Supplemental Material for

"Heme Reversibly Damps PERIOD2 Rhythms in Mouse Suprachiasmatic Nucleus Explants"

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Supplemental Table

Treatment	n	Damping	Period (h)		Washout RI	Arrhythmic	
			Pre	Post		Pre	Post
SCN							
Controls	24	0.262 ± 0.026	24.54 ± 0.09	23.72 ± 0.17	0.572 ± 0.025	1	0
170 μM Na_3PO_4	18	0.266 ± 0.032	24.58 ± 0.10	24.02 ± 0.11	0.585 ± 0.030	1	0
.2 % DMSO + 170 μM Na_3PO_4	6	0.253 ± 0.048	24.46 ± 0.20	22.85 ± 0.48	0.530 ± 0.042	0	0
Heme, 3 μM	15	0.382 ± 0.023	24.27 ± 0.18	23.81 ± 0.23	0.533 ± 0.036	0	0
Heme, 30 μM	12	0.557 ± 0.052	25.04 ± 0.14	24.14 ± 0.20	0.576 ± 0.035	0	1
Heme, 100 μM	4	0.676 ± 0.077	24.95 ± 0.48	23.52 ± 0.65	0.630 ± 0.021	1	1
Protoporphyrin IX, 30 μM	5	0.247 ± 0.039	24.81 ± 0.08	23.80 ± 0.64	0.504 ± 0.052	1	0
SnPP, 30 μM	7	0.582 ± 0.056	24.61 ± 0.13	23.84 ± 0.26	0.572 ± 0.033	1	2
Keto, 10 μM	6	0.610 ± 0.102	23.59 ± 0.19 *	24.52 ± 0.35	0.565 ± 0.024	0	0
SnPP, 30 μM + Heme, 30 μM	9	0.878 ± 0.076	25.16 ± 0.32	23.19 ± 0.44	0.574 ± 0.030	1	0
Keto, 10 μM + Heme, 30 μM	5	0.518 ± 0.042	25.38 ± 0.21	23.87 ± 0.34	0.610 ± 0.019	1	0
$Fe(NH_4)_2(SO_4)_2$, 30 μM	5	0.325 ± 0.063	24.72 ± 0.23	24.03 ± 0.50	0.598 ± 0.024	0	1
Biliverdin, 3 μM	7	0.344 ± 0.035	24.66 ± 0.08	24.22 ± 0.17	0.579 ± 0.040	0	0
Biliverdin, 30 μM	9	0.503 ± 0.061	24.57 ± 0.19	23.23 ± 0.38	0.550 ± 0.032	1	0
CO, 30 μM	5	0.448 ± 0.068	24.13 ± 0.35	24.80 ± 0.81	0.387 ± 0.068	0	1
CO, 100 μM	7	0.390 ± 0.060	24.55 ± 0.28	25.12 ± 0.85	0.419 ± 0.053	0	0
Air	9	0.439 ± 0.079	24.35 ± 0.18	24.91 ± 0.68	0.421 ± 0.061	0	0
N-methylprotoporphyrin IX, 10 μM	5	0.298 ± 0.043	24.68 ± 0.10	23.73 ± 0.47	0.513 ± 0.039	1	0
N-methylprotoporphyrin IX, 50 μM	6	0.379 ± 0.030	25.57 ± 0.10 *	23.00 ± 0.26	0.560 ± 0.042	1	1
Esophagus							
Control (170 μM Na_3PO_4)	7	0.605 ± 0.097	22.89 ± 0.24	23.34 ± 0.20	0.600 ± 0.011	0	0
Heme, 30 μM	8	0.544 ± 0.051	23.16 ± 0.28	23.62 ± 0.13	0.596 ± 0.020	0	0
Heme, 100 μM	7	0.702 ± 0.094	23.60 ± 0.43	23.79 ± 0.35	0.549 ± 0.037	0	0
SnPP, 30 μM	6	0.560 ± 0.045	23.61 ± 0.23	23.20 ± 0.10	0.618 ± 0.016	0	0
Heme, 30 μM + SnPP, 30 μM	5	0.608 ± 0.025	24.07 ± 0.13	23.87 ± 0.37	0.558 ± 0.022	1	0
Thymus							
Control (170 μM Na_3PO_4)	6	0.786 ± 0.126	24.56 ± 0.30	23.49 ± 0.14	0.471 ± 0.035	0	0
Heme, 30 μM	6	0.762 ± 0.074	25.06 ± 0.26	23.77 ± 0.11	0.502 ± 0.014	0	0
SnPP, 30 μM	6	0.934 ± 0.079	23.71 ± 0.15	23.56 ± 0.19	0.494 ± 0.035	0	0
Heme, 30 μM + SnPP, 30 μM	6	0.858 ± 0.060	24.84 ± 0.33	23.82 ± 0.18	0.514 ± 0.019	0	0
Spleen							
Control (170 μM Na_3PO_4)	6	0.539 ± 0.052	23.10 ± 0.20	24.48 ± 0.38	0.598 ± 0.009	0	0
Heme, 30 μM	6	0.664 ± 0.079	23.54 ± 0.26	24.20 ± 0.40	0.574 ± 0.028	0	0
SnPP, 30 μM	6	0.722 ± 0.063	23.37 ± 0.17	23.68 ± 0.12	0.597 ± 0.032	0	0
Heme, 30 μM + SnPP, 30 μM	6	0.942 ± 0.106	23.70 ± 0.16	23.57 ± 0.13	0.624 ± 0.027	0	0

Summary of the number of samples excluded from each group due to arrhythmia (rightmost column) either before ("Pre") or after ("Post") the media change, the numbers of samples included in the final analysis (n; those tissues excluded due to arrhythmia are not counted), and the mean (± SEM) damping constant, pre-media change periods, post-media change periods, and rhythmicity indices (RI) following the media change for each group. (*, for period, significantly different from control of the same tissue, Tukey's HSD, $p < .05$; significant differences for damping and RI are discussed in the Results in the main text); Keto, ketoconazole; SnPP, tin-protoporphyrin IX; CO, carbon monoxide; DMSO, dimethylsulfoxide

