Fig. 1A

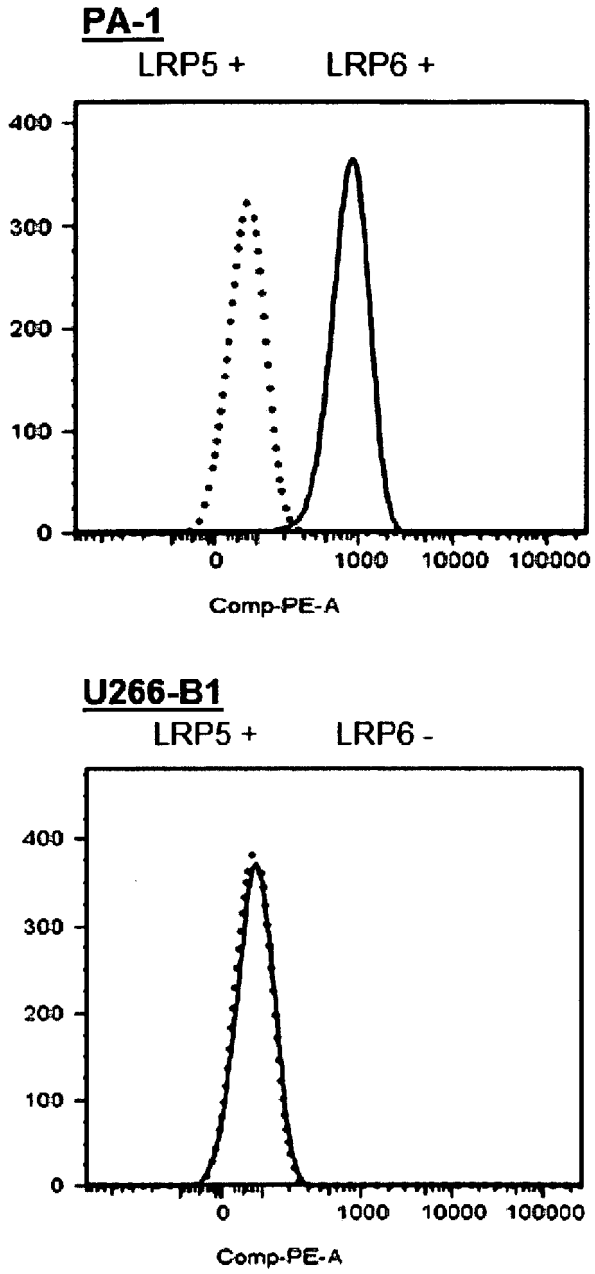
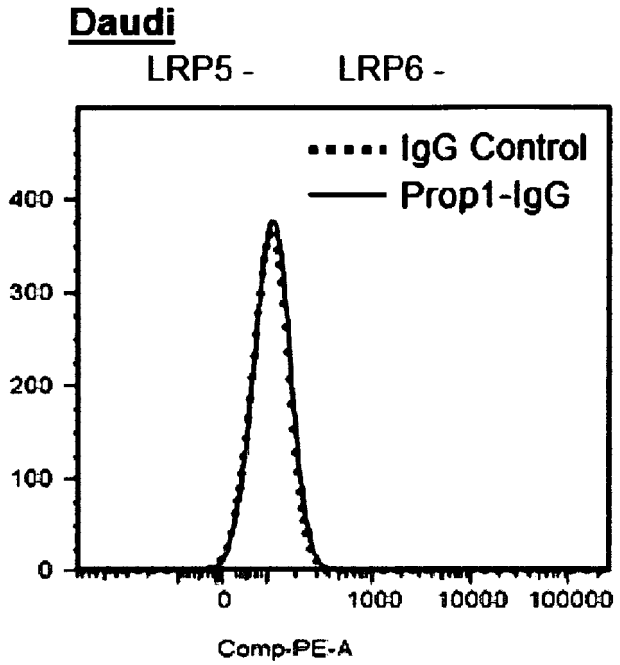

PA-1
LRP5 +
LRP6 +
400
300
200
100
0
0
1000
10000
100000
Comp-PE-A
U266-B1
LRP5 +
LRP6 -
400
300
200
100
0
0
1000
10000
100000
Comp-PE-A

Fig. 1A continued



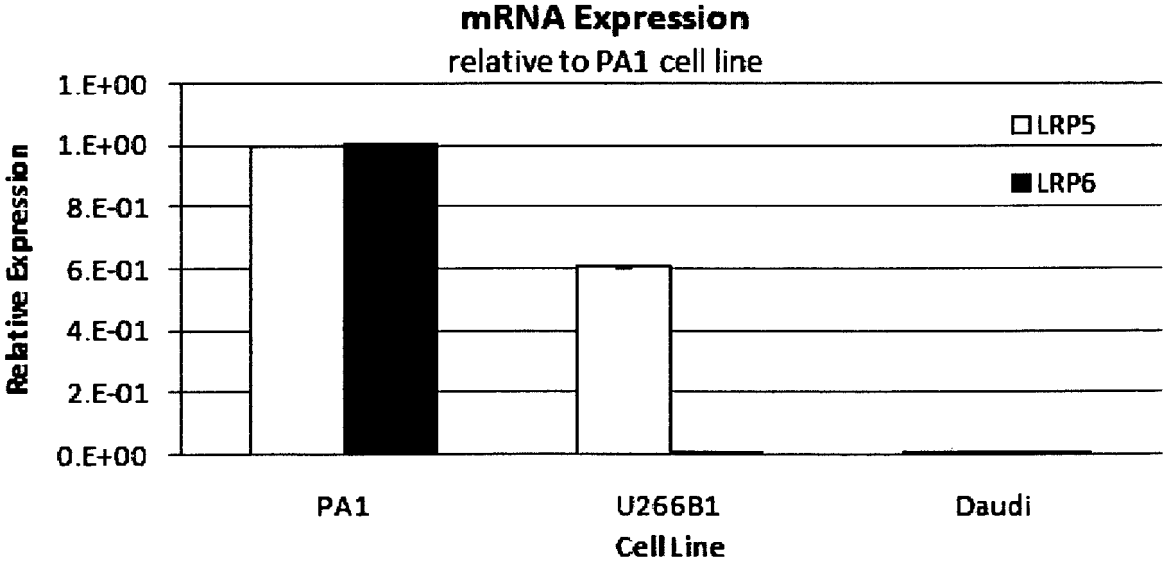
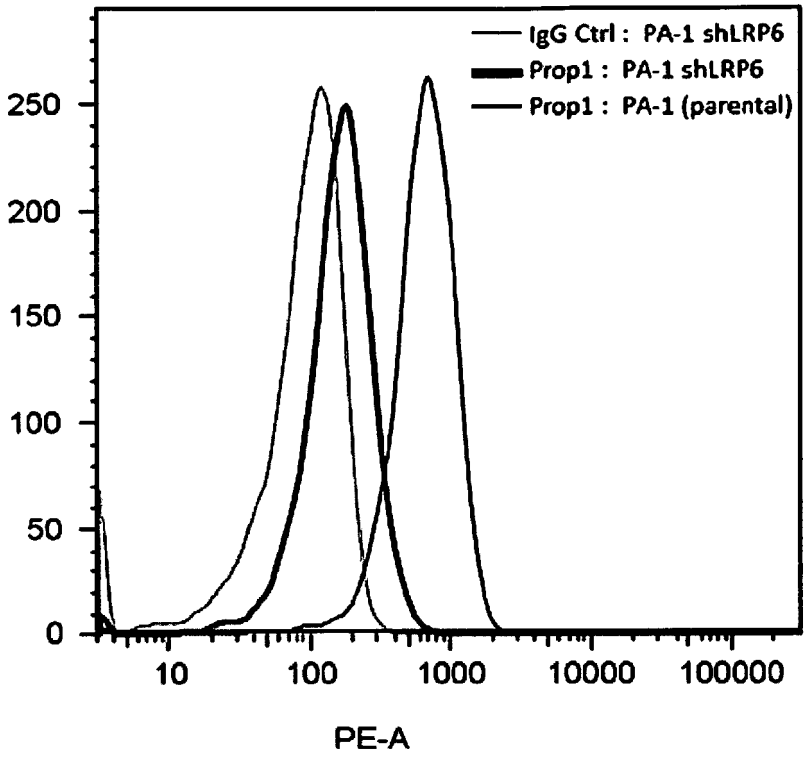


Fig. 1B


IgG Ctrl : PA-1 shLRP6
Prop1 : PA-1 shLRP6
Prop1 : PA-1 (parental)
250
200
150
100
50
0
10
100
1000
10000
100000
PE-A

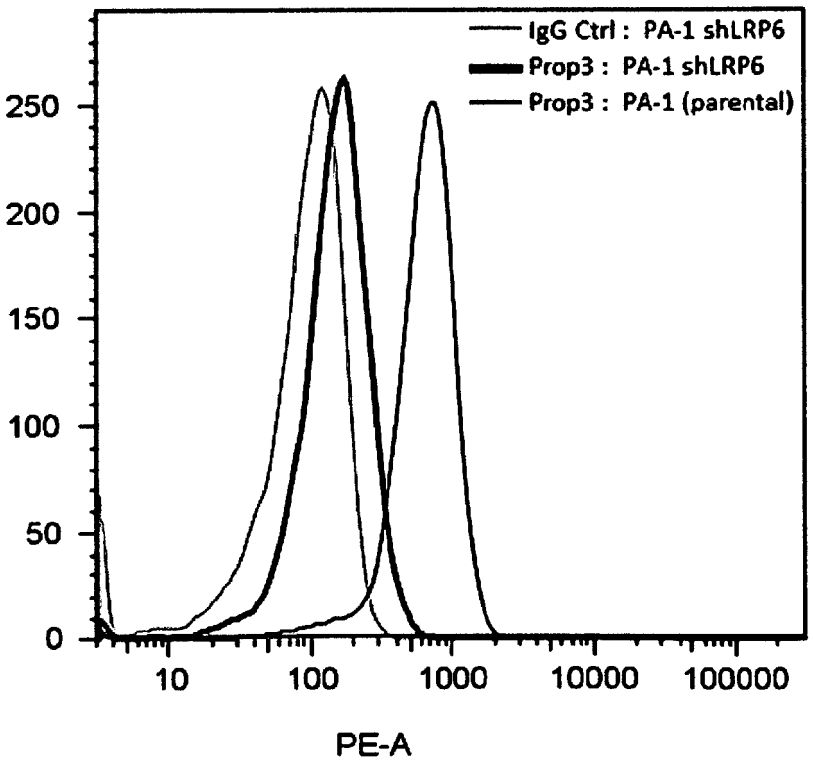

IgG Ctrl : PA-1 shLRP6
Prop3 : PA-1 shLRP6
Prop3 : PA-1 (parental)
250
200
150
100
50
0
10
100
1000
10000
100000
PE-A

Fig. 1B continued

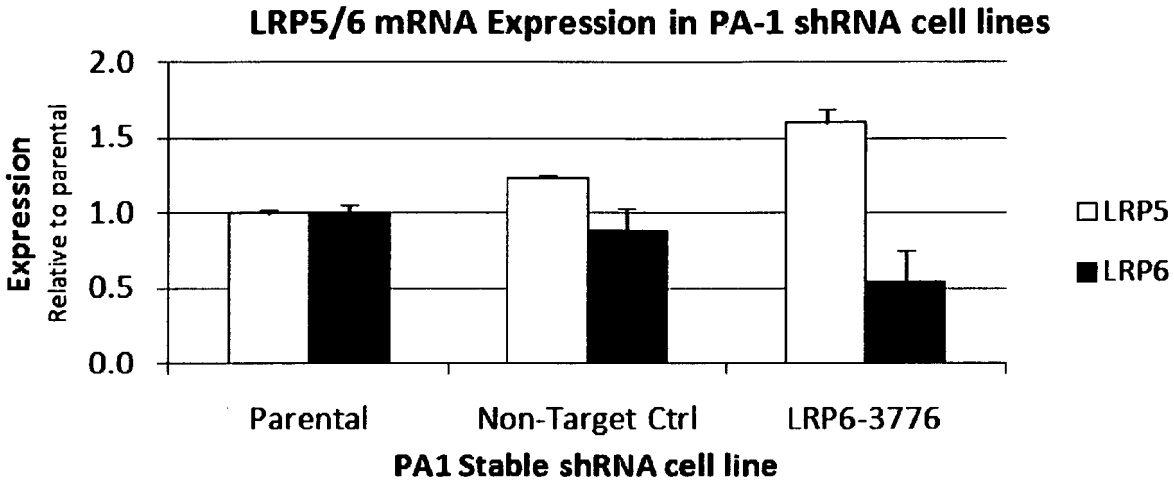


Fig. 2A

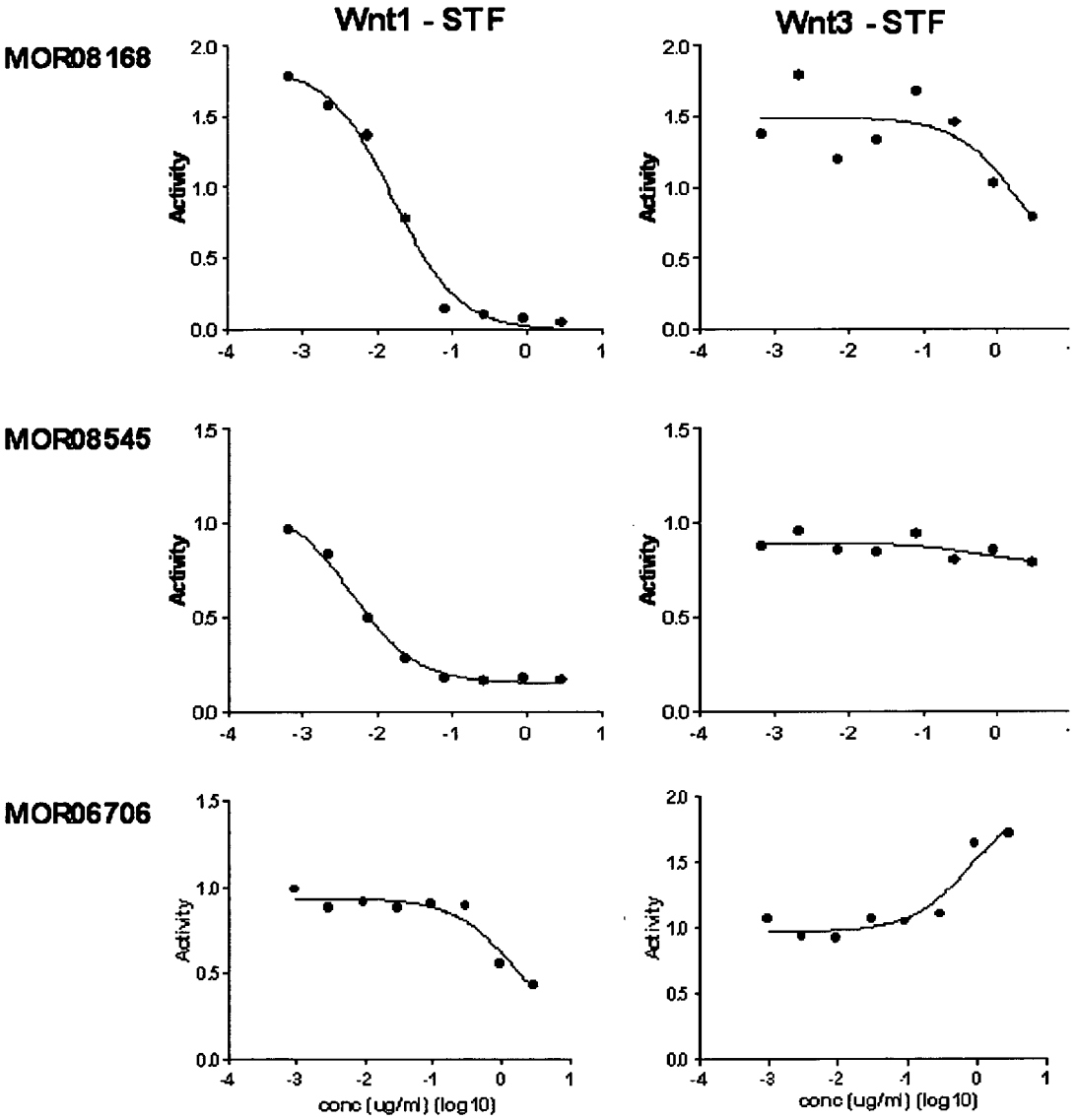

Wnt1 - STF
Wnt3 - STF
MOR08168
MOR08545
MOR06706
Activity
conc (ug/ml) (log10)

