Adipose tissue-derived mesenchymal stem cells mediate PD-L1 overexpression in white adipose tissues of obese individuals, resulting in T-cell dysfunction

Assia Eljaafari[1,2,,3]*, Julien Pestel[1,2], Brigitte Le Magueresse-Battistoni[1,2], Stephanie Chanon[1,2],

Julia Watson[1,2], Maud Robert[1,2, 4], Emmanuel Disse[1,2,5,] Hubert Vidal[1,2]

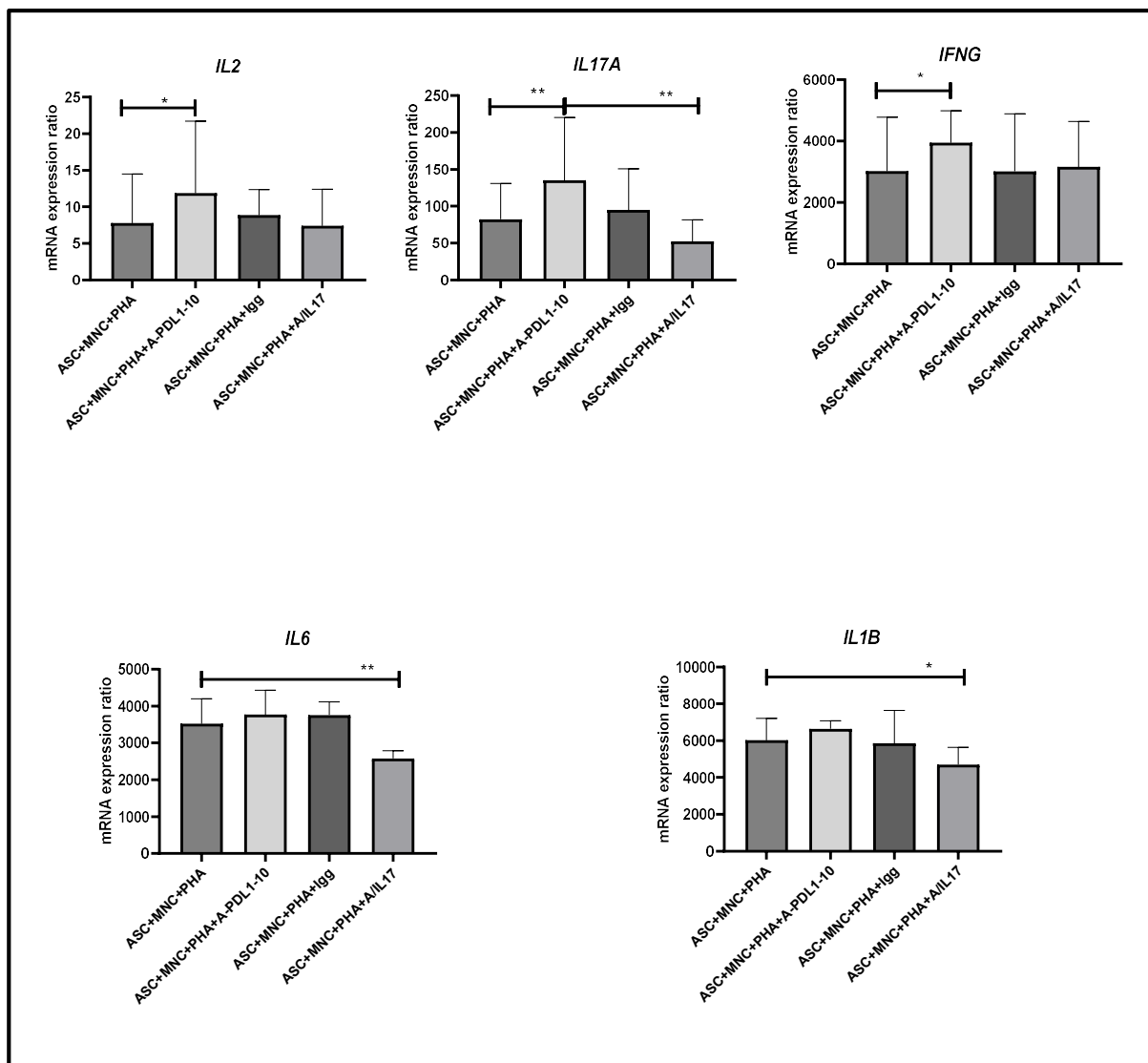## Supplementary Informations :

Supplementary Figure S1

Figure S1*: Specificity of PD-L1 blockade, as compared with IL-17A blockade.* MNC were activated by PHA in the presence of ob-ASC and anti-PD-L1mAbs (R&D Systems, AF156,10

