Supporting Information

Green et al. 10.1073/pnas.1303671110

SI Materials and Methods

Cell Culture. SUM190PT and SUM225CWN were obtained from the University of Michigan and cultured in Ham's F12 with 5% (vol/vol) FCS, 5 μg/mL insulin, 1 μg/mL hydrocortisone, Ciprofloxacin (Cipro), and fungizone. CAL-51 was obtained from the German Tissue Repository DSMZ and cultured in DMEM with 10% (vol/vol) FCS, Cipro, and fungizone. All other cell lines were obtained from the ATCC and propagated in DMEM with 10% FCS, Cipro, and fungizone (MDA-MB-231, BT549, Hs578T) or RPMI with 10% FCS, Cipro, and fungizone (HCC-1395, MDA-MB-468, MCF7, T47D, SKBR3, BT474, HCC-1419, HCC1187, HCC1937). To make FRED and WILMA double transgenic cell lines, cells were transduced simultaneously with both vectors and selected with 1 μg/mL Puromycin. Puro-resistant cells were treated with 50 nM GSK-3 inhibitor XV (Calbiochem). Twenty-four hours later, mLumin$^+$ GFP$^+$ cells were collected by FACS, expanded in vitro, and used for subsequent experiments. All transgenic cell lines underwent Puro selection and sorting, except SUM190PT-FW, SUM225CWN-FW, BT549-FW, and HCC1187-FW, which were expanded and analyzed shortly after transduction. Immortalized human RMFs were kindly provided by C. Kupperwasser (Tufts University School of Medicine, Boston, MA) and cultivated in DMEM 10% FCS. Transduced RMFs were selected with 5 μg/mL Blasticidin (Life Technologies).

Lentiviral Vector Construction. p156RRLsinPPTCMVGFPPRE (1) was used as the viral backbone for all lentiviral constructs by replacement of the CMV-eGFP sequence. Virus was produced by the Salk GT3 core facility by cotransfecting 293T cells with the pMDLg/pRRE, pRSV.REV, and pMD2.G helper plasmids. WILMA (pJG042) comprises 12 TCF binding sites, obtained from the pBAR plasmid (2), upstream of a click-beetle red luciferase-eGFP fusion protein (3). FRED (pJG045) contains the constitutive EF1α/HTLV promoter upstream of mLumin (4), followed by the T2A cleavage peptide from *Thoseaasigna virus* (5), followed by a truncated CD4 (amplified from pMACS 4.1; Miltenyi Biotec), followed by the P2A cleavage peptide from *Porcine teschovirus-1* (5), followed by the Puromycin resistance gene.

Real-Time Wnt Reporter Assay. Twenty thousand MB231-W cells were seeded into a 96-well white opaque plate (Corning) with 20,000 Wnt-producing or control iCHO cells in phenol red-free DMEM-F12 (Life Technologies), 10% (vol/vol) serum, 100 μM D-Luciferin (Biosynth), and 250 ng/mL Dox (to induce Wnt expression by the iCHO cells). Real-time luminescence counts from six replicate wells were collected every 30 min by a temperature-controlled luminometer (Tecan M200) set to 37 °C.

PDX Processing and Passaging. PDX lines were originally initiated by implantation of a fresh patient tumor fragment into the mammary fat pad of recipient SCID/Beige mice (to be described elsewhere) and were maintained by serial passage in vivo at intervals characteristic for each line, and in accordance with Institutional Animal Care and Use Committee requirements.

PDX tumors were excised, minced, and incubated at 37 °C for 1–3 h in digestion media [DMEM, 2% (vol/vol) FCS, 1× Pen-Step, 10 mM Hepes] with DNase, collagenase, and hyaluronidase. The suspension was then triturated and passed over a 40-μm cell strainer. Blood cells were lysed with ACK lysis buffer (Life Technologies). Cells were washed with HF buffer (Hank's Balanced Salt Solution, 2% FCS, 10 mM Hepes) and subjected to density gradient centrifugation using Optiprep (Sigma) to remove dead cells.

