Supplemental Materials

184AA3: a xenograft model of ER+ breast adenocarcinoma

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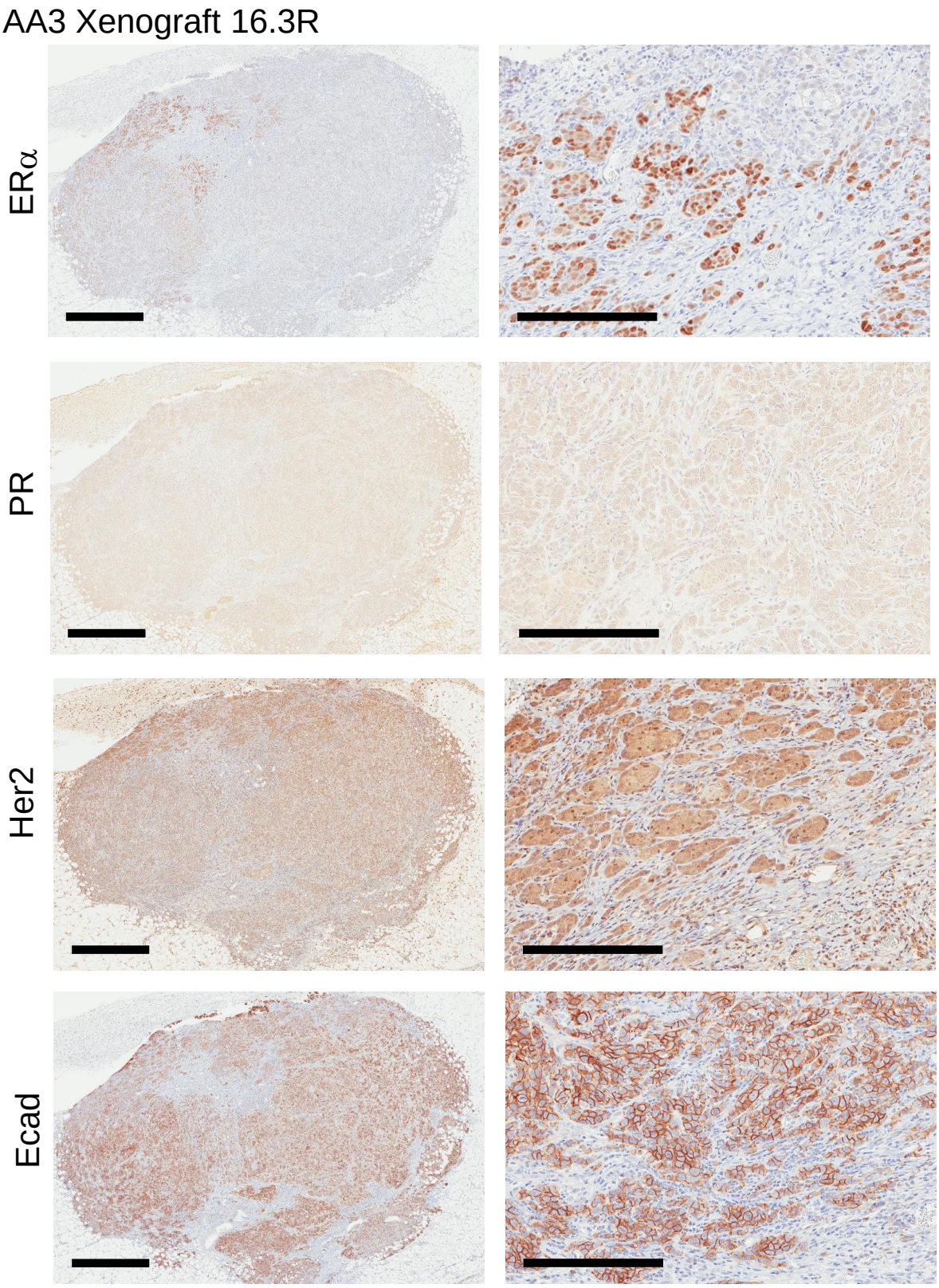
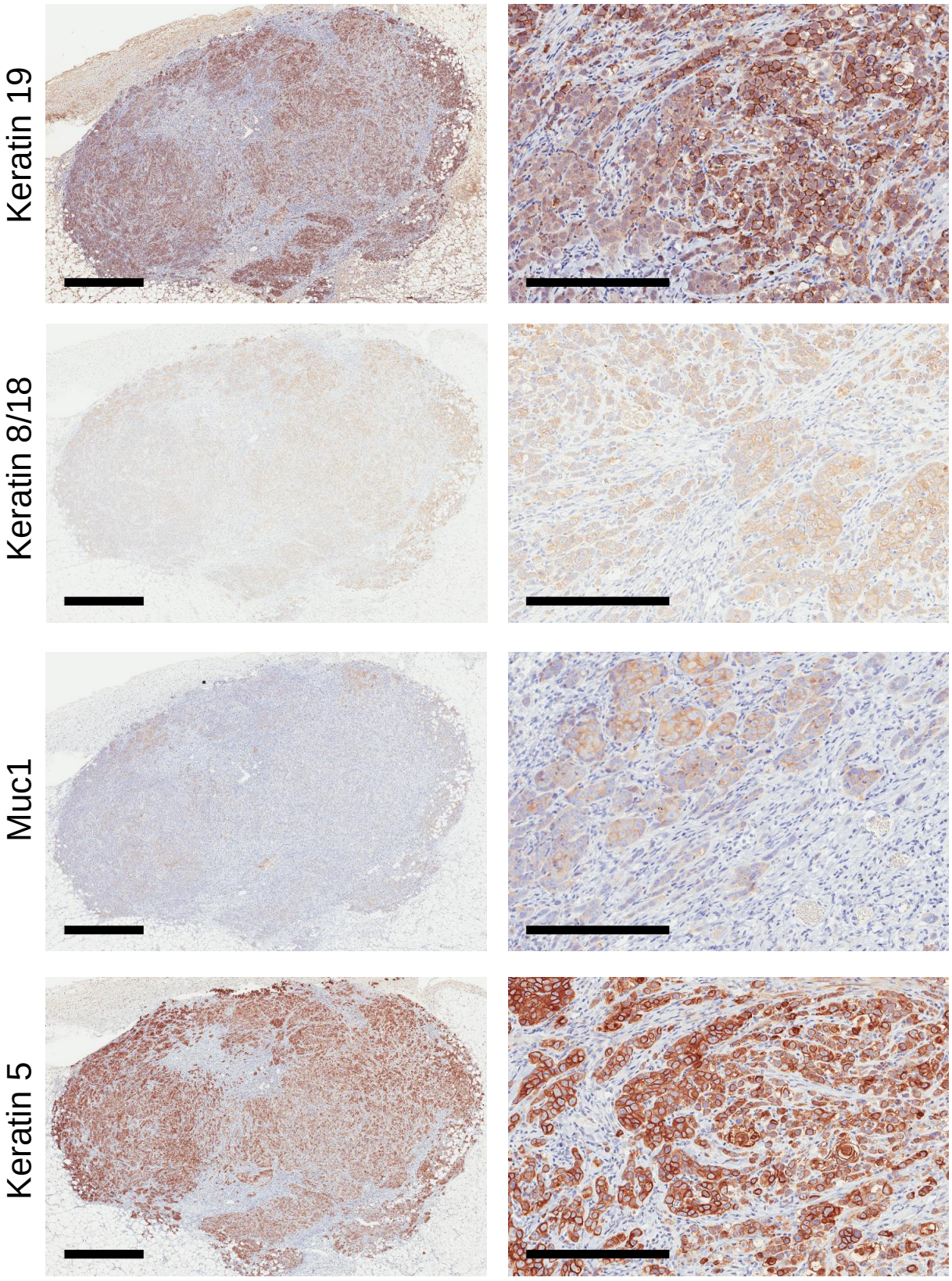
Figure S1a



