

18 97

PR8 DTICIGYHANNSTDTVDTVLEKNTVTVTHSVNLLDSDHNGKLCRLKGIAPLQLGKCNIA GWLLGNPECDPLLPVRSWSYIV
Ca107 DTLICIGYHANNSTDTVDTVLEKNTVTVTHSVNLLDSDHNGKLCRLKGVAPLHLGKCNIA GWILGNPECESLSTASSWSYIV
H5 DQICIGYHANNSTEQVDTIMEKNTVTVTHAQDILERTHNGKCLDNLGVKPLILRDCSVAGWLLGNPMCDDEFINVPESWSYIV
H6 DKICIGYHANNSTQVDTILEKNTVTVTHSVELENNQKEERFKILNKAFLDLRGCTIEGWILGNPQCDLLLDGQDWSYIV
H8 DRICIGYQSNNSTDTVNTLIEQNVPTQTMELVETEKHYPAYCNTDLGAPLELRDCKIEAVIYGNPKCDIHLKDQGSYIV
H9 DKICIGHQSTNSTETVDTLLETNVPVTHAKELLHTEHNGMLCATSLGHPLILDCTIEGLVYGNPSCDLLLGGREWSYIV
H11 DEICIGYLSNNSTDKVDTIENNVVTSSVELVETEHTGSFCSINGKQPISLGDSCSFAGWILGNPMCDLIGKTSWSYIV
H13 DRICVGYLSTNSSERVDTLLENVPTSSVDLVE TNHTGTYSLGGISPVHLGDCSFEGWIVGNPACASNLGIREWSYLI

98 173

PR8 ETPNSENGICYPGDFIDYEEELREQLSSVSSFERFEI FPKeSSWPNNH-TN-GVTAACSH-EGKSSFYRNLLWLTE-KEGS
Ca107 ETPSSDNGTCYPGDFIDYEEELREQLSSVSSFERFEI FPKtSSWPNNH-SNkGVTAACPH-AGAKSFYKNLIWLVK-KGNS
H5 EKASPNALCYPGDFIDYEEELKHLLSRISHFEKIQIIPK-SSWSNHdaSS-GVSSACPY-LGKSSFFRNVVLIK-KNST
H6 ERPTAQNICYPGALNEVEELKALIGSSERVEFEMFPK-STWTGVD-TSsGVTKACPY-NSGSSFYRNLLWI IKTKSAA
H8 ERPSAPGMCYPGSIENLEELRFVFSASYSKIRLFDY-SRWNVTR-SG--TSKACNAsTGQSFYRSINWLPKPKPDT
H9 ERSSAVNGTCYPGNVENLEELR TLFSSASSYQRIQIFPD-TTWNVTY-TG--TSRACSG----SFYRSMRWLTQKS-GF
H11 EKPNTNGICYPGILENEEELRLKFSGVLEFNKFEAFTS-NGWGAVN-SGaGVTAACKF-GSSNSFFRNMIWLHQ-SGT
H13 EDPSAPHGLCYPGELDNNGLRHLFSGIRSFSTRTELIAP-TSWGAVN-DG--VSSACQD-KGASSFYRNLVWFVERG-NK

174 253

PR8 YPKLKNSYVNKKGKEVLVWLGIIHPPNSKEQQNLYQENAYVSVVTSNYNRRFTPEIAERPKVDRQAGRMNYYWTLKPG
Ca107 YPKLSKSYINDKGKEVLVWLGIIHPPNSTADQQSLYQNADAYVFGSSRYSKFKPEIAIRPKVDRQEGRMNYYWTLVEPG
H5 YPTIKRSYNNNTQEDLLVWLGIIHPPNDAAEQTKLYQNPTTYSVGTSTLNQRLVPEIATRPKVNGQSGRIEFFWTILKPN
H6 YPVIKGTYNNTGSQLYFVWGVHPPDTEQNTLYGSGDRYVRMGTESMNFAKSPEIAARPAVNGQRGRIDYYSVLKPG
H8 YDFNFTYVNNEDGDIIFLWGIHPPDTEQNTLYKNANTLTSVTNTINRNFOQNI GPRPLVRGQDPCRYWGLKRG
H9 YPVQDAQYTNNGKSI LFWGIIHPPPTYEQNTLYIRNDTTTSVTTEDLNRTFKPVI GPRPLVNGLQGRIDYYSVLKPG
H11 YPVIKRTFNNTGRDVLVWVGIIHPPATLKEHQDLYKKDSSYVAVGSETYNRRFTPEI STRPKVNGQAGRMTFYWTIVKPG
H13 YPVIRGTYNNTGRDVLVWVGIIHPPVSTDEARKLYVNDNPTLVSTSSWSRKYNLEIGIRPGYNGQKSWMKIYWYLMHPG

