Manuscript title: Establishing a panel of chemo-resistant mesothelioma models for investigating chemo-resistance and identifying new treatments for mesothelioma.

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Supplementary information (SI) table 1: Genes found on the SABiosciences Drug resistance and metabolism PCR array arranged in functional groups.

Functional gene group	Gene abbreviations
Drug Resistance	*Abcb1 (Mdr-1), Abcb1b, Abcb4, Abcc1 (Mrp1), Abcc2 (Mrp2), Abcc3 (Mlp-2), Abcc5 (Mrp5), Abcc6 (Mrp6), Abcg2 (Bcrp), Bax, Bcl2, Bcl2l1 (Bcl-x), Mvp, Rb1, Top1, Top2a, Top2b, Tp53.*
Drug Metabolism	*Arnt, Blmh, Crabp1, Cyp1a1, Cyp1a2, Cyp2b2, Cyp2c, Cyp2c13, Cyp2c22, Cyp2c37, Cyp2c7, Cyp2c79, Cyp2d4v1, Cyp2e1, Cyp3a2, Dhfr, Ephx1, Gstm1 (Mgst1), Nat2, Sult1e1 (Ste), Sod1, Ugcg.*
DNA repair	*Apc, Atm, Brca1, Brca2, Ercc3 (Xpb), Xpa, Xpc.*
Cell Cycle	*Ccnd1, Ccne1, Cdk2, Cdk4, Cdkn1a (p21Waf1), Cdkn1b (p27Kip1), Cdkn2a (p16Ink4a), Cdkn2d (p19ink4d).*
Growth Factor Receptors	*Egfr, Erbb2 (Neu, Her2), Erbb3, Erbb4, Fgf2 (bFGF), Met*
Hormone Receptors	*Ar, Esr1 (ERa), Esr2 (ERß-cx), Igf2r, Ppara, Ppard, Pparg, Rara, Rarb, Rxra, Rxrb*
Transcription Factors	*Ahr, Ap1s1, Elk1, Fos, Gabpa, Hif1a, Mafb, Myc, Nfkb1, Nfkb2, Nfkbib (Trip9), Nfkbie*

SI table 2: Primary antibodies for immunohistochemical analysis.

Antibody	Description	Source	Dilution	Identifying Cellular Localization
WT1, clone 6F-H2	Expressed in normal mesothelium and regarded as a useful marker of malignant mesothelioma[1, 2]	Dako	1:100	Nucleus
Calretinin, rabbit polyclonal	Expressed in normal mesothelial cells and considered key identifier of malignant mesothelioma, especially epithelioid[3-5]	Zymed (Life Technologies)	1:100	Must be nucleus
Podoplanin, hybridoma supernatant ***NB: clone D2-40 did not recognize rat tissue***	Useful marker especially for sarcomatoid mesothelioma[6-8]	Angiobio	1:50	Membrane and cytoplasm
Mesothelial Cell, clone HBME-1	Found in normal mesothelium and used as a marker of malignant mesothelioma[4, 9]	Dako	1:500	Membrane
Cytokeratin 5/6, clone D5/16B4	Considered a useful marker of malignant mesothelioma, especially the epithelioid subtype[5, 10, 11]	Chemicon (Merck Millipore)	1:100	Cytoplasm
Cytokeratin, clone MNF116	Considered useful marker of malignant mesothelioma[6, 12]	Dako	1:50	Cytoplasm
E-Cadherin, clone 36	Decreased expression indicative of epithelial to mesenchymal transition[13]	BD transduction systems	1:1000	Membrane
Vimentin, clone EPR3776	Increased expression indicative of epithelial to mesenchymal transition[13]	Epitomics	1:200	Cytoplasm
Ki67, clone SP6	Expressed in proliferating cells[14]	Thermo Scientific	1:100	Nucleus
CD15, clone C3D-1	Negative marker of MM (carcinoma-related)[7, 15]	Santa Cruz	1:100	Membrane
TTF-1, clone 8G7G3/1	Negative marker of MM (carcinoma-related)[11]	Dako	1:100	Nucleus
CEA, clone II-7 (*did not recognize rat tissue)*	Negative marker of MM (carcinoma-related)[3, 5]	Dako	1:50	Cytoplasm

SI table 3: Antibody panel for rat leukocyte cell detection.

