

Expanded View Figures

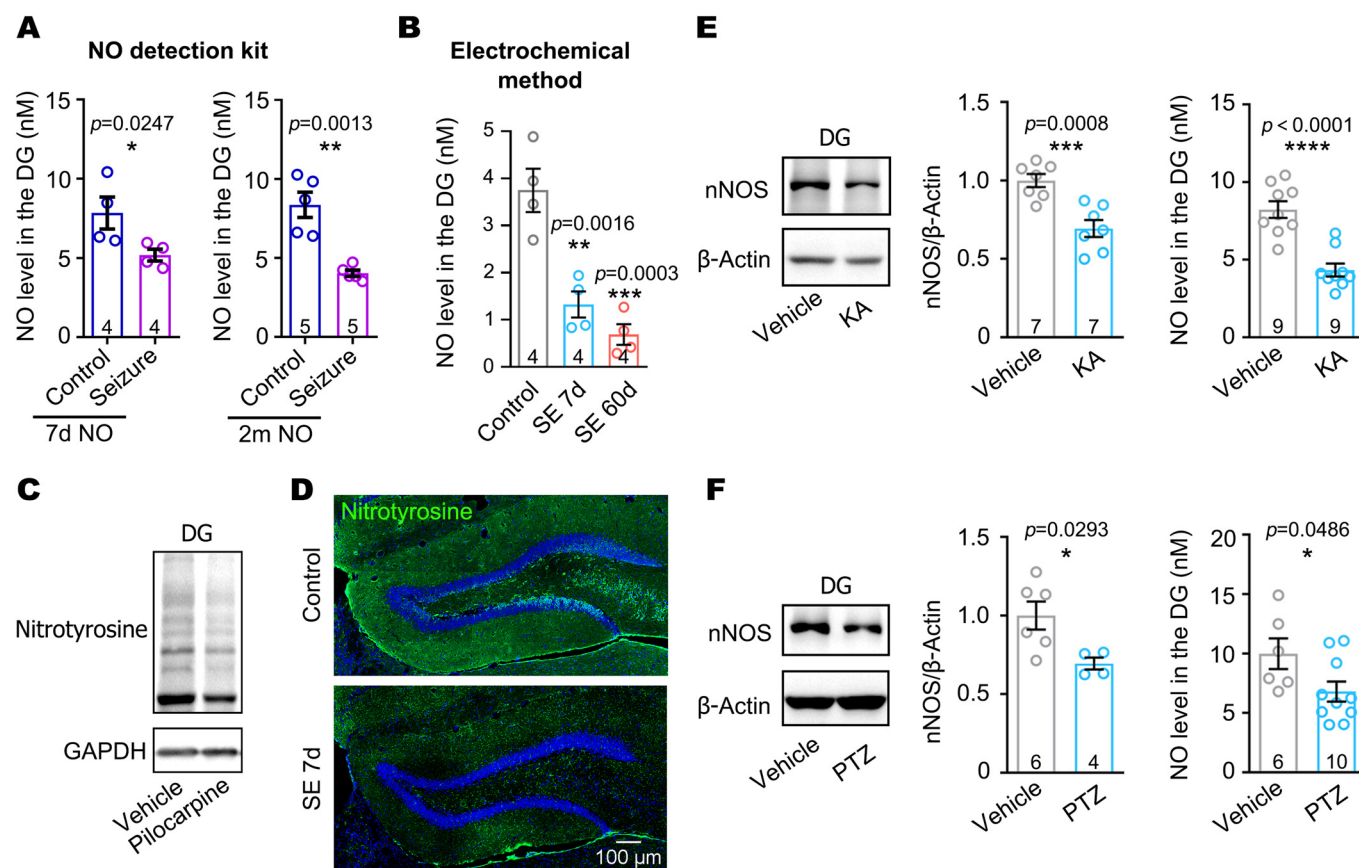


Figure EV1. NO content decreases in TLE mouse models.

