Supplementary Figures

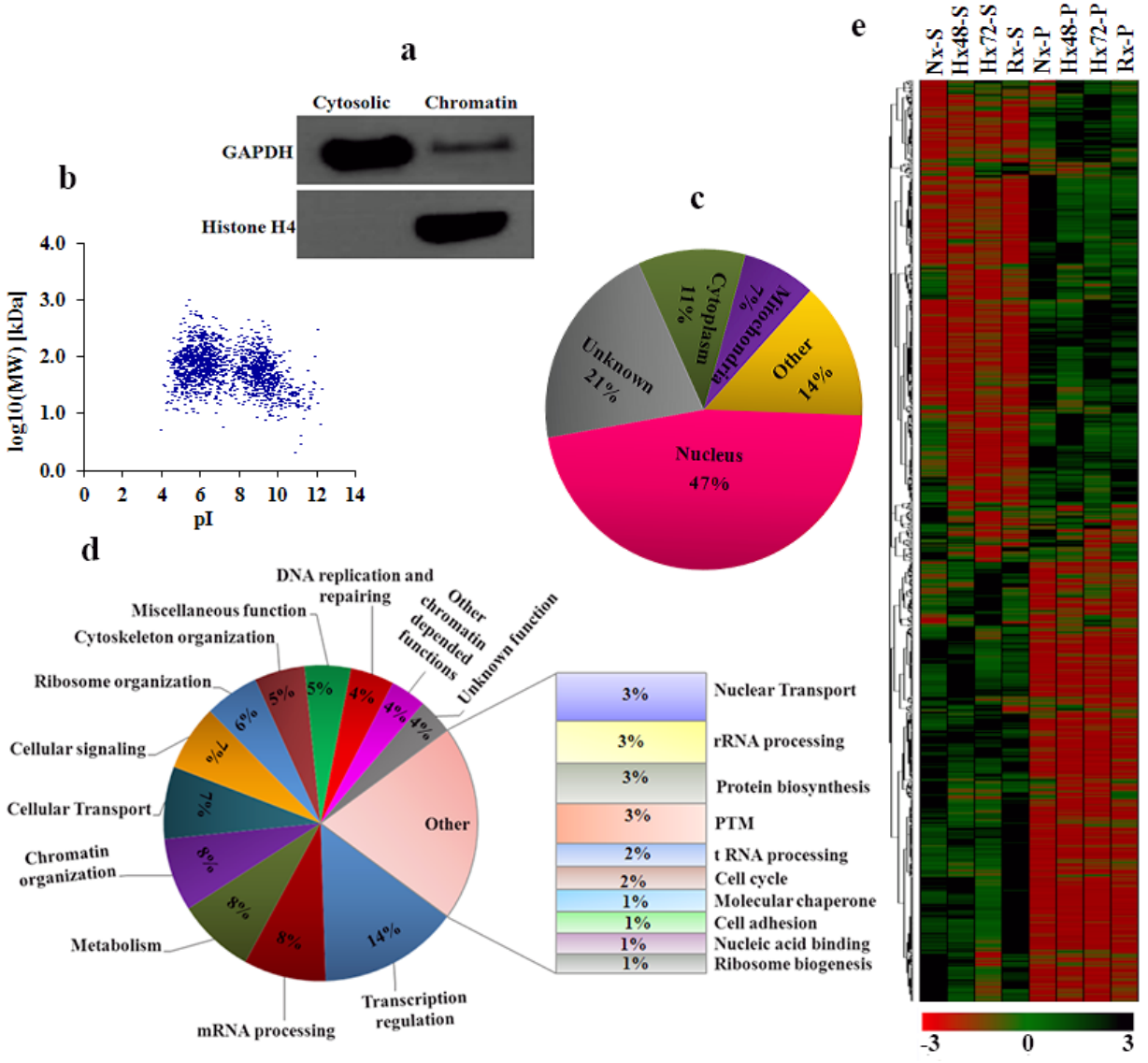

Figure S1: Characterization of identified proteins. a: Western blot of GAPDH and histone H4 in cytosolic and chromatin fractions respectively. b: Physicochemical distribution of the identified proteins according to molecular weight (MW) and isoelectric point (pI). c: Classification of the identified proteins according to their subcellular localization. d: Functional classification of the identified proteins. e: Heat-map showing relative protein abundance in the different chromatin digests. iTRAQ values of different chromatin digests were used for the cluster analysis.

