Expanded View Figures

Fig EV1. A gain of function screen identifies USP8 as a metastasis-activator.

A DUB activity profiling in cells by using biotin-Ub-VME pull-down assay.
B Biotin ABP analysis of USP8 in breast cancer cell lines (left) and patients tumor samples (right).
C Oncomine data analysis of USP8 expression in Melanoma, IBC (Invasive Breast Carcinoma), TBC (Tubular Breast Carcinoma) vs. normal tissues.
D Viable cell number counts in control (Co.) and USP8 stably depleted-MDA-MB-231 cells (with two independent shRNA: #1 and #2).
E Left panel: representative pictures of transwell assay performed in control or USP8 stably depleted-MCF10A-RAS (MII) cells. Quantification of migrated cells was shown in the right panel.
F Quantification of the $CD44^{high}/CD24^{low}$ population in control (Co.) and USP8 stably depleted-MDA-MB-231 cells (with two independent shRNA: #1 and #2) ($n = 3$ per group).
G A representative flow cytometric analysis of control (Co.) and USP8 stably depleted-MDA-MB-231 cells (with two independent shRNA: #1 and #2) stained for CD24 and CD44.

Data information: $*P < 0.05$ (two-tailed Student's *t* test (C, E, F)). Data are shown as mean + SD (E) or as means ± SD (C, D, F).

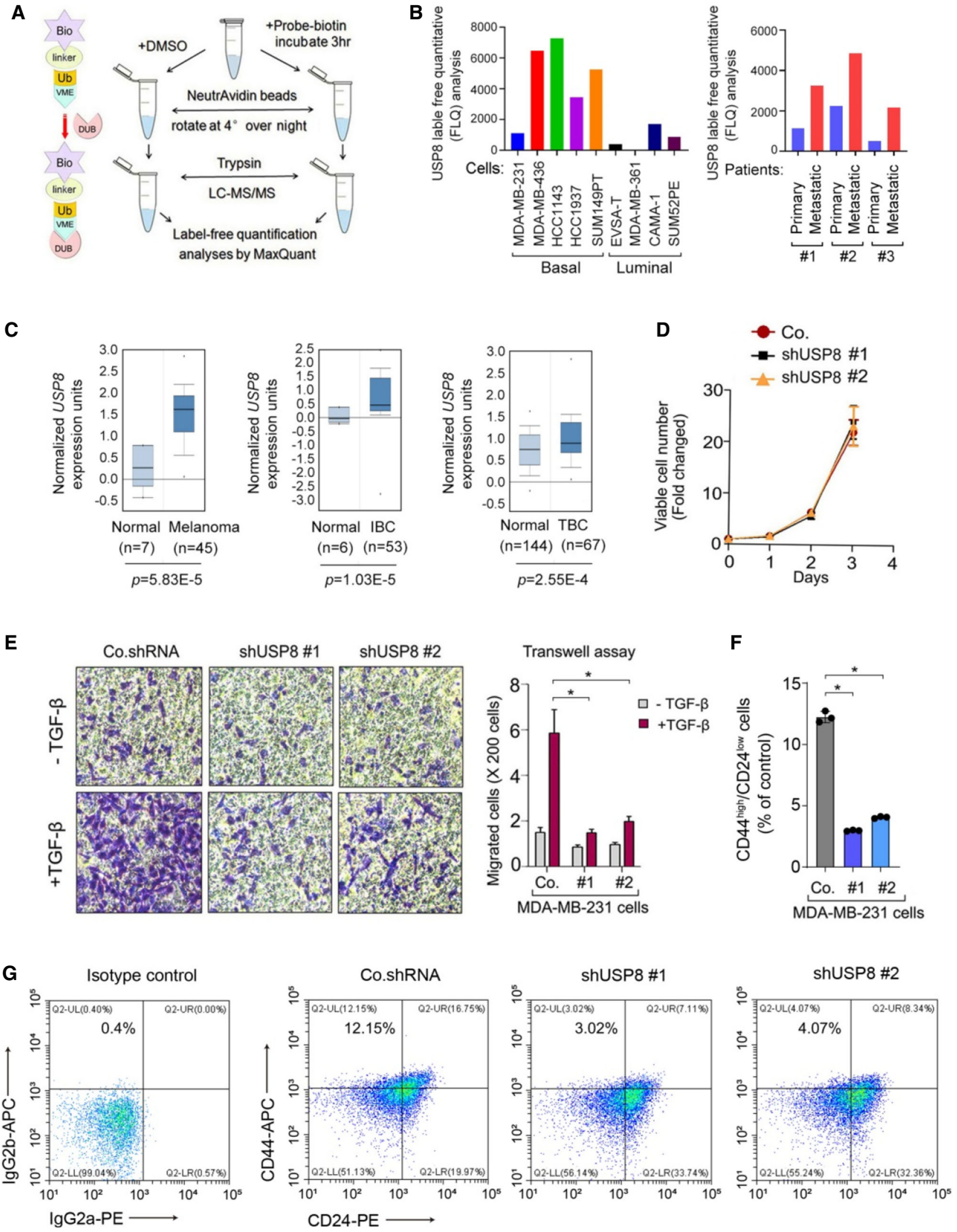
A
Bio
linker
Ub
VME
DUB
+DMSO
+Probe-biotin
incubate 3hr
NeutrAvidin beads
rotate at 4° over night
Trypsin
LC-MS/MS
Label-free quantification
analyses by MaxQuant
B
USP8 lable free quantitative (FLQ) analysis
Cells:
MDA-MB-231
MDA-MB-436
HCC1143
HCC1937
SUM149PT
EVSA-T
MDA-MB-361
CAMA-1
SUM52PE
Basal
Luminal
Patients:
Primary
Metastatic
#1
#2
#3
C
Normalized USP8 expression units
Normal Melanoma
(n=7) (n=45)
p=5.83E-5
Normal IBC
(n=6) (n=53)
p=1.03E-5
Normal TBC
(n=144) (n=67)
p=2.55E-4
D
Co.
shUSP8 #1
shUSP8 #2
Viable cell number (Fold changed)
Days
E
Co.shRNA
shUSP8 #1
shUSP8 #2
- TGF-β
+TGF-β
Transwell assay
Migrated cells (X 200 cells)
MDA-MB-231 cells
F
CD44high/CD24low cells (% of control)
MDA-MB-231 cells
G
Isotype control
Co.shRNA
shUSP8 #1
shUSP8 #2
IgG2b-APC
IgG2a-PE
CD44-APC
CD24-PE
0.4%
12.15%
3.02%
4.07%

