

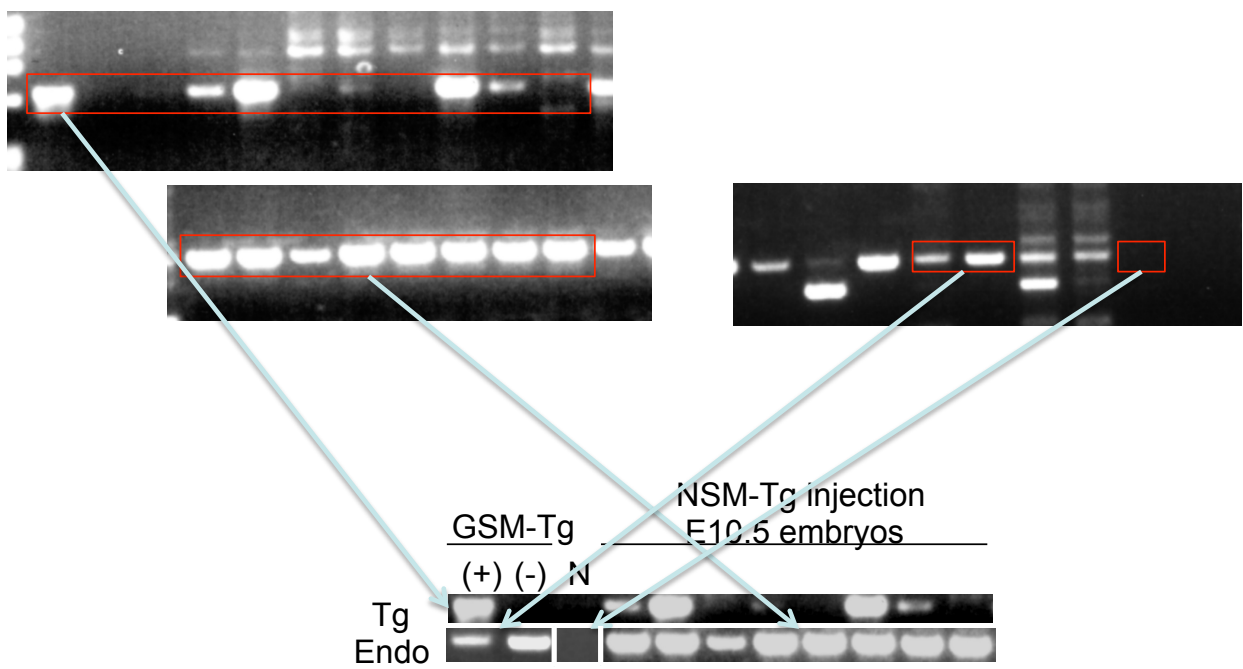
1 **Title: Transgenic expression of the N525S-tuberin variant in *Tsc2* mutant (Eker) rats causes**
2 **dominant embryonic lethality**

