

Different cryo-formulations to cryopreserve wild-type iCART cells

Cryo-formulation Number (#)	Number of Mice (n)	Activation/Transduction Day	Treatment Groups
	5		Nalm6 cells in PBS (Control)
	5		Primary CART cells
	4	4/7	Fresh CART cells
1	3	4/8	CS10
2	1	4/8	Stem Cellbanker
3	2	4/8	BamBanker hRM
4	1	4/8	50% CS10 + 50% EM* (IMDM + 15% Human serum, w/ Cytokines#)
5	1	4/8	50% CS10 + 50% EM* (IMDM + 15% Human serum)
6	2	4/8	50% CS10 + 50% EM* (IMDM + 5% Human serum albumin)
7	1	4/8	80% Human Serum + 10%DMSO + 10% EM* (IMDM + 15% Human serum, w/ Cytokines#)
8	1	4/8	80% Human Serum + 10%DMSO + 10% EM* (IMDM + 15% Human serum)
9	2	4/10	50% CS10 + 50% EM* (IMDM + 15% Human serum, w/ Cytokines#)
10	2	4/8	50% CS10 + 50% Human Serum w/Cytokines
11	2	4/8 (different source)	50% CS10 + 50% EM* (IMDM + 5% Human serum albumin)

FIG. 1

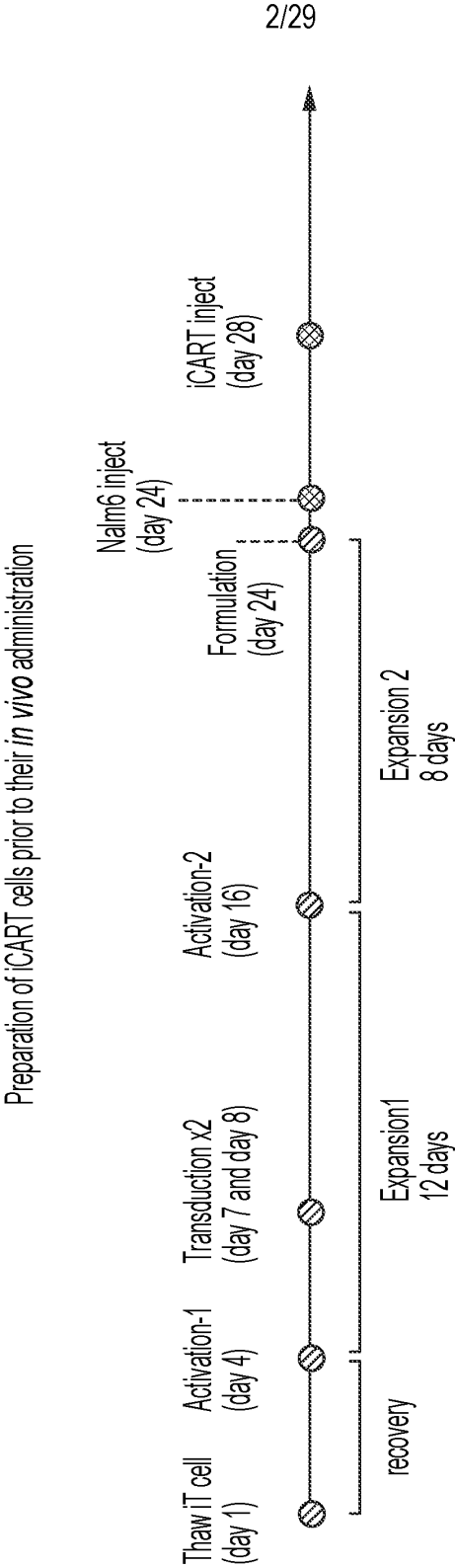


FIG. 2

In vivo efficacy of wild-type iCART cells cryopreserved using cryo-formulation #2, cryo-formulation #6, and cryo-formulation #10

Cryo-formulation number (#)	Number of Mice (n)	Activation/Transduction Day	Treatment Groups	1st Dose 1E7 CAR+ (15-Nov-2019)	2nd Dose 1E7/5E6 CAR+ (19-Nov-2019)
	5		Primary CART cells	1.00E+07	N/A
	4	4/7	Fresh CART cells	1.00E+07	N/A
2	1	4/8	Stem Cellbanker	1.00E+07	1.00E+07
6	2	4/8	50% CS10 + 50% EM* (IMDM + 5% Human serum albumin)	1.00E+07	1E7 (1 mouse)
10	2	4/8	50% CS10 + 50% Human Serum w/Cytokines	1.00E+07	N/A

FIG. 3A

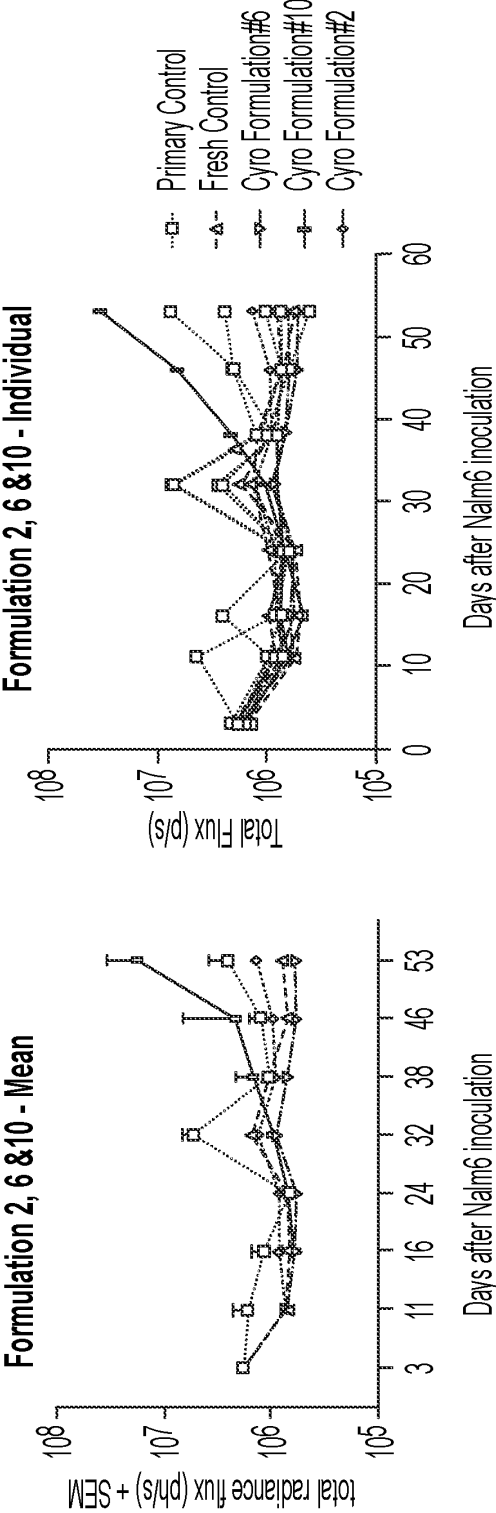


FIG. 3C

FIG. 3B

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Experimental design to evaluate the effect of washing of cryo-formulations on the *in vivo* efficacy of wild-type iCART cells

Group	Treatment Groups	Cryo-formulations	Dose	Route/Regimen	Target cell	n
1	PBS control		0	iv/1shot	Nalm6-luc/iv	5
2	Primary CART control		5.0 E6	iv/1shot	Nalm6-luc/iv	5
3	Formulation 1, no wash	50% CS10 + 50% Expansion medium (IMDM + 15% Human serum + Cytokines*)	1.5 E7	iv/1shot	Nalm6-luc/iv	5
4	Formulation 1, low	50% CS10 + 50% Expansion medium (IMDM + 15% Human serum + Cytokines*)	1.5 E7	iv/1shot	Nalm6-luc/iv	5
5	Formulation 1, high	50% CS10 + 50% Expansion medium (IMDM + 15% Human serum + Cytokines*)	4.0 E7	iv/1shot	Nalm6-luc/iv	5
6	Formulation 2, no wash	50% CS10 + 50% Expansion medium (IMDM + 5% HSA + Cytokines*)	1.5 E7	iv/1shot	Nalm6-luc/iv	5
7	Formulation 2, low	50% CS10 + 50% Expansion medium (IMDM + 5% HSA + Cytokines*)	1.5 E7	iv/1shot	Nalm6-luc/iv	5
8	Formulation 2, high	50% CS10 + 50% Expansion medium (IMDM + 5% HSA + Cytokines*)	4.0 E7	iv/1shot	Nalm6-luc/iv	5
9	Formulation 3, no wash	STEM-CELLBANKER® GMP grade	1.5 E7	iv/1shot	Nalm6-luc/iv	5
10	Formulation 3, low	STEM-CELLBANKER® GMP grade	1.5 E7	iv/1shot	Nalm6-luc/iv	5
11	Formulation 3, high	STEM-CELLBANKER® GMP grade	4.0 E7	iv/1shot	Nalm6-luc/iv	5