(A) Data graph showing the concentration of NO in DG 7 days or 2 months after pilocarpine-induced SE using NO detection kit. Students' *t*-test, $n = 4$ mice. (B) NO measurements using electrochemical methods. One-way ANOVA, $n = 5$ mice. (C) Representative images showing decreased nitrotyrosine-modified proteins in the DG 2 months after pilocarpine administration. The same result was observed in 5 mice. (D) Representative photos of immunofluorescence of nitrotyrosine in the DG 7 days after pilocarpine-induced SE. (E) Representative image and data graph showing nNOS expression in DG 14 days after KA-induced SE. Students' *t*-test, $n = 7-9$ mice. (F) Representative image and data graph showing nNOS expression in DG 28 days after PTZ-induced SE. Students' *t*-test, $n = 4-10$ mice. Error bars correspond to $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ***** $P < 0.00001$.

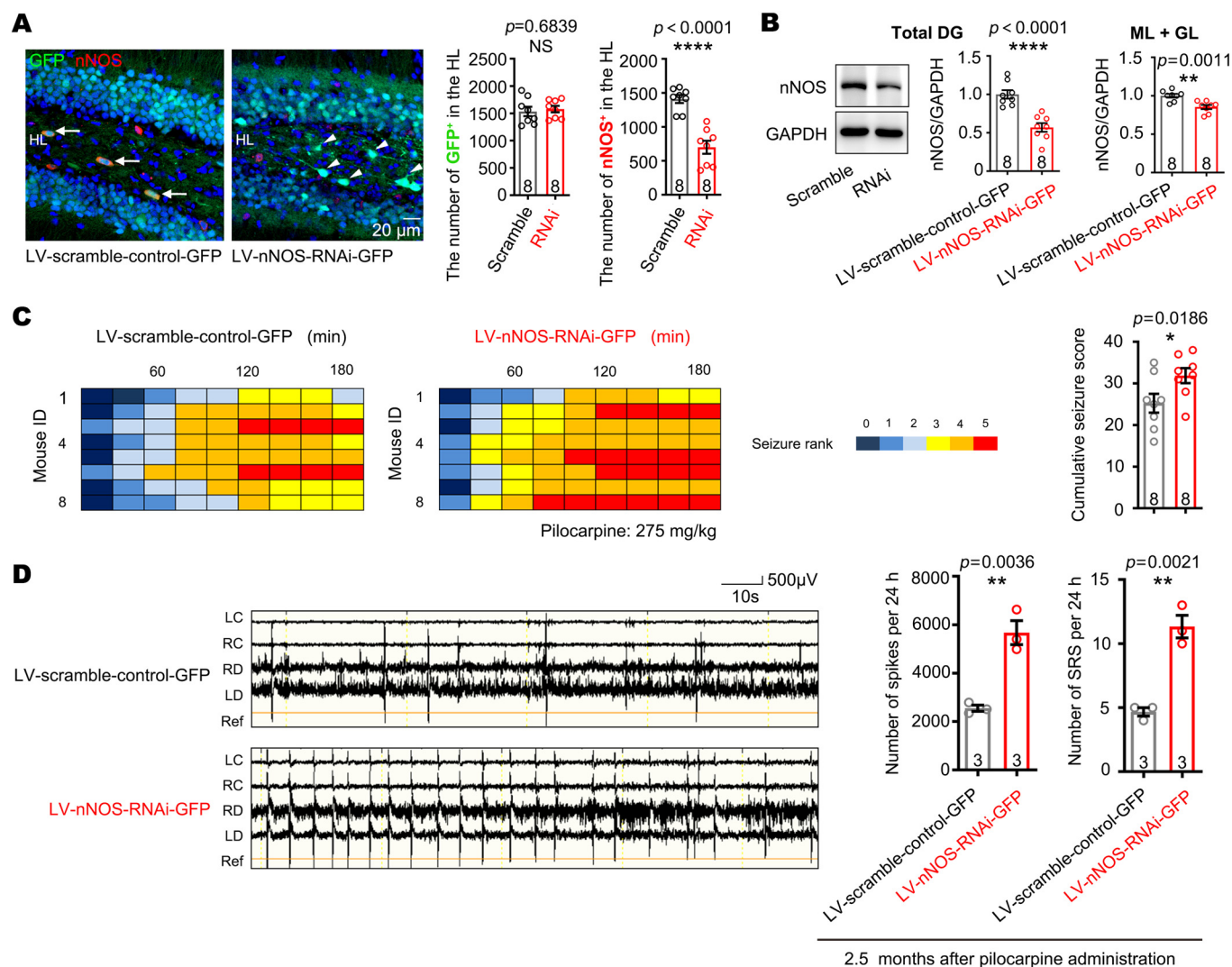


Figure EV2. Knockdown of nNOS increases susceptibility of inducing status epilepticus and aggravates epileptogenesis in the pilocarpine mouse model of TLE.

