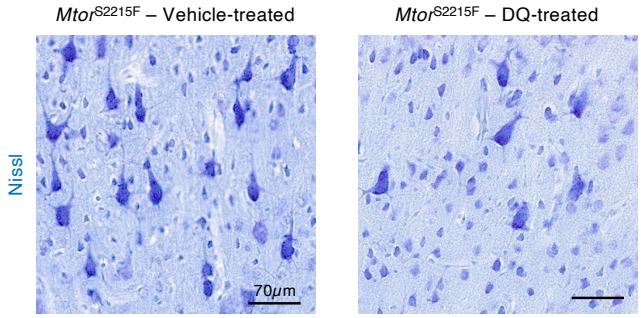
nature neuroscience

Article

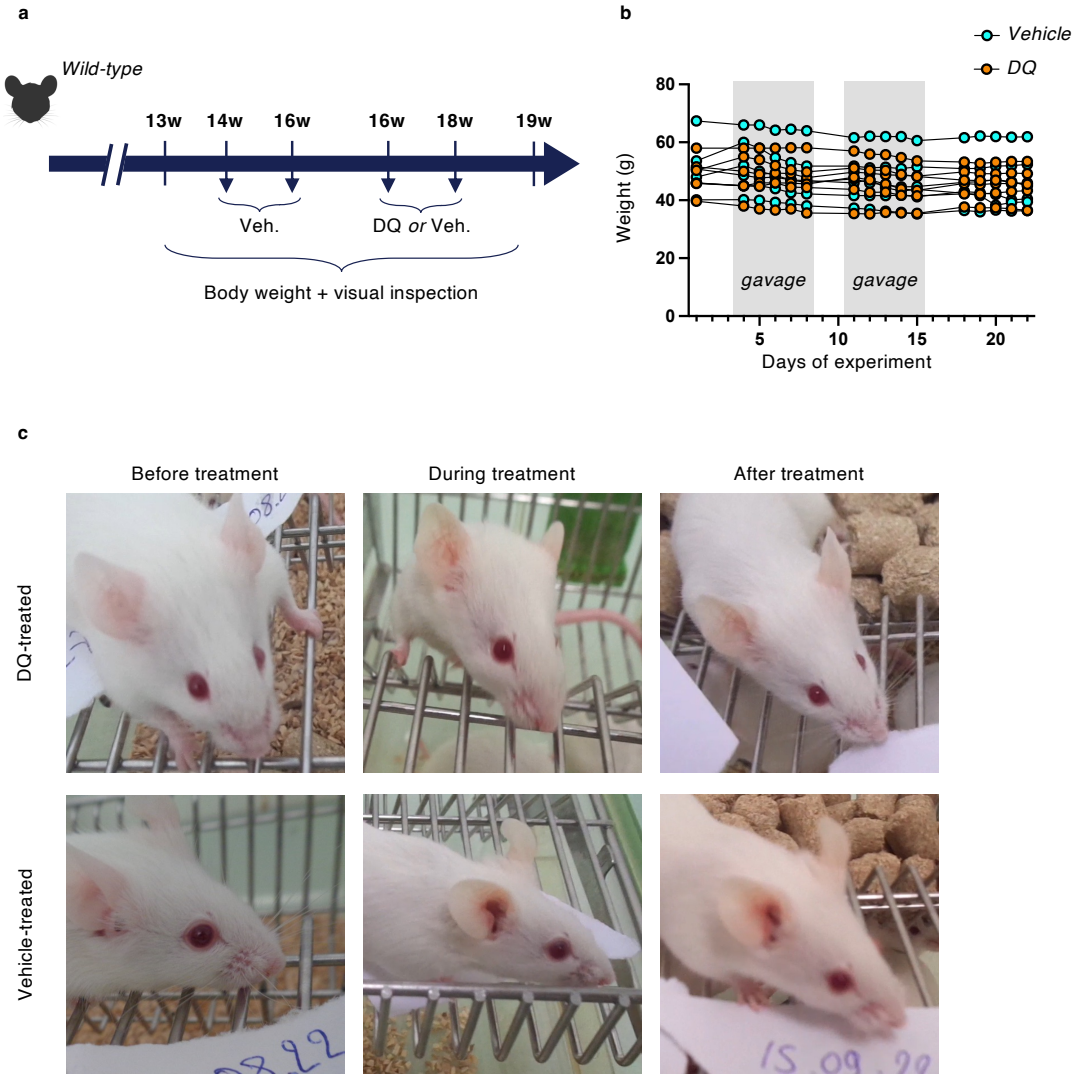
https://doi.org/10.1038/s41593-024-01634-2

