

SUPPLEMENTARY INFORMATION

Dysregulation of macrophage PEPD in obesity determines adipose tissue fibro-inflammation and insulin resistance

V Pellegrinelli et al

Corresponding authors: vp332@medschl.cam.ac.uk(VP); ajv22@medschl.cam.ac.uk (AV-P).

Supplementary Information Guide

Supplementary Tables (10)

Table S1: RNA-Seq reads mapped to the ENSEMBLE version GRCm38.p5 of the mouse reference genome sequence (GRCm38.p5) using STAR v2.5.

Table S2: Differentially expressed genes (DEGs) in GnW (a) and liver (b) from *pepd* HET mice versus *pepd* WT mice fed HFD 45%.

Table S3: Differentially expressed genes (DEGs) in GnW (a) and liver (b) from *pepd* KO mice versus *pepd* WT mice fed HFD 45%.

Table S4: Pathway enrichment analyses of the DEGs in GnW (a) and liver (b) from *Table S2*, performed with PIANO, using the gene set collection C2 retrieved from the Molecular Signatures Database (MSigDB).

Table S5: Pathway enrichment analyses of the DEGs in GnW (a) and liver (b) from *Table S3*, performed with PIANO, using the gene set collection C2 retrieved from the Molecular Signatures Database (MSigDB).

Table S6: Differentially expressed genes (DEGs) in GnW (a) and liver (b) from *pepd* WT mice fed HFD 45% versus *pepd* WT mice fed chow diet.

Table S7: Differentially expressed genes (DEGs) in GnW (a) and liver (b) from *pepd* HET mice fed HFD 45% versus *pepd* HET mice fed chow diet

Table S8: Differentially expressed genes (DEGs) in GnW (a) and liver (b) from *pepd* KO mice fed HFD 45% versus *pepd* KO mice fed chow diet

Table S9: Reagents and resources used in the study

Supplementary Table 10: Mouse and human gene primers used for RT-qPCR.

Table S2-S8 are available online as separate files. Statistical analysis of the RNAseq data is detailed in the methods section.

Table S1: RNA-Seq reads mapped to the ENSEMBLE version GRCm38.p5 of the mouse reference genome sequence (GRCm38.p5) using STAR v2.5.

Sample	Diet	Genotype	PEPD ID	Total Reads	Uniquely Mapped Reads	Uniquely Mapped Reads %	Multi Mapped Reads	Multi Mapped Reads %
D707_D501	Chow	KO	PEPD 301	8735979	7360900	84,25%	1035473	11,85%
D707_D502	Chow	HET	PEPD 306	8043290	6846911	85,12%	1002534	12,46%
D707_D503	45%HFD	KO	PEPD 312	8130998	7052352	86,73%	855073	10,51%
D707_D504	45%HFD	HET	PEPD 313	8367299	7184651	85,86%	862205	10,30%
D707_D505	45%HFD	HET	PEPD 314	7747695	6698573	86,45%	789286	10,18%
D707_D506	45%HFD	WT	PEPD 322	8104649	7015226	86,55%	855592	10,55%
D707_D508	45%HFD	HET	PEPD 324	8639457	7343993	85,00%	916753	10,61%
D708_D501	Chow	HET	PEPD 339	8456056	6960791	82,31%	1058178	12,51%
D708_D502	45%HFD	KO	PEPD 340	6802037	5761678	84,70%	713189	10,48%
D708_D503	Chow	HET	PEPD 341	7709896	6688513	86,75%	772386	10,01%
D708_D504	Chow	KO	PEPD 346	7923319	6620298	83,55%	997556	12,59%
D708_D505	Chow	WT	PEPD 347	7830084	6691002	85,45%	812673	10,37%
D708_D506	Chow	HET	PEPD 355	8211241	7039867	85,73%	931646	11,34%
D708_D507	Chow	HET	PEPD 357	7362327	6323510	85,89%	841495	11,42%
D708_D508	Chow	KO	PEPD 358	8119382	6962802	85,75%	871184	10,72%
D709_D501	45%HFD	WT	PEPD 360	7608713	6450883	84,78%	808492	10,62%
D709_D502	45%HFD	KO	PEPD 361	7409409	6293567	84,94%	870649	11,75%
D709_D503	45%HFD	KO	PEPD 374	6666850	5745494	86,18%	743072	11,14%
D709_D504	45%HFD	WT	PEPD 376	6469272	5591603	86,43%	676126	10,45%
D709_D505	45%HFD	HET	PEPD 382	7380531	6297858	85,33%	738923	10,01%
D709_D506	45%HFD	WT	PEPD 383	6836072	5901656	86,33%	781248	11,42%
D709_D507	45%HFD	HET	PEPD 391	7184285	6161343	85,76%	770128	10,71%

