Driver mutations, immune microenvironment, and response to immune checkpoint blockade in clear cell renal cell carcinoma

DOD/KCRP and AACR-Kure It
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Study description/goals

- Determine how some epigenetic driver mutations in ccRCC might shape the immune milieu and possibly the response to immunotherapy

Abbreviated prelim data

- *PBRM1* and *SETD2* loss is associated with/causative of immune signaling through pathways associated with response to immune checkpoint blockade
  - Isogenic cell lines
  - TCGA analysis

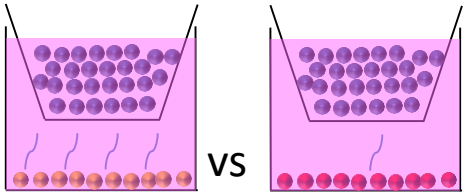
Aim 1 hypothesis: SETD2 loss causes inflammation through novel neoantigen generation mech

- SETD2 is required for proper intron splicing
- SETD2 loss causes intron retention

- Hypothesis: retained introns are translated into peptides, generating neoantigens, which cause inflammation, and drive an unfolded protein response

Measure effect of *SETD2* and *PBRM1* loss on immune exhaustion and neoantigen generation

Model *SETD2*/*PBRM1* loss on inflammation and Tcell biology/exhaustion



vs

IFNγ or TNFα/NF-κB effects on Tcell exhaustion

PBRM1, *SETD2* isogenic cell lines

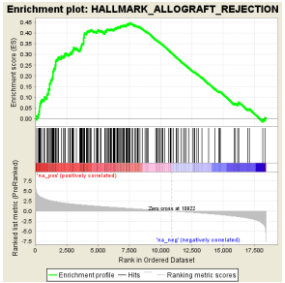
FACS

Immunoblot

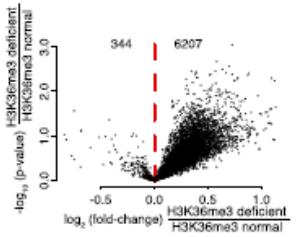
IFNγ outputs
PD-L1/2
MHC-I

RNAseq

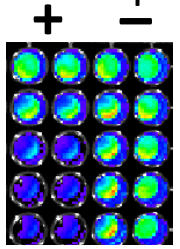
Intron retention

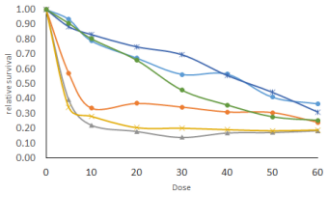


GSEA



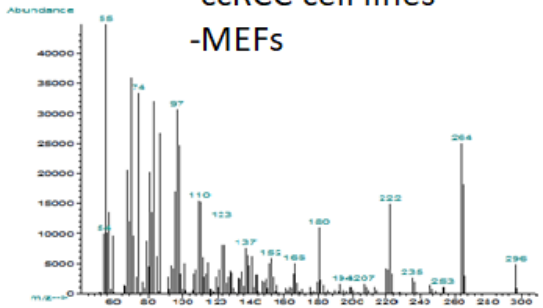
Therapeutic vulnerabilities (Unfolded protein resp)

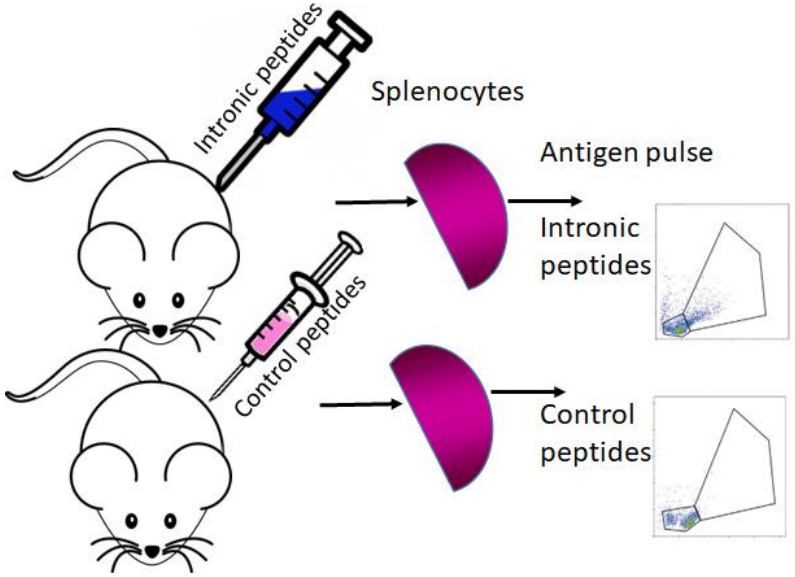




Determine if *SETD2* loss- induced intron retention generates immunogenic neoantigens

