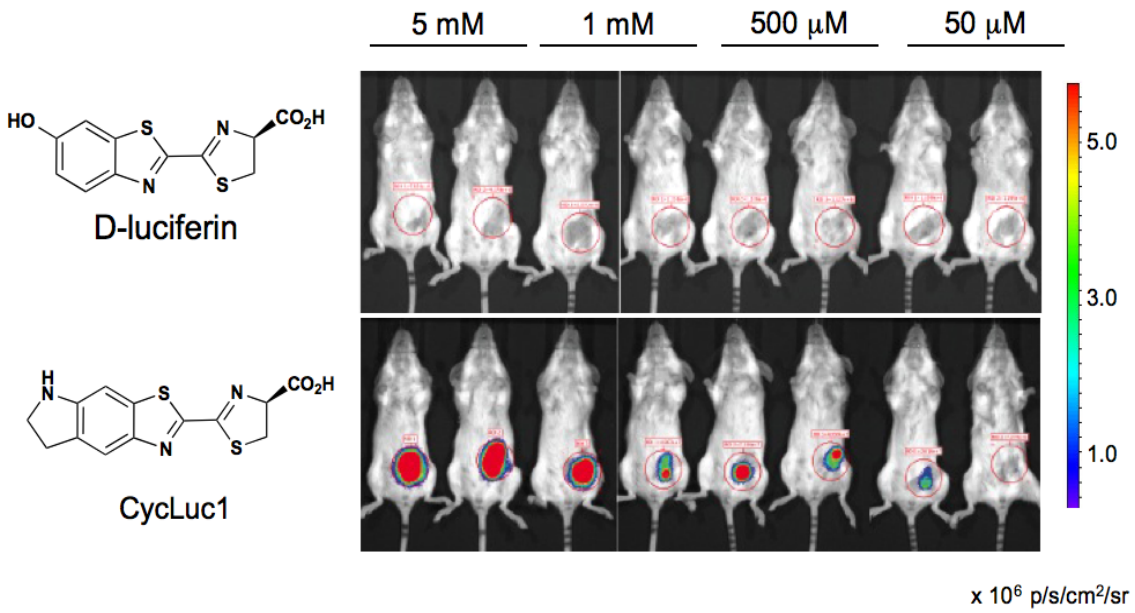
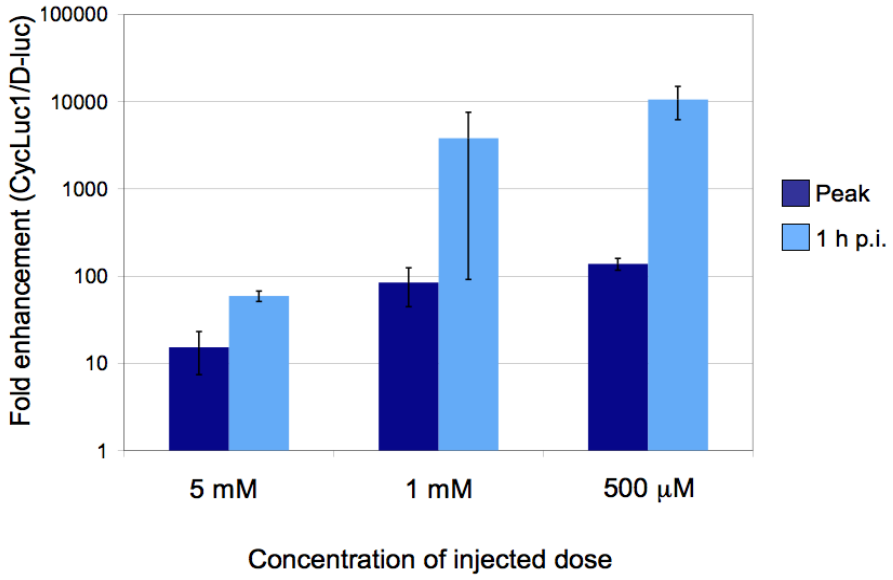
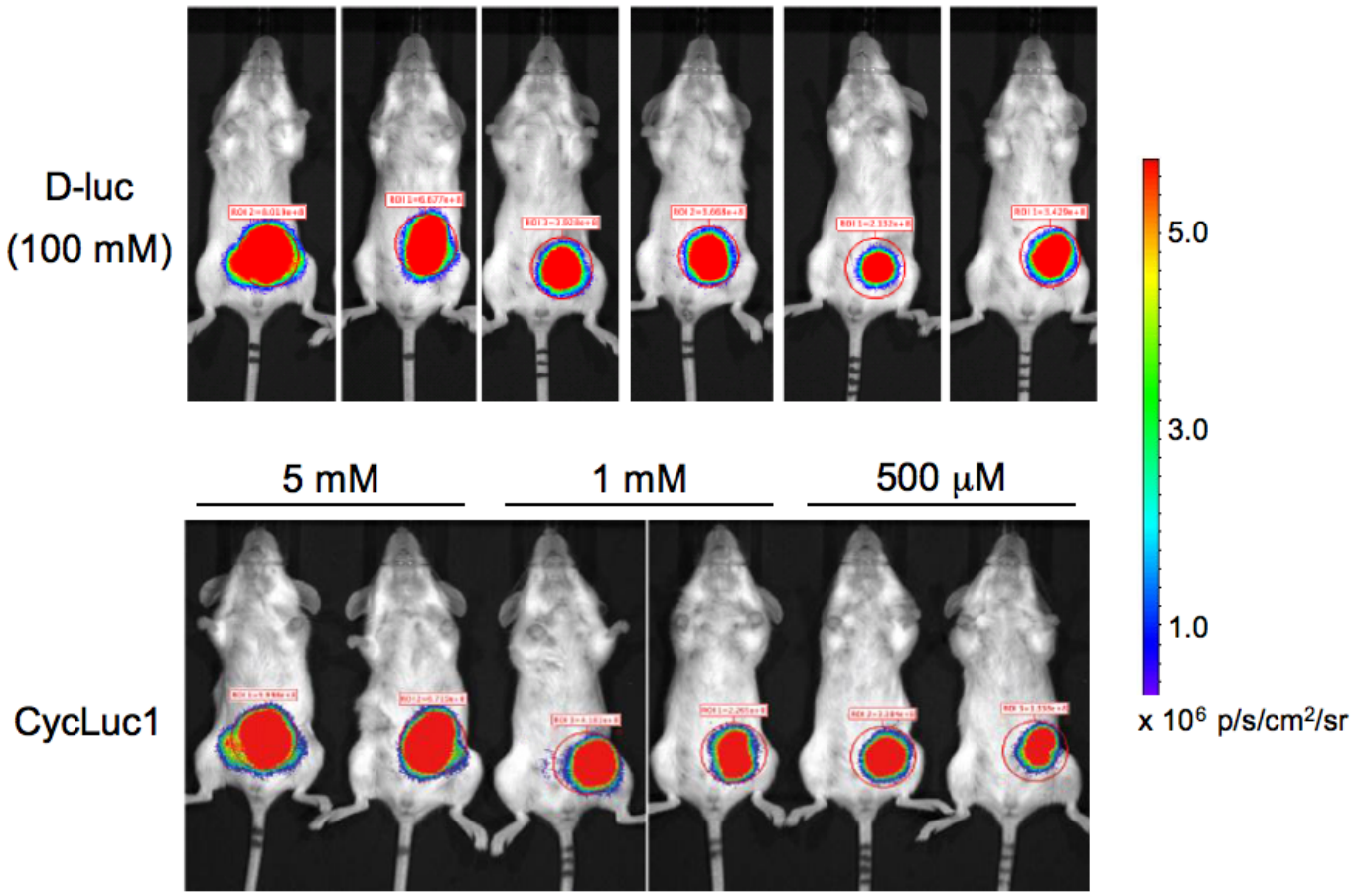
Supplementary information

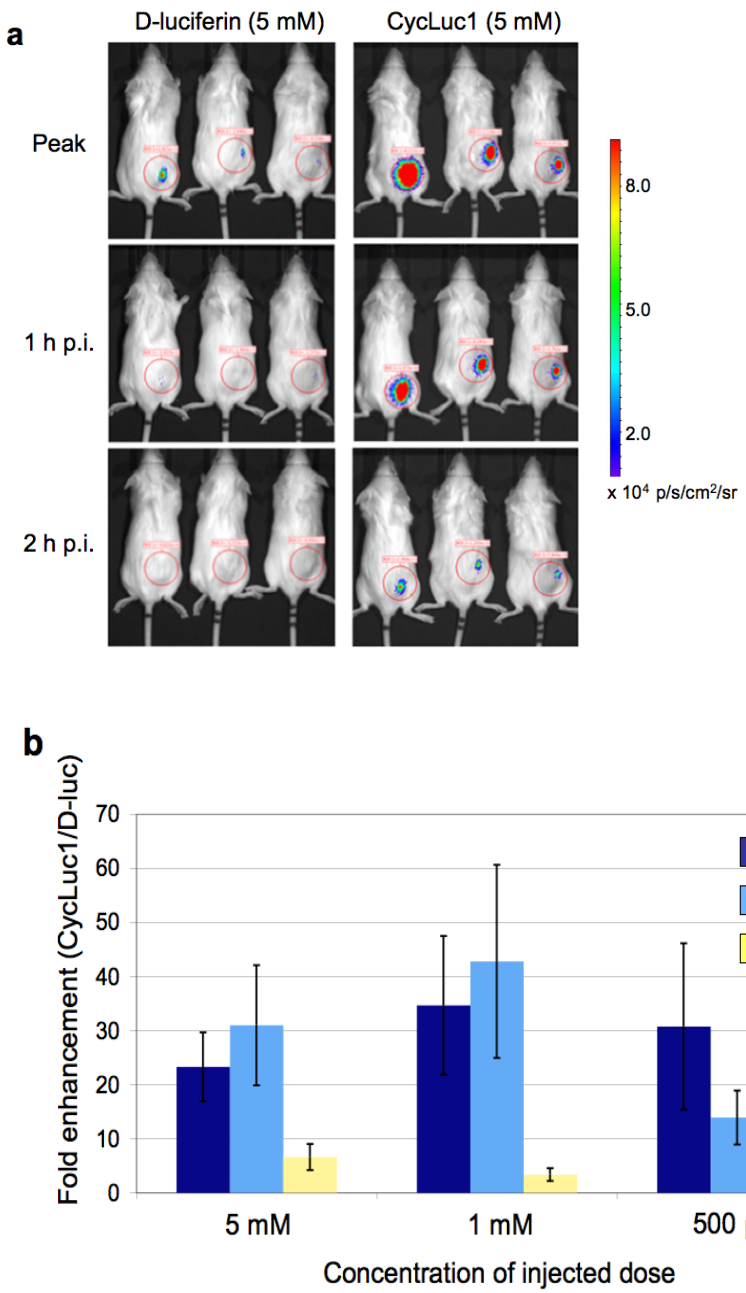




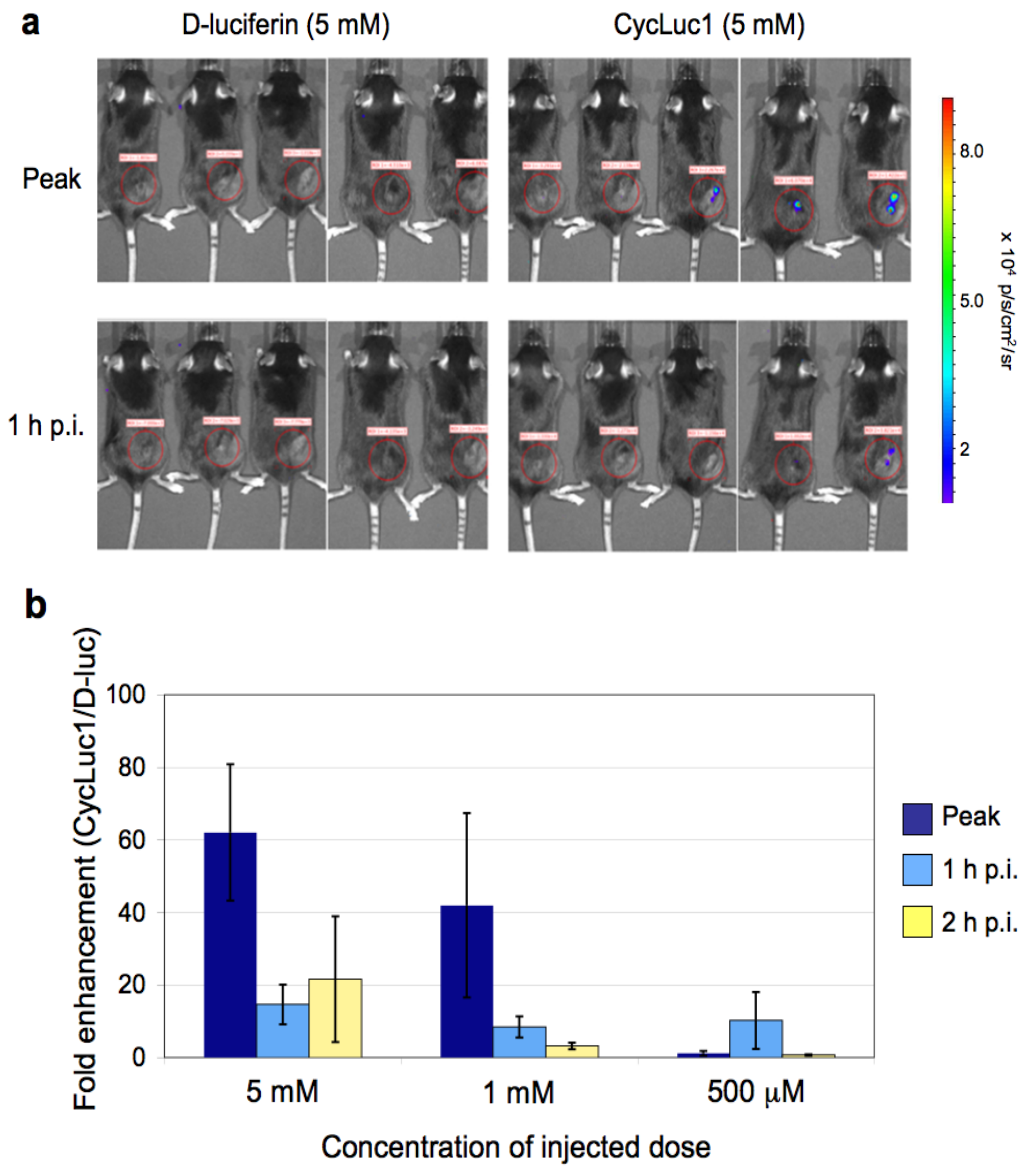