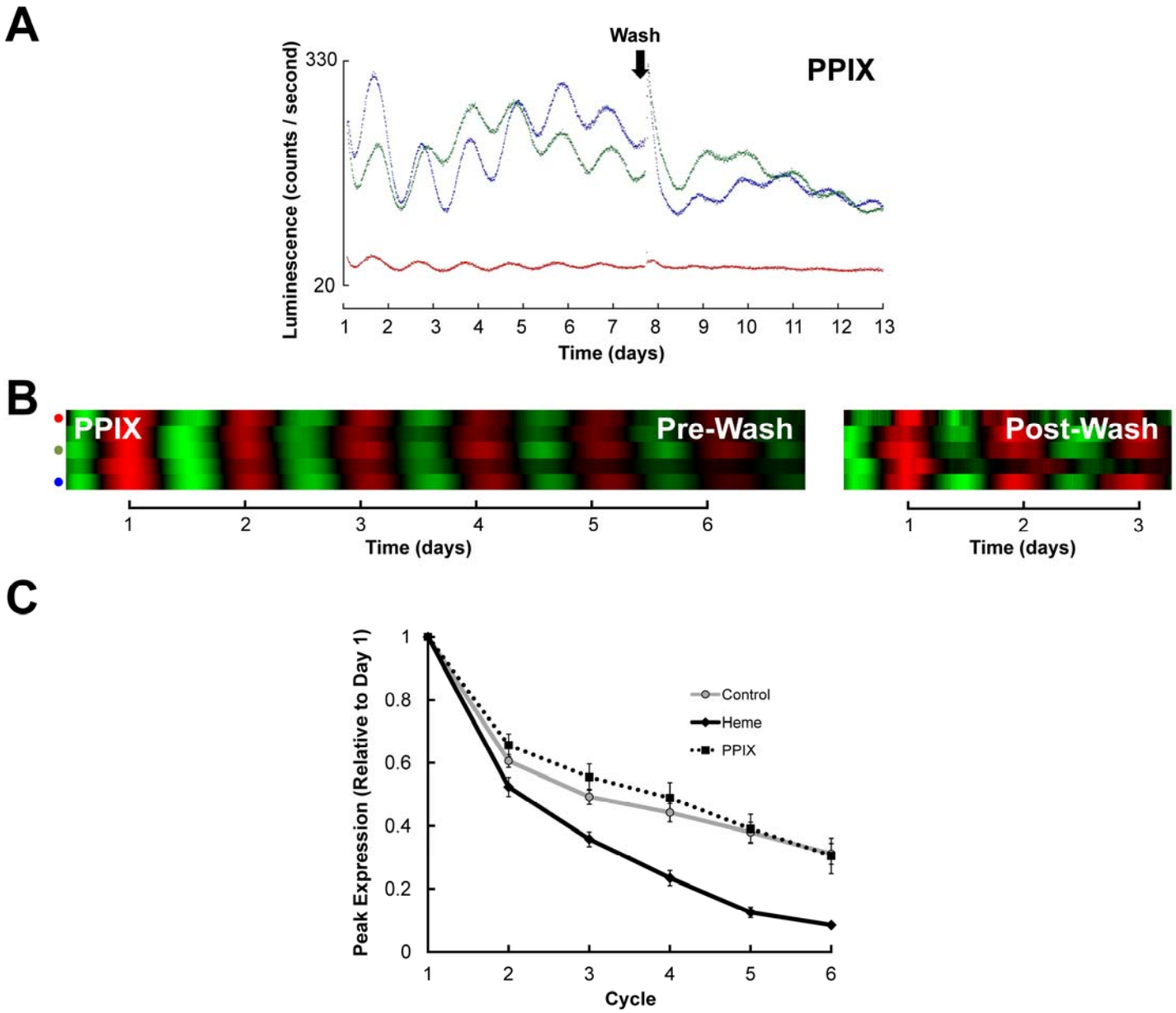

Figure S1. Effects of 30 µM protoporphyrin IX (PPIX) on mouse SCN PER2::LUCIFERASE rhythms. **A**, Three representative luminescence traces from SCN slices treated with PPIX from dissection time (Day 0); downward arrow indicates time of media change. **B**, Heatmap representing baseline-subtracted and smoothed traces from all PPIX-treated SCN explants. Each heatmap row represents a separate sample, the x-axis represents time, and color represent normalized luminescence (green indicates low luminescence levels, red indicates high luminescence levels, black indicates near-baseline luminescence; see key to Figure 1 in the main text). Traces before the media change (left panel) were normalized independently of traces after the media change (right panel). Red, blue, and green dots to the left of the heatmap identify heatmap rows corresponding to the red, blue, and green raw traces in part A. **C**, Mean peak expression of each circadian cycle, normalized to the peak expression of the cycle on the day after dissection (Cycle 1), for control SCN explants (gray, solid line) and for explants treated with 30 µM heme (black, solid line) or with 30 µM PPIX (black, dotted line). A two-way repeated measures ANOVA indicated significant effects of treatment ($F_{(2,38)} = 12.933$, $p < .001$) and of day ($F_{(4,152)} = 415.313$, $p < .001$) and a significant treatment X day interaction ($F_{(8,152)} = 9.062$, $p < .001$) with Tukey's HSD's revealing that PPIX did not differ from the control group ($p = .679$) but did differ from the heme group ($p = .001$). In this and subsequent figures, error bars indicate ± SEM.

