**Supplemental Figure Legend:**

**Figure S1. Kaplan–Meier plot of breast cancer patient survival when stratified according to LGR5–8 mRNA expression.**

A. LGR5: OS, overall Survival ($n_{high}$ = 557, $n_{low}$ = 560); DMFS, Distant Metastasis Free Survival ($n_{high}$ = 793, $n_{low}$ = 816); RFS, Relapse Free Survival ($n_{high}$ = 1741, $n_{low}$ = 1813); PPS, post progression survival ($n_{high}$ = 172, $n_{low}$ = 179).

B. LGR6: OS ($n_{high}$ = 260, $n_{low}$ = 262); DMFS ($n_{high}$ = 331, $n_{low}$ = 333); RFS ($n_{high}$ = 830, $n_{low}$ = 830), PPS ($n_{high}$ = 70, $n_{low}$ = 70).

C. LGR7: OS ($n_{high}$ = 260, $n_{low}$ = 262); DMFS ($n_{high}$ = 331, $n_{low}$ = 333); RFS ($n_{high}$ = 806, $n_{low}$ = 854); PPS($n_{high}$ = 70, $n_{low}$ = 70).

D. LGR8: OS ($n_{high}$ = 255, $n_{low}$ = 267); DMFS ($n_{high}$ = 324, $n_{low}$ = 340); RFS($n_{high}$ = 815, $n_{low}$ = 845); PPS($n_{high}$ = 69, $n_{low}$ = 71). OS: Overall Survival; RFS: Relapse Free Survival; DMFS: Distant Metastasis Free Survival, and PPS: Post-Progression Survival.

**Figure S2. LGR4 expression was correlated with the invasive ability of different cell lines.**

A. Heatmap of LGR family mRNA expression in 45 different immortalized normal breast or breast cancer cell lines. Data (GEO Accession Number GSE10890) values in log2 of expression signal were sorted and analyzed. Scale bar: red indicates high expression and green represents low mRNA expression.

B-C. Invasive abilities of 6 breast cancer cell lines identified by Boyden chamber invasion assay in vitro. The invasive cells were stained followed by photography (B), and quantitation (C). As shown in the blue by red arrow, invasive potential weak and strong were divided by the average of different invasive cell number. Experiment was independently repeated three times and graphs show the means ± S.D. Scale bars, 100 μm.

D. *LGR4* expression in 6 human breast cancer cell lines of differing invasiveness, as determined by qPCR. Experiment was repeated three times.

**Figure S3. *Lgr4* heterozygosity delays mammary tumor onset and progression in MMTV-*PyMT* transgenic mice.**

A. *Lrg4* mRNA levels in mammary tumors from PyMT–*Lgr4*$^{+/+}$, –*Lgr4*$^{+/–}$ and –*Lgr4*$^{–/–}$ mice at 12 weeks, as identified by qPCR.

B-C. *Lrg4* heterozygosity delayed tumor formation in MMTV-*PyMT* transgenic mice. Representative images of H & E stained sections from mammary glands of indicated mice (C). Classification of mammary gland lesions in 9-, 13- and 15-week-old MMTV-*PyMT* mice with indicated *Lgr4* genotype (B). Scale bars, 500 μm. IDC: Invasive ductal carcinoma; DCIS: Ductal carcinoma in situ; ADH: Atypical ductal hyperplasia; PL: Proliferative lesion without atypia; N: Normal.

D. PCNA staining of tumors from 9-, 13- and 15-week-old MMTV-*PyMT* mice of indicated *Lgr4* genotype. Scale bars, 200 μm.

**Figure S4. *Lgr4* regulates the breast tumor onset and metastasis to the lungs in MMTV-*PyMT* mice and transfection efficiency testing in different BrCa cell migration and invasion assay.**

A. Whole mount of indicated mice at 3.5 weeks of age.

B. Kaplan–Meier plot showing palpable tumor-free mice until 120 days of age. PyMT–*Lgr4*$^{+/+}$ ($n$ = 11) and PyMT–*Lgr4*$^{+/–}$ ($n$ = 13). Log-rank test was used to determine $P$ values.

C. *Lgr4* heterozygosity reduced the number of tumors per mouse in MMTV-*PyMT* mice. Error bars are mean ± S.D. *$P$ < 0.05. $n$ = 7 per group.

