Inhibition of cleavage of human complement component C5 and the R885H C5 variant by two distinct high affinity anti-C5 nanobodies

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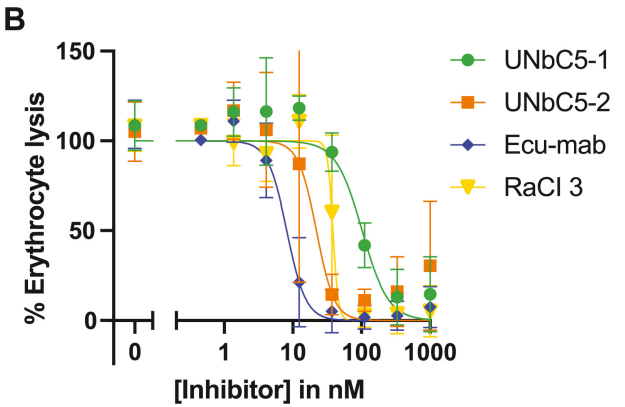
Attention, la **Figure 1** originale contient un panel C mais nous vous demandons de commenter seulement les panels A, B et D représentés ici.

A

Clone	a.a.	Framework 1	CDR1	Framework 2	CDR2
UNbC5-1	1	EVQLVESGGGLVQAGGSLRLSCAASGFTFD	DYAIG	WFRQAPGKEREGVS	CISTSDGSTYYADSVKG
UNbC5-2	1	EVQLVESGGGLVQPGGSLRLSCAASGRTFS	TNTMG	WFRQAPGQEREFVA	LISGNGRILDYSDSAKG

		Framework 3	CDR3	Framework 4
UNbC5-1	67	RFTISSDNAKNTVYLQMNSLKPEDTAVYYCAA	DPYLPIRGRGIESTDFGS	WGQGTQVTVSS
UNbC5-2	67	RFTISRDNAKNTVYLQMNSLKPEDTGVYFCAA	EF----RGRTL----ASY	WGQGTQVTVSS



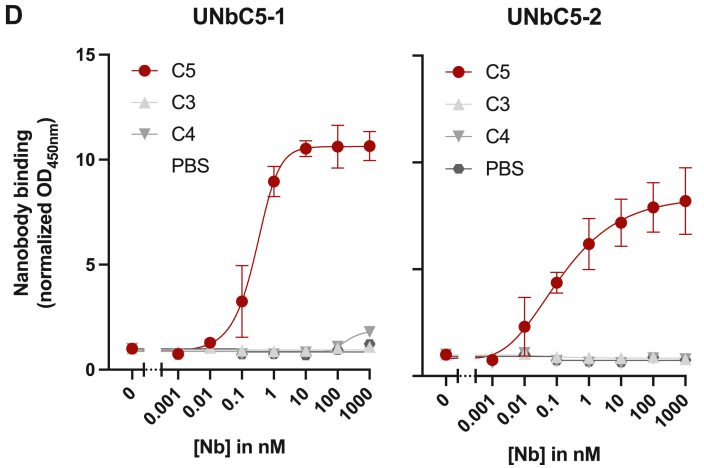


Figure 1. Identification of two C5-targeting nanobodies that interfere with complement. *A*, protein sequence alignment of UNbC5-1 and UNbC5-2. Frameworks 1 to 4 and CDRs 1 to 3 are indicated. Conserved amino acids are indicated in *black* and unique amino acids are indicated in *red*. *B*, CP mediated hemolysis of antibody-coated sheep erythrocytes incubated with 2.5% normal human serum and a titration of our nanobodies UNbC5-1 and UNbC5-2 and known complement inhibitors RaCI3 and Ecu-mab. The A_{405} values of the supernatants were measured; the % erythrocyte lysis was calculated using the 0% lysis (buffer) and 100% lysis (milliQ water) control samples.

D, nanobody bind to complement protein C5 and not homologs C3 and C4. Microtiter plates were coated with complement proteins and incubated with increasing concentrations of UNbC5-1 and UNbC5-2. Nanobody binding was measured using polyclonal rabbit-anti-VHH QE19 antibodies and donkey-anti-rabbit-HRP antibodies, at an absorbance of 450 nm. Coating with PBS was taken along as a negative control. Data information: (*A*), sequences were aligned using T-coffee (42). *B–D*, data represent mean ± SD of three individual experiments (*B* and *D*) curves were fitted and IC_{50} and EC_{50} values were obtained. CDR, complementarity determining regions; CP, classical pathway; HRP, horseradish peroxide.