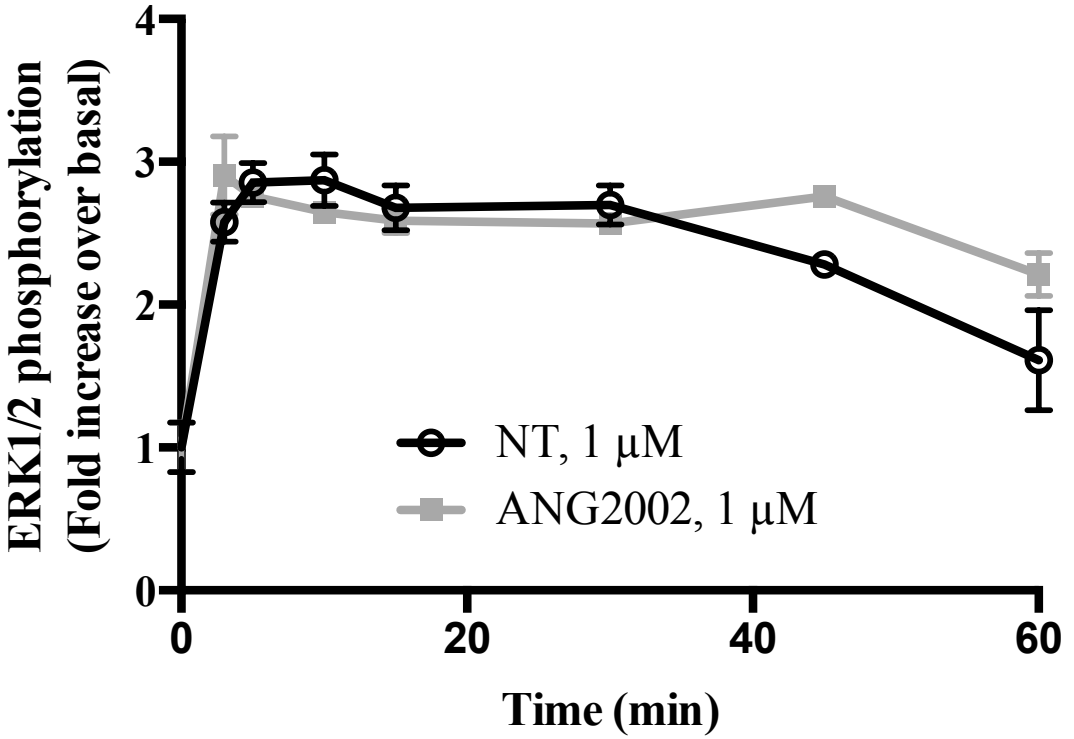
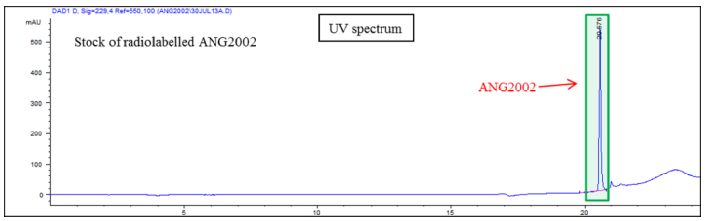
Supplemental Figure 1. Time course of NTS2-mediated ERK1/2 phosphorylation. Phosphorylation of ERK1/2 was examined in 1321N1 cells stably expressing NTS2 following stimulation with 1 μM of ANG2002 (filled square) or native NT (open circles). Results were expressed in fold increase over basal phosphorylation. The values are the means ± SEM of two separate experiments carried out in triplicate.

Supplemental Figure 2. Detection of intact ANG2002 in mouse brain homogenate. **A.** HPLC chromatogram of radiolabeled [^{125}I]-ANG2002 showed that the radiolabeled molecule used for in situ brain perfusion was highly pure. **B.** 99% of ^{125}I eluted radioactivity was associated to elution fractions corresponding with the ANG2002 peak. **C.** To determine the efficacy of the extraction conditions and the quality of recovery, [^{125}I]-ANG2002 was spiked at a specific cpm amount into a mouse brain homogenate. After extraction, radioactivity quantification by HPLC analysis revealed than 96% of the recovered radioactivity was found associated to the ANG2002 elution fractions. **D.** Integrity of [^{125}I]-ANG2002 after a 4-min in situ brain perfusion. After tracer extraction from brain tissues, more than 80% of ^{125}I radio-eluted was found associated to ANG2002 elution fractions, indicating that ANG2002 molecule remained stable during the perfusion and can be significantly recovered from the perfused brain in an intact form. Only 8% for the recovered radioactivity was found associated to an elution fraction at around 5 min.



Supplemental Figure 1

A



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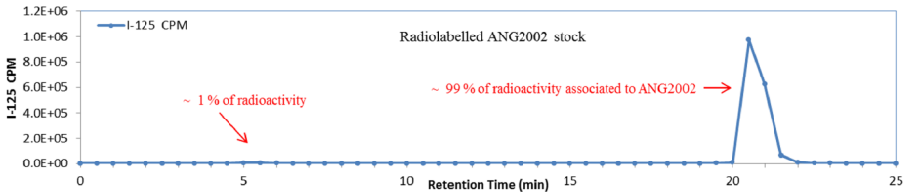

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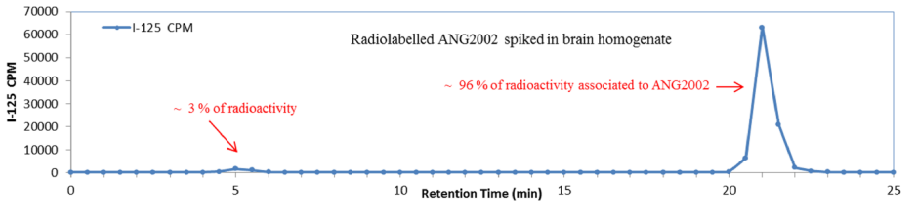

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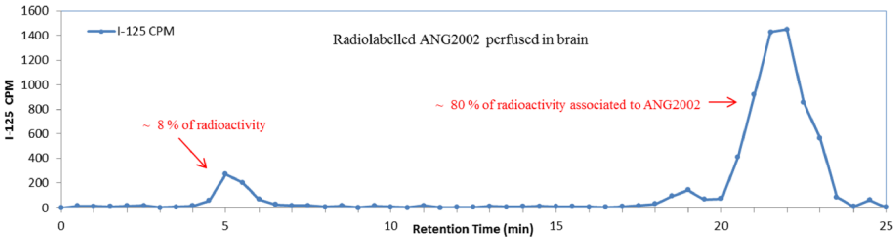

Supplemental Figure 2

Incubation time at 37°C in rat plasma	NT	ANG2002
5 min **10 min** **30 min**	**R^8/R^9**	No cleavage observed
1h **3h** **5h**	No further cleavage observed	No cleavage observed
7h	No further cleavage observed	**R^8/R^9**

Supplemental Table 1. Degradation kinetics of the NT (1-13) sequence in the native (NT) and conjugated (ANG2002) forms in rat plasma. The profiles of degradation were analyzed by HPLC-MS.