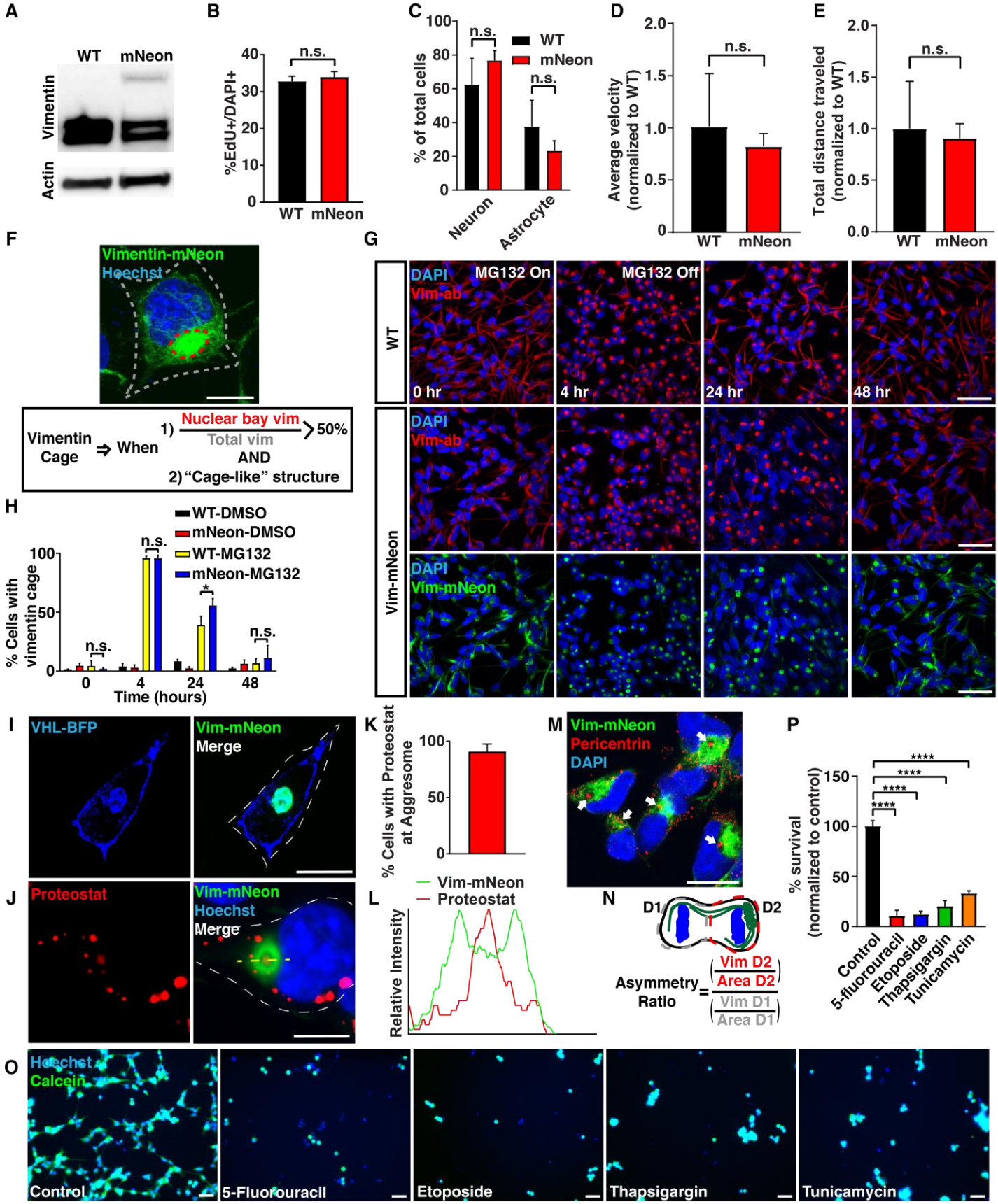

Figure S1. Vimentin cages the aggresome in response to disruptions in proteostasis, Related to Figure 1. A) Protein was extracted from WT and vimentin-mNeon NSCs and run on a western blot probing for vimentin and β-actin levels. B)

