

## Supplementary Information

### Establishment of a novel human CIC-DUX4 sarcoma cell line, Kitra-SRS, with autocrine IGF-1R activation and metastatic potential to the lungs

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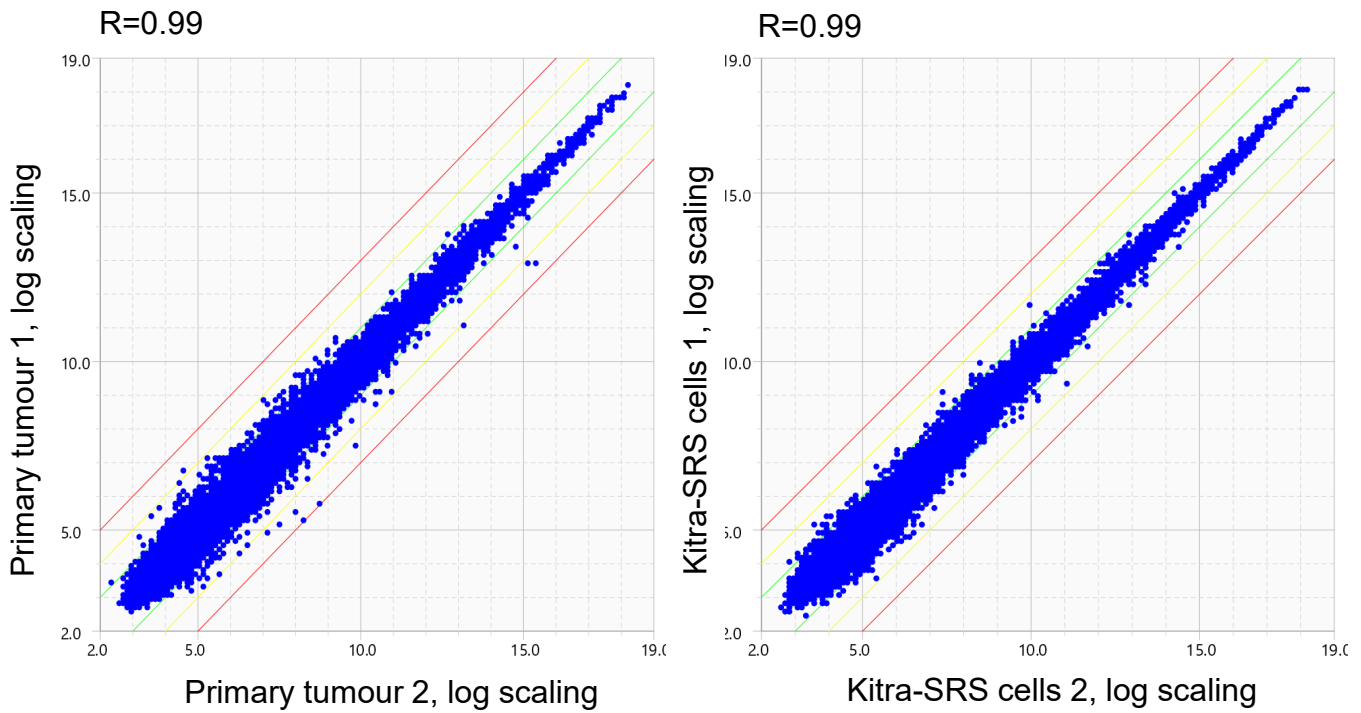
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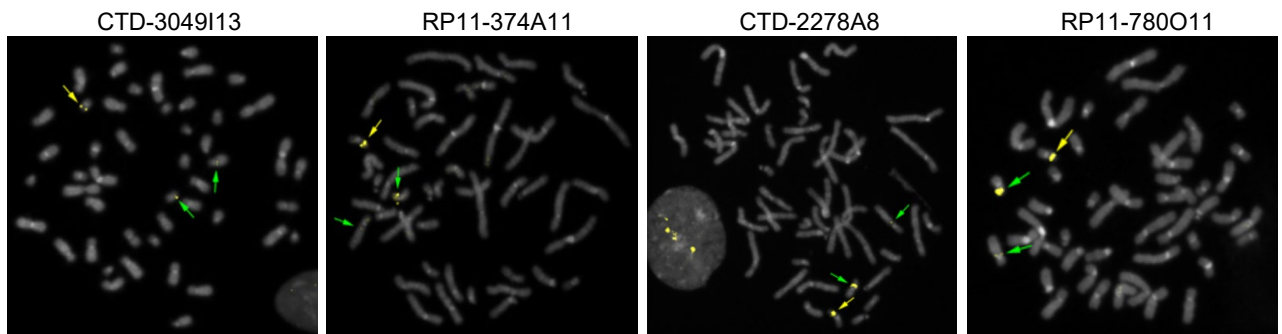
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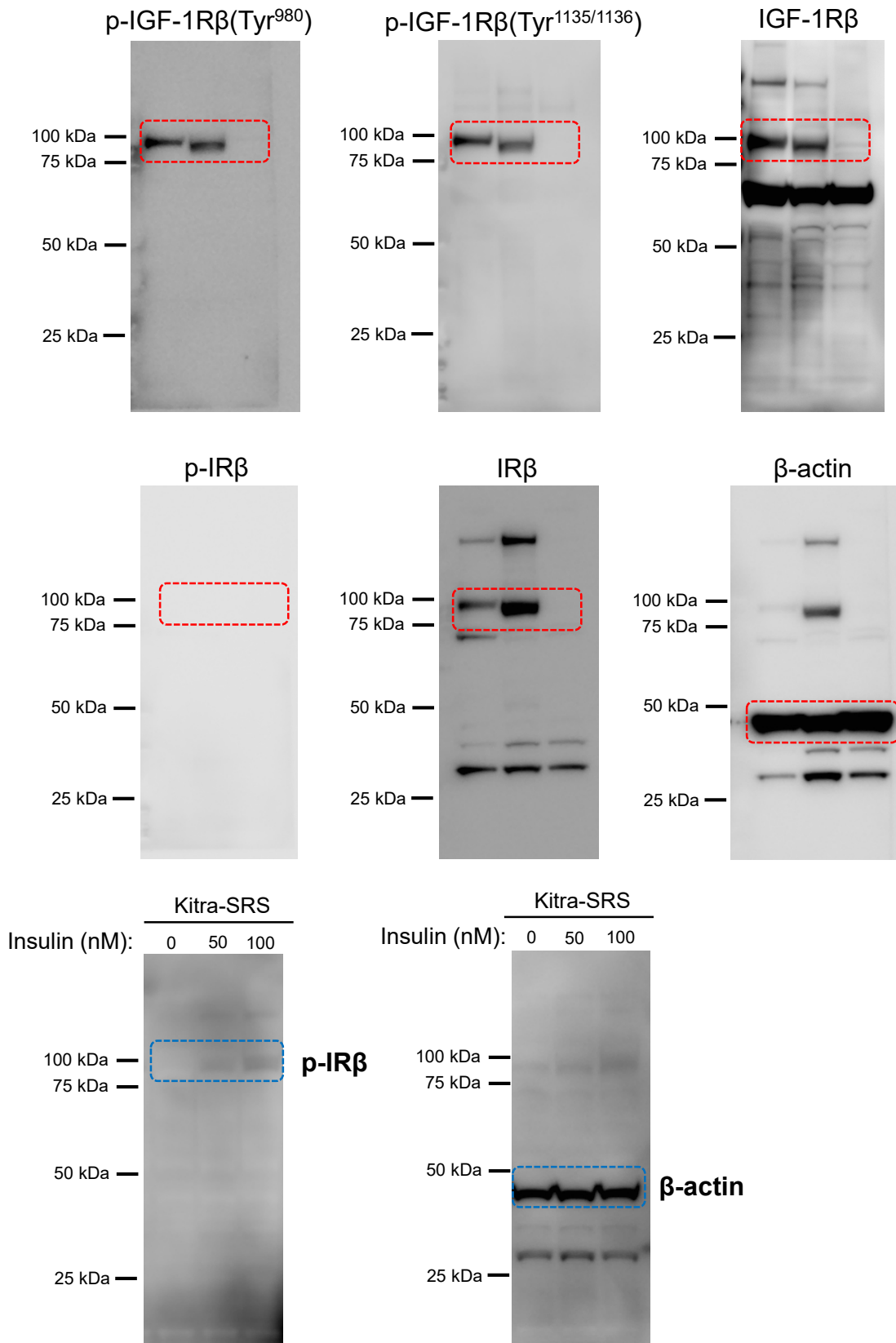
E-mail: s.takenaka.0816@ort.med.osaka-u.ac.jp



