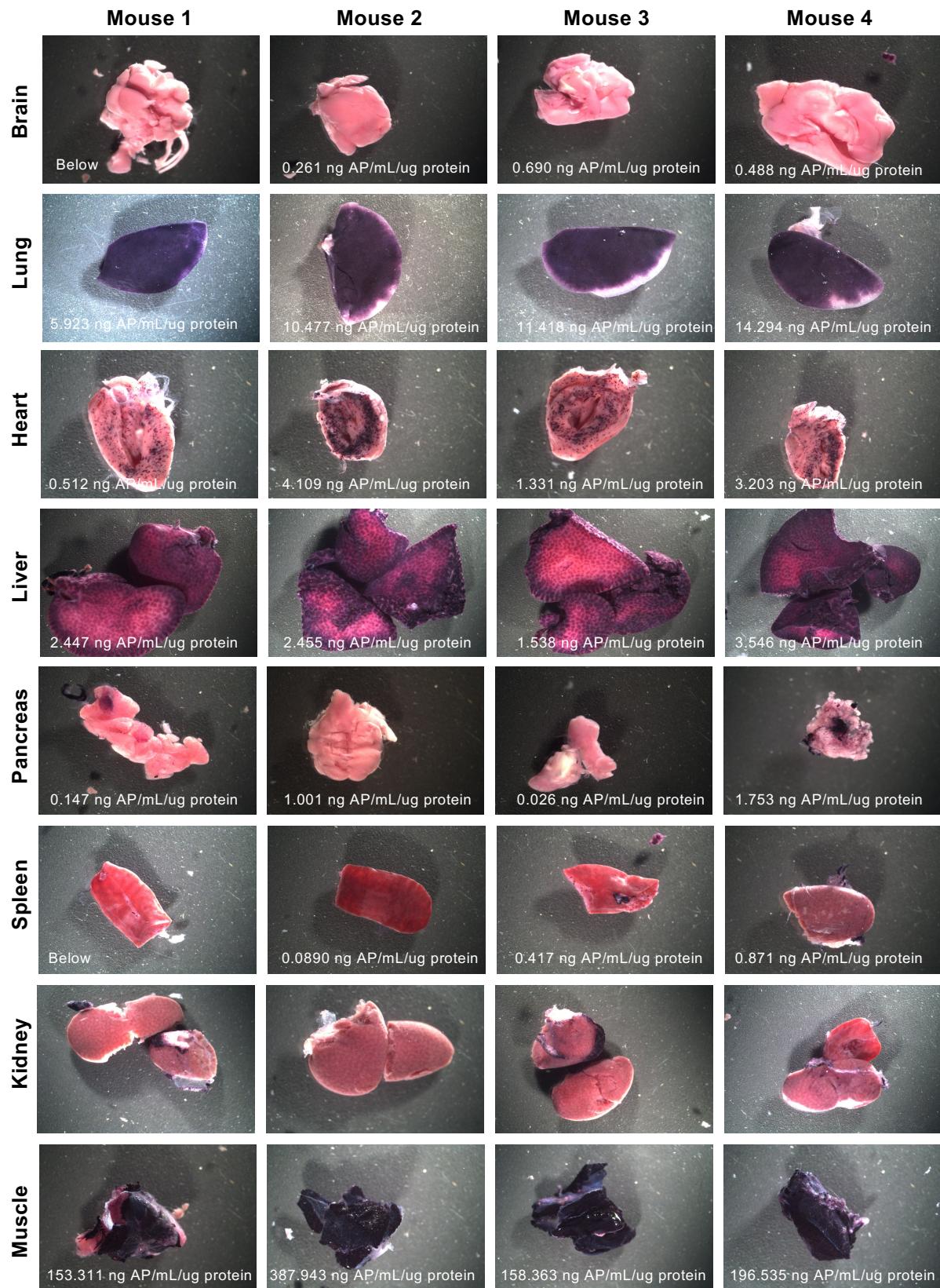
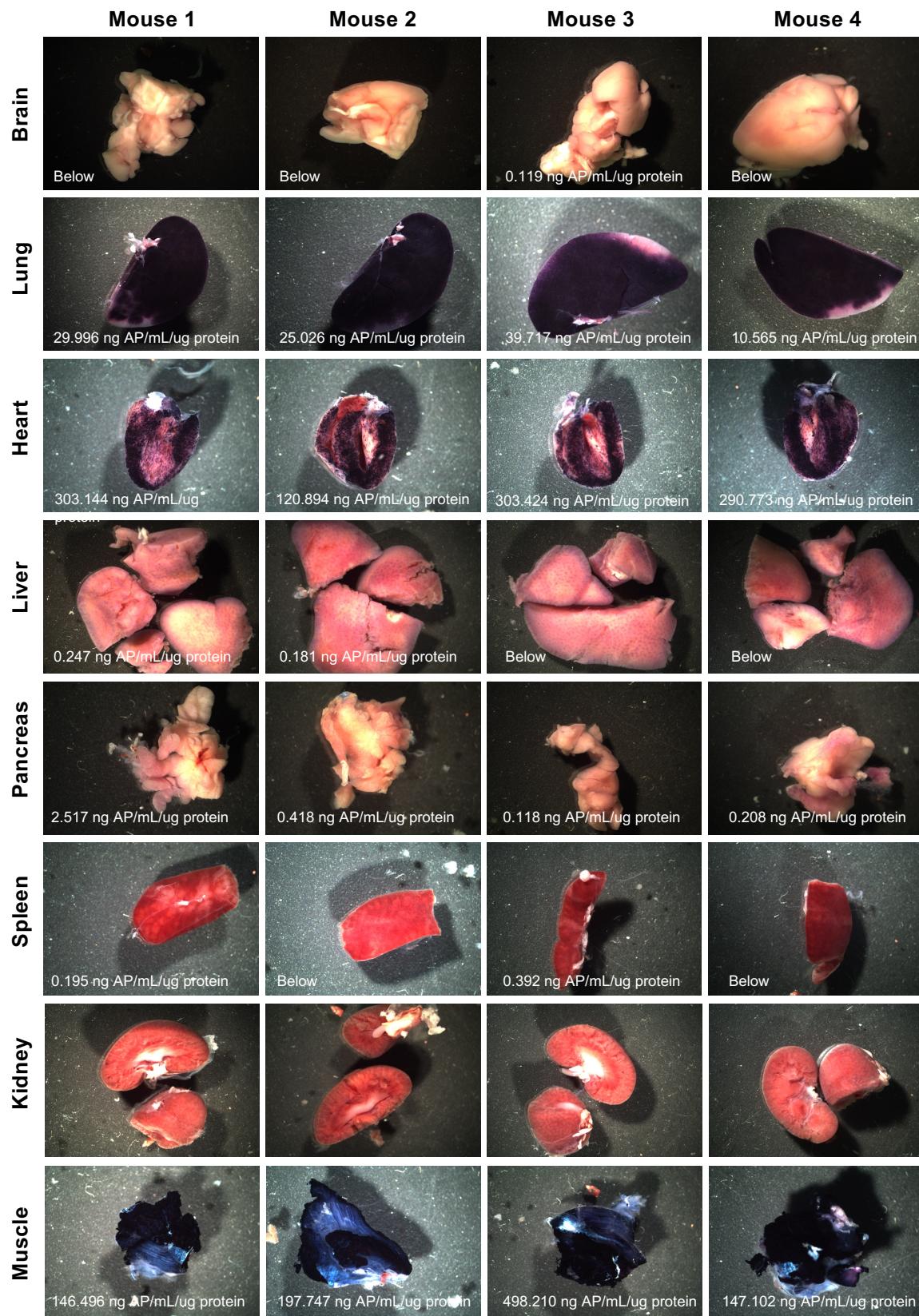


**AAV6.2FF-Promoter 1 Delivered IN, IM, and IP**



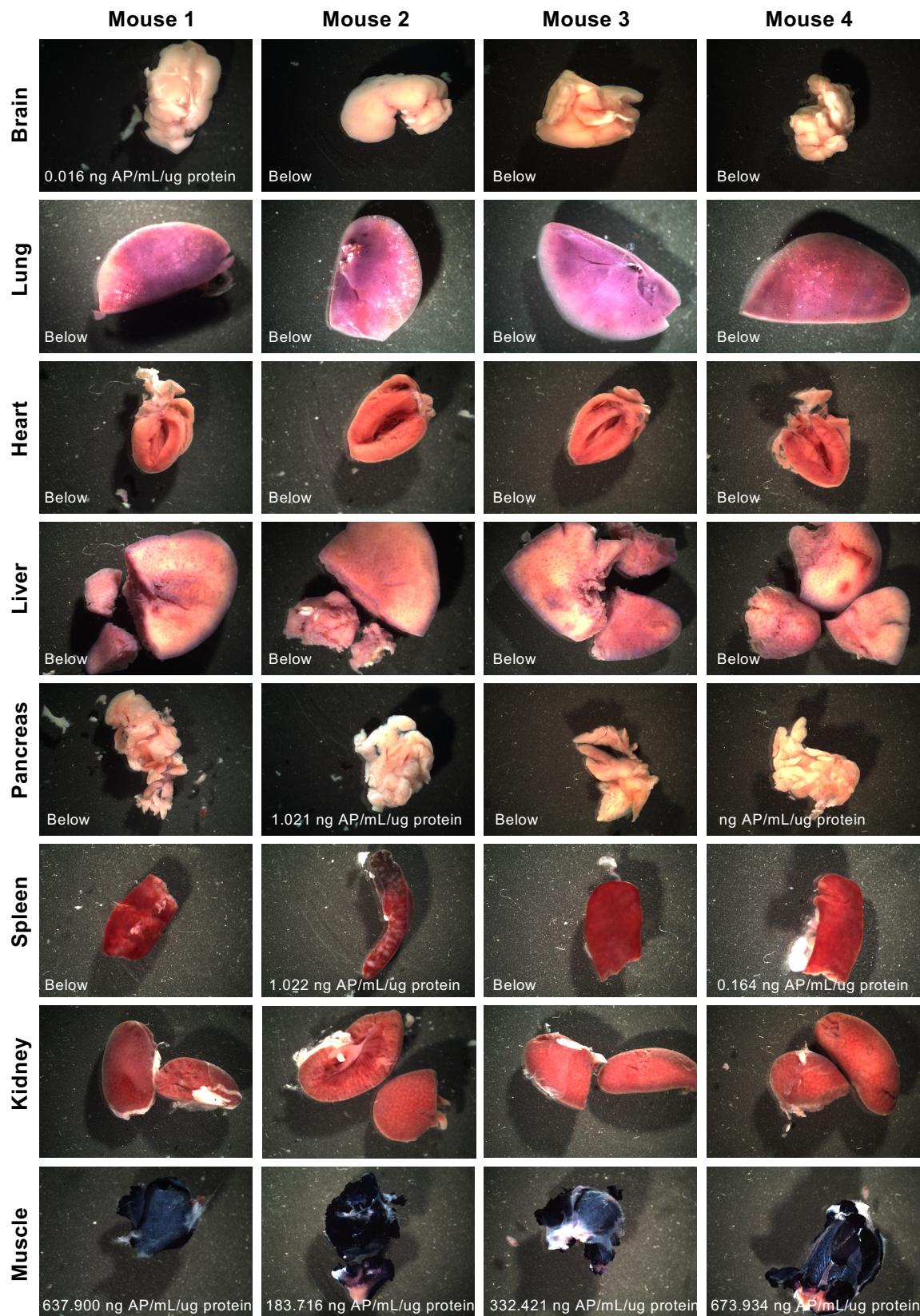
**Figure S1. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 1 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 1 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 2 Delivered IN, IM, and IP**



