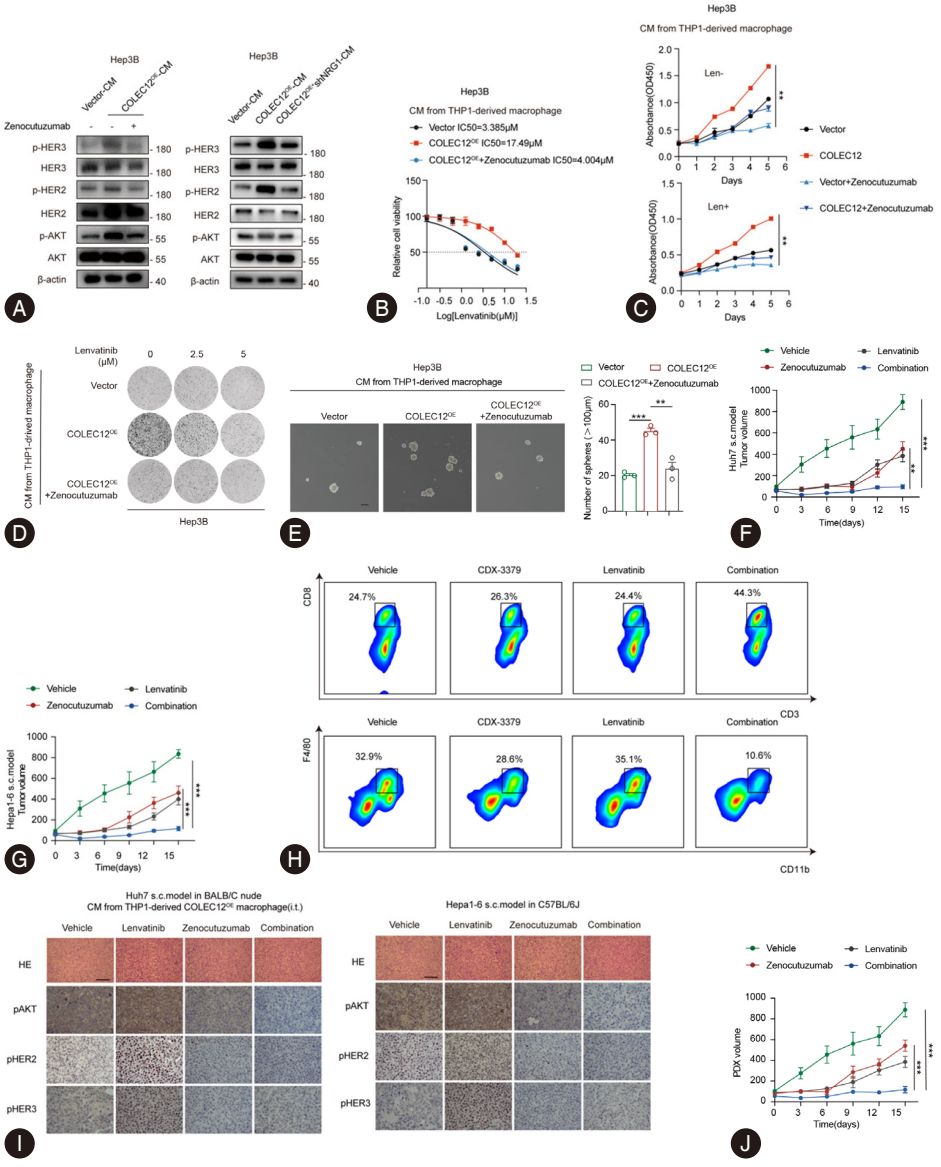

Supplementary Figure 5. NRG1 activates cancer cell-intrinsic HER2/HER3 signaling to drive lenvatinib resistance and stemness in HCC. (A) Immunoblots of phosphorylated and total HER3, HER2, and AKT in Hep3B cells cultured with Vector CM or COLEC12^{OE} CM with or without zenocutuzumab, or CM from COLEC12^{OE} macrophages transduced with shNRG1 (n=3). (B) IC50 values of Hep3B cells cultured with Vector CM, COLEC12^{OE} CM, or COLEC12^{OE} CM plus zenocutuzumab (0.01 μM) and treated with increasing concentrations of lenvatinib (n=3). (C) Proliferation of Hep3B cells cultured with CM from THP-1-derived macrophages, with or without zenocutuzumab (0.01 μM), in the absence or presence of lenvatinib (2.5 μM), as assessed by CCK-8 assay (n=3). (D) Representative images of colony formation assays in Hep3B cells treated with Vector CM, COLEC12^{OE} CM, or COLEC12^{OE} CM plus zenocutuzumab (0.01 μM), in the presence of lenvatinib (0, 2.5, or 5 μM). (E) Representative brightfield images (left) and quantification (right) of tumorspheres (>100 μm) formed by Hep3B cells in the indicated CM. Sphere numbers were significantly reduced by zenocutuzumab (0.01 μM) (treatment n=3). Scale bar, 100 μm. (F) Tumor growth curves of Huh7 subcutaneous xenografts treated with vehicle, lenvatinib, zenocutuzumab, or the combination (n=6). (G) Tumor growth curves of Hepa1-6 subcutaneous xenografts treated with vehicle, lenvatinib, zenocutuzumab, or the combination (n=6). (H) Flow cytometry analysis of intratumoral immune cells from Hepa1-6 tumors in C57BL/6 mice treated with vehicle, CDX-3379, lenvatinib, or the combination. The combination therapy markedly increased CD3^{+}CD8^{+} T-cell infiltration (top) and reduced CD11b^{+}F4/80^{+} tumor-associated macrophages (bottom). (I) Representative images of haematoxylin and eosin (H&E), p-AKT, p-HER2 and p-HER3 staining in Huh7 and Hepa1-6 xenografts. Scale bars, 50 μm. (I) Growth of patient-derived xenograft (PDX) tumors under the same treatment regimens (n=6). Data are presented as mean±standard error of the mean. Statistical significance was assessed by two-tailed unpaired Student's t-test or one-way ANOVA with multiple comparisons. *P<0.05, **P<0.01, ***P<0.001. NRG1, Neuregulin 1; HCC, hepatocellular carcinoma; CM, conditioned medium; COLEC12, Collectin Subfamily Member 12; COLEC12^{OE}, COLEC12-overexpressing; PDX, patient-derived xenograft. Source data are provided as a Source Data file.

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