Supplementary data for

Delivery of *LOXL1-AS1*-siRNAs using targeting peptide-engineered extracellular vesicles with focused ultrasound to suppress medulloblastoma metastasis

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This file includes additional tables and figures:

Supplementary Table S1. Information on peptides used in the study.

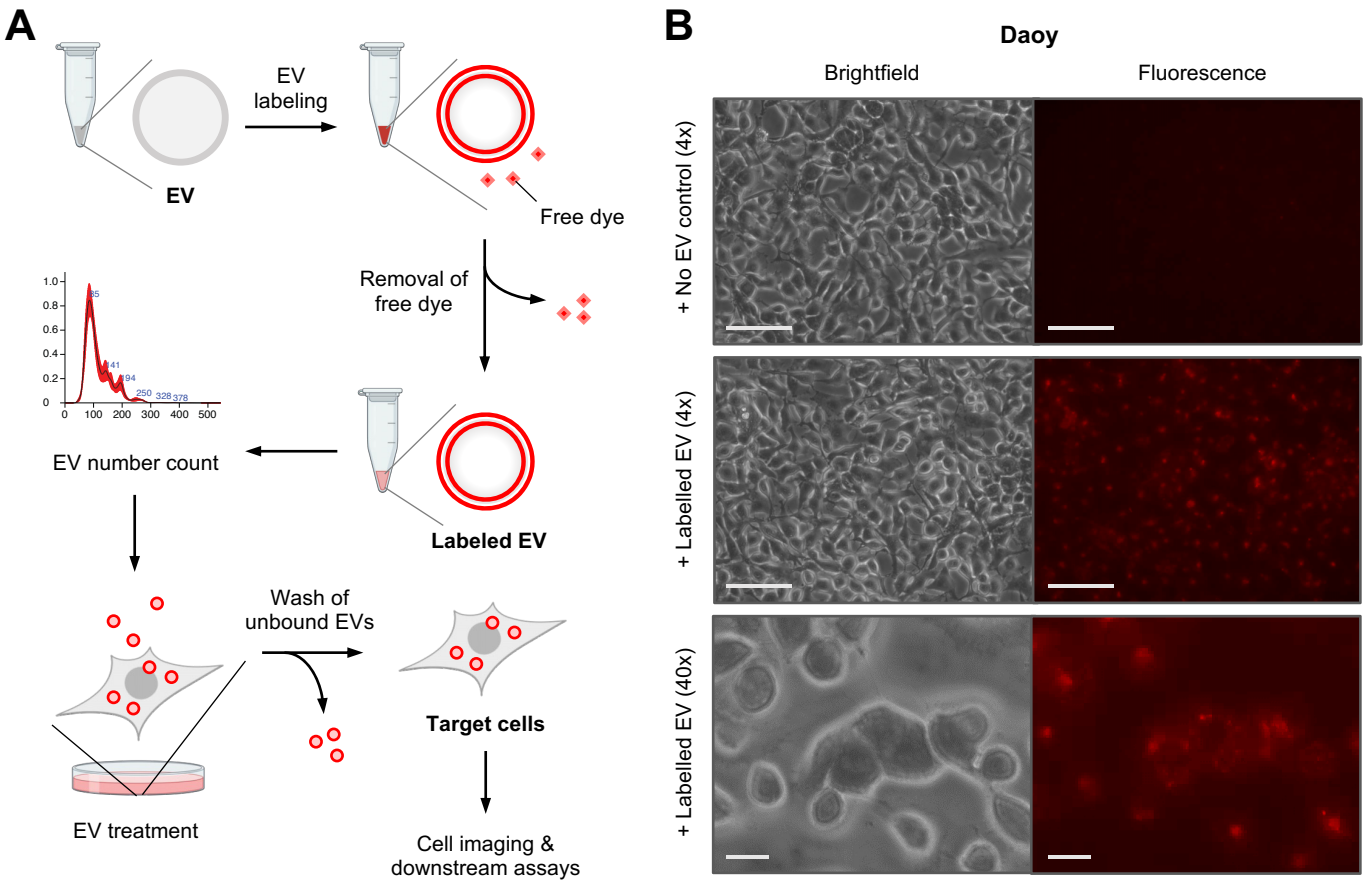
Name	Peptide sequence	Coding sequence (5’ → 3’)
E1-3 peptide	FSRPAFL	TTC AGC AGG CCC GCC TTC CTG
NC peptide	YDEEGGGE	TAC GAC GAG GAG GGC GGC GGC GAG
V5 epitope (V5-tag)	GKPIPNPLLGLDST	GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG

Supplementary Table S2. Information on siRNAs used in the study.

