Figure 1

SPR analysis of humanized 8A6 WT and N297A variants, GB3 WT and ch8A6 WT

	k_{off} [s^{-1}]
hu8A6_wt	9.5×10^{-4}
hu8A6_N297A	8.8×10^{-4}
ch8A6_wt	4.9×10^{-4}
chGB3_N297A	1×10^{-2}

Figure 1: Surface Plamon Resonance analysis of humanized 8A6 (hu8A6_VH10+VL6) according to SEQ. ID. No. 3 and 4 in either Wildtype or N297A format, ch8A6_WT (according to SEQ. ID. NO. 1 and 2) and chGB3_N297A.

Figure 2

Sequence of hu8A6_wt and hu8A6-N297A

The aminoacids of the variable regions are just numbered regardless to any numbering scheme. For a better understanding of the aminoacid changes in the Fc-domain the EU-numbering was chosen.

Heavy chain / humanized 8A6_wt (glycosylated)

VH domain (variant VH10)

```
1  QVQLVESGGG VVQPGRSLRL SCAASGFTFS DYYMAWVRQA PGKGLEWVAS ISYDGSNKYY
61 GDSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARPG DYWGQGTLVT VSS (SEQ ID
NO. 3)
```

Fc-domain (glycosylated N297 / allotyp G1m17 containing K214; E356; M358; A431 / without C-terminal Lys; according to Eu-numbering)

```
118 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
178 GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG
238 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
298 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
358 MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
418 QQGNVFSCSV MHEALHNHYT QKSLSLSPG- (SEQ ID NO. 6)
```

Heavy chain / humanized 8A6_N297A (deglycosylated)

VH domain see above

Fc-domain (N297A variant / allotyp G1m17 containing K214; E356; M358; A431 / without C-terminal Lys; according to Eu-numbering)

```
118 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
178 GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG
238 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYA
298 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
358 MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
418 QQGNVFSCSV MHEALHNHYT QKSLSLSPG- (SEQ ID NO. 28)
```

Figure 2: Sequences of hu8A6_wt and hu8A6_N297A variants showing position of N to A aminoacid modification in N297A format

Figure 3

FACS analysis showing non-blocking characteristics of ch8A6_N297A

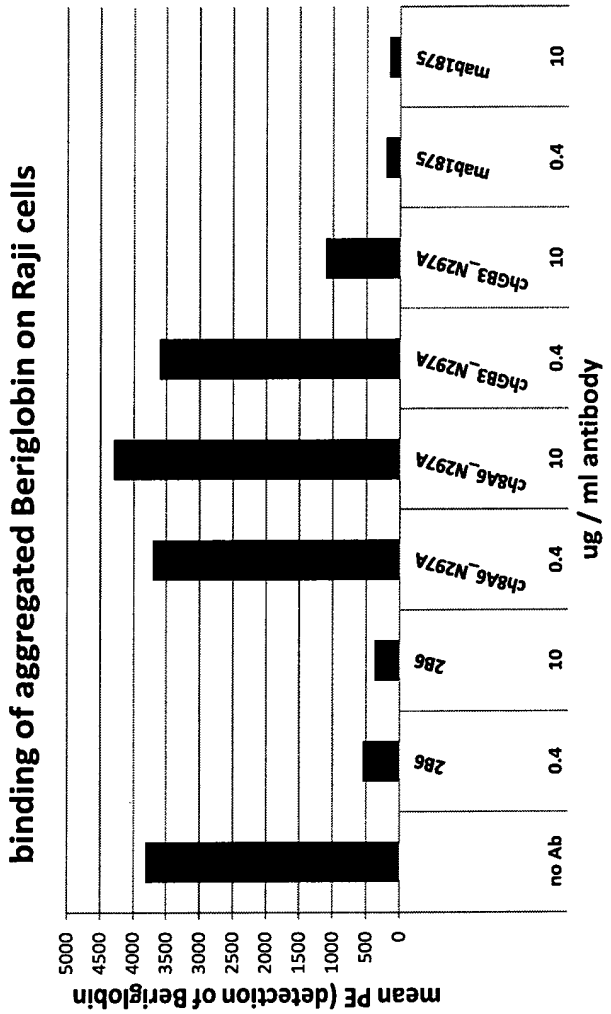


Figure 3: Non-blocking characteristic of ch8A6_N297A. Raji cells were incubated with a set amount of aggregated human IgG and varying amounts of ch8A6_N297A, chGB3_N297A or blocking antibodies 2B6 or mab1875 (R&D). The antibodies according to the invention are non-blocking.

