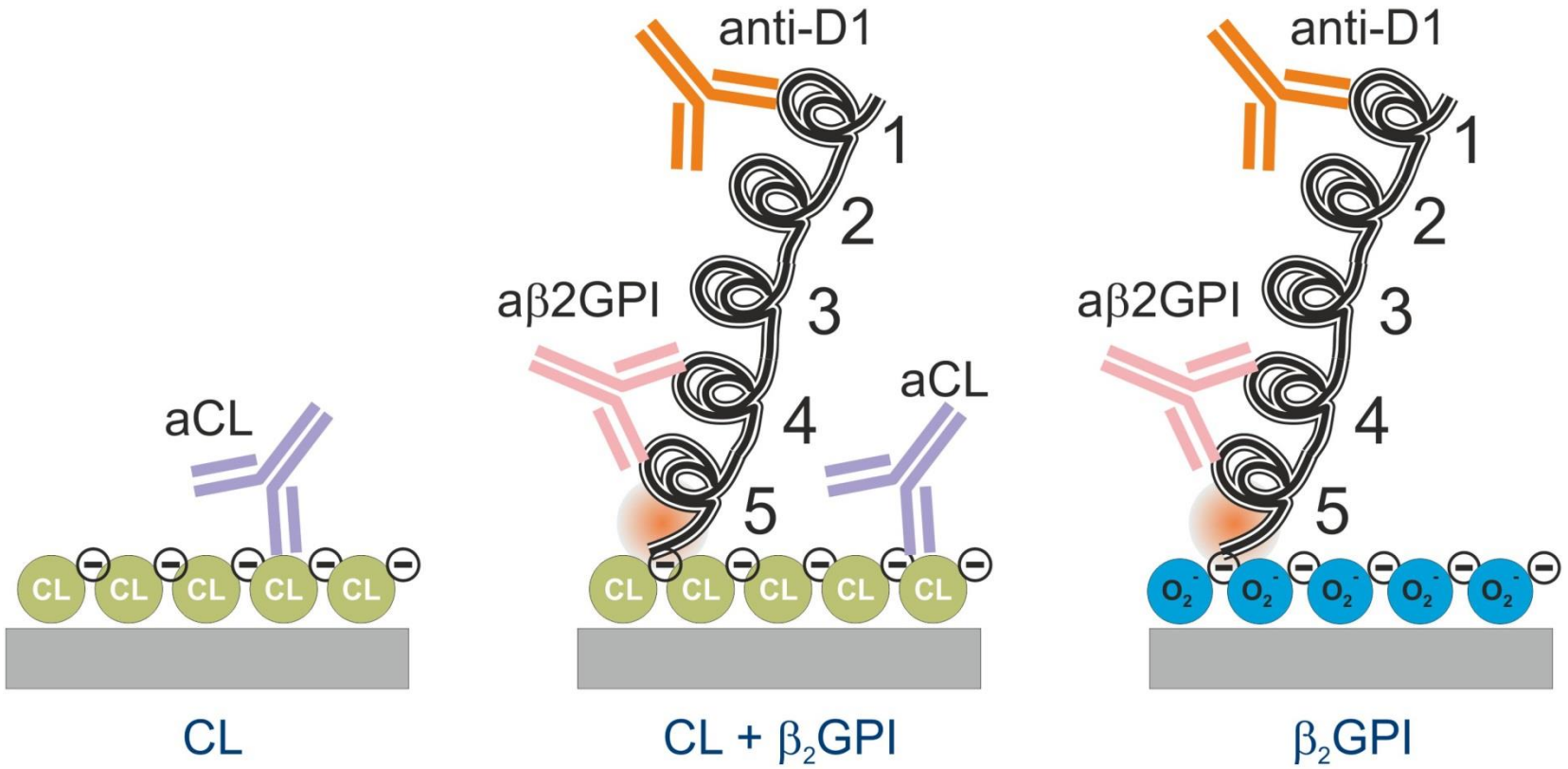
Antiphospholipid antibody-induced cellular responses depend on epitope specificity

Karl J. Lackner

Are all aPL Equal ?

- Can we differentiate aPL by diagnostic assays?
- Can we differentiate aPL by their effects?
- Do lipid reactive, cofactor independent aPL have a role in APS?

