nature cell biology

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a

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GAUSSIAN SUMMARY:
Mean Diameter   = 5739.3 nm            Chi Squared        = 115.733
Stnd. Deviation = 6159.2 nm (107.3 %)  Baseline Adj.      = 0.220 %
Coeff. of Var'n = 1.073                Mean Diff. Coeff. = 8.10E-010 cm2/s
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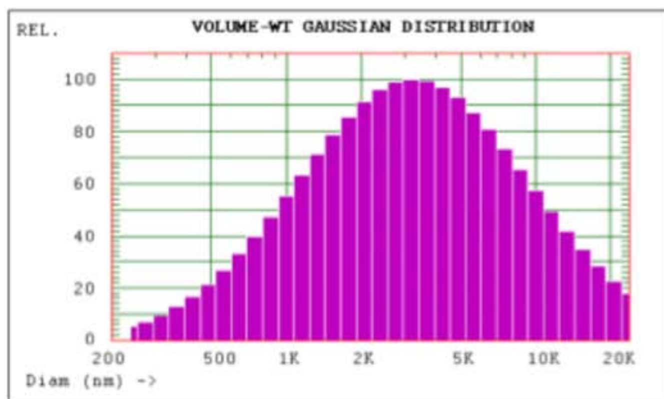

LULIP.1

Cumulative Result:

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25 % of distribution < 1454.4 nm
50 % of distribution < 2930.8 nm
75 % of distribution < 5838.1 nm
90 % of distribution < 10421.6 nm
99 % of distribution < 20507.5 nm
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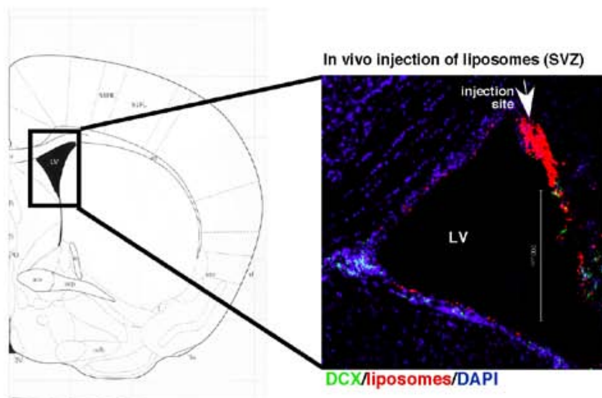
b



c

In vivo uptake of PtdSer liposomes (SGZ)

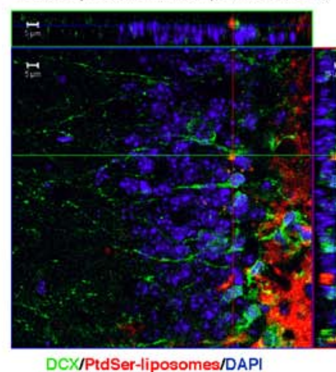


Figure S1 Physical properties of the liposomes and their intracranial injection. **(a)** Gaussian volume distribution data for the liposome preparation was obtained using dynamic laser light scattering performed with a Nicomp 370 instrument (Accucomp, Santa Barbara, CA). The median size of the particle was 5.7 μm with > 75% of the particles being over 1.5 μm in diameter. Briefly, the methodology used was as follows: an aliquot of liposomes was dispersed in normal saline and placed in a round cuvette and the measurement performed at room temperature. Particle counting using Coulter principle was performed on a Multisizer 3 instrument (Beckman-Coulter, Hialeah, FL). The instrument was calibrated with standard polystyrene latex beads and was operated in volumetric mode, with 0.5 ml sample taken through a 50 μm orifice. Volume distribution data is presented. **(b)** (left) Coronal image from the Mouse Brain Atlas indicating the location of the lateral ventricles and the injection site of liposomes. (right) The phagocytic activity of DCX cells near the injection site was examined in the area within approximately 200 μm (as indicated). **(c)** Wild type mice intracranially injected with fluorescently labeled phosphatydilserine (PtdSer) liposomes were examined immunohistochemically in the subgranular zone (SGZ) for DCX^+ cells with phagocytosed liposomes. Representative confocal micrograph is shown. Data from one representative experiment out of 3 independent experiments is shown (n > 10 fields analyzed in each experiment; n = 4 mice/group). Scale bar = 5 μm.

