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Supplementary Materials for

PSMA redirects cell survival signaling from the MAPK to the PI3K-AKT pathways to promote the progression of prostate cancer

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Published 14 March 2017, *Sci. Signal.* **10**, eaag3326 (2017)
DOI: 10.1126/scisignal.aag3326

This PDF file includes:

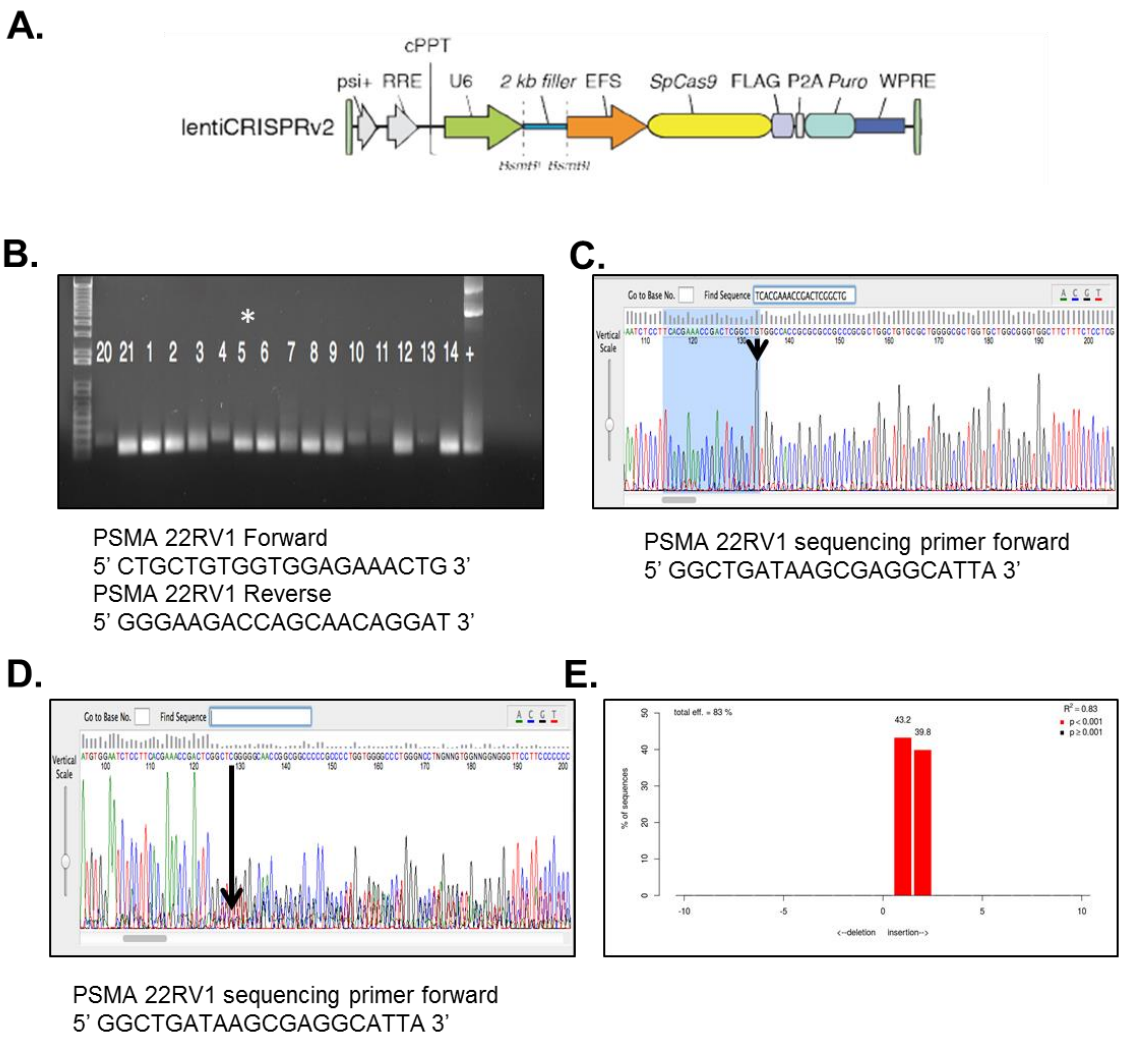

Fig. S1. CRISPR knockout of PSMA in 22RV1 cells. (A) LentiCRISPRv2 plasmid #52961 (Image from Addgene, Cambridge, MA with permission. The annealed gRNAs were cloned in at BSMB1. **(B)** PCR verification of transduction of the lentiCRISPRv2 containing the PSMA gRNA into cells. Clones 1-14, 10, 21 and positive control were all tested. A strong PCR band indicates that lenti-guide virus transduced cell. (**C and D)** Sanger sequencing of PCR products using a nested primer. Chromatographs of (C) 22RV1 wild-type and (D) 22RV1-CRISPR-PSMA^{KO} verified alteration of the genome as indicated by the black arrows. **(E)** Tracking of Indels by Decomposition (TIDE) analysis of 22RV1-CRISPR-PSMA^{KO} indicated a +1,+2 insertion on the 2 alleles (https://tide-calculator.nki.nl/). Data in panels D and E are from clone 5. This clone was used in all experiments.

