
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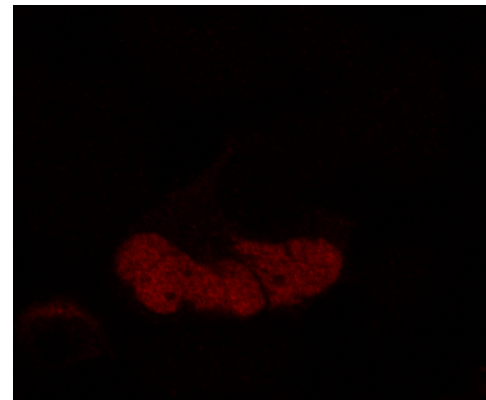
RAR α



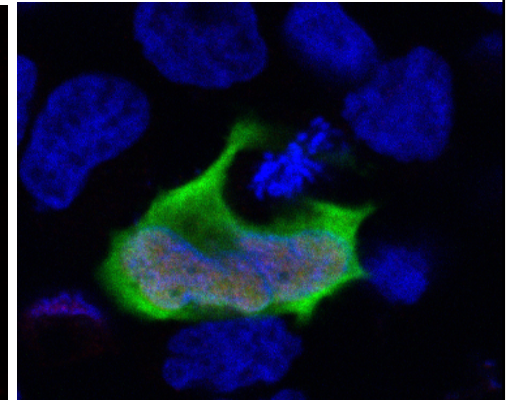
S100A3+RAR α



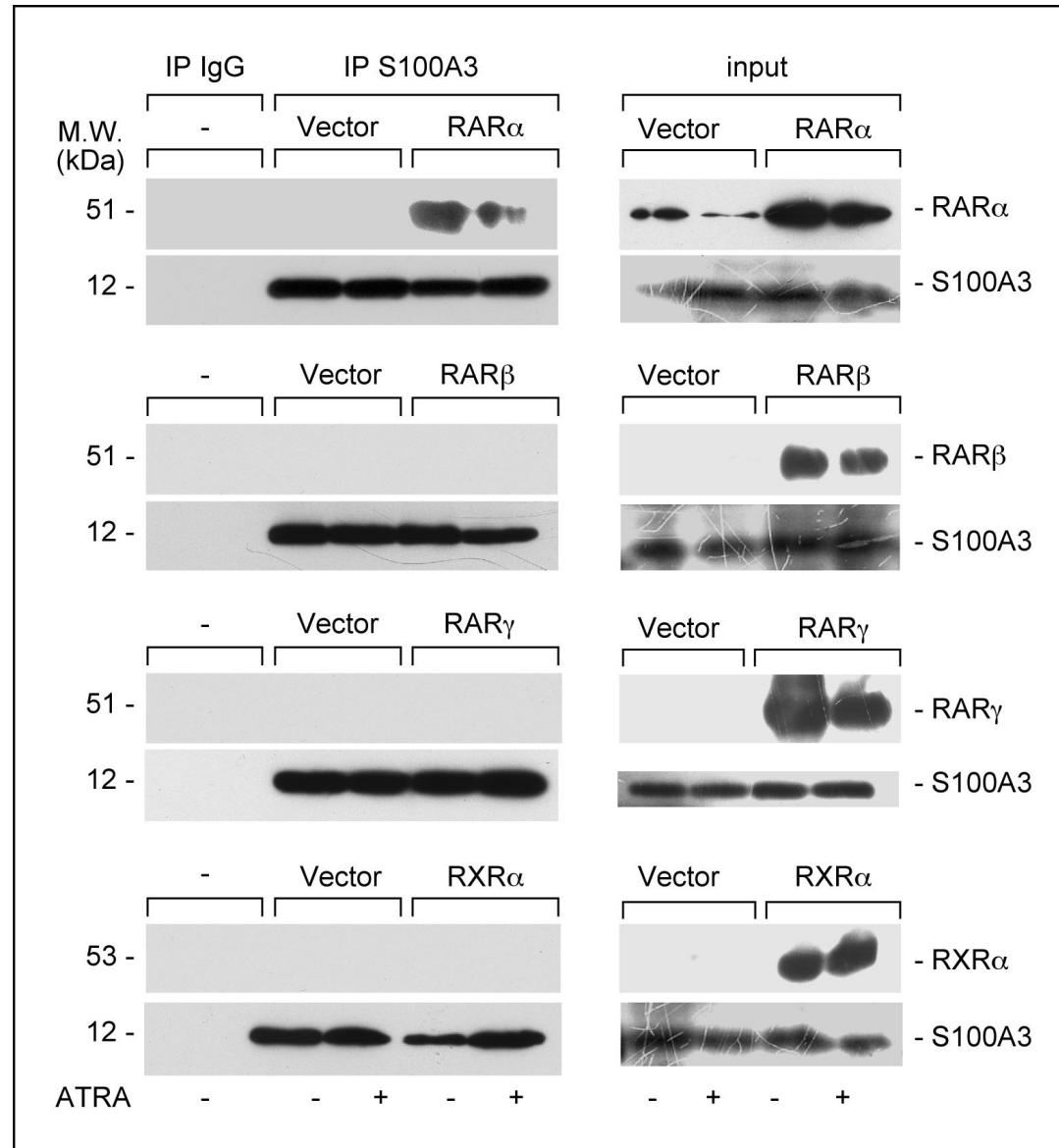
RAR α



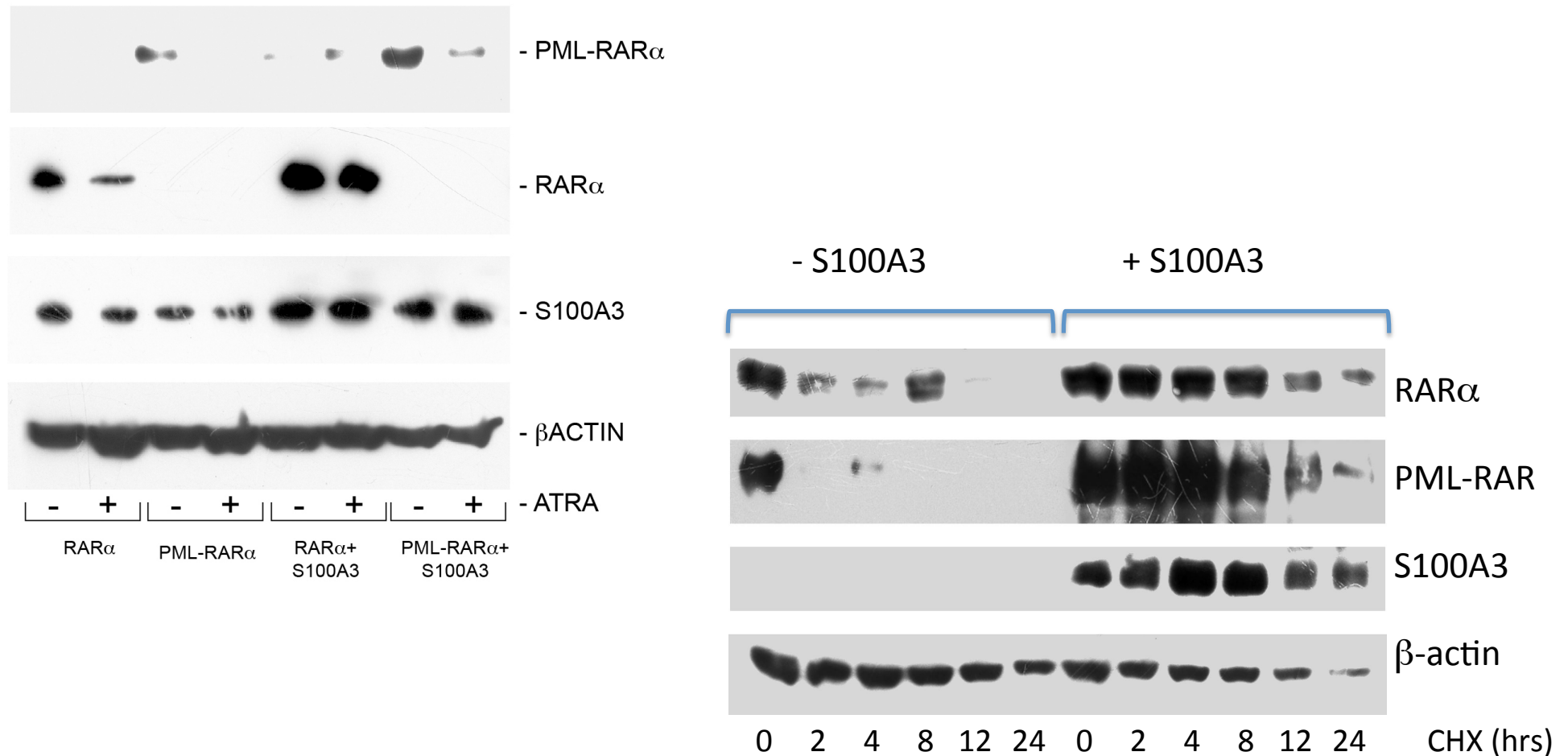
S100A3+RAR α



