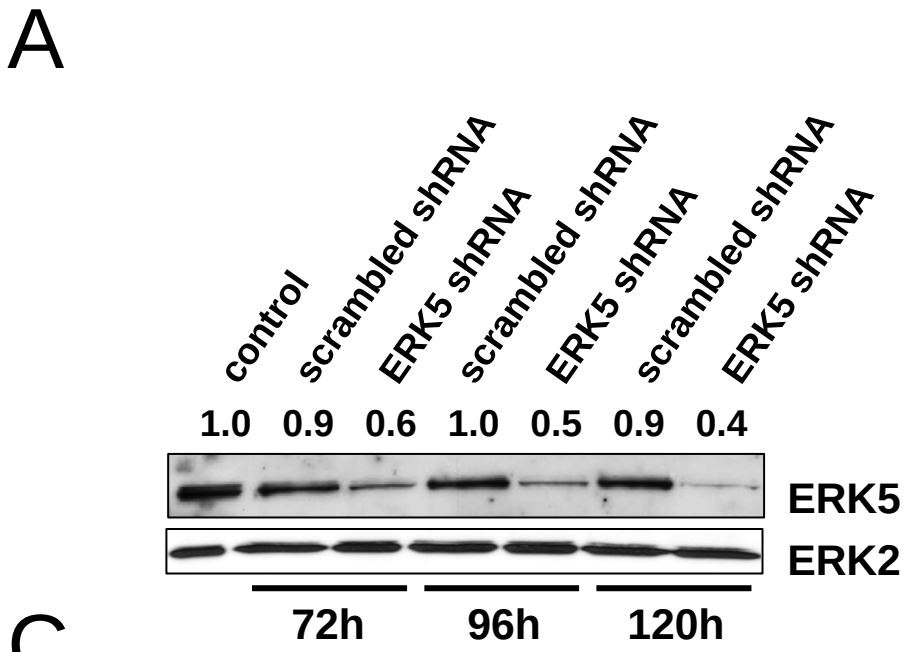
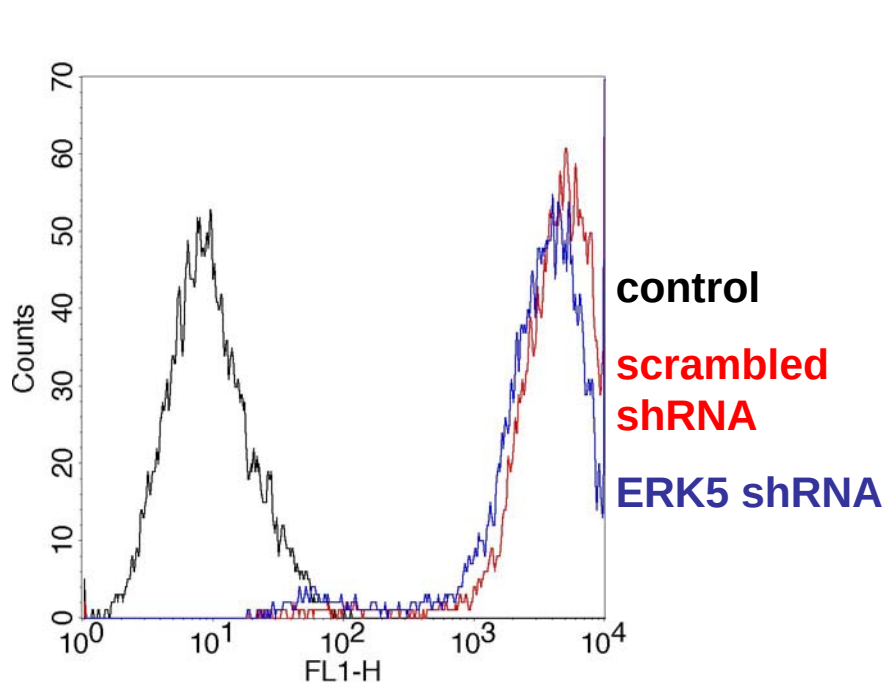