*Cytokines: IL-7 and IL-15 at the final concentration of 5ng/mL

FIG. 4

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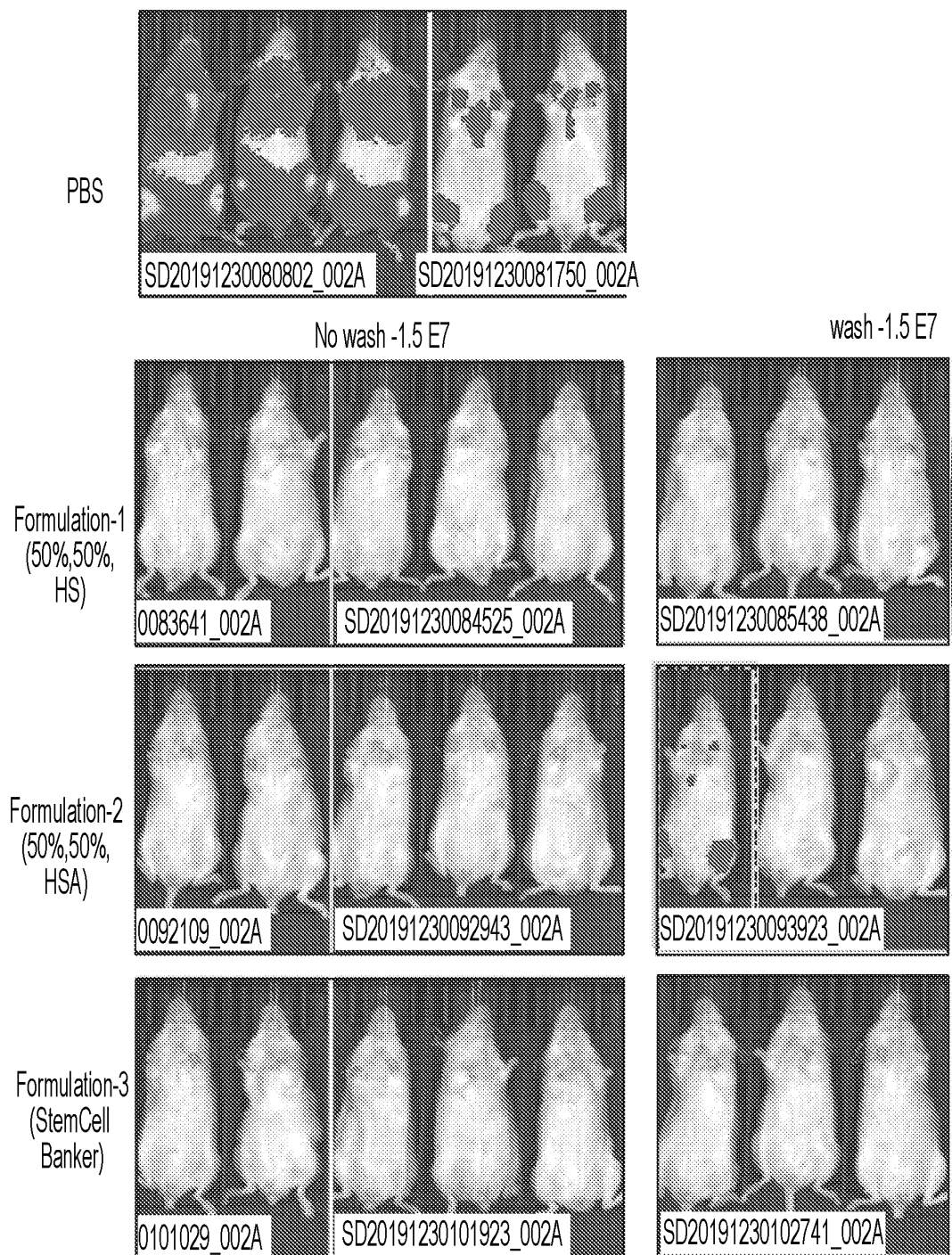
In vivo efficacy of wild-type iCART cells: animal imaging at Week 1

FIG. 5

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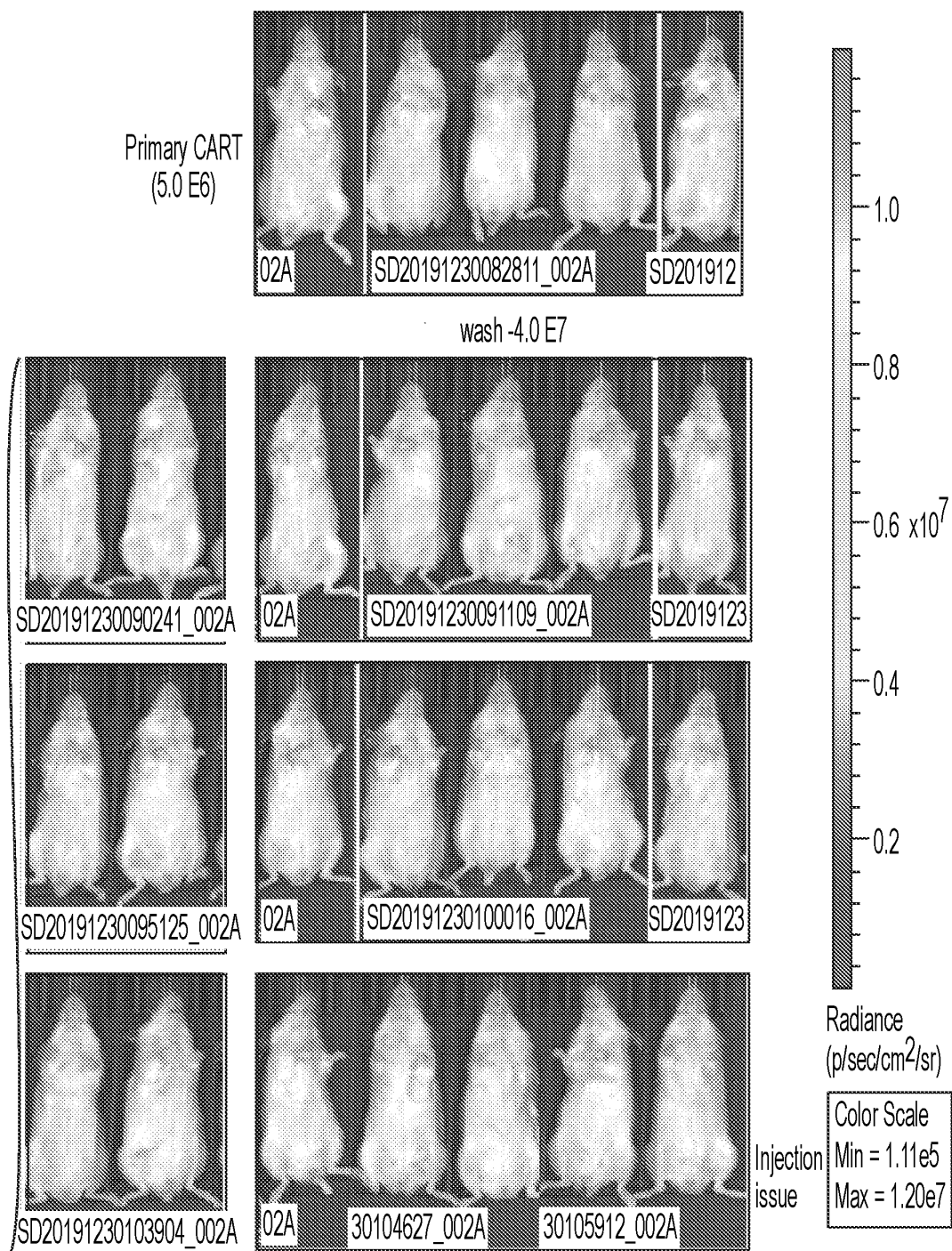


FIG. 5 CONT.

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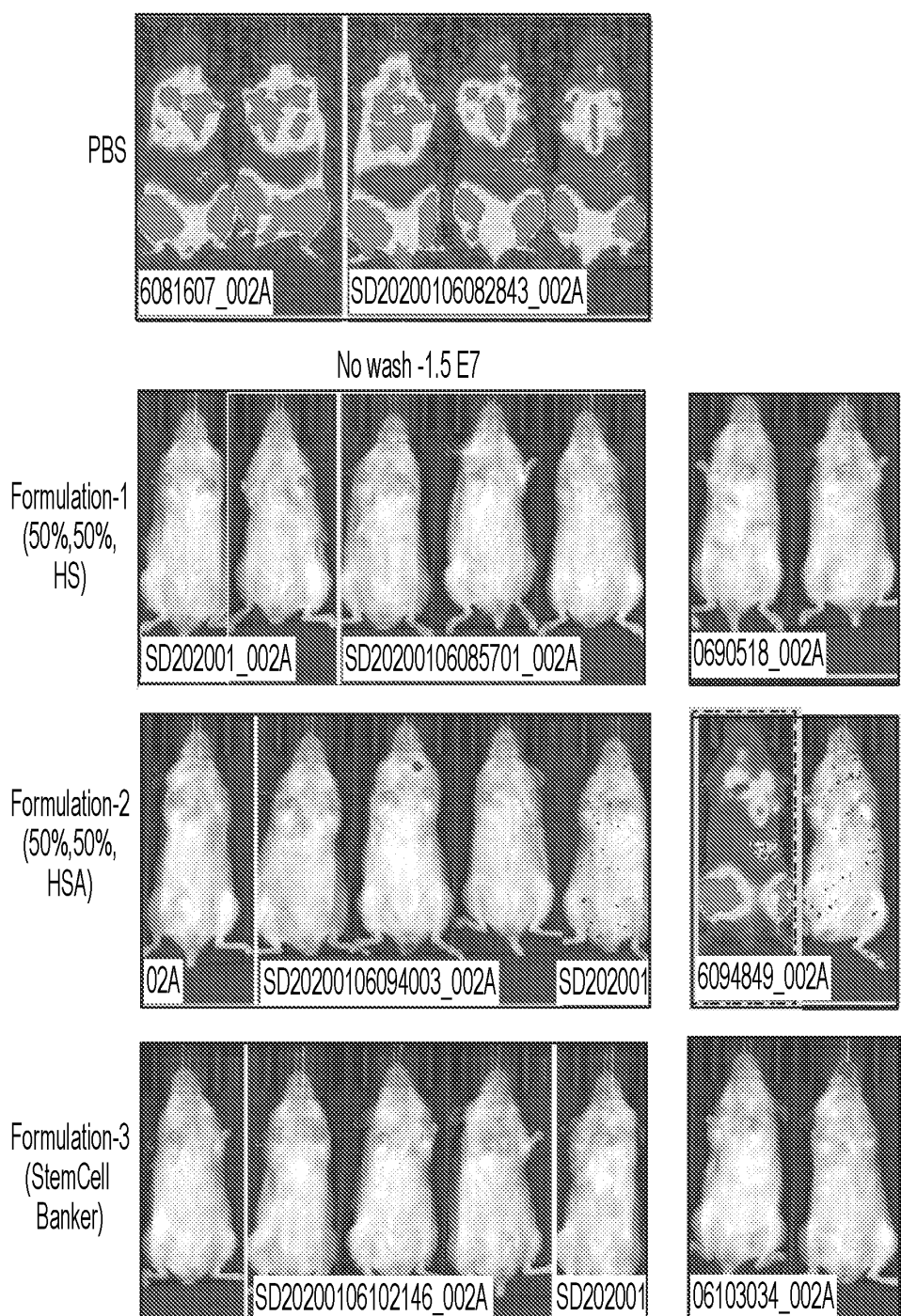
In vivo efficacy of wild-type iCART cells: animal imaging at Week 2

