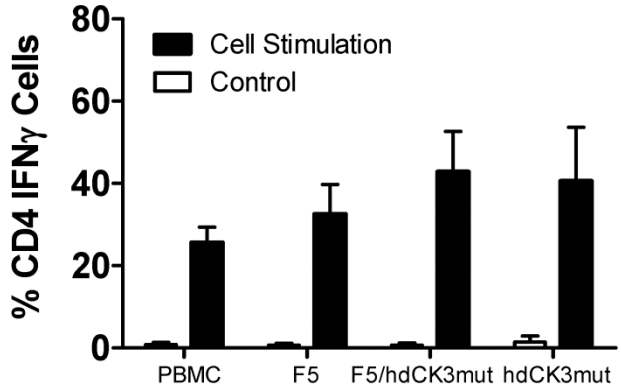
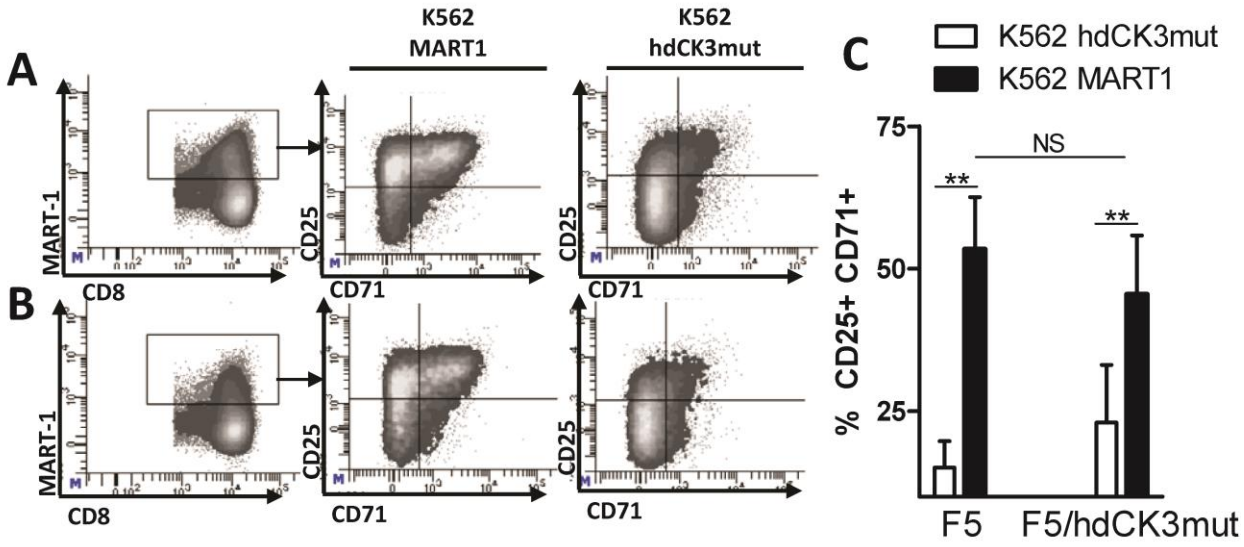
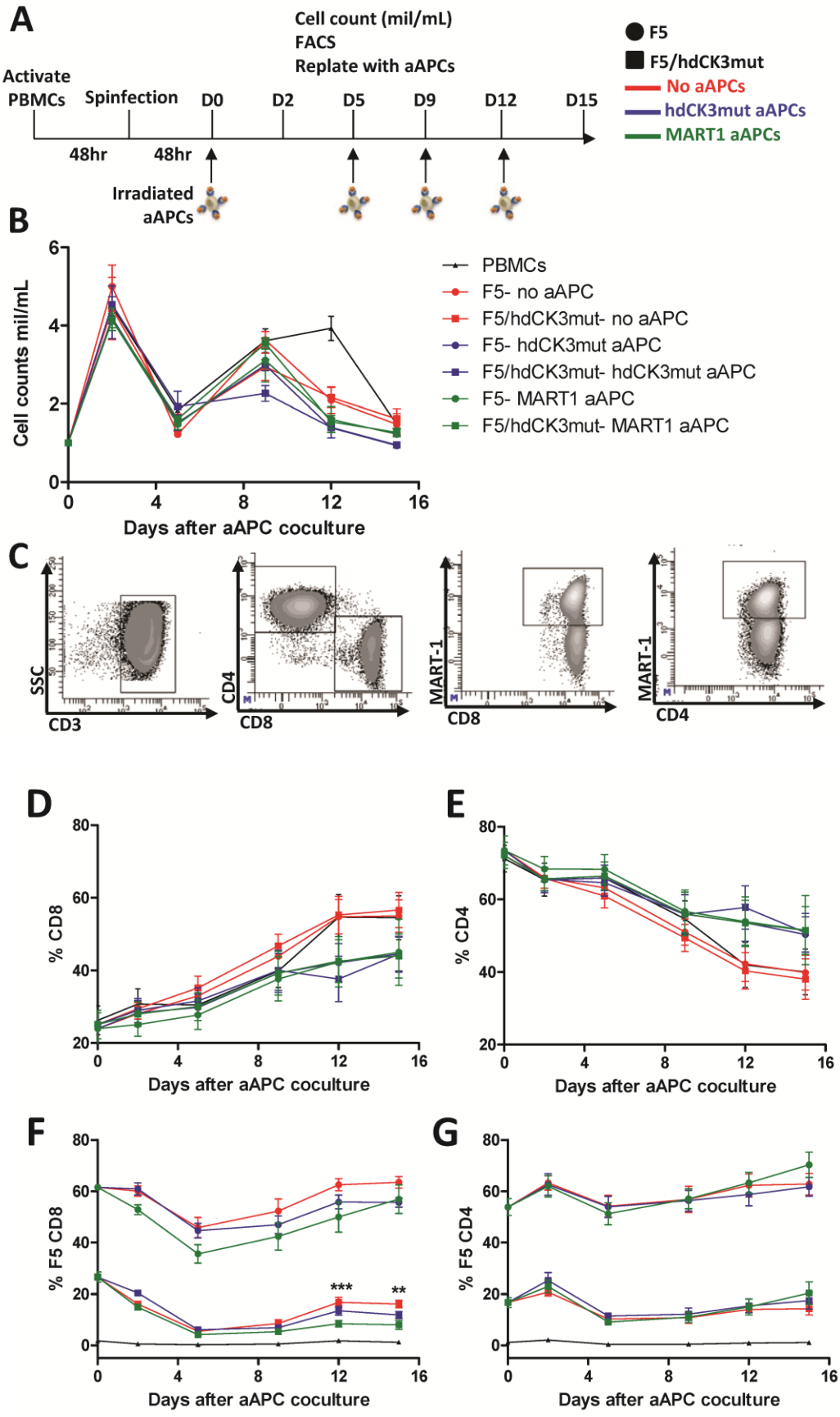


