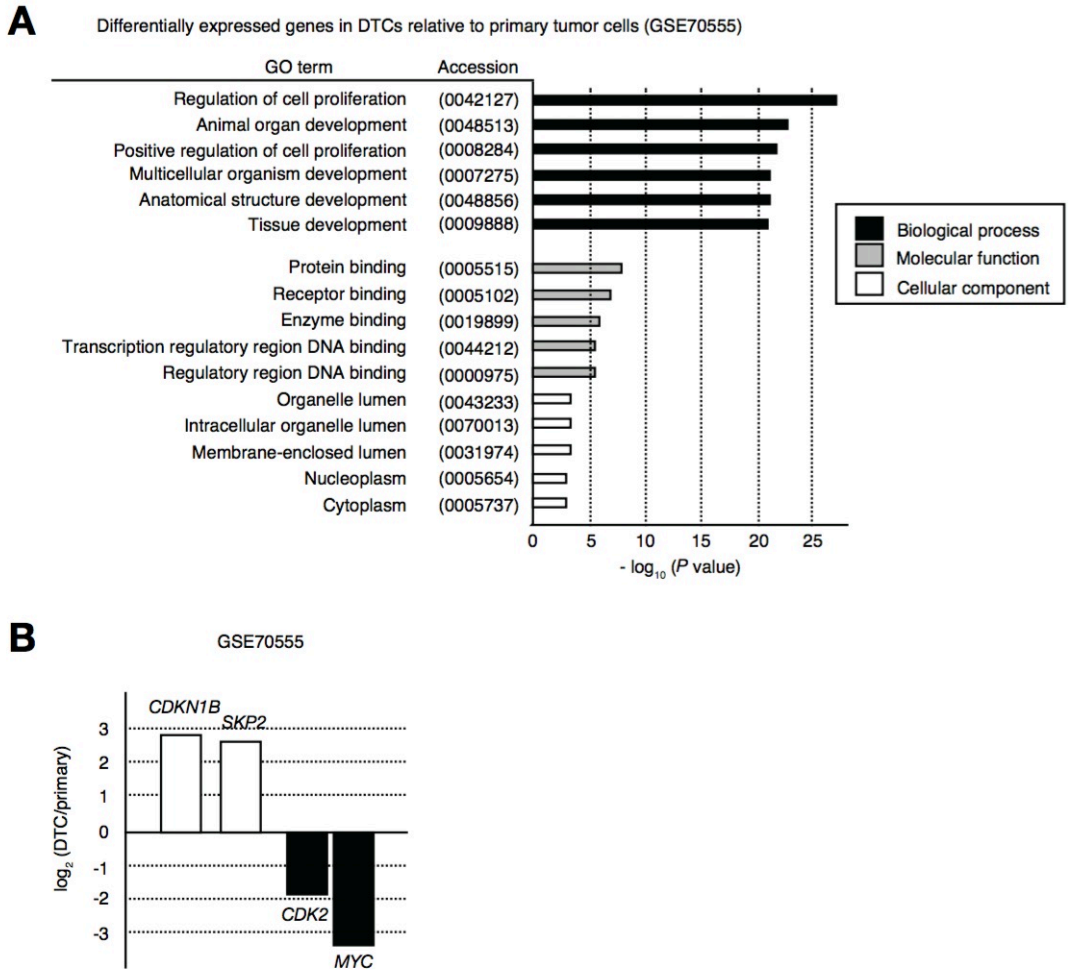
Supplemental Material

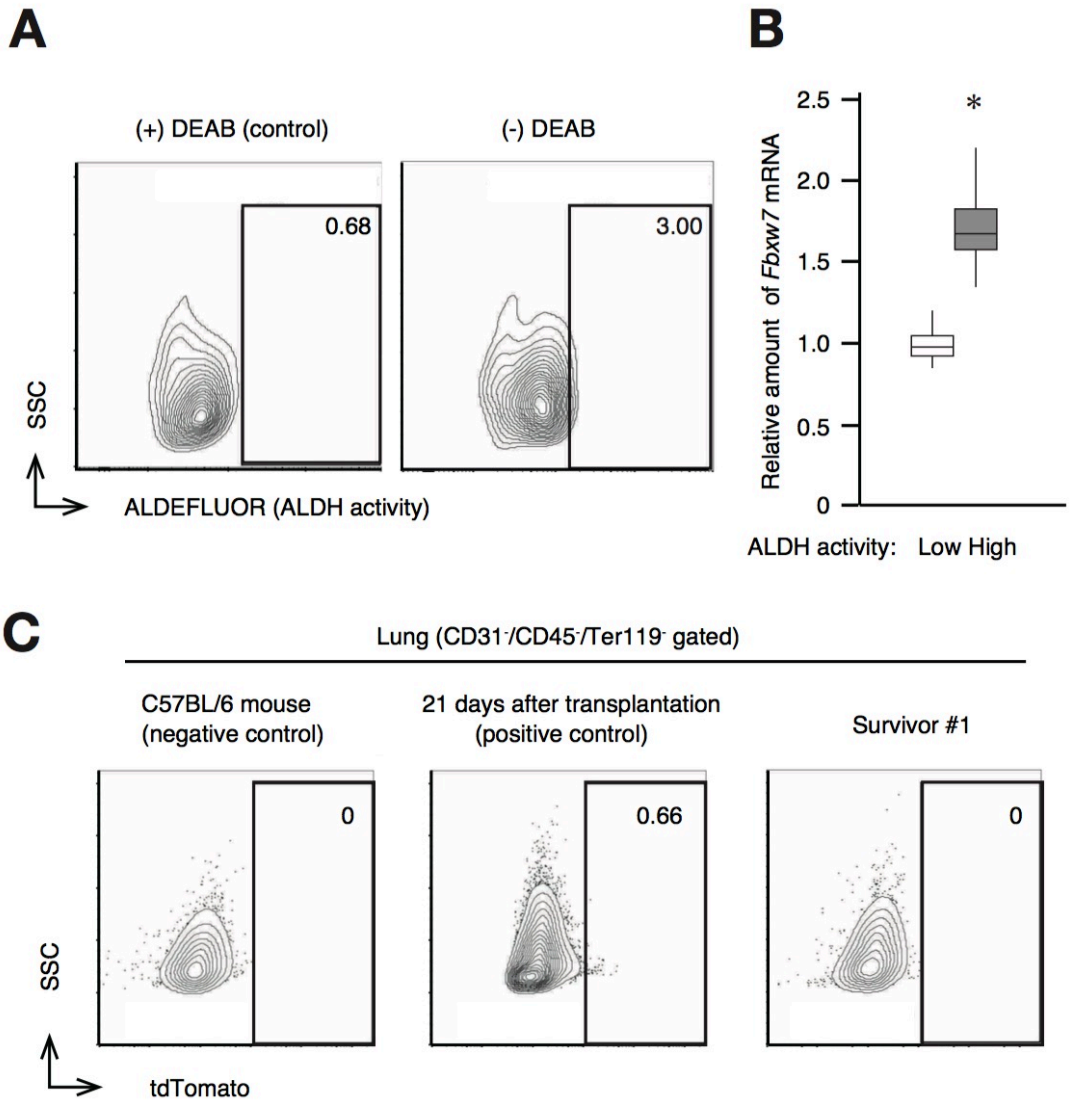
Prevention of cancer dormancy by Fbxw7 ablation eradicates disseminated tumor cells

Hideyuki Shimizu, Shoichiro Takeishi, Hirokazu Nakatsumi, and Keiichi I. Nakayama



