Supporting Information for:

Gd-Labeled Microparticles in MRI: *In Vivo* Imaging of Microparticles after Intraperitoneal Injection

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1. Reagents

Unless otherwise noted, reagents were purchased from commercial sources and used as received. Triethylamine, toluene, and methylene chloride were distilled prior to use, and acetonitrile (MeCN) was stored over molecular sieves.

2. Syntheses of APMS functionalized on its external surface with TEG

APMS. Cetyltrimethylammonium bromide (CTAB, 1.8 g, 4.94 mmol) was dissolved in an aqueous acidic solution composed of deionized water (39.6 g), ethanol (200 proof, 11.1 g, 241 mmol), and concentration HCl (36.5wt%, 4.4 g, 44 mmol). After TEOS (4.0 g, 19.2 mmol) was added into this solution and stirred at 800 rpm for 5 minutes, the NaF (0.5 M aqueous solution, 4.76 g, 2.33 mmol) was added and the mixture was stirred until a white precipitate was observed (~90 s), at which point it was immediately transferred to a 125 ml Teflon bottle and placed into a 100 ºC oven for 40 min. The resulting mixture was then cooled to room temperature, and the white precipitate was collected by vacuum filtration and rinsed with 10 ml of deionized water twice, and dried under heat and vacuum for 48 hours to yield as-synthesized APMS. The surfactant was further removed by calcination at 550 ºC for 6 h under air to produce the product designated as calcined APMS.

APMS functionalized on its external surface with secondary amines (APMS-NHMe). After as-synthesized (uncalcined) APMS (0.60 g) was dried under vacuum for 48 hrs at 80°C, it was suspended in 20 mL of dry hexanes. Under an atmosphere of dry N_2, 3-(*N*-methylamino)propyltrimethoxysilane (MAPTMS, 0.4 mmol, 77.4 g, 78.8 µl) was added via syringe. The reaction mixture was refluxed 2 hrs, then the powder was recovered by filtration, washing, and air-dried. The surfactant in the functionalized APMS was removed by refluxing in large excess (200 mL per gram of APMS) of acidified ethanol (0.1 M HCl) for 4 hrs. The extraction process was repeated twice. The functionalized APMS was filtered off, washed with absolute ethanol several times, and finally dried under vacuum.

Monotosyl tetraethylene glycol (12-(p-Tosylsulfonyl)-3,6,9,12-tetraoxadodecanol, TEG-Ts). Monotosyl tetraethylene glycol was prepared by a modified reaction described by Bartz et al. 12 g of tetraethylene glycol (TEG, 0.062 mol) and 2.530 g (3.48 ml, 0.025 mol) of anhydrous triethylamine were dissolved in 75 ml of anhydrous acetonitrile. A solution of *p*-toluenesulfonyl chloride (TsCl, 4.766g, 0.025 mol) in 20 ml of dry acetonitrile was added dropwise over 1 h at 0°C. The reaction mixture was then stirred for 14 h at room temperature under an inert atmosphere. Precipitated triethylamine hydrochloride was filtered off and washed with acetonitrile, the solution was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and loaded onto a silica gel column, and then eluted with hexane/ethyl acetate ((1:1, v/v) -> (0:1, v/v)). The remaining TsCl eluted first (0.26 g, 0.00136 mol, R_f = 0.89 in ethyl acetate). Tetraethylene glycol ditosylate (colorless oil) eluted followed by tetraethylene glycol tosylate (colorless

oil). The second faction was the disulfonate (0.85 g, 0.00169 mol, R_f = 0.75 in ethyl acetate, 6.8% yield based on *p*-toluenesulfonyl chloride). The third faction was the monosulfonate (4.71 g, 0.0135 mol, R_f = 0.24~0.27 in ethyl acetate, 65% yield based on *p*-toluenesulfonyl chloride). ^{1}H NMR of the monotosylate (500 MHz, $CDCl_3$): δ = 7.792 (2H, dd, *J* = 8.29, 2.67 Hz, H-Ar), 7.335 (2H, dd, *J* = 7.95, 2.66 Hz, H-Ar), 4.163 (2H, t, *J* = 4.86 Hz, Ts-O$\underline{CH_2}$), 3.697 (2H, t, *J* = 4.85 Hz, TsOCH_2-$\underline{CH_2}$-O), 3.640 (2H, t, OCH_2-$\underline{CH_2}$-OH), 3.584~3.604 (10H, m, O-$\underline{CH_2}$), 2.442 (3H, s, $\underline{CH_3}$-ph), 2.385 (1H, br, OH). ^{13}C NMR of the monotosylate (125 MHz, $CDCl_3$): δ = 144.85, 132.96, 129.85, 127.93, 72.50, 70.67, 70.60, 70.42, 70.28, 69.31, 68.64, 61.62, 21.60.

APMS functionalized on its external surface with TEG (APMS-TEG). To attach TEG to APMS, 1.0g of APMS-NHMe was suspended in 30 ml of anhydrous acetonitrile that contained triethylamine (300 μL, 2 mmol) and TEG-Ts (520 mg, 1.5 mmol). After the mixture was refluxed for 6 hours under an inert atmosphere, the resulting APMS-TEG was captured by filtration, washed with ethanol, and dried under vacuum.

3. Syntheses of Gd-Doped MCM-41

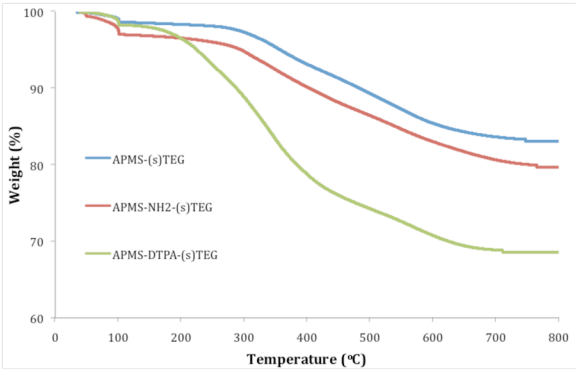
This material was synthesized as reported by M. Grun, K. K. Unger, A. Matsumoto, K. Tsutsumi, *Microporous Mesoporous Mat*. **1999**, *27*, 207, with the addition of $GdCl_3$ at the beginning of the preparation. CTAB (0.60 g) and $GdCl_3$•$6H_2O$ (150 mg) were dissolved in 30 mL of deionized water. To this solution was added aqueous ammonia (37 %, 2.45 mL) and the heat was raised to 30 °C. After thermal equilibration for several minutes, TEOS (2.5 g) was added, and the solution was stirred at this temperature for 24 h after which it was hydrothermally treated at 90 °C for another 24 hr. The slurry was then filtered and washed with water, and the resulting powder was calcined at 550 °C for 6 hr.