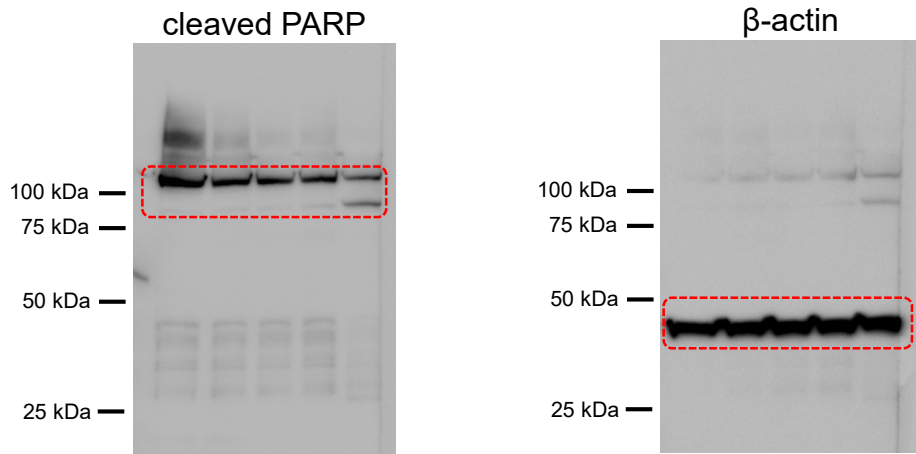
**Supplementary Figure S1.** Scatter plots showing the correlation of gene expression between samples of the primary tumour (left) and the correlation between samples of Kitra-SRS cells (right). R represents the correlation coefficient. Green and red lines indicate absolute log-fold-change  $>1$  and  $>3$ , respectively.