Antibody	Leukocyte detected	Source
Anti-rat CD4-FITC	CD4+ T cells	Biolegend
Anti-rat CD8a-PE	CD8+ T cells	Biolegend
Anti-rat CD45-PE/Cy7	All leukocytes	Biolegend
Anti-rat CD3-APC[1]	T cells	BD
Anti-rat CD45 RA-FITC[1]	B cells	BD
Anti-CD161a-PE[1]	Natural Killer cells	BD

[1]Components of the BD Rat T/B/NK Cocktail.

Figure S1: Gene expression in parental and chemo-resistant mesothelioma cells.

Fold change in excision repair cross complementation group 1 (*Ercc1*), ribonucleotide reductase M1 (*Rrm1*) and thymidylate synthetase (*Tyms*) gene expression was calculated using the $2^{-\text{delta-delta Ct}}$ method after normalizing to a panel of housekeeping genes (*Rplp1*, *Actb* and *Gapdh*). *Bars* show the means and standard error in gene expression relative to parental II-45 cells from at least three independent experiments. P-values were calculated from at least three independent experiments using a one-way Anova test with a value of less than 0.05 indicating significance. Parental mesothelioma cells, II-45; cisplatin resistant II-45 cells, CisR; pemetrexed resistant II-45 cells, PemR; combination (cisplatin + pemetrexed) resistant II-45 cells, ComboR; gemcitabine resistant II-45 cells, GemR and vinorelbine resistant II-45 cells, VLBR.

Figure S2: Glutathione and its contribution to chemo-resistance.

Cell viability of parental (II-45), cisplatin resistant (CisR) and combination resistant (ComboR) cells following cisplatin treatment in absence or presence (+ BSO) of buthionine sulfoximine was determined using MTT assays. The drug dose causing 50% growth inhibition (IC_{50} drug dose) was determined and the results are expressed as a percentage of the parental cells. The p-value was calculated by comparing the IC_{50} values from at least three independent experiments using a one-way Anova test with a value of less than 0.05 indicating significance.

Figure S3: CGH schematic for rat Chromosome 5.

Genomic alterations for chromosome 5 are depicted. Figure generated using Agilent Genomic Workbench v7 program. A homozygous deletion of approximately 4 megabases covering the *Cdkn2a/2b* and *Mtap* gene loci was identified in both the parental (replicates presented by blue lines) and combination resistant (replicates presented by orange lines) II-45 cell lines.

Figure S4: Chemo-resistance is maintained *in vivo*

Rats were injected with 1 x 10^6 parental (II-45) or cisplatin resistant (CisR) II-45 cells sub-dermally into the flank (n = 2 per group). Cisplatin was given intraperitoneally on days 3, 14, 21 and 36 at a dose of 2 mg/kg. Rats were euthanised at ethically approved endpoints and survival was plotted using a log-rank (Mantel-Cox) Gehan-Breslow-Wilcoxon graph (a). *Ex-vivo* transplants were taken and cell viability was assessed using MTT assays (b). *Points* show the mean and standard error of triplicate wells with the different lines indicating individual rats. The drug dose causing 50% growth inhibition (IC_{50} drug dose; c) was determined and the p-value was calculated by comparing the IC_{50} values from at least three independent experiments using a student's t-test test with a value of less than 0.05 indicating significance. *** $p<0.001$.

Figure S5: Representative images of positive immunohistochemical labelling of pleural mesothelioma.

