Fig 1 suppl

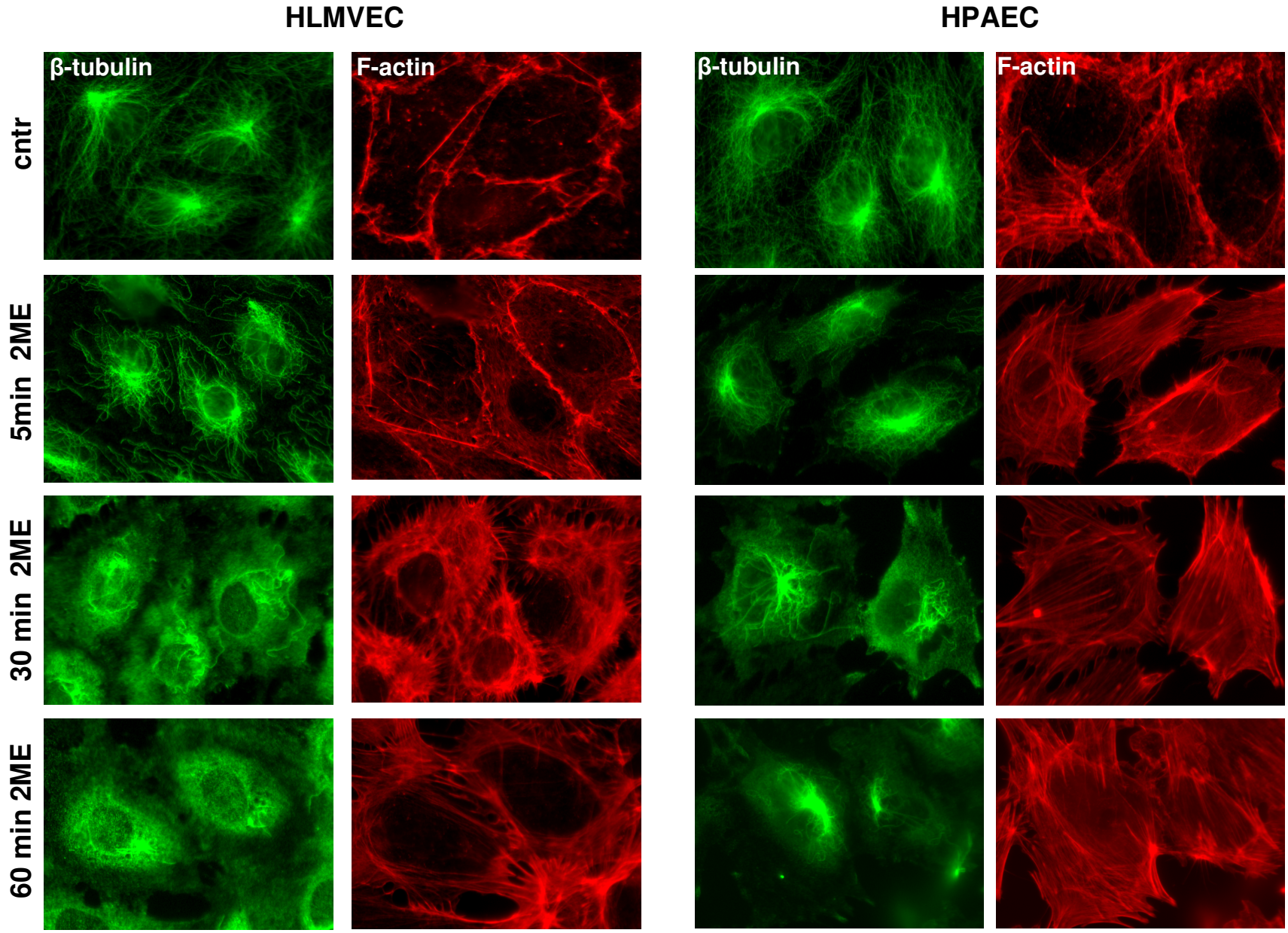
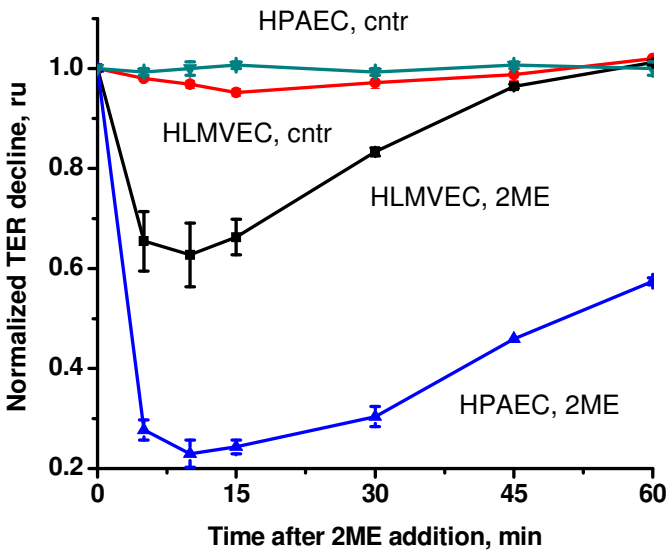
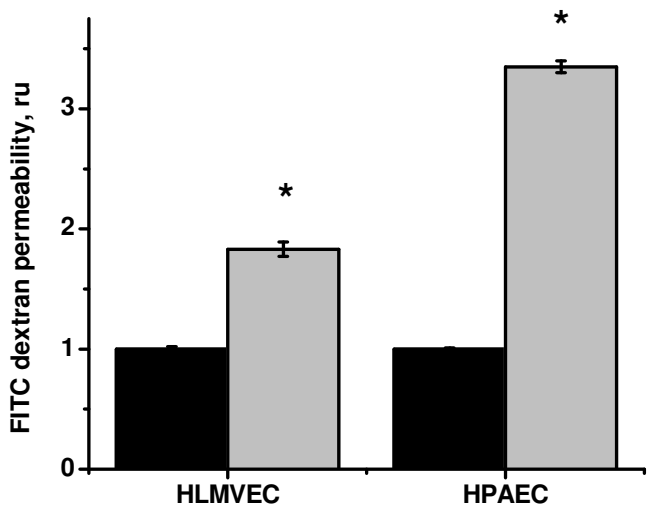