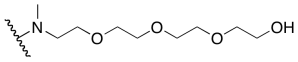
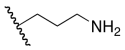
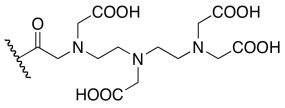
4. Measurement of Gd^{3+} in Aqueous Solution

The Gd^{3+} was measured by colorimetric titration using xylenol orange as an indicator and ethylenediamine tetraacetic acid (EDTA) disodium salt as titrant. Briefly, samples containing Gd^{3+} were adjusted to pH ~ 4.5 with sodium acetate (100 mM), and several drops of xylenol orange (in water) were added, producing a purple/violet color. This analyte solution was then titrated against EDTA disodium salt (1 mM in water) until the solution turned a pale yellow color at the equivalence point. The concentration of Gd^{3+} was then determined, given that it forms a 1:1 complex with EDTA. Titrations were performed in triplicate for each sample.

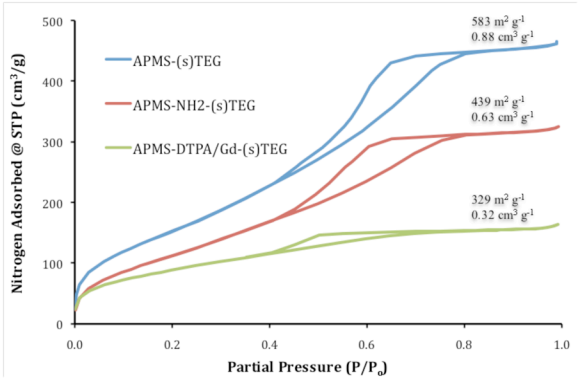
5. Characterization of Particles

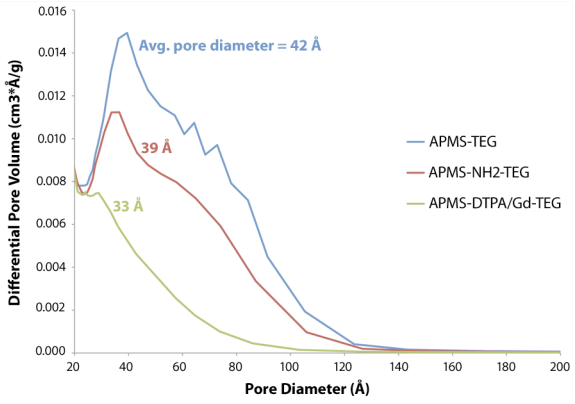
a. Thermogravimetric Analysis (**Figure S1**).



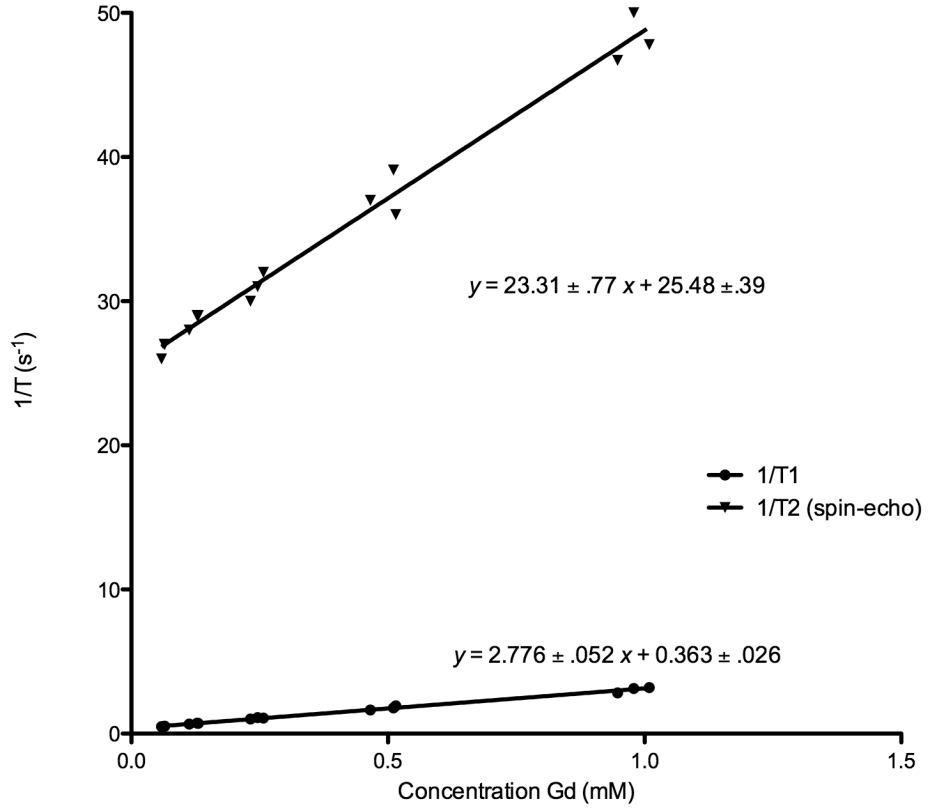
Sample	Weight loss $\frac{W_{800°C} - W_{100°C}}{W_{100°C}}$	Weight Difference $W_{modified} - W_{starting\ material}$	Organic Group Lost	MW (g/mol)	Loading (mmol/g)
APMS-TEG	-0.15757	-	residual surfactant, 	-	-
APMS-NH_2-TEG	-0.17854	-0.02097		58	0.362
APMS-DTPA-TEG	-0.30218	-0.12364		377	0.328

b. Nitrogen Physisorption Isotherms and Pore Size Distributions (**Figure S2**).





c. Relaxivity curves of APMS-DTPA/Gd-TEG suspended in agar at a field of 3.0 T (**Figure S3**).



