SUPPLEMENTAL DATA

SUPPLEMENTAL TABLE 1. SPR Req binding responses of chimeric anti-CEA scFv-Fc variants to immobilized mFcRn.

Fragment	Req (mFcRn) 110 RU[a]	Req (mFcRn) 900 RU[a]	Average relative binding[b]
WT	54	212.8	1.0
H435R	57.1	236.8	1.09
H435Q	16.9	73.4	0.33
I253A	5	34.3	0.13
H310A	2.3	11.7	0.05
H310A/H435Q	NB[c]	2.1	0.01[d]

[a] Resonance unit (RU) responses of duplicate injections of the scFv-Fc variants over levels of immobilized recombinant soluble mFcRn at two densities.

[b] Relative binding based on the equilibrium binding response (Req) average from two different immobilized densities of mFcRn.

[c] No binding, (NB).

[d] High density surface only.

SUPPLEMENTAL TABLE 2. SPR Req binding responses of chimeric anti-CEA scFv-Fc variants to immobilized hFcRn.

Fragment	Req (hFcRn) 320 RU[a]	Req (hFcRn) 1200 RU[a]	Average relative binding[b]
WT	105	244.1	1.0
H435R	110	306.6	1.15
H435Q	NB[c]	NB	-
I253A	NB	9.1	0.04[d]
H310A	NB	NB	-
H310A/H435Q	NB	NB	-

[a] Resonance unit (RU) responses of duplicate injections of the scFv-Fc variants over immobilized recombinant soluble hFcRn at two densities.

[b] Relative binding based on the equilibrium signal level (Req) average from two different immobilized densities of hFcRn.

[c] No binding (NB).

[d] High density surface only.

SUPPLEMENTAL TABLE 3. Statistics (2-way ANOVA analysis) of the blood activity curves of ^{123}I-labeled scFv-Fc fragments in human FcRn transgenic mice.

WT Balb/c	hFcRnTg	P value	Significance <0.01 (99%)
WT	**WT**	0,0068	YES
H435R	**H435R**	< 0.0001	YES
H435Q	**H435Q**	< 0.0001	YES
I253A	**I253A**	0,0004	YES
H310A	**H310A**	0,0038	YES
H310A/H435Q	**H310A/H435Q**	0,051	NO

```
hFcRn           MGVPRPQPWALGLLLFLLPGSLGAESHLSLLYHLTAVSSPAPGTPAFWVSGWLGPQQYLS 60
mFcRn           MGMP--LPWALSLLLVLLPQTWGSETRPPLMYHLTAVSNPSTGLPSFWATGWLGPQQYLT 58
                **:*   ****.***.*** : *:*:: .*:*******.*:.* *:**.:*********:

hFcRn           YNSLRGEAEPCGAWVWENQVSWYWEKETTDLRIKEKLFLEAFKALG--GKGPYTLQGLLG 118
mFcRn           YNSLRQEADPCGAWMWENQVSWYWEKETTDLKSKEQLFLEALKTLEKILNGTYTLQGLLG 118
                ***** **:*****:****************: **:*****:*:*    :*.********

hFcRn           CELGPDNTSVPTAKFALNGEEFMNFDLKQGTWGGDWPEALAISQRWQQQDKAANKELTFL 178
mFcRn           CELASDNSSVPTAVFALNGEEFMKFNPRIGNWTGEWPETEIVANLWMKQPDAARKESEFL 178
                ***..**:***** *********:*: : *.* *:***:  ::: * :* .**.**  **

hFcRn           LFSCPHRLREHLERGRGNLEWKEPPSMRLKARPSSPGFSVLTCSAFSFYPPELQLRFLRN 238
mFcRn           LNSCPERLLGHLERGRRNLEWKEPPSMRLKARPGNSGSSVLTCAAFSFYPPELKFRFLRN 238
                * ***.**  ****** ****************...* *****:*********::*****

hFcRn           GLAAGTGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQHAGLAQPLRVELESPAKSSV 298
mFcRn           GLASGSGNCSTGPNGDGSFHAWSLLEVKRGDEHHYQCQVEHEGLAQPLTVDLDSSARSSV 298
                ***:*:*: . ***.****** * * ** ****** * *:* ****** *:*:*.*:***

hFcRn           LVVGIVIGVLLLTAAAVGGALLWRRMRSGLPAPWISLRGDDTGVLLPTPGEAQDADLKDV 358
mFcRn           PVVGIVLGLLLVVVAIAGGVLLWGRMRSGLPAPWLSLSGDDSGDLLPGGNLPPEAEPQGA 358
                 *****:*:**:..* .**.*** **********:** ***:* ***  . . :*: :..

hFcRn           NVIPATA 365
mFcRn           NAFPATS 365
                *.:***:
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SUPPLEMENTAL FIGURE 1. CLUSTAL multiple amino acid alignment of mouse and human FcRn HC. Amino acids E115, E116 and W131 are highlighted in red (human numbering). Alignment score: 66%.

A.

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hIgG1           -----------------------------------------------EPKSCDKTHTCPP 13
hIgG3           ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR 60
hIgG2           -----------------------------------------------ERKCC---VECPP 10
hIgG4           -----------------------------------------------ESKYG---PPCPS 10
                                                               * *       **

hIgG1           CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK 73
hIgG3           CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAK 120
hIgG2           CPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK 69
hIgG4           CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK 70
                **** . * ******************************:*****:*:************

hIgG1           TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV 133
hIgG3           TKLREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV 180
hIgG2           TKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV 129
hIgG4           TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV 130
                ** ****:***:********:*****************.**:.*******:*********

hIgG1           YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS 193
hIgG3           YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYNTTPPMLDSDGSFFLYS 240
hIgG2           YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYS 189
hIgG4           YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS 190
                ******:************************************:****:***********

hIgG1           KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 232
hIgG3           KLTVDKSRWQQGNIFSCSVMHEALHNRYTQKSLSLSPGK 279
hIgG2           KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 228
hIgG4           RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK 229
                :*********:**:************:********* **
```

