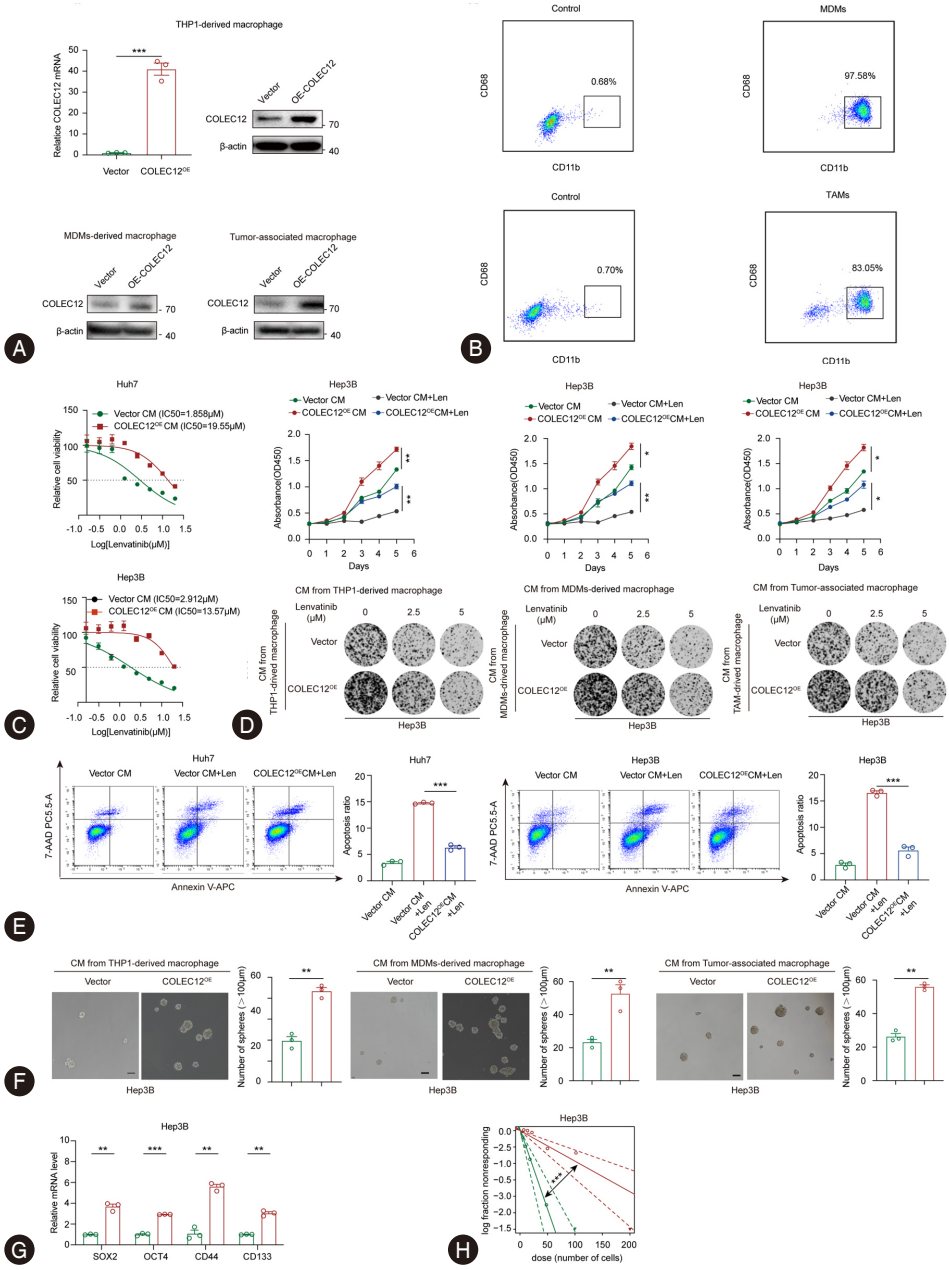

Supplementary Figure 2. COLEC12^{high} TAMs drive lenvatinib resistance through paracrine-mediated stemness reprogramming. (A) qRT-PCR analysis confirming successful overexpression of COLEC12 in THP-1-derived macrophages, MDMs and TAMs. Western blot analysis verifying COLEC12 expression in THP-1-derived macrophages following lentiviral transduction (n=3). (B) Flow cytometric identification of CD11b^{+}CD68^{+} macrophages in MDMs and TAMs. Representative dot plots showing CD11b versus CD68 staining in control cells (left panels) and in (top right) MDMs and (bottom right) TAMs. (C) IC50 values of lenvatinib in Huh7 cells and Hep3B cells treated with COLEC12^{OE} CM or Vector CM (n=3). (D) Cell proliferation was assessed by CCK-8 assay in the presence of 2.5 μM lenvatinib, and clonogenic potential was evaluated through colony formation assays with varying concentrations of lenvatinib (0, 2.5, and 5 μM). (E) Representative flow cytometry plots and quantification by flow cytometry in Huh7 and Hep3B cells treated with 2.5 μM lenvatinib (n=3). (F) Sphere formation assay of Hep3B cells cultured with COLEC12^{OE} CM or Vector CM (n=3). Scale bar, 100 μm. (G) qRT-PCR analysis of stemness markers SOX2, OCT4, CD44, and CD133 in Hep3B cells after exposure to COLEC12^{OE} CM or Vector CM (n=3). (H) In vitro limiting dilution assay of Hep3B cells treated with COLEC12^{OE} CM or Vector CM. Data are presented as stem cell frequency in chart form (n=3). Data are presented as mean±standard error of the mean. Statistical significance was determined using Student's t-test for two-group comparisons or one-way ANOVA for multiple groups. Kruskal–Wallis test for tumor initiation assays (M, N). COLEC12, Collectin Subfamily Member 12; TAMs, tumor-associated macrophages; MDMs, monocyte-derived macrophages; COLEC12^{OE}, COLEC12-overexpressing; CM, conditioned medium. *P<0.05, **P<0.01, ***P<0.001. Source data are provided as a Source Data file.