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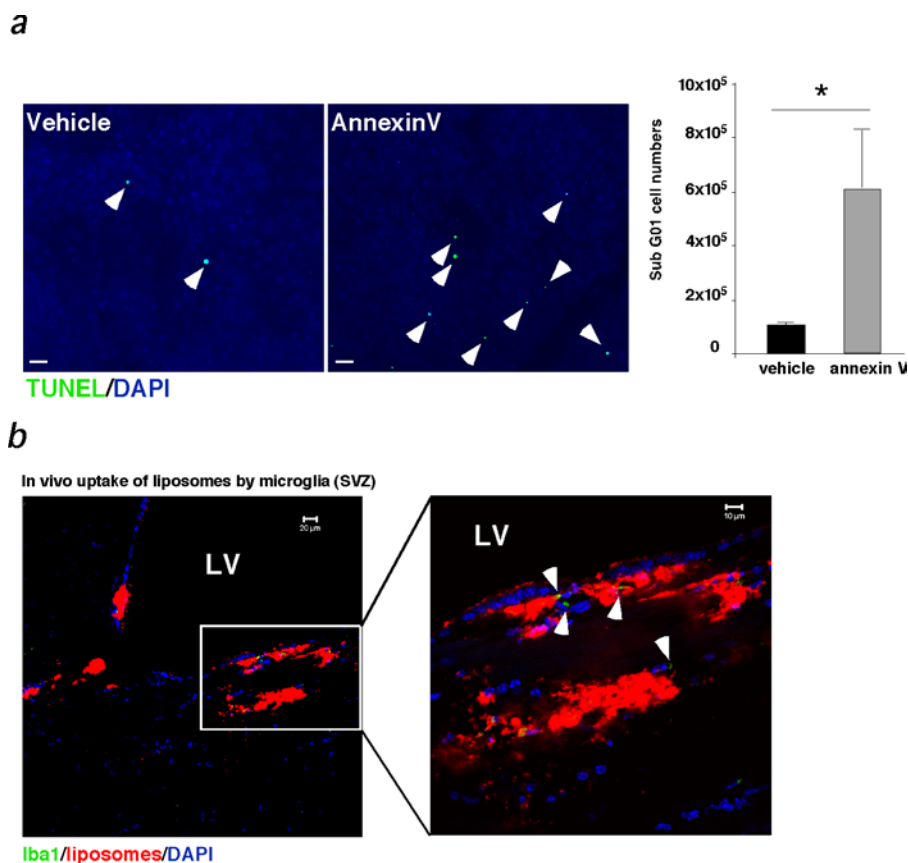

Figure S2 Accumulation of apoptotic debris in the thymus and microglia activity in the brain in annexinV-treated mice. **(a)** B6 mice (aged 5weeks) were given two i.v. injections of 20ug annexin V or vehicle at t=0 and t=3h. At t=6h, mice were euthanized and thymus collected for either histology or prepared for analysis flow cytometry. The rationale was that normal thymus has a high turnover of immature thymocytes that undergo apoptosis and that blocking their uptake via annexin V (which masks PtdSer dependent engulfment) would lead to increased number of uncleared corpses. Representative fluorescent images of accumulated apoptotic nuclei via TUNEL staining (indicated by arrowheads) in the thymus after short-term annexin V (or vehicle) treatment. Scale bar = 20 μm. Bar graph represents a quantification of apoptotic (sub G0) thymocytes as analyzed by flow cytometry (average ± s.e.m.). Student's t-test statistical analysis was performed from n = 3 mice in each group (*, $p < 0.05$). **(b)** Wild type mice intracranially injected with fluorescently labeled liposomes were examined immunohistochemically in the subventricular zone (SVZ) for Iba1^{+} cells. Only few Iba1+ cells with were determined along the ventricular walls (indicated by arrow heads). Representative confocal micrographs (of different magnifications) are shown. Representative experiment out of 3 independently performed (n > 20 fields analyzed in each experiment; n = 4 mice/group). Scale bar = 10 μm.