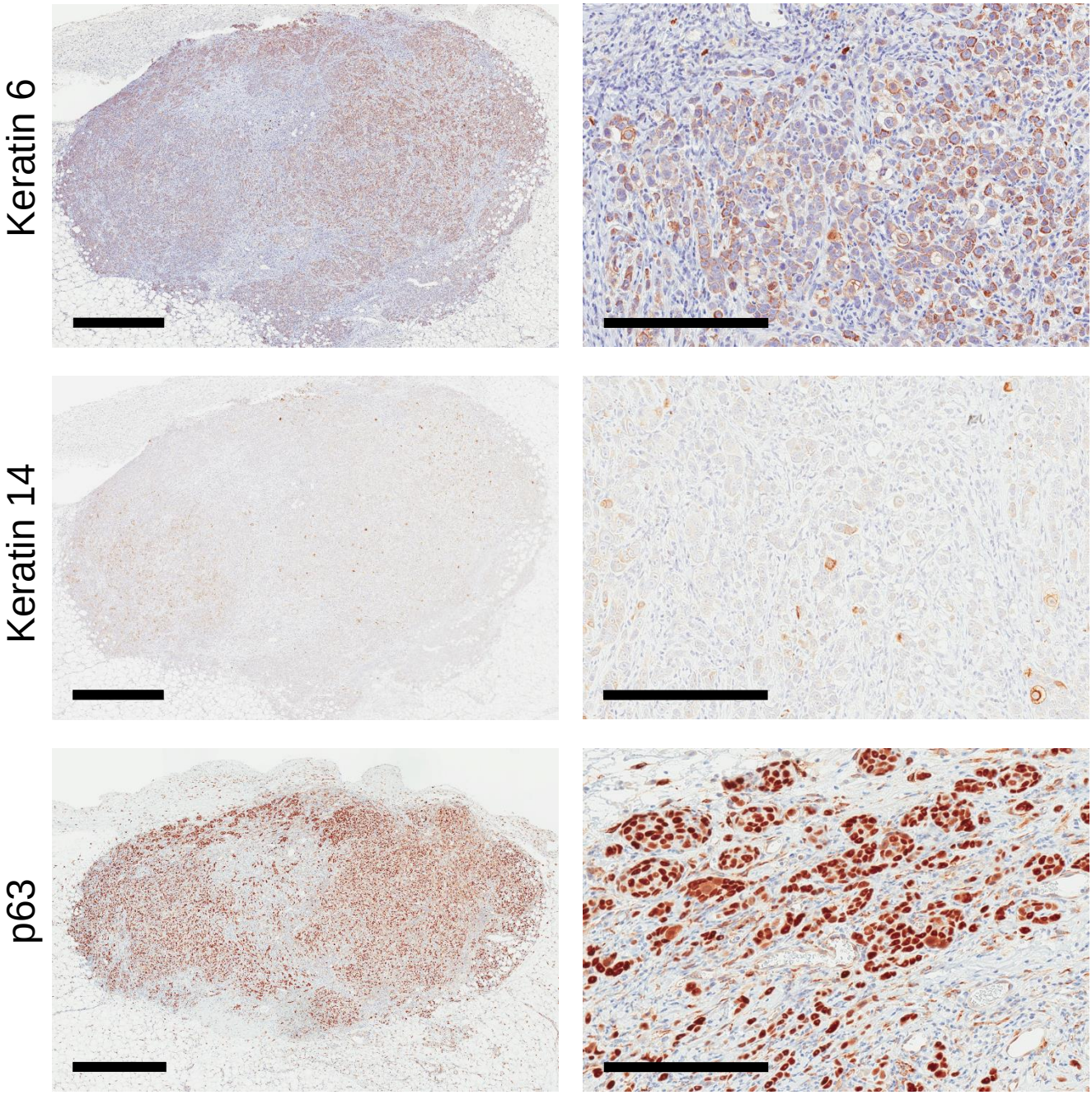
Supplemental Figure 1a. Characterization of 184AA3 xenografts. 184AA3-derived xenograft tumor immunostained with antibodies specific for Estrogen Receptor (ER), Progesterone receptor (PR), Her2, and E-cadherin (Ecad). Scale bars = 800μm (low magnification image, left) and 200mm (high magnification image, right).

