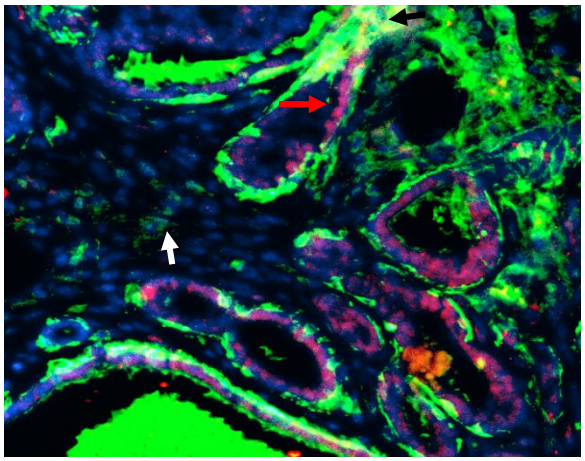
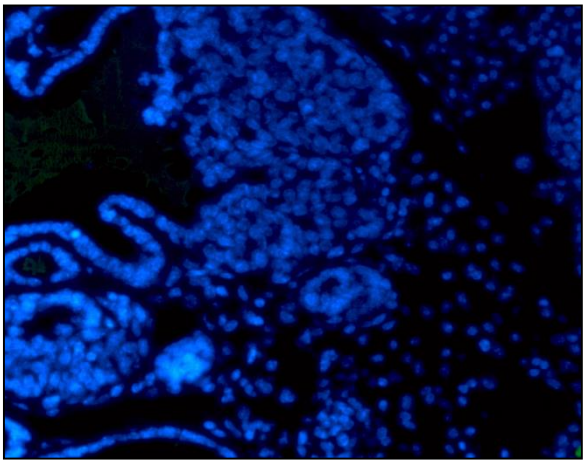
p63/α-sma

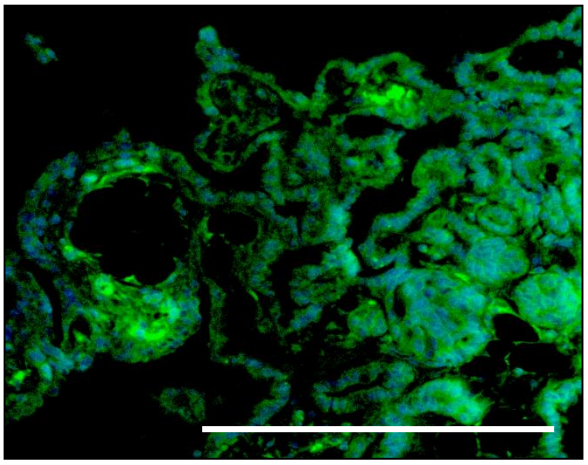
Anti-rabbit-AlexaFluor488/
anti-mouse-Alexa658

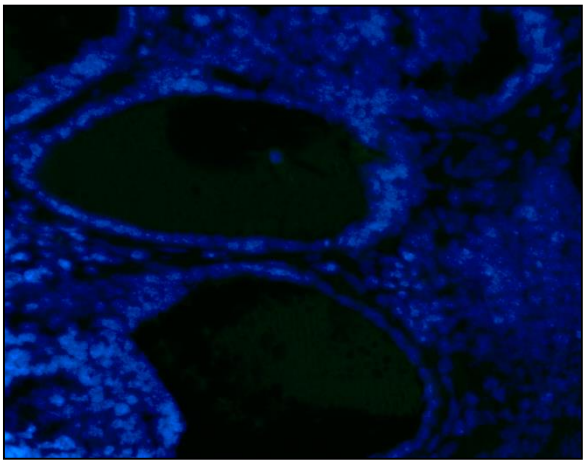




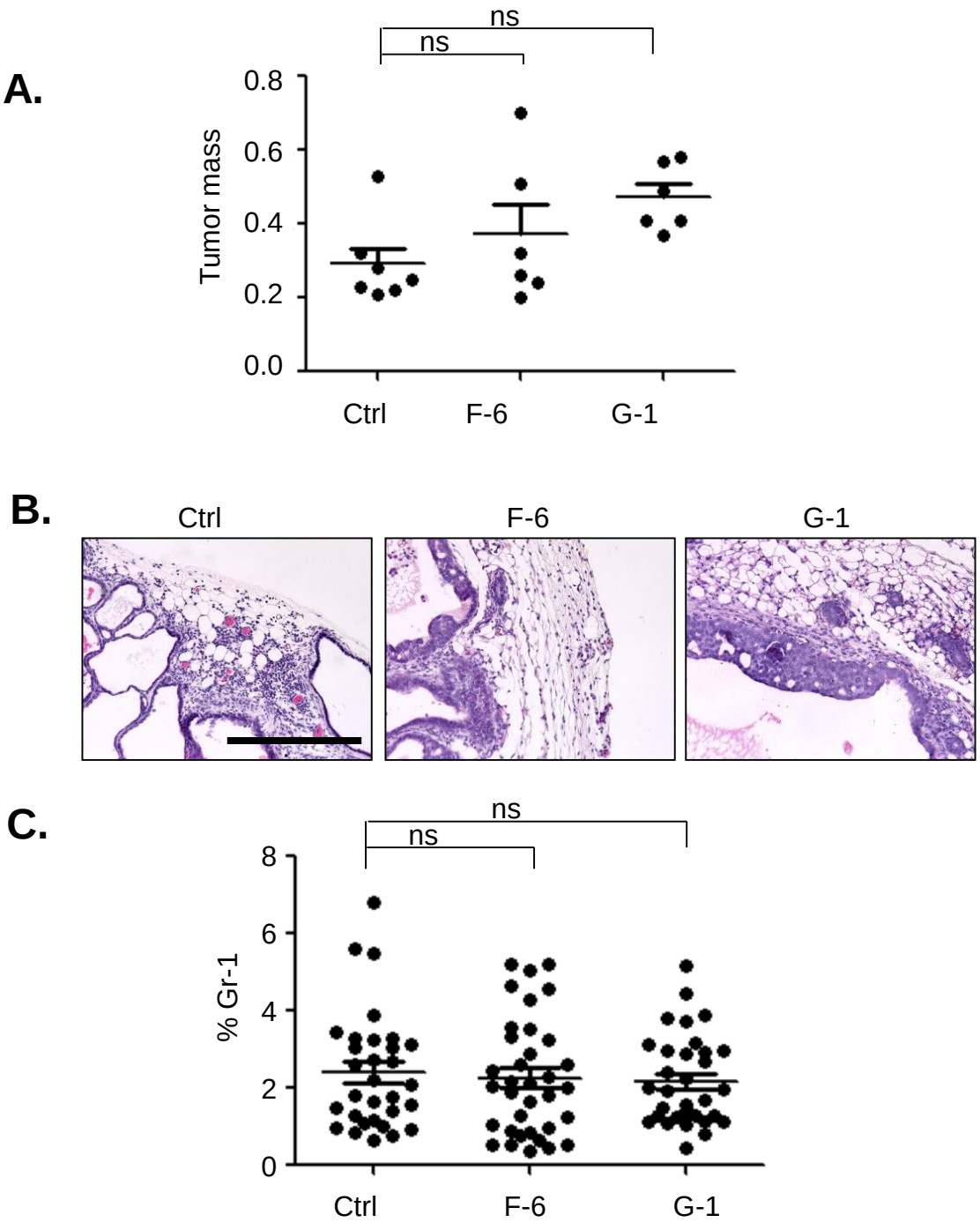
CK5/6

Anti-mouse-AlexaFluor488

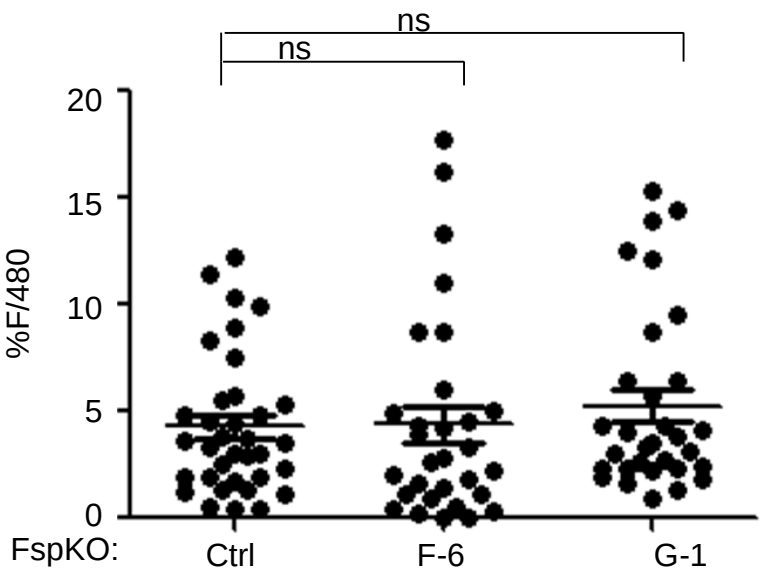




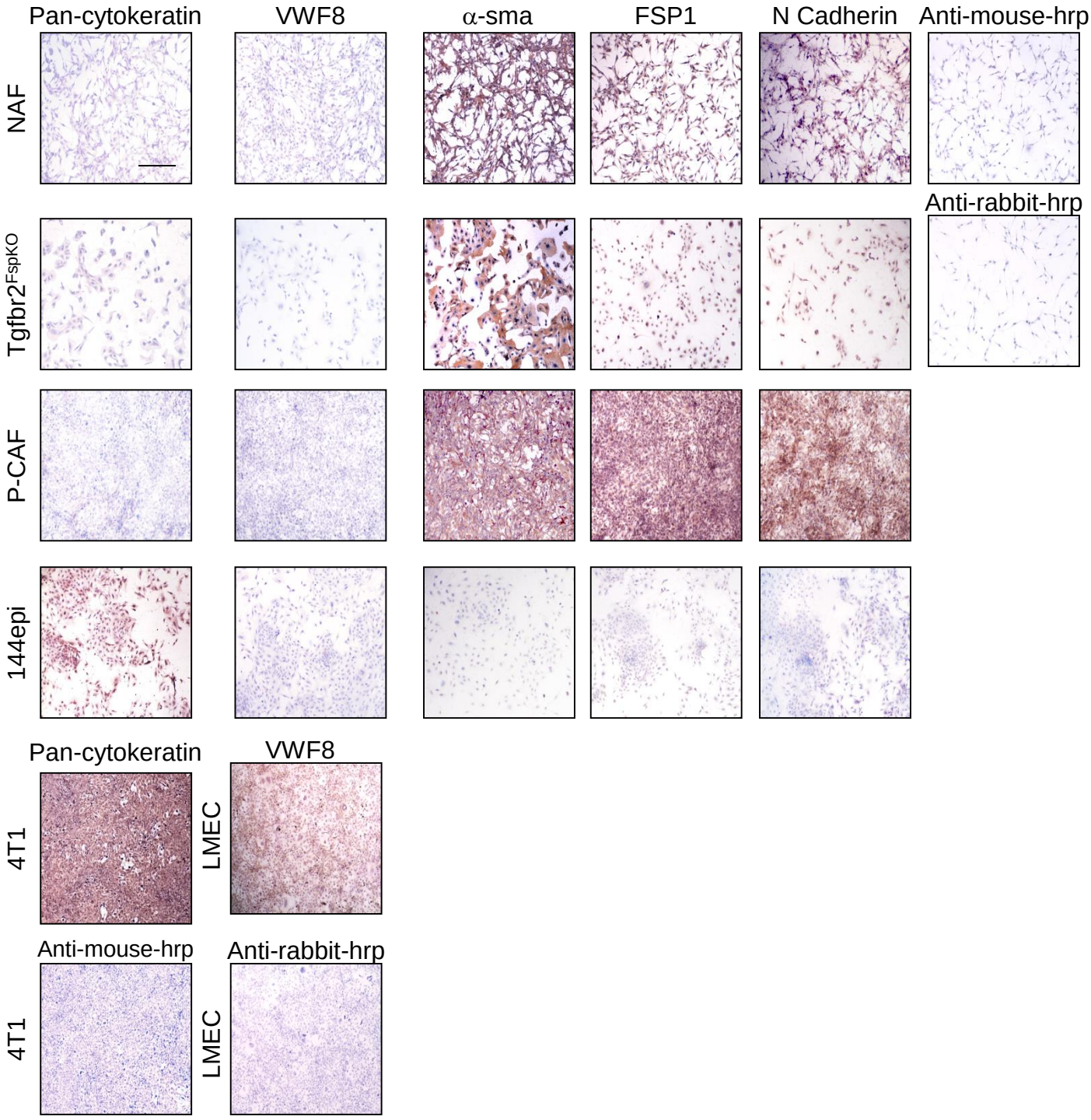
Supplemental Fig 1. Expression of p63, α-sma and CK5/6 in in papillary lesions derived from MMTV-PyVmT/C57Bl/6 mice. Mammary lesions from 15 week old MMTV-PyVmT/C57Bl/6 mice were immunofluorescence stained