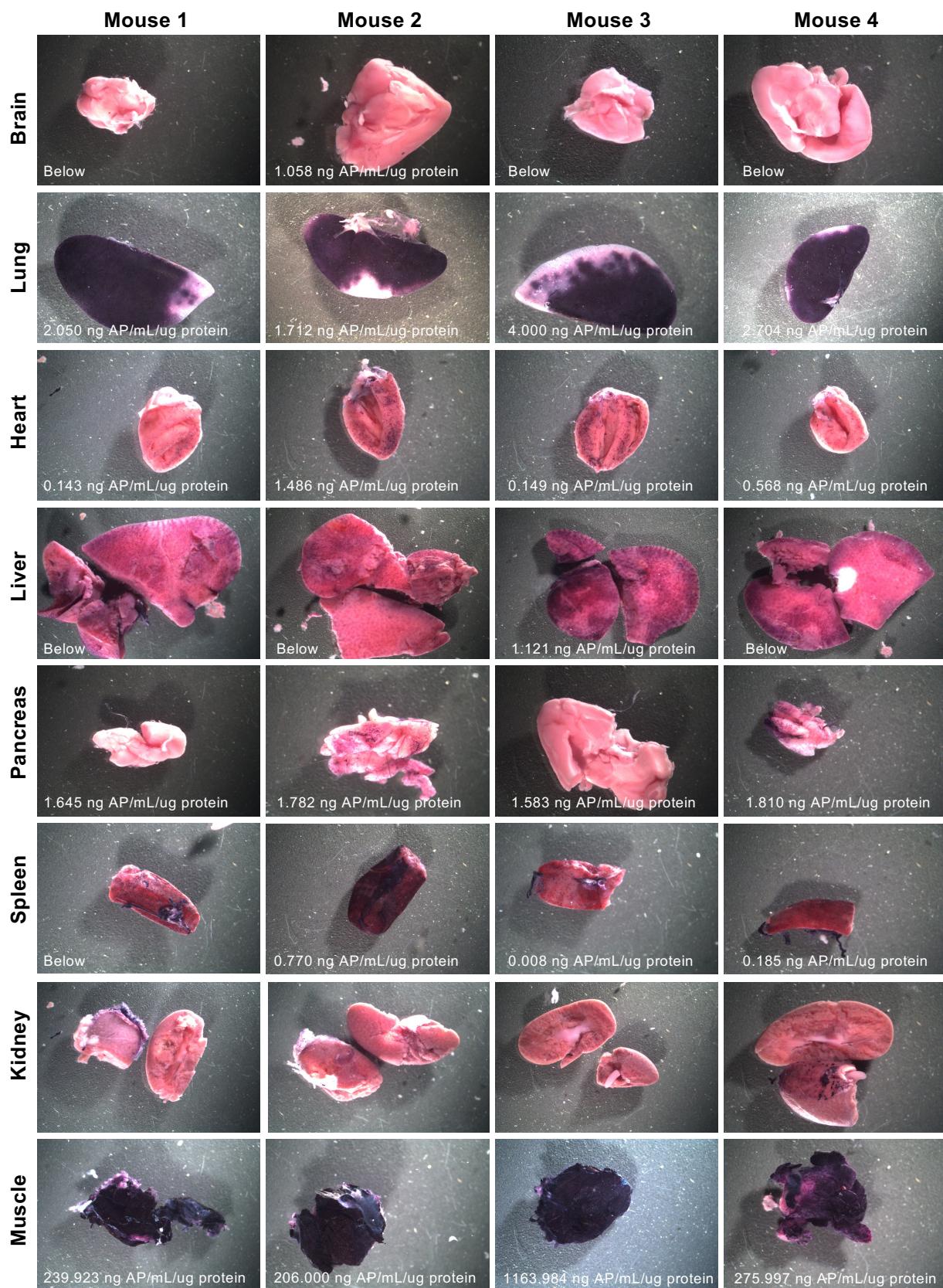
**Figure S2. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 2 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 2 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 3 Delivered IN, IM, and IP**



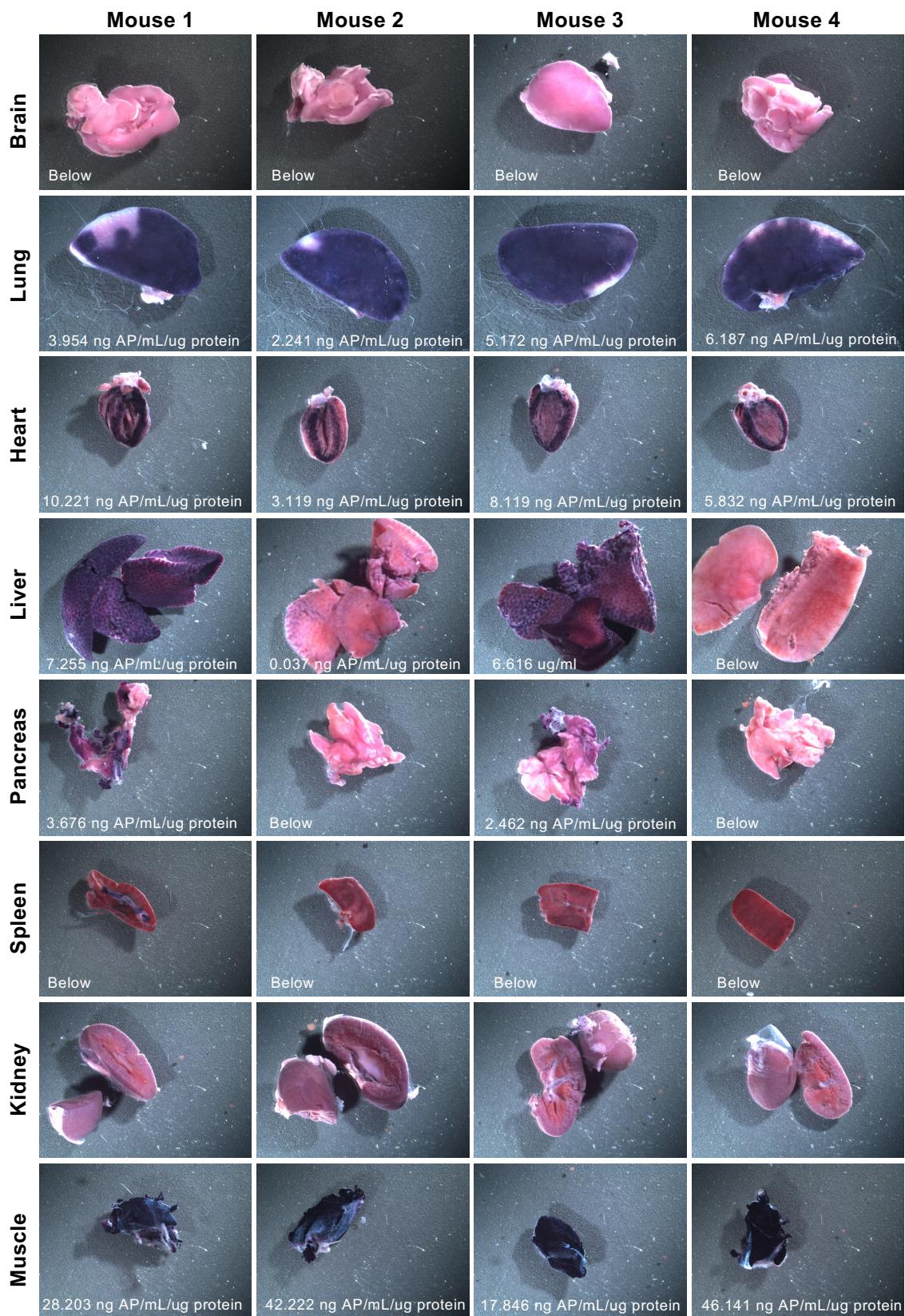
**Figure S3. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 3 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 3 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 4 Delivered IN, IM, and IP**



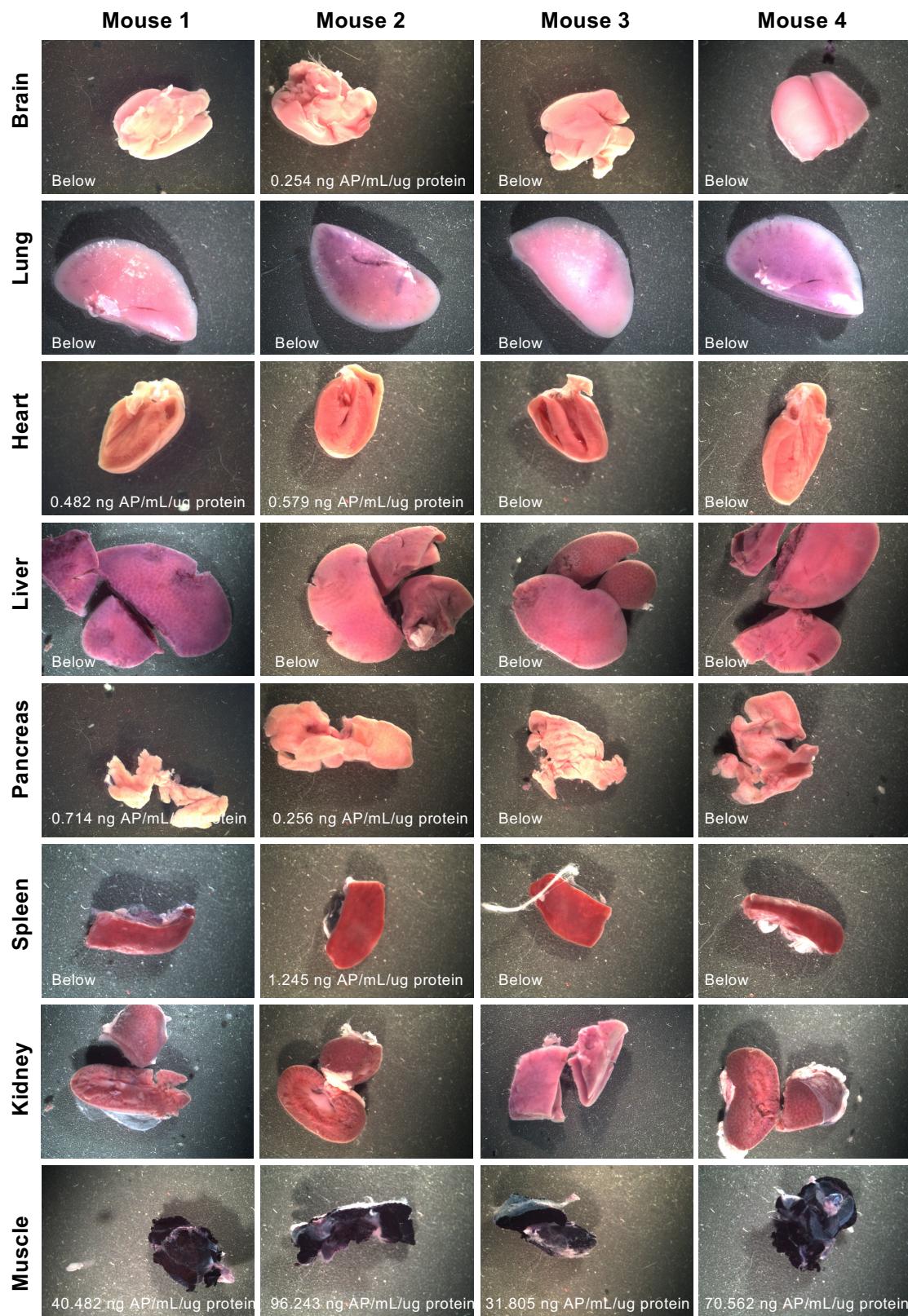
**Figure S4. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 4 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 4 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 5 Delivered IN, IM, and IP**