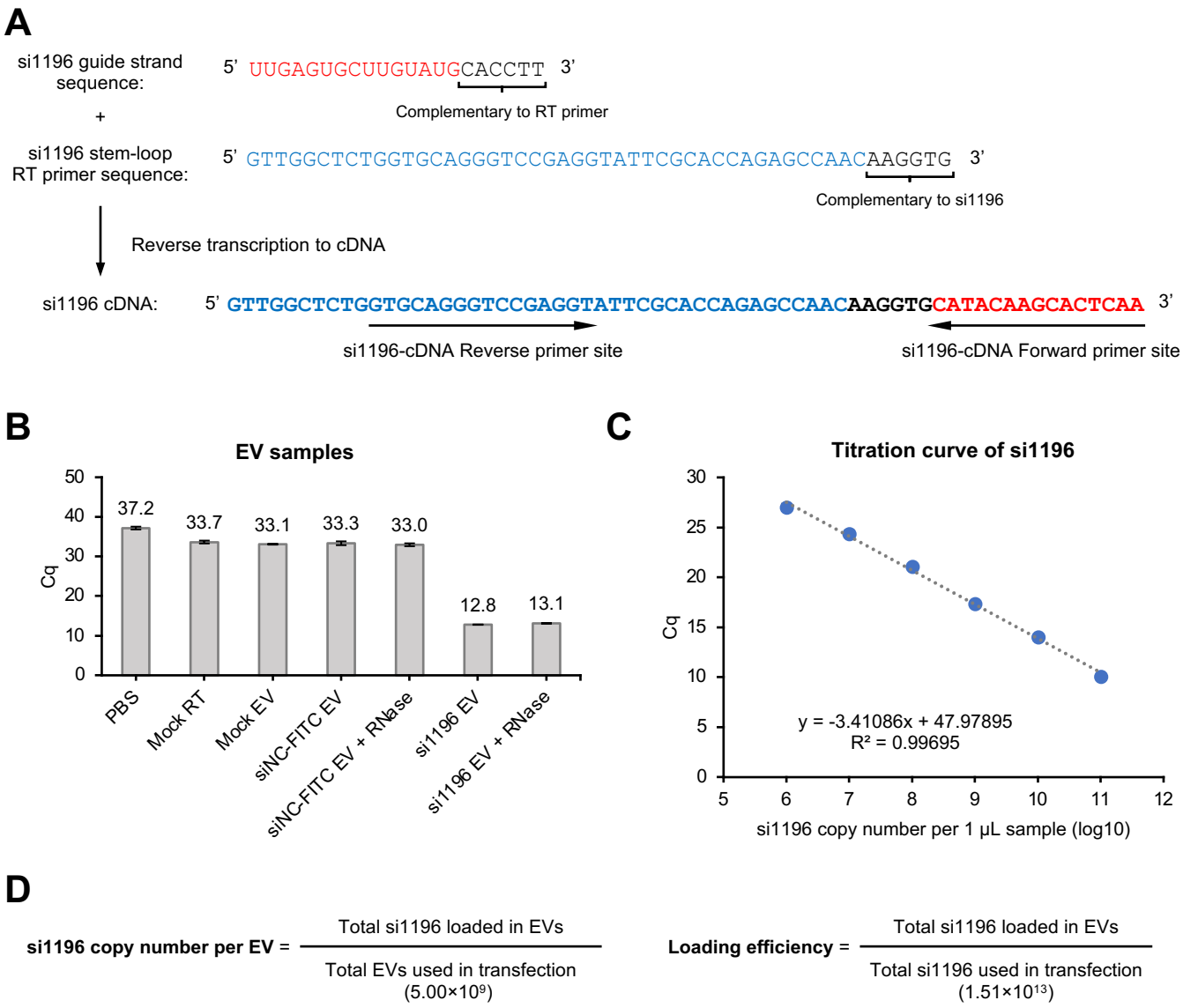
siRNA details	siRNA sequence	Target gene
siRNA name: **siLOXL1-AS1 #1196 (si1196)** Target sequence: GGTGCATACAAGCACTCAA	5′ GGUGCAUACAAGCACUCAATT TTCCACGUAUGUUCGUGAGUU 5′	*LOXL1-AS1* (NR_040066.1)
siRNA name: **Negative control siRNA (siNC)** Target sequence: TTCTCCGAACGTGTCACGT	5′ UUCUCCGAACGUGUCACGUTT TTAAGAGGCUUGCACAGUGCA 5′	(None)

Supplementary Table S3. Gene-specific primer pairs for qRT-PCRs in this study.

