SEQ ID NO: 2 Amino acid sequence of the wild-type alpha chain variable domain. CDRs are underlined and CDR1, 2, 3 are designated SEQ ID NO: 41, 42 and 43, respectively. The constant region is shown in italics. The FM2 and FM3 regions are in bold and are designated SEQ ID NO: 47 and 48, respectively.

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPY**LFWYVQYPNRGLQFLLK**YITGDNLV**KGSY**
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDINSGAGSYQLTFGKGTKLSVIP*NIQNP*
DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWS
NKSDFACANAFNNSIIPEDTFFPSPESS

SEQ ID NO: 3 Amino acid sequence of the wild-type beta chain variable domain. CDRs are underlined and are designated SEQ ID NO: 44, 45 and 46, respectively. The constant region is shown in italics. The FM2 and FM3 regions are in bold and are designated SEQ ID NO: 49 and 50, respectively.

SAVISQKPSRDICQRGTSLTIQCQVDSQVTM**MFWYRQQPGQSLTLIAT**ANQGSEA**TYESGF**
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL*EDLKNVF*
PPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPA
LNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA
D

FIG. 1

SEQ ID NO: 4 Amino acid sequence of the soluble extracellular region of a native alpha chain of the invention. CDRs are underlined. The constant region is shown in italics and the non-native cysteine residue is shown in bold (at position 48 of constant region).

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYITGDNLVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDINSGAGSYQLTFGKGTKLSVIP*NIQNP*
*DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDK**C**VLDMRSMDFKSNSAVAWS*
NKSDFACANAFNNSIIPEDTFFPSPESS

SEQ ID NO: 5 Amino acid sequence of the soluble extracellular region of a native beta chain of the invention. CDRs are underlined. The constant region is shown in italics and the 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.

SAVISQKPSRDICQRGTSLTIQCQVDSQVTMMFWYRQQPGQSLTLIATANQGSEATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL*EDLKNVF*
*PPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGV**C**TDPQPLKEQPA*
*LNDSRY**A**LSSRLRVSATFWQ**D**PRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA*
D

FIG. 2

Amino acid sequences of mutated TCR alpha chain variable regions of the invention

SEQ ID NO: 6 mutant alpha chain (a12) CDRs are underlined, **mutations in bold**

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYITGDNLVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRD**SDQH**AGSYQLTFGKGTKLSVIP

SEQ ID NO: 7 mutant alpha chain (a24) CDRs are underlined, **mutations in bold**

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKY**LGDSA**LVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDINSGAGSYQLTFGKGTKLSVIP

SEQ ID NO: 8 mutant alpha chain (a24l) CDRs are underlined, **mutations in bold**

AQSVAQPED**L**VNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKY**LGDSA**LVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDINSGAGSYQLTFGKGTKLSVIP

SEQ ID NO: 9 mutant alpha chain (a28) CDRs are underlined, **mutations in bold**

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYITGDNLVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVR**SSRQH**AGSYQLTFGKGTKLSVIP

SEQ ID NO: 10 mutant alpha chain (a78l) CDRs are underlined, **mutations in bold**

AQSVAQPED**L**VNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKY**LGDSA**LVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDI**D**SGAGSYQLTFGKGTKLSVIP

SEQ ID NO: 11 mutant alpha chain (a82l) CDRs are underlined, **mutations in bold**

AQSVAQPED**L**VNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKY**LGDSA**LVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDI**R**SGAGSYQLTFGKGTKLSVIP

SEQ ID NO: 12 mutant alpha chain (a82) CDRs are underlined, **mutations in bold**

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKY**LGDSA**LVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDI**R**SGAGSYQLTFGKGTKLSVIP

SEQ ID NO: 51 mutant alpha chain (a86) CDRs are underlined, **mutations in bold**

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKY**LTD**DNLVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDINSGAGSYQLTFGKGTKLSVIP

