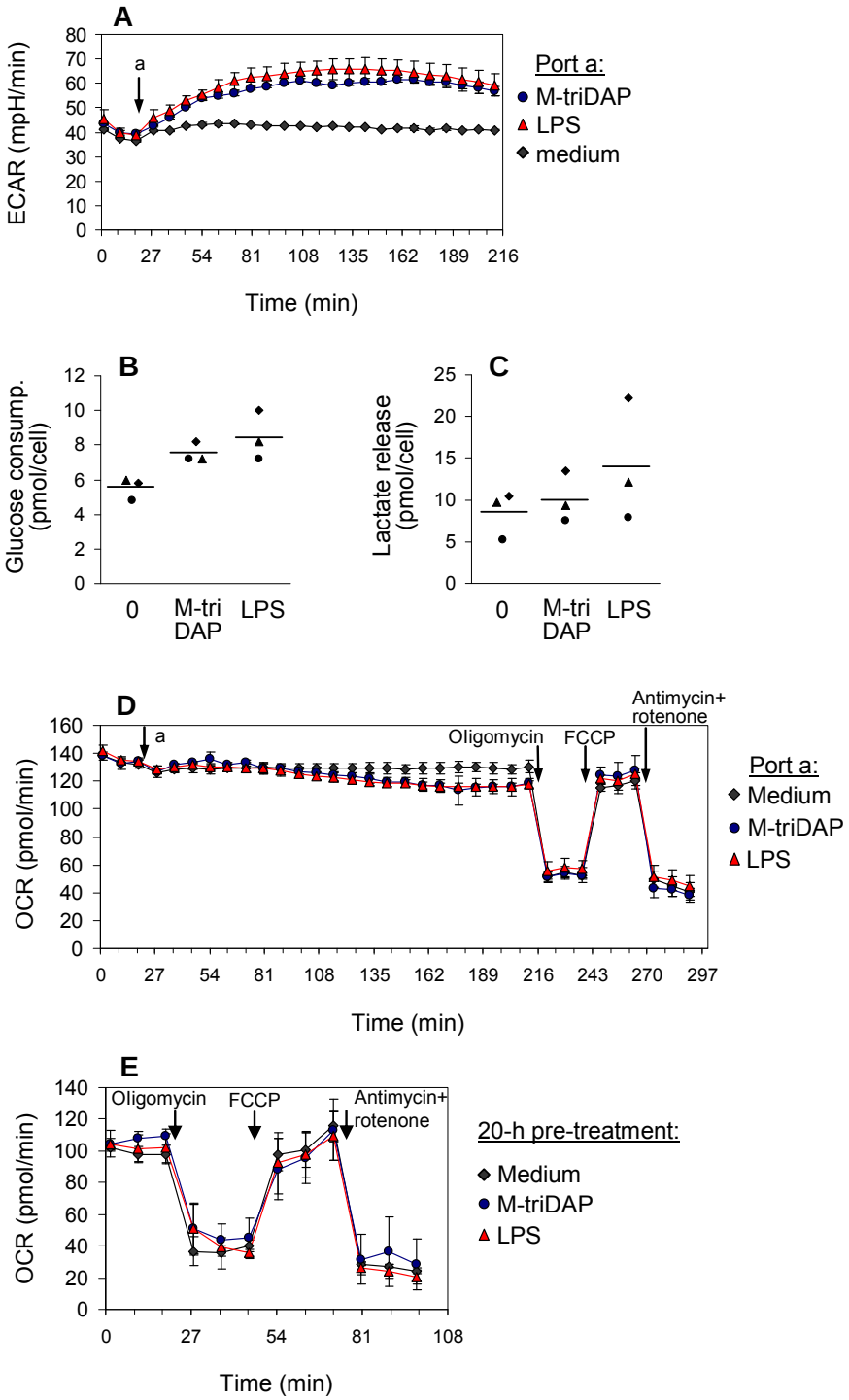

Figure S1. Additional characteristics of glucose and oxygen metabolism in MDM generated with M-CSF or GM-CSF. **A-C, c**haracteristics of glycolysis in MDM generated with M-CSF (M-MDM). M-MDM were stimulated with M-triDAP or LPS and assessed for ECAR (**A**), 24-h glucose consumption (**B**) and 24-h lactate release (**C**). 3 independent donors (one representative donor is shown in A). **D** and **E**, mitochondrial stress test in GM-CSF-generated MDM, untreated or treated by M-triDAP or LPS for 3 h (D) or 20 h (E). Mitochondrial stress test was performed by measuring OCR upon sequential injections of oligomycin, FCCP and antimycin A + rotenone as described (Everts et al, 2012). MDM show almost no spare respiratory capacity, because OCR after addition of FCCP does not exceed baseline OCR, and use most of oxygen consumed to sustain mitochondrial respiration, because OCR after addition of antimycin A and rotenone is around 30% of baseline OCR.