Figure 4

FACS analysis of binding specificity of humanized 8A6 variants

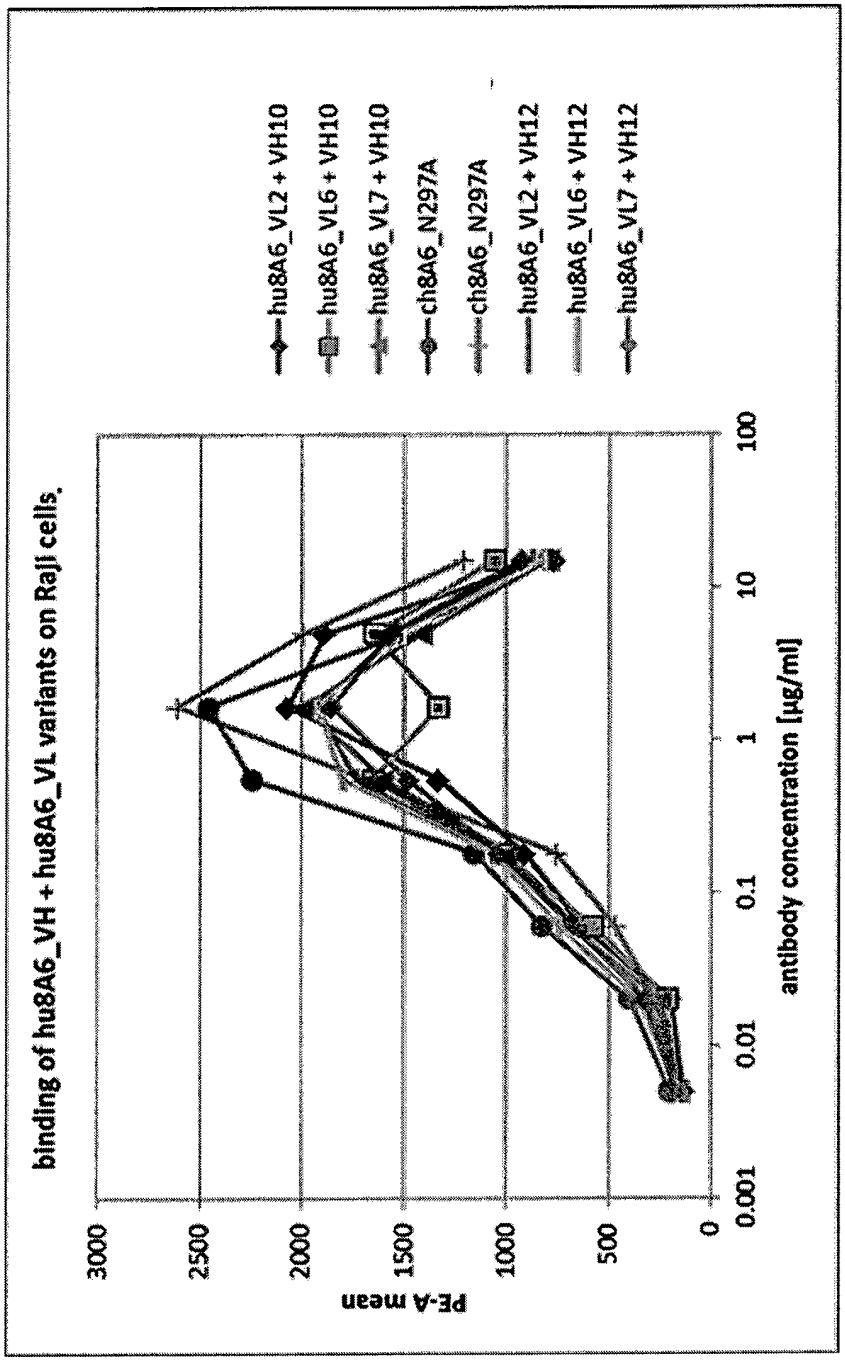


Figure 4: Binding from 15 µg/ml to 0.005 µg/ml of Protein A purified antibody (hu8A6_VL + hu8A6_VH and ch8A6_N297A to native FcγRIIB expressed on Raji cells. Humanized 8A6 variants bind with high avidity to FcγRIIB expressed on Raji cells.