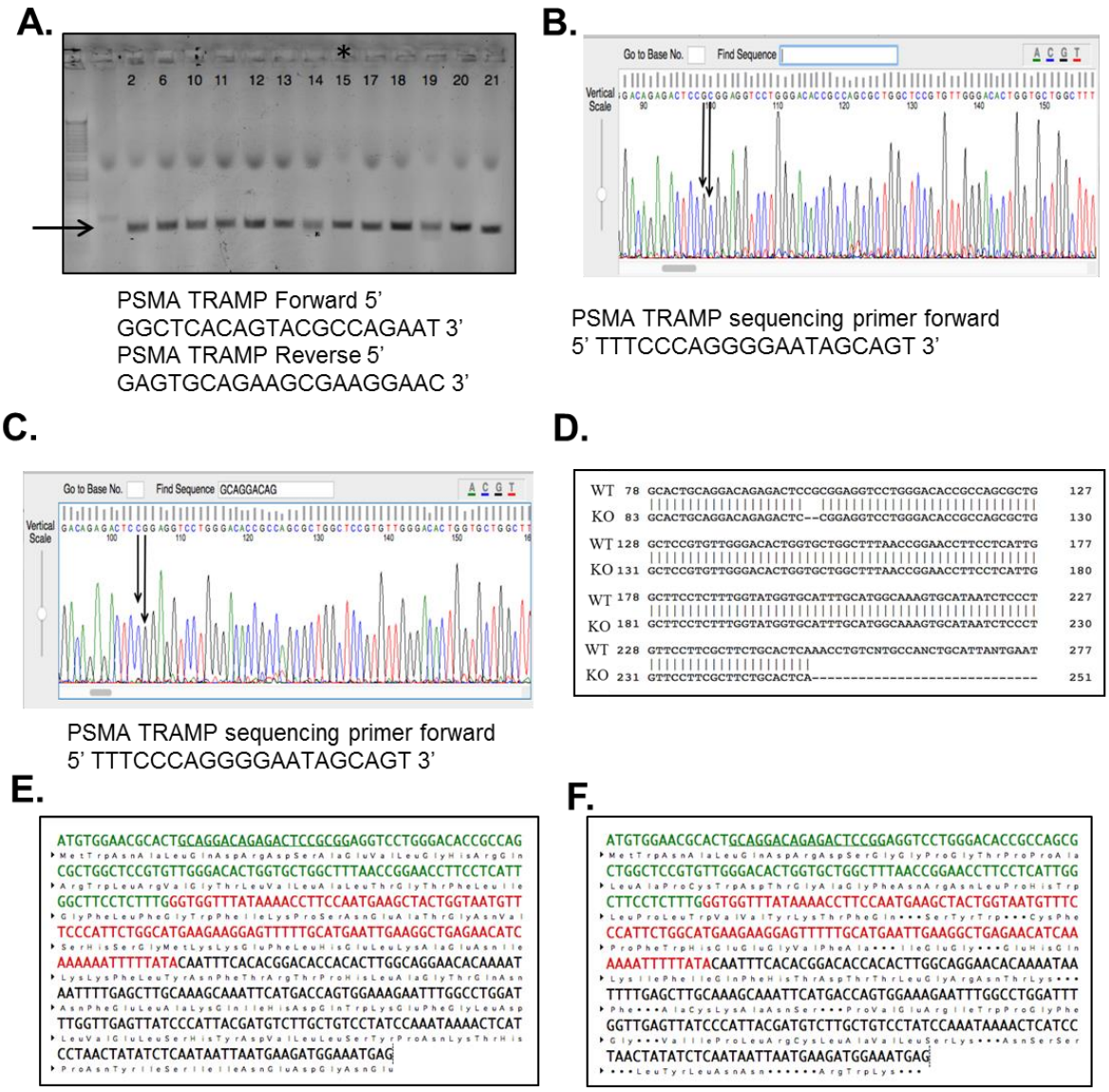

Fig. S2. CRISPR knockout of PSMA in TRAMP-C1 cells. (A) PCR verification of transduction of the lentiCRISPRv2 containing the PSMA gRNA into cells. Clones 2,5, 10-21 were all tested. A strong PCR band indicates that lenti-guide virus transduced cell (indicated by arrow)**. (B and C)** Sanger sequencing of PCR products using a nested primer. Chromatographs for (B) TRAMP wild-type and (C) TRAMP-CRISPR-PSMA^{KO} verified alteration of the genome as indicated by the dual black arrows. **(D)** Emboss pairwise sequence alignment (http://www.ebi.ac.uk/Tools/psa/) of TRAMP wild-type and of TRAMP-CRISPR-PSMA^{KO} reveals a 2bp frame shift mutation in the first exon leads to a premature stop codon in the second exon after splicing making a truncated protein**. (H and I)** Alteration in the protein was verified with the ExPasy Translation Tool (http://web.expasy.org/translate/). The gRNA is underlined. The deletion is in the first exon (green) after Ser and the stop codon appears in the second exon (red) after the 2 bp deletion. Data in panels B, C, D and F are from clone 15. This clone was used in all experiments.

