Supplementary Information

Agarose Spot as a Comparative Method for *in situ* Analysis of Simultaneous Chemotactic Responses to Multiple Chemokines

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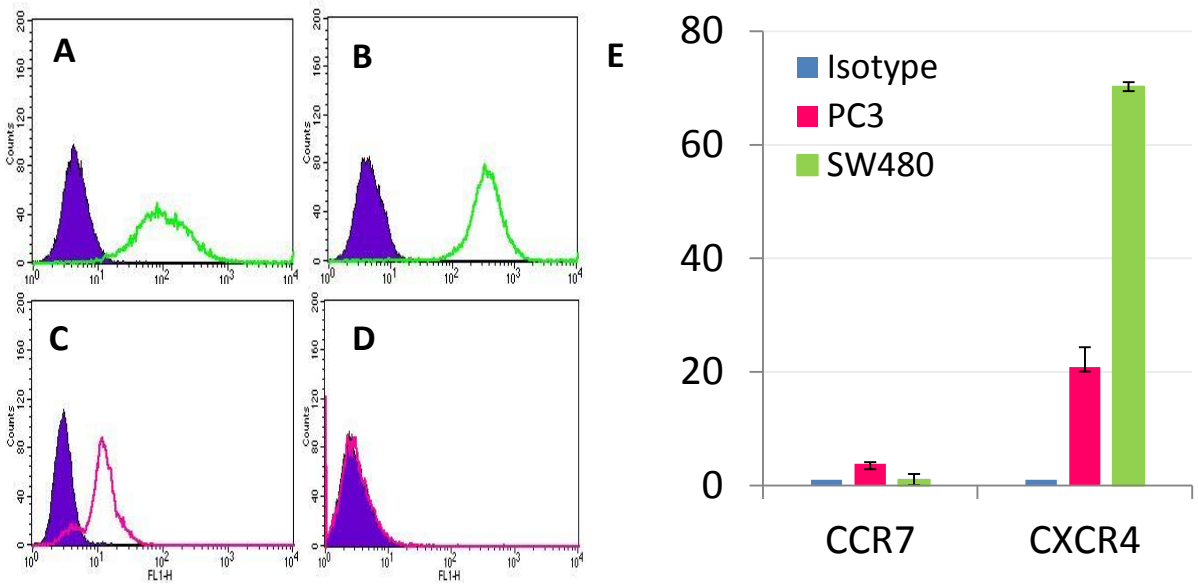
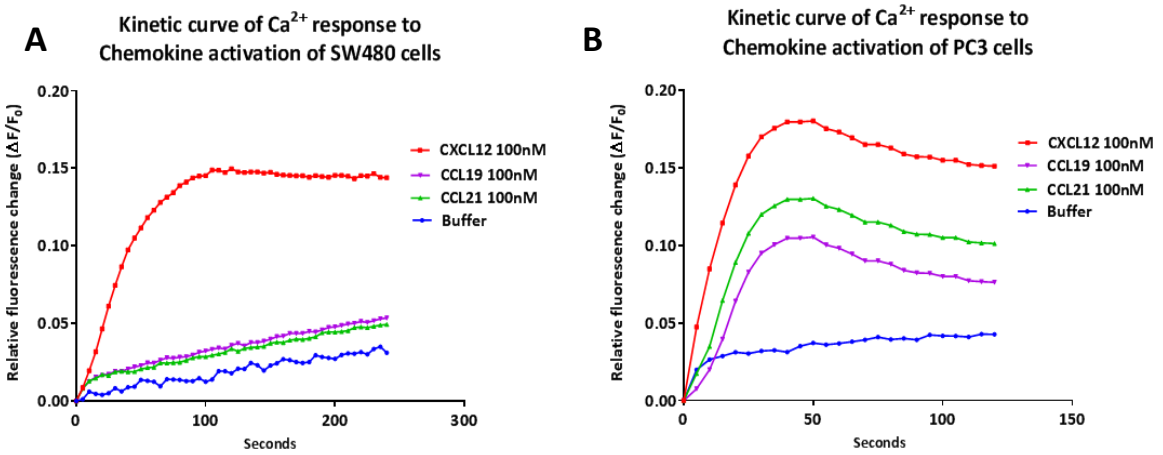
Supplementary Information S1. (**A**) Video showing movement of PC-3 cells under an agarose spot containing CCL21. (**B**) Video showing tracking of PC-3 cells movements adjacent to an agarose spot containing CCL21. (**C**) Video showing tracking of PC-3 cells movements under an agarose spot containing CCL21.

