



Figure S2

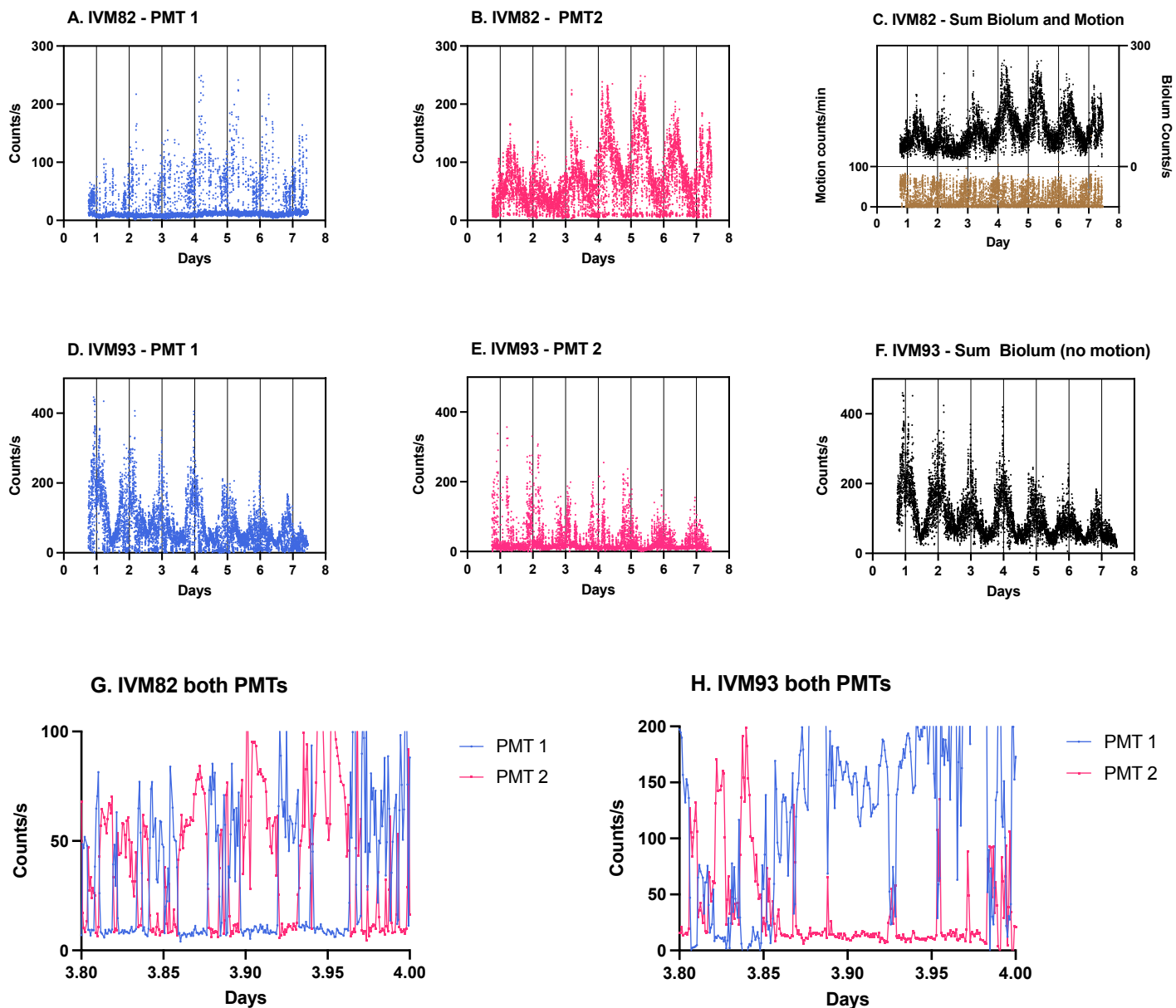


Figure S2. Raw data for cases shown in Figure 2. For each animal, IVM82 and IVM93, the incoming counts/s detected by each of the two photomultiplier tubes (PMTs) is plotted over 7 days of recording. We then sum the counts collected each second from PMT1 and PMT2 for the final raw data files, plotted in C and F. In the case of IVM82, we also collected locomotor activity from a motion sensor; this is shown as counts/min in brown below bioluminescence (black) in C. Such data was not available for IVM93 due to software malfunction. In G and H we show on a shorter time base how the signal switched between PMT1 and PMT2, presumably as the animal moved around in the cage.



Figure S3

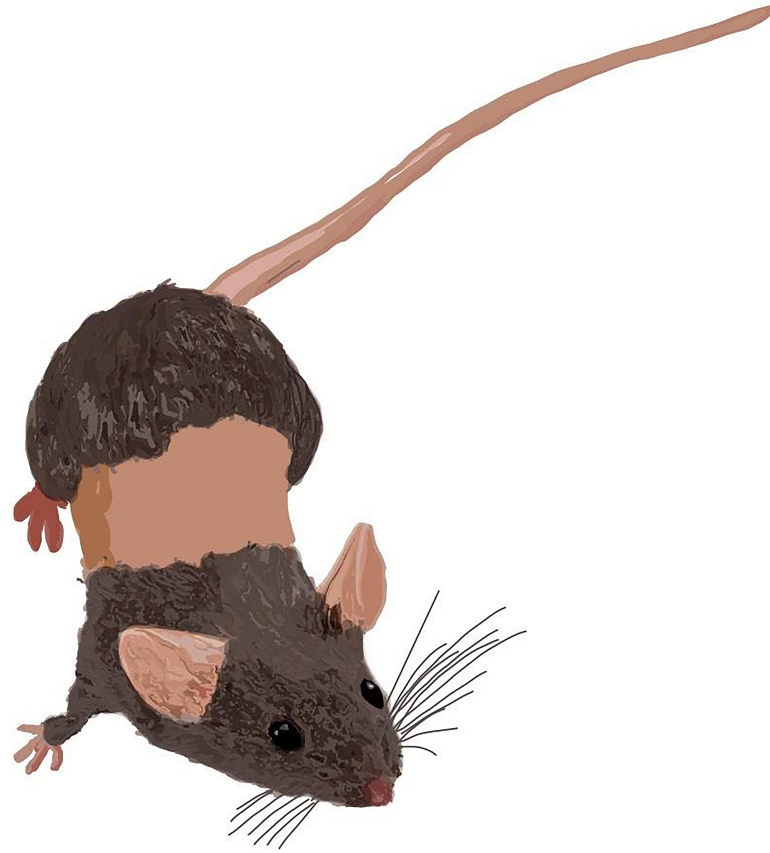


Figure S3. Drawing to demonstrate the extent of the shave administered prior to experiments in mice with black fur. The mouse was shaved in a band that extended from dorsal to ventral surface.