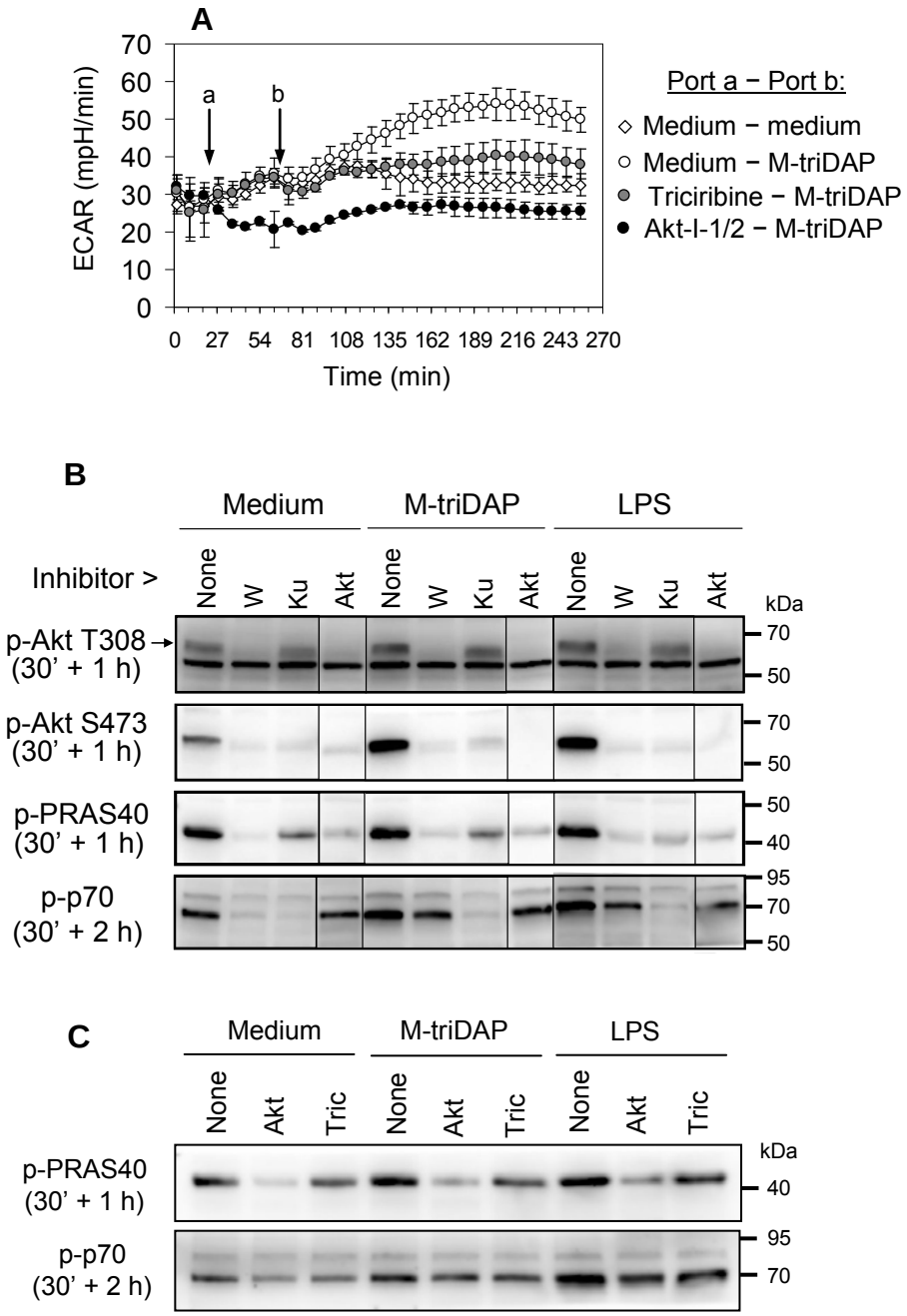

Figure S2. To the role of Akt in M-triDAP- or LPS-induced glycolytic responses of MDM. A, measurements of ECAR after addition of Akt-I-1/2 (10 μM) or triciribine (20 μM) and subsequent stimulation with M-triDAP. Mean ± s.d. of quadruplicate wells, one representative donor out of 4. **B and C,** inhibition of Akt, PRAS40 and p70 phosphorylation in resting and stimulated MDM. Cells were pre-incubated for 30 min with medium, wortmannin (W, 100 nM), Ku 0063794 (Ku, 1 μM), Akt-I-1/2 (Akt, 10 μM) or triciribine (Tric, 20 μM), then cultured for 1 h (p-Akt, p-PRAS40) or 2 h (p-p70) with medium, M-triDAP (10 μg/ml) or LPS (100 ng/ml), whereafter phosphorylated kinases were assessed. One representative experiment out of 2 (B) or 3 (C). Arrow indicates specific bands for Akt/p-Akt (60 kD) and p-p70 (70 kD), vertical lines in B indicate individual gel boundaries.

