a Orthotopic injection (lung)

N.S.

Luciferase activity [× 10^4]

8.0
6.0
4.0
2.0
0

Wt   *Ask1*^{-/-}

b Ectopic injection (dorsal skin)

N.S.

Tumor size [cm^3]

0.4
0.3
0.2
0.1
0

Wt   *Ask1*^{-/-}

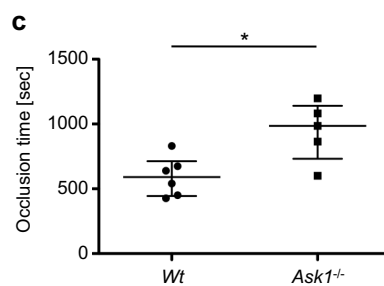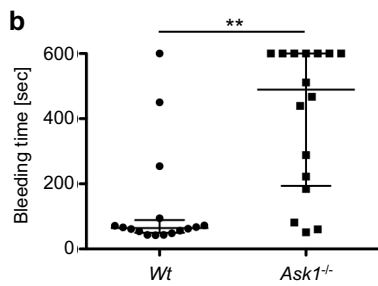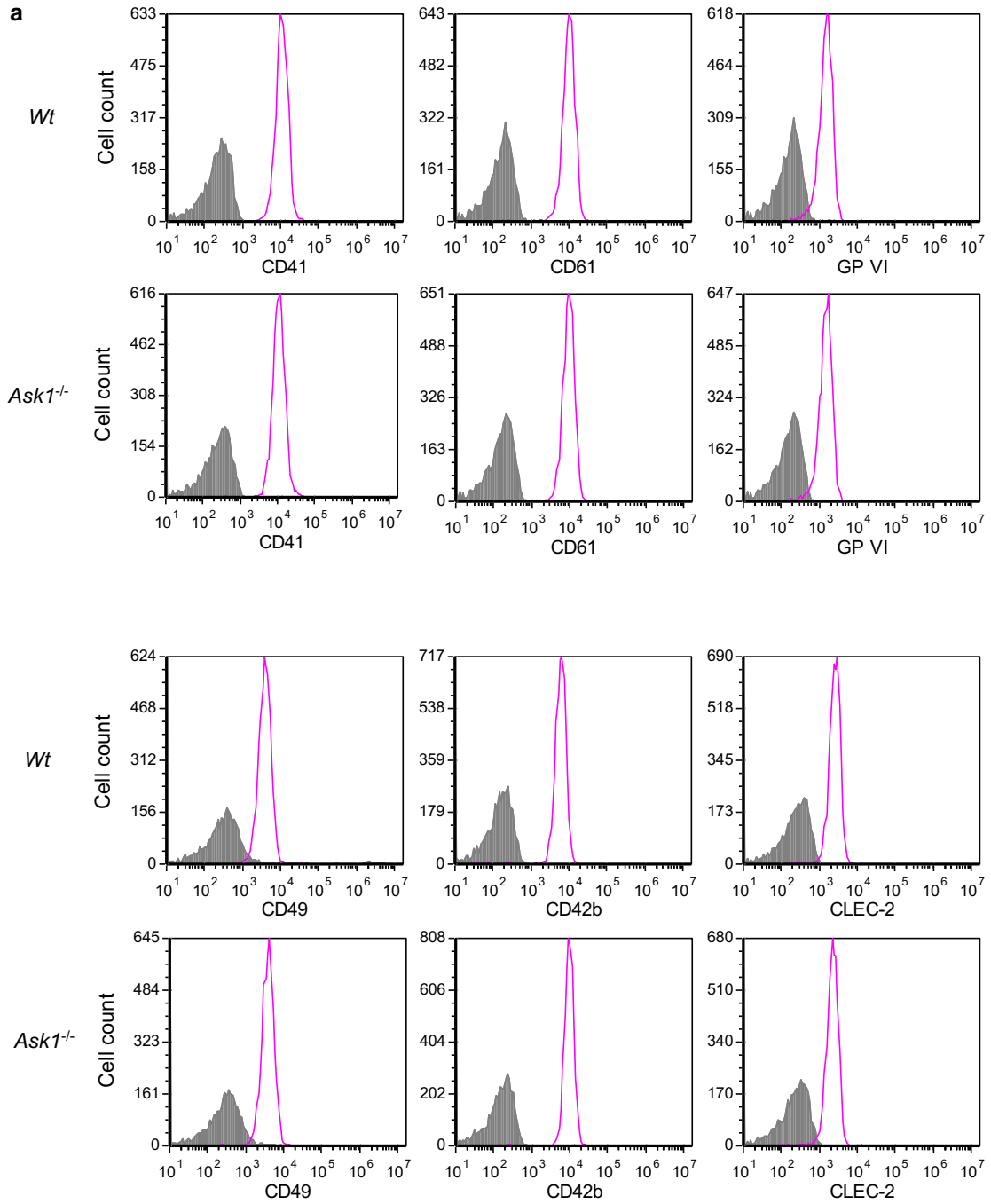**Supplementary Figure 1** Characterization of ASK1 in mice in the context of tumor