D. The volume of primary tumors until 12 weeks between PyMT–*Lgr4*$^{+/+}$ and PyMT–*Lgr4*$^{+/–}$ mice. Error bars are mean ± S.D. **$P$ < 0.01; ***$P$ < 0.001. $n$ = 7 per group.

E. H & E staining of lung sections from 15-week-old mice of indicated genotype. Scale bars, 500 μm.

F. Graph shows quantitation of metastatic foci in lungs of MMTV-*PyMT Lgr4*$^{+/+}$ and MMTV-*PyMT Lgr4*$^{+/–}$ mice. $n$ = 6 mice per group. Error bars are mean ± S.D. ***$P$ < 0.001.

G-J. *LGR4* knockdown or overexpression efficiency related to Fig. 4A-4D, respectively,

tested by qPCR analysis. Experiment was independently repeated three times and representative graphs show the means ± S.D. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

**Figure S5. LGR4 did not regulate cAMP-CREB signaling in breast cancer**

A. The adenosine 3',5'-cyclic monophosphate (cAMP) level was examined by ELISA in PyMT–*Lgr4* CKO and PyMT–*Lgr4*$^{fl/fl}$ tumors cells (n=3 mice for each group, experiments conducted in triplicate).

B. Active phosphoSer133-CREB in PyMT–*Lgr4* CKO mice tumors was examined using a specific anti-phospho-CREB antibody by Western blot analysis.

C. Treated with 50 μM forskolin activated cAMP in MDA-MB-231 knockdown LGR4 cells. p-FAK was examined by Western blot analysis. Experiment was independently repeated three times.

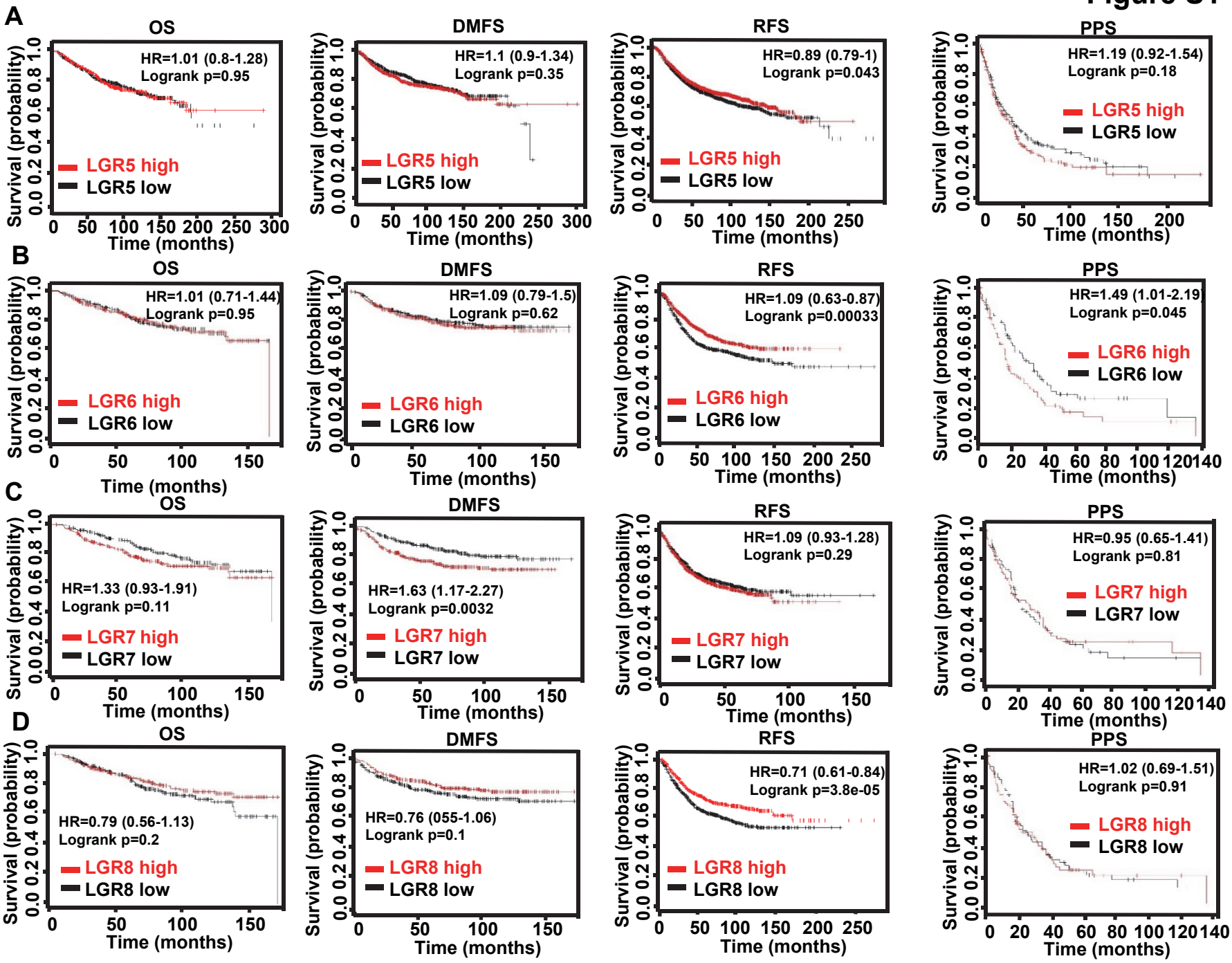
**Supplemental Table 1: Primer sequences used in this study.**

