Supplemental Materials

Molecular Biology of the Cell

Mooren et al.

Supplemental Figure 1. A) WAVE2 expression was decreased by treatment with siRNA for 72 hrs. We observed a similar level of KD at 48 hrs post-siRNA treatment. B) Latrunculin A disrupts cell-cell adhesion in endothelial monolayers. Monolayers were treated with LatA (1 μM, 15 min), then stained for ICAM-1 and F-actin. C) Loss of WAVE2 increases endothelial monolayer permeability, measured with fluorescent dextran and plotted as normalized permeability index. Data is derived from experiments on three or more days, with three or more repetitions on each day. Plotted values are the mean of all the data points, and error bars are standard deviation of the mean ($N \geq 9$).

Supplemental Figure 2. S1P causes membrane ruffling at cell-cell contacts in endothelial monolayers. Monolayers were treated with S1P (0.5 μM, 5 min), then fixed and stained for cortactin.

Supplemental Figure 3. WAVE2 depletion from endothelial monolayer has little to no effect on ZO-1. A) WAVE2-depleted endothelial monolayers contain many gaps that are devoid of ZO-1 staining (bottom row). Cell-cell junctions that persist in WAVE2-knockdown monolayers contain ZO-1 at levels comparable to those of junctions in control monolayers. The yellow outlines denote gaps in the WAVE2-depleted monolayers. B) Immunoblot for ZO-1 in control and WAVE-2 depleted ECs.

Supplemental Figure 4. WAVE2 is important for junctions to re-assemble following calcium depletion / repletion. Calcium was removed from the medium with EGTA, to disassemble cadherin-based junctions, and then replaced, to induce re-assembly. Control and WAVE2-depleted monolayers stained for VE-cadherin and F-actin are shown. Images are representative, selected from five fields of view in each of three separate samples. Scale bar = 50 μm.

Supplemental Figure 5. A) WAVE2 influences the organization of myosin in endothelial cells. Control and WAVE2-depleted monolayers were fixed and stained for myosin-IIA. In the WAVE2-knockdown monolayer, spread cells display increased circumferential myosin staining. Cells that are rounded up and blebbing (red asterisks) do not show myosin-II circumferential fibers. B) WAVE2 depletion from ECs does not affect F-actin levels. Immunoblot of detergent-resistant F-actin cytoskeletons.

SUPPLEMENTAL MOVIE LEGENDS

Supplemental Movie 1. Lymphocyte undergoing the transcellular route of transmigration. EC expresses membrane-tagged GFP. Images collected every 10 s for 15 min. Movie plays at 7 frames per second (fps).

Supplemental Movie 2. WAVE2 is not recruited to during early phase of the paracellular route of lymphocyte transmigration. ECs express WAVE2-GFP. Images collected every 20 s for 30 min. Movie plays at 7 fps.

Supplemental Movie 3. WAVE2 localizes to protrusive membrane waves that repair paracellular transmigration gaps. This membrane wave repairs the paracellular gap seen in Supplemental Movie 2. ECs express WAVE2-GFP. Images collected every 20 s for 30 min. Movie plays at 7 fps.

Supplemental Movie 4. WAVE2 is important for cell-cell adhesion during lymphocyte transmigration. Monolayer of WAVE2-depleted ECs following lymphocyte addition. Images collected every 20 s for 30 min. Movie plays at 7 fps.

