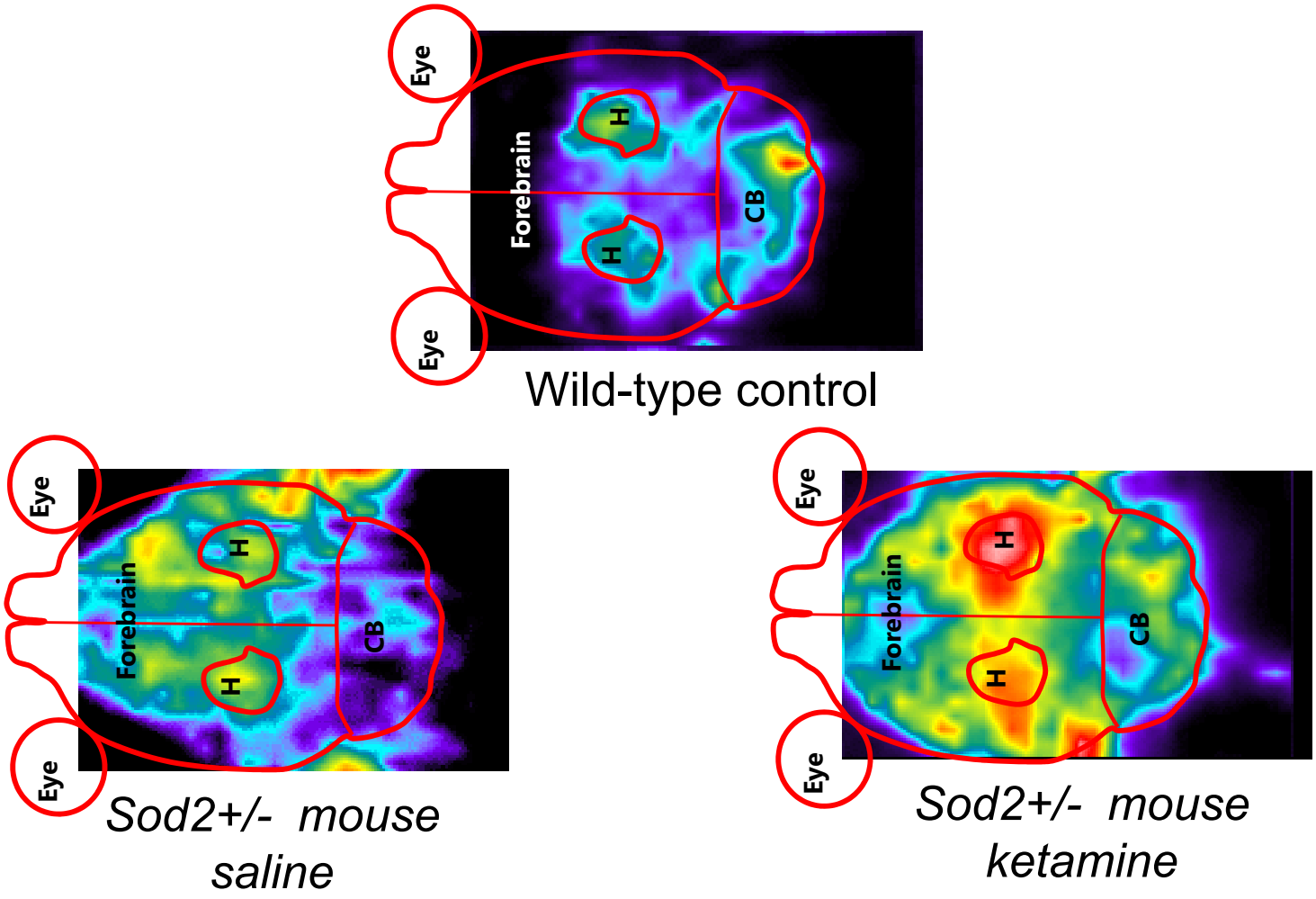
Supplemental Figure 1 ***In vivo*** **imaging reveals that regional superoxide levels differ between treatment groups.** Images of ox-DHE fluorescence in (a) wild-type C57BL6 control mouse, (b) age-matched *Sod2*$^{+/-}$ mouse with increased mitochondrial superoxide production, (c) age-matched *Sod2*$^{+/-}$ with additional increased cytosolic superoxide production from NADPH oxidase (Nox2) induced by repeated ketamince injections (Behrens et al., 2007). There is a substantial increase in ox-DHE throughout the brain in the untreated *Sod2*$^{+/-}$ mouse compared to the control that correlates well with ox-DHE fluorescence in slices prepared post-mortem from these mice. In contrast, ketamine injection produced a different pattern of ox-DHE fluorescence, with a much greater increase in fluorescence in the hippocampal formation and the cerebellum compared to the forebrain cortical hemispheres. These results correspond well to prior results using confocal imaging of fixed brain slices prepared from *in vivo* DHE-injected mice (Behrens et al., 2007; Behrens et al., 2008).

