Figure 1 The kappa light chain sequence of 2c3 mouse antibody. The CDRs are labelled.

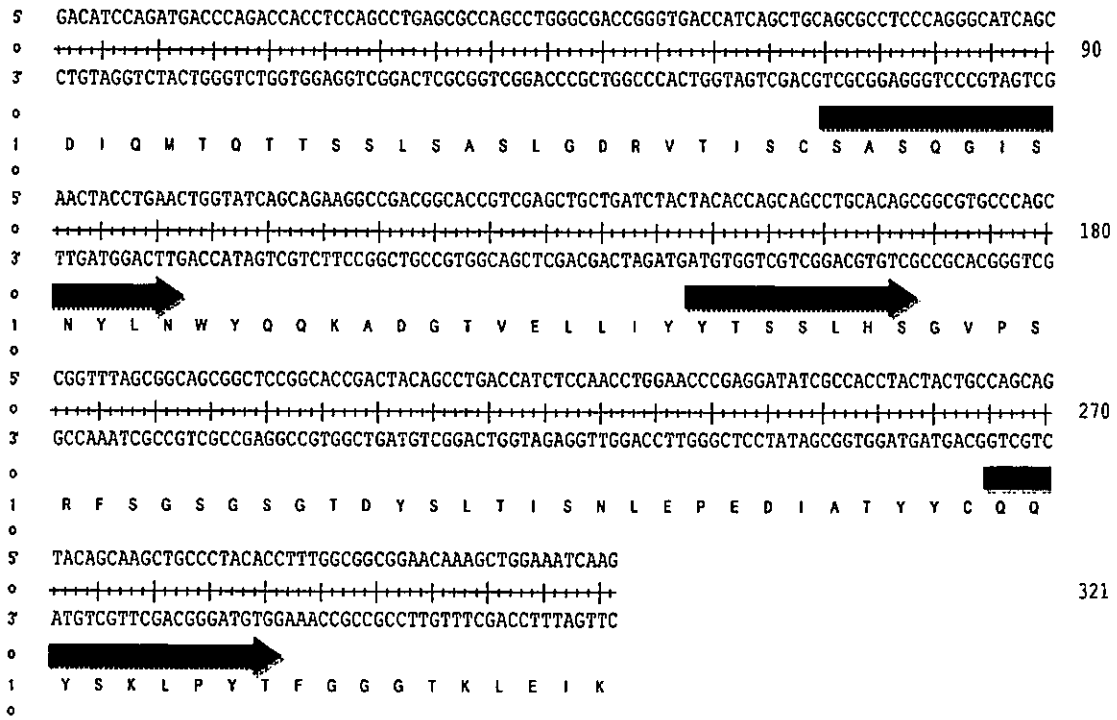


Figure 2 The heavy chain sequence of 2c3 mouse antibody. The CDRs are labelled.

