SUPPLEMENTARY DATA

Altered Extracellular Vesicle Concentration, Cargo and Function in Diabetes Mellitus

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Supplementary Table S1. Primer sequences for RT-qPCR

CRADD	GGGCAGGTTCCCTAACAGTCA	TACTTGTTTGTCTCTGGCCTCCAT
CXCL5	GGAGTTCATCCCAAAATGATCAGT	CAAATTTCCTTCCCGTTCTTCA
DDIT3	AGAACCAGGAAACGGAAACAGA	TCTCCTTCATGCGCTGCTTT
GSTP1	CCTGGTGGACATGGTGAATG	CCGCCTCATAGTTGGTGTAGATG
HPRT	AGATGGTCAAGGTCGCAAGCT	GGGCATATCCTACAACAAACTTGTC
SOD2	GCCCTGGAACCTCACATCAA	CCAACGCCTCCTGGTACTTC
UBC	ATTTGGGTCGCGGTTCTTG	TGCCTTGACATTCTCGATGGT

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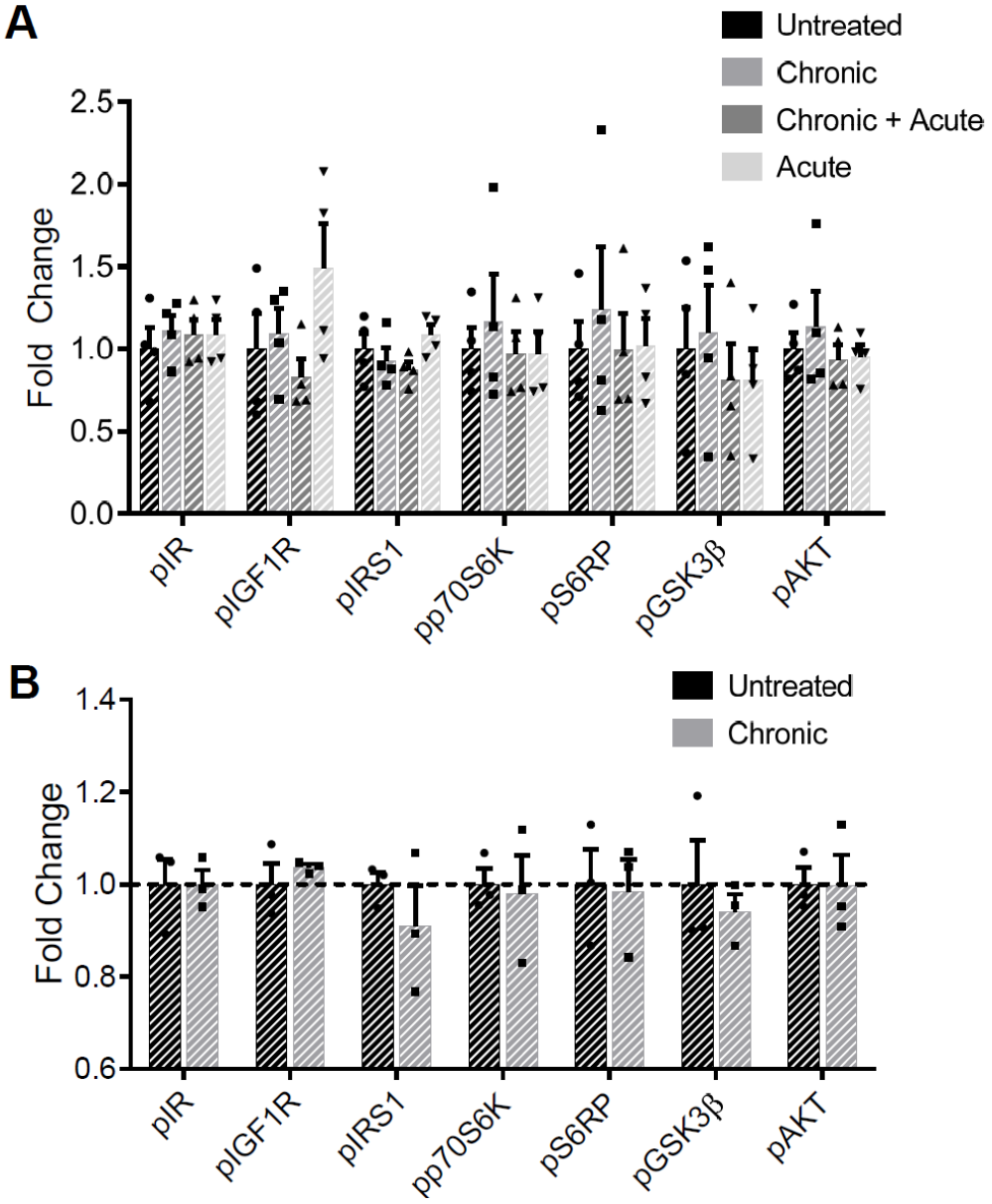
Supplementary Figure S1. EV neuronal cargo after insulin treatment. **(A)** EVs were isolated from primary cortical neurons treated with either no insulin (untreated), insulin for 48 hours (chronic), insulin for 48 hours followed by 30 minutes with fresh insulin (chronic + acute) or no insulin for 48 hours followed by 30 minutes with fresh insulin (acute). **(B)** Primary cortical neurons were incubated with fresh media for 48 hours either containing no insulin (untreated) or 200nM of insulin (chronic). The vesicles were lysed and protein content was analyzed using the AKT signaling panel II and insulin signaling kits from MesoScale Diagnostics. The histograms represent the mean + SEM (n=4 for A and n=3 for B).



