Supplementary Material for

Inflammation converts human mesoangioblasts into targets of alloreactive immune responses: implications for allogeneic cell therapy of DMD

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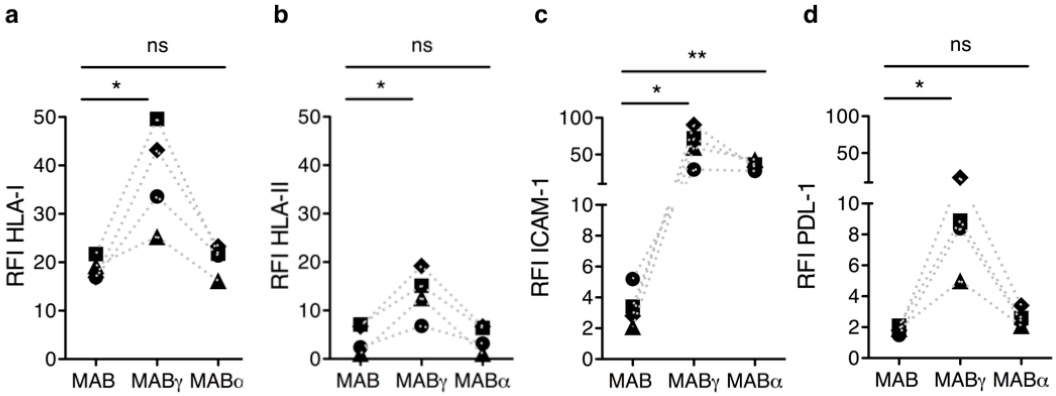
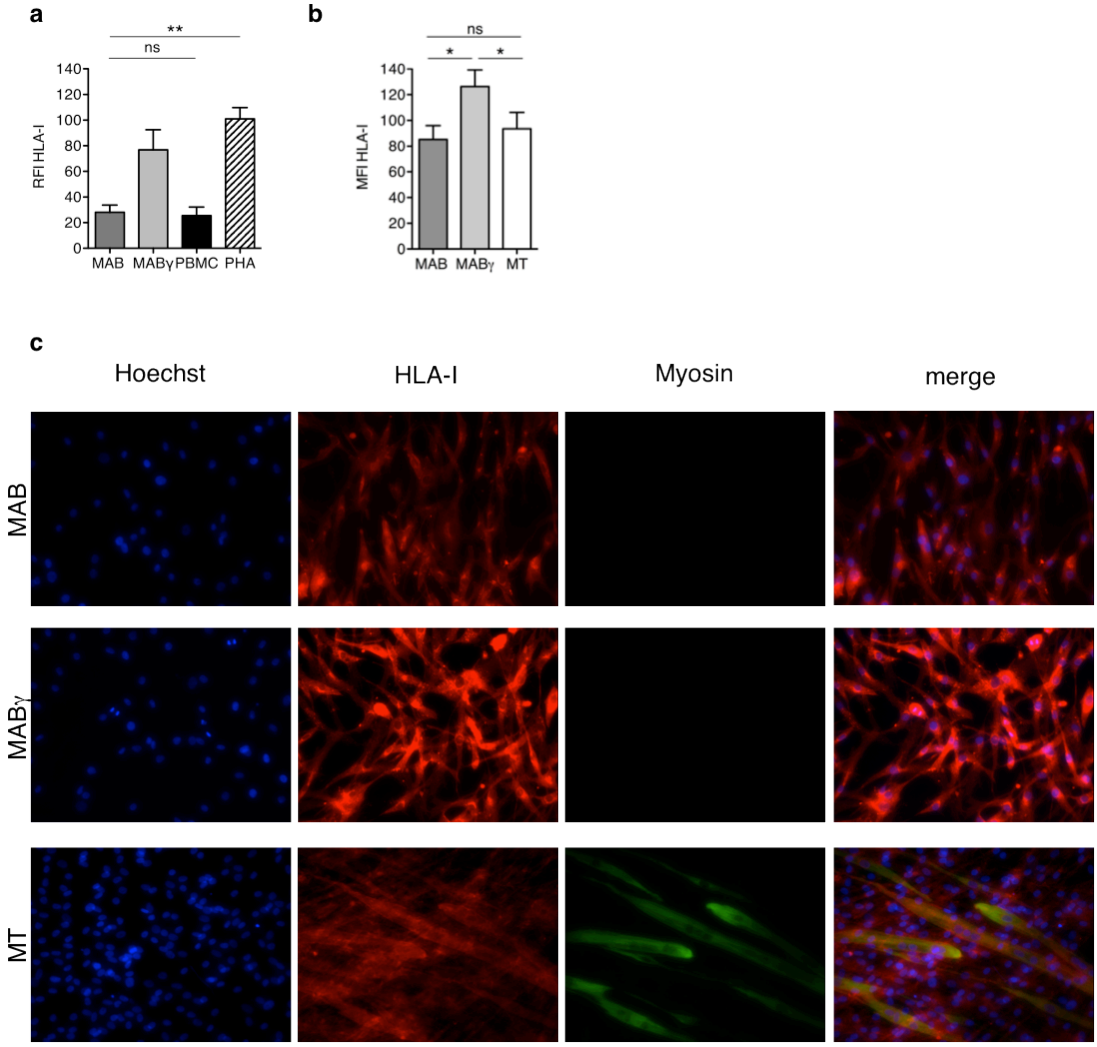
Supplementary Figure 1



