Supplementary Information for

Crosstalk between Mutant p53 and p62/SQSTM1 Augments Cancer Cell Migration by Promoting the Degradation of Cell Adhesion Proteins

Saptaparna Mukherjee¹, Martino Maddalena¹†, YiQing Lü²-³†, Sebastien Martinez²-³, Nishanth Beugali Nataraj⁴, Ashish Noronha⁴, Sansrity Sinha⁵, Katie Teng²-³, Victoria Cohen-Kaplan⁶, Tamar Ziv⁷, Sharathchandra Arandkar¹⁸, Ori Hassin¹, Rishita Chatterjee⁴, Anna-Chiara Pirona¹, Michal Shreberk-Shaked¹, Anat Gershoni¹, Yael Aylon¹, Zvulun Elazar⁵, Yosef Yarden⁴, Daniel Schramek²-³ and Moshe Oren¹,*

† These authors contributed equally to this work

* To whom correspondence should be addressed.

Email: moshe.oren@weizmann.ac.il.

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### Table S1. Interactome analysis of p53<sup>R273H</sup> in PANC-1 cells

| Protein name                             | Gene name | p-value | Ratio (log2) PANC-1/KO | subcellular localization                                                                 |
|------------------------------------------|-----------|---------|------------------------|-----------------------------------------------------------------------------------------|
| Dynein light chain 1, cytoplasmic        | DYNLL1    | 0.027   | 5.28                   | Cytoskeleton Mitochondria Nucleus                                                        |
| Sequestosome-1                           | SQSTM1    | 0.006   | 4.78                   | Nucleus Endosome Lysosome Endoplasmic reticulum Cytosol                                  |
| Keratin, type I cytoskeletal 19          | KRT19     | 0.015   | 3.87                   | Cytoskeleton Extracellular region or secreted Plasma membrane                             |
| Tumor suppressor p53-binding protein 1   | TP53BP1   | 0.011   | 3.45                   | Nucleus                                                                                 |
| 40S ribosomal protein S4, X isoform      | RPS4X     | 0.004   | 2.58                   | Cytoplasm                                                                               |
| Nucleoporin NUP35                        | NUP35     | 0.046   | 1.91                   | Nucleus                                                                                 |
| Annexin A2                               | ANXA2; ANXA2P2 | 0.035 | 0.90                   | Extracellular region or secreted Melanosome                                             |
Table S2. Proteins differentially regulated by p53<sup>R273H</sup> and p62 in PANC-1 cells