Fig EV1.

Fig EV2. Loss of USP8 increases the percentage of $CD8^+$ T cells from lymph nodes and spleen.

A FACS analysis (left) and Quantification of TβRII in purified EVs from various types of cancer cell lines: metastatic bresat cancer cells (MDA-MB-231), colorectal cancer cells (MC38 and HCT116), melanoma B16 or lung cancer cells (A549).

B ELISA analysis of TβRII in purified EVs from various types of cancer cell lines: metastatic bresat cancer cells (MDA-MB-231), colorectal cancer cells (MC38 and HCT116), melanoma B16 or lung cancer cells (A549).

C EVs concentration by nanosight of 4T1 and MDA-MB-231 cells infected with lentivirus encoding control shRNA (Co.sh) or USP8 shRNA.

D Quantification of the percentage of $T\beta RII^+$ EVs in plasma samples from mice at day 42.

E–G Experimental analysis *in vivo*: BALB/c mice were nipple injected with control and USP8 stably depleted-4T1 cells (5×10^5 cells per mouse), followed by tail vein-injection of Co.EVs or $T\beta RII^+$ EVs (50 μg per mouse every other day) for 3 weeks ($n = 5$ per group) (E). Bright view of primary tumor from each group at week 3 (left) and tumor volume measured in time (right) (F). Percentage of lung metastasis area of all mice (left) and representative HE stained lung sections (right) from each group at week 3 (G). Scale bar, 1 mm.

Data information: $^*P < 0.05$ (two-tailed Student's *t* test (A, C, D, G) or two-way ANOVA (B, F)). Data are shown as mean + SD (A, C) or as means ± SD (B, D, F, G).