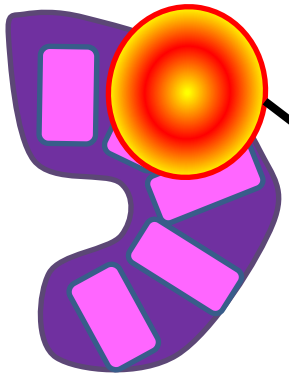
LC-MS/MS *SETD2*-isogenics
-ccRCC cell lines
-MEFs





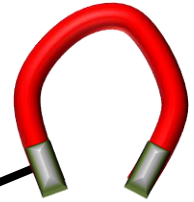
Aim2

Determine if *PBRM1* and *SETD2* mutations associate with exhaustion signatures in T cells in localized RCCs.



CD3-based magnet separation

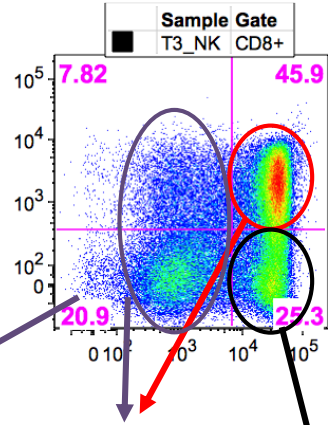
Surgery
Disaggregation



CD3$^+$ cells

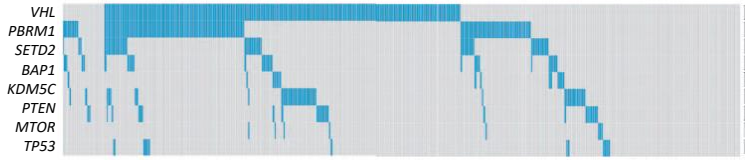
CD3$^-$ cells

FACS Deplete
CD31 (endothelial)
FAP (stroma)
CD45 (immune)



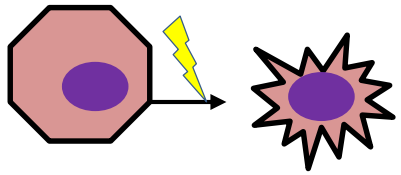
FACS Analyze
CD8$^+$T cells
Exhaustion
Activation
Ki-67/IFNγ

ccRCC cells

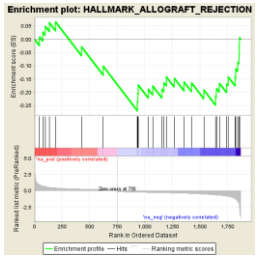


Targeted exome sequencing
Mutation group assignment

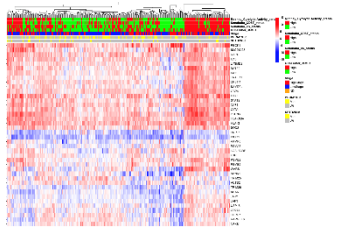
Ex vivo manipulation
Cytolysis assays
Reversibility?



