Supplementary Information

Mapping of m^{6}A and Its Regulatory Targets in Prostate Cancer Reveals a METTL3-low Induction of Therapy Resistance

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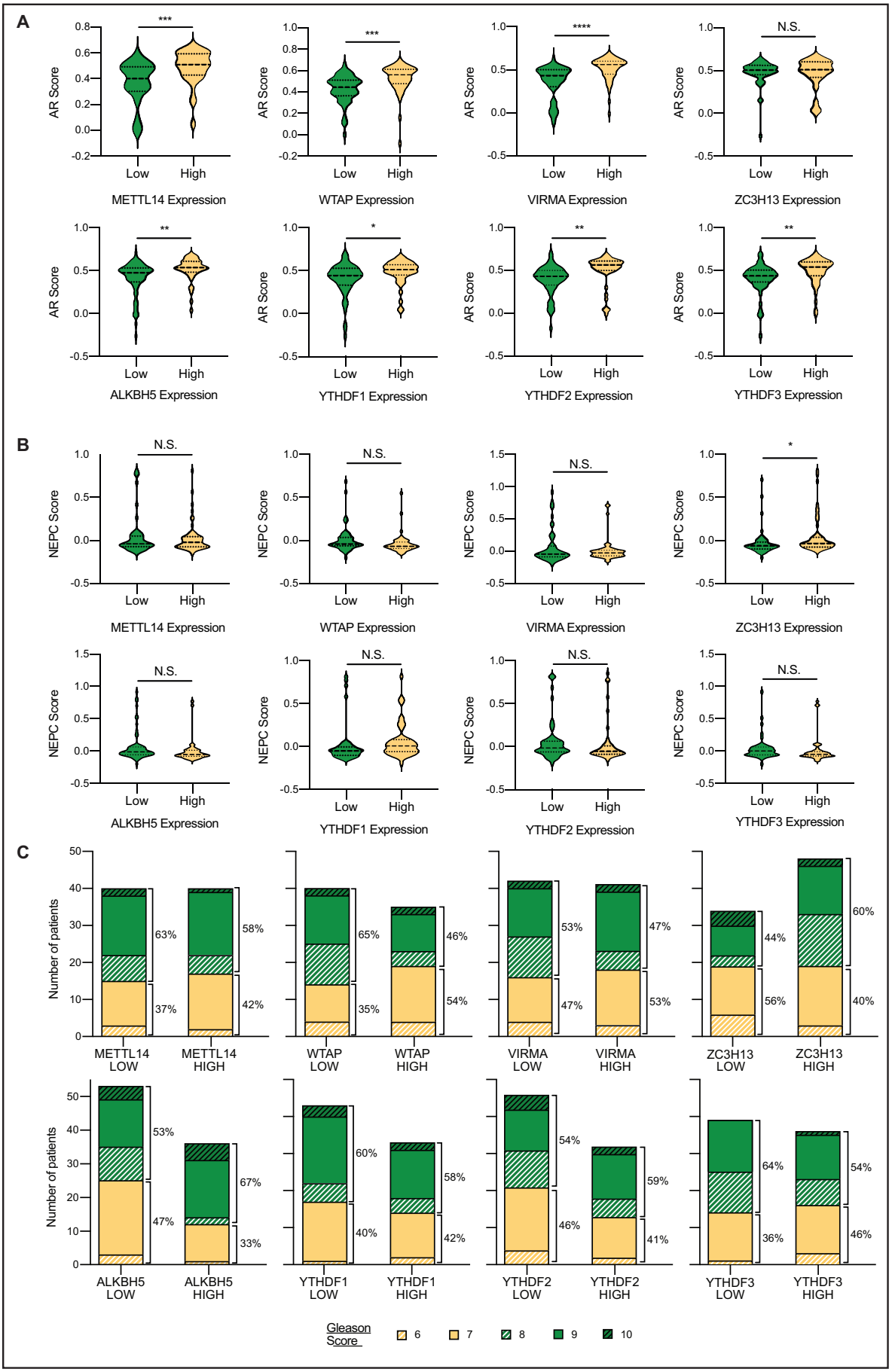
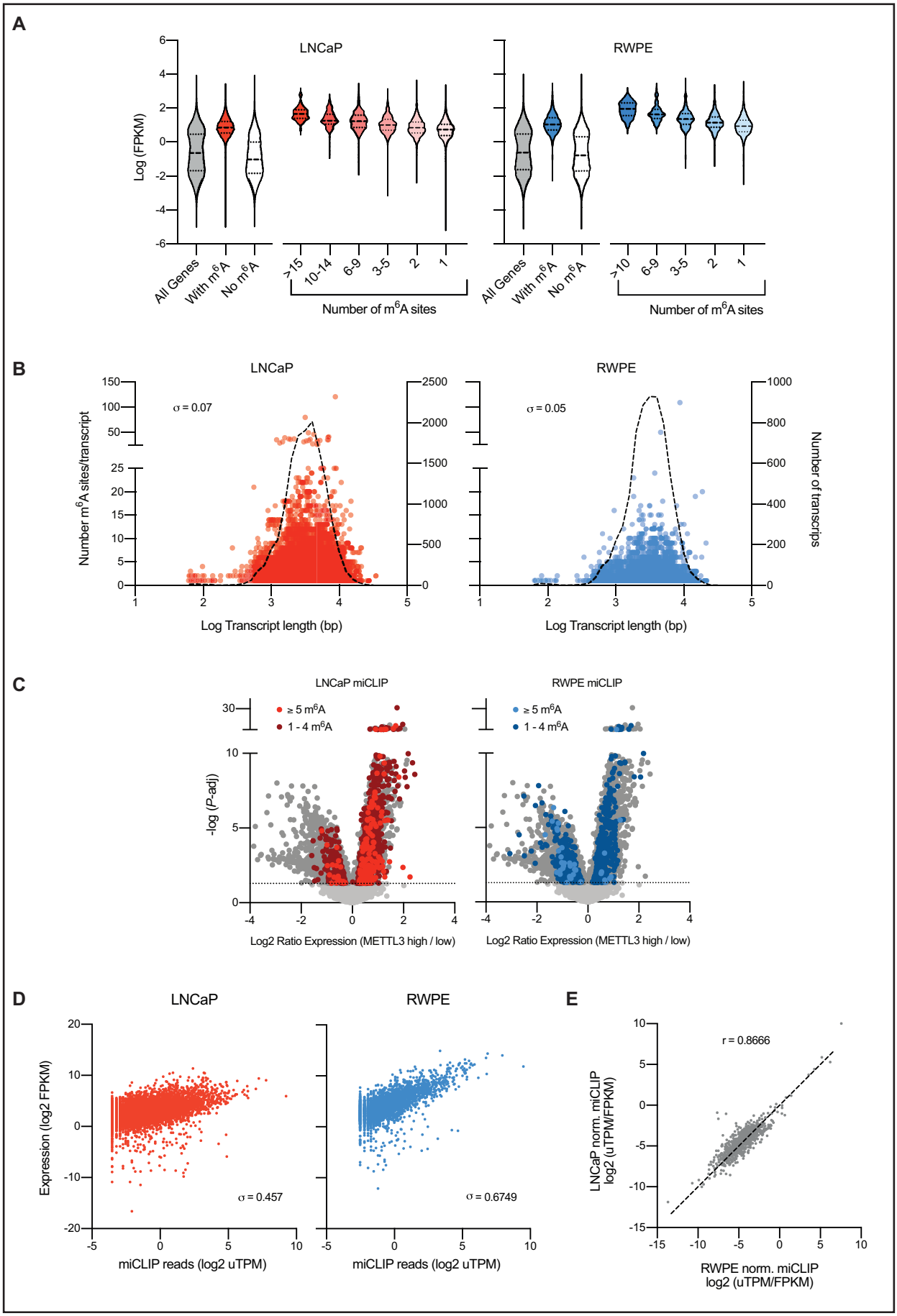
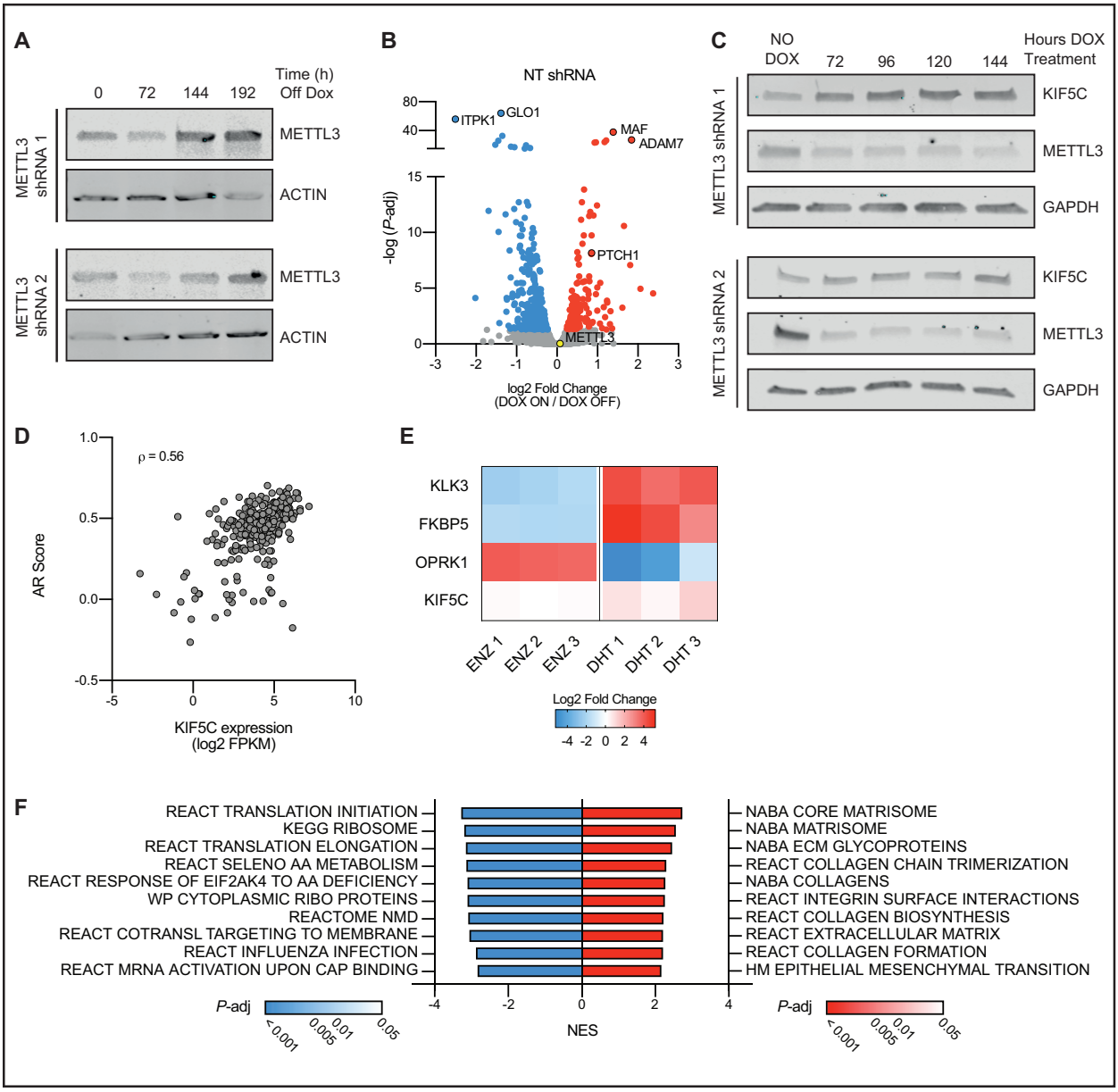

