Supplementary Materials for

Whole-body fluorescence cryotomography identifies a fast-acting, high-contrast, durable contrast agent for fluorescence-guided surgery

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Figures S1 to S5
Table S1

Figure S1.

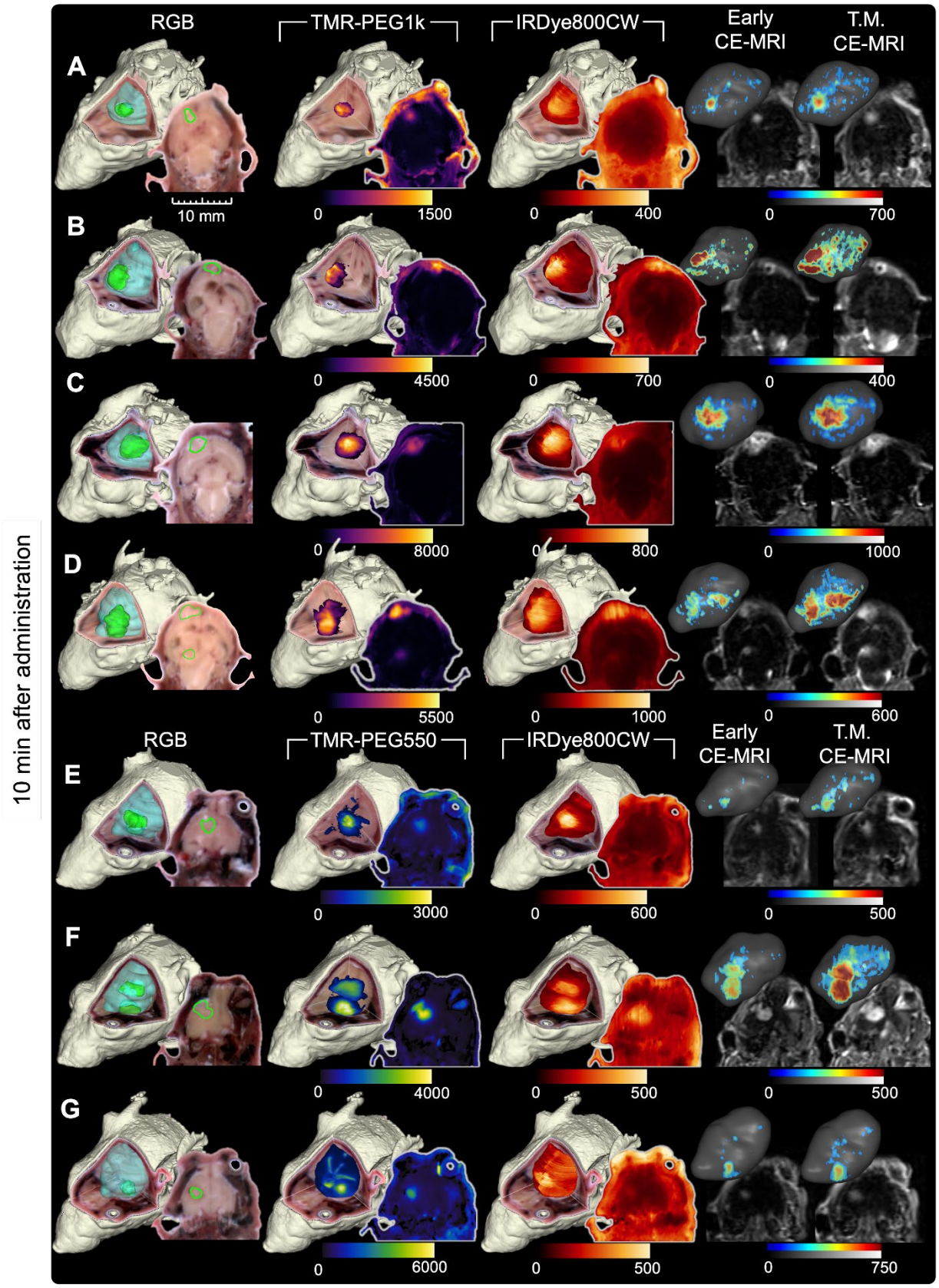
RGB
TMR-PEG1k
IRDye800CW
Early CE-MRI
T.M. CE-MRI
A
10 mm
0 1500
0 400
0 700
B
0 4500
0 700
0 400
C
0 8000
0 800
0 1000
D
0 5500
0 1000
0 600
10 min after administration
RGB
TMR-PEG550
IRDye800CW
Early CE-MRI
T.M. CE-MRI
E
0 3000
0 600
0 500
F
0 4000
0 500
0 500
G
0 6000
0 500
0 750

Figure S1. Cryo-imaging and CE-MRI image volumes of the remaining animals with U251 tumors 10 min after agent administration (described in Figure 2).

