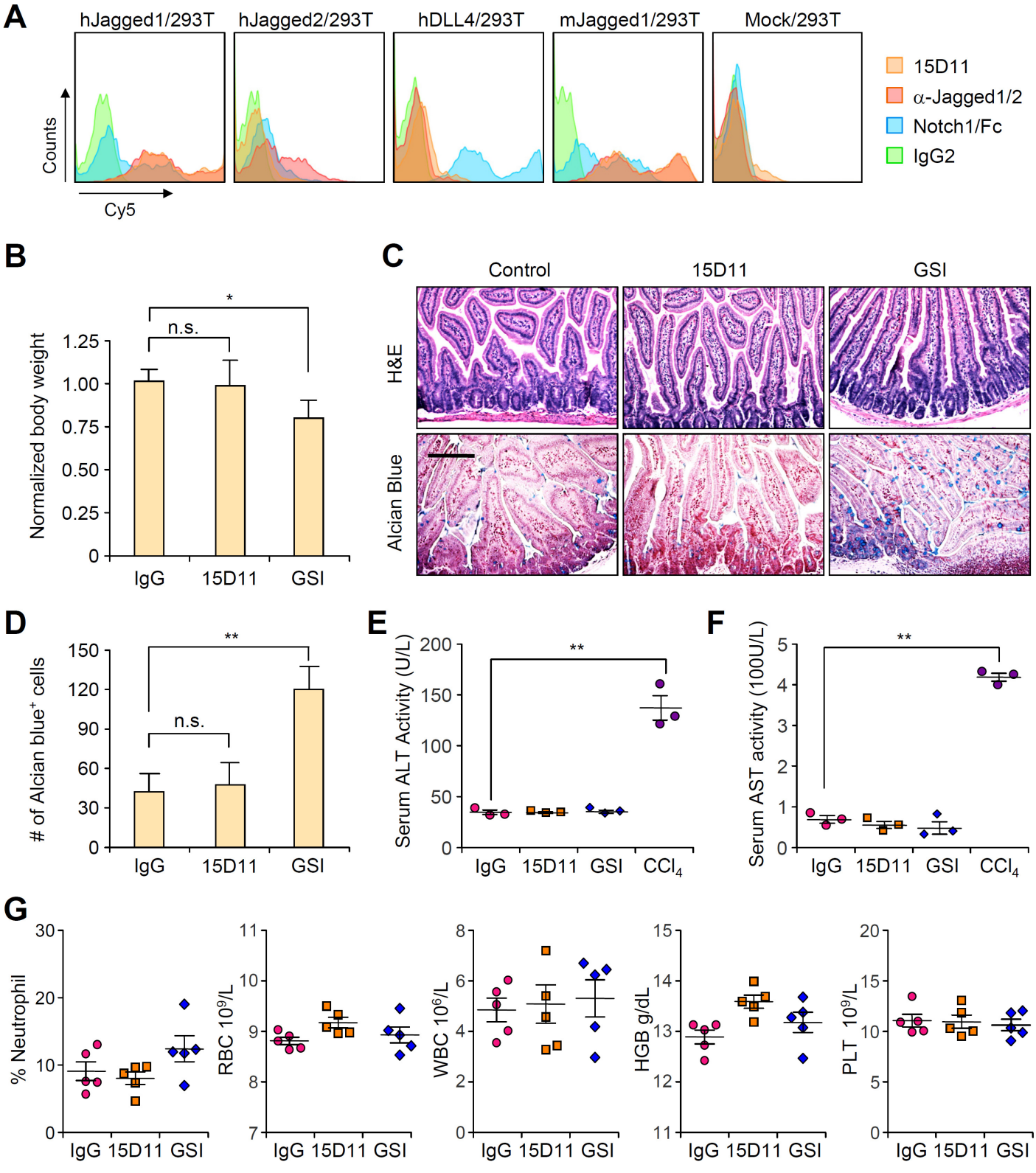
A
hJagged1/293T
hJagged2/293T
hDLL4/293T
mJagged1/293T
Mock/293T
Counts
Cy5
15D11
α-Jagged1/2
Notch1/Fc
IgG2
B
Normalized body weight
n.s.
*
IgG
15D11
GSI
C
Control
15D11
GSI
H&E
Alcian Blue
D
# of Alcian blue+ cells
n.s.
**
IgG
15D11
GSI
E
Serum ALT Activity (U/L)
**
IgG
15D11
GSI
CCl4
F
Serum AST activity (100U/L)
**
IgG
15D11
GSI
CCl4
G
% Neutrophil
RBC 10^9/L
WBC 10^6/L
HGB g/dL
PLT 10^9/L
IgG 15D11 GSI

