

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

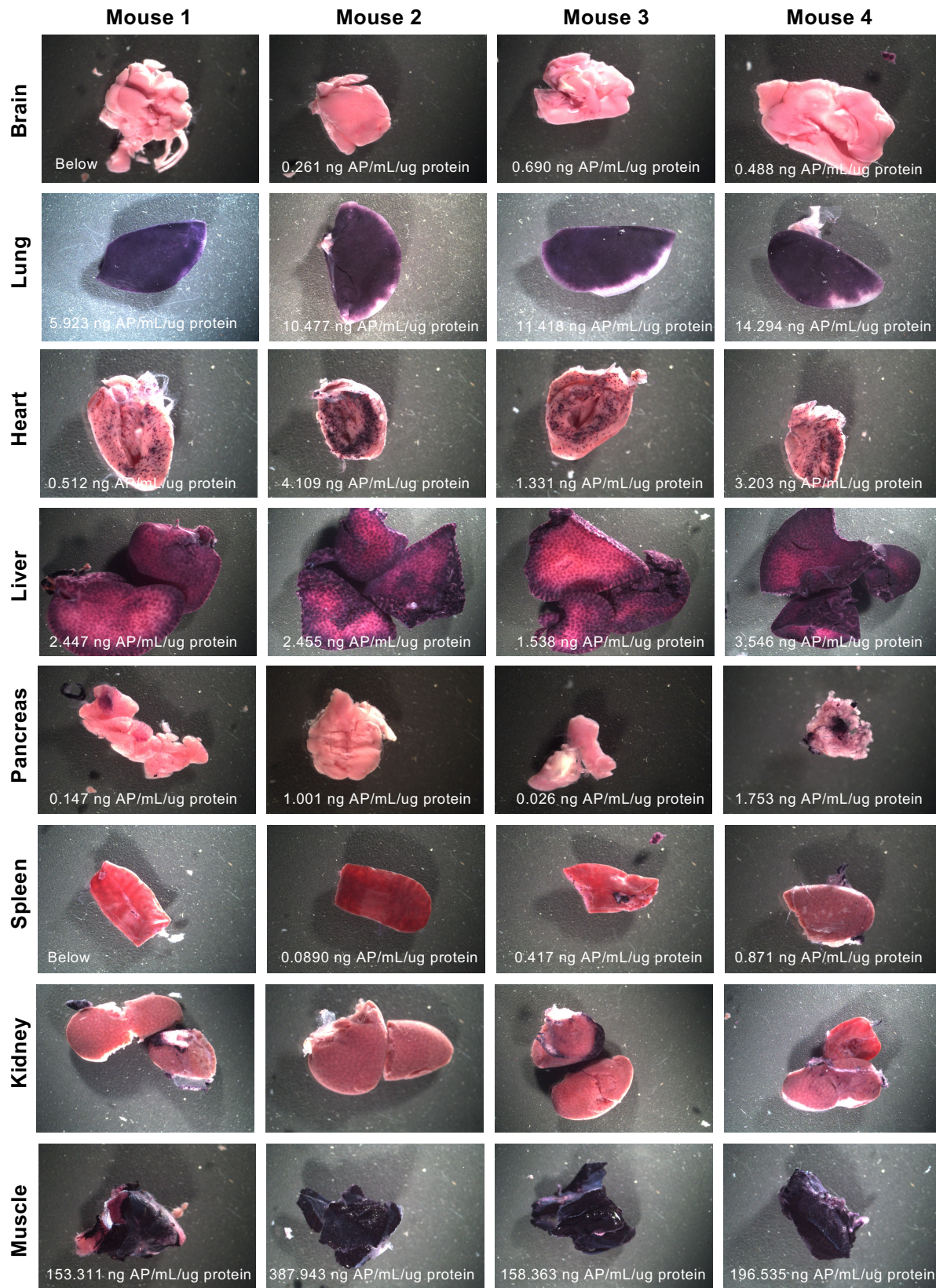


Figure S1. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 1 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 1 via 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

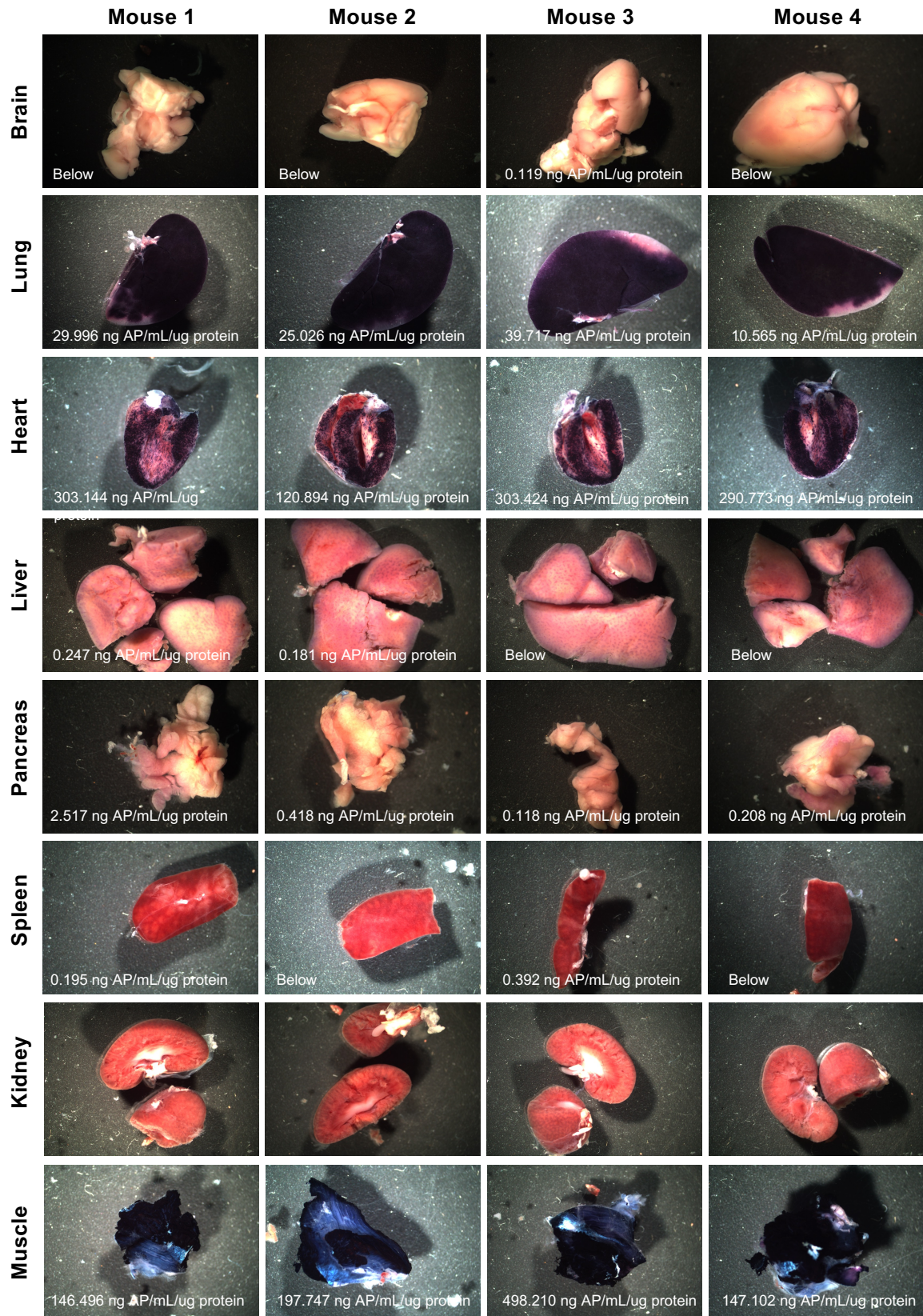


Figure S2. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 2 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 2 via 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/ μ g protein) for each tissue.

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

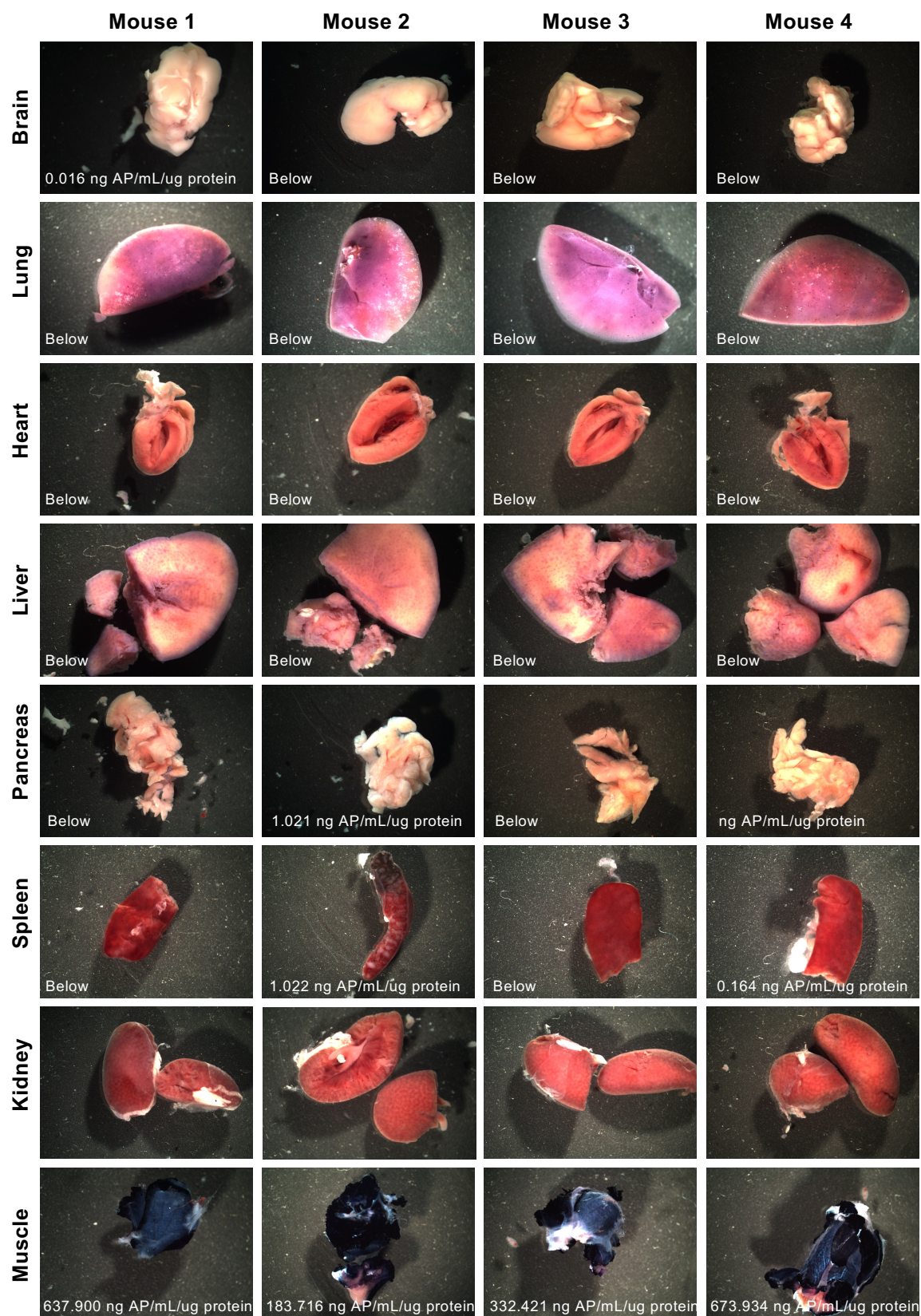


Figure S3. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 3 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 3 via 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

