Supplementary Methods

Flow cytometry analysis

FACS antibodies used are CD45 PerCP, PECAM-1-PE (MEC 13.3), PECAM-1-APC (MEC 13.3), c-kit-APC (2B8), VEGFR-2-PE (Avas 12α1), VEGFR-1-PE (Clone 141515), VE-Cadherin (11D4.1), Sca-1-PE (E13–161.7), Gr-1- (8C5), CD11b- (M1/70), CD3e- (2C11), NG2 (Chondroitin Sulfate Proteoglycan) and DAPI (Sigma) for 7-AAD (7-amino-actinomycin D) or viability. All monoclonal antibodies, their appropriate isotype controls were purchased from BD Pharmingen, exept for anti-VEGFR-1 antibodies which were obtained from R&D systems and Ng2 from Chemicon.

Immunohistochemistry

For β-galactosidase (βgal) staining mice were perfused with 0.2% glutaraldehyde, 2 mM MgCl2, 5 mM ethyl- enediaminetetraacetic acid [EDTA] in phosphate buffer saline [PBS] to preserve βgal expression. Vibrotome sections (300um) made were post-fixed at 4°C in 0.2% glutaraldehyde, 2mM $MgCl_2$, 5mM EDTA in PBS, washed three times for 30 min at 37°C with detergent buffer (0.01% sodium deoxycholate, 0.02% Nonidet P-40, 2 mM MgCl2 in PBS, pH 7.3), and then stained overnight in detergent buffer containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 1 mg/ml X-Gal. X-Gal was prepared at 25 mg/ml stock-solution in dimethylformamide. For histological examination, stained samples were washed twice in PBS and post-fixed overnight in 1% paraformaldehyde at 4°C. After washes with PBS, samples were dehydrated and embedded in paraffin wax, and 300 um sections were cut and counterstained with Nuclear fast red (Vector Labs). Bone marrow was embedded in Histogel (American Master Tech Scientific, Inc.) before dehydration and paraffin embedding. Deparaffinized sections of Xgal stained embryos were incubated with rat anti-mouse CD31 (BD Pharmingen) 1:100, secondary antibody of biotinylated rabbit anti-rat IgG 1:100, and ABC Elite with NovaRED peroxidase stain (Vector laboratories).

Activation of Notch

As positive control for Notch activation, EYFP cells were FACS isolated from animals that were treated with either vehicle or cisplatin and seeded on plates that were previously coated with either IgG (Fcg-specific) or JagFc at 20ug/ml (as previously described[1]). Cells were activated in this form for 30min, after which time, RNA was isolated and RT-PCR was performed as described below.

Expression Analysis

Assessment of expression in distinct subpopulations of FACS-isolated cells was performed by real-time PCR. cDNA was reversed transcribed from total RNA and expression of specific genes was quantified with real-time PCR using SYBR Premix ExTaq (Takara, Japan). Reactions were performed on an ABI 7300 system (ABI, Foster City, CA). For each reaction, a melting curve was generated to test primer dimmer formation and false priming. Subsequently relative expression of each transcript was determined using the double standard curve and GAPDH or Hprt was used for normalization as indicated in the graph. A list of the primers used is shown below:

Notch 1	F: AACGTGGTCTTCAAGCGTGAT
	R: AGCTCTCCACACGGTTCATC
PECAM	F: TGCGATGGTGTATAACGTCACCTC
	R: TGCACCTTCACCTCGTACTCAATC
VE-CAD	F: TAGGGAAAGAGTCCATTGTGTGCAGG
	R: TCACACACTTTAGGTTCGTAGGGC
VEGFR1	F: ATGGAGCTCAAGAAAGAGAGCCTG
	R: GGCTGCTTGGAGATCTCACTGTAA
VEGFR2	F: GAGATGCAGGAAACTACACGGTCA
	R: TCCATAGGCGAGATCAAGGCTTTC
VCAM	F: TGCGAGTCACCATTGTTCTCAT
	R: CATGGTCAGAACGGACTTGGA
ICAM	F: TGGAGGTGGCGGGAAAG
	R: TCCAGCCGAGGACCATAC
DLL4	F: CAGTTGCCCTTCAATTTCACCTGG
	R: TTGGCTGATGAGAGAGTTTCCTGG
Tie-2	F: CTTGCAAGAACAATGGAGTCTGCC
	R: ACTTGCATCCTTCTGGTCCACTAC
MRC-1	F: TCTTTTACGAGAAGTTGGGGTCAG
	R: ATCATTCCGTTCACCAGAGGG
Hes 1	F: GCCAATTTGCCTTTCTCATC