Fig. 2B

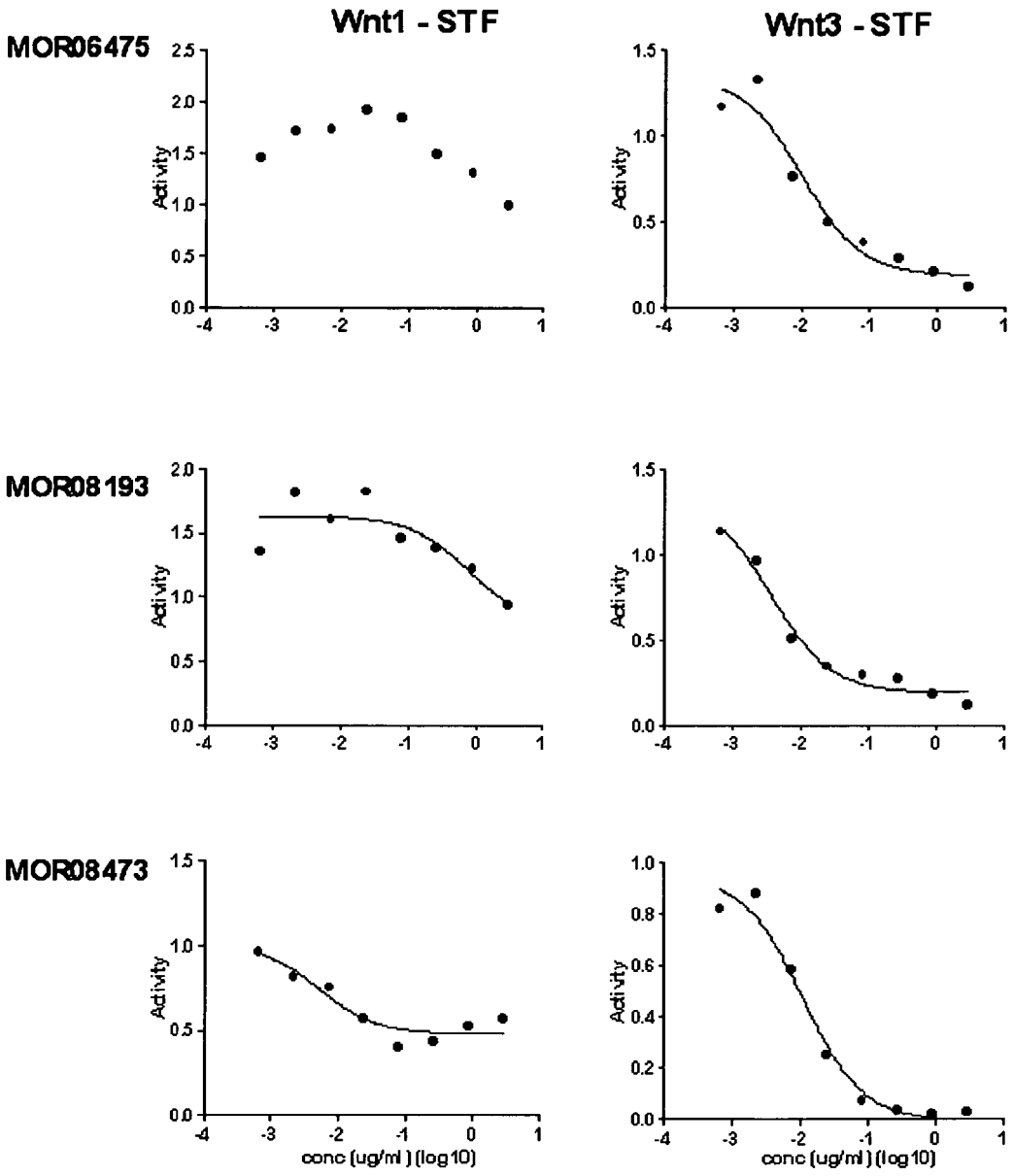

Wnt1 - STF
Wnt3 - STF
MOR06475
MOR08193
MOR08473
Activity
conc (ug/ml) (log10)

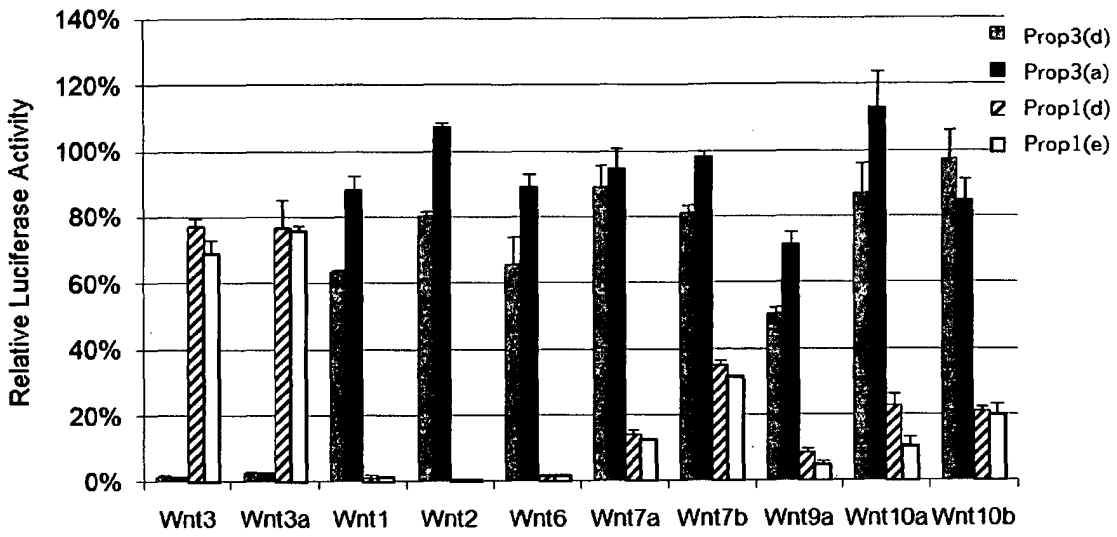
Fig. 3

	Prop 1			Prop3		
	MOR08168	MOR08545	MOR06706	MOR06475	MOR08193	MOR08473
human LRP6	0.28	0.09	0.14	0.31	0.36	0.74
mouse LRP6	0.12	0.06	n/a	0.96	0.08	0.46
cynomolgus LRP6	0.88	0.30	0.46	0.22	0.20	0.52

EC_{50} values expressed in nM.

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Fig. 4



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Fig. 5

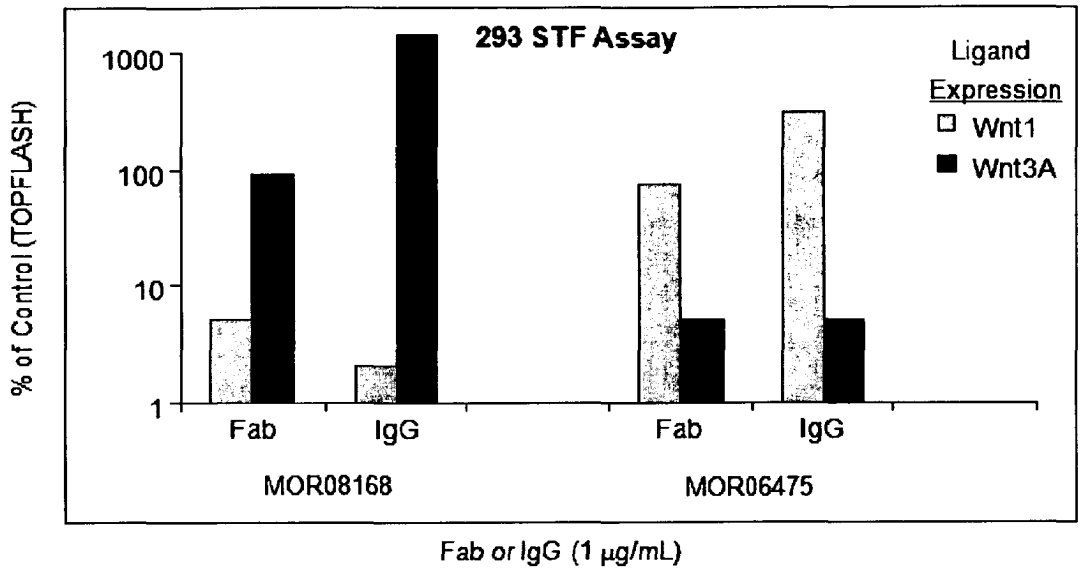

293 STF Assay
Ligand Expression
Wnt1
Wnt3A
% of Control (TOPFLASH)
1000
100
10
1
Fab
IgG
Fab
IgG
MOR08168
MOR06475
Fab or IgG (1 µg/mL)

Fig. 6

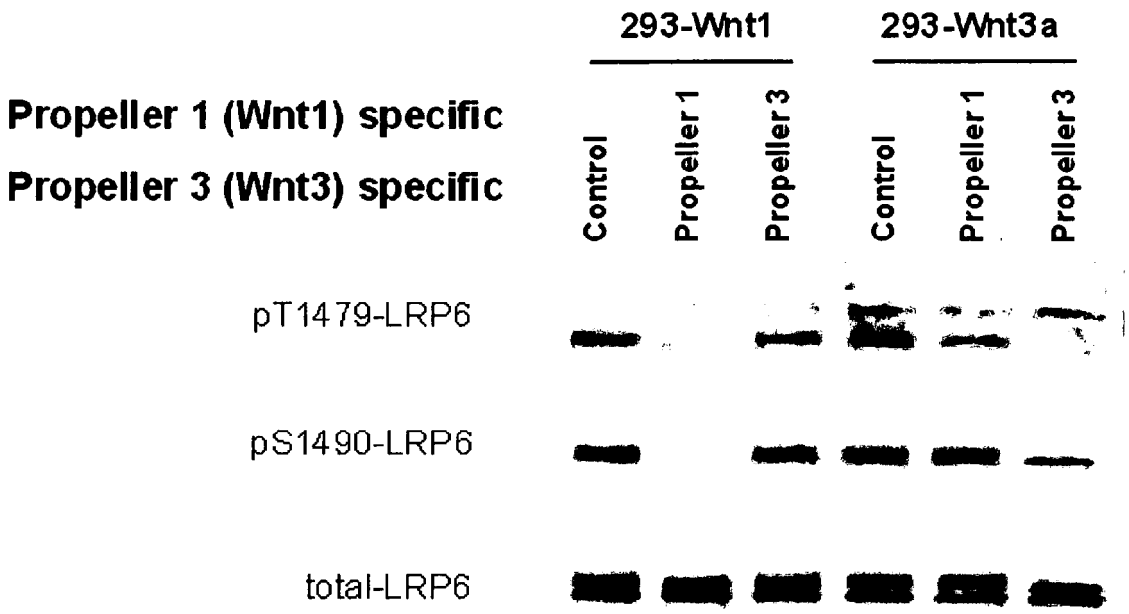
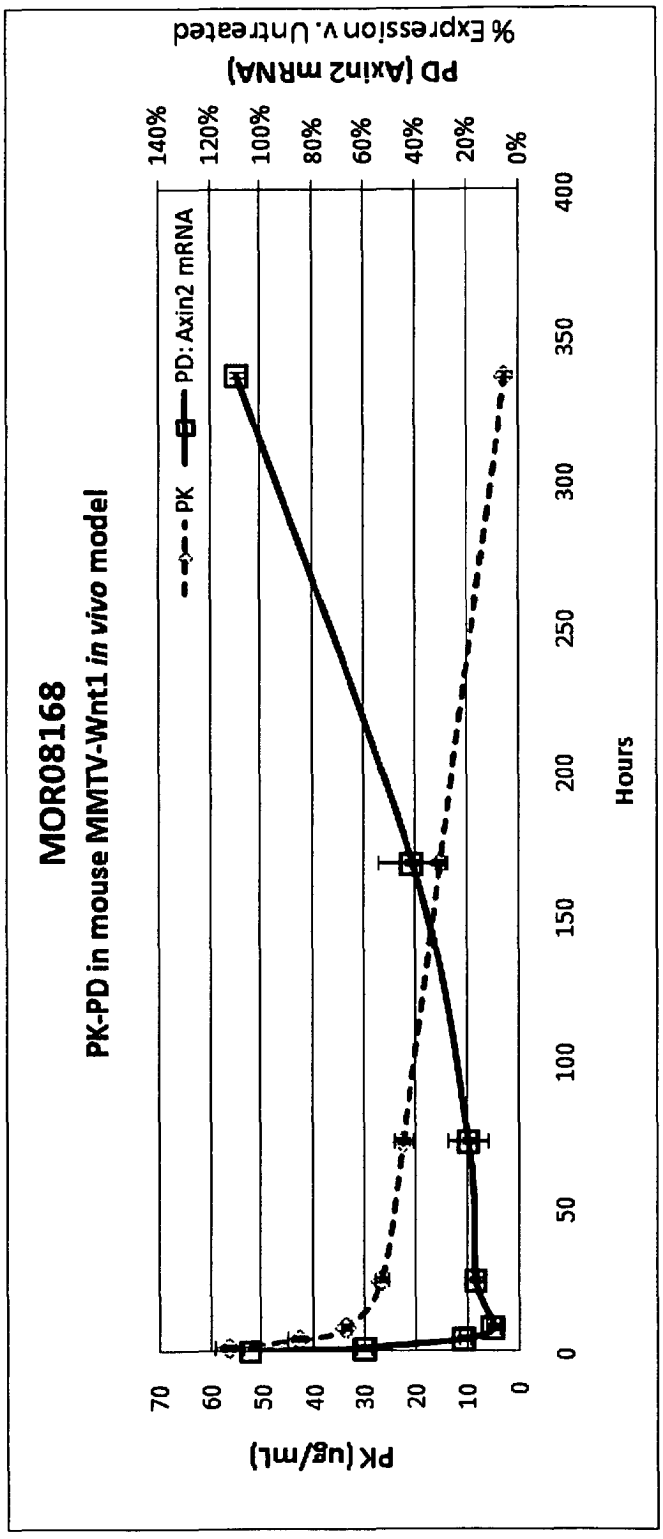

293-Wnt1
293-Wnt3a
Propeller 1 (Wnt1) specific
Propeller 3 (Wnt3) specific
Control
Propeller 1
Propeller 3
Control
Propeller 1
Propeller 3
pT1479-LRP6
pS1490-LRP6
total-LRP6

