# Supplementary Materials

# **Co-inflammatory roles of TGFβ1 in the presence of TNFα drive a pro-inflammatory fate in mesenchymal stem cells**

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A. Experimental setup



### B. Unsupervised mRNA clustering



### Supplementary Figure 1

# Experimental setup of MSC stimulation by TNFa and TGFB1 for mRNA arrays, and data of unsupervised clustering

Human BM-derived MSCs were stimulated with TNF $\alpha$  (50ng/ml) or TGF $\beta$ 1 (10ng/ml), or treated by a vehicle control. Cytokine concentrations were selected as described in "Materials and methods". (**A**) For mRNA arrays, MSCs of Donor #1 were stimulated by the cytokines for the indicated time points, in two separate biological repeats ("*i*, *ii*"). qPCR validations of 18 up-regulated mRNAs were performed in MSCs of Donors #2 and #3, as demonstrated in Figure 3. (**B**) Unsupervised clustering of total mRNAs included in the array.

### **METABRIC:** Target correlations



### **Supplementary Figure 2**

## Coordinated expression of CCL2, CXCL8 and Cox-2 in patient samples of the METABRIC dataset

The METABRIC dataset, consisting of 1992 samples of breast cancer patients, was used to determine the correlations between CCL2, CXCL8 and PTGS2 (Cox-2) expression levels. a.u, Arbitrary Units. Similar analysis performed with the TCGA dataset of patient samples is demonstrated in Figure 4. Details on statistical analysis are provided in Figure 4B.



### Supplementary Figure 3

### TGF $\beta$ 1 stimulation elevates the protein expression levels of CCL2 in MSCs

Human BM-derived MSCs were stimulated with TGF $\beta$ 1 (10ng/ml) for 24 hours, or treated by a vehicle control (--). CCL2 levels in cell supernatants were determined by ELISA, at the linear range of absorbance. The figure demonstrates fold change of CCL2 release, independently determined in MSCs of 6 different donors. The bar represents mean  $\pm$  SD and each dot represents the fold change value obtained in one donor out of the 6 different MSCs donors.

### A. p65 siRNA: Efficacy



# B. Smad3 siRNA: Efficacy



# C.



#### C1. p65 siRNA: Efficacy

### **Supplementary Figure 4**

### Efficacy of p65 or Smad3 down-regulation following siRNA transfection

The figure complements the data of Figure 6 by demonstrating the efficacy of siRNA-mediated down-regulation of p65 and Smad3 expression, as appropriate. (A) Efficacy of p65 down-regulation by p65 siRNA, in TNFα-stimulated MSCs used in Figure 6A. (B) Efficacy of Smad3 down-regulation by Smad3 siRNA, in TGF<sup>β</sup>1-stimulated MSCs used in Figure 6B. (C) Efficacies of p65 and Smad3 downregulations by siRNA to p65 and Smad3, in TNF $\alpha$ +TGF $\beta$ 1-stimulated MSCs used in Figure 6C.



### **Supplementary Figure 5**

Α.

Roles of p65 and Smad3 in regulating TNF $\alpha$ +TGF $\beta$ 1-cooperatively-induced CCL2/CXCL8 and Cox-2 expression in MSCs – Another donor The figure complements the findings presented in Figures 6C and Supplementary Figure 4C, by showing similar findings in another donor (out of total n=3 independent experiments). The different experimental parts of the figure were performed in parallel, using MSCs of the same donor. \*p $\leq$ 0.05, \*\*p $\leq$ 0.01, \*\*\*p $\leq$ 0.001. ###p $\leq$ 0.001 compared to vehicle-treated, control siRNA-transfected cells. NS, Not Significant.

# A. TAK1 siRNA: Efficacy in signaling exps (10 min stim.) and impact on p38 activation

A1. TAK1 siRNA: Efficacy





### B. TAK1 siRNA: Efficacy in functional exps (24 hr stim.)



### Supplementary Figure 6

The impact of TAK1 down-regulation on p38 activation and efficacy of TAK1 siRNA in the different relevant experiments The figure complements the data of Figure 7. (A) TAK1 siRNA in signaling experiments. (A1) Efficacy of TAK1 down-regulation in TNF $\alpha$ +TGF $\beta$ 1-stimulated MSCs used in Figure 7B (I $\kappa$ B $\alpha$  expression and p65 and Smad3 activation). Following the siRNA transfection procedure, MSCs were stimulated by the cytokines for 10 minutes. (A2) Following TAK1 down-regulation by siRNA, as demonstrated in Supplementary Figure 6A1, p38 activation was determined in the same MSCs. The findings are representatives of n=3 independent experiments, performed with MSCs of 2 different donors, which have shown similar results. (B) TAK1 siRNA in functional assays in which following siRNA transfection, the MSCs were stimulated for 24 hours with TNF $\alpha$ +TGF $\beta$ 1 (no rapid signaling events are expected at this later time point). The findings were obtained with the MSCs used in Figure 7C. (B1) TAK1 down-regulation in analyses of CCL2 and CXCL8 expression (shown in Fig. 7C1, 6C2). (B2) TAK1 down-regulation in analyses of Cox-2 expression (shown in Fig. 7C3).

### A. TAK1 siRNA: Efficacy in signaling exps and impact on IκBα expression and on p65, Smad3 and p38 activation (10 min stim.)

