Transcriptional Perturbation of Protein Arginine Methyltransferase-5 exhibits MTAP-selective oncosuppression

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SUPPLEMENTARY TABLES

Supplementary table I. Patient characteristics

|                      | UK cohort (n=79) | AUS cohort (n=100) |
|----------------------|------------------|-------------------|
|                      | MTAP CN loss (n=33) | MTAP WT (n=46) | MTAP CN loss (n=63) | MTAP WT (n=37) |
| Age at diagnosis     | 66 (61-74)       | 63 (55-70)       | 69 (62-74)       | 64 (57-74)       |
| (median (IQR))       |                  |                  |                  |                  |
| Sex (N (%))          |                  |                  |                  |                  |
| M                    | 29 (88)          | 38 (83)          | 53 (84)          | 28 (76)          |
| F                    | 4 (12)           | 8 (17)           | 10 (16)          | 9 (24)           |
| Survival status      |                  |                  |                  |                  |
| (N (%))              |                  |                  |                  |                  |
| Alive                | 1 (3)            | 0 (0)            | 4 (6)            | 6 (16)           |
| Dead                 | 32 (97)          | 46 (100)         | 59 (94)          | 31 (84)          |
| Overall survival (months) (median (95% CI)) | 12.5 (95%CI: 6.8-18.2) | 17.6 (95%CI: 6.5-28.7) | 8.7 (95%CI: 4.6-12.8) | 22.7 (95% CI: 11.4-33.9) |
| IMIG Stage (N (%)) | | | | |
| 1 | 0 (0) | 1 (2) | 2 (3) | 3 (8) |
| 2 | 4 (12) | 7 (15) | 24 (38) | 14 (38) |
| 3 | 24 (73) | 28 (61) | 30 (48) | 17 (46) |
| 4 | 5 (15) | 10 (22) | 7 (11) | 3 (8) |
| Histology (N (%)) | | | | |
| Epithelioid | 28 (85) | 43 (93) | 37 (59) | 26 (70) |
| Biphasic | 5 (15) | 3 (7) | 18 (28) | 4 (11) |
| Sarcomatoid | 0 (0) | 0 (0) | 8 (13) | 7 (19) |
| Neoadjuvant chemotherapy administered (N (%)) | 1 (3) | 4 (9) | 1 (2) | 0 (0) |
### Supplementary table 2 GSEA analysis