Fig. 7



Time points: 1, 4, 8 and 24 hours, 3, 7 and 14 days

Fig. 8A

Probe ID	Gene Symbol	Gene Title	Entrez Gene ID	log(2)fold change 0 h vs. 8 h	adj.P.Val 0 h vs. 8 h
1435154_at	AU018091	expressed sequence AU018091	245128	2.51	0.008168
1418743_a_at	LOC100047138 /// Tesc	similar to Tescalcin /// tescalcin	100047138 /// 57816	2.42	0.000707
1418744_s_at	LOC100047138 /// Tesc	similar to Tescalcin /// tescalcin	100047138 /// 57816	2.41	0.001168
1447891_at	---	---	---	2.35	0.00122
1417956_at	Cidea	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	12683	2.32	0.006174
1436279_at	---	---	---	2.25	0.000742
1451139_at	Slc39a4	solute carrier family 39 (zinc transporter), member 4	72027	2.04	0.000336
1417130_s_at	Angptl4	angiopoietin-like 4	57875	1.94	0.000722
1434918_at	Sox6	SRY-box containing gene 6	20679	1.79	0.002107
1451612_at	Mt1	metallothionein 1	17748	1.74	0.004696
1441915_s_at	2310076L09Rik	RIKEN cDNA 2310076L09 gene	66968	1.71	0.0025
1423860_at	Ptgds	prostaglandin D2 synthase (brain)	19215	1.66	0.001237
1428283_at	Cyp2s1	cytochrome P450, family 2, subfamily s, polypeptide 1	74134	1.64	0.004778
1439036_a_at	Atp1b1	ATPase, Na+/K+ transporting, beta 1 polypeptide	11931	1.63	0.00705
1451410_a_at	Crip3	cysteine-rich protein 3	114570	1.62	0.000223
1459898_at	Sbsn	suprabasin	282619	1.60	0.002259
1433888_at	Atp2b2	ATPase, Ca++ transporting, plasma membrane 2	11941	1.58	0.009975
1450435_at	L1cam	L1 cell adhesion molecule	16728	1.57	0.001168

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Fig. 8A continued

Probe ID	Gene Symbol	Gene Title	Entrez Gene ID	log(2)fold change 0 h vs. 8 h	adj.P.Val 0 h vs. 8 h
1455506_at	Slc25a34	solute carrier family 25, member 34	384071	1.55	7.26E-05
1422820_at	Lipe	lipase, hormone sensitive	16890	1.52	0.003431
1447655_x_at	Sox6	SRY-box containing gene 6	20679	1.51	0.003772
1451204_at	Scara5	scavenger receptor class A, member 5 (putative)	71145	1.51	0.001792
1451828_a_at	Acsl4	acyl-CoA synthetase long-chain family member 4	50790	1.48	6.46E-05
1451331_at	Ppp1r1b	protein phosphatase 1, regulatory (inhibitor) subunit 1B	19049	1.44	0.002536
1418453_a_at	Atp1b1	ATPase, Na+/K+ transporting, beta 1 polypeptide	11931	1.43	0.004956
1419706_a_at	Akap12	A kinase (PRKA) anchor protein (gravin) 12	83397	1.42	0.000118
1418911_s_at	Acsl4	acyl-CoA synthetase long-chain family member 4	50790	1.40	0.003297
1439630_x_at	Sbsn	suprabasin	282619	1.38	0.00074
1438261_at	Cited4	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4, mRNA (cDNA clone IMAGE:3670674)	56222	1.36	0.006783
1429952_at	Mospd4	motile sperm domain containing 4	72076	1.36	0.005578
1435595_at	1810011O10Rik	RIKEN cDNA 1810011O10 gene	69068	1.34	0.000592
1429352_at	Mocos	molybdenum cofactor sulfurase	68591	1.32	0.009036
1459897_a_at	Sbsn	suprabasin	282619	1.32	0.001447
1447845_s_at	Vnn1	vanin 1	22361	1.31	0.004465
1421841_at	Fgfr3	fibroblast growth factor receptor 3	14184	1.29	7.26E-05
1424937_at	2310076L09Rik	RIKEN cDNA 2310076L09 gene	66968	1.29	0.000118
1449403_at	Pde9a	phosphodiesterase 9A	18585	1.27	0.009729
1424218_a_at	Creb3l4	cAMP responsive element binding protein 3-like 4	78284	1.21	0.002044

SUBSTITUTE SHEET (RULE 26)

Fig. 8A continued

Probe ID	Gene Symbol	Gene Title	Entrez Gene ID	log(2)fold change 0 h vs. 8 h	adj.P.Val 0 h vs. 8 h
1424763_at	Rsph9	radial spoke head 9 homolog (Chlamydomonas)	75564	1.21	0.006783
1420521_at	Papln	papilin, proteoglycan-like sulfated glycoprotein	170721	1.21	0.004502
1434442_at	Stbd1	starch binding domain 1	52331	1.19	0.004723
1438033_at	Tef	thyrotroph embryonic factor	21685	1.17	0.001335
1442335_at	---	---	---	1.16	0.006174
1448227_at	Grb7	growth factor receptor bound protein 7	14786	1.14	0.001261
1416596_at	Slc44a4	solute carrier family 44, member 4	70129	1.14	0.008898
1439620_at	Car13	carbonic anhydrase 13	71934	1.13	0.00323
1418780_at	Cyp39a1	cytochrome P450, family 39, subfamily a, polypeptide 1	56050	1.12	0.006174
1460409_at	Cpt1a	carnitine palmitoyltransferase 1a, liver	12894	1.10	0.003297
1449146_at	Notch4	Notch gene homolog 4 (Drosophila)	18132	1.10	0.008351
1443841_x_at	Uap1l1	UDP-N-acteylglucosamine pyrophosphorylase 1-like 1	227620	1.08	0.00764
1417273_at	Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	27273	1.08	0.006174
1430278_a_at	Dqx1	DEAQ RNA-dependent ATPase	93838	1.07	0.0025
1426065_a_at	Trib3	tribbles homolog 3 (Drosophila)	228775	1.04	0.002859
1438038_at	4930402H24Rik	RIKEN cDNA 4930402H24 gene	228602	1.03	0.00599
1427537_at	Eppk1	epiplakin 1	223650	1.02	0.00114
1416457_at	Ddah2	dimethylarginine dimethylaminohydrolase 2	51793	1.01	0.005738

Fig. 8B

Probe ID	Gene Symbol	Gene Title	Entrez Gene	log(2)fold change 0 h vs. 8 h	adj.P.Val 0 h vs. 8 h
1449451_at	Serpinb11	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 11	66957	-3.95	7.37E-07
1451129_at	Calb2	calbindin 2	12308	-3.14	0.006783
1436845_at	Axin2	axin2	12006	-2.92	4.53E-07
1416468_at	Aldh1a1	aldehyde dehydrogenase family 1, subfamily A1	11668	-2.83	0.000506
1427508_at	Arsi	arylsulfatase i	545260	-2.55	0.000592
1418601_at	Aldh1a7	aldehyde dehydrogenase family 1, subfamily A7	26358	-2.55	0.005578
1432592_at	Pappa	pregnancy-associated plasma protein A	18491	-2.55	6.46E-05
1425985_s_at	Masp1	mannan-binding lectin serine peptidase 1	17174	-2.48	0.001787
1434802_s_at	Ntf3	neurotrophin 3	18205	-2.43	0.000914
1425978_at	Myocd	myocardin	214384	-2.39	0.00374
1436221_at	Ildr2	immunoglobulin-like domain containing receptor 2	100039795	-2.36	0.00296
1418678_at	Has2	hyaluronan synthase 2	15117	-2.31	0.000996
1436894_at	Ildr2	immunoglobulin-like domain containing receptor 2	100039795	-2.3	0.0025
1427633_a_at	Pappa	pregnancy-associated plasma protein A	18491	-2.21	0.002187
1436293_x_at	Ildr2	immunoglobulin-like domain containing receptor 2	100039795	-2.17	0.002799
1433959_at	Zmat4	zinc finger, matrin type 4	320158	-2.14	0.00638
1427600_at	---	---	---	-2.12	6.46E-05
1432591_at	Pappa	pregnancy-associated plasma protein A	18491	-2.07	0.001159

Fig. 8B continued

Probe ID	Gene Symbol	Gene Title	Entrez Gene	log(2)fold change 0 h vs. 8 h	adj.P.Val 0 h vs. 8 h
1448397_at	Gjb6	gap junction protein, beta 6	14623	-2.06	0.002796
1449926_at	Cd70	CD70 antigen	21948	-2.05	0.006174
1441807_s_at	---	---	---	-2.02	0.004956
1438602_s_at	Masp1	mannan-binding lectin serine peptidase 1	17174	-2.01	0.00255
1425212_a_at	Tnfrsf19	tumor necrosis factor receptor superfamily, member 19	29820	-1.94	6.46E-05
1428223_at	Mfsd2	major facilitator superfamily domain containing 2	76574	-1.93	0.006536
1420006_at	Bmp15	Bone morphogenetic protein 15 (Bmp15), mRNA	12155	-1.91	0.000707
1429506_at	Nkd1	naked cuticle 1 homolog (Drosophila)	93960	-1.91	0.001459
1433894_at	Jazf1	JAZF zinc finger 1	231986	-1.9	0.000592
1422699_at	Alox12	arachidonate 12-lipoxygenase	11684	-1.89	0.001168
1460449_at	Anks1b	ankyrin repeat and sterile alpha motif domain containing 1B	77531	-1.87	0.000592
1417275_at	Mal	myelin and lymphocyte protein, T-cell differentiation protein	17153	-1.87	0.002349
1449169_at	Has2	hyaluronan synthase 2	15117	-1.8	0.000707
1420005_s_at	Bmp15	bone morphogenetic protein 15	12155	-1.78	0.002107
1449033_at	Tnfrsf11b	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	18383	-1.76	0.00402
1433990_at	Lhfpl3	lipoma HMGIC fusion partner-like 3	269629	-1.73	0.001503
1431229_at	Ipw	imprinted gene in the Prader-Willi syndrome region	16353	-1.71	0.0022
1429592_at	Lhfpl3	lipoma HMGIC fusion partner-like 3	269629	-1.7	0.004465

Fig. 8B continued

Probe ID	Gene Symbol	Gene Title	Entrez Gene	log(2)fold change 0 h vs. 8 h	adj.P.Val 0 h vs. 8 h
1457617_at	---	---	---	-1.64	0.008658
1434265_s_at	Ank2	ankyrin 2, brain	109676	-1.64	0.006936
1449634_a_at	Anks1b	ankyrin repeat and sterile alpha motif domain containing 1B	77531	-1.6	0.005666
1455436_at	Diras2	DIRAS family, GTP-binding RAS-like 2	68203	-1.56	0.00813
1457429_s_at	Gm106	gene model 106, (NCBI)	226866	-1.56	0.000118
1425425_a_at	Wif1	Wnt inhibitory factor 1	24117	-1.55	0.000336
1415921_a_at	Tnfrsf19	tumor necrosis factor receptor superfamily, member 19	29820	-1.53	8.15E-05
1457948_at	Gas7	Growth arrest-specific 7-cb protein (Gas7-cb)	14457	-1.53	0.006196
1439954_at	6430514M23Rik	RIKEN cDNA 6430514M23 gene	399595	-1.52	0.006504
1417709_at	Cyp46a1	cytochrome P450, family 46, subfamily a, polypeptide 1	13116	-1.52	0.004364
1445247_at	C530044C16Rik	RIKEN cDNA C530044C16 gene	319981	-1.52	0.000604
1428665_at	Pfn4	profilin family, member 4	382562	-1.51	0.006174
1448147_at	Tnfrsf19	tumor necrosis factor receptor superfamily, member 19	29820	-1.47	2.14E-08
1456335_at	Gm106	gene model 106, (NCBI)	226866	-1.47	0.001787
1440546_at	9630002D21Rik	RIKEN cDNA 9630002D21 gene	319560	-1.44	0.003664
1450506_a_at	Aen	apoptosis enhancing nuclease	68048	-1.43	0.005422
1453041_at	Ano9	anoctamin 9	71345	-1.41	0.000592
1453700_s_at	4933403O03Rik /// EG245263	RIKEN cDNA 4933403O03 gene /// predicted gene, EG245263	245263 /// 74399	-1.41	0.004956

Fig. 8B continued

Probe ID	Gene Symbol	Gene Title	Entrez Gene	log(2)fold change ; adj.P.Val 0 h vs. 8 h	0 h vs. 8 h
1449478_at	Mmp7	matrix metallopeptidase 7	17393	-1.39	0.009569
1452938_at	Anks1b	ankyrin repeat and sterile alpha motif domain containing 1B	77531	-1.37	0.006568
1444541_at	---	---	---	-1.37	0.000828
1450728_at	Fjx1	four jointed box 1 (Drosophila)	14221	-1.36	0.00323
1452728_at	Kirrel3	kin of IRRE like 3 (Drosophila)	67703	-1.36	0.005549
1430118_at	2700046A07Rik	RIKEN cDNA 2700046A07 gene	78449	-1.31	0.008169
1441272_at	Matr3	Matrin 3, mRNA (cDNA clone MGC:28206 IMAGE:3989914)	17184	-1.29	0.002269
1419539_at	Irx4	Iroquois related homeobox 4 (Drosophila)	50916	-1.26	0.005098
1442456_at	Spata5	spermatogenesis associated 5	57815	-1.26	0.006174
1456672_at	---	---	---	-1.24	0.006783
1439026_at	Trpm3	transient receptor potential cation channel, subfamily M, member 3	226025	-1.23	0.006174
1440446_at	---	---	---	-1.21	0.001125
1426139_a_at	Ccrl1	chemokine (C-C motif) receptor-like 1	252837	-1.2	0.009707
1435941_at	Rhbdl3	rhomboid, veinlet-like 3 (Drosophila)	246104	-1.18	0.004465
1453645_at	2700046A07Rik	RIKEN cDNA 2700046A07 gene	78449	-1.18	0.000692
1444905_at	D1Ertd705e	DNA segment, Chr 1, ERATO Doi 705, expressed	51940	-1.17	0.003297
1422733_at	Fjx1	four jointed box 1 (Drosophila)	14221	-1.14	0.007138
1423957_at	Aen	apoptosis enhancing nuclease	68048	-1.07	0.000391

Fig. 8B continued

Probe ID	Gene Symbol	Gene Title	Entrez Gene	log(2)fold change 0 h vs. 8 h	adj.P.Val 0 h vs. 8 h
1433084_at	4930402C16Rik	RIKEN cDNA 4930402C16 gene	73812	-1.07	0.006802
1421498_a_at	2010204K13Rik	RIKEN cDNA 2010204K13 gene	68355	-1.07	0.007469
1426981_at	Pcsk6	proprotein convertase subtilisin/kexin type 6	18553	-1.06	0.000506
1446846_at	---	---	---	-1.05	0.004364
1429861_at	Pcdh9	protocadherin 9	211712	-1.04	0.006174
1447958_at	---	---	---	-1.02	0.000592
1435608_at	LOC631806 /// Znrf3	similar to Goliath homolog precursor (Ring finger protein 130) (R-goliath) /// zinc and ring finger 3	407821 /// 631806	-1.02	0.000118
1456266_at	---	---	---	-1.02	0.002282
1421341_at	Axin2	axin2	12006	-1.02	0.001447
1418495_at	Zc3h8	zinc finger CCCH type containing 8	57432	-1.01	0.000233