µg/ml), anti-IL17A Ab (Secukinumab, Novartis Diagnostics, Pharma S.A.S., 92506, Rueil-Malmaison, France; 50µg/ml) or irrelevant polyclonal goat IgG (Santa-Cruz Biotechnology/INC, Europe; sc-8828, 10µg/ml). Cytokine mRNA expression levels were measured by RT-qPCR, as mentioned in the Materials and Methods section. Results were expressed as a ratio relative to the *TBP* house-keeping gene. Non parametric *t* tests were used. Data are the mean ± SD of n=4. **,*represent a p value <0.01, or <0.05, respectively.
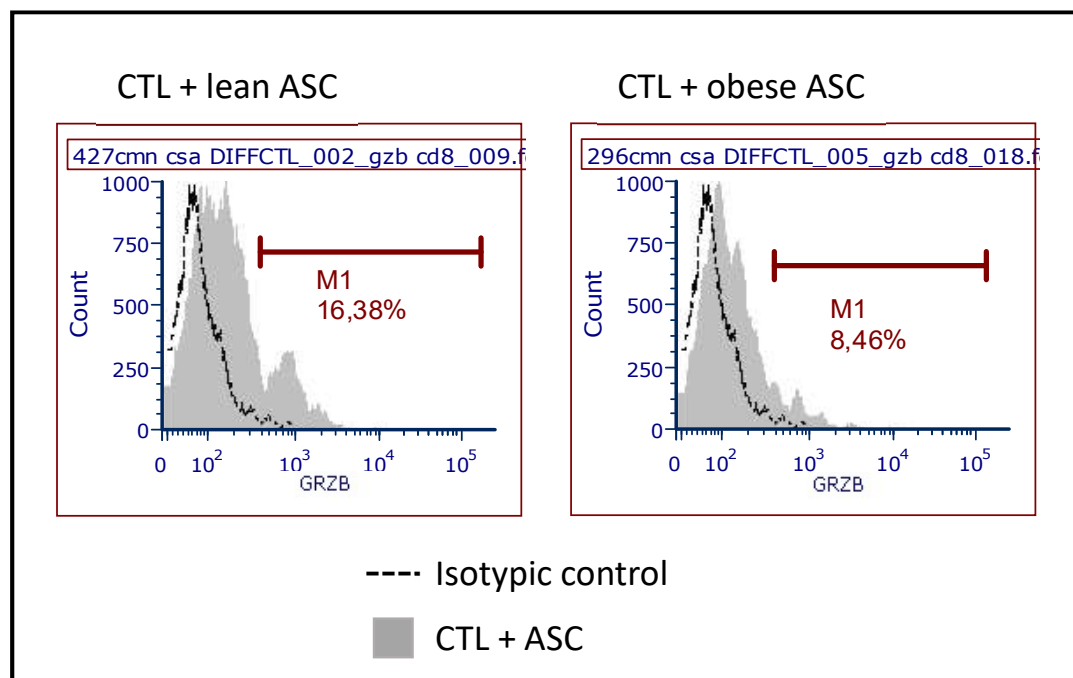
Supplementary Figure S2

Figure S2: *CTL activity is reduced in the presence of obese versus lean ASC,*

CTL were obtained following co-culture with obese or lean ASC in the presence of PHA for 72 hours, washed away from PHA, rested for one day and and expanded in the presence of IL-2 (R&D Systems, Minneapolis, MN, USA); 20UI/ml) for 8 additional days. In the meanwhile, lean or obese adipocytes were differentiated from the same ASC used in the co-culture experiments for 8 days, using the protocol described in the Materials and Section of the paper. After 12 days, cells were then restimulated with the differentiated adipocytes for 6 hours before being permeabilized and stained for granzyme B. Intra-cellular secretion of the enzyme was measured by cytofluorometry. This figure is representative of 2 experiments using different ASC

Table S1 *List of primers used in RT-qPCR*

Human primers		
Gene	**Forward primer**	**Reverse primer**
TBP	AGACCATTGCACTTCGTGCC	CCTGTGCACACCATTTTCCC
CD274 (PDL1)	CTGCAGGGCATTCCAGAAAG	GTTCAGCAAATGCCAGTAGG
PDCD1	CCCAAGGCGCAGATCAA	GCACTTCTGCCCTTCTCTCTGT
TNF	AGCCCATGTTGTAGCAAACC	GAGGTACAGGCCCTCTGATG
IL2	CAAACTCACCAGGATGCTCA	GCACTTCCTCCAGAGGTTTG
IFNG	GATGACCAGAGCATCCAAAAG	CATGTATTGCTTTGCGTTG
IL17A	ACCAATCCCAAAAGGTCCTC	TGGTAGTCCACGTTCCCATC
IL1B	GGCAATGAGGATGACTTGTT	TGTAGTGGTGGTCGGAGATT
IL6	AGCCCTGAGAAAGGAGACATGTAACAAG	TTCTGCAGGAACTGGATCAGGACTTT

Murine primers		
Gene	**Forward primer**	**Reverse primer**
Tbp	TGGTGTGCACAGGAGCCAAG	TTCACATCACAGCTCCCCAC
Cd274 (Pdl1)	AATGTGACCAGCAGTCTGAG	AAGCACCCAGTGAGTCCTGT
Pdcd1	CTAGCTGTCTTCTGCTCAAC	GGAAGTCCAGCTCCTCATAG