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Figure S3 Accumulation of apoptotic debris in SVZ in annexinV-treated mice. Accumulation of apoptotic nuclei (indicated by arrowheads) in the subventricular zone (SVZ) was determined via TUNEL staining after short-term annexin V treatment. Representative images are shown (n = 3 mice in each group with at least 8 slices analyzed for each mouse). Quantification of TUNEL positive cells per subventricular zone (average ± s.e.m.) is an extrapolation based on 12 brain slices from each mouse (approx. 200μm apart) with n = 4 mice/group. Scale bar = 50 μm.

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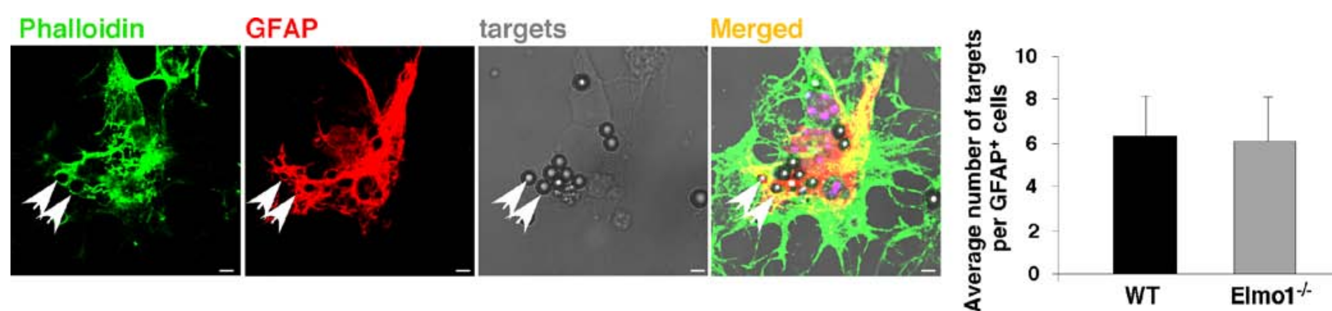

Figure S4 Elmo1 in astrocyte phagocytic activity. Representative images of astrocytes from wild type and *Elmo1*$^{-/-}$ mice engulfing simplified targets (negatively charged carboxyl-modified beads). Bar graphs represents quantification of total phagocytosed targets by astrocytes from wild type and *Elmo1*$^{-/-}$ mice (average ± s.e.m.). Scale bar = 3 μm. Representative experiment out of 3 independently performed (n > 10 fields analyzed in each experiment). Scale bar = 10 μm.

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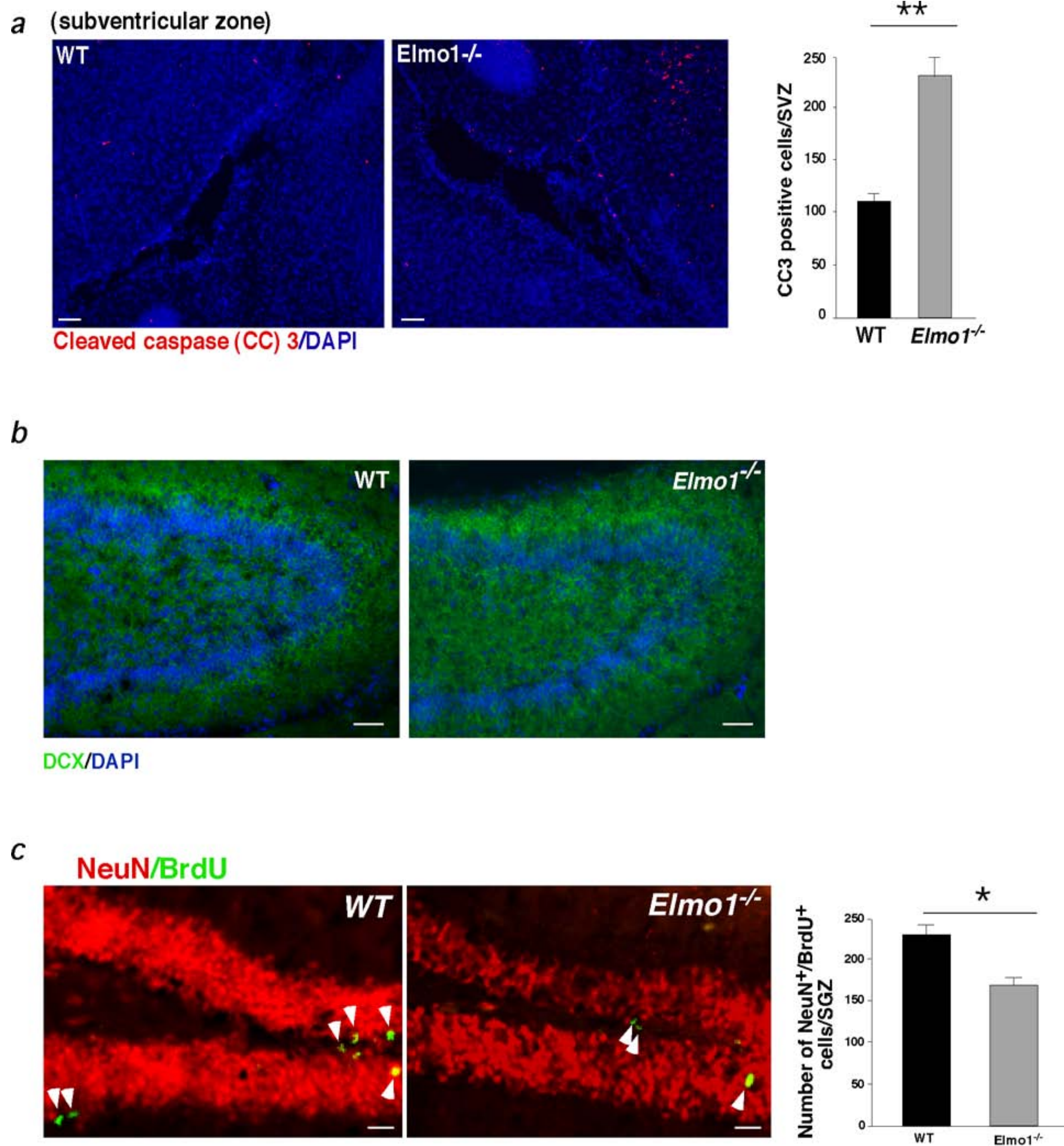