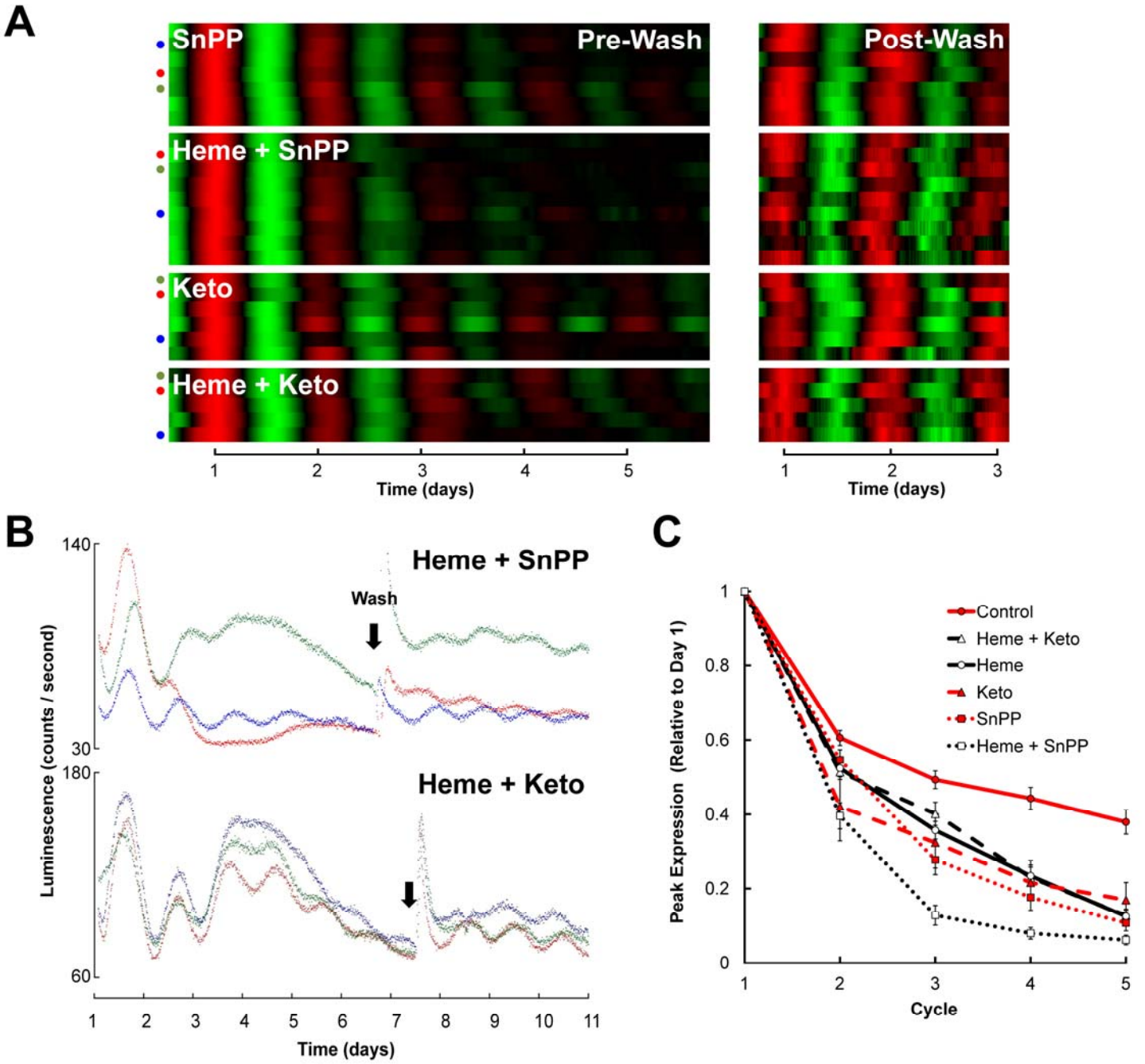

Figure S2. Representative raw traces (**B**) and all baseline-subtracted, smoothed, and normalized traces (**A**) for SCNs treated with 30 μM tin-protoporphyrin IX (SnPP; top) or 10 μM ketoconazole (Keto; bottom) in the presence or absence of 30 μM heme (see Fig. 4 in the main text). **C**, Peak PER2::LUC expression (normalized to peak expression on the first cycle *in* vitro) for SCNs treated as in part A. A three-way (Day X Heme X Inhibitor) repeated-measures ANOVA indicated significant effects of inhibitor ($F_{(2,57)} = 17.45$, $p < .0001$) and of heme ($F_{(1,57)} = 8.503$, $p = .0051$) and a significant inhibitor X heme interaction ($F_{(2,57)} = 4.550$, $p = .0147$); all possible within-subjects effects and interactions were also significant (p's < .001).