Relaxivities ($mM^{-1}s^{-1}$) at 1.5 T suspended in agar. Because of the large dimensions of the particles, the per-particle relaxivities were large, surpassing 10^9 mM^{-1} s^{-1}. See the per-Gd calculations on the next page.

Sample	**r_1 (per Gd)**	**r_1 (per particle)**	**r_2 (per Gd)**	**r_2 (per particle)**
APMS/Gd	2.8 ± .1	~ 3 x 10^9	23 ± 1	~ 2 x 10^{10}
DTPA/$Gd_{(aq)}$	2.9 ± .2	-	2.9 ± .2	-

Comparison of relaxation times for APMS-DTPA/Gd-TEG and control APMS-TEG particles (data taken from one trial).

Sample	**Concentration (mg particle/mL)**	**T_1 (ms)**	**T_2 cpmg (ms)**	**T_2 spin echo (ms)**
APMS-DTPA/Gd-TEG	4.66	319 ± 3	58.02 ± 0.06	20.0 ± 0.4
APMS-TEG	4.65	3370 ± 20	117.8 ± 0.1	34 ± 2
water	0	3320 ± 20	146.9 ± 0.1	36 ± 2

Per-particle gadolinium loading calculation

$$\bar{\rho}_{solid} = f_{w/w,silica} \cdot \rho_{silica} + f_{w/w,organic} \cdot \rho_{organic}$$
$$= (0.735)(2.2\ g\ cm^{-3}) + (0.265)(1.0\ g\ cm^{-3}) = 1.88\ g\ cm^{-3}$$

$$f_{porosity} = \frac{\bar{V}_{pore}}{\bar{V}_{solid} + \bar{V}_{pore}} = \frac{(0.32\ cm^3\ g^{-1})(1\ g)}{\left(\frac{1\ g}{1.88\ g\ cm^{-3}}\right) + (0.32\ cm^3\ g^{-1})(1\ g)} = 0.376$$

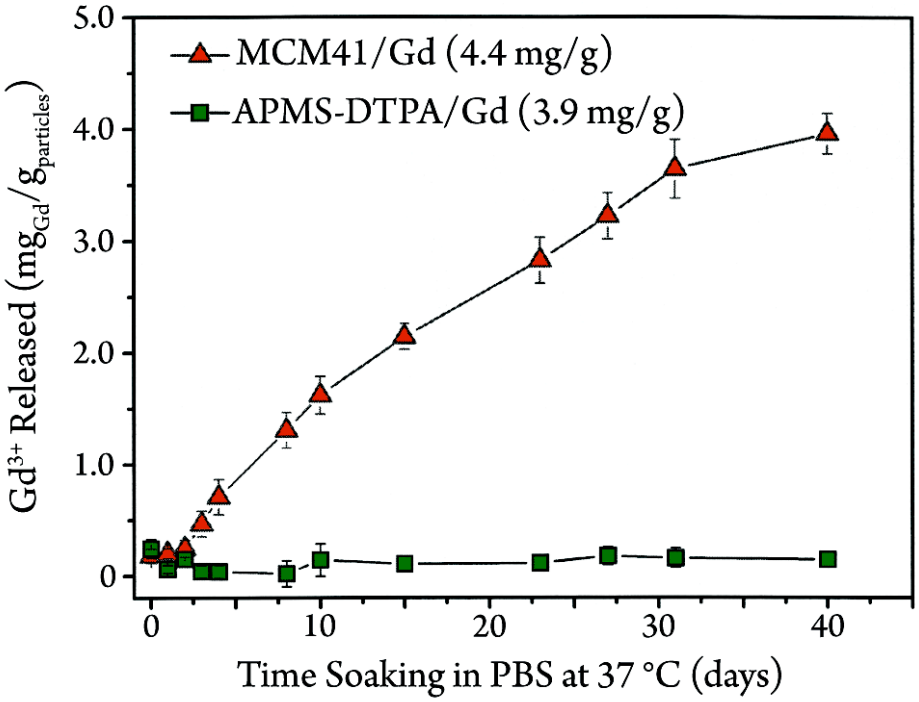
$$\bar{V}^{total}_{particle} = \frac{4}{3}\pi\left(\frac{1.8 \times 10^{-6} m}{2}\right)^3 = 3.05 \times 10^{-18} m^3$$

$$\bar{V}^{solid}_{particle} = \bar{V}^{particle}_{total} \cdot (1 - f_{porosity}) = (3.1 \times 10^{-18} m^3)(1 - 0.376) = 1.91 \times 10^{-18} m^3$$

$$\bar{m}_{particle} = \bar{V}_{solid} \cdot \bar{\rho}_{solid} = \left(\frac{1.93 \times 10^{-18} m^3}{particle}\right)\left(\frac{1.88\ g}{cm^3}\right)\left(\frac{1 \times 10^6 cm^3}{1\ m^3}\right) = \frac{3.58 \times 10^{-12} g}{particle}$$