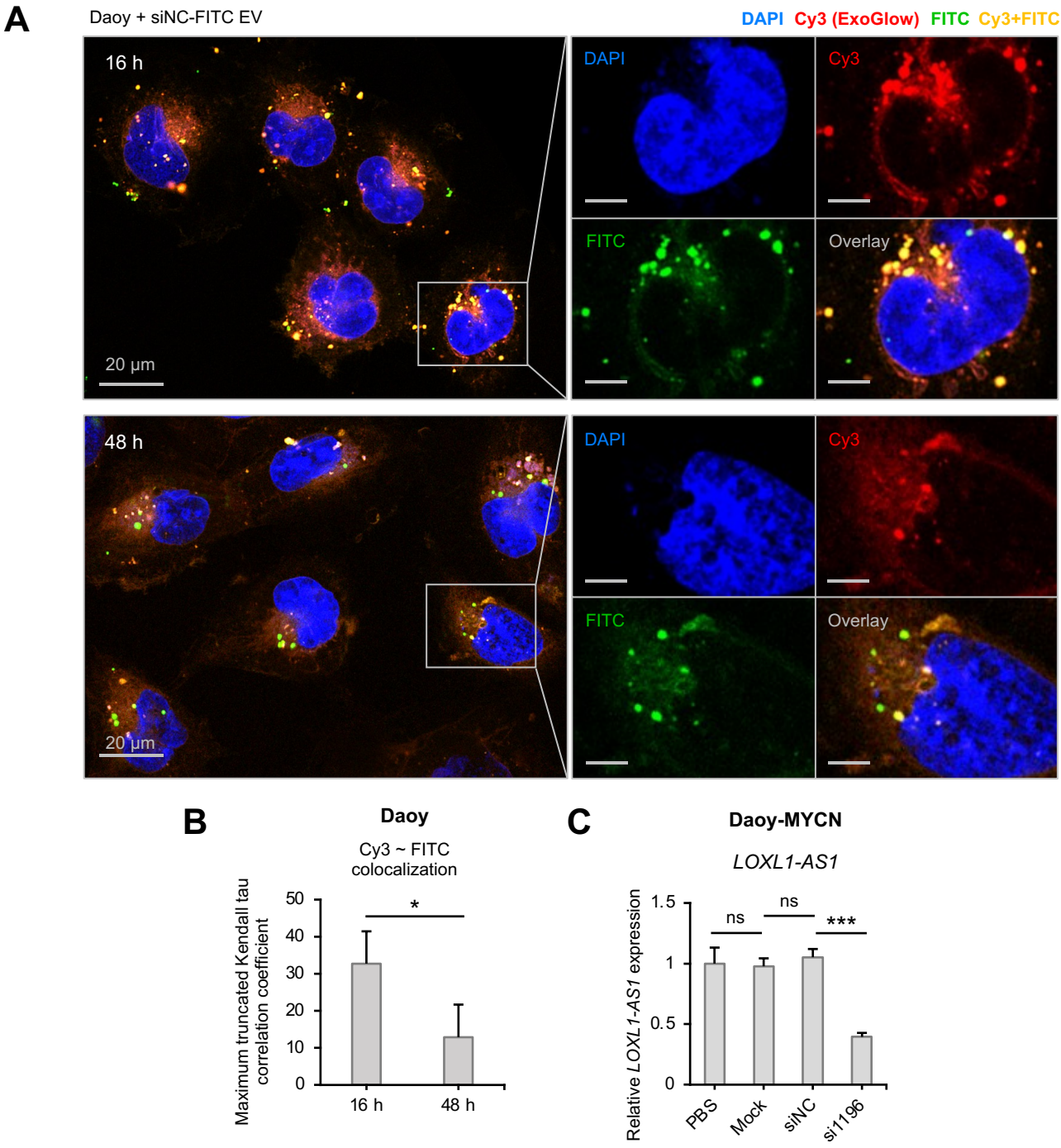
Gene name	Forward and reverse primers (5’ → 3’)
BMI1	Forward: AATCCCCACCTGATGTGTGT Reverse: GCTGGTCTCCAGGTAACGAA
HSPCB	Forward: AGCCTACGTTGCTCACTATTACG Reverse: GAAAGGCAAAAGTCTCCACCT
Lamp2-E1-3	Forward: CAGGCCCGCCTTCCTGGTTA Reverse: GTGCCATGGTCTGAATGGTTACAG
Lamp2-V5	Forward: TAACCCTCTCCTCGGTCTCGATT Reverse: GTGCCATGGTCTGAATGGTTACAG
LOXL1-AS1	Forward: AGTCCACAAATCCTAGGTGTA Reverse: CTCGTTTCCGATCCAGCCAGG
OCT3/4	Forward: ATCGAGAACCGAGTGAGAGG Reverse: CACTCGGACCACATCCTTCT
SOX2	Forward: TTGCTGCCTCTTTAAGACTAGGA Reverse: TAAGCCTGGGGCTCAAACT
NANOG	Forward: CTCCAACATCCTGAACCTCAGC Reverse: CGTCACACCATTGCTATTCTTCG
TGFB2	Forward: ACACTCAGCACAGCAGGGTCCT Reverse: TTGGGACACGCAGCAAGGAGAAG
si1196-cDNA	Forward: TTGAGTGCTTGTATGCACC Reverse: GTGCAGGGTCCGAGGT