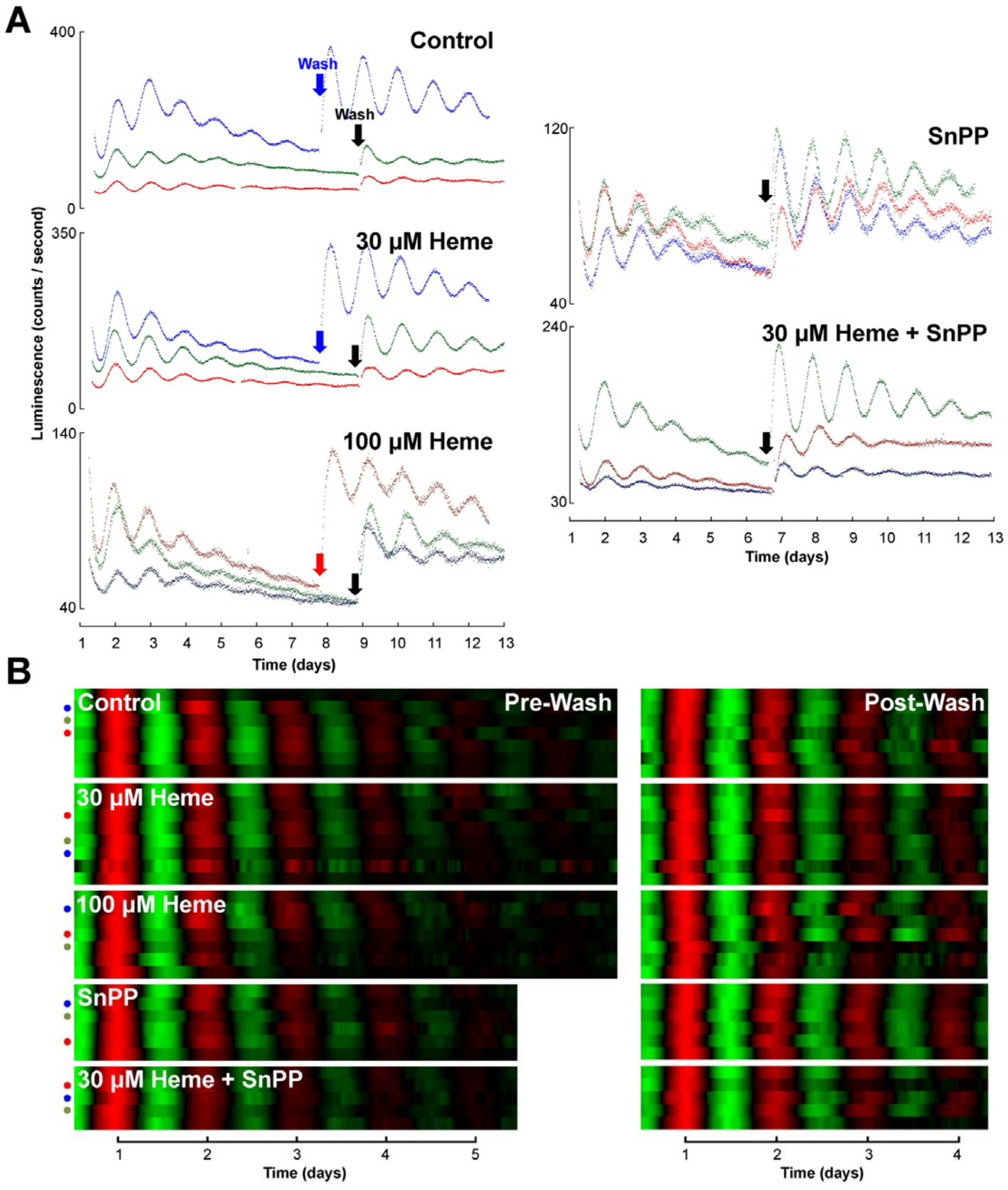

Figure S3. Representative raw traces (**A**) and all baseline-subtracted, smoothed, and normalized traces (**B**) for control esophagus explants and for esophagus explants treated with 30 μM or 100 μM heme or with 30 μM tin-protoporphyrin IX (SnPP) in the presence or absence of 30 μM heme.

