SUPPLEMENTARY MATERIALS & METHODS

Isolation, Culture and Expansion of IFP-MSC

IFP tissue (<20ml) was mechanically dissected and washed repeatedly with Dulbecco's Phosphate Buffered Saline (DPBS; Sigma), followed by enzymatic digestion using 235 U/ml Collagenase I (Worthington Industries, Columbus, OH) diluted in DPBS and 1% bovine serum albumin (Sigma) for 2 hours at 37^{o}C with agitation. Cell digests were inactivated with complete media [DMEM low glucose GlutaMAX (ThermoFisher Scientific, Waltham, MA) +10% fetal bovine serum (FBS; VWR, Radnor, PA], washed and seeded at a density of 1x10^{6} cells/175 cm^{2} flask in three different complete media: hPL, Chemically-reinforced (Ch-R), DMEM/10%FBS. Complete hPL medium was prepared by supplementing DMEM low glucose GlutaMAX with 10% hPL solution (PL Bioscience, Aachen, Germany) and 0.024 mg/ml xeno-free heparin (PL Bioscience, Aachen, Germany). Complete chemically-reinforced medium was prepared by mixing Mesenchymal Stem Cell Growth Medium 2 with supplement provided according to manufacturer's instructions (PromoCell, Heidelberg, Germany).

All MSC were cultured at 37°C 5% (v/v) CO_2 until 80% confluent (denoted as P0), then passaged at a 1:5 ratio until P3 detaching them with TrypLE™ Select Enzyme 1X (Gibco, ThermoFisher Scientific) and assessing cell viability with 0.4% (w/v) Trypan Blue (Invitrogen, Carlsbad, CA).

Immunophenotype

2.0 x 10^{5} cells were labelled with monoclonal antibodies specific for: CD10, CD44, CD56, CD73, CD90, CD105 (Biolegend, San Diego, CA), CD146, LepR (Miltenyi Biotec, Auburn, CA), CD166, CD271, NG2, HLA-DR (BD Biosciences, San Jose, CA), CD200, CXCR4 (Invitrogen) and the corresponding isotype controls. All samples included a Ghost Red Viability Dye (Tonbo Biosciences, San Diego, CA). Data were acquired using a Cytoflex S (Beckman Coulter, Brea, CA) and analysed using Kaluza analysis software (Beckman Coulter).

Quantitative real-time PCR (qPCR)

RNA extraction was performed using the RNeasy Mini Kit (Qiagen, Frederick, MD) according to manufacturer's instructions. Total RNA (1µg) was used for reverse transcription with SuperScript™ VILO™ cDNA synthesis kit (Invitrogen).

10 ng cDNA were analysed by qPCR using QuantiFast SYBR Green qPCR kit (Qiagen) and a StepOne Real-time thermocycler (Applied Biosystems, Foster City, CA). For each target, human transcript primers were selected using PrimerQuest (Integrated DNA Technologies, San Jose, CA). All samples (n=3) were analysed in triplicate. Mean values were normalized to

GAPDH, expression levels were calculated using the $2^{-\Delta\Delta}$Ct method and represented as the relative fold change of the primed cohort to the naïve (=1).

A pre-designed 28 gene Taqman low density cytokine array (TLDA, Applied Biosystems) was performed (n=3) using 1000 ng cDNA per IFP-MSC sample and processed using StepOne Real-time thermocycler (Applied Biosystems, LLC). Data analysis was performed using DataAssist software v2.0 (Applied Biosystems, LLC). Mean values were normalized to GAPDH, expression levels were calculated using the $2^{-\Delta Ct}$ method. Values were represented in a heatmap of transcript expression levels (with 34 cycles cut-off point) using Pearson's correlation distance method and complete linkage clustering method between the different samples. Also, values were represented in a separate fold change heatmap as the relative fold change of the hPL or Ch-R to DMEM/10%FBS medium (reference sample) expanded IFP-MSC
($2^{-\Delta Ct}$= X sample/X reference sample).