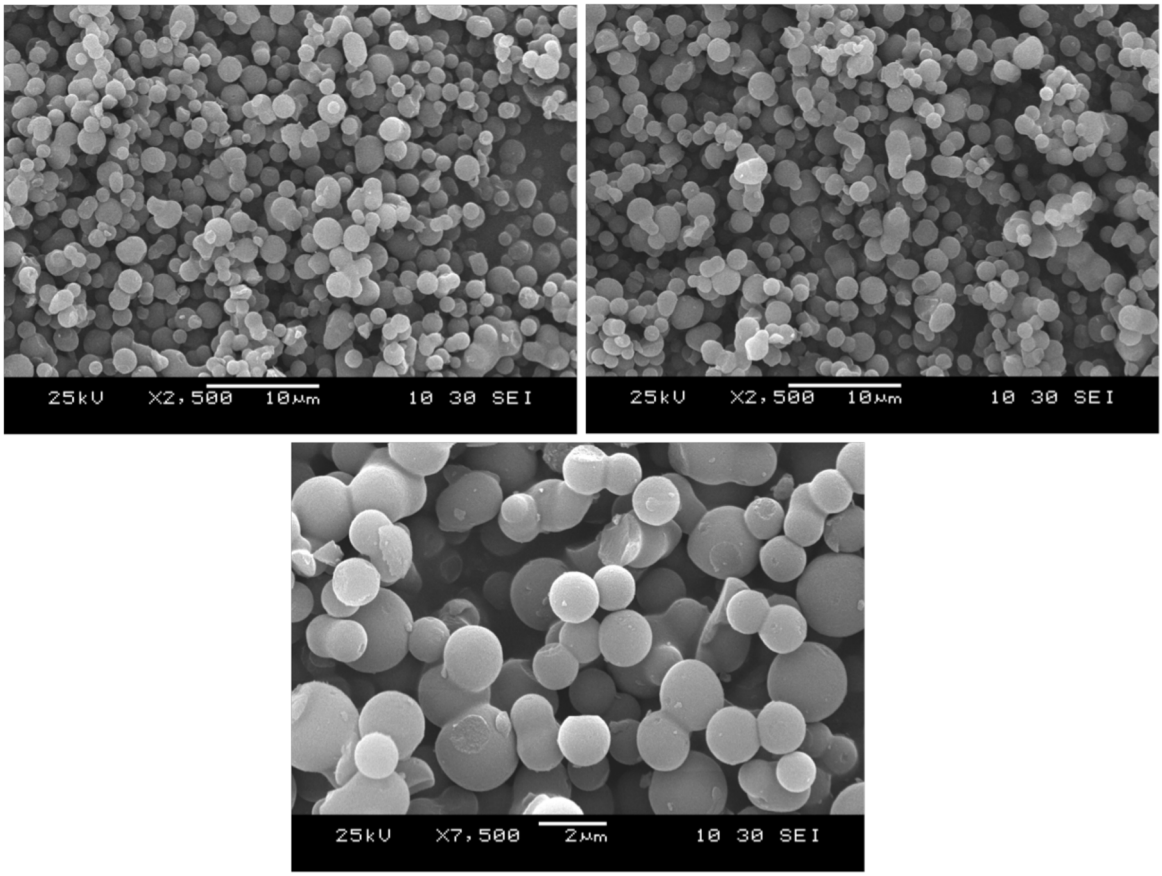
$$n^{Gd}_{particle} = \left(\frac{3.58 \times 10^{-12} g}{particle}\right)\left(\frac{0.067 g\ Gd}{1\ g\ particles}\right)\left(\frac{1\ mol\ Gd}{157\ g\ Gd}\right)\left(\frac{6.022 \times 10^{23}\ Gd}{1\ mol\ Gd}\right) = \frac{9.2 \times 10^8\ Gd}{particle}$$

d. Leaching of Gd^{3+} from APMS-DTPA/Gd-TEG and MCM-41 doped with Gd (**Figure S4**).



d. Scanning Electron Micrographs (**Figure S5**).