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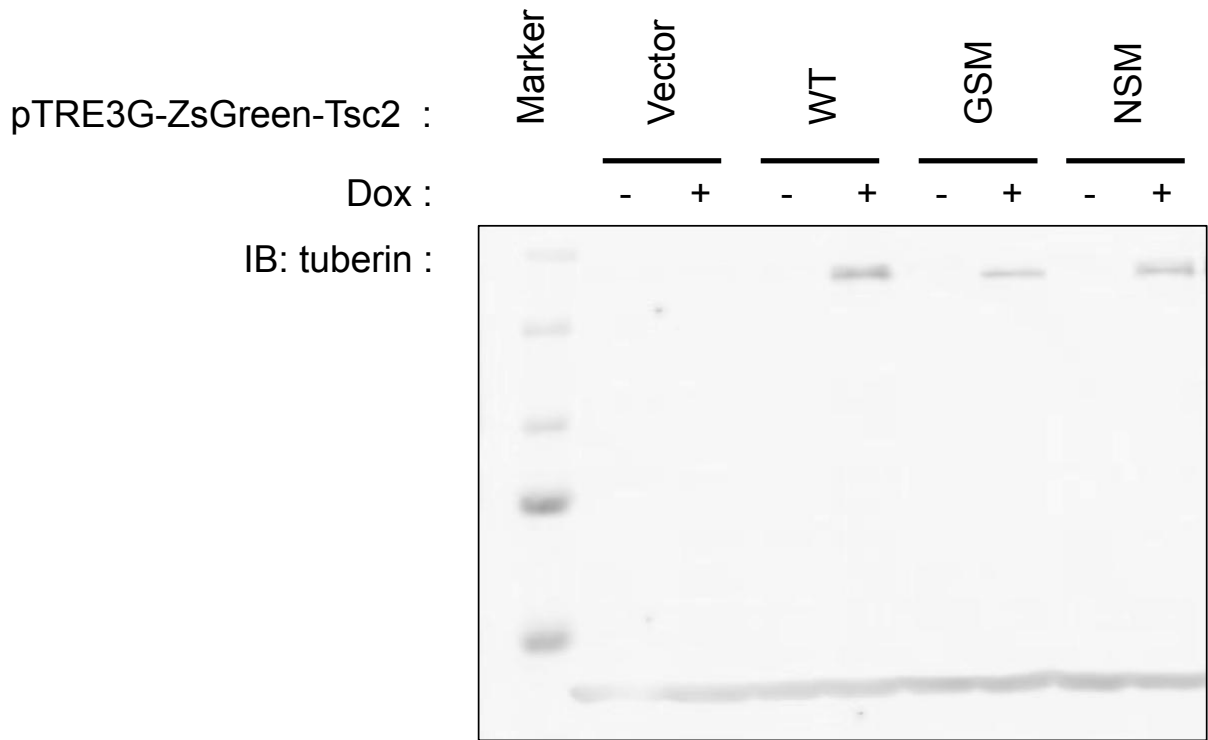
4 *Authors:* Masatoshi Shiono, Toshiyuki Kobayashi, Riichi Takahashi, Masatsugu Ueda, Chikashi
5 Ishioka, Okio Hino

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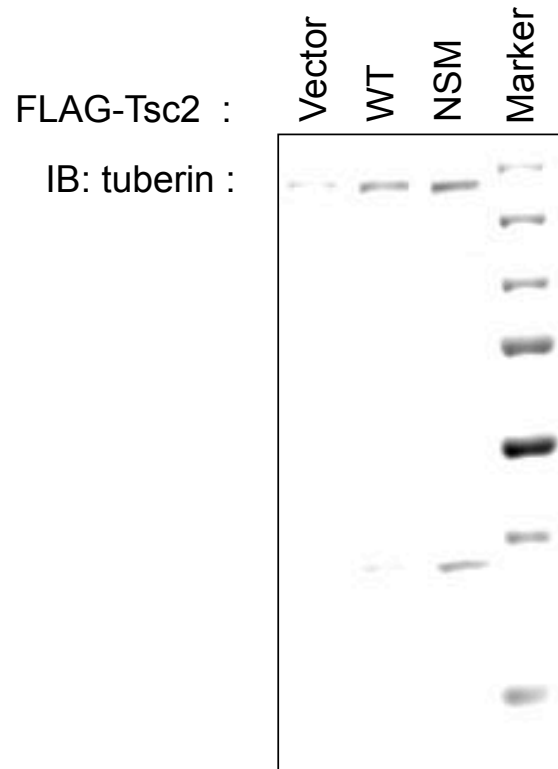
- 1 **Supplementary Figure and figure legends**
- 2 **Supplementary Figure S1. Full-length gels for Fig. 1b.**



1 **Supplementary Figure S2. Full-length blots for Fig. 2a.**



1 **Supplementary Figure S3. Full-length blots for Fig. 4a.**



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1 **Supplementary Note S1. Targets in Phospho-RTK array analysis**

2 Akt 1/2/3 (S473), Akt 1/2/3 (T308), AMPK alpha1 (T183), AMPK alpha2 (T172), beta-Catenin,
3 Chk-2 (T68), c-Jun (S63), CREB (S133), EGF R (Y1068), eNOS (S1177), ERK1/2 (T202/Y204,
4 T185/Y187), FAK (Y397), Fgr (Y412), Fyn (Y420), GSK-3 alpha/beta (S21/S9), Hck (Y411),
5 HSP27 (S78/S82), HSP60, JNK 1/2/3 (T183/Y185, T221/Y223), Lck (Y394), Lyn (Y397),
6 MSK1/2 (S376/S360), p27 (T198), p38 alpha (T180/Y182), p53 (S15), p53 (S392), p53 (S46),
7 p70 S6 Kinase (T421/S424), PDGF R beta (Y751), PLC gamma-1 (Y783), PRAS40 (T246),
8 Pyk2 (Y402), RSK1/2/3 (S380), Src (Y419), STAT2 (Y689), STAT3 (S727), STAT3 (Y705),
9 STAT5a (Y699), STAT5a/b (Y699), STAT5b (Y699), STAT6 (Y641), TOR (S2448), WNK-1
10 (T60), Yes (Y426)