Differentiation of aPL by Immunoassays

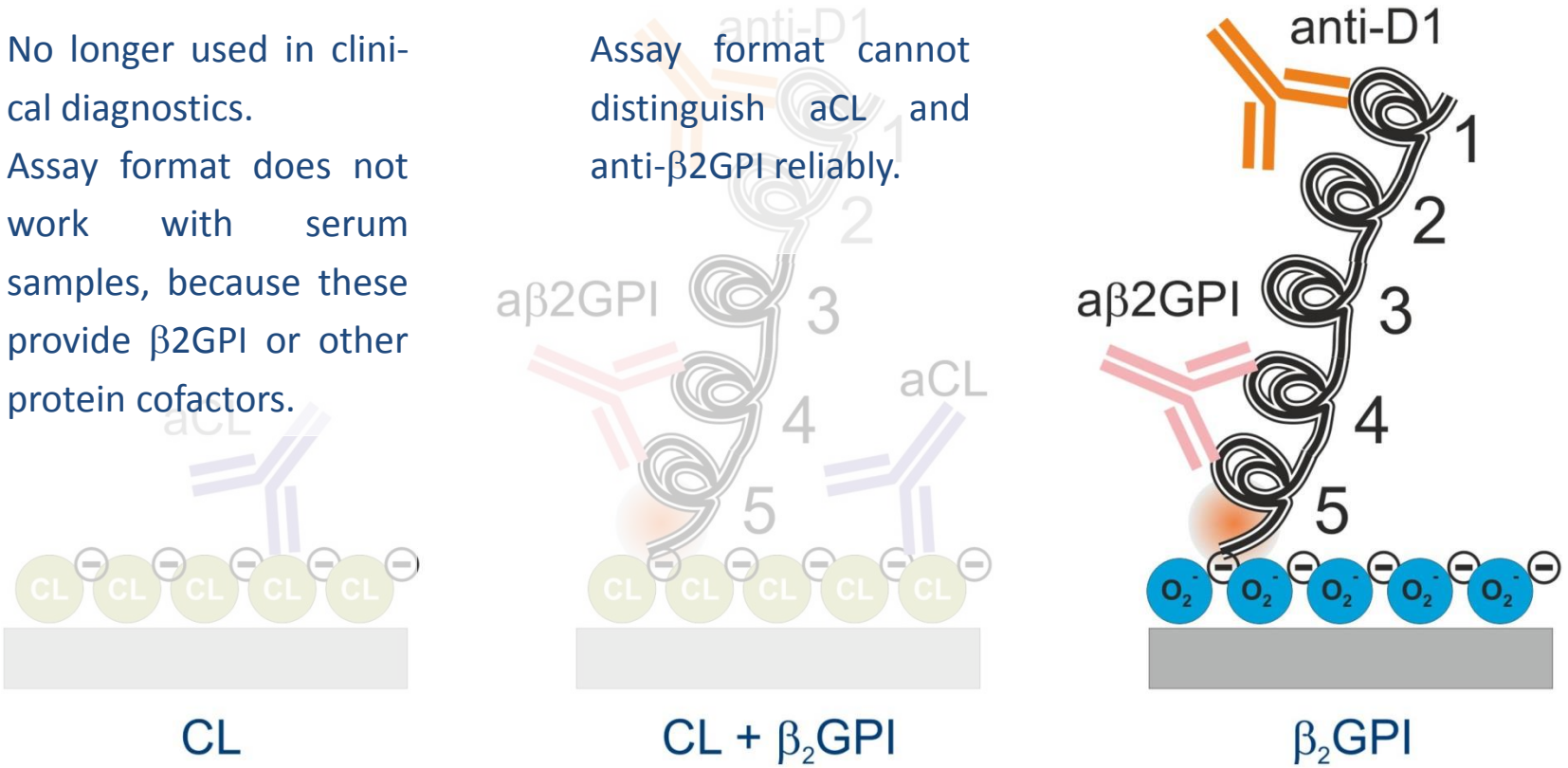


Differentiation of aPL by Immunoassays

No longer used in clinical diagnostics.

Assay format does not work with serum samples, because these provide β2GPI or other protein cofactors.

Assay format cannot distinguish aCL and anti-β2GPI reliably.