Supplementary Figure 1. Comparative BLI of tumor xenografts with D-luciferin and CycLuc1. BALB/c mice harboring 4T1-luc2 tumors were injected i.p. with the indicated dose of D-luciferin (top images) or CycLuc1 (bottom images) and imaged 1 h post injection. All images are plotted on the same scale. Red circles outline the regions of interest (ROIs) used to quantify photon flux (below). In the bar graphs, photon flux values were calculated for all ROIs, and the fold increase in bioluminescent signal achieved with CycLuc1 relative to D-luciferin (at varying concentrations) is plotted. Error bars represent the standard error of the mean.



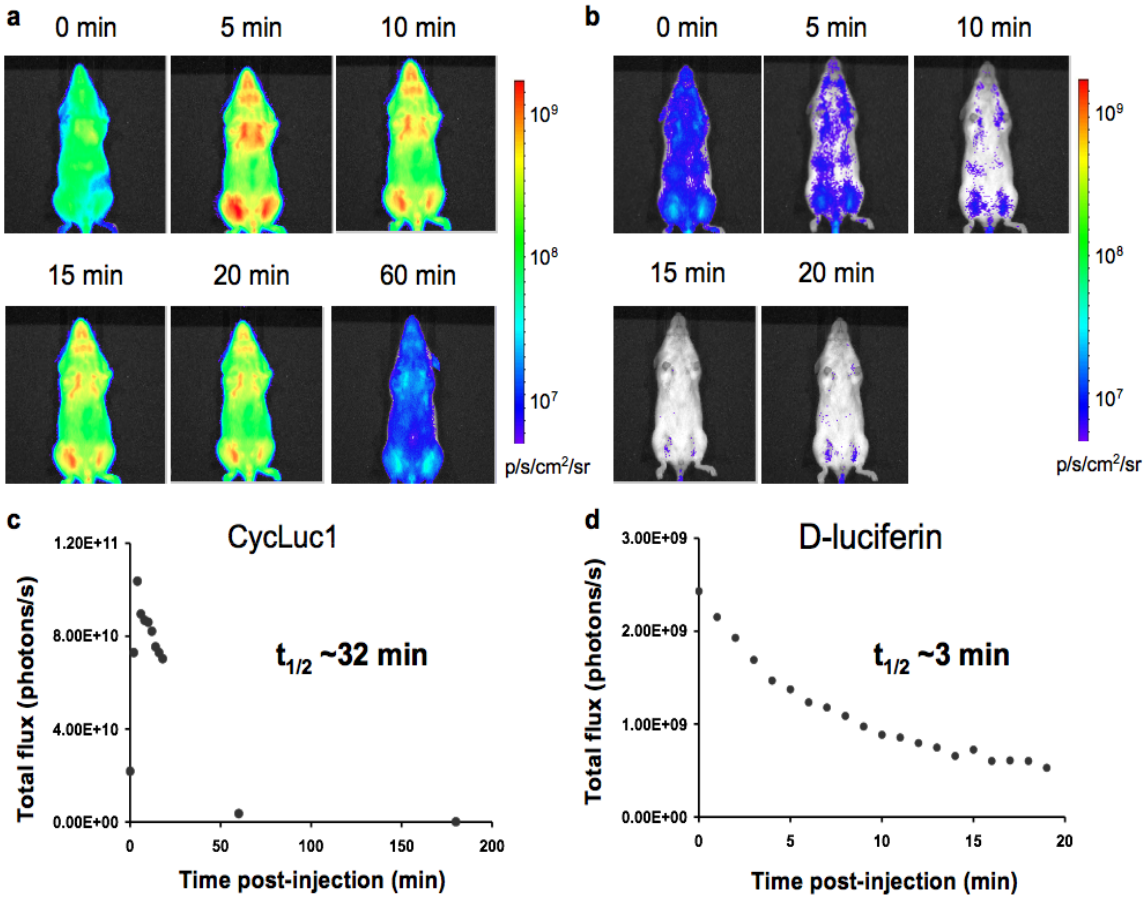
Supplementary Figure 2. Bioluminescence images of 4T1-luc2 cells in BALB/c mice injected i.p. with 100 μl of 100 mM D-luciferin (standard BLI conditions) or the indicated dose of CycLuc1.