| Target Gene for qRT-PCR | Forward primer 5’ -> 3’ | Reverse primer 5’ -> 3’ |
|---|---|---|
| Human *LGR4* | 5’ACCTGGAGACCTTAGACTTG3’ | 5’CCACGAATGACTAGGGAATG3’ |
| Human *Oct4* | 5’CTGCAGAAGGAGCTAGAACAGT3’ | 5’CTCGAAGCGACAGATGGTG3’ |
| Human *Sox2* | 5’TACAGCATGTCCTACTCGCAG3’ | 5’GAGGAAGAGGTAACCACAGGG3’ |
| Human *Axin2* | 5’TAGGTTCTGGCTATGTCTTTG3’ | 5’GTATCGTCTGCGGGTCTT3’ |
| Human *Snail1* | 5’ATCGGAAGCCTAACTACAGC3’ | 5’TTTCCCACTGTCCTCATCTG3’ |
| Human *Slug* | 5’CGAACTGGACACACATACAGTG3’ | 5’CTGAGGATCTCTGGTTGTGGT3’ |
| Human *Twist* | 5’ GCAAGATTCAGACCCTCAAG3’ | 5’AGTTATCCAGCTCCAGAGTC3’ |
| Human *N-cadherin* | 5′GTGCCATTAGCCAAGGGAATTCAGC3′ | 5′TGGCACGTCTTGACCTTGAA3′ |
| Human *E-cadherin* | 5′TCCATTTCTTGGTCTACGCC3′ | 5′CACCTTCAGCCAACCTGTTT3′ |
| Mouse *Lgr4* | 5’AAGATAACAGCCCCCAAGAC3’ | 5’AGGCAGTGATGAACAAGACG3’ |

**Supplemental Table 2: Antibody information in this study.**

| Antibody Target | Supplier | Catalog Number | Application | Dilution |
|---|---|---|---|---|
| **Cofilin** | Cell Signaling Technologies (CST) | 3312 | immunoblot | 1:1000 |
| **phosphoCofilin** | CST | 3313 | immunoblot | 1:1000 |
| **Cyclin D1** | Abcam | ab134175 | immunoblot | 1:1000 |
| **E-cadherin** | CST | 3195 | immunoblot | 1:1000 |
| **Vimentin** | CST | 5741 | immunoblot | 1:1000 |
| **ZEB1** | CST | 3399 | immunoblot | 1:1000 |
| **phosphoERK** | CST | 4377 | immunoblot | 1:1000 |
| **ERK** | CST | 9102 | immunoblot | 1:1000 |
| **phosphoFAK** | CST | 3283 | immunoblot | 1:1000 |
| **FAK** | Santa Cruz | sc-557 | immunoblot | 1:200 |
| **P-CREB** | CST | 9198 | immunoblot | 1:1000 |
| **CREB** | CST | 9197 | immunoblot | 1:1000 |
| **GAPDH** | CST | 5174 | immunoblot | 1:1000 |
| **β-actin** | Sigma | A5441 | immunoblot | 1:1000 |
| **phosphoSrc** | CST | 2101 | immunoblot | 1:1000 |
| **Src** | CST | 2108 | immunoblot | 1:1000 |
| **IRDye® 800CW Goat anti-Mouse IgG** | LI-COR | 925-32210 | immunoblot | 1:10000 |
| **IRDye® 800CW Goat anti-Rabbit IgG** | LI-COR | 925-32211 | immunoblot | 1:10000 |
| **Rhodamine Phalloidin** | Thermo-Molecular Probes™ | R415 | immunofluorescence | 1:20 |
| **Vinculin** | Santa curz | sc-55465 | immunofluorescence | 1:200 |
| **PCNA** | Santa curz | sc-7907 | immunohistochemical | 1:200 |
| **β-catenin** | CST | 9562 | immunohistochemical | 1:200 |
| **Cyclin D1** | Abcam | ab134175 | immunohistochemical | 1:200 |
| **Human LGR4 antibody** | Dr. Qingyun (Jim) Liu | | immunohistochemical | 1:200 |
| **Goat anti-Rat IgG Secondary Antibody** | Thermo Fisher | 62-9520 | immunohistochemical | 1:1000 |

