Fig. 1A

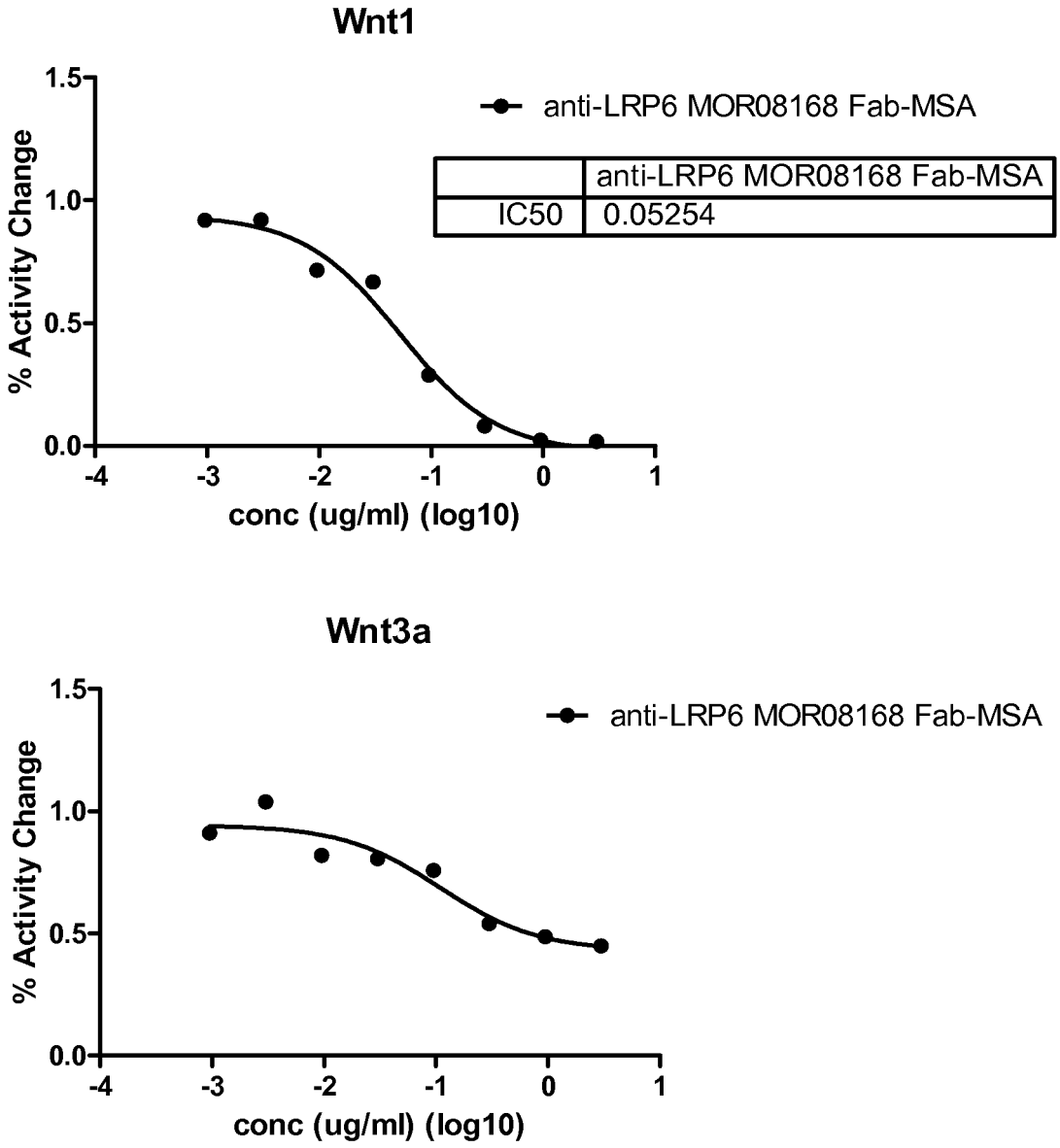

Wnt1
anti-LRP6 MOR08168 Fab-MSA
anti-LRP6 MOR08168 Fab-MSA
IC50
0.05254
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)
Wnt3a
anti-LRP6 MOR08168 Fab-MSA
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)

Fig1B

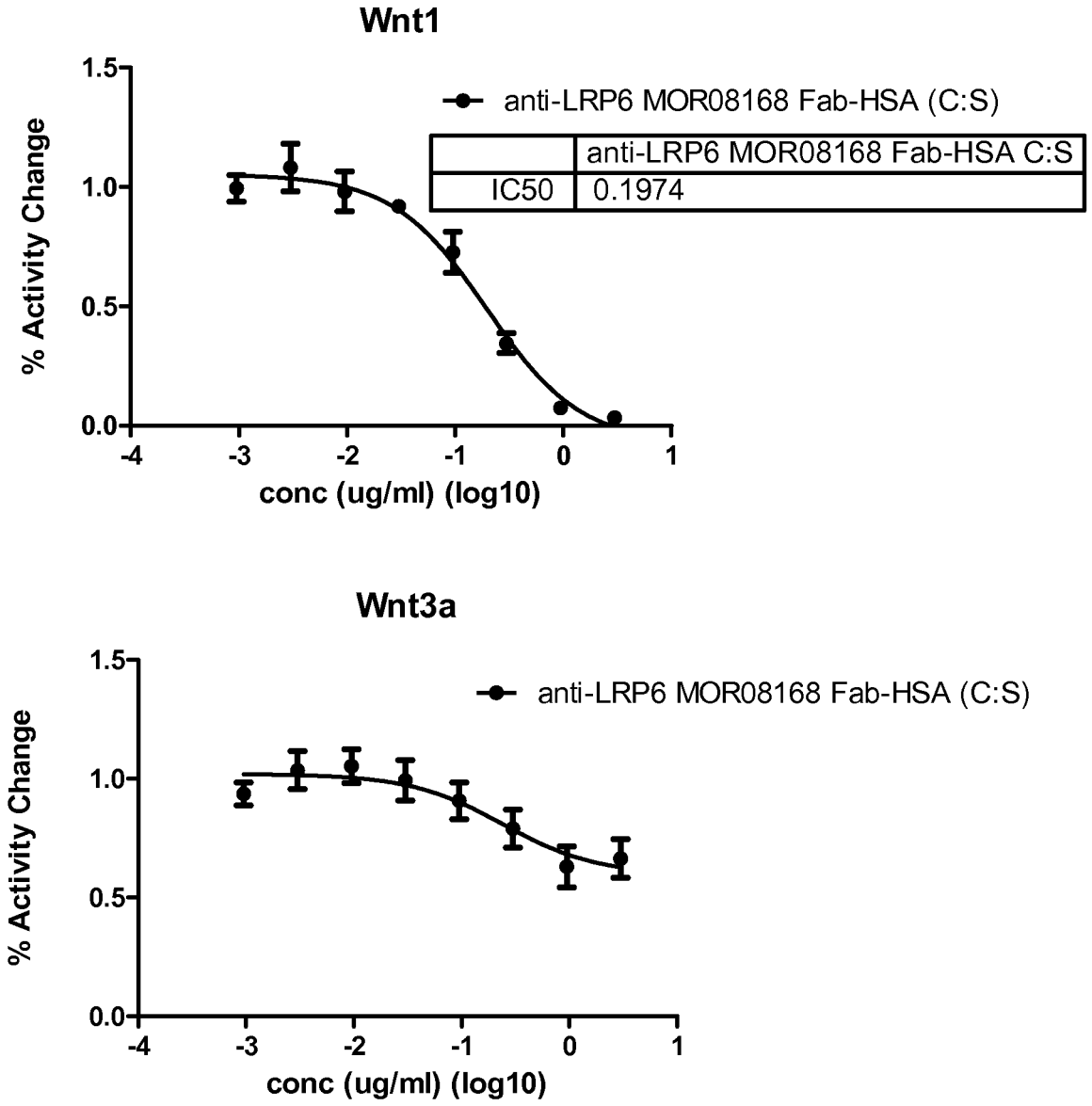

Wnt1
anti-LRP6 MOR08168 Fab-HSA (C:S)
anti-LRP6 MOR08168 Fab-HSA C:S
IC50
0.1974
% Activity Change
conc (ug/ml) (log10)
Wnt3a
anti-LRP6 MOR08168 Fab-HSA (C:S)
% Activity Change
conc (ug/ml) (log10)

Fig 1C

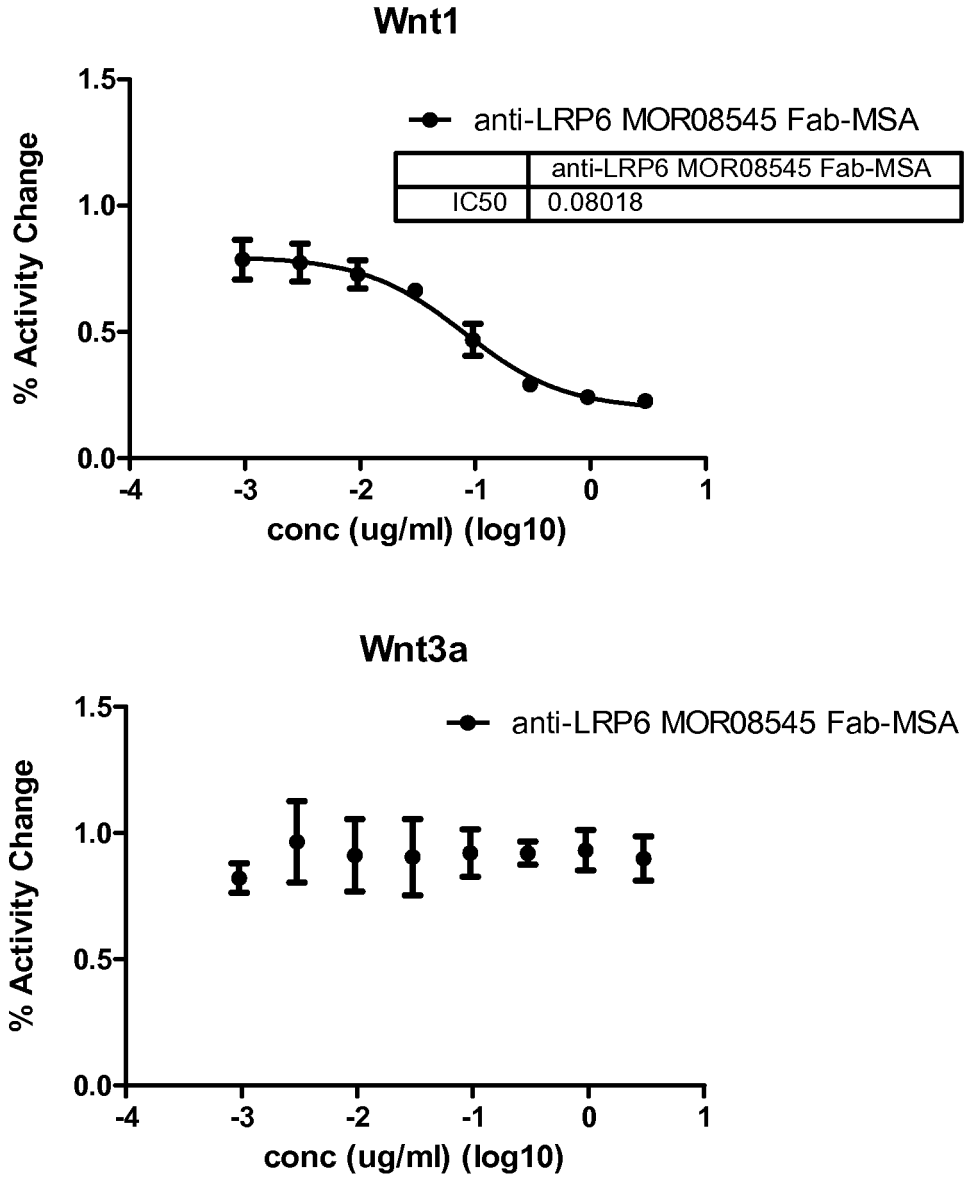
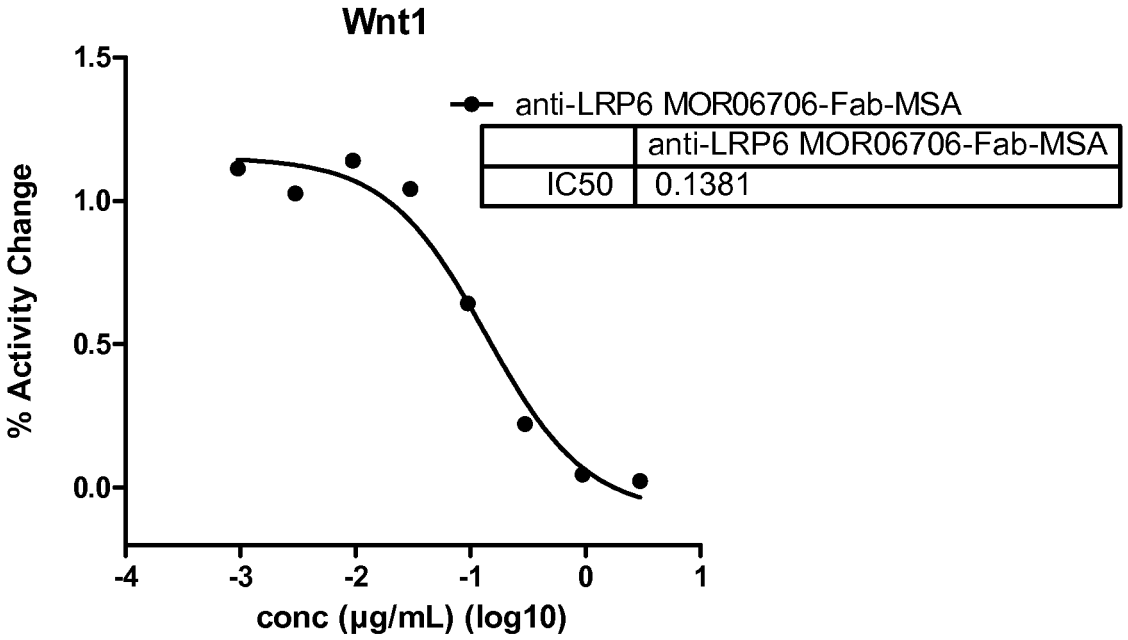

Wnt1
anti-LRP6 MOR08545 Fab-MSA
anti-LRP6 MOR08545 Fab-MSA
IC50 0.08018
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)
Wnt3a
anti-LRP6 MOR08545 Fab-MSA
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)

Fig1D



	anti-LRP6 MOR06706-Fab-MSA
IC50	0.1381

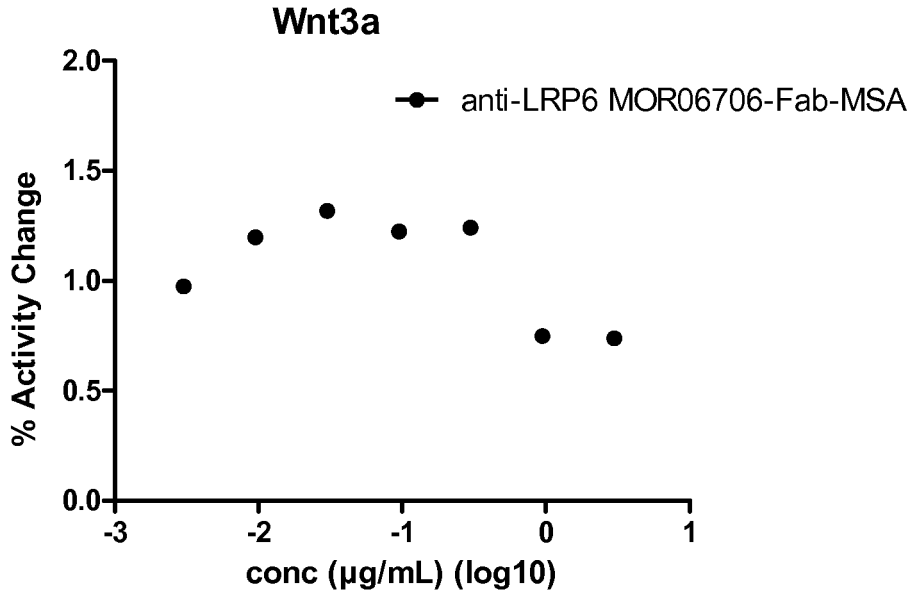
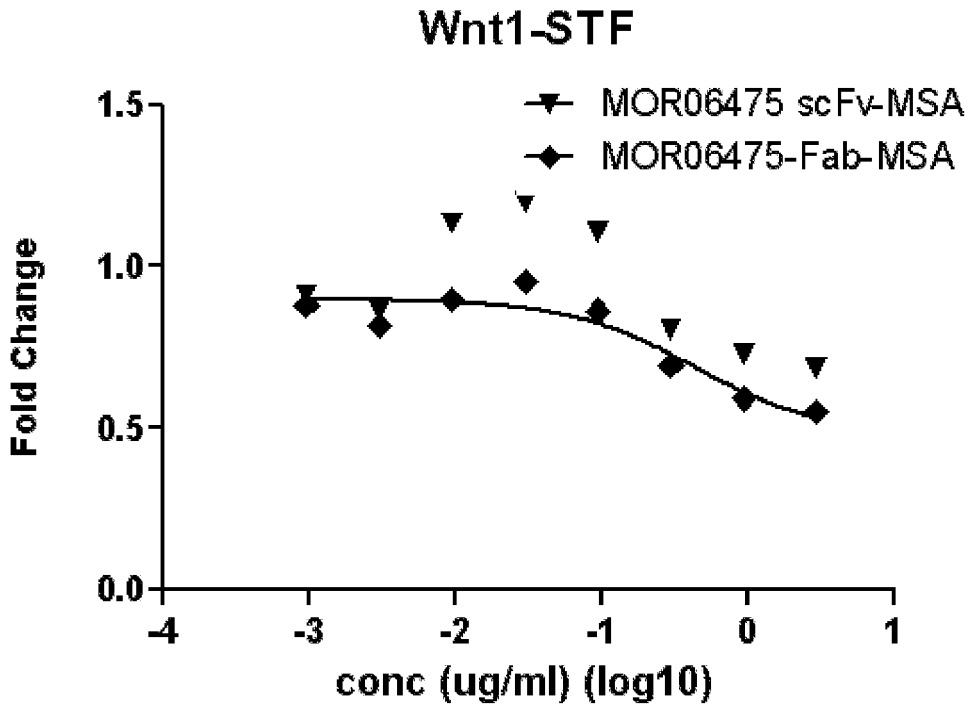
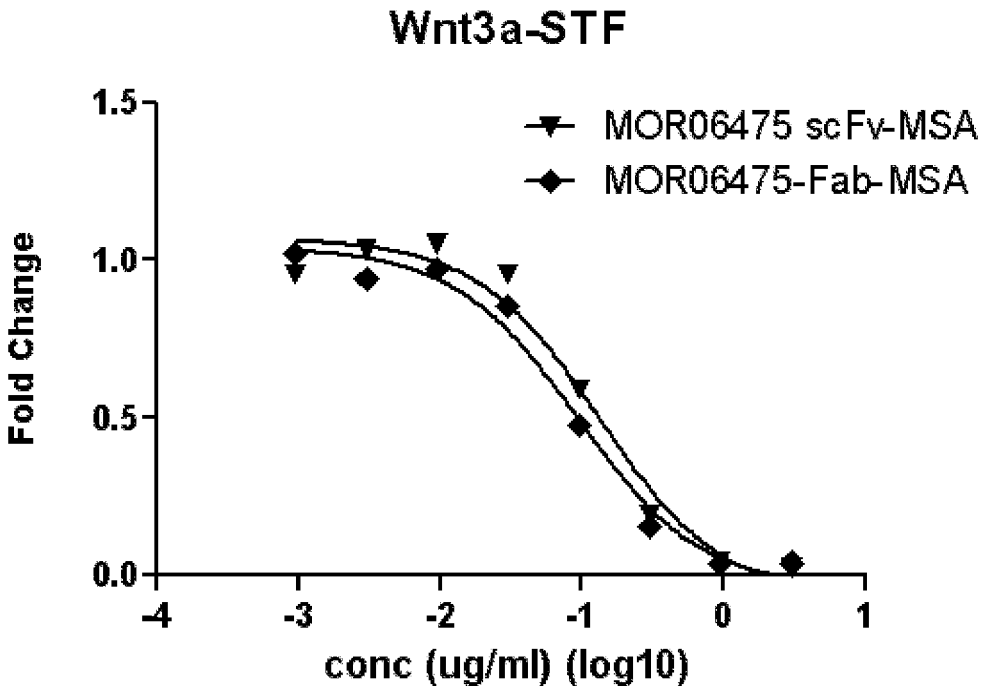


