

Figure 1

SEQ ID NO: 2 Amino acid sequence of the scaffold alpha chain extracellular region. CDRs are underlined. The extracellular constant region is shown in italics. A non-native cysteine residue is shown in bold (at position 48 of constant region)

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      10          20          30          40          50          60
AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI YSNGDKEDGR
      70          80          90         100         110         120
FTAQLNKASQ YVSLLRDSQ PSDSATYLCA VRNYNTDKLI FGTGTRLQVF PNIQNPDPAV
      130         140         150         160         170         180
YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD SDVYITDKCV LDMRSMDFKS NSAWAWSNKS
      190
DFACANAFNN SIIPEDT

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SEQ ID NO: 3 Amino acid sequence of the scaffold beta chain extracellular region. CDRs are underlined. The extracellular constant region is shown in italics. A non-native cysteine residue is shown in bold (at position 57 of constant region). Additional non-native amino acids at position 75 and position 89 of the constant region are also shown in bold.

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      10          20          30          40          50          60
NAGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV GAGITDQGEV
      70          80          90         100         110         120
PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASSYATGGT GELFFGEGSR LTVLEDLKNV
      130         140         150         160         170         180
FPPEVAVFEP SEAEISHTQK ATLVCLATGF YPDHVELSWW VNGKEVHSGV CTDPQPLKEQ
      190         200         210         220         230         240
PALNDSRYAL SSRLRVSATF WQDPRNHFRC QVQFYGLSEN DEWTQDRAKP VTQIVSAEAW
GRAD

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Figure 2

Amino acid sequences of mutated TCR alpha chain variable regions. CDRs are underlined and mutations are in bold

SEQ ID NO: 4 mutant alpha chain (a01)

AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI YSNGDKEDGR
FTAQLNKASQ YVSL**L**IRDSQ PSDSATYLCA ARNY**K**TD**LLI** FGTGTRLQVF P

SEQ ID NO: 5 mutant alpha chain (a13)

AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI YSDGDKEDGR
FTAQLNKASQ YVSL**L**IRDSQ PSDSATYLCA ARNY**K**TD**LLI** FGTGTRLQVF P

SEQ ID NO: 6 mutant alpha chain (a19)

AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GK**G**SPELIMSI YSDGDKEDGR
FTAQLNKASQ YVSL**L**IRDSQ PSDSATYLCA ARNY**K**TD**LLI** FGTGTRLQVF P

Figure 3

Amino acid sequences of mutated TCR beta chain variable regions. CDRs are underlined. Mutations are in bold

SEQ ID NO: 7 mutant beta chain (b02)

NAGVTQTPKF QVLKTGQSMT LQCAQD**LNHG** YMSWYRQDPG MGLRLIHYSV GAGITDQGEV
PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASSYATGGT GVLFFGEGSR LTVL

SEQ ID NO: 8 mutant beta chain (b03)

NAGVTQTPKF QVLKTGQSMT LQCAQD**SHG** YMSWYRQDPG MGLRLIHYSV GAGITDQGEV
PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASSYATGGT GDLFFGEGSR LTVL

SEQ ID NO: 9 mutant beta chain (b04)

NAGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV GAGITDQGEV
PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASSYATGGT GLLFFGEGSR LTVL

SEQ ID NO: 10 mutant beta chain (b05)

NAGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYS**L** GAGITDQGEV
PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASSYATGGT GDLFFGEGSR LTVL

SEQ ID NO: 11 mutant beta chain (b09)

NAGVTQTPKF QVLKTGQSMT LQCAQD**LSHG** YMSWYRQDPG MGLRLIHYSV GAGITDQGEV
PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASSYATGGT GDLFFGEGSR LTVL

Figure 4

Amino acid sequences of TCR-anti-CD3 fusions comprising mutated TCR alpha and beta variable domains

Fusion 1 (a19b03)

SEQ ID NO: 12 alpha chain (a19)

AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKGPELIMSI YSDGDKEDGR
 FTAQLNKASQ YVSLIIRDSQ PSDSATYLCA **ARNYKTDLLI** FGTGTRLQVF PNIQNPDPAV
 YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD SDVYITDKCV LDMRSMDFKS NSAWAWSNKS
 DFACANAFNN SIIPEDT

