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Supplemental Information

The Leucine-Rich Repeat Region of CARMIL1 Regulates IL-1-Mediated ERK Activation, MMP Expression, and Collagen Degradation

Qin Wang, Karambir Notay, Gregory P. Downey, and Christopher A. McCulloch

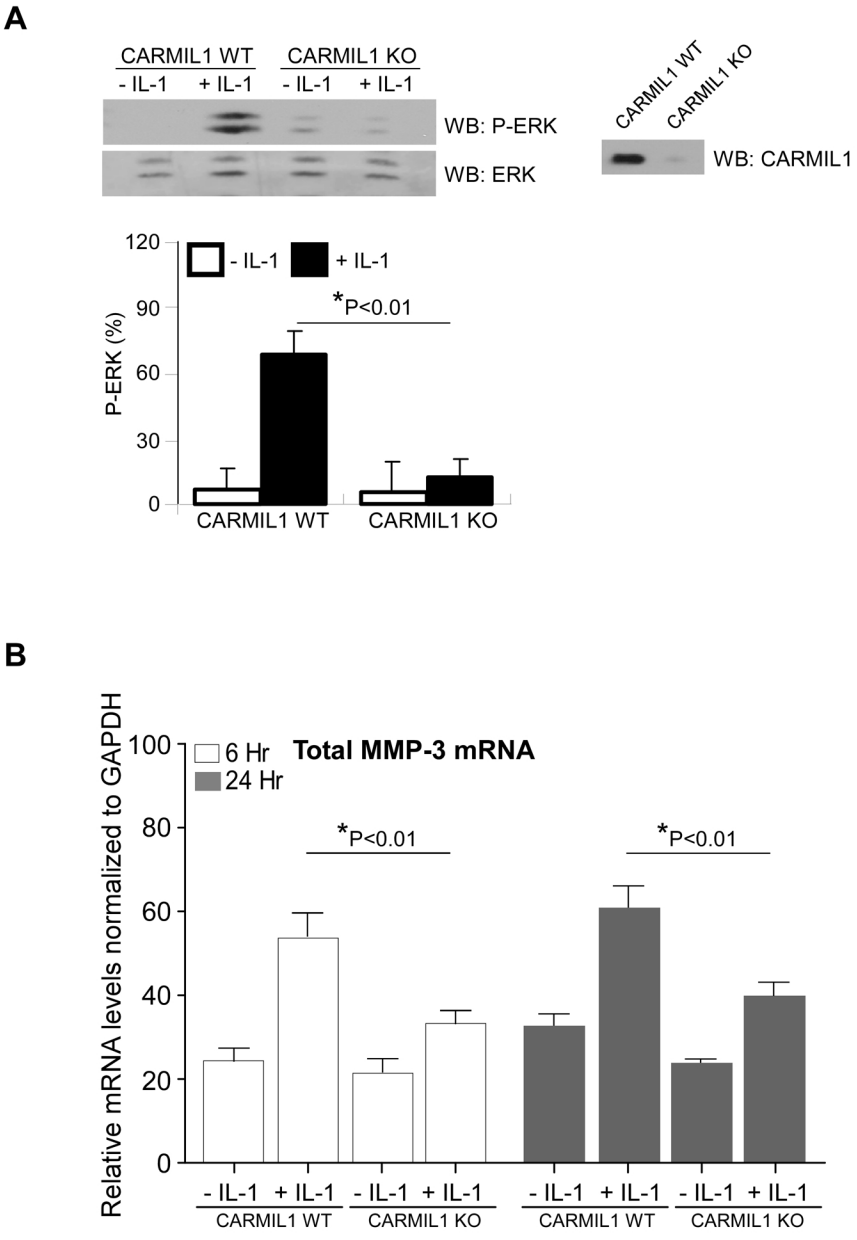
Supplementary Figure 1. IL-1-induced ERK phosphorylation and MMP3 expression are CARMIL1-dependent. Related to STAR Methods. *A*) Immortalized Human gingival fibroblasts- hTERT (CARMIL1 WT) and CARMIL1 deletion by using CRISPR/Cas9 genome editing technology (CARMIL1 KO) were plated on fibronectin (*FN*) for 24 hr, serum starved for 6 hrs., then treated without or with IL-1 (20 ng/ml). Whole cell lysates were immunoblotted for p(T202/Y204)-ERK and total ERK. Lysates of CARMIL1 WT and CARMIL1 KO cells were also immunoblotted for CARMIL1 to confirm CARMIL1 KO, β-Actin was used for loading control. Ratios of p(T202/Y204)-ERK to ERK were quantified by densitometry. Densitometry data were assessed by ANOVA and are expressed as means ± SE. *B*) CARMIL1 WT and CARMIL1 KO cells were treated without or with IL-1 (20 ng/ml) for 6 hr and 24 hr. Total RNA was extracted, and MMP-1, MMP-3, MMP-9 and MMP-14 mRNA levels were quantified by qRT-PCR analysis. Values are mean ± SE from $n = 3$ independent experiments.