Lentiviral Transduction. Mouse cells were depleted from live tumor cell suspensions (above) before infection to maximize viral titer. Cell suspensions were incubated with biotinylated anti-mouse H2k antibodies (BD), followed by incubation with magnetic particles conjugated to anti-biotin magnetic microbeads (Miltenyi) and then passage over a magnetic column as per manufacturer's instructions (Miltenyi). A total of 500,000 live human cells, taken from the flow-through, were mixed with FRED and WILMA lentivirus at estimated multiplicities of infection of 200 or 400, respectively, in the presence of 0.8 mg/mL polybrene and subjected to centrifugation at 900 × *g* for 45 min. The ratio of FRED:WILMA infectious particles was 1:2 to increase the likelihood that cells transduced with FRED would also carry WILMA. After spinfection, cells were washed thrice with HF buffer to remove free virus and mixed with untransduced cells (250,000 to 1×10^6, depending on availability) to promote tumor take and then injected into new recipient mice in 50 μL of 50:50 matrigel:digestion media. Transduced cells (CD4$^+$) were enriched from resulting tumors by magnetic separation with anti-CD4 magnetic microbeads (Miltenyi) and injected into new recipient mice for expansion.

Imaging. Mice and tissues were imaged using the IVIS Spectrum instrument by Caliper Life Sciences. Mice were anesthetized with 2% (vol/vol) isofluorane, injected i.p. with 100 μL of 30 mg/mL D-luciferin (Biosynth) and incubated for 10–15 min before image acquisition. For ex vivo imaging, fresh tissues were incubated in 0.3 mg/mL D-luciferin in PBS for 2–3 min before acquisition. Fluorescence images were acquired using the default instrument settings for mCherry. Data were analyzed using Living Image 4.1 software (Caliper Life Sciences).

Immunohistochemistry. After ex vivo imaging, tumors were placed in 10% (vol/vol) neutral buffered formalin overnight, then transferred to 30% (wt/vol) sucrose the next day until saturated, embedded in paraffin, and sectioned. For antigen retrieval, slides were boiled for 10 min in 0.01 M citrate buffer (pH 6.0). Sections were then blocked in 0.3% hydrogen peroxide for 30 min, then blocked for endogenous biotin per manufacturer's instructions (Vector Laboratories #SP-2001), then stained with anti-Her2 (CST 2165) 1:400 in 1% BSA, anti-CK10 (Abcam #ab76318) 1:250 in 1% BSA, anti-GFP (Abcam #ab13970) 1:800 in 1% BSA, or anti-Ki67 (Abcam # ab15580) 1:200 in 1% BSA, followed by biotinylated goat anti-rabbit IgG (Jackson #0016-065-045) 1:500 or biotinylated donkey anti-chicken IgY (Jackson #703-065-155), followed by HRP-streptavidin (Jackson #0016-030-084) 1:500 and finally developed with AEC chromagen from Vector Laboratories (#SK4200). The proliferative index was calculated by counting 1,000 cells in well-labeled areas and determining the percentage of positively labeled cells. Six different tumors were scored.

1. Follenzi A, Ailles LE, Bakovic S, Geuna M, Naldini L (2000) Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. *Nat Genet* 25(2):217–222.

2. Biechele TL, Moon RT (2008) Assaying beta-catenin/TCF transcription with beta-catenin/TCF transcription-based reporter constructs. *Methods Mol Biol* 468: 99–110.

3. Nervi B, et al. (2007) Factors affecting human T cell engraftment, trafficking, and associated xenogeneic graft-vs-host disease in NOD/SCID beta2mnull mice. *Exp Hematol* 35(12):1823–1838.
4. Perou CM, et al. (2000) Molecular portraits of human breast tumours. *Nature* 406(6797): 747–752.
5. Donnelly ML, et al. (2001) The 'cleavage' activities of foot-and-mouth disease virus 2A site-directed mutants and naturally occurring '2A-like' sequences. *J Gen Virol* 82(Pt 5): 1027–1041.