D710_D501	Chow	WT	PEPD 393	8564670	7296485	85,19%	922458	10,77%
D710_D502	45%HFD	KO	PEPD 398	8227166	7008007	85,18%	927791	11,27%
D710_D503	45%HFD	HET	PEPD 400	6182948	5307086	85,83%	664276	10,74%
D710_D504	45%HFD	KO	PEPD 545	7938676	6796124	85,60%	839014	10,56%
D710_D505	45%HFD	KO	PEPD 554	7391470	6166893	83,43%	772445	10,45%
D710_D506	45%HFD	KO	PEPD 555	8324283	7094824	85,23%	908872	10,91%
D710_D507	45%HFD	HET	PEPD 556	7639865	6449993	84,42%	772104	10,10%
D710_D508	45%HFD	HET	PEPD 557	8099599	6866629	84,77%	851036	10,50%
D711_D501	Chow	WT	PEPD 565	7448830	6237064	83,73%	778531	10,45%
D711_D502	Chow	WT	PEPD 568	8210613	6855045	83,49%	823816	10,03%
D711_D503	Chow	KO	PEPD 569	6002149	4729458	78,79%	779978	12,99%
D711_D504	45%HFD	KO	PEPD 585	7866984	6665392	84,72%	951001	12,08%
D711_D505	45%HFD	WT	PEPD 586	8457272	7164456	84,71%	929763	10,99%
D711_D506	45%HFD	WT	PEPD 587	8334038	7240383	86,87%	938666	11,26%
D711_D507	45%HFD	HET	PEPD 588	7792220	6733692	86,41%	836633	10,73%
D711_D508	45%HFD	WT	PEPD 589	8414976	7262246	86,30%	831210	9,88%
D712_D501	45%HFD	WT	PEPD 590	7408145	6344196	85,63%	790868	10,67%
D712_D502	45%HFD	HET	PEPD 591	7459845	6403981	85,84%	774005	10,37%
D712_D503	Chow	KO	PEPD 592	7463886	6273586	84,05%	782412	10,48%
D712_D504	Chow	HET	PEPD 595	7315992	6171088	84,35%	763890	10,44%
D712_D505	Chow	HET	PEPD 596	7364463	6043417	82,06%	817355	11,09%
D712_D507	Chow	HET	PEPD 597	7199724	6041573	83,91%	737897	10,24%