RNA-seq
Authentication

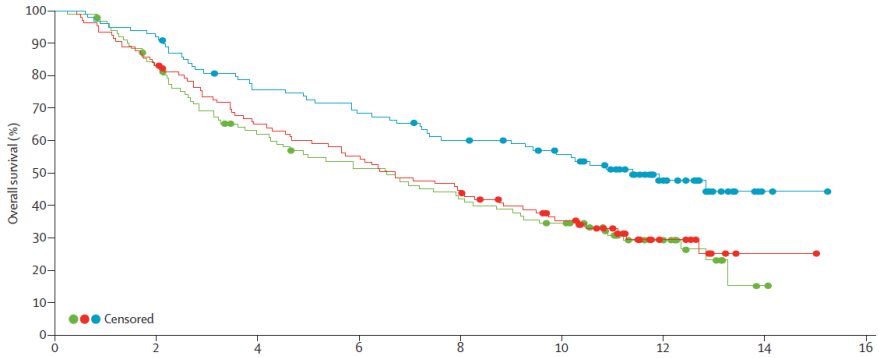


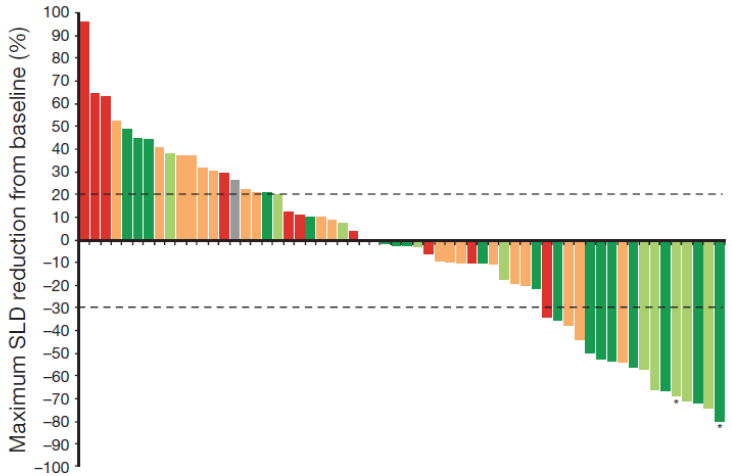
Mutant vs WT
GSEA & DESeq
Exhaustion signatures
Immune infiltrate



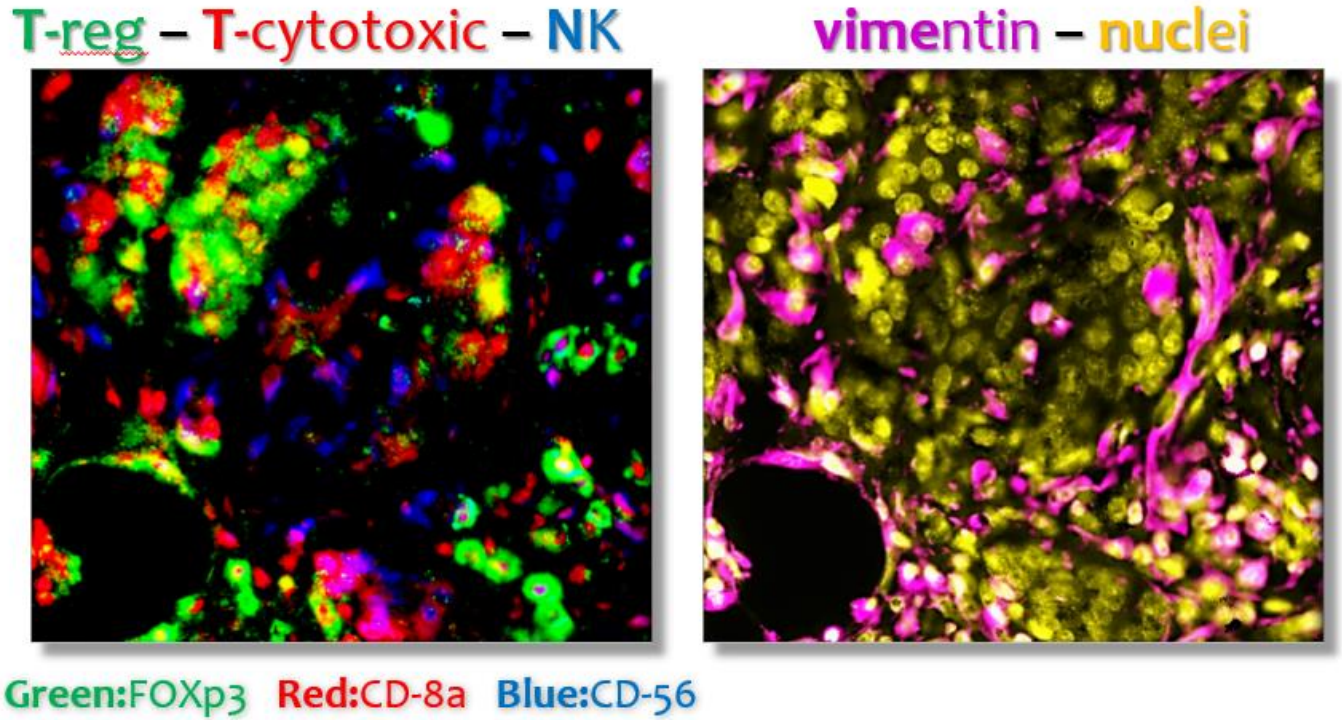
Aim 3: To determine if *PBRM1* and *SETD2* mutations are biomarkers of ICB outcomes

