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- Supplemental Figure 1. Using high accuracy mass spectrometry to profile the urine proteomes
 of childhood kidney tumors reveals markers of tissue injury and hematuria.
- 4 (A). The 30 proteins which were most highly enriched in kidney tumors as compared with healthy
- 5 controls and children with abdominal pain; RTK = Rhabdoid tumor of the kidney, CCSK = Clear cell
- 6 sarcoma of the kidney, RCC = Renal cell carcinoma.
- 7 (B-E). The most highly enriched proteins in the urine of patients with rhabdoid tumors of the
- 8 kidney (B), clear cell sarcomas of the kidney (C), renal cell carcinomas (D), and Wilms tumors (E)
- 9 as compared with other pediatric renal tumors and controls.





11 Supplemental Figure 2. Elevated urine PHB/Cr ratio at diagnosis is a specific biomarker of

12 relapse in favorable histology Wilms tumors.

- 13 (A-B). Diagnostic urine creatinine levels (mg/dL) (A) and PHB/creatinine (Cr) levels (B) in favorable
- 14 histology Wilms tumor patients who relapsed (Red, n = 49) are compared with those who were
- 15 cured (Blue, n = 50) and normal controls (Black, n = 40).
- 16 (C). A receiver operating characteristic curve demonstrates the prognostic power of diagnostic
- 17 urine prohibitin to predict relapse in favorable histology Wilms tumors at different sensitivity and
- 18 specificity with an area under the curve of 0.75 (95% confidence interval 0.65-0.85).





21 Supplemental Figure 3. Tissue microarray immunohistochemistry standards and non-Wilms

22 tumor controls

23 The tissue microarray included several control tissues which did not express any PHB as shown.

- 24 These were scored as 0+.
- 25 (A). Pre-malignant nephrogenic rests did not demonstrate PHB staining via IHC.
- 26 (B). Embryonal Rhabdomyosarcoma did not demonstrate PHB staining via IHC.
- 27 (C). Clear cell sarcoma did not demonstrate PHB staining via IHC.
- 28 (D). Wilms tumor tissues with low but evident expression of PHB were scored a 1+.
- 29 (E). Wilms tumor tissues with moderate expression of PHB were scored a 2+.
- 30 (F). Wilms tumor tissues with high expression of PHB were scored a 3+.

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35 Wilms tumors with diffuse anaplasia

36 Normalized PHB mRNA expression is compared to *TP53* gene status, separated by those with

37 TP53 mutations (N = 27), copy number (CN) loss (N = 4), or wild type (N = 7) in 38 diffusely

38 anaplastic Wilms Tumors. Box blots overlaid in black demonstrate median and standard

39 deviation.



42 Supplemental Figure 5. PHB expression in Wilms tumors compared with adjacent normal 43 kidney tissues.

(A). Percentage of cells with 0+ to 3+ PHB expression as measured by Halo imaging analysis
 software.

- 46 (B). A comparison of the average area of normal kidney (black diamonds) and Wilms tumor (red
- 47 diamonds) whole cells, cytoplasm, and nuclei with overlaid median and standard deviations
- 48 shown.
- 49



51 Supplemental Figure 6. BH3 profiling reveals decreased apoptotic priming with PHB 52 overexpression

53 (A-B). Cytochrome c loss in response to treatment with different pro-apoptotic peptides

54 comparing Wild type (Black), with Empty (Gray), and PHB overexpressing cells (Red, Brown) in BJ

control fibroblast cells (A) and WiT49 Wilms tumor cells (B).

	· · · · ·	Cured WT	Relapsed WT
Patients	N	50	49
Gender (Male)	N (%)	17 (34%)	24 (49%)
Age at diagnosis (in Months)	Median (Range)	43 (6-217)	52 (5-129)
Histology	Favorable (%)	50 (100%)	49 (100%)
	Anaplastic (%)	0 (0%)	0 (0%)
Stage	I (%)	8 (16%)	4 (8%)
	II (%)	18 (36%)	22 (45%)
	III (%)	24 (48%)	23 (47%)
	IV (%)	0 (0%)	0 (0%)
	V (%)	0 (0%)	0 (0%)
Chemotherapy	Vincristine, Dactinomycin (%)	23 (46%)	23 (45%)
	Vincristine, Dactinomycin, Doxorubicin (%)	25 (50%)	23 (47%)
	Other/Unknown (%)	2 (4%)	4 (8%)

57 Supplemental Table 1. Characteristics of Wilms tumor patients in the validation ELISA cohort

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Patients	Ν	59
Gender (Male)	N (%)	26 (44%)
Age at diagnosis (in Months)	Median (Range)	43 (6-120)
Histology	Favorable (%)	59 (100%)
	Anaplastic (%)	0 (0%)
Stage	I (%)	11 (19%)
	II (%)	19 (32%)
	III (%)	20 (34%)
	IV (%)	6 (10%)
	V (%)	3 (5%)
Chemotherapy	Vincristine, Dactinomycin (%)	0 (0%)
	Vincristine, Dactinomycin, Doxorubicin (%)	0 (0%)
	Other/Unknown (%)	59 (100%)

Patients	Ν	38
Gender (Male)	N (%)	14 (37%)
Age at diagnosis (in Months)	Median (Range)	50 (10-236)
Histology	Favorable (%)	23 (61%)
	Anaplastic (%)	15 (39%)
Stage*	I (%)	3 (11%)
	(%)	5 (18%)
	(%)	6 (21%)
	IV (%)	11 (39%)
	V (%)	3 (11%)
Chemotherapy*	Vincristine, Dactinomycin (%)	8 (29%)
	Vincristine, Dactinomycin, Doxorubicin (%)	16 (57%)
	Other/Unknown (%)	4 (14%)

Supplemental Table 3. Characteristics of Wilms tumor patients in the Halo analysis cohort

65 *10 patients with limited clinical data available, so the stage and chemotherapy data only includes 28
66 patients for whom this data was available.