Shimizu et al., Supplementary Figure S1

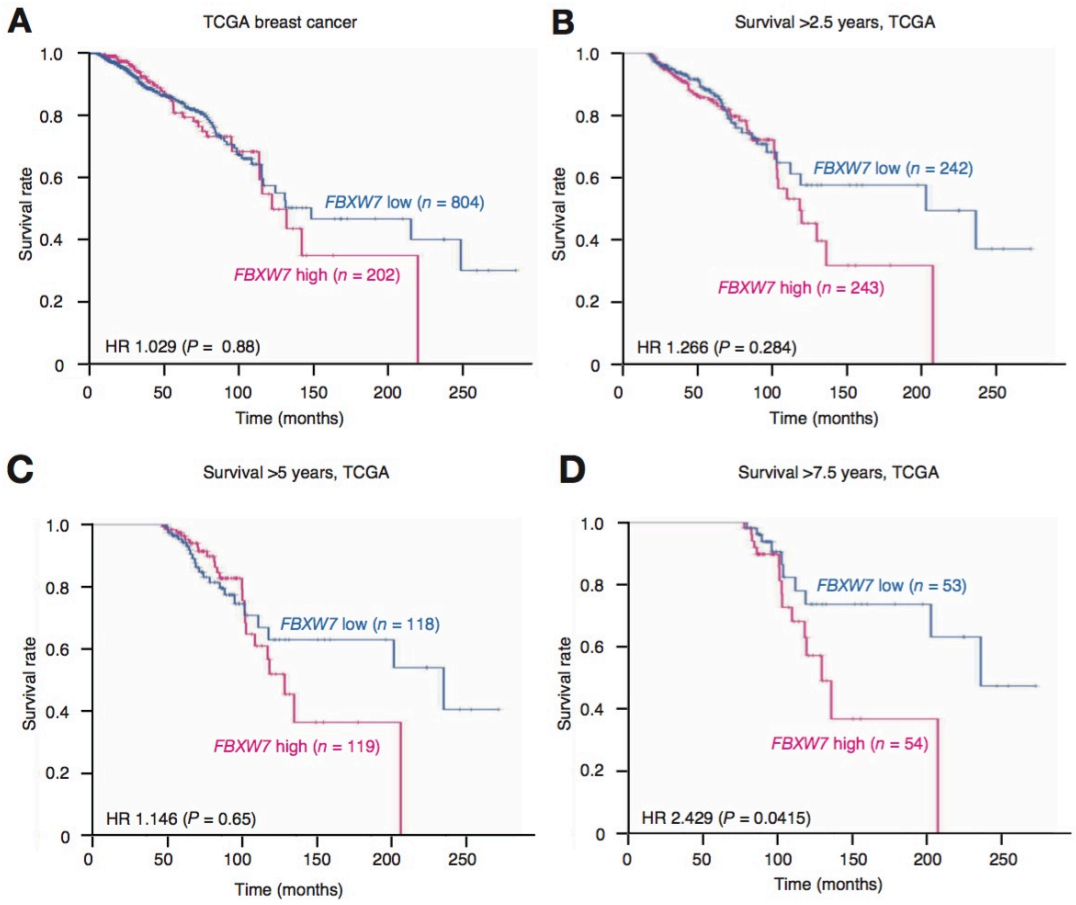
Supplemental Figure 1. Single-cell analysis reveals that genes related to cell cycle regulation are the most differentially expressed in DTCs relative to primary breast tumor cells. (**A**) GO term analysis for differentially expressed genes in DTCs relative to primary tumor cells in patient-derived xenograft models of breast cancer (GSE70555). (**B**) Expression of representative genes in GO 0042127.



