Discoidin Domain Receptor 2 Regulates Neutrophil Chemotaxis in 3D Collagen Matrices

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SUPPLEMENTAL INFORMATION

I- MOVIE LEGENDS

Movie 1 – Neutrophil migration on a 2D collagen coated surface.
EZ-Taxiscan chemotaxis of primary human neutrophils on a collagen coated surface (treated or not with rDDR) to 500 nM IL-8. Frames were taken every 30 s for 20 min.

Movie 2 – Neutrophil chemotaxis in a 3D collagen lattice.
Neutrophils were embedded in collagen I. Neutrophil chemotaxis to 50 nM IL-8 was recorded. Frames were taken every 30 s for 15 min.

Movie 3 – Neutrophil chemotaxis in a 3D collagen lattice treated with rDDR.
Neutrophils were embedded in collagen I treated with 15 μg/mL rDDR. Neutrophil chemotaxis to 50 nM IL-8 was recorded. Frames were taken every 30 s for 15 min.

Movie 4 – Neutrophil chemotaxis in a 3D collagen lattice treated with GM6001.
Neutrophils were embedded in collagen I. Neutrophil chemotaxis to 50 nM IL-8, in the presence of 25 μM GM6001 was recorded. Frames were taken every 30 s for 15 min.

II- SUPPLEMENTAL FIGURES

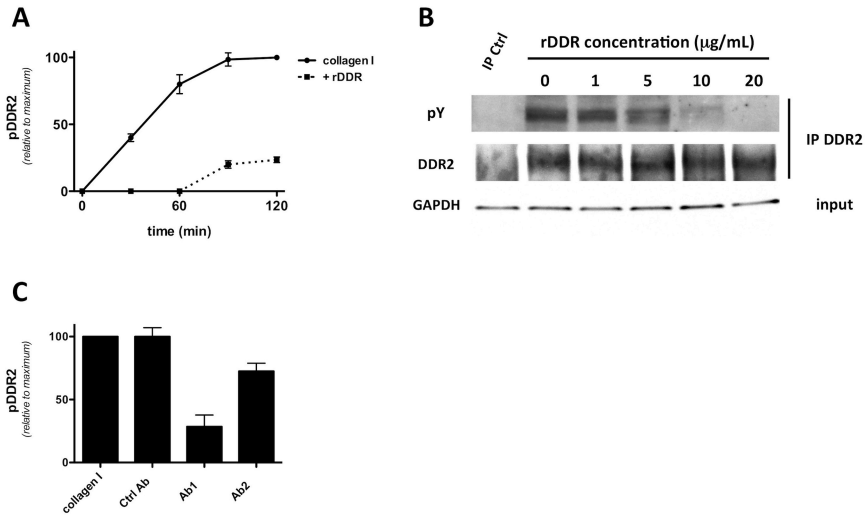

Figure S1 – Related to Figure 1.

A- Kinetics of DDR2 activation

Human primary neutrophils were cultured in the presence of collagen I (pretreated with rDDR or not). DDR2 was immunoprecipitated from cell lysates at different times of collagen treatment. Results represent the normalized western blot intensity (average ± SEM) of 3 independent experiments.

B- DDR2 activation in inhibited in a dose-dependent manner by rDDR

Human primary neutrophils were embedded for 1 h in collagen I, pretreated with different concentrations of rDDR. Cells were lysed and DDR2 was immunoprecipitated.

C- DDR2 blocking antibodies inhibit DDR2 phosphorylation

Human primary neutrophils were pretreated for 30 min with blocking DDR2 antibodies, and cultured in collagen I for 1h. DDR2 was immunoprecipitated from cell lysates. Results represent the normalized western blot intensity (average ± SEM) of 3 independent experiments.

A

	Collagen I	+ rDDR
CI	0.78 ± 0.05	0.75 ± 0.04
V (μm/min)	13.4 ± 2.1	12.8 ± 1.9

B

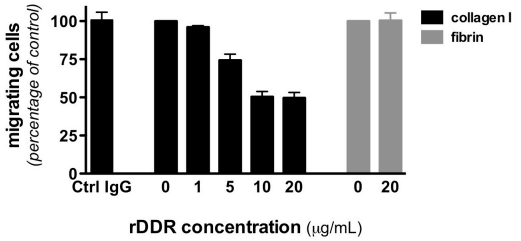

Figure S2 – Related to Figure 2

A- Neutrophil migration on a collagen-coated surface (EZ-Taxiscan) is not altered by rDDR treatment

Neutrophil migration to 500 nM IL-8 on a collagen I-coated surface (pretreated or not with rDDR) was measured in a EZ-Taxiscan assay. Results represent the speed and chemotaxis index (average ± SEM) of 3 independent experiments.

