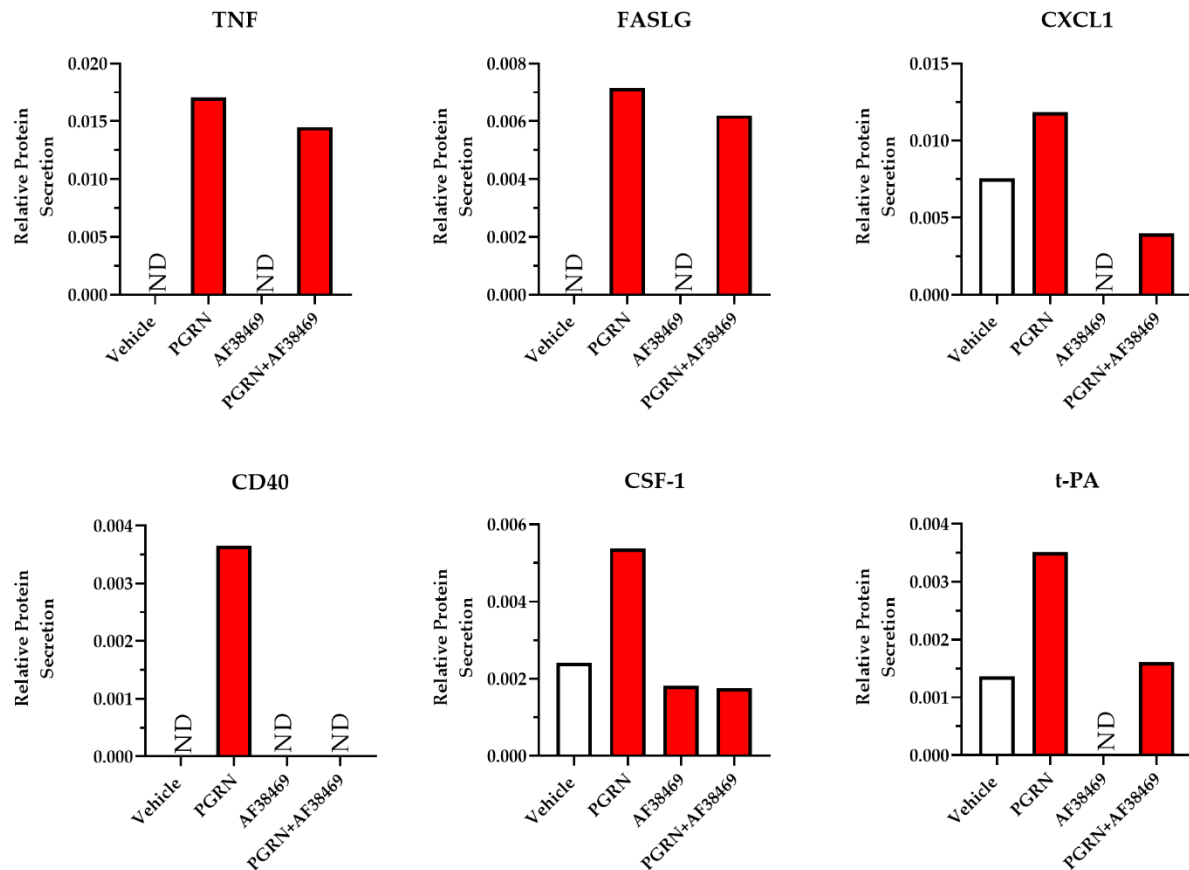