lung metastasis. (**a**) Luciferase activity of the lung lysates at 7 days after orthotopic

injection of 3LL-Luc2 cells (*Wt* mice: n = 25, *Ask1*^{-/-} mice: n = 25). (**b**) Tumor volume

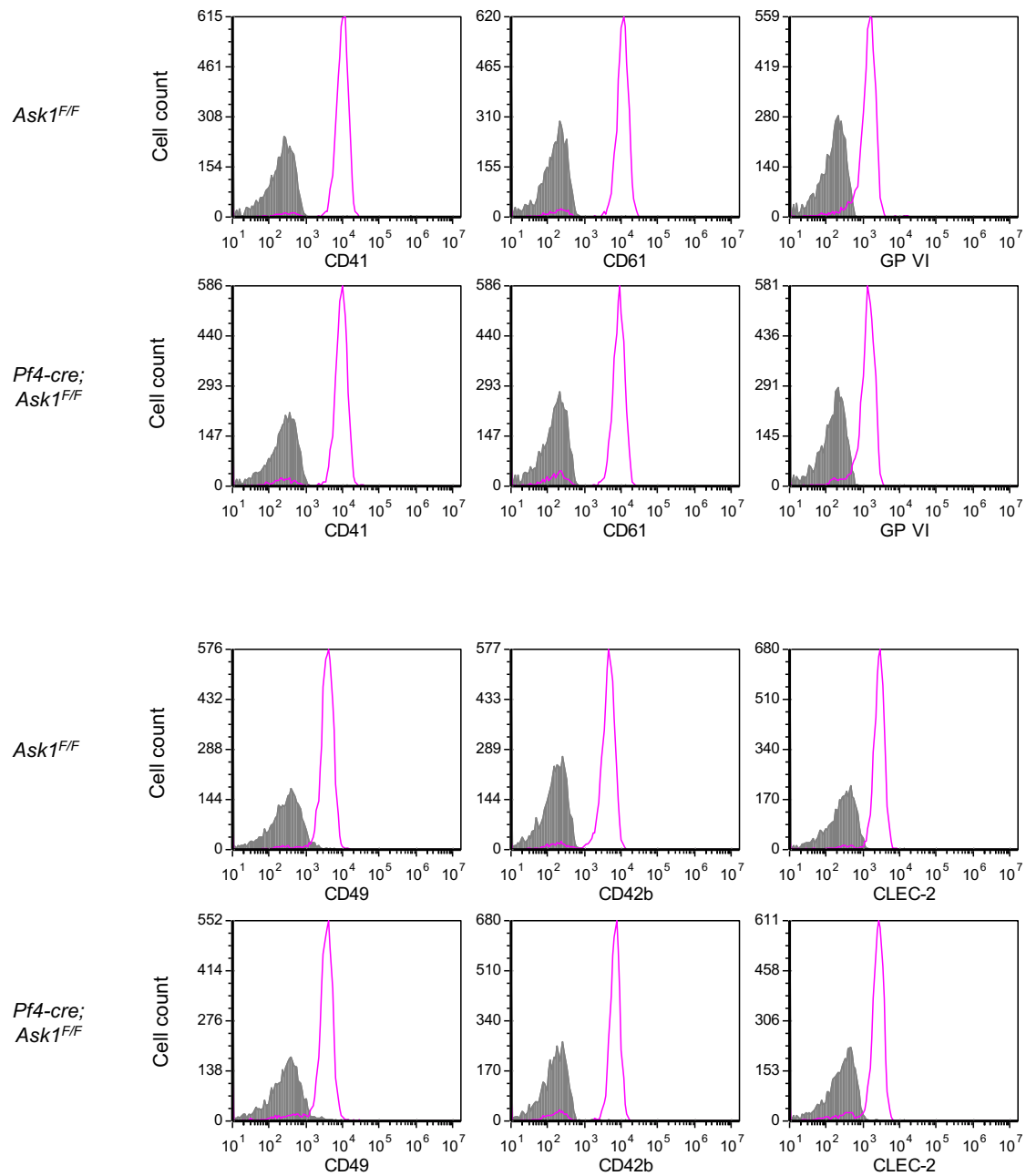at 28 days after subcutaneous injection of 3LL-Luc2 cells (*Wt* mice: n = 9, *Ask1*^{-/-} mice:

