

Supplement Figure

S1 Screening of anti-PAI-1mAbs

A. The specificity of the reaction between anti-PAI-1 antibodies and GST-PAI-1 proteins detected by ELISA. GST and GST-PAI-1 prokaryotic protein (1 µg/mL) were coated in the 96-well plate at 100 µl/well. Anti-PAI-1 mAb were added to the wells at a concentration of 2 µg/mL and incubated for 2 h, followed by detection using TMB.

B. Immunoprecipitation of PAI-1 from KYSE30luc cells with mAbs.

C. Detection of eukaryotic PAI-1 protein (His-PAI-1) and prokaryotic PAI-1 protein (GST-PAI-1) with mAbs by Western blot.

D. Detection of eukaryotic PAI-1 protein (His-PAI-1) and prokaryotic PAI-1 protein (GST-PAI-1) with mAb-2E3 by Western blot.

S2 Identification of His-PAI-1 protein activity

A-B. Identification of His-PAI-1 protein activity by Z-GGR-AMC system (**A**) and S-2251 system (**B**).

S3 mAb pairing for detection of PAI-1 by sandwich ELISA

A. mAb-1E2 and mAb-2E3-HRP were respectively identified as capture and detection antibodies in double-antibody sandwiched ELISA for the detection of PAI-1.

B. Standard curve line of the PAI-1 detection.

S4 Effects of PAI-1 on cell proliferation.

A. Effect of PAI-1 overexpression on proliferation.

B. Effects of exogenous human recombinant His-PAI-1 (100 ng/mL) on proliferation

** $P < 0.01$, ns ≥ 0.05 .

S5 PAI-1 mAb inhibits the migration and invasion of KYSE30lm3 and KYSE450lm2 in a dose-dependent manner

A. mAb-2E3 showed stronger ability in preventing migration compared to mAb-1E2.

B-C. Effects of mAb-1E2 and mAb-2E3 on the motility of KYSE30lm3 and KYSE30luc cells in Wound healing assay (**B**) and Transwell chamber assay (**C**).

D-F. PAI-1 in conditioned medium (CM) of EC9706 cells promotes migration, which is attenuated by immunodepletion of PAI-1. **(D)** Detection of PAI-1 protein overexpression in EC9706 cells. **(E)** Immunodepletion of PAI-1 in the CM of EC9706 cells. **(F)** Western blot analysis of PAI-1 in the CM of EC9706 cells after immunodepletion. **(G)** Immunodepletion of PAI-1 in the CM of EC9706 cells attenuated mAb-1E2 and mAb-2E3' inhibitory effects on migration.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $P \geq 0.05$.

S6 PAI-1 mAb inhibits proliferation and colony formation of PAI-1^{hi} cells

A. Effects of PAI-1 mAbs on proliferation of KYSE30lm and KYSE450lm2 cells.

B. Effects of PAI-1 mAbs on colony formation of KYSE450lm2, KYSE30lm3 and YES-2 cells.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $P \geq 0.05$.

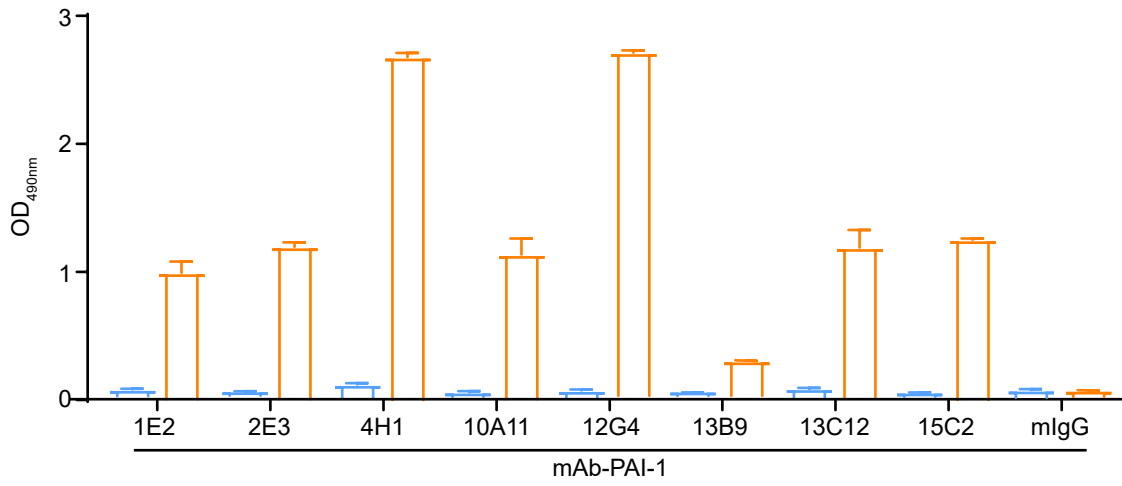
S7 mAb-2E3 did not active in blocking PAI-1 site which binds to PLAU

