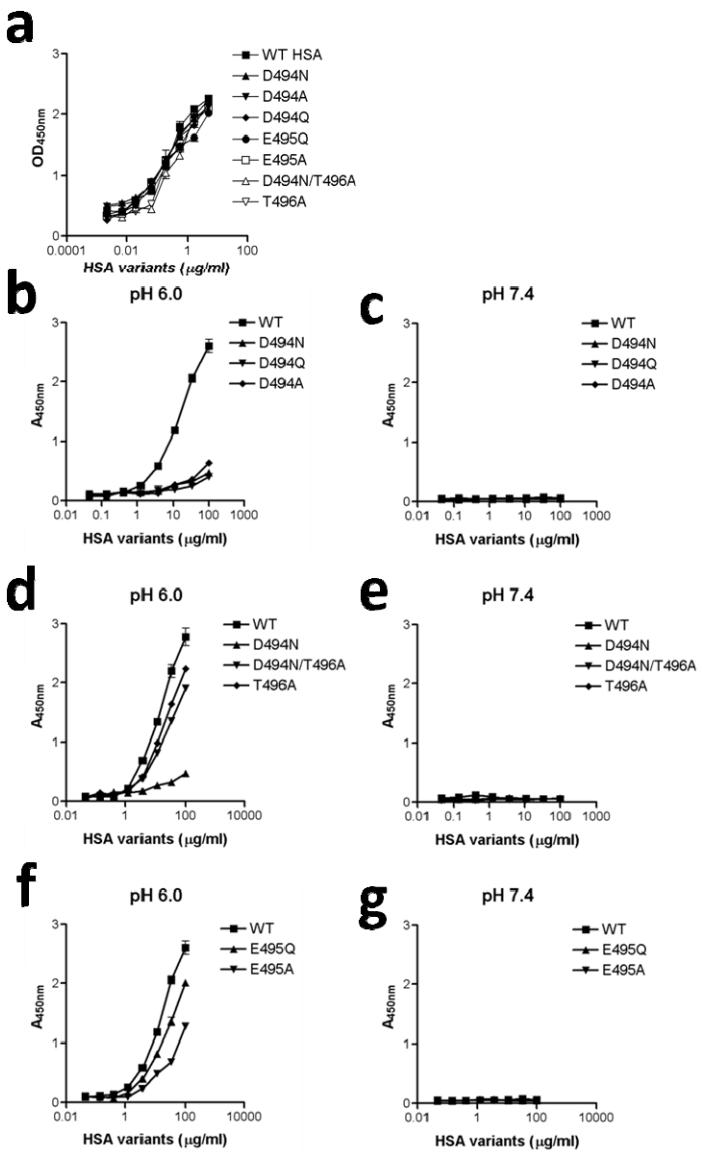
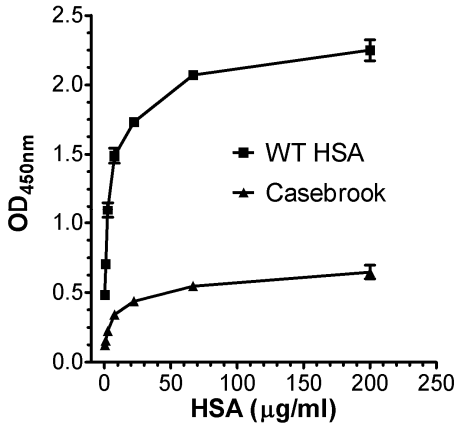
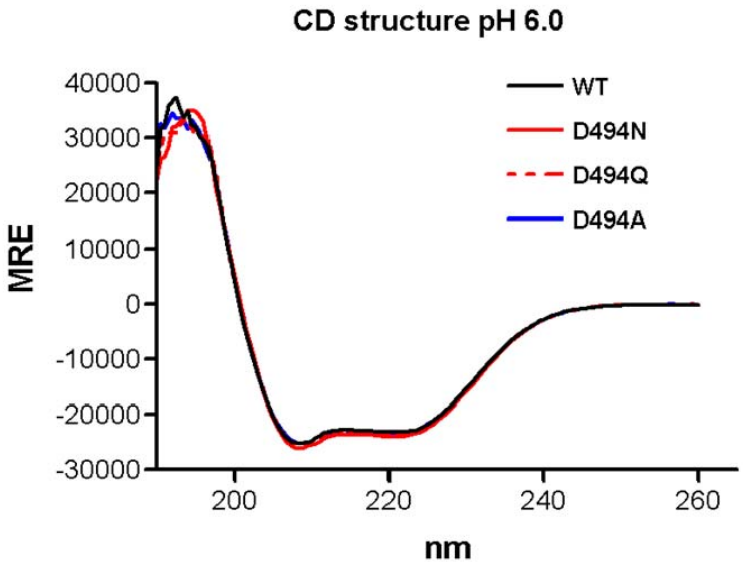