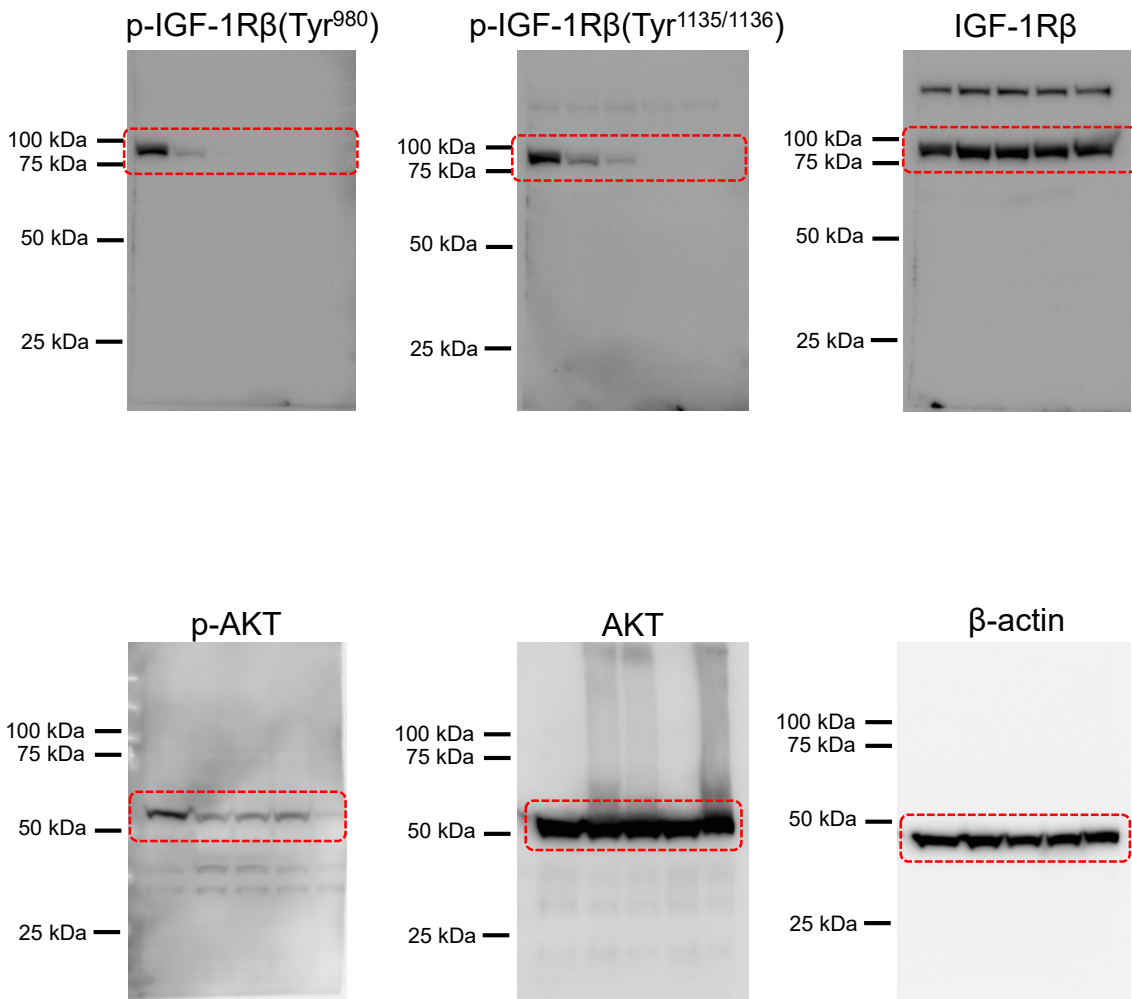
**Supplementary Figure S2.** FISH analysis of Kitra-SRS cells. The yellow arrows indicate probes for the 19q13.2 region; the green arrows, a split of one copy of each probe.