Tumors derived from parental II-45 cells. (a) Nuclear positivity for Wilms Tumor 1 protein (WT-1) demonstrating extensive labelling with approximately 90% of tumor cells displaying prominent nuclear labelling; (b) Very sparse focal nuclear labelling with only the occasional

positive cell and limited cytoplasmic immunolabelling was observed for calretinin; (c) HBME-1 labelling showing membranous cell localization around the periphery of the tumor; (d) Cytoplasmic immunolabeling for cytokeratin MNF116 was observed at the periphery of the tumors with occasional positive cells in the near vicinity (a similar pattern was seen with immunolabeling for cytokeratin 5/6); (e) Distinct membranous immunolabeling for E-cadherin; (f) Vimentin immunolabeling showing diffuse cytoplasmic staining; (g) Granular nuclear positivity for Ki67. Insert: 2x higher magnification; Scale bar = 50 μm.

Figure S6: Cytokine biomarkers in endpoint plasma samples.

Rats were injected with 100 μL serum free media or serum free media containing 5 x 10^5 parental or chemo-resistant II-45 cells directly into the pleural cavity. At ethical endpoint, rats were euthanized and plasma samples were analysed for cytokine levels relative to rats with parental II-45 mesothelioma. (a) IL-1β; (b) IL-4; (c) IL-5; (d) IL-6; (e) IL-7; (f) IL-12; (g) IL-13; (h) IL-18; (i) G-CSF); (j) GM-CSF; (k) GRO/KC; (l) IFN-λ; (m) MIP-1α; (n) VEGF. Normal control rats, Controls; parental mesothelioma cells, II-45; cisplatin resistant II-45 cells, CisR; pemetrexed resistant II-45 cells, PemR; combination (cisplatin + pemetrexed) resistant II-45 cells, ComboR; gemcitabine resistant II-45 cells, GemR; vinorelbine resistant II-45 cells, VLBR. P-values were calculated using a one-way Anova test with a value of less than 0.05 indicating significance. *$p<0.05$, **$p<0.01$, ***$p<0.001$ relative to rats with parental II-45 mesothelioma.