Figure S2.

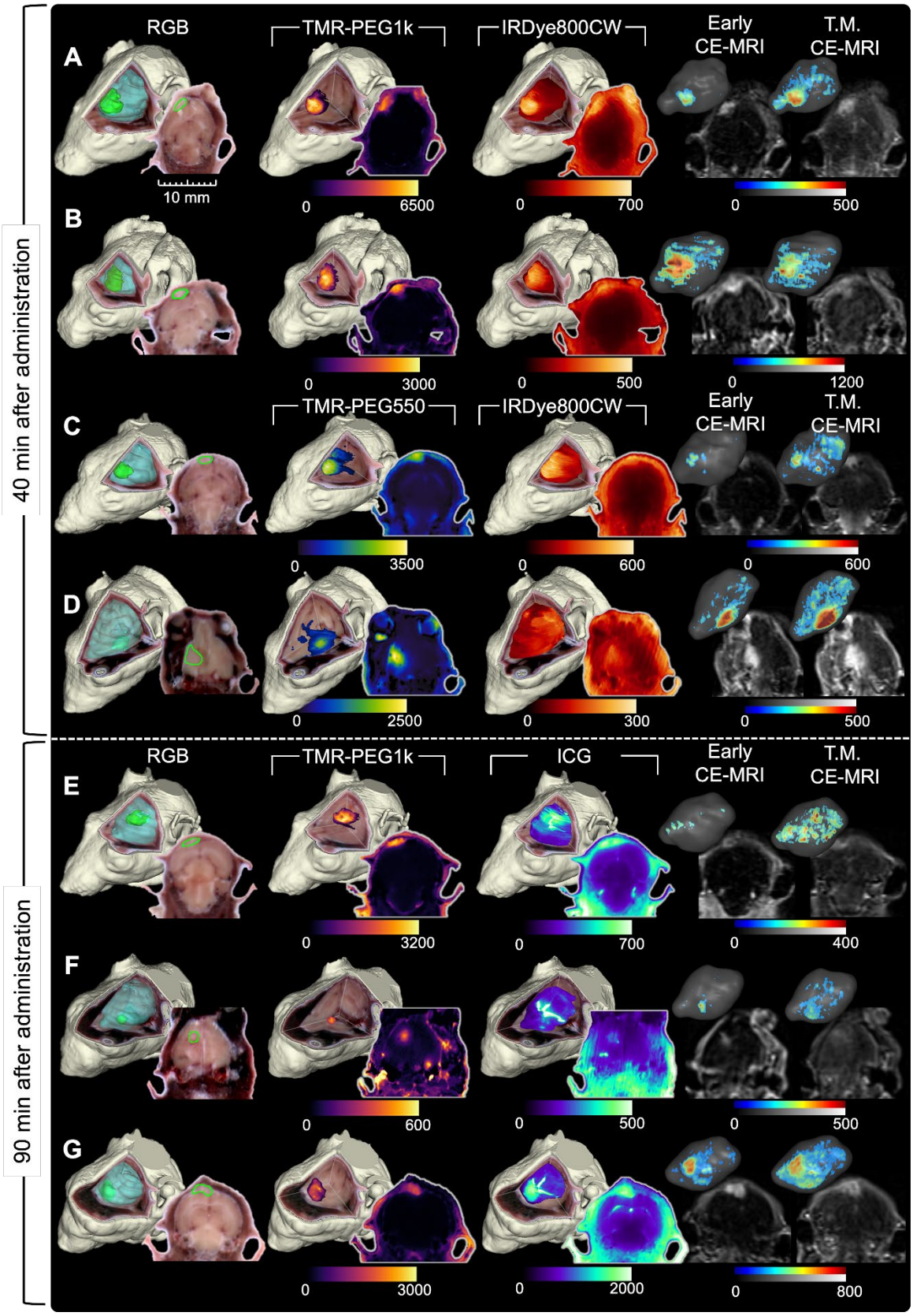
RGB
TMR-PEG1k
IRDye800CW
Early CE-MRI
T.M. CE-MRI
A
10 mm
0
6500
0
700
0
500
B
0
3000
0
500
0
1200
TMR-PEG550
IRDye800CW
C
0
3500
0
600
0
600
D
0
2500
0
300
0
500
40 min after administration
RGB
TMR-PEG1k
ICG
E
0
3200
0
700
0
400
F
0
600
0
500
0
500
G
0
3000
0
2000
0
800
90 min after administration

Figure S2. Cryo-imaging and CE-MRI image volumes of the remaining animals with U251 tumors 40 and 90 min after agent administration (described in Figure 3).