# Figure S1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LGR5 low
DMFS
HR=1.1 (0.9-1.34)
Logrank p=0.35
LGR5 high
LGR5 low
RFS
HR=0.89 (0.79-1)
Logrank p=0.043
LGR5 high
LGR5 low
PPS
HR=1.19 (0.92-1.54)
Logrank p=0.18
LGR5 high
LGR5 low
B
OS
HR=1.01 (0.71-1.44)
Logrank p=0.95
LGR6 high
LGR6 low
DMFS
HR=1.09 (0.79-1.5)
Logrank p=0.62
LGR6 high
LGR6 low
RFS
HR=1.09 (0.63-0.87)
Logrank p=0.00033
LGR6 high
LGR6 low
PPS
HR=1.49 (1.01-2.19)
Logrank p=0.045
LGR6 high
LGR6 low
C
OS
HR=1.33 (0.93-1.91)
Logrank p=0.11
LGR7 high
LGR7 low
DMFS
HR=1.63 (1.17-2.27)
Logrank p=0.0032
LGR7 high
LGR7 low
RFS
HR=1.09 (0.93-1.28)
Logrank p=0.29
LGR7 high
LGR7 low
PPS
HR=0.95 (0.65-1.41)
Logrank p=0.81
LGR7 high
LGR7 low
D
OS
HR=0.79 (0.56-1.13)
Logrank p=0.2
LGR8 high
LGR8 low
DMFS
HR=0.76 (055-1.06)
Logrank p=0.1
LGR8 high
LGR8 low
RFS
HR=0.71 (0.61-0.84)
Logrank p=3.8e-05
LGR8 high
LGR8 low
PPS
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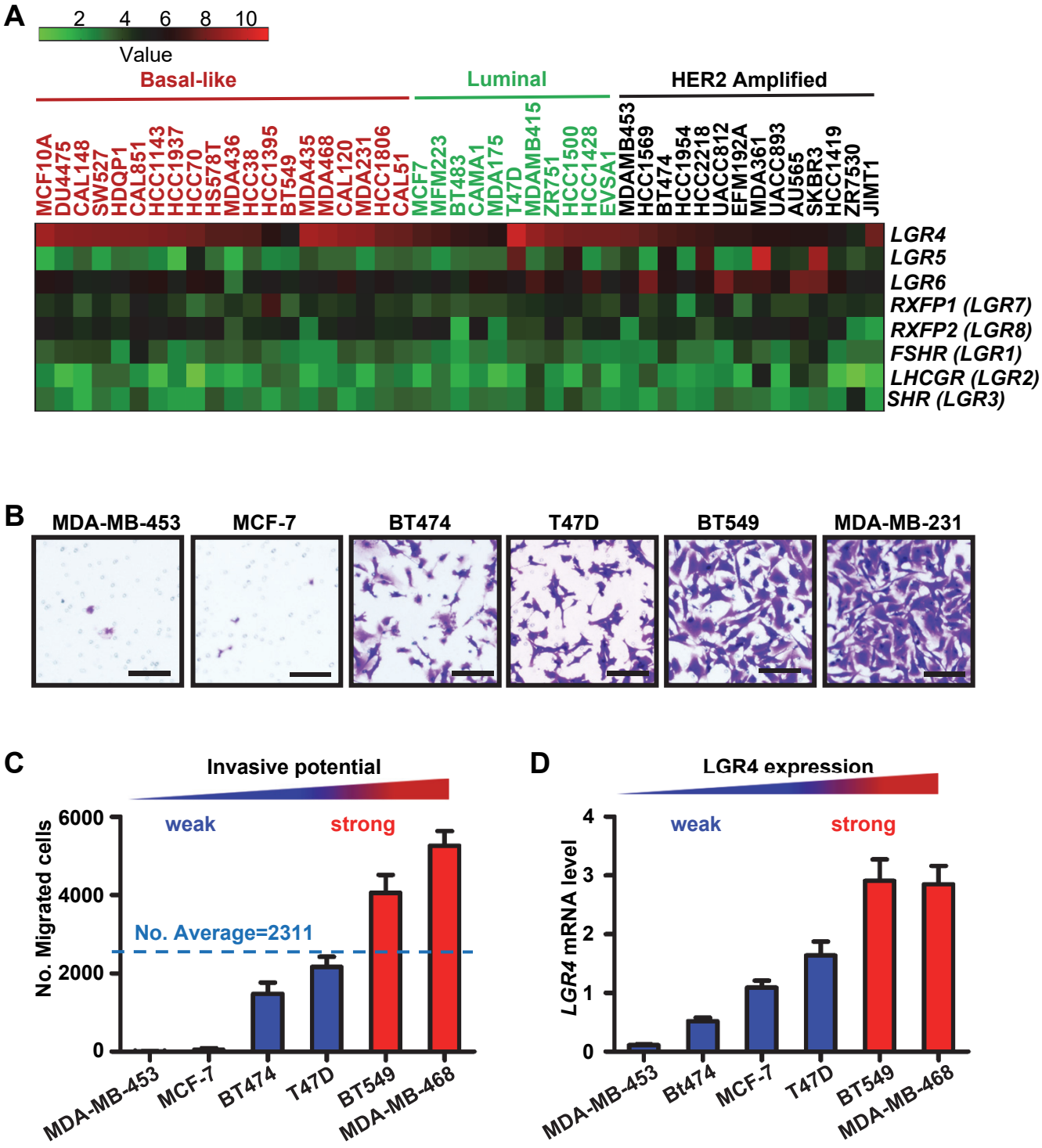
Logrank p=0.91
LGR8 high
LGR8 low
Survival (probability)
Time (months)