A3. TAK1 siRNA: p65

(10 min)

AL.

p-p65

(65 kDa)

(65 kDa)

GAPDH (36 kDa)

siRNA:

Total-p65

### A4. TAK1 siRNA: Smad3 and p38



### A2. TAK1 siRNA: ΙκΒα





A1. TAK1 siRNA: Efficacy

- B. TAK1 siRNA: Efficacy in CCL2/CXCL8 exps and impact on CCL2/CXCL8 expression (24 hr stim.)
- B1. TAK1 siRNA: B2. TAK1 siRNA: CCL2 and CXCL8 Efficacy

- C. TAK1 siRNA: Efficacy in Cox-2 exp. and impact on Cox-2 expression (24 hr stim.)
- C1. TAK1 siRNA: C2. TAK1 siRNA: Cox-2 Efficacy



### **Supplementary Figure 7**

**Roles of TAK1 in regulating TNF** $\alpha$ +**TGF** $\beta$ **1-cooperatively-induced CCL2/CXCL8 and Cox-2 expression in MSCs** – **Another donor** The figure complements the findings presented in Figure 7 and in Supplementary Figure 6, by showing similar findings in another donor (out of total n=3 independent experiments). The different experimental parts of the figure were performed in parallel. More details are provided in the legends of Figure 7 and of Supplementary Figure 6 (note details about stimulation time).

# CM of MSCs: Morphological changes in MDA-MB-231 breast tumor cells

### 1. Control medium



3. CM of Control-MSCs





## 4.CM of $T\alpha + T\beta$ -stimulated MSCs





### **Supplementary Figure 8**

Factors released by TNF $\alpha$ +TGF $\beta$ 1-stimulated MSCs induce cellular elongation in MDA-MB-231 breast cancer cells – Another experiment The figure complements the findings of Figure 8A by showing higher magnification of cells (Scale bar = 50 $\mu$ m), in another independent experiment (out of total n>3 independent experiments).