Shimizu et al. Supplementary Figure S2

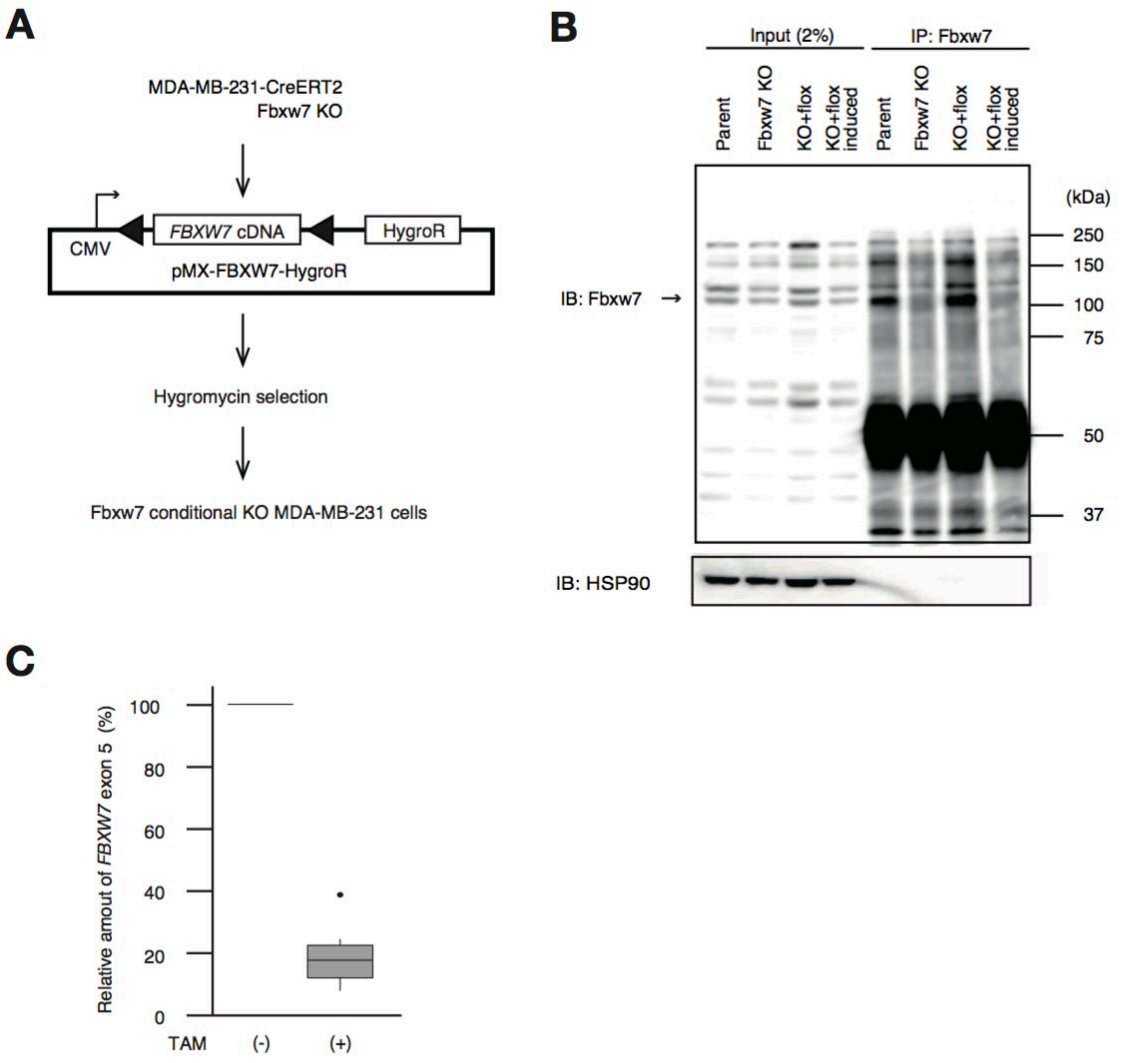
Supplemental Figure 2. *Fbxw7* is expressed preferentially in quiescent mouse breast cancer cells. (**A**) FACS analysis of E0771 cells stained with ALDEFLUOR for isolation of slow-cycling, ALDH^{high} cells. Cells were treated with the ALDH inhibitor *N*,*N*-diethylaminobenzaldehyde (DEAB) as a negative control. (**B**) RT and real-time PCR analysis of *Fbxw7* mRNA abundance in ALDH^{low} and ALDH^{high} fractions of E0771 cells isolated as in (**A**). Box-and-whisker plots show the median, the lower and upper quartiles, and 1.5× the interquartile range. **P* < 0.05 versus ALDH^{low} (Mann-Whitney U test, *n* = 4