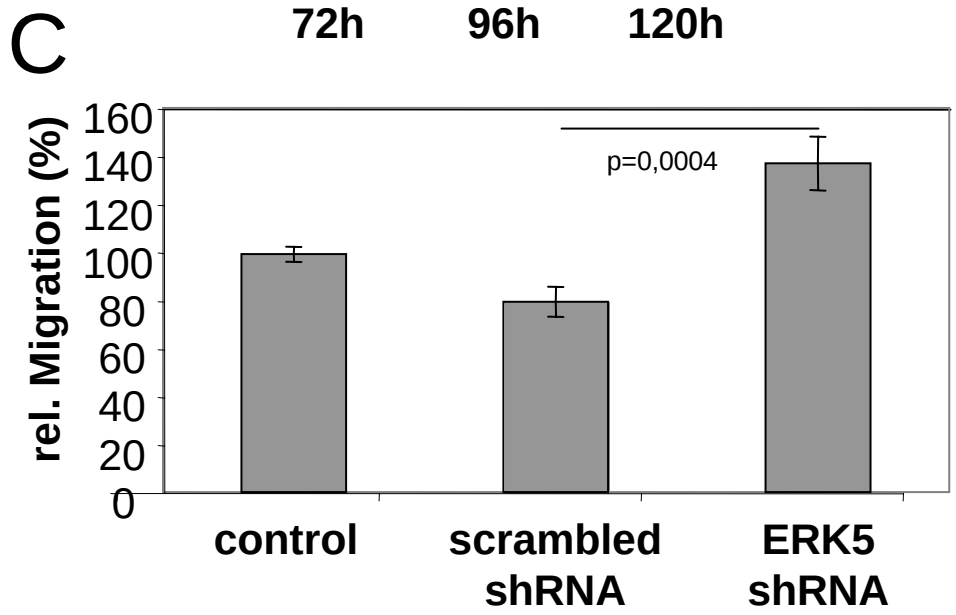
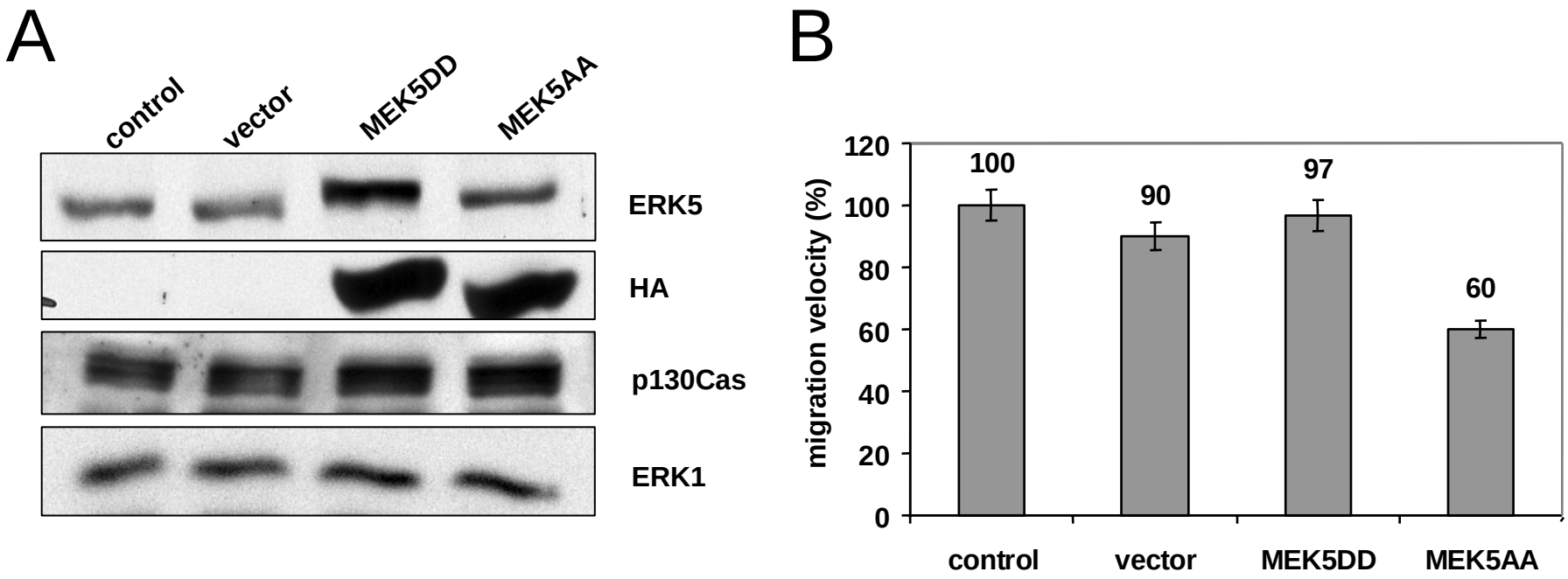


