
Supplementary file

Pyrotinib Targeted EGFR-STAT3/CD24 Loop-Mediated Cell Viability in TSC

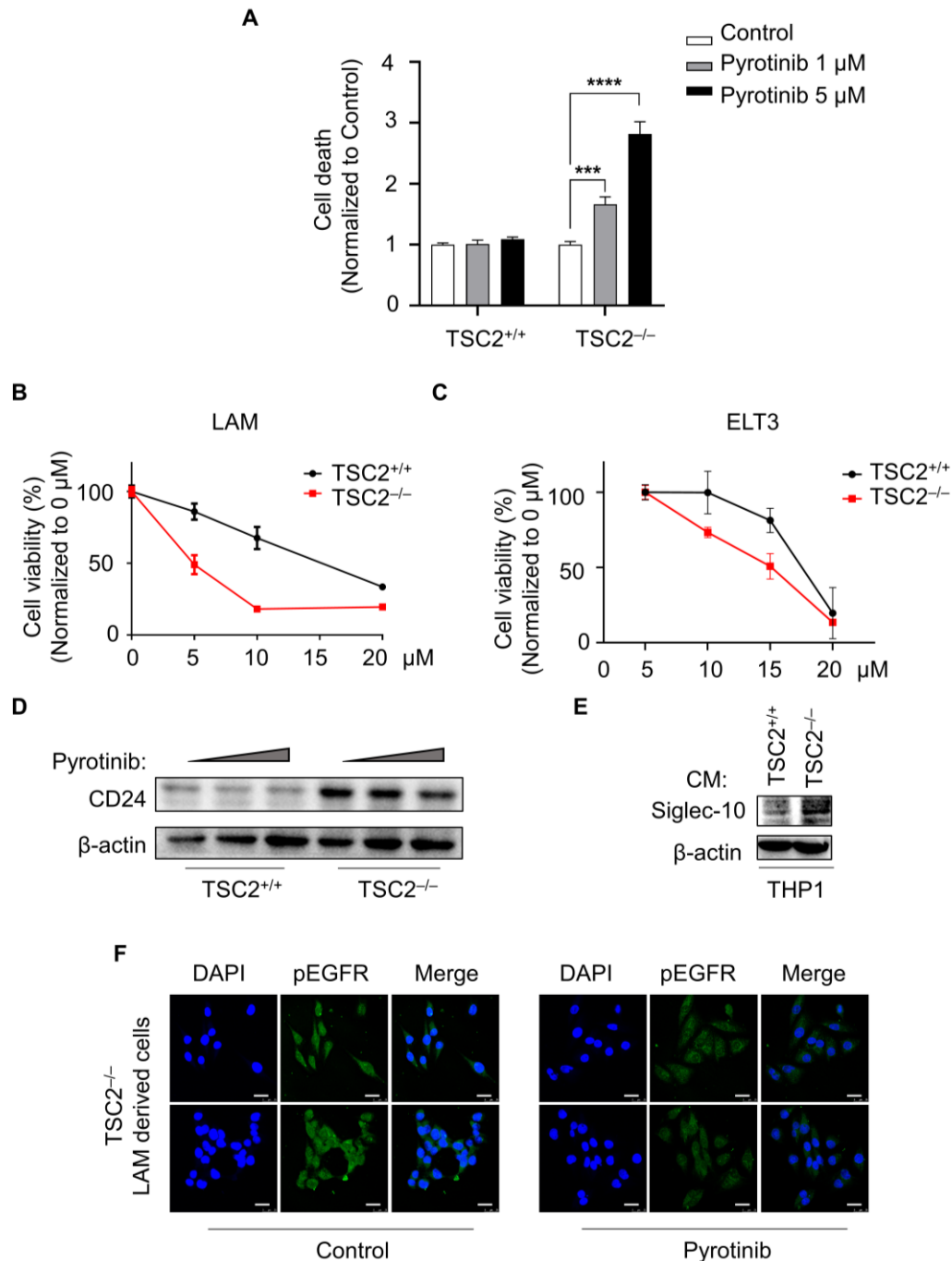


Figure S1. Targeting efficiency of Pyrotinib in MEF/LAM/ELT3-derived cells. **A**, MEF derived cells treated with or without pyrotinib (1 μ M or 5 μ M) for 48 h. Cell death was determined with the PI assay ($n=5$). *** $P < 0.001$, control versus pyrotinib (1 μ M). **** $P < 0.001$, control versus pyrotinib (5 μ M), Student t test. **B and C**, LAM derived cells (**B**) and ELT3 derived cells (**C**) were treated with different concentration of pyrotinib for 48 h, and cell viability were detected by CCK8 assay, and IC₅₀ of LAM derived TSC2-deficient cells was 4.404 μ M, as while as 3.338 μ M in ELT3 derived TSC2-deficient cells. **D**, Immunoblot analysis of CD24 in LAM derived cells treated with or without pyrotinib (1 μ M or 5 μ M). **E**, Macrophages of THP1 was co-incubated with culture supernatant of LAM with or without TSC2-deficient cells for 6 h, and THP1 cells were lysed, and siglec-10 was detected by immunoblot. **F**,

Localization and expression of pEGFR detected by immunofluorescence staining in TSC2-deficient LAM derived cells treated with or without pyrotinib (5 μ M) (magnification 630 \times , bar = 25 μ m).