Supplementary Figures

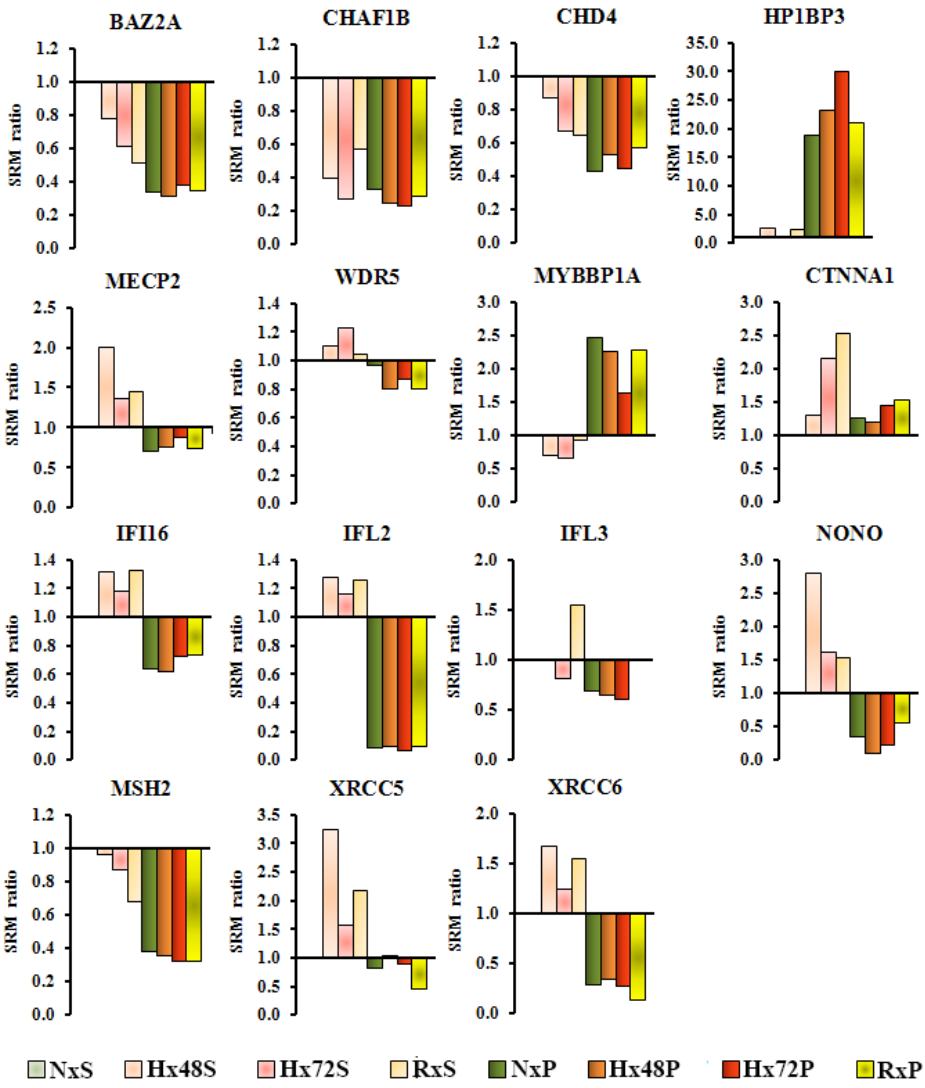

Figure S2: Validation of quantitative iTRAQ results by SRM based quantification. SRM ratio represents the relative protein abundance in different nuclease digests. Chromatins from different conditions were partially digested by DNase I and proteins were quantified by SRM based proteomic approach. Supernatant fractions NxS, Hx48S, Hx72S and RxS and pellet fractions NxP, Hx48P, Hx72P and RxP were obtained by partial DNase I digestion of respective chromatin extracted from normoxic, 48h hypoxic, 72h hypoxic and reoxygenated A431 cells.