| Entrez Gene ID | Gene Symbol | Gene Description |
|---------------|-------------|------------------|
| 2029          | ENSA        | endosulfine alpha |
| 11479         | ELFN2       | extracellular leucine-rich repeat and fibronectin type III domain containing 2 |
| 26472         | PPP1R14B    | protein phosphatase 1, regulatory (inhibitor) subunit 14B |
| 23193         | GANAB       | glucosidase, alpha; neutral AB |
| 22927         | HABP4       | hyaluronan binding protein 4 |
| 85014         | TMEM141     | transmembrane protein 141 |
| Gene ID | Gene Symbol | Gene Name                      | Description                                                                 |
|---------|-------------|--------------------------------|-----------------------------------------------------------------------------|
| 11054   | OGFR        | opioid growth factor receptor  |                                                                             |
| 84717   | HDGFRP2     | hepatoma-derived growth factor-related protein 2 |                                                                             |
| 84263   | HSDL2       | hydroxyysteroid dehydrogenase like 2 |                                                                             |
| 85364   | ZCCHC3      | zinc finger, CCHC domain containing 3 |                                                                             |
| 56993   | TOMM22      | translocase of outer mitochondrial membrane 22 homolog (yeast) |                                                                             |
| 11487   | OSBPL59     | oxysterol binding protein-like 5 |                                                                             |
| 84331   | FAM195A     | family with sequence similarity 195, member A |                                                                             |
| 90317   | ZNF616      | zinc finger protein 616 |                                                                             |
| 84687   | PPP1R9B     | protein phosphatase 1, regulatory subunit 9B |                                                                             |
| 11570   | EVI5L4      | ecotropiviral integration site 5-like |                                                                             |
| 2801    | GOLGA2      | golgin A2 |                                                                             |
| 57659   | ZBTB4       | zinc finger and BTB domain containing 4 |                                                                             |
| 5532    | PPP3CB      | protein phosphatase 3, catalytic subunit, beta isozyme |                                                                             |
| 23258   | DENND5A     | DENN/MADD domain containing 5A |                                                                             |
| 26000   | TBC1D10B    | TBC1 domain family, member 10B |                                                                             |
| 23061   | TBC1D9B     | TBC1 domain family, member 9B (with GRAM domain) |                                                                             |
| 23299   | BICD2       | bicaudal D homolog 2 (Drosophila) |                                                                             |
| 4720    | NDUFS2      | NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa |                                                                             |
| Gene Symbol | Description |
|-------------|-------------|
| 10811 NOXA1 | NADPH oxidase activator 1 |
| 5886 RAD23A | RAD23 homolog A (S. cerevisiae) |
| 59 ACTA2 | actin, alpha 2, smooth muscle, aorta |
| 11193 WBP4 | WW domain binding protein 4 (formin binding protein 21) |
| 29924 EPN1 | epsin 1 |
| 57804 POLR2D | polymerase (DNA-directed), delta 4 |
| 37492 C19orf68 | OMA1 homolog, zinc metalloprotease (S. cerevisiae) |
| 29123 ANKRD11 | ankyrin repeat domain 11 |
| 23373 CRTC1 | CREB regulated transcription coactivator 1 |
| 25875 LETMD1 | LETM1 domain containing 1 |
| Gene ID | Gene Symbol | Description |
|---------|-------------|-------------|
| 79144   | PPDPF       | pancreatic progenitor cell differentiation and proliferation factor homolog (zebrafish) |
| 54760   | PCSK4       | proprotein convertase subtilisin/kexin type 4 |
| 9790    | BMS1        | BMS1 homolog, ribosome assembly protein (yeast) |
| 8665    | EIF3F       | eukaryotic translation initiation factor 3, subunit F |
| 7586    | ZKSCAN1     | zinc finger with KRAB and SCAN domains |
| 1632    | ECI1        | enoyl-CoA delta isomerase 1 |
| 166     | AES         | amino-terminal enhancer of split |
| 23265   | EXOC7       | exocyst complex component 7 |
| 55624   | POMGN1      | protein O-linked mannose beta1,2-N-acetylg glucosaminyl transferase |
| 3913    | LAMB2       | laminin, beta 2 (laminin S) |
| 8733    | GPAA1       | glycosylphosphatid ylinositol anchor attachment protein 1 homolog (yeast) |
| 2036    | R3HCC1      | R3H domain and coiled-coil containing 1 |
| 23351   | KHNYP       | KH and NYN domain containing |
| 23229   | ARHGEF9     | Cdc42 guanine nucleotide exchange factor (GEF) 9 |
| 60343   | FAM3A       | family with sequence similarity 3, member A |
| 25870   | SUMF2       | sulfatase modifying factor 2 |
| 593     | BCKDH       | branched chain keto acid dehydrogenase E1, alpha polypeptide |
| Index | Gene ID | Description                                                                 |
|-------|---------|------------------------------------------------------------------------------|
| 14434 | ZNF664  | zinc finger protein 664                                                       |
| 79090 | TRAPPC 6A | trafficking protein particle complex 6A                                       |
| 80854 | SETD7   | SET domain containing (lysine methyltransferase) 7                           |
| 6425  | LOC6428 | uncharacterized LOC6428                                                       |
| 57647 | DHX37   | DEAH (Asp-Glu-Ala-His) box polypeptide 37                                    |
| 7637  | ZNF84   | zinc finger protein 84                                                        |
| 11367 | SDSL    | serine dehydratase-like                                                      |
| 60686 | C14orf93| chromosome 14 open reading frame 93                                          |
| 23408 | SIRT5   | sirtuin 5                                                                    |
| 55420 | JPX     | JPX transcript, XIST activator (non-protein coding)                          |
| 90321 | ZNF766  | zinc finger protein 766                                                       |
| 93621 | MRFAP1  | Morf4 family associated protein 1                                             |
| 79088 | ZNF426  | zinc finger protein 426                                                       |
| 10013 | LOC1001 | uncharacterized LOC1001                                                       |
| 1564  | 31564   |                                                                              |
### Supplementary Table 3 Connectivity map analysis

