

Stress-inducible SCAND factors suppress stress response and are biomarkers for enhanced prognosis in cancers

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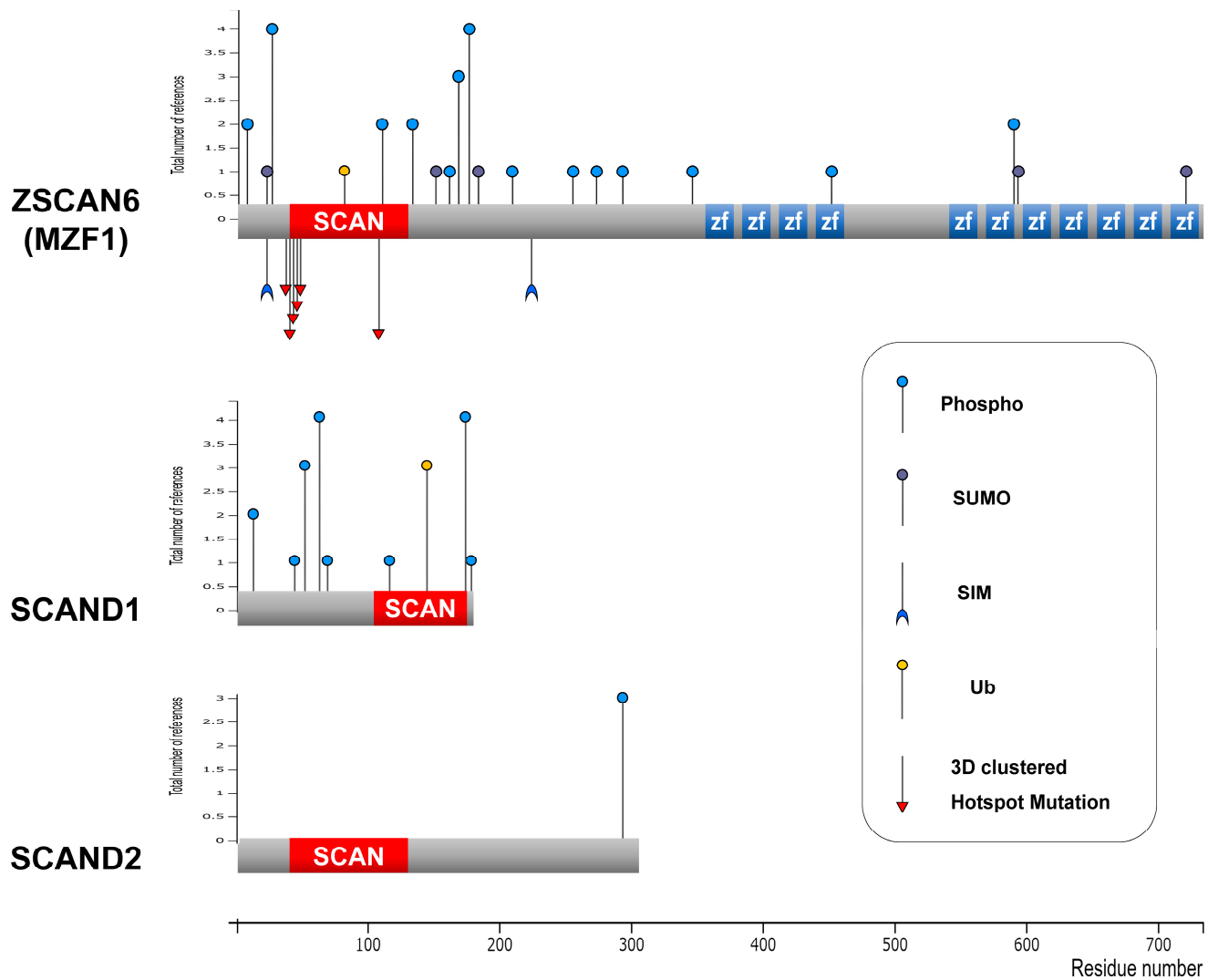


Figure S1. Protein structures of MZF1(ZSCAN6), SCAND1, and SCAND2. Post-translational modifications (PTM) and hotspot mutation were mapped. SCAN, SCAN domain. zf, zinc finger domain. Phospho, phosphorylation site. SUMO, sumoylation site. SIM, SUMO interaction motif. Ub, ubiquitination site. Some PTMs were shown in a ref. (Eguchi T et al., 2015, J Cell Biochem 116). This figure was shown as a supplemental item in ref. (Eguchi T et al., 2022, Cells 11).

