

Figure 1: Sequences of ALFA and CLDN6 bispecific constructs

A

C = constant domain
V = variable domain
H = heavy chain
L = light chain
VHH = variable heavy chain of heavy chain only antibody

a) Secretion signal aCLDN6(IMAB027)VH-CH1-gs-linker-aALFA-VHH-gs-His-Tag:

MDWTRVFCLLAVAPGAHSEVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWWKQSHGKNLEWIGLINPYNGGTIYNQKFKGKATLTVDKSSS
TAYMELLSITSEDSAVYYCARDYGFVLDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVWTVPSSSLGTQTYICNVNHPKPSNTKVDKKVEPKSCggsgggsEVQLQESGGGLVQPGGSLRLSCTASGVTTISALNAMAMGWYRQAPGER
RVMVAASERGNAMYRESVQGRFTVTRDFTNKMVSLQMDNLKPEDTAVYYCHVLEDRVDSFHDYWGQGTTQTVTVSSgggsHHHHHH**

b) Secretion signal aALFA-VHH-gs-linker-aCLDN6(IMAB027)VH-CH1-gs-His-Tag:

MDWTRVFCLLAVAPGAHSEVQLQESGGGLVQPGGSLRLSCTASGVTTISALNAMAMGWYRQAPGERRMVAASERGNAMYRESVQGRFTVTR
DFTNKMVSLQMDNLKPEDTAVYYCHVLEDRVDSFHDYWGQGTTQTVTVSSggsgggsEVQLQSGPELVKPGASMKISCKASGYSFTGYTMNWWKQ
SHGKNLEWIGLINPYNGGTIYNQKFKGKATLTVDKSSSTAYMELLSITSEDSAVYYCARDYGFVLDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVWTVPSSSLGTQTYICNVNHPKPSNTKVDKKVEPKSCgggsHHHHHH**

c) Secretion signal aCLDN6(IMAB027)-VH-CH1-CH2-CH3-gs-linker-aALFA-VHH-gs-His-Tag:

MDWTRVFCLLAVAPGAHSEVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWWKQSHGKNLEWIGLINPYNGGTIYNQKFKGKATLTVDKSSS
TAYMELLSITSEDSAVYYCARDYGFVLDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVWTVPSSSLGTQTYICNVNHPKPSNTKVDKKVEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKCKVSNKALPAPIEKTSKAGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVQGVFSCVMHEALHNHYTQKSLSLSPGggsgggsEVQLQESGGGLVQPGGSL
RLSCTASGVTTISALNAMAMGWYRQAPGERRMVAASERGNAMYRESVQGRFTVTRDFTNKMVSLQMDNLKPEDTAVYYCHVLEDRVDSFHDY
WGQGTTQTVTVSSgggsHHHHHH**

C = constant domain
V = variable domain
H = heavy chain
L = light chain
VHH = variable heavy chain of heavy
chain only antibody

Figure 1: Sequences of ALFA and CLDN6 bispecific constructs
B

d) Secretion signal aALFA-VHH-gs-linker-aCLDN6(IMAB027)VH-CH1-CH2-CH3-gs-His-Tag:

MDWTWRVFCLLAVAPGAHSEVQLQESGGGLVQPGGSLRLSCTASGVTISALNAMAMGWYRQAPGERRVMVAASERGNAMYRESVQGRFTVTR
DFTNKMVSLQMDNLKPEDTAVYCHVLEDRVDSFDYWGQGTQVTVSSggggggssEVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWNVK
QSHGKNLEWIGLINPYNGGTIYNQKFKGKATLTVDKSSSTAYMELLSLTSEDSAVYYCARDYGFVLDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDIKRVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKgggHHHHHH**

e) Leader Signal-VL-CL (IMAB027):

MHFQVQIFSFLLISASVIMSRGQIVLTQSPAIMASAPGEKVTITCSASSSVYLHWFAQKPGTSPKLWVYSTNLP SGV~~PARFGSGSGTSYSLTISR~~MEA
EDAATYYCQQRSIYPPWTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*