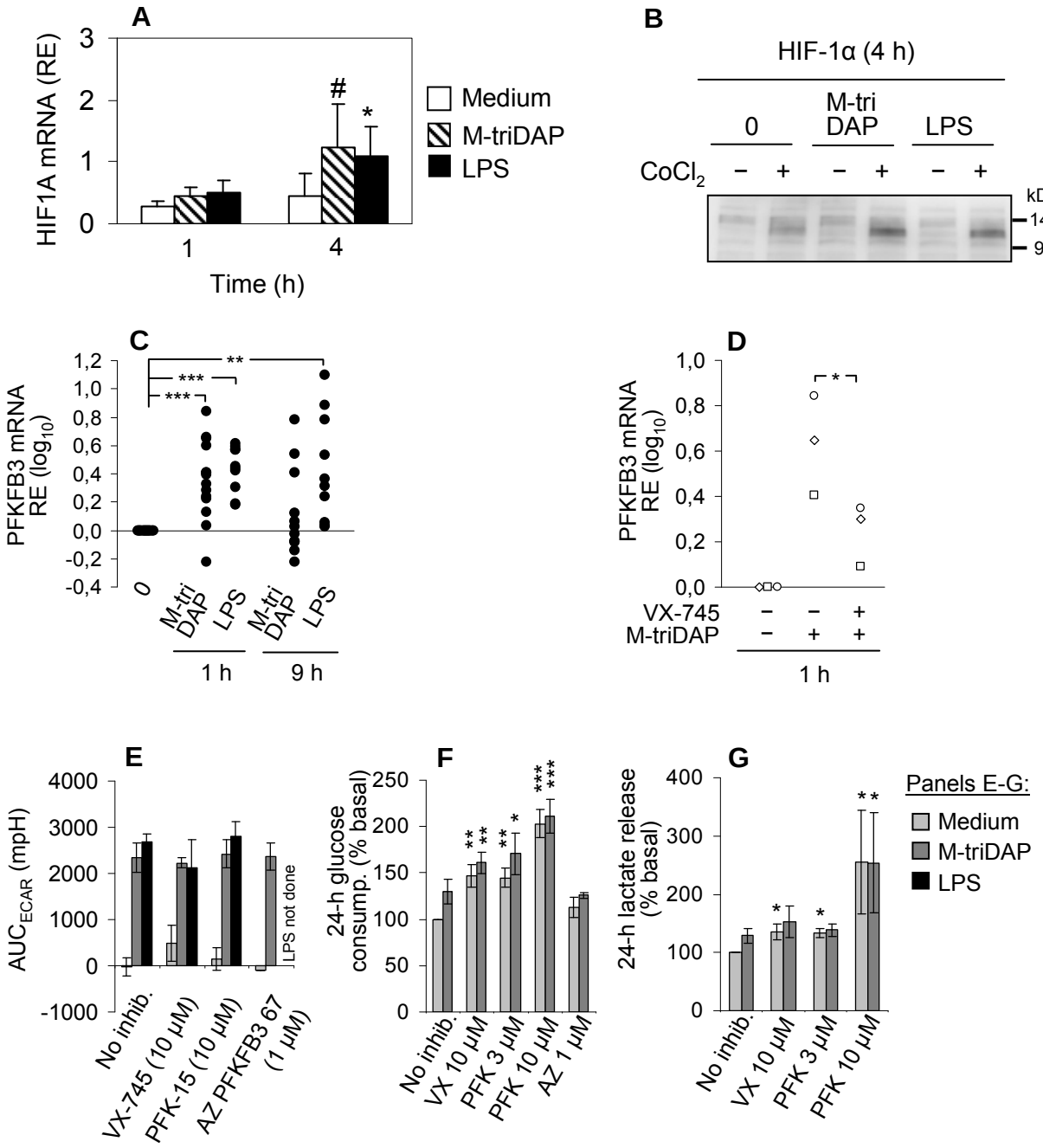

Figure S3. Induction of HIF1-α and PKFKB3 expression in MDM after triggering of NOD1 or TLR4. **A,** kinetics of HIF-1A mRNA expression in MDM stimulated with M-triDAP (10 μg/ml) or LPS (100 ng/ml). Mean ± SD, 5 to 8 independent experiments per data point. * $p < 0.05$, # $p = 0.06$ compared to basal HIF1A mRNA expression. **B,** HIF-1α protein in MDM stimulated for 4 h with M-triDAP or LPS with or without addition of CoCl2 (100 μM) to mimick hypoxia. HIF-1α gives a band of around 120 kD. **C,** PFKFB3 mRNA expression in MDM upon stimulation with M-triDAP or LPS (11 donors). **D,** effect of p38 inhibitor pre-treatment (VX-745, 10 μM) on PFKFB3 mRNA expression 1 h after addition of M-triDAP (3 experiments). **E, F and G,** effects of p38 (VX-745) and PFKFB3 inhibitors (AZ PFKFB3 67, PFK-15) on ECAR (E), 24-h glucose consumption (F) and lactate release (G) in basal conditions and upon M-triDAP stimulation (mean ± SD, 4 donors). In F and G, asterisks denote p-values in relation to cells treated with M-triDAP without inhibitors.