A

Homo sapiens **myeloid zinc finger 1 (MZF1)**, transcript variant 2, mRNA
NCBI Reference Sequence: NM_198055.2 2666 bp mRNA

```

841 atgcaggaat caccactggg cctgcaggtg aaagaggagt cagaggttac agaggactca
      -----F1----->
901 gatttctcgg agtctgggcc tctagctgcc acccaggagt ctgtaccac cctcctgcct
    Ex3||Ex4
961 gaggaggccc agagatgtgg gaccgtgtg gaccagatct ttccccacag caagactggg
      Ex4||Ex5               <-----R1-----

```

B

Homo sapiens **SCAN domain containing 1 (SCAND1)**, transcript variant 3, mRNA
NCBI Reference Sequence: NM_001385710.1 1732 bp mRNA

```

841 cgccgacctg gacgcagaga agccagagac tttcgcttc ggctgccga ggcgcttcgc
      -----F1----->
901 tgggtcaggt aagctccgca cactctcggc cgggtccgag tccgactccc tcaagggtga
961 cgcgagctct gccctttaac cggaaacgtc tccctgctca cccaccccc gcgcagacgc
1021 agtgtcgagc acacagctac cggacaaaga gtgacgcccc gagctggagt tatggcggct
      <-----R1-----

```

C

Homo sapiens **SCAND2 mRNA, complete cds** GenBank: AF229246.1

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421 gagtgcagtt gcaaagctct tctctgacct gaacaatggc tgtagctgtg gaccaacaaa
    Ex1              5'-UTR|CDS
481 tccagactcc ttcagtacaa gatctccaaa tagttaaact ggaagaagat tccactggg
541 agcaggaaat ttcccttcaa gggaattacc ctggaccaga gacatcctgc cagagctttt
      -----F2----->
601 ggcatttccg ttaccaagaa gcatcacgac cccgagaggc cctcctccag ctccagaagc
      -----F1----->
661 tctgttgtca gtggctaagg ccagagaagt gtacaaaaga gcagatcctg gagttgctgg
      <-----R2-----
721 tcctagaaaca gttccggact gtccttctcc aggagatcca gatctgggtc agacagcagc
--R2--
781 atccggagag tggagaggag gcagtggccc tgggtgaaga cttgcagaaa gaacctggaa
<-----R1-----
841 gacagaggct ggagcctcgg gcgaggccgt ccggccgcac ccctcctgct cagctgcggt
:      Ex1||Ex2
:
1261 gtccccgcct cgcgcgtccc cgggccggac aaaagccatg gctgcttgtt atccggtcaa
      -----F3----->
1321 tggaacgggt tatgtacgtc atgttgataa tccaaatgga gacggaagac gtgtgaatg
      CDS||3'-UTR
1381 tattacatta cgtaaagaa cgggatgcca aggtaagtgg aggtatactt cgaatttttc
1441 tagaaggtaa agcctagttt gctgacattg aacccaaatt tgatagactg ctgtttttct
1501 ggtctgacca tcgcaaccct catgaagtac aaccagcata tgctacaaag tacgcaataa
      <-----R3-----

```

D

Homo sapiens **SCAN domain containing 2 pseudogene (SCAND2P)**, transcript variant 1, non-coding RNA
NCBI Reference Sequence: NR_004859.1 4509 bp RNA

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541 ttcaagggaa ttaccctgga ccagagacat cctgccagag cttttggcat ttccgttacc
      -----F2----->
601 aagaagcatc acgaccccga gaggcctcc tccagctcca gaagctctgt tgtcagtggc
      -----F1----->
661 taaggccaga gaagtgtaca aaagagcaga tcctggagtt gctggctcta gaacagttcc
      <-----R2-----
721 cgactgtcct tctccaggag atccagatct gggtcagaca gcagcatccg gagagtggag
      <-----R1-----
781 aggagggagc ggccctgggt gaagacttgc agaaagaacc tggaagacag aggctggagc
:      -----
:      Ex1||Ex2
1261 gtccccgggc cggacaaaag ccatggctgc ttgttatccg gtcaatggaa cgggttatgt
      -----F3----->
1321 acgtcatgtt gataatccaa atggagacgg aagacgtgtg aaatgtatta cattacgtta

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Figure S2. Primer design for human MZF1(ZSCAN6), SCAND1, and SCAND2(SCAND2P) gene sequences. (A) MZF1 transcript variant 2 mRNA: NM_198055.2. (B) SCAND1 transcript variant 3 mRNA: NM_001385710.1. (C) SCAN domain containing 2 pseudogene (SCAND2P) transcript variant 1 non-coding RNA: NR_004859.1. (D) SCAND2 mRNA complete CDS: AF229246.1.

Table S1. Primer sequences for qRT-PCR.

| Primer name | Sequences (5' to 3') | Refseq ID | Location | Position of the 5' of the primer |
|--------------------|-------------------------|----------------|----------|----------------------------------|
| MZF1 h F1 | TGCAGGTGAAAGAGGAGTCA | NM_198055.2 | Exon 3 | 863 |
| MZF1 h R1 | AGTCTTGCTGTGGGGAAAGA | NM_198055.2 | Exon 5 | 1017 |
| SCAND1 h F1 | CGCAGAGAAGCCAGAGACTT | NM_001385710.1 | Exon 3 | 853 |
| SCAND1 h R2 | CGG GCG TCA CTC TTT GTC | NM_001385710.1 | Exon 3 | 1060 |
| SCAND2 h F1 | CCTCCTCCAGCTCCAGAAG | NR_004859 | Exon 1 | 626 |
| SCAND2 h R1 | CTCCTCTCCACTCTCCGGAT | NR_004859 | Exon 1 | 785 |
| SCAND2 h F2 | CCAGAGACATCCTGCCAGAG | NR_004859 | Exon 1 | 561 |
| SCAND2 h R2 | CTAGGACCAGCAACTCCAGG | NR_004859 | Exon 1 | 711 |
| SCAND2 h F3 | CATGGCTGCTTGTTATCCGG | AF229246.1 | CDS* | 1297 |
| SCAND2 h R3 | CTTCATGAGGGTTGCGATGG | AF229246.1 | 3'-UTR** | 1527 |
| HSP90AA1 h F1 | GAGCAGTACGCTTGGGAGTC | NM_001017963.3 | Exon 4 | 1183 |
| HSP90AA1 h R1 | TCCACGACCCATAGGTTTAC | NM_001017963.3 | Exon 5 | 1260 |
| RNA18SN5 hmr F1245 | GACTCAACACGGGAAACCTC | NR_003286.4 | n.a.*** | 1245 |
| RNA18SN5 hmr R1364 | AGACAAATCGCTCCACCAAC | NR_003286.4 | n.a.*** | 1364 |

*Coding DNA sequence. **3'-untranslated region, *** not applicable.

