

## *Supplementary Methods*

### *Expression of human recombinant fH-derived proteins*

Human recombinant fH-derived proteins hSCR18-20 and hSCR16-17 were produced using the EasySelect™ *Pichia* Expression Kit (Invitrogen) according to the manufacturer's instructions. DNA fragments encoding fH-derived domains were amplified by nested PCR from the cDNA of full-length fH (OriGene, True Clone full length cDNA clone TC120040 NM\_000186) and cloned into the expression vector pPICZα A using EcoRI and XbaI restriction sites (Figure 1A). Supernatants of the expression cultures were harvested and recombinant proteins were purified by affinity chromatography using 6×His Ni-NTA column. Purification of the recombinant SCR fragments was analyzed by separation of all fractions on reducing SDS-gels followed by Western blot analysis. Proteins were detected by HRP-conjugated mouse anti-His monoclonal Ab (Sigma) and showed the expected band for hSCR18-20 (33kDa) and hSCR16-17 (25kDa), respectively (Figure 1B).

### *Heparin-binding activity of human recombinant fH-derived proteins*

Affinity chromatography was performed to investigate the capacity of fH-derived domains to bind to Heparin. Therefore, recombinant proteins were diluted in 10mM Sodium phosphate-buffer and applied on a 1ml HiTrap Heparin HP (GE Healthcare) column. After two washing steps, bound protein was eluted by high salt (10mM Sodium phosphate buffer with 1M NaCl). Flow-through (FT), wash (W1, W2) and elution fractions (E1, E2) were collected and analyzed by SDS-PAGE and Western Blot (Figure 1C). Detection of the recombinant fragments was carried out as described above. Affinity chromatography revealed the Heparin-binding activity of recombinant hSCR18-20. As shown in Figure 1C, hSCR18-20 was detected in the elution fraction (E1), which proves the Heparin-binding capacity of this

domain. In contrast, hSCR16-17 appeared mainly in the Flow-through fraction (FT) and did not bind to the column.

#### *Primary CLL cells*

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient centrifugation (GE Healthcare) and cultured overnight in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS) (GIBCO), 5mM glutamine (GIBCO) and 0.5 µg/ml LPS (Sigma). The B cell fraction of PBMCs was determined by flow cytometry measuring the frequency of CD20-positive cells.

#### *Immunofluorescence*

To measure CD20 expression, PBMCs from patients were incubated with anti-CD20 mAb (rituximab) for 30 min at 4°C. After washing, cells were stained with FITC-conjugated rabbit anti-human IgG. In addition, CD55 and CD59 expression was also analyzed in patient samples. Therefore, cells were incubated with either mouse anti-human CD55 (clone IA10, BD Pharmingen) or mouse anti-human CD59 (clone p282, BD Pharmingen) monoclonal Abs for 30 min at 4°C. After washing, cells were stained with FITC-conjugated polyclonal goat anti-mouse IgG. Samples were analyzed on a FACS Canto II cytometer (Becton Dickinson).