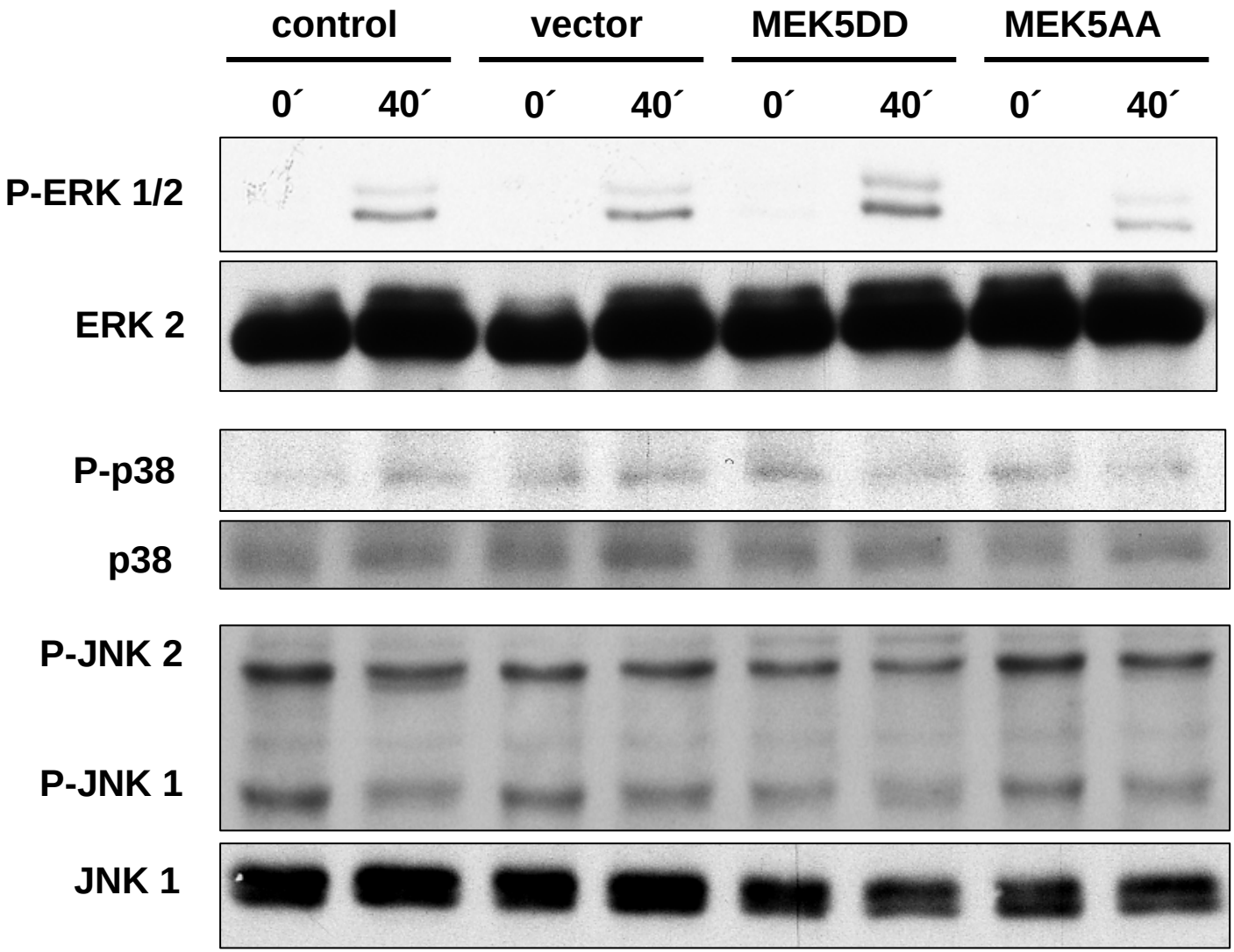




Supplementary Figure 1. **Knock down of ERK5 increases EC migration capacity.** *A*, HUVEC were transduced with lentiviruses containing either scrambled or ERK5 specific shRNA and the efficiency of ERK5 knock down was verified at 72-120 h post-infection by Western blot analysis with an ERK5 specific antiserum (upper panel). Immunoblotting of ERK2 served as loading controls. *B*, The efficiency of lentiviral shRNA transfer was measured 48 h after transduction by flow cytometry of co-expressed EGFP. *C*, Lateral migration was studied 120 h after transduction. Relative mean migration velocity from three independent experiments normalized to control cells ± SD is depicted.