Correlation of mutation status with PFS, OS, ORR (NCT03172754, axi/nivo)



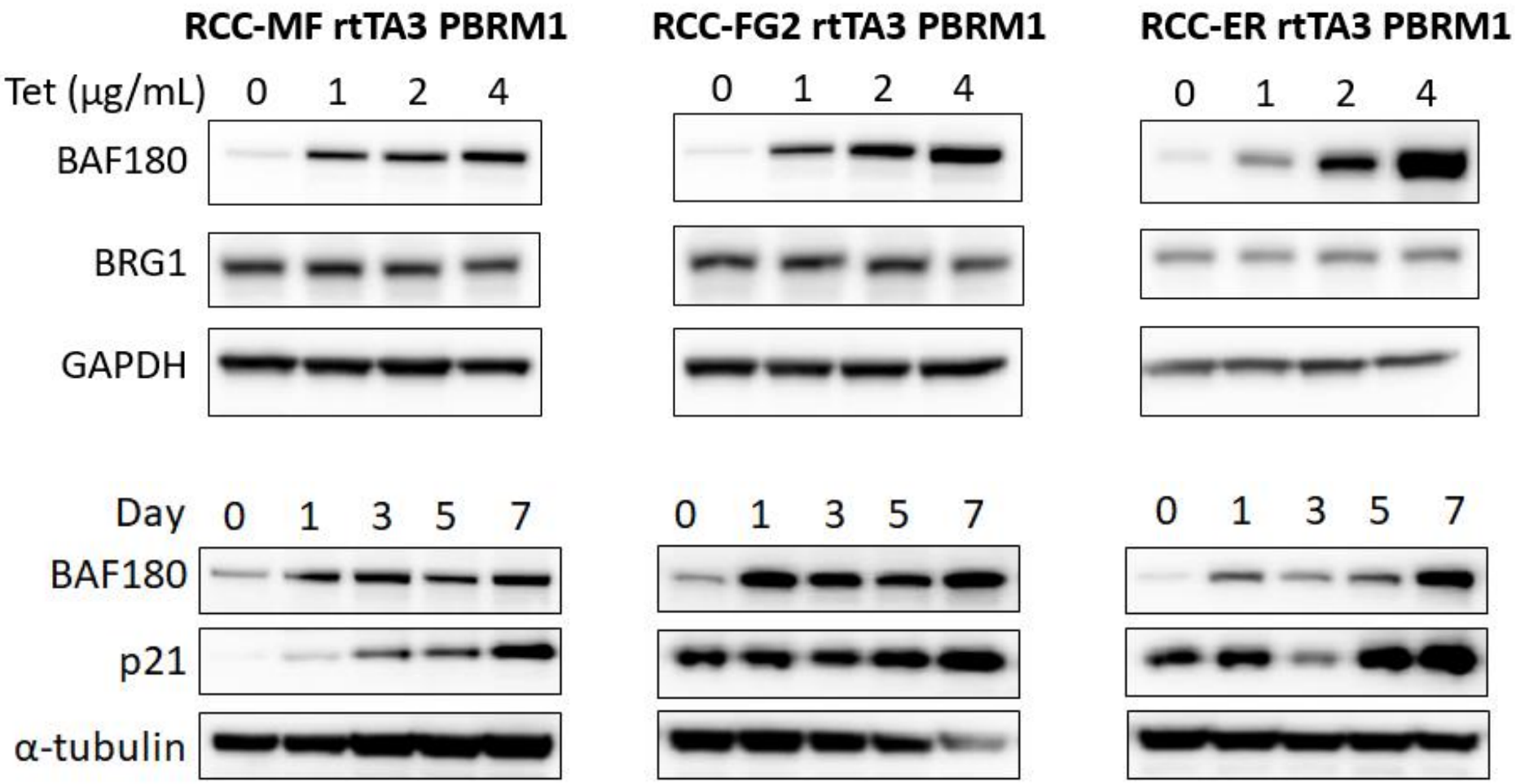


Correlation of mutation status with pre-treatment immune infiltration



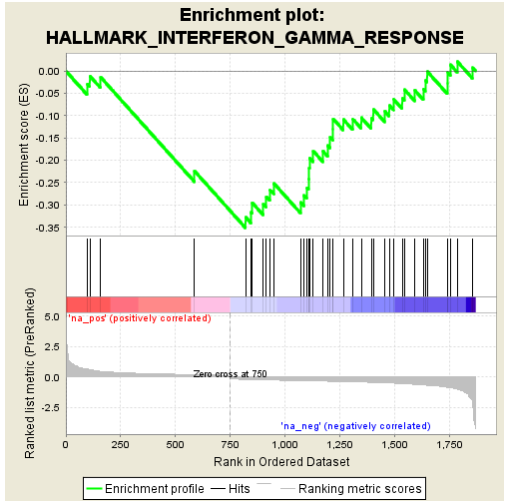
ADDITIONAL DATA IF QUESTIONS ARISE

