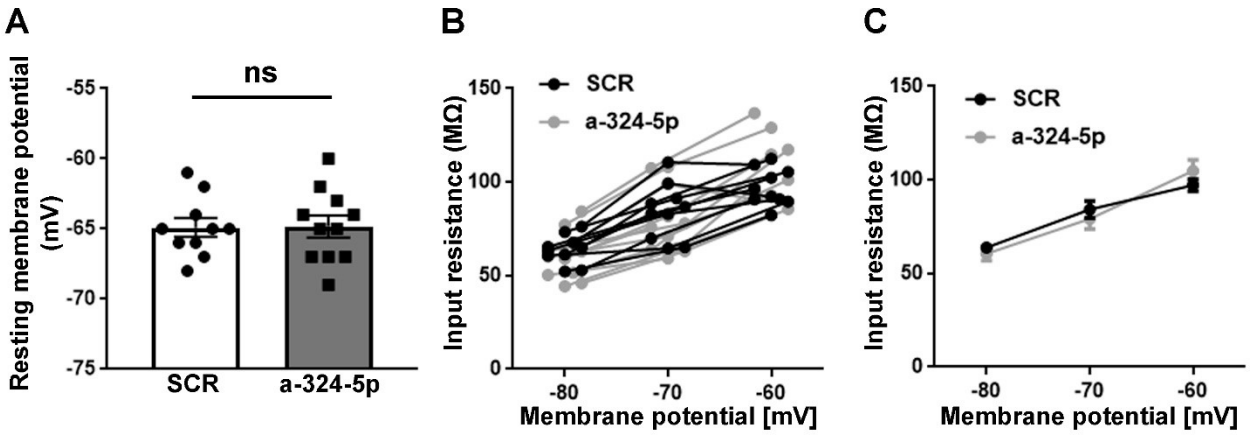

Figure S1: Resting membrane potential and input resistance are unchanged after miR-324-5p inhibition.

(A) Resting membrane potential is not significantly different in mice injected with scrambled or miR-324-5p-specific antagomir (SCR: n=10, a-324-5p: n=11, from 3 mice each, unpaired two-tailed t-test, t(19)=0.078, p=0.938). **(B,C)** Input resistance is not significantly different measured at multiple membrane potentials (SCR: n=10, a-324-5p: n=11, from 3 mice each, 2-way ANOVA, antagomir: F(1,19)=0.001, p=0.973; interaction antagomir X membrane potential: F(2,37)=4.034, p=0.026; Sidak's multiple comparisons post hoc tests p>0.5 for all three membrane potentials). Individual experiments shown in **B**, cumulative values shown in **C**. Error bars represent SEM.