independent experiments). (**C**) A mouse injected with Fbxw7 KO–tdTomato E0771 cells in Figure 4H that survived until 150 days after cell transplantation was analyzed for residual tumor cells in the lung. A mouse at 21 days after transplantation of Fbxw7 KO–tdTomato cells was analyzed as a positive control.



Shimizu et al. Supplementary Figure S3

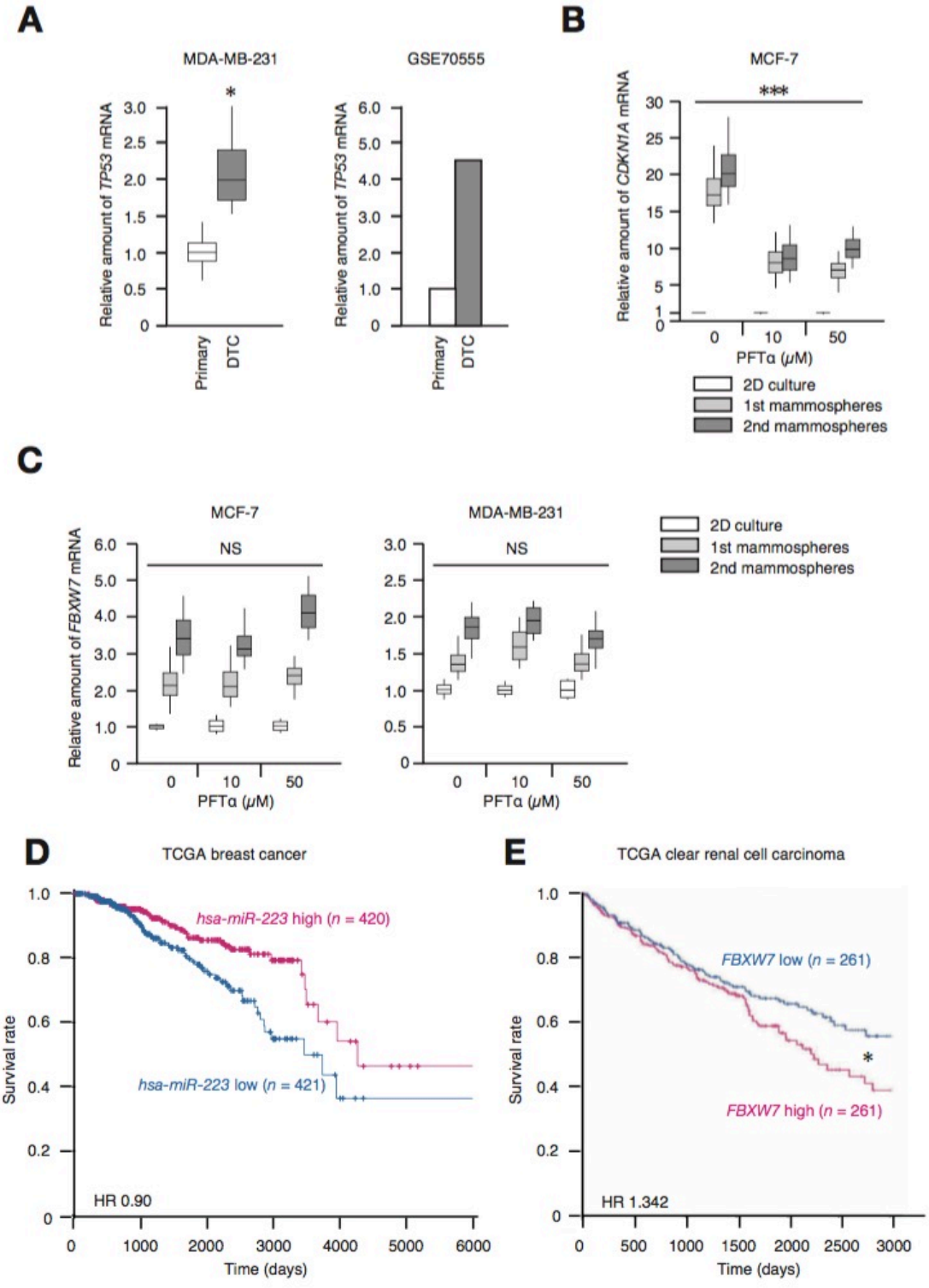
Supplemental Figure 3. Breast cancer patients with a low *FBXW7* expression level in their tumors survive longer than those with a high *FBXW7* expression level. (**A**) Kaplan-Meier curves for breast cancer patients in the TCGA cohort classified in the top 20% or bottom 80% for *FBXW7* expression level. (**B** to **D**) Kaplan-Meier curves for 2.5-year (**B**), 5.0-year (**C**), or 7.5-year (**D**) survivors among breast cancer patients in the TCGA cohort with an *FBXW7* expression level lower or higher than the median.