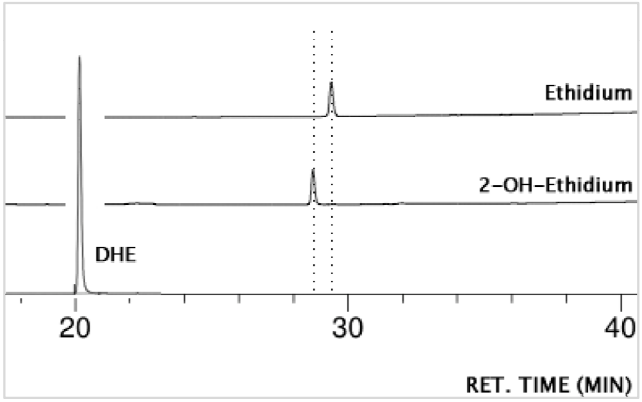
Supplemental Figure 2 HPLC analysis of purified 2-OH ethidium. HPLC analysis of ethidium and 2-hydroxyethidium (2-OH Et) showing (top) a sample spiked with authentic ethidium and 2-OH ethidium, and (bottom) HPLC analysis of 2-OH-Et synthesized and purified as described in the methods showing a single peak. The purified 2-OH Et exhibits the characteristic absorbance spectrum of 2-OH-Et (inset), and the expected molecular mass (330 g/mol), determined by LC-MS (Agilent quadripole LC-MS-Trap). HPLC conditions were as previously reported (Zielonka et al, 2009) with minor modifications, and elution of compounds was monitored concurrently by fluorescence (Ex λ=490 nm, Em λ1=567 nm, Em λ2=596 nm) and by absorbance using a diode array detector.

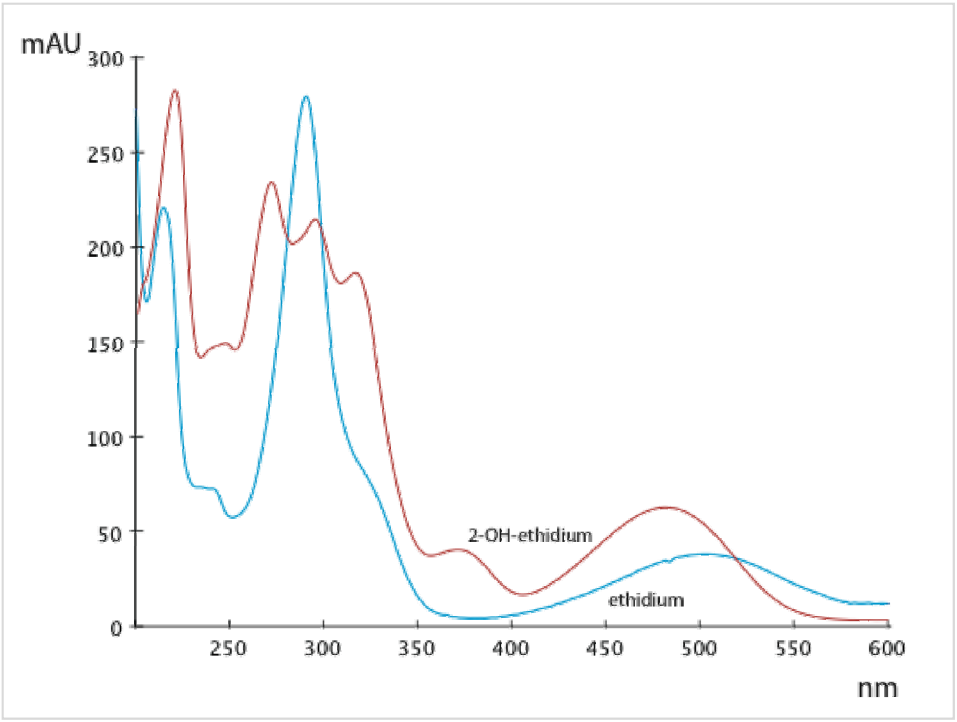
Supplemental Figure 3 Use of fluorescence lifetime ***ex vivo*** **and** ***in vivo*** **to identify oxidation product of DHE** ***in vivo*** **in the intact mouse.** Agar phantom brains containing ethidium, 2-hydroxyethidium (2-OH Et), or no compound (salmon sperm DNA alone) were placed in the Optix scanner, and imaged for 40 minutes using the filter settings previously employed for live animal *in vivo* imaging. All three conditions (control, ethidium, 2-hydroxyethidium) were imaged simultaneously. No change in fluorescence intensity or lifetime was observed throughout the imaging session, suggesting minimal photooxidation with the pulsed settings employed. (a) Fluorescence lifetimes for phantom brains were < 0.5 ns (control), 7 ± 0.2 ns