CO.Dp ID   CO.Dp Tree   Co.Dp Tree <thco.dp th="" tree<="">   Co.Dp Tree   Co.Dp Tree</thco.dp>				3	h	7	h	14	lh	24	lh
CO-000425remarcinde NA-templated18321.00 <th>GO.bp ID</th> <th>GO.bp Term</th> <th>Size</th> <th>TNFα</th> <th>TGF<sub>β1</sub></th> <th>TNFα</th> <th>TGFβ1</th> <th>TNFα</th> <th>TGF<sub>β1</sub></th> <th>TNFα</th> <th>TGFβ1</th>	GO.bp ID	GO.bp Term	Size	TNFα	TGF <sub>β1</sub>	TNFα	TGFβ1	TNFα	TGF <sub>β1</sub>	TNFα	TGFβ1
Condensity   regular matchedic process   [143]   2712   3 M224   3 M244   3 M224   3 M244   3 M224   3 M244	GO:0006351	transcription-DNA-templated	1838	2.34E-16	1.97E-11	1.35E-13	1.44E-14	3.10E-05	3.73E-07		
02.000105.   angle incontriguing one present income into a system in a system in a system into a system in a system in a system into	GO:0044281	small molecule metabolic process	1483	2.72E-03	8.49E-03	6.52E-10	1.90E-09	4.73E-16	1.39E-12	1.93E-20	2.23E-12
C30007153   signal randomicanic   988   out   5307-00   5307-00   5307-00   5720-00   12042   5720-00   12042   5720-00   12042-00   5720-00   12042-00   5720-00   12042-00   5720-00   12042-00   5720-00   12042-00   5720-00   12042-00   5720-00   12042-00   20460-00   5720-00   12042-00   20460-00   5720-00   12040-00   20460-00   5720-00   12040-00   20460-00   12040-00   20460-00   12040-00   20460-00   12040-00   20400-00	GO:0006355	regulation of transcription-DNA-templated	1312	1.22E-07	2.55E-06	4.22E-05	5.40E-07		1.56E-03		
CD:004.007   Res.10   7.812-01   2.412-11	GO:0007165	signal transduction	988			5.03E-03		5.25E-05	1.71E-04	1.94E-03	2.29E-04
GO-001497   mec expression   881   IAILE0   200760   207820   207820   207820   208820   1   2048200   1   2	GO:0045944	positive regulation of transcription from RNA polymerase II promoter	969	7.01E-10	7.22E-11	2.44E-11	2.26E-12	7.07E-05	7.85E-10	1.31E-04	5.54E-05
GO-004708   image immune response   638   image imag	GO:0010467	gene expression	881	1.81E-07	2.20E-08	9.70E-15	2.73E-21	6.36E-12	4.80E-16	1.87E-07	1.55E-07
G2.0007128   G2.0007128   G2.0007123   G2.0007123   G2.0007124   G2.0007	GO:0045087	innate immune response	838			1.99E-07	8.71E-03		2.46E-03		
G0:000122   length regulation of transcription fram RAA polymerse II promoter   75   56.60   1976-06   3328.00   1976-10   4988-00   1976-06     G0:0001224   inclinal CTrasse medical signal transformation   653   6446.00   572.00   1976-00   7712-01   4971-00	GO:0007186	G-protein coupled receptor signaling pathway	837	3.57E-07		7.59E-06	1.62E-10	1.06E-05	4.18E-04		3.30E-04
G0:000734   would CTP are mediated signal random density in a second	GO:0000122	negative regulation of transcription from RNA polymerase II promoter	725	5.66E-09	1.79E-06	3.33E-08	1.91E-11	6.80E-03	7.79E-05		
63:004:127   cellular protein metabolic process   64:01   29:004   13:13:20   4.067:00   31:16:0   4.067:00   31:16:0   4.067:00   31:16:0   4.067:00   31:16:0   4.067:00   31:16:0   4.067:00   31:16:0   4.067:00   4.07:00:00:00:00:00:00:00:00:00:00:00:00:0	GO:0007264	small GTPase mediated signal transduction	653	8.64E-05	8.73E-06	8.27E-04	8.71E-11		4.04E-04		
G0:000051 applotic process 610 661268 1.282-04 400-09 312-04 200-09 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-00 320-04 220-04 320-04 220-04 320-04 220-04 320-04 220-04 320-04 2	GO:0044267	cellular protein metabolic process	640	2.99E-03		1.35E-10	9.17E-11	4.02E-10	6.56E-15	8.95E-05	3.76E-12
G0:005114 exidation-reduction process 552 1 1312-08 320.40 (72.80) 137.800 152.00 137.800 152.00	GO:0006915	apoptotic process	610	6.61E-05	1.25E-04	4.69E-09	3.41E-08	4.69E-09	2.78E-09	1.61E-08	2.24E-04
Gl-001032   trial process   566   4062-06   LEXE-00   2560-00   5552-08   202E-01   3482-00   AUZE-00     GO-0006564   transcription framscription-DNA-templated   510   407-00   327E-08   1502-00   7.232-00   1502-00   7.232-00   1522-00   2.27E-04   1.22E-04   8.00E-06   7.232-00   1.22E-04   8.00E-06   1.22E-04   8.00E-07   1.22E-04 </td <td>GO:0055114</td> <td>oxidation-reduction process</td> <td>582</td> <td></td> <td></td> <td></td> <td>1.31E-03</td> <td>8.20E-04</td> <td>6.29E-06</td> <td>9.74E-06</td> <td>2.28E-07</td>	GO:0055114	oxidation-reduction process	582				1.31E-03	8.20E-04	6.29E-06	9.74E-06	2.28E-07
Gd:0000560 irmscription from KNA polymerase II promoter 554 9.3116-00 2.344-65 7.376-00 1.336-68 1.432-68 - 7.372-00 1.336-68 7.322-00 7.326-00 1.336-68 - 7.322-00 7.326-00 7.3	GO:0016032	viral process	566	4.06E-06		1.82E-09	2.56E-09	6.55E-08	2.92E-14	3.41E-07	1.98E-05
61:00:0711   absolution   1   12:00:00 <t< td=""><td>GO:0006366</td><td>transcription from RNA polymerase II promoter</td><td>554</td><td>9.31E-09</td><td>4.60E-04</td><td>2.34E-05</td><td>7.37E-08</td><td>1.50E-03</td><td>1.33E-05</td><td></td><td>3.42E-03</td></t<>	GO:0006366	transcription from RNA polymerase II promoter	554	9.31E-09	4.60E-04	2.34E-05	7.37E-08	1.50E-03	1.33E-05		3.42E-03
G0:004330   no:live:regulation of transcription-DNA:templated   511   1426-06   2278-06   1.782-06   2782-04   1     G0:004306   neighive:regulation of componitic process   468   0.080-05   1.812-06   2.782-04   1.022-06   1.258-06   1.022-08   2.558-05   1   0.090-07   0.000-07   0.000-07   0.000-07   0.000-07   1.022-08   1.022-08   1.022-08   0.000-07   0.000	GO:0007411	axon guidance	540			7.39E-03	1.45E-06				7.42E-03