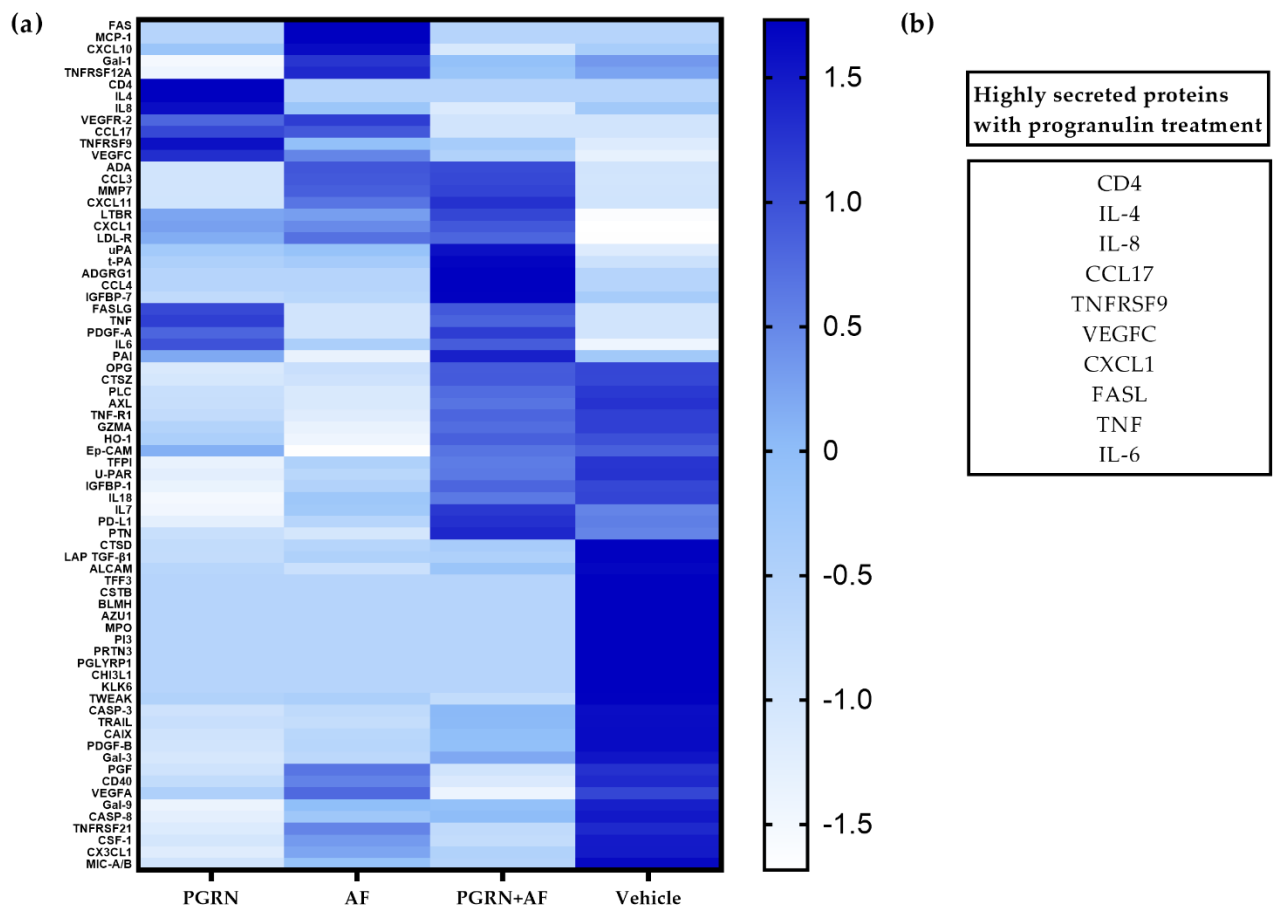


