Supplementary Files

Tspan8 and Tspan8/CD151 knockout mice unravel the contribution of tumor and host exosomes to tumor progression.

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Table S1
Primers

Tspan8 genotyping reverse primer 1	AGTGTGCCTTTCAGCCACTTCC
Tspan8 genotyping forward primer 2	CCCGGAGATGAGGAAGAGGAG
Tspan8 genotyping forward primer 3	TTTGAAGCGTGCAGAATGCC
CD151 genotyping reverse primer 1	CAGCTTAGGACCTCTTCTCA
CD151 genotyping forward primer 2	GCTCCATGTTCCTGTACACT
CD151 genotyping forward primer 3	GCCTCTGTTCCACATACACT
CD151 genotyping forward primer 4	ATGATAACCCACCATGTGTC

Table S2
Reagents
Table S2A
Antibodies

Antibody	**Species**[a]	**Supplier**
ADAM10	rabbit	Santa Cruz, HD, G
ADAM17 (TACE)	rabbit	Santa Cruz, HD, G
ALDH1/2	rabbit	Santa Cruz, HD, G
Alix	rabbit	Santa Cruz, HD, G
β-actin	mouse	Biozol, Munic, G
β-catenin	rabbit	Becton Dickinson, HD, G
BAD	hamster	Becton Dickinson, HD, G
pBAD	rabbit	Cell Signaling, Frankfurt, G
BAX	rabbit	Becton Dickinson, HD, G
Bcl2	rabbit	Becton Dickinson, HD, G
BclXl	rabbit	Cell Signaling, Frankfurt, G
Casp3	rabbit	Becton Dickinson, HD, G
Casp8	rabbit	Becton Dickinson, HD, G
clv.Casp9	rabbit	Cell Signaling, Frankfurt, G
CD9	rat	Becton Dickinson, HD, G
CD11b (ITαM) (YBM6.6.10)	rat	EACC[a]
CD13	rabbit	Santa Cruz, HD, G
CD24 (M1.69)	rat	EACC
CD29 (ITβ1) (Itgβ1) (KMI6)	rat	ATCC[b]
CD31 (PECAM1)	rat	Becton Dickinson, HD, G
CD34	rat	Becton Dickinson, HD, G
CD38	sheep	R&D Systems, G
CD41 (ITα2b)	rat	Becton Dickinson, HD, G
CD44 (IM7)	rat	EACC
CD47 (IAP)	rat	Becton Dickinson, HD, G
CD49a (ITα1)	monocl.hamster	Becton Dickinson, HD, G
CD49b (Itgα2)	monocl.hamster	Becton Dickinson, HD, G
CD49c (Itgα3)	goat	R&D Systems, G
CD49d (ITα4) (PS/2)	rat	ref 1[c]
CD49e (ITα5)	rat	Becton Dickinson, HD, G
CD49f (ITα6)	rat	ImmunoTools, Friesoythe, G
CD54 (ICAM1)	monocl.hamster	Becton Dickinson, HD, G
CD56 (NCAM1)	rat	ImmunoTools, Friesoythe, G
CD61 (ITβ3)	monocl.hamster	Becton Dickinson, HD, G
CD62E (selectin E)	rat	Becton Dickinson, HD, G
CD62L (selectin L)	rat	ImmunoTools, Friesoythe, G
CD62P (selectin P)	rat	Becton Dickinson, HD, G
CD63 (Tspan30)	rabbit	Santa Cruz, HD, G
CD81 (Tspan28)	monocl.hamster	ImmunoTools, Friesoythe, G
CD95	monocl.hamster	Becton Dickinson, HD, G
CD95L	monocl.hamster	Becton Dickinson, HD, G
CD102 (ICAM2)	rat	Becton Dickinson, HD, G
CD103 (ITαE)	rat	Becton Dickinson, HD, G
CD104 (ITβ4)	rat	Becton Dickinson, HD, G
CD105 (Endoglin)	rat	Becton Dickinson, HD, G
CD106 (VCAM1)	rat	Becton Dickinson, HD, G
CD117 (c-KIT)	rat	Becton Dickinson, HD, G
CD121a (IL1RI)	rat	Becton Dickinson, HD, G
CD123 (IL3R)	rat	Becton Dickinson, HD, G
CD133	goat	SantaCruz, HD, G
CD138 (Syndecan)	rat	Becton Dickinson, HD, G
CD142 (Tissue factor)	rabbit	Becton Dickinson, HD, G
CD144 (cadherin5)	rat	Becton Dickinson, HD, G
CD151 (Tspan24)	rat	R&D Systems, G

Table S2A continued

Antibody	Species[a]	Supplier
CD253 (TRAIL)	rat	Biozol, Munic, G
CD254 (TRANCE)	rat	Biozol, Munic, G
Coll I	rabbit	Rockland, Gilbertsville, USA
Coll IV	rabbit	Rockland, Gilbertsville, USA
CXCL12 (SDF1)	rabbit	abcam, Cambridge, UK
CXCR4 (CD184)	rat	Becton Dickinson, HD, G
EphA4	rabbit	SantaCruz, HD, G
FGFR3	rabbit	Cell Signaling, Frankfurt, G
FN	mouse	Becton Dickinson, HD, G
FOXO3	rabbit	SantaCruz, HD, G
FRIZZLED	rabbit	SantaCruz, HD, G
GMCSF	rat	Becton Dickinson, HD, G
Gr1	rat	ImmunoTools, Friesoythe, G
HSP70	rabbit	BioTrend, Cologne, G
HSP90	mouse	BioTrend, Cologne, G
IL1β	rat	BioTrend, Cologne, G
IL3	rat	Becton Dickinson, HD, G
Lamp1	rat	Becton Dickinson, HD, G
LIF	rabbit	SantaCruz, HD, G
LNα1	rabbit	Rockville, Gilbertsville, PA
LNγ2	rabbit	Rockville, Gilbertsville, PA
Lyve	rabbit	SantaCruz, HD, G
MCSFR (CD115)	rat	Bio Rad, Munich, G
MDR1	rabbit	SantaCruz, HD, G
MMP2	rabbit	Dianova, Hamburg, G
MMP3	rabbit	SantaCruz, HD, G
MMP7	rabbit	SantaCruz, HD, G
MMP9	rabbit	Dianova, Hamburg, G
MMP14	rabbit	SantaCruz, HD, G
Nanog	rabbit	SantaCruz, HD, G
N-Cadherin	mouse	Becton Dickinson, HD, G
NOTCH	mouse	Biolegend, San Diego, Ca, US
Oct3	rabbit	SantaCruz, HD, G
OPN	rabbit	BioTrend, Cologne, G
Podoplanin	hamster	Biozol, Munic, G
S100A4	rabbit	abcam, Cambridge, UK
SCA1 (Ly6A)	rat	Becton Dickinson, HD, G
SCF (KITL)	rat	Bio Rad, Munich, G
Slug	rabbit	SantaCruz, HD, G
Snail	rabbit	SantaCruz, HD, G
Sox2	rabbit	SantaCruz, HD, G
Ter119	rat	Becton Dickinson, HD, G
TGFβ1	rat	Becton Dickinson, HD, G
Thrombospondin	rabbit	SantaCruz, HD, G
TNFα	rat	Becton Dickinson, HD, G
TNFRI (CD120a)	hamster	Becton Dickinson, HD, G
TNFRII (CD120b)	rabbit	SantaCruz, HD, G
TSG101	rabbit	SantaCruz, HD, G
TSP1	rabbit	SantaCruz, HD, G
Tspan8	rabbit/rat	home made/R&D Systems, G
Twist	rabbit	Becton Dickinson, HD, G
uPA	rabbit	American Diagnostica, Stamford, G
uPAR	rabbit	American Diagnostica, Stamford, G
VEGFR1	rabbit	Dianova, Hamburg, G
VEGFR2 (CD309) (FLK1)	rabbit	Dianova, Hamburg, G
VEGFR3 (FLT4)	rat	Becton Dickinson, HD, G
Vimentin	mouse	Becton Dickinson, HD, G
vWF	rabbit	abcam, Cambridge, UK ZAP70

Table S2A continued

Antibody	**Species**[a]	**Supplier**
Wnt1	rabbit	Santa Cruz, Heidelberg, G
Wnt5a	rabbit	Santa Cruz, Heidelberg, G
ZEB1	rabbit	SantaCruz, HD, G
dye or biotin labeled secondary antibodies /Streptavidin		Dianova, Becton Dickinson, Amersham