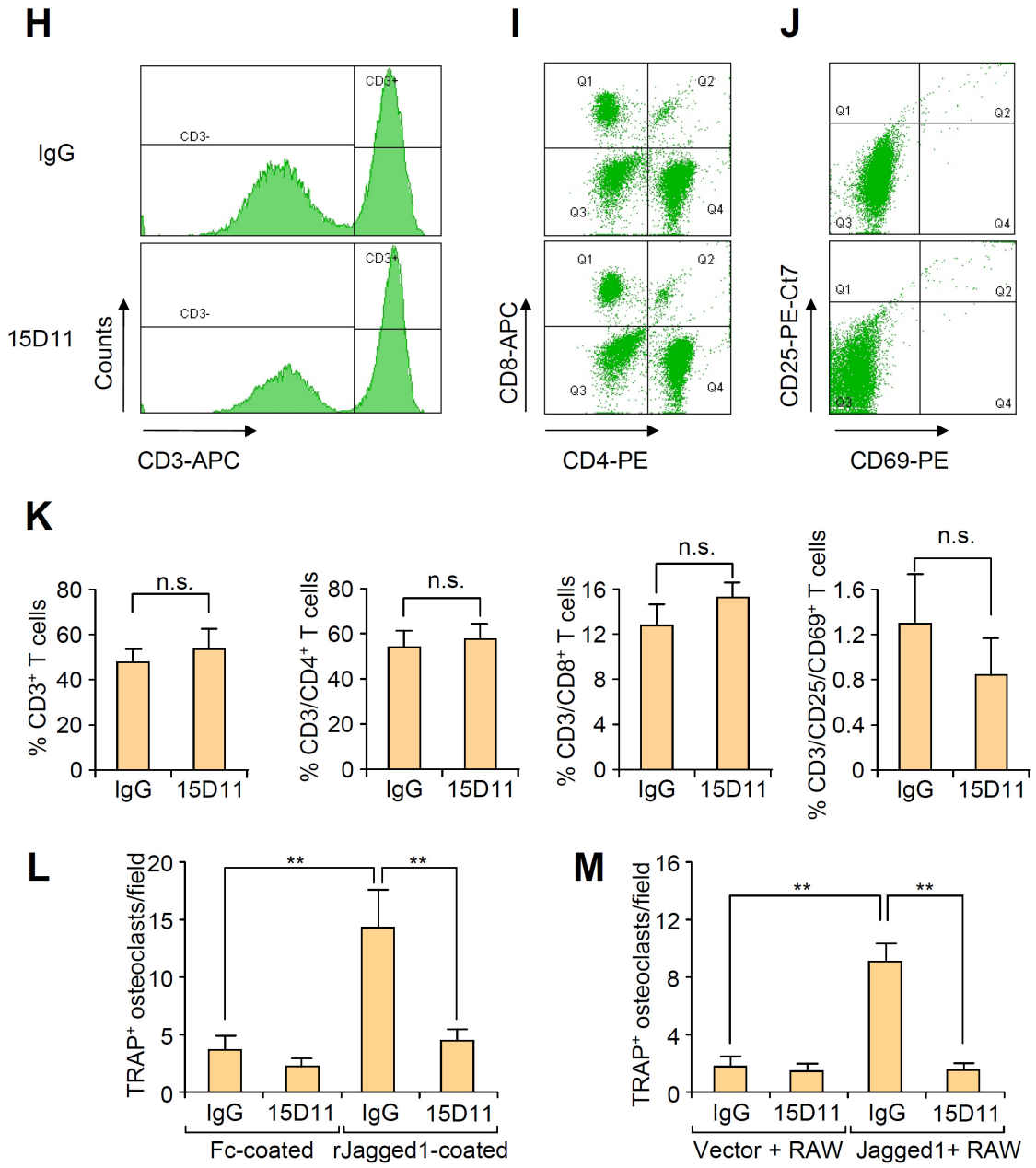

Figure S1, related to Figure 1. ***In vivo*** **administration of 15D11 causes no adverse effect. (A)** Flow cytometric screen to test 15D11's binding affinity to HEK-293T cells transiently transfected individually with human Jagged1, human Jagged2, human Dll4, mouse Jagged1 or mock transfected with empty plasmid. Ligand or receptor expression was confirmed using human Notch-1/Fc chimera, human (IgG2) anti-human Jagged1/2 antibody, and 15D11 antibody. (**B**) 2-3 month old female FVB mice were treated with respective agents at the dosage of 10 mg/kg for IgG/15D11 (by i.p. injection), and 100 mg/kg for GSI (by oral gavage) twice a week for 6 weeks. The relative mouse weight

was quantified before and after the treatment. n = 10. *p<0.05 by Student's t-test. n.s., not significant. Data is presented as mean ± SEM. (**C**) Small intestine samples were obtained from mice in experiment in A. H&E and Alcian blue staining was performed on processed, sliced samples. Scale bar: 200 μm. (**D**) Quantification of Alcian blue staining results from B. n = 6. **p<0.01 by Student's t-test. Data is presented as mean ± SEM. (**E**) Balb/c mice were treated with respective agents twice a week for 6 weeks or acutely treated with 200 μl 8% CCl_4 in vegetable oil by i.p. injection. Serum ALT activity was measured following the standard protocol of an ALT assay kit (Sigma). n = 3. **p<0.01 by Student's t-test. Data is presented as mean ± SEM. (**F**) Serum samples from mice in C were used to measure AST activity following the standard protocol of an AST assay kit (Sigma). n = 3. **p<0.01 by Student's t-test. Data is presented as mean ± SEM. (**G**) 2-3 month old female FVB mice were treated with respective agents for two times in a week before the blood samples were draw directly from the heart. Blood cell counts were performed using the Sysmex XN-3000 Hematology System (Sysmex America, Inc.). n = 5. Data is presented as mean ± SEM. (**H-K**) T cell profiling in peripheral blood after treatment with 15D11 or control IgG. 6-week-old female FVB mice were treated with IgG and 15D11 antibody twice a week for two weeks at the dosage of 10 mg/kg. Peripheral blood was stained with T cell profiling kit (catalog# 55843, BD Biosciences) and with T cell activation kit (catalog# 557908, BD Biosciences) and then subject to FACS analysis of CD3 (H) CD4/CD8 gated from $CD3^+$ cells (**I**) and CD69/CD25, gated from $CD3^+$ cells (**J**). Results were quantified in K. (**L**) Quantification of $TRAP^+$ osteoclasts from experiment in Figure 1E. n = 3. **p<0.01 by Student's t-test. Data is presented as mean ± SEM. (**M**) Quantification of $TRAP^+$ osteoclasts from experiment in Figure 1F. n = 3. **p<0.01 by Student's t-test. Data is presented as mean ± SEM.