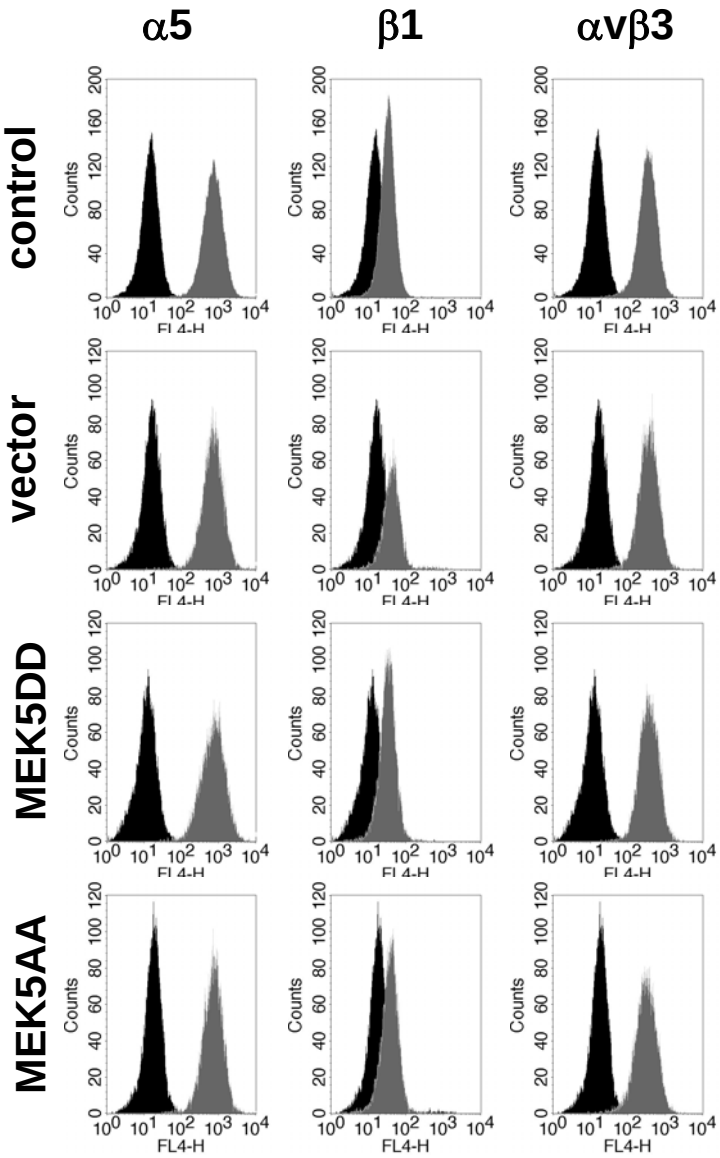


Supplementary Figure 2. **Constitutive activation of ERK5 does not alter cell migration of NIH3T3 murine fibroblasts.** Fibroblasts were transduced with retroviruses containing either MEK5 mutants or empty vector as indicated, selected for zeocin resistance and studied for: *A*, expression and activation of endogenous ERK5 by gel retardation analysis and B, lateral migration capacity. The expression of recombinant MEK5 and endogenous p130Cas were verified by anti-HA- and anti-p130Cas specific antibodies, respectively. Immunoblotting of ERK1 served as loading controls. The migration velocity is shown as mean values from three independent experiments normalized to control cells ± SD. ,