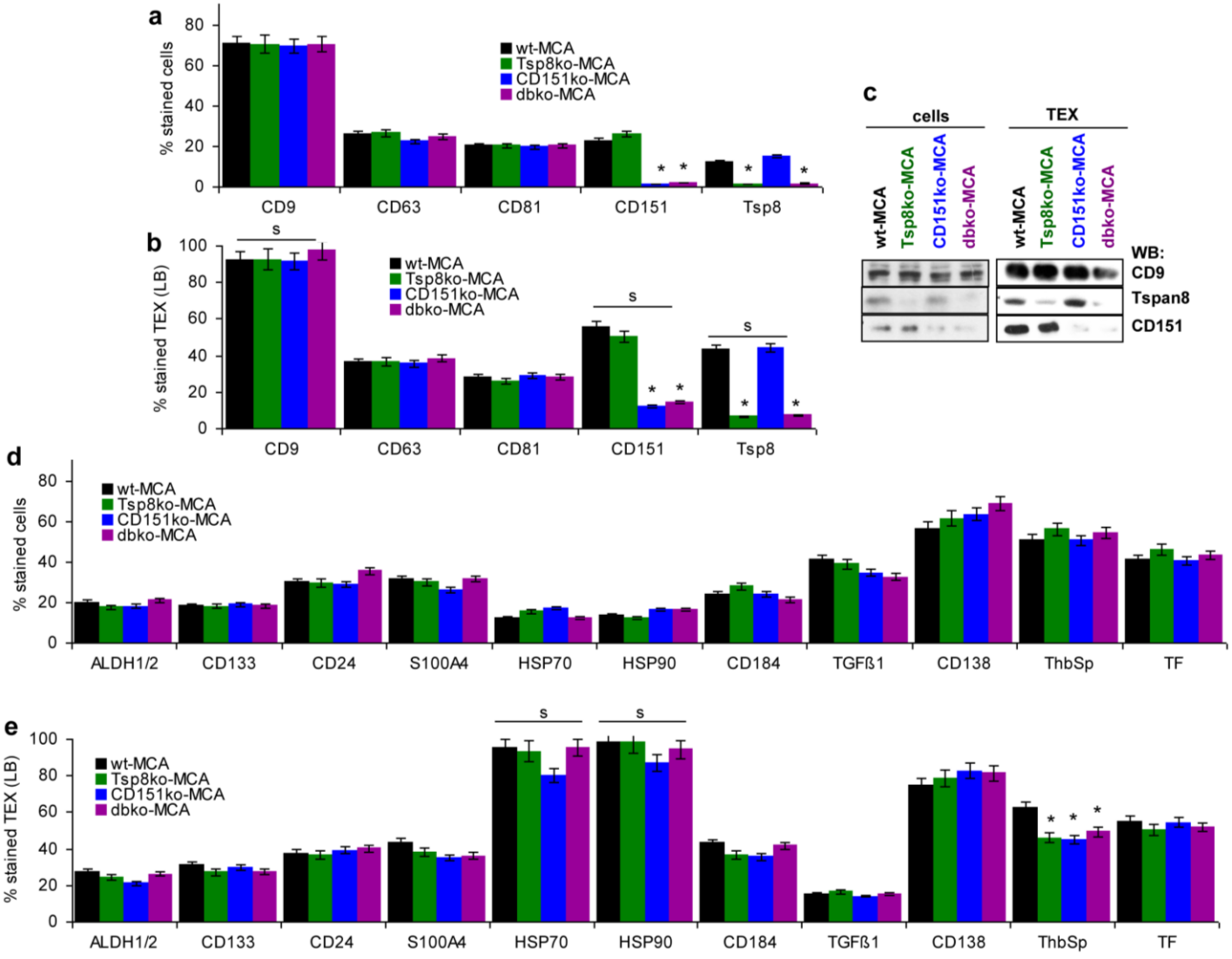
Table S2B
Chemicals

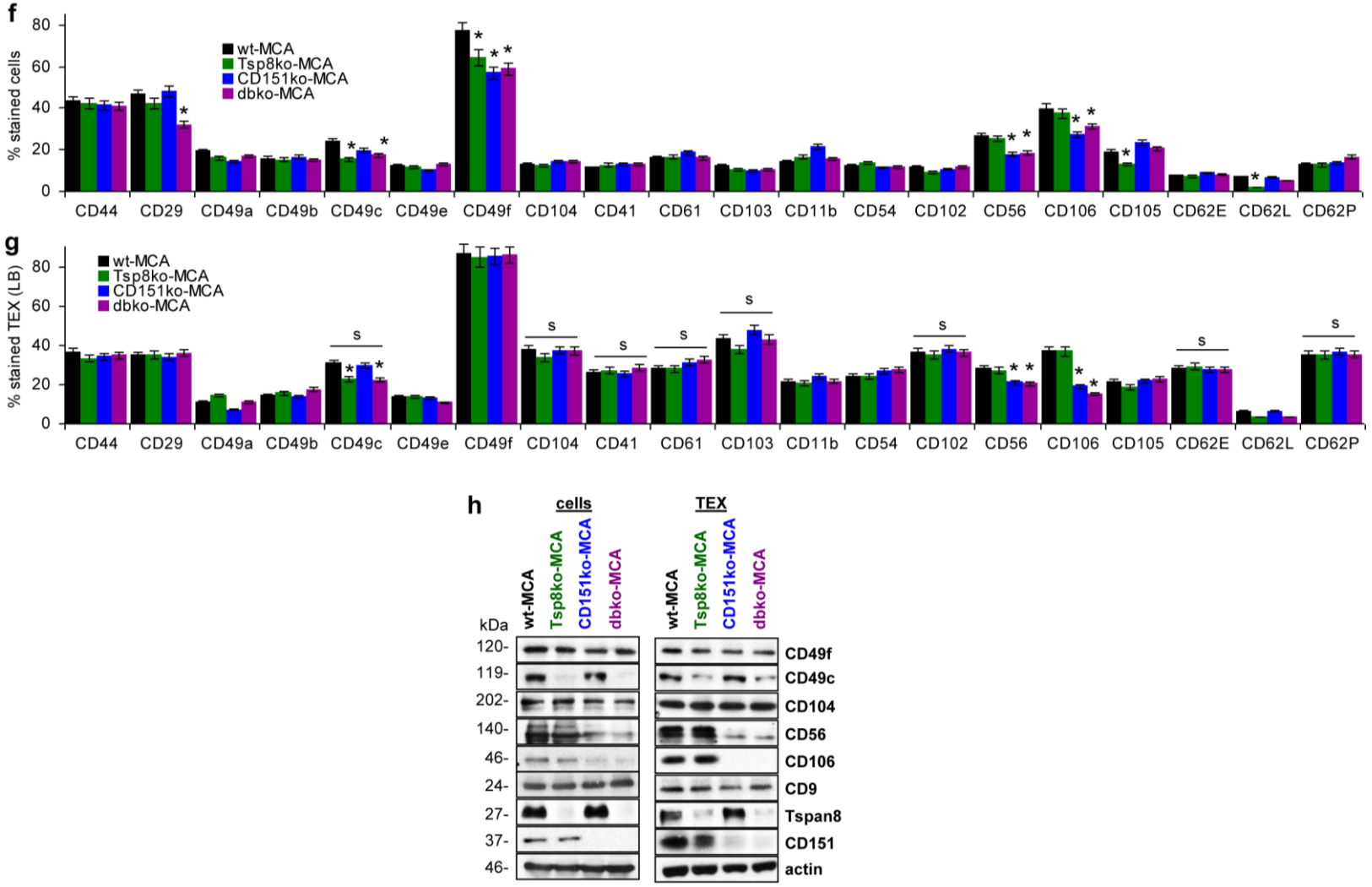
Matrix proteins	**Dose**	**Supplier**
Coll I	10µg/ml	Sigma, Munic, G
Coll IV	10µg/ml	Sigma, Munic, G
FN (Fibronectin)	2µg/ml	Sigma, Munic, G
LN 111	1µg/ml	Sigma, Munic, G
LN332	10µg/ml	804G, exosome-depleted culture supernatant w/o FCS (ref.2)

Chemicals	**Dose**	**Supplier**
AnnexinV-FITC / APC	0.2µl-0.5µl	Becton Dickinson, HD, G
Heparin	10U/ml	Radiopharm, Ulm, G
Latex beads 4µm		Invitrogen, Karlsruhe, G
Lymphoprep		GIBCO, Darmstadt, G
MCA (3-methylcholanthrene)	30µl, 0.5% sol., i.m.	Sigma Aldrich, Heidelberg, G
Matrigel	1:5 dilution	Becton Dickinson, HD, G
PI	0.08µg	Becton Dickinson, HD, G
SP-Dio_{18}(3)	1:1000 dilution	Invitrogen, Karlsruhe, G
Transferrin	1:100	Sigma, Munic, G

[a] EACC: European Collection of Animal Cell Cultures
[b] ATCC: American Type Cell Culture Collection
[c] References

1. Hession C, Moy P, Tizard R, Chisholm P, Williams C, Wysk M, et al. Cloning of murine and rat vascular cell adhesion molecule-1. Biochem Biophys Res Commun. 1992;183:163-9.
2. Izumi K, Hirao Y, Hopp L, Oyasu R. In vitro induction of ornithine decarboxylase in urinary bladder carcinoma cells. Cancer Res. 1981;41:405-9.


a
% stained cells
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
CD9
CD63
CD81
CD151
Tsp8
b
% stained TEX (LB)
c
cells
TEX
WB:
CD9
Tspan8
CD151
d
ALDH1/2
CD133
CD24
S100A4
HSP70
HSP90
CD184
TGFß1
CD138
ThbSp
TF
e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% stained cells
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
CD44 CD29 CD49a CD49b CD49c CD49e CD49f CD104 CD41 CD61 CD103 CD11b CD54 CD102 CD56 CD106 CD105 CD62E CD62L CD62P
g
% stained TEX (LB)
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
CD44 CD29 CD49a CD49b CD49c CD49e CD49f CD104 CD41 CD61 CD103 CD11b CD54 CD102 CD56 CD106 CD105 CD62E CD62L CD62P
h
cells
TEX
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
kDa
120-
119-
202-
140-
46-
24-
27-
37-
46-
CD49f
CD49c
CD104
CD56
CD106
CD9
Tspan8
CD151
actin

