Supporting Information For:

Reagents for Astatination of Biomolecules. 2. Conjugation of Anionic Boron Cage Pendant Groups to a Protein Provides a Method for Direct Labeling that is Stable to In Vivo Deastatination.

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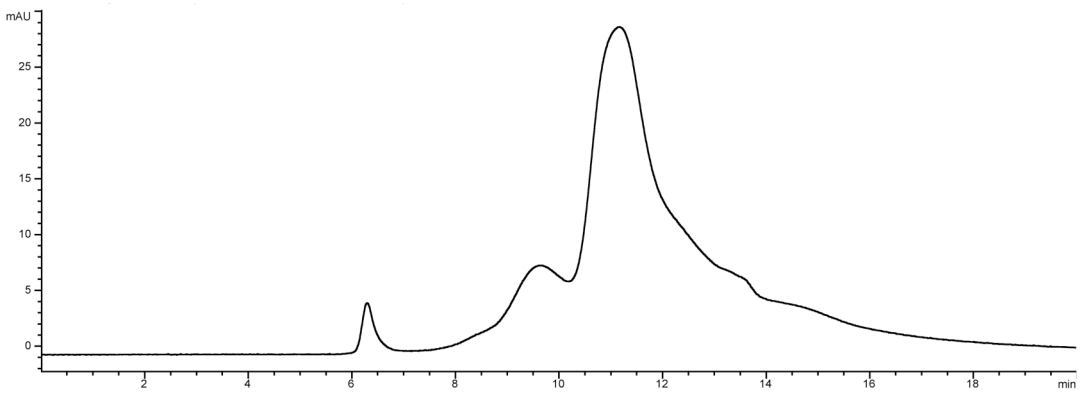
PAGES:

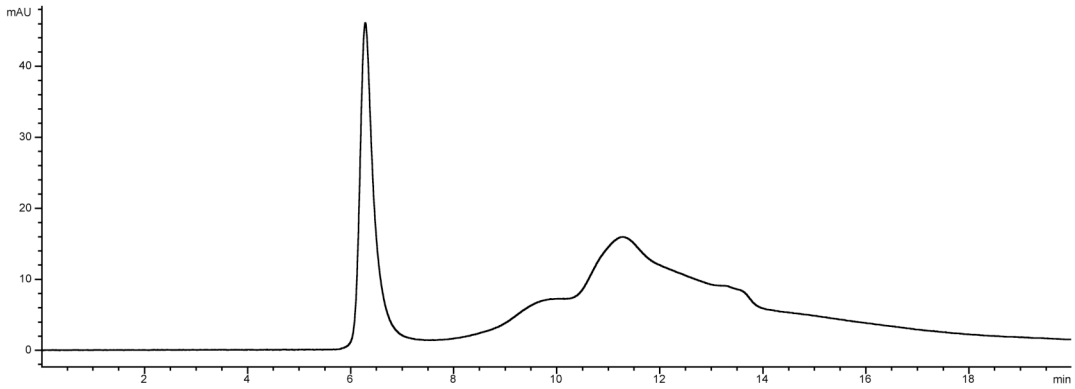
Figure S1:

SE-HPLC chromatograms for the reactions of 107-1A4 Fab´-NEM with 10 equiv. (panel A), 20 equiv. (panel B) and 50 equiv. (panel C) of *nido*-carboranyl derivative **2**. Note the growth of high molecular weight species (t_R = 6.3 min) and broadened Fab´-NEM peak (e.g. t_R = 10-11 min) with increased amounts of **2** in the reaction.