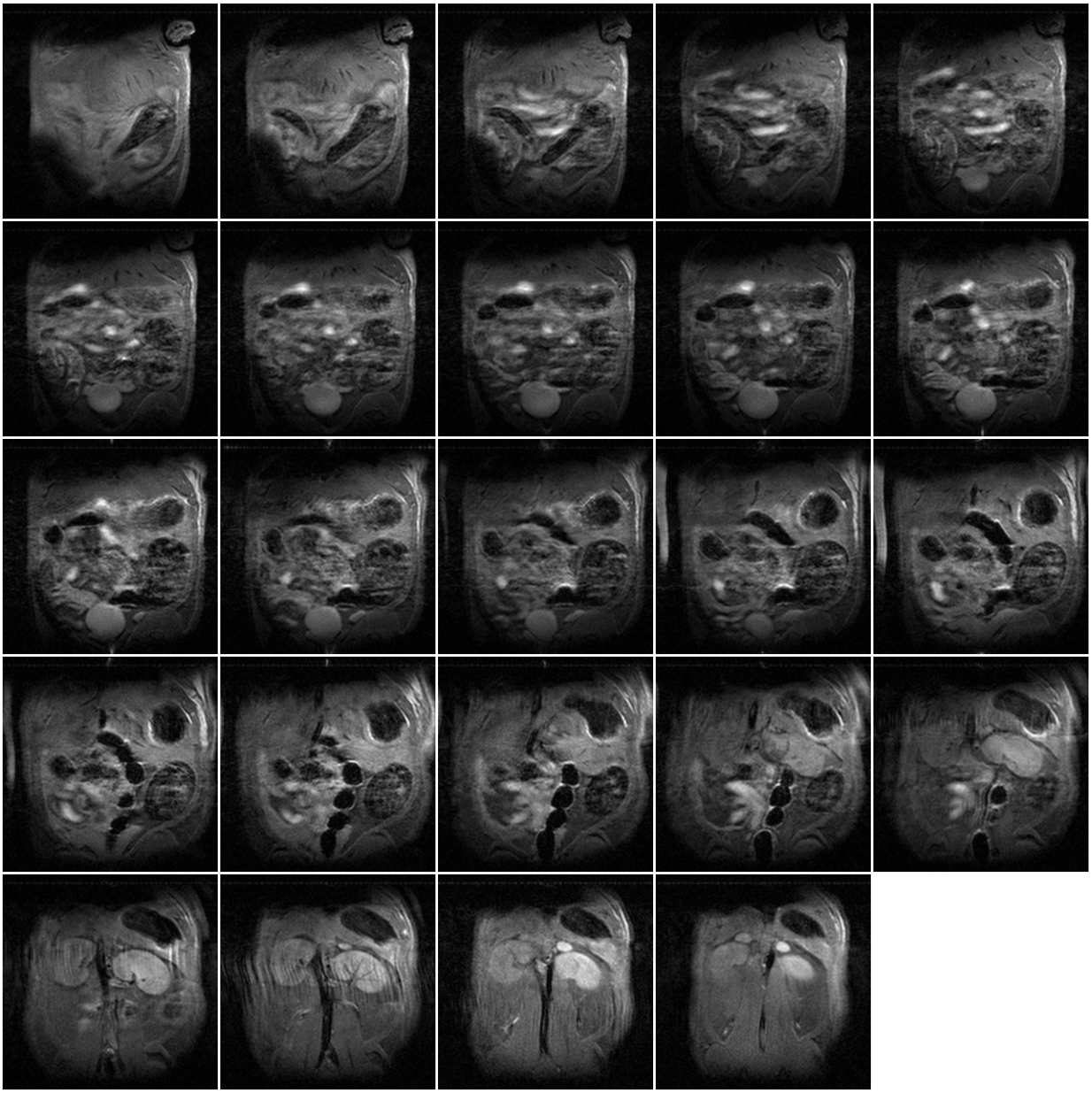
APMS-DTPA/Gd-TEG



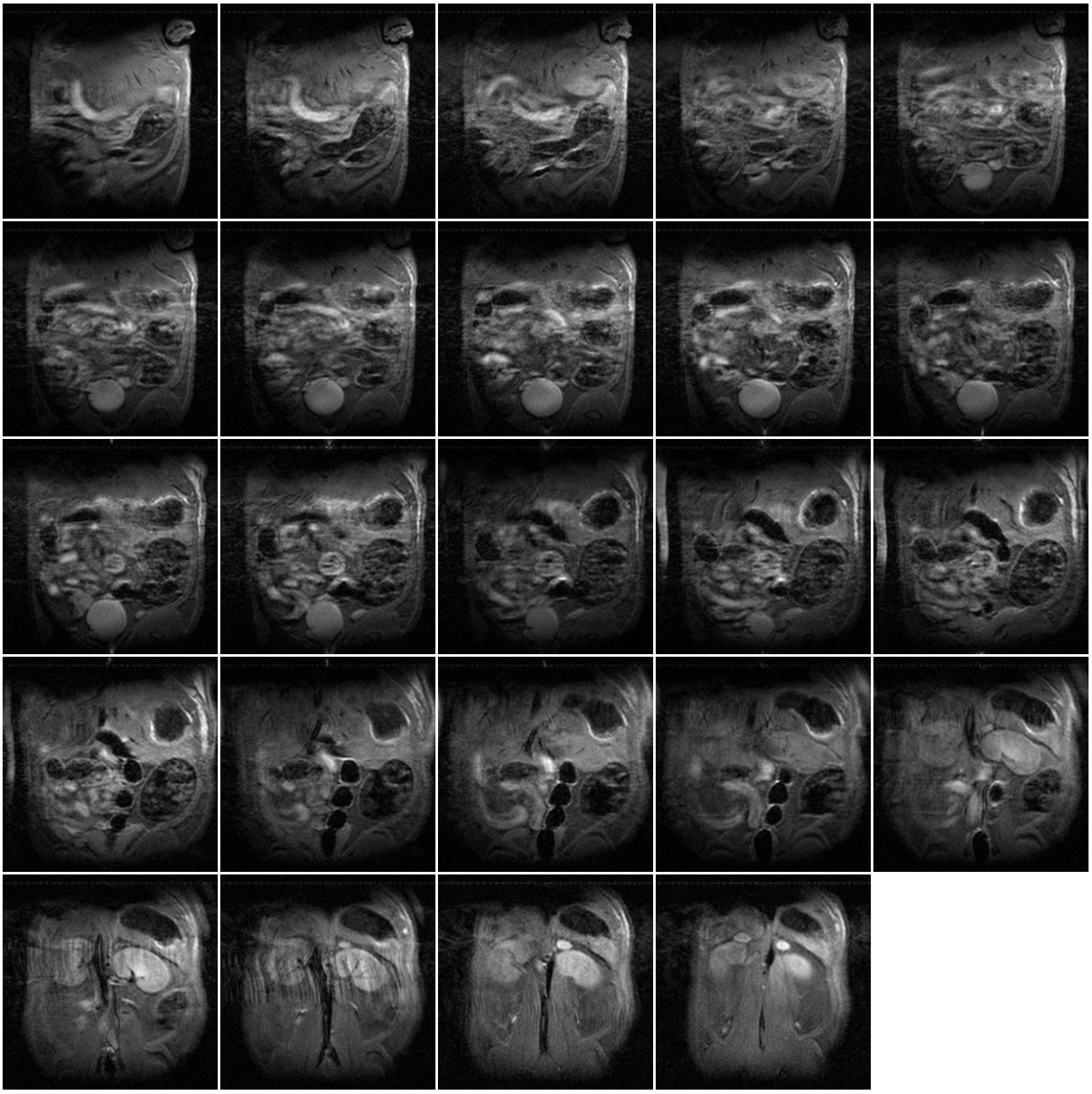
6. Additional MR Images of the Rat Shown in Figure 2.

All images are T1-weighted using a fat-suppression algorithm. The coronal image slices below are in sequence from anterior to posterior starting in the upper left, then across and down. These MR images constitute a subset of the data we possess, and a full paper is in preparation that will include data from more animals and additional experiments (Lathrop, 2010, in preparation; ref 33 in manuscript).