Table S9: Reagents and resources used in the study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-collagen I	Abcam	ab21286, RRID:AB_446161
anti- α SMA 1A4	Sigma	A2547, RRID:AB_476701
anti- PEPD	Abcam	ab86507, RRID:AB_1952335
anti-phospho-NF- κ B p65 (Ser536) (93H1)	Cell signaling	#3033, RRID:AB_331284
anti-NF- κ B p65	Cell signaling	#3034, RRID:AB_330561
anti-beta Actin	Abcam	ab8227, RRID:AB_2305186
anti-GADPH (D4C6R)	Cell signalling	#97166S, RRID:AB_2756824
anti- β -Tubulin	Cell signalling	#2146S, RRID:AB_2210545
anti- phospho-AKT (Ser473) (D9E)	Cell signalling	#4060, RRID:AB_2315049
anti-AKT	Cell signalling	#9272, RRID:AB_329827
MACS mouse CD11b microbeads	Miltnyibiotec	130-049-601, RRID:N/A
Anti-rabbit IgG, HRP-linked	Cell signaling	#7074, RRID:AB_2099233
Anti-mouse IgG, HRP-linked	Cell signaling	#7076, RRID:AB_330924
Alexa555-conjugated anti-rabbit	Life technologies	A21428, RRID:AB_141784
Alexa488-conjugated anti-mouse	Life technologies	A21202, RRID:AB_141607
Chemicals, siRNAs, Peptides, and Recombinant Proteins		
Prolidase from porcine kidney	Sigma-aldrich	P6675-250UN; CAS:9025-32-5
N-Benzyloxycarbonyl-L-proline	Alfa Aesar	B21672.30; CAS: 1148-11-4
Erlotinib hydrochloride	Sigma-aldrich	SML2156, 183319-69-9
Lipopolysaccharide	Sigma-aldrich	L4391
Dexamethasone	Sigma-aldrich	D1756; CAS: 50-02-2
Animal-free recombinant murine interleukin 4	Peptotech	AF-214-14
Insulin (Actrapid 100 IU/ml)	Novo Nordisk	1331415
Bodipy 493/503	Life science	D-3922
Phalloidin-iFluor 594 Reagent	Abcam	ab176757
Hoechst 33342	Thermofisher	H3570
45% HFD	Research Diets	D12451
58% HFD		D12331
L-Glycyl-L-Proline	Sigma-aldrich	5765594-5MG
L-Proline	Sigma-aldrich	P0380
Triton-X-100	Sigma-aldrich	T-9284

STAT60	AMS Biotechnology	CS-502
Collagenase from Clostridium histolyticum type 2	Sigma-aldrich	C6885
Bovine serum albumin	Sigma-aldrich	A8412
Lipofectamine™ LTX Reagent with PLUS™	Thermofisher	15338030
ON-TARGETplus Mouse Egfr siRNA	Horizon (Dharmacon)	L-040411-00-0010
ON-TARGETplus Non-targeting Control Pool	Horizon (Dharmacon)	D-001810-10-20
Critical Commercial Assays		
Free glycerol assay	Sigma-aldrich	F6428-40mL
LDH-Cytotoxicity Calorimetric Assay Kit II	BioVision	#K313
Human PEPD (Peptidase D) ELISA Kit	Elabsciences	E-EL-H5575.96
Proteome profile mouse phospho-RTK	RD system	ARY014
Mouse Xaa-Pro dipeptidase (PEPD) ELISA kit	CUSABIO	CSB-EL017784MO
RNeasy minikit (250)	Qiagen	74106
RNA 6000 Nano Kit	Agilent	5067-1511
TruSeq Stranded mRNA HT Sample Prep Kit	Illumina	20020595
Deposited Data		
RNAseq data mouse	This paper	N/A
Proteomic Data iPS	PRIDE repository	PXD001953
RNAseq data iPS	European Genome-phenome Archive (EGA)	EGAS00001000563
Experimental Models: Cell Lines		
3T3L1 Cells	Zen-Bio	SPL1F
RAW264.7 cells	ATCC	TIB-71
Experimental Models: Organisms/Strains		
C57Bl/6J	Charles River	JAX™ Strain, RRID:IMSR_JAX:000664
<i>C57BL/6J-Lepob</i>	Charles River	JAX™ Strain, RRID:IMSR_JAX:000632
<i>Pepdtm1a(KOMP)Wtsi</i>	Wellcome Trust Sanger Institute	MFPW; EPD0224_1_C04
Oligonucleotides		