WT (black bar) and vimentin-mNeon (red bar) NSCs were pulsed with EdU for 1 hour and then fixed and analyzed for percent EdU+ cells. (n=3; Student's t-test; mean ± SD). C) WT or vimentin-mNeon NSCs were differentiated for 14 days by removing growth factors, and then quantified for generation of neurons (MAP2ab) or astrocytes (GFAP). (n=3; Student's t-test; mean ± SD). D-E) WT and vimentin mNeon-NSC timelapse images were analyzed for average velocity or total distance traveled to quantify cell motility. (N=3; Student's t-test; mean ± SD). F-H) WT and Vimentin-mNeon NSCs (green) were treated with 5 μM MG132 for 4 hours to induce a transient loss of proteostasis, allowed to recover for up to 48 hours, and stained with a vimentin antibody to analyze the percentage of vimentin cages. (N=3; Two-way ANOVA with post-hoc Tukey's test; mean ± SD). I) Vimentin-mNeon NSCs (green) were electroporated with a construct expressing the mutant tumor suppressor Von Hippel-Lindau (VHL; blue) and imaged 24 hours later. J-K) Vimentin-mNeon NSCs were stressed with 5 μM MG132 for 4 hours and then immunostained for vimentin (green), stained with Proteostat (red), and quantified for the percentage of cells that had Proteostat puncta present at or within the vimentin cage. L) Intensity histogram along the yellow dotted line in J depicting vimentin and Proteostat fluorescence. M) Vimentin-mNeon NSCs were stressed with 5 μM MG132 for 2 hours and then immunostained for pericentrin (centrosome; red). White arrows indicate centrosomes. N) Schematic depicting quantitation of asymmetry during mitosis. O-P) Images and quantification of survival (living cells labeled by Calcein AM; green) of WT NSCs after modification of drug treatment stress paradigms that did not induce vimentin cage formation in initial experiments (50 nM 5-fluorourucil for 48 hours, 100 nM etoposide for 48 hours, 1 μg/mL tunicamycin for 9 hours, and 1 μM thapsigargin for 9 hours). (n=3; Two-way ANOVA with post-hoc Tukey's test; mean ± SD). Scale bars, 10 μm (I, J, M), 50 μm (G, O). Nuclei were labeled with DAPI or Hoechst (blue). White line denotes edge of the cell. *$p<0.05$, ****$p<0.0001$.