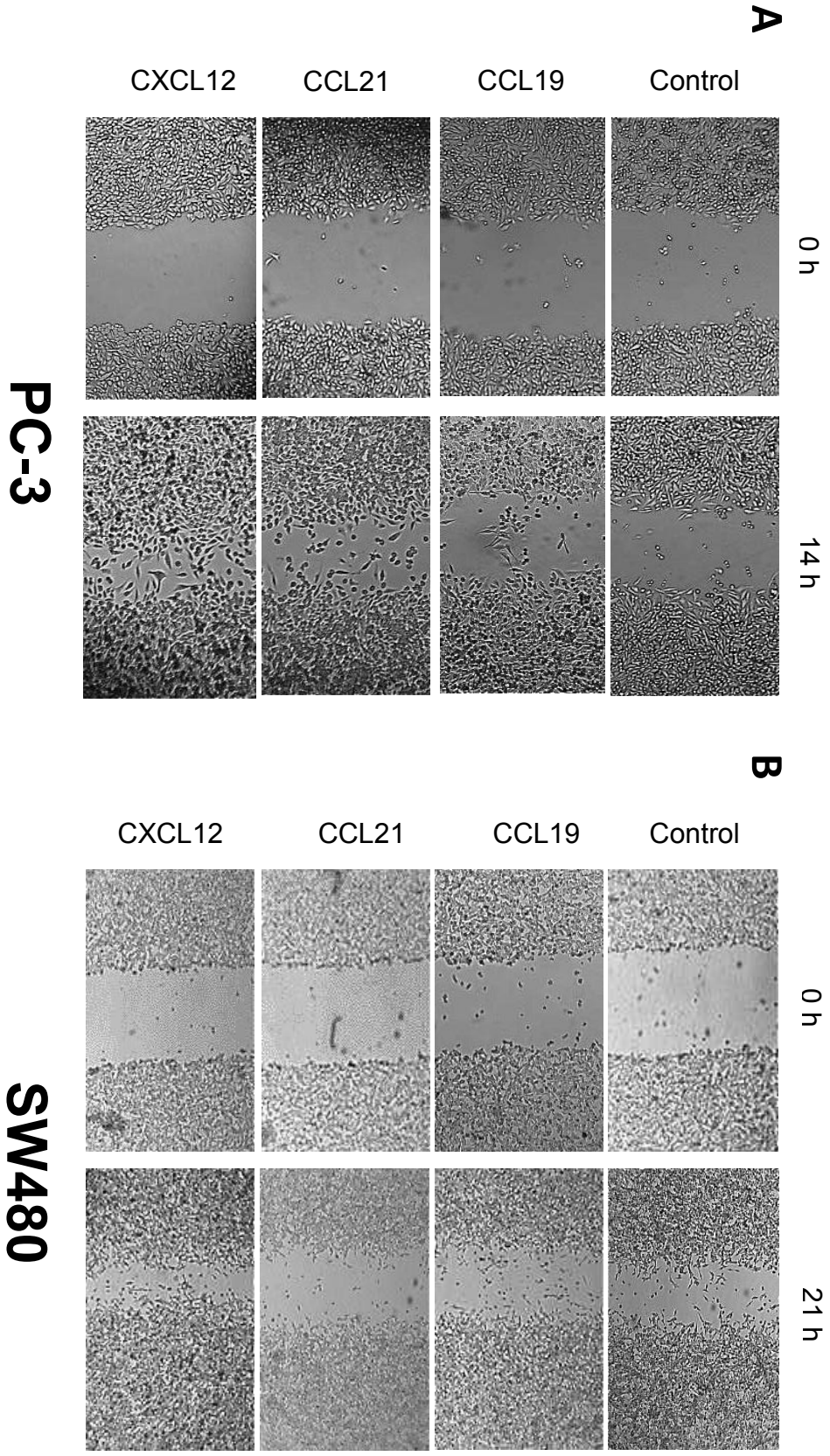
Supplementary Information S2. A video tutorial for agarose spot assay



Supplementary Information S3. Expression of CXCR4 (green) compared with isotype (filled purple) in: (**A**) PC-3 cells; (**B**) SW480 cells; Expression CCR7 (cyan) compared with isotype (filled purple) in: (**C**) PC-3 cells; (**D**) SW480 cells; (**E**)Relative expression of CCR7 and CXCR4 relative to isotype control.



Supplementary Information S4. (**A**) Calcium mobilisation (Ca^{2+} flux) assay shows SW480 cells respond to 100 nM CXCL12 (red), but not to CCL21 (green), and CCL19 (purple). (**B**) calcium mobilisation (Ca^{2+} flux) assay shows PC-3 cells respond to 100 nM CXCL12 (red), CCL21 (green), and CCL19 (purple). Control (assay buffer, blue) shown for comparison.



Supplementary Information S5. (**A**) Scratch assay showing the responses to CXCL12, CCL21, CCL19 (10nM) and control for PC-3 cells. (**B**) Scratch assay showing the responses to CXCL12, CCL21, CCL19 (10nM) and control for SW480 cells.