

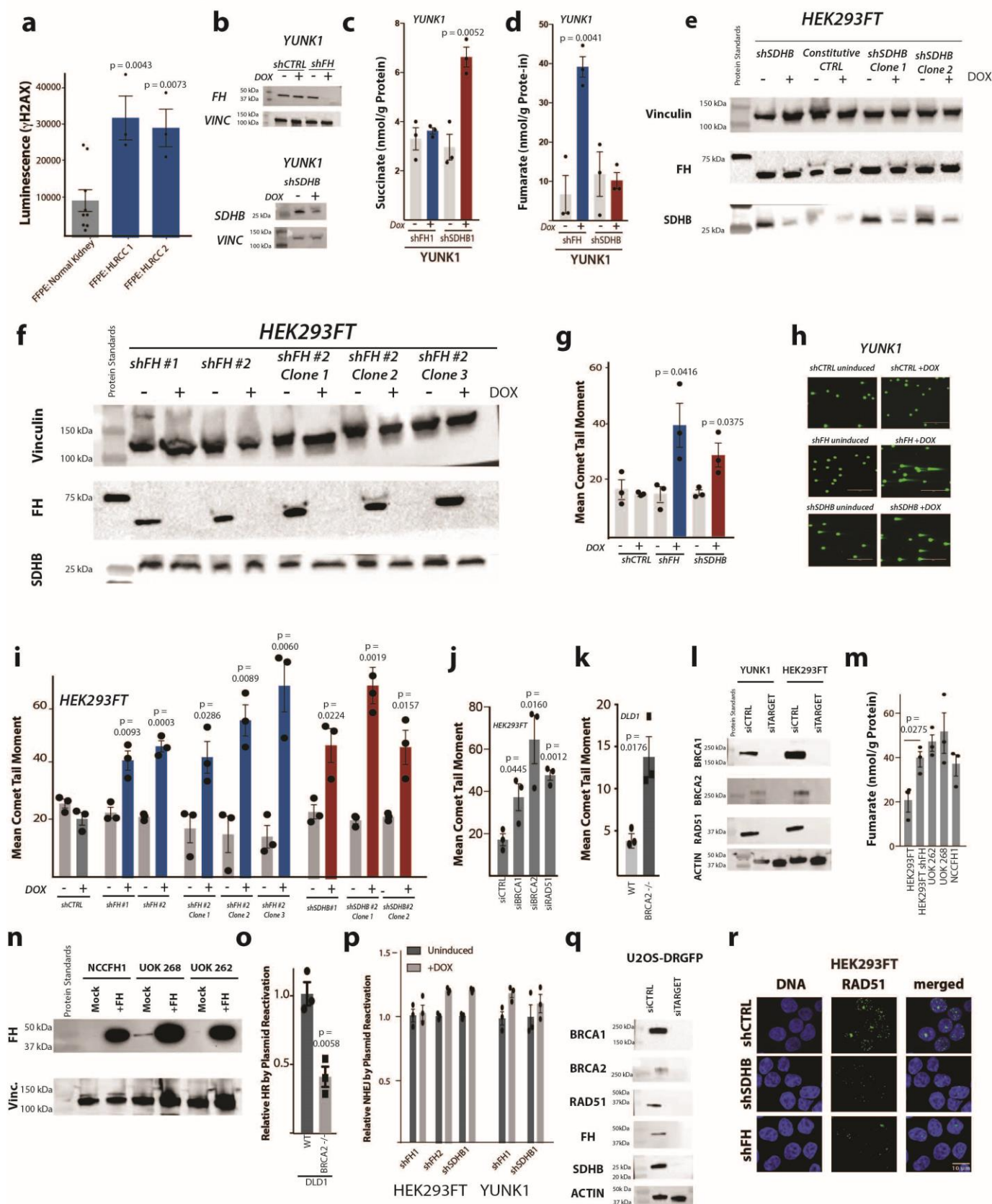
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Krebs-cycle-deficient hereditary cancer syndromes are defined by defects in homologous-recombination DNA repair