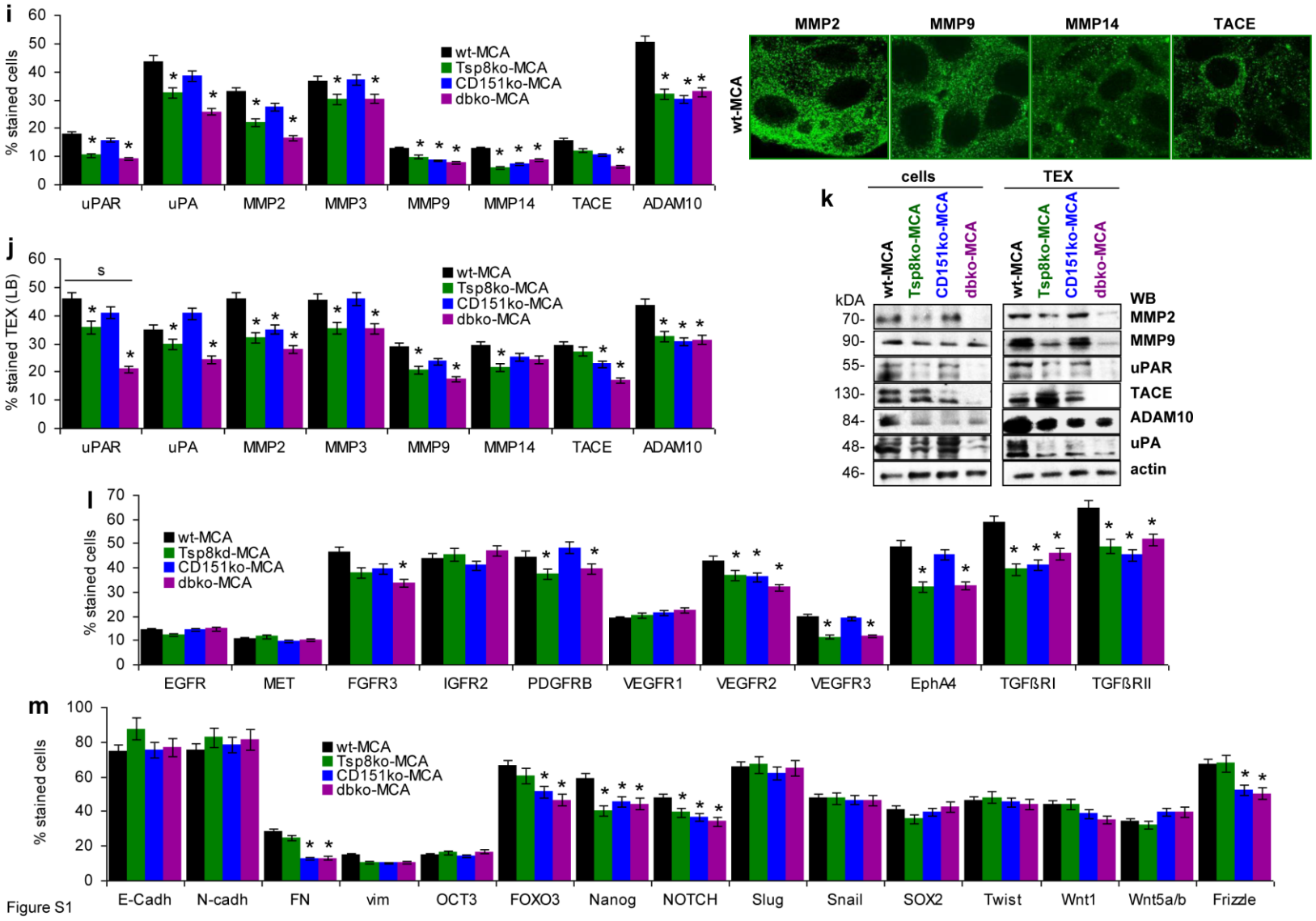
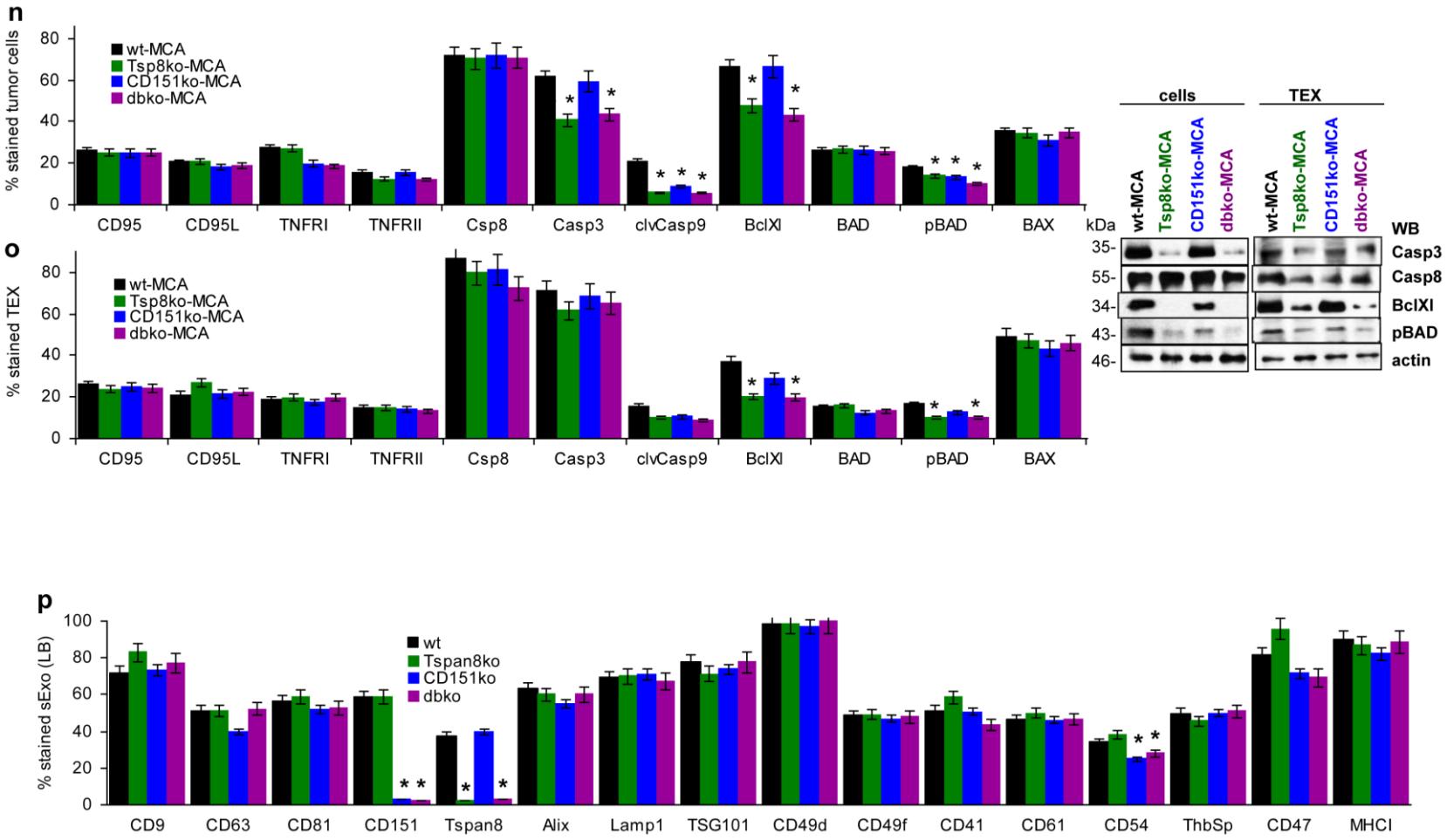

i
% stained cells
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
uPAR
uPA
MMP2
MMP3
MMP9
MMP14
TACE
ADAM10
MMP2
MMP9
MMP14
TACE
wt-MCA
j
% stained TEX (LB)
s
k
cells
TEX
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
kDA
WB
MMP2
MMP9
uPAR
TACE
ADAM10
uPA
actin
l
% stained cells
wt-MCA
Tsp8kd-MCA
CD151ko-MCA
dbko-MCA
EGFR
MET
FGFR3
IGFR2
PDGFRB
VEGFR1
VEGFR2
VEGFR3
EphA4
TGFßRI
TGFßRII
m
% stained cells
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
E-Cadh
N-cadh
FN
vim
OCT3
FOXO3
Nanog
NOTCH
Slug
Snail
SOX2
Twist
Wnt1
Wnt5a/b
Frizzle

Figure S1


n
% stained tumor cells
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
CD95
CD95L
TNFRI
TNFRII
Csp8
Casp3
clvCasp9
BclXl
BAD
pBAD
BAX
o
% stained TEX
cells
TEX
kDa
WB
Casp3
Casp8
BclXl
pBAD
actin
35-
55-
34-
43-
46-
p
% stained sExo (LB)
wt
Tspan8ko
CD151ko
dbko
CD9
CD63
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CD49f
CD41
CD61
CD54
ThbSp
CD47
MHCI