HLMVEC
HPAEC
β-tubulin
F-actin
β-tubulin
F-actin
cntr
5min 2ME
30 min 2ME
60 min 2ME

Fig 2 suppl

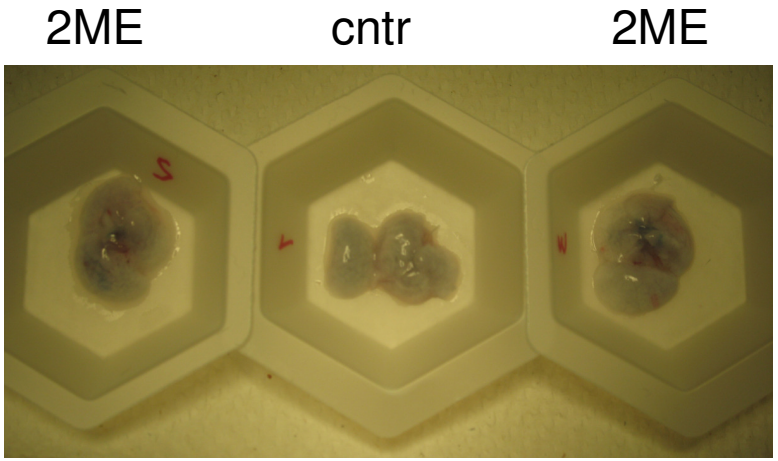




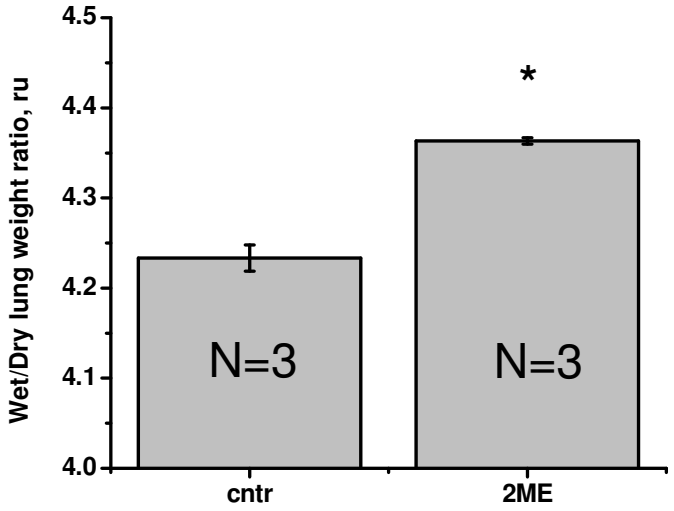
The effect of 2ME on macrovascular (HPAEC) and microvascular (HLMVEC) endothelial barrier.
A. EC were grown to confluence on gold microelectrodes. Media was changed to serum-free media 1h prior the experiment. TER was normalized to the point preceding the addition of vehicle or 50µM 2ME. Shown are mean±SEM (n=3).
B. EC were grown to confluence on collagenated nylon transwell inserts. FITC-dextran was added to the upper chamber and cells were stimulated with vehicle or 50µM 2ME for 2h. Fluorescence of the media in the lower chamber was assessed and expressed as folds of control. *$p<0.05$ compared with corresponding controls. 2ME evokes more significant and sustained increase of permeability across HPAEC monolayers.