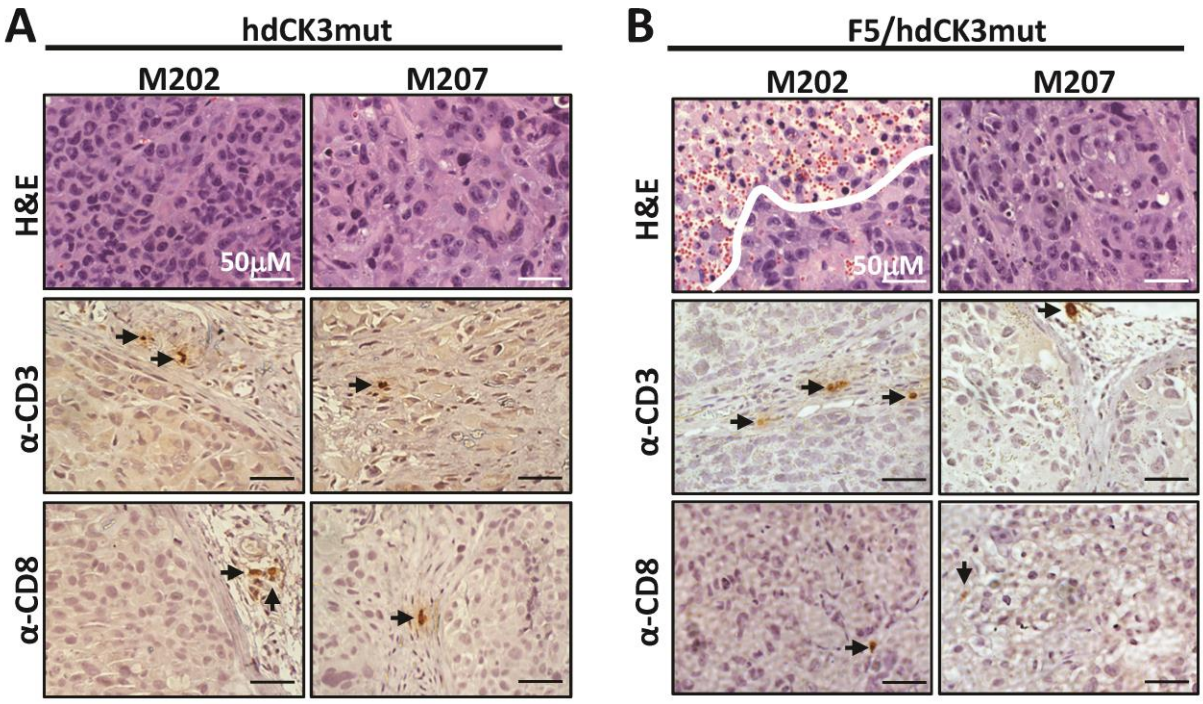
Supplemental Figure S1: hdCK3mut expressing CD4 T cells are capable of IFN-γ cytokine production. Percentage of CD4 cells that produce IFN-γ shown as mean with +/- SD. (n=4)