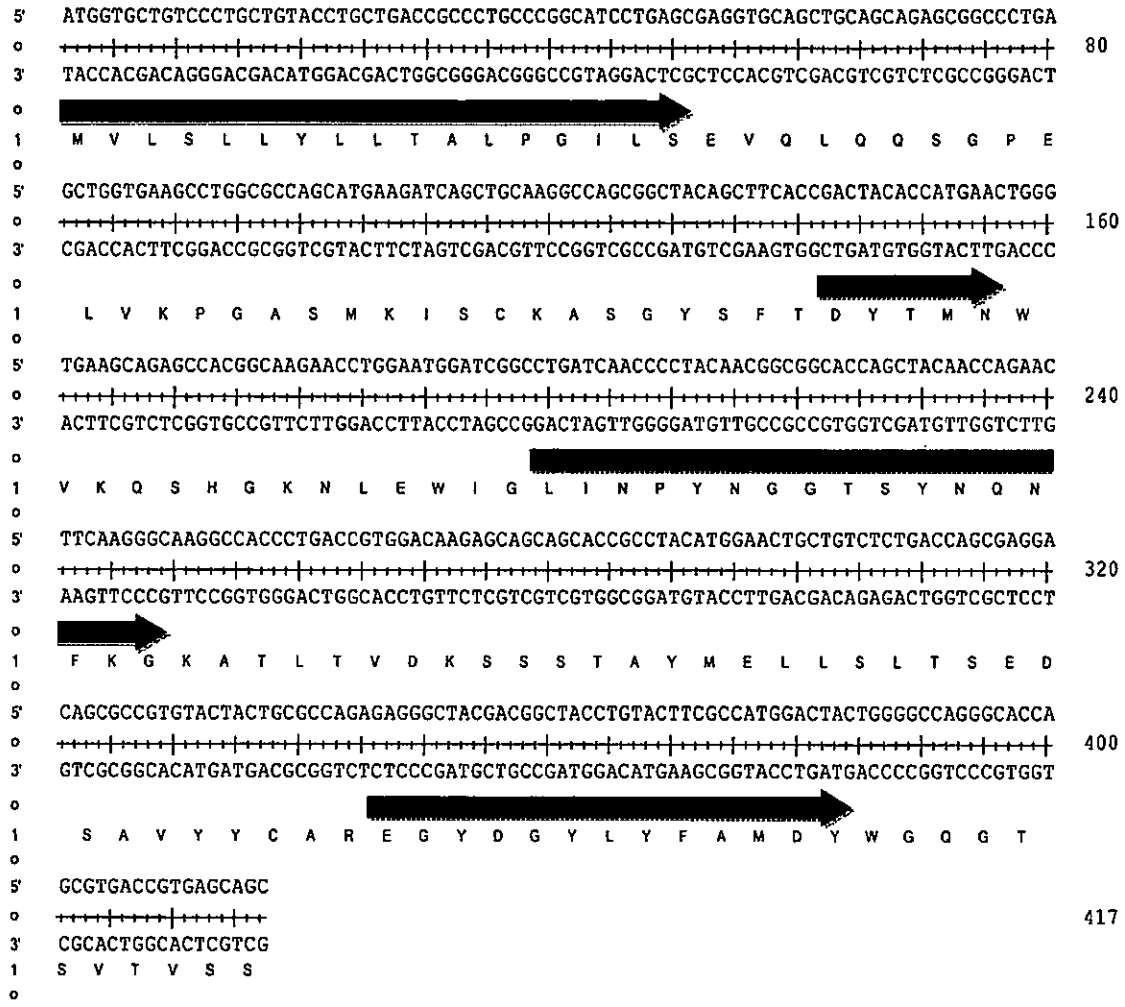


Figure 3 DNA and amino acid sequence of AY393094.

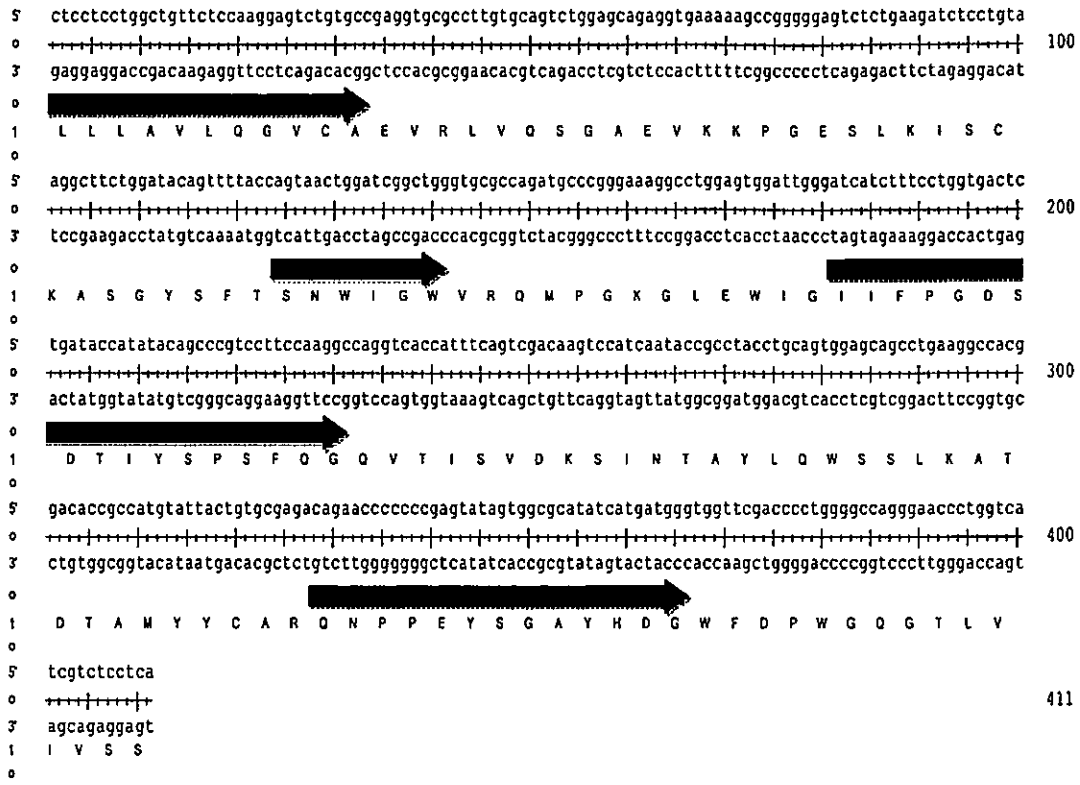


Figure 4 DNA and amino acid sequence of humanised 2c3 RHA.