| Drug                                           | Replicate | p value     | Z score     |
|------------------------------------------------|-----------|-------------|-------------|
| BRD-K71103788 _duloxetine hcl                  | 48        | 1.85E-10    | -6.2659907 |
| BRD-K75641298 _METOCLOPRAMIDE HYDROCHLORIDE    | 29        | 2.77E-09    | -5.8298123 |
| BRD-K36862742 _hydro-flumethiazide              | 141       | 3.04E-08    | -5.4165957 |
| BRD-K99964838 _S1014                           | 66        | 6.16E-08    | -5.2888016 |
| BRD-A45889380 _QUINACRINE HYDROCHLORIDE        | 206       | 8.03E-08    | -5.240004  |
| BRD-K91699951 _benzonatate                     | 234       | 1.39E-07    | -5.1383754 |
| BRD-K71879491 _ATRA                            | 205       | 2.17E-07    | -5.0531872 |
| BRD-A51820102 _econazole                       | 36        | 2.22E-07    | -5.0489293 |
| BRD-K37270826 _mifepristone                    | 125       | 2.62E-07    | -5.0175175 |
| BRD-K70487031 _cis-(Z)-FLUPENTHIXOL            | 27        | 3.03E-07    | -4.9893544 |
| BRD-K63675182 _triflupromazine                 | 179       | 5.12E-07    | -4.8868451 |
| BRD-K989375097 _pirenzepine                    | 36        | 6.96E-07    | -4.826062  |
| BRD-K19706299 _MRE-269                         | 99        | 7.54E-07    | -4.8102311 |
| BRD-K32164935 _TOLAZAMIDE                      | 353       | 1.17E-06    | -4.7219067 |
| BRD-K28936683 _ketotifen                       | 85        | 1.18E-06    | -4.7204909 |
| BRD-A23723433 _paclitaxel                      | 48        | 1.22E-06    | -4.7138292 |
| BRD-K39621635 _artemether                      | 34        | 1.25E-06    | -4.7081455 |
| BRD-K81418486 _SAHA_trt_poscon                | 12        | 1.36E-06    | -4.6910799 |
| BRD-K92093830 _doxorubicin                     | 159       | 1.58E-06    | -4.6599437 |
| BRD-A49160188 _donepezil                       | 73        | 1.74E-06    | -4.6404777 |
| BRD-K70487031 _flupentixol                     | 37        | 2.14E-06    | -4.5971052 |
| BRD-K10916986 _S1527                          | 67        | 2.20E-06    | -4.5915996 |
| BRD-K92778217 _mefenamic-acid                  | 160       | 2.39E-06    | -4.5741376 |
| BRD-K13514097 _S1120                          | 58        | 3.79E-06    | -4.4764562 |
| BRD-K45995181 _Auranofin                       | 3         | 3.84E-06    | -4.474083  |
| BRD-K66175015 _S1011                          | 59        | 4.68E-06    | -4.4316279 |
| BRD-K53737926 _amitryptiline                  | 137       | 4.95E-06    | -4.4194573 |
| BRD-K52075040 _cerulenin                       | 210       | 5.16E-06    | -4.4103748 |
| BRD-K20075662 _betazole                        | 143       | 5.27E-06    | -4.4059091 |
| BRD-K33106508 _cytarabine                      | 48        | 5.52E-06    | -4.395691  |
| BRD-K91290917 _Amodiaquin dihydrochloride dihydrate | 57    | 5.65E-06    | -4.3905396 |
| BRD-K81418486 _vorinostat                      | 1303      | 6.50E-06    | -4.360225  |
| BRD-K93754473 _tamoxifen                       | 843       | 6.59E-06    | -4.3571007 |
| BRD-K25433859 _maprotiline                    | 37        | 9.22E-06    | -4.2829289 |
| BRD-K39339537 _epirizole                       | 217       | 1.16E-05    | -4.2316941 |
| BRD-A45889380 _Quinacrine dihydrochloride dihydrate | 10    | 1.58E-05    | -4.1619013 |
| BRD-K81528515 _HY-10159                        | 66        | 1.60E-05    | -4.1585908 |
SUPPLEMENTARY METHODS

Clonogenic assays

5000 cells per well were seeded in 12 well plates and left untreated or treated with EPZ015666 (1 µM, 5 µM, 10 µM). The PRMT5 inhibitor EPZ015666 was obtained from Selleckchem (Ely, UK).