Figure S1b

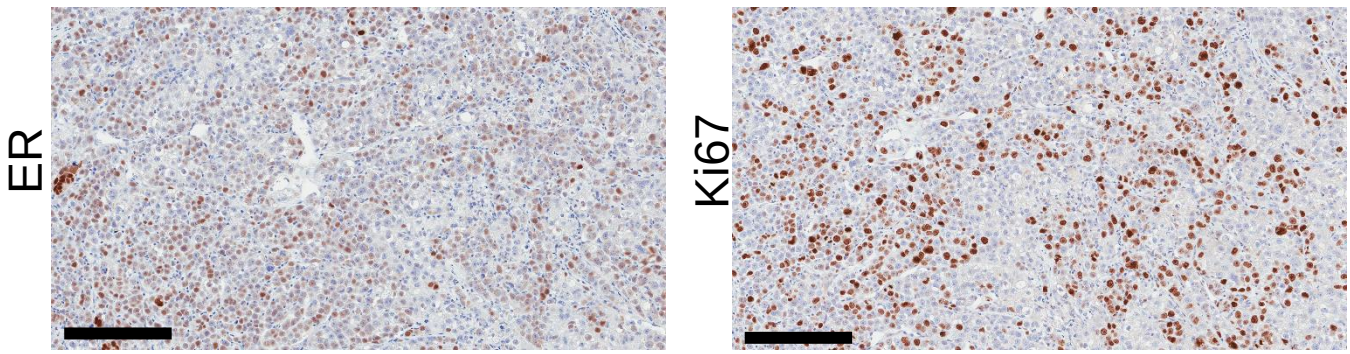


Supplemental Figure 1b. Characterization of 184AA3 xenografts. 184AA3-derived xenograft tumor immunostained with antibodies specific for Keratin 19, Keratin 8/18, Muc1, and Keratin 5. Scale bars = 800µm (low magnification image, left) and 200µm (high magnification image, right).