Fig 1E





MOR06475 scFv-MSA	0.1340
MOR06475-Fab-MSA	0.09372

Fig. 2

Construct	Wnt1-STF IC50 (nM)	Wnt3a-STF IC50 (nM)
anti-LRP6_MOR08168 Fab-MSA (C:S) MOR06475 scFv	**0.48**	**0.37**
anti-LRP6_MOR08168 Fab-HSA (C:S) MOR06475 scFv	**0.47**	**0.39**
anti-LRP6_MOR08168 Fab-HSA (C:S) MOR06475 scFab	**0.98**	**7.9**
anti-LRP6_MOR08168 hIgG1LALA (mut Hinge F:T; Y:D) MOR06475 scFv	**0.28**	**0.40**
anti-LRP6_MOR08168 hIgG1LALA 2(CH2_CH3) (mut Hinge F:T; Y:D) MOR06475 scFv	**0.42**	**0.25**

Fig 3

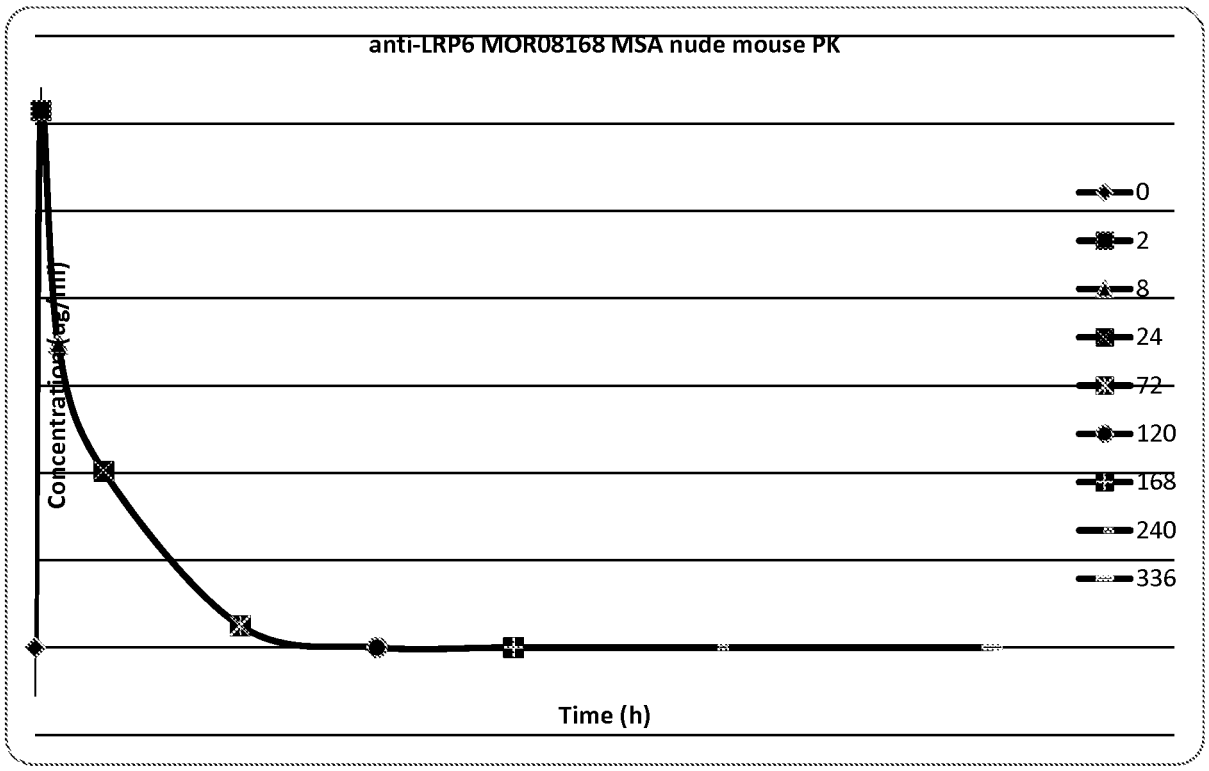

anti-LRP6 MOR08168 MSA nude mouse PK
Concentration (ug/ml)
Time (h)
0
2
8
24
72
120
168
240
336

Fig 4

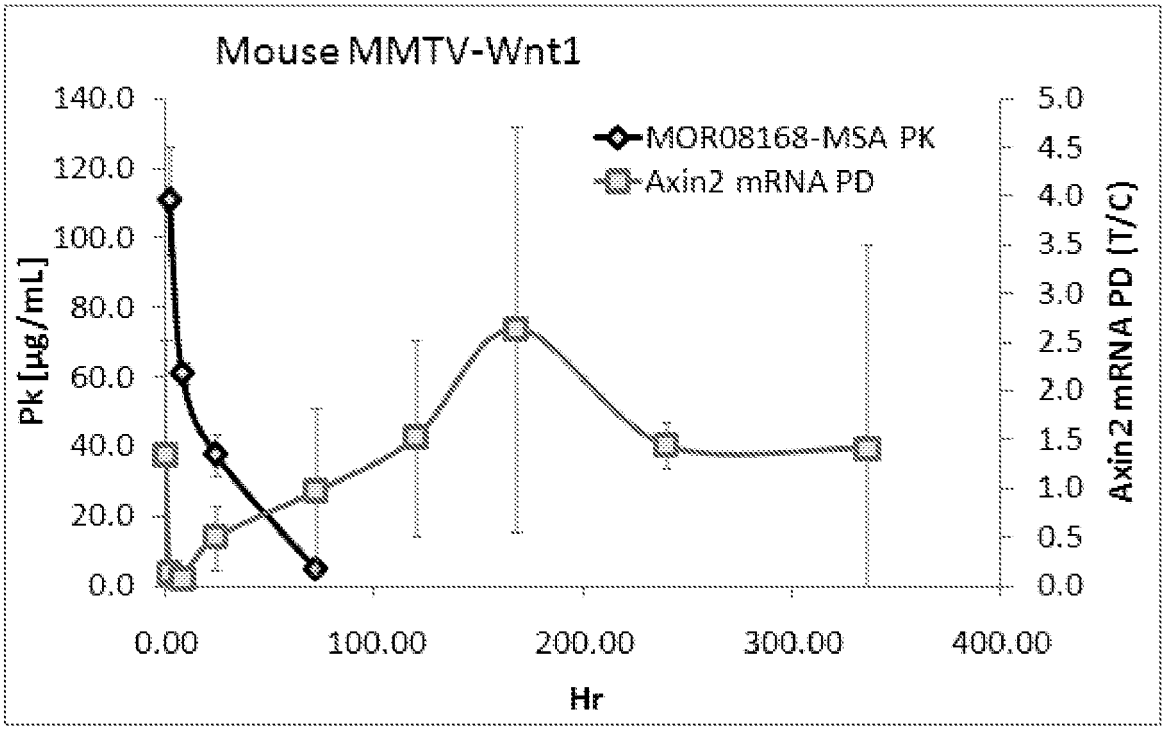

Mouse MMTV-Wnt1
MOR08168-MSA PK
Axin2 mRNA PD
Pk [µg/mL]
Axin2 mRNA PD (T/C)
Hr
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.00
100.00
200.00
300.00
400.00

Fig 5

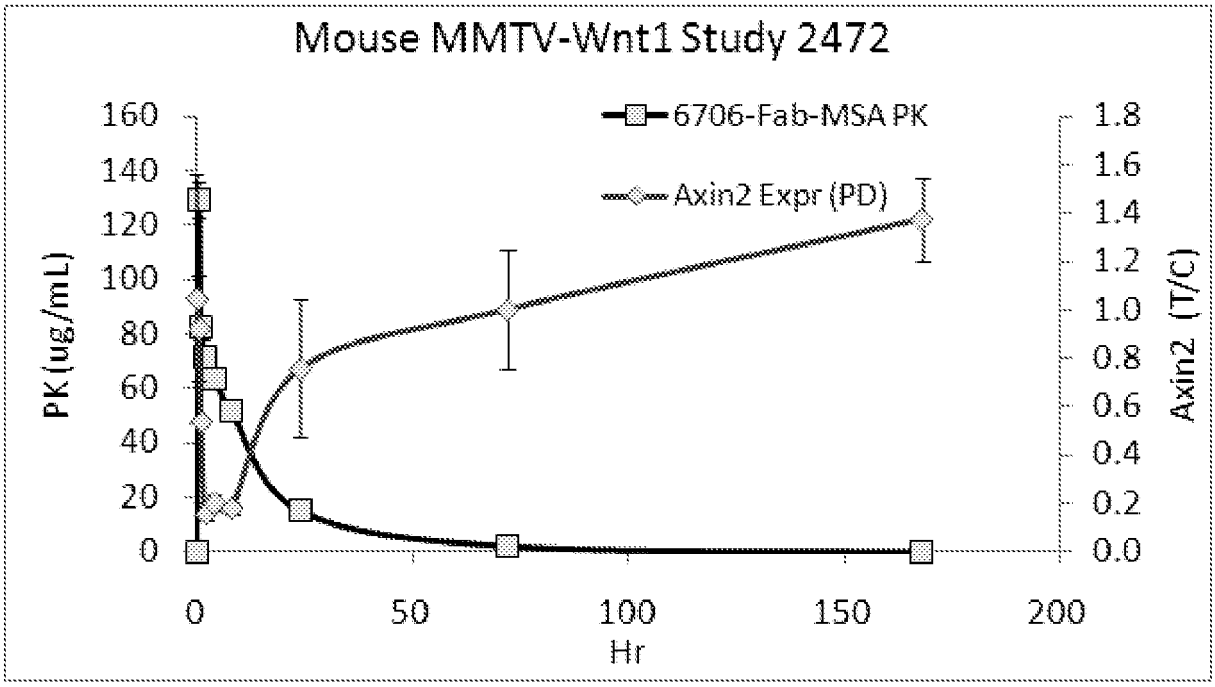

Mouse MMTV-Wnt1 Study 2472
6706-Fab-MSA PK
Axin2 Expr (PD)
PK (ug/mL)
Axin2 (T/C)
Hr
160
140
120
100
80
60
40
20
0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200

Fig 6

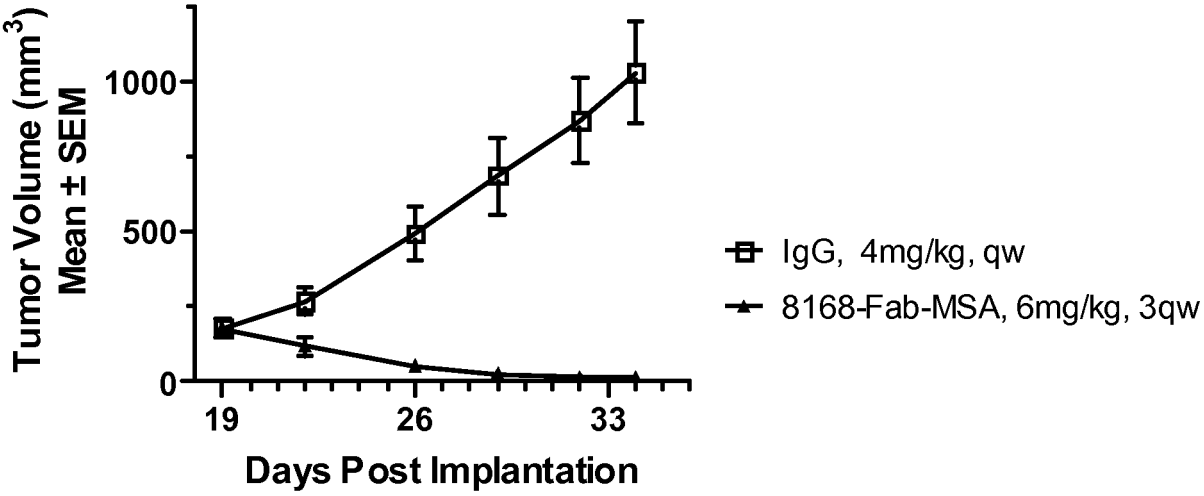

Tumor Volume (mm3)
Mean ± SEM
1000
500
0
19
26
33
Days Post Implantation
IgG, 4mg/kg, qw
8168-Fab-MSA, 6mg/kg, 3qw

Fig 7

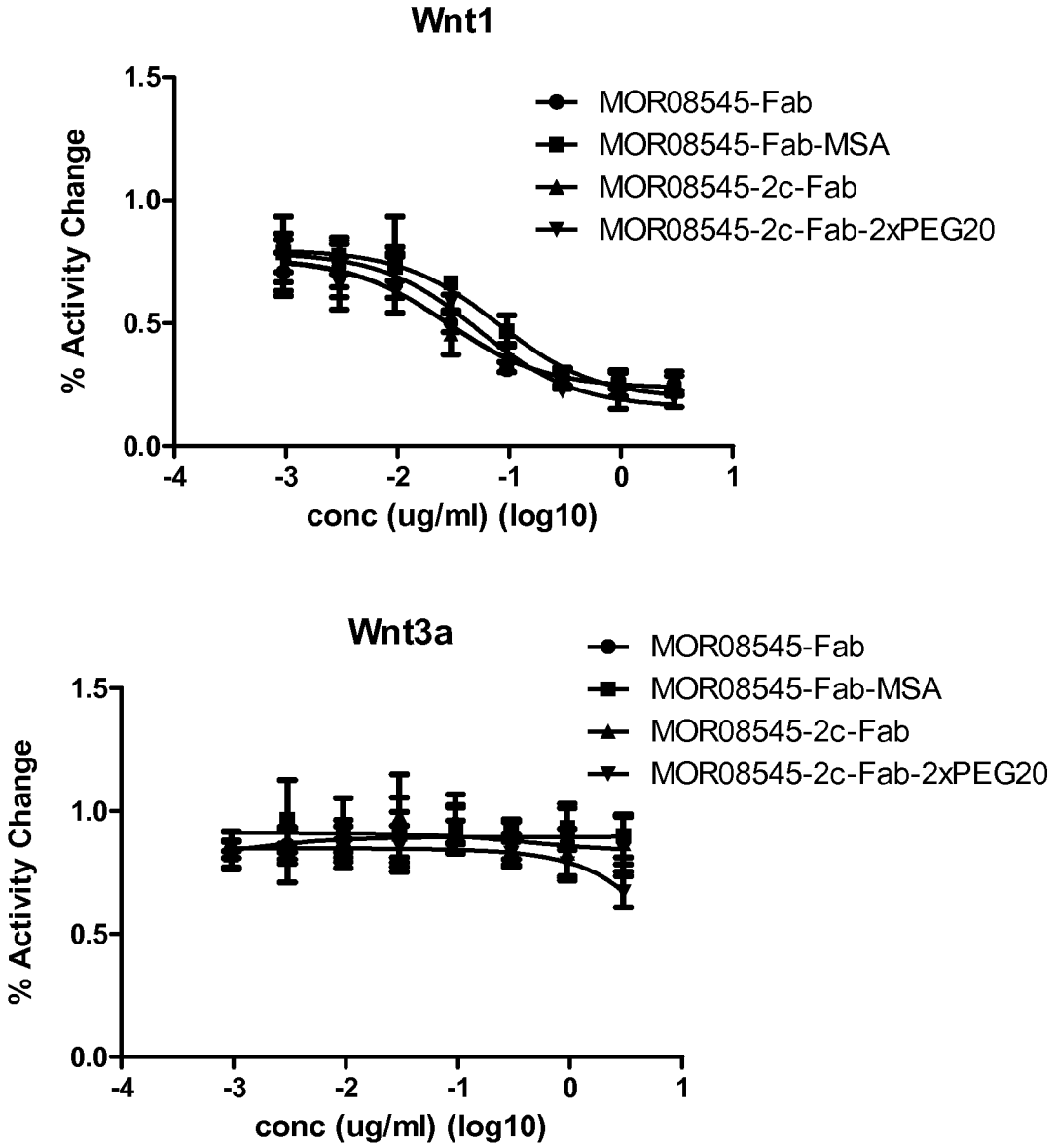

Wnt1
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)
MOR08545-Fab
MOR08545-Fab-MSA
MOR08545-2c-Fab
MOR08545-2c-Fab-2xPEG20
Wnt3a
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)
MOR08545-Fab
MOR08545-Fab-MSA
MOR08545-2c-Fab
MOR08545-2c-Fab-2xPEG20

