

Absence of NKG2D ligands defines leukaemia stem cells and mediates their immune evasion

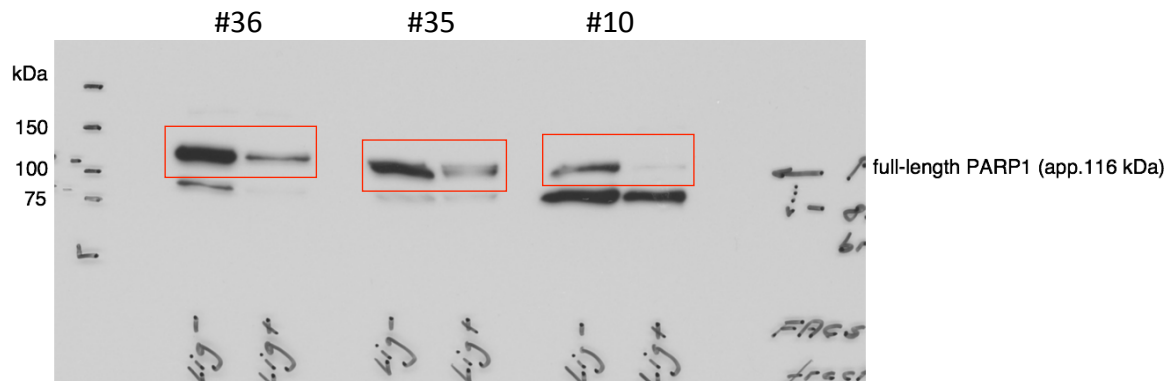
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Supplementary Figure 1: Immunoblot data. Uncropped scans of western blots with size marker indication.

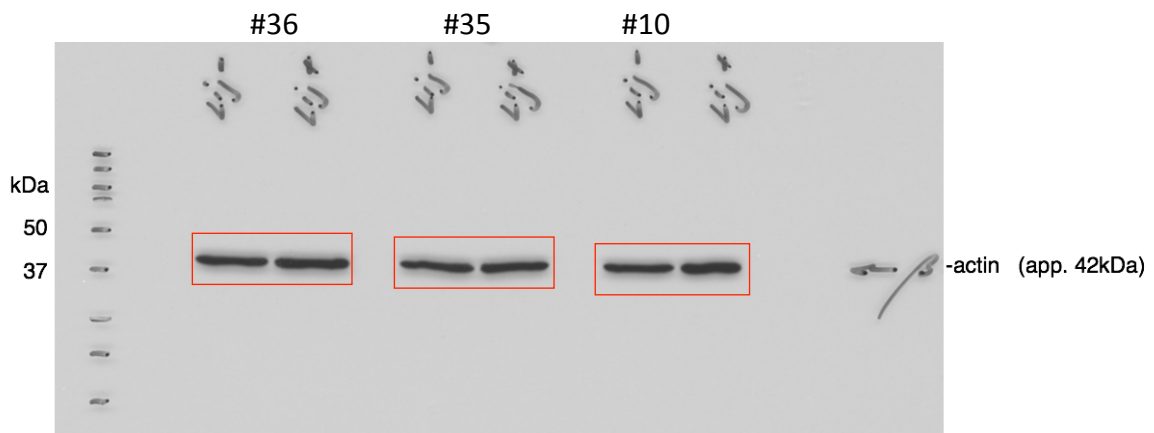
a) Figure 4b

Anti-PARP1 (CST #9542)