63:00:306   incgaive regulation of 2ppot(ic process)   486   0067:05   151E-07   4.26:09   267E-09   1.27E-04   8.90E-06     60:00:30537   positive regulation of CTase activity   485   267E-03   3.27E-03   1.14E-06   57E-03   2.27E-04   1.27E-04   1.27E-	GO:0045893	positive regulation of transcription- DNA-templated	511	1.42E-06	2.67E-08	2.12E-06	1.74E-08	9.19E-05	2.76E-04		
G0:004547   positive regulation of CTPase activity   452   2016;08	GO:0043066	negative regulation of apoptotic process	486	9.08E-05		1.51E-07	4.26E-09	2.67E-09	1.72E-04	8.90E-06	
G0:004382   increditver regulation of transcription-DNA-templated   442   0.885e.04   1.142.66   5914.03   2.534.63     G0:00400278   inutrotic cell cycle   454   1.885e.06   1.192.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.102.08   1.002.11   1.005.12   0.0000278   1.005.103   0.105.108   0.105.1	GO:0043547	positive regulation of GTPase activity	485	2.02E-03	2.32E-03						
G0:000278   mintotic-cell cycle   4.94   1.88E-06   1.18E-08   376E-12   1.91E-08   1.09E-12     G0:00145011   meteoritorphin TRK receptor signaling pathway   377   1   1.62E-08   5.00E-07   2   1.7E-04   3.4E-04     G0:00155071   ordical stimulus involved in sensory perception of smell   360   1.42E-08   3.46E-01   1.25E-06   5.00E-00   1.25E-04   3.4E-04     G0:0015301   ordical biguitation   361   2.44E-03   1.35E-06   1.07E-04   1.62E-08   5.05E-06   1.58E-04   1.62E-08   5.05E-06   1.58E-04   1.62E-08   5.05E-06   1.58E-04   1.62E-08   5.05E-06	GO:0045892	negative regulation of transcription-DNA-templated	482	6.85E-04		1.14E-06	5.91E-03		2.53E-05		
G0:0048011   iserrortophin TRX receptor signaling pathway   397   iserror 1.966.00   Sout-20   iserror 1.976.00   iserror 1.976.00<	GO:0000278	mitotic cell cycle	434	1.85E-06	1.19E-04	1.12E-09	8.76E-12	1.91E-08		1.09E-12	
G2:0050911 detection of chemical stimulus involved in sensory perception of smell 360 16:20:00 46:82:00 1:392:01 507:068 2012:05 1:21:04 3:201:05 0 6:331:00 3:31:00 5:352:06 1:582:04 2:31:04 2:31:04 3:30:02 3:31:00 5:352:06 1:582:04 1:31:04 1:32:04 3:33:04 1:32:04 1:32:04 3:33:04 1:32:04 1:32:04	GO:0048011	neurotrophin TRK receptor signaling pathway	397			1.96E-08	5.00E-07				
G0:0016567   protein nbiguitination   362   3.246.00   8.586.03   2.848.07   2.176.06	GO:0050911	detection of chemical stimulus involved in sensory perception of smell	380	1.62E-08		4.68E-07	1.39E-12	5.07E-08	2.01E-09	1.12E-04	3.34E-04
G0:000823   cell proliferation   355   3.480.0   6.50:03   5.082-06   1.582-04   1.282-04	GO:0016567	protein ubiquitination	363	2.34E-03	1.51E-03	6.83E-03	2.84E-07		2.17E-05		
G0:001031 protein transport 346 1.35E.06 1.07E.06 8.20E.04 1.29E.04 1.29E.04 1.29E.04 1.29E.04 2.72E.07   G0:0001301 cpidtrmal growth factor receptor signaling pathwav 324 1 1.47E.04 1.65E.03 1.27E.04 2.72E.07   G0:0003198 reracellular matrix organization 310 1 4.38E.06 9.58E.04 1.07E.06 8.20E.04 4.04E.07   G0:0003198 Fe-epsilon receptor signaling pathway 301 2.78E.04 2.17E.04 2.17E.04 1.37E.07 7.32E.04 4.04E.07   G0:00003095 Fe-epsilon receptor signaling pathway 301 2.21E.06 7.40E.04 2.37E.07 7.32E.04 4.04E.05   G0:0006310 NA repair 2.22 2.21E.06 1.96E.04 3.32E.03 3.41E.04 1.05E.04 2.21E.06 7.40E.04 2.21E.06 7.40E.04 2.21E.06 7.40E.04 2.21E.06 5.22E.04 1.05C.04 2.21E.06 7.40E.04 2.21E.06 7.40E.04 2.21E.05 1.05E.04 2.02E.03 2.00E.03 2.00C.0003.03 2.00C.0006.36 2.21E.05 1.05E.04 2.21E.06 1.05E.0	GO:0008283	cell proliferation	355	2.34E-03		6.50E-03	5.05E-06	1.58E-04	2.34E-03	2.30E-04	
G0:001301 cell division 331 2.552.08 2.77E.04 2.162.07 1.72E.06 2.72E.04 CO.000713   G0:0007173 post-translational protein modification 316 4.33E.05 9.58E.03 C 7.32E.05   G0:0007105 extracellular matrix organization 310 2.75E.04 2.75E.04 4.04E.07 CO.0000305   G0:0000705 receptor signaling pathway 298 4.09E.03 4.91E.05 7.37E.05 1.37E.07 7.32E.03 4.80E.05   G0:00006301 DXA repair 292 4.166.04 0.02E.04 2.21E.04 7.40E.04 8.28E.05   G0:0006321 DXA repair 291 1.55E.06 0.23E.05 1.37E.04 8.02E.05 1.37E.04 4.00E.05 1.47E.04 6.02 1.47E.04 1.65E.04 1.45E.04 1.65E.04 1.45E.05 1.37E.04 1.00E.04 3.38E.03 1.4E 1.65E.04 1.45E.04 1.45E.05 1.37E.04 1.00E.04 3.38E.03 1.4EE.05 1.87E.06 5.72E.04 1.00C.0000365 1.45E.05 1.47E.04 1.35E.03 4.00E.05 1.47E.04 1.35E.03 4.02E.05 1.35E.04	GO:0015031	protein transport	346			1.35E-06	1.07E-06	8.20E-04	1.39E-04	1.92E-03	6.53E-06
G0:0001717   endermal growth factor receptor signaling pathway   324   1.748-04   1.658-03   Image: Constraint of the constraint o	GO:0051301	cell division	331	2.55E-05		2.77E-04	2.16E-07		1.27E-06	2.72E-07	
G0:0043687   post-translational protein modification   113     4.33E-65   9.58E-03    6   7.32E-05     G0:000109   extracellular matrix organization   313     8.47E-05   3.92E-04   4.44E-07     G0:0000596   recpsilon receptor signaling pathway   301    2.79E-04   2.17E-05   7.32E-03   4.48E-03     G0:0006976   organetic organization   229   2.81E-06   1.62E-04   1.35E-03   3.41E-04   8.37E-04   8.37E-04 <td>GO:0007173</td> <td>epidermal growth factor receptor signaling pathway</td> <td>324</td> <td></td> <td></td> <td>1.74E-04</td> <td>1.65E-03</td> <td></td> <td></td> <td></td> <td></td>	GO:0007173	epidermal growth factor receptor signaling pathway	324			1.74E-04	1.65E-03				