Supplementary Figures

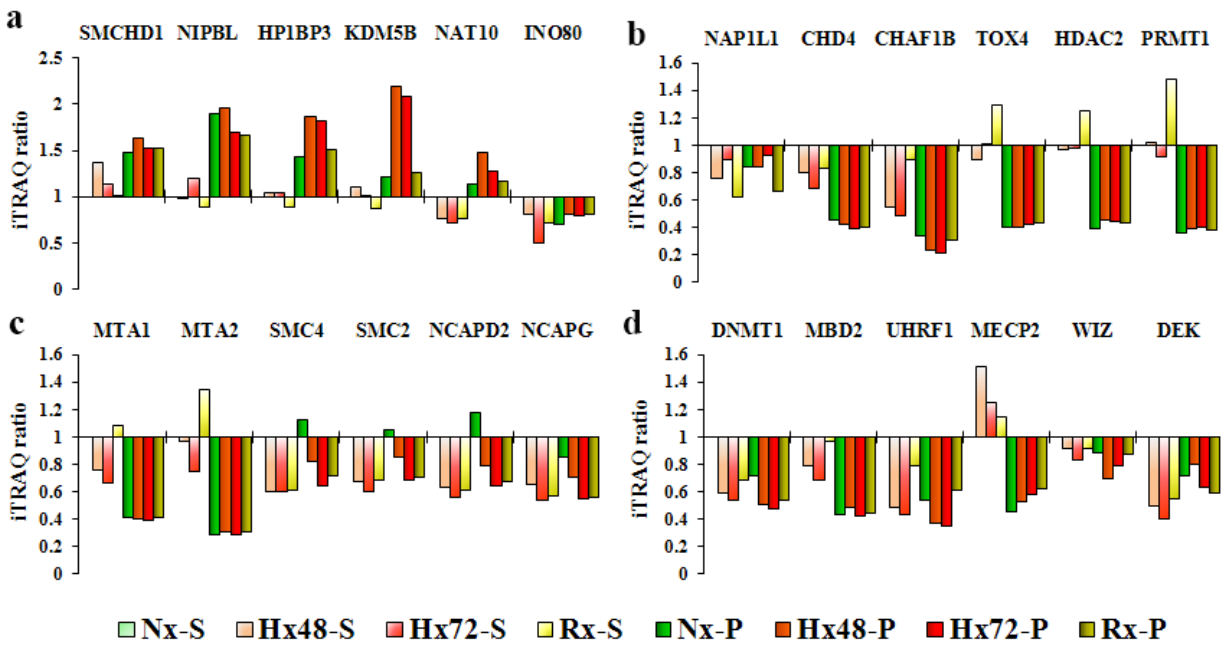

Figure S3: Chromatin organizer proteins exhibit differential association with chromatin during hypoxia and re-oxygenation. A431 cancer cells were cultured under conditions of normoxia (Nx), 48h hypoxia (Hx48), 72h hypoxia (Hx72), or 48h hypoxia followed by 24h re-oxygenation (Rx). Chromatin was then extracted from the cells and subjected to partial DNase I digestion to release euchromatin-binding proteins into the supernatant (suffix S) and leave heterochromatin-bound proteins behind in the undigested pellet (suffix P). Shown are iTRAQ ratios indicating the relative abundance of proteins in the different chromatin digests (a-d).

Supplementary Figures

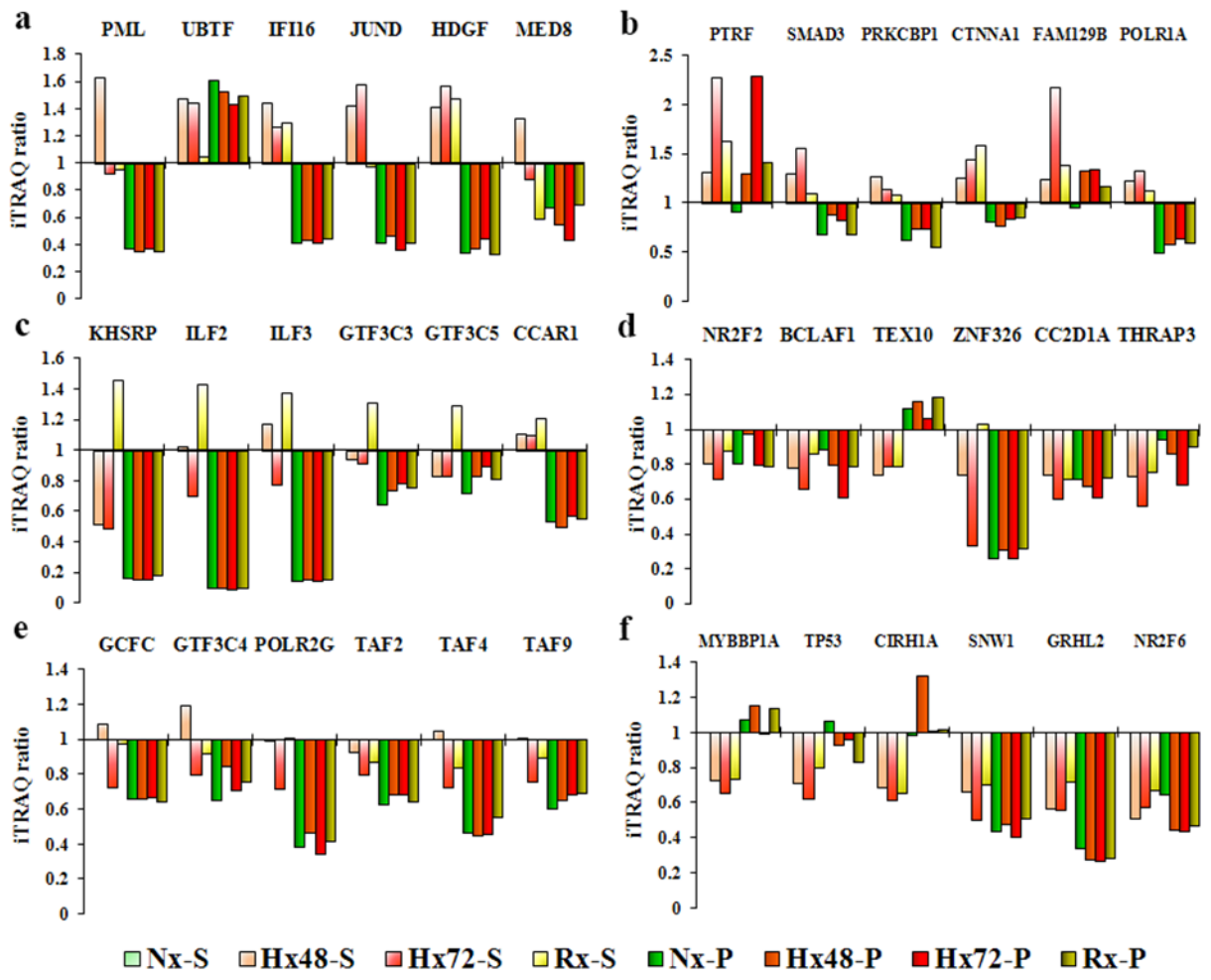

Figure S4: Chromatin association of transcriptional regulators is modulated by hypoxia/re-oxygenation. A431 cancer cells were cultured under conditions of normoxia (Nx), 48h hypoxia (Hx48), 72h hypoxia (Hx72), or 48h hypoxia followed by 24h re-oxygenation (Rx). Chromatin was then extracted from the cells and subjected to partial DNase I digestion to release euchromatin-binding proteins into the supernatant (suffix S) and leave heterochromatin-bound proteins behind in the undigested pellet (suffix P). Shown are iTRAQ ratios indicating the relative abundance of proteins in the different chromatin digests (a-f).