Trilineage differentiation

Chondrogenic differentiation ($0.25x10^6$ IFP-MSC/pellet) was induced for 21 days with serum-free MesenCult-ACF differentiation medium (STEMCELL Technologies Inc, Vancouver, Canada). Harvested pellets were cryosectioned and 6-μm frozen sections stained with 1% toluidine blue (Sigma) for semi-quantitative assessment of chondrogenic differentation. Osteogenic differentiation (5000 IFP-MSC/cm^2) was induced for 21 days with StemPro Osteogenesis differentiation kit (ThermoFisher Scientific) and semi-quantitative assessment of mineralization was performed using 1% Alizarin Red S (Sigma). Adipogenic differentiation (40000 IFP-MSC/cm^2) was induced for 15 days with StemPro Adipogenesis kit (ThermoFisher Scientific) and semi-quantitative assessment of lipid accumulation within the cell cytoplasm was performed using 0.5% Oil Red (Sigma). Quantitative real-time PCR (qPCR) (n=3) to evaluate transcript expression for all three differentiation lineages tested is described above.

Secretome analysis

Protein array of 41 growth factors (GFs) (RayBio® C-Series, RayBiotech, Peachtree Corners, GA) was used to determine secreted levels obtained from IFP-MSC expanded in all three culturing conditions. For each population, 1 mL of conditioned media obtained from 2 donors, was prepared and used for each assay following the manufacturer's instructions. Data shown represent 40 sec exposure in FluorChem E chemiluminescence imaging system (ProteinSimple, San Jose, CA). Results were generated by quantifying the mean spot pixel density of each array using protein array analyser plugin using ImageJ software (Fiji/ImageJ, NIH website). The signal intensities were normalized with the background whereas separate signal intensity results represent the

average pixel density of two spots per protein. The signal intensity for each protein spot is proportional to the relative concentration of the antigen in the sample.

Pathway analysis

Putative interactomes were generated by Search Tool for Retrieval of Interacting Genes/Proteins (STRING 11.0; available from: http://string-db.org) database using interaction data from experiments, databases, neighbourhood in genome, gene fusions, co-occurrence across genomes, co-expression and text-mining. An interaction confidence score of 0.4 was imposed to ensure high interaction probability. K-means clustering algorithm was used to organize proteins into 3 separate clusters per condition tested, discriminated by colours. Venn diagrams were used to demonstrate all possible relations between naïve and/or TIC-primed IFP-MSC cultured in all three different conditions for the significantly ($p<0.05$) altered proteins. Functional enrichments related to biological process, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and reactome pathways were presented in radar graphs for all conditions tested.

Substance P *in vitro* assay

SP was then quantified in centrifuged (1500 rpm; 5 minutes) conditioned media (in technical triplicates run in duplicates within the membrane) obtained from IFP-MSC cultures: i) in baseline cultures (*i.e.,* endogenous MSC-derived SP); ii) after exogenous addition of substance P (834 pg/ml) for 35 minutes to the cell-free supernatant (*i.e.,* supernatant group); and iii) after addition of SP (834 pg/ml) for 35 minutes to the cells in fresh medium (*i.e.,* cells group). SP final levels were determined by subtracting measured optical densities of individual wells at 450nm and 540nm (SpectraMax M5 spectrophotometer, Molecular Devices, San Jose, CA), and converted into concentrations using the reference standard curve run with the assay, and contrasted to samples with only exogenously-added SP to the medium (*i.e.,* no cells and no supernatant).

CD10 immunolocalization

IFP-MSC groups were fixed with 4% paraformaldehyde, washed with PBS, followed by incubation with tris buffered saline (TBS; Sigma-Aldrich) containing 0.05% Triton X-100 solution (Sigma-Aldrich) for 30 minutes. Groups were then incubated with blocking solution composed of TBS with 10% normal goat serum (NGS) for 1 hour. Goat anti-human CD10 polyclonal antibody (R&D Systems) was prepared in TBS containing 1% NGS and added to samples for 1 hour incubation at room temperature with gentle agitation. Samples were washed with TBS and incubated for 1 hour with secondary antibody containing AlexaFluor594 conjugated rabbit anti-goat IgG antibody combined with DAPI (Thermo Fisher Scientific) at room temperature with gentle agitation in the dark. TBS was used to wash cells, and microscope images were acquired using Leica DMi8 microscope with Leica X software (Leica).