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12 **Supplementary Note S2. Targets in RPPA analysis**

13 Angiogenesis: p-eNOS (Ser1177)

14 Apoptosis: p-Bad (Ser112), p-Bad (Ser136), p-Bad (Ser155), p-Bcl-2 (Ser70), p-Bcl-2 (Thr56),
15 p-Bim (Ser69), p-NDRG1 (Thr346)

16 Cell cycle: p-Aurora A (Thr288), p-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198),
17 p-Cyclin D1 (Thr286), p-Rb (Ser780), p-Rb (Ser795), p-Rb (Ser807/811), p-Wee1 (Ser642)

18 Cytoskelton: p-AP2M1 (Thr156), p-Cofilin (Ser3), p-Ezrin (Thr567)/Radixin (Thr564)/Moesin
19 (Thr558), p-MARCKS (Ser167/170), p-MYPT (Ser507), p-MYPT (Ser668), p-MYPT (Thr853),
20 p-VASP (Ser157), p-VASP (Ser239)

21 DNA damage: p-ATM (Ser1981), p-ATR (Ser428), p-BAP1 (Ser592), p-BRCA1 (Ser1524),
22 p-Chk1 (Ser296), p-Chk1 (Ser317), p-Chk1 (Ser345), p-Chk2 (Ser19), p-Chk2 (Ser33/35),
23 p-Chk2 (Thr68), p-p53 (Ser15), p-p53 (Ser20), p-p53 (Ser37), p-p53 (Ser392), p-p53 (Ser46),
24 p-p53 (Ser6), p-p53 (Ser9)

1 Energy homeostasis: p-Acetyl-CoA carboxylase (Ser79), p-AMPK α (Thr172), p-AMPK β 1
2 (Ser108), p-LKB1 (Ser428)
3 Forkhead: p-FoxO1 (Ser256), p-FoxO1 (Thr24)/FoxO3a (Thr32), p-FoxO1 (Thr24)/FoxO3a
4 (Thr32)/FoxO4 (Thr28), p-FoxO3a (Ser253), p-FoxO3a (Ser318/321)
5 Insulin receptor: p-IRS-1(Ser307), p-IRS-1(Ser612), p-IRS-1(Ser636/639)
6 Mitosis marker: p-Histone H2A.X (Ser139), p-Histone H3 (Ser10), p-Histone H3 (Ser28),
7 p-Histone H3 (Thr11), p-Histone H3 (Thr3), p-NPM (Ser4), p-TACC3 (Ser558)
8 NF κ B: p-I κ B- α (Ser32/36), p-NF κ B p65 (Ser468), p-NF κ B p65 (Ser536), p-RelB (Ser552),
9 p-TBK1/NAK (Ser172)
10 p38 MAPK: p-HSP27 (Ser82), p-MAPKAPK-2 (Thr222), p-MAPKAPK-2 (Thr334),
11 p-MKK3/MKK6 (Ser189/207), p-MSK1 (Thr581), p-p38 MAPK (Thr180/Tyr182)
12 PI3K-AKT-mTOR: Non-p-4E-BP1 (Thr46), p-4E-BP1 (Ser65), p-4E-BP1 (Thr37/46), p-4E-BP1
13 (Thr70), p70-S6 kinase (Thr389), p-Akt (Ser473), p-Akt (Thr308), p-eEF2k (Ser366), p-eIF4B
14 (Ser422), p-eIF4E (Ser209), p-eIF4G (Ser1108), p-GSK-3 α (Ser21), p-GSK-3 α / β (Ser21/9)
15 GSK-3 α preferred, p-GSK-3 β (Ser9), p-mTOR (Ser2448), p-PDK1 (Ser241), p-PKD/PKC μ
16 (Ser744/748), p-PKD/PKC μ (Ser916), p-PRAS40 (Thr246), p-PTEN (Ser380), p-Raptor
17 (Ser792), p-S6Rb (Ser235/236), p-Tuberin/TSC2 (Ser939), p-Tuberin/TSC2 (Thr1462),
18 p-Tuberin/TSC2 (Thr1571)
19 Ras-Raf: p-A-Raf (Ser299), p-B-Raf (Ser445), p-c-Raf (Ser259), p-c-Raf (Ser289/296/301),
20 p-c-Raf (Ser338), p-CREB (Ser133), p-MEK1/2 (Ser217/221), p-Mnk1 (Thr197/202), p-p44/42
21 MAP kinase (Thr202/Tyr204), p-p90RSK (Ser380), p-p90RSK (Thr359/Ser363), p-p90RSK
22 (Thr573), p-PAK1 (Ser144)/PAK2 (Ser141), p-PAK1 (Ser199/204)/PAK2 (Ser182/197),
23 p-PAK1 (Thr423)/PAK2 (Thr402), p-PAK2 (Ser20), p-RSK2 (Ser227), p-RSK3(Thr356/Ser360)
24 RTK: Non-p-Src (Tyr416), Non-p-Src (Tyr527), p-ALK (Tyr1282/1283), p-c-Kit(Tyr703),
25 p-EGF Receptor (Tyr1045), p-EGF Receptor (Tyr1068), p-EGF Receptor (Tyr992), p-FLT3