B- Neutrophil migration through a collagen-coated transwell is inhibited in a dose-dependent manner by rDDR

Transwells were coated with collagen I (black) or fibrin (grey), pretreated with different concentrations of rDDR. Migration of human primary neutrophils to 1 μM LTB_4 was determined after 6 h. Results represent the average ± SEM of 3 independent experiments.

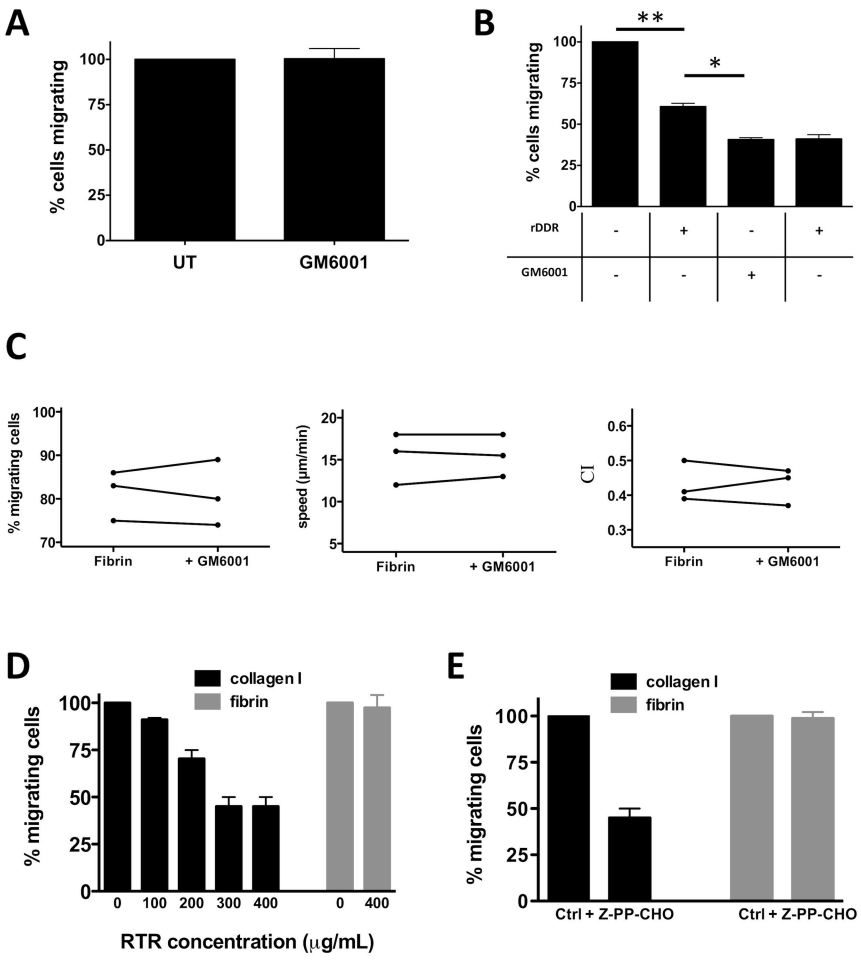

Figure S3 – Related to Figure 5.

A- GM6001 treatment does not affect neutrophil migration through fibrin-coated transwells

Human primary neutrophils were treated with 25 mM GM6001 and migration to 500 nM IL-8 through fibrin-coated transwells was determined after 6 h. Results represent the relative percentage of migrating cells (average ± SEM) of 3 independent experiments.

B- DDR2-dependent neutrophil migration correlates with MMP activity

The number of neutrophils (treated or not with 25 mM GM6001) migrating through collagen-coated transwells (pretreated or not with rDDR) was determined after 6 h.

Results represent the relative percentage of migrating cells (average ± SEM) of 4 independent experiments. *$p < 0.05$, Friedman test; Dunn's post hoc test.

C- GM6001 treatment does not affect neutrophil chemotaxis in a fibrin matrix

Human primary neutrophils were pretreated with 25 mM GM6001 for 30 min and embedded in fibrin. Migration of neutrophils to 50 nM IL-8 (in the presence of the inhibitor) was recorded. The percentage, average speed and CI of neutrophils migrating were determined from 3 independent experiments.

D- Neutrophil migration through a collagen-coated transwell is inhibited in a dose-dependent manner by RTR

Transwells were coated with collagen I (black) or fibrin (grey). Migration of human primary neutrophil to 500 nM IL-8 in the presence of RTR was determined at 6 h. Results represent the average ± SEM of 3 independent experiments.

E- PE inhibitor reduces neutrophil migration through collagen, but not fibrin

Human primary neutrophil were pretreated with 100 mM Z-PP-CHO for 30 min and migration to 500 nM IL-8 through collagen I- of fibrin-coated transwells was determined after 6 h. Results represent the average ± SEM of 3 independent experiments.