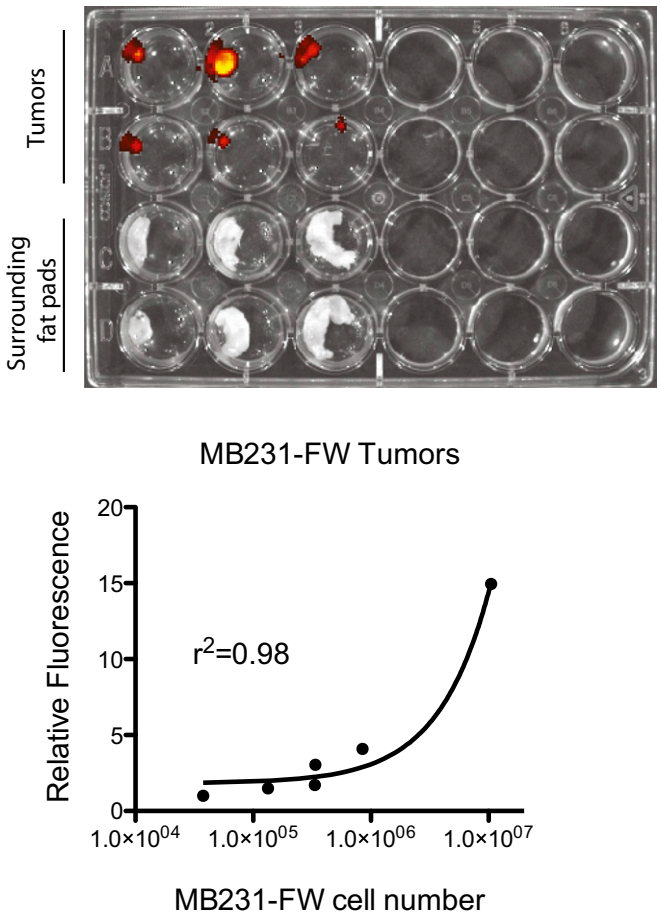

Fig. S1. Red fluorescence is a surrogate for -FW (double transgenic) cell number. Six tumors were initiated with 40,000 MB231-FW cells and 4×10^6 reduction mammary fibroblast (RMF)-GFP cells. After 6 wk the tumors were harvested and placed in a dish for ex vivo imaging (rows A and B, columns 1–3). The surrounding fat pad was also excised and imaged to confirm complete removal of the tumor (rows C and D, columns 1–3). After imaging, the tumors were dissociated and the total cell number was estimated by counting with a hemocytometer. The percentage of red fluorescent cells was determined by flow cytometry and used to calculate the total number of red fluorescent cells.