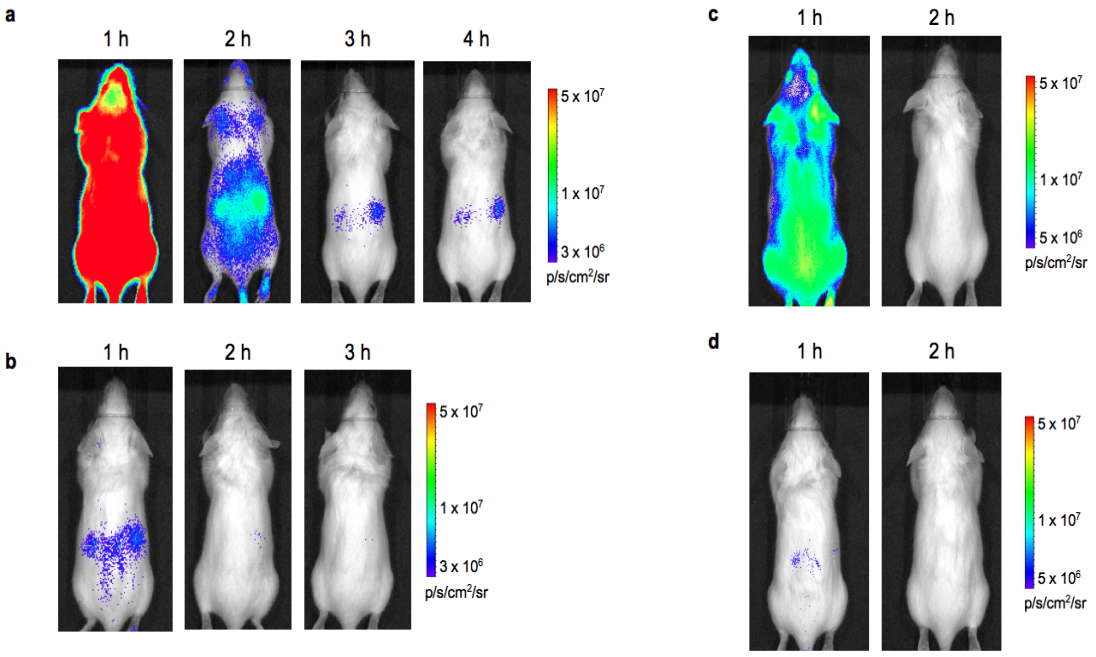
Supplementary Figure 3. BLI of DB7-luc cells in FVB mice. DB7-luc cells were implanted into the right flanks of FVB mice prior to imaging with D-luciferin (5 mM–500 μM) or CycLuc1 (5 mM–500 μM). (a) Representative images from mice treated with 5 mM solutions are shown at peak emission intensities (top panels), one hour post-injection (middle panels) and two hours post-injection (bottom panels). All images are plotted on the same relative scale, and the red circles outline the regions of interest (ROIs) used to quantify relative photon flux from the animals. (b) The fold increase in bioluminescent signal achieved with CycLuc1 relative to D-luciferin (at various concentrations) is plotted. Error bars represent the standard error of the mean.