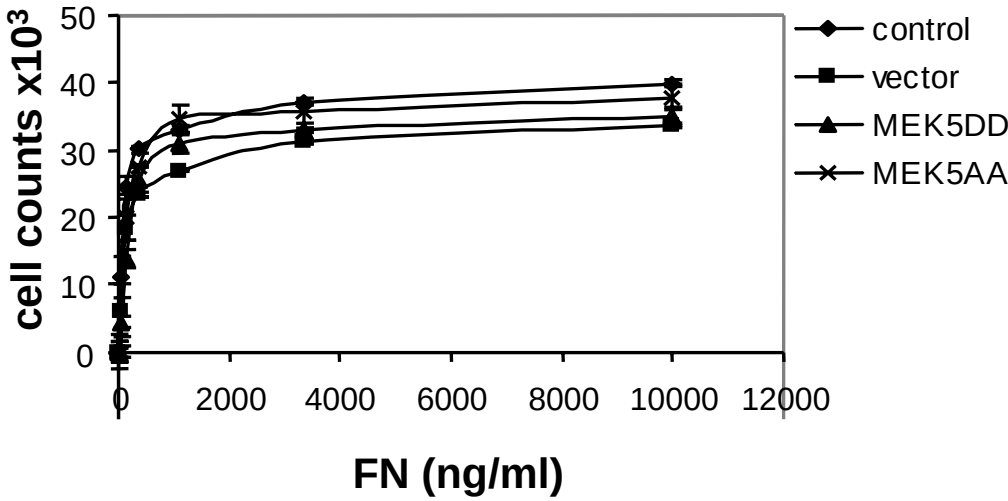


Supplementary Figure 3. **Expression of MEK5DD does not alter activation of p38 and JNK, but slightly activates ERK1/2.** Activation of ERK1/2, p38 and JNK was verified by Western blot analysis with phospho-specific antibodies as indicated. The appropriate loading controls were verified immunoblotting of ERK1/2, p38 or JNK, respectively.