Figure S3.

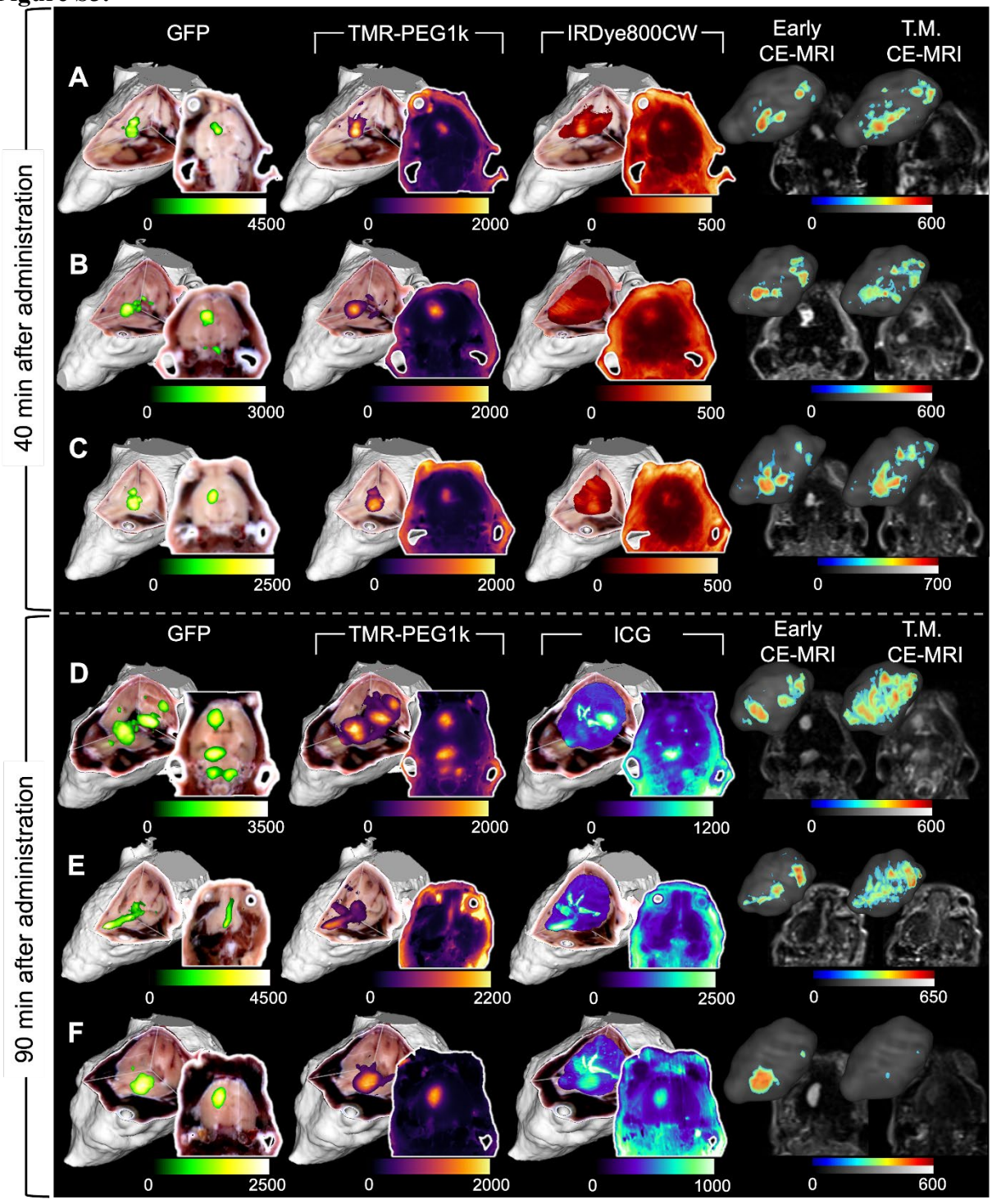

Figure S3. Cryo-imaging and CE-MRI image volumes of the remaining animals with U87 tumors 40 and 90 min after agent administration (described in Figure 6).

Figure S4.