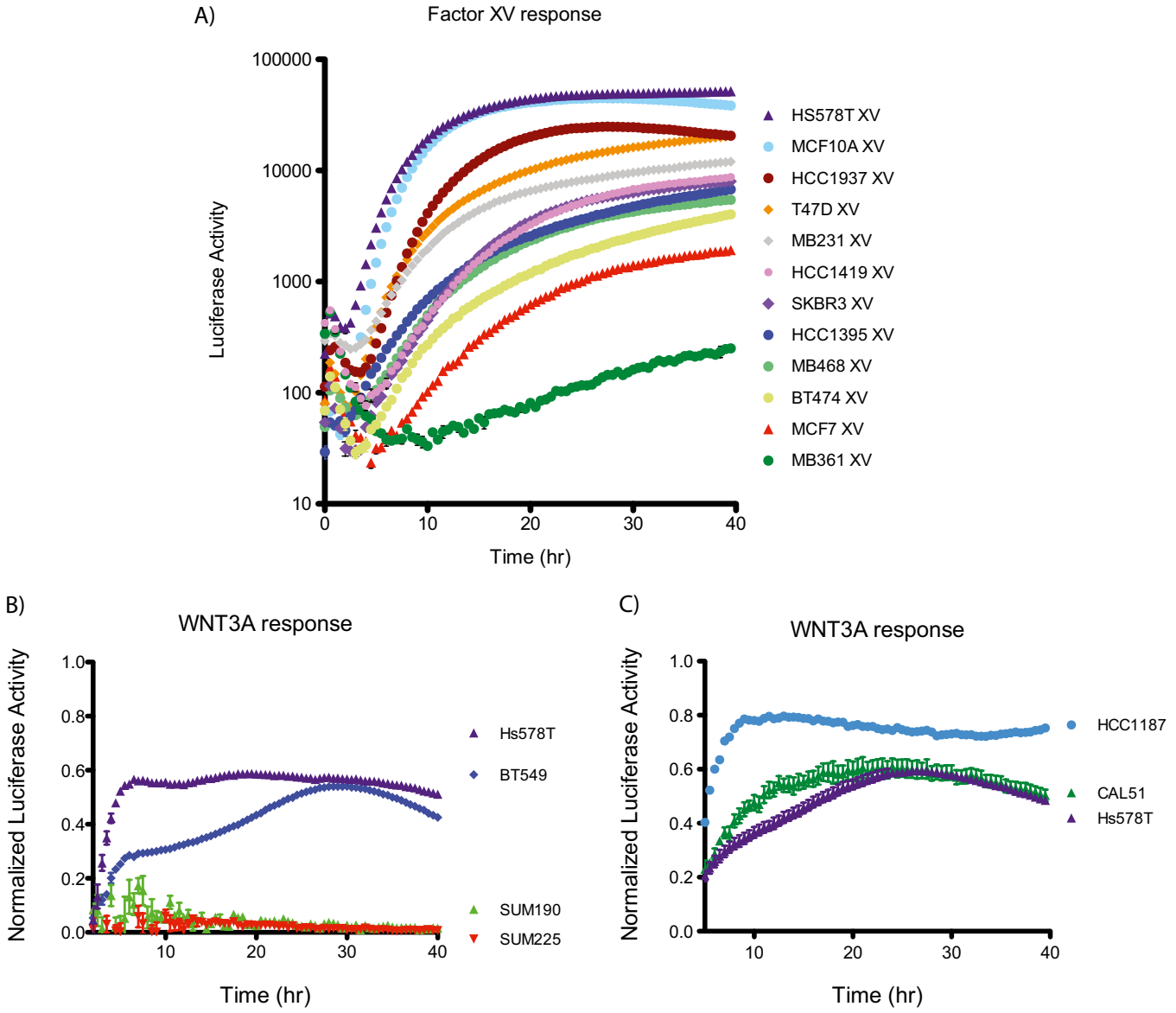

Fig. S2. Wnt responsiveness of additional breast cancer cell lines. Real-time bioluminescence monitoring assay. (*A*) Cells were treated with the GSK-3 inhibitor Factor XV. The Wnt-inducible luciferase marker (WILMA) reporter was robustly induced in all cell lines except MB361, suggesting that WILMA transduction may not have been successful in this line. MB361 was thus excluded from further analysis. BT549, SUM-190, SUM-225, HCC-1187, and CAL-51 were also induced by XV (data not shown). (*B* and *C*) Transgenic breast cancer cells were cocultured with parental (control) or WNT3A-producing inducible CHO (iCHO) cells, and luminescence was measured every 30 min for 40 hours. To adjust for variable transduction efficiency of WILMA, data were normalized by subtracting absorbance in the presence of control from absorbance in the presence of WNT3A, and the remainder was divided by absorbance in the presence of GSK-3 inhibitor XV.