Figure S4 (A,B)

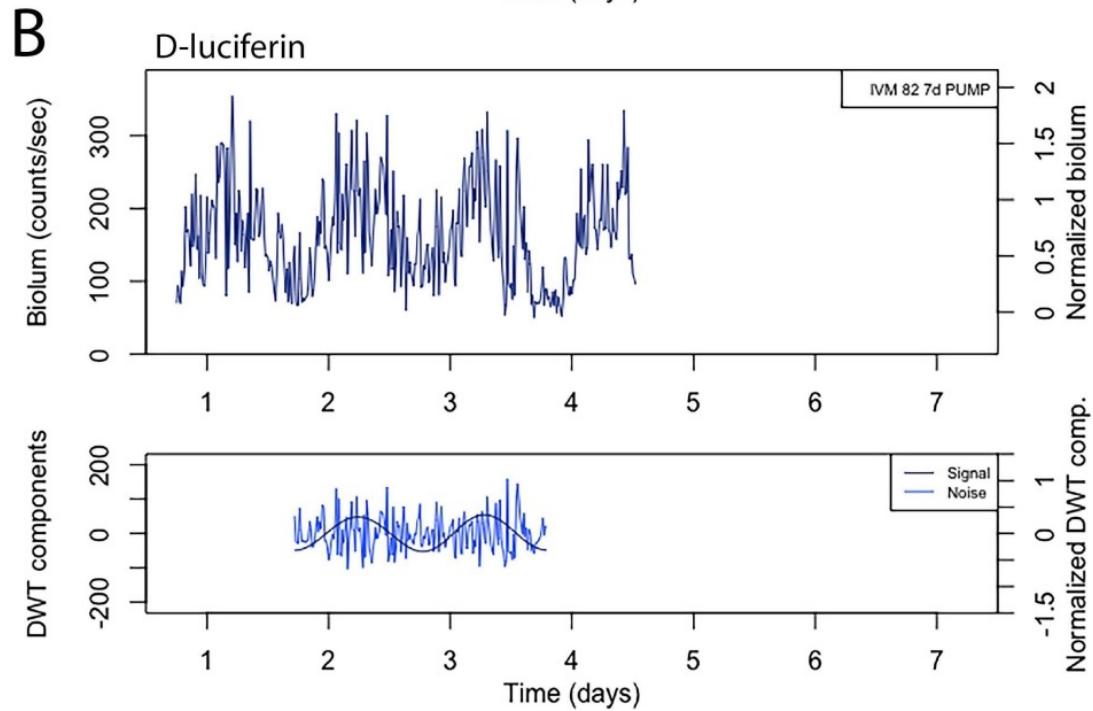
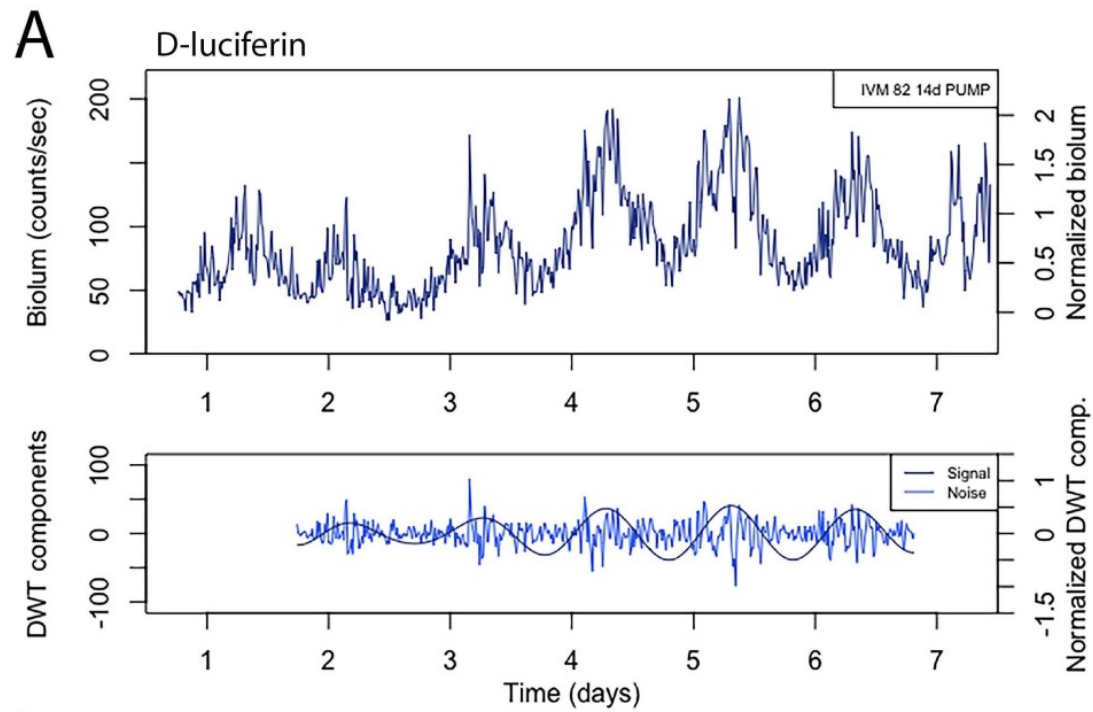


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red - CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.

Figure S4 (C, D)