(A) Representative photos and data graphs of nNOS-positive/GFP-positive cells in the hilus 1 month after injection 1 μ L of LV-scramble-control-GFP or LV-nNOS-RNAi-GFP into the hilus. Notably, less nNOS⁺ cells were observed after injection of LV-nNOS-RNAi-GFP. Arrows indicated transduced hilar nNOS-positive interneurons and arrowheads indicated transduced hilar nNOS-negative interneurons. The same observation was repeated in 5 mice. Student's *t*-test, $n = 8$ mice. (B) Western blot showing decreased content of nNOS protein in the DG and in the ML + GL regions 1 month after injection 1 μ L of LV-nNOS-RNAi-GFP into the hilus. Student's *t*-test, $n = 8$ mice. (C) Heat map and data graph showing cumulative seizure score for 3 h after pilocarpine treatment at a dose of 275 mg/kg in mice. The mice received injection 1 μ L of LV-scramble-control-GFP or LV-nNOS-RNAi-GFP into the hilus 1 month before pilocarpine administration. Student's *t*-test, $n = 12$ mice. (D) Representative images and data graphs of EEG recordings showing brain waves of mice 2.5 months after pilocarpine-induced SE. The mice received injection 1 μ L of LV-scramble-control-GFP or LV-nNOS-RNAi-GFP into the hilus 1 month before pilocarpine administration. Student's *t*-test, $n = 3$ mice. GL granule layer, ML molecular layer, HL hilus, LC left cortex, RC right cortex, RD right DG, LD left DG, Ref Reference. Error bars correspond to $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, ***** $P < 0.0001$, NS, not significant.