GO:000198   extracellular matrix organization   11   2.78E-04   2.78E-04   2.78E-04   2.77E-05   4.78E-05   4	GO:0043687	post-translational protein modification	316			4.33E-05	9.58E-03				7.32E-05
GO:0003095 Fe-epsion receptor signaling pathway 301 2.79E-04 2.77E-05 1.37E-07 7.32E-03 4.80E-05   GO:0000560 organelic organelic organization 292 2.81E-06 1.64E-04 3.02E-03 2.21E-06 7.40E-04 8.28E-05   GO:0000521 DNA repair 292 2.81E-06 1.64E-04 1.53E-04 4.80E-05 CO:0008543   GO:00045210 vascular endobleilal growth factor receptor signaling pathway 284 6.33E-04 3.41E-04 . .   GO:0004305 positive regulation of apoptotic process 281 6.79E-05 6.23E-03 6.44E-08 4.30E-05 1.14E-05 1.87E-06 5.72E-04 4.00E-03   GO:00005367 transcription initiation from RNA polymerase II promoter 260 2.60E 7.67E-04 1.39E-03 6.33E-05 3.62E-03 .	GO:0030198	extracellular matrix organization	313					8.47E-05	3.92E-04	4.04E-07	
G0:0000996   organization   298   4.09E-03   4.91E-05   7.774-05   1.37E-07   7.32E-05   4.80E-05     G0:00002543   fibroblast growth factor receptor signaling pathway   291   1   1.54E-04   1.35E-03   2.1E-06   7.40E-04   8.28E-05     G0:00002543   fibroblast growth factor receptor signaling pathway   291   1   1.54E-04   1.35E-03   3.41E-04   2.0E   6.0E   7.40E-04   8.28E-05   1.40E-04   8.28E-05   1.40E-05   7.40E-04   8.28E-05   1.40E-05   7.40E-04   8.28E-04   0.0E.03   6.41E-04   2.0E   5.72E-04   0.0E.03   1.33E-05   1.41E-05   8.77E-04   4.00E-03   0.0E.03   0.0E.03 <td>GO:0038095</td> <td>F c-epsilon receptor signaling pathway</td> <td>301</td> <td></td> <td></td> <td>2.79E-04</td> <td>2.17E-03</td> <td></td> <td></td> <td></td> <td></td>	GO:0038095	F c-epsilon receptor signaling pathway	301			2.79E-04	2.17E-03				
GO:0006251   DNA repair   2/21   ZM1E-06   1/24E-06   7/24E-06   7/24E-06   7/24E-06   7/24E-06   7/24E-06   7/24E-07   2/24E-07   0<	GO:0006996	organelle organization	298	4.09E-03	1.0 (7) 0.1		4.91E-05	7.77E-05	1.37E-07	7.32E-03	4.80E-05
GO:0008543   Inbrobast growth factor receptor signaling pathway   291   1.54E-04   1.55E-03   0   0   0     GO:0008500   vascular endbelial growth factor receptor signaling pathway   281   6.37E-06   6.32E-03   3.41E-04   1.35E-03   0   0     GO:00043005   positive regulation of apoptotic process   281   6.79E-05   6.23E-03   6.44E-08   4.30E-05   1.41E-05   1.87E-06   5.72E-04   0     GO:00005307   transcription initiation from RNA polymerase II promoter   260   2.08E-03   2.27E-03   4.00E-03   3.87E-04   2.27E-03   4.00E-03   3.87E-04   2.27E-04   4.00E-03   0	GO:0006281	DNA repair	292	2.81E-06	1.96E-04	3.02E-03	2.21E-06		7.40E-04	8.28E-05	
GO:0048010   Vascular endothenial growth factor receptor signaling pairway   244   6   3.88E-03   3.41E-03   1.41E-03   1.47E-04   5.72E-04     GO:0043000   positive regulation of apoptotic process   260   2.08E-03   6.27E-03   6.44E-08   4.30E-05   1.14E-05   1.87E-06   5.72E-04     GO:0007265   Ras protein signal transduction   244   8   8.21E-03   3.88E-03   0	GO:0008543	fibroblast growth factor receptor signaling pathway	291			1.54E-04	1.35E-03				
GO:00043005 positive regulation of apoptotic process 251 n./9E405 6.24E408 4.30E405 1.14E405 1.37E404 5.7E244   GO:0006367 transcription initiation from RNA polymerase II promoter 260 208E403 2.27E+03 4.00E404 2.27E+03 4.00E403 1.33E+05 5.65E+07 2.99E+03 6.38E+03 2.07E+03 4.99E+03 2.99E+03 6.65E+03 0 2.99E+03 6.65E+03 0 2.99E+03 6.65E+03 0 0 2.99E+03 6.65E+03 0 0 2.99E+03 6.65E+03 0 0 0 0.0001505 8.99E+04 4.91E+05 1.92E+04 6.45E+03 0	GO:0048010	vascular endotnellal growth factor receptor signaling pathway	284	( 50T 05	C 227 02	3.38E-03	3.41E-04	1.145.05	1.055.04	5 535 04	
CO-000330   RNA splicing   210   220E-03   227E-03   4.00E-03     GO-000330   RNA splicing   251   7.12E-03   1.08E-04   7.91E-11   8.79E-04      GO-000330   RNA splicing   251   7.12E-03   1.08E-04   7.91E-11   8.79E-04      GO:0007067   mitotic nuclear division   241   1.62E-05   7.67E-04   1.39E-03   1.33E-05   5.65E-07     GO:0007067   mitotic nuclear division   235   8.58E-04   2.21E-06   1.52E-09   2.99E-03     GO:000186   activation of MAPKK activity   232   3.09E-03   1.66E-03        GO:0001701   in utero embryonic development   216   1.09E-04   1.74E-03   3.07E-06	GO:0043065	positive regulation of apoptotic process	281	6./9E-05	6.23E-03	0.44E-08	4.30E-05	1.14E-05	1.87E-06	5.72E-04	
GO:0000530   RAX splitting   231   7.12E-03   1.08E-04   7.9E-11   R.79E-04   1     GO:0007265   Ras protein signal transduction   241   1.62E-05   7.67E-04   1.39E-03   1.33E-05   5.65E-07     GO:0007067   mitotic nuclear division   235   8.58E-04   2.21E-06   1.52E-09   2.99E-03     GO:0000166   activation of MAPKK activity   232   3.09E-03   1.66E-03        GO:00001525   angiogenesis   227   9.53E-03   4.11E-03   5.66E-04       GO:00007608   sensory perception of smell   213   3.80E-07   1.53E-03   4.91E-05   1.92E-04   6.45E-03       GO:00007608   sensory perception of smell   213   3.80E-07   1.53E-03   4.91E-05   1.92E-04   6.45E-03     1.08E-03     1.08E-04   1.01E-04   1.08E-04   1.01E-04   1.08E-04   1.01E-04   1.08E-04   1.01E-04   1.08E-04   1.08E-04   1.08E-04   1.08E-04	GO:0006367	DNA selising	200	<b>7 13</b> 102		1.005.04	2.08E-03		2.2/E-03	4.00E-03	