In COS-7 cells, S100A3 interacts with RAR α only .

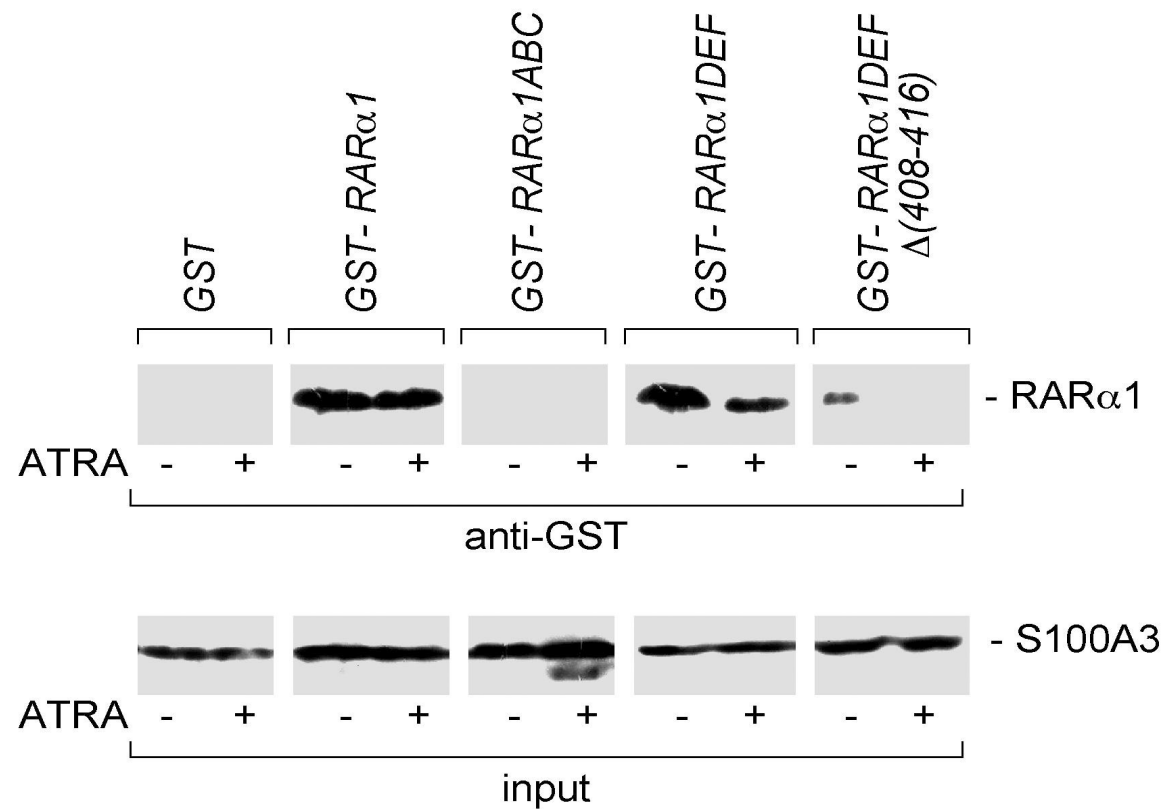


- In COS-7 cells , over-expression of S100A3 induces an increase in RAR α and PML-RAR expression
- S100A3-induced increase of RAR α and PML-RAR is the result of a **decreased degradation** of the two receptors.