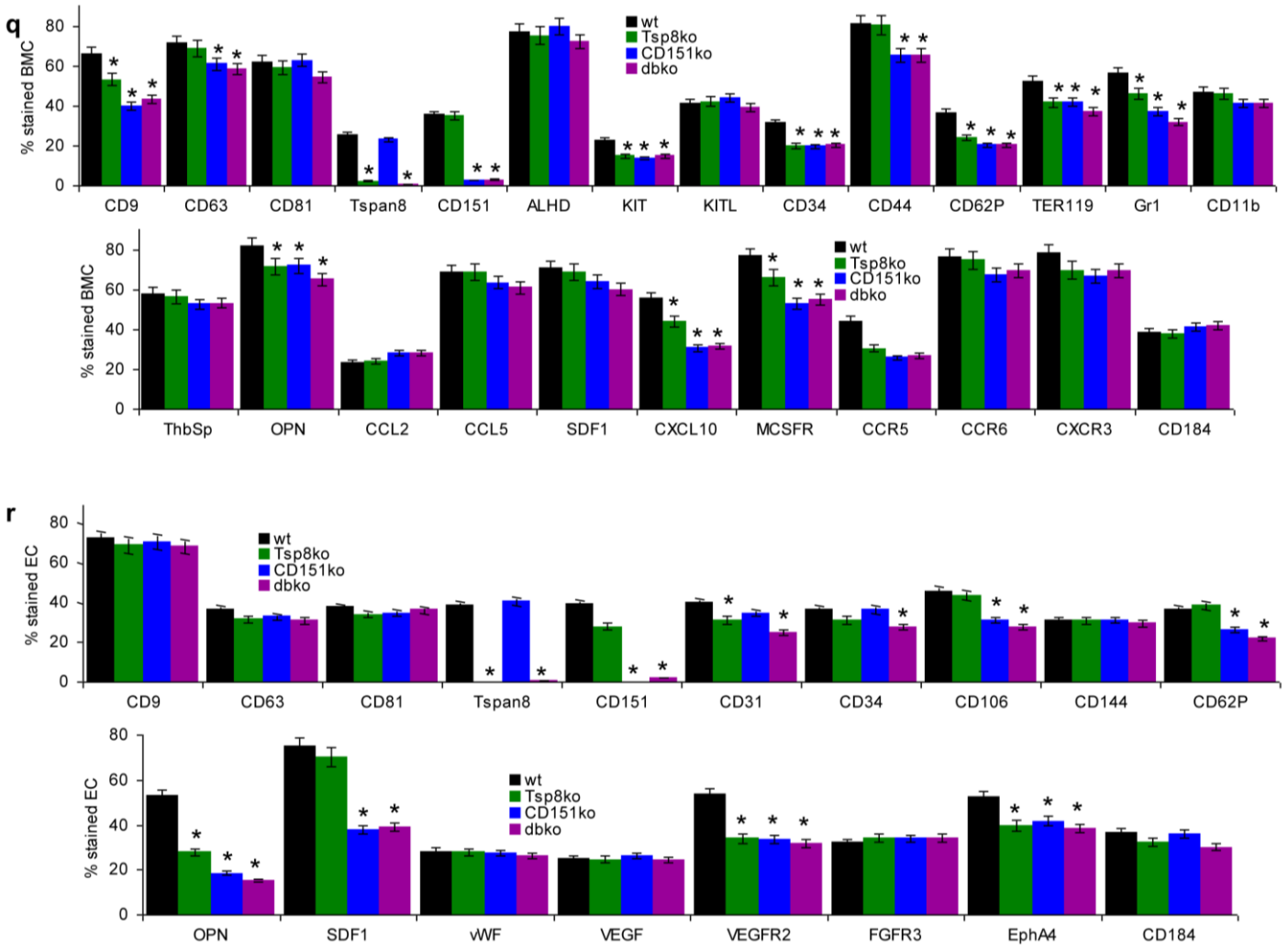
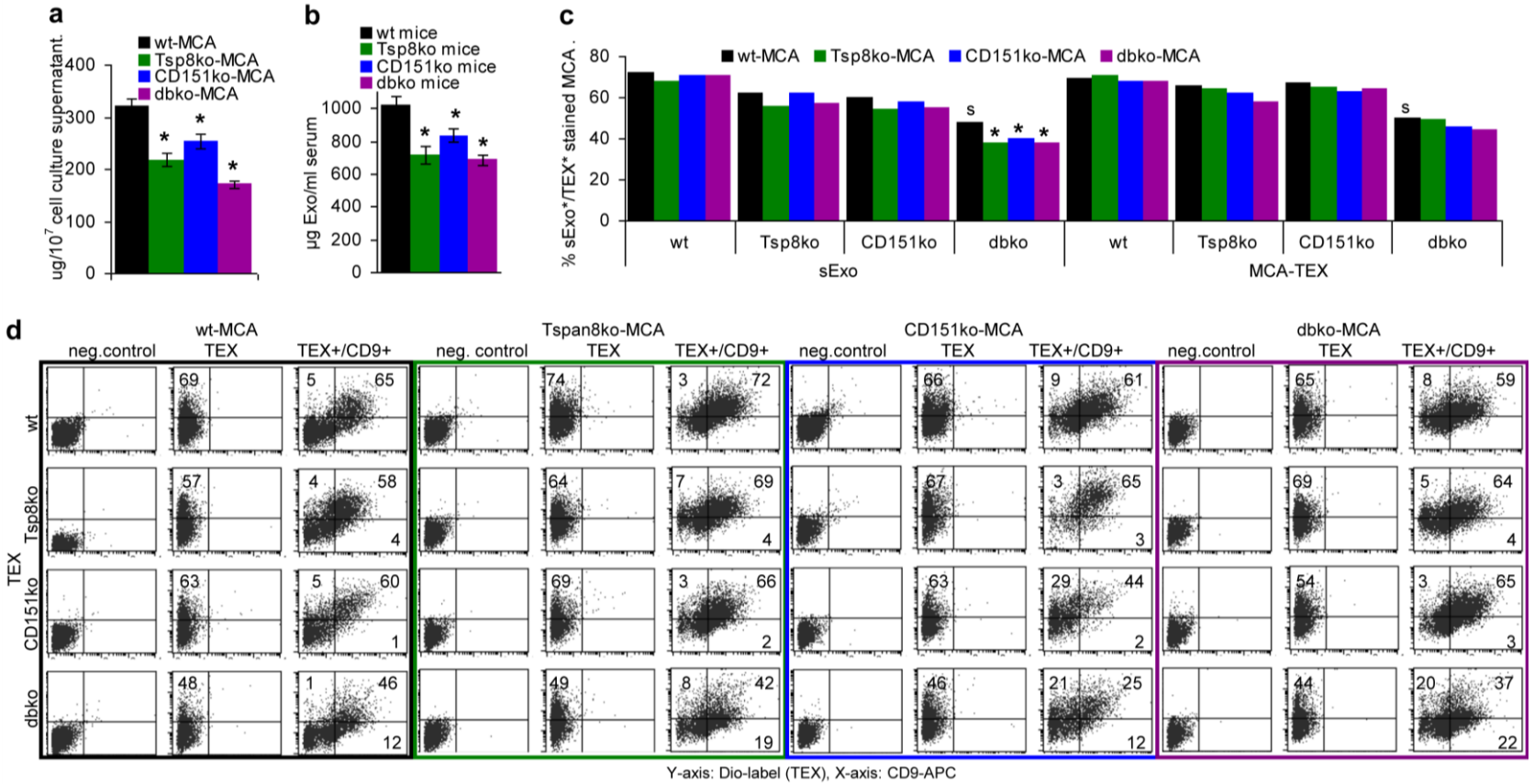


Figure S1 **Characterization of MCA tumors, bone marrow cells and endothelial cells from Tspan8ko- and/or CD151ko-mice.** (a,b) Flow-cytometry evaluation of tetraspanin expression in wt- and ko-MCA tumor cells and TEX and (c) confirmation by WB; (d,e) flow-cytometry evaluation of common tumor markers in wt- and ko-MCA tumor cells and TEX; (f,g) flow-cytometry evaluation of adhesion molecules in wt- and ko-MCA tumor cells and TEX and (h) confirmation by WB; (i,j) flow-cytometry evaluation of proteases in wt- and ko-MCA tumor cells and TEX including examples of confocal microscopy and (k) confirmation by WB; (l) flow-cytometry evaluation of oncogenic receptors in wt- and ko-MCA tumor cells; (m) flow-cytometry evaluation of EMT markers and transcription factors in wt- and ko-MCA tumor cells; (n,o) flow-cytometry evaluation of apoptosis markers in wt- and ko-MCA tumor cells and TEX and confirmation by WB; (p) flow-cytometry evaluation of tetraspanins and Exo markers in sExo; (q) flow-cytometry evaluation of tetraspanins and hematopoietic markers in wt- and ko-BMC; (r) flow-cytometry evaluation of tetraspanins and endothelial cell markers in wt- and ko-EC. Flow cytometry values represent the mean % stained cells / TEX or sExo±SD (3assays); significant differences between wt- and ko-cells, TEX and sExo: *, significant differences between cells and TEX: s.
Tumor marker and sExo marker expression are not severely affected by the Tspan8ko and/or CD151ko. Only ThbSp expression is slightly reduced in ko-TEX. Ko MCA cells and TEX differed in few adhesion molecules, only CD56 and CD106 expression being reduced in CD151ko- and dbko- and CD49c in Tspan8ko- and dbko-MCA cells and TEX. Oncogenic receptors, EMT and apoptosis markers were hardly affected, but protease expression frequently was reduced in ko-MCA cells and TEX. In BMC differences in hematopoietic progenitor markers were dominating. In EC some angiogenic factors and receptors were recovered at a reduced level in ko cells. Differences in expression were mostly weak, but observed in cells and TEX. Only some of the differences were restricted to either Tspan8ko- or CD151ko-cells / TEX.


a
ug/10^7 cell culture supernatant.
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
b
µg Exo/ml serum
wt mice
Tsp8ko mice
CD151ko mice
dbko mice
c
% sExo+/TEX+ stained MCA.
wt-MCA
Tsp8ko-MCA
CD151ko-MCA
dbko-MCA
wt
Tsp8ko
CD151ko
dbko
sExo
MCA-TEX
d
neg.control
wt-MCA TEX
TEX+/CD9+
neg. control
Tspan8ko-MCA TEX
TEX+/CD9+
neg.control
CD151ko-MCA TEX
TEX+/CD9+
neg.control
dbko-MCA TEX
TEX+/CD9+
TEX
wt
Tsp8ko
CD151ko
dbko
Y-axis: Dio-label (TEX), X-axis: CD9-APC