SEQ ID NO: 13 beta chain (b03)

AIQMTQSPSS LSASVGDRVT ITCRASQDIR NYLNWYQQKP GKAPKLLIYY TSRLESGVPS
 RFGSGSGSTD YTLTISSLQP EDFATYYCQQ GNTLPWTFGQ GTKVEIKGGG GSGGGGSGGG
 GSGGGGSGGG SEVQLVESGG GLVQPGGSLR LSCAASGYSF TGYTMNWVRQ APGKGLEWVA
 LINPYKGVST YNQKFKDRFT ISVDKSKNTA YLQMNSLRAE DTAVYYCARS GYYGDSWYF
 DVWGQGTILVT VSSGGGGSNA GVTQTPKFQV LKTGQSMTLQ CAQDMSHGYM SWYRQDPGMG
 LRLIHYSVGA GITDQGEVPN GYNVSRSTTE DFPLRLLSAA PSQTSVYFCA SSYATGGTGD
LFEGEGSRLT VLEDLKNVFP PEVAVFEPSE AEISHTQKAT LVCLATGFYP DHVELSWWVN
 GKEVHSGVCT DPQPLKEQPA LNDSTRYALSS RLRVSATFWQ DPRNHFRQV QFYGLSENDE
 WTQDRAKPVT QIVSAEAWGR AD

Fusion 2 (a13b03)

SEQ ID NO: 14 alpha chain (a13)

AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI YSDGDKEDGR
 FTAQLNKASQ YVSLIIRDSQ PSDSATYLCA **ARNYKTDLLI** FGTGTRLQVF PNIQNPDPAV
 YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD SDVYITDKCV LDMRSMDFKS NSAWAWSNKS
 DFACANAFNN SIIPEDT

SEQ ID NO: 13 beta chain (b03)

AIQMTQSPSS LSASVGDRVT ITCRASQDIR NYLNWYQQKP GKAPKLLIYY TSRLESGVPS
 RFGSGSGSTD YTLTISSLQP EDFATYYCQQ GNTLPWTFGQ GTKVEIKGGG GSGGGGSGGG
 GSGGGGSGGG SEVQLVESGG GLVQPGGSLR LSCAASGYSF TGYTMNWVRQ APGKGLEWVA
 LINPYKGVST YNQKFKDRFT ISVDKSKNTA YLQMNSLRAE DTAVYYCARS GYYGDSWYF
 DVWGQGTILVT VSSGGGGSNA GVTQTPKFQV LKTGQSMTLQ CAQDMSHGYM SWYRQDPGMG
 LRLIHYSVGA GITDQGEVPN GYNVSRSTTE DFPLRLLSAA PSQTSVYFCA SSYATGGTGD
LFEGEGSRLT VLEDLKNVFP PEVAVFEPSE AEISHTQKAT LVCLATGFYP DHVELSWWVN
 GKEVHSGVCT DPQPLKEQPA LNDSTRYALSS RLRVSATFWQ DPRNHFRQV QFYGLSENDE
 WTQDRAKPVT QIVSAEAWGR AD

Fusion 3 (a01b03)**SEQ ID NO: 15** alpha chain (a01)

AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI YSNGDKEDGR
FTAQLNKASQ YVSLIIRDSQ PSDSATYLCA ARNYKTDLLI FGTGTRLQVF PNIQNPDPAV
YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD SDVYITDKCV LDMRSMDFKS NSAVAWSNKS
DFACANAFNN SIIPEDT

SEQ ID NO: 13 beta chain (b03)

AIQMTQSPSS LSASVGDRVT ITCRASQDIR NYLNWYQQKP GKAPKLLIYY TSRLESGVPS
RFGSGSGSTD YTLTISSLQP EDFATYYCQQ GNTLPWTFGQ GTKVEIKGGG GSGGGGSGGG
GSGGGGSGGG SEVQLVESGG GLVQPGGSLR LSCAASGYSF TGYTMNWVRQ APGKGLEWVA
LINPYKGVST YNQKFKDRFT ISVDKSKNTA YLQMNSLRAE DTAVYYCARS GYYGDSWYF
DVWGQGTLVTVSSSGGGGNA GVTQTPKFQV LKTGQSMTLQ CAQDMSHGYM SWYRQDPGMG
LRLIHYSVGA GITDQGEVPN GYNVSRSTTE DFPLRLLSAA PSQTSVYFCA SSYATGGTGD
LFEGEGSRLT VLEDLKNVFP PEVAVFEPSE AEISHTQKAT LVCLATGFYP DHVELSWWVN
GKEVHSGVCT DPQPLKEQPA LNSRYALSS RLRVSATFWQ DPRNHFRQV QFYGLSENDE
WTQDRAKPVTVQIVSAEAWGR AD