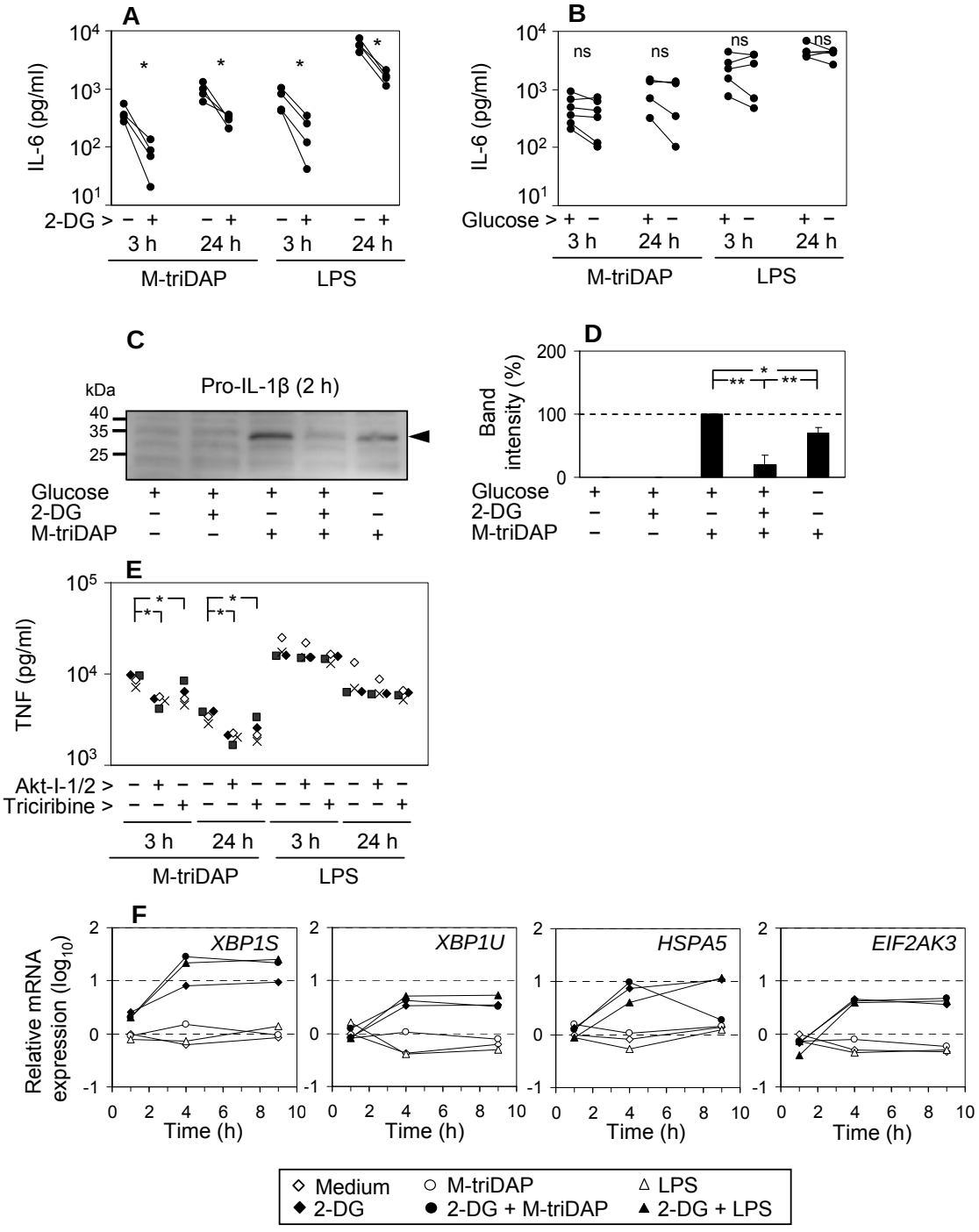

Figure S4. Additional data illustrating alterations of cytokine production by MDM under 2-DG or Akt inhibitor treatment or glucose starvation. **A and B,** effects of 2-DG (A) and glucose-free medium (B) on M-triDAP- or LPS-induced IL-6 production by MDM from individual donors. * $p < 0.05$, ns, non significant (paired t-test). **C and D,** cells were pre-treated with 2-DG (50 mM) or glucose-free medium for 30 min, then cultured with or without M-triDAP for 2 h, whereafter levels of pro-IL-1β were assessed (C, a representative experiment; D, densitometry, mean ± SD of 5 donors). **E,** effects of Akt-I-1/2 and triciribine on TNF levels in supernatants 3 or 24 h after addition of M-triDAP or LPS (4 experiments, each designated by a unique symbol; * $p < 0.05$ by paired t-test). **F,** effects of 2-DG without or with PRR agonists on mRNA levels of UPR markers in MDM from a representative donor.