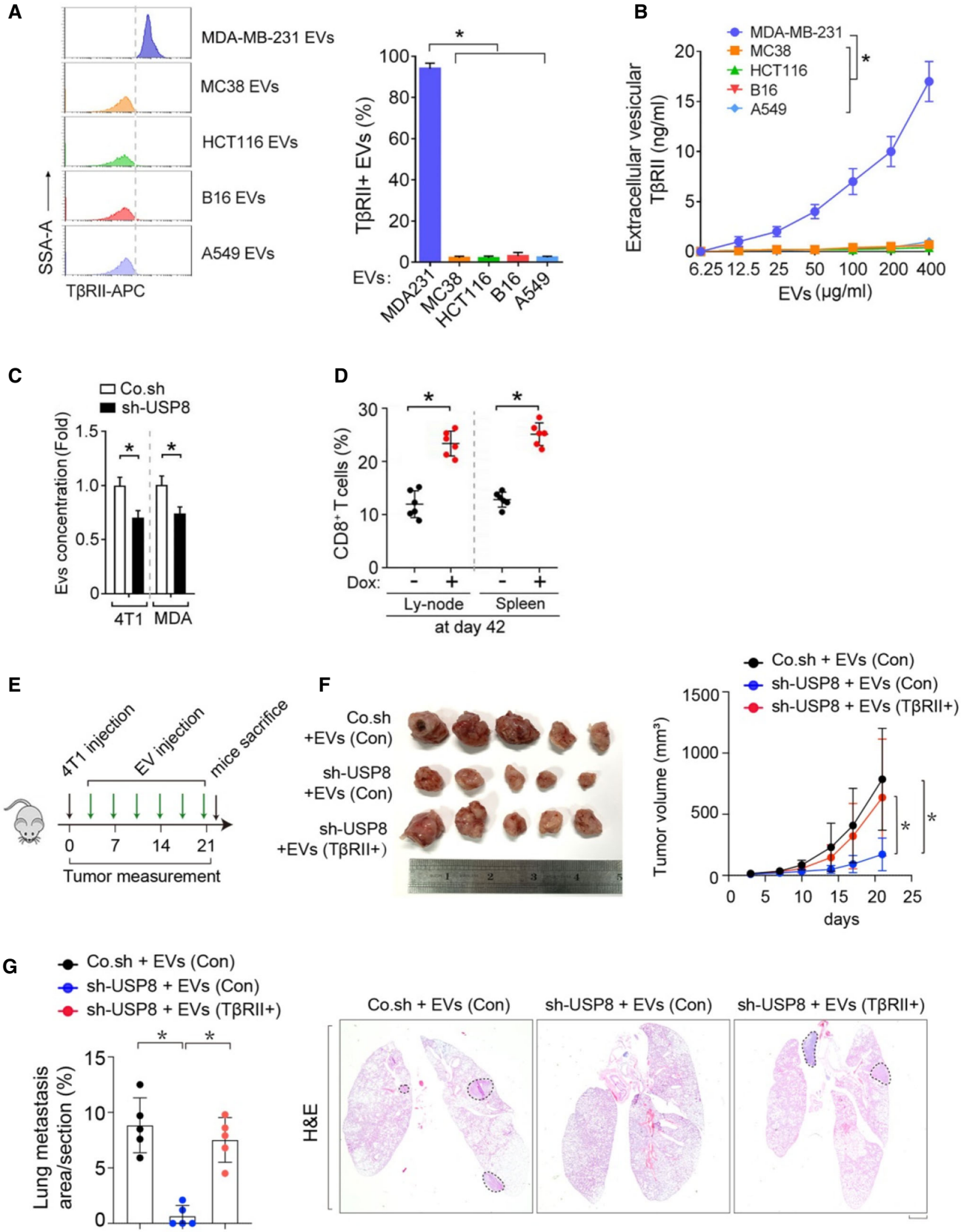
A
MDA-MB-231 EVs
MC38 EVs
HCT116 EVs
B16 EVs
A549 EVs
SSA-A
TβRII-APC
TβRII+ EVs (%)
EVs:
MDA231
MC38
HCT116
B16
A549
B
MDA-MB-231
MC38
HCT116
B16
A549
Extracellular vesicular TβRII (ng/ml)
EVs (μg/ml)
C
Co.sh
sh-USP8
Evs concentration (Fold)
4T1
MDA
D
CD8⁺ T cells (%)
Dox:
Ly-node
Spleen
at day 42
E
4T1 injection
EV injection
mice sacrifice
Tumor measurement
F
Co.sh +EVs (Con)
sh-USP8 +EVs (Con)
sh-USP8 +EVs (TβRII+)
Co.sh + EVs (Con)
sh-USP8 + EVs (Con)
sh-USP8 + EVs (TβRII+)
Tumor volume (mm³)
days
G
Co.sh + EVs (Con)
sh-USP8 + EVs (Con)
sh-USP8 + EVs (TβRII+)
Lung metastasis area/section (%)
H&E

