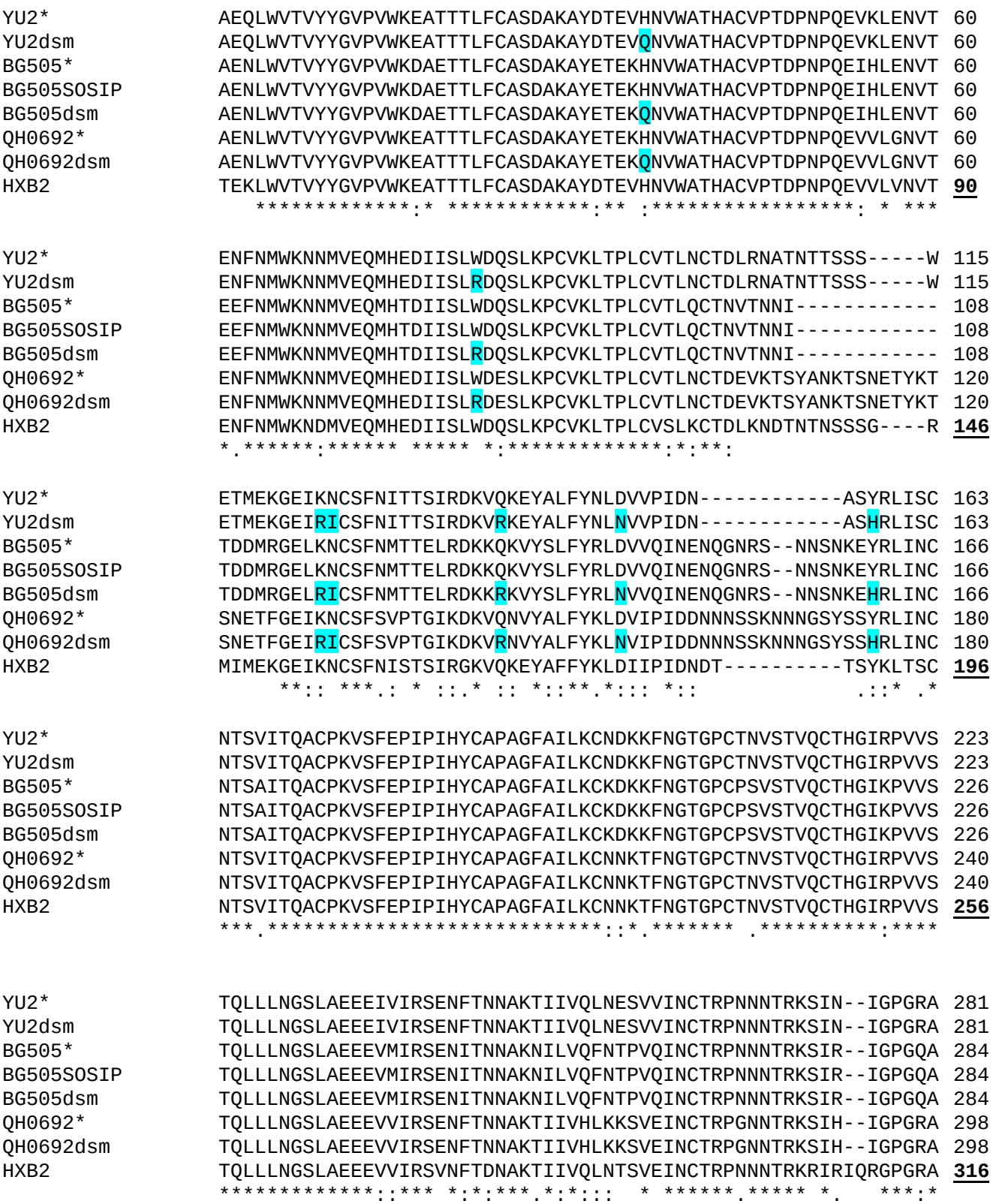
S1 Figure.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BG505* AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVT 60
BG505SOSIP AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVT 60
BG505dsm AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKQNVWATHACVPTDPNPQEIHLENVT 60
QH0692* AENLWVTVYYGVPVWKEATTTLFCASDAKAYETEKHNVWATHACVPTDPNPQEVVLGNVT 60
QH0692dsm AENLWVTVYYGVPVWKEATTTLFCASDAKAYETEKQNVWATHACVPTDPNPQEVVLGNVT 60
HXB2 TEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLVNVT 90
YU2* ENFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDLRNATNTTSSS-----W 115
YU2dsm ENFNMWKNNMVEQMHEDIISLRDQSLKPCVKLTPLCVTLNCTDLRNATNTTSSS-----W 115
BG505* EEFNMWKNNMVEQMHTDIISLWDQSLKPCVKLTPLCVTLQCTNVTNNI------------ 108
BG505SOSIP EEFNMWKNNMVEQMHTDIISLWDQSLKPCVKLTPLCVTLQCTNVTNNI------------ 108
BG505dsm EEFNMWKNNMVEQMHTDIISLRDQSLKPCVKLTPLCVTLQCTNVTNNI------------ 108
QH0692* ENFNMWKNNMVEQMHEDIISLWDESLKPCVKLTPLCVTLNCTDEVKTSYANKTSNETYKT 120
QH0692dsm ENFNMWKNNMVEQMHEDIISLRDESLKPCVKLTPLCVTLNCTDEVKTSYANKTSNETYKT 120
HXB2 ENFNMWKNDMVEQMHEDIISLWDQSLKPCVKLTPLCVSLKCTDLKNDTNTNSSSG----R 146
YU2* ETMEKGEIKNCSFNITTSIRDKVQKEYALFYNLDVVPIDN-----------ASYRLISC 163
YU2dsm ETMEKGEIRICSFNITTSIRDKVRKEYALFYNLNVVPIDN-----------ASHRLISC 163
BG505* TDDMRGELKNCSFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRS--NNSNKEYRLINC 166
BG505SOSIP TDDMRGELKNCSFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRS--NNSNKEYRLINC 166
BG505dsm TDDMRGELRICSFNMTTELRDKKRKVYSLFYRLNVVQINENQGNRS--NNSNKEHRLINC 166
QH0692* SNETFGEIKNCSFSVPTGIKDKVQNVYALFYKLDVIPIDDNNNSSKNNNGSYSSYRLINC 180
QH0692dsm SNETFGEIRICSFSVPTGIKDKVRNVYALFYKLNVIPIDDNNNSSKNNNGSYSSHRLINC 180
HXB2 MIMEKGEIKNCSFNISTSIRGKVQKEYAFFYKLDIIPIDNDT----------TSYKLTSC 196
YU2* NTSVITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCTNVSTVQCTHGIRPVVS 223
YU2dsm NTSVITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCTNVSTVQCTHGIRPVVS 223
BG505* NTSAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVS 226
BG505SOSIP NTSAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVS 226
BG505dsm NTSAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVS 226
QH0692* NTSVITQACPKVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVS 240
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BG505* TQLLLNGSLAEEEVMIRSENITNNAKNILVQFNTPVQINCTRPNNNTRKSIR--IGPGQA 284
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QH0692dsm TQLLLNGSLAEEEVVIRSENFTNNAKTIIVHLKKSVEINCTRPGNNTRKSIH--IGPGRA 298
HXB2 TQLLLNGSLAEEEVVIRSVNFTDNAKTIIVQLNTSVEINCTRPNNNTRKRIRIQRGPGRA 316