Fig. 9A

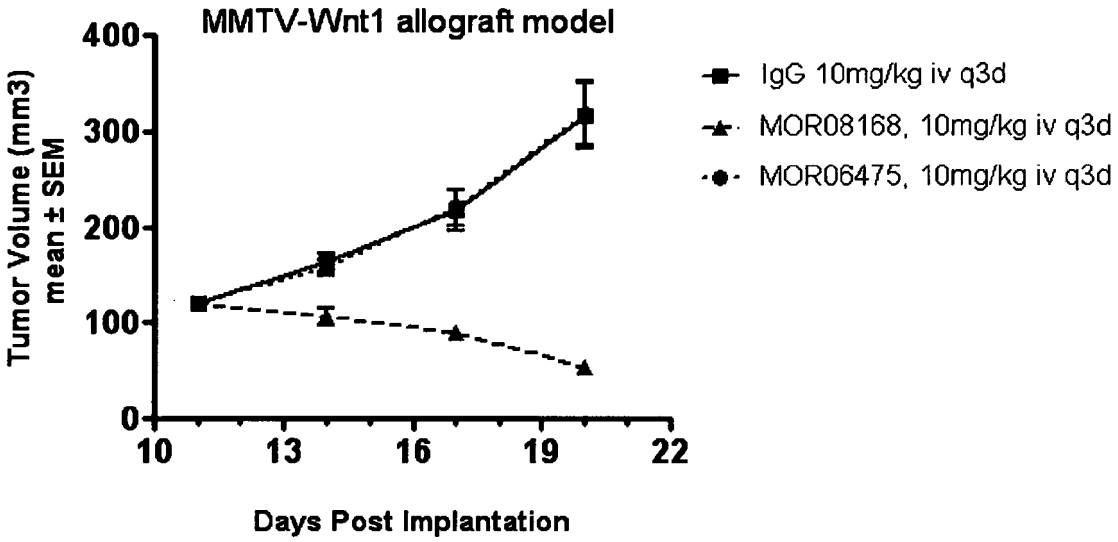

MMTV-Wnt1 allograft model
Tumor Volume (mm3)
mean ± SEM
400
300
200
100
0
10
13
16
19
22
Days Post Implantation
IgG 10mg/kg iv q3d
MOR08168, 10mg/kg iv q3d
MOR06475, 10mg/kg iv q3d

Fig. 9B

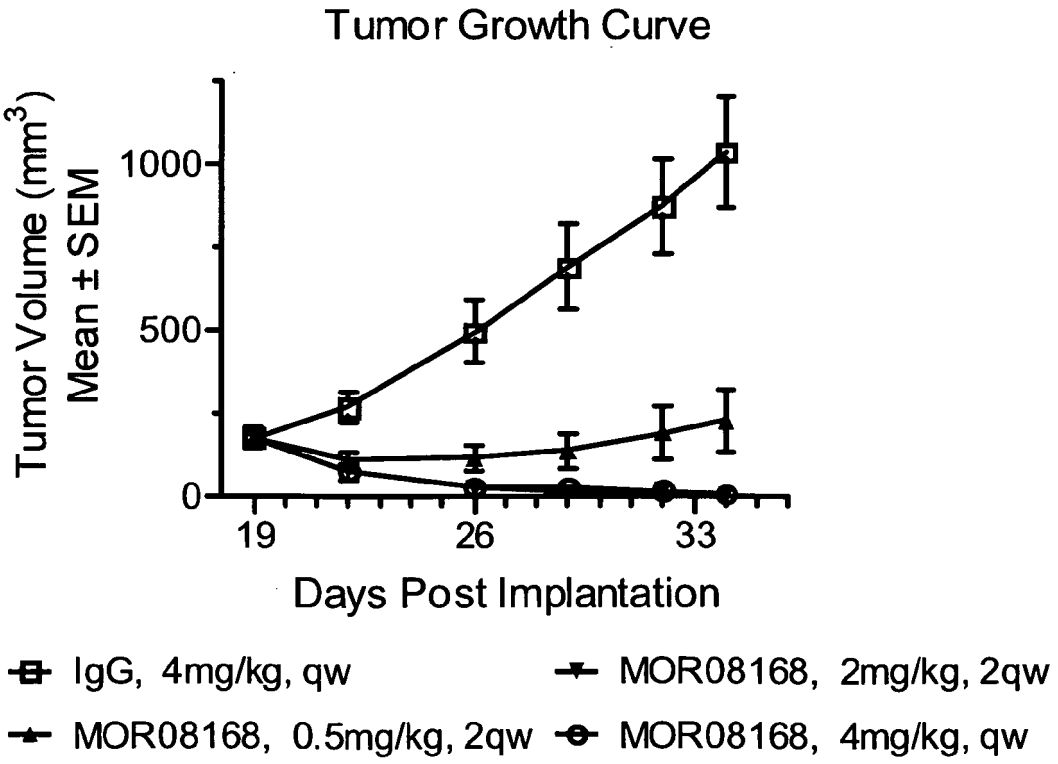

Tumor Growth Curve
Tumor Volume (mm3)
Mean ± SEM
1000
500
0
19
26
33
Days Post Implantation
IgG, 4mg/kg, qw
MOR08168, 2mg/kg, 2qw
MOR08168, 0.5mg/kg, 2qw
MOR08168, 4mg/kg, qw

Fig. 10

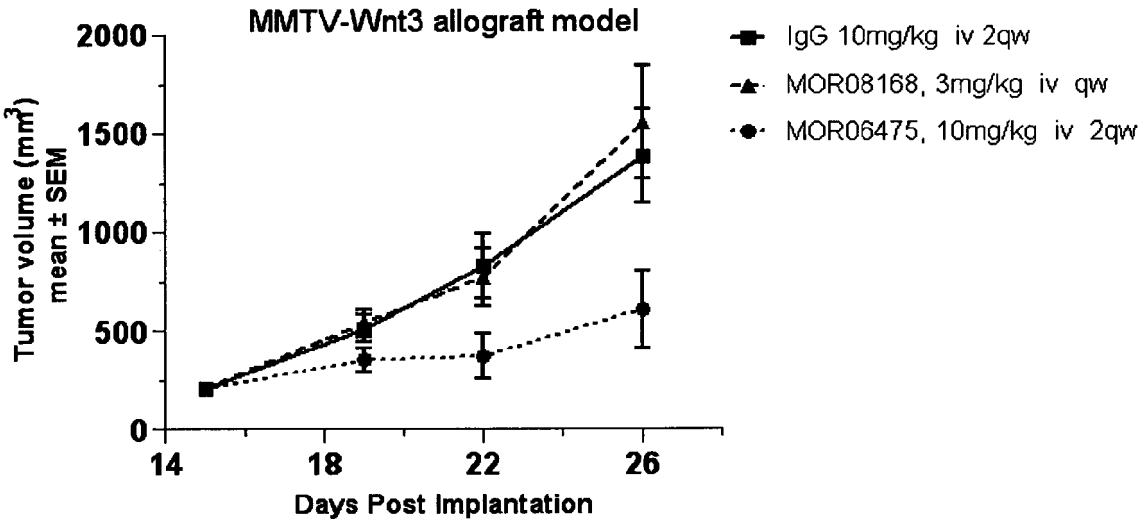

MMTV-Wnt3 allograft model
Tumor volume (mm3) mean ± SEM
2000
1500
1000
500
0
14
18
22
26
Days Post Implantation
IgG 10mg/kg iv 2qw
MOR08168, 3mg/kg iv qw
MOR06475, 10mg/kg iv 2qw

Fig. 11

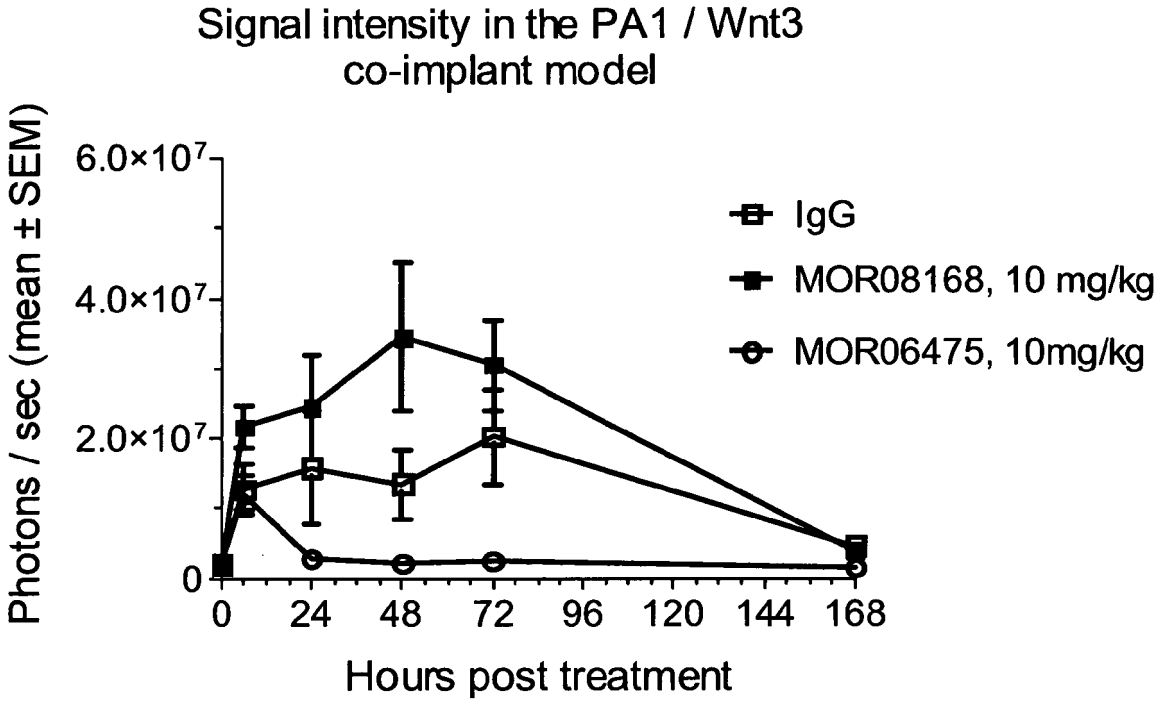

Signal intensity in the PA1 / Wnt3 co-implant model
Photons / sec (mean ± SEM)
6.0×10⁷
4.0×10⁷
2.0×10⁷
0
0
24
48
72
96
120
144
168
Hours post treatment
IgG
MOR08168, 10 mg/kg
MOR06475, 10mg/kg

Fig. 12A

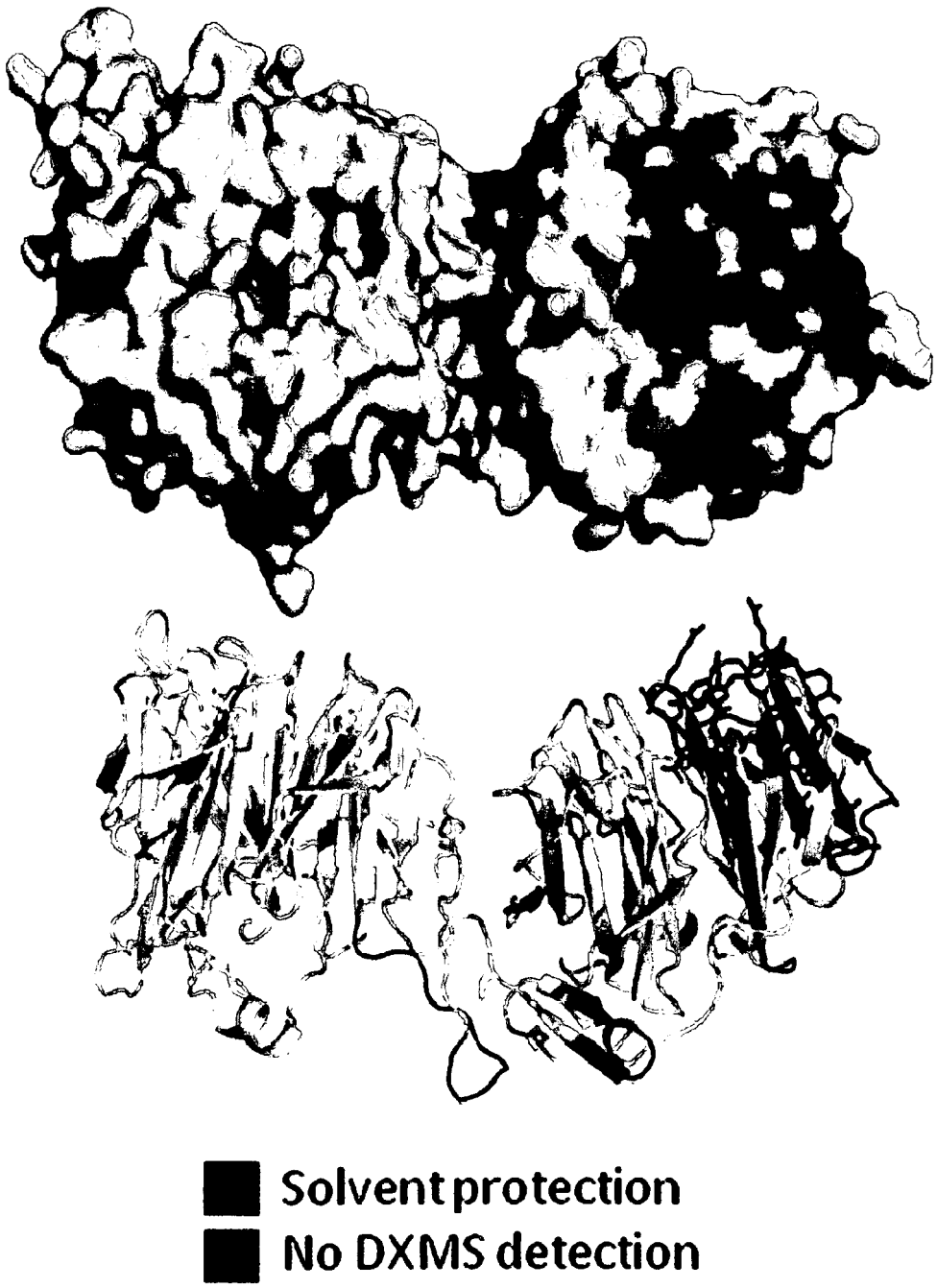


Fig. 12B

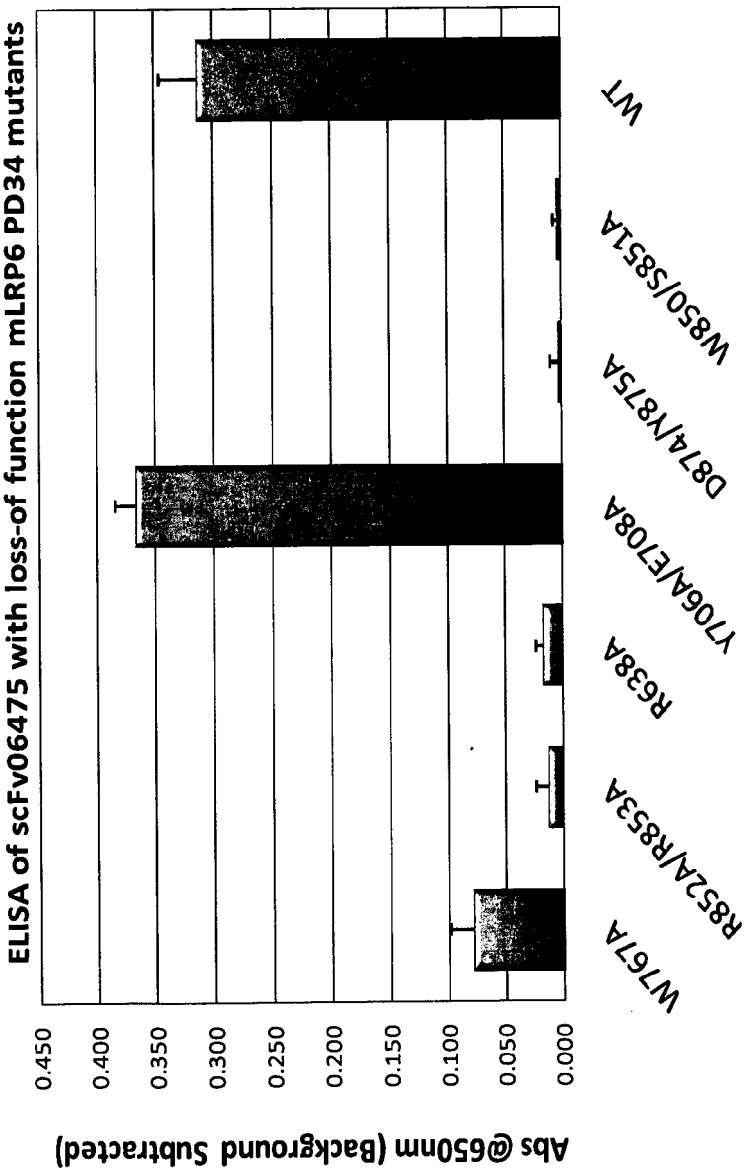
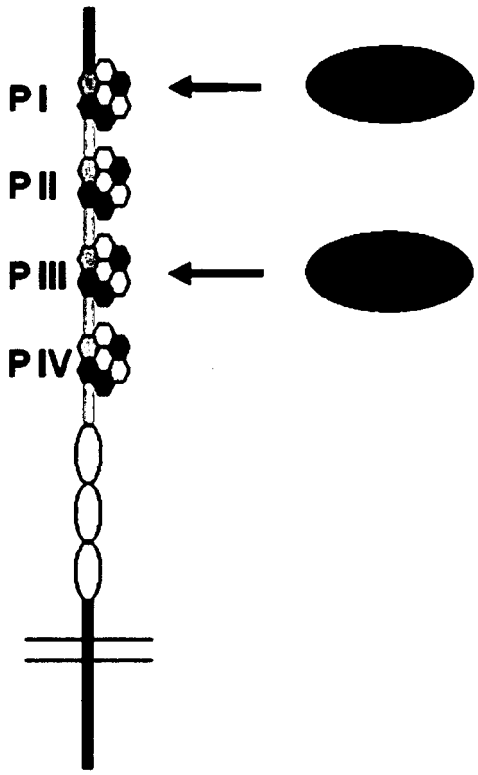