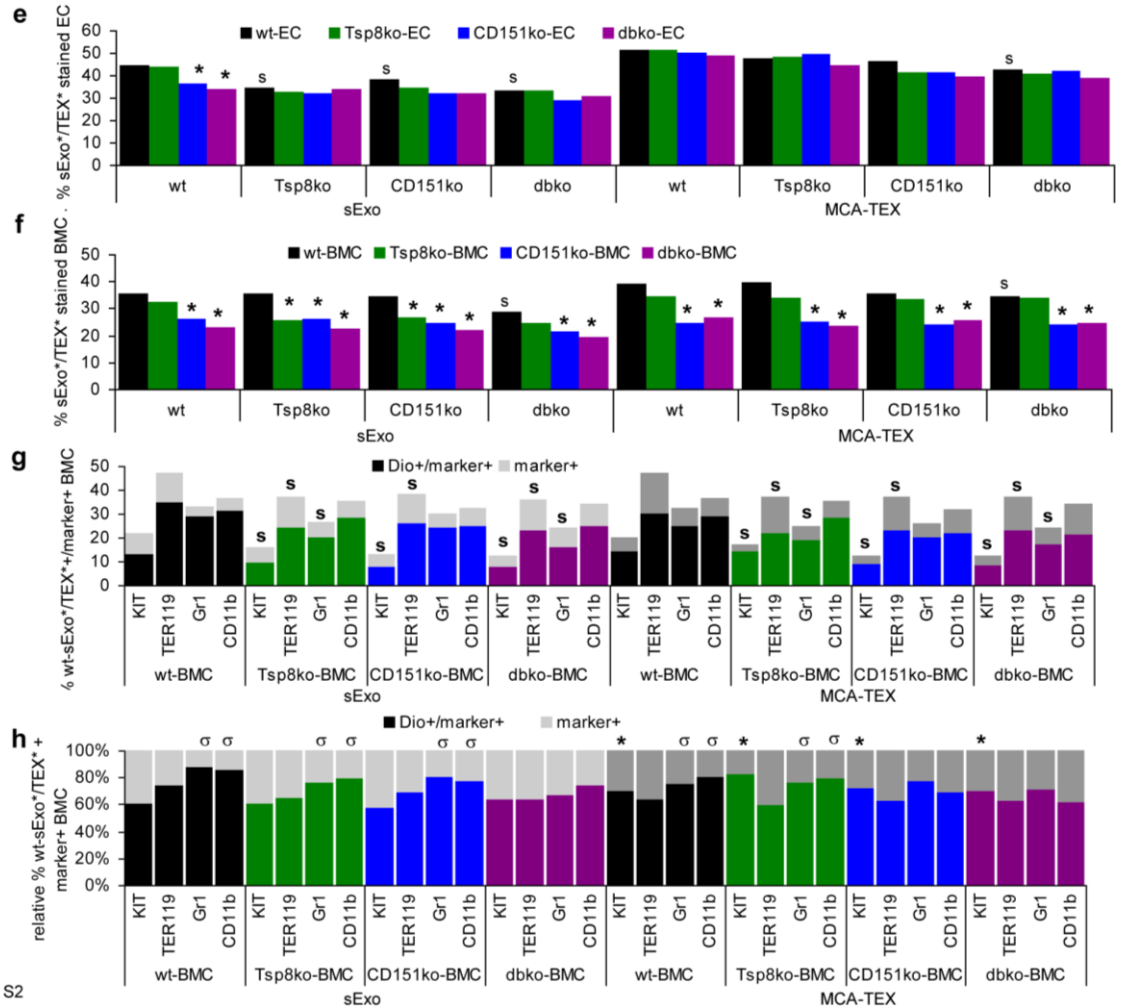
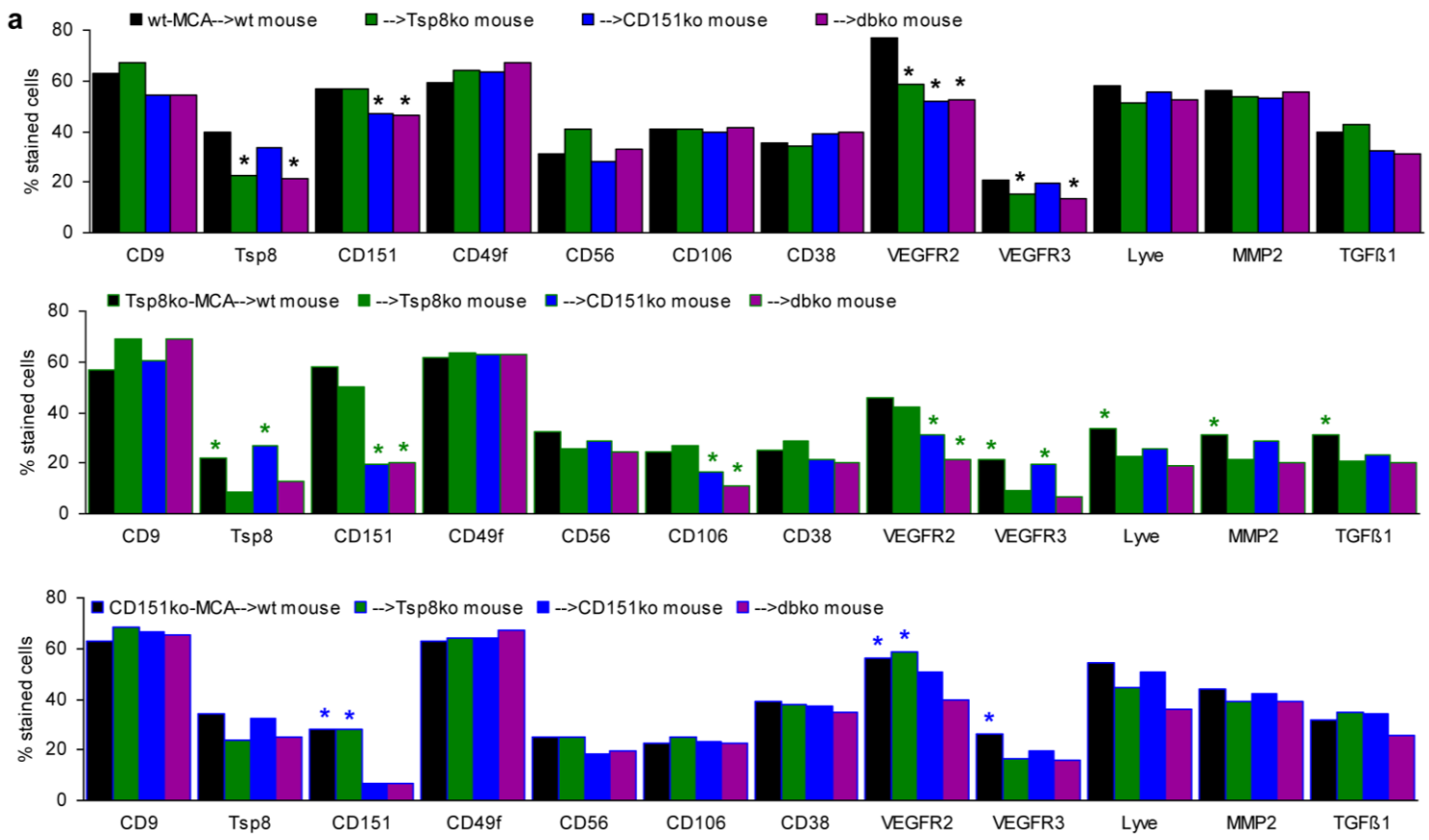
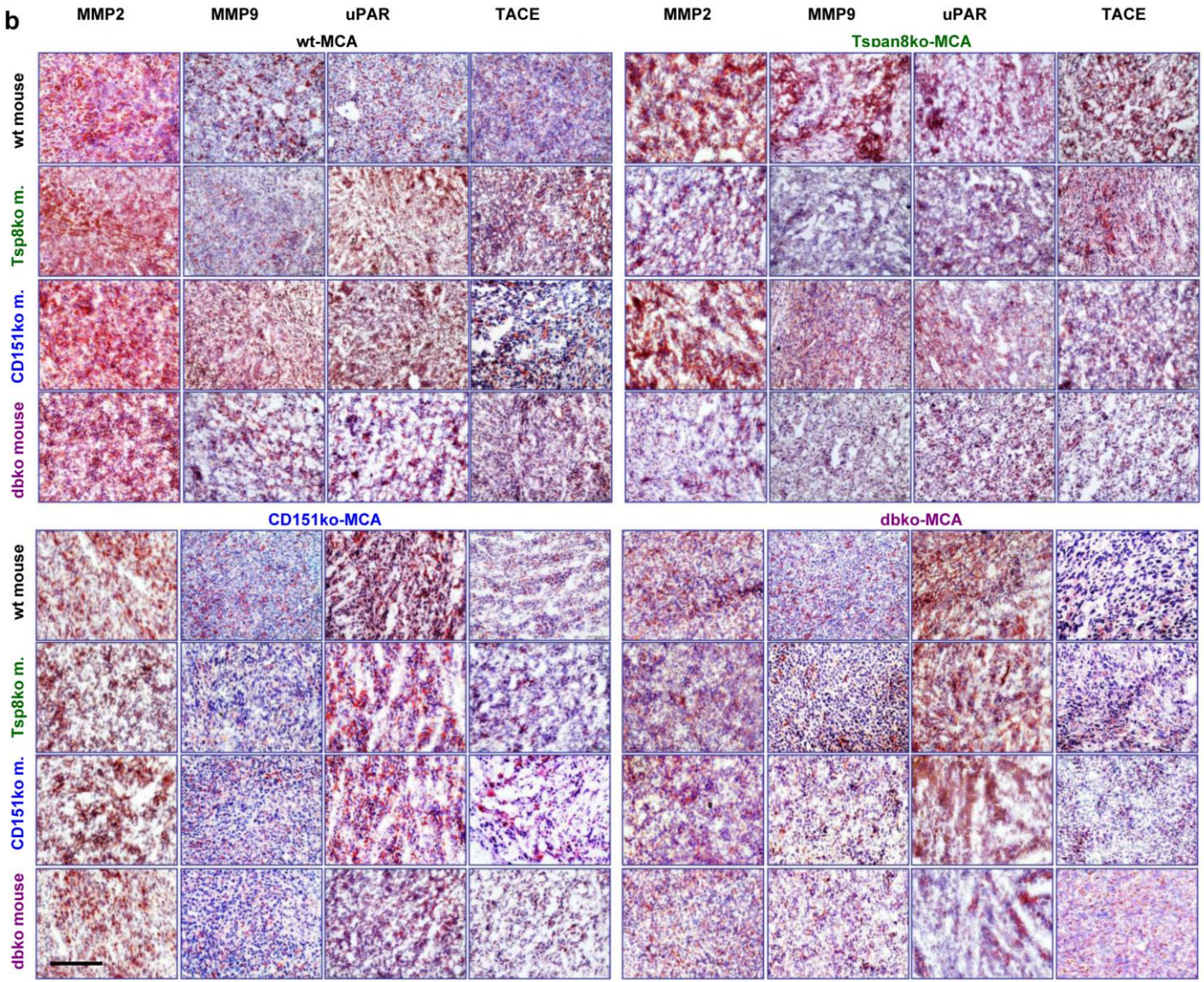


Figure S2

Figure S2 **Tspan8- and CD151-dependence of TEX and sExo delivery and uptake**. (a) TEX were collected from wt- and ko-MCA tumors after 48h culture in the absence of FCS; the amount of TEX/10^7 cells was evaluated by Bradford; mean µg/10^7 cells±SD of 3 cultures; significant differences between wt- and ko-TEX: *; (b) wt and ko mice were bled by heart puncture. Plasma was separated from cells by centrifugation and was subjected to Exo purification; mean µg/ml±SD of 5 samples/group; significant differences between wt- and ko-sExo: *; (c-h) sExo and TEX uptake was evaluated by flow-cytometry after 2h-3h coculture with Dio-labeled sExo/TEX; (c,e,f) Dio-labeled sExo and TEX uptake by MCA tumor cells, EC and BMC; the mean percent (triplicates) sExo+/TEX+ cells is shown; significant differences between wt- and ko-sExo/TEX are indicated by s; significant differences between wt- versus ko-acceptor cells are indicated by *; (d) wt- and ko-MCA were incubated with Dio-labeled wt- and ko-TEX and counterstained with anti-CD9-APC; representative examples and the mean % of Dio+/CD9+ and Dio-/CD9+ cells are shown; (g,h) wt- and ko-BMC were incubated with Dio-labeled wt-sExo and -TEX and counterstained for the indicated markers; in (g) the percent of Dio+marker+ and Dio-marker+ BMC is shown; differences in the uptake by ko-BMC compared to wt-BMC: s; (h) the experiment shown in (g) was evaluated according to the percent of Dio-labeled subpopulations, differences between subpopulations: σ, differences between sExo and TEX: s.