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BG505SOSIP      FYATGDIIGDIRQAHCNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHS 344
BG505dsm        FYATGDIIGDIRQAHCNVSKATWNETLGKVVKQLRKHYGNNTIIRFANSSGGDLEVTTHS 344
QH0692*         FYATGDIIGDIRQAHCNLSSVQWNDTLKQIVIKLGEQFGTNKTIAFNQSSGGDPEIVMHS 358
QH0692dsm       FYATGDIIGDIRQAHCNLSSVQWNDTLKQIVIKLGEQYGTNKTIAFNQSSGGDPEIVMHS 358
HXB2            FVTIGK-IGNMRQAHCNISRAKWNNTLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTHS 375
                : : *. **::*****.:* . *::** ::. :* :::*.*. * *  ***** *:. **

YU2*            FNCGGEFFYCNSTQLFT--WNDT-----RKLN-NTGRNITLPCRIKQIINMWQEVGKAMY 393
YU2dsm          FNCGGEFFYCNSTQLFT--WNDT-----RKLN-NTGRNITLPCRIKQINNMWQEVGKAMY 393
BG505*          FNCGGEFFYCNTSGLFNSTWISNTSV-QGSNSTGSNDSITLPCRIKQIINMWQRIGQAMY 403
BG505SOSIP      FNCGGEFFYCNTSGLFNSTWISNTSV-QGSNSTGSNDSITLPCRIKQIINMWQRIGQAMY 403
BG505dsm        FNCGGEFFYCNTSGLFNSTWISNTSV-QGSNSTGSNDSITLPCRIKQINNMWQRIGQAMY 403
QH0692*         FNCGGEFFYCNTTQLFNSTWEFHGNWTRSNFTESNSTTITLPCRIKQIVNMWQEVGKAMY 418
QH0692dsm       FNCGGEFFYCNTTQLFNSTWEFHGNWTRSNFTESNSTTITLPCRIKQINNMWQEVGKAMY 418
HXB2            FNCGGEFFYCNSTQLFNSTWFNSTWSTEGSNNTEGSDTITLPCRIKQIINMWQKVGKAMY 435
                ***********:: **.  *         . .   . .********** ****.:*:***

YU2*            APPIRGQIRCSSNITGLLLTRDGGKDTNGTEIFRPGGGDMRDNWRSELYKYKVVKIEPLG 453
YU2dsm          APPIRGQIRCSSNITGLLLTRDGGKDTNGTEIFRPGGGDMRDNWRSELYKYKVVKIEPLG 453
BG505*          APPIQGVIRCVSNITGLILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLG 463
BG505SOSIP      APPIQGVIRCVSNITGLILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLG 463
BG505dsm        APPIQGVIRCVSNITGLILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLG 463
QH0692*         APPIRGQIRCSSNITGLLLTRDGGVNG-TRETFRPGGGDMRDNWRSELYKYKVVKIEPLG 477
QH0692dsm       APPIRGQIRCSSNITGLLLTRDGGVNG-TRETFRPGGGDMRDNWRSELYKYKVVKIEPLG 477
HXB2            APPISGQIRCSSNITGLLLTRDGGNSNNESEIFRPGGGDMRDNWRSELYKYKVVKIEPLG 495
                **** * *** ******:******     * ****************************


YU2*            VAPTKAKRRVVQREK--RAVGLGALFLGFLGAAGSTMGAASITLTVQARQLLSGIVQQQN 511
YU2dsm          VAPTKCQRRVVQKREAEAATSTGATFSGFSGSAGSTMGATSITLTVQARQLLSGIVQQQN 513
BG505*          VAPTRAKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQS 523
BG505SOSIP      VAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQS 523
BG505dsm        VAPTRCQRRVVGKREAEAATSTGATFSGFSGSAGSTMGATSMTLTVQARNLLSGIVQQQS 523
QH0692*         VAPTKAQRRVVQKREAEAATSTGATFSGFSGSAGSTMGATSITLTVQARQLLSGIVQQQN 537
QH0692dsm       VAPTKCQRRVVQKREAEAATSTGATFSGFSGSAGSTMGATSITLTVQARQLLSGIVQQQN 537
HXB2            VAPTKAKRRVVQREK--RAVGIGALFLGFLGAAGSTMGAASMTLTVQARQLLSGIVQQQN 553
                ****:.:**** :..    .. ** * ** *:*******:*:******* *********.