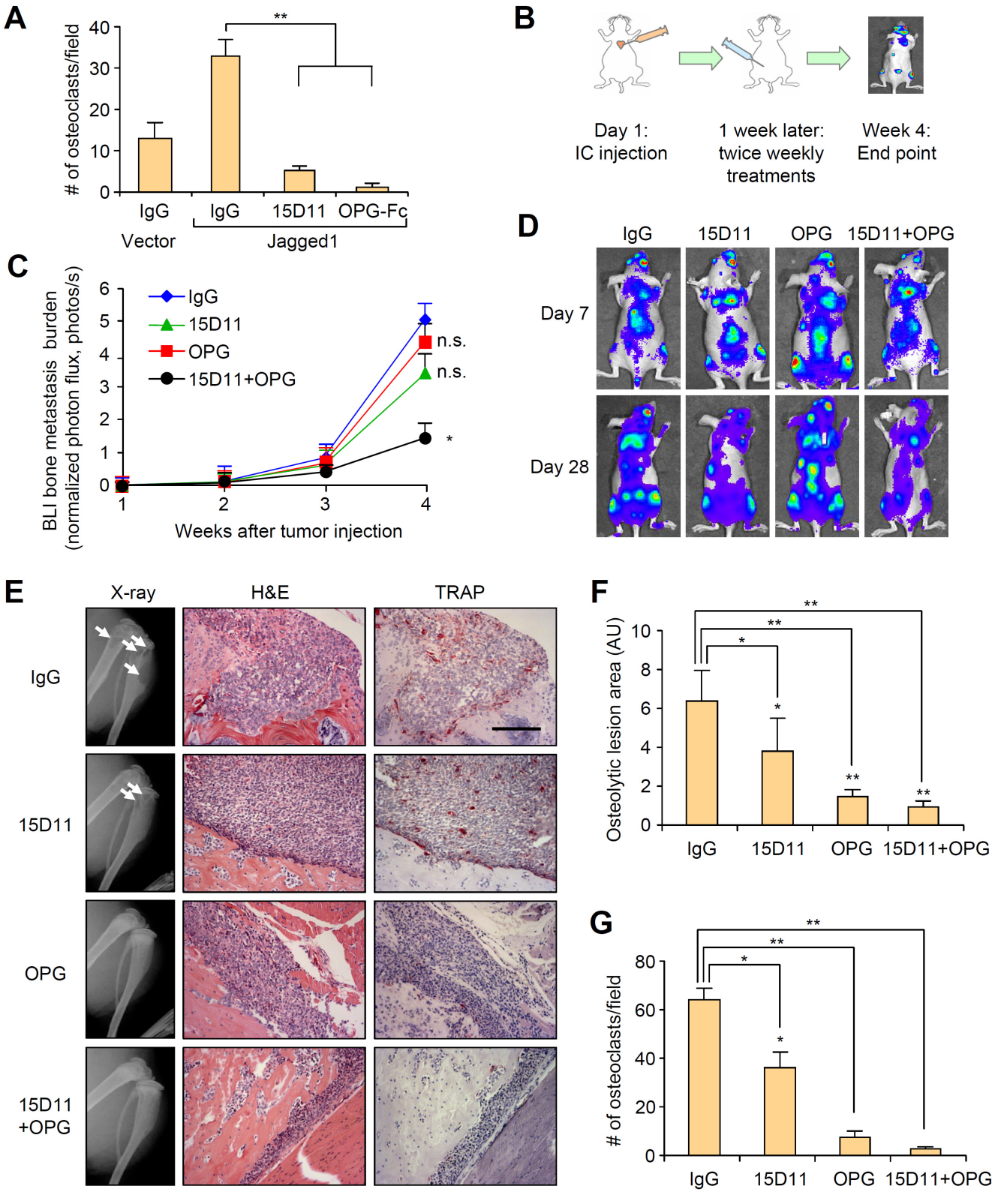
A
# of osteoclasts/field
40
30
20
10
0
**
IgG
IgG
15D11
OPG-Fc
Vector
Jagged1
B
Day 1:
IC injection
1 week later:
twice weekly
treatments
Week 4:
End point
C
BLI bone metastasis burden
(normalized photon flux, photos/s)
6
5
4
3
2
1
0
IgG
15D11
OPG
15D11+OPG
n.s.
n.s.
*
1
2
3
4
Weeks after tumor injection
D
IgG
15D11
OPG
15D11+OPG
Day 7
Day 28
E
X-ray
H&E
TRAP
IgG
15D11
OPG
15D11
+OPG
F
Osteolytic lesion area (AU)
10
8
6
4
2
0
**
**
*
*
**
**
IgG
15D11
OPG
15D11+OPG
G
# of osteoclasts/field
80
60
40
20
0
**
**
*
*
IgG
15D11
OPG
15D11+OPG

Figure S2, related to Figure 2. **Administration of 15D11 and OPG inhibits bone metastasis of highly bone-metastatic SCP2 cell line with high endogenous Jagged1 expression. (A)** Quantification of $TRAP^+$ cells from Figure 2F. n = 10. **p<0.01 by Student's t-test. Data is presented as mean ± SEM. (**B**) Schematic representation of the experimental procedure. In brief, SCP2 cells were IC injected into 4-6 weeks female athymic nude mice to generate bone metastases. One week later, mice were randomized into four groups. Bone metastasis progression was monitored by weekly BLI imaging until the experimental endpoint (4 weeks after injection). Mice were treated one week after the tumor cell injection and continued twice a week at the dosage of 10 mg/kg for IgG/15D11, and 3 mg/kg for OPG-Fc. (**C**) Quantification of bone metastasis burden each week based on BLI imaging from the experiment. n = 10. *p<0.05 by Mann-Whitney test. Data is presented as mean ± SEM. (**D**) Representative BLI images on Day 7 right after randomization and in week 4 right before the end point of the experiment. (**E**) Representative X-ray images, H&E staining images, and TRAP staining images of hind limb bones from experiment in A were shown. Scale bar: 200 μm. (**F**) Quantification of osteolytic lesions based on X-ray images. AU: arbitrary unit. n=10. *p<0.05, **p<0.01 by Student's t-test. Data is presented as mean ± SEM. AU: arbitrary units. (**G**) Quantification of $TRAP^+$ osteoclasts per field. n = 5. *p<0.05, **p<0.01 by Student's t-test. Data is presented as mean ± SEM.