Supplementary Figure S1. Binding of HSA mutants to hFcRn. (a) The levels of titrated amounts of HSA variants (5.0-0.002 μg/ml) directly coated in the wells at pH 6.0 were controlled using a horseradish peroxidase conjugated anti HSA antibody preparation. ELISA responses of HSA WT, D494N, D494Q and D494A binding to hFcRn at (b) pH 6.0 and (c) pH 7.4. ELISA responses of HSA WT, D494N, D494N/T496A and T496A binding to hFcRn at (d) pH 6.0 and (e) pH 7.4. ELISA responses of HSA WT, E495Q and E495A binding to hFcRn at (f) pH 6.0 and (g) pH 7.4. In all FcRn binding experiments titrated amounts (100-0.4 μg ml^{-1}) of the HSA variants were coated in wells followed by addition of a constant amount of hFcRn-GST (0.5 μg ml^{-1}). Bound receptor was detected using an HRP-conjugated anti-GST antibody. The values represent the average of triplicates. (a-g) *n*=3-4. All data are presented as mean ± s.d.

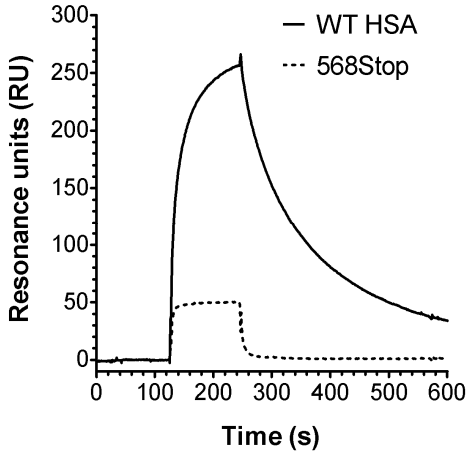


Supplementary Figure S2. Binding of WT and Casebrook HSA to hFcRn. ELISA responses showing binding of WT and the Casebrook HSA variant isolated from a heterozygous individual to hFcRn at pH 6.0. Titrated amounts of the HSA variants (200-0.3 μg ml^{-1}) were coated in wells followed by adding of a constant amount of hFcRn-GST (0.5 μg ml^{-1}). Bound receptor was detected using an HRP-conjugated anti-GST antibody. The values represent the average of triplicates. *n*=3. All data are presented as mean ± s.d.

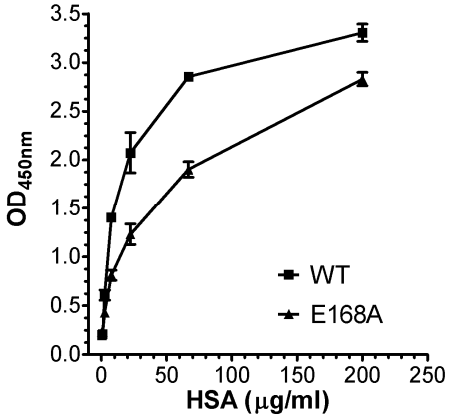


Supplementary Figure S3. CD spectra for HSA variants. Representative CD spectra of WT and HSA mutants obtained by CD measurements at pH 6.0. MRE; mean residual ellipticity. The secondary structural elements for each of the HSA variants are summarized in Supplementary Table S1.