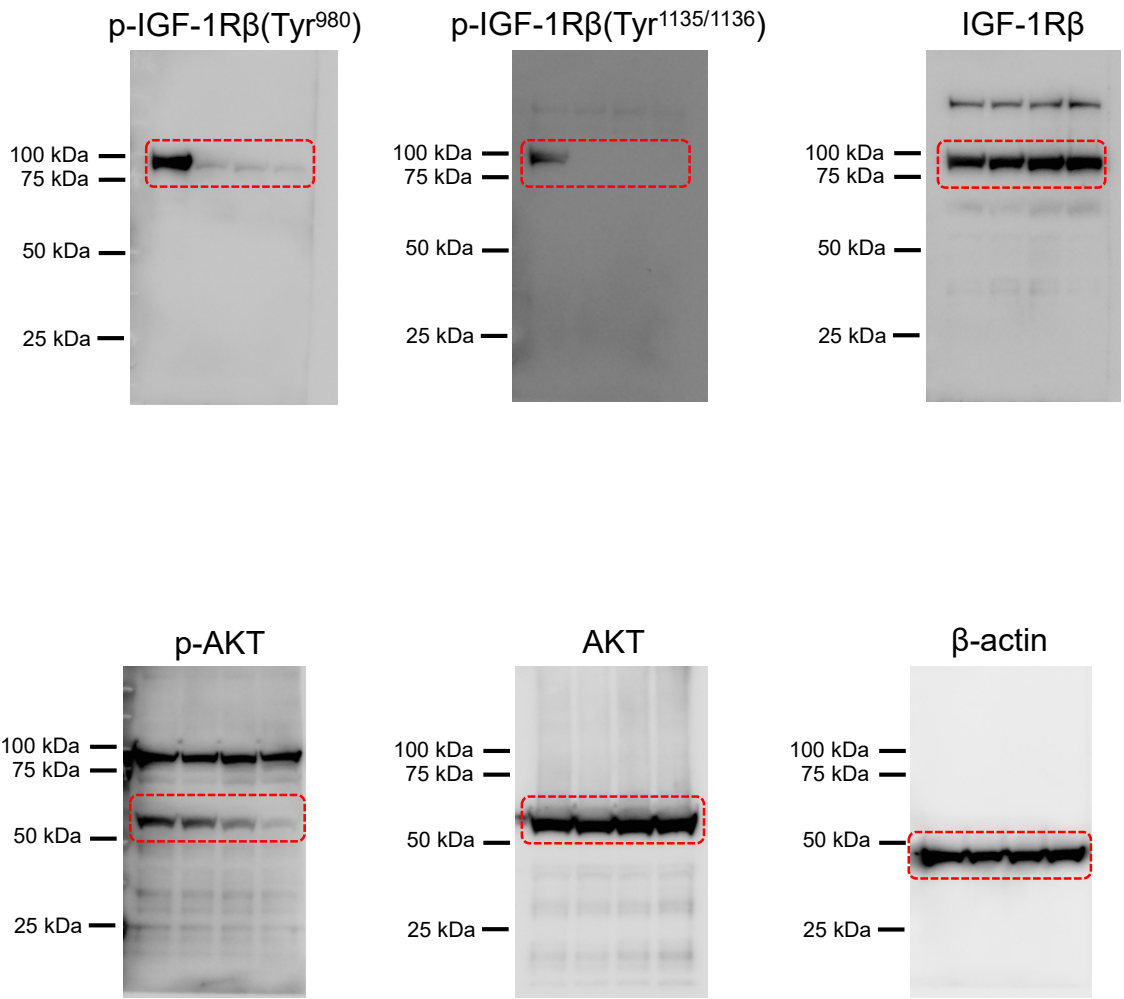
**Supplementary Figure S3.** Full-length blots of p-IGF-1Rβ(Tyr<sup>980</sup>), p-IGF-1Rβ(Tyr<sup>1135/1136</sup>), IGF-1Rβ, p-IRβ, IRβ and β-actin protein expression for Figure 5b. Kitra-SRS cells were treated with 0-100 nM insulin for 10 min and then subjected to western blotting with the anti-p-IRβ antibody. Insulin was used as a positive control.



**Supplementary Figure S4.** Full-length blots of cleaved PARP and  $\beta$ -actin protein expression for Figure 6c.

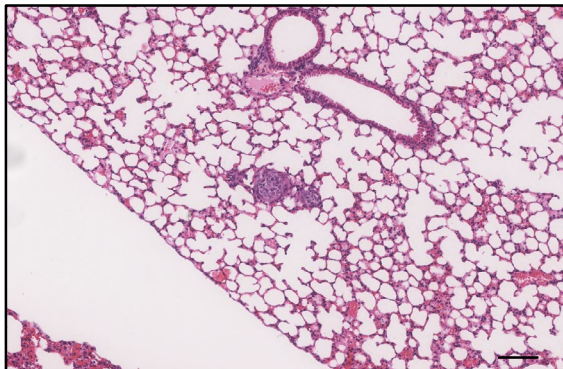


**Supplementary Figure S5.** Full-length blots of p-IGF-1Rβ(Tyr<sup>980</sup>), p-IGF-1Rβ(Tyr<sup>1135/1136</sup>), IGF-1Rβ, p-AKT, AKT and β-actin protein expression for Figure 6d.