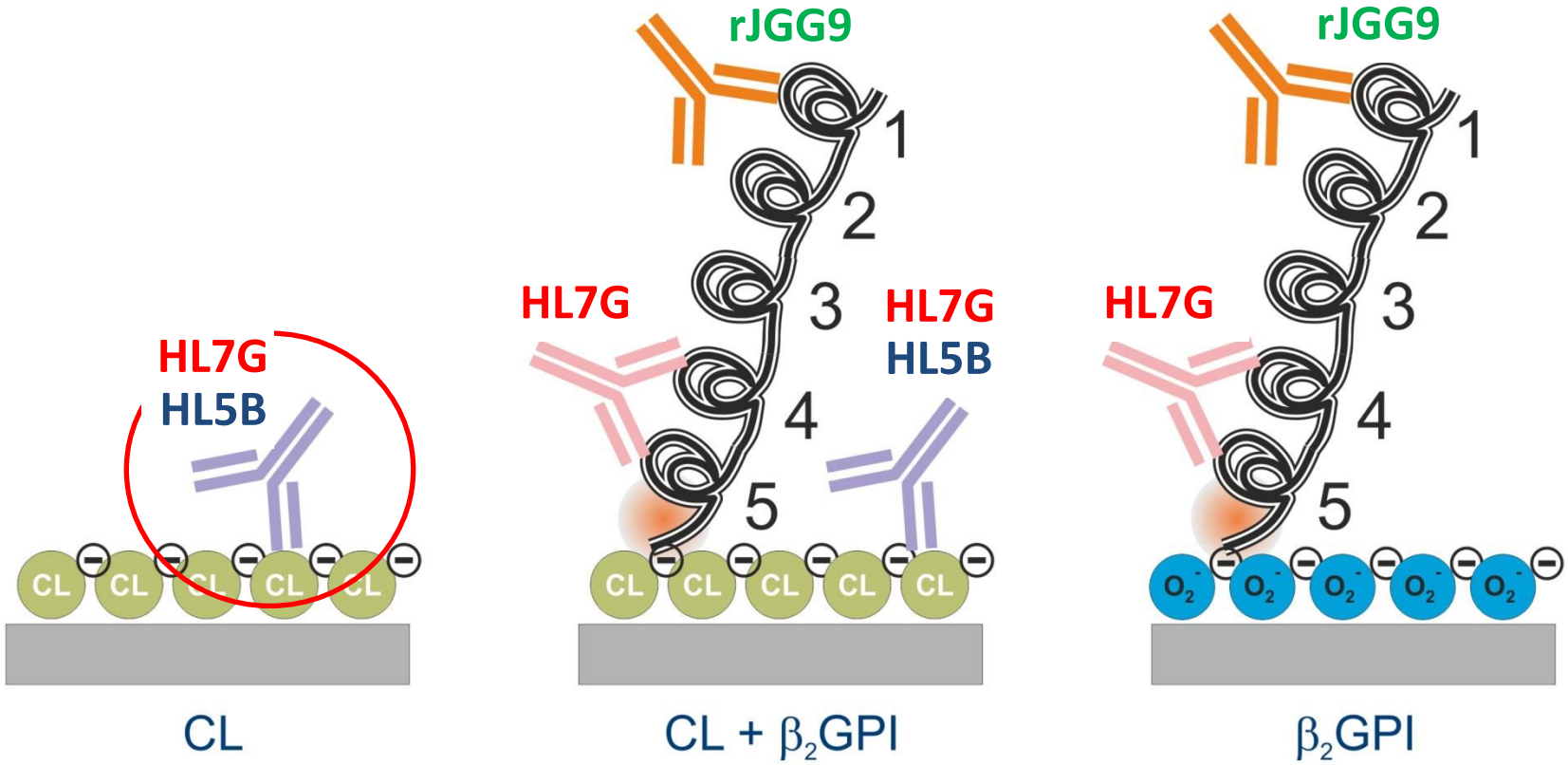


Human Monoclonal aPL

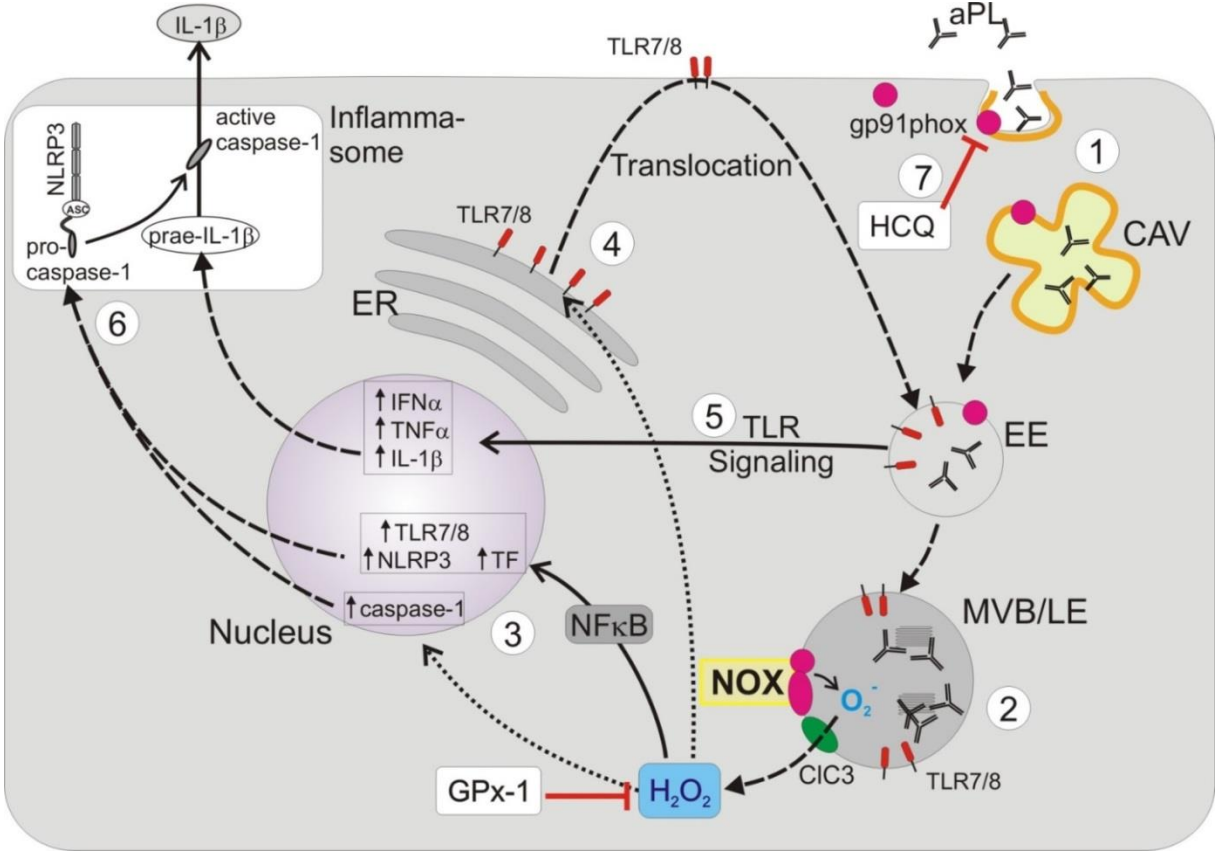
Antibody	Isotype	Somatic Mutations	Cardio-lipin	Cofactor Dependency	β2GPI
HL5B	IgG	++	+	no	-
RR7F	IgG	∅	+	no	-
HVA2	IgM	+	+	no	-
HL7G	IgG	++	+	no*	+
JGG9	IgM	++	+	yes	+ (D1)
rJGG9	IgG	++	+	yes	+ (D1)

*) binding is increased by β2GPI

Human Monoclonal aPL



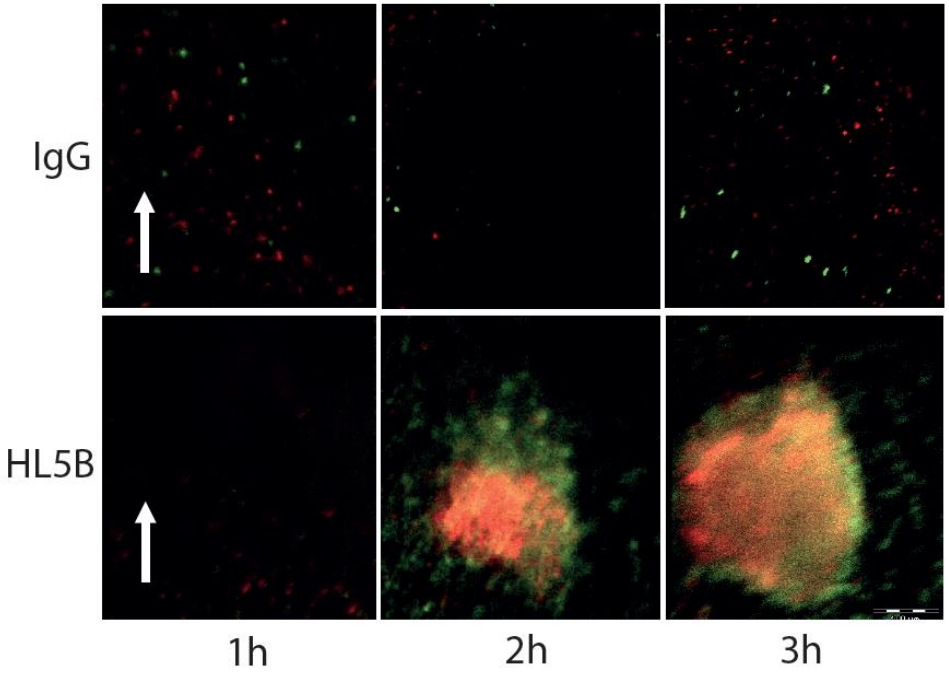
Cellular Effects of “Anticardiolipin“ Antibodies

