JBC Supporting Information

Optimized serum stability and specificity of an αvβ6 integrin-binding peptide for tumor targeting

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Table S1. Original, cyclized, and modified A20FMDV2 peptide sequences used for binding and serum stability studies.

Peptide	Sequence	Weight (g/mol)
A20FMDV2	biotin-NAVPNLRGDLQVLAQKVARTK-amide	2517.0
C1C18 DFBP	biotin-CAVPNLRGDLQVLAQKVCRTK-amide	2832.2
C1C19 DFBP	biotin-CAVPNLRGDLQVLAQKVACTK-amide	2747.1
C1C20 DFBP	biotin-CAVPNLRGDLQVLAQKVARCK-amide	2802.1
C2C18 DFBP	biotin-NCVPNLRGDLQVLAQKVCRTK-amide	2875.2
C2C19 DFBP	biotin-NCVPNLRGDLQVLAQKVACTK-amide	2790.1
C6C17 DFBP	biotin-NAVPNCRGDLQVLAQKCARTK-amide	2805.1
C1C18 S-S	biotin-**C**AVPNLRGDLQVLAQKV**C**RTK-amide	2536.1
C2C18 R_DTKA_D DFBP	biotin-NCVPNLRGDLQVLAQKVCR_DTKA_D-amide	2946.3
C2C18 CitTKA_D DFBP	biotin-NCVPNLRGDLQVLAQKVCCitTKA_D-amide	2947.3
C2C18 P_h R_DTKA_D DFBP	biotin-NCVP_hNLRGDLQVLAQKVCR_DTKA_D-amide	2962.3
C1C18 A_D $R_DTK_DA_D$ DFBP	biotin-CA_DVPNLRGDLQVLA_DQKVCR_DTK_DA_D-amide	2903.2
C2C18 $_A$FGD RTKA_D DFBP	biotin-NCVPNL$_A$FGDLQVLAQKVCRTKA_D-amide	2952.3
C2C18 CitGD DFBP	biotin-NCVPNLCitGDLQVLAQKVCRTK-amide	2876.2
C2C18 $_G$FGD R_DTKA_D DFBP	biotin-NCVPNL$_G$FGDLQVLAQKVCR_DTKA_D-amide	2994.3

C, DFBP-cyclized; **C**, disulfide-cyclized; R_D, D-arginine; A_D, D-alanine; Cit, Citrulline; P_h, hydroxyproline; K_D, D-lysine; $_A$F, 4-aminophenylalanine; $_G$F, 4-guanidinophenylalanine.

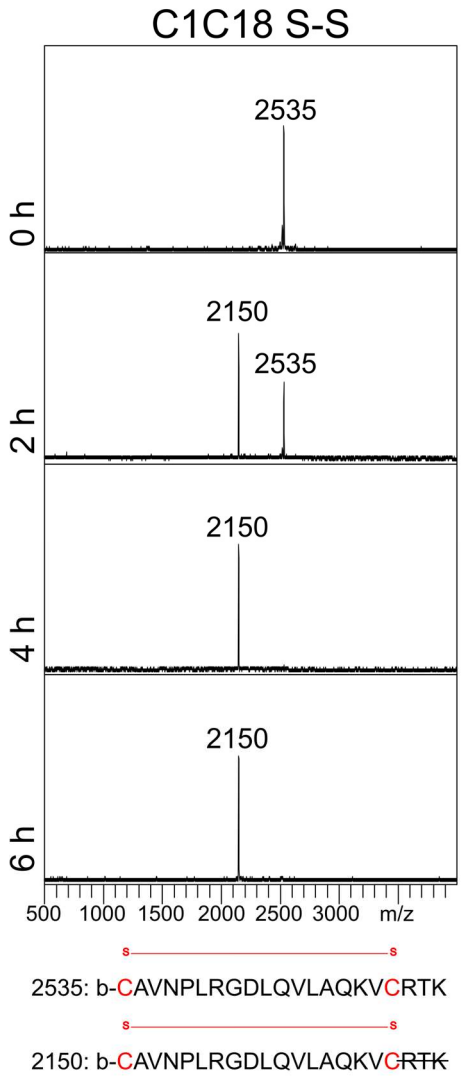

Figure S1. Disulfide cyclization provides less enzymatic stability than DFBP cyclization for the C1C18 peptide. MALDI-ToF spectra of disulfide-cyclized C1C18 S-S incubated in normal mouse serum for 0, 2, 4, and 6 h at 37 °C. Molecular weights of prominent peaks are shown. *Bottom*: predicted amino acid sequences of degradation products based on measured molecular weights.

