

Supplementary Material

Signal Regulatory Protein Beta 2 is a novel positive regulator of innate anticancer immunity

Nienke Visser, Levi Collin Nelemans, Yuan He, Harm Jan Lourens, Macarena González Corrales, Gerwin Huls, Valerie R. Wiersma, Jan Jacob Schuringa, Edwin Bremer[#]

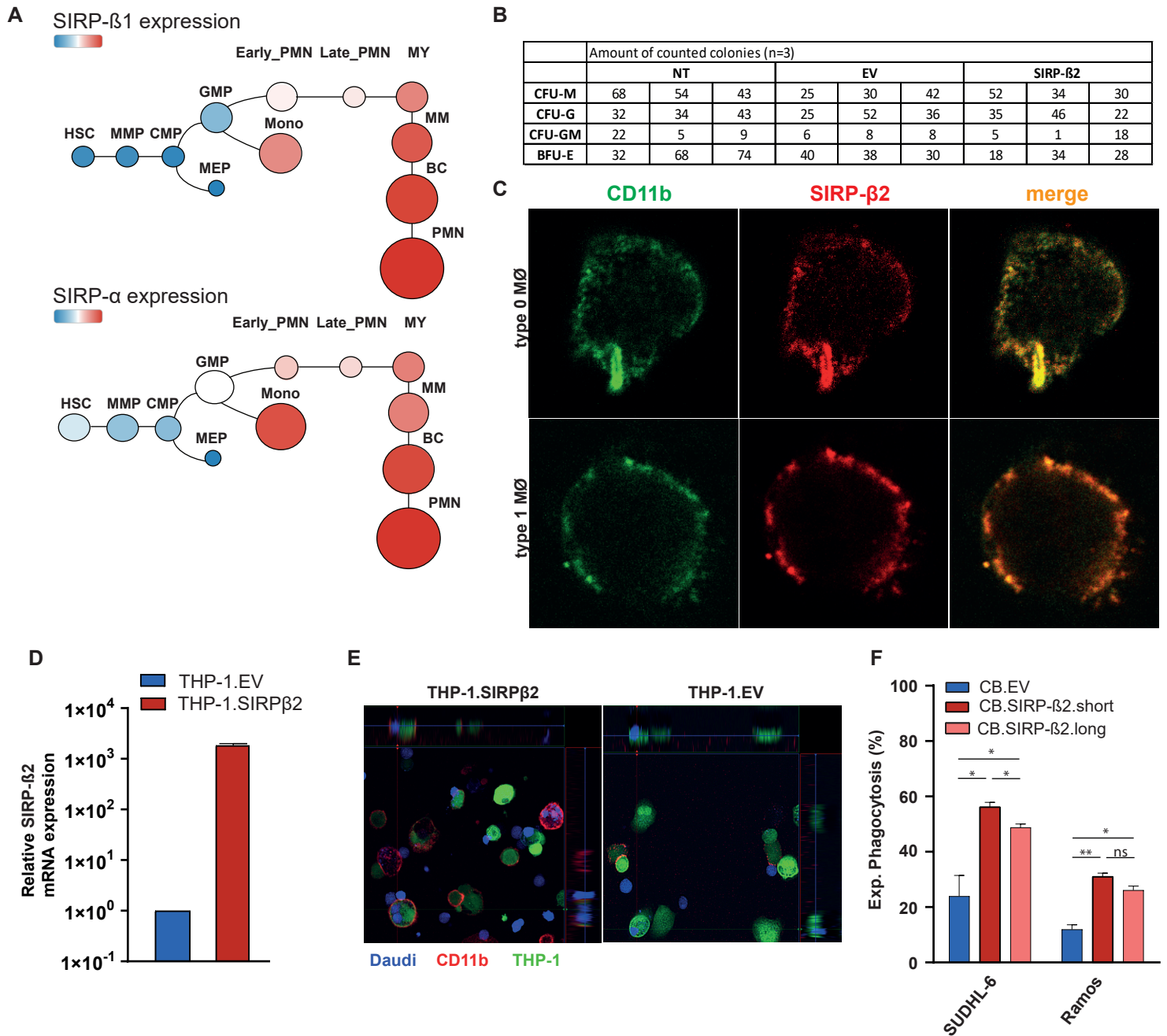
Author affiliations

Department of Hematology, University of Groningen, University Medical Center Groningen (UMCG), Groningen, The Netherlands