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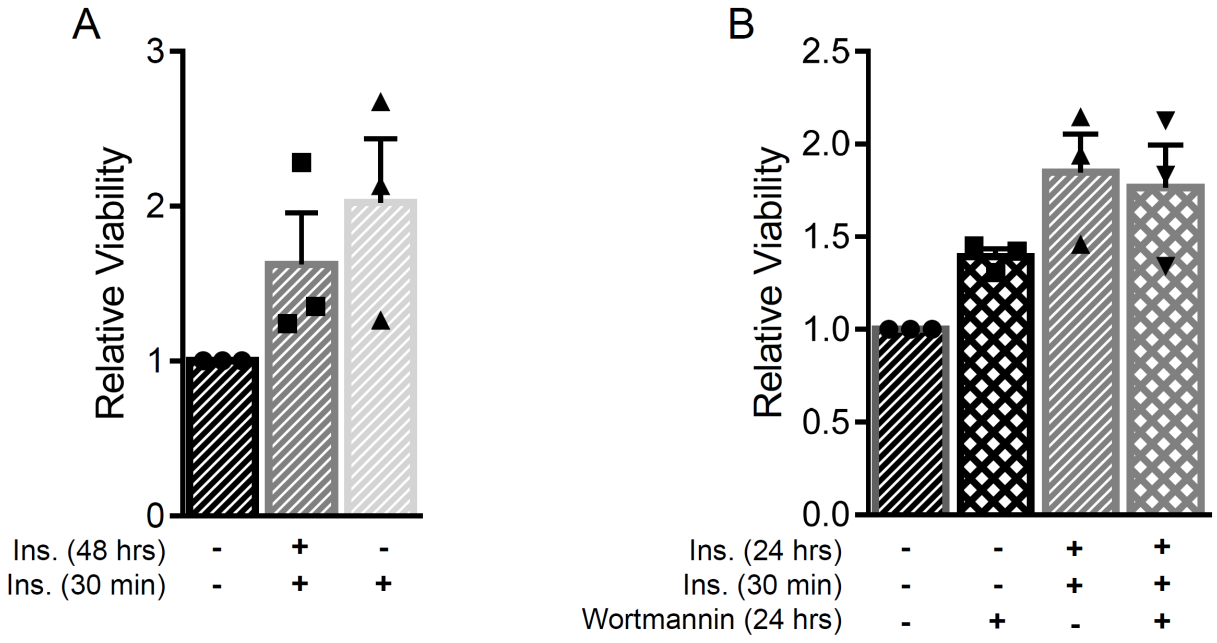
Supplementary Figure S2. Cell viability of primary cortical neurons. **(A)** Primary cortical neurons were treated with insulin (200nM) for the indicated times. Cell viability was measured using a MTT assay. **(B)** Neurons were treated as in (A) in the absence or presence of wortmannin. Each experiment was performed in triplicate. The histogram represents the mean + SEM from 3 independent experiments.