Supplementary Figures

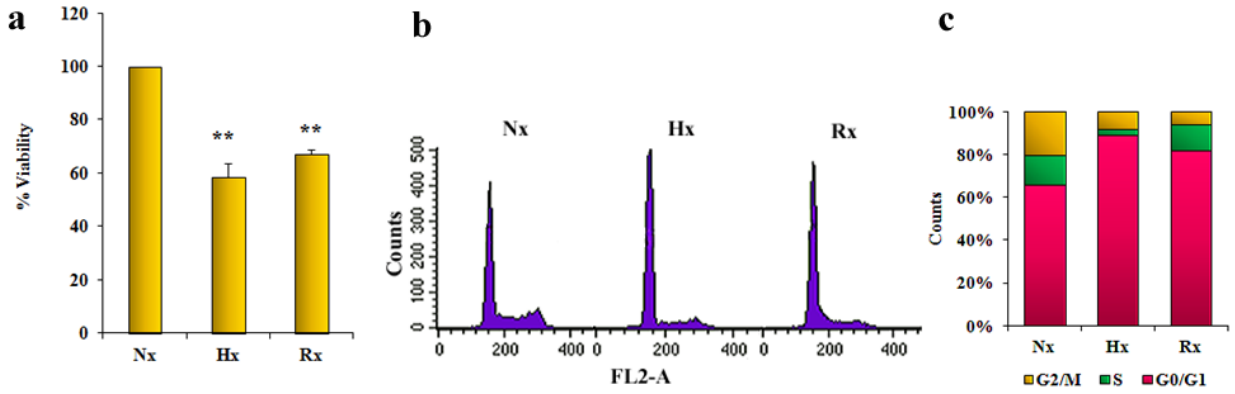

Figure S5: Viability and cell cycle progression of A431 cells under hypoxic conditions. a: Percentage of viable A431 cells as assessed by MTT assay after culture in normoxia, hypoxia, or hypoxia/re-oxygenation (n=3 biological replicates, **P<0.005). b: Cell cycle analysis of normoxic, hypoxic and re-oxygenated cells after staining with propidium iodide and measurement of DNA content using flow-cytometry. c: Phase distribution of cells cultured under normoxic, hypoxic or re-oxygenated conditions.

Supplementary Figures

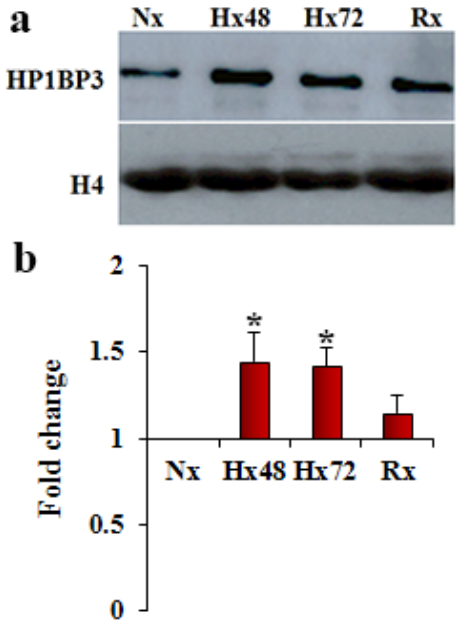

Figure S6: Chromatin association of HP1BP3 during different hypoxic conditions. a: Western blot images showing the differential association of HP1BP3 with chromatin during hypoxia and re-oxygenation. Chromatin extract from different conditions were used for the western blot analysis. b: Change in HP1BP3 chromatin association level under variable oxygenation. The chromatin association level of HP1BP3 is expressed relative to that of histone 4 (n=5 experimental replicates, *P<0.05).

Supplementary Figures

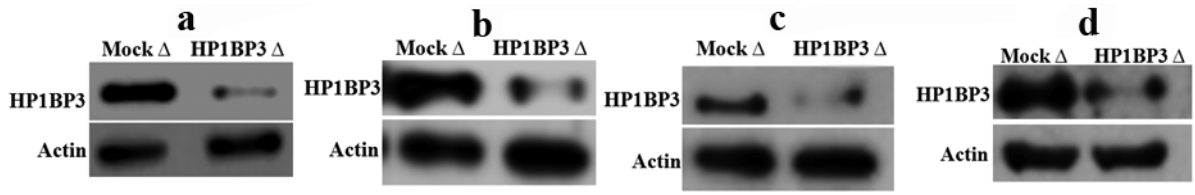

Figure S7: Confirmation of HP1BP3 knockdown. Western blots confirming successful shRNA-knockdown of HP1BP3 expression in A431 cancer cells using shRNA-1(a), shRNA-2 (b) and shRNA-3(c). HP1BP3 knockdown in U2OS cells using shRNA-1(d).

