Supplemental material

Ma et al., https://doi.org/10.1084/jem.20190351

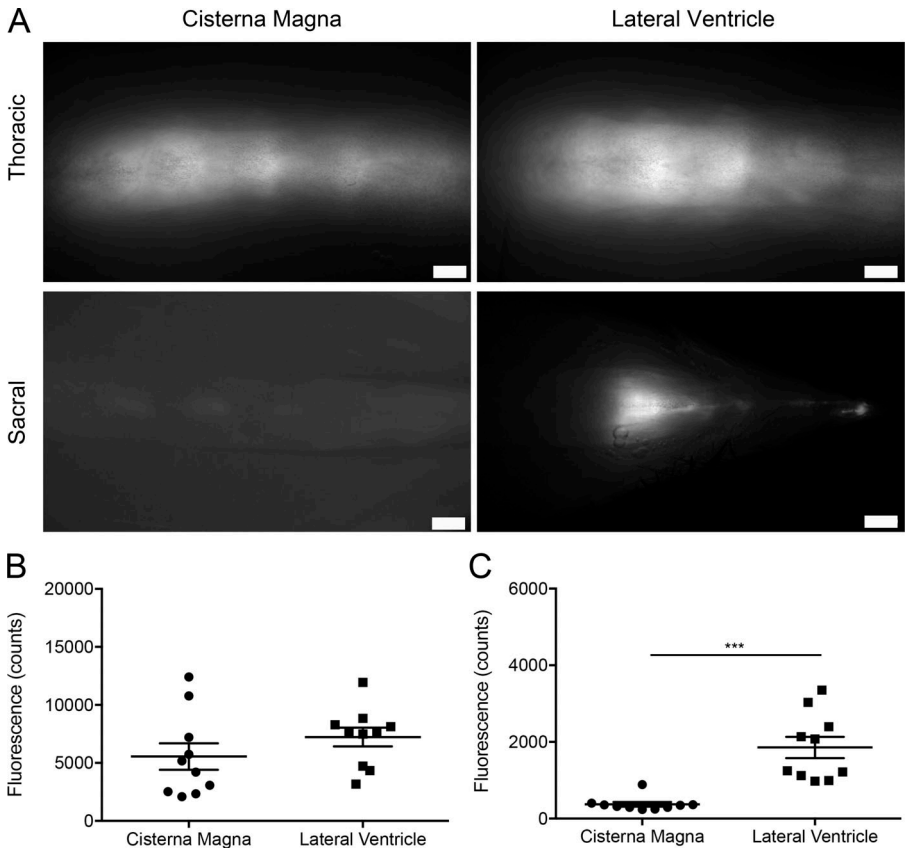


Figure S1. **Transport of tracers occurs to the sacral region after lateral ventricle infusion but not after cisterna magna infusion. (A)** P40D680 tracer was infused into either the lateral ventricle (2.5 µl of 200 µM) or cisterna magna (5.0 µl of 100 µM) of mice (n = 10 per infusion site), and the distribution at 60 min was assessed by near-infrared imaging of the thoracic and sacral regions of the spine immediately postmortem. **(B)** Quantification of tracer signal within the thoracic region. Data are mean ± SD (two-tailed Student's t test). **(C)** Quantification of tracer signal within the sacral region. Data are mean ± SD (two-tailed Student's t test; ***, P < 0.001). Scale bars, 1 mm. Data are representative of two independent experiments.