Fig 8

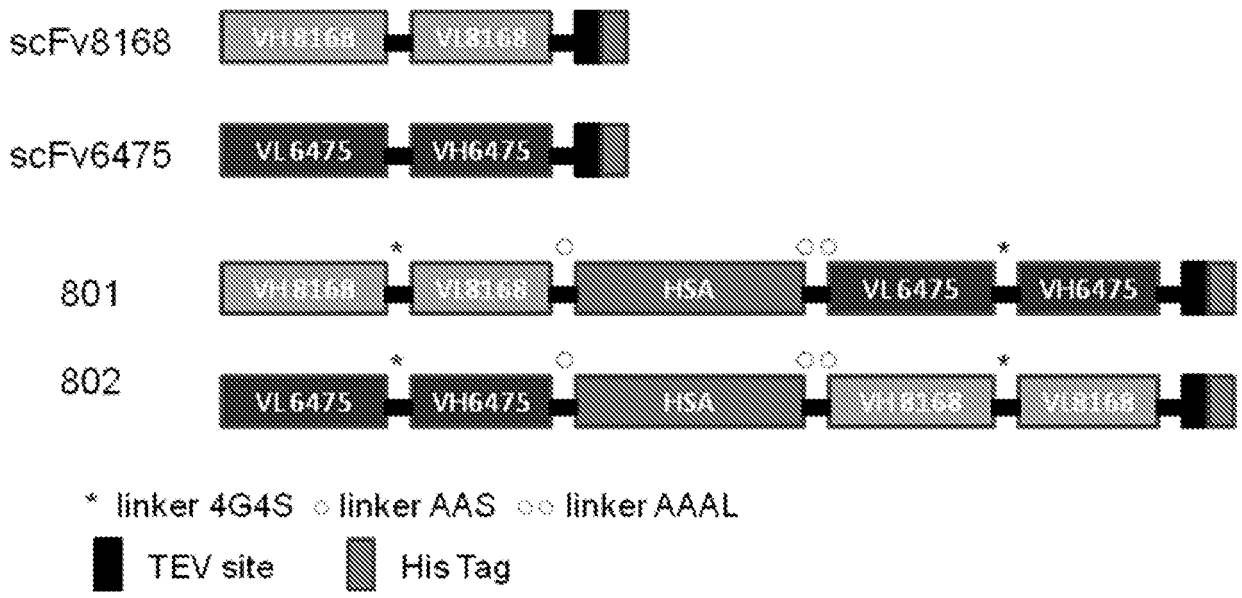

scFv8168
VH8168
VL8168
scFv6475
VL6475
VH6475
801
HSA
802
* linker 4G4S
linker AAS
linker AAAL
TEV site
His Tag

Fig 9

HSA fusion molecule structures

Construct nickname	module1	Module 2	Module 3	Linker1/2
801T	scFv8168	HSA	scFv6475	AAS/AAAL
802T	scFv6475	HSA	scFv8168	AAS/AAAL
803T	scFv8168	HSA	scFv6475	G4SGG/3G4S
804T	scFv6475	HSA	scFv8168	G4SGG/3G4S
801T-no tag (NT)	scFv8168	HSA	scFv6475	AAS/AAAL
802T-no tag (NT)	scFv6475	HSA	scFv8168	AAS/AAAL
802T-no linker (NL)	scFv6475	HSA	scFv8168	No linker
808T	scFv6475	HSA	scFv8168	KTHT/KTHT
812T	scFv6487	scFv8168		3G4S
812T-HSA	scFv6487	scFv8168	HSA	3G4S/DKTHT
809T	scFV8168	HSA		G4S
810T	scFv6475	HSA		G4S
801TF	scFv8168	HSA	scFv6475	No linker
802TF	scFv6475	HSA	scFv8168	No linker

Fig 10A

Purification of 801T and 802T using Ni-NTA

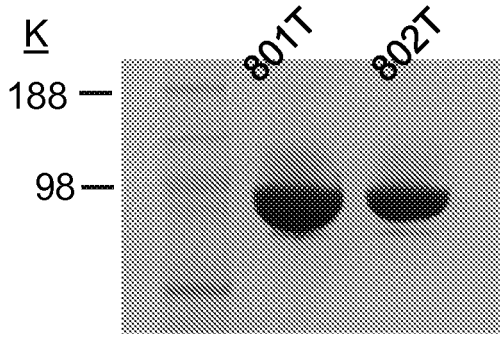


Fig 10B

Purification of 801T-no tag and 802T-no tag

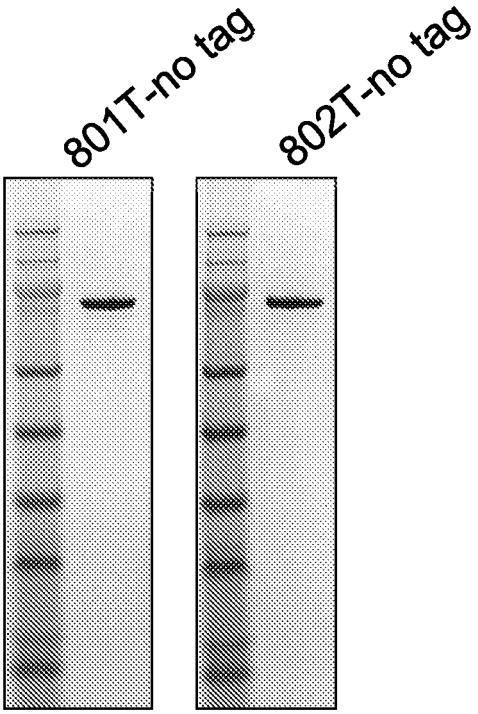


Fig 10C

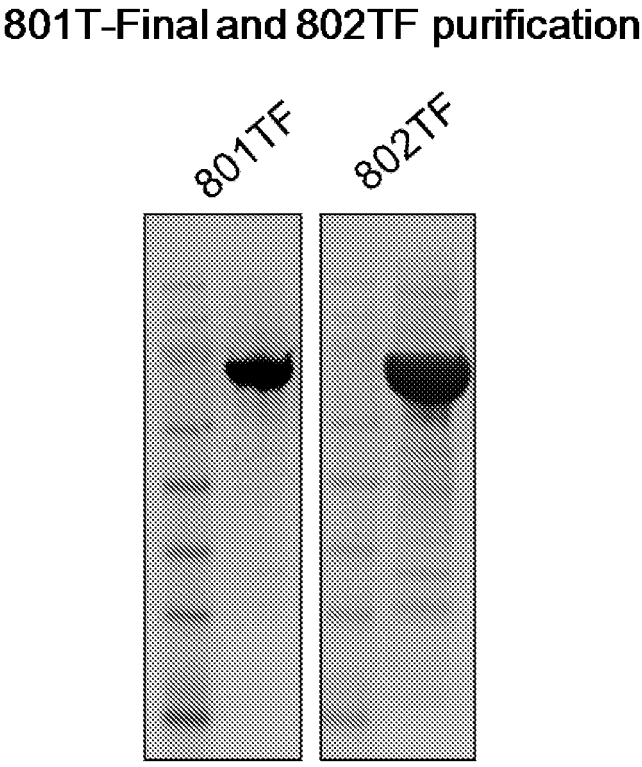


Fig 11

Thermostability measurement of HSA fusion based biparatopic molecules

	T_m by DSF (ProteoSTAT)	T_m by DSC
scFv8168-HSA-scFv6475 (801)	55, 69	53.7, 57.8, 71.4
scFv8168-HSA-scFv6475 (801T)	62	62.4, 71.3
scFv6475-HSA-scFv8168 (802)	53.5, 60	51.0, 65, 72.0
scFv6475-HSA-scFv8168 (802T)	65.5	64.5, 72.6

Fig 12A

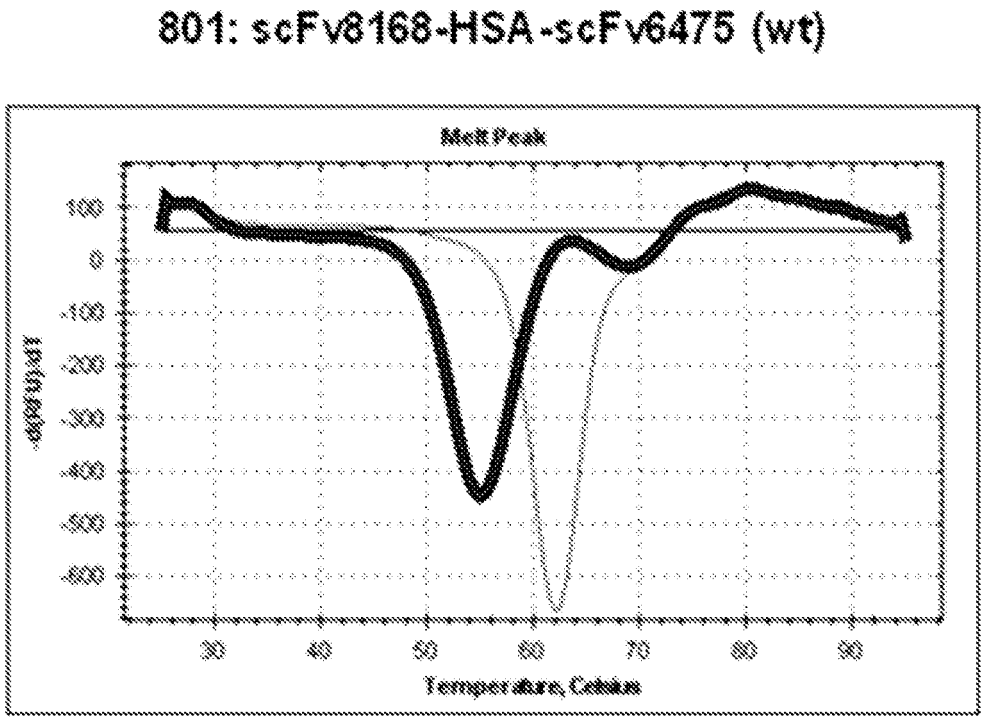

801: scFv8168-HSA-scFv6475 (wt)
Melt Peak
100
0
-100
-200
-300
-400
-500
-600
30
40
50
60
70
80
90

Fig 12B

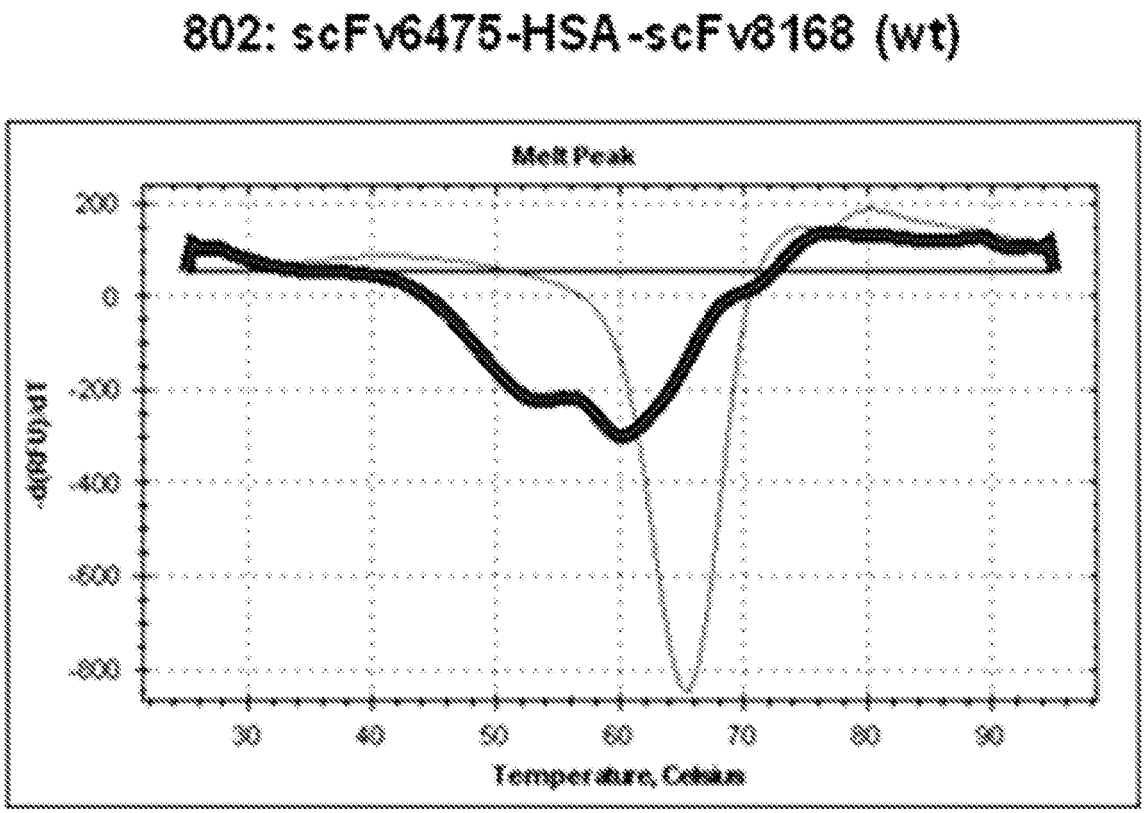

802: scFv6475-HSA-scFv8168 (wt)
Melt Peak
200
0
-200
-400
-600
-800
30
40
50
60
70
80
90
Temperature, Celsius

Fig 13A

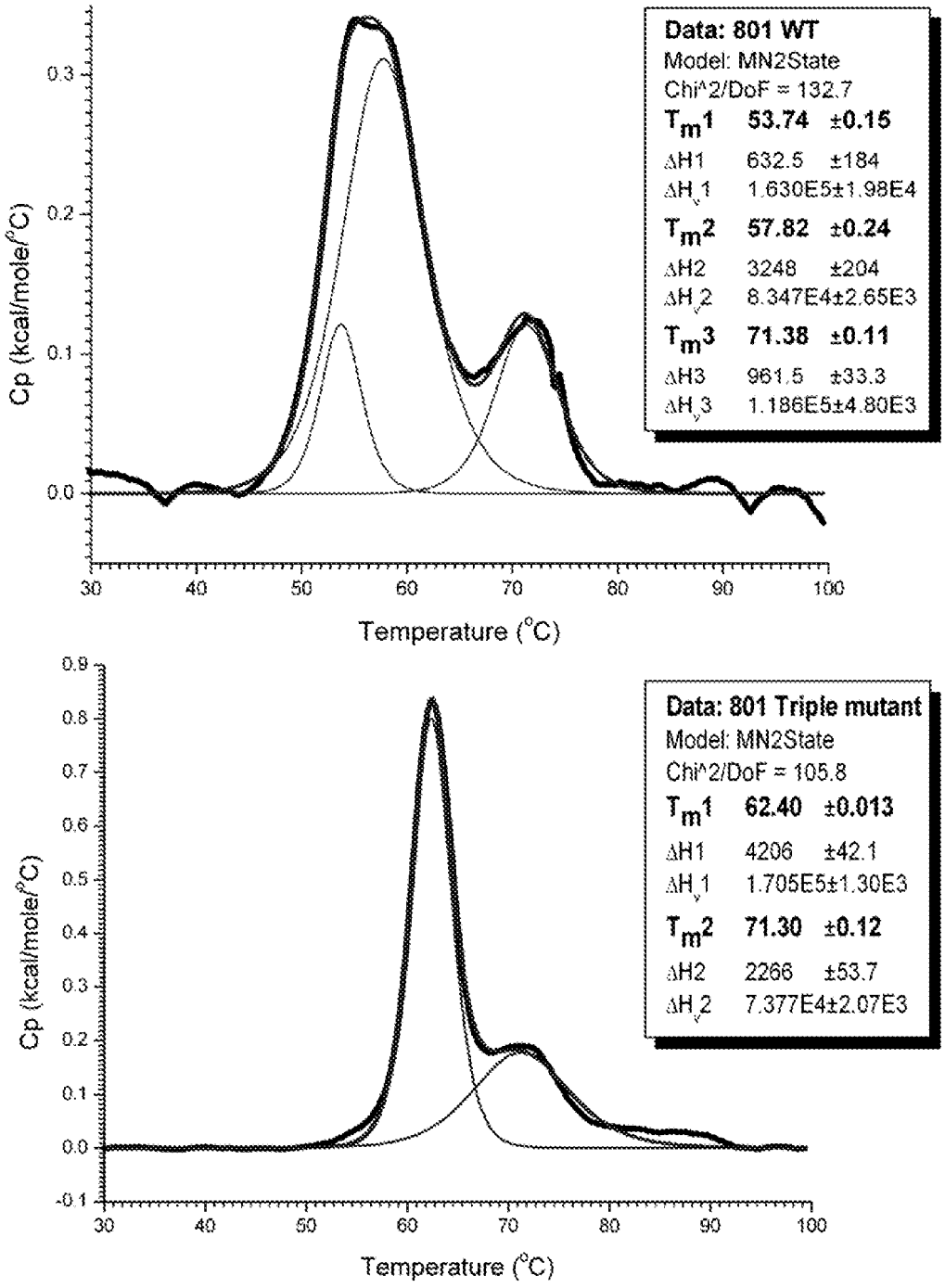

Data: 801 WT
Model: MN2State
Chi^2/DoF = 132.7
Tm1 53.74 ±0.15
ΔH1 632.5 ±184
ΔHv1 1.630E5±1.98E4
Tm2 57.82 ±0.24
ΔH2 3248 ±204
ΔHv2 8.347E4±2.65E3
Tm3 71.38 ±0.11
ΔH3 961.5 ±33.3
ΔHv3 1.186E5±4.80E3
Cp (kcal/mole/°C)
Temperature (°C)
Data: 801 Triple mutant
Model: MN2State
Chi^2/DoF = 105.8
Tm1 62.40 ±0.013
ΔH1 4206 ±42.1
ΔHv1 1.705E5±1.30E3
Tm2 71.30 ±0.12
ΔH2 2266 ±53.7
ΔHv2 7.377E4±2.07E3
Cp (kcal/mole/°C)
Temperature (°C)