PBRM1 reintroduction into PBRM1-/- cells reactivates PBRM1-target genes

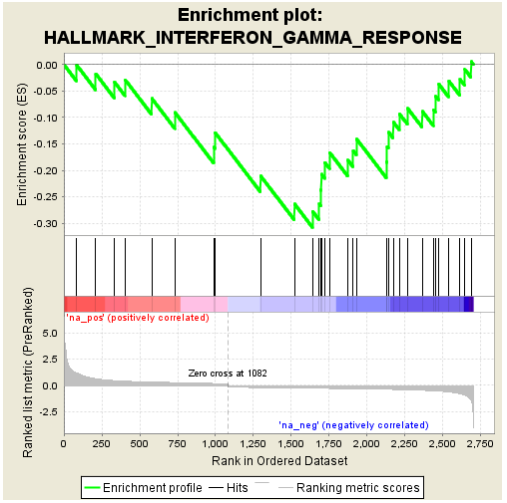


PBRM1 deficiency causes IFNγ and TNFα/NF-κB signaling

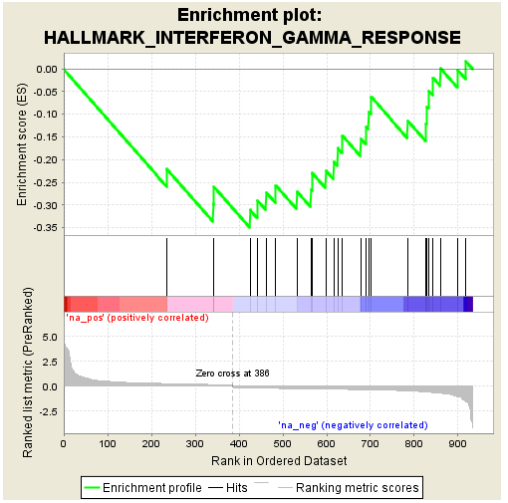
IFN- γ signaling



RCC-MF ± *PBRM1*
(N496del1bp)

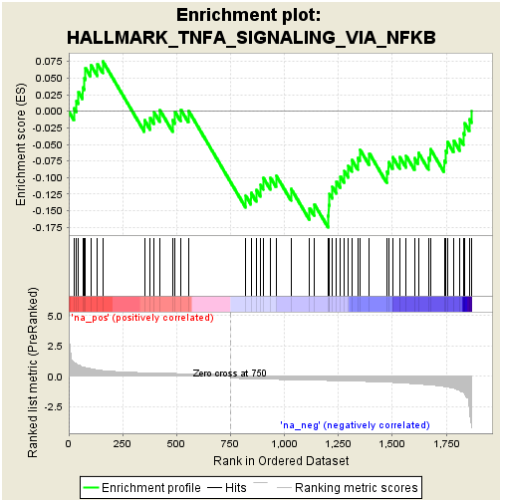


RCC-ER ± *PBRM1*
(D251del4bp)