	R: AGCCACTGGAAGGTGACACT
Hey 1	F: AATGGCCCACGGGAACCGCTGG
	R: CACCACGGGAAGCACCGGTC
GAPDH	F: TATGACTCTACCCACGGCAAGT
	R: ATACTCAGCACCAGCATCACC

Supplementary References

1. Hofmann, J.J., Zovein, A.C., Koh, H. et al. Jagged1 in the portal vein mesenchyme regulates intraphepatic bile duct development: insights into Alagille syndrome. Development 2010;137(23):4061-72.

Supplementary figure and table legends

Supplementary figure 1

A,B. FACS results showing EYFP+ cells in the bone-marrow of the constitutive **(A)** and CIVE **(B)** mice. **C.** Immunohistochemical analysis of βgal expression in bone marrow sections of inducible VE-Cadherin Cre ER^{T2}/R26R (CIVE) mice after tamoxifen induction **(a,b)** or oil control **(c,d)**. **D.** Tangential section of blood vessel in CIVE mouse after tamoxifen induction, showing patch expression of βgal. **E.** Immunohistochemical analysis of βgal expression in tumor sections of inducible VE-Cadherin Cre ER^{T2}/R26R (CIVE) mice after tamoxifen induction or oil control.

Supplementary figure 2

A. Tumor growth of C26 cells in balb/c mice, either untreated, treated with cisplatin or with paclitaxel. **B.** Number of vascular structures per field in LLC tumors eight days after start of treatment, quantified by confocal microscopy. **C.** Number of EYFP+ cells per field in LLC tumors eight days after start of treatment, quantified by confocal microscopy. **D,E.** Percentage of EYFP+ cells in the blood of in Bl/6 mice, transplanted with CIVE bone marrow, respectively one **(D)** or eight **(E)** days after start treatment. **F.** Percentage of EYFP+ cells in the tumor compared to different organs eight days after cisplatin. * $p<0.05$, ** $p< 0.01$ compared to vehicle control.

Supplementary figure 3

A. Distribution of EYFP+ cells in the tumor, 2 independent areas were defined: areas with no vascular structures and vascular rich areas and the number of EYFP+ cells were counted per field. **B.** The number of EYFP+ cells per vascular structure were quantified using confocal microscopy. **C,D.** Percentage of bone marrow derived (endothelial) cells, EYFP+ **(C)** or EYFP+PECAM+ **(D)** in subcutaneous growing LLC cells in Bl/6 mice, transplanted with constitutive VE-Cadherin Cre/EYFP bone morrow, eight days after start treatment. * $p<0.05$, ** $p< 0.01$, *** $p<0.001$ compared to vehicle control.

Supplementary figure 4

A. Pericyte coverage of vessels, quantified by the number of vascular structures surrounded by SMA+ cells on confocal microscopy. **B.** Representative confocal images of EYFP+ cells in the LLC tumors (Blue: TOPRO, Red: PECAM, Orange: SMA, Green: EYFP). * $p<0.05$ compared to vehicle control.

Supplementary figure 5

PCR from bone marrow after transplantation demonstrating full lox/lox reconstitution of the bone marrow showing genetic reconstitution by the donor. Lane 1 – lox/lox marrow to be transplanted. Lane 2 – wild-type marrow. Lane 3 – transplanted marrow in wt mouse.

Supplementary figure 6

Quantitative PCR of Notch, DLL4, Hes-1, Hey-1 in the EYFP+ cells after treatment with either vehicle control or cisplatin with and without stimulation byJag-1.