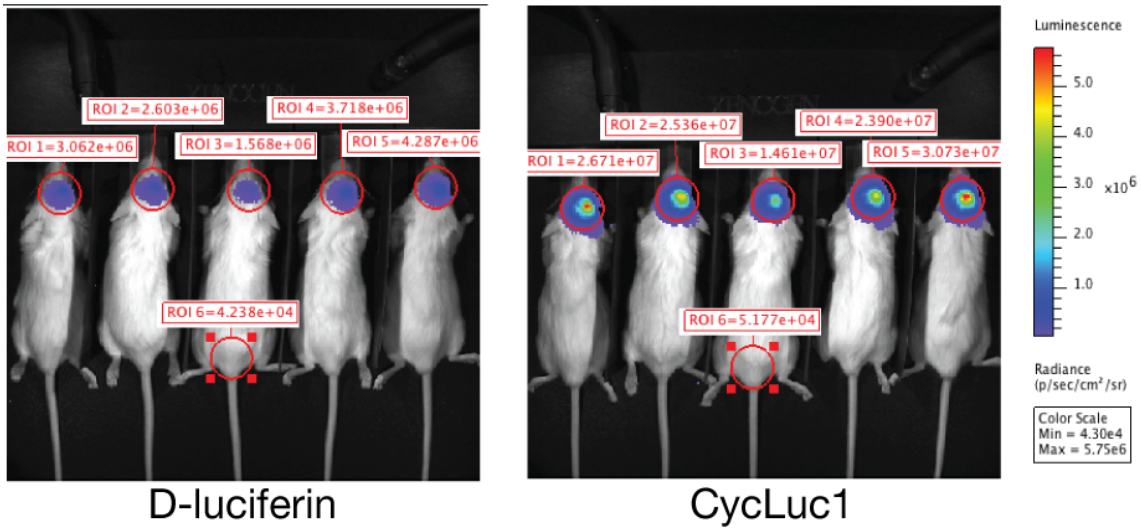
Supplementary Figure 4. BLI of CMT-64-luc cells in C57BL/6 mice. CMT-64-luc cells were implanted into the right flanks of FVB mice prior to imaging with D-luciferin (5 mM–500 μM) or CycLuc1 (5 mM–500 μM). (a) Representative images from mice treated with 5 mM solutions are shown at peak emission intensities (top panels), one hour post-injection (middle panels) and two hours post-injection (bottom panels). All images are plotted on the same relative scale, and the red circles outline the regions of interest (ROIs) used to quantify relative photon flux from the animals. (b) The fold increase in bioluminescent signal achieved with CycLuc1 relative to D-luciferin (at various concentrations) is plotted. Error bars represent the standard error of the mean.



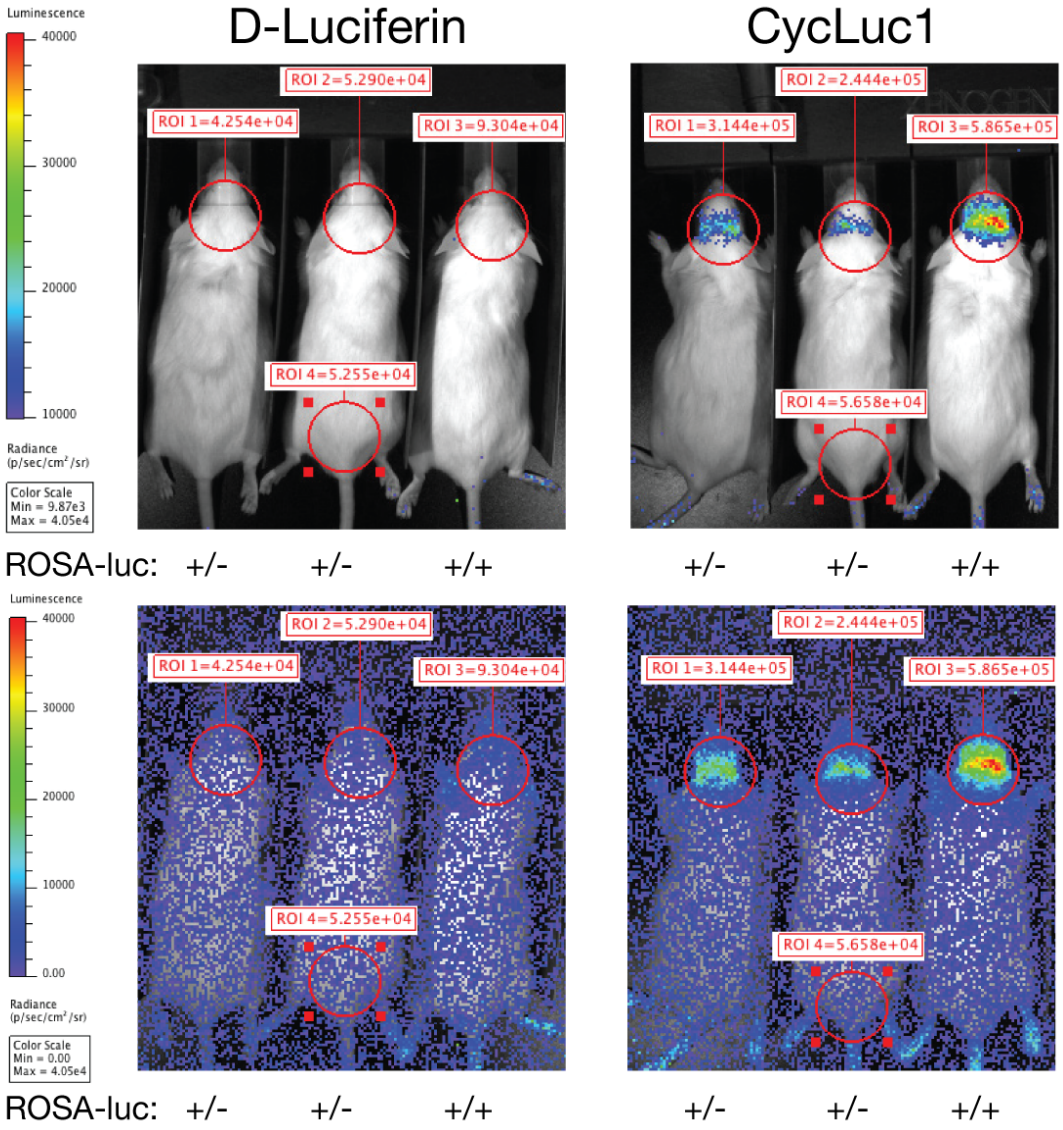
Supplementary Figure 5. Comparison of D-luciferin and CycLuc1 in luciferase-expressing transgenic mice. L2G85-FVB mice were injected i.v. with 100 μl of 5 mM (a) CycLuc1 