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Supplementary Figure 1

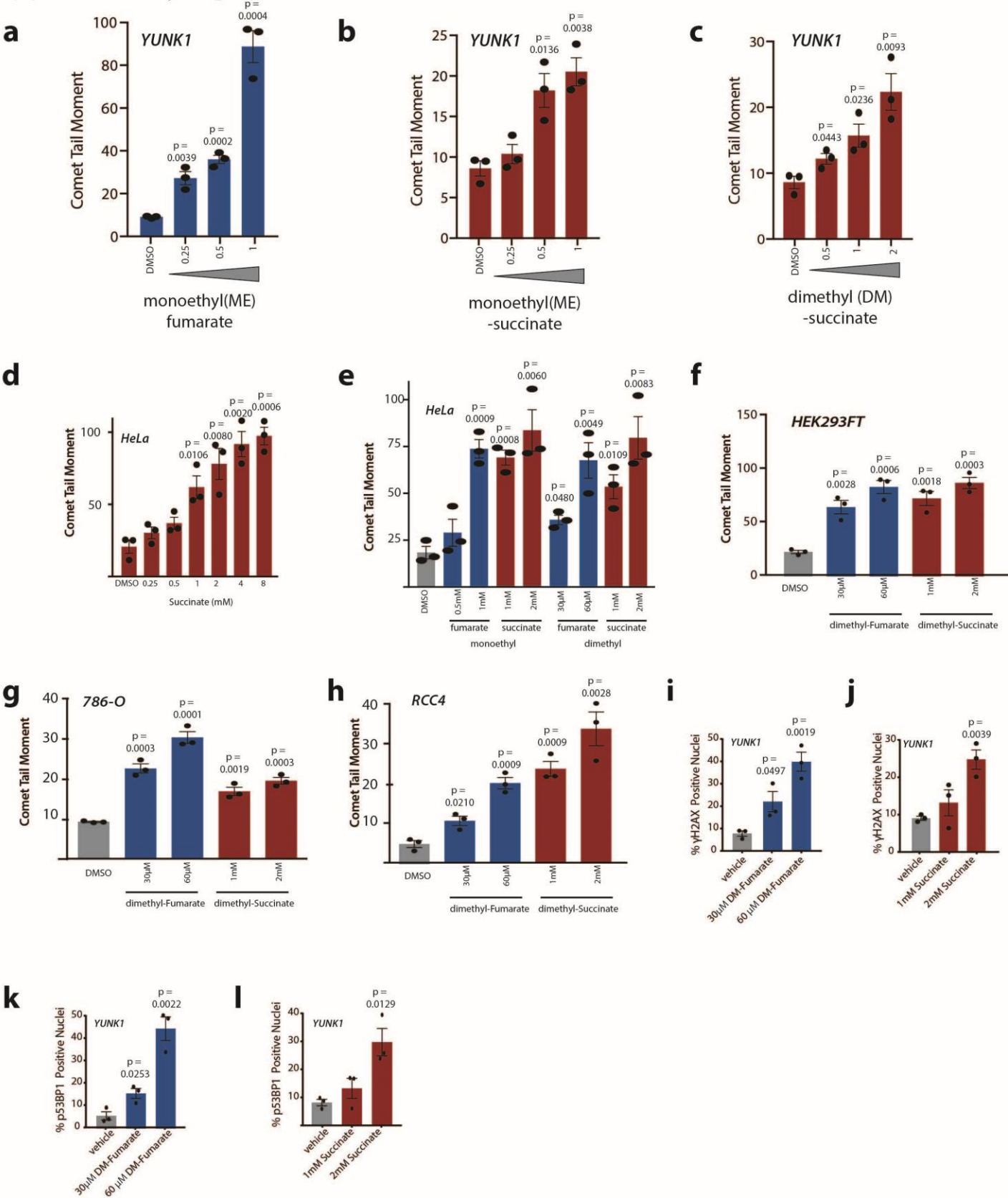


Supplementary Figure 1

γ H2AX ELISA and additional validation data for cell lines and reporter assays.

