

Title

**Signaling Inhibitors Accelerate the Conversion of mouse iPS Cells into
Cancer Stem Cells in the Tumor Microenvironment**

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Results of five standard experiments without inhibitors											
Exp.	No. 1		No. 2		No. 3		No. 4		No. 5		Overall
	<i>GFPi</i> ^{*1} <i>X10</i> ⁻³	RFi ^{*2}	<i>GFPi</i> <i>X10</i> ⁻³	RFi	<i>GFPi</i> <i>X10</i> ⁻³	RFi	<i>GFPi</i> <i>X10</i> ⁻³	RFi	<i>GFPi</i> <i>X10</i> ⁻³	RFi	
	6.58	0.89	8.43	1.01	6.59	0.85	9.03	1.22	9.08	1.24	
	6.97	0.94	13.88	1.66	10.16	1.31	7.03	0.95	10.3	1.41	
	8.24	1.12	6.39	0.77	8.05	1.04	5.63	0.76	6.16	0.84	
	9.53	1.29	5.71	0.68	5.76	0.74	7.08	0.96	7.38	1.01	
	5.7	0.77	7.41	0.89	10.12	1.3	8.61	1.17	5.79	0.79	
	7.27	0.98	8.33	1	6	0.77	6.92	0.94	5.21	0.71	
average ^{*3}	7.38	1	8.36	1	7.78	1	7.38	1	7.32	1	1
error	1.34	0.18	2.91	0.35	1.99	0.26	1.24	0.17	2	0.27	0.24 ^{*4}

GFP fluorescence intensity (*GFPi*^{*1}) depicted the read from each well of miPSCs present in CM and the average of the reads were calculated for each experiment. Then the relative fluorescence intensity (RFi^{*2}) was depicted in the table after division by each average value^{*3}. From the table above the thresholds to distinguish the results were determined as 1.00 ± 0.24 ^{*4} to show the following: positive > 1.24; $0.76 \leq$ no significant difference ≤ 1.24 ; negative < 0.76.

Table S 1. Thresholds to distinguish positive and negative results.

Name	CAS No.	Company	Target of signaling pathway	GFP fluorescence	optimal concentration (μ M)
				(P, N, NSD)	
Masitinib	790299-79-5	AdooQ BioScience	Kit, PDGF α/β	P	6.25
Quizartinib	950769-58-1	AdooQ BioScience	FLT3	P	1.25
Alk5 Inhibitor II	446859-33-2	STEMGENT	TGF- β	P	10
Bafetinib (INNO-406)	859212-16-1	Selleck Chemicals	BCR/ABL	P	1.25
BIRB-796	285983-48-4	AdooQ BioScience	p38-MAPK	P	5
CHIR99021	252917-06-9	Selleck Chemicals	GSK3	P	2.5
Cycbpamine	4449-51-8	STEMGENT	Hedgehog	P	5
Dasatinib	302962-49-8	AdooQ BioScience	BCR/ABL, Src	P	1.25
DCC-2036 (Rebastinib)	1020172-07-9	Selleck Chemicals	Src, ABL1	P	2.5
DMAXX (vadimezan)	117570-53-3	Selleck Chemicals	VDAs	P	0.63
Fingolimod (FTY720)	162359-56-0	Selleck Chemicals	TGF- β	P	1
GZD824	1421783-64-3	Selleck Chemicals	BCR/ABL	P	0.63
imatinb mesylate	220127-57-1	Selleck Chemicals	BCR/ABL	P	25
Imatinib	152459-95-5	AdooQ BioScience	BCR/ABL	P	6.25
KAAD-Cycbpamine	306387-90-6	STEMGENT	GLI1	P	5
PD0325901	391210-10-9	Selleck Chemicals	MEK	P	5
ponatinib	943319-70-8	Selleck Chemicals	ABL, PDGFR, VEGFR, FGFR, Src	P	0.63