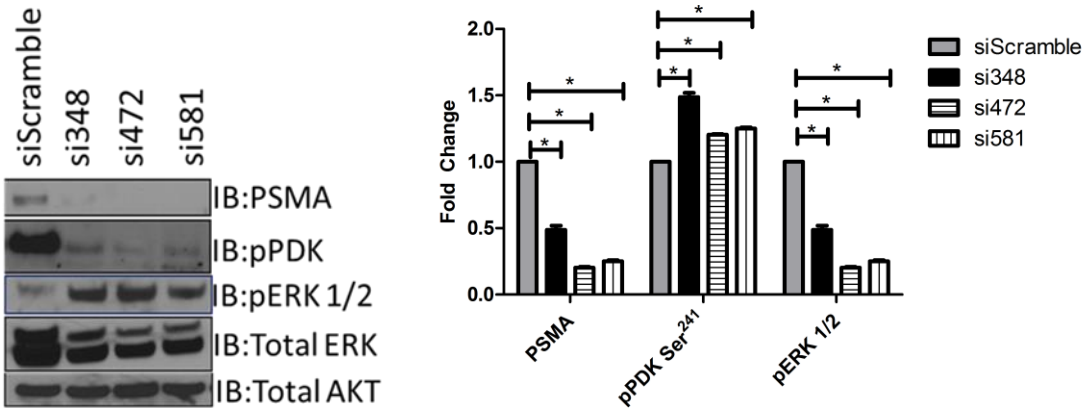

Fig. S3. PSMA knockdown increases the phosphorylation of ERK1/2 and decreases that of PDK in TRAMP-C1 cells. Western blotting for the indicated proteins in lysates from TRAMP-C1 cells transfected with one of three siRNAs against mouse PSMA or a control (siScramble). Blots are representative and data are mean ± SE of n=3 blots for each experimental condition, each with 3 experimental replicates normalized to either total ERK or AKT and presented as fold change relative to control. *$P < 0.05$, paired Student's *t*-test.

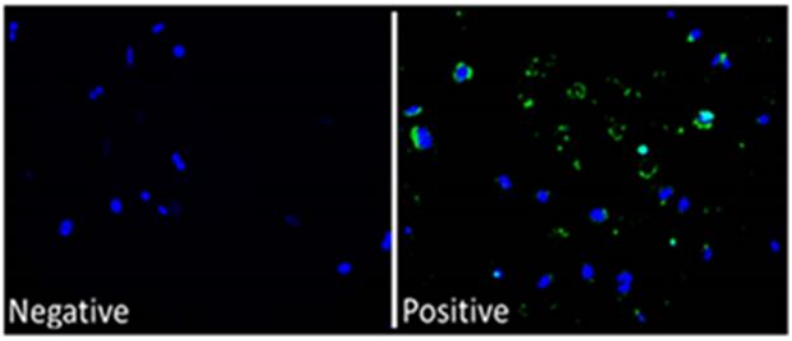

Fig. S4. Cotransfection of TRAMP-C1 cells with FITC and PSMA blocking peptide. Representative image of co-transfection of a positive control fluorescein isothiocyanate (FITC)-containing protein to ensure appropriate transfection efficiency of the PSMA scramble and blocking peptides in TRAMP-C1 cells (20X). Data are representative of n=3 for 3 experiments.