or (b) D-luciferin. For (a)-(b), photon intensities are shown in units of photons/s/cm^2/steradian and plotted on logarithmic scales. (c) Total photon output from L2G85-FVB luc mice treated with CycLuc1 (i.v.) or (d) D-luciferin (i.v.). The apparent half-life of bioluminescent signal ($t_{1/2}$) is shown. (Note: the animals were placed on the imaging bed immediately after injection and the start of signal acquisition.)



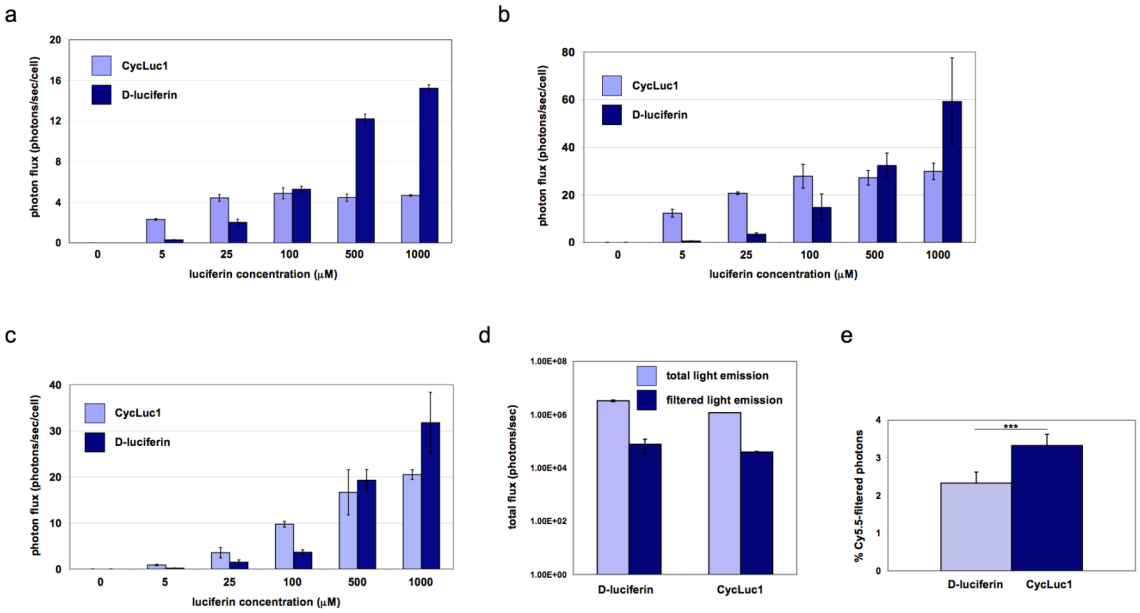
Supplementary Figure 6. Biodistribution of D-luciferin and CycLuc1 in luciferase-expressing transgenic mice. Mice were injected with 100 μl of 5 mM (a) CycLuc1 or (b) D-luciferin and dorsal images were acquired until the bioluminescent signal returned to background levels (180 min for D-luciferin, 240 min for CycLuc1). Images were also acquired following intravenous (i.v.) injection of 100 ml of 5 mM (c) CycLuc1 or (d) D-luciferin. For (a)-(d), photon intensities are shown in units of photons/s/cm^2/steradian and plotted on logarithmic scales.