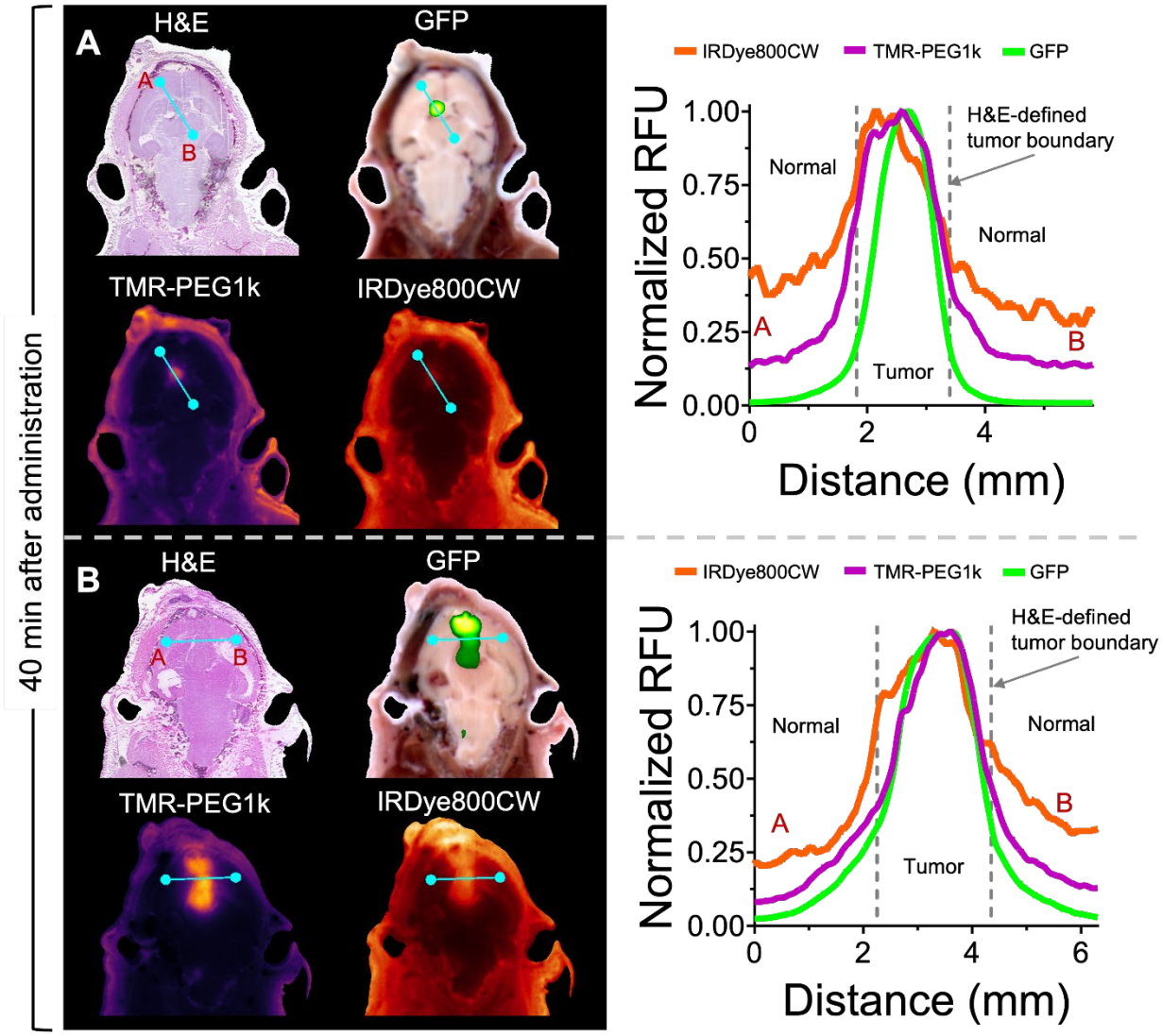

Figure S4. Co-registered H&E, GFP and contrast agent fluorescence for one section, and normalized fluorescence intensity profiles of GFP and contrast agent fluorescence for two animals (**A**-**B**) with U87 tumors 40 minutes after agent administration (described in Figure 8E-I).

Figure S5.