Supplementary Figures

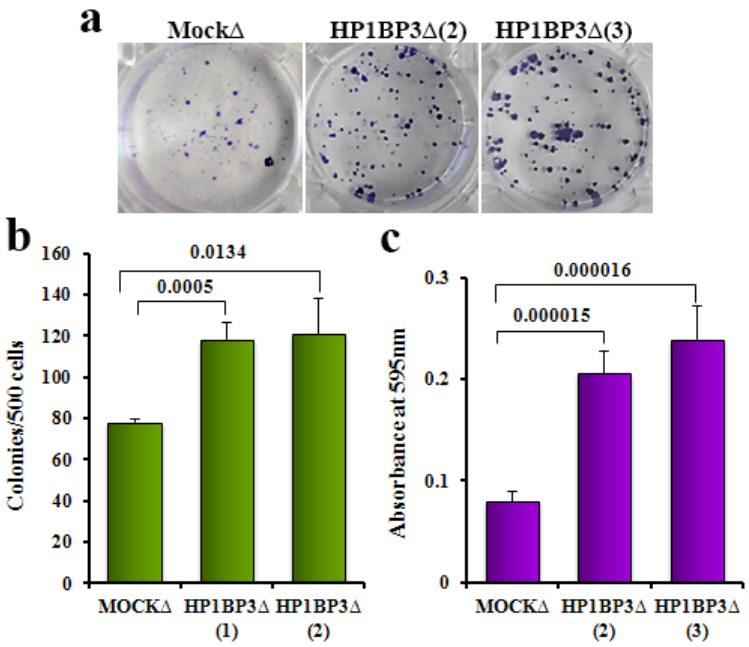

Figure S8: Validation of on target effect of HP1BP3 shRNA during clonogenic assay. Representative images (a) and quantification of crystal violet staining in HP1BP3-depleted A431 phenotypes obtain by using shRNA-2 and shRNA-3 and mock-depleted control cells (b and c). Each experiment was performed in triplets.

Supplementary Figures

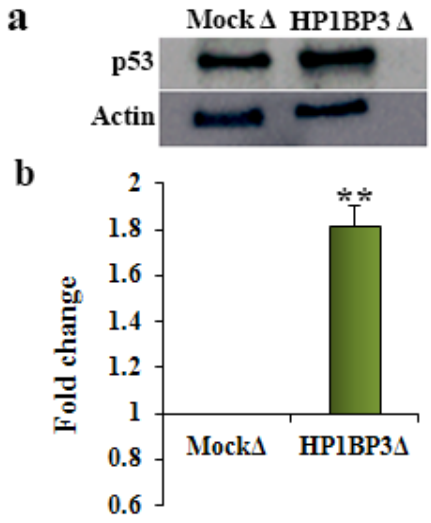

Figure S9: HP1BP3 depletion is associated with increased p53 expression in A431 cells. a: Analysis of p53 expression level in HP1BP3-depleted A431 cells and mock-depleted control cells by Western blot (a) or quantification expressed relative to actin staining (b) (n=5 experimental replicates, **P<0.005).

Supplementary Figures

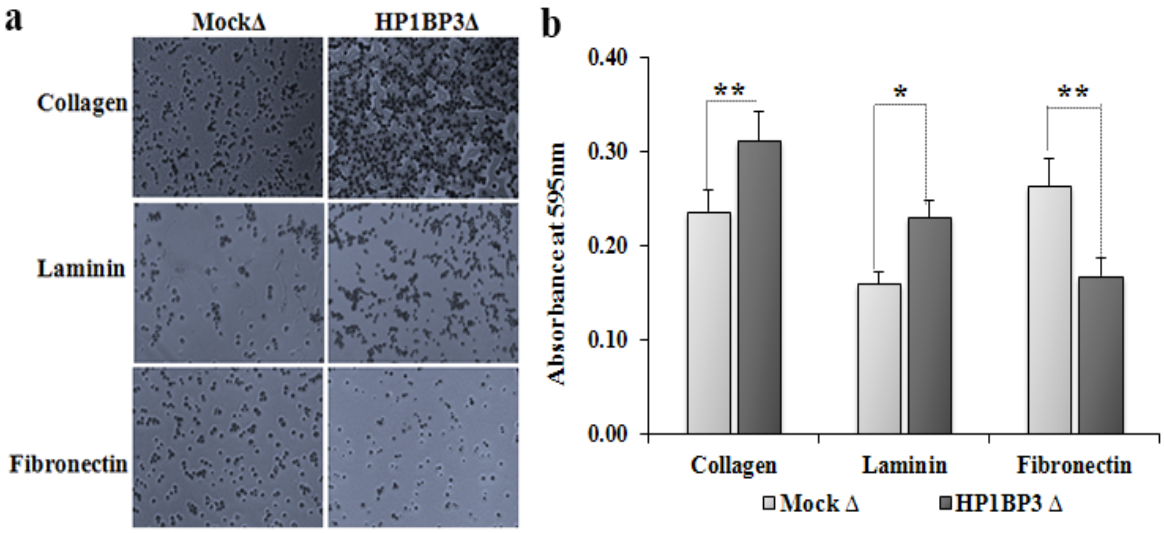

Figure S10: HP1BP3-mediated regulation of cell adhesion. Adhesion of HP1BP3-depleted A431 cells to ECM proteins collagen, laminin or fibronectin, compared with mock-depleted control cells (*P<0.05, **P<0.005).