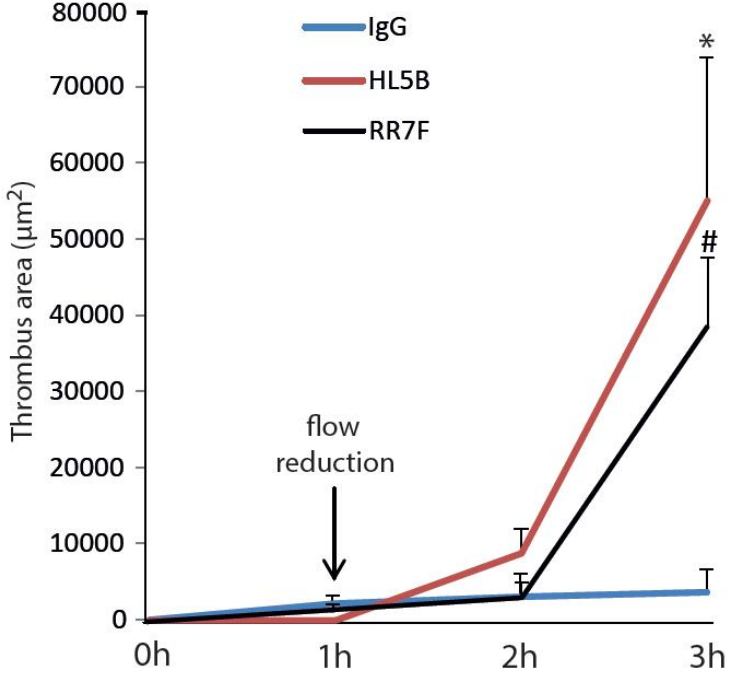


Lackner KJ et al. Curr Rheumatol Rep 2017

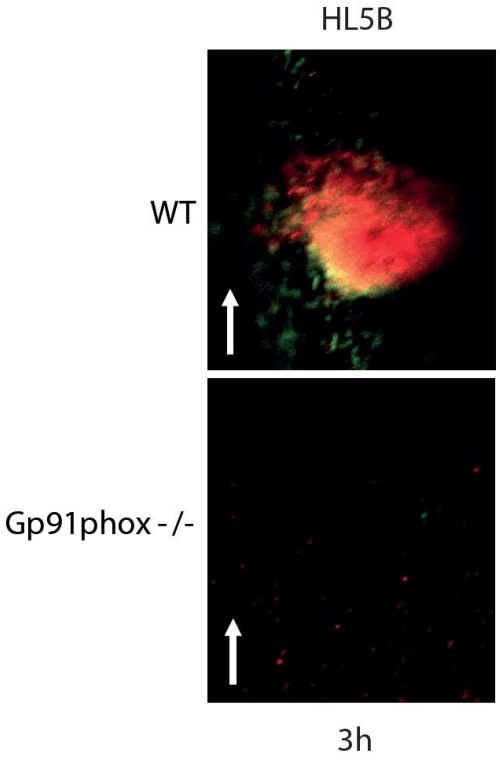
(1-5) Prinz et al. Blood 2011, Thromb Haemost 2013; (5,6) Müller-Calleja et al. Thromb Haemost 2015; (7) Müller-Calleja et al. Ann Rheum Dis 2017

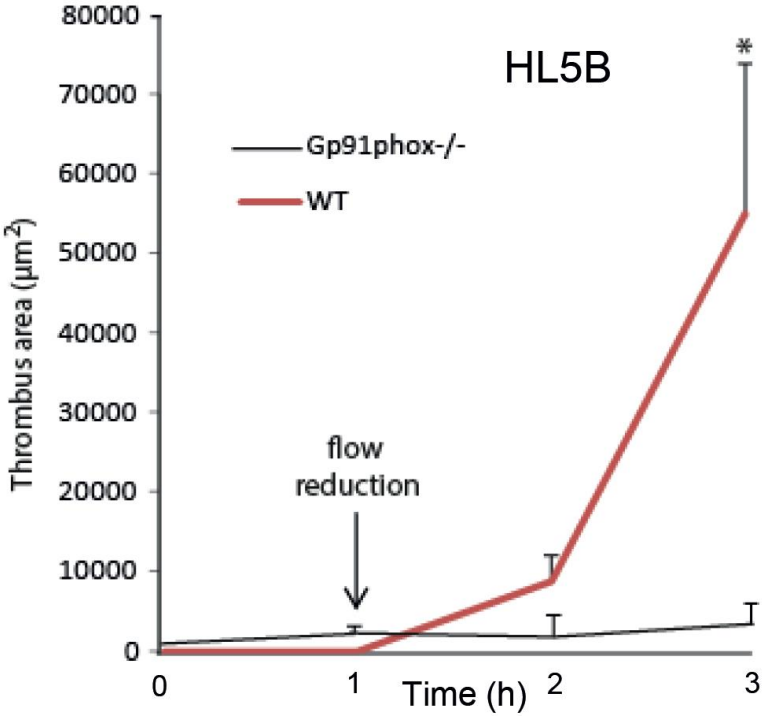
In vivo Thrombosis accelerated by anti-CL





In vivo Thrombosis Acceleration by anti-CL





In vivo Thrombosis Acceleration by anti-CL

- Lipid reactive, cofactor independent aPL accelerate thrombosis in an *in vivo* thrombosis model.
- This effect depends on the signal transduction pathway delineated *in vitro*.
- This pathway is activated *in vitro* by all anti-CL positive IgG-fractions isolated from APS patients (n = 18 / 20).