Figure 2: Sequences of ALFA and CD3 bispecific constructs

C = constant domain
V = variable domain
H = heavy chain
L = light chain
VHH = variable heavy chain of heavy chain only antibody

a) Secretion signal-aALFA-VHH-gs-linker-aCD3-VHH (F04)-gs-His-Tag:

MMIDWTTWRVFCLLAVAPGAHSEVQLQESGGGLVQPGGSLRLSTASGVTISALNAMAMGWYRQAPGERRVMVAAVSERGNAMYRESVQGRFTVTRDFTNKIM
VSLQMDNLKPEDTAVYCHVLEDRVDSFDHYWGQGTQTVTSS*gggsggs*EVQLVESGGGPVQAGGSLRLSCAASGRTYRGYSMAWFRQSPGKEREFVAAI
VWSDGNTYYEDFVKGRFTISDSAKNTLYLQMTNLKPEDTALYYCAAKIRPIYFIKAGQDYWGGQGTQTVTSS*ggs*HHHHHHH**

b) Secretion signal-aCD3-VHH (F04)-gs-linker-aALFA-VHH-gs-His-Tag:

MMDDWTTWRVFCLLAVAPGAHSEVQLVESGGGPVQAGGSLRLSCAASGRTYRGYSMAWFRQSPGKEREFVAIVWSDGNTYYEDFVKGRFTISRDSAKNTLYLQ
MTNLKPEDTALYYCAAKIRPIYFIKIQGVDYWGGGTQVTSSGGGGGGGGSEVQLQESGGGLVQPGGSLRLCTASGTISALNAMAMGWYRQAPGERRVMVA
AVSERGNAMYRESVQGRFTVTRDFTNKMVSLQMDNLKPEDTAVYYCHVLEDRVDSFHDYWGQGTQVTVSSGGSHHHHHH**

c) Secretion signal-aALFA-VHH-gs-linker-VL(TR66)-gs-linker-VH(TR66)-gs-His-Tag:

VSLQMDNLKPEDTAVYVCHVLEDRVDSFHDYWGQGTQTVTVSSggggsggsQIVLTQSPAIMASPGKEVTMTCRASSVSYMWNWYQKSGTSPKRWIYDTSK
 V/ASGVVPV/FSGSGSGT/SYSLT/ISSMEAEADATY/CQQW/SSNPLTFGAGTKLELKGggsgggsgggsgggsgggsggVQLQQSGAEALRPGASVKMSCKTSGYT
 FTRYTMHWVWKQRPQGGLIEWIGYINPSRGVTNYNQKFKDKATLTDDKSSSTAVMQLSSLTSEDSAVYVCARYDDHDHYSLDYWGQGTTLTVSSggsHHHHHHH**

d) Secretion signal-VL/TR66)-gs-linker-VH(TR66)-gs-linker-aALFA-VHH-gs-His-Tag:

[illegible]