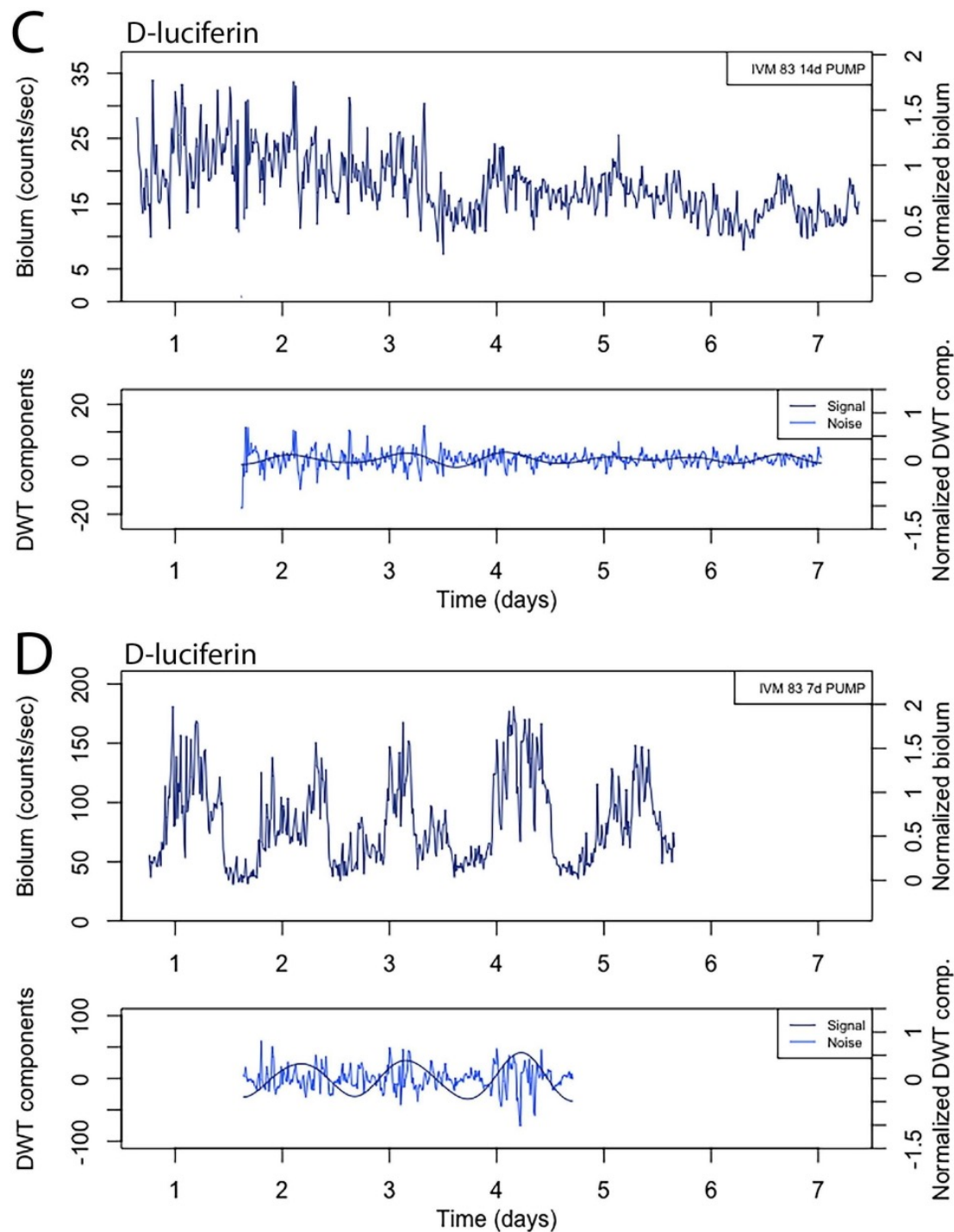


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red - CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.

Figure S4 (E, F)

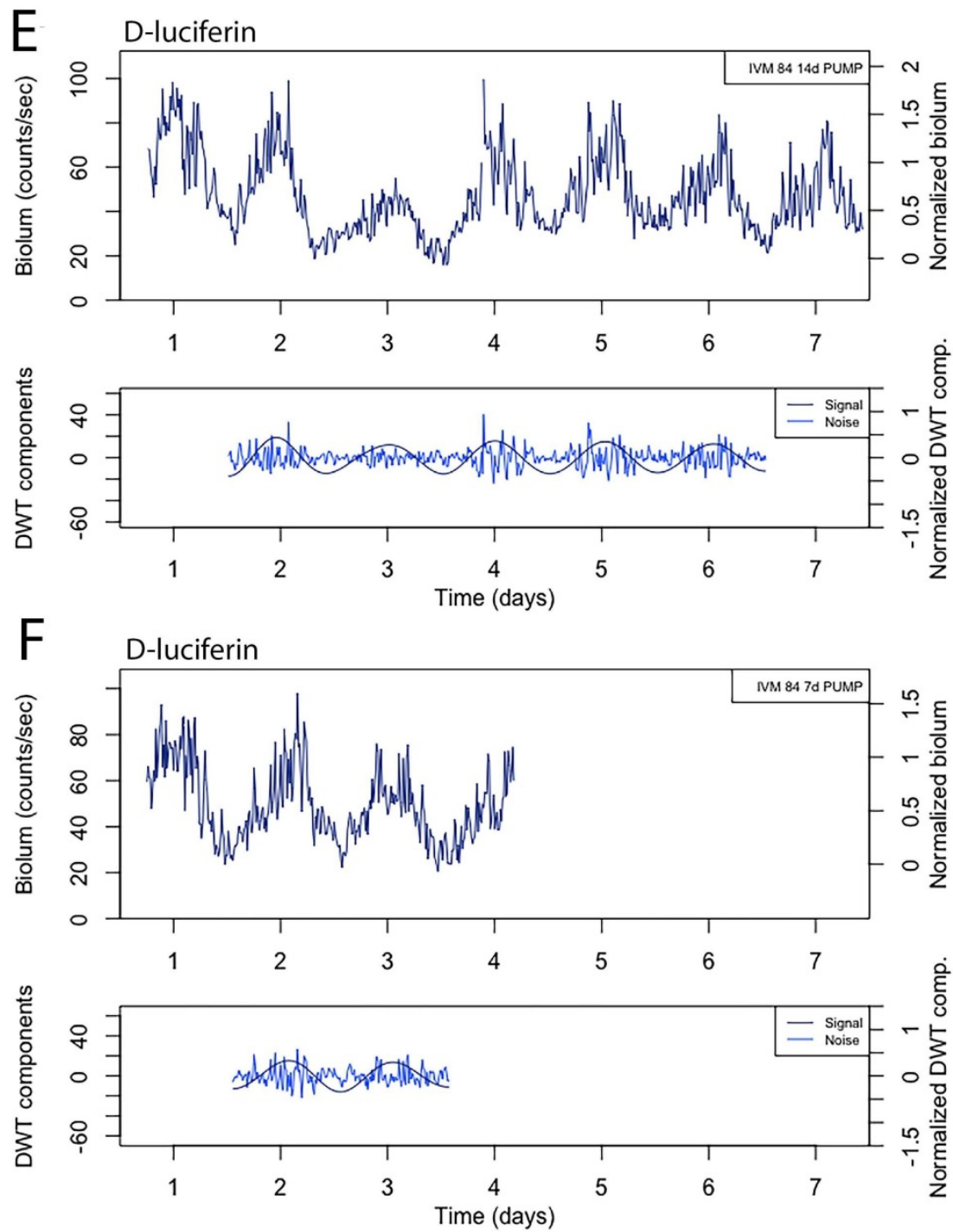


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red - CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.



Figure S4 (G, H)