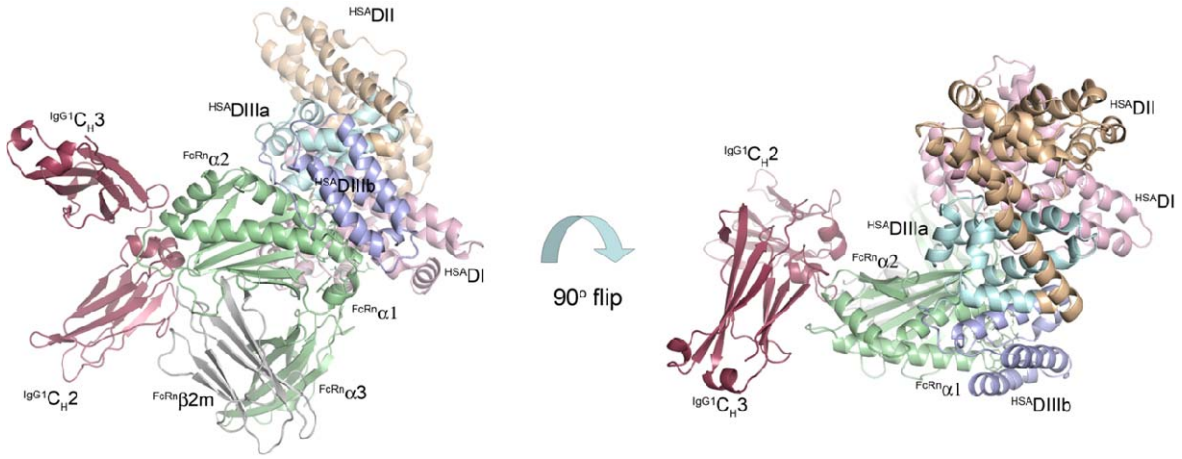
Supplementary Figure S4. SDS-PAG analysis of HSA variants. Equal amounts (1 µg) of HAS variants (H440Q, H464Q, H510Q and H535Q) were applied on a NuPAGE 4-12% Bis-Tris gel. Electrophoresis was performed for 50 min at 200 V. Gels were stained with InstantBlue protein stain (Expedeon).



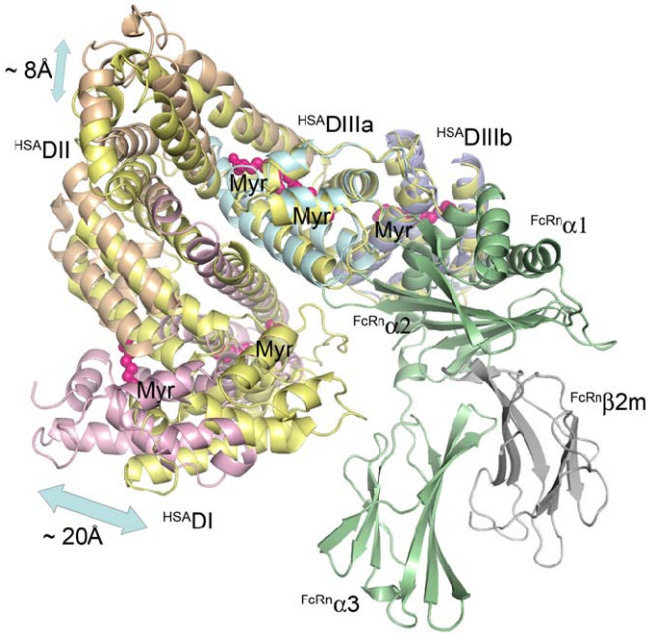
Supplementary Figure S5. SPR binding responses of HSA variants. Representative SPR responses obtained when 1 μM of WT HSA and HSA 568Stop (truncated variant that lacks the last 17 a.a.) were injected over immobilized hFcRn (2000 RU) at pH 6.0. Injections were performed at 25 °C with flow rate of 50 μl min^{-1}.