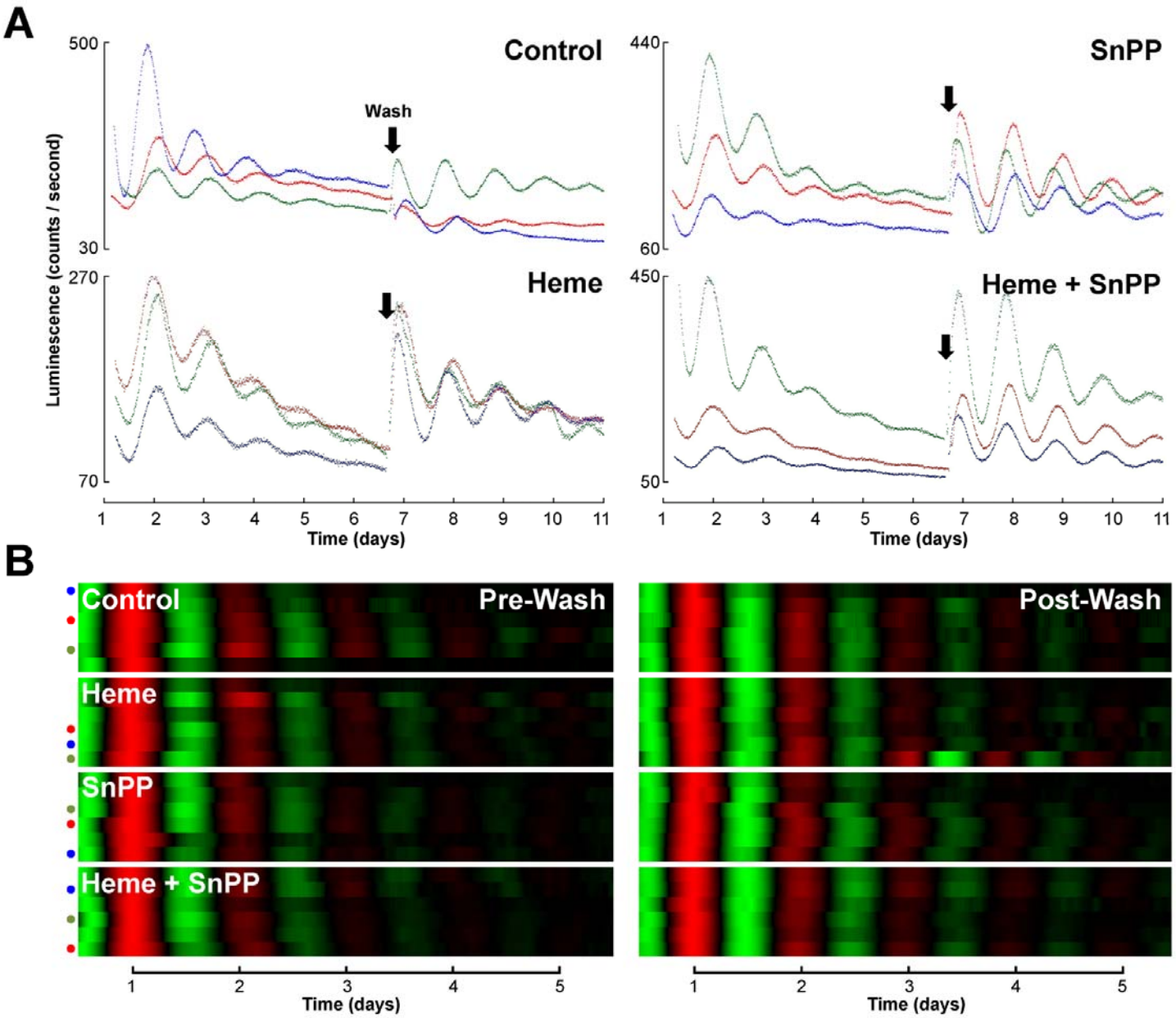

Figure S4. Representative raw traces (**A**) and all baseline-subtracted, smoothed, and normalized traces (**B**) for control thymus explants and for thymus explants treated with 30 μM tin-protoporphyrin IX (SnPP) or 30 μM heme alone or together.

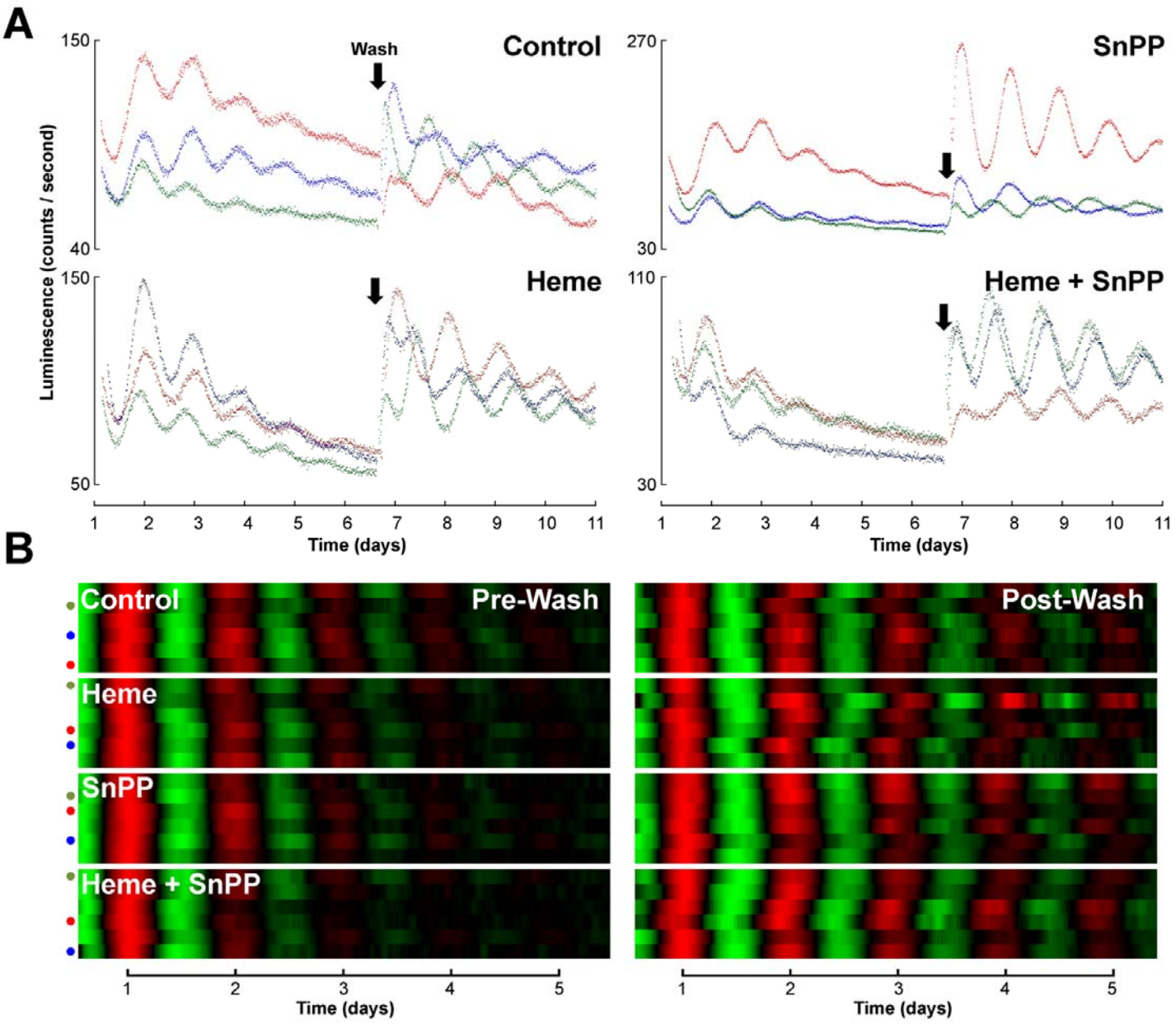

Figure S5. Representative raw traces (**A**) and all baseline-subtracted, smoothed, and normalized traces (**B**) for control spleen explants and for spleen explants treated with 30 μM tin-protoporphyrin IX (SnPP) and 30 μM heme alone or together.