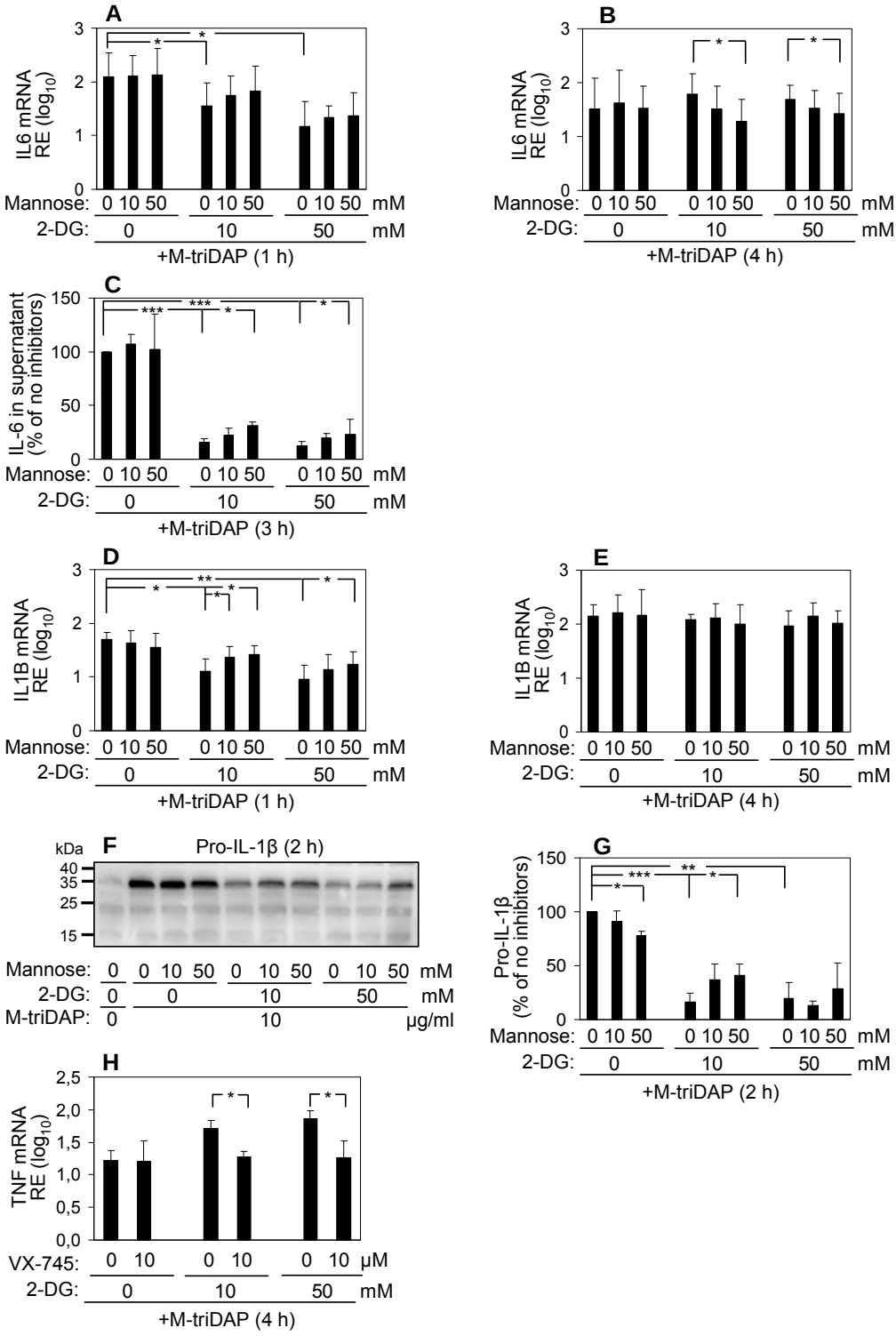

Figure S5. Effects of 2-DG, mannose and VX-745 on IL-6 and IL-1β production by M-triDAP-stimulated MDM. Cells were pre-treated for 30 min with 2-DG and/or D-mannose (A-G) or with VX-745 (H) at indicated concentrations in ordinary medium, stimulated with M-triDAP for indicated time periods, whereafter levels of IL-6 mRNA (A and B), IL-6 in supernatant (C), IL1B mRNA (D and E), intracellular pro-IL-1β (F and G) and TNF mRNA (H) were assessed. 3 experiments per data point, p-values by paired t-test. In C and G, results were normalized to cells treated with M-triDAP without 2-DG or mannose.