# Figure S2


A
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Value
Basal-like
Luminal
HER2 Amplified
MCF10A
DU4475
CAL148
SW527
HDQP1
CAL851
HCC1143
HCC1937
HCC70
HS578T
MDA436
HCC38
HCC1395
BT549
MDA435
MDA468
CAL120
MDA231
HCC1806
CAL51
MCF7
MFM223
BT483
CAMA1
MDA175
T47D
MDAMB415
ZR751
HCC1500
HCC1428
EVSA1
MDAMB453
HCC1569
BT474
HCC1954
HCC2218
UACC812
EFM192A
MDA361
UACC893
AU565
SKBR3
HCC1419
ZR7530
JIMT1
LGR4
LGR5
LGR6
RXFP1 (LGR7)
RXFP2 (LGR8)
FSHR (LGR1)
LHCGR (LGR2)
SHR (LGR3)
B
MDA-MB-453
MCF-7
BT474
T47D
BT549
MDA-MB-231
C
Invasive potential
weak
strong
No. Migrated cells
6000
4000
2000
0
No. Average=2311
MDA-MB-453
MCF-7
BT474
T47D
BT549
MDA-MB-468
D
LGR4 expression
weak
strong
LGR4 mRNA level
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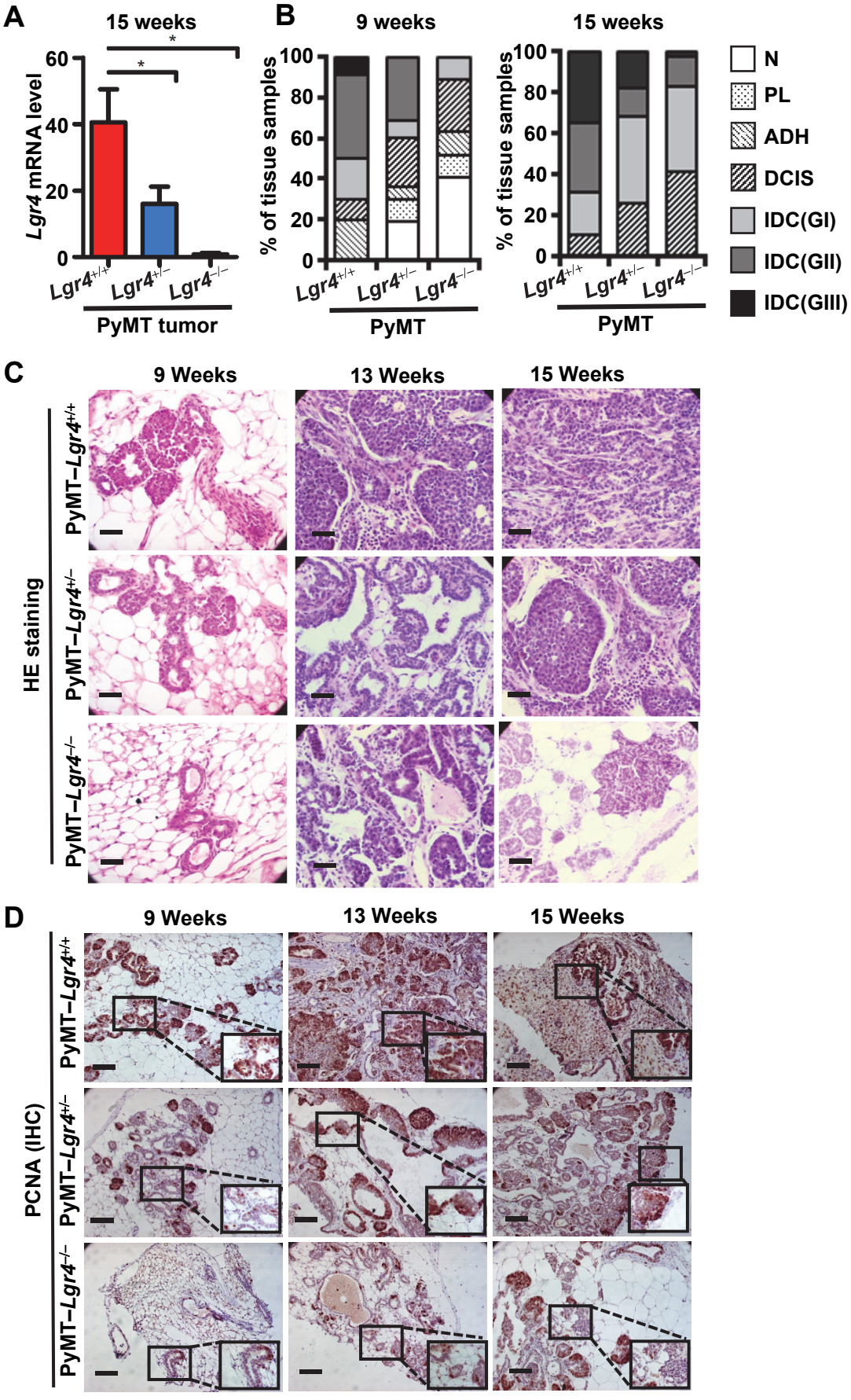
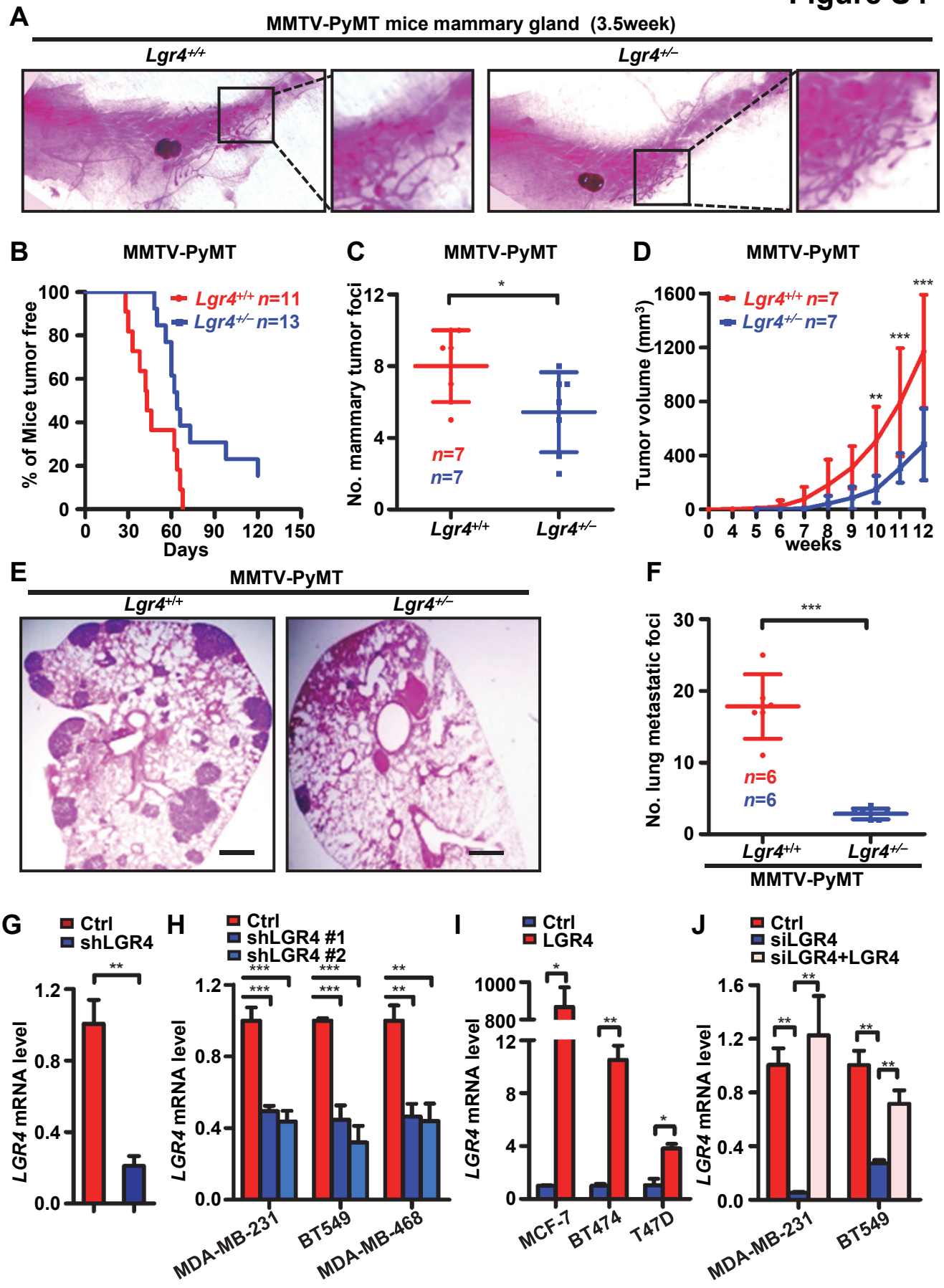
3
2
1
0
MDA-MB-453
Bt474
MCF-7
T47D
BT549
MDA-MB-468