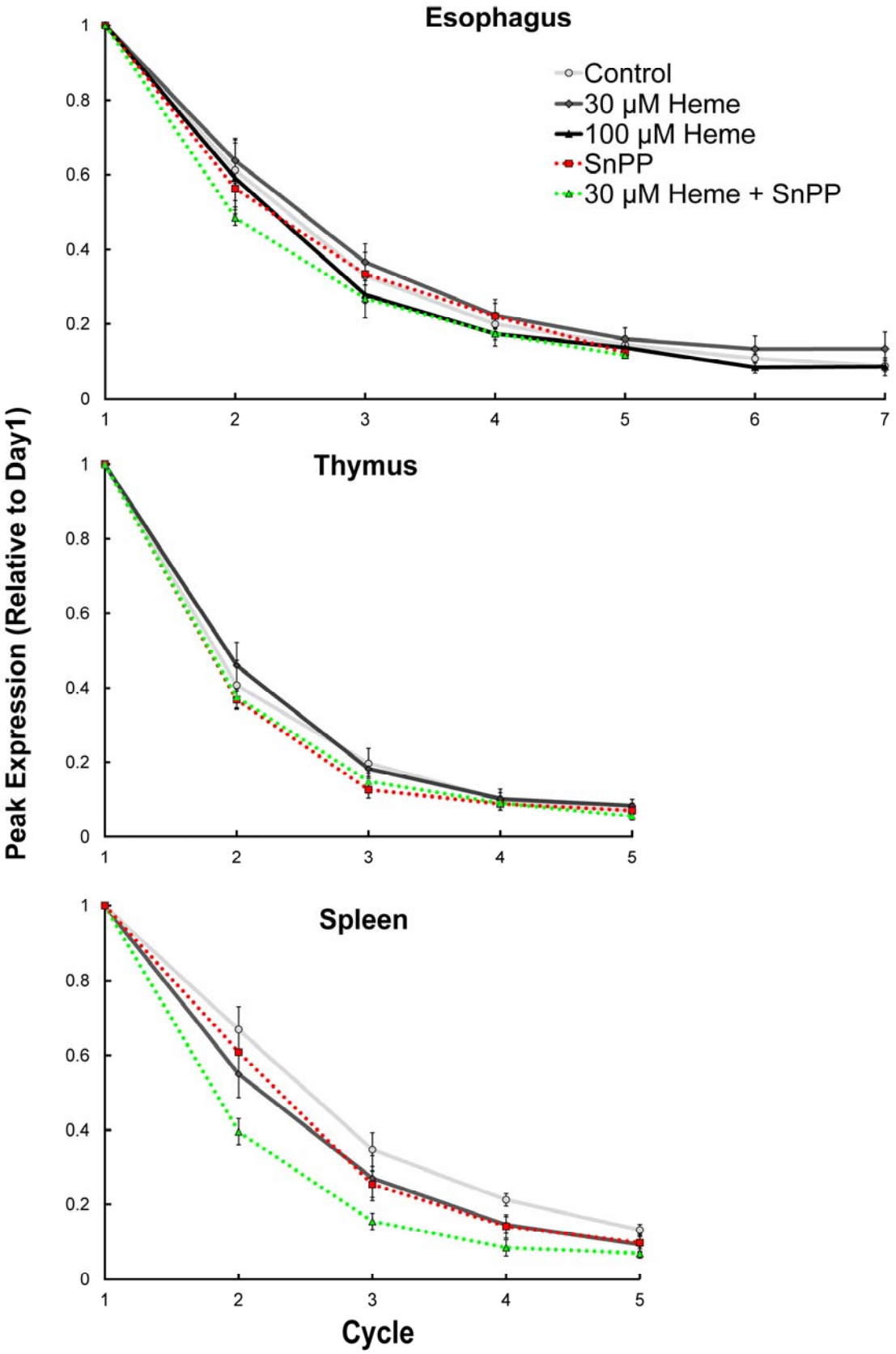

Figure S6. Effects of 30 μM heme, 30 μM SnPP, or both on relative peak PER2::LUC expression in esophagus (top), thymus (middle), and spleen (bottom) explants. A three-way repeated measures ANOVA (Heme X Inhibitor X Cycle) indicated no effect of heme or SnPP in esophagus or thymus explants (p's for all between-subjects factors and interactions > .165). However, in spleen explants, a similar analysis indicated a significant effect of heme (p = .010) and of SnPP (p = .007) but no heme X SnPP interaction (p = .984).