FIG. 3

Amino acid sequences of mutated TCR beta chain variable regions of the invention

SEQ ID NO: 13 mutant beta chain (b5) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVDSQVTMMFWYRQQPGQS**P**TLIATA**WT**G**G**EATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 14 mutant beta chain (b12) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLAL**MFWYRQQPGQS**P**TLIATA**WT**G**G**EATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 15 mutant beta chain (b52) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLAL**MFWYRQQPGQS**P**TLIATA**WT**G**G**EATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSG**A**ADTQYFGPGTRLTVL

SEQ ID NO: 16 mutant beta chain (b56) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLA**MMFWYRQQPGQS**P**TLIATA**WT**G**G**EATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 17 mutant beta chain (b56l) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLA**MMFWYRQQPGQSLTLIATA**WT**G**G**EATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 18 mutant beta chain (b65) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLAL**MFWYRQQPGQS**P**TLIATA**WT**GSEATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 19 mutant beta chain (b65l) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLAL**MFWYRQQPGQSLTLIATA**WT**GSEATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 20 mutant beta chain (b67) CDRs are underlined, **mutations in bold**

FIG. 4

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLA**MMFWYRQQPGQS**P**TLIATA**WT**GG**G**EATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSG**A**ADTQYFGPGTRLTVL

SEQ ID NO: 21 mutant beta chain (b67l) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLA**MMFWYRQQPGQSLTLIATA**WT**GG**G**EATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSG**A**ADTQYFGPGTRLTVL

SEQ ID NO: 22 mutant beta chain (b68) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLA**MMFWYRQQPGQS**P**TLIATA**WT**GSEATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 23 mutant beta chain (b68l) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVD**KRLA**MMFWYRQQPGQSLTLIATA**WT**GSEATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGPGTRLTVL

SEQ ID NO: 52 mutant beta chain (b71) CDRs are underlined, **mutations in bold**

SAVISQKPSRDI**K**QRGTSLTIQCQVDSQV**A**MMFWYRQQPGQS**P**TLIATA**W**QGSEATYESGF
VIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSG**A**ADTQYFGPGTRLTVL

FIG. 4 Cont'd

Amino acid sequences of alpha and beta chains of ImmTAC molecules of the invention

ImmTAC1 (S2C)

SEQ ID NO: 32 ImmTAC alpha chain (comprising SEQ ID NO: 7(a24) and the constant domain of SEQ ID NO: 4, truncated by 8 amino acids)

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYLGDSALVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDINSGAGSYQLTFGKGTKLSVIPNIQNP
DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWS
NKSDFACANAFNNSIIPEDT

SEQ ID NO: 33 ImmTAC beta chain (comprising an anti-CD3 scFv (bold) fused via a linker (italics) to a TCR beta chain comprising SEQ ID NO: 15(b52) and the constant domain of SEQ ID NO: 3)

AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGS
GGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALIN
PYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWG
QGTLVTVSS*GGGGS*SAVISQKPSRDIKQRGTSLTIQCQVDKRLALMFWYRQQPGQSPTLIA
TAWTGGEATYESGFVIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGAADTQYFGP
GTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHS
GVCTDPQPLKEQPALNDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAK
PVTQIVSAEAWGRAD

FIG. 5

Amino acid sequences of alpha and beta chains of ImmTAC molecules of the invention

ImmTAC2 (S2E)

SEQ ID NO: 35 ImmTAC alpha chain (comprising SEQ ID NO: 7(a24) and the constant domain of SEQ ID NO: 4, truncated by 8 amino acids)

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYLGDSALVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDINSGAGSYQLTFGKGTKLSVIPNIQNP
DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWS
NKSDFACANAFNNSIIPEDT

SEQ ID NO: 36 ImmTAC beta chain (comprising an anti-CD3 scFv (bold) fused via a linker (italics) to a TCR beta chain comprising SEQ ID NO: 18(b65) and the constant domain of SEQ ID NO: 5)

AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGS
GGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALIN
PYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWG
QGTLVTVSS*GGGGS*SAVISQKPSRDIKQRGTSLTIQCQVDKRLALMFWYRQQPGQSPTLIA
TAWTGSEATYESGFVIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGP
GTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHS
GVCTDPQPLKEQPALNDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAK
PVTQIVSAEAWGRAD

