

# **Suppression of nuclear factor-kappa B activation and inflammation in microglia by a physically-modified saline**

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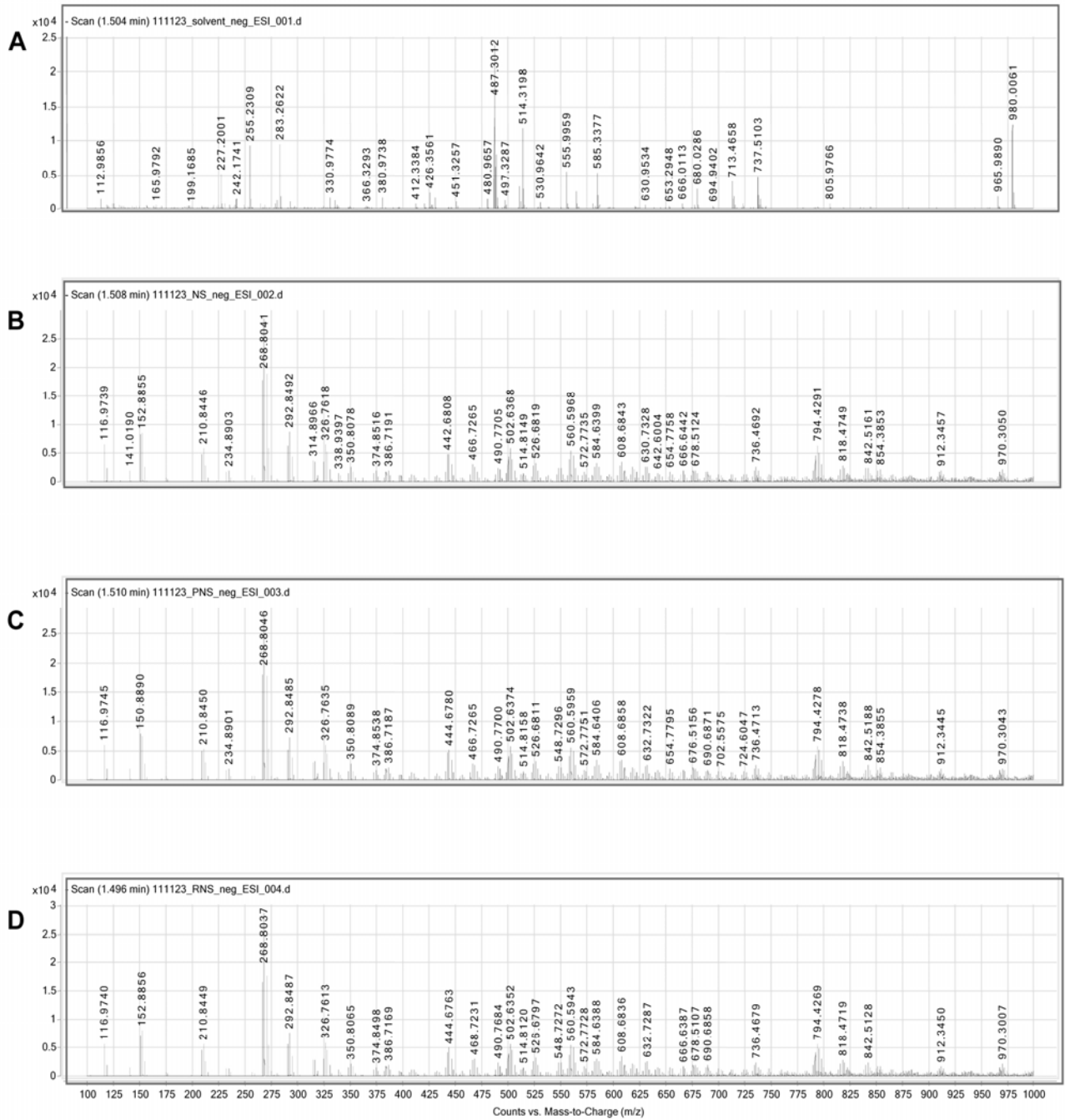
## **Legends to supplementary figures**

Supplementary Figure 1. **Mass spectrometric analyses of NS, PNS60 and RNS60.** To examine compositional differences in NS, PNS60 and RNS60, the LC-Q-TOF system was configured with an electrospray ionization interface (ESI) and the analysis was performed in both positive and negative modes. To facilitate visual comparison, the 100 to 1000  $m/z$  scan range for each sample was separated into 9 segments of 100  $m/z$  each and printed as part of the study data. The extracted segments from each of PNS60 and RNS60 were compared to the corresponding extracted segments for NS. Only a part of it is shown (A, solvent; B, NS; C, PNS60; D, RNS60).