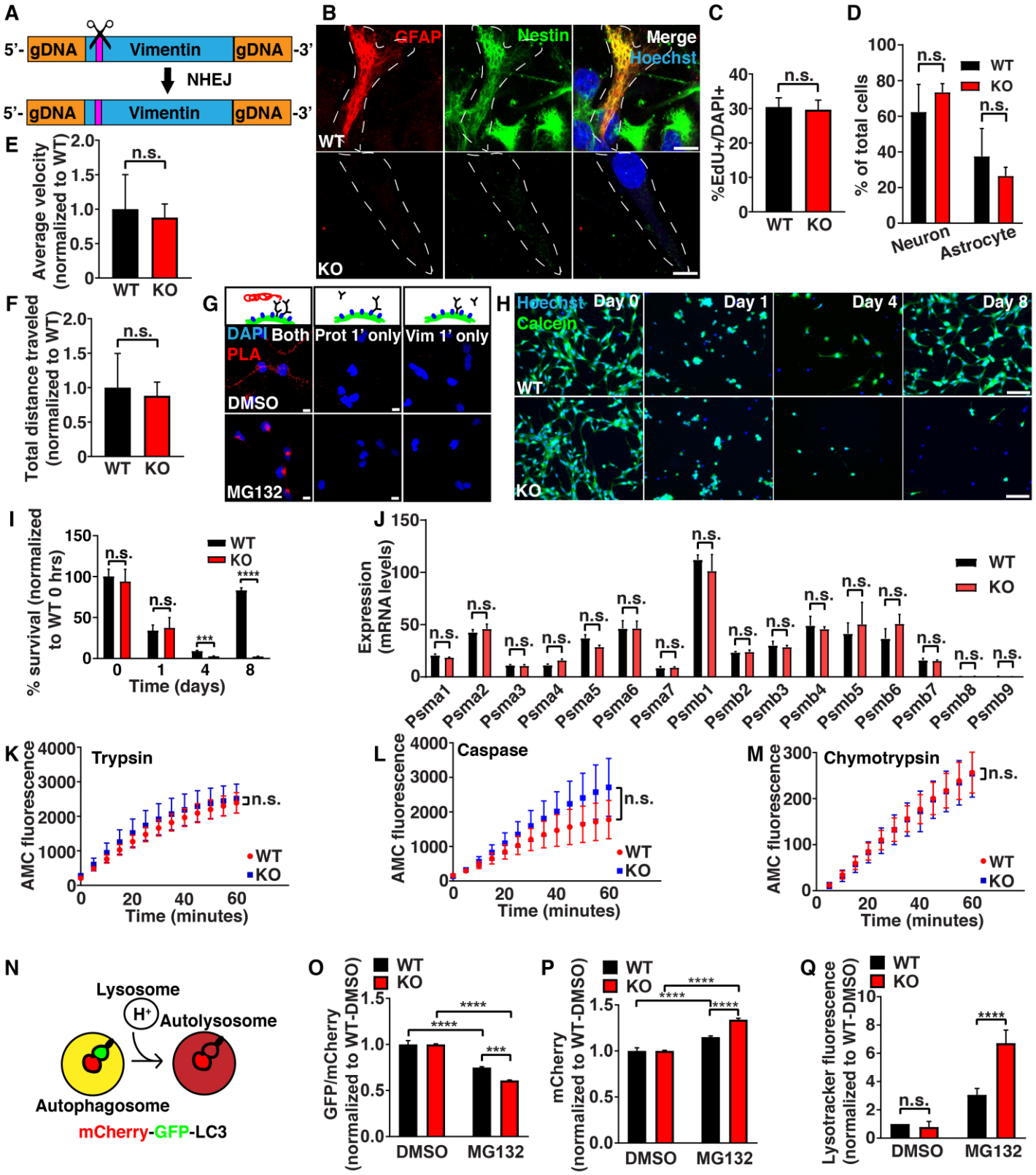

Figure S2. Vimentin KO disrupts proteasome localization to the aggresome and decreases resilience to proteostasis disruption, Related to Figure 2. A) Schematic of CRISPR/Cas9-based vimentin KO NSC generation strategy. B) WT and vimentin KO NSCs were immunostained for GFAP (red) and nestin (green). C) WT (black bar) and vimentin KO (red bar) NSCs were pulsed with EdU for 1 hour and then fixed and analyzed for percent EdU+ cells. (N=3; Student's t-test; mean ±

SD). D) WT or vimentin KO NSCs were differentiated for 14 days and then quantified for generation of neurons (MAP2ab) or astrocytes (GFAP). (n=3; Student's t-test; mean ± SD). E-F) WT and vimentin mNeon-NSCs live-cell imaging timelapses were analyzed for average velocity or total distance traveled to quantify cell motility. (N=3; Student's t-test; mean ± SD). G) (Controls for Fig. 2G) WT NSCs were treated with 0.05% DMSO or 5 μM MG132 for 4 hours and then fixed and probed with a proximity ligation assay (red) against vimentin and the α5 subunit of the proteasome, with negative controls shown here using probes for only one antibody. H-I) WT (black bar) and vimentin KO (red bar) NSCs were treated transiently with 5 μM MG132 for 8 hours and then were monitored for cell survival over the course of the following week (normalized to day 0). Living cells are labeled by calcein (green). (N=3; Two-way ANOVA with post-hoc Tukey's test; mean ± SD). J) Proteasome subunit mRNA levels from total RNA sequencing data generated from WT and vimentin KO NSCs (n=3; Two-way ANOVA with post-hoc Tukey's test; mean ± SD). K-M) Proteasome activity assays performed on protein lysates generated from WT (red) or vimentin KO (blue) NSCs measuring caspase-like, trypsin-like and chymotrypsin-like proteasome activity (N=3; Student's t-test; mean ± SD). N-P) WT (black bar) and vimentin KO (red bar) NSCs were lentivirally transduced with mCherry-GFP-LC3. 7 days after viral transduction both cell types were treated either with 0.02% DMSO or 2 μM MG132 for 24 hours and then were allowed to recover for 1 hour without drug before analyzing mCherry and GFP fluorescence. (N=3; Two-way ANOVA with post-hoc Tukey's test; mean ± SD). Q) WT (black bar) and vimentin KO (red bar) NSCs were treated with 0.05% DMSO or 5 μM MG132 for 4 hours and then allowed to recover for 2 hours before staining with the dye Lysotracker (lysosomes; red) (N=3; Two-way ANOVA with post-hoc Tukey's test; mean ± SD). Nuclei were labeled with Hoechst (blue). Scale bars, 10 μm (B), 100 μm (H). White lines denote edge of the cell. ***$p<0.001$, ****$p<0.0001$.