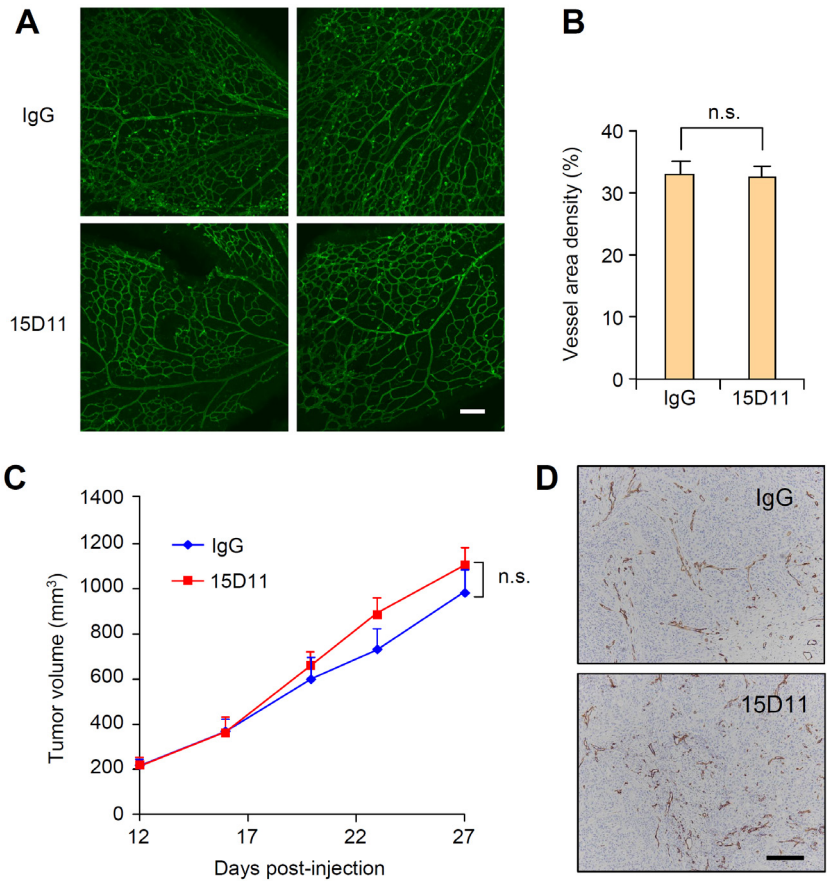

Figure S3, related to Figure 2. 15D11 has no effect on angiogenesis *In Vivo*. (**A**) Retinas from neonate mouse pups (post-natal day 6.5) were treated with anti-Jagged1 antibody (15D11) or human IgG (50 μg/dose) as a negative control. Stained retinas were mounted on slides and vasculature of the retinal whole-mounts was imaged using a Zeiss LSC confocal microscope. Scale bar: 200 μm. (**B**) Quantification of vessel density from A. n = 3. n.s.: no significant difference between two groups by Student's t-test. Data is presented as mean ± SEM. (**C**) Colo205 colorectal cancer cells were inoculated into athymic nude mice subcutaneously and randomized on Day 11 before treatment. Mice were treated with IgG or 15D11 antibody at the dosage of 300 μg/mouse for twice a week. Tumor volume was monitored by palpation. n = 9. n.s.: no significant difference between two groups by Student's t-test. Data is presented as mean ± SEM. (**D**) Histomorphometry of Colo205 tumors from mice treated for 96 hr with 500 μg of IgG, or 15D11 antibody. Intra-tumor blood vessels were visualized by CD31 staining and counterstained with hematoxylin. (n = 3 per group). No vascular differences between groups were observed. Scale bar: 200 μm.