GO:0007265   Rasp forein signal ransport   244   6   8.21E-00   3.85E-03   1.33E-05   5.65E-07     GO:0007067   mitotic nuclear division   211   1.62E-05   7.67E-04   1.39E-03   1.33E-05   5.65E-07     GO:0007067   mitotic nuclear division   213   1.62E-05   7.67E-04   1.39E-03   1.52E-09   2.99E-03     GO:0000186   activation of MAPKK activity   232   3.09E-03   1.66E-03        GO:0001701   in utero embryonic development   216   1.09E-04   1.74E-03   1.05E-03   3.07E-06 <td>GO:0008380</td> <td>RNA splicing</td> <td>231</td> <td>7.12E-05</td> <td></td> <td>1.08E-04</td> <td>7.91E-11</td> <td></td> <td>8./9E-04</td> <td></td> <td></td>	GO:0008380	RNA splicing	231	7.12E-05		1.08E-04	7.91E-11		8./9E-04		
GO:000700 <sup>+</sup> Informe interne arrysion 241 L362E-495 L35E-03 L35E-03 L35E-05 S.05E-07   GO:00006886 intracellular protein transport 235 8.58E-04 2.21E-06 L35E-03 2.99E-03   GO:0000186 activation of MAPKK activity 232 3.09E-03 L66E-03     GO:0000186 activation of MAPKK activity 232 9.53E-03 4.11E-03 5.66E-04    GO:00001701 in utero embryonic development 216 1.09E-04 1.74E-03 1.03E-03 3.07E-06     GO:0000708 sensory perception of smell 213 3.80E-07 1.53E-03 4.91E-05 1.92E-04 6.45E-03    GO:0000398 mRNA splicing- via spliceosome 209 1.39E-03 1.33E-09 1.51E-07 1.33E-09   GO:0000022 GI/S transition of mitotic cell cycle 155 1.74E-04 3.01E-04 1.06E-04 1.60E-04 1.60E-03   GO:0000886 G2/M transition of mitotic cell cycle 141 3.02E-03 1.51E-04 1.60E-03 1.60E-03  60:0004247 antigen processing and presentation of p	GO:0007203	mitotia nuclean division	244	1 (2E 05		8.21E-03	3.88E-03		1 225 05	5 (5E 07	
GO:0000036   Intractedual protein transport   233   6.55E-04   1.52E-03   1.52E-03   1.52E-03   2.99E-03     GO:0000186   activation of MAPKK activity   232   3.09E-03   1.66E-03        GO:00001701   in utero embryonic development   216   1.09E-04   1.74E-03   1.03E-03   3.07E-06        GO:00007608   sensory perception of smell   213   3.80E-07   1.53E-03   4.91E-05   1.92E-04   6.45E-03       GO:0000398   mRNA splicing- via spliceosome   209   1.39E-03   1.33E-09   1.51E-07   1.33E-09     GO:0000082   G1/S transition of mitotic cell cycle   155   1.74E-04   3.01E-04   1.08E-04     GO:000086   G2/M transition of mitotic cell cycle   155   1.74E-04   3.01E-04   1.66E-03    1.66E-03    1.66E-04   1.61E-04   2.15E-03   60:0004260   DNA replication   1.66E-04   1.61E-04   2.15E-03   60:0002474   antigen processing and presentation of peptide antigen via MHC class I   99 <td>GO:0007007</td> <td>introtic nuclear division</td> <td>241</td> <td>1.02E-05</td> <td></td> <td>7.0/E-04</td> <td>1.39E-03</td> <td></td> <td>1.53E-05</td> <td>5.05E-07</td> <td>2.005.02</td>	GO:0007007	introtic nuclear division	241	1.02E-05		7.0/E-04	1.39E-03		1.53E-05	5.05E-07	2.005.02
GO:0000160   activation of MAY RK activity   232   6   3.092-03   1.662-03   6	GO:0000380	nativation of MARKK activity	233			3.58E-04	2.21E-00		1.52E-09		2.99E-03
CO:000125   angugenesis   227   2352-33   4.11E-03   5.05E-04   4.11E-05   5.06E-04   1     GO:0001701   in utero embryonic development   216   1.09E-04   1.74E-03   1.03E-03   3.07E-06   1	GO:000180		232			0.52E.02	1.00E-03		5 66E 04		
GO:0007601 Indicidentity for development 210 1.032-04 1.032-03 3.072-00 6.452-03   GO:0007608 sensory perception of smell 213 3.802-07 1.532-03 4.912-05 1.922-04 6.452-03   GO:0000398 mRNA splicing-via spliceosome 209 1.332-09 1.532-03 4.912-05 1.922-04 6.452-03   GO:0001024 membrane organization 159 6.882-03 2.090 1.012-04 1.008-04   GO:0000082 G1/S transition of mitotic cell cycle 155 1.74E-04 3.012-04 1.10E-04 0   GO:0000086 G2/M transition of mitotic cell cycle 153 1.852-03 4.382-04 1.662-04 1.612-04 2.152-04   GO:000086 G2/M transition of mitotic cell cycle 141 3.022-03 1.652-03 1.602-03   GO:00006260 DNA replication 134 8.50E-03 9.50E-04 7.71E-03 1.51E-03 1.60E-03   GO:0002474 antigen processing and presentation of peptide antigen via MHC class I 99 2.15E-03 8.54E-03 0 0   GO:0002474 antigen processing and presentation of peptide antigen v	GO:0001525	angiogenesis	216	1.000 04	1 74E 02	9.55E-05	4.11E-05		5.00E-04		
CO:0000000   status   Precipitorial statu   Precipitor	GO:0007608	sensory percention of smell	210	2 80E 07	1./4E-03	1.03E-03	4.01E.05	1 02E 04	6 45E 03		
CO:0000020 Interva spinctosome 200 1.352-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00 4.912-00 1.052-00	GO:0007308	mBNA splicing, via spliceosome	210	3.00E-07		1 20E 02	4.71E-05	1.741-04	1.51E.07		1 22E 00