Supplementary Figures

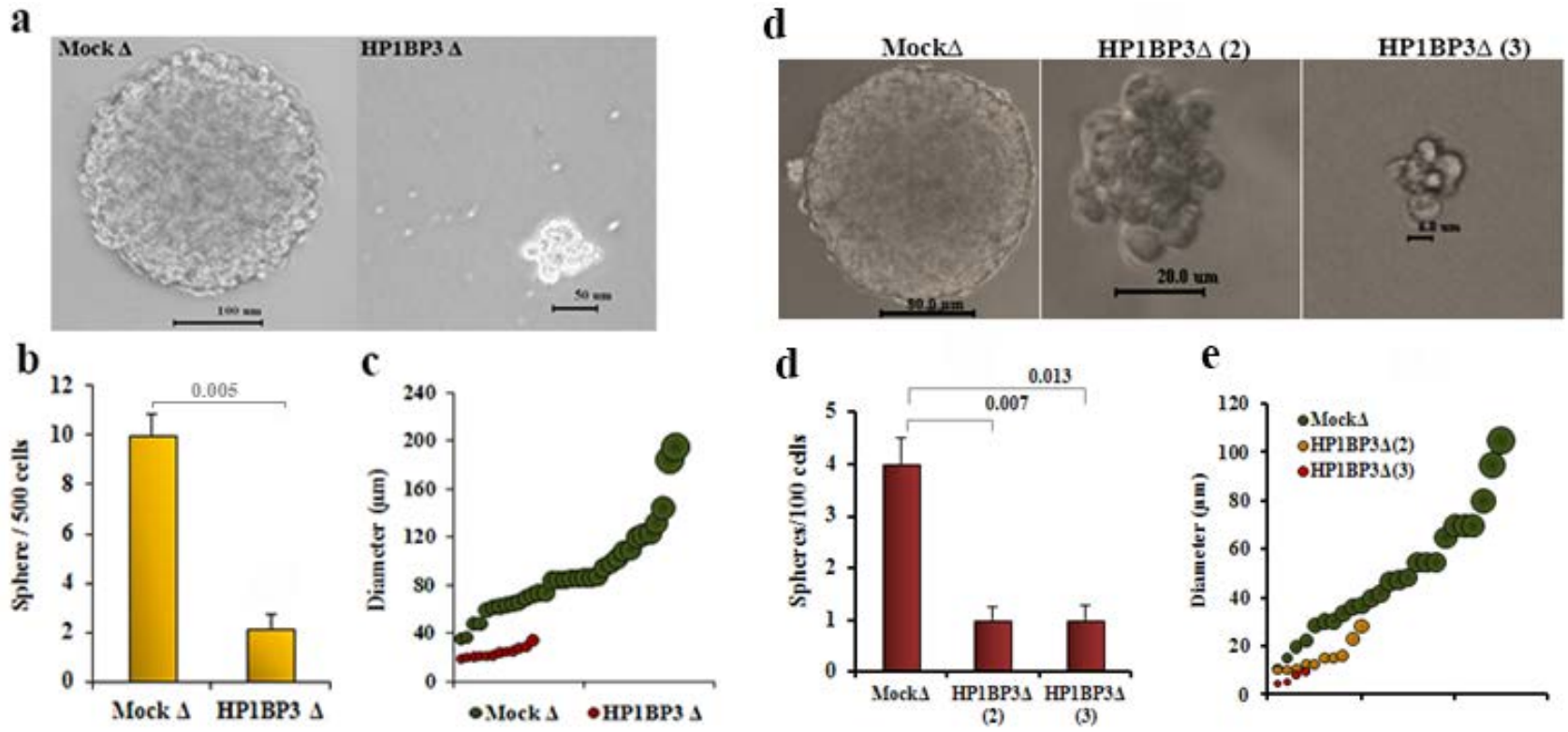

Figure S11: HP1BP3 depletion impairs cancer cell self-renewal. a: Images of tumor spheres formed by HP1BP3-depleted different A431 phenotypes obtained by using different shRNAs (HP1BP3Δ [shRNA-1], HP1BP3Δ(2) [shRNA-2] and HP1BP3Δ(3) [shRNA-3]) or mock-depleted controls(a). Number of tumor spheres formed/specified number of cells seeded (b and d). Size distribution of tumor spheres formed by HP1BP3 depleted A431 phenotypes and mock control A431 cells (c and e). Each experiment was performed in triplets.