A
AR Score
***
***
****
N.S.
**
*
**
**
Low
High
METTL14 Expression
WTAP Expression
VIRMA Expression
ZC3H13 Expression
ALKBH5 Expression
YTHDF1 Expression
YTHDF2 Expression
YTHDF3 Expression
B
NEPC Score
N.S.
*
C
Number of patients
63%
37%
58%
42%
65%
35%
46%
54%
53%
47%
47%
53%
44%
56%
60%
40%
METTL14 LOW
METTL14 HIGH
WTAP LOW
WTAP HIGH
VIRMA LOW
VIRMA HIGH
ZC3H13 LOW
ZC3H13 HIGH
53%
47%
67%
33%
60%
40%
58%
42%
54%
46%
59%
41%
64%
36%
54%
46%
ALKBH5 LOW
ALKBH5 HIGH
YTHDF1 LOW
YTHDF1 HIGH
YTHDF2 LOW
YTHDF2 HIGH
YTHDF3 LOW
YTHDF3 HIGH
Gleason Score
6
7
8
9
10

Supplementary Figure 1. Association between m^{6}A "writer", "eraser", and "reader" proteins and markers of advanced-stage disease. A) Castration resistant prostate cancer samples[31] were grouped based on *METTL14*, *WTAP*, *VIRMA*, *ZC3H13*, *ALKBH5*, *YTHDF1*, *YTHDF2*, or *YTHDF3* expression: low, z-score > 1, n = 46, 50, 52, 48, 41, 42, 58, and 48 respectively; high, z-score < -1, n = 57, 50, 55, 52, 66, 53, 45, and 45 respectively. **A)** AR score in high vs. low samples. Median and quartiles are indicated with a dotted line, statistical significance is indicated as determined by t-test. **B)** NEPC score in in high vs. low samples. Median and quartiles are indicated with a dotted line, statistical significance is indicated as determined by t-test. **C)** Gleason score in high vs. low samples. No statistically significant differences were detected by Fischer's exact test between samples with Gleason 6-7 and Gleason 8+ scores.

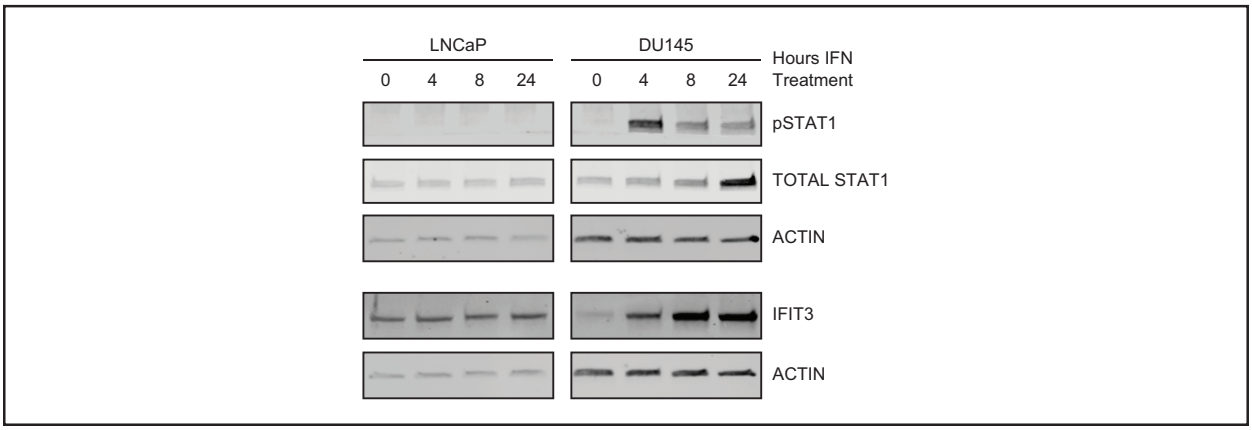

A
LNCaP
RWPE
Log (FPKM)
All Genes
With m6A
No m6A
>15
10-14
6-9
3-5
2
1
>10
Number of m6A sites
B
LNCaP
RWPE
σ = 0.07
σ = 0.05
Number m6A sites/transcript
Number of transcrips
Log Transcript length (bp)
C
LNCaP miCLIP
RWPE miCLIP
≥ 5 m6A
1 - 4 m6A
-log (P-adj)
Log2 Ratio Expression (METTL3 high / low)
D
LNCaP
RWPE
Expression (log2 FPKM)
σ = 0.457
σ = 0.6749
miCLIP reads (log2 uTPM)
E
r = 0.8666
LNCaP norm. miCLIP
log2 (uTPM/FPKM)
RWPE norm. miCLIP
log2 (uTPM/FPKM)