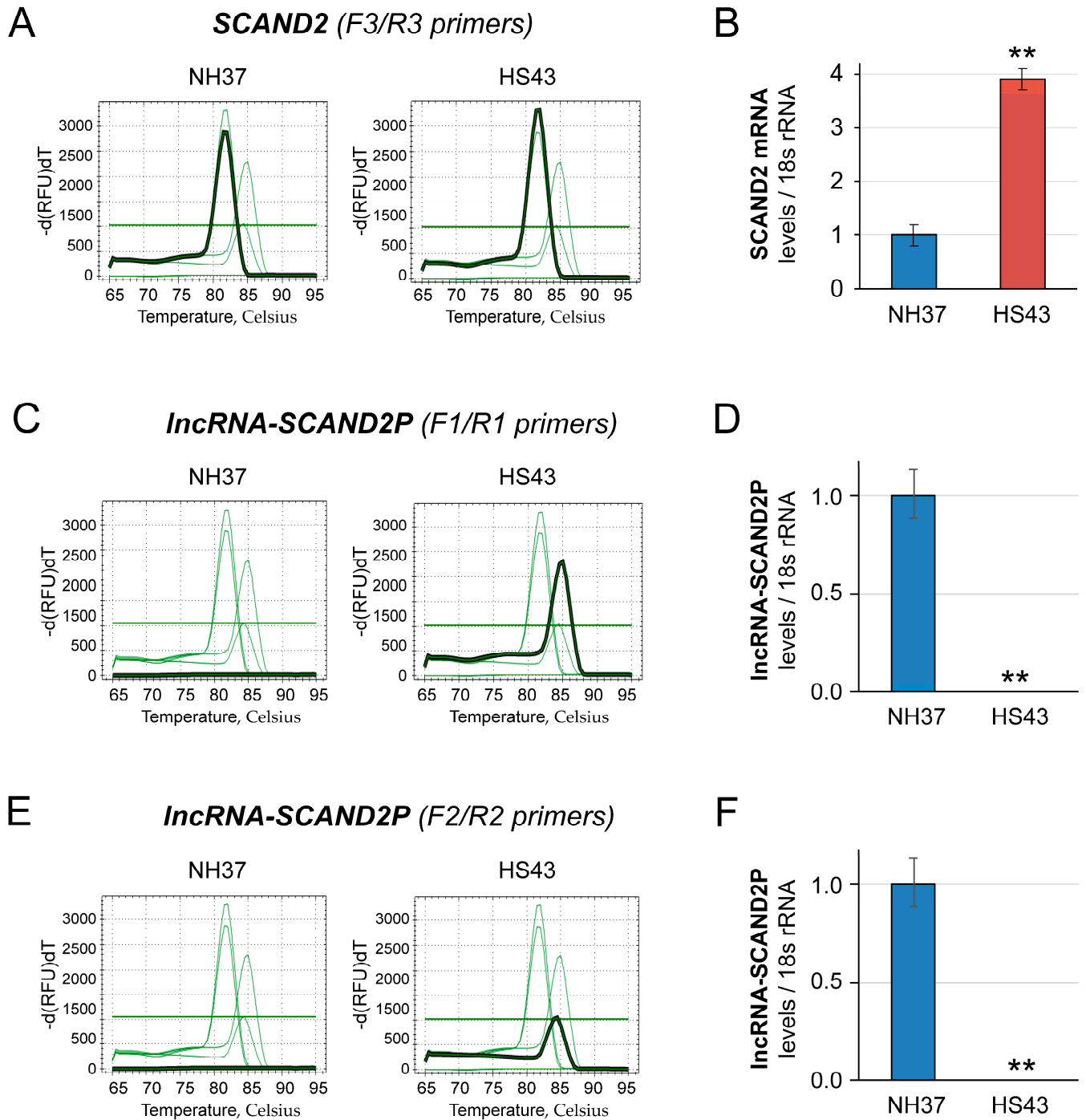
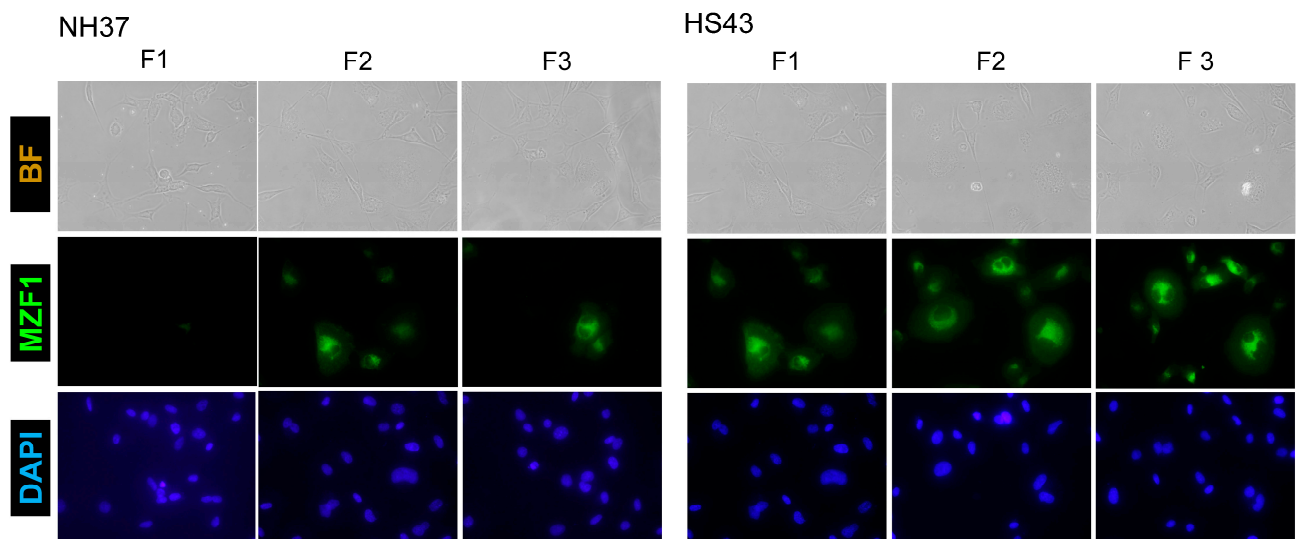


Figure S3. Melting curve analysis and qRT-PCR for SCAND2 mRNA and lncRNA-SCAND2P. We examined the correlation between heat shock stress (HSS) and the expression of SCAND2 mRNA (AF229246.1) (A, B) and its pseudogene variant lncRNA-SCAND2P (NR_004859.1) (C–F) in DU-145 prostate cancer cells. (A, C, E) Melting curves of PCR amplicons, indicating single amplicons in each PCR. (B, D, F) qRT-PCR analyses. (A, B) A region of the complete coding sequence of SCAND2 mRNA, using a primer pair (SCAND2 h F3 and R3), was upregulated with HSS. (C–F) Regions