Supplemental Methods

Cell Lines

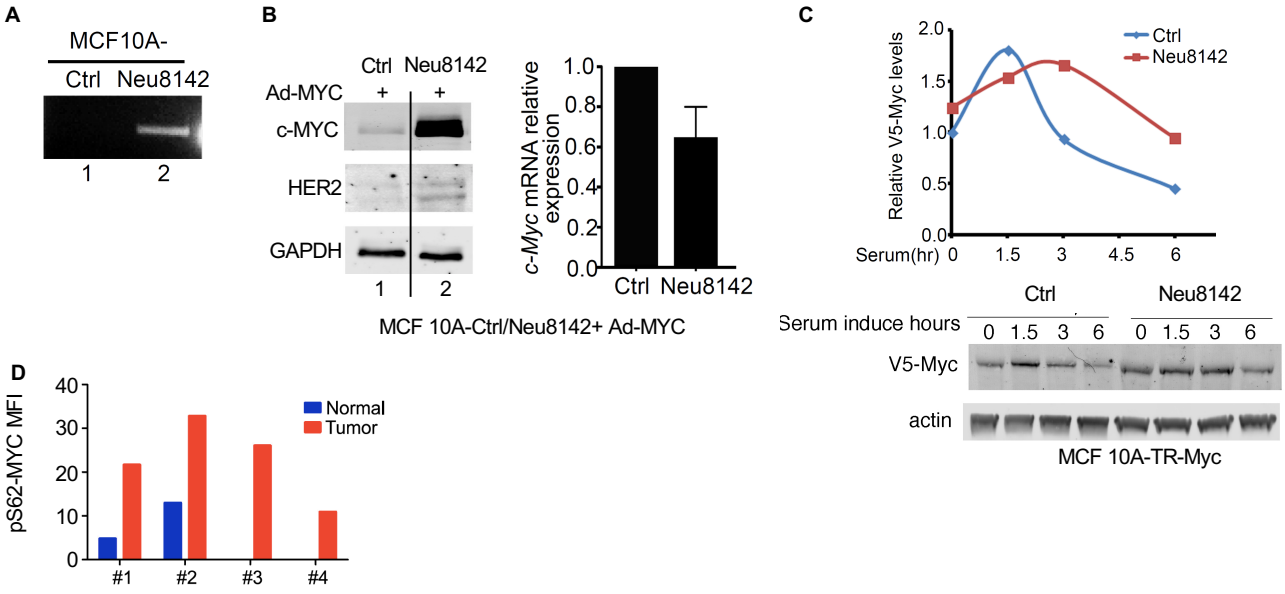
The MCF10A cell line was purchased from American Type Culture Collection (ATCC). Media used was as follows. MCF10A, 45% DMEM, 45% F-12 Ham's. 5% horse serum, 2.5 mM L-glutamine, 20 ng/mL EGF, 10 ug/mL insulin, 500 ng/mL hydrocortisone, and 100 ng/mL cholera toxin. MCF10A-TR-MYC cells were generated by infecting a 100-mm dish of MCF10A cells with a lentivirus (approximate multiplicity of infection of 10) encoding the tet-repressor, pLenti4/TO/V5-Dest-Myc (Invitrogen), in 5 mL MCF10A modified media (MCF10A media with 5% defined FBS instead of horse serum) and 6 μg/mL Polybrene for 12 h. Media was changed to 10mL modified media for 24 h. Cells were then split at 1:10 dilution and maintained in modified media supplemented with 5 μg/mL Blasticidin (Invitrogen) for 10 d until distinct colonies formed. Six colonies were picked, expanded, and screened for their ability to only express V5-MYC when treated with 1 μg/mL doxycycline. The best clone was then used for further experiments and continually maintained in modified media with 5 μg/mL Blasticidin and 200 μg/mL Zeocin (28). MCF10A-TR-Myc-Neu8142 cells were generated by transfecting MCF10A-TR-Myc with pcDNA3-Neu8142 plasmid. MCF10A-TR-Neu8142 cells were generated by transfecting MCF10A-TR parental cells (28) with pLenti6/TR-Neu8142 plasmid. MCF10A-Ctrl and MCF10A-Neu8142 stable cells were generated by transfecting MCF10A cells with pcDNA3 or pcDNA3-Neu8142 (contains the Neu8142 active mutant) plasmid. 24 hours after transfection, cells were split and cultured in Neomycin containing media for about 2 weeks until distinct colonies formed. 4 colonies from each transfection were picked and tested for expression of Neu and representative colony was chosen for study.