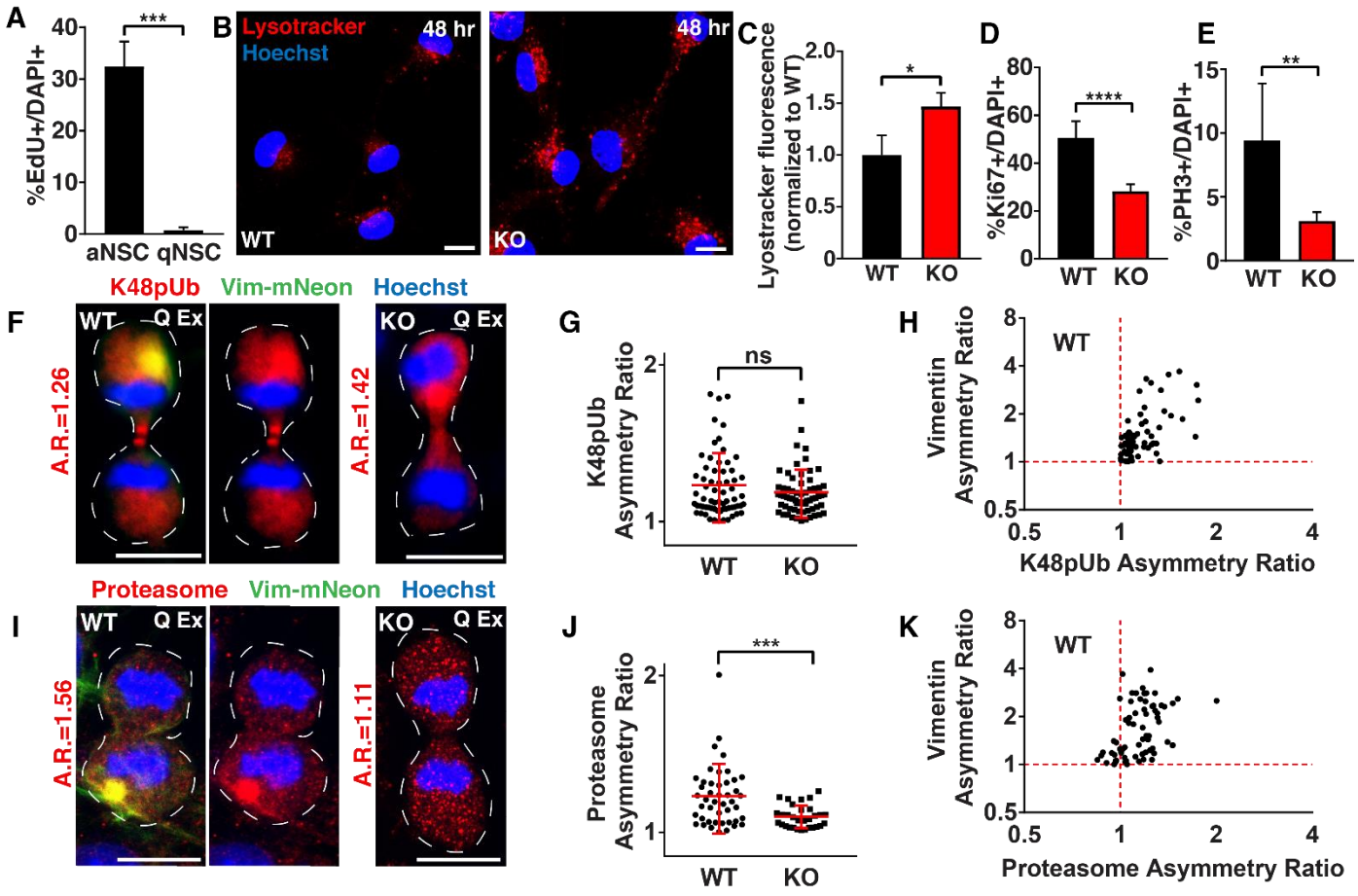

Figure S3. Vimentin KO NSCs have a reduced capacity to clear protein and exit quiescence, and fail to asymmetrically segregate proteasomes during mitosis, Related to Figure 3. A) aNSCs or qNSCs were pulsed with EdU for 1 hour and then fixed and analyzed for percent EdU+ cells. (n=3; Student's t-test; mean ± SD). B-C) WT or vimentin KO NSCs exiting quiescence for 48 hours were stained with the dye Lysotracker (lysosomes; red). (N=3; Student's t-test; mean ± SD). D-E) WT and vimentin KO NSCs exiting quiescence for 48 hours were fixed and immunostained for Ki67 (cells not in G_0), and phosphohistone H3 (PH3; late G2/M phase). (n=3; Student's t-test; mean ± SD). F-N) Vimentin-mNeon (WT; green) NSCs and vimentin KO NSCs exiting quiescence were immunostained for either F-H) K48pUb or I-K) proteasome. Asymmetry