FIG. 6

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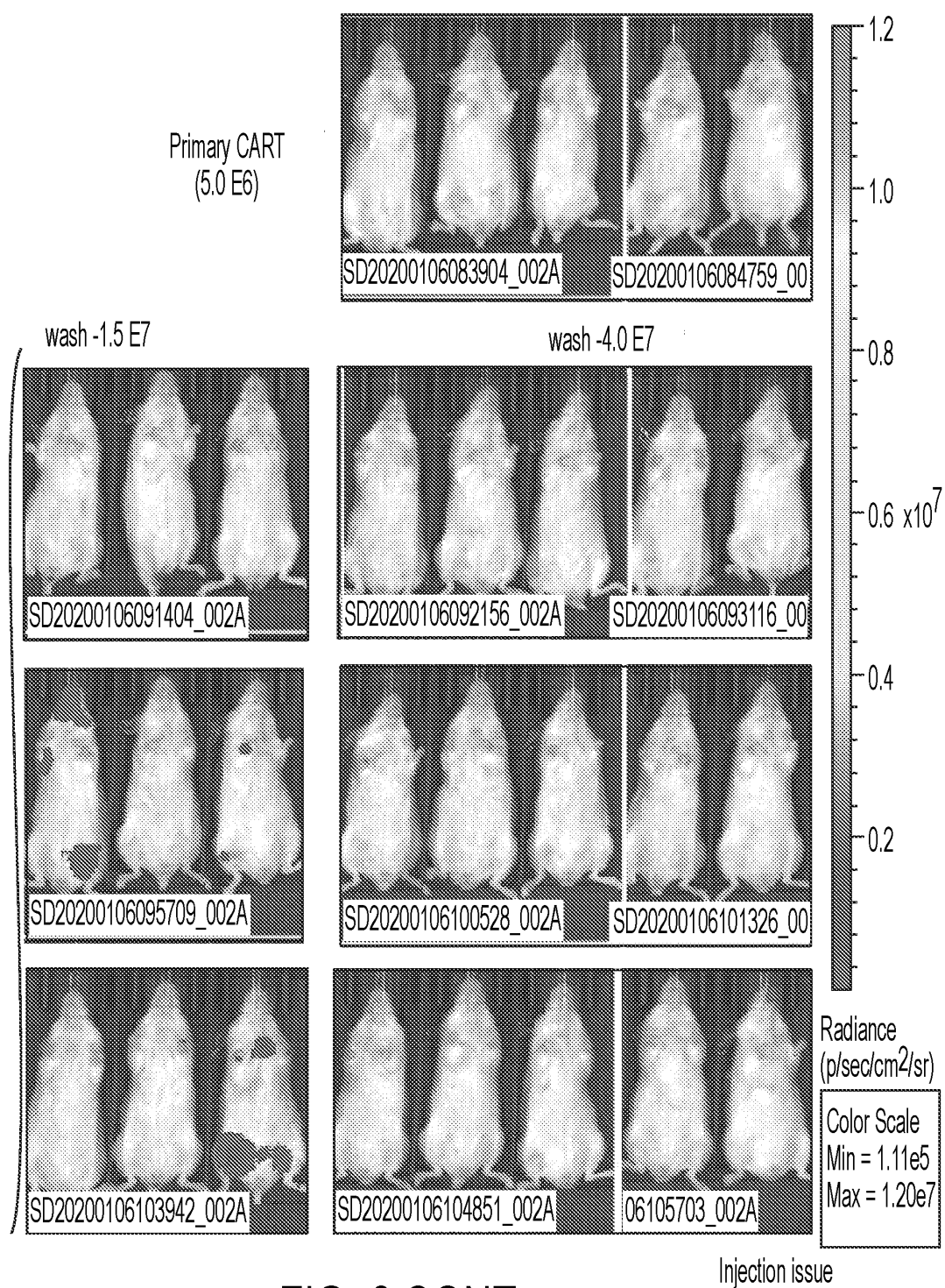


FIG. 6 CONT.

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Condition #	Conditions
1	50% CS-10, 50% culture media (expansion media with cytokine and 15% human serum)
2	80% human serum and 10% culture media (expansion media with cytokine and 15% human serum) and 10% DMSO containing 5% (w/v) sucrose
3	CS10
4	90% CS-10, 10% culture media (expansion media with cytokine and 15% human serum)
5	Stem Cell Banker GMP

FIG. 7A

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Group	effector cell	Condition #	Frozen sock term	Description	dose	n
Gr-1	Control		-	Control		5
Gr-2	iCART, fresh	1	7 days	Expanded for 7 days. iCART cells collected and administrated without additional formulation.	1×10^7	5
Gr-3	iCART, cryostock	1	14 days	Cultured for 3 days. iCART cells collected and administrated without additional formulation.	1×10^7	5
Gr-4	iCART, fresh	2	7 days	Expanded for 7 days. iCART cells collected and administrated without additional formulation.	1×10^7	5
Gr-5	iCART, cryostock	2	14 days	Cultured for 3 days. iCART cells collected and administrated without additional formulation.	1×10^7	5 $\rightarrow$ 4
Gr-6	iCART, fresh	3	7 days	Collected and administrated without additional formulation.	1×10^7	5
Gr-7	iCART, cryostock	3	14 days	Collected and administrated without additional formulation.	1×10^7	5
Gr-8	iCART, cryostock	4	14 days	Cultured for 3 days. iCART cells collected and administrated without additional formulation.	1×10^7	5
Gr-9	iCART, cryostock	5	14 days	Cultured for 3 days. iCART cells collected and administrated without additional formulation.	1×10^7	3 $\rightarrow$ 1
Gr-10	iCART, cryostock	3	17 days	Washed and administrated.	1×10^7	4 $\rightarrow$ 1
Gr-11	iCART, cryostock	4	17 days	Washed and administrated.	1×10^7	4 $\rightarrow$ 1
Gr-12	Primary CART (cryo)		-	100% CS10	5×10^6	5

FIG. 7B

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Experimental design to evaluate and compare the *in vivo* efficacy of fresh iCART cells vs cryostock iCART cells

