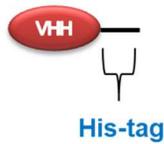


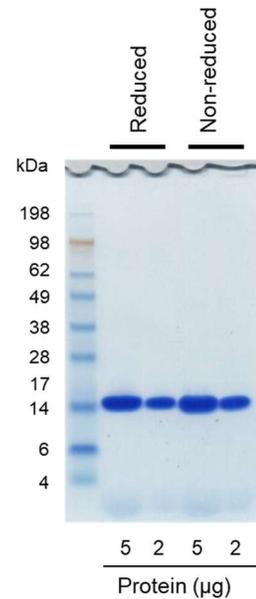
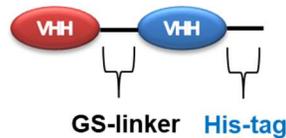
a**Anti-mCTLA-4 VHH (H11)**

His tag: highlighted in blue

Signal Sequence: highlighted in red

MKYLLPTAAAGLLLLAAQPAMAQVQLVESGGGLAQP
 GGSRLS**C**AASG**S**TISSVAVGWYRQTPGNQREWVA
 TSSTSSTTATYADSVKGRFTISRDN**A**KNTIY**L**QMNSL
 KPEDTAVYYCKTGLTNWGRGTQVT**S**AAAH**H**HHHHH
 HHHHHH

Molecular weight: 13.6 kDa

b**c****Anti-mCTLA-4 VHH (H11)****Anti-serum albumin VHH**

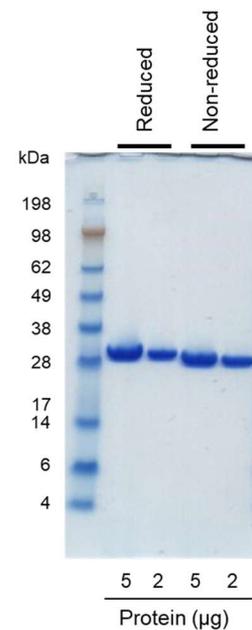
His tag: highlighted in blue

Signal Sequence: highlighted in red

Linker: highlighted in purple

METD**T**LLLL**W**LLLL**W**VP**G**ST**G**QVQLVESGGGLAQP**G**GS**L**
 RL**S**CAASG**S**TISSVAVGWYRQTPGNQREWVATSSTSSTT
 ATYADSVKGRFTISRDN**A**KNTIY**L**QMNSLKPEDTAVYYCK
 TGLTNWGRGTQVT**S**SS**G**GS**G**SG**S**GG**S**GG**S**GG**S**GG**S**GG
SGA**V**QLVESGGGLVQPGNSLRL**S**CAASGFTFRSFGMSW
 VRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDN**A**
 KTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQVT**S**
 SAAAH**H**HHHHHHHHHHH

Molecular weight: 27.2 kDa

d

Supplementary Fig. 1 Generation of anti-mouse CTLA-4 VHH (H11) and half-life extended H11 linked to anti-serum albumin VHH (H11-HLE). Amino acid sequence and SDS-PAGE of H11 (**a** and **b**) and H11-HLE (**c** and **d**)

a

Signal Sequence: highlighted in red

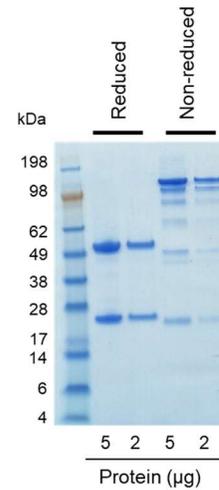
Ipilimumab mIgG2a heavy chain

MGWSCII~~FLVATATGVHS~~QVQLVESGGGVVQPGRSLRLSCAAS
 GFTFSSYTMHWVRQAPGKGLEWVTFISYDGNKYYADSVKGRF
 TISRDNKNTLYLQMNSLRAEDTAIYYCARTGWLGPFDYWGGQT
 LVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTL
 TWN~~SGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNV~~
 AHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFI~~FP~~PKI
 KDVL~~MISLSP~~IVTCVVVDVSEDDPDVQISW~~FVNNVEVHTAQ~~TQTH
 REDYN~~STLRVVSALPIQH~~QDWMSGKEFKCKV~~NNKDL~~PAPIERTI
 SKPKGSVRAPQVYVLPPEEEEMTKKQVTLTCMVTD~~FMPEDIYVE~~
 WTNNGKTELNYKNTEPVLDS~~GSYFMYSKLRVEKKNW~~VERNSY
 SCSVVHEGLHNHHTTKSFSRTPGK

Ipilimumab mIgG2a light chain

MGWSCII~~FLVATATGVHS~~EIVLTQSPGTL~~SLSPGERATL~~SCRASQ
 SVGSSYLAWYQKPGQAPRLLIYGAFSRATGIPDRFSGSGSGTD
 FTLTISRLEPEDFAVYYCQYQGS~~SPWTFGQGT~~KVEIKRADAAPT
 V SIFPPSSEQLTSGGASVVCFLN~~NFYPKDINVKWKIDG~~SERQNGVL
 NSWTDQDSK~~DSTYSMSSTLT~~LTKDEYERHNSY~~TCEATHKTST~~SPI
 VKSFNRNEC