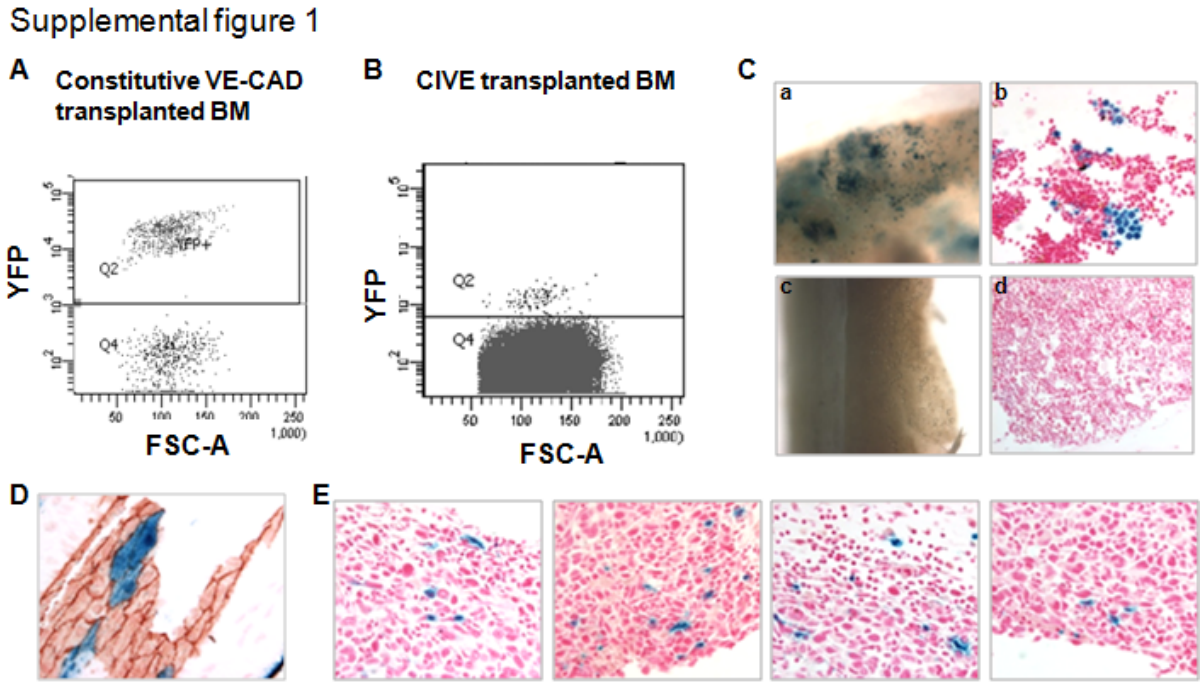
Supplementary figure 7

Percentage of NG2+ cells from total cells in subcutaneously growing LLC cells in Bl/6 mice, that were transplanted respectively with CIVE or CIVE-Notch KO bone marrow, eight days after start treatment.

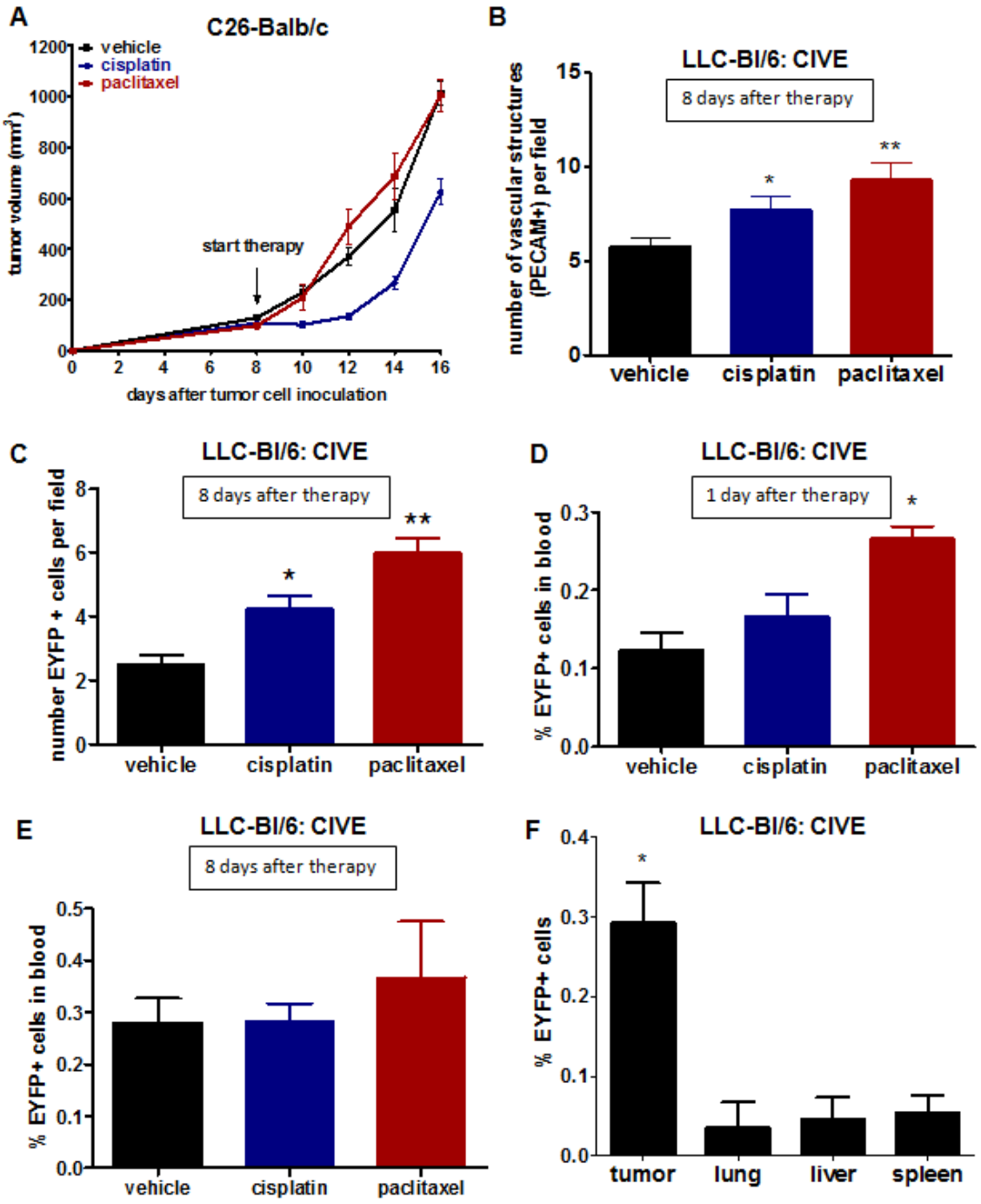
Supplementary table 1. Used transgenic mouse models.