Figure 5

FACS analysis of binding specificity of humanized 8A6 variants

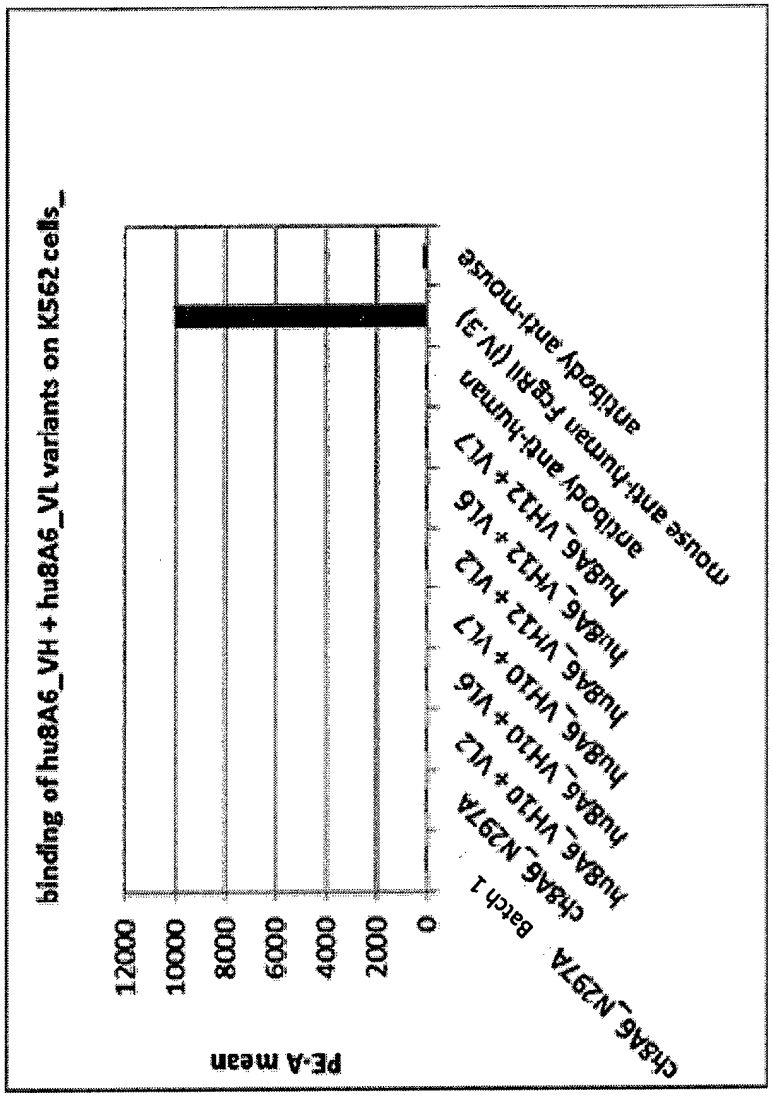


Figure 5: Binding of 15 µg/ml of Protein A purified antibody (hu8A6_VH + hu8A6_VL and ch8A6_N297A) and ch8A6_N297A to native FcγRIIA expressed on K562 cells. Antibodies according to the invention do not bind to FcγRIIA on K-562.