R(+)-Bay K8644	98791-67-4	Selleck Chemicals	L-type Ca ²⁺ channel activator ^{1,2}	P	5
R406	841290-80-0	Cayman Chemical Company	Syk	P	10
Thiazovivin	1226056-71-8	STEMGENT	RHO/ROCK	P	15
2-Methoxyestradiol (2-MeOE2)	362-07-2	Selleck Chemicals	TGF- β	NSD	—
AG-013736	319460-85-0	Selleck Chemicals	VEGFR	NSD	—
AST-487	630124-46-8	Adipogen Life Sciences	FLT3	NSD	—
AT9283	896466-04-9	Selleck Chemicals	Aurora B	NSD	—
AZD-6244/ARRY-886	606143-52-6	Selleck Chemicals	VEGFR	NSD	—
BI-2536	755038-02-9	AdooQ BioScience	Plk1	NSD	—
BIBF-1120 (derivative)	656247-17-5	AdooQ BioScience	VEGFR, PDGF, FGFR	NSD	—
BIBW-2992	850140-72-6	AdooQ BioScience	Her2, EGFR	NSD	—
BIO	667463-62-9	Cayman Chemical Company	GSK3	NSD	—
BIX01294	935693-62-2	STEMGENT	Histone lysine methyltransferase	NSD	—
BMS-345541	547757-23-3	AdooQ BioScience	NF- κ B	NSD	—
BMS-387032/SNS-032	345627-80-7	AdooQ BioScience	CDKs	NSD	—
BMS-540215	649735-46-6	AdooQ BioScience	VEGFR	NSD	—
CEP-701	111358-88-4	Focus Biomolecules	FLT3, JAK2, TrkA	NSD	—
CHIR-258/TKI-258	405169-16-6	AdooQ BioScience	FGFR	NSD	—
CHIR-265/RAF-265	927880-90-8	AdooQ BioScience	RAF, VEGFR	NSD	—
CI-1033	289499-45-2	Cayman Chemical Company	ErbBR	NSD	—

Crizotinib	877399-52-5	LC Laboratories, Inc	ALK	NSD	—
Danuserib (PHA-739358)	827318-97-8	Selleck Chemicals	FGFR, ABL	NSD	—
Degrasy (WP1130)	856243-80-6	Selleck Chemicals	BCR/ABL	NSD	—
DMF	1968/12/2	SIGMA	prepare solution	NSD	—
DMSO	67-68-5	SIGMA	prepare solution	NSD	—
Dorsomorphin	866405-64-3	STEMGENT	AMPK	NSD	—
Doxycycline hyclate	24390-14-5	Selleck Chemicals	Inhibit the inflammatory	NSD	—
Erlotinib	183321-74-6	Cayman Chemical Company	EGFR	NSD	—
FG-4592	808118-40-3	Selleck Chemicals	HIF-PH	NSD	—
Forskolin	66428-89-5	STEMGENT	MAPK	NSD	—
GDC-0879	905281-76-7	AdooQ BioScience	CDK	NSD	—
GDC-0941	957054-30-7	AdooQ BioScience	CLASS I PI3K	NSD	—
Gefitinib	184475-35-2	AdooQ BioScience	EGFR	NSD	—
GSK-690693	937174-76-0	Synkinase Pty Ltd	AK1/2/3	NSD	—
GW-2580	870483-87-7	Cayman Chemical Company	cFMSR	NSD	—
HKI-272	698387-09-6	AdooQ BioScience	EGFR	NSD	—
IDE-1	11160927-48-9	STEMGENT	Inducer of definitive endoderm	NSD	—
IDE-2	N/A	STEMGENT	TGF- β	NSD	—
JNJ-28312141	885692-52-4	AdooQ BioScience	CSF1R	NSD	—
KW-2449	1000669-72-6	AdooQ BioScience	FLT3, STAT5	NSD	—