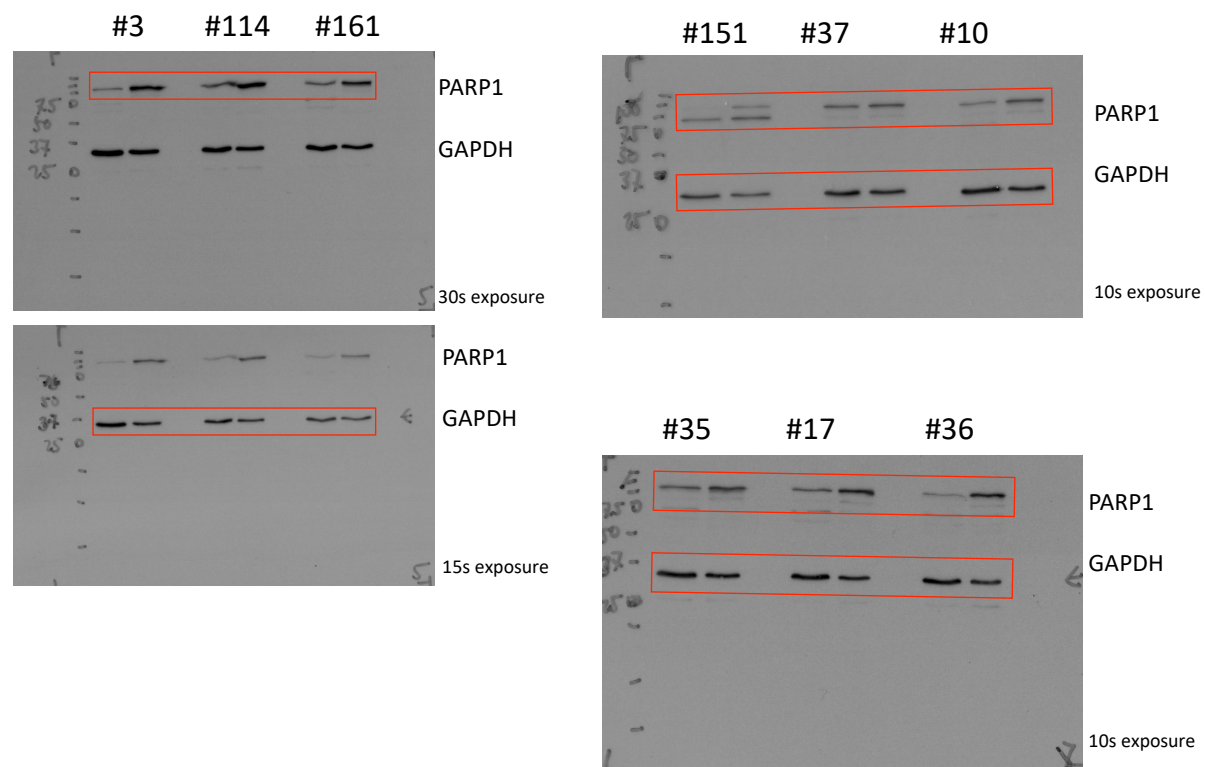
anti PARP1 Ab # 9542 by Cell Signaling Technology detects full-length PARP1 protein at an apparent size of app. 116 kDa and derived breakdown products of which (e.g. a 89 kDa fragment as found here), see product data sheet for reference

Anti beta-Actin (CST #3700, processing control)



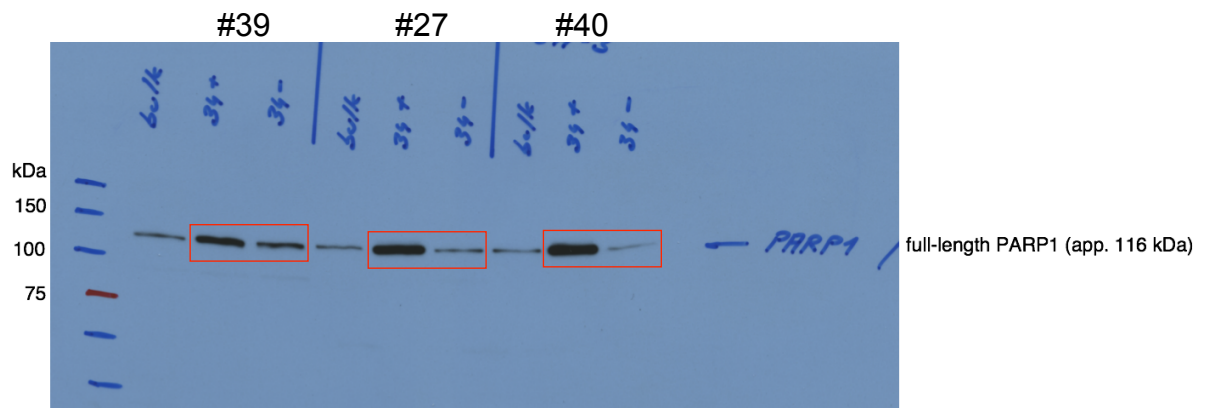
b) Extended Data Figure 8b

Anti-PARP1 (CST #9542) and anti-GAPDH (#5174P, loading control)