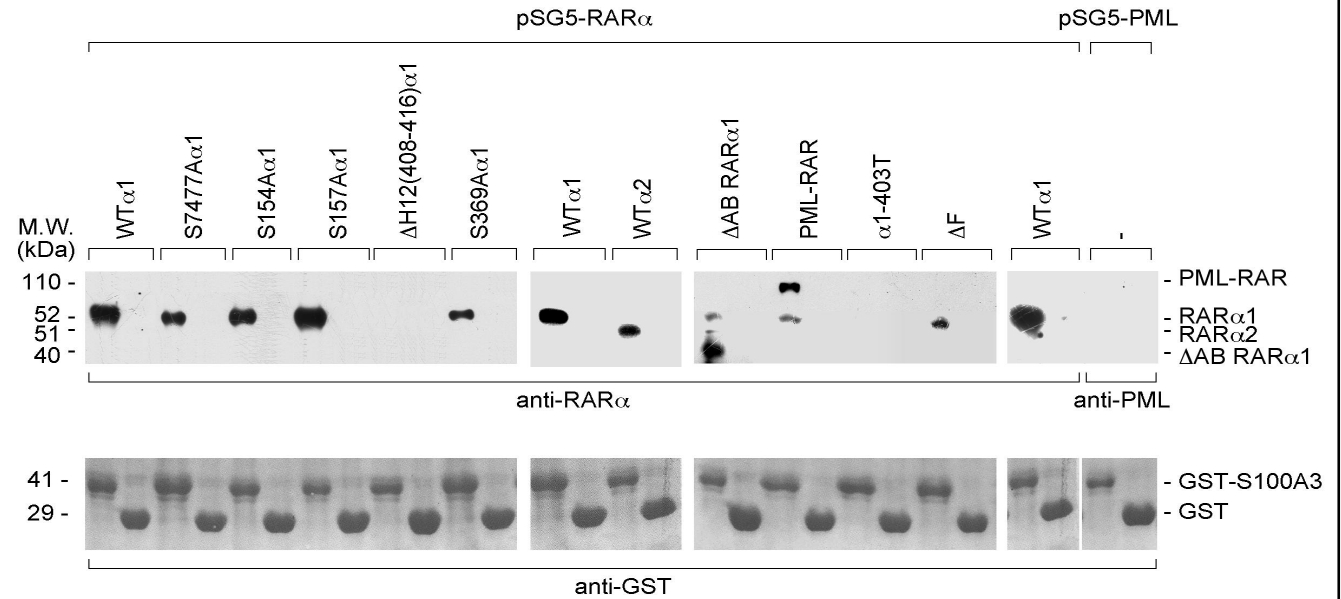
S100A3 interacts directly with RAR α

Far-Western

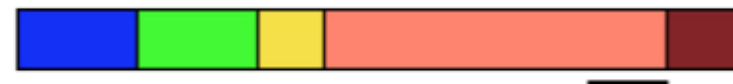
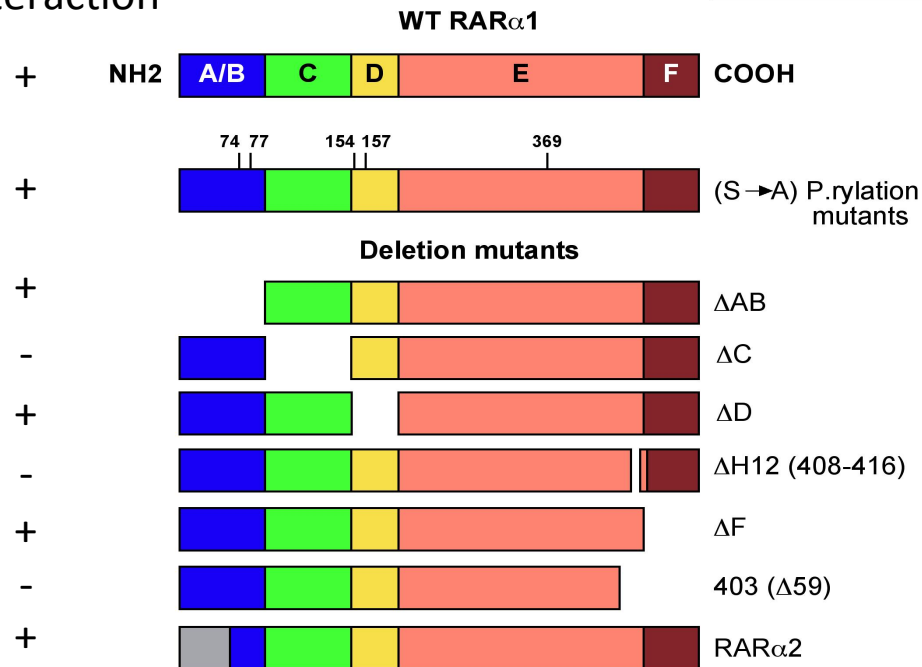


