A

Gi/o-GPCRs expression heatmap across breast cancer subtypes (Basal, HER2, Luminal A, Luminal B). Rows: ADORA1, ADORA3, ADRA2A, ADRA2B, ADRA2C, AGTR2, APLNR, C3AR1, C5AR1, CASR, CCR1, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, ACKR4, CCRL2, CHRM2, CHRM4, CNR1, CNR2, CX3CR1, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, ACKR3, DRD2, DRD3, DRD4, EDNRB, F2RL1, F2RL3, FFAR2, FFAR3, FPR1, FPR2, FPR3, GABBR1, GALR1, GALR2, GALR3, GPER1, GRM2, GRM3, GRM4, GRM6, GRM7, GRM8, HCAR1, HCAR2, HRH3, HRH4, HTR1A, HTR1B, HTR1D, HTR1E, HTR1F, HTR5A, LHCGR, LPAR1, LPAR2, LPAR3, LPAR4, LTB4R, LTB4R2, MCHR1, MRGPRD, MTNR1A, MTNR1B, NPBWR1, NPBWR2, NPFFR2, NPR1, NPR2, NPR3, NPY1R, NPY2R, NPY5R, OPRD1, OPRK1, OPRL1, OPRM1, OXTR, P2RY12, P2RY13, PTAFR, PTGER3, RXFP1, RXFP2, RXFP3, RXFP4, S1PR1, S1PR3, S1PR4, S1PR5, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, TSHR, XCR1, XCR1. Legend: Hight (red), Low (blue), No change (grey).

1

B Gs-GPCRs

Basal | HER2 | Luminal A | Luminal B

ADORA2A
ADORA2B
ADRB1
ADRB2
ADRB3
AVPR2
CALCR
CALCRL
CCKAR
CCKBR
CRCP
CRHR1
CRHR2
DRD1
DRD5
EDNRA
FSHR
GCGR
GHRHR
GIPR
GLP1R
GLP2R
GPBAR1
GPER1
GPR101
GPR119
GPR132
GPR174
HRH2
HTR4
HTR6
HTR7
LHCGR
LPAR3
MC1R
MC2R
MC3R
MC4R
MC5R
NPSR1
P2RY11
PTGDR
PTGER2
PTGER4
PTH1R
PTH2R
RXFP1
RXFP2
S1PR2
S1PR3
S1PR4
SCTR
TAAR1
TSHR
VIPR1
VIPR1

Hight | Low | No change

C

Gq-GPCRs

Basal | HER2 | Luminal A | Luminal B

ADRA1A
ADRA1B
ADRA1D
AGTR1
AVPR1A
AVPR1B
NMBR
GRPR
BDKRB1
BDKRB2
BRS3
CASR
CCKAR
CHRM1
CHRM3
CHRM5
CYSLTR1
CYSLTR2
EDNRA
EDNRB
F2R
F2RL1
FFAR1
FFAR2
GALR2
GHSR
GNRHR
GPR139
GRM1
GRM5
GRPR
HCRTR1
HCRTR2
HRH1
HTR2A
HTR2B
HTR2C
KISS1R
LHCGR
LPAR1
LPAR2
LPAR3
LPAR4
LPAR5
LTB4R
LTB4R2
MAS1
MCHR1
MCHR2
MRGPRX1
MRGPRX3
MRGPRX4
NMBR
NMUR1
NMUR2
NPFFR1
NPSR1
NTSR1
NTSR2
FFAR4
OXTR
P2RY1
P2RY11
P2RY14
P2RY2
P2RY4
P2RY6
PTAFR
PTGER1
PTGFR
PTH1R
PTH2R
S1PR2
S1PR3
TACR1
TACR2
TACR3
TBXA2R
TRHR
TSHR
UTS2R