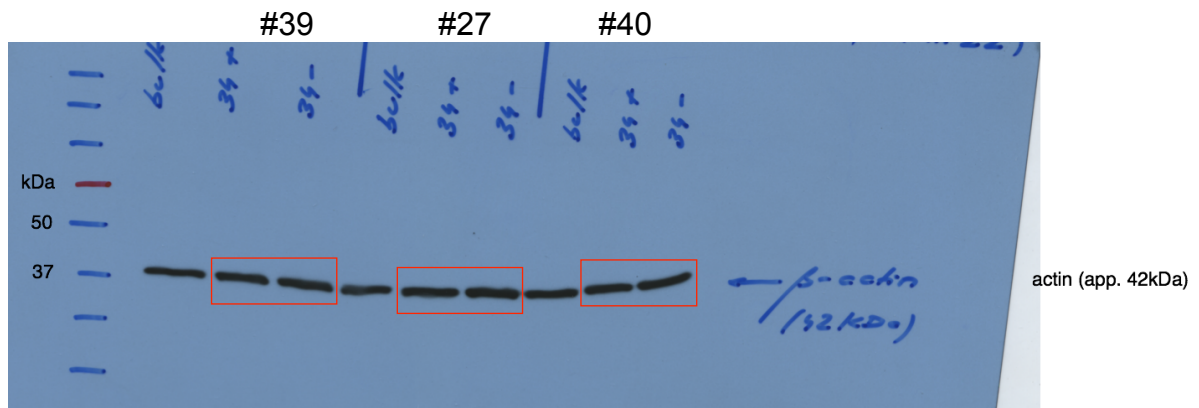


c) Extended Data Figure 8c

Anti-PARP1 (CST #9542)

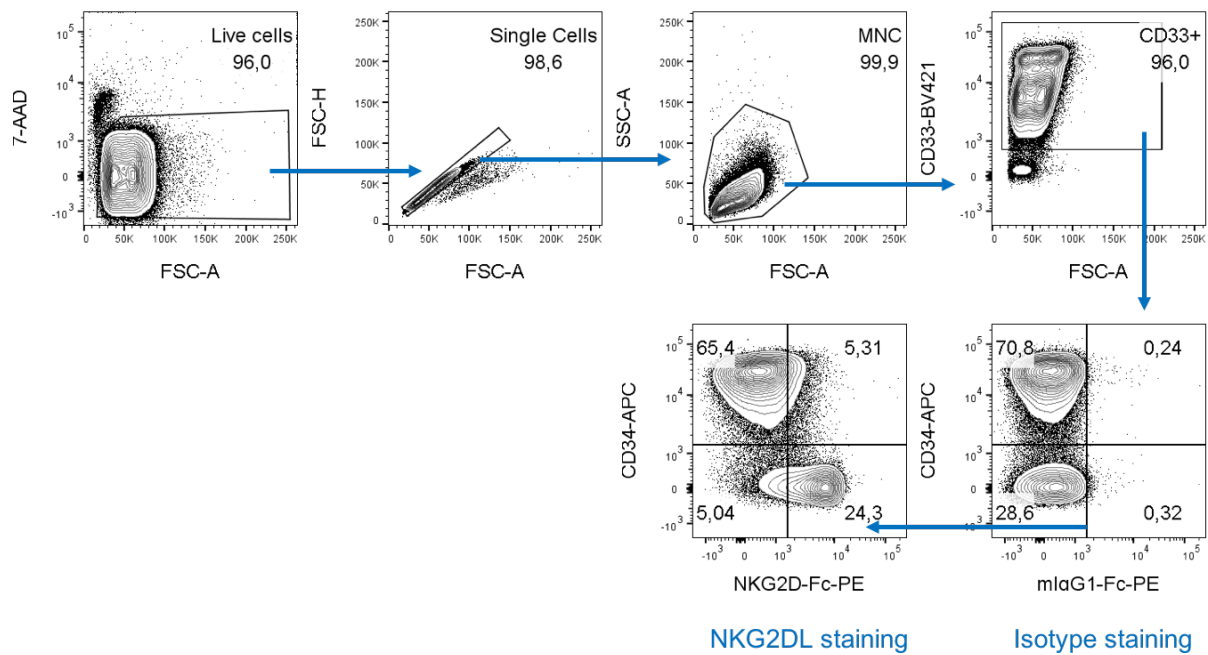


Anti beta-Actin (CST #3700, loading control)