Figure S1c

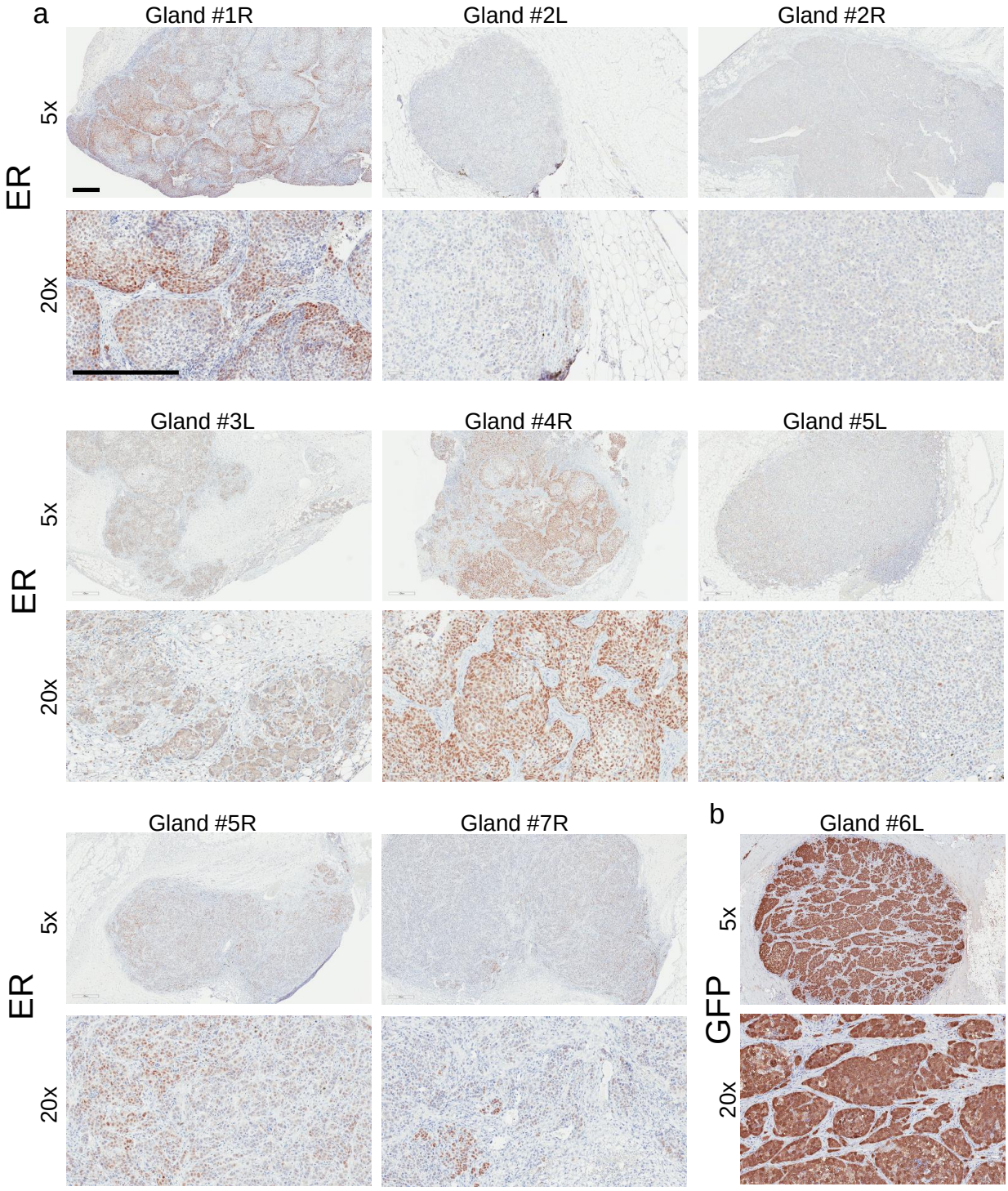


S1d



Supplemental Figure 1c. Characterization of 184AA3 xenografts. 184AA3-derived xenograft tumor Immunostained with antibodies specific for Keratin 6, Keratin 14, p63.
d)184AA3 xenograft #25.12R, immunostained for ER and Ki-67. Scale bars = 800µm (low magnification image, left) and 200µm (high magnification image, right).

Figure S2

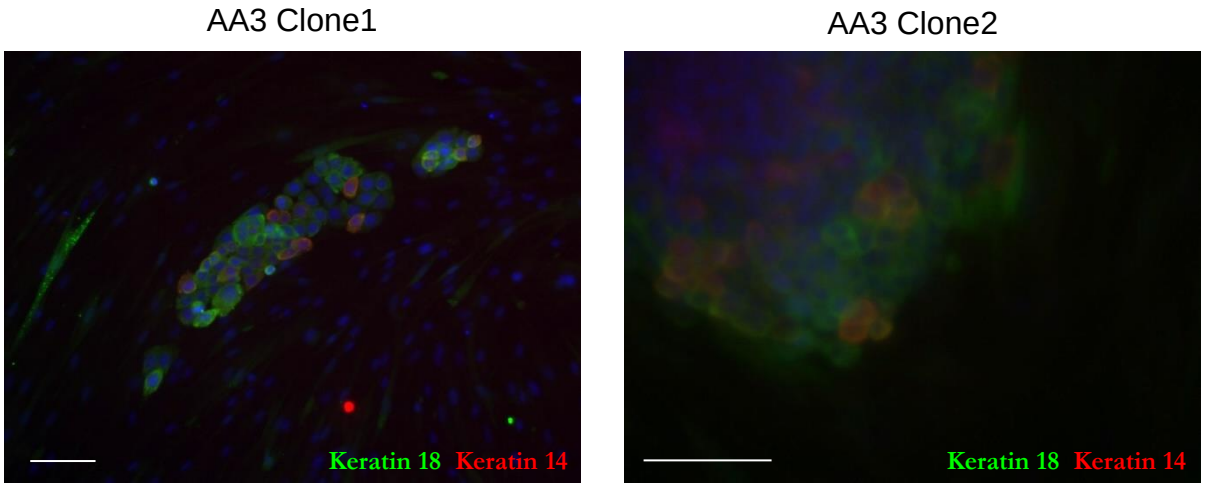


Supplemental Figure 2. ER expression in 184AA3 xenografts.

(a) ER immunostained sections of the 8 tumors formed in Experimental group HM20. H&E staining and growth curves of these tumors are provided in Figure 2.