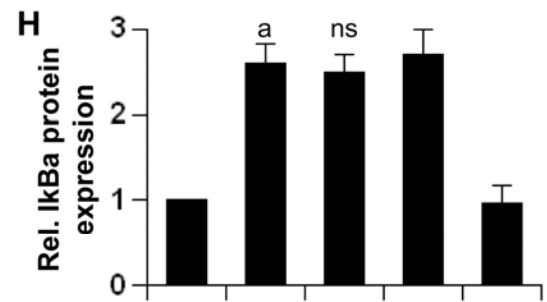
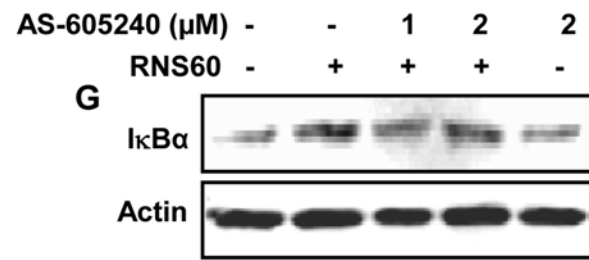
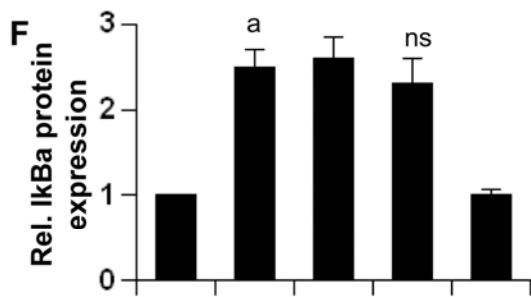
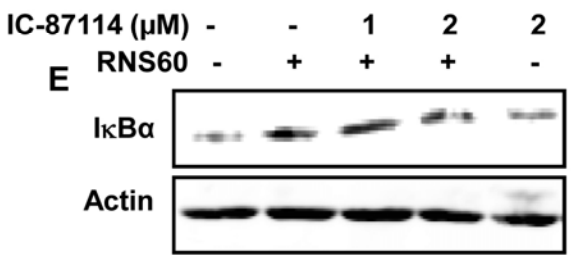
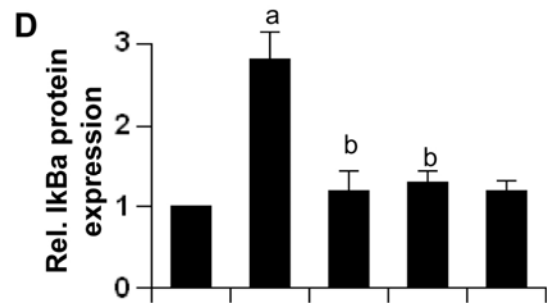
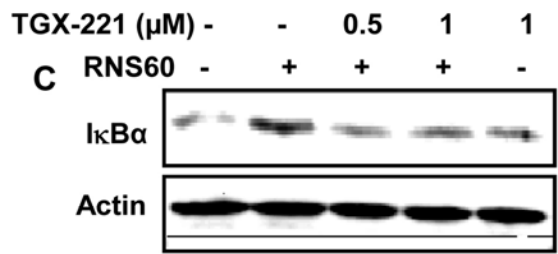
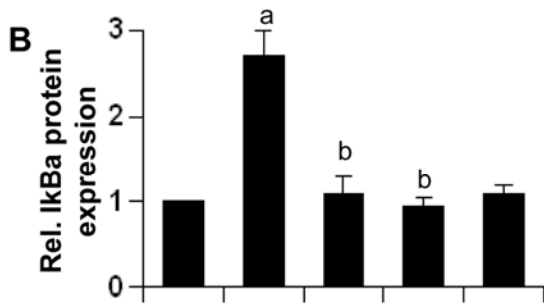
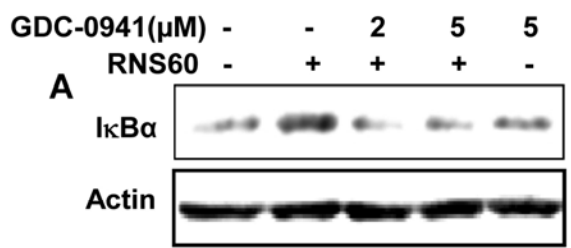
Supplementary Figure 2. **Effect of isoform-selective PI-3 kinase inhibitors on RNS60-mediated upregulation of I $\kappa$ B $\alpha$  in mouse microglial cells.** Mouse BV-2 microglial cells preincubated with different concentrations of GDC-0941 (A&B), TGX-221 (C&D), IC-87114 (E&F), and AS-605240 (G&H) for 30 min were treated with RNS60 (10% v/v) under serum-free condition. After 1 h of treatment, the level of I $\kappa$ B $\alpha$  was monitored by Western blot (A, C, E, & G). Bands were scanned and results presented as protein expression relative to Actin (B, D, F, &H). Results represent mean  $\pm$

SD of three separate experiments. <sup>a</sup>*p* < 0.001 vs control; <sup>b</sup>*p* < 0.001 vs RNS60; ns, non-significant.