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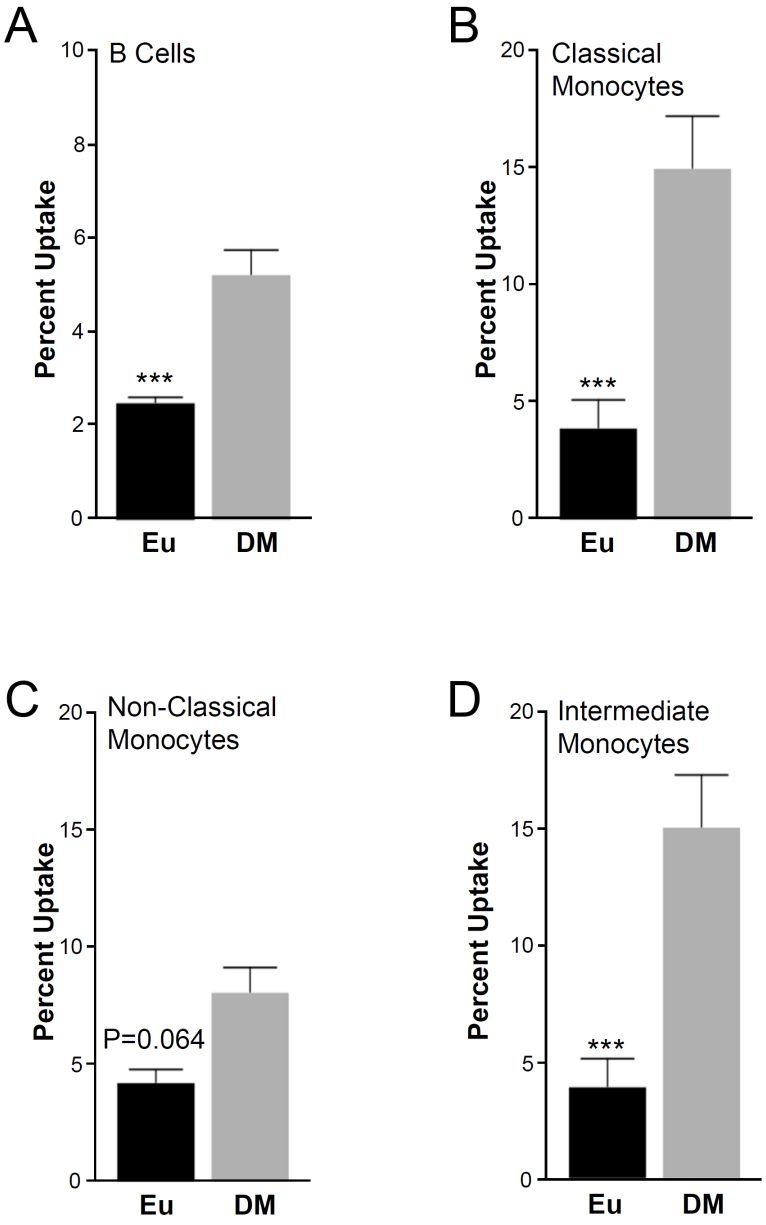
Supplementary Figure S3. PBMC internalization of EVs from diabetic and euglycemic individuals. **(A)** PBMCs (~200,000 cells) were incubated with plasma EVs (3 x 10^8) from diabetic (DM, n=39) and euglycemic (Eu, n=19) for 24 hours. Cells were sorted by FACS into B cells that had internalized EVs ($CD19^+PKH^+$) **(B)** classical monocytes that had internalized EVs ($CD14^{++}CD16^-PKH^+$) **(C)** non-classical monocytes that had internalized EVs ($CD14^-CD16^+PKH^+$) and **(D)** intermediate monocytes that had internalized EVs ($CD14^{++}CD16^+PKH^+$). The histograms represent the mean + S.E.M. The data was log transformed and significance was calculated using linear mixed model regression taking into account matching. ****P*<0.001