FIG. 6

Amino acid sequences of alpha and beta chains of ImmTAC molecules of the invention

ImmTAC3 (S2G)

SEQ ID NO: 37 ImmTAC alpha chain (comprising SEQ ID NO: 12(a82) and the constant domain of SEQ ID NO: 4, truncated by 8 amino acids)

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYLGDSALVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDIRSGAGSYQLTFGKGTKLSVIPNIQNP
DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWS
NKSDFACANAFNNSIIPEDT

SEQ ID NO: 38 ImmTAC beta chain (comprising an anti-CD3 scFv (bold) fused via a linker (italics) to a TCR beta chain comprising SEQ ID NO: 15(b52) and the constant domain of SEQ ID NO: 5)

AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGS
GGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALIN
PYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWG
QGTLVTVSS*GGGGS*SAVISQKPSRDIKQRGTSLTIQCQVDKRLALMFWYRQQPGQSPTLIA
TAWTGGEATYESGFVIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGAADTQYFGP
GTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHS
GVCTDPQPLKEQPALNDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAK
PVTQIVSAEAWGRAD

FIG. 7

Amino acid sequences of alpha and beta chains of ImmTAC molecules of the invention

ImmTAC4 (S2I)

SEQ ID NO: 39 ImmTAC alpha chain (comprising SEQ ID NO: 12(a82) and the constant domain of SEQ ID NO: 4, truncated by 8 amino acids)

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYLGDSALVKGSY
GFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDIRSGAGSYQLTFGKGTKLSVIPNIQNP
DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWS
NKSDFACANAFNNSIIPEDT

SEQ ID NO: 40 ImmTAC beta chain (comprising an anti-CD3 scFv (bold) fused via a linker (italics) to a TCR beta chain comprising SEQ ID NO: 18(b65) and the constant domain of SEQ ID NO: 5)

AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGS
GGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALIN
PYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWG
QGTLVTVSS*GGGGS*SAVISQKPSRDIKQRGTSLTIQCQVDKRLALMFWYRQQPGQSPTLIA
TAWTGSEATYESGFVIDKFPISRPNLTFSTLTVSNMSPEDSSIYLCSVGGSGGADTQYFGP
GTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHS
GVCTDPQPLKEQPALNDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAK
PVTQIVSAEAWGRAD

FIG. 8

Functional testing of ImmTAC molecules of the invention (Exp. No. PCA006)