Figure 3: Overview of synthesized ALFA-Dye peptides

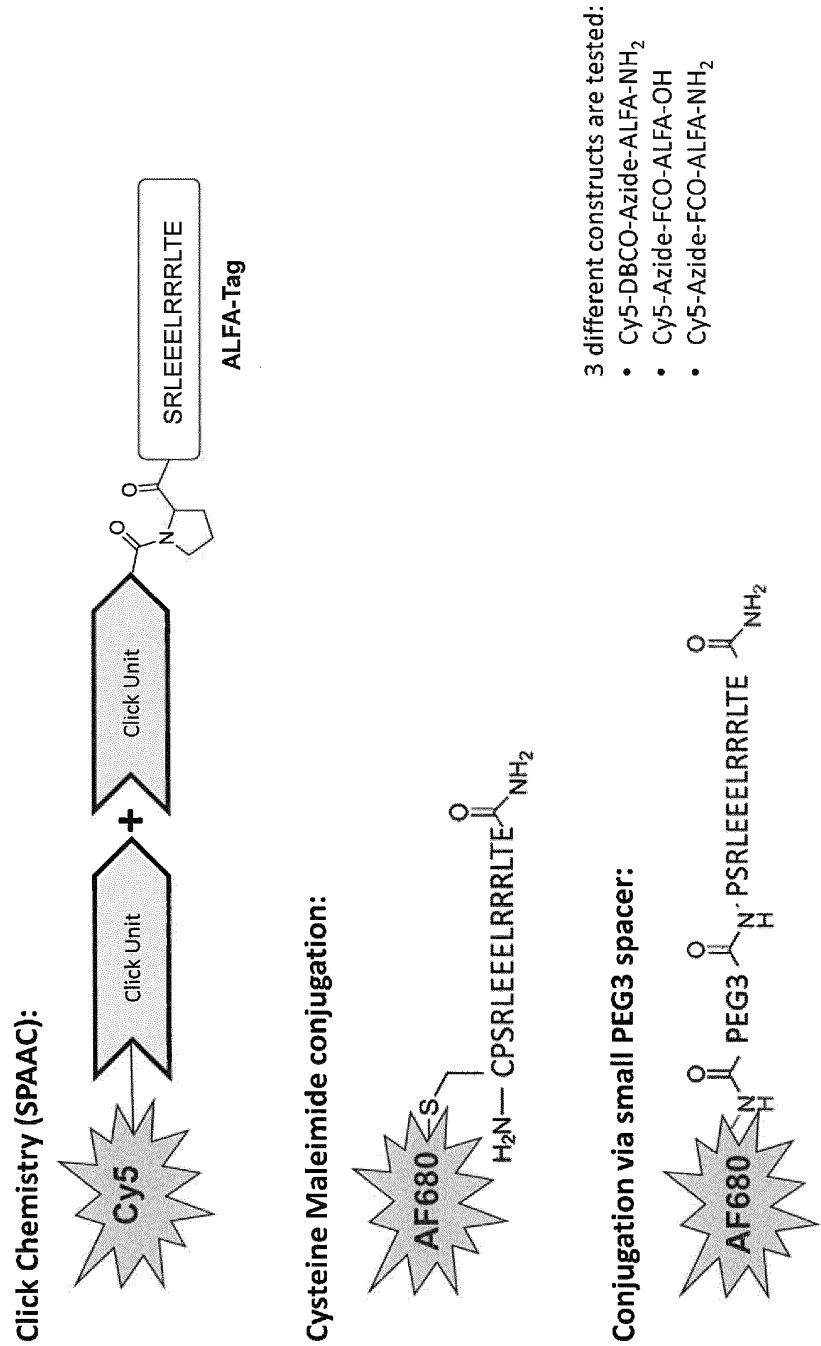