A. Expression of PAI-1 ligands.

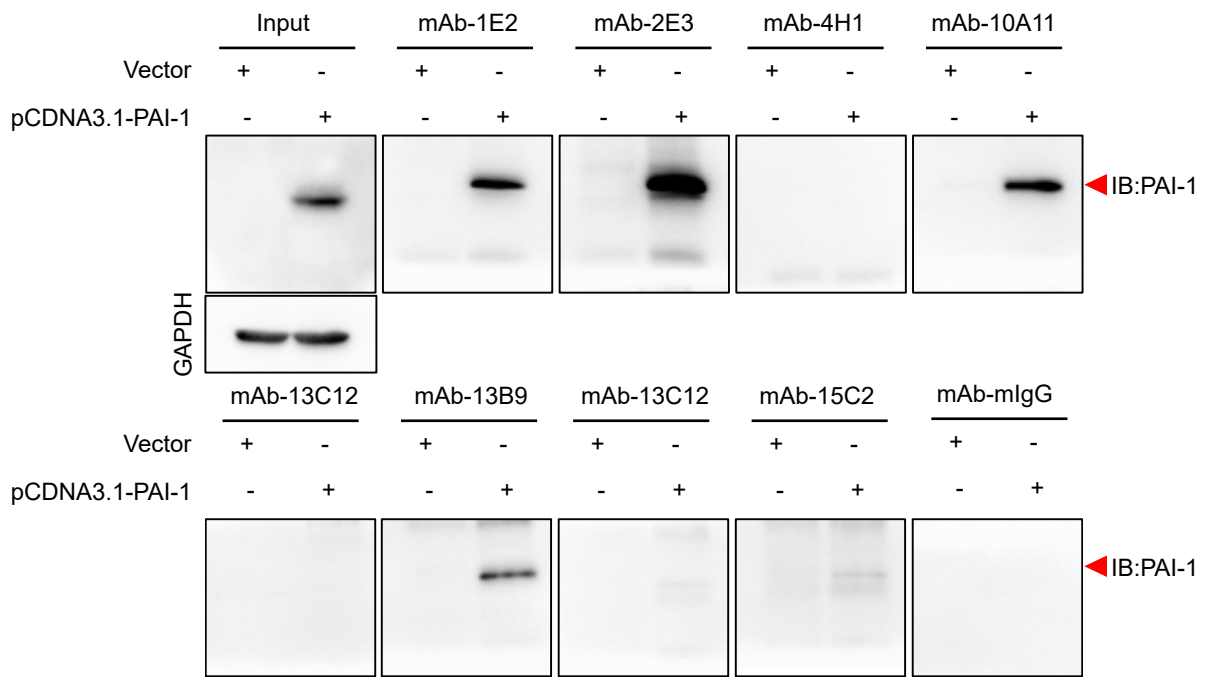
B. The mAb-2E3 did not affect PAI-1 activity as detected in S-2251 system, indicating that mAb-2E3 failed to block the binding of PAI-1 and uPA.

Fig.S1.