Lapatinib	231277-92-2	AdooQ BioScience	Her2	NSD	—
LY-317615	170364-57-5	AdooQ BioScience	PKC- β	NSD	—
LY-333531	169939-93-9	Cayman Chemical Company	PKC- β	NSD	—
MLN-120B	783348-36-7	MedChemexpress Co., limited	IKK- β	NSD	—
MLN-518	387867-13-2	AdooQ BioScience	FLT3, cKit, PDGFR	NSD	—
MLN-8054	869363-13-3	AdooQ BioScience	Aurora A	NSD	—
Nilotinib	641571-10-0	AdooQ BioScience	BCR/ABL	NSD	—
PD-173955	260415-63-2	Synkinase Pty Ltd	Src/ Abl	NSD	—
PHA-665752	477575-56-7	AdooQ BioScience	c-Met/HGF/SF	NSD	—
PI-103	371935-79-4	AdooQ BioScience	PI3K	NSD	—
Pifithrin-alpha	63208-82-2	STEMGENT	p53	NSD	—
plinabulin(NPI-2358)	714272-27-2	Selleck Chemicals	Angiogenesis and Tumor vasculature	NSD	—
PLX-4720	918505-84-7	Cayman Chemical Company	B-raf	NSD	—
PP-242	1092351-67-1	AdooQ BioScience	mTOR	NSD	—
PTK-787	212141-51-0	AdooQ BioScience	VEGFR	NSD	—
pumorphamine	483367-10-8	STEMGENT	Hedgehog	NSD	—
R547	741713-40-6	AdooQ BioScience	CDKs	NSD	—
RG108	48208-26-0	STEMGENT	DNA methyltransferase	NSD	—
ROCK II Inhibitor	N/A	STEMGENT	ROCK	NSD	—
Saracatinib (AZD0530)	379231-04-6	Selleck Chemicals	Src	NSD	—

SB431542	301836-41-9	STEMGENT	TGF- β	NSD	—
SC1(Pluripotin)	839707-37-8	STEMGENT	ERK1	NSD	—
SGX-523	1022150-57-7	AdooQ BioScience	MET	NSD	—
Sorafenib	475207-59-1	AdooQ BioScience	VEGFR	NSD	—
Staurosporine	62996-74-1	Cayman Chemical Company	Prevent ATP binding to the kinase	NSD	—
SU-14813	627908-92-3	AdooQ BioScience	VEGFR, PDGF, FGFR	NSD	—
Sunitinib	341031-54-7	Cayman Chemical Company	VEGFR	NSD	—
TAE-684	761439-42-3	Synkinase Pty Ltd	ALK	NSD	—
TG-100-115	677297-51-7	AdooQ BioScience	PI3K	NSD	—
TG-101348	936091-26-8	AdooQ BioScience	JAK	NSD	—
Tranlycypromine hydrochloride	1986-47-6	STEMGENT	MAO	NSD	—
Vandetanib	443913-73-3	Cayman Chemical Company	VEGFR, EGFR	NSD	—
Veliparib (ABT-888)	912444-00-9	Selleck Chemicals	PARP	NSD	—
Wnt inhibitor iwp-2	686770-61-6	STEMGENT	Wnt	NSD	—
Wnt inhibitor iwp-3	N/A	STEMGENT	Wnt	NSD	—
Wnt inhibitor iwp-4	N/A	STEMGENT	Wnt	NSD	—
Y27632	146986-50-7	STEMGENT	ROCK	NSD	—
A-674563	552325-73-2	AdooQ BioScience	PKA, CDK2, AKT	N	12.5
A83-01	909910-43-6	Selleck Chemicals	TGF- β , ALK5	N	10
ABT-869	796967-16-3(4)	AdooQ BioScience	RTK, VEGF, PDGF	N	6.25

All-Trans Retinoic Acid	302-79-4	Selleck Chemicals	Ligand for the retinoic acid receptor	N	1
AMG-706	857876-30-3	AdooQ BioScience	VEGFR	N	10
DAPT	208255-80-5	STEMGENT	Notch	N	10
IOX2	931398-72-0	Selleck Chemicals	PHD	N	0.63
IPA3	42521-82-4	STEMGENT	Pak1	N	2.5
LDN-193189	1062368-24-4	STEMGENT	BMP	N	5
Nitatinib (AMN-107)	641571-10-0	Selleck Chemicals	ERK1/2, PDGF	N	0.31
NVP-BHG712	940310-85-0	Selleck Chemicals	RTK	N	15
Olaparib (AZD2281, KU-0059436)	763113-22-0	Selleck Chemicals	BRCA1, BRCA2	N	1
PD173034	219580-11-7	STEMGENT	FGFR	N	5
Pifithrin-μ	64984-31-2	STEMGENT	p53	N	10
SMO antagonist	N/A	STEMGENT	Hedgehog	N	5
Tanespimycin (17-AAG)	75747-14-7	Selleck Chemicals	Her2, AKT	N	0.5