CO:0001024   Infinitiation of maintainin   1.52   0.0886403   2.200205   4.910-05   1.060-06     GO:0000082   GI/S transition of mitotic cell cycle   155   1.74E-04   3.01E-04   1.10E-04   1.06E-04     GO:0000086   G2/M transition of mitotic cell cycle   153   1.85E-03   1.85E-03   4.38E-04   1.66E-04   1.61E-04   2.15E-04     GO:0000086   G2/M transition of mitotic cell cycle   141   3.02E-03   1.65E-03   1.60E-03   1.60E-03     GO:00006260   DNA replication   134   8.50E-03   9.50E-04   7.71E-03   1.51E-03   1.60E-03     GO:0002474   antigen processing and presentation of peptide antigen via MHC class I   99   2.15E-03   8.54E-03   4.08E-04   4.08E-03     GO:0006364   rRNA processing   94   3.85E-06   9.40E-04   3.92E-04   4.08E-03     GO:0031308   politive regulation of protein ubiquitination   63   6.05E-03   9.47E-03   2.96E-03   4.08E-03     GO:0034084   IRE1-mediated unfolded protein response   55   5.96E-03   9.47E-03 <td>GO:0000520</td> <td>membrane organization</td> <td>159</td> <td></td> <td></td> <td>6 88E 03</td> <td>2 00E 05</td> <td></td> <td>1.51E-07</td> <td></td> <td>1.08E 04</td>	GO:0000520	membrane organization	159			6 88E 03	2 00E 05		1.51E-07		1.08E 04
GO:0000062 GVIS transition of informed centretic 153 1.742-04 3.012-04 1.102-04 GO:0000425   GO:0000086 G2/M transition of mitotic cell cycle 153 1.85E-03 4.38E-04 1.66E-04 1.61E-04 2.15E-04   GO:0000086 G2/M transition of mitotic cell cycle 141 3.02E-03 1.65E-03 1.60E-03   GO:0000260 DNA replication 134 8.50E-03 9.50E-04 7.71E-03 1.51E-03 1.60E-03   GO:0002474 antigen processing and presentation of peptide antigen via MHC class I 99 2.15E-03 8.54E-03 0 60:0000636 G0:00034138 toll-like receptor 3 signaling pathway 81 9.90E-03 5.75E-06 9.10E-03 2.96E-03 0   GO:0031398 positive regulation of protein ubiquitination 63 6.05E-03 9.47E-03 0 0   GO:0031398 positive regulation of protein ubiquitination 63 6.05E-03 9.47E-03 3.42E-03 3.42E-03	GO:0001024	$C_{1/S}$ transition of mitotic coll cycle	155			0.00E-03	2.90E-05	1 10E 04	4.91E-05		1.00E-04
GO:0000086   G2/M transition of mitotic cell cycle   135   160E-03   1.60E-03   1.60E-04   1.61E-04   2.18E-04     GO:0000086   G2/M transition of mitotic cell cycle   141   3.02E-03   1.65E-03   1.60E-03   1.6	GO:000002	cellular linid metabolic process	153			1.74E-04	1.85E.02	1.10E-04	1665.04	1.61E.04	2 15E 04
GO:000260   DNA replication   134   8,50E-03   9,50E-04   7,71E-03   1,51E-03   1,00E-03     GO:0006260   DNA replication   134   8,50E-03   9,50E-04   7,71E-03   1,51E-03	GO:0000086	G2/M transition of mitotic cell cycle	141			3.02F-02	1.65E-03	4.5012-04	1.0012-04	1.60E-02	2.1312-04
GO:00030968 endoplasmic reticulum unfolded protein response 101 1.46E-03 5.20E-03 7.4E-03 1.40E-03 5.21E-04   GO:00030968 endoplasmic reticulum unfolded protein response 101 1.46E-03 5.21E-06 5.48E-05 1.40E-03 5.72E-04   GO:0002474 antigen processing and presentation of peptide antigen via MHC class I 99 2.15E-03 8.54E-03 4.08E-03   GO:00036364 rRNA processing 94 3.85E-06 9.40E-04 3.92E-04 4.08E-03   GO:0034138 toll-like receptor 3 signaling pathway 81 9.90E-03 5.75E-06 9.10E-03 2.96E-03 4.08E-03   GO:0031398 positive regulation of protein ubiquitination 63 6.05E-03 9.47E-03 4.74E-03 4.40E-03   GO:0031398 IBEL-mediated unfolded protein response 55 5.96E-03 9.47E-03 4.42E-03	GO:0000000	DNA replication	134		8 50E 02	9.50E 04	7.71E 02	1.51E.02		1.00E-03	
GO:0002474 antigen processing and presentation of peptide antigen via MHC class I 99 2.15E-03 8.54E-03 101 172E-04   GO:0002474 antigen processing and presentation of peptide antigen via MHC class I 99 2.15E-03 8.54E-03 101   GO:00036364 rRNA processing 94 3.85E-06 9.40E-04 3.92E-04 4.08E-03   GO:0034138 toll-like receptor 3 signaling pathway 81 9.90E-03 5.75E-06 9.10E-03 2.96E-03 101   GO:0031398 positive regulation of protein ubiquitination 63 6.05E-03 9.47E-03 101 140E-03   GO:003498 IRE1-mediated unfolded protein response 55 5.96E-03 3.42E-03 3.42E-03	GO:0030968	endonlasmic reticulum unfolded protein response	101	146E-02	0.30E-03	5.21E-06	5.48E-05	1.51E-03	5 19F-05		7 72E-04
GO:0006364   rRNA processing   94   3.85E-06   9.40E-04   3.92E-04   4.08E-03     GO:0006364   rRNA processing   94   3.85E-06   9.40E-04   3.92E-04   4.08E-03     GO:0031398   positive regulation of protein ubiquitination   63   6.05E-03   9.47E-03   9.47E-03     GO:0031398   IREL-mediated unfolded protein response   55   5.96E-03   3.42E-03	GO:0002474	antigen processing and presentation of pentide antigen via MHC class I	99	1.4012-05		2 15E-03	8 54E-03	1.4012-03	5.171-05		111212-04
GO:0034138   toll-like receptor 3 signaling pathway   81   9.90E-03   5.75E-06   9.10E-03   2.96E-03   4.06E/03     GO:0031398   positive regulation of protein ubiquitination   63   6.05E-03   9.47E-03   9.47E-03   9.47E-03     GO:0031498   IRE1-mediated unfolded protein response   55   5.96E-03   9.47E-03   3.42E-03	GO:0006364	rRNA processing	94	1		2.131-03	3.85E-06	9.40E-04	3.92E-04		4.08E-03
GO:0031398 positive regulation of protein ubiquitination 63 6.05E-03 9.47E-03 00.0031398 IRE-1-mediated unfolded protein response 55 5 5 9.67E-03 3.47E-03 3.47E-03	GO:0034138	toll-like recentor 3 signaling nathway	81	9 90E-03		5 75E-06	9 10F-03	2.96E-03	0.741-04		10012-00
CO:003498 IREL-mediated unfolded protein response 55 506 506 706 707 707 707 707 707 707 707 707 7	GO:0031398	positive regulation of protein ubiquitination	63	6.05E-03		5.751-00	9.47E-03	2.7012-03			
	GO:0036498	IRE1-mediated unfolded protein response	55			5.96E-03					3.42E-03