Identification of the domain of RAR α protein interacting with S100A3

GST-pull down

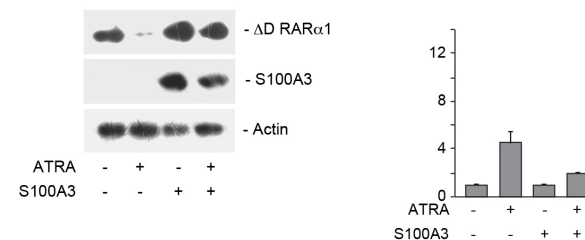
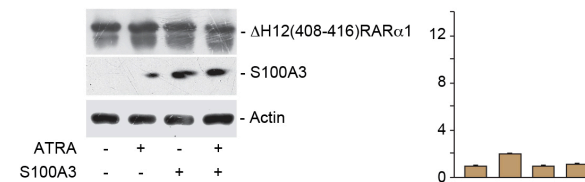
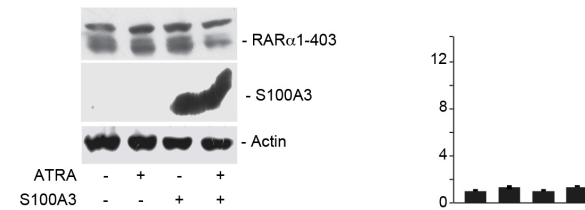
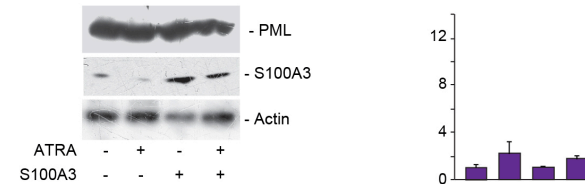
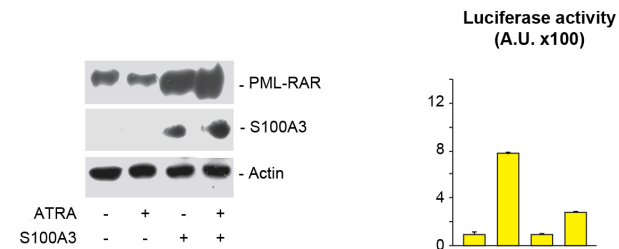
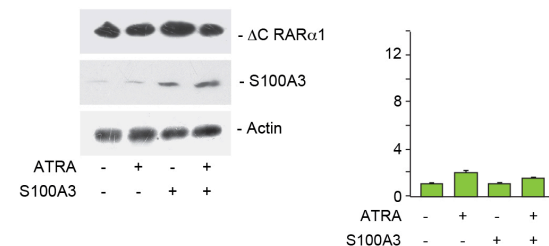
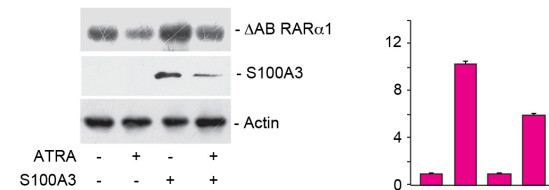
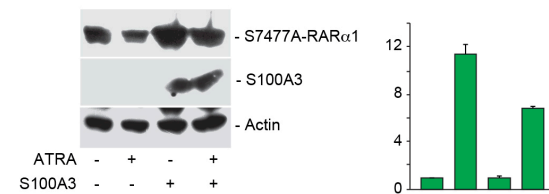
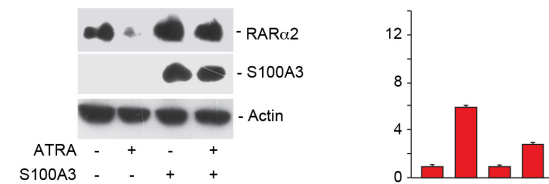
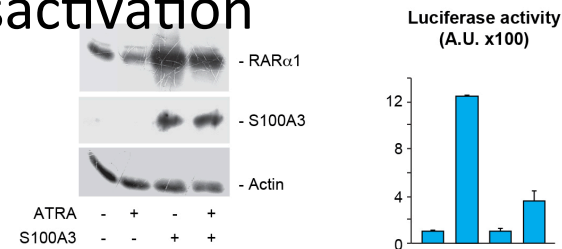


interaction

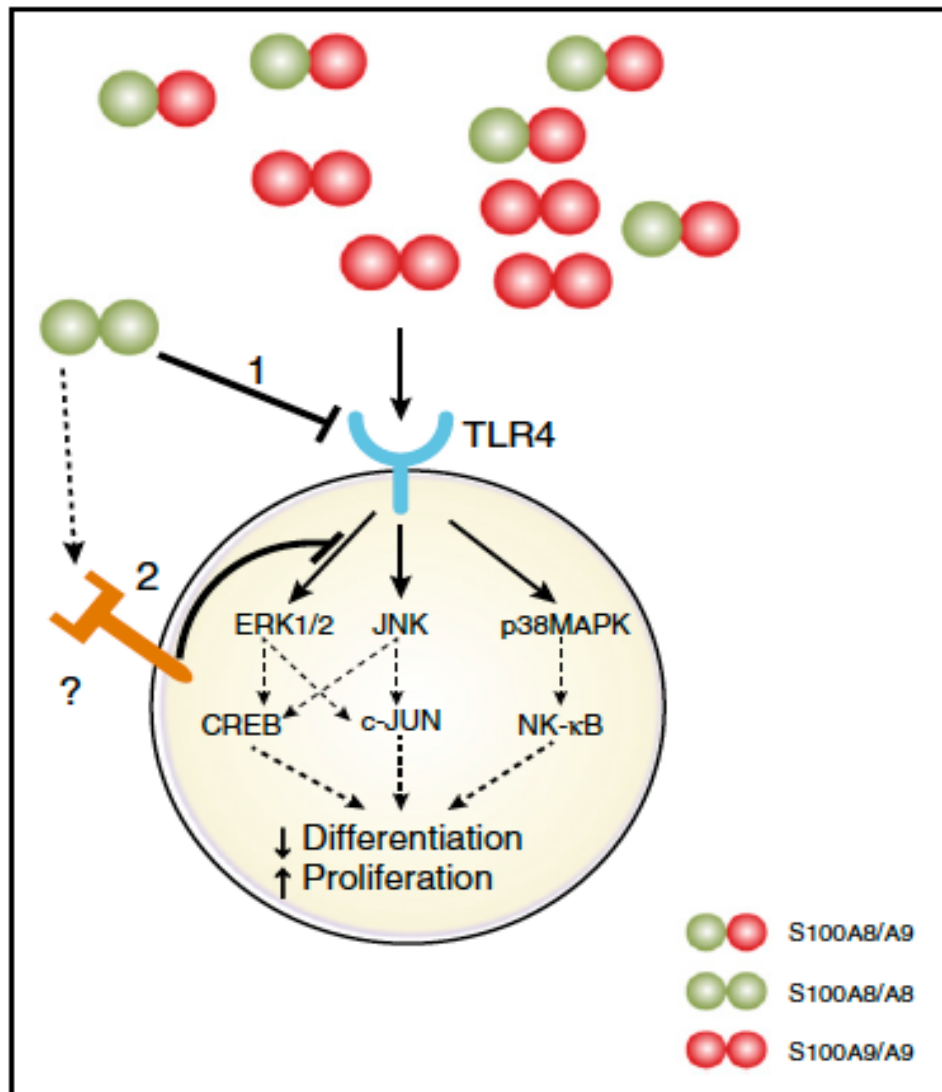


Part of RAR α E region interacting with S100A3

S100A3 stabilization correlates with a decrease of RAR α or PML-RAR-mediated transactivation



