Supplementary Videos

Supplementary Video 1. **Real-time *in vivo* imaging of leptomeninges in Nalm6-GFP leukemic mice 10 minutes post-engraftment (merged channels).** Intravital confocal microscopy performed through a thinned-skull window in a mouse approximately 10 minutes post-intravenous engraftment of Nalm6-GFP ALL cells. Circulating Nalm6-GFP cells (green) are visible in the lumen of the leptomeningeal and superficial cerebral vasculature (red), but do not extravasate. Examples of cells in circulation are best seen at time marks 00.33, 00.58, 01.03, 01.21 (mm.ss). See Supplementary Video 2 for green channel only (n = 12 mice imaged on day 0 post-engraftment).

Supplementary Video 2. Real-time *in vivo* imaging of leptomeninges in Nalm6-GFP leukemic mice 10 minutes post-engraftment (green channel). Intravital confocal microscopy performed through a thinned-skull window in a mouse approximately 10 minutes post-intravenous engraftment of Nalm6-GFP ALL cells. Circulating Nalm6-GFP cells (green) are visible in the lumen of the leptomeningeal and superficial vasculature (red), but do not extravasate. Examples of cells in circulation are best seen at time marks 00.33, 00.58, 01.03 and 01.21 (mm.ss). See Supplementary Video 1 for merged red and green channels (n = 12 mice imaged on day 0 post-engraftment).

Supplementary Video 3. Real-time *in vivo* imaging of leptomeninges in Nalm6-GFP leukemic mice 45 minutes post-engraftment. Intravital confocal microscopy performed through a thinned-skull window in a mouse approximately 45 minutes post-intravenous engraftment of Nalm6-GFP ALL cells. Nalm6-GFP cells (green) are visible attached within or circulating through the lumen of the leptomeningeal and superficial cerebral vasculature (red), but they do

not extravasate. Examples of cells in circulation are best seen at time marks 00.01, 00.03 and 00.07 (mm.ss). Video play rate is slowed to facilitate viewing of cells in circulation (*n* = 12 mice imaged on day 0 post-engraftment).

Supplementary Video 4. *In vivo* imaging of leptomeninges in Nalm6-GFP leukemic mice 12 days post-engraftment. Z-stack video (10X magnification) of intravital confocal microscopy images obtained through a thinned-skull window in a mouse 12 days post-intravenous engraftment of Nalm6-GFP ALL cells. No Nalm6-GFP ALL cells (green) are detected in or around the leptomeningeal or superficial cerebral vasculature (red) (*n* = 4 mice imaged at intermediate time points post-engraftment).

Supplementary Video 5. *In vivo* imaging of leptomeninges in Nalm6-GFP leukemic mice 37 days post-engraftment. Z-stack video (20X magnification) of intravital confocal microscopy images obtained through a thinned-skull window in a mouse 37 days post-engraftment of Nalm6-GFP ALL cells. At the beginning of the Z-stack, Nalm-6-GFP+ cells (green) are visible within the BM at the edge of the skull window. In subsequent images, leptomeningeal vasculature (red) comes into focus and Nalm6-GFP ALL cells can be seen within the leptomeningeal tissue. A Nalm6-GFP cell is visible in the lumen of the CNS vasculature, but does not extravasate (timemark: 00.05 mm.ss) (*n* = 7 mice imaged at end stage disease).

Supplementary video 6. 3D reconstruction videos of emissary vessels within control SCID vertebral sections (example 1). 3D reconstruction video of H&E stained histologic specimens shows emissary vessels bridging the bone marrow (BM) and subarachnoid space (SA). Arrows indicate emissary vessel (*n* = 3 mice).

Supplementary Video 7. 3D reconstruction videos of emissary vessels within control SCID vertebral sections (example 2). 3D reconstruction video of H&E stained histologic specimens shows emissary vessels bridging the bone marrow (BM) and subarachnoid space (SA). Arrows indicate emissary vessel (*n* = 3 mice).

Supplementary video 8. 3D reconstruction videos of Nalm-6 migration into the CNS along bone channels within vertebral sections (example 1). 3D reconstruction video of H&E stained histologic specimens from endpoint mice shows leukemic cells in transit through bony channels (arrows) bridging the BM and SA space (*n* = 3 mice).

Supplementary video 9. 3D reconstruction videos of Nalm-6 migration into the CNS along bone channels within vertebral sections (example 2). 3D reconstruction video of H&E stained histologic specimens from endpoint mice shows leukemic cells in transit through bony channels (arrows) bridging the BM and SA space (*n* = 3 mice).

Supplementary video 10. 3D reconstruction videos of RCH-ACV migration into the CNS along bone channels within vertebral sections of endpoint mice (example 1). 3D reconstruction video of H&E stained histologic specimens from endpoint mice shows leukemic cells in transit through bony channels (arrows) bridging the BM and SA space (*n* = 3 mice).

Supplementary video 11. 3D reconstruction videos of RCH-ACV migration into the CNS along bone channels within vertebral sections of endpoint mice (example 2). 3D

reconstruction video of H&E stained histologic specimens from endpoint mice shows leukemic cells in transit through bony channels (arrows) bridging the BM and SA space (n = 3 mice).