Primers for quantitative PCR

ROSA-MYC genotype primers were as described previously (30)

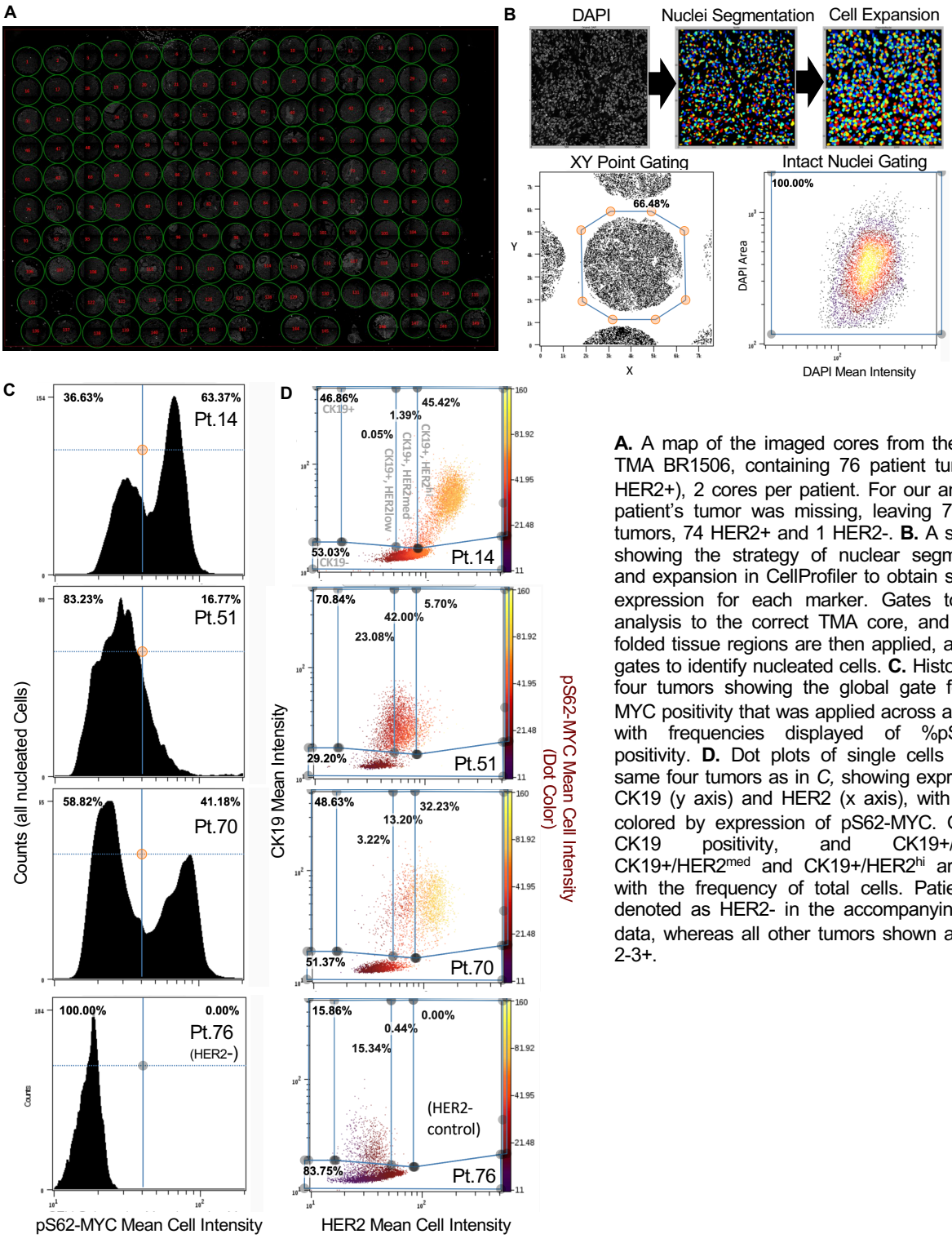
Gene	Forward	Reverse
NeuNT genotype	5’-TTCCGGAACCCACATCAGGCC	5’-GTTTCCTGCAGCAGCCTACGC
ROSA recombination	5'-AAAGTCGCTCTGAGTTGTTAT	5'-GCAGCGAGTCCGAGGAAGG 5'-GCCTTGGGAAAAGCGCCTC
Rat *Neu*	5’-GCCAGGAGTGTGTGGAGGAGT	5’-CCAGATGGGCATGTAGGAGAG
Mouse *Actin*	5’-TCATCACCAACTGGGACGACA	5’-CGCCTGGATAGCCACATACAT