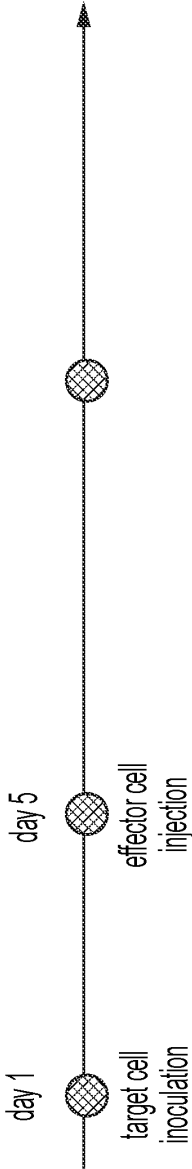


FIG. 7C

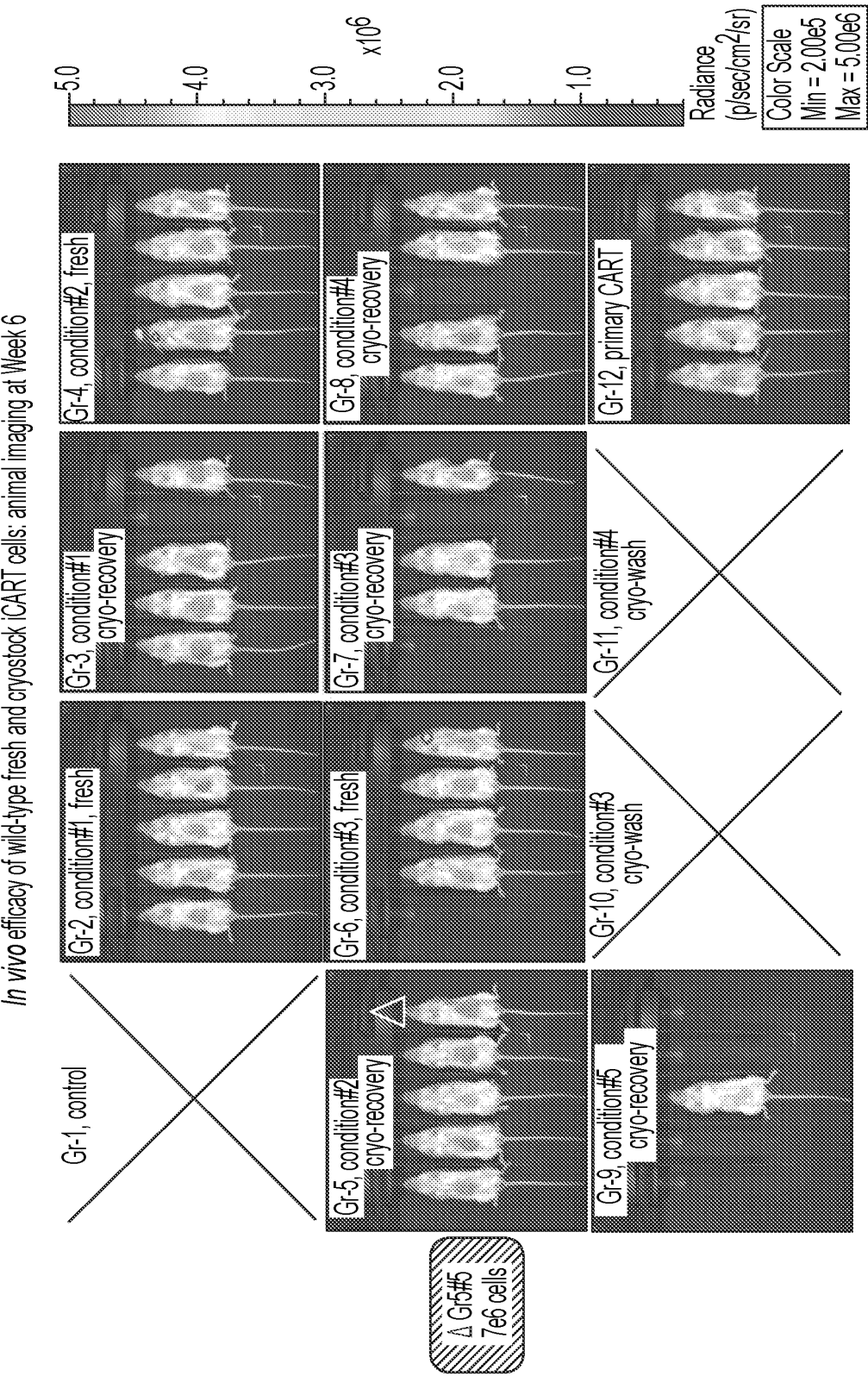


FIG. 8

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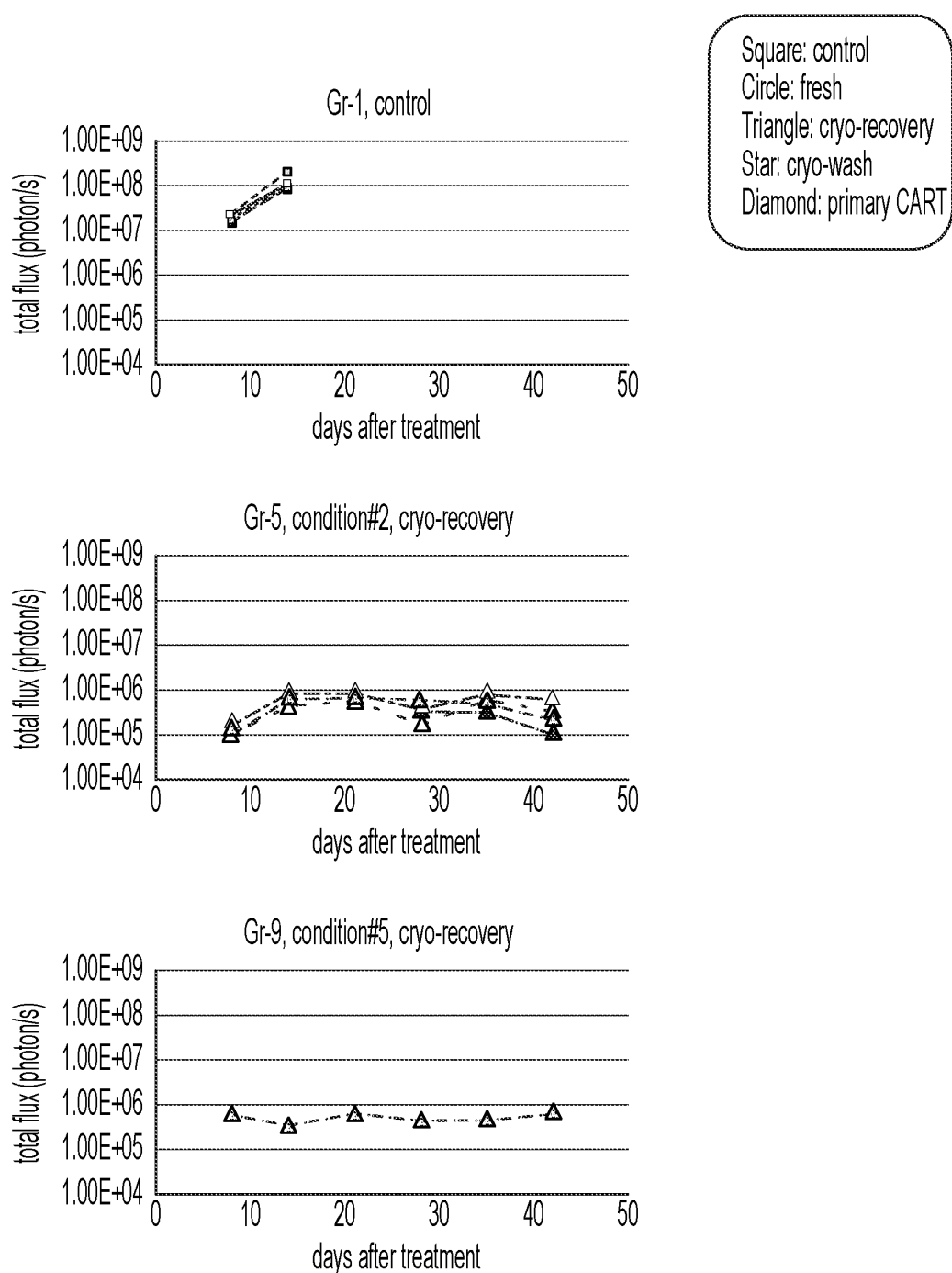
In vivo efficacy of wild-type fresh and cryostock iCART cells: animal imaging at Week 6

FIG. 9

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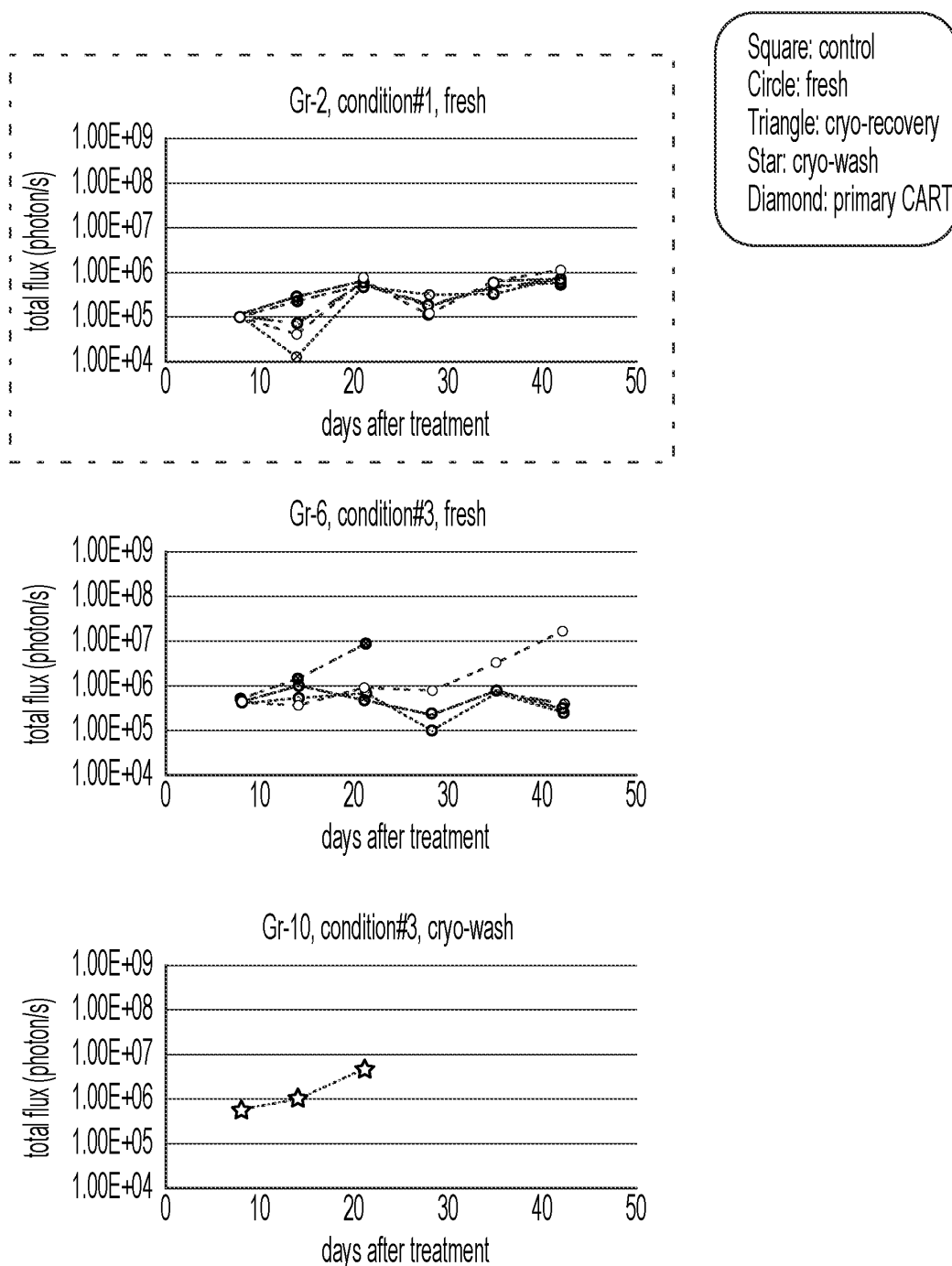
In vivo efficacy of wild-type fresh and cryostock iCART cells: animal imaging at Week 6

FIG. 9 CONT.