Fusion 4 (a13b09)**SEQ ID NO: 14** alpha chain (a13)

AKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI YSDGDKEDGR
FTAQLNKASQ YVSLIIRDSQ PSDSATYLCA ARNYKTDLLI FGTGTRLQVF PNIQNPDPAV
YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD SDVYITDKCV LDMRSMDFKS NSAVAWSNKS
DFACANAFNN SIIPEDT

SEQ ID NO: 16 beta chain (b09)

AIQMTQSPSS LSASVGDRVT ITCRASQDIR NYLNWYQQKP GKAPKLLIYY TSRLESGVPS
RFGSGSGSTD YTLTISSLQP EDFATYYCQQ GNTLPWTFGQ GTKVEIKGGG GSGGGGSGGG
GSGGGGSGGG SEVQLVESGG GLVQPGGSLR LSCAASGYSF TGYTMNWVRQ APGKGLEWVA
LINPYKGVST YNQKFKDRFT ISVDKSKNTA YLQMNSLRAE DTAVYYCARS GYYGDSWYF
DVWGQGTLVTVSSSGGGGNA GVTQTPKFQV LKTGQSMTLQ CAQDLSHGYM SWYRQDPGMG
LRLIHYSVGA GITDQGEVPN GYNVSRSTTE DFPLRLLSAA PSQTSVYFCA SSYATGGTGD
LFEGEGSRLT VLEDLKNVFP PEVAVFEPSE AEISHTQKAT LVCLATGFYP DHVELSWWVN
GKEVHSGVCT DPQPLKEQPA LNSRYALSS RLRVSATFWQ DPRNHFRQV QFYGLSENDE
WTQDRAKPVTVQIVSAEAWGR AD