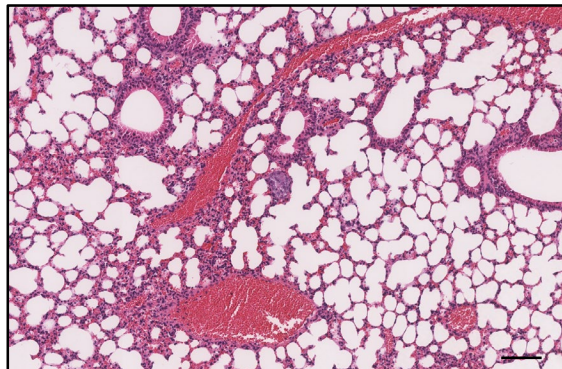


**Supplementary Figure S6.** Full-length blots of p-IGF-1Rβ(Tyr<sup>980</sup>), p-IGF-1Rβ(Tyr<sup>1135/1136</sup>), IGF-1Rβ, p-AKT, AKT and β-actin protein expression for Figure 6e.

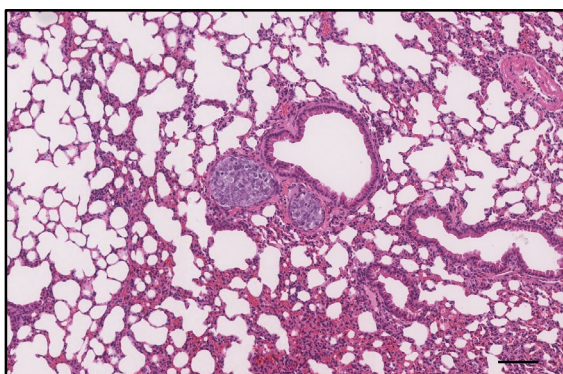
Vehicle mouse 1



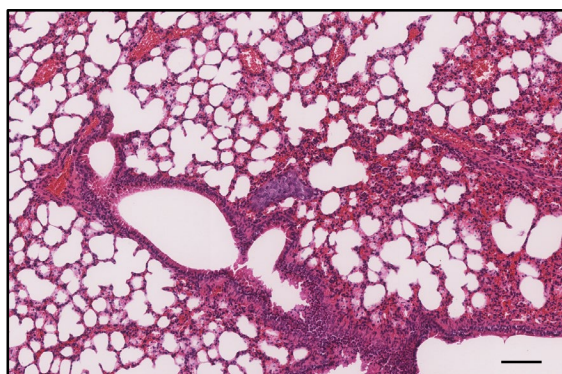
Vehicle mouse 2



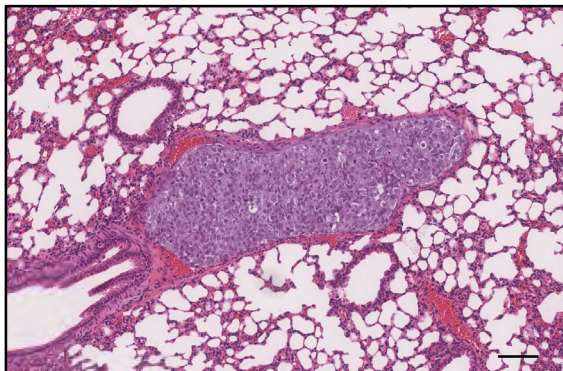
Vehicle mouse 3



Vehicle mouse 4

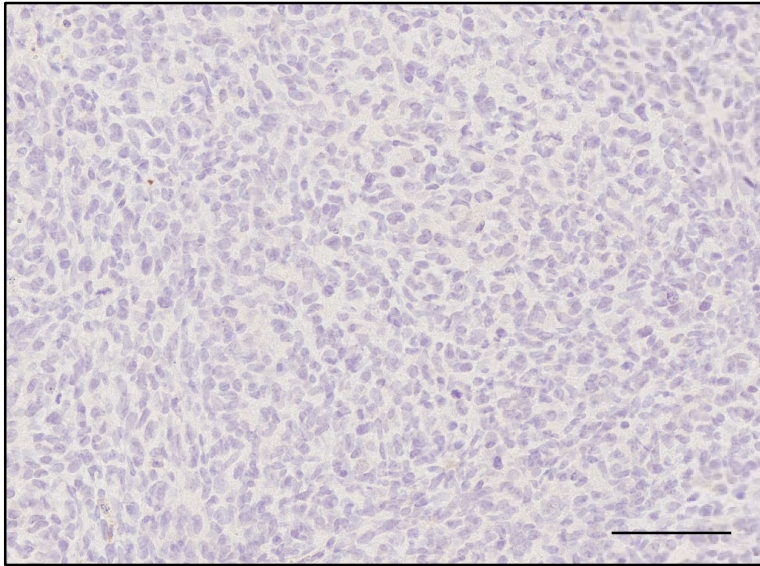