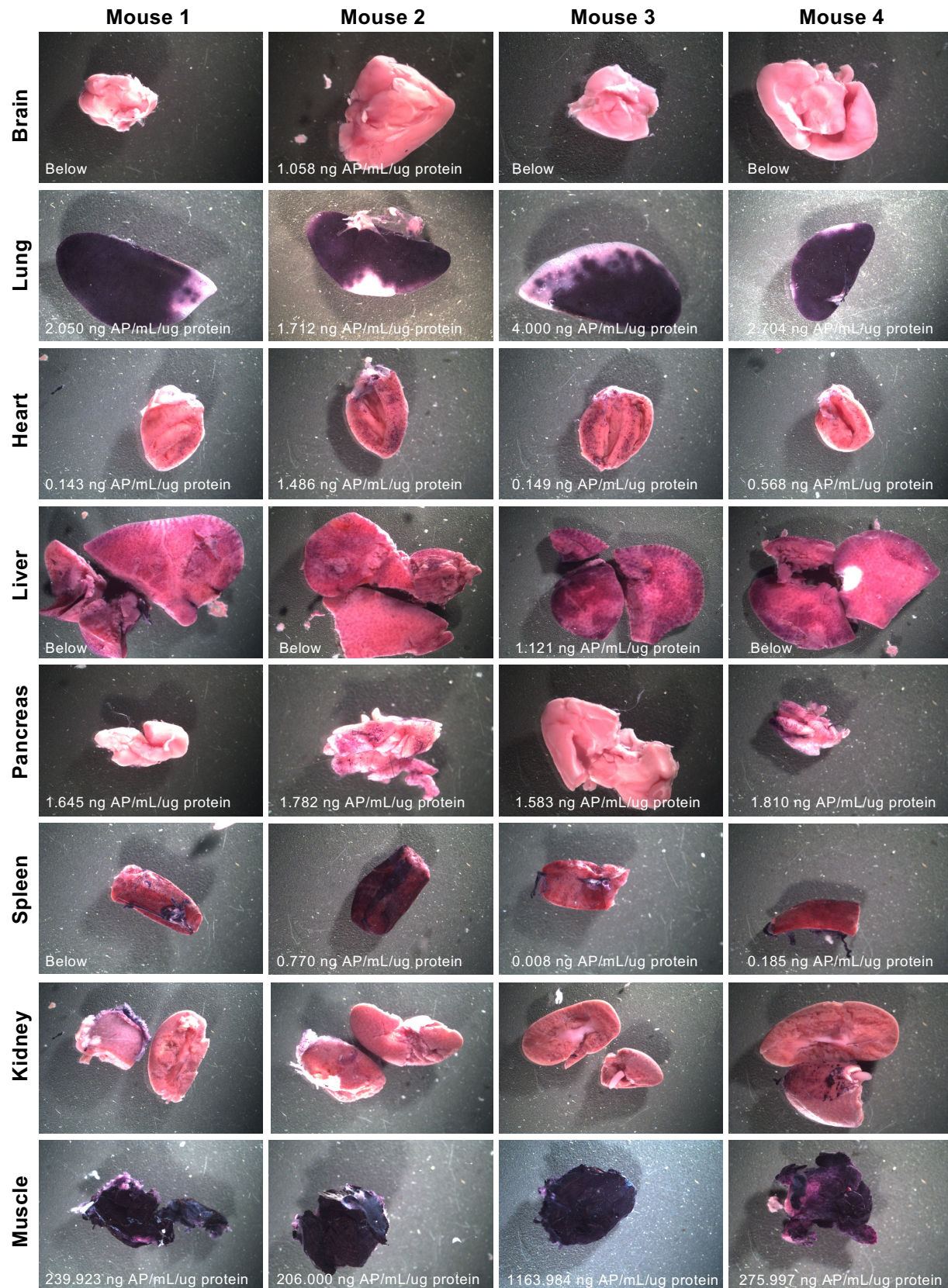


Figure S4. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 4 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 4 via 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

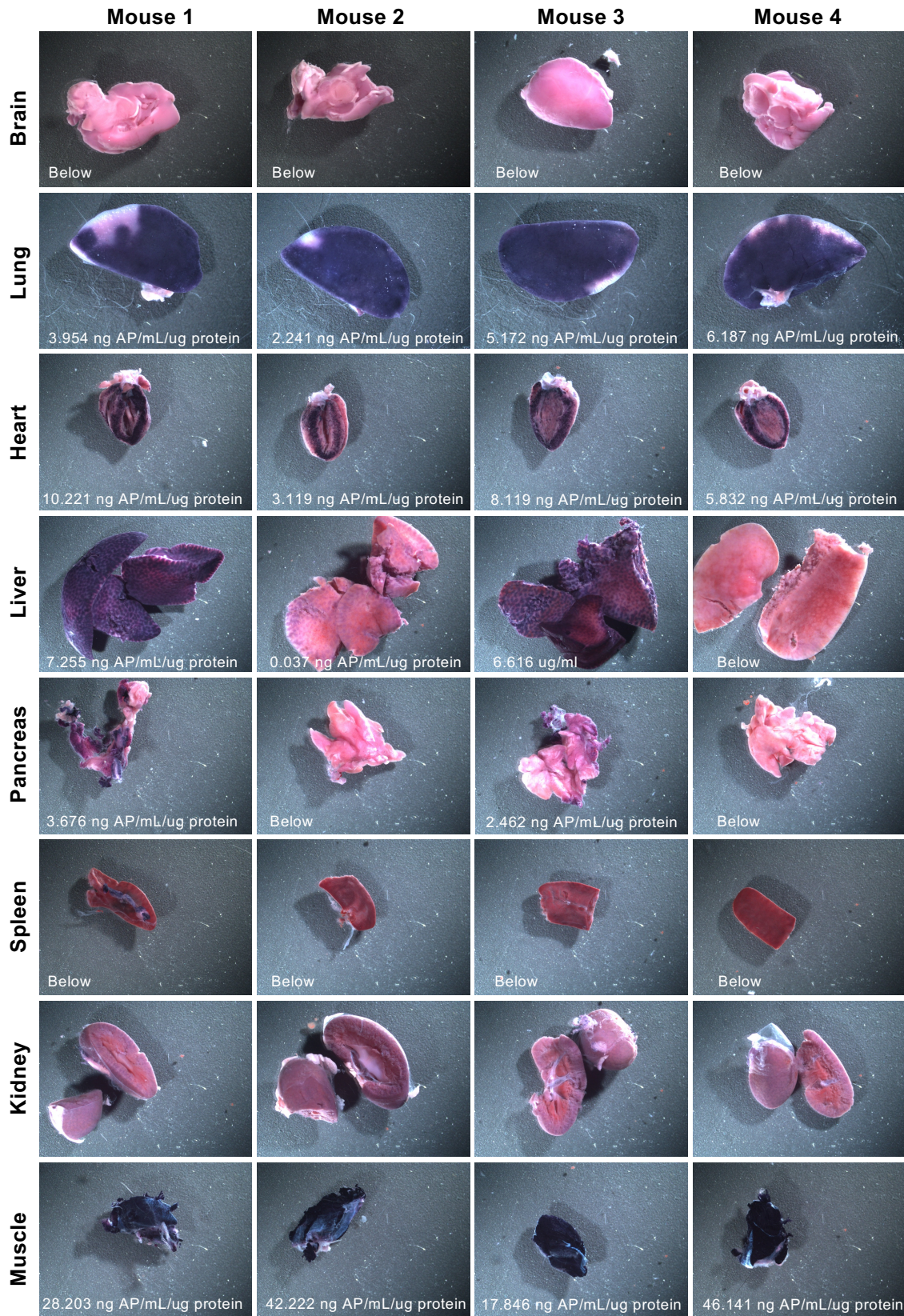


Figure S5. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 5 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 5 via 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