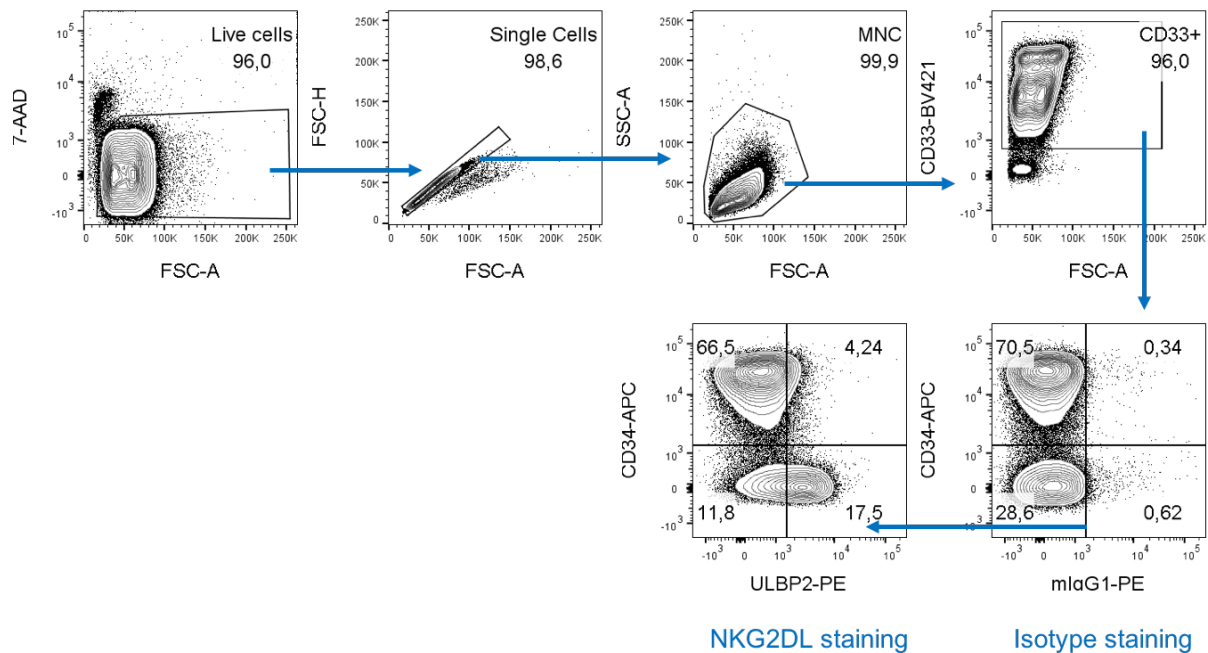


Supplementary Figure 2: Gating Strategies

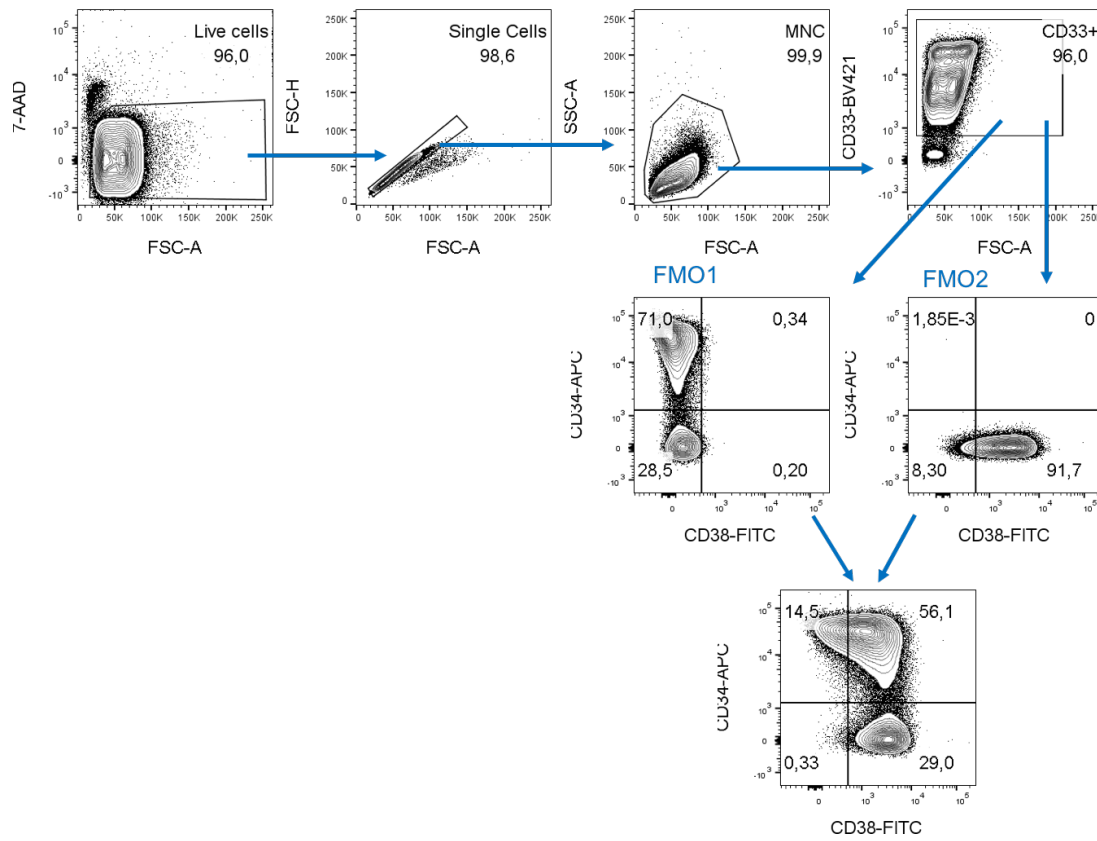
a) NKG2DL staining: NKG2D-Fc chimera and streptavidin-PE