ratio (A.R.) of proteasome or K48pUb is indicated beside each image. (n≥39; Mann-Whitney test; mean ± SD). Nuclei are labeled by Hoechst. Scale bars, 10 μm. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

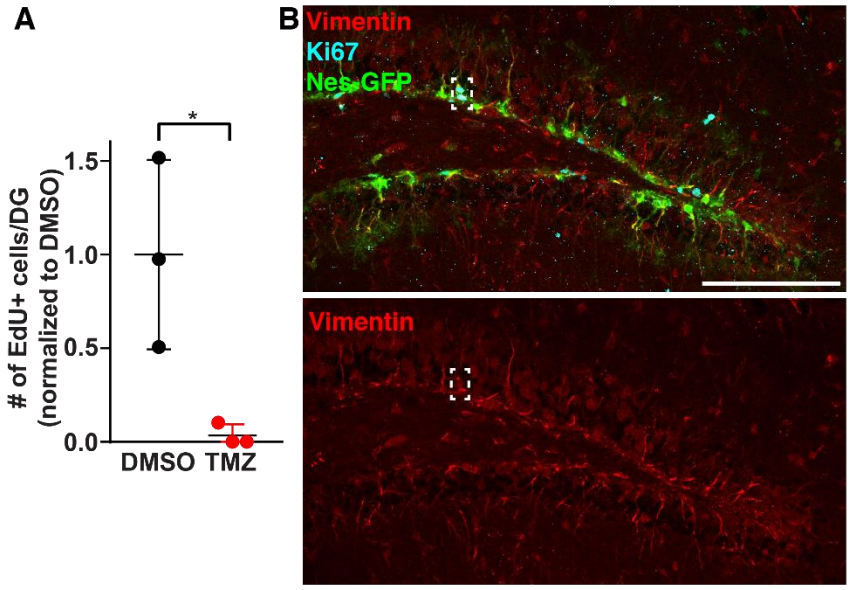

Figure S4. Validation of temozolomide assay and vimentin immunostaining in the brain, Related to Figure 4. A) WT mice were injected i.p. with DMSO (black bar) or 25 mg/kg TMZ (red bar) once per day for 3 consecutive days. They were allowed to recover for 2 hours before being injected with 50 mg/kg EdU once, and then were sacrificed 3 hours following the EdU injection and analyzed for proliferative (EdU+) NSCs. (n=3, Student's t-test; mean ± SD). B) 7 week old Nestin-GFP mouse brain sections were immunostained for GFP (NSCs; green), vimentin (red) and Ki67 (cyan). Scale bar, 100 μm. *p<0.05.