Fig EV2.

Fig EV3. USP8 inhibitor represses cancer progression and metastasis.

A Normalized TβRII mRNA expression from MDA-MB-231 breast cancer cells treated with USP8 inhibitor and TGF-β (2.5 ng/ml) as indicated.

B Pre-ranked gene set enrichment analysis (GSEA) in MDA-MB-231 cells treated with control DMSO versus USP8 inhibitor (2 μM) for 24 h. The consensus signature of the genes correlated with various breast tumor characteristics that enriched in cells with normal USP8 activity (control) or cells with suppressed USP8 activity (USP8 inhibitor) were shown.

C–F Experimental analysis *in vivo*: MDA-MB-231-Luc cells (1×10^5 per mouse) were intracardially injected into nude mice ($n = 5$ for each group). After 3 weeks, USP8 inhibitor (1 mg/kg) was injected via intraperitoneal injection every the other day for three weeks. BLI signals (left panel) and BLI imaging of three representive nude mice from each group at week 6 (right panel) were shown (C). Number of bone metastasis nodules (D). Percentage of $T\beta RII^+$ crEVs in plasma samples from mice (E). Immunoblot analysis of metastatic tumor nodule in mice (F).

Data information: $^*P < 0.05$ (two-tailed Student's *t* test (A, D, E) or two-way ANOVA (C)). Data are shown as mean ± SD (A, C, D, E).