CD10 immunomagnetic separation

Briefly, IFP-MSC were suspended in staining buffer containing PBS with 0.5% bovine serum albumin (BSA) and 2 mM EDTA and then incubated with biotinylated anti-human CD10 (Miltenyi Biotech, Inc., Auburn, CA) at RT for 20 minutes. Invitrogen™ CELLection Dynabeads™ Biotin Binder Kit (Thermo Fisher Scientific) were used according to manufacturer's instructions for magnet-activated cell sorting resulting in the POS and NEG subpopulations. POS and NEG IFP-MSC were directly plated in culture to obtain relevant numbers for the *in vivo* study yielding the CD10^{bright} and CD10^{dim} IFP-MSC populations.

Substance P immunolocalization

Rat knee joints were harvested by cutting the femur and tibia/fibula 1 cm above and below the joint line, muscles were removed and joints were fixed with 10% neutral buffered formalin (Sigma-Aldrich) for 14 days at room temperature. Knee joints were decalcified, cut at sagittal plane in half, embedded in paraffin and serial 4 µm sections were obtained. Hematoxylin and Eosin (H&E) staining was performed to evaluate the structure and morphology of knee joints.

For anti-substance P immunofluorescence staining, sections were incubated with 1x citrate buffer solution at 60 °C overnight for antigen retrieval, permeabilised with 1x PBS + 0.2% Triton X-100 for 20 minutes at room temperature, and incubated with blocking buffer (1x PBS + 0.1% Triton X-100 with 10% rabbit serum) for 1 hour at room temperature. In between different treatments sections were washed with 1x PBS. Rabbit anti-rat substance P polyclonal antibody (Millipore) was prepared in blocking buffer (1:100) and sections were incubated at 4 °C overnight. Sections were washed with 1x PBS + 0.01% Triton X-100 and incubated for 1 hour with secondary antibody containing Alexa Fluor594 conjugated goat anti-rabbit IgG antibody (Thermo Fisher Scientific) at room temperature. Controls were incubated with secondary antibody only. All sections were rinsed with 1x PBS, mounted in prolong gold antifade reagent with DAPI (Invitrogen), and microscope images were acquired using Leica DMi8 microscope with Leica X software (Leica).

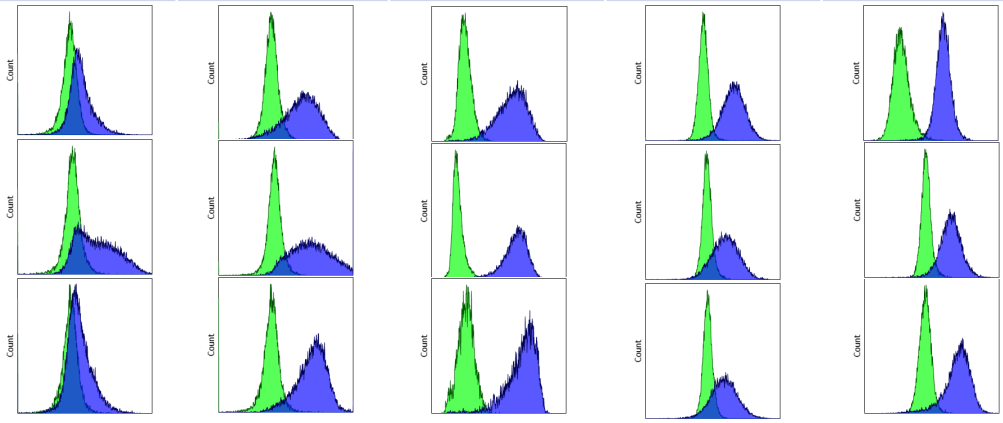
Appendix Table A1. Transcripts and primers.