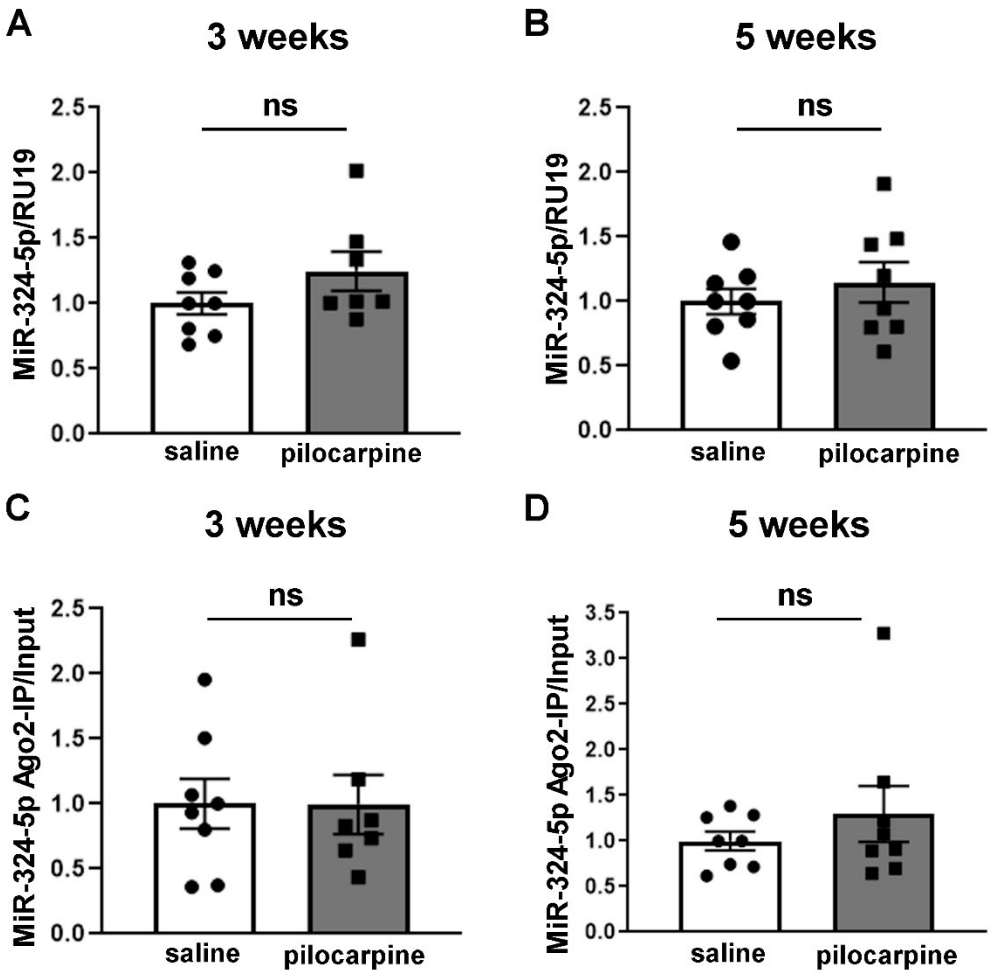

Figure S2: MiR-324-5p levels or association with the RISC are not significantly changed three and five weeks after pilocarpine-induced *status epilepticus*.

(A,B) qRT-PCR did not show significant changes in miR-324-5p levels normalized to RU19 at 3 weeks (**A**: saline: n=8, pilocarpine: n=7, unpaired two-tailed t-test, t(13)=1.496, p=0.159) and 5 weeks (**B**: n=8, unpaired two-tailed t-test, t(14)=0.808, p=0.433) after pilocarpine treatment. **(C,D)** Association of miR-324-5p with Ago2 was also unchanged at both time points (**C:** saline: n=8, pilocarpine: n=7, two-tailed Mann-Whitney test, p=0.867; **D**: n=8, two-tailed Mann-Whitney test, p=0.858). MiR-324-5p in Ago2-IPs was normalized to input levels. Error bars represent SEM.