Fig3 suppl

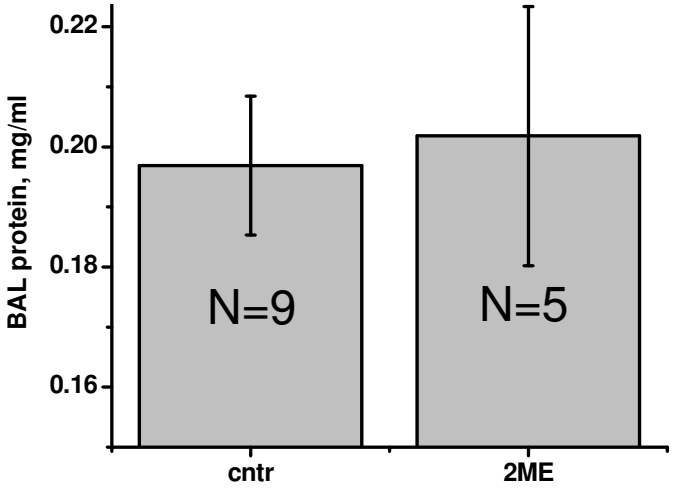
A



B

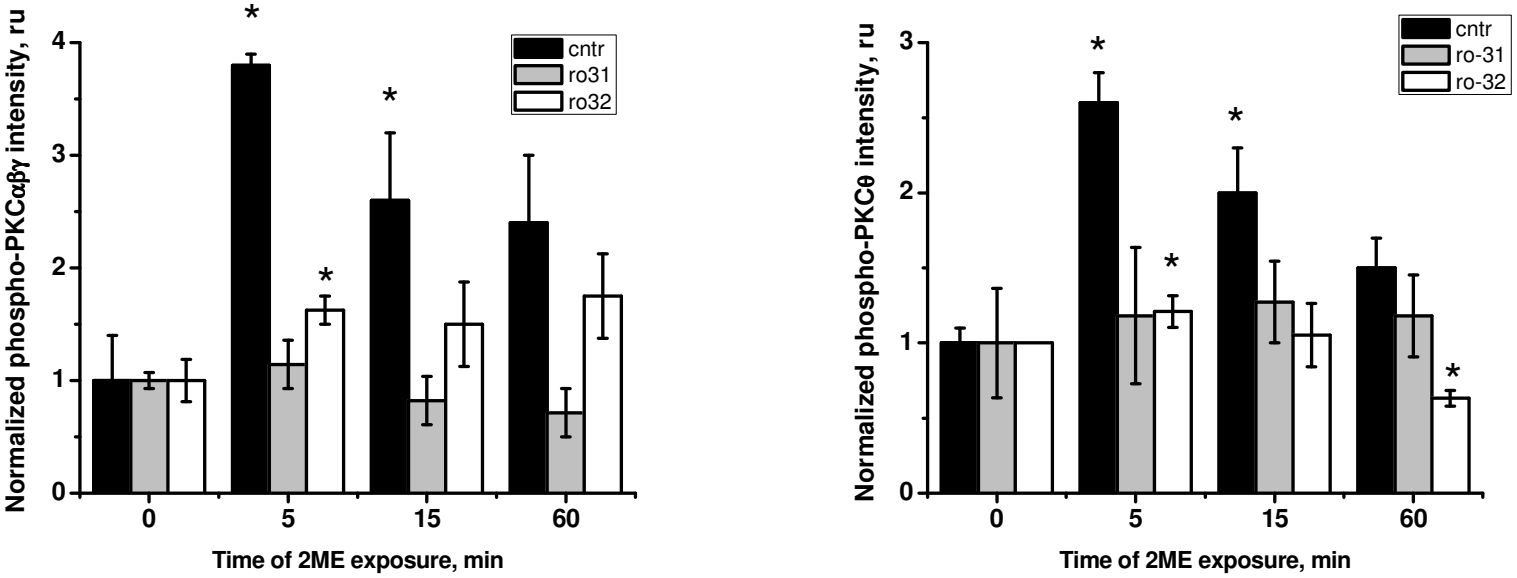


C



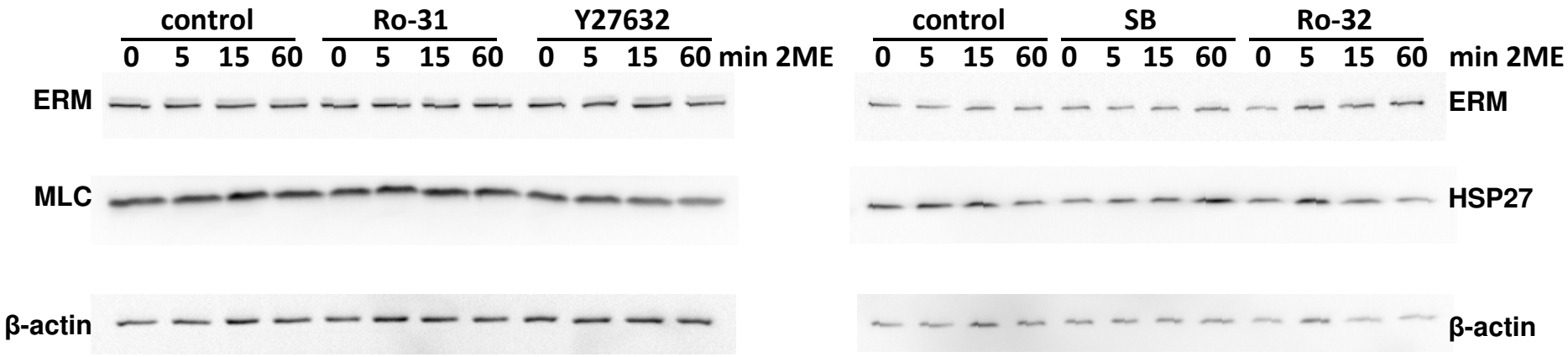
Lungs of mice stimulated with 10 mg/kg 2ME or vehicle for 3h and subjected to intravenous Administration of Evans Blue-albumin were extracted, weighed, photographed (A), and dried for 48h at 65°C. Lungs were weighed again, and wet/dry lung ratio was expressed as mean±SEM (B). Alternatively, lungs were perfused with HBSS to collect bronchoalveolar lavage fluid. After lysing red blood cells and spinning, protein concentration was assessed in supernatant (C). *$p<0.05$ compared with corresponding controls. Note that 2ME causes marked accumulation of Evans Blue in lung tissue and lung edema. Increase of protein concentration in BAL did not reach significant level.

Fig 4 suppl



Western blots described in the legend of Fig 2D were analyzed with phosphoPKCαβγ (left panel), phospho PKCθ (right panel), and β-actin antibodies. Normalized to β-actin phosphoPKC intensities are presented as folds of corresponding controls (in the presence of vehicle or inhibitor, in the absence of 2ME). *p<0.05 compared with corresponding controls.
Note that Ro-31-7549 and Ro-32-0432 pretreatment suppresses the ability of 2ME to increase PKC αβγ and PKC θ phosphorylation.

Fig 5 suppl



Samples from Fig 4A, B were analyzed by Western blot with indicated above antibodies to demonstrate that Ro-31-7549, Ro-32-0432, SB-203580 and Y27632 pre-treatment does not affect the expression of ERM, MLC, or HSP27 in EC. Therefore, the effects of the inhibitors seen on Fig 4A, B should be attributed to the effects on ERM, MLC, and HSP27 phosphorylation.