(a) Quantification of γ H2AX ELISA performed on FFPE normal kidney samples and FFPE HLRCC cases. $n=3$ for HLRCC samples and $n=9$ for Normal kidney samples $\pm$ SEM. Statistical analysis by two tailed t-test, $df=10$. Dots represent technical replicates. (b) Western blot analysis of FH and SDHB in doxycycline-inducible shRNA models. Cells were collected 96 h after doxycycline induction of shRNA expression. Vinculin is used as a loading control. (c-d) LC/MS quantification of (c) succinate and (d) fumarate in YUNK1 doxycycline inducible shRNA models. $n=3 \pm$ SEM. Western blot analysis of (e) SDHB and (f) FH expression in pooled selected populations using two independent shFH shRNA sequences and 3 independent shFH clonal cell lines, and a single pooled population and two independent clones for shSDHB in HEK293FT shRNA models. (g) Quantification and (h) representative images of neutral comet assays performed in YUNK1 doxycycline inducible shRNA models for shFH and shSDHB. $n=3 \pm$ SEM. (i) Quantification of neutral comet assay performed 96 h after doxycycline addition to media, for the independent sequences and clones of shFH and shSDHB in the HEK293FT cells. $n=3 \pm$ SEM. (j) Quantification of neutral comet assay performed 72h post siRNA transfection of siBRCA1, siBRCA2 and siRAD51 in HEK293FT cells. $n=3 \pm$ SEM. (k) Quantification of neutral comet assay performed in parental DLD1 and BRCA2 $-/-$ DLD1 cells. $n=3 \pm$ SEM. (l) Western blot analysis of siRNA knockdown of BRCA1, BRCA2 and RAD51 in YUNK1 and HEK293FT cells. (m) Quantification by LC/MS of fumarate levels in patient-derived HLRCC cell lines UOK 262 and UOK 268 compared to HEK293FT and HEK293FT expressing shRNA targeting FH (clone 1). UOK 262, UOK268 and NCCFH1 fumarate quantification data is also presented in figure 2c. $n=3 \pm$ SEM. (n) Western blot analysis of FH expression in the NCCFH1, UOK 268, and UOK 262 patient-derived cell lines transiently transfected with an FH expressing vector. Vinculin is used as a loading control. (o) Quantification of luciferase reactivation by HR in parental DLD1 and BRCA2 $-/-$ DLD1 cells. $n=3 \pm$ SEM. (p) Quantification of luciferase reactivation by non-homologous end-joining (NHEJ) in the doxycycline-inducible shRNA models of FH and SDHB knockdown in HEK293FT and YUNK1 cells. $n=3 \pm$ SEM. (q) Western blot analysis of siRNA knockdowns of FH, SDHB, BRCA1, BRCA2, and RAD51 in U2OS cells. (r) Representative images of RAD51 foci formation upon 2Gy IR treatment of HEK293FT cells with or without shRNA suppression of FH or SDHB compared to non-targeted control shRNA. For c, d, g, i, j, k, m, and o statistical analyses were by two-sided t-test with $df=4$, and bars represent mean $\pm$ SEM. For g,h,l,m,n and q images were cropped around the known molecular weight of the band of interest and these are representative blots, with each blot repeated independently 3 times with similar results. Full blots appear in Supplementary Figure 6.