of the non-coding RNA transcript variant 1 of SCAND2P, using primer pairs (SCAND2 h F1/R1 (C, D) or SCAND2 h F2/R2 (E, F)) were detected under the non-heated condition but became under detection limit after HSS. ** $P < 0.01$, $n = 3$.

A MZF1 expression



B SCAND2 expression

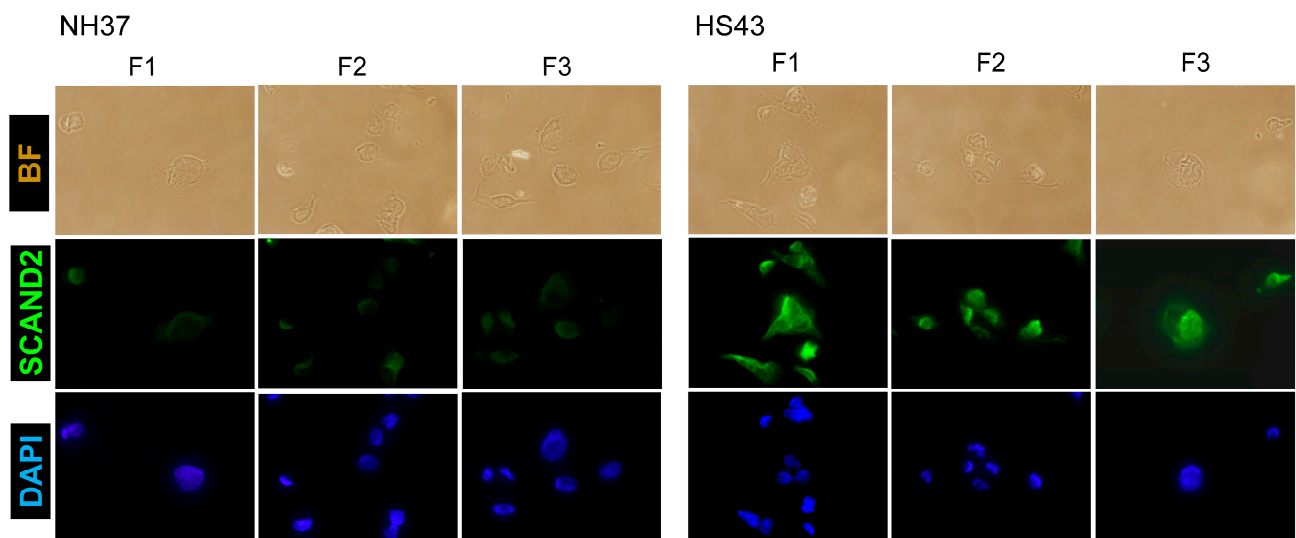


Figure S4. Immunocytochemistry for MZF1 (A) and SCAND2 (B) expression in DU-145 cells with or without heat shock. Cells were cultured under two conditions; non-heated at 37°C (NH37) and heat-shocked at 43°C (HS43) for 30 min. Three different fields (F1, F2, and F3) for each condition were shown. BF, bright field.