Supplementary table 2. Phenotype of EYFP+ cells in the bone marrow of mice transplanted with the CIVE bone marrow.

Figures


Supplemental figure 1
A
Constitutive VE-CAD transplanted BM
YFP
FSC-A
YFP+
Q2
Q4
B
CIVE transplanted BM
YFP
FSC-A
Q2
Q4
C
a
b
c
d
D
E

Supplemental figure 2



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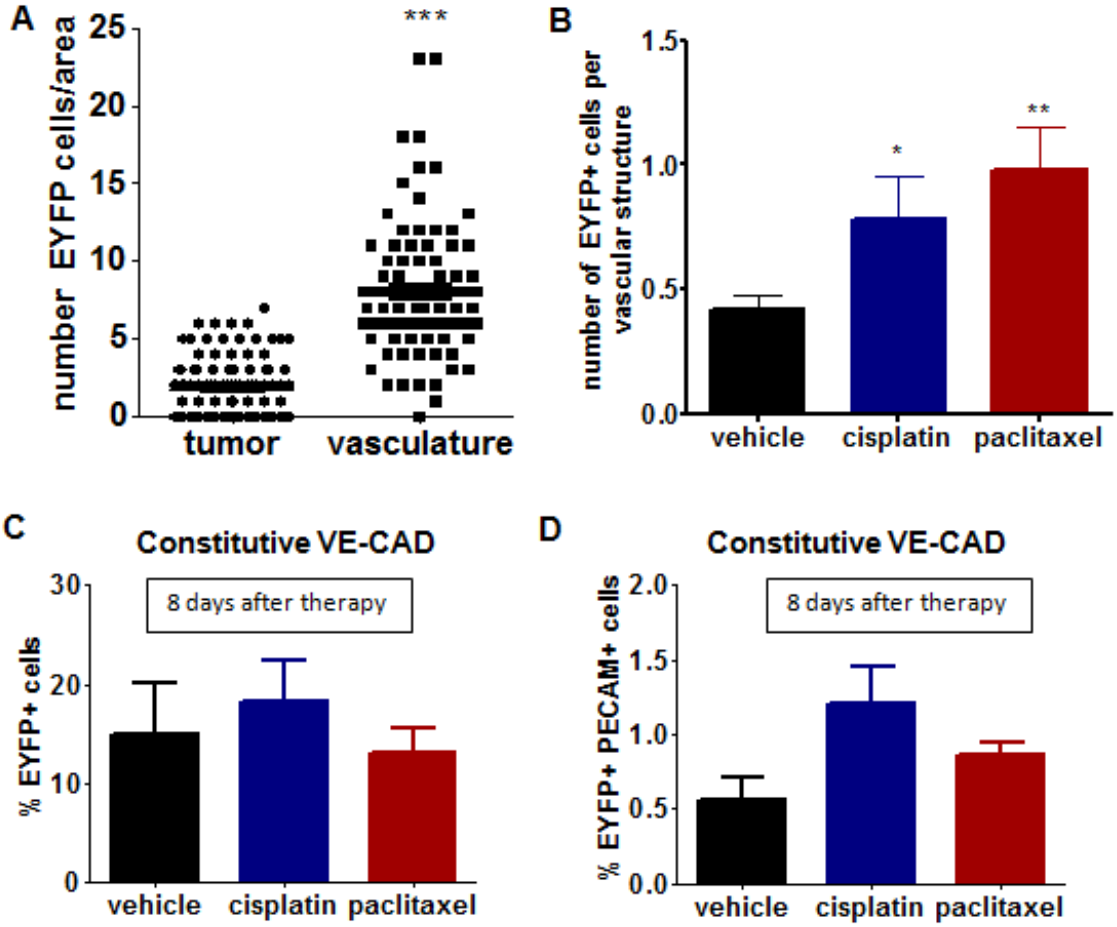