Supplementary Figure 2

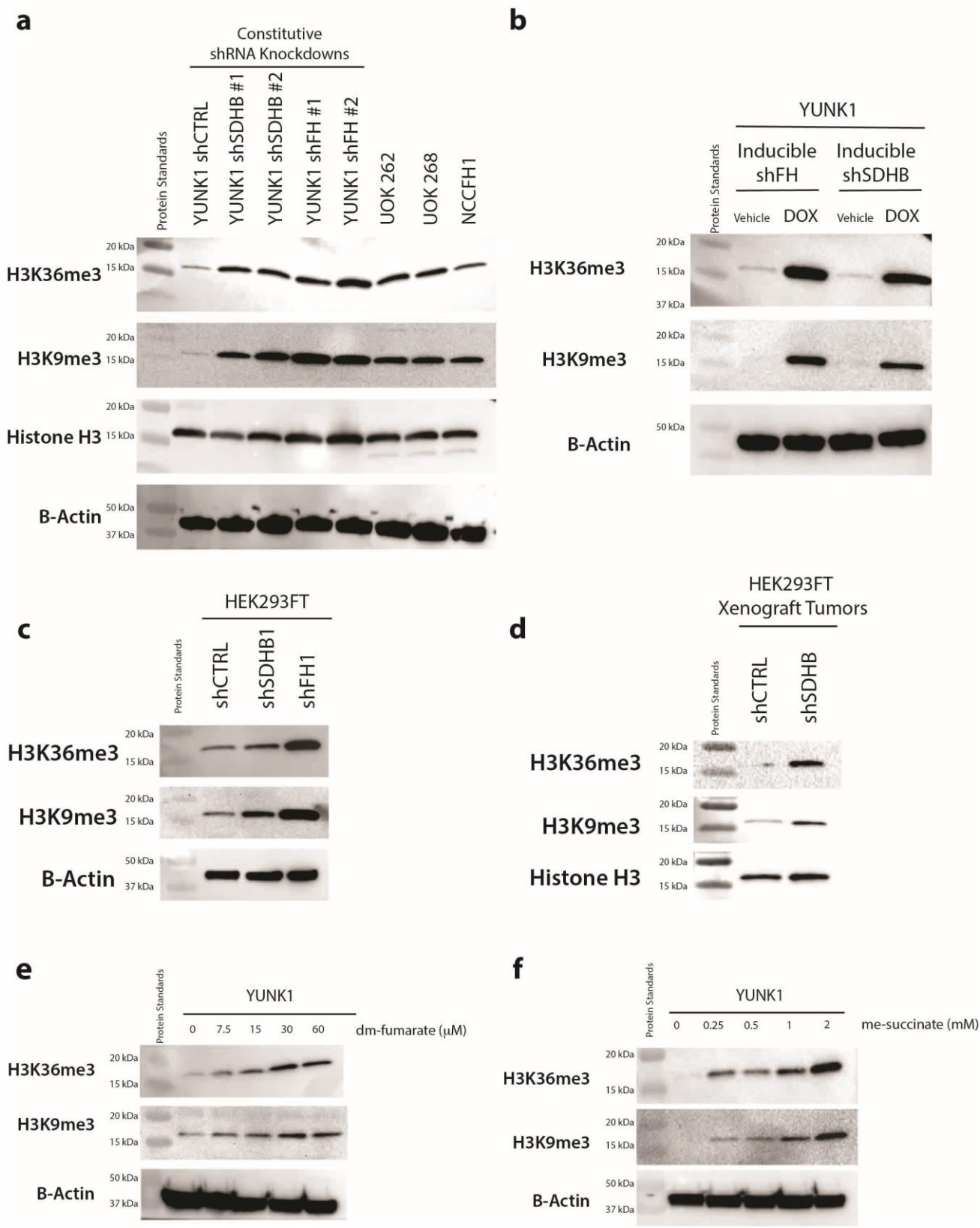


Supplementary Figure 2

Quantification of neutral comet assay results after metabolite treatment of cell lines.

(a-c) Quantification of neutral comet assay performed in YUNK1 cells 24 h after the addition of indicated doses of (a) monoethyl-fumarate, (b) monoethyl-succinate and (c) dimethyl-succinate to the cell culture medium. (d) Quantification of neutral comet assay performed in HeLa cells 24 h after addition of the indicated doses of succinate to the cells. (e-h) Quantification of neutral comet assay performed in (e) HeLa, (f) HEK293FT, (g) 786-O, and (h) RCC4 cells 24 h after the addition of indicated concentrations of metabolites. - (i-j) Quantification of immunofluorescent γ H2AX foci in YUNK1 cells 24 h after the addition of the indicated doses of (i) dimethyl-fumarate and (j) succinate. (k-l) Quantification of immunofluorescent p53BP1 foci in YUNK1 cells 24 h after the addition of the indicated doses of (k) dimethyl-fumarate and (l) succinate. For all panels n=3, bars represent mean $\pm$ SEM and statistical analysis was by two-sided t-test and df=4.