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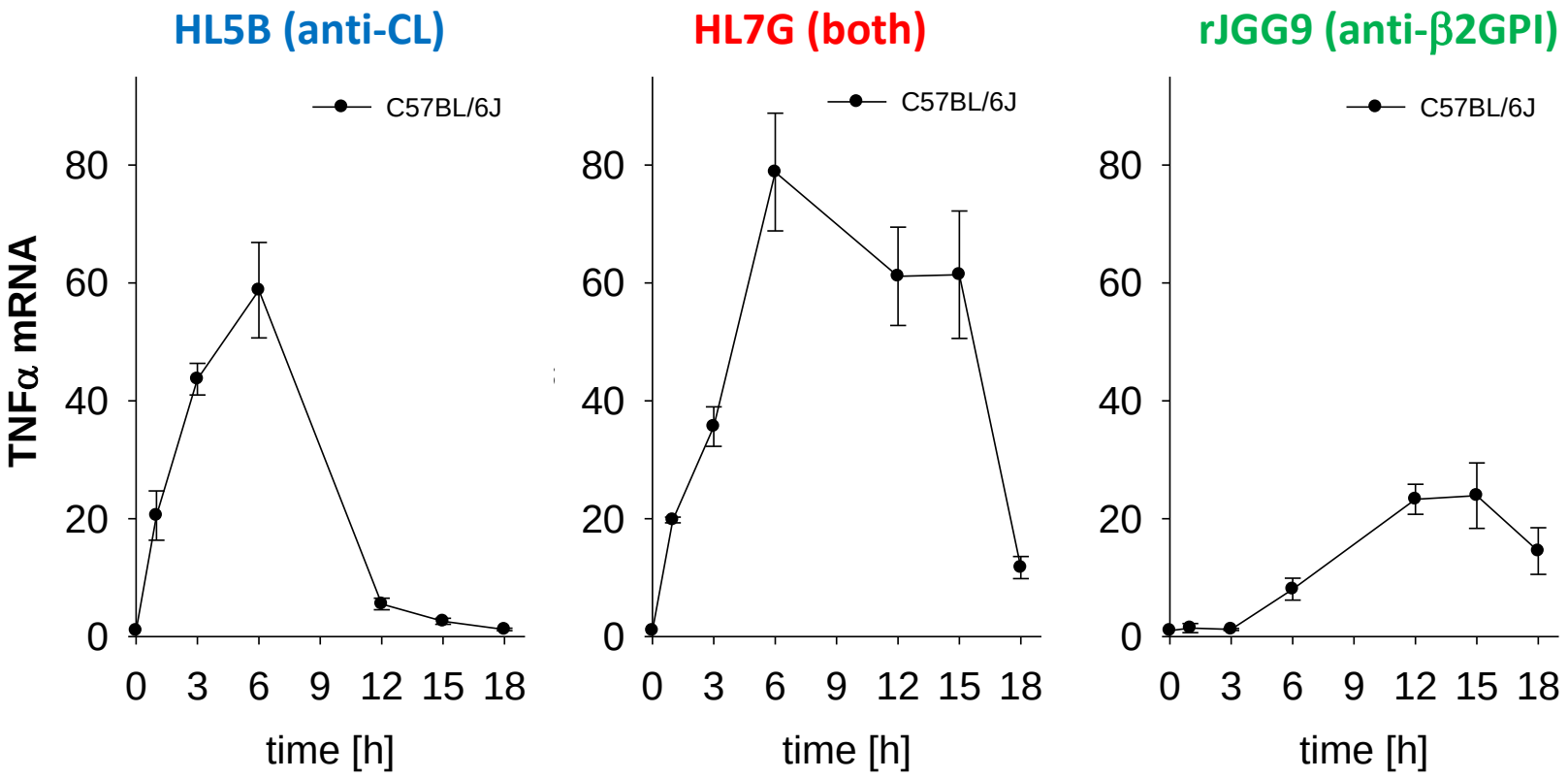
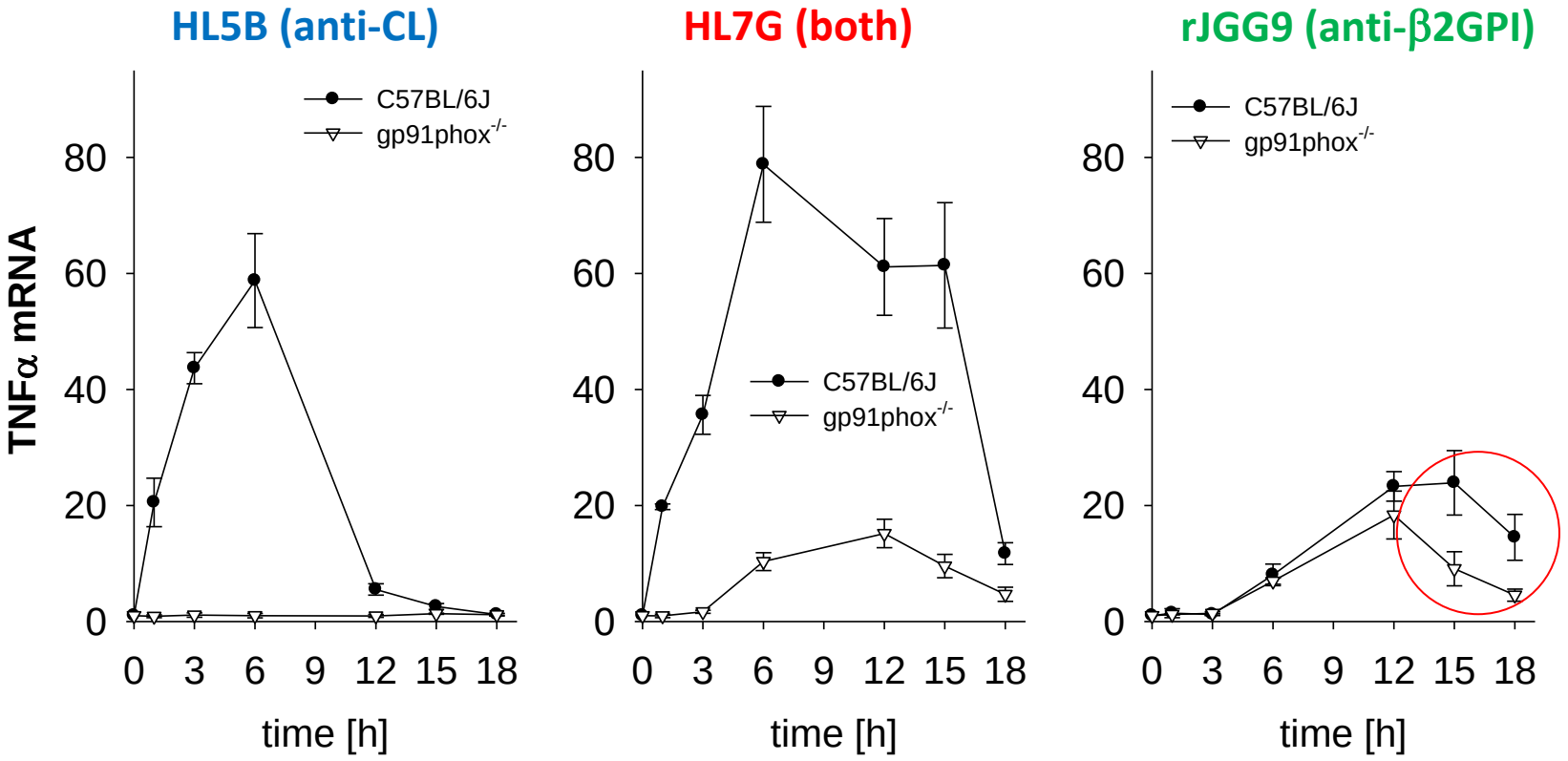
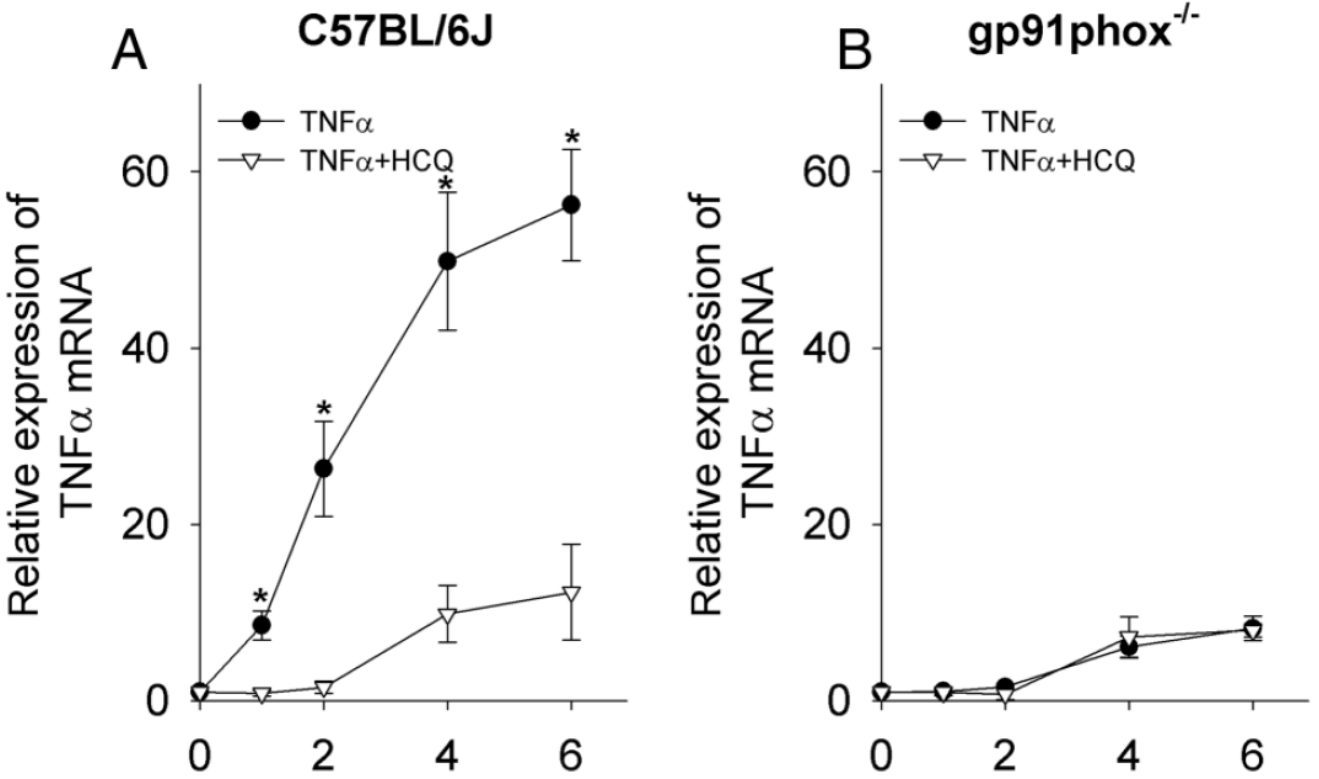
Monocyte Actviation by Different aPL