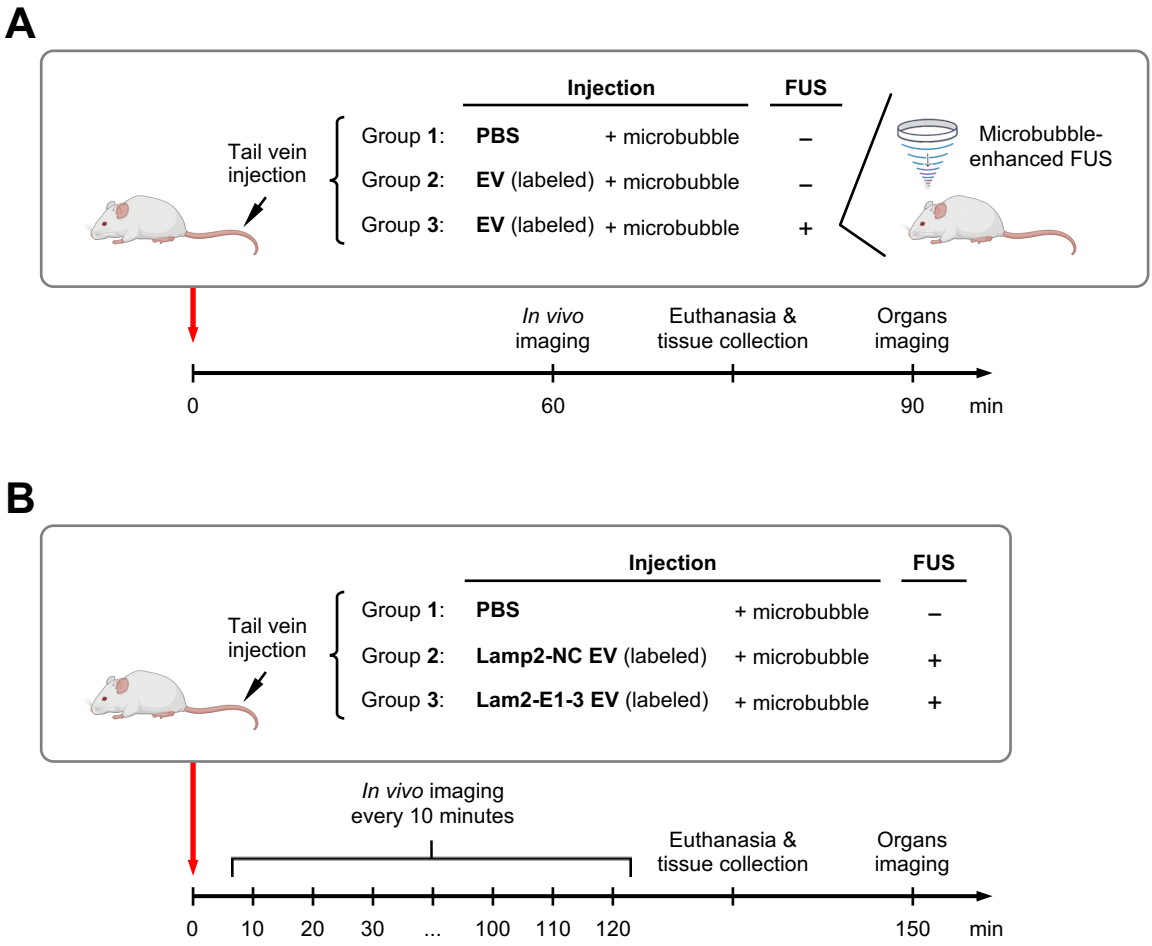
Supplementary Figure S1. EV labeling for treatment on Daoy cells *in vitro*. (A) Schematic illustration of EV labeling process and EV treatment on target cells. (B) Microscopic images of Daoy cells in normal monolayer culture after treatment with labeled EVs for 16 h and washed three times with 1× PBS. A no-EV control sample was included during the EV labeling process and was treated on Daoy cells to compare fluorescence signal. Scale bars, 20 μm (4x) and 500 μm (40x). See also Figure 3A-B.