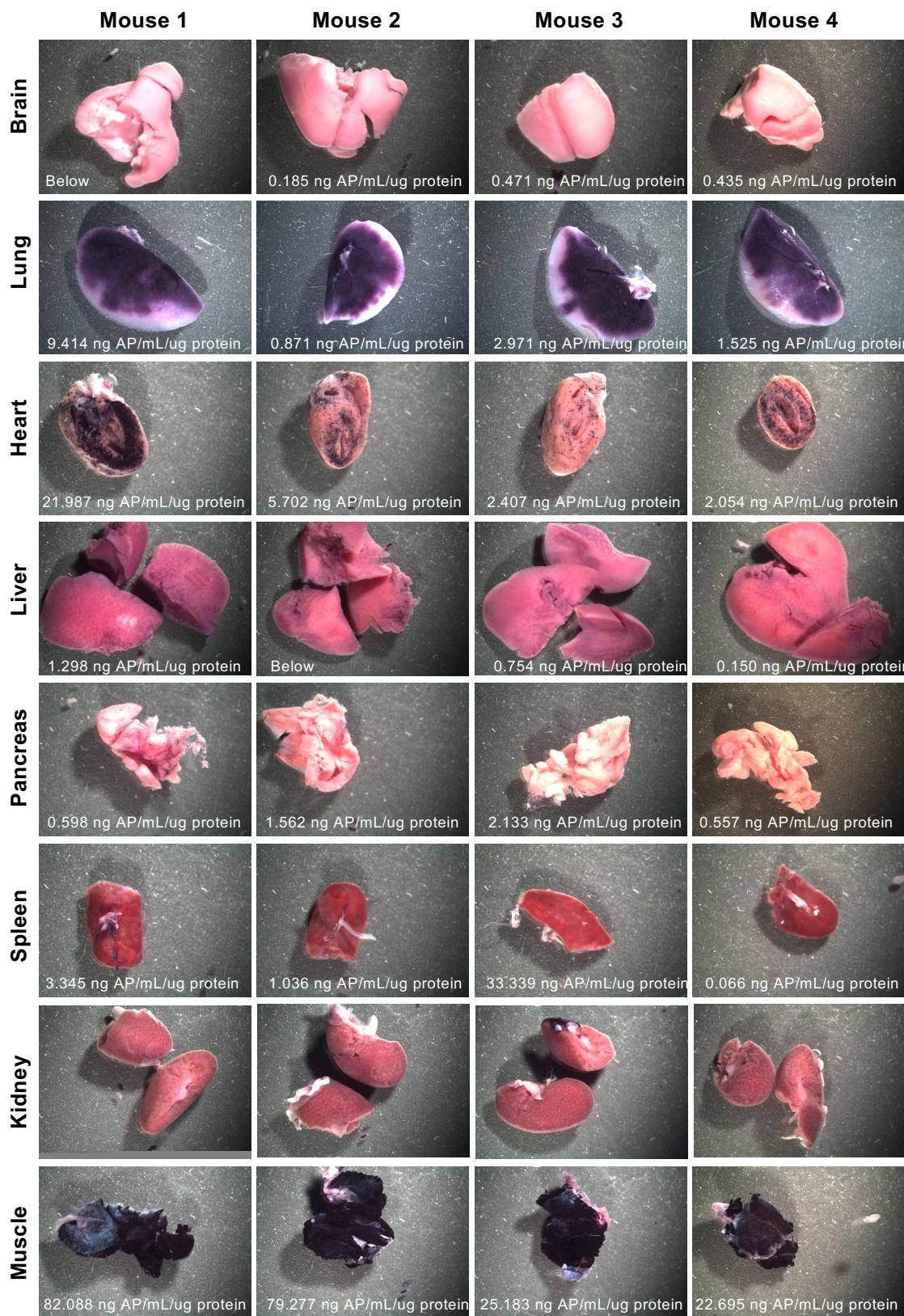
**Figure S5. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 5 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 5 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 6 Delivered IN, IM, and IP**



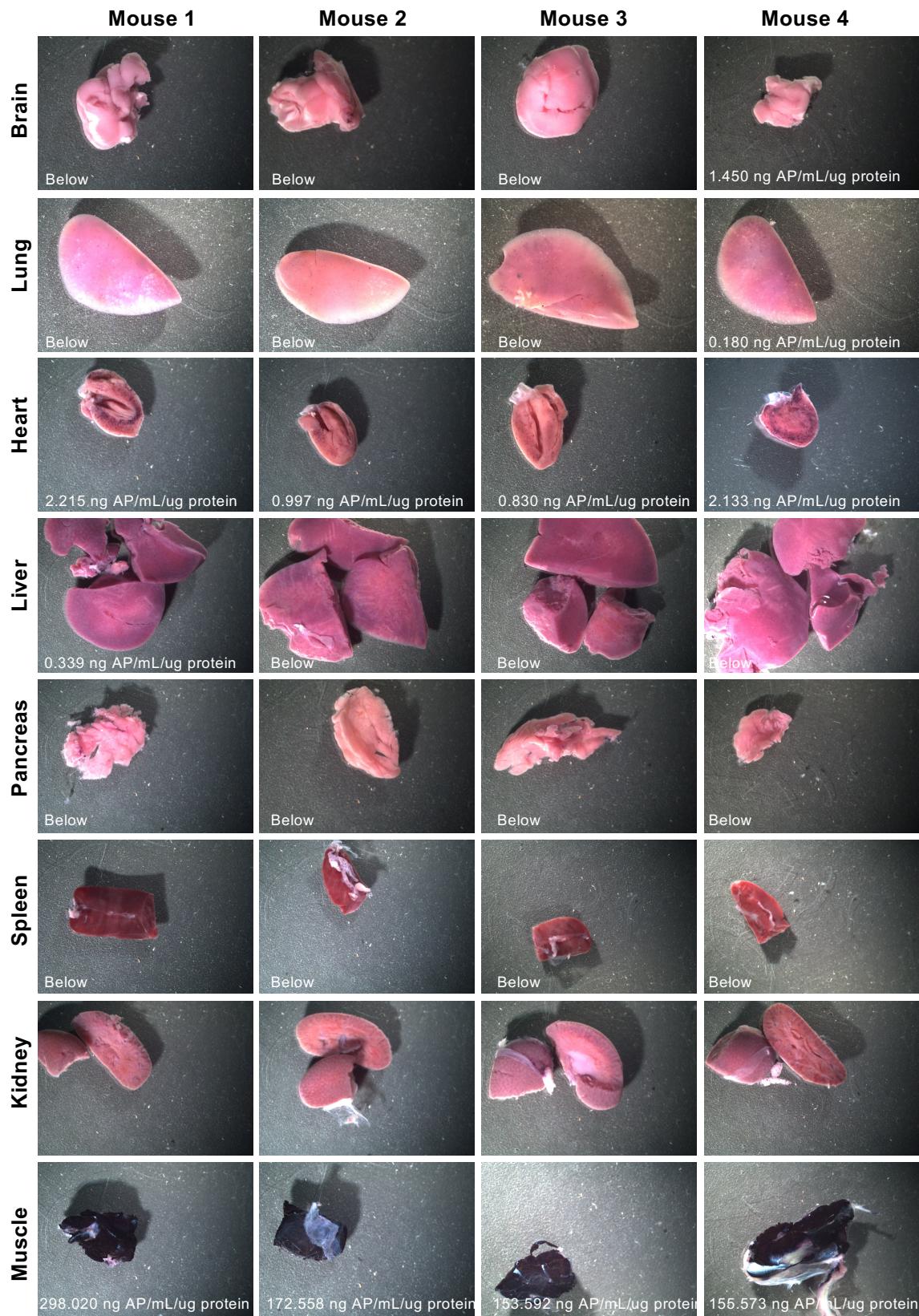
**Figure S6. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 6 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 6 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 7 Delivered IN, IM, and IP**