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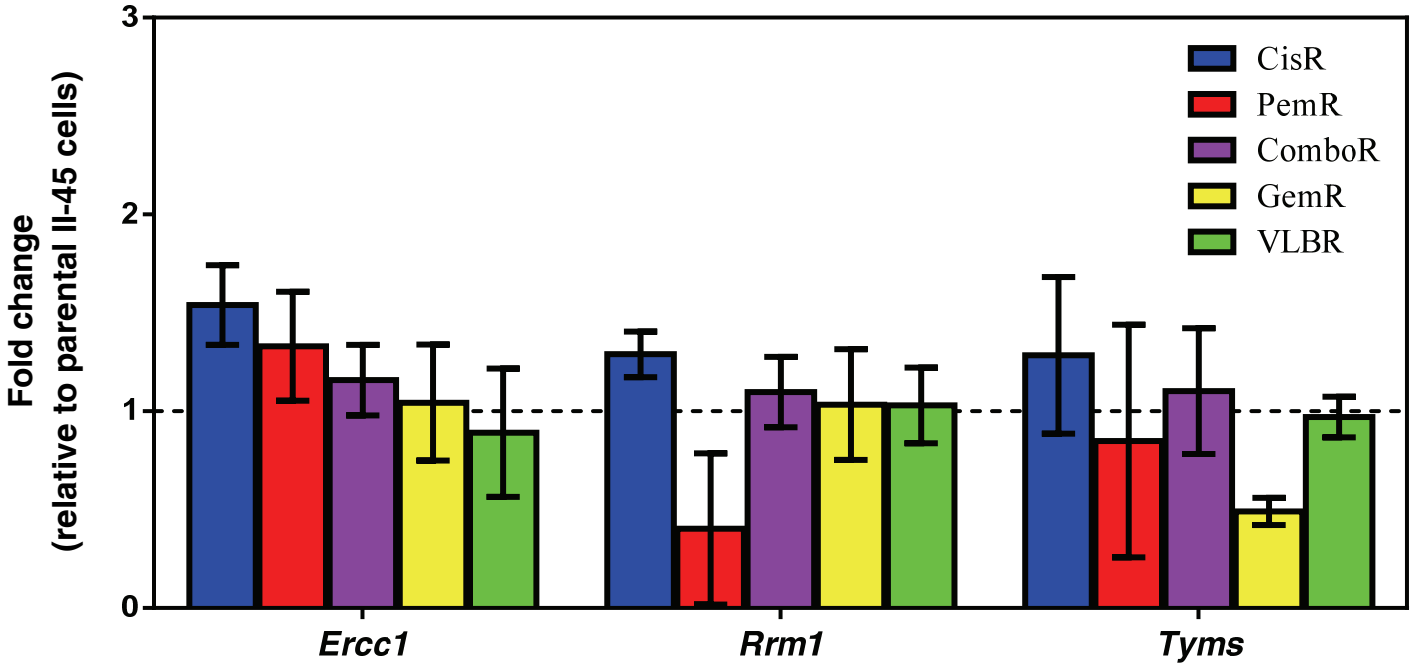
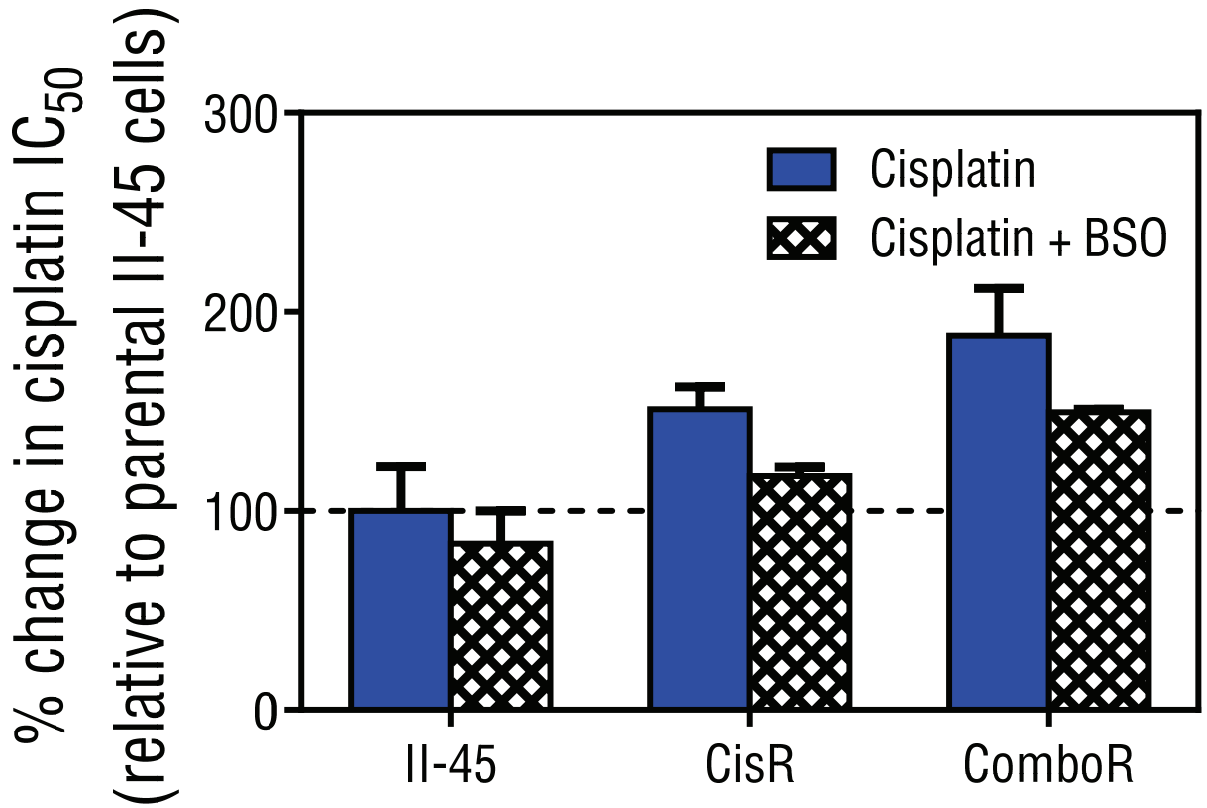