Supplementary Figure S6. Binding of hFcRn variants to HSA. ELISA responses showing binding of (0.5 μg ml^{-1}) hFcRn WT and hFcRn E168A to titrated amounts of the WT HSA (200-0.3 μg ml^{-1}) coated in wells. The ELISA was performed at pH 6.0 and bound receptors were detected using an HRP-conjugated anti-GST antibody. The values represent the average of triplicates. *n*=3. All data are presented as mean ± s.d.



Supplementary Figure S7. A structural model of IgG and HSA bound to hFcRn. The ternary model was made by superpositioning of the crystal structure of rat FcRn in complex with IgG2a Fc (Pdb code 1FRT) onto the hFcRn-HSA complex from the ZDOCK docking, followed by replacement of rat IgG2a Fc with the corresponding human IgG1 Fc (Pdb code 1l6x). The crystal model of the ternary hIgG1-hFcRn-HSA complex was designed using PyMOL (DeLano Scientific) and is shown in two orientations. The hFcRn HC in green, β2m in gray, and the three HSA α-helical domains DI in pink, DII in orange and DIII in cyan/blue. The HSA DIII is split into DIIIa (cyan) and DIIIb (blue). The two domains (C_H2 and C_H3) of half of the IgG1 Fc molecule are shown in maroon.



Supplementary Figure S8. Predicted conformational change in HSA-hFcRn upon binding of fatty acids. The docked HSA without fatty acids (yellow) binds to the α1 and α2 domains of hFcRn (green) mainly via HSA DIII and DI. Upon binding of fatty acids (5 myristate (Myr) molecules shown in magenta spheres), DI (pink) and DII (orange) undergoes a larger conformational change relative to DIII (cyan/blue), with up to 20Å displacements. HSA DI moves away from hFcRn. The crystal structure figure was designed using PyMOL (DeLano Scientific) with the crystallographic data of HSA as previously described (Pdb codes 1bm0 and 1bj5). The hFcRn HC is shown in green and β2m in gray.

Supplementary Table S1. Secondary structural elements determined by CD

HSA variant	Structural elements pH 7.4					Structural elements pH 6.0				
	Helix	Antiparallel	Parallel	Beta turn	Random coil	Helix	Antiparallel	Parallel	Beta turn	Random coil
WT	72	0	0,7	7,3	20	**64,8**	0,5	1,7	10,1	22,4
D494N	65,6	0,4	1,7	10,3	22	**67,9**	0,2	1,4	9,3	21,2
D494A	62,2	1,4	2,5	12,7	21,2	**65,9**	0,5	1,7	10,3	21,7
E495Q	65,3	0,3	1,7	10,4	22,2	**65**	0,6	1,8	10,5	22,1
E495A	64,8	0,5	1,8	10,6	22,2	**65,1**	0,5	1,7	10,5	22,2
D494Q	66,1	0,3	1,6	10,1	21,8	**67,1**	0,3	1,5	9,5	21,6

Supplementary Table S2. CLUSTAL multiple amino acid alignment of albumin sequences