Supplementary Figure 2. Relation of miCLIP results to transcript length and expression. **A)** Comparing the number of m^6A sites per transcript to the transcript expression as determined by paired RNA-Seq. **B)** Comparing the number of m^6A sites per transcript (circles, plotted on the left y-axis) to the transcript length shows no correlation (Spearman). The distribution is similar to the overall number of transcripts of a given length (dotted line, plotted on the right y-axis). **C)** Comparing the number of m^6A sites per transcript (colored dots) to the changes in transcript expression detected between METTL3-high and -low patient samples (fold difference on x-axis and significance on y-axis), **D)** Normalizing m^6A levels per transcript (unique tags per million, uTPM) by the transcript expression from RNA-seq (FPKM). **E)** Correlation between the normalized m^6A levels per site (uTPM/FPKM) for the sites common to both LNCaP and RWPE cells lines (n = 889) (Pearson r = 0.937).

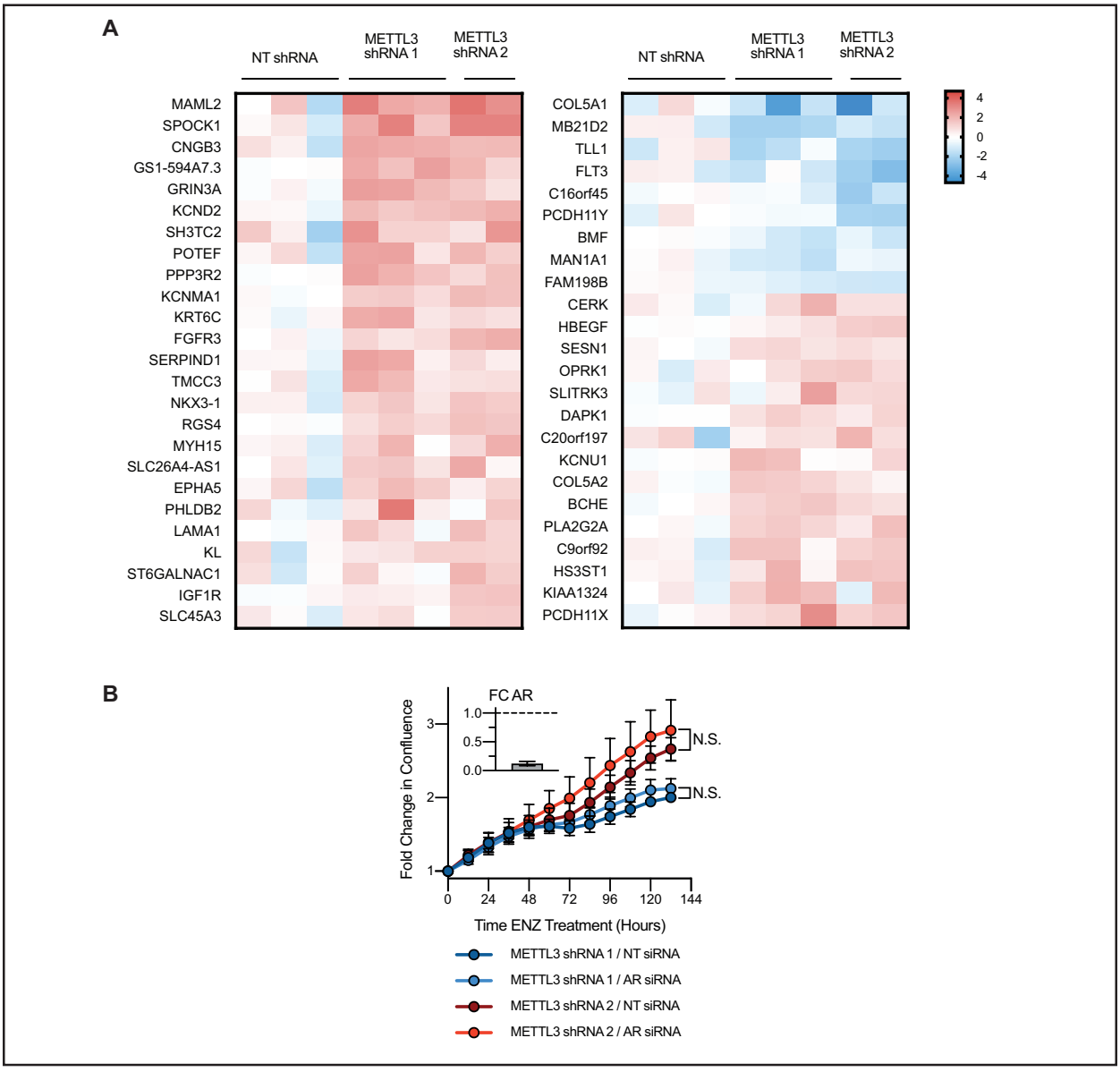


Supplementary Figure 3. Reversible knockdown of *METTL3* in LNCaP cells upregulates KIF5C. A) Doxycycline induction of *METTL3* shRNA is reversible. Cells were treated with doxycycline for 96 hours, and then changed to media without doxycycline for the indicated amount of time followed by Western blot analysis for METTL3. **B)** Significantly (adjusted *P*-value < 0.05) differentially expressed genes with induction of the NT shRNA (n = 3). Common genes also induced in the two *METTL3* shRNA lines are highlighted with a black border. **C)** Western blot analysis of the upregulation of KIF5C with METTL3 knockdown. Cells were treated with

doxycycline for the indicated amount of time. **D)** *KIF5C* expression is correlated with AR score in castration resistant prostate cancer samples[31] (n = 264) (Spearman's ρ = 0.56). **E)** Fold change in expression of the *KIF5C* and the AR-regulated genes *KLK3, FKBP5* and *OPRK1* with DHT or ENZ treatment of LNCaP cells in published RNA-seq datasets (GSE110903, GSE147250, GSE135879, GSE130534, GSE115395). **F)** Gene set enrichment analysis of genes ranked by TE as determined by Riborex analysis of Ribo-seq data.