Hight | Low | No change

3

D

G12/13 GPCRs

| | Basal | HER2 | Luminal A | Luminal B |

CASR
F2R
LPAR1
LPAR2
LPAR4
LPAR5
LPAR6
RXFP1
S1PR2
S1PR4
S1PR5
TSHR

■ Hight   ■ Low   ■ No change

Supplemental Supplemental Figure 1. Expression of GPCRs in TCGA invasive breast cancer dataset. A, $G_{i/o}$-coupled GPCRs; B, G_s-coupled GPCRs; C, $G_{q/11}$-coupled GPCRs; D, $G_{12/13}$-coupled GPCRs; E, GPCRs with unknown G-protein linkage. The expression levels (high, low and no change) of GPCR mRNA relative to diploid samples are organized based on the molecular subtypes of breast cancer shown on the top.
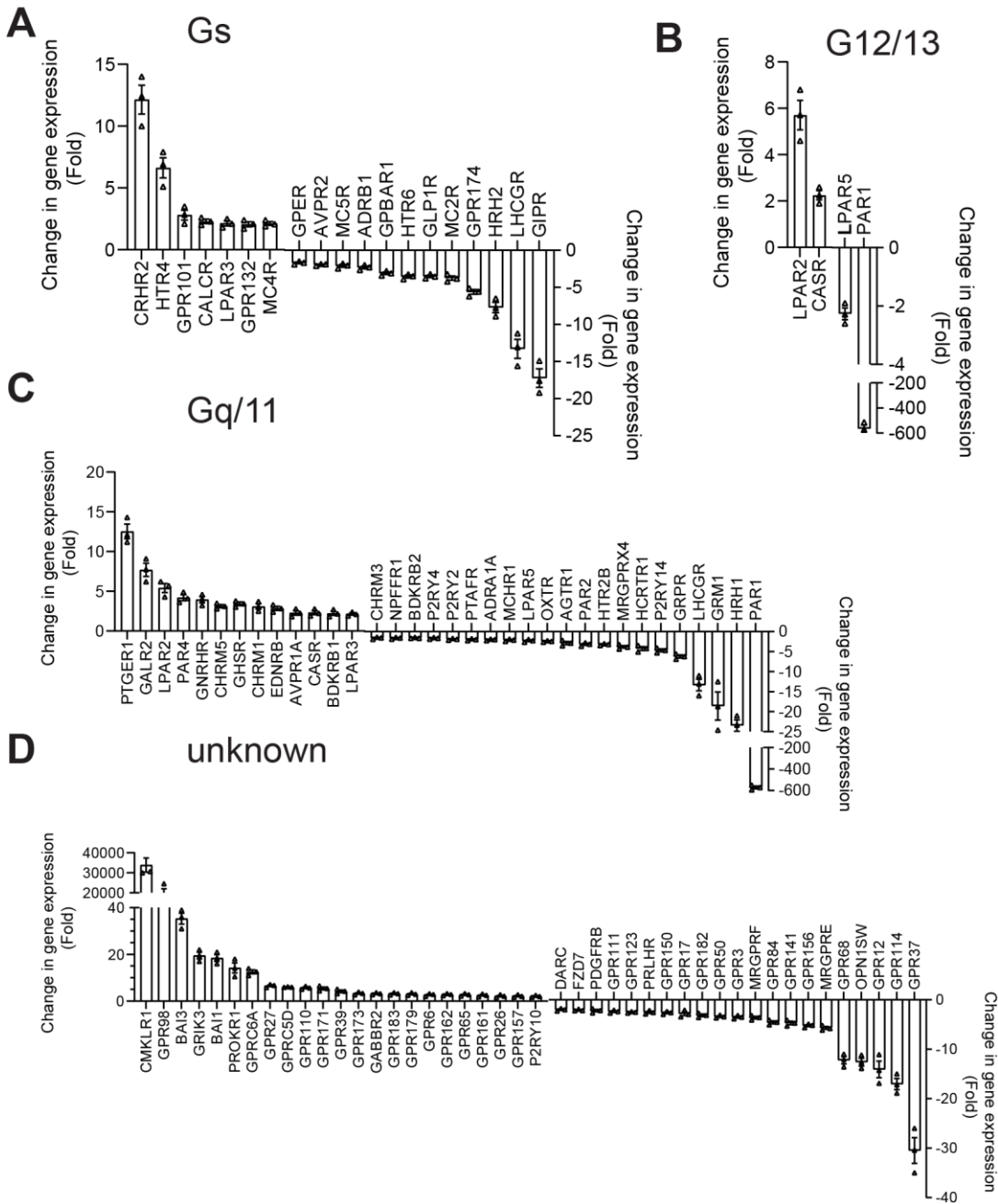
Figure 2. Altered GPCR expression in Neu cells. A, G_s-coupled GPCRs; B, $G_{q/11}$-coupled GPCRs; C, G_s-coupled GPCRs; D, GPCRs with unknown G-protein linkage that show more than 2-fold change of expression in Neu cells as compared to control cells. The data are expressed as mean±SEM, n=3.