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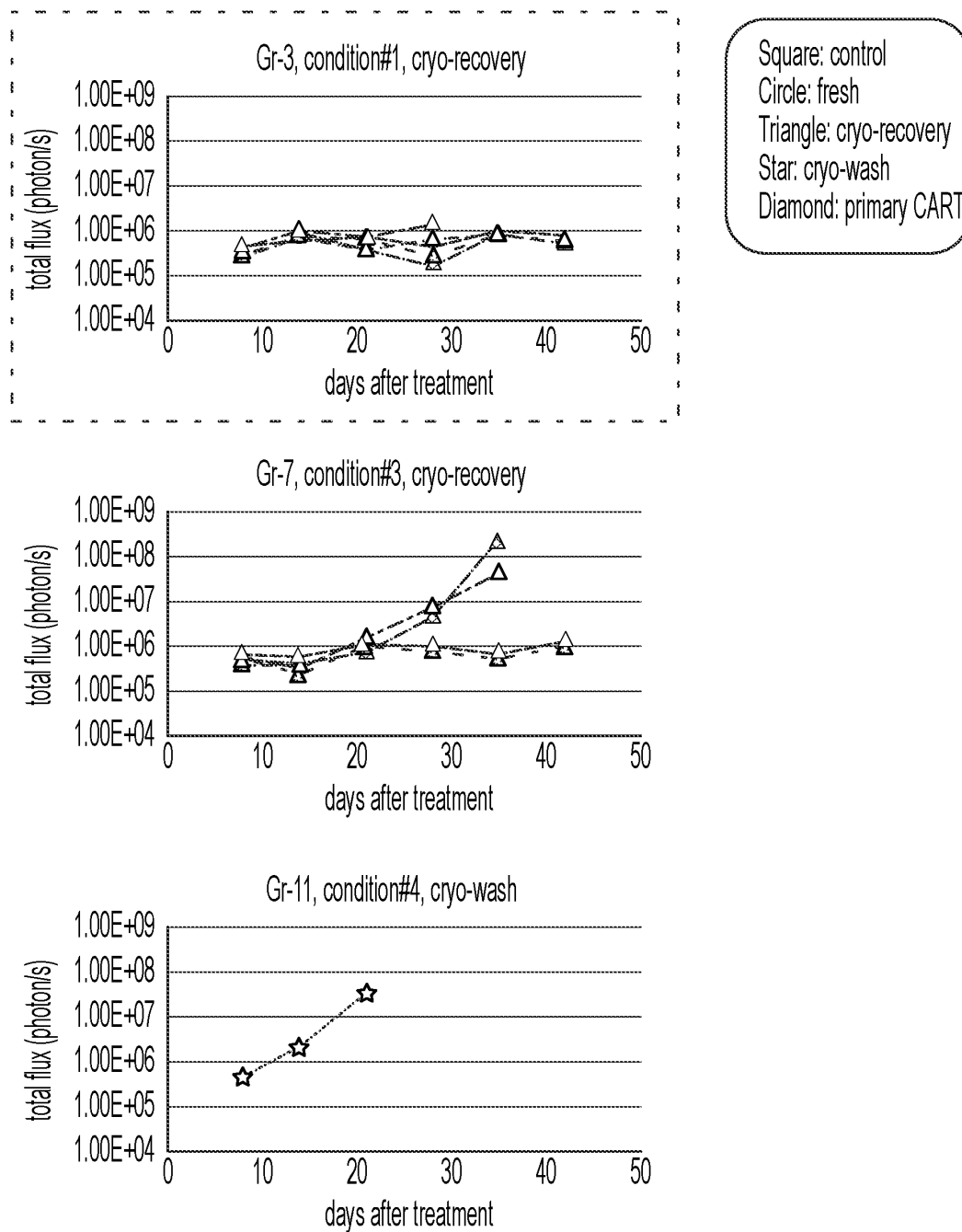
In vivo efficacy of wild-type fresh and cryostock iCART cells: animal imaging at Week 6

FIG. 9 CONT.

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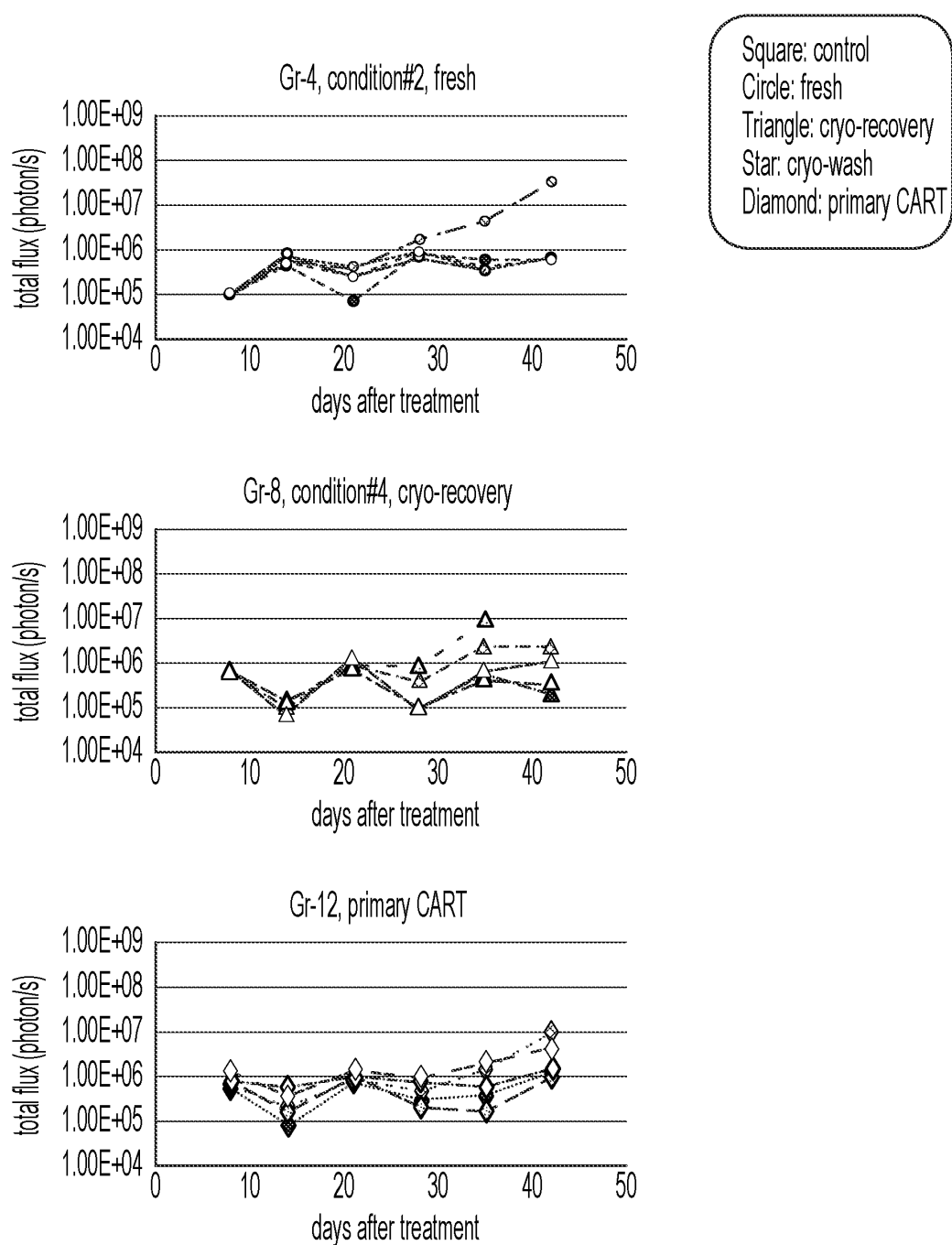
In vivo efficacy of wild-type fresh and cryostock iCART cells: animal imaging at Week 6

FIG. 9 CONT.

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Cryo-formulations to cryopreserve iCART cells

Formulation #	Formulations
#1	80%HS+10%EM+10%DMSQ w/ Cytokine
#2	80%HS+10%EM_10%DMSO w/o Cytokine
#3	CS10:EM (HS)=1:1 w/ Cytokine
#4	CS10:EM (Albminar)=1:1 w/o Cytokme
#5	CS10:EM (HS)=1:1 w/o Cytokme
#6	CS10:WB=2:1
#7	CS10:WB=1:1
#8	StemCell Banker:WB = 2:1
#9	BamBanker hRM:WB = 2:1
#10	CS10:HS=1:1 w/ Cytokine
#11	CS10:HS=1:1 w/o Cytokine
#12	StemCell Banker
#13	BamBanker hRM

FIG. 10

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Group	Group name	Formulation	dose	route/regimen	target cell	n
Gr-1	Control			iv/1 shot	Nalm6-luc/iv	5
Gr-2	Fresh, 1e7	Fresh	1e7	iv/1 shot	Nalm6-luc/iv	5
Gr-3	Primary CART, 5e6	CS10, cryo	5e6	iv/1 shot	Nalm6-luc/iv	5
Gr-4	Formulation#1, 2e7	80%HS+10%EM+10%DMSO w/ Cytokine	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-5	Formulation#1, 3e7	80%HS+10%EM+10%DMSO w/ Cytokine	3e7	iv/1 shot	Nalm6-luc/iv	5
Gr-6	Formulation#2, 2e7	80%HS+10%EM+10%DMSO w/o Cytokine	2e7	iv/1 shot	Nalm6-luc/iv	5→4
Gr-7	Formulation#3, 2e7	CS10:EM (HS)=1:1 w/ Cytokine	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-8	Formulation#3, 3e7	CS10:EM (HS)=1:1 w/ Cytokine	3e7	iv/1 shot	Nalm6-luc/iv	5
Gr-9	Formulation#4, 2e7	CS10:EM (Albminar)=1:1 w/o Cytokine	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-10	Formulation#4, 3e7	CS10:EM (Albminar)=1:1 w/o Cytokine	3e7	iv/1 shot	Nalm6-luc/iv	5

FIG. 11A

Gr-11	Formulation#5, 2e7	CS10:EM (HS)=1:1 w/o Cytokine	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-12	Formulation#6, 2e7	CS10:WB=2:1	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-13	Formulation#6, 3e7	CS10:WB=2:1	3e7	iv/1 shot	Nalm6-luc/iv	5
Gr-14	Formulation#7, 2e7	CS10:WB=1:1	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-15	Formulation#8, 2e7	StemCell Banker:WB=2:1	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-16	Formulation#9, 2e7	BamBanker hRM:WB =2:1	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-17	Formulation#10, 2e7	CS10:HS=1:1 w/ Cytokine	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-18	Formulation#11, 2e7	CS10:HS=1:1 w/o Cytokine	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-19	Formulation#12, 2e7	StemCell Banker	2e7	iv/1 shot	Nalm6-luc/iv	5
Gr-20	Formulation#13, 2e7	BamBanker hRM	2e7	iv/1 shot	Nalm6-luc/iv	5

FIG. 11A CONT.