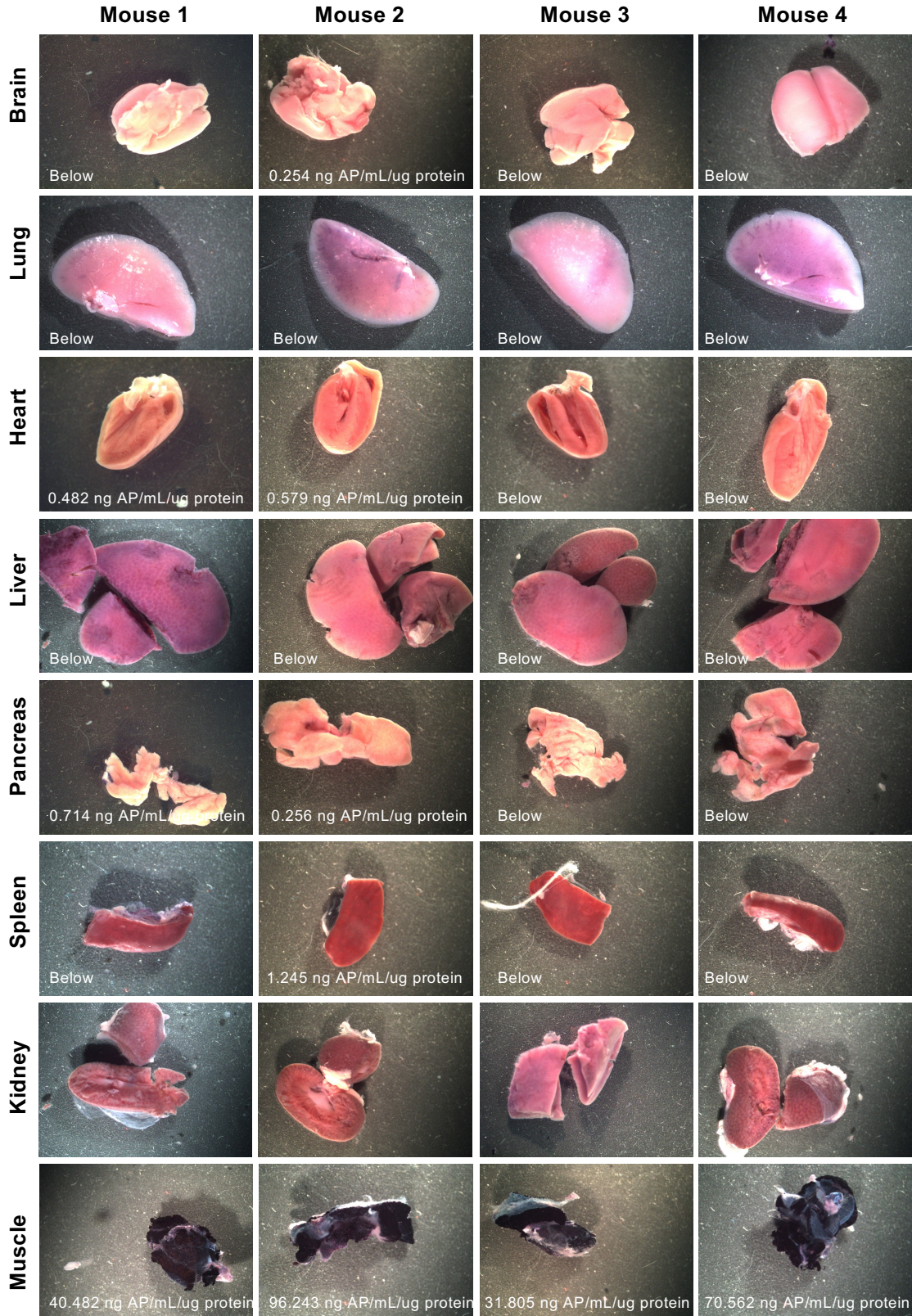


Figure S6. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 6 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 6 via 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

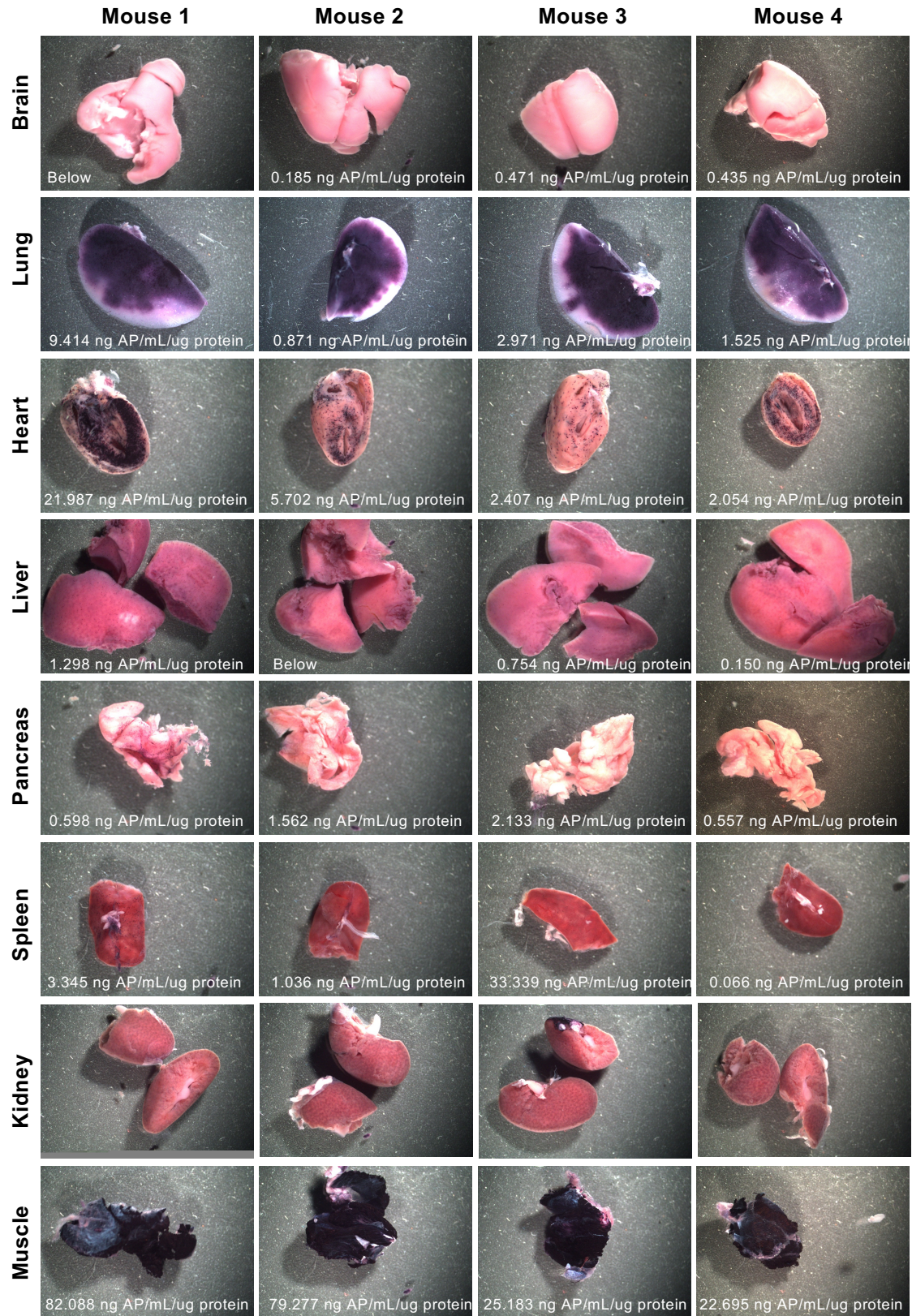


Figure S7. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 7 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 7 via 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

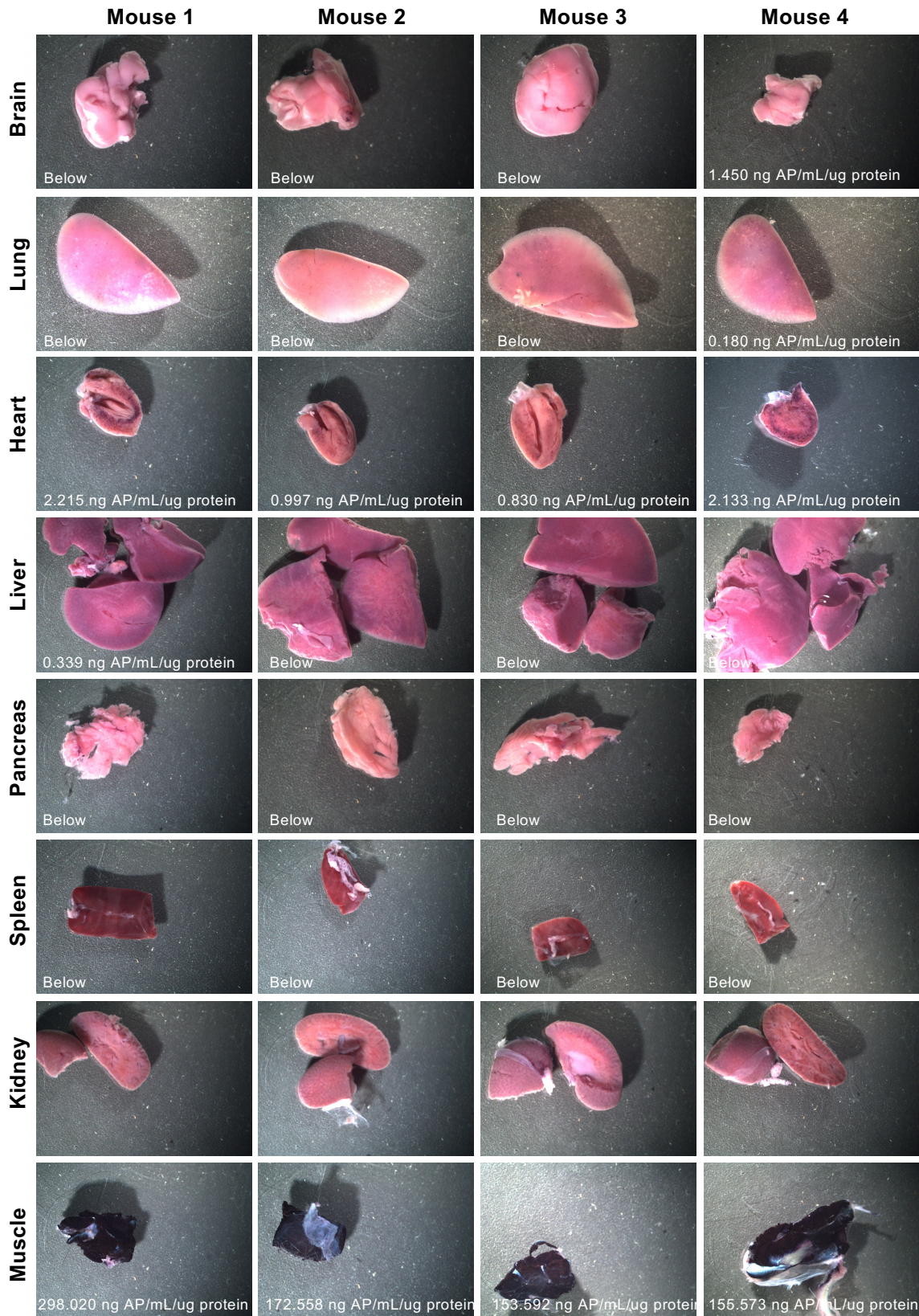


Figure S8. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 8 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 8 via 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/ μ g protein) for each tissue.