Supplementary Figure 3

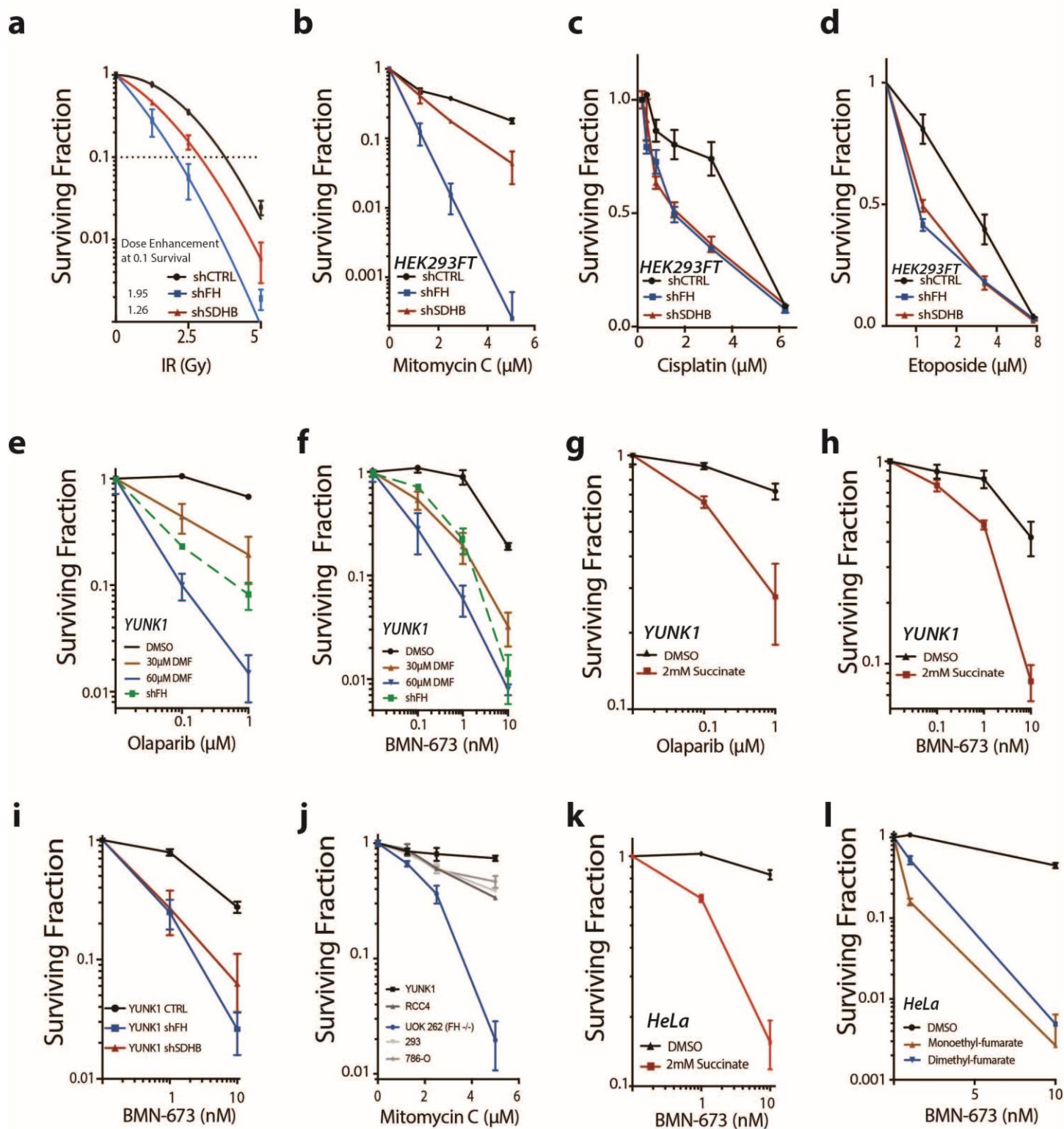


Supplementary Figure 3

Histone hypermethylation in cells deficient in Krebs-cycle enzymes, in xenografts and in metabolite-treated cells.