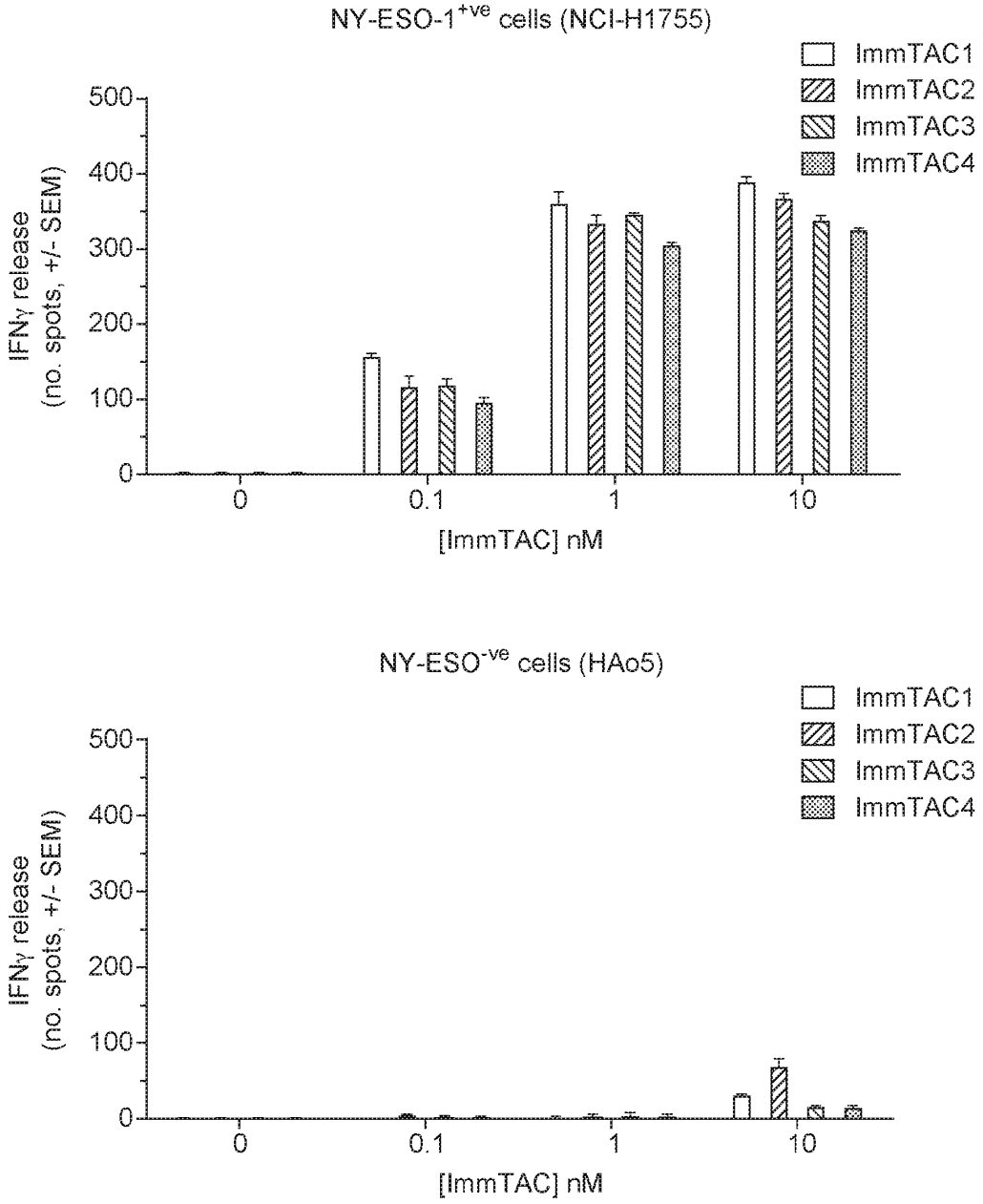


FIG. 9

Further functional testing of ImmTAC molecules of the invention (Exp. No. PCA005)