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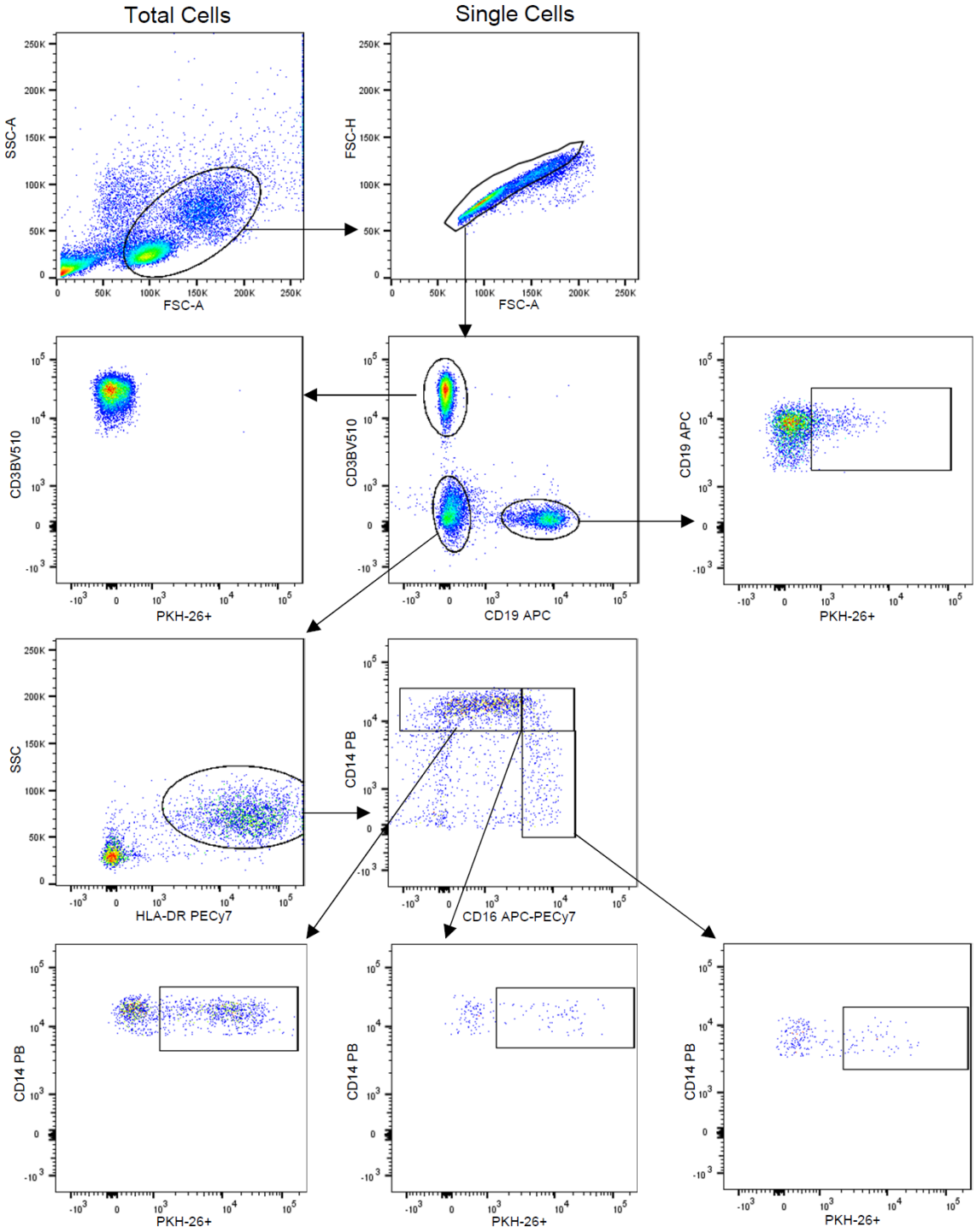
Supplementary Figure S4. FACS gating for EV internalization assays. PBMCS were incubated with PKH26-labeled EVs as described in Research Design and Methods. Cells were sorted into PKH+ and PKH- T Cells ($CD3^{+}$), B Cells ($CD19^{+}$), intermediate monocytes ($CD14^{++}CD16^{+}$), classical monocytes ($CD14^{++}CD16^{-}$) and nonclassical monocytes ($CD14^{-}CD16^{+}PKH^{+}$). Representative FACS plots are shown.



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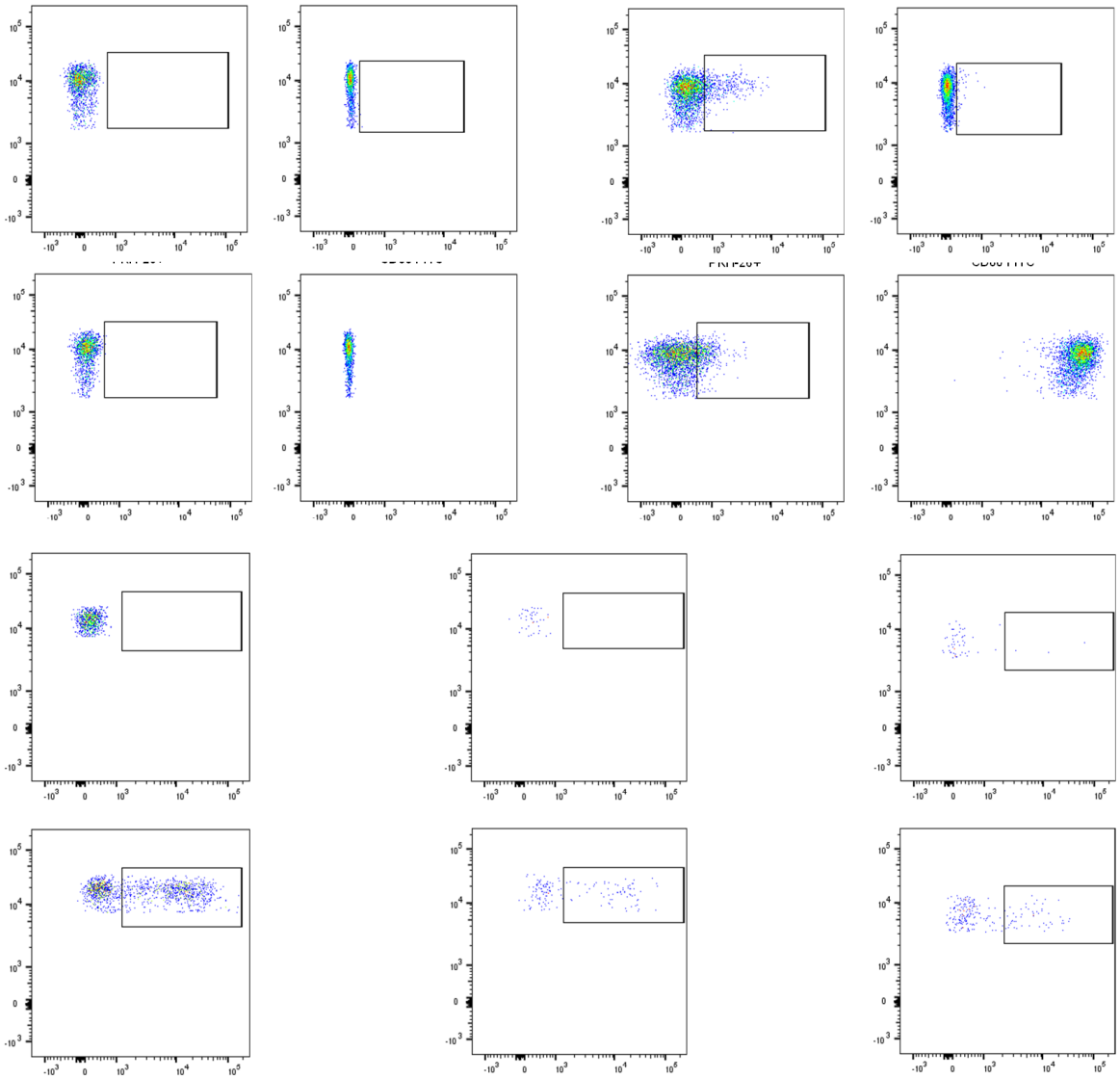
Supplementary Figure S5. FACS sorting controls for EV internalization assay. **(A,B)** Representative FACS plots of B Cells ($CD19^{+}$) sorted for either PKH-26, CD80, CD25 or MHC-II are shown. Samples were stained for IgG control (A) or specific antibodies (B). **(C-E)** Representative FACS plots of monocytes (CD14+) sorted into (C) classical monocytes ($CD14^{++}CD16^{-}$), (D) intermediate monocytes ($CD14^{++}CD16^{+}$) or (E) non-classical monocytes ($CD14^{-}CD16^{+}$). Cells were incubated with either PBS and stained with a CD14 isotype control or incubated with PKH-labeled EVs and stained with a Pacific Blue conjugated CD14 antibody as denoted on the plots.



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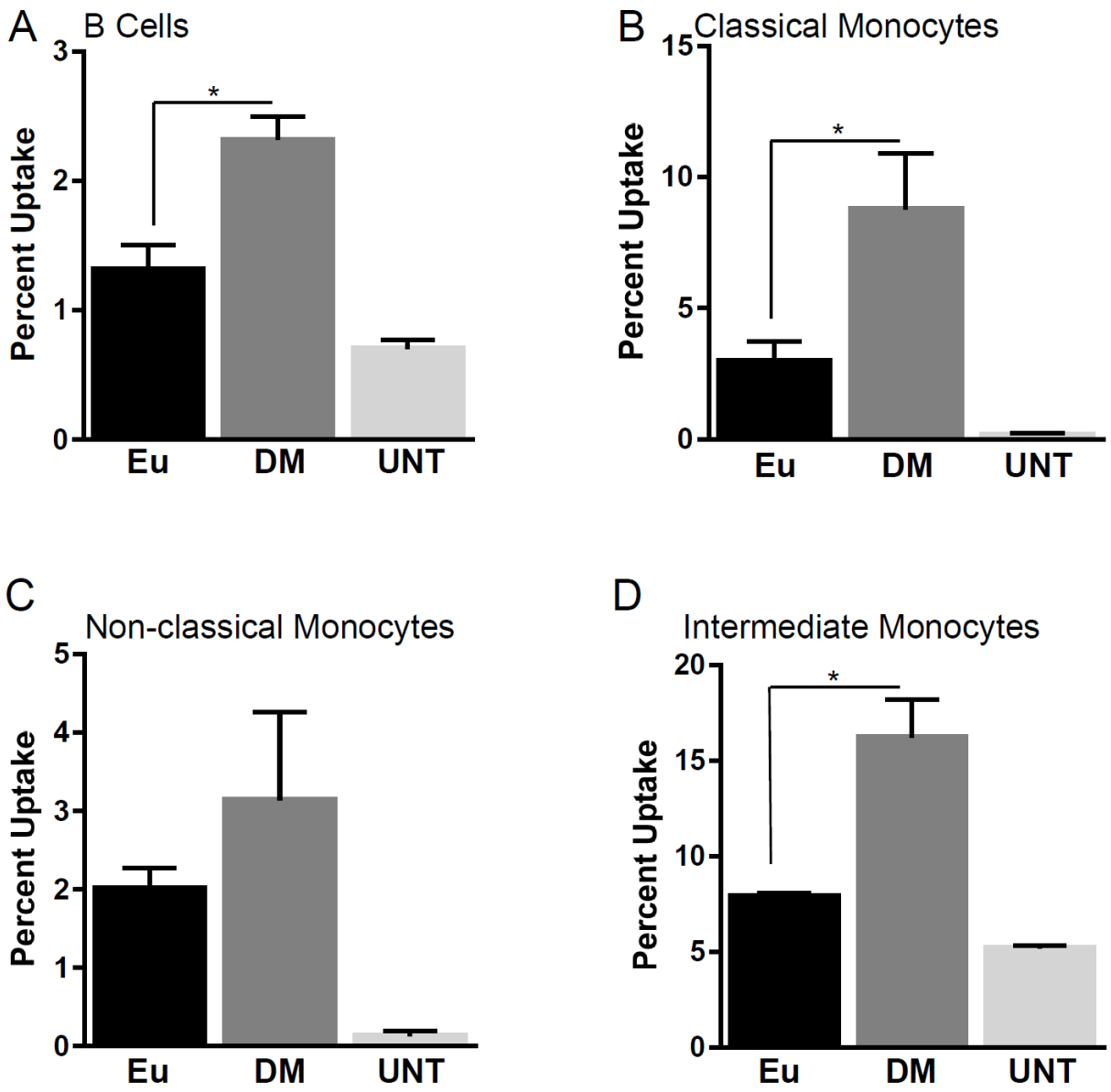
Supplementary Figure S6. Monocyte internalization of diabetic and euglycemic EVs from the microarray experiment. **(A)** PKH26 labeled EVs from several individuals were pooled (4.5×10^{11}) and grouped as either diabetic (DM, n=3) or euglycemic (Eu, n=3) and incubated with PBMCs for 24 hours. PBS with PKH26 was used as a negative control (UNT, n=3). A small aliquot was taken for FACS analysis while RNA from the remaining cells was isolated and used for microarray. The FACS aliquot was sorted into B cells that had internalized EVs ($CD19^{+}PKH^{+}$) **(B)** classical monocytes that had internalized EVs ($CD14^{++}CD16^{-}PKH^{+}$) **(C)** non-classical monocytes that had internalized EVs ($CD14^{-}CD16^{+}PKH^{+}$) and **(D)** intermediate monocytes that had internalized EVs ($CD14^{++}CD16^{+}PKH^{+}$). Histograms represent the mean + SEM. Statistical significance was calculated using student's T test. *P<0.05.



