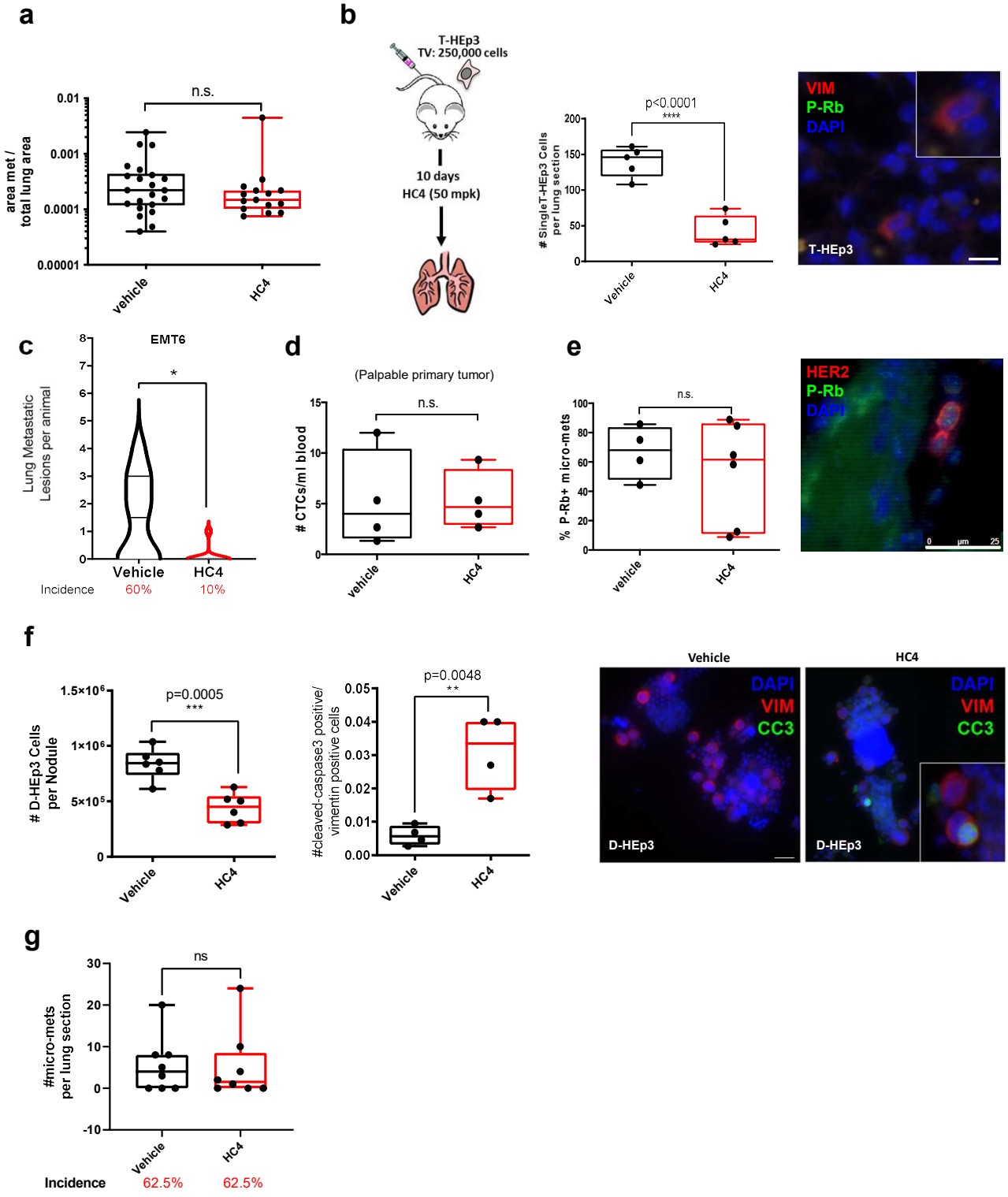
a
n.s.
area met / total lung area
0.01
0.001
0.0001
0.00001
vehicle
HC4
b
T-HEp3
TV: 250,000 cells
10 days
HC4 (50 mpk)
p<0.0001
****
SingleT-HEp3 Cells per lung section
200
150
100
50
0
Vehicle
HC4
VIM
P-Rb
DAPI
T-HEp3
c
EMT6
*
Lung Metastatic Lesions per animal
8
7
6
5
4
3
2
1
0
Vehicle
HC4
Incidence
60%
10%
d
(Palpable primary tumor)
n.s.
CTCs/ml blood
15
10
5
0
vehicle
HC4
e
n.s.
% P-Rb+ micro-mets
100
80
60
40
20
0
vehicle
HC4
HER2
P-Rb
DAPI
0
µm
25
f
p=0.0005
***
D-HEp3 Cells per Nodule
1.5×10⁶
1×10⁶
5×10⁵
0
Vehicle
HC4
p=0.0048
**
#cleaved-caspase3 positive/ vimentin positive cells
0.05
0.04
0.03
0.02
0.01
0.00
Vehicle
HC4
Vehicle
HC4
DAPI
VIM
CC3
D-HEp3
DAPI
VIM
CC3
D-HEp3
g
ns
#micro-mets per lung section
30
20
10
0
-10
Vehicle
HC4
Incidence
62.5%
62.5%

Supplementary Figure S2. (a) Normalized area of single macro-metastases in lung in vehicle (N=22) and HC4-treated animals (N=15). P by Mann-Whitney test. (b) $2.5x10^5$ HNSCC T-Hep3 cells as single cell suspensions were injected via tail vein in Balb/c nu/nu mice and 24 h after injection animals were treated with vehicle or HC4 (50 mg/kg) for 10 days. Frequency of T-HEp3 DCCs in the lung detected via a human specific anti-vimentin antibody was scored. 5 lung sections/animal ± s.d (N=5 per group). Scale bar, 10 μm. P by Mann-Whitney test. (c) Balb/c syngeneic EMT6 mice were treated with HC4 at 10 mg/kg for 17 days. Metastatic lesions were detected by H&E staining and quantified in 5 lung section/animal. P by Mann-Whitney test. (d) Quantification of circulating tumor cells/ml blood by HER2 staining of cytospins (N=4 per group) P by Mann-Whitney test. (e) Percentage P-Rb+ micro-metastasis per lung section/animal in vehicle (N=4) and HC4-treated animals (N=6). Scale bar, 25 μm. P by Student's t test. (f) Number of D-Hep3 cells per nodule in vehicle and HC4-treated (20 μM, 6 days) CAM tumors (N=6 per group) (left); quantification and representative images of D-HEp3 CAM tumors stained for cleaved caspase-3 (green), vimentin (red), and DAPI (blue) (right). Approximately 1000 cells per group were assessed, p by Mann-Whitney test. Scale bars, 25 μm. (g) Mice were injected with MMTV-HER2 cells from primary tumor lesions via the tail vein, allowed them to expand into established metastatic lesions for 3 weeks and treated with vehicle or HC4 (50 mg/kg) for 2 weeks. Micro-metastasis were detected by H&E or IF (HER2 detection) staining and quantified in 5 lung section/animal ± s.d (N=8 per group). P by Mann-Whitney test.