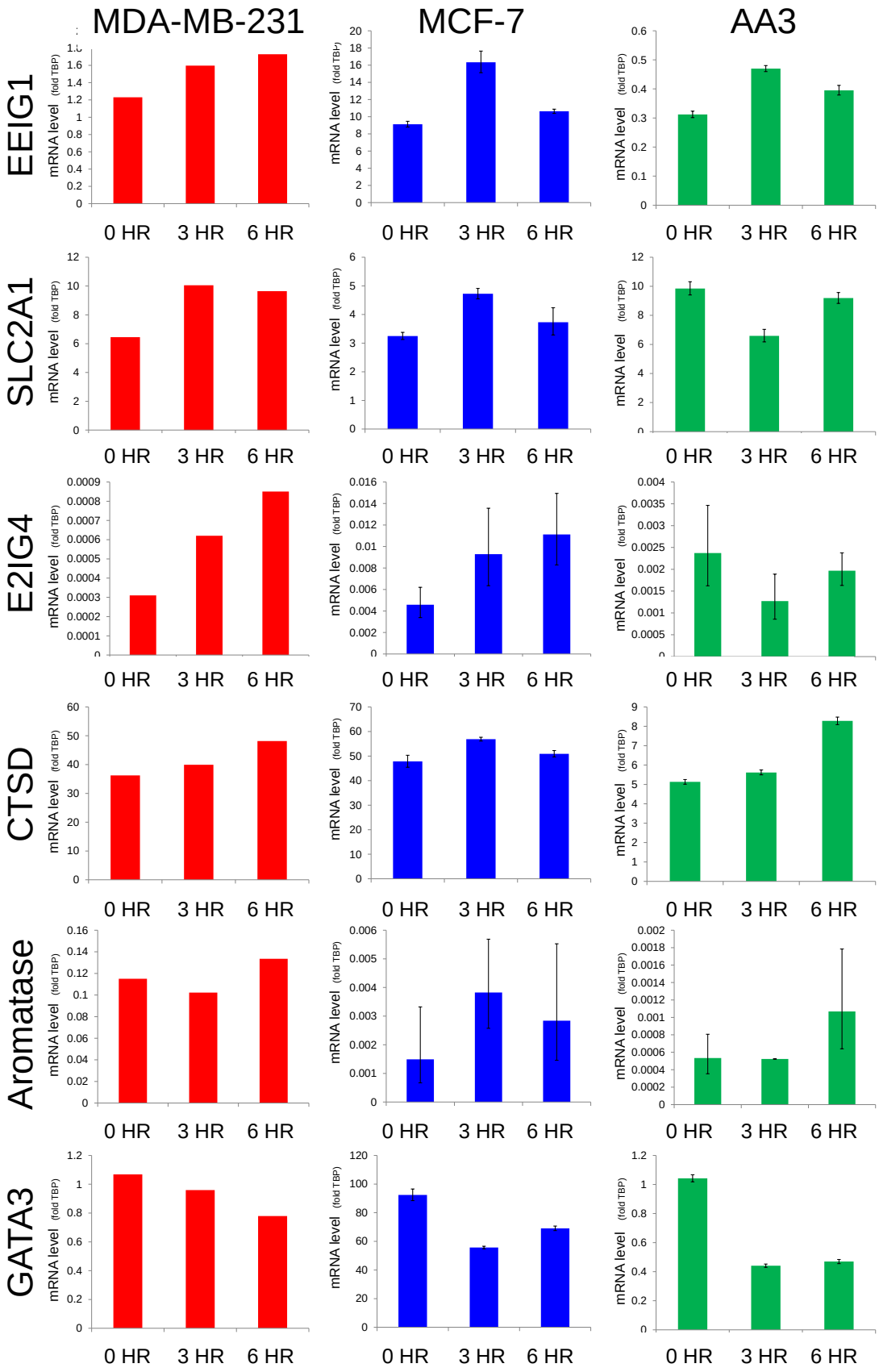
(b) Xenograft HM29.6, formed by 184AA3-H2b-GFP labeled cells. GFP was detected in paraffin sections by immunohistochemistry. Scale bars = 400µm (5x zoom) and 100µm (20x zoom).

