SUPPLEMENTAL FIGURES

Figure S1. KO cells were validated by DNA sequencing. WT allele is indicated with the guide DNA sequence. Deletion or insertion in each clone is shown below the WT allele.

Figure S2. The correlation of TNKS and RNF146 based on published data. (A) The correlation of TNKS and RNF146 in normal (up plot) and tumor (down plot) tissue based TCGA mRNA calculated in GEPIA2. (B) The protein expression of RNF146 (up plot) and TNKS (down plot) in normal and tumor tissue based on CPTAC data calculated in UALCAN. (C) the cell growth curve of cells used in this study.

Figure S3. The protein changed by XAV939 treatment. (A and B) Volcano plot shows the significant change in differentially expressed proteins. Each dot represents a protein. The brown and olive dots indicate significantly up-regulated and down-regulated proteins, respectively. The known substrates of TNKS1/2 are labeled with gene names. Y axis is the –Log p-value, and X axis is the Log2 fold change. Tests were performed using a t-test filtered by p-value ≤ 0.01, a fold change cut-off of 2 and unique peptide ≥ 2. (C) Biological process enrichment analysis by Metascape for proteins upregulated by XAV939 treatment. (D) The protein upregulated by XAV939 treatment were compared with high-confidence predicted substrates of TNKS1/2 from Guettler et al's data [25] and high-confidence interaction proteins from Xu et al's data [26].

Figure S4. Double knockout of TNKS1/2 significantly changed the proteome compared with XAV939 treatment. (*A* and *B*) the correlation of protein change between TP53TNKS1/2TKO vs TP53KO and TP53TNKSTKO+XAV939 vs TP53KO or TP53TNKS1/2TKO vs TP53KO+XAV939. Each dot represents a protein. The brown and olive dots indicate significantly up-regulated and down-regulated proteins in TP53TNKS1/2TKO vs TP53KO, respectively. The known substrates of TNKS1/2 and validated proteins were labeled with gene names. (C) Proteins upregulated by double knockout of TNKS1/2 were compared with high-

confidence predicted substrates of TNKS1/2 from Guettler et al's data [25] and high-confidence interaction proteins from Xu et al's data [26]. (D) Immunoblot assay of whole cell extracts for NFATC2 in 293A and RNF146KO cells plus XAV939 treatment (10 μM, 24 h). TNKS1/2 was used as positive controls, and β-actin was used as the loading control. (E) The correlation between the quantified proteome and transcriptome changes of each gene product in TP53TNKS1/2TKO vs TP53KO. The brown and olive dots indicate significantly up-regulated and down-regulated proteins in TP53TNKS1/2TKO vs TP53KO, respectively. The cross dot means gene products with adjusted p value≤0.01 in transcriptome analysis. The dash line shows the cutoff of fold change 2 in transcriptome analysis. The known substrates of TNKS1/2, proteins validated by WB in this study and proteins with significant up-regulation in transcriptome were labeled with gene names.

Figure S5. (A) Proteins upregulated by knockout of RNF146 were compared proteins upregulated by double knockout of TNKS1/2 and proteins upregulated by XAV939 treatment. (*B*) Protein levels of OTUD5 treated with XAV939 (TNKSi) or olaparib (PARP1 inhibitor) in different cells as indicated. (C) The correlation between the quantified proteome and transcriptome changes of each gene product in RNF146KO vs 293A. The brown and olive dots indicate significantly up-regulated and down-regulated proteins in RNF146KO vs 293A, respectively. The cross dot means gene products with adjusted p value≤0.01 in transcriptome analysis. The dash line shows the cutoff of fold change 2 in transcriptome analysis. The known substrates of RNF146, proteins validated by WB in this study and proteins with significantly up-regulated in transcriptome were labeled with gene names.