Fig 13B

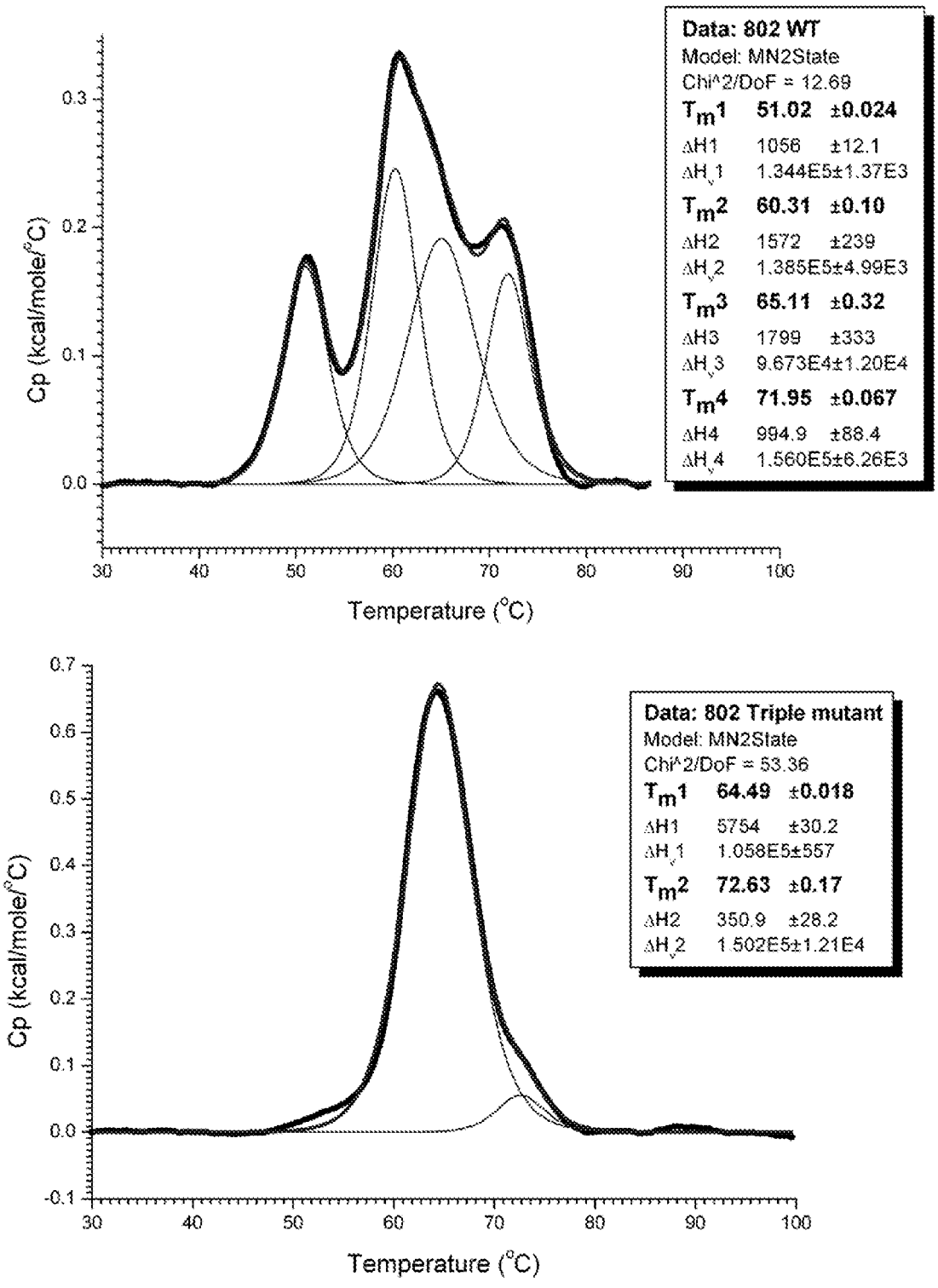

Data: 802 WT
Model: MN2State
Chi^2/DoF = 12.69
Tm1 51.02 ±0.024
ΔH1 1056 ±12.1
ΔHv1 1.344E5±1.37E3
Tm2 60.31 ±0.10
ΔH2 1572 ±239
ΔHv2 1.385E5±4.99E3
Tm3 65.11 ±0.32
ΔH3 1799 ±333
ΔHv3 9.673E4±1.20E4
Tm4 71.95 ±0.067
ΔH4 994.9 ±88.4
ΔHv4 1.560E5±6.26E3
Cp (kcal/mole/°C)
Temperature (°C)
Data: 802 Triple mutant
Model: MN2State
Chi^2/DoF = 53.36
Tm1 64.49 ±0.018
ΔH1 5754 ±30.2
ΔHv1 1.058E5±557
Tm2 72.63 ±0.17
ΔH2 350.9 ±28.2
ΔHv2 1.502E5±1.21E4
Cp (kcal/mole/°C)
Temperature (°C)

Fig 14

Thermostability by DSF	
	Tm
801T	**62**
802T	**65.5**
803T	**63**
804T	**66**
801T-no tag	**63**
802T-no tag	**65**
802T-no linker	**64.5**
808T	**66**
812T	**55**
812T-HSA	**60, 64.5**
801TF	**64**
802TF	**66**
911T	**53.5, 60**
912T	**52.5, 62**

Fig 15A

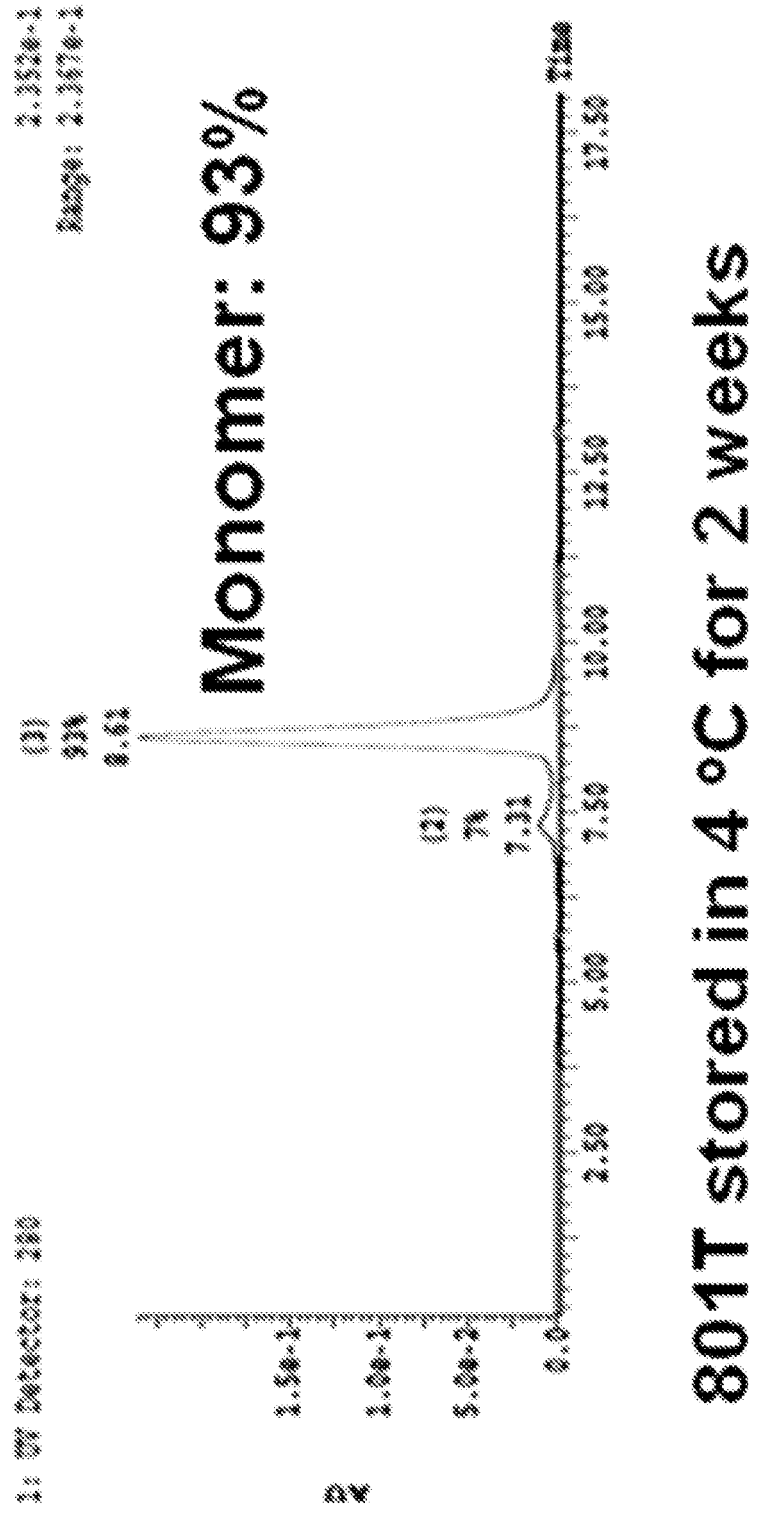

801T stored in 4 °C for 2 weeks
Monomer: 93%
1: CH Detector, 180
2.50
5.00
7.50
10.00
12.50
15.00
17.50
Time
0.0
5.0e-2
1.0e-1
1.5e-1
nV
(1)
93%
8.61
(2)
7%
7.31

Fig 15A contd.

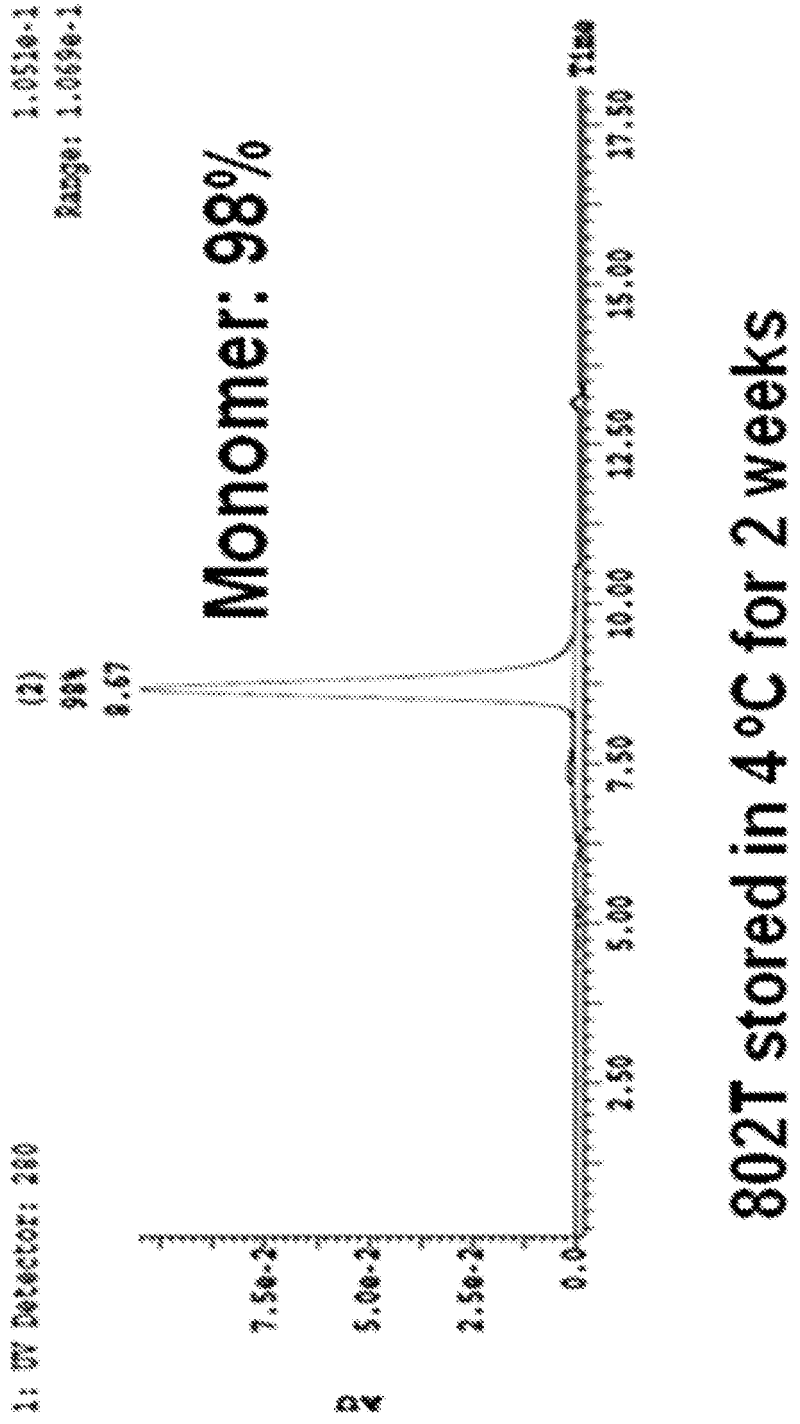


Fig 15B

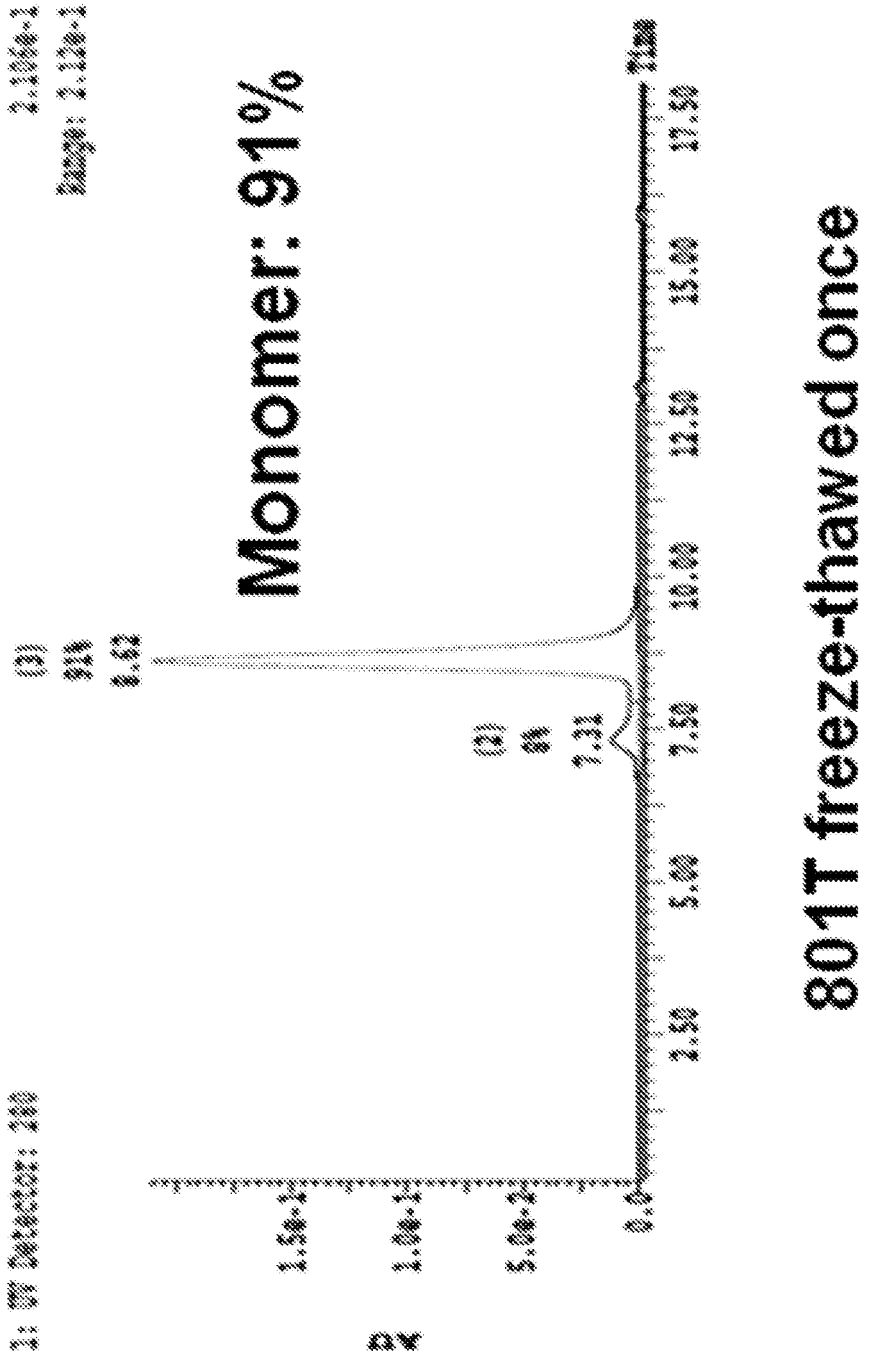

801T freeze-thawed once
Monomer: 91%
1: RI Detector: 180
nRIU
2.50
5.00
7.50
10.00
12.50
15.00
17.50
Time
1.62
7.31
2.106e-1
Isocpc: 2.112e-1

Fig 15B contd.

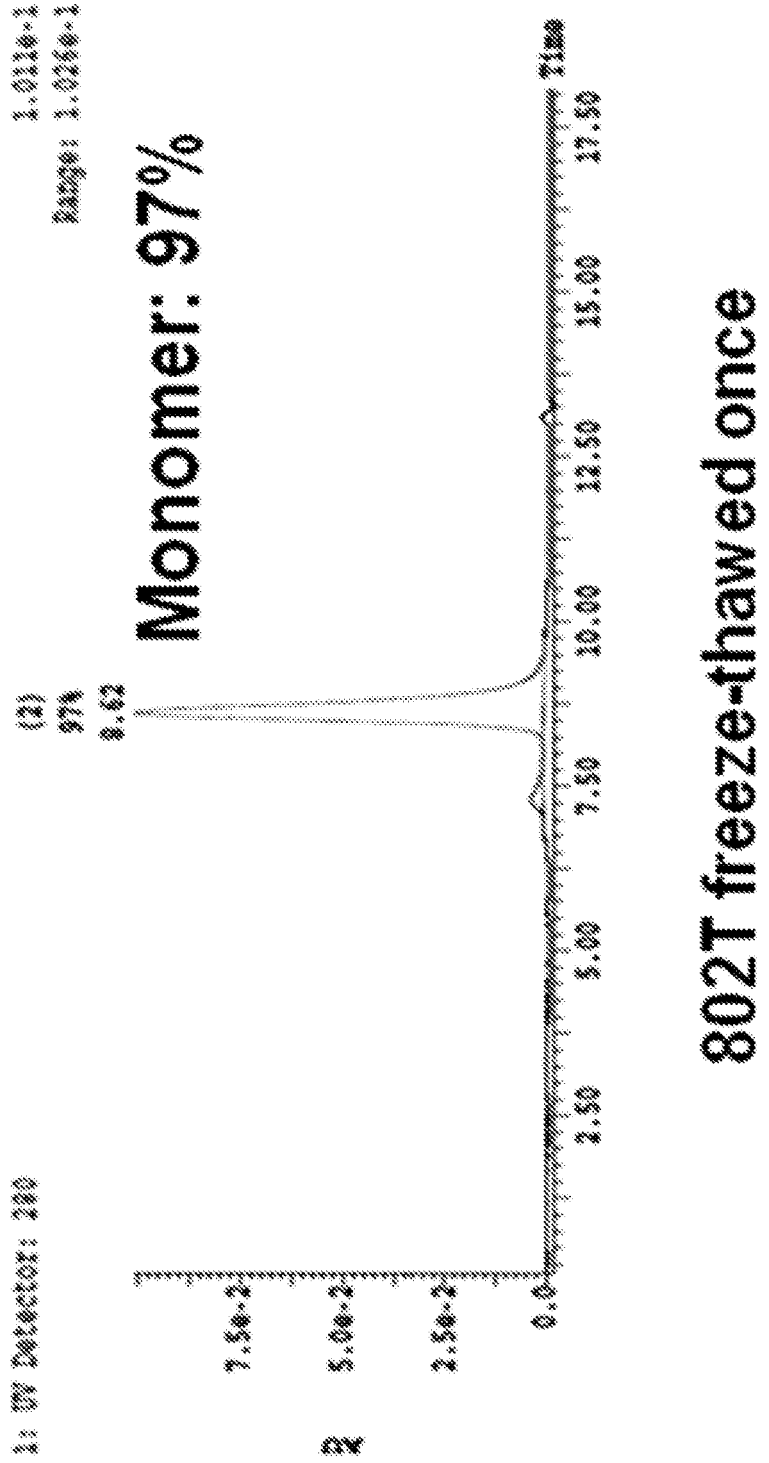
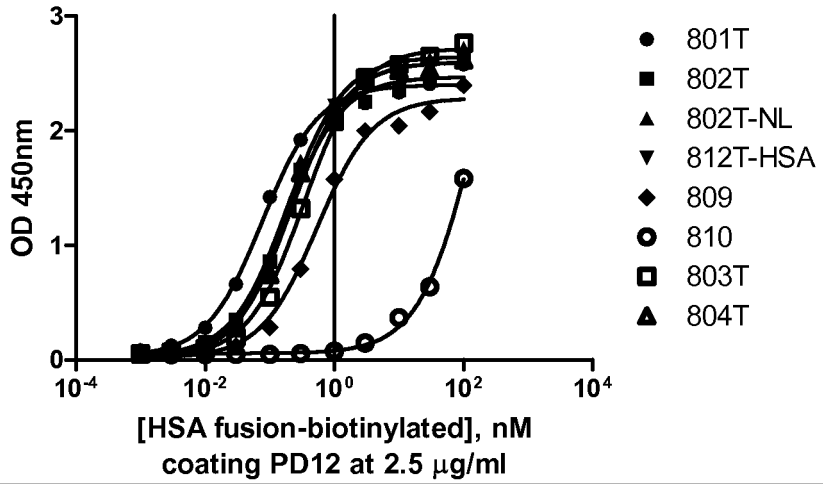


