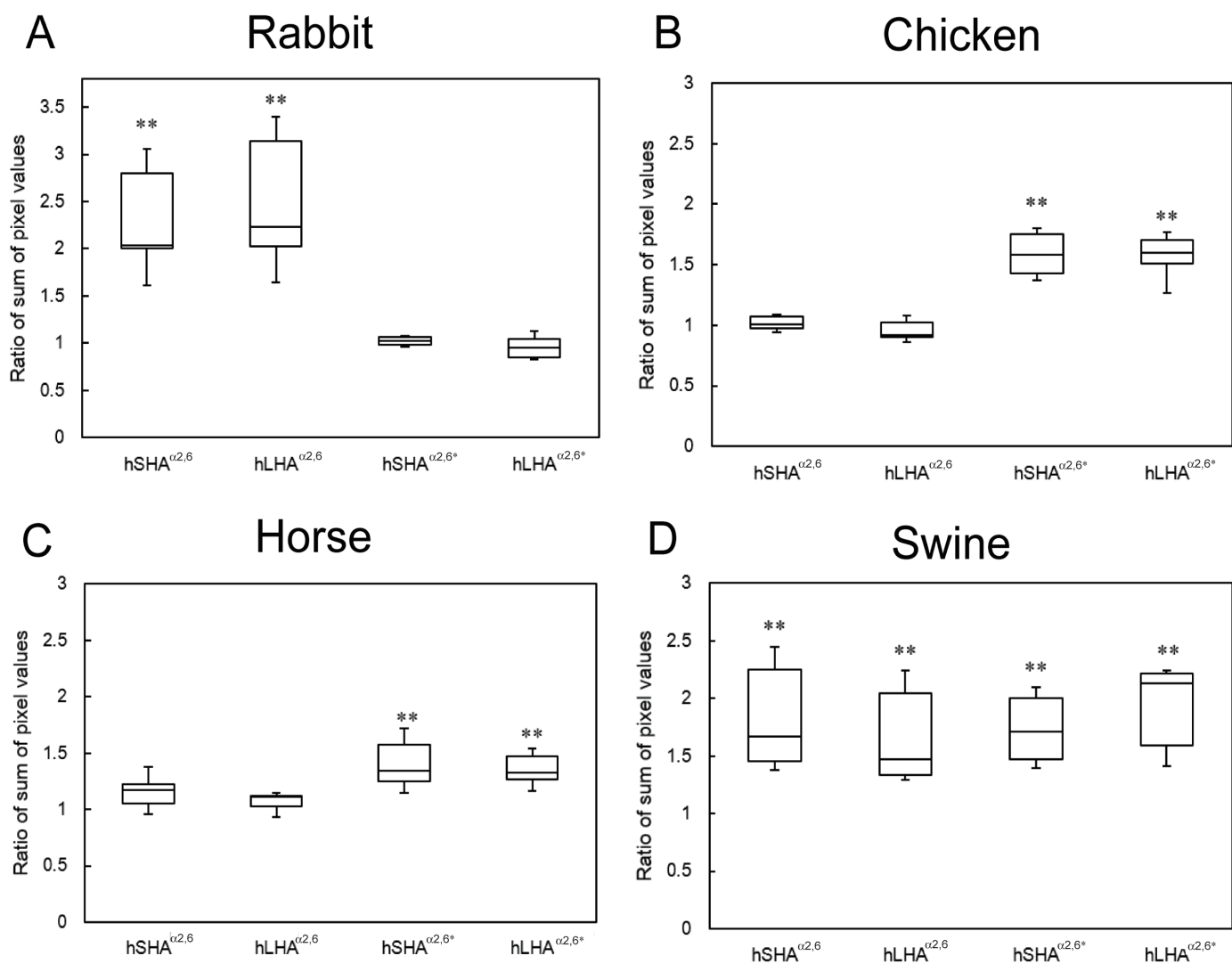


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Table S1 List of primers

Name	Sequence (5' to 3')
human short HA Forward	TTGCTCGTTTCTGCCCTGTGTCTGCTGAAAGGCATTGC
human short HA Reverse	GTAATCAGATCCACCTGTCTGACACTTGGCATCACACTC
human long HA Forward	TTGCTCGTTTCTGCCAACAAACAGCACAGACACAGTGGACAC
human long HA Reverse	GTAATCAGATCCACCCTGGTAGACTCCATACTCTCCAATTTAC
N190E Forward	GAGTGCATCACCCCTCCAAACATTGGAG <u>AA</u> CAGAGGGCTCTACCACACAGAGAATGCC
N190E Reverse	GGCATTCTCTGTGTGGTAGAGAGCCCTCTG <u>TTCT</u> CCAATGTTTGGAGGGTGATGCACTC
D225G Forward	CCTGAGATTGCCAAGAGACCAAAGGTGAGGG <u>GT</u> CAGGAGGGCAGGATAAACTACTACTGGACC
D225G Reverse	GGTCCAGTAGTAGTTTATCCTGCCCTCCTG <u>ACC</u> CCTCACCTTTGGTCTCTTGCAATCTCAGG

The underlines indicate mutation point.