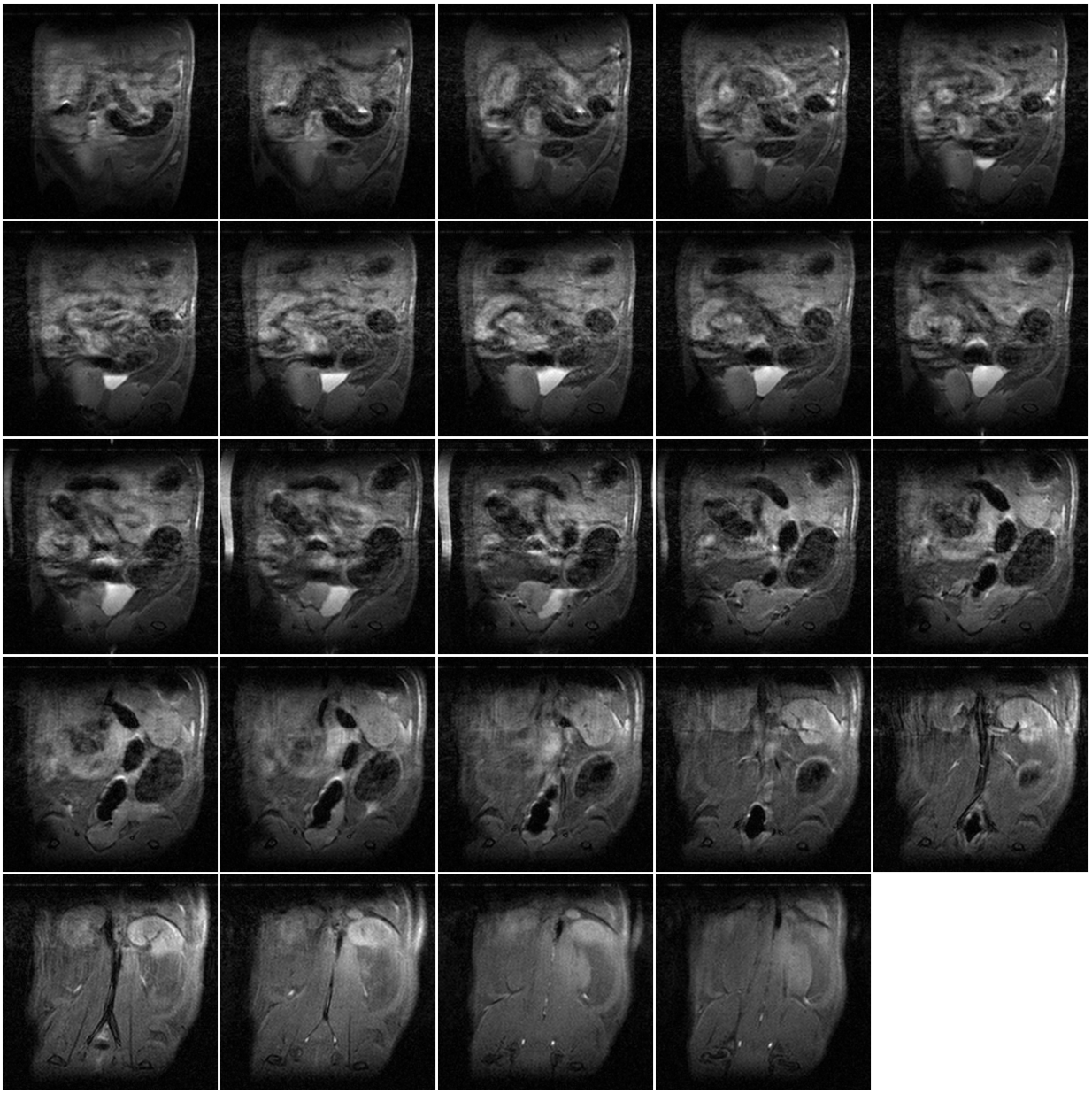
Preinjection (**Figure S6**).



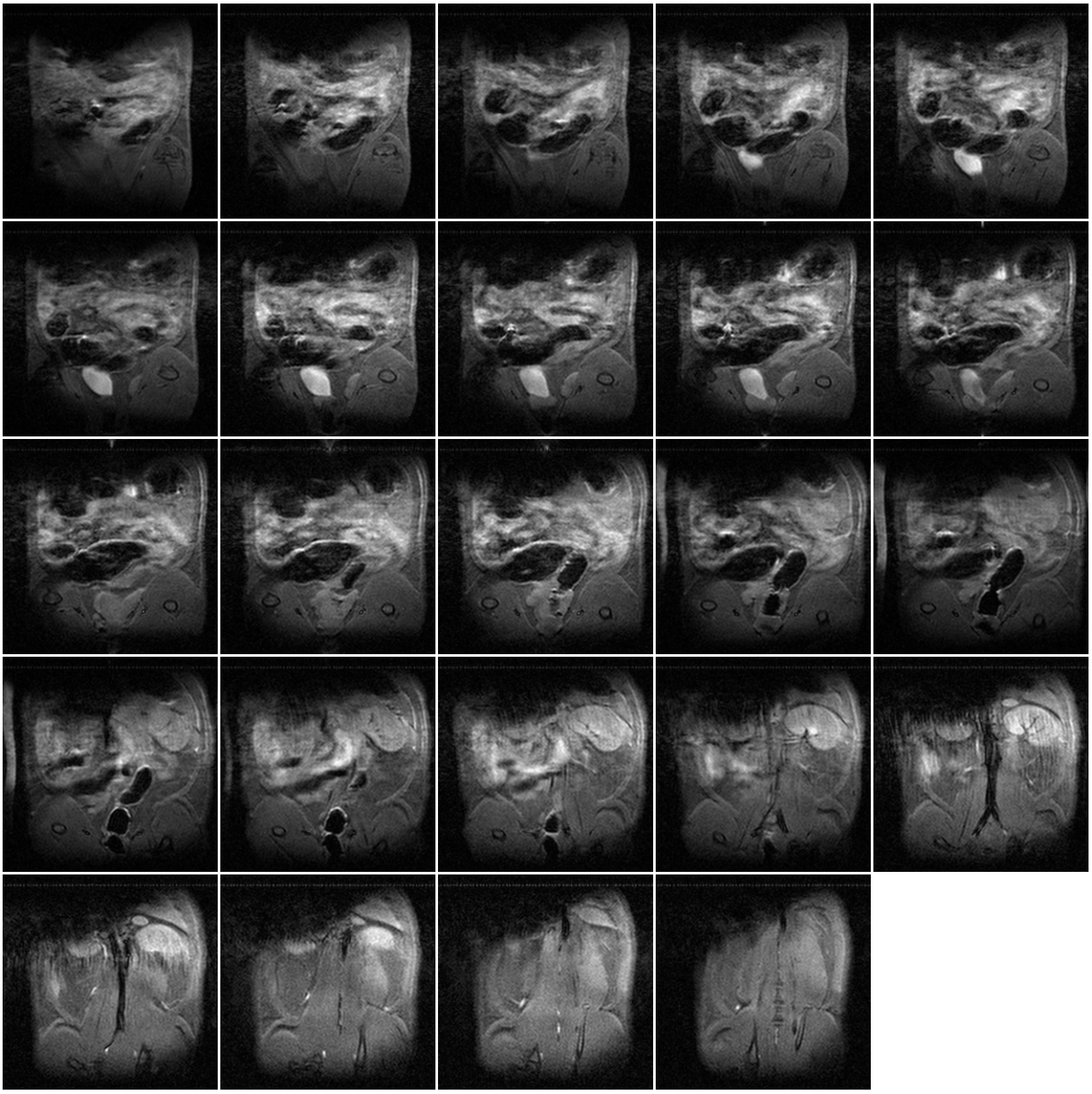
Immediately Post-Injection (0 hr) (**Figure S7**).