B.

```
mIgG1           ---VP--RDCGCKPC----ICTVPEVS---SVFIFPPKPKDVLTITLTPKVTCVVVDISK 48
mIgG3           EPRIP--KPSTPPGS----SCPPGNILGGPSVFIFPPKPKDALMISLTPKVTCVVVDVSE 54
mIgG2a          EPRGP--TIKPCPPC----KCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSE 54
mIgG2b          EPSGPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSE 60
                    *         .     *.  ::    *******: **.* *:*:* *******:*:

mIgG1           DDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAF 108
mIgG3           DDPDVHVSWFVDNKEVHTAWTQPREAQYNSTFRVVSALPIQHQDWMRGKEFKCKVNNKAL 114
mIgG2a          DDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL 114
mIgG2b          DDPDVQISWFVNNVEVHTAQTQTHREDYNSTIRVVSTLPIQHQDWMSGKEFKCKVNNKDL 120
                ***:*:.****:: ***** **.:. ::***:* ** *** ****: ******:**.  :

mIgG1           PAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPA 168
mIgG3           PAPIERTISKPKGRAQTPQVYTIPPPREQMSKKKVSLTCLVTNFFSEAISVEWERNGELE 174
mIgG2a          PAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTE 174
mIgG2b          PSPIERTISKIKGLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTE 180
                *:***:**** **  ::**** :*** *::::..*:***::..* .  * ***  **.

mIgG1           ENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK 227
mIgG3           QDYKNTPPILDSDGTYFLYSKLTVDTDSWLQGEIFTCSVVHEALHNHHTQKNLSRSPGK 233