Figure S3



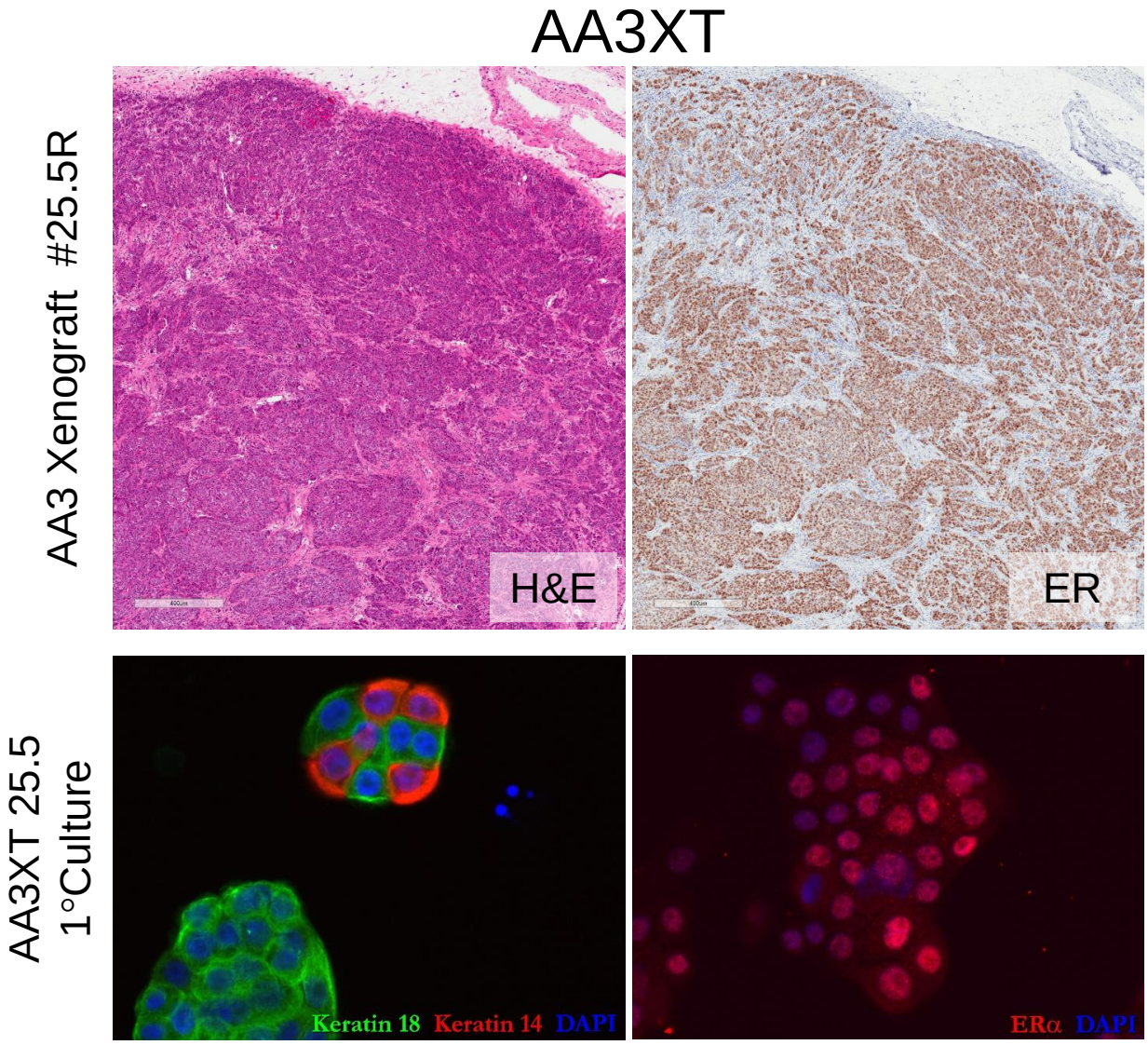
Supplemental Figure 3. 184AA3 clonal colonies. Keratin 18 (green) and Keratin 14 (red) immunostained colonies derived from single cells. Single 184AA3 cells were seeded directly into wells of a 96 well dish, each containing a subconfluent monolayer of ZsGreen-labeled primary breast fibroblasts. Cells were immunostained after 2 weeks in culture. Scale bars = 100µm.

Figure S4



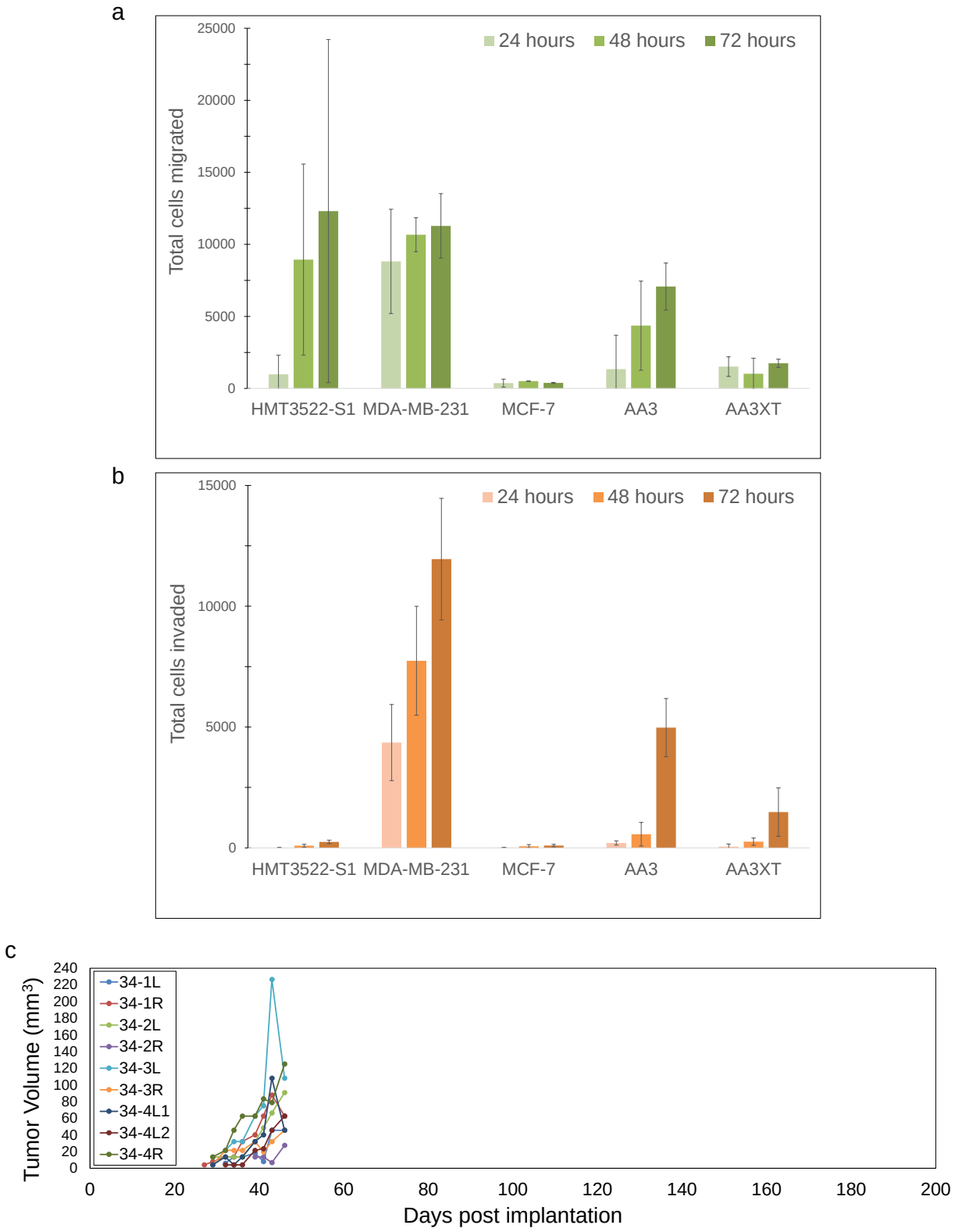
Supplemental Figure 4. ER functionality in 184AA3. Gene expression changes resulting from 3-hour and 6-hour estradiol stimulation of 184AA3 and reference cell lines MDA-MB-231 and MCF-7. Three of the nine genes measured by qRT-PCR are shown in Figure 5c. Shown here are: EEIG1, SLC2A1, E2IG4, CTSD, Aromatase, and GATA3. Transcripts were measured by qRT-PCR and are expressed as fold differences relative to TBP.