Figure S1

293A_TP53 KO cell

TP53 sgRNA: GGTAAAGAGGCTGGTGGACG

```
WT genome    TTCCTGAAAACAACGTTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG
KO allele#1  TTCCTGAA-------TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG
KO allele#2  TTCCTGAAAACAACG----------------------------------GGCTGG
```

293A_RNF146 KO cell

RNF146 sgRNA: TGAGCGCACTAGTAGAGAGC

```
WT genome    AATGGGTGGTGGCAGTACGATGAGCGCACTAGTAGAGAGCTGGAAGATGCTTTTTCCAAA
KO genome    AATGGGTGGTGGCAGTACGATGAGCGCACTAGT--AGAGCTGGAAGATGCTTTTTCCAAA
```

293A_TP53TNKS1/2 TKO cell

TNKS sgRNA: GGTAAAGAGGCTGGTGGACG

```
WT genome    GGGTAAAGAGGCTGGTGG   ACGCGGCAAACGTAAATGCA
KO allele#1  GGGTAAAGAGACTGGTGGG  ACGCGGCAAACGTAAATGCA (+1nt and one mutation)
KO allele#2  GGGTAAAGAGACTGGTGGG..ACGCGGCAAACGTAAATGCA (+169nt and one mutation)
```

TNKS2 sgRNA: TCCAATTATCTCGAGCATTG

```
WT genome    ATGGTGCAGACCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAATTAAA
KO allele#1  ATGGTGCAGACCCCAATGCTCGA--TAATTGGA----TA-TCC---------AGCTGCAATTAAA
KO allele#2  ATGGTGCAGACCCCAATGCTC----TAATTGGA----TA-TCCTCTCCATGAAGCTGCAATTAAA
```

TP53 sgRNA: GGTAAAGAGGCTGGTGGACG

```
WT genome    TTCCTGAAAACAACGTTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG
KO allele#1  TTCCTGAA-------TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG
KO allele#2  TTCCTGAAAACAACG----------------------------------GGCTGG
```