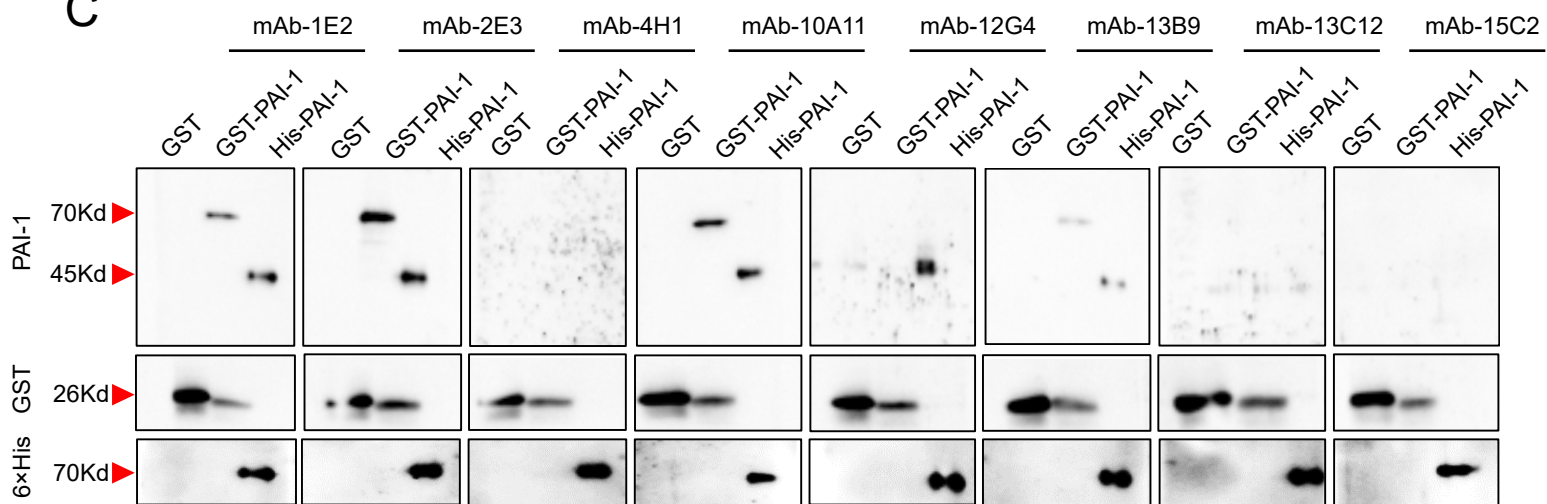
A



B



C



D

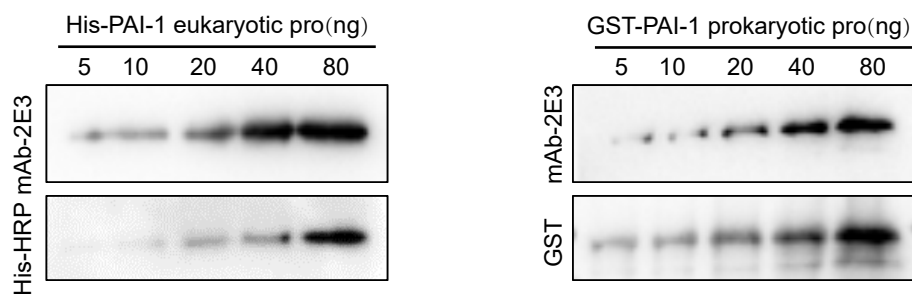
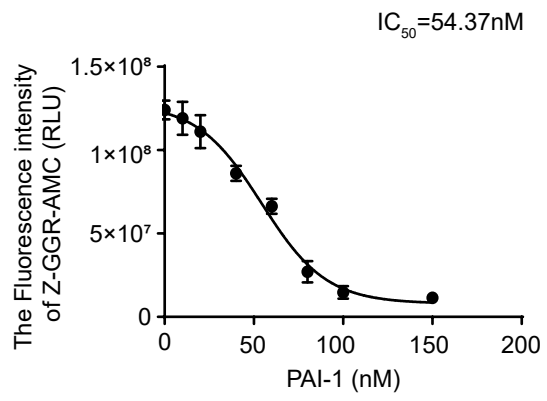


Fig.S2.