A: 10 equivalents of **2**



B: 20 equivalents of **2**



C: 50 equivalents of **2**

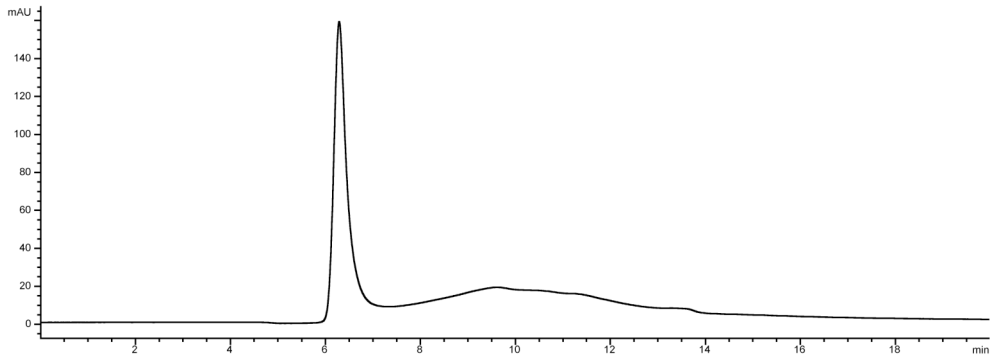
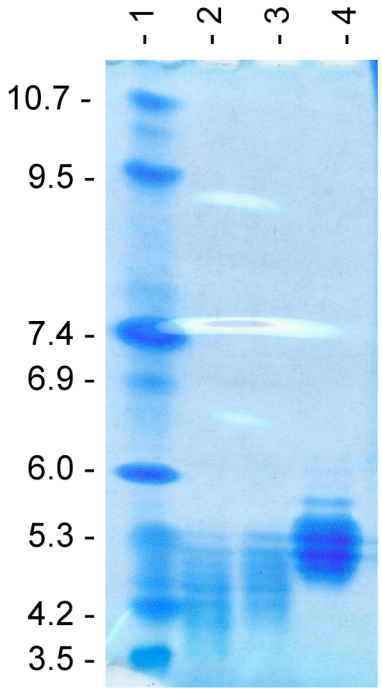


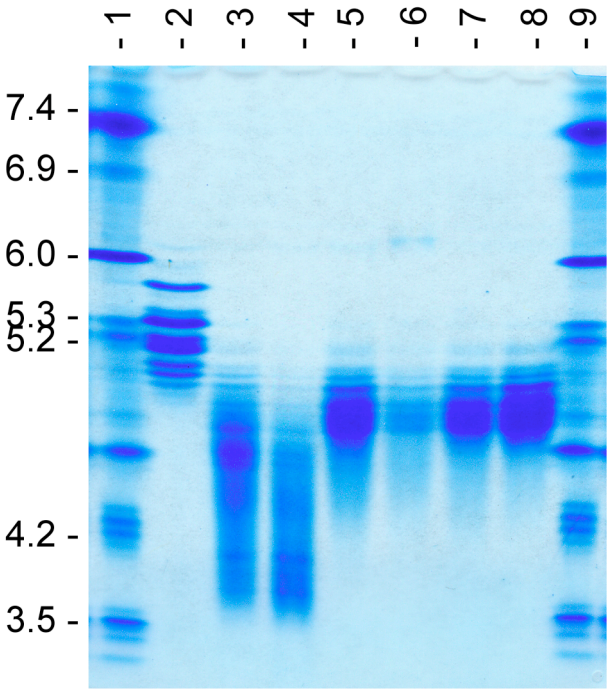
Figure S2:
Digitized representations of isoelectric focusing gels running conjugation reaction products of borane reagents **3**,**4**,**5**,**6** and **14** with Fab´-NEM. Standards used are described in manuscript. Inclusion of Fab´-NEM allows comparison to starting material. If no bands remain for starting material, it is thought that every Fab´ has at least one conjugate. Note that IEF in panel A is run from pI of 10.7 to 3.5, whereas in panel B the pI range is 7.4 to 3.5.

A:



Lanes:
1: IEF Standards
2: 4-Fab´ (5 equiv)
3: 4-Fab´ (10 equiv)
4: Fab´-NEM

B:



Lanes:
1: IEF Standards
2: Fab´-NEM
3: 6-Fab´ (10 equiv)
4: 6-Fab´ (20 equiv)
5: 5-Fab´ (10 equiv)
6: 5-Fab´ (20 equiv)
7: 14-Fab´ (10 equiv)
8: 14-Fab´ (20 equiv)
9: IEF Standards

Table S1: Tissue Distribution of [^{125}I]Fab´-NEM in BALB/c nu/nu mice.[a]

Tissue	1 h[b,c]	4 h	24 h
	[^{125}I]Fab´	**[^{125}I]Fab´**	**[^{125}I]Fab´**
blood	14.76 ± 0.06	9.10 ± 1.50	1.25 ± 0.20
muscle	0.99 ± 0.11	1.09 ± 0.01	0.21 ± 0.01
lung	7.33 ± 1.09	5.01 ± 0.78	0.86 ± 0.09
kidney	47.58 ± 5.14	22.99 ± 5.79	4.61 ± 0.64
spleen	3.33 ± 0.61	2.51 ± 0.32	0.54 ± 0.10
liver	3.30 ± 0.13	2.18 ± 0.27	0.84 ± 0.26
intestine	2.10 ± 0.25	1.75 ± 0.20	0.22 ± 0.03
neck	6.84 ± 0.50	11.58 ± 3.79	0.99 ± 0.13
stomach	3.97 ± 0.81	8.79 ± 2.32	0.59 ± 0.07
urine[d]	16.22 ± 2.70	53.48 ± 24.36	4.34 ± 2.68

