Supplementary figure 1



Supplementary Figure 1. **miR-9 knockdown and overexpression have no effect on apoptosis.** Expression of the apoptotic marker proteins cleaved PARP and cleaved caspase-3 was assessed by Western blotting using lysates of HSC3 and H357 parental cells and HSC3 miR-9 overexpressing and H357 miR-9 knockdown cells.

Supplementary figure 2



Supplementary Figure 2. **miR-9 knockdown affects cell cycle profile.** Cell cycle analysis of **a.** HSC3 miR-9 overexpression and **b.** H357 miR-9 knockdown cells was conducted using PI staining and flow cytometry. Data represents mean \pm SEM for three independent (n=3) experiments. Asterisks (*) show statistical significance as follows: * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001. Representative cell cycle profiles are shown.



Supplementary Figure 3. miR-9 modulation in HNSCC cells affects proliferation, cell cycle, colony formation and invasion. a. b. Cell proliferation of HN30 and HN5 miR-9 knockdown and overexpression cells was assessed by generating growth curves over 5 days. c. d. Cell cycle analysis of miR-9 knockdown and overexpressing cells was conducted using PI staining followed by flow cytometry. e. f. The ability of the miR-9 knockdown and overexpression cells to form colonies was tested using the soft agar assay. Data represents mean \pm SEM for three independent (n=3) experiments. Asterisks (*) show statistical significance as follows: * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001.



Supplementary Figure 4. **CXCR4 modulation in HNSCC cells affects cell cycle.** Cell cycle analysis of **b.** CXCR4 knockdown and **b.** overexpressing cells was conducted using PI staining and analysed by flow cytometry. Data represents mean \pm SEM for three independent (n=3) experiments. Asterisks (*) show statistical significance as follows: * = p < 0.05, ** = p < 0.01, **** = p < 0.001. Representative cell cycle profiles are shown.



Supplementary Figure 5. Plerixafor titration on CXCR4 overexpressing and miR-9 knockdown cells. a. b. Effectiveness of plerixafor assessed by MTT assay in miR-9 knockdown and CXCR4 overexpression cells. Data represents mean \pm SEM for three independent (n=3) experiments. Asterisks (*) show statistical significance as follows: * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001.

Supplementary figure 6



Supplementary Figure 6. Plerixafor blocks CXCL12 induced increase in proliferation in miR-9 knockdown cells. Cells were grown in media supplemented with CXCL12. Plerixafor was added on day 1-5 and the effect on cell growth was observed. Data represents mean \pm SEM for three independent (n=3) experiments.





Supplementary Figure 7. Effect of plerixafor on cell cycle profile. Cell cycle analysis of **a**. miR-9 knockdown and **b**. CXCR4 overexpressing cells after addition of plerixafor was conducted using PI staining and analysed by flow cytometry. Data represents mean ±SEM for three independent (n=3) experiments. Asterisks (*) show statistical significance as follows: * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.001. Exemplary cell cycle profiles are shown.