(a) Western Blot analysis of histone 3 lysine 36 trimethylation (H3K36me3) and histone 3 lysine 9 trimethylation (H3K9me3), along with total histone 3, in YUNK1 cells with constitutive shRNA knockdown of FH and SDHB, as well as in HLRCC patient derived cell lines, UOK 262, UOK 268, and NCCFH1. Actin was as used as a loading control. (b-c) Western blot analysis of H3K36me3 and H3K9me3 levels in doxycycline-inducible shRNA knockdowns of FH and SDHB in (b) YUNK1 cells and (c) HEK293FT cells treated with doxycycline for 96 h before collection of cells for western blot analysis. Actin was used as a loading control. (d) Western blot analysis of H3K36me3 and H3K9me3, along with total H3, in HEK293FT tumor xenografts harvested for analysis when the tumors were 80 mm³ in size. (e-f) Western blot analysis of H3K36me3 and H3K9me3 in YUNK1 cells treated with the indicated concentrations of (e) dimethyl-fumarate or (f) monoethyl-succinate for 24 h. Actin is used as a loading control. Each blot was independently performed 3 times with similar results. For all panels, images were cropped around the known molecular weight of the band of interest. Full blots appear in Supplementary Figure 6.

Supplementary Figure 4

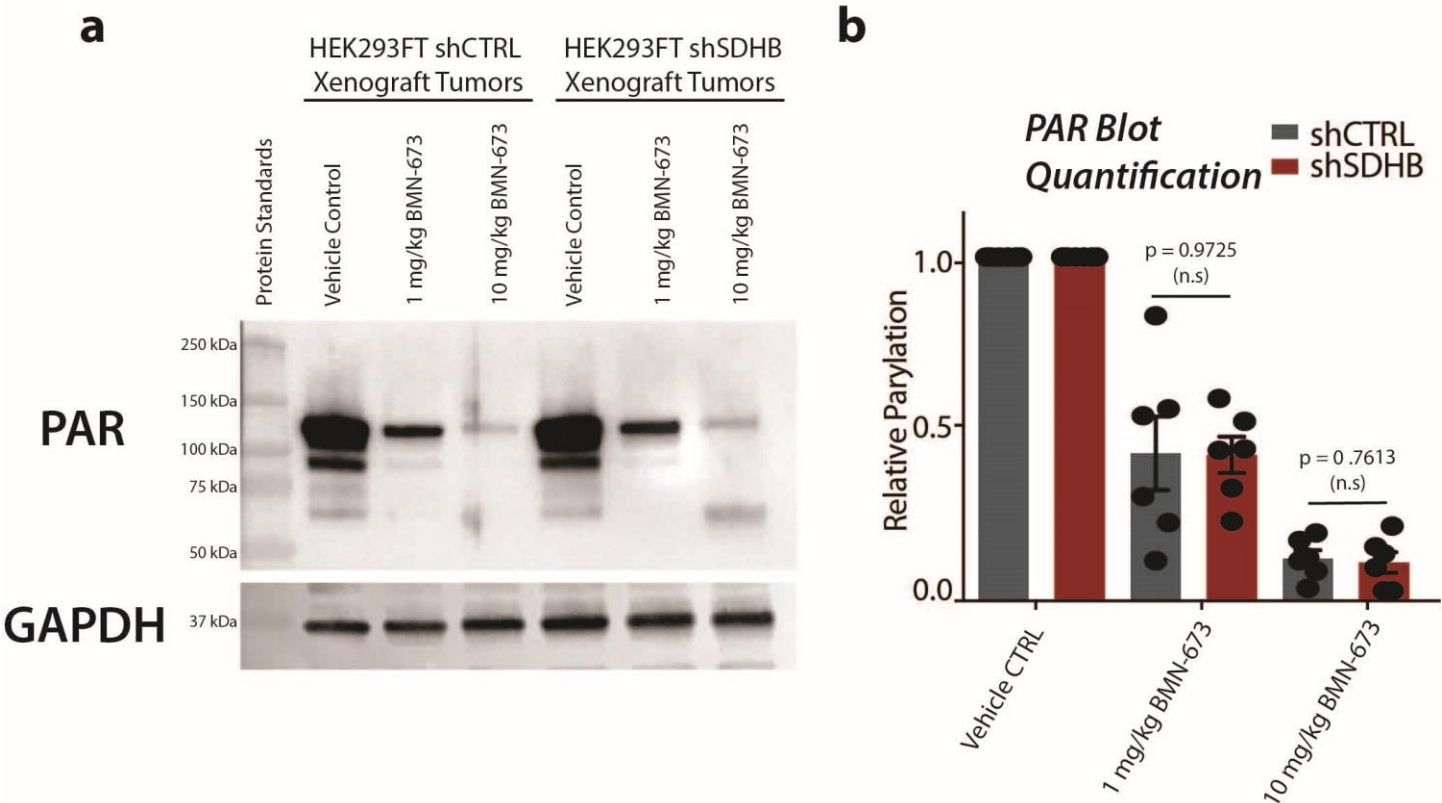


Supplementary Figure 4

DNA-repair-inhibitor and DNA-damaging-agent sensitivity in cells deficient in Krebs-cycle enzymes.