Vehicle mouse 5



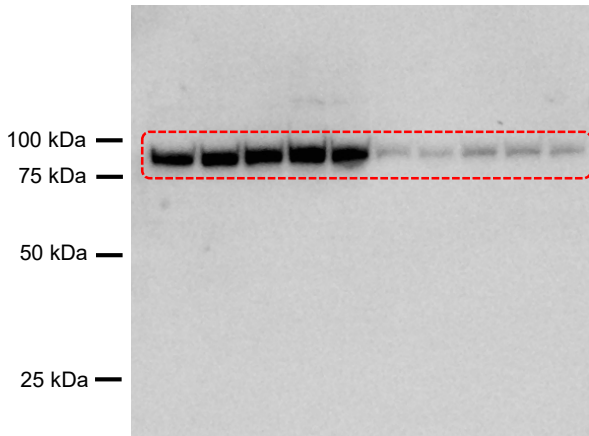
**Supplementary figure S7.** HE staining of lungs in vehicle-treated mice. Scale bars: 100 $\mu$ m.



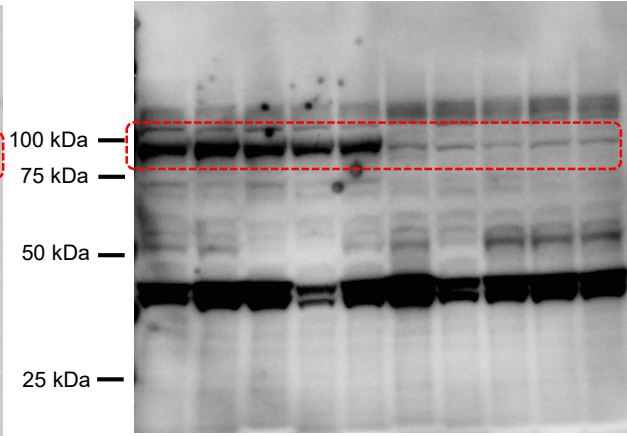


**Supplementary Figure S8.** A negative control for Ki-67 immunostaining.

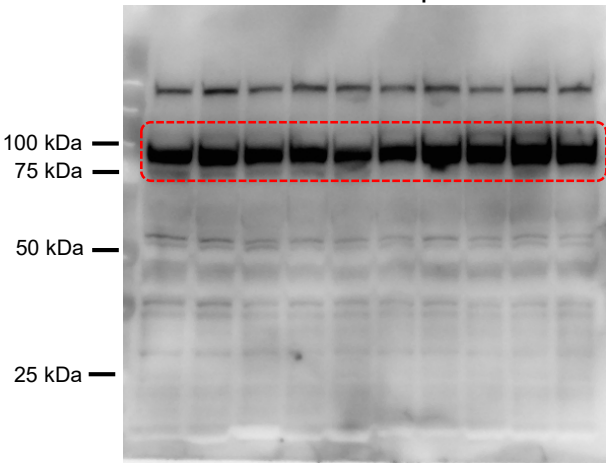
p-IGF-1R $\beta$ (Tyr<sup>980</sup>)



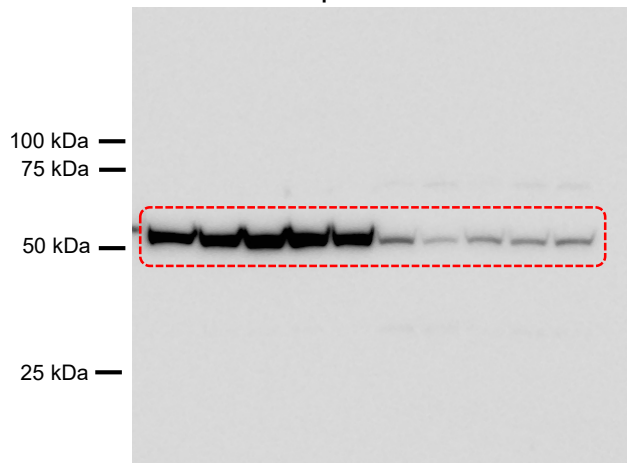
p-IGF-1R $\beta$ (Tyr<sup>1135/1136</sup>)



