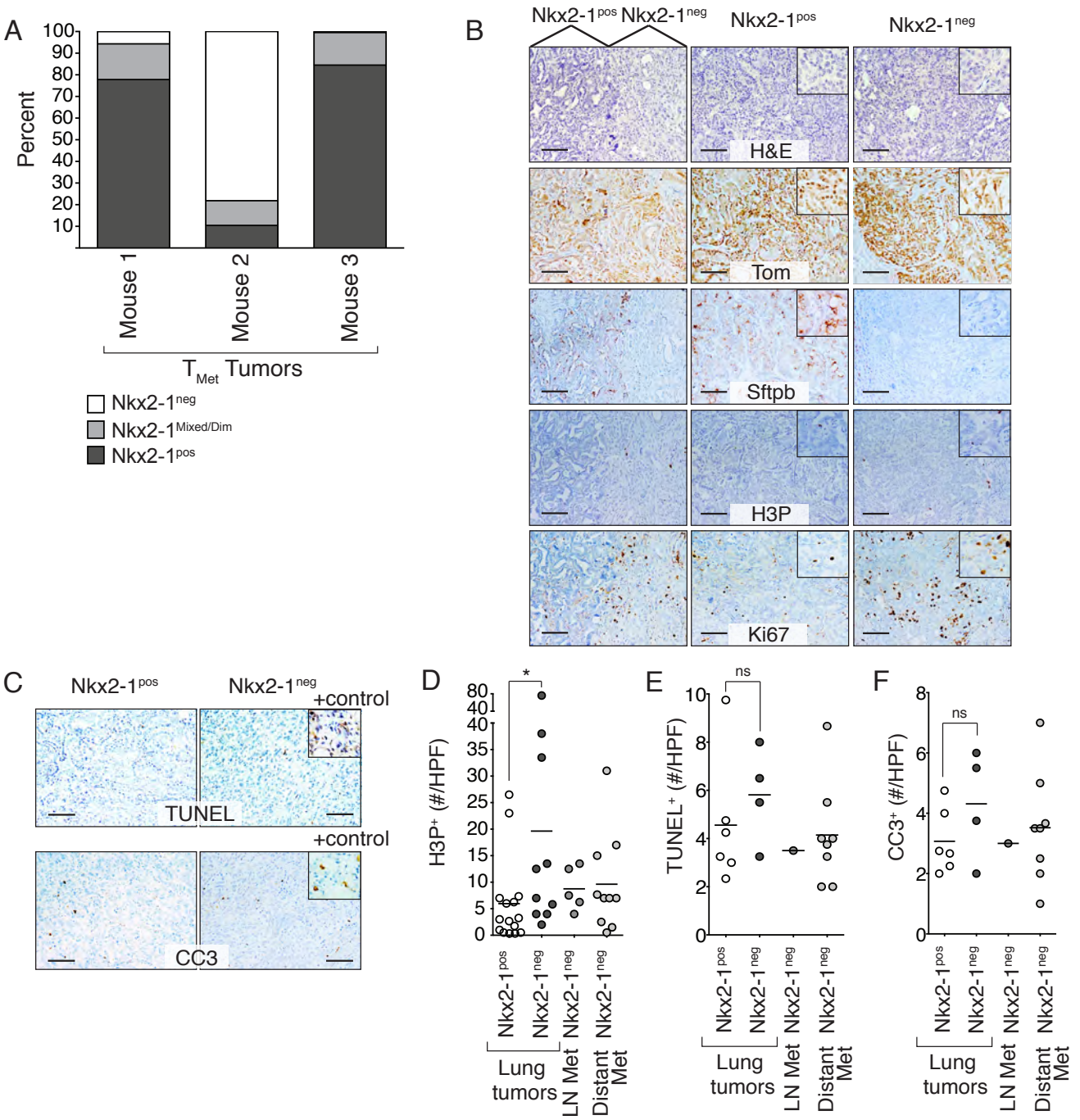
Caswell et al, Supplemental Figure 8



Supplemental Figure 8: Nkx2-1^{neg} areas have a selective advantage in primary T_{Met} tumors with significantly higher levels of proliferation and insignificant changes in levels of cell death.

A. Quantification of Nkx2-1^{pos}, Nkx2-1$^{Mixed/Dim}$, and Nkx2-1^{neg} areas in T_{Met} tumors. T_{Met} tumors within the mouse contain Nkx2-1^{neg} areas that comprise a variable fraction of the total tumor but cell lines derived from T_{Met} tumors are Nkx2-1^{neg} as assessed by western blotting, qPCR or microarray analysis (Winslow *et al,* 2011). This suggests that as these cell lines are selected during culture the Nkx2-1^{neg} subpopulation outcompetes the Nkx2-1^{pos} population. This selection could be driven by increased proliferation of cells within the Nkx2-1^{neg} state or other aspects of the *in vitro* environment that select for the metastatic subclone.

B. Representative H&E and IHC images of Nkx2-1^{neg} and Nkx2-1^{pos} areas in *KPT*-Late tumors. Tumors were stained for tdTomato, the Nkx2-1 target surfactant protein B (Sftpb), phospho-histone H3 (H3P) and Ki67. Scale bar is 100μm in all panels. Inset shows higher magnification.

C. Representative IHC images for TUNEL and cleaved caspase 3 (CC3) staining. Very few positive cells can be detected in Nkx2-1^{pos} or Nkx2-1^{neg} areas. Inset shown positive staining on involuting breast tissue.

D-F. Quantification of H3P^{+}(D), TUNEL^{+}(E), and CC3^{+}(F) cells in *KPT*-Late tumors. Each dot represents a tumor area and the bar represents the mean. Data is the number of positive cells per high power (40X) field (#/HPF). *p-value < 0.025. ns = not significant.