Primers for mRNA expression	This paper	See Table S10
Human peptidase D (<i>PEPD</i>)	Applied Biosystems Inc.	Hs00944654_m1
Fatty acid synthase (<i>FASN</i>)		Hs00188012_m1
Solute carrier family 2 member 4 (<i>GLUT4</i>)		Hs00168966_m1
Adiponectin (<i>ADIPOQ</i>)		Hs00605917_m1
Peroxisome proliferator activated receptor gamma (<i>PPARG</i>)		Hs00234592_m1
Cd68 (<i>cd68</i>)		Hs00154355_m1
Tumor necrosis factor (<i>TNF</i>)		Hs00174128_m1
Collagen type VI alpha 3 chain (<i>COL6A3</i>)		Hs00915125_m1
Collagen type VI alpha 6 (<i>COL6A6</i>)		Hs01029204_m1
Collagen type IV alpha 1 chain (<i>COL4A1</i>)		Hs00266237_m1
Epidermal growth factor receptor (<i>EGFR</i>)		Hs01076090_m1
Software and Algorithms		
GraphPad Prism7 and 8	GraphPad Software	RRID: SCR_002798
Fiji	Fiji	RRID: SCR_002285
Zeiss LSM 510 Meta Confocal microscope with LSM 3D software	Carl Zeiss	N/A
`piano` R package	Väremo et al., 2013 ¹	N/A
STAR v2.5.1b	Dobin et al., 2013 ²	N/A
featureCounts v1.5	Liao et al, 2013 ³	N/A
Customized Python and R scripts	Haak et al., 2018 ⁴ ; https://github.com/bpucker/RNA-Seq_analysis	N/A
DESeq2	Love et al., 2014 ⁵	N/A

Table S10: Mouse and human gene primers used for RT-qPCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Probe (5'-3')	Reporter /quencher
Murine				
<i>18s</i>	CGG CTA CCA CAT CCA AGG AA	GCT GGA ATT ACC GCG GCT	GAG GGC AAG TCT GGT GCC AG	FAM/TAMRA
<i>36b4</i>	AGA TGC AGC AGA TCC GCA T	GTT CTT GCC CAT CAG CAC C		SYBR
<i>Bactin</i>	GCT CTG GCT CCT AGC ACC AT	GCC ACC GAT CCA CAC AGA GT	ATC AAG ATC ATT GCT CCT GAG CGC	FAM/TAMRA
<i>B2m</i>	ACT GAT ACA TAC GCC TGC AGA GTT	TCA CAT GTC TCG ATC CCA GTA GA		SYBR
<i>Adrge1</i>	CAG ATA CAG CAA TGC CAA GCA	GAT TGT GAA GGT AGC ATT CAC AAG TG	6TGC AGG GCA GGG ATC TTG GTT ATG C0	FAM/TAMRA
<i>Mrc1</i>	GCA TGG GTT TTA CTG CTA CTT GAT T	CAG GAA TGC TTG TTC ATA TCT GTC TT		SYBR
<i>Pparγ2</i>	GAT GCA CTG CCT ATG AGC ACT T	AGA GGT CCA CAG AGC TGA TTC C	AGA GAT GCC ATT CTG GCC CCA CCA ACT T	FAM/TAMRA
<i>Tnfa</i>	CATCTTCTCAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC	CACGTCGTAGCAAACCACC AAGTGGA	FAM/TAMRA
<i>Pepd</i>	CGGCATGCGCCACACCTCTT	TCGTCCGGTCATTGGGGGCT		SYBR
<i>Mmp9</i>	GCCGACTTTTGTGGTCTTCC	GGTACAAGTATGCCTCTGCCA		SYBR
<i>asma</i>	TCCTGACGCTGAAGTATCCGAT	GGTGCCAGATCTTTTCCATGTC		SYBR
<i>Col1a2</i>	CATGCTCAGCTTTGTGGATACG	CGTACTGATCCCGATTGCAA		
<i>Col3a1</i>	AAGGGCGAAGATGGCAAAGA	AGCCACTAGGACCCCTTCT		
<i>Col4a1</i>	GGCCCCAAAGGTGTTGATG	AGGTTCTCCCTTTTGACCTTGAG		
<i>Col4a2</i>	CCCATCTGACATCACACTTGTG	TGAGATTACGCCGGGTATCC		
<i>Col6a1</i>	GATGAGGGTGAAGTGGGAG A	CAGCACGAAGAGGATGTCAA		
<i>Fn</i>	GAGTGGAAGTGTGAGCGACA	GGTGAGTCTGCGTTGGTAA		SYBR
<i>Cox2</i>	CCCTGAAGCCGTACACATCA	GTCAGTGTAGAGGGCTTTCAAT TCT	TTGAAGAACTTACAGGAG AGAAGGAAATGGCTG	SYBR
<i>Glut4</i>	ACTCATTCTTGGACGGTTCCT C	CACCCGAAGATGAGTGGG		
<i>Adipone ctin</i>	CAGTGGATCTGACGACACCA A	TGGGCAGGATTAAGAGGAACA	AGG GCT CAG GAT GCT ACT GTT GCA AGC	FAM/TAMRA
<i>Leptin</i>	AGCATCCACTGCTATGGTAGC A	TCTTCTAGTCCCAAGCATTTTGG		