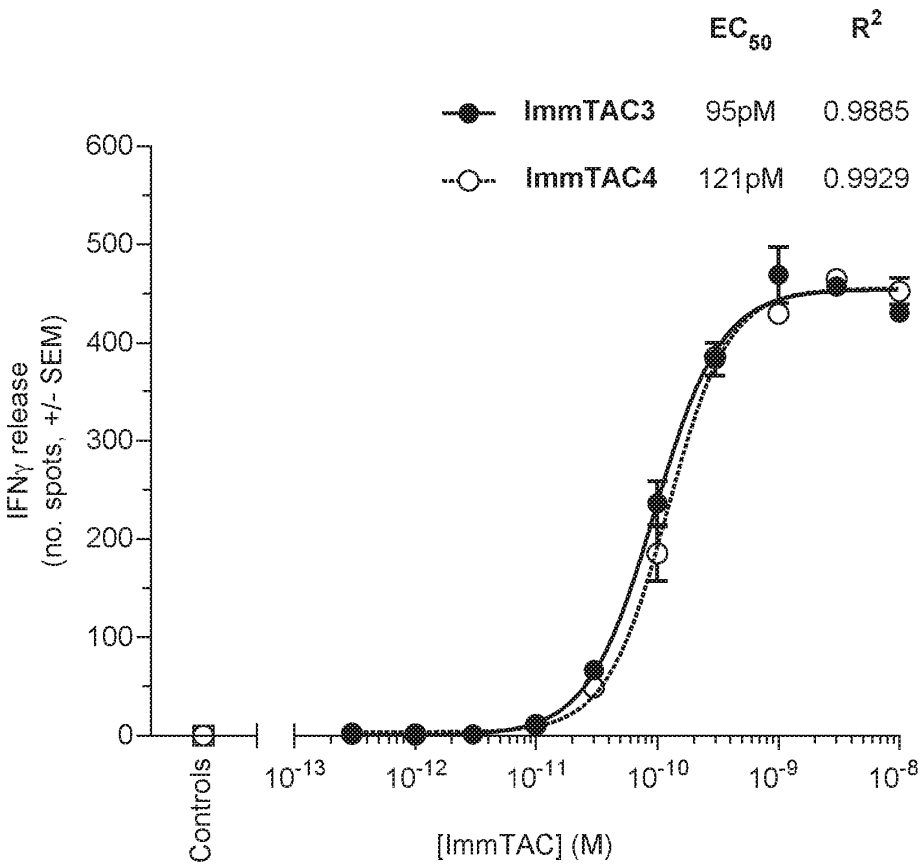
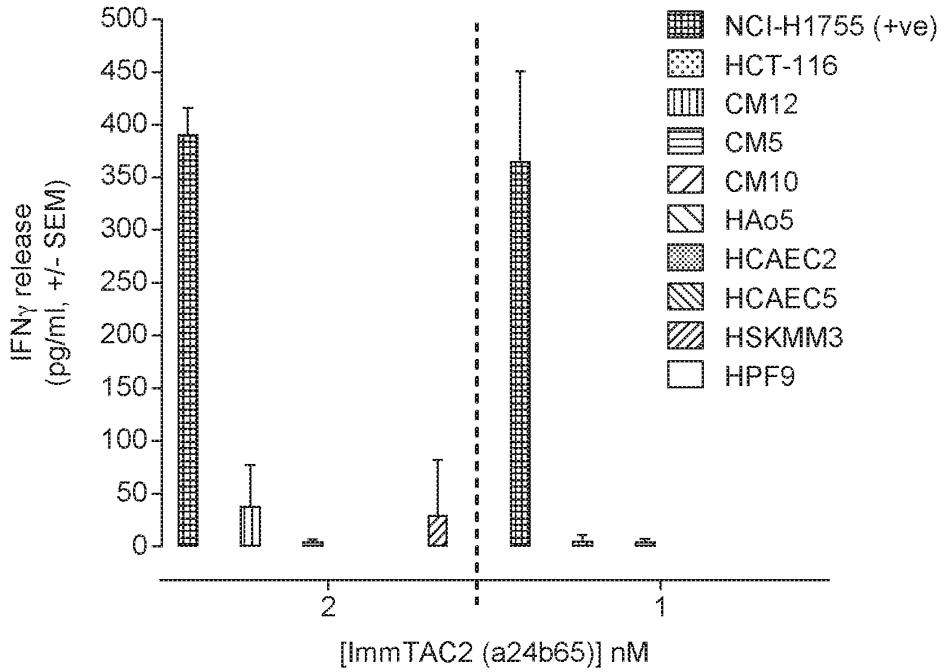


FIG. 10

Further specificity testing of ImmTAC molecules of the invention (Exp. No. PCA002/3)