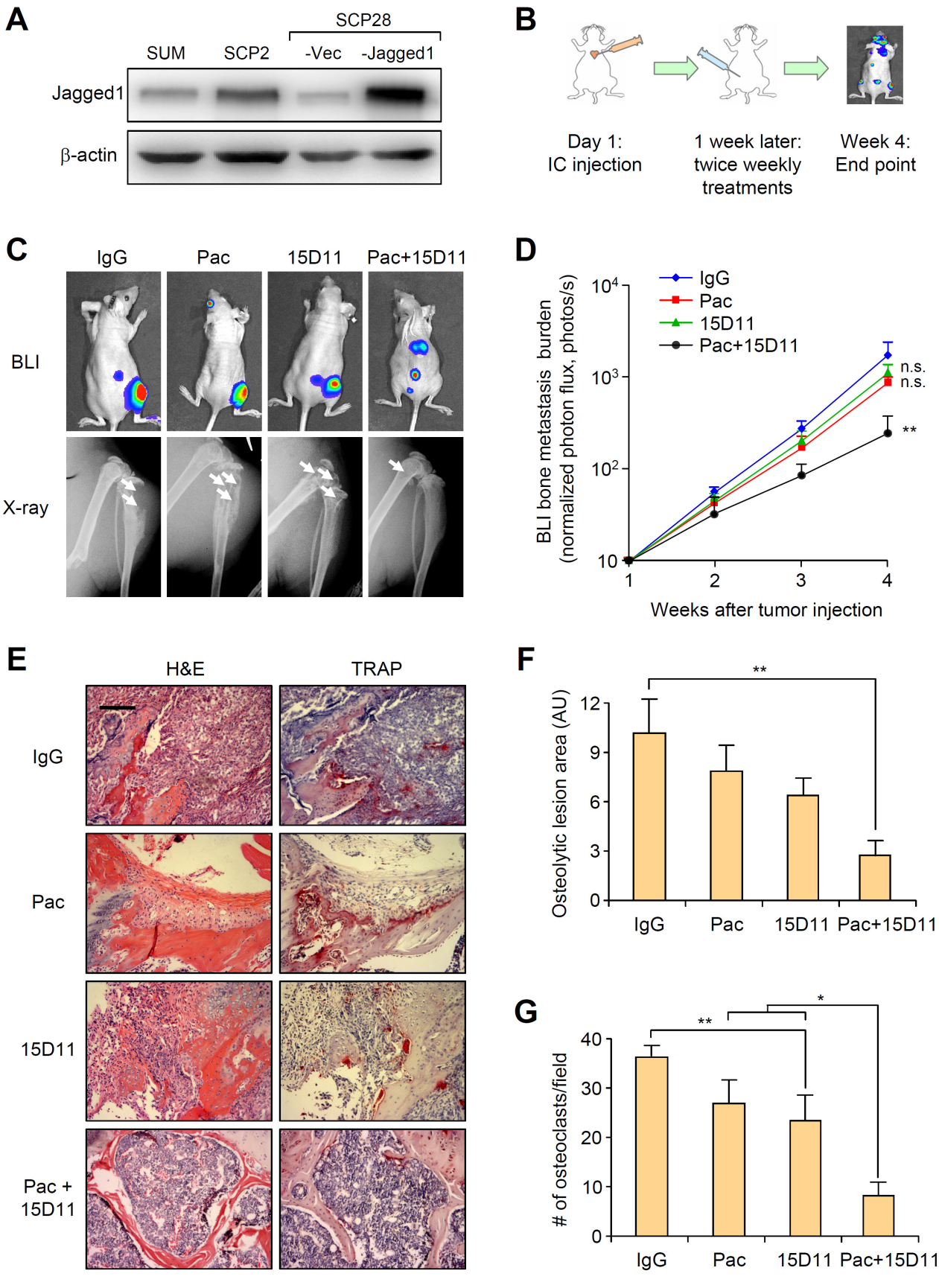
A
SCP28
SUM
SCP2
-Vec
-Jagged1
Jagged1
β-actin
B
Day 1:
IC injection
1 week later:
twice weekly
treatments
Week 4:
End point
C
IgG
Pac
15D11
Pac+15D11
BLI
X-ray
D
IgG
Pac
15D11
Pac+15D11
BLI bone metastasis burden
(normalized photon flux, photos/s)
Weeks after tumor injection
n.s.
n.s.
**
E
H&E
TRAP
IgG
Pac
15D11
Pac +
15D11
F
Osteolytic lesion area (AU)
**
IgG
Pac
15D11
Pac+15D11
G
# of osteoclasts/field
**
*
IgG
Pac
15D11
Pac+15D11

Figure S4, related to Figure 4. 15D11 synergizes with chemotherapy to inhibit bone metastasis in the SUM1315-M1B1 model. (**A**) Western blot analysis of Jagged1 protein expression level in SUM1315-M1B1, SPC2, SCP28-Vector, and SCP28-Jagged1 cells. β-actin was used as loading control. (**B**) Schematic representation of the experimental procedure. In brief, SUM1315-M1B1 cells were IC injected into 4-6 weeks female athymic nude mice to generate bone metastases. One week later, mice were randomized into four groups. Bone metastasis progression was monitored by weekly BLI imaging until the experimental endpoint (4 weeks after injection). Treatments were initiated one week after the injection and continued twice a week at the dosage of 10 mg/kg for IgG/15D11, and 20 mg/kg for paclitaxel. (**C**) Representative BLI and X-ray images of mice in week 4 right before euthanizing the mice. (**D**) Quantification of bone metastasis burden each week based on BLI imaging from the experiment. n = 10. $p<0.01$ by Mann-Whitney test. Data is presented as mean ± SEM. (**E**) Representative H&E staining and TRAP staining images of hind limb bones from experiment in A were shown. Scale bar: 100 μm. (**F**) Quantification of osteolytic lesions based on X-ray images. AU: arbitrary unit. n = 10. **$p<0.01$ by Student's t-test. Data is presented as mean ± SEM. (**G**) Quantification of the number of $TRAP^+$ osteoclasts per field. n = 5. **$p<0.01$ by Student's t-test. Data is presented as mean ± SEM.