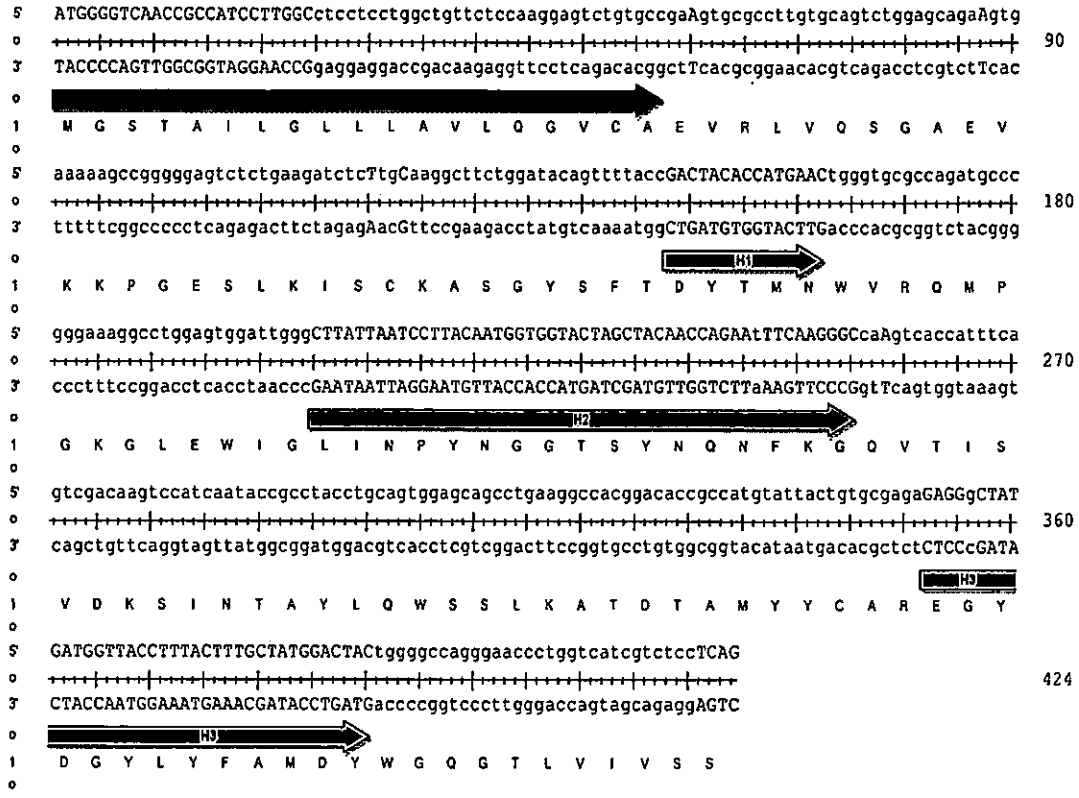


Figure 5 DNA and amino acid sequence of AY510106.