Supplementary Figure 2. Effect of CARMIL1 KD on adhesion formation. Related to STAR Methods. HGFs were transfected with siRNA Control and siRNA CARMIL1 and plated on FN for 20 min, 40 min, 1 hr and 2 hr in the presence of IL-1 and coimmunostained for CARMIL1 and paxillin. *A*) Confocal microscopy images of immunofluorescence localization of CARMIL1 (red) and paxillin (green) in cells spreading on FN. *B)* number of focal adhesions/cell in the cell periphery (<5 μm from the cell membrane, $n = 10$-15 cells/group, upper panel), focal adhesions area/cell ($n = 10$-15 cells/group, middle panel), and length of focal adhesions in cell periphery ($n = 10$-15 cells/group, lower panel) were evaluated by ImageJ from the paxillin images. Data were quantified using ImageJ. Differences between groups were measured by ANOVA.

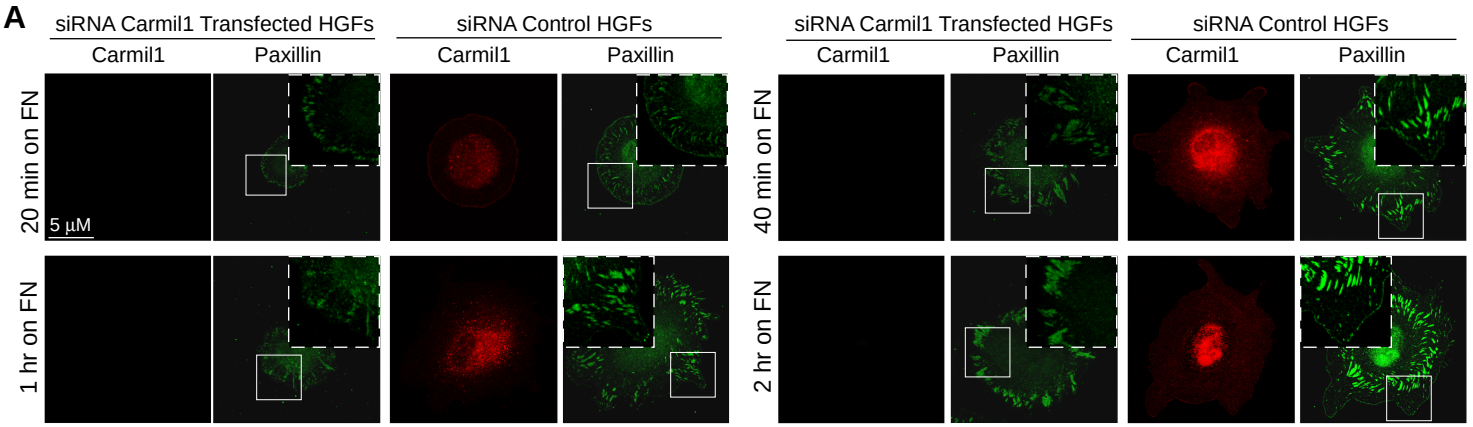
Supplementary Figure 3. Effect of CARMIL1 knock down on cell spreading and extension formation. Related to STAR Methods. HGFs were transfected with siRNA Control and siRNA CARMIL1 and plated on FN for 20 min, 40 min, 1 hr and 2 hr in the presence of IL-1 and coimmunostained for CARMIL1 and phalloidin. *A*) Confocal microscopy images of immunofluorescence of CARMIL1 (red) and phalloidin (actin signals, blue) in cells spreading on FN. *B)* Cell surface area ($n = 10$-15 cells/group, upper panel), number of extensions/cell ($n = 10$-15 cells/group , middle panel), and length of cell extension ($n = 10$-15 cells/group, lower panel) were evaluated by ImageJ from the phalloidin images. Data were quantified using ImageJ. Differences between groups were measured by ANOVA.