A



B

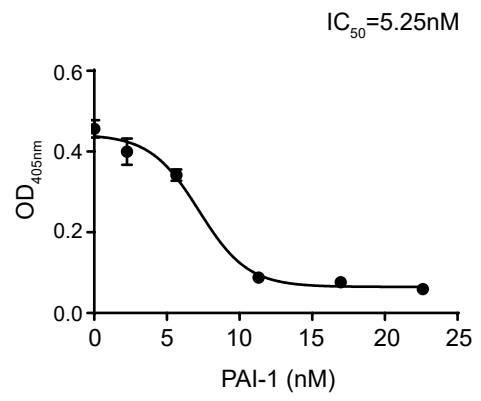
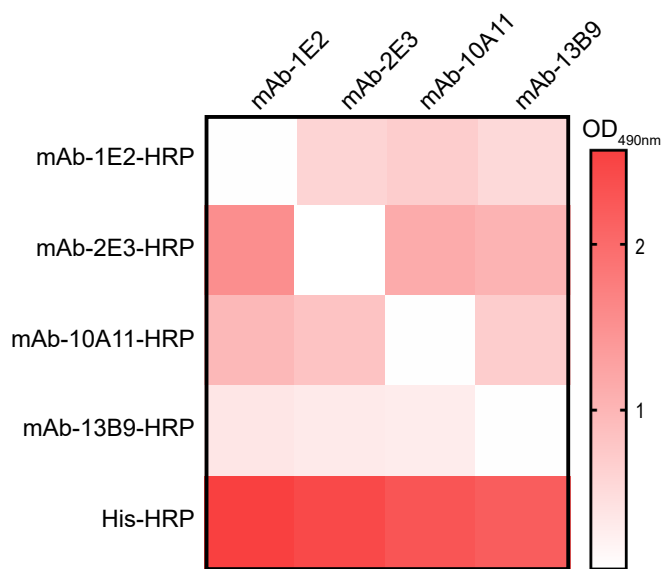


Fig.S3.

A



B

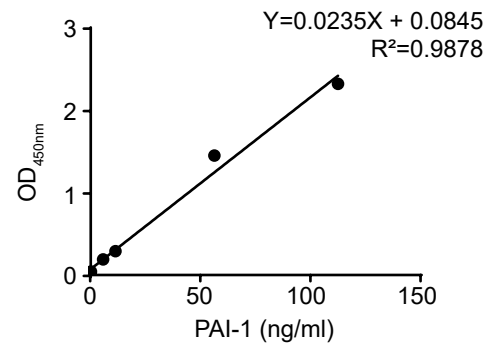
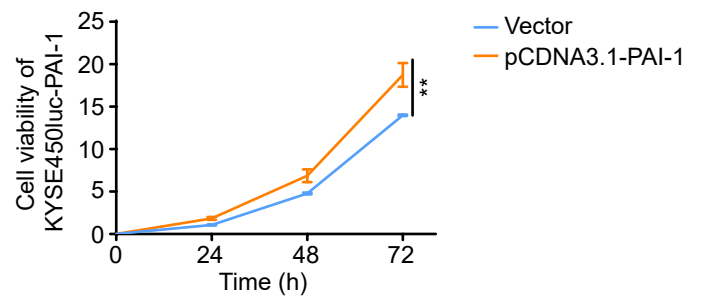
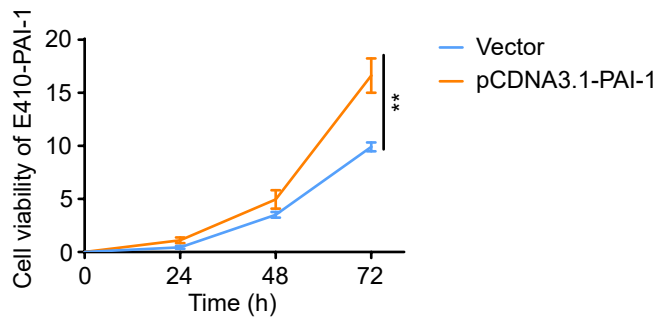


Fig.S4.