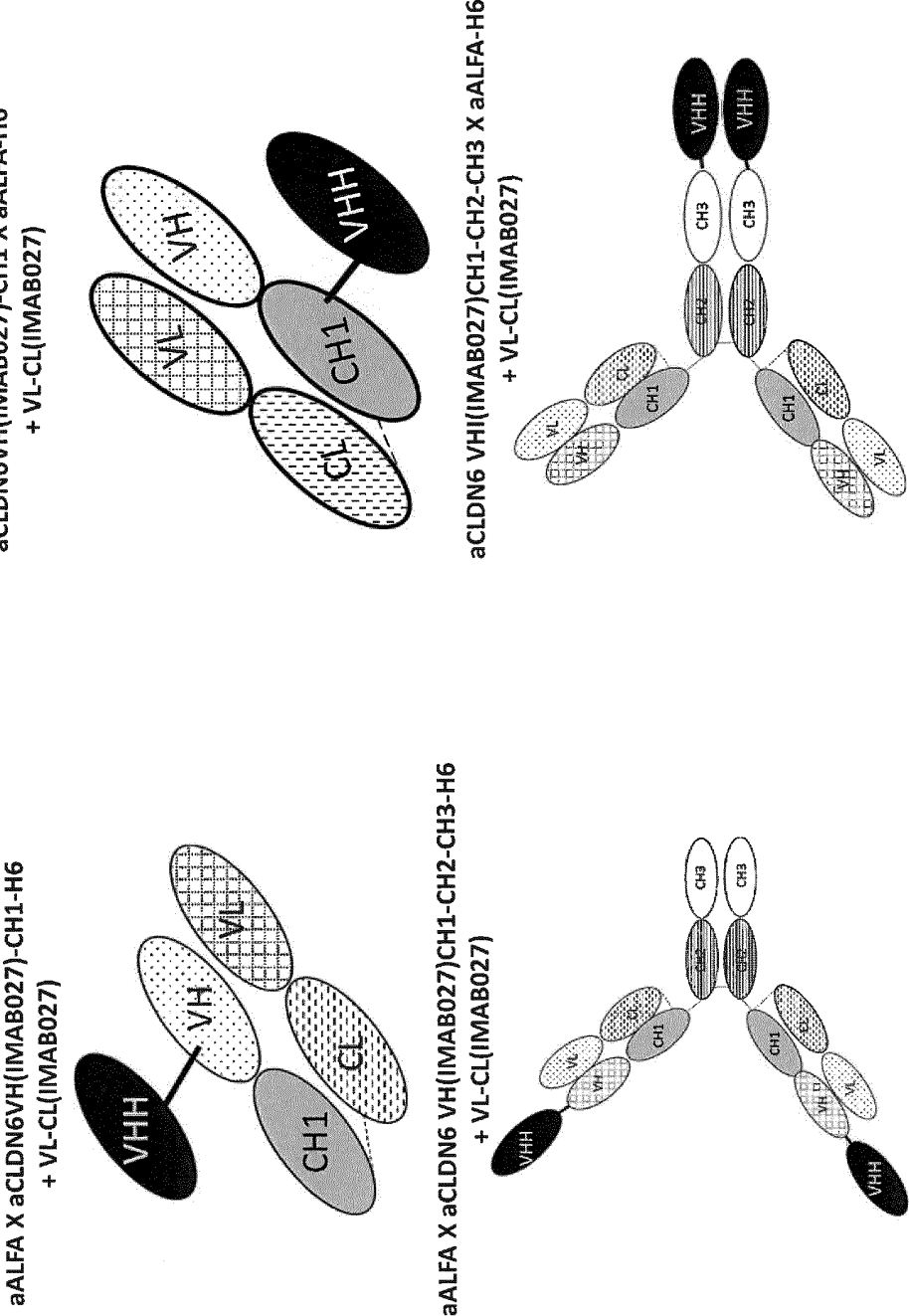
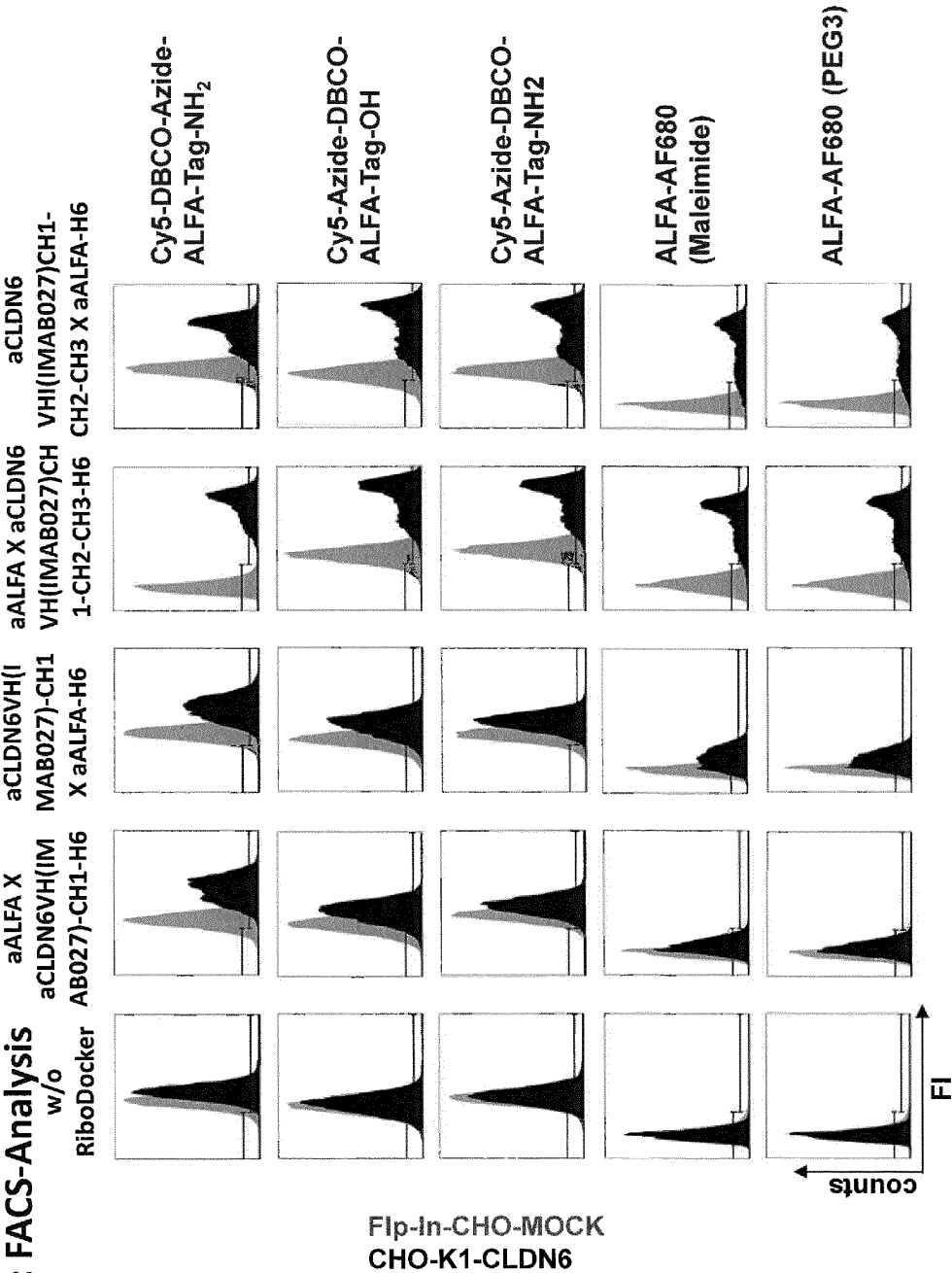