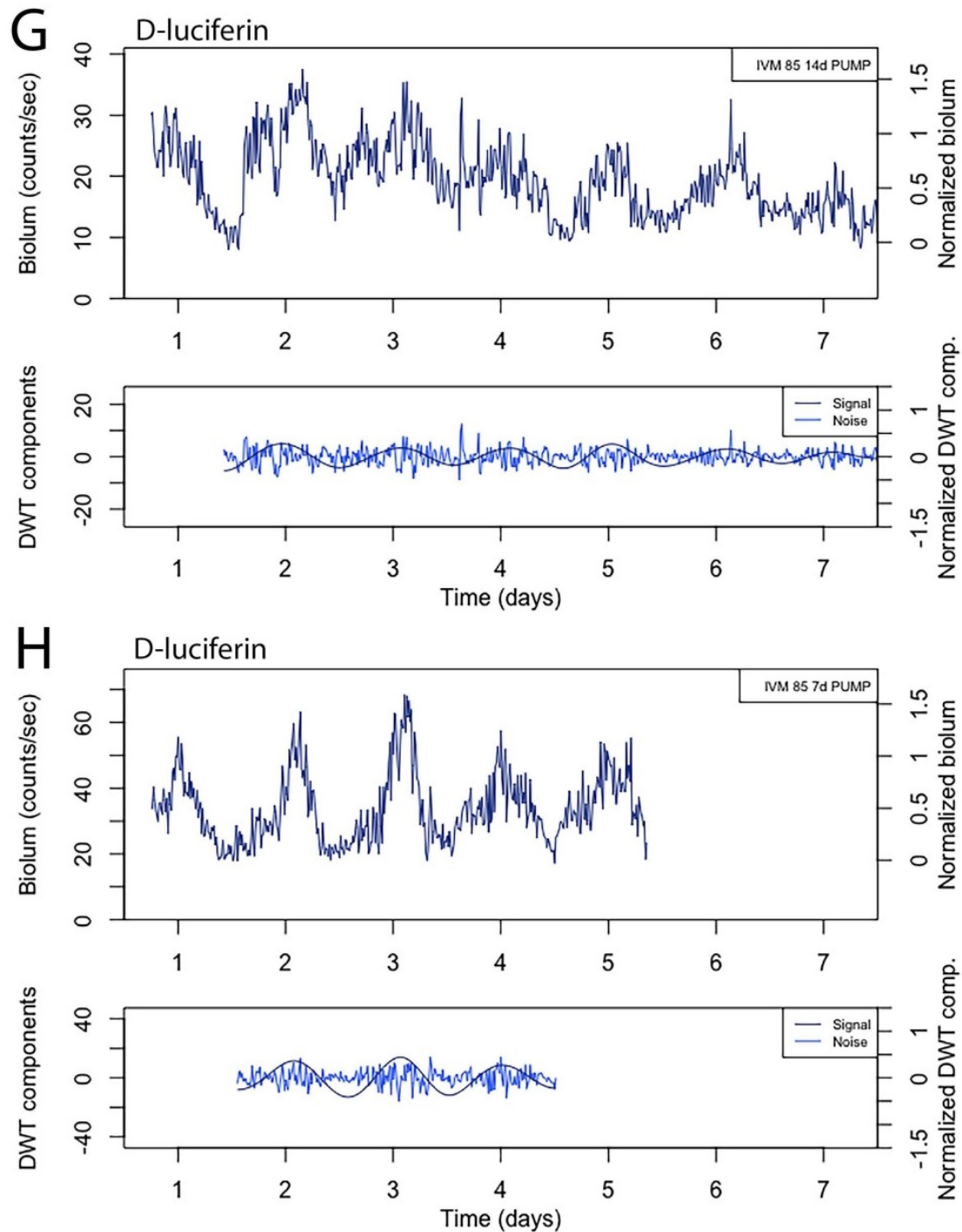


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red- CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.

Figure S4 (I, J)

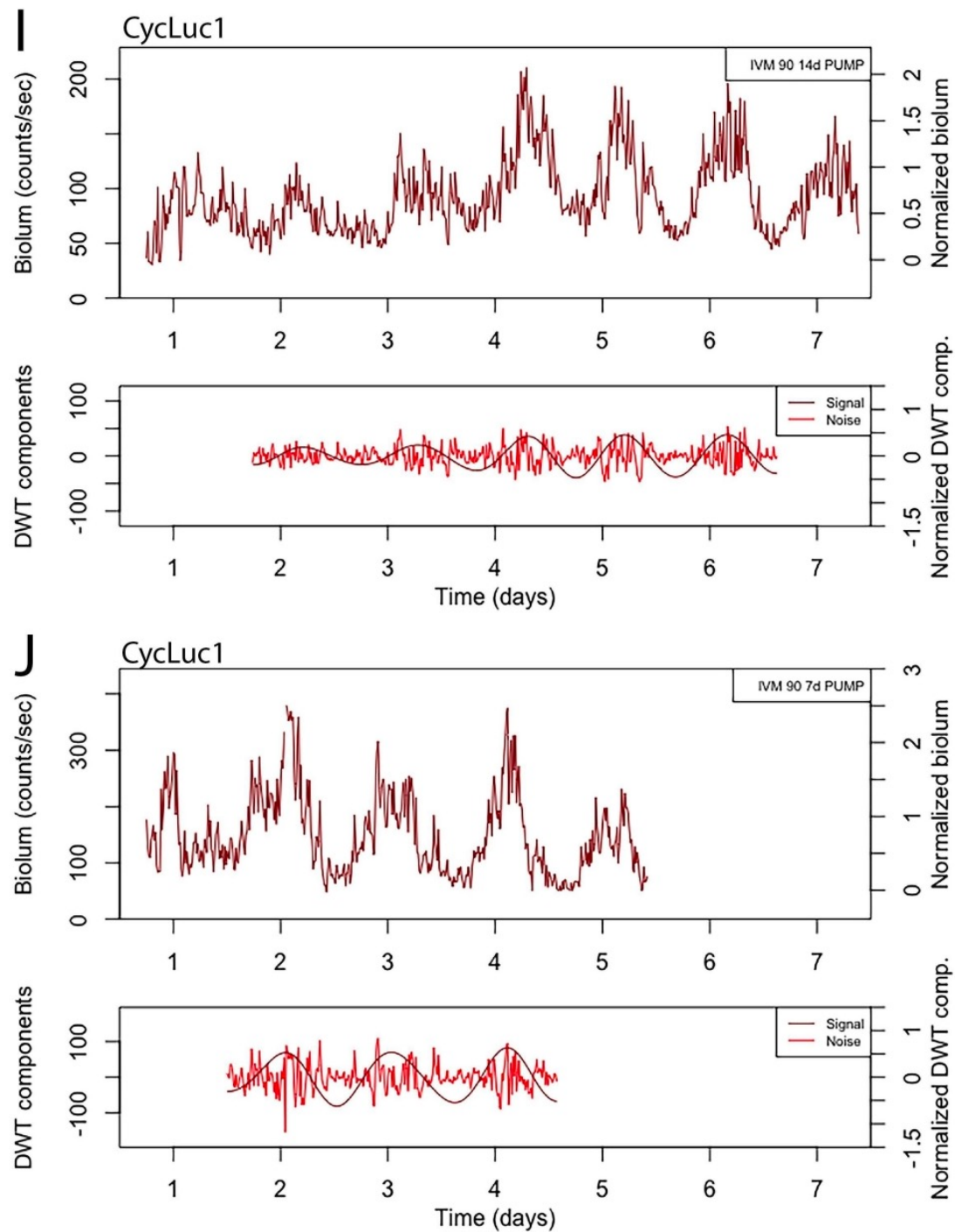


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red - CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.

Figure S4 (K, L)