Figure S2

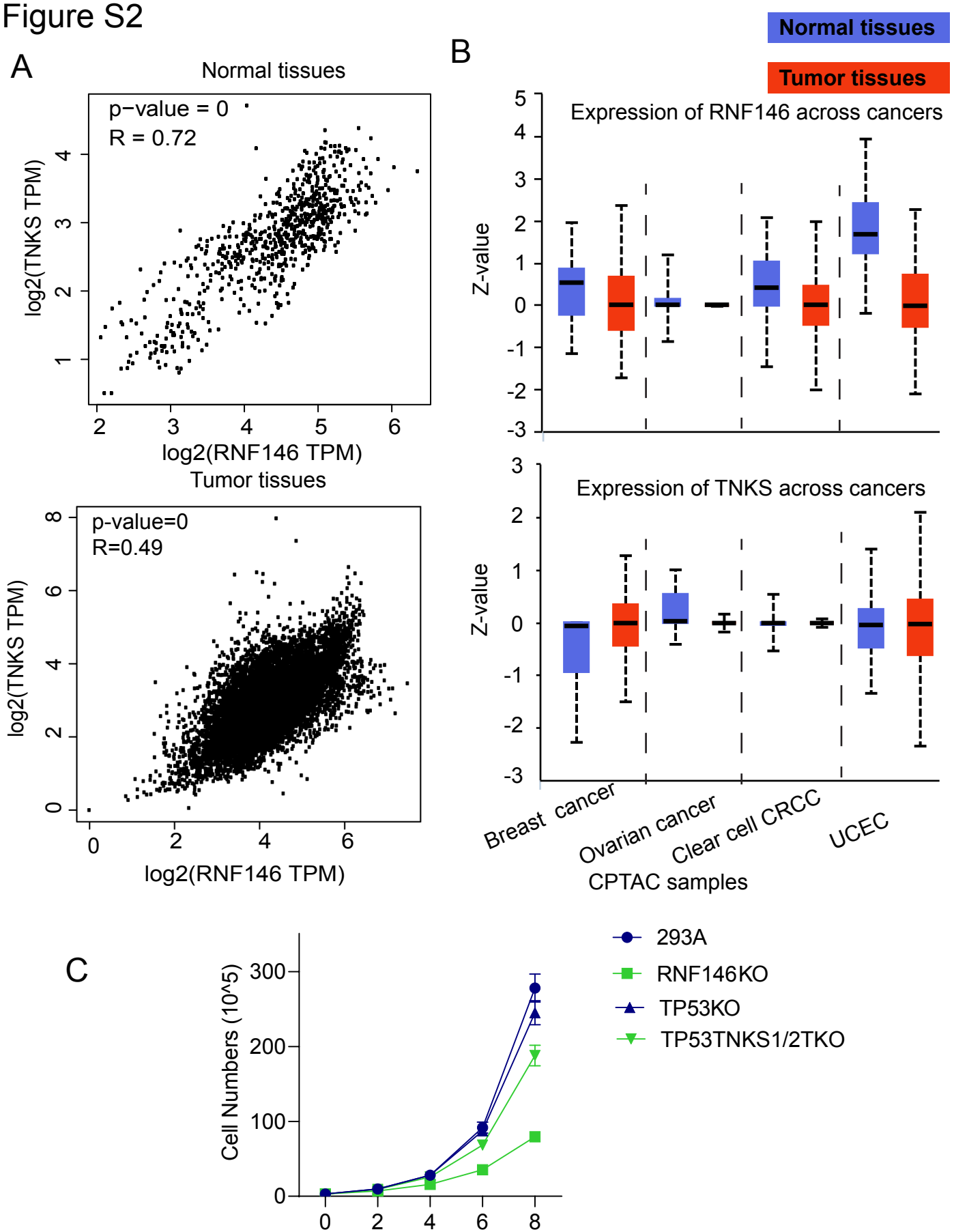
