Supplementary Methods for:

Two-Photon Calcium Imaging of Evoked Activity from L5 Somatosensory Neurons in vivo.

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Supplementary figure legends:

**Supplementary Figure 1.** Calcium indicator expression targeted to Layer 5 and imaged using RAMM. (a) Two images of one section from one experiment showing GCaMP3-loaded layer 5 neurons (left) and an overlay of the loaded neurons and DAPI-stain for the same section (right). The DAPI staining of the section shows the loaded neurons as being located just beneath the dense layer of cell bodies in layer 4. (b) Two-photon microscope image (side projection) from a z-stack (2 μm steps). L5 neurons labeled with GCaMP3 (green) and superficial astrocytes labeled with SR101 (red) in an anesthetized mouse. Note that the deep-layer red is most likely due to fluorescence from bright astrocyte labeling restricted to superficial layers.

**Supplementary Figure 2.** Functional calcium imaging at 800 μm below pia

(a) MP image of layer 5 neuronal somata at 800 μm below the pia. Ca^{2+} traces from the outlined neurons are shown in b.

**Supplementary Figure 3.** So/Su ratios for 162 neurons as a function of depth of the neuronal soma. Individual neurons (black circles) and mean for all neurons with a single field of view (red bar) are shown. Numbered neurons highlighted in red at 680μm indicated in Fig 2a, and those at 740μm indicated in Fig. 2b.
**Supplementary Figure 4.** Simultaneously imaging activity from large populations of L5 dendrites. (a) MP image of GCaMP3 labeled apical dendrites and L2/3 somas taken at 240 µm below the pia. (b) 80 Ca²⁺ traces simultaneously recorded from dendrites shown in a.

**Supplementary Figure 5.** Dendritic transients from thin tufted L5 neuron

(a) y-z side projection from a z-stack taken at 2 micron steps showing populations of L5 neurons and ascending apical dendrites containing GCaMP3 calcium indicator (b) with x-y frames from three different depths indicated (white dashed lines on y-z projection) and (c) location of soma (arrow). (d) Spontaneous calcium transients recorded from apical dendrites at different depths from pia (indicated in a, b). (e) Reconstructed apical dendrite showing common source of dendritic activity shown in d with corresponding depths of transients depicted (dashed colored lines).

**Supplementary Movie** Z-stack from pia to layer 5b

Z-stack movie containing 256 x 256 pixel images, single channel, 2 micron steps, 500 images starting at the pial surface. L5 neurons (green) expressing the calcium indicator GCaMP3.
Supplementary Figure 1
Mittmann_Wallace_Czubayko_Herb_Schaefer_Looger_Denk_Kerr
Supplementary Figure 2

Mittmann_Wallace_Czubayko_Herb_Schaefer_Looger_Denk_Kerr
Supplementary Figure 3
Mittmann_Wallace_Czubayko_Herb_Schaefer_Looger_Denk_Kerr
Supplementery Figure 5

Mittmann_Wallace_Czubayko_Herb_Schaefer_Looger_Denk_Kerr