YU2*            NLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICTTTVPWNTS 571
YU2dsm          NLLRAPEAQQHLLQLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCTTVPWNTS 573
BG505*          NLLRAIEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICTTNVPWNSS 583
BG505SOSIP      NLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCTNVPWNSS 583
BG505dsm        NLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCTNVPWNSS 583
QH0692*         NLLRAIEAQQHMLQLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICTTAVPWNAS 597
QH0692dsm       NLLRAPEAQQHMLQLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCTAVPWNAS 597
HXB2            NLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQQLLGIWGCSGKLICTTAVPWNAS 613
                ***** *****:*:************:******* *************** * ****:*
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YU2*              WSNKSLNEIWDNMTWMKWEREIDNYTHIIYSLIEQSQNQQEKNEQELLALDKWASLWNWF  631
YU2dsm            WSNKSLNEIWDNMTWMKWEREIDNYTHIIYSLIEQSQNQQEKNEQELLALDKWASLWNWF  633
BG505*            WSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDKWASLWNWF  643
BG505SOSIP        WSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDKWASLWNWF  643
BG505dsm          WSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDKWASLWNWF  643
QH0692*           WSNKSQDYIWNNMTWMQWDKEINNYTNLIYSLLEDSQNQQEKNEHELLELDKWASLWNWF  657
QH0692dsm         WSNKSQDYIWNNMTWMQWDKEINNYTNLIYSLLEDSQNQQEKNEHELLELDKWASLWNWF  657
HXB2              WSNKSLEQIWNHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF  673
                  ***:. . **:: **::*::**.***  *: *:*:**********::** **********

YU2*              DITKWLWYIK   641
YU2dsm            DITKWLWYIK   643
BG505*            DISNWLWYIK     653
BG505SOSIP        DISNWLWYIK   653
BG505dsm          DISNWLWYIK   653
QH0692*           DITRWLWYI    666
QH0692dsm         DITRWLWYI    666
HXB2              NITNWLWYIK   683
                  :*:.****
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S1 Fig. Alignment of Env amino acid sequences used in this work. N-terminal signal sequences have been removed, since these were replaced by the signal sequence from Aga2p. The hydrophilic fusion peptide sequence (highlighted in green) and the optimized Kex2p-cleavage site (highlighted in purple) are as described [1]. Additional "stabilizing" mutations as described by Grimm et al., are highlighted in cyan. "SOSIP" mutations [2] are highlighted in grey and the original BG505 SOSIP sequence is shown for reference. The sequence of Env from strain HXB2 (NCBI AAB50262.1) to reference the standard numbering scheme starting from the first codon in the signal sequence of HXB2 (numbers shown in bold and underlined). Other sequences are numbered with reference to the first codon after the Aga2p signal sequence in the yeast expression constructs. Alignment was performed using Clustal Omega [3]. Asterisks indicate positions where all sequences are identical, colons indicate strong conservation, periods indicate weak conservation. Positions T605 (C605 in the QH0692dsm and YU2dsm sequences), W610, W614, L619 (Q619 in QH0692), W623, W628, W631, I635, Y638, I642, L646, W666, I682, and K683 are highlighted in red on the HXB2 sequence.

References for S1 Figure.

1. Grimm SK, Battles MB, Ackerman ME. Directed evolution of a yeast-displayed HIV-1 SOSIP gp140 spike protein toward improved expression and affinity for conformational antibodies. PLoS One. 2015;10(2):e0117227.
2. Schulke N, Vesanen MS, Sanders RW, Zhu P, Lu M, Anselma DJ, et al. Oligomeric and conformational properties of a proteolytically mature, disulfide-stabilized human immunodeficiency virus type 1 gp140 envelope glycoprotein. J Virol. 2002;76(15):7760-76.
3. Sievers F, Higgins DG. Clustal Omega, accurate alignment of very large numbers of sequences. Methods Mol Biol. 2014;1079:105-16.