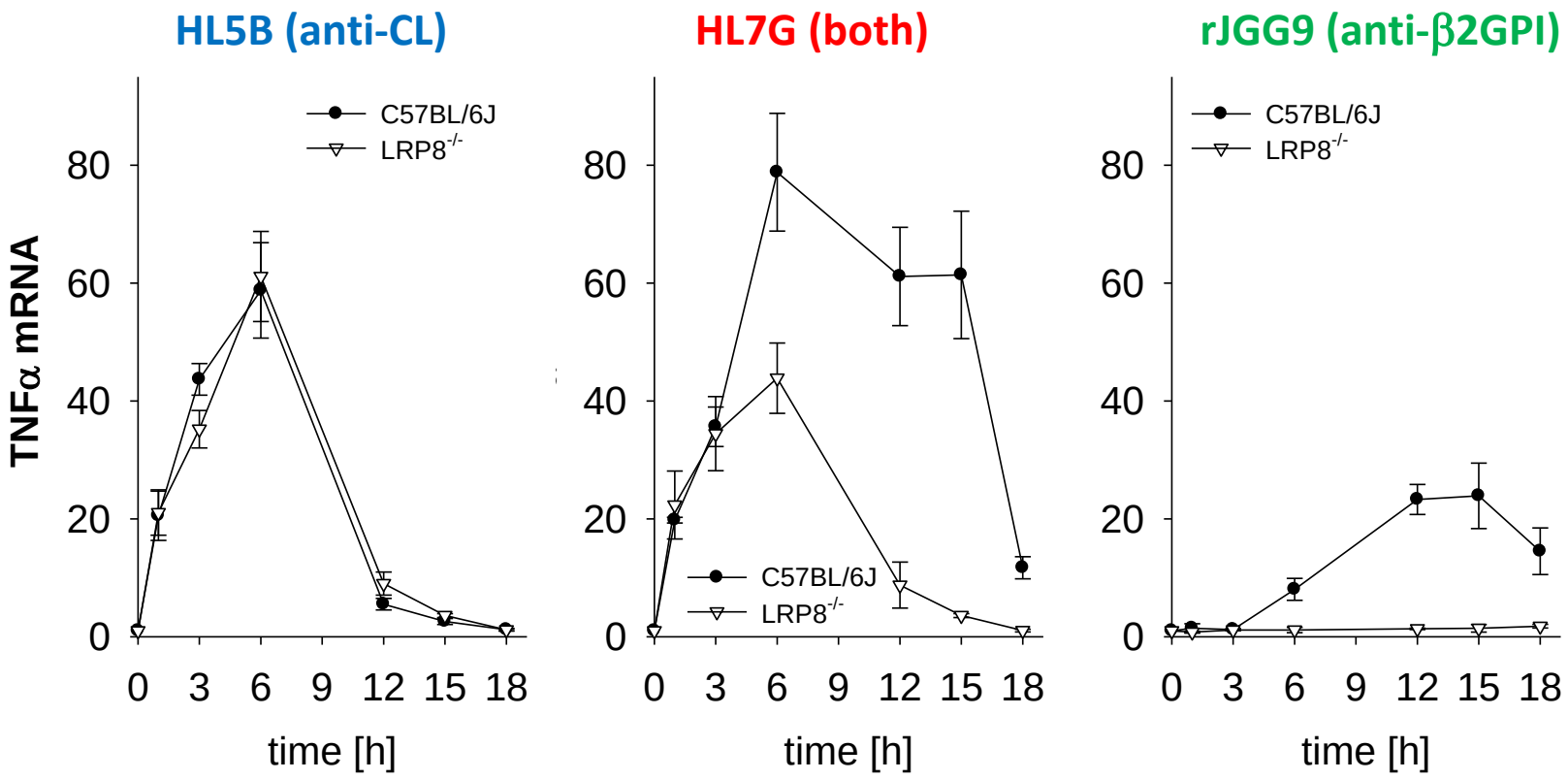
Monocyte Actviation by aPL – gp91phox$^{-/-}$ mice