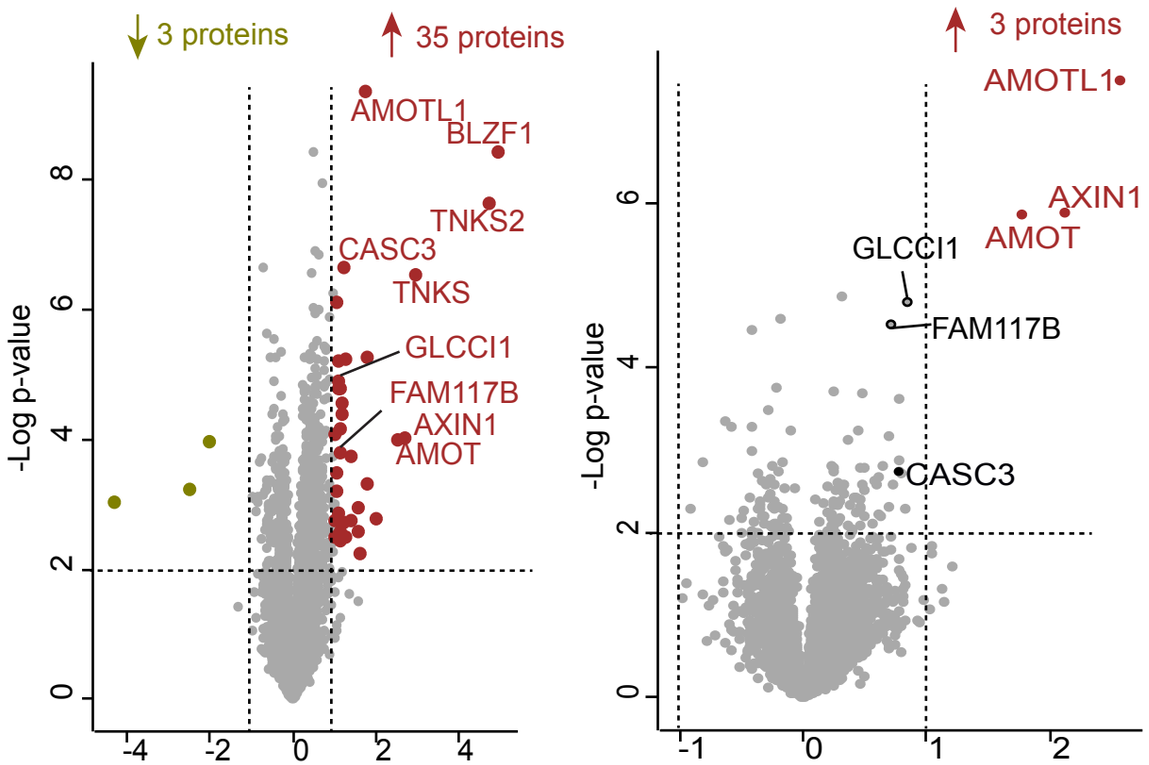
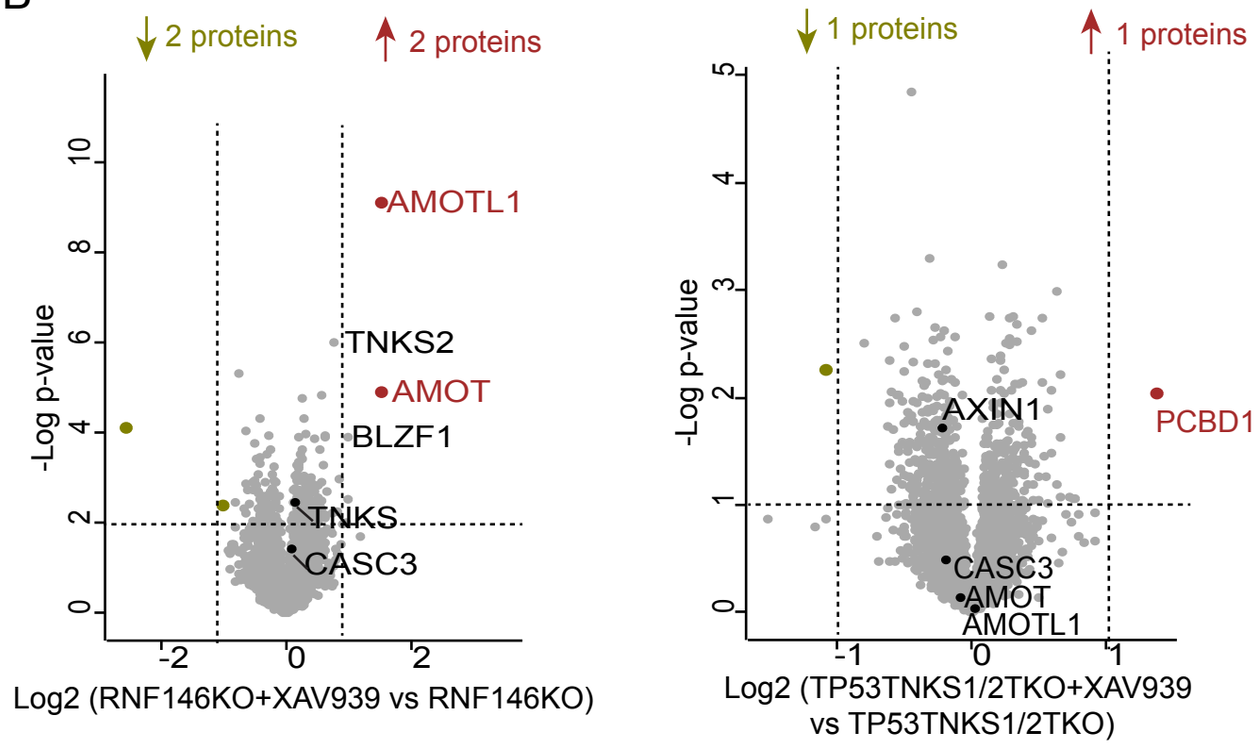