# Interleukin-6 induces stem cell propagation through liaison with the sortilin-progranulin axis in breast cancer

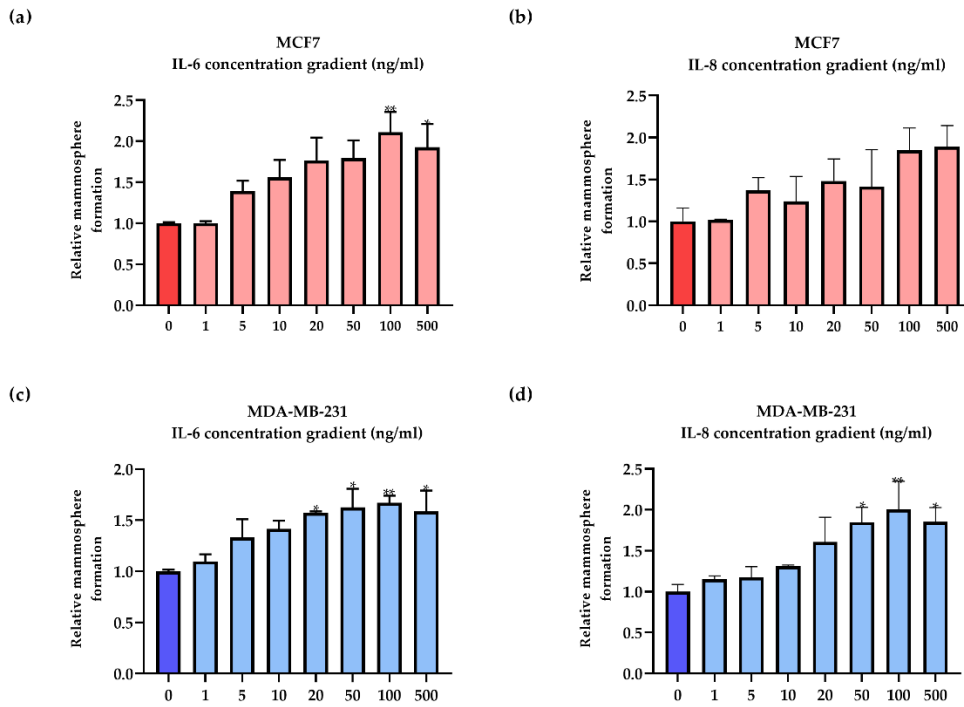
## Supplemental figures



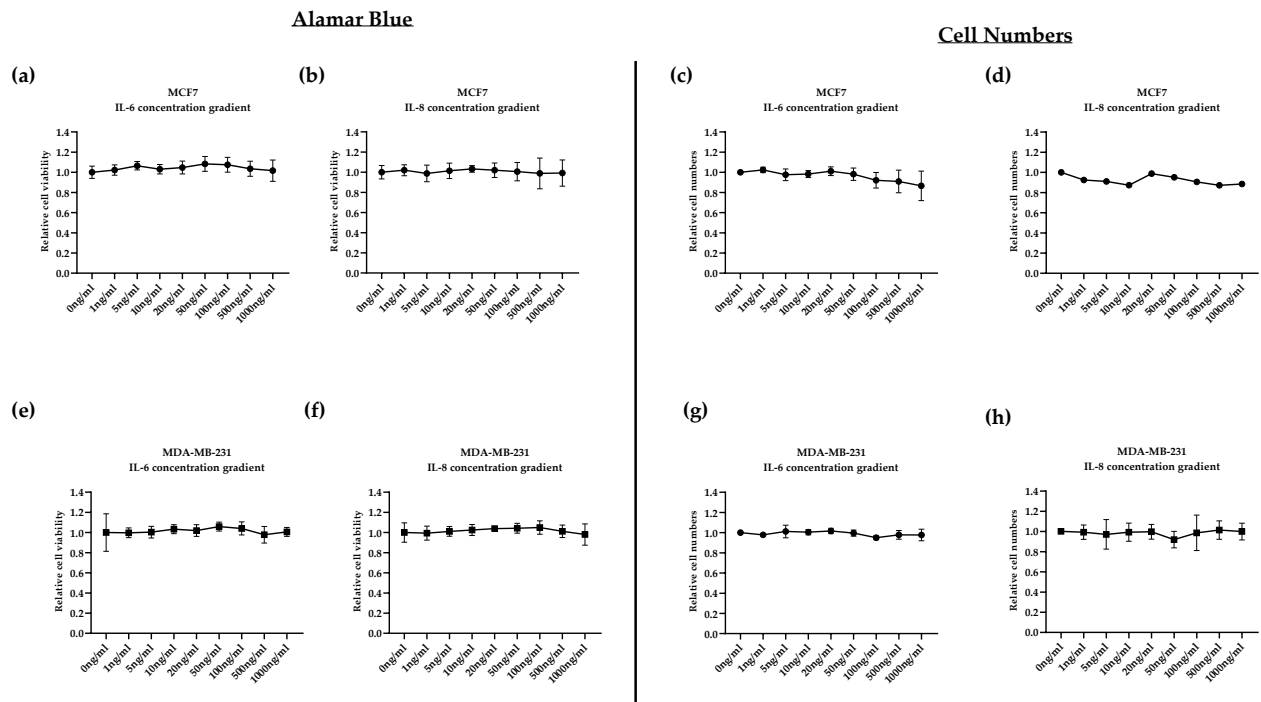
**Figure S1.** Changes in protein secretion after treatment with progranulin and AF38469 in MCF7 cells. MCF7 cells were untreated (vehicle), treated with 1  $\mu$ g/ml progranulin or 2  $\mu$ g/ml AF38469, or a combination, for 48 h. Bar charts showing increased secretion of TNF, FASLG, CXCL1, CD40, CSF-1 and t-PA in MCF7 cells treated with progranulin. Protein secretion was reduced in CXCL1, CD40, CSF-1 and t-Pa after treatment with AF38469. Data includes one biological replicate, ND: not detected, PGRN: progranulin, CXCL1: Chemokine (C-X-C motif) ligand 1, CD40: cluster of differentiation 40, TNF: tumor necrosis factor, FASL: tumor necrosis factor ligand superfamily member 6, CSF-1: colony stimulating factor 1, tPa: tissue plasminogen activator.