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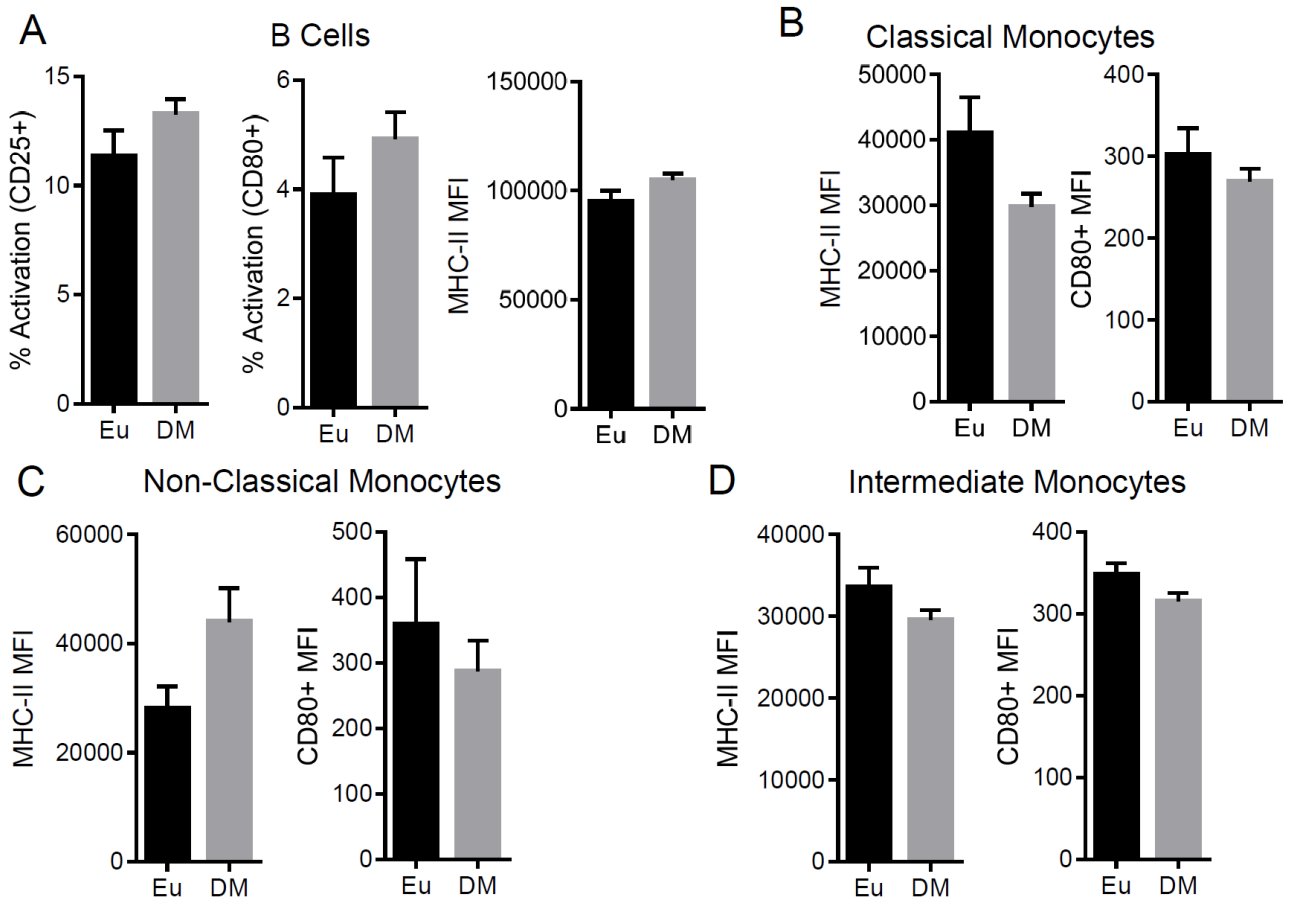
Supplementary Figure S7. PBMC activation levels following EV internalization. PBMCs (~200,000 cells) were incubated with plasma EVs (3 x 10^8) from diabetic (DM, n=39) and euglycemic (Eu, n=19) for 24 hours and cells were sorted by FACS. **(A)** B cells that had internalized EVs expressing either CD25 ($CD19^+PKH^+CD25^+$), CD80 ($CD19^+PKH^+CD80^+$) or MHC-II ($CD19^+PKH^+MHC\text{-}II^+$) . **(B-D)** Monocytes were subdivided into either (B) classical monocytes that had internalized EVs ($CD14^{++}CD16^-PKH^+CD25^+$) (C) non-classical monocytes that had internalized EVs ($CD14^-$ CD16+PKH+) and (D) intermediate monocytes that had internalized EVs ($CD14^{++}CD16^+PKH^+$) and the mean fluorescent intensity (MFI) for MHC-II and CD80 were measured.