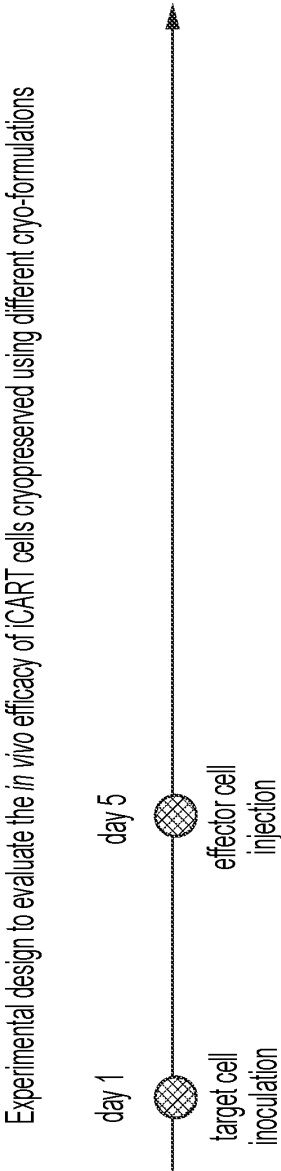


FIG. 11B

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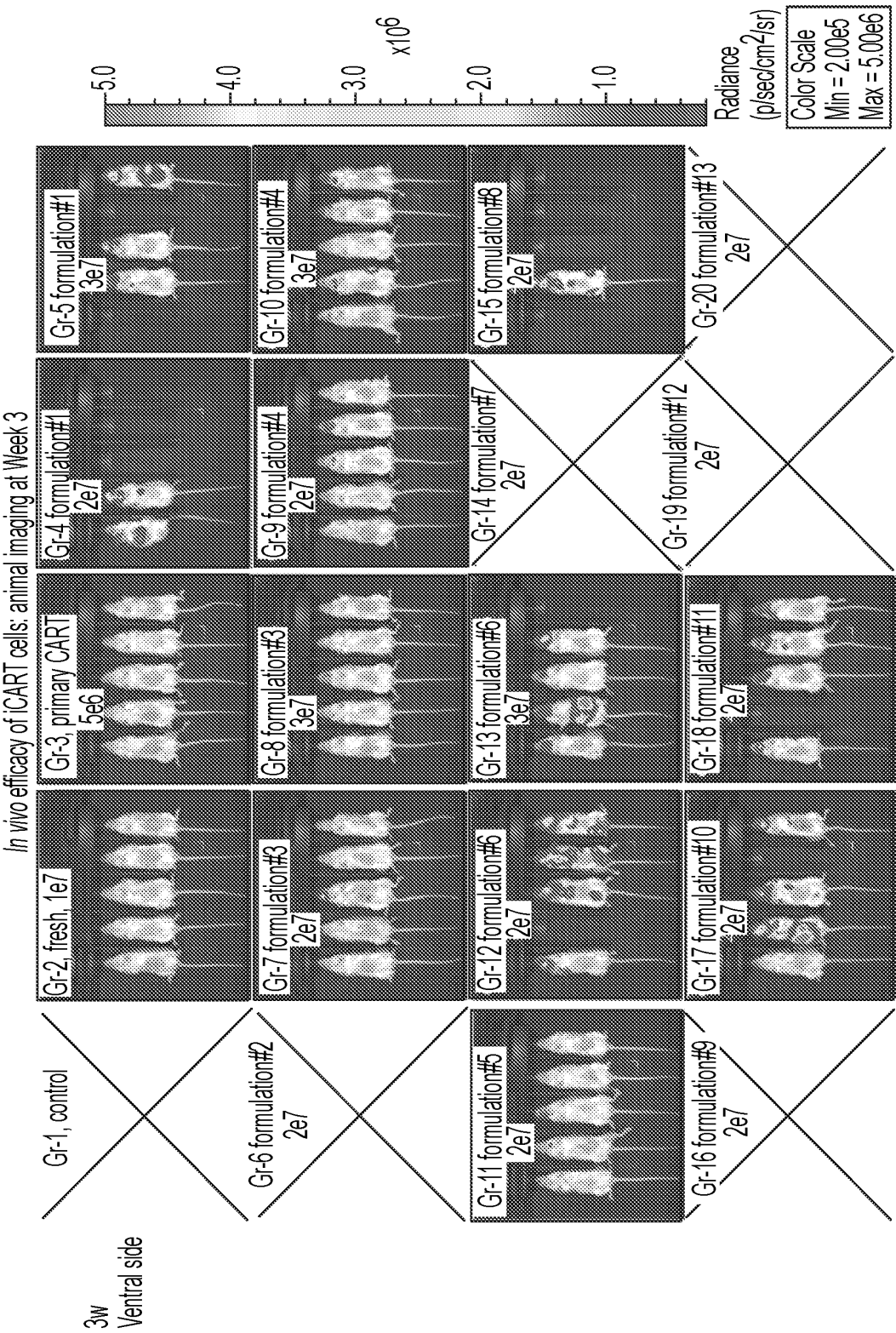


FIG. 12

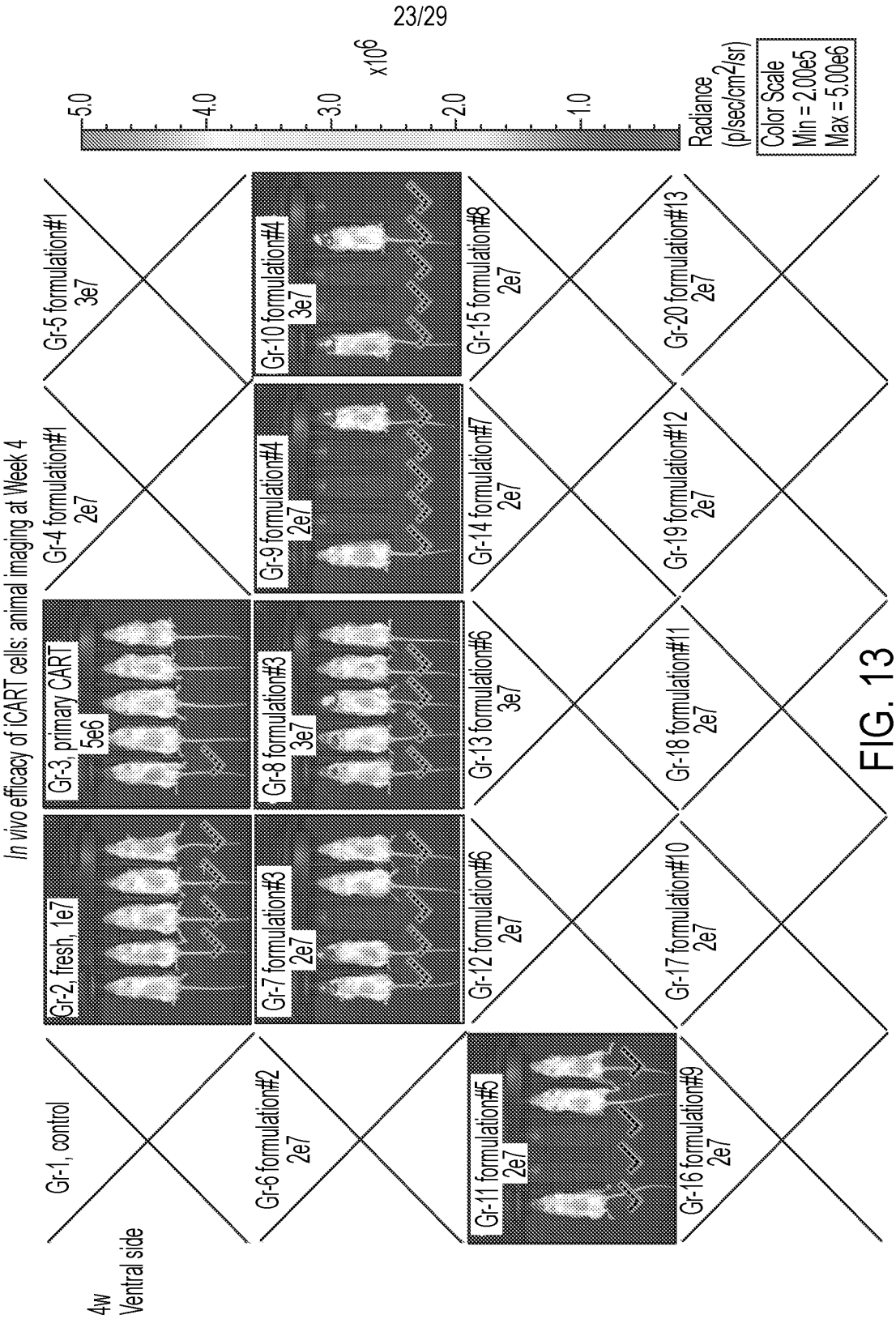


FIG. 13

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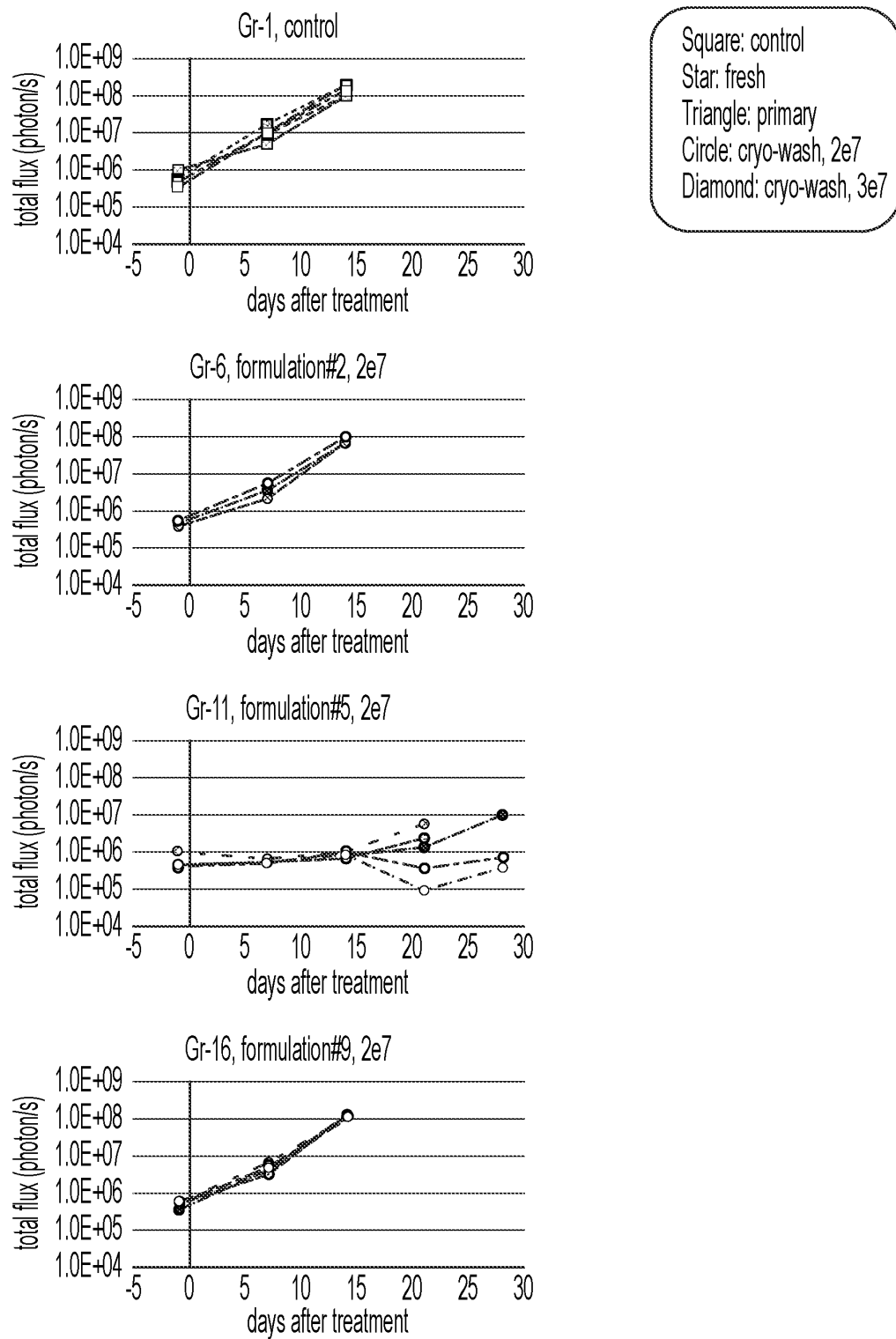
In vivo efficacy of iCART cells at Week 4