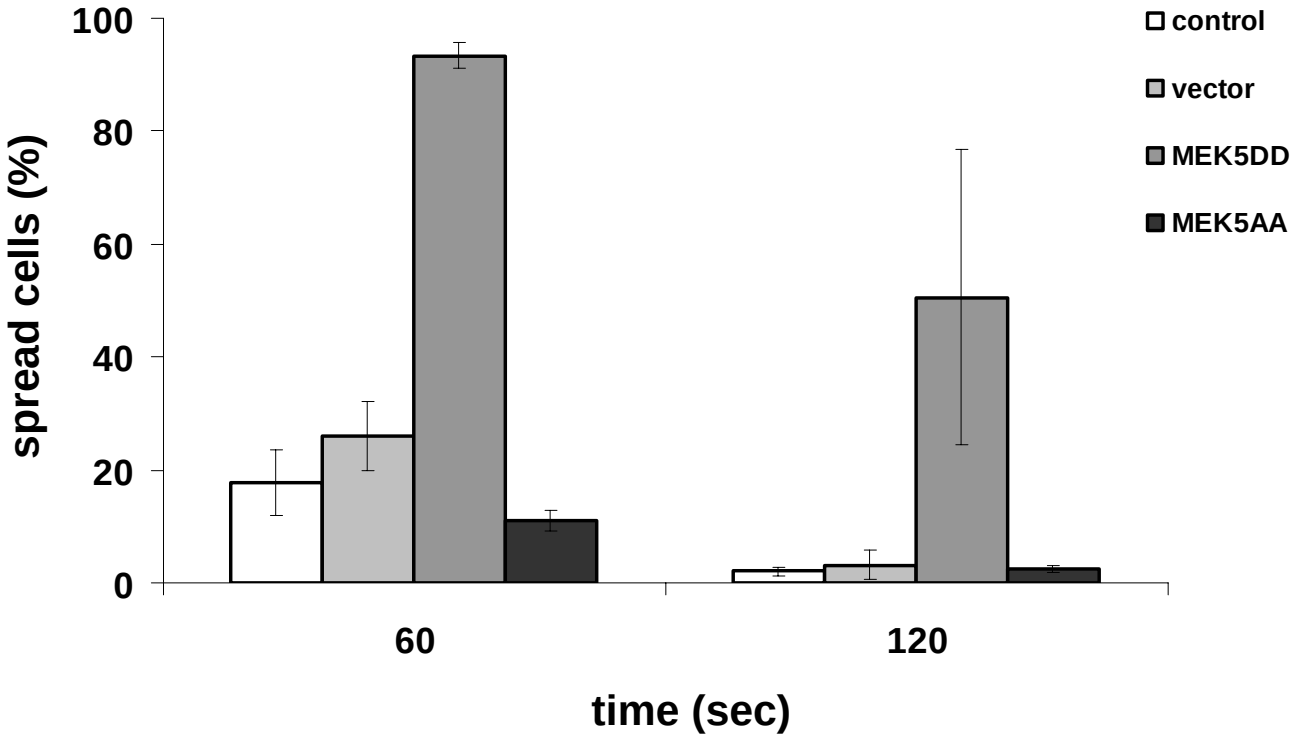
A



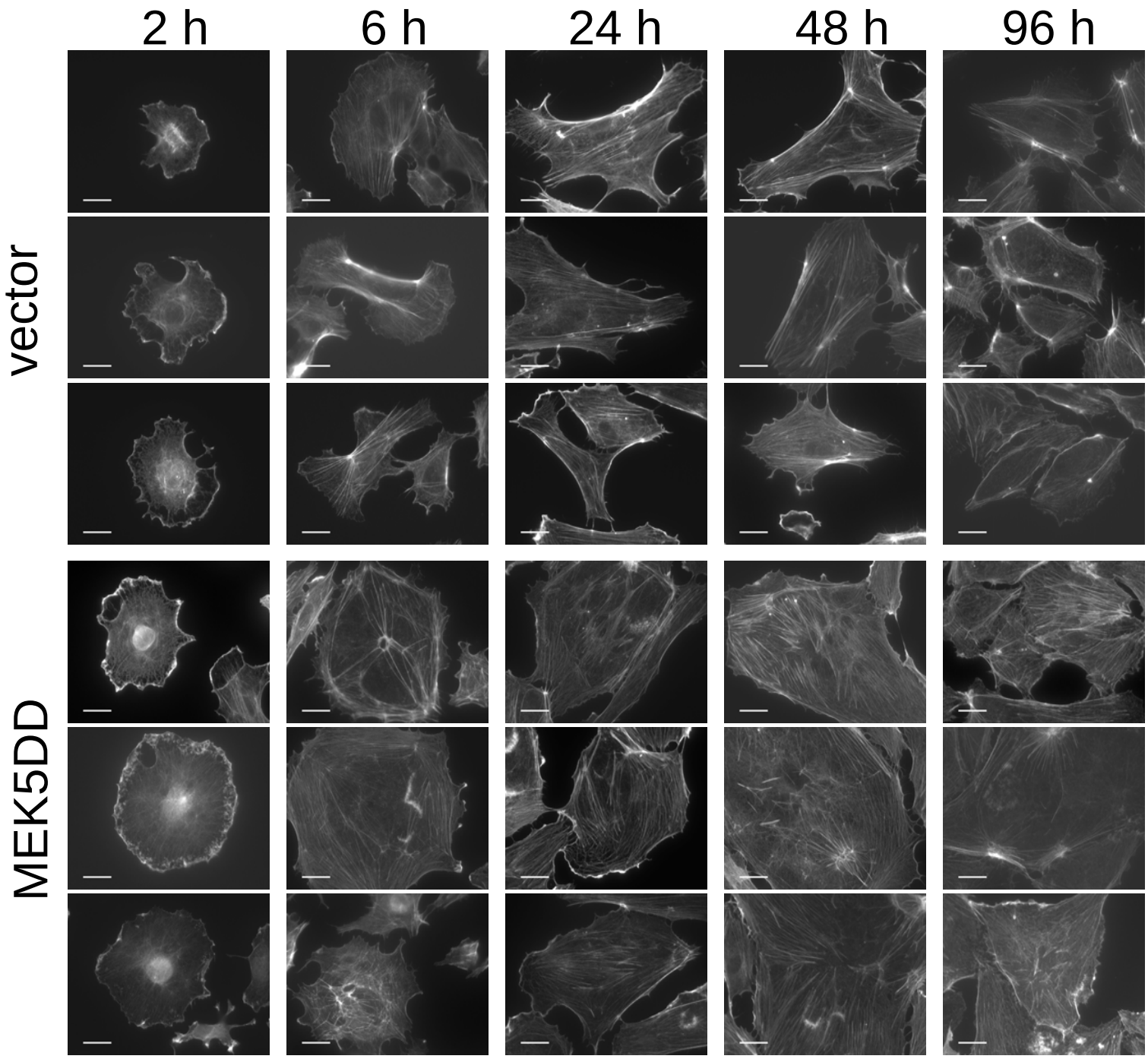
B



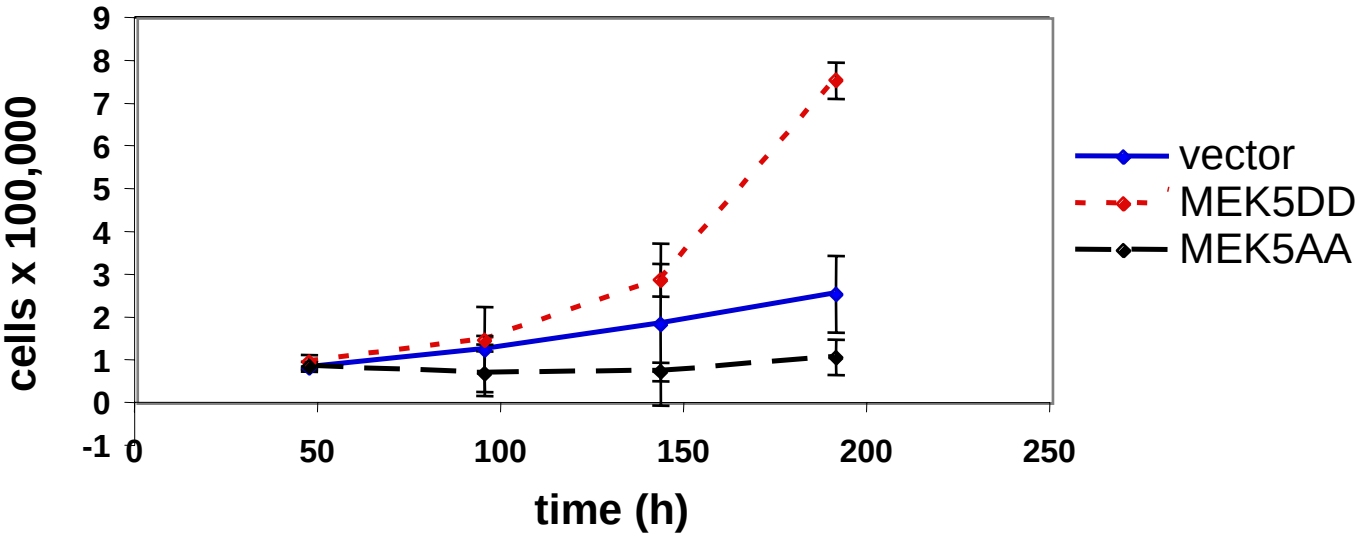
Supplementary Figure 4. **Constitutive activation of ERK5 does not alter surface expression of representative integrins or initial attachment of endothelial cells to a given substrate.** *A*, Expression of human integrins α5 (left panel), β1 (middle panel) or αvβ3 (right panel) was detected by flow cytometry in control HUVEC and EC transduced with retroviruses containing empty vector or MEK5 mutants. 104 cells were analyzed. Isotype control is indicated by black curves, while specific antibodies by grey curves. *B*, Trypsinized EC were plated onto fibronectin coated dishes and the attachment efficiency was measured 1 h later. Mean numbers of attached EC ± SD of three independent experiments are depicted versus concentration of fibronectin.



Supplementary Figure 5. **Expression of MEK5DD leads to reduced cell detachment**. The delayed detachment of MEK5DD transduced cells was measured in comparison to control or vector and MEK5AA transduced cells. 2x104 cells were plated onto gelatin-precoated 24-well plate dishes and incubated in HUVEC medium for 24h. After washing of cells with PBS, 0.5 ml of trypsin/EDTA solution was added to each well and cells were incubated for additional 60 or 120 seconds at room temperature. After that cells were fixed by addition of 0.5 ml of 4% glutharaldehyde, stained with crystal violet and the percentage of spread cells was scored. The experiment was repeated three times and the median values ± SD are shown.