Effects of S100A8 and S100A9 on AML cell differentiation and proliferation

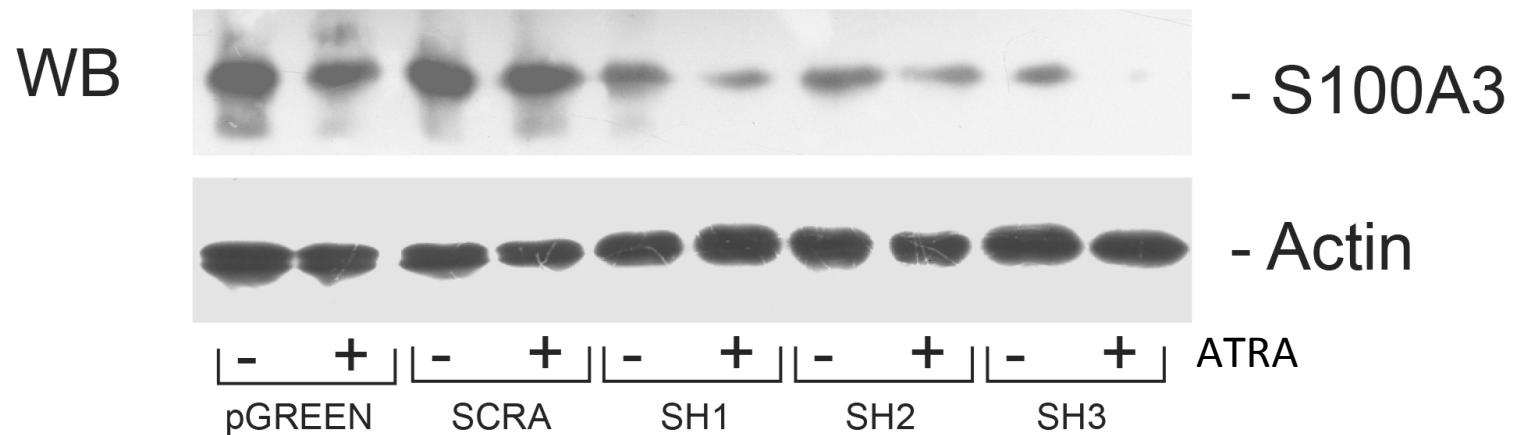


- S100A9 induces differentiation and growth arrest of AML cells via TLR4.
- S100A8 regulates S100A9 activity and sustains AML immature phenotype
- ATRA-mediated expression of S100A9 in APL cells promotes apoptosis and growth inhibition

NB4 cells infected with shRNA against S100A3

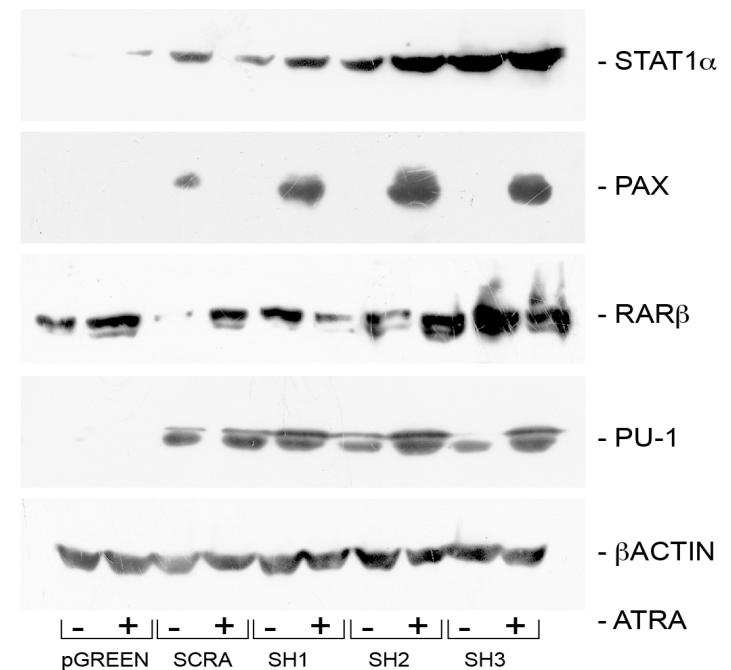
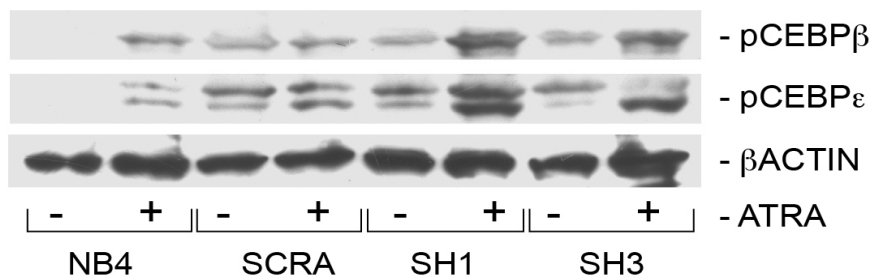
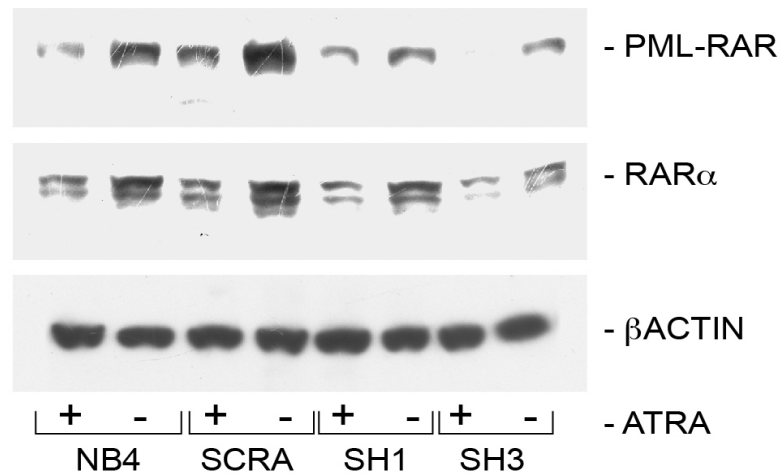
5 cell population were selected and expanded:

- pGreen (empty vector)
- SCRA (sh scramble)
- SH1 (SH1-S100A3)
- SH2 (SH2-S100A3)
- SH3 (SH3-S100A3)