<i>Il6</i>	ACA CAT GTT CTC TGG GAA ATC GT	AAGTGCATCATCGTTGTTTCATA CA	ATGAGAAAAGAGTTGTGC AATGGCAATTCTGA	FAM/ TAMR A
<i>Mgl1</i>	TGCTGGTATTTGCCCATGAC	CCGACACCACCATCCTCTCT		SYBR
<i>Il18</i>	TGGGCCTCAAAGGAAAGAAT	CAGGCTTGTGCTCTGCTTGT		SYBR
<i>Arg1</i>	CATTTGGGTGGATGCTCACA	TGGTACATCTGGGAACCTTCCTT T		SYBR
<i>Fizz1</i>	GGGATGACTGCTACTGGGTG	TCAACGAGTAAGCACAGGCA		SYBR
<i>Clec10a</i>	TGAGAAAGGCTTTAAGAACT GGG	GACCACCTGTAGTGATGTGGG		SYBR
<i>Adrp</i>	TCATCCAGA AAGCTGGAGCCA GCAGTC	GCAGTCTTTCCTCCATCCTGTC		SYBR
<i>Fasn</i>	GCCCAGACAGAGAAGAGGCA	CTGACTCGGGCAACTTCCC	GGAGGAGGTGGTGATAGC CGGTATGTC	FAM/ TAMR A
<i>Myo D</i>	AGCACTACAGTGGCGACTCA	GGCCGCTGTAATCCATCA		
<i>Myogeni n</i>	CCTTGCTCAGCTCCCTCA	TGGGAGTTGCATTCACTGG		
<i>Myostat in</i>				
<i>Ttn</i>	GAGGTGCCGAAGAAACTTGT	TTGGGTGCTTCCGGTACTT		
<i>Cdkn1a</i>	GAACATCTCAGGGCCGAAAA	CAATCTGCGCTTGGAGTGATAG		
<i>Ccnd1</i>	CATCCATGCGGAAAATCGT	TCTACGCACTTCTGCTCCTCA		
<i>Tgfb1</i>	GCAGTGGCTGAACCAAGGA	AGAGCAGTGAGCGCTGAATC		
Human cohort 1				
PLIN1	AAGTTGAAGCTTGAGGAGCG AGG	GCTCGCGATGGGAACGCTGA		SYBR
CIDEA	GAGGTCCAACGCAGTCCAGC TG	GTACGCACTGACACATGCCTGG AG		SYBR
ADRP	GAGTCGTCTTCGGGACGCGC	TTGGCAACTGCAATTTGCGGCT		SYBR
TIP47	TGCAGAGCGGCGTGGACAAG	CGACCTGCTCTGGCTTGGGC		SYBR
Human cohort 2				
18s	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT	GAGGGCAAGTCTGGTGCC AGCA	FAM/ TAMR A
PEPD	TTCCGCCAGGAGTCCTTCTT	GTCGTCCACGGCATACTTCT		SYBR