(a) Clonogenic survival assay in HEK293FT shSDHB clone 1 (+ doxycycline), HEK293FT shFH clone 1 (+ doxycycline) and HEK293FT shCTRL (+ doxycycline) after treatment with the indicated doses of ionizing radiation. Dose enhancement ratio at 0.1 survival is indicated. (b, c, and d) Clonogenic survival assay in HEK293FT shSDHB clone 1 + doxycycline, HEK293FT shFH clone 1 +doxycycline, and HEK293FT shCTRL + doxycycline with the indicated doses of (b) mitomycin C (c) cisplatin and (d) etoposide. (e and f) Clonogenic survival assay with the indicated doses of (e) Olaparib and (f) BMN-673 for YUNK1 cells also treated or not with 30 μ M or 60 μ M of dimethyl fumarate, as indicated, or with doxycycline to induce FH knockdown. (g and h) Clonogenic survival assay with the indicated doses of (g) Olaparib and (h) BMN-673 for YUNK1 cells also treated or not with 2 mM succinate. (i) Clonogenic survival assay in YUNK1 cells with constitutive shRNA knockdown of FH and SDHB in response to BMN-673. (j) Clonogenic survival assay of cell lines of renal origin treated with indicated doses of mitomycin C. (k-l) Clonogenic survival assay in response to BMN-673 in HeLa cells also treated or not with (k) 2 mM succinate or (l) 1mM monoethyl-fumarate or 60 μ M dimethyl-fumarate. For all panel n=3, dots represent mean $\pm$ SEM.

