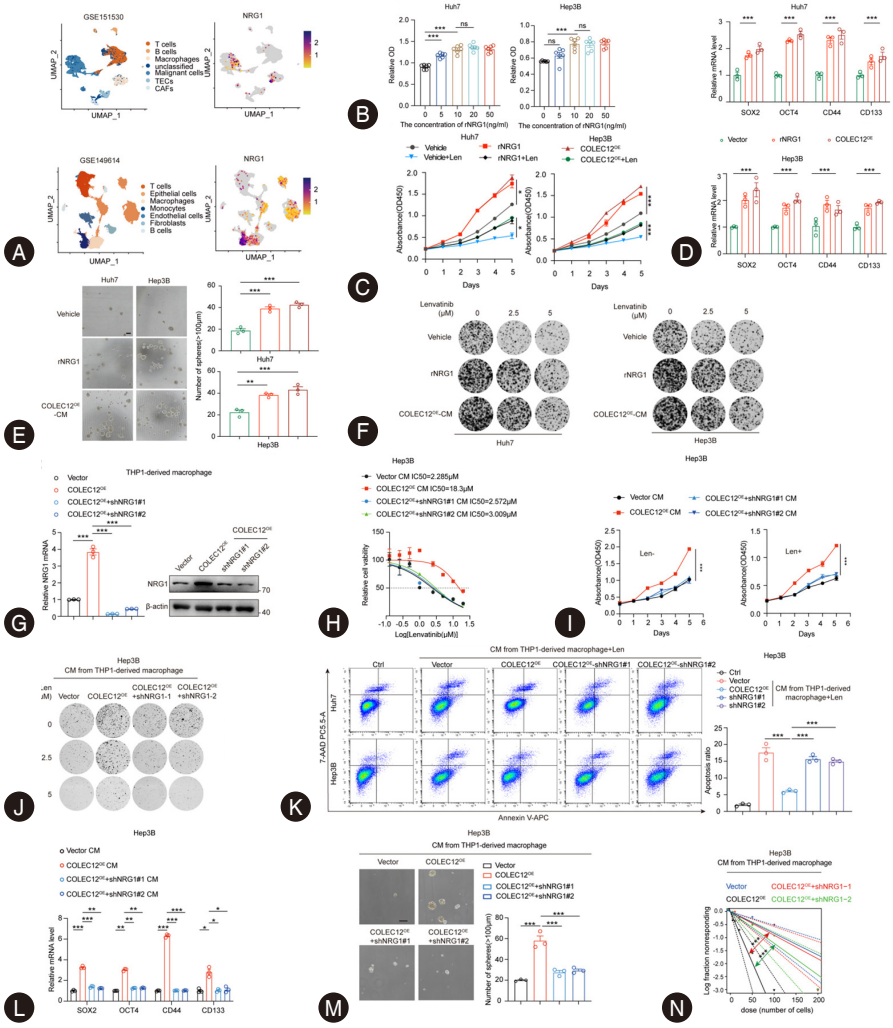

Supplementary Figure 4. NRG1 mediates COLEC12^{high} TAMs-induced stemness and therapeutic resistance. (A) Single-cell RNA-seq analysis of NRG1 expression across HCC tumor microenvironmental cell types. (B) Proliferation response of Huh7 and Hep3B cells to recombinant human NRG1 (rNRG1; 0–50 ng/mL), assessed by CCK-8 assay (n=6). (C) Cell proliferation curves for Huh7 and Hep3B exposed to rNRG1 (10 ng/mL) or COLEC12^{OE} CM±lenvatinib (2.5 μM), measured via CCK-8 assay (n=3). (D) qRT-PCR of stemness markers in Huh7 and Hep3B cells treated with rNRG1 (10 ng/mL) or COLEC12^{OE} CM (n=3). (E) Tumorsphere formation assays demonstrating increased sphere-forming capacity after rNRG1 (10 ng/mL) or COLEC12^{OE} CM exposure (n=3). (F) Colony formation assay in Huh7 and Hep3B cells treated with rNRG1 (10 ng/mL) or COLEC12^{OE} CM in lenvatinib treatment (0, 2.5, and 5 μM) (n=3). (G) Validation of NRG1 knockdown efficiency in COLEC12^{OE} macrophages via qRT-PCR and Western blot (n=3). (H) IC50 values of lenvatinib in Hep3B cells treated with Vector CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM (n=3). (I) Proliferation of Hep3B cells in response to Vector CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM, with or without lenvatinib (2.5 μM), measured by CCK-8 assay (n=3). (J) Colony formation of Hep3B cells treated with Vector CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM (n=3). (K) Left: Representative flow cytometry plots of Huh7 and Hep3B cells treated with Vector CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM with 2.5 μM lenvatinib. Right: Quantification of apoptosis in Hep3B cells by flow cytometry after 2.5 μM lenvatinib treatment in the corresponding Vector CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM (n=3). (L) qRT-PCR analysis of stemness markers (SOX2, OCT4, CD44, and CD133) in Hep3B cells after treatment with Vector-CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM (n=3). (M) Tumorsphere formation assay of Hep3B cells treated with Vector CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM (n=3). (N) In vitro limiting dilution assay demonstrating altered stem cell frequency in Hep3B cells treated with Vector-CM, COLEC12^{OE} CM, COLEC12^{OE}+shNRG1#1 CM, or COLEC12^{OE}+shNRG1#2 CM (n=3). Data are presented as stem cell frequency in chart form. Data are presented as mean±standard error of the mean. Statistical significance was assessed by Student's t-test for comparisons between two groups and one-way ANOVA for comparisons among multiple groups. Tumor initiation assays were analyzed using the Kruskal–Wallis test. *P<0.05, **P<0.01, ***P<0.001. Source data are provided as a Source Data file. NRG1, Neuregulin 1; COLEC12, Collectin Subfamily Member 12; TAMs, tumor-associated macrophages; HCC, hepatocellular carcinoma; COLEC12^{OE}, COLEC12-overexpressing; CM, conditioned medium.