TRANSCRIPT NAME	PRIMERS
BGLAP	Forward: *CAG CGA GGT AGT GAA GAG AC* Reverse: *TGA AAG CCG ATG TGG TCA G*
OMD	Forward: *ACG ATG ATC CTG ACA ATG CTC* Reverse: *GTA TAG GTT TTG TGA AGT CGT AAG TG*
FABP4	Forward: *AAG AAG TAG GAG TGG GCT TTG* Reverse: *TCA ACG TCC CTT GGC TTA TG*
PPARγ	Forward: *GCC TGC ATC TCC ACC TTA TT* Reverse: *AGC GGG AAG GAC TTT ATG TAT G*
ACAN	Forward: *TGT GGG ACT GAA GTT CTT GG* Reverse: *AGC GAG TTG TCA TGG TCT G*
COMP	Forward: *GAC AGT GAT GGC GAT GGT ATA G* Reverse: *TCA CAA GCA TCT CCC ACA AA*
GAPDH	Forward: *TAC GTC GTG GAG TCC ACT GG* Reverse: *GCC AAC GTG TCA GTG GTG GA*

SUPPLEMENTARY FIGURES

Appendix Figure A1: CD10 expression levels in crude and CD10 immunomagnetically-sorted non-induced and TIC-primed IFP-MSC. Crude naïve FBS show low and variable CD10 levels that are significantly enriched upon culturing in regulatory-complaint media in all cell groups tested (crude and CD10 immuno-selected without and with TIC-priming). All experiments were performed independently (n=3).

	FBS	Regulatory-compliant medium			
	Non-induced	Non-induced	Primed	CD10^{-} re-expanded	CD10^{+}
	2	86.36	88.97	83.47	95.84
	29.91	86.58	91.32	70.51	88.12
	7.19	93.66	98.07	69.87	93.42
AVE	**13.0**	**88.9**	**92.8**	**74.6**	**92.5**
SD	*14.84*	*4.15*	*4.72*	*7.67*	*3.95*



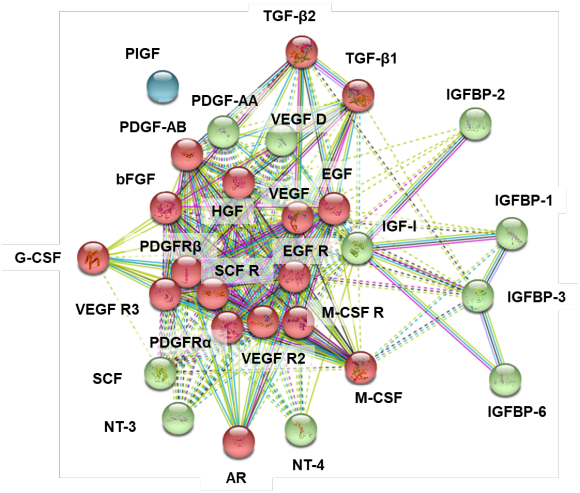
Appendix Figure A2: Inflammation-related molecular signature of non-induced hPL, FBS, and Ch-R IFP-MSC. Real time quantitative PCR (RT qPCR) showing expression of inflammatory cytokine with relative fold changes calculated ($2^{-\Delta\Delta Ct}$) using the FBS-grown data as reference (set up as 1).

Gene name	Reference sample (FBS medium)	hPL/ reference sample (fold change)	Ch-R/ reference sample (fold change)
IFN-α-1	1	0.6	0.5
IFN-α-16	1	0.8	0.6
IFN-α-17	1	0.8	0.6
IFN-α-2	1	0.8	0.6
IFN-α-6	1	0.8	0.6
IFN-α-7	1	0.8	0.6
IFN-α-8	1	0.6	0.4
IFN-β-1	1	0.8	0.7
IFN-γ	1	0.8	0.6
IL-10	1	0.8	0.6
IL-12-α	1	0.7	0.2
IL-12-β	1	0.4	0.4
IL-13	1	0.8	0.6
IL-15	1	0.7	0.6
IL-16	1	0.4	1.0
IL-17-α	1	0.8	0.6
IL-18	1	0.1	0.0
IL-1-α	1	0.7	3.1
IL-1-β	1	0.8	0.1
IL-2	1	0.8	0.6
IL-3	1	0.8	0.6
IL-4	1	0.7	0.4
IL-5	1	0.8	0.6
IL-6	1	1.2	0.6
IL-8	1	6.8	8.6
IL-9	1	0.8	0.6
LTA	1	0.8	0.7
TNF-α	1	1.2	0.5