Targeting pathological cells with senolytic drugs reduces seizures in neurodevelopmental mTOR-related epilepsy

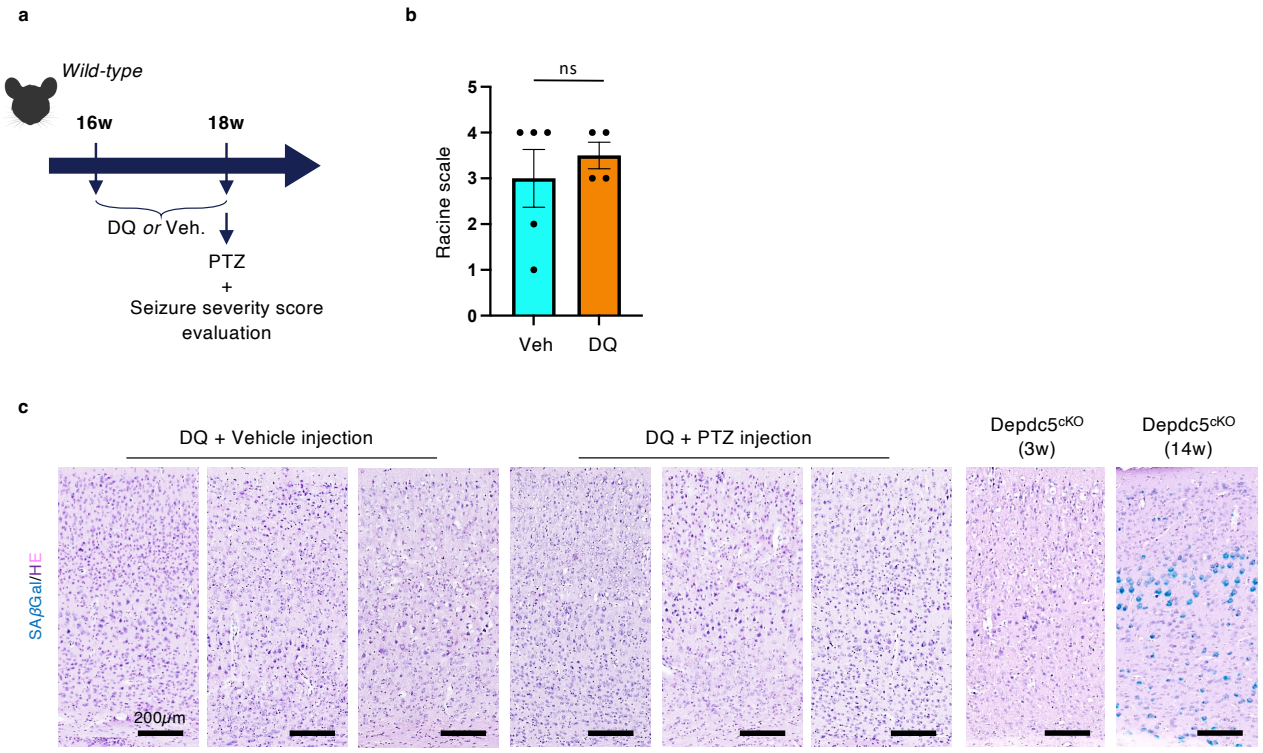
In the format provided by the authors and unedited



Supplementary Fig. 1. Histological Nissl coloration of cortical slices from DQ-treated and Vehicle-treated *Mtor*^{S2215F} mice. Coloration was repeated three times on n=3 animals.



Supplementary Fig. 2. Absence of major side-effects in DQ-treated wild-type animals. (a) Study design for evaluation of potential deleterious effects following DQ administration. **(b)** Evolution of mouse body weight following DQ or vehicle administration by gavage over the course of two weeks on n=6 animals receiving either DQ or vehicle. **(c)** Representative images of facial indicators to assess animal well-being according to the mouse grimace scale.



Supplementary Fig. 3. Absence of antiepileptic effects of DQ administration in a mouse model of pentylenetetrazol-induced seizures. (a) Study design for evaluation of potential antiepileptic effects following DQ administration. **(b)** Maximum seizure severity score reached based on Racine scale in n=5 animals who received vehicle and n=4 animals who received DQ (1: sudden behavioral arrest, 2: head nodding, 3: forelimb clonus with rearing, 4: generalized tonic-clonic seizure, 5: lethal seizure). Two-tailed Mann-Whitney test. ns: P=0.8413 **(c)** SAβGal colorimetric assay in pentylenetetrazol (PTZ)-induced seizures following vehicle or DQ administration. SAβGal colorimetric assay on a negative control (3 weeks-old Depdc5^{cKO}) and a positive control (14 weeks-old Depdc5^{cKO}). Scatter dot plots are presented as mean ± SEM. Colorimetric assay was repeated at least two times.