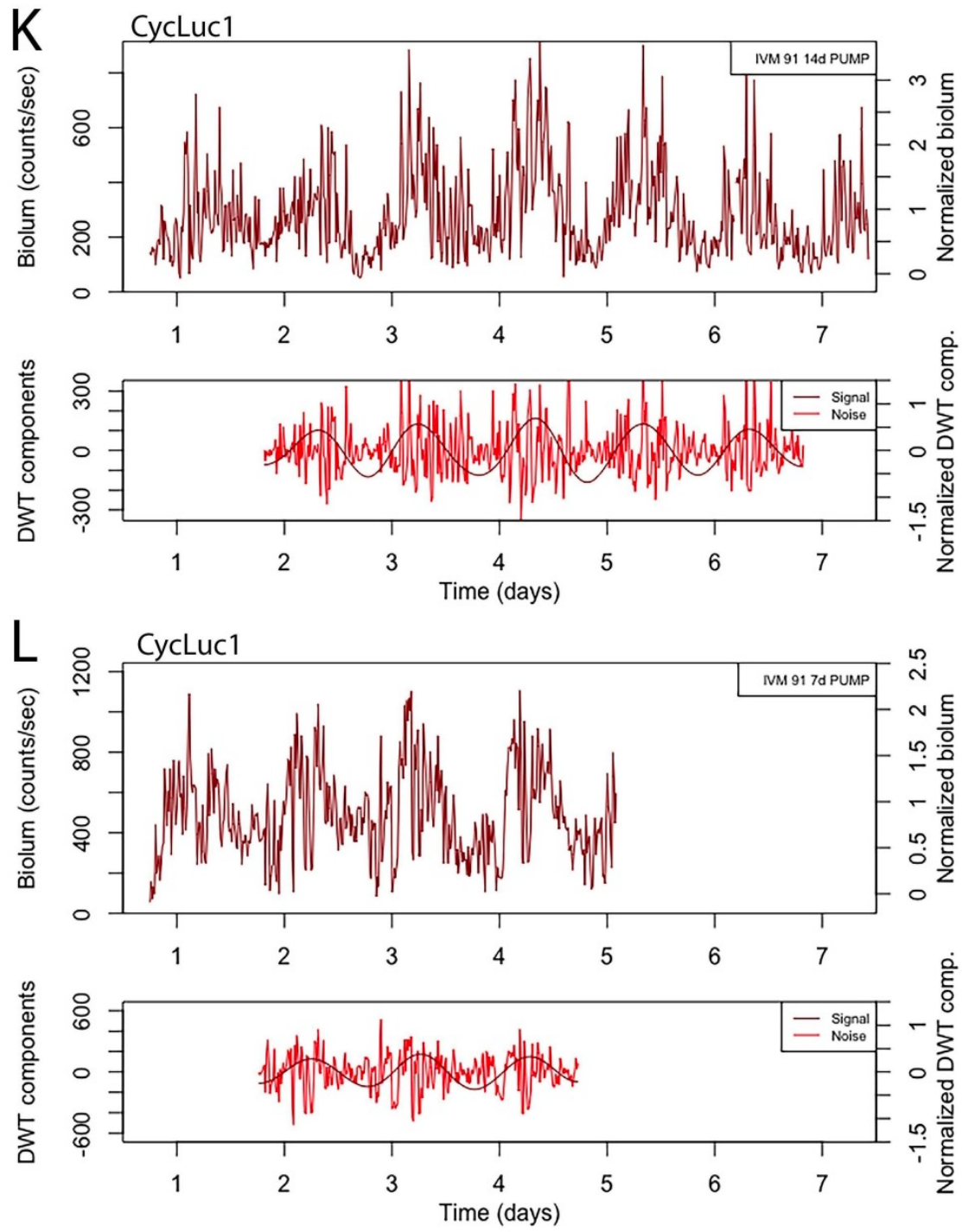


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red - CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.

Figure S4 (M, N)

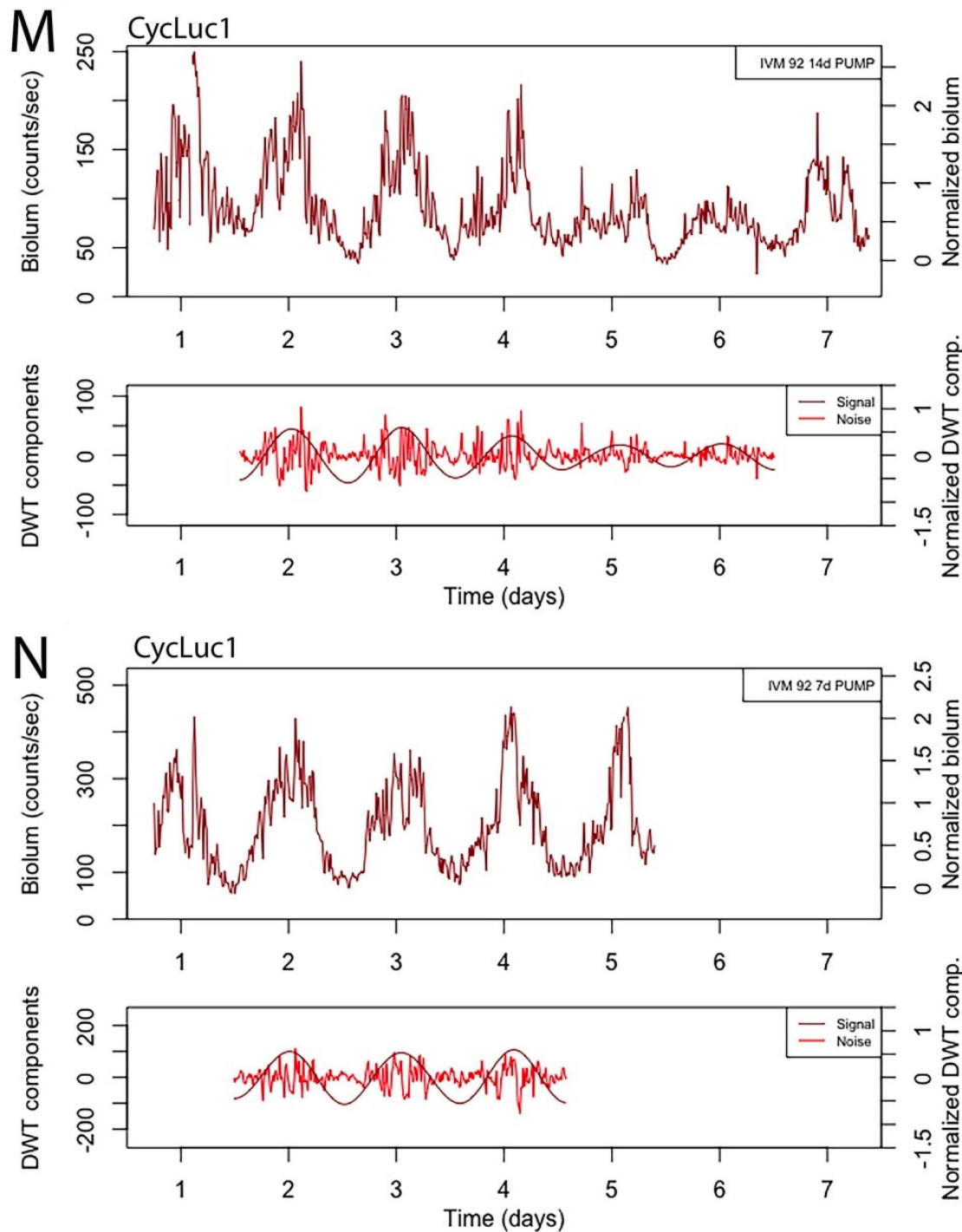


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red - CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.



Figure S4 (O, P)