Figure 6

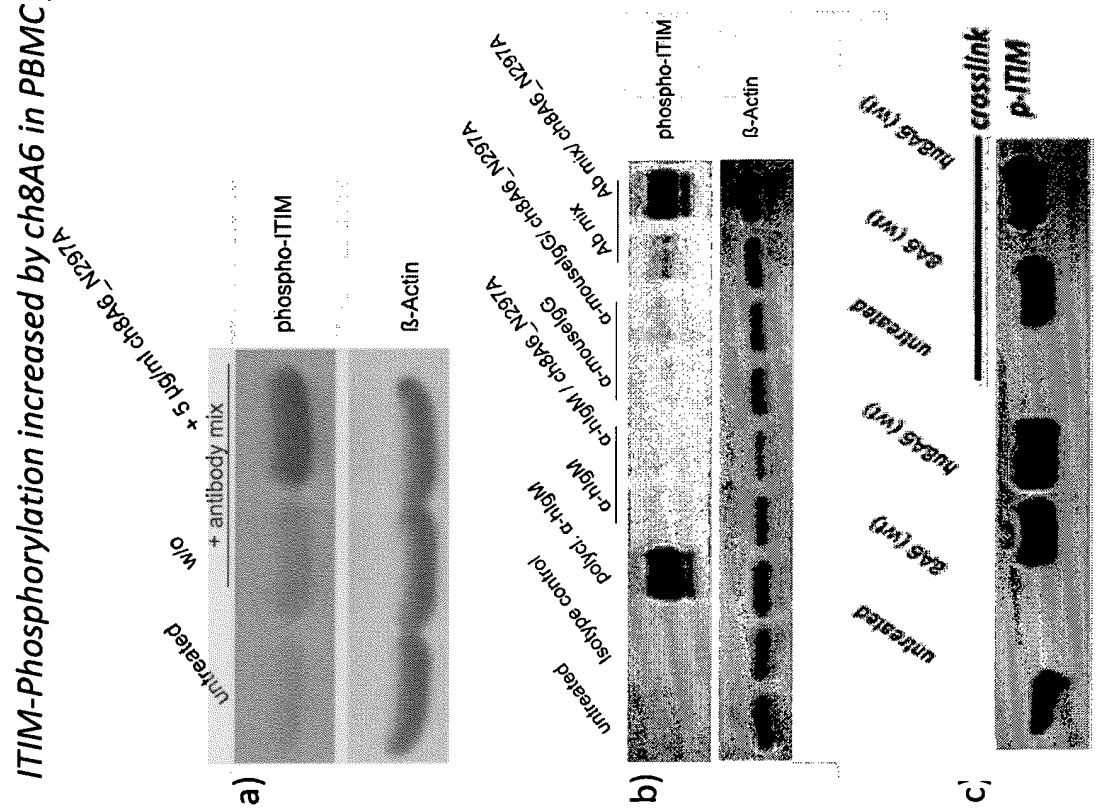


Figure 6 a:
ITIM-Phosphorylation Assay. PBMC from healthy donor were isolated using Ficoll seperation and subsequently left untreated or incubated for 25 minutes with an antibody mix containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit). Subsequently the cells were either treated 20 minutes with 5µg/mL ch8A6_N297A or buffer as control (w/o). Cells were harvested after incubation and lysed according to protocol. Lysates were subjected to WB-analysis. ß-Actin = loading control.

Figure 6 b:

Control experiment for ITIM-Phosphorylation. Daudi cells were left untreated or treated for 25 minutes with an isotype control antibody, polyclonal anti-human anti-IgM (polycl. anti-hIgM), monoclonal anti human IgM (anti-hIgM), anti-hIgM + 5µg/mL ch8A6_N297A, anti-mouse IgG from rabbit (amouseIgG), amouseIgG+5µg/mL ch8A6, mix of anti-hIgM and amouseIgG (Ab mix) or Ab mix + 5µg/mL ch8A6_N297A). ß-Actin = loading control.

Figure 6c:

8A6 (wt) as well as hu8A6 showed strong phosphorylation of Fcgamma RIIB without the need of preceding coligation of BCR and FcgRIIB. After crosslinking of the receptors by the antibody mix, 8A6 (wt) and hu8A6 were - as expected - able to induce ITIM phosphorylation