# Supplementary Table 1

# Kinetics-based shifts in transcriptional programs induced by both $TNF\alpha$ and $TGF\beta1$ in MSCs

Gene ontology (GO) enrichment analysis of pathways that were modified by both TNF $\alpha$  and TGF $\beta$ 1 at one time point or more (p $\leq$ 0.01, after BY correction for multiple-testing, compared to vehicle-treated cells). The table indicates the p values of change of each GO term, at each specific time point following stimulation by TNF $\alpha$  or TGF $\beta$ 1. White squares – The response did not pass the required cut-off. Size = Number of genes that are included in the transcriptional program.

# Supplementary Table 2: Primers used in qPCR analyses

Gene	Primer direction (fw=forward, rv=reverse)	Sequence (5' -> 3')
PTCS2	PTGS2_fw	actctggctagacagcgtaa
11052	PTGS2_rv	agatcatctctgcctgagtatc
CV2CL1	CX3CL1_fw	ccaccttctgccatctgac
CAJULI	CX3CL1_rv	atgttgcatttcgtcacacc
EDST11	EPSTI1_fw	aggcaaaagtcaaccaggtg
LESIII	EPSTI1_rv	tgaaggccagataggagtcaa
ANCDTI 4	ANGPTL4_fw	gtggaccctgaggtccttc
ANOI 114	ANGPTL4_rv	ccaccttgtggaagagttgc
DTHII	PTHLH_fw	ctcggtggagggtctcag
1 1111111	PTHLH_rv	tggatggacttccccttgt
	PLAU_fw	ttgctcaccacaacgacatt
FLAU	PLAU_rv	ggcaggcagatggtctgtat
DDCO	RPS9_fw	aacttatgtgaccccgcgga
NF 37	RPS9_rv	cagcttcagctcttggtcga

### Supp. Table 2B

Gene	Primer direction (fw=forward, rv=reverse)	Sequence (5' -> 3')	UPL Probe	
CCL2	CCL2_fw	agtctctgccgcccttct	#40	
CCL2	CCL2_rv	gtgactggggcattgattg	π40	
CXCL8	CXCL8_fw	agacagcagagcacacaagc	#72	
	CXCL8_rv	atggttccttccggtggt	#12	
NGF	NGF_fw	tccggacccaataacagttt	#32	
	NGF_rv	ggacattacgctatgcacctc	π32	
ЦС	IL6_fw	gatgagtacaaaagtcctgatcca	#40	
IL0	IL6_rv	ctgcagccactggttctgt	π <b>-</b> 0	
LIF	LIF_fw	tgccaatgccctctttattc	- #26	
	LIF_rv	gtccaggttgttggggaac		
HBEGF	HBEGF_fw	tggggcttctcatgtttagg	- #55	
	HBEGF_rv	catgcccaacttcactttctc		
CSE2	CSF2_fw	tctcagaaatgtttgacctcca	- #1	
0512	CSF2_rv	gcccttgagcttggtgag		

MMP1	MMP1_fw	gctaacctttgatgctataactacga	#7	
	MMP1_rv	tttgtgcgcatgtagaatctg		
MMP3	MMP3_fw	caaaacatatttctttgtagaggacaa	#36	
	MMP3_rv	ttcagctatttgcttgggaaa	#30	
VEGFC	VEGFC_fw	tgccagcaacactaccacag	#27	
	VEGFC_rv	gtgattattccacatgtaattggtg	#21	
FGF1	FGF1_fw	accaagtggattctgcttcc	#56	
	FGF1_rv	cttgtggcgctttcaagac	#30	
IL12A	IL12A_fw	cactcccaaaacctgctgag	#50	
	IL12A_rv	caatctcttcagaagtgcaagg	#30	
GAPDH	GAPDH_fw	agccacatcgctcagacac	#60	
	GAPDH_rv	gcccaatacgaccaaatcc	#00	
HPRT	HPRT_fw	tgaccttgatttattttgcatacc	#72	
	HPRT_rv	cgagcaagacgttcagtcct	#/3	