Fold change
(relative to parental II-45 cells)
3
2
1
0
CisR
PemR
ComboR
GemR
VLBR
Ercc1
Rrm1
Tyms


% change in cisplatin IC_{50}
(relative to parental II-45 cells)
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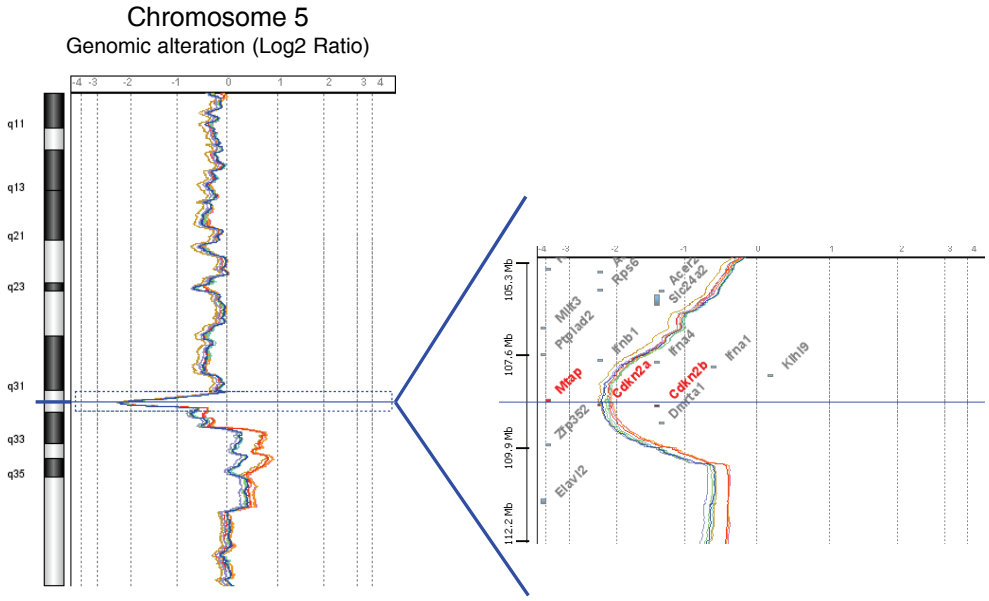
200
100
0
Cisplatin
Cisplatin + BSO
II-45
CisR
ComboR