Ko MCA cells deliver less TEX and ko mice deliver less sExo. Only dbko-sExo and -TEX uptake is impaired in MCA, cells, EC and BMC. BMC subpopulations differ slightly in uptake being strongest in myeloid progenitors. Furthermore, KIT+ cells take up TEX more readily than sExo.


a
wt-MCA-->wt mouse
-->Tsp8ko mouse
-->CD151ko mouse
-->dbko mouse
% stained cells
0
20
40
60
80
CD9
Tsp8
CD151
CD49f
CD56
CD106
CD38
VEGFR2
VEGFR3
Lyve
MMP2
TGFß1
Tsp8ko-MCA-->wt mouse
-->Tsp8ko mouse
-->CD151ko mouse
-->dbko mouse
% stained cells
CD151ko-MCA-->wt mouse
-->Tsp8ko mouse
-->CD151ko mouse
-->dbko mouse
% stained cells


b
MMP2
MMP9
uPAR
TACE
wt-MCA
Tspan8ko-MCA
CD151ko-MCA
dbko-MCA
wt mouse
Tsp8ko m.
CD151ko m.
dbko mouse

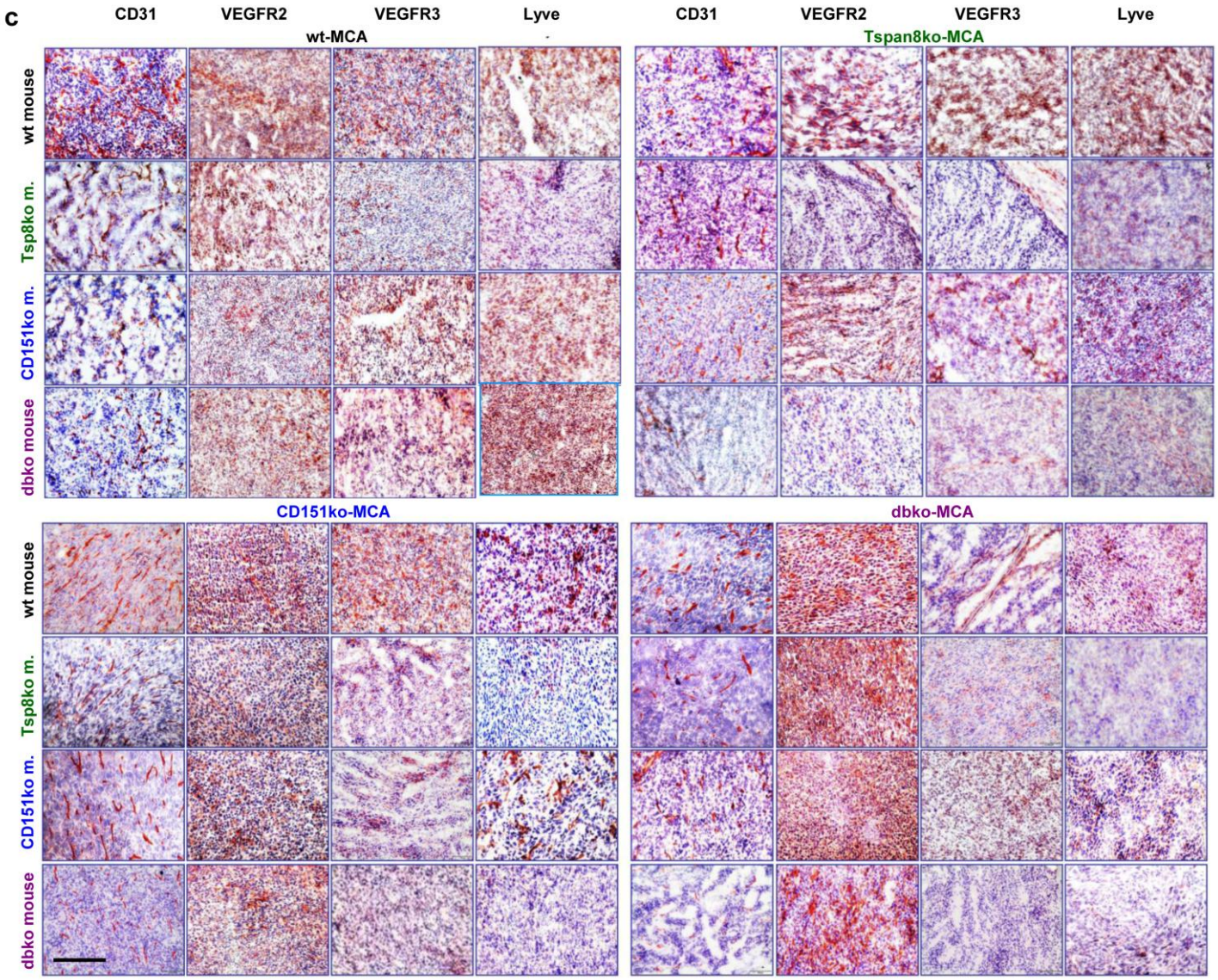


Figure S3 **Ex vivo analysis of tetraspanin, adhesion molecule and angiogenic factor expression in MCA tumors**. (a) At autopsy, wt, Tspan8ko and CD151ko tumor tissue after growth in wt- and ko-mice was meshed and analyzed by flow-cytometry for expression of the indicated markers. Mean % stained cells (3 mice/group) are shown; significant differences to the syngeneic, non-autochthonous host: *. (b,c) Immunohistochemistry examples of protease and angiogenesis-related marker expression in shock frozen tumor sections (scale bar: 100µm).
Flow-cytometry uncovered an impact of the host on Tspan8, CD151, VEGFR2, VEGFR3, Lyve, MMP2 and TGFβ1 expression. The impact of the host was most pronounced in Tspan8ko MCA tumors. Immunohistochemistry confirmed the impact of the host on protease with a particularly strong increase in MMP2 and MMP9 in Tspan8ko-MCA grown in wild type mice. Angiogenesis was most strongly increased in CD151ko-MCA grown in wt mice and VEGFR3 and Lyve in Tspan8ko-MCA grown in wt mice.

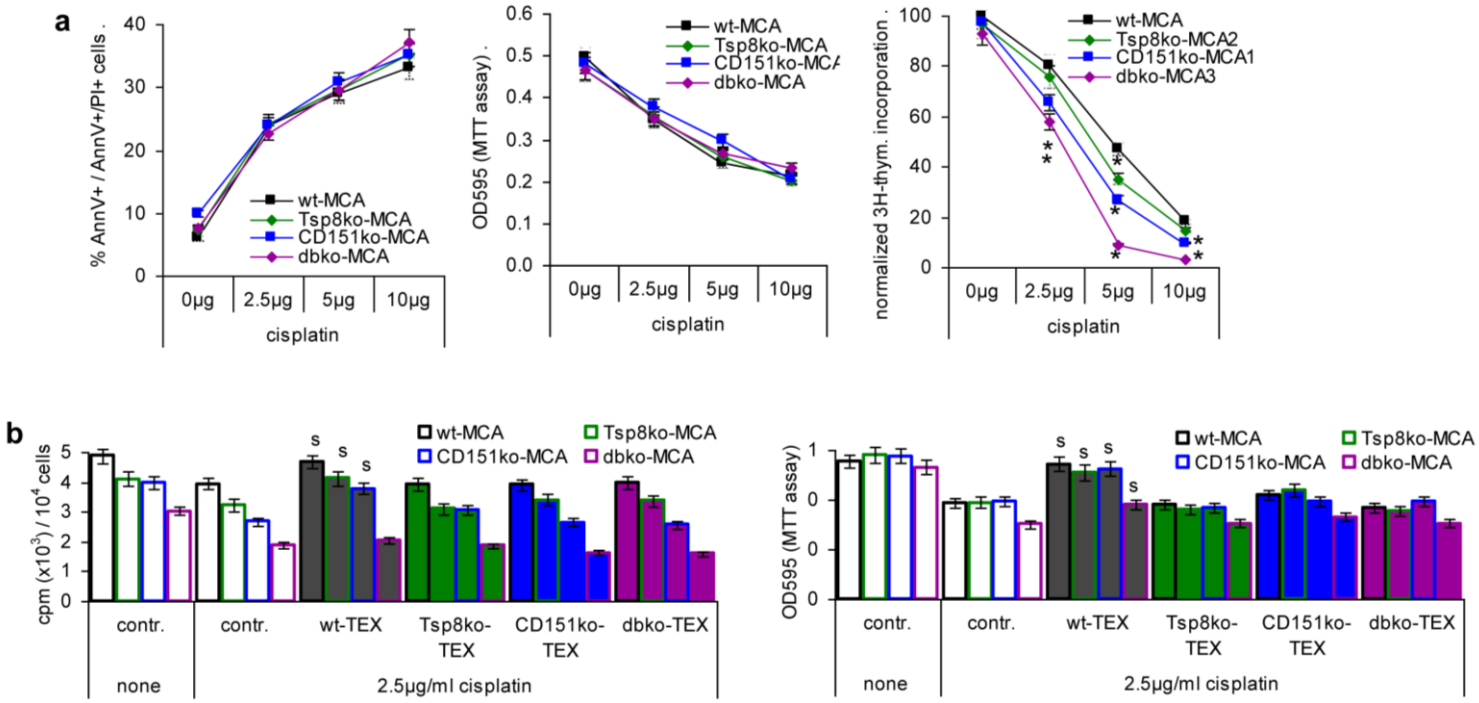


Figure S4 **TEX and apoptosis resistance of wt- and ko-MCA tumor cells**. (a) Apoptosis susceptibility of wt- and ko-MCA cells was evaluated after 48h culture in the presence of increasing doses of cisplatin by AnnV and PI staining (% AnnV+ and AnnV+/PI+ cells), by the MTT assay (mitochondrial integrity) and 3H-thymidine uptake; mean values±SD of triplicates are shown; significant differences between wt- and ko-MCA cells: *; (b) proliferation and MTT assay of cisplatin-treated (2.5µg/ml) wt- and ko-MCA cells cultured in the absence or presence of wt- and ko-TEX; mean cpm/10^4 cells±SD and mean OD±SD (triplicates); significant differences in the presence of TEX: s.
High apoptosis susceptibility of these MCA tumors is not significantly affected by a Tspan8ko and/or a CD151ko and only wt-TEX support wt-, Tspan8ko- and CD151ko-MCA tumor cell proliferation in the presence of cisplatin.