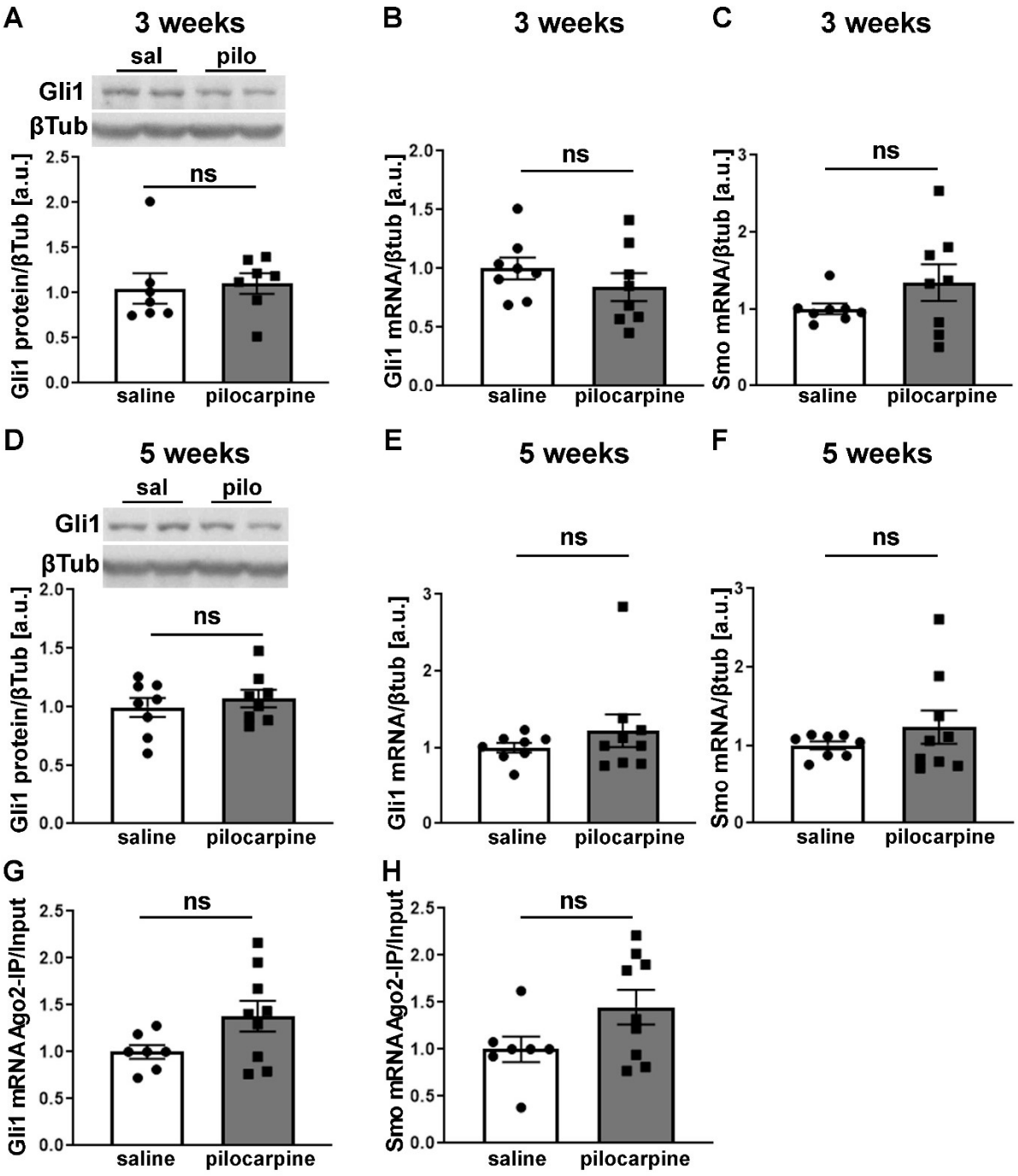

Figure S3: MicroRNA-induced silencing of Smo or Gli1 mRNA are not altered in epilepsy.

(A) Gli1 protein levels are unchanged 3 weeks after pilocarpine treatment in mice (n=7, two-tailed Mann-Whitney test, p=0.259). Example western blot shown at top. **(B,C)** Gli1 (**B**)- and Smo (**C**)-specific qRT-PCR analyses showed that mRNA levels were likewise unchanged 3

weeks after pilocarpine treatment (**B**: n=8, unpaired two-tailed t-test, t(14)=1.053, p=0.310, **C**: n=8, two-tailed Mann-Whitney test, p=0.505). **(D)** Five weeks after pilocarpine treatments, Gli1 protein levels are not different from saline control (n=8, unpaired two-tailed t-test, t(14)=0.687, p=0.503). Example western blot shown at top. **(E,F)** No significant changes in Gli1 (**E**) or Smo (**F**) mRNA levels were detected 5 weeks following pilocarpine treatment (**E**: saline: n=8, pilocarpine: n=9, two-tailed Mann-Whitney test, p=0.673; **F**: saline: n=8, pilocarpine: n=9, two-tailed Mann-Whitney test, p=0.963). **(G,H)** Ago2 association in chronically seizing mice (5 weeks after pilocarpine) is not significantly changed for Gli1 mRNA (**G**: saline: n=7, pilocarpine: n=9, unpaired two-tailed t-test, t(14)=1.917, p=0.076) or Smo mRNA (**H**: saline: n=7, pilocarpine: n=9, unpaired two-tailed t-test, t(14)=1.847 p=0.086). For western blots, Gli1-specific bands were normalized to βTubulin on the same blot, and samples were loaded in duplicates. For qRT-PCRs, Gli1 and Smo mRNA levels were normalized to βtubulin mRNA. Ago IPs were normalized to input. Error bars represent SEM.

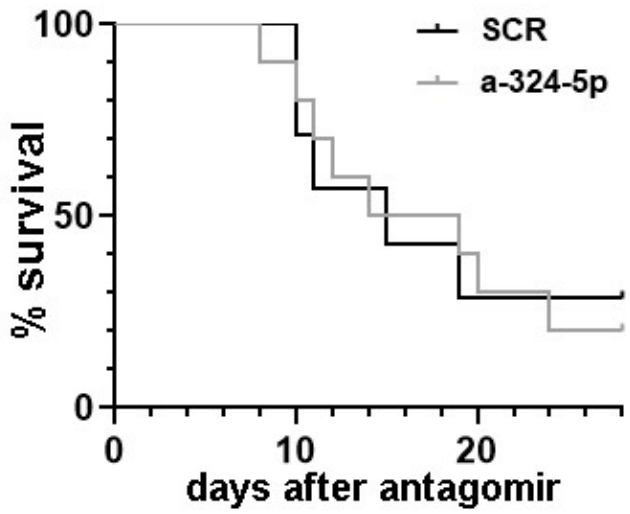

Figure S4: Survivability of epileptic mice is not affected by antagomir treatment.