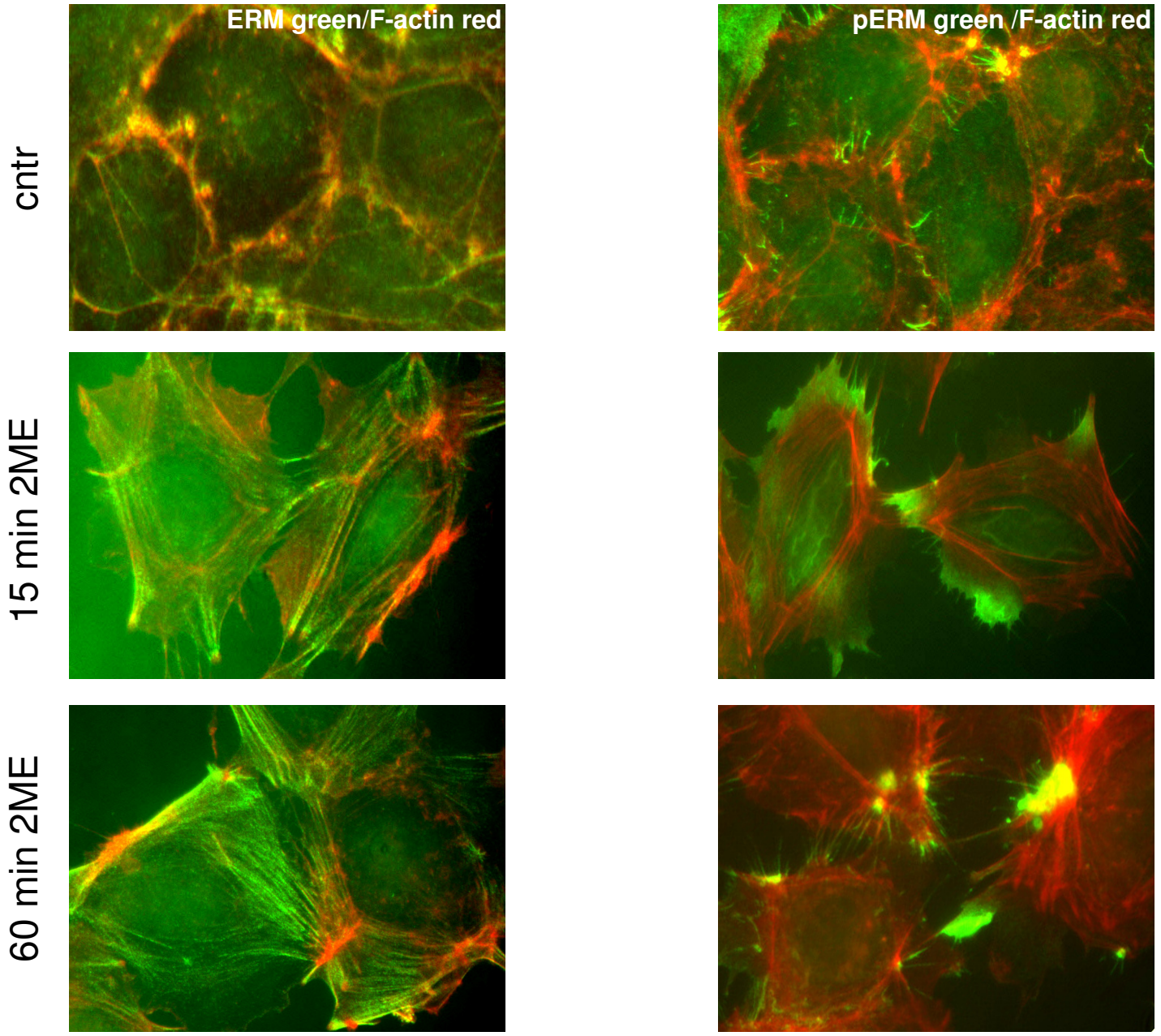

ERM green/F-actin red
pERM green /F-actin red
cntr
15 min 2ME
60 min 2ME

Fig6 Suppl