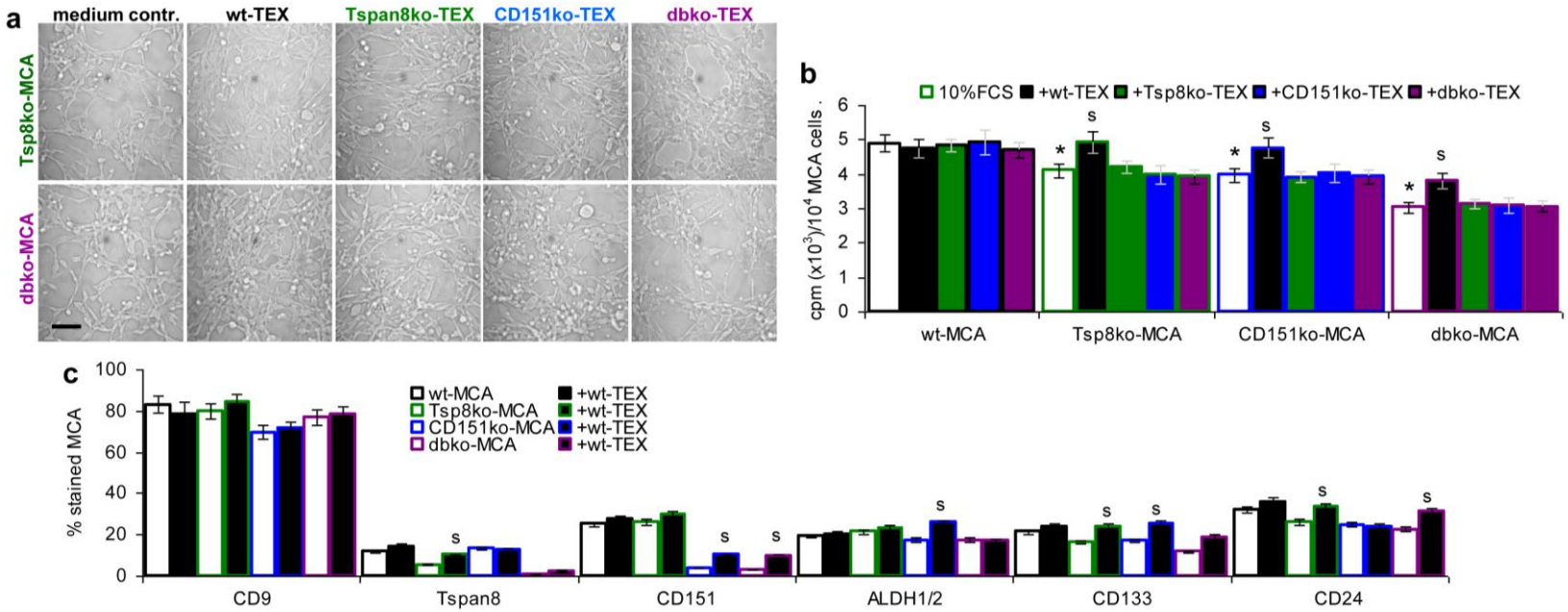
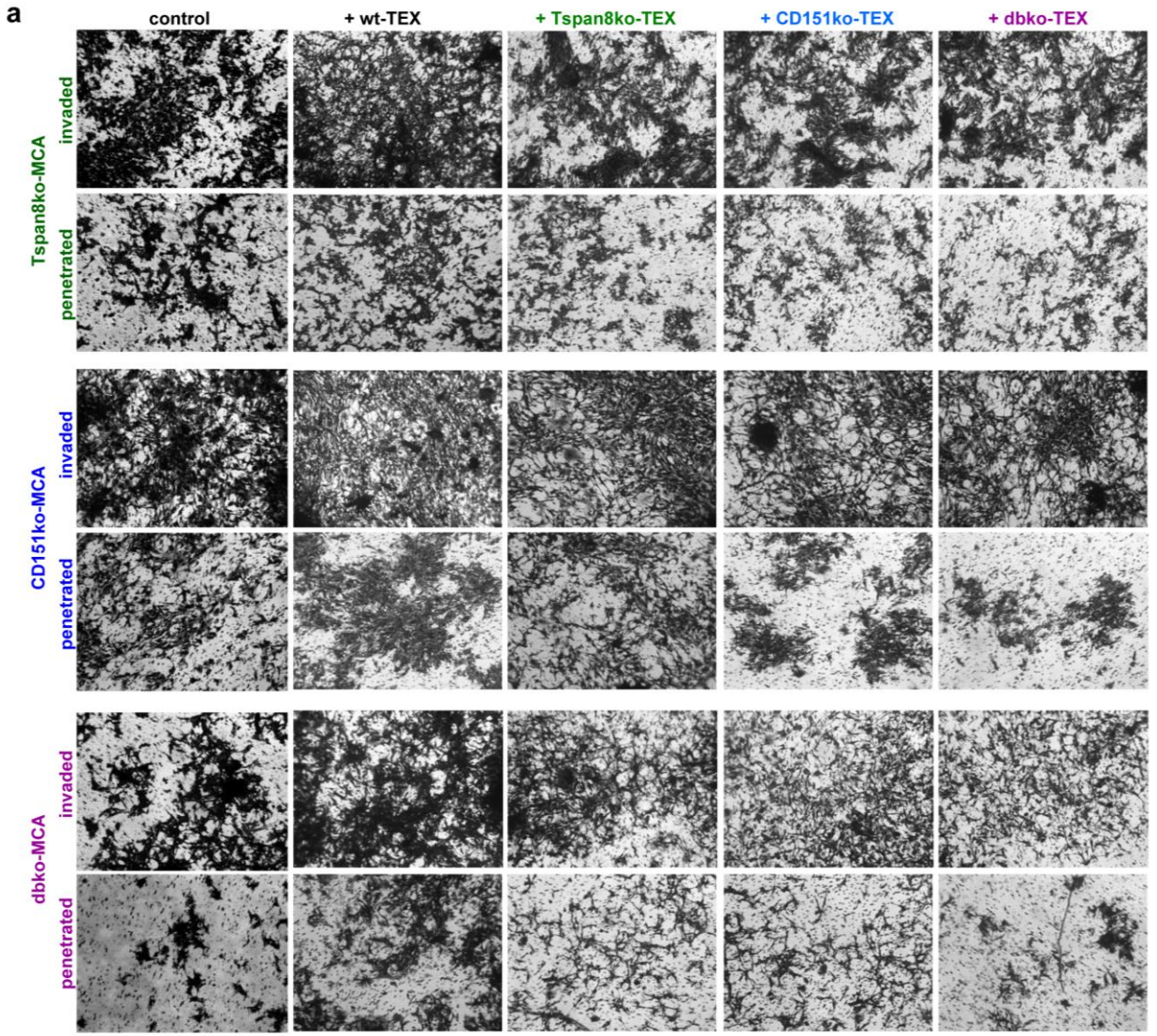


Figure S5 **The impact of TEX on EMT**. (a) Tspan8ko- and dbko-MCA cells were seeded at low density in 6-well plates in the absence or presence of wt- and ko-TEX. Cell density and morphological appearance was evaluated after 72h by light microscopy (scale bar: 100µm); (b) Proliferation (3H-thymidine incorporation) of wt- and ko-MCA cells in the presence or absence of wt- and ko-TEX (30µg/ml); mean cpm/10^4 cells±SD (triplicates); significant differences between wt- and ko-MCA cells: *, significant differences in the absence versus presence of TEX: s. (c) WT- and ko-MCA cells were cocultured with wt-TEX for 48h; flow-cytometry analysis of tetraspanins and stem cell markers expression; the mean % stained cells±SD (3 replicates); significant differences by coculture with TEX: s.
Despite a slight shift towards a more fibroblastoid phenotype, proliferative activity does not strongly vary between wt- and ko-MCA tumors and only wt-TEX slightly promote proliferation. Only wt TEX support stem cell marker CD133 and CD24 expression.


a
control
+ wt-TEX
+ Tspan8ko-TEX
+ CD151ko-TEX
+ dbko-TEX
Tspan8ko-MCA
invaded
penetrated
CD151ko-MCA
invaded
penetrated
dbko-MCA
invaded
penetrated