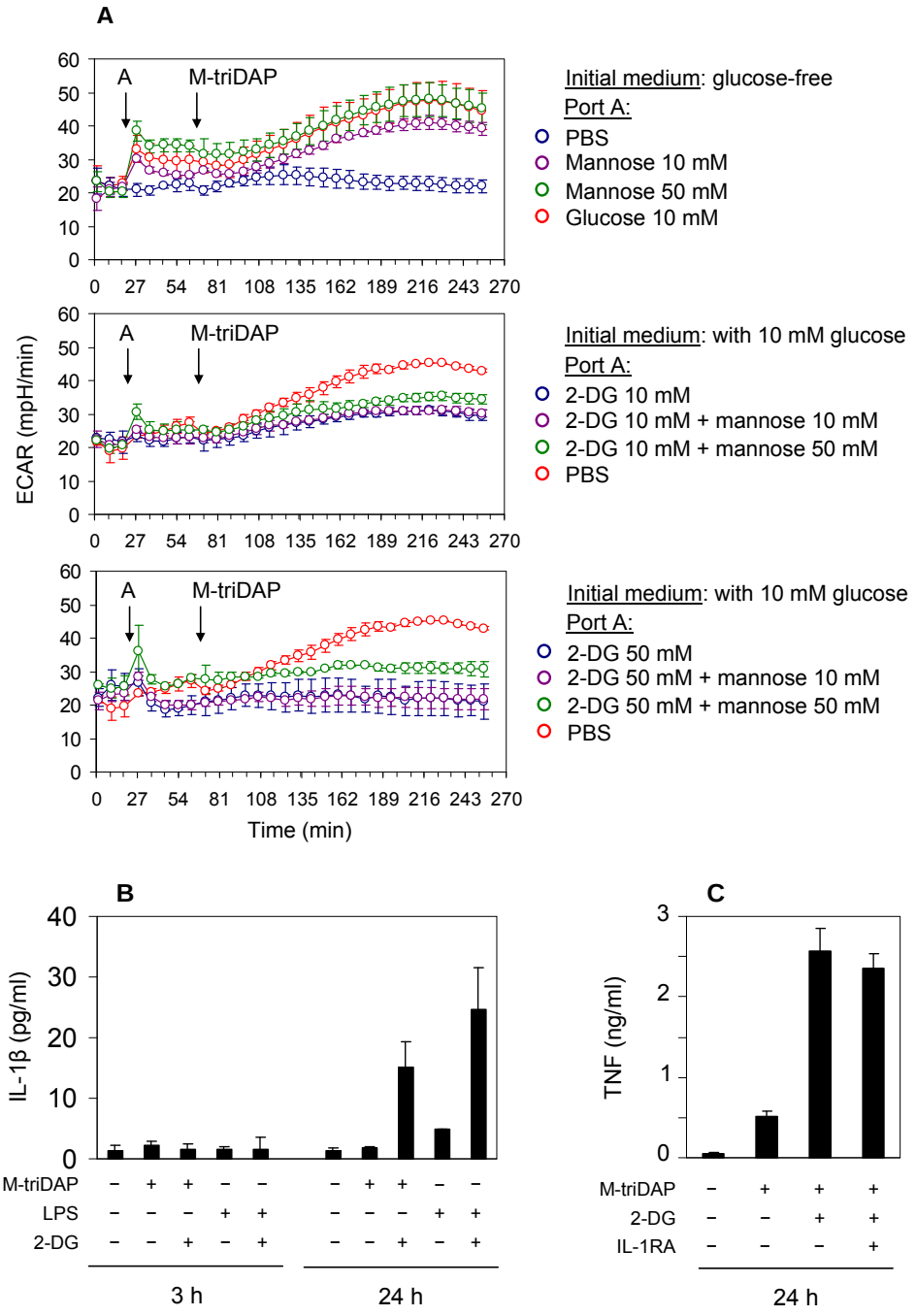

Figure S6. To the mechanisms of the modulatory effects of 2-DG and D-mannose on glycolysis and cytokine production by MDM. **A,** effects of D-mannose and 2-DG on glycolytic reprogramming of MDM. Cells were plated in glucose-free medium upper plot or ordinary medium (middle and lower plots). PBS, D-mannose and/or 2-DG were injected through ports A to indicated final concentrations, then M-triDAP was injected through ports B to 10 μg/ml. Mean ± SD of quadruplicate wells, one experiment out of 2 with similar results. **B,** effect of 2-DG (50 mM) on levels of IL-1β in supernatants secretion during 3-hour and 24-hour stimulation with M-triDAP. Mean ± SD of triplicate wells, one experiment out of 2 with similar results. **C,** effect of IL-1RA (500 ng/ml) on 2-DG-mediated enhancement of TNF production induced by M-triDAP, mean ± SD of 3 independent donors.