254 331

PR8 DTIIFEANGNLIAPMYAFA-LSRFGSGIITS-NASMHECNTKCQTPLGAINSSLPYQNIHPVTIGCEPKYVRS AKLRMV
Ca107 DKITFEATGNLVPRYAFA-MERNAGSGIITS-DTPVHDCNTTCQTPKGAINSLPFOQNIHPITIGKCPKYVRS TKLRLA
H5 DAINFESNGNFIAPYAYK-IVKKGDSIMKS-ELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGCEPKYVRSNRLVLA
H6 ETLNVESNGNLIAPWYAYKFVSTNNKGAIFKS-NLPIENC DATCQTIAGVLR TNKTFQNVSPWIGCEPKYVRS ESLRLA
H8 ETLKIRTNGLIAPFEGYL-LKGESHGRIIQNEdPIIGNCNTKCQTYAGAINSSKPFQNASRHYMGCEPKYVRS KASLRLA
H9 QTLRVRNGLIAPWYGHV-LSGGSHGRILKT-DLKGNCVVCQTEKGGNLSLTPFHNI SKYAFGTCPKYVRS VNSLKL A
H11 ESITFESNGAFLAPRYAFA-IVSVGNKLFRS-ELNIESCS TKCQTEVGGINTNKS FHSVHRNTIGDCKPYVNSVKS LKLA
H13 ESISFESNGGLLAPKYGYI-IEEYKGRIFQS-RIRIAKCN TKCQTSVGGINTNKTFOQNIERNALGDCKPYIKSGQLKLA

332 411

HA1 --- HA2

PR8 TGLRNIPSIQSRGLFAGIAGFIEGGWTGMIDGWYGYHHQNEQSGYAADQKSTQNAINGITNKVNTVIEKMNIOFTAVGK
Ca107 TGLRNIPSIQSRGLFAGIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDEITNKVNSVIEKMNIOFTAVGK
H5 TGLRNAPQREKRGFLFAGIAGFIEGGWQGMVDGWYGYHHSNEQSGYAADQESTQKAI DGVNTKVN SI INKMNTQFEAVGK
H6 TGLRNVPQIETRGLFAGIAGFIEGGWTGMIDGWYGYHHSNSQSGYAADKESTQKAI DGITNKVNSI IDKMNTQFEAVDH
H8 VGLRNTPSVEPRGLFAGIAGFIEGGWGMIDGWYGYHHSNSEGTGMAADQKSTQEAIDKITNKVNNI VDKMNRFEVVDH
H9 VGLRNVPARSSRGLFAGIAGFIEGGWGLVAGWYGFQHSNANTLSDQVGMADRDSTQKAI DKITNKVNNI VDKMNYEIIDH
H11 TGLRNVPAIASRGLFAGIAGFIEGGWGLINGWYGFQHRNEEGTGI AADKESTQKAI DQITSKVNNI VDRMNTNFESVQH
H13 TGLRNVPAISKRGLFAGIAGFIEGGWGLINGWYGFQHQNEQCVGMADKESTQKAI DQITTKNINIEKMNIGNYDSIRG