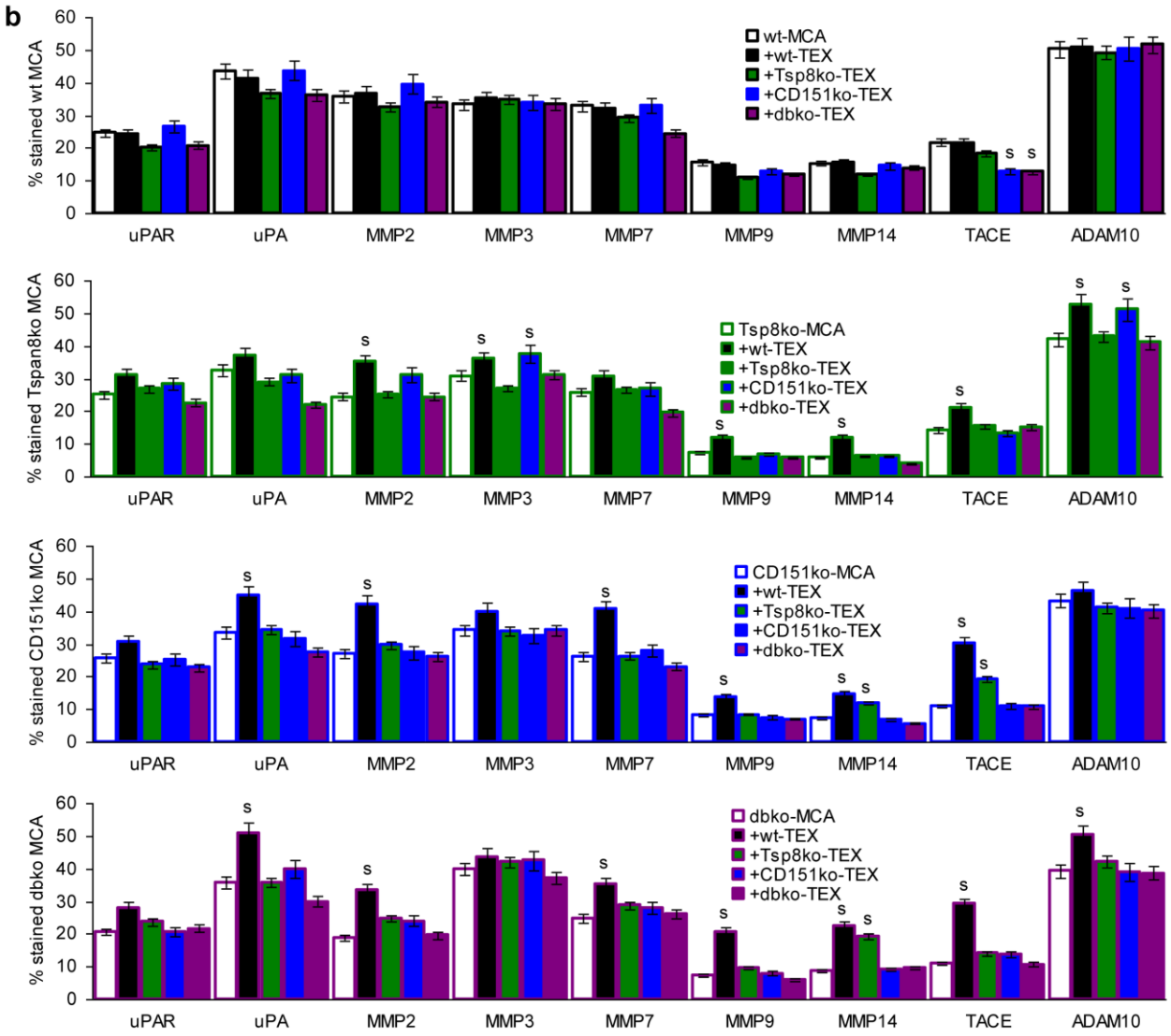
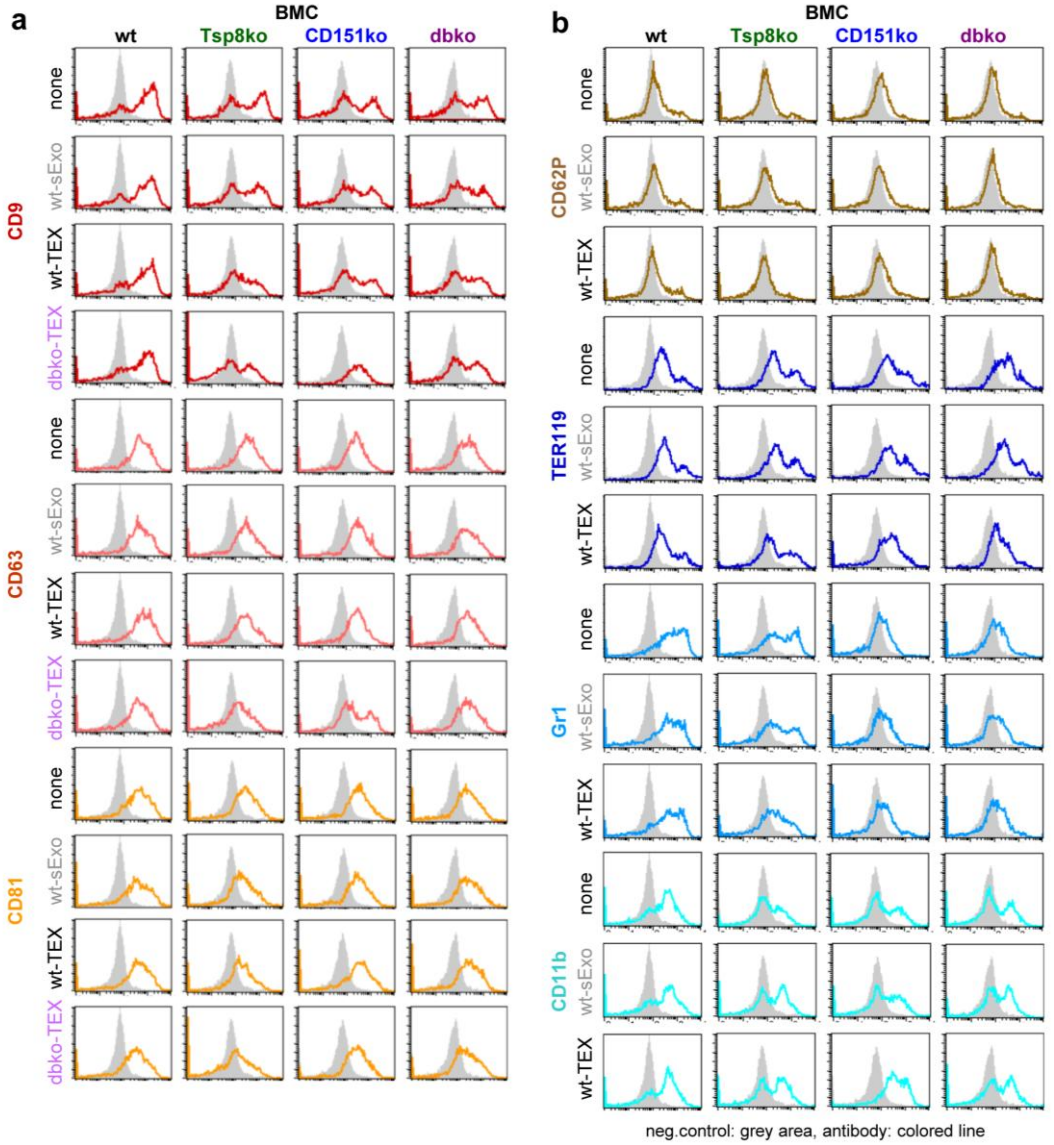


Figure S6 **TEX proteases and invasion of Tspan8ko and/or CD151ko MCA cells**. (a) Tspan8ko-, CD151ko- and dbko-MCA cells were seeded on matrigel containing 50µg/ml TEX. After 48h cells on top of the matrigel were removed and cell within the matrigel (invading) and cells on the lower membrane site were stained with crystal violet; representative examples are shown; (b) wt- and ko-MCA cells were cocultured with wt- and ko-TEX. After 48h expression of proteases was evaluated by flow-cytometry; mean % stained cells±SD (triplicates) are shown; significant changes in protease expression in TEX-treated cells: s.
Only wt-TEX strongly support ko-MCA cell invasion, which is accompanied by upregulation of MMPs, ADAM10 and TACE mostly in Tspan8ko-MCA cells; only in CD151ko- and dbko-MCA cells uPA expression also becomes upregulated.


a
BMC
wt
Tsp8ko
CD151ko
dbko
CD9
CD63
CD81
none
wt-sExo
wt-TEX
dbko-TEX
b
CD62P
TER119
Gr1
CD11b
neg.control: grey area, antibody: colored line

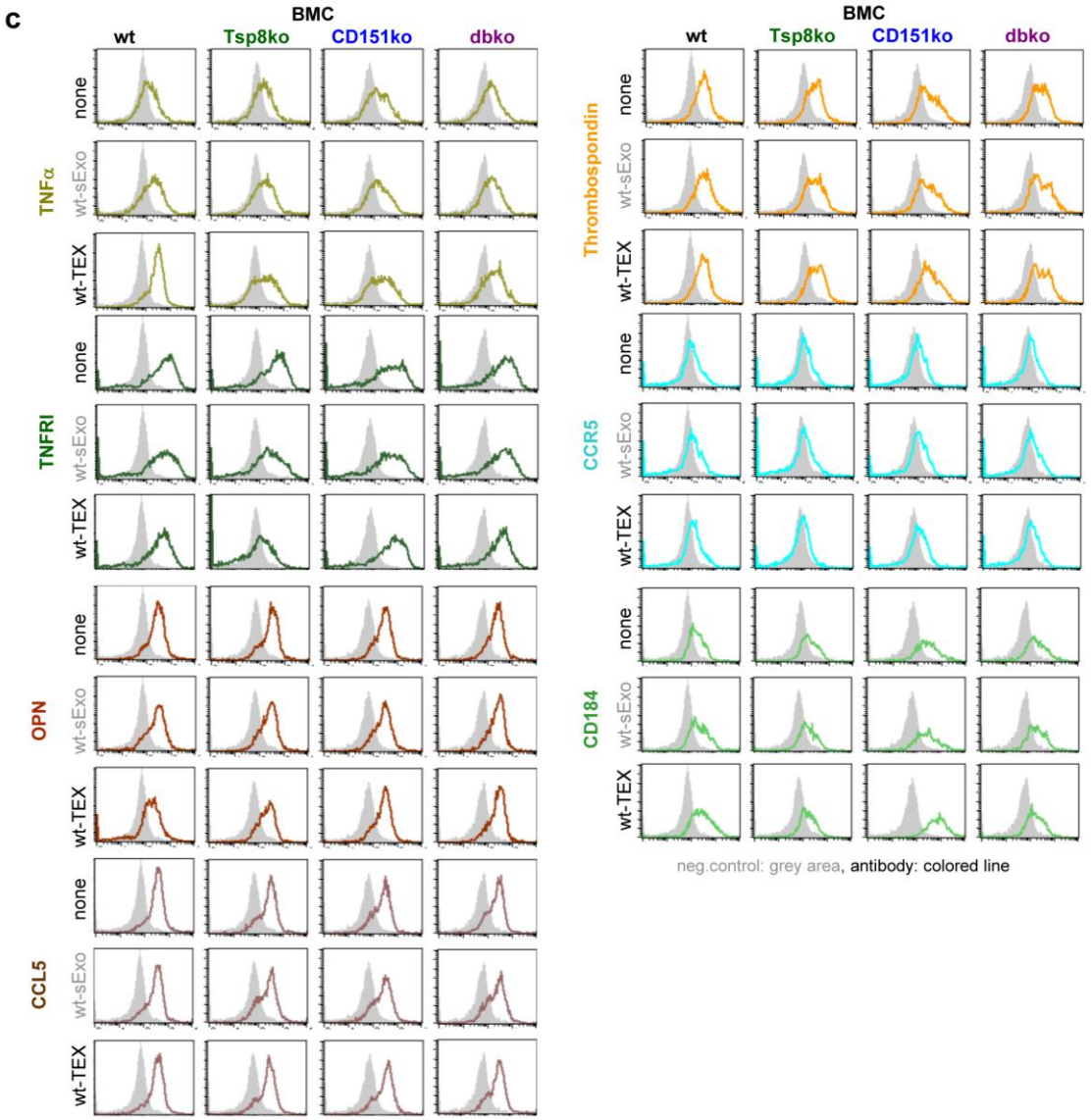


Figure S7 **Flow-cytometry examples on the impact of sExo and TEX uptake on BMC subpopulations.** BMC were incubated with wt-sExo and wt- and ko-TEX for 48h-72h. Recovery of BMC subpopulations was evaluated by flow-cytometry; representative examples of overlays with negative controls are shown for (a) CD9, CD63, CD81, (b) CD62P, TER119, Gr1, CD11b; (c) TNFα, TNFRI, OPN, CCL5, ThbSp, CCR5 and CD184.
Only in Tspan8ko-BMC CD9, CD63 and TNFRI expression is slightly reduced by wt-TEX; low CD62P and TER119 expression is impaired by coculture with TEX; CD11b and CD184 expression is unchanged or increased in wt-TEX-treated BMC.