SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Induction of a mesenchymal phenotype in HCC827 cells promotes overexpression of CXCR7.

A. GSEA of HCC827 cells chronically exposed to TGFβ1 (top), HCC827 cells constitutively expressing shRNA against E-cadherin (CDH1, middle), and SNAIL-V5 overexpressing HCC827 cells (bottom). The normalized enrichment score (NES) and the nominal P values are indicated. **B**. Annexin V assay showing HCC827 cells but not the cells chronically exposed to TGFβ1 (p=0.0039, Student's t-test, top), HCC827 cells overexpressing shRNA against non-target (ΔNT) but not the cells overexpressing shRNA against CDH1 (ΔCDH1) (p=0.0106, Student's t-test, middle), and HCC827 cells overexpressing LacZ-V5 but not the cells overexpressing SNAIL-V5 (p=0.006, Student's t-test, bottom) undergo significant apoptosis upon 48 hour 1μmol/L gefitinib treatment. Average of three independent assays. Bars, S.D. Representative flow histograms of Annexin V apoptosis assays are displayed. Quantification of apoptosis assay by Annexin V assay. **C.** HCC827 cells were chronically treated with vehicle (HCC827) or 10ng/mL TGFβ1(+TGFβ). HCC827 cells were transduced with pLKO.1 lentiviral vector coding for non-target (ΔNT) or CDH1 (ΔCDH1). HCC827 cells were transduced with pEXP304 lentiviral vector coding for LacZ-V5 or V5-tagged SNAIL (SNAIL-V5) and stable cell lines were established. Lysates were made from the exponentially growing cells and subject to immunoblots with antibodies indicated. Gel image is a representative of three independent experiments. **D.** Representative single cells flow cytometry images from Amnis Imagestream. Yellow staining represents CXCR7.

Supplementary Figure 2. CXCR7 is overexpressed in acquired EGFR TKI-resistant GEMM models with EGFR$^{Del\ E746_A750/T790M}$.

A. Representative overall survival (OS) analyses in lung adenocarcinoma patients with high or low

CXCR7 expression using PROGgeneV2. Significance was determined by the log-rank test. **B**. Schematic representation of the WZ4002 treatment in $EGFR^{Del\ E746\_A750/T790M}$ (TD) mice. **C.** Representative MRI images from 2 of 8 $EGFR^{Del\ E746\_A750/T790M}$ (TD) mice, TD125 and TD122. Arrows: tumors. H: Heart. **D.** Changes in tumor volume over time in TD mice (n = 8) treated with WZ4002 for indicated time. Error bars: S.D. **E**. Immunohistochemical analyses using anti-CXCR7 antibody of tumors from TD mice untreated and WZ4002 treated (2 weeks and 12 weeks) mice. Scale bars 50μm. **F**. Immunohistochemical analyses using anti-CDH1 (E-cadherin), anti-CDH2 (N-Cadherin) or anti-VIM (Vimentin) antibodies of tumors from TD mice untreated and WZ4002 treated (2 weeks and 12 weeks) mice. Scale bars 50μm.

Supplementary Figure 3. Depletion of CXCR7 in mesenchymal HCC4006Ge-R cells.

A. Lysates prepared from mass culture of osimertinib resistant HCC4006 cells (O-R) were subjected to immunoblot with antibodies indicated. A representative image from three independent experiments. **B.** Lysates from HCC4006O-R cells treated with DMSO or 100nM osimertinib for 24 hours were applied to phospho-receptor tyrosine kinase arrays. Duplicate spots in the corners are phospho-tyrosine controls. A representative image from three independent experiments. **C.** O-R cells were treated with gefitinib or erlotinib or osimertinib at the indicated concentrations for 72 hours and viable cells were quantified in CCK8 assay. The percentage of viable cells is shown relative to untreated controls. Data points are average of duplicate wells from three independent assays. Error bars, SD. **D**. GSEA of mesenchymal HCC4006Ge-R cells compared to epithelial HCC4006 cells. The normalized enrichment score (NES) and the nominal P values are indicated. **E.** Taqman qRT-PCR using *CXCR7* probe in HCC4006Ge-R cells transduced with lentiviruses coding for shRNA against non-target (∆NT), or two different sequences of shRNAs against CXCR7 (∆CX#1 and ∆CX#2). Samples in triplicates and average of three independent assays. Error bars: S.D. ****p≤0.0001 ANOVA and Tukey's multiple comparison test). **F**. Representative flow cytometric assay outputs for the quantification of CXCR7 (top)

or CXCR4 (bottom) expressed on the surface of HCC4006 cells, HCC4006Ge-R cells, and HCC4006GeSB-R cells transduced with lentiviruses coding for shRNAs against non-target (ΔNT) or CXCR7 (ΔCX). **G.** Taqman qRT-PCR using *CXCR7* or *CXCR4* probes in HCC4006 cells or HCC4006Ge-R cells transduced with lentiviruses coding for shRNA against non-target (ΔNT), or shRNA against CXCR7 (ΔCX). Results were normalized for the endogenous *GUSB*. Samples in triplicates and average of three independent assays. Error bars: S.D. ****p≤0.0001 ANOVA and Tukey's multiple comparison test).