1 (Tyr591), p-Gab1 (Tyr627), p-Gab2 (Tyr452), p-HER2/ErbB2 (Tyr1221/1222), p-HER2/ErbB2
2 (Tyr1248), p-HER4/ErbB4 (Tyr984), p-IGF-I Receptor (Tyr1131)/Insulin Receptor (Tyr1146),
3 p-IGF-I Receptor (Tyr1135/1136), p-IGF-I Receptor (Tyr980), p-Met (Tyr1234/1235), p-PDGF
4 Receptor- β (Tyr1009), p-PDGF Receptor- β (Tyr1021), p-PDGF Receptor- β (Tyr740), p-PDGF
5 Receptor- β (Tyr751), p-PDGF Receptor- β (Tyr771), p-PKC(pan) β II (Ser660), p-PKC α/β
6 (Thr638/641), p-PKC δ (Thr505), p-PKC δ/θ (Ser643/676), p-PKC ζ/λ (Thr410/403), p-PKC θ
7 (Thr538), p-PKM2 (Tyr105), p-PLC γ (Tyr1217), p-PLC γ (Tyr759), p-PLC γ (Tyr783), p-Ret
8 (Tyr905), p-Shc (Tyr239), p-Shc (Tyr317), p-SHIP2 (Tyr1135), p-SHP-2 (Tyr580), p-Src Family
9 (Tyr416), p-Src (Tyr527), p-Syk (Tyr323), p-Syk (Tyr525/526), p-VEGF Receptor 2 (Tyr1059),
10 p-VEGF Receptor 2 (Tyr1175), p-VEGF Receptor 2 (Tyr951), p-Zap-70 (Tyr319)/Syk (Tyr352)
11 SAPK/JNK: p-ATF-2 (Thr71), p-c-Fos (Ser32), p-c-Jun (Ser63), p-c-Jun (Ser73),
12 p-SEK1/MKK4 (Thr261), p-SAPK/JNK(Thr183/Tyr185)
13 STAT: p-Jak2 (Tyr1007/1008), p-Stat 1(Tyr701), p-Stat 2 (Tyr690), p-Stat 3 (Ser727), p-Stat 3
14 (Tyr705), p-Stat 5 (Tyr694), p-Stat 6 (Tyr641), p-Tyk2 (Tyr1054/1055)
15 TGF- β : p-Smad1/5 (Ser463/465), p-Smad2 (Ser465/467), p-Smad2 (Ser465/467)/Smad3
16 (Ser423/425), p-TAK1 (Thr184/187)
17 Wnt: p-LRP6 (Ser1490), p- β -catenin (Ser33/37/Thr41), p- β -catenin (Ser45)
18 Housekeeping: γ -tubulin
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