Supplemental Figure Legends

Supplemental Figure 1: Validation of siRNA knockdown efficiency. Effects of the E3 ligase knockdowns on their respective mRNA levels, determined by qRT-PCR (mean+/-SEM, n=3-4 cultures per siRNA). All 4 Spsb genes were knocked down compared to negative controls (p-value shown, ANOVA, Sidak's multiple comparisons test.

Supplemental Figure 2: Comparison of U2OS cell rhythms after Dexamethasone or Serum Shock synchronization. A) qRT-PCR of *Per2* (normalized to *Gapdh*) mRNA levels in normal U2OS cells after synchronization with the agents indicated, within the same experiment. Time 0 = immediately prior to dexamethasone (dex) or 50% horse serum (serum shock) exposure. Data are plotted relative to the average signal produced over the entire collection for each exposure. B) Representative luciferase activity rhythms produced by the stably transfected *Bmal1-Luc* U2OS cell line used in our experiments, in response to each synchronizing agent. Time 0 = start of dex, or the end of the 2-hour treatment with 50% horse serum (thus the rhythm is advanced by ~2 hours relative to the dex treated cells. After ~12 hours, both dex and serum shock produce rhythms with similar circadian characteristics.

Supplemental Figure 3: Spsb4 selectively degrades RevErbα. A) Representative western blots of AD293 cell extracts transfected with constructs expressing Flag-tagged core clock proteins and either an empty Sport6 vector or Spsb4. Cells were treated with Cycloheximide for the indicated hours before lysis. Cell lysates were analyzed for immunoblotting for the levels of flag-tagged clock proteins as well as GAPDH as a loading control. B) Quantitation of blots shown on the left panel plotted relative to time 0. Means +/- SEM, n=3-7 independent experiments are shown. Asterisks indicates differences at individual time points p=0.036, ***p =0.0006, ****p<0.0001 by two-way ANOVA, Sidak's multiple comparisons test. Time x E3 ligase interaction p-values are shown for each graph.

Supplemental Figure 4: Spsb2 and Spsb3 do not degrade Flag-RevErbα in AD293 cells. AD293 cells were co-transfected with Flag-RevErbα, Sport6 control, Spsb2-HA, Spsb3-HA and Spsb4-HA plasmids. Cells were treated with CHX for the indicated hours before lysis. Cell lysates were analyzed for immunoblotting for the levels of flag-tagged RevErbα, HA tagged-Spsb proteins and β-tubulin as a loading control. A representative western blot of two independent experiments.

Supplemental Figure 5: Spsb1 and Spsb4 degrade Flag-RevErbα in U2OS cells. U2OS cell lysates were co-transfected with Flag-RevErbα and Sport6, Spsb1 or HA tagged Spsb4 plasmids. Cells were treated with CHX for the indicated hours before lysis. Cell lysates were analyzed for immunoblotting for the levels of flag-tagged RevErbα, HA tagged-Spsb4, as well as GAPDH as a loading control. A representative western blot of two independent experiments is shown.

Supplemental Figure 6: The combined siRNA knockdown of Spsb2 and Spsb3 is not additive. Average period lengths of *Bmal1-Luc* U2OS cells combined from two independent experiments (mean +/-SEM, n=4-6 cultures per siRNA) ANOVA, Sidak's multiple comparisons test.

Supplemental Figure 7: Spsb1 and Spsb4 depletion causes a delay in the mRNA expression of Bmal1 and Cry1. Quantification of RevErba protein abundance (mean+/- SEM, n=4) profiles overlaid on Bmal1 and Cry1 mRNA profiles of synchronized U2OS cells collected every 2 hours from Figure 5. Data are plotted as described for Figure 5.