Chromosome 5
Genomic alteration (Log2 Ratio)
-4 -3 -2 -1 0 1 2 3 4
q11
q13
q21
q23
q31
q33
q35
105.3 Mb
107.6 Mb
109.9 Mb
112.2 Mb
Mtap
Cdkn2a
Cdkn2b
Dmrta1
Ifnb1
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Ifna1
Klhl9
Mlkl3
Ptplad2
Rps6
Zfp352
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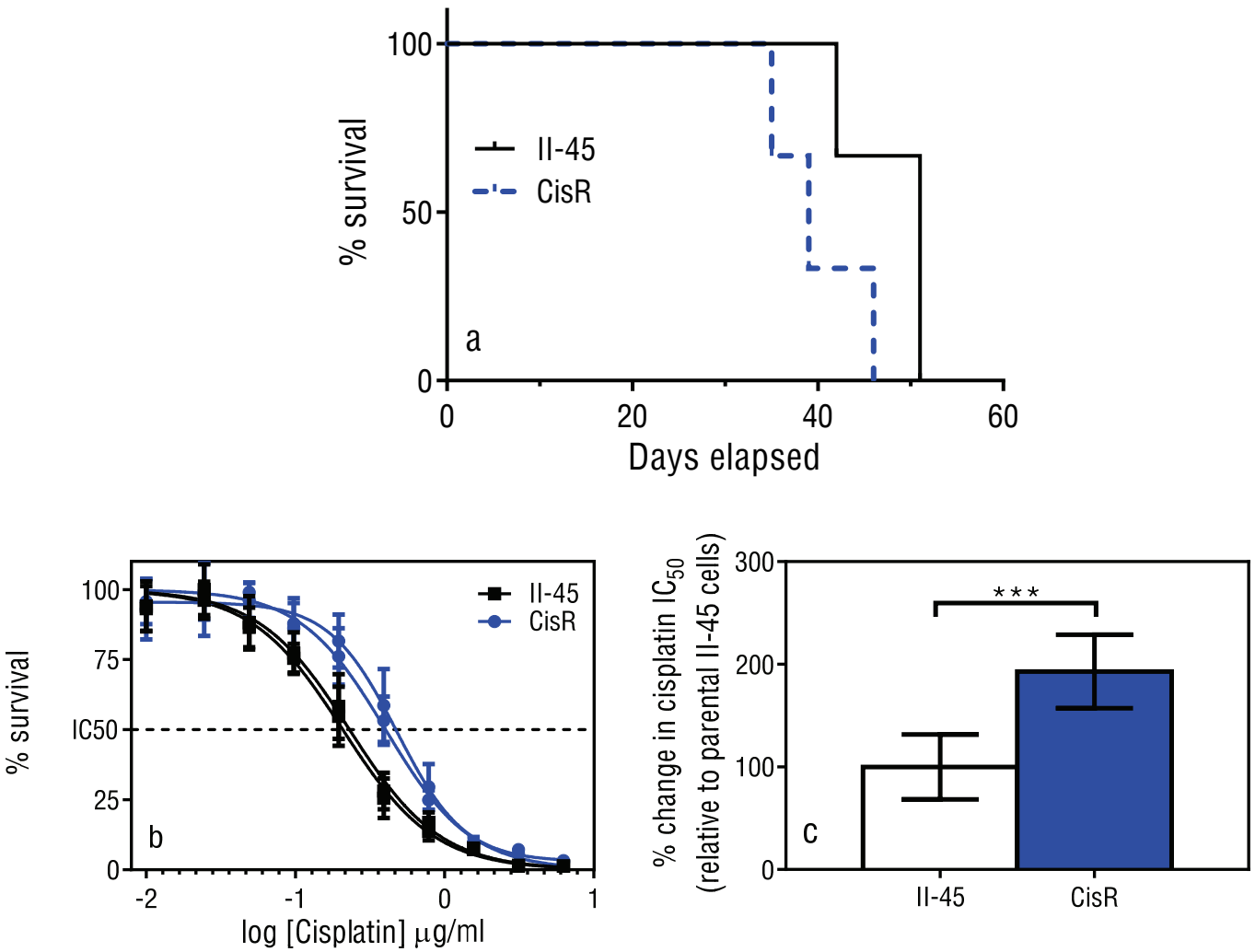
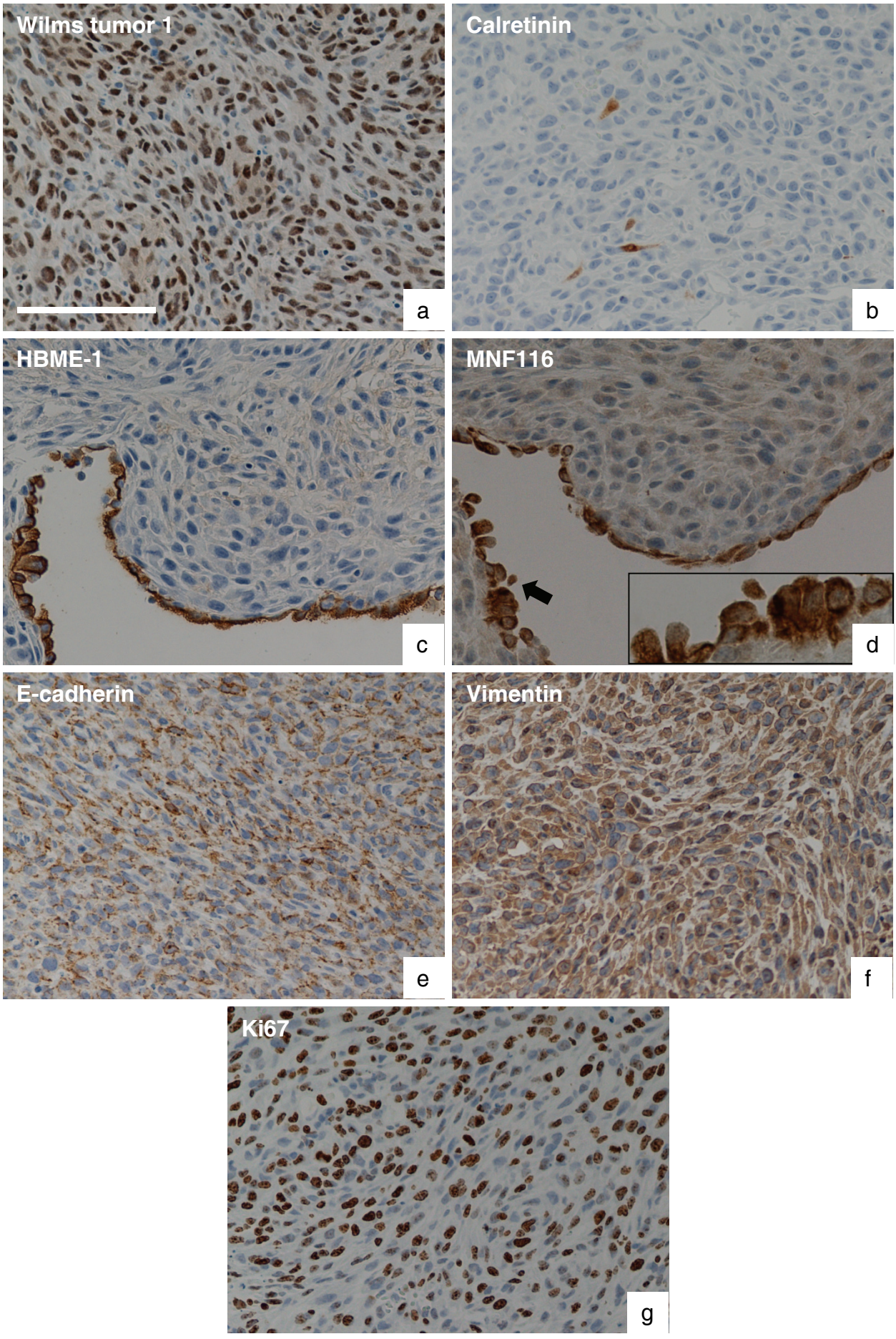