[a]Values shown are % injected dose / gram ± standard deviation. Fab´-SH is capped with N-ethylmaleimide. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for n = 3 mice at 1 h, 4 mice at 4 h and 5 mice at 24 h; average animal weight for all groups is 26.32 ± 2.18 G; Injectate for each animal had 5 μCi/15 μg of [^{125}I]Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice.

Table S2: Tissue Distribution of Co-injected [^{125}I]1b-Fab´ and [^{211}At]1c-Fab´ in BALB/c nu/nu mice.[a]

Tissue	1 h[b,c]		4 h	
	[^{125}I]**1b-Fab´**	[^{211}At]**1c-Fab´**	[^{125}I]**1b-Fab´**	[^{211}At]**1c-Fab´**
blood	19.21 ± 1.17	13.55 ± 0.97	7.41 ± 0.53	6.65 ± 0.54
muscle	0.76 ± 0.13	1.12 ± 0.10	0.82 ± 0.09	0.90 ± 0.04
lung	6.72 ± 0.62	14.32 ± 1.04	4.29 ± 0.24	11.67 ± 0.50
kidney	33.61 ± 6.43	20.83 ± 3.03	30.89 ± 5.30	16.95 ± 2.35
spleen	3.04 ± 0.50	9.43 ± 1.71	1.68 ± 0.13	10.49 ± 1.39
liver	4.20 ± 0.24	3.89 ± 0.16	2.90 ± 0.18*	2.70 ± 0.23*
intestine	1.86 ± 0.60*	2.47 ± 0.53*	6.51 ± 3.09	3.02 ± 0.34
neck	3.01 ± 0.40	14.66 ± 2.05	2.68 ± 0.10	29.41 ± 4.80
stomach	1.94 ± 0.35	12.09 ± 2.43	2.36 ± 0.13	17.19 ± 1.84
urine[d]	69.66 ± 78.58*	20.75 ± 30.57*	27.41 ± 15.54*	20.67 ± 21.23*

[a]Values shown are % injected dose / gram ± standard deviation. Fab´-SH is capped with N-ethylmaleimide. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for n = 5 mice at 1 h and n = 5 mice at 4 h; average animal weight for 1 h group, 30.93 ± 3.81 g, and for 4 h group, 30.02 ± 1.65 g; Injectate for each animal had 3 μCi/15 μg of [^{211}At]**1c**-Fab´ and 8 μCi/15 μg of [^{125}I]**1b**-Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. *Denotes tissues/fluids that are **not** statistically different based on paired Student-t test ($p>0.05$).

Table S3: Tissue Distribution of Co-injected [^{125}I]2-Fab´ and [^{211}At]2-Fab´ in BALB/c nu/nu mice.[a]

Tissue	1 h[b,c]		4 h	
	[^{125}I]**2-Fab´**	[^{211}At]**2-Fab´**	[^{125}I]**2-Fab´**	[^{211}At]**2-Fab´**
blood	33.68 ± 3.49*	27.03 ± 2.71*	22.86 ± 1.73*	19.06 ± 1.39*
muscle	0.72 ± 0.15	0.76 ± 0.10	1.11 ± 0.13	0.98 ± 0.14
lung	10.88 ± 3.16	11.47 ± 1.99	8.55 ± 0.98	9.08 ± 0.49
kidney	12.84 ± 1.51	11.07 ± 1.33	11.89 ± 1.71	11.96 ± 1.66
spleen	4.45 ± 0.24*	7.59 ± 0.81*	3.96 ± 0.39*	5.94 ± 0.40*
liver	7.71 ± 1.07	7.72 ± 0.96	5.93 ± 0.62	6.43 ± 0.71
intestine	2.17 ± 0.49	2.52 ± 0.57	3.94 ± 0.14*	5.81 ± 0.22*
neck	3.19 ± 0.55*	5.98 ± 0.94*	5.72 ± 1.22*	9.07 ± 2.35*
stomach	1.84 ± 0.30*	3.87 ± 0.64*	3.96 ± 0.38*	5.36 ± 0.91*
urine[d]	3.71 ± 0.23	3.73 ± 0.50	28.26 ± 3.52*	17.05 ± 2.85*

