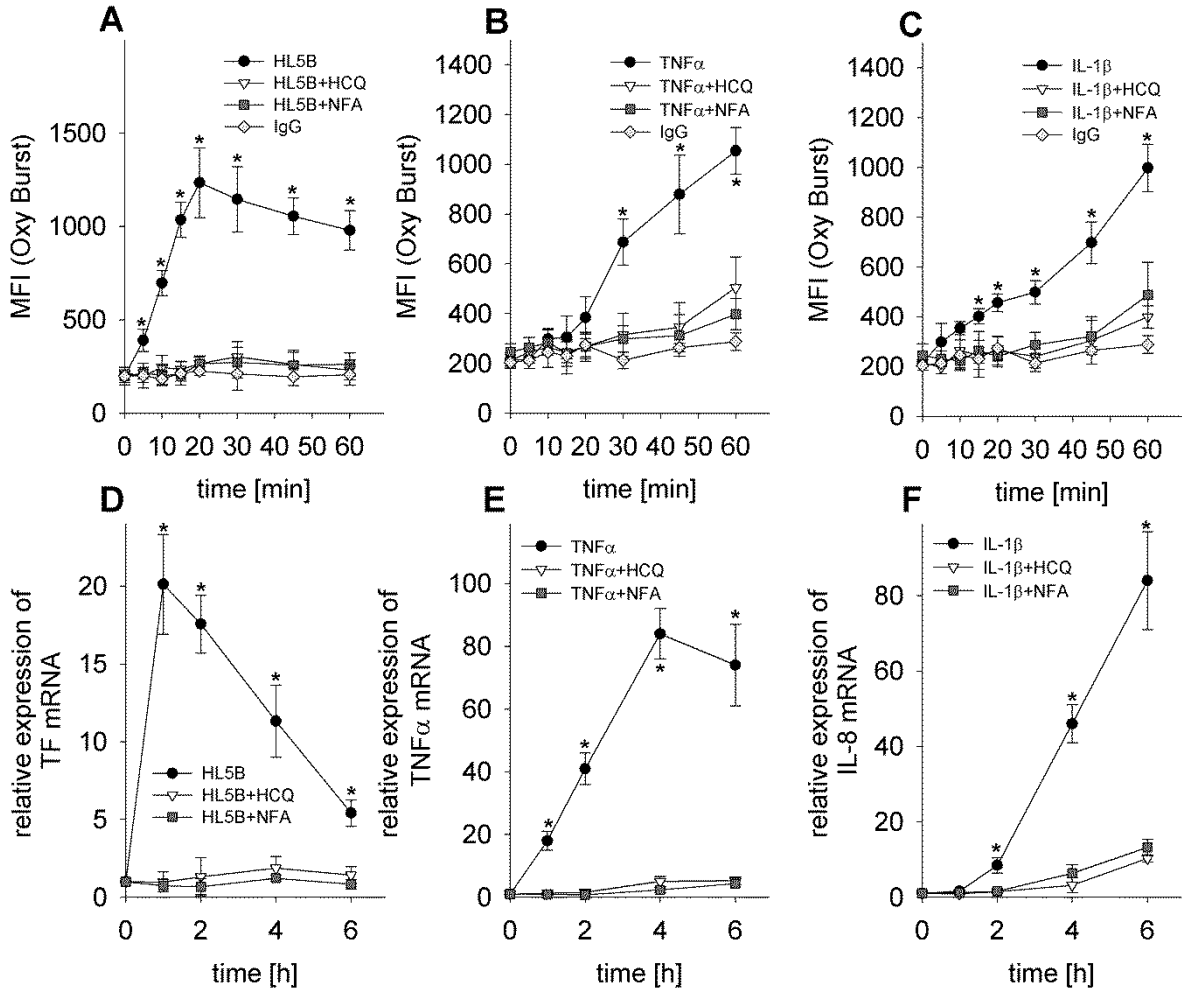
