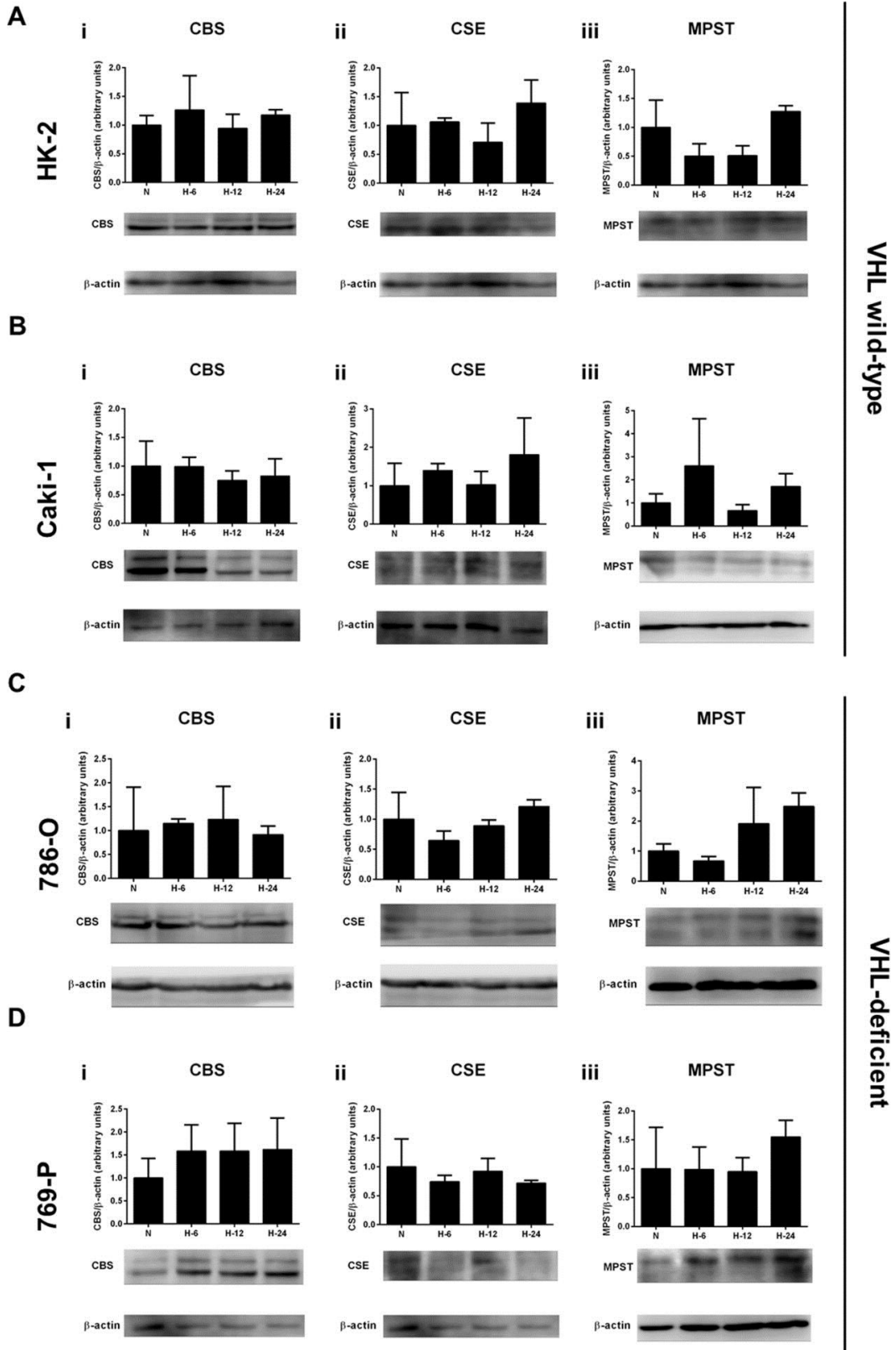


Supplementary Fig. 1.

Baseline normoxic expression of H₂S-producing enzymes is unaltered in VHL-deficient ccRCC cell lines when compared to malignant and non-malignant VHL wild-type renal cell lines. Protein was isolated from 80 to 90% confluent cell cultures grown in normoxia (21% O₂) using RIPA buffer. SDS-PAGE was carried out on 40–50 μ g of whole cell lysate using 10–12% poly-acrylamide gels and western blots were performed using PVDF membranes. Blots were probed for (A) CBS (63 kDa), (B) CSE (45 kDa) and (C) MPST (33 kDa) while β -actin (43 kDa) served as a loading control against which protein expression was normalized. Expression of CBS, CSE and MPST in renal cancer cell lines (Caki-1, 786-O, 769-P) was normalized to expression of these same enzymes in the non-malignant renal epithelial cell line HK-2. Error bars represent standard error of the mean (SEM), n = 3. One-way ANOVA and Tukey's multiple comparisons test revealed no statistically significant differences in CBS, CSE nor MPST expression between cell lines.



Supplementary Fig. 2.

Hypoxic induction of H₂S-producing enzymes in malignant, and non-malignant renal cell lines. Protein was isolated from 80 to 90% confluent cell cultures grown in normoxia (N; 21% O₂) or hypoxia (1% O₂) for 6, 12 or 24 h (H-6, H-12, H-24) using RIPA buffer. SDS-PAGE was carried out on 40–50 µg of whole cell lysate using 10–12% poly-acrylamide gels and western blots were performed using PVDF membranes. Hypoxic induction of CBS (63 kDa), MPST (33 kDa) and CSE (45 kDa) was evaluated in **(A)** HK-2, **(B)** Caki-1, **(C)** 786-O and **(D)** 769-P cell lines. β-actin (43 kDa) served as a loading control against which protein expression was normalized. Error bars represent standard error of the mean (SEM), n = 3. One-way ANOVA and Tukey's multiple comparisons test revealed a lack of statistically significant differences in CBS, CSE and MPST expression between treatments in all cell lines.²