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

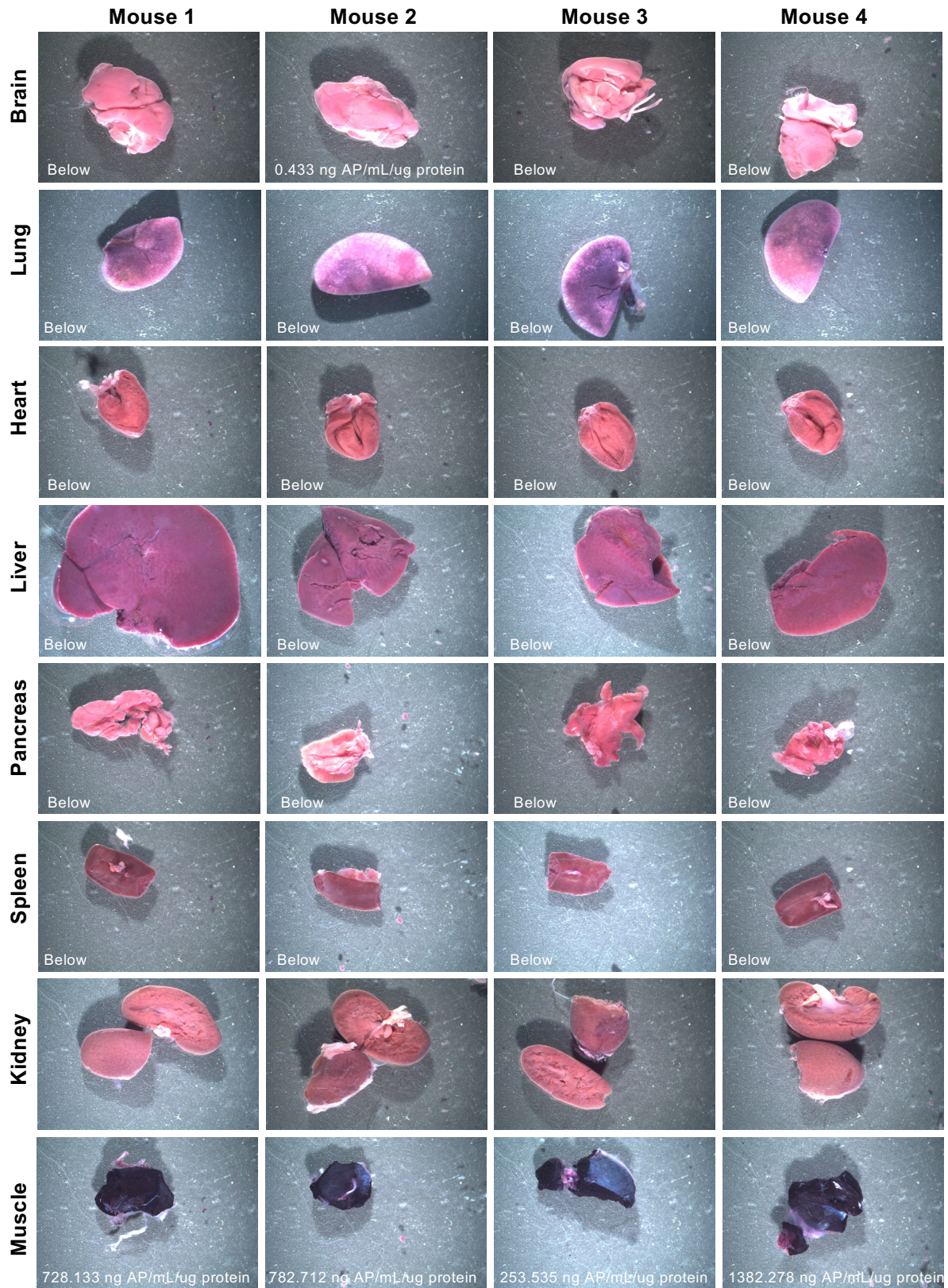


Figure S9. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 9 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 9 via 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

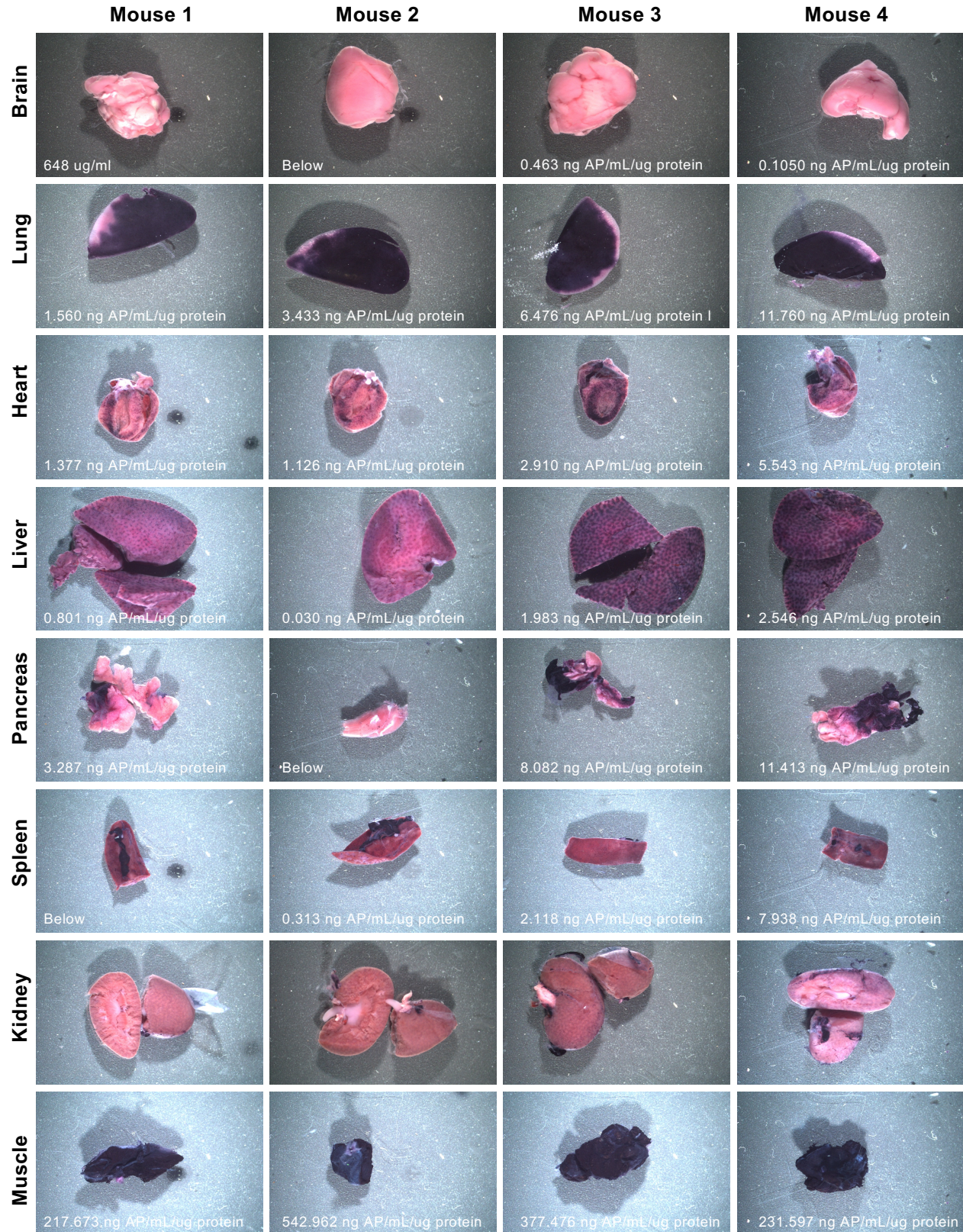


Figure S10. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 10 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 10 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

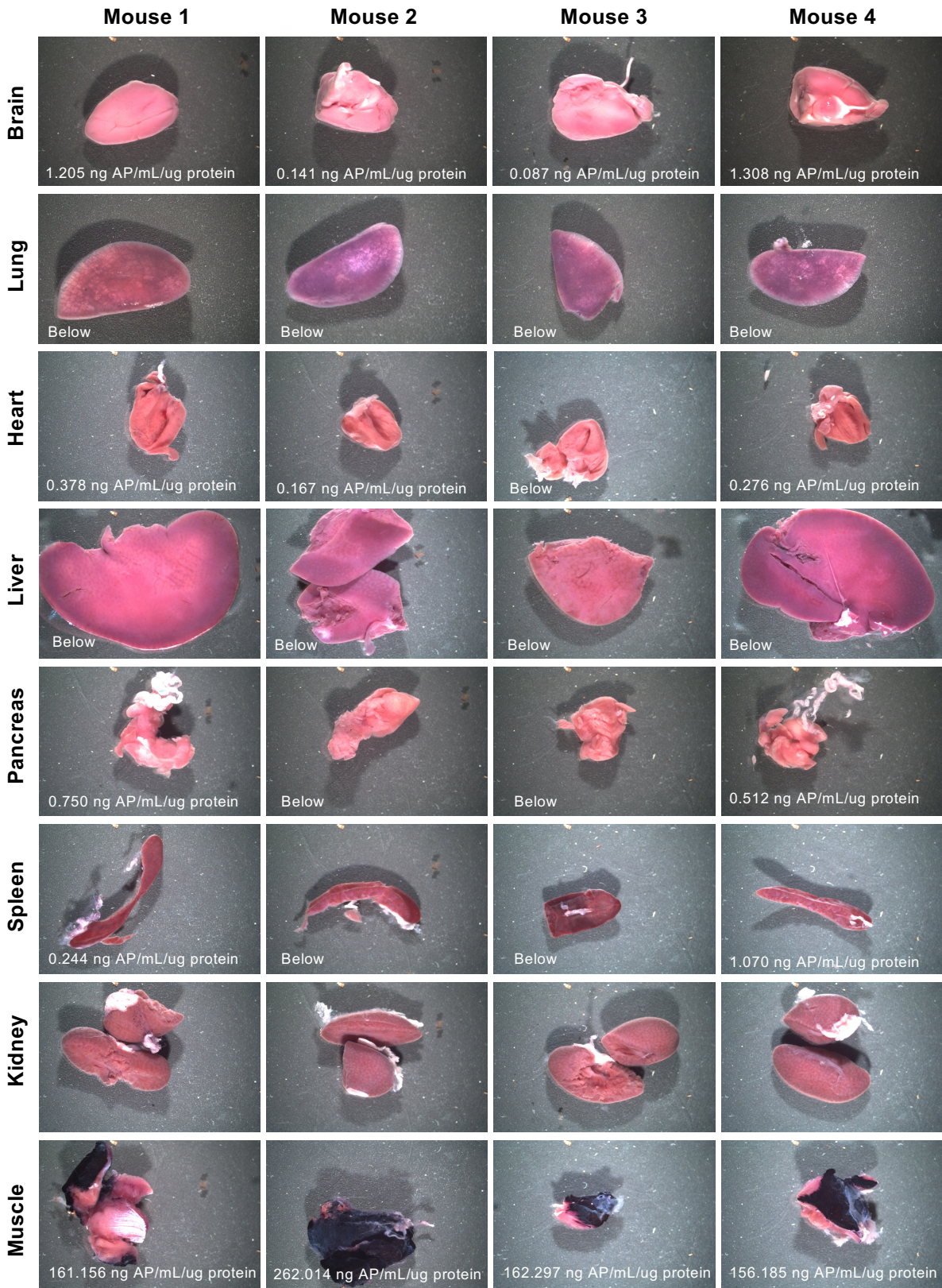


Figure S11. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 11 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 11 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