A



B

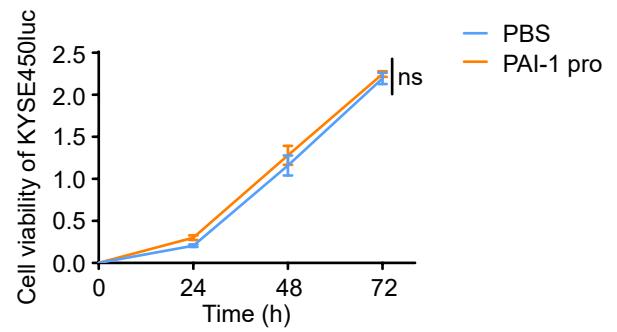
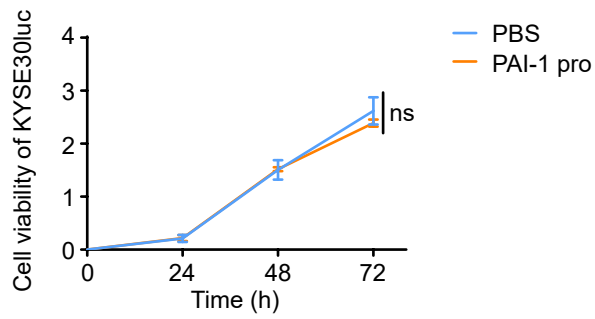
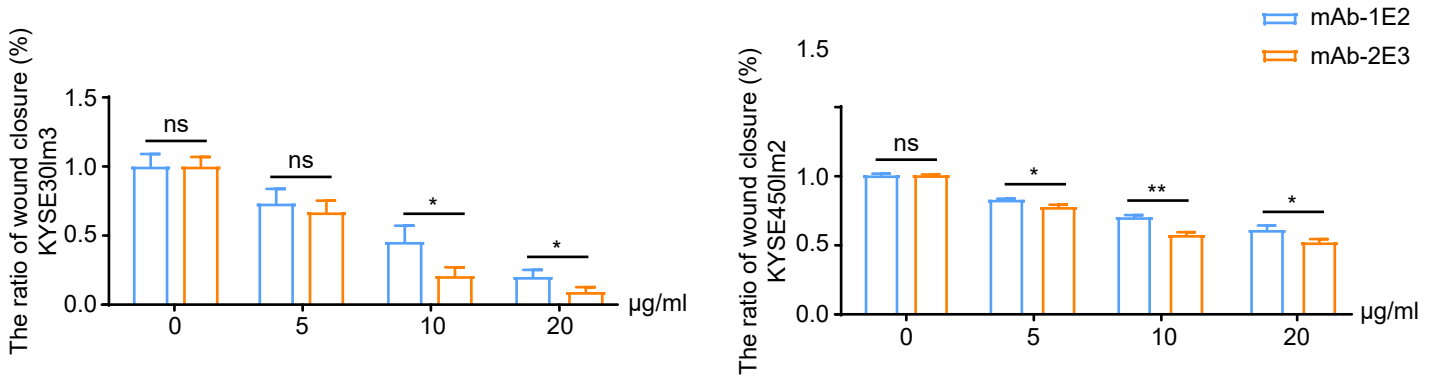
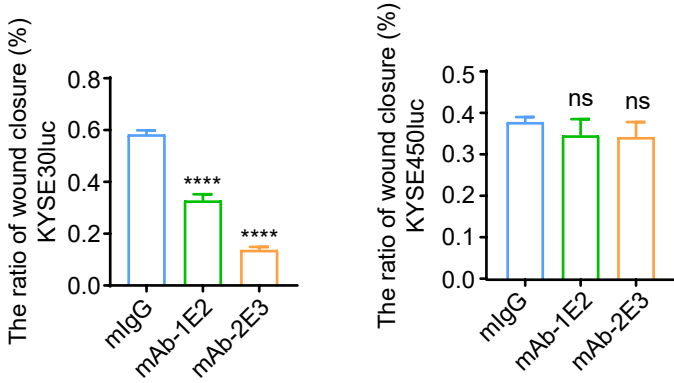


Fig.S5.