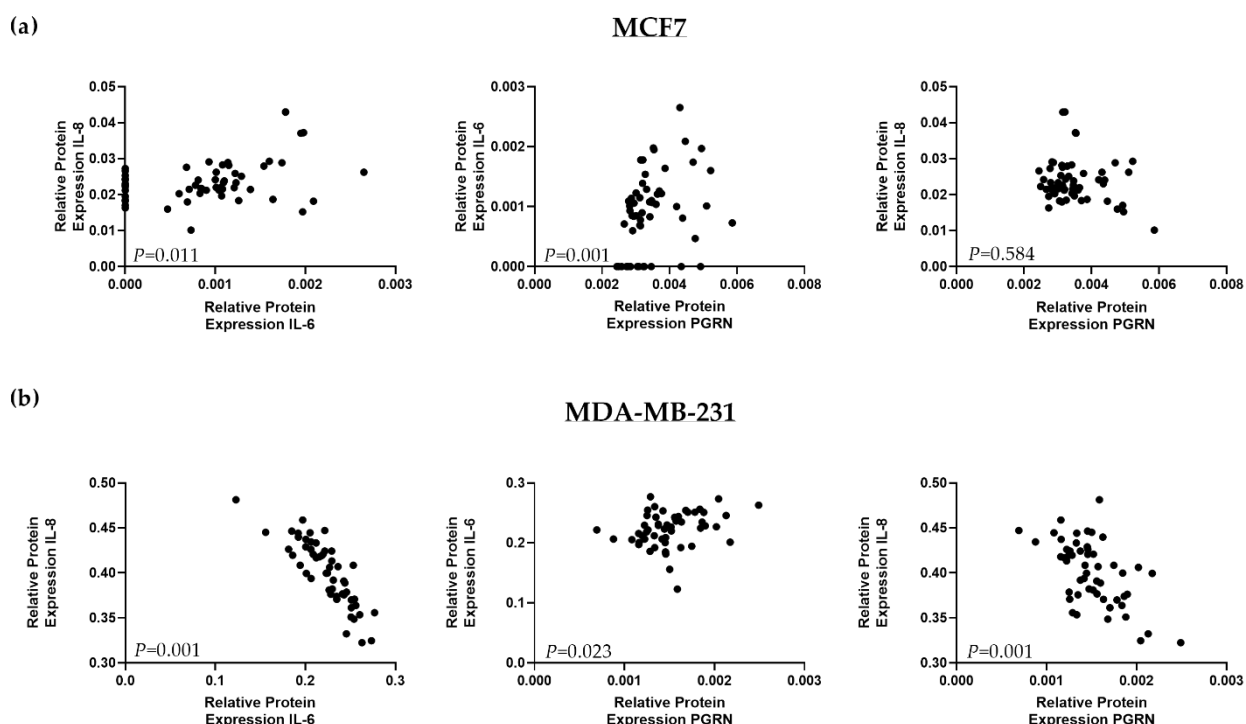
**Figure S2.** Progranulin treatment increased IL-6 and IL-8 secretion in breast cancer cells. (a) Heat map showing secreted proteins and cytokines in MDA-MB-231 cells. The cells were untreated (Vehicle), treated with 1  $\mu\text{g/ml}$  progranulin or 2  $\mu\text{g/ml}$  AF38469, or a combination, for 48 h. The data is auto-scaled and each protein is normalized to the total secretion of each sample. (b) Table of highly secreted proteins with progranulin treatment. Data represents one biological replicate. PGRN: progranulin, IL: interleukin.