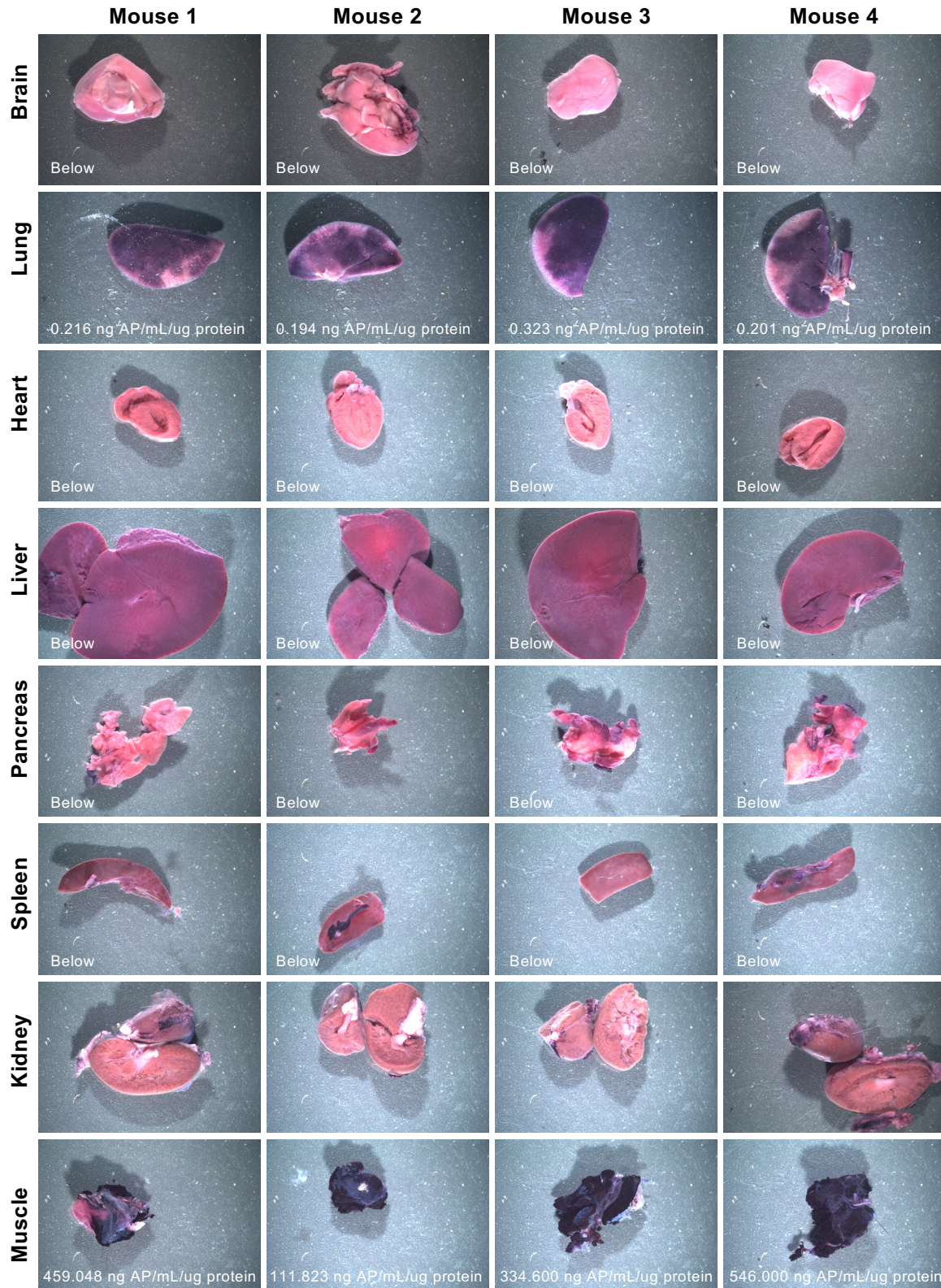


Figure S12. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 12 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received 1×10^{11} vg (per route) of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 12 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.

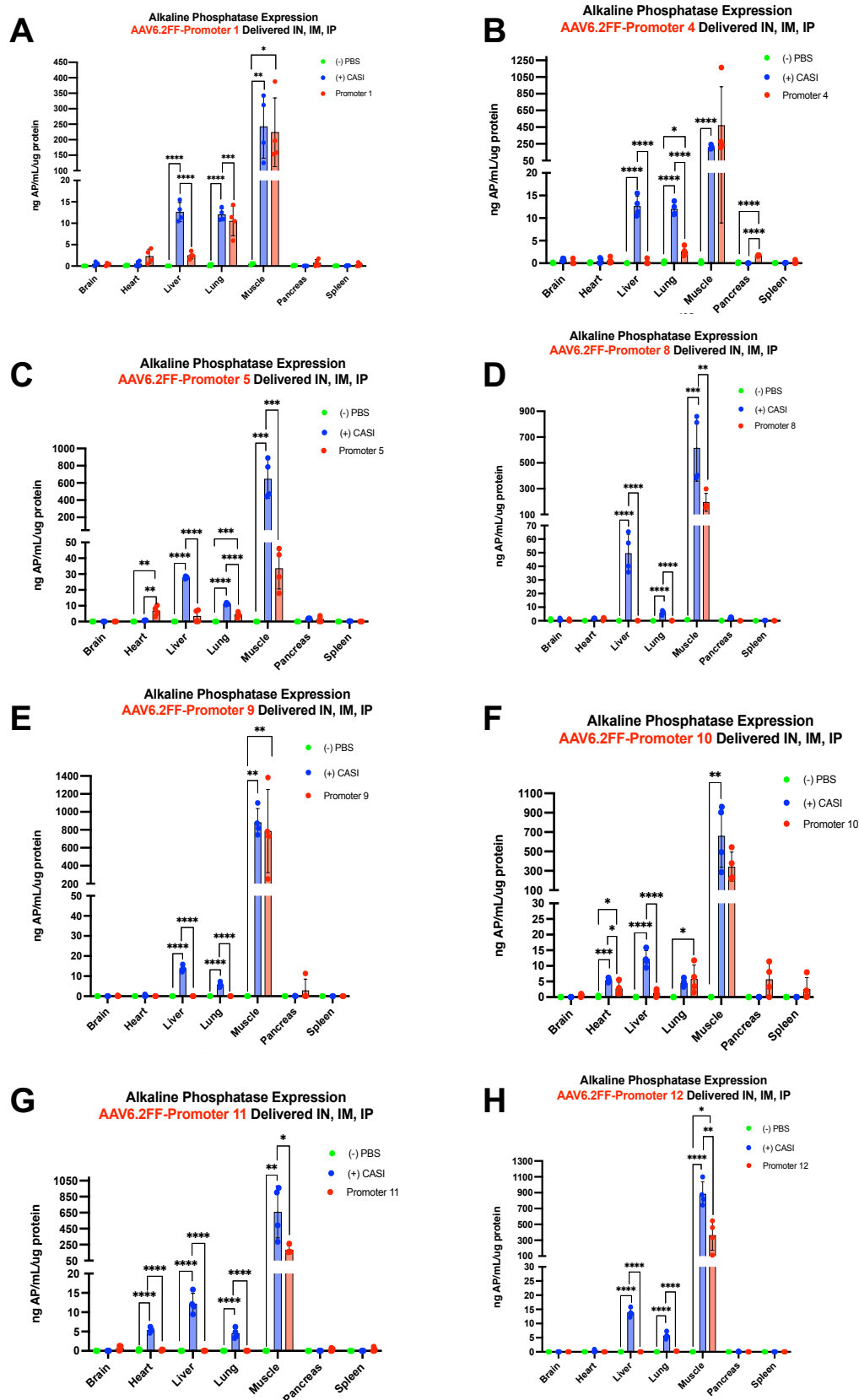


Figure S13. Quantitative analysis of tissue-wide AAV6.2FF-mediated AP expression driven by promoters 1, 4, 5, 8, 9, 10, 11 and 12 delivered intranasally, intramuscularly, and intraperitoneally. Mice received AAV6.2FF-AP driven by promoters (A) 1, (B) 4, (C) 5, (D) 8, (E) 9, (F) 10, (G) 11, or (H) 12, intranasally, intramuscularly and intraperitoneally at a dose of 1×10^{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