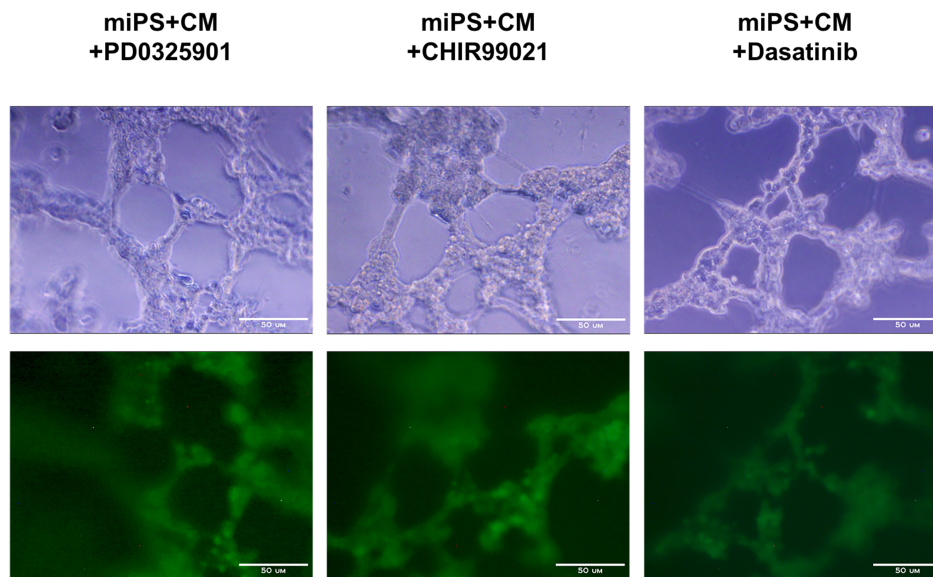
P: Positive compound, N: Negative compound, NSD: No Significant Difference compound

Table S 2. The list of chemical compounds (110).

NO.	Names	ACCESSION	Forward Primer Sequence 5'- 3'	Reverse Primer Sequence 5'- 3'
1	mouse Oct3/4 endogeneous	NM_013633.2	<i>TCTTTCCACCAGGCCCGGCTC</i>	<i>TGCGGGCGGACATGGGGAGATCC</i>
2	mouse Sox2 endogeneous	NM_011443.3	<i>TAGAGCTAGACTCCGGGCGATGA</i>	<i>TTGCCTTAAACAAGACCACGAAA</i>
3	mouse Klf4 endogeneous	NM_010637.3	<i>GGACTTACAAAATGCCAAGGGGTG</i>	<i>TCGCTTCCTCTTCTCCGACACA</i>
4	mouse c-myc endogeneous	NM_010849.4	<i>TGACCTAACTCGAGGAGGAGCTGGAATC</i>	<i>AAGTTTGAGGCAGTTAAAATTATGGCTGAAGC</i>
5	mouse Oct3/4 total	NM_013633.2	<i>CTGAGGGCCAGGCAGGAGCACGAG</i>	<i>CTGTAGGGAGGGCTTCGGGCACTT</i>
6	mouse Sox2 total	NM_011443.3	<i>GGTTACCTCTTCTCCCACTCCAG</i>	<i>TCACATTGTCGACAGGGGCAG</i>
7	mouse Klf4 total	NM_010637.3	<i>CACCATGGACCCGGGCGTGGCTGCCAGAAA</i>	<i>TTAGGCTGTTCTGGGCCGGGGCCACGA</i>
8	mouse c-myc total	NM_010849.4	<i>CAGAGGAGGAACGAGCTGAAGCGC</i>	<i>TTATGCACCAGAGTTTCGAAGCTGTTTCG</i>
9	mouse Oct3/4 transgene	M34381.1	<i>TTGGGCTAGAGAAGGATGTGGTTC</i>	<i>TTATCGTCGACCACTGTGCTGCTG</i>
10	mouse Sox2 transgene	NM_003106.4	<i>GGTTACCTCTTCTCCCACTCCAG</i>	<i>TTATCGTCGACCACTGTGCTGCTG</i>
11	mouse Klf4 transgene	XM_021200389.1	<i>GCGAACTCACACAGGCGAGAAACC</i>	<i>TTATCGTCGACCACTGTGCTGCTG</i>
12	mouse c-myc transgene	NM_010637.3	<i>CAGAGGAGGAACGAGCTGAAGCGC</i>	<i>TTATCGTCGACCACTGTGCTGCTG</i>
13	mouse Nanog	NM_028016.3	<i>AGGGTCTGCTACTGAGATGCTCTG</i>	<i>CAACCACTGGTTTTTCTGCCACCG</i>
14	Pik3ca	NM_008839.2	<i>GCCACAGACACTACTGCGTA</i>	<i>CACCGAACAGCAAACTCCG</i>
15	Pik3cb	NM_029094.3	<i>CTGATTTTACGGCGGCATGG</i>	<i>TGAGGGCCTCGTCAAACTTC</i>
16	Pik3cg	NM_001146201	<i>ACCTGTGCCTTCTGCCTTAC</i>	<i>TGCGGCCTGAACTTTTCTTC</i>
17	Pik3r1	NM_001024955.2	<i>AGCGGAGAACCTATTGCGAG</i>	<i>ACTTCGCCGTCTACCACTAC</i>
18	Pik3r5	NM_177320.2	<i>AAGTCCTTTGTCAGCAGTCCC</i>	<i>CTGGTAAACCTGCAGCAACAC</i>
19	Pik3r6	NM_001004435.3	<i>TGAGACGACCACATCTCCC</i>	<i>TCCACATGCCCTGATTGCTC</i>
20	Pik3ap1	NM_031376.4	<i>GAAGGCCATTTCTGAAGATTCTGG</i>	<i>TCTCGTCCAGCTTGCATCTC</i>
21	Pten	NM_001304718.2	<i>TGAAGACCATAACCCACCACAG</i>	<i>AGCATCTTGTCTGTTTGTGGAAG</i>