A
Normal tissues
p−value = 0
R = 0.72
log2(TNKS TPM)
log2(RNF146 TPM)
Tumor tissues
p-value=0
R=0.49
log2(TNKS TPM)
log2(RNF146 TPM)
B
Normal tissues
Tumor tissues
Expression of RNF146 across cancers
Z-value
Expression of TNKS across cancers
Z-value
Breast cancer
Ovarian cancer
Clear cell CRCC
UCEC
CPTAC samples
C
Cell Numbers (10^5)
293A
RNF146KO
TP53KO
TP53TNKS1/2TKO

Figure S3

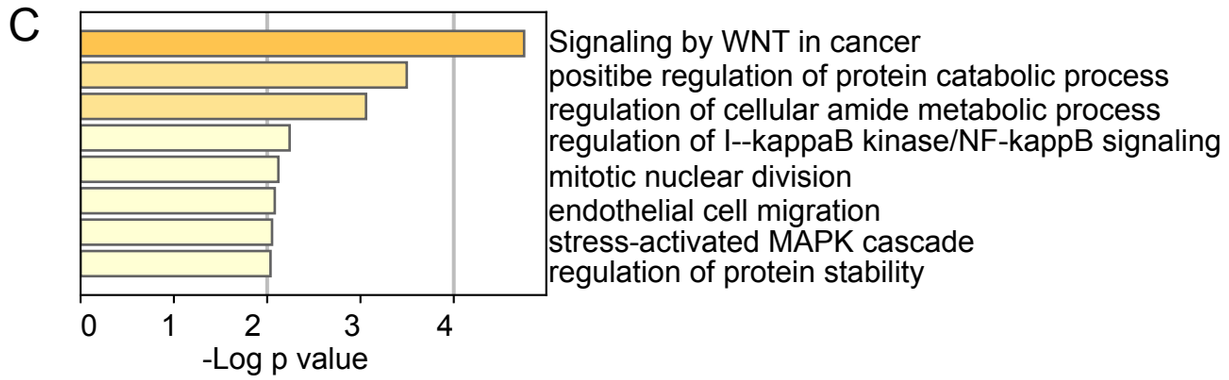
A



B



C



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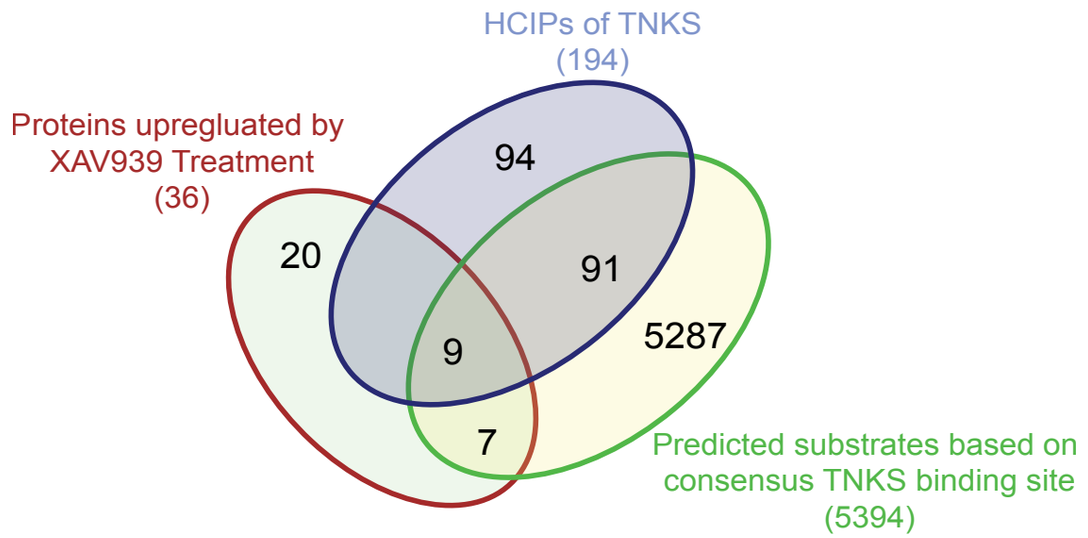