ELISA of scFv06475 with loss-of function mLRP6 PD34 mutants
Abs @650nm (Background Subtracted)
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
WT
W850/S851A
D874/W875A
Y706A/E708A
R638A
R852A/R853A
W767A

Fig. 13



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Fig. 14

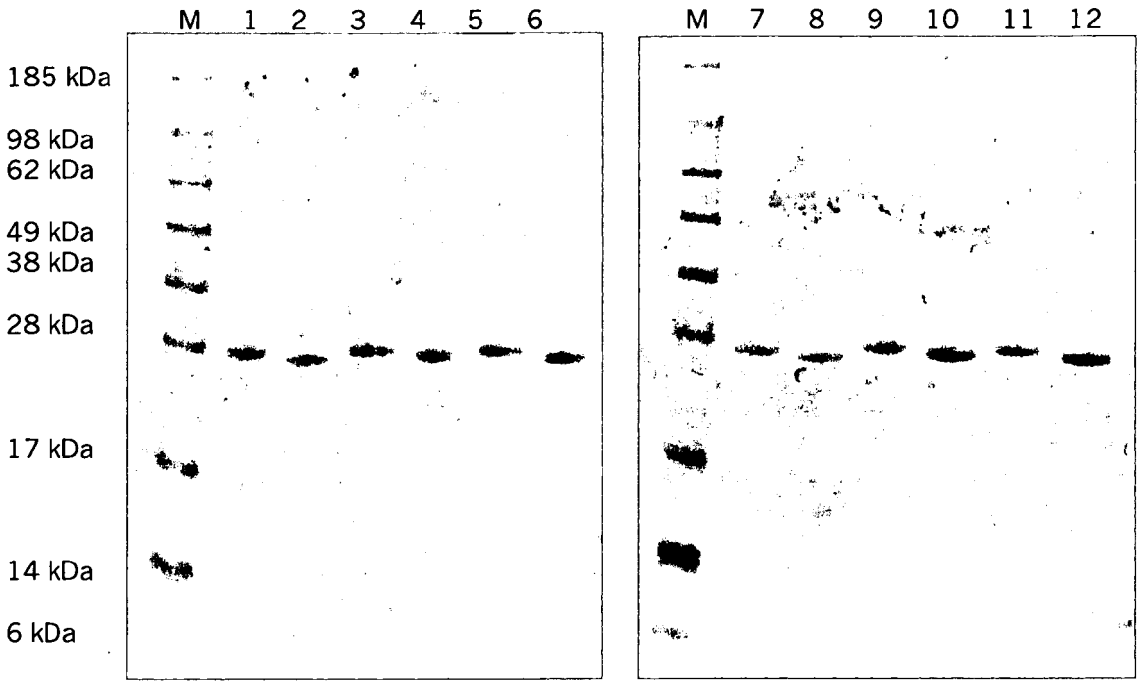

M 1 2 3 4 5 6
M 7 8 9 10 11 12
185 kDa
98 kDa
62 kDa
49 kDa
38 kDa
28 kDa
17 kDa
14 kDa
6 kDa

Fig. 15

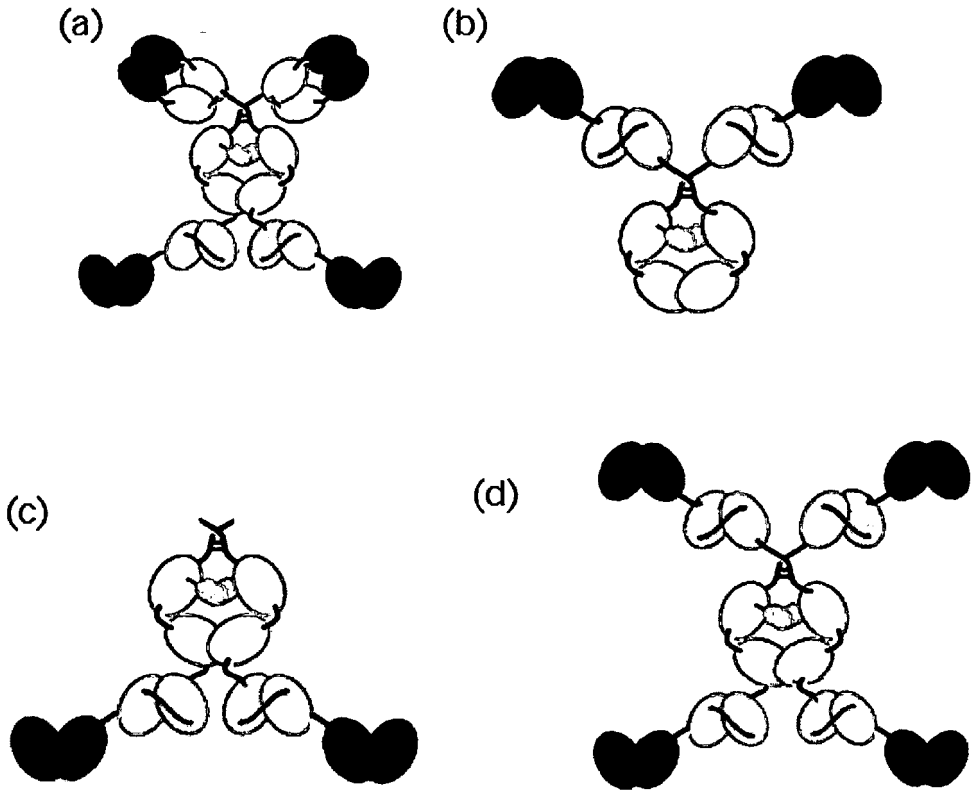


Fig. 16

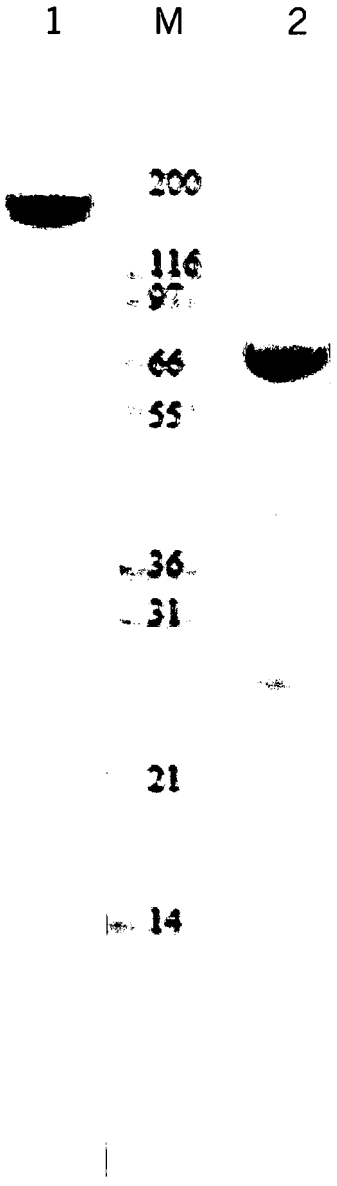


Fig. 17

Wnt1-STF

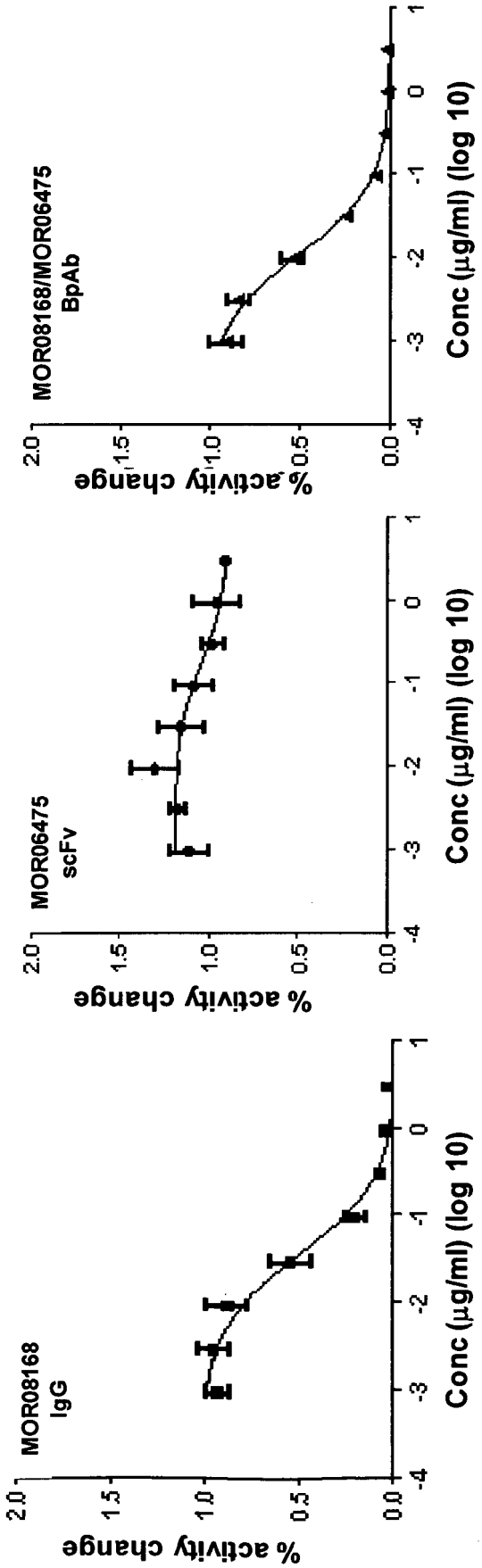


Fig. 17 continued

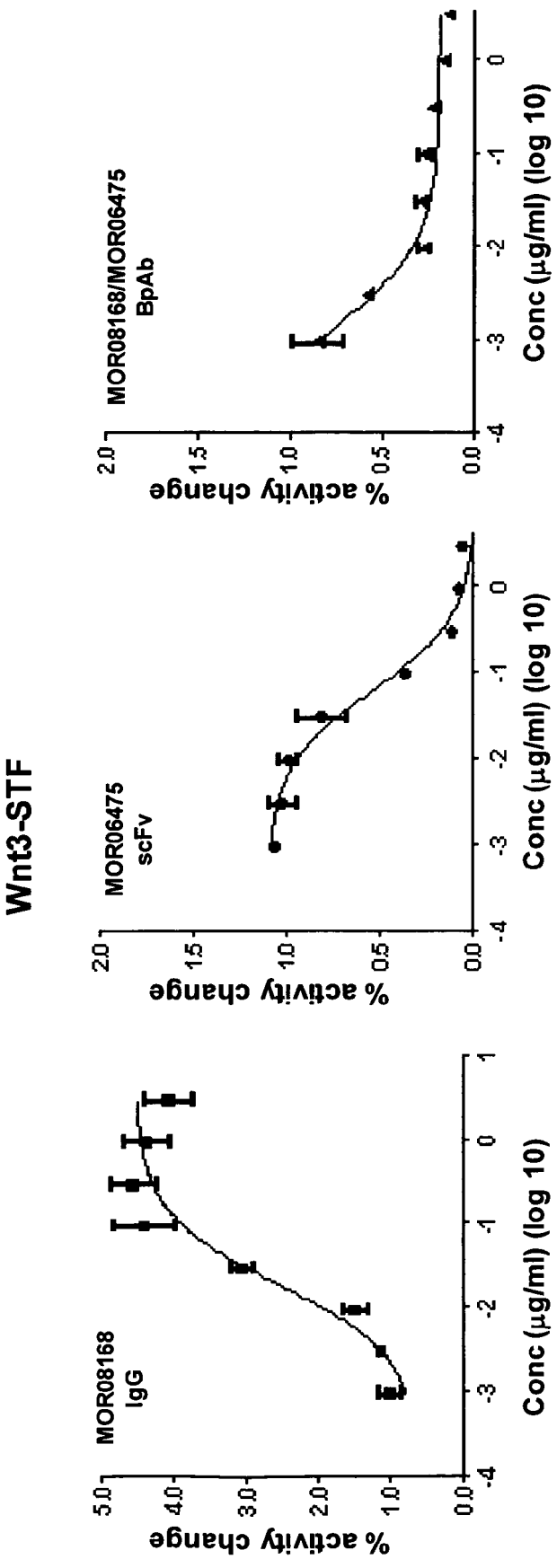


Fig. 18

	HEK STF IC50 (µg/ml) WNT1	WNT3a
MOR06475-sc-fv-Vl-Vh	n/a	0.07
MOR06475-sc-fv-Vh-Vl	n/a	0.06
MOR06475-sc-fv-Vl-Vh-(GGGGS)3	n/a	0.08
MOR06475-sc-fv-Vh-Vl-(GGGGS)3	n/a	0.08
MOR06475-Fab	n/a	0.11
MOR08168-sc-fv-Vl-Vh	0.05	n/a
MOR08168-sc-fv-Vh-Vl	0.06	n/a
MOR08168-sc-fv-Vl-Vh-(GGGGS)3	0.07	n/a
MOR08168-sc-fv-Vh-Vl-(GGGGS)3	0.1	n/a
MOR08168-Fab	0.15	n/a
MOR08545-sc-fv-Vl-Vh	0.53	n/a
MOR08545-sc-fv-Vh-Vl	0.06	n/a
MOR08545-sc-fv-Vl-Vh-(GGGGS)3	n/a	n/a
MOR08545-sc-fv-Vh-Vl-(GGGGS)3	0.05	n/a
MOR08545-Fab	0.05	n/a
anti-LRP6_MOR08168_hIgG1 LALA_6475scfv_at_VL	0.013	0.0071
anti-LRP6_MOR06475_hIgG1 LALA_8168scfvatCH3_(VH-3-VL)	0.013	0.0082
anti-LRP6_MOR06475_hIgG1 LALA_8168scfvatCH3_(VH-4-VL)	0.016	0.0094
anti-LRP6_MOR08168_hIgG1 LALA_6475scfvatCH3_opt_DPtoDA	0.02	0.0076
anti-LRP6_MOR08168_hIgG1 LALA_6475scfvatCH3_opt_DPtoTA	0.026	0.0098
anti-LRP6_MOR08168_hIgG1 LALA_6475scfvatCH3_opt_w/o-K	0.015	0.0064
MOR06475 IgG	n/a	0.0039
MOR08168 IgG	0.025	n/a
MOR08168hIgG1LALA 6475 scfv	0.016	0.011

