

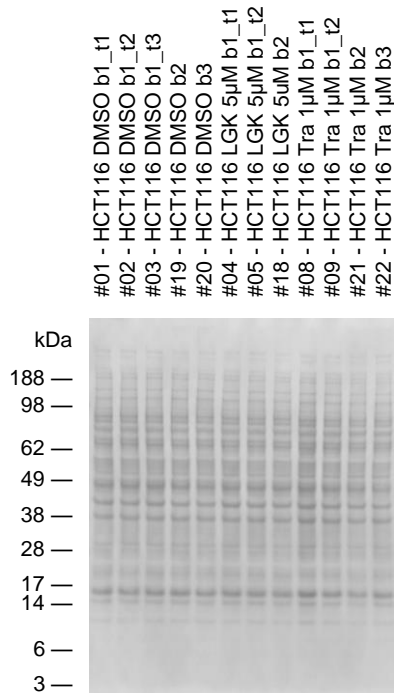


23 September 2015

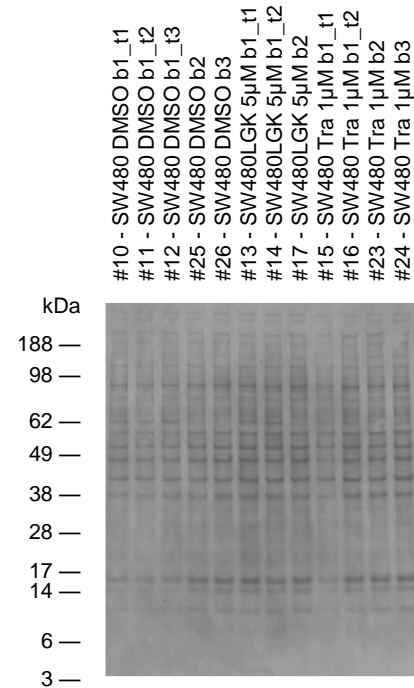
DKFZ samples

Natural and Medical
Sciences Institute
at the University of Tübingen

PonceauS

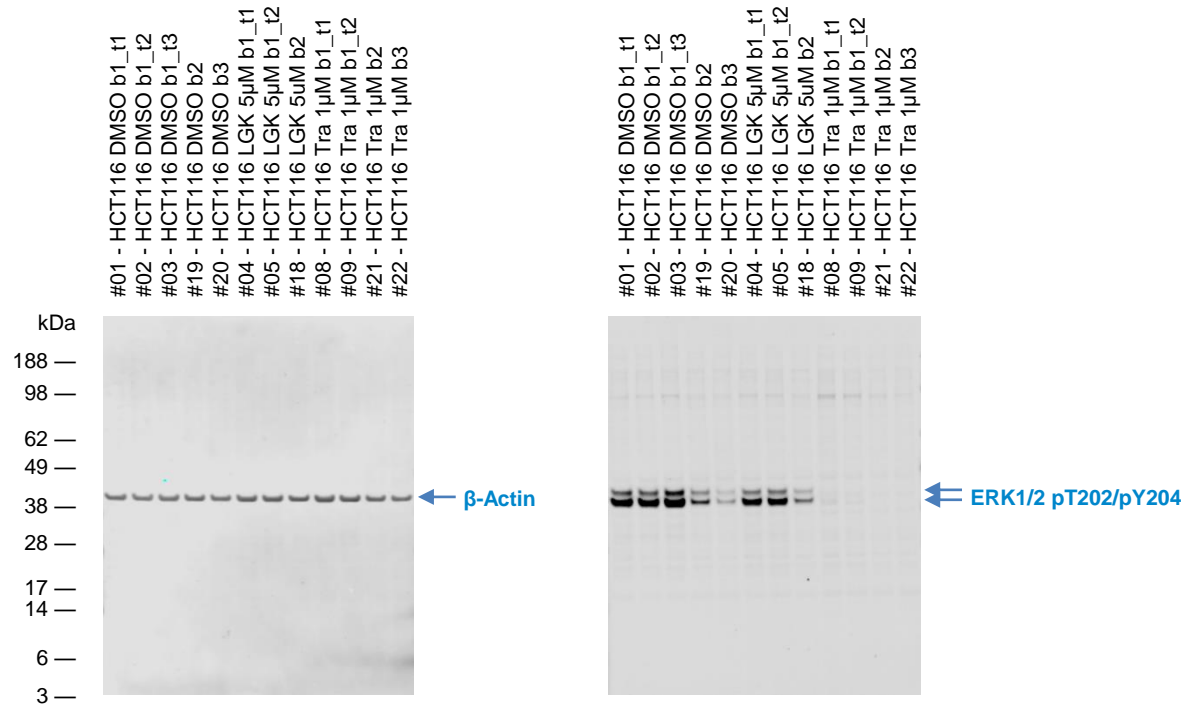


- 100 µl 2X LDS lysis buffer, 10 min 95 °C
- 8.6µg per sample loaded on gel
- NuPAGE® Novex® 4-12% Bis-Tris protein gel, 1.0 mm, 15 well
- 150 V, 90 min
- Blotted on nitrocellulose membrane
- 30 V, 75 min
- PonceauS stain