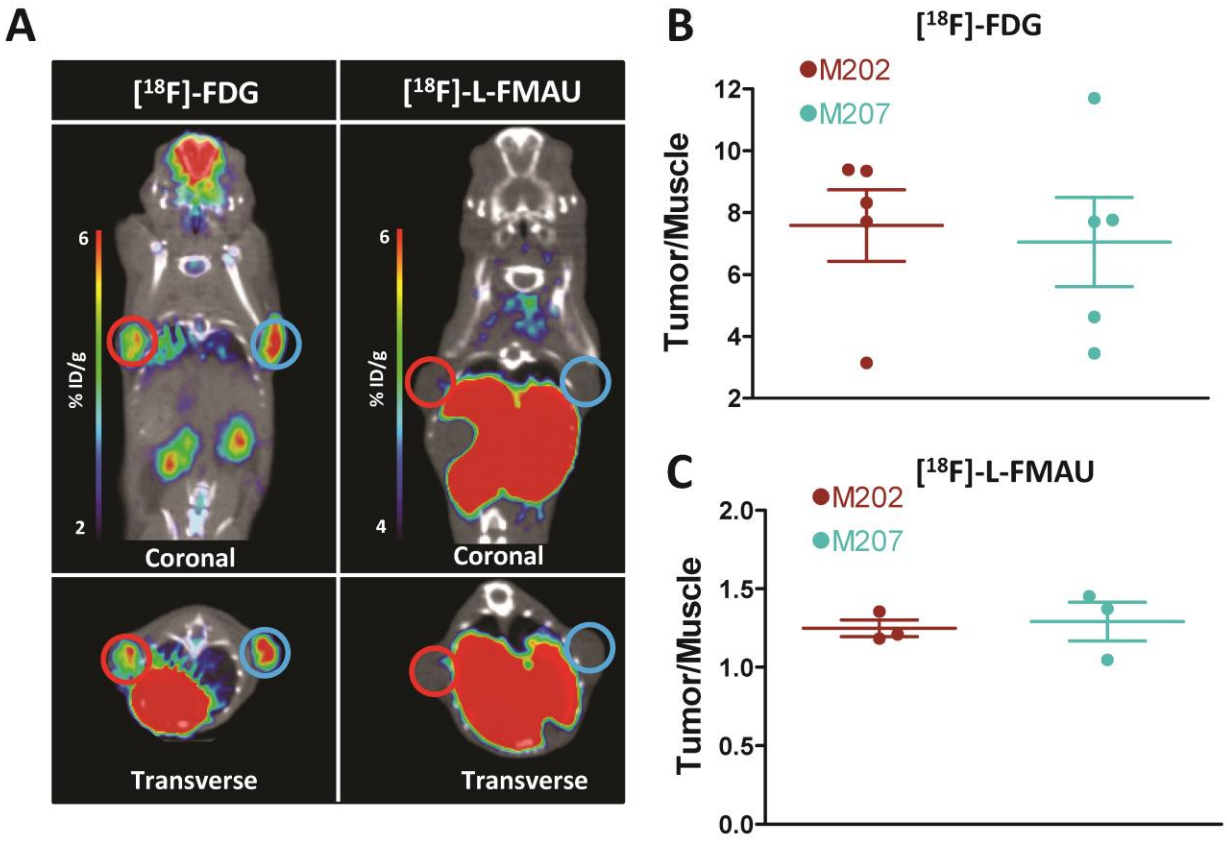
Supplemental Figure S2: Expression of hdCK3mut does not alter T cell activation. aAPC (K562) and transduced PBMC T cells were co-cultured for 72hrs. Representative flow cytometry plots from T cells expressing F5 (A) or F5/hdCK3mut (B) co-cultured with MART-1 expressing aAPCs or hdCK3mut. First panel gated showing F5 T cells, activated F5 T cells are detected after 72hr coculture with aAPCs (CD25+, CD71+) expressing MART1-second panel, or hdCK3mut-third panel. (C) Quantification of activated F5 T cells. White bars are cells cultured with hdCK3mut aAPCs, black bars are cells cultured with MART-1 aAPCs ($P<0.005$ for differing aAPCs or NS by T test for F5 and F5/hdCK3mut) shown as mean +SD (n=4).