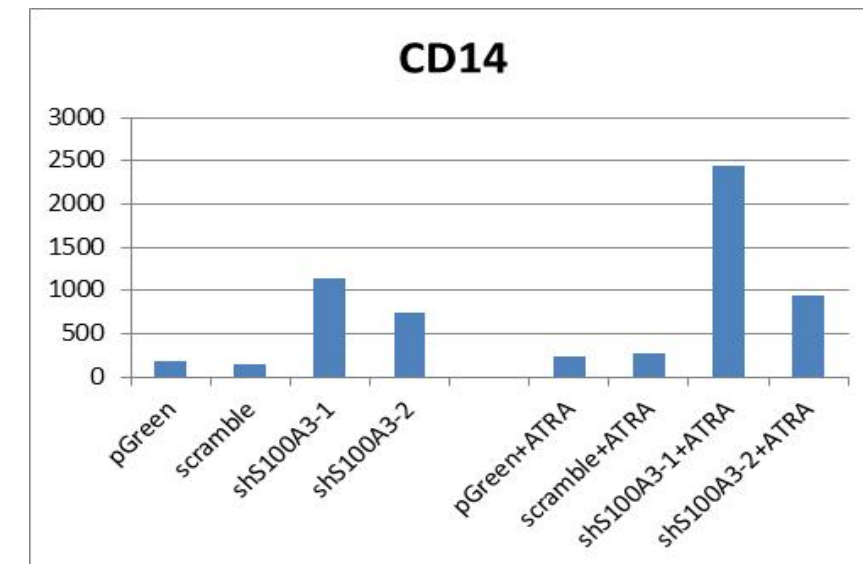
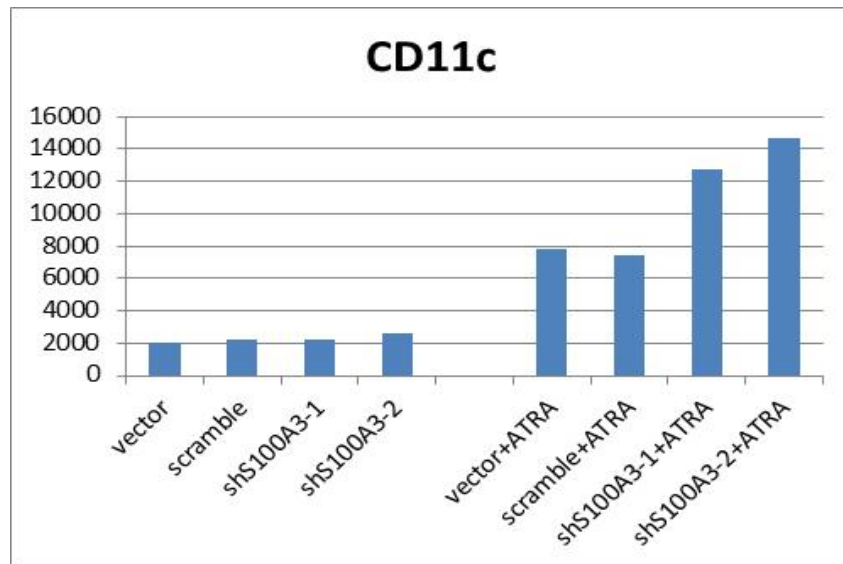
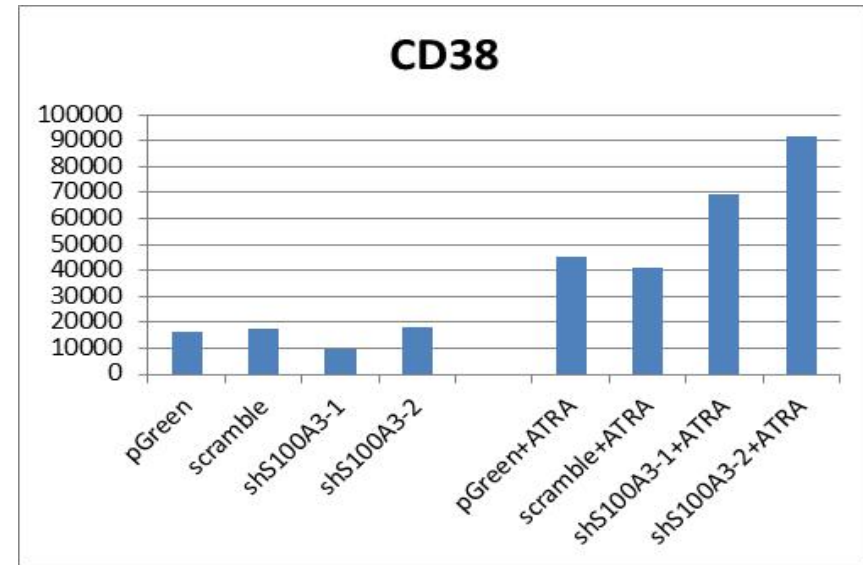
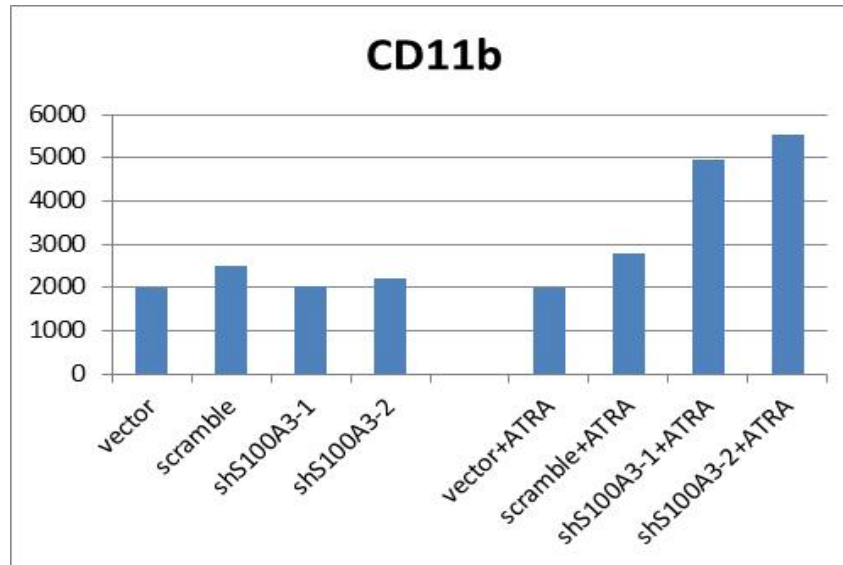