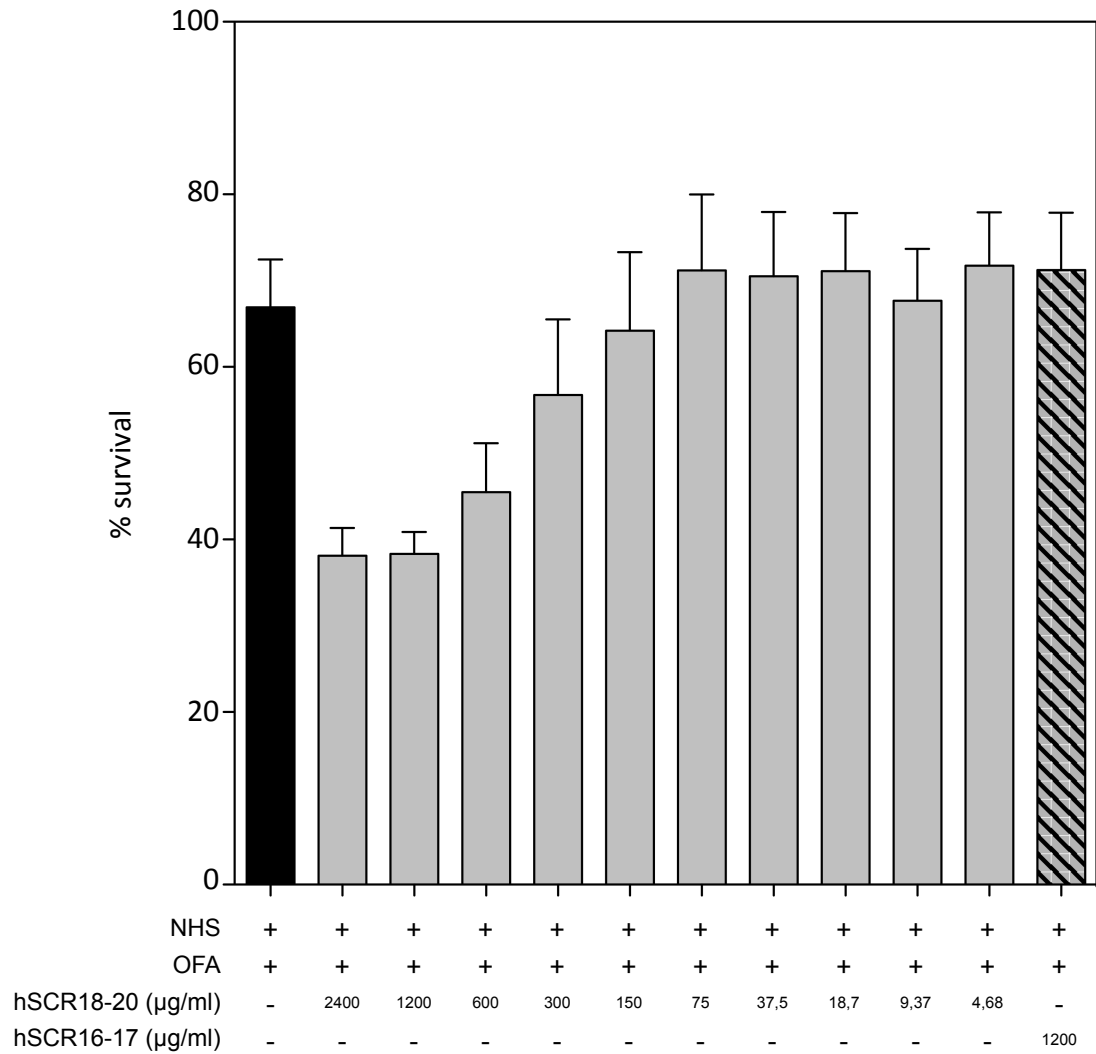
#### *Complement-dependent cytotoxicity assay*

To analyze the specificity of enhanced CDC induced by OFA in the presence of hSCR18-20, we mixed PBMCs from a CLL patient with PBMCs from a healthy donor to obtain a mixture of cells with a balanced CD3<sup>+</sup> T cell and CD19<sup>+</sup> B cell population (Figure 2A, dot plot). CDC assays were then performed with this heterogeneous cell suspension. The samples were treated with OFA (20 µg/ml) or hSCR18-20 (1200 µg/ml) alone or in combination under standard conditions (in 25% NHS or hiNHS for 1h at 37°C). Following incubation, cells were

stained with APC-Cy7-conjugated anti-human CD3 (clone HIT3a) (BioLegend) and APC-conjugated anti-human CD19 (clone HIB19) (BioLegend) mAbs for 30 min at 4°C. After washing, PI staining was performed and cells were analyzed on a FACS Canto II cytometer. PI-negative viable cells were counted in the CD19<sup>+</sup> B cell and the CD3<sup>+</sup> T cell fraction for 60 seconds at constant flow rate. The PI-negative, viable cell count in the hiNHS control sample was set as 100% survival and survival rates were calculated as described above.

*Western blot analysis to determine C3 fragments*

CLL cells ( $2 \times 10^5$ ) were incubated with OFA (2  $\mu$ g/ml) and NHS in the presence or absence of hSCR18-20. This OFA concentration allowed about 20%-30% lysis of cells (data not shown). As controls, CLL cells were treated with hiNHS or NHS alone. After different time points of incubation, C3 processing was stopped by adding ice cold 50 mM of EDTA. Cells were washed twice with PBS and the pellet was lysed. Cell lysates were analyzed on an 8% gel under reducing conditions. Following transfer, the blot was developed with a polyclonal, goat anti-human C3 Ab (Complement Technology Inc.).



The optimal concentration of hSCR18-20 (1200mg/ml) was determined by titration of the SCRs in lysis assays. Normal human serum (NHS) (25%) as a source of active complement or heat-inactivated NHS (hiNHS) was added and samples were incubated for 1 hour at 37°C. Prior to analysis dead cells were stained with propidium iodide (PI). Analysis was performed on a FACS Canto II cytometer measuring the PI-negative viable cell counts in the samples