Figure 7

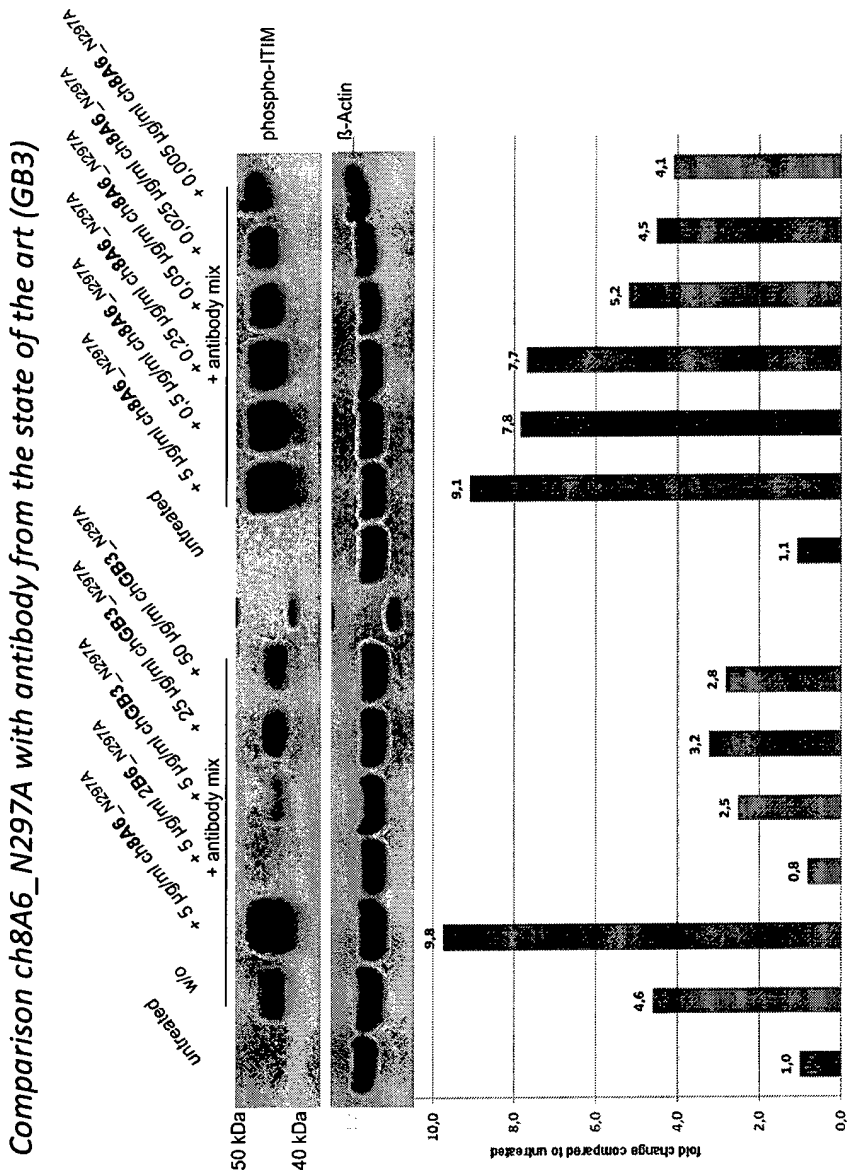


Figure 7:
ITIM-Phosphorylation Assay. Daudi cells left were either left untreated or incubated for 25 minutes with an antibody mix containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit). Subsequently the cells were either treated 20 minutes with varying amounts of chGB3_N297A or ch8A6_N297A or buffer as control (w/o). Cells were harvested after incubation and lysed according to protocol. Lysates were subjected to WB-analysis. ß-Actin = loading control.

Figure 8

Comparison of the effect of the humanized variant hu8A6_N297A and chGB3_N297A and ch8A6_N297A on ITIM phosphorylation in primary PBMCs

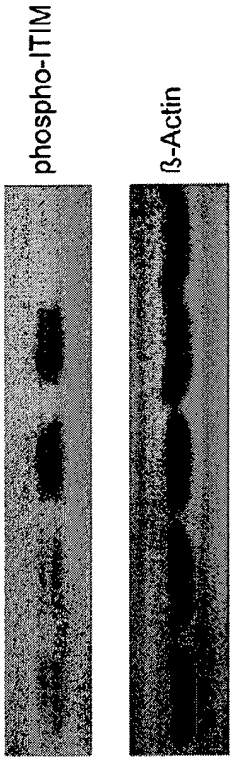


Figure 8: Comparison of the effect of the humanized variant hu8A6_N297A and chGB3_N297A and ch8A6_N297A on ITIM phosphorylation in primary PBMCs. After crosslinking of BCR and FcgRIIB by the antibody mix, the different antibodies were added at 5 µg/ml and Western Blot analysis for ITIM phosphorylation was conducted. ß-Actin = loading control.