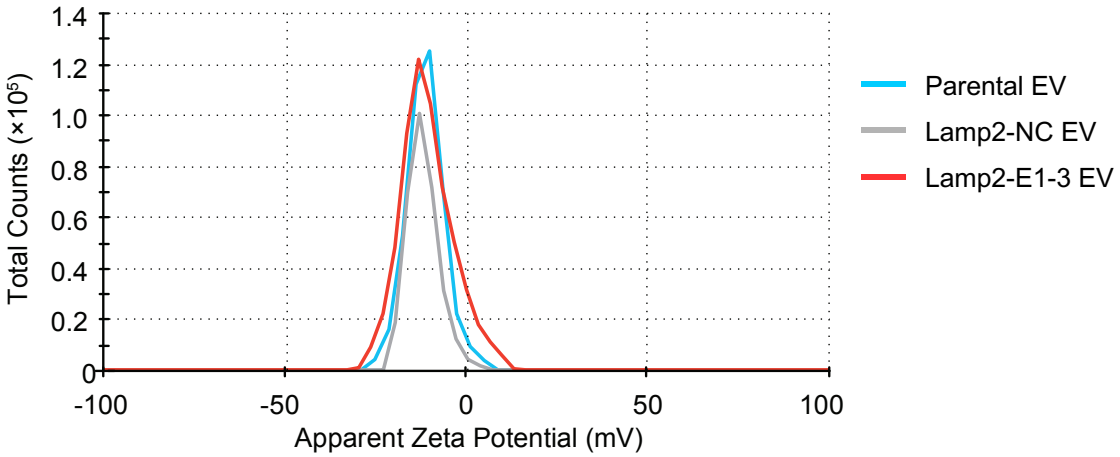
Supplementary Figure S2. RT-qPCR-based quantification of *LOXL1-AS1* siRNA #1196 (si1196). (A) Design of the stem-loop RT primer used to anneal to the si1196 guide strand. The resulting extended cDNA product serves as a template for RT-qPCR using specific forward and reverse primers designed at the indicated sites. (B) RT-qPCR analysis of EV samples showing mean Cq values of si1196 EVs with and without RNase treatment compared to control samples. (C) Titration curve of si1196, plotting known si1196 copy number (x-axis) against the corresponding Cq values (y-axis). Simple linear regression was used to determine the equation (y = f(x)) and R^2 value, which were used to calculate total si1196 copy number in each EV sample from (B). (D) Calculation of si1196 copy number per EV and loading efficiency. See also Figure 3F-G.