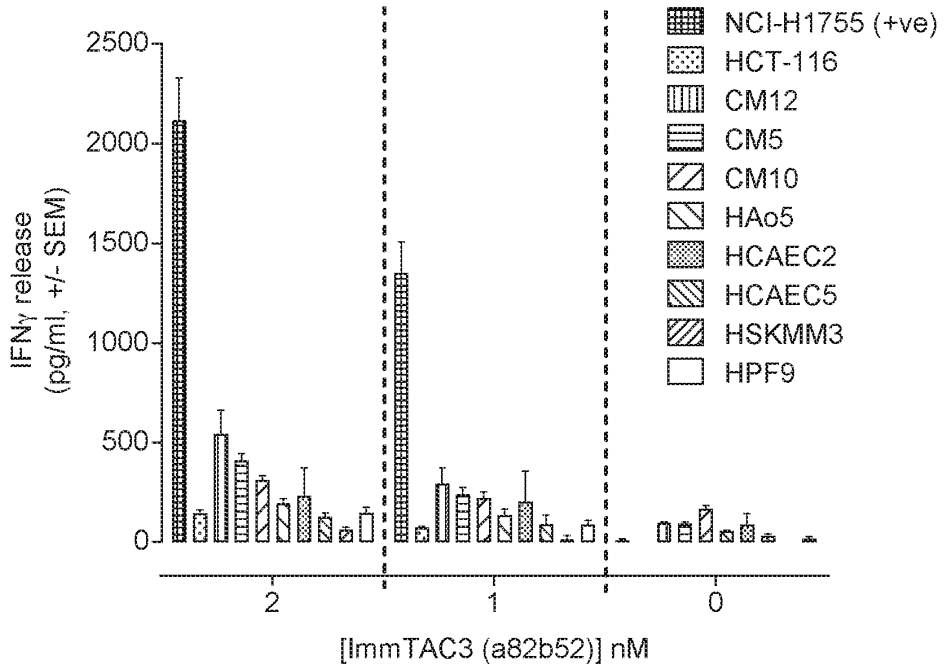


FIG. 11

Further specificity testing of ImmTAC molecules of the invention (Exp. No. PCA002/3)

