

**Supplemental information**

**A novel AAV9-dual microRNA-vector targeting**

***GRIK2* in the hippocampus as a treatment**

**for mesial temporal lobe epilepsy**

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**Table S1.** Results from RNAseq analysis performed on Glutaneurons.

Gene Name	Ensembl	Fold Change	padj
<i>na</i>	ENSG00000289417	0.584976744	0.042674
<i>na</i>	ENSG00000289040	0.587813896	0.028982
KCNIP4-IT1	ENSG00000280650	0.611832012	0.028654
NRG1-IT1	ENSG00000253974	0.638090163	0.039094
<i>na</i>	<i>ENSG00000249049</i>	0.638252995	0.005491
<i>na</i>	<i>ENSG00000289013</i>	0.65867056	0.037389
ANKRD10-IT1	ENSG00000229152	0.672551731	0.047556
NDC1	ENSG00000058804	0.68200984	0.028982
<i>na</i>	<i>ENSG00000288105</i>	0.695764586	0.028982
FSIP2	ENSG00000188738	0.704872887	0.013427
HFM1	ENSG00000162669	0.713636375	0.038847
USP15	ENSG00000135655	0.718046855	0.01593
GRIK2	ENSG00000164418	0.718792499	0.001842
GABRA2	ENSG00000151834	0.719712814	0.016328
DNAH14	ENSG00000185842	0.721835947	0.039273
UGGT2	ENSG00000102595	0.739745091	0.028089
ELOVL5	ENSG00000012660	0.740752789	0.001152
LINC00645	ENSG00000258548	0.741343409	0.04085
FEM1C	ENSG00000145780	0.742443437	0.024065
ANKRD36	ENSG00000135976	0.743004827	0.022738
ZBTB41	ENSG00000177888	0.749210234	0.029039
NKTR	ENSG00000114857	0.750529916	0.027781
RAP1B	ENSG00000127314	0.750584278	0.042315
CCDC82	ENSG00000149231	0.755563404	0.02954
ZDHHC21	ENSG00000175893	0.756978785	0.037389
DPY19L4	ENSG00000156162	0.758862327	0.048406
SOCS5	ENSG00000171150	0.759772472	0.016568
LRRTM4	ENSG00000176204	0.760496539	0.032675
SYT14	ENSG00000143469	0.763136563	0.028982
PUM2	ENSG00000055917	0.763653523	0.013427
ANO5	ENSG00000171714	0.767461618	0.038477
FER	ENSG00000151422	0.772463258	0.039067
FAR1	ENSG00000197601	0.781913179	0.038477
MBD5	ENSG00000204406	0.796519485	0.048964
MATR3	ENSG00000015479	0.799667835	0.0417
CALY	ENSG00000130643	1.20065345	0.021593
<i>na</i>	<i>ENSG00000218426</i>	1.204330118	0.035061
<i>na</i>	<i>ENSG00000287763</i>	1.211661418	0.013006
UNCX	ENSG00000164853	1.215027617	0.010911
VWA1	ENSG00000179403	1.223826241	0.017154
RPS6KA4	ENSG00000162302	1.224465901	0.032485
TPGS1	ENSG00000141933	1.233523394	0.028654