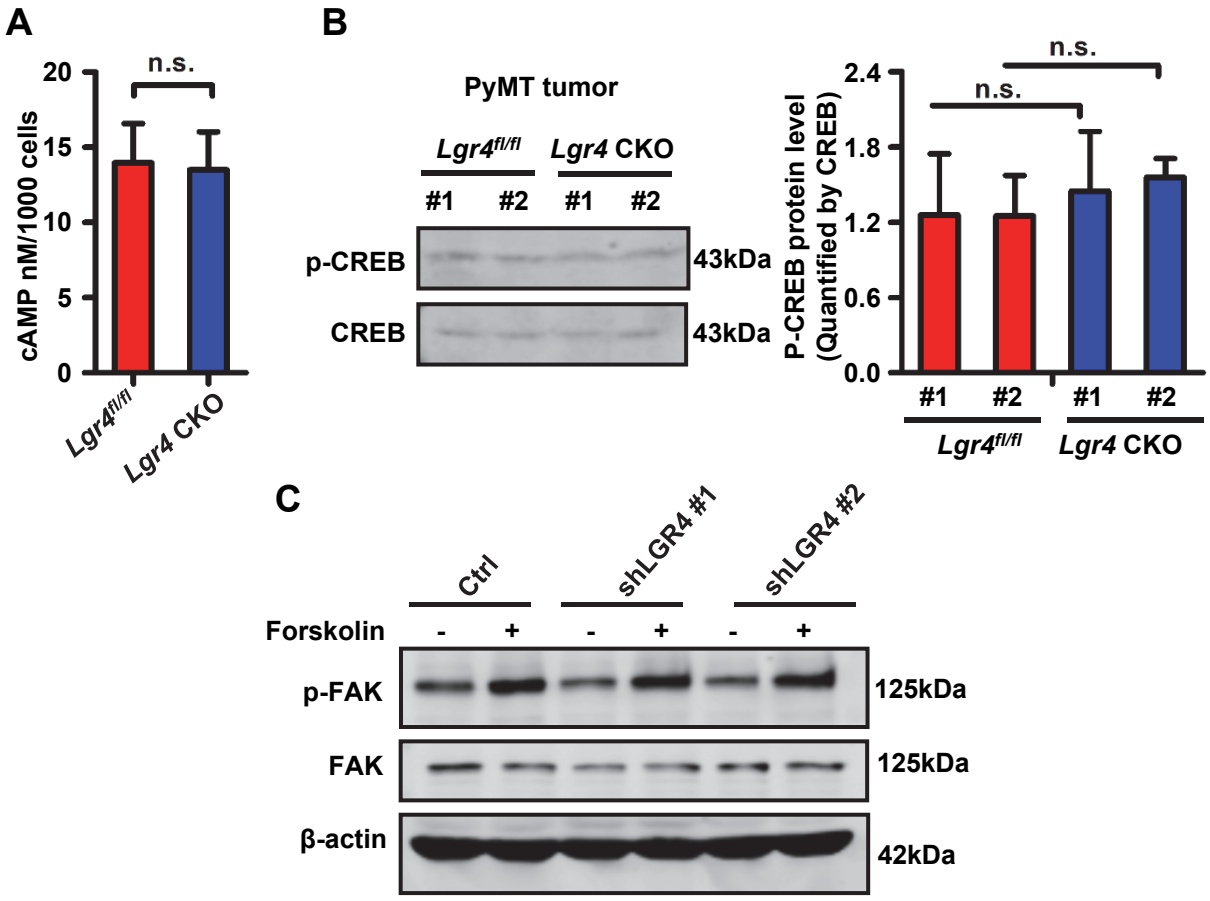
Figure S3


A
15 weeks
Lgr4 mRNA level
60
40
20
0
*
*
Lgr4+/+
Lgr4+/−
Lgr4−/−
PyMT tumor
B
9 weeks
% of tissue samples
100
80
60
40
20
0
Lgr4+/+
Lgr4+/−
Lgr4−/−
PyMT
15 weeks
% of tissue samples
100
80
60
40
20
0
Lgr4+/+
Lgr4+/−
Lgr4−/−
PyMT
N
PL
ADH
DCIS
IDC(GI)
IDC(GII)
IDC(GIII)
C
9 Weeks
13 Weeks
15 Weeks
HE staining
PyMT–Lgr4+/+
PyMT–Lgr4+/−
PyMT–Lgr4−/−
D
9 Weeks
13 Weeks
15 Weeks
PCNA (IHC)
PyMT–Lgr4+/+
PyMT–Lgr4+/−
PyMT–Lgr4−/−

# Figure S4


A
MMTV-PyMT mice mammary gland (3.5week)
Lgr4+/+
Lgr4+/–
B
MMTV-PyMT
% of Mice tumor free
Lgr4+/+ n=11
Lgr4+/– n=13
Days
C
MMTV-PyMT
No. mammary tumor foci
n=7
n=7
Lgr4+/+
Lgr4+/–
D
MMTV-PyMT
Tumor volume (mm3)