for p63 (red), α-sma (green), or CK5/6 (green), with DAPI counterstain. White arrow points to α-sma+ fibroblasts. Red arrow points to p63+ epithelium. Black arrow points to p63+/α-sma+ myoepithelium.



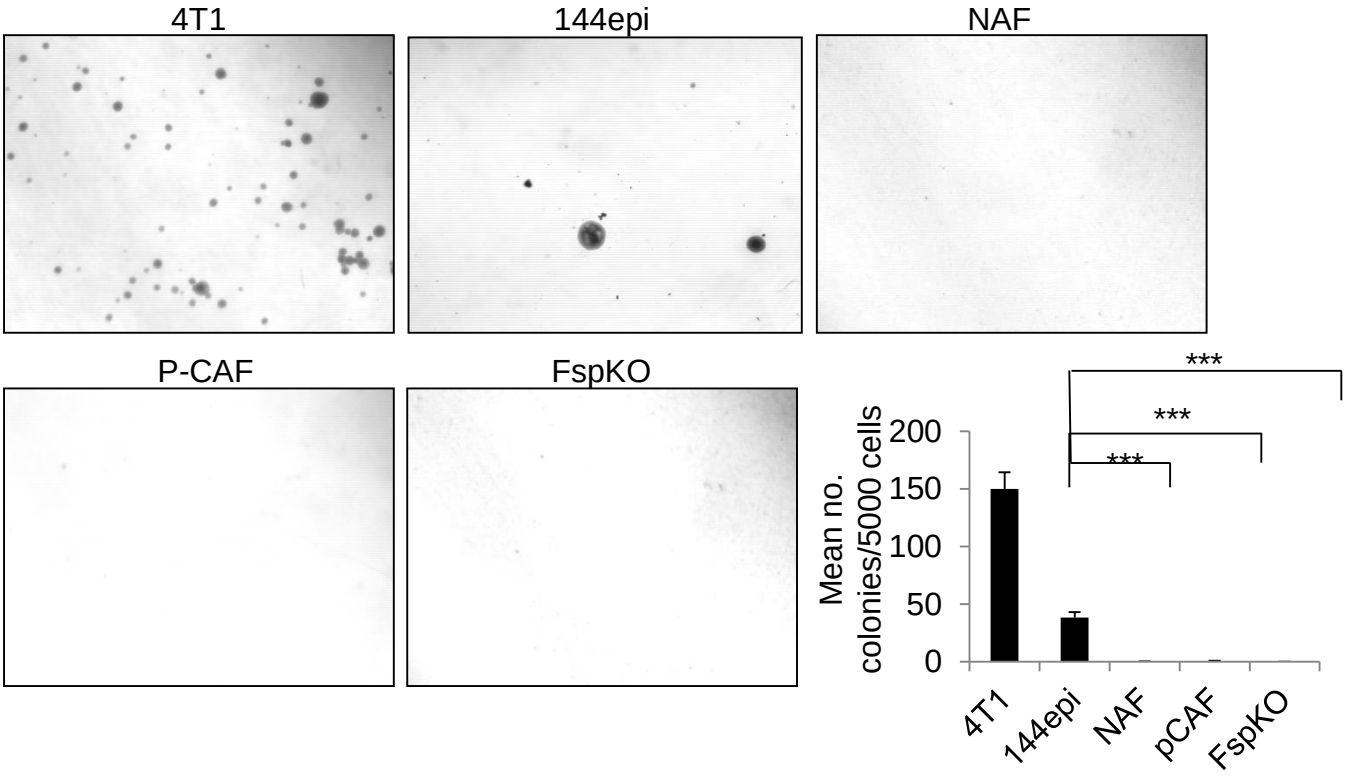
Supplemental Fig 2. CXCR2 knockdown does not affect tumor growth of 144epi cells transplanted alone. 144epi cells expressing control or CXCR2 shRNAs (F-6, G-1) were orthotopically transplanted into C57Bl/6 mice for 60 days. Mammary tissues were analyzed for **A.** tumor mass, **B.** H&E and **C.