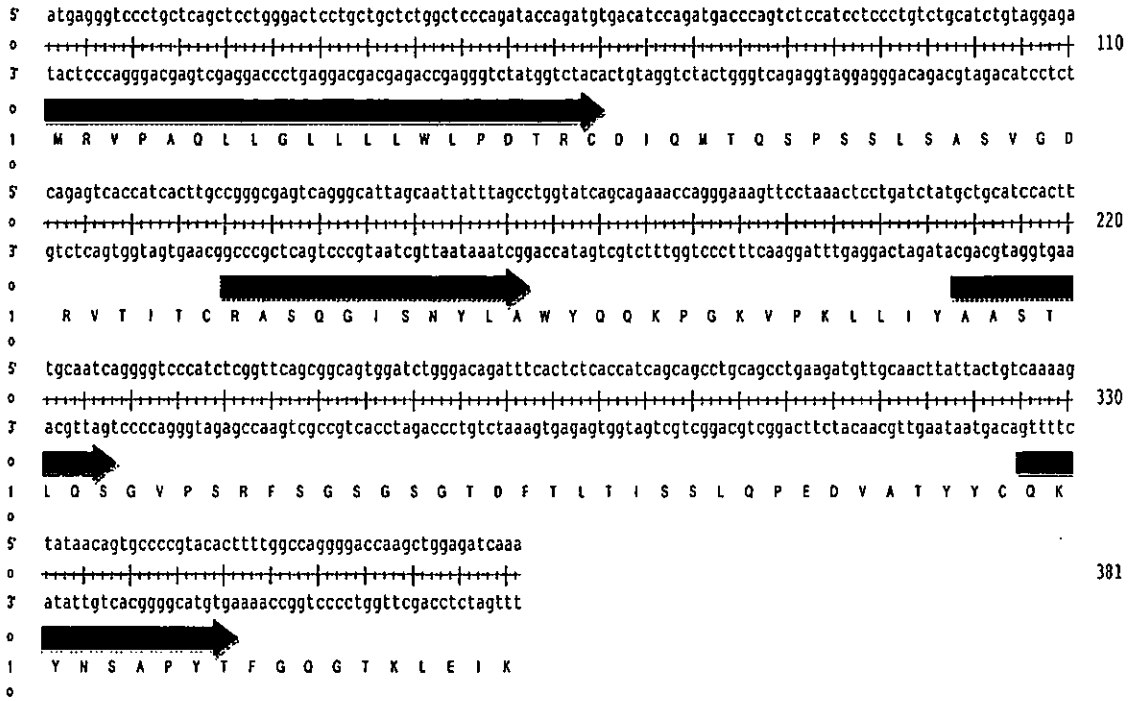


Figure 6 The sequence of the humanised kappa light chain 2c3 RKA.

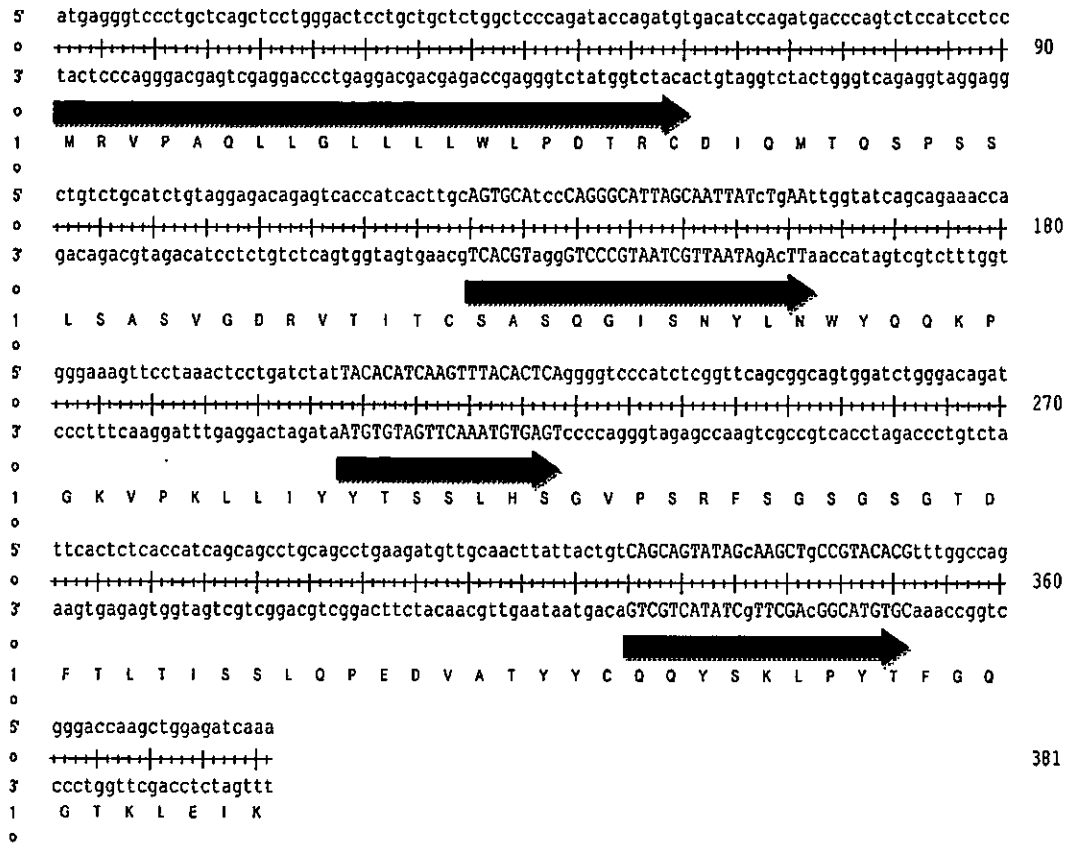
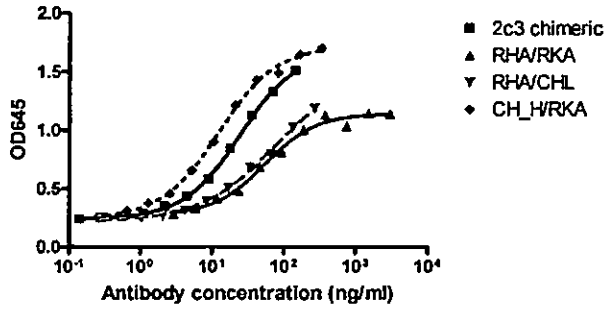
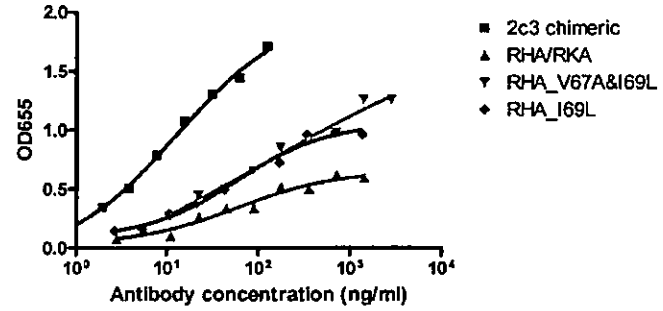


