Supplementary Figure 1. The amino acid sequences of m610.27 (A) and m630.3 (B) in alignment with their corresponding germlines of human antibody V genes and/or wild types. The complementarity-determining regions (CDRs) and framework regions (FRs) are indicated according to the ImMunoGeneTics annotation (http://imgt.cines.fr/). The somatic mutations in the V regions of the antibodies and the mutations induced by random mutagenesis are highlighted with gray background.

Supplementary Figure 2. Schematic representation of antibody structures and SDS-PAGE analysis. (A) Fab m610.27 was converted to a standard IgG1 format. (B) m630.3Fc was constructed by joining eAd m630.3 to the N terminus of human IgG1 Fc through a hinge linker. (C) The bispecific antibody, m660, was generated by fusing scFv m610.27 and eAd m630.3 to the N termini of the heavy and light chain constant regions of a human IgG1, respectively, via a linker composed of three repeats of the G_4S motif. (D) Reducing and nonreducing SDS-PAGE of the antibodies purified from 293 free style cell cultures.

Supplementary Figure 1

(A)

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                   FR1                     CDR1          FR2                CDR2          FR3                                      CDR3            FR4
              _________________________  ___________  ________________  __________  ______________________________________  _____________  ___________
              1        10        20       30          40        50        60          70        80        90        100
              .........|.........|......  ...|........ .|.........|..... ....|.....  ....|.........|.........|.........|....
m610 VH       QVQLVQSGA.EVKKPGASVKVSCKAS  GYTF....TSYY MHWVRQAPGQGLEWMGI INPS..GGST  SYAQKFQ.GRVTMTRDTSTSTVYMELSRLRSDDTAVYYC ARDVQWLAYGMDV  WGQGTTVTVSS
IGHV1-46*01   QVQLVQSGA.EVKKPGASVKVSCKAS  GYTF....TSYY MHWVRQAPGQGLEWMGI INPS..GGST  SYAQKFQ.GRVTMTRDTSTSTVYMELSSLRSEDTAVYYC AR
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                   FR1                     CDR1          FR2               CDR2          FR3                                      CDR3         FR4
              _________________________  ___________  ________________  _________  _____________________________________  __________  __________
              1        10        20       30          40        50        60         70        80        90        100
              .........|.........|......  ...|........ .|.........|..... ....|.....  ....|.........|.........|.........|....
m610 VL       DIQMTQSPSPLSASVGDRVTITCRAS  QSI......SSY LNWYQQKPGKAPKLLIY AA.......S  SLQSGVP.SRFSGSG..SGTDFTLTISSLQPEDFATYYC QQSYSTPLTF  GGGTKVEIKR
IGKV1-39*01   DIQMTQSPSSLSASVGDRVTITCRAS  QSI......SSY LNWYQQKPGKAPKLLIY AA.......S  SLQSGVP.SRFSGSG..SGTDFTLTISSLQPEDFATYYC QQSYSTP
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                   FR1                     CDR1          FR2               CDR2          FR3                                      CDR3         FR4
              _________________________  ___________  ________________  _________  _____________________________________  __________  __________
              1        10        20       30          40        50        60         70        80        90        100
              .........|.........|......  ...|........ .|.........|..... ....|.....  ....|.........|.........|.........|....
m610 VL       DIQMTQSPSPLSASVGDRVTITCRAS  QSI......SSY LNWYQQKPGKAPKLLIY AA.......S  SLQSGVP.SRFSGSG..SGTDFTLTISSLQPEDFATYYC QQSYSTPLTF  GGGTKVEIKR
m610.27 VL    DIQMTQSPSSLSASVGDRVTITCRAS  QSI......SSY LNWYQQKPGRAPDLLIN AA.......S  SLQSGVP.SRFSGSG..SGTDFTLTISSLQPEDFATYFC QQSYSLPFTF  GGGTKVEIKG
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(B)

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                FR1                      CDR1          FR2                CDR2          FR3                                        CDR3                    FR4
         _________________________  ___________  _________________  __________  ______________________________________  _____________________  ___________
         1        10        20       30          40        50        60          70        80        90        100
         .........|.........|......  ...|........ .|.........|..... ....|.....  ....|.........|.........|.........|....
m630     QVQLVQSGG.GLVQPGGSLRLSCAAS  SFDF....DYYE MSWVRQAPGQRLEWVAY ISKS..GGTK  MYADSVK.GRFTISRDNSKNTLYLQMNTLRAEDTAMYYC AKDRATCSGGSCYSFYYGMDV  WGQGTLVTVSS
m630.1   QVQLVQSGG.GLVQPGGSLRLSCAAS  SFDF....DYYE MSWVRQAPGQRLEWVAY ISKS..GGTK  MYADSVK.GRFTISRDNSRNTLYLQMNTLRAEDTAMYYC AKDRATCRGGSCYSFYHGMDV  WGQGTLVTVSS
m630.3   QVQLVQSGG.GLVQPGGSLRLSCAAS  SFDF....DYYE MSWVRQAPGQRLEWVAY ISKS..GGTK  MYADSVK.GRFTISRDNSKNTLYLQMNTLRAEDTAMYYC AKDRATCRGGRCYSFYYGMDV  WGQGTLVTVSS
m630.4   QVQLVQSGG.GLVQPGGSLRLSCAAS  SFDF....GYYE MSWVRQAPGQRLEWVAY ISKS..GGTK  MYADSVK.GRFTISRDNSKNTLYLQMNTLRAEDTAMYYC AKDRATCSGGRCYSFYYGMDV  WGQGTLVTVSS
m630.9   QVQLVQSGG.GLVQPGGSLRLSCAAS  SFDF....DYYE MSWVRQAPGQRLEWVAY ISKS..GGTK  MYADSVK.GRFTISRDNSKNTLYLQMNTLRAEDTAMYYC AKDRATCSGGRCYSFYYGMDV  WGQGTLVTVSS
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Supplementary Figure 2