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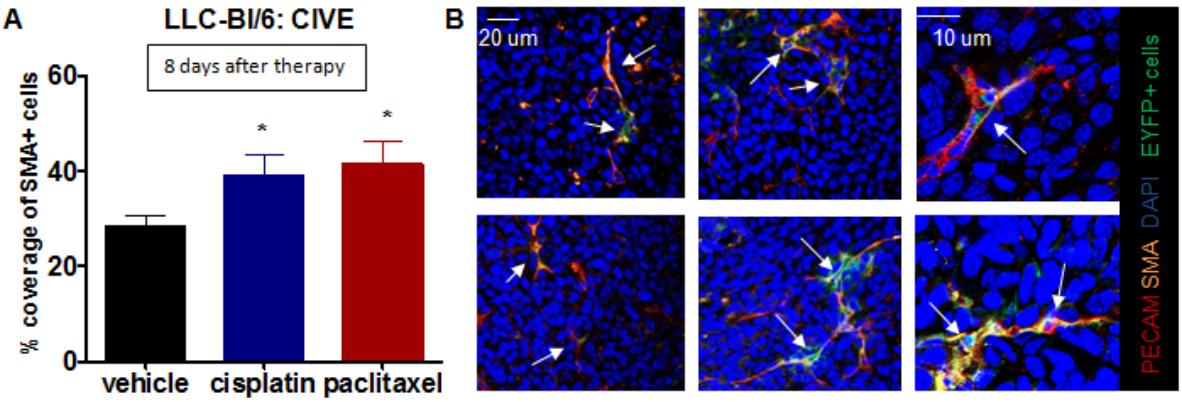