Figure 7 Comparison of humanised antibody 2c3 RHA/RKA and variants with chimeric 2c3.

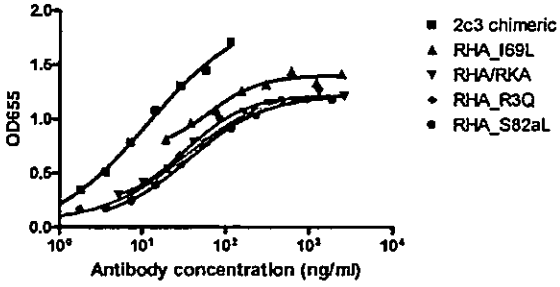
A



B



C



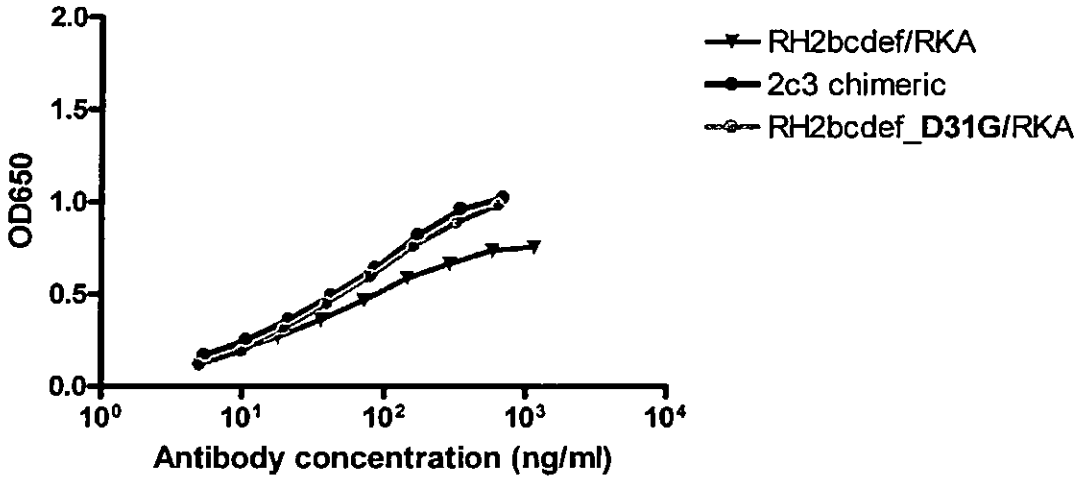
Comparison of IL-25 binding by recombinant 2c3 antibodies A. encoded by 2c3 RHA or 2c3 RKA co-expressed with chimeric 2c3 heavy chain (CH_H) or the 2c3 kappa light chain (CHL) compared with the fully chimeric 2c3 antibody (CH_H + CHL) B. encoded by 2c3 RHA mutants 2c3 RH_V67A&I69L or 2c3 RH_I69L co-transfected with 2c3 RKA and compared to chimeric 2c3 or unmutated humanised 2c3 RHA/RKA antibodies C. encoded by RHA mutants I69L, R3Q, S82aL co-transfected with 2c3 RKA and compared to the chimeric 2c3 or unmutated humanised 2c3 RHA/RKA antibodies. IL-25 binding was measured by ELISA.

Figure 8 Framework AJ399823 used in the design of humanised 2c3 RH2.