Figure 5

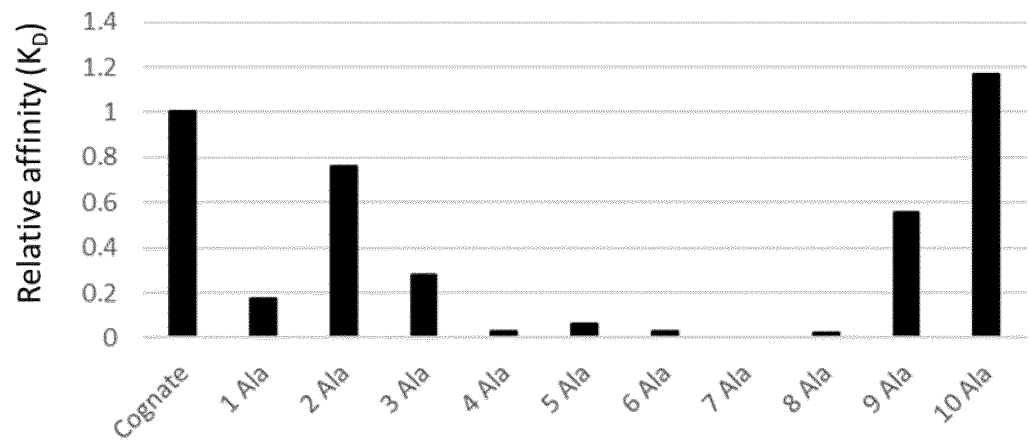


Figure 6

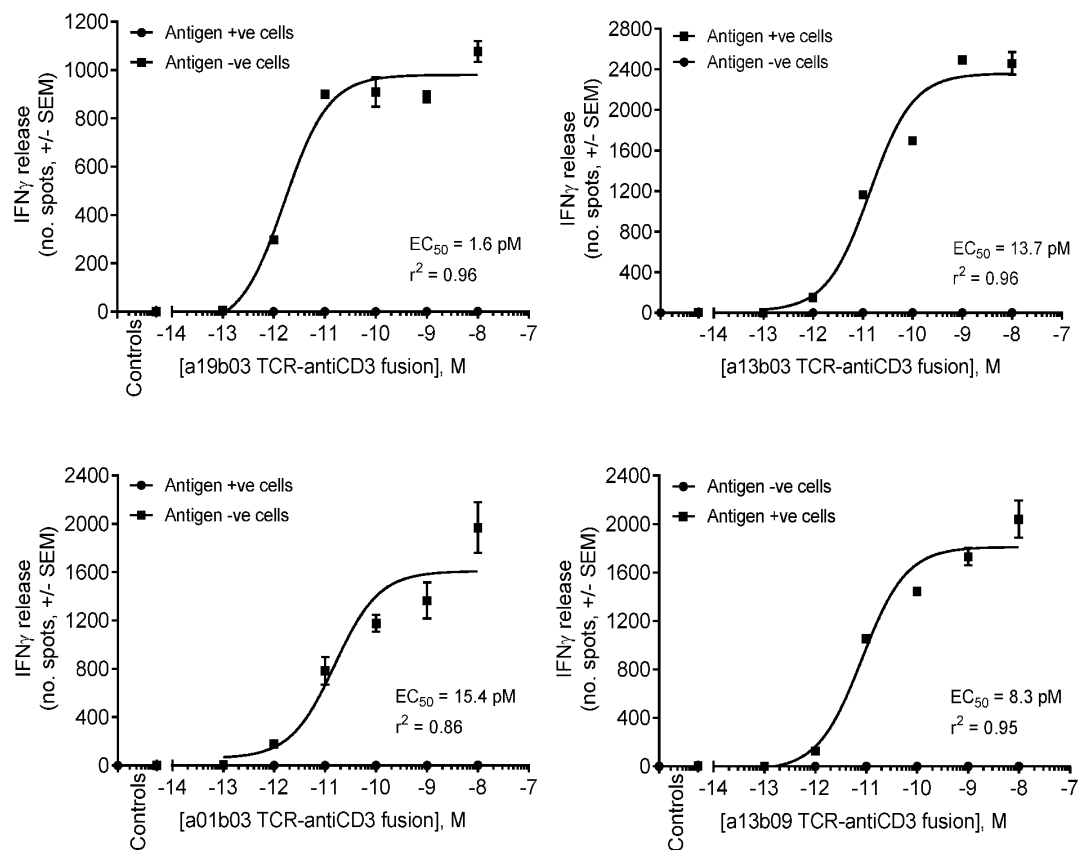


Figure 7