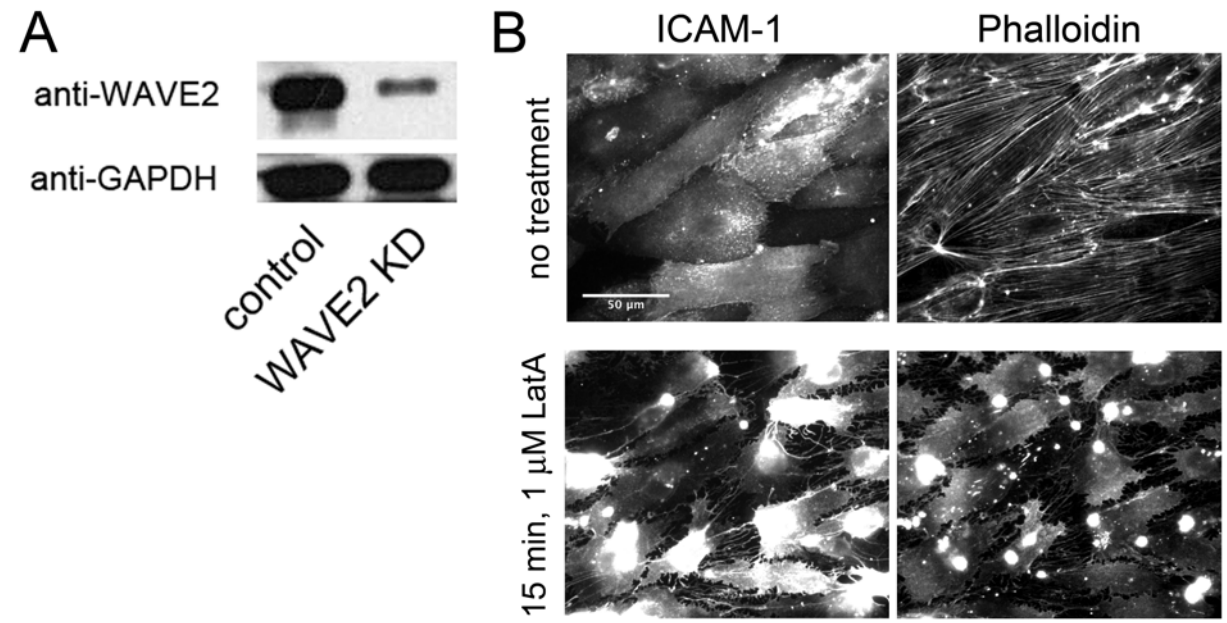
Supplemental Movie 5. Dynamic reorganization of cell-cell contacts during lymphocyte transmigration. Lymphocytes added to control cell monolayers. Images collected every 20 s for 30 min. Movie plays at 7 fps. Scale bar applies for Movies 6 and 7.

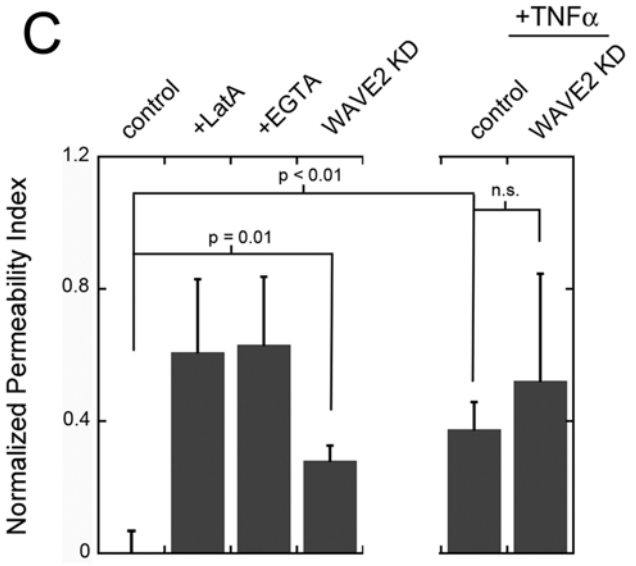
Supplemental Movie 6. Rescue of cell-cell junctions by expression of siRNA-resistant WAVE2 in WAVE2-knockdown EC monolayer. At time zero, the culture medium was replaced. Images collected every 20 s for 30 min. Movie plays at 7 fps.

Supplemental Movie 7. Endothelial cell-cell junctions display dynamic ruffling within a monolayer. Arrow and arrowhead denote two examples of the beginning of a junctional membrane ruffle. Images collected every 10 s for 30 min. Movie plays at 7 fps.