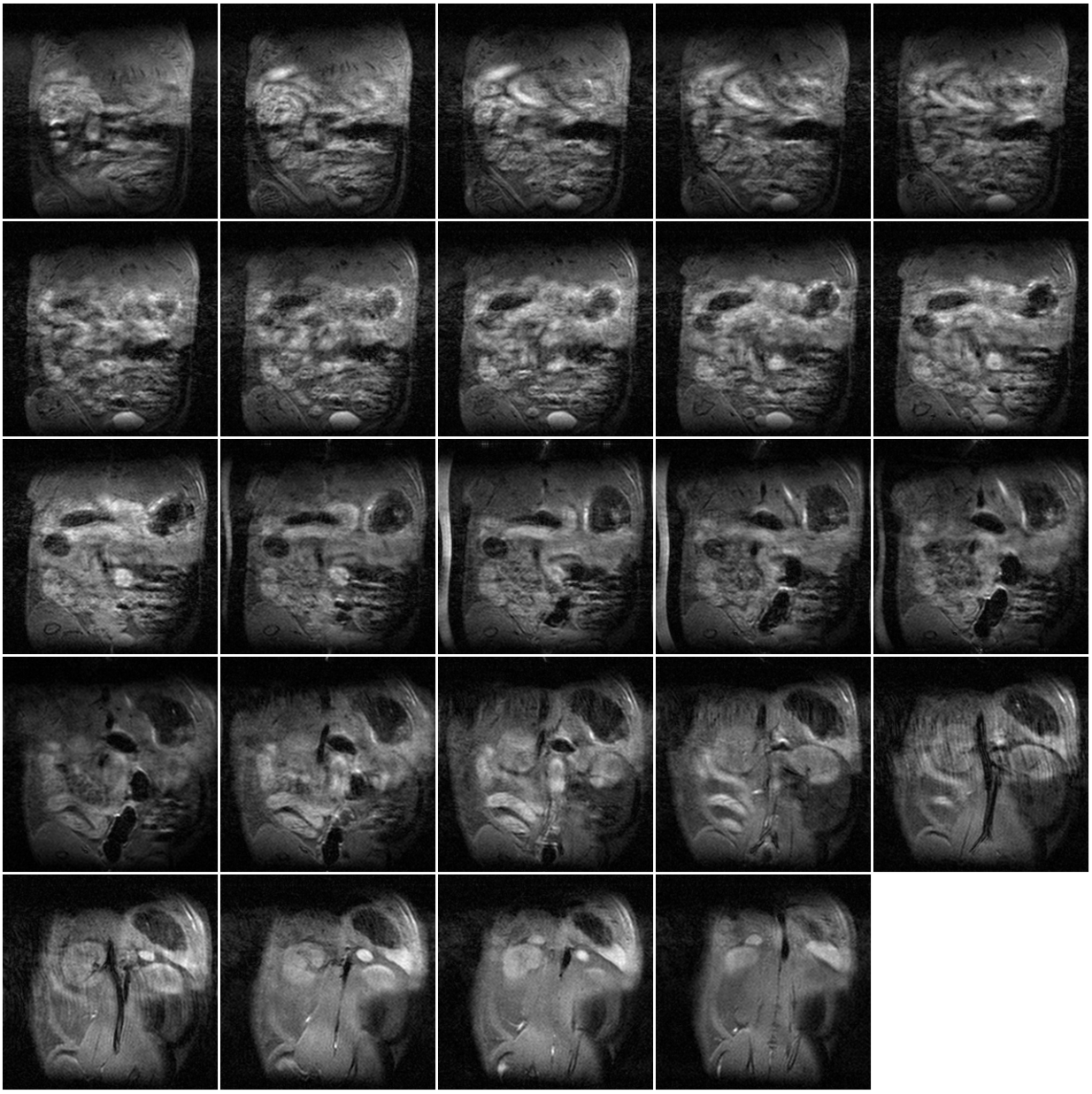
2 hr Post-Injection (**Figure S8**).