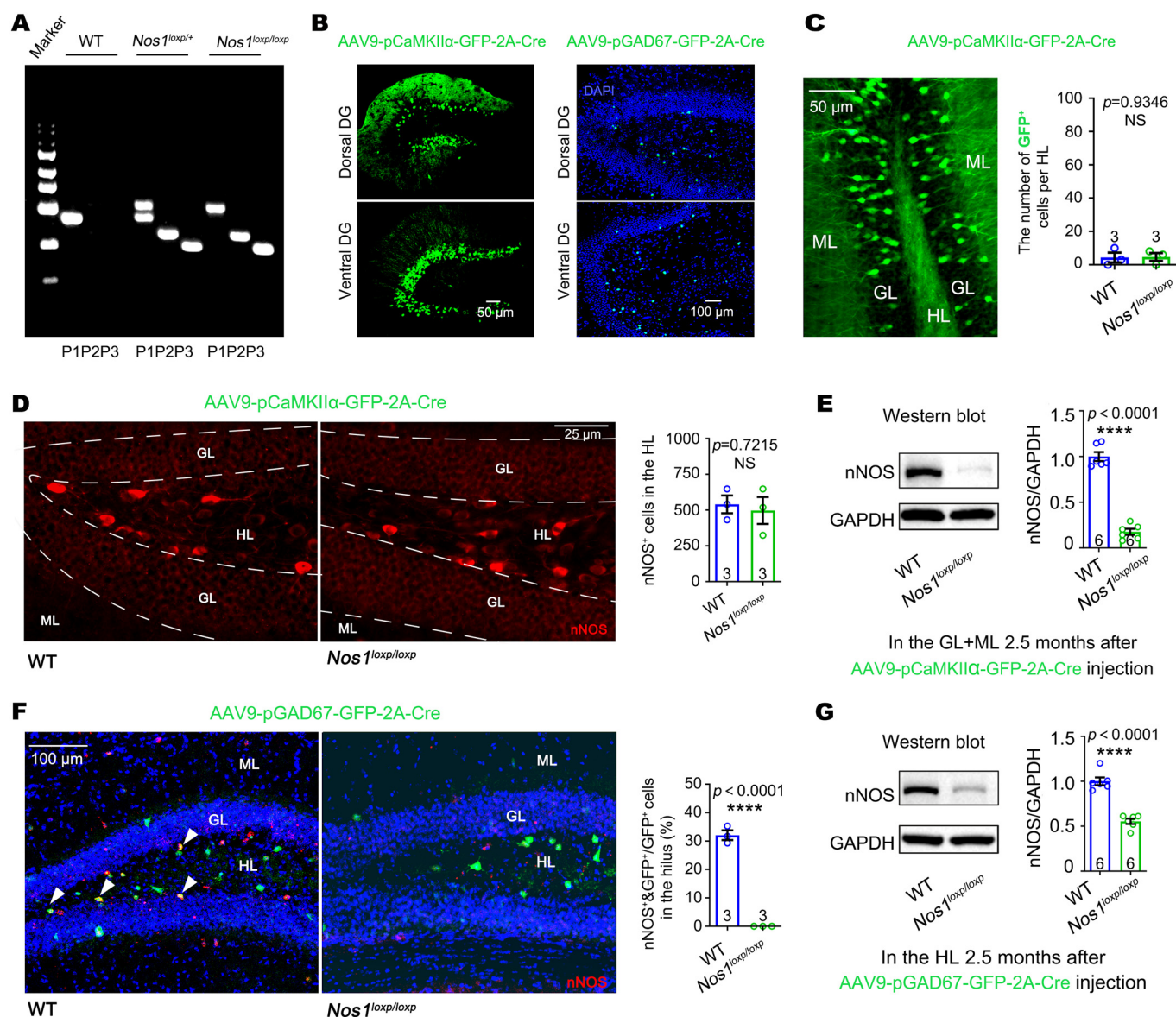


Figure EV3. Increased susceptibility to SE induction after deletion of nNOS from hilar interneurons but not from DGCs.

(A) Representative genotyping of *Nos1^{loxP/loxP}* mice. P1, P2, and P3 showed productions synthesized by three primers, respectively. (B) The distribution of AAV9-pCaMKII α -GFP-2A-Cre virus or AAV9-pGAD67-GFP-2A-Cre virus transduced neurons along the DG dorsal-ventral axis. The same pattern was observed in all injected mice. (C) Representative photo of AAV9-pCaMKII α -GFP-2A-Cre transduction and data graph showing the number of GFP⁺ cells in the hilus were not altered. Student's *t*-test, *n* = 3 mice. (D) Representative photos and data graph showing nNOS-positive cells in the hilus 1 month after injection of AAV9-pCaMKII α -GFP-2A-Cre into the hilus of WT or *Nos1^{loxP/loxP}* mice. No significant difference was observed. Student's *t*-test, *n* = 3 mice. (E) The expression of nNOS protein in the granule layer and molecular layer were reduced after injection of AAV9-pCaMKII α -GFP-2A-Cre virus into the hilus of *Nos1^{loxP/loxP}* mice. Student's *t*-test, *n* = 6 mice. (F) Representative photos and data graphs showed that the number of nNOS⁺ cells in the hilus was reduced and no granule neurons were transduced. Arrowheads indicated transduced hilar nNOS⁺ neurons. Student's *t*-test, *n* = 3 mice. (G) The expression of nNOS protein in the hilus was reduced after injection of AAV9-pGAD67-GFP-2A-Cre virus into the hilus of *Nos1^{loxP/loxP}* mice. Student's *t*-test, *n* = 6 mice. GL granule layer, ML molecular layer, HL hilus. Error bars correspond to $\pm$ s.e.m. *****P* < 0.0001, NS, not significant.