CDKN2D	ENSG00000129355	1.234297209	0.028089
NDUFB7	ENSG00000099795	1.234771055	0.020021
FGFBP3	ENSG00000174721	1.236284626	0.032485
TMEM160	ENSG00000130748	1.237051683	0.028654
FAM171A2	ENSG00000161682	1.241676003	0.000256
ZNF580	ENSG00000213015	1.241776638	0.00534
CORO1B	ENSG00000172725	1.246745544	0.048964
ANTKMT	ENSG00000103254	1.252902021	0.032485
TMEM158	ENSG00000249992	1.254685013	0.028982
RPLP1	ENSG00000137818	1.255973098	0.018191
ZNF579	ENSG00000218891	1.256683491	0.000434
SNORA73B	ENSG00000200087	1.257005807	0.048141
ATP5F1D	ENSG00000099624	1.258443646	0.000358
MRPL55	ENSG00000162910	1.288422298	0.039067
MEX3D	ENSG00000181588	1.29308729	1.16E-05
FAM131C	ENSG00000185519	1.302537751	0.002716
SLC10A3	ENSG00000126903	1.31026559	0.041166
PRR7	ENSG00000131188	1.312897567	0.013006
DPY19L2P3	ENSG00000291213	1.326112905	0.039067
CBARP	ENSG00000099625	1.326192851	0.000169
ZNF771	ENSG00000179965	1.343661485	0.000132
TP53TG5	ENSG00000124251	1.363059961	0.048964
GLTPD2	ENSG00000182327	1.366936269	0.028982
TTC9B	ENSG00000174521	1.387000408	0.00134
CITED4	ENSG00000179862	1.392920211	0.001347
TRIM8-DT	ENSG00000272933	1.407589183	3.26E-05
CHCHD10	ENSG00000250479	1.440721155	1.28E-05
PCSK1N	ENSG00000102109	1.452825586	0.021692
C11orf96	ENSG00000187479	1.462711768	0.005348
CEBPB	ENSG00000172216	1.48512436	2.52E-07
BTBD17	ENSG00000204347	1.511385821	0.025646
HES4	ENSG00000188290	1.515355793	1.81E-05
C1QTNF4	ENSG00000172247	1.571064154	3.77E-09
INAFM1	ENSG00000257704	1.705244732	1.19E-07
C4orf48	ENSG00000243449	2.0300481	0.010838

**Table S2.** Result of *in silico* analysis

**Table S3.** Mean *GRIK2* decrease in human organotypic slices treated by AAV9aGRIK2 vs non transduced slice, per patient.

Type	Gender	Age (in years) at resection	(n responding slices / n tested slices)
Not available	Male	40	50% (4/5)
IIa	Male	44	84%
IIIb	Female	46	25%
glioblastoma	Female	52	23%
Ia	Female	51	25%
IIa	Male	49	25%
IIa	Male	40	36%
Ia	Female	11	41%
Ia	Male	49	35%

**Table S4.** Pathological features of patients with refractory mTLE who donated their resection for electrophysiology studies

Pathology	Gender	Age (in years) at resection
Cryptogenic refractory epilepsy, epileptogenic region located on the right temporal lobe and associated with the anterior insula	Female	41
Refractory temporal epilepsy located on the left lobe with hippocampal sclerosis	Female	44
Refractory temporal epilepsy located on the left lobe with possible focal cortical dysplasia	Female	52
Refractory temporal epilepsy located on the anterior right lobe with minimal hippocampal sclerosis	Male	41
Left anterior temporal focal epilepsy with hypertrophic appearance of the left amygdala nucleus, Loss of hippocampal digitations without other signs of sclerosis	Male	36

**Table S5.** Summary of incidence and average severity of histopathology findings in NHP GLP toxicity study.

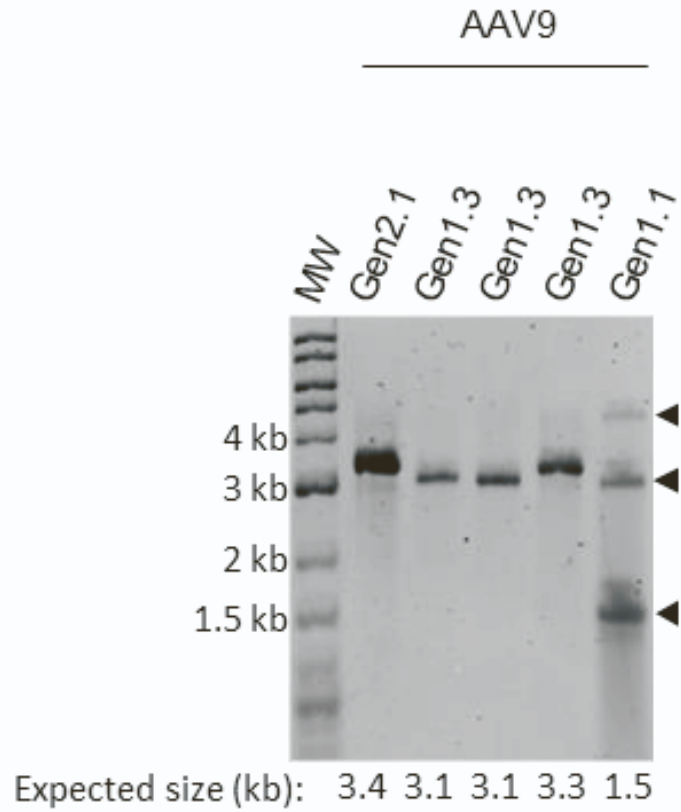
	incidence	average severity	incidence	average severity	incidence	average severity
Dose (vg/hippocampus)	6.00E+10		2.40E+11		1.20E+12	
microgliosis	2/6	0.67	5/6	1.33	6/6	2
astrocytosis	2/6	0.67	5/6	1.67	6/6	1.83
gliosis	3/6	0.83	3/6	1	6/6	2
infiltrate, mononuclear cells	2/6	0.33	4/6	1	6/6	1.67