In NB4 silencing of S100A3

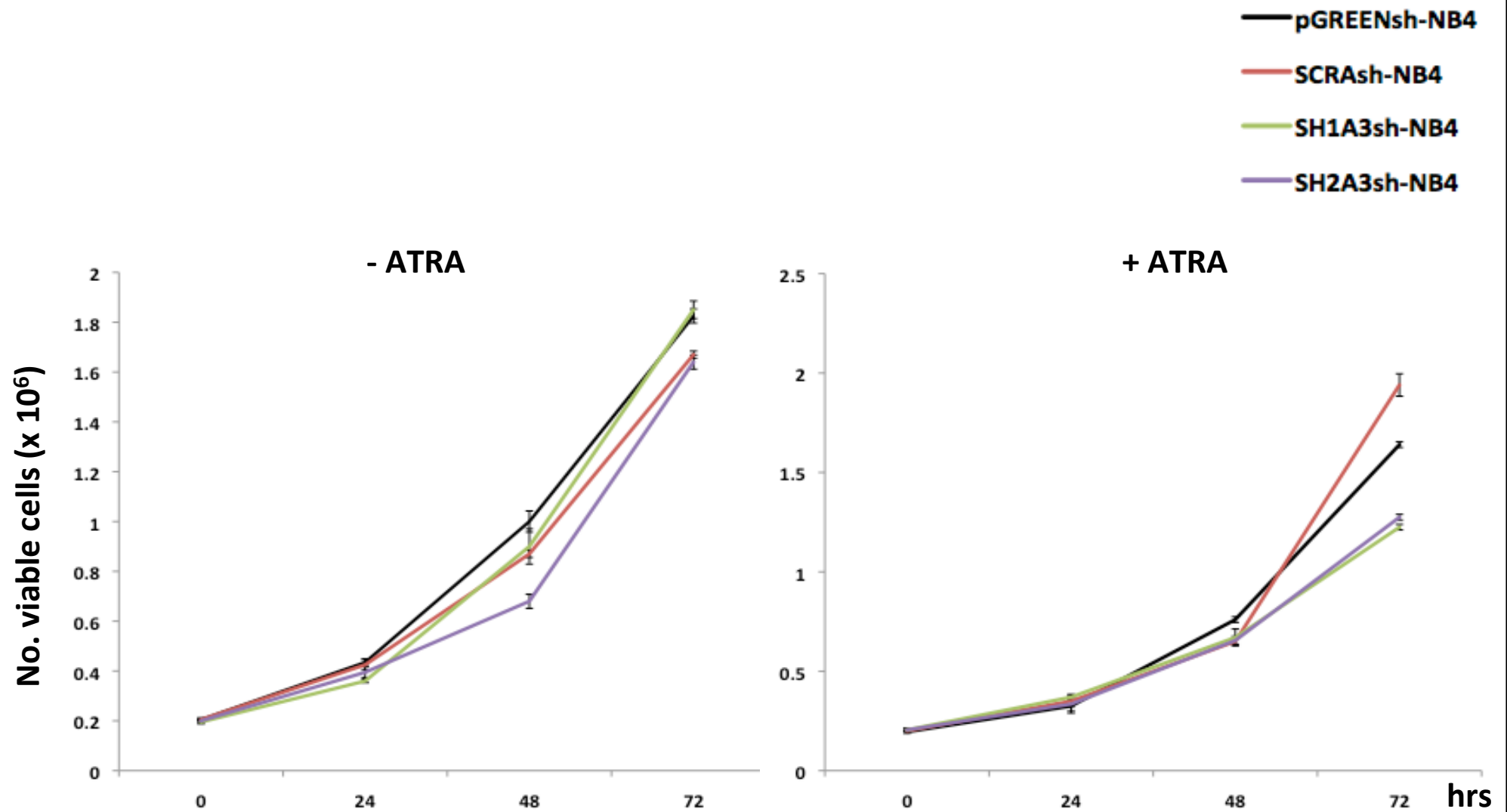
- decrease RAR α and PML-RAR expression
- increase responsiveness of different markers of NB4 ATRA-mediated differentiation



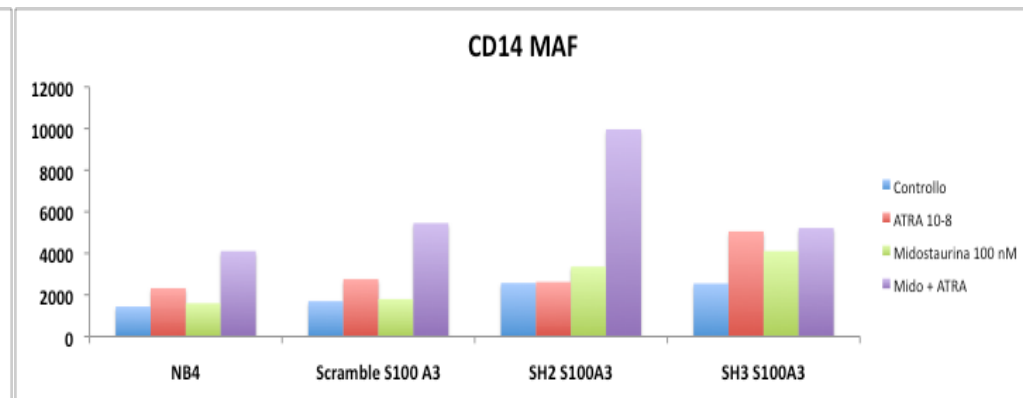
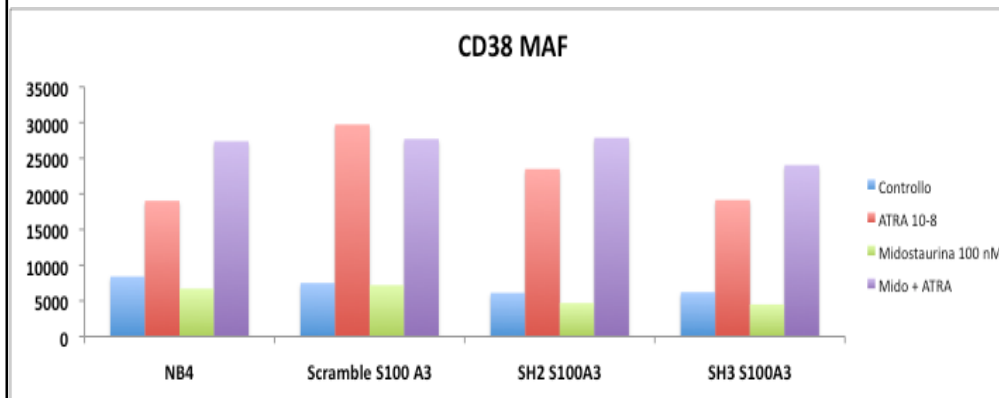
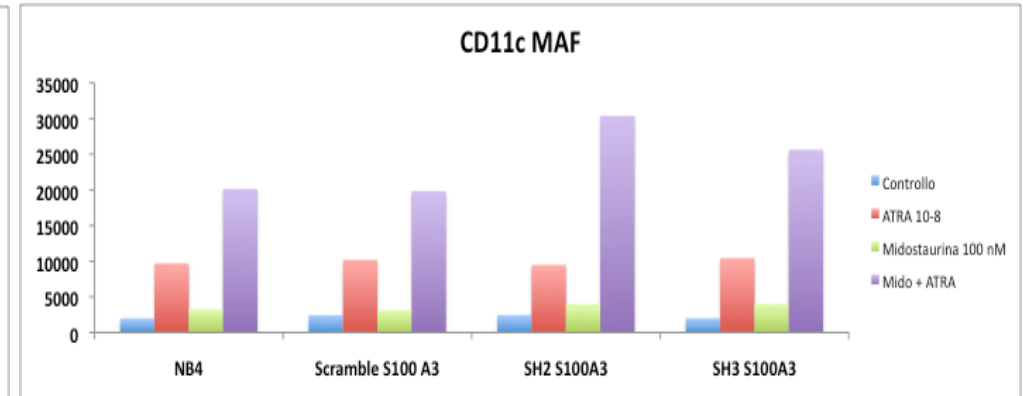
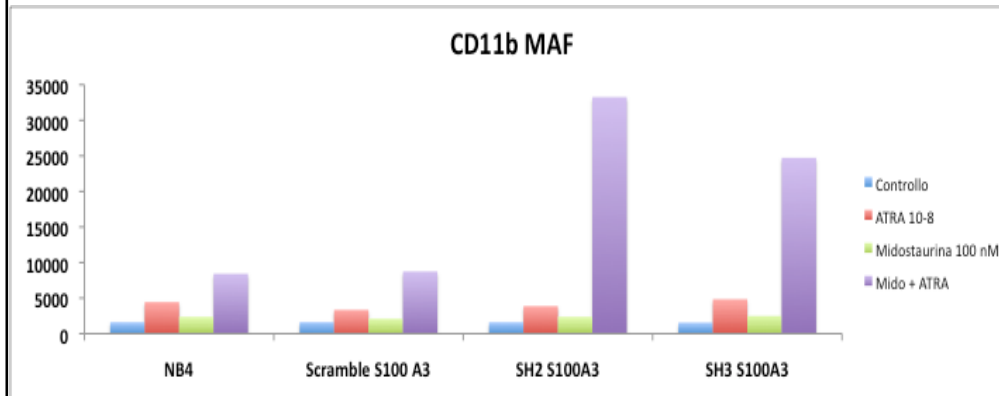
Expression of granulocytic and monocyte differentiation markers in S100A3 silenced NB4 cell lines