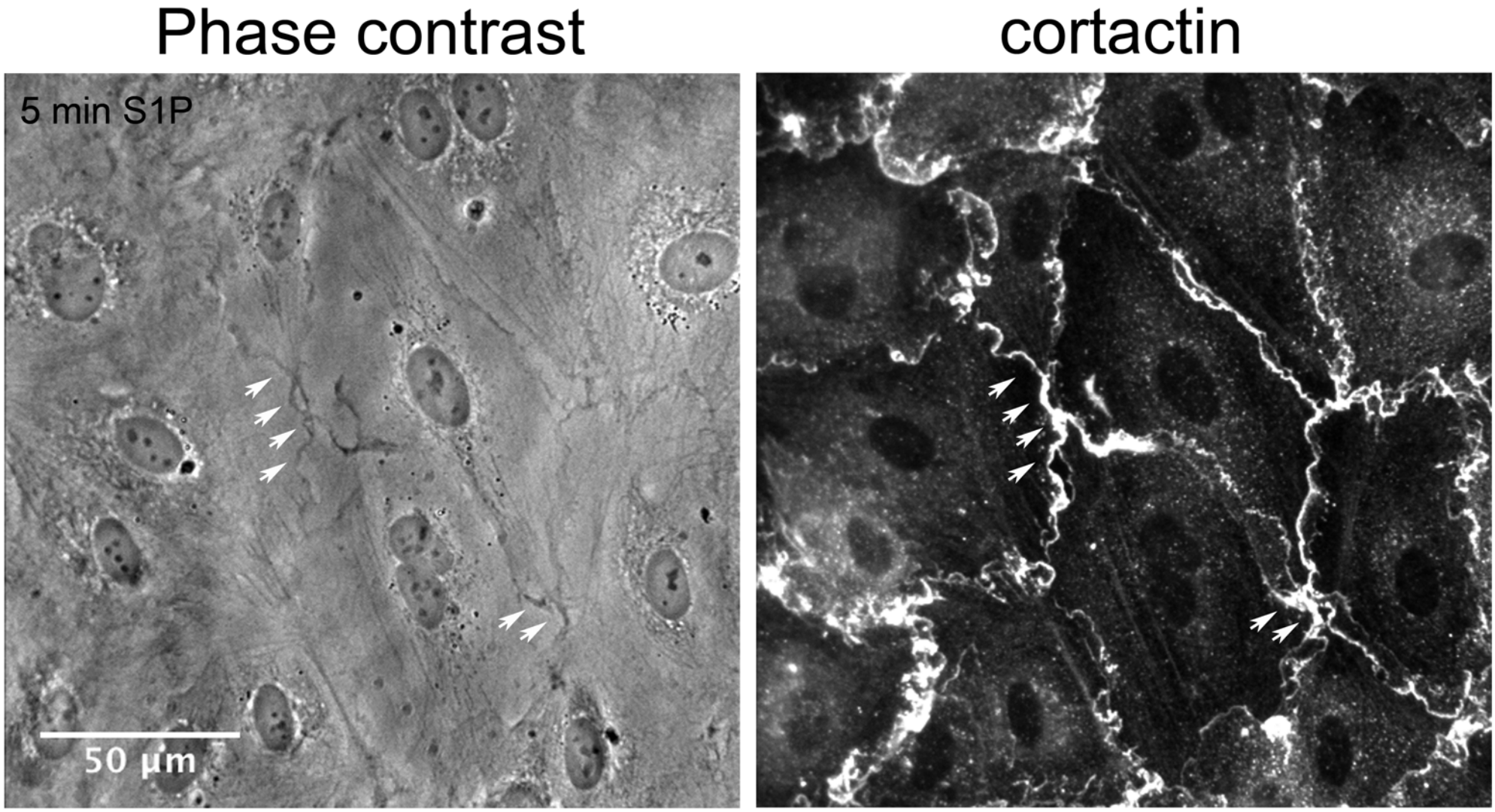
Supplemental Movie 8. ECs display WAVE2 localized to dynamic ruffles at edges along sites of cell-cell contact following lymphocyte addition. PBLs were added 2 minutes prior to the movie collection. Images collected every 20 s for 8 min. Movie plays at 7 fps.