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human           DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAE 60
dog             EAYKSEIAHRYNDLGEEHFRGLVLVAFSQYLQQCPFEDHVKLAKEVTEFAKACAAEESGA 60
sheep           DTHKSEIAHRFNDLGEENFQGLVLIAFSQYLQQCPFDEHVKLVKELTEFAKTCVADESHA 60
goat            DTHKSEIAHRFNDLGEENFQGLVLIAFSQYLQQCPFDEHVKLVKELTEFAKTCVADESHA 60
cattle          DTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEHVKLVNELTEFAKTCVADESHA 60
Donkey          DTHKSEIAHRFNDLGEKHFKGLVLVAFSQYLQQCPFEDHVKLVNEVTEFAKKCAADESAE 60
Rabbit          EAHKSEIAHRFNDVGEEHFIGLVLITFSQYLQKCPYEEHAKLVKEVTDLAKACVADESAA 60
mouse           EAHKSEIAHRYNDLGEQHFKGLVLIAFSQYLQKCSYDEHAKLVQEVTDFAKTCVADESAA 60
rat             EAHKSEIAHRFKDLGEQHFKGLVLIAFSQYLQKCPYEEHIKLVQEVTDFAKTCVADENAE 60
Hamster         DAHKSEIAHRFKDLGEQHFKGLVLIAFSQFLQKCPYEEHVKLVNEVTDFAKTCVADESAE 60
Guinea          EAHKSEIAHRFNDLGEGHFKGLVLITLSQHLQKSPFEEHVKLVNEVTDFAKACVADESAQ 60
                :::***:***::*:** :* .***::::*.**:..:::* **.:*:*::** *.*:*.

human           NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEV 120
dog             NCDKSLHTLFGDKLCTVASLRDKYGDMADCCEKQEPDRNECFLAHKDDNPGFPPLVAPEP 120
sheep           GCDKSLHTLFGDELCKVATLRETYGDMADCCEKQEPERNECFLNHKDDSPDLPKLK-PEP 119
goat            GCDKSLHTLFGDELCKVATLRETYGDMADCCEKQEPERNECFLKHKDDSPDLPKLK-PEP 119
cattle          GCEKSLHTLFGDELCKVASLRETYGDMADCCEKQEPERNECFLSHKDDSPDLPKLK-PDP 119
Donkey          NCDKSLHTLFGDKLCTVATLRATYGELADCCEKQEPERNECFLTHKDDHPNLPKLK-PEP 119
Rabbit          NCDKSLHDIFGDKICALPSLRDTYGDVADCCEKKEPERNECFLHHKDDKPDLPPFARPEA 120
mouse           NCDKSLHTLFGDKLCAIPNLRENYGELADCCTKQEPERNECFLQHKDDNPSLPPFERPEA 120
rat             NCDKSIHTLFGDKLCAIPKLRDNYGELADCCAKQEPERNECFLQHKDDNPNLPPFQRPEA 120
Hamster         NCDKSLHTLFGDKLCAIPTLRDSYGELADCCAKKEPERNECFLKHKDDHPNLPPFVRPDA 120
Guinea          NCGKAIATLFGDKVCAIPSLRETYGELADCCAKEDPDRVECFLQHKDDNPNLPPFERPEP 120
                .* *::  :***::* :..** .**::**** *::*:* **** **** *.:* :  *:

human           DVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLP 180
dog             DALCAAFQDNEQLFLGKYLYEIARRHPYFYAPELLYYAQQYKGVFAECCQAADKAACLGP 180
sheep           DTLCAEFKADEKKFWGKYLYEVARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLP 179
goat            DTLCAEFKADEKKFWGKYLYEVARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLP 179
cattle          NTLCDEFKADEKKFWGKYLYEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLP 179
Donkey          DAQCAAFQEDPDKFLGKYLYEVARRHPYFYGPELLFHAEEYKADFTECCPADDKAGCLIP 179
Rabbit          DVLCKAFHDDEKAFFGHYLYEVARRHPYFYAPELLYYAQKYKAILTECCEAADKGACLTP 180
mouse           EAMCTSFKENPTTFMGHYLHEVARRHPYFYAPELLYYAEQYNEILTQCCAEADKESCLTP 180
rat             EAMCTSFQENPTSFLGHYLHEVARRHPYFYAPELLYYAEKYNEVLTQCCTESDKAACLTP 180
Hamster         EAMCTSFQENAVTFMGHYLHEVARRHPYFYAPELLYYAEKYSAIMTECCGEADKAACITP 180
Guinea          EALCTAFKENNDRFIGHYLYEVSRRHPYFYAPELLYYAEKYKNALTECCEAADKAACLTP 180
                :. *  *: :   *  :**:*::*******.****:.*:.*.  : :**   ** .*: *