100
50
0
% survival
II-45
CisR
a
0
20
40
60
Days elapsed
100
75
IC50
25
0
% survival
II-45
CisR
b
-2
-1
0
1
log [Cisplatin] μg/ml
300
200
100
0
% change in cisplatin IC50
(relative to parental II-45 cells)
***
c
II-45
CisR


Wilms tumor 1
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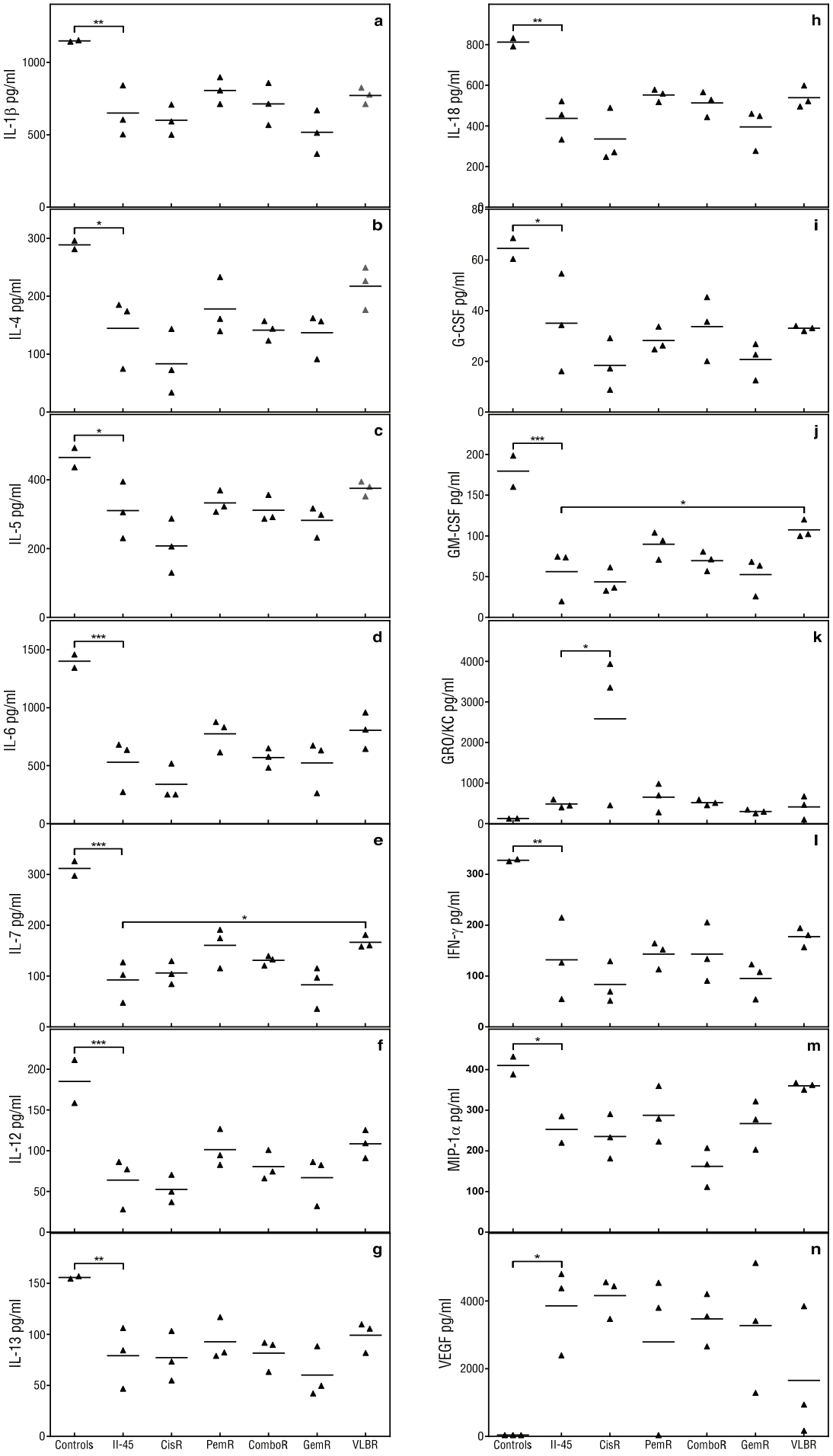
Calretinin
b
HBME-1
c
MNF116
d
E-cadherin
e
Vimentin
f
Ki67
g


a
IL-1β pg/ml
b
IL-4 pg/ml
c
IL-5 pg/ml
d
IL-6 pg/ml
e
IL-7 pg/ml
f
IL-12 pg/ml
g
IL-13 pg/ml
h
IL-18 pg/ml
i
G-CSF pg/ml
j
GM-CSF pg/ml
k
GRO/KC pg/ml
l
IFN-γ pg/ml
m
MIP-1α pg/ml
n
VEGF pg/ml
Controls
Il-45
CisR
PemR
ComboR
GemR
VLBR