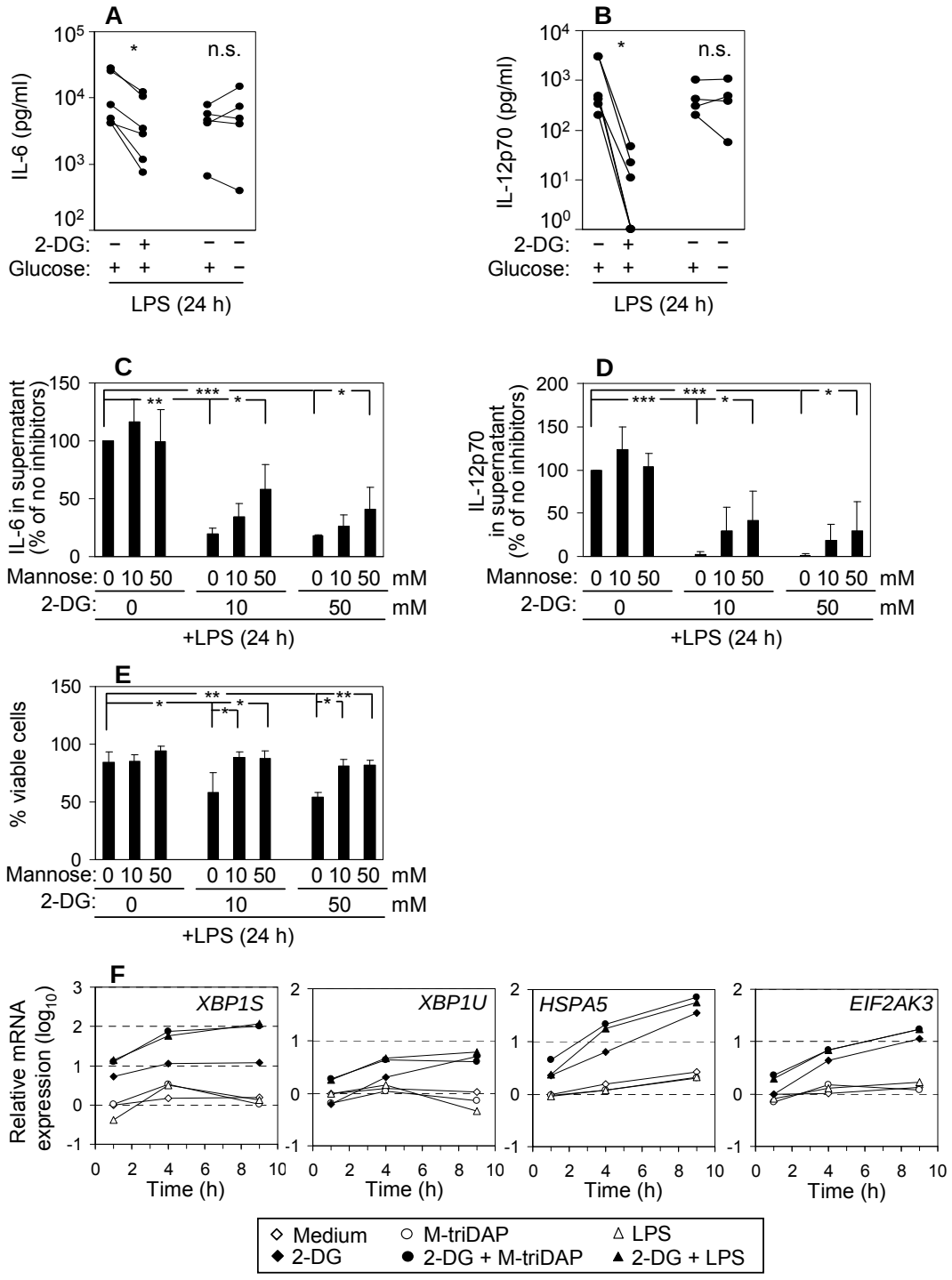

Figure S7. Additional data illustrating alterations of cytokine production by MDDC under 2-DG treatment or glucose starvation. **A and B,** effects of 2-DG and glucose-free medium on IL-6 (A) and IL-12p70 (B) production by MDDC upon M-triDAP or LPS treatment. **C and D,** influence of 2-DG and mannose on IL-6 (C) and IL-12p70 (D) production by LPS-stimulated MDDC (3 experiments per data point, results were normalized to cells treated with LPS without 2-DG or mannose, p-values by paired t-test). **E,** effects of 2-DG and mannose on viability of LPS-stimulated MDDC (3 experiments). Percentages of live cells were assessed by trypan blue exclusion. **F,** effects of 2-DG without or with PRR agonists on mRNA levels of UPR stress markers in MDDC from a representative donor.

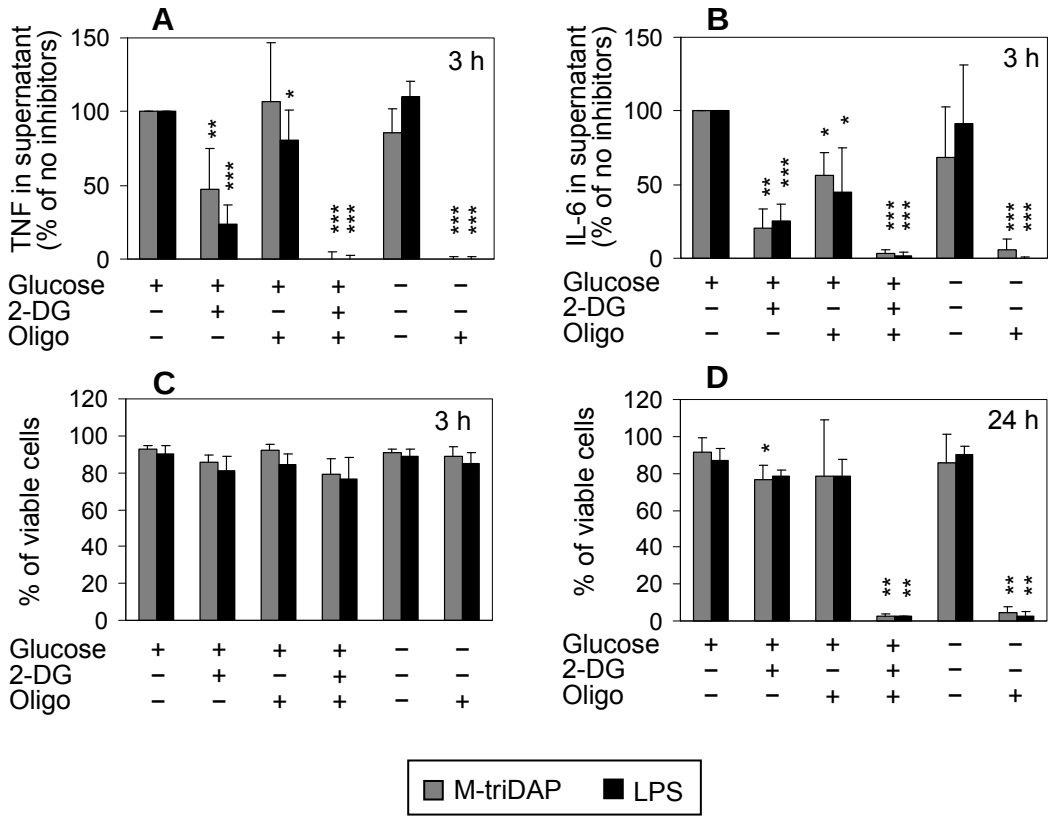

Figure S8. Cytokine production and viability of MDM in hypoxia-like conditions. MDM were pre-treated with 2-DG (50 mM), glucose-free medium and/or oligomycin (2 μM) for 30 min, then activated with M-triDAP or LPS. TNF (A) and IL-6 (B) production was assessed at 3 h, viability of trypsinized cells was assessed by trypan-blue exclusion at 3 h (C) and 24 h (D). Mean ± SD, p-values by paired t-test. Three to 10 independent experiments per data point.