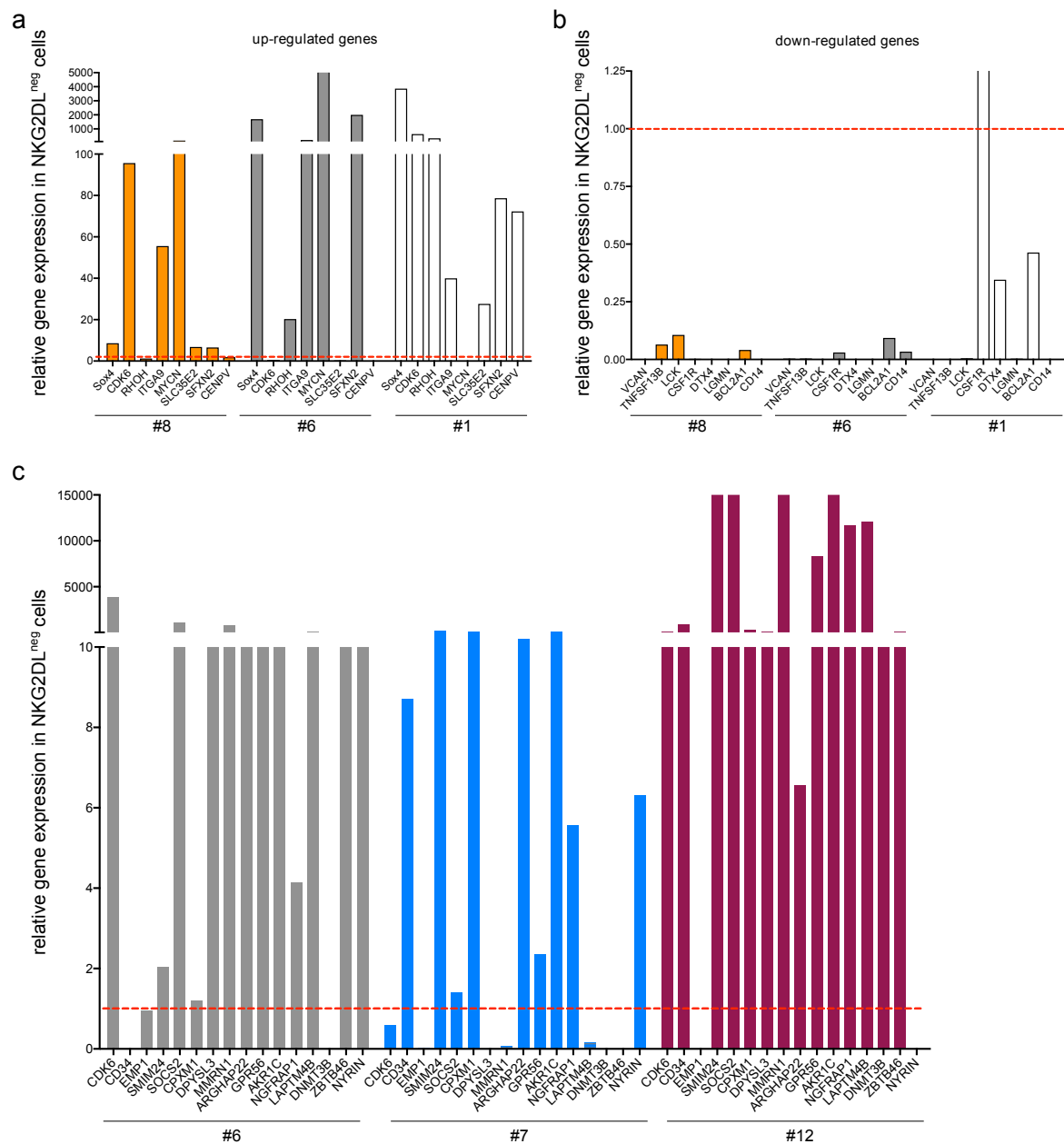


b) Single ligand staining (MICA, MICB, ULBP1, ULBP2/5/6, here exemplified for ULBP2)

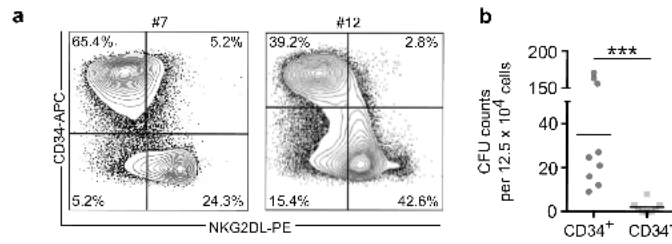


c) CD34/CD38 staining





Supplementary Figure 3: Validation of differential gene expression by quantitative real-time PCR. (a-b) To validate differential expression in NKG2DL⁻ versus NKG2DL⁺ subpopulations, qRT-PCR analysis was performed on genes found up- (a) or down-regulated (b) in the microarray data set; shown are results of n=3 AML (#8, #6, #1), analysed each in technical triplicates; the dotted line represents gene expression in NKG2DL⁺ subpopulations. (c) qRT-PCR verification of the 17 gene stemness score; analysis was performed in the same manner as in a-b.



Supplementary Figure 4: Investigation of CD34⁺ and CD34⁻ AML subpopulations in CFU assays. (a) Representative flow cytometry analysis and (b) analysis of sorted subpopulations in CFU (b: #7, #8, #12, technical triplicates). A Mann-Whitney Test was performed for statistical analysis. Note that CD34⁺ subpopulations display lower percentages of NKG2DL⁺ cells and have increased stemness properties. (* p < 0.05, ** p < 0.01, *** p < 0.001).

a

>Parp1 binding motif

GTGGGATTTGAAATACGCTTGAAGGAGCTGAC**TTTGACTC**CCGGAGATGATGGGGAAGAGAAATCAGAAGGGACCAAGGATGGTGATGTTCTTAAGAGAACTGAGGAGGAAGAGAGGATGATATGGTGCGACAGCTATAGAGAGTCTT < 150
V G L K * R * R S * L * L P E M M G K E E I R R D Q G W * C S * E K L R R K R G * Y G G R R I E S L
W D * N S V E G A D F D S R R * W G K R K S E G T K D G D V L K R N * G G R E D D M V A D V * R V F
G I E I A L K E L T L T P G D * D G E R G N Q K C P R H V M F L R E T E F E R M I W N Q T V Y R E S L
CACCTTAACCTTTATCGGAACCTCCCTGACT**AAACTGAGGG**CCCTCTACTACCCCTTCTCCCTTTAGTCTCTCCCTGATCTCTGAACTACAAGAAATCTCTTTGACTCCCTCTCTCTCTCTATACACACCGCTGCATATCTCTCAGAA
10 20 30 40 50 60 70 80 90 100 110 120 130 140