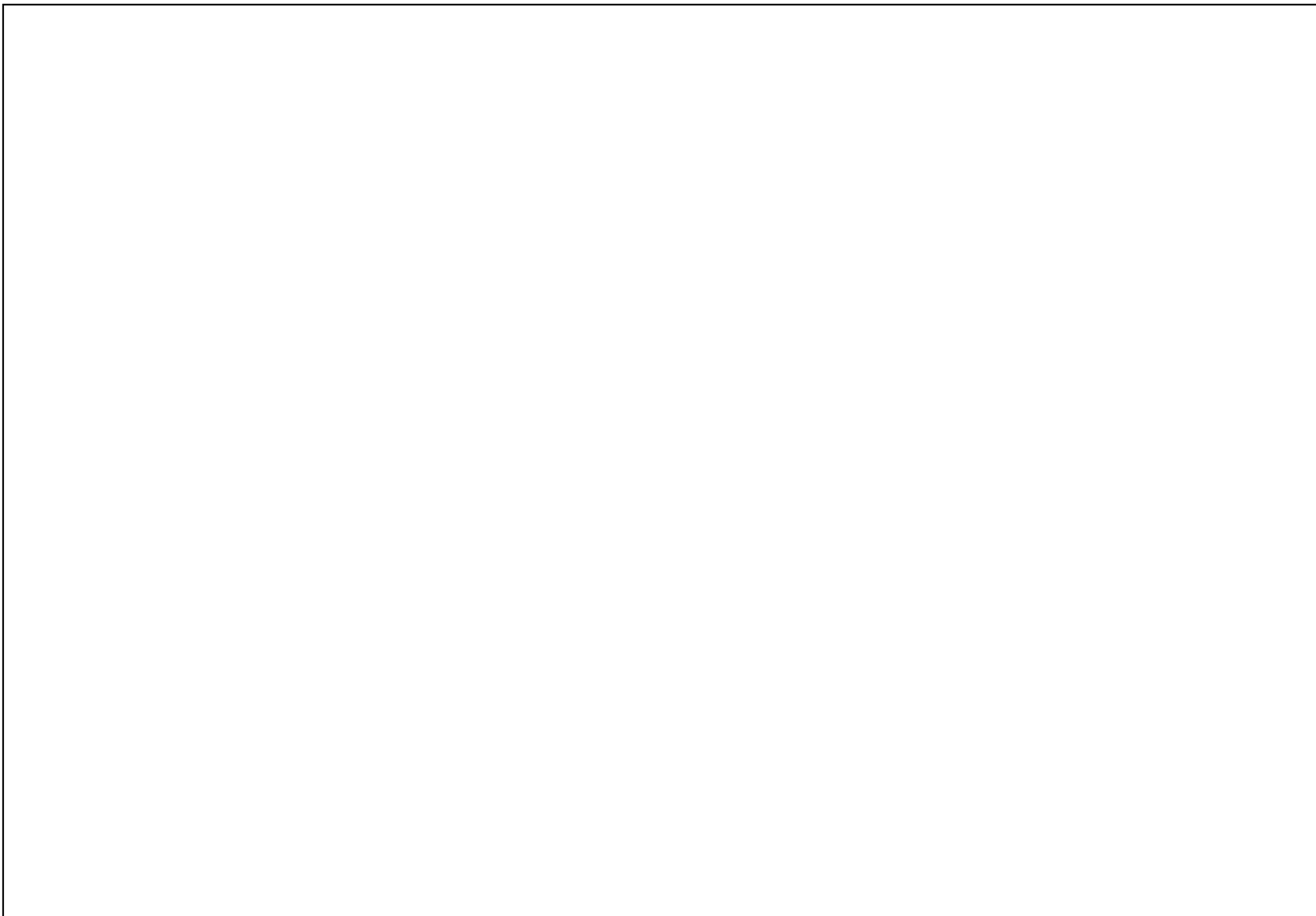


Silencing of S100A3 in NB4 cells decrease ATRA-dependent cell growth



Synergistic effect of Midostaurin (inhibitor of FLT3) and ATRA in NB4 cells





RAR α interactome

Flow chart of the experiment