** Gr-1 expression by immunostaining. Immunostaining was quantified by Image J. Statistical analysis was determined by One Way ANOVA followed by Bonferonni post-hoc analysis. Statistical significance was determined by $p < 0.05$. *$p<0.05$; ns=not significant. Scale bar=200 microns. N=6 per group.



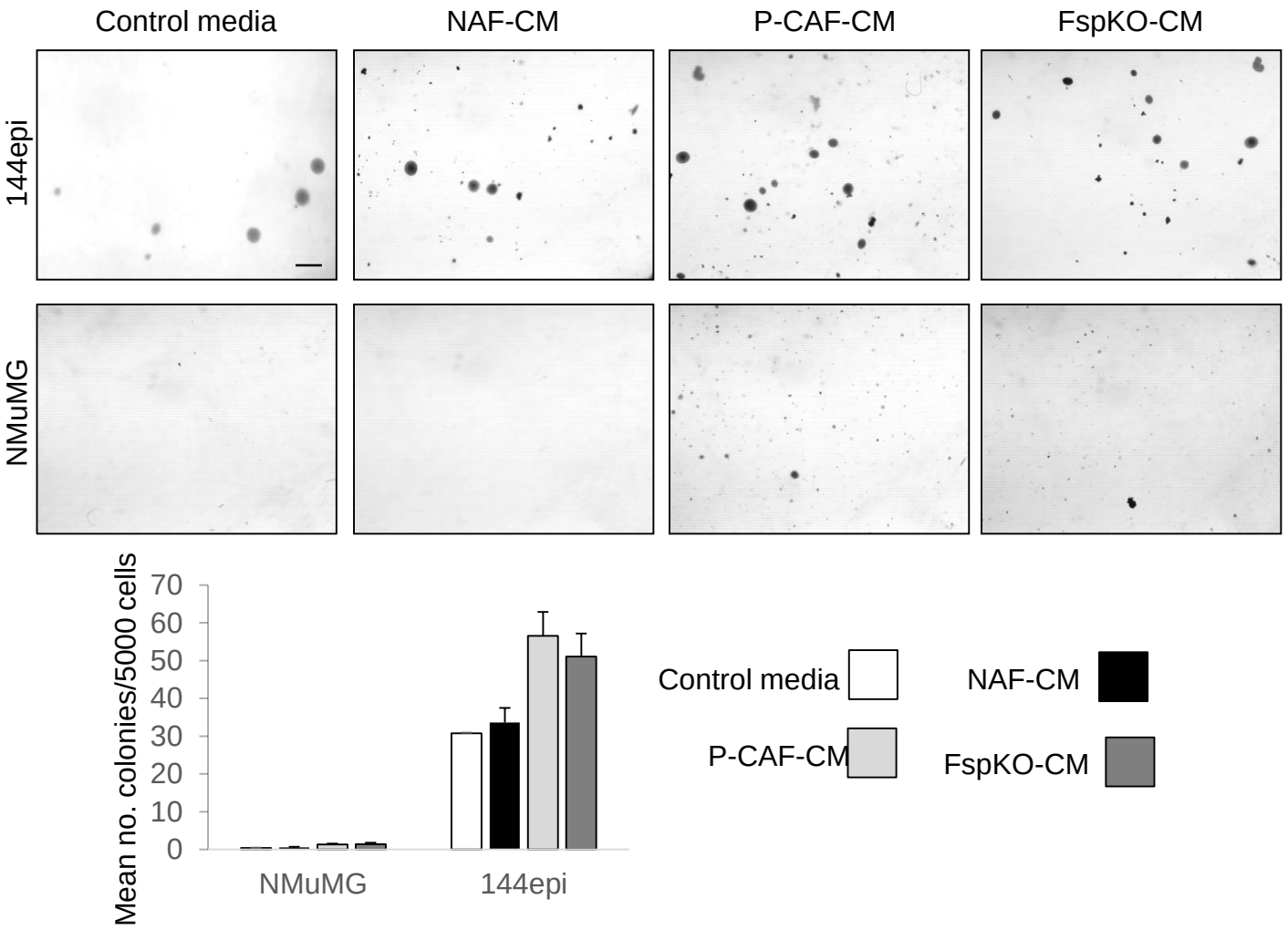
Supplemental Fig 3. CXCR2 knockdown does not affect recruitment of F4/80+ cells mediated by Tgfbr2^{FspKO} fibroblasts. Mammary lesions derived from Tgfbr2^{FspKo} fibroblasts transplanted with control shRNA (Ctrl) or CXCR2 deficient 144epi cells (F-6, G-1) were immunostained for F4/80. Expression