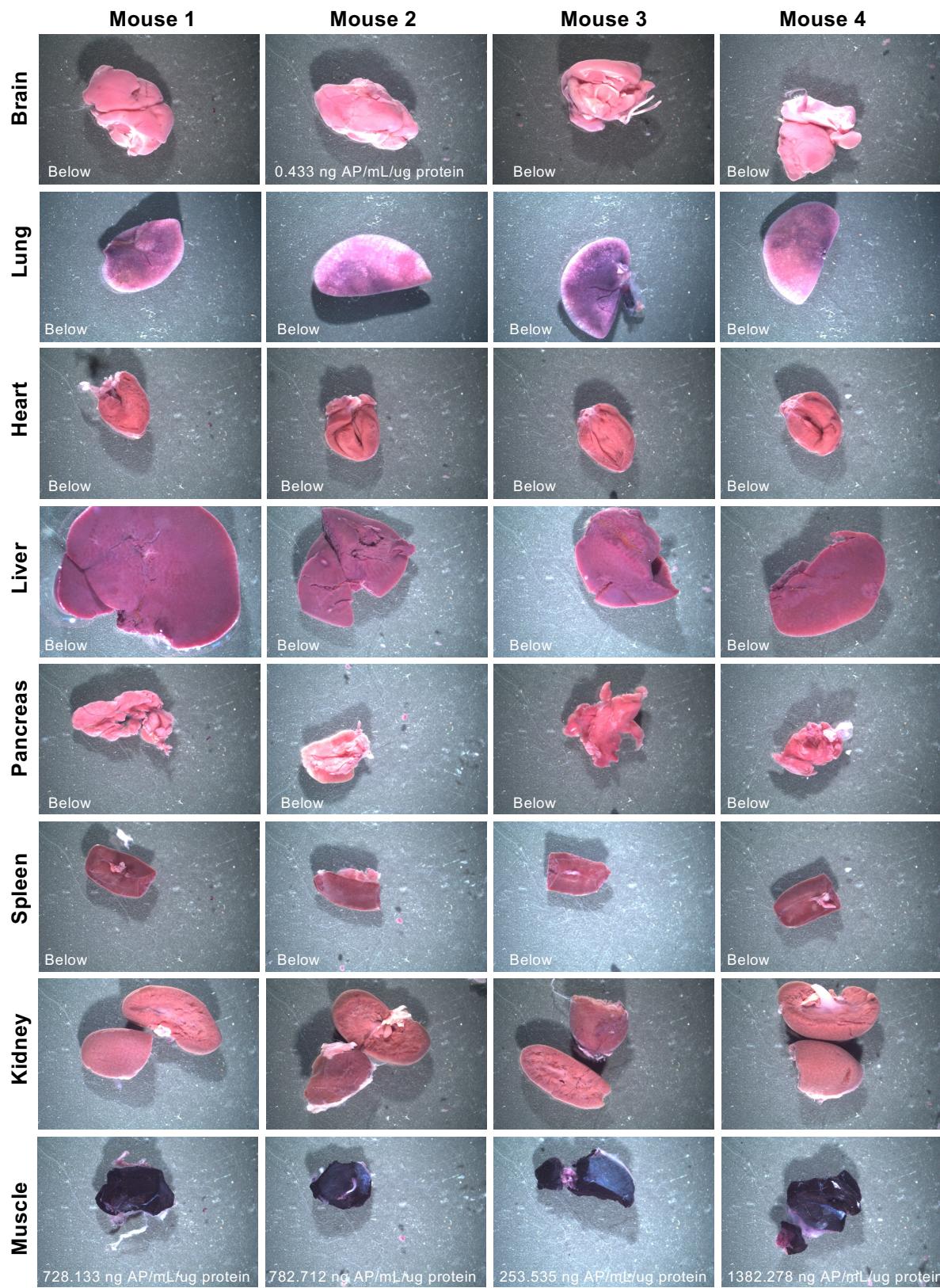
**Figure S7. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 7 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 7 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 8 Delivered IN, IM, and IP**



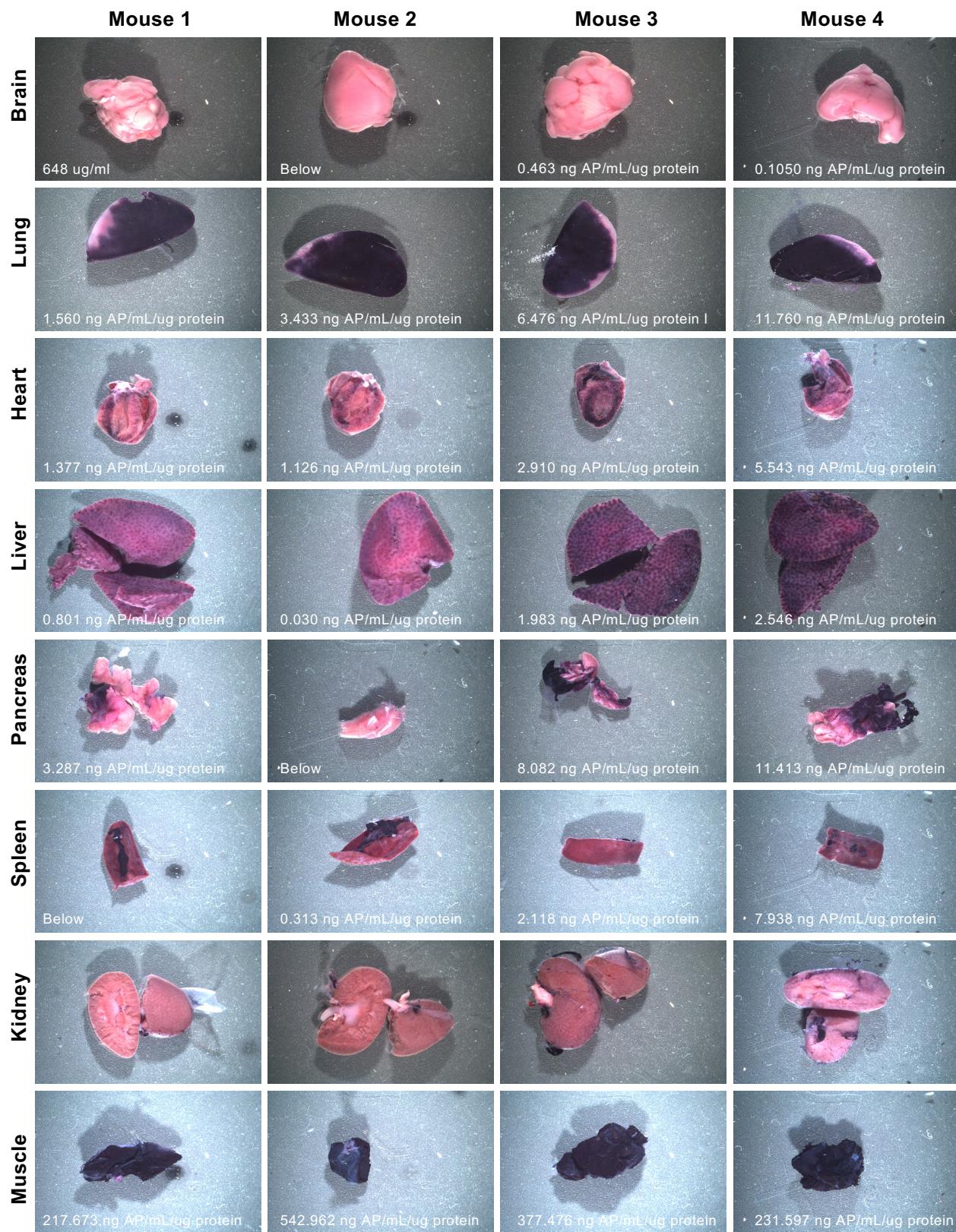
**Figure S8. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 8 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 8 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 9 Delivered IN, IM, and IP**



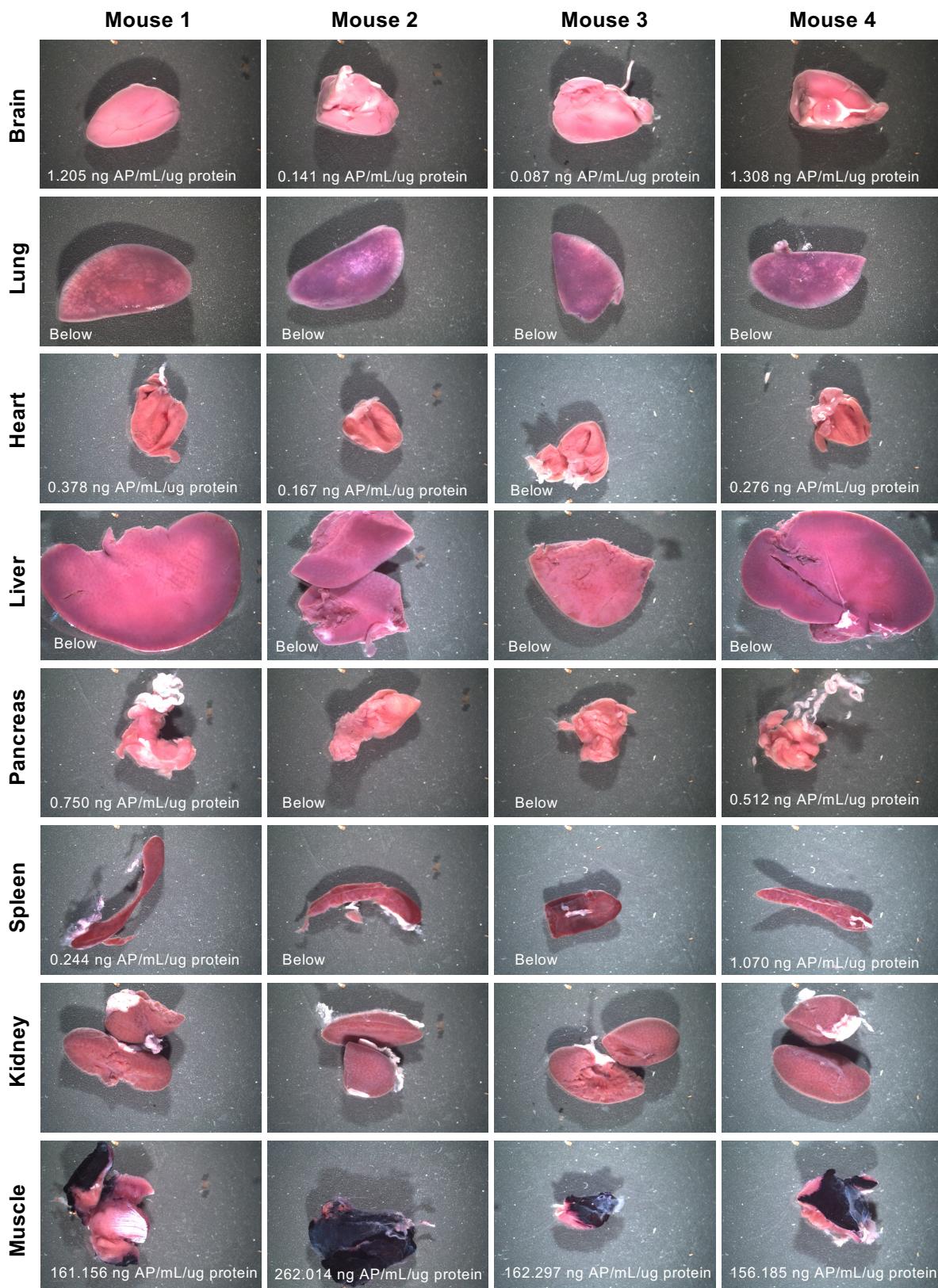
**Figure S9. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 9 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 9 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 10 Delivered IN, IM, and IP**



