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"N-acetylated Proline-Glycine-Proline induced G-protein dependent chemotaxis of neutrophils is independent of CXCL8 release."

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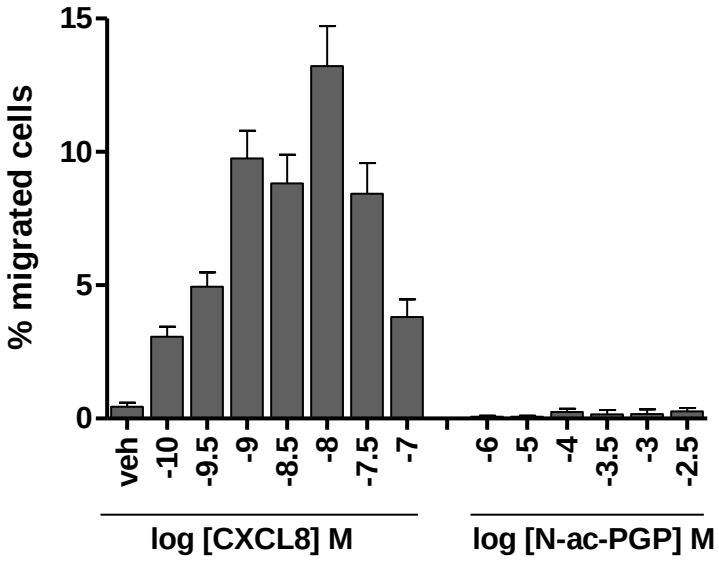

Suppl. Fig. 1: Chemotactic response to CXCL8 or N-a-PGP of L1.2 cells expressing human CXCR2.

Cell culturing and transfection

The murine pre-B L1.2 cells (kindly provided by Dr. Pease, Imperial College London, London, UK) were grown in RPMI 1640 medium with GlutaMax-I and 25 mM HEPES, supplemented with 10% heat-inactivated certified FBS, penicillin, streptomycin, glutamine, nonessential amino acids, 2-mercaptoethanol, and sodium pyruvate. L1.2 cells were transfected with 10 µg receptor/1x10^7 cells using a Bio-Rad Gene Pulser Xcell (330 V and 975 µF), and subsequently grown overnight in culture medium supplemented with 10 mM sodium butyrate.

Chemotaxis L1.2 cells

Twenty-four hours after transfection, migration of L1.2 cells towards CXCL8 or N-ac-PGP was determined using 5-µm pore ChemoTx 96-well plates (Neuro Probe). First the lower wells of the ChemoTx plates were blocked for 30 min using RPMI 1640 medium with GlutaMAX-I and 25 mM HEPES supplemented with 1% (w/v) BSA. CXCL8 or N-ac-PGP was diluted in the same medium supplemented with 0.1% (w/v) BSA, and dispensed in the bottom wells of the chemotaxis plate after removing the blocking buffer. The membrane was placed on top of these wells and 2.5 $x10^5$ cells in the same buffer were applied to the upper surface (total 31 ul) and incubated for 4 h in a humidified chamber at 37°C in the presence of 5% CO_2. The number of cells that traversed the 5-µm pore membrane and migrated into the bottom wells was quantified on the Victor2 1420 multilabel plate reader upon the incorporation of the Calcein AM dye (Invitrogen). Data are shown as the percentage migrated cells.