A CRITICAL ROLE OF YB-1 IN THE GENESIS AND PROGRESSION OF KRAS-MUTATED HUMAN BREAST CANCER

Supplementary Figure Legends

Supplementary Figure 1. High levels of YB-1 are associated with metastatic human breast cancers and *KRAS*-amplified tumors. **a-b,** *YBX1* mRNA expression according to the ER (**a**) or metastatic status (**b**) of invasive breast carcinomas in the TCGA dataset. **c** *YBX1* mRNA expression according to gene copy numbers of *ERBB2* (left panel), *PIK3CA* (middle panel) and *AKT1* (right panel) in invasive breast carcinomas in the TCGA dataset. All *YBX1* values are shown as RPKMs.

Supplementary Figure 2. Transplants of primary human mammary cells expressing *myrAKT1* produce DCIS-like structures. **a-b,** Representative IHC images of sections stained for ER, PR, and Ki67 (**a**); or SMA, CK14, CK8-18 and p63 (**b**) in tumors derived from transplants of either BCs or LPs-transduced with *myrAKT1*. Scale bar, 100 μm. **c,** Representative FACS profile (left panel) and Western blots (right panel) of human mammary MCF10A cells expressing inducible YB1-2A-KO, cultured with or without doxycycline. **d,** Representative IHC images of sections stained for YB-1 in tumors generated from LPs (left) or BCs (right) transduced with an inducible *myrAKT1* vector, and transplanted into mice given water with or without doxycycline.

Supplementary Figure 3. YB-1 is required for dissemination of intravenously injected MDA-MB-231 cells. **a,** Dot plot showing levels of bioluminescence of tumors derived from MDA-MB-231 cells stably expressing a shScr or a shYB-1 cDNA (described in Figure 5H) and assessed 47 days post-transplant. **b,** Comparison of YB-1 levels (staining intensity) in tumors shown in A. **c,** Western blots of MDA-MB-231 cells transfected with *siScr, siYB-1#2* or *siYB-1#6*. **d,** Representative pictures of bioluminescence signals obtained in mice 45 days after being injected intravenously with MDA-MB-231 cells transfected with *siScr, siYB-1#2* or *siYB-1#6*. **e,** Representative H&E or YB-1 stained photomicrographs of sections of lungs of mice injected intravenously with MDA-MB-231 cells transfected with *siScr, siYB-1#2* or *siYB-1#6*.

Supplementary Figure 4. Inhibition of YB-1 expression downregulates expression of *PPIF*, *SLC3A2* and *SLC7A1*. **a-b,** Genes whose transcript and protein expression in MDA-MB-231 cells after shYB-1 transduction was either upregulated (**a**) or down-regulated (**b**) by comparison to shScr-transduced (control) cells, based on an analysis of matched pairs of RNAseq and proteomics data. **c-d,** *SLC3A2* and *SLC7A1* mRNA expression according to *KRAS* (**c**) or *YBX1* (**d**) copy number status in invasive breast carcinomas in the TCGA data. Values for *SLC3A2* and *SLC7A1* are shown as RPKMs.

Supplementary Figure 5. *KRAS* amplification is associated with a YB-1-regulated HIF1α response. **a,** Representative images of CAIX-stained sections of tumors produced from shYB-1- or shScr-transduced MDA-MB-231 cells. Scale bar, 100 μm. Bar graph shows measured levels of CAIX (staining intensity). **b,** Western blots showing HIF1α

and YB-1 levels (relative to GRB2) in shYB-1- and shScr-transduced MDA-MB-231 cells maintained for up to 24 hours in 1% O_2. **c,** *HIF1A* (left panel), *CAIX* (middle panel), and *VEGFA* (right panel) transcript levels (RPKM values) shown according to *KRAS* copy number status in the invasive breast carcinoma data set in METABRIC. **d,** *HIF1A* (left panel), *CAIX* (middle panel), and *VEGFA* (right panel) transcript levels are shown according to *YBX1* copy number status in the TCGA invasive breast carcinoma dataset. All transcript values are shown as RPKMs.