Figure 9

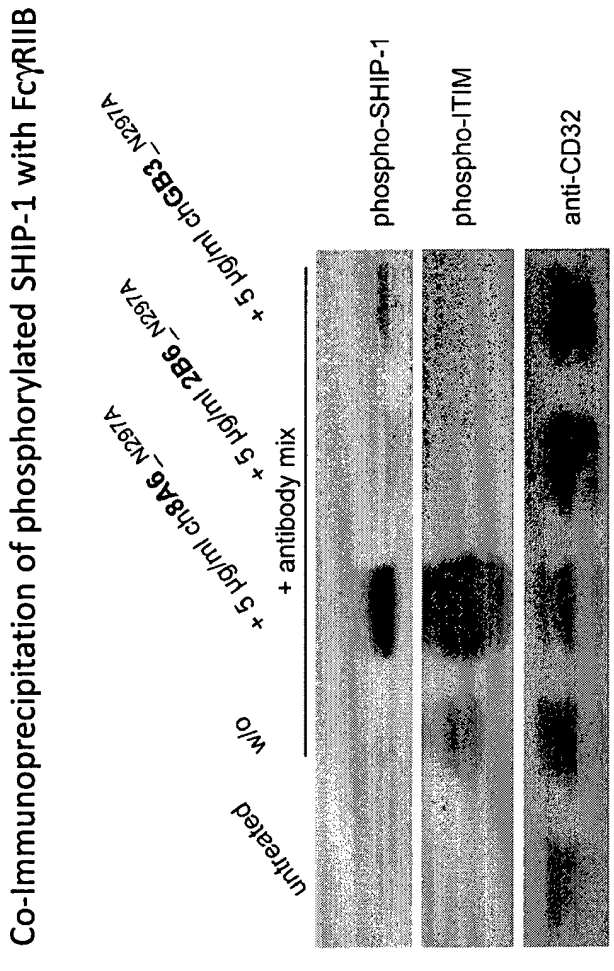


Figure 9: Co-Immunoprecipitation of phosphorylated SHIP-1 with FcγRIIB ITIM. After stimulation of Daudi cells with the antibody mix and either ch8A6_N297A, blocking anti-FcγRIIB antibody 2B6 or chGB3_N297A (5µg /mL), FcγRIIB was precipitated from cell lysates and Western Blot analysis was performed for the phosphatase SHIP-1. anti-CD32 using pan anti-CD32 antibody (AF1330) = loading control.

Figure 10

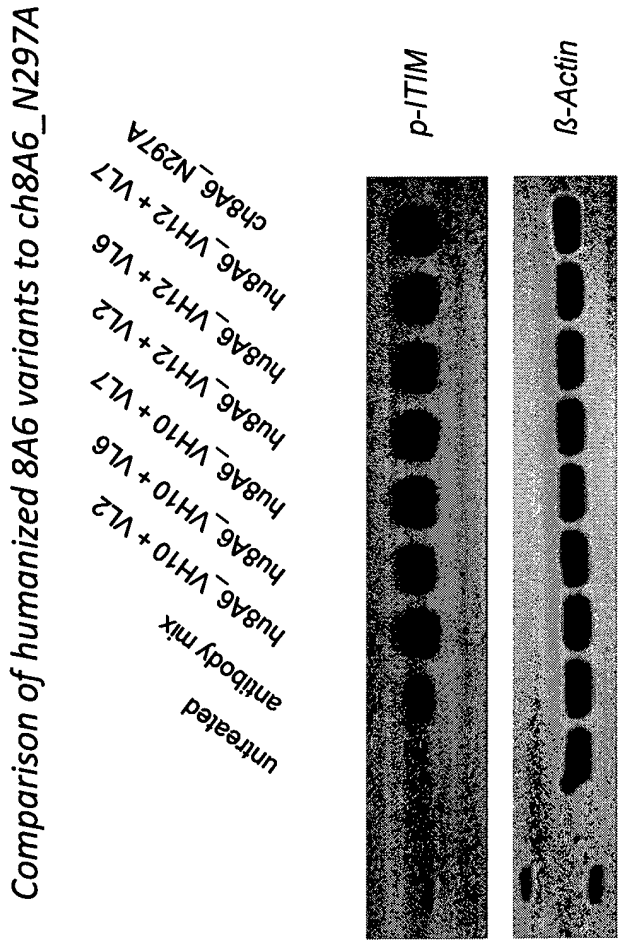


Figure 10:
ITIM-Phosphorylation Assay. Daudi cells were left incubated for 25 minutes with buffer (untreated) or a mix (antibody mix) containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit). Subsequently the cells were either treated 20 minutes with 0.25µg/mL of ch8A6_N297A or humanized 8A6 variants hu8A6_VH10+VL2/VH10+VL6/ VH10+VL7/VH12+VL2/VH12+VL6/VH12+VL7. Cells were harvested after incubation and lysed according to protocol. Lysates were subjected to WB-analysis. ß-Actin = loading control.