Figure S5



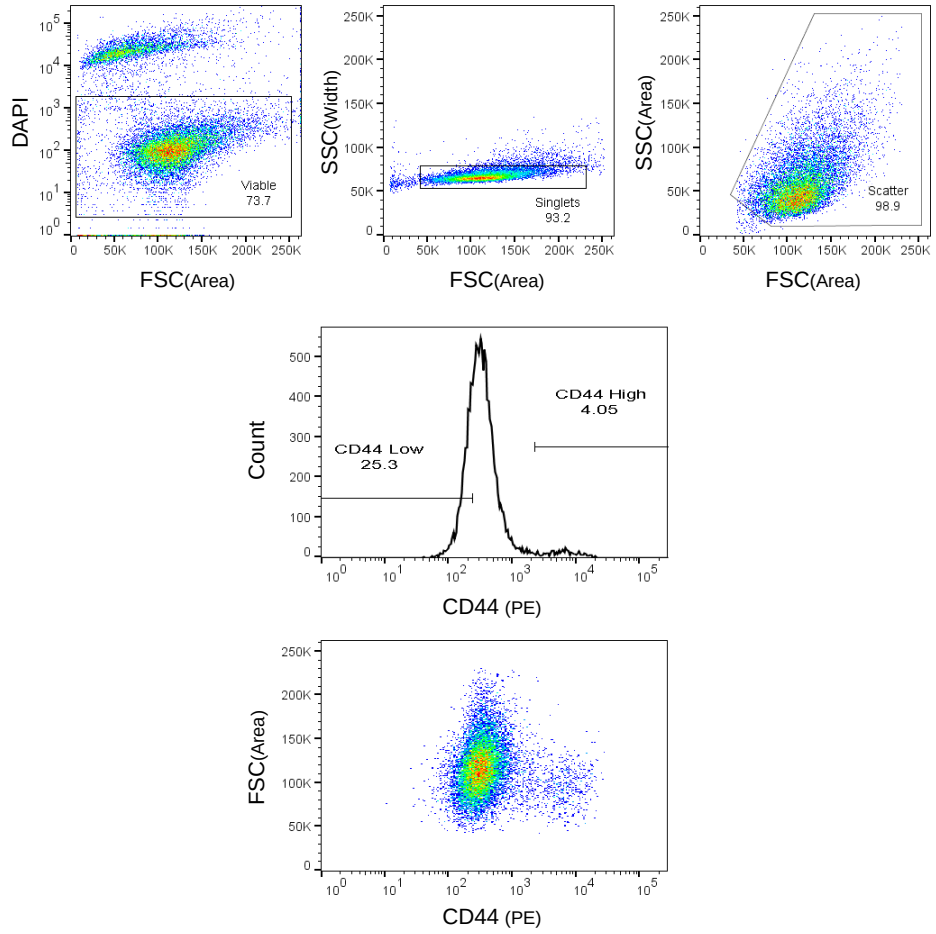
Supplemental Figure 5. 184AA3XT derivation and characterization. The 184AA3XT strain was derived from a primary culture of an 184AA3-derived tumor, HM25.5R. The tumor was minced and initially embedded into 3D matrigel. 184AA3XT cells were subsequently harvested from the matrigel culture and seeded onto a dish coated with a thin layer of Collagen I (Purecol, Advanced Biomatrix). Cultures were immunostained with keratin 14, keratin 18, and ER.

Figure S6



Supplemental Figure 6. 184AA3XT migration, invasion and in vivo growth. **(a)** Migration through Boyden chamber (top) or invasion through matrigel coated chamber (bottom). The functional ability of 184AA3XT and 184AA3 cells to migrate and invade through matrigel was measured at 24-, 48, and 72-hours after cells were seeded into the top of a Boyden chamber with 8µm pores. **(b)** 184AA3XT were grafted into NSG mice as described in methods. Appearance and growth of the tumors was vastly accelerated compared to the parent line (Compare growth to that shown in Figure 2a)

Figure S7



Supplemental Figure 7. CD44 FACS sorting gates. Full backgating illustrating how CD44High and CD44Low 184AA3 cells were FACS sorted.