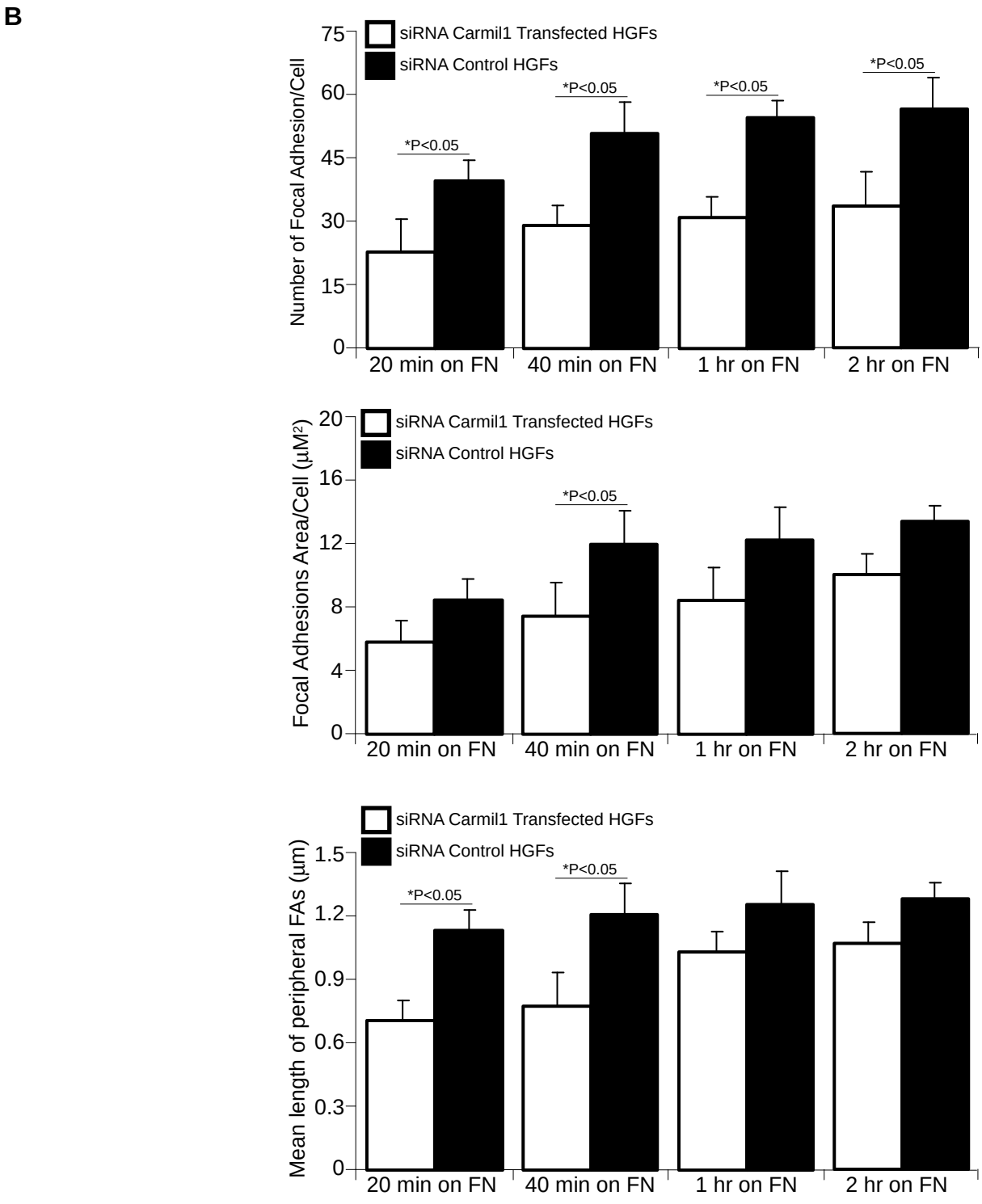
Supplementary Figure 4. Effect of CARMIL1 knock out on adhesion formation and cell spreading. Related to STAR Methods. CARMIL1 deletion by using CRISPR/Cas9 genome editing technology (CARMIL1 KO) and immortalized Human gingival fibroblasts- hTERT (CARMIL1 WT) and were plated on fibronectin (*FN*) for 30 min, 2 hr, and 5 hr in the presence of IL-1 and coimmunostained for CARMIL1, paxillin and phalloidin. *A*) Confocal microscopy images of immunofluorescence localization of CARMIL1 (red) paxillin (green) in cells spreading on fibronectin. *B)* Number of focal adhesions/cell in the cell periphery (<5 μm from the cell membrane, $n = 10$-15 cells/group, left upper panel), length of focal adhesions in cell periphery ($n = 10$-15 cells/group, right upper panel), number of extensions/cell ($n = 10$-15 cells/group, left lower panel), and length of cell extension ($n = 10$-15 cells/group, right lower panel), were evaluated by ImageJ from the paxillin images. Data were quantified using ImageJ. Differences between groups were measured by ANOVA.