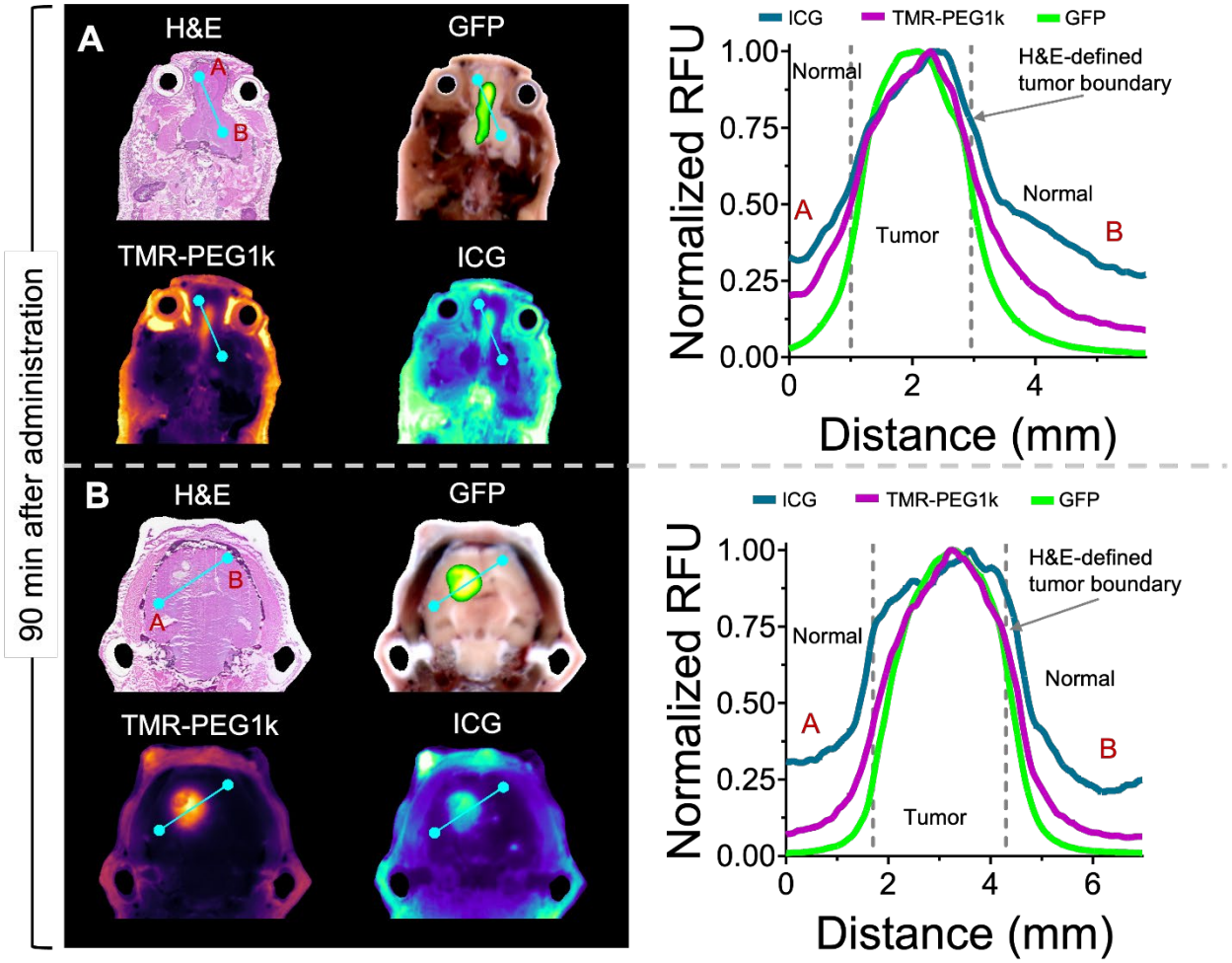

Figure S5. Co-registered H&E, GFP and contrast agent fluorescence for one section, and normalized fluorescence intensity profiles of GFP and contrast agent fluorescence for two animals (**A-B**) with U87 tumors 90 minutes after agent administration (described in Figure 8E-I).

Table S1.

Table S1. P-values for comparing TBR and CNR for paired fluorescent contrast agents injected in the same animal.

Paired TBR Contrast Agents	**10 min**	**40 min**	**90 min**
TMR-PEG1k vs. IRDye800CW	0.002^{**}	0.028^{*}	---
TMR-PEG1k vs. ICG	---	---	0.020^{*}
TMR-PEG550 vs. IRDye800CW	0.132^{ns}	0.174^{ns}	---
Paired CNR Contrast Agents			
TMR-PEG1k vs. IRDye800CW	0.040^{*}	0.078^{ns}	---
TMR-PEG1k vs. ICG	---	---	0.040^{*}
TMR-PEG550 vs. IRDye800CW	0.089^{ns}	0.125^{ns}	---

Note: $^{****}p \leq 0.0001$, $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$, $^{ns}p \geq 0.05$.