Shimizu et al. Supplementary Figure S4

Supplemental Figure 4. Generation of Fbxw7 conditional KO MDA-MB-231-mVenus cells. (**A**) Strategy for establishment of Fbxw7 conditional KO cells. Conventional Fbxw7 KO MDA-MB-231-mVenus cells were infected with retroviruses for Cre-ERT2 or containing human *FBXW7* cDNA flanked by loxP sequences (triangles). CMV, cytomegalovirus promoter. (**B**) Confirmation of Fbxw7 depletion in the Fbxw7 conditional KO cells. Lysates prepared from parental or conventional Fbxw7 KO cells as well as from Fbxw7 conditional KO (KO+flox) cells treated (induced) or not with 10 μM

4-hydroxytamoxifen for 48 h were subjected to IP with antibodies to Fbxw7. The resulting precipitates as well as the original cell lysates (2% of input) were subjected to IB analysis with antibodies to Fbxw7. (**C**) RT and real-time PCR analysis of *FBXW7* mRNA abundance in DTCs isolated from BM of mice ($n = 6$) as in Figure 6A that were treated (or not) with tamoxifen (but not with paclitaxel). Box-and-whisker plots show the median, the lower and upper quartiles, 1.5× the interquartile range. *$P < 0.05$ versus (–)tamoxifen (Mann-Whitney U test).



Shimizu et al. Supplementary Figure S5

Supplemental Figure 5. p53 is not directly responsible for up-regulation of *FBXW7* expression in DTCs. (**A**) RT and real-time PCR analysis of *TP53* mRNA abundance in MDA-MB-231 primary tumors and DTCs ($n = 4$ recipients). *$P < 0.05$ (Mann-Whitney U test). (**B**) RT and real-time PCR analysis of *CDKN1A* mRNA abundance in mammospheres formed by MCF-7 cells during incubation with or without pifithrin-α (PFTα) ($n = 4$ independent experiments). ***$P < 0.001$ (Two-way ANOVA). (**C**) RT and real-time PCR analysis of *FBXW7* mRNA abundance in mammospheres formed by MCF-7 or MDA-MB-231 cells during incubation with or without PFTα ($n = 4$ independent experiments). All box-and-whisker plots show the median, the lower and upper quartiles, 1.5× the interquartile range, and outliers. NS, not significant (Two-way ANOVA). (**D**) Kaplan-Meier curves for breast cancer patients in the TCGA data set with tumors expressing *hsa-miR-223* at levels lower or higher than the median. (**E**) Kaplan-Meier curves for clear cell renal cell cancer patients in the TCGA data set with tumors expressing *FBXW7* at levels lower or higher than the median. *$P < 0.05$ (log-rank test).