| Downregulated by mutp53 (increased upon mutp53 silencing) | Upregulated by mutp53 (decreased upon mutp53 silencing) |
|-----------------------------------------------------------|--------------------------------------------------------|
| RABEP1, F2RL1, ALPPL2;ALPP, TGM2, PADI2, MVP, CSTB, CRIP2, S100A4, MARCKS, MET, VAT1, FASN, CRIP1, ANPEP, CKB, NECAP2, ACAP2, DBI, AKAP12, RAB27B, CPNE2, RPS6KA3, TUBB3, HSPA2, LGALS3, LIMS1, ACSF2, HAGH, UAP1, LXN, GLRX2, APPL1, PYCARD, GJA1, EIF4G3, COG4, BID, FLNA, BZW2, HSPB1, VCL, SWAP70, TUBB2B, TRAPPC3, SOD2, CHMP2B, COMMD4, TRIM47, ZYX, NES, TMSB4X, VPS11, TPM2, PRR14L, IL18, IDH2, SNX9, CAP2, HSPA14, BZW1, SYAP1, MEA1,PP1R2;PPP1R2P3 | ACSS1,ZMYM4, TP53,PRIM1, C18orf25, FTL, HPSE, ABCG2, GNAO1, COX11, LAMA5, GPRC5C, EPCAM, PLD3, NXT2, COQ9, MICU2, CES1, DDX52, ABHD6, BPTF, NVL, ANXA3 |
| Downregulated by p62 (increased upon p62 silencing) | Upregulated by p62 (decreased upon p62 silencing) |
|---------------------------------------------------|--------------------------------------------------|
| RABEP1, F2RL1, HABP4, NDUFAF6, SIPA1, MYL9, SO2, TMEM189, CYP1B1, GLIPR2, MAN2B2, CTSV, LGALS3, GLB1, C17orf75, GM2A, CCM2, GPR64, GLRX2, I CAM1, PADI2, HSDL1, YIF1A, MARCKS, GJA1, MARC2, MARC1, LRRC1, PLL, PDLIM5, GDAP1, IL18, ALDH1B1, EPPK1, ANXA3, LIPA, GSTK1, CTBS, NMS1, AGA, ANXA1, SERPINH1, TMEM41B, HMG2A, NTPCR, CYB5A, RAB27B, CTSB, STX3, TRMT11, SL2C25A4, EPS15, NHLRC3, PRCP, MGFGE8, GPHN, IGF1R, ACSF2, DNAS E2, RPS6KA3, EHHADH, GOSR2, ERO1L, SEPTIN8, M VP, HRSP12, TMEM167A, SP100, LIMA1, FLNA, PNPO, SNX9, STBD1, GPRC5A, FLNB, CLIC4, AK3, RWDD1, AGO2, TAP1, E124, MYPN, HDHD3, SIAE, OSBP1L10, NP EPPS, Q6ZSR9, ARL6IP5, DERL2, PSMB8, H6PD, SEC31A, ETHE1, ATP13A3, LACTB2, ACTA1, ACTC1, ACT G2, ACTA2, SYNRG2, LXN, ARF4, ZYX, SYTL2, EXD2, DBT, REEP3, ARMC10, MISP, SMAP1, MTG2, PISD, SL C25A20, TMX4, NIPSAP3A, TMLHE, COBP1, ACN9, TMUB2, REQL4, FKBP3, POFU12, NADK2, SRPK2, GR R89B, GPR89A, CKMT1A, CSTB, VPS11, BID, UGP2, S100A6, VCL, YBX3, CTSZ, GSTM3, RAB32, MOSPD1, ET FDH, GALNS, TAOK1, HIBADH, APP1, ACA1, TGM2, CHMP4A, MFF, HSPA2, UGDH, CCNYL1, LANCL1, AGK, ODR4, CHMP2B, SNF8, CC2D1B, TMEM9B, LAMTOR4, ANXA4, HIBCH, KRT18, Gox8, TOR4A, COP B2, COPA, NEU1, CD70, ABCG2, RAP1B, ESCO2, ANXA 5, CMAS, CRYZ, BPHL, ASCC3, CKAP4, PROSC, PRKR A, LAMC1, COG1, CHP1, GNPAT, ARCN1, EML4, CNP, AGPAT9, DHRS4, SEC22B, CAT, CSR1P1, MIPEP, CLU, EZR, KEAP1, ARMCX3, CTS1, COPE, FAM98A, YIPF5 | RPIA, WASL, SQSTM1, HSPA13, PRI M1, NCEH1, LBR, ANPEP, UAP1, SP AG5, TRPV2, EPHB2, SL2C26A2, TGO LN2, KNSTRN, KNCMA1, NT5E, CAT2, TYSND1, EEF1A2, SL2C20A1, I TGA3, PAEBP1, MCM, SOAT1, TM EM87A, TYMS, PLAU, C8orf33, CTN NB1, CDKN2AIP, POLD3, ACADVL, GTF2E1, S100A16, C18orf25, CES1, PDXDC1, XPOT, FAR1, KIF20A, TSN, TPRG1L, AFAP1L2, AXL, WDR70, A IM1, BLVRB, COQ9, ANXA6, PHIP, A URKA, PPI4, HLF, SLC29A1, NCA PG, RPRD2, CBS, CDC33, ISYNA1, Y BX2, NMD3, LEO1, LAMA5, LMNB1, MORF4L1, ITGB1, STUB1, PHAX, SI RT1, TSNAX, SL2C5A3, UQCR10, MOPF4L2, SAP30, DDX39A, NOLC1, NO L7, PHF6, MAP1B, MRGBP, ECE1, PP IL3, SARS2, HIST1H1C, HIST2H2BE ;HIST1H2BB; HIST1H2BO; HIST1H 2BJ, CYR61, LIG1, LOXL2, FAM96B, AKAP8, RNASEH2C, NVL, HP1BP3, TOMM34, ZNF185, ZNF593, TIMM17B, LUC7L, H2AFX; HIST1H2AA, CTPS1, POLD2, YTHDC1, SL2C25A15, M DC1 |
Table S3. Enrichment analysis by DAVID of p62 regulated proteins in PANC-1 cells.

