Figure S1. Tumorspheres cultures.

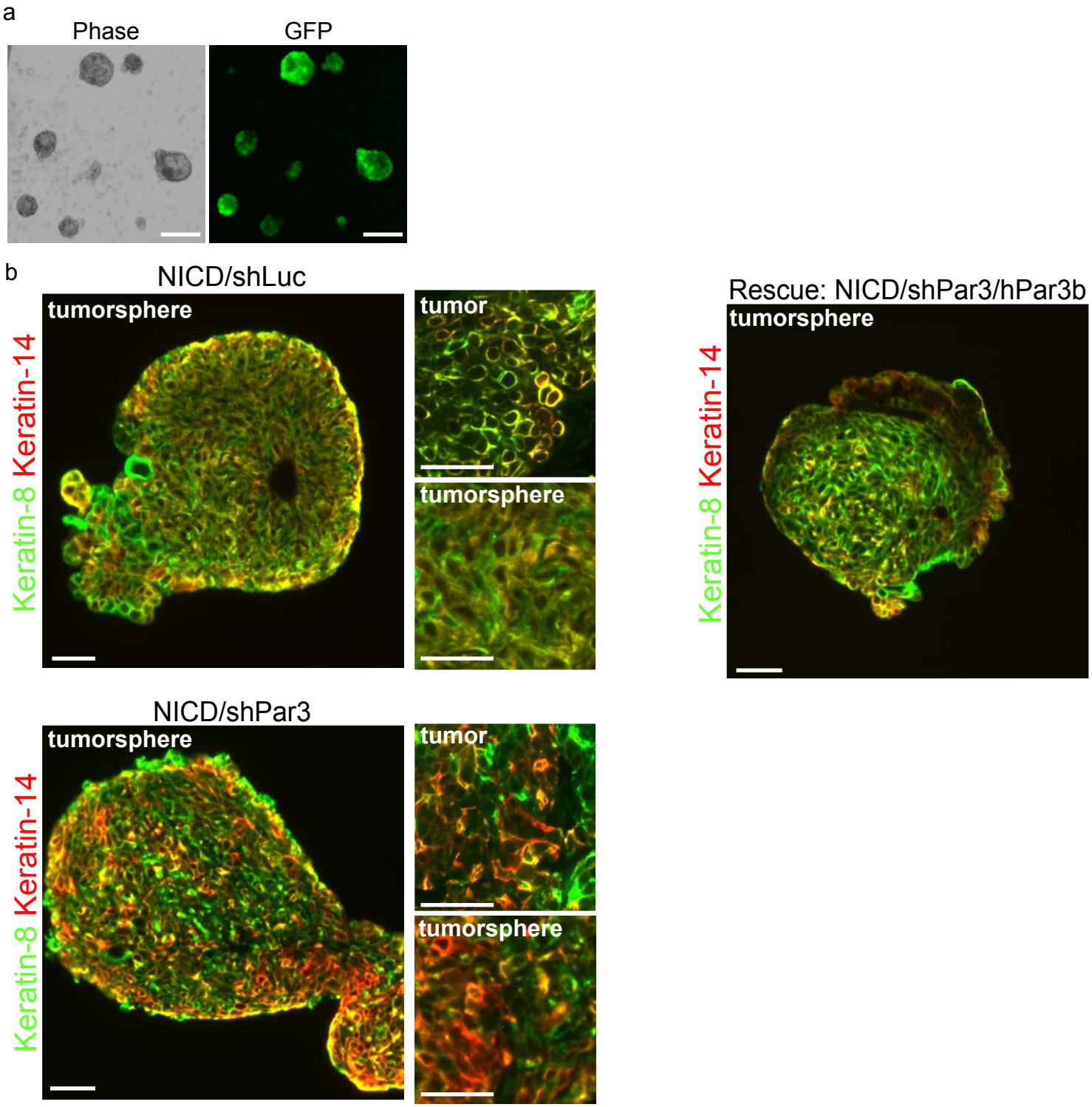
(a) Representative phase contrast and fluorescent micrograph of tumorspheres showing high transduction efficiency of lentiviral infection with a GFP marker. **(b)** Primary tumors and tumorspheres were stained for Keratin-8 (1:250, University of Iowa Developmental Studies Hybridoma Bank) and Keratin-14 (1:500, Covance). Human Par3b (hPar3b) is resistant to the mouse-specific Par3 shRNA (McCaffrey and Macara, 2009). Insets at the right show a magnified image of tumorspheres (lower) and sections from NICD/shLuc and NICD/shPar3 tumors. Scale bars = 100μm (a) and 25μm (b).