[a]Values shown are % injected dose / gram ± standard deviation. Fab´-SH is capped with N-ethylmaleimide. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for $n = 5$ mice at 1 h and $n = 5$ mice at 4 h; average animal weight for 1 h group, 28.34 ± 2.38 g, and for 4 h group, 28.58 ± 2.16 g; Injectate for each animal had 5 μCi/10 μg of [^{211}At]**2**-Fab´ and 5 μCi/10 μg of [^{125}I]**2**-Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. *Denotes tissues/fluids that are statistically different based on paired Student-t test ($p>0.05$).

Table S4: Tissue Distribution of Co-injected [^{125}I]3-Fab´ and [^{211}At]3-Fab´ in BALB/c nu/nu mice.[a]

Tissue	1 h[b,c]		4 h	
	[^{125}I]**3-Fab´**	[^{211}At]**3-Fab´**	[^{125}I]**3-Fab´**	[^{211}At]**3-Fab´**
blood	13.77 ± 0.90*	10.51 ± 0.66*	9.04 ± 0.62*	6.63 ± 0.45*
muscle	0.46 ± 0.09	0.58 ± 0.08	0.57 ± 0.07*	0.37 ± 0.10*
lung	4.73 ± 0.32*	7.79 ± 0.31*	4.79 ± 0.39*	5.52 ± 0.35*
kidney	4.12 ± 0.69	4.57 ± 0.30	4.04 ± 0.14	3.72 ± 0.32
spleen	13.97 ± 7.46	17.35 ± 9.11	13.77 ± 2.34	16.82 ± 2.48
liver	26.92 ± 1.21	25.57 ± 1.24	33.96 ± 3.87	32.15 ± 3.66
intestine	2.49 ± 3.91	3.38 ± 5.33	8.28 ± 1.90	8.46 ± 1.91
neck	3.45 ± 0.37*	6.14 ± 1.15*	4.49 ± 1.03*	8.59 ± 1.49*
stomach	1.75 ± 0.13*	3.70 ± 1.03*	3.28 ± 0.89*	7.26 ± 0.58*
urine[d]	2.48 ± 0.61	2.77 ± 0.76	16.48 ± 6.97	10.11 ± 1.12

[a]Values shown are % injected dose / gram ± standard deviation. Fab´-SH is capped with N-ethylmaleimide. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for n = 5 mice at 1 h and n = 5 mice at 4 h; average animal weight for 1 h group, 28.38 ± 1.47g, and for 4 h group, 26.72 ± 1.69 g; Injectate for each animal had 2 μCi/5 μg of [^{211}At]**3**-Fab´ and 2 μCi/5 μg of [^{125}I]**3**-Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. *Denotes tissues/fluids that are statistically different based on paired Student-t test ($p>0.05$).

Table S5: Tissue Distribution of Co-injected [^{125}I]4-Fab´ and [^{211}At]4-Fab´ in BALB/c nu/nu mice.[a]

Tissue	1 h[b,c]		4 h	
	[^{125}I]**4-Fab´**	[^{211}At]**4-Fab´**	[^{125}I]**4-Fab´**	[^{211}At]**4-Fab´**
blood	25.09 ± 1.71	26.61 ± 1.73	11.69 ± 2.53	13.87 ± 2.99
muscle	0.84 ± 0.07	0.84 ± 0.11	0.72 ± 0.10	0.82 ± 0.14
lung	8.57 ± 0.62*	9.95 ± 0.73*	5.41 ± 1.33	6.94 ± 1.47
kidney	23.80 ± 3.50	24.62 ± 3.87	27.41 ± 6.43	29.55 ± 6.79
spleen	5.44 ± 0.62*	6.58 ± 0.93*	3.73 ± 0.32*	4.98 ± 0.18*
liver	10.35 ± 0.91	11.61 ± 1.07	10.78 ± 1.82	11.93 ± 1.99
intestine	2.23 ± 0.32	2.59 ± 0.46	2.58 ± 0.46*	4.96 ± 1.30*
neck	7.51 ± 1.16*	3.72 ± 0.80*	6.51 ± 1.97*	4.12 ± 0.71*
stomach	5.43 ± 0.92*	2.72 ± 0.21*	4.86 ± 1.34*	3.20 ± 0.23*
urine[d]	41.52 ± 19.43	37.13 ± 17.58	47.34 ± 10.63*	27.62 ± 8.51*