Downregulated by p62 (FDR≤0.05):

| Category                  | Term                                | Count | %    | P-Value   | Benjamini |
|---------------------------|-------------------------------------|-------|------|-----------|-----------|
| GOTERM_CC_DIRECT          | extracellular exosome               | 87    | 45.8 | 1.4E-23   | 3.8E-21   |
| GOTERM_CC_DIRECT          | mitochondrion                       | 46    | 24.2 | 4.9E-13   | 6.7E-11   |
| GOTERM_CC_DIRECT          | intracellular membrane-bound organelle | 26  | 13.7 | 5.4E-10   | 5.0E-8    |
| GOTERM_CC_DIRECT          | lysosome                             | 16    | 8.4  | 1.1E-8    | 7.8E-7    |
| GOTERM_CC_DIRECT          | membrane                             | 51    | 26.8 | 2.7E-8    | 1.5E-6    |
| GOTERM_CC_DIRECT          | COPI vesicle coat                    | 6     | 3.2  | 2.0E-7    | 8.9E-6    |
| GOTERM_CC_DIRECT          | mitochondria                         | 17    | 8.9  | 2.6E-7    | 1.0E-5    |
| GOTERM_CC_DIRECT          | endoplasmic reticulum membrane       | 26    | 13.7 | 2.4E-6    | 8.2E-5    |
| GOTERM_CC_DIRECT          | focal adhesion                       | 17    | 8.9  | 2.7E-6    | 8.2E-5    |
| GOTERM_CC_DIRECT          | transport vesicle                    | 9     | 4.7  | 5.8E-6    | 1.6E-4    |
| GOTERM_CC_DIRECT          | mitochondrial outer membrane         | 10    | 5.3  | 2.3E-5    | 5.6E-4    |
| GOTERM_CC_DIRECT          | cell-cell adherens junction          | 14    | 7.4  | 2.9E-5    | 6.5E-4    |
| GOTERM_CC_DIRECT          | perinuclear region of cytoplasm      | 19    | 10.0 | 6.9E-5    | 1.5E-3    |
| GOTERM_CC_DIRECT          | peroxisome                           | 8     | 4.2  | 9.5E-5    | 1.9E-3    |
| GOTERM_CC_DIRECT          | lysosomal lumen                      | 7     | 3.7  | 2.3E-4    | 4.3E-3    |
| GOTERM_CC_DIRECT          | actin cytoskeleton                   | 10    | 5.3  | 4.1E-4    | 7.1E-3    |
| GOTERM_CC_DIRECT          | actin filament                       | 6     | 3.2  | 5.3E-4    | 8.5E-3    |
| GOTERM_CC_DIRECT          | melanosome                           | 7     | 3.7  | 5.9E-4    | 9.0E-3    |
| GOTERM_CC_DIRECT          | cytosol                              | 53    | 27.9 | 6.6E-4    | 9.5E-3    |
| GOTERM_CC_DIRECT          | Golgi membrane                       | 16    | 8.4  | 1.1E-3    | 1.5E-2    |
| GOTERM_CC_DIRECT          | cytoplasm                            | 73    | 38.4 | 1.9E-3    | 2.4E-2    |
| GOTERM_CC_DIRECT          | mitochondrial inner membrane         | 13    | 6.8  | 2.0E-3    | 2.4E-2    |
| GOTERM_CC_DIRECT          | stress fiber                         | 5     | 2.6  | 2.2E-3    | 2.6E-2    |
| GOTERM_CC_DIRECT          | ER to golgi transport vesicle        | 4     | 2.1  | 2.3E-3    | 2.6E-2    |
| GOTERM_CC_DIRECT          | extracellular space                  | 26    | 13.7 | 2.6E-3    | 2.9E-2    |
| GOTERM_CC_DIRECT          | cell body                            | 5     | 2.6  | 3.9E-3    | 4.1E-2    |

Upregulated by p62 (FDR≤0.05):

| Category                  | Term      | Count | %    | P-Value   | Benjamini |
|---------------------------|-----------|-------|------|-----------|-----------|
| GOTERM_CC_DIRECT          | nucleoplasm | 43    | 39.4 | 5.0E-10   | 1.1E-7    |
| GOTERM_CC_DIRECT          | nucleosome | 8     | 7.3  | 1.1E-6    | 1.2E-4    |
| GOTERM_CC_DIRECT          | nucleolus  | 16    | 14.7 | 1.0E-4    | 7.4E-3    |
| GOTERM_CC_DIRECT          | nucleus    | 48    | 44.0 | 5.8E-4    | 3.2E-2    |
Fig S1.