Molecular weight: 146 kDa

b**c**

Signal Sequence: highlighted in red

Mutation in Fc region: highlighted in blue

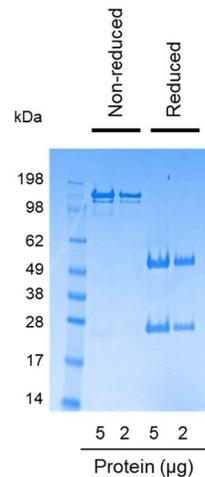
Ipilimumab mIgG2a L234A_L235A_P329G heavy chain

MGWSCII~~FLVATATGVHS~~QVQLVESGGGVVQPGRSLRLSCAAS
 GFTFSSYTMHWVRQAPGKGLEWVTFISYDGNKYYADSVKGRF
 TISRDNKNTLYLQMNSLRAEDTAIYYCARTGWLGPFDYWGGQT
 LVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTL
 TWN~~SGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNV~~
 AHPASSTKVDKKIEPRGPTIKPCPPCKCPAPN**AA**GGPSVFI~~FP~~PKI
 KDVL~~MISLSP~~IVTCVVVDVSEDDPDVQISW~~FVNNVEVHTAQ~~TQTH
 REDYN~~STLRVVSALPIQH~~QDWMSGKEFKCKV~~NNKDL~~**G**APIERTI
 SKPKGSVRAPQVYVLPPEEEEMTKKQVTLTCMVTD~~FMPEDIYVE~~
 WTNNGKTELNYKNTEPVLDS~~GSYFMYSKLRVEKKNW~~VERNSY
 SCSVVHEGLHNHHTTKSFSRTPGK

Ipilimumab mIgG2a L234A_L235A_P329G light chain

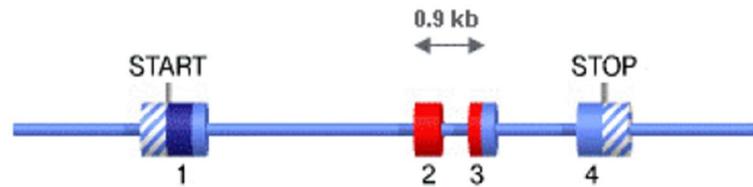
MGWSCII~~FLVATATGVHS~~EIVLTQSPGTL~~SLSPGERATL~~SCRASQ
 SVGSSYLAWYQKPGQAPRLLIYGAFSRATGIPDRFSGSGSGTD
 FTLTISRLEPEDFAVYYCQYQGS~~SPWTFGQGT~~KVEIKRADAAPT
 V SIFPPSSEQLTSGGASVVCFLN~~NFYPKDINVKWKIDG~~SERQNGVL
 NSWTDQDSK~~DSTYSMSSTLT~~LTKDEYERHNSY~~TCEATHKTST~~SPI
 VKSFNRNEC

Molecular weight: 146 kDa

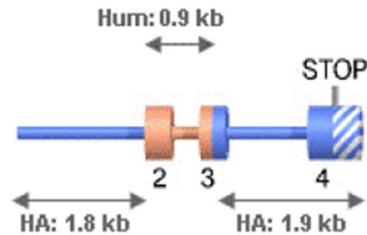
d

Supplementary Fig. 2 Generation of Ipilimumab mIgG2a (Ipi-WT) and Ipilimumab mIgG2a LALAPG (Ipi-LALAPG). Amino acid sequence and SDS-PAGE of Ipi-WT (**a** and **b**) and Ipi-LALAPG (**c** and **d**)

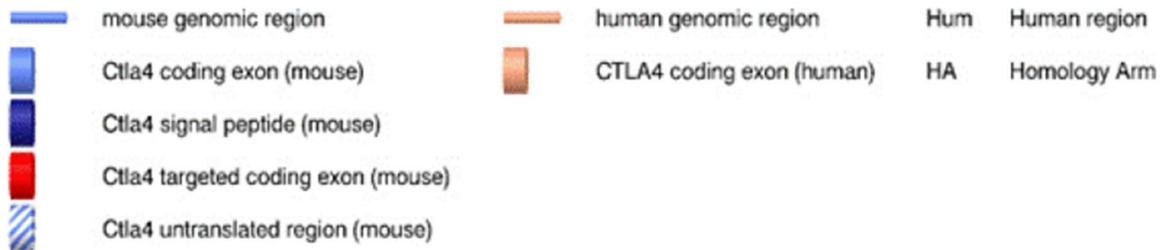
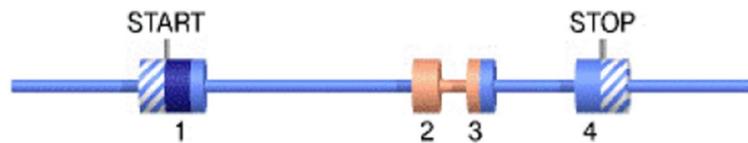
Mouse genomic locus



Targeting vector



Humanized allele (after CRISPR/Cas9-mediated gene editing)



Supplementary Fig. 3 Humanization of the Ctla4 gene via CRISPR/Cas9-mediated gene editing. The targeting construct replaced the exons coding for the extracellular (exon 2) and part of transmembrane (exon 3) domains of murine CTLA-4 with those of human CTLA-4.