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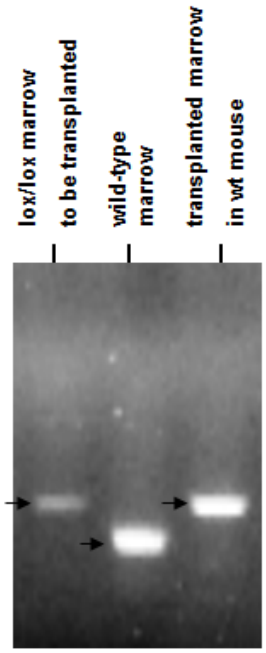
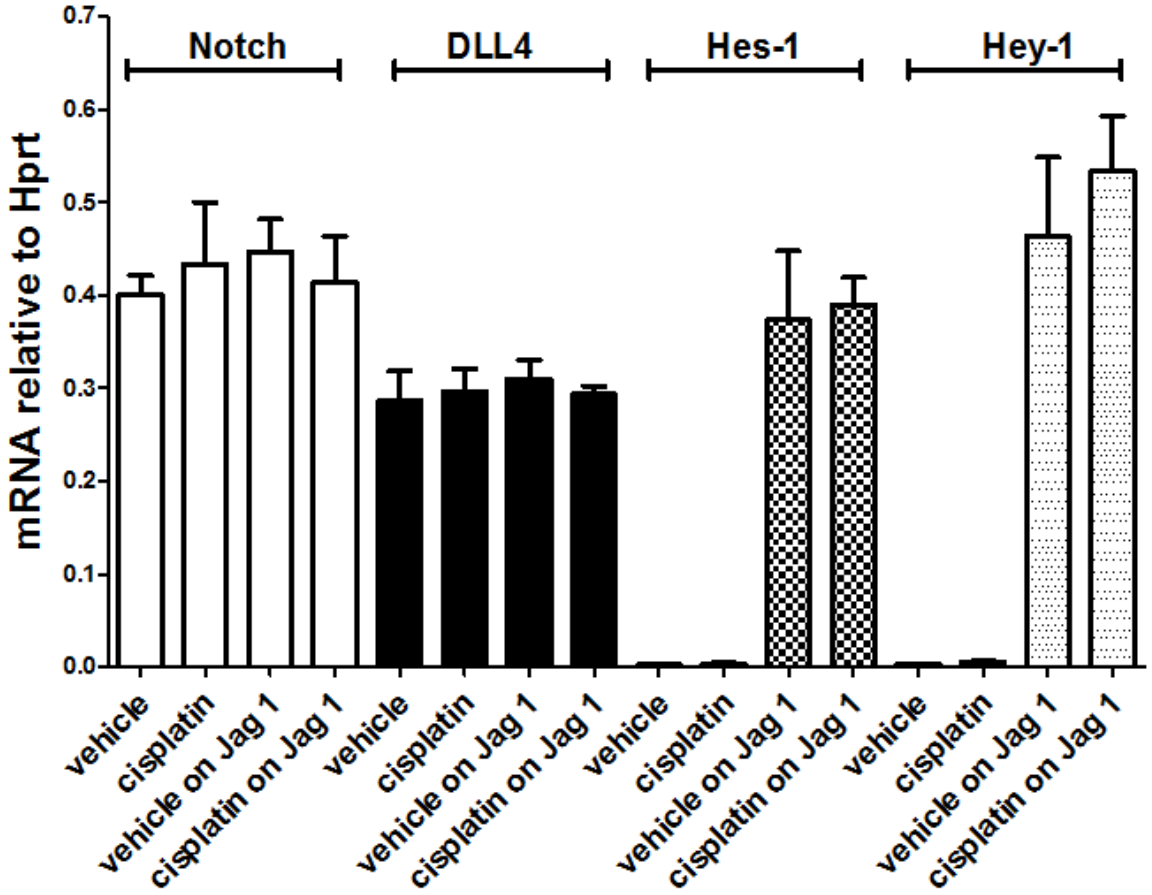
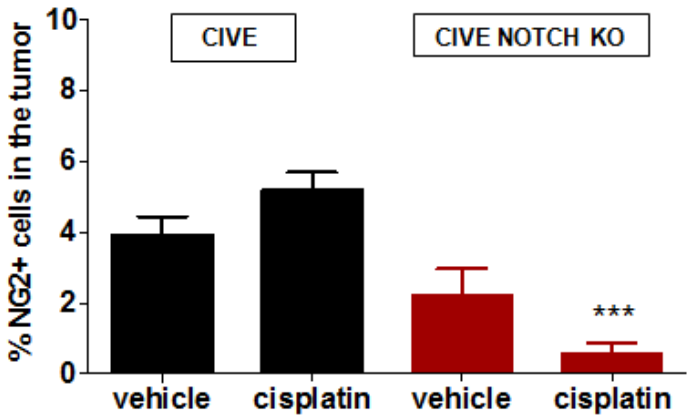


Supplemental figure 6



Supplemental figure 7



Supplemental table 1

Mouse model	Transgene	Reference
VE-CAD Cre	VE-CAD promoter driving the Cre-recombinase cDNA	Alva et al. Dev Dyn 2006 (28)
VE-CAD Cre ERT2	VE-CAD promoter driving the Cre-recombinase cDNA fused to the ERT2 sequence for response to tamoxifen	Monvoisin et al. Dev Dyn 2006 (29)
Notch lox/lox	Flox Notch1 gene	Radtke et al Immunity 1999 (30)
Rosa 26 – LacZ	Rosa – neo stop cassette – LacZ	Soriano et al. Nat Genet 1999 (26)
Rosa 26 – EYFP	Rosa – neo stop cassette – YFP	Srinivas et al. BMC Dev Biol 2001 (25)

Supplemental table 2

Surface marker	% positive (mean) in YFP+ cells in the BM
CD45+	79
PECAM+	38
CD45-PECAM+	1,7
VEGFR2+	19
CD45-VEGFR2+	0,58
PECAM+ VEGFR2+	4,6
VE-cadherin+	46
VEGFR1+	1,4
CD11b+ Gr1+	59
Sca-1+	4,00
CD117/c-kit+	6,2
CD3c+	3,6
NG2+	1,3

Phenotype of the EYFP+ cells in the transplanted CIVE bone marrow