FIG. 14

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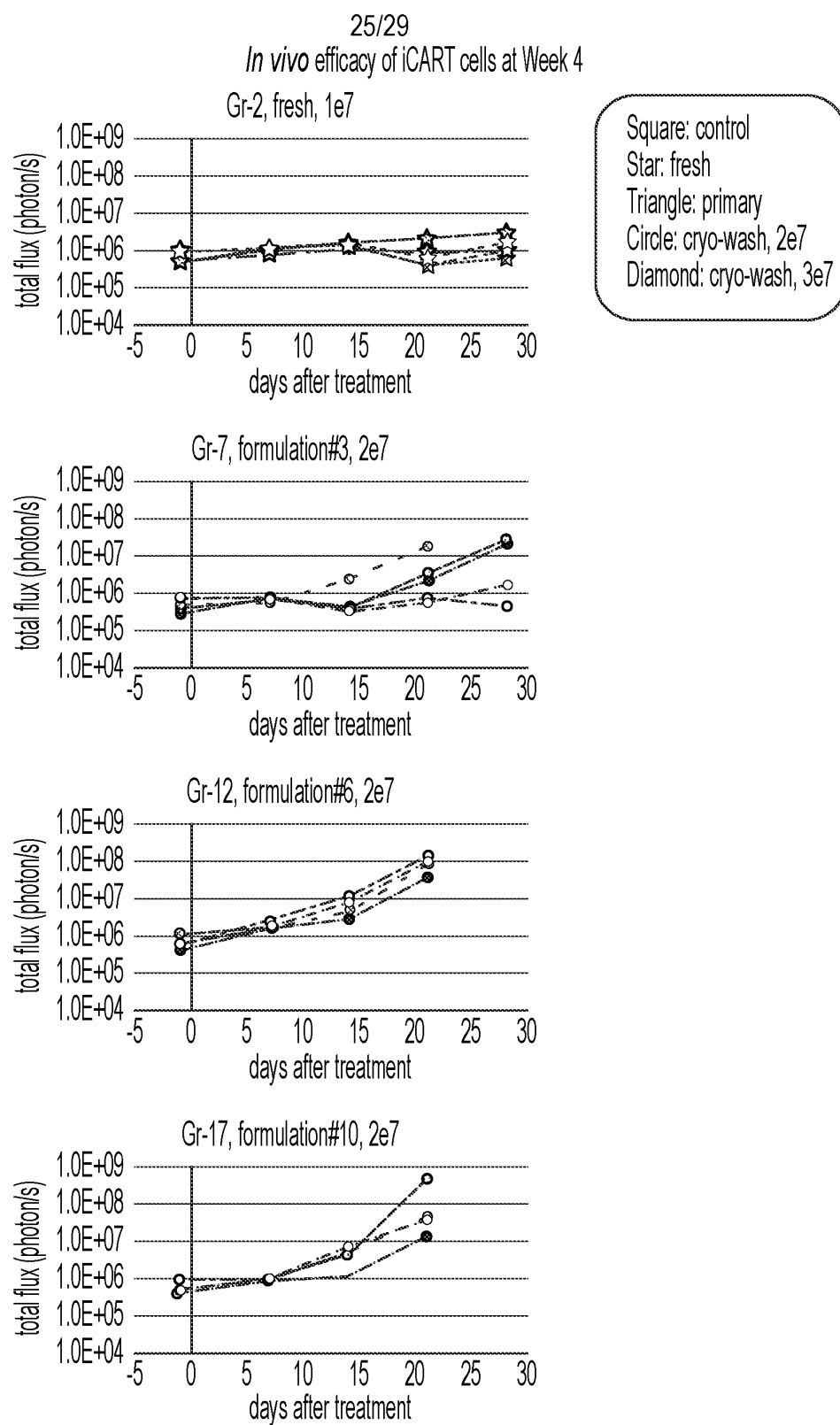


FIG. 14 CONT.

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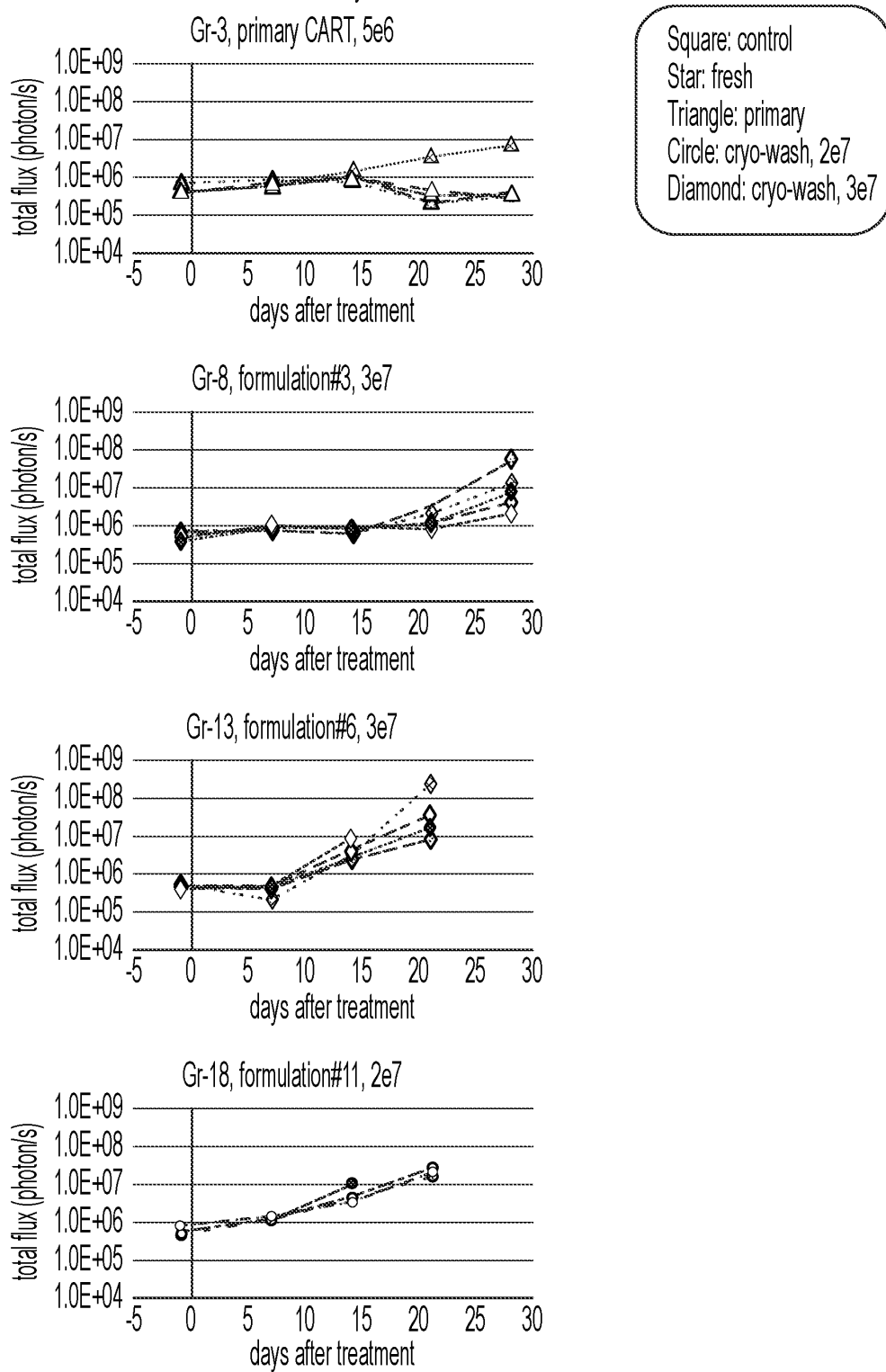
In vivo efficacy of iCART cells at Week 4

FIG. 14 CONT.