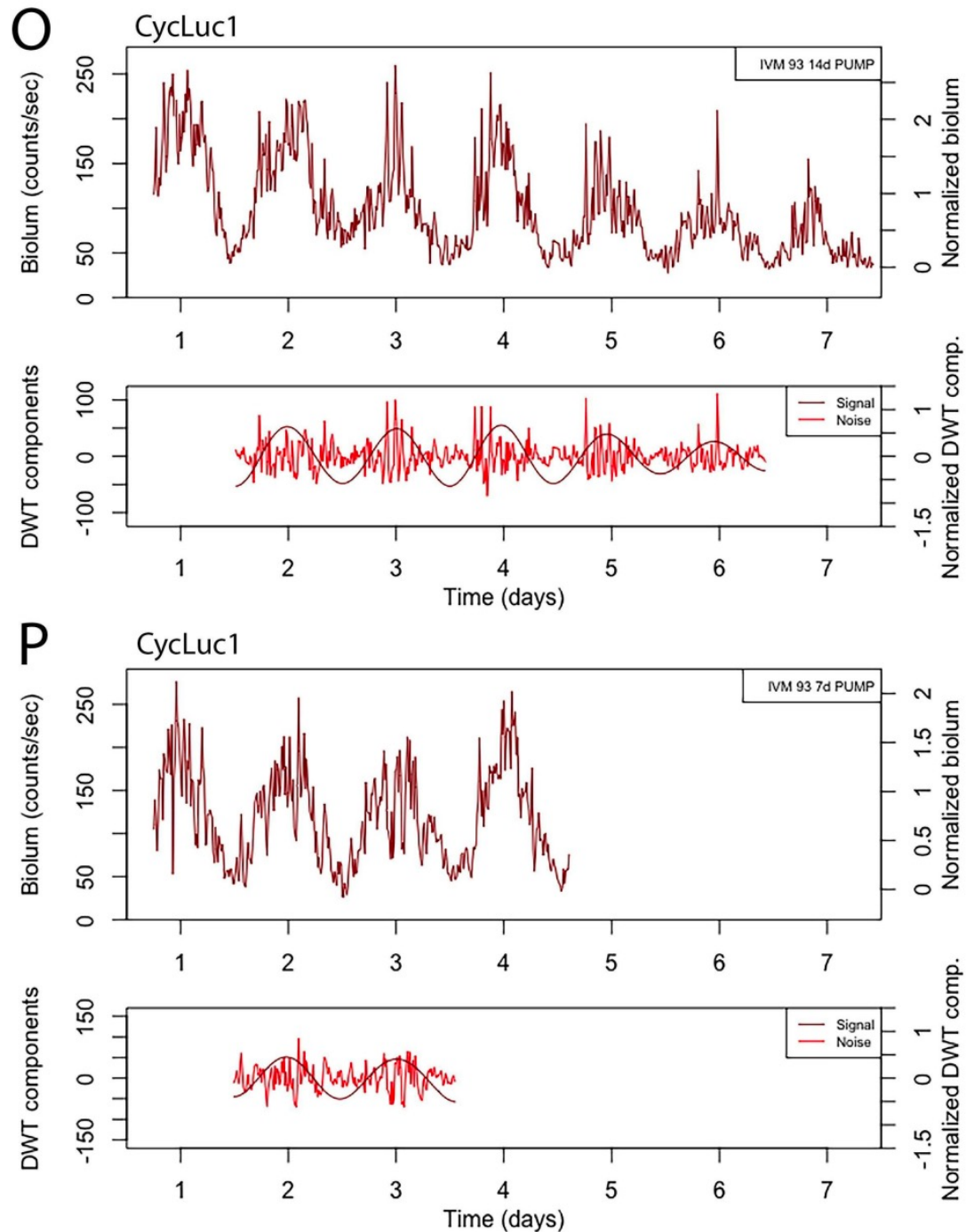


Figure S4. All results of bioluminescence data with either 7-day or 14-day D-luciferin or CycLuc1 pumps. Top graph for each example: 15-minute median binned trace with counts/sec scale on the left and normalized scale on the right (subtract first percentile then divide by median, so min is mapped to zero and median mapped to 1; using first percentile reduces the effect of outliers). Bottom graph for each example: For the signal-to-noise ratio analysis, the DWT-calculated circadian component D6 is treated as the signal and the summed components D1-D4 are treated as the noise. The data before the first trough and after the last trough are discarded to avoid edge effects. The 14 day pumps delivered 0.25 uL/h of 100 mM D-luciferin or 5 mM CycLuc1. The 7 day pumps delivered 0.50 uL/h of 100 mM D-luciferin or 5 mM CycLuc1 (blue - D-luciferin pumps, red- CycLuc1 pumps). Individual animals are identified by the “IVMxx” number.

Figure S5

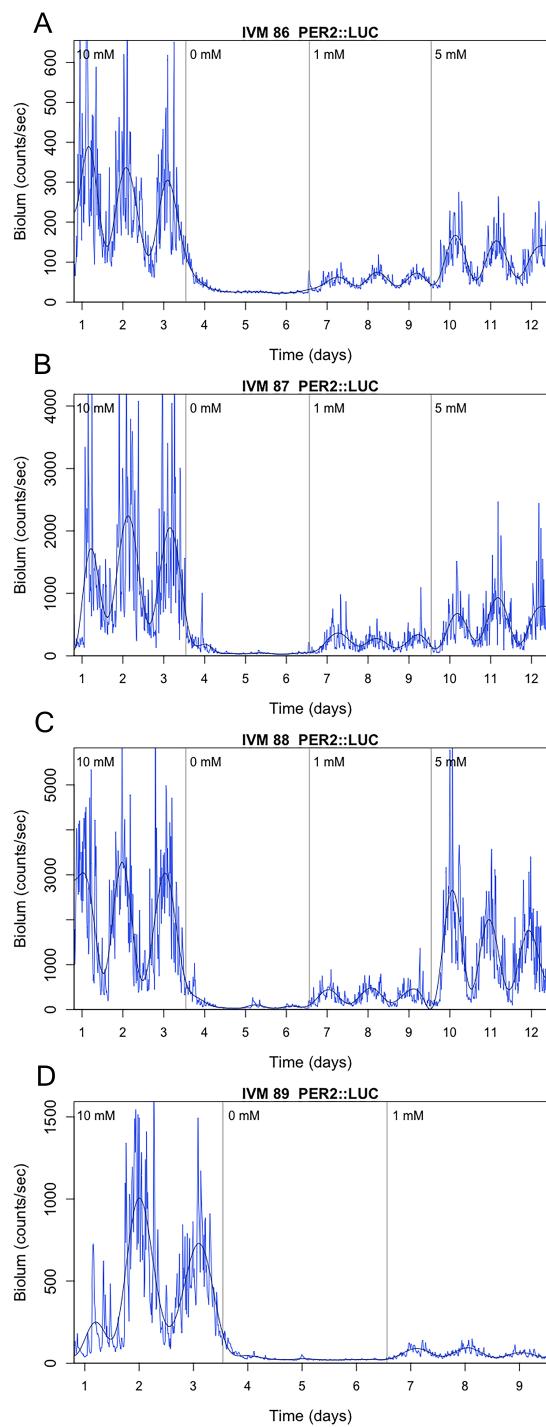


Figure S5. All drinking dose response data. Individual animals are identified by the “IVMxx” number. Final data for IVM 89 (D) was lost due to equipment failure.