Supplemental Figure S1 Box plots showing the ratio of sum of pixel values for (A) rabbit, (B) chicken, (C) horse, and (D) swine erythrocytes.

hSHA^{α2,6*} and hLHA^{α2,6*} represent the hSHA^{α2,6} and hLHA^{α2,6} species with two point mutations each (N190E and D225G). In each box plot, the y-axis represents as a relative ratio to negative controls. The center bar shows the median value, the box denotes the 25 and 75% centiles, the upper and lower bars represent the range, and the whiskers indicate the 5 and 95% quantiles. *P*-values were determined by the Steel-Dwass test (**P* < 0.05, ***P* < 0.01).

hSHA ^{α2,6}	-----CLLKGIAPLQLGNCVAGWILGNPECEL
hLHA ^{α2,6}	NNSTDTVDTVLEKNVTVTHSVNLLLEDShNGKLCCLLKGIAPLQLGNCVAGWILGNPECEL
hSHA ^{α2,6*}	-----CLLKGIAPLQLGNCVAGWILGNPECEL
hLHA ^{α2,6*}	NNSTDTVDTVLEKNVTVTHSVNLLLEDShNGKLCCLLKGIAPLQLGNCVAGWILGNPECEL
	<hr/>
	HA1
hSHA ^{α2,6}	LISKESWSYIVETPNPENGTCYPGYFADYEELREQLSSVSSFERFEIFPKESSWPNTVTVT
hLHA ^{α2,6}	LISKESWSYIVETPNPENGTCYPGYFADYEELREQLSSVSSFERFEIFPKESSWPNTVTVT
hSHA ^{α2,6*}	LISKESWSYIVETPNPENGTCYPGYFADYEELREQLSSVSSFERFEIFPKESSWPNTVTVT
hLHA ^{α2,6*}	LISKESWSYIVETPNPENGTCYPGYFADYEELREQLSSVSSFERFEIFPKESSWPNTVTVT
	<hr/>
	HA1
hSHA ^{α2,6}	GVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWG ¹⁹⁰ VHHPPNIGNQRA
hLHA ^{α2,6}	GVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWG ¹⁹⁰ VHHPPNIGNQRA
hSHA ^{α2,6*}	GVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWG ¹⁹⁰ VHHPPNIGNQRA
hLHA ^{α2,6*}	GVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWG ¹⁹⁰ VHHPPNIGNQRA
	<hr/>
	HA1
hSHA ^{α2,6}	LYHTENAYVSVVSSHYSRRFTPEIAKRPKVRD ²²⁵ QEGRINYYWTLLEPGDTIIFEANGNLIA
hLHA ^{α2,6}	LYHTENAYVSVVSSHYSRRFTPEIAKRPKVRD ²²⁵ QEGRINYYWTLLEPGDTIIFEANGNLIA
hSHA ^{α2,6*}	LYHTENAYVSVVSSHYSRRFTPEIAKRPKVRD ²²⁵ QEGRINYYWTLLEPGDTIIFEANGNLIA
hLHA ^{α2,6*}	LYHTENAYVSVVSSHYSRRFTPEIAKRPKVRD ²²⁵ QEGRINYYWTLLEPGDTIIFEANGNLIA
	<hr/>
	HA1
hSHA ^{α2,6}	PWYAFALSRGFGSGIITSNAPMDEC-----
hLHA ^{α2,6}	PWYAFALSRGFGSGIITSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRSA
hSHA ^{α2,6*}	PWYAFALSRGFGSGIITSNAPMDEC-----
hLHA ^{α2,6*}	PWYAFALSRGFGSGIITSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRSA
	<hr/>
	HA1
hSHA ^{α2,6}	-----
hLHA ^{α2,6}	KLRMVTGLRNIPSIQSRGLFGAIGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQN
hSHA ^{α2,6*}	-----
hLHA ^{α2,6*}	KLRMVTGLRNIPSIQSRGLFGAIGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQN
	<hr/>
	HA1 HA2
hSHA ^{α2,6}	-----
hLHA ^{α2,6}	AINGITNKVNSVIEKMNTQFTAVGKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLE
hSHA ^{α2,6*}	-----
hLHA ^{α2,6*}	AINGITNKVNSVIEKMNTQFTAVGKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLE
	<hr/>
	HA2
hSHA ^{α2,6}	-----
hLHA ^{α2,6}	NERTLDFHDSNVKNLYEKVKSQ ²²⁵ LKNNAKEIGNGCFEFYHKCNNECMESVKN ²²⁵ GT ²²⁵ YDYPKYS
hSHA ^{α2,6*}	-----
hLHA ^{α2,6*}	NERTLDFHDSNVKNLYEKVKSQ ²²⁵ LKNNAKEIGNGCFEFYHKCNNECMESVKN ²²⁵ GT ²²⁵ YDYPKYS
	<hr/>
	HA2
hSHA ^{α2,6}	-----
hLHA ^{α2,6}	EESKLNREKIDGVKLESMGVYQ
hSHA ^{α2,6*}	-----
hLHA ^{α2,6*}	EESKLNREKIDGVKLESMGVYQ
	<hr/>
	HA2

Supplemental Figure S2 Sequence alignment of hHAs^{α2,6} and hHAs^{α2,6*}.

The full-length sequences of hSHA^{α2,6}, hLHA^{α2,6}, hSHA^{α2,6*}, and hLHA^{α2,6*} are shown. Multiple sequence alignments were generated using the ClustalW program (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). The underlined sequence labelled HA1 represents the head region that binds to sialic acid side chains on surface glycoproteins (Skehel and Wiley 2000), while the underlined sequence labelled HA2 represents the stem region required for membrane fusion activity and infectivity (Skehel and Wiley 2000). The shaded amino acids show the amino acids mutated to generate hHAs^{α2,6*}.