Fig 16

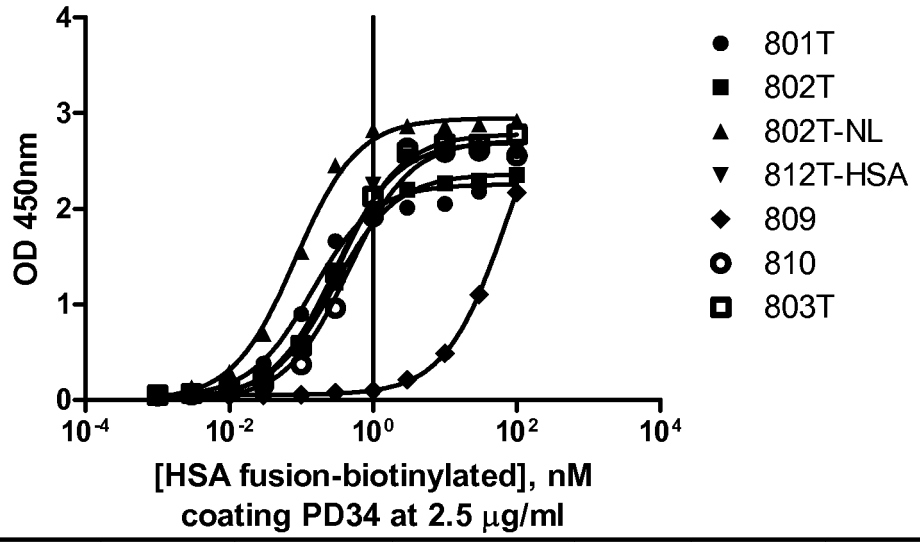
Aggregation by analytical SEC after multiple freeze-and-thaws (FTs)	
Sample	**Percent monomer**
802T-2 FTs	99
802T-3 FTs	99
802T-4 FTs	99
802T-nolinker-2 FTs	94
802T-nolinker-3 FTs	94
802T-nolinker-4 FTs	94
804T-2 FTs	100
804T-3 FTs	100
804T-4 FTs	97
808T-2 FTs	100
808T-3 FTs	94
808T-4 FTs	94

Fig 17A



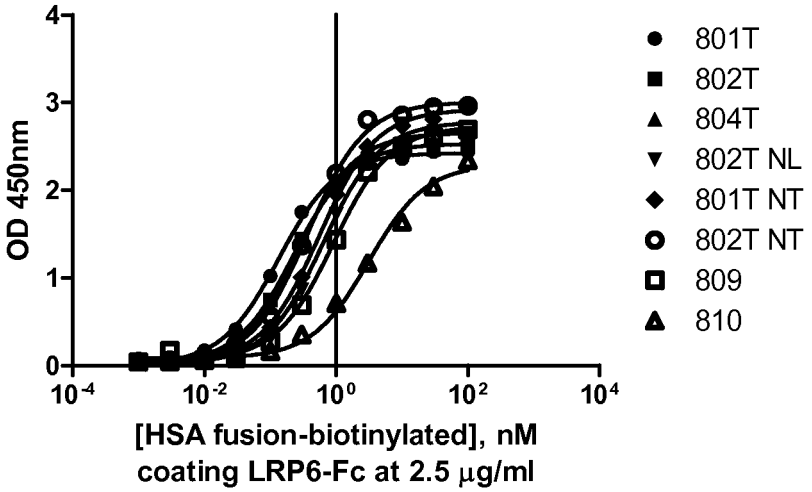
	801T	802T	802T-NL	812T-HSA	809	810	803T	804T
EC50	0.07767	0.1793	0.1871	0.2104	0.5388	157.6	0.3388	0.2105

Fig 17B



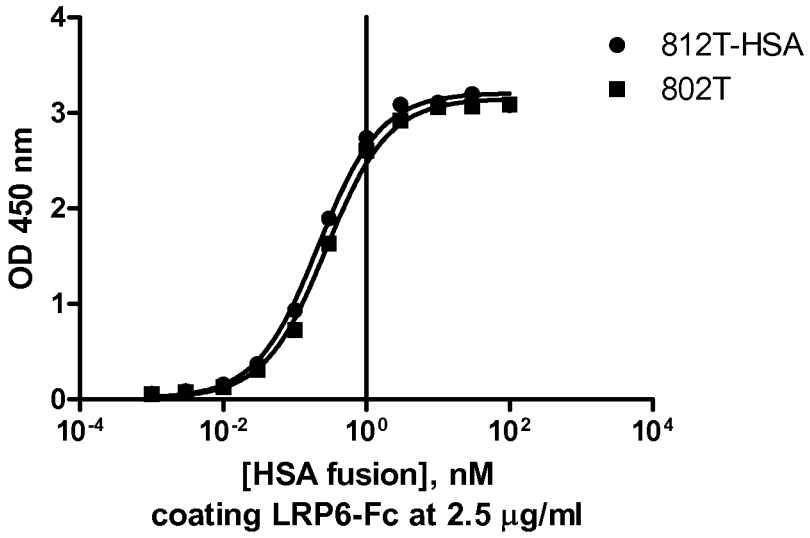
	801T	802T	802T-NL	812T-HSA	809	810	803T
EC50	0.1436	0.2885	0.08363	0.2928	75.50	0.4578	0.3360

Fig 17C



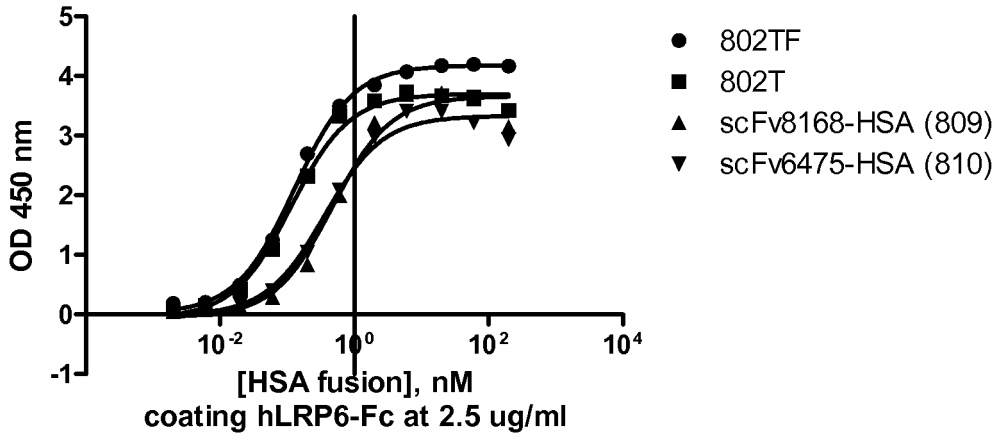
	801T	802T	804T	802T NL	801T NT	802T NT	809	810
EC50	0.1343	0.2324	0.2712	0.6371	0.5545	0.3739	0.9083	3.127

Fig 17D



	812T-HSA	802T
EC50	0.2147	0.2768

Fig 17E



	802TF	802T	scFv8168-HSA (809)	scFv6475-HSA (810)
EC50	0.1269	0.1153	0.4716	0.3595

Fig 18A

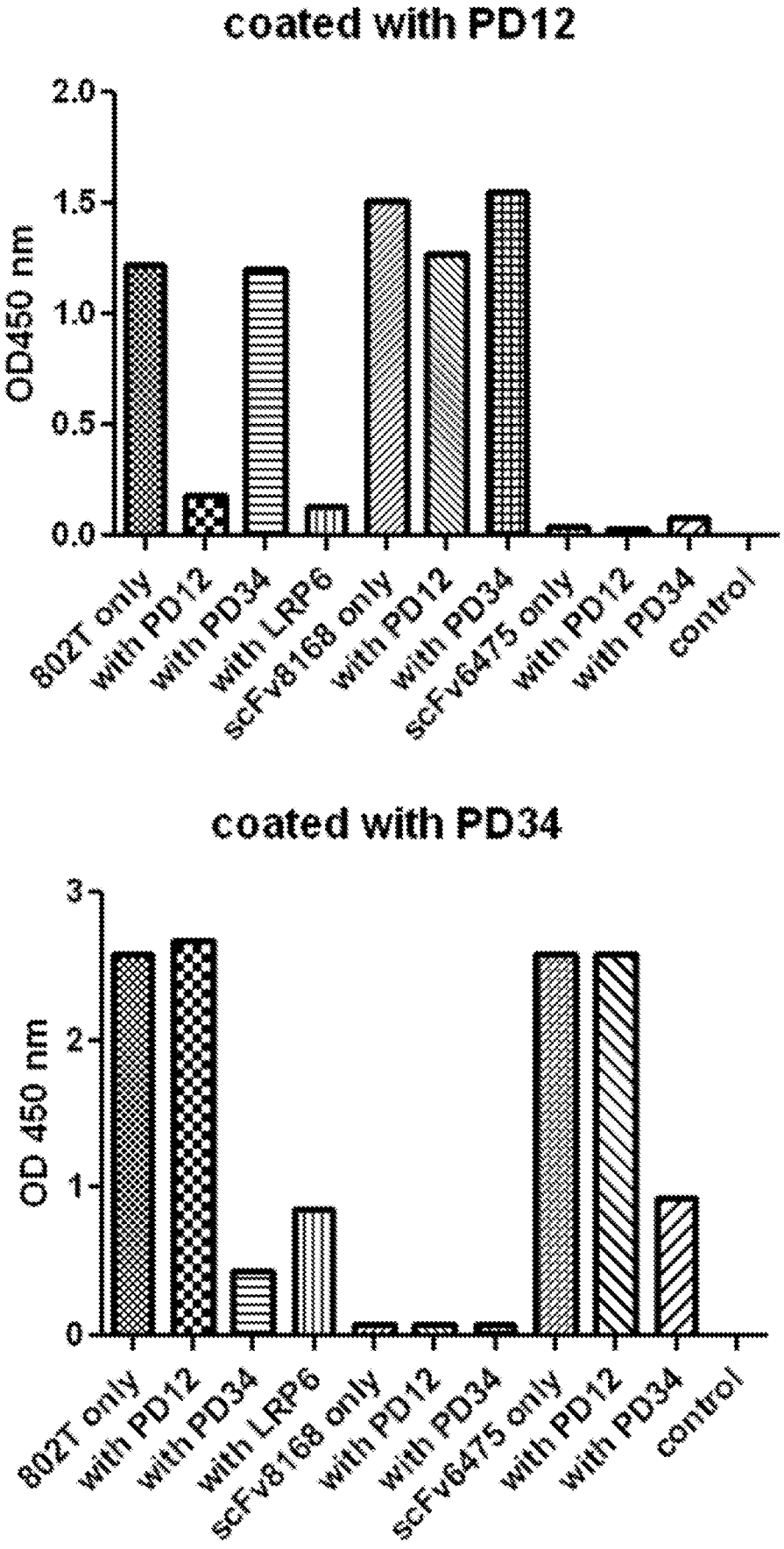

coated with PD12
OD450 nm
2.0
1.5
1.0
0.5
0.0
802T only
with PD12
with PD34
with LRP6
scFv8168 only
with PD12
with PD34
scFv6475 only
with PD12
with PD34
control
coated with PD34
OD 450 nm
3
2
1
0
802T only
with PD12
with PD34
with LRP6
scFv8168 only
with PD12
with PD34
scFv6475 only
with PD12
with PD34
control

Fig 18B

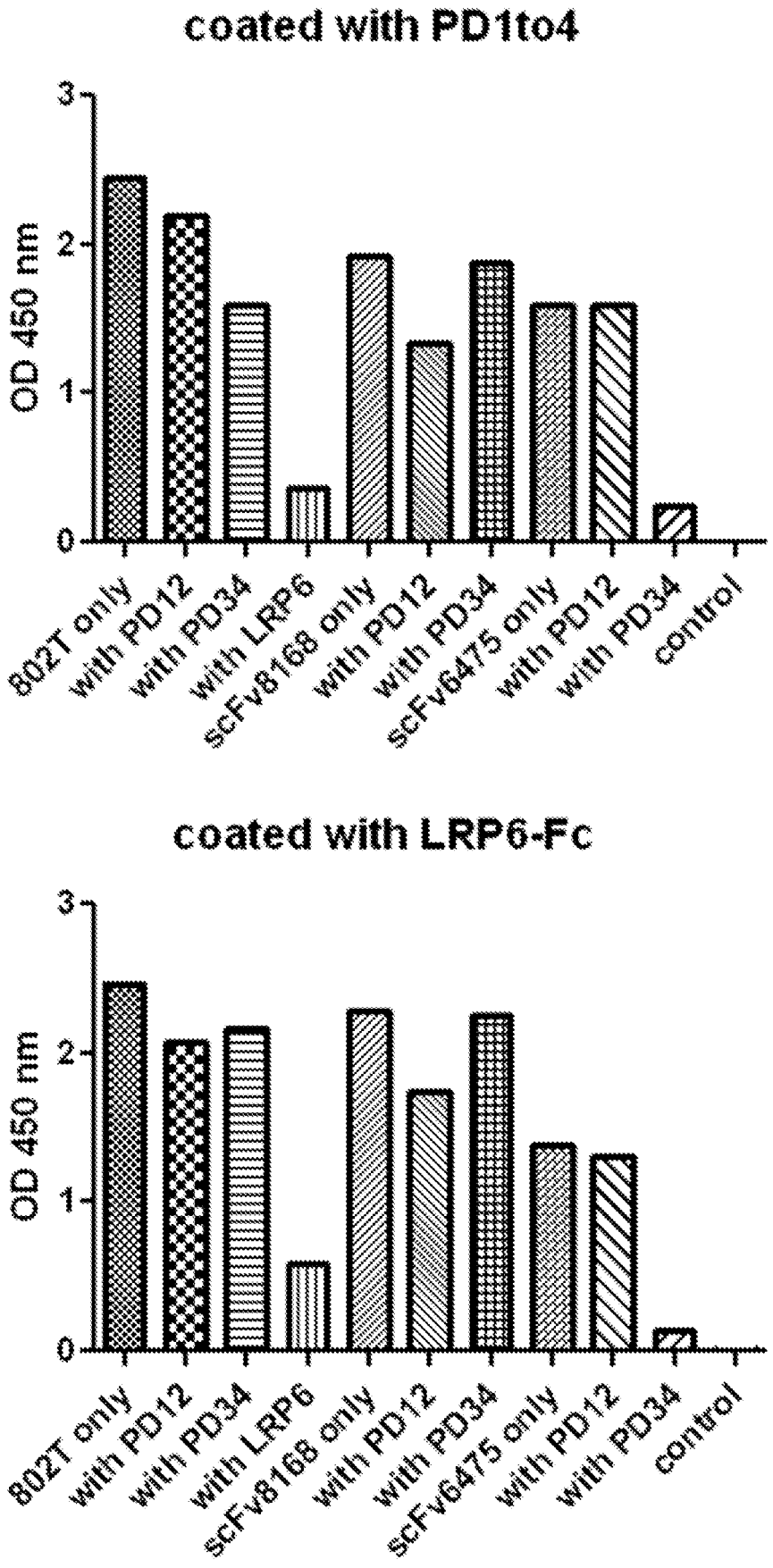

coated with PD1to4
OD 450 nm
0
1
2
3
802T only
with PD12
with PD34
with LRP6
scFv8168 only
with PD12
with PD34
scFv6475 only
with PD12
with PD34
control
coated with LRP6-Fc
OD 450 nm
0
1
2
3
802T only
with PD12
with PD34
with LRP6
scFv8168 only
with PD12
with PD34
scFv6475 only
with PD12
with PD34
control