Figure S6

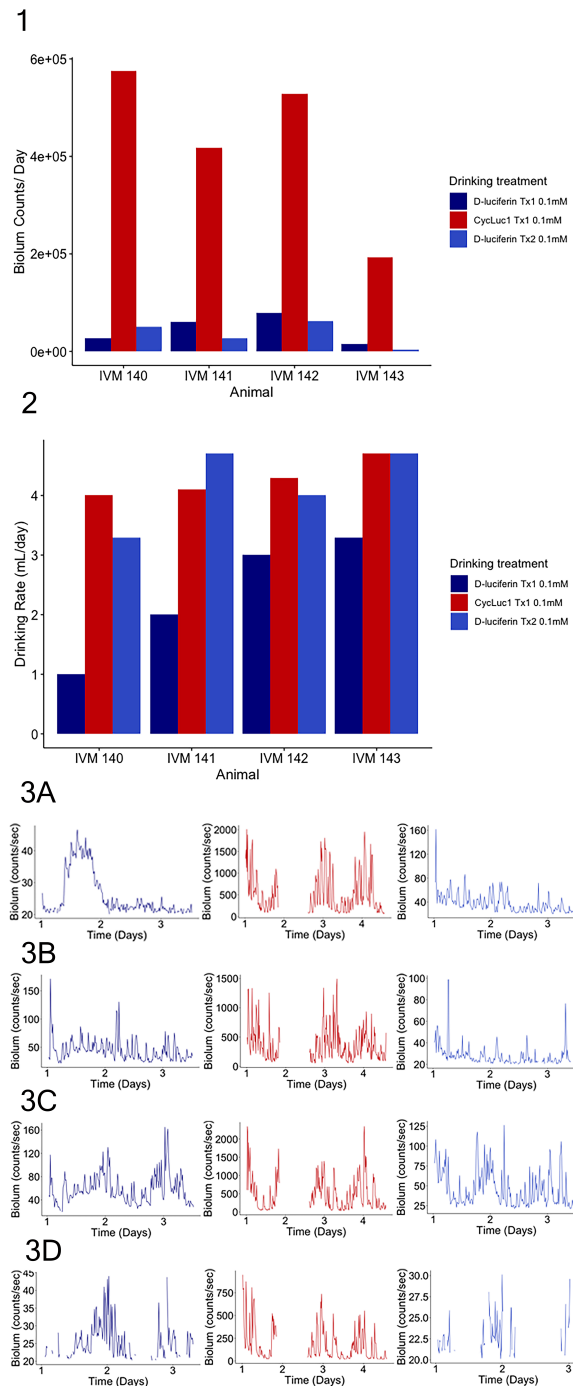


Figure S6. A) Drinking rate results for CycLuc1 vs D-luciferin comparison data. Drinking rate per day for each treatment was calculated by dividing the total volume of substrate consumed by the number of days. B) Bioluminescence records for all animals (Left - 0.1 mM D-luciferin treatment 1, center - 0.1 mM CycLuc1 treatment, right - 0.1 mM D-luciferin treatment 2). Individual animals are identified by the “IVMxx” number.

Figure S7

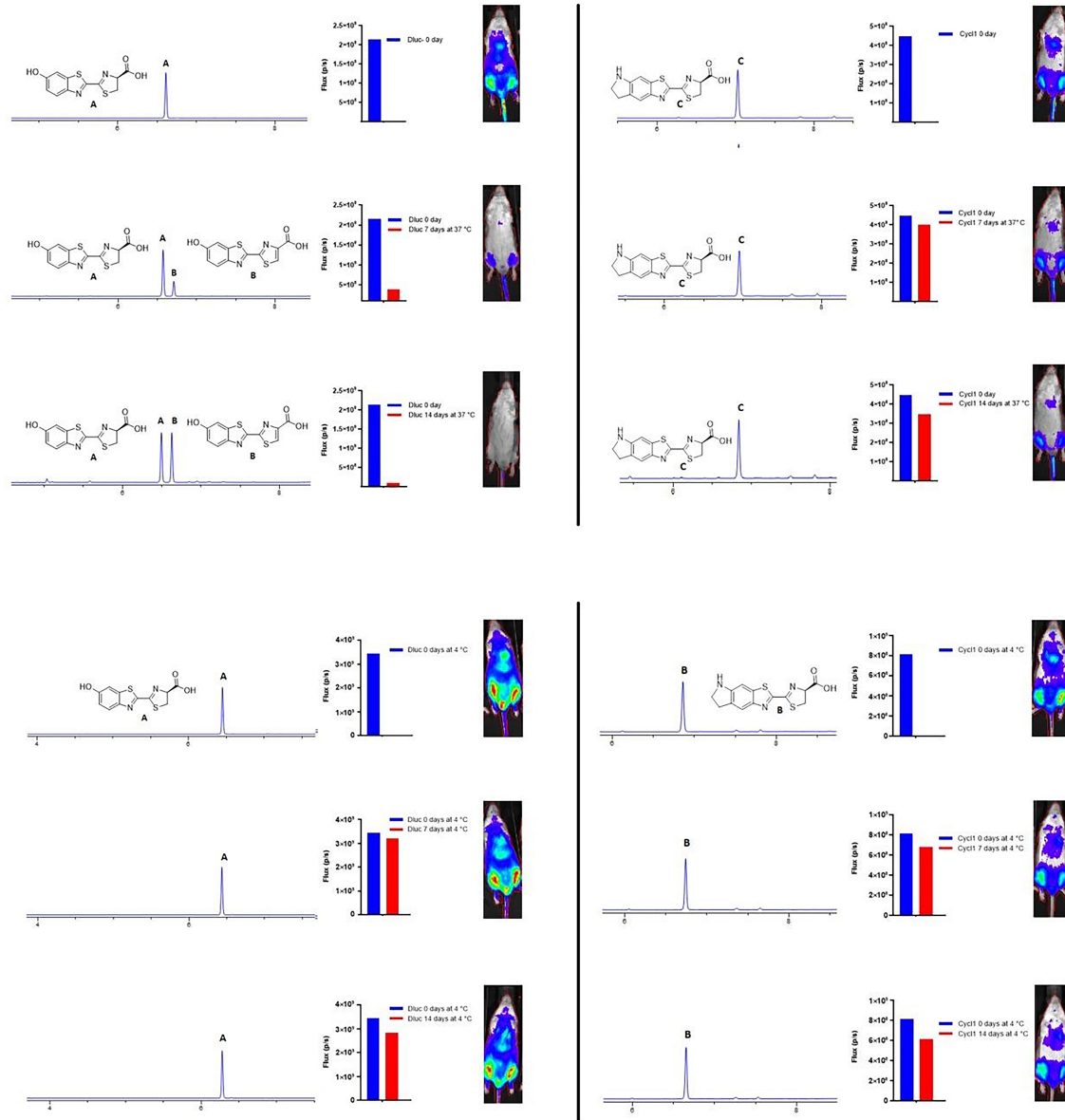


Figure S7. Evaluation of ip D-luciferin and CycLuc1 after incubation at 4 °C or 37 °C. LC/MS and in vivo bioluminescence imaging after incubation in aqueous buffer at the specified temperature for 0, 7, or 14 days in FVB/NJ mice previously receiving iv injection of AAV9-CMV-WTluc2 as previously described ([Mofford et al. 2015](#)). Shown are results from the 4 male mice tested; results were replicated with 4 female mice (not shown).