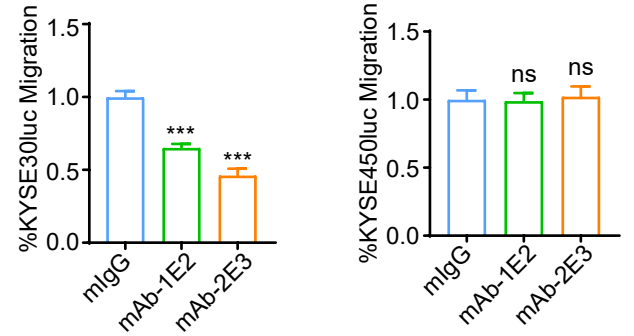
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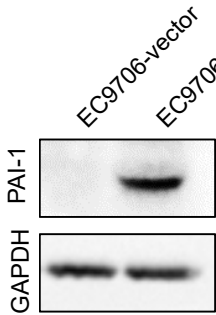
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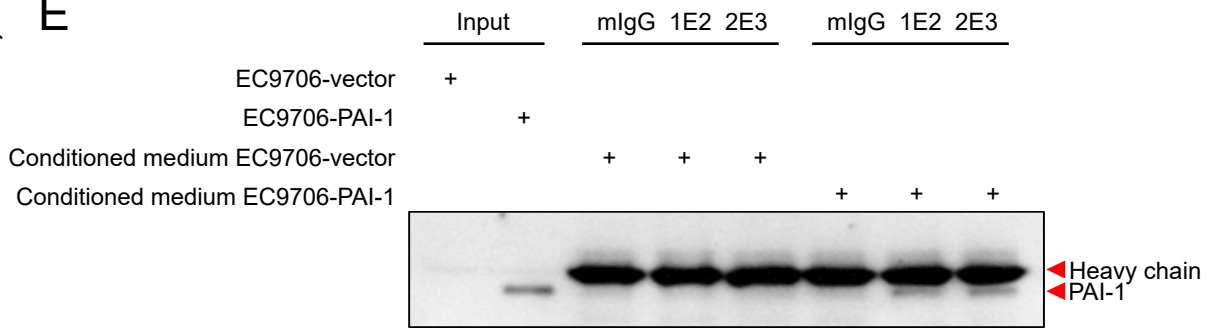
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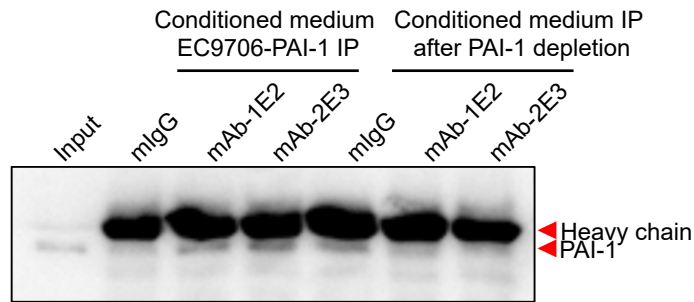
D