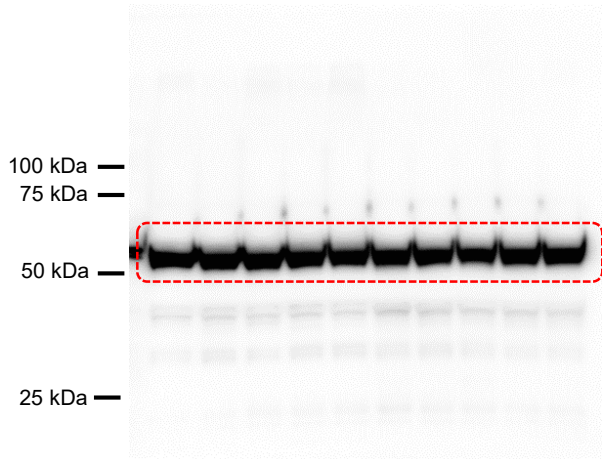
IGF-1R $\beta$



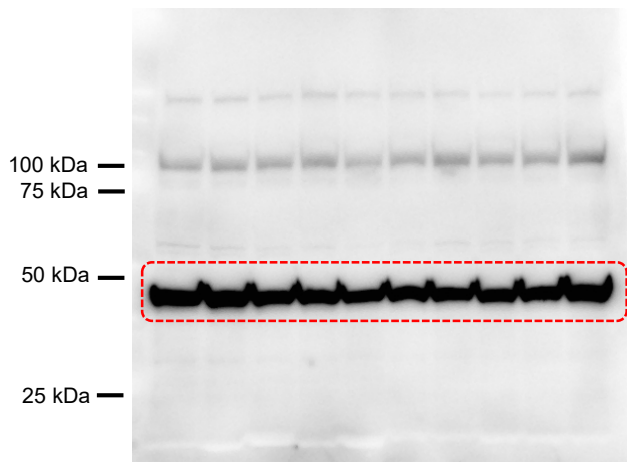
p-AKT



AKT



$\beta$ -actin



**Supplementary Figure S9.** Full-length blots of p-IGF-1R $\beta$ (Tyr<sup>980</sup>), p-IGF-1R $\beta$ (Tyr<sup>1135/1136</sup>), IGF-1R $\beta$ , p-AKT, AKT and  $\beta$ -actin protein expression for Figure 7g.

**Supplementary Table S1.** STR analysis.

Microsatellite (Chromosome)	Kitra-SRS (P50)	Tumor Tissue
Amelogenin	X, X	X, X
D21S11	29, 32.2	29, 32.2
D5S818	10, 12	10, 12
D13S317	8, 10	8, 10
D7S820	11, 12	11, 12
D16S539	9	9
vWA	16, 18	16, 18
TH01	7, 9	7, 9
TPOX	8, 9	8, 9
CSF1PO	12	12

Cells were compared to a tumor tissue from the patient.

P indicates a passage number of a cell line.

**Supplementary Table S2.** Detection of *CIC-DUX4* from RNA-seq data.

	<i>CIC(4525)-DUX4L2(1075)</i>
Kitra-SRS	<i>CIC(4376)-DUX4L4(1014)</i>
	<i>CIC(4505)-DUX4L12(977)</i>

Numbers in parentheses represent the nucleotide position at the breakpoint junction in the reference cDNA sequence of each gene; *CIC* (NM\_015125), *DUX4L2* (NM\_001127386), *DUX4L4* (NM\_001177376), and *DUX4L12* (NG\_012776).

**Supplementary Table S3.** Primer sequences for the RT-PCR.

<b>Primer name</b>	<b>Primer sequence (5'→3')</b>
<b>CIC4120(ref)</b>	TGAGTTGCCTGAGTTTCG
<b>DUX4RTr2</b>	TGAGGGGTGCTTCCAGCG
<b>CIC-fl</b>	ACCATGTATTTCGGCCCCACAGG
<b>DUX4-fl</b>	TCCTAAAGCTCCTCCAGCAGAG
<b>pENTER1A-F</b>	CTACAAACTCTTCCTGTTAGTTAG
<b>pENTER1A-R</b>	ATGGCTCATAACACCCCTTG
<b>CIC770F</b>	AAGGAGAAGCAGAAGTACCACGAC
<b>CIC1522F</b>	GTTTTCACCTGTGATCCGTTCTC
<b>CIC2309F</b>	CCTGCCACTGTCACTAACCTACTG
<b>CIC3046F</b>	CAGAATCACCTATGTGCAGTCAGC
<b>CIC3838F</b>	CAGCAAATTCCCCAGCTCATCTTC
<b>CIC4523F</b>	GCCCGCTATGCAGACATCTTTC

**Supplementary Table S4.** *DUX4* pseudogenes identical to the *DUX4* component of *CIC-DUX4* transcript of Kitra-SRS cells from Ensemble Genome Browser 95: <http://www.ensembl.org/index.html>.