Table S2. Co-expression correlation of SCAN-TF genes vs. HSPs in prostate adenocarcinoma specimens.

| vs MZF1 | | | | vs SCAND1 | | | | vs SCAND2 | | | |
|------------------|-------------|----------|----------|------------------|-------------|-----------|----------|------------------|-------------|----------|----------|
| Correlated | Spearman's | | | Correlated | Spearman's | | | Correlated | Spearman's | | |
| Gene | Correlation | p-Value | q-Value | Gene | Correlation | p-Value | q-Value | Gene | Correlation | p-Value | q-Value |
| HSPBP1 | 0.504 | 8.89E-33 | 2.82E-31 | HSPBP1 | 0.778 | 2.90E-100 | 1.81E-97 | HSPBP1 | 0.159 | 4.34E-04 | 1.54E-03 |
| HSPA13 | -0.448 | 1.75E-25 | 3.29E-24 | HSPA13 | -0.572 | 7.79E-44 | 1.05E-42 | HSPA13 | -0.357 | 4.18E-16 | 9.13E-15 |
| HSPA4 | -0.358 | 3.41E-16 | 2.70E-15 | HSPA4 | -0.303 | 7.97E-12 | 2.47E-11 | HSPA4 | -0.394 | 1.52E-19 | 4.80E-18 |
| HSPBAP1 | 0.354 | 6.99E-16 | 5.37E-15 | HSPBAP1 | 0.347 | 2.94E-15 | 1.09E-14 | HSPBAP1 | 0.281 | 2.62E-10 | 2.80E-09 |
| HSPB11 | 0.352 | 1.08E-15 | 8.16E-15 | HSPB11 | 0.516 | 1.36E-34 | 1.20E-33 | HSPB11 | 0.248 | 2.99E-08 | 2.35E-07 |
| HSPA4L | -0.326 | 1.58E-13 | 9.53E-13 | HSPA4L | -0.411 | 2.66E-21 | 1.32E-20 | HSPA4L | -0.0341 | 0.452 | 0.571 |
| HSP90AA1 | -0.321 | 3.63E-13 | 2.11E-12 | HSP90AA1 | -0.188 | 2.86E-05 | 5.87E-05 | HSP90AA1 | -0.32 | 4.34E-13 | 6.62E-12 |
| HSPH1 | -0.3 | 1.42E-11 | 6.96E-11 | HSPH1 | -0.191 | 2.20E-05 | 4.55E-05 | HSPH1 | -0.312 | 1.67E-12 | 2.37E-11 |
| HSPA7 | 0.298 | 1.69E-11 | 8.21E-11 | HSPA7 | 0.241 | 7.45E-08 | 1.84E-07 | HSPA7 | 0.277 | 4.54E-10 | 4.67E-09 |
| HSPB9 | 0.278 | 4.05E-10 | 1.70E-09 | HSPB9 | 0.342 | 7.59E-15 | 2.76E-14 | HSPB9 | 0.269 | 1.64E-09 | 1.56E-08 |
| HSP90B3P | -0.246 | 3.47E-08 | 1.20E-07 | HSP90B3P | -0.403 | 1.78E-20 | 8.51E-20 | HSP90B3P | -0.242 | 6.29E-08 | 4.72E-07 |
| HSPE1 | 0.216 | 1.38E-06 | 3.98E-06 | HSPE1 | 0.392 | 2.07E-19 | 9.38E-19 | HSPE1 | -0.124 | 6.20E-03 | 0.0161 |
| HSPB8 | -0.21 | 2.79E-06 | 7.77E-06 | HSPB8 | -0.0333 | 0.463 | 0.528 | HSPB8 | 0.0593 | 0.191 | 0.293 |
| HSPB7 | -0.203 | 6.01E-06 | 1.61E-05 | HSPB7 | -0.0237 | 0.602 | 0.66 | HSPB7 | 0.0263 | 0.563 | 0.672 |
| HSPA12A | -0.203 | 6.43E-06 | 1.72E-05 | HSPA12A | -0.149 | 9.59E-04 | 1.69E-03 | HSPA12A | 0.0673 | 0.138 | 0.225 |
| HSP90AB4P | 0.184 | 4.27E-05 | 1.03E-04 | HSP90AB4P | 0.189 | 2.55E-05 | 5.25E-05 | HSP90AB4P | 0.149 | 9.91E-04 | 3.21E-03 |