Supplemental Figure 1. MYC expression and stability is enhanced in Neu8142 expressing breast cells



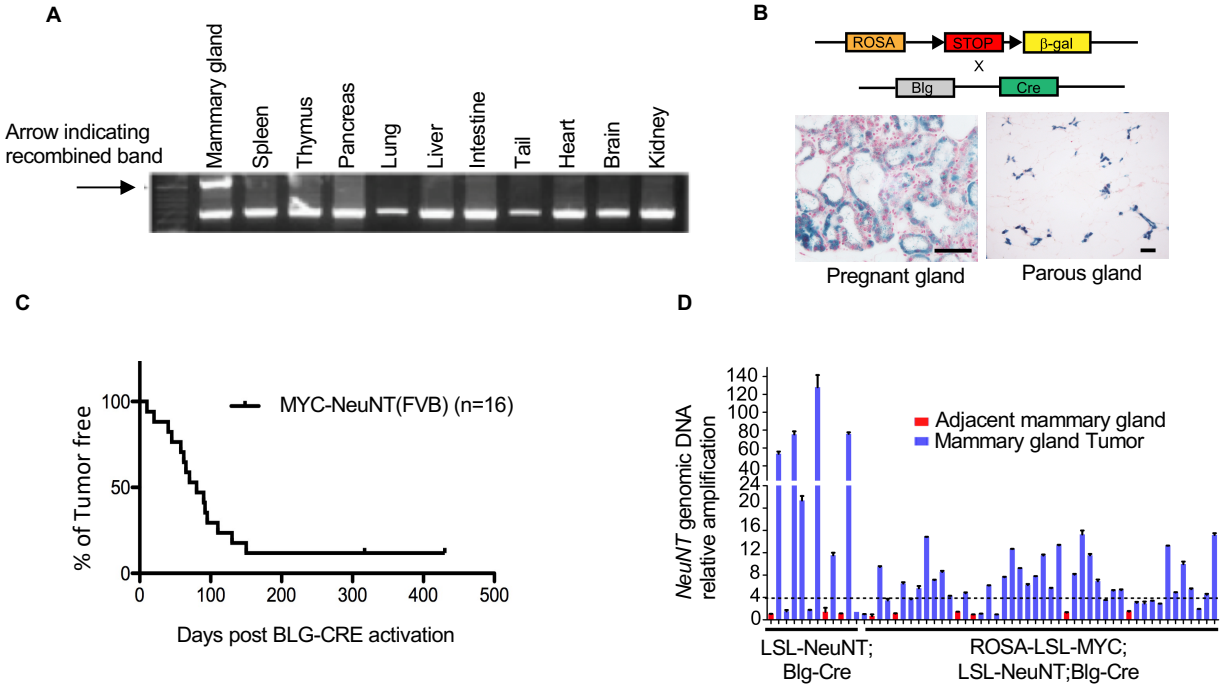
A. RT-PCR analysis of Neu expression in MCF10A-Ctrl and MCF10A-Neu8142 cell lines. cDNA from MCF10A-Ctrl or MCF10A-Neu8142 cell lines was used as a template for PCR. **B**. Western and qRT-PCR analysis of MCF10A-Neu8142 and MCF10A-Ctrl cells infected with Ad-MYC showing the expression of MYC, HER2 and GAPDH proteins (left) and *Myc* mRNA (right graph, means +/- SEM, n=3). **C.** Western blots of cell lysates from MCF10A-TR-MYC-Neu8142 and MCF10A-TR-MYC-Ctrl cells stimulated with serum for indicated time. The ratio of MYC vs. Actin was calculated, normalized to the control cell line at the 0 hour time point, and graphed. **D**. The average mean fluorescent intensity (MFI) of pS62-MYC in tumor cells or adjacent normal mammary gland cells is shown for 4 HER2+ patient tumors. Two patients did not have adjacent normal regions for analysis.