Supplementary Figure 1

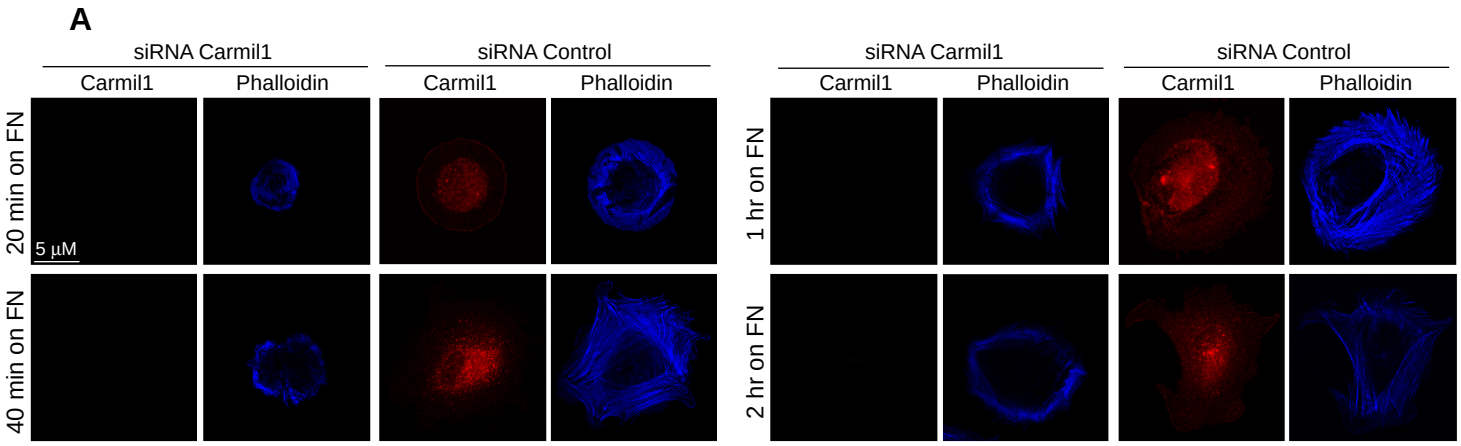


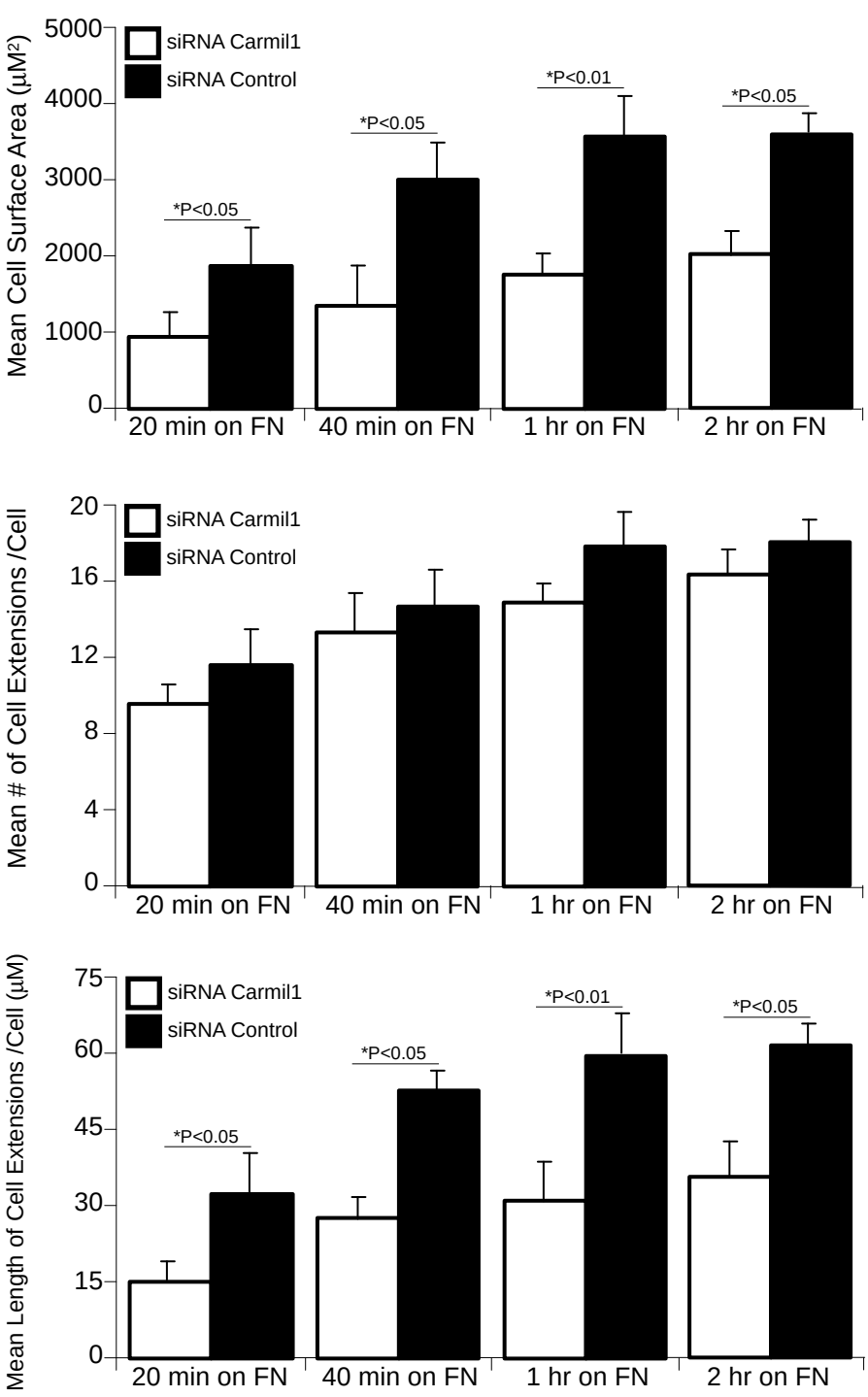
Supplementary Figure 2





Supplementary Figure 3





Supplementary Figure 4