Dependency of TNFα-Signaling on NADPH-Oxidase



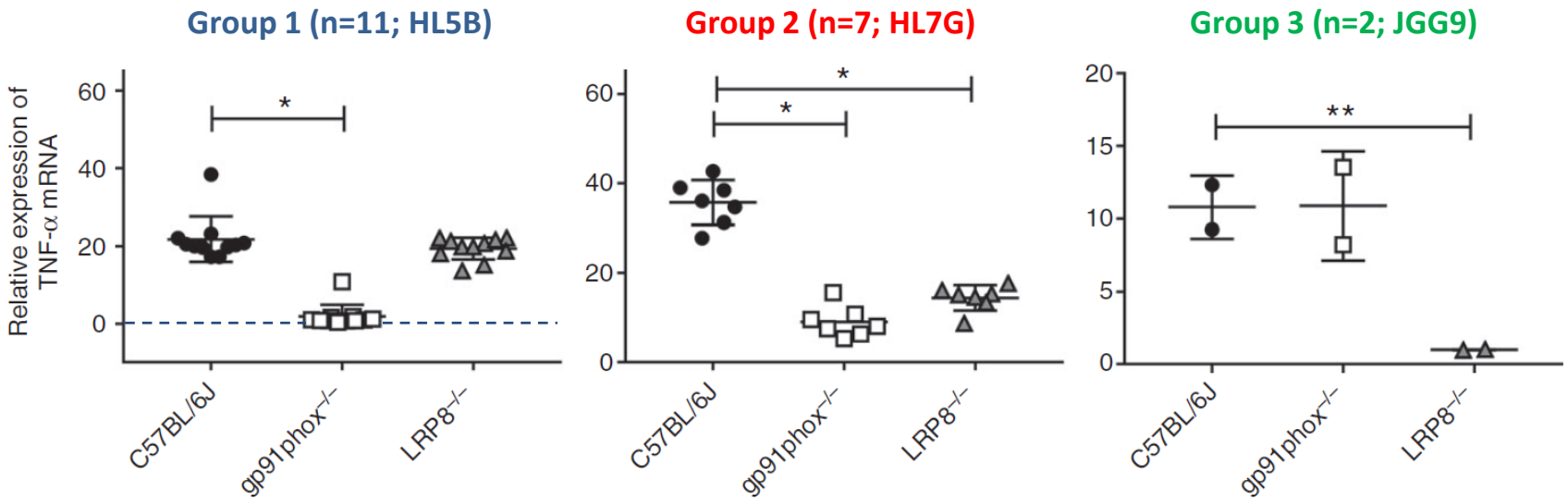
Monocyte Actviation by aPL – LRP8$^{-/-}$ mice



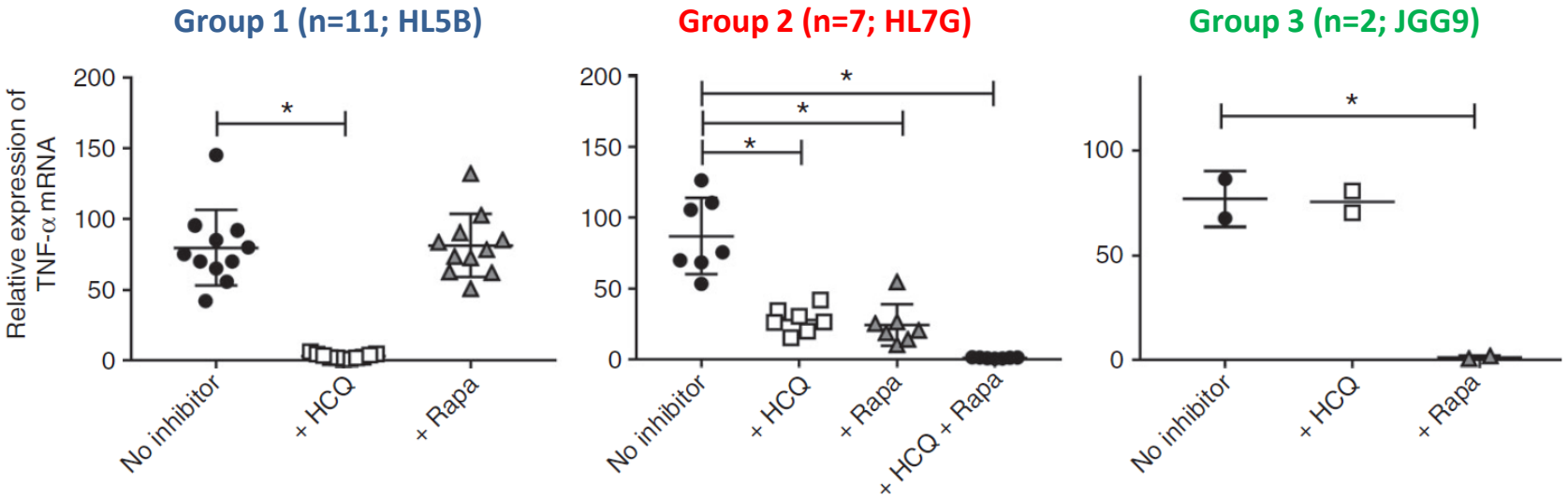
APS Patients

Sex	Age	Underlying disease	Clinical manifestations	IgG characteristics			Pattern
				LA	aCL only	anti-β2GPI	
f	37	SLE	Pulmonary embolism	+	+	0	1 – HL5B
m	42	SLE	Sinus vein thrombosis	+	+	0	1 – HL5B
m	61	SLE	Deep vein thrombosis; pulmonary embolism	+	+	0	1 – HL5B
f	57		Deep vein thrombosis	+	+	0	1 – HL5B
m	51	SLE	Deep vein thrombosis	+	+	0	1 – HL5B
m	64	SLE	Deep vein thrombosis	+	+	0	1 – HL5B
f	42		Thrombosis of inferior v. cava	+	+	0	1 – HL5B
f	61	SLE	Deep vein thrombosis	0	+	0	1 – HL5B
f	43	SLE	Recurrent abortions	+	+	0	1 – HL5B
f	52		Recurrent TIA	0	+	0	1 – HL5B
f	37		Deep vein thrombosis; Budd-Chiari Syndrome	0	+	0	1 – HL5B
m	26		Deep vein thrombosis	+	+	+	2- HL7G
f	63	SLE	Deep vein thrombosis; pulmonary embolism	+	+	+	2- HL7G
f	43	SLE	Deep vein thrombosis	+	+	+	2- HL7G
f	65	SLE	Deep vein thrombosis; pulmonary embolism	+	+	+	2- HL7G
f	44	SLE	Deep vein thrombosis	+	+	+	2- HL7G
f	45	SLE	2 abortions	+	+	+	2- HL7G
m	31		Deep vein thrombosis; pulmonary embolism	+	+	+	2- HL7G
f	36	SLE	Deep vein thrombosis; pulmonary embolism	+	0	+	3 – JGG9
f	43		Deep vein thrombosis	+	0	+	3 – JGG9