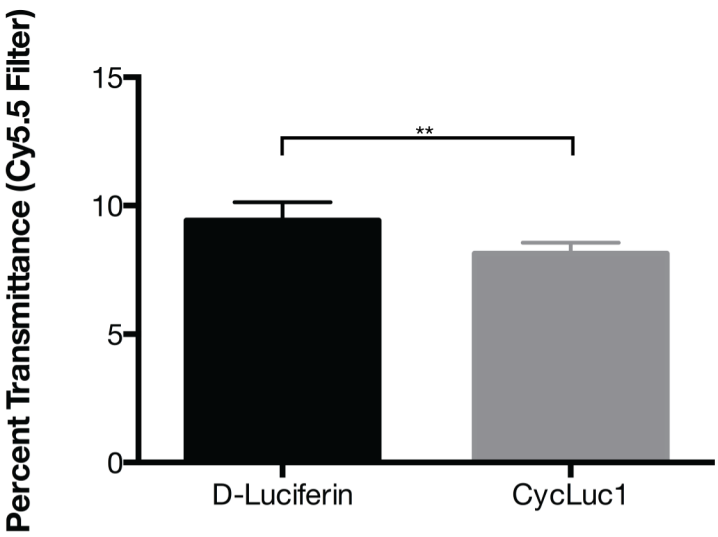
Supplementary Figure 7. Comparison of photon flux from AAV9-luc2 mice. Five female FVB mice were treated with adeno-associated virus 9 (AAV9) as described in the Methods to express codon-optimized *luc2* luciferase in the brain striatum. Nine weeks after surgery, the mice were injected i.p. with the standard imaging dose of D-luciferin (0.1 ml of 100 mM) and imaged ten minutes post-injection. The next day, the same five mice were injected i.p. with 0.1 ml of 5 mM CycLuc1 and imaged ten minutes post-injection. Both images are plotted on the same scale, and the red circles outline the regions of interest (ROIs) used to quantify relative photon flux. ROI6 was used to measure background signal.



Supplementary Figure 8. Comparison of photon flux from *Dat-luc* mice. *Dat-luc* mice were injected i.p. with the standard imaging dose of D-luciferin (0.1 ml of 100 mM) and imaged ten minutes post-injection. The next day, the same mice were injected i.p. with 0.1 ml of 5 mM CycLuc1 and imaged ten minutes post-injection. The first two mice are heterozygous for the *Rosa26-luc* allele, the third is homozygous. Both images are plotted on the same scale, and the red circles outline the regions of interest (ROIs) used to quantify relative photon flux. ROI4 was used to measure background signal. The top images were autoscaled by the Living Image software. In the bottom images, the same data is displayed with the minimum set to zero to show all signal, including camera noise.



Supplementary Figure 9. Bioluminescence imaging of (a) DB7-luc, (b) 4T1-luc2, and (c) CMT-64-luc cells treated with the indicated concentration of CycLuc1 (light blue bars) or D-luciferin (dark blue bars). (d) Light output from 50,000 4T1-luc cells treated with 1 mM D-luciferin or CycLuc1. Photons were collected either with an open filter (light blue bars) or after passage through a Cy5.5 filter (dark blue bars). The percent filtered light captured in each case is shown in (e), ***P < 0.001 (t-test). For (a)-(e), error bars represent the standard error of the mean for three independent experiments.



Supplementary Figure 10. Photon flux from AAV9-luc2 mice through a Cy5.5 filter. Mice were imaged as described for Supplementary Figure 7, with and without a Cy5.5 emission filter (695–770 nm bandpass). The ratio of Cy5.5-filtered photon flux to unfiltered photon flux is expressed as a percentage (D-luciferin: 9.4 ± 0.3 %; CycLuc1: 8.1 ± 0.2 %). Error bars are S.E.M. (n = 5 mice for each luciferin). **P < 0.01 (t-test).