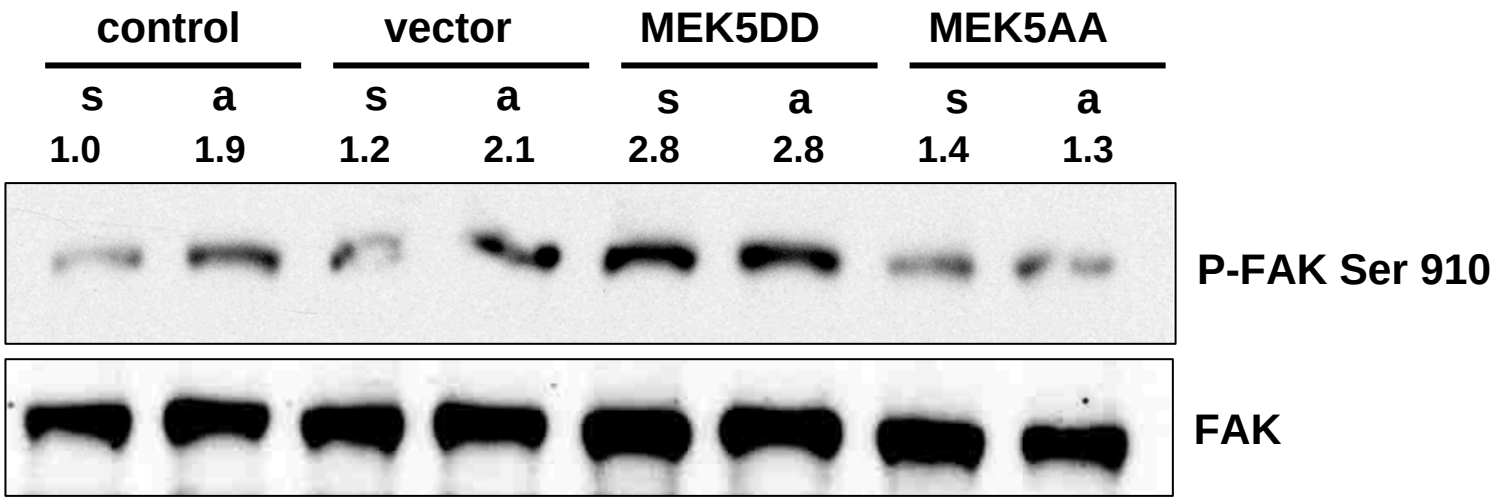

2 h
6 h
24 h
48 h
96 h
vector
MEK5DD

Supplementary Figure 6. **Constitutive activation of ERK5 alters F-actin organization.** HUVEC were plated on non-coated glass coverslips for times indicated, fixed with PFA and stained for F-actin with phalloidin-TRITC. As HUVEC vary significantly in their morphology, several cell images are shown. The upper three panel sets represent vector transduced cells and the lower three, the MEK5DD transduced cells. Scale bars: 20μm.



Supplementary Figure 7. **Constitutive activation of ERK5 enhances EC proliferation.** Equal amounts of EC transduced with empty vector or MEK5 mutants were plated in HUVEC medium into 6-well dishes and incubated for times indicated. After that cells were trypsinized and counted. Mean cell numbers of three independent experiments ± SD are depicted.

A



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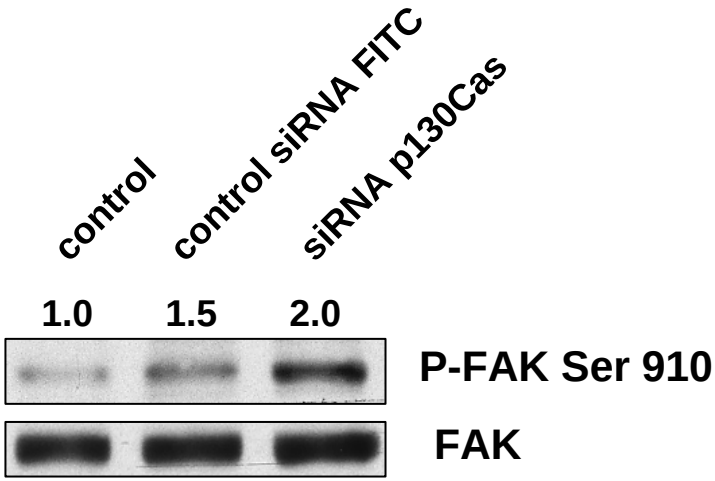

Supplementary Figure 8. **Constitutive activation of ERK5 results in hyperphosphorylation of FAK at S910.** Phosphorylation of FAK was analyzed by Western blot analysis with S910 phospho-specific antibodies (upper panel). Immunoblotting with a FAK-specific antibody served as loading controls (lower panel). Phosphorylation intensity was estimated densitometrically and presented as relative intensities of the phospho-bands to the appropriate loading controls.