n = 11). The data are shown as the mean ± s.e.m. N.S., non-significant. Unpaired

Student's *t*-tests (**a** and **b**).

a

Wt

| CD41 | CD61 | GP VI |

Ask1⁻ᐟ⁻

| CD41 | CD61 | GP VI |

Wt

| CD49 | CD42b | CLEC-2 |

Ask1⁻ᐟ⁻

| CD49 | CD42b | CLEC-2 |

b

Bleeding time [sec]

**

Wt   *Ask1⁻ᐟ⁻*

c

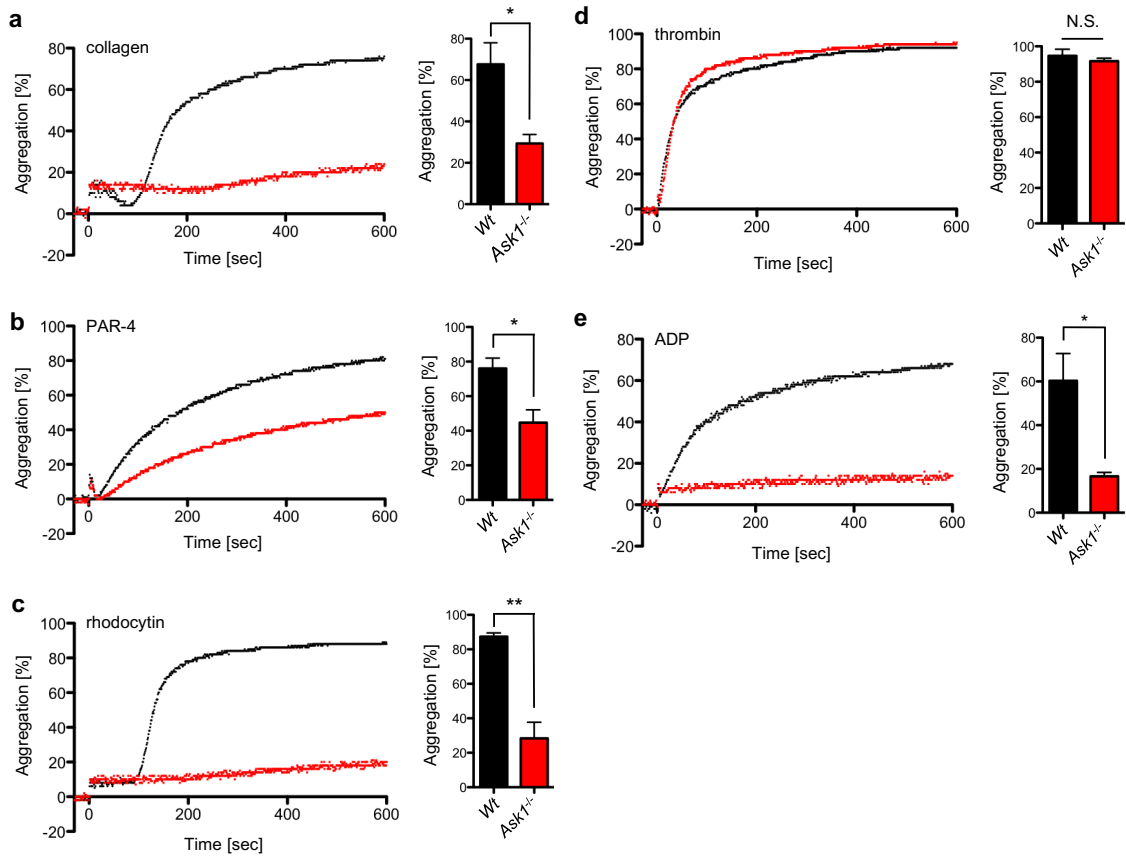Occlusion time [sec]