Figure S2. Rac activity and knockdown of Tiam1 in mammary epithelial cells.

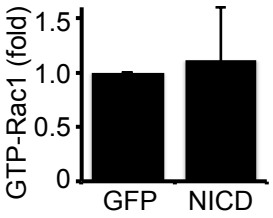
(a) Levels of active Rac1-GTP were measured in GFP and NICD spheres using a GLISA assay kit (Cytoskeleton Inc.), n=4. **(b)** Immunoblot of cell lysates from primary MECs expressing shLuc (control) or Tiam1 shRNAs (shRNA sequences: shTiam1-1 5'-ggaacagattctcaagcta; shTiam1-2 5'-cgagttccttaagactcta; shTiam1-3 5'-cgatgactttatatttata; shTiam1-4 5'-tttcgtgctatgatgaatc) for Tiam1 (1:500, Santa Cruz). E-cadherin (1:2000, BD Biosciences) was used as a loading control.

Figure S3. JNK activity controls tumorsphere growth downstream of Tiam1 and Rac.

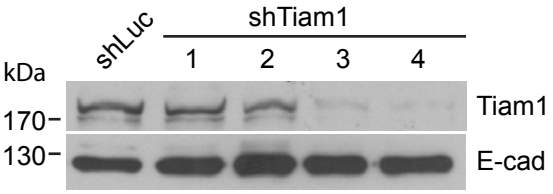
(a) Tumorsphere cultures of MECs expressing GFP (control) or Tiam1 grown in the presence or absence of 25μM JNK inhibitor (SP600125). **(b)** Quantification of tumorsphere sizes in (a), n=3. **(c)** Tumorsphere cultures of MECs expressing GFP (control) or constitutively active $Rac1^{G12V}$ grown in the presence or absence of 25μM JNK inhibitor (SP600125). **(d)** Quantification of tumorsphere sizes in (c), n=3. **(e)** **I**mmunoblot of cell lysates from primary MECs expressing NICD/shLuc or NICD/shPar3 for phospho-ERK1/2 (1:1000, Cell Signaling Technology). Tubulin was used as a loading control. Scale bars = 1mm.