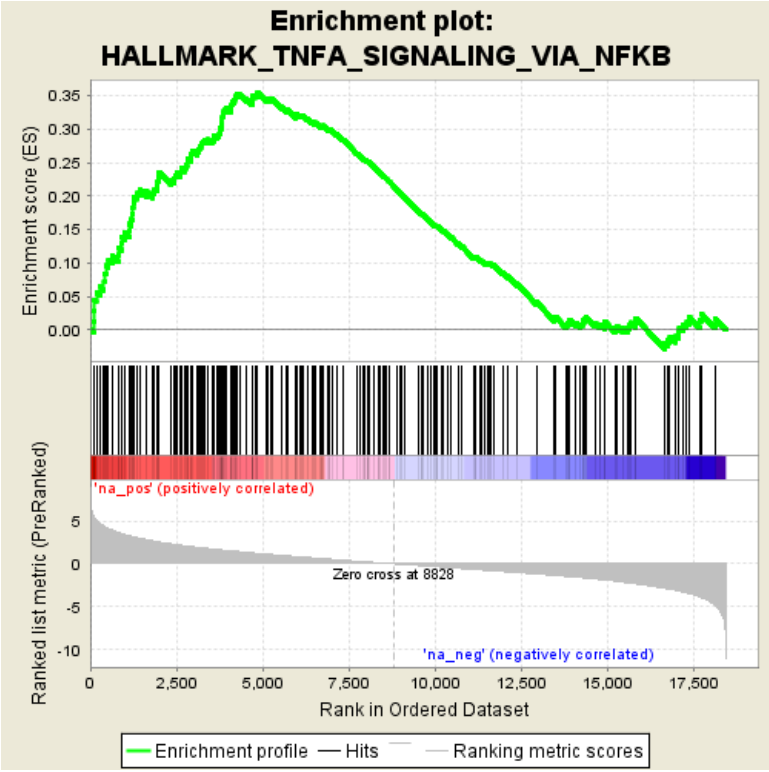
RCC-FG2 ± *PBRM1*
(Q445*)

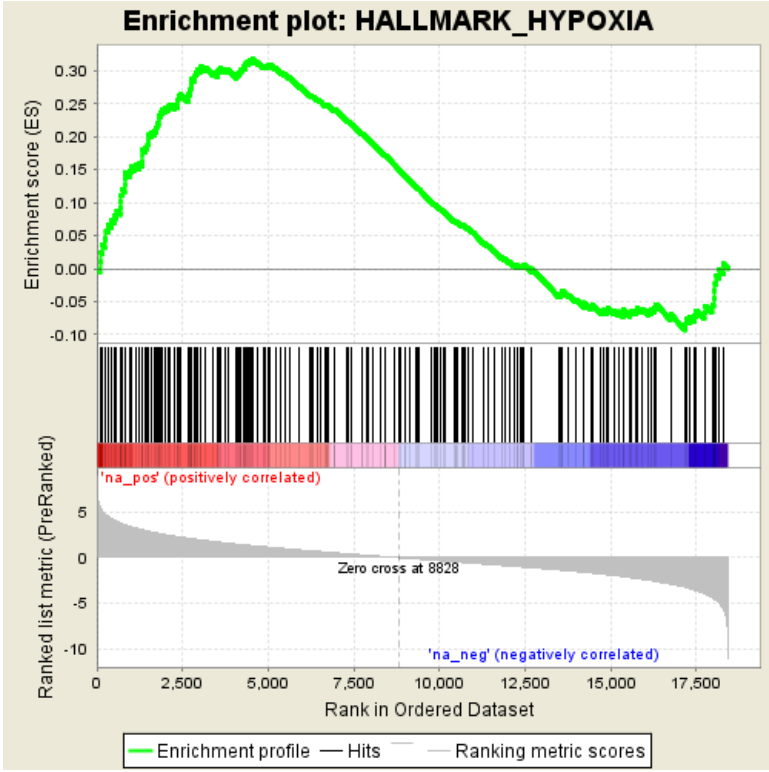
TNFα/NF-κB signaling



RCC-MF ± *PBRM1*
(N496del1bp)