Supplementary Figure 5

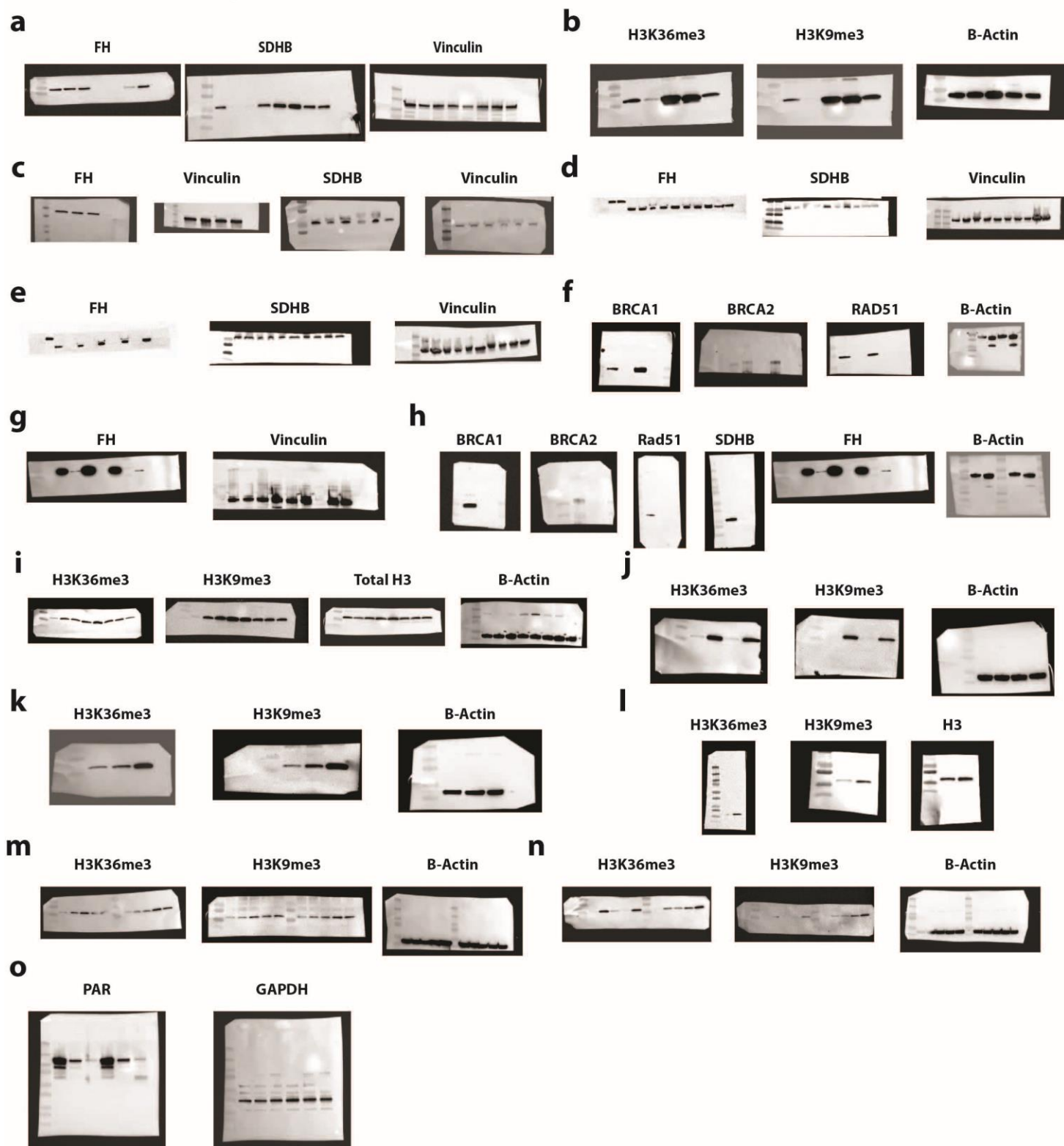


Supplementary Figure 5

PAR levels determined by western blotting.

(a) Western blot image and (b) quantification of total cellular poly-ADP-Ribose (PAR) levels in HEK293FT tumor samples 24 h after treatment with the indicated concentrations of BMN-673. n=6 replicates; bars represent mean +/- SEM. Statistical analysis by two-sided t-test with df=10. For a, images were cropped around the known molecular weight of the band of interest. Full blots appear in Supplementary Figure 6.

Supplementary Figure 6



Supplementary Figure 6

Full-length, uncropped western blots.

Full length, uncropped western blots probed with the indicated antibodies as shown in (a) Figure 2a, (b) Figure 3i, (c) Supplementary Figure 1b, (d) Supplementary Figure 1e, (e) Supplementary Figure 1f, (f) Supplementary Figure 1l, (g) Supplementary Figure 1n, (h) Supplementary Figure 1q, (i) Supplementary Figure 3a, (j) Supplementary Figure 3b, (k) Supplementary Figure 3c, (l) Supplementary Figure 3d, (m) Supplementary Figure 3e, (n) Supplementary Figure 3f, and (o) Supplementary Figure 5a.