mIgG2a          LNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK 233
mIgG2b          ENYKDTAPVLDSDGSYFIYSKLNMKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK 239
                 :**:* *::*:**:**:**** :....*   : ::*.* **.*:*::  *.:*::***
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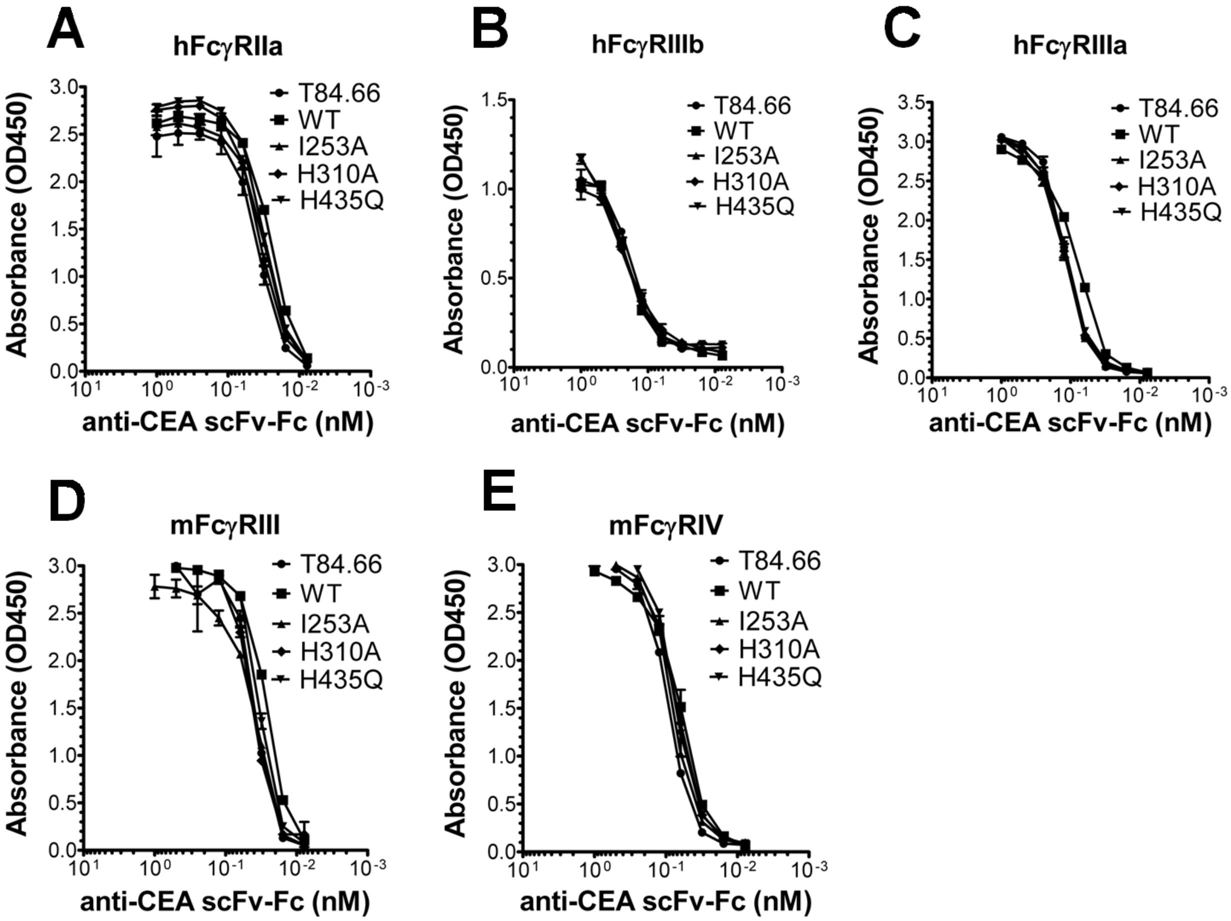
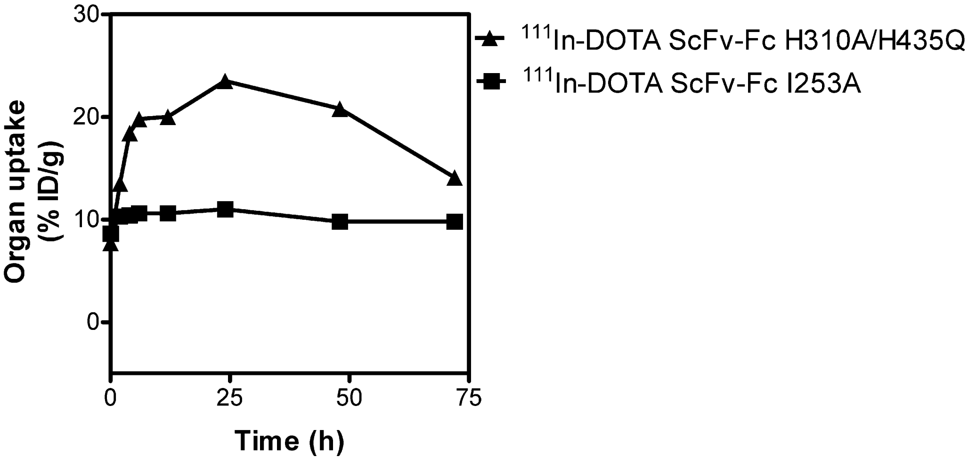
SUPPLEMENTAL FIGURE 2. CLUSTAL multiple amino acid alignment of mouse and human IgG Fc C_H2-C_H3. Amino acid sequences corresponding to the hinge region are highlighted in bold and red while sequence areas involved in binding to classical Fcγ receptors are shown in bold black. The three key amino acid residues involved in binding to FcRn, I253, H310 and H435 are highlighted in bold green.



SUPPLEMENTAL FIGURE 3. ELISA measurements of FcγR binding to scFv-Fc variants. Binding of (A) hFcγRIIa, (B) mFcγRIIIb, (C) hFcγRIIIa, (D) mFcγRIII and mFcγRIV to titrated amounts of T84.66 and the scFv-Fc variants (WT, I253A, H310A and H435Q). The numbers given represent the mean of triplicates.



SUPPLEMENTAL FIGURE 4. (B) Liver accumulation in LS174T xenografted athymic nude mice of ^{111}In-DOTA labeled anti-CEA scFv-Fc H310A/H435Q and I253A post injection. Liver uptake is expressed as percent injected dose per gram (% ID/g). The Figures were constructed using the biodistribution data previously reported (36).