[a]Values shown are % injected dose / gram ± standard deviation. Fab´-SH is capped with N-ethylmaleimide. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for n = 5 mice at 1 h and n = 5 mice at 4 h; average animal weight for 1 h group, 29.92 ± 1.23g, and for 4 h group, 25.11 ± 3.62 g; Injectate for each animal had 2 μCi/15 μg of [^{211}At]**4**-Fab´ and 5 μCi/15 μg of [^{125}I]**4**-Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. *Denotes tissues/fluids that are statistically different based on paired Student-t test ($p>0.05$).

Table S6: Tissue Distribution of Co-injected [^{125}I]5-Fab´ and [^{211}At]5-Fab´ in BALB/c nu/nu mice.[a]

Tissues	1h[b,c]		4h		24h	
	[^{125}I]**5-Fab´**	[^{211}At]**5-Fab´**	[^{125}I]**5-Fab´**	[^{211}At]**5-Fab´**	[^{125}I]**5-Fab´**	[^{211}At]**5-Fab´**
blood	24.84 ± 4.08*	17.67 ± 1.85*	12.50 ± 3.10	11.86 ± 2.65	2.69 ± 0.45	3.07 ± 0.51
muscle	0.56 ± 0.11	0.54 ± 0.11	0.44 ± 0.10	0.48 ± 0.12	0.38 ± 0.10	0.37 ± 0.12
lung	9.15 ± 1.83	9.48 ± 1.97	5.18 ± 1.46	6.00 ± 1.66	1.87 ± 0.41	2.37 ± 0.45
kidney	10.26 ± 2.03	10.85 ± 2.06	6.63 ± 1.48	8.08 ± 1.82	4.53 ± 0.83	5.71 ± 1.06
spleen	7.46 ± 1.73	8.19 ± 1.92	9.91 ± 2.30	10.38 ± 2.29	17.59 ± 5.35	18.01 ± 5.66
liver	9.72 ± 1.42*	7.45 ± 0.59*	11.28 ± 2.47*	6.67 ± 1.08*	14.48 ± 3.08*	9.20 ± 1.14*
intestine	1.98 ± 0.60	2.43 ± 0.87	1.31 ± 0.36	1.63 ± 0.46	0.89 ± 0.12*	1.18 ± 0.18*
neck	5.93 ± 0.79*	2.76 ± 0.41*	5.00 ± 1.05*	3.30 ± 0.64*	2.97 ± 0.76	3.80 ± 1.04
stomach	2.71 ± 0.66*	1.34 ± 0.29*	2.79 ± 0.38*	1.99 ± 0.45*	1.63 ± 0.37	1.90 ± 0.50
urine[d]	12.24 ± 6.02	11.26 ± 7.32	15.54 ± 3.43*	6.19 ± 1.47*	4.78 ± 0.74*	3.42 ± 0.99*

[a]Values shown are % injected dose / gram ± standard deviation. Fab´-SH is capped with N-ethylmaleimide. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for n = 5 mice at 1 h and n = 5 mice at 4 h; average animal weight for 1 h group, 26.81 ± 1.10 g, for 4 h group, 31.71 ± 3.90 g, and for 24 h group, 26.66 ± 2.23 g; Injectate for each animal had 15 μCi/15 μg of [^{211}At]**5**-Fab´ and 15 μCi/15 μg of [^{125}I]**5**-Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. *Denotes tissues/fluids that are statistically different based on paired Student-t test ($p>0.05$).

Table S7: Tissue Distribution of Co-injected [^{125}I]6-Fab´ and [^{211}At]6-Fab´ in BALB/c nu/nu mice.[a]