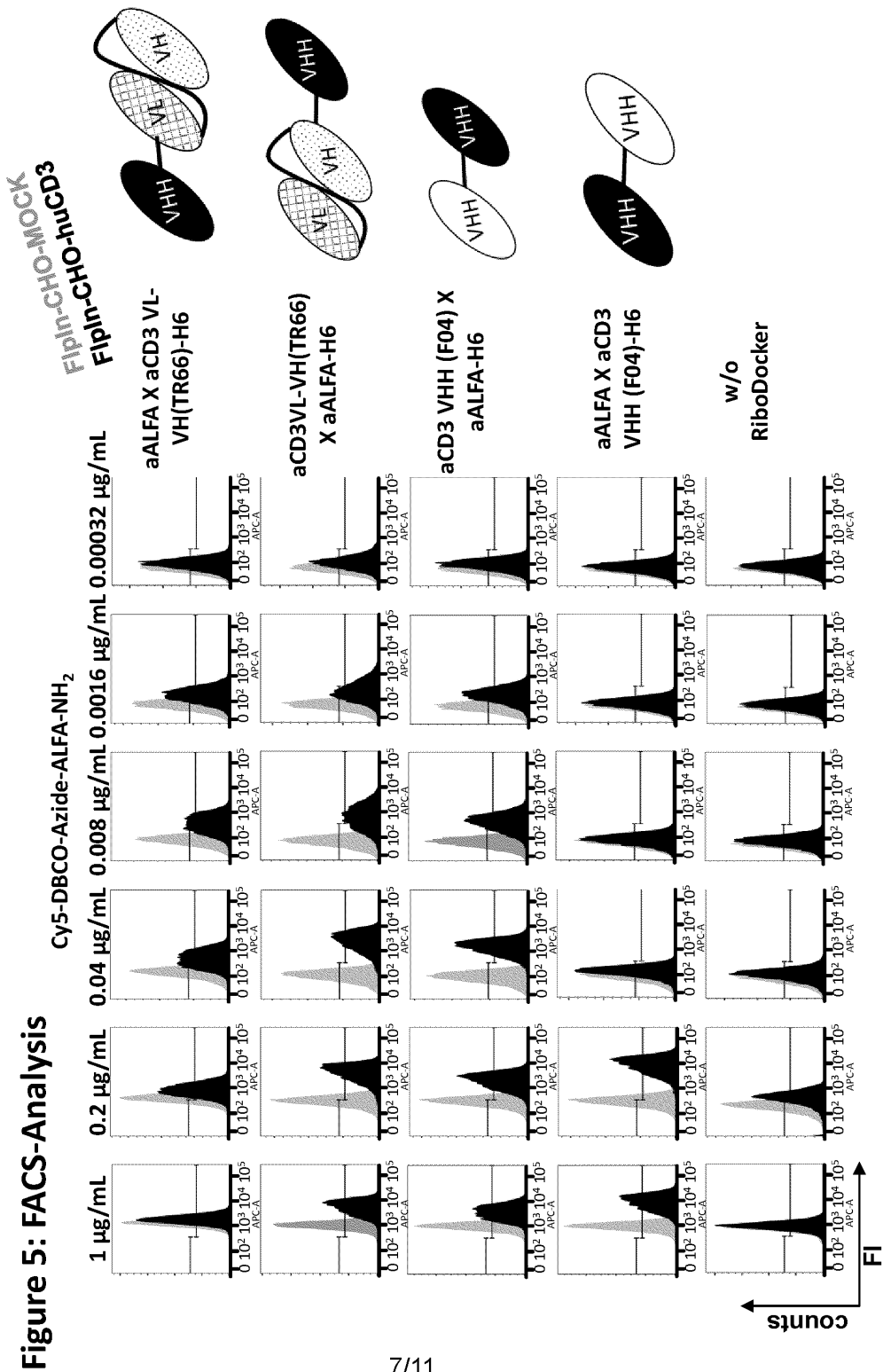