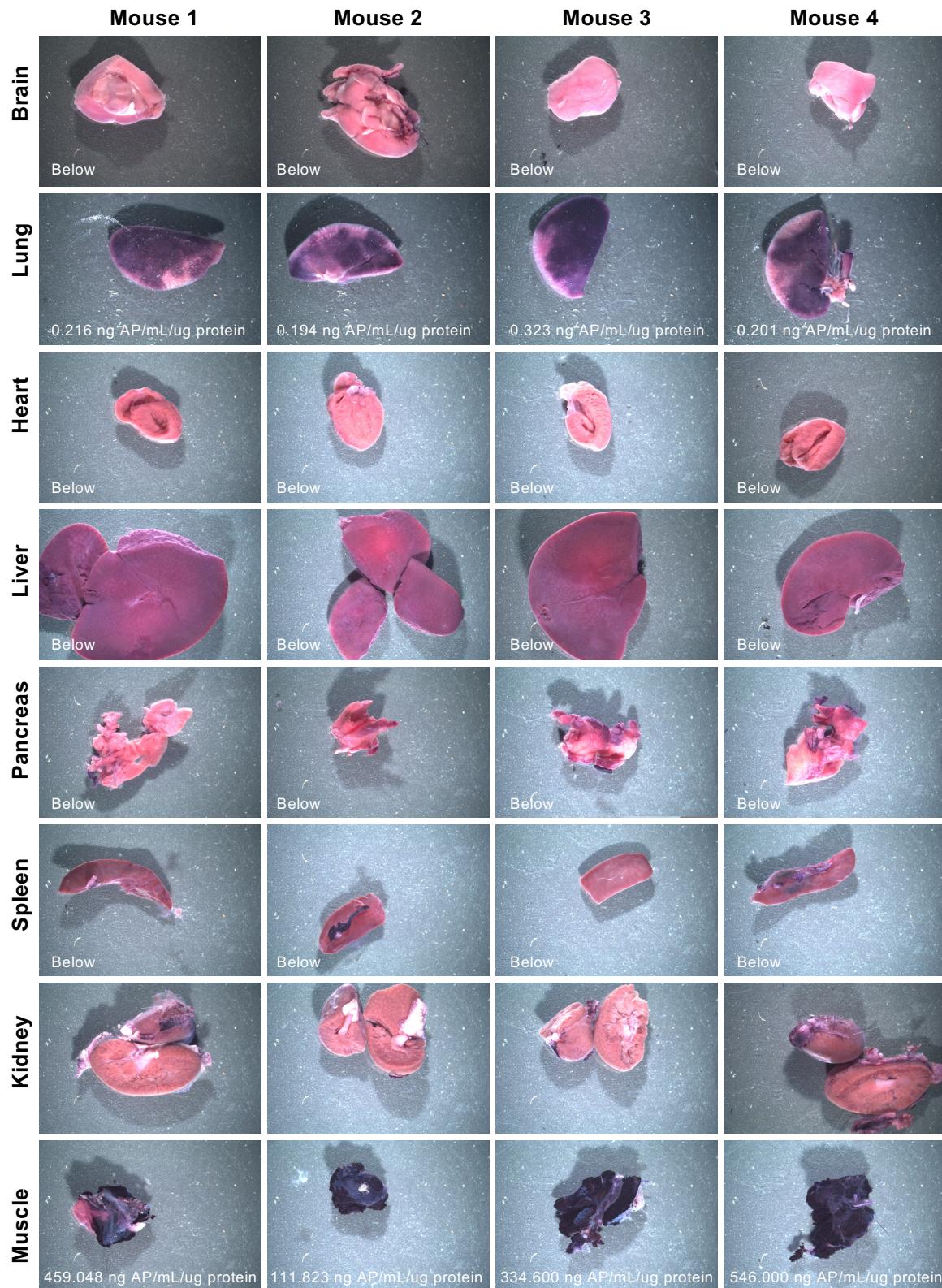
**Figure S10. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 10 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 10 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 11 Delivered IN, IM, and IP**

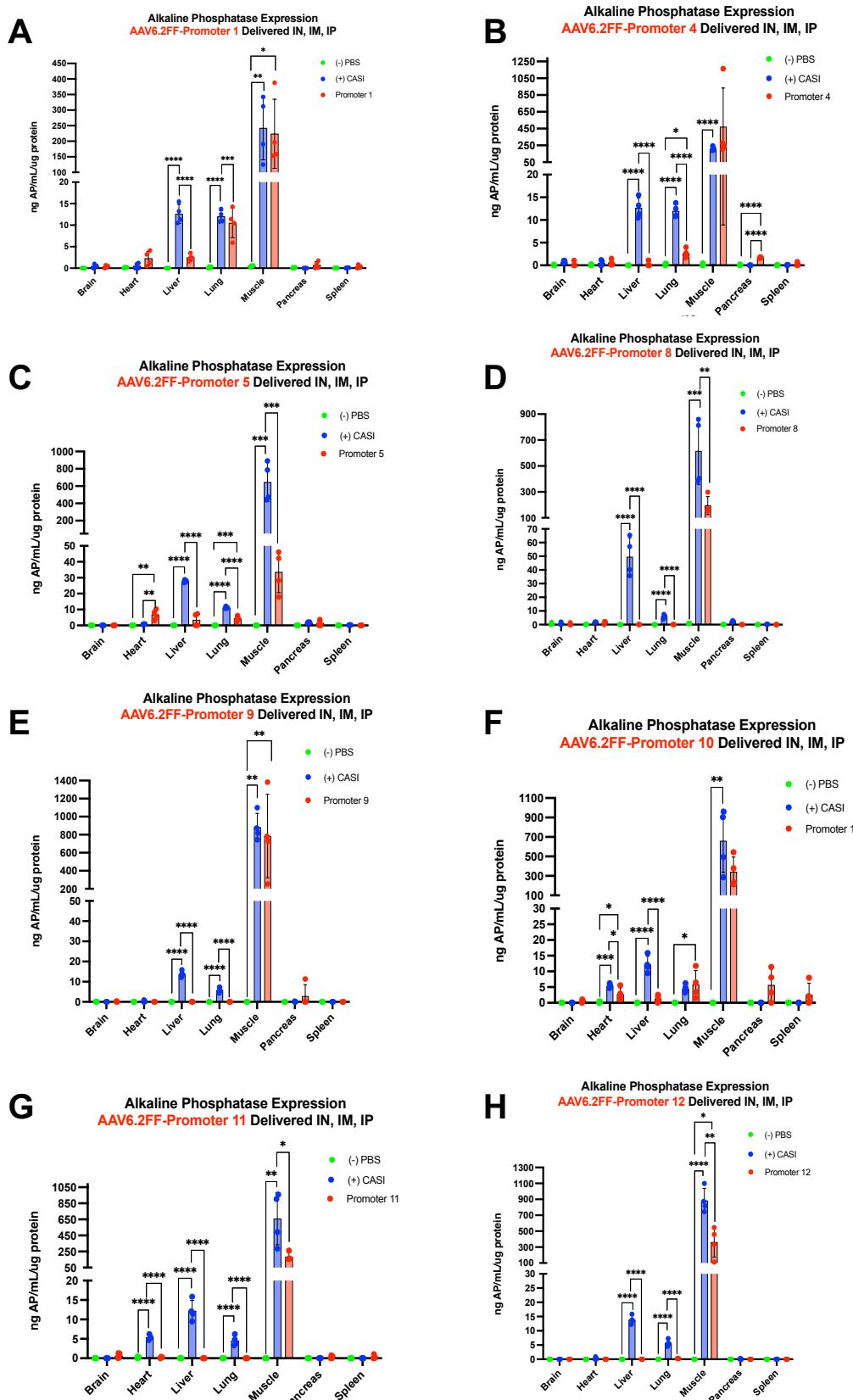


**Figure S11. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 11 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 11 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 12 Delivered IN, IM, and IP**

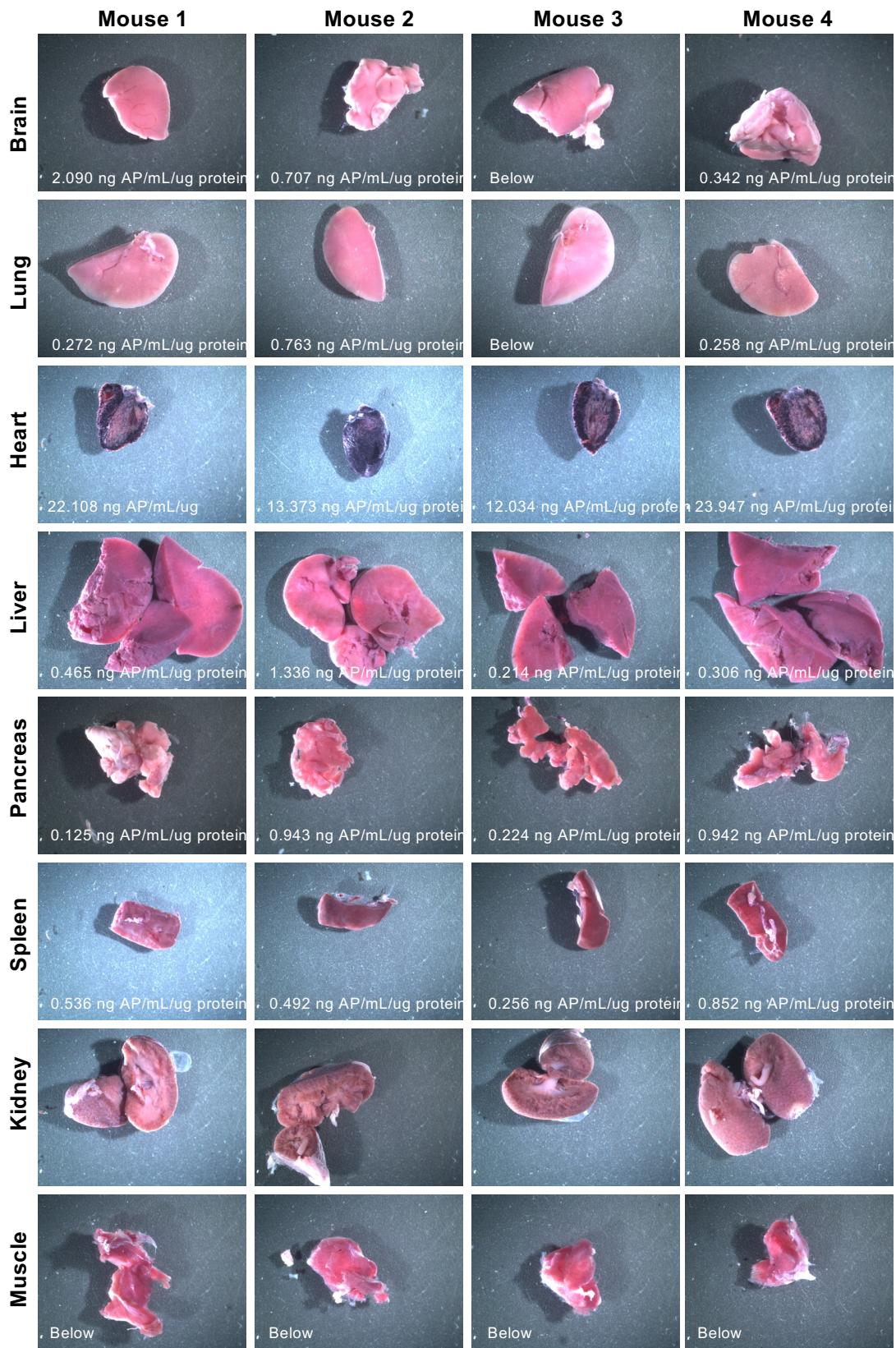


**Figure S12. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 12 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 12 via intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.