*

Wt   *Ask1⁻ᐟ⁻*

Supplementary Figure 2 *Ask1*$^{-/-}$ mice is normal in surface expression of platelet

receptors on platelets but exhibits unstable hemostasis. (**a**) The surface expression of

platelet receptors was determined by flow cytometry (n = 3). The isotype controls are

noted in gray. (**b**) Bleeding time in the tail bleeding assay (*Wt* mice: n = 16, *Ask1*$^{-/-}$

mice: n = 16). (**c**) Occlusion time in the femoral artery in the $FeCl_3$-induced thrombosis

assay (*Wt* mice: n = 6, *Ask1*$^{-/-}$ mice: n = 5). The data are presented with the median and

interquartile range. **p < 0.01; *p < 0.05. Mann-Whitney *U*-test (**b** and **c**).

Supplementary Figure 3 Surface expression of platelet receptors on platelets from

Ask1^{F/F} and *Pf4-cre; Ask1^{F/F}* mice. The surface expression of platelet receptors was

determined by flow cytometry (n = 3). The isotype controls are noted in gray.

Supplementary Figure 4 *Ask1*^{-/-} mice shows defects in ADP-dependent platelet aggregation. (**a-e**) Platelet aggregation of *Wt* and *Ask1*^{-/-} mice induced by 1 μg/ml collagen (**a**), 50 μM PAR-4 (**b**), 1 nM rhodocytin (**c**), 0.025 U/ml thrombin (**d**) and 10 μM ADP (**e**). Representative traces (left) and the maximal aggregation percentage within 10 minutes of measurement (right) are presented. The data are expressed as the mean ± s.e.m. (n = 3 each). **p < 0.01; *p < 0.05; N.S., non-significant. Unpaired Student's *t*-tests (**a-e**).

Analyzed parameters [unit]	Wt	Ask1$^{-/-}$	Ask1$^{F/F}$	Pf4-cre; Ask1$^{F/F}$
Leukocyte [10^3 /μl]	1.19 ± 0.31	1.36 ± 0.13	1.48 ± 0.04	1.41 ± 0.36
Erythrocyte [10^4 /μl]	1.07 ± 0.05	1.00 ± 0.03	1.04 ± 0.04	0.98 ± 0.02
Hemoglobin [g/dl]	1.50 ± 0.12	1.40 ± 0.06	1.53 ± 0.03	1.43 ± 0.03
Hematocrit [%]	5.00 ± 0.26	4.73 ± 0.15	4.90 ± 0.21	4.70 ± 0.12
Platelet [10^3 /μl]	92.3 ± 4.37	89.7 ± 4.48	109.7 ± 8.65	105.0 ± 6.51
MCV [fl]	46.5 ± 0.44	47.3 ± 0.22	47.2 ± 0.37	47.8 ± 0.35
MCH [pg]	14.0 ± 0.50	14.0 ± 0.24	14.8 ± 0.23	14.6 ± 0.24
MCHC [g/dl]	29.9 ± 0.82	29.6 ± 0.34	31.4 ± 0.69	30.5 ± 0.38

Supplementary Table 1

Hematological parameters. Data are mean ± s.e.m. (n = 3).