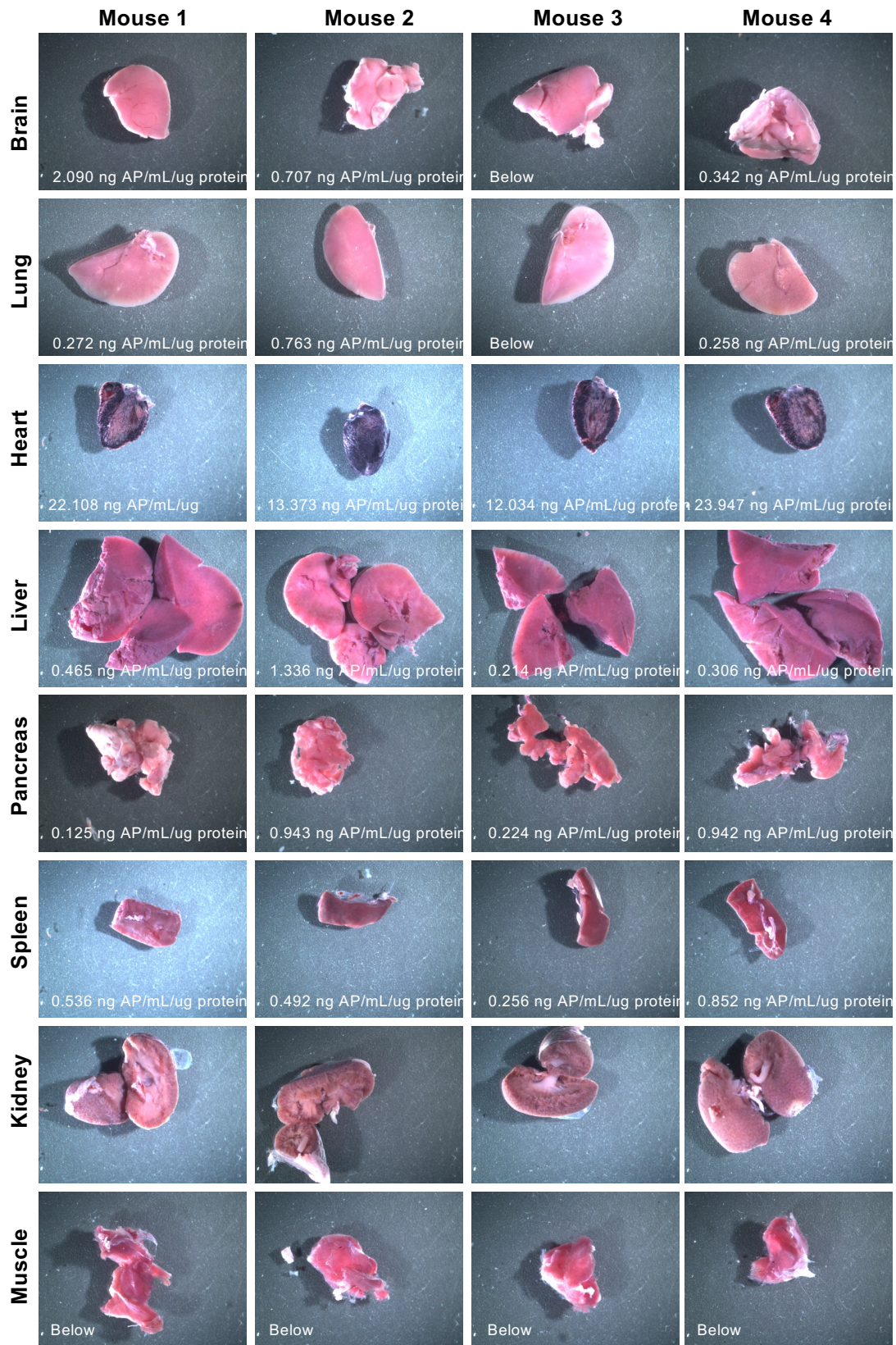


Figure S14. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 2 delivered intraperitoneally. C57BL/6 mice received 1×10^{11} vg of AAV6.2FF expressing alkaline phosphatase (AP) driven by promoter 2 via intraperitoneal (IP) administration only. At 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/ μ g protein) for each tissue.

AAV9-Promoter 2 Delivered IP

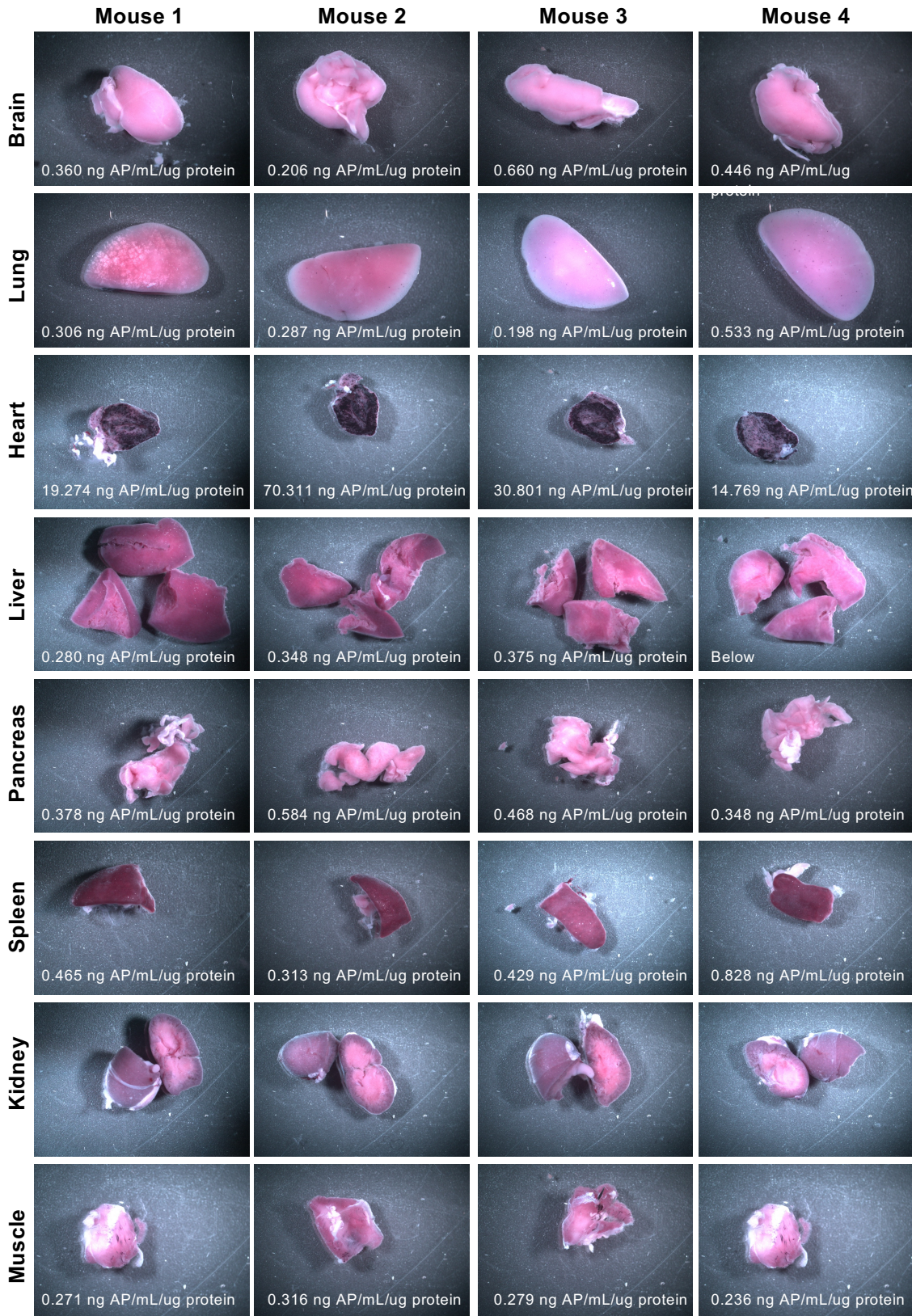


Figure S15. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 2 delivered intraperitoneally. C57BL/6 mice received 1×10^{11} vg of AAV9 expressing alkaline phosphatase (AP) driven by promoter 2 via intraperitoneal (IP) administration only. At three weeks 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


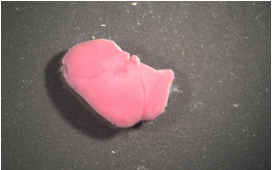
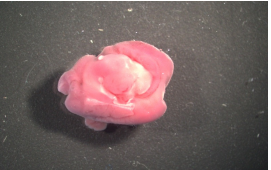









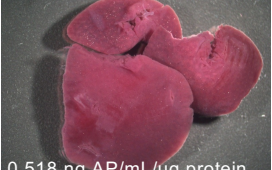



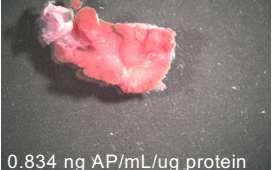
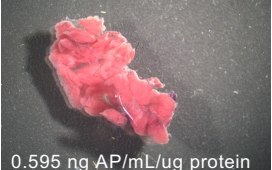
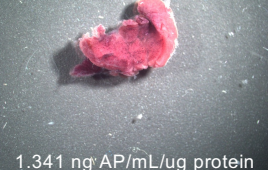
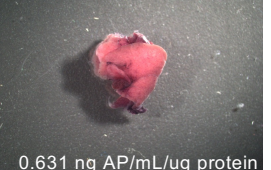
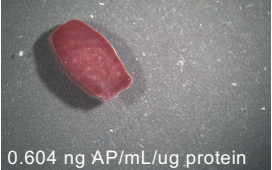
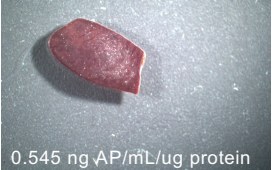

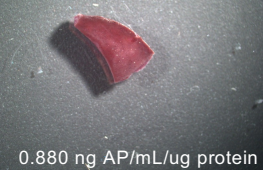




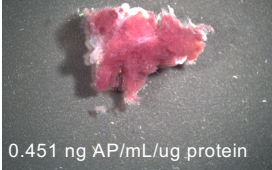
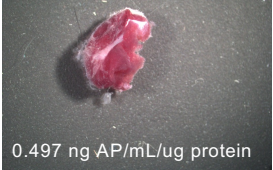

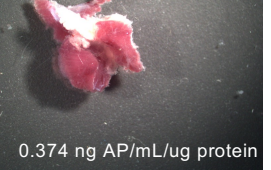
	Mouse 1	Mouse 2	Mouse 3	Mouse 4
Brain	 0.683 ng AP/mL/ug protein	 0.504 ng AP/mL/ug protein	 1.103 ng AP/mL/ug protein	 Below
Lung	 0.543 ng AP/mL/ug protein	 0.596 ng AP/mL/ug protein	 0.466 ng AP/mL/ug protein	 0.347 ng AP/mL/ug protein
Heart	 1.328 ng AP/mL/ug	 3.486 ng AP/mL/ug protein	 7.840 ng AP/mL/ug protein	 6.037 ng AP/mL/ug protein
Liver	 0.518 ng AP/mL/ug protein	 0.498 ng AP/mL/ug protein	 0.568 ng AP/mL/ug protein	 0.546 ng AP/mL/ug protein
Pancreas	 0.834 ng AP/mL/ug protein	 0.595 ng AP/mL/ug protein	 1.341 ng AP/mL/ug protein	 0.631 ng AP/mL/ug protein
Spleen	 0.604 ng AP/mL/ug protein	 0.545 ng AP/mL/ug protein	 0.705 ng AP/mL/ug protein	 0.880 ng AP/mL/ug protein
Kidney	 	 	 	
Muscle	 0.451 ng AP/mL/ug protein	 0.497 ng AP/mL/ug protein	 0.439 ng AP/mL/ug protein	 0.374 ng AP/mL/ug protein

Figure S16. Tissue-wide macroscopic and quantitative analysis of AAV6.2FF-mediated AP expression driven by promoter 7 delivered intraperitoneally. C57BL/6 mice received 1×10^{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/ μ g protein) for each tissue.