*tissue severity grade: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.*

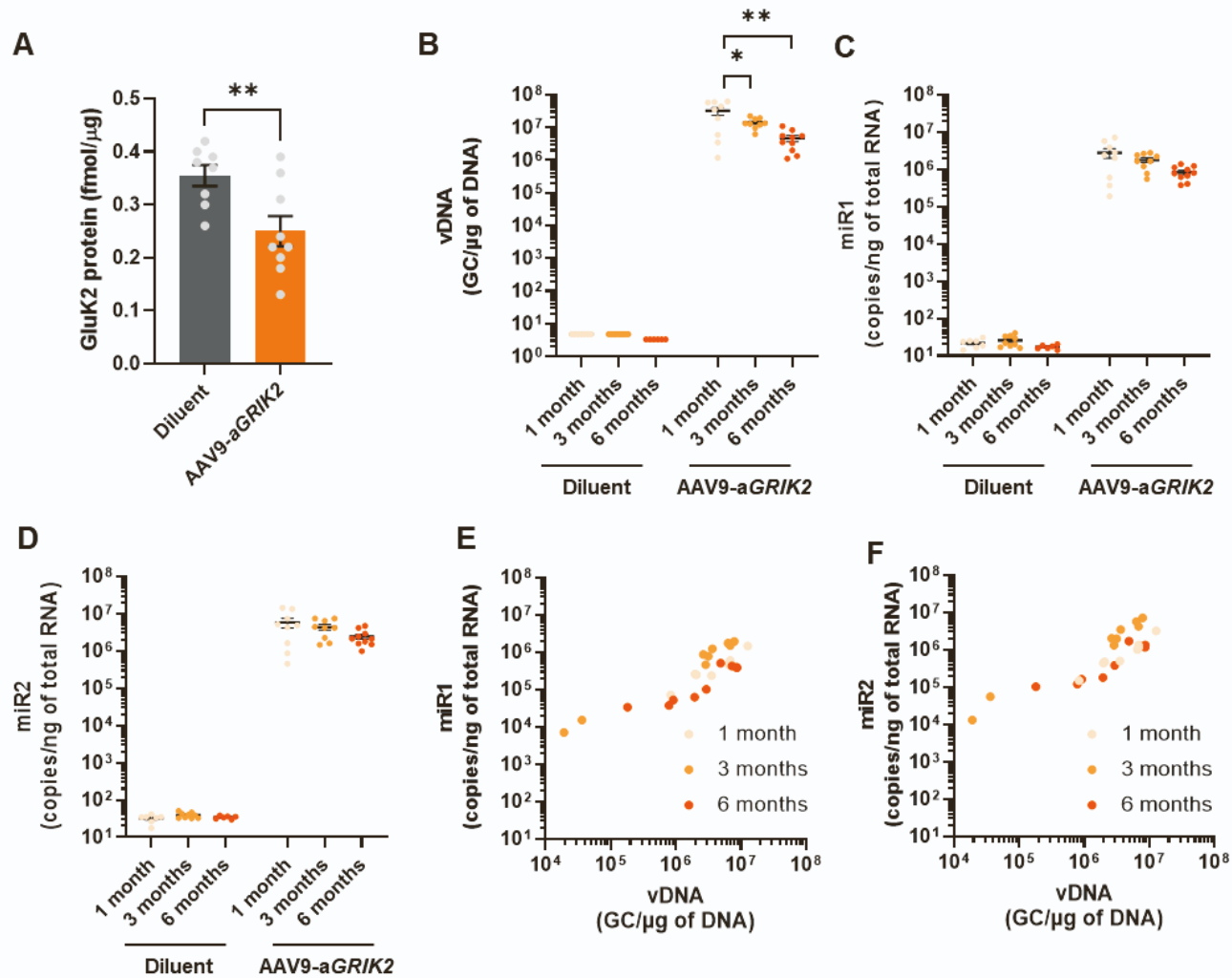
**Table S6:** Reporting of unscheduled death across studies investigating the effect of AAV-*aGRIK2* on electrographic seizures.