Five days after treatment, cells were fixed on ice in methanol for 20 minutes. Cells were then stained with crystal violet (Sigma, Gillingham, UK) for 10 minutes. Colonies were dissolved in 30% acetic acid to allow quantification. Each treatment condition was measured in triplicate.

siRNA transfections

The non-silencing control (NT) and siRIOK, were obtained from and Qiagen (Manchester, UK). siRNA transfections (20 nM) were performed using the RNAiMAX transfection reagent (Invitrogen, Paisley, UK) according to manufacturer’s instructions.
Flow Cytometry

Samples were analysed on a BD FACS Calibur flow cytometer machine, using Cell Quest Pro software (Becton Dickinson, Oxford, UK). Cells were fixed after 16, 32, 72 and 120 hours of treatment with quinacrine, EPZ015666 or after silencing of PRMT5 and stained with propidium iodide (Sigma).

Gene expression

NCI-H2591 and NCI-H2052 cells were transfected with non-silencing control (NT), siPRMT5 Qiagen and siPRMT5 Dharmacon. RNA was extracted 120h after transfection with the RNeasy Mini Kit (Qiagen, Manchester, UK) and 100 ng of RNA were hybridised on the SurePrint G3 Human Gene Expression v3 8x60k array (Agilent, Cheadle, UK).

Gene expression dataset was analysed using Limma in R/Bioconductor software package. Comparisons were performed siQ vs siNT, siD vs siNT. Gene lists were then filtered by fold change ≥2, unadjusted p value ≤ 0.05. Webgestalt (http://www.webgestalt.org/) was used to perform Gene Ontology, KEGG pathway, Pathway Common, Wiki Pathway. GSEA Molecular Signatures Database (http://software.broadinstitute.org/gsea/msigdb/index.jsp) was used to investigate 85 upregulated gene set.
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. A) Cells were transfected with siNT or siRIOK1 20 nM. Cell proliferation was measured by clonogenic assay. Data were normalized to siNT controls (NCI-H2591: p= n.s.; NCI-H2052: p=0.0001; NCI-H2452: p=n.s.; MPP89: p= n.s.). The levels of PRMT5 expression and H4 arginine 3 symmetrical di-methylation (H4R3me2S) were measured by western blot 72 hours after transfection. These gels have been cropped and the full length gels are presented in Supplementary Figure 3 M-O. B) Flow cytometry plot showing cells transfected with siNT or siPRMT5 for 120 hours. C) Cells were left untreated or treated with EPZ015666 1µM, 5 µM and 10µM. Cell proliferation was measured by clonogenic assay 5-7 days after treatment. Data were normalized to untreated controls (NCI-H2591: 1 µM p=0.0176; 5 µM p=0.0001; 10 µM p=0.0001; NCI-H2052: 1 µM p=n.s.; 5 µM p= n.s. 10 µM p=0.0033; MPP89: 1 µM p=n.s.; 5 µM p= n.s. 10 µM p= n.s.). D) Flow cytometry plot showing cells left untreated or treated with EPZ015666 5 µM and 10µM for 72 hours.

Supplementary Figure 2. Flow cytometry plot showing cells left untreated or treated with quinacrine 1 µM for 120 hours. B) Flow cytometry plot showing cells transfected with siNT, sic-JUN 20 nM, for 120 hours.

Supplementary Figure 3. Uncropped images of full length gels used throughout the manuscript. A-C: Full length gels of cropped gels in Figure 1C (A: NCI-H2591; B: NCI-H2052; C: MPP89); D-F: Full length gels of cropped gels in Figure 1D (D: NCI-H2591; E: NCI-2052; F: MPP89); G-I: Full length gels of cropped gels in Figure 3C (G: NCI-H2591; H: NCI-H2052; I: MPP89); J-K: Full length gels of cropped gels in Figure 4B (J: NCI-H2591; K: NCI-H2052); L: Full length gel of cropped gel in Figure 4E; M-O:
Full length gels of cropped gels in Supplementary Figure 1A (M: NCI-H2591; N: NCI-H2052; O: MPP89).

Supplementary Figure 1.
Supplementary Figure 2.
Supplementary Figure 3.