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Fig. 19

Antibody	Kd [nM] binding of Ab's to FcRn			
FcRn	**huFcRn, pH6.0**	**huFcRn, pH7.4**	**cynoFcRn, pH6.0**	**cynoFcRn, pH7.4**
MOR08168	0.023	LLB	0.28	LLB
MOR08168/6475 BpAB	0.021	LLB	0.12	LLB

LLB: low level binding

Fig. 20

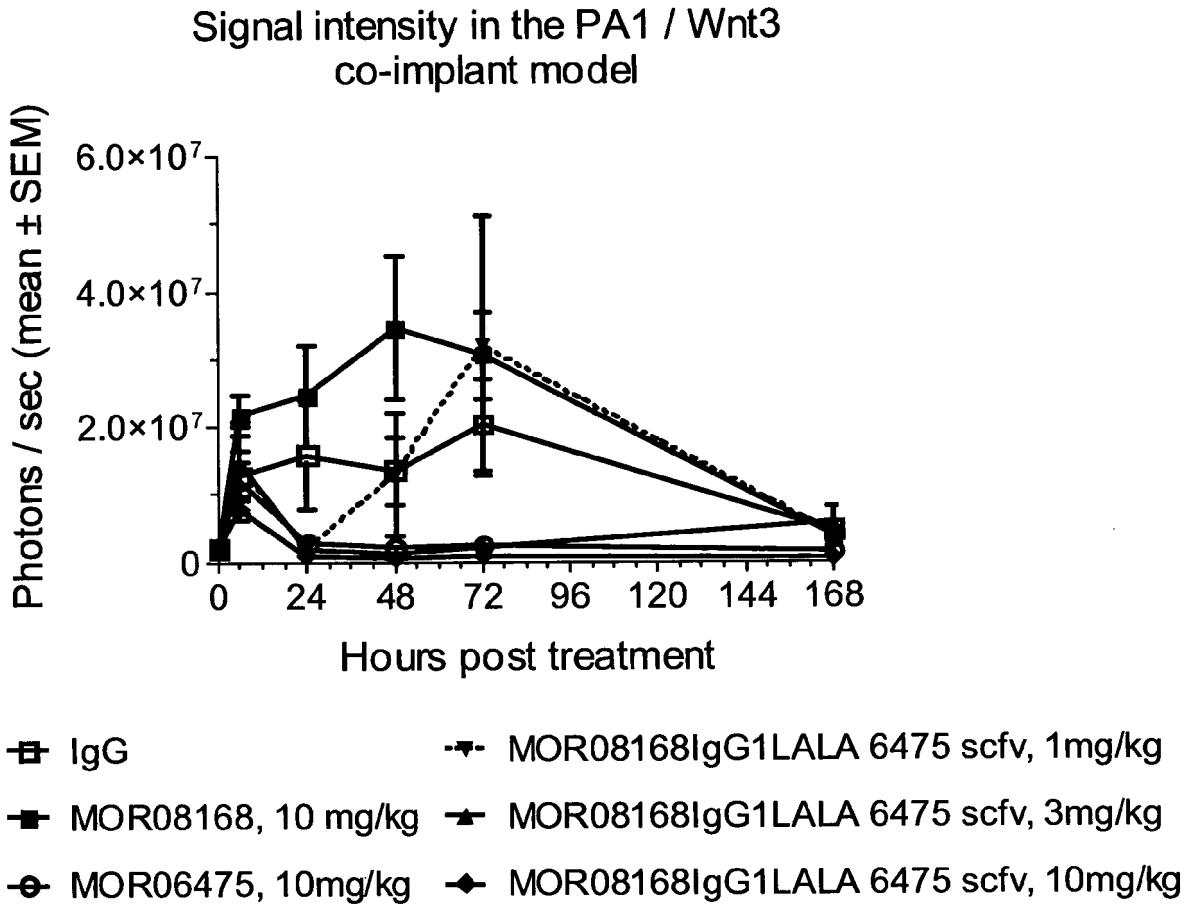

Signal intensity in the PA1 / Wnt3 co-implant model
Photons / sec (mean ± SEM)
6.0×10⁷
4.0×10⁷
2.0×10⁷
0
0
24
48
72
96
120
144
168
Hours post treatment
IgG
MOR08168, 10 mg/kg
MOR06475, 10mg/kg
MOR08168IgG1LALA 6475 scfv, 1mg/kg
MOR08168IgG1LALA 6475 scfv, 3mg/kg
MOR08168IgG1LALA 6475 scfv, 10mg/kg

Fig. 21

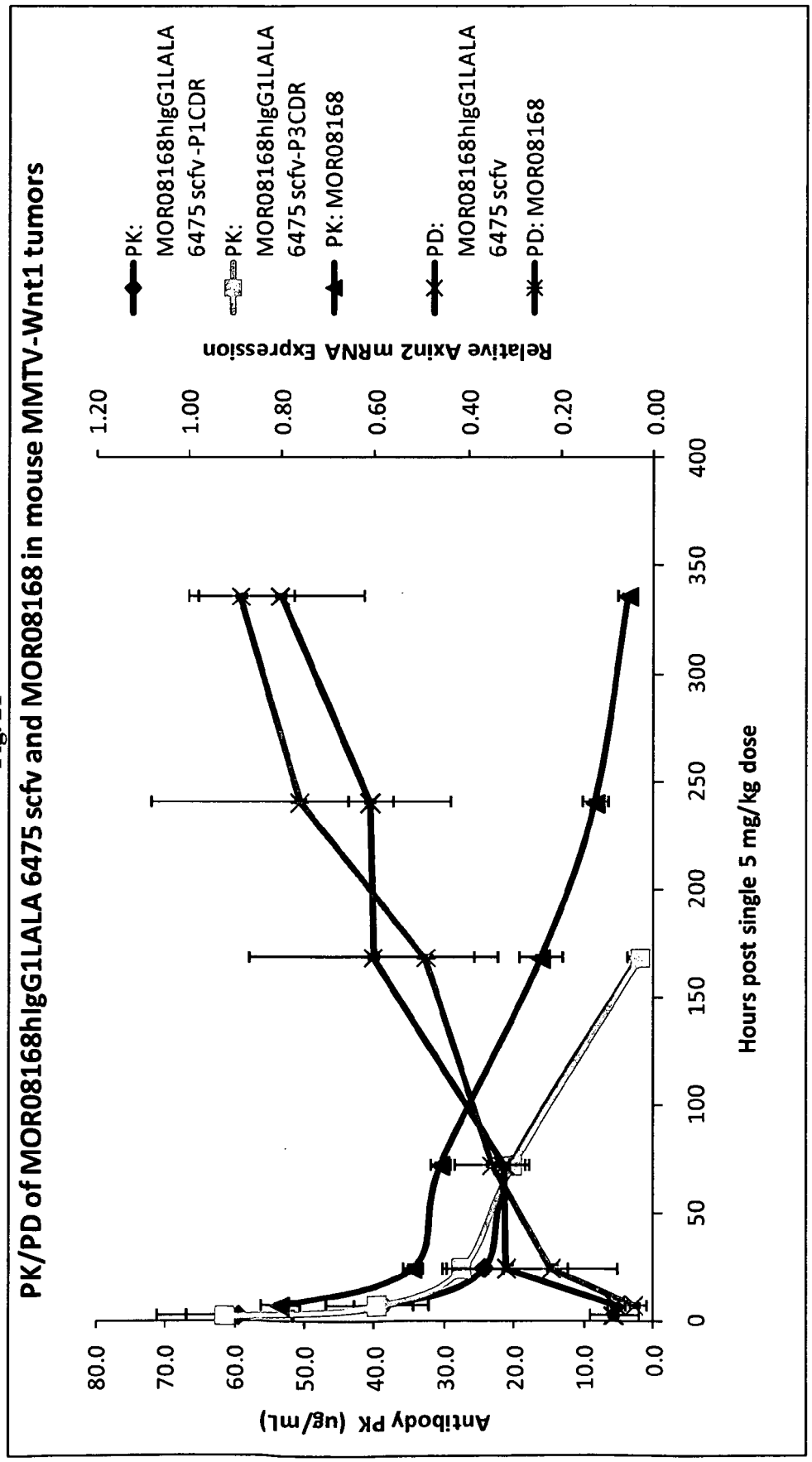

PK/PD of MOR08168hIgG1LALA 6475 scfv and MOR08168 in mouse MMTV-Wnt1 tumors
PK: MOR08168hIgG1LALA 6475 scfv -P1CDR
PK: MOR08168hIgG1LALA 6475 scfv-P3CDR
PK: MOR08168
PD: MOR08168hIgG1LALA 6475 scfv
PD: MOR08168
Antibody PK (ug/mL)
Relative Axin2 mRNA Expression
Hours post single 5 mg/kg dose
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
0.00
0.20
0.40
0.60
0.80
1.00
1.20
0
50
100
150
200
250
300
350
400

Fig. 22

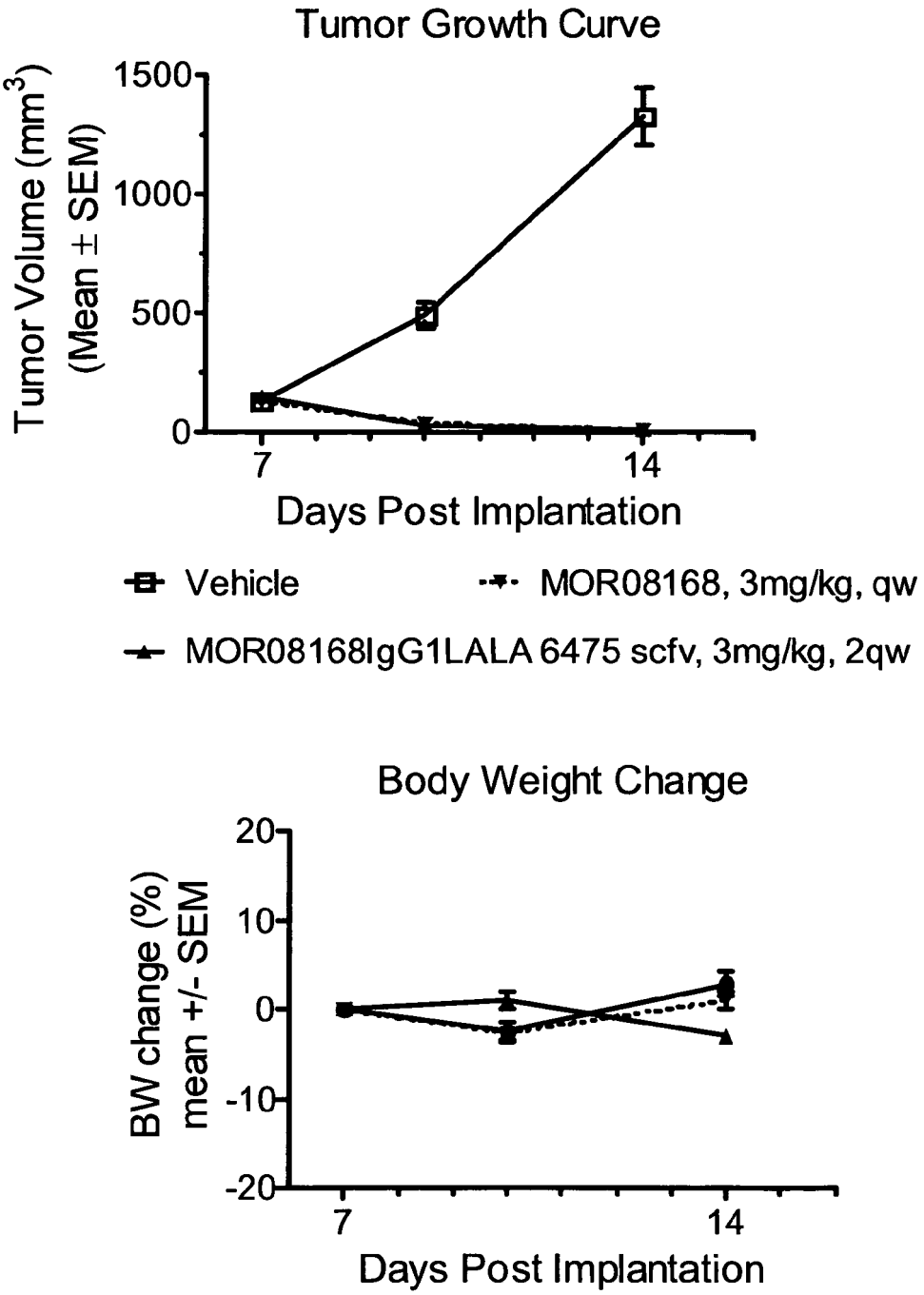

Tumor Growth Curve
Tumor Volume (mm3)
(Mean ± SEM)
1500
1000
500
0
7
14
Days Post Implantation
Vehicle
MOR08168, 3mg/kg, qw
MOR08168IgG1LALA 6475 scfv, 3mg/kg, 2qw
Body Weight Change
BW change (%)
mean +/- SEM
20
10
0
-10
-20
7
14
Days Post Implantation

Fig. 23

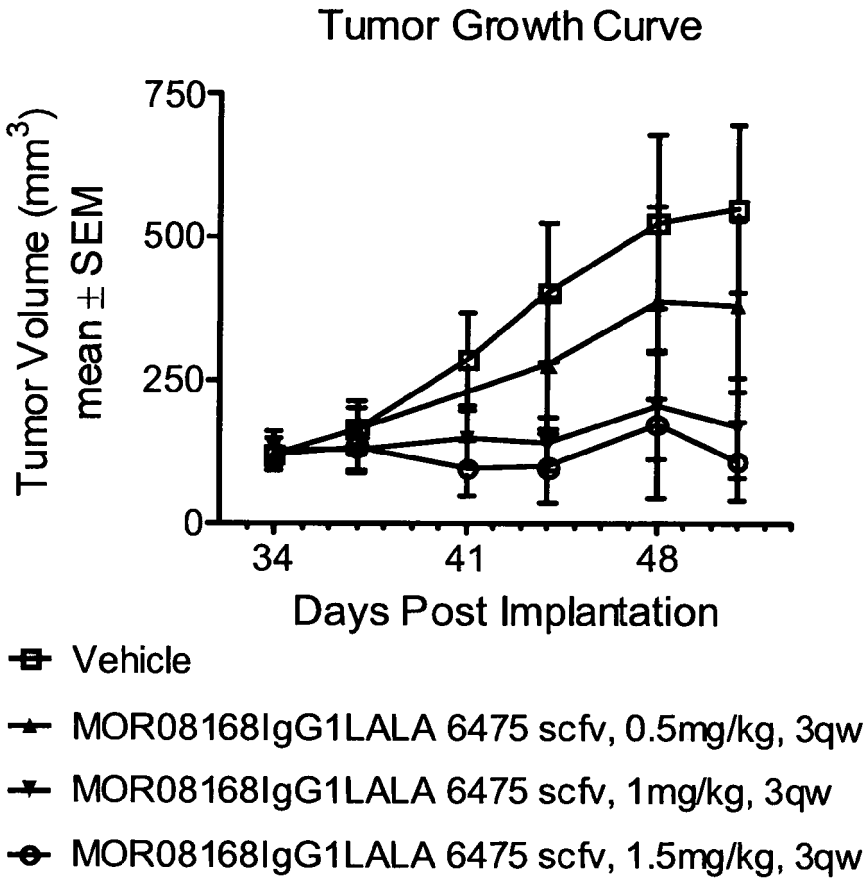

Tumor Growth Curve
Tumor Volume (mm3) mean ± SEM
750
500
250
0
34
41
48
Days Post Implantation
Vehicle
MOR08168IgG1LALA 6475 scfv, 0.5mg/kg, 3qw
MOR08168IgG1LALA 6475 scfv, 1mg/kg, 3qw
MOR08168IgG1LALA 6475 scfv, 1.5mg/kg, 3qw