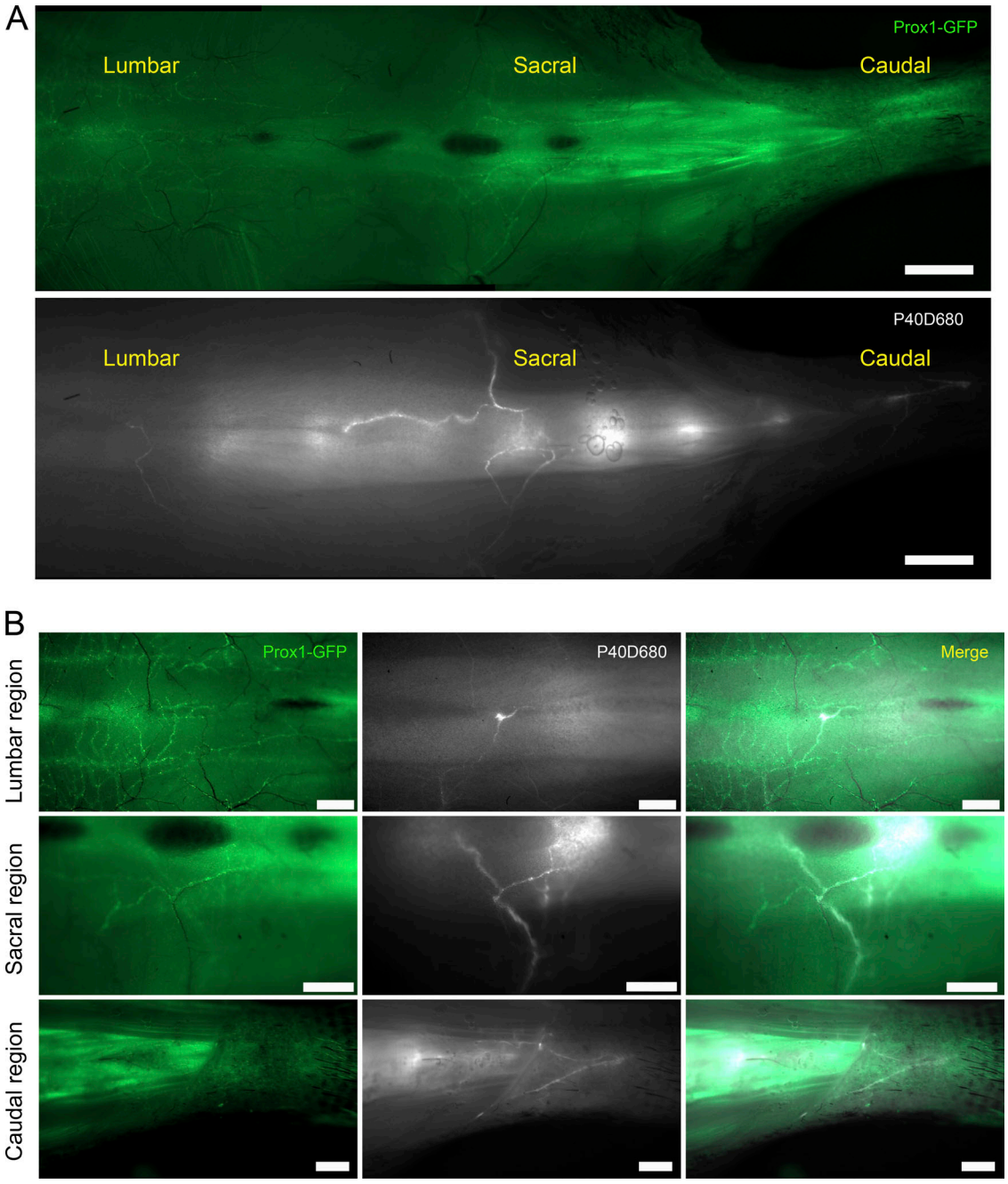


Figure S2. **Lymphatic outflow routes from the dorsal aspect of the caudal spine.** P40D680 tracer was infused into the lateral ventricle (2.5 µl of 200 µM), and the mice were allowed to recover and move about the cage normally. The distribution at 60 or 90 min was assessed by near-infrared imaging of the sacral region of the spine immediately postmortem. **(A)** Representative stitched image of three CSF outflow regions to lymphatic vessels from the caudal spine at 60 min. Weak outflow from the lumbar region is only rarely observed. More regular outflow occurs at several intervertebral sites in the sacral region toward the sacral LNs. Commonly, outflow from the caudal spine proceeds to subcutaneous lymphatic vessels of the tail, which then drain toward inguinal LNs. Scale bars, 2 mm. **(B)** Further representative examples at closer magnification at 90 min of the outflow routes at the three regions. Scale bars, 1 mm. Data are representative of three independent experiments.