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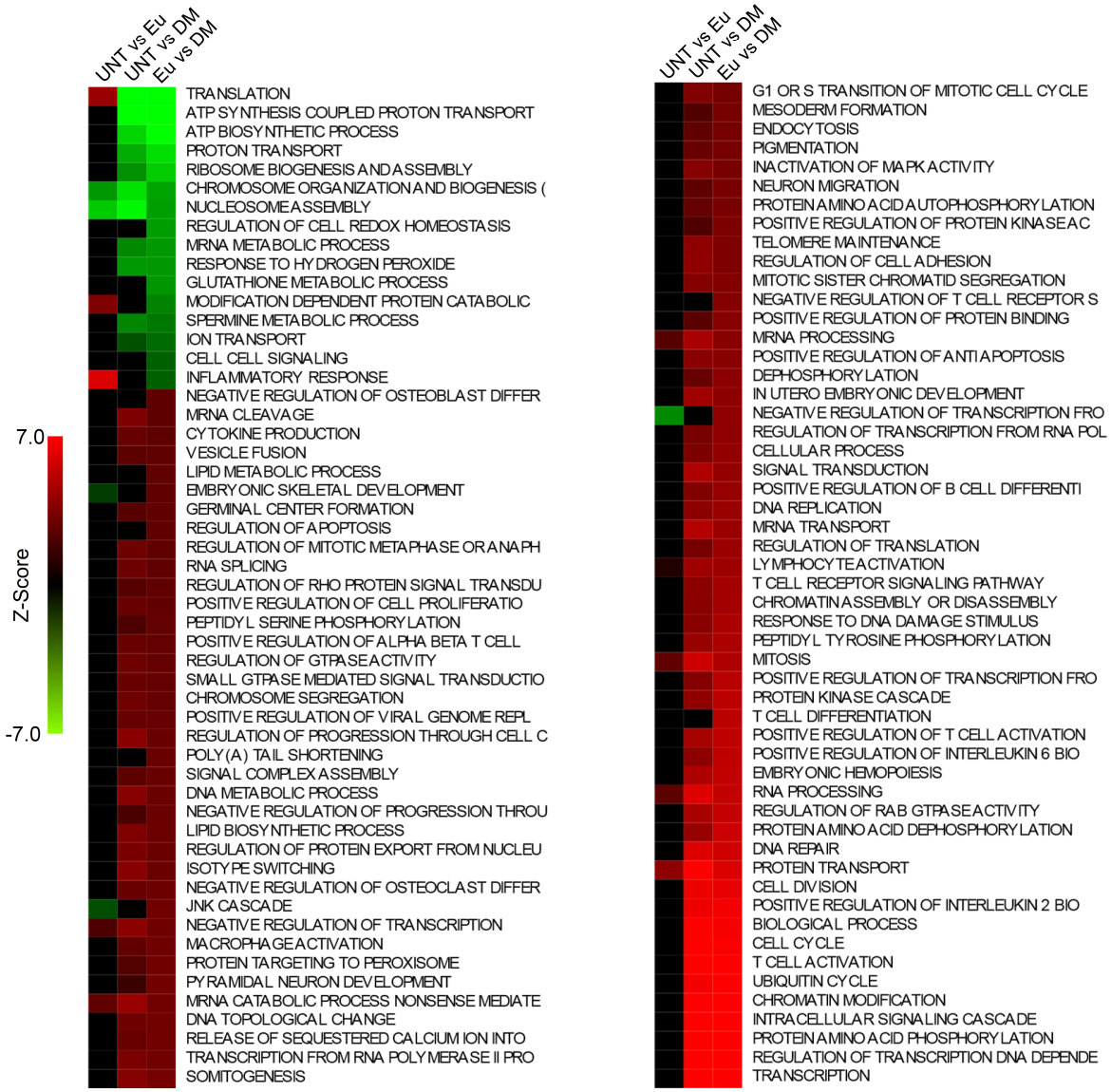
Supplementary Figure S8. Significant biological pathways altered in monocytes by EVs from diabetic individuals. Plasma EVs (4.5 x 10^{11}) from diabetic (DM, n=2) or euglycemic (Eu, n=3) individuals were incubated with PBMCs for 24 hours or untreated (UNT). Monocytes were isolated using FACS and gene expression was assessed via microarray. Significant biological pathways (P<0.01) are visualized on the heat map.



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Supplementary Figure S9. EVs are internalized by cells. Similar EV internalization assays were performed as we previously reported (Eitan E. et al., NPJ Aging Mech.Dis. 2016; Zhang, S. et al., Neuro. Biol. of Aging 2018). Primary cortical neurons were incubated with PKH26-labeled EVs in a ratio ~300 vesicles per neuron for 2 hours. Neurons were washed twice with PBS and fixed in 4% paraformaldehyde/PBS for 20 min. Fixed cells were incubated in blocking solution (0.3% Triton X-100 and 10% normal goat serum in PBS) for 30 min, and then incubated overnight at 4°C with antibodies against the neuronal marker MAP2 (Hm2, Sigma M9942). Cells were washed three times and incubated with Alexa Fluor 488 tagged anti-mouse secondary antibodies (Invitrogen) in blocking solution for 1 hr. The cells were then washed twice with PBS and nuclei were stained with DAPI (Sigma #32670). Coverslips were washed and mounted on slides in an anti-fade medium (Vector Laboratories, Burlingame, CA, USA). Images were acquired using a Zeiss LSM 510 confocal microscope with a 40 × objective. A representative confocal image of EV internalization is shown.