Supplemental Figure 2: Analysis of HER2 expression and MYC phosphorylation in HER2+ patient tumors



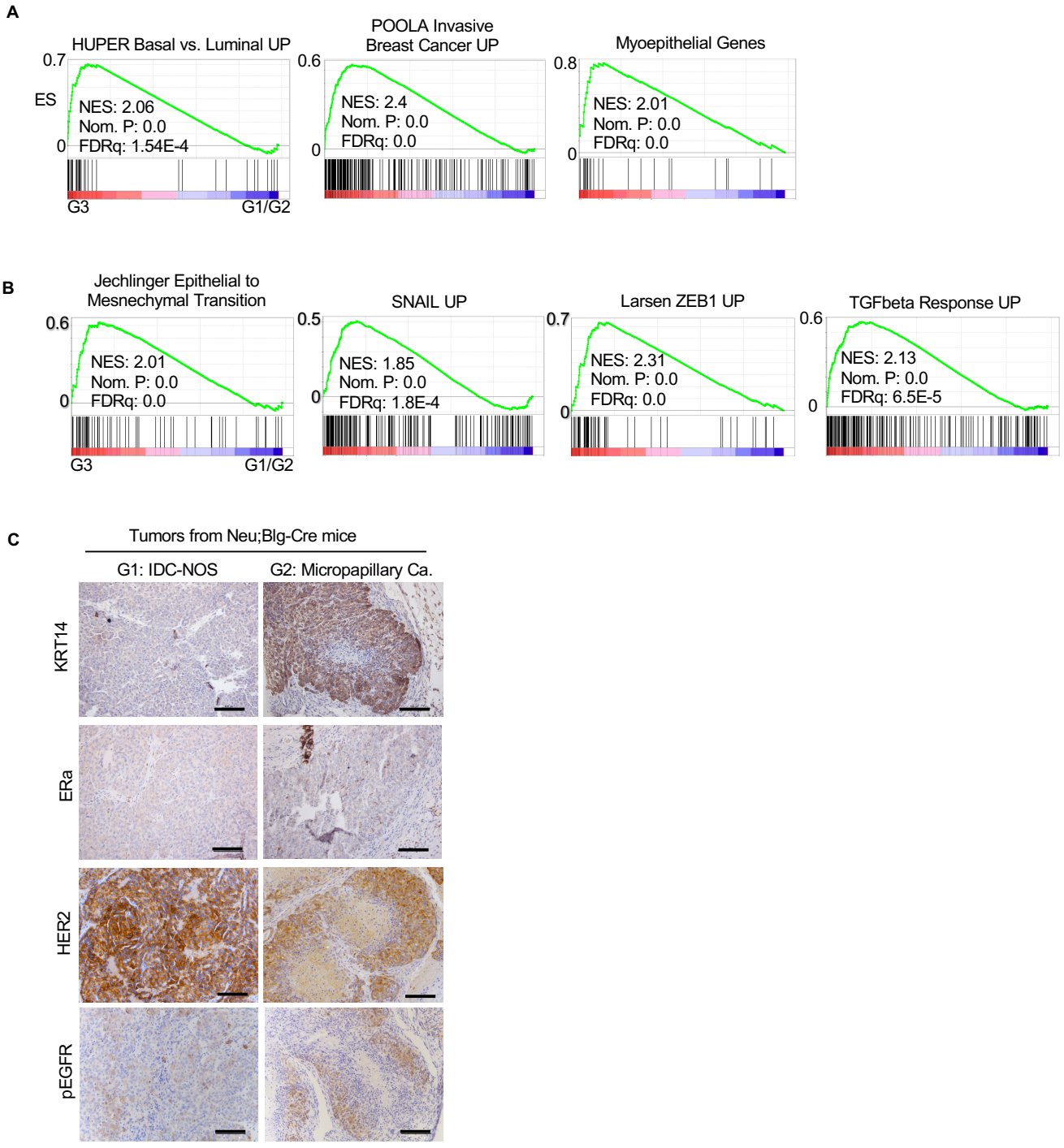
A. A map of the imaged cores from the Biomax TMA BR1506, containing 76 patient tumors (75 HER2+), 2 cores per patient. For our analysis, 1 patient's tumor was missing, leaving 75 patient tumors, 74 HER2+ and 1 HER2-. **B.** A schematic showing the strategy of nuclear segmentation, and expansion in CellProfiler to obtain single cell expression for each marker. Gates to restrict analysis to the correct TMA core, and to avoid folded tissue regions are then applied, as well as gates to identify nucleated cells. **C.** Histograms of four tumors showing the global gate for pS62-MYC positivity that was applied across all tumors, with frequencies displayed of %pS62-MYC positivity. **D.** Dot plots of single cells from the same four tumors as in *C,* showing expression of CK19 (y axis) and HER2 (x axis), with the dots colored by expression of pS62-MYC. Gates for CK19 positivity, and CK19+/HER2^{low}, CK19+/HER2^{med} and CK19+/HER2^{hi} are shown with the frequency of total cells. Patient 76 is denoted as HER2- in the accompanying patient data, whereas all other tumors shown are HER2 2-3+.