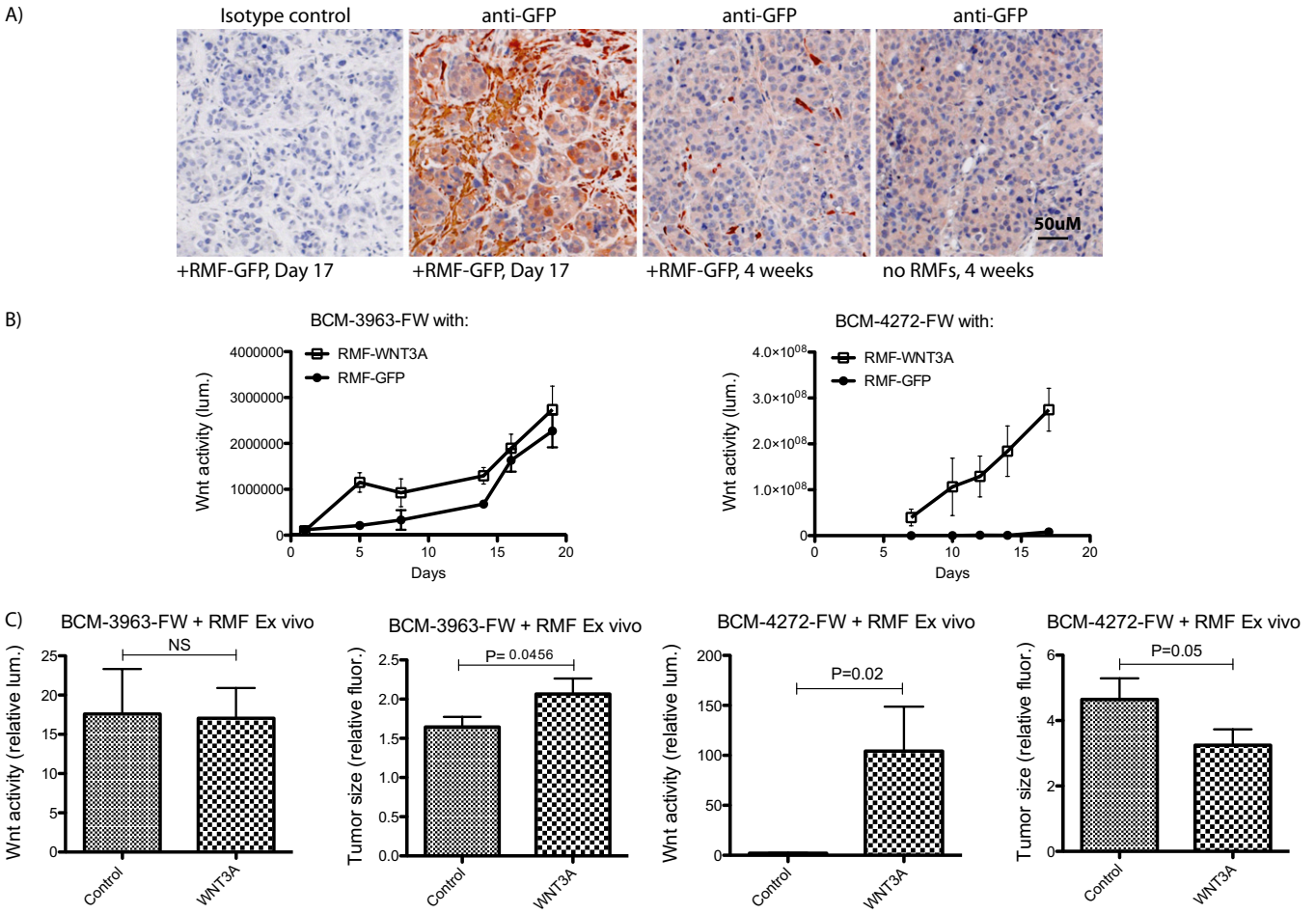

Fig. S3. Repeats of PDX recombination assays. (*A*) BCM-4272-FW cells were injected alone or with RMF-GFP. Tumors were harvested at the indicated time-points, fixed, and stained with anti-GFP to detect RMFs. (*B*) Repeat of recombination experiment as in Fig. 5 using BCM-3963-FW and BCM-4272-FW, $n = 10$ mice in each group. Bioluminescence was measured weekly during tumor formation. (*C*) Tumors from *B* were harvested at day 19 (BCM-3963-FW) or day 21 (BCM-4272-FW), and luminescence and fluorescence were measured ex vivo.