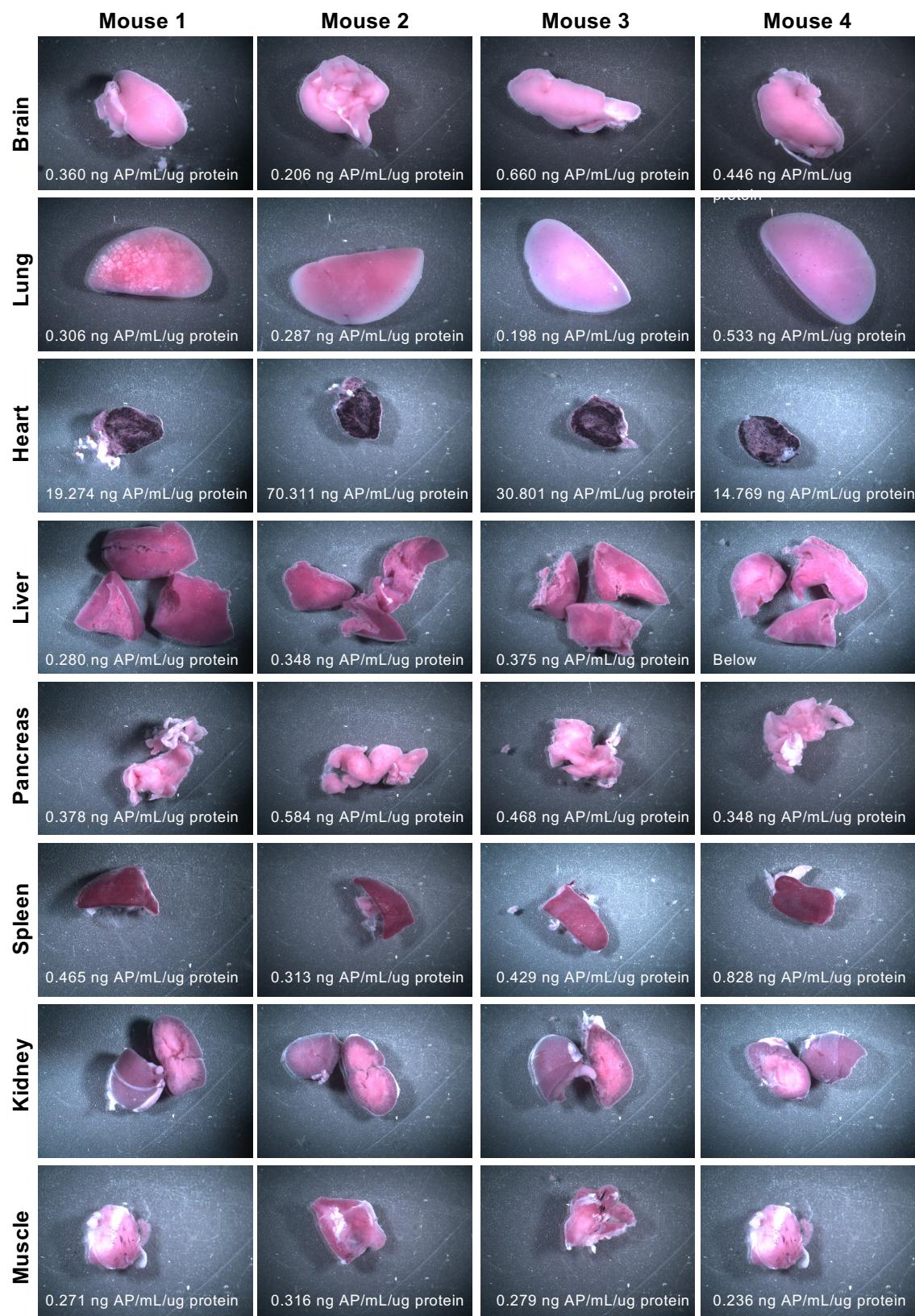
**Figure S13. Quantitative analysis of tissue-wide AAV6.2FF-mediated AP expression driven by promoters 1, 4, 5, 8, 9, 10, 11 and 12 delivered intranasally, intramuscularly, and intraperitoneally.** Mice received AAV6.2FF-AP driven by promoters (A) 1, (B) 4, (C) 5, (D) 8, (E) 9, (F) 10, (G) 11, or (H) 12, intranasally, intramuscularly and intraperitoneally at a dose of  $1 \times 10^{11}$  vg for each route of administration. Positive and negative control groups received AAV6.2FF-CASI-AP and PBS, respectively. Mice were euthanized 21 days post-AAV administration, tissues were collected, and a portion of each tissue was homogenized. Alkaline phosphatase expression was quantified across tissues using an enzymatic AP activity assay. Individual one-way ANOVAs were performed to compare the groups within each tissue. \*P  $\leq 0.05$ , \*\*P  $\leq 0.01$ , \*\*\*P  $\leq 0.001$ , and \*\*\*\*P  $\leq 0.0001$ .

**AAV6.2FF-Promoter 2 Delivered IP**



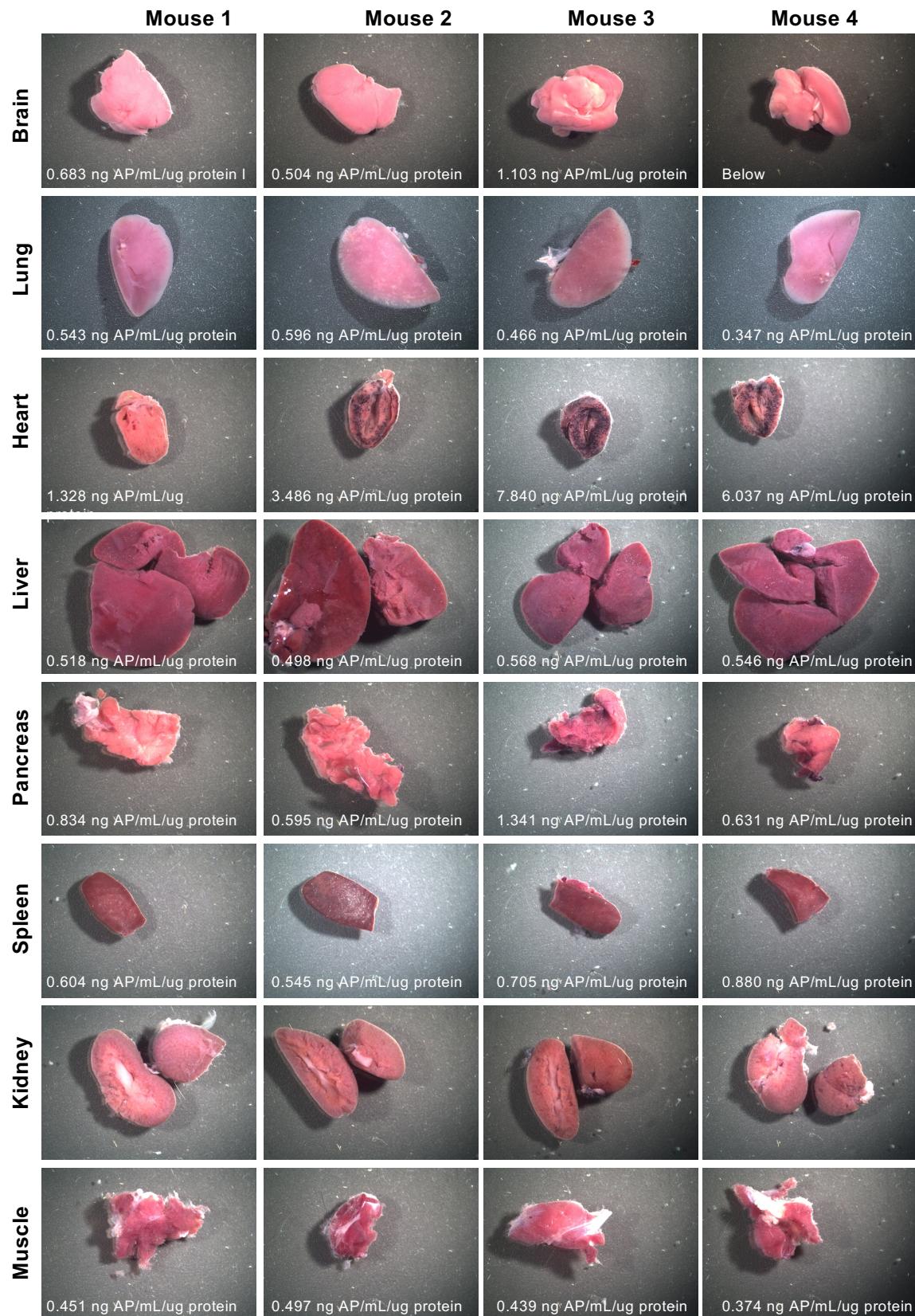
**Figure S14. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 2 delivered intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 2 via intraperitoneal (IP) administration only. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV9-Promoter 2 Delivered IP**