Fig 19A

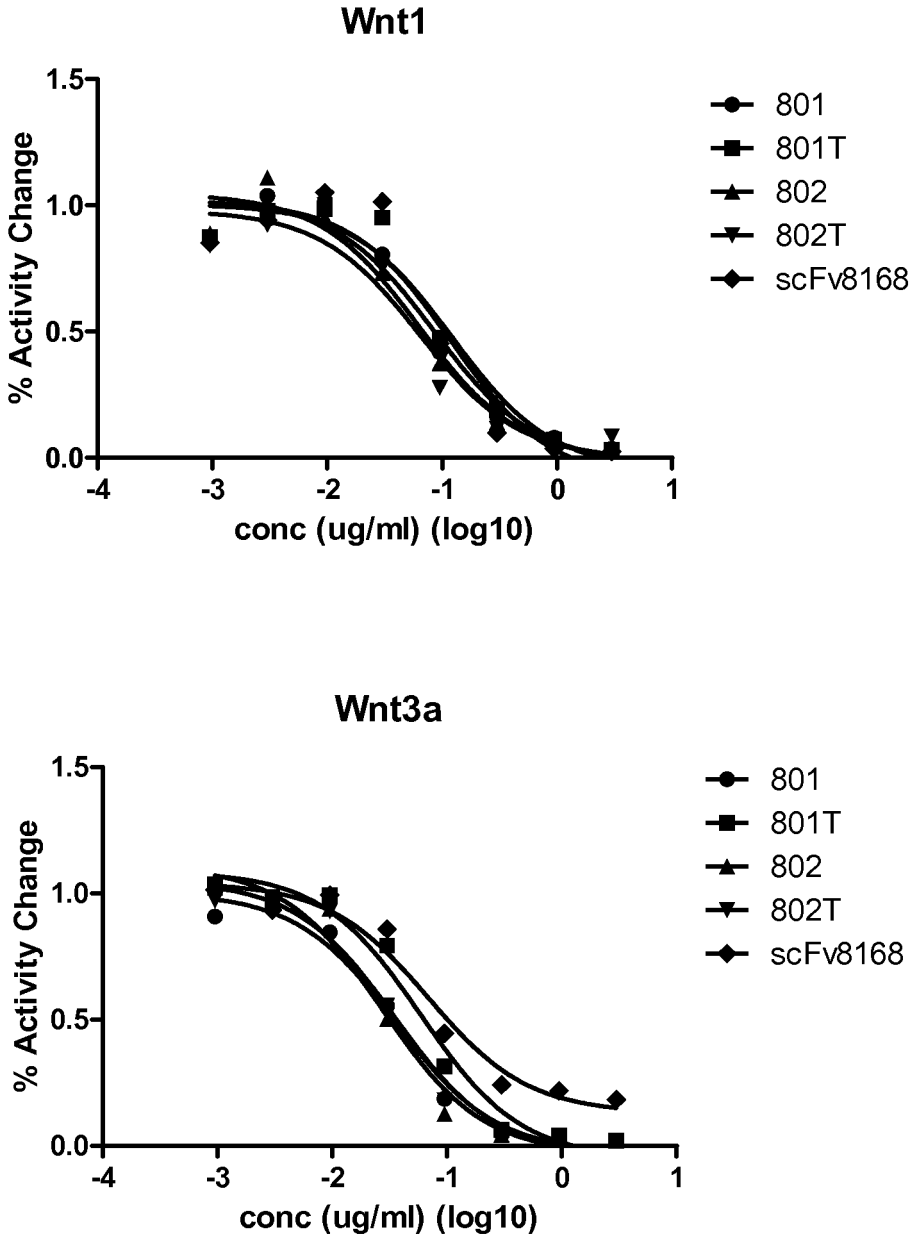

Wnt1
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)
801
801T
802
802T
scFv8168
Wnt3a
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
conc (ug/ml) (log10)
801
801T
802
802T
scFv8168

Fig 19B

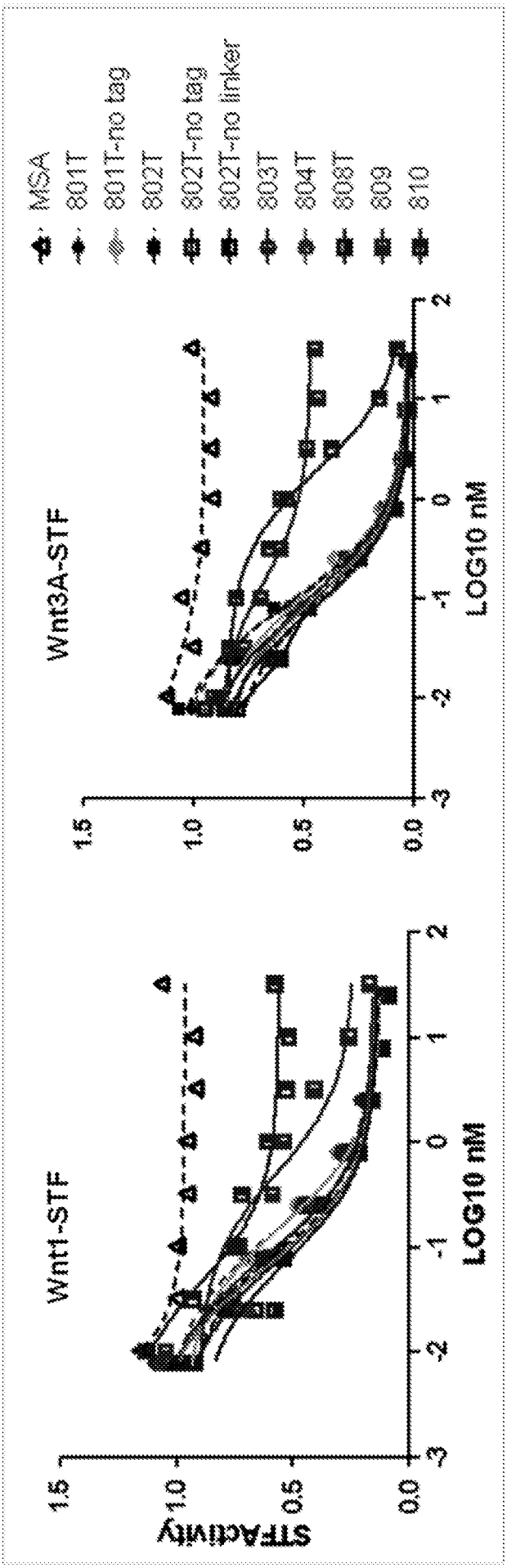

MSA
801T
801T-no tag
802T
802T-no tag
802T-no linker
803T
804T
808T
809
810
Wnt3A-STF
Wnt1-STF
STFActivity
LOG10 nM
1.5
1.0
0.5
0.0
-3
-2
-1
0
1
2

Fig 19C

ID	Desc	His Tag	Linker	IC50 (nM) Wnt1	Wnt3A
801T	scFv8168-HSA-scFv6475	Y	AAS/AAAL	0.133	0.098
802T	scFv6475-HSA-scFv8168	Y	AAS/AAAL	0.102	0.087
801T-no tag	scFv8168-HSA-scFv6475	N	AAS/AAAL	0.160	0.118
802T-no tag	scFv6475-HSA-scFv8168	N	AAS/AAAL	0.074	0.084
803T	scFv8168-HSA-scFv6475	Y	G4SGG/3G4S	0.082	0.117
804T	scFv6475-HSA-scFv8168	Y	G4SGG/3G4S	0.062	0.099
802T-no linker	scFv6475-HSA-scFv8168	Y	-	0.071	0.097
808T	scFv6475-HSA-scFv8168	Y	KTHT	0.074	0.076
809	ScFv8168 D1-HSA (VH: I34M, S49A)	Y		0.500	N.D.
810	ScFv6475 S8-HSA (VH: M100F)	Y		N.D.	1.930

"T" = Mutation: 8168VH-S49A, I34M / 6475VH-M95F

Fig 19D

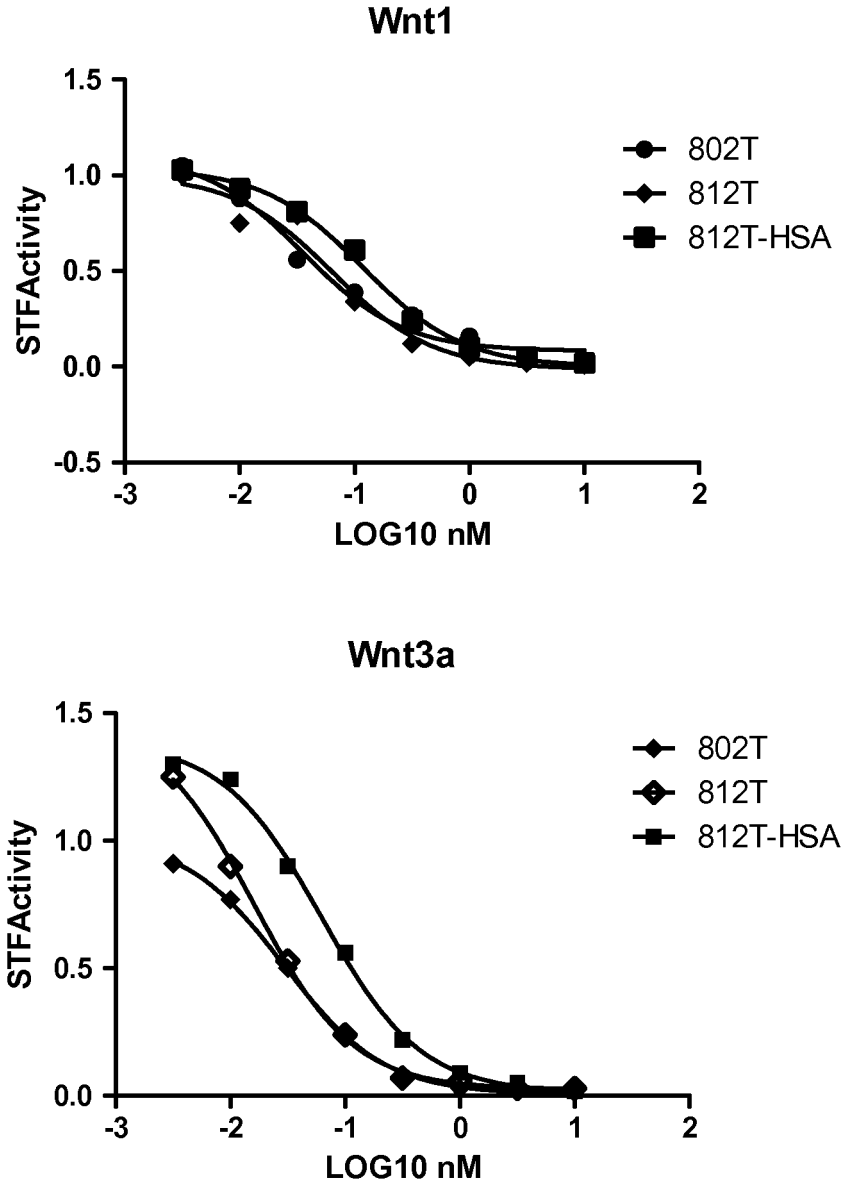

Wnt1
STFActivity
1.5
1.0
0.5
0.0
-0.5
-3
-2
-1
0
1
2
LOG10 nM
802T
812T
812T-HSA
Wnt3a
STFActivity
1.5
1.0
0.5
0.0
-3
-2
-1
0
1
2
LOG10 nM
802T
812T
812T-HSA

Fig 19E

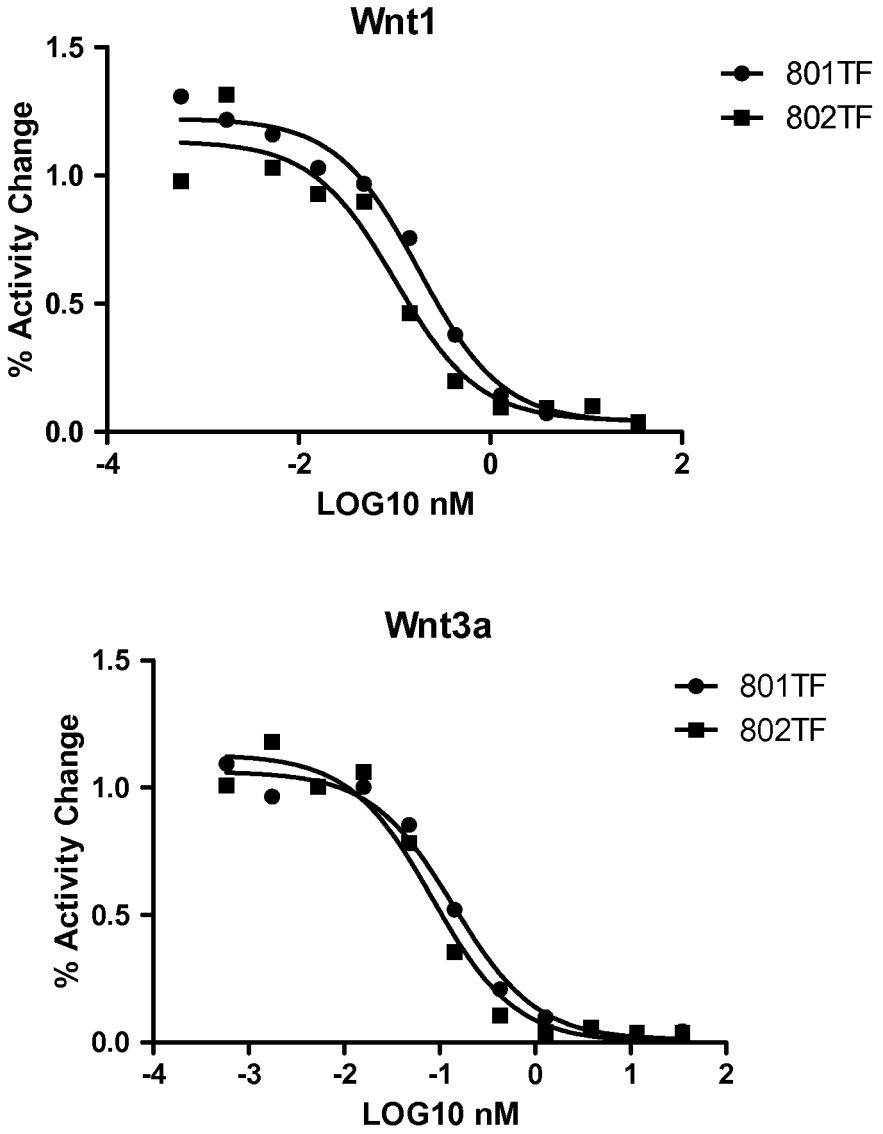

Wnt1
801TF
802TF
% Activity Change
1.5
1.0
0.5
0.0
-4
-2
0
2
LOG10 nM
Wnt3a
801TF
802TF
% Activity Change
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
2
LOG10 nM

Fig 20

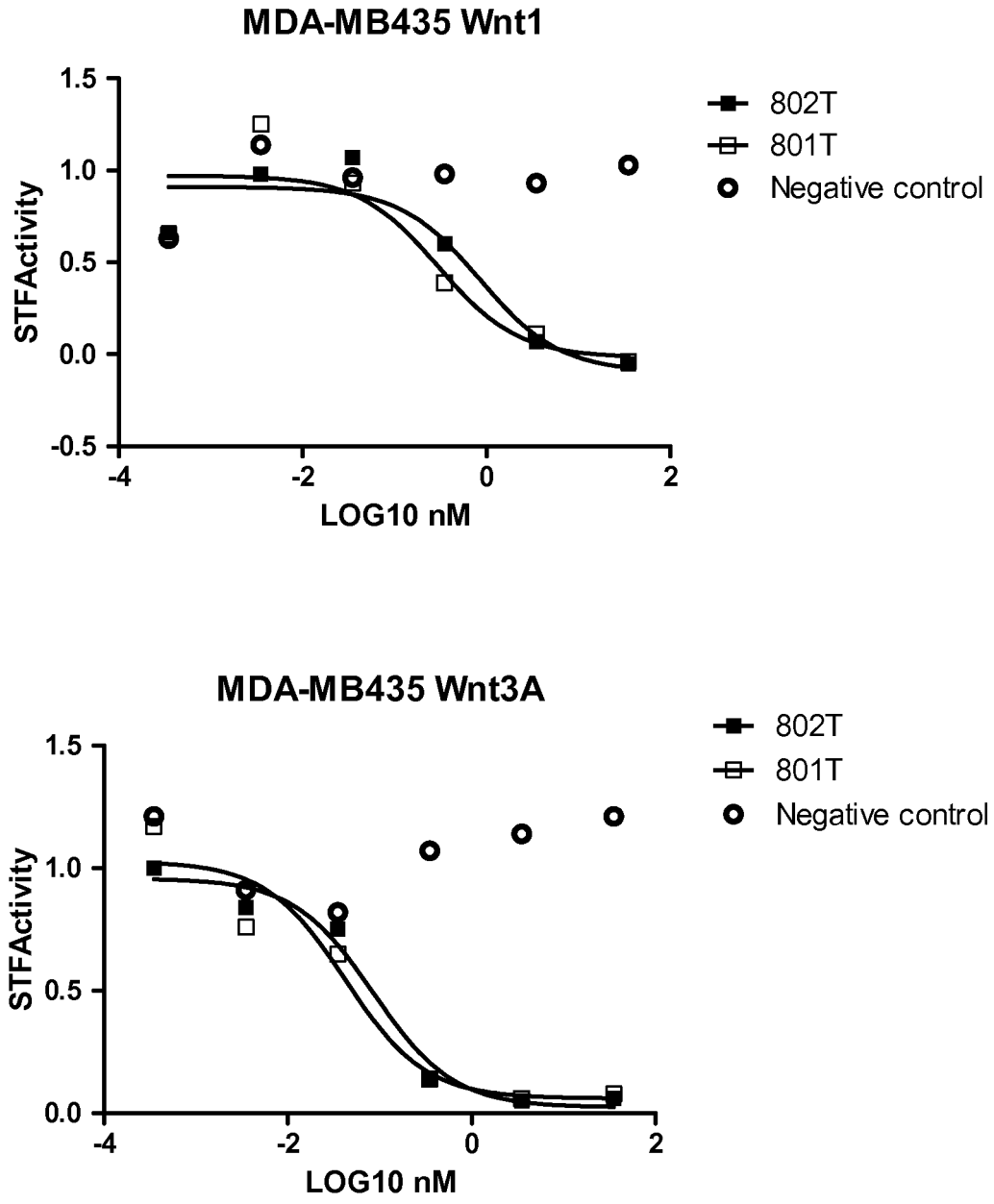

MDA-MB435 Wnt1
STFActivity
1.5
1.0
0.5
0.0
-0.5
-4
-2
0
2
LOG10 nM
802T
801T
Negative control
MDA-MB435 Wnt3A
STFActivity
1.5
1.0
0.5
0.0
-4
-2
0
2
LOG10 nM
802T
801T
Negative control

Fig 21A

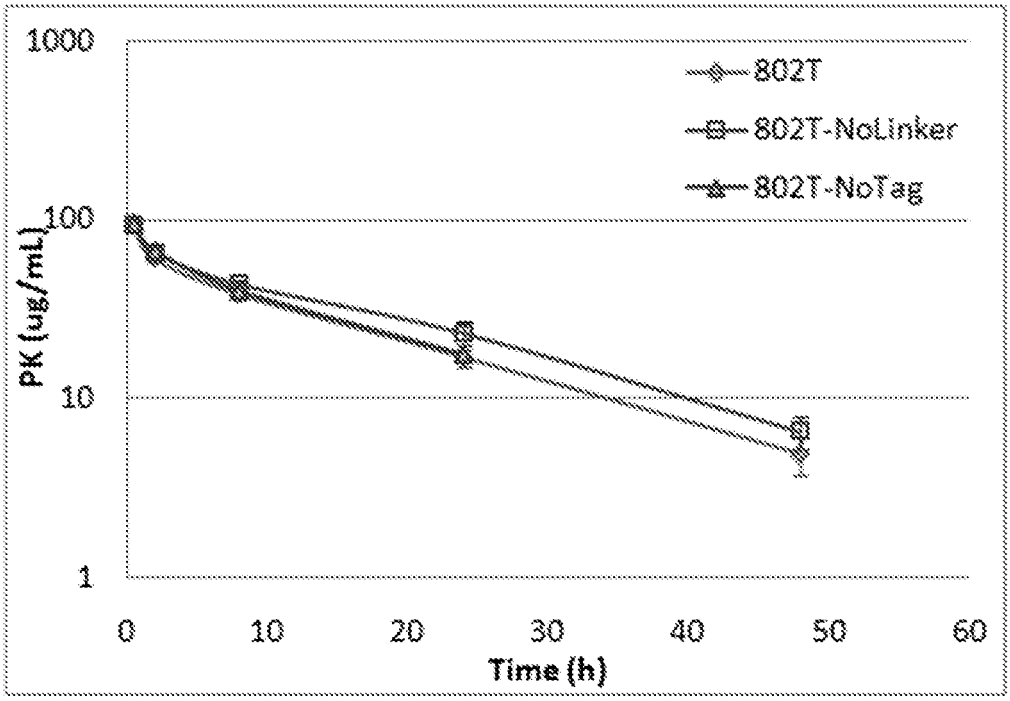

1000
100
10
1
PK (ug/mL)
0
10
20
30
40
50
60
Time (h)
802T
802T-NoLinker
802T-NoTag