Lgr4+/+ n=7
Lgr4+/– n=7
weeks
E
MMTV-PyMT
Lgr4+/+
Lgr4+/–
F
No. lung metastatic foci
n=6
n=6
Lgr4+/+
Lgr4+/–
MMTV-PyMT
G
Ctrl
shLGR4
LGR4 mRNA level
H
Ctrl
shLGR4 #1
shLGR4 #2
LGR4 mRNA level
MDA-MB-231
BT549
MDA-MB-468
I
Ctrl
LGR4
LGR4 mRNA level
MCF-7
BT474
T47D
J
Ctrl
siLGR4
siLGR4+LGR4
LGR4 mRNA level
MDA-MB-231
BT549

# Figure S5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n.s.
20
15
10
5
0
cAMP nM/1000 cells
Lgr4fl/fl
Lgr4 CKO
B
PyMT tumor
Lgr4fl/fl
Lgr4 CKO
#1
#2
#1
#2
p-CREB
43kDa
CREB
43kDa
n.s.
n.s.
2.4
1.8
1.2
0.6
0.0
P-CREB protein level
(Quantified by CREB)
#1
#2
#1
#2
Lgr4fl/fl
Lgr4 CKO
C
Ctrl
shLGR4 #1
shLGR4 #2
Forskolin
-
+
-
+
-
+
p-FAK
125kDa
FAK
125kDa
β-actin
42kDa