Supplementary Figures

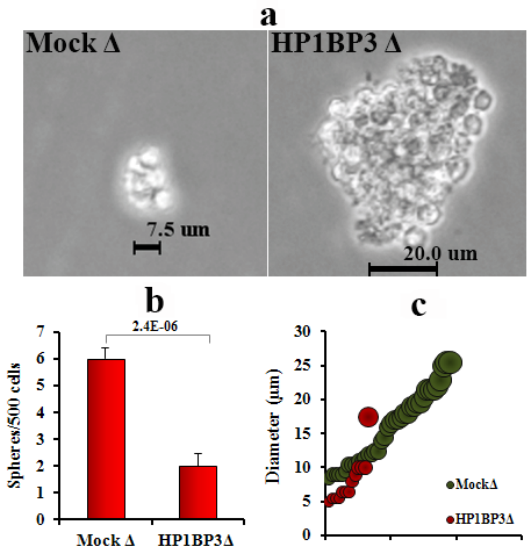

Figure S12: Effect of HP1BP3 depletion upon self-renewal property of U2OS cells. a: Images of spheres formed by mock and HP1BP3 depleted U2OS cells. b: Number of tumorospheres formed/500 cells seeded c: Size distribution of mock and HP1BP3 depleted U2OS spheres. Each experiment was performed in triplets.

Supplementary Figures

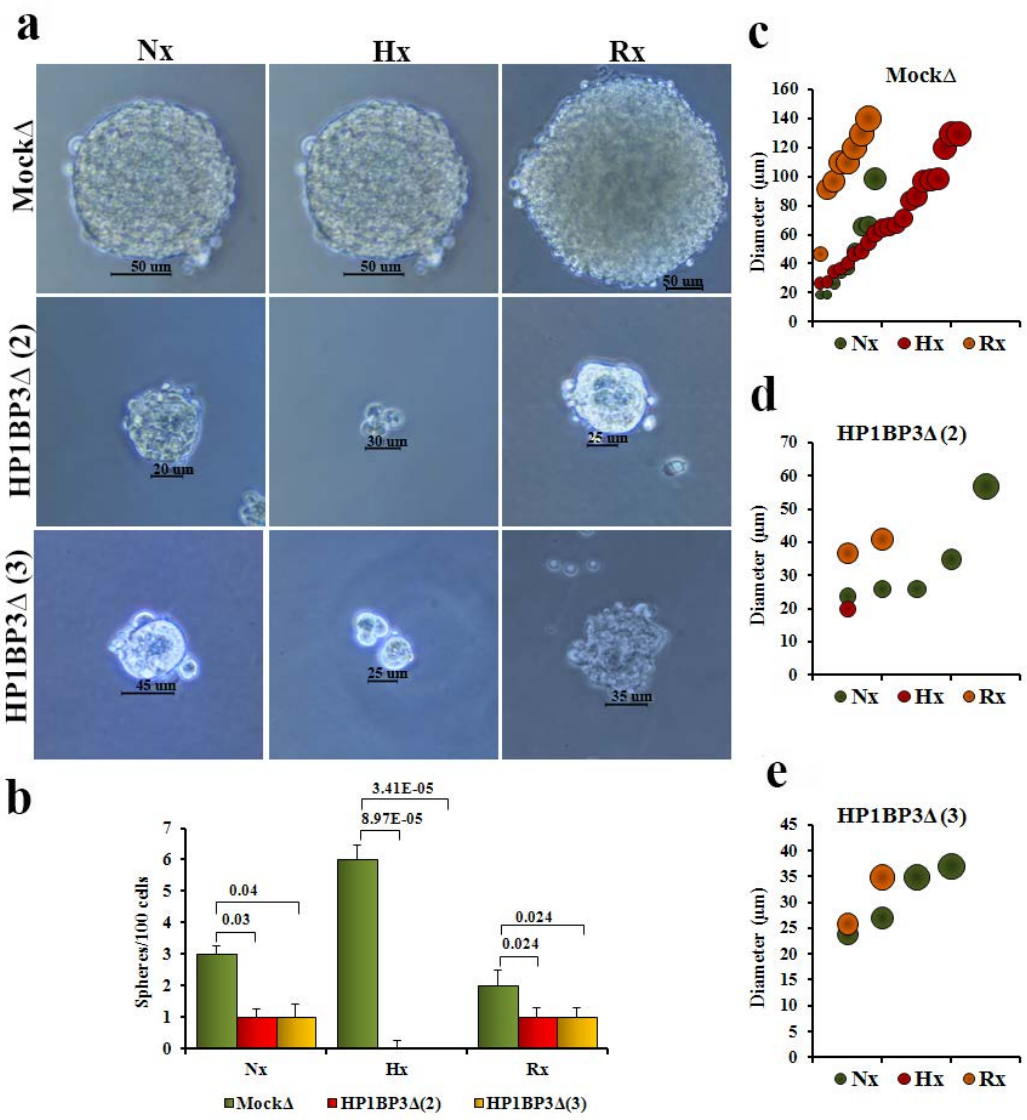

Figure S13: Effect of HP1BP3 depletion upon self-renewal property of cancer cells during hypoxia/re-oxygenation. HP1BP3-depleted A431 cells were cultured for 48h in normoxia (Nx), 48h hypoxia (Hx), or 24h hypoxia/24h re-oxygenation (Rx), and then assessed for tumor sphere formation over 10d of culture (a and b). Size distribution of tumor spheres formed by HP1BP3-depleted A431 cells subjected to the same conditions (c-e). Each experiment was performed in triplets. Different HP1BP3 depleted phenotypes were obtained from stable transfection of different shRNAs (shRNA-2 and shRNA-3) in A431 cells.