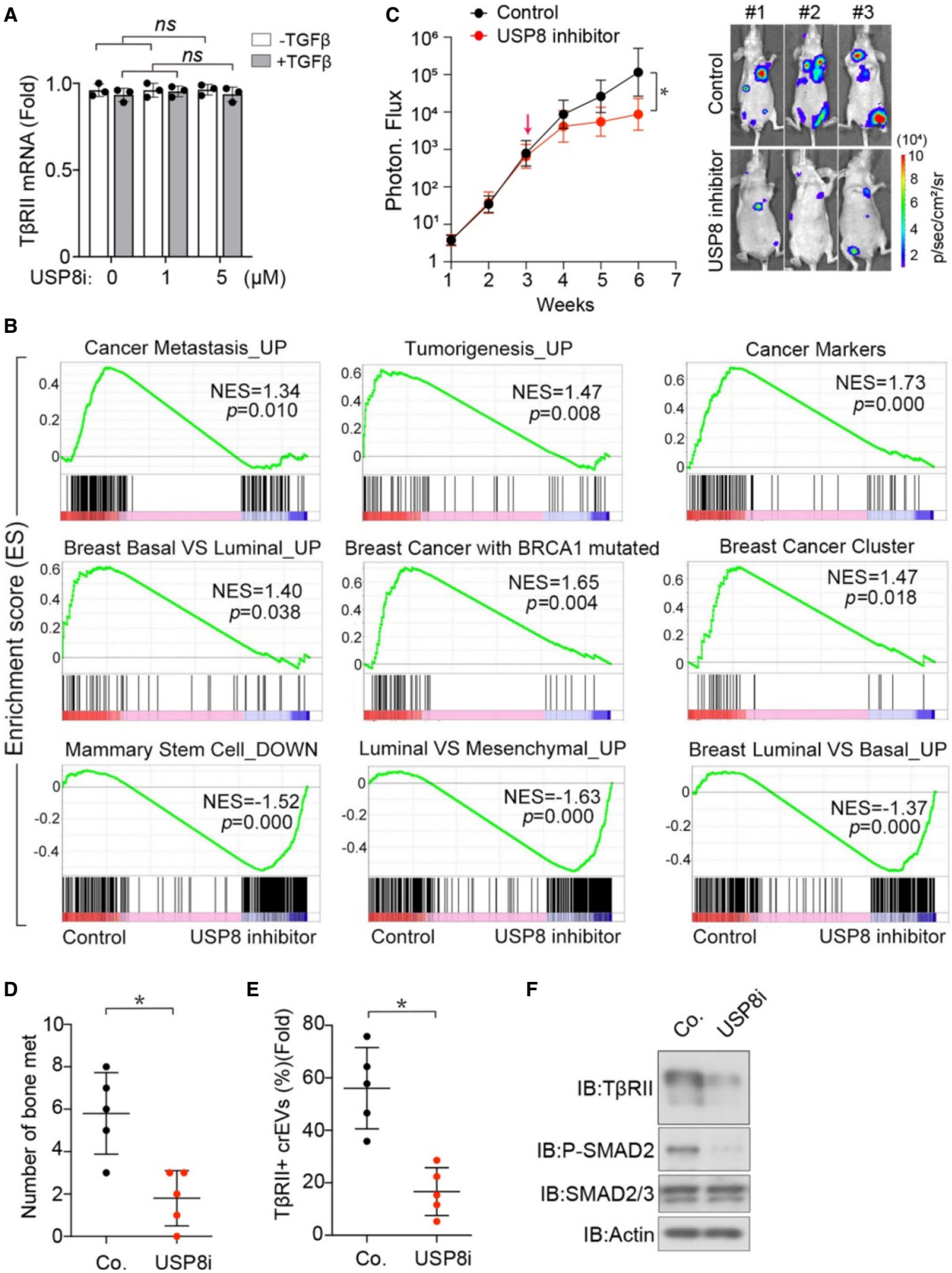

Fig EV3.

Fig EV4. TβRII$^+$ EVs increase tumor growth and metastasis *in vivo*.

A, B Transwell migration assay (A) and transwell invasion assay (B) using MDA-MB-231 cells co-cultured with Co.EVs or TβRII$^+$ or TβRII$^-$ EVs (50 μg) for 48 h. Representative figures from three independent experiments are shown. The quantification of migrated cells per field is shown in the bar graphs; mean ± SD.

C The cellular proliferation of CD8$^+$ T cells was analyzed using a 4-day CFSE dilution assay.

D T cell-mediated cancer cell killing assay: MDA-MB-231 cells co-cultured with or without activated T cells for 48 h were subjected to crystal violet staining (left). MDA-MB-231-to-T cell ratio = 1:3. Quantification of cytotoxicity of T cells (right).

E–H Experimental analysis in vivo: BALB/c mice were nipple injected with 4T1 cells (5 × 105 cells per mouse), followed by tail vein-injection of Co.EVs or TβRII$^+$ or TβRII$^-$ EVs (50 μg per mouse every other day) for 3 weeks (n = 5 per group) (E). Tumor volume measured in time (F). Percentage of TβRII$^+$ crEVs in plasma of all mice in each group at week 3 (G). Percentage of lung metastasis area of all mice (left) and representative HE stained lung sections (right) from each group at week 3 (H). Scale bar, 1 mm.

Data information: *P < 0.05 (two-tailed Student's t test (A, B, D, G, H) or two-way ANOVA (F)). Data are shown as mean ± SD (A, B, D, F, G, H).