AAV9-Promoter 7 Delivered IP

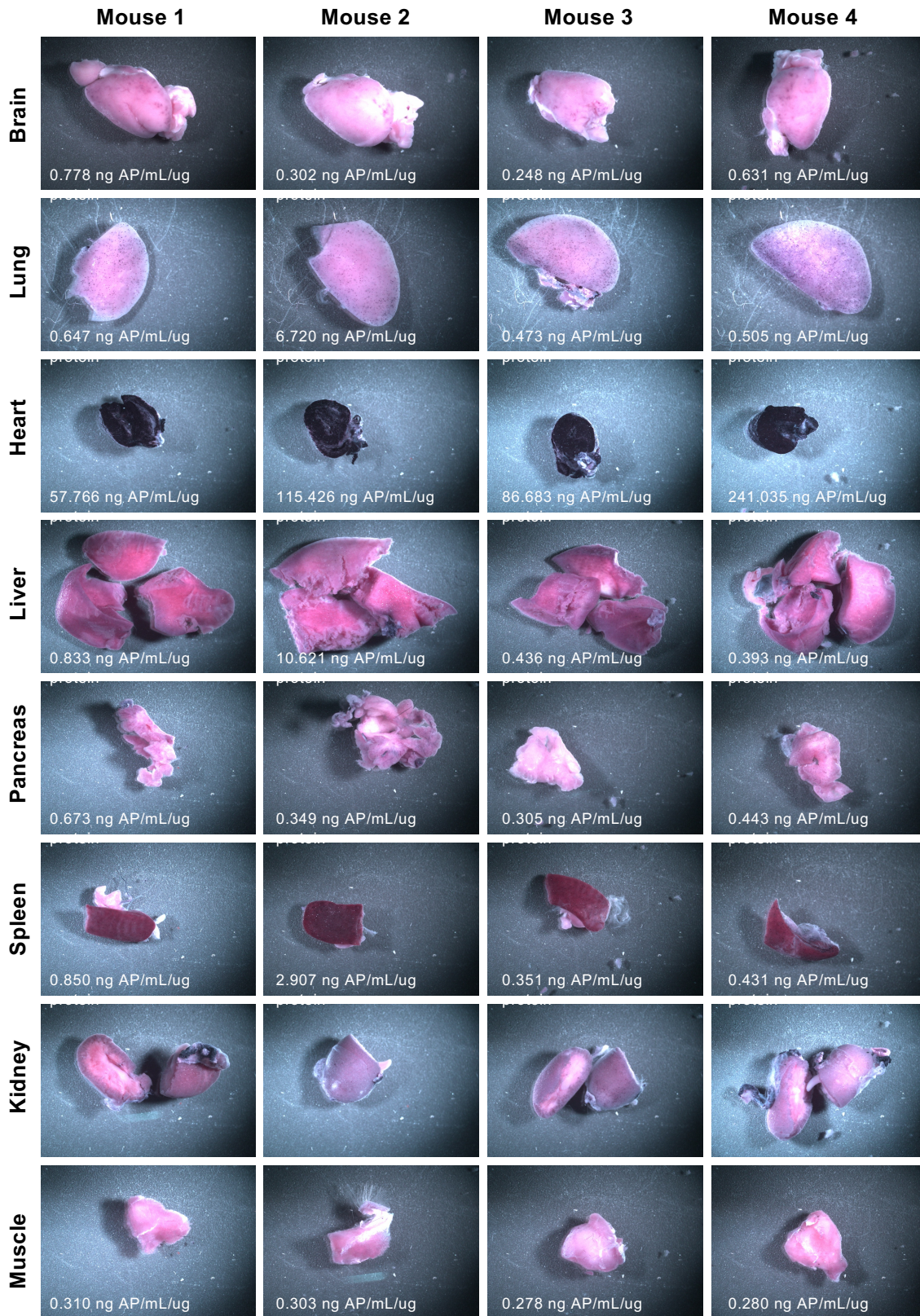


Figure S17. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 7 delivered intraperitoneally. C57BL/6 mice received 1×10^{11} vg of AAV9 expressing alkaline phosphatase (AP) driven by promoter 7 via intraperitoneal (IP) administration only. At three weeks 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.

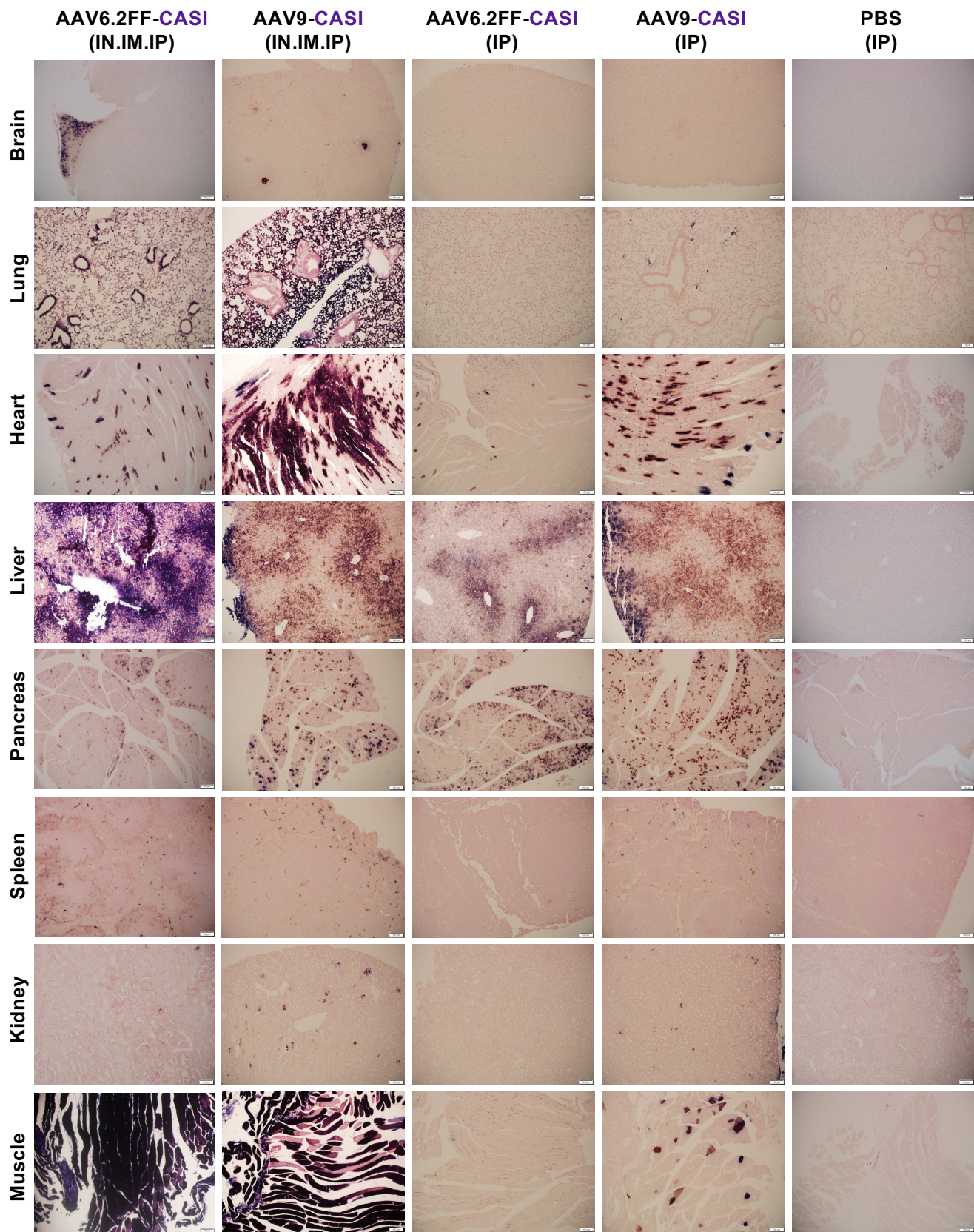


Figure S18. Histological analysis of AAV-mediated AP expression driven by the CASI promoter across different AAV serotypes and different routes of administration. Mice received either AAV6.2FF-AP or AAV9-AP driven by the CASI promoter administered via either combined routes [intranasal (IN), intramuscular (IM), and intraperitoneal (IP)] or a single intraperitoneal injection at a dose of 1×10^{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