Figure 11

Comparison of humanized 8A6 variants to ch8A6_N297A, 2B6 and chGB3_N297A

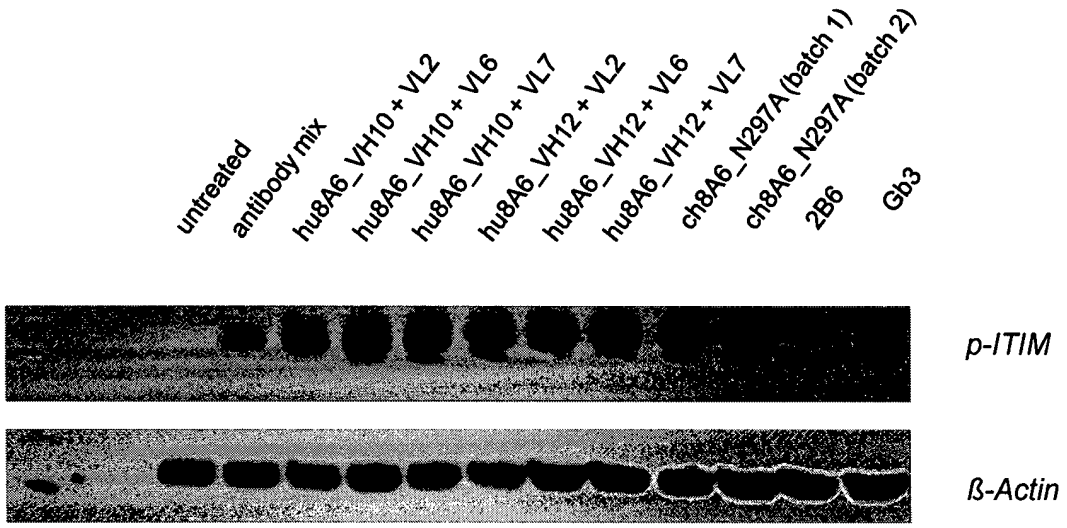


Figure 11:
ITIM-Phosphorylation Assay. Daudi cells were left incubated for 25 minutes with buffer (untreated) or a mix (antibody mix) containing anti-IgM (anti-human, mouse) and anti-mouse IgG (rabbit). Subsequently the cells were either treated 20 minutes with 0.25µg/mL of ch8A6_N297A, humanized 8A6 variants hu8A6_VH10+VL2/VH10+VL6/ VH10+VL7/VH12+VL2/VH12+VL6/VH12+VL7, blocking 2B6 or chGB3_N297A. Cells were harvested after incubation and lysed according to protocol. Lysates were subjected to WB-analysis. ß-Actin = loading control.

Figure 12

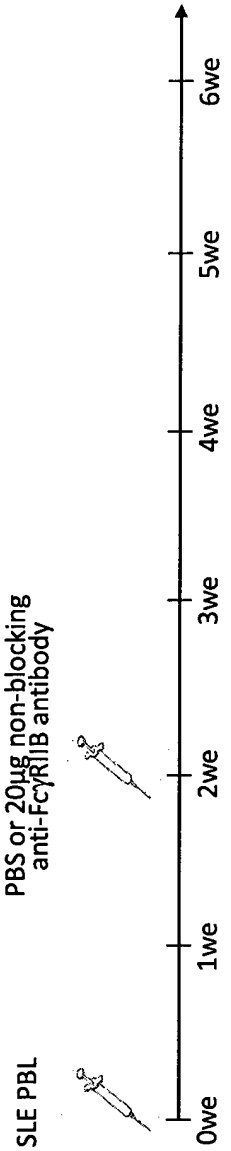


Figure 12: Experimental setup for SLE-PBL mouse model. PBL from human SLE patients are transfered into immuno-compromised mice. PBL cells are engrafted and mice are subsequently treated with control (PBS) or anti-FcγRIIb ch8A6_N297A antibody according to the invention.