Fig. 24A

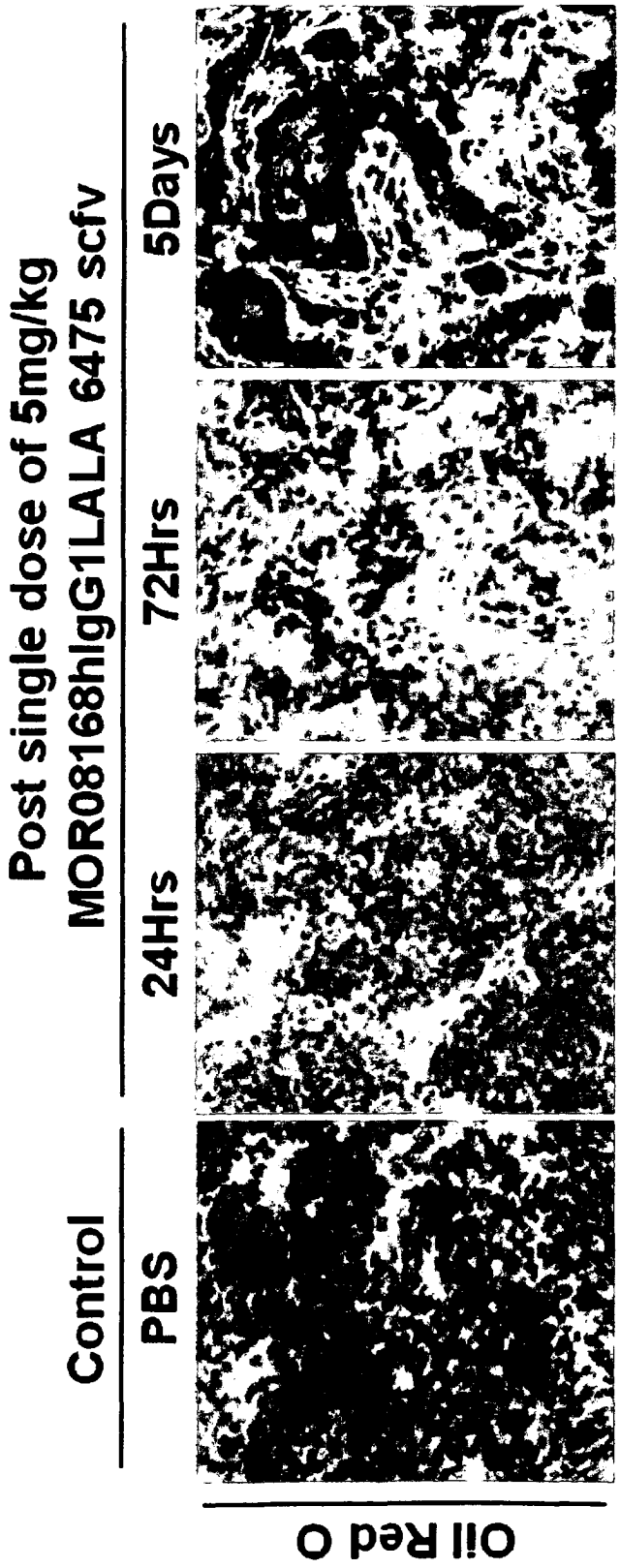

Post single dose of 5mg/kg
MOR08168hIgG1LALA 6475 scfv
Control
PBS
24Hrs
72Hrs
5Days
Oil Red O

Fig. 24B

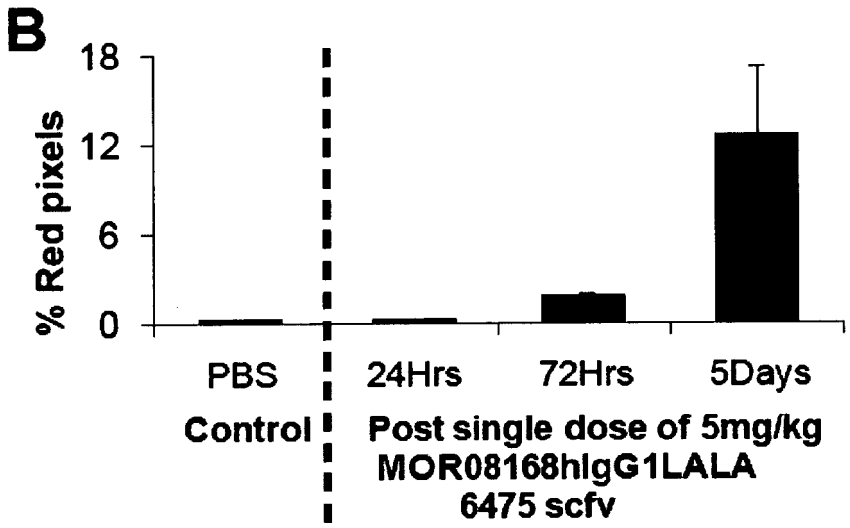
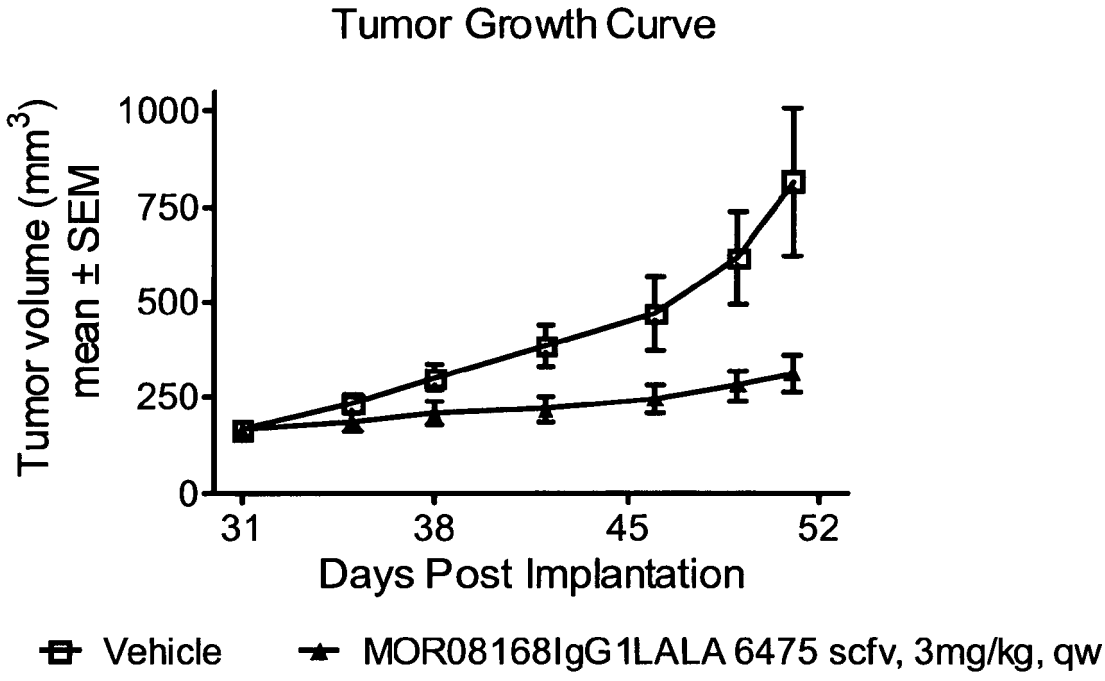

B
18
12
6
0
% Red pixels
PBS
24Hrs
72Hrs
5Days
Control
Post single dose of 5mg/kg
MOR08168hIgG1LALA
6475 scfv

Fig. 25


Tumor Growth Curve
Tumor volume (mm3) mean ± SEM
1000
750
500
250
0
31
38
45
52
Days Post Implantation
Vehicle
MOR08168IgG1LALA 6475 scfv, 3mg/kg, qw

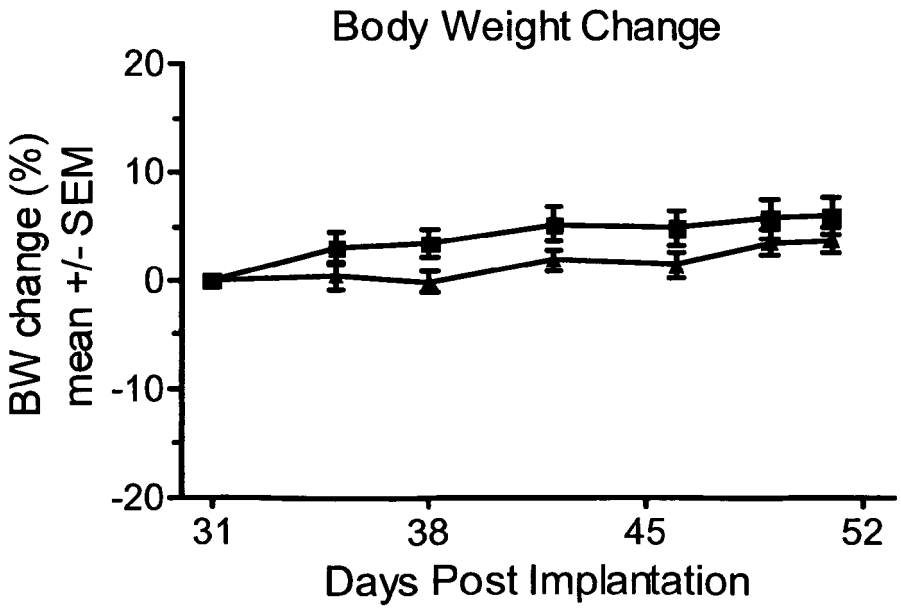

Body Weight Change
BW change (%) mean +/- SEM
20
10
0
-10
-20
31
38
45
52
Days Post Implantation

Fig. 26A

Summary of KD measurement of MOR08168 IgG LALA 6475scFv, MOR08168 and MOR06475 binding to PD1/2 and PD3/4

Binding to PD1/2	Ka (1/Ms)	Kd (1/s)	KD (M)	N
MOR08168 IgG LALA 6475scFV	1.27E+05	6.10E-05	8.68E-10	3
MOR08168	8.72E+04	9.05E-05	1.08E-09	4
Binding to PD3/4	Ka (1/Ms)	Kd (1/s)	KD (M)	N
MOR08168 IgG LALA 6475scFV	2.11E+05	3.14E-04	1.93E-09	3
MOR06475	2.01E+05	2.17E-04	1.13E-09	4

Fig. 26B

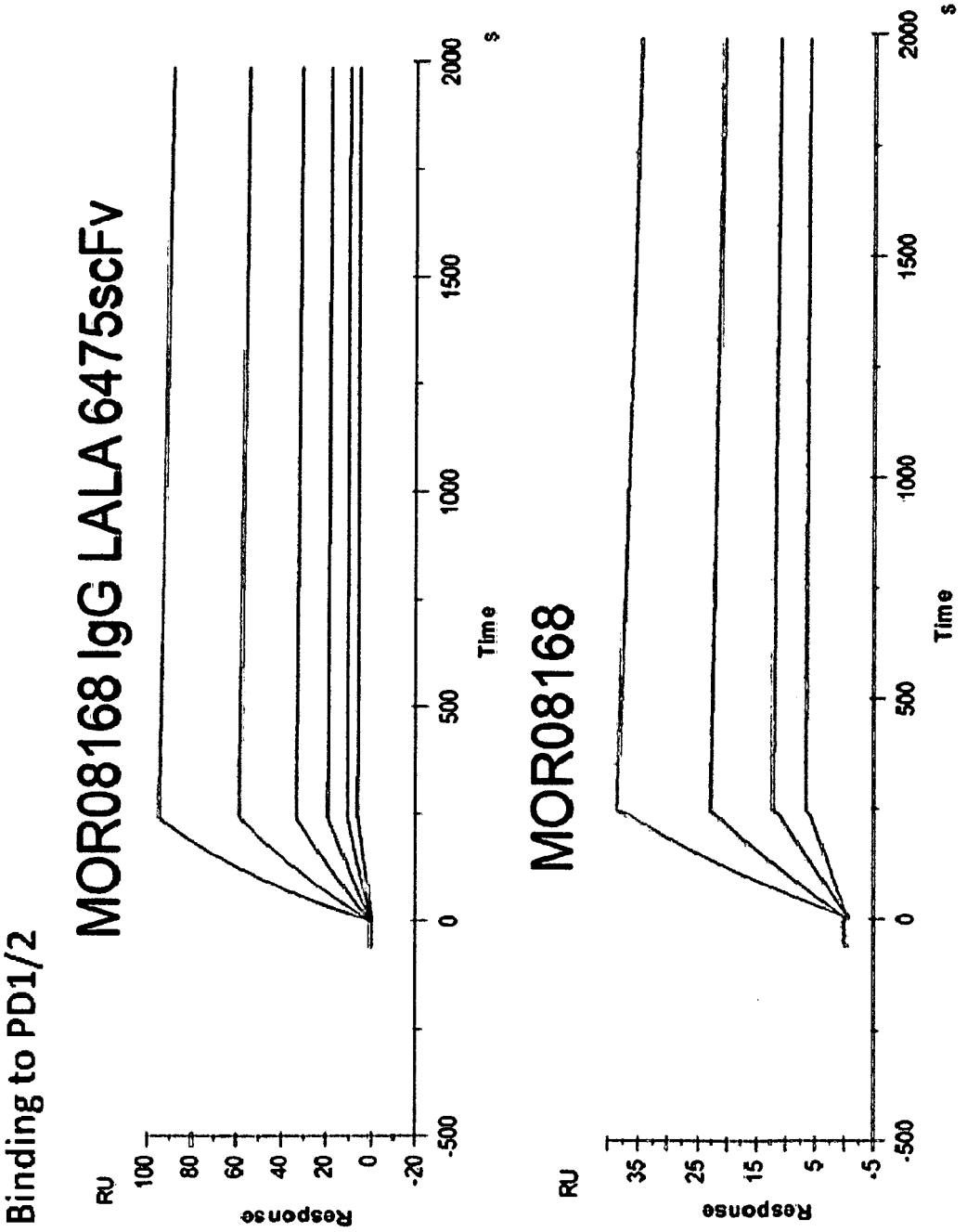

Binding to PD1/2
MOR08168 IgG LALA 6475scFv
RU
Response
100
80
60
40
20
0
-20
-500
0
500
1000
1500
2000
s
Time
MOR08168
RU
Response
35
25
15
5
-5
-500
0
500
1000
1500
2000
s
Time