Supplementary Figures

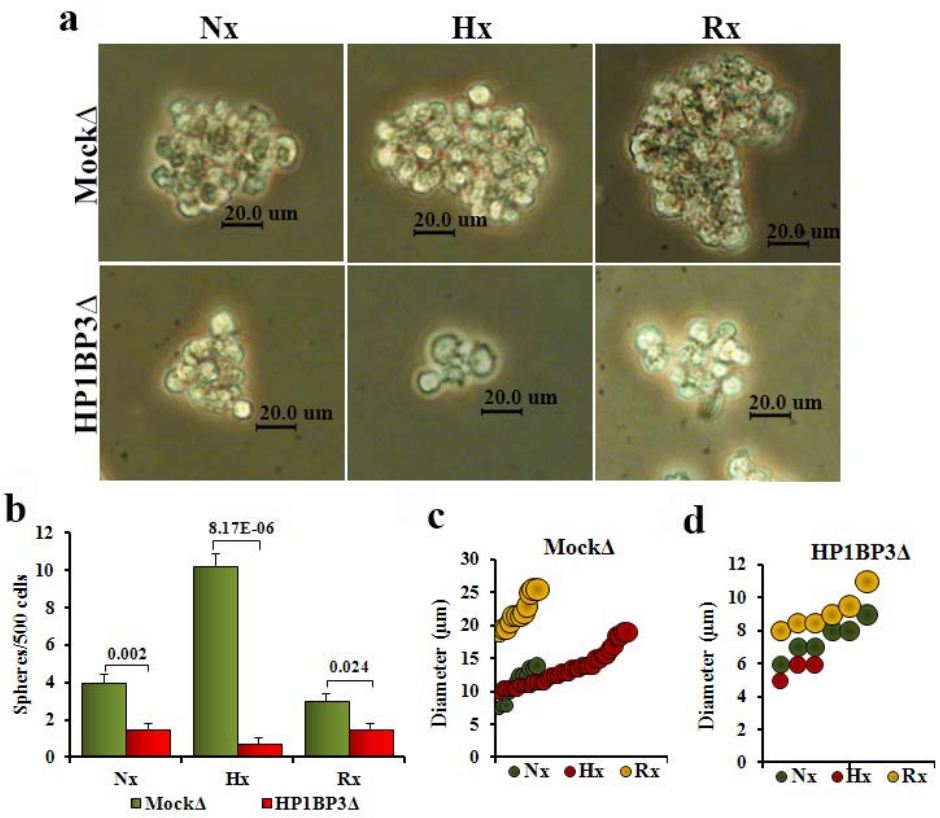

Figure S14: HP1BP3 depletion inhibits self-renewal property of U2OS cells during hypoxia/re-oxygenation. HP1BP3-depleted U2OS cells were cultured for 48h in normoxia (Nx), 48h hypoxia (Hx), or 24h hypoxia/24h re-oxygenation (Rx), and then assessed for tumor sphere formation over 10d of culture (a and b). Size distribution of tumor spheres formed by HP1BP3-depleted U2OS cells subjected to the same conditions (c). Each experiment was performed in triplets.

Supplementary Figures

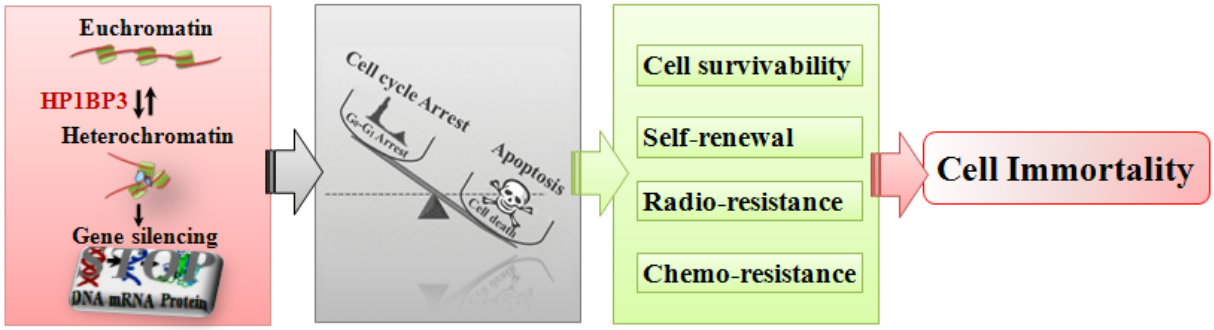

Figure S15: HP1BP3-induced immortalization of cancer cells during hypoxia.