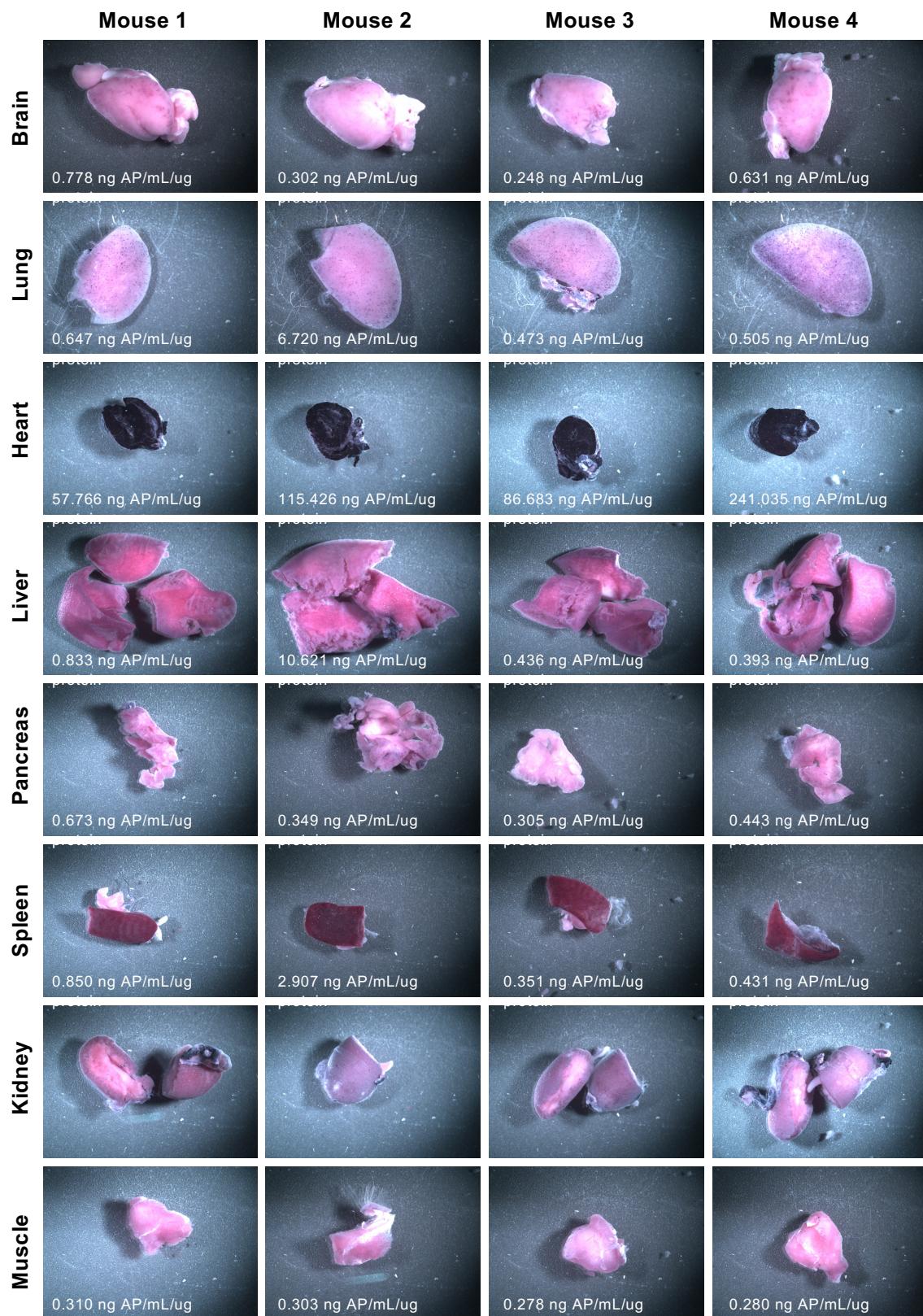
**Figure S15. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 2 delivered intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg of AAV9 expressing alkaline phosphatase (AP) driven by promoter 2 via intraperitoneal (IP) administration only. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV6.2FF-Promoter 7 Delivered IP**

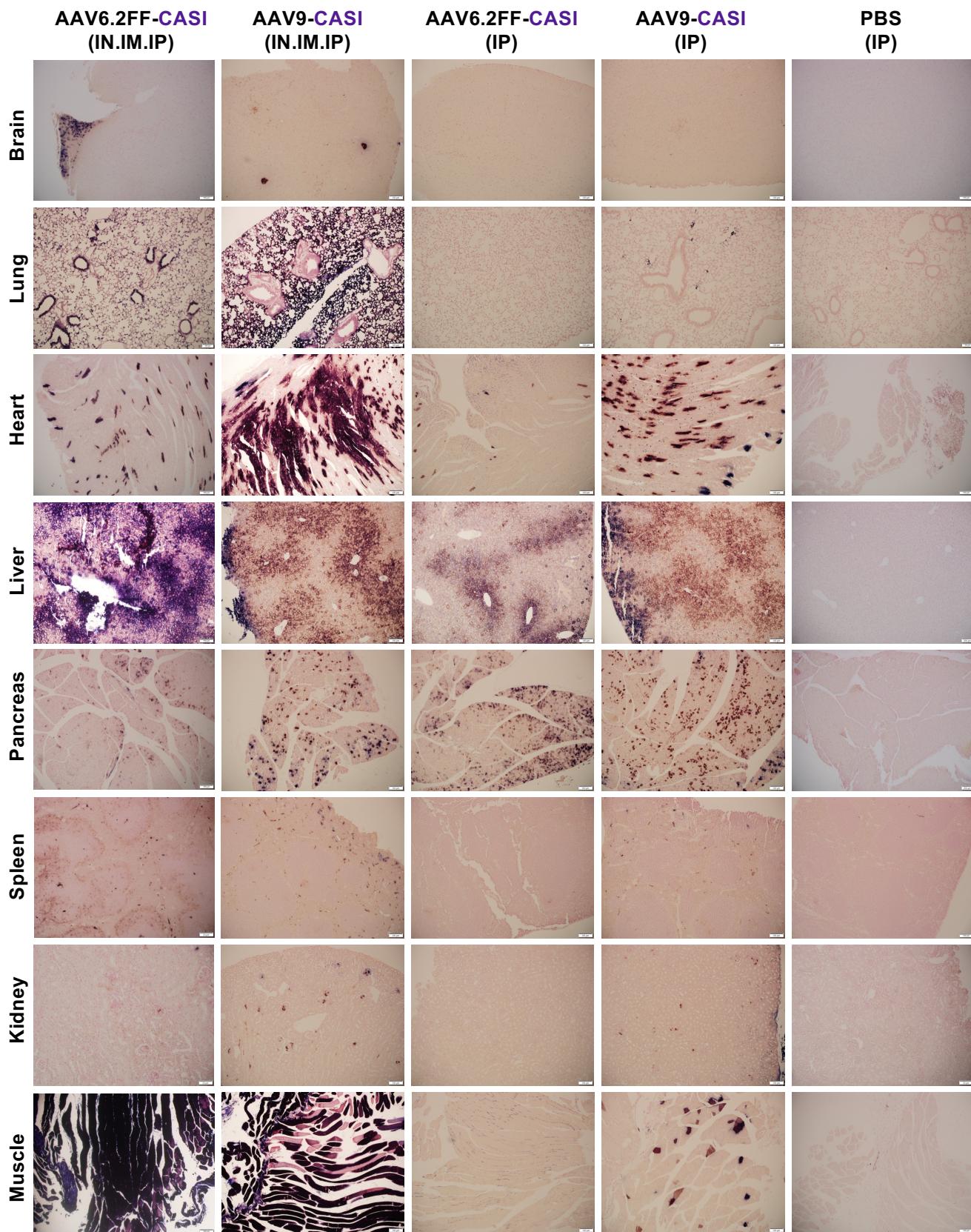


**Figure S16. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 7 delivered intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 7 via intraperitoneal (IP) administration only. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV9-Promoter 7 Delivered IP**

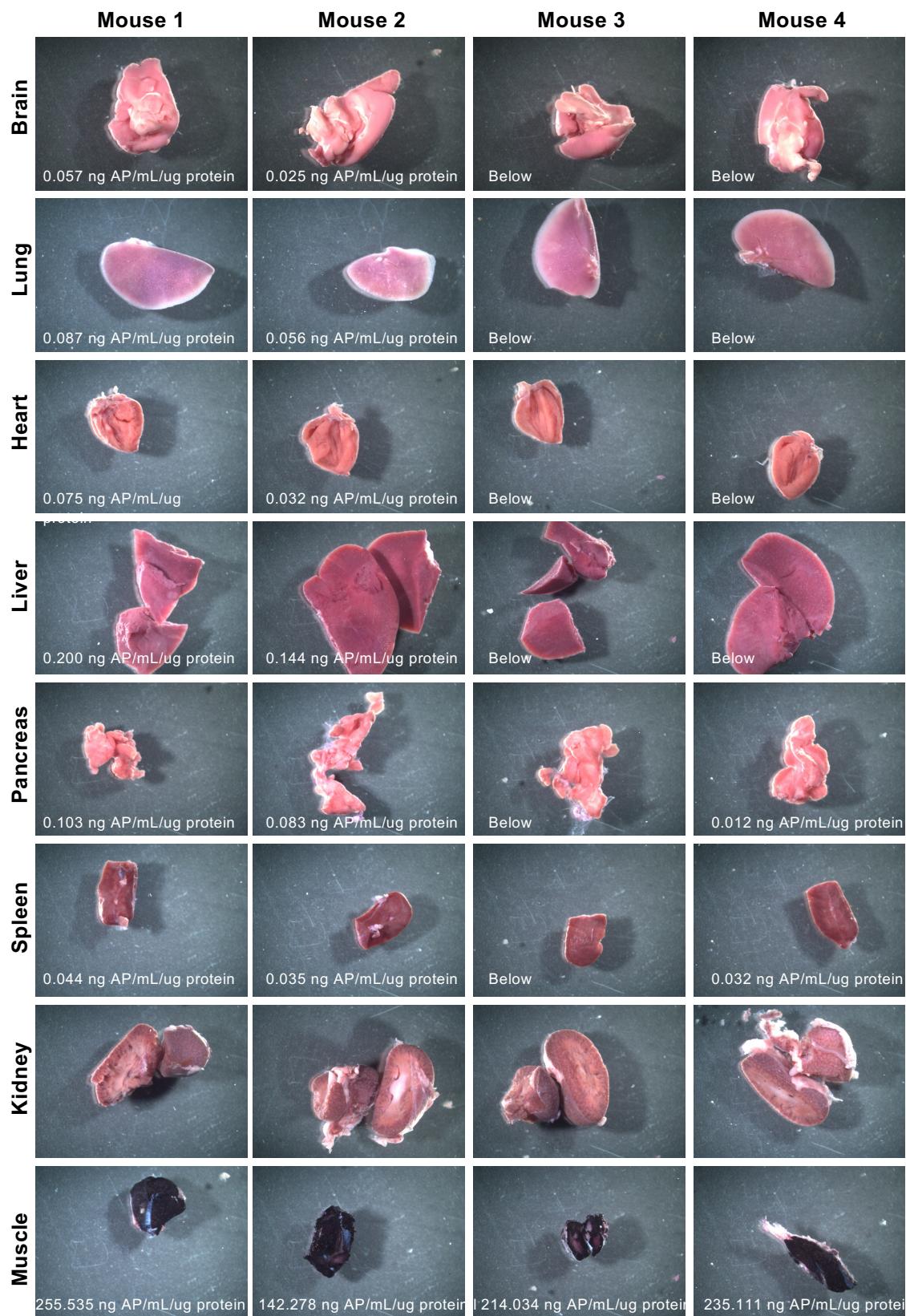


**Figure S17. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 7 delivered intraperitoneally.** C57BL/6 mice received  $1 \times 10^{11}$  vg of AAV9 expressing alkaline phosphatase (AP) driven by promoter 7 via intraperitoneal (IP) administration only. At three weeks post-AAV administration, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.