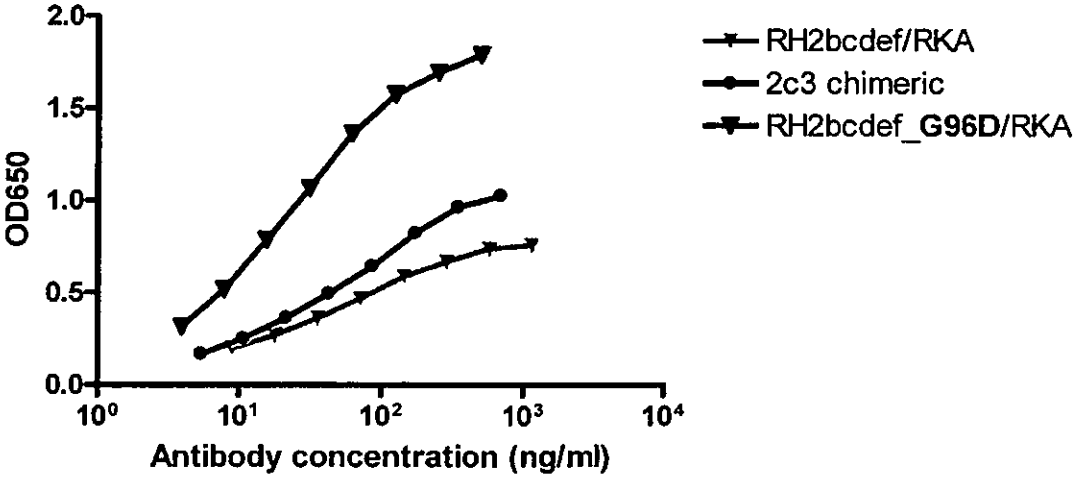


Figure 9 Effect of specific CDR mutations to 2c3 RH2bcdef binding to IL-25.

A

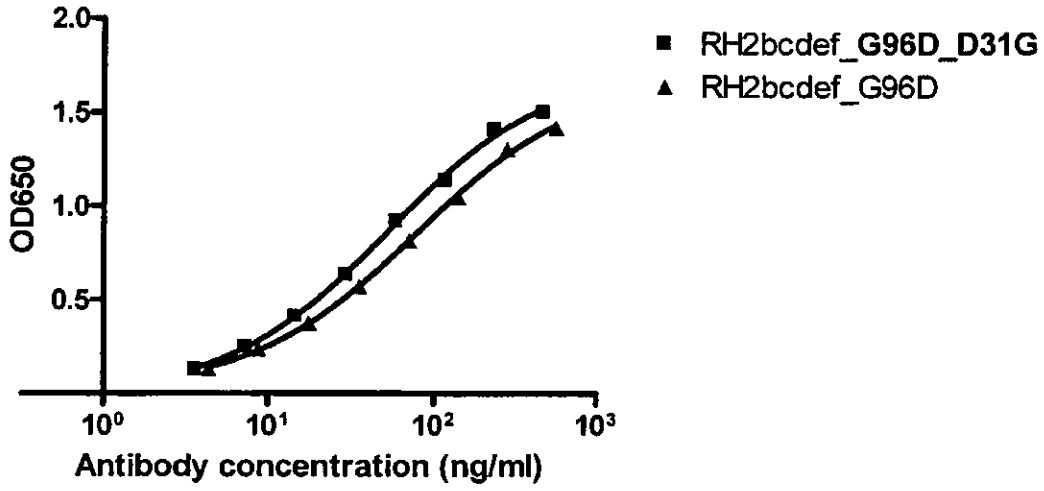


B



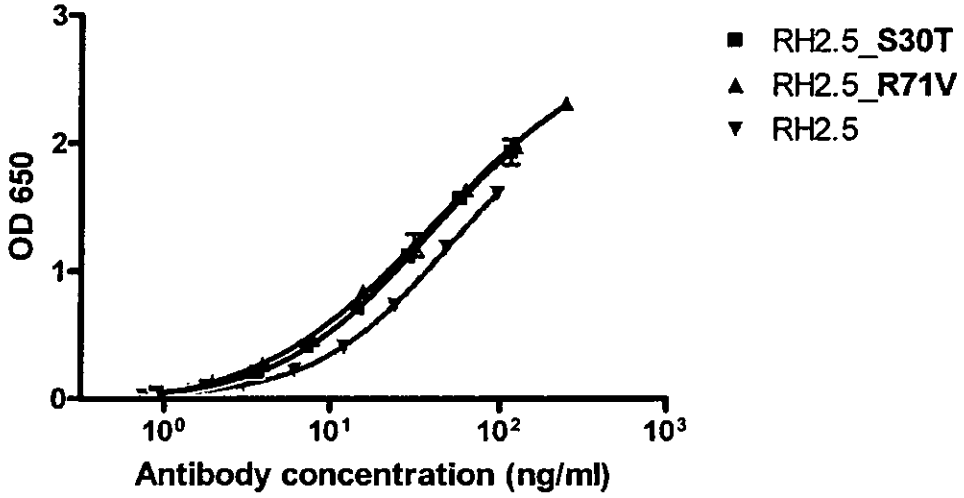
The mutation of the 2c3 RH2bcdef heavy chain at two positions in its CDRs. HEK 293T cells were co transfected with either 2c3 RH2bcdef/RKA or 2c3 chimeric constructs plus **A.** CDR mutation at position 31 from aspartate to glycine (2c3 RH2bcdef_D31G) or **B.** CDR mutation at position 96 from glycine to aspartate. Binding to human IL-25 was measured by ELISA