Supplementary Figure 4. Characterization of CXCR7 signaling.

A. Cell surface expression of CXCR7 or CXCR4 in cells expressing shRNAs against non-target (ΔNT), CXCR4 (ΔCX4) or CXCR7 (ΔCX7). Average of four independent assays, ****p≤0.0001 ANOVA and Tukey's multiple comparison test, N.S., not significant. (Right, Top) Representative flow cytometric assay outputs for the quantification of CXCR7 on the surface of HCC4006Ge-R cells transduced with lentiviruses coding for shRNAs against non-target (red) or CXCR7 (blue). (Right, Bottom) Same with HCC4006Ge-R cells transduced with lentiviruses coding for shRNAs against non-target (red) or CXCR4 (blue). **B.** HCC4006Ge-R cells were transduced with lentiviruses coding for shRNA against non-target (ΔNT), or two different sequences of shRNAs against CXCR7 (ΔCX#1 and ΔCX#2 as shown in Fig.S3C) and the cells were treated with 500nM gefitinib for the times indicated. Lysates were prepared and subjected to Western blot with antibodies indicated. A representative image from three independent experiments. **C**. HCC827 cells stably transduced with pEXP304 vector coding for V5-tagged SNAIL (SNAI1-V5) were transduced with shRNA against non-target (ΔNT) or CXCR7 (ΔCX7). The cells were treated with DMSO (D) or 500nmol/L gefitinib (G) for 4 hours. Lysates were made and immunoblots were performed with antibodies indicated. VIM:vimentin. CDH2: N-cadherin. A representative immunoblots from four independent experiments. **D.** The expression of β-arrestins was assessed by immunohistochemical (IHC) staining of $EGFR^{T790M/Del19}$ lung tumors prior to the treatment, 2 weeks and

12 weeks post WZ4002-treatments. Photos shown are representative fields from two mice in each group in low and high magnification. Scale bars measure 50 μm. **E.** HCC4006 cells and HCC4006Ge-R cells transduced with lentivirus coding for shRNA against non-target (∆NT) or *CXCR7* (∆CX). The cells were treated with DMSO (-) or gefitinib (+) for 15 minutes prior to lysate preparation. The lysates were subject to immunoblot with antibodies indicated. Gel image representative of three independent experiments. **F.** HCC4006 or Ge-R cells stably expressing shRNA targeting non-target (ΔNT) or shRNA against CXCR7 (ΔCX) were treated with DMSO or 500nmol/L osimertinib (EGFRi) for 48 hours and Annexin V assay was performed. Representative flow histograms from three independent assays are displayed.

Supplementary Figure 5. Mesenchymal NSCLCs are exclusively sensitive to MEK inhibition.

A. Representative flow cytometric assay outputs for the quantification of CXCR7 on the surface of HCC4006 cells ectopically expressing V5-tagged LacZ (LacZ-V5, blue) or V5-tagged CXCR7 (CXCR7-V5, red). **B.** Phosphorylation of ERK, and AKT were assessed by immunohistochemical (IHC) staining of $EGFR^{T790M/Del19}$ lung tumors prior to the treatment, 2 weeks and 12 weeks post WZ4002-treatments. Photos shown are representative fields from two mice in each group in low and high magnification. Scale bars measure 50 μm. **C.** HCC4006 cells and HCC4006Ge-R cells were treated with DMSO or 500nmol/L osimertinib (EGFRi) or 500nmol/L trametinib (MEKi) or 500nmol/L buparlisib (PI3Ki) for 72 hours and Annexin V assay was performed. Representative flow histograms from three independent Annexin V apoptosis assays are displayed. **D.** HCC4006 cells and mass culture of gefitinib resistant HCC4006Ge-R cells were treated with osimertinib (EGFRi) or trametinib (MEKi) or buparlisib (PI3Ki) at the indicated concentrations for 72 hours and viable cells were quantified in CCK8 assay. The percentage of viable cells is shown relative to untreated controls. Data points are average of duplicate wells from three independent assays. Error bars, SD. **E.** HCC4006 cells, HCC4006Ge-R cells, and HCC4006O-R cells were treated with DMSO or 500nmol/L osimertinib (EGFRi) or 500nmol/L trametinib