Supplementary Figure 3. **Effect of siRNA knockdown of p110 $\alpha$  and p110 $\delta$  isoforms of PI-3 kinase on RNS60-mediated upregulation of I $\kappa$ B $\alpha$  in mouse microglial cells.** Mouse BV-2 microglial cells were transfected with control siRNA, p110 $\alpha$  siRNA or p110 $\delta$  siRNA (Santa Cruz, CA). After 48 h of transfection, the knockdown of p110 $\alpha$  and p110 $\delta$  was monitored by Western blot (A). After 48 h, cells were stimulated with RNS60 for 1 h followed by monitoring the level of I $\kappa$ B $\alpha$  by Western blot (B). Bands were scanned and results presented as protein expression relative to Actin (C). Results represent mean  $\pm$  SD of three separate experiments. <sup>a</sup>*p* < 0.001 vs control; <sup>b</sup>*p* < 0.001 vs cont siRNA-RNS60.

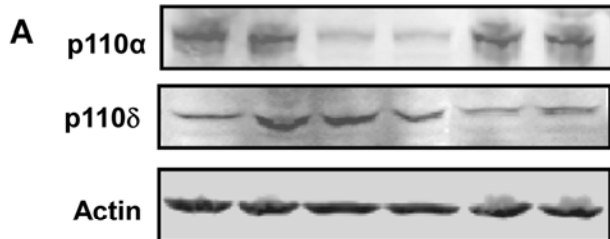


**Supplementary Fig. 1**

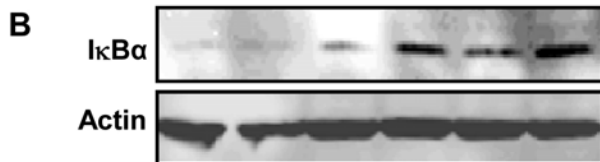


**Supplementary Figure 2**

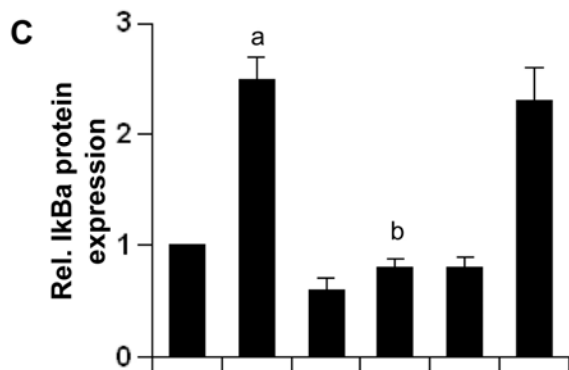
Control SiRNA	+	+	-	-	-	-
p110- $\delta$ SiRNA	-	-	-	-	+	+
p110- $\alpha$ SiRNA	-	-	+	+	-	-



Cont SiRNA	-	-	-	-	+	+
p110- $\delta$ SiRNA	-	-	+	+	-	-
p110- $\alpha$ SiRNA	+	+	-	-	-	-
RNS60	-	+	-	+	-	+



Cont SiRNA	+	+	-	-	-	-
p110- $\delta$ SiRNA	-	-	-	-	+	+
p110- $\alpha$ SiRNA	-	-	+	+	-	-
RNS60	-	+	-	+	-	+



**Supplementary Figure 3**

## Supplementary Table 1

**Table 1**

Test Material	Ba <sup>d</sup>	Ni	K	TOC
Bottled RNS60 <sup>a</sup>	0.0024 (0.0004)	0.0059 (0.0008)	0.6 (2)	35
Bottled PNS60 <sup>a,b</sup>	0.0028 (0.0004)	0.0043 (0.0008)	0.2 (2)	43
Source saline <sup>c</sup>	0 (0.0004)	0.0045 (0.0008)	0.6 (1) <sup>e</sup>	21

### Table 1 Legend

ICP-MS results for the three tested metals (out of 26 total) that were above the minimal detection limits, presented as ppm (mg/L) above the detection limit (provided in parentheses). Total Organic Carbon (TOC) values are shown in ppb. <sup>a</sup>In glass bottles, <sup>b</sup>This saline contacted the same device surfaces as RNS60 and was bottled in the same way, <sup>c</sup>Supplied in polypropylene bottles, <sup>d</sup>Barium is known to leach from glass, <sup>e</sup>The smaller detection limit for control saline resulted from large analysis volumes.