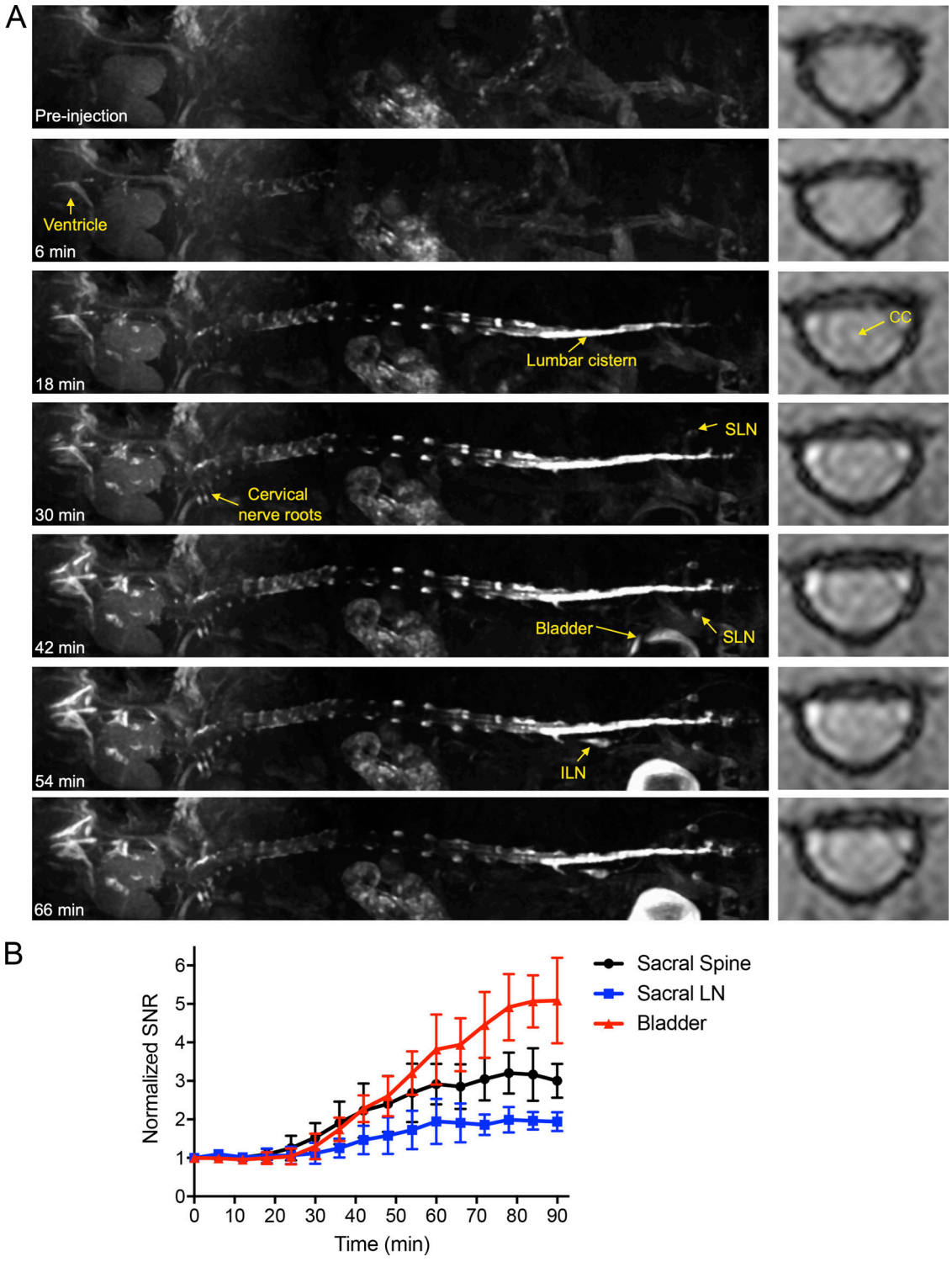
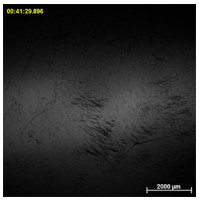
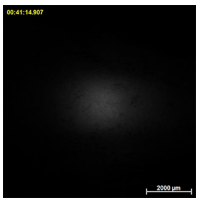


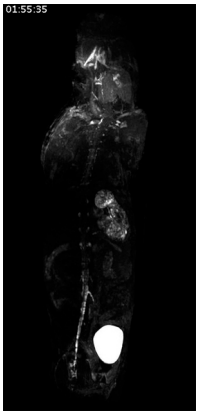
Figure S3. **Full-spine MRI demonstrates dynamics of spread of contrast agent with outflow to sacral and iliac LNs. (A)** Left panels: Visualization of tracer spread after low rate intraventricular infusion (0.1 µl/min) of a Gadospin D solution at 25 mM gadolinium; data acquired with a series of T1-weighted MRI measurements (3D time-of-flight gradient recalled echo sequence). Representative signal dynamics using maximum-intensity projections visualizing the ventricular system and the entire spine column. Enhancement of signal in the sacral LN (SLN) is detectable as early as 30 min and in the iliac LN (ILN) as early as 42 min following the beginning of contrast agent infusion. Images are representative of n = 5 mice. Right panels, representative signal dynamics using 2D transverse sections through the spinal column (n = 5). Enhancement of signal in the CC of the cervical spinal cord is detectable at 18 min following the beginning of contrast agent infusion. **(B)** Quantification of the dynamics of contrast agent distribution in the spine, sacral LNs, and bladder over time. Data are plotted as mean ± SD of signal-to-noise ratio (SNR) of n = 5 mice. Data are representative of three independent experiments.



Video 1. **Near-infrared imaging of the dynamics of CSF spread of P40D680 to the thoracic region of the spine.** Noninvasive imaging of thoracic spine at 24× magnification starting at 5 min after intraventricular infusion of 2.5 µl of 200 µM P40D680. Enhancement of thoracic spine is apparent at *t* = ∼25 min (corresponding to 30 min after the completion of the infusion). Quantification is shown in Fig. 1 C. Images were acquired at 1 frame per 15 s until 60 min after the infusion.



Video 2. **Near-infrared imaging of the dynamics of CSF spread of P40D680 to the sacral region of the spine.** Noninvasive imaging of sacral spine at 24× magnification starting at 5 min after intraventricular infusion of 2.5 µl of 200 µM P40D680. Enhancement of thoracic spine is apparent at *t* = ∼16 min (corresponding to ∼21 min after the completion of the infusion). Quantification is shown in Fig. 1 C. Images were acquired at 1 frame per 15 s until 60 min after the infusion.



Video 3. **MRI of CSF contrast agent spread and outflow in the spine.** Maximum-intensity projections video (representative of *n* = 5 mice) showing spread of tracer after low-rate intraventricular infusion (0.1 µl per min) of a Gadospin D solution at 25 mM gadolinium. Enhancement of signal in the sacral LN is detectable as early as 30 min and in the iliac LN as early as 42 min following the beginning of contrast agent infusion. Images were acquired at 1 frame per 6.05 min.