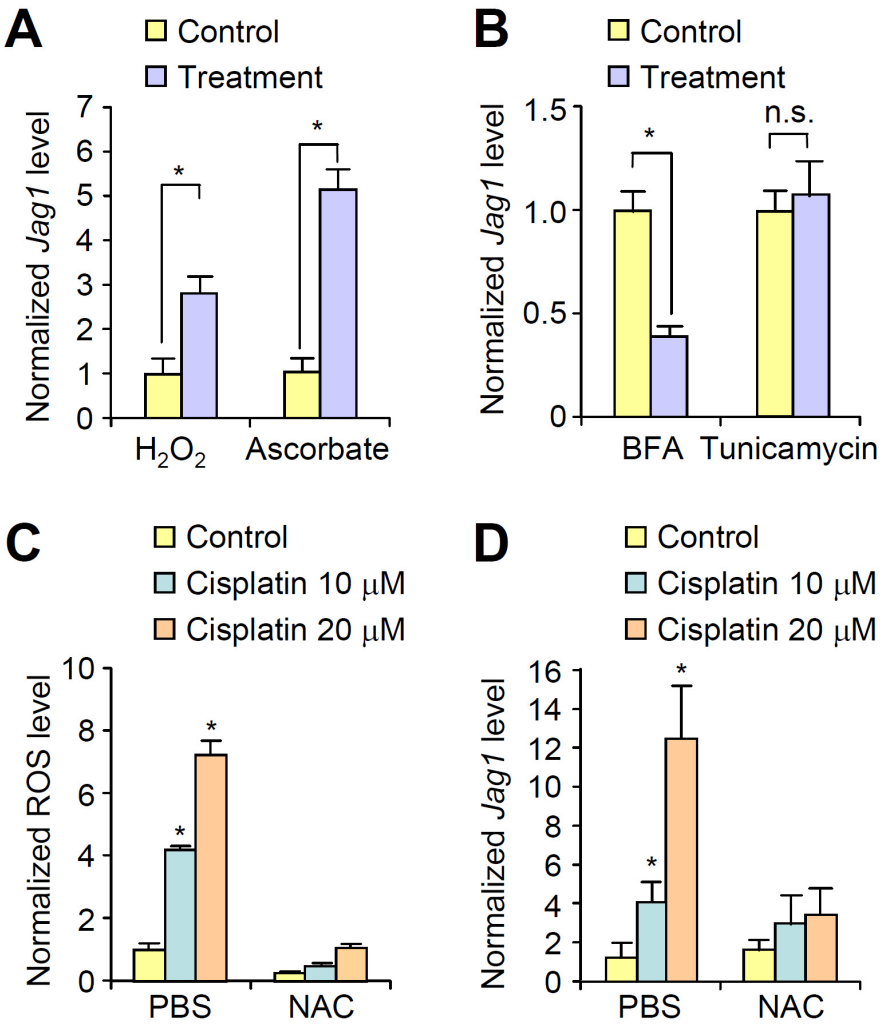

Figure S5, related to Figure 5. Chemotherapy induces Jagged1 expression through the ROS pathway in MSCs. (**A**) 2x10^5/well MSC cells were seeded in 6-well plate and treated with two independent agents inducing ROS responses. H_2O_2 was administrated at the concentration of 200 μM and ascorbate was administrated at the concentration of 2 mM. mRNA was collected 24 hr after treatments and subjected to real time PCR analysis to determine *Jag1* mRNA level. *Gapdh* mRNA severed as internal control. n = 3. *p<0.05 by Student's t-test. Data is presented as mean ± SEM. (**B**) 2x10^5/well MSC cells were seeded in 6-well plate and treated with agents inducing ER stress. Brefeldin A was administrated at 1 μM and Tunicamycin was administrated at 10 μg/ml. mRNA was collected 24 hr after treatments and subjected to real time PCR analysis to determine *Jag1* mRNA level. *Gapdh* mRNA severed as internal control. n =

3. *p<0.05 by Student's t-test. Data is presented as mean ± SEM. (**C**) 2x10^5/well MSC cells were seeded in 6-well and treated with increasing amount of cisplatin in the absence or presence of 2 mM NAC (ROS inhibitor). Cell lysate was collected and the ROS activity was determined by a lipid peroxidation (MDA) assay kit (Sigma, Cat# MAK085-1KT). n = 3. *p<0.05 by Student's t-test. Data is presented as mean ± SEM. (**D**) 2x10^5/well MSC cells were seeded in 6-well plate and treated with increasing amount of cisplatin in the absence or presence of 2 mM NAC. mRNA was collected 24 hr after treatments and subjected to real time PCR analysis to determine *Jag1* mRNA level. *Gapdh* mRNA severed as internal control. n = 3. *p<0.05 by Student's t-test. Data is presented as mean ± SEM.