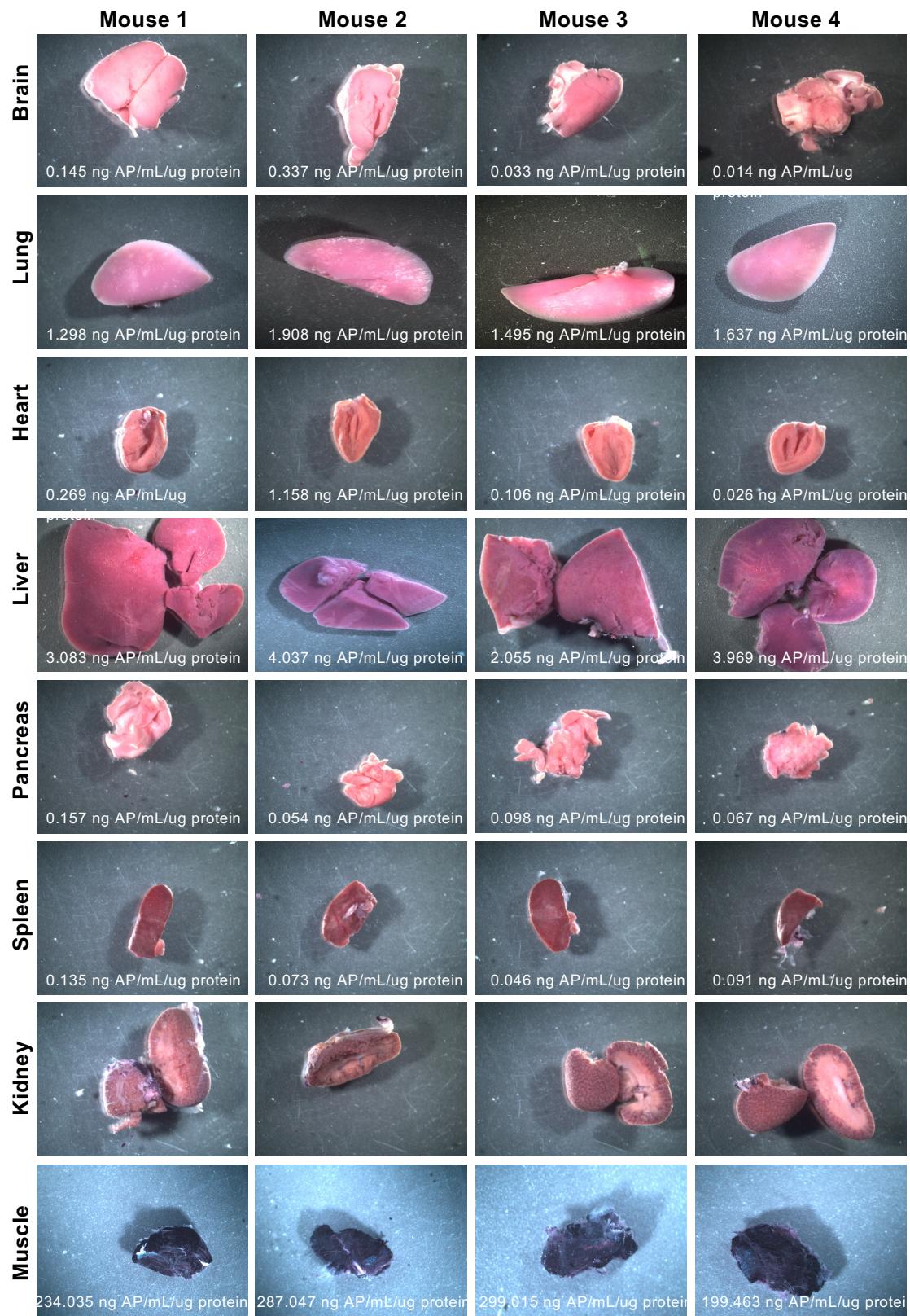
**Figure S18. Histological analysis of AAV-mediated AP expression driven by the CASI promoter across different AAV serotypes and different routes of administration.** Mice received either AAV6.2FF-AP or AAV9-AP driven by the CASI promoter administered via either combined routes [intranasal (IN), intramuscular (IM), and intraperitoneal (IP)] or a single intraperitoneal injection at a dose of  $1 \times 10^{11}$  vg per route. Mice were euthanized 21 days post AAV administration, and tissues were collected, fixed and stained for AP. Tissues were subsequently embedded in paraffin, sectioned and stained to detect AP expression, with nuclei counterstained using nuclear fast red. Representative images taken at 10x magnification are shown.

**AAV9-Promoter 3 Delivered IN, IM and IP**

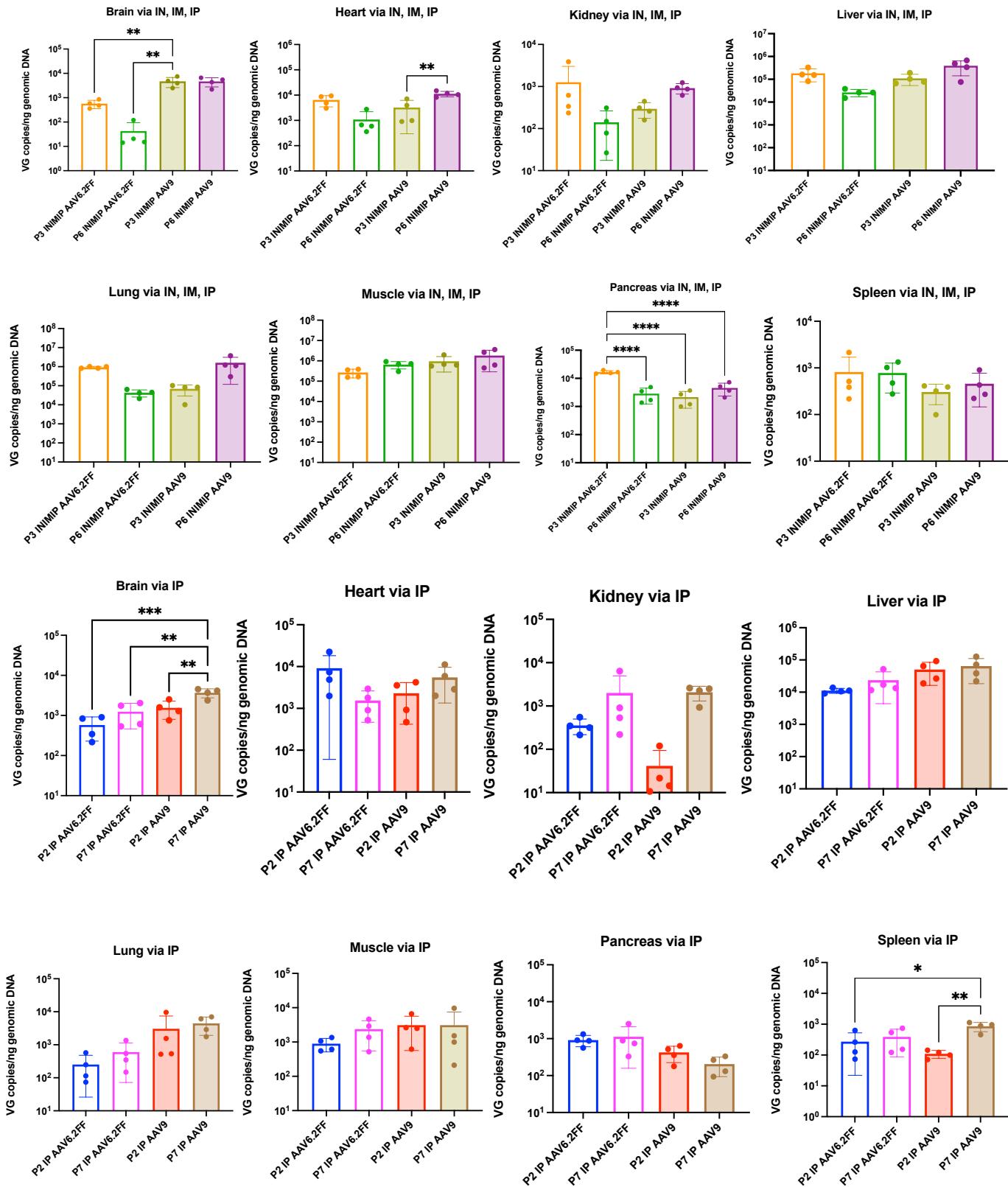


**Figure S19. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 3 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received AAV9 expressing alkaline phosphatase (AP) driven by promoter 3 via intranasal (IN), intramuscular (IM) and intraperitoneal (IP) administration at a dose of  $1 \times 10^{11}$  vg per route. Three weeks post-AAV, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.

**AAV9-Promoter 6 Delivered IN, IM and IP**



**Figure S20. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 6 delivered intranasally, intramuscularly, and intraperitoneally.** C57BL/6 mice received AAV9 expressing alkaline phosphatase (AP) driven by promoter 6 via intranasal (IN), intramuscular (IM) and intraperitoneal (IP) administration at a dose of  $1 \times 10^{11}$  vg per route. Three weeks post-AAV, mice were euthanized, and tissues were collected for either macroscopic AP staining or quantitative AP activity analysis. Shown are gross images from all mice and corresponding AP expression levels (ng AP/mL/ug protein) for each tissue.



**Figure S21. Tissue-wide AAV vector genome quantification.** DNA was extracted from paraffin-embedded tissue sections, and vector genome copies were quantified by qPCR, normalized to total genomic DNA (vg/ng) and corrected by subtracting background levels measured in each negative control tissue sample. The shaded bars represent mice that received the AAV9 capsid expressing alkaline phosphatase (AP) from promoters 2, 3, 6, or 7 by either combined intranasal (IN), intramuscular (IM), and intraperitoneal (IP) administration or IP administration only, at a dose of  $1 \times 10^{11}$  vg per route. Unshaded bars represent mice that received AAV6.2FF expressing AP from promoters 2, 3, 6 or 7 by either combined IN, IM, IP administration or IP administration only, at a dose of  $1 \times 10^{11}$  vg per route. Individual one-way ANOVAs were performed to compare the groups within each tissue. \*P  $\leq 0.05$ , \*\*P  $\leq 0.01$ , \*\*\*P  $\leq 0.001$ , and \*\*\*\*P  $\leq 0.0001$ .