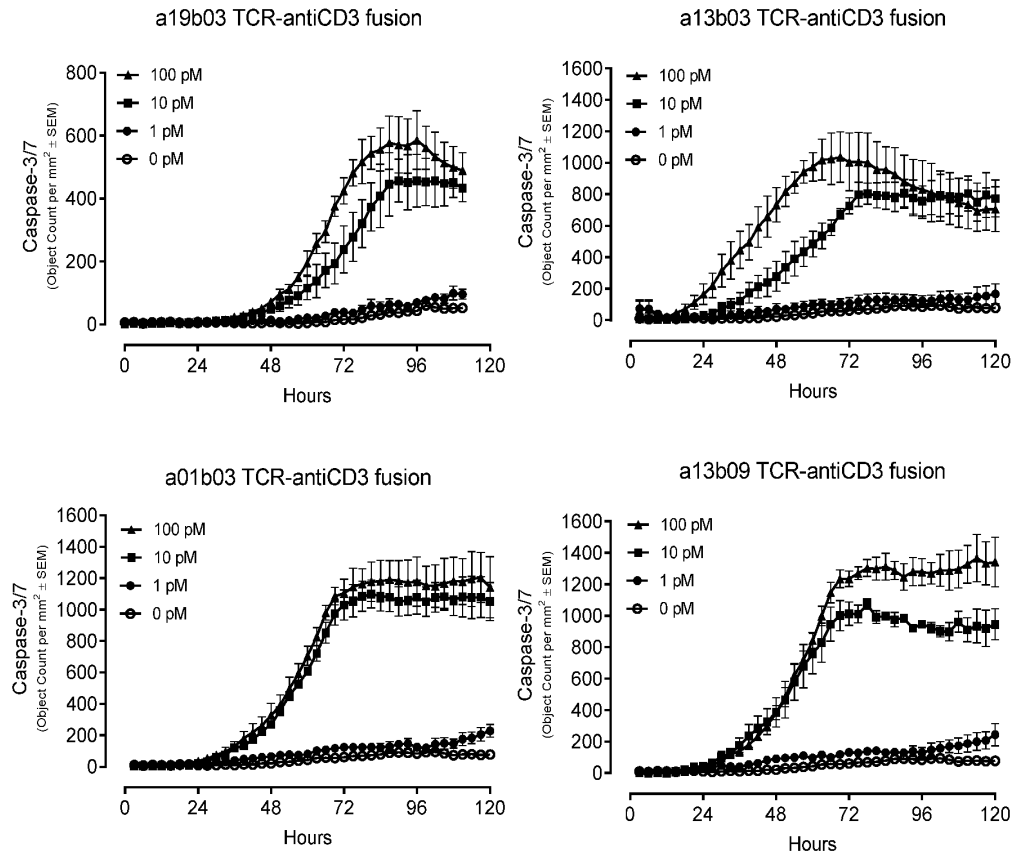
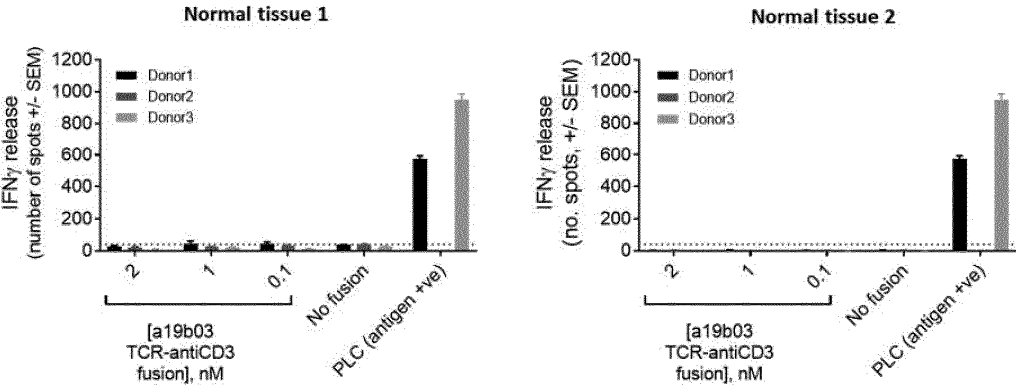


Figure 8

a)



b)

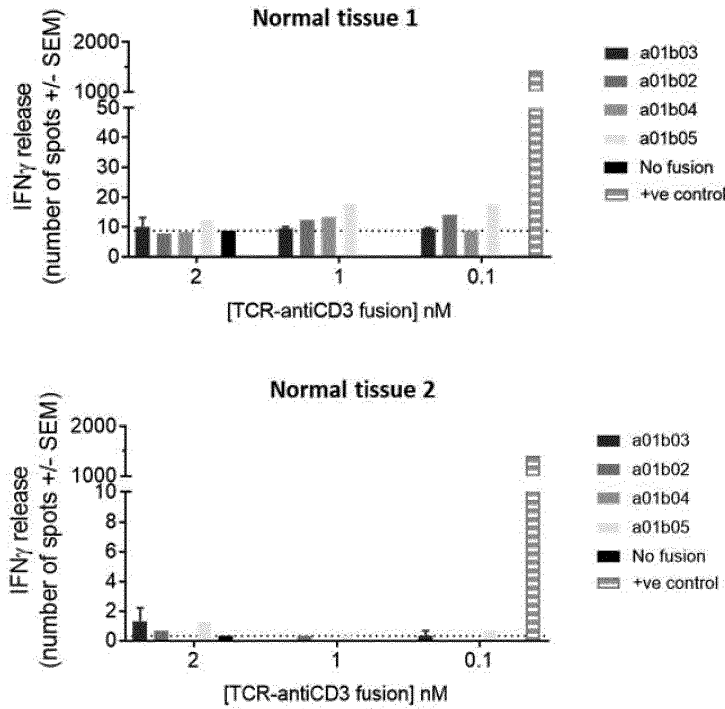


Figure 9