Figure S5 Roles of ELMO1 in neurogenesis. **(a)** Representative images of cleaved caspases 3 labeling in the SVZ from wild type and *Elmo1*$^{-/-}$ mice. Bar graph represents quantification of CC3 positive cells per subventricular zone, which is an extrapolation based on 12 brain slices from each mouse (approx. 200μm apart) with n = 4 mice/group (average ± s.e.m.). Scale bar = 50 μm. **(b)** No significant difference in general DCX fluorescence or in gross anatomy was evident between neonatal wild type and *Elmo1*$^{-/-}$ mice (n = 4 mice/group). Scale bar = 20 μm. **(c)** Representative images from *Elmo1*$^{-/-}$ mice labeled for NeuN (red) and BrdU (green) 30 days after BrdU injection. Bar graph represents quantification of NeuN$^+$BrdU$^+$ cells in SGZ of wild type and *Elmo1*$^{-/-}$ mice (average ± s.e.m.). Student's t-test statistical analysis was performed from n = 6 mice in each group with at least 10 slices analyzed for each mouse (*, $p < 0.05$). Scale bar = 20 μm.

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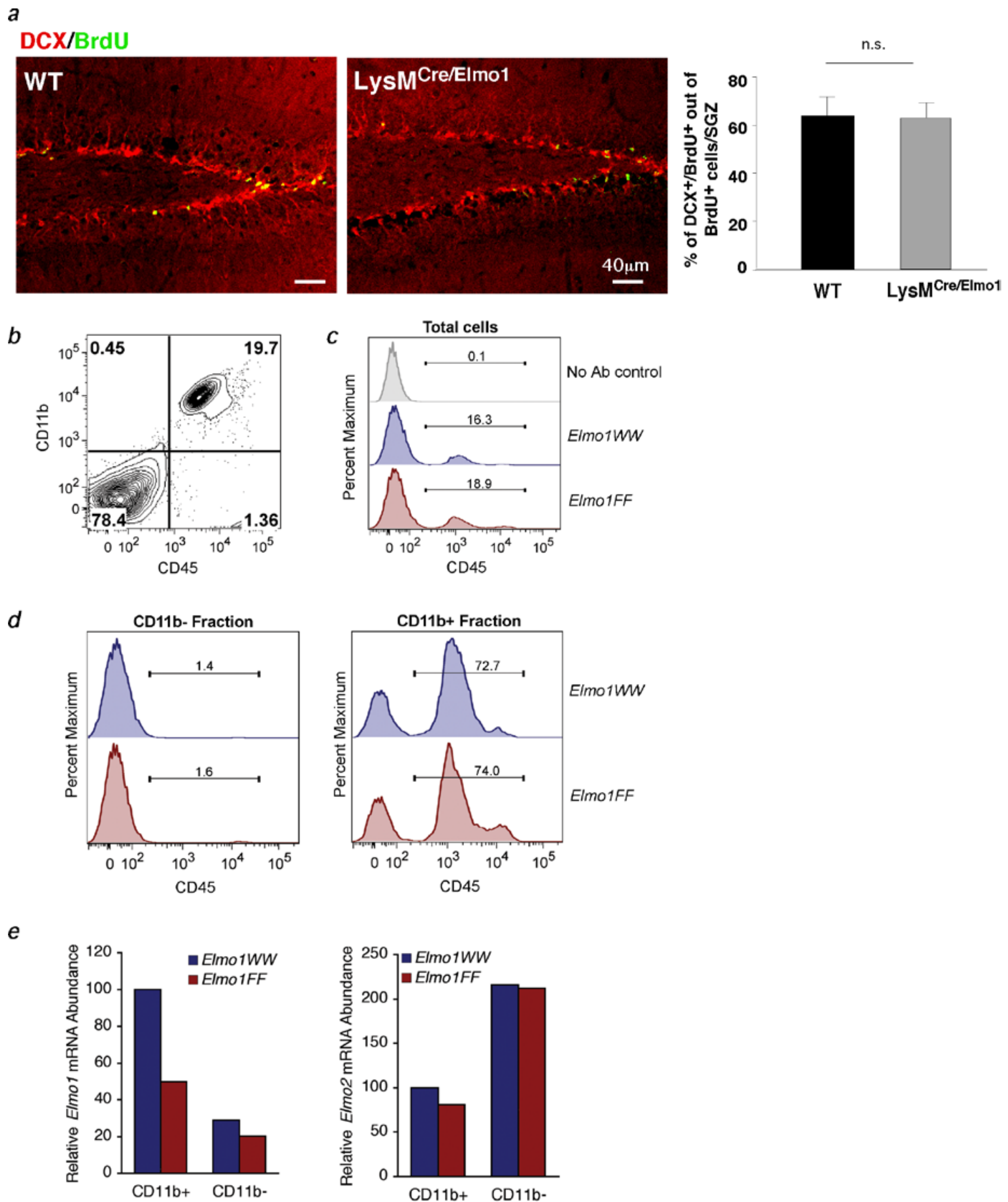

Figure S6 LysM$^{Cre/Elmo1}$ mice do not exhibit any apparent impairment in adult neurogenesis. **(a)** Representative confocal images from LysM$^{Cre/Elmo1}$ mice labeled for DCX (red) and BrdU (green) 7 days after BrdU injection. Bar graphs