human           KLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTK 240
dog             KIEALREKVLLSSAKERFKCASLQKFGDRAFKAWSVARLSQRFPKADFAEISKVVTDLTK 240
sheep           KIDAMREKVLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKADFTDVTKIVTDLTK 239
goat            KIETMREKVLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKADFTDVTKIVTDLTK 239
cattle          KIETMREKVLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVTDLTK 239
Donkey          KLDALKERILLSSAKERLKCSSFQKFGERAFKAWSVARLSQKFPKADFAEVSKIVTDLTK 239
Rabbit          KLDALEGKSLISAAQERLRCASIQKFGDRAYKAWALVRLSQRFPKADFTDISKIVTDLTK 240
mouse           KLDGVKEKALVSSVRQRMKCSSMQKFGERAFKAWAVARLSQTFPNADFAEITKLATDLTK 240
rat             KLDAVKEKALVAAVRQRMKCSSMQRFGERAFKAWAVARMSQRFPNAEFAEITKLATDLTK 240
Hamster         KLDALKEKALASSVNQRLKCSSLQRFGQRAFKAWAVARMSQKFPKADFAEITKLATDLTK 240
Guinea          KLDAIKEKALVSSAQQRLKCASLQKFGERAFKAWSVARLSQKFPKAEFAEISTIVTSLTK 240
                *:: :. .   ::..:*::*:*:*:**:** ***::.*:** **:*:*.:::.:.*.***

human           VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPA 300
dog             VHKECCHGDLLECADDRADLAKYMCENQDSISTKLKECCDKPVLEKSQCLAEVERDELPG 300
sheep           VHKECCHGDLLECADDRADLAKYICDHQDALSSKLKECCDKPVLEKSHCIAEVDKDAVPE 299
goat            VHKECCHGDLLECADDRADLAKYICDHQDTLSSKLKECCDKPVLEKSHCIAEIDKDAVPE 299
cattle          VHKECCHGDLLECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCIAEVEKDAIPE 299
Donkey          VHKECCHGDLLECADDRADLTKYICEHQDSISGKLKACCDKPLLQKSHCIAEVKEDDLPS 299
Rabbit          VHKECCHGDLLECADDRADLAKYMCEHQETISSHLKECCDKPILEKAHCIYGLHNDETPA 300
mouse           VNKECCHGDLLECADDRAELAKYMCENQATISSKLQTCCDKPLLKKAHCLSEVEHDTMPA 300
rat             INKECCHGDLLECADDRAELAKYMCENQATISSKLQACCDKPVLQKSQCLAEIEHDNIPA 300
Hamster         LTEECCHGDLLECADDRAELAKYMCENQASISSKLQACCDKPVLKKSHCLSEVENDDLPA 300
Guinea          VTKECCHGDLLECADDRQELAKYMCEHQDSISSKLKECCVKPTLQKAHCILEIQRDELPT 300
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human           DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKC 360
dog             DLPSLAADFVEDKEVCKNYQEAKDVFLGTFLYEYARRHPEYSVSLLLRLAKEYEATLEKC 360
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sheep           NLPPLTADFAEDKEVCKNYQEAKDVFLGSFLYEYSRRHPEYAVSVLLRLAKEYEATLEDC 359
goat            NLPPLTADFAEDKEVCKNYQEAKDVFLGSFLYEYSRRHPEYAVSVLLRLAKEYEATLEDC 359
cattle          NLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEYEATLEEC 359
Donkey          DLPALAADFAEDKEICKHYKDAKDVFLGTFLYEYSRRHPDYSVSLLLRIAKTYEATLEKC 359
Rabbit          GLPAVAEEFVEDKDVCKNYEEAKDLFLGKFLYEYSRRHPDYSVVLLLRLGKAYEATLKKC 360
mouse           DLPAIAADFVEDQEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKC 360
rat             DLPSIAADFVEDKEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKC 360
Hamster         DLPSLAADFVEDKEVCKNYAEAKDVFLGTFLYEYARRHPDYSVALLLRLAKKYEATLEKC 360
Guinea          ELPDLAVDFVEDKEVCKNFAEAKDVFLGTFLYEYSRRHPEYSIGMLLRIAKGYEAKLEKC 360
                 ** :: :*.*.:::**:: :*** *** *****:****:*:: :***:.* **:.*:.*