(ethidium), and 12 ± 0.3 ns (2-OH-Et). (b) Fluorescence lifetime imaging in live animals with (left) no DHE injection or (right) mouse with robust DHE oxidation due to induction of Nox2-dependent superoxide (see methods for additional details). Lifetime of the fluorescent product in the control (uninjected) mouse was <0.5 ns and in the DHE-injected mouse was 7 ± 0.3 ns. No DHE-injected mice exhibited a fluorescence lifetime higher than this value, thus indicating that the oxidation product of DHE *in vivo* correlates with ethidium.

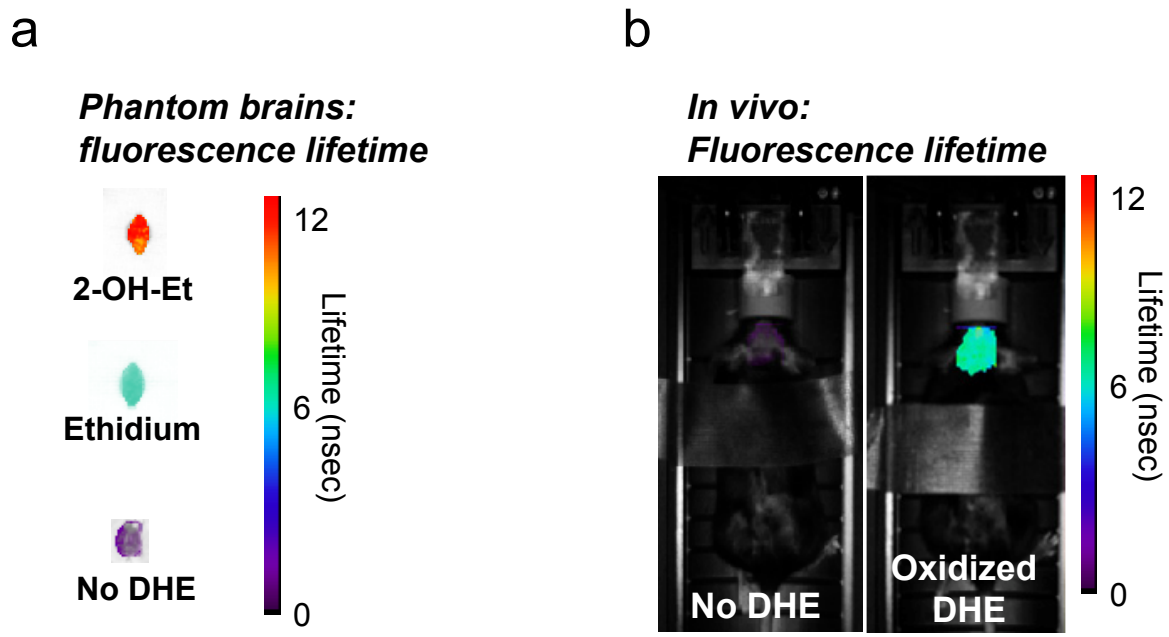


Supplemental Figure 1





Supplemental Figure 2



Supplemental Figure 3