Subject name	Gene	Genomic Location
ENST00000611059.1	<i>DUX4L15</i>	10:133760834-133761125
ENST00000554103.2	<i>DUX4L13</i>	10:133754225-133754516
ENST00000622460.1	<i>DUX4L10</i>	10:133744307-133744598
ENST00000619712.1	<i>DUX4L20</i>	10:133684645-133684936
ENST00000622058.1	<i>DUX4L21</i>	10:133681346-133681637
ENST00000618238.1	<i>DUX4L22</i>	10:133678036-133678327
ENST00000615195.1	<i>DUX4L23</i>	10:133674726-133675017
ENST00000617576.2	<i>DUX4L24</i>	10:133671427-133671718
ENST00000624915.1	<i>DUX4L25</i>	10:133668128-133668419
ENST00000566884.2	<i>DUX4L4</i>	4:190082095-190082386

**Supplementary Table S5.** Karyotype results of 10 metaphase cells on Kitra-SRS cells at passage 20 in M-FISH analysis.

Karyotype results	Cell number
48, XX, del(1)(p32), +8, t(12;19)(q13;q13), +20	6
48, XX, +8, t(12;19)(q13;q13), +20	2
47, XX, del(1)(p32), +8, t(12;19)(q13;q13), -15, +20	1
47, XX, del(1)(p32), t(12;19)(q13;q13), +20	1

**Supplementary Table S6.** Karyotype results of 20 metaphase cells on Kitra-SRS cells at passage 100 in G-banding.

Karyotype results	Cell number
47,XX,del(1)(p?),+8,der(12)add(12)(p13)t(12;19)(q13;q13.1), der(19)t(12;19)(q13;q13.1)	15
49,XX,+1,del(1)(p?)x2,+8,t(12;19)(q13;q13.1),+20	1
48,XX,del(1)(p?),+8,t(12;19)(q13;q13.1),+20	1
48,XX,del(1)(p?),+8,+der(12)t(12;19)(q13;q13.1), t(12;19)(q13;q13.1),+20	1
48,XX,del(1),+3,+8,der(12)add(12)t(12;19)(q13;q13.1), add(17)(q11.2),der(19)t(12;19)(q13;q13.1)	1
49,XX,del(1),+8,der(12)add(12)t(12;19)(q13;q13.1), +?19,der(19)t(12;19),+20	1

**Supplementary Table S7.** Incidence of lung metastasis in Kitra-SRS-inoculated mice.

	Metastasis +
$1 \times 10^7$ cells (n=5)	4/5 (80%)
$1 \times 10^8$ cells (n=5)	3/5 (60%)

**Supplementary Table S8.** Seventeen drugs that inhibited more than 80 % cell viability in Kitra-SRS cells among 1134 FDA-approved drugs.

Drug name	Cell viability (%)
	Kitra
Mitoxantrone HCl	-3.2
Penfluridol	0.1
Alexidine HCl	0.5
Auranofin	0.5
Terfenadine	1.2
Ponatinib (AP24534)	2
Carfilzomib (PR-171)	2.3
Emetine	2.3
Idarubicin HCl	3.2
Daunorubicin HCl	3.5
Bortezomib (PS-341)	3.6
9-Aminoacridine	3.9
Crystal Violet	4.2
Doxorubicin (Adriamycin)	6.6
Epirubicin HCl	7
Topotecan HCl	7.8
Camptothecin	8.3

## Supplementary Table S9. Antibodies.

Target	Clone/ product name	Source	Supplier	Concentration/ Dilution	Blocking buffer
<b><i>Immunohistochemistry</i></b>					
CD99	O13	mouse monoclonal	Nichirei Biosciences	a diluted antibody	-
bcl-2	124	mouse monoclonal	Nichirei Biosciences	a diluted antibody	-
WT1	#83535	rabbit monoclonal	Cell Signaling Technology	1:100	-
Ki-67	#9027	rabbit monoclonal	Cell Signaling Technology	1:400	-
normal rabbit IgG	148-09551	-	Wako	1:1000	-
normal mouse IgG	sc-2025	-	Santa Cruz	1:1000	-
<b><i>Immunoblotting</i></b>					
IGF-1R $\beta$	#3027	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
phospho-IGF-1R $\beta$ (Tyr980)	#4568	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
phospho-IGF-1R $\beta$ (Tyr1135/1136)	#3024	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
IR $\beta$	#3025	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
phospho-IR $\beta$ (Tyr1185)	ab62321	rabbit monoclonal	Abcam	1:2000	5% milk in TBST
AKT	#4691	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
phospho-AKT	#4060	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
cleaved PARP	#9542	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
$\beta$ -actin	#4970	rabbit monoclonal	Cell Signaling Technology	1:1000	5% milk in TBST
horseradish peroxidase (HRP)-coupled goat anti-rabbit IgG	#7074	-	Cell Signaling Technology	1:1000	-
HRP-coupled horse anti-mouse IgG	#7076	-	Cell Signaling Technology	1:1000	-