Fig 21B

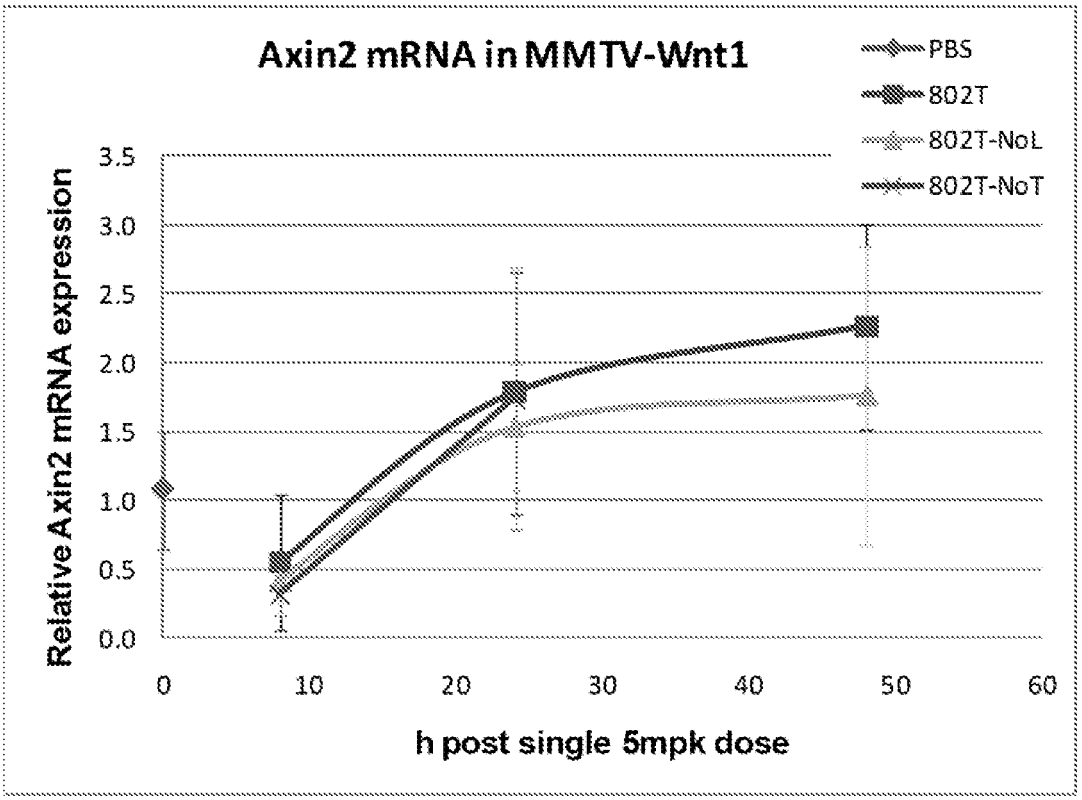

Axin2 mRNA in MMTV-Wnt1
PBS
802T
802T-NoL
802T-NoT
Relative Axin2 mRNA expression
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
h post single 5mpk dose

Fig 22

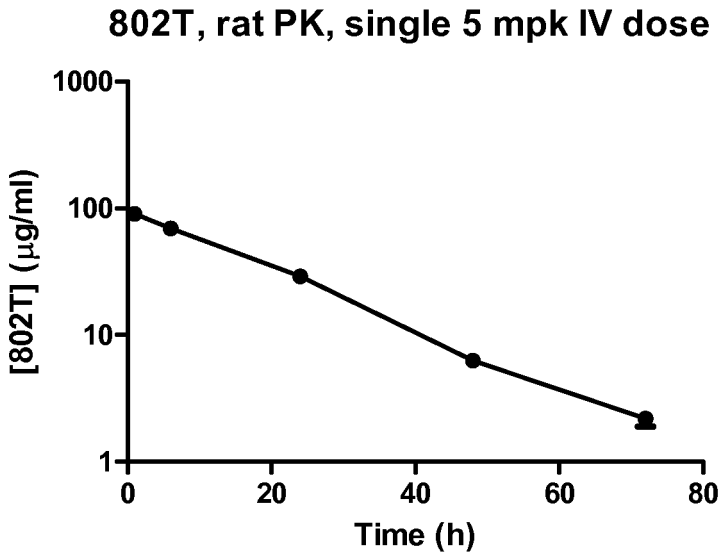

802T, rat PK, single 5 mpk IV dose
[802T] (μg/ml)
1000
100
10
1
0
20
40
60
80
Time (h)

Fig 23A

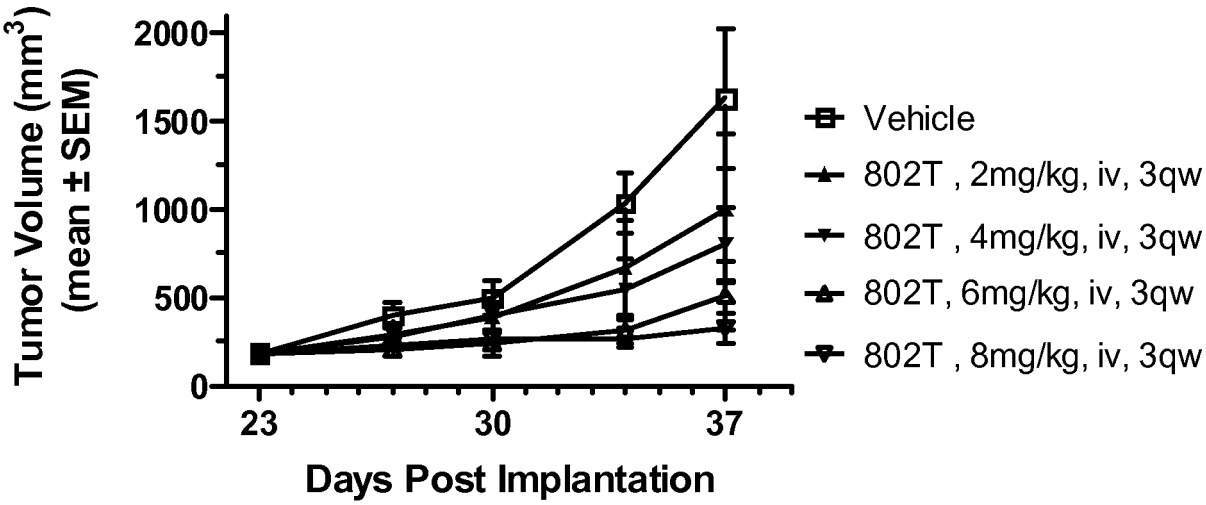

Tumor Volume (mm3)
(mean ± SEM)
2000
1500
1000
500
0
23
30
37
Days Post Implantation
Vehicle
802T , 2mg/kg, iv, 3qw
802T , 4mg/kg, iv, 3qw
802T, 6mg/kg, iv, 3qw
802T , 8mg/kg, iv, 3qw

Fig 23B

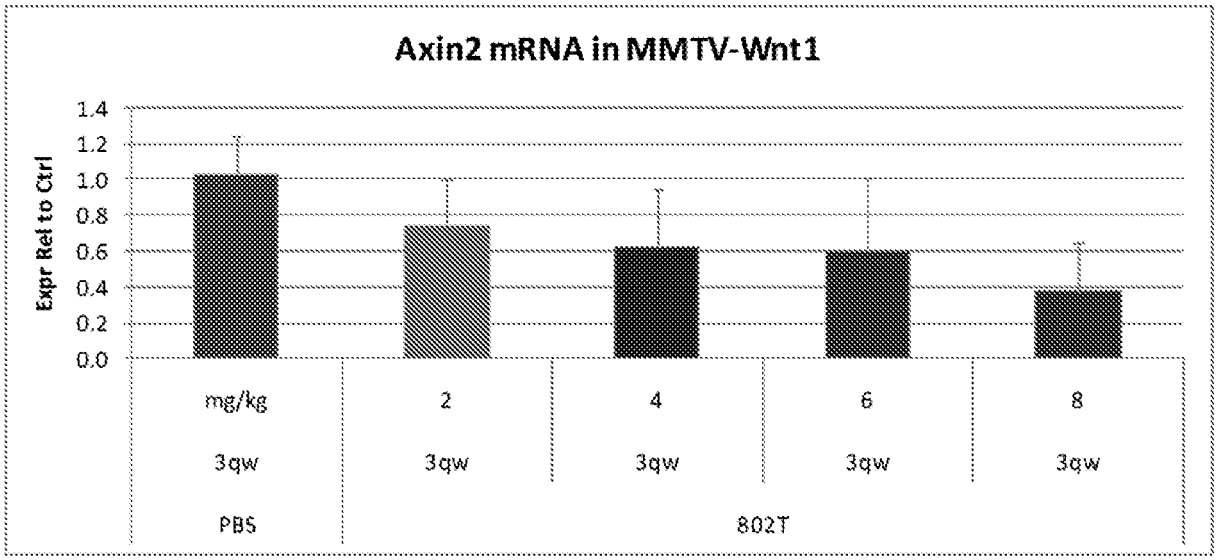

Axin2 mRNA in MMTV-Wnt1
Expr Rel to Ctrl
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
2
4
6
8
3qw
3qw
3qw
3qw
3qw
PBS
802T

Fig 24A

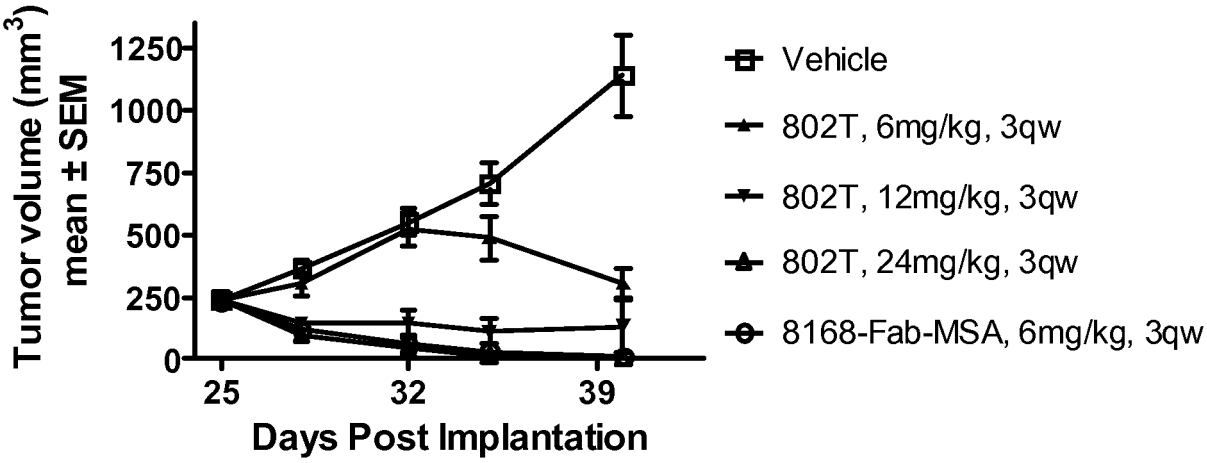

Tumor volume (mm3)
mean ± SEM
1250
1000
750
500
250
0
25
32
39
Days Post Implantation
Vehicle
802T, 6mg/kg, 3qw
802T, 12mg/kg, 3qw
802T, 24mg/kg, 3qw
8168-Fab-MSA, 6mg/kg, 3qw

Fig 24B

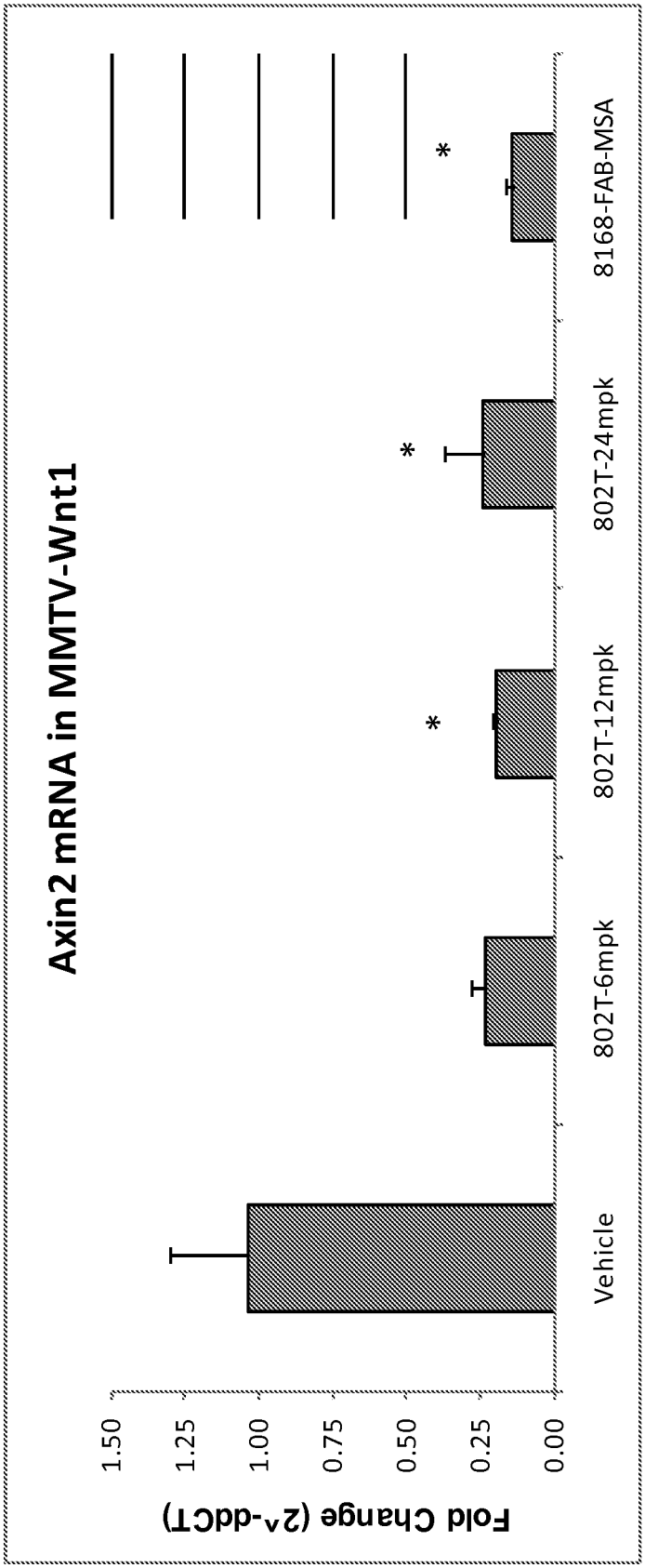

Axin2 mRNA in MMTV-Wnt1
Fold Change (2^-ddCT)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Vehicle
802T-6mpk
802T-12mpk
802T-24mpk
8168-FAB-MSA
*
*
*

Fig 24C

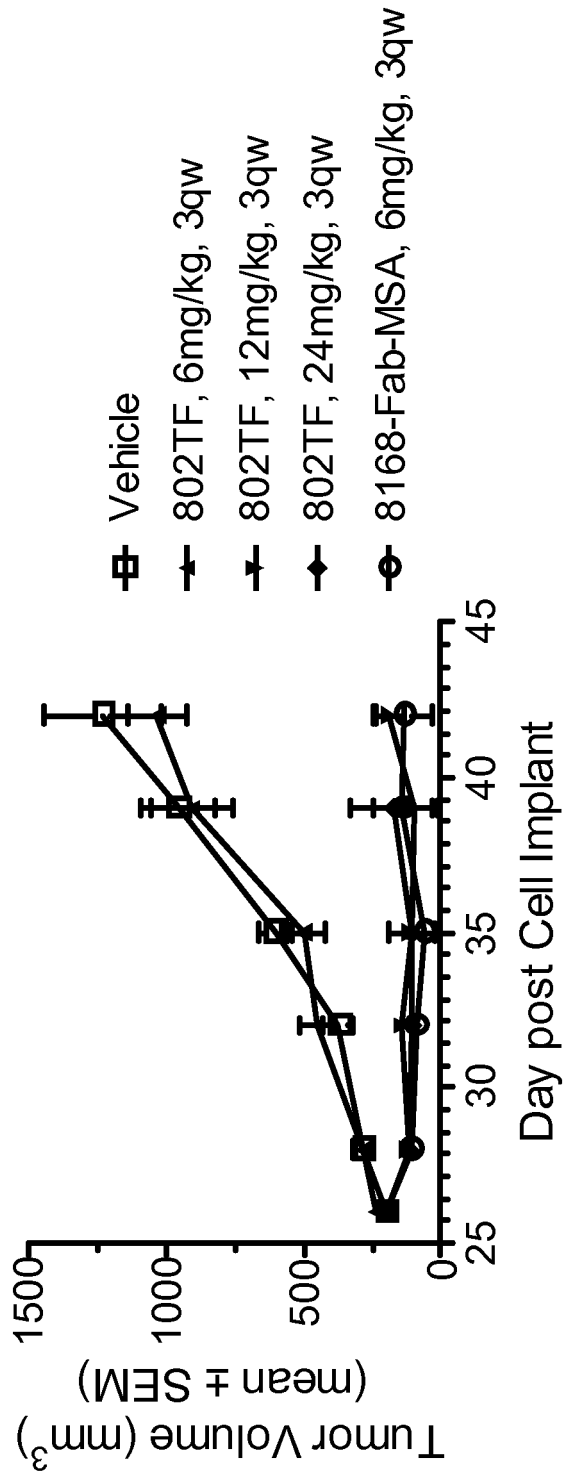
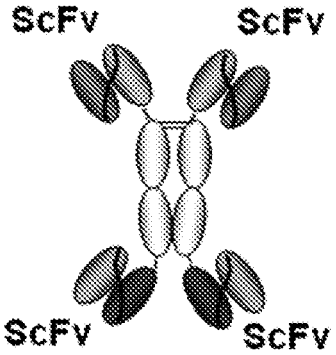