O.L. Mooren et al., Suppl. Figure 1

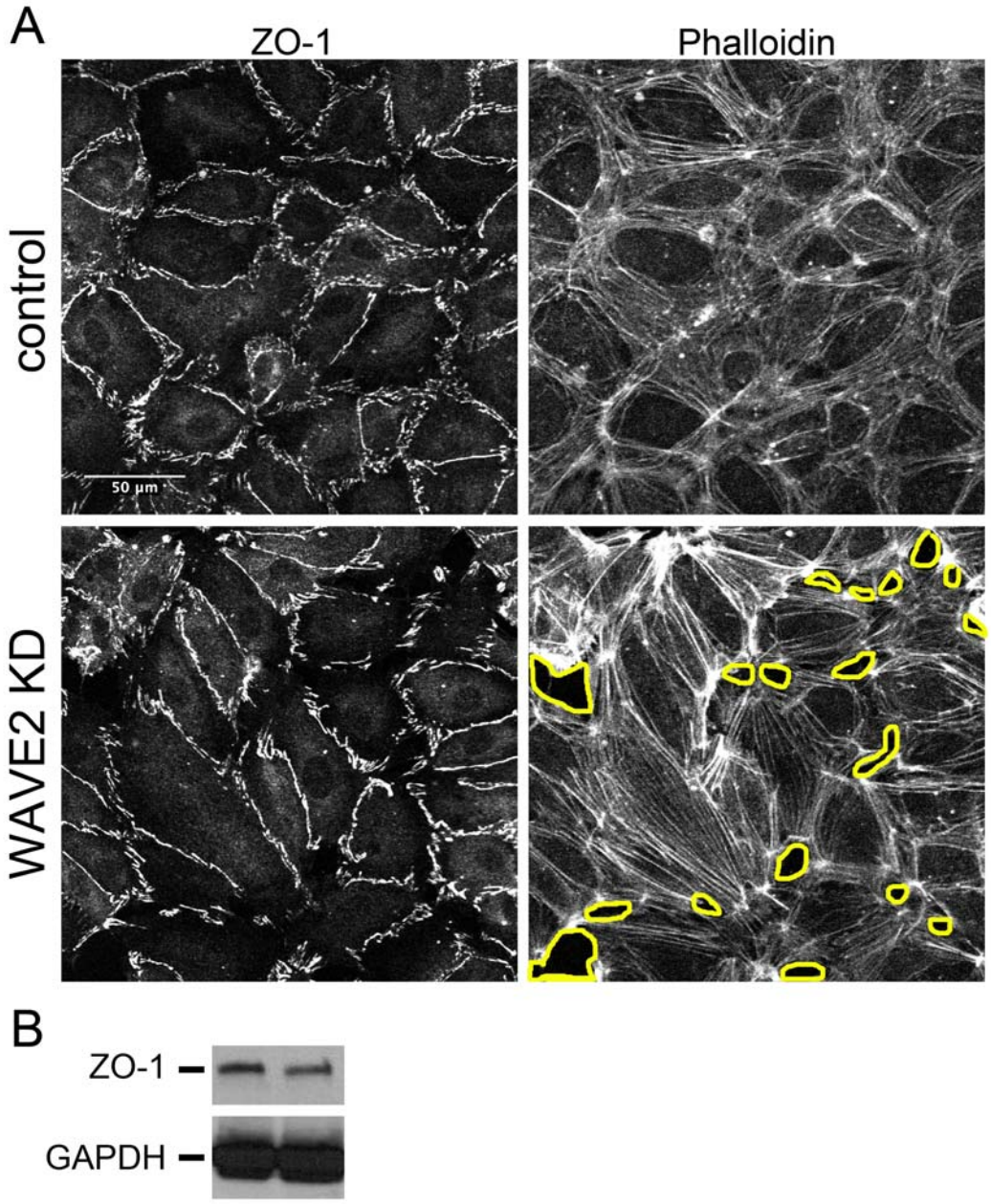




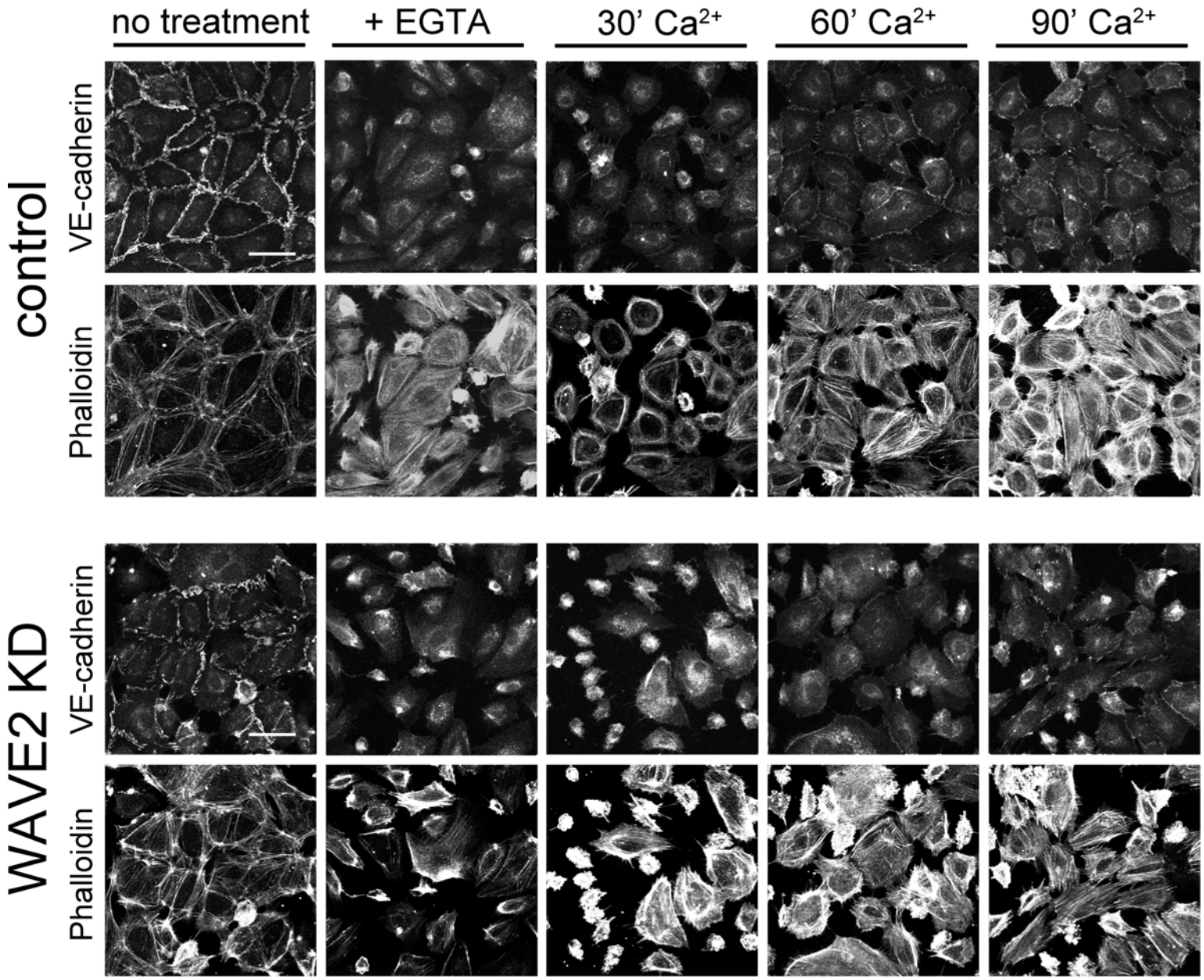
O.L. Mooren et al., Suppl. Figure 2



O.L. Mooren et al., Suppl. Figure 3



O.L. Mooren et al., Suppl Figure 4



O.L. Mooren et al., Suppl. Figure 5