Fig. 26B continued

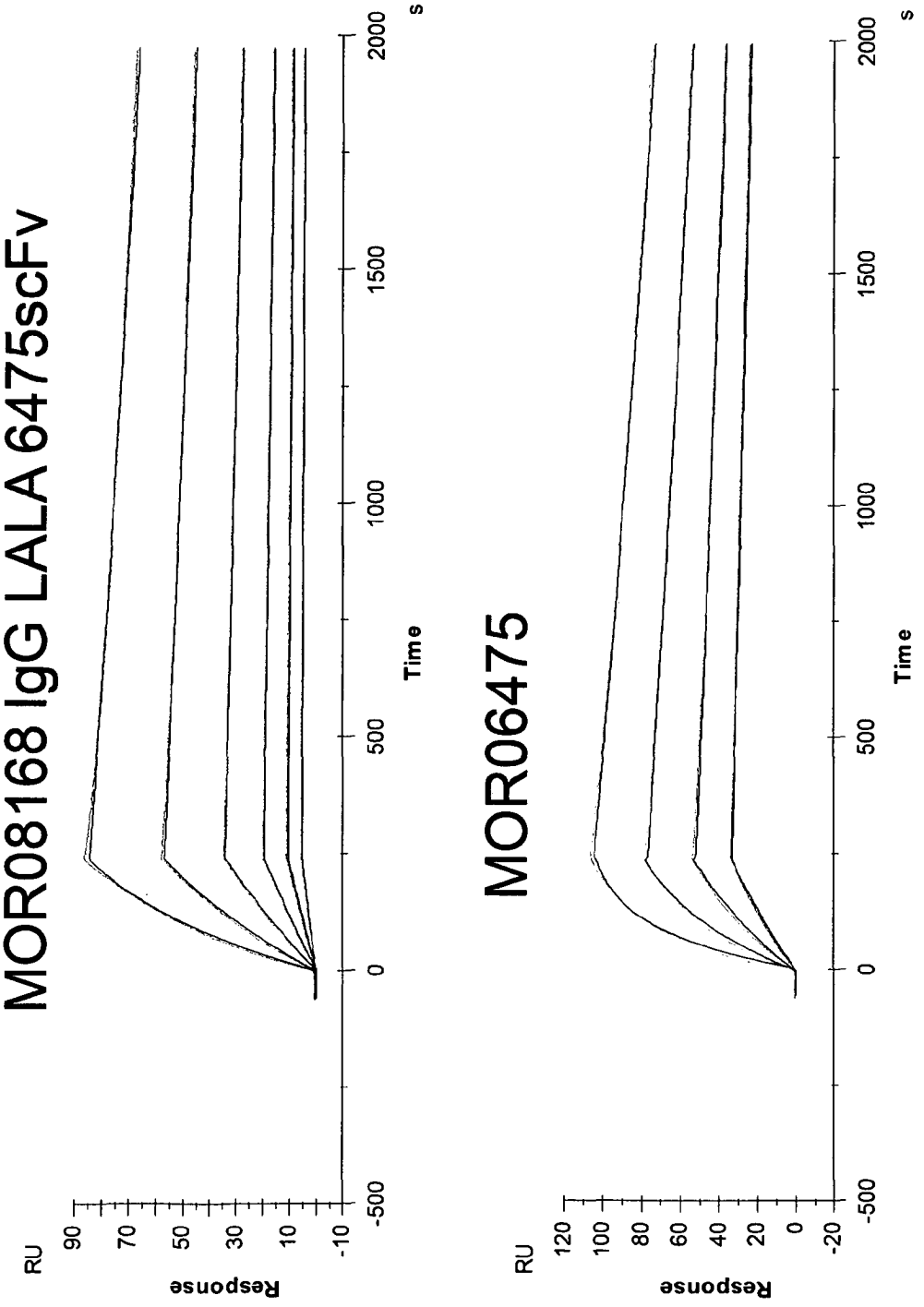


Fig. 26C

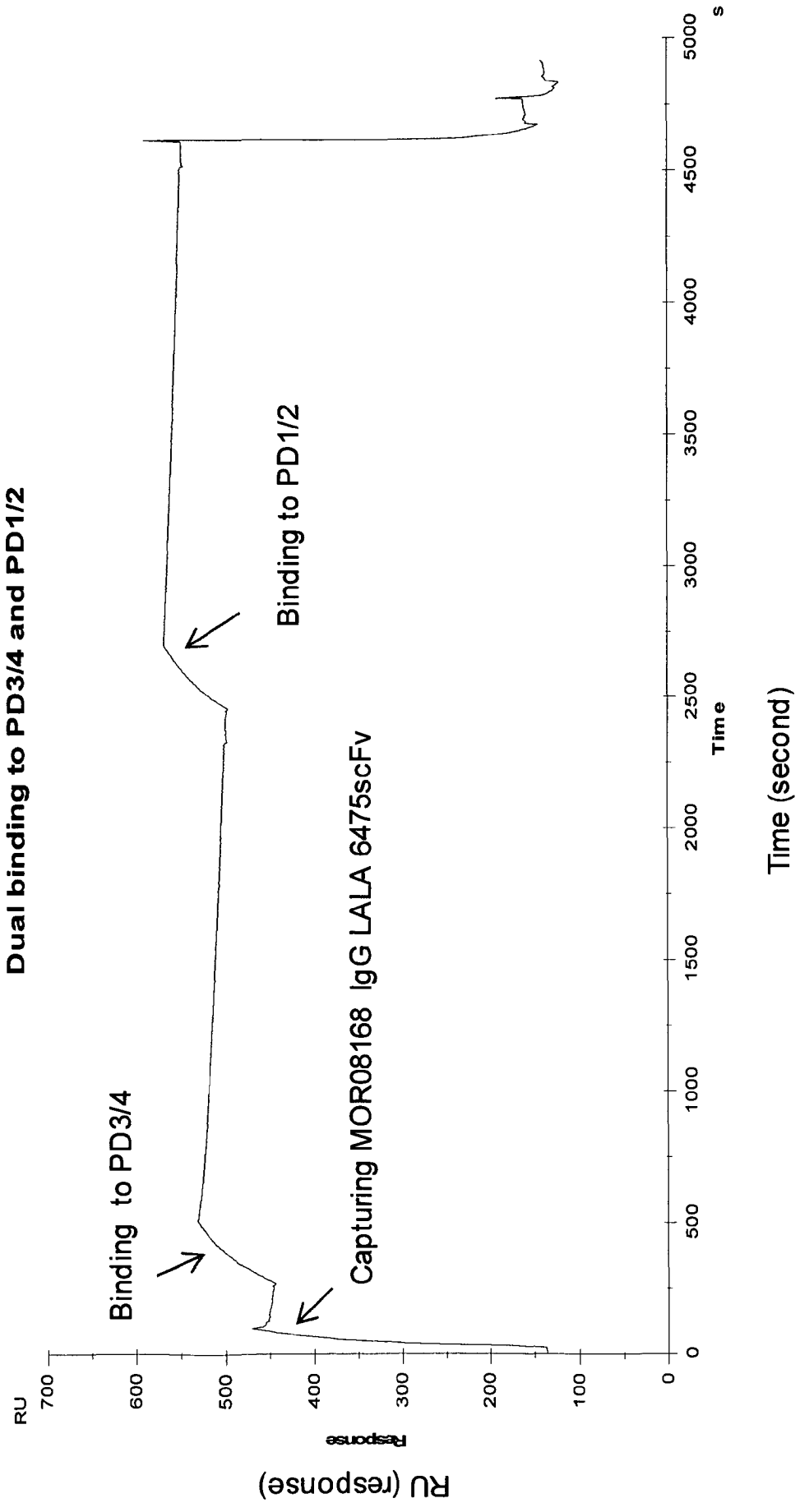

Dual binding to PD3/4 and PD1/2
Binding to PD3/4
Capturing MOR08168 IgG LALA 6475scFv
Binding to PD1/2
RU
Response
RU (response)
Time
s
Time (second)
0
100
200
300
400
500
600
700
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000

Fig. 27

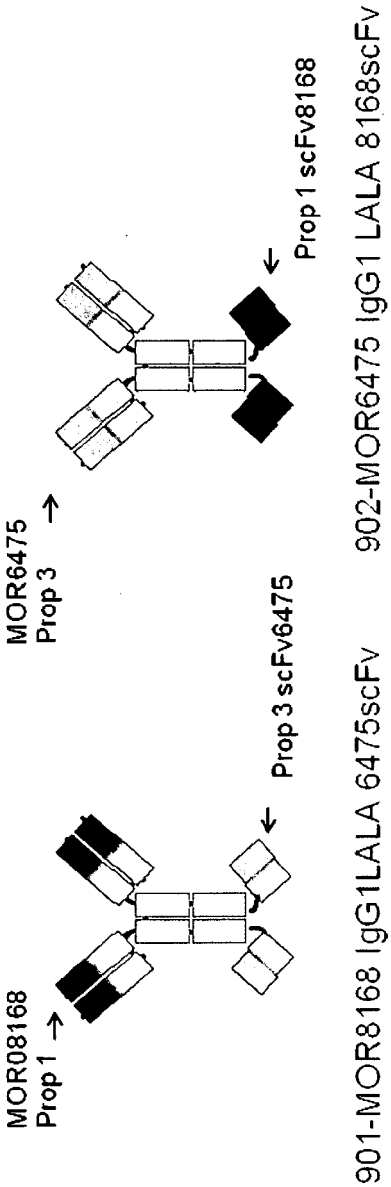

MOR6475
Prop 3
Prop 1 scFv8168
902-MOR6475 IgG1 LALA 8168scFv
MOR08168
Prop 1
Prop 3 scFv6475
901-MOR8168 IgG1LALA 6475scFv

Fig. 28

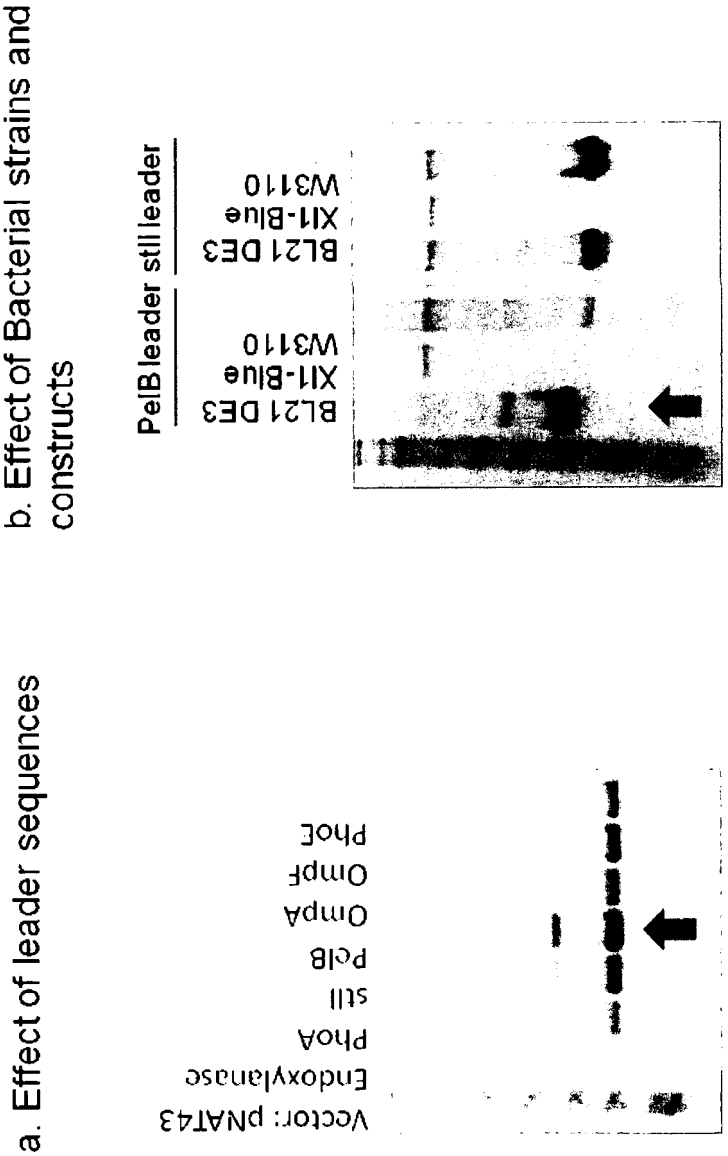

a. Effect of leader sequences
Vector: pNAT43
Endoxylanase
PhoA
stII
PelB
OmpA
OmpF
PhoE
b. Effect of Bacterial strains and constructs
PelB leader stII leader
BL21 DE3
XI1-Blue
W3110
BL21 DE3
XI1-Blue
W3110

Fig. 29

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. 30

Tm of single point mutations in scFv08168

Construct ID	mutation	T_m on protein from E.coli'
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. 31

Tm of single vs double mutations in scFv08168

Construct ID	Mutation	T_m on protein from E.coli[1]	T_m on protein from mammalian[1]	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		

[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. 31 continued

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.

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Fig. 32

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	-

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Fig. 33

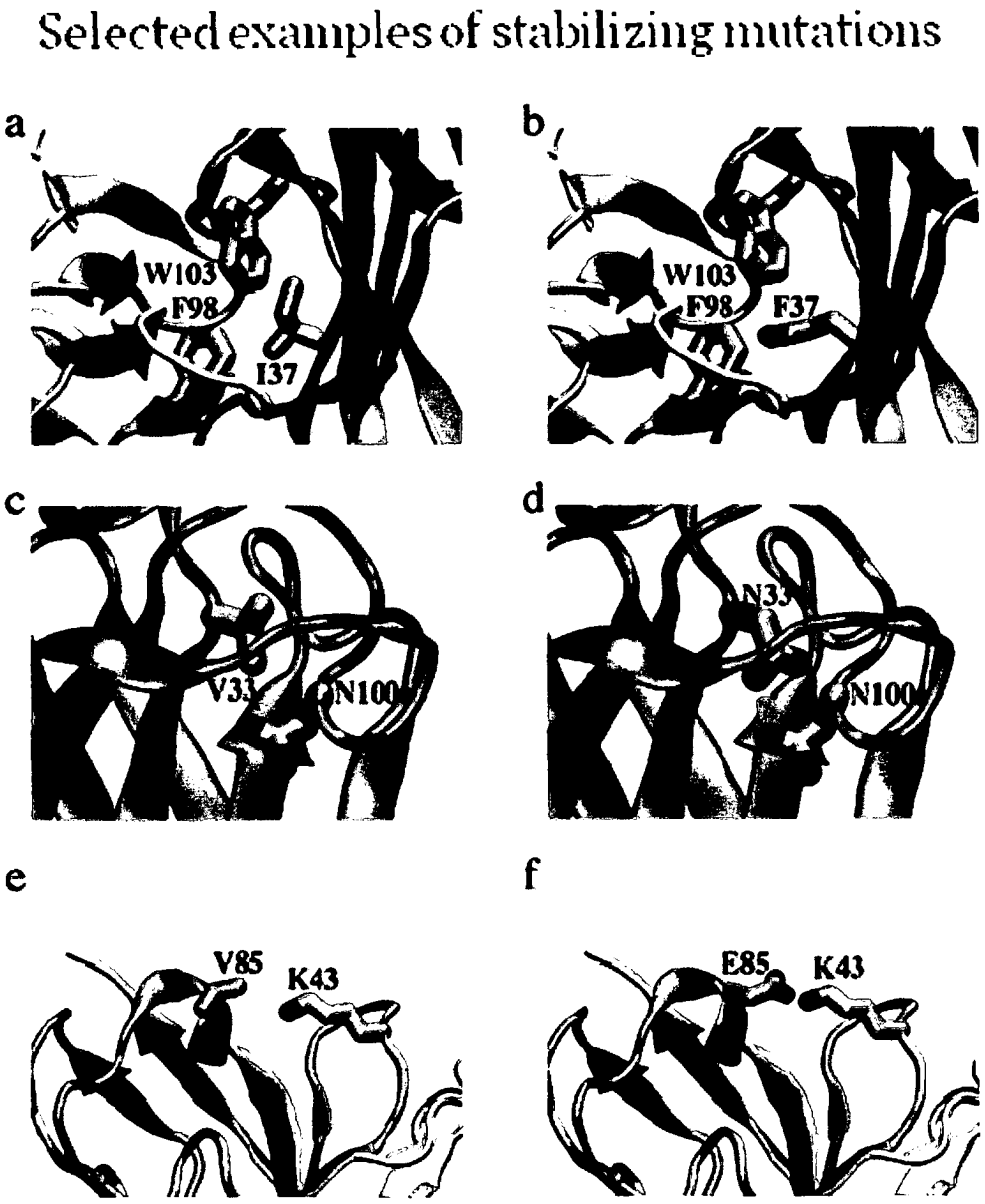

Selected examples of stabilizing mutations
a
W103
F98
I37
b
W103
F98
F37
c
V33
N100
d
N33
N100
e
V85
K43
f
E85
K43

Fig. 34

Thermostability measurement of HSA fusion and IgG based biparatopic molecules		
	T_m by DSF (ProteoSTAT)	T_m by DSC
MOR6475 IgG1-scFv8168 (902wt)	47, 72.5	ND
MOR6475 IgG1-scFv8168 (902 mutant)	62, 76	ND

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