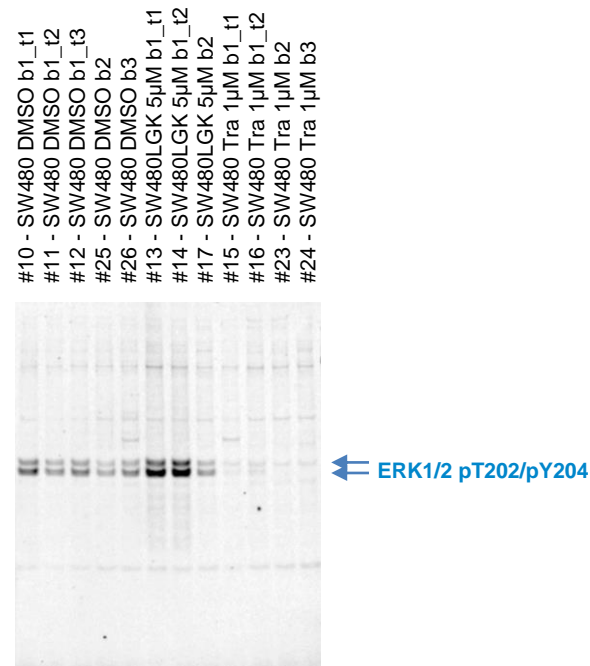
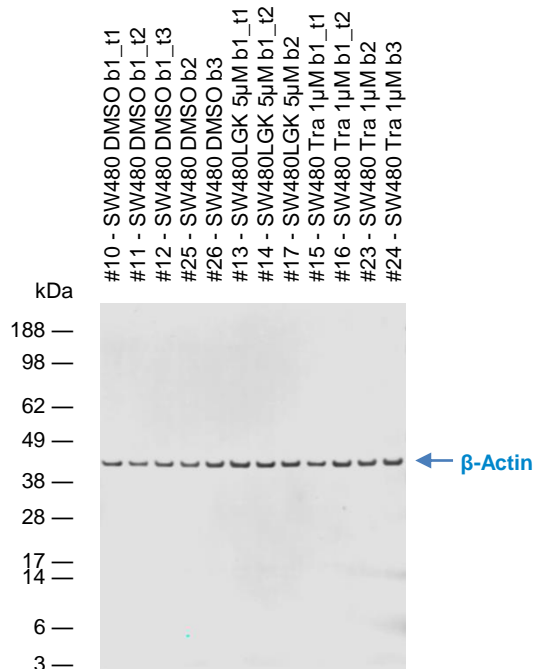
- 100 µl 2X LDS lysis buffer, 10 min 95 °C
- 5 µg per sample loaded on gel, except #12 here only 3 µg loaded
- NuPAGE® Novex® 4-12% Bis-Tris protein gel, 1.0 mm, 15 well
- 150 V, 90 min
- Blotted on nitrocellulose membrane
- 30 V, 75 min
- PonceauS stain

Western Blot – HCT116



- 100 μl 2X LDS lysis buffer, 10 min 95 °C
- 8.6μg per sample loaded on gel
- NuPAGE® Novex® 4-12% Bis-Tris protein gel, 1.0 mm, 15 well
- 150 V, 90 min
- Blotted on nitrocellulose membrane
- 30 V, 75 min
- PonceauS stain
- Membrane blocked with 5 % milk in TBST
- Primary antibody incubation in 1% BSA in TBST
 - rabbit anti-ERK1/2 pT202/pY204
CST #4370, 1:1000, 42/44 kDa, over night at 4°C
 - mouse anti-β-Actin
Sigma-Aldrich #A1978, 1:50.000, 42 kDa, over night at 4°C
- Secondary antibody incubation 1h at RT in 5 % milk in TBST
 - IRDye® 800CW donkey anti-rabbit IgG (H+L)
LI-COR #926-32213, 1:10.000
 - IRDye® 680 donkey anti-mouse IgG (H+L)
LI-COR #926-32222, 1:10.000
- Readout on LI-COR Odyssey® CLx Infrared Imaging System

Western Blot – SW480



- 100 μ l 2X LDS lysis buffer, 10 min 95 °C
- 5 μ g per sample loaded on gel, except #12 here only 3 μ g loaded
- NuPAGE® Novex® 4-12% Bis-Tris protein gel, 1.0 mm, 15 well
- 150 V, 90 min
- Blotted on nitrocellulose membrane
- 30 V, 75 min
- PonceauS stain
- Membrane blocked with 5 % milk in TBST
- Primary antibody incubation in 1% BSA in TBST
 - rabbit anti-ERK1/2 pT202/pY204
CST #4370, 1:1000, 42/44 kDa, over night at 4°C
 - mouse anti- β -Actin
Sigma-Aldrich #A1978, 1:50.000, 42 kDa, over night at 4°C
- Secondary antibody incubation 1h at RT in 5 % milk in TBST
 - IRDye® 800CW donkey anti-rabbit IgG (H+L)
LI-COR #926-32213, 1:10.000
 - IRDye® 680 donkey anti-mouse IgG (H+L)
LI-COR #926-32222, 1:10.000
- Readout on LI-COR Odyssey® CLx Infrared Imaging System