Figure S8

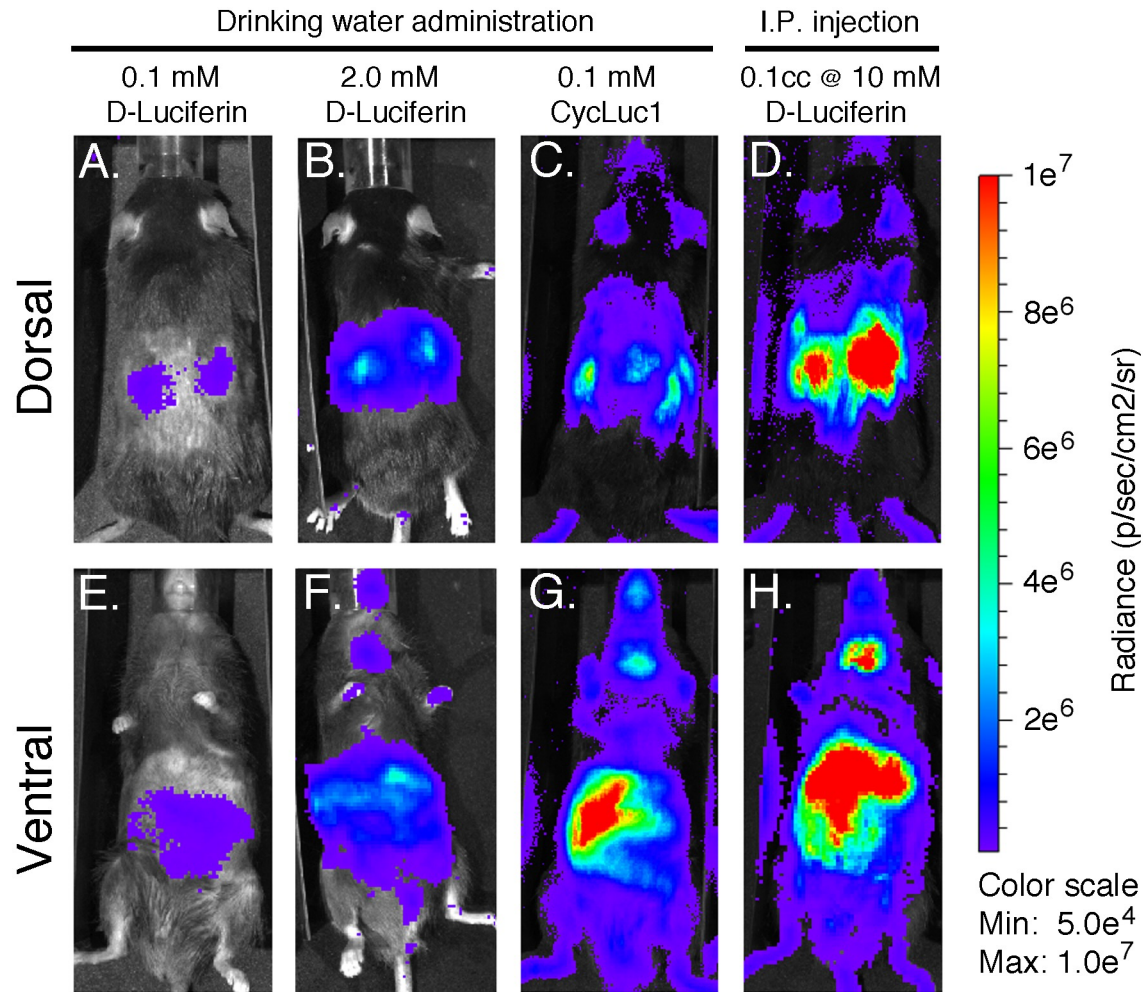


Figure S8. IVIS imaging to assess the source of bioluminescence in *Per2<sup>LucSV/+</sup>* mice. Images show paired dorsal (A-D) and ventral (E-H) images from a representative male mouse from each treatment group. Mice were imaged after drinking 0.1 mM D-luciferin (A,E), 2 mM D-luciferin (B,F) or CycLuc1 (0.1 mM; C,G), or after injection of 0.1mL D-luciferin (10mM; D,H). All images are adjusted to the bioluminescence scale bar shown at right. Results shown are representative of each group (n=6-10 per group).

Figure S9

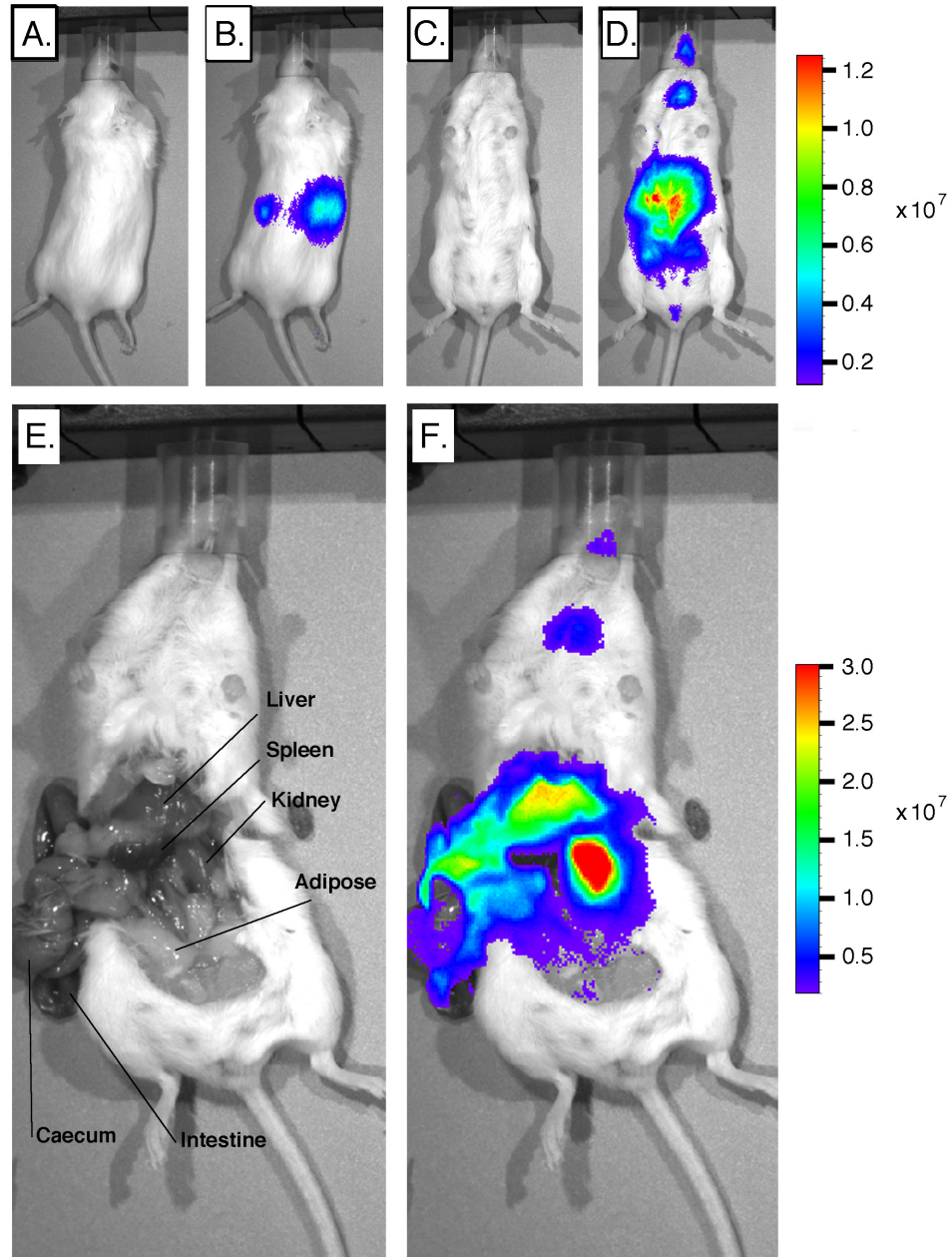


Figure S9. Images show a representative result from imaging experiments that included dissections. Photographs of the dorsal (A) and ventral (C) views are paired with bioluminescence images in B and D. Following dissection, the photograph (E) and IVIS image (F) show the majority of signal arising from the kidney. Images adjusted to the bioluminescence scale bars shown at right.