**SUPPLEMENTARY METHODS TO:**

*LONG-TERM, HIGH-RESOLUTION IMAGING IN THE MOUSE NEOCORTEX THROUGH A CHRONIC CRANIAL WINDOW*

*A. Holtmaat, T. Bonhoeffer, D. Chow, J. Chuckowree, V. De Paola, S. B. Hofer, M. Hübener, T. Keck, G. Knott, W-C. A. Lee, R. Mostany, T. D. Mrsic-Flogel, E. Nedivi, C. Portera-Cailliau, K. Svoboda, J.T. Trachtenberg, L. Wilbrecht*

**Thinned skull preparation**

For comparison with chronic cranial windows we also imaged and tracked dendritic spines in the somatosensory cortex in GFP-M mice through thinned skull (6 cells; 6 mice, age 6 -10 weeks; **Supplementary Fig. 2**).

*Methods*. Mice are deeply anesthetized with 4% isoflurane in 100% oxygen. Once down, anesthesia is maintained at 1.5%-2% isoflurane in 100% oxygen. Core body temperature is monitored and maintained using a regulated heating pad. The hair on the scalp is removed with an electric razor. The scalp is then sterilized with three alternating swabs of alcohol and betadine. An incision is made along the midline of the scalp to expose the skull overlying the cortical region of interest. Any fascia overlying the skull is scraped away with a scalpel blade. A 1mm diameter region of skull is then thinned using a high speed micro drill (Fine Science Tools, Item Number 18000-17) and a stainless steel burr (Fine Science Tools, Item Number 19008-07). Drilling is halted every few seconds to prevent heating and bone dust is removed using a compressed air canister. Care is taken not to deflect the skull during drilling. Drilling continues until the fine vasculature of the dura is visible. At this point, thinning continues by hand using a microsurgical blade (Surgistar; part number 6900 & 1310). The mouse is then placed under the laser scanning microscope and GFP-labeled neurons are imaged. If the images are dim and the dendritic structure is poorly defined, the mouse is removed from the microscope and skull thinning continues using the microsurgical blade. This process is repeated until image clarity is maximized. Animals showing any signs of damage, such as subdural or epidermal bleeding or axonal/dendritic blebbing are discarded from any study. When imaging is complete, the wound margins of the scalp are sutured together using #5-O nylon suture. Mice are given a bolus of warm saline for rehydration and are allowed to recover from anesthesia on a water-circulating heating pad.