Table S1. Enzyme inhibitors used in the study.

Inhibitor	**Target**	**Supplier**	**Final concentration**
2-deoxy-D-glucose (2-DG)	Glycolysis (glucose-6-phosphate isomerase), N-linked protein glycosylation	Sigma	2-50 mM
Akt-I-1/2	Akt	Tocris	10 μM
Antimycin A	Mitochondrial complex III	Sigma	500 nM
AZ PFKFB3 67	PFKFB3	Tocris	1 μM
Carbonyl cyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP)	Mitochondrial electron transport chain	Sigma	2 μM
Ku 0063794	mTOR (both mTORC1 and mTORC2)	Tocris	1 μM
Oligomycin	Mitochondrial ATP synthase	Sigma	2 μM
PFK-15	PFKFB3	Tocris	1-10 μM
Rapamycin	mTORC1	Merck Millipore	10 nM
Rotenone	Mitochondrial complex I	Sigma	500 nM
Triciribine	Akt	Tocris	20 μM
VX-745	p38 MAPK	Tocris	10 μM
Wortmannin	PI-3 kinase	Sigma	100 nM

Table S2. PCR primers used in the study.

Gene	Gene full name	Primer	Sequence (5' → 3')
ACOD1 (IRG1)	Aconitate decarboxylase 1	Forward	TGCAAGGCCGATTACTGCAT
		Reverse	GAGCTTCTCGGCACTTTGTC
ACTB	Actin beta	Forward	GCACAGAGCCTCGCCTTT
		Reverse	CACGATGGAGGGGAAGACG
EIF2AK3 (PERK)	eukaryotic translation initiation factor 2 alpha kinase 3	Forward	TTCAGCACTCAGATGGAGAGAG
		Reverse	CGTTCCATGGGGGATGGAGA
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Forward	CAGCCTCCCGCTTCGCTCTC
		Reverse	ACCAGGCGCCCAATACGACC
HIF1A	Hypoxia inducible factor 1, alpha subunit	Forward	AGCCCTAACGTGTTATCTGTCG
		Reverse	TCCATTTTTCGCTTTCTCTGAGC
HK1	Hexokinase 1	Forward	CAGCTCCTGGCCTATTACTTCA
		Reverse	CCGGGAGAGGCCATTCTTC
HK2	Hexokinase 2	Forward	CCTGAGGACATCATGCGAGG
		Reverse	TGGACTTGAATCCCTTGGTCC
HK3	Hexokinase 3	Forward	CATTGGGTCTTCAGGGTTGC
		Reverse	TGTAGCTGTGCCCTTGTCAC
HSPA5 (BiP)	Heat shock protein family A (Hsp70) member 5	Forward	CATCAACGAGCCTACGGCAG
		Reverse	TGGCCACAACTTCGAAGACA
IL1B	Interleukin 1B	Forward	GAGCTCGCCAGTGAAATGATG
		Reverse	TGGTGGTCGGAGATTCGTAG
IL6	Interleukin 6	Forward	GCCACTCACCTCTTCAGAACG
		Reverse	TCAGCCATCTTTGGAAGGTTCA
LDHA	Lactate dehydrogenase A	Forward	TGCCTGTATGGAGTGGAATGAA
		Reverse	CCAGGATGTGTAGCCTTTGAGT
PFKFB3	6-phosphofructo-2-kinase / fructose-2,6-bisphosphatase 3	Forward	AAACTGACGCCTGTCGCTTA
		Reverse	CTCATGAGCGGGTTAGGTCC
PFKL	Phosphofructokinase, liver type	Forward	GGGTGCCAAAGTCTTCCTCAT
		Reverse	GGATGATGTTGGAGACGCTCA
PFKM	Phosphofructokinase, muscle	Forward	GCCATCAGCCTTTGACAGAAT
		Reverse	ACCTGGACACATTCCATGAGG
PKM1	Pyruvate kinase, isoform M1	Forward	AGTGATGTGGCCAATGCAGTC
		Reverse	AAACAGCTTGCGGTGGAAC
PKM2	Pyruvate kinase, isoform M2	Forward	CTATCCTCTGGAGGCTGTGC
		Reverse	TCTGTGGGGTCGCTGGTAA
SLC2A1 (GLUT1)	Solute carrier family 2, member 1	Forward	ATTGGCTCCGGTATCGTCAA
		Reverse	ATGGCCACGATGCTCAGATA
SLC2A3 (GLUT3)	Solute carrier family 2, member 3	Forward	GACCCAGAGATGCTGTAATGGT
		Reverse	TGGCAAATATCAGAGCTGGGG
TNF	Tumor necrosis factor	Forward	TCGGCCCCCAGAGGGAAGAG
		Reverse	CGGCGGTTCAGCCACTGGAG
XBP1S	X-box binding protein 1 (spliced mRNA)	Forward	CTGAGTCCGCAGCAGGTG
		Reverse	CTCTGGGGAAGGGCATTTGA
XBP1U	X-box binding protein 1 (unspliced mRNA)	Forward	TGGCCTTGTAGTTGAGAACCA
		Reverse	TGCACGTAGTCTGAGTGCTG