A
Cell count (mil/mL)
FACS
Replate with aAPCs
F5
F5/hdCK3mut
No aAPCs
hdCK3mut aAPCs
MART1 aAPCs
Activate PBMCs
Spinfection
D0
D2
D5
D9
D12
D15
48hr
48hr
Irradiated aAPCs
B
Cell counts mil/mL
Days after aAPC coculture
PBMCs
F5- no aAPC
F5/hdCK3mut- no aAPC
F5- hdCK3mut aAPC
F5/hdCK3mut- hdCK3mut aAPC
F5- MART1 aAPC
F5/hdCK3mut- MART1 aAPC
C
SSC
CD3
CD4
CD8
MART-1
CD8
MART-1
CD4
D
% CD8
Days after aAPC coculture
E
% CD4
Days after aAPC coculture
F
% F5 CD8
***
**
Days after aAPC coculture
G
% F5 CD4
Days after aAPC coculture

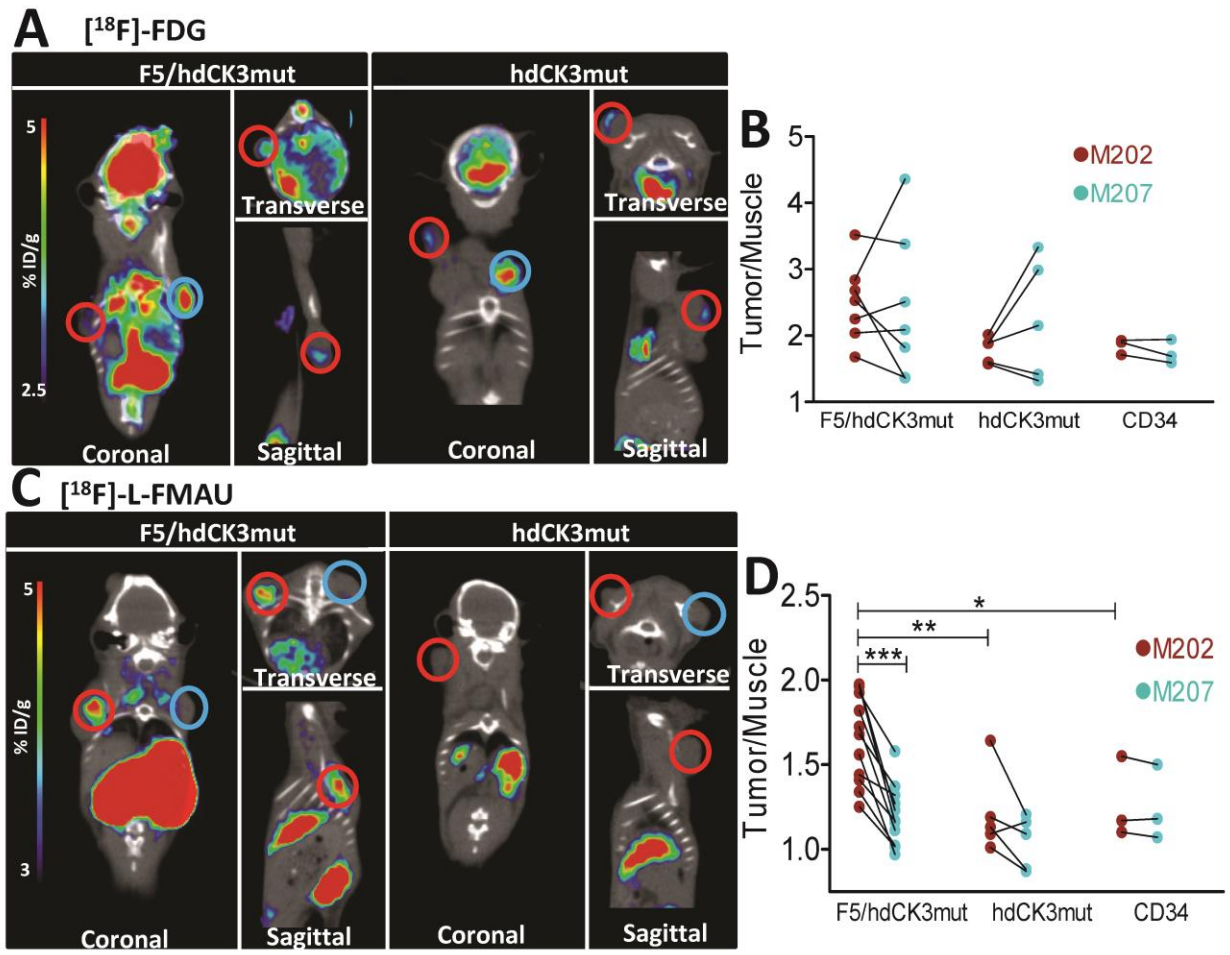
Supplemental Figure 3: Repeated aAPC coculture with F5 or F5/hdCK3mut PBMCs. (A) Schematic of experiment. PBMCs were activated 48hr, infected and analyzed 48hr after. Total PBMCs were counted and plated at $1x10^6$ cells/mL with irradiated aAPC plated at 5:1 T cell:aAPC. Time points of analysis indicated as days after initial aAPC coculture. (B) Total cell counts in million/mL. Black-mock PBMCs, Red- no aAPCs, Blue- hdCK3mut aAPCs, Green-MART1 aAPCs, Circles- F5 transduced, Squares- F5/hdCK3mut transduced. NS by Two-Way ANOVA. (C) Representative FACS plots of transduced PBMCs. Initial gating on CD3, then CD4 or CD8 with F5 TCR identified by MART-1 tetramer. Total percentage of CD8 (D), CD4 (E), F5 CD8 (F), and F5 CD4 (G) are displayed over time. Statistics calculated by Two-Way ANOVA. N=3 unique PBMC donors.