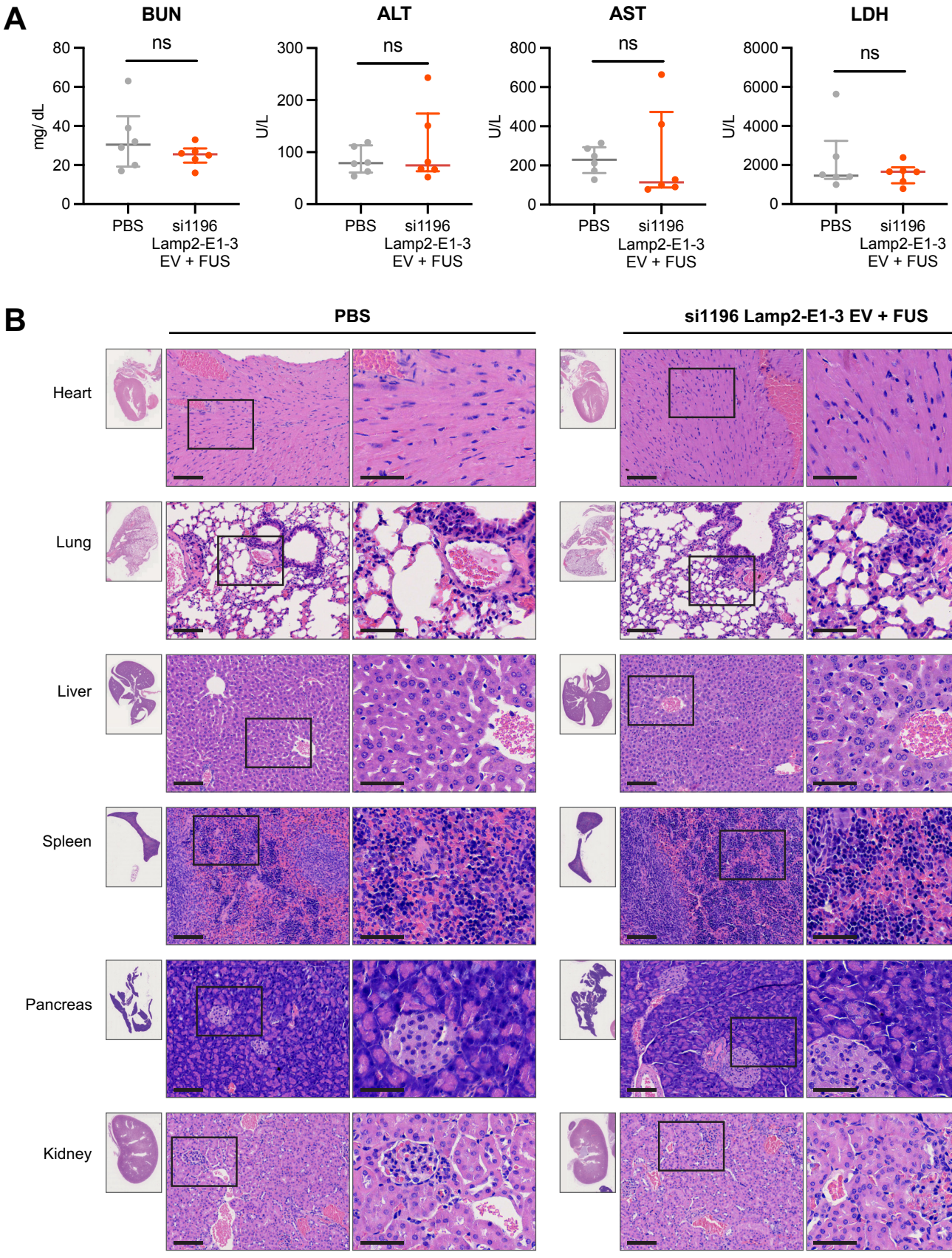
Supplementary Figure S3. Change in EV and siRNA signals in Daoy cells after EV treatment. (A) Confocal microscopic images of Daoy cells treated with siNC-FITC EVs at 16-h and 48-h time points. Blue = cell nucleus. Red = labeled EV. Green = siNC-FITC. Yellow = EV~siNC-FITC colocalization. Scale bars, 20 µm (left) and 10 µm (right). See also Figure 3H. (B) Co-localization scores of Cy3 signal (EV) and FITC signal (siNC) using the maximum truncated Kendall tau correlation coefficient. (C) RT-qPCR analysis of *LOXL1-AS1* expression in Daoy-MYCN cells 48 h after transfection with siRNAs using a liposome-based commercial kit. Values are relative to the PBS group. See also Figure 3I. Quantitative data are presented as the mean ± SD of replicates from three independent experiments. ns, non-significant. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