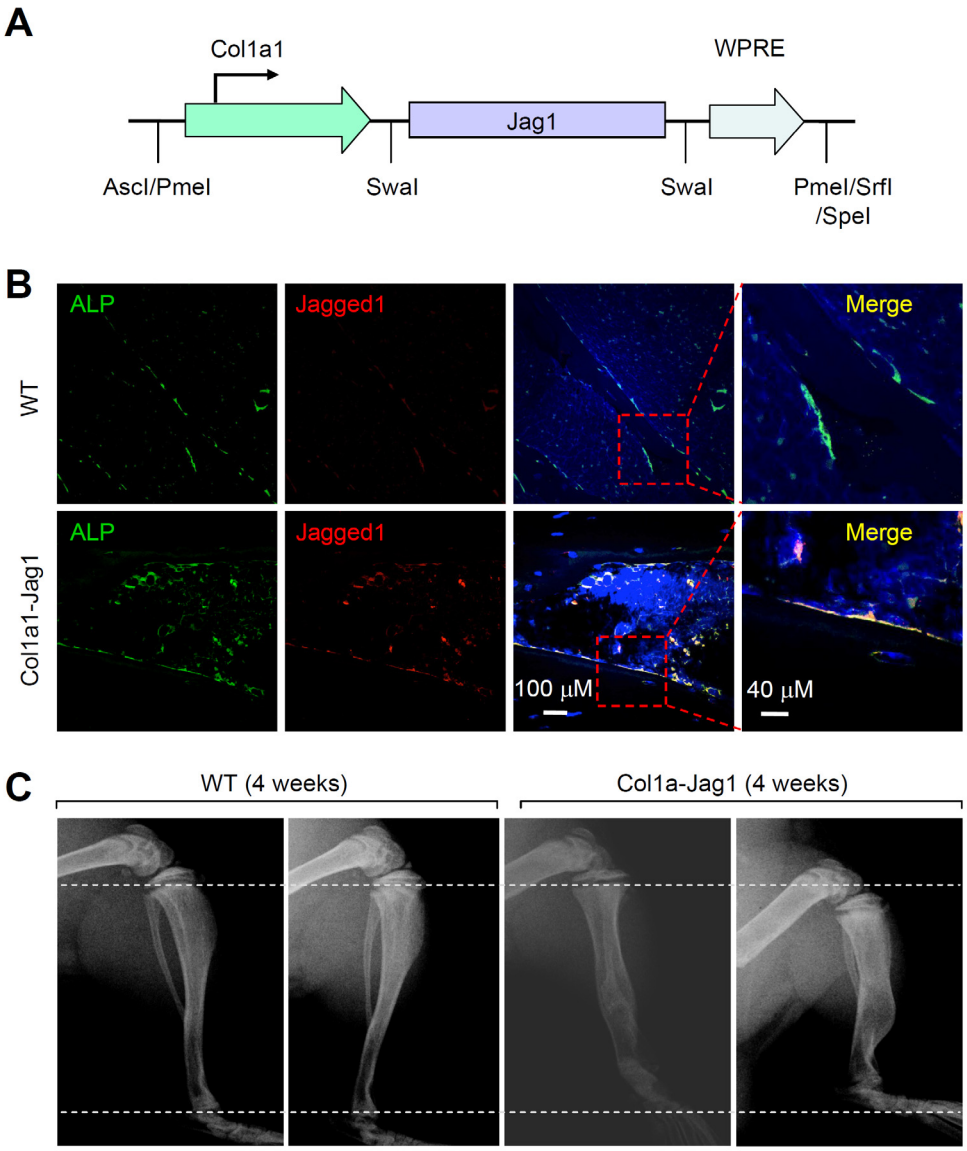

Figure S6, related to Figure 6. Generation of osteoblast-specific Jagged1 expression mice. (**A**) DNA construct for generating the Col1a1-Jag1 transgenic mice. Mouse Jagged1 coding sequence was cloned from FVB background cDNA library and inserted into the SwaI site of pTyr-Col1a1 vector. (**B**) 8-week old WT or Col1a1-Jag1 mice were euthanized for hind-limb bone collection, followed by cryo-staining of Jagged1 and ALP. Scale bars: 100 μm and 40 μm respectively. (**C**) X-ray imaging analysis of the femur and tibia of 4-week old WT and Col1a1-Jag1 mice. Note the shortened, but swallowed tibia bone with decreased bone density in Col1a1-Jag1 mice.

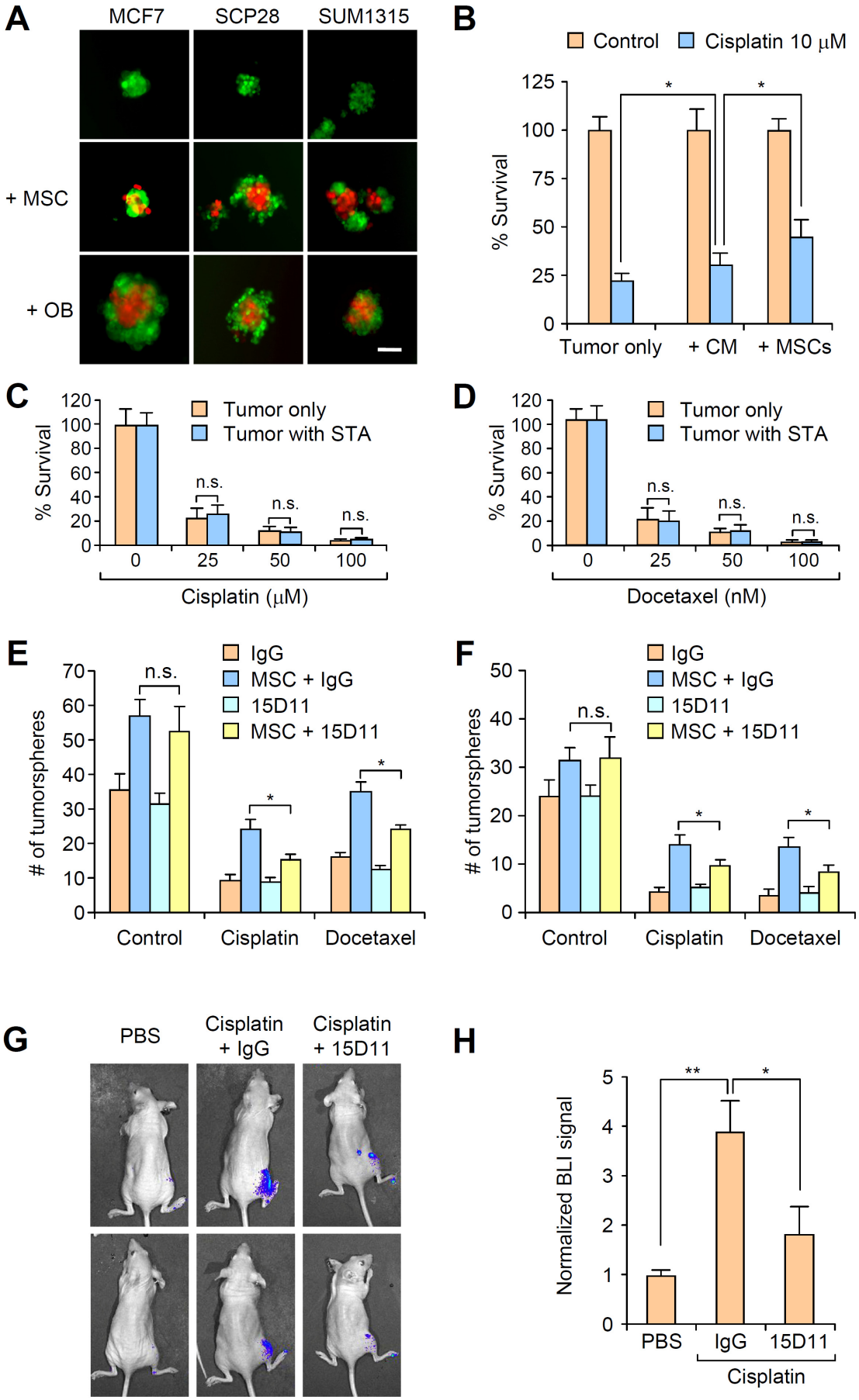
A
MCF7
SCP28
SUM1315
+ MSC
+ OB
B
Control
Cisplatin 10 μM
% Survival
Tumor only
+ CM
+ MSCs
C
Tumor only
Tumor with STA
% Survival
n.s.
Cisplatin (μM)
D
Tumor only
Tumor with STA
% Survival
n.s.
Docetaxel (nM)
E
IgG
MSC + IgG
15D11
MSC + 15D11
# of tumorspheres
n.s.
Control
Cisplatin
Docetaxel
F
IgG
MSC + IgG
15D11
MSC + 15D11
# of tumorspheres
n.s.
Control
Cisplatin
Docetaxel
G
PBS
Cisplatin + IgG
Cisplatin + 15D11
H
Normalized BLI signal
PBS
IgG
15D11
Cisplatin