E



F



G

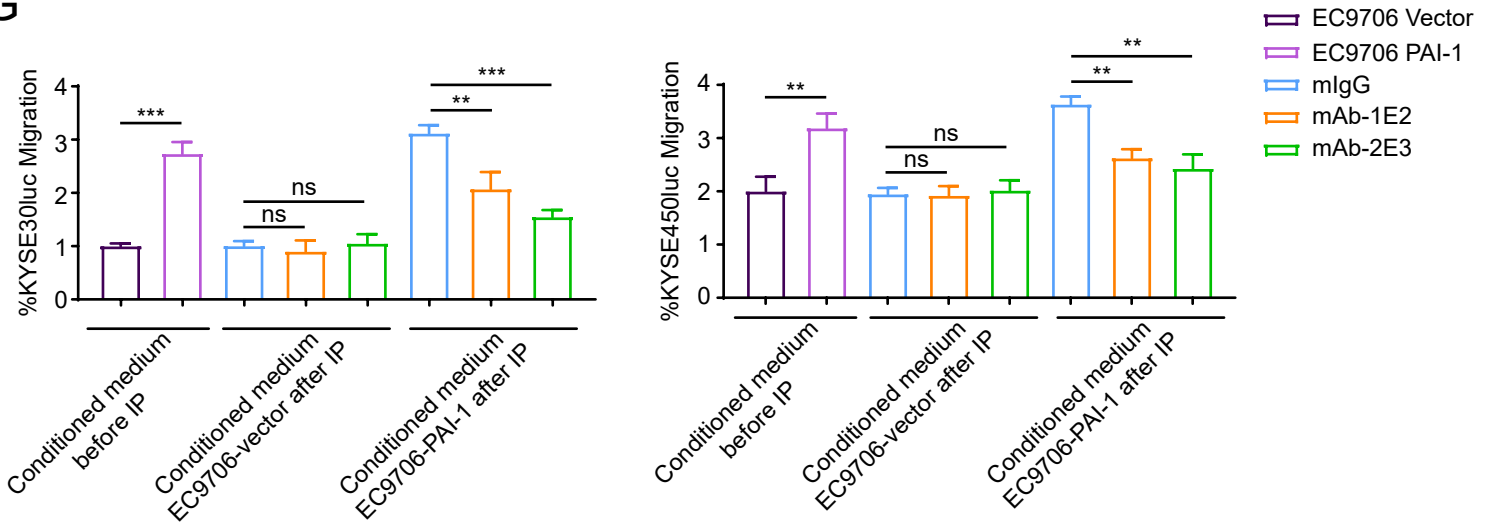
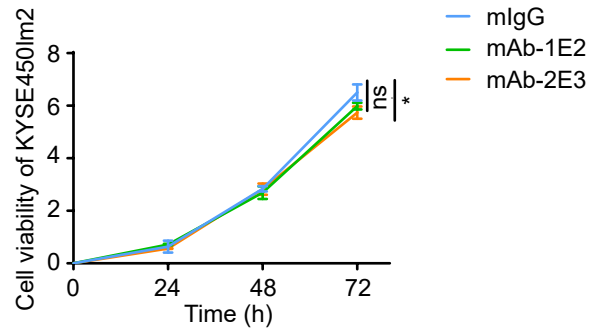
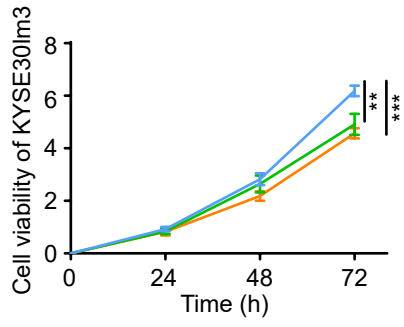


Fig.S6.

A



B

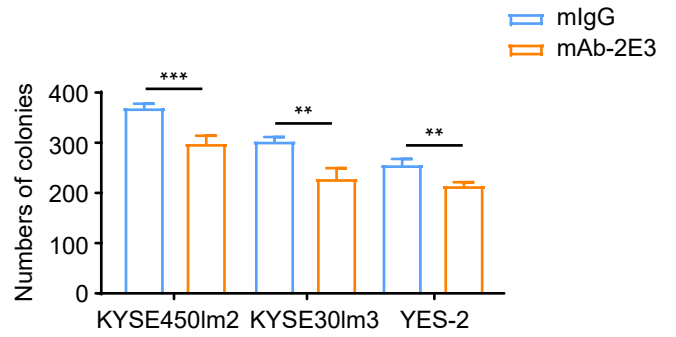
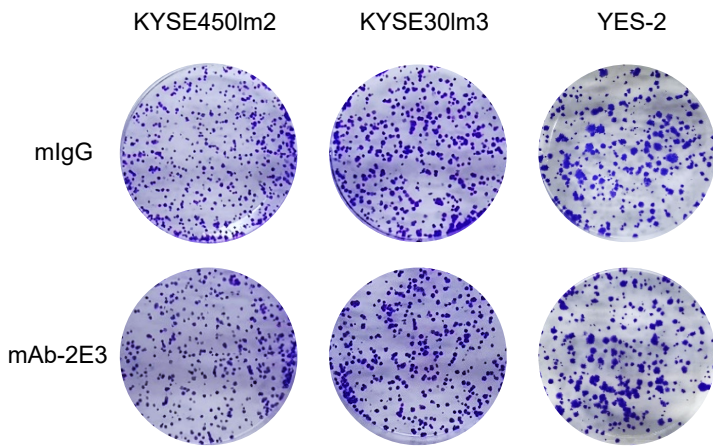


Fig.S7.