Supplemental Figure 3. Further characterization of the ROSA-LSL-Myc;LSL-NeuNT;Blg-Cre mouse model



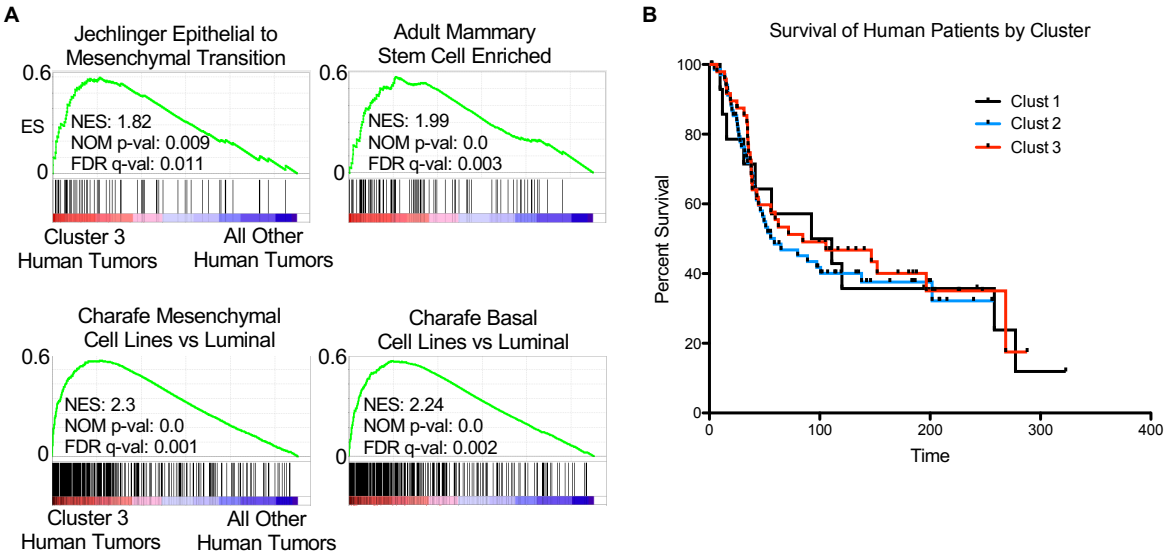
A. Extracted DNA from the indicted tissues from MYC;NeuNT mouse after Blg-Cre activation, Cre-mediated recombination was only detected in mammary gland by RT-PCR. **B**. Diagram showing the generation of ROSA-LSL-ß-gal;Blg-Cre reporter mice by breeding the ROSA-LSL-Myc;Blg-Cre and ROSA-LSL-ß-gal reporter mice. ß-gal staining showed that Blg-Cre was activated in pregnant mammary gland and parous gland. Scale bar in left one = 50 µm, in right side = 100 µm. **C.** Mammary gland tumor incidence in MYC;NeuNT (ROSA-LSL-Myc;LSL-NeuNT;Blg-Cre) in 10 generation backcrossed FVB genetic background mice (compare to Fig. 2B in mixed 129/BL6/FVB background). **D**. qPCR of genomic DNA from mammary gland tumors or adjacent normal tissues in 9 NeuNT and 40 MYC;NeuNT mice showed amplification of *NeuNT* gene in these tissues. The amplification of *NeuNT* was set to 4 in one of tumor samples and the relative amplification in the rest of the samples was calculated and graphed.

Supplemental Figure 4. Further characterization of Group 3 MYC;NeuNT tumors and NeuNT tumors



A. The gene set enrichment analysis (GSEA) of G3 tumors compare to G1&G2 tumors from MYC;NeuNT mice. **B.** Additional GSEA results examining genesets related to epithelial-to-mesenchymal transition. **C.** Immunohistochemistry staining with KRT14, anti-ERα, HER2 and pEGFR in G1 and G2 NeuNT tumors. Scale bar = 100μm.

Supplemental Figure 5. Further characterization of human HER2+ ER- tumor groups



A. Additional gene-set enrichment analysis (GSEA) of the human tumors from cluster 3, which was enriched for Claudin-low tumors, compare to all others. **B.** Overall survival between patients from these different clusters of HER2^{+} ER^{-} tumors observed no significant differences in outcome.