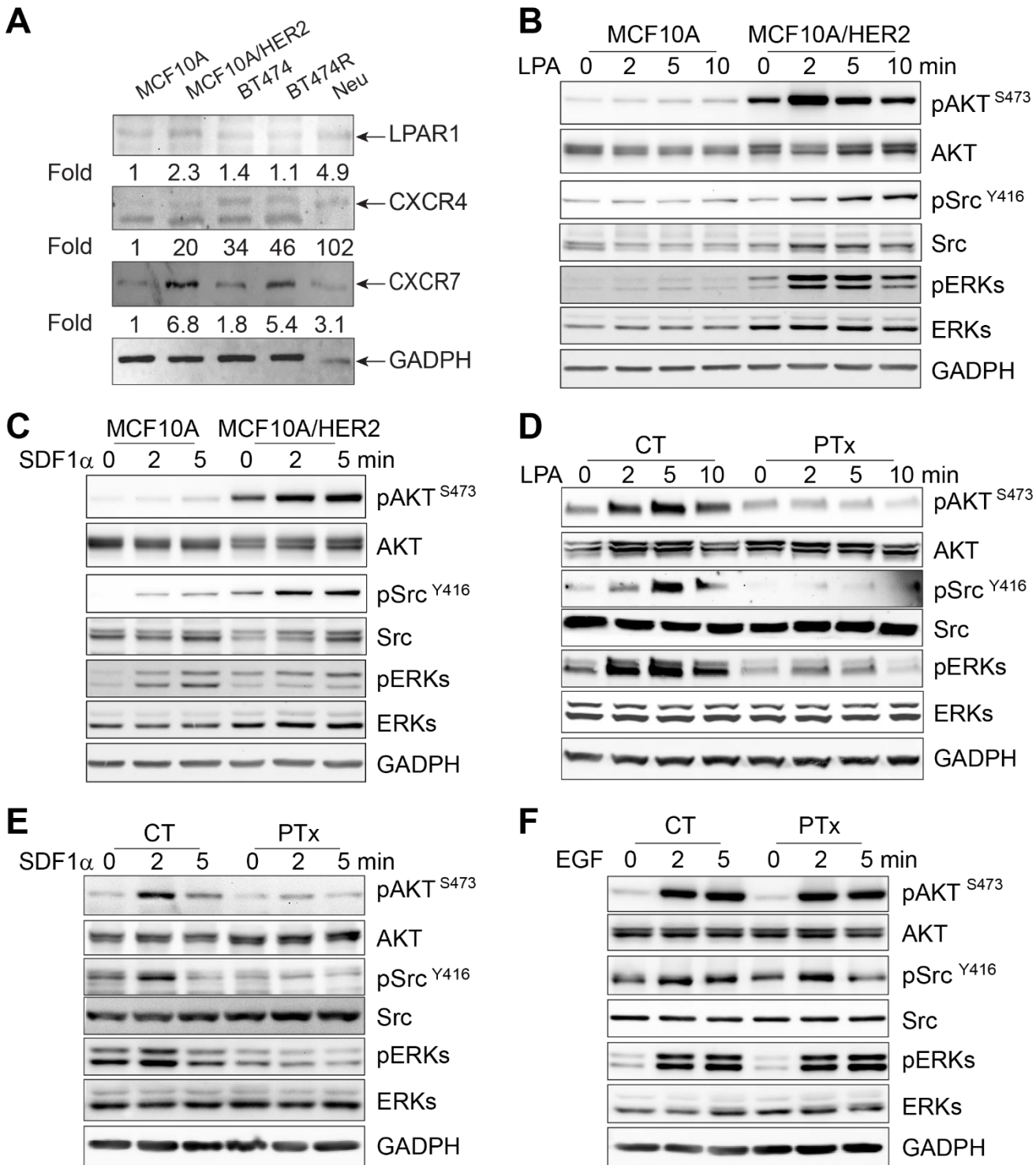
Supplemental Figure 3. Upregulated Gi/o-GPCR signaling in MCF10A/HER2 cells. Western blotting showing A, the expresson of LPAR1, CXCR4 and CXCR7 in the indicated cells. The levels of LPAR1, CXCR4 and CXCR7 in each cell line were quantified as the ratio of the protein to GADPH and expressed as the fold increase over MCF10A, which is indicated underneath the images; B, the response of MCF10A and MCF10A/HER2 to LPA (B)- and SDF1α (C)-stimulated AKT^{S473} and Src^{Y416} phosphorylation; D-F, the effect of PTx treatment on LPA (D)-, SDF1α (E)- and EGF (F)-stimulated AKT^{S473} and Src^{Y416} phosphorylation in MCF10A/HER2 cells. The images are representative of at least three independent experiments and were assembled from multiple blots run with samples from the same experiments.