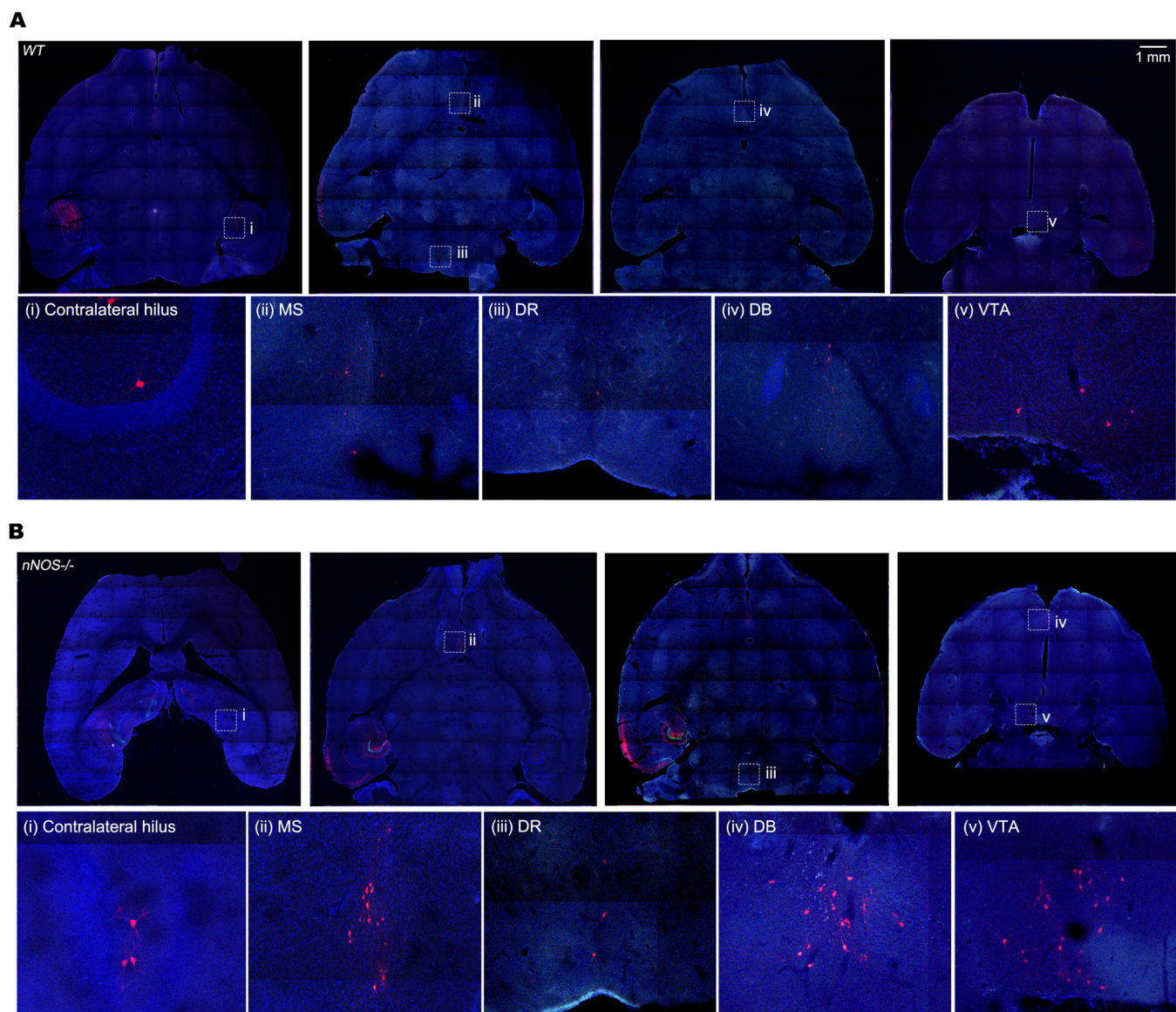


Figure EV4. Distribution of traced cells projecting to DGCs.

(A) Representative photos showing the distribution of traced cells projecting to DGCs in the brain of WT mice. (B) Representative photos showing the distribution of traced cells projecting to DGCs in the brain of *Nos1*^{-/-} mice. (i–v) were zoomed photos from white squares, respectively. MS medial septum, DR dorsal raphe, DB diagonal band, VTA ventral tegmental area.