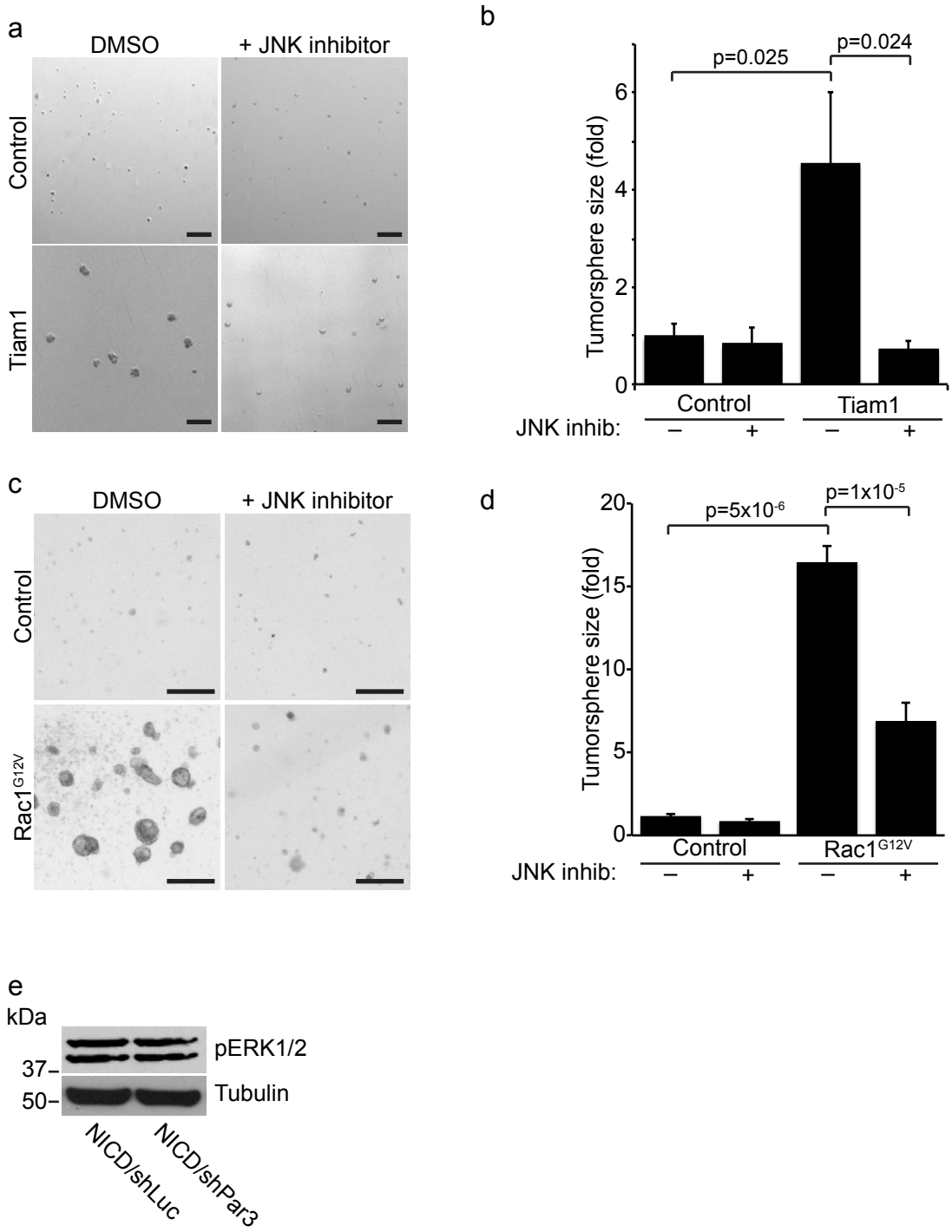

a
Phase
GFP
b
NICD/shLuc
tumorsphere
tumor
tumorsphere
Keratin-8 Keratin-14
Rescue: NICD/shPar3/hPar3b
tumorsphere
Keratin-8 Keratin-14
NICD/shPar3
tumorsphere
tumor
tumorsphere
Keratin-8 Keratin-14

a


GTP-Rac1 (fold)
1.5
1.0
0.5
0
GFP
NICD

b


shLuc
shTiam1
1
2
3
4
kDa
170
130
Tiam1
E-cad


a
DMSO
+ JNK inhibitor
Control
Tiam1
b
p=0.025
p=0.024
Tumorsphere size (fold)
0
2
4
6
Control
Tiam1
JNK inhib:
–
+
–
+
c
DMSO
+ JNK inhibitor
Control
Rac1G12V
d
p=5x10-6
p=1x10-5
Tumorsphere size (fold)
0
5
10
15
20
Control
Rac1G12V
JNK inhib:
–
+
–
+
e
kDa
37–
50–
pERK1/2
Tubulin
NICD/shLuc
NICD/shPar3