Figure S4

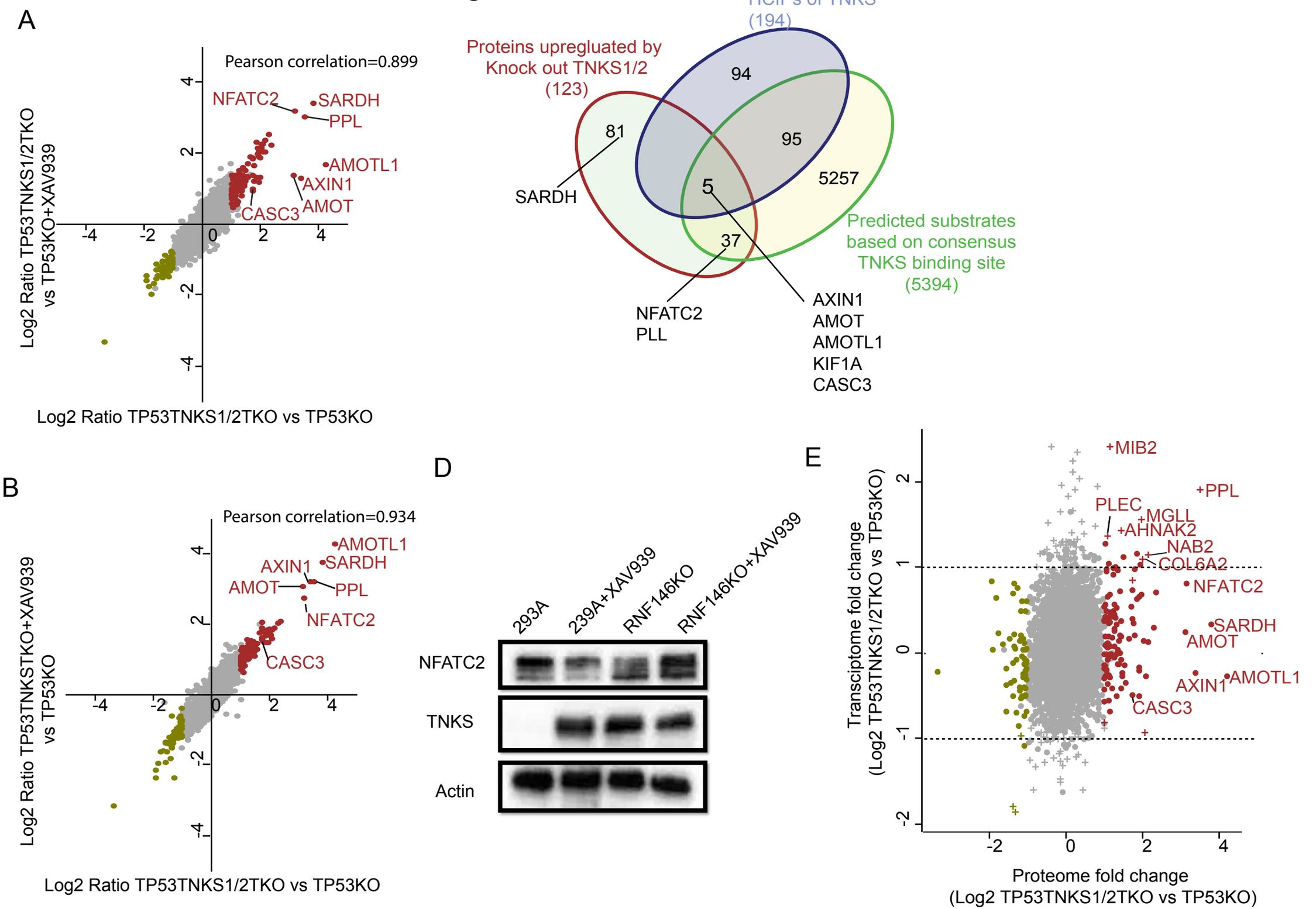
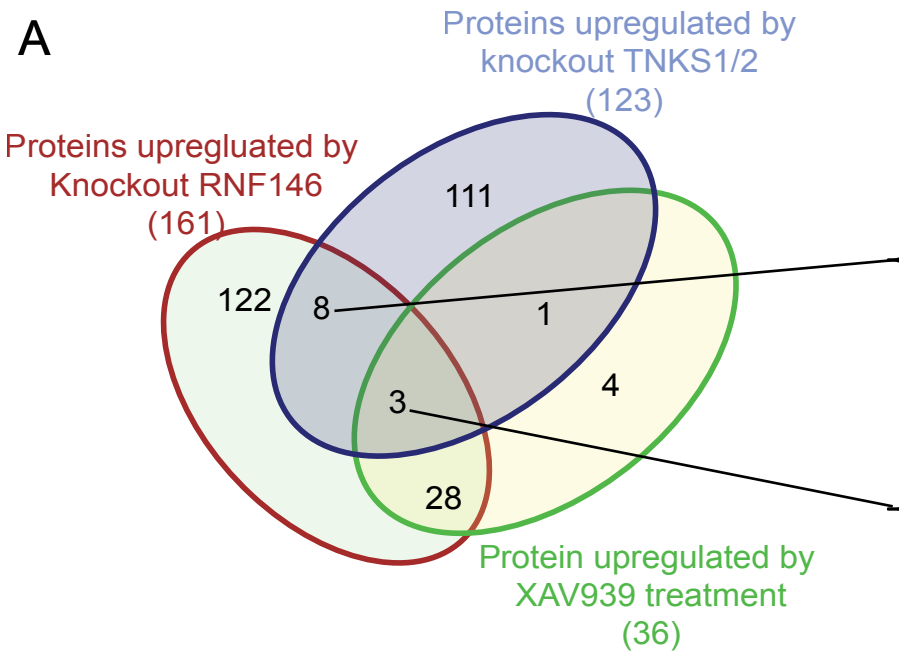