A

| PANC-1 | KO |
|--------|----|
| Input  | IP | Input | IP |
| p53    |    | GAPDH |

B

| p53 | TAD | PRD | DBD | TD | CTD |
|-----|-----|-----|-----|----|-----|
| 1   | 61  | 94  | 292 | 325| 356 |

RMSD: 3.03 Å

C

P359-H368

wtp53

model3_p53R273H

RMSD: 3.03 Å

D

E

p53

TAD PRD DBD TD RD

331-335

F

Original

CAGATCCGTTGGCGCT
GlnIleArgGlyArg

Mutated to

GCCGCCGCTGCCGCT
AlaAlaAlaAlaAlaAla

Penta Ala substitution: 5A

PANC-1

3'UTR shp53

R273H - FL 5A

V5

GAPDH
p53R273H interacts with p62 via its C terminal domain

(A) Lysates of control and p53 knockout (KO) PANC-1 cells were subjected to immunoprecipitation with anti-p53 antibodies (PAb1801+PAb421), followed by Western blot analysis with-HRP conjugated anti-p53 antibodies.

(B) Domain organization of the human p53 and SQSTM1/p62 proteins. The PDB IDS representing the available crystal structures of different regions of the two proteins are indicated.

(C) Cartoon representation of the pairwise structural alignment of the full-length modelled structures of wtp53 (green) and p53R273H (cyan). Red circles indicate regions that differ between wt p53 and p53R273H.

(D) Representative images from molecular docking of p62 (red) with wtp53 (teal). The putative interacting residues of the two proteins are shown in stick form in the structure. The putative interactions are shown in black dotted lines.

(E) Cartoon of alanine substitution of residues 331-335 of p53R273H to generate p53R273H-5A (DNA and protein sequence).

(F) Western blot analysis of stable expression of V5 tagged intact p53R273H (FL) and p53R273H-5A (5A) in 3’UTR shp53 PANC-1 cells. Tagged p53 was visualized with anti-V5 antibody; GAPDH served as loading control.
Fig S2. p62 promotes migration of cancer cells harboring p53\textsuperscript{R273H}

(A) RT-qPCR analysis of TP53 and SQSTM1 mRNA levels in PANC-1 cells without (CTRL) or with shRNA-mediated knockdown of p53 or/and p62. Values were normalized to GAPDH mRNA. Mean + SEM from four biological repeats.

(B) Gap closure assay of PANC-1 cells without (CTRL) or with shRNA-mediated knockdown of p53 or/and p62. Cells were seeded in 12-well dishes containing ibidi culture inserts. Inserts were removed at time (t=0), and cells were allowed to migrate for 18h, at which time the cultures were photographed; representative pictures are shown. Right: quantification of % of gap area covered, from three biological repeats, 3 fields per condition. Error bars = SEM. Statistical significance was determined using one-way ANOVA and Tukey’s post hoc test of the indicated comparisons, *P-value<0.05; **P-value<0.01.

(C) Western blot analysis of p62-GFP, expressed stably in control (CTRL) and p53-silenced PANC-1 cells.

(D) Representative images of gap closure assay with the same cells as in (C).

(E) Western blot analysis of control HCT116 cells (wt), p53 knockout HCT116 cells (KO), and p53 KO cells stably overexpressing either p53\textsuperscript{R273H} or p53\textsuperscript{R175H}, without (CTRL) or with stable silencing of p62 (shp62).

(F) Representative images of gap closure assay with the same cells as in E.