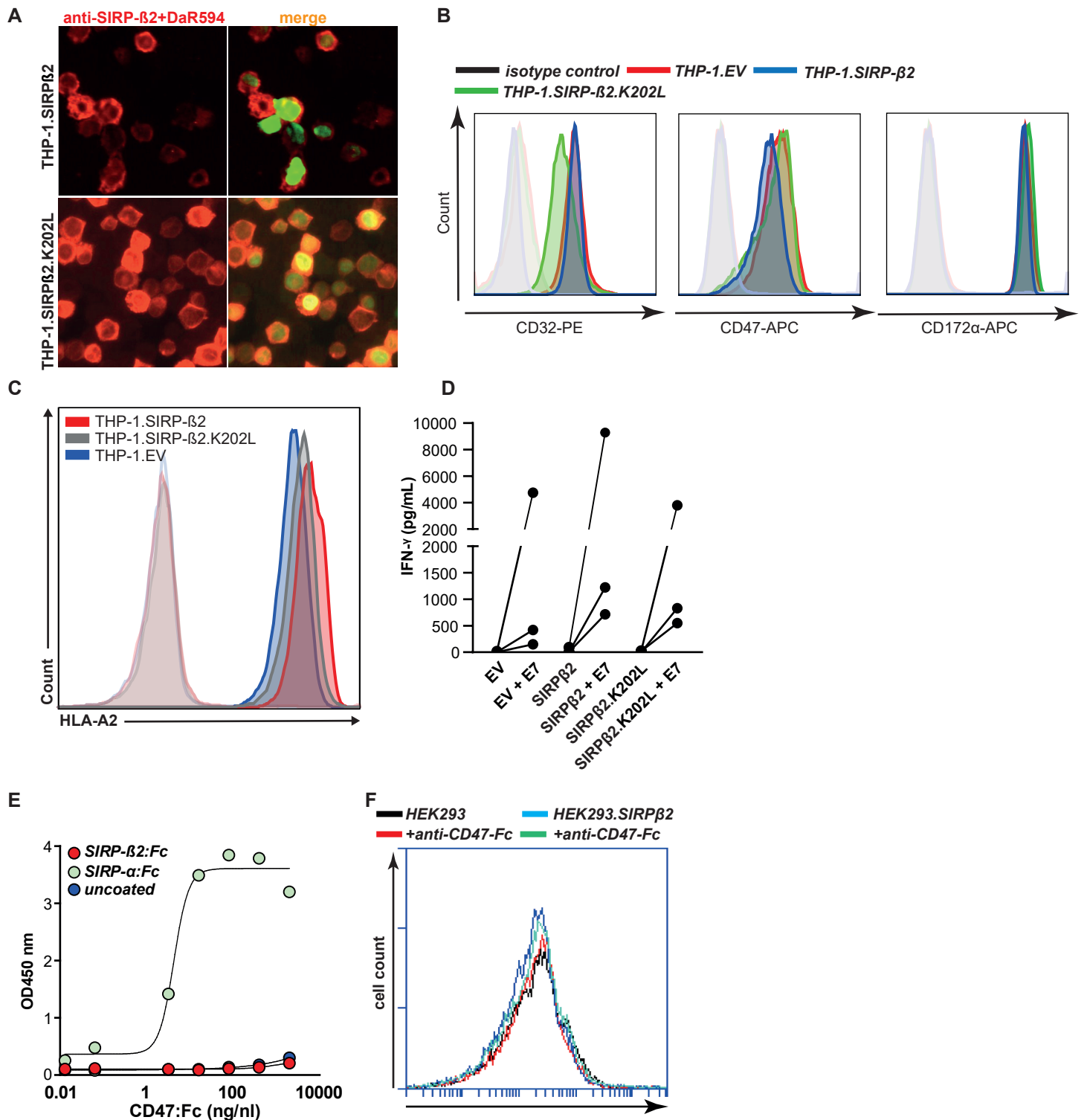
Key words: SIRP- β 2; CD47; phagocytosis; macrophage; antigen presentation

[#]Correspondence to:

Prof. Dr. E Bremer, University Medical Center Groningen (UMCG), Groningen 9713 EZ, The Netherlands, e.bremer@umcg.nl



Suppl. Fig. 1. Expression pattern and functional phagocytosis activity of SIRP- β 2 (A) mRNA SIRP- β 1 (above) and SIRP- α (below) expression in hierarchical differentiation tree. (B) Original data derived from counted colonies of the Colony Formation Unit assay after 14days, NT, EV and SIRP- β 2. (C) Representative confocal images of the surface expression of CD11b and SIRP- β 2 on type 0 and type 1 macrophages. (D) Relative SIRP- β 2 mRNA expression in THP-1.EV and THP-1.SIRP- β 2 (n=2). (E) Representative orthogonal images of THP-1 mediated phagocytosis incubated with Daudi cells for 3h, treated with RTX (1 μ g/ml). (F) Experimental phagocytosis of CB derived macrophages expressed EV control, SIRP- β 2 and SIRP- β 2.K202L co-cultured with SUDHL-6 and Ramos, 3h (n=3)



Suppl. Fig. 2. Interactome analyses of SIRP- β 2 identified DAP12 as signaling component and a strong association of SIRP- β 2 with MHC-class I and potentiate HPV-E7-specific T cell responses. (A) Representative confocal microscopy images of SIRP- β 2 surface expression on THP-1.SIRP- β 2 and THP-1.SIRP- β 2.K202L. (B) Cell surface expression of CD32, CD47 and CD172a of THP-1.EV, THP-1.SIRP- β 2 and THP-1.SIRP- β 2.K202L, determined by flow. (C) HLA-ABC expression of THP-1.EV, THP-1.SIRP- β 2 and THP-1.SIRP- β 2.K202L determined by flow cytometry. (D) Quantitative IFN- γ secretion by primary E7-TCR specific T cells in co-culture with THP-1.EV, THP-1.SIRP- β 2 and THP-1.SIRP- β 2.K202L and with or without E7 peptide for 48 h (n=3). (E) Potential binding of the known SIRP- α receptor CD47 was evaluated by detecting binding of CD47:Fc on SIRP- β 2:Fc and SIRP- α :Fc-coated ELISA plates. (F) Histogram of binding of CD47:Fc to wild-type HEK293T cells and HEK293T cells transduced with SIRP- β 2.