Group	Dose (gc/ Hippocampus)	Number of animals			
		Enrolled	Surviving	Unscheduled death	
				Post-AAV injection	Post electrode implantation
non-injected		7	7	n.a.	0
diluent		24	13	n.a.	11
AAV9 control	5.00E+09	22	16	2	6
AAV- <i>aGRIK2</i>	5.00E+07	12	8	0	4
AAV- <i>aGRIK2</i>	5.00E+08	36	22	2	12
AAV- <i>aGRIK2</i>	5.00E+09	46	35	3	8
AAV- <i>aGRIK2</i>	5.00E+10	14	9	0	5
% survival of AAV injected mice			69.23077	94.61538	73.07692
Total % survival			68.32298	95.65217	71.42857

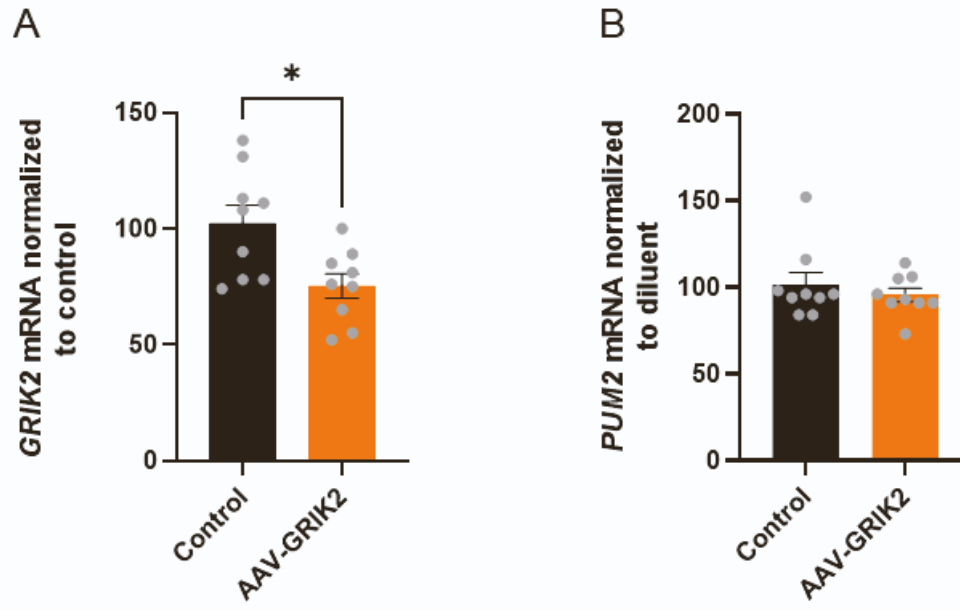




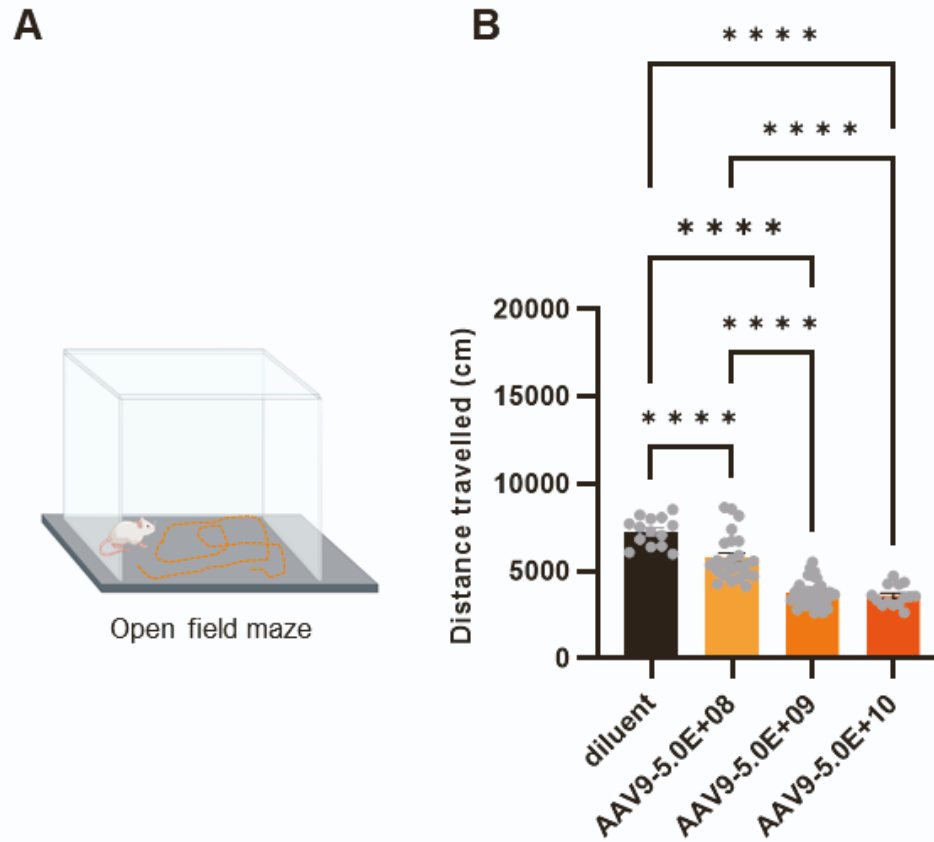
**Fig. S1.** Incorporation of a DNA stuffer to increase expected genome length limits genome packaging to a single copy of the intended genome length. Alkaline gel analysis demonstrating the length of packaged Gen1.1, Gen1.3, and Gen2.1 vector genomes.



**Fig. S2.** (A) GluK2 protein expression at 6 months after administration of AAV9-aGRIK2 or diluent in non-epileptic mice. (B) Quantification of vDNA, (C) miR1 and (D) miR2 in the hippocampus of epileptic mice administered with AAV9-aGRIK2 or the diluent. (E) Correlation between vector copies and miR1 or (F) miR2 expression in pilocarpine treated mice up to six months after administration of AAV9-aGRIK2. (A) \*\*  $P < 0.01$ , unpaired t-test. (B to F) \*  $P < 0.05$  and \*\*  $P < 0.01$ , Kruskal Wallis test followed by Dunn's multiple comparison. Results are presented as mean  $\pm$  SEM.



**Fig. S3.** AAV9-a*GRIK2* administration in epileptic mice reduces hippocampal expression of *GRIK2* mRNA but not of *PUM2* mRNA expression. (A) *GRIK2* mRNA expression levels. (B) *PUM2* mRNA expression levels. Results are presented as mean  $\pm$  SEM. \*  $P < 0.05$ , unpaired t-test.



**Fig. S4.** AAV9-*aGRIK2* treatment reduces the pathological hyperlocomotion in epileptic mice. (A) Schematic representation of the open field maze. (B) Distance traveled in the open-field 3 weeks after administration of AAV9-*aGRIK2* or diluent. Results are presented as mean  $\pm$  SEM. \*\*\*\*  $P < 0.0001$ , one-way ANOVA followed by Tukey's multiple comparisons test.