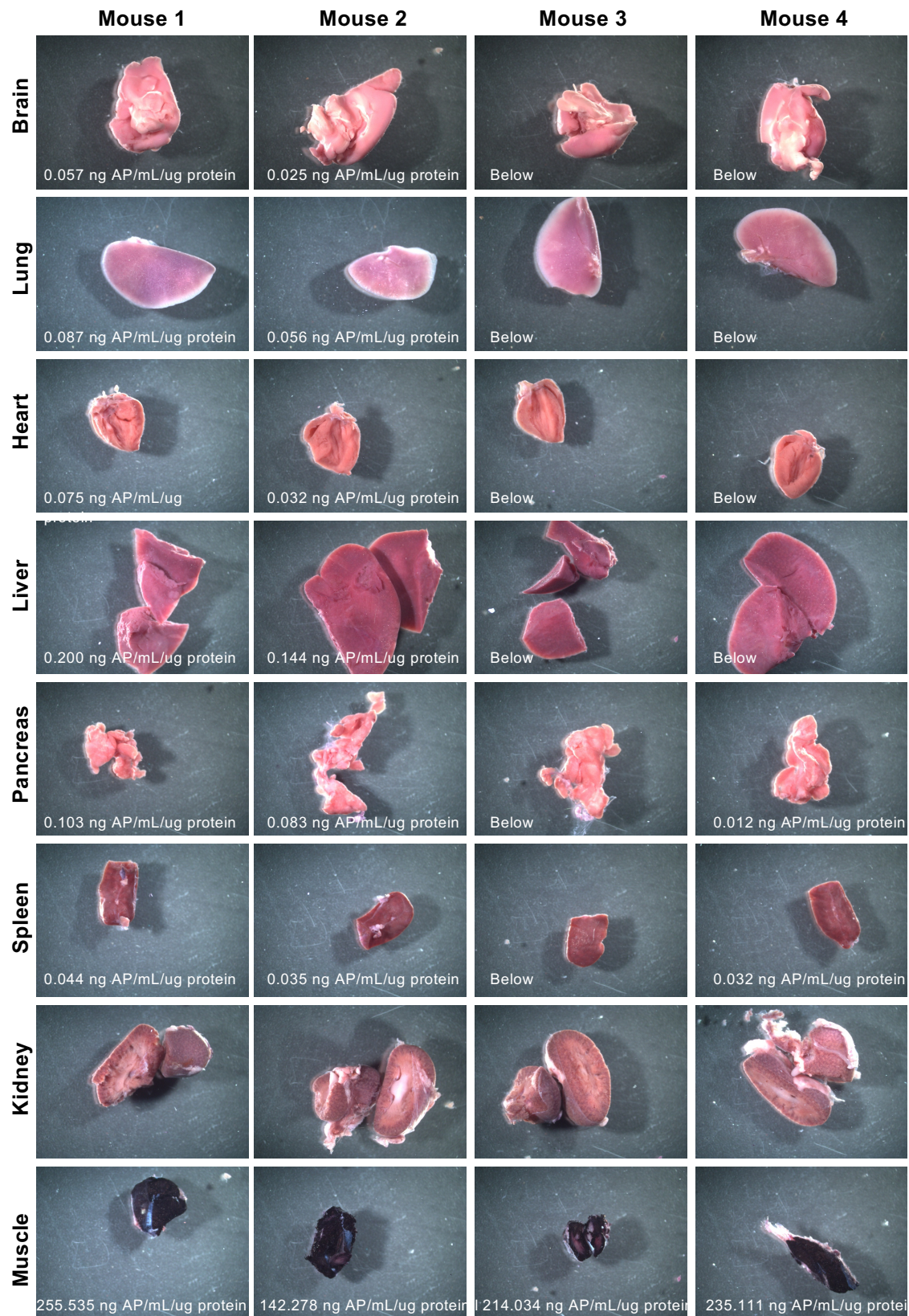


Figure S19. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 3 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received AAV9 expressing alkaline phosphatase (AP) driven by promoter 3 via intranasal (IN), intramuscular (IM) and intraperitoneal (IP) administration at a dose of 1×10^{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/ μ g protein) for each tissue.

AAV9-Promoter 6 Delivered IN, IM and IP

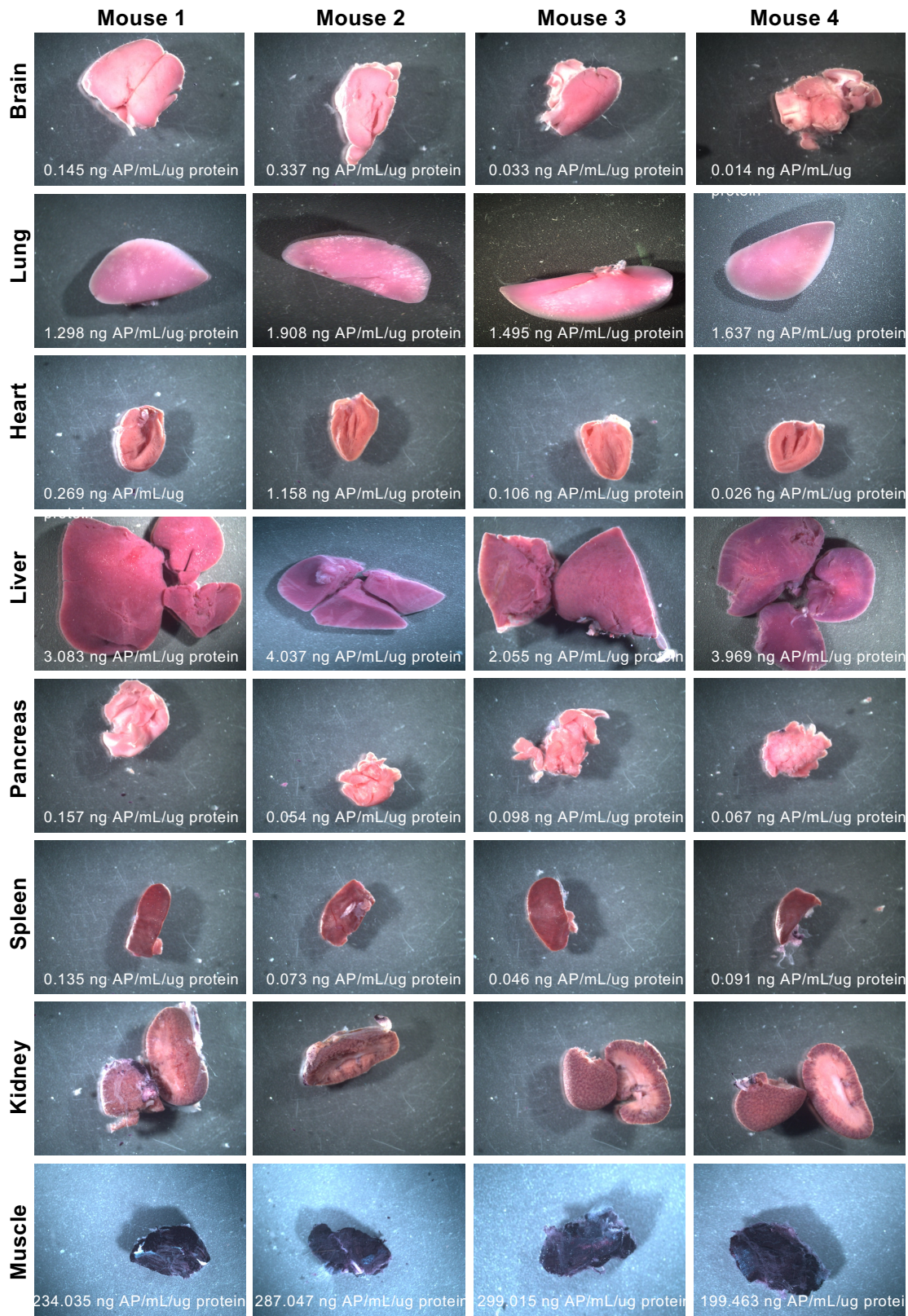


Figure S20. Tissue-wide macroscopic and quantitative analysis of AAV9-mediated AP expression driven by promoter 6 delivered intranasally, intramuscularly, and intraperitoneally. C57BL/6 mice received AAV9 expressing alkaline phosphatase (AP) driven by promoter 6 via intranasal (IN), intramuscular (IM) and intraperitoneal (IP) administration at a dose of 1×10^{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.

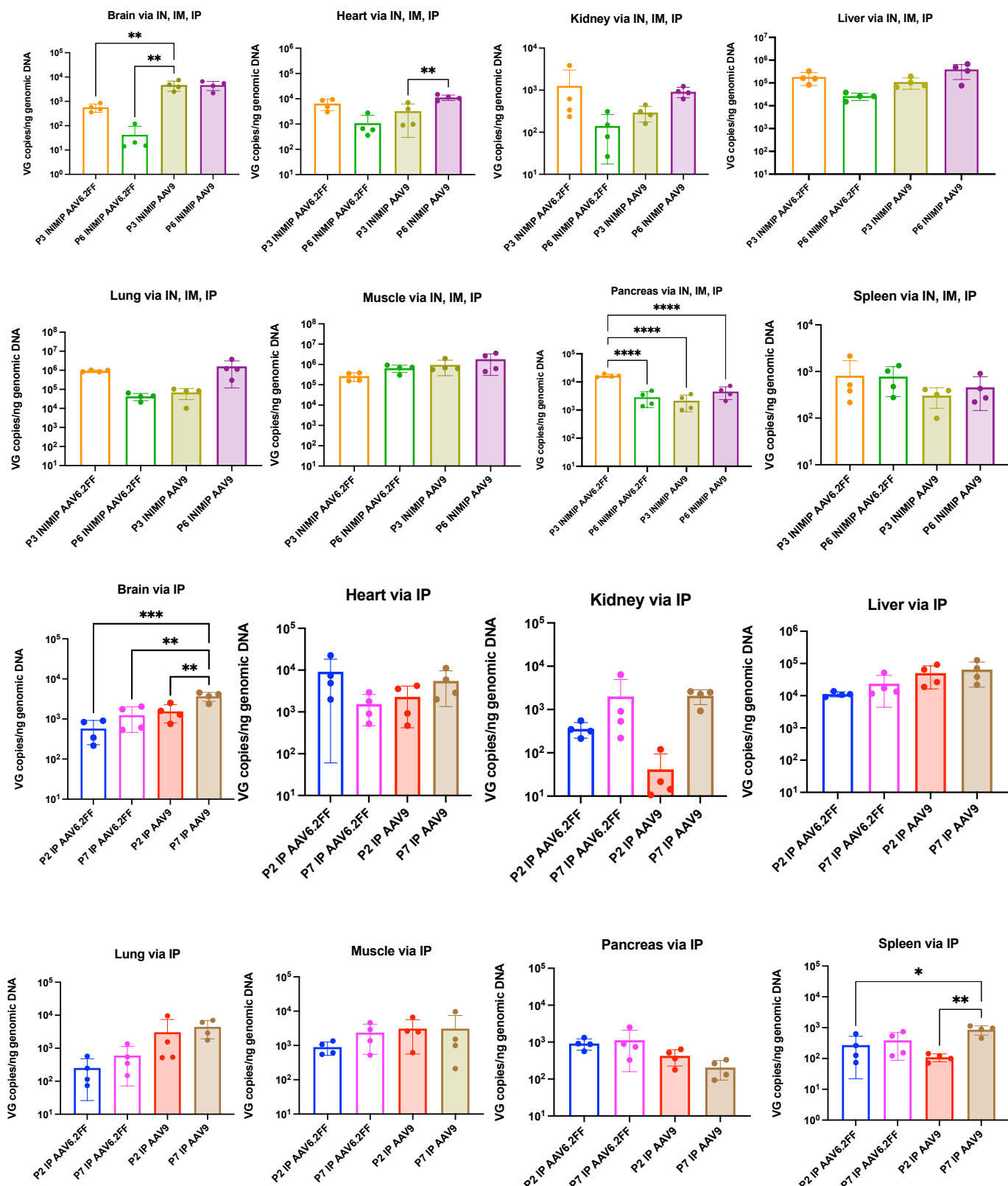


Figure S21. Tissue-wide AAV vector genome quantification. DNA was extracted from paraffin-embedded tissue sections, and vector genome copies were quantified by qPCR, normalized to total genomic DNA (vg/ng) and corrected by subtracting background levels measured in each negative control tissue sample. 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 1×10^{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 1×10^{11} vg per route. Individual one-way ANOVAs were performed to compare the groups within each tissue. *P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001..