Supplementary Table 1. Detailed clinical features of the patient cohort

Patient ID	Neuropathology	Sex	Seizure frequency	Duration of epilepsy	Engel score	Anti-seizure medication	Used for experiments
Cases							
1 (FCD-64)	FCDIIb	M	Daily	2.5y	1	VPA, CBZ	IHC, SAβGal
2 (FCD-33)	FCDIIa	M	Weekly	5.6y	1	CBZ	IHC, SAβGal
3 (FCD-56)	FCDIIb	F	Daily+	15.4y	1	ZNS, TPM, OXC, LTG	IHC, SAβGal, LCM, EM
4 (FCD-57)	FCDIIb	M	Daily+	8.7y	3	LTG, OXC	EM
5 (FCD-59)	FCDIIb	M	Daily+	11.7y	2	CLB, OXC	IHC, SAβGal
6 (FCD-61)	FCDIIb	M	Weekly	6.5y	2	CBZ, STP, VGB	IHC, SAβGal
7 (FCD-70)	FCDIIb	M	Daily	13.6y	2	CLB, CBZ, TPM, FBM	EM
8	FCDIIb	M	Daily	N/A	1	LEV, VPA, CBZ, CLB	IHC, MEA
9	FCDIIb	M	Daily	28y	1	CBZ, LCS, CLB	MEA
10 (FCD-36)	HME/IIa	F	Daily+	2.4m	3	CBZ, LEV, VGB	IHC, SAβGal
11 (HME-73)	HME/IIa	F	Daily+	2.4m	1	CBZ, ZNS, LKZ, LEV	EM
12 (HME-74)	HME/IIa	M	Daily+	1.3y	1	LCS, VPA	SAβGal, EM
13 (HME-77)	HME/IIa	M	Daily	6m	1	TPM, VGB, CLB	IHC, SAβGal, LCM
14 (HME-79)	HME/IIb	M	Daily+	4.8m	1	VPA, TPM, CBZ, CLB	IHC, SAβGal
21	FCDIIa	M	Daily	7y	1	CBZ, LEV	IHC, SAβGal
22	FCDIIb	F	Daily	4y	2	LCS, OXC	IHC, SAβGal
23	FCDIIb	M	Weekly	6.6y	1	LCS, OXC, ZNS	IHC, SAβGal
24	FCDIIb	F	Daily	3.7y	1	CBZ, LCS	IHC, SAβGal
25	FCDIIb	M	Daily	6.3y	1	CBZ	IHC, SAβGal
26	FCDIIb	M	Daily	4y	3	TPM, RIS	IHC, SAβGal
27 (FCD-65)	FCDIIb	M	Daily	2.8y	1	LTG, VPA	IHC, SAβGal
28	FCDIIb	F	Daily	5y	1	LEV, VGB	IHC, SAβGal
29	FCDIIa	F	Daily	3.4y	1	OXC, TPM, VGB	IHC, SAβGal
30	FCDIIb	F	Daily	11y	1	CBZ, TPM	IHC, SAβGal
Epileptic controls							
15 (FCD-16)	FCDI	F	Daily+	2y	2	OXC, LEV	IHC, SAβGal
16 (FCD-13)	mMCD	M	Weekly	8.8y	3	VPA	IHC, SAβGal

17 (FCD-18)	FCDI	F	Daily	3.4y	1	OXC, LTG, CLB	IHC, SAβGal
18 (FCD-7)	mMCD	F	Daily+	5.3y	2	CLB, CBZ	IHC, SAβGal
19 (FCD-12)	FCDI	M	Weekly	3.5y	3	LCS, OXC	IHC, SAβGal
20 (FCD-6)	mMCD	F	Daily+	1.7y	3	VPA, LEV, VGB	IHC, SAβGal
31 (FCD-17)	FCDI	F	Daily	2m	1	CZP, VPA	IHC, SAβGal
32 (FCD-5)	mMCD	F	Daily	3y	1	CLB, LCS, OXC	IHC, SAβGal
33 (FCD-8)	mMCD	F	Daily	9.7y	2	CBZ	IHC, SAβGal
34 (FCD-15)	mMCD	M	Daily	6.3y	1	OXC, VPA	IHC, SAβGal
35	mMCD	F	Daily	3.1y	3	LEV, VGB	IHC, AβGal
36	mMCD	M	Daily	6y	1	CBZ, STP	IHC, SAβGal
37 (FCD-10)	FCDI	M	Daily	5.1y	1	LCS, OXC	IHC, SAβGal