Supplementary Table 1. Antibodies

Antibody specificity	Clone	Conjugate	Source	Dilution	Figure
Muc1 (CD227)	HMPV	FITC and unlabeled	BD Biosciences 559774	1:100	Fig.1b,3b,3c
Thy1 (CD90)	5E10	PE/Cy5	BioLegend 328112	1:40	Fig.3b,3c
E-Cadherin	67A4	Alexafluor488	BioLegend 324110	1:40	Fig.1b,3b,3c
CALLA (CD10)	HI10a	Brilliant Violet 421	BioLegend 312217	1:20	Fig.3b
CD24	ML5	PE	BioLegend 311106	1:40	Fig.3b,3c
CD44	515	PE	BD Biosciences 550989	1:40	Fig.3b,3c,4a,S3b
EpCAM	9C4	FITC	BioLegend 324203	1:40	Fig.3b,3c
α6 Integrin (CD49f)	GoH3	Brilliant Violet 421	BioLegend 313624	1:40	Fig.3b,3c
Keratin 14	Rabbit polyclonal	None	Thermo RB-9020	1:400	Fig.1b,3a,3c,S3a,S6
Keratin 19	A53-B/A2.26	None	Thermo MS-198	1:400	Fig.3c
Keratin 18	DC10	None	Thermo MS142P0	1:400	Fig.1b,3a,3c,S3a,S6
P63	4A4	None	Abcam ab32353	1:100	Fig.3c
Estrogen Receptor (ER)	SP1	None	Thermo RM-9101	1:50	Fig.1b,3c,5b
Estrogen Receptor (ER)	HC20	None	Santa Cruz SC543	1:100	FigS5
Progesterone Receptor (PR)	PgR636	None	Dako M356929-2	1:100	Fig.3c
Smooth muscle actin	1A4	None	Thermo MS-113	1:100	Fig.1b
Vimentin	Polyclonal	None	NeoMarkers RB-9063	1:200	Fig.1b
CD56	HCD56	FITC	BioLegend 318303	1:40	Fig.3c
Ki67	Ki-67	None	BioLegend Rb-1510	1:100	Fig. S1d
αtubulin	DM1A	None	Santa Cruz Biotechnology	1:200	Fig. 5b
Center for Comparative Medicine, UC Davis					
Antibody specificity	**Clone**	**Conjugate**	**Source**	**Dilution**	**Figure**
Her2	EP1045Y	None	ThermoScientific RM-2112	1:1000	Fig.S1a
Ecad	Rabbit polyclonal	None	Abcam, ab53033*	1:1200	Fig.1b,S1a
K19	EPNCIR127B	None	Epitomics, 3863	1:1200	Fig.S1a
K8/18	Guinea pig polyclonal	None	Fitzgerald, 20R-CP004	1:2000	Fig.1b,S1a
Muc1	HMPV	None	Pharmingen, 550486	1:400	Fig.1b,S1a
K5	EP1601Y	None	Abcam, ab52635	1:1000	Fig.S1a
K6	Rabbit polyclonal	None	Abcam, ab24646*	1:2000	Fig.S1a
K14	Rabbit polyclonal	None	Neomarker, Rb-9020	1:1000	Fig.S1a
P63	EPR5701	None	Epitomics, 5353	1:2000	Fig.S1a
ER	SP1	None	ThermoScientific, RM-9101	1:100	Fig.1b,2b,6d,S1a,S1d,S2a
ER	HC-20	None	Santa Cruze Biotechnology	1:100	Fig. 6b
PR	Rabbit polyclonal	None	Dako, A0098	1:500	Fig.S1a
Ki-67	Ab4	None	ThermoScientific, RB-1510	1:800	Fig.6d
GFP	Goat Polyclonal	None	Abcam, ab6673	1:800	Fig.S2b

*No longer available

Supplementary Table 2. qRT-PCR Primers

Gene	Forward Primer	Reverse Primer
TBP	5’ CACGAACCACGGCACTGATT	5’ TTTTCTTGCTGCCAGTCTGGAC
ER (ESR1)	5’ TGATTGGTCTCGTCTGGCG	5’ CATGCCCTCTACACATTTTCCC
PDZK1	5’ CAGACTTCACTGTTGGTGGTAG	5’ CATTGGGCAGTTCTTGACTTTG
B4GALT1	5’ CGCCACTCAAGAGACAAGAA	5’ GAGTGAGTTCAAACCATCAGAGA
EEIG1 (FAM102A)	5’ GAGGTTCACCTTCGTGTGTAAG	5’ CGAAGCCCAGCTTGGAATAA
SLC2A1	5’ CAGATGATGCGGGAGAAGAA	5’ CGAAGATGCTCGTGGAGTAATA
E2IG4 (TSKU)	5’ GACCCAATGCACTTTCTTG	5’ GCTGCTACTCCTGTGCTAAT
CTSD	5’ AGTGCTTCACAGTCGTCTTC	5’ GGACTTGTCGCTGTTGTACT
Aromatase (CYP19A1)	5’ GTCTGGATCTCTGGAGAGGAAA	5’ GCCGAATCGAGAGCTGTAATG
GATA3	5’ ACACTCTGGAGGAGGAATGCCA	5’ TTCGGTTTCTGGTCTGGATGCCTT