represent percentage of neuronal differentiation of all BrdU positive cells seven days after BrdU injection in wild type and LysM$^{Cre/Elmo1}$ mice (average ± s.e.m.). Representative experiment out of 3 independently performed (n = 5 mice/group). Scale bar = 10 µm. Scale bar = 40 µm. **(b-e)** Total cells were isolated from the brains of adult *LysM*-Cre+ mice carrying wild-type (*WW*) or floxed (*FF*) *Elmo1* alleles and enriched by anti-CD11b column separation. **(b)** Non-enriched (total) cells from wild-type mice were stained with antibodies to CD45 and CD11b and analyzed by flow cytometry. Note that >90% of CD45+ cells are also CD11b^{+}. (**c-d**) The enrichment of the populations obtained from the column separation was determined by anti-CD45 staining of the non-enriched (**c**), CD11b-depleted (**d,** left) and CD11b-enriched (**d**, right) fractions from *Elmo1WW* and *Elmo1FF* mice. Non-viable cells were excluded from the analysis by propidium iodide staining. (**e**) Relative mRNA levels of *Elmo1* (left) and *Elmo2* (right) in *Elmo1WW* and *Elmo1FF* mice were determined in the indicated cell fractions by quantitative PCR analysis. In **c-e**, total cells were pooled from two littermates per genotype prior to analysis and column enrichment.

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