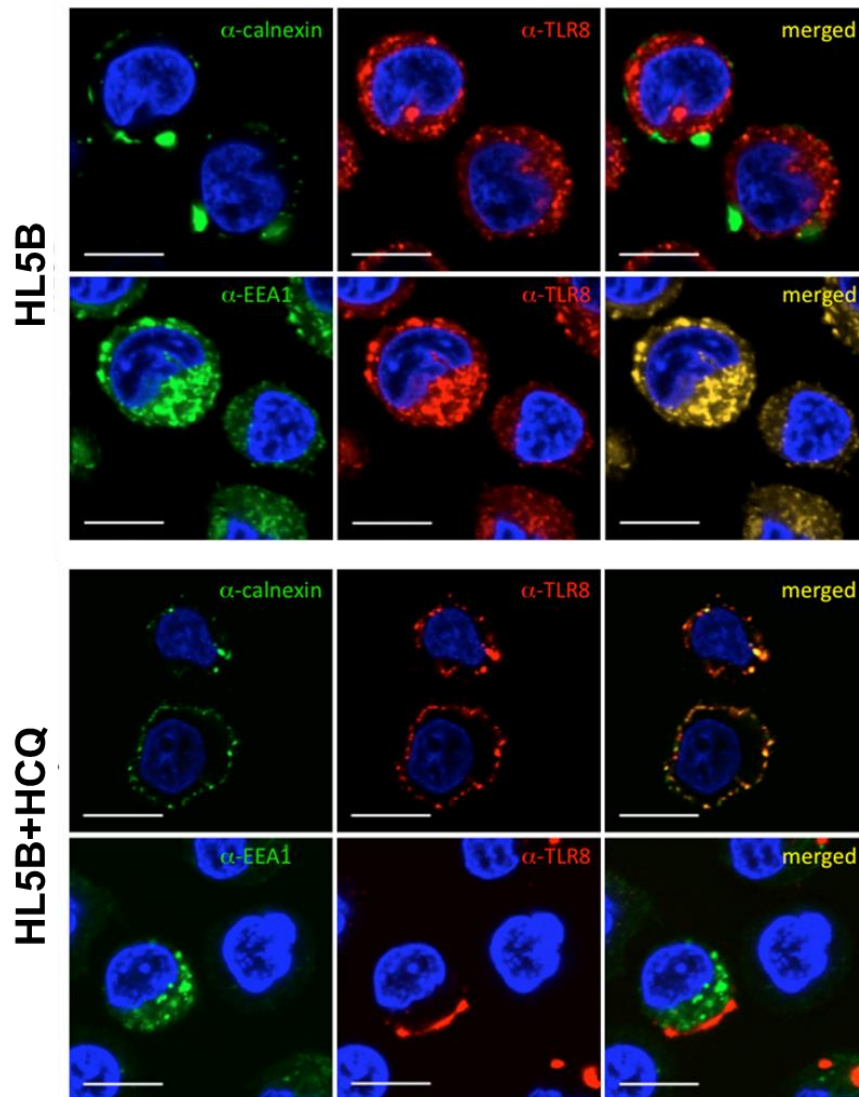