Vehicle
802TF, 6mg/kg, 3qw
802TF, 12mg/kg, 3qw
802TF, 24mg/kg, 3qw
8168-Fab-MSA, 6mg/kg, 3qw
Tumor Volume (mm3)
(mean ± SEM)
1500
1000
500
0
25
30
35
40
45
Day post Cell Implant

Fig 25

scFv-Fc-scFv fusion molecule structure			
	Module 1	Module 2	Module 3
911T	scFv8168	Fc	scFv6475
912T	scFv6475	Fc	scFv8168

Fig 26



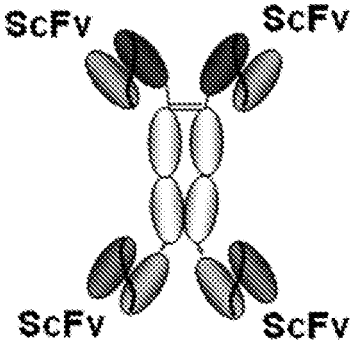


Fig 27

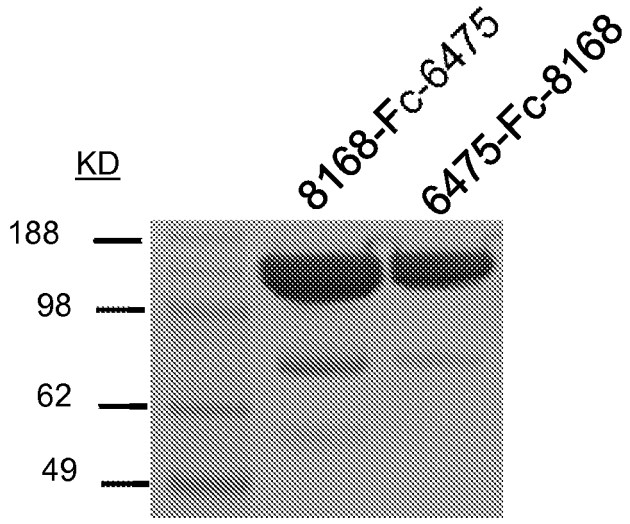


Fig 28

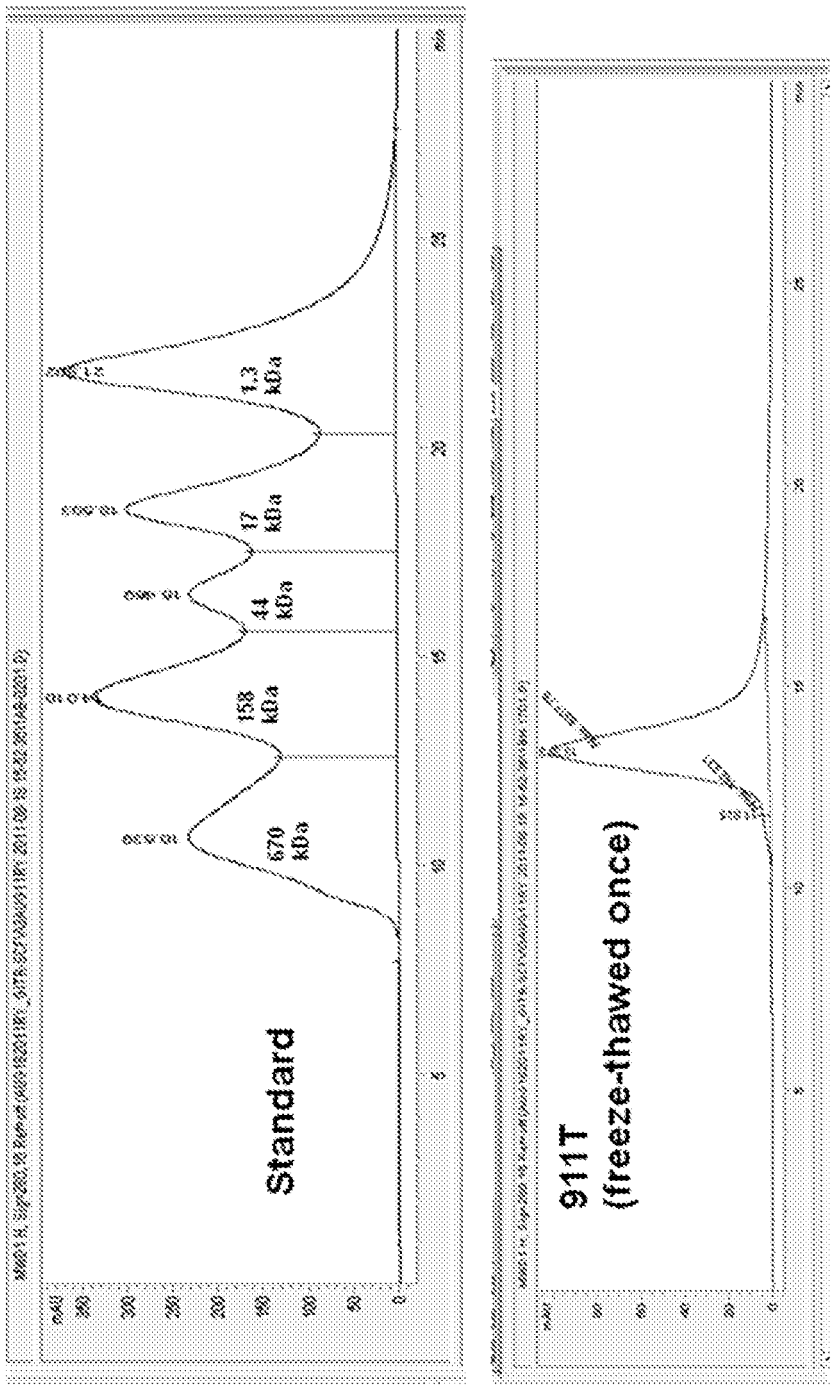

Standard
670 kDa
158 kDa
44 kDa
17 kDa
1.3 kDa
911T
(freeze-thawed once)

Fig 29

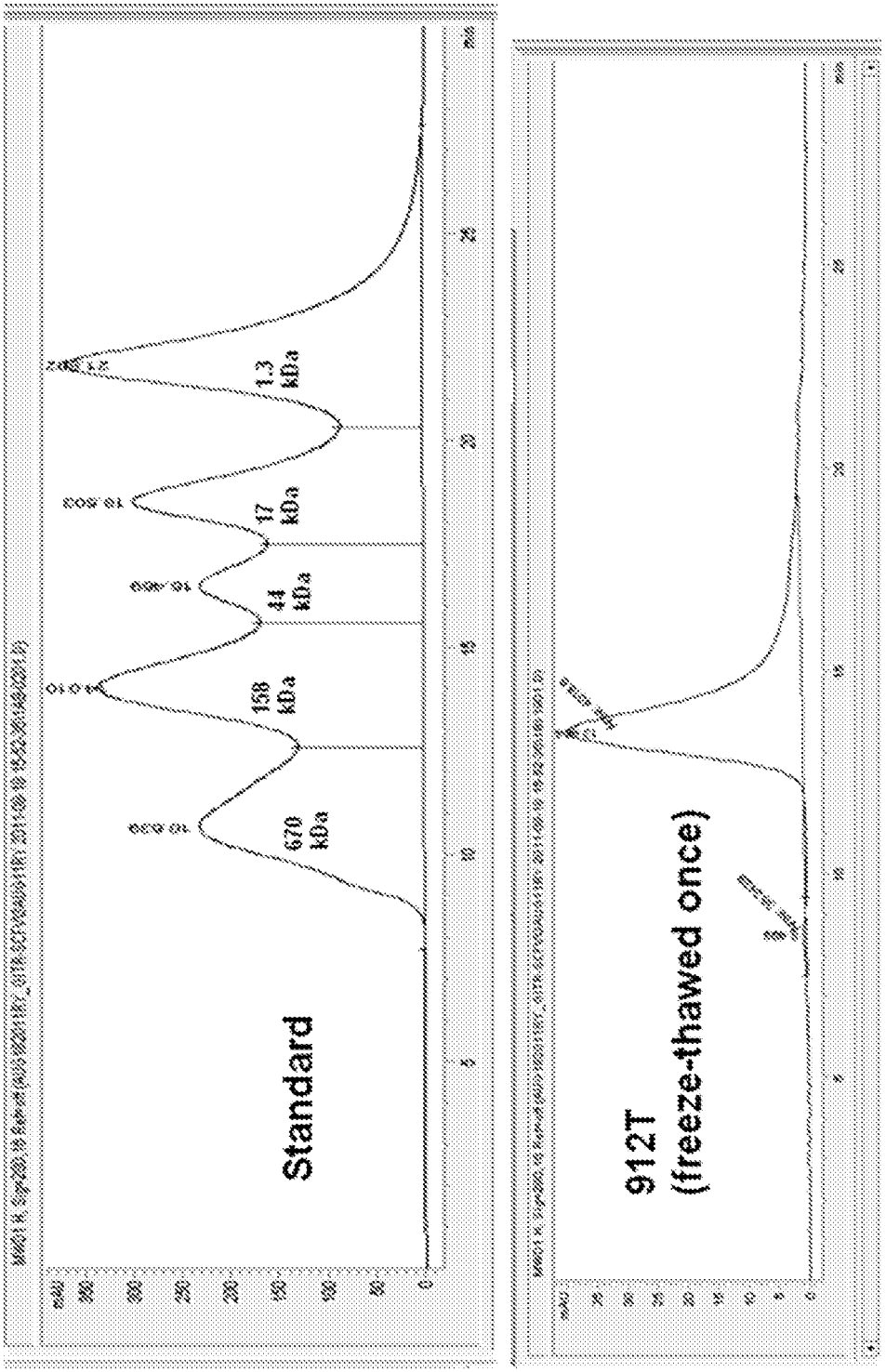

Standard
670 kDa
158 kDa
44 kDa
17 kDa
1.3 kDa
912T
(freeze-thawed once)

Fig 30

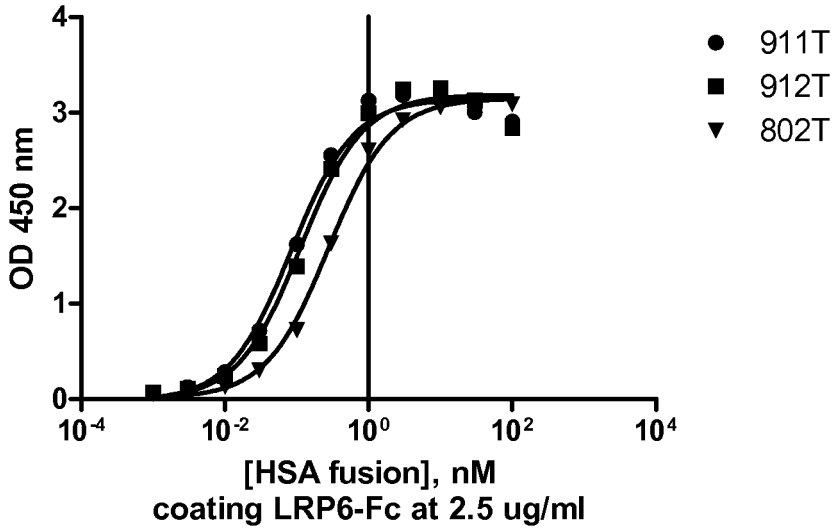

OD 450 nm
4
3
2
1
0
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
[HSA fusion], nM
coating LRP6-Fc at 2.5 ug/ml
911T
912T
802T

Fig 31

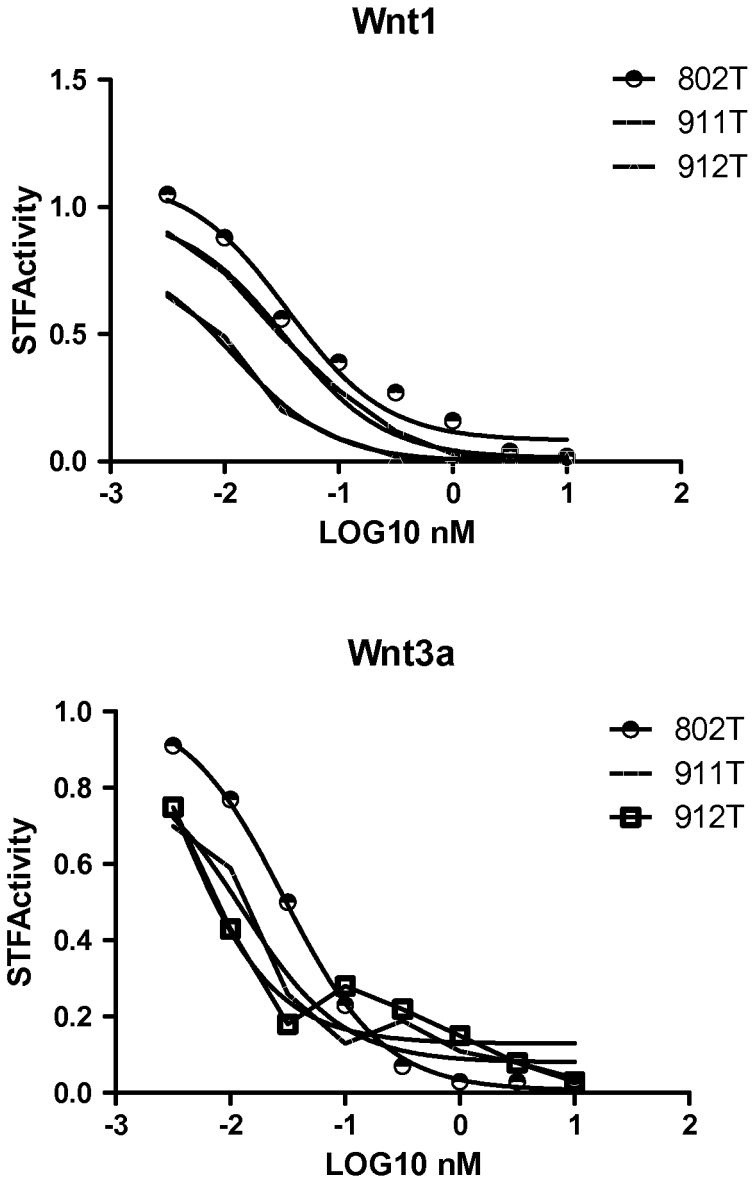

Wnt1
802T
911T
912T
STFActivity
1.5
1.0
0.5
0.0
-3
-2
-1
0
1
2
LOG10 nM
Wnt3a
802T
911T
912T
STFActivity
1.0
0.8
0.6
0.4
0.2
0.0
-3
-2
-1
0
1
2
LOG10 nM

Fig 32

Tm of single point mutations in scFv06475

Construct ID	mutation[a]	T_m on protein from E.coli	T_m on protein from mammalian
scFv06475 WT		59	61
6475-S2	VH:G34V	61	64
6475-S3	VH:I37F	61	64.5
6475-S6	VH:V85E	60	62.5
6475-S8	VH:M95F	61.5	64.5
6475-S9	VL:D93N	59.5	60.5

[a]Both kabat and Chothia numbering system have been used. The numbering is the same for all mutations but VH: G34V in scFv6475. This would be VH: G32V in Chothia numbering system. The numbering system in the text is Kabat system.

Fig 33

Tm of single point mutations in scFv08168

Construct ID	mutation	T_m on protein from E.coli[a]
WT	WT	48.50
B02	VH:V033N	50.50
B03-S1	VH:I034M	56.00
B04	VH:I034F	52.50
C05	VH:S049A	54.00
C07	VH:G050S	51.50
C08	VH:W052aG	55.50
C10	VH:H058Y	52.00
F11	VL:V047L	51.00
G02	VL:G064V	50.50
G07	VL:T078V	51.00

[a]Proteins were expressed from Acella strain.Samples were analyzed by DSF without removal of imidazole.

Fig 34

Tm of single vs double mutations in scFv08168

Construct ID	Mutation	T_m on protein from E.coli[a]	T_m on protein from mammalian[b]	T_m on protein from mammalian
scFv08168 WT	WT	48.50	49	49
scFv08168 B02	VH:V33N	50.50		
scFv08168 B03-S1	VH:I34M	56.00	57	56.5
scFv08168 B04	VH:I34F	52.50		
scFv08168 C05	VH:S49A	54.00		
scFv08168 C07	VH:G50S	51.50		
scFv08168 C10	VH:H58Y	52.00		
scFv08168 F11	VL:V47L	51.00		
scFv08168 G02	VL:G64V	50.50		
scFv08168 G07	VL:T78V	51.00		

Fig 34 contd

Tm of single vs double mutations in scFv08168

Construct ID	Mutation	T_m on protein from E.coli[a]	T_m on protein from mammalian[b]	T_m on protein from mammalian
scFv08168.D1	VH S49A, I34M	61	61.5	62.5
scFv08168.D2	VH S49A, I34F	57.5	58	58.5
scFv08168.D4	VH I34M, G50S	59.5	59.5	60
scFv08168.D5	VH I34M, H58Y	59	59	59
scFv08168.D6	VH I34M, V48I	57	56	57.5
scFv08168.D7	VH I34M, VL S22T	57	57	58
scFv08168.D8	VH I34M, VL V47L	57.5	56	58.5
scFv08168.D9	VH I34M, VL G64V	57.5	58.5	57.5

[a]Proteins were expressed from Acella strain.Samples were analyzed by DSF without removal of imidazole.
[b]Protein expressed from 293T suspension cells. Samples were analyzed by DSF without removal of imidazole.

Fig 35

Activity of scFv06475, scFv08168 and the variants in different assays

Construct ID	Mutations	EC50 by ELISA nM	Affinity by Proteon nM	IC50 by STF assay for Wnt1 inhibition (nM)	IC50 by STF assay for Wnt3a inhibition (nM)
scFv06475 WT		0.76	-	-	1.48
scFv06475-S2	VH:G34V	27.4	-	-	-
scFv06475-S3	VH:I37F	4.3	-	-	-
scFv06475-S6	VH:V85E	0.73	-	-	1.33
scFv06475-S8	**VH:M95F**	**1.0**	-	-	**0.96**
scFv06475-S9	VL:D93N	0.88	-	-	
scFv08168 WT		2.04	3.82	7.41	-
scFv08168 B03-S1	VH:I34M	0.98	-	5.19	-
scFv08168-D1	**VH S49A, I34M**	**1.61**	**2.55**	**2.44**	**-**
scFv08168-D2	VH S49A, I34F	1.68	-	2.59	-
scFv08168-D4	VH I34M, G50S	1.47	-	5.56	-
scFv08168-D5	VH I34M, H58Y	1.22	-	-	-
scFv08168-D6	VH I34M, V48I	1.24	-	0.74	-
scFv08168-D7	VH I34M, VL S22T	1.15	-	4.81	-
scFv08168-D8	VH I34M, VL V47L	0.91	-	-	-
scFv08168-D9	VH I34M, VL G64V	1.26	-	11.11	-