miR-33a is a tumor suppressor microRNA that is decreased in prostate cancer

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: MiR-33a level in prostate cancer cells after overexpression or knockdown of miR-33a. Relative expression of miR-33a in LNCaP cells transfected with mimic miR-33a (A) or miR-33a inhibitor (B). Relative expression of miR-33a in LNCaP cells transfected with mimic miR-33a (C) or miR-33a inhibitor (D). MiRNA levels were normalized to RNU43, *P < 0.05, **P < 0.01, ***P < 0.001.
Supplementary Figure 2: MiR-33a target nucleotide sequence on PIM1 3’ UTR. Predicted miR-33a target nucleotide sequence on PIM1 3’ UTR and mutated base pairs as a result of site directed mutagenesis.

| PIM1 3’UTR | CCAAAUAUGCACAACAAAUGCAAA |
| miR-33a | ACGUUACGUUGAUGUUGUG |
| miR-33a Mut | ACGUUACGUUGAUGUUGUG |
| PIM1 3’UTR Mut | CCAAAUAUGCACAACAGUTGCAAA |

Supplementary Figure 3: PIM1 level in mRNA and protein level in miR-33a mimic transfected control and PIM1 overexpressing LNCaP cells. Relative mRNA (A) and protein (B) level of PIM1 in control and PIM1 overexpressing LNCaP cells transfected with mimic miR-33a. Gene expression levels were normalized to β-actin, *P < 0.05, **P < 0.01, ***P < 0.001.
Supplementary Figure 4: PIM1 3’ UTR sequence controls PIM1 expression and phenotype mediated by miR-33a. Relative PIM1 mRNA levels in LNCaP cells stably overexpressing PIM1 with mutant 3’UTR (PIM1 Mut) and controls following MiR-33a mimic transfection (A) *P < 0.05 versus control without PIM1. Proliferation (B), soft agar colony formation (C) and invasion (D) were measured in PIM1 Mut and controls treated with exogenous miR-33a. *P < 0.05 versus control without PIM1. †P < .05 PIM1 Mut versus PIM1 wild type 3-UTR.

Supplementary Figure 5: Correlation of SREBF2 mRNA and mRNAs of genes associated lipid oxidation and synthesis in metastatic prostate cancer. Correlation of (A) CPT1A vs. SREBF2, (B) HADHA vs. SREBF2, (C) HADHB vs. SREBF2, and (D) SREBF1 vs. SREBF2 mRNAs in metastatic PCas in the Taylor dataset using cBioPortal. Pearson r² is shown above each plot.
Supplementary Table 2: QRT-PCR primer sequences and PIM1 3′UTR cloning and mutagenesis primer sequences

| Gene             | Sequence                                                                 |
|------------------|--------------------------------------------------------------------------|
| Beta-actin-F     | 5′-GCCTGCCTTTGGCCGATC-3′                                                |
| Beta-actin-R     | 5′-CCCACGATGGAGGGGAAG-3′                                                |
| PIM1-F           | 5′- CCGTCTACACGGACTTCGAT-3′                                             |
| PIM1-R           | 5′- CTGGCCCGATCTCCTTT-3′                                                |
| CPT1A-F          | 5′- GGCAAGTTTTGCTCACATACG-3′                                            |
| CPT1A-R          | 5′- GAACAACTTCAGCCTGTTCC-3′                                             |
| HADHB-F          | 5′- AAACCAAGGTTGGATTGCCT-3′                                             |
| HADHB-R          | 5′- CACTATCATAGCATGGGCCTG-3′                                            |
| YWHAH-F          | 5′- GGTGACAGAGCTGAATGAAAC-3′                                            |
| YWHAH-R          | 5′- TCATTGCAAAACTTCTCCAGC-3′                                            |
| ABDE1-F          | 5′- GTTACACCACAGGCAAATTGA-3′                                            |
| ABDE1-R          | 5′- GGGATAGGCAACCTTGGAAG-3′                                             |
| EIF5A2-F         | 5′- CACCATGGAAGACGAATTGA-3′                                             |
| EIF5A2-R         | 5′- CAAACAAAGTGAAACCTTGGC-3′                                            |
| LDHA-F           | 5′- GTTGCTGTTGCTCTCCTGAA-3′                                             |
| LDHA-R           | 5′- TCCAATAGCCAGATGTGT-3′                                               |
| CDK16-F          | 5′- AGGACATCAACAAGCCTCCTA-3′                                            |
| CDK16-R          | 5′- GTTTCCAAAGCCAATTCAG-3′                                              |
| FRS2-F           | 5′- GAAAGACATGGAAGCTG-3′                                               |
| FRS2-R           | 5′- TCACGTTGGGATGTATAA-3′                                               |
| PIM1 ORF+UTR-F   | 5′- CCTCAGTTGTCCTCGACTC-3′                                             |
| PIM1 ORF+UTR-R   | 5′- GAGAGATCTGGAAGGCAAC-3′                                             |
| Mutagenesis-F    | 5′-ACATTTACAGTTTTCTGTTGATTGAACTGTTTGTGATTTTTTTTGTTGTTGTTG-3′           |
| Mutagenesis-R    | 5′-CACACACACACACCAATGCAACACACGTTCAATCAACAGAAGCTGTAATGT-3′               |