levels were quantified by Image J. Statistical analysis was determined by One Way ANOVA followed by Bonferonni post-hoc analysis. Statistical significance was determined by $p < 0.05$. *$p<0.05$; ns=not significant. n=7 for 144epi:Control shRNA, n=6 for 144epi:F-6, n=6 for 144epi:G-1.



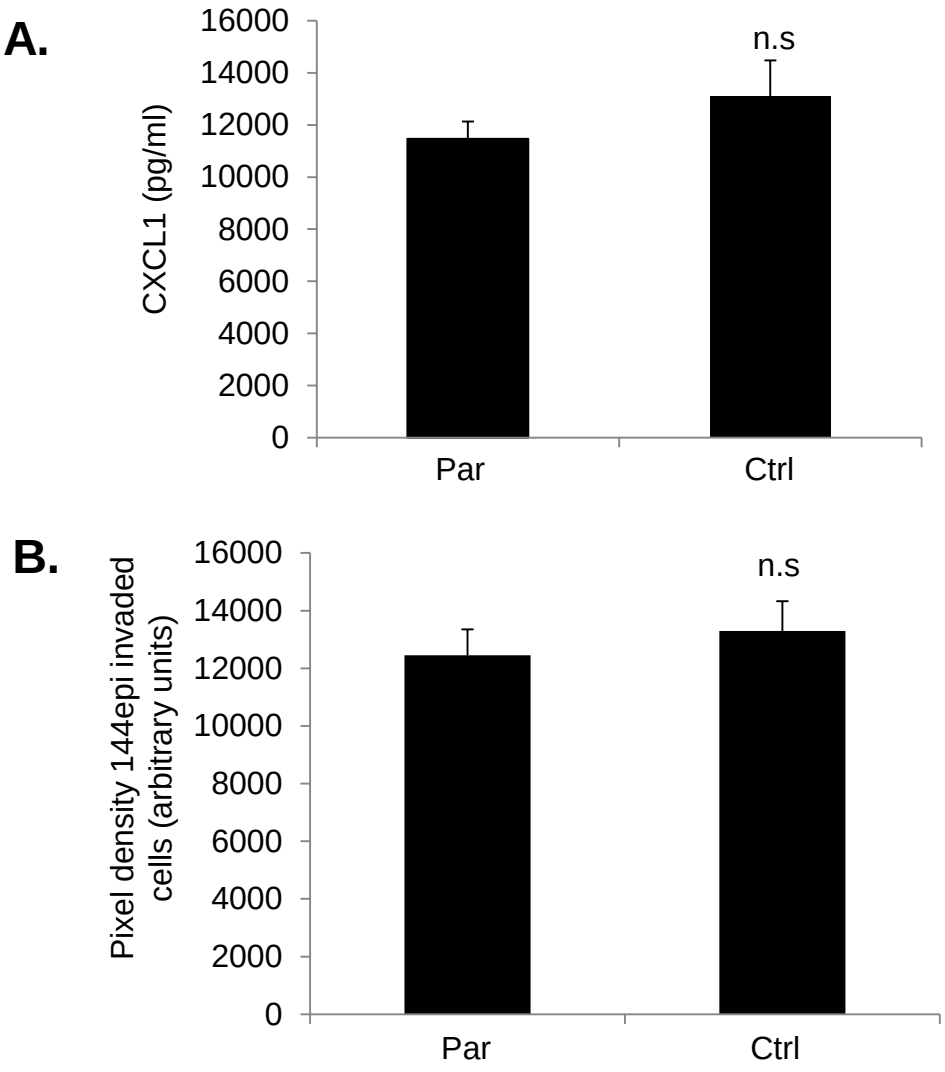
Supplemental Fig 4. Molecular Characterization of fibroblasts and 144epi cells. Cultured NAFs, Tgfbr2^{FspKO} fibroblsts, P-CAFs and 144epi cells were immunostained for expression of the indicated proteins and counterstained with hematoxylin. 