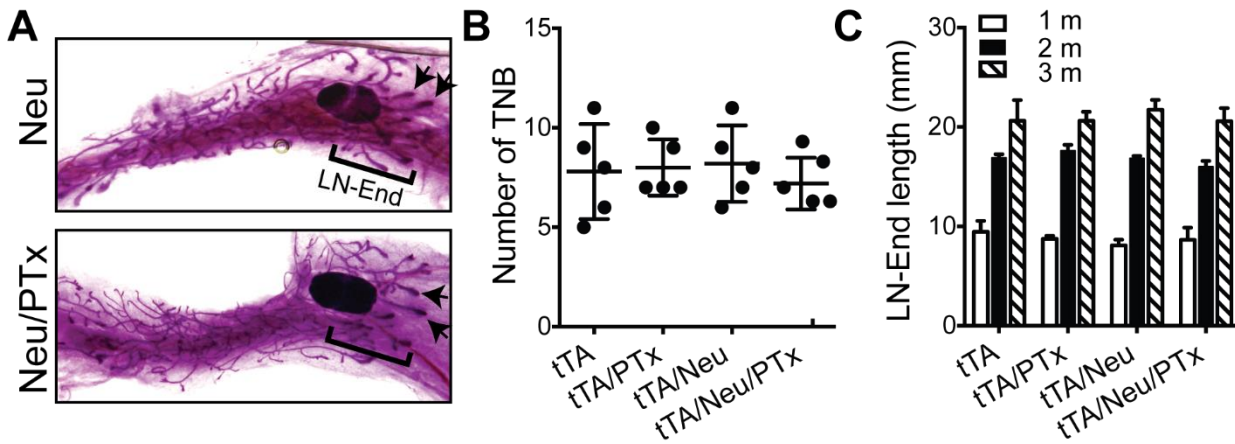
Supplemental Figure 4. PTx expression does not affect mammary gland development. A, Whole-mount *in situ* staining showing the terminal end buds (TNBs, indicated by arrows) and the length of ductal distance, measured from the lymph node (LN) to the end of TNBs (LN-End), in the mammary glands from 1-month old Neu and Neu/PTx mice. B-C, quantitative data showing the number of TNBs (B) and the length of LN-End (C) in mammary glands from transgenic mice at different ages. M: month.
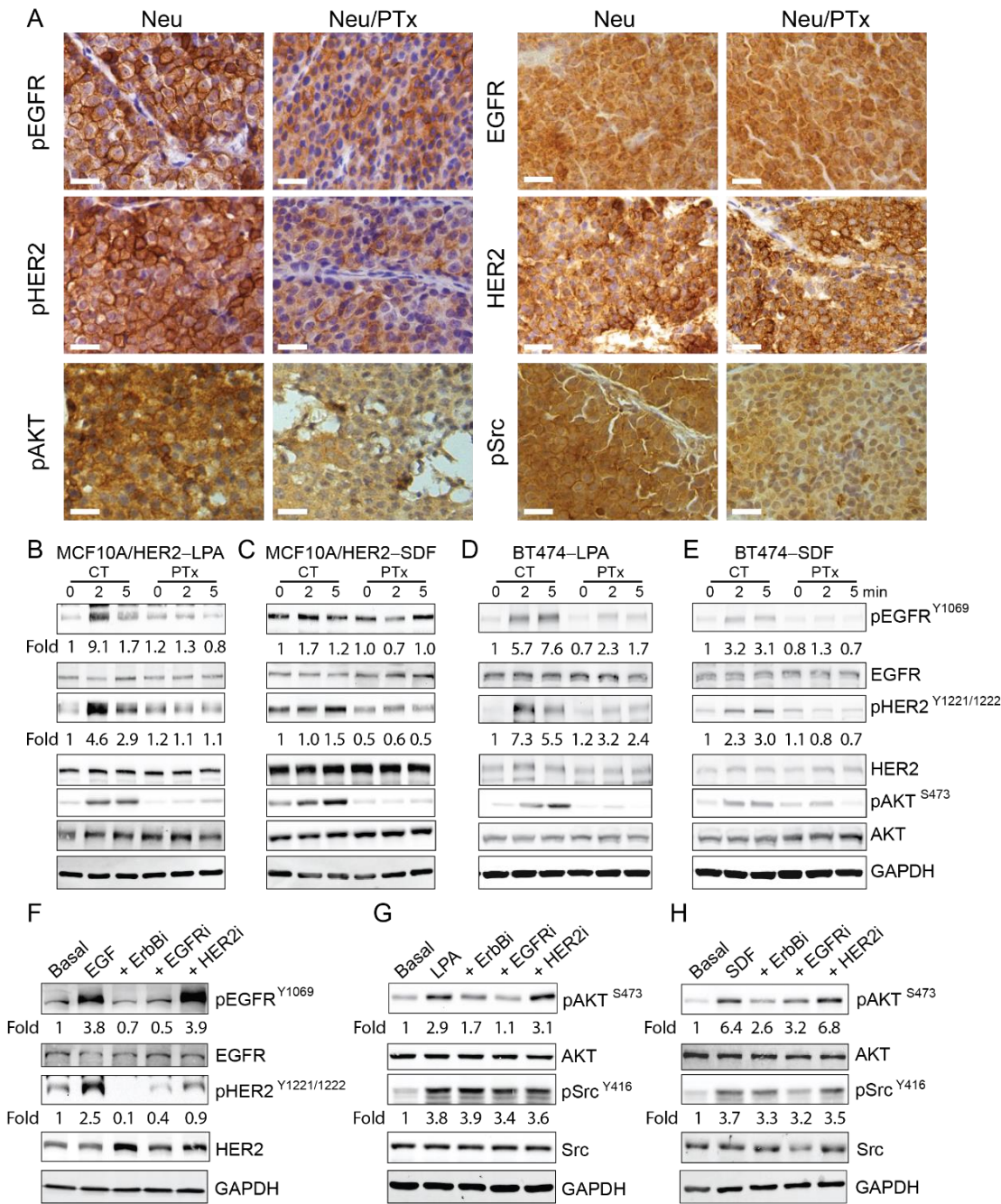
Supplemental Figure 5. Cross-talk between $G_{i/o}$-GPCRs and EGFR/HER2. A. IHC staining showing phospho-EGFR^{Y1069} (pEGFR), total EGFR, phospho-HER2$^{Y1221/1222}$ (pHER2), total HER2, phospho-AKT^{S473} (pAKT), and phospho-Src^{Y416} (pSrc), in