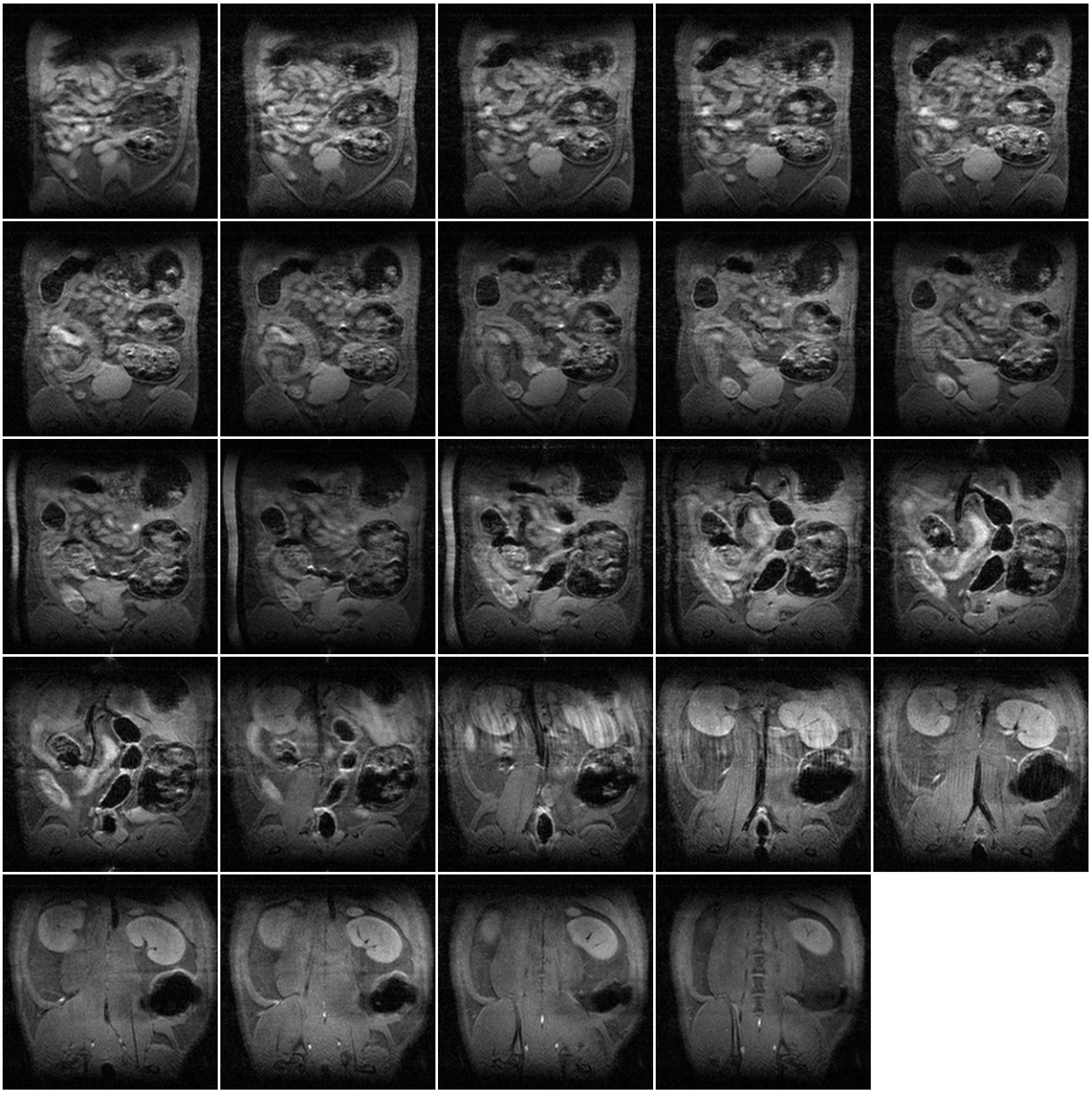
4 hr Post-Injection (**Figure S9**).



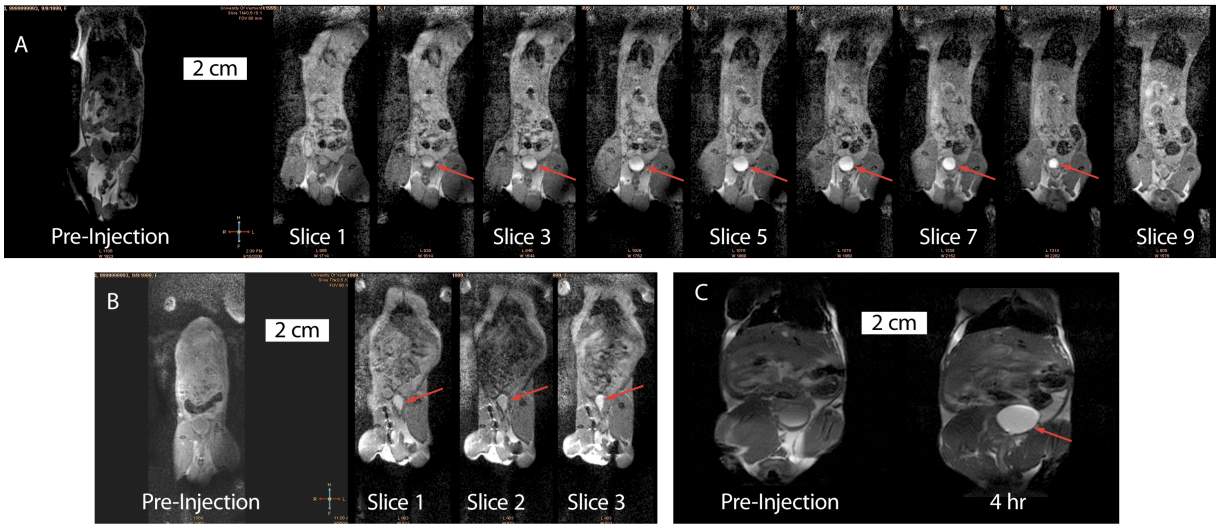
25 hr Post-Injection (**Figure S10**).



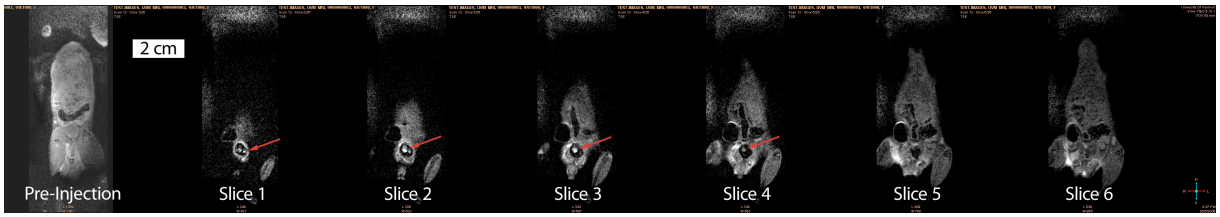
144 hr Post-Injection (**Figure S11**).



MR Images Showing Contrast in the Bladders of Three C57BL/6 mice 1 hr (A, B) and 4 hr (C) post-injection (**Figure S12**).



MR Images Showing Punctate Contrast in the Bladder of a C57BL/6 Mouse roughly 5 minutes post-injection (**Figure S13**).



Additional SEM and EDS Data of Urine Collected 2 hr Post-Injection and Treated with Proteinase-K and Detergents, Leaving Salty Residue (**Figure S14**).