TGTAGATCTCTCACATTGGAGGGGACTATGTCGAGGTCACAGATGTCCTAAGCAGGCTGGAAAAGGAGCTTGGTGTGTAGTGAACACAGGAGCGCCCTCTCTGGCCCTGTGATCACCCAGGAGCTGAATAGAG < 300
C R S L T L E G T H V G G T D V L R Q A G K G S L E R A W C C S E P Q G A A S L A L * S P R D * I E
V D L S H W R G L W S E V Q M S * G R L E K G V W R E L G V V V N H R E P P P W P C D H P G T E * R
* I S H I G G D Y G R R Y R C P K A G W K R E S G E S L V L * * T T G S R L L L G P V I T Q G L N R E
ACATCTAGAGAGTGAACCTCCCTGATACAGCCTCCATGTCACAGGATTCGTCGAGCTTTCCCTCAGACCTCTCTGAACTACAAGAAATCTCTTTGACTCCCTCTGGCGGAGGAACCGGACACTGTGGGCTCTGACTTATCTC
160 170 180 190 200 210 220 230 240 250 260 270 280 290

>Parp1 binding motif >Parp1 binding motif

AGGCGCCCTGGGAGACTTCAGACACTAGAGGATATAAGGGGGTGAAGGGGGGCTGGCTGG**TTTGAGTCA**AAGGGAGGAGAAGGAGATTATAAGCTGAAACGTCTAAGAGAG**TTTGTGGT**CTGAGCGGTTCTACTGCGGAGGCTGCTTC < 450
R R P W E T S D T * R I * G G E R G A W L * V K G R R R R L * S * N V * E S L W S E R F Y C G R C F
G G P G R L O T L R G Y K G V K G G P G F E S K G G E C D Y K A E T S K R V C O L S G S T A A G A S
A A L G D F R H L E D I R G * K G G L A L S Q R E E K E I I K L K R L R E F V V * A V L L R R Q V V L L
TCCCGGGAGCCCTCTGAAGTCTGTGAATCTCTATATTTCCCACTTTCCCCCGGAGCC**GAACCTCAGT**TTCCCTCTCTCTCTCTAATATTTGACTTTTCAGATTCTCT**AAACAC**CCAGACTGCCAAGATGACCGCTCCACGAAG
310 320 330 340 350 360 370 380 390 400 410 420 430 440

TGAGAGGACAGAGTGGCTGAGATCTGGAACAGGCTGCAAAATCTGGTCACTGGTCTCATTGCCAGTAACCTGTGCGCGGTTGAGGAGTGTGTTGGAGAATAGCCACCGCTGTCTCTCTCTGGAAGAAACAAGCAGTGAGAGCGGG < 600
* F A E V A E I W K Q V C K S G H W S H C Q * R C A R L R E C V G R I A T R C L S W K E Q A S F S R
E R Q R W L R S G N R S A N L V T G L I A S N A V R G * G S V L G E * P R V V C P G R N K P V R A G
R G R G G * D L E T G L Q I W S L V S L P V T L C A V E G V C W E N S H A L S V L E G T S Q * E P V
ACTCTCGCTCTCCAGGACTCTAGACCTTTCTCAGAGCTTTGACACGAGTACAGGTGTCATTGACACGCGCACTCCCTCAGACACCTCTTATCGGTGGCAACACAGAGACCTCTCTTTTGGTCACTCTCGGCC
460 470 480 490 500 510 520 530 540 550 560 570 580 590

b

GTGGTGGGAGAGCTGTAGAGAGCTCTTGTAGATCTGTCTATATTGAAGGGGACTATGGTCCAGAGGTACAGATGTCTAAACAGGCTGGAAGAGGAGCTCTGAGAGAGCTTGGTGTGTGAATGAACCATGGGAGCCGCTCGTTGGC < 150
V V G D V * R V L V D L S Y * R G L W S Q R Y R C P K T G W K R E S G E S L V L * * T M G S R L V G
W W E T C R E S L * I C H I E G D Y G P R G T D V L K Q A G K G S L E R A W C N E P W G A A S L A
G C R R V E S P C R S V I L K G T H V P E V Q M S * H R L E K G V W R E L G V V M N H G E P P R W P
CACCACCCCTGACATCTCTCAGGAACATCTAGACAGTATAAATCTCCCTGATACAGGGTCTCCATGCTACAGGATTTGTCGACCTTTCCCTCAGACCTCTCTCGAACCAACATTACTTGTGTACCCCTCGCGGAGCAACCG
10 20 30 40 50 60 70 80 90 100 110 120 130 140