Gene Symbol	TSC2 ^{+/+}	TSC2 ^{-/-}	Log2Fold-Change (TSC2 ^{-/-} vs. TSC2 ^{+/+})	Pvalue	FDR
Cd24a	0.2118336	5.5011925	5.2893589	5.00E-05	0.0004
Cd1d1	1.7205636	0.6158119	-1.104752	0.0316	0.12835
Cd81	8.3645793	6.7701456	-1.594434	5.00E-05	0.0004
Cd300lb	2.822726	0.0548058	-2.76792	0.0424	0.162439
Cd200	3.8694135	0.802024	-3.067389	5.00E-05	0.0004
H2-K1	0.8743655	1.8896489	1.0152835	0.00615	0.0322113
Ildr2	0.018636	2.5172616	2.4986256	0.04235	0.162317
Cd1d1	1.7205636	0.6158119	-1.104752	0.0316	0.12835
Il6st	4.0059483	2.1793718	-1.826577	0.0007	0.0046575
Igsf10	1.0435959	0.0079094	-1.035687	0.00705	0.0362655
Pdgfra	2.53234	1.1211969	-1.411143	0.0002	0.0014727
Tapbp	4.935779	6.4111536	1.4753746	5.00E-05	0.0004
Mr1	3.4339084	0.3484316	-3.085477	5.00E-05	0.0004
Cxcl12	0.5216163	5.746977	5.2253606	5.00E-05	0.0004
Ccl20	0.5982164	4.25587	3.6576536	0.00295	0.0169383
Bmp6	0.0982659	2.5554994	2.4572335	5.00E-05	0.0004

Figure S2. Table of analysis of RNA-seq results in MEF cells. The RNA-seq results was obtained from reference 13 (PMID: 34853810).

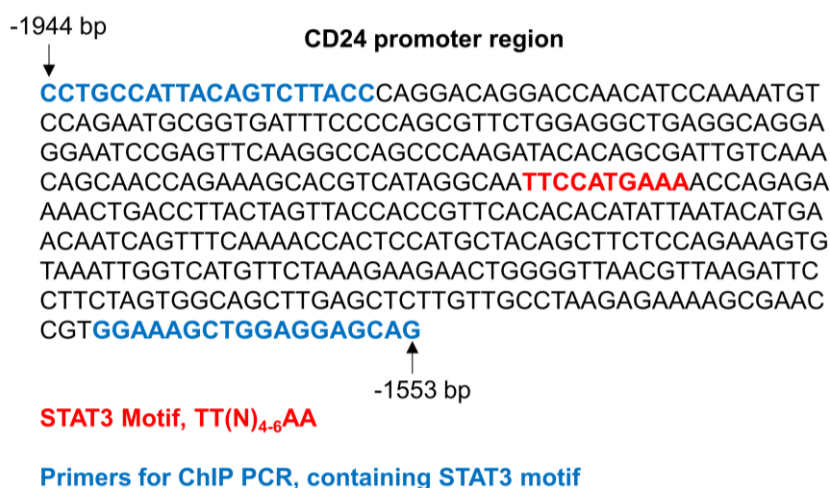


Figure S3. ChIP PCR of CD24 promoter region designed for this study. The full length of construct contained one important STAT3 binding motifs (-1778/-1769 bp), and primers for ChIP PCR sequence was designed by forward sequence: CCTGCCATTACAGTCTTACC and revised sequence: CTGCTCCTCCAGCTTTCC, including the promoter region of -1944/-1553

bp.

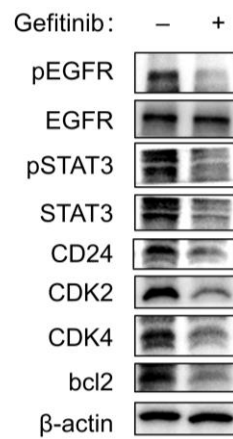


Figure S4. Gefitinib used to validate the EGFR/STAT3/CD24 signaling. Immunoblot analysis of MEF derived cells treated with or without Gefitinib (50 μM).

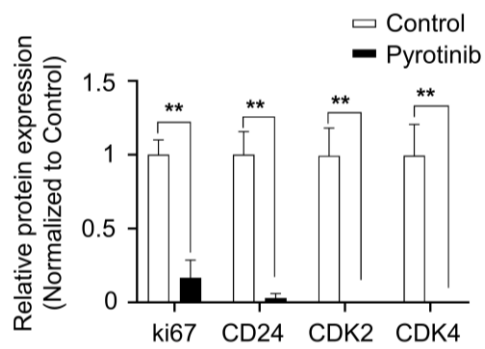


Figure S5. Semi-quantified the proteins in Figure 5E.