SUPPLEMENTARY FIGURE 1. IFN-γ and TNF-α treatment of MAB results in upregulation of molecules involved in the immunological synapse. The levels of expression of HLA class I (**a**), HLA class II (**b**), ICAM-1 (**c**) and PDL-1 (**d**) was quantified by cytofluorimetric analysis on mesoangioblasts untreated (MAB), treated with IFN-γ (MABγ) or treated with TNF-α (MABα). Relative fluorescence intensity (RFI) was measured as the ratio of the mean fluorescence intensity (MFI) of specific markers to the MFI of isotype controls. Each symbol represents the RFI of HLA class I, HLA class II, ICAM-1 and PDL-1 in individual donors. Cells harvested from individual donors are represented in all plots by the same symbol.

*p<0.05; **p<0.01.

Supplementary Figure 2



SUPPLEMENTARY FIGURE 2. Levels of expression of HLA class I on target cells. (**a**) The level of expression of HLA class I was measured on mesoangioblasts untreated (MAB) or treated with IFN-γ (MABγ), PBMC and PHA T-cell lines (PHA) by FACS analysis. Relative fluorescence intensity (RFI) was measured as the ratio of the mean fluorescence intensity (MFI) of specific markers to the MFI of isotype controls. Averages + SEM are shown. N=4 (**b**) The level of expression of HLA class I was measured on MAB, MABγ and myotubes differentiated from MAB (MT) by immunofluorescence for myosin heavy chain (green), HLA-I (red) and Hoechst (blue). Averages of mean fluorescence intensity (MFI) + SEM are shown. N=3 (**c**) Results from a representative experiment are shown.

*p<0.05; **p<0.01.

Supplementary Table 1. Immunological characterization of untreated, IFN-γ treated and TNFα treated mesoangioblasts.

Function	Marker	MAB (RFI) [a]	MABγ (RFI) [a]	MABα (RFI) [a]
HLA MOLECULES	HLA-I	20.5 (11.6-80.6)	64.8 (23.3-220.4) [d]	21.6 (16.2-23.3)
	HLA-II	2.1 (0.7-79.5)	16.0 (1.8-276.5) [d]	4.8 (1.1-6.7)
COSTIMULATORY MOLECULES	CD80	1.0 (0.5-1.1)	0.9 (0.8-1.3)	0.9 (0.9-1.0)
	CD86	1.0 (0.9-1.2)	1.0 (0.8-1.3)	1.0 (0.9-1.1)
	CD70	1.0 (0.5-1.5)	1.0 (0.3-1.3)	1.0 (0.9-1.2)
	CD40	1.0 (0.6-1.3)	1.0 (0.7-1.2)	1.1 (0.9-1.2)
	PDL-1	1.7 (1.0-2.1)	4,5 (0.5-16.7) [b]	2.5 (2.1-3.4)
ADHESION MOLECULES	ICAM-1	2.6 (1.0-5.8)	39.2 (1.6-90.5) [d]	34.9 (27.8-43.0) [c]
	LFA-3	3.0 (1.0-12.0)	2.9 (1.0-13.0)	2.2 (1.9-2.5)

[a] Median and range

[b] $p<0.05$

[c] $p<0.01$

[d] $p<0.001$