(G) PANC-1 cells expressing control shRNA, p53 shRNA or p62 shRNA, were injected orthotopically into the pancreas of NOD-SCID-gamma mice (1 million cells/mouse). Tumor-free survival is plotted for 8 mice in each group (control, shp53 and shp62). Right: p-values by log-rank (Mantel-Cox) test.
Fig S3. The p53^{R273H}-p62 axis downregulates proteins associated with cell adhesion

(A) PANC-1 cells, without (CTRL) or with stable knockdown of p53 (shp53), were subjected to shRNA-mediated silencing of p62 with two different shRNA constructs. Shown is a western blot analysis of the indicated proteins in the various cell pools.

(B) PANC-1 cell lysates, without (CTRL) or with stable knockdown of either p53 (shp53) or p62 (shp62) were subjected to western blot analysis of Zyxin. GAPDH served as loading control.

(C) Western blot analysis of the indicated proteins in PANC-1 cells upon stable knockdown of p53 using two different shRNA constructs. The Connexin 43 and GAPDH panels are from a separate gel, using the same cell lysates.

(D,E) RT-qPCR analysis of Connexin 43 (GJA1) and Filamin A (FLNA) mRNA levels in PANC-1 cells, without (CTRL) or with stable knockdown of p53 (shp53) or p62 (shp62). Values were normalized to GAPDH mRNA. Mean + SEM from three biological repeats.

(F) Western blot analysis of Connexin 43 levels in 3’UTR shp53 PANC-1 cells stably expressing V5-tagged intact p53^{R273H} (FL) or p53^{R273H-5A} (5A). Connexin 43 band intensities, corrected for GAPDH in the same lane, were calculated relative to the control cells and are shown below the lanes.

(G) Immunofluorescence staining of ZO-1 (red) in PANC-1 cells, without (CTRL) or with stable knockdown of p53 (shp53) or p62 (shp62). Cells were counterstained with DAPI (blue).

(H) Western blot analysis of Connexin 43 in control (CTRL), p53-silenced and p62-silenced PANC-1 cells, without or with transient (48 hrs) overexpression of Connexin 43. RASGAP was used as loading control.

(I) Immunofluorescence staining of Connexin 43 (red) and ZO-1 (green) in PANC-1 cells, without (CTRL) or with transient (48 hrs) overexpression of Connexin 43. Scale bar= 10 μm.

(J) Western blot analysis of Connexin 43 in control (CTRL), p53-silenced and p62-silenced PANC-1 cells, without or with siRNA-mediated knockdown of Connexin 43. GAPDH served as loading control.
Fig S4.

A

B

C

D

E

F
**Fig S4. p53**<sup>R273H</sup> **and p62 promote proteasomal degradation of Connexin 43**

(A) Quantification of PLA foci of fig. 4D based on counting of 15 cells. Error bars = SEM. One-way ANOVA and Tukey’s post hoc test of the indicated comparisons. **P-value<0.01, ***P-value<0.001, NS- not significant.

(B) PANC-1 cells, stably expressing shRNA against the 3’UTR of p62 mRNA, were transiently transfected with FLAG-tagged full length (FL) p62 or p62ΔUBA. 48 hrs hours later, cell lysates were subjected to Western blot analysis with antibodies against the indicated proteins; anti-FLAG antibody was employed to visualize ectopic p62.

(C) PANC-1 cells, stably expressing shRNA against the 3’UTR of p62 mRNA, were transiently transfected with FLAG-tagged full length (FL) p62. 48 hrs hours later, cells were fixed and subjected to immunofluorescence staining of Connexin 43 (red) and p62 (green). Anti-FLAG antibody served to visualize ectopic p62.

(D) Immunofluorescence staining of Connexin 43 (red) and p115 (green) in PANC-1 cells, non-treated (NT) or treated with MG132 (10 µM) for the indicated durations (in hours). Counterstaining with DAPI. Scale bar = 10 μm.

(E) Immunofluorescence staining of Zyxin (ZYX; red) in PANC-1 cells, non-treated (NT) or treated with MG132 (10 µM) for the indicated durations (in hours). Counterstaining with DAPI. Scale bar = 10 μm.

(F) Immunofluorescence staining of K48- or K63-based polyubiquitin chains (red) and p62 (green) in PANC-1 cells, using a limiting amount of p62 antibody (1: 700 dilution). Scale bar = 10 μm.