(MEKi) or 500nmol/L buparlisib (PI3Ki) for 5 days and remaining cells were stained with crystal violet. A representative image from three independent experiments. **F.** HCC827 cells were transduced with pEXP304 lentiviral vector coding for LacZ-V5 or V5-tagged SNAIL (SNAIL-V5). The cells were treated with DMSO or 500nmol/L osimertinib (EGFRi) or 500nmol/L trametinib (MEKi) or 500nmol/L buparlisib (PI3Ki) for a week and remaining cells were stained with crystal violet. **G.** HCC827 cells overexpressing pBabe (vector) or pBabe-MEKDD (MEKDD) were treated with DMSO or 100nmol/L osimertinib or 100nmol/L crizotinib or osimertinib/crizotinib combination for 48 hours and Annexin V assay was performed. Representative flow histograms from three independent Annexin V apoptosis assays are displayed.

Supplementary Figure 6. CXCR7 depletion in EGFR mutant cells attenuates ERK reactivation following EGFR TKI treatment.

A. HCC4006 cells were stably transfected with vectors coding for the indicated single-guided RNA (sgRNA). The exponentially growing mass culture of cells were lysed and the lysates were subject to immunoblots with anti-CXCR7 antibody. The image is representative of three independent immunoblots. **B.** HCC827 cells stably expressing shRNA targeting non-target (ΔNT) or shRNA against CXCR7 (ΔCX) were treated with DMSO or 100nmol/L osimertinib or 100nmol/L crizotinib for the indicated hours. Cells were lysed, and CXCR7 and phosphorylation of ERK1/2, EGFR, and MET was detected by Western blot. The image is representative of four independent experiments. **C.** (Left) Taqman qRT-PCR using *CXCR7* probe in PC9 cells transduced with lentiviruses coding for shRNA against non-target (∆NT), or two different sequences of shRNAs against CXCR7 (∆CX#1 and ∆CX#2). Samples in triplicates and average of three independent assays. Error bars: S.D. ****p≤0.0001 ANOVA and Tukey's multiple comparison test). *GUSB* was used as the reference control. (Right) PC9 cells stably expressing shRNA targeting non-target (ΔNT) or two different sequences of shRNAs against CXCR7 (∆CX#1 and ∆CX#2) were treated with 100nM osimertinib for indicated times. Lysates were subjected to Western blot

antibodies indicated. Gel image representative of three independent experiments. The image is representative of three independent immunoblots. **D.** (Left) Taqman qRT-PCR using *CXCR7* probe in NCI-H1975 cells transduced with lentiviruses coding for shRNA against non-target (∆NT), or shRNA against CXCR7 (∆CX#1). Samples in triplicates and average of three independent assays. Error bars: S.D. ****p≤0.0001 ANOVA and Tukey's multiple comparison test). *GUSB* was used as the reference control. (Right) NCI-H1975 cells stably expressing shRNA targeting non-target (ΔNT) or shRNA against CXCR7 (ΔCX) were treated with DMSO or 100nmol/L osimertinib for the indicated hours. Cells were lysed, and CXCR7 and phosphorylation of ERK1/2 and EGFR were detected by immunoblotting. The image is representative of three independent immunoblots. **E.** (Left) Lysates made from HCC827 cells ectopically expressing V5-tagged LacZ (LacZ-V5) or V5-tagged CXCR7 (CXCR7-V5), treated with DMSO (D) or a mix of 100nmol/L osimertinib and 100nmol/L crizotinib (OC) for 24 hours, were subject to immunoblots with antibodies indicted. (Right, top) Cell surface expression of CXCR7 in HCC827 cells ectopically expressing V5-tagged LacZ (LacZ-V5) or V5-tagged CXCR7 (CXCR7-V5). Average of five independent assays with duplicate runs, ****p≤0.0001, ***p≤0.001ANOVA and Tukey's multiple comparison test. (Right, bottom) Representative flow cytometric assay outputs for the assay to quantify cell surface expression of CXCR7. **F.** HCC4006 cells stably expressing shRNA targeting non-target (ΔNT) or shRNA against CXCR7 (ΔCX) were treated with DMSO or 100nmol/L osimertinib for 24 hours and Annexin V assay was performed. Representative flow histograms from three independent Annexin V apoptosis assays are displayed. **G.** HCC827 cells stably expressing shRNA targeting non-target (ΔNT) or shRNA against CXCR7 (ΔCX) were treated with DMSO or a combination of 10nmol/L osimertinib and 10nmol/L crizotinib (OC) for 72 hours and Annexin V assay was performed. Representative flow histograms from three independent Annexin V apoptosis assays are displayed.