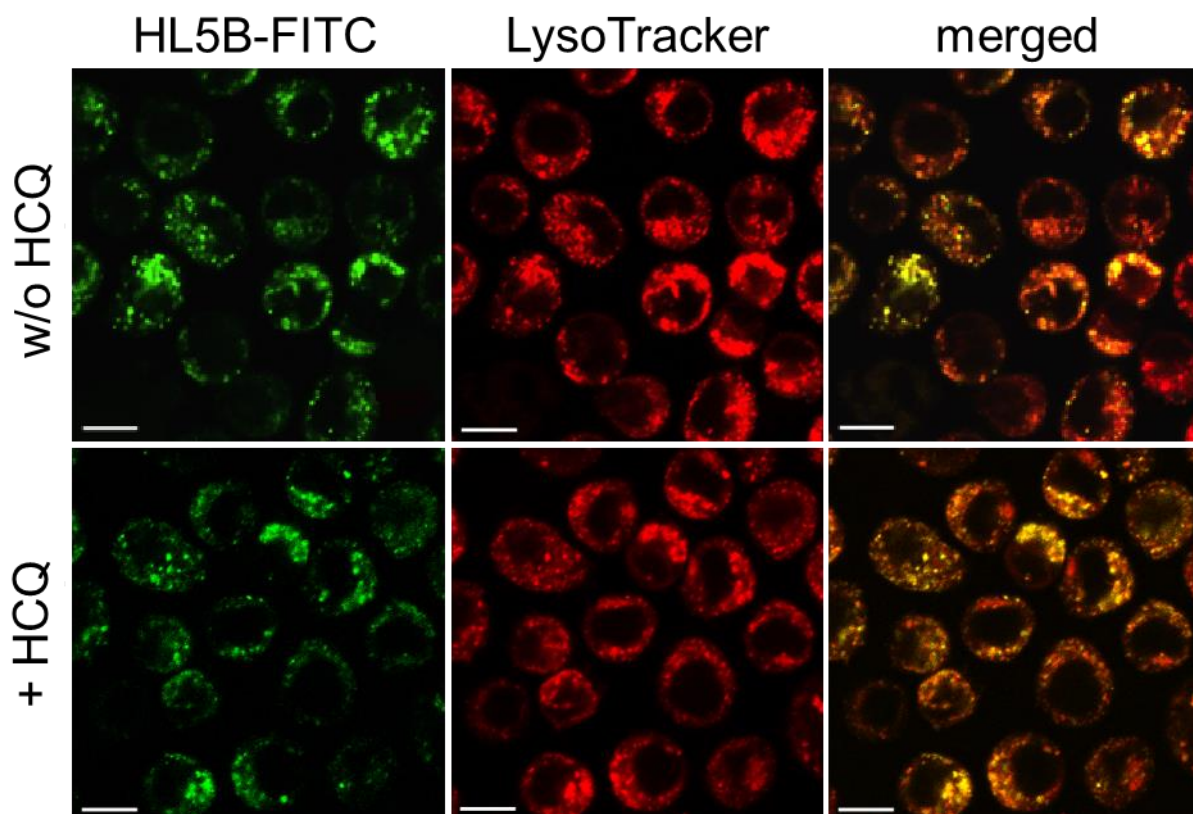
Supplementary Figures



Suppl. Fig. 1: Human CD14⁺ monocytes were isolated from buffy coats as described [24] and stimulated with 100ng/ml HL5B or IgG (A, D), 10ng/ml TNF α (B, E), or 10ng/ml IL-1 β (C, F) either alone or together with HCQ (10 μ M) or NFA (0.1mM). (A-C) To measure ROS production, cells were loaded with OxyBurst before stimulation. (D-F). Relative expression of TF mRNA (D), TNF α mRNA (E) and IL-8 mRNA (F) was normalized to IgG stimulated (D) or unstimulated cells (E+F) and β -actin expression. Data are from 3 independent experiments with 3 different donors measured in duplicate. *) p < 0.01 agonist vs. agonist + HCQ



Suppl. Fig. 2: HCQ prevents aPL mediated TLR8 translocation. Confocal images of MM1 cells stained with α -TLR8 (red) and either α -calnexin or α -EEA1 (green). Nuclei were visualized with DAPI (blue). Before fixation cells were stimulated for 30 minutes with HL5B (100 ng/mL) either alone or in the presence of HCQ (10 μ M). While after incubation with HL5B alone TLR8 colocalizes with EEA1, it colocalizes with calnexin if HCQ is added. This indicates that the previously described translocation of TLR8 from the ER to the endosome [22] is prevented by HCQ.



Suppl. Fig. 3: aPL uptake in the presence of HCQ. MM1 cells were stimulated for 15 minutes with FITC labeled HL5B (100 ng/ml) either alone or together with HCQ (10 μ M). The endosomal compartment was visualized using the pH sensitive dye LysoTracker (50nM). Cells were imaged by confocal fluorescence microscopy under exactly identical instrument settings to allow comparison of fluorescence of HL5B in the absence or presence of HCQ. The addition of HCQ did not affect antibody uptake. Bar = 10 μ m

Cofactor-dependent IgG fractions						
Sex	Age	Clinical manifestation	aPL in blood sample for purification			Underlying disease
			LA	CL	β2GPI	
M	26	DVT	+	IgG	IgG	0
W	63	DVT; PE	+	IgG	IgG	SLE
W	43	DVT	+	IgG	IgG	SLE
W	65	DVT; PE	+	IgG/IgM	IgG/IgM	SLE
W	44	DVT	+	IgG	IgG/IgM	SLE
Cofactor-independent IgG fractions						
W	37	PE	+	IgG	0	SLE
M	42	Sinus vein thrombosis	+	IgG	IgM	SLE
M	61	DVT; PE	+	IgG/IgM	IgM	SLE
W	57	DVT	+	IgG	0	0
M	51	DVT	+	IgG	0	SLE
M	64	DVT	+	IgG	0	SLE
W	42	V.cava. inf. thrombosis	+	IgG	0	0
W	61	DVT	0	IgG	0	SLE
W	43	Recurrent abortions	+	IgG	0	SLE
W	52	Recurrent TIA	0	IgG	0	0

Suppl. Table 1: Characteristics of the APS patients tested