B
CD4⁺T

412 491

PR8 EFNKLEKRMENLNKKVDDGFLDIWTYNAELLVLENERLDFHDSNVKNLYEKVKSQ LKNNAKEI GNGCFEFYHKCDNEC
Ca107 EFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRSQ LKNNAKEI GNGCFEFYHKCDNTEC
H5 EFNLEERRIENLNKKMEDGFLDVWTYNAELLVLENERLDFHDSNVKNLYDKVRLQLRDN AKELGNGCFEFYHKCDNEC
H6 EFSNLEERRIDNLNKRMEDGFLDVWTYNAELLVLENERLTDLHDANVKNLYEKVKSQ LRDNANDLNGNGCFEFWYHKCDNEC
H8 EFSEVEKRINMINDKIDDQIEDLWAYNAELLVLENKTLDEHDSNVKNL FDEVKRRLSANA IDAGNGCFDILHKCDNEC
H9 EFSEVETRLNMINNKIDDQIQDVWAYNAELLVLENKTLDEHDANVNNLYNKV KRALGSNAMEDGKGFELYHKCDQDC
H11 EFSEIEERINRLSKHVDDSVDDIWSYNAQLLVLENEKTLDLHDSNVNRLHERVRRMLKDN AKDEGNGCFTFYHKCDNEC
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CD4⁺T

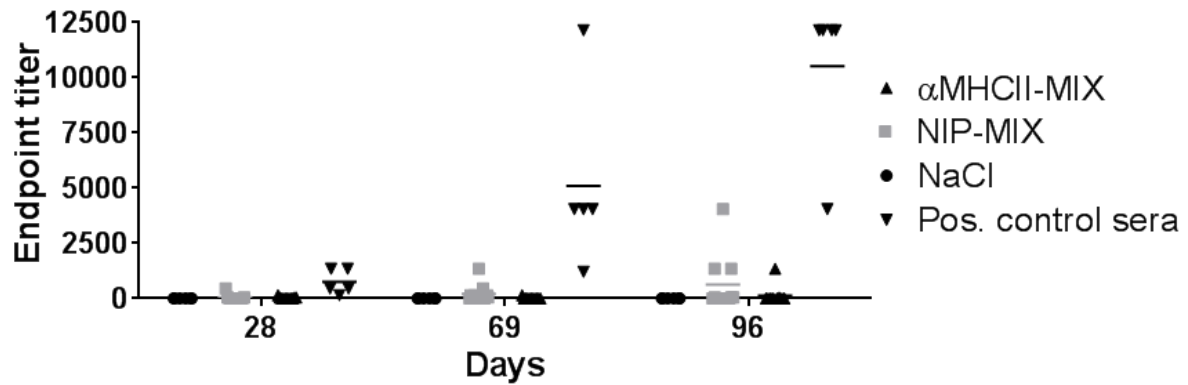
492 541

PR8 MESVRNGTYDYPKYSEESKLNREKVDGVKLES MG-IYQILAIYSTVASSL
Ca107 MESVKNGTYDYPKYSEEA KLNREEDIGVKLESTR-IYQILAIYSTVASSL
H5 MESVKNGTYDYPQYSEEARLNREIEISGVKLES MG-TYQ-----
H6 IESVKNGTYDYPKYQDES KLNREQEIESVKLDNLG-VYQILAIYSTVSSSL
H8 METIKNGTYNHKEYEEAKLERSKINGVKLEENT-TYKILSIYSTVAASL
H9 METIRNGTYNRRKYREESRLERQKIEGVKLESEG-TYKILT IYSTVASSL
H11 IEKVRNGTYDHKEFEKESKINRQEI EGVKLDSSGnVYKILSIYSCIASSL
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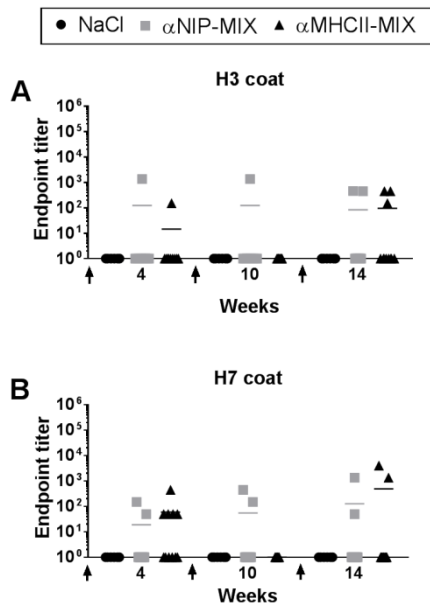
CD8⁺