Figure 10 Effect on IL-25 binding by combining CDR mutations D31G and G96D.



The humanised heavy chain 2c3 RH2bcdef was mutagenised and the mutants D31G and G96D were combined. HEK 293T cells were co transfected with either 2c3 RH2bcdef_G96D_D31G/RKA or 2c3 RH2bcdef_G96D/RKA and supernatants collected after 3 days. Antibody binding to human IL-25 was measured by ELISA.

Figure 11 Comparison of 2c3 RH2.5_S30T and 2c3 RH2.5_R71V binding to IL-25.



RH2.5 is the codon optimised version of RH2 plus the mutations D31G and G96D. The one remaining untested VCI residue, S30T, was examined and its binding to IL-25 by ELISA was compared with RH2.5_R71V and RH2.5. HEK 293T cells were co-transfected with the RH2.5_S30T or RH2.5_R71V or RH2.5 and the light chain construct RKA. After three days supernatants were harvested and binding to IL-25 measured by ELISA.

Figure 12 *In vivo* mouse model of AHR.

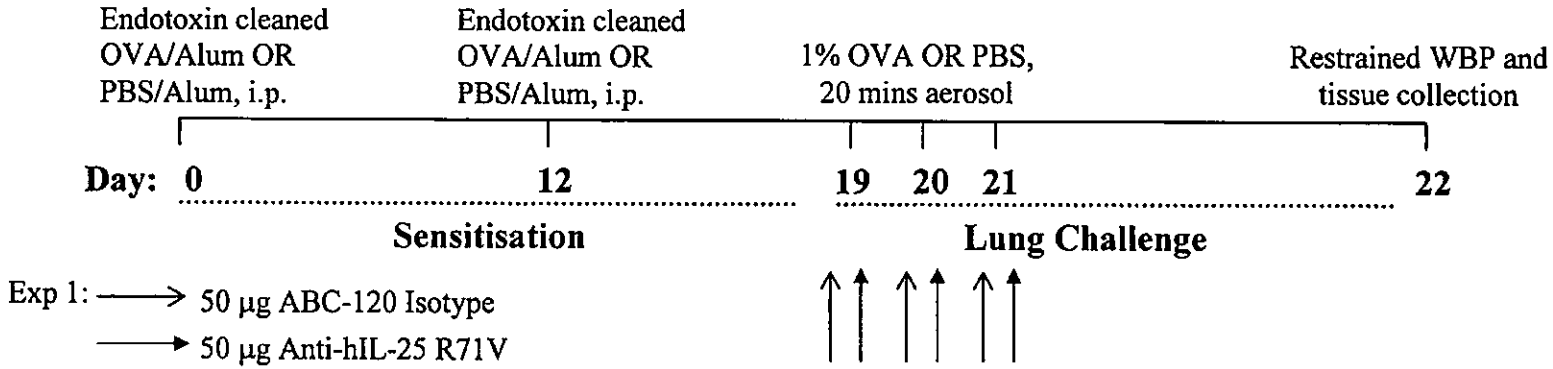
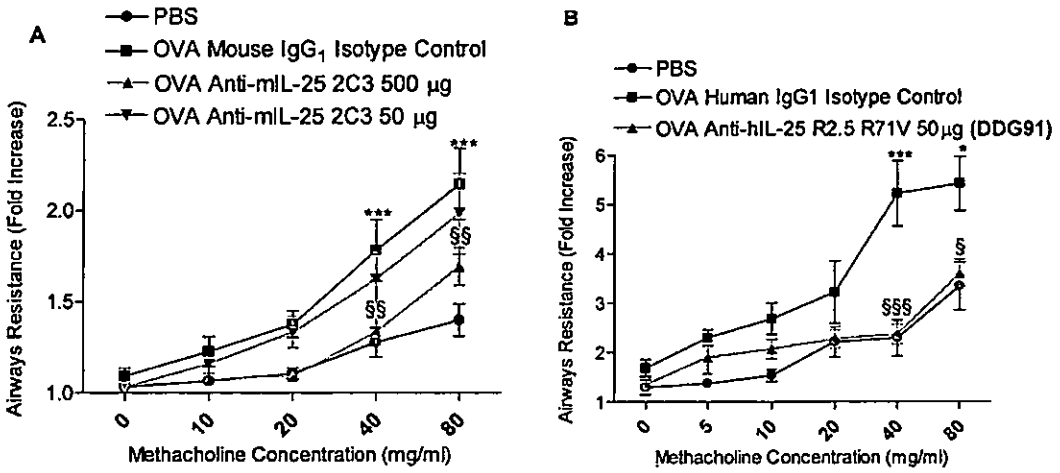