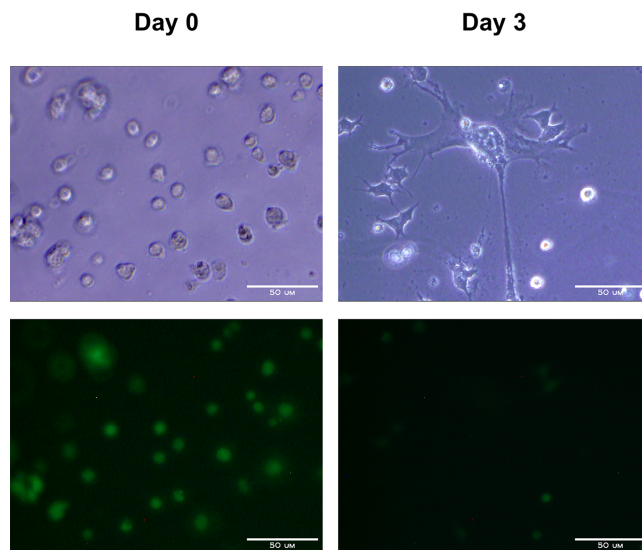
Table S 3. List of Primers Used in the Experiments.

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43 Fig. S1 Tube formation assays in miPSCs and other three converted cells after 20h of culture on
 44 Matrigel. Differentiation potential is another property of CSCs, along with self-renewal. miPSCs and
 45 other three converted cells were assessed for the potential to differentiate into endothelial-like cells
 46 forming capillary-like tubes on Matrigel. Capillary-like tubes were formed by these cells indicating
 47 pro-angiogenic properties in tumorigenesis. These cells showed high potential of tube formation.



miPSCs without LIF

Fig. S2 miPSCs in adherent culture without LIF. Left: miPSCs with high expression of GFP. Right: after 3 days, most of the cells differentiated and no expression of GFP. After 1 week no cells survived.