Figure 4A: FACS-Analysis constructs







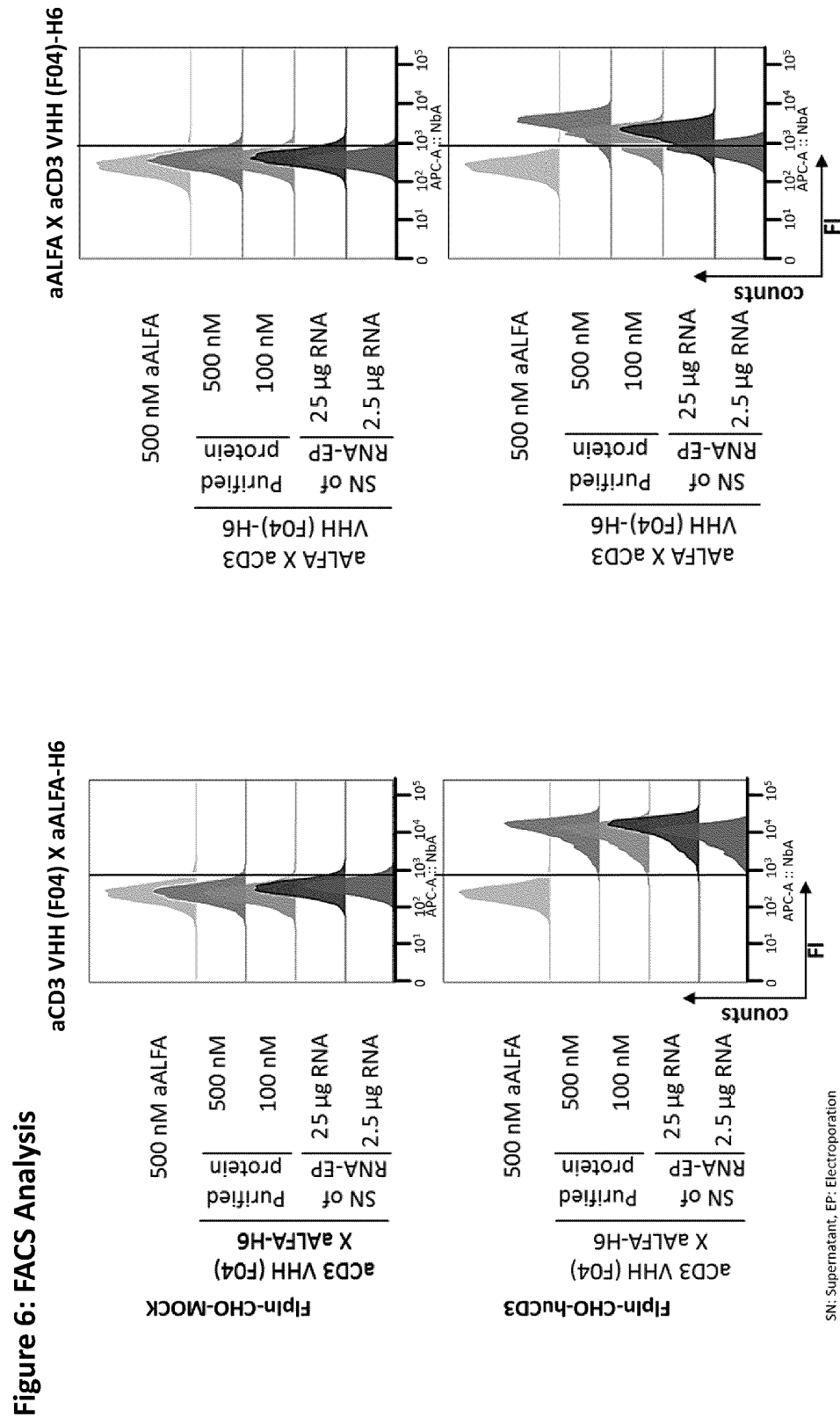


Figure 7: Modular, bispecific antibodies for cancer therapy

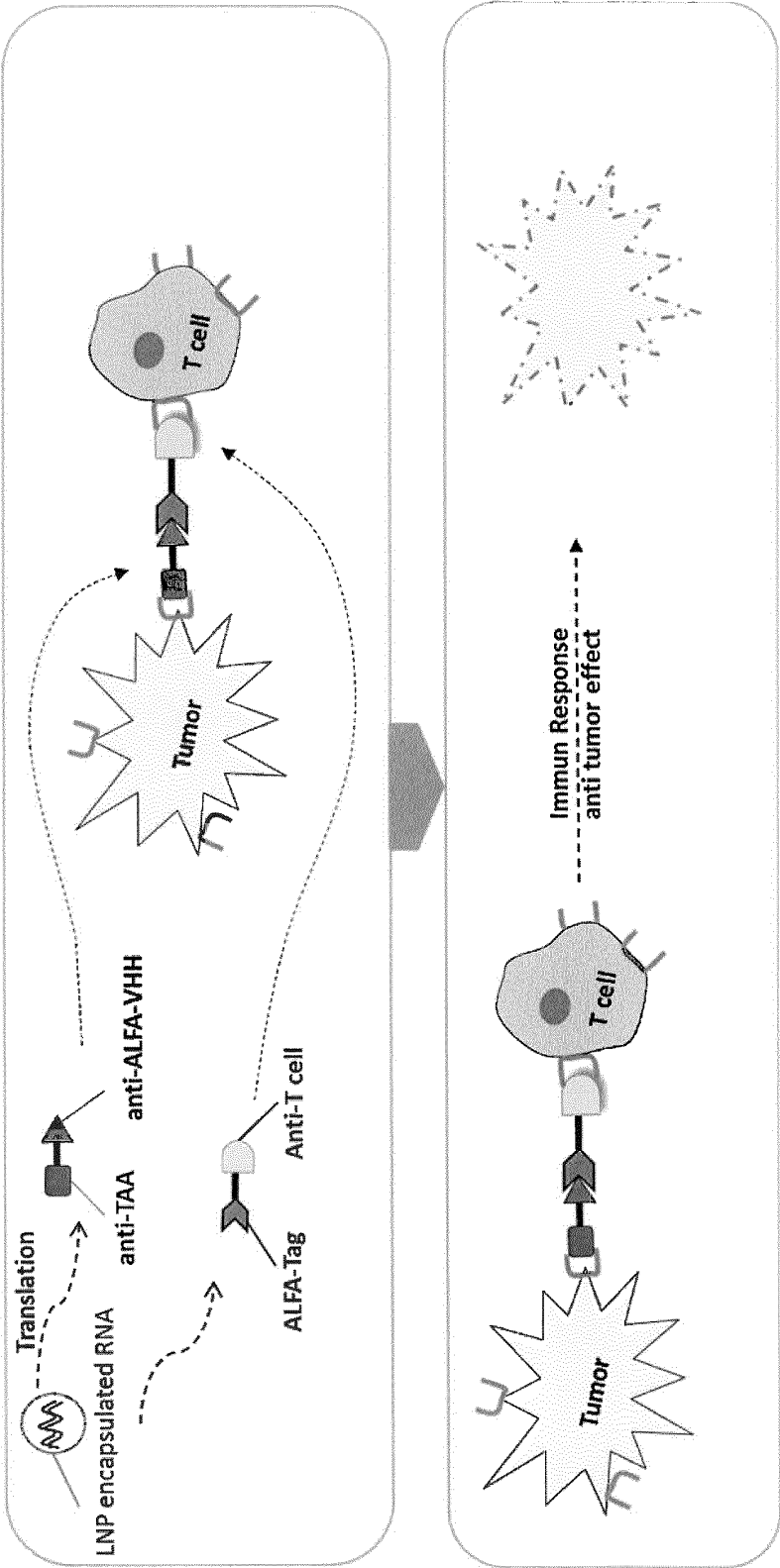


Figure 8: Modular CAR-T cell approach