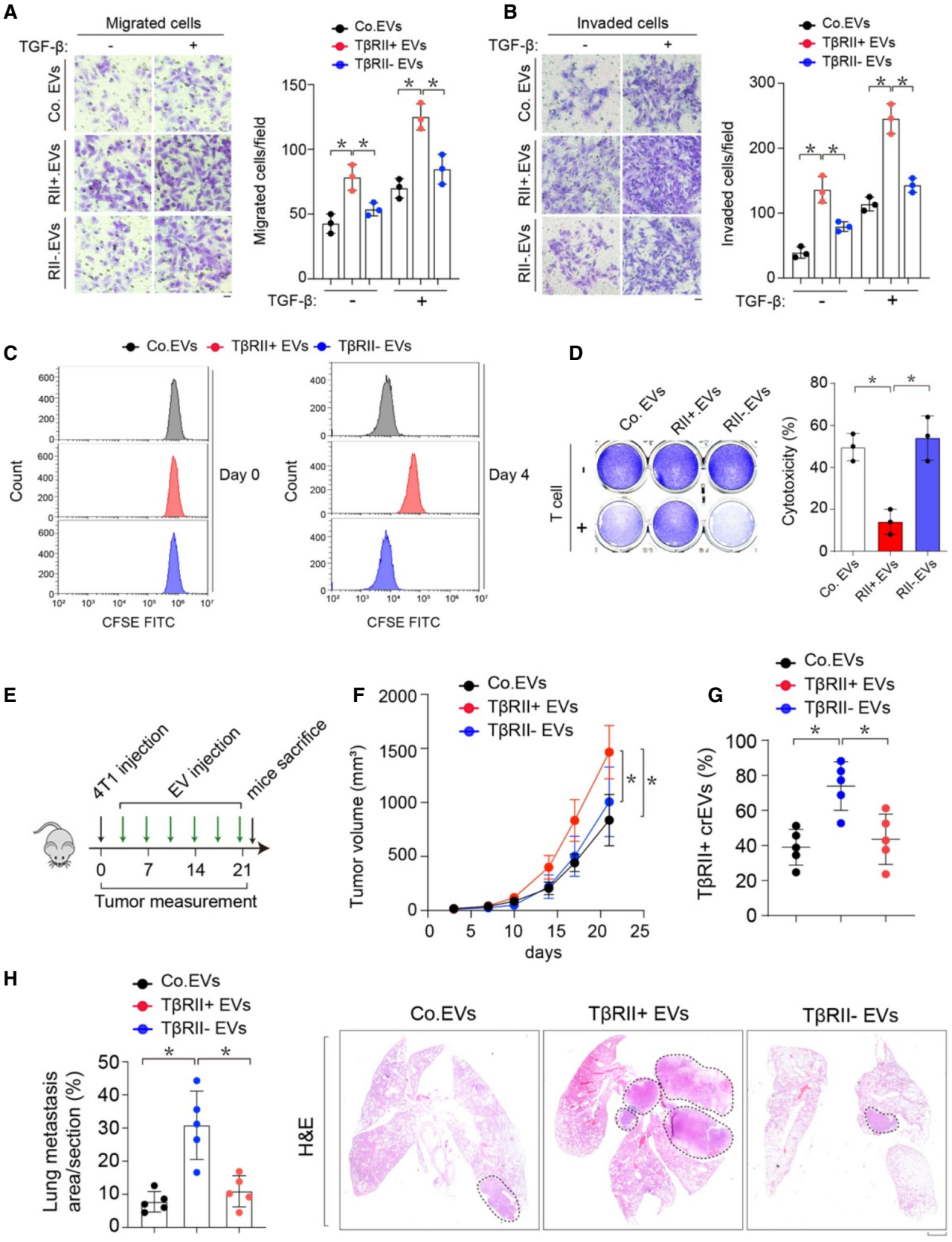
A
Migrated cells
TGF-β:
Co. EVs
RII+.EVs
RII-.EVs
Co.EVs
TβRII+ EVs
TβRII- EVs
Migrated cells/field
B
Invaded cells
Invaded cells/field
C
Count
Day 0
Day 4
CFSE FITC
D
T cell
Cytotoxicity (%)
E
4T1 injection
EV injection
mice sacrifice
Tumor measurement
F
Tumor volume (mm³)
days
G
TβRII+ crEVs (%)
H
Lung metastasis area/section (%)
H&E

Fig EV4.