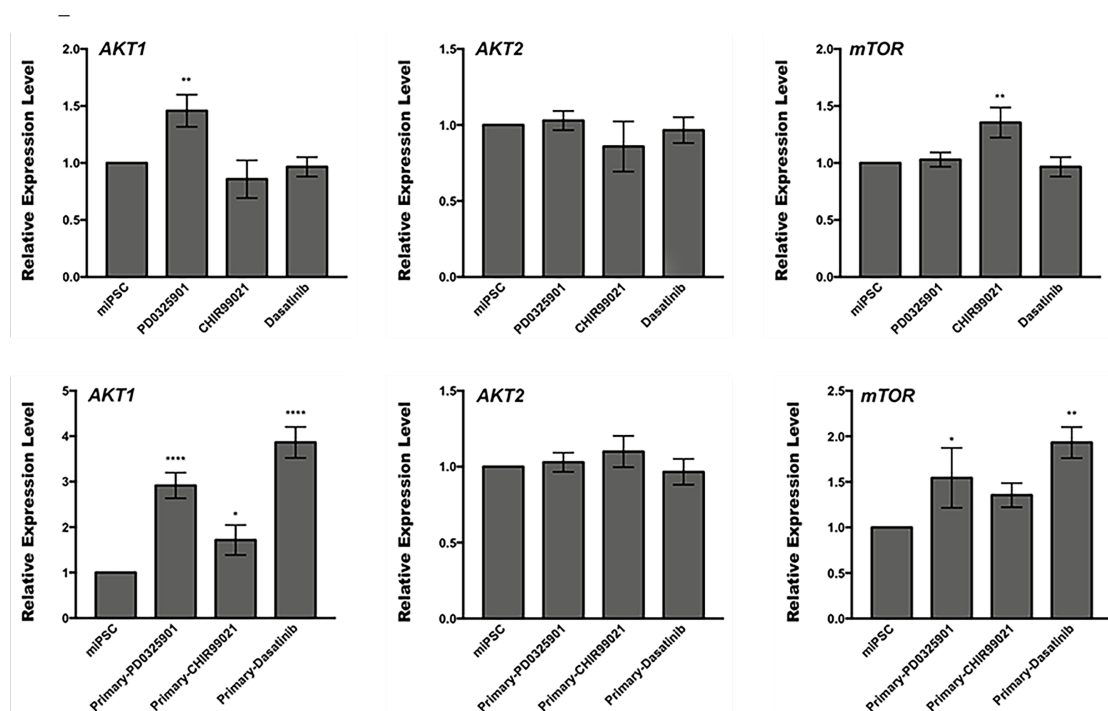
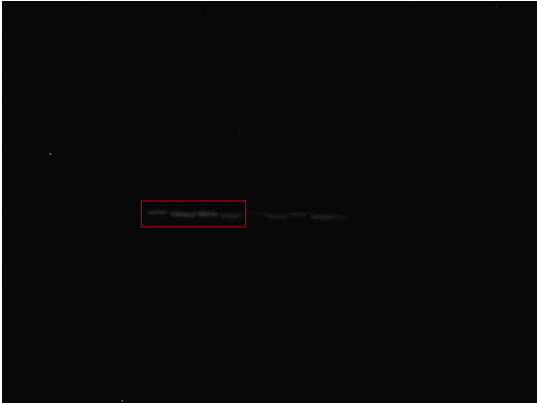


Fig. S3 RT-qPCR analysis of AKT1, AKT2 and mTOR.

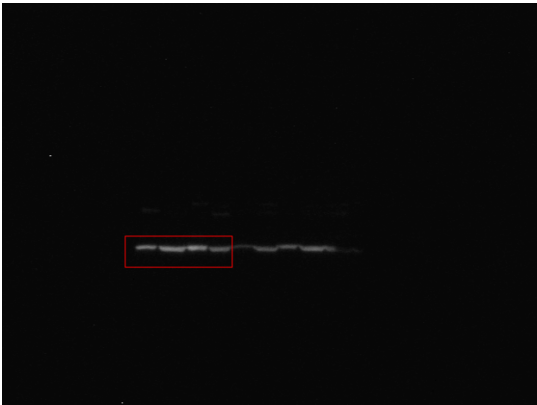
Figure 6b (Full unedited gels of Western blots)

miPS-LLCcm-PDpr
miPS-LLCcm-CHpr
miPS-LLCcm-Da1pr
miPSC

p-AKT



AKT



β -actin

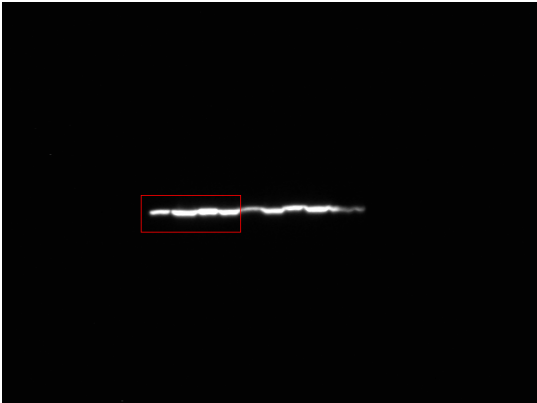


Figure 6c (Full unedited gels of Western blots)