**Figure S3.** IL-6 and IL-8 induced mammosphere formation in MCF7 and MDA-MB-231 cells. Mammosphere-forming capacity in (a, b) MCF7 and (c, d) MDA-MB-231 cells treated with IL-6 or IL-8 for 48 h. The bar charts represent the relative number of spheres formed in control samples against various concentrations of IL-6 or IL-8, five days after cell seeding in non-adherent polyHEMA-coated 6-well plates. Results are presented as relative mammosphere formation  $\pm$  SEM normalized to the control (0 ng/ml). Statistical significance was calculated using one-way ANOVA adjusted for multiple comparison ( $n=3$ ), where  $*P < 0.05$  and  $**P < 0.01$  IL: interleukin.



**Figure S4.** Alamar blue viability assay. Dose response curves of IL-6 and IL-8 treatment for 48 h were generated using the Alamar Blue assay or absolute cell count for (a, b, c, d) MCF7 and (e, f, g, h) MDA-MB-231 cells. Values are represented relative to proliferation status before treatment and to the control (0 ng/ml). No significant difference in viability could be detected. Data is represented as mean  $\pm$  SEM (n=3-4).



**Figure S5.** Scatterplot of the correlation between secreted IL-6, IL-8 and progranulin in the PDS model system.  $P$ -values calculated using Spearman correlation.  $P < 0.05$  is considered statistically significant. For MCF7 cells grown on patient-derived scaffolds,  $n=57$  and for MDA-MB-231 cells grown on patient-derived scaffolds,  $n=53$ . PGRN: progranulin, IL-6: interleukin-6, IL-8: interleukin-8.

**Table S1.** Amino acid sequence of synthesized secreted sortilin (sSORT).

Amino acid sequence of secreted sortilin, after removal of signal peptide and pro-peptide (N-terminal is not confirmed experimentally).	SAPGEDEECGRVDRDFVAKLANNTHQHVFDDLGRGSVLSWVG DSTGVILVLTTFHVPLVIMT FGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEVSGGSRGGRIFRSSD FAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWVSKNFGGKWEEIHKAVCLAKWG SDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFASVMADKDTTRR IHVSTDQGD TWSMAQLPSVGQE QFYSLAANDDMVFMHVDEPGDTGFGTIFTSDDRGIVYS KSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRKPENSECDAT AKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGD AISVMVPDVYISDDGGYS WTKMLEGPHYYTILDSGGIIVAIEHSSRPINVIKFSTDEGQCWQTYTFRDPYFTGLASEPGAR SMNISIWGFTESFLT SQWVSYTIDFKDILERNCEEKDYTIWLAHSTD PEDYEDGCILGYKEQFLR LRKSSVCQNGRDYVVT KQPSICLCSLEDFLCDFGYR PENDSKCVEQPELKGHDLEFCLYGREE HLTTNGYRKIPGDKCQGGVN PVREVKDLKKKCTSNFLSPEKQNSKSNSTGHHHHHHH
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