Abbreviations: HME: hemimegalencephaly; mMCD: mild malformation of cortical development with excessive heterotopic neurons; m: months; y: years; duration of epilepsy: duration between seizure onset and surgery; Daily+: multiple per day. Seizure frequency is based on long-term EEG recordings. Anti-seizure medication abbreviations: CBZ: carbamazepine; CLB: clobazam; CZP: clonazepam; FBM: felbamate; LEV: levetiracetam; LCS: lacosamide; LKZ: licozam; LTG: lamotrigine; OXC: oxcarbazepine; RIS: risperidone; STP: stiripentol; VGB: vigabatrin; VPA: valproic acid; TPM: topiramate; ZNS: zonisamide. IDs in parentheses refer to patients previously reported in Baldassari et al [11]. Column 'Used for exp' indicates for which type of experiment samples were used; ICC: immunohistochemistry, SAβGal: SaβGal colorimetric assay; EM: electron microscopy; LCM: laser-capture microdissection followed by ddPCR; MEA: multielectrode array.

Supplementary Table 2. Descriptive statistics of the patient cohort

	Age at surgery	Age at seizure onset	Duration of epilepsy	Seizure frequency
Cases	8.45y (9.22y)	1.99y (3.22y)	6.45y (6.35y)	2.17 (0.64)
Controls	7.67y (4.78y)	3.22y (4.11y)	4.47y (2.75y)	2.08 (0.64)
P value	P=0.7364	P=0.3586	P=0.2042	P=0.6871

Descriptive statistics of four clinical parameters in the patient (n = 37) cohort. Data are presented as mean and SD between parentheses. For seizure frequency, a score of 1 was attributed for 'Weekly', a score of 2 for 'Daily' and a score of 3 for 'Daily+'. No statistical difference was observed between cases and controls by two-tailed unpaired t-test with Welch's correction.

Supplementary Table 3. List of differentially produced cytokines in *Depdc5*^{cKO} mice

Analytes	Canonical SASP	mTOR dependent	Diff. produced cKO vs. WT	
			5 weeks	10 weeks
IL1b	**X**	**X**	P=0.3939 ns	**P=0.0022 ****
IL6	**X**	**X**	P=0.2576 ns	**P=0.0303 ***
CXCL1	**X**	**X**	P=0.5887 ns	**P=0.0022 ****
CXCL2	**X**	**X**	P=0.1320 ns	**P=0.0043 ****
CXCL10	**X**	**X**	**P=0.0260 ***	**P=0.0022 ****
CCL2	**X**	**X**	P=0.6991 ns	**P=0.0022 ****
CCL20	**X**	**X**		**P=0.0195 ***
CCL3	**X**		P=0.3095 ns	**P=0.0022 ****
IL16			P>0.9999 ns	**P=0.0260 ***
IL17A			P=0.4848 ns	P=0.4156 ns
IL17C			P=0.2403 ns	**P=0.0368 ***
IL21			P=0.0568 ns	**P=0.0173 ***
IL22			P=0.3723 ns	**P=0.0152 ***
IL33				
TNFa				**P=0.0238 ***

The following analytes (n =14/29) were not detected in electrochemiluminescent assay: IL15, IFNg, IL2, IL4, IL5, IL9, IL10, IL12, IL17E, IL17F, IL23, IL27, IL30, IL31. Statistics: two-tailed Mann-Whitney test.

Supplementary Table 4. List of differentially produced cytokines in Mtor^{S2215F} mice

Analytes	Canonical SASP	mTOR dependent	Diff. produced mTOR^{S225F} vs. GFP
IL1b	**X**	**X**	**P=0.0022 ****
IL6	**X**	**X**	**P=0.0173 ***
CXCL1	**X**	**X**	**P=0.0022 ****
CXCL2	**X**	**X**	**P=0.0022 ****
CXCL10	**X**	**X**	**P= 0.0022 ****
CCL2	**X**	**X**	**P=0.0022 ****
CCL3	**X**		**P=0.0260 ***
IL16			**P=0.0022 ****
IL17A			P=0.9372 ns
IL17C			P=0.6688 ns
IL33			P=0.0649 ns
TNFa			**P=0.0022 ****

The following analytes (n = 17/29) were not detected in electrochemiluminescent assay: CCL20, IL15, IFNg, IL2, IL4, IL5, IL9, IL10, IL12, IL17E, IL17F, IL21, IL22, IL23, IL27, IL30, IL31. Statistics: two-tailed Mann-Whitney test.