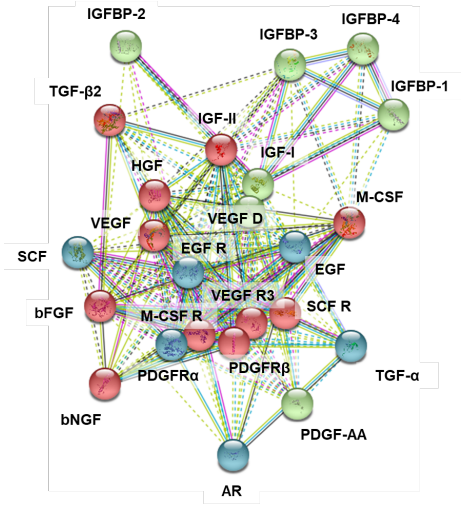
Appendix Figure A3: Protein association network of non-induced and TIC-primed hPL and Ch-R expanded IFP-MSC. STRING analysis of the proteins with statistical differences between hPL/Ch-R and FBS in naïve or TIC- priming conditions (total of 41) was performed using all available interaction sources and 0.4 as a confidence interaction score. K-means algorithm revealed high protein-protein interaction (PPI) enrichment (p-value <1.0e-16) with all GFs clustered into 3 groups indicated with different colors. All experiments were performed independently (n=2).

Non-induced IFP-MSC

hPL

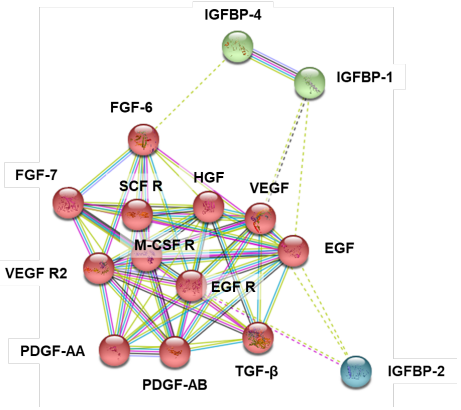


Ch-R

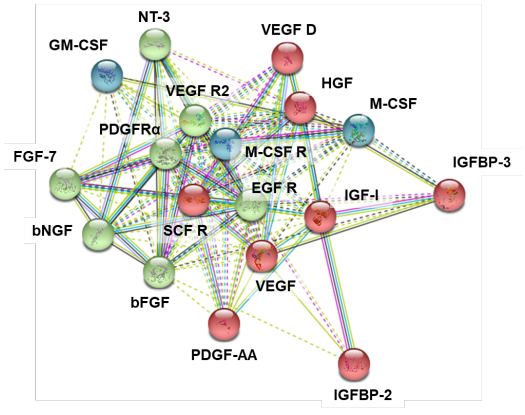


TIC-primed IFP-MSC

hPL



Ch-R



Appendix Figure A4: Protein interactomes of non-induced and TIC-primed hPL and Ch-R expanded IFP-MSC. (A and B) Biological processes and KEGG/reactome pathways analyses revealed different type and number of proteins affected in naïve and TIC-primed IFP-MSC. (B) Biological processes and interactome pathways analysis of the three different groups compared showed similar protein involvement except few differences. Radar graph percentages represent the number of proteins involved in a specific pathway/function, related to the total amount of proteins differentially expressed between hPL or Ch-R and FBS. All experiments were performed independently (n=2).