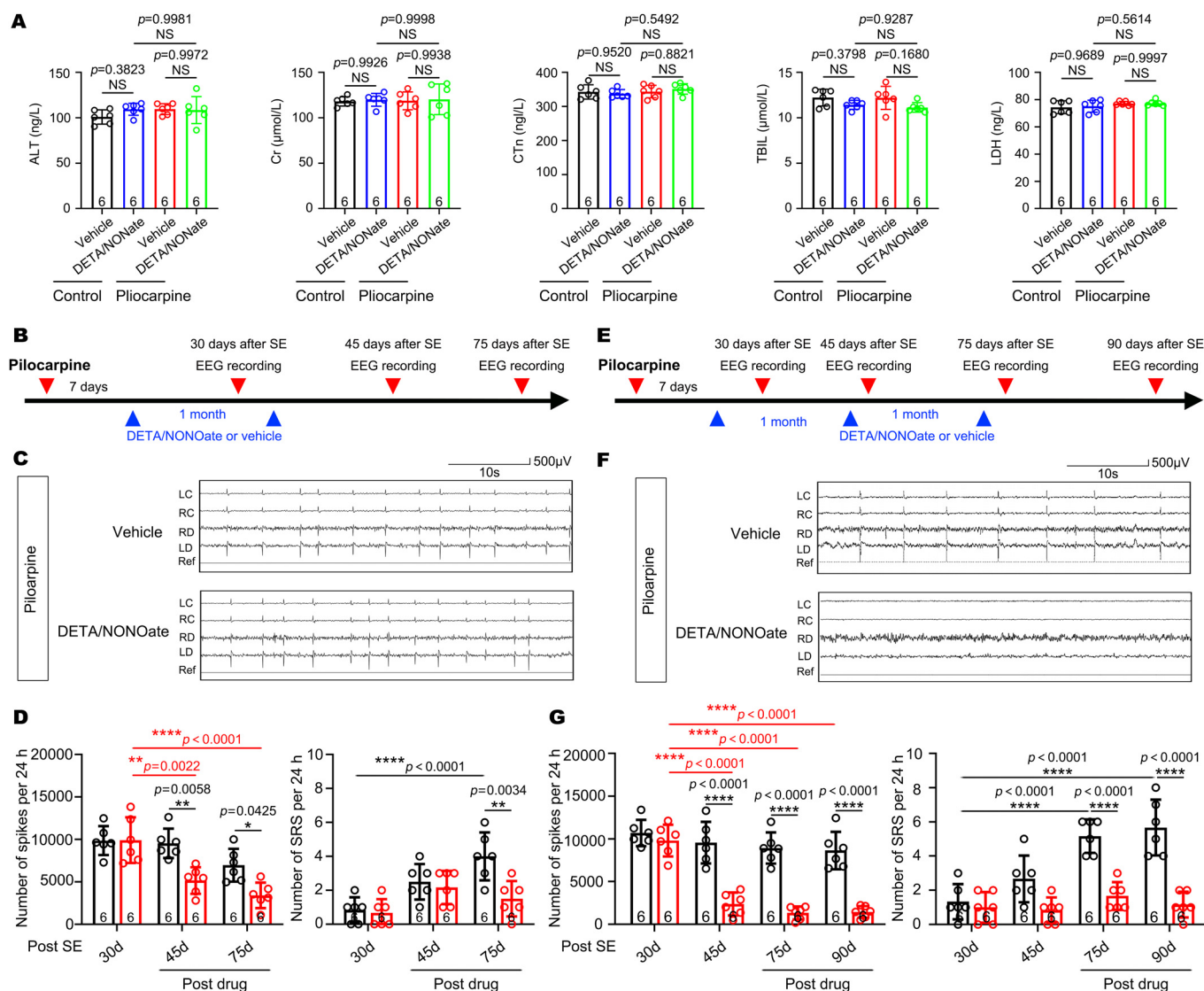


Figure EV5. Chronic treatment with NO Donor prevents epileptogenesis with long-lasting effect.

(A) Quantification of plasmatic ALT, TBIL, Cr, LDH, and CTn using ELISA kits. Two-way ANOVA, $n = 6$ mice. (B–D) Experimental paradigm (B), representative EEG recordings (C), and data graphs (D), showing chronic treatment with DETA/NONOate (1 mg/kg, i.p., 1 time per day) for 1 month after pilocarpine-induced SE for 7 days prevented the development of SPKs and SRS. Two-way ANOVA, $n = 6$ mice. (E–G) Experimental paradigm (E), representative EEG recordings (F), and data graphs (G), showing chronic treatment with DETA/NONOate (1 mg/kg, i.p., 1 time per day) for 1 month after pilocarpine-induced SE for 37 days prevented the development of SPKs and SRS. Two-way ANOVA, $n = 6$ mice. ALT alanine aminotransferase, TBIL total bilirubin, Cr creatinine, LDH lactate dehydrogenase, CTn cardiac troponin. Error bars correspond to $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, NS, not significant.