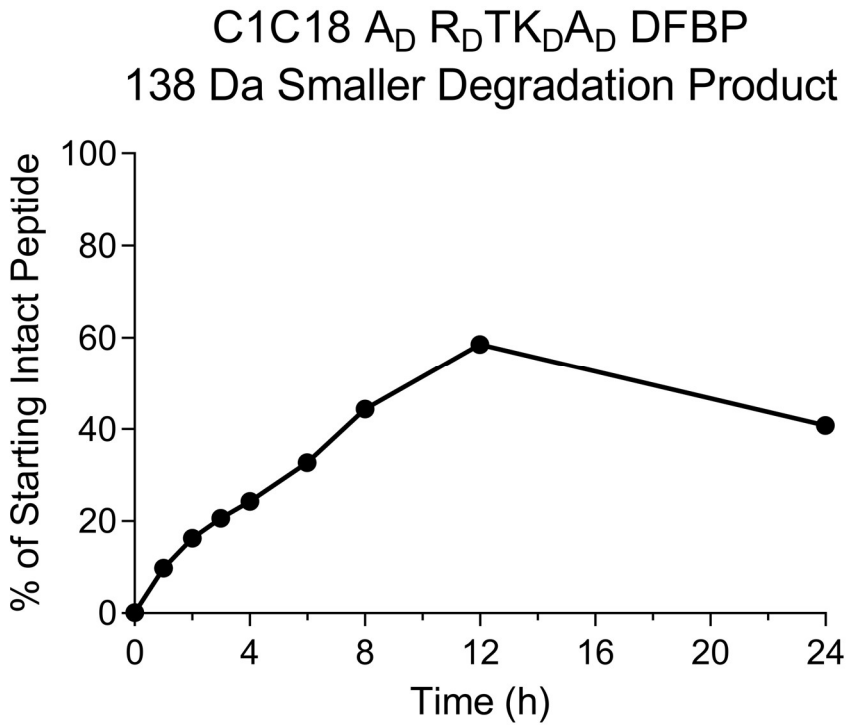

Figure S2. DFBP-cyclized C1C18 A_D R_DTK$_D$$A_D$ is degraded into a stable 138 Da smaller product over serum incubation. Accumulation of a 138 Da smaller degradation product from the DFBP-cyclized C1C18 A_D R_DTK$_D$$A_D$ peptide over a 24-h incubation in normal mouse serum, as measured by LC-MS. Values are normalized to the 0 h timepoint for the intact peptide.

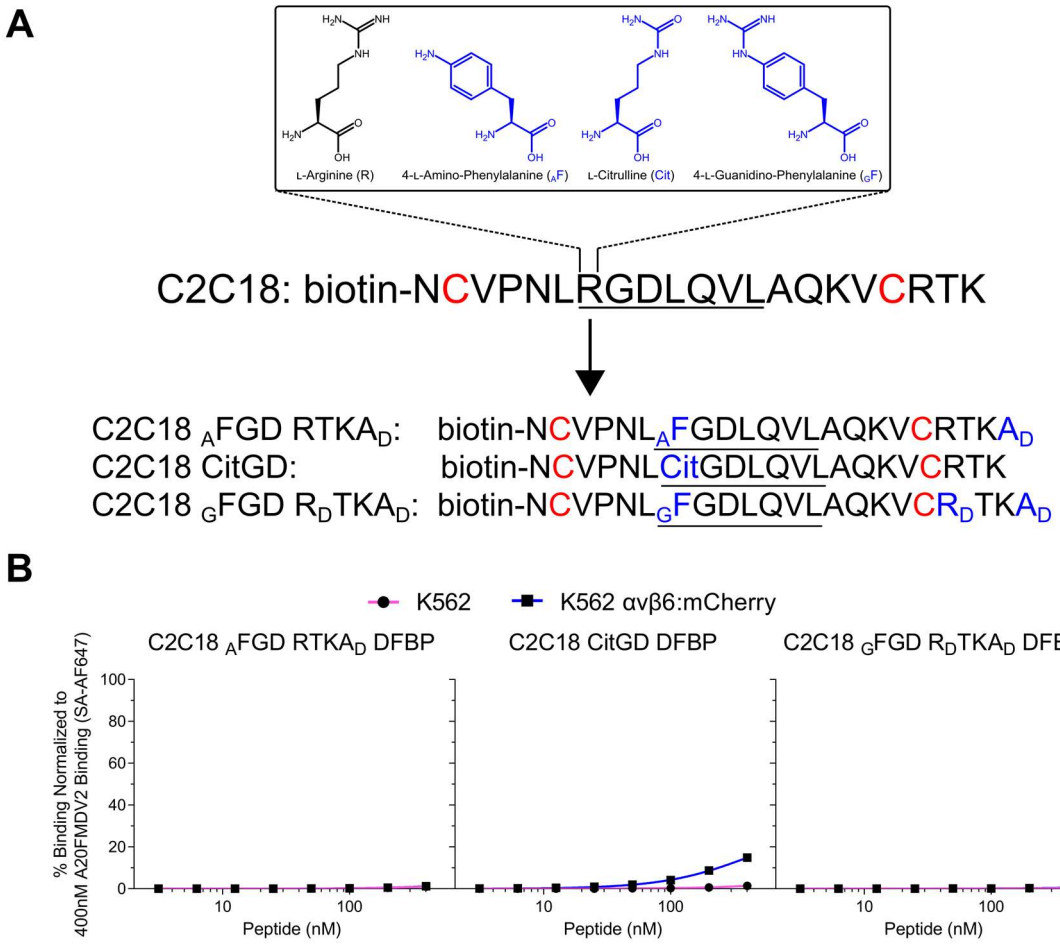

Figure S3. DFBP-cyclized C2C18 peptides with arginine mimetic-modified RGD motifs fail to bind αvβ6$^+$ cancer cells. *A*, schematic of mimetic substitutions made to the sequence of C2C18 DFBP to replace arginine in the RGD motif. Chemical structures of arginine (*black*) and mimetics (*blue*) are shown for comparison. The resulting mimetic-substituted peptide sequences are also listed, with cysteine substitutions for DFBP cyclization shown in *red* and substitutions and C-terminal modifications shown in *blue*. The RGDLXXL motif that is important for αvβ6 recognition is *underlined* in all sequences. *B*, flow cytometry binding curves of mimetic-substituted peptides to K562 and K562 αvβ6:mCherry cells, normalized to 400 nM A20FMDV2 binding to K562 αvβ6:mCherry cells. The curves represent a nonlinear regression of one independent experiment in which binding data are fitted to a Hill equation. SA-AF647, streptavidin Alexa Fluor 647.