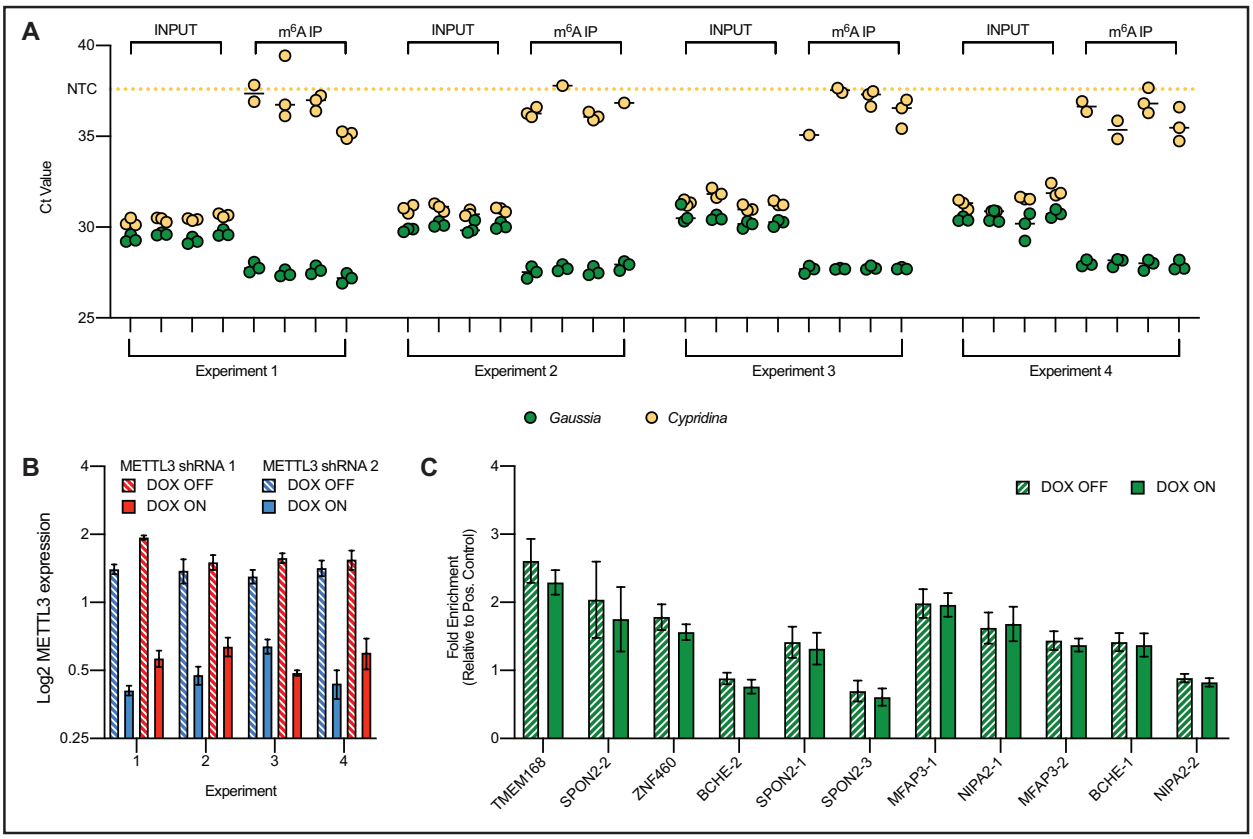


Supplementary Figure 4. LNCaP cells do not respond to IFN. Western blot analysis of IFN response as determined by the upregulation of STAT1 and IFIT3 in LNCaP and DU145 cells in response to IFN-α treatment.



Supplementary Figure 5. A subset of ENZ-regulated genes are differentially expressed with METTL3 knockdown and ENZ resistance is AR-independent. A) Data is expressed as the fold-change over NT shRNA for all three lines with 24 hours of 10 μM ENZ treatment. On the left and right are genes down-and up-regulated by ENZ respectively. **B)** Dual knockdown of *METTL3* and *AR* does not change the ability of the cells to proliferate in the presence of 10 μM ENZ (n = 3, error bars = SEM). Plotted is the fold change in confluency compared to time zero.

Sufficient *AR* knockdown (88%) was confirmed in tandem by qPCR and is plotted in the top left as fold change over NT siRNA (n = 3).



Supplementary Figure 6. m^{6}A-immunoprecipitation and qPCR of selected candidate genes. A) Levels of spike-in control luciferase mRNAs in both input (5%) and IP pools for 4 independent experiments. The positive control *Gaussia* mRNA is synthesized to contain m^{6}A while the negative control *Cypridina* mRNA contains no m^{6}A. Plotted are the triplicate Ct values as determined by qPCR. In all experiments the levels *Cypridina* mRNA in the IP fractions are approximate to those seen in no template control reactions indicating no enrichment. **B)** Changes in the expression of *METTL3* in each experiment in response to doxycycline as measured by qPCR. Error bars indicate technical triplicates. **C)** Enrichment with m^{6}A-IP over input for selected m^{6}A sites on candidate genes normalized to the *Gaussia* luciferase positive control mRNA in response to *METTL3* knockdown (n = 8).

