Identification of novel natural substrates of fibroblast activation protein-alpha by differential degradomics and proteomics

Hui Emma Zhang, Elizabeth J Hamson, et al

Supplemental Figure Legends

Supplemental Figure 1: FACS plots showing the gating strategy for CSF-1 staining in the FAP e+ and e- MEFs. Based on the cell size, a gate was firstly set on the Forward Scatter (FSC) vs Side Scatter (SSC) plot to exclude debris and dead cells, allowing analysis of viable cells. To exclude the doublets and cell clumps, another gate was set on the FSC-Area vs FSC-Height plot, allowing analysis of viable and single cells for CSF-1 positivity based on the normal sheep IgG control (R660 C).

Supplemental Figure 2: MEF characterisation: DPP4, DPP8 and DPP9 mRNA expression and prolyl endopeptidase (PEP) activity in FAP e+, FAP e- and empty vector control MEFs. (A) mRNA was isolated from MEFs for qPCR analysis. mRNA levels were normalised to the house keeping gene β-actin. (B) Endopeptidase activity in the secretomes of FAP e+ and FAP e- MEFs using the fluorogenic post-proline endopeptidase substrate Z-Gly-Pro-AMC (1 mM) in the presence or absence of an inhibitor of FAP, DPP4, DPP8 and DPP9 (PT100 / Val-boro-Pro; 50 μM) and a PEP-selective inhibitor (S17092) (1 μM). Mean ± SD; n = 3.

Supplemental Figure 3: Log-transformed fold-change distributions. Distribution of TAILS data before (A) and after (B) normalization shown in box and whiskers plot in the style of Tukey (three replicates). Distribution of quantitative peptide / protein alterations before (C) and after (D) normalization shown in box and whiskers plot in the style of Tukey (five replicates).