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In vivo efficacy of iCART cells at Week 4

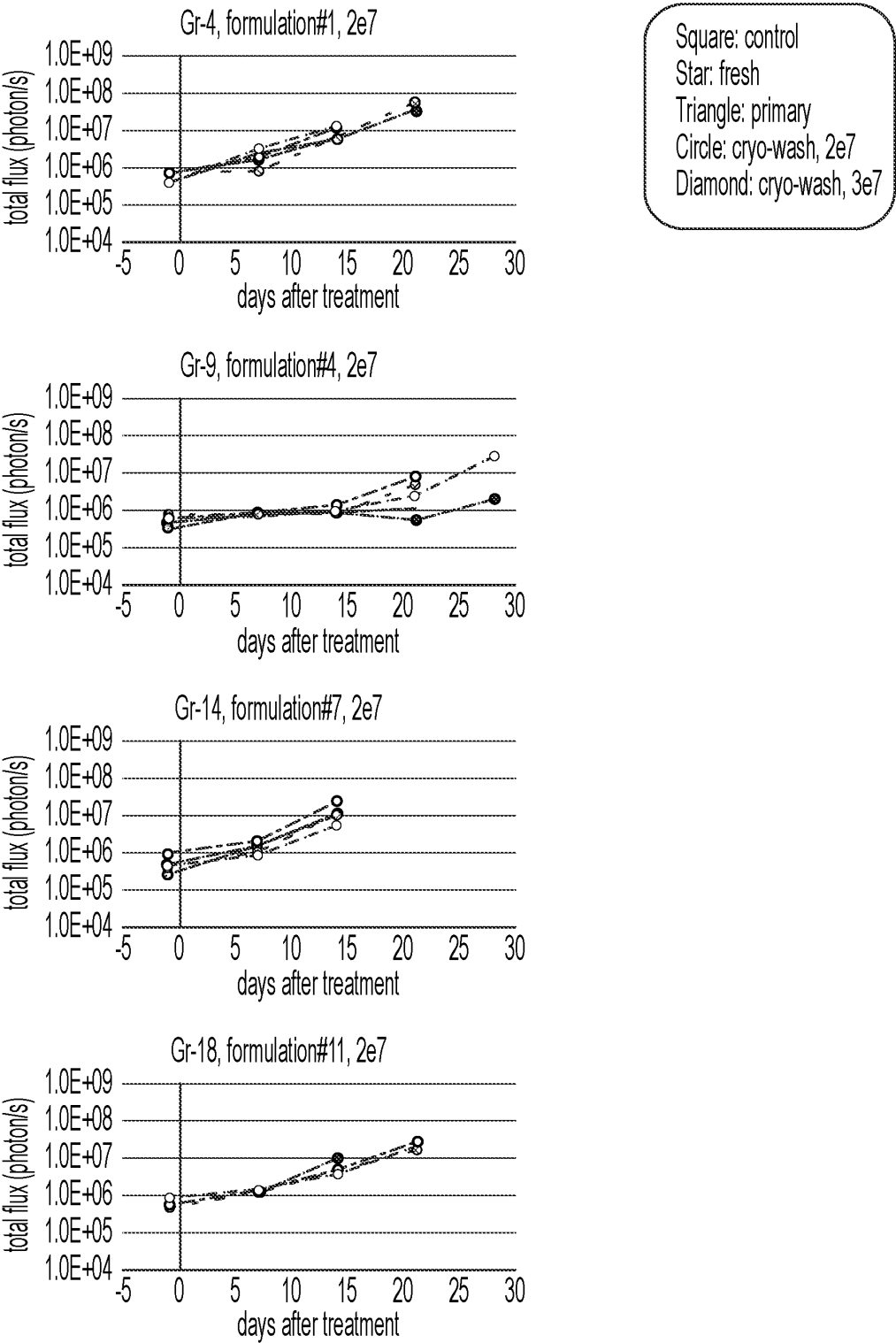


FIG. 14 CONT.

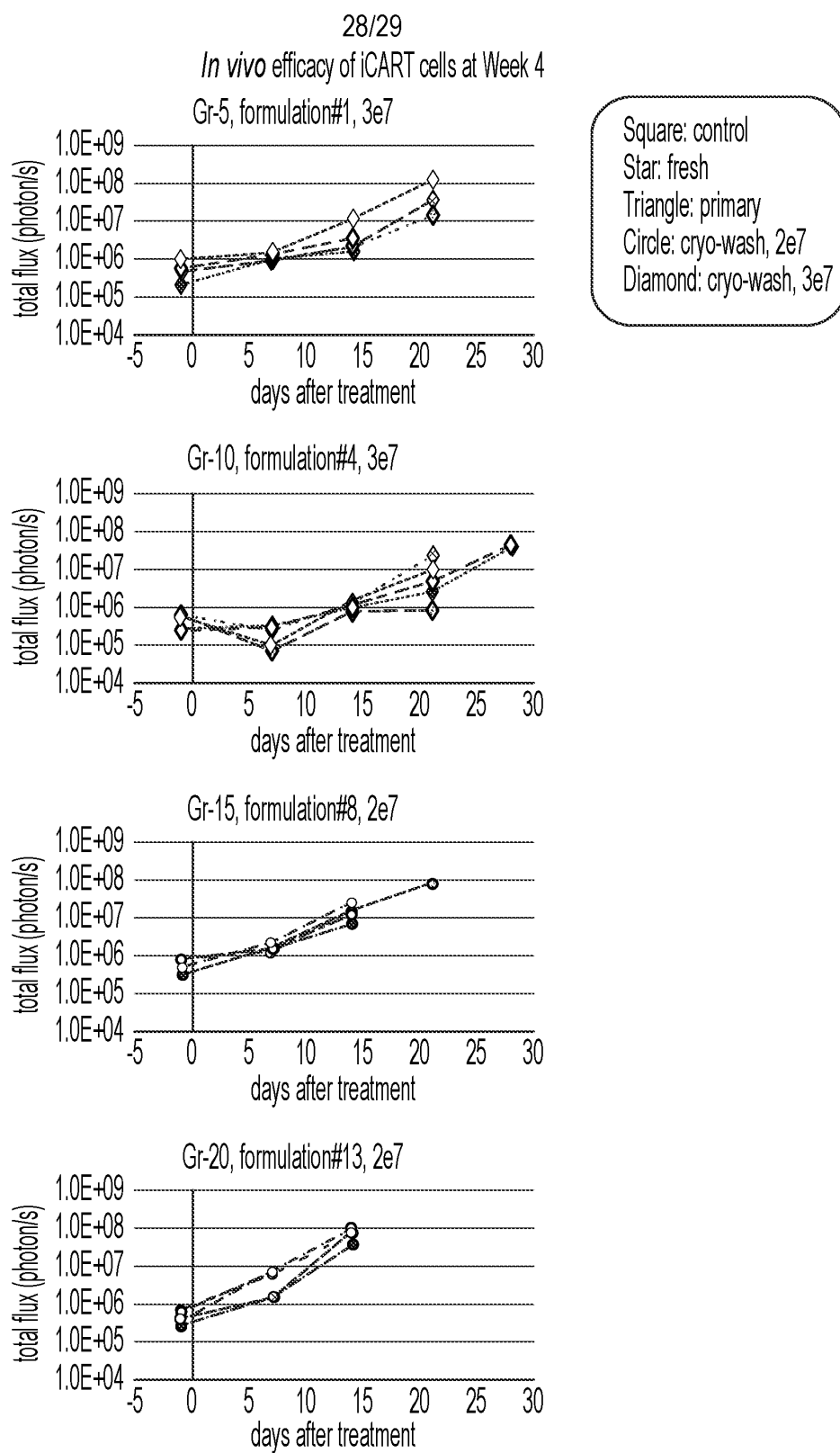


FIG. 14 CONT.

In vivo efficacy of iCART cells: cellular kinetics

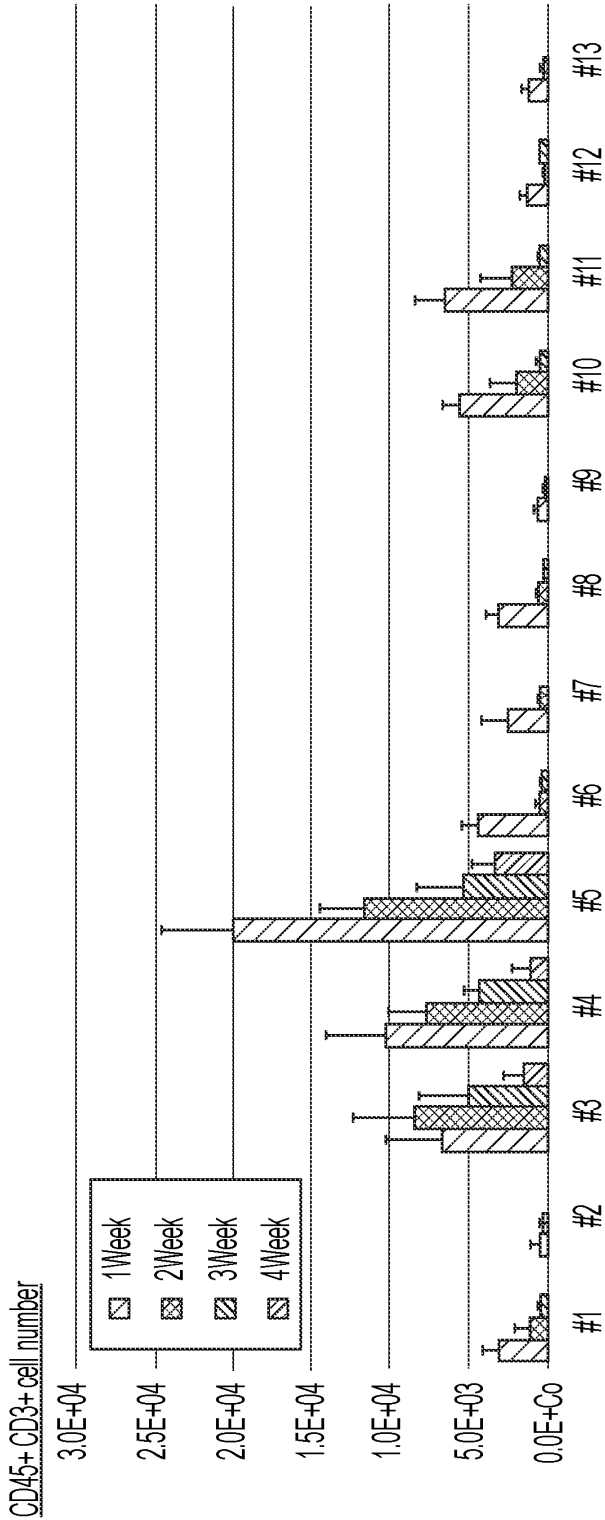


FIG. 15