| HSPA1B | 0.176 | 9.23E-05 | 2.14E-04 | HSPA1B | 0.274 | 7.90E-10 | 2.19E-09 | HSPA1B | -0.0374 | 0.41 | 0.531 |
| HSPA5 | -0.174 | 1.11E-04 | 2.54E-04 | HSPA5 | -0.212 | 2.41E-06 | 5.40E-06 | HSPA5 | -0.29 | 6.94E-11 | 8.09E-10 |
| HSPA8 | -0.157 | 5.07E-04 | 1.06E-03 | HSPA8 | -0.352 | 1.07E-15 | 4.07E-15 | HSPA8 | -0.274 | 7.08E-10 | 7.10E-09 |
| HSPG2 | -0.151 | 7.93E-04 | 1.62E-03 | HSPG2 | -0.282 | 2.42E-10 | 6.88E-10 | HSPG2 | -0.0246 | 0.587 | 0.693 |
| HSP90AB2P | -0.15 | 8.69E-04 | 1.76E-03 | HSP90AB2P | -0.268 | 1.92E-09 | 5.20E-09 | HSP90AB2P | -0.202 | 6.80E-06 | 3.55E-05 |
| HSPA6 | 0.145 | 1.36E-03 | 2.69E-03 | HSPA6 | 0.163 | 2.94E-04 | 5.49E-04 | HSPA6 | 0.0642 | 0.157 | 0.25 |
| HSPA1A | 0.137 | 2.49E-03 | 4.72E-03 | HSPA1A | 0.279 | 3.51E-10 | 9.90E-10 | HSPA1A | -0.0836 | 0.0651 | 0.121 |
| HSPB6 | -0.134 | 2.95E-03 | 5.54E-03 | HSPB6 | 0.102 | 0.0244 | 0.0364 | HSPB6 | 0.0603 | 0.183 | 0.283 |
| HSPA14 | -0.117 | 9.41E-03 | 0.0163 | HSPA14 | -0.277 | 5.08E-10 | 1.42E-09 | HSPA14 | -0.0762 | 0.0928 | 0.163 |
| HSP90B1 | -0.112 | 0.0132 | 0.0223 | HSP90B1 | -0.167 | 2.04E-04 | 3.87E-04 | HSPB1 | 0.034 | 0.454 | 0.572 |
| HSPB3 | -0.112 | 0.0137 | 0.023 | HSPB3 | 0.0262 | 0.563 | 0.625 | HSPB3 | -0.0508 | 0.263 | 0.377 |
| HSPA1L | 0.0931 | 0.0397 | 0.0615 | HSPA1L | 0.0935 | 0.039 | 0.0564 | HSPA1L | 0.156 | 5.28E-04 | 1.84E-03 |
| HSP90AB1 | -0.0746 | 0.0996 | 0.142 | HSP90AB1 | -0.11 | 0.0155 | 0.0238 | HSP90AB1 | -0.276 | 5.67E-10 | 5.76E-09 |
| HSPB2 | -0.0639 | 0.159 | 0.215 | HSPB2 | 0.177 | 8.44E-05 | 1.66E-04 | HSPB2 | 0.109 | 0.0159 | 0.0366 |
| HSPB1 | 0.0573 | 0.207 | 0.271 | HSPB1 | 0.45 | 9.36E-26 | 5.66E-25 | HSP90B1 | -0.244 | 4.93E-08 | 3.74E-07 |
| HSPA9 | -0.0491 | 0.279 | 0.351 | HSPA9 | -0.208 | 3.71E-06 | 8.17E-06 | HSPA9 | -0.246 | 3.54E-08 | 2.76E-07 |
| HSPA2 | -0.042 | 0.354 | 0.431 | HSPA2 | 0.204 | 5.40E-06 | 1.18E-05 | HSPA2 | -0.0254 | 0.576 | 0.684 |
| HSPD1 | -0.0358 | 0.43 | 0.508 | HSPD1 | -0.0162 | 0.721 | 0.767 | HSPD1 | -0.26 | 5.75E-09 | 5.05E-08 |
| HSPB1P1 | 0.0191 | 0.675 | 0.736 | HSPB1P1 | 0.406 | 9.63E-21 | 4.65E-20 | HSPB1P1 | 0.0196 | 0.665 | 0.757 |
| HSPA12B | 0.0161 | 0.723 | 0.778 | HSPA12B | 0.0806 | 0.0754 | 0.104 | HSPA12B | 0.135 | 2.77E-03 | 7.98E-03 |

Spearman's correlation < -0.25 were shown in red. p-Values < 1E-10 were shown in yellow. q-Values < 1E-10 were shown in green.