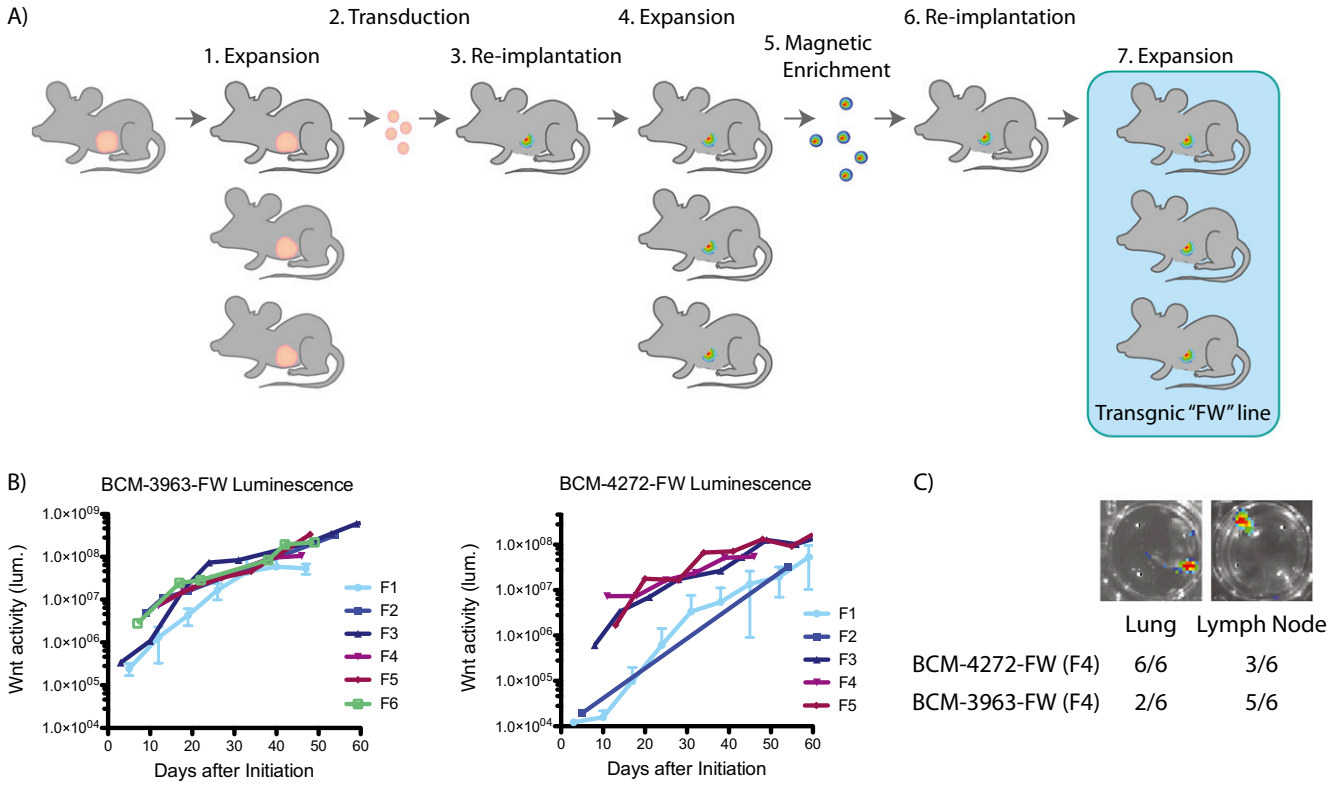

Fig. S4. Wnt signaling in patient-derived xenograft (PDX) lines. (*A*) Flowchart depicting generation of transgenic PDX lines. Tumors were collected from stable transplantable lines, dissociated, and infected with high-titer fast recovery and discrimination (FRED) and WILMA lentiviruses. After infection, cells were expanded in new hosts. The resulting tumors, composed of a mixture of transduced and nontransduced cells, were dissociated, and transduced cells were recovered with magnetic anti-CD4 microbeads. These cells then underwent another expansion cycle resulting in a polyclonal enriched transgenic "FW" line. (*B*) Bioluminescence of transgenic lines was measured weekly for multiple generations. In BCM-4272-FW, the elevated signal observed in the later generations coincides with magnetic enrichment of transduced cells. (*C*) Bioluminescence of lungs and lymph nodes was measured ex vivo after collection of tumors from three mice of each transgenic PDX line in the F4 generation.