4T1 mammary carcinoma cells were used as a positive control for Pan-cytokeratin staining. Lung microvascular endothelial cells (LMEC) were used as a positive control for VWF8 staining. Scale bar=400 microns.



Supplemental Fig 5. Growth of 144epi cells and fibroblasts in soft agar. 4T1 mammary carcinoma cells (positive control), 144epi cells, NAFs, P-CAF or Tgfbr2^{FspKO} fibroblasts were plated in soft agar in triplicate wells for 14 days s. Colonies were counted using Image J software. Statistical analysis was determined by One Way ANOVA followed by Bonferonni post-hoc analysis. Experiments were performed 3 times. Statistical significance was determined by p <0.050***p<0.001. Scale bar= 1 mm.



Supplemental Figure 6. Effect of fibroblast conditioned medium on 144epi and NMuMG cell growth in soft agar. NMuMG and 144epi mammary epithelial cells were plated in soft agar in triplicate wells in the presence or absence of control DMEM/10% FBS medium or conditioned medium from NAFs, P-CAF or Tgfbr2^{FspKO} fibroblasts for 14 days. Colonies were counted using Image J software. Statistical analysis was determined by One Way ANOVA followed by Bonferonni post-hoc analysis. Experiments were performed 3 times. Statistical significance was determined by p <0.050***p<0.001. Scale bar= 1 mm.



Supplemental Fig 7. Effects of control siRNA transduction in P-CAF on CXCL1 expression and 144epi invasion. A. P-CAFs were transfected with control siRNAs (Ctrl) and analyzed for CXCL1 expression by ELISA **B**. P-CAF were coated on the understide of Matrigel coated Transwells and analyzed for 144epi invasion to the underside. Statistical analysis was determined by Two Tailed T-test. Statistical significance was determined by $p < 0.05$. n.s= not significant. Mean$\pm$SEM values are shown.