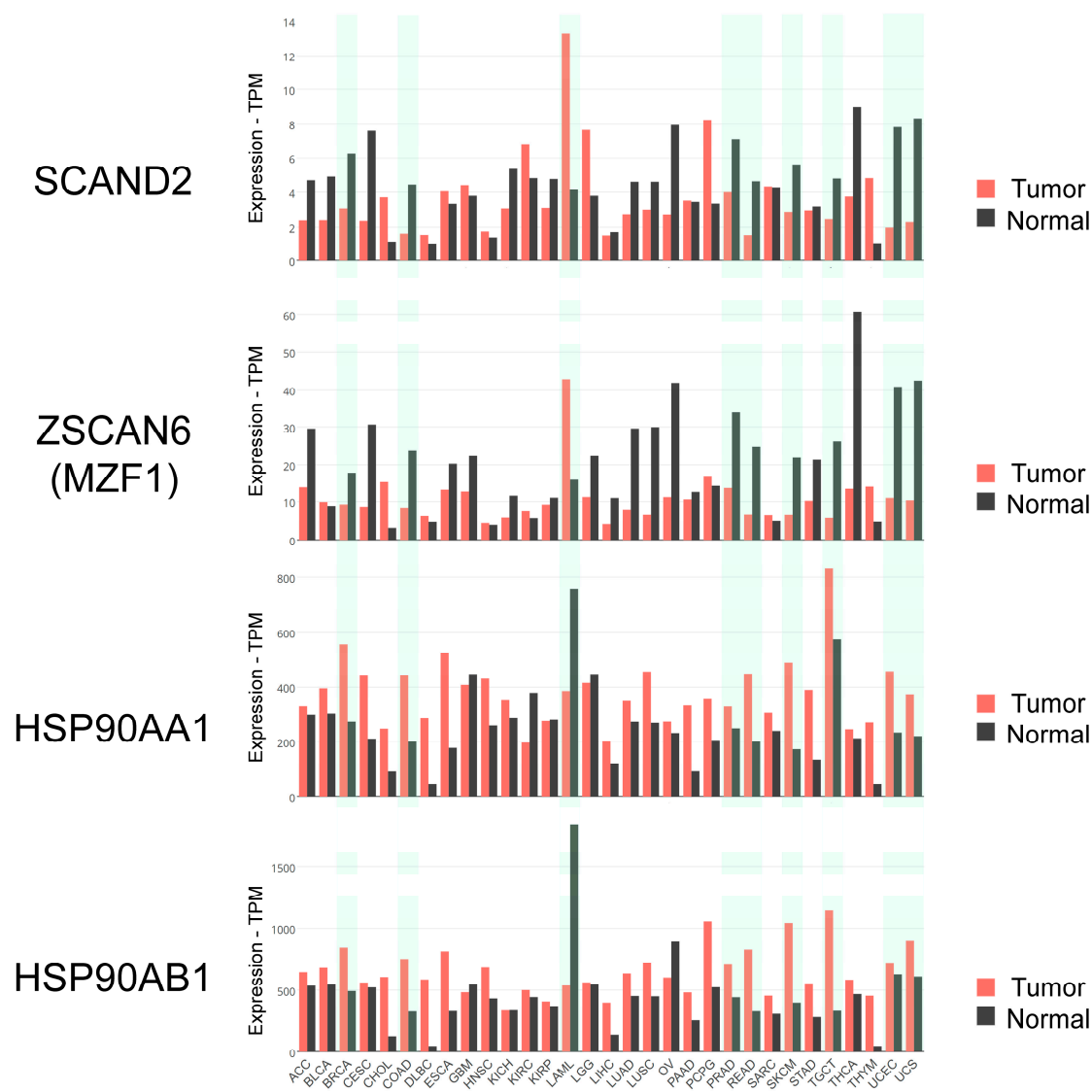


Figure S5. Gene expression profiling of SCAND2, ZSCAN6(MZF1), and HSP90 genes in various tumor types vs. paired normal tissues. Data were from TCGA PanCancer Atlas.

Table S3. Patients-derived tumor vs. normal tissue samples from TCGA PanCancer Atlas and GTEx data

| TCGA | Detail | Tumor | Normal | GTEx | Num |
|------|--|-------|--------|---------------|-----|
| ACC | Adrenocortical carcinoma | 77 - | | Adrenal Gland | 128 |
| BLCA | Bladder Urothelial Carcinoma | 404 | 19 | Bladder | 9 |
| BRCA | Breast invasive carcinoma | 1085 | 112 | Breast | 179 |
| CESC | Cervical squamous cell carcinoma and endocervical adenocarcinoma | 306 | 3 | Cervix Uteri | 10 |
| CHOL | Cholangio carcinoma | 36 | 9 - | | - |
| COAD | Colon adenocarcinoma | 275 | 41 | Colon | 308 |
| DLBC | Lymphoid Neoplasm Diffuse Large B-cell Lymphoma | 47 - | | Blood | 337 |
| ESCA | Esophageal carcinoma | 182 | 13 | Esophagus | 273 |
| GBM | Glioblastoma multiforme | 163 - | | Brain | 207 |
| HNSC | Head and Neck squamous cell carcinoma | 519 | 44 - | | - |
| KICH | Kidney Chromophobe | 66 | 25 | Kidney | 28 |
| KIRC | Kidney renal clear cell carcinoma | 523 | 72 | Kidney | 28 |
| KIRP | Kidney renal papillary cell carcinoma | 286 | 32 | Kidney | 28 |
| LAML | Acute Myeloid Leukemia | 173 - | | Bone Marrow | 70 |
| LGG | Brain Lower Grade Glioma | 518 - | | Brain | 207 |