Supplemental Figure S4: IHC of M202 and M207 Tumors from hdCK3mut and F5/hdCK3mut recipient animals. (A) H&E, α-CD3 and α-CD8 from representative sections of M202 (HLA-A*0201+) and M207 (HLA-A*0201-) xenografts from hdCK3mut recipients. (Scale bar is 50μM) (B) Additional IHC of M202 and M207 Tumors from F5/hdCK3mut recipient animals. H&E from M202 (HLA-A*0201+) and M207 (HLA-A*0201-) a white border is drawn to distinguish viable tumor from tumor necrosis. α-CD3 and α-CD8 from representative sections of M202 or M207 xenografts. Black arrows point to T cells. (Scale bar is 50μM)



Supplemental Figure S5: Engraftment and PET imaging of melanoma xenografts M202 and M207 in control NSG recipients. (A) [18F]-FDG and [18F]-L-FMAU images from NSG animals implanted with M202 and M207 xenografts. M202 xenografts are circled in red with M207 xenografts circled in aqua. (B) Quantification of the tumor/muscle ratio from [18F]-FDG images (NS by T test) shown as mean +/- SD (n=5). (C) Quantification of the tumor/muscle ratio from [18F]-L-FMAU images (NS by T test) shown as mean +/- SD (n=3).



Supplemental Figure S6: Additional PET images from BLT animals. (A) [18F]-FDG scans from F5/hdCK3mut and hdCK3mut recipient animals. M202 tumors are circled in red, M207 tumors are circled in aqua. (B) Quantification of [18F]-FDG scans. Matched tumors are connected by black lines. (C) Additional [18F]-L-FMAU scans from F5/hdCK3mut and hdCK3mut recipient animals. M202 tumors are circled in red, M207 tumors are circled in aqua. (D) Quantification of [18F]-L-FMAU scans. Matched tumors are connected by black lines. (n=3 to n=11) Statistics by one way ANOVA.

	F5/hdCK3mut		hdCK3mut		CD34	
Probe	# animals	Mean Diff.	# animals	Mean Diff.	# animals	Mean Diff.
FDG	4/7	0.09	2/5	-0.32	2/3	0.04
L-FMAU	11/11	0.44	4/5	0.17	2/3	0.02

Supplemental Table S1: Difference in M202/M207 signal in BLT recipient animals. Mean difference is average fold change in M202 subtracting average fold change in M207.