Shown is a Kaplan-Meier survival curve over the 28-day post-injection EEG-recording period of all mice that were implanted with electrodes and injected with antagomir. Ten mice were injected with miR-324-5p antagomir, and 8 were injected with scrambled antagomir. Only 2 in each group survived the entire 28 days. No significant difference in survivability was detected (a-324-5p: n=10; SCR: n=8; log-rank test p=0.914).

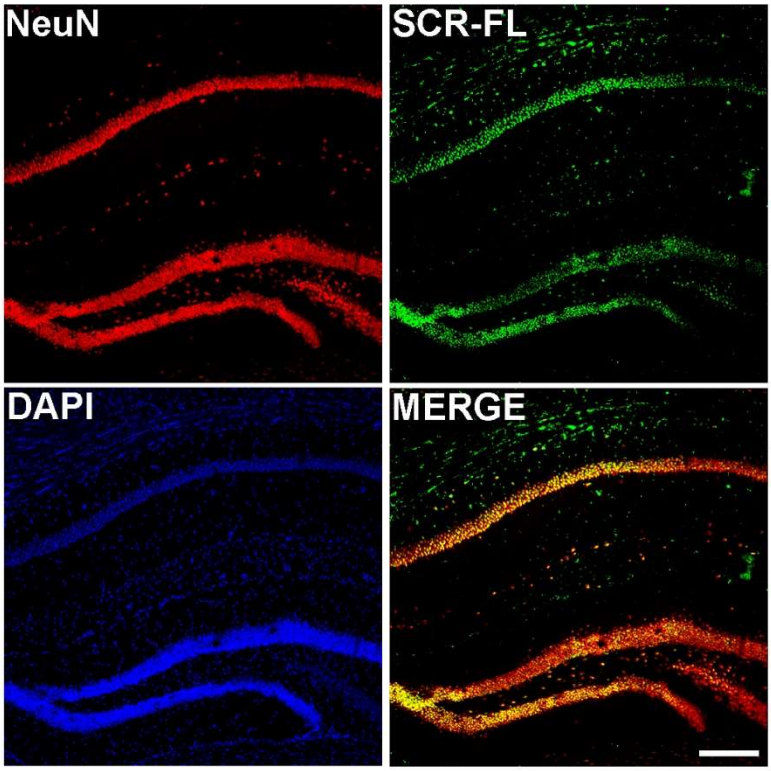

Figure S5: Antagomirs are stable for a prolonged time in the mouse brain.

An example image of a hippocampus from a mouse ICV-injected with a fluorescein-tagged scrambled antagomir (*SCR-FL*, LNA-modified, Qiagen) shows green fluorescent signal in hippocampal neurons 10 days after injection. Staining with a NeuN antibody (*NeuN*, ab177487, Abcam, RRID: AB_2532109, red) was used to label neurons, DAPI staining (*DAPI*, blue) was used to label all cell nuclei. Yellow signal in a merged image of NeuN and fluorescein labeling (*MERGE*) indicates neurons that have taken up the antagomir. Note the yellow signal in the CA1 region, where Kv4.2 is mostly expressed, indicating neuronal expression of the antagomir. Shown are images from male offspring of a C57BL/6J X FVB/NJ breeding, the same genotype that was used in all pilocarpine experiments. The scale bar is 200 µm.

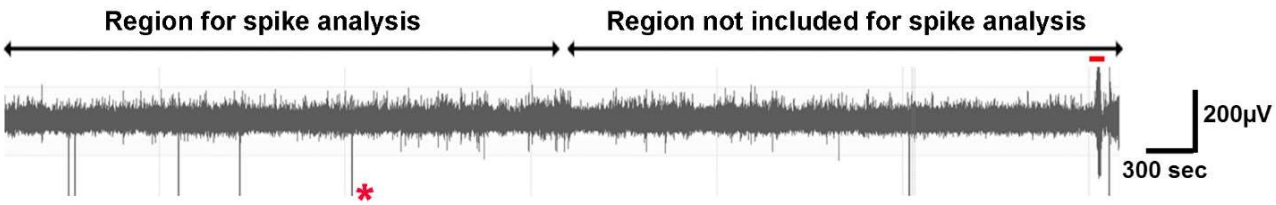

Figure S6: Extended EEG trace used for interictal epileptiform spike detection

The example shows an EEG trace over 2 hours from a mouse injected with scrambled antagomir. The trace includes a seizure at the very end (red bar) and indicates the 1-hour period before the seizure that was not included in spike analysis. The red asterisk marks an example of an EEG artifact. EEG artifacts were manually removed before analysis.