Supplementary Table 1. Oligonucleotide Sequences

qPCR	GAPDH	Fwd	GAC AGT CAG CCG CAT CTT CT
qPCR	GAPDH	Rev	TTA AAA GCA GCC CTG GTG AC
qPCR	HMBS	Fwd	AGC TTG CTC GCA TAC AGA CG
qPCR	HMBS	Rev	AGC TCC TTG GTA AAC AGG CTT
qPCR	ACTB	Fwd	TGA CGT GGA CAT CCG CAA AG
qPCR	ACTB	Rev	CTG GAA GGT GGA CAG CGA GG
qPCR	METTL3	Fwd	TTG TCT CCA ACC TTC CGT AGT
qPCR	METTL3	Rev	CCA GAT CAG AGA GGT GTA G
qPCR	TMPRSS2	Fwd	GTC CCC ACT GTC TAC GAG GT
qPCR	TMPRSS2	Rev	CAG ACG ACG GGG TTG GAA G
qPCR	KLK3	Fwd	TGG GGA CCA CCT GCT ACG CC
qPCR	KLK3	Rev	TCG GTG ATC AGA ATG ACC CAC GAG
qPCR	FKBP5	Fwd	GCA ACA GTA GAA ATC CAC CTG
qPCR	FKBP5	Rev	CTC CAG AGC TTT GTC AAT TCC
qPCR	NKX3.1	Fwd	CCC ACA CTC AGG TGA TCG AG
qPCR	NKX3.1	Rev	GAG CTG CTT TCG CTT AGT CTT
qPCR	AR	Fwd	GTG TCA AAA GCG AAA TGG GC
qPCR	AR	Rev	GCT TCA TCT CCA CAG ATC AGG
qPCR	NR5A2	Fwd	CTT TGT CCC GTG TGT GGA GAT
qPCR	NR5A2	Rev	GTC GGC CCT TAC AGC TTC TA
M6A IP qPCR	SPON2_1	Fwd	AAG AAG AGG CTG AGT GCG TC
M6A IP qPCR	SPON2_1	Rev	TTC AGT GCA GAG ATG GTC GG
M6A IP qPCR	SPON2_2	Fwd	CAA GAG CAG GAC TCG CTA CG
M6A IP qPCR	SPON2_2	Rev	AGA CGC AGT TAT CAG GGA CG
M6A IP qPCR	SPON2_4	Fwd	CTC CCA CGT GGT TGC AGA TA
M6A IP qPCR	SPON2_4	Rev	TCC GAA ACC GCC CCA TTT AT
M6A IP qPCR	MFAP3_1	Fwd	CAG AGT CTC AAG GCA GCA GTC
M6A IP qPCR	MFAP3_1	Rev	GCT GAC AGT TTT CAT ATG CCC C
M6A IP qPCR	MFAP3_2	Fwd	AGC CCC AGA TAA ACA TGA TGG G
M6A IP qPCR	MFAP3_2	Rev	ACC AGA AGA GAA ACC ATG GGA C
M6A IP qPCR	ZNF460	Fwd	CAT CTC TGT GGG AAA ACC TGT
M6A IP qPCR	ZNF460	Rev	CCA CTG GGT CTC TTT CAT GGG
M6A IP qPCR	TMEM168	Fwd	AGT GCA AGG AGC AGA GTT GA
M6A IP qPCR	TMEM168	Rev	TGC GTC CCT TTT CAG TCC AG
M6A IP qPCR	BCHE_1	Fwd	TCC AGG AGT GAG TGA GTT TGG
M6A IP qPCR	BCHE_1	Rev	AGG CCT CAC GGT AGT TTT CAG
M6A IP qPCR	BCHE_2	Fwd	GGA AAG CAG GAT TCC ATC GC
M6A IP qPCR	BCHE_2	Rev	ACC CAC ACA ACT TTC TTT CTT GC
M6A IP qPCR	NIPA_1	Fwd	GGA GTG GCA AGA TAT GCC TGT
M6A IP qPCR	NIPA_1	Rev	TGC CAT TCA TTG CTT TCT CGT
M6A IP qPCR	NIPA_2	Fwd	AGG AGT GGC AAG ATA TGC CT
M6A IP qPCR	NIPA_2	Rev	GGC ATG CAA CAA GAA TAT CCC C
shRNA	METTL3 1	Fwd	CCGGGCAAGTATGTTCACTATGAAACTCGAGTTTCATAG TGAACATACTTGCTTTTTG
shRNA	METTL3 1	Rev	AATTCAAAAAGCAAGTATGTTCACTATGAAACTCGAGTTT CATAGTGAACATACTTGC

shRNA	METTL3 2	Fwd	CCGGGCTGCACTTCAGACGAATTATCTCGAGATAATTCG TCTGAAGTGCAGCTTTTTG
shRNA	METTL3 2	Rev	AATTCAAAAAGCTGCACTTCAGACGAATTATCTCGAGATA ATTCGTCTGAAGTGCAGC
shRNA	GFP	Fwd	CCGGTACAACAGCCACAACGTCTATCTCGAGATAGACGT TGTGGCTGTTGTATTTTTG
shRNA	GFP	Rev	AATTCAAAAATACAACAGCCACAACGTCTATCTCGAGATA GACGTTGTGGCTGTTGTA