Figure S7, related to Figure 7. Administration of 15D11 sensitizes bone metastatic tumor cells to chemotherapy *in vitro* and *in vivo*. (**A**) A 3D co-culture model for breast cancer cells and osteoblasts or MSCs in low attachment plates. At the presence of osteoblasts or MSCs (red), tumor cells (green) formed hybrid tumorspheres in close contact with osteoblast/MSCs. Scale bar: 100 μm (**B**) MSCs were cultured in regular media on 10 cm dishes and treated with 10 μM cisplatin for 24 hr. Cells were then switched to serum free media for another 24 hr before the CM were collected. Luciferase-labelled SUM1315-M1B1 cells were seeded alone, with MSC CM, or with MSCs and treated with 10 μM cisplatin. The relative number of surviving tumor cells after 48 hr were quantified by luciferase assay. n = 3. *p<0.05 by Student's t-test. Data is presented as mean ± SEM. (**C-D**) 3D tumorsphere culture of SUM1315-M1B1 cells, alone or in co-cultured with STA endothelial cells, were treated with indicated concentrations of cisplatin or docetaxel. Two days after the chemotherapy, the surviving tumor spheres were counted under the fluorescent microscope. n = 3. n.s., p>0.05 by Student's t-test. Data is presented as mean ± SEM. (**E**) In SCP28 and MSC 3D co-culture model, established tumorspheres were treated with respective chemotherapy agent in combination with 5 μg/ml IgG or 15D11. The surviving tumorspheres were counted under the fluorescence microscope (for GFP^+ tumorspheres). n = 3. *p<0.05 by Student's t-test. Data is presented as mean ± SEM. (**F**) In SUM1315-M1B1 and MSC 3D co-culture model, established tumorspheres were treated with respective chemotherapy agent in combination with 5 μg/ml IgG or 15D11. The surviving GFP^+ tumorspheres were counted under the fluorescence microscope. n = 3. *p<0.05 by Student's t-test. Data is presented as mean ± SEM. (**G**) Female athymic nude mice were i.p. injected with PBS or Cisplatin one day before tumor cell injection. 10^5 SUM1315-M1B1 cells were IIA injected into these nude mice. Cisplatin treated mice were randomly assigned into two groups and treated with either IgG or 15D11 antibody at the time of tumor cell injection. The representative BLI images from Day 4 were shown. (**H**) Quantification of BLI signal at Day 4 from experiment in E. n = 5. *p<0.05, **p<0.01 by Student's t-test. Data is presented as mean ± SEM.

Table S1, related to STAR Methods. Primers for real-time quantitative PCR.

Gene	Forward 5'-3'	Reverse 5'-3'
JAG1	GAGCTATTTGCCGACAAGGC	GGAGTTTGCAAGACCCATGC
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
HES2	AGAACTCCAACTGCTCGAAGCT	CGGTCATTTCCAGGACGTCT
HEY1	AGCCGAGATCCTGCAGATGA	GCCGTATGCAGCATTTTCAG
HEY2	AGATGCTTCAGGCAACAGGG	CAAGAGCGTGTGCGTCAAAG
Jag1	AAACAAAACACAGGGATTGCCC	CATCTCTGGGACGACAGAACT
Gapdh	TCCCACTCTTCCACCTTCGATGC	GGGTCTGGGATGGAAATTGTGAG
Hes1	CCCCAGCCAGTGTCAACAC	TGTGCTCAGAGGCCGTCTT
Hes2	GCTACCGGACCAAGGAAGTTC	GAGCTAGACTGTTCTCAAAGTGAGTGA
Hey1	GGGAGGGTCAGCAAAGCA	GCTGCGCATCTGATTTGTCA
Hey2	CACATCAGAGTCAACCCCATGT	GTGAGGAGAGCAGAGCCATGA
HeyL	AGATGCAAGCCCGGAAGAA	CGCAATTCAGAAAGGCTACTGTT
Il6	CCTCTCTGCAAGAGACTTCCATCCAG	AGGCCGTGGTTGTCACCAGC