>Parp1 binding motif >Parp1 binding motif

CCTGTGATTACCCAGGAAGTGAATAGAGAGGGGGCCCTGGGAGACCTCAGACACTTAGAGGATATAAGGGGGTGAAGGGGGGACTGGCT**TTTGAGT**CGAAGGGAGGAGAGGAGATTTATATAGCTGAAACGTCTAAGAGAA**TTTGTGAT** < 300
P V I T Q E L N R E G A L G D L R H L E D I R G * K G G P G F E S K G G E G D Y I A E T S K R I C D
L * L P R N * I E R G P W E T S D T * R I * G G E R G D L A L S R R E E K E I I * L K R L R E F V I
C D Y P G T E * R G G P G R P Q T L R G Y K G V K G G T W L * V E G R R R R R L Y S * N V * E N L * S
GGACACTAATGGGTCTTGACTTATCTCTCCCGGGAGCCCTCTGGAGTCTGTGAATCTCTATATTTCCCACTTTCCCCCTGGAGCC**AAACTCAG**CTTCCCTCTCTCTCTCTAATATATCGACTTTTCAGATTTCTT**AAACACTA**
160 170 180 190 200 210 220 230 240 250 260 270 280 290

CTGAGCGTTTCTACTGGGGCAAGTGCCTTGAAGGCAGAGGGGCTGAGATCTGGAACAGGCTGCAAAATCTGGTCACTGGTCTCATTGCACTAAGCTGTGCGCGGTTGAGGGAGTGATTTGGGAGAAAACACCGGTTGCTGTGCT < 450
L S V S T G A S A S E R Q R R L R S G N R S A N L V T G L I A V T L C A V E G V Y W E K N H A L S V
* A F L L G Q V L L K G R G G * D L E T G L Q I W S L V S L O * R C A R L R E C I G R K T T R C L S
E R F Y V G K C F * K A E A A E I W K Q V C K S G H W S H C S N A V R G * G S V L G E K P R V V C P
GACTCGAAGATGACCCGTTACGAAGACTTTCCGTCTCCCGGACTCTAGACCTTTGTCAGACGTTTAGACAGCTGACAGGTAACGTATTGCGACACCGCCAACTCCCTCACATAACCTCTTTTGGTGGCAACACAGACAG
310 320 330 340 350 360 370 380 390 400 410 420 430 440

CCGGAAGGAACAAGCCAGTGAGAGCGGGCTGATGGAGGACCGGCGAAAGGGGCTTGGTGAAGCCCGCGCTCCTTTGGGGTGGGAATGCGGGGATGGGGTGGTGCAGATGCAAGGAGGGCGGACAGGGTCCAGGTCTGCTCTATAAGTTT < 600
P E G T S Q * E P A * W E D R R K G L G E A R A P W G W E C G D G V V A H Q C G R O G P C R A H K F
R K E Q P E S R P D G R T G E R G L V K P A L L G C G C N A C M G W S R C R E G C D R V Q V V L I S L
G R N K P V R A G L M G G P A K G A W * S P R S L G V G M R G W G G R D A G R A T G S R S C S * V W
GGCCTTCCTTGTTCGGTCACTCTCGGCGGACTACCTCTCGGCGCTTCCCGGAACCACTTCGGGCGGAGGAACCCACCTTACGCCCTACCCACAGCGGTACGTCCCTCCCGCTGTCCCGAGGTCCAGCAGGATTTCAAA
460 470 480 490 500 510 520 530 540 550 560 570 580 590

Supplementary Figure 5: *In silico* analysis of PARP1 binding to the MICA (a) and the MICB promoter (b). Shown are potential binding sites for PARP1.