Tissue	1 h[b,c]		4 h	
	[^{125}I]**6-Fab´**	[^{211}At]**6-Fab´**	[^{125}I]**6-Fab´**	[^{211}At]**6-Fab´**
blood	23.31 ± 1.85	21.82 ± 1.59	13.17 ± 2.41	12.38 ± 2.19
muscle	0.72 ± 0.08	0.66 ± 0.08	0.73 ± 0.16	0.63 ± 0.14
lung	7.94 ± 0.50	8.51 ± 0.67	5.79 ± 0.55	5.90 ± 0.50
kidney	39.61 ± 5.58	37.55 ± 4.76	30.88 ± 2.20	26.41 ± 1.64
spleen	5.56 ± 0.58*	7.62 ± 0.92*	4.29 ± 0.66	4.50 ± 0.62
liver	10.58 ± 0.89*	13.58 ± 1.36*	13.41 ± 1.98	11.81 ± 1.85
intestine	2.06 ± 0.32	2.24 ± 0.48	3.18 ± 1.07	4.91 ± 2.11
neck	3.53 ± 0.15	3.36 ± 0.10	9.13 ± 2.66*	4.53 ± 0.84*
stomach	2.07 ± 0.12	2.00 ± 0.19	4.17 ± 2.43	2.09 ± 1.06
urine[d]	68.37 ± 27.01	65.10 ± 24.83	99.48 ± 124.10	41.90 ± 45.66

[a]Values shown are % injected dose / gram ± standard deviation. Fab´-SH is capped with N-ethylmaleimide. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for n = 5 mice at 1 h and n = 5 mice at 4 h; average animal weight for 1 h group, 27.98 ± 3.22g, and for 4 h group, 28.59 ± 2.11 g; Injectate for each animal had 4 μCi/15 μg of [^{211}At]**6**-Fab´ and 4 μCi/15 μg of [^{125}I]**6**-Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. *Denotes tissues/fluids that are statistically different based on paired Student-t test ($p>0.05$).

Table S8: Tissue Distribution of Co-injected [^{125}I]7-Fab´ and [^{211}At]7-Fab´ in BALB/c nu/nu mice.[a]

Tissues	1h[b,c]		4h		24h	
	[^{125}I]**7-Fab´**	[^{211}At]**7-Fab´**	[^{125}I]**7-Fab´**	[^{211}At]**7-Fab´**	[^{125}I]**7-Fab´**	[^{211}At]**7-Fab´**
blood	17.62 ± 2.22*	21.74 ± 2.47*	10.15 ± 2.86	14.20 ± 3.76	1.35 ± 0.25*	2.33 ± 0.29*
muscle	0.95 ± 0.24	0.97 ± 0.26	0.60 ± 0.18	0.71 ± 0.18	0.26 ± 0.02	0.29 ± 0.11
lung	7.53 ± 2.22	9.36 ± 2.91	3.76 ± 1.13	5.29 ± 1.85	0.96 ± 0.12*	1.58 ± 0.31*
kidney	26.84 ± 1.21*	31.55 ± 1.69*	31.02 ± 4.74	37.22 ± 5.10	17.87 ± 3.23*	23.09 ± 3.66*
spleen	4.01 ± 0.47*	5.38 ± 0.54*	3.31 ± 0.91	4.87 ± 1.33	2.94 ± 0.80*	4.58 ± 1.22*
liver	6.60 ± 0.53*	9.03 ± 0.61*	6.84 ± 1.42*	10.19 ± 1.96*	5.97 ± 0.74*	9.69 ± 1.04*
intestine	1.94 ± 0.36	2.40 ± 0.55	1.93 ± 0.72	2.71 ± 1.21	0.51 ± 0.11*	0.88 ± 0.25*
neck	9.47 ± 1.43*	3.08 ± 0.20*	9.70 ± 3.58*	5.31 ± 1.75*	1.21 ± 0.33	1.81 ± 0.51
stomach	5.36 ± 1.41*	2.27 ± 0.28*	6.77 ± 0.71*	3.42 ± 0.44*	0.68 ± 0.09*	1.05 ± 0.33*
urine[d]	46.03 ± 32.23	44.32 ± 30.61	38.33 ± 42.37	12.64 ± 27.78	5.90 ± 3.46	5.20 ± 2.52

[a]Values shown are % injected dose / gram ± standard deviation. [b]Time of sacrifice from injection of radioactivity. [c]Data were obtained for n = 5 mice at 1 h and n = 5 mice at 4 h; average animal weight for 1 h group, 26.81 ± 1.10 g, for 4 h group, 31.71 ± 3.90 g, and for 24 h group, 26.66 ± 2.23 g; Injectate for each animal had 10 μCi/8 μg of [^{211}At]**7**-Fab´ and 10 μCi/8 μg of [^{125}I]**7**-Fab´ in approximately 100 μL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. *Denotes tissues/fluids that are statistically different based on paired Student-t test ($p>0.05$).