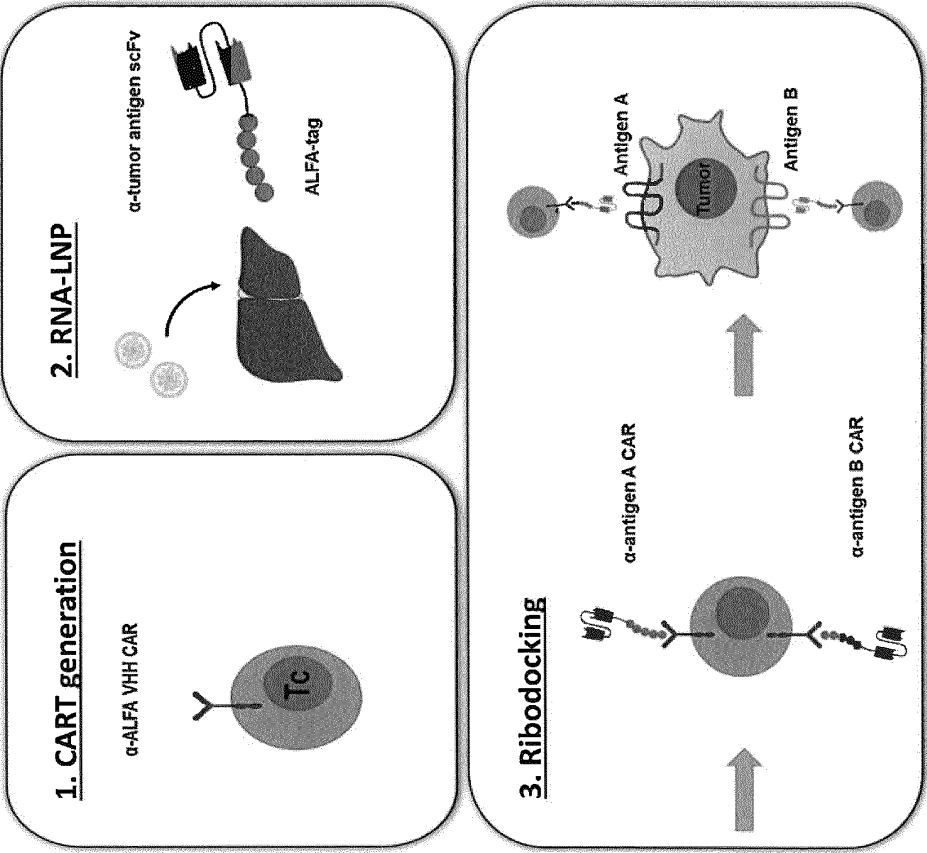


Figure 9: RiboDocker for targeting of ALFA peptide presenting nanoparticles

N/P	Description	Purpose
1	ALFA coated PLX	Sample
2	Non coated PLX	
3	ALFA coated PLX	Negative control
4	Non coated PLX	