A
Pearson correlation=0.899
NFATC2
SARDH
PPL
AMOTL1
AXIN1
AMOT
CASC3
Log2 Ratio TP53TNKS1/2TKO vs TP53KO+XAV939
Log2 Ratio TP53TNKS1/2TKO vs TP53KO
B
Pearson correlation=0.934
AMOTL1
AXIN1
SARDH
AMOT
PPL
NFATC2
CASC3
Log2 Ratio TP53TNKSTKO+XAV939 vs TP53KO
Log2 Ratio TP53TNKS1/2TKO vs TP53KO
C
HCIPs of TNKS (194)
Proteins upregulated by Knock out TNKS1/2 (123)
Predicted substrates based on consensus TNKS binding site (5394)
94
81
95
5257
5
37
SARDH
NFATC2
PLL
AXIN1
AMOT
AMOTL1
KIF1A
CASC3
D
293A
239A+XAV939
RNF146KO
RNF146KO+XAV939
NFATC2
TNKS
Actin
E
+MIB2
+PPL
PLEC
+MGLL
+AHNAK2
NAB2
COL6A2
NFATC2
SARDH
AMOT
AXIN1
AMOTL1
CASC3
Transciptome fold change (Log2 TP53TNKS1/2TKO vs TP53KO)
Proteome fold change (Log2 TP53TNKS1/2TKO vs TP53KO)

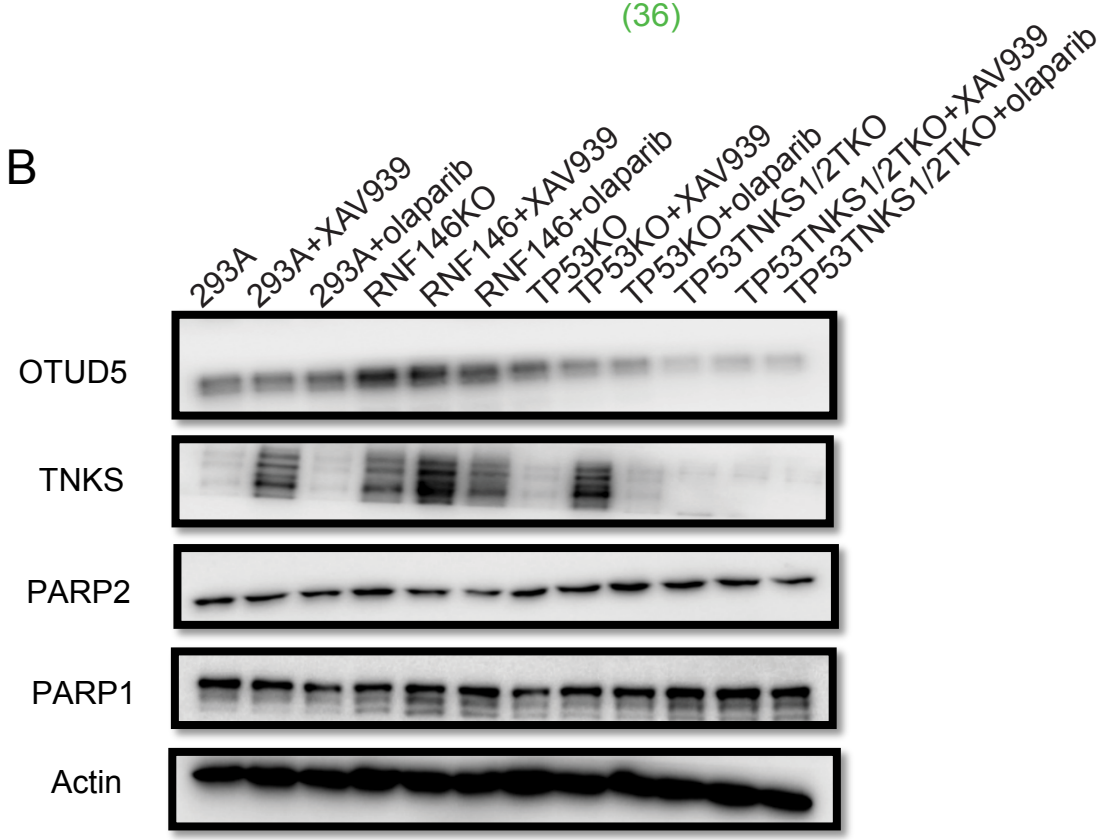
Figure S5

A



Gene names	Log2 RNF146KO vs 293A	Log2 TP53TNKS1/2TKO vs TP53KO	Known substrates	Predicted substrate based on consensus TNKS binding site
MGLL	1.79	1.99		
SARDH	1.76	3.81		
KIF1A	1.45	1.08		+
TIMP1	1.36	1.94		
CY7B1	1.30	1.24		
POMP	1.21	1.11		
TAGLN	1.17	1.93		
ATL1	1.14	1.38		
AXIN1	2.73	3.4	+	+
AMOT	2.26	3.13	+	+
CASC3	1.60	1.75	+	+

B



C