human           CAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVST 420
dog             CATDDPPTCYAKVLDEFKPLVDEPQNLVKTNCELFEKLGEYGFQNALLVRYTKKAPQVST 420
sheep           CAKEDPHACYATVFDKLKHLVDEPQNLIKKNCELFEKHGEYGFQNALIVRYTRKAPQVST 419
goat            CAKEDPHACYATVFDKLKHLVDEPQNLIKKNCELFEKHGEYGFQNALIVRYTRKAPQVST 419
cattle          CAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRYTRKVPQVST 419
Donkey          CAEADPPACYATVFDQFTPLVEEPKSLVKKNCDLFEEVGEYDFQNALIVRYTKKAPQVST 419
Rabbit          CATDDPHACYAKVLDEFQPLVDEPKNLVKQNCELYEQLGDYNFQNALLVRYTKKVPQVST 420
mouse           CAEANPPACYGTVLAEFQPLVEEPKNLVKTNCDLYEKLGEYGFQNAILVRYTQKAPQVST 420
rat             CAEGDPPACYGTVLAEFQPLVEEPKNLVKTNCELYEKLGEYGFQNAILVRYTQKAPQVST 420
Hamster         CAEADPSACYGKVLDEFQPLVEEPKNLVKANCELFEKLGEYGFQNALIVRYTQKAPQVST 420
Guinea          CAEADPHACYAKVFDELQPLIDEPKKLVQQNCELFDKLGEYGFQNALAVRYTQKAPQVST 420
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human           PTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES 480
dog             PTLVEVSRKLGKVGTKCCKKPESERMSCAEDFLSVVLNRLCVLHEKTPVSERVTKCCSES 480
sheep           PTLVEISRSLGKVGTKCCAKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTKCCTES 479
goat            PTLVEISRSLGKVGTKCCAKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTKCCTES 479
cattle          PTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTKCCTES 479
Donkey          PTLVEIGRTLGKVGSRCCKLPESERLPCSENHLALALNRLCVLHEKTPVSEKITKCCTDS 479
Rabbit          PTLVEISRSLGKVGSKCCKHPEAERLPCVEDYLSVVLNRLCVLHEKTPVSEKVTKCCSES 480
mouse           PTLVEAARNLGRVGTKCCTLPEDQRLPCVEDYLSAILNRVCLLHEKTPVSEHVTKCCSGS 480
rat             PTLVEAARNLGRVGTKCCTLPEAQRLPCVEDYLSAILNRLCVLHEKTPVSEKVTKCCSGS 480
Hamster         PTLVEAARNLGKVGSKCCVLPEAQRLPCVEDYISAILNRVCVLHEKTPVSEQVTKCCTGS 480
Guinea          PTLVEYARKLGSVGTKCCSLPETERLSCTENYLALILNRLCILHEKTPVSERVTKCCTES 480
                ***** .*.** **::**  ** :*:.* *:.::  **::*:********:::****: *