Supplementary Tables

Table S1. Intermediate filament expression in WT and vimentin KO NSCs. Related to Figure 2.

Gene	Name	WT Average	WT Stan. Dev.	KO Average	KO Stan. Dev.	Significant
Nes	Nestin	23.57	6.96	19.14	6.09	no
Gfap	Glial fibrillary acidic protein	42.82	0.92	14.91	1.19	yes
Vim	Vimentin	76.99	0.95	239.17	29.65	yes
Des	Desmin	0.09	0.07	0.07	0.05	no
Nefl	Neurofilament-L	0.00	0.00	0.02	0.02	no
Nefm	Neurofilament-M	0.03	0.02	0.03	0.02	no
Nefh	Neurofilament-H	0.09	0.05	0.04	0.03	no
Lmna	Lamin A	2.66	0.62	2.17	0.17	no
Lmnb1	Lamin B1	33.84	6.60	32.21	2.59	no
Lmnb2	Lamin B2	5.80	0.27	7.22	0.97	no
Synm	Synemin	0.49	0.14	0.42	0.06	no
Ina	Internexin	0.93	0.09	0.70	0.09	no
Bfsp1	Filensin	0.01	0.01	0.02	0.01	no
Bfsp2	Phakinin	3.25	0.20	1.37	0.45	no
Sync	Syncoilin	0.36	0.19	0.25	0.04	no
Prph	Peripherin	0.12	0.09	0.04	0.03	no
Krt1	Keratin 1	0.02	0.02	0.07	0.04	no
Krt10	Keratin 10	1.31	0.25	1.16	0.13	no
Krt13	Keratin 13	0.00	0.00	0.00	0.00	no
Krt17	Keratin 17	0.00	0.00	0.03	0.02	no
Krt18	Keratin 18	0.02	0.02	0.00	0.00	no
Krt2	Keratin 2	0.00	0.00	0.01	0.01	no
Krt20	Keratin 20	0.02	0.03	0.00	0.00	no
Krt222	Keratin 222	0.22	0.14	0.13	0.04	no
Krt24	Keratin 24	0.00	0.00	0.02	0.02	no
Krt25	Keratin 25	0.00	0.00	0.00	0.00	no
Krt26	Keratin 26	0.00	0.00	0.01	0.02	no
Krt28	Keratin 28	0.40	0.14	0.18	0.01	no
Krt33b	Keratin 33B	0.00	0.00	0.00	0.00	no
Krt34	Keratin 34	0.00	0.00	0.00	0.00	no
Krt35	Keratin 35	0.00	0.00	0.00	0.00	no
Krt40	Keratin 40	0.01	0.01	0.00	0.00	no
Krt7	Keratin 7	0.00	0.00	0.00	0.00	no
Krt73	Keratin 73	0.00	0.00	0.05	0.02	no
Krt78	Keratin 78	0.00	0.00	0.01	0.01	no
Krt8	Keratin 8	0.02	0.03	0.00	0.00	no
Krt80	Keratin 80	0.01	0.01	0.02	0.02	no
Krt82	Keratin 82	0.00	0.00	0.03	0.04	no
Krt83	Keratin 83	0.00	0.00	0.01	0.02	no
Krt84	Keratin 84	0.01	0.01	0.00	0.00	no

Krt86	Keratin 86	0.00	0.01	0.00	0.00	no
Krt87	Keratin 87	0.05	0.07	0.00	0.00	no
Krt80	Keratin 80	0.01	0.01	0.02	0.02	no
Krt82	Keratin 82	0.00	0.00	0.03	0.04	no
Krt83	Keratin 83	0.00	0.00	0.01	0.02	no
Krt84	Keratin 84	0.01	0.01	0.00	0.00	no
Krt86	Keratin 86	0.00	0.01	0.00	0.00	no
Krt87	Keratin 87	0.05	0.07	0.00	0.00	no

Table S2. Proteins co-precipitated by vimentin-mNeon in either a DMSO or MG132 treated condition, followed by LC-MS/MS (attached as excel file). Related to Figure 2.

Table S3. Total RNA sequencing data from untreated WT and vimentin KO NSCs (attached as excel file). Related to Figure 2.