Neu and Neu/PTx tumors. Scale bar=10 μm. The images are represtative of 6 to 9 tumors. B-E, Western blotting showing phosphorylation of EGFR, HER2, and AKT in MCF10A/HER2 (B-C) and BT474 (D-E) cells stimulated with 10 μM LPA (B) and 50 nM SDF1α (C) and treated with vehicle (CT) or PTx (200ng/ml). D-F, the effect of 1 μM of the pan-ErbB-, EGFR-, and HER2-selective inhibitors on the activation of EGFR and HER2 by EGF in Neu cells (D) and the phosphorylation of AKT^{S473} and Src^{Y416} by LPA (E) and SDF1 α (F) stimulation in MCF10A/HER2 cells. The phosphorylation of EGFR^{Y1068}, HER2$^{Y1221/1222}$, AKT^{S473}, and Src^{Y416} was quantified as the ratio of the phosphorylated to total protein and expressed as the fold increase over basal, which is indicated underneath the images. All of the blotting images are representative of at least three independent experiments and assembled from multiple blots run with the samples from the same experiments.
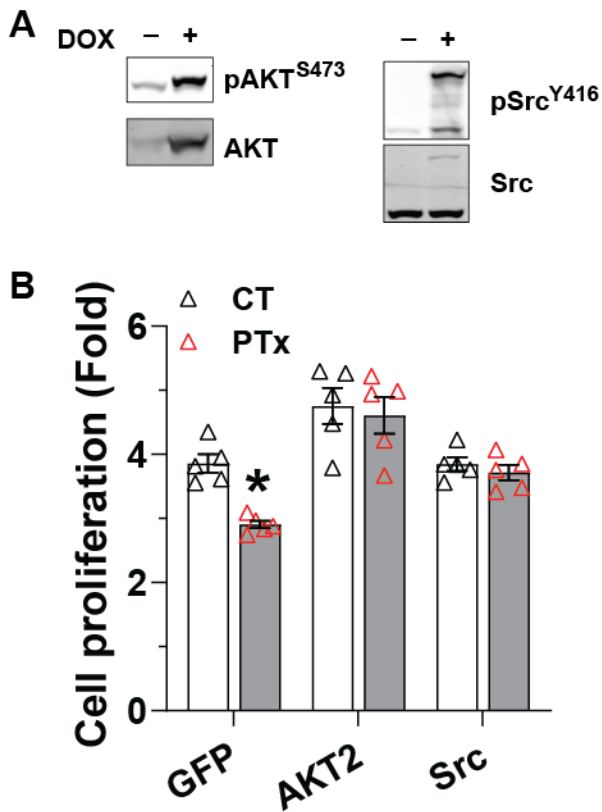
Supplemental Figure 6. $G_{i/o}$-GPCRs signal through AKT and Src pathways to regulate Neu cell growth.
A, Western blotting showing the expression of myristoylated AKT2 and GFP-tagged Src/Y527F in Neu cells induced by doxycylcine (DOX, 0.5 µg/ml for AKT2 and 0.1 µg/ml for Src) for three days. The images were assembled from multiple blots run with the samples from the same experiment. **B**, the effect of induced myristoylated AKT2 and GFP-tagged Src/Y527F expression on PTx-mediated inhibition of Neu cell growth. Neu cells expressing the indicated constucts were treated with doxycyline in the presence of vehicle (CT) or PTx (200ng/ml) for 5 days. Two-tail unpaired Student's *t* test, *$p<0.05$, n=5.