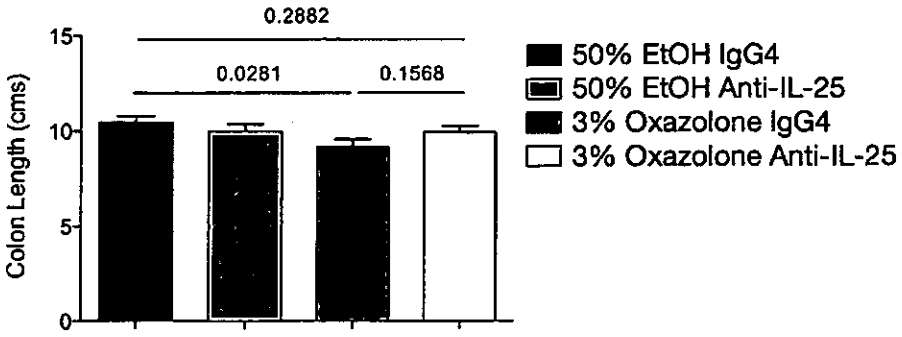
Figure 13 Effect of administering 2c3 RH2.5_R71V to pulmonary resistance in response to methacholine.



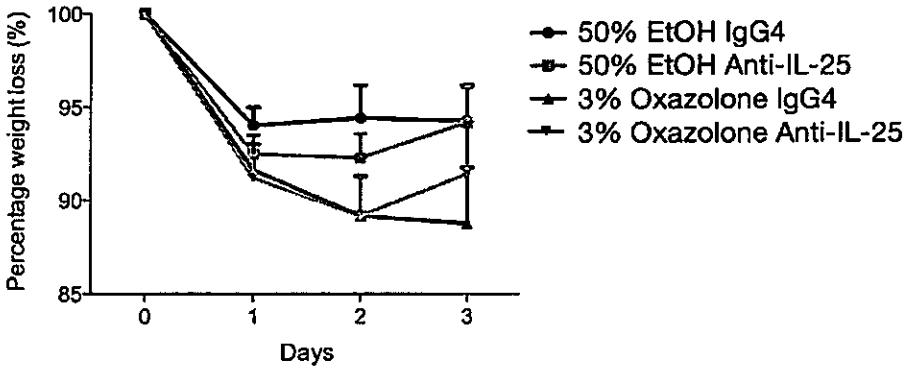
Treatment with anti-mIL-25 2c3 or anti-hIL-25 2c3 RH2.5 R71V during the lung challenge phase in an acute experimental mouse asthma model blocks AHR. Airways resistance was measured by restrained whole body plethysmography in response to PBS and then to increasing concentrations of methacholine. *(A)* Mice received anti-mIL-25 2c3 prior to each OVA aerosol challenge at either a 500 µg or a 50 µg dose. *(B)* Mice received anti-hIL-25 RH2.5 R71V prior to each OVA aerosol challenge at a 50 µg dose. A two way ANOVA with Bonferroni post-test was used to assess statistical differences. *, **, and *** denotes a significance value of $p=<0.05$, $p=<0.01$, and $p=<0.001$ between PBS and OVA IgG_1 isotype treated mice. §, §§, and §§§ denotes a significance value of $p=<0.05$, $p=<0.01$, and $p=<0.001$ between OVA IgG_1 isotype and anti-IL-25 treated mice.

Figure 14

A


0.2882
0.0281
0.1568
Colon Length (cms)
15
10
5
0
50% EtOH IgG4
50% EtOH Anti-IL-25
3% Oxazolone IgG4
3% Oxazolone Anti-IL-25

B


Percentage weight loss (%)
100
95
90
85
0
1
2
3
Days
50% EtOH IgG4
50% EtOH Anti-IL-25
3% Oxazolone IgG4
3% Oxazolone Anti-IL-25