Supplementary figure 1: Alignments of protein sequences from vaccine components. Sequences from the different vaccine inserted HAs were aligned using the NCBI Basic

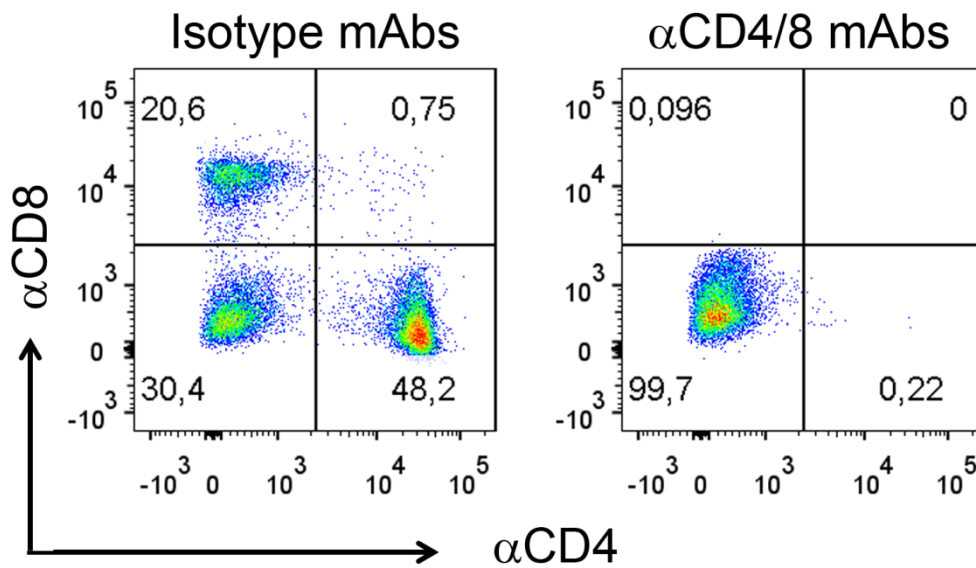
Logical Alignment Search Tool (BLAST). Amino acids that are conserved in at least 5 of the selected strains are marked with red. Known conserved B- and T-cell epitopes are underlined. Top row numbering is according to PR8.



Supplementary figure 2: Induction of stem-reactive antibodies in sera of vaccinated mice. BALB/c mice [n=6 (NaCl), or 10/group] were immunized thrice (week 0, 4 and 7) with 25 μ g DNA/EP of the indicated vaccines. As positive control, a group of mice (n=5) was immunized with vaccines encoding only the stem region of HA. Sera from indicated time points after vaccination were evaluated in an ELISA coated with Phox-BSA, and then recombinant proteins expressing Phox-specific scFv linked to the HA stem. HA stem reactive IgG in sera was then evaluated. Responses are given for individual mice, with the mean indicated.



Supplementary figure 3: Vaccine-induced cross-reactivity against HAs from group 2 influenzas. BALB/c mice [n=6 (controls) or 10/group] were immunized thrice (week 0, 4 and 7) with 25 μ g DNA/EP of the vaccines indicated. Serum IgG specific for HA from A/Hong Kong/1/1968 (H3N2) (a) or HA from A/Shanghai/1/2013 (H7N9) (b) were measured in sandwich ELISAs. Responses from individual mice are shown, with the mean indicated by a horizontal line.



Supplementary figure 4: Efficacy of T cell depletion. Mice were immunized thrice with DNA/EP, and treated every other day from day 12 after the third immunization with depleting antibodies against CD4 (GK1.5) and CD8 (TIB105), or isotype controls. Shown are stained splenocytes that were harvested at the termination of the experiment (day 7 after viral challenge) to assess the degree of depletion in mice receiving either isotype control mAbs (left panel) or depleting mAbs (right panel). The samples are representative within groups.