Figure 13

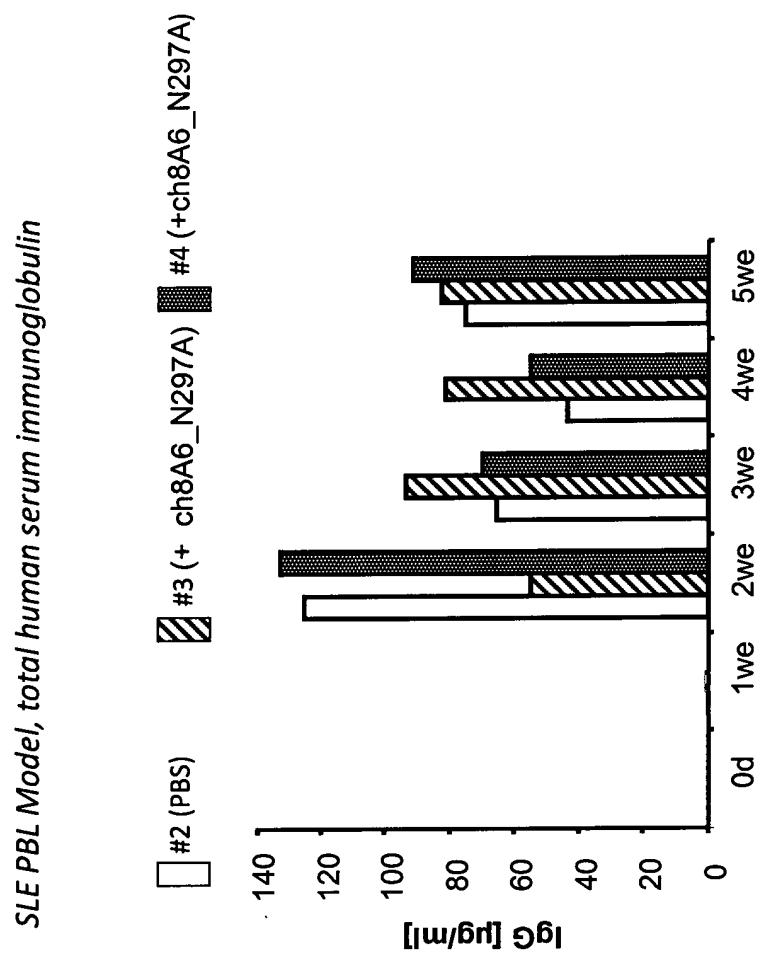


Figure 13:
Total human IgG level [µg/mL] in mice grafted with PBL from human donors suffering from SLE. Depicted are mice treated with control (#2, PBS) or chimeric 8A6 = ch8A6_N297A (#3 and #4, anti-FcγRIIB antibody, N297A-Format). No significant difference in total human IgG between PBS or anti-FcγRIIB.

Figure 14

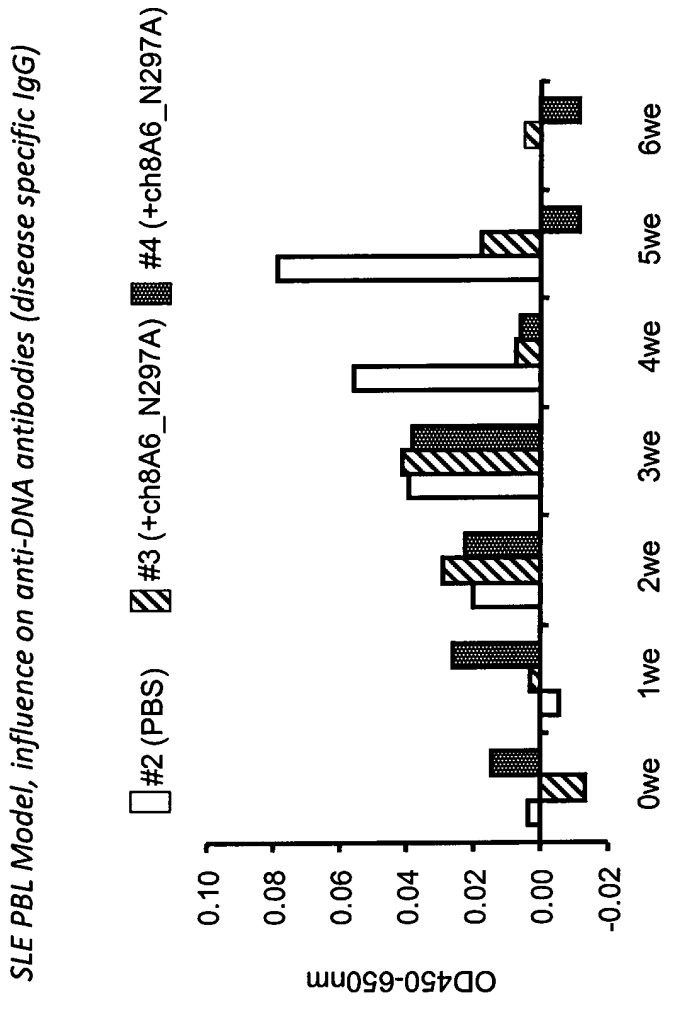


Figure 14:
Reduction of disease specific human anti-DNA IgG in ch8A6_N297A treated mice starting week 4 post SLE-PBL transfer/grafting. Depicted are anti-DNA IgG titers in two different mice, #3 and #4 (treated with ch8A6_N297A), #2 shows PBS control