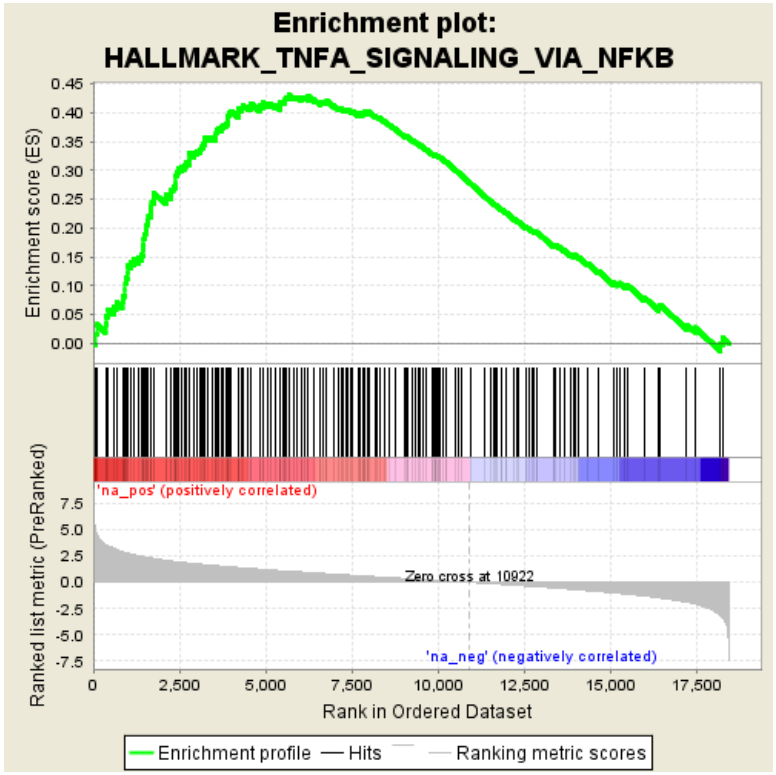
TNFα/NF-κB signaling is also present in *PBRM1* ccRCCs in TCGA

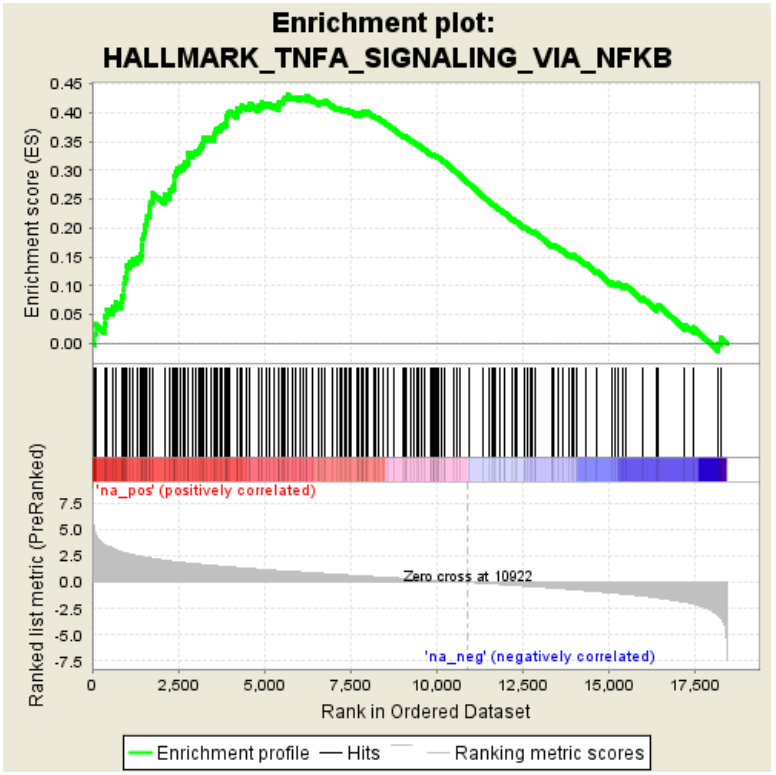




**Echoes work of Gao/Kaelin

Curiously, *SETD2* TCGA tumors also have high IFNγ and TNFα/NF-κB signatures





Also present and relevant:
Allograft rejection
Inflammatory response
IL6/JAK/STAT3 signaling
Unfolded protein response**