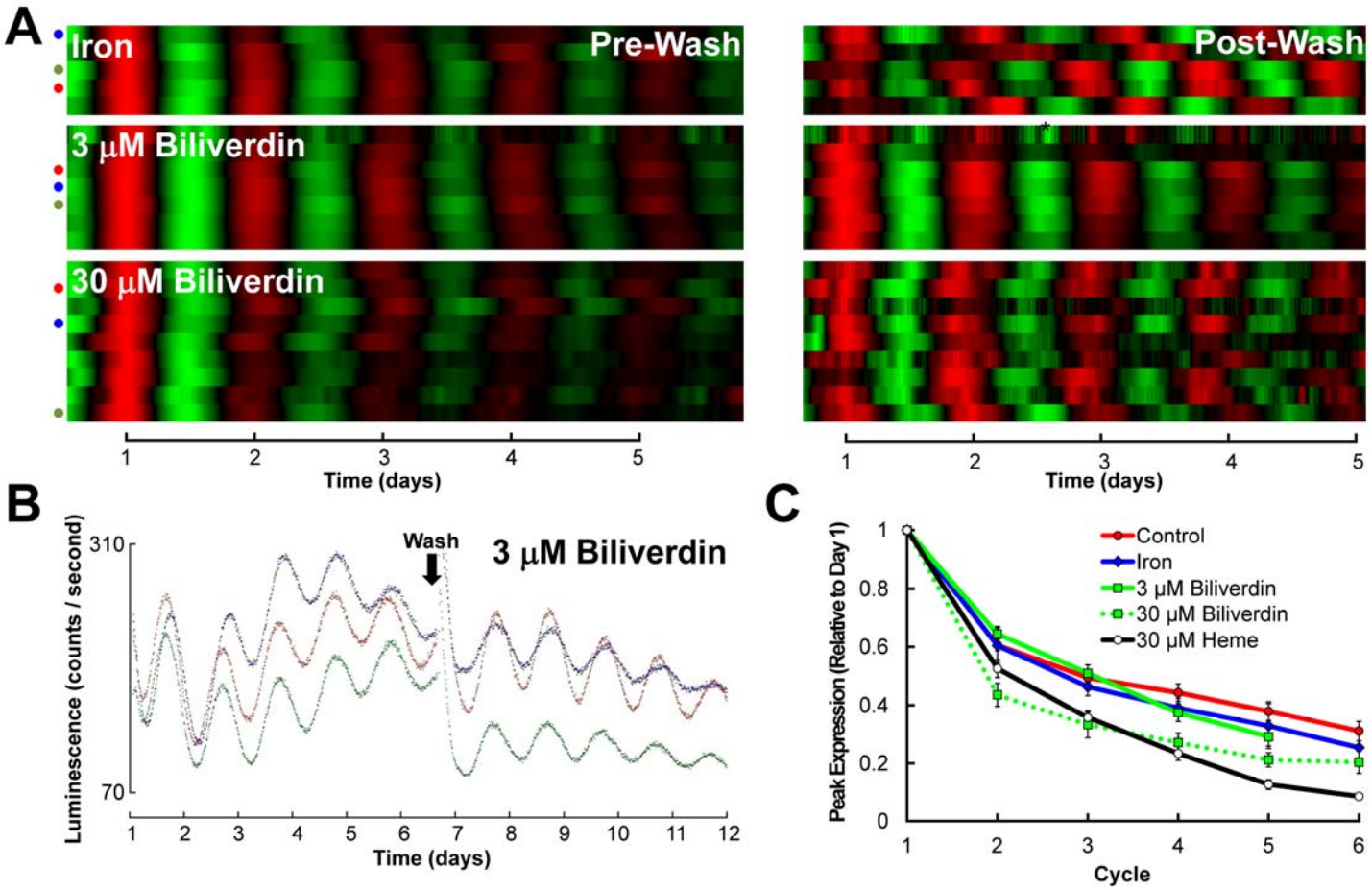

Figure S7. **A**, All baseline-subtracted, smoothed, and normalized data for SCNs treated with iron and biliverdin. Colored dots to the left of the rows indicate which traces are presented in part B and in Fig. 6A. **B**, Three representative raw traces of SCNs treated with 3 μM biliverdin. **C**, Mean peak PER2::LUC expression for each cycle (normalized to the first cycle) for control SCNs (red) and for SCNs treated with 30 μM iron (blue), 3-30 μM biliverdin (green), or 30 μM heme (black). We found a significant effect of treatment (two-way repeated measures ANOVA, $F_{(4,52)} = 8.818$, $p < .0001$) and a significant treatment X cycle interaction ($F_{(16,208)} = 5.838$, $p < .0001$); 30 μM biliverdin and 30 μM hemin groups but not the Fe^{2+} or 3 μM biliverdin groups differed from the control (Tukey's HSD: control vs. 3 μM biliverdin, $p = .973$; control vs. Fe2+, $p = .956$; control vs. 30 μM biliverdin, $p = .0006$; control vs. 30 μM hemin, $p = .0001$).