human           LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT 540
dog             LVNRRPCFSGLEVDETYVPKEFNAETFTFHADLCTLPEAEKQVKKQTALVELLKHKPKAT 540
sheep           LVNRRPCFSDLTLDETYVPKPFDEKFFTFHADICTLPDTEKQIKKQTALVELLKHKPKAT 539
goat            LVNRRPCFSDLTLDETYVPKPFDGESFTFHADICTLPDTEKQIKKQTALVELLKHKPKAT 539
cattle          LVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTEKQIKKQTALVELLKHKPKAT 539
Donkey          LAERRPCFSALELDEGYIPKEFKAETFTFHADICTLPEDEKQIKKQSALAELVKHKPKAT 539
Rabbit          LVDRRPCFSALGPDETYVPKEFNAETFTFHADICTLPETERKIKKQTALVELVKHKPHAT 540
mouse           LVERRPCFSALTVDETYVPKEFKAETFTFHSDICTLPEKEKQIKKQTALAELVKHKPKAT 540
rat             LVERRPCFSALTVDETYVPKEFKAETFTFHSDICTLPDKEKQIKKQTALAELVKHKPKAT 540
Hamster         VVERRPCFSALPVDETYVPKEFKAETFTFHADICSLPEKEKQMKKQAALVELVKHKPKAT 540
Guinea          LVNRRPCFSALHVDETYVPKPFHADSFTFHADICTLPEKEKQVKKQMALVELVKHKPKAS 540
                :.:****** *  ** *:** *. . ****:*:*:*.: *:::*** **.**:****:*:

human           KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL 585
dog             DEQLKTVMGDFGAFVEKCCAAENKEGCFSEEGPKLVAAAQAALV- 584
sheep           DEQLKTVMENFVAFVDKCCAADDKEGCFVLEGPKLVASTQAALA- 583
goat            DEQLKTVMENFVAFVDKCCAADDKEGCFLLEGPKLVASTQAALA- 583
cattle          EEQLKTVMENFVAFVDKCCAADDKEACFAVEGPKLVVSTQTALA- 583
Donkey          KEQLKTVLGNFSAFVAKCCGAEDKEACFAEEGPKLVASSQLALA- 583
Rabbit          NDQLKTVVGEFTALLDKCCSAEDKEACFAVEGPKLVESSKATLG- 584
mouse           AEQLKTVMDDFAQFLDTCCKAADKDTCFSTEGPNLVTRCKDALA- 584
rat             EDQLKTVMGDFAQFVDKCCKAADKDNCFATEGPNLVARSKEALA- 584
Hamster         GPQLRTVLGEFTAFLDKCCKAEDKEACFSEDGPKLVASSQAALA- 584
Guinea          EEQMKTVMGDFAAFLKKCCDADNKEACFTEDGPKLVAKCQATLA- 584
                  *:::*: :*  :: .** * :*: **  :* :**   : :*
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Supplementary Table S3. HSA plasmids

HSA mutein	Sub-cloning plasmid	Disintegration plasmid
Wild type	pDB2243	pDB2244
D494N	pDB3876	pDB3887
D494A	pDB3877	pDB3888
E495Q	pDB3878	pDB3889
E495A	pDB3879	pDB3890
D494Q	pDB3880	pDB3891
D494N, T496A	pDB3881	pDB3892
T496A	pDB3882	pDB3893

Supplementary Table S4. HSA plasmids

Amino acid substitution in HSA	**Plasmid**
HSA H440Q	pDB3986
HSA H464Q	pDB3987
HSA H510Q	pDB3988
HSA H535Q	pDB3989
HSA Q417A	pDB4084
HSA P499A	pDB4085
HSA K500A	pDB4086
HSA K536A	pDB4087
HSA P537A	pDB4088
HSA E501A	pDB4108
HSA K568*	pDB4557

* = a stop codon was engineered into the DNA sequence encoding HSA to terminate the polypeptide sequence at amino acid 567.