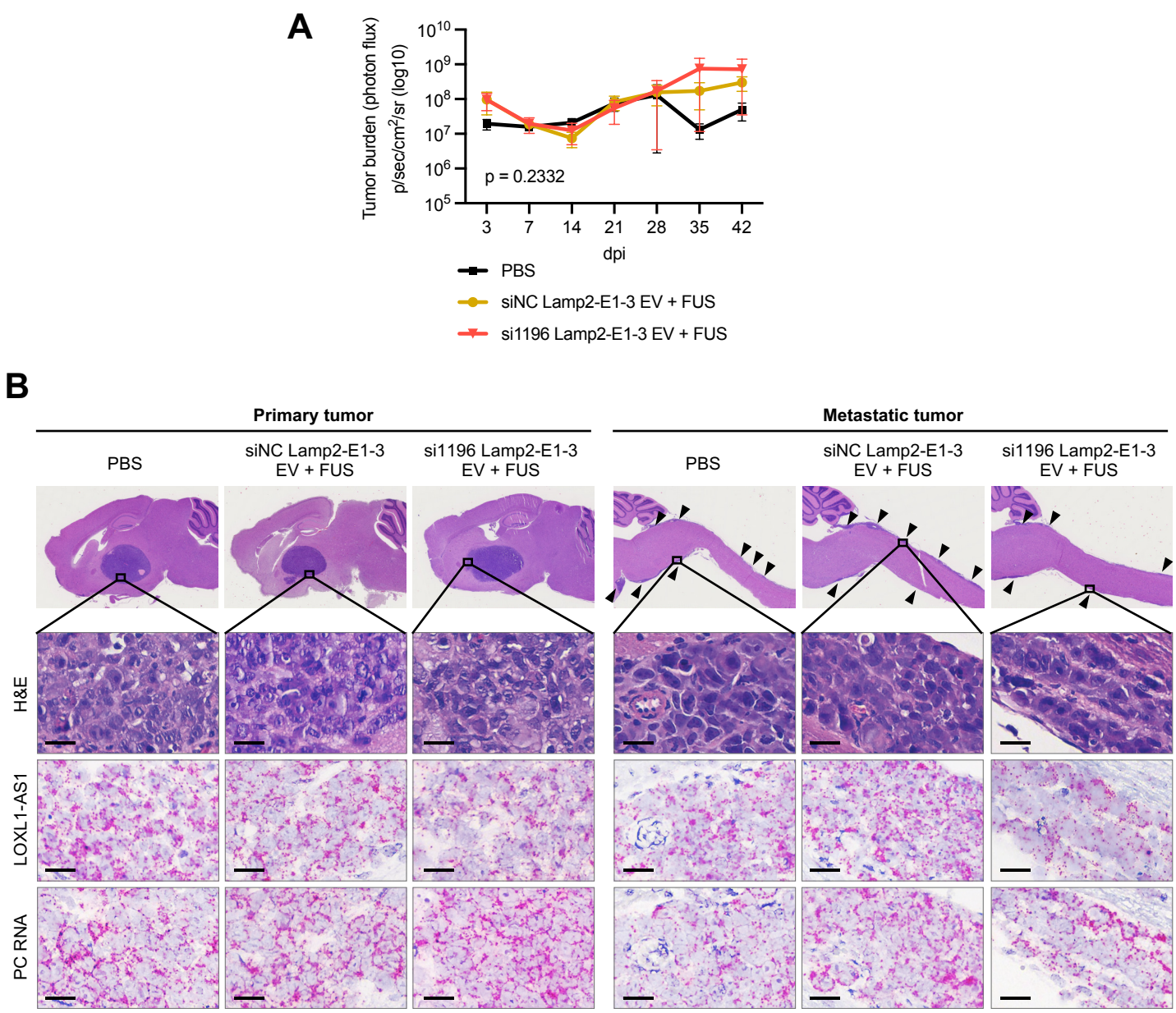
Supplementary Figure S4. Animal study of EV distribution and delivery to the brain. (A) Schematic outline of the animal study in Figure 6A-B. Fluorescence-labeled Lamp2-E1-3 EVs were mixed with microbubbles and injected into the tail vein, followed immediately by focused ultrasound (FUS). Fluorescence imaging, euthanasia, tissue collection, and imaging of dissected organs were performed at the indicated time point. (B) Schematic outline of the animal study in Figure 6C-F. Mice were injected either Lamp2-NC EVs or Lamp2-E1-3 EVs together with microbubbles into the tail vein and were treated with FUS. Serial fluorescence imaging was performed every 10 min until 120 min after injection, followed by euthanasia, tissue collection, and imaging of dissected organs at the indicated time point. See also Figure 6.