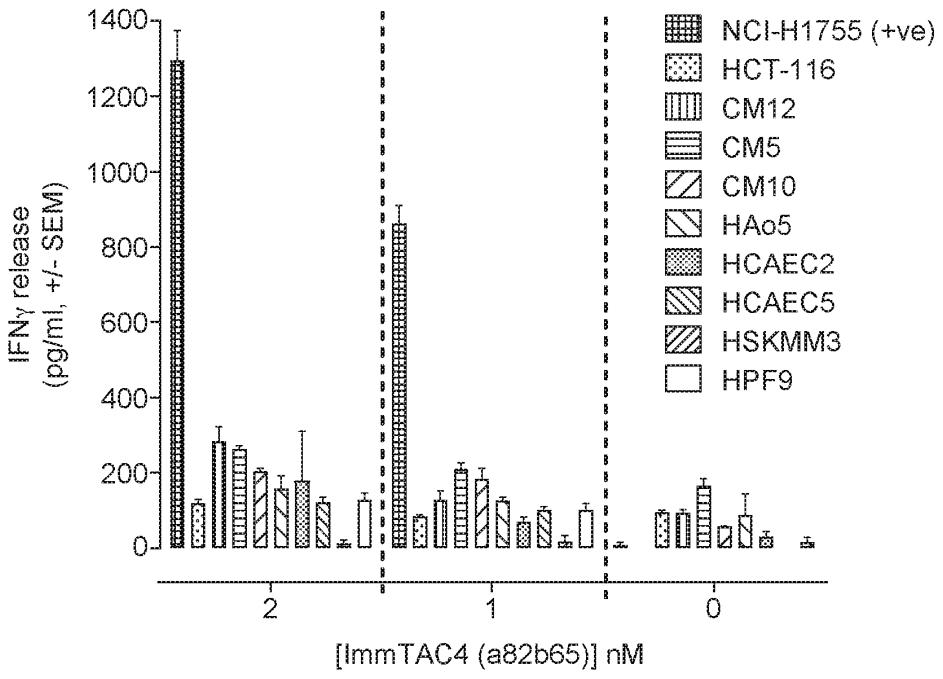


FIG. 11 Cont'd

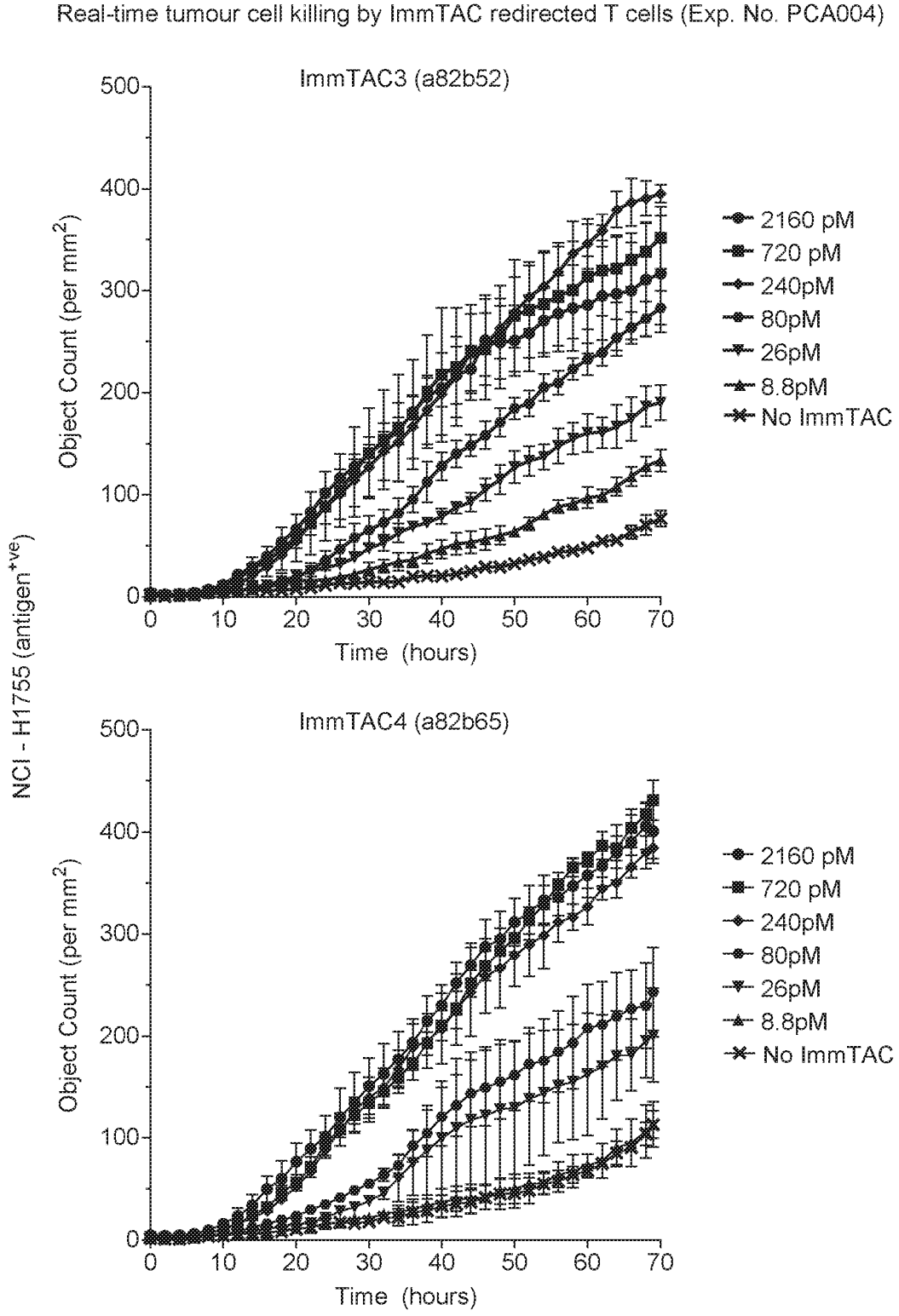

Real-time tumour cell killing by ImmTAC redirected T cells (Exp. No. PCA004)
ImmTAC3 (a82b52)
ImmTAC4 (a82b65)
NCI - H1755 (antigen+ve)
Object Count (per mm2)
Time (hours)
0
100
200
300
400
500
0
10
20
30
40
50
60
70
2160 pM
720 pM
240pM
80pM
26pM
8.8pM
No ImmTAC

FIG. 12

Real-time tumour cell killing by ImmTAC redirected T cells (Exp. No. PCA004)