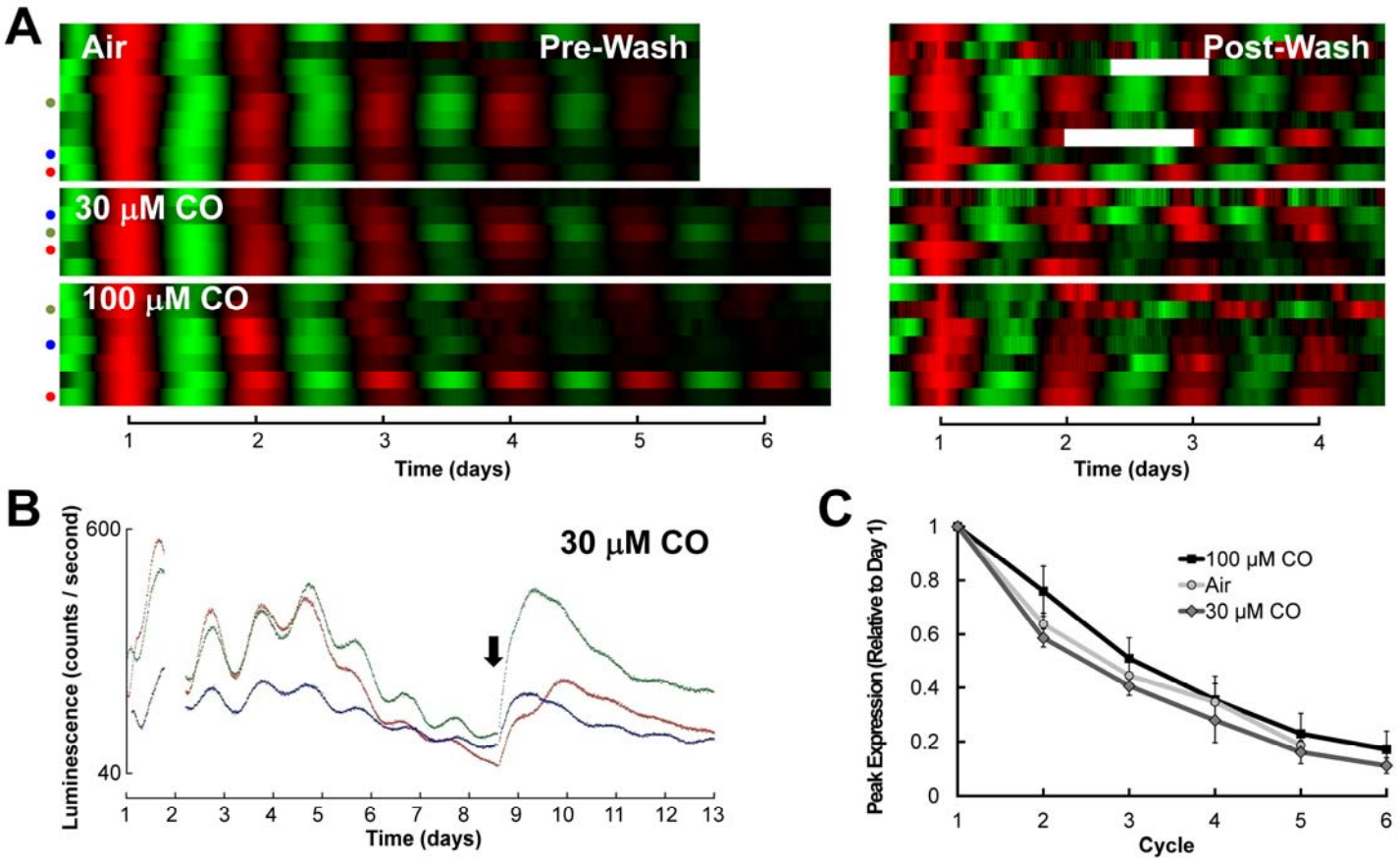

Figure S8. A, All baseline-subtracted, smoothed, and normalized data for SCNs sealed with carbon monoxide (CO) or under air. Colored dots to the left of the rows indicate which traces are presented in part B and in Fig. 6C. **B**, Three representative raw traces of SCNs treated with 30 μM CO. **C**, Mean peak PER2::LUC expression for each cycle (normalized to the first cycle) for air- and CO-treated SCNs. We observed no differences between SCN slices sealed under air and those sealed under 30 μM or 100 μM carbon monoxide (two-way repeated measures ANOVA: treatment, $F_{(2,18)} = .915$, $p = .418$; day X treatment, $F_{(8,72)} = .503$, $p = .850$).

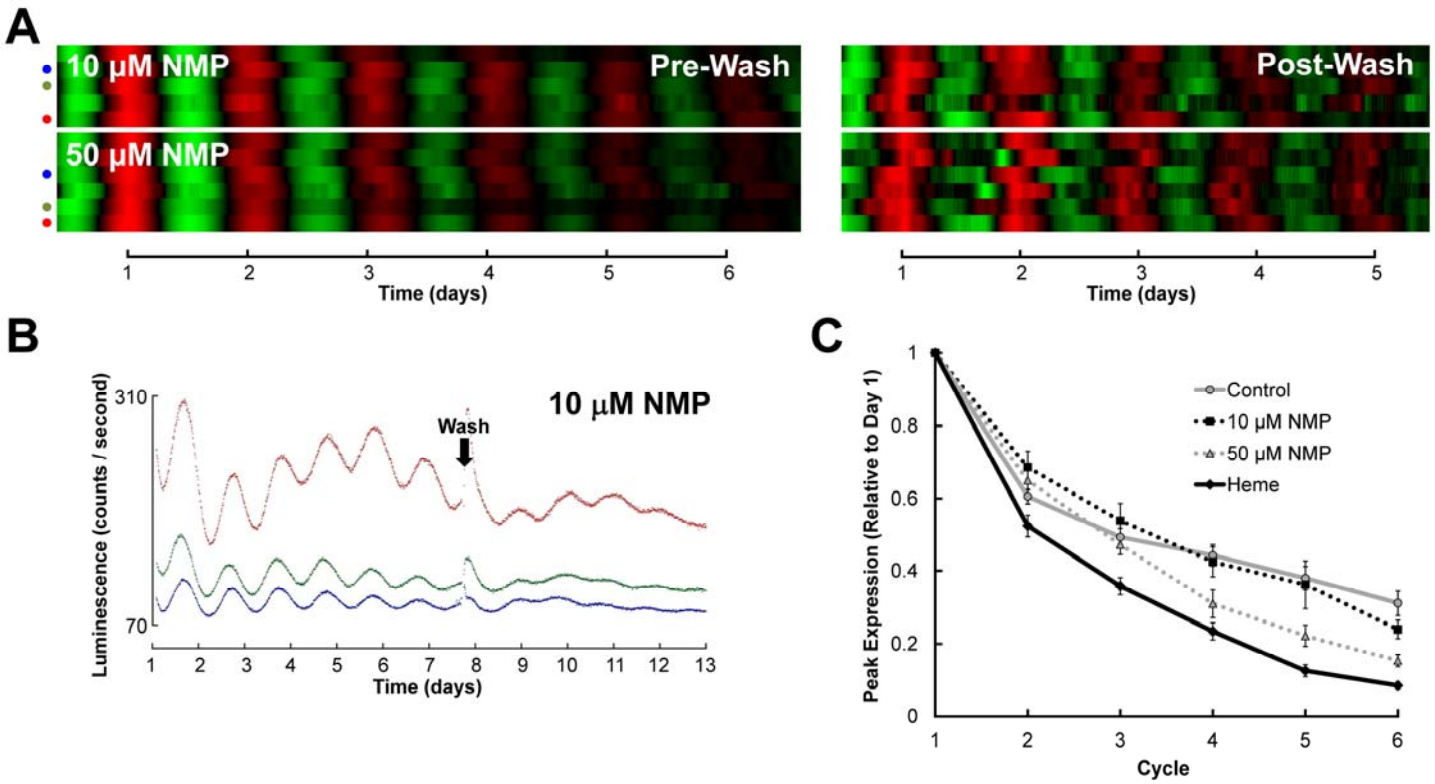

Figure S9. A, All baseline-subtracted, smoothed, and normalized data for SCNs treated with 10 µM or 50 µM N-methylprotoporphyrin IX (NMP). Dots to the left of a heatmap row indicate which raw traces are represented in part B or in Fig. 7A. **B**, Three representative raw traces of PER2::LUC activity for SCNs treated with 10 µM NMP. **C**, We found a significant effect of treatment ($F_{(3,37)} = 6.235$, $p = .002$) and a significant treatment X cycle interaction ($F_{(15,185)} = 5.07$, $p < .0001$); post-hoc tests suggested that these effects were due to a difference between 30 µM heme and control groups ($p = .001$) rather than to a difference between the control and the NMP groups ($p = 1.00$ and $p = .240$ for 10 µM and 50 µM, respectively).