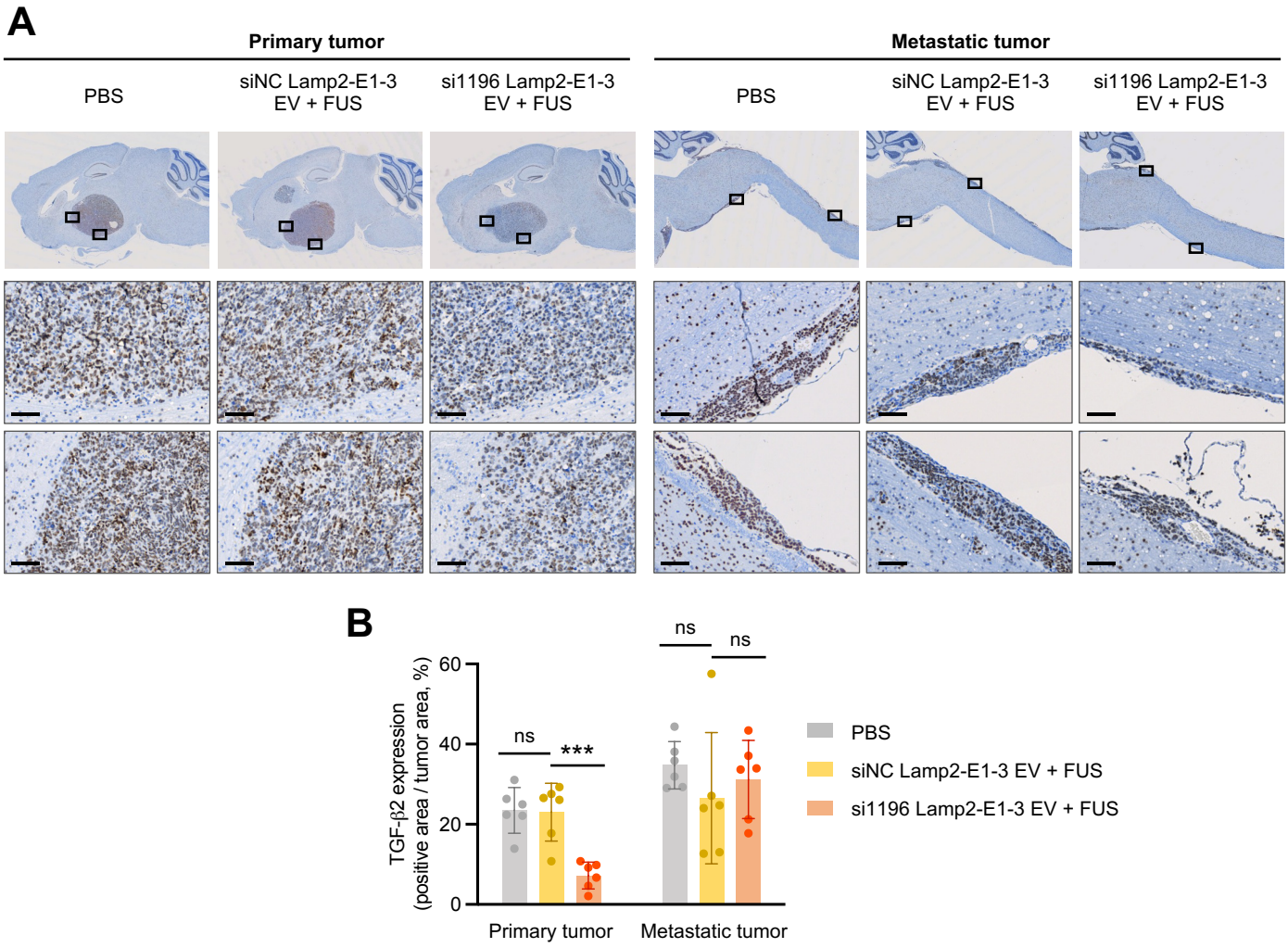
Supplementary Figure S5. Zeta potentials of peptide-expressing EVs. Combined zeta potential analyses of three EV types: non-modified EVs (parental EV), EVs expressing the non-cell-penetrating peptide (Lamp2-NC EV), and EVs expressing the MB-targeting peptide (Lamp2-E1-3 EV).



Supplementary Figure S6. Assessment of systemic toxicity of intravenously administered EVs. (A) Levels of serum biomarkers BUN, ALT, AST, and LDH in mice of the indicated treatment groups at the time point of euthanasia. (B) Representative hematoxylin and eosin (H&E)-stained sections of major organs. Scale bars, 100 μm (low magnification) and 50 μm (high magnification). ns, non-significant.



Supplementary Figure S7. *In vivo* EV-mediated *LOXL1-AS1* gene silencing in MB xenograft tumors. (A) Quantification of photon flux from bioluminescence imaging data in Figure 7C, reflecting tumor burden in Daoy-MYCN-Luc xenografts. Statistical differences among the three groups were assessed by two-way ANOVA. (B) Histopathological analysis of mouse brain and spinal cord tissues using hematoxylin and eosin (H&E) staining and *in situ* hybridization for *LOXL1-AS1* and *PPIB* (as positive control, PC). Arrowheads indicate metastatic tumor tissue. Scale bars, 20 μm. See also Figure 7F.



Supplementary Figure S8. Downregulation of TGF-β2 in MB xenograft tumors with *in vivo* EV-mediated *LOXL1-AS1* gene silencing. (A) Immunohistochemistry (IHC) analysis of TGF-β2 expression in mouse brain and spinal cord tissues. All sections were counterstained with hematoxylin. Scale bars, 100 μm. (B) Quantification of TGF-β2 expression in (A) as the percentage of tumor area with positive IHC signal. Data are presented as the mean ± SD of multiple tumor lesions from at least three mice per group. ns, non-significant. *** $p<0.001$. See also Figure 7F and Supplementary Figure S7B.