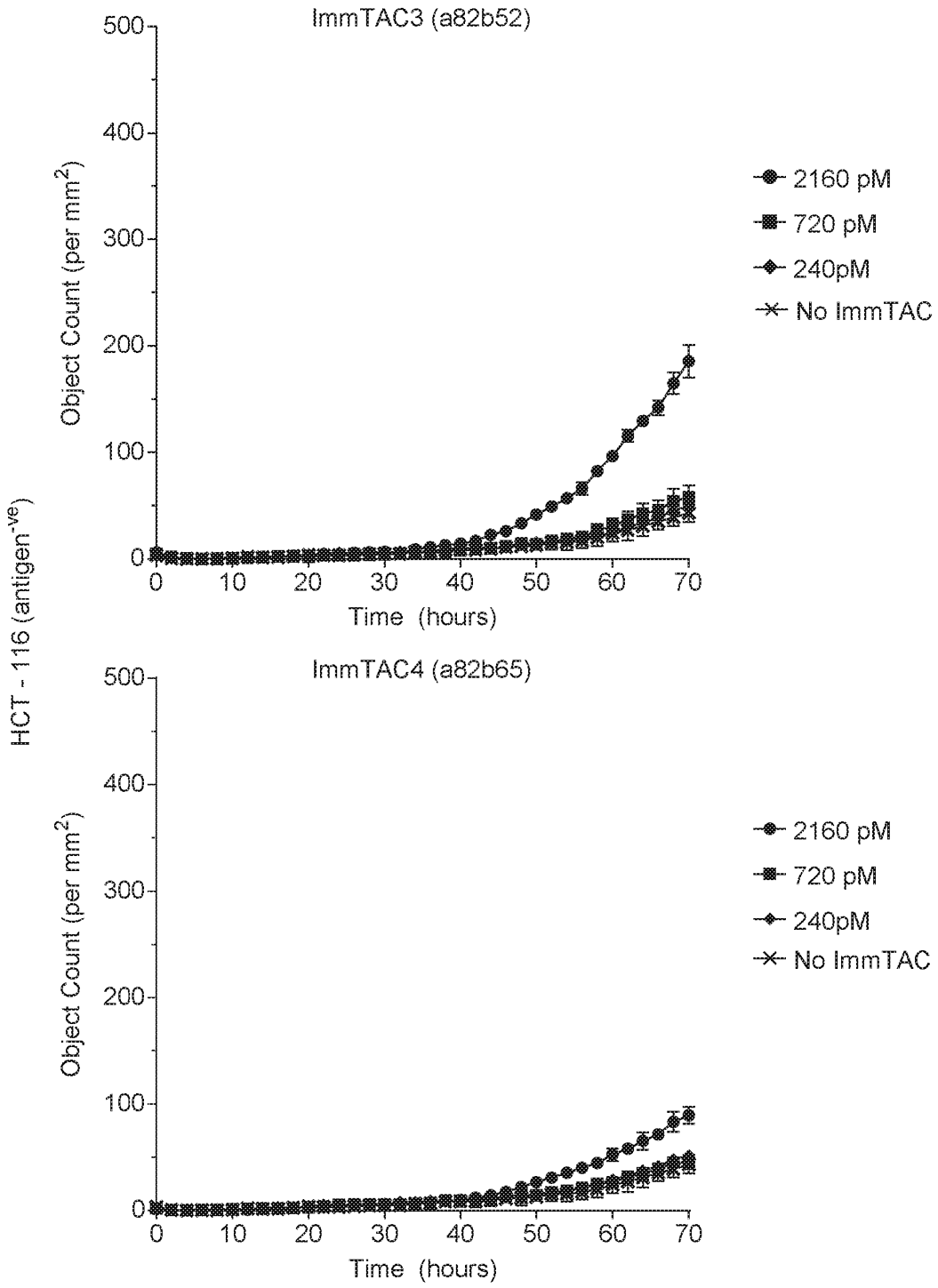


FIG. 12 Cont'd