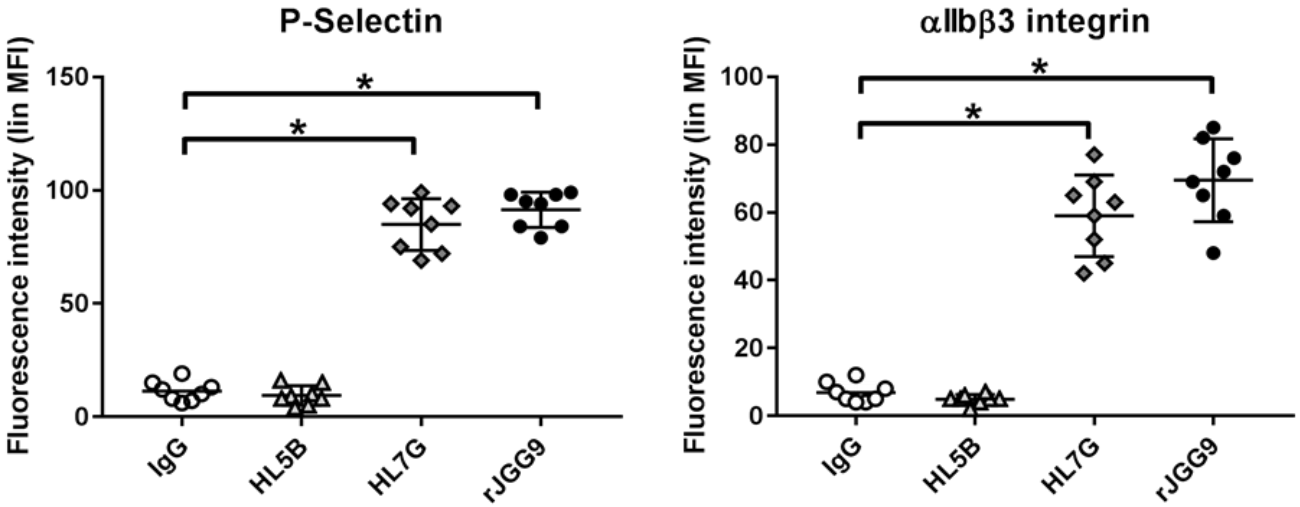
Monocyte Activation by Patient aPL



Monocyte Activation by Patient aPL



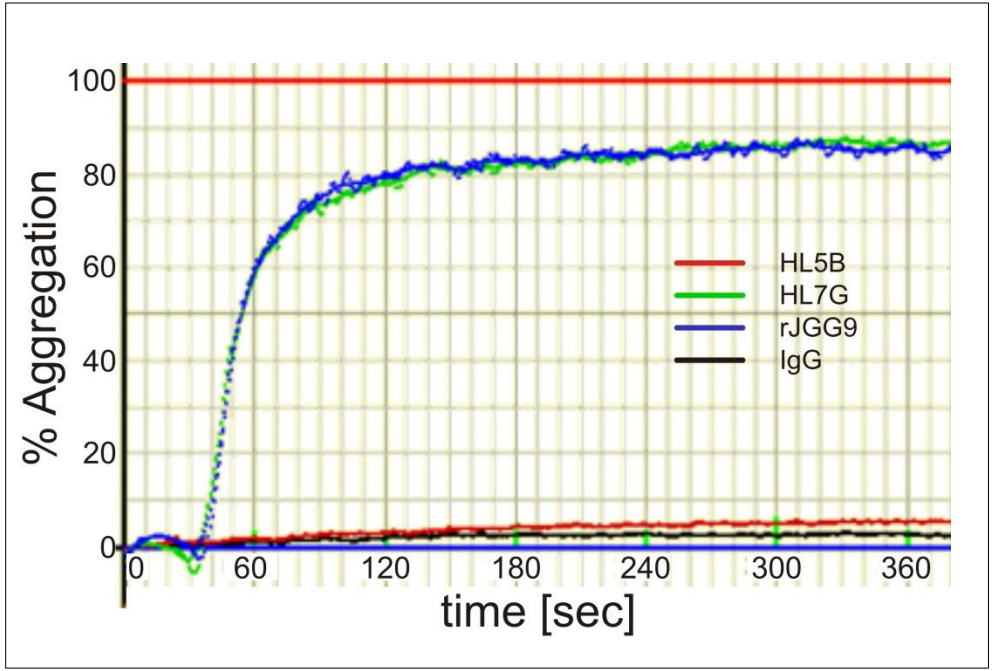
Induction of Platelet Activation Markers by aPL



Hollerbach et al - unpublished

Induction of Platelet Aggregation by aPL

Monoclonal aPL



Patient IgG

Antibody [50 µg/ml]	Donors	% aggregation after 3 min	P value vs. IgG control
control	3	8 ± 2	
Patient 16 (HL5B)	3	7 ± 1	n.s.
Patient 17 (HL7G)	3	89 ± 5	< 0.001
Patient 18 (JGG9)	5	90 ± 6	< 0.001

Hollerbach et al - unpublished

Are anti-CL and anti-β2GPI Related?

- HL5B and HL7G were cloned from the same APS patient.
- They are both IgG_2-λ
- HL5B is heavily mutated from germline with 14 amino acid exchanges in its heavy chain CDRs plus an insertion of 14 amino acids not present in the germline sequence.
- In the light chain CDRs of HL5B there are another 4 amino acid exchanges.
- The variable regions of the heavy chains of HL5B and HL7G are identical.
- HL7G has 5 additional amino acid exchanges in its light chain CDRs.

Differences between HL5B and HL7G Light Chains

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CDR 1 (λ – chain)

HL5B	T	G	T	S	S	D	V	G	G	Y	N	Y	V	S
	ACT	GGA	ACC	AGC	AGT	GAC	GTT	GGT	GGT	TAT	AAC	TAT	GTC	TCC
HL7G	T	G	T	S	S	D	V	G	G	Y	**H**	Y	V	S
	ACT	GG**C**	ACC	AGC	AGT	GAC	GTT	GGT	GGT	TAT	**C**AC	TAT	GTC	TC**T**

CDR 2 (λ – chain)

HL5B	E	V	**I**	N	R	P	**P**
	GAG	GTC	A**T**T	AAT	CGG	CCC	**C**CA
HL7G	**A**	V	**I**	**H**	R	**A**	**P**
	G**C**G	GTC	A**T**T	**C**AT	CGG	**G**CC	**C**CA

CDR 3 (λ – chain)

HL5B	S	S	Y	T	**T**	**R**
	AGC	TCA	TAT	ACA	A**C**C	AG**G**
HL7G	**G**	S	Y	T	**T**	**R**
	GT	TCA	TAT	ACA	A**C**C	AG**G**

This pair of monoclonal aPL from one APS patient provides evidence that anticardiolipin antibodies may convert to anti-β2GPI by a limited number of somatic mutations.

Summary

- aPL induce a proinflammatory and/or procoagulant state by at least two distinct mechanisms.
- Due to overlapping effects of aPL the use of monoclonal aPL is mandatory if mechanisms of action shall be identified.
- Our data show that the majority of (if not all) aPL isolated from APS patients activate either one of two or both cellular pathways.
- Binding specificity correlates with cellular effects.
- It is proposed that the high risk observed with triple positivity is related to the presence of different pathogenic aPL which may act synergistically to induce pathology.

Thank you for your attention