| LIHC | Liver hepatocellular carcinoma | 369 | 50 | Liver | 110 |
| LUAD | Lung adenocarcinoma | 483 | 59 | Lung | 288 |
| LUSC | Lung squamous cell carcinoma | 486 | 50 | Lung | 288 |
| MESO | Mesothelioma | 87 - | - | | - |
| OV | Ovarian serous cystadenocarcinoma | 426 - | | Ovary | 88 |
| PAAD | Pancreatic adenocarcinoma | 179 | 4 | Pancreas | 167 |
| PCPG | Pheochromocytoma and Paranganglioma | 182 | 3 - | | - |
| PRAD | Prostate adenocarcinoma | 492 | 52 | Prostate | 100 |
| READ | Rectum adenocarcinoma | 92 | 10 | Colon | 308 |
| SARC | Sarcoma | 262 | 2 - | | - |
| SKCM | Skin Cutaneous Melanoma | 461 | 1 | Skin | 557 |
| STAD | Stomach adenocarcinoma | 408 | 36 | Stomach | 175 |
| TGCT | Testicular Germ Cell Tumors | 137 - | | Testis | 165 |
| THCA | Thyroid carcinoma | 512 | 59 | Thyroid | 278 |
| THYM | Thymoma | 118 | 2 | Blood | 337 |
| UCEC | Uterine Corpus Endometrial Carcinoma | 174 | 13 | Uterus | 78 |
| UCS | Uterine Carcinosarcoma | 57 - | | Uterus | 78 |
| UVM | Uveal Melanoma | 79 - | - | | - |

Lung adenocarcinoma

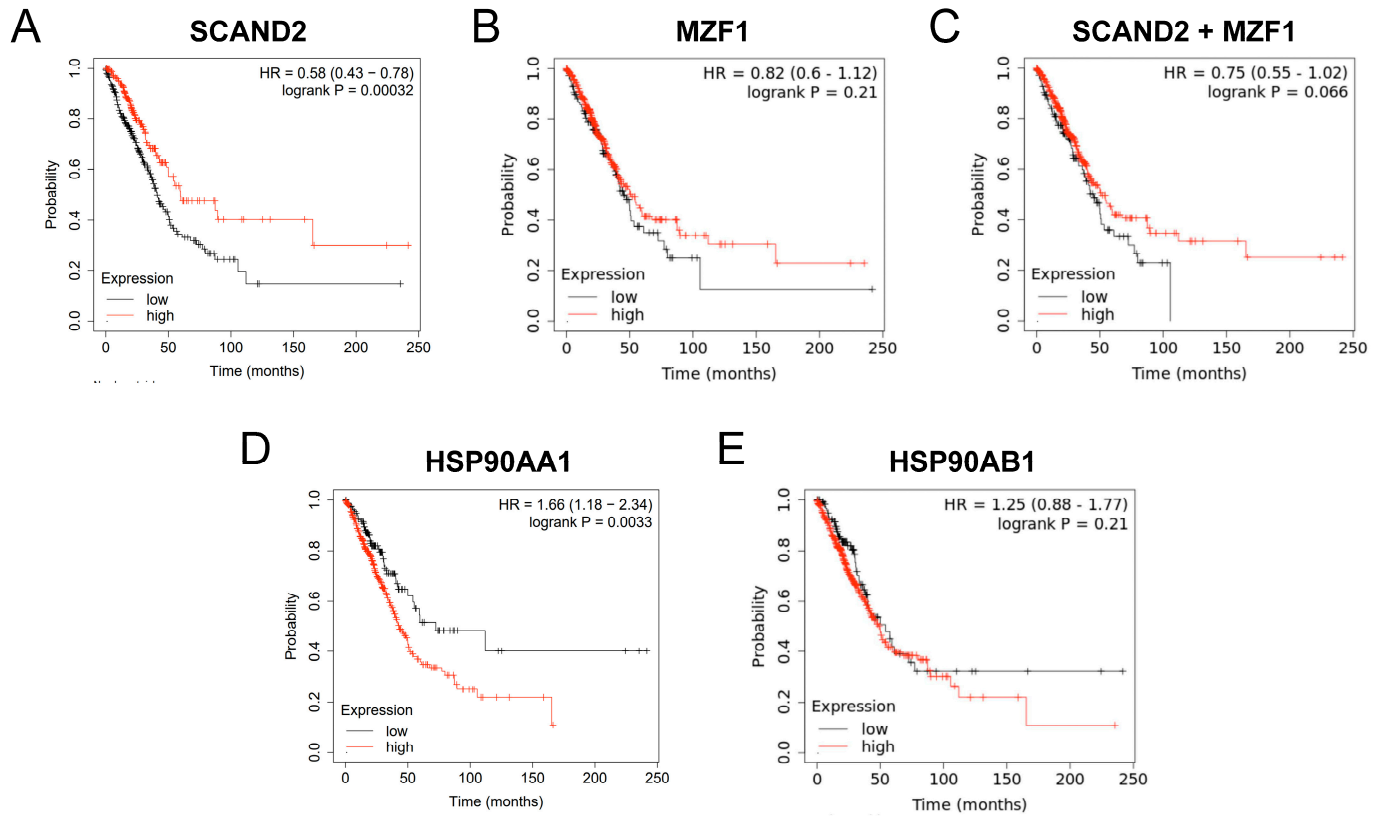


Figure S6. Kaplan-Meier analysis of SCAN-TFs and HSP90 expression in lung adenocarcinoma. Data were from TCGA PanCancer Atlas, lung adenocarcinoma (LUAD), n=504. SCAND2 and MZF1 expression are correlated with a better prognosis, whereas HSP90 expression is correlated with a poor prognosis of lung adenocarcinoma.