Data file S1. R script document for analysis of GEO data set GSE32571.

```
# Version info: R 3.2.3, Biobase 2.30.0, GEOquery 2.36.0, limma 3.26.8
# R scripts generated  Tue Apr 5 12:19:07 EDT 2016

################################################################
#   Differential expression analysis with limma
library(Biobase)
library(GEOquery)
library(limma)

# load series and platform data from GEO

gset <- getGEO("GSE32571", GSEMatrix =TRUE)
if (length(gset) > 1) idx <- grep("GPL6947", attr(gset, "names")) else
idx <- 1
gset <- gset[[idx]]

# make proper column names to match toptable
fvarLabels(gset) <- make.names(fvarLabels(gset))

# group names for all samples
gsms <- paste0("021020000121110110101020000011022211010101002121 10",
        "111120011000220112220010212221020210220122000220")
sml <- c()
for (i in 1:nchar(gsms)) { sml[i] <- substr(gsms,i,i) }

# log2 transform
ex <- exprs(gset)
qx <- as.numeric(quantile(ex, c(0., 0.25, 0.5, 0.75, 0.99, 1.0),
na.rm=T))
LogC <- (qx[5] > 100) ||
          (qx[6]-qx[1] > 50 && qx[2] > 0) ||
          (qx[2] > 0 && qx[2] < 1 && qx[4] > 1 && qx[4] < 2)
if (LogC) { ex[which(ex <= 0)] <- NaN
  exprs(gset) <- log2(ex) }

# set up the data and proceed with analysis
sml <- paste("G", sml, sep="")    # set group names
fl <- as.factor(sml)
gset$description <- fl
design <- model.matrix(~ description + 0, gset)
colnames(design) <- levels(fl)
fit <- lmFit(gset, design)
cont.matrix <- makeContrasts(G2-G0, G1-G0, G2-G1, levels=design)
fit2 <- contrasts.fit(fit, cont.matrix)
fit2 <- eBayes(fit2, 0.01)
tT <- topTable(fit2, adjust="fdr", sort.by="B", number=250)

# load NCBI platform annotation
gpl <- annotation(gset)
platf <- getGEO(gpl, AnnotGPL=TRUE)
ncbifd <- data.frame(attr(dataTable(platf), "table"))

# replace original platform annotation
tT <- tT[setdiff(colnames(tT), setdiff(fvarLabels(gset), "ID"))]
```

```
tT <- merge(tT, ncbifd, by="ID")
tT <- tT[order(tT$P.Value), ]  # restore correct order

tT <- subset(tT,
select=c("ID","adj.P.Val","P.Value","F","Gene.symbol","Gene.title"))
write.table(tT, file=stdout(), row.names=F, sep="\t")
########################################################
#   Boxplot for selected GEO samples
library(Biobase)
library(GEOquery)

# load series and platform data from GEO

gset <- getGEO("GSE32571", GSEMatrix =TRUE)
if (length(gset) > 1) idx <- grep("GPL6947", attr(gset, "names")) else
idx <- 1
gset <- gset[[idx]]

# group names for all samples in a series
gsms <- paste0("02102000012111011010102000001102221101010100212110",
        "11112001100022011222001021222102021022012200022O")
sml <- c()
for (i in 1:nchar(gsms)) { sml[i] <- substr(gsms,i,i) }
sml <- paste("G", sml, sep="")  set group names

# order samples by group
ex <- exprs(gset)[ , order(sml)]
sml <- sml[order(sml)]
fl <- as.factor(sml)
labels <- c("Benign","Tumor+Low+Gleason","Tumor+High+Gleason")

# set parameters and draw the plot
palette(c("#dfeaf4","#f4dfdf","#f2cb98", "#AABBCC"))
dev.new(width=4+dim(gset)[[2]]/5, height=6)
par(mar=c(2+round(max(nchar(sampleNames(gset)))/2),4,2,1))
title <- paste ("GSE